CHAPTER VI
SUMMARY AND CONCLUSION

The thesis entitled “In silico design, synthesis and biological evaluation of novel 1,2,4-triazole derivatives” submitted by the author is divided into 6 chapters.

Chapter 1 deals with an introduction wherein details about the design of drugs, Molecular modeling, cancer, tuberculosis and inflammation have been highlighted.

Cancer is a group of more than 100 diseases characterized by uncontrolled cellular growth as a result of changes in the genetic information of cells. Cells and tissues are complex systems with critical stages and checkpoints to ensure normal growth, development, and function. Normally the division, differentiation, and death of cells are carefully regulated. All cancers start as a single cell that has lost power over its normal growth and reproduction processes. Human adults are made up of around $10^{13}$ cells, which are renewed and replaced constantly. About 5–10 percent of cancers result directly from inheriting genes associated with cancer, but the majority involves alterations or damage accumulated over time to the genetic material within cells. The causes of damage are both endogenous (internal) and exogenous (environmental). Food, nutrition, and physical activity are important environmental factors in the development of cancer.

Chapter 2 deals with review of literature about the molecular modeling and the compounds synthesized using the model, which can be used for the treatment of cancer, tuberculosis and anti-inflammatory activity. It also discussed the different types of heterocyclic compounds used for the expected in vitro and in vivo anti-cancer, in vitro anti-tuberculosis and anti-inflammatory activity.

Chapter 3 deals with the scope and objectives of the study and the aim to design, synthesis, characterization and evaluation of in vitro and in vivo anticancer activities, in vitro anti-tuberculosis and anti-inflammatory activity. The objectives of the research work are

- To perform in silico molecular modelling studies of proposed 1,2,4-triazole derivatives
- To select the derivatives based on the results of in silico studies
- To synthesize the selected derivatives by conventional method
To characterize the synthesized compounds using IR, \(^1\)HNMR, \(^{13}\)CNMR, Mass spectra and elemental analysis data

To evaluate the pharmacological activity of synthesized compounds:
- Toxicity study
- \textit{In vitro} anticancer activity
- \textit{In vivo} anticancer activity
- \textit{In vitro} antitubercular activity
- \textit{In vivo} anti-inflammatory activity

Chapter 4 deals with materials and methods. The complete list of materials, chemicals, equipments and instruments were used for the present studies have been listed. The methodology of research has been described:

All the computational procedures of the present work were carried out by Intel\(^\circledR\)Core (\textsuperscript{TM}) 2 Duo processor with Microsoft Windows XP. Software used in this research work are mentioned.

\textit{In silico} molecular modifications of 55 proposed derivatives of 1,2,4-triazole were done by using various software. The derivatives selected with the help of these selection parameters were taken to wet lab synthesis. The selected compounds were synthesized by conventional method through a series of four steps.

ACD Lab Chemsketch 10.00 was employed for 3-D drawing, optimizing and calculating various physicochemical descriptors of the proposed molecules. Molinspiration was used for calculating the log \(P\) values, drug likeness and Lipinski’s rule of five. PASS software was utilized for the forecast of pharmacological activities of the proposed moieties. Schrodinger (Maestro) Version 9.6 – QIKPROP was employed to predict the ADME properties of the proposed moieties. Schrodinger (Maestro) Version 9.6 - Glide XP was utilized for molecular docking of the proposed molecules.

The computational modeling investigation relied upon the GLIDE (Grid-based Ligand Docking from Energetics) program (Glide, version 5.0, Schrodinger, LLC New York, 2008) for the docking assignment. Crystallographic structures of the target of interests (receptors/enzymes) were obtained from the PDB (protein data bank) and saved in a standard 3D co-ordinate format. Protein targets and their PDB ID are Tyrosine protein kinase: 3 KRR; Protein kinase –C:1 ZRZ; Heat Shock Protein:2CCU; Tubulin: 3M89; \textit{Mycobacterium tuberculosis} pantothenate synthetase: 3 COZ;
Glutamine synthetase from *Mycobacterium tuberculosis*: 1HTO; Mitogen-activated protein kinase: 3 CTQ.

Novel 1,2,4-triazole derivatives were selected through target based drug design and then prepared and characterization was performed by Fourier Transformed Infra Red, Proton NMR, Carbon-13 NMR and Mass spectroscopy. The toxicity evaluation was done in accordance with the procedure described by Sita Sharan Patel, *et al.*, 2011 and Shirish Sadashiv Pingale, *et al.*, 2011. Anticancer activities of selected compounds were evaluated against SKMEL (Human malignant melanoma cell line), MCF7 (Breast cancer cell line) and Hep2 (He La derivative) cell lines by the MTT assay method. The *in vivo* anticancer activity of selected compounds was evaluated with the procedure of Mathan, *et al.*, 2013 and antitubercular activity of selected compounds was evaluated by Resazurin micro titer assay (REMA) method by the procedure described by Reham, *et al.*, 2013. The anti-inflammatory activity of selected compounds was evaluated by carrageenan-induced paw edema method and was carried out as per the procedure of Sanjay Yadav, *et al.*, 2012.

Chapter 5 deals with a detailed account of results with corresponding interpretation and discussion on the outcome of each phase of experimentation. 55 different proposed derivatives of 1,2,4-triazole were subjected to *in silico* molecular modifications by using different software. 3D-drawing, optimizing and calculating various molecular descriptors of proposed derivatives were done by using ACD Lab Chemsketch software.

The molinspiration software was used to study the LogP values, violation of Lipinski’s rule of five and drug likeness by comparing with already existing standard drugs. Analysis of Lipinski’s rule of five revealed that 13 out of 55 proposed derivatives did not follow the Lipinski’s rule and hence those 13 compounds were not suitable for the biological evaluation. The PASS software was used to predict the biological activities of proposed molecules. The result of the prediction is presented as the list of activities with appropriate ‘Pa’ (Probability to be active) and ‘Pi’(Probability to be inactive) sorted in descending order of the difference (Pa-Pi) > 0. ‘These values vary from 0.000 to 1.000. If Pa > 0.7, the compound is very likely to produce this activity in experiments, but the chance of being the derivative of the known therapeutic agents for this compound is also high. If 0.5 < Pa < 0.7, the compound is likely to exhibit its
activity in experiments, but this probability is less, and the compound is not so similar to the known therapeutic agents. If $P_a < 0.5$, the compound is unlikely to show its activity in experiments, but if the activity is confirmed in the compound, it might be a new chemical substance.

Schrodinger Glide XP software was used for predicting the protein-ligand binding modes. In this study, the compound having high (-) value is considered to possess significant therapeutic activity. The proposed 1,2,4-triazole analogues were subjected to four different anticancer proteins, namely Tyrosine protein kinase, Protein kinase-C, Heat shock protein and Tubulin to explore their effectiveness as agents against cancer. The analogues compound 17 and compound 18 presented good binding interaction in terms of glide score with all the four proteins. Hence the analogues, Compound 17 and Compound 18 can be proposed to have better anticancer activity and therefore selected for their synthesis. The compounds having significant docking scores for anticancer activity are compound 17, 18 & 20 to Tyrosine protein kinase (3KRR); compound 17 & 18 to Protein Kinase – C (1ZRZ); compound 17 & 18 to Heat shock protein (2CCU); compound 17 & 18 to Tubulin (3M89); compound 17 & 18 to 3KRR by Discovery studio.

In case of docking study for antitubercular activity, the analogues compound 10 and compound 54 exhibited highest glide score on binding to antitubercular proteins namely 3 COZ and 1 HTO. Where as compound 7 exhibited glide score only with 1HTO. Hence, these three analogues were selected for synthesis. Regarding with the docking study for anti-inflammatory activity, the docking with 3CTQ, the analogues compound 17 and compound 18 presented profound binding interaction and found to show highest glide score, implying the prospective efficiency as agents for inflammatory diseases. Hence, these two designed analogues are considered for future use.

In this evaluation the synthesized compounds 7, 10, 17, 18 and 54 were subjected to acute toxicity ($LD_{50}$), and sub acute toxicity studies. The $LD_{50}$ study was performed and the dose fixed for the synthesized compound 17 and 18 was 10mg.kg$^{-1}$bw as evaluation dose. Among the five derivatives, the compound 17 and 18 were selected for the in vitro anticancer evaluation based on their Schrodinger Glide XP score. The selected compounds were evaluated against SKMEL, MCF7 and Hep2 cell
lines by the MTT assay method. The tested derivatives showed cytotoxic activity against the three tested cancer cell lines. But the compound 17 showed significant anticancer activity in both Hep2 and MCF7 cell lines with IC\textsubscript{50} values of 125\textmu g/ml and 128\textmu g/ml respectively.

Since the compound 17 has shown better anti-cancer activity by \textit{in vitro} method, it was selected for the evaluation of \textit{in vivo} anticancer activity. Two doses of test derivative 10 and 20 mg.kg\textsuperscript{-1}bw were utilized for the anticancer evaluation. The 5-fluorouracil injection in the dose of 20mg.kg\textsuperscript{-1}bw was used for the comparative evaluation. Body weight, life span, cell count, hematological and biochemical parameters of treated animals were analyzed and compared.

In case of average life span, the tumor control animals showed the life span of 48\%, whereas in group 4 and 5, animals treated with the compound 17 at the dose of 10 and 20mg/kg. bw showed the life span of 75\% and 78\% respectively. The animal group treated with 5-fluorouracil showed the life span of 92\%.

There was increased cancer cell count in the group 2 tumor control animals. In DLA tumor bearing, a regular rapid increase in ascitic tumor volume was observed. The results showed that treatment with the tested compound at a dose of 10 and 20 mg/kg.bw inhibit the tumor volume and viable tumor cell count. Treatment with synthesized compound 17 at the dose 10 and 20mg/kg.bw was found significantly reduced (p < 0.001) in the level of WBC in comparison with tumor rats (group 2). However, group 5 synthesized compound 17 at the dose 20mg/kg body weight showed that the WBC was restored to near normal as that of group 3 (5-fluorouracil). Treatment with synthesized compound 17 at the dose 10 and 20mg/kg.bw significantly increased in the level of RBC count, hemoglobin level, platelets and packed cell volume in comparison with tumor rats (group 2). From the results, it was observed that the treatment with this compound brought back the hemoglobin level, RBC and platelet count to normal levels significantly.

Among the five derivatives, the compound 10 and 54 were selected for the \textit{in vitro} antitubercular evaluation based on their Schrodinger Glide XP score. The selected couple of derivatives showed antitubercular activity, but the compound 10 showed significant antitubercular activity comparing with the compound 54.
Based on the results of *in silico* screening, compound 17 and 18 were selected for the evaluation of anti-inflammatory activity. From the results it was clear that both the selected compounds exhibited anti-inflammatory activity. Among the two compounds, the compound 18 proved to be effective anti-inflammatory agent.

The synthesized compound 10: (2- [(Diethyl amino) methyl] -5- (4-hydroxy phenyl) -4- {((4-nitro phenyl) methylidene) amino}]-1,2,4-triazolin-3-thione) possesses significant antitubercular activity and the synthesized compound 17 (2- (piperidin-1-ylmethyl) -5- (4-hydroxy phenyl) -4- {((4-dimethyl amino) phenyl methylidene) amino}]-1,2,4-triazolin-3-thione) is having better *in vitro* and *in vivo* anticancer activity. The synthesized compound 18: (2- (morpholin-4-ylmethyl) -5- (4-hydroxy phenyl) -4- {((4-dimethyl amino) phenyl methylidene) amino}]-1,2,4-triazolin-3-thione) has distinctive anti-inflammatory activity. These effects may be due to the bridging of 1, 2, 4- trizole derivatives with suitable heterocyclic or aromatic ring systems.
SCOPE FOR FUTURE STUDY

The present study scientifically established the design, synthesis, characterization and evaluation of in vitro and in vivo anticancer activity, anti-tubercular activity and anti-inflammatory activity. The synthesized compounds can be further investigated on

✓ A detailed pharmacodynamic and pharmacokinetic studies of the compound 17 - 2- (piperidin-1-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione for anticancer activity and compound 18 - 2- (morpholin-4-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione for anti-inflammatory activity.

✓ Clinical studies for the synthesized compounds 17 (Phase I, Phase II and Phase III) 2- (piperidin-1-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione for anticancer activity and compound 18 2-(morpholin-4-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione for anti-inflammatory activity.

✓ Formulation and evaluation of synthesized compound 17 2-(piperidin-1-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione.

169