5. Conclusions
The thesis includes applications of computer-aided drug design, synthetic chemistry and biological evaluation approaches for cancer research. The major purpose of the current work is to discover some potential lead molecules against potential biological targets especially involved in cancer related diseases. The work was started with design of some molecules from the hypotheses of 2D QSAR analyses [128]. The work is based on the fact that every molecule contains some structural features for which to be active against some biological targets for phenylacetyl isoglutamines. Therefore it is also important to find out the potential biological targets for the designed molecules. Exhaustive literature review as well as in silico analyses helped in the identification of MMPs as the most potential biological targets. The designed compounds were synthesized based on these predictions and both enzymatic and cellular assays were conducted to justify the predictions. Some of these compounds showed potential activities against MMP-2, the most validated MMP target against cancer invasion and metastases. In addition, cytotoxicity assays conducted on multiple human cancer cell lines confirmed that the designed compounds are non-cytotoxic as well as non-apoptotic in nature. However, the molecules are predicted to be active against other two metalloenzymes, HDAC-8 and APN, both of them are important targets for the inhibition of cancer invasion and metastases. The current work further explores the compounds as dual MMP-2/HDAC-8 inhibitors and investigates the involvements of such dual inhibitors for the inhibition of human lung carcinoma migration and invasion. Therefore, some potentially active MMP-2 inhibitors were tested against HDAC-8 and nuclear extract of HDACs (rich in HDAC-1 and 2). The enzymatic assays confirmed that designed molecules are active against HADC-8 but less potent against nuclear HDACs, indicating that they may act as selective HDAC-8 inhibitors. The enzymatic assays performed on MMPs and HDACs provided the opportunity to understand the contributions and influences of different isoforms for inhibition of migration and invasion of human lung carcinoma A549 cells. These cellular assays revealed that apart from MMP-2 and HDAC-8, MMP-9 plays an important role in these processes. Compounds inhibiting MMP-2, MMP-9 and HDAC-8 showed the higher rate of inhibitions comparing those inhibiting any two isoforms.

The dissertation also includes detailed molecular modeling analyses of these phenylacetyl isoglutamines as APN inhibitors. This in silico study may be utilized for
Conclusion

the design of novel APN inhibitors. The investigation insinuated that the designed molecules (D1-D76) may be developed as potential APN inhibitors. After confirming that isoglutamine fragments contain specific fingerprints for selective MMP inhibition, some drug designing methodologies, like fragment-based lead preparation and de novo lead modification were adopted to design some potent as well as selective MMP-2 inhibitors. The best active designed MMP-2 inhibitor had IC$_{50}$ value of 24 nM whereas the best selective inhibitor (IC$_{50} = 51$ nM) showed at least 4 times specificity to MMP-2 against all tested MMPs. Active derivatives are non-cytotoxic against human lung carcinoma cell - A549. At non-cytotoxic concentrations, the inhibitors reduced intracellular MMP-2 expression up to 78% and also exhibited satisfactory anti-migration and anti-invasive properties against A549 cells.