CHAPTER – 4

SUMMARY

OF

THE PRESENT THESIS
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In summary, the present thesis entitled “Conventional and Microwave Assisted Synthesis of Quinazolinone-Sulfonamide Linked Hybrid Molecules derived from Amino Acids and their Biological Studies” is divided into four chapters. This chapter-4 includes the summary of all the investigations carried out for the present thesis.

The chapter-1 deals with a brief introduction of 4(3H)-quinazolinones and sulfonamides in the point of synthetic methods and their medicinal importance, followed by which, an overview for the incorporation of amino acids and sulfonamide in the quinazolinone ring system, and an overview to hybrid molecules by structural hybridization as well as the background of microwaves in organic synthesis are discussed.

Based on the objectives, several 4-(actamido/amino)-N-[(4-oxo-3-substituted aryl-3,4-dihydroquinazolin-2-yl)alkyl]benzenesulfonamides \{G, A, V, L, F -(1-19)- Ac/Am\} with the diversity in their molecular structure were designed to assimilate 4–quinazolinone and sulphonamide moieties in a single molecular framework.

Various attempts had been carried out in the laboratory to establish the easy and milder methodologies to derive entitled quinazolinone-linked-sulfonamide hybrid entities in good amount of yields from commercially available appropriate amino-acids. The detailed synthetic aspects to derive entitled hybrid molecules from amino acids using conventional and microwave irradiation method is described in the Chapter-2. In beginning, the optimization of synthetic methodologies for the synthesis of entitled compounds, followed by the experimental protocols and data of synthesized compounds are described.

Two approaches were adopted for the successful synthesis of desired hybrid molecules from the amino acids without involving group protecting strategies.

[a] An easy, efficient, milder and rapid multi–step synthetic approach was developed from unprotected $\alpha$-amino acids, which involves milder conditions for emphasizing steps viz., (i) preparation of 4-(acetamidobenzenesulfonyl)amino acids by the reaction in aqueous–media, (ii) phosphazo–method of condensation for the preparation of methyl N–acylanthranilates, (iii) base mediated selective ester–cleavage of previously prepared esters to produce N–acylanthranilic acids, along with (iv) key–step, rapid and improved Grimmel’s hetero–cyclization method for the formation of quinazolinone ring tolerating desired substitutions at position-2.

[b] A convenient and convergent one-pot synthetic approach was also established for the synthesis of entitled compounds in the presence of triphenyl phosphite as a
condensing reagent to proceed the formation of quinazolinone ring by the cyclo-condensation of primary amines with the important intermediate 2-substituted-1,3-benzoazin-4-ones. The required intermediate was prepared by the reaction of \( N-(4\)acetamidobenzesulfonyl)amino acids and anthranilic acid, which upon condensation lead to the formation of \( N\)-acylanthranilic acids to produce appropriate benzoazinones in a one-pot reaction fashion.

The conventional as well as microwave-assisted approach was explored for the synthesis of compounds. It was observed that under the microwave irradiation conditions, reaction time was improved with a little effect on the yields of compounds.

The structures of so-called synthesized compounds were confirmed with the help of physico-chemical analyses, and spectroscopic techniques like FT-IR, \(^1\)H-NMR, \(^{13}\)C-NMR and Mass spectroscopy.

Chapter-3 deals with the biological activity of 4-\((3H)\)-quinazolinones, and it includes two sections: Section [A] *in silico* screening of all the synthesized compounds, and Section [B] *in vitro* evaluations of the filtered compounds.

As described in Section [A], *in silico* virtual screening of the library of newly designed ligands was carried out to explore computer-assisted chemistry. Accordingly, QSAR screening of the library of ligands (190 entitled compounds) was carried to find out the drug-like candidates based on the ADMET screening using various filters like Lipinski rule of five, mutagenicity and carcinogenicity profile. The docking was performed with the help of GOLD programme to identify the lead compound and to study the Ligand-Receptor interaction of entire set of filtered hits candidates (12 molecules) against 3D-protein structures of the receptors of antimalarial, antituberculosis and anti-HIV activity. All the compounds have shown Ligand-Binding interactions as indicated by the dock fitness score. Further, virtual screening of 12 hits by docking with various receptors of antimalarial (**G12Am; G15Am; G13Am**), antitubercular (**G13Am; G12Am; G15Am; V13Am**) and anti-HIV (**L13Am; G13Am; V13Am; G15Am**) activity resulted with five compounds **G12Am, G13Am, L13Am, V13Am and G15Am** among the screened hits with drug like property out of possible derivatives. Of which, the most promising lead compound **G13Am** has shown apparent antitubercular activity with 1EYE (GoldScore : 43.6151) and anti-HIV activity with 1IKV (GoldScore : 36.0201), while **G12Am** found to have strong potential of antimalarial activity with 1RL4 (GoldScore : 44.4362). These novel and potent lead compounds could serve as advanced leads for further optimization as well as *in vitro* exploration of antimalarial and antituberculosis activity.
In vitro antimicrobial, antimalarial and antituberculosis activity of twelve filtered hits, quinazolinone-sulfonamide molecules (selected by the in silico screening) is discussed in the section [B] of Chapter-3.

In vitro antibacterial and antifungal activity were carried out against four bacteria (S. aureus, B. subtilis, S. mutans, E. coli) and two fungi (A. niger, C. albicans) by the broth dilution method. From the above results, it was observed that compounds G15Am and G13Am showed excellent activity, followed by G7Ac, G12Am, V13Am, L12Am and L14Am showed good to better activity against all the bacteria and fungi with comparable MIC values to the standard drugs.

In vitro antituberculosis activity was measured against M. tuberculosis H37Rv in L-J media by conventional method. Amongst the compounds tested, five compounds namely G13Am, G15Am, V13Am, L16Am and L13Am have exhibited more than 90 % growth inhibition in a primary screening. From the compounds screened for antituberculosis activity, compound G13Am found to be most potent, while compounds G15Am, V13Am, L16Am and L13Am have displayed better activity than the remainders.

Further, these twelve quinazolinone-sulfonamide hybrid molecules were screened for their in vitro antimalarial activity by pLDH assay and MTT-assay against P. falciparum and P. vivax (field isolates). The results of pLDH assay for both P. vivax and P. falciparum, indicate that compound L13Ac and V13Am followed by G15Am, G12Am and L14Am have shown to better activity with the IC_{50} values of ≤ 100 ppm. The result of MTT-assay also shows that, compounds G12Am, G15Am, L13Ac, V13Am and L14Am have shown EC_{50} values < 120 ppm for both the parasites, which indicates that these compounds have better % protection of RBCs, as well as, all the compounds were founds to have least toxicity against P. falciparum, while few compounds have shown moderate to low toxicity against P. vivax in terms of their CC_{50} values.

Based on the above in vitro study, it is to be concluded that the bio-activities of the compounds were influenced by the substitutions at the 2nd and 3rd position of quinazolinone ring. At position-2, compounds with side chain of amino acids in the order: Glycine > Valine ≥ Leucine, while the presence of electron-releasing or electron-attracting groups on the aryl substitution at 3rd position drive the activity of test compounds. Compounds with a p-chloro/bromo group on aryl ring exhibited better activity followed by the other substitutions like m-chloro, bromo, (p≈m>o), p-methoxy groups on aryl ring.

It is to state that the in silico screening helped us to find twelve drug like candidates amongst the synthesized library of several quinazolinone-sulfonamide hybrids. Further,
compounds $G_{13}A_{m}$, $G_{15}A_{m}$ and $V_{13}A_{m}$ have demonstrated good agreement in the performance of \textit{in silico} and \textit{in vitro} anti-TB activity. However, better performers of \textit{in silico} screening (compounds $G_{12}A_{m}$ and $G_{15}A_{m}$ and $G_{13}A_{m}$) have been agreed more or less with the results of \textit{in vitro} antimalarial activity.

In the conclusion, the established synthetic protocol might be used as a more general method for the synthesis of various 2,3-disubstituted-4(3$H$)-quinazolinone derivatives. Further, the newly designed and explored scaffolds may be taken for future development in order to find the leads with potential medicinal applications.