ORIGINAL ARTICLE

Study of Molecular Basis of Interaction Between B12 Enzymes and Flavin Derivatives Using Molecular Docking Technique

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ABSTRACT

Objective: The kinetic studies indicate that riboflavin acts as a sensitizer in the photolysis of cyanocobalamin and in this way promotes the degradation of the molecule. Many microorganisms use light for pathogenesis. The present work describes the interaction of vitamin B_2 and B_{12} at molecular level in bacterial enzymes which could delineate the possible mechanism of inhibiting the disease producing bacteria.

Methodology: The molecular basis of interaction between the flavin derivatives and the B_{12}- dependent enzymes was studied using molecular modeling software, the MVD.. A series of nineteen flavin derivatives and three B_{12} containing enzymes; *glutamate mutase, diol dehydratase* and *methionine synthase* were taken for the study. The potential binding affinity between flavin derivatives and B_{12} enzymes was checked on the basis of lowest docking score, number of hydrogen bonds and favorable binding modes.

Results: We found riboflavin, 2-ketoflavin and 4-ketoflavin as the best interacting compounds with each of the three enzymes taken. The regions containing lone pair of electrons are critical for the maximum number of binding conformations.

Conclusion: It may be concluded that flavin derivatives may influence the activity of B_{12} enzymes. Therefore, the understanding of molecular basis of this interaction and the identification of the key factors involved, might be useful to design new molecules with enhanced selectivity towards B_{12} dependent enzymes. This can lead to the screening and discovery of new compounds as useful antibiotics and the active natural drugs.

Key words: B_{12}-enzymes, photosensitivity, flavin derivatives, molecular interaction studies, docking technique.

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INTRODUCTION

Cyanocobalamin (vitamin B_{12}) is a photosensitive compound and is highly susceptible to reducing agents. Recent advances in X-ray crystal structural determination of several B_{12} containing enzymes have played a major role in B_{12} enzymology and has revealed the amino acid sequences and motifs responsible for the interaction and recognition of the cofactor[1,2]. All the organometallic B_{12} forms are coenzymes in metabolically important enzymes i.e. methylcobalamin and adenosylcobalamin and they share a homology in their amino acid sequences as determined by their conserved domains[3,4]. Adenosylcobalamin serves as a source of free radicals for a group of enzymes that catalyzes the rearrangement reactions. Radicals play an important role in enzyme catalysis due to their specificity and high reactivity[5]. On the other hand, riboflavin (vitamin B_2) is a well known photosensitizer and an active participant in a series of proton coupled electron transfer reactions[6], which is associated with the breakdown and synthesis of macromolecules. Riboflavin is widely used in the therapeutic medicine due to intensive research in the chemistry of series of alloxazine and isoalloxazine compounds and the detection of their biological activity. Both of its subunits, ribitol and isoalloxazine possess radical scavenging ability[7,8].

Light is an abundant signal which is used by many organisms and has been linked to diseases[9]. Recently,

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disease causing protein photosensors have been found in pathogenic bacteria and fungi[10,11]. It was also found that flavins make non-covalent bonds with histidine residues in most of the bacteria[12]. Kinetic studies indicate that riboflavin act as a sensitizer in the photolysis of cyanocobalamin and in this way promotes the degradation of the molecule[13].

In view of these facts, present work was conducted to evaluate the mode of action of flavins in photodegradation of cyanocobalamin in disease producing bacteria at molecular level and the utilization of same photoreceptors in pathogenic bacteria for their degradation which was previously involved in pathogenesis. This study may be used as an important tool for the screening and discovery of the new vitamin B12 -based drugs and may possibly give rise to a new insight of vitamin B12.

Computational studies make it easier to elucidate the reaction mechanisms at the molecular level from ligand binding to the substrate activation and reaction[14]. Computer-based molecular modeling aims to speed up drug discoveries by predicting potential effectiveness of ligand-protein interactions prior to laborious experiments and costly preclinical trials.

In the present work, docking study has been carried out to examine the affinity of flavin derivatives for the substrate binding sites of B12-dependent enzymes. Molecular docking techniques are used to predict how a protein interacts with small or large molecules. It is an in-silico tool to simulate processes where a ligand position is determined in a predicted and predefined binding site in the receptor molecule. Docking algorithms have a common feature of using energy-based scoring function for the determination of most active conformation of the ligand.

A sophisticated molecular modeling software, the MolDock or Molegro Virtual Docker (MVD) 2011.5.0 for Windows was used for the study. MolDock is based on a new heuristic or hybrid search algorithm that handles all aspects of the docking processes from preparation of the molecule to determination and prediction of potential binding sites of the target protein and binding modes of the ligands. It is the implementation of evolutionary algorithm, focused on molecular docking simulations. It offers high quality docking based on a novel optimization technique as well as built in tools for advanced data analysis visualization[15].

Molegro Virtual Docker software yielded higher docking accuracy than other docking programs by docking flexible ligands to 77 protein targets; the accuracies were MVD, 87%; Glide, 82%; Surflex, 75%; and FlexX, 58%[15].

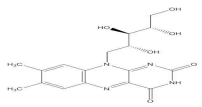
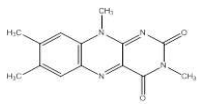
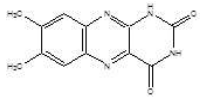
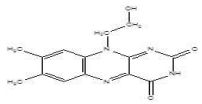
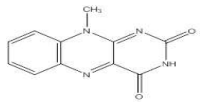
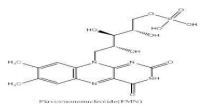
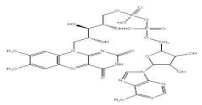
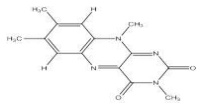
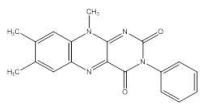
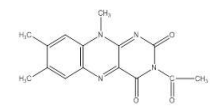
In this study, the parts of the riboflavin molecule and its derivatives, which are in intimate contact with the binding pocket of the coenzyme B12- dependent enzymes, have been identified which may provide a route for investigation on the possible mode of action of these compounds on the catalysis of B12- dependent enzymes.

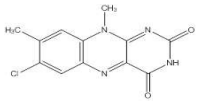
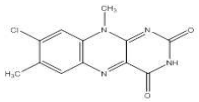
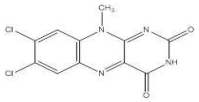
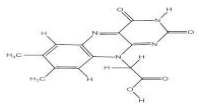
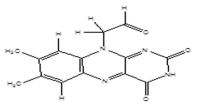
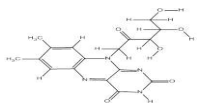
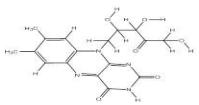
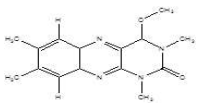
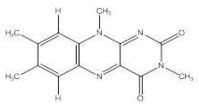
MATERIALS & METHODS

Test Set and Settings

Nineteen flavin derivatives[16,17] were selected for the study (Table 1).

Table 1. Structures of flavin derivatives

S. no	Ligands	Structure
1	Riboflavin	
2	Lumiflavin	
3	Lumichrome	
4	10-Hydroxyethylflavin	
5	10-Methylisoalloxazine	
6	FMN	
7	FAD	
8	3- Methyllumiflavin	
9	3-Benzyllumiflavin	
10	3-Acetyllumiflavin	

11	7-Chlorolumiflavin	
12	8-Chlorolumiflavin	
13	7,8-Dichlorolumiflavin	
14	Carboxymethylflavin	
15	Formylmethylflavin	
16	2-Ketoflavin	
17	4-Ketoflavin	
18	1,3,7,8-Tetramethy l-4-methoxyalloxazine	
19	3,10-Dimethylisoalloxazine	

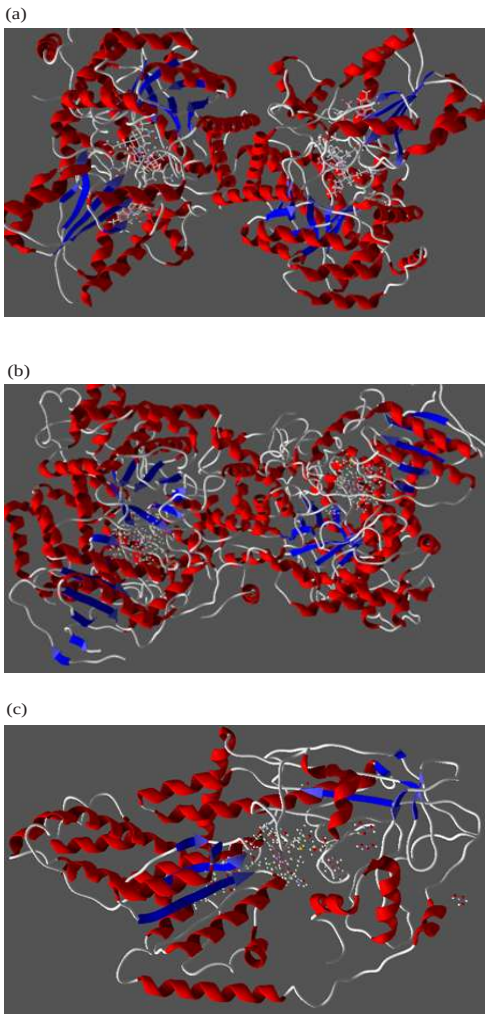


Fig. 1: Three-dimensional crystal structures of enzymes: (a) glutamate mutase PDB code: 1CCW (b) diol dehydratase PDB code: 1EGM (c) methionine synthase PDB code: 3IVA. Beta sheets are shown in blue, alpha helices in red and bound ligands; cobalamin and (TAR in a, PGO in b and SAH in c) are seen in ball and stick in element colors.

MVD requires 3D structures of both ligand and protein. Chem Bio draw ultra ver. 12.0 was used to build three dimensional structures of nineteen flavin derivatives selected for docking. The structures were drawn manually, cleaned in 3D format and saved as structure data file (SDF) files. The X-ray 3-D crystallographic structures of the protein targets used were extracted from the Protein Data Bank (PDB)[18]. These protein targets were Glutamate Mutase (GM), PDB code: 1CCW[19] (fig.1a), Diol Dehydratase (DD), PDB code: 1EGM[20] (fig.1b) and Methionine Synthase (MS), PDB code: 3IVA[21] (fig1c) complexed with co-crystallized ligands D-tartaric acid (TAR), propanediol (PGO) and s-adenosylhomocystein (SAH) respectively and bound co-enzyme B_{12} as shown in Figure 1.

Automated Preparation and Cavity Detection

In docking processes using MolDock, charges and protonation states are automatically assigned in combination with the automated prediction of cavities.

Search Algorithms and Scoring Functions

MVD is implemented with three search algorithms: MolDock optimizer, MolDock SE (simplex evolution) and Iterated simplex. In the present study, the MolDock SE was used with maximum iterations of 1500 and population size of 50. The scoring function was derived from the piecewise linear potential (PLP) scoring function[15].

Table 2: Binding energies calculated for flavin derivatives with the enzyme glutamate mutase by MVD with different scoring parameters

S. No	Name of the Ligand (kcal/ mol)	MolDock Score (kcal/ mol)	Rerank Score (kcal/ mol)	H bond Score (kcal/ mol)
1	Riboflavin	-101.13	-77.62	-13.90
2	2-Ketoflavin	-92.76	-72.39	-10.75
3	4-Ketoflavin	-105.39	-89.83	-12.27
4	Lumiflavin	-97.27	-88.73	-11.31
5	3-Benzyllumiflavin	-90.34	-61.14	-3.51
6	Lumichrome	-81.08	-67.78	-6.82
7	10-Hydroxyethylflavin	-92.29	-71.42	-11.98
8	FMN	-25.53	141.81	-9.63
9	FAD	-103.26	51.12	-8.52
10	3-Methyllumiflavin	-93.93	-83.39	-3.53
11	3,10-Dimethylisoalloxazine	-93.98	-83.43	-3.55
12	3-Acetyllumiflavin	-83.46	-71.97	-4.92
13	7-Chlorolumiflavin	-89.14	-78.32	-3.07
14	8-Chlorolumiflavin	-76.19	-68.99	-4.27
15	7,8-Dichloro lumiflavin	-87.40	-76.00	-3.59
16	Carboxymethylflavin	-92.27	-66.02	-2.45
17	Formylmethylflavin	-86.03	-71.93	-4.53
18	3,10-Dimethylisoalloxazine	-82.85	-74.23	-3.53
19	1,3,7,8-Tetramethyl -4-methoxyalloxazine	-87.97	-49.27	-4.77

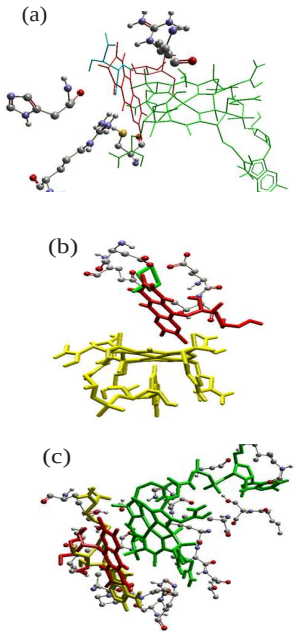


Fig. 2: Superimposition of riboflavin (B_2) on co-crystallized ligands of enzymes: (a) glutamate mutase; B_2 is seen in red, TAR in blue and coenzyme B_{12} in green (b) diol dehydratase; B_2 is seen in red, PGO in green and B_{12} in yellow (c) methionine synthase; SAH is seen in yellow, B_2 in red and B_{12} in green.

Re-Ranking Procedure

In MolDock, a re-ranking procedure is applied to the highest ranked poses i.e. with lowest MolDock score to further increase docking accuracy.

Docking Methodology

Flexible docking was performed following the standard protocol implemented in MVD 2011.5.0 for Windows. The enzymes from protein data bank and structures of nineteen flavin derivatives, were imported and cavities were detected by the cavity prediction algorithm of MVD to predict the potential binding sites for the proteins.

For each complex, 10 independent runs were conducted with the guided differential evolution algorithm and one pose was returned for each run. The most stable pose was selected according to the best MolDock score and re-rank score for each crystal structure.

RESULTS

The docking results obtained with each of the three enzymes are summarized in tables II, III and IV, selected on the basis of highest ranked pose i.e. lowest MolDock score. Highest binding affinity of the compounds has been identified on the basis of lowest docking energy. The superimposition of riboflavin on the co-crystallized ligands present in the active site cavity of the enzymes is shown in figure 2.

Docking of the top ranked inhibitors for each of the studied protein targets are shown in figures 3, 4 and 5 respectively. Hydrogen bond interactions and the H - bond distances between the atoms of active site residues and the selected ligands are given in table 5.

DISCUSSION

In this study, the parts of the riboflavin molecule and its derivatives, which are in intimate contact with the binding pocket of the coenzyme B_{12}- dependent enzymes, have been identified and we observed that flavin derivatives made non-covalent bonds with most of the "histidine" residues present in enzymes which has an important impact on the inhibition of disease producing bacteria. The discussions of the docking results for each of the three enzymes are as follows:

Glutamate Mutase

The crystal structure (figures 1a) shows that the enzyme is a heterodimer consisting of two identical functional halves. Each half possesses a B_{12} cofactor, E and S subunits and a co- crystallized ligand tartarate ion (TAR). Only one part of the heterodimer was taken for the present study.

The part of the enzyme where a TAR is located was presumed to be the active site of the enzyme to which the tartarate ion is connected via strong hydrogen bonds to the amino acid residues Arg 100, Arg 66, Arg 149,

Table 3: Binding energies calculated for flavin derivatives with the enzyme diol dehydratase by mvd with different scoring parameters.

S. No	Name of the Ligand	MolDock Score (kcal/ mol)	Rerank Score (kcal/ mol)	H bond Score (kcal/ mol)
1	Riboflavin	-110.33	-101.09	-12.76
2	2-Ketoflavin	-107.30	-59.97	-10.98
3	4-Ketoflavin	-102.12	-25.66	-8.01
4	Lumiflavin	-75.36	13.13	-4.98
5	Lumichrome	-65.46	77.76	-4.17
6	10-Hydroxyethylflavin	-68.93	-45.14	-6.26
7	3,10-Dimethylisoalloxazine	-65.60	-19.92	-4.53
8	FMN	-55.22	-5.78	-10.21
9	FAD	-31.74	73.47	-8.74
10	3-Methyllumiflavin	-71.01	-65.90	-0.62
11	3-Benzyllumiflavin	-94.72	-30.18	-0.30
12	3-Acetyllumiflavin	-83.38	45.27	-2.50
13	7-Chlorolumiflavin	-76.99	-2.571	-4.47
14	8-Chlorolumiflavin	-72.30	-63.35	-5.32
15	7,8-Dichloro lumiflavin	-70.60	-45.35	-0.78
16	Carboxymethylflavin	-91.43	-22.90	-6.78
17	Formylmethylflavin	-90.64	13.90	-6.53
18	1,3,7,8-Tetramethyl-4-methoxyalloxazine	-84.80	-76.15	-1.45
19	3,10-Dimethylisooalloxazine	-54.32	-49.98	-0.87

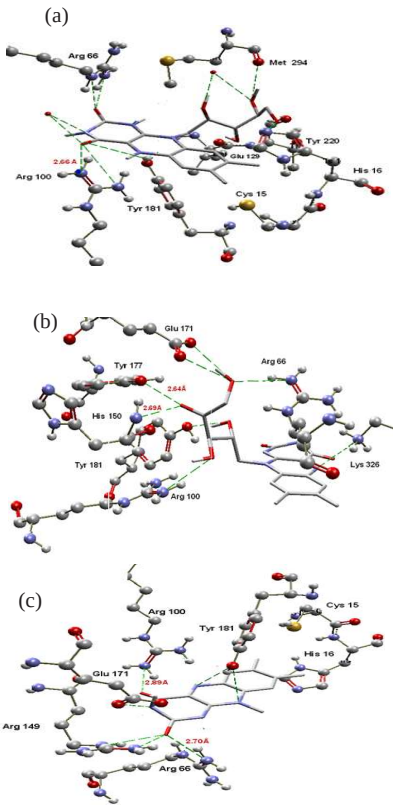


Fig. 3: Docking of flavin derivatives into the active site of glutamate mutase: (a) riboflavin (b) 4-ketoflavin (c) lumiflavin. Hydrogen bond interactions are shown in green dashed lines whereas hydrogen bond distances are given in Å in red. Flavin derivatives are represented in thick sticks and proteins in ball and stick model in element colors.

Glu 171, Tyr 181 and His 150.[19] The best docking conformations for the enzyme GM have been obtained with the compounds riboflavin, 4-ketoflavin and lumiflavin (figure 3).

In figure 3(a) riboflavin is connected with the binding pocket of enzyme via three hydrogen bonds and two occluded water molecules and seemed to occupy the space closer to the substrate binding site. The strong interaction was seen (O4) of the riboflavin and (NH2) of Arg100. While in figure 3(b) the docking space occupied by 4'-ketoflavin was almost seemed to overlap the space where the molecule TAR was located. The hydrogen bond interactions also showed the same amino acid residues as found within the active site cavity. The lowest MolDock score and rerank score was also observed with this compound (table 2). Lumiflavin occupied somewhat different position as compared to riboflavin and 4'-ketoflavin probably due to the absence of the ribityl chain (figure 3c). The other flavin derivatives also seemed to favor the interaction except FMN and FAD as indicated by their MolDock score and re-rank score with relatively small number of or no interactions and were therefore, not described in detail.

Diol Dehydratase

Crystal structure of Dioldehydratase (DD) reconstituted with cyanocobalamin (figure 1b) was taken and monomer alpha of heterodimer was selected for the study. The á subunit constitutes the active site having (â/á)8 TIM barrel domain that occupies the substrate.

The co-crystallized ligand, propanediol (PGO) and K^+ ion are deeply buried inside the barrel attached via hydrogen bonding to the residues Ser 362, Glu 170, His 143, Asp 335 and Gln 296.The best docking conformations were obtained with riboflavin, 2'-ketoflavin and 4-ketoflavin and are shown in figure 4.

In figure 4(a), riboflavin was almost superimposed on the ligand PGO complexed with diol dehydratase and the oxygen (O5) of hydroxyl group of riboflavin made three hydrogen bonds. The lowest MolDock score and re-rank score was observed for this compound as well as lowest H- bond score. Studies showed that His143 and Asp335 participate in dehydration process for product formation[22]. The high re-rank score for 2'-ketoflavin and 4'-ketoflavin (table 3) suggested that the compounds might not accommodate themselves completely within the binding pocket of the enzyme probably due to some repulsions between the charges. The remaining derivatives did not exhibit any significant binding conformation.

Methionine Synthase

The cobalamin cofactor serves as both acceptor and donor of methyl group during which it oxidizes once

Table 4: Binding energies calculated for flavin derivatives with the enzyme methionine synthase by MVD with different scoring parameters

S. No	Name of the Ligand	MolDock Score (kcal/ mol)	Rerank Score (kcal/ mol)	H bond Score (kcal/ mol)
1	Riboflavin	-100.92	-87.75	-13.18
2	2-Ketoflavin	-106.33	-85.64	-6.79
3	4-Ketoflavin	-107.70	-89.33	-8.47
4	Lumiflavin	-86.22	-75.93	-2.22
5	Lumichrome	-84.10	-73.57	-2.31
6	10-Hydroxyethylflavin	-97.13	-83.35	-5.61
7	10-Methylisoalloxazine	-86.58	-76.39	-2.38
8	FMN	-80.63	-68.58	-9.31
9	FAD	-68.50	-9.63	-6.103
10	3-Methyllumiflavin	-82.17	-71.31	-1.55
11	3-Benzyllumiflavin	-94.63	-84.77	-0.95
12	3-Acetyllumiflavin	-89.84	-79.65	-0.63
13	7-Chlorolumiflavin	-79.39	-67.38	-1.29
14	8-Chlorolumiflavin	-87.58	-73.14	-2.13
15	7,8-Dichloro lumiflavin	-88.22	-4.67	0
16	Carboxymethylflavin	-95.70	-78.15	-2.75
17	Formylmethylflavin	-96.55	-80.36	-2.5
18	3,10-Dimethylisoalloxazine	-81.87	-70.72	-0.43
19	1,3,7,8-Tetramethyl-4-methoxyalloxazine	-90.18	-80.74	-2.45

in every 2000 catalytic cycles and must be reactivated in aerobic organisms[21].

In the present study the X-ray crystal structure of methionine synthase reconstituted with cob (II) alamin and s-adenosyl homocystein (SAH) was used (figure 1c).

The structure reveals the positioning of s-adenosyl homocystein with respect to the cobalamin cofactor in the reactivation complex[21]. The SAH binds with the active site through hydrogen bonds to the residues Ala 1141, Tyr 1190, Arg 1134, Arg 1094 and Asp 946. The best conformations have been found with riboflavin, 2'-ketoflavin and 4'-ketoflavin and are shown in figure 5.

Riboflavin in figure 5(a), appears to be easily superimposed on the co-crystallized ligand but did not seemed to favor the interaction with the amino acid residues present in the large binding pocket of enzyme methionine synthase. Only one strong H- bond was formed with Arg1134. In figure 5(b), the 2'-carbonyl of 2'-ketoflavin did not show any interaction. Similarly in figure 5(c), 4-ketoflavin showed one H-bond interaction with Arg1134. The observed MolDock score and Re-rank score showed somewhat significant interaction.

The ineffective participation functional groups of the derivatives such as 7or 8-chlorolumiflavin, 3, 10-dimethylalloxazine, 1,3,7, 8-tetramethyl-4-methoxyalloxazine etc. did not show any effect in the interaction.

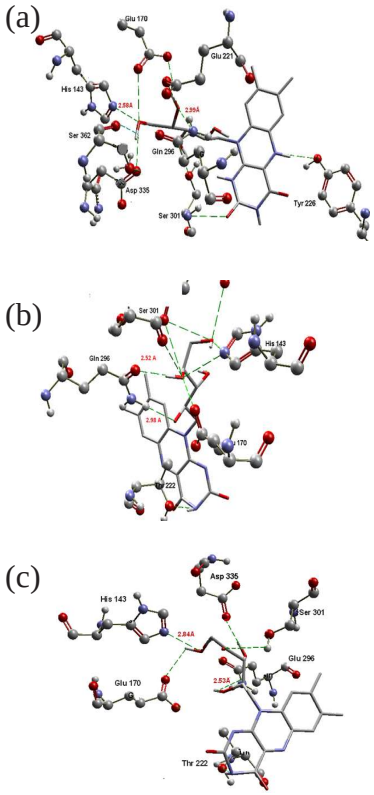


Fig. 4. Docking of flavin derivatives into the active site of enzyme diol dehydratase: (a) riboflavin (b) 2-ketoflavin (c) 4-ketoflavin. Hydrogen bond interactions are shown in green dashed lines whereas hydrogen bond distances are given in Å in red.

CONCLUSION

The present work suggests that multiple binding modes of riboflavin and the selected derivatives are possible with the B12 containing enzymes and therefore, they can interfere the function of coenzyme B12 dependent enzymes in disease producing microorganisms as observed from results showing the non-covalent bond formation with the histidine residues in catalytic activity.

As we know that cobalamin dependent enzymes need radicals to catalyze the biological reactions. Such radicals can be generated from either enzyme bound coenzymes or proteins possibly due to light reactions. Modifications at the radical center of coenzyme B12 may intensely affect the radical formation and stability and thus provide a rationale for the inhibition of the enzyme catalysis by the substrate analog or inhibitor. This fact can be taken into account to investigate the radical scavenging property of riboflavin in radical initiating enzyme catalyzed reactions of adenosyl and methylcobalamin dependent enzymes. Radical based enzyme reactions are difficult to investigate experimentally except by the use of Electron Spin Resonance (ESR) Spectroscopy and thus require theoretical studies for its understanding.

Table 5: List of hydrogen bonding between ligand atoms and amino acid residues in the active site cavity of enzyme glutamate mutase, diol dehydratase and methionine synthase.

S. No	Enzyme name	Ligand name	Ligand atom	Amino acid residue atom	H- bond distance (Å)
1	Glutamate Mutase(1CCW)	Riboflavin	O4	Arg100 (NH_2)	2.66
		4-Ketoflavin	O4	Tyr 177 (OH) His 150 (NH)	2.64 2.69
		Lumiflavin	O4 O2	Arg100 (NH_2) Arg 66 (NH_2)	2.89 2.70
2	Diol Dehydratase (1EGM)	Riboflavin	O5' O4'	His 143 (NE2) Gln 296 (NE2)	2.58 2.99
		2-Ketoflavin	O2 O4'	Gln 296 (NE2) Gln296 (OE1)	2.98 2.52
		4-Ketoflavin	O5' O2'	HIS 143 (NE2) Gln 296 (NE2)	2.84 2.53
3	Methionine Synthase (3IVA)	Riboflavin	O4'	Arg 1134 (NH_2)	2.91
		2-Ketoflavin	O4'	Arg1134 (NH_2)	2.81
		4-Ketoflavin	O4'	Arg1134 (NH_2)	2.51

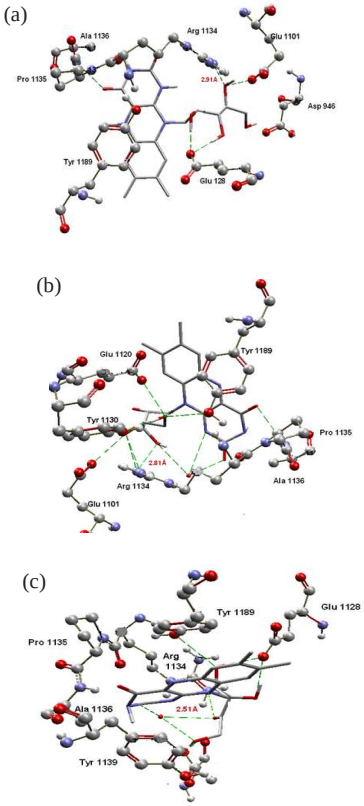


Fig. 5: Docking of flavin derivatives into the active site of enzyme methionine synthase: (a) riboflavin (b) 2-ketoflavin (c) 4-ketoflavin. Hydrogen bond interactions are shown in green dashed lines. Hydrogen bond distances are given in red in Å.

Further experimental approaches can be adopted to probe the effects of structural alterations of flavins in the catalytic properties of coenzyme B_{12}- dependent enzymes.

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