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Anti-malarial drug therapy employing falcipain2 inhibitors

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ABSTRACT

Malaria is one of the most common infectious diseases affecting 300-500 million people every year. The increased resistance of the parasitic strains to anti-malarial drugs have made it all the more difficult to combat the disease at an early stage. Therefore, there is an immediate need to come up with novel strategies to arrest the spread of infection. We have been able to conclude that falcipain2, cysteine protease from P.falciparum is mainly responsible for degradation of haemoglobin and thus inhibitors of falcipain2 will block the hydrolysis of haemoglobin, preventing the further spread of infection. This article mainly focuses on the identification of new drug targets for antimalarial therapy and characterization of falcipain2 inhibitors by carrying out docking studies and statistical analysis. We have used docking softwares to identify the specific conformations of ligands that show the maximum binding affinity with the target and further analysed these results by generating descriptors manually to perform QSAR. We have made use of regression studies to perform similarity analysis by generating optimum models using statistical software. The models were generated with r^2 =0.99, r^2_{cv}= 0.99, s= 0.002 when Ds and GATS8s descriptors were correlated. The main classes of falcipain2 inhibitors include peptides comprising of vinyl sulphones, aldehydes and ketone groups[7]. These peptides were tested for anti-malarial activity and two potential hits were identified.

Keywords: *Malaria, Falcipain2 Inhibitors, QSAR, Docking*

1. INTRODUCTION

Malaria is caused by protozoan parasites, belonging to *Plasmodium* type. *Plasmodium falciparum* is mainly responsible for severe cases of malarial infection whereas *Plasmodium ovale, Plasmodium vivax, Plasmodium malariae* and other groups generally cause a milder form of the infection[5]. It is transmitted by the bite of a female infected Anopheles mosquito which introduces parasites in the blood stream of humans leading to the extensive rupture and consequent degradation of red blood cells. In people lacking immunity, the progression of the disease is very rapid which can even pose a high risk of death. The body's immune system comprising of the complement system and antibodies counter this adverse situation by limiting parasite growth and proliferation[1]. However, our immune system is not always able to clear the pathogen infected red blood cells from the blood stream and thus anti-malarial drugs are required to cure the disease. The two most commonly used anti-malarial drugs are chloroquine and quinine[8]. It has been observed that in due course of time, parasite strains develop resistance to these medications thereby hindering further diagnosis and treatment.

Thus, there is a need to identify new biochemical drug targets and cysteine protease falcipain2 from *Plasmodium falciparum* has proven to be one of the promising target enzyme[1]. Inhibitors suppress the hemoglobin degradation activity of falcipain genes and through our study we have been able to generate the models of two such compounds which fulfil biological parameters like solubility (logS), cardio-toxicity (hERG2) and metabolizing capacity (2C9pK_i) necessary for drug development. These compounds have been further analysed by Auto dock[13] and LeadIT[12] softwares and based on their docking properties like dock score, binding efficiency, ligand efficiency it can be predicted that they are crucial in anti-malarial drug design.

2. REVIEW OF LITERATURE

Not long ago, chloroquine and quinine[8] were the two most widely used anti-malarial drugs. Both these drugs inhibited the conversion of extremely toxic heme to non-toxic haemozoin[6], thereby causing the death of parasite due to increased levels of toxicity. Nevertheless, the increased level of mutations in the parasitic strains have made it almost impossible to counter its development by the application of these drugs. Recently, Artemisinin-based combination therapies (ACTs) gained importance due to its enhanced overall efficiency in lowering the transmission of the disease[2], it has proven to be highly effective in controlling the proliferative rate of the disease after the appearance of initial symptoms. However, the fact that it is very expensive and not available in remote locations makes it unfavourable to be used extensively in drug formulations[2]. The limited knowledge, public acceptance and most

importantly safety of these combination therapies have led to their decreased production and supply. Due to less availability and expensive nature of ACTs, rapid antigen-detection diagnostics tests (RDTs) gained popularity in several South African countries[3]. It has been established as an efficient approach to address the issue of expense as this anti-malarial treatment is offered free of cost to affected children in government clinics. Of late, peptidyl cysteine protease inhibitors have been employed to treat the disease at an early stage as they prevent erythrocytic rupture and degradation. Falcipain-2 inhibitors have proven to be very effective in anti-malarial drug therapy[4] and research is currently being carried out to extensively study the biological properties of these ligand compounds. Several *In-silico* studies have been carried out to analyse the docking properties and structure activity relationships (SAR) of these compounds yet all the necessary parameters for drug development have not been completely resolved. Hence, to predict the anti-malarial activity of the inhibitors and to analyse their therapeutic properties, we have considered a set of 32 compounds and evaluated their inhibitory activities by performing QSAR studies and two hit compounds have shown promising result in this regard.

3. MATERIALS AND METHODS

A set of 32 compounds was selected based on literature survey and a common biological assay. All these compounds showed inhibitory activities against cysteine protease falcipain2. DMSO assay was taken into consideration during the collection of ligand molecules. A worksheet was prepared taking into account the IUPAC name, IC50 value and the bio-assay of these ligands. The crystal structure of falcipain2 of Plasmodium falciparum was retrieved from Protein Data Bank (PDB ID 2GHU[9]). Structures of the ligand molecules were prepared using MarvinSketch[15] software in both 2D and 3D form. Further analysis of the conformational data was carried out by generating descriptors applying PaDel[11] software. Another dataset was prepared for detailed study on targeted ligands keeping in mind its interaction with other amino acids, bio-availability and bio-assay.

The interaction of targeted ligands with the protein (2GHU[9]) was determined by performing docking employing LeadIT[12] software. The protein molecule and ligands (in sdf format) were loaded and the residues were selected based on protein data analysis for docking. The two ligands which had interaction with Cys42 residue were considered for further docking studies using Auto-dock[13] tool. The dock score for these compounds were noted down separately. The protein to be studied was retrieved in .pdb format and crystallographic water molecules and other heteroatoms were removed from the pdb file. The ligand molecules were exported from LeadIT[12] in .mol2 format. These ligands were converted to their respective pdb forms using specific commands and Openbabel[10] software. Docking was carried out by taking the protein and specific ligand file as the input, the ligand was suitably fit within the grid box so generated and required changes were saved. The docking parameters were entered and Genetic Algorithm was employed for optimization studies. Lamarckian[16] algorithm was used for generating the output and the number of iterations was set to 10. Auto-grid and auto-dock commands were entered to generate the glg and dlg files respectively. The grid and dock files so produced helped during result analysis.

This step was followed by the manual generation of descriptors for QSAR study. The dataset was divided into Test set, Training set and Validation sets for statistical analysis using MyStat[17] software and the combination of descriptors that established maximum r^2 and optimum levels of p-value and standard error were chosen to be evaluated for log activity. The log activity of these compounds were calculated based on the constant values (β) of descriptors. Further, a comparative analysis was carried out with the previously obtained log IC_{50} values from literature survey. The data was then represented in graphical form (scatter plot) and trendline was added in the excel sheet to obtain the respective r^2 vales of training and test sets.

The compounds were further tested for their ADME properties employing StarDrop[14] software to generate a drug model having high solubility, and minimum levels of cardio-toxicity and metabolizing capacity. Docking studies were aimed at analysing protein-ligand interactions, QSAR study was carried out to generate the best descriptors for optimum model development and StarDrop[14] was employed to study the biological parameters necessary for drug formulation. We expect to come up with the best ligand molecules that will fulfil the aforementioned criteria and can likely be used as anti-malarial drugs.

4. RESULTS

1) a) Molecular docking by Auto-dock[13]

The docking result was analysed based on binding energy, ligand efficiency, hydrogen bond interactions and the two compounds S2000001 and S1000005 showed the best results with a binding energy of -9.1kJ/mol and -10.95 kJ/mol respectively. The following interactions have been observed:
H44: GLY83, H44: GLU14

b) Molecular docking by LeadIT[12]

The docking result was analysed based on dock score and interaction of ligands with Cys42 residue. The two compounds that showed the most compelling results are S2000001 and S1000005 with a dock score of -21.16 and -17.82 respectively.

2) QSAR model prediction

The best combination of descriptors derived from training set and test set was Ds and GATS8s. The r^2, p-value and standard error of estimate of the training set model were determined to be 0.999, 0.864 and 0.002 respectively. The r^2, p-value and standard error of estimate of the test set model were determined to be 0.998, 0.234 and 0.003 respectively. The q^2 values of training set and test set were 0.998 and 0.997 respectively. The descriptors considered in this study assisted in the generation of 3D-molecular indices and it further improved the validation of the model by analysing the structural features of the compounds that are responsible for their physical, chemical and biological properties.

3) ADME property prediction

ADME property prediction was carried out using StarDrop[14] software and the compounds that showed optimum solubility (logS), cardio-toxicity (herg2) and metabolizing capacity (2C9pK_i) were chosen as the most suitable drugs for anti-malarial therapy. A higher value of intrinsic solubility is desired so that the drug readily solubilizes in the body and it is one of the most sought after characteristic in the oral administration of drugs. A comparative lower value of herg2 parameter is desired so that the cardio-toxicity can be kept at a minimum value. Similarly, the metabolizing capacity should also be low inorder to prevent liver damage.
Based on the above observations, S2000001 and S1000005 were identified as the hit compounds.

5. DISCUSSION

We have analysed the compounds based on the dock score, IC_{50} values, binding energy and ADME properties. Now, on one hand S2000001 showed an IC_{50} value of 2.2µM, a dock score of -21.16 and binding energy of -9.1KJ/mol and on the other hand, S1000005 showed an IC_{50} value of 13.2 µM, dock score of -17.82 and binding energy of -10.95 KJ/mol. QSAR analysis also generated optimum models with a r^2 value of 0.999 which proves that the descriptors generated were highly accurate in predicting the biological activity of the compounds. However, both of these compounds have lesser solubility and optimum cardio-toxicity and metabolizing capacity. It can be concluded that the ligands are fitting appropriately in the pockets of protein molecule that has led to higher dock scores and binding efficiency. The minimum IC_{50} values of both the compounds suggests that very less concentration is required for maximum inhibition activity. The solubility of these compounds can be increased by computationally altering their functional groups. Thus, we have come to a conclusion that compounds S2000001 and S1000005 have promising activity towards anti-malarial drug therapy.

Fig 1 shows the docking result, Fig 2 and Fig 3 show QSAR analysis for training and test sets respectively.

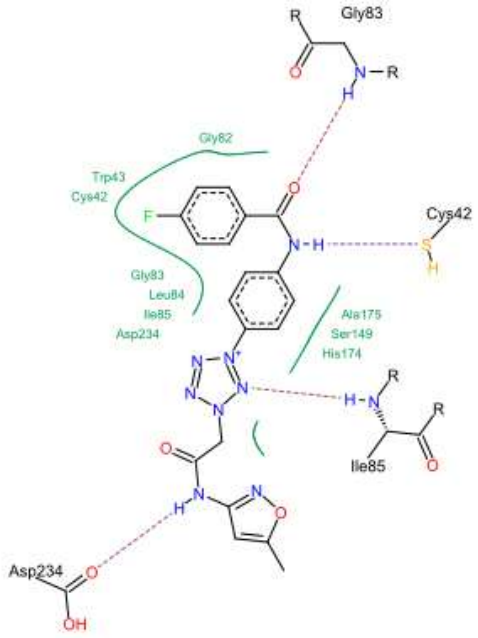

Fig 1: Proposed interaction of ligand with Cys42 residue is depicted

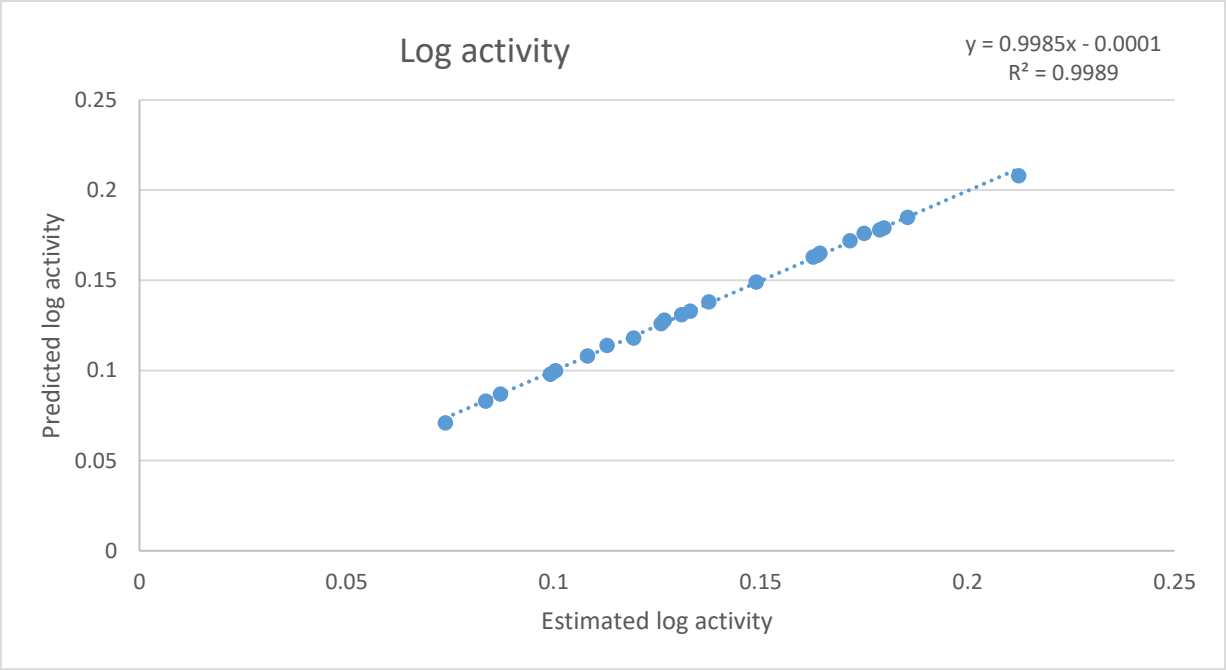

Fig 2: Graph of predicted log values vs experimentally derived log values for training set

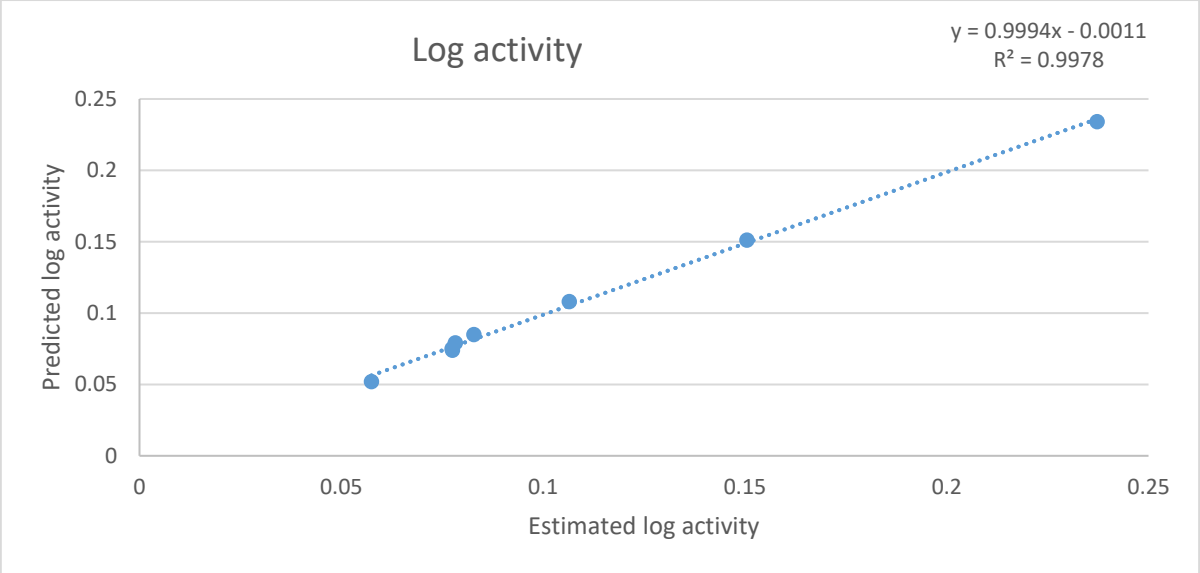

Fig 3: Graph of predicted log values vs experimentally derived log values for test set

6. CONCLUSION

A total of 32 small molecules were taken into account during this study and two such compounds have been identified as potential inhibitors against falcipain2. The solubility of hit compounds can be enhanced by altering their functional groups for better oral drug administration. The drugs so developed should have appropriate bio-availability, metabolic activity and toxicity profile. It is believed that the prospective inhibitors which have been discussed here can assist in finding potent molecules capable of arresting the degradation activity of falcipain2 towards haemoglobin. The small molecules will further undergo optimization to aid in identification of promising lead compounds.

7. FUTURE WORK

Following hit confirmation, several compounds are chosen according to their specific characteristics to produce lead compounds based on selectivity, affinity, lipophilicity, efficacy, cytotoxicity and other physio-chemical parameters. In our case, further analysis is necessary to validate the *In-silico* results of the generated hits. If the lead compounds show favourable results with respect to clinical trials and obtain regulatory approvals, they can be further marketed as potential anti-malarial drugs.

8. REFERENCES

[1] Li, H. *et al.* Identification of Novel Falcipain-2 Inhibitors as Potential Antimalarial Agents through Structure-Based Virtual Screening. 4936–4940 (2009). doi:10.1021/jm801622x.
[2] Mutabingwa, T. K. Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! *Acta Trop.* **95,** 305–315 (2005).
[3] Hamer, D. H. *et al.* Improved Diagnostic Testing and Malaria Treatment Practices in Zambia. *Jama* **297,** 2227 (2007).
[4] Lee, B. J. *et al.* Antimalarial Activities of Novel Synthetic Cysteine Protease Inhibitors. *Society* **47,** 3810–3814 (2003).
[5] PAUL, Uttam Kumar et al. Perceptions about malaria among the Bedia tribal people in Uttar-Dinajpur district of West Bengal, India, **International Journal of Research in Medical Sciences.**
[6] Nadjm B, Behrens RH (2012). "Malaria: An update for physicians". *Infectious Disease Clinics of North America*. **26** (2): 243–59.
[7] Harris, F. & Pierpoint, L. PhotodynamicTherapy Based on 5-Aminolevulinic Acid and Its Use as an Antimicrobial Agent. *Med. Res. Rev.* **29,** 1292–1327 (2012).
[8] White, N. J. Artesunate versus quinine for treatment of severe falciparum malaria: A randomised trial. *Lancet* **366,** 717–725 (2005).
[9] Hogg, T. *et al.* Structural and functional characterization of falcipain-2, a hemoglobinase from the malarial parasite Plasmodium falciparum. *J. Biol. Chem.* **281,** 25425–25437 (2006).
[10] O'Boyle, N. M. *et al.* Open Babel: An open chemical toolbox. *J. Cheminform.* **3,** 33 (2011).
[11] Yap, C. W. PaDEL-descriptor: An open source software to calculate molecular descriptors and fingerprints. *J. Comput. Chem.* **32,** 1466–1474 (2011).
[12] Kramer, B., Rarey, M. & Lengauer, T. Evaluation of the FLEXX incremental construction algorithm for protein-ligand docking. *Proteins Struct. Funct. Genet.* **37,** 228–241 (1999).
[13] Morris, G. M., Huey, R. & Olson, A. J. Using AutoDock for Ligand-Receptor Docking. in *Current Protocols in Bioinformatics* **24,** 8.14.1-8.14.40 (John Wiley & Sons, Inc., 2008).
[14] Peach, M. L. *et al.* Computational tools and resources for metabolism-related property predictions. 1. Overview of publicly available (free and commercial) databases and software. *Future Med. Chem.* **4,** 1907–1932 (2012).
[15] Csizmadia, P. MarvinSketch and MarvinView: molecule applets for the World Wide Web. *ECSOC-3, Third Int. Electron. Conf. Synth. Org. Chem.* 367–369 (1999).
[16] Brooks, B. R. *et al.* CHARMM: The Biomolecular Simulation Program B. *J. Comput. Chem.* **30,** 1545–1614 (2009).
[17] Jadhav, S., Nikam, K., Gandhi, A., Shinde, N. & Salunkhe, K. Applications of computer science in pharmacy: An overview. *Natl. J. Physiol. Pharm. Pharmacol.* **2,** 1–9 (2012).

