IN-SILICO STUDY, SYNTHESIS AND BIOLOGICAL SCREENING OF SOME NOVEL CURCUMIN ANALOGUES

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Chapter 7
Conclusions

7. Conclusions

This present study is critically focused on the development of novel curcumin analogues against both microbial & malarial infections since the humans in deep quest for such antimicrobial and anti-malarial agents.

By analyzing the experimental results, it has been concluded that the compounds **A7, CP10, CP11 and CP12** are the most active members of this study as antibacterial agent with (zone of inhibition up to 21 mm, 19mm, 18mm and 21mm respectively). The rationale behind the anti-microbial activity of the compounds may be attributed to the electron donating group (methoxy viz **A7)**. Hence, methoxy substitution may be the reason for the high activity of Compound **A7** among tested compounds. It is juvenile to arrive at the conclusion on structure activity aspect of these compounds and further assessment is desirable to use them for clinical study. Molecular docking studies also revealed that compound **A7** has minimum binding/ docking energy (-10.2 kcal/mol) and may be considered as good inhibitor of glucosamine 6-phosphate (GlcN-6-P).

The result of antifungal screening indicated that compounds, **A2**, **A3**, **A4**, **A7** and **A8** showed moderate to excellent antifungal activity. Compounds **A2**, **A3** and **A4** showed moderate activity against *C. albicans.* Interestingly, compound **A3** also showed moderate activity against three fungal strains viz. *C. albicans, C. tropicalis, C. krusie.* Among the halogenated derivatives chloro substitution at *ortho* or *para* position increased antifungal potency appreciably (compound **A2** zone of inhibition = 14.80mm), (compound **A4** zone of inhibition =13.37mm) but chloro substitution at both *ortho* and *para* positions caused a marginal decrease in potency (compound **A5** zone of inhibition = 12.67mm). This may be attributed to successful interactions of the compound with target protein(s) when chloro substitution occurred either at *ortho* or *para* postion.

CP10, **CP11** and **CP12** showed good antibacterial activity when compared with ciprofloxacin used as standard. Particularly, compound **CP10** showed maximum antibacterial activity (Zone of inhibition up to 21mm) against *E.coli* and *S aureus*. The substitution with chloro group at *ortho* and *para* positions led to slender decrease in potency (compound **CP12** zone of inhibition =19mm).

Comparative docking of GlcN-6-P synthase with the pyrazole analogues of curcumin and the standard drug flucanozole revealed that the docked energy for the compound **CP10**, **CP11**, **CP12** was -10.85, -10.58, -11.12 kcal/mol at active site 1 and -12.18, -12.32, -11.35 kcal/mol at active site 2 with an estimated inhibition constant of 1.08×10^{-7}, 1.44×10^{-7},

5.23 $x10^{-8}$ and 1.44 x 10^{-8}, $8.31x10^{-9}$, $5.35x10^{-8}$ respectively. The docked energy of the Flucanozole was only -5.36 at active site 1 and -6.57 kcal/mol at active site 2 with an inhibition constant of 5.53 $x10^{-5}$ and 4.7 x 10^{-5} respectively. In the first active site the geometry of compounds **CP10**, **CP11** and **CP12** are "frozen" in the binding pocket due to strong and stable hydrogen bonds formed between the amido moiety of the inhibitor and the His77 amino acid residue present in the binding site and well conserved in GlcN-6-P synthase sequences from various organisms.

In conclusion, the desirable improvement in antimicrobial activity in synthesized compounds requires electron releasing groups and chloro substitution and hence may be the reason for higher activity of **CP10**, **CP11 and CP12**. However, none of the newly synthesized compounds were found to be superior over the reference drugs. However, additional evaluation is enviable to use them for clinical study. Docking studies were carried out in order to rationalize the pharmacological results.

The results of antifungal screening indicated that compounds **CP2**, **CP7**, **CP11**, **CP13** showed moderate to excellent antifungal activity. Compounds **CP2**, **CP7** and **CP11** showed moderate activity against *C. albicans*. Interestingly, compound **CP13** showed good activity against two fungal strains viz. *C. albicans* and *C. krusie*. Methoxy substituition (compound **CP13** zone of inhibition =16mm) increased antifungal potency significantly. Among the halogenated analogues chloro substitution at *para* position increased antifungal activity appreciably (compound **CP11** zone of inhibition=13mm). The increase in potency of *para* substituted may be attributed to appropriate orientation of chloro to fit in binding site. Out of fourteen compounds synthesized, majority of them showed substantial anti-microbial activity against tested strains.

Samples **A3**, **A5**, **A8** and **B2** were active. Sample **CP2** was moderately active and sample **CP3** was weakly active. The remaining samples were inactive. The anti-malarial drug chloroquine was used as standard drug and is considered very active with IC_{50} = 0.135 μg/ml.

The results of anti-malarial screening revealed that among the compounds screened **A3**, **A5**, **A8** & **B2** displayed significant anti-malarial activity. Among the halogenated derivatives (**A3**-**A5**) the chloro derivatives (**A3** IC_{50} =9.47μg/ml, **A5** = IC_{50} =7.09 μg/ml) were the active ones. This is in accordance with presence of chloro group in diverse active anti-malarial molecules including chloroquine, pyronalidine and others. It is noticed that mono substitution either at *ortho* or *para* position increased the anti-malarial potency

conspicuously (IC_{50}=9.47μg/ml) with respect to unsubstituted. While chloro substitution at both *ortho* and *para* position caused a trivial augment in potency (**A5**, IC_{50} =7.09 μg/ml). The addition of methoxy group in an endeavor to increase electron density has decreased the potency of unsubstituted derivatives (IC_{50} =8.91 μg/ml) when the methoxy group is at *para* position. Tri substituted methoxy group increased the potency **A8** (IC_{50} =5.19μg/ml). In case of electron withdrawing (**A6**, Nitro, IC_{50}> 50μg/ml) and electron releasing (**A9**, methylated amine, IC_{50}> 50μg/ml) substituent were found to be inactive. Among Phenyl pyrazoline analogues (**B1**-**B5**) *para* chloro substitution on phenyl rings retrieved and enhanced the potency (Compound **B2** IC_{50} =8.91 μg/ml,) signifying that substitutions may result in restitution of potency. Whereas, methoxy (Compound **B4** IC_{50}>50μg/ml) and N, N dimethylamino (**B5** IC_{50}>50μg/ml) substitutions exhibited low effectiveness. Phenyl pyrazoline analogues (**B1**-**B5**) did not significantly increase the anti-malarial potency except *para* chloro substitution (**B2**). The most active compounds (IC_{50}<10 μg/ml i.e **A3**, **A5**, **A8**, **A11** and **B2**) were further evaluated for their cytotoxicity against HepG2 cell line.

All tested compounds except **A3** were found to be devoid of cytotoxicity at inhibitory concentrations indicated by their safety. Additionally, these compounds were found to possess good selectivity index (SI =8.67) showing their selectivity towards malaria parasite. The selectivity index (SI) was calculated based on ratio of CC_{50} and IC_{50} values.

The statistical details of the QSAR model given above speak for its good statistical quality. The R^2 value is above 0.8, which suggest that a good percentage of the total variance in biological activity is accounted by the model. Low value of standard error of estimate (< 0.3) indicates the accuracy of the statistical fit.

From the molecular descriptors incorporated in the QSAR model, one may conjecture that molecular flexibility and hydrophobicity predominantly govern the anti-bacterial inhibitory activity of curcumin analogues under study.

The equations indicate that thermodynamic parameter (Standard Gibbs Free Energy) and steric parameters (diameter) shows positive contribution while Electronic parameter (LUMO Energy) shows negative contribution towards the activity.

HOMO is the highest occupied molecular orbital called frontier orbital and determines the way it interacts with other species. HOMO is the orbital that could act as an e-donor. Since it is outermost (highest energy), the positive contribution of HOMO energy suggested that substitution of group at with electron donating groups are favourable for the antibacterial activity of compounds.

Total connectivity is a stearic parameter which determines the total compactness of molecule at particular conformation. Positive contribution of total connectivity suggests that substitutions or fractions of molecules which contribute for compactness of molecule are favourable for antibacterial activity.

Ovality is the ratio of the molecular surface area to the minimum surface area. The minimum surface area is the surface area of a sphere having a volume equal to the solvent excluded volume of the molecule. The positive contribution of the ovality indicates the activity will be increase with bulky substituent.

The present study gives rise to QSARs with good statistical significance and predictive capacity for the antibacterial inhibitory activity of curcumin analogues.

The study also attempts a QSAR study on curcumin derivatives. Applying Lipinski's Rule of five to curcumin derivatives to evaluate druglikeness (absorption, distribution, metabolism and excretion), there was no violation of the rule determining drugs pharmacological activity in the body. These studies are expected to provide useful insights into the roles of various substitution patterns on the curcumin derivative and also help to design more potent compounds. The docking studies and QSAR indicate that substitution of electron–rich compounds may lead to improved biological activity of curcumin derivatives.

Summary

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The rapid evolution of antibiotic resistance pose a grave threat to the therapy of many bacterial infections and necessiciate imperative and scrupulous efforts to develop next generation of antibiotics. The availability of complete microbial genome sequence has led to devise concerted strategy that is used to look at novel anti-bacterials, nevertheless in spite of the identification of many new potential drug targets, novel antimicrobial agents have been sluggish to emerge from these efforts.

The outburst of drug resistant microbial strains necessitates the studies for synergistic effects of antibiotics in combination with plant's derivatives to develop the antimicrobial cocktail with a wider spectrum of activity and reduction of adverse side effects of antimicrobial agents. The promising results for antimicrobial activity of curcumin analogues in our present study will be an ideal candidate to enhance the inhibitory effect of existing antimicrobial agents through synergism.

All the most effective drugs that we have had in the last few decades have been one by one rendered useless by the remarkable ability of malaria parasite to mutate and develop resistance.

Recent research has suggested that Artemisinin and Curcumin showed an additive interaction in killing *Plasmodium falciparum* in culture. The Curcumin-Artemisinin combination may prove superior from several perspectives. Both are from natural sources of long-term use, and as such, no resistance is known to curcumin that is present in a dietary supplement. although curcumin is reported to manifest low bioavailability and rapid metabolism in rodents and humans but the development of oral and parenteral curcumin formulations or curcumin analogues with improved bioavailability while retaining their immunomodulatory properties and possibly more potent anti-malarial activity deserves investigation.

In our present study we reported promising activity of our curcumin analogues against *P. falciparum*. Recent report also has suggested antimalarial synergism between Artemisinin and Curcumin in killing *P. falciparum* culture. The Curcumin-Artemisinin combination may prove superior from several perspectives. Both are from natural sources of long-term use, and as such, no resistance is known to curcumin that is present in a dietary supplement. We need to delve deep into computer-assisted docking to predict molecular interaction and binding affinity of Artemisinin-Curcumin hybrid and its derivatives with *Plasmodium falciparum* Ca^{2+}-ATPase (PfATP6) to endorse hybrid molecules as the next-generation antimalarial drugs. With no credible malaria vaccine in sight, there is an urgent need to

develop new drugs with different mechanisms of action to help preclude issues of cross-resistance.

Matrix Metalloproteinases (MMPs) are family of enzymes responsible for degradation of extracellular matrix. MMP-9 (gelatinase B) is one of the common matrix metalloproteinase that is associated with tissue destruction in a number of disease states such as rheumatoid arthiritis, fibrotic lung disease, dilated cardiomyopathy, as well as cancer invasion and metastasis. MMP-9, in particular seems to be a key protease associated with tumor progression. Therefore, development of inhibitors of MMP-9 is an exigent task which can have therapeutic benefit for patients suffering from various cancers. Curcumin, a natural yellow pigment from turmeric, has become focus of interest with regard to its role in regulation of matrix metalloproteinases (MMPs).

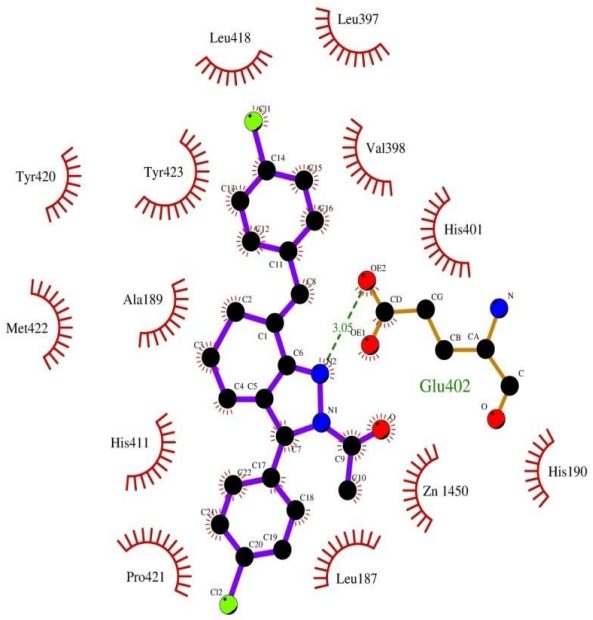

Figure 8.1. 2D interaction diagram for the complex protein-ligand: after docking interaction between 1GKC and A4

Further, all compounds were docked onto the active site of Matrix metalloproteinase-9(PDB: 1GKC) using Auto dock 4.2. Docking analysis revealed deep engulfment of the molecules into the inner groove of MMP-9 active site by making stable ligand-receptor poses. Compound A4 was found to be most active with minimum binding energy (-10.05 kcal/mol). Analysis of these docked ligands with the proteins brought in focus some

important interactions operating at the molecular level. Only hexahydroindazole analogues were able to show good binding energy another series of molecules failed to show promising results.

These studies are expected to provide useful insights into the roles of various substitution patterns on the curcumin derivative and also help to design more potent compounds in near future. The docking studies indicate that substitution of electron–rich compounds may lead to design of novel analogues for potent MMP-9 inhibitors