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

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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 ( g/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. 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. 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.<sup>1</sup> 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.<sup>5</sup> The name “Chalcones” was given by Kostanecki and Tambor.<sup>6</sup> Chalcones are the bichromophoric molecules separated by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids<sup>7</sup> exhibiting a wide spectrum of biological activities include, antiulcer,<sup>8</sup> anticonvulsant<sup>9</sup>, antifertility<sup>10</sup>, antibacterial<sup>11-12</sup>, antiviral,<sup>13</sup> antifungal<sup>14</sup>, anti-allergic<sup>15-16</sup>, hypoglycemic<sup>17</sup>, antioxidant<sup>18</sup> and anti-inflammatory activity<sup>19-21</sup>, anticancer<sup>22-23</sup>. 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<sup>24</sup>. 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'-piperazinoacetophenone (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<sup>31-32</sup>. 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 some 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 1H-NMR (CDCl<sub>3</sub>).

(c) Characterization of piperazine nucleus containing novel chalcones RC-1 to RC-8.
1- (4’-piperazinyl phenyl)-3-(3’-bromo phenyl)-2-propene-1-one (RC-1). IR (KBr) cm<sup>-1</sup>: N-H str --- 3415.7, C=O str --- 1647, C=C str --- 1595.7. 1H-NMR (CDCl<sub>3</sub>) (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=H), 8.0 (1H, d, J=8.8Hz, C-2H), 6.76-7.86 (8H aromatic protons), 7.36 (1H, s, C-2'H).

1- (4’-piperazinyl phenyl)-3-(4’-nitro phenyl)-2-propene-1-one (RC-2). IR(KBr) cm<sup>-1</sup>: N-H str --- 3437.84, C=O str --- 1648.39, C=C str --- 1597.11, N-O str --- 1519.03. 1H-NMR (CDCl<sub>3</sub>) (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-3'H), 7.99 (1H, d, J=8.8Hz, C-2'H), 7.51-8.16 (8H, aromatic protons).

MATERIALS AND METHODS
Materials used for this experiment are of analytical grade. TLC: Merck silica gel 60 F<sub>254</sub> 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<sub>2</sub>SO<sub>4</sub> showed a pink color. FeCl<sub>3</sub> test: treatment of the same compounds showed violet color. IR Spectra: Perkin-Elmer 377 spectrophotometer. KBr pressed pellet technique. <sup>1</sup>H NMR Spectra: Bruker AV 400 spectrometer (1H: 400 MHz in CDCl<sub>3</sub>); chemical shifts δ in ppm, J in Hz; TMS as internal standard.

(a) Synthesis of compounds
A mixture of 4’-piperazinoacetophenone (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. 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.
FIGURE 1: Scheme: Synthesis of novel chalcone derivatives

1-(4'-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. ¹H-NMR (CDCl₃) (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- (4'-piperazinyl phenyl)-3-(4'-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. ¹H-NMR (CDCl₃) (δ ppm): 2.87 (1H, bs, aliphatic N-H), 3.05 (4H, piperazinyl protons), 3.35 (4H, piperazinyl protons), 2.38 (3H, s, benzyllic 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- (4'-piperazinyl phenyl)-3-(4'-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. ¹H-NMR (CDCl₃) (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), 7.2-7.99 (8H, aromatic protons).

1-(4'-piperazinyl phenyl)-3-(2',4'-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. ¹H-NMR (CDCl₃) (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- (4'-piperazinyl phenyl)-3-( 2'-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. ¹H-NMR (CDCl₃) (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- (4'-piperazinyl phenyl)-3-( 4'-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. ¹H-NMR (CDCl₃) (δ ppm): 2.01 (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)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Molecular formula</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
</tr>
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<tr>
<td>RC-1</td>
<td>3'-bromo phenyl</td>
<td>C₁₉H₁₉ON₂Br</td>
<td>120</td>
<td>79.2</td>
</tr>
<tr>
<td>RC-2</td>
<td>4'-nitro phenyl</td>
<td>C₁₉H₁₀O₃N₃</td>
<td>132-134</td>
<td>72</td>
</tr>
<tr>
<td>RC-3</td>
<td>anthracenyl</td>
<td>C₂₇H₂₄O₃</td>
<td>128-130</td>
<td>69.5</td>
</tr>
<tr>
<td>RC-4</td>
<td>4'-methyl phenyl</td>
<td>C₂₀H₂₂O₃</td>
<td>161-162</td>
<td>80.7</td>
</tr>
<tr>
<td>RC-5</td>
<td>4'-chloro phenyl</td>
<td>C₁₉H₁₉ON₂Cl</td>
<td>190</td>
<td>74</td>
</tr>
<tr>
<td>RC-6</td>
<td>2',4'-dichloro phenyl</td>
<td>C₁₉H₁₈ON₂Cl₂</td>
<td>148-150</td>
<td>66.6</td>
</tr>
<tr>
<td>RC-7</td>
<td>2'-chloro phenyl</td>
<td>C₁₉H₁₀ON₂Cl</td>
<td>125-126</td>
<td>73.9</td>
</tr>
<tr>
<td>RC-8</td>
<td>4'-fluoro phenyl</td>
<td>C₁₉H₁₉ON₂F</td>
<td>185-187</td>
<td>71.5</td>
</tr>
</tbody>
</table>
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). 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% CO₂ incubator.

(b) MTT assay:
The MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide) assay developed by Mosmann 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 5x10³ cells/well in growth medium and cultured at 37°C in 5% CO₂ 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.

\[
\% \text{ Viability} = \frac{\text{corrected OD of sample}}{\text{Control OD}} \times 100
\]

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 IC₅₀ value both standard and test on different cancer cell lines.

<table>
<thead>
<tr>
<th>Type of cell line</th>
<th>Standard IC₅₀ values</th>
<th>Test sample RC-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 (Breast cancer)</td>
<td>72.55± 0.43</td>
<td>104.4± 0.82</td>
</tr>
<tr>
<td>Liver carcinoma (HepG₂)</td>
<td>3.68 ±0.10</td>
<td>230.2 ±4.41</td>
</tr>
<tr>
<td>HeLa Cervix Cancer</td>
<td>31.51 ± 0.39</td>
<td>104.9 ± 0.65</td>
</tr>
<tr>
<td>Brain cancer cell line</td>
<td>36.98 ± 0.36</td>
<td>109.8 ± 0.14</td>
</tr>
<tr>
<td>Colon cancer cell line</td>
<td>38.97 ± 0.24</td>
<td>73.72 ± 0.24</td>
</tr>
</tbody>
</table>
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.
RESULTS AND DISCUSSION

Table 2 showed that IC\textsubscript{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\textsubscript{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\textsuperscript{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\textsuperscript{36}. Many clinical studies explain the tamoxifen application in various kinds of malignamant diseases\textsuperscript{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\textsuperscript{38}. 

IC\textsubscript{50} values of RC 7 and tamoxifen on different cell lines

![Graph showing IC\textsubscript{50} values](image)

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

![Images showing before and after treatment](image)

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 256C onc.µg/ml) showed unchracterized cell. (Photographed under inverted microscope).
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 haloclines 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. Suvitha Syam et al., reported apoptosis induction of chalcones in MCF-7 cells by activating caspase-7, caspase-8, and caspase-9. Retinoid chalcone hybrids showed anticancer activity against colon cancer cell lines HT-29, and synthetic chalcones antitumor activity in brain tumor cell lines is mediated by c-Myc-mediated reactive oxygen species production.

**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 derivatives to get new leads for the treatment of cancer.

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**REFERENCES**


