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Phase Assisted 3D QSAR, Microwave Enhanced Synthesis and Evaluation of Quinoline based Antimalarial Agents

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Abstract:- The ligand-based 3D-Quantitative structure activity relationship study was performed using pharmacophore techniques which include PHASE module version 3.2 of Schrodinger, LLC, New York, 2010. PHASE i.e. Pharmacophore alignment search engine is a module developed by Schrodinger for p'cophore generation. PHASE has many uses like it is comprehensive, self-contained system for pharmacophore perception, for developing 3D quantitative structure-activity relationships model and 3D screening of database. A series of 23 novel 4-aminoquinoline rhodanine derivatives and 16 novel ketoenaminechalcone having β–hematin inhibitory activity were selected from literature survey and biological activities thus obtained were reported as IC50 (nM).These compounds were used to develop a 3D QSAR model which is based on its p'cophoric features. The synthesis, characterization and evaluation for antimalarial activity of these compounds were performed and compared with chloroquine.

Keywords- *PHASE, 3D QSAR, Anti malarial Activity, Schrodinger.*

I. INTRODUCTION

Malaria is a worldwide occurring disease and a leading cause of death. Its severity depends on the interaction of the parasite with the Anopheles mosquito vector, the human host body and the environmental factors.[1] Almost all the human deaths are generally caused by *P.Falciparum* species. The pharmacological action of 4-aminoquinolines causes detoxification of free haem, which is produced at the time of degradation of Hb. Chloroquine (CQ) which leads to a complex formation with haem and reduces haem polymerization leading to a build-up of toxic haem (FpIX) molecules.[2]

➢ *Drug Resistance in Malaria*

Resistance to antimalarial drugs has lead to challenging problems in controlling malaria in variuous parts of the world. Resistance to antimalarial drugs has been a particular problem with P.falciparumin which widespread resistance to chloroquine, sulphadoxine-pyrimethamine and mefloquine has been observed.[3]

➢ *CHLOROQUINE Resistance Development Mechanism*

It has already been proved that the amount of chloroquine into the digestive vacuole decreases in parasite-resistant strains which reduces the strong accumulation mechanism of chloroquine and therefore showing low efficient, also suggests mutations in transporter proteins in these resistant strains. chloroquine – ferriprotoporphyrin (FP) binding affinity in the digestive vacuole is reduced of the Resistant isolates, therefore CQ-resistant isolates have generated a process by which the excess of chloroquine to FP is reduced.[4]

➢ *PFCRT – A Carrier of Chloroquine*

The PfCRT i.e malaria parasite's CRT is an essential protein of membrane present in the parasite's acidic digestive vacuole. The chloroquine resistant transporter role within the parasite is not known and the protein was described as a transporter as it possesses 10 transmembrane domains. Mutations in CRT can lead to a reduction in intravacuolar amount of chloroquine and therefore causes resistance against chloroquin. [5]

➢ *Structural Modification in Chloroquine to Overcome Resistance*

The structure chloroquine includes a 7-chloroquinoline-substituted ring system with a amino side chain which is flexible. The haem-binding template, 7-chloro- and terminal amino group all are mandatory for antimalarial activity. At the 4-amino group in the chloroquine structure, aliphatic side chain is present, it has been studied that by replacement of this aliphatic chain with phenyl ring the resistance against chloroquine has been reduced and by introducing a phenyl ring which enhances activity against chloroquine resistant parasite. It also has been studied that in the aliphatic side chain reduction from 4 carbon atoms to 2 or 3 carbons enhances activity and resistance against parasite reduces resistance. One of the analogues of chloroquine which is a short-chain aminoquinoline antimalarial (AQ13) has undergone Phase I clinical trials.[5]

➢ *Computer Aided Drug Design*

CADD is an inventive tool of discovering lead structural molecules based on the knowledge and study of a biological target available CADD uses computational chemistry to identify, improve selectivity or decrease toxicity associated with lead. [6]

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- *Structure-based drug design (Molecular Docking)*

When there is sufficient information available regarding structure of the biological target for a disease and its binding sites, then structure based approach can be used, in which specific ligand–protein interactions are studied which might be helpful to design new molecules, which shows good activity towards the target with minimal side effects is called as structure-based drug design. [6]

- *Ligand-based Drug Design (Pharmacophore Development)*

When there is very less knowledge about the structure of target, but a large number of active ligands have been detected already, then ligand-based drug design gives the alternative way of applying the available information into models that can be used in identifying new active moieties. The ligand-based design mainly includes many computational methods that depends only on the structure of known compounds and have become highly similar to pharmacophore modelling methodology and quantitative structure-activity relationships (QSAR) techniques.[6]

II. 3-D QSAR BASED PHARMACOPHORE MODEL GENERATION BY PHASE

- *Pharmacophore Modeling*

A pharmacophore may be defined as a set of groups that carry spatial arrangement of structural features needed for a compound for possesing pharmacological activity. Modelling of compounds is done by using various approaches like denovo, virtual screening for optimization of best set of molecules [7]

- *PHASE*

PHASE is a process to design , which provides some advantageous information like flexibility and feedback, focusing the user as an integral part of the p'cophore development process. It is not aimed to provide information like a single model that is proved to be the best by some predetermined measure, but rather it suggest a set of possible number of models that can be further evaluated by different aspects, whose relevance is assessed by the user..[8]

- *Statistical parameters of 3-D Quantitative Structure Activity Relationship model generated by PHASE*

The prediction of the biological activity is the main purpose of developing Quantitative Structure Activity Relationship model. If a "good" correlation is provided (as indicated by a high correlation coefficient), then the Quantitative Structure Activity Relationship can be applied to give reliable predictions of biological activity. [9]

III. EXPERIMENTAL

- *3D- Quantitative Structure Activity Relationship based pharmacophore model generation by PHASE methodology*

This study mainly emphasizes on elucidation of the 3D structural features of beta-hematin inhibitor and also to get predictive 3D- Quantitative Structure Activity Relationship models, which may guide further in the rational drug design and synthesis of novel inhibitor compounds.

- *Software and hardware requirements*

PHASE version 3.2 module present in the Maestro 9.1 modeling package from Schrodinger, Molecular Modeling Interface Inc., LLC, New York, USA installed on Pentium IV (2.80) GHz, with Window XP operating system were used to carry out 3D- Quantitative Structure Activity Relationship studies. The ligand structures were prepared using structure drawing tool in the Schrodinger Molecular Modelling software.

- *Procedure*

The data of 23 compounds of novel 4-aminoquinoline rhodanine and 16 compounds of novel keto-enaminechalcone-chloroquine showing beta-hematin inhibitory activity were taken from the literature survey. The ligand structures were drawn using ChemDraw ultra 8.0.The in vitro biological activity data was reported as IC50. The IC50 values were converted to pIC50 using the formula (pIC50= −log IC50). The total sets of inhibitors (49 compounds) were bifurcated randomly into a training set (35 compounds) for generation of 3D-QSAR models and a test set (14 compounds) for validation of the developed model.

- *Preparing Ligands:*

Ligprep Version 2.4 is used as a cleaning step for structures in which we have attached hydrogen, converted the structure from 2 dimensional to 3 dimensional, generated stereoisomer, and neutralized charged structure or determines the most probable ionization state at a user-defined pH. Conformers for each ligand were generated using ConfGen by using OPLS-2005 force field for generation of pharmacophore model development,.

- *Creating P'cophore Sites*

The p'cophore model process includes each protein structure which is depicted as a set of points in 3D space, which combines with different chemical features that may facilitate non-covalent attachment between the ligand molecule and its target protein. The characterization of p'cophore sites which are evaluated on the basis of type, location and directionality. Once the conformers are generated, the chemical features of a ligand were defined by using built-in four pharmacophoric features: H-bond acceptor (A), H-bond donor (D), hydrophobic group (H), and aromatic ring (R).

➢ *Scoring and Perceiving Common Pharmacophores*

The resulting p'cophore groups were scored and ranked thereafter. The generated p'cophore hypotheses were evaluated on the basis of 'Survival active' and 'Survival-inactive' scores. Out of 20 hypotheses generated, the best pharmacophore hypothesis (ADHRR).91 was selected on basis of highest survival score. The selected 3D p'cophore hypothesis contains the following features: one H-bond acceptor (red sphere; A2 with two arrow), and two aromatic ring (R10 and Ring11; gray circle), one hydrophobic group (green sphere; H6), one hydrogen bond donor (D4). The 2D representation of the ADHRR hypothesis shows the key pharmacophoric elements: Oxygen of ketoenaminechalcone (sphere A2 with one arrow), aromatic ring of quinoline nucleus (R10 and R11), chloro group of quinoline nucleus (H6), one amino group (D4).

➢ *Building 3D– Quantitative Structure Activity Relationship Models*

The selected hypothesis were used to carry out QSAR modeling, by partitioning the dataset into a training set (70%) and a test set (30%). All the common p'cophore hypotheses generated successfully and scored in the above manner were used to generate atom-based 3D-QSAR models by correlating the predicted and estimated activity for the set of 35 training molecules using Partial least square analysis. The PLS regression with good statistics was carried out using PHASE V3.2 with a maximum of N/5 PLS factors. Validation of all models was done by predicting activity of the set of 14 test set molecules.

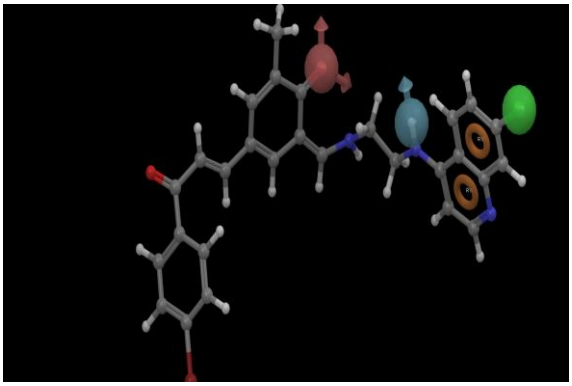

Fig 1:- Pharmacophore Generated From The Best PHASE Hypothesis

IV. CHEMISTRY

A series of 4-aminoquinoline derivatives (P1-P18) were synthesized according to reaction and its conditions. Compounds I1 and I2 were prepared by performing nucleophilic substitution at the 4th position of 4, 7-dichloroquinoline with ortho-phenylenediamine or pera-phenylenediamine in presence of hydrochloric acid. Target compounds (P1-P18) are synthesized by condensation of free amino group present in compounds I1 or I2 with aldehyde group of various heterocyclic aromatic aldehydes in the presence of hydrochloric acid which acts as an oxidising agent and thus imines formed by removal of water molecule.

➢ *General Procedure of Synthesis of 4-Anilinoquinolines*

A solution of 4, 7-dichloroquinoline (1.0 equiv.), p-phenylenediamine or o-phenylenediamine (1.0 equiv.) and concentrated hydrochloric acid (25µl) in methanol (500µl) was heated at 130°C by microwave irradiation for 15 min. The solvent was evaporated and the residue was dissolved in water, which was made alkaline with aqueous saturated sodium bicarbonate solution and extracted twice with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuum to get desired 4 anilinoquinoline derivatives such as N1-(7-chloroquinolin-4-yl)benzene-1,4-diamine (I1), N1-(7-chloroquinolin-4-yl)benzene-1,2-diamine (I3) with excellent yields.

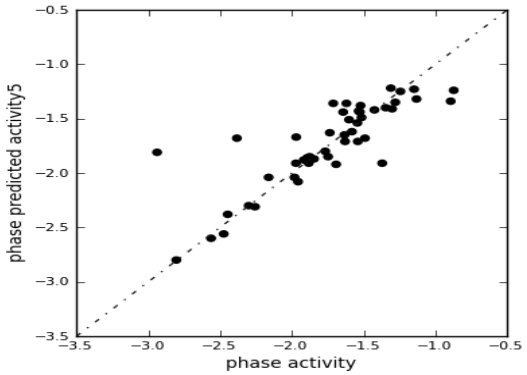

Fig 2:- PHASE activity Vs PHASE predicted activity scatter plot of 49 compounds

➢ *General Procedure of Synthesis of 4-Aminoquinoline Derivatives (P1-P18)*

Equimolar mixture of 4-anilinoquinolines (I1 and I2)(0.01 mol) and various aromatic heterocyclic aldehydes (Ar-CHO) (0.01 mol), was dissolved in 10ml of methanol with few drops of sulphuric acid. The resultant mixture was refluxed under microwave for 10min at 90oC. The solvent was removed and the crude product thus obtained was crystallized in ethanol. [10]

V. RESULTS

A 3D– Quantitative Structure Activity Relationship analysis was performed on the series of 4-aminoquinoline derivatives to observe the effect of spatial arrangement of structural features on their beta-hematin inhibition. The large value of F (54.7) indicated a statistically significant regression model, which was further supported by the small value of the variance ratio P (2.20), an indication of a high degree of confidence interval. Further, the small value of SD (0.15) and Root-Mean-Square Error RMSE (0.40) established that the data used for model generation was best suitable for the Quantitative Structure Activity Relationship analysis. (Table no.1)[

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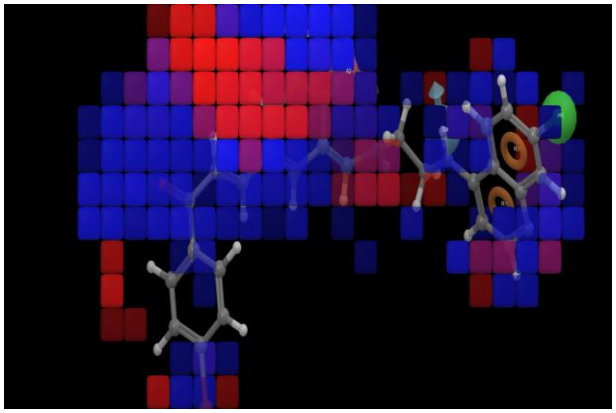


Fig 3:- PHASE 3D plots of crucial pharmacophore region.
Blue cubes:Positive coefficient favored areas.
Red cubes: Negative coefficient favored areas.

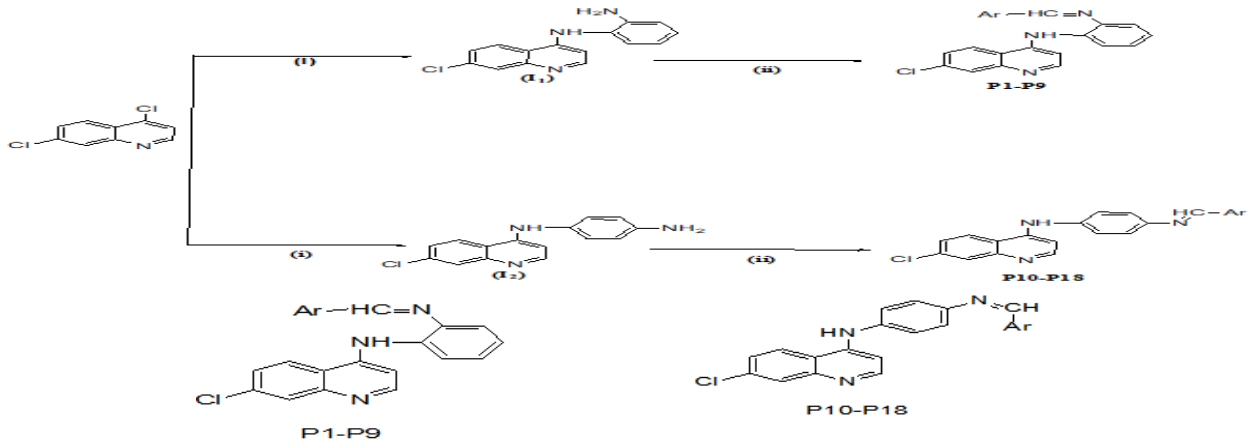
From Fig. 3, we can infer that the chloro group at the 7th position of the quinoline ring represent Hydrophobic group (H6), the benzene ring of quinoline nucleus represent Aromatic rings (R10 and R11) and at the 4th position of the quinoline ring amino group is present which represent Hydrogen bond donor (D4) and all these three features are essential for the beta-hematin inhibitory activity. Blue cubes near the amino group at 4th position attached to the 4-aminoquinoline suggests that the presence of H-bond donors (D4) will contribute positively and any substitution to this position will leads to increase in activity.

ROW	ID	# FACTORS	Std Dvtn	Reg^2	F	P	STABILITY	RMSE	Q^2	P-R
		1	0.2886	0.5963	48.7	5.54e-008	0.9714	0.4105	0.1623	0.4445
11	ADHRR	2	0.2303	0.7508	48.2	2.205e-010	0.8435	0.4072	0.1756	0.423
		3	0.1956	0.8258	49	7.187e-012	0.7018	0.4201	0.1226	0.3781
		4	0.1711	0.8710	50.6	6.421e-013	0.6125	0.4221	0.1142	0.373
		5	0.15	0.9042	54.7	6.975e-014	0.5879	0.4139	0.1484	0.4161

Table 1:- The Partial Least Square statistical parameters of the selected 3D– Quantitative Structure Activity Relationship model

Note- Std Dvtn: Standard deviation of the regression, Reg: squared value of R2 for the regression, F denotes Variance Ratio. P denotes significance level of variance ratio, RMSE: Root-mean-square error, Q denotes squared value of Q2 for the predicted activities, P-R: Pearson R value for the correlation between the predicted and observed activity for the test set.

VI. REACTION SCHEME



Scheme 1. Synthesis scheme of 4-aminoquinoline derivatives: Reagents and conditions applied: (i) 4-anilinoquinolines, methanol, hydrochloric acid, MW, 15min, 130°C (ii) Aromatic heterocyclic aldehydes (Ar-CHO), methanol, few drops of hydrochloric acid, MW, 10min, 90°C.

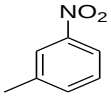
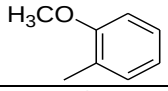
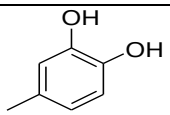
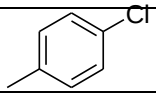
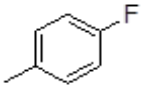
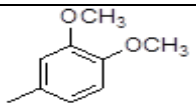
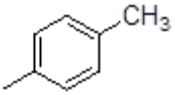
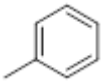
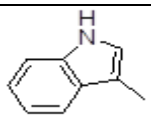
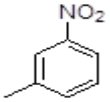
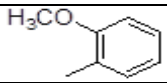
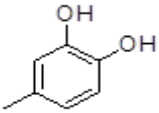
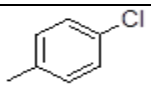
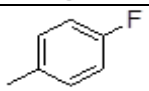
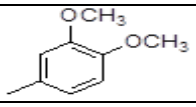
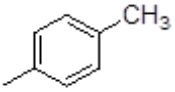
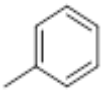
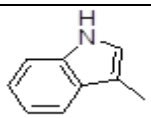
Code	Ar
P1	
P2	
P3	
P4	
P5	
P6	
P7	
P8	
P9	
P10	
P11	
P12	
P13	
P14	
P15	
P16	
P17	
P18	

Table 2:- Structure and IUPAC Name of 4-aminoquinoline Derivatives

VII. CHARACTERIZATION

- *Characterization of Synthesized Product was Done by Following Methods:*

Thin Layer Chromatography (TLC), Melting Point Determination, Solubility Determination, ClogP Determination, Ultraviolet-Visible Spectroscopy (UV), 1HNMR Spectroscopy, Mass Spectroscopy (MS), IR Spectroscopy (FTIR).

- *In Vitro Biological Evaluation*

The antimalarial activity of the synthesized compounds was evaluated by SYBR Green I-based assay method on Plasmodium. falciparum at Microcare Laboratories, Surat, Gujarat.

Procedure: Chloroquine sensitive (3D7) strains were maintained in human O+ve erythrocytes suspended in RPMI 1640 medium with albumax II, Na_2CO_3, and Gentamicin. Cultures containing predominantly early ring stages of *P. falciparum* were utilized for testing. Typically, wells were run in triplicate and drugs were tested against (Pf3D7) strains at different concentrations. After addition of sample solutions to 96-well microtiter plates in triplicate, various dilutions were prepared with Roswell park memorial institute 1640 medium and infected erythrocytes were added to give a final volume of 100 µl with 2 % hematocrit and 1% parasitemia. Plates were transferred into a modular incubator gassed with 95% nitrogen, 5% oxygen and 5% carbon dioxide and incubated at 37°C for 48 h. After 48 hours, a 100 µl SYBR green- I solution (Dye + Lysis buffer) was added and further incubated at 37 °C. The lysis buffer was added and formulated in an attempt to achieve the broadest possible utility and the greatest speed of the assay. Plates were exposed to Plate Reader for fluorescence to determine the parasitemia from fluorescence measured. Overall growth inhibition was assessed by comparison of the growth in the treated wells with that in the control wells, to which no drug was added.

VIII. SUMMARY AND CONCLUSION

Malaria is a very serious worldwide health challenge in spite of rigorous efforts to control the disease. *P. falciparum* is main cause of for most of the severe clinical cases of malaria, which results in twenty lakhs deaths per year.

Chloroquine is a molecule which has chances for chemical modification compared to other p'cophoric molecules as it has various advantages like good clinical efficacy, limited toxicity in host, easy to use and has simple cost-effective synthesis.

Phase module of Schrodinger was utilized for the designing of compounds for the development of p'cophore based 3D Quantitative Structure Activity Relationship model.

Considering the structural requirements as per the detailed literature review and the derived p'cophore model, 4-aminoquinoline derivatives (P1-P18) were designed and synthesized. The reaction completion was determined by TLC. Finally, the structures of compounds were confirmed by infra red spectroscopy, 1H NMR technique, Ultraviolet spectroscopy and Mass spectrometry.

After confirming the designed molecules with anticipated structures, the antimalarial activity of these molecule was evaluated in vitro by SYBR green I assay method against *Plasmodium falciparum*. The studies were carried out in the Microcare Laboratories, Surat, Gujarat.

Among the 18 evaluated molecules of the series (P1-P18) against *Plasmodium falciparum* showed IC_{50} values having the range in between 0.60µg/ml-1.32µg/ml. Nine compounds (P1,P3, P4, P6, P9, P11, P13, P14 and P15) displayed malarial inhibitory activity in the range 0.6-0.94 µg/ml. However, the results of activity mainly suggest that among the different substituted compounds with aromatic heterocyclic aldehydes (P1-P18), the 4-chloro substituted compound P4 is shown to be most potent (IC50 = 0.6 µg/ml). Infact compound P4 when compared to flouro substituted compound (P5, IC50 = 1.32 µg/ml) having same phenyl linker is two times more active then P5.

Based on the detailed p'cophore 3D- Quantitative Structure Activity Relationship study and literature study it is expected that all of the new drug candidates will boost up the current Structure-based drug design of new antimalarial discovery and lay down milestone in treatment of malaria. The structural flexibility of these compounds allows the possibility of elaborating the present work by increasing the number of derivatives and by altering the positions of substitutions on root nucleus followed by SAR studies. This in turn would open up new ways for developing novel malarial inhibitors to overcome malaria which threatens a major part of the world population.

Compound name	IC_{50} (µg/ml)
P1	**0.87**
P2	0.96
P3	0.84
P4	0.60
P5	1.32
P6	0.87
P7	1.16
P8	1.12
P9	0.86
P10	1.16
P11	0.85
P12	0.98
P13	0.91
P14	0.71
P15	0.62
P16	1.20
P17	0.96
P18	1.00

Table 3:- *In-vitro* Malarial inhibitory activity of synthesized moleules against *P.falciparum*

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