International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.1, pp 284-293, Jan-Mar 2013

Synthesis, Screening And in vitro Anticancer Activity Of Piperazine Nucleus Containing Novel Chalcones On Different Cell Lines

Pusapati Madan Ranjit[2], Shaik Abdul Rahaman[1]*, Kola Phani Kumar[2], Yalamanchali Rajendra Prasad[3], Thotakura Santhipriya[2], Gurram Chinna Venkata Sai Manikanta[2], Nissankara rao Lakshmi Sudeepthi[4]

[1]Nirmala College of Pharmacy, Mangalagiri, Guntur AP, India.
[2]NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna District, A.P, India.
[3]University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India.
[4]KVSR Siddhartha College of pharmaceutical sciences, Vijayawada, Krishna District, A.P, India.

***Corres. author: rahaman_pharma@rediffmail.com**
Tel: + 91 9849702527

Abstract: Chalcones and their derivatives have been shown to have potent anticancer activity. However, the exact mechanisms of cytotoxic activity remain to be established. In this study, we have synthesized a series of novel piperazine nucleus containing chalcone derivatives of 1- (4″-piperazinyl phenyl)-3-(substituted phenyl)-2-propene-1-one, by Claisen-Schimdt reaction in which piperazine acetophenone condensed with various aromatic aldehydes. The structures of new compounds were confirmed by FT-IR and ^{1}H-NMR ($CDCl_3$). Out of the total 8 compounds we are selected RC-7 because it showed significant growth inhibition action against brine shrimp, when compared with other compounds. RC-7 showed cytotoxic activity on selected cell lines like MCF-7, HepG-2, Hela, Brain and colon against tamoxifen used as standard. The results indicated that RC-7 showed cytotoxic activity on all cell line with IC_{50} values (µg/ml) 73.72 ± 0.24,230.2 ±4.41,104.9 ± 0.65,109.8 ± 0.14, 104.4± 0.82 respective cell lines mention above. As per the results, our conclusion is piperazine nucleus containing novel chalcone showed anticancer properties. We will further study, regarding the mechanism, site of action of anticancer activity of this compound on substitutions of other functional groups.
Keywords: Chalcones, Piperazine nucleus, Anticancer activity, Cell lines.

INTRODUCTION

Cancer is the most leading cause of mortality in India, is a chronic disorder involved in various cell signaling pathways and disorganized cell functions like irregular cell proliferation with disturbed apoptosis[1-2]. Worldwide reports on cancer supported that among all the types of cancers breast cancer, blood cancer, liver cancer, lung cancer, brain cancer, colon cancer, prostate cancer, cervical cancer and ovarian cancer etc. plays a vital role in the mortality[3]. Clinically chemotherapeutic agents showed beneficial effects in cancer treatment. These

chemical compounds exhibited fatal adverse effects like bone marrow depression and some drugs produces alopecia[4]. Even though we had well developed scientific knowledge, till today development of anticancer agents without any adverse effects and with lowest possible cost is a potential research area for pharmaceutical industry in worldwide.

Experimental works supported that chemical compounds with nitrogen containing heterocyclic's and chalcones showed anticancer activity against various cell lines[5]. The name "Chalcones" was given by Kostanecki and Tambor[6].Chalcones are the bichromophoric molecules separated by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids[7] exhibiting a wide spectrum of biological activities include, antiulcer[8], anticonvulsant[9] , antifertility[10], antibacterial[11-12], antiviral[13], antifungal[14], anti-allergic[15-16] , hypoglycemic[17], antioxidant[18] and anti-inflammatory activity[19-21], anticancer[22-23].They considered as the precursor of flavonoids and isoflavonoids. Chemically they consisted of open-chain flavonoid by a three carbon α β-unsaturated carbonyl system[24]. The presence of a reactive α, β-unsaturated keto functional group in chalcone is found to be responsible for their broad spectrum activity, which may be altered depending on the type and position of substituent on the aromatic rings.

It is evident from the literature that there is no work has been reported on 4′-piperazino aromatic nucleus containing chalcones and their applications. Keeping in this view, we have proposed to synthesize novel chalcone derivatives of piperazine moiety (RC_1 – RC_8) to evaluate their anti cancer activities. Previous studies have indicated that chalcones and their derivatives demonstrate anticancer activity in various tumor cells. Natural and synthetic chalcones have been shown to have strong anti proliferative effects in both primary and established ovarian cancer cells[25] and in gastric cancer HGC-27 cells[26].

Piprerazine containing chalcones are the novel compounds exhibited wide range of pharmacological activities includes antihistamine[27], antioxidant, anti-inflammatory[28], antimicrobial[29], and anticancer properties[30]. Scanty information was available on piperazine containing chalcone as antiprolifaratory agent. The present study was designed for anticancer activity evaluation of piperazine containing chalcones.

MATERIALS AND METHODS

Materials used for this experiment are of analytical grade. TLC: *Merck* silica gel 60 F_{254} Al-backed plates; solvent system using methanol: ethyl acetate (1:1) and tested under UV lamp at 254 nm staining with phosphomolybdic acid. Wilson test: using conc. H_2SO_4 showed a pink color. $FeCl_3$ test: treatment of the same compounds showed violet color. IR Spectra: *Perkin-Elmer 377* spectrophotometer. KBr pressed pellet technique. ^{1}H NMR Spectra: *Bruker AV 400* spectrometer (^{1}H: 400 MHz in $CDCl_3$); chemical shifts δ in ppm, *J* in Hz; TMS as internal standard.

(a) Synthesis of compounds

A mixture of 4′-piperaizinoacetophenone (0.001 M) and aryl aldehyde (0.001 M) was stirred in methanol (10.0 ml) and to it 5 mM of 40% KOH was added [31-32]. The mixture was kept for 24 h and it was acidified with 1:1 HCl and water then it was filtered through vacuum by washing with water and crystallized from a mixture of ethyl acetate and methanol (8:2) to afford compounds from RC-1 to RC-8.

(b) Chemistry

We have developed **s**ome novel piperazine nucleus containing chalcone derivatives (RC-1 to RC-8) synthesized by Claisen-Schimdt reaction in which piperazine acetophenone was condensed with various aromatic aldehydes as shown in Scheme. The yields of synthesized chalcones were obtained between 66.6 and 80.7% (Table 1). Their chemical structure was elucidated by means of FT-IR and ^{1}H-NMR (CDC l_3).

(c)Characterization of piperazine nucleus containing novel chalcones RC-1 to RC-8.

1- (4ð-piperazinyl phenyl)-3-(3å-bromo phenyl)-2-propene-1-one **(RC-1)**. IR (KBr) cm^{-1}: N-H str --- 3415.7, C=O str --- 1647, C=C str --- 1595.7. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.87 (1H, bs, aliphatic N-H), 2.58 and 3.48 (8H, piperazinyl protons), 6.91 (1H, d, J=8.4Hz, C-3H), 8.0 (1H, d, J=8.8Hz, C-2H), 6.76-7.86 (8H aromatic protons), 7.36 (1H, s, C-2^{1}H).

1- (4ð-piperazinyl phenyl)-3-(4å-nitro phenyl)-2-propene-1-one **(RC-2).** IR(KBr) cm^{-1}: N-H str --- 3437.84,C=O str --- 1648.39,C=C str --- 1597.11, N-O str --- 1519.03. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.33 (1H, bs, aliphatic N-H), 2.59 and 3.07 (8H, piperazinyl protons), 6.91 (1H, d, J=8.8Hz, C-3H), 7.99 (1H, d, J=8.8Hz, C-2H), 7.51-8.16 (8H, aromatic protons).

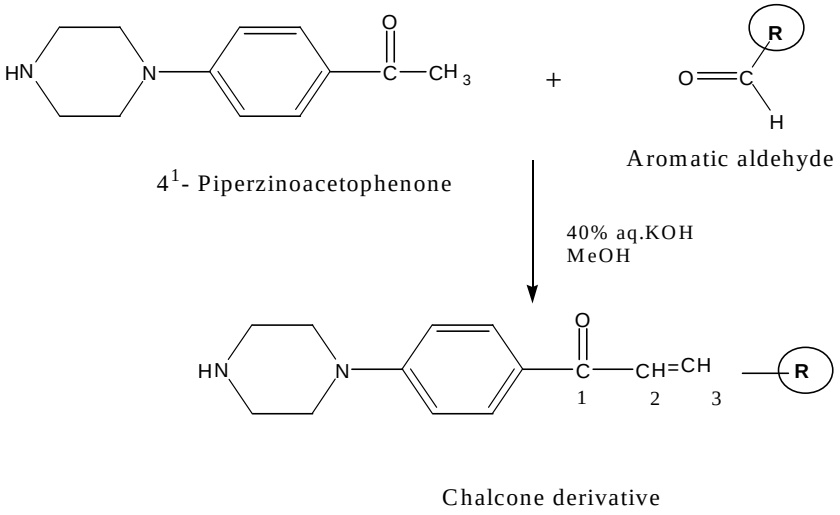

FIGURE 1: Scheme: Synthesis of novel chalcone derivatives

1-(4ð-piperazinyl phenyl)-3-anthracenyl-2-propene -1-one **(RC-3).** IR (KBr) cm^{-1}: N-H str --- 3431.7,C=O str --- 1648.3,C=C str --- 1600.7. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.35 (1H, bs, aliphatic N-H), 3.36 (4H, piperazinyl protons), 3.59 (4H, piperazinyl protons),6.92(1H, d, J=8.8Hz, C-3H), 7.56 (1H, d, J=7.2Hz, C-2H), 7.2-8.73 (13H, aromatic protons).

1- (4ð-piperazinyl phenyl)-3-(4å-methyl phenyl)-2-propene-1-one **(RC-4).** IR (KBr) cm^{-1}: N-H str --- 3427.91, C=O str --- 1647.27, C=C str --- 1602.42. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.87(1H, bs, aliphatic N-H), 3.05 (4H, piperazinyl protons), 3.35 (4H, piperazinyl protons), 2.38 (3H, s, benzylic protons), 6.90 (1H, d, J=9.2Hz, C-3H), 7.20 (1H, d, J=8.0Hz, C-2H), 7.2-8.0 (8H, aromatic protons).

1- (4ð-piperazinyl phenyl)-3-(4å-chloro phenyl)-2-propene-1-one **(RC-5).** IR(KBr) cm^{-1}: N-H str --- 3429.27, C=O str --- 1649.56, C=C str --- 1599.44. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.25 (1H, bs, aliphatic N-H), 3.02 (4H, piperazinyl protons), 3.34 (4H, piperazinyl protons), 6.89 (1H, d, J=8.8Hz, C-3H), 7.36 (1H, d, J=8.8Hz, C-2H),7.26-7.99(8H, aromatic protons).

1-(4ð-piperazinyl phenyl)-3-(2å,4å-dichloro phenyl)-2-propene-1-one **(RC-6).** IR(KBr) cm^{-1}: N-H str --- 3437.79, C=O str --- 1654.99, C=C str --- 1595.03. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.19 (1H, bs, aliphatic N-H), 2.62 and 3.36 (8H, piperazinyl protons), 7.20 (1H, d, J=7.4Hz, C-3H), 7.85 (1H, d, C-2H), 6.76-8.08 (7H, aromatic protons).

1- (4ð-piperazinyl phenyl)-3-(2å-chloro phenyl)-2-propene-1-one **(RC-7).** IR(KBr) cm^{-1}: N-H str --- 3423.9, C=O str --- 1659.4, C=C str --- 1595.6. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.01(1H, bs, aliphatic N-H), 3.09 and 3.43 (8H, piperazinyl protons), 6.92 (1H, d, J=8.8Hz, C-3H), 7.23 (1H, d, J=7.2Hz, C-2H).

1- (4ð-piperazinyl phenyl)-3-(4å-fluoro phenyl)-2-propene-1-one **(RC-8).** IR(KBr) cm^{-1} : N-H str --- 3353.26, C=O str --- 1650.48, C=C str --- 1606.18. ^{1}H-NMR ($CDCl_3$) (δ ppm): 2.0(1H, bs, aliphatic N-H), 3.01 and 3.33 (8H, piperazinyl protons), 6.92 (1H, d, J=9.2Hz, C-3H), 7.72 (1H, d, J=8.8Hz, C-2H), 7.07-7.76 (8H, aromatic protons).

TABLE 1: Physical data of synthesized compounds (RC-1 to RC-8)

Compound	R	Molecular formula	m.p. (°C)	Yield (%)
RC-1	3′-bromo phenyl	$C_{19}H_{19}ON_2Br$	120	79.2
RC-2	4′-nitro phenyl	$C_{19}H_{19}O_3N_3$	132-134	72
RC-3	anthracenyl	$C_{27}H_{24}ON_2$	128-130	69.5
RC-4	4′-methyl phenyl	$C_{20}H_{22}ON_2$	161-162	80.7
RC-5	4′-chloro phenyl	$C_{19}H_{19}ON_2Cl$	190	74
RC-6	2′,4′-dichloro phenyl	$C_{19}H_{18}ON_2Cl_2$	148-150	66.6
RC-7	2′-chloro phenyl	$C_{19}H_{19}ON_2Cl$	125-126	73.9
RC-8	4′-fluoro phenyl	$C_{19}H_{19}ON_2F$	185-187	71.5

SCREENING OF ANTI CANCER ACTIVITY:

Preliminary growth inhibitory activity of synthesized chalcone derivatives RC-1 to RC-8 was evaluated against brine shrimp (*Artemia salina:* preliminary results are not showed)[33]. Among all the compounds RC 7 showed significant growth inhibition action against brine shrimp after 24 hours of incubation when compare with other compounds. Further, growth inhibitory properties of RC-7 was studied by using the MTT assay on five human cancer cell lines, including MCF-7 (breast), liver carcinoma HepG2, carcinoma of cervix–HeLa cells, carcinoma of brain, and carcinoma of colon against standard drug tamoxifen.

(a) Cell culture and growth medium

Carcinoma of Breast – MCF-7 cells, Liver carcinoma – HepG2, cervix (HeLa), Brain and colon cancer cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

(b) MTT assay:

The MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide) assay developed by Mosmann [34] was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of both standard (Tamoxifen) and test compound RC-7 (8, 16, 32, 64, 128 and 256 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer. Both standard and test maintained in triplicate. The percent cell viability was determined with respect to control, is calculated using formula.

% Viability = corrected OD of sample /Control OD * 100 and percentage of inhibition was determined by using formula, % Inhibition = 100-%viability.

(c) Statistical analysis

Data were represented as Mean ± SD. $^*P< 0.05$ was considered as significant when compared with tamoxifen (t- test) by using Graph pad prism 5 version.

TABLE 2 Mean ± S.D of IC_{50} value both standard and test on different cancer cell lines.

Type of cell line	IC_{50} values	
	Standard	Test sample RC-7
MCF-7 (Breast cancer)	72.55± 0.43	104.4± 0.82
Liver carcinoma ($HepG_2$)	3.68 ±0.10	230.2 ±4.41
HeLa Cervix Cancer	31.51 ± 0.39	104.9 ± 0.65
Brain cancer cell line	36.98 ± 0.36	109.8 ± 0.14
Colon cancer cell line	38.97 ± 0.24	73.72 ± 0.24

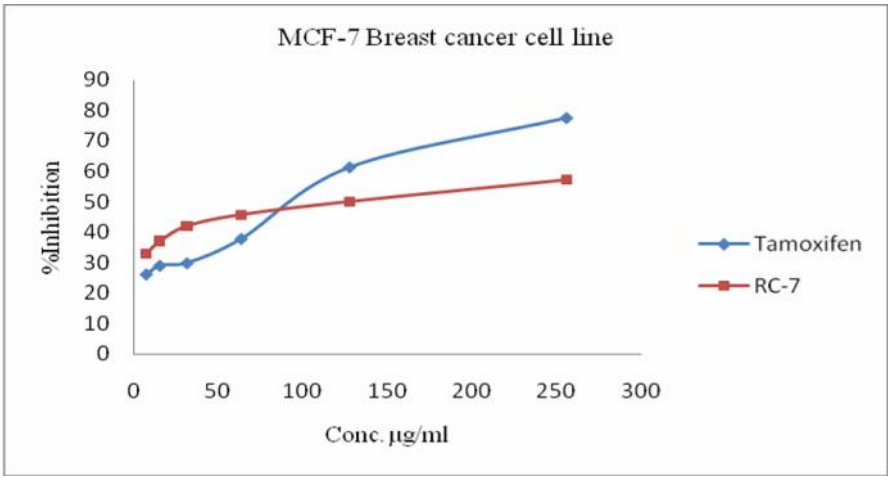

FIGURE 2: Showed % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against MCF-7 cancer cell line

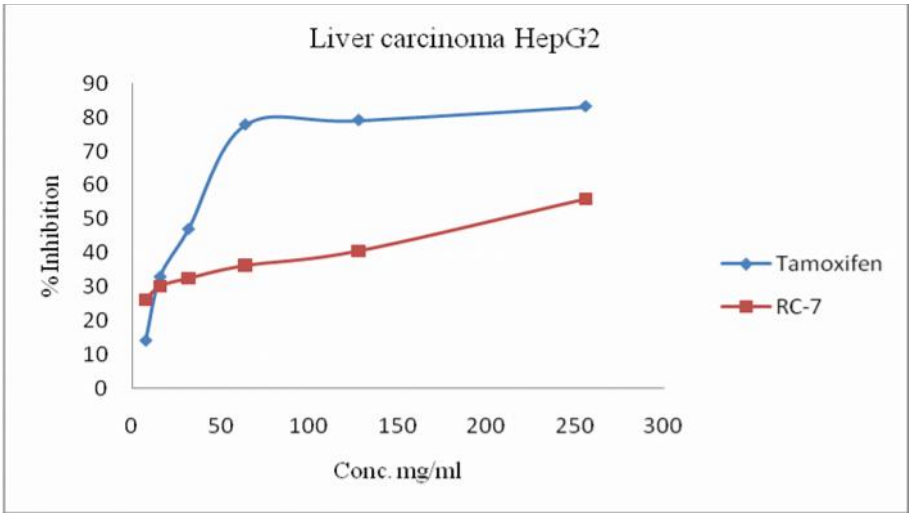

FIGURE 3: Showed % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against liver carcinoma HepG2 cells line

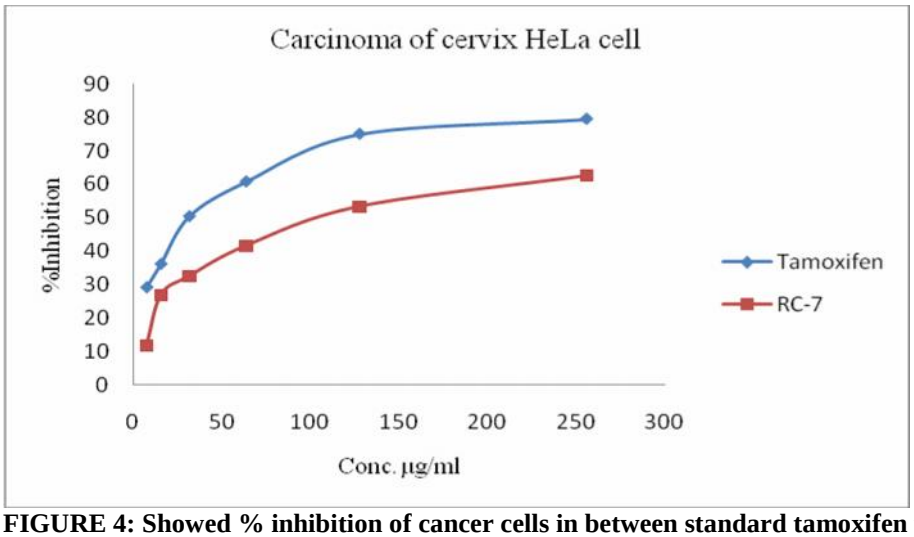

FIGURE 4: Showed % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against carcinoma of cervix HeLa cells

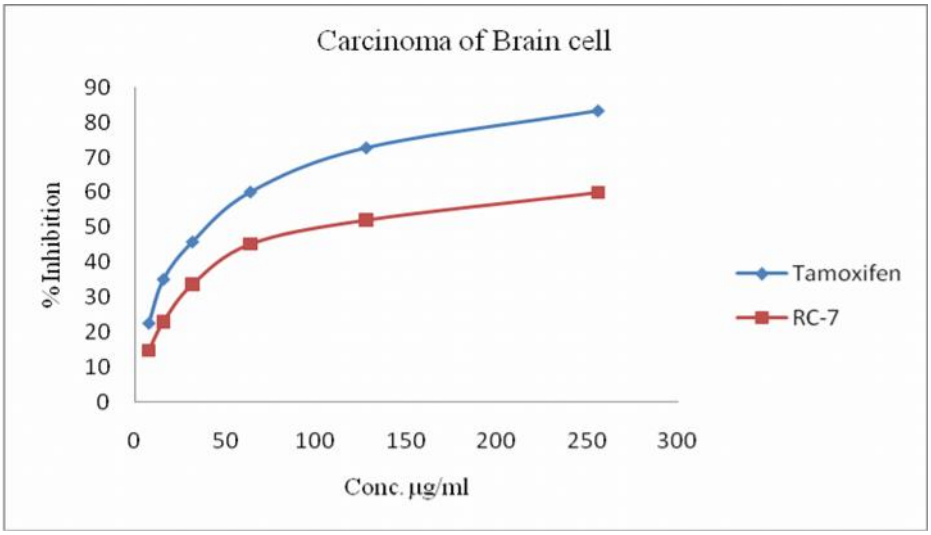

FIGURE 5: Showed % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against carcinoma of Brain cells

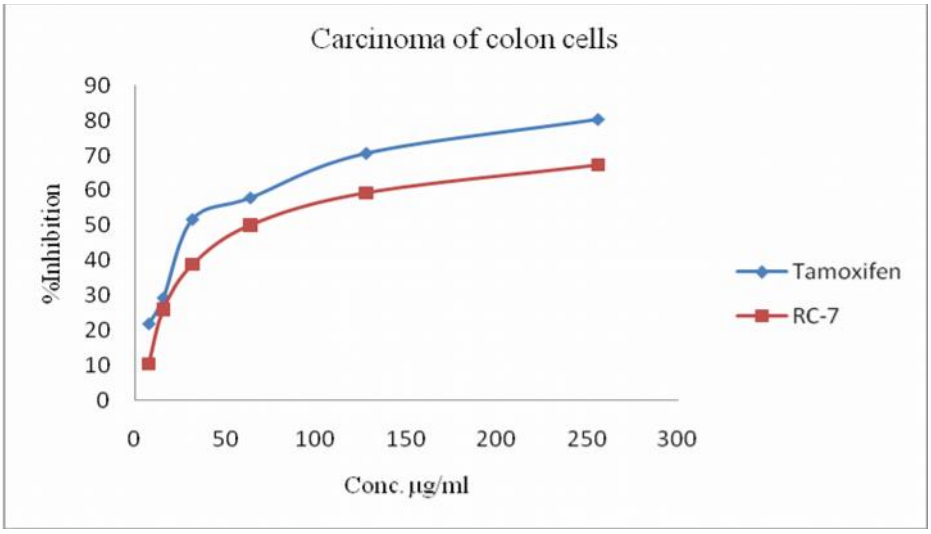

FIGURE 6: Showed % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against carcinoma of colon cells

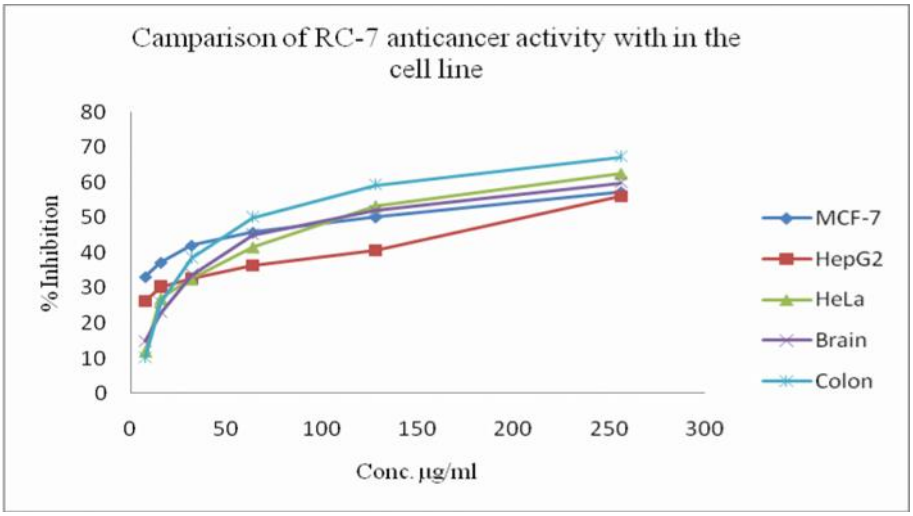

FIGURE 7: Showed comparison of % inhibition of cancer cells in between standard amoxifen *vs.* RC-7 against various cancer cell lines.

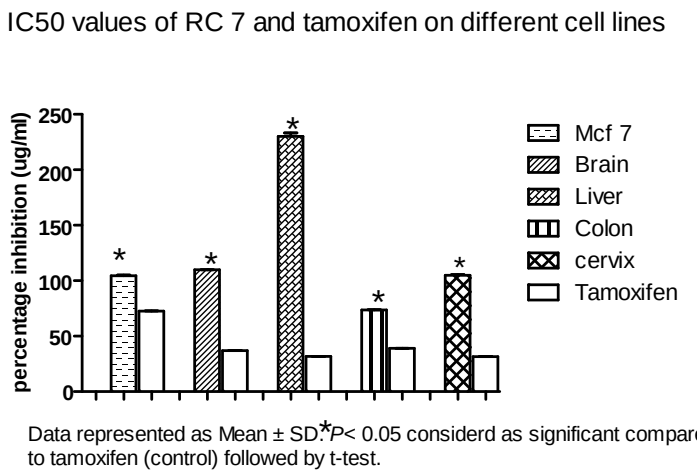

FIGURE 8: IC_{50} values of RC-7 and tamoxifen on different cell lines

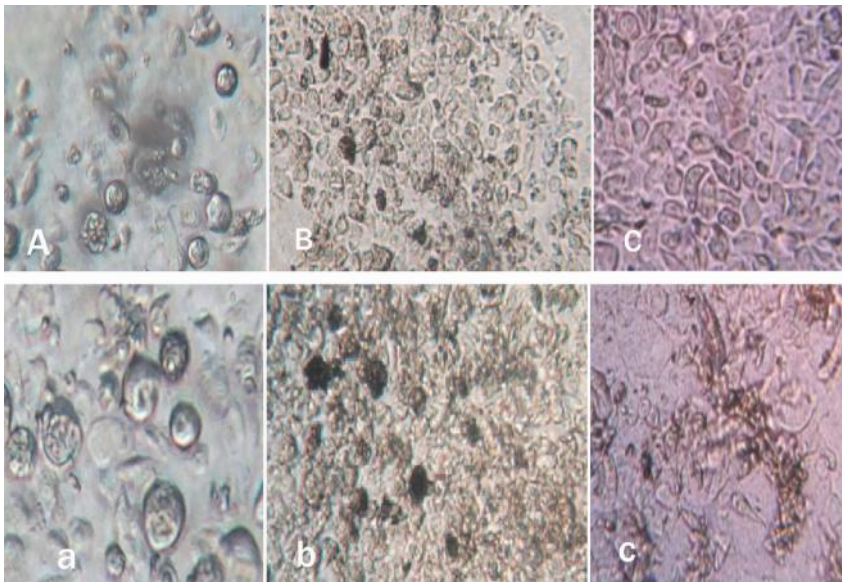

FIGURE 9: Showed that A, B and C are the MCF-7, HepG2 cell, HeLa cells before treatment showing actively dividing cells. a, b, and c are the MCF-7, HepG2 cell, HeLa cells after the treatment with test sample RC-79 (at 256Conc.µg/ml) showed unchracterized cell.(Photographed under inverted microscope).

RESULTS AND DISCUSSION

Table 2 showed that Ic_{50} concentrations (µg/ml) of both standard and RC-7 against MCF-7, liver carcinoma HepG2, carcinoma of cervix HeLa cells, carcinoma of Brain, and carcinoma of colon cells. Figure 2, 3, 4, 5, and 6 showed that percentage of inhibition of MCF-7 (breast), liver carcinoma HepG2, carcinoma of cervix HeLa cells, carcinoma of brain, and carcinoma of colon cells against test sample RC-7 when compared with standard. Figure 7 and 8 showed the comparison of % inhibition of cancer cells in between standard tamoxifen *vs.* RC-7 against various cancer cell lines. IC_{50} values (µg/ml) of RC-7 and tamoxifen on different cell lines respectively calculated. This is a first report of piperazine nucleus containing novel chalcones showed anticancer properties. RC-7 showed good cytotoxic property on colon (73.72 ± 0.24, $*P < 0.05$) cell line when compared with other cancer cell lines.

The primary antitumor activity of tamoxifen by inhibition protein kinase C[35] and also ability to facilitate the apoptosis in cancer cell not expressing estrogen receptor is due to generation of oxidative stress resulting in thiol depletion and activation of the transcriptional factor NF-kappaB [36]. Many clinical studies explain the tamoxifen application in various kinds of maligamant diseases[37]. Novotny et al., reviews the application of tamoxifen in various cancer like melanoma, small cell lung carcinoma, pancreatic, other endocrine and soft tissue cancers[38].

Tamoxifen is clinically used for treatment of breast cancer, so it was used as standard against MCF-7 cancer cell lines. According to review of literates, tamoxifen was used as standard for other cell lines mentioned above.

Reports of previous works on revealed the anticancer activity of chalcones against various cancer cell lines. S. Halide Akbas investigated role of quercetin in combination Cytotoxicity in MCF-7 and MDA-MB 231 Human Breast Cancer Cells reported quercetin showed better activity in all the cells[39]. Suvitha Syam et al, reported apoptosis induction of chalcones in MCF-7 cells by activating caspase-7, caspase-8, and caspase-9 [40], retinoid chalcone hybrids showed anticancer activity against colon cancer cell lines HT-2[41], and synthetic chalcones antitumor activity in brain tumor cell lines is mediated by c-Myc-mediated reactive oxygen species production[42].

CONCLUSION

We are reported that, piperazine nucleus containing novel chalcone showed anticancer properties and have ability to inhibit the various types of cancer cells. In our research we are randomly selected the RC-7 form a series of synthetic chalcone derivatives and screen the cytotoxic activates on various cell line showed activity. Further, we will screen the cytotoxic properties of other piperazine nucleus containing chalcone derivates and also know the influence the cytotoxic properties of these compounds. We want to know the effects of these compounds on further substitution with other functional groups or other nucleus, including its molecular mechanisms of action of cytotoxic properties. This research provides an approach to develop potent new chalcone derivates to get new leads for the treatment of cancer.

ACKNOWLEDGEMENTS

The authors are thankful to the management Nirmala College of Pharmacy, Mangalagiri, NRI College of pharmacy, Pothavarappadu, Agiripalli mandal for providing the research facilities and also to the Research Gateway for Biosciences, Dwarakanagar, Visakhapatnam, A.P. India, for carryout cell line culture work.

REFERENCES

1. Hiss, DC, and Gabriels, GA, "Implications of endoplasmic reticulum stress, the unfolded protein response and apoptosis for molecular cancer therapy. Part I: targeting p[53], Mdm2, GADD153/CHOP, GRP78/BiP and heat shock proteins," *Expert Opin. Drug Discov*, 2009; vol.4, pp 799–821.
2. Jemal, A. Bray, F. Center, MM. Ferlay, J, Ward, E, and Forman, D, "Global cancer statistics", *CA Cancer J. Clin*, 2011; vol.61(2), pp 69-90.
3. Johnstone, RW. Ruefli, AA, and Lowe, SW, "Apoptosis-A Link between Cancer Genetics and Chemotherapy", *Cell*, 2002; vol. 108, pp153–164.
4. http://pharmalicensing.com/company/ dispcompany/2098.
5. Babasaheb P. Bandgara, B, Shrikant S. Gawandeb, Ragini G. Bodadec, Jalinder V. Totred, and Chandrahas N. Khobragadec., "Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents" *Bioorganic & Medicinal Chemistry*, 2010; vol. 18, pp1364–1370.
6. Katritzky. AR. and AF. Pozharskii, "Handbook of Heterocyclic Chemistry" , 2nd edition, Pergamon Press, New York. 2000
7. Yesuthangam Y, Pandian S, Venkatesan K, Gandhidasan R, and Murugesan R.,. "Photogeneration of reactive oxygen species and photoinduced plasmid DNA cleavage by novel synthetic chalcones", J. *Photochem. Photobiol B: Biol*, 2011; vol.102, pp200-208.
8. Lucky, Okunrobo, Cyril, Usifoh and john Uwaya., "Anti-inflammatory and gastro protective properties of some Chalcones", *Acta poloniae pharmaceutica -drug research*, 2006; vol. 63, pp195-199.
9. Nikhil D. Amnerkar, and Kishore P. Bhusari, "Synthesis, anticonvulsantactivity and 3D-QSAR study of some prop-2-eneamido and 1-acetyl-pyrazolin derivatives of amino benzothiazole". *European Journal of Medicinal Chemistry*, 2010; vol. 45, pp149–159.
10. Dennis jacob and Kaul DK., "Oestrogenic and antifertility effects of chalcone derivatives" *Acta Endocrinol*, 1973; vol. 74, pp371-378.
11. Balkrishna Tiwari, Pratapwar AS, Tapas AR, Butle SR and VatkarK BS, and Mallikarjun G., "Synthesis and Antimicrobial Activity of Some Chalcone Derivatives", *International Journal of ChemTech Research*, 2010; vol.2, pp499-503,.
12. Sivakumar PM, Seenivasan SP, Kumar V, and Doble M., "Synthesis, antimycobacterial activity evaluation, and QSAR studies of chalcone derivatives", *Bio org Med Chem Lett.* 2007; vol.15, 17(6), pp 1695-700.

13. Trivedi JC, Bariwal JB, Upadhyay KD, Naliapara YT, Joshi S.K, and Pannecouque CC., "Improved and rapid synthesis of new coumarinyl chalcone derivatives and their antiviral activity", *Tetrahedron Lett*, 2007; vol. 48, pp 8472–8474.
14. Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM, and Sibirny AA., "Antifungal activity of chalcones: a mechanistic study using various yeast strains", *Eur J Med C*hem, 2008; vol. 43(10), pp 2220-2208.
15. Yamamoto T, Yoshimura M, Yamaguchi F, Kouchi T, Tsuji R, Saito M, Obata A, and Kikuchi M., "Anti-allergic activity of naringenin chalcone from a tomato skin extract", *Bio sci Biotechnol Biochem*, 2004; vol. 68, pp1706-1711.
16. Chiung-Yun Chang,A Li-Jiau Huang, A Jih-Pyang Wang,A,B Che-Ming Teng,C Sheng-Chih Chen,A and Sheng-Chu Kuo, "Synthesis and Anti-platelet, Anti-inflammatory and Anti-allergic Activities of Methoxyisoflavanquinone and Related Compounds", *Chem. Pharm. Bull*, 2000; vol.48, pp 964-973.
17. Rosangela Guollo Damazioa, Ana Paula Zanattaa, Luisa Helena Cazarollia, Alessandra Mascarellob, Louise Domeneghini Chiaradiab, Ricardo José Nunesb, Rosendo Augusto Yunesb and Fátima Regina Mena Barreto Silvaa, "Nitrochalcones Potential *in vivo* insulin secretagogue", *Biochimie*, 2009; Vol. 9, pp1493–1498.
18. Frederique A.A. van Acker, Jos A. Hageman, Guido RM, M. Haenen, Wim JF. van der Vijgh, Aalt Bast, and Wiro MPB. Meng., "Synthesis of Novel 3,7-Substituted-2-(3‘,4‘-dihydroxyphenyl)flavones with Improved Antioxidant Activity", *J. Med. Chem*, 2000; vol. 43, pp 3752–3760.
19. Herencia F, Ferrándiz ML, Ubeda A, Guillén I, Dominguez JN, Charris JE, Lobo GM, and Alcaraz MJ, "Novel anti-inflammatory chalcone derivatives inhibit the induction of nitric oxide synthase and cyclooxygenase-2 in mouse peritoneal macrophages", *FEBS let*, 1999; pp129-134,.
20. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. *Eur J Med Chem*, 2007; 42: 125-127.
21. Kim Y. H, Kim J, Park H, and Kim HP, "Anti-inflammatory Activity of the Synthetic Chalcone Derivatives: Inhibition of Inducible Nitric Oxide Synthase-Catalyzed Nitric OxideProduction from Lipopolysaccharide-Treated RAW 264.7 Cells", *Biol Pharm Bull*, 2007; 30, pp1450-1455.
22. Leung BP, Shauna C, Alastair Gracie J, David Hunter, Canetti CA, Carol C, Fernando C, Liew FY and McInnes IB., "A role for IL-18 in neutrophil activation", *J Immunol*, 2001; 167, pp 2879–2886.
23. Modzelewska A, Pettit C, Achanta G, Davidson N E, Huang P, and Khan S R., "Anticanceractivities of novel chalcone and bis-chalcone derivatives", *Bioorg Med Chem*, 2006; 14, pp 3491-3495.
24. Ruby John A, Sukumarana, K, Girija K, Rao MNA, Subbarajuc V, and Ramadasan K, "Anticancer and antioxidant activity of synthetic chalcones and related compounds", *Cancer Letters*, 1995; 97, pp 33–37.
25. De Vincenzo R, Scambia G, and Mancuso S., "Effect of synthetic and naturally occurring chalcones on ovarian cancer cell growth: structure-activity relationships", *Anticancer Drug Des*, 1995; 10, pp 481–490,.
26. Shibata S., "Anti-tumorigenic Chalcones", *Stem Cells*, 1994; 12:44–52.
27. Rahaman SA., Y.Ragjendra prasad, K. Bhuvaneswari, and Phani K kumar.,"Synthesis and antihistaminic activity of novel pyrazoline derivatives", *Int.J. ChemTech Res.*, 2010; 2(1).
28. Bandgar BP, Patil SA, Gacche RN, Korbad BL, Hote BS, Kinkar SN, and Jalde SS., "Synthesis and biological evaluation of nitrogen-containing chalcones as possible anti-inflammatory and antioxidant agents". *Bio org Med Chem Lett*., 20(2), pp 730-3, 2010.
29. Tomar V, Bhattacharjee G, Kamaluddin, Kumar A, *Bio org Med Chem Lett*, 2007Oct 1; 17(19):5321-4.
30. Rosanna Filosa , Antonella Peduto, Paolo de Caprariis,Carmela Saturnino, Michela Festa, Antonello Petrella, Amedeo Pau,Ge´rard Aime´ Pinna, Paolo La Colla, Bernardetta Busonera, and Roberta Loddo, "Synthesis and antiproliferative properties of N3/8-disubstituted 3,8-diazabicyclo[3.2.1]octane analogues of 3,8-bis[2-(3,4,5- trimethoxy phenyl)pyridin-4-yl]methyl-piperazine", *European journal of medicinal chemistry*, 2007; 42 (3), pp 293-306,.
31. Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM, and Sibirny AA., " Antifungal activity of chalcones: a mechanistic study using various yeast strains", *Eur J Med Chem*, 2008; 43(10), pp2220-2208.
32. Koblyakov VA., "Free radicals and inflammation (progress in inflammation research series)". *Biochemistry*, 2001; 66, pp 937–938.
33. Evidente A, Andolfi A, Vurro M, Zonno M C, and Motta A, "Cytochalasins Z4, Z5, and Z6,

three new 24-Oxa [14] cytochalasans produced by Phoma exigua var. heteromorpha". *J Nat Prod,* 2003; 66(12), pp1540-1544,.

34. Mosmann T, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays". *J. Immunol. Methods,* 1983; 65.
35. Gelman, E.P, "Tamoxifen for the treatment of malignancies other breast and endometrial carcinoma". *Semin. Oncol*, Suppl.1.24, pps165-s170, 1997.
36. Ferlini,C,Scambia,G.,Marone,M.,Distefano, M.,Gaggini,C.,Ferrandina,G.,Fattotossi,A.,Isola,G., Benedetti Panici, P.,and Mancuso, S, "Tamoxifen induces oxidative stress and apotosis in oestrogen receptor-negative human cancer cell line", *Br.J.Cancer,* 1999; 79, pp 257-263.
37. CI PDQ Clinical trial Search. Bethesda, USA, National Cancer Institute. 1999.
38. Novotny L, P.Rauko, A.Vachalkova, and M.Peterson-Biggs., "Tamoxifen in cancer therapy". *Neoplasma,* 2000. 47, pp1,
39. Akbas S.H, Timur M, and Ozben T., "The effect of quercetin on topotecan cytotoxicity in MCF-7 and MDA-MB 231 human breast cancer cells", *J Surg Res*, 2005; 125(1), pp 49-55.
40. Suvitha Syam, Siddig Ibrahim Abdelwahab, Mohammed Ali, Al-Mamary and Syam Mohan., "Synthesis of Chalcones with Anticancer Activities", *Molecules,* 2012; 17, pp 6179-6195.
41. Mizuno CS, Paul S, Suh N, and Rimando AM., "Synthesis and biological evaluation of retinoid-chalcones as inhibitors of colon cancer cell growth", *Bioorg Med Chem Lett*, 2010; 15,l 20(24), pp7385-7.
42. Kim TH, Seo WD, Ryu HW, Seo HR, Jin YB, Lee M, Ji YH, Park KH, and Lee YS., "Anti-tumor effects by a synthetic chalcone compound is mediated by c-Myc-mediated reactive oxygen species production", *Chem Biol Interact*, 2010; 188(1), pp111-118.

*****