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RESEARCH ARTICLE

BIOINFORMATICS

IN SILICO ANALYSIS AND DEVELIPMENT OF ANTI- MALARIAL COMPOUNDS AGAINST DIHYDROOROTATE DEHYDROGENASE USING CHEM-BIOINFORMATIC TOOLS

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ABSTRACT

Computational studies have been developed to unravel the mechanism of action of the anti-malarial drugs and to give guidelines for the development of new derivatives with improved efficiency. In this study, dihydroorotate dehydrogenase (DHOD) a key enzyme in *de novo* pyrimidine biosynthesis and the major source of electrons for the mitochondrial electron transport chain of intraerythrocytic malaria parasites was selected as a target for potential inhibitors. Beginning with the natural inhibitors found in crystallographed molecules representing *.pdb file, a general similarity search were done in PDB, NCI and PUBMED databases resemble probable antimalarial compounds. This made it possible to dock these ligand libraries with DHOD by FlexX 3.2, 2007 software and comparing the inter-molecular interactions and scores which are given to them by the software. The highest absolute value of interactions energies were considered be the best and proper ligands. These ligands were used for second 95% similarity search in PUBCHEM for proposed antimalarial compound which have the same mechanism as A26. As an interesting point among 400 found molecules was presence of quinolines. This could propose a new mechanism for these important anti-malarial agents.

KEYWORDS

Dihydroorotate dehydrogenase, *Plasmodium falciparum,* Docking, Anti-malarial, infectious agents.

BACKGROUND

Malaria is a major infectious disease in the tropics, with more than 300 million clinical cases reported annually. A vaccine for malaria does not exist, making the use of drugs for disease prophylaxis and treatment seems to be the only option available[1]. The two most widely used anti-malarial drugs, chloroquine (CQ) and sulphadoxinepyrimethamine (SP, commonly available as Fansidar; Roche), are failing at an accelerating rate in most malaria endemic regions, with consequent increases in malaria-related morbidity and mortality[2]. To combat malaria, new drugs are desperately needed, but traditional mechanisms for drug development have provided few drugs to treat diseases of the developing world. Today, CQ resistance has spread to the vast majority of malaria-endemic areas, rendering this drug increasingly ineffective. Moreover, because the high likelihood of emerging resistance to the current first-line drugs, artemisinin-based combination therapies, there is currently great interest in finding the next generation of anti-malarial drugs[3]. While, malaria affects many countries with poor public health resources, attributes of an ideal treatment for malaria are different from those for diseases of industrialized countries. An ideal anti-malarial should be inexpensive to synthesize, have good oral bioavailability, have short treatment regimens, be well tolerated by the patient, and be stable at room temperature. One approach to the discovery of such anti-malarial agents involves the identification of new therapeutic targets that then form the basis for chemical screens to identify small molecules that modulate the target's activity *in vivo*. Although such an approach has been highly productive in general, it has not worked well for many infectious agents. In many cases, these target-based screens reveal small molecules with potent activity against an enzyme but that are still unable to clear an infection, either because the target is not really essential to the microbe's viability in the host or because the compound is unable to inhibit the target in the *in vivo* environment[4]. An alternative and more traditional approach is to perform cell-based screens directly against living organisms in which a small molecule is tested in an unbiased fashion against all targets required for viability simultaneously. The disadvantage is that once a compound with potent cellular activity is discovered, lead optimization is hindered without knowing which protein target the compound inhibits. Computational (virtual) screening of drug-like compounds simultaneously against the molecular structures of multiple protein targets, taking into account protein-inhibitor dynamics, might help to identify lead inhibitors more efficiently, particularly for complex drug-resistant diseases like malaria. Here we apply the potential benefits of this approach, using *Plasmodium falciparum* dihydroorotate dehydrogenase (DHOD) enzyme. The flavin enzyme dihydroortate dehydrogenase is a key enzyme in *de novo* pyrimidine biosynthesis and the major source of electrons for the mitochondrial electron transport chain of intraerythrocytic malaria parasites. Pyrimidine biosynthesis presents an attractive drug target in malaria parasites due to the absence of pyrimidine salvage[5]. A26 (Figure 1) is a noncompetitive inhibitor of this enzyme. Ligands similar to this compound obtained from different databases by similarity searching and docked to inhibitor site of A26. Ten compounds of top slice of series were selected by top scores of the

software. There was close relationship between our docked best scores and quinolins compounds.

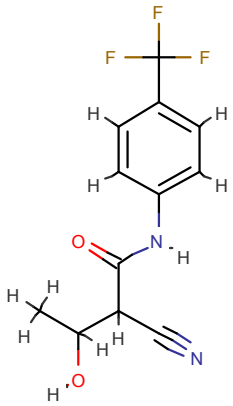

Figure 1
A26 structure

METHODS

In first step the proper protein PDB ID[6] were found within literature as 1TV5 (PfDHOD) Dihydroorotate Dehydrogenase. Inhibiting this enzyme as a key step in pyrimidine synthesis pathway will produce novel drug candidates for anti-malarial agents. This enzyme could have 2 classes of inhibitors, competitive compounds such as orotic acid analogs and noncompetitive ones like A26 which was crystallographed with DHOD. As the first one has high risks for metabolite competition among other pathways, it was decided to screen compounds resemble A26. Having found the A26 binding domain in protein, ligands were searched at PUBCHEM[11] for any resemblance. On the other hand, about 1000 compounds with more than 80% resemblance was catch from ENHANCED NCI[7] ligand database by PASS PREDICTION[12] order for their hypothetical anti-malarial activity. This had made a good database for analytical usage of docking software FlexX 3.2, 2006 (http://www.biosolveit.com) because it can flexibly dock a huge *.SDF database with "Fast Flexible *ab initio* Docking" Model. After docking by defaults, 10 compounds of top slice of series were chosen by top scores of the software. NCI[7], PUBMED[10] and PDB[6] molecules are mentioned in table 1 and 2 with their components of docking scores. Passing the first screening phase, the second similarity search with more tightened conditions had done for real world molecules resemble to the best docked ones. This time similarity search occurred in more than 95% for the same structural compounds in PUBCHEM[11] that could imitate the same role as best docked molecules. This resulted in 400 molecules which were proposed to have anti DHOD activity. Then the driven molecules were searched in literature database SCIRUS[9] and PUBMED[10] for having anti-malarial activity.

RESULTS

The first scans on PfDHODH showed that the best docking site for A26 compounds is an alpha-turn-alpha-beta pocket at the late N-terminal of the protein which is too near to prostetic site of FMN, as an oxidoreductase agent in the active site of enzyme. After data download of NCI[7], PUBMED[10] and PDB[6] websites, all the molecules were screened by docking process which reveals the best interactions with that pocket. Passing this

phase, 10 molecules remained that are shown in final figures with their docking best scores in table 1 and table 2. In phase two a new similarity search with more than 95% precision was carried out in PUBCHEM[11] structural search for whole world resemble compounds which have the same functional groups and total structures. By scanning more than 400 downloaded compounds within literature searches through their names, smiles, synonyms, structural activities and IDs in PUBMED[10], SCIRUS[9] and GOOGLE[8], so interesting information have been obtained which shows the close relationship between our docked best scores and quinolines. A vast range of compounds of anti-malarial quinolines were in the similarity searches of the second best score molecule (Figure 2).

Table 2
PUBMED & PDB top docked molecules and scores

Ligand	Rank	Score	Match	Lipo	Ambig	Clash
(1) 1550	1	-7.0615	-14.1	-0.8168	-3.1446	0
(6) 9883147	1	-3.0062	-13.438	-0.9123	-2.4559	0
(7) 10356498	1	-12.4429	-13.9529	-12.4869	-5.3771	8.374
(10) 16756177	1	-4.8509	-7.7809	-14.0064	-5.4548	9.9912
(11) 17753809	1	-7.0615	-14.1	-0.8168	-3.1446	0
(13) 19361933	1	-9.9448	-12.9999	-13.7498	-4.9769	7.9817
(14) 20601424	1	-5.3241	-13.7828	-0.4597	-2.0817	0
(16) 21594338	1	-6.0426	-14.1	-2.1111	-2.4855	0.254

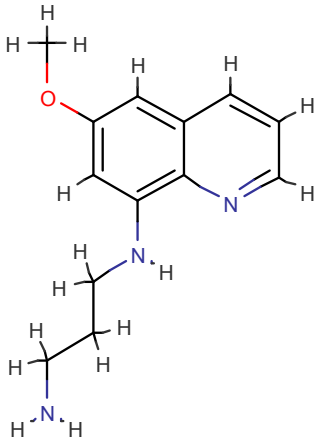

Figure 2
Second best docked enhanced NCI (CID: 114099)

Table 1
Enhanced NCI docked ligande and scores

	Ligand	Rank	Score	Match	Lipo	Ambig	Clash
1	(1) NSC2452 N~1~,N~1~-diethyl-N~6~-(6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine	1	-9.4218	-16.6104	-21.8194	-7.5862	17.1942
2	(2) NSC3589 N-(6-methoxy-8-quinolinyl)dicarbonimido/ic diamide/imido	1	-20.8872	-18.0315	-10.8262	-7.5428	8.7134
3	(3) NSC3613 N~3~-(4-chlorophenyl)-N~1~-isopropyl-1,3-butanediamine	1	-9.3654	-11.0365	-11.9664	-4.3299	5.5673
4	(4) NSC4377 (8-chloro-2-(4-chlorophenyl)-4-quinolinyl)(2-piperidinyl)methanol	1	-5.2783	-12.9121	-0.9679	-1.052	0.0536
5	(5) NSC4378 1-(7-chloro-2-(4-chlorophenyl)-4-quinolinyl)-2-(diethylamino)ethanol	1	-0.8693	-9.4363	-3	-2.919	0.686
6	(6) NSC4379 1-(8-chloro-2-(4-chlorophenyl)-4-quinolinyl)-2-(diethylamino)ethanol	1	-2.5098	-10.4827	-4.0723	-2.9061	1.1514
7	(7) NSC5364 cinchonan-9-ol	1	-10.1385	-9.0627	-17.1932	-5.1668	10.2842
8	(8) NSC5367 N-(2-chloropropyl)-N,N-dimethylamine	1	-4.9184	-6.4451	-7.1506	-2.574	3.0513
9	(9) NSC6176 cinchonan-9-ol	1	-10.1385	-9.0627	-17.1932	-5.1668	10.2842
10	(10) NSC7182 N~1~-(6-methoxy-8-quinolinyl)-1,3-propanediamine	1	-19.2404	-21.238	-12.2497	-5.6014	8.8487
11	(11) NSC7444 N~1~,N~1~-diethyl-N~3~-(6-methoxy-8-quinolinyl)-1,3-propanediamine	1	-14.6009	-16.9798	-17.1297	-6.6633	10.9718
12	(12) NSC7455 1-(4-chloro-1-naphthyl)-2-(dibutylamino)ethanol	1	5.7463	-9.529	-2.7865	-2.9248	1.5866
13	(13) NSC8358 N~1~,N~1~-diethyl-N~4~-(7-methyl-4-quinolinyl)-1,4-pentanediamine	1	2.5588	-8.8621	-1.6432	-2.8597	0.7238
14	(14) NSC8583 N~1~-isobutyl-N~1~-isopropyl-N~2~-(6-methoxy-8-quinolinyl)-1,2-propanediamine	1	-1.7714	-13.4655	-1.3721	-2.6029	0.469
15	(15) NSC10310 1-fluoro-4-(trichloromethyl)benzene	1	-4.0466	-5.0231	-8.3003	-2.0313	4.508
16	(16) NSC10644 9-phenanthryl(2-piperidinyl)methanol	1	-3.5744	-11.4791	-0.5244	-1.1709	0
17	(17) NSC10647 1-(5-chloro-1-naphthyl)-2-(dibutylamino)ethanol	1	5.2763	-9.5511	-2.5707	-3.1719	1.17

18	(18) NSC10649 N~4~-(4-(diethylamino)-1-methylbutyl)-N~6~,N~6~-dimethyl-4,6-quinolinediamine	1	1.5881	-4.4421	-8.6737	-1.5348	1.0386
19	(19) NSC10809 7-chloro-N-(4-(diethyl(methyl)-lambda~5~-azanyl)-1-methylbutyl)-4-quinolinamine	1	1.5599	-9.0372	-2.1088	-3.5604	1.0664
20	(20) NSC12260 8-((7-chloro-4-quinolinyl)amino)-6-quinolinol	1	-10.2246	-13.7828	-0.8453	-2.5411	0.1446
21	(21) NSC13001 N~4~-(6-chloro-2,3-dimethoxy-9-acridinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine	1	6.0096	-8.1033	-5.1796	-1.7099	1.6024
22	(22) NSC13027 8-((3-(diethylamino)propyl)amino)-2,6-quinolinediol	1	-13.6772	-18.0895	-15.3952	-6.5575	9.765
23	(23) NSC13046 1-(6,8-dichloro-2-(4-chlorophenyl)-4-quinolinyl)-2-(diethylamino)ethanol	1	-2.4489	-10.4827	-4.0576	-2.8896	1.181
24	(24) NSC13054 2-(dibutylamino)-1-(6,8-dichloro-2-(2-pyridinyl)-4-quinolinyl)ethanol	1	7.6008	-7.9193	-4.2716	-0.8512	1.2429
25	(25) NSC13254 N~4~-(7-chloro-4-quinolinyl)-N~1~-ethyl-1,4-pentanediamine	1	-12.0156	-16.0458	-14.8818	-6.1659	11.2779
26	(26) NSC13258 8-((3-(diethylamino)propyl)amino)-6-quinolinol	1	-15.4864	-18.1364	-16.5387	-5.9026	9.8914
27	(27) NSC13266 N~1~-ethyl-N~4~-(6-methoxy-8-quinolinyl)-1,4-pentanediamine oxalate	1	-12.2841	-15.6849	-16.8716	-7.2576	12.33
28	(28) NSC13267 N~1~-(5,6-dimethoxy-8-quinolinyl)-1,6-hexanediamine	1	-3.7435	-11.0969	-14.4227	-6.8974	12.0735
29	(29) NSC13269 N~1~,N~1~-diethyl-N~4~-[1,5]naphthyridin-4-yl-1,4-pentanediamine	1	-13.1695	-16.624	-15.1932	-5.989	9.4367
30	(30) NSC13276 N~4~-(5-chloro-4-quinolinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine	1	-6.5111	-12.8921	-16.7219	-6.5458	14.4487
31	(31) NSC13278 N~4~-(6-methoxy-8-quinolinyl)-N~1~-propyl-1,4-pentanediamine oxalate	1	-10.907	-18.1067	-14.9944	-6.9562	12.5503
32	(32) NSC13279 N~1~-isopropyl-N~4~-(6-methoxy-8-quinolinyl)-1,4-pentanediamine oxalate	1	-3.5116	-13.7374	-2.3919	-3.0596	0.4772
33	(33) NSC13281 N~1~-(2,6-dimethoxy-8-quinolinyl)-N~3~,N~3~-diethyl-1,3-propanediamine	1	-17.3014	-18.3082	-20.266	-7.8638	12.5367
34	(34) NSC13288 N~4~-(6,7-dimethyl-4-quinolinyl)-	1	1.4017	-9.0011	-1.8041	-3.4379	0.4448

	N~1~,N~1~-diethyl-1,4-pentanediamine						
35	(35) NSC13314 N~4~-(5,6-dimethoxy-8-quinolinyl)-N~1~-isopropyl-1,4-pentanediamine oxalate	1	-3.0216	-13.7785	-2.7881	-3.4984	0.4434
36	(36) NSC13382 N~4~-(6,8-dichloro-4-quinolinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine	1	-5.9205	-10.9283	-16.4685	-5.9802	12.2565
37	(37) NSC13447 N~5~-(7-chloro-4-quinolinyl)-N~1~,N~1~-diethyl-1,5-hexanediamine	1	2.417	-10.2762	-2.3374	-3.4819	1.9124
38	(38) NSC13477 N~4~-(7-chloro-3-phenyl-4-quinolinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine	1	3.2982	-5.9212	-6.6977	-1.0584	0.3755
39	(39) NSC13478 N~1~,N~1~-diethyl-N~4~-(6-phenoxy-4-quinolinyl)-1,4-pentanediamine	1	3.038	-9.7461	-2.972	-2.7965	0.5526
40	(40) NSC13594 N~1~-isopropyl-N~2~-(6-methoxy-8-quinolinyl)-1,2-propanediamine	1	-10.0145	-13.6155	-14.5082	-6.825	12.5343
41	(41) NSC13595 N~1~,N~1~-diethyl-N~2~-(4-(methylthio)-8-quinolinyl)-1,2-ethanediamine	1	-9.8134	-13.3477	-14.9559	-6.5367	11.2268
42	(43) NSC13608 N~1~,N~1~-diethyl-N~4~-(7-fluoro-4-quinolinyl)-1,4-pentanediamine	1	-9.3345	-11.8304	-19.4786	-6.3909	13.1655
43	(44) NSC13619 N~1~-(3-(diethylamino)propyl)-N~3~-(6-methoxy-8-quinolinyl)-1,3-propanediamine	1	3.3583	-13.0465	-2.0202	-3.1121	0.7371
44	(45) NSC13620 (6,8-dichloro-2-(4-chlorophenyl)-4-quinolinyl)(2-piperidinyl)methanol	1	-3.5505	-11.4573	-0.5169	-1.1763	0
45	(46) NSC13713 N~1~-(5,6-dimethoxy-8-quinolinyl)-N~3~,N~3~-diethyl-1,3-propanediamine	1	-15.5059	-18.898	-18.553	-6.6064	11.9516
46	(47) NSC13716 N~1~,N~1~-diethyl-N~4~-(7-(trifluoromethyl)-4-quinolinyl)-1,4-pentanediamine	1	4.0172	-9.0828	-1.7416	-3.079	1.3206
47	(48) NSC13719 N~2~-(5-chloro-6-methoxy-8-quinolinyl)-N~1~,N~1~-diisobutyl-1,2-propanediamine	1	-1.5133	-13.5674	-2.5133	-3.3406	1.308
48	(49) NSC13727 N~4~,N~7~-bis(4-(diethylamino)-1-methylbutyl)[1,10]phenanthroline-4,7-diamine	1	10.7046	-8.4095	-2.8177	-3.6505	0.5822
49	(50) NSC13822 N~1~-(5,6-dimethoxy-8-quinolinyl)-1,6-	1	-3.7435	-11.0969	-14.4227	-6.8974	12.0735

	hexanediamine						
50	(52) NSC14199 N~4~-(7-chloro-4-quinolinyl)-N~1~-methyl-1,4-pentanediamine	1	-15.0234	-17.3644	-12.8292	-4.6619	7.4321
51	(53) NSC14663 (trichloromethyl)benzene	1	-5.2981	-5.5099	-7.4465	-1.5277	2.3859
52	(54) NSC14753 N~1~-(7-chloro-4-quinolinyl)-N~3~-(6-methoxy-8-quinolinyl)-1,3-propanediamine	1	-13.5777	-10.4657	-17.7404	-8.5893	10.8177
53	(55) NSC15016 N~1~,N~1~-diethyl-N~4~-(6-methoxy-8-quinolinyl)-1,4-pentanediamine	1	-6.4925	-11.2732	-16.3763	-6.6127	11.1697
54	(56) NSC15023 (6-chloro-1-naphthyl)(2-piperidinyl)methanol	1	-10.2549	-7.7891	-14.9427	-4.7723	7.6492
55	(57) NSC15030 1-(8-chloro-2-phenyl-4-quinolinyl)-2-(diethylamino)ethanol	1	-0.8314	-9.4036	-2.9463	-2.918	0.6365
56	(58) NSC15994 N~1~-(5-chloro-6-methoxy-8-quinolinyl)-N~3~,N~3~-diethyl-1,3-propanediamine	1	-11.5744	-15.8846	-16.2974	-6.4385	11.8461
57	(59) NSC15995 N~4~-(5,7-dichloro-4-quinolinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine	1	3.4028	-9.0828	-1.9882	-3.1616	2.4353
58	(60) NSC16001 (7-chloro-2-phenyl-4-quinolinyl)(2-piperidinyl)methanol	1	-3.6086	-11.5123	-0.5335	-1.1628	0

DISCUSSION

As it is evident in the graphs of the docking pocket, it has a completely dipolar hydropathical structure and it means that the docked molecules should have a kind of dipole nature (Figure 3, Figure 4). A26 with its phenol ring and H donor/acceptor tail with a high flexibility range has such structure. The novel searched structure NCI3589 with docking score of -20.8872 (FlexX scores) compare to A26 (NCI1550) with score of -7.0615 (FlexX scores) has much higher affinity to hydrophobic half for its isoquinoline part and other half for flexible long H-donor tail which has close interactions with pocket residues as it is shown in figures (Figures 5-6,7). A26 has an interaction with an inter-protein water molecule and hydrogen bonds with amino/Kenton backbone groups respectively through its F atoms and H-acceptor/donor parts of the tail. Other docked compounds have the same situation too. But the big gap between the 3589 and 1550 is because tail length and the isoquinoline functional group of 3589 that matches so much better than other with hydrophilic first and hydrophobic end of the pocket respectively. Among the final similarity searches there were many used anti-malarial agents like D/L Primaquines, Chinocides, Pentaquines, Plasmocides and Plasmoquines that structurally and conformationally resemble the second best docked molecule (Figure 2), this means that these molecules have the same flexibility, functional groups in tail and bipolarity in whole molecule.

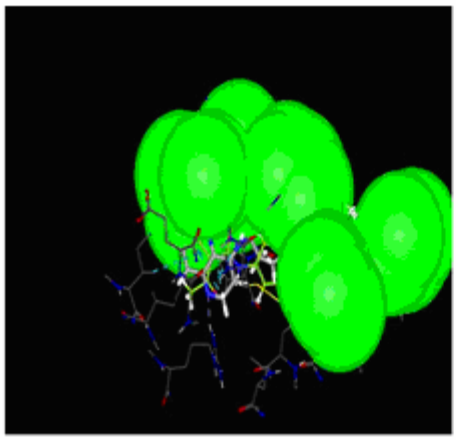

Figure 3
Aromatic part of the pocket of docking.

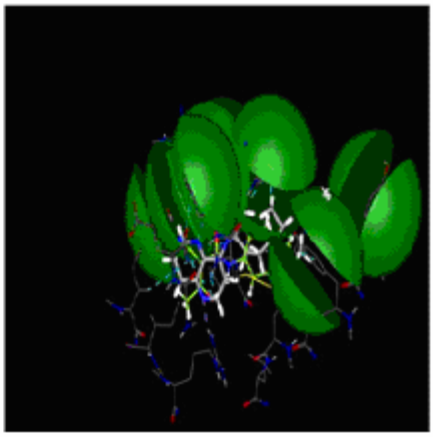

Figure 4
Phenyl centers of the docking pocket

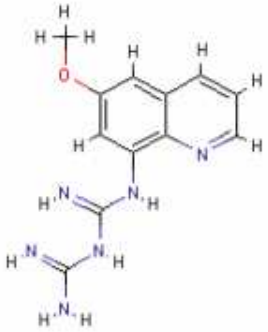

Figure 5
Best docked ENHANCED NCI (CID: 276800)

Figure 6
Best docked PUBMED and PDB (CID: 10356498)

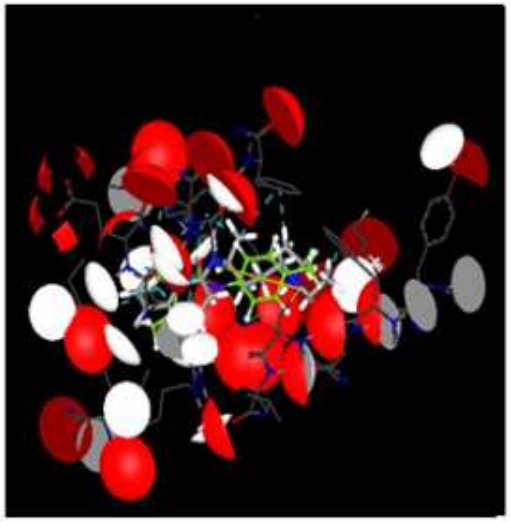

Figure 7: H-donor (white) and acceptors (red) in opposite site of the docking pocket

CONCLUSION

There are so many hypothesizes which predict the mechanisms of quinoline family inhibitors such as, respiratory pathway inhibition with electron attraction, respiratory pathway inhibition with radical molecule production, DHOD inhibition through interaction with FMN channel during orotic acid oxidation. As the other descriptions are mentioned before these new ligands which have high structural similarity to the quinolines, could have the same anti-malarial activity that has not been checked *In-vitro*. On the other hand resemblance of these structures to best docked molecules reveals a close relationship in their functional activities of parasite killing process. It would be possible that we confirm the new mechanism for quinolines which let design of new novel anti malarial agents.

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