International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.2, pp 995-1004, 2017

Synthesis and Screening for Anticancer Activity of a series of Novel Chalcone derivatives on MCF-7 Cell Line

S. Sathya[1]*, D. Reuben jonathan[2],S. Pandiammal[3], B. K. Revathi[4] and G. Usha[5]*

[1]PG Department of Physics, Bhaktavatsalam Memorials College, Chennai-80, India
[2]PG and Research Department of Chemistry, Madras Christian College, Chennai-59, India
[3]PG and Research Department of Zoology, Sir Theagaraya College, Chennai-21, India
[4]PG and Research Department of Physics, Madras Christian College, Chennai-59, India
[5*] PG and Research Department of Physics, Queen Mary's College (A), Chennai-4, India

Abstract : In recent past the study of anti-cancer activity of number of chemical compounds has gained momentum. It is our observation that the chalcone containing compounds exhibit potent anti-cancer activities. In our attempt to understand chalcone derivatives as inhibitors of cancerous cells, a series of four new and novel chalcone compounds namely (E)-3-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one(**HMHP-I**),(2E)-1-(4-hydroxy-3- methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one(**HMHP-II**),4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl benzoate(**MPEB**) and 3-(4-methoxyphenyl)-5-(4-benzyloxyphenyl)-6-methyl-2-cyclohexen-1-one(**MBMC**) have been synthesized by Claisen-Schimdt reaction in which acetophenone condensed with various aromaticaldehydes. All the four derivatives were screened for their anti-cancer effect by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method and found to be an excellent inhibitor on MCF-7 cell line.

Keywords: chalcone; cytotoxicity; MCF-7 cells; cell lines;MTT assay method, % Cell viability.

1. Introduction

Cancer is one of the most dangerous, fast propagating with quite high mortality rate disease of present century even in the developed countries. The situation is even worse in the under developed countries due to lack of knowledge, poverty and non-availability of quality drugs[1].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[2], including antibacterial[3], antifungal[4], antimicrobial[5],antitumor[6],anticancer[7],antimalarial[8],anti-inflammatory[9],antileishmanial[10]and antioxidant[11] activities. Compounds containing chalcone moiety are identified as precursor of flavonoids which are abundant in edible plants. Substituted chalcones are of particular interest for various studies because of their vital role as precursor in the biosynthesis of flavanoids abundantly available in plant kingdom. These bichromophoric molecules separated by a keto-vinyl chain are very useful as substrate for the synthesis of biologically very important heterocyclic compounds like cyclohexenone derivatives and pyrazoline derivatives. The scope of introducing variations in the structure of chalcones by changing various substituents has created an interest among the scientists of different fields. Besides the different traditional methods used for synthesizing these

molecules such as base catalyzed (NaOH) and acid catalyzed (dry HCl gas) in the presence of suitable solvent, many new eco-friendly methods like use of ultrasonic radiations[12], microwave assisted[13,14], solvent free synthesis by grinding[15,16] etc., have been developed. The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers[17].The cytotoxic study throws light on the fact that the chalcone is potent anti-proliferative agent against human breast cancer cells without being significantly cytotoxic to normal cells[18.] The chemical structure of chalcone (1,3-diphenyl-2-propen-1-ones) consists of two aromatic rings joined by a three carbon α, β- unsaturated carbonyl system. The prepared compounds are α, β- unsaturated ketones (chalcones) containing the keto-ethylenic group (–CO–CH=CH-). In this communication, we report the anticancer activity of four as grown crystals through Claisen-Schimdt reaction.

2. Reaction procedure- Synthetic Method of Aromaticchalcones

2.1. Synthesis of Chalcones

In the synthesis procedure using Claisen-Schimdt reactionthe compound **(E)-3-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one**was prepared from 4-hydroxyacetophenone and vanillin, the compound***(2E)-1-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one*** from 4-hydroxy-methoxyacetophenone and 4-hydroxybenzaldehyde, the compound ***4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl benzoate*** from 4-hydroxyacetophenone and4-methoxybenzaldehyde, the compound ***3-(4-methoxyphenyl)-5-(4-benzyloxyphenyl)-6-methyl-2-cyclohexen-1-one*** from1,1'- benzene -1,4-diyldiethanone and 4-(benzyloxy) benzaldehyde, respectively.

In addition triethylamine and benzoylchloride were added to the compound MPEBby esterification reaction and the compound MBMCwas added with ethyl methyl ketoneand 10% NaOH, respectively.

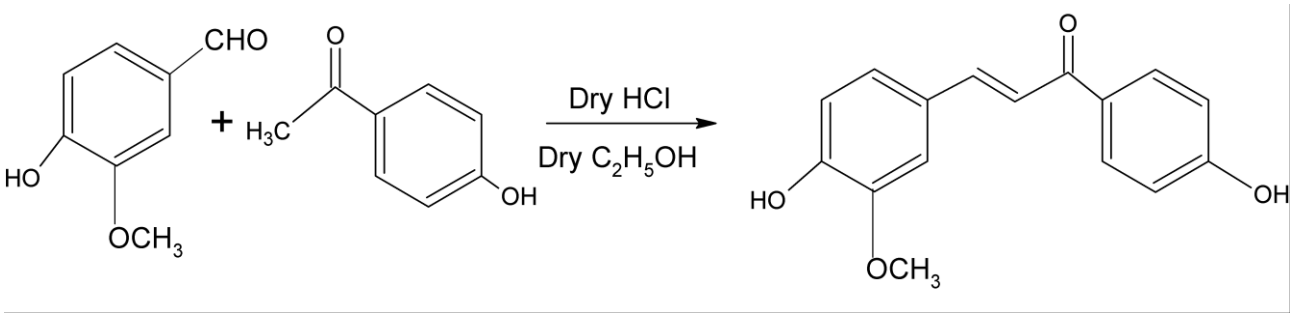


Figure 1: Reaction scheme for HMHP-I

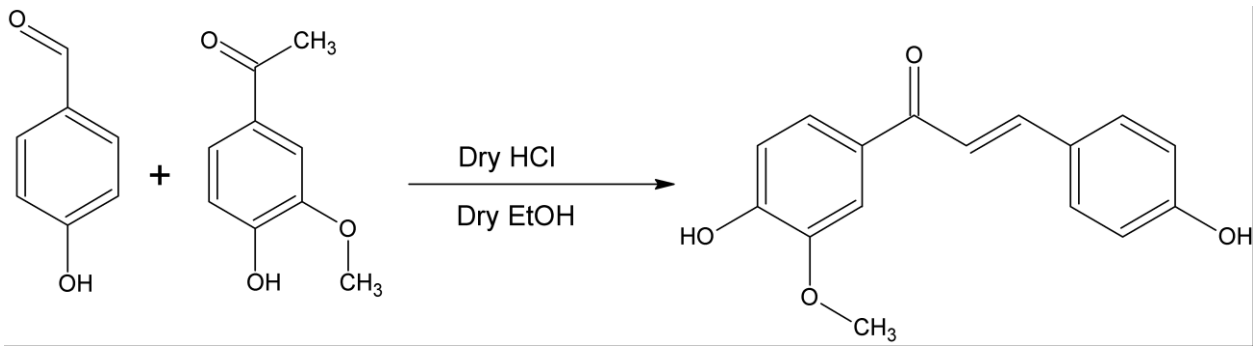


Figure 2: Reaction scheme for HMHP-II

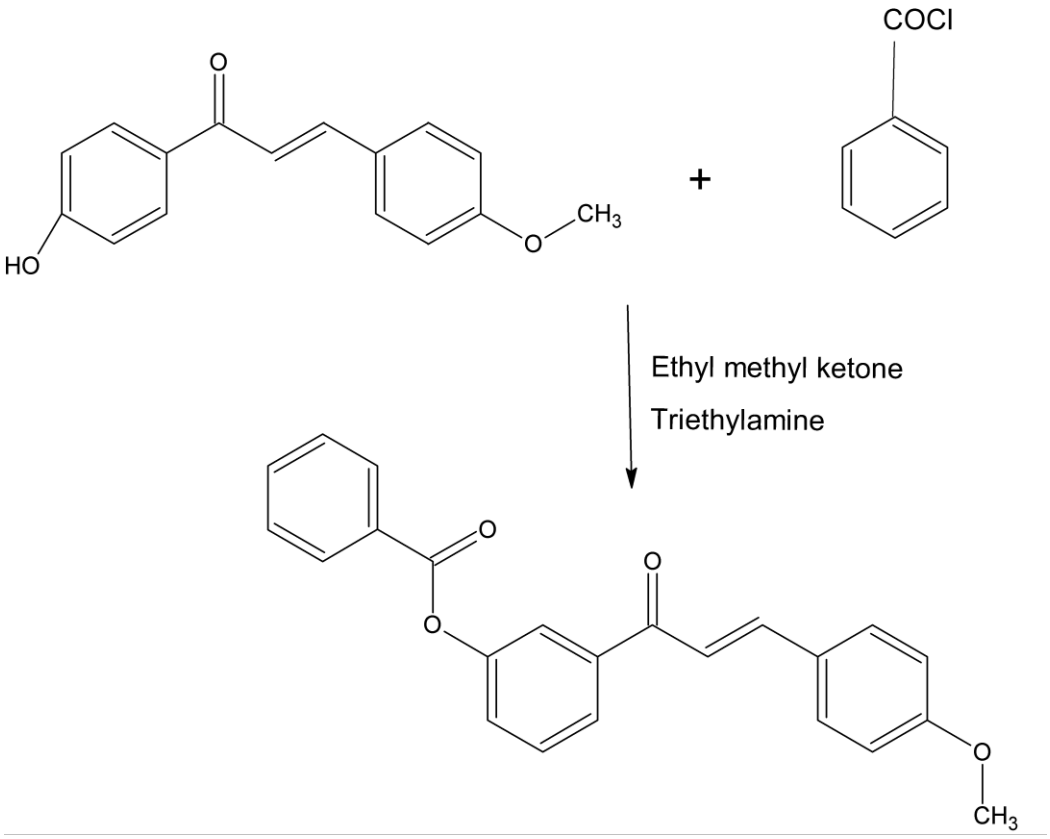
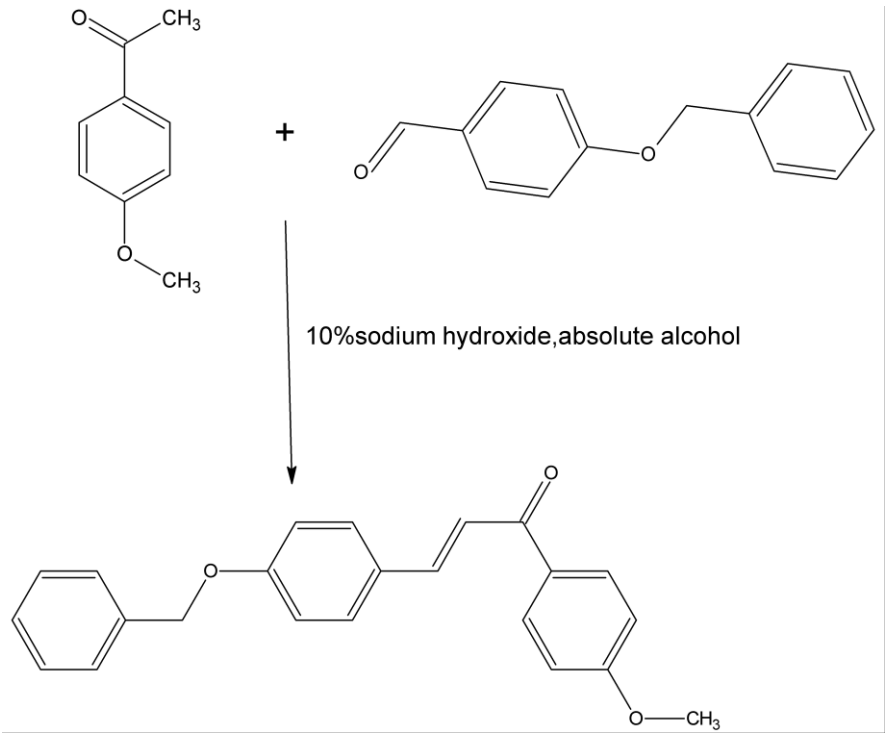


Figure 3: Reaction scheme for MPEB



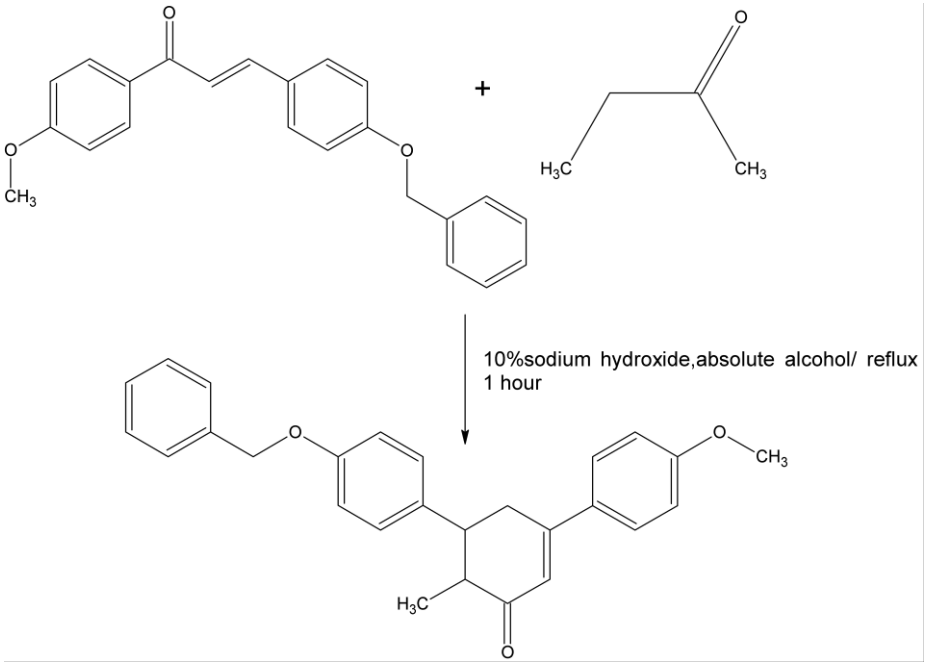


Figure 4: Reaction scheme for MBMC

3. MTT Assay Method

The magnitude of cytotoxicity of the grown compounds namely HMHP-I, HMHP-II, MPEB and MBMC were determined by MTT assay method[23]. In the procedure,Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C under 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,. and 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank and the concentration required for a 50% inhibition (IC_{50}) was determined graphically.

The percentage of cell viability was calculated using the following formula:

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

Graphs are plotted against % of cell viability versus concentration of the sample. Cell control and sample control is included in each assay to compare the full cell viability assessments.

4. Results and discussion

A series of four new chalcone derivatives HMHP-I, HMHP-II, MPEB and MBMC were synthesized by slow evaporation solution method. The 3D structures were elucidated from XRD analysis and the results were reported[19-22]. Cytotoxic activity of the grown crystals was studied by the MTT assay method.

Cytotoxic report on the crystal HMHP-I, HMHP-II, MPEB and MBMC

The organic crystal HMHP-I, HMHP-II, MPEB and MBMC were investigated for their anti-cancer activity on *MCF-7* cell lines by the MTT assay method[24-32]. The Figure 4(a) & 4(b) shows the image of the normal *MCF-7* cell lines and cytotoxic effect of HMHP-1, HMHP-II, MPEB and MBMC at 15.6µg/ml and 7.8 µg/ml concentrations, respectively. The % of cell viability at different concentrations of the sample is listed in Table 2. The plot of % of cell viability of HMHP-I, HMHP-II, MPEB and MBMC versus concentration in µg/ml is shown in Figure 4(c). The comparative IC_{50}values are shown in bar graph (Figure (4d)).

The concentration required for a 50% of viability (IC_{50}) was determined graphically and found that the compounds HMHP-I & MPEB were with 50.79% cell viability and HMHP-II & MBMC were with 49.2% at 15.6µg/ml & 7.8µg/ml, respectively. Scientific analysis of the dataleads to the conclusion that the compoundsHMHP-I, HMHP-II and MPEB, may be most suitable material for pharmaceutical application with the concentration less than 15.6µg/ml. It is of our understanding that the grown compounds exhibit excellent inhibition effect on **MCF-7 Cell line,** so that they may be used for cancer (breast) treatment.

Conclusion

A series of four new and novel chalcones containing compounds were synthesized and screened for their anti-cancer activity from MTT assay method. The compound HMHP-I and MPEB show 50.79% inhabitation of viability at a concentration less than 15.6µg/ml on MCF-7 cell lines and the compounds HMHP-II and MBMC exhibit 49.2% inhabitation of viability at concentration of 15.6 and 7.8µg/ml on MCF-7 cell lines, respectively. Chalcone containing compounds have been observed to acts as potent inhibitor of cancer cells; our studies on the same class of compounds also confirm the observed behavior beyond doubt. Attempts will be made to synthesis more compounds in this series to achieve lead novel drugs for a treatment of cancer.

Table 1: Characteristic features of the compounds

S.No	IUPAC Name	Empirical Formula	Melting point°C	Yield %
1	(E)-3-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	$C_{16}H_{14}O_4$	229	85
2	(2E)-1-(4-hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	$C_{16}H_{14}O_4$	199	95
3	4-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]phenyl benzoate	$C_{23}H_{18}O_4$	153	95
4	3-(4-methoxyphenyl)-5-(4-benzyloxyphenyl)-6-methyl-2-cyclohexen-1-one	$C_{27}H_{26}O_3$	170	80

Table 2: Anti-cancer activity of HMHP-I, HMHP-II, MPEB and MBMC onMCF-7 cell line

Compound	Concentration (µg/ml)									Cell control
		1000	**500**	**250**	**125**	**62.5**	**31.2**	**15.6**	**7.8**	
HMHP-I	**Dilutions**	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	-
	Absorbance (O.D)	0.03	0.10	0.15	0.18	0.23	0.28	0.32	0.37	0.63
	Cell viability (%)	4.76	15.87	23.80	28.57	36.50	44.44	50.79	58.73	100
HMHP-II	**Dilutions**	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	-
	Absorbance (O.D)	0.05	0.12	0.17	0.20	0.25	0.29	0.31	0.33	0.63
	Cell viability (%)	7.93	19.04	26.98	31.74	39.68	46.03	49.20	52.38	100
MPEB	**Dilutions**	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	-
	Absorbance (O.D)	0.08	0.14	0.19	0.24	0.27	0.30	0.32	0.34	0.63
	Cell viability (%)	12.69	22.22	30.15	38.09	42.85	47.61	50.79	53.96	100
MBMC	**Dilutions**	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	-
	Absorbance (O.D)	0.02	0.06	0.09	0.11	0.16	0.21	0.26	0.31	0.63
	Cell viability (%)	3.17	9.52	14.28	17.46	25.39	33.33	41.26	49.20	100

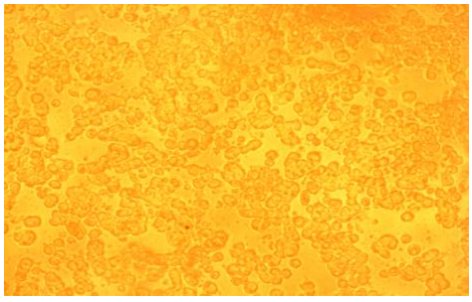
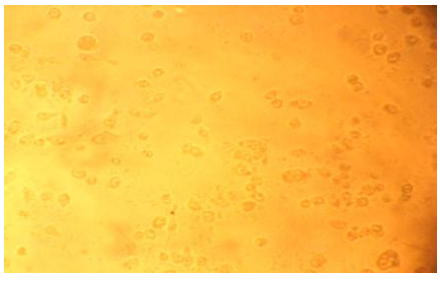
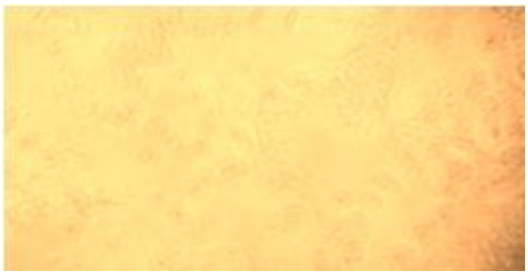


Figure 4(a). Normal MCF-7 Cell line

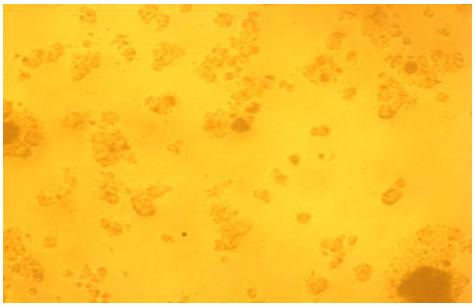
Cytotoxic effect of HMHP-I at 15.6µg/ml concentration



Cytotoxic effect of HMHP-II at 15.6µg/ml concentration

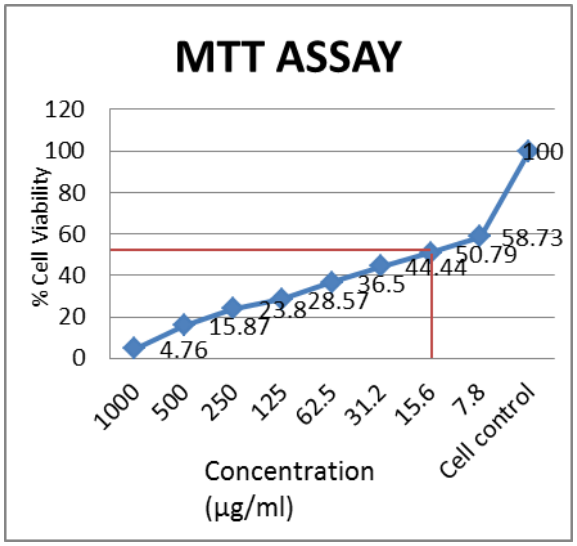


Cytotoxic effect of MPEB at 15.6µg/ml concentration

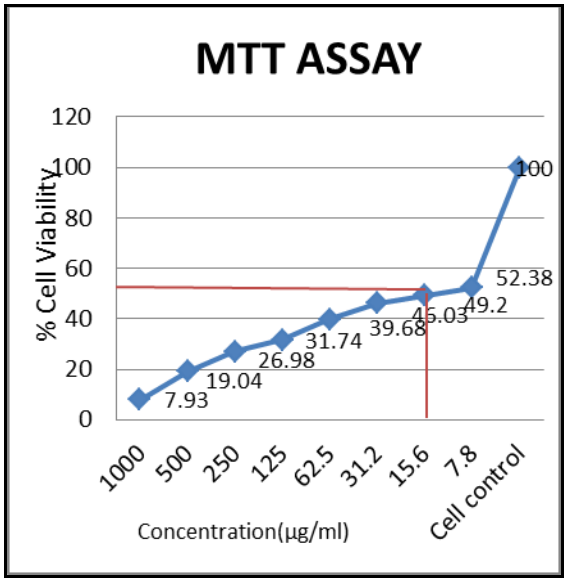


Cytotoxic effect of MBMC at 7.8µg/ml concentration

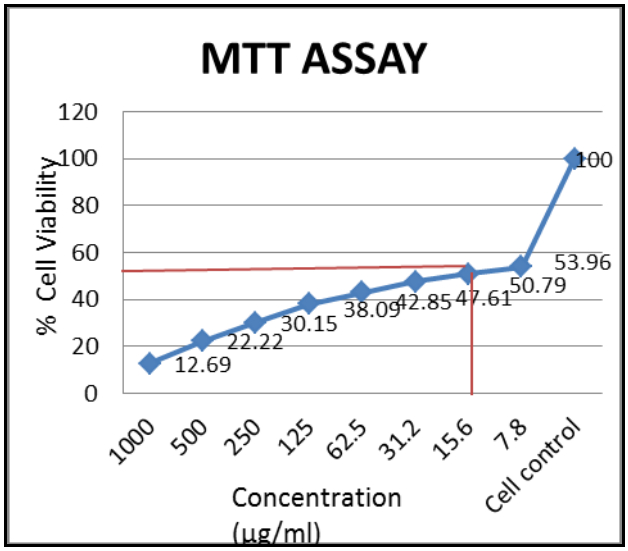
Figure 4(b). Inhibitory effect of the grown crystals on MCF-7 Cell line



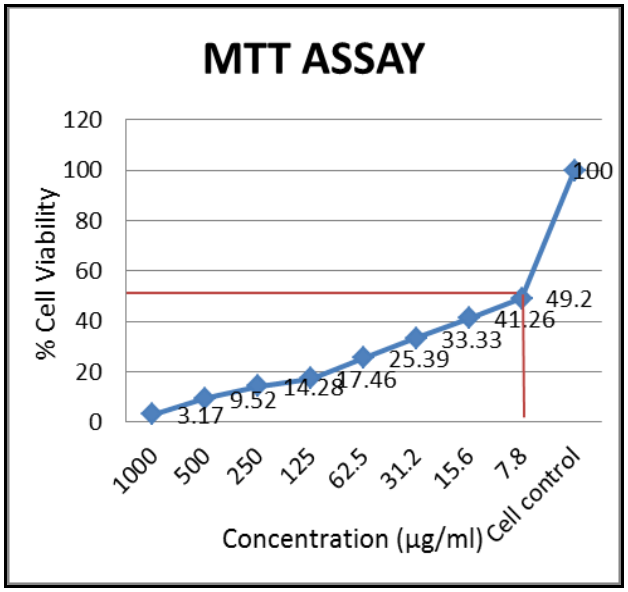
HMHP-I



HMHP-II



MPEB



MBMC

Figure 4(c). Plot of % Cell viability versus Concentration with cell control

Table 3: IC_{50}Values of the Compounds

COMPOUNDS	IC_{50}(µg/ml)
HMHP-I	15.6
HMHP-II	15.6
MPEB	15.6
MBMC	7.8

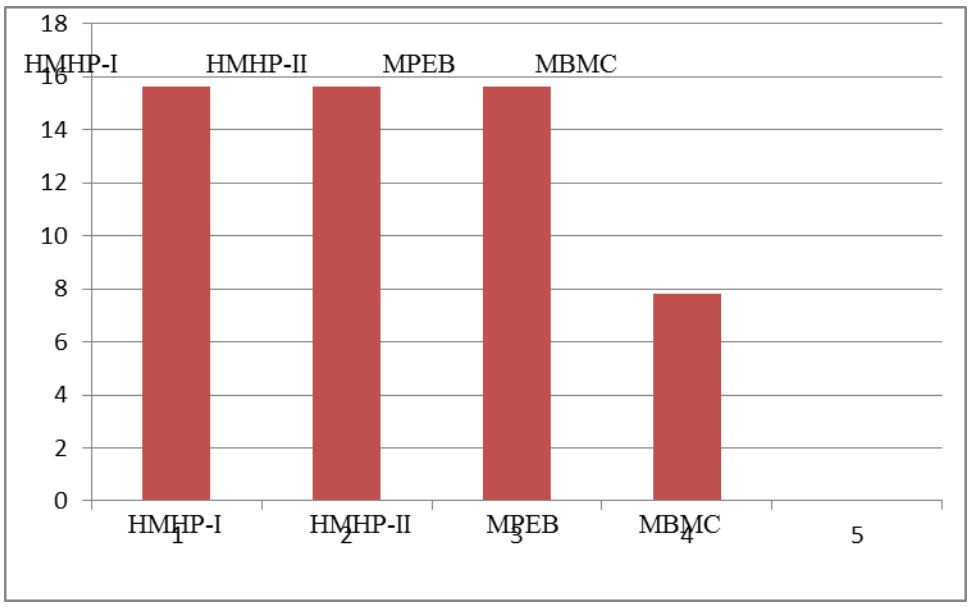


Figure 4(d). Comparative IC_{50} Values

References

1. Wolff ME. Burger Medicinal Chemistry and Drug Discovery, 5th edition, (1994) pp-611.
2. Nowakowska, Z. A review of anti-infective and anti-inflammatory chalcones, Eur. J. Med. Chem., (2007), 42: 125-137.
3. Ravi Sethi andMamta 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. 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.
5. FarukAlam, 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.
6. Park, E. J., Park, H. R., Lee, J. S., Kim, J., Licochalcone A: an inducer of cell differentiation and cytotoxic agent from Pogostemoncablin, Planta Med., 1998, 64(5), 464–466.
7. 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.
8. Ugwu, David. , Ezema, Benjamin, E ., Eze, Florence, U OnoabedjeEfeturi, A , Ezema, Chidimma G, Ekoh, Ogechi, C and Ayogu, Jude. Synthesis and Antimalarial Activities of ChalconeDerivatives , International Journal of ChemTech Research., 2014-2015, 7(4): 1966-1984.
9. 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.
10. Nielsen, S. F., Christensen, S. B., Cruciani, G., Kharazmi, A., Liljefors, T. Antileishmanialchalcones: statistical design, synthesis, and three-dimensional quantitative structure-activity relationship analysis, J. Med. Chem., 1998, 41: 4819-4832.
11. 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.
12. Chtourou, M., Abdelhédi, R., Frikha, M. H., Trabelsi, M., 2009. Ultrason.Sonochem.17, 246.
13. (a) Abdel-Aziz, H. A., Al-Rashood, K. A., ElTahir, K. E. H.; Ibrahimca, H. S. 2011. J. Chin. Chem. Soc., 58, 863.
14. Kakati, D., Sarma, J. C. 2011.Chemistry Central Journal, 5, 1.
15. Rateb, N. M. ,Zohdi, H. F. 2009. Synth.Commun.39, 2789.
16. Rajendra, K., Saini, K., Choudhary, S. A.; Joshi, Y. C.; Joshi, P. 2005. E-Journal of Chemistry.2, 224.

17. Heffeter P., Jakupec M. A., W. Orner, S. Wild, N. G. Keyserlingk, L. Elbling, H. Zorbas, A. Korynevska, S. Knasmuller, H. Sutterluty, M. Micksche, B. K. Keppler, W. Berger. (2006). Biochem.Pharmacol.71, 426.
18. Anticancer And Molecular Docking Studies of Chalcone Derivatives Vasanthi, R. Reuben jonathan, D. Usha, G. International Journal of ChemTech Research, 2016,9(9),pp 419-428.
19. 19.Sathya, S., Reuben Jonathan, D., Prathebha, K., Usha, G and Jovita, J . 2014. ActaCryst, 70(5), o593-o594
20. Sathya, S., Reuben Jonathan, D., Prathebha, K., Jovita, J., and Usha, G. 2014. ActaCryst. E70, o1158–o1159
21. Sathya, S., Reuben Jonathan, D., Prathebha, K., Jovita, J and Usha, G. 2014. ActaCryst. E70, o1007
22. Sathya, S., Reuben Jonathan, D., Sidharthan, J.,Vasanthi, R and Usha, G. 2015. ActaCryst. E71, 16–18
23. Mosmann, T. 1983. J. Immunol. Methods, 65(1-2), 55–63.
24. Ahmad, A., Wang, Z., Ali, R., Kong, D., Banerjee, S., Padhye, S., Sarkar, F.H. Apoptosis inducing effect of garcinol is mediated by NF-κB signaling in breast cancer cells. 2010. J. Cell. Biochem.109, 1134–1141.
25. Syam, S., Abdul, A.B., Sukari, M.A., Mohan, S., Abdelwahab, S.I., Wah, T.S. The Growth Suppressing Effects of Girinimbine on Hepg2 Involve Induction of Apoptosis and Cell Cycle Arrest. 2011. Molecules. 16, 7155–7170.
26. Ye, N., Qin, J., Shi, W., Liu, X., Lin, B. Cell-based high content screening using an integrated microfluidic device. 2007. Lab Chip 7, 1696–1704.
27. 27.Mantena, S.K., Sharma, S.D., Katiyar, S.K. Berberine inhibits growth, induces G1 arrest and apoptosis in human epidermoid carcinoma A431 cells by regulating Cdki–Cdk-cyclin cascade, disruption of mitochondrial membrane potential and cleavage of caspase 3 and PARP. 2006. Carcinogenesis. 27, 2018–2027.
28. Luo, X., Budihardjo, I., Zou, H., Slaughter, C., Wang, X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. 1998.Cell. 94, 481–490.
29. Hsu, Y.L., Kuo, P.L., Tzeng, W.S., Lin, C.C. Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis. 2006. Food Chem. Toxicol.44, 704–713.
30. Suvitha Syam, Siddig Ibrahim Abdelwahab, Mohammed Ali Al-Mamary and Syam Mohan. Synthesis of Chalcones with Anticancer Activities. 2012. Molecules.17, 6179-6195
31. Pusapati Madan Ranjit, Shaik Abdul Rahaman, Kola Phani Kumar, Yalamanchali Rajendra Prasad, ThotakuraSanthipriya, Gurram ChinnaVenkata Sai Manikanta, Nissankararao Lakshmi Sudeepthi. Synthesis, Screening Andin vitro Anticancer Activity Of Piperazine Nucleus Containing Novel Chalcones On Different Cell Lines. 2013. IJPRIF. Vol.5, No.1, pp 284-293.
32. Jovita, J. V., Reuben Jonathan, D., Chidambaravinayagam, S., Ramanand, A and Sagayaraj, P. 2014. J. Chem. Pharm. Res., 6(6):608-614.

*****