2 LITERATURE REVIEW

Chalcones are the class of compounds gained much attraction because of the wide variety of biological responses and the active α, β unsaturated system. Chalcones can provide large number of derivatives by incorporating various heterocyclic rings into the enone system. Various methods were developed for the synthesis of chalcones and evaluated their biological activity. Different methods were reported for the synthesis of chalcones. Chalcones, Pyrazolines, Xanthenediones and Acridinediones were reported to possess diverse biological activity and marked cytotoxic nature against diverse cell lines.

Scott H. Kaufmann and William C. Earnshaw, 2000 (Kaufmann and Earnshaw, 2000) reported the apoptosis induction nature of various chemotherapeutic agents employed in cancer chemotherapy. The review illustrates various chemotherapeutic agents exhibiting their anticancer effect via inducing apoptosis. Drugs like Bleomycin, Cisplatin, Chloambucil, Adriyamycin, Doxorubicin and Cytarabine etc are well known apoptosis inducers.

Maioral et al., 2013 reported the apoptotic inducing nature of naphthyl chalcones in human acute leukemia cells lines. The naphthyl chalcones, 13 were screened for their cytotoxic potential against human acute myeloid leukemia (K562), and Jurkat cells. The most cytotoxic chalcones were screened for cell cycle analysis and found that the increased the proportion of cells in sub G0/G1 phase (Maioral et al., 2013).

Lou et al., 2010 reported the apoptosis induction nature of 2’, 4’-dihydroxychalcone, 14 in human gastric cancer cells, MGC 803. 2’, 4’-dihydroxychalcone
well known for it’s *in vitro* cytotoxic nature against a variety of cell lines. The antitumor mechanism of 2’, 4’-dihydroxychalcoec was performed against MGC 803. The Hoechst staining techniques illustrates the presence of characteristic morphological changes of apoptosis. 2’, 4’-dihydroxychalcoec caused a cell cycle arrest at G2/M phase was demonstrated by flow cytometric analysis (Lou et al., 2010).

Winter *et al.*, 2010 demonstrated the apoptosis induction, caspases activation nature of naphthyl chalcones on leukemic cell lines. Various naphthyl chalcones were studied about its mode of cytotoxicity against lymphoblastic leukemia cell line (L1210). Chalcones 15, 16, and 17 were found to possess time dependant cytotoxicity and apoptosis induction due to mitochondrial damage and oxidative stress (Winter *et al.*, 2010).

Keute Victor and Louis P Sandjo. 2012 reviewed the biological potential of Isobavachalcone (IBC) 18, a prenylated chalcones belongs to class of flavonoids. IBC shows marked cytotoxicity against wide range of cell lines. IBC known for the apoptosis
induction nature against neuroblastoma cell lines, IMR-32 and NB-39 by intrinsic pathway (Kuete and Sandjo, 2012).

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Synthesis of heterocyclic derivatives}
\end{figure}}
\]

Raja Solomon and Hoyun Lee, 2012 reported the synthesis of heteroaryl chalcones, 20 (a-v) employing Clasien-Schmidt condensation reactions from 5-(4-methoxyphenyl) thiophene/furan-2-carbaldehyde, 19 (Scheme 1) and evaluated the cytotoxic potential against breast cancer cells, MDA-MB231 and MDA-MB468 and noncancerous epithelial cells (184B5). The cytotoxicity nature of the compounds was studies by Sulphorhodamine B assay method. The study revealed that the methyl substitution on the phenyl ring showed excellent antiproliferative activity against MDA-MB231 and MDA-MB4. The introduction of 3-bromo-4-fluorophenyl group led to substantial reduction of antiproliferative activity on both MDA-MB231 and MDA-MB468 cells (Solomon and Lee, 2012).

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Synthesis of heterocyclic derivatives}
\end{figure}}
\]

Reaction Conditions: (i) Ethanol/ Sod. Hydroxide at 0°C

Scheme 1: Synthesis of heterocyclic derivatives

(E)-a-benzylthio chalcones, 23 were prepared as novel inhibitors of BCR-ABL kinase by (Reddy et al., 2010) The Claisen Schmidt condensation was performed in ethanol with sodium hydroxide in room temp. for 8 hours. The synthesized compounds
were assessed for their cytotoxicity activity against K562, a BCR-ABL positive leukemic cell line.

\[
\begin{align*}
\text{S} & \quad \text{O} \\
\text{S} & \quad \text{O} \\
\end{align*}
\]

Reaction Conditions: (i)NH4OAc, AcOH, reflux, 8 h; (ii)PhCH2NH2, AcOH, reflux, 8 h.

Scheme 2: Method for the synthesis of (E 2) α- aryl thiochalcones

(Kumar et al., 2003) synthesized various novel boronic acid chalcones, 27 and evaluated their cytotoxic potential against breast cancer cell lines. The study was aimed to identify the MDM2 as a drug target breast cancer therapy by inhibiting the expression of MDM2. It is found that the boronic acid chalcones were more toxic than normal chalcones towards breast cancer cells.

\[
\begin{align*}
\text{CHO} & \quad \text{CHO} \\
\text{CHO} & \quad \text{CHO} \\
\end{align*}
\]

Reaction and conditions: a NaOH, Methanol, reflux b NaH, Pinacol Boronate, THF c NaOH, H2O
Scheme 3: Synthesis of boronic acid chalcones

(Sarda et al., 2009) synthesized various substituted 1, 3-diaryl-2-propene-1-ones, 30 (a-j) by solvent free method using Al₂O₃/NaOH support. Different aldehydes, 28, substituted acetophenones, 29 (a-j) and NaOH were adsorbed on neutral alumina and heated and the substituted chalcones were formed. The solvent free approach for the synthesis of chalcones was performed successfully and reported to have more 90% yield.

![Reaction scheme](image)

Reaction and condition: Al₂O₃/NaOH, 60°C

Scheme 4: Synthesis of chalcones by solvent free methods

This method offered a simple, efficient and mild protocol for the synthesis of chalcones by Clasien – Schmidt condensation in a solvent free technique.

Synthesis of chalcones, 35 by Suzuki couplings between phenyl boronic acid, 31 and activated cinnamic acid, 32, activated benzoic acid, 27 and phenyl vinyl boronic acid, 34 by (Eddarir et al., 2003).

![Suzuki reaction scheme](image)

Scheme 5: Synthesis of chalcones by Suzuki reaction
(Zangade et al., 2011) reported the synthesis of chalcones by efficient environmentally friendly way without catalyst under mild reaction conditions by shorter reaction time. 2-Acetyl naphthol, 36 (a-j) substituted benzaldehydes, 37 (a-j) grinded with pot. Hydroxide for several minutes yielded chalcones, 38 (a-j) with higher percentage of yield and purity.

![Reaction and conditions: a KOH, grinding](image)

Scheme 6: Synthesis of chalcones by grinding method

(Lakshmi Kantam et al., 2005) synthesized chalcones, 41 by simple effect reaction by condensation of aldehydes, 39 and ketones, 40 in the presence of solid catalyst, Mg-Al-Otsu hydrotalcite (HT-OtBu). The reaction was simple; efficient gave a yield of 75 to 87% of chalcones.

![Reaction and condition; a HT-O’ Bu Catalyst, Toluene, Reflux](image)

Scheme 7: Synthesis of chalcones by solid base catalyst
Chalcones were prepared by Iodine catalysed solvent free grinding method elaborated by (Wang and Zeng, 2009). Substituted acetophenones, 42 were reacted with diversely substituted aldehydes, 43 under solvent free conditions in presence of 10 mol% of iodine for five minutes resulted 91% of chalcones 44 (a-l).

![Diagram of chalcone synthesis](image)

R₁ - H, R₂ – H, R₁- H, R₂ – 4-CH₃, R₁- H, R₂ – 3-NO₂, R₁- H, R₂ – N(CH₃)₂, R₁- H, R₂ –2OH,

R₁ – 4-CH₃, R₂ – H, R₁ – H, R₂ – 3-NO₂,

Reaction and condition: 10 mol% Iodine, Grinding

Scheme 8: Synthesis of chalcones by grinding method

Sylvie Ducki et al., reported 2009 the structural and cytotoxic relation as tubulin polymerization inhibitors between chalcones and Combretastain. A series of α alkoxy chalcones, 48 were prepared and reported the IC 50 values against K 562 cells (Ducki et al., 2009).

![Diagram of alkoxy chalcone synthesis](image)

Reactions and Conditions:   
i) Br₂ / hv, dry ethanol, 0°C, 1h  

ii) ROH, Ag₂CO₃, BF₃, Et₂O, rt, 2 days  

iii) ArBCHO, NaOH, MeOH, rt, overnight

Scheme 9: Synthesis of alkoxy chalcones
The IC 50 values of the chalcones were summarized in Table 1.

**Table 1: IC 50 values of alkoxy chalcones against K562**

<table>
<thead>
<tr>
<th>No</th>
<th>Entry</th>
<th>IC 50 values µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48a</td>
<td>0.0015</td>
</tr>
<tr>
<td>2</td>
<td>48b</td>
<td>0.0026</td>
</tr>
<tr>
<td>3</td>
<td>48c</td>
<td>0.0037</td>
</tr>
<tr>
<td>4</td>
<td>48d</td>
<td>0.011</td>
</tr>
<tr>
<td>5</td>
<td>48e</td>
<td>0.020</td>
</tr>
<tr>
<td>6</td>
<td>48f</td>
<td>0.22</td>
</tr>
<tr>
<td>7</td>
<td>48g</td>
<td>0.23</td>
</tr>
<tr>
<td>8</td>
<td>48h</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Sylvie Ducki *et al.*, 2009 elaborated chalcones as important anti mitotic agents have same mechanism of action as that of Combretastain in their cytotoxic nature. Structure Activity Relationship studies were performed and identified the chalcones which exhibiting tubulin polymerization by interacting with Colchicine binding site. α-methyl chalcones, 49 was found to be the most active with an IC 50 (inhibition of tubulin assembly) 1.8µM with cytotoxicity profile in nanogram level (Ducki *et al.*, 2009).

![Structural formula of 49](image)

(Pilatova *et al.*, 2010) illustrated the cytotoxicity and antiangiogenic profile. Four chalcones, 4- hydroxychalcones, 50, 2-(4-methoxybenzylidene)-1-benzosuberone, 51, 52 E-2-(X-benzylidene)-1-tetralones, 53 performed their cytotoxicity potential against three cell lines, HeLa, HUVEC and Jurkat cell lines. The cell cycle analysis, apoptosis analysis by Annexin V /PI staining, Western blotting, cell migration assay was performed to understand the role of chalcones in cancer prevention. The chalcones, 50, 52 and 53 significantly decreased the survival of Jurkat cell lines. At lower concentration none of the compounds showed significant effect. He chalcones were studied for their apoptosis induction nature against HeLa and Jurkat cells at 1.0µM/L concentration for 24, 48 and 72 h.
(Bellina et al., 2006) reported the imidazole modification of Combretastain analogues and evaluated the cytotoxicity and tubulin polymerization inhibition at Colchicine binding site. In this work the authors described the importance of E/Z configuration of molecules in the biological response. To maintain the potency of Combretastain the Z configuration was found to be important and achieves by bioisosteric replacement of the double bond by imidazole ring. Compounds, 1, 5-Diaryl-1H-imidazoles, \( 56 \) (a-e) were synthesized by regioselectively by coupling of 1-aryl-1H-imidazoles, \( 54 \) (a-c) with aryl iodides, \( 55 \) (a, b, d).

\[ \begin{align*}
\text{Reagents and conditions: a, } &Pd(OAc)_2 \text{ (5 mol%), CuI (2 equiv), DMF, 140 °C, 48–60 h.}
\end{align*} \]

Scheme 10: Synthesis of various imidazole modifications of chalcones.

(Sánchez Maya et al., 2005) explained the replacement of 3-hydroxy, 4-methoxy phenyl ring \( 6 \), ring B of Combretastain with 2-napthyl moiety, \( 57 \) with zero reduction of biological activity. A study using different carbocyclic and heterocyclic moieties for the replacement of ring A and ring B in Combretastain shows 2-napthyl system is a good alternate for 3-hydroxy, 4-methoxy phenyl ring. The cytotoxic activity against four different cell lines was performed and naphthyl derivatives exhibited same or better level than that of Combretastain.

(Syam et al., 2012) reported the synthesis of various chalcones and their cytotoxic effects on a choice of cell lines for lung cancer (A549), Breast cancer (MCF7), prostrate
(PC3), colorectal (HT29), and liver (WRL68). Diversely substituted acetophenones, 58 (a-e) reacted with various substituted benzaldehydes, 59 (a-j) resulted the formation of chalcones 60 (a-x).

\[
\text{R}_1\text{CHO} + \text{R}_2\text{O} + \text{R}_2\text{R}_1 \rightarrow \text{R}_1\text{R}_2
\]

R1- H R2- H, R1-4CH3 R2- H, R1- OCH3, R2- H, R1- H R2- 2Cl, R1-4CH3, R2- 4Cl, R1- OCH3 R2-2-Cl, R1- H R2- 4CH3 R1- 4CH3 R2- 4CH3

Reagents and conditions: Grinding with NaOH, 10 mins

Scheme 11: Synthesis of chalcones by grinding technique

The cytotoxicity study was performed by MTT assay method and IC 50 values were calculated. The results showed the compounds exhibited well cytotoxic activity against all cell lines except A549. The mode of cytotoxic nature was not studied.

(Sivakumar et al., 2010a) illustrated the synthesis of 1, 3, 5-triphenyl-2-pyrazoline from chalcones and evaluated their biological activity. Here chalcones were synthesized by Clasien Schmidt condensation reaction and further treated with phenyl hydrazine in acetic acid to yield 1, 3, 5-triphenyl-2-pyrazoline.

\[
\text{R}_1\text{CHO} + \text{R}_2\text{R}_1 \rightarrow \text{R}_1\text{R}_2\text{N} = \text{N} + \text{R}_2\text{R}_1
\]

R1: -C6H5CH2O, -Cl, R2: - OCH2-CH3, -F, -Br, -OCH3,

Reagents and conditions: a) KOH/Ethanol, stirring rt b) phenyl Hydrazine, Acetic acid, reflux, 2-7 h

Scheme 12: Synthesis of 1, 3, 5-triphenyl-2-pyrazolines
The efficient, simple method for the synthesis of acetylated pyrazolines from chalcones was developed and pyrazolines were obtained in high yield and purity.

(Rathish et al., 2009) reported the synthesis and biological evaluation of 1, 3, 5-trisubstituted pyrazolines possessing benzene sulphonamide. Chalcones, 59 on treatment with 4-hydrazinobenzensulfonamide hydrochloride in ethanol resulted the formation of 3, 5-diaryl-2-pyrazolines bearing benzene sulfonamide moiety, 67 (a-s).

\[
\begin{align*}
58 & \quad + \quad 65 \\
& \quad \xrightarrow{\text{i}} \quad 66 (a-s) \\
& \quad \xrightarrow{\text{ii}} \quad 67 (a-s)
\end{align*}
\]

Reagents and conditions: (i) 30% aq NaOH, EtOH, 0–5°C, 12 h; (ii) 4-hydrazinobenzensulfonamide hydrochloride, EtOH, reflux 12–18 h.

**Scheme 13:** Synthesis of 1, 2, 3 trisubstituted pyrazolines

(Havrylyuk et al., 2009), synthesized several novel thiazolone-based compounds containing 5-Aryl-3-phenyl-4, 5-dihydro-1Hpyrazol-1-yl molecular structure and screened for in vitro anticancer activity. Most of them displayed anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, and prostate and breast cancer cell
lines. The most efficient anticancer compound, 68 was found to be active with selective influence on colon cancer cell lines, especially on HT 29 (log GI50 = -6.37).

(Manna and Agrawal, 2009) synthesized a series of substituted pyrazolines, 69 and evaluated them for their anticancer activity and for their ability to inhibit P-glycoprotein mediated multidrug resistance by direct binding to a purified protein domain containing an ATP-binding site and a modulator interacting region. Compounds found to bind to P-glycoprotein with greater affinity.

(Johnson et al., 2007a) designed and synthesized new pyrazolines derivatives, 70 which are analogous to combretastatin-A4 and tested for anticancer activity against verity of cell lines.
(Yar et al., 2007) reported a series of 5-(-(4-(substituted)phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-toluidino methane thione and 5-(substituted)phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-2-methoxy anilino methane thiones were synthesized and examined against human lung tumor cell line (A549) in vitro. Among those tested, 5-(4-fluorophenyl)-3-(4-hydroxy-3-methylphenyl)-4, 5-dihydro-1H-1-pyrazolyl-2-toluidino methane thione, $71$ & 5-(4-chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4, 5-dihydro-1H-1-pyrazolyl-2-methoxy anilino methane thione, $72$ showed more potent cytotoxicity than the other synthesized compounds.

Heravi et al., 2009, reported the synthesis of various Xanthene-1, 8-dione, $75$ and Acridine-1, 8-dione, $76$ using heteropoly acid catalysts from dimidone, $73$ and aromatic aldehydes $74$. Heteropoly acid is considered as green, inexpensive easily available reusable catalyst under solvent free conditions.

Reagents and conditions: i) $\text{H}_{14}\text{[NaP}_5\text{W}_{30}\text{O}_{110}]$ (0.7 mol %), 110°C
Scheme 14: Synthesis of Xanthenes 1,8-dions

(Kantevari et al., 2006) reported the synthesis of 9-aryl-1,8-dioxooctahydroxanthene, 77 by simple efficient way using dimidone, 73 substituted benzaldehydes, 67. In this methodology the authors performed refluxing along with MeCN and TMSCl.

Scheme 15: Synthesis of 9-aryl-1,8-dioxooctahydroxanthene

(Dabiri et al., 2008), reported the one pot synthesis of Xanthenedions, 79 under solvent free conditions using MK10 as a catalyst. In the method the authors performed the synthesis by heating the mixture of 5, 5-dimethyl-1, 3-cyclohexanedione, 73, 4-chlorobenzaldehyde, 78 at 100 °C long with montmorillonite K10 for 1.2 h.
Reagents and conditions: MK10, heating at 100°C

Scheme 16: Synthesis of Xanthenedions by solvent free methods

(Mulakayala et al., 2012) synthesized and evaluated the anticancer property of various 1,8-dioxo-octahydroxanthenes, 80 using molecular iodine as the catalyst. All the compounds exhibited significant cytotoxic potential and 9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione, 81 found to be promising in cytotoxic nature.

Reagents and conditions: Iodine, Isopropyl alcohol, 70 - 80°C

Scheme 17: Synthesis of Xanthenedions catalysed by molecular iodine
The compound, 82 showed better cytotoxic activities against all the cell lines employed for the study, K 562 (23 µM), Colo205 (38 µM), IMR 32 (23 µM) respectively.

(Das et al., 2007) reported the efficient simple method for the synthesis of 1, 8-Dioxo-octahydroxanthenes, 83 from 5, 5-dimethyl-1, 3-cyclohexanedione, 73 and aromatic aldehydes, 74 in the presence of silica supported sodium hydrogen sulfate (NaHSO₄·SiO₂).

Reagents and conditions: i) NaHSO₄·SiO₂, CH₃CN, 6-6.5 h, reflux

Scheme 18: Synthesis of 1,8-Dioxo-octahydroxanthenes by heterogeneous catalysis

(Pore et al., 2010) demonstrated the synthesis of 1-oxo-hexahydroxanthenes, 85 by an environmentally friendly green method. This is achieved by reacting 5, 5-dimethyl-1, 3-cyclohexanedione, 73 and salicylaldehyde derivatives, 84 under reflux in water for an hour without catalyst.

Reagents and conditions: Water, Reflux

Scheme 19: Synthesis of 1-oxo-hexahydroxanthenes in water
(Das et al., 2006) reported the usage of Amberlyst-15 for the synthesis of various 1, 8-dioxo-octahydroxanthenes, 86 (a-o) and 1, 8-dioxo-decahydroacridines, 87(a-o) in excellent yields.

Reagents and conditions: i) RNH₂, Amberlyst-15, CH₃CN, Reflux, 6h
ii) Amberlyst-15, CH₃CN, Reflux, 5h

Scheme 20: Synthesis of xanthenediones and Acridinediones

(Giri et al., 2010) reported the synthesis and cytotoxic study of various xanthenes derivatives against various cell lines, DU145, MCF17 and HeLa. Compound, 88 ([N,N-diethyl]-9-hydroxy-9-(3-methoxyphenyl)-9H-xanthene-3-carboxamide), was found to possess an IC₅₀ values ranging from 36 to 50 µM across all three cancer cell lines.

(Bhattacharya et al., 2009); reported the cytotoxic potential and the synthesis of 14-aryl-14H-dibenzo[a.j]xanthenes, 90 (a-n) by one pot condensation reaction of 2-Naphthol, 89 with various aryl aldehydes, 80 by TaCl₅ under solvent free conditions. The methodology was found to be simple efficient solvent free condition and solvent free conditions. All the compounds were tested their cytotoxic potential against six cell lines. Compounds 91, showed an IC₅₀ of 37.9 and 41.3 µM against Colo-205 and 502713, respectively, whereas 92 showed IC₅₀ of 41.9 µM against Colo-205.
The detailed survey of literature made clarifications regarding the need of synthesis of various polymethoxy substituted chalcones and it’s heterocyclic derivatives as anticancer agents. The role of chalcones as anticancer agents is well understood from the available reports. But the involvement of polymethoxy substituted methoxy chalcones and its acetylated pyrazolines derivatives as an anticancer agents and induction of apoptosis nature is not well studied. The molecular interaction studies of chalcones, pyrazolines xanthenediones and acridinediones on tubulins was also not yet studied well to understand the mechanism of inhibition.

In the present scenario medicinal chemists are in search of simple small molecules which can synthesize, and purify easily as therapeutic agent. In this regards chalcones, and its heterocyclic analogues, xanthenediones and acridinediones gains a momentum in cancer research. In the present study owns the objectives as synthesizing chalcones and its derivatives, xanthenediones, Acridinediones as analogues of Combretastain with

i. To study the molecular interaction between the tubulin and the synthesized analogues by \textit{in silico}. 

\[ 
\begin{align*}
\text{CHO} & \quad R + 2 \quad \text{OH} \\
80 \text{ (a-n)} & \quad 89 \quad \rightarrow \quad 90 \text{ (a-n)} \\
\end{align*} \]

\textbf{R: -4Cl, -2Cl, -4-OCH}_3, -\textbf{NH}_2

\textbf{Reagents and Conditions: TaCl}_5 (10 \text{ mol %}), \text{ Heating}

\textbf{Scheme 21: Synthesis of benzoxanthenes by condensation reaction}
ii. The modifications of two aromatic rings by increasing the ring size. Vary the number and position of methoxy groups in the both the rings.

iii. Increasing the carbon chain between two aromatic centers. Introducing rings on the bridge and restricting the configuration.

iv. To assess the anticancer activity of the synthesized analogues by *in vitro* and the apoptosis inducing nature of the promising molecules.

v. Develop a QSAR between the descriptors and quantified cytotoxicity value.