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Research Article

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Molecular docking studies of lichen metabolites as malarial protein inhibitors: Plasmepsin II protease and dihydro folate reductase

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ABSTRACT

Plasmepsin II (PL II), an aspartyl protease inhibitor of P. falciparum, plays a key role in the haemoglobin degradation inside the food vacuole. Besides, dihydrofolate reductase (DHFR), a small enzyme that plays a critical role in the building of DNA and other processes, is found to be involved in the reproduction of the parasites, P. falciparum and P. vivax. Recently more focus has been arrived in the treatment and cure of malaria through inhibition of these key enzymes, PL II protease and DHFR. The main aim of the present study is to find out the binding mode and hydrogen bond interactions of the lichen metabolites; depsides viz. atranorin (ATR) & lecanoric acid (LA), depsidone, salazinic acid (SA) and dibenzofuran, usnic acid (USA) derivatives with PL II protease and DHFR proteins through molecular docking simulations using glide module v5.5 (Schrodinger suite 2009) by flexible docking method. The docking results indicate that the dibenzofuran USA derivatives are showing impressive hydrogen bond and hydrophobic interactions with PL II protease, whereas the depsidone, SA shows satisfactory interactions and the depsides namely, ATR and LA with least interactions. With respect to the DHFR, depsides; ATR and LA are showing effective H-bond interactions with the DHFR protein active sites of the two species in different docking grids. Further, SA and USA derivatives are showing flat interactions with the active residues of the DHFR protein. From the above results, it is clearly evident that further studies can be attempted in designing similar such structure-based novel molecules with PL II and DHFR inhibitory activity.

Keywords: Lichen metabolites, Plasmepsin II, DHFR, molecular docking, atranorin, lecanoric acid, salazinicacid, dibenzofuran derivatives

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INTRODUCTION

The most notable area of multi collaborative effort after the genome project is now in the field of anti-malarial drugs. It is estimated that up to 40% of the world's population lives in regions where malaria is endemic. Of the four different types of species of *Plasmodium*, *Plasmodium falciparum* is the most dangerous form of all, responsible for more than 95% malaria related deaths [1]. The increasing resistance of *Plasmodium falciparum* to existing therapies such as chloroquine, mefloquine, fansidar etc [2, 3] has urged the need to develop newer leads of chemotherapeutic significance.

Recently, increased attention has been paid on the plasmepsin II aspartyl protease inhibitors of *Plasmodium falciparum* [4] as potential anti-malarial leads in drug design. This key enzyme is one among the four catalytically active one in aspartyl protease family involved in the haemoglobin degradation inside the food vacuole. Similarly, dihydrofolate reductase is a small enzyme that plays a supporting role, but an essential role, in the building of DNA

and other processes. This enzyme is involved in the reproduction of the parasites, *Plasmodium falciparum* and *Plasmodium vivax*. Hence, in our research study, we tried to find the suitable analogues with high binding affinity, which could be a possible lead, with respect to the key enzymes plasmepsin II and dihydrofolate reductase. Most of the works reported so far are on the peptidyl inhibitors with modest activity in cell culture (IC_{50}> 20 μM) [5]. In combating the dreadful disease of this type, particular attention was paid to natural compounds that are produced in high quantities and easily accessible or to synthetic modification of moieties that are of economic significance. Particular attention was paid to a few acylphloroglucinols reported to possess putative anti-plasmodial activity [6, 7]. To permit meaningful investigations at molecular level, isolated compounds were focused that are of significance in developing libraries targeting the human cysteine proteases of malaria parasite, PL II.

Herein, we report the application of combinatorial chemistry and structure-based design methods to rapidly identify potent and selective low molecular weight non-peptidyl inhibitors of malarial parasites, *Plasmodium falciprum* and *Plasmodium vivax*. In this regard, the largest lichen metabolite, (+)-usnic acid, a dibenzo-furandione acylphloroglucinol reported to exhibit weak anti-malarial activity [8] was used as scaffold to design newer chemical entities with pronounced anti-malarial activity. The hypothesis for design was based on the modification of the triketone moiety that has been apparently responsible for its activity/toxicity [9], the strong intramolecular hydrogen bonding contributing its lipophilic nature thereby making it as membrane disrupter [10, 11] and the keto-enol tautomer strongly suggest the molecule appropriate for chemical modification in the triketone moiety without disturbing the pharmacophore architecture of the parent nucleus [12]. In addition, the other secondary lichen metabolites reported to exhibit protease inhibitor effect namely the depsides (atranorin and lecanoric acid) and depsidones (salazinic acid) were also taken up for the purpose of the present study [13]. Thus, Usnic acid, its derivatives, the depsides and depsidones were subjected to molecular docking simulations using glide module v5.5 (Schrodinger suite 2009) against distinctive malarial targets, plasmepsin II and dihydro folate reductase for the development of newer leads in anti-malarial drug design.

EXPERIMENTAL SECTION

Dataset

The dataset molecules, atranorin, lecanoric acid, salazinic acid and dibenzofuran (usnic acid) derivatives, and reference ligands (Artesunate & Mefloquine shown in Fig. 1) were sketched using MDL ISIS Draw and then applied into a LigPrep module (incorporated in Maestro 9.0) for further alterations.

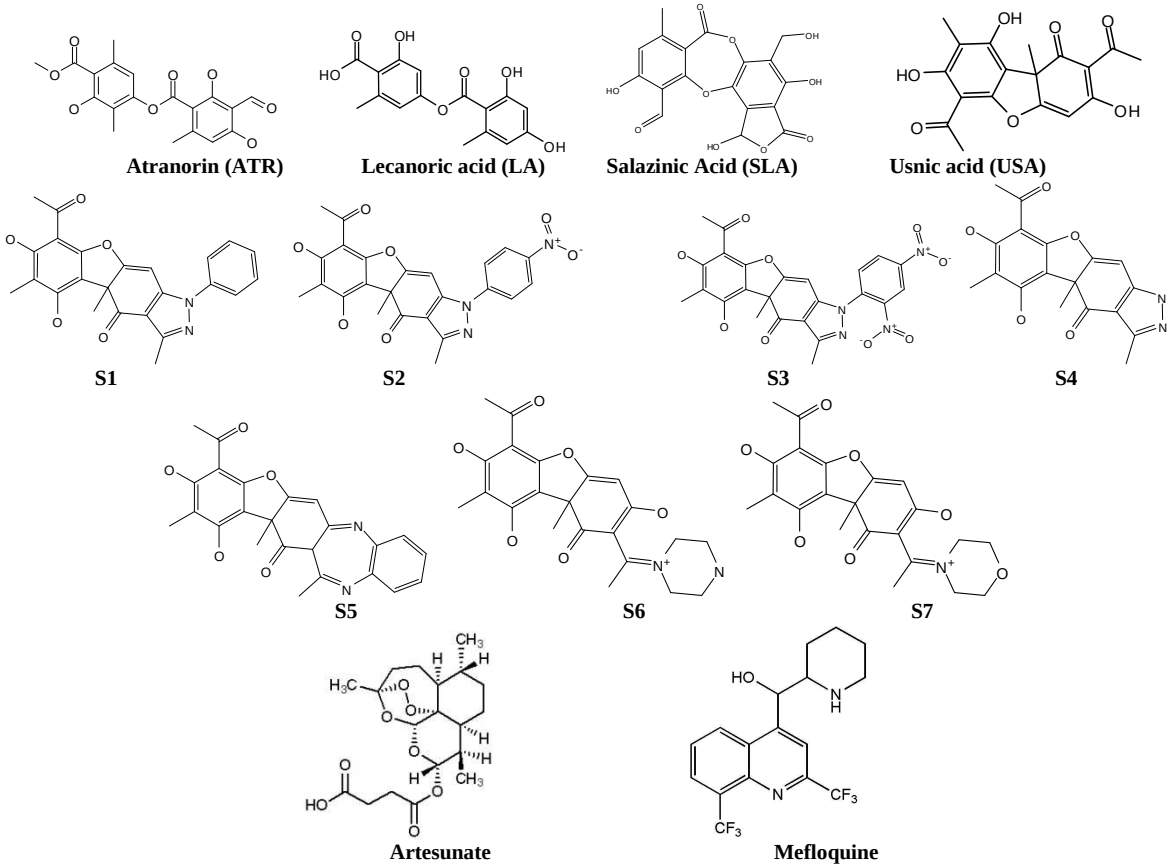


Figure 1. Chemical structure of lichen metabolites and dibenzofuran (usnic acid) derivatives

Ligand Preparation

LigPrep module v2.3 [14] was used to convert the sketched molecules present in 2D form to 3D, hydrogens were added and further the unfavourable bond lengths and bond angles were corrected by subjecting each ligand to a full length minimization using OPLS-2005 as force field. While performing the ionization states of the molecules, we retained the pH range as 5-9 with the help of Epik (Sophisticated algorithm and performs ionization and tautomerization together) in LigPrep. For each ligand, the tautomeric and stereoisomeric forms were generated; where one of the isomeric forms will have strong interactions with the binding sites. Decisively, lowest energy conformation as one per ligand was generated.

Protein preparation

The X-ray crystal structure of the proteins Plasmepsin II, *Plasmodium falciparum* dihydro folate reductase and *Plasmodium vivax* dihydro folate reductase were obtained from protein data bank [15-22] and further modified for glide docking calculations. Later, the proteins were prepared by employing the protein preparation wizard of the Schrödinger suite 2009. The H-atoms were added to the proteins at necessary positions and the co-crystallized ligands, water molecules were identified and removed from the structure. Further minimization was performed by applying an OPLS-2005 as force field and RMSD value of 0.30 Å.

Receptor grid generation

After the protein preparation, the receptor energy docking grid was generated using the receptor grid generation panel. The plasmepsin II protein (**PDB ID: 1LEE**) was associated with a ligand (R36), using this, the active site position and size of the active site was determined. Assuming the centre of the docking grid set by co-crystallized ligand, the residues of the protein were enclosed within the 10 Å from that centroid. The dataset of depsides (atranorin & lecanoric acid), depsidones (salazinic acid), dibenzofuran (usnic acid) derivatives and reference ligands (Artesunate & Mefloquine) were docked in the kinase domain of plasmepsin II protein, using glide 5.5 module in extra precision mode (XP) by the application of MCSA (Monte Carlo Based Simulated Algorithm) based minimization. The above process was repeated for the development of distinctive receptor docking grids of the specified protein molecules of interest in the present study.

Docking Validation

For validating the glide dock program, all the proteins were redocked with the native ligand and the reference ligands, artesunate and mefloquine, the results of which were represented and listed in Table 1.

Table 1.Glide docking results of lichen metabolites and dibenzofuran derivatives with respective target proteins

Comp.	Poses[a]	G. score (XP)	G. energy (Kcal/mol)	H-bonds[b]	Interacting Residues
Plasmepsin II; PDB ID: 1LEE					
ATR	4	-5.69	-39.98	2	Val78, Tyr192
S1	6	-4.65	-36.12	1	Tyr192
S2	6	-4.79	-31.56	1	Ala38
S3	4	-4.21	-41.55	1	Ser79
S4	12	-4.6	-24.24	2	Gly36, Ser79
S5	12	-4.12	-32.35	1	Ser218
S6	2	-3.76	-38.17	2	Asp214, Tyr192
S7	15	-4.2	-37.29	2	Tyr192, Ser79
SLA	8	-5.83	-44.52	4	Asp34, Thr217, Ser79, Ser218
USA	26	-5.18	-37.82	2	Asp214, Asp34
Mefloquine	4	-6	-37.36	2	Asp214, Asp34
Artesunate	1	-3.82	-29.49	-	-
R36	1	-7.71	-69.34	3	Gly36, Asn76, Ser79
Plasmepsin II; PDB ID: 1W6H					
ATR	4	-7.85	-40.72	2	Ser79, Ser37
S1	6	-3.22	-36.96	1	Ser79
S2	6	-3.08	-41.18	1	Gly216
S3	4	-3.11	-33.52	1	Asp34
S4	12	-4.72	-43.9	2	Asp34, Gly216
S5	12	-4.73	-34.03	1	Ser218
S6	2	-4.44	-39.47	1	Thr217
S7	15	-4.63	-39.62	1	Thr217
SLA	8	-7.79	-53.36	4	Thr217, Ser218, Val78, Asp34
USA	26	-5.04	-32.41	1	Thr217
Mefloquine	4	-6.42	-36.03	5	Ser79, Ser218, Asp214, Asp34 (2)
Artesunate	1	-3.86	-23.87	-	-
TIT	1	-5.72	-80.71	3	Tyr192, Ser79, Asp34
Plasmepsin II; PDB ID: 1LF3					
ATR	4	-6.88	-42.73	1	Val78
LA	10	-6.91	-44.09	4	Tyr192, Gly216, Ser79, Gly36

S1	6	-4.44	-29.45	1	Tyr192
S2	6	-4.36	-38.23	2	Tyr192, Ser218
S3	4	-3.75	-42.68	2	Gly216, Asp214
S4	12	-5.11	-39.19	4	Ser79, Ser118, Ser218
S5	12	-3.8	-40.28	1	Asp214
S6	2	-2.51	-34.99	1	Gly36
S7	15	-4.78	-38.26	3	Ser79 (2), Val78
SLA	8	-6.15	-46.21	5	Ser218 (2), Val78, Asp34, Thr217
USA	26	-5.51	-43.55	4	Gly216, Asp34, Ser218 (2)
Mefloquine	4	-7.11	-36.15	2	Asp214, Asp34
Artesunate	1	-3.2	-29.57	2	Ser79 (2)
EH58	5	-8.09	-76.24	5	Gly216, Tyr192, Ser79 (2), Val78
Plasmepsin II; PDB ID: 1ME6					
ATR	4	-4.13	-43.42	1	Tyr192
S1	6	-4.02	-39.8	-	-
S2	6	-4.77	-43.14	2	Ser218, Gly216
S3	4	-4.08	-39.26	1	Thr217
S4	12	-4.85	-33.7	1	Gly216
S5	12	-4.6	-36.96	2	Asn288, Pro243
S6	2	-4.24	-29.93	1	Ser79
S7	15	-4.47	-26.83	1	Thr217
SLA	8	-6.27	-51.13	3	Ser218, Thr217, Asp34
USA	26	-5.4	-34.89	2	Gly216, Ser218
Mefloquine	4	-6.97	-40.8	2	Asp214, Asp34
Artesunate	1	-0.88	-29.38	2	Val78, Ser218
IVS	1	-4.23	-58.06	3	Asp438, Glu453 (2)
Plasmepsin II; PDB ID: 2IGX					
ATR	4	-6.89	-33.25	2	Leu131, Trp41
S1	6	-7.14	-43.51	2	Asp214, Tyr192
S2	6	-5.91	-45.52	2	Asp214, Gly36
S3	4	-5.32	-46.93	1	Asp34
S4	12	-5.08	-27.97	2	Gly216, Ser218
S5	12	-6.44	-34.97	-	-
S6	2	-4.62	-40.84	2	Asp214, Gly36
S7	15	-6.17	-37.79	2	Asp214, Gly36
SLA	8	-7.38	-38.31	4	Ala117, Thr114, Tyr77 (2)
USA	26	-6.64	-41.75	3	Asp214, Ala38, Try92
Mefloquine	4	-8.52	-28.19	2	Asp34, Trp41
Artesunate	1	-2.61	-28.38	2	Ala38, Tyr77
A1T	1	-5.72	-63.21	1	Trp41
Plasmepsin II; PDB ID: 2BJU					
ATR	4	-6.42	-40.4	3	Asp34, Ser218, Gly216
S1	6	-6.35	-40.99	1	Thr217
S2	6	-5.01	-37.83	1	Tyr77
S3	4	-5.04	-50.15	1	Thr217
S4	12	-5.96	-34.76	3	Tyr192, Ala38, Leu131
S5	12	-6.31	-34.22	2	Asp34, Tyr192
S6	2	-4.81	-27.29	1	Tyr77
S7	15	-5.43	-34.33	1	Gly36
SLA	8	-4.78	-45.47	4	Ser218, Thr217, Asp34 (2)
USA	26	-5.33	-33.12	2	Leu131, Ala38
Mefloquine	4	-6.36	-33.53	1	Gly216
Artesunate	1	-3.36	-32.65	2	Ala38, Tyr192
IH4	1	-10.59	-59.22	1	Gly216
***Plasmodium falciparum*, Dihydro folate reductase; PDB ID: 1J3I**					
ATR	4	-7.66	-55.08	3	Ser111, Asp54, Ile154
S1	6	-8.13	-51	1	Tyr170
S2	6	-8.06	-58.4	1	Tyr170
S3	4	-8.39	-63.76	1	Tyr170
S4	12	-7.16	-43.1	1	Ser111
S5	10	-8.01	-49.83	1	Ile164
S6	1	-6.97	-43.01	1	Ile164
S7	15	-8.35	-31.84	1	Leu40
SLA	8	-8.62	-47.49	2	Ser108, Asp54
USA	26	-8.51	-43.09	3	Ser167 (2), Ile164
Mefloquine	4	-6.27	-37.62	1	Ile164
Artesunate	1	-5.1	-38.87	1	Ser167
WRA	10	-9.69	-46.35	4	Ile14, Ile164, Asp54 (2)
***Plasmodium falciparum*, Dihydro folate reductase; PDB ID: 1J3J**					
ATR	4	-7.67	-46.6	2	Asp54, Ile164
LA	10	-9.01	-48.48	3	Asp54, Ile164, Asn108

S1	6	-8.57	-55.59	2	Asn108, Tyr170
S2	6	-7.77	-50.17	1	Asn108
S3	3	-9.21	-59.94	2	Asn108, Tyr170
S4	12	-8	-44.95	2	Asn108, Tyr170
S5	12	-8.16	-46.95	1	Leu40
S6	2	-8.13	-50.71	2	Leu40, Ser111
S7	15	-7.65	-48.23	1	Leu40
SLA	8	-8.89	-50.82	3	Asp54, Ile164, Asn51
USA	26	-8.22	-47.24	2	Asn108, Tyr170
Mefloquine	4	-7.38	-36.62	2	Asn108, Ile164
Artesunate	1	-6.84	-36.46	2	Arg122, Arg59
CP6	2	-8.53	-35.34	5	Ile14, Ile164, Asp54 (2), Cys15
***Plasmodium vivax*, Dihydro folate reductase; PDB ID: 2BLA**					
ATR	4	-8.82	-47.28	4	Tyr179, Ile173, Asn117 (2)
LA	10	-8.29	-43.92	3	Asp53, Met54, Arg131
S1	6	-7.29	-45.63	2	Asn117, Ala15
S2	6	-5.91	-47.72	2	Arg58, Thr44
S3	4	-6.27	-52.34	2	Asn117, Ala15
S4	12	-7.39	-39.34	1	Leu39
S5	12	-6.79	-44.61	1	Ile173
S6	2	-6.01	-44.35	2	Asn117, Ile173
S7	15	-6.32	-29.82	2	Ile173, Asp53
SLA	8	-7.4	-45.36	3	Ile173, Asp53, Trp47
USA	26	-7.67	-34.86	2	Ser120, Arg131
Mefloquine	4	-6.23	-40.41	2	Asp53 (2)
Artesunate	1	-7.48	-36.96	1	Arg131
CP6	6	-8.47	-36.01	5	Ile13, Ile173, Asp53 (2), Cys14
***Plasmodium vivax*, Dihydro folate reductase; PDB ID: 2BLC**					
ATR	4	-9.58	-45.47	3	Asn117, Asp53, Ile173
S1	6	-6.73	-46.99	2	Asn117, Tyr179
S2	6	-7.89	-51.56	2	Arg131, Ala15
S3	4	-7.57	-54.12	1	Ala15
S4	12	-7.31	-38.71	2	Ala15, Asn117
S5	12	-7.58	-45.68	1	Leu39
S6	2	-7.87	-48.99	1	Leu39
S7	15	-7.62	-38.14	1	Ile173
SLA	8	-9.47	-49.51	4	Asn50, Asp53, Ile173, Trp47
USA	26	-7.68	-43.45	1	Leu39
Mefloquine	4	-7.26	-39.24	2	Asn117, Ile173
Artesunate	1	-7.33	-32.84	2	Arg131 (2)
CP7	8	-9.83	-33.85	3	Ile13, Ile173, Asp53
***Plasmodium vivax*, Dihydro folate reductase; PDB ID: 2BL9**					
ATR	4	-6.73	-51.4	3	Ile173, Asp53, Asn50
S1	6	-7.83	-49.97	1	Tyr179
S2	6	-6.66	-47.13	1	Tyr179
S3	4	-7.88	-57.4	1	Tyr179
S4	12	-7.12	-39.51	2	Ala15, Leu39
S5	12	-7.49	-47.62	1	Ile173
S6	2	-7.82	-45.34	2	Leu39, Ser120
S7	15	-7.85	-44.04	2	Leu39, Ser120
SLA	8	-8.05	-50.09	4	Asn50, Asp53, Trp47, Ile173
USA	26	-7.3	-36.45	1	Ser120
Mefloquine	4	-6.53	-36.88	2	Ile173 (2)
Artesunate	1	-4.28	-35.59	1	Trp47
CP6	4	-8.45	-35.4	4	Ile13, Ile173, Asp53 (2)

[a]Number of poses generated per each ligand; [b]Number of Hydrogen bonds

RESULTS AND DISCUSSION

Molecular docking simulations were performed with the lichen metabolites; atranorin, lecanoric acid, salazinic acid and dibenzofuran (usnic acid) derivatives as malarial target ligands on plasmepsin II (PL II) and dihydro folate reductase (DHFR). The purpose of the docking studies is to identify the binding mode of lichen metabolites and the molecular interactions between the ligands and the target proteins PL II and DHFR protein. Hence, the aspartic proteinase PL II was retrieved from 5 different sources (*Plasmodium falciparum*, PDB IDs: 1LEE, 1W6H, 1LF3, 1ME6, 2IGX & 2BJU) with effectively bound co-crystallized ligands and each single ligand was docked with 5 PDB IDs of the same protein in order to find out the effective binding residues present in PL II protein and the pharmacophore groups of the ligands. Also, we retrieved DHFR from two different species and different sources, respectively, (*Plasmodium falciparum*, PDB IDs: 1J3I & 1J3J and *Plasmodium vivax*, PDB IDs: 2BLA, 2BLC &

2BL9). The docking studies were performed using glide v 5.5 in flexible mode and the results represented in Table 1.

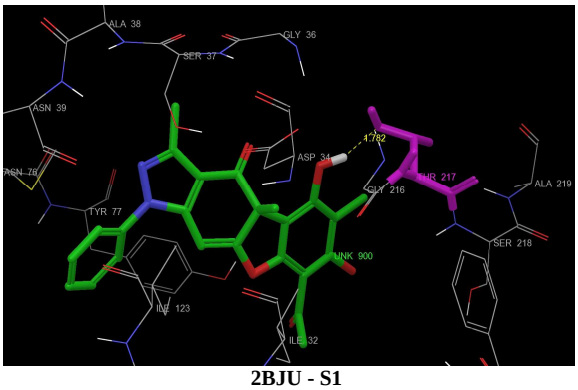
The docking results describes that the lichen metabolites atranorin, lecanoric acid, salazinic acid and dibenzofuran (usnic acid) derivatives are showing good hydrogen bond interactions with PL II with glide score ranging from -7.85 to -2.51. It is evident that the docking ligands have effective H-bond interactions and hydrophobic contact with the active residues viz. Thr217, Gly36, Ser79, Asp214, Ser218, Tyr192, Gly216, and Tyr77 present in the PL II protein. While looking into the docking studies, it is clearly evident that the lichen metabolites have better binding interactions with the above specified active residues of PL II.

Of the docking ligands, the depside, atranorin (ATR) has shown good hydrogen bond interactions with many of the active residues in each docking grid, like Tyr192, Ser79, Ser218 and Gly216, with a glide score ranging from -7.85 to -4.13 and the ligand, lecanoric acid (LA) has shown good H-bond interactions with the residues viz. Ser79, Tyr192, Gly216, and Gly36, with a glide score range of -6.91.

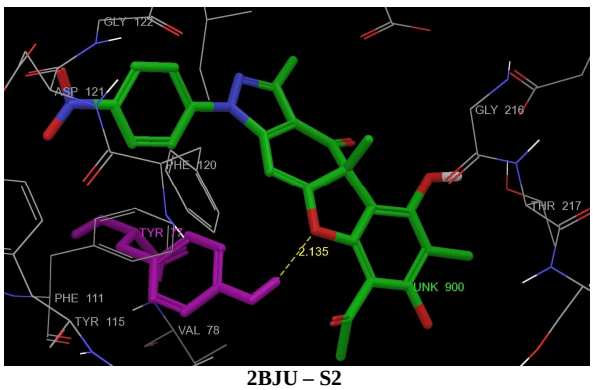
The depsidone molecule salazinic acid has shown effective hydrogen bond interactions with active residue Thr217, Ser79, Ser218, and Tyr77, with a glide score ranging from -7.79 to -4.78. The dibenzofuran molecule usnic acid has shown hydrogen bond interactions with Asp214, Thr217, Gly216, and Ser218, with a glide score ranging from -6.64 to -5.04. The dibenzofuran derivatives S1-S7 molecules have shown acceptable hydrogen bond interactions with plasmepsin II. In that, the ligands S2, S3, S4, S6 and S7 have shown good H-interactions with the active residues present in the respective grids but the compounds S1 and S2 have not showing any type of interaction with the residues present in the 1ME6 and 2IGX docking grids. As well, the reference ligand, artesunate has not shown any type of interaction with the residues present in the energy grid of PDB IDs, 1LEE, 1W6H and 2BJU.

Similarly, the glide score illustrates that all the docking ligands are showing effective hydrogen bond interactions with dihydro folate reductase protein of *Plasmodium falciparum*. The docking ligands are showing acceptable H-bond interactions with residues Asp54, Ile164, Asn108, Tyr170 and Leu40 present in dihydro folate reductase protein of 1J3I and 1J3J docking grids. Of the depsides, atranorin (ATR) and lecanoric acid (LA) are showing good hydrogen bond interactions with residues Asp54 and Ser108 and Ile154 of 1J3I docking grid and with residues Asp54 and Ile164 of 1J3J docking grid respectively. Similarly, salazinic acid shows good hydrogen bond interactions with Ser108, Asp54 and Ile164 present in DHFR protein of 1J3I and 1J3J docking grids. The rest of the dibenzofuran derivatives are also showing good hydrogen bond interactions with active residues of DHFR protein.

Likewise, docking results of certain ligands against DHFR of *Plasmodium vivax* were impressive with its effective binding mode against residues Ile173, Asp53, Ile13 and Asn117. In these ligands, ATR and LA are showing hydrogen bond interactions with Asp53, Ile173, Asn117, and Asn50 in 2BLA, 2BLC and 2BL9 docking grids, respectively. The depsidone moiety SA also shows acceptable hydrogen bond interactions with the residues Ile173, Asp53 and Trp47 in three docking grids. Further, of the dibenzofuran derivatives; USA is showing hydrogen bond interactions with Ser120, Arg131 and Leu39 in three docking grids and the ligands S1-S7 are showing satisfactory H-bond interactions with the residues Asp53, Asn117, Ile173, Tyr179, and Leu39 in the same docking grids, wherein the reference ligands docking results are found to be supporting and are evidences to the respective lichen metabolites and dibenzofuran derivatives. The binding mode of lichen metabolites with PL II (2BJU docking grid) and DHFR (2BLC docking grid) are specified in Figure 2 and Figure 3, respectively.



2BJU - S1



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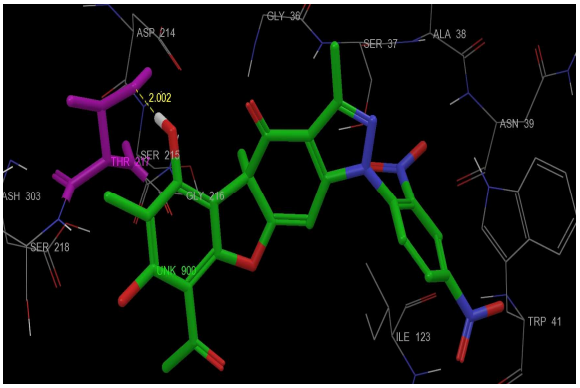

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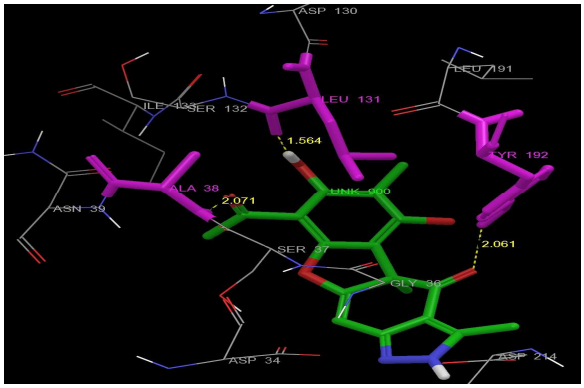
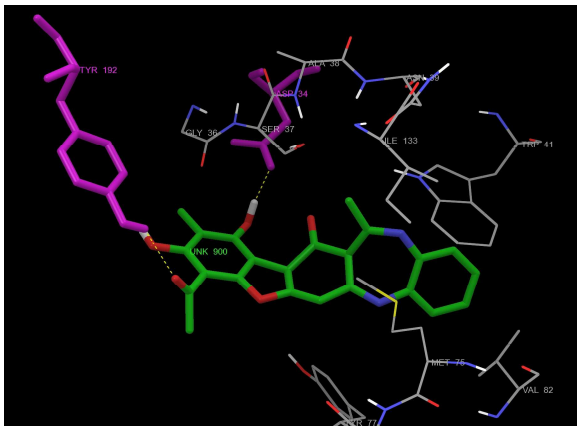
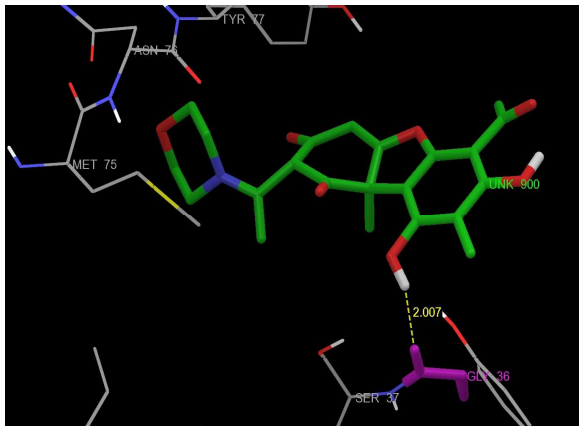


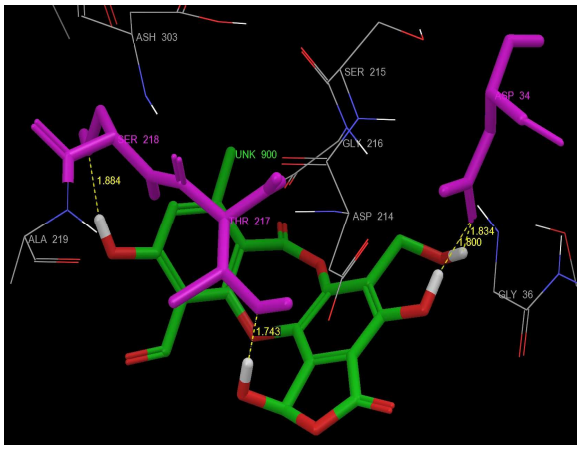
2BJU – S4



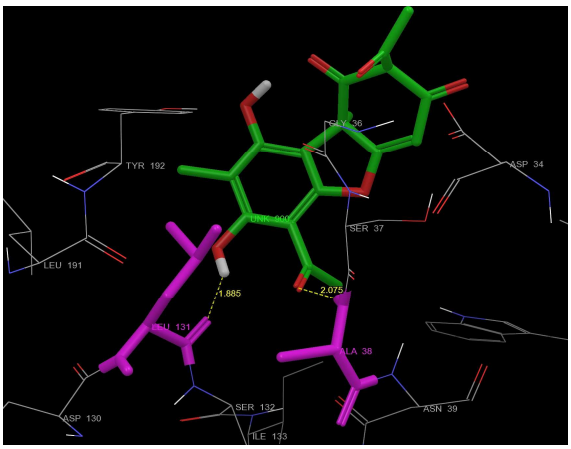
2BJU – S5



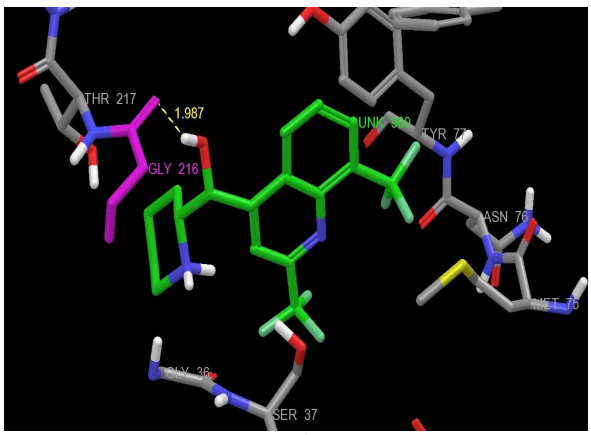
2BJU – S7



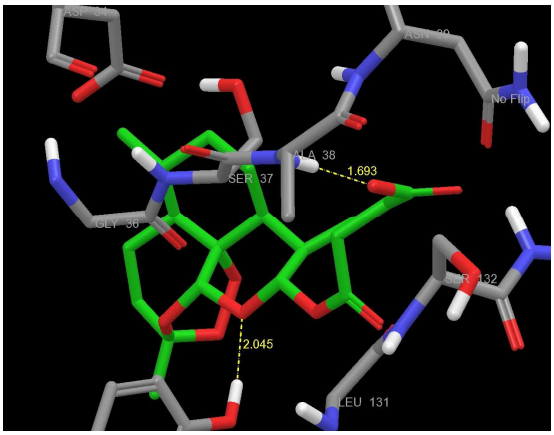
2BJU – SLA



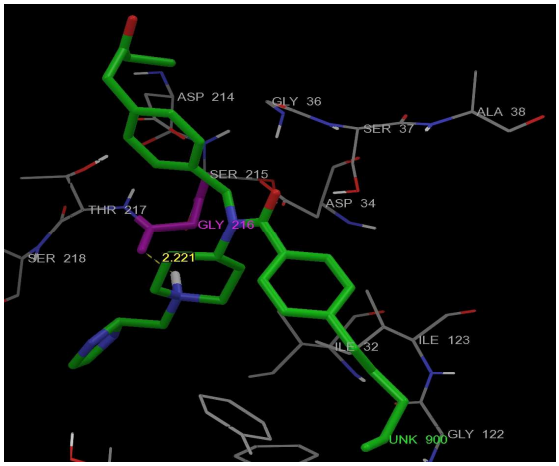
2BJU – USA



2BJU - Mefloquine

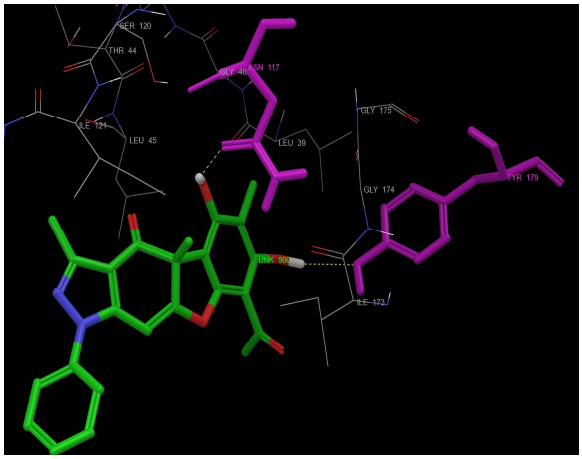


2BJU - Artesunate

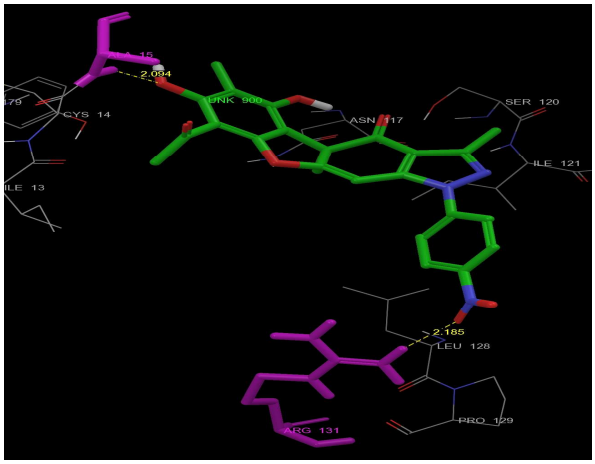


2BJU – IH4

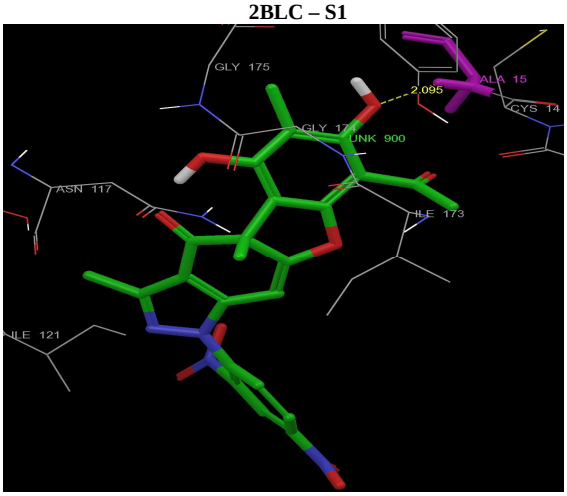
Figure 2. Binding mode of dibenzofuran (usnic acid) derivatives S1-S5 & S7, Salazinic acid (SLA), Usnic acid (USA), Mefloquine and Artesunate with Plasmepsin II (2BJU docking grid)



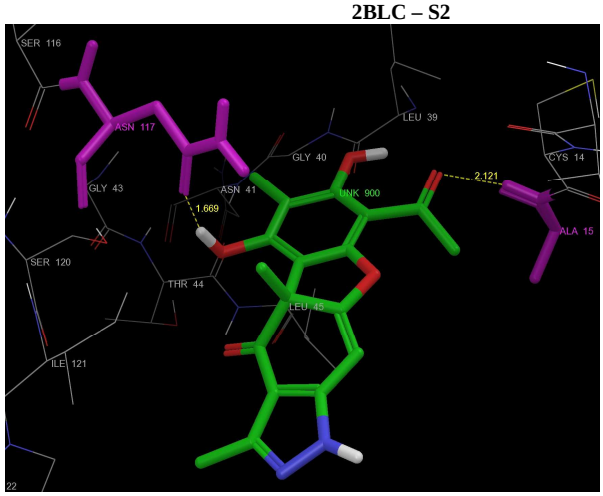
2BLC – S1



2BLC – S2



2BLC – S3



2BLC – S4

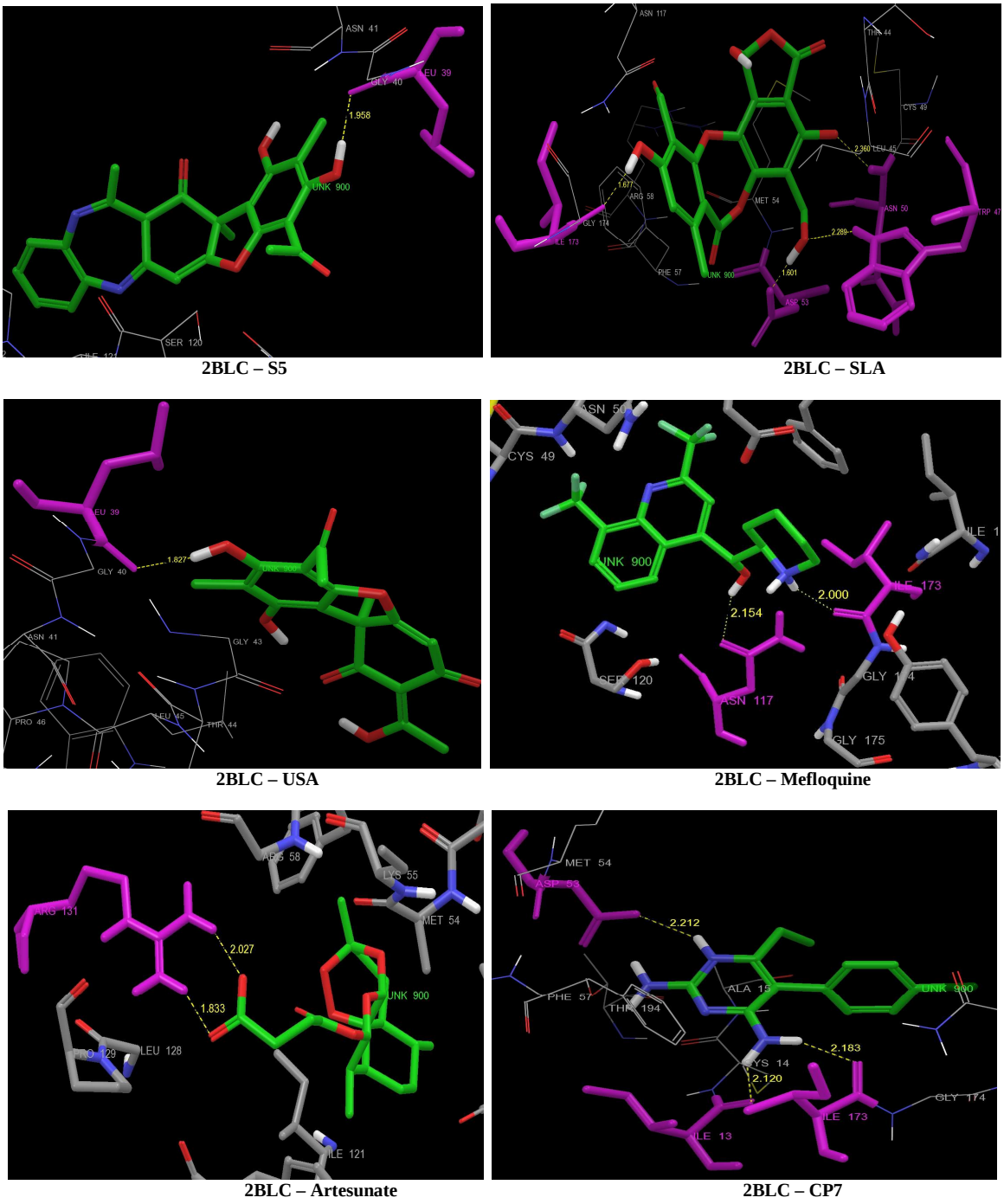


Figure 3. Binding mode of dibenzofuran (usnic acid) derivatives S1-S5, Salazinic acid (SLA), Usnic acid (USA), Mefloquine and Artesunate with Dihydro folate reductase (2BLC docking grid)

On comparison of the binding mode of lichen metabolites; depsides (ATR & LA), depsidone (SA) and dibenzofuran derivatives (USA) on the target protein PL II, the dibenzofuran derivatives have shown effective hydrogen bond interactions with the active residues present in the five different docking energy grids, followed by moderate interactions in the depsidone moiety salazinic acid having good hydrogen bond interactions of the active residues respective of the five energy grids. Further, of the depsides, atranorin (ATR) and lecanoric acid (LA), LA is showing more effective H-bond interactions with PL II protein.

With respect to dihydro folate reductase (DHFR) of *Plasmodium falciparum*, the depsides moieties, ATR and LA are showing satisfactory H-bond interactions with active residue Asp54 in the 1J3I and 1J3J energy grid and Ile154 & Ile164 of the two energy grids, respectively. Similarly, the depsidone moiety SLA is showing moderate H-bond interactions with the active residues of the target protein. Furthermost, the dibenzofuran derivatives are also showing moderate binding interactions with the DHFR protein. In case of DHFR of *Plasmodium vivax*, the depsides moieties

ATR and LA are showing better H-bond interactions when compared to the depsidone moiety SA and dibenzofuran derivatives. Salazinic acid followed by dibenzofuran derivatives is worthful in the docking studies against the target protein DHFR.

After analyzing the docking reports of the lichen metabolites atranorin, lecanoric acid, salazinic acid and dibenzofuran (usnic acid) derivatives against plasmepsin II (PL II) and dihydro folate reductase (DHFR) malarial proteins, it's concluded that in the case of PL II protein, the dibenzofuran (usnic acid) derivatives are showing effective hydrogen bond interactions with residues Thr217, Gly36, Ser79, Asp214, Ser218, Tyr192, Gly216, and Tyr77 with satisfactory glide score of the five docking grids. With respect to DHFR, it's revealed that the depsides, ATR and LA are showing effective binding interactions with residues Asp54, Ile164, Asn108, Tyr170 & Leu40 (*Plasmodium falciparum*) and Ile173, Asp53, Ile13 & Asn117 (*Plasmodium vivax)* with good glide score.

CONCLUSION

In the current study, several molecular docking simulations were carried out using lichen metabolites atranorin, lecanoric acid, salazinic acid and dibenzofuran derivatives (usnic acid) against aspartic proteinase plasmepsin II-PL II (*Plasmodium falciparum*) and dihydrofolate reductase – DHFR (*P. falciparum, P. vivax)*, to determine the binding mode, binding energy and hydrogen bond interactions of ligands with respect to the PL II and DHFR proteins. The docking studies were carried out using glide module v5.5 of Schrödinger suite 2009. For this, we retrieved distinctive PDB IDS for a single protein (PL II; PDB IDs: 1LEE, 1W6H, 1LF3, 1ME6, 2IGX &2BJU, DHFR; PDB IDs: 1J3I &1J3J (*P. falciparum*) and 2BLA, 2BLC & 2BL9 (*P. vivax*). The active residues were finalized based on the co-crystallized ligands interactions present in the retrieved PDB IDs and on the reference ligands interactions. Based on this active residues analysis and the docking results of the docked ligands, we conclude that the dibenzofuran derivatives (usnic acid) are showing effective hydrogen bond and hydrophobic interactions with the target PL II protein. The depsidone molecule salazinic acid shows moderate interactions compared to the depsides atranorin (ATR) and lecanoric acid (LA). With respect to the dihydro folate reductase, depsides; ATR and LA are showing satisfactory binding mode, binding energy and H-bond interactions with the active residues present in the DHFR protein active sites of the two species in different docking grids. Further, salazinic acid and dibenzofuran derivatives are showing flat interactions with the active residues of the DHFR of different energy grids. These docking results are expected to be helpful in designing new chemical entities and further structural development of atranorin, lecanoric acid, salazinic acid and dibenzofuran (usnic acid) analogues against aspartic proteinase plasmepsin II and dihydro folate reductase proteins.

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Glossary of abbreviations

A1T 5-pentyl-N-{[4'-(piperidin-1-ylcarbonyl)biphenyl-4-yl]methyl}-N-[1-(pyridin-2-ylmethyl)piperidin-4-yl]-pyridine-2-carboxamide
ATR Atranorin
CP6 5-(4-chloro-phenyl)-6-ethyl-pyrimidine-2,4-diamine
CP7 6-ethyl-5-phenylpyrimidine-2,4-diamine
EH5 N-(1-benzyl-3-{[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionyl]-[2-(hexahydro-benzo[1,3]dioxol-5-yl)-ethyl]-amino}-2-hydroxy-propyl)-4-benzyloxy-3,5-dimethoxy-benzamide
IH4 N-(R-carboxy-ethyl)-alpha-(S)-(2-phenylethyl)
IVS 3-hydroxy-6-methyl-4-(3-methyl-2-(3-methyl-2-(3-methyl-butyrylamino)-butyrylamino)-butyrylamino)-hepatonoic acid ethyl ester
LA Lecanoric acid
R36 4-amino-N-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide
TIT N-((3S,4S)-5-[(4-bromobenzyl)oxy]-3-hydroxy-4-{[N-(pyridin-2-ylcarbonyl)-L-valyl]amino}pentanoyl)-L-alanyl-L-leucinamide
SLA Salazinic acid
WRA 6,6-dimethyl-1-[3-(2,4,5-trichlorophenoxy)propoxy]-1,6-dihydro-1,3,5-triazine-2,4-diamine
USA Usnic acid