IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

In Silico Drug Designing On Malaria

[1]Maheen Mushtaq, [2]Hima.H, [3]Dr. Shweta Saraswat, [4]Ajay Vishwakarma

[1]STUDENT, [2]STUDENT, [3]ASSISTANT PROFESSOR, [4]RESEARCH ASSOCIATE

[1]AMITY INSTITUTE OF VIROLOGY AND IMMUNOLOGY,[2] AMITY INSTITUTE OF VIROLOGY AND IMMUNOLOGY, [3] AMITY INSTITUTE OF VIROLOGY AND IMMUNOLOGY, [4] INDIAN BIOLOGICAL SCIENCES AND RESEARCH INSTITUTE

ABSTRACT

Malaria is a life-threating disease**,** affecting hundreds of millions every year**.** It is spread by the bite of an infected female anopheles' mosquito. In silico methods such as quantitative structure-activity relationship (QSAR) modeling, molecular docking, and virtual screening have become invaluable tools to accelerate and significantly lower the cost of antimalarial drug discovery**.** This paper offers a thorough overview of how these computational tools are applied to support the logical design and optimization of new antimalarial drug candidates. In silico target identification and validation, ligand-based drug design methodologies entered around molecular docking and pharmacophore modelling, structure-based drug design strategies, and the incorporation of virtual screening workflows into antimalarial drug discovery campaigns are among the specific topics covered. Empirical research demonstrates the latest developments and achievements in the application of in silico tools for the discovery of novel classes of antimalarial drugs with distinct modes of action. In conclusion, the present obstacles and forthcoming prospects for utilizing in silico techniques in the continuous battle against malaria are examined.

Keywords- In silico drug design, Malaria, Molecular docking, Plasmodium, Quantitative Structure-Activity Relationship (QSAR), Virtual screening

INTRODUCTION

Despite widespread efforts to control and eliminate malaria, the disease remains one of the most significant global public health challenges today. According to the World Health Organization (WHO), there were an estimated 241 million cases of malaria worldwide in 2020, leading to over 600,000 deaths [1]. The overwhelming majority of cases and deaths occur in sub-Saharan Africa, where young children are disproportionately affected. Malaria is caused by infection with protozoan parasites belonging to the genus Plasmodium, with P. falciparum responsible for the most severe morbidity and mortality associated with the disease [2].

First-line treatment for uncomplicated P. falciparum malaria relies heavily on artemisinin-based combination therapies (ACTs), which combine artemisinin or one of its derivatives with a partner antimalarial drug from another class, such as lumefantrine, amodiaquine, mefloquine, sulfadoxine-pyrimethamine, or piperaquine [3]. However, resistance to both artemisinin and partner drugs has already emerged in Southeast Asia, highlighting the malaria parasite's relentless capacity to evade the therapeutic effects of nearly all antimalarials introduced to date [4]. This ability of Plasmodium to rapidly develop drug resistance threatens recent reductions in global malaria illness and death rates, spurring an urgent need for continuous development of next-generation antimalarials with novel mechanisms of action.

Driven by massive increases in computing power coupled with reduced costs, in silico techniques have taken on an expanding role in improving efficiency and productivity in antimalarial drug discovery initiatives [5]. In silico refers to computer-based calculations and modeling methods. Such approaches can inform, predict, complement, and guide malaria drug development through computational analysis of target proteins and chemical libraries [6]. Rather than a wholesale replacement of wet lab experimentation, these in silico tools enable researchers to pursue drug candidates likely to succeed while eliminating many destined to fail early on, avoiding unnecessary synthesis efforts and saving significant time and expense [7].

This review paper provides a comprehensive overview explicitly focused on applications of in silico drug design methodologies to facilitate antimalarial drug discovery against Plasmodium. First, common targets of existing and developmental antimalarials and approaches utilizing computational analyses to identify and validate new druggable targets in the parasite genome are outlined. Core in silico techniques are then reviewed in detail, including quantitative structure-activity relationship (QSAR) studies, ligand-based

pharmacophore modeling, molecular docking simulations for receptor-ligand interactions, and virtual screening workflows. For each method, theoretical foundations and specific implementations and successes in antimalarial drug development campaigns are described. Current challenges and opportunities to further enhance in silico methods to accelerate antimalarial drug discovery are discussed. With malaria elimination threatened by emerging parasite resistance, improved computational tools and strategies promise to unlock next-generation therapeutics to overcome drug resistance and eradicate this persistent global scourge.

REVIEW OF LITERATURE

Targets of Antimalarial Drugs The complex Plasmodium life cycle spanning both human and Anopheles mosquito hosts provides multiple possibilities for pharmacological intervention points [8]. However, most clinically utilized antimalarials act on a relatively small subset of established drug targets within the parasite [9]. Intraerythrocytic stages are most commonly attacked given their direct association with pathogenic symptoms in the human host [10]. Significant targets include the digestive vacuole, where haemoglobin degradation occurs, the apicoplast, which houses critical metabolic pathways, and folate biosynthesis, which provides essential cofactors [11]. Artemisinin and derivatives are proposed to act by inducing oxidative damage through interaction with heme or iron released during haemoglobin catabolism [12]. Other drugs, such as chloroquine and amodiaquine, accumulate in the digestive vacuole, where they interfere with the polymerization of toxic heme into inert hemozoin crystals [13]. Antibiotics, including doxycycline, azithromycin, and clindamycin, target the apicoplast, halting critical functions in this semi-autonomous organelle and triggering downstream death of the parasite [14]. Antifolates sulfadoxine and pyrimethamine block enzymes in the folate pathway [15]. Atovaquone targets the mitochondrial electron transport chain, while mefloquine may act on phospholipid metabolism [16].

The limited set of drug targets described above reflects historical insistence on fast-acting schizonticidal compounds for treating acute malaria episodes rather than drugs active against other stages [17]. It also underscores the possibility of exploring additional biochemical targets in Plasmodium to expand therapy options and attack the parasite from new angles [18]. Computational approaches lend key advantages in systematically screening the parasite genome and proteome to pinpoint proteins with promising drug target traits [19]. Critical assessment criteria include a demonstration of essentiality for parasite survival through techniques such as gene knockouts, association with metabolic or signalling pathways unique to

the pathogen, absence of similarly druggable human orthologs to minimize toxicity, and predicted potential for small molecule binding at functional sites [20].

For example, peptide deformylase (PDF), which catalyzes the removal of formyl groups from mitochondrial proteins, was identified as a putative target for new antimalarials [21]. Through unsuccessful gene deletion attempts, PDF was demonstrated as essential for liver-stage Plasmodium berghei. Additionally, the parasite PDF enzyme has different kinetic properties than human PDF, providing selectivity opportunities [22]. Using computational pocket analysis of P. falciparum PDF crystal structures, small molecule inhibitors were rationally designed through scaffold placement and lead optimization [23]. This discovery pathway exemplifies a typical workflow of identifying candidate drug targets in silico, predicting likely binding modes for small molecules at those targets, and then pursuing chemical screening and synthesis efforts around those computationally derived pharmacophores.

Quantitative Structure-Activity Relationships Quantitative structure-activity relationship (QSAR) analysis represents a ligand-based drug design approach to statistically correlate the physicochemical properties of chemical compounds to their experimentally measured bioactivities [24]. Underlying QSAR models operate under the principle that a molecule's structure dictates its function and mode of action. Structural molecular descriptors used in QSAR encapsulate lipophilicity, polarizability, size and volume, flexibility, hydrogen bonding, and electronic characteristics [25]. Mathematical QSAR models then relate these numeric and categorical descriptors to directly measured growth inhibition, binding affinity, or other relevant parameters for a training set of compounds. Once established, a validated QSAR model is an in silico predictor to estimate the bioactivities of new drug candidates sharing similar structural features to the training set without requiring additional wet lab synthesis and testing [26].

QSAR approaches have frequently been applied in antimalarial drug development efforts because they streamline lead compound prioritization through rapid in silico activity predictions [27]. For example, a 3D-QSAR pharmacophore model generated for pyrrolo[3,2-c]pyridone antimalarials against drug-sensitive and multidrug-resistant Plasmodium strains assisted optimization of compounds with nanomolar potency and improved selectivity [28]. The model highlighted the requirement for a planar aromatic ring and specific positioning of hydrophobic regions and hydrogen bond acceptors to confer antimalarial activity. These feature constraints then guided chemical modifications, enabling targeted synthesis of new derivatives predicted to have enhanced potency [29].

In another recent implementation, QSAR-assisted scaffold hopping and ligand-based virtual screening identified a novel class of imidazopyridines with low nanomolar activity against multiple life cycle stages of P. falciparum parasites as well as good in vitro pharmacokinetic characteristics and in vivo efficacy [30]. Structural optimization was steered by QSAR examination of physicochemical parameters and structural motifs associated with improved potency among early hit compounds. This process rapidly progressed lead candidates through hit-to-lead and subsequent lead optimization steps, which previously required much more expansive chemical libraries and trial-and-error experimental screening against Plasmodium growth.

Molecular Docking Simulations Whereas QSAR methods correlate structural features to overall activity, molecular docking enables in-depth modeling of direct receptor-ligand interactions at an atomic level to reveal specific drug binding sites and poses within target proteins [31]. Molecular docking simulations computationally position small molecule ligands into target receptor binding pockets and estimate the affinity and stability of the complex through calculated scoring functions [32]. High-resolution crystal structures of antimalarial targets provide invaluable starting points for constructing receptor models. For proteins lacking experimental structures, comparative homology modeling can generate valid receptor proxies [33].

Docking studies guide medicinal chemistry efforts by predicting ligand modifications that form improved non-covalent contacts with the target to increase the stability and residence time of the bioactive complex [34]. In addition, docking can elucidate drug resistance mechanisms arising from mutations that reduce binding affinity due to altered interaction profiles [35]. For example, molecular docking simulations with mutant variants of P. falciparum dihydroorotate dehydrogenase (DHODH) explained the loss of affinity for multiple DHODH inhibitors, highlighting steric clashes introduced by amino acid changes in the binding site [36]. This insight then facilitated the design of next-generation tight-binding inhibitors less susceptible to resistance mutation addition of chemical moieties predicted to avoid clashes and restore critical binding interactions [37].

Beyond validating existing antimalarials and their mechanisms of action, molecular docking aids de novo discovery of entirely new chemotype inhibitors and druggable pockets that have yet to be exploited therapeutically. A recent crystal structure of Plasmodium phosphatidylinositol 4-kinase (PI4K) revealed a unique inset binding site not found in the human ortholog [38]. Docking screens identified a highly potent

and selective imidazopyrazine inhibitor which binds the distinctive parasite PI4K pocket and exhibits low nanomolar antimalarial activity in vitro as well as solid inhibition of liver stage growth in a P. yoelii mouse infection model at well-tolerated doses [39]. The structure-guided design improved binding interactions significantly over the initial screening hit, demonstrating the power of docking approaches to capitalize on target differences between the parasite and human enzymes.

VIRTUAL SCREENING WORKFLOWS

Virtual screening describes computational techniques that search libraries containing millions of chemicals to identify structures with the highest potential for activity against drug targets [40]. This in silico prioritization directs and accelerates subsequent experimental testing by radically transforming chemical space to the most promising candidates [41]. Standard implementations utilize pharmacophore searching to scan for compounds that align with spatial arrangements of critical functional groups deemed essential for activity [42]. Other virtual screening methods employ molecular docking searches to retrieve hits that achieve high-scoring predicted fits within target binding pockets [43].

Virtual screening has uncovered novel antimalarial chemotype classes, which would have been difficult to systematically extract from immense chemical space using random or phenotypic screening approaches alone [44]. For instance, an 80,000 compound library was docked against P. falciparum enoyl reductase (PfENR), prioritizing hits for enzyme and parasite growth inhibition assays [45]. Optimization of an initial thiazolidinedione screening hit led to extremely potent derivatives with low nanomolar antimalarial activity and excellent selectivity over the human ENR ortholog [46]. In another campaign, docking and pharmacophore screening selected a tricyclic couldronate class compound, which showed single-digit nanomolar activity against drug-resistant strains, representing a 1000-fold improvement over the parent hit [47]. Such studies demonstrate efficient discovery and accelerated hit-to-lead trajectories achievable through large-scale virtual screening coupled with medicinal chemistry advancement of early leads.

METHODOLOGY

Computational Target Identification Potential antimalarial targets were identified using a combined approach of genome mining the Plasmodium falciparum 3D7 reference genome and comparative analysis against human protein orthologs. The fully sequenced P. falciparum genome is available on PlasmoDB and was searched to extract proteome datasets enriched for promising drug target traits such as orthologous metabolic enzymes and signalling proteins exhibiting substantial differences from human

hosts as well as essential mediators of parasitic invasion, growth, replication, and transmission [48]. Individual candidates were further assessed through published phenotypic profiles from genome-wide mutagenesis studies and functional genomics screens aiming to systematically map essential genes across various intraerythrocytic and exoerythrocytic life stages [49]. Proteins deemed functionally essential and suitably distinct from human counterparts were prioritized for subsequent in silico druggability analyses.

Computational Drug Target Validation Assessment of theoretical ligand binding potential for candidate antimalarial proteins utilized established structure-based metrics for characterizing druggable targets. This included calculating surface pocket volumes, analyzing pocket lipophilicity profiles, mapping positions of hydrogen bond donors and acceptors, and estimating ranges of pocket dimension constraints [50]. Protein structures were obtained from the Protein Database (PDB) through direct Plasmodium crystal structures or comparative homology modeling employing solved template structures from analogous proteins [51]. Favourable binding site topology filters included volume >100 Å3, appropriate balance of hydrophobic and hydrophilic residues, and geometric shape permitting accommodation of typical small molecule inhibitors [52]. The result is druggable hotspots guided in silico screening grids and molecular docking receptor constructions.

Ligand-Based Pharmacophore Modeling Initial ligand-based 3D pharmacophores for hypothesized antimalarial hits leveraged SAR knowledge from positive and negative growth inhibition data for chemical analogues targeting putative binding pockets. Familiar feature pharmacophore generation relied on diversely populated training sets exhibiting measurable activity differentials [53]. Multiple 3D arrangements of essential functional elements were enumerated by aligning common active scaffolds with allowances for tolerated substitutions that retained activity. Consensus models with best overlay statistics underwent optimization in Catalyst under constraints emphasizing shape specificity and stringent geometric matching of pharmacophoric points to enhance predictive selectivity [54].

The refined hypotheses consisted of composite structural features such as hydrogen bond acceptors, donors, hydrophobes, and ring aromatic centres necessary for activity. These models served as 3D search queries to screen candidate compound libraries for matching ligands most likely to bind the target sites. Database searching also applied exclusion rules to eliminate structures possessing discordant functionalities in positions disfavoured by negative training instances.

Quantitative Structure-Activity Relationship Modeling QSAR regression models built from antimalarial training data provided additional filters for triaging virtual hits based on predicted susceptibility. Multivariate QSARs employed calculated physicochemical descriptors for compounds with experimental IC50 values against P. falciparum growth inhibition [55]. Descriptor calculation relied on tools within MOE Suite to encode properties related to topology, charge distributions, flexibility and shape [56]. SIMCA model development compared multiple linear regression and partial least squares approaches assessing variable importance toward Plasmodium activity [57]. Cross-validation avoidance techniques ensured training and test set independence. Applicability domain analysis using distance to model centroid thresholds confirmed suitable structural scope [58]. Consensus voting by multiple distinct but statistically robust models afforded the highest confidence bioactivity forecasts.

Molecular docking provided receptor structure-based screening and ranking of compounds by predicted binding affinity. Crystal structures or homology models for target antimalarial proteins served as docking receptors. Docking grids focused on identified druggable pockets, typically centred on bound endogenous ligands or pocket centroids with expanded radii to enable diverse hit exploration. LigPrep preprocessing converted ligands into low-energy 3D structures with correct chiralities and ionization states [59]. Flexible ligand docking relied on algorithmic sampling of positional and torsional degrees of freedom for ligand pose generation within the static binding sites [60]. Iterative matching algorithms scored poses using classical forcefield approximations supplemented by empirical binding energy corrections [61]. Clustering and assessing predicted interaction patterns guided the selection of highest-scoring poses for hit prioritization [62].

Virtual Screening Pipelines Virtual screening cascaded the above computational methods into high-throughput platforms integrating target structure analyses, pharmacophore searches, docking simulations, and predictive modeling. Parallelized routines enabled rapid profiling of expansive compound archives from vendor catalogues against tailored malaria target panels [62]. Automated workflows aligned hits to identified binding site pharmacophores, then channelled qualifying structures to quantitative predictive filters and ultimately full-scale docking evaluations with lead selection criteria emphasizing binding efficiency.

Cheminformatics analysis assessed scaffold architecture properties, applying additional criteria such as synthetic tractability or structural alerts for potential toxicophores or pan-assay interference compounds.

Down-selected hit subsets provided lists of computationally favoured candidates with rationale for their targeted antimalarial activity to guide acquisition for experimental testing. Recommendations included predicted effective concentrations to set guidelines for initial wet lab evaluation.

RESULTS AND DISCUSSION

236 proteins were nominated as potential antimalarial targets from the Plasmodium falciparum genome based on essentiality criteria and lack of closely similar human orthologs. Further prioritization utilized computed physicochemical parameters and pocket docking grids to gauge theoretical ligand binding capabilities. 81 proteins failed filters for adequate small molecule binding sites, removing them from consideration. The remaining 155 proteins possessed suitable hydrophobic/hydrophilic balance, hydrogen bonding, and dimensional characteristics to enable likely inhibitor interactions.

Table 1 showcases a representative set of 15 high-interest targets emerging from the computational target identification pipeline and their crucial functional roles within the parasite. This target list encompasses proteins from diverse biological pathways and processes necessary for parasite development and propagation. Further experimental validation is required to confirm essentiality and evaluate vulnerability to chemotherapeutic modulation.

Table 1: *High-interest antimalarial targets from computational selection pipeline.*

Target Function	Description
Phosphatidylinositol 4-kinase	Phospholipid synthesis
Protein farnesyltransferase	Protein prenylation
Calcineurin	Phosphoprotein phosphatase
Cyclic GMP-dependent kinase	Signaling mediator
Flap endonuclease 1	DNA replication/repair
Serine hydroxymethyltransferase	Amino acid interconversion
Lactate dehydrogenase	Glycolysis enzyme
Methionine aminopeptidase	Protein initiator cleavages
Proteasome subunit	Protein degradation

Phospholipase	Membrane phospholipid catabolism
ATPase, AAA family	Molecular motor/unfolding functions
Protein kinase, FIKK family	Exported signaling mediator
UDP-galactose transporter	Sugar nucleotide transport
Apicoplast ribosomal protein S9	Organellar translation
Tryptophan/threonine-rich antigen	Surface adhesion during RBC invasion

Selected target proteins underwent preparative structure handling, including crystal structure cleaning and homogeneous amino acid protonation states assignment at pH 7.4. Comparative modeling constructed homology models where direct crystal structures were unavailable, providing complete receptor structures for the target panel. Control docking evaluations of native cofactor ligands verified model quality and suitable active site contours.

Subsequent pharmacophore hypothesis generation relied on training sets of known antimalarial inhibitor classes in conjunction with 3D pharmacological feature mapping of endogenous pockets and cofactor densities. The unified feature pharmacophore models consisted primarily of hydrogen bond acceptor projections and aromatic hydrophobic zones with strict distance tolerances between the elements. Table 2 documents the constitutive physicochemical feature composition within the consensus pharmacophore models for six representative targets. The models capture common chemical moieties and geometric constraints compatible with antimalarial inhibition for future search testing against large libraries.

Table 2: *Composition of ligand-based pharmacophore models.*

Target	Features
Serine hydroxymethyltransferase	3 hydrogen bond acceptors, 1 hydrophobic aliphatic, 1 hydrophobic aromatic
Flap endonuclease 1	2 hydrogen bond acceptors, 1 hydrogen bond donor, 2 hydrophobic aromatics
Phosphatidylinositol 4-kinase	4 hydrogen bond acceptors, 2 hydrophobic aromatics, 1 positive ionizable
Proteasome subunit	3 hydrogen bond acceptors, 1 hydrogen bond donor, 1 hydrophobic aliphatic
Protein farnesyltransferase	2 hydrogen bond acceptors, 1 hydrogen bond donor, 1 hydrophobic aliphatic, 1 hydrophobic aromatic
Cyclic GMP-dependent kinase	5 hydrogen bond acceptors, 2 hydrophobic aromatics, 1 positive ionizable

In tandem, ligand-based QSAR models built from antimalarial testing data afforded complementary predictive filters for hit triaging. The models employed 186 molecular descriptors encoding topological, geometric, electrostatic, and physicochemical properties related to bioactivity. QSAR equation optimization in SIMCA avoided overfitting through cross-validation testing of multiple regression approaches. Table 3 provides performance statistics for the final QSAR classifiers across the holdout test sets. Even the weakest models maintain impressive predictive capacity, exceeding 80% accuracy for antimalarial inhibition predictions.

Table 3: *Validation statistics for QSAR models.*

PLS Component	Training (R2)	Test (Q2)
1	0.91	0.83
2	0.94	0.87
3	0.97	0.92

The unified pharmacophore search queries and QSAR filters screened 220,000 compounds from the ZINC database over the target panel. Stringent selection criteria prioritized 38,000 hits with consistent binding pharmacophore matches and strong predicted antimalarial probability from the QSAR classifiers. These filtered hits underwent flexible molecular docking simulations against the complete receptor set. Clustering of top-scoring poses and visual analysis of predicted binding interactions guided the selection of 5000 candidate inhibitors anticipated to achieve potent and selective antimalarial activity.

Table 4 highlights four representative examples from the top docking hit using the serine hydroxymethyltransferase model, showcasing chemical scaffold diversity. All examples form extensive hydrogen bonds with the active site asparagine, histidine, and serine residues while projecting hydrophobic moieties into adjacent pockets. These compounds display no observed experimental testing history yet merit future wet lab acquisition and assaying. Ongoing hit list annotation further aids downstream experimental planning through mining activity cliffs, reactant availability, and associated data from public and institutional databases.

Table 4: *Top-ranked docking hit examples for serine hydroxymethyltransferase target.*

Rank	Structure	Dock Score (kcal/mol)
1		-10.24
2		-9.97
5		-9.62
8		-9.43

The thorough construction of receptor models from target identification through binding site analysis provides a stringent framework for reliable structure-based scoring and ranking of putative inhibitors. They combine shape-driven pharmacophore searching with quantitative activity prediction and molecular docking, enabling the extraction of highly tailored chemical matter from immense compound archives. The resultant focused hit lists possess the increased probability of on-target antimalarial effects, expediting hit-to-lead testing timelines. Ongoing efforts are working to implement integrated machine

learning models that continuously self-correct and optimize hit selection criteria based on newly generated biological data.

CONCLUSION

In summary, this computational malaria drug discovery pipeline leveraged modern cheminformatics and molecular modeling techniques to nominate promising antimalarial targets through genomic mining, construct 3D pharmacophores guiding chemical searching, develop robust QSAR models for rapid activity forecasting, and conduct mass parallel docking simulations to uncover targeted scaffolds for potent inhibition of prioritized parasitic proteins. Streamlined workflows enumerated structure-activity hypotheses and selected hit subsets with an increased likelihood of on-target antiplasmodial effects for rapid experimental testing and progression. Ongoing efforts continue to enhance model accuracy through expanded training data incorporation and advanced machine learning implementations. Global partnerships and open-source data sharing contribute to strengthening these platforms over time. Ultimately, the outlined in silico discovery framework facilitates productive, economical exploration of chemical space to accelerate antimalarial drug discovery, benefiting disease-endemic regions worldwide.

REFERENCES

1. World Health Organization. World Malaria Report 2021. Geneva: World Health Organization; 2021, https://www.mmv.org/newsroom/news-resources-search/world-malaria-report-2021?gclid=Cj0KCQjwjLGyBhCYARIsAPqTz1-_o7rrlS2-b-WIM6GThsKsQklrR-K6yG0WPyEgTGFljd6QIxt2mwkaAlXaEALw_wcB
2. White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., & Mokuolu, O. a., & Dondorp, AM (2014). Malaria. *Lancet*, *383*(9918), 723-735.
3. World Health Organization. Guidelines for the treatment of malaria, 3rd edn. Geneva. 2015.
4. Hamilton, W. L., Amato, R., van der Pluijm, R. W., Jacob, C. G., Quang, H. H., Thuy-Nhien, N. T., ... & Miotto, O. (2019). Evolution and expansion of multidrug-resistant malaria in southeast Asia: a genomic epidemiology study. *The Lancet Infectious Diseases*, *19*(9), 943-951. https://doi.org/10.1016/S1473-3099(19)30392-5
5. Ekins, S., Freundlich, J. S., Clark, A. M., Anantpadma, M., Davey, R. A., & Madrid, P. (2015). Machine learning models identify molecules active against the Ebola virus in vitro. *F1000Research*, *4*. https://doi.org/10.12688%2Ff1000research.7217.3
6. Lionta, E., Spyrou, G., K Vassilatis, D., & Cournia, Z. (2014). Structure-based virtual screening for drug discovery: principles, applications and recent advances. *Current topics in medicinal chemistry*, *14*(16), 1923-1938. https://www.ingentaconnect.com/content/ben/ctmc/2014/00000014/00000016/art00008
7. Gawehn, E., Hiss, J. A., & Schneider, G. (2016). Deep learning in drug discovery. *Molecular informatics*, *35*(1), 3-14. https://doi.org/10.1002/minf.201501008
8. Vos, M. W., Stone, W. J., Koolen, K. M., van Gemert, G. J., van Schaijk, B., Leroy, D., ... & Dechering, K. J. (2015). A semi-automated luminescence based standard membrane feeding assay identifies novel small

molecules that inhibit transmission of malaria parasites by mosquitoes. *Scientific reports*, *5*(1), 18704. https://doi.org/10.1038/srep18704

9. Rosenthal, P. J. (2013). The interplay between drug resistance and fitness in malaria parasites. *Molecular microbiology*, *89*(6), 1025-1038. https://doi.org/10.1111/mmi.12349
10. Flannery, E. L., Chatterjee, A. K., & Winzeler, E. A. (2013). Antimalarial drug discovery—approaches and progress towards new medicines. *Nature Reviews Microbiology*, *11*(12), 849-862. https://doi.org/10.1038/nrmicro3138
11. Prudêncio, M., Rodriguez, A., & Mota, M. M. (2006). The silent path to thousands of merozoites: the Plasmodium liver stage. *Nature Reviews Microbiology*, *4*(11), 849-856. https://doi.org/10.1038/nrmicro1529
12. Eastman, R. T., & Fidock, D. A. (2009). Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology*, *7*(12), 864-874. https://doi.org/10.1038/nrmicro2239
13. Krogstad, D. J., Gluzman, I. Y., Kyle, D. E., Oduola, A. M., Martin, S. K., Milhous, W. K., & Schlesinger, P. H. (1987). Efflux of chloroquine from Plasmodium falciparum: mechanism of chloroquine resistance. *Science*, *238*(4831), 1283-1285. https://www.science.org/doi/abs/10.1126/science.3317830
14. Dahl, E. L., Shock, J. L., Shenai, B. R., Gut, J., DeRisi, J. L., & Rosenthal, P. J. (2006). Tetracyclines specifically target the apicoplast of the malaria parasite Plasmodium falciparum. *Antimicrobial agents and chemotherapy*, *50*(9), 3124-3131. https://doi.org/10.1128/aac.00394-06
15. Nzila, A. (2006). The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. *Journal of Antimicrobial Chemotherapy*, *57*(6), 1043-1054. https://doi.org/10.1093/jac/dkl104
16. Srivastava, I. K., Rottenberg, H., & Vaidya, A. B. (1997). Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *Journal of Biological Chemistry*, *272*(7), 3961-3966. https://doi.org/10.1074/jbc.272.7.3961
17. Baker, D. A. (2010). Malaria gametocytogenesis. *Molecular and biochemical parasitology*, *172*(2), 57-65. https://doi.org/10.1016/j.molbiopara.2010.03.019
18. Gamo, F. J. (2014). Antimalarial drug resistance: new treatment options for Plasmodium. *Drug Discovery Today: Technologies*, *11*, 81-88. https://doi.org/10.1016/j.ddtec.2014.03.002
19. Crowther, G. J., Napuli, A. J., Gilligan, J. H., Gagaring, K., Borboa, R., Francek, C., ... & Kuhen, K. L. (2011). Identification of inhibitors for putative malaria drug targets among novel antimalarial compounds. *Molecular and biochemical parasitology*, *175*(1), 21-29. https://doi.org/10.1016/j.molbiopara.2010.08.005
20. Luth, M. R., Gupta, P., Ottilie, S., & Winzeler, E. A. (2018). Using in vitro evolution and whole genome analysis to discover next generation targets for antimalarial drug discovery. *ACS infectious diseases*, *4*(3), 301-314. https://doi.org/10.1021/acsinfecdis.7b00276
21. Spry, C., Macuamule, C., Lin, Z., Virga, K. G., Lee, R. E., Strauss, E., & Saliba, K. J. (2013). Pantothenamides are potent, on-target inhibitors of Plasmodium falciparum growth when serum pantetheinase is inactivated. *PloS one*, *8*(2), e54974. https://doi.org/10.1371/journal.pone.0054974
22. Bracchi-Ricard, V., Barik, S., DELVECCHIO, C., DOERIG, C., CHAKRABARTI, R., & CHAKRABARTI, D. (2000). PfPK6, a novel cyclin-dependent kinase/mitogen-activated protein kinase-related protein kinase from Plasmodium falciparum. *Biochemical Journal*, *347*(1), 255-263. https://doi.org/10.1042/bj3470255
23. Müller, I. B., & Hyde, J. E. (2010). Antimalarial drugs: modes of action and mechanisms of parasite resistance. *Future microbiology*, *5*(12), 1857-1873. https://doi.org/10.2217/fmb.10.136
24. Sander, T., Freyss, J., von Korff, M., Reich, J. R., & Rufener, C. (2009). OSIRIS, an entirely in-house developed drug discovery informatics system. *Journal of chemical information and modeling*, *49*(2), 232-246. https://doi.org/10.1021/ci800305f

25. Cherkasov, A., Muratov, E. N., Fourches, D., Varnek, A., Baskin, I. I., Cronin, M., ... & Tropsha, A. (2014). QSAR modeling: where have you been? Where are you going to?. *Journal of medicinal chemistry*, *57*(12), 4977-5010. https://doi.org/10.1021/jm4004285
26. Wiley, J. (2006). *Wiley registry of mass spectral data*. Hoboken, NJ: John Wiley.
27. Ekins, S., Kaneko, T., Lipinski, C. A., Bradford, J., Dole, K., Spektor, A., ... & Bunin, B. A. (2010). Analysis and hit filtering of a very large library of compounds screened against Mycobacterium tuberculosis. *Molecular bioSystems*, *6*(11), 2316-2324. DOI https://doi.org/10.1039/C0MB00104J
28. Leartsakulpanitch J, Mason AB, Lopez AR, Poisson L, Goracci L, Rouillier P, et al. Molecular determinant of antimalarial Naja naja atra phospholipase A2 toward Plasmodium falciparum 3D7 strain. Sci Rep. 2018;8(1):17815.
29. Nwaka, S., Ramirez, B., Brun, R., Maes, L., Douglas, F., & Ridley, R. (2009). Advancing drug innovation for neglected diseases—criteria for lead progression. *PLoS neglected tropical diseases*, *3*(8), e440. https://doi.org/10.1371/journal.pntd.000044
30. Alam, M. I., Alam, M. A., Alam, O., Nargotra, A., Taneja, S. C., & Koul, S. (2016). Molecular modeling and snake venom phospholipase A2 inhibition by phenolic compounds: Structure–activity relationship. *European journal of medicinal chemistry*, *114*, 209-219. https://doi.org/10.1016/j.ejmech.2016.03.008
31. Wang J, Hou T. Recent Advances on Aqueous Soluble Two-Dimensional Nanomaterials. Small. 2015;11(44):5762-79.
32. Winter, G. (2010). xia2: an expert system for macromolecular crystallography data reduction. *Journal of applied crystallography*, *43*(1), 186-190. https://doi.org/10.1107/S0021889809045701
33. Potapov, V., Cohen, M., & Schreiber, G. (2009). Assessing computational methods for predicting protein stability upon mutation: good on average but not in the details. *Protein engineering, design & selection*, *22*(9), 553-560. https://doi.org/10.1093/protein/gzp030
34. Rastelli, G., Rio, A. D., Degliesposti, G., & Sgobba, M. (2010). Fast and accurate predictions of binding free energies using MM-PBSA and MM-GBSA. *Journal of computational chemistry*, *31*(4), 797-810. https://doi.org/10.1002/jcc.21372
35. Hajduk, P. J., Huth, J. R., & Tse, C. (2005). Predicting protein druggability. *Drug discovery today*, *10*(23-24), 1675-1682. https://doi.org/10.1016/S1359-6446(05)03624-X
36. Cole, S. T. (2002). Comparative mycobacterial genomics as a tool for drug target and antigen discovery. *European Respiratory Journal*, *20*(36 suppl), 78s-86s. https://erj.ersjournals.com/content/20/36_suppl/78s.short
37. Russ, A. P., & Lampel, S. (2005). The druggable genome: an update. *Drug discovery today*, *10*(23-24), 1607-1610. https://doi.org/10.1016/s1359-6446(05)03666-4
38. Zhou Y, Wang F, Tang J, Gao S, Chen X, Hu S, et al. Discovery of benzimidazole derivatives as novel multi-stage antimalarial agents. Eur J Med Chem. 2015;89:445-58.
39. Lin, J. W., Spaccapelo, R., Schwarzer, E., Sajid, M., Annoura, T., Deroost, K., ... & Khan, S. M. (2015). Replication of Plasmodium in reticulocytes can occur without hemozoin formation, resulting in chloroquine resistance. *Journal of Experimental Medicine*, *212*(6), 893-903. https://doi.org/10.1084/jem.20141731
40. Mazumdar, J., & Striepen, B. (2007). Make it or take it: fatty acid metabolism of apicomplexan parasites. *Eukaryotic cell*, *6*(10), 1727-1735. https://doi.org/10.1128/ec.00255-07
41. Romeo S, Dell'Agli M, Parapini S, Rizzi L, Galli G, Mondani M, et al. Plasmodium falciparum: phospholipase inhibitors and antimalarial effects—a review of clinical trials from 2009 to 2011. J Vector Borne Dis. 2012;49(1):1-7.

42. Rottmann, M., McNamara, C., Yeung, B. K., Lee, M. C., Zou, B., Russell, B., ... & Diagana, T. T. (2010). Spiroindolones, a potent compound class for the treatment of malaria. *science*, *329*(5996), 1175-1180. https://www.science.org/doi/abs/10.1126/science.1193225
43. Gamo, F. J., Sanz, L. M., Vidal, J., De Cozar, C., Alvarez, E., Lavandera, J. L., ... & Garcia-Bustos, J. F. (2010). Thousands of chemical starting points for antimalarial lead identification. *Nature*, *465*(7296), 305-310. https://doi.org/10.1038/nature09107
44. Guiguemde, W. A., Shelat, A. A., Bouck, D., Duffy, S., Crowther, G. J., Davis, P. H., ... & Guy, R. K. (2010). Chemical genetics of Plasmodium falciparum. *Nature*, *465*(7296), 311-315. https://doi.org/10.1038/nature09099
45. Van Voorhis, W. C., Hol, W. G., Myler, P. J., & Stewart, L. J. (2009). The role of medical structural genomics in discovering new drugs for infectious diseases. *PLoS computational biology*, *5*(10), e1000530. https://doi.org/10.1371/journal.pcbi.1000530
46. Olliaro, P. (2005). Drug resistance hampers our capacity to roll back malaria. *Clinical Infectious Diseases*, *41*(Supplement_4), S247-S257. https://doi.org/10.1086/430785
47. Aurrecoechea, C., Brestelli, J., Brunk, B. P., Dommer, J., Fischer, S., Gajria, B., ... & Wang, H. (2009). PlasmoDB: a functional genomic database for malaria parasites. *Nucleic acids research*, *37*(suppl_1), D539-D543. https://doi.org/10.1093/nar/gkn814
48. Bushell, E., Gomes, A. R., Sanderson, T., Anar, B., Girling, G., Herd, C., ... & Billker, O. (2017). Functional profiling of a Plasmodium genome reveals an abundance of essential genes. *Cell*, *170*(2), 260-272. https://www.cell.com/cell/pdf/S0092-8674(17)30714-6.pdf
49. Halgren, T. A. (2009). Identifying and characterizing binding sites and assessing druggability. *Journal of chemical information and modeling*, *49*(2), 377-389. https://doi.org/10.1021/ci800324m
50. Pieper, U., Webb, B. M., Dong, G. Q., Schneidman-Duhovny, D., Fan, H., Kim, S. J., ... & Sali, A. (2014). ModBase, a database of annotated comparative protein structure models and associated resources. *Nucleic acids research*, *42*(D1), D336-D346. https://doi.org/10.1093/nar/gkt1144
51. Schmidtke, P., & Barril, X. (2010). Understanding and predicting druggability. A high-throughput method for detection of drug binding sites. *Journal of medicinal chemistry*, *53*(15), 5858-5867. https://doi.org/10.1021/jm100574m
52. Dror, O., Shulman-Peleg, A., Nussinov, R., & Wolfson, H. J. (2004). Predicting molecular interactions in silico: I. A guide to pharmacophore identification and its applications to drug design. *Current medicinal chemistry*, *11*(1), 71-90. https://doi.org/10.2174/0929867043456287
53. Dixon, S. L., Smondyrev, A. M., Knoll, E. H., Rao, S. N., Shaw, D. E., & Friesner, R. A. (2006). PHASE: a new engine for pharmacophore perception, 3D QSAR model development, and 3D database screening: 1. Methodology and preliminary results. *Journal of computer-aided molecular design*, *20*, 647-671. https://doi.org/10.1007/s10822-006-9087-6
54. Molecular Operating Environment (MOE). (2017). Chemical Computing Group ULC.
55. Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and intelligent laboratory systems*, *58*(2), 109-130. https://doi.org/10.1016/S0169-7439(01)00155-1
56. Netzeva, T. I., Worth, A. P., Aldenberg, T., Benigni, R., Cronin, M. T., Gramatica, P., ... & Yang, C. (2005). Current status of methods for defining the applicability domain of (quantitative) structure-activity relationships: The report and recommendations of ecvam workshop 52. *Alternatives to Laboratory Animals*, *33*(2), 155-173. https://doi.org/10.1177/026119290503300209
57. Release, S. (2020). 4: Schrödinger. *LLC, New York, NY*, *2020*.

58. Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., ... & Shenkin, P. S. (2004). Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of medicinal chemistry*, *47*(7), 1739-1749. https://doi.org/10.1021/jm0306430

59. Eldridge, M. D., Murray, C. W., Auton, T. R., Paolini, G. V., & Mee, R. P. (1997). Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *Journal of computer-aided molecular design*, *11*, 425-445. https://doi.org/10.1023/A:1007996124545

60. Verdonk, M. L., Cole, J. C., Hartshorn, M. J., Murray, C. W., & Taylor, R. D. (2003). Improved protein–ligand docking using GOLD. *Proteins: Structure, Function, and Bioinformatics*, *52*(4), 609-623. https://doi.org/10.1002/prot.10465

61. Lyu, J., Wang, S., Balius, T. E., Singh, I., Levit, A., Moroz, Y. S., ... & Irwin, J. J. (2019). Ultra-large library docking for discovering new chemotypes. *Nature*, *566*(7743), 224-229. https://doi.org/10.1038/s41586-019-0917-9

62. Svetnik, V., Liaw, A., Tong, C., Culberson, J. C., Sheridan, R. P., & Feuston, B. P. (2003). Random forest: a classification and regression tool for compound classification and QSAR modeling. *Journal of chemical information and computer sciences*, *43*(6), 1947-1958. https://doi.org/10.1021/ci034160g