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Active site binding interactions of β-carboline derivative for HIV reverse transcriptase, protease and integrase

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Abstract— Recently Anti-HIV activity of β-carboline derivatives is reported. The highly active compound 1-Formyl-beta-carboline-3-carboxylic acid methyl ester which has anti-HIV activity (IC_{50} = 2.9 µM) was docked into the active sites of HIV reverse transcriptase (RT), integrase (IN) and protease (PR). The compound was showing good binding energy score and binding interactions with RT as compared to PR and IN after comparison of docking results. The compound showed two H bonding interactions with Lys103 residue and good binding free energy score -8.63 Kcal/mol at temperature 298.15 K for HIV RT protein.
Keywords: β-carboline derivative, HIV, Reverse transcriptase, Integrase, Protease

1. Introduction

The human immunodeficiency virus (HIV) is the causative agent for the acquired immunodeficiency syndrome (AIDS). The *pol* gene of HIV encodes three key enzymes to propagate its life cycle. (1) Reverse transcriptase (RT), also known as RNA-dependent DNA polymerase, (2) Integrase (IN) and (3) HIV protease (PR). RT, PR and IN are found important targets for the treatment of AIDS. During infection, HIV integrates its RNA into host genome by fusion of viral particle with cell membrane. The viral RNA is being used as a template for synthesis of double stranded DNA using RT enzyme. The viral cDNA is integrated into host genome in the form of provirus. Depending upon the site of integration, the provirus can be constitutively transcribed if it is integrated near an active promoter, or remain silent until a stress response triggers transcription. The new virus particles are released from host by using host machinery for transcription followed by translation, packaging, fusion and maturation. These enzymes are potential drug targets for chemotherapeutics [1-3].

Natural products and its derivatives are rich source for biologically active compounds. Many natural and its derivatives products have been used as lead molecules for further modifications to improve their anti-HIV activities [4-6]. β-carboline occur naturally in the seeds of *Peganum harmala.* β-carboline derivatives have shown a diverse range of biological activities including anti-HIV activity [7-9]. Recently, anti-HIV activity of beta-carboline derivatives has been reported using human CD4+ T cell line (CEM-GFP) infected with HIV-1 $NL_{4.3}$. The Objective of the present study is to explore the binding mode of highly active β-carboline derivative with HIV RT, PR and IN using computational docking methodology. The compound 1-Formyl-beta-carboline-3-carboxylic acid methyl ester showed good anti-HIV activity in a human CD4+ T cell line (IC_{50}= 2.9 µM) [10] but it is not specified for which particular HIV drug target. Hence, this compound was selected for docking studies against active sites of HIV-1 RT, PR and IN proteins. The objective of this study is to explore the active site binding mode with binding energy and interactions for HIV drug targets RT, PR and IN proteins. Prediction of interactions between small molecules and proteins is a crucial step to decipher many biological processes and plays a critical role in drug discovery. When detailed 3D structure of the protein target is available, protein-ligand complexes are obtained with binding affinity and interactions using computational methodology [10-15]. Binding energy calculated from programs like AutoDock, Dock, Gold program etc is reliable.

2. Methods

Docking studies of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester were carried out using AutoDock 4.2 program [16]. AutoDock 4.2 has a free-energy scoring function that is based on a linear regression analysis, the AMBER force field and Monte Carlo simulated annealing (SA) method. Crystal structures of HIV RT, IN and PR proteins were used for docking studies, their PDB ID's are 3FFI, 1QS4 [17] and 1HSG [18], respectively. Highly active β-carboline derivative, 1-Formyl-beta-carboline-3-carboxylic acid methyl ester compound was built using SYBYL7.1 molecular modeling package [19] installed on a Silicon Graphics Fuel Work station running IRIX 6.5. Minimization of compound was done using Tripos force field, Gasteiger Huckel, partial atomic charges [20] and powell's conjugate gradient method with energy gradient convergence criteria of 0.05 kcal/mol [21]. AutoDock Tool was used for preparation of all protein structures for docking. Co-crystallized ligands and all water molecules were removed from protein structures. Also, Polar hydrogen's were added and non polar hydrogen's were merged, finally atom type parameter and Kallman united atom charge was added. Grid sizes for all proteins were adjusted such that it includes active site and a significant portion of the surrounding surface. For docking study Lamarckian genetic search algorithm was employed and docking run was set to 30. All other parameters were set to default Value: maximum number of energy evaluation 25,

000, 00 per run; maximum number of generation in the genetic algorithm was increased to 27,000 [16].

3. Docking Study

Brahmbhatt, K. G. et al., studied the inhibtion of HIV by β-carboline derivatives and reported that β-carboline derivative 1-Formyl-beta-carboline-3-carboxylic acid methyl ester inhibits HIV with inhibition rate of 78.17% at non-cytotoxic concentration [10]. The docking result of HIV RT indicates that 1-Formyl-beta-carboline-3-carboxylic acid methyl ester fits well into the active site of HIV RT protein as illustrated in Fig. (**1**). The H-bonding interactions were observed with Lys103 which have been reported to be important for inhibition [22]. Docking results of HIV RT are shown in Table 1. The indole group of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester compound was found in close proximity of Lys102, Lys103, Lys104, Val106, Pro225, Phe227 and Pro236 residues. These residues exhibited hydrophobic interactions and also strengthens the binding of inhibitor to the active site. H-bonding interactions were observed in NH--O=C Lys103 and C=O--HN Lys103. Presence of hydrophobic residues in the active site and multiple ring system inhibitors strengthen the hypothesis that hydrophobic interactions are important for inhibition [23, 23]. 3-Carboxylic acid methyl ester portion observed near Leu100 and Leu234 residues and 1-Formyl-beta-carboline-3-carboxylic acid methyl ester was observed to be well superimposed on the co-crystallized ligand as shown in Fig. (**1**). The compound showed good binding free energy -8.63 Kcal/mol at temperature 298.15 K. On the basis of the above findings the docked conformation of the inhibitor can be considered as the most favorable conformation.

The docking results of HIV-1 PR showed H-bonding interaction with active site residues described in Table 1. The H-bonding interaction exhibited by C=O group of 3-carboxylic acid group i.e. C=O--HN Ile50, and C=O--HN Ile50'. The indole portion of this compound is surrounded by Ala28', Gly27', Asp29', Val32' Ile50, Pro81' and Val82' which showed hydrophobic interaction. 3-carboxylic acid methyl ester portion of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester is surrounded by Ile50', Ile50 and Gly47' as illustrated in Fig. (**2**). This docked conformation was not well superimposed with co-crystallized ligand and also the binding free energy was observed to be -5.86 Kcal/mol at temperature 298.15 K.

The docking result of β-carboline derivative 1-Formyl-beta-carboline-3-carboxylic acid methyl ester into the active site of HIV-1 IN protein is illustrated in Fig. (**3**) and Table 1. The molecule exhibits H-bonding interactions with His67, Lys156, and Lys159 residues. As reported in various literatures [25-32], most of the compounds consistently exhibited H-bonding towards catalytic residues Asp64 and Asp116. These residues are important for integrase activity. But this compound does not show any H-bonding with these residues. The indole portion of the compound was observed to be surrounded by Asp64, Glu152, and Lys156 residues. 3-carboxylic acid methyl ester portion of the compound was located near Thr66, His67 Lys156 and Lys159 which exhibited hydrophobic interactions to these residues. The highly electronegative atoms (oxygen of –OH and oxygen of -C=O groups) formed H bond with His67 Lys156 and Lys159. The docking analysis revealed that compounds bound differently to the active site than 5-CITEP. On the basis of experimental data (Anti HIV-1 activity of that compound) and docking results, we can hypothesize that this compound may be active against HIV RT.

4. Conclusion

The docking studies of β-carboline derivative, 1-Formyl-beta-carboline-3-carboxylic acid methyl ester were performed into the active sites of HIV-1 RT, IN and PR. We concluded from these docking studies that compound showed binding interactions and good binding energy with HIV RT protein as compared to PR and IN protein. It was also found that compound oriented similar to co-crystallized ligand, pyridone diaryl ether non-nucleoside inhibitor into the active site of RT.

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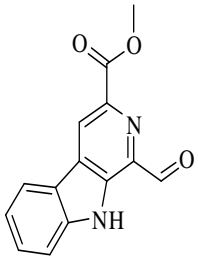
Table 1- *H-bonding interactions and estimate total binding energy observed between compounds and active site of RT, PR, and IN*

Compound	RT H-Boning	PR H-Boning	IN H-Boning
 Estimate binding free energy (Kcal/mol at temperature 298.15 K)	NH--O=C Lys103 (2.24) C=O--HN Lys103 (1.91) -8.63	C=O--HN Ile50 (2.14) C=O--HN Ile50' (2.68) -5.86	HO--HN His67 (2.05) C=O--HN Lys156 (2.18) HO--HN Lys159 (2.05) -4.62

The value in parenthesis is H-bonding distance between donor/acceptor atom of ligand and protein acceptor/donor atoms and ' indicate B chain of PR.

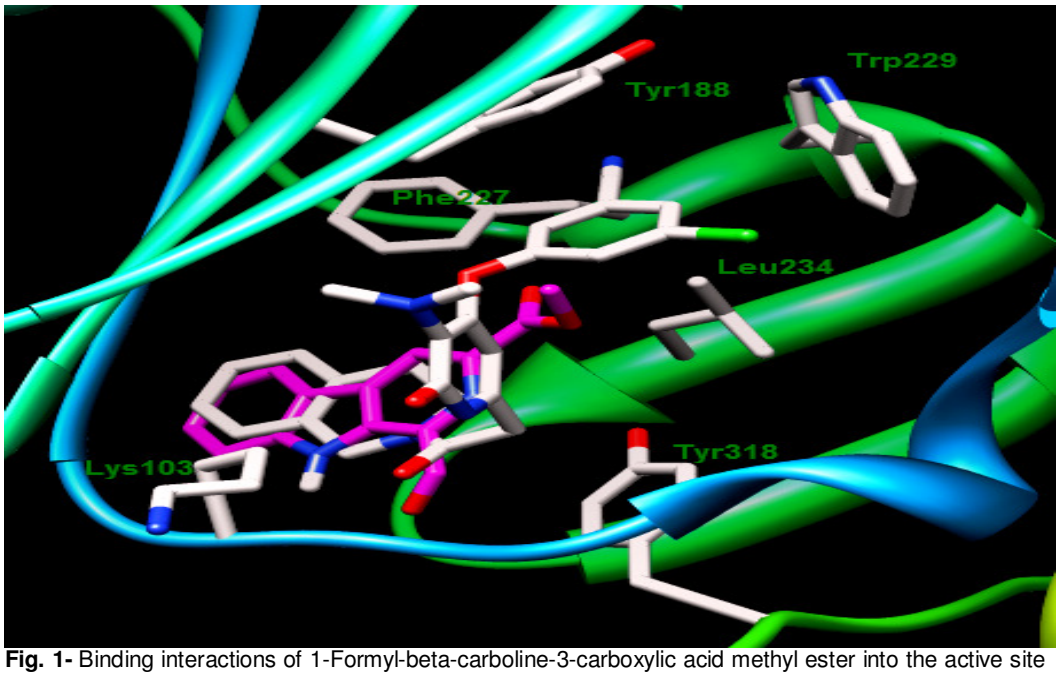


Fig. 1- Binding interactions of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester into the active site of RT (3FFI) as obtained by docking studies. Magenta color sticks for docked conformation of compound; white color sticks for co-crystallized ligand.

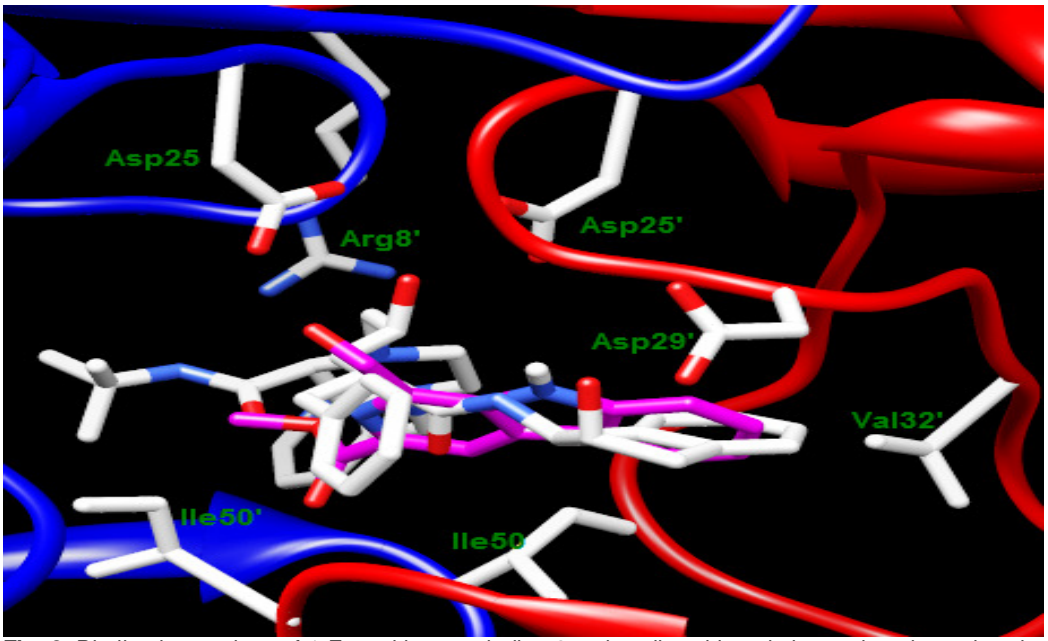


Fig. 2- Binding interactions of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester into the active site of PR (1HSG) as obtained by docking studies. Blue color represent chain A, Red color represent chain B; Magenta color sticks for docked conformation of compound; white color sticks for co-crystallized ligand.

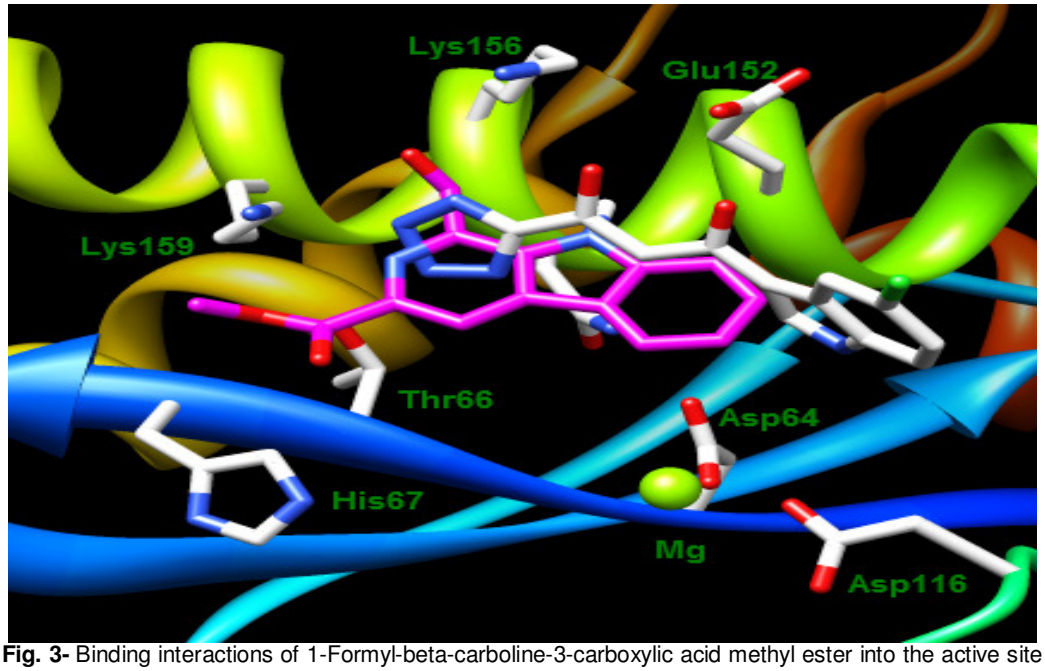


Fig. 3- Binding interactions of 1-Formyl-beta-carboline-3-carboxylic acid methyl ester into the active site of IN (1QS4) as obtained by docking studies; Magenta color sticks for docked conformation of compound; white color sticks for co-crystallized ligand; Green ball for magnesium ion.