http://dx.doi.org/10.4314/jpb.v12i1.9
Vol. 12 no. 1, pp. 60-76 (March 2015)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Design and docking studies of inhibitors for the chorismate synthase from *Streptococcus pneumoniae* using 5-enol pyruvylshikimate 3-phosphate (EPSP) analogues

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Received 17th December 2014; Accepted 28th January 2015

Abstract

Streptococcus pneumoniae is one of the common causes of community-acquired bacterial infection. It causes respiratory tract infections and is increasingly becoming resistant to antibacterial agents available for its treatment/ management. There is therefore an urgent need for new antibacterial agents, particularly those that act on novel targets, or improvements on the agents available. Chorismate synthase (CS) is a key enzyme in the catalysis of 5-enolpyruvylshikimate 3-phosphate (EPSP) to chorismate, which is the final step in the shikimate pathway. This pathway has been shown to be absent in humans, thus making it an attractive target for potential antibacterial development. *In silico* docking studies were carried out on analogues of EPSP in four different active site models of CS from *Streptococcus pneumoniae*. Active site model 3 was found to be most appropriate for docking studies as it contained conserved active site arginines and water molecules required for EPSP binding. Four EPSP analogues were found to have higher docking scores than the natural substrate and were used to design inhibitors for CS based on substitutions on the C1 and C3- phosphate positions of CS. C3-phosphate monosubstituted molecules and C1 monosubstituted molecule were seen to have higher docking scores than the lead molecules. Disubstitution on both C1 and C3-phosphate positions resulted in molecules showing greater inhibitory potentials from their docking scores. These series of analogues will provide a starting point for further investigations into the identification of inhibitors of chorismate synthase.

Keywords: Chorismate synthase (CS); Docking studies; 5-enolpyruvylshikimate-3- phosphate (EPSP); *Streptococcus pneumoniae*; Flavin mononucleotide (FMN)

INTRODUCTION

Chorismate synthase catalyzes the seventh and final step in the main trunk of the shikimate pathway, which is the trans-1,4 elimination of phosphate from 5-enolpyruvylshikimate 3-phosphate (EPSP) to yield chorismate. The shikimate pathway links metabolism of carbohydrates to biosynthesis of aromatic compounds and is a major feature in the metabolic processes of prokaryotes, plants, fungi and some forms of eukaryotes as well as apicomplexan parasites but is absent in mammals (Roberts *et al.*, 2002). Chorismate is of eminent importance to the biosynthesis of many compounds and metabolites. It is the end product of the shikimate pathway and lies at a metabolic node that makes it the precursor of the three aromatic amino acids and the precursor for five distinct pathways. These pathways are

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ISSN 0189-8442

necessary for the production of folate, para-aminobenzoic and other cyclic metabolites such as ubiquinone and menaquinone (Maclean & Ali, 2003). The reaction requires the presence of a reduced flavin mononuclotide (FMN) as a cofactor which is directly involved in the mechanism of the reaction. The flavin serves as an electron donor to EPSP, initiating C-O bond cleavage thereby introducing the second of the three double bonds of the benzene ring (Fig 1) (Herrman & Weaver, 1999). The flavin intermediate decays after EPSP is converted to chorismate and after phosphate is released from the enzyme (Herrman & Weaver, 1999). No net redox change occurs as the flavin molecule is not consumed during the reaction (Ahn *et al.*, 2004).

There are different sources of chorismate synthase, but they show a high degree of sequence conservation. Depending on the organism, chorismate synthase is either monofunctional, requiring "chemically or enzymatically reduced flavin" addition to *in vitro* enzyme assays (usually only active in an anaerobic environment); or bifunctional, requiring a "second enzymatic activity, an NAD(P)H-driven flavin reductase" within the same polypeptide chain (Rev 1999). Chorismate synthases from *Neurospora crassa* and *Saccharomyces cerevisiae* possess an additional NADPH:FMN oxidoreductase activity (Macheroux 1999) and are thus referred to as bifunctional enzymes. The active site responsible for this additional activity appears to overlap with that of chorismate synthase activity, as shown in the works of Kitzing, *et al.,* (2001), that a common binding site exists for both EPSP and NADPH. Chorismate synthases from many other organisms, including *Streptococcus pneumoniae, Escherichia coli, Thermatoga maritima, Plasmodium falciparum,* and *Staphylococcus aureus* do not show such activity and are termed monofunctional (Bornemann, 2002).

Streptococcus pneumoniae is the most common cause of community-acquired bacterial and respiratory tract infections such as pneumoniae. Over the decades, increasing resistance has been developed to β-lactams (Appelbaum, 2002.), macrolides (Lynch & Martinez, 2002), and fluoroquinolones (Hooper, 2002) by *Streptococcus pneumoniae,* thereby causing a great challenge for health care providers in treating this common community-acquired pathogen. For these reasons, there is an urgent need for new antibacterial agents, particularly those that act on novel targets (American Society of Health-System Pharmacists, 2004). As the work of Maclean and Ali (2003) has availed the structure of the chorismate synthase enzyme from the pathogen *Streptococcus pneumoniae,* major new opportunities in structure-based drug design of inhibitors of this enzyme will arise from knowledge of this structure and docking studies. A number of inhibitors of the enzyme are known as shown in the works of Thomas *et al.,* (2003) and Osborne *et al.,* (2000) and more are likely to be found using combinatorial, and virtual screening methods. These inhibitors could, for example, compete with the substrate and cofactor in binding to the enzyme, lock the enzyme in unproductive conformations, or undergo suicide radical chemistry (Bornemann, *et al.,* 2003) or in the formation of false products which can lead to the termination of the pathway.

In effect, the aim of this study is to use docking studies method and molecular dynamics studies to obtain and understand the mechanism of inhibition using analogues of the natural substrate of chorismate synthase-EPSP and also development of new molecules which will likely show inhibitory activities to the enzyme.

EXPERIMENTAL

All methods used in this study were entirely *in silico,* making use of several web

interfaces, online databanks and software applications.

Identification of molecular target. The crystal structure of chorismate synthase was downloaded from the Protein Databank page (Berman H.M., *et al.*, 2000), with pdb code: 1QXO.pdb. The enzyme is a large tetrameric structure with four active sites. The structures of the A chain, the ligand, the cofactor and water molecules within 5 Angstroms of the ligand were taken from the large protein structure. Using CAChe workspace, the hybridization and valence of the structure was beautified. The added hydrogens were relaxed using MM2 geometry optimization (Locking everything except the hydrogens). The substrate is residue 5001 which is located in the active site together with an oxidized form of the cofactor, residue 4001. From this structure, four models were prepared for docking studies.

Preparation of Model 1 (5Å around substrate, waters included). Starting from the optimized structure of 1QXO.pdb, a 5-Angstrom sphere (Residues, Waters, HETs) around and including the substrate was selected. And an active site group was created from the residues after deleting the substrate. This was saved and labelled model 1.

Preparation of Model 2 (8Å around substrate, waters deleted). Starting from the optimized structure of 1QXO.pdb, the water molecules were deleted from it. An 8-Angstrom sphere (Residues, Waters, HETs) around and including the substrate was selected. The substrate was deleted and the remaining residues defined as the active site groups. The structure was saved and labelled model 2.

Preparation of Model 3 (8Å around substrate, waters included and after MD). Starting from the optimized structure of 1QXO.pdb, the whole structure was locked and a 10-Angstrom sphere (Residues, waters, HETs) around and including the substrate was selected and unlocked. A molecular dynamics run (MM2) at 300K for 100ps with an equilibration time of 0.5ps was performed. Three snapshots of low energy structures after 50ps were selected and validated. To define the active site in the structure, an 8-Angstrom sphere (Residues, Waters, HETs) around and including the substrate was selected. The substrate was deleted and the remaining residues grouped as active site residues. The structure was saved and labelled Model 3.

Preparation of Model 4 (8Å around substrate, waters deleted and after MD). Starting from the optimized structure of 1QXO.pdb,the water molecules were deleted. The whole structure was locked and a 10-Angstrom sphere (Residues, waters, HETs) around and including the substrate was selected and unlocked. A molecular dynamics run (MM2) at 300K for 100ps with an equilibration time of 0.5ps was performed. Three snapshots of low energy structures after 50ps were selected and validated. To define the active site in the structure, an 8-Angstrom sphere (Residues, Waters, HETs) around and including the substrate was selected. The substrate was deleted and the remaining residues grouped as active site residues. The structure was saved and labelled Model 4.

Validation of the models. Validation of the models was done using Ramachandran plots in PROCHECK (Laskowski, 1993).

Preparation of the ligand structures. Analogues of the natural substrate (EPSP) were drawn in CAChe workspace paying particular attention to the stereochemistry and a molecular mechanics optimisation using MM2 was performed on each structure.

Docking studies. The series of substrate analogues and natural substrate were docked into the four active site models of 1QXO.pdb using project leader in CAChe (FSQ Poland, 2008). The flexible active site side chains and flexible ligands type docking in 3000 generation, using Amber van der Waals was

performed on each model a minimum of three times.

RESULTS AND DISCUSSION

Binding affinity. Compound 1, 3, 9, and10 were found to have higher binding affinity as compared with the binding affinity of the natural substrate in all four active site models (table 2). Compounds 1 and 3 show similar binding affinities, which could be attributed to having the same substituent on the C1 of EPSP. This can also be said for compounds 9 and 10, with compound 9 having a methyl substituent on the O1P of EPSP and compound 10 having a methyl substituent on both O1P and O2P of EPSP. This led to the modification of these lead compounds, focusing on substitutions on the C3-phosphate oxygens and C1 positions of EPSP. The new analogue's binding affinities shows compounds B, C, E, F and L to have a higher binding affinity than the lead compounds and the natural substrate (Table 3). C3-phosphate monosubstituted molecules B and L, and C1 monosubstituted molecule C were seen to have higher docking scores than the lead molecules. Disubstitution on both C1 and C3-phosphate positions resulted in molecules E and F showing great inhibitory potentials from their docking scores.

Active site model validation. Validation data from Ramachandran plots for the four active site models indicates that models 1, 2 and 4 are of good quality (Fig 5). However, upon additional optimisation of model 3, no improvements were noticed in the area of residues in the most favourable regions.

The binding of chorismate synthase to the natural substrate (EPSP) and co-factor (Reduced FMN) is strongly coordinated by the presence of water in its active site. Therefore, models 2 and 4 would not depict the proper interactions *in vivo* because the water molecules have been deleted from them. Active site model 3 shows a close resemblance to that described in the work of Maclean & Ali (2003), showing appropriate interactions with active site residues as it is seen to contain conserved arginines and water molecules required for EPSP binding.

Active site model 3 can said to be the appropriate for the sake of this docking study. Active site model 3 and 4 are seen to contain conserved arginines (Arg48, Arg101, Arg107, Arg125, and Arg337) which are important for EPSP binding, but unlike model 4, model 3 still contains important water molecules. Though model 1 can be seen to still contain conserved water molecules, the important amino acid residues Arginine 337 and 107 are missing.

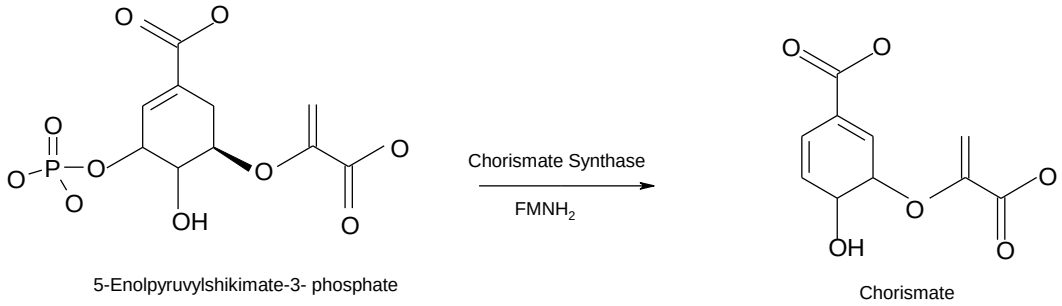
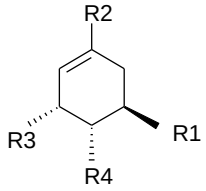


Figure 1: Conversion of 5-enolpyruyvl shikimate-3-phosphate to chorismate by chorismate synthase enzyme using reduced FMN as a co-factor.

Table 1: 5-Enolpyruvyl shikimate-3- phosphate (EPSP) analogues



Compound	R1	R2	R3	R4
1	$-OC(CH_2)COO^-$	$-CH_2OPO^{-}_3$	$-OPO^{2-}_3$	-OH
2	-OC(CH2)CO-	$-COO^-$	$-OPO^{2-}_3$	-O-
3	$-OC(CH_2)PO^{2-}_3$	$-CH_2OPO^{2-}_3$	$-OPO^{2-}_3$	-OH
4	$-OC(CH_2)COO^-$	$-COO^-$	$-OSO^{-}_3$	-OH
5	$-OC(CH_2)COO^-$	$-SO^{-}_3$	$-OPO^{2-}_3$	-OH
6	$-OC(CH_2)SO^{-}_3$	$-SO^{-}_3$	$-OPO^{2-}_3$	-OH
7	$-OC(CH_2)SO^{-}_3$	$-COO^-$	$-OPO^{2-}_3$	-OH
8	$-OC(CH_2)SO^{-}_3$	$-SO^{-}_3$	$-SO^{-}_4$	-OH
9	$-OC(CH_2)COO^-$	$-COO^-$	$-OPO^{2-}_3CH_3$	-OH
10	$-OC(CH_2)COO^-$	$-COO^-$	$-OP(OCH_3)_2O$	-OH
21	$-OC(CH_2)COO^-$	$-COO^-$	$-OPO^{2-}_3$	-H
22	$-OC(CH_2)COO^-$	$-COO^-$	-OH	-OH
23	$-OC(CH_2)COO^-$	$-COO^-$	-H	-OH
24	$-OC(CH_2)COO^-$	$-COO^-$	-H	-OH
25	$-OC(CH_2)COCH_3$	$-COO^-$	$-OPO^{2-}_3$	-OH
26	-OCH3	$-COO^-$	$-OPO^{2-}_3$	-OH
27	-OH	$-COO^-$	$-OPO^{2-}_3$	-OH
28	-H	$-COO^-$	$-OPO^{2-}_3$	-OH
29	$-OC(CH_2)COO^-$	$-COCH_3$	$-OPO^{2-}_3$	-OH
30	$-OC(CH_2)COO^-$	$-CH_2OH$	$-OPO^{2-}_3$	-OH
A	$-OC(CH_2)COO^-$	$-C_2H_5OPO^{2-}_3$	$-OPO^{2-}_3$	-OH
B	$-OC(CH_2)COO^-$	$-COO^-$	$-OP(OC_2H_5)_2O$	-OH
C	$-OC(CH_2)COO^-$	$-C(CH_3)CH_2OPO^{2-}_3$	$-OPO^{2-}_3$	-OH
D	$-OC(CH_2)COO^-$	$-COO^-$	$-CH_2OPO^{2-}_3$	-OH
E	$-OC(CH_2)COO^-$	$-CH_2OPO^{2-}_3$	$-OP(OCH_3)_2O$	-OH
F	$-OC(CH_2)COO^-$	$-OP(OCH_3)_2O$	$-C_2H_4COO^-$	-OH
G	$-OC(CH_2)COO^-$	$-CH_2OCH_3$	$-OPO^{2-}_3$	-OH
H	$-OC(CH_2)COO^-$	$-COO^-$	$-OP(OCH_2Cl)O^{-}_2$	-OH
I	$-OC(CH_2)COO^-$	$-OC_2H_2$*	$-OPO^{2-}_3$	-OH
J	$-OC(CH_2)COO^-$	$-C_3H_3OH$*	$-OPO^{2-}_3$	-OH
K	$-OC(CH_2)COO^-$	$-CH_2OPO^{-}_3$	$-OPO^{2-}_3$	-OH
L	$-OC(CH_2)COO^-$	$-COO^-$	$-OP(OCH_3)_2O$	-OH
M	$-OC(CH_2)PO^{2-}_3$	$-CH_2OPO^{2-}_3$	$-OPO^{2-}_3$	-OH
N	$-OC(CH_2)COO^-$	$-COO^-$	$-OPO^{2-}_3CH_3$	-OH
O	$-OC(CH_2)COO^-$	$-CH_2OH$	$-OPO^{2-}_3$	-OH
Natural substrate	$-OC(CH_2)COO^-$	$-COO^-$	$-OPO^{2-}_3$	-OH

N.B. compound 2 is cyclic between R1 and R4. * Ring closure between C1 and C2
Compounds K, L, M, N and O are enantiomers of compounds 1, 10, 3, 9 and 30 respectively.

Table 2: Binding affinity of EPSP analogues to the choristmate synthase active site models1- 4 using their docking scores.

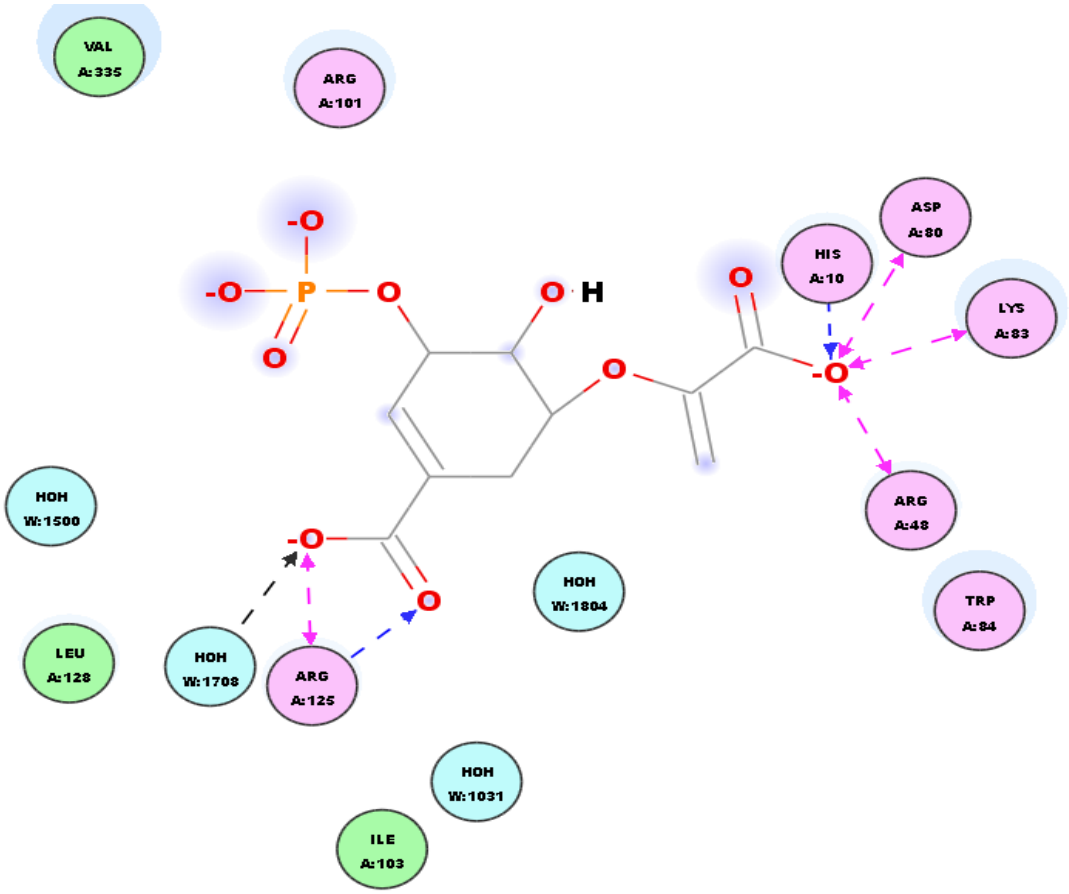
Chemical Sample	MODEL 1 DOCKING SCORES			MODEL 2 DOCKING SCORES		
	Dock 1	Dock 2	Dock 3	Dock 1	Dock 2	Dock 3
ligandSample1	-404.091	-380	-374.664	-460.558	-483.154	-502.068
ligandSample10	-405.853	-446.898	-429.364	-500.534	-522.212	-506.303
ligandSample2	-309.06	-307.703	-134.077	-358.115	-387.675	-360.398

ligandSample21	-349.161	-318.468	-323.714	-408.992	-382.646	-390.665
ligandSample22	-478.903	-325.714	-334.751	-376.313	-405.064	-247.489
ligandSample23	-300.285	-356.997	-308.58	-359.609	-391.281	-362.826
ligandSample24	-297.462	-289.365	-274.147	-341.364	-352.885	-341.878
ligandSample25	-384.635	-387.136	-342.293	-434.471	-467.687	-426.948
ligandSample26	-333.385	-313.596	-327.251	-422.567	-290.048	-412.138
ligandSample27	-279.671	-286.959	-441.552	-335.01	-356.27	-369.404
ligandSample28	-282.24	-280.388	-278.881	-343.221	-348.67	-328.019
ligandSample29	-352.311	-345.024	-358.713	-327.369	-475.41	-435.255
ligandSample3	-386.566	-357.406	-382.638	-515.283	-515.842	-482.483
ligandSample30	-362.679	-398.806	-346.759	-486.872	-282.657	-432.324
ligandSample4	-319.498	-304.55	-331.429	-426.762	-420.715	-400.564
ligandSample5	-325.636	-342.009	-328.176	-430.055	-446.75	-412.353
ligandSample6	-331.269	-329.798	-302.069	-442.58	-429.969	-418.497
ligandSample7	-347.184	-331.835	-350.044	-439.344	-413.883	-384.929
ligandSample8	-326.603	-334.024	-335.683	-400.284	-412.733	-400.376
ligandSample9	-390.082	-443.01	-382.001	-468.819	-458.348	-504.571
naturalsubSample1	-342.092	-352.149	-380.683	-433.547	-239.977	-455.958

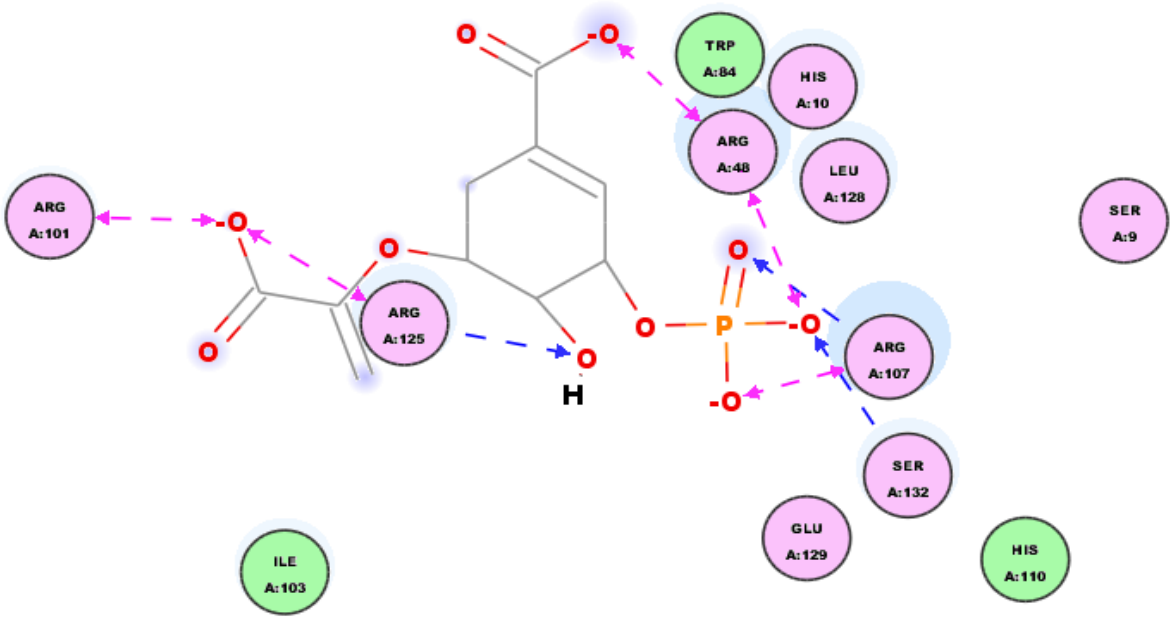
Chemical Sample	MODEL 3 DOCKING SCORES			MODEL 4 DOCKING SCORES		
	Dock 1	Dock 2	Dock 3	Dock 1	Dock 2	Dock 3
ligandSample1	-393.395	-582.314	-314.57	-378.254	-395.912	-406.731
ligandSample10	-360.016	-534.443	-432.294	-414.898	-449.941	-245.401
ligandSample2	-263.05	-387.756	-256.407	-205.96	-143.483	-133.157
ligandSample21	-90.174	-469.112	-290.121	-334.046	-320.88	-341.787
ligandSample22	-304.504	-452.286	-93.942	-313.259	-298.077	-319.265
ligandSample23	-256.415	-508.77	-213.785	-302.372	-154.249	-307.506
ligandSample24	-212.377	-367.295	-241.373	-270.72	-146.744	-280.622
ligandSample25	-374.133	-515.341	-317.381	-189.041	-334.193	-371.32
ligandSample26	-323.553	-475.903	-270.853	-194.944	-314.114	-346.938
ligandSample27	-145.322	-513.113	-253.696	-156.424	-312.094	-278.657
ligandSample28	-239.629	-392.252	-227.787	-274.996	-263.32	-270.728
ligandSample29	-338.18	-505.176	-347.187	-259.724	-338.509	-244.651
ligandSample3	-389.564	-574.878	-364.006	-417.303	-415.625	-394.269
ligandSample30	-335.983	-513.968	-329.62	-373.131	-390.548	-226.12
ligandSample4	-316.797	-454.019	-297.731	-332.468	-333.443	-151.708
ligandSample5	-302.195	-492.753	-287.308	-311.971	-229.438	-324.684
ligandSample6	-107.025	-432.501	-288.046	-344.097	-341.409	-331.562
ligandSample7	-310.285	-504.656	-261.02	-344.224	-351.74	-216.709
ligandSample8	-323.659	-457.32	-264.211	-317.729	-151.094	-321.318
ligandSample9	-356.552	-521.11	-365.633	-376.523	-365.362	-208.052
naturalsubSample1	-320.031	-485.663	-295.517	-331.646	-172.8	-326.518

Table 3: Binding affinity of new analogues of compound 1,3,9 and 10 to the choristmate synthase active site models 1- 4 using their docking scores.

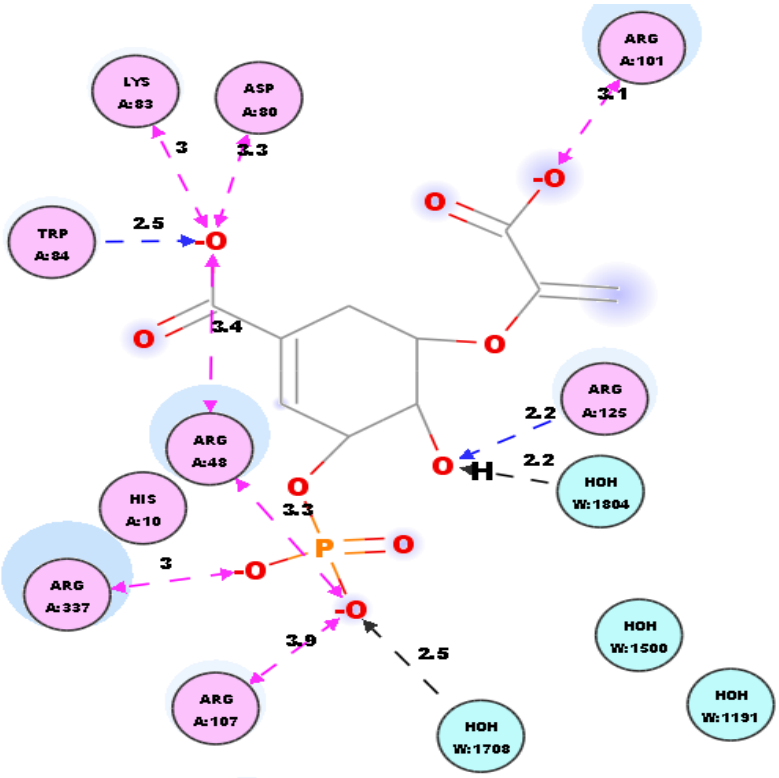
Compound	Model 1	Model 2	Model 3	Model 4
F	-457.855	-553.873	-495.655	-423.987
E	-426.186	-564.438	-471.569	-467.764
B	-475.812	-560.692	-443.142	-479.906
L	-430.882	-525.650	-413.760	-257.165
C	-428.856	-432.324	-404.142	-460.544
2	-350.00	-502.068	-375.024	-383.632
3	-425.90	-509.404	-393.529	-404.350
9	-384.349	-466.897	-168.630	387.598
10	-429.00	-551.345	-378.132	-412.141
Natural substrate	-337.00	-427.576	-332.574	-337.968



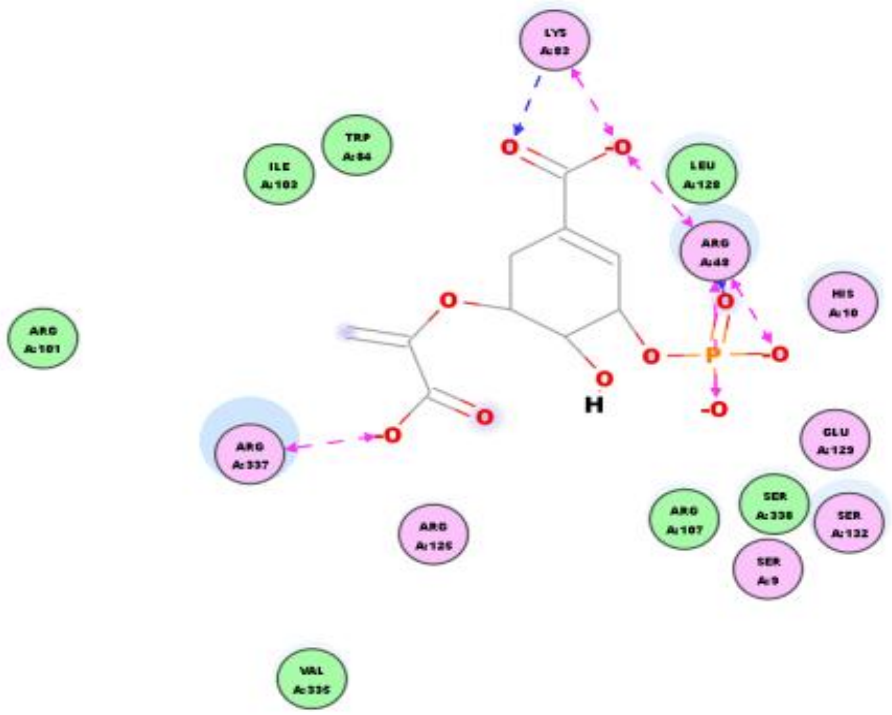
Model 1 Active site



Model 2 Active Site



Model 3 Active Site



Model 4 Active Site

Figure 2: Representation of active site models with bound natural substrate. The bound ligand is shown in stick form while the active site amino acid residues are shown in disc form. Blue disc shown in models 1 and 3 represents conserved water molecules.

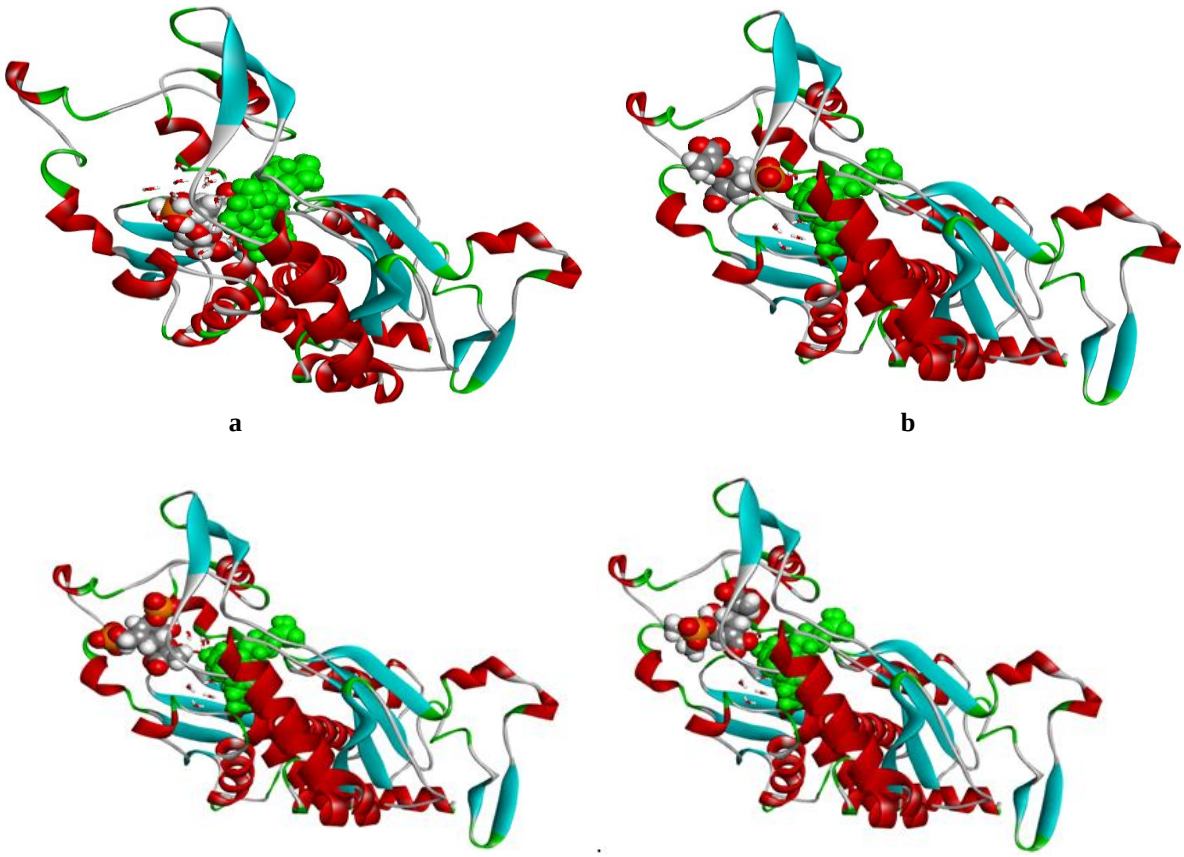
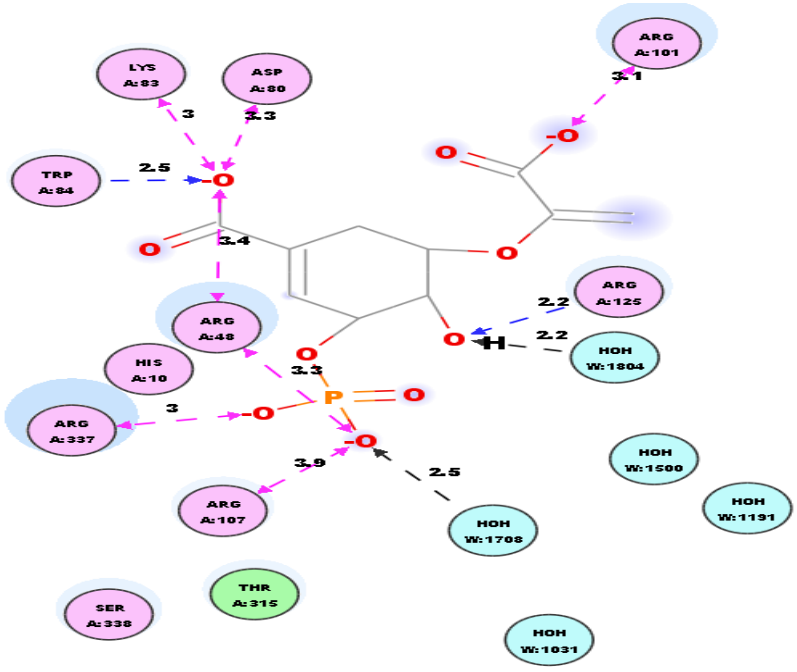
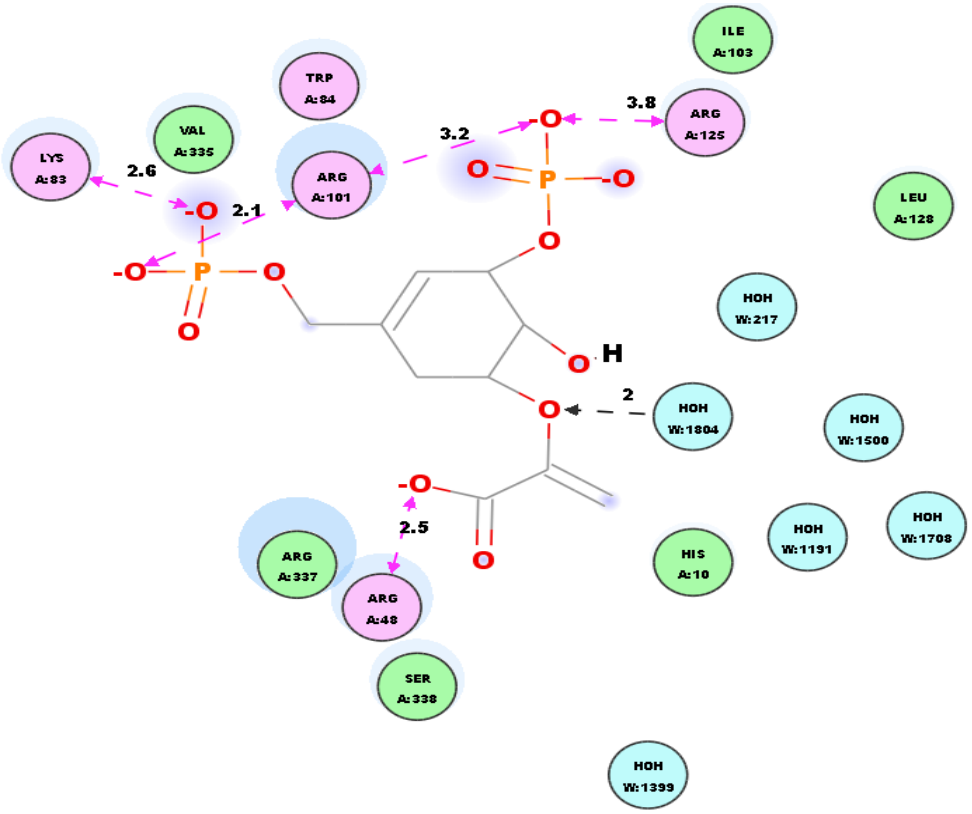


Figure 3: Ribbon representation of the CS structure in complex with co-factor FMN in green spheres and Ligand in coloured spheres with different degree of active site opening. Water molecules are represented in the stick form. (a) 1QXO.pdb; (b) natural substrate- EPSP; (c) compound 1 and (d) compound 10.



a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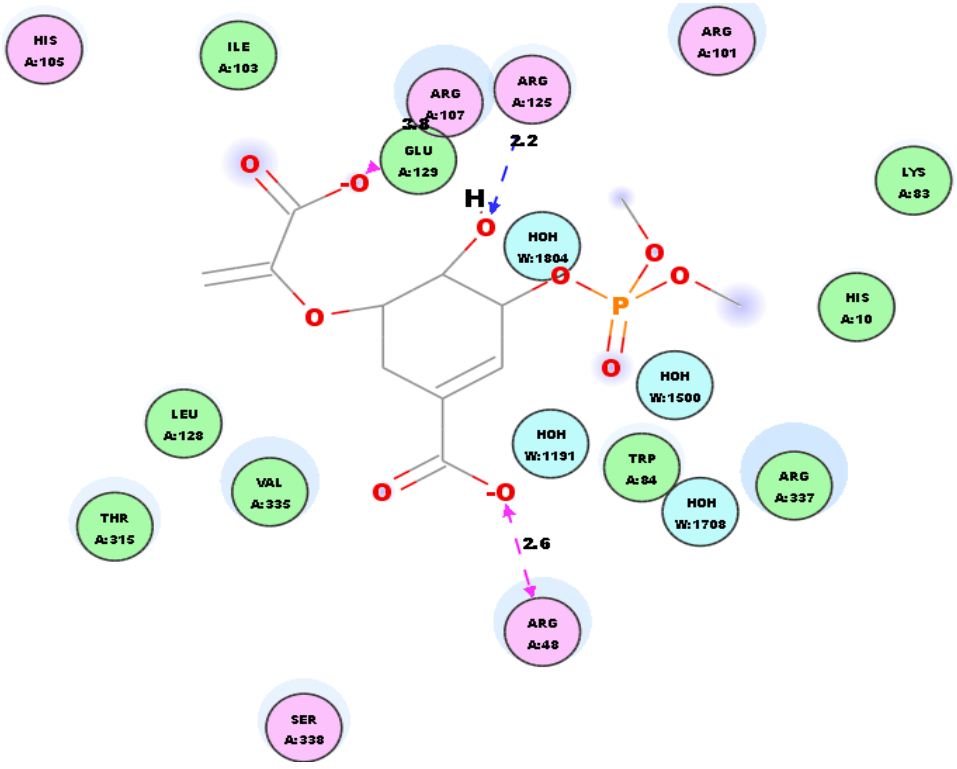
2.1
3.2
3.8
2
2.5

b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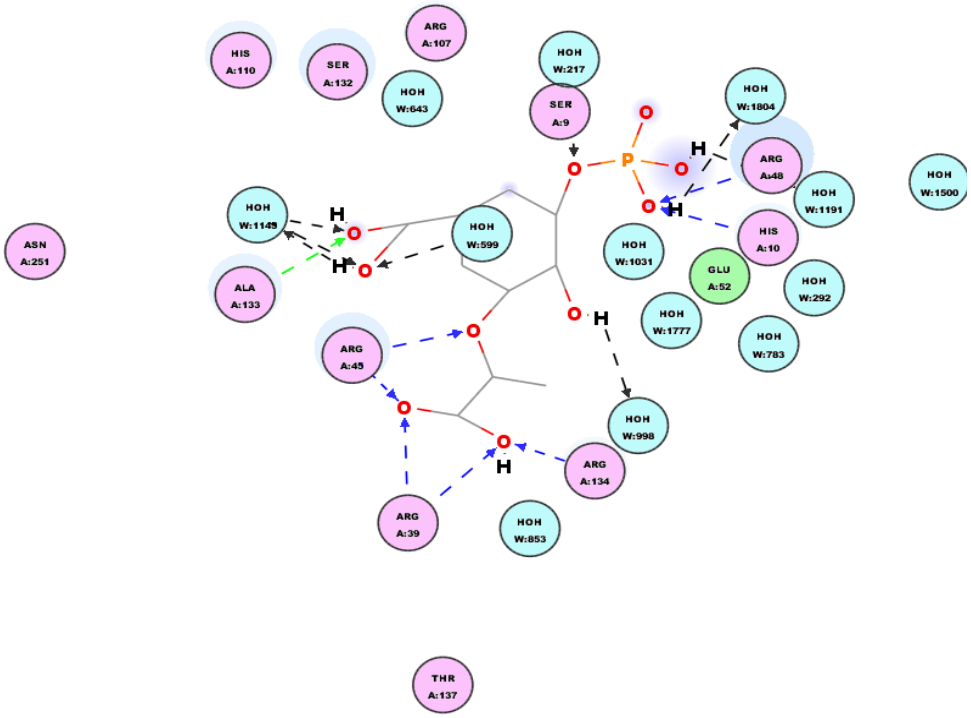
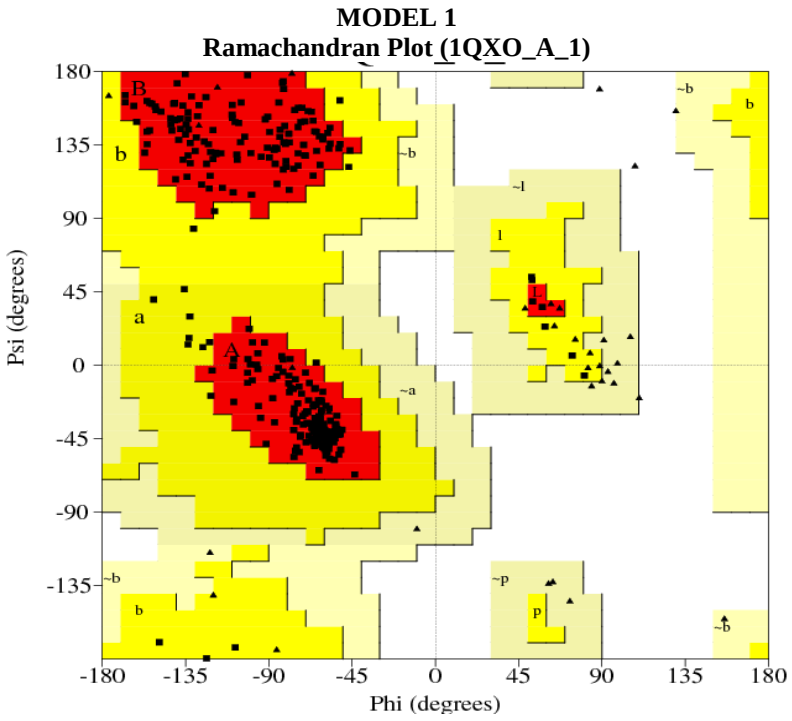
ARG A:48
SER A:338
2.2
2.6

c

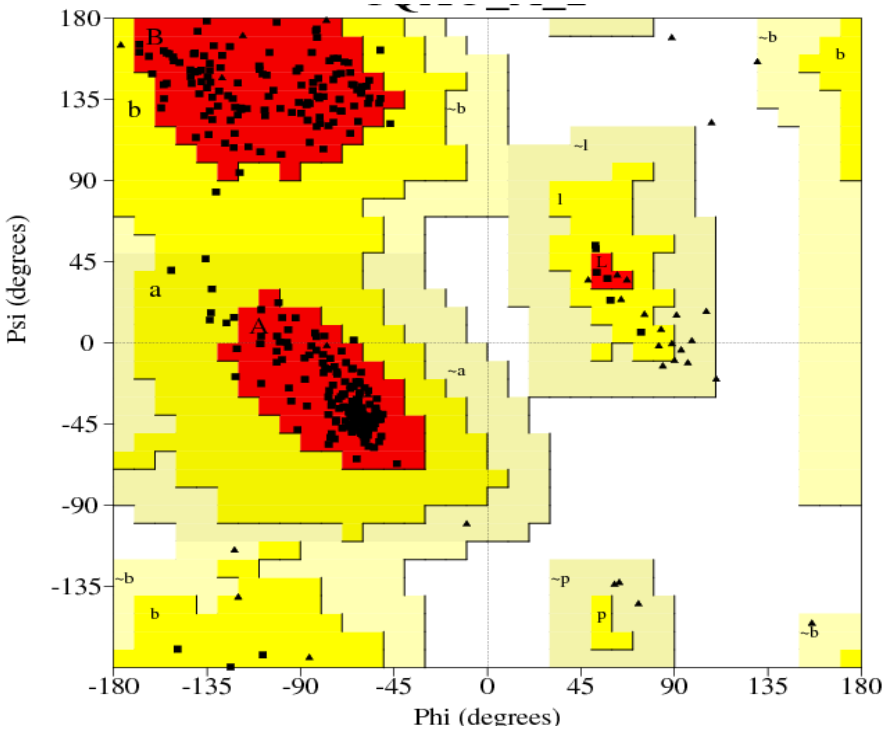


d

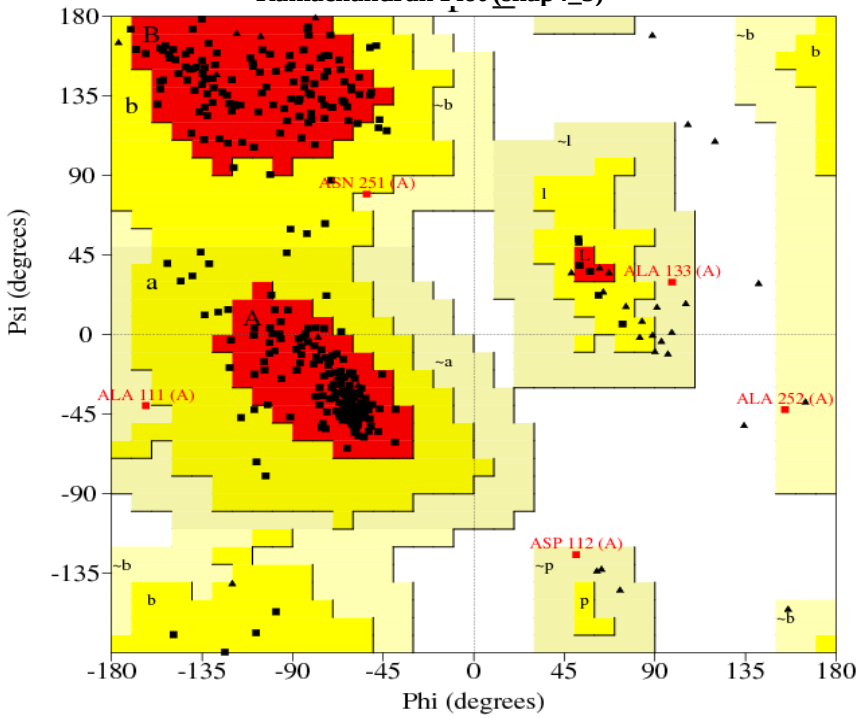
Figure 4: Binding pattern of natural substrate (a); compound 1 (b); compound 10 (c); and EPSP from 1QXO.pdb (d) ligands are shown in the stick form while amino acid residues and water molecules are shown in the disc forms. Discs in green indicate atoms showing Van der Waal's interaction while that in magenta indicates polar, charge and hydrogen –bond interactions. Water molecules are indicated in blue. Charge-charge interactions are shown in pink dash lines with arrows on both ends; blue dash lines indicates hydrogen bond interactions with amino acid side chains with arrow towards hydrogen bond acceptor; green dash lines indicate hydrogen bond interaction with amino acid main chain; black dash lines indicates hydrogen bond interaction with non- amino acid atoms.

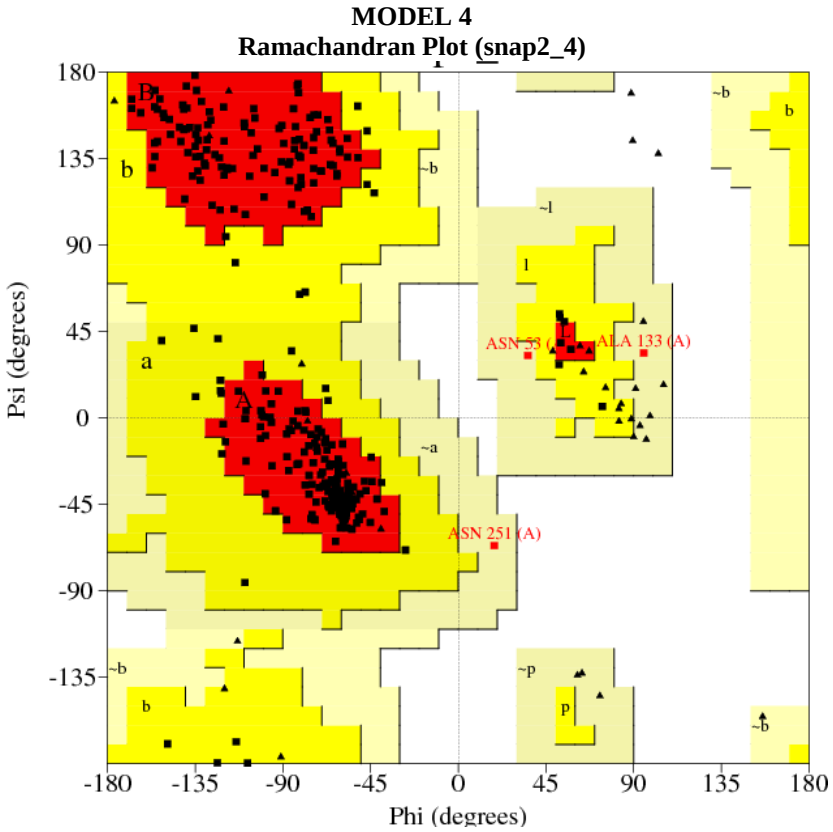


MODEL 2
Ramachandran Plot (1QXO_A_2)



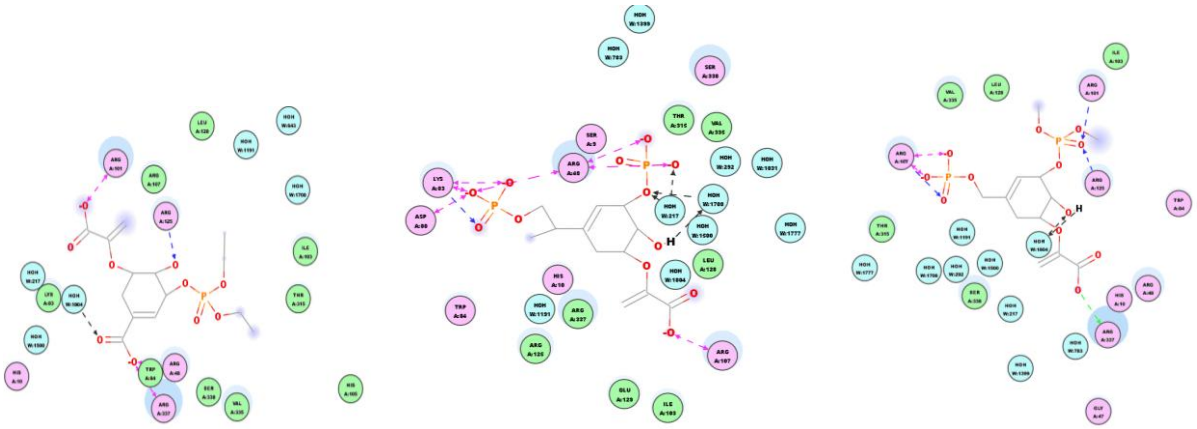
MODEL 3
Ramachandran Plot (snap4_3)



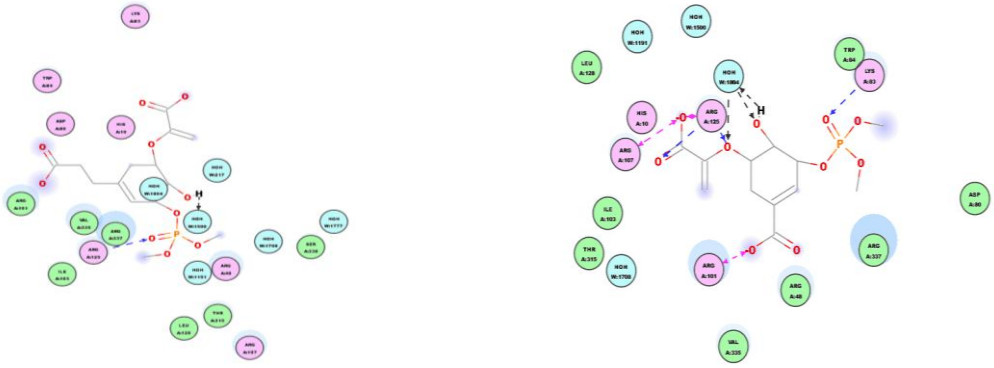


	Model 1		Model 2		Model 3		Model 4	
Residues in most favoured regions [A,B,L]	277	93.0%	278	93.3%	264	87.4%	272	90.1%
Residues in additional allowed regions [a,b,l,p]	21	7.0%	20	6.7%	33	10.9%	27	8.9%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0%	0	0%	5	1.7%	3	1.0%
Residues in disallowed regions	0	0%	0	0%	0	0%	0	0%
No. of non-glycine and non-proline residues	298	100%	298	100%	302	100%	302	100%
No. of end residues (excl. Gly and Pro)	25		25		21		21	
No. of glycine residues (shown as triangles)	38		38		38		38	
Total no. of proline residues	15		15		15		15	
Total No. of residues	376		376		376		376	

Figure 5: Plot Statistics based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%. A good quality model would be expected to have over 90% in the most favored regions.



Compound B Compound C Compound E



Compound F Compound L

Figure 6: Binding pattern of EPSP analogues. Ligands are shown in the stick form while amino acid residues and water molecules are shown in the disc forms. Discs in green indicate atoms showing Van der Waal's interaction while that in magenta indicates polar, charge and hydrogen –bond interactions. Water molecules are indicated in blue. Charge-charge interactions are shown in pink dash lines with arrows on both ends; blue dash lines indicates hydrogen bond interactions with amino acid side chains with arrow towards hydrogen bond acceptor; green dash lines indicate hydrogen bond interaction with amino acid main chain; black dash lines indicates hydrogen bond interaction with non- amino acid atoms.

Mechanism of chorismate synthase. The cofactor FMN is deeply buried into the active site (Fernandes, *et al.*, 2007) upon binding of EPSP, there is a tightening of the active site, preventing solvent access to FMN with EPSP blocking any possible exit of FMN from the active site (Fig 3a). In this study, analogues of EPSP were seen to be making interactions that left various degrees of opening of the active site, making it FMN solvent accessible and likely to exit the binding site (Fig 3 b-d).

Binding pattern of EPSP analogues. Binding interactions of substrates to enzyme's active sites including ionic, hydrogen and van der Waals bonding are of great importance. These interactions must be strong enough to hold substrate in place, just long enough for reaction to take place and weak enough to release the product. Therefore, an inhibitor would be expected to bind more strongly than the natural substrate, causing clogging of the enzyme active site and blocking the natural substrate's access to the enzyme site thereby stopping enzymatic reaction (Patrick, 2001).

The chorismate synthase crystal structure; 1QXO.pdb, from the *Streptococcus pneumoniae* is in the inactive state because it is bound to oxidised FMN, thereby inhibiting the conversion of ESPS to chorismate.

Therefore, any ligand which will bind to this conformation with a higher affinity than the natural substrate (EPSP) will be of great interest as a potential competitive inhibitor of the chorismate synthase enzyme. The EPSP analogues, compounds 1, 10 (Fig 4) and compounds B, C, E, F and L (Fig 6) show significant higher binding affinity to the enzyme. They also show conformational changes that put them far away from direct interaction with residues important for the elimination of phosphate from EPSP (Fig 4). The binding of phosphate is determined by the conformations of the residues between Tyr 331 and Pro 340. In the close conformation a salt-bridge interaction is formed between Arg 337 side chains and O1P and O2P and a hydrogen bond between its main chain carbonyl and O1P, phosphate has to be protonated by His 10 to make it a better leaving group (Fig 4d) and EPSP carboxylate group has to be in a conformation as to interact with His 110 (Maclean & Ali, 2003) which is lost on binding of compounds B, C, E, F and L (Fig 6).

Conclusion

The target of novel pathways, such as one observed in the final step of the shikimate pathway can be fully taken advantage of in the design of new therapeutic antibacterials in human medicine. This study chose to target this step by observing the enzyme's natural substrate analogues using docking studies. Twenty ESPS analogues were docked alongside EPSP (natural substrate) into Chorismate Synthase to identify potential inhibitors of Chorismate Synthase from their binding affinities and docking scores. Four analogues, compound 1, 3, 9 and 10 were observed to have higher binding affinities to CS over the natural substrate. This showed that modifications at C1 and C3-Phosphate Oxygens led to higher binding affinities. Bearing this in mind, further modifications were carried out on these two sites, this yielded compounds A, B,C, E and F having higher binding affinities than the leads and the natural substrate. Compounds E and F generally ranked higher because modifications were carried out on both sites i.e. C1 and C3-Phosphate Oxygens.

Four active site models were taken into consideration in this study, but active site model 3 was chosen as the most appropriate for docking studies as it contained conserved water molecules in the active site and it contained active site residues within $8A^{o}$.

Further optimisation could be carried out on these series of analogues to better understand their binding and subject to further studies of substitution about the C1 and phosphate oxygens.

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