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COMPUTATIONAL DRUG RE-POSITIONING: AN APPROACH TO DISCOVER NOVEL ANTIMALARIALS

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Abstract- In this study molecular docking method was used to select potential inhibitors of the Plasmodium targets and the antimalarial activity of these inhibitors (drugs) was confirmed using Plasmodium berghei induced mice (in vivo). One hundred commercially available drugs not used for malaria were selected from DrugBank and the potential binding affinities of the selected drugs against the Plasmodium targets were analysed using molegro virtual docker software. Ten drugs with the best affinities (Fluconazole, indomethacin, loratidine, lisinopril, meloxicam, promethazine, nifedipine, clarithromycin, piroxicam and flucloxacillin) were selected and the antimalarial activity of these drugs were confirmed using Plasmodium berghei mice model. Nifedipine, clarithromycin, flucloxacillin and lisinopril produced significant suppressive, curative and prophylactic activity while meloxicam and piroxicam produced only significant suppressive and curative activity. All the tested drugs produced significant suppressive activity. Nifedipine, clarithromycin, flucloxacillin and lisinopril are therefore potential chemoprophylatic and chemothrerapeutic agents whilemeloxicam and piroxicam are potential chemotherapeutic agents.

Keywords- Plasmodium berghei, Antimalarial, Computational repositioning;Mice.

I. INTRODUCTION

Malaria is a major public health problem in Nigeria affecting more than 100 million people annually(1). In 2010, about 216 million people were infected with 655,000 mortality worldwide. African region accounts for 81% and 91% of the cases and deaths respectively, with 86% of the mortalities observed among theunder-fives. Nigeria, Cote d'Ivoire, the democratic republic of Congo, Mozambique, Burkina Faso and Mali accounted for 60% of malaria deaths in 2010(1)The emergence of drug-resistant strains has compromised the efficacy of several antimalarial drugs, including artemisinin, thus necessitating the need for discovering of other novel antimalarial(2).Repositioning of known drugs for a new therapeutic use can be employed alternatively to the traditional/conventional process of drug development. The conventional process of drug development requires identification and optimization of a lead compound, preclinical studies and clinical trials, hence time-consuming and very expensive(3). An average expenditure for developing a new drug totaling to 400 million U.S dollars with an average duration of 17-20 years(4). An alternative to the traditional/conventional process of drug development is repositioning of known drugs for a new therapeutic use. This is cost-effective and time-efficient; therefore, many pharmaceutical companies are currently moving from de-novo drug discovery to repositioning of known drugs for a new therapeutic use. The estimated time required for repositioning of a known drug for a new clinical indication is 3-12 years (5). There are several examples of such successful repositioning of drugs. For example, Thalidomide, a drug released into the market in 1957 for treatment of morning sickness but later withdrawn in 1961 due to its teratogenic effect has been repositioned for use in erythema nodosum leprosum and multiple myeloma (5). Another well-known example is sildenefil, a phosphodiesterase inhibitor, initially developed for managing angina, but later repositioned for erectile dysfunction (5).

Others include the antihypertensive agent minoxidil, currently approved for male baldness (5), the anti-cancer agent raloxifene was later repositioned for use in osteoporosis (5), the antiretroviral drug plerixafor subsequently repositioned for multiple myeloma and tretinoin, an anti-acne drug that was later developed for acute promyelocytic leukaemia(APL) (5). Nsanzabana and Rosenthal (6)demostrated synergism of HIV Aspartate protease inhibitor, lopinavir with lumefantrine against P. *falciparum invitro.*

Computational drug repositioning using molecular docking method can be employed to develop a virtual screening platform to predict binding affinities between a library of known therapeutic agents and the protein targets(5) Molecular docking remains the principal computational technique widely adopted in drug discovery (3). It is a structure based virtual screening that predict the binding orientation and binding affinity of a small molecule (potential drug) and a protein target (3). This technique was pioneered in early 1960(s) and remains the generally acceptable method in drug discovery. The rapid rise in the number of the known three dimensional structures of *Plasmodium falciparum*

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protein targets through X-ray crystallography has made structure based virtual screening more prominent in drug discovery (3). The advantage of molecular docking virtualscreening over the traditional experimental method is that it saves time and resources(3) In a study by, Computational drug repositioning has been attempted by many researchers. For instance, Li, An (7) employed the approachto identify nilotinib as a potent inhibitor of MAPK14 drug target (a protein responsible for inflammation) and Penna-Coutinho, Cortopassi (8) used the method to select three (3) drugs with best affinity for *Plasmodium falciparumlactate dehydrogenase* (PfLDH) and the antimalarial activity of the selected drugs were then confirmed using *Plasmodium berghei* mice model.

Is it possible to use computational method to discover known drugs (not used for malaria) with activity against multiple*Plasmodium* targets? This question is examined in the current study.

Abbreviations: PDB, Protein data bank; NCBI, National center for biotechnology information; MVD, Molegro virtual docker; MST, Mean survival time.

II. MATERIALS AND METHODS

1) A. Development of local data base of molecular targets in *Plasmodium* from PDB (Protein data bank)

The three dimensional structure of ten (10) important *Plasmodium* targets (in PDB text format) necessary for its survival and multiplication were selected and downloaded from the protein data bank(PDB) website (www.rcsb.org) and a local data base was created for the plasmodium targets in a personal computer.

2) B. Criteria for target selection

The *Plasmodium* protein targets were selected based on validated selection criteria and scoring. Targets with score of at least 80 out of 115 were selected and considered critical in the survival and multiplication of the parasite. The criteria include: involvement in a critical pathway necessary for the survival and replication of plasmodium, confirmed or putativetargets of known antimalarial,absenceof significant cross talk from National center for biotechnology information(NCBI) blast search, drugability of the target (easily accessible binding site) and site or location of the protein target within parasite (membrane/cytoplasm).

3) C. Development of local data base of the potential ligands from DrugBank

One hundred (100) commercially available drugs, used for indications other than malaria, were selected.The three dimensional structures of the drugs were downloaded from DrugBank website (www.drugbank.ca) and saved in SDF format. A local data base for the downloaded drugs was created in a personal computer.

4) D. Criteria for drug selection

Drugs with excellent safety profile (LD50>2000mg/kg), easily available (out of patent), inexpensive, with no reported pharmacokinetic interaction with any known antimalarial were selected for the work.

5) E. Insilico docking and selection ofbestten (10) drugs

Docking simulation of the drugs against the *Plasmodium* targets was ran using molegro virtual docker version 5.5 (CLC Bio in Denmark).It start with identification of binding sites of the protein targets. Molegro virtual docker (MVD) was used topredict various orientations or conformations of the drugs against the protein targets. The conformations with the least binding energies were selected and saved. The average binding energies was calculated for each ligand after ten simulations with MVD. A protein fixed and ligand flexible docking methodcalled Lamarkian genetic algorithm was employed. The Hydrogen bond score, Number of Hydrogenbond and interacting residues of the protein with the ligands were also analysed using the software. Ten(10) best drugs with the least binding energies against the *Plasmodium* targets were selected for confirmation of activity in the wet laboratory using *Plasmodium berghei* mice model (*in vivo*).

6) F. Experimental Animals

Six to eight weeks-old male albino mice (20±8g) obtained from the animal house of Institute for Advanced Medical Research and Training (IMRAT) university college hospital Ibadan, Nigeria were used for this study. The animals were housed in cages with free access to water and food pellets and allowed to adapt for a week before commencement of the study. The animals were maintained at a temperature 22 $\pm 1^{o}$C,relative humidity of 14 ± 1% and under 12 hour light and 12 hour dark cycle. The experiment was performed in accordance with the guidelines of university committee on the use and care of animals.

7) G. *Plasmodium berghei* parasite

The *NK65* chloroquine sensitivestrain of *Plasmodium berghei*was purchased from National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The parasite was conserved in the Department of pharmacology laboratory, Usmanu Danfodiyo University Sokoto via passage of blood from infected into healthy mice.

8) H. Drugs and Chemicals

The ten (10) selected drugs were purchased from the Pharmacy of Usmanu Danfodiyo University teaching Hospital (UDUTH) Sokoto, Nigeria. The drugs were dissolved in distilled water and 10ml/kg of the dissolved drug was given to each mouse, at mice equivalent dose.

9) I. Parasite Induction

Blood containing infected red cells (from infected donor mice) were diluted with normal saline so that 0.2mlswill contain 1×10^{6} parasitized red cells. The mice were inoculated with 0.2 mls of the infected blood intraperitoneally, using hypodermic needle fitted to 1-ml syringe.

10) J. Antiplasmodial studies

Design of the antimalarial study

1. The study is divided into three experiment;
   (a) Experiment 1 is the suppressive test
   (b) Experiment 2 is the curative test and estimation of mean survival time

(c) Experiment 3 is the prophylactic test

2. Each of the experiment;

a) Seventy two-infected mice were randomly divided into 12 groups of 6 mice each.
b) Two control groups (positive and negative) were dosed with 10mls/kg of chloroquine phosphate (5mg/Kg) and distilled water respectively.
c) The remaining ten groups were administered with 10mls/kg of the respective drugs at mice equivalent dosages

3. The mice equivalent dosage was calculated by multiplying the minimum recommended human dose by 12 (9).
4. The mouse in each of the group was orally treated daily for 4 consecutive days. For experiment 1 and 3, treatment begins at day 1 while for experiment 2, the treatment begins at day 4.
5. The mouse in each group wasinoculated intraperitoneally with 0.2mls of blood containing 10^6*Plasmodium berghei* infected red cells. For experiment 1 and 2, the inoculation was at day 1 while for experiment 3, inoculation was at day 5.

11) K. Evaluation of schizontocidal activity of selected drugs *on*early infection (4-day Suppressive Test)

A 4-day Peter suppressive test against chloroquine sensitive *Plasmodium berghei* mice model was used(10). On day 5 of the experiment (a day-post treatment), tail blood was collected from each mouse and thin films of the samples were stained with leishman. Buffered water ("PH" 6.8) was added to the film, kept for 8-10 minutes, cleaned with cotton wool and air dried, before it was viewed at ×100 magnification of the microscope.Theaverage percentage parasitaemia and percentage of parasite suppression were calculated in each of the group as shown below (8):

Percentage Parasitaemia= Number of infected red cells X100
Total no. of red cells examined

% suppression =PC-PTG/PC

Where PC is the parasitaemia in the untreated group,
PTG is the parasitaemia in the test group.

Drugs that reduce parasitaemia by 29-40% were consideredpartially active antimalarials, while agents that produce greater than 40% reduction in parasitaemia were considered active.

L. Evaluation of schizontocidal activity of selecteddrugson established infection (Curative or rane test)

The method of Ryley and Peters (10)was employed to evaluate the curative potential of the tested drugs.On day 8 of the experiment (a day after completion of treatment), the average percentage parasitaemia and percentage of parasite suppression were calculated for each of the group as shown above.

12) M. Determination of mean survival time

The duration of 28 days survival was recorded for each mouse. Mean survival time (MST) was calculated using the following formula (10)

MST= Sum of survival time of all mice in a group (days)
Total no. of mice in that group

***13)* N. Evaluation of prophylactic activity of selected drugs(Repository test)**

The evaluation of the prophylactic potential of the ten selected drugs was conducted according to the method ofRyley and Peters (10). Thin film of blood smears were made from each mouse seventy-two hours after inoculation (day 8). The percentages of parasitaemia and suppression of parasitaemia were recorded as earlier stated.

14) O. Statistical analysis/ Data presentation

Data was presented in tables and charts formats as appropriate and expressed as mean ± standard error of mean. Statistical analysis of the data was performed using one-way analysis of variance and posthoc test (Bonferroni) with P-value ≤ 0.05 considered statistically significant.

III. RESULTS

15) A. Selected drugs andprotein targets

Table 1 shows the list of ten (10) selected protein targets and one hundred (100) selected drugs.

Table 1 List and scores of the ten selected *Plasmodium* protein targets and one hundred selected drugs
(A)List and scores of the ten (10) selected Plasmodium protein targets

S/N	Plasmodium protein targets	Critical in the pathway(50)	Putative antimalarial drug target(30)	No significant crosstalk(15)	Drugability of the target(10)	Location of target in plasmodium(10)	Total Score(115)
1	*Plasmodium falciparum* merozoite surface protein (MSP)	50	0	15	10	Surface(10)	85
2	*Plasmodium falciparum* lactate dehydrogenase (PFLDH)	50	0	15	10	Nucleus(5)	80
3	*Plasmodium falciparum* dihydroorotate dehydrogenase (PFDODH)	50	0	15	10	Nucleus(5)	80
4	Falcipain 2	50	0	15	10	Cytoplasm(10)	85
5	Plasmepsin	50	0	15	10	Cytoplasm(10)	85
6	Sporozoite protein	50	0	15	10	Surface(10)	85
7	*Plasmodium falciparum* erythrocyte membrane protein 1 (PFEMP1)	50	0	15	10	Surface(10)	85
8	Plasmodium falciparum hypoxanthine Guanine phosphoribosyl transferase (PFHGPT)	50	0	15	10	Nucleus(5)	80
9	*Plasmodium falciparum* Thymidilate synthase-Dihydrofolate reductase (PFTS-DFR)	50	30	15	10	Nucleus(5)	105
10	*Plasmodium falciparum* erythrocyte binding antigen (PFEBA)	50	0	15	10	Surface(10)	85

(B) **List of one hundred selected drugs**

S/N	Name of Drug	S/N	Name of Drug	S/N	Name of Drug	S/N	Name of Drug
1.	Ascorbic acid	26.	Penicillin	51.	Sulindac	76.	Terbutaline
2.	Thiamine	27.	Cyproheptidine	52.	Nafcillin	77.	Granisetron
3.	Pyridoxine	28.	Allupurinol	53.	Chloroquine	78.	Ondansetron
4.	Baclofen	29.	Ceftazidime	54.	Mebendazole	79.	Tinidazole
5.	Tramadol	30.	Trimethoprim	55.	Sumatriptan	80.	Amantadine
6.	Fluconazole	31.	Lansoprazole	56.	Cefixime	81.	Metronidazole
7.	Erythromycin	32.	Loratidine	57.	Nitrofuratoin	82.	Buprenorphine
8.	Azithromycin	33.	Nabumetone	58.	Oxacillin	83.	Misoprostol
9.	Pantoprazole	34.	Ketorolac	59.	Nedocromil	84.	Meclofenamic acid
10.	Doxycycline	35.	Quinine	60.	Lisinopril	85.	Aspirin
11.	Clotrimazole	36.	Tenoxicam	61.	Thiabendazole	86.	Hydrocodone
12.	Flucloxacillin	37.	Celocoxib	62.	Esomeprazole	87.	Salbutamol
13.	Acetaminophen	38.	Dicloxacillin	63.	Meclizine	88.	Ketoprofen
14.	Piperacillin	39.	Cefotaxime	64.	Scopolamine	89.	Sulfamethoxazole
15.	Indomethcin	40.	Cimetidine	65.	Naprozen	90.	Ketoconazole
16.	Omeprazole	41.	Albendazole	66.	Rivabarin	91.	Ibuprofen
17.	Parazinamide	42.	Piroxicam	67.	Meloxicam	92.	Penicillin G
18.	Doxylamine	43.	Voriconazole	68.	Fosfomycin	93.	Praziquantel
19.	Amlodipine	44.	Enalapril	69.	Penicilamine	94.	Amoxycillin
20.	Ampicillin	45.	Diclofenac	70.	Ranitidine	95.	Promethazine
21.	Diphenhydramine	46.	Nifedipine	71.	Ceftriazone	96.	Colchicine
22.	Atorvastin	47.	Rabenprazole	72.	Metochlorpramide	97.	Lopinavir
23.	Fluvastin	48.	Cloxacillin	73.	Posaconazole	98.	Arthemeter
24.	Cefuroxime	49.	Itraconazole	74.	Cefazolin	99.	Lumefantrine
25.	Chlorpheniramine	50.	Chloroprocaine	75.	Mg hydroxide	100.	Clarithromycin

B. Docking studies

Table 2 and 3 shows the binding affinities, Hydrogen-bond energies and amino acids residues interactions of the ten drugs and chloroquine against the target proteins

Table 2Binding energies of the best ten drugs and chloroquine against the ten *Plasmodium* targets

S/N	Drugs	Merozoite surface protein	PFLDH	PFDODH	Falcipain 2	Plasmepsin	Sporozite protein	PFEMP 1	PFHGPT	PFDHFR-TS	PFEBA
1	Fluconazole	-169.528	-162.943	-203.62	-148.608	-178.629	-142.949	-113.542	-162.509	-125.415	-167.272
2	Indomethacin	-211.266	-195.039	-243.57	-197.014	-215.165	-179.2	-208.31	-204.057	-175.942	-202.314
3	Loratidine	-213.411	-195.732	-232.575	-213.734	-218.903	-182.206	-204.54	-221.9	-194.153	-210.121
4	Lisinopril	-206.055	-198.978	-244.199	-194.493	-182.522	-198.41	-215.164	-233.852	-183.46	-196.431
5	Meloxicam	-185.454	-179.845	-235.51	-182.036	-200.498	-163.7	-188.523	-205.166	-164.043	-140.035
6	Promethazine	-173.074	-165.283	-200.677	-155.645	-175.063	-143.112	-160.935	-187.904	-157.042	-164.507
7	Nifedipine	-207.389	-196.2	-233.892	-189.161	-211.003	-179.447	-195.773	-209.313	-186.923	-196.725
8	Clarithromycin	–361.698	-314.057	-371.46	-354.144	-281.432	-283.146	-315.257	-394.390	-295.246	-325.652
9	Piroxicam	-175.568	-155.591	-208.884	-164.714	-171.031	-139.219	-154.514	-165.864	-145.933	-181.514
10	Flucloxacillin	-224.739	-216.659	-256.099	-219.854	-233.398	-198.12	-223.128	-240.587	-199.896	-231.538
11	Chloroquine	-175.747	-170.637	-216.351	-166.785	-177.321	-155.506	-127.880	-200.861	-156.112	-184.923

Table 3 Moldoc score,Hbond, No. of Hbond and interacting residues of Merozoite surface protein against chloroquine and the best ten(10) drugs

S/N	Drugs	MolDoc Score ($Kcalmol^{-1}$)	Hbond score ($Kcalmol^{-1}$)	No. of H bond	Residues of binding site interacting with ligands
1	Fluconazole	-169.528	-7.398	15	ASP57,GLY55,CYS56,LYS29 & Ala58
2	Indomethacin	-211.266	-9.013	8	GLY54,GLY55,Asn53,CYS56,Ala58,Asp57,Lys29 &Ile90
3	Loratidine	-213.411	-0.149	4	Ala58,Asp57,Asn53,Cys56,Asn52&Gly55
4	Lisinopril	-206.055	-4.958	20	Lys29,Leu32,Gly54,Gly55,Ala58,Cyst50,Asp57,Asp59,Asn53,Gly54,thr48,Asn50&cys90
5	Meloxicam	-185.454	-8.437	4	Arg198,Asn115&Asp104
6	Promethazine	-173.074	-0.017	3	Gly54,Lys29,Gly55&Asn53
7	Nifedipine	-207.389	-12.209	16	Cys56,Gly56,Asn53,Gly54,Cys49,Ile90,Lys29&Asn52,Glu51
8	Clarithromycin	-361.698	-6.313	5	Leu22,His21,Phe19,Ala58&Asp59
9	Piroxicam	-175.568	-4.757	10	Asn53,Cys56,Gly54&lys29
10	Flucloxacillin	-224.739	-4.657	13	Gly55,lys29,Asp57,Cys56,Asn53&Asn52
11	Chloroquine	-175.747	-1.35	4	Asn53,Ala58,Asn52,Asn50,Cys49,Thr48&Glu51

16) C. Recommended humanand mice equivalent dosages of chloroquine and the ten selected drugs

Table 4 shows the minimum recommended human and the mice equivalent dosagesofthe ten selected drugs and chloroquine

Table 4 Recommended human dosages of chloroquine and ten selected drugs and their mice equivalent dosages

S/N	Drugs	Recommended dosage/70kg/day	Mice (20mg) equivalent dosage/kg/day
1	Fluconazole	1.4mg	16.8 mg
2	Indomethacin	0.7mg	8.4mg
3	Loratidine	0.1mg	1.2mg
4	Lisinopril	0.1mg	1.2mg
5	Meloxicam	0.1mg	1.2mg
6	Promethazine	0.4mg	4.8mg
7	Nifedipine	0.3mg	3.6 mg
8	Clarithromycin	7mg	84mg
9	Piroxicam	0.1mg	1.2mg
10	Cloxacillin	3.5mg	42mg
11	Chloroquine	5mg	60mg

17) D. Results of animal studies

18) Suppressive antiplasmodial activity

Compared to untreated control group, a statistically significant ($P<0.05$) suppression of the parasites (>40%) was observed in each of the treated groups (table 5). Chemosuppressive activity by either indomethacin (81.5%), fluconazole (80.7%), clarithromycin (77.6%), flucloxacillin (77.6%), loratidine (76.9) or nifedipine (73.8%) did not differ significantly ($P>0.05$) from that observed in pos itive control (chloroquine-treated mice (90.8%; table 5).

19) E. Curative antiplasmodial activity and the mean survival time

The suppression (>40%) of parasiteswas observed to be high in the groups treated with chloroquine, nifedipine, meloxicam, lisinopril,flucloxaci llin,clarithromycin and piroxicam, but only mild (29-40%) in the groups treated with promethazine, loratidine and fluconazole. There was no significant difference ($P>0.05$) in chemosuppression between the mice administered with nifedipine and the chloroquine-treated group. On the other hand, mice treated with indomethacin did not differ significantly ($P>0.05$) in chemosuppression from the vehicle-treated controlgroup (table 6). Compared with vehicle-treated animals,the groups of mice treated with chloroquine, meloxicam, nifedipine and lisinopril exhibited a highly significant increase ($P<0.01$), while promethazine produced a slightly significant increase ($P<0.05$) in mean survival time (table 6). On the other hand, compared to the negative control group,no statistically significant difference ($P>0.05$) was observed in the mean survival time of the groups of mice treated with either clarithromycin, fluconazole, flucloxacillin, loratidine or indomethacine (table 6 and graph 2)

20) F. Prophylactic Antiplasmodial activity

Pre-treatment with either nifedipine, flucloxacillin, clarithromycin or lisinopril resulted in astatistically significanthigh degree of suppression (>40%) of the parasites compared to the vehicle-treated group. However, pre-treatment with loratidine (39.5%), piroxicam (34.8%), promethazine (34.8) or meloxicam produced only mild suppression (29-40%) of the parasites, though statistically significant ($P<0.05$).The degree of chemosuppression did not differ significantly ($P>0.05$) between the positive control (chloroquine) and the groups treated with either nifedipine (48.8%), flucloxacillin (44.1%), clarithromycin(41.8%),lisinopril (41.8%), loratidine(39.5%), piroxicam (34.8%), promethazine (34.8%) or meloxicam (32.5%).Furthermore, no significant difference ($P>0.05$) in chemosuppression was observed in indomethacin-treated group (13.9%) compared to the vehicle-treated control group (negative control) (Table 7 and figure 1).

Table 5Suppressive effect of *selected drugs* and chloroquine against *P. berghei berghei* infection in mice.

S/N	Treatment Groups	Parasite count	% Suppression
1	Fluconazole	$2.08\pm0.15^{*}$	80.7
2	Indomethacin	$2.00\pm0.18^{*}$	81.5
3	Loratidine	$2.50\pm0.18^{*}$	76.9
4	Lisinopril	$3.08\pm0.24^{**}$	71.5
5	Meloxicam	$3.17\pm0.31^{***}$	70.8
6	Promethazine	$3.33\pm0.28^{**}$	69.5
7	Nifedipine	$2.83\pm0.31^{*}$	73.8
8	Clarithromycin	$2.42\pm0.15^{*}$	77.6
9	Piroxicam	$3.33\pm0.61^{**}$	69.2
10	Flucloxacillin	$2.41\pm0.24^{*}$	77.6
11	Chloroquine	$1.00\pm0.13^{*}$	90.8
12	Distilled water	10.83 ± 0.98	-

Table 6 Curative effect and MST of *selected drugs* and chloroquine against *P. berghei berghei* infection in mice.

S/N	Treatment Groups	Parasite count	% Cure	Mean Survival Time (MST)
1	Fluconazole	$2.17\pm0.21^{**}$	36.3	9.17 ± 0.87
2	Indomethacin	3.00 ± 0.41	11.8	6.33 ± 0.62
3	Loratidine	$2.08\pm0.15^{**}$	38.7	$8.17\pm0.54^{**}$
4	Lisinopril	$1.58\pm0.27^{**}$	53.4	$15.67\pm1.09^{**}$
5	Meloxicam	$1.58\pm0.20^{**}$	53.4	$16.50\pm1.31^{**}$
6	Promethazine	$2.08\pm0.24^{**}$	38.8	$12.33\pm0.62^{**}$
7	Nifedipine	$1.50\pm0.00^{*}$	55.8	$15.83\pm0.40^{**}$
8	Clarithromycin	$1.83\pm0.17^{**}$	46.1	9.67 ± 0.42
9	Piroxicam	$2.00\pm0.18^{**}$	41.2	9.67 ± 1.12
10	Flucloxacillin	$1.83\pm0.17^{**}$	46.1	$8.33\pm0.62^{*}$
11	Chloroquine	$0.50\pm0.00^{*}$	85.2	27.17 ± 0.54
12	Distilled water	3.42 ± 0.20	-	7.17 ± 0.31

Table 7Prophylactic effect of *selected drugs* and chloroquine against *P. bergheiberghei* infection in mice

S/N	Treatment Groups	Parasite count	% Prophylaxis
1	Fluconazole	2.58±0.27**	27.90
2	Indomethacin	3.08±0.42	13.90
3	Loratidine	2.17±0.11*	39.50
4	Lisinopril	2.08±0.08*	41.80
5	Meloxicam	2.42±0.33*	32.50
6	Promethazine	2.33±0.11*	34.80
7	Nifedipine	1.83±0.11*	48.80
8	Clarithromycin	2.08±0.08*	41.80
9	Piroxicam	2.25±0.11*	34.80
10	Flucloxacillin	2.00±0.13*	44.10
11	Chloroquine	1.58±0.08*	55.80
12	Distilled water	3.58±0.15	-

Values are expressed as mean ± SEM, n = 6
*Values of the group with superscript * are statistically significant (p<0.05) compared to negative control group.*
*Values with superscript ** are statistical significant (p<0.05) compared to negative and positive control groups.*

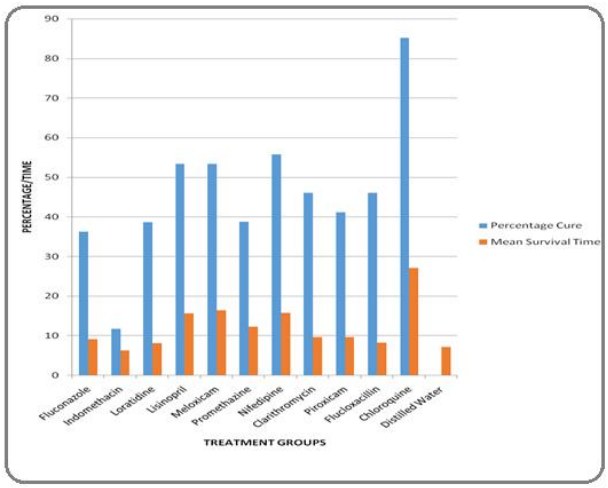

Figure 1 Graphical representation of chemosuppression and mean survival time in curative test

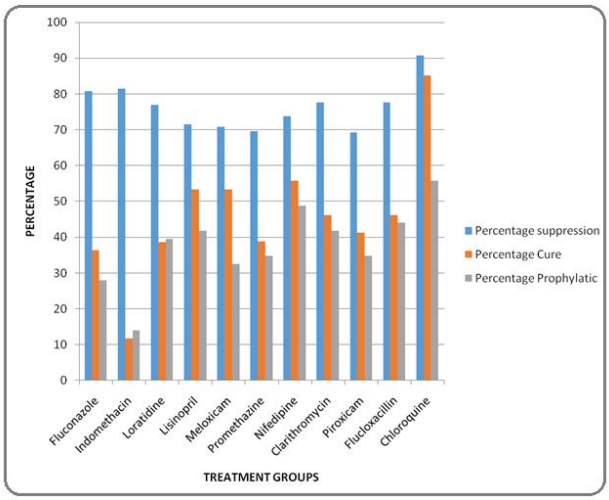

Figure 2 Graphical representation of chemosuppression

1V. DISCUSSION

The main objective of this work was to employ computational repositioning method to identify drugs which may have the potential to interfere with critical pathways in the life cycle of *plasmodium falciparum* through molecular docking simulation. The rodent model was employed to confirm the anti-malarial activity of the drugs, because it takes into account potential prodrug effect and immune system effect in combating infection (11). Clarithromycin, Nifedipine, Lisinopril and Flucloxacillin have the highest predicted overall binding affinities against the ten protein targets. The 4 drugs demonstrated higher values against *plasmodium falciparum merozoite surface protein, Plasmodium falciparum dihydroorotate dehydrogenase,and Plasmodium falciparum lactate dehyrogenase* and *Plasmodium falciparum hypoxanthine Guanine phosphoribosyl transferase*. These findings correspond to the fact that the drugs produce significant chemo suppressant, chemotherapeutic and chemo prophylactic effects against *Plasmodium berghei* mice model. The mechanisms of action of these drugs might be related to activity on multiple protein targets with more affinity for the 4 target proteins. Previous study by Ekland, Schneider (12) also identified Clarithromycin to produce antimalarial effect by inhibiting *plasmodium falciparum apicoplast*. Nifedipine a calcium channel inhibitor, enhances plasma level of quinine by inhibiting metabolism of quinine (13). This study was the first to identify lisinopril and flucloxacillin as potential antimalarial drugs.

This study was the first to demonstrate the antimalarial effect of meloxicam and piroxicam. The 2 drugs exhibited strongestbinding affinities against*Plasmodium falciparum dihydroorotate dehydrogenase* and a relatively lesser value for *merozoite surface protein* and*Plasmodium falciparum lactate dehydrogenase.* Meloxicam and piroxicam (cyclooxygenase, COX inhibitors) produce significant suppressive and curative effect only, without prophylactic effect. This might be due to their lesser affinity for *merozoite surface protein* and*Plasmodium falciparum lactate dehyrogenase*. Ketoprofen, a similar COX inhibitor, was demonstrated to have an inherent antimalarial effect in *invivo* and *in vitro* studies against chloroquine resistance strain of *Plasmodium yoeli yoeli (14)*

Indomethacin and loratidine have similar docking results with meloxicam and piroxicam but both produce only suppressive effect. This might be due to reasons related to pharmacokinetic or other invivo differences not evaluated by molecular docking. Previous study by (15) also demonstrated that Indomethacin potentiates the antimalarial effect of sub-curative doses of Chloroquine in *plasmodium berghei* and *P. vinkei* infected mice by forming conjugates with glutathione. Though Cyproheptidine, an antihistamine, showed an inherent antimalarial effect against Chloroquine resistance strains of *plasmodium yoeli yoeliin vivo* and *in vitro (14)*, this study was the first to identify loratidine as a potential antimalarial drug.

Fluconazole and Promethazine have the least predicted binding affinitiescompared to the first two groups. This is consistent with the drugs showing only suppressant effect in animal studies. Promethazine, a first generation-antihistamine, was demonstrated to enhance parasite clearance when combined with chloroquine in Aotus

monkeys infected with Chloroquine resistance *Plasmodium falciparum*(16). Fluconazole is an azole antifungal agents like Posaconazole and Itraconazole. This was the first study that demonstrated the antimalarial effect of fluconazole, though posaconazole and itraconazole were identified as inhibitors of *Plasmodium falciparum lactate dehyrogenase (8).*

It is interesting that all the ten drugs that produced significant suppressant effects against *plasmodium berghei* mice model also had the best binding affinities against *Plasmodium falciparum dihydroorotate dehydrogenase* postulating this target as responsible for their suppressive effects.

The findings that clarithromycin and flucloxacillin produce significant effects in the three tests for antimalarial activity while piroxicam showed only suppressive and curative activity is interesting, but that these did not translate to increase in mean survival time compared to the negative control; suggest caution and further evaluation before being recommended for use. This is a paradox because, especially, curative effect should result in survival. A possible explanation is that these drugs were toxic to mice at the dose administered, thus reducing the survival period even at a low parasite load.These paradoxes may need to be explored in further studies.While this may not be surprising for indomethacin, fluconazole and loratidine as they produce only mild curative effect.

V. CONCLUSION

The result of the study showed that all the tested drugs produced significant activity against *Plasmodium* parasite in suppressive test, with four of the 10 drugs being effective in all the 3 experimental models used in the current study and 6 drugs being effective in 2 of the 3 experiments. This study suggest that molecular docking simulation is an important strategy for repositioning of approved drugs towards new indications. This approach is more practical and less expensive than discovery of novel compounds that will require toxicity studies, especially for neglected tropical diseases such as malarial infection.

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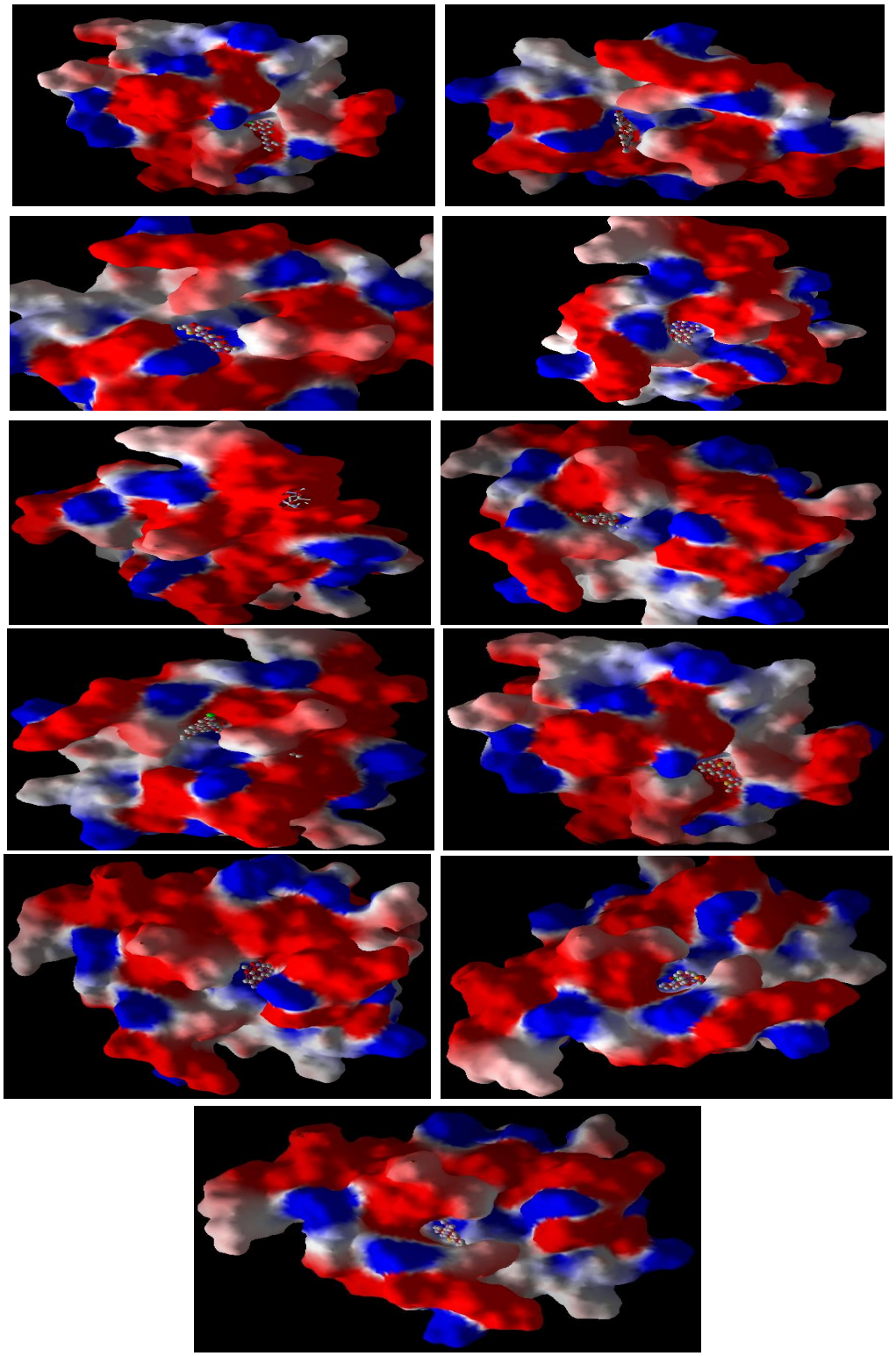
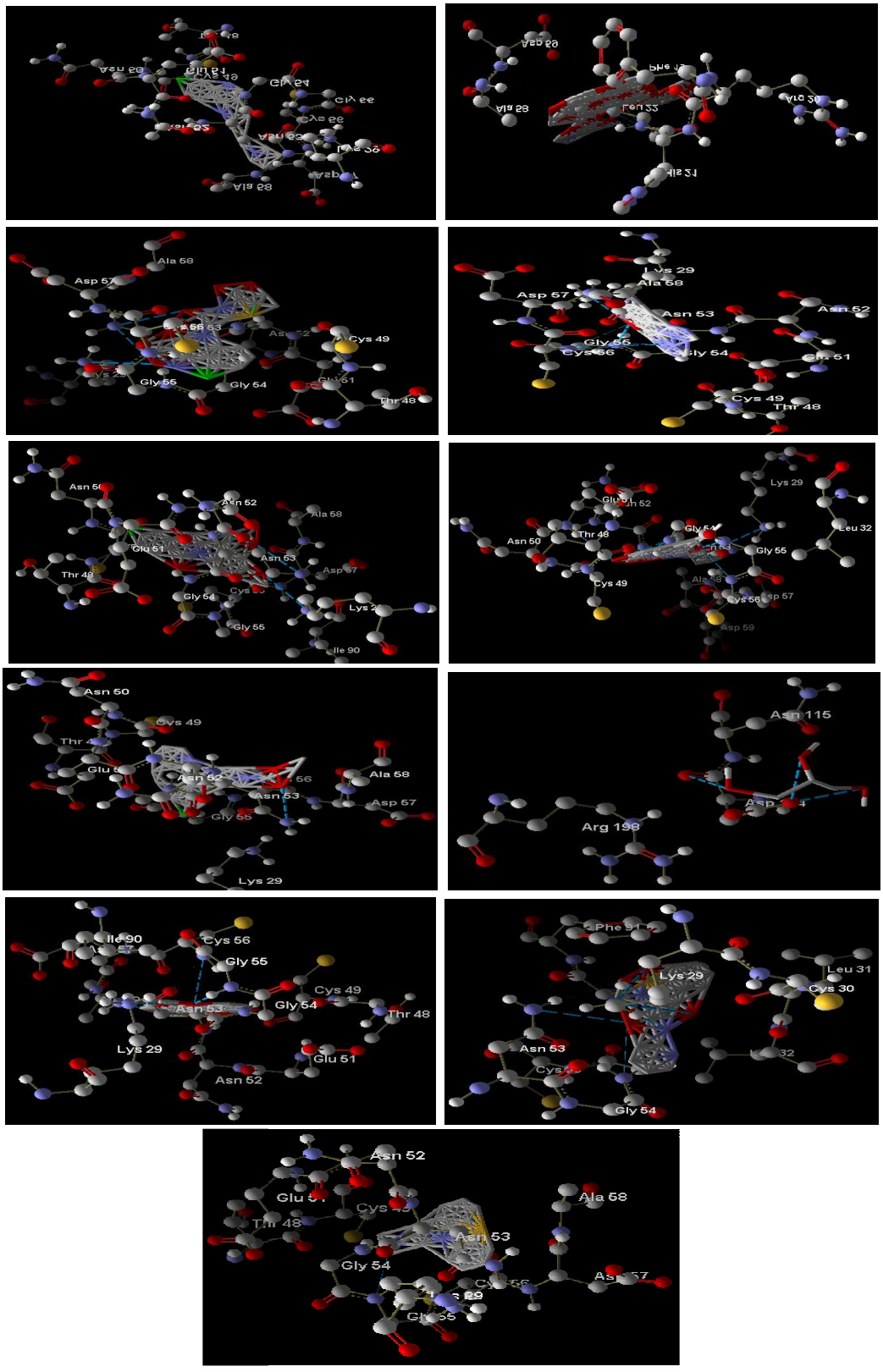
APPENDICES



Appendix A: Drugs in the active pocket of merozoite protein



Appendix B: Active site of merozoite surface protein interacting with the tested drugs