5 CONCLUSION

The present study was aimed to synthesis various polymethoxy substituted chalcones, N-acetyl pyrazolines, xanthenediones and Acridinediones by different routes. Three different methods of synthesis were identified for chalcones and performed a comparative study about all the three methods. The methods identified were, Clasien – Schmidt condensation, grinding method and nanoparticle catalysed synthesis. All the three methods offered chalcones in good yield and high purity. The grinding and nanoparticles catalysed methodology satisfied the principle of green chemistry in terms of solvent free synthesis. Consider the reaction time and reusability of the catalyst the nano mediated synthesis had an upper hand over other two methodologies adopted. All the chalcones were converted in to N-acetyl pyrazolines by reaction with hydrazine hydrate in the acetic acid in ethanol under reflux. A series polymethoxy substituted xanthenediones and acridinediones were synthesized by reacting cyclohexanedione with polymethoxy benzaldehydes. The reaction conditions were optimized and identified a better catalyst, gallic acid for the conversion. All the synthesized compounds were characterized by FTIR, $^{1}$HNMR, $^{13}$CNMR and HRMS techniques.

A molecular docking study was performed for all the molecules on tubulin (1FFX) using GLIDE module of Schrodinger suit. The docking results were analyzed using XP visualizer. The interaction study was compared with a standard Colchicine since the ligands were allowed to bind on Colchicine binding site of tubulin. All the chalcones and pyrazolines were found to have a better interaction but acridinediones and xanthenediones were showed poor interaction with the site. Re-docking was performed for all the ligands and found to have a RMSD value of zero.

A representative series of chalcones and acetylpyrazolines were screened for their antioxidant and free radical scavenging nature by four different methods. All the study was performed at five different concentrations ranging from 10µg/mL to 100µg/mL. Compound, 3-(2,4-Dimethoxyphenyl)-1-(2-naphthyl) prop-2-en-1-one, (98d) showed a scavenging activity of 22.1% at 10 µg/mL concentration against DPPH radical. Acetyl pyrazolines showed satisfactory scavenging activity against DPPH radical. Hydrogen
peroxide scavenging activity was performed and among chalcones compound, 3-(2, 3, 4-Trimethoxyphenyl)-1-(2-naphthyl) prop-2-en-1-one, (98b) and 1-Acetyl-5-(2,3,4-trimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole, (102b) exhibited 26.7% and 44.8% respectively. Both chalcones and pyrazolines showed comparatively poor scavenging effect against Fe$^{2+}$ ions. When the analysis of NO scavenging activity was done it was found that all the chalcones and pyrazolines documented high percentage of scavenging effect. 3-(2, 3, 4-Trimethoxyphenyl)-1-(2-naphthyl) prop-2-en-1-one, (98b) produced a scavenging activity of 32.1% against NO free radicals. A generalized conclusion can be made for the antioxidant study was both chalcones and pyrazolines were proved as good scavengers for free radicals except Fe$^{2+}$. The chalcones and pyrazolines possessing methoxy groups at position 3 of ring B were found have better scavenging activity.

Cytotoxicity potential by MTT assay of all the synthesized compounds were performed against a panel of three cell lines and IC 50 values were documented. Chalcone, 1-(2-Fluorophenyl)-3-(3,5-dimethoxyphenyl) prop-2-en-1-one (97e) had an IC 50 value of 0.0209µM against HCT-15, 0.0436 µM against A-549 and 0.0629 µM against HeLa. 3-(3,4,5-Trimethoxyphenyl)-1-(2-naphthyl) prop-2-en-1-one, (98c) showed an IC 50 value of 0.019 µM against HeLa, 0.020 µM against HCT-15 and 0.022µM against A-549. Other chalcones, 99b and 99h were also found to have cytotoxicity value of 0.0209 and 0.0167 against HCT-15 and with an IC 50 value of 0.0195 against A-549. Chalcone, 101h had a cytotoxic profile with an IC 50 value of 0.0266 µM, against HeLa and 0.0430 µM against A-549. All the chalcones showed promising cytotoxic activity against all the three cell lines employed for the study. A generalized conclusion can be made from the observations that those chalcones possessing a methoxy group at third position either with another one or two methoxy groups showed critical activity. This indicated the position 3 in the ring B of chalcones have much importance in interacting the binding sites to produce a cytotoxic effect.

Those compounds produced significant cytotoxic nature alone considered for cell cycle analysis study using flow cytometry. Compounds at their IC 50 values were treated with HeLa and A-549 for the cell cycle study. This study reveals compounds 1-(2-
Fluorophenyl)-3-(2,4–dimethoxyphenyl)prop-2-en-1-one \(97c\), 1-(2-Fluorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one \(97e\), 1-(2-Fluorophenyl)-3-(2,5-dimethoxyphenyl) prop-2-en-1-one \(97f\) showed an indication of apoptosis on A-549 cells. Compound \(97e\) was found to induce apoptosis the most against A-549 in comparison with other compounds. Chalcone \(98\) showed better apoptotic nature against HeLa and other compounds were satisfactory in nature.

DNA damage of the cells was performed by alkaline comet assay and calculations were performed using Tri-Tek cometscore. The highest DNA damage was found to be in 3-(3,4,5-Trimethoxyphenyl)-1-(2-naphthyl) prop-2-en-1-one \(98\) where 77.6% of DNA was found in the tail of the comet. Hoechst 33342 DNA staining for scoring apoptosis was also performed qualitatively and compound \(98\) showed profound activity.

A QSAR study was performed using the IC 50 values of A 549 and four variables. The study revealed that 2,4 or 3,4 or 2,5-dimethoxyphenyl groups in compounds make QSAR models more predictive when RDF110V (fourth variable) is introduced. It was observed that inclusion of RDF110V to four variable non-linear QSAR model acclaims estimated responses very close to those of the observed ones. Effect of other substituent was found to be silent in comparison to strong correlation between 2, 4 and 2, 5-dimethoxyphenyl group substitution and contribution of RDF110V and RDF060. Absence of 2,4 or 2,5 dimethoxyphenyl groups or presence of 2,3,4 or 2,4,5 or 2,4,5 or 2,4,6-trimethoxyphenyl groups make the effect or contribution of RDF110V silent in non-linear QSAR model. Presence of -1-(naphtalen-2-yl) in molecules has recorded a flat response from addition of RDF110V, RDF060 and RDF05e.

The present study reveals various simple and efficient techniques for the synthesis of chalcones, pyrazolines and xanthenediones and acridinediones. The role of chalcones and other analogues as anticancer agents were well established \textit{in vitro} and there are more areas to explore the role of these compounds \textit{in vivo}.  

154