PREFACE

The thesis entitled "*In silico* Study, Synthesis and Biological Screening of Some Novel Curcumin" submitted for the award of the degree of Doctor of Philosophy (Ph.D.) contains the research work carried out during the period of July 2010 to October 2015, at the Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi, India under the supervision of Prof. (Dr.) Sushil Kumar Singh. This thesis contains introduction, literature review, the detailed procedures for the synthesis of designed compounds, their characterization by physico-chemical and spectroscopic methods. Biological evaluation includes antimicrobial and antimalarial activities of the compounds. The antimicrobial results were presented with MIC values. The anti-malarial activity was performed by the method of Trager and Jensen with minor modifications.

Three series of novel curcumin analogues, eg. Hexahydroindazole (A1-B5), Pyrazole (CP1-CP14) and Cycloheptanone (C1-C14) were prepared by coupling of substituted benzaldehydes with respective cyclopentanone/cyclohexanone and cycloheptanone (in ratio of 2: 1) in a base catalyzed Claisen-Schmidt condensation where temperature was maintained between 15-25˚C. The reaction mixture was further refluxed (15-20h) with hydrazines (hydrazine hydrate, phenyl-hydrazine) and acetic acid. The progress of reactions and purity were monitored by thin layer chromatography (TLC). The compounds were obtained in good yield (48 - 86%). The structures of newly synthesized compounds were ascertained on the basis of their analytical and spectral profiles.

Antimicrobial activity of newly synthesized compounds were first screened by disc diffusion method against various Gram positive and Gram negative human pathogenic bacteria viz. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27893, *S. typhi* MTCC 3216, *E. faecalis* and *Staphylococcus aureus* ATCC 25323 and different fungal strains of *Candida* according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Fresh grown bacteria were mixed in sterile saline (0.85%) and the turbidity was matched with McFarland No. 2 system to achieve concentration of 10^7 CFU/ml. Sterile petri plates containing 20 ml of Mueller Hinton agar (MHA, Hi-Media) were used for all bacterial culture. and Sabouraud's dextrose agar (SDA)/Potato dextrose agar (PDA) (Hi-Media) were used for all fungal culture.

The synthesized compounds were screened for *in vitro* antimalarial activity against CQ-resistant (W2 clone) of *Plasmodium falciparum* using [3H] hypoxanthine incorporation assay.

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 and on searching the rationale behind the compound's antimicrobial activity, it is hypothesized that the electron donating group (methoxy viz A7) has commanding role in it. Hence methoxy substitution may be the reason for highest 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. Automated docking was used to determine the orientation of inhibitors bound to the active site of GlcN-6-P synthase. A genetic algorithm method, implemented in the program AutoDock4.2, was employed 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).

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*. Double substitution with chloro group at *ortho* and para position led to slender decrease in potency (compound Cp12 zone of inhibition =19mm). In summary, 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, CP11and CP12. However, none of the newly synthesized compounds were found to be superior over the reference drugs. It is infantile to draw conclusion on structure activity aspect of these compounds and additional evaluation is enviable to use them for clinical study. 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 1.08x10-7, 1.44 x10-7, 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.

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} = 9.47µM, A5 = IC_{50} = 7.09 µM) 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} = 9.47) with respect to unsubstituted. While chloro substitution at both *ortho* and *para* position caused a trivial augment in potency (A5, IC_{50} = 7.09 µM). The addition of methoxy group in an endeavour to increase electron density has decreased the potency of unsubstituted derivatives (IC_{50} = 8.91 µM) when the methoxy group is at *para* position. Tri substituted methoxy group increased the potency A8 (IC_{50} = 5.19µM). In case of electron withdrawing (A6, Nitro, IC_{50}> 50µM) and electron releasing (A9, methylated amine, IC_{50}> 50µM) 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 µM,) signifying that substitutions may result in restitution of potency. Whereas, methoxy (Compound B4 IC_{50}>50µM) and N, N dimethylamino (B5 IC_{50}>50µM) 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 µM i.e A3, A5, A8, A11and 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. This indicates their safety in the mammalian system. 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 Cycloheptanone analogues (C1-C14) of curcumin did not show any promising antimalarial activity against *P. falciparum* clone W2 (Chloroquine-resistant). It might be due to presence of bulky group (cycloheptanone). These analogues need to be further explored for the treatment of malaria.

In summary, this study allows us to conclude that enviable improvement of anti-malarial activity in synthesized analogues of curcumin requires (a) electron donating group (methoxy) at the *ortho*, *meta* and *para* positions, (b) electron withdrawing (chloro) group at the *ortho* and *para* postions. Although, it is infantile to arrive at the conclusion on detailed comprehensive structure activity aspect of these compounds and further assessment is desirable to use them for clinical study. Due to straightforward synthesis, these curcumin analogues embody a novel scaffold for developing new, affordable and effective anti-malarial and antimicrobial drugs with less chance of developing resistance.

In the end relevant references are included along with list of papers presented & published and reprint of publications from the present study is also included.

The contents of this thesis may be useful for medicinal chemists working on curcumin analogues and open new vista in firmament of designing and developing novel antimicrobial and antimalarial agent.