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Anticancer and Molecular Docking Studies of Chalcone Derivatives

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Abstract: Chalcone derivatives namely (2E)-3-(4-hydroxy-3-ethoxyphenyl)-1-(4- hydroxyl phenyl) prop-2-en-1-one – (HEHP) and (2E)-3-(3,4 dimethoxy phenyl)-1-(1-hydroxy-2 napthyl)prop-2-en-1-one – (DHNP) were synthesized by acid/ base catalyzed Claisen-Schmidt reaction. The cytotoxic activities of the compounds against normal Vero cell and breast cancer MCF7 cell lines were assessed by MTT assay method. Since cytotoxicity is inversely proportional to the cell viability, higher IC_{50} values with the Vero cell line suggests that the compounds are greatly non- toxic to normal Vero cell line. The IC_{50} values of 7.8 and 62.5µg/ml, exhibited against the MCF7 cells by the derivatives, HEHP and DHNP suggest the significant anticancer activity of the compound HEHP. Induced fit docking analysis was also carried out for the compounds and is compared with the same co-crystal ligand. The exemestane (co-crystal) has docked well at the active site of target protein O-H...N and O-H...O hydrogen bonds involving oxygen atom of the co-crystal with the nitrogen atom of the amino acid residue MET 374 and Oxygen atom of the residue Thr310, at a distance of 2.86 and 2.90Å, respectively, with the glide score of -9.473 and glide energy of -48.055Kcal/mol. The compounds HEHP and DHNP have been observed to be at the active site of target protein with the glide score of –8.505, -8.330 and glide energy of - 41.503, -50.661 Kcal/mol, respectively, which are comparable with the corresponding values of the co-crystal.
Keywords: Chalcone, Anticancer activity, cytotoxicity, Docking study, IC_{50} value, glide score, glide energy.

Introduction

Chalcones are a major class of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff and have been extensively studied for their broad spectrum of biological activities[1], including antibacterial[2,3], antifungal[4,5], antimicrobial[6,7], antitumor[8], anticancer [9,10,11], antimalarial [12] ,anti-inflammatory[13], antileishmanial[14] and antioxidant[15,16] activities. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new class of drugs having improved potency and lesser toxicity. In view of the extended scope of biological applications, the compounds HEHP & DHNP of chalcone have been synthesized.

Breast Cancer, is the second most proliferated cancer after the lung cancer. Estrogens are suggested to cause breast cancer by stimulating cell growth and proliferation through receptor- mediated processes and via their genotoxic metabolites[17, 18]. Therefore, inhibition of estrogen production/ effect is a common practice for breast cancer treatment[19] and can be inhibited by aromatase inhibitors (AI). Women who started having periods earlier or entered menopause later than usual have a high risk of developing breast cancer, because their bodies

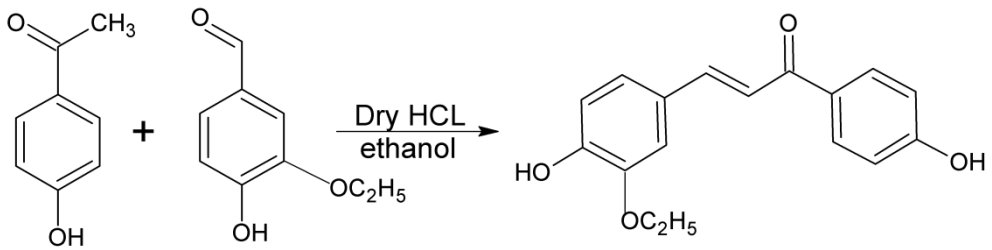
have been exposed to estrogen for longer time. Post-menopause, obese and overweight women may have a high risk of developing breast cancer. The breast cells multiply in an orderly fashion and new cells are made to replace the died ones. But in cancer, the cells multiply uncontrollably and there are too many cells progressively more and more than there should be. The breast cells are positive and more likely to respond hormonal therapies with Tamoxifen, Raloxifene, Toremifene. Tamoxifen is a drug, taken orally as a tablet, which interferes with the activity of estrogen but with serious side effects such as blood clots, stroke, uterine cancer, and cataracts. Other reactions include leg swelling/pain, breathing trouble, chest pain, vision changes. These side effects restrict the usage of the synthetic anticancer drugs and prompted the researcher to find new and natural anticancer compounds.

Experimental

(i) Synthesis of the Compounds

Synthesis of (2E)-3-(4-hydroxy-3-ethoxyphenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one – (HEHP)

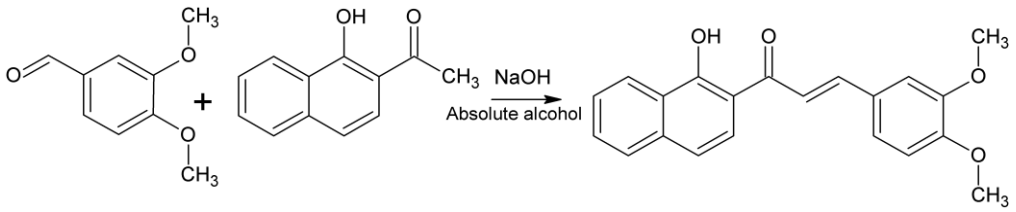
The compound is synthesized using the acid catalyzed Claisen-Schmidt reaction[20]. Dry HCl gas was made to pass through a well-cooled and stirred solution of 4-hydroxyacetophenone (0.05mol) and 4-hydroxy-3-ethoxybenzaldehyde (0.05mol) in 120ml of absolute alcohol taken in a 250ml round-bottomed flask for a time frame of one hour. Wine red coloured solution was formed. On addition of sufficient quantity of ice cold water yellow coloured precipitate of (2E)-3-(4-hydroxy-3-ethoxyphenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one was formed (Scheme.1). It was filtered, then washed with double distilled water and finally allowed to dry. The dried product was re-crystallized from hot ethanol [yield is 80%; Melting Point: 220°C]



Scheme.1 .Reaction Scheme of HEHP

Synthesis of (2E)-3-(3, 4 dimethoxy phenyl)-1-(1-hydroxy-2 napthyl)prop-2-en-1-one –(DHNP)

The compound (DHNP) is also synthesized following the base catalyzed Claisen-Schmidt reaction [21]. In a 250 ml round-bottomed flask 2-acetyl-1-naphthol (0.05mol) and 3, 4-dimethoxybenzaldehyde (0.05mol) were taken to which 120ml of absolute alcohol was added and stirred at room temperature for a span of 5 minutes. Then 20ml of 10% sodium hydroxide solution was added and the mixture was stirred for a time span of 8 hours. The reddish purple coloured precipitate formed by adding sufficient amount of cold dilute hydrochloric acid was filtered, washed with distilled water and dried. The formed crude chalcone derivative was recrystallized twice from absolute alcohol [yield: 90%; Melting Point: 110°C]. The reaction scheme is given in Scheme.2



Scheme. 2. Reaction Scheme of DHNP

(ii) Evaluation of Biological Activity of the Compounds

The cytotoxic activity of the synthesised compounds was evaluated against normal Vero (African green monkey kidney) and MCF-7 (human breast cancer) cell lines by MTT Assay method. Microculture Tetrazolium (MTT) assay, the colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

In the MTT assay procedure, the cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Presence of viable cells was visualized by the development of purple colour due to formation of formazan crystals which is proportionate to the viable cell number. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula

% cell viability = [A570 of treated cells / A570 of control cells] × 100

Graphs are plotted using the % of Cell viability along Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

The Cytotoxic Activity of the Compounds on Normal Vero Cell Line

The results of cytotoxicity evaluation of the compounds with concentration ranging from 1000 to 7.8 µg/ml are shown in Figures (1 ,2) and Table 1. It is noted from the results that the cell viability decreases as the concentration of the compounds increases. Even for higher concentration as 1000µg/ml of the compounds, percentage of cell viability for HEHP and DHNP are 80% and 77.77%, respectively. Since the cell viability is greater than 50% for both the compounds, the IC_{50} value may be obtained for concentration greater than 1000 µg/ml and so it cannot be calculated from the present graph drawn. As cytotoxicity is inversely proportional to the cell viability, higher IC_{50} values suggests that the compounds are almost non- toxic to normal Vero cell line. At 7.8 µg/ml concentration, the cell viability is noted as 95.55% for HEHP and 97.77% for DHNP that shows low cytotoxic effect.

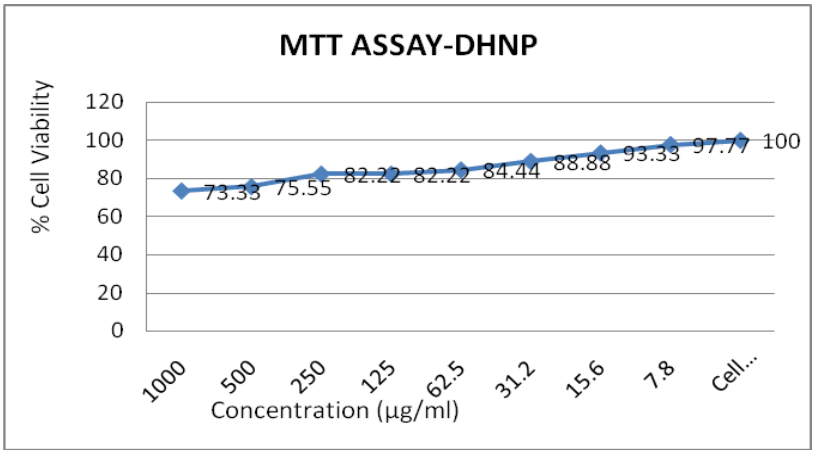


Fig.1 Cytototoxic Activity of Compound HEHP on Vero Cell Line

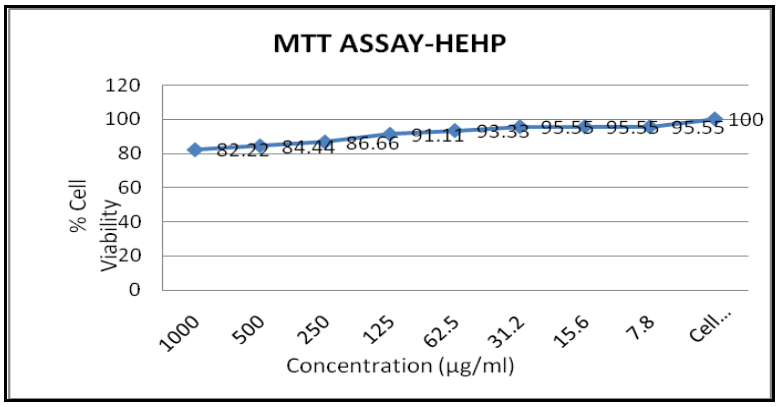


Fig.2 Cytototoxic Activity of Compound DHNP on Vero Cell Line

Table 1. Cytotoxic Activity of Compounds on Vero Cell Line

Concentration (µg/ml)	Dilutions	Compound HEHP		Compound DHNP	
		Absorbance (O.D)	Cell Viability (%)	Absorbance (O.D)	Cell Viability (%)
1000	Neat	0.37	82.22	0.33	73.33
500	01:01	0.38	84.44	0.34	75.55
250	01:02	0.39	86.66	0.37	82.22
125	01:04	0.41	91.11	0.37	82.22
62.5	01:08	0.42	93.33	0.38	84.44
31.2	01:16	0.43	95.55	0.4	88.88
15.6	01:32	0.43	95.55	0.42	93.33
7.8	0.08611	0.43	**95.55**	0.44	**97.77**
Cell control	-	0.45	100	0.45	100

Table 2. Anticancer Effects of the Compounds on Mcf7 Cell Line

Concentration (µg/ml)	Dilutions	Compound HEHP		Compound DHNP	
		Absorbance (O.D)	Cell Viability (%)	Absorbance (O.D)	Cell Viability (%)
1000	Neat	0.05	8.47	0.11	18.64
500	01:01	0.07	11.86	0.15	25.42
250	01:02	0.1	16.94	0.18	30.5
125	01:04	0.12	20.33	0.26	44.06
62.5	01:08	0.16	27.11	0.3	**50.84**
31.2	01:16	0.21	35.59	0.38	64.4
15.6	01:32	0.25	42.37	0.42	71.18
7.8	0.08611	0.31	**52.54**	0.44	74.57
Cell control	-	0.59	100	0.59	100

Anticancer Activity of the Compounds on Mcf7 Cell Line

The grown compounds were subjected to the anticancer activity studies and the results are shown in Figures(3,4) and Table 2. The recorded results reveals that the cell viability decreases as the concentration of the compounds increases, The concentration corresponding to the IC_{50} values of the compounds HEHP and DHNP are 7.8, and 62.5 µg/ml, respectively, and suggests that the compound HEHP is having good anticancer effect on breast cancer cell line.

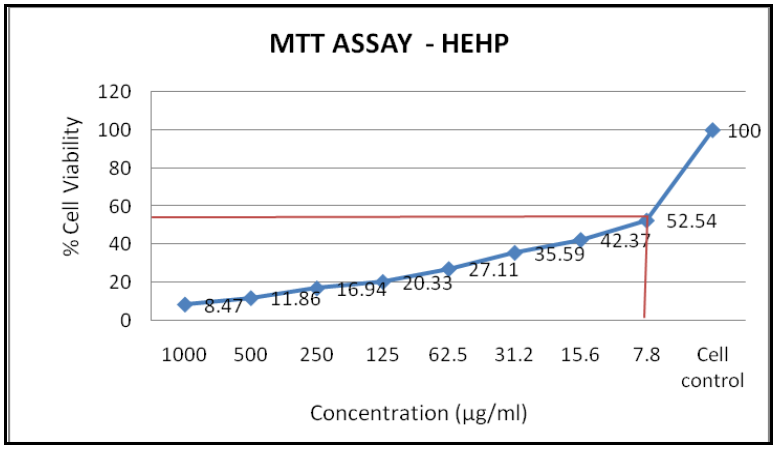


Fig. 3. Anticancer Activity of Compound HEHP on Mcf7 Cell Line

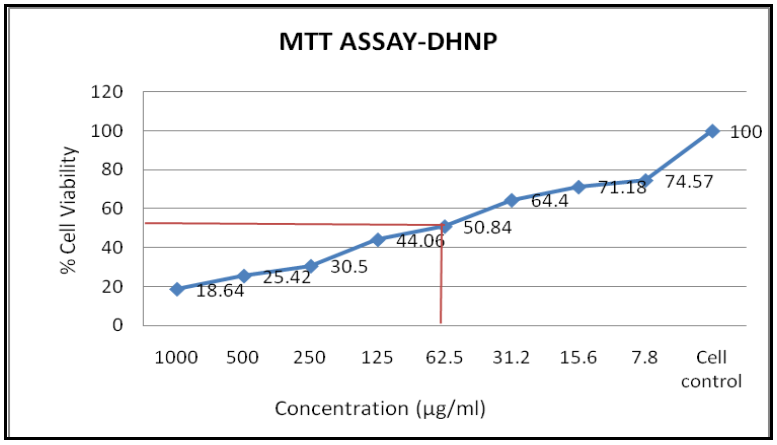


Fig. 4. Anticancer Activity of Compound DHNP on Mcf7 Cell Line

Results

The inhibitory effect of the compounds on MCF7 cancer cells shows that the cells experienced a significant decrease in viability at very low concentration of the compounds. For the compound HEHP, IC_{50} value on MCF-7 cell line is 7.8µg/ml, while on Vero cell line is greater than 1000 µg/ml, which suggests that the compounds have strong inhibition against the MCF-7 cell lines and weak inhibition on normal healthy body cell. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio guided studies, if it exerts an IC_{50} value ≤ 30 µg/ml [22]. IC_{50} value obtained for the compound HEHP is satisfying the said condition(less than 30µg/ml), and it may be used for pharmaceutical applications as potential anticancer compound based on their ability to induce cytotoxicity in cancer cells with relatively low toxicity to normal cells. Compound DHNP is found to possess moderate anticancer activity. Further studies with *in vivo* and clinical trials needs to be conducted to establish these as safe agents for cancer therapy.

(iii) Docking Studies

Molecular docking studies of the synthesized compounds has been carried out to understand the binding mechanisms of these bioactive compounds with human aromatase protein (receptor) using XP docking program of Maestro, Schrödinger software and to evaluate whether these molecules can be used as potential drug candidates. Estrogens are female sex hormones involved in the development and growth of breast tumors. Aromatase is expressed at a higher level in human breast cancer tissue than in normal breast tissue. Aromatase inhibitors, which stop the production of estrogen in postmenopausal women, have become useful in the management of patients with breast cancer whose lesion was found to be estrogen receptor positive. Twenty to thirty percent of the patients who fail anti-estrogen treatment respond to aromatase-inhibitor treatment. Exemestane (AROMASINR), 6-methylideneandrosta-1,4-diene-3,17-dione, a rationally designed, selective,

orally active, long-lasting and safe hormonal drug, has demonstrated impressive pharmacological and clinical properties in improving the treatment of breast cancer patients

Protein, Ligand Preparation and Induced Fit Docking

The three dimensional crystal structure of human Aromatase complexed with co-crystal ligand exemestane, ($C_{16}H_{14}F_3NO_3S$) (PDB id: 3S7S) was downloaded from the Protein Data Bank (PDB). All computational works were performed using the molecular modeling software Maestro8. GLIDE-5.5 (Grid-based Ligand Docking with Energetics) performs flexible (IFD) docking between the ligand molecules with a macromolecule. The ligand preparation is to prepare the three dimensional structure of drug like molecules in maestro format. The impact module performs conversions, apply corrections to the structures, generate variations on the structures and optimize the structures. The structures were minimized using impact energy minimization with 1000 cycles of Steepest Descent and 5000 cycles of Conjugate gradient.

Results and Discussion

Molecular docking study has shown that the compounds bind well at the active site of human aromatase. The exemestane (co-crystal) has docked well at the active site of target protein with the glide score of -9.473 and glide energy of -48.055 Kcal/mol. Oxygen atom of the co-crystal interacts with nitrogen group of MET 374 and Oxygen atom of THR 310 by forming hydrogen bonds at a distance of 2.86 and 2.90Å, respectively (Figure.5). In the compound HEHP, the oxygen atom O1 in the ethoxyphenyl group of the compound interacts with the nitrogen atom of MET 374 (O-H...N) at a distance of 3.11 Å and the nitrogen atoms of ARG 115 interact with the oxygen atom O4 (N-H...O) of the carbonyl group of the compound at a distance of 3.26 and 3.00 Å with the glide score of - 8.505 and glide energy of 41.503 Kcal/mol(Figure.6). In the compound DHNP, the oxygen atoms O3 and O4 in the dimethoxyphenyl group of the compound interacts with the oxygen atoms of THR 310(O-H...O) and SER 314(O-H...O) at a distance of 3.23 and 3.15 Å, respectively with the glide score of -8.330 and glide energy of -50.661Kcal Kcal/mol(Figure.7). Both the compounds HEHP and DHNP, show more number of hydrogen bond interactions (Table 3) and exhibit good glide score and glide energy compared to that of the co-crystal, hence can act as potential inhibitors of human Aromatase enzyme.

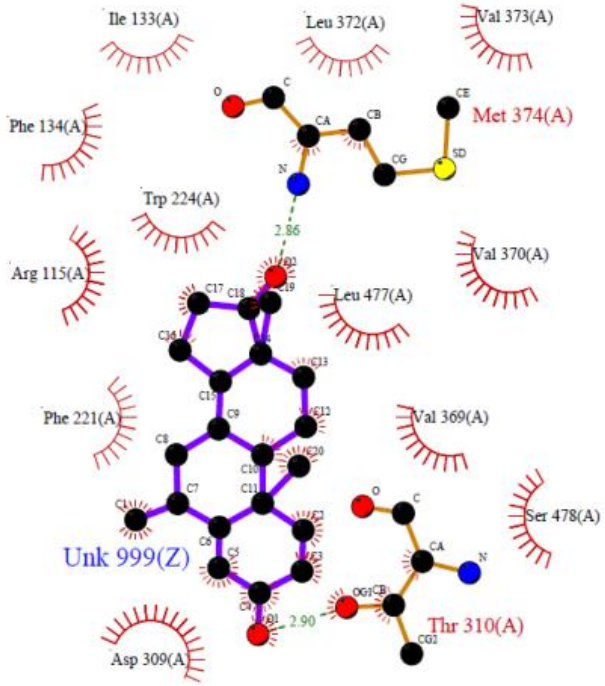


Fig.5 Ligplot Showing the Interactions of Cocrystal Ligand at The Active Site and Hydrophobic Residues.

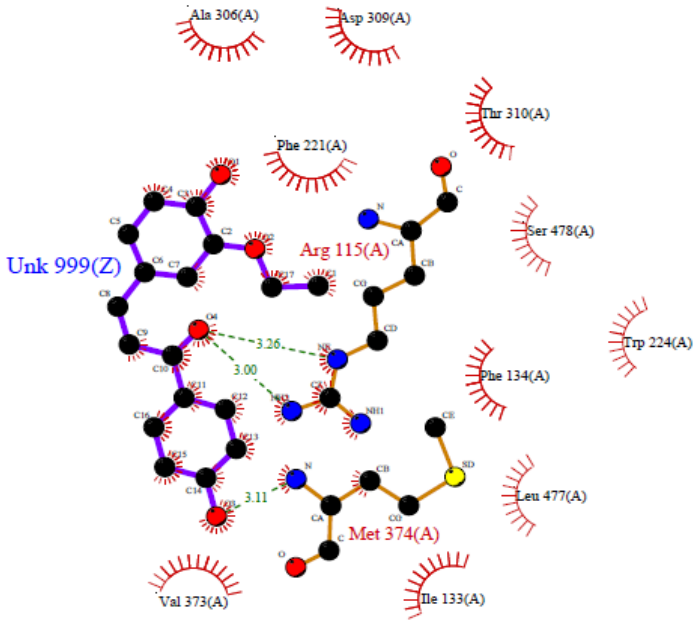


Fig. 6 .Ligplot Showing the Interactions of the Compound HEHP at the Active Site and Hydrophobic Residues.

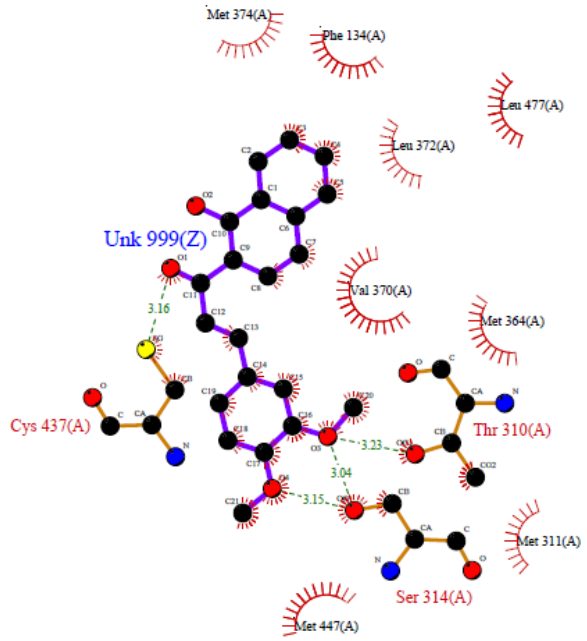


Fig.7 Ligplot Showing the Interactions of the Compound DHNP at the Active Site and Hydrophobic Residues.

Table 3. Induced Fit Docking Studies of the Compounds with the Human Aromatase Enzyme as Protein Target

Ligand	No of poses	H-bond interaction D-H…A	Distance (Å)	Glide score	Glide Energy Kcal/mol
Co-crystal	3	1.(MET 374)N-H…O (THR 310)O-H…O	2.86 2.90	9.473	48.055
		2.(MET 374)N-H…O (ASH 309)O-H…O	2.95 3.16	-8.278	-41.263
		3.(ARG 115)N-H…O	3.17	-6.933	-45.834
HEHP	4	1.(MET 374)O-H…N (ARG 115)N-H…O (ARG 115)N-H…O	3.11 3.26 3.00	-8.505	-41.503
		2.(ARG 145)O-H…N (ALA 438)O-H…O (ASP 309)O-H…O (LEU 477)O-H…O	2.73 3.09 3.23 2.84	-.8.042	-41.757
		3.(ARG 145)O-H…N (LEU 477)O-H…O	2.80 2.81	-7.644	-40.840
		4.(VAL 369)N-H…O (MET 307)O-H…S	3.29 3.22	-7.187	-48.628
DHNP	2	1.(CYS 437)S -H…O (SER 314)O-H…O (SER 314)O-H…O (THR 310)O-H...O	3.16 3.15 3.04 3.23	-8.331	-50.661
		2.(MET 374)N-H…O (LEU 372)O-H…O (CYS 437)O-H…O	3.27 2.91 3.22	-8.149	-47.050

Conclusion

Two new chalcone derivatives were synthesised and screened for cytotoxicity on MCF7 cancer cell and normal Vero cell lines. The cytotoxic study throws light on the fact that the chalcone HEHP is potent anti-proliferative agent against human breast cancer cells without being significantly cytotoxic to normal cells and DHNP exhibits moderate anticancer activity. The docking studies of the compounds show good docking score and hydrogen bonding interactions and are comparable to that of the co-crystal. This study may lead to development of new therapeutic agents in our fight against cancer.

Acknowledgement

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References

1. Nowakowska, Z., A review of anti-infective and anti-inflammatory chalcones, Eur. J. Med. Chem., (2007), 42: 125-137.
2. Birendra kumar, Binita Kumari, Neha Singh, Ram,.B and Balram. B., Synthesis, Antibacterial and Antifungal Activity of Flavanones and Chalcones Derived From 2-Hydroxy-Acetonaphthone, Journal of Applicable Chemistry., 2014, 3 (4): 1468-1474.
3. Ravi Sethi and Mamta Ahuja, Synthesis, Characterization and Antibacterial Activity of Cobalt Complex of 2-Pyrazoline with Pyridinyl Moiety , International Journal of PharmTech Research., 2016, 9(1): 35-40.
4. Lahtchev, K. L., Batovska, D. I, Parushev, S. P, Ubiyvovk, V.M., Sibirny A.A., Antifungal activity of chalcones: a mechanistic study using various yeast strains,European Journal of Medicinal Chemistry., 2008, 43:2220-2228.
5. Ravindra S., Dhivare , S., Rajput ,S., Microwave assisted solvent free synthesis and antifungal evaluation of3, 5-bis-(4-hydroxy-3-methoxybenzylidene)-Nphenylpiperidine-2, 6-dionederived from N-phenyl glutarimides ,International Journal of ChemTech Research., 2016, 9(3) : 325-331.
6. Balkrishna Tiwari, AS Pratapwar, AR Tapas, SR Butle and BS Vatkar, Synthesis and Antimicrobial Activity of Some Chalcone Derivatives, International Journal of ChemTech Research., 2010, 2(1): 499-503 .
7. Faruk Alam, A Study on the Antimicrobial and Antioxidant Activities of Some New 1, 3, 4-Thiadiazole Derivatives, International Journal of ChemTech Research., 2014-2015, 7(5): 2520-2531.
8. Park, E. J., Park, H. R., Lee, J. S., Kim, J., Licochalcone A: an inducer of cell differentiation and cytotoxic agent from Pogostemon cablin, Planta Med., 1998, 64(5), 464–466.
9. Anto, R. J., Sukumaran, K., Kuttan, G., Rao, M. N. A., Subbaraju, V., Kuttan R., Anticancer and antioxidant activity of synthetic chalcones and related compounds, Cancer Lett.,1995, 97(1), 33–37.
10. Sharma Poonam , Majee Chandana, A Review on Anticancer Natural Drugs, International Journal of PharmTech Research., 2015, 8(7): 131-141.
11. Vedavalli, S., Srinivasan, T., Suhitha, S., Velmurugan, D., Kandaswamy, M., Molecular docking study of chalcone derivative with Human estrogen receptor as target protein and its anti-cancer activity against HepG2 Cells , International Journal of PharmTech Research., 2014 , 6(5): 1580-1583.
12. Ugwu, David. , Ezema, Benjamin, E ., Eze, Florence, U Onoabedje Efeturi, A , Ezema, Chidimma G, Ekoh, Ogechi, C and Ayogu, Jude. Synthesis and Antimalarial Activities of Chalcone Derivatives , International Journal of ChemTech Research., 2014-2015, 7(4): 1966-1984.
13. Hesieh, H. K., Tsao, L. T., Wang, J. P., Lin, C. N. S., Synthesis and anti-inflammatory effect of chalcones, J. Pharm. Pharmacol., 2000, 52: 163–171**.**
14. Nielsen, S. F., Christensen, S. B., Cruciani, G., Kharazmi, A., Liljefors, T. Antileishmanial chalcones: statistical design, synthesis, and three-dimensional quantitative structure-activity relationship analysis, J. Med. Chem., 1998, 41: 4819-4832.
15. Ravichandran R, Rajendran. M, D., Devapiriam, Studies on Chalcone Derivatives Antioxidant and Stability Constant , J. Chem. Bio. Phy. Sci., Sec. A., 2013, 3 (4); 2446-2458.
16. Shendarkar, G.R., DSavant ,M., Badole K.D. , Waghmare G.S., Synthesis and Pharmacological Evaluation of Some Chalcone Derivatives, International Journal of PharmTech Research., 2012 ,4(3): 1129-1135.
17. Cavalieri, E., chakravarti, D., Guttenplan ,J., Hart, E., Ingle, J., Jankowiak, R., Muti, P.,Rogan, E., Russo, J., Santen, R., Sutter, T., Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention, Biochim Biophys Acta.,2006, 1766:63-78.
18. Yager, J.D., Davidson ,N.E., Estrogen carcinogenesis in breast cancer, N Engl J Med., 2006, 354:270-282.
19. Czajka-Oraniec I, Simpson E.R., Aromatase research and its clinical significance, Endokrynol Pol ., 2010, 61:126-134.
20. Sathya, S., Reuben Jonathan, D., Prathebha, K., Usha, G and Jovita, J., (E)-3-(4-Hydroxy-3-methoxyphenyl)-1- (4-hydroxyphenyl)prop-2-en-1-one , Acta Cryst., (2014), E70, o593--o594.
21. Sathya, S., Reuben Jonathan, D., Prathebha, K., Jovita, J., & Usha, G., Crystal structure of 4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl benzoate , Acta Cryst., (2014), E70, o1007.

22. Suffness ,M., and Pezzuto, JM., Assays related to cancer drug discovery. In Methods in Plant Biochemistry: Assays for Bioactivity Hostettmann K (Ed), Academic Press, London, 1990, 6: 71–133.

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