Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/9.1.20230219

Research article
2023 | Volume 9 | issue 1 | Pages 25-30

ARTICLEINFO

Received
January 04, 2023
Revised
February 25, 2023
Accepted
March 27, 2023

***Corresponding Author**
Samar Shahzadi
E-mail
samar.iiui@gmail.com

Keywords
Bioinformatics
In silico structure prediction
Hepatitis B virus
Genotype A2
HBeAg

How to Cite
Shahzadi S, Tahir Q, Khan MI, Raza A, Khan MF, Larra SA. *In silico* structure prediction and molecular docking analyses to reveal potential binding domain of Hepatitis B virus genotype A2. Biomedical Letters 2023; 9(1):25-30.

Scan QR code to see this publication on your mobile device.

Open Access

In silico structure prediction and molecular docking analyses to reveal potential binding domain of Hepatitis B virus genotype A2

Samar Shahzadi[1,*], Qanta Tahir[1], Muhammad Imran Khan[2], Ali Raza[2], Muhammad Fardeen Khan[3], Sajjad Ahmad Larra[4]

[1]Department of Bioinformatics, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Department of Microbiology and Molecular Genetics, University of Okara, Okara, Pakistan
[3]Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[4] National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

Abstract

Hepatitis B Virus (HBV) infects the hepatocytes to cause serious liver diseases. HBeAg regulates the response of immune system to the intracellular capsid act as T-cell tolerogen. The immune response regulation may predispose to chronicity during perinatal infections to prevent the severe liver injuries. Various *in silico* approaches including comparative modeling, threading approach and *ab initio* approach were employed for the prediction of 3D structures of the selected protein followed by the validation of the predicted structures through Errat, Procheck and Anolea. The predicted 3D structure of HBeAg revealed overall quality factor of 95.9184%. Interestingly, it was observed that only 1.97% residues were present in outlier region while 98.03% in favored and allowed region. Molecular docking analyses were performed and the attempt was for the identification of novel ligands for HBeAg. The reported compound may regulate the activity and act as regulator of HBeAg. Interestingly, least binding energy of -7.1 Kcal/mol was observed in the reported compound and high binding affinity to predict the binding residues (Asp-51, Phe-53, Val-56, Arg-57, Met-95, Ala-98, Asn-103, Arg-111, Asp-112, Val-115, Val-118 and Asn-119). The function determination of the selected target protein is due to the identification of effective binding sites in protein structures. The reported compound may act as potent molecule and the predicted structure is reliable for the functional studies and structural insights.

Introduction

Hepatitis B virus (HBV) infects hepatocytes, cell type of parenchymal tissues of liver to cause liver diseases. HBV life cycle is complex and has the ability to develop numerous antiviral agents. The life cycle of HBV has relaxed-circular partially double-stranded genomic DNA (rcDNA), converts into a molecular template DNA to enhance the viral RNA intermediate. Chronic infection results due to high stability of HBV which is poorly curable [1]. 250 million people are assumed to be chronically effected by HBV including one million deaths yearly. HBV uses numerous ways to tackle the host innate immunity to increase its replication includes taking advantage of the growing immune system of young children to smooth its persistence. It can also use maternal viral E antigen to guide the immunity of the offspring to support its perseverance after vertical transmission [2]. HBV is one of the familiar worldwide blood-borne pathogen. The chronic hepatitis B leads to an inactive carrier state which results in cirrhosis and fatal liver cancer. HBV surface-antigen vaccine is productive, but medicaments are now not curative [3].

HBeAG is an external core antigen protein with 214 amino acids. Main function of HBeAG is in regulating immune response to intracellular capsid. By having immune regulatory effect it acts as a T-cell tolerant to avert demolition of contaminated cells by cytotoxic T-cells. This immune regulation may incline to chronicity through perinatal infections and stop acute liver injury during adult infections [4].

A non-particulate fervid protein named HBeAg is a HBV replication marker. HBeAg is the antigen of HBV and has the ability to cross the placenta towards specific insensitiveness of helper T cells to the capsid protein and HBeAg in newborns. HBeAg plays the role of tolerogen after birth as it is tolerated in utero. The HBeAg-positive mothers showed continual prenatal transmission while it is less incessant HBeAg-negative mothers. The genotypes and sub-genotypes of HBV may have perceptible geographical distribution leads to different mutations near to HBV genome coding for HBeAg. The distinct genotypes of HBV can be in charge of multiple infection natural history and different ways of transmission in children, present in numerous areas of the world, where different genotypes prevail [5].

Progressive improvements have been observed in immuno-informatics [6-10] and computational drug designing [11-21] from last decade. Numerous problems of biology have been resolved through applying various approaches of bioinformatics [14]. The present work demonstrates the molecular docking analyses to explore the compound against HBeAg. The ligands of vast structural entities and common structural features were explored. The experimental resolved 3D structure of HBeAg through X-ray crystallography and Nuclear Magnetic Resonance (NMR) was not available yet. The 3D reliable structure of HBeAg was modelled by applying the crystal structure.

Materials and Methods

The canonical sequence of HBeAg having accession number O91532 was retrieved from Uniprot Knowledgebase database in FASTA format. In present effort, the 3D structure prediction of the target protein was performed followed by the molecular docking studies on DELL Core-I-7 workstation. The amino acid sequences of the target protein was retrieved and subjected to BLASTp to identify the suitable template by utilizing the Protein Data Bank (PDB) [22]. MODELLER 9.15 [23], an automated program of protein modeling was used for the three dimensional (3D) structure prediction of the target protein by satisfying the spatial restraints. The retrieved templates were utilized to predict the structures through homology modeling approach [24, 25]. Various evaluation tools such as ERRAT [26], ProCheck [27], Rampage [28] and Anolea [29] were employed to evaluate the generated models. Moreover, the predicted structures of the target protein were further evaluated by using MolProbity evaluation tool [30].

ZINC library was utilized to analyze and evaluate the binding pockets of the target protein. The ligands were used and protein–ligand molecular docking analyses were performed by utilizing AutoDock tools [31]. The drug like properties including number of rotatable bonds, H-bond acceptors and H-bond donors were calculated by utilizing PubChem [32]. The Lipinski's rule of five [33] was calculated for the selected ligand by utilizing mCule servers. The mutagenesis and carcinogenicity of the selected compounds were also calculated and no carcinogenic and mutagenic risks were observed. The objective to perform the molecular docking analyses was to identify the binding pattern of the target protein against the selected ligands. The geometrical optimization of the predicted 3D structure of the target protein and the selected ligands were performed by using UCSC Chimera and ChemDraw Ultra respectively. The generated docked complexes were visualized and analyzed by employing the VMD, PyMol, Ligplot and

Chimera 1.6. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the selected ligands were analyzed by employing the admetSAR.

Results and Discussion

The aim of this work was depends on the relation of Hepatitis B virus and *in silico* analyses to identify the binding pockets. The aligned templates against the selected target protein having E-value, maximum score, identity, and query coverage were selected for homology modeling (**Table 1**). The scrutinized suitable templates were employed for 3D structure prediction of the selected protein. The scrutinized templates showed reliable evaluation analyses. The 3D predicted structures were modeled comparatively by utilizing the crystal structures of the selected templates retrieved from PDB.

The utilized evaluation tools revealed the reliability and efficacy of the selected 3D predicted structures (**Fig. 1 A-C**). The favored, allowed and outlier regions of the predicted structures were calculated in Ramachandran plot. 98.03% residues were observed in favored and allowed region however, only 1.97% residues were observed in outlier region. The overall quality factor was observed 95.91% for the predicted structure and seems reliable for further analyses.

Moreover, the molecular docking studies of the reported ligands revealed fluctuation and variation in the binding energy. Initially, the molecular docking analyses were done having twenty poses, and 90 runs were saved, out of which the suitable effective poses of the complexes having least binding energy were selected. It was observed that the reported ligand effectively binds to the selected target protein (**Table 2**).

Table 1: The selected templates for External Core Antigen sorted by their E-values, overall quality, max score, query coverage and identity

Description	Maximum score	Total score	Query coverage	Per identity	E-value
Chain B capsid protein	353	353	83%	94.94%	3e-125
Hepatitis e-antigen	313	313	74%	94.34%	3e-110
F97L Hepatitis B protein	302	302	69%	97.32%	2e-105
CryoEM	310	310	69%	100%	2e-108
Hepatitis B viral capsid	307	307	69%	100%	7e-108

The molecular docking analyses were analyzed on the basis binding affinity, properties of the selected ligand, drug properties and least binding energy (**Table 3**). The selected ligand was observed as a cyclic compound (**Fig. 2**) having important and acceptable biological properties. The reported ligand may be considered as potent anti HBV agents by targeting HBeAg.

Table 2: Molecular docking analyses including ligand efficiency and binding residues of the selected target protein

Properties	HBeAg
Final intermolecular energy (kcal/mol)	-10.87
Estimated free energy of binding (kcal/mol)	-7.1
Estimated inhibition constant, Ki (μM)	37.76
Unbound system's energy (kcal/mol)	-0.52
Torsional free energy (kcal/mol)	4.04
Ligand efficiency	-0.47
Binding residues	Asp-51, Phe-53, Val-56, Arg-57, Met-95, Ala-98, Asn-103, Arg-111, Asp-112, Val-115, Val-118 and Asn-119

The molecular docking tool was employed and top ranked complexes of the selected target protein of HBV with least binding energies were chosen for further analyses. Different variation in least binding energy was observed however, the stability of the selected ligand depends on the conserved binding affinities of the docked complexes.

Interestingly, it was observed that the docked complexes of the selected target protein with the selected ligand showed reliable results by satisfying the selected parameters and filters. It was further revealed that the reported ligand molecule binds at the conserved region of the selected target protein leads to explore the binding residues. Extensive *in silico* analyses suggested that the observed binding residues and their combination may lead to the least binding energy of the docked complexes of the selected protein and reported ligand molecules (**Fig. 3**).

The drug design process is a costly and time consuming [15]. Therefore, various computational approaches and techniques have been applied to design compounds [17]. The emergence of bioinformatics approaches have significance in decreasing the required time with minimum side effects [34].

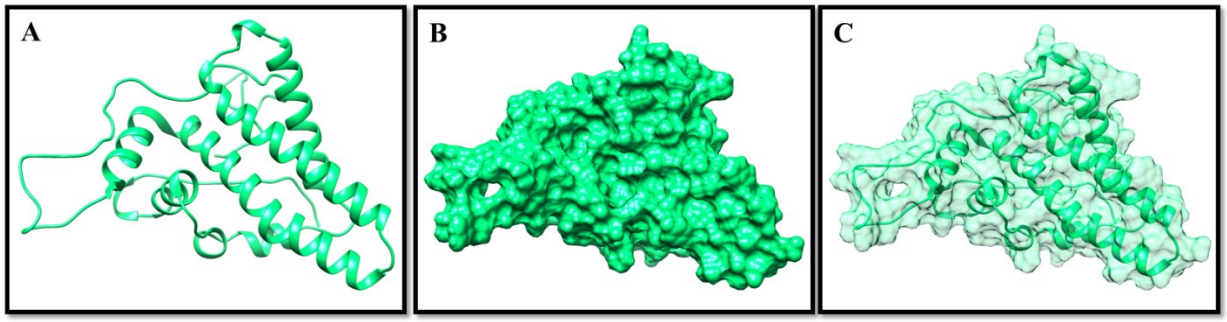

Fig. 1: 3D structure of the target protein HBeAg **A)** Ribbon structure **B)** Surface view **C)** Surface view having 80% transparency.

The structures of the selected ligands were evaluated for their oral bioavailability and efficacy [35]. ADMET properties of the selected ligand were analyzed. Various mathematical models including carcinogens, acute oral toxicity, Ames toxicity, cytochrome P450 2D6 inhibition, honey bee toxicity, aqueous solubility [LogS], blood–brain barrier penetration, human intestinal absorption, fish toxicity and Caco2 permeability were calculated. Numerous toxicities were predicted (**Table 3**). The observed toxicities help to evaluate the metabolites, intermediates and pollutants [12].

Table 3: The drug properties of the selected ligand (MLCB-0696)

Ligand properties	MLCB-0696
Hydrogen bond donor	08
cLogP	-1.16
Hydrogen bond acceptor	10
Molecular weight (g/mol)	515.64
Rotatable bonds	03
Blood–brain barrier (BBB) (probability)	0.9000
Human intestinal absorption (HIA) (probability)	0.6995
Caco2 permeability (probability)	0.8570
CYP450 2D6 inhibitor (probability)	0.9366
Carcinogens (probability)	0.9400
Acute oral toxicity (probability)	0.4931
Aqueous solubility (LogS)	-2.935
Fish toxicity (LC50, mg/L)	0.5828
Honey bee toxicity (HBT) (probability)	0.7079

The predicted aqueous solubility (at 25°C) of the selected ligand showed solubility of the compound in the water. The reported compound showed less LogP value leads to follows the Lipinki's rule of five. It was observed that the compound could be easily be absorbed by the human intestine evaluated through mathematical model of intestinal human absorption. Toxicity and carcinogenicity risk assessment were also calculated, and it was observed that the selected ligand was non-carcinogenic.

Extensive literature survey and *in silico* analyses suggested that the suitable compound must be the one

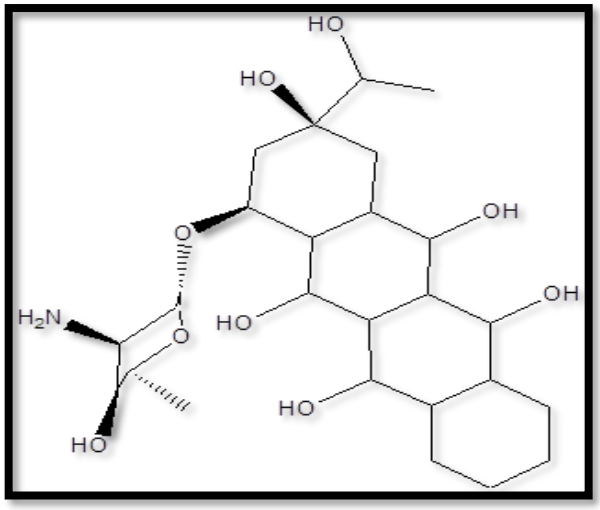

Fig. 2: The 2D structure of the selected ligand (MLCB-0696)

that undergoes from the selected parameters to satisfy the drug properties, least binding energy and effective binding affinity values. By applying the selected parameters, it is suggested that the reported compound has potential to use against HBV by targeting HBeAg. The generated molecular docking results suggested that the observed binding residues (Asp-51, Phe-53, Val-56, Arg-57, Met-95, Ala-98, Asn-103, Arg-111, Asp-112, Val-115, Val-118 and Asn-119) were crucial.

Conclusion

In conclusion, the reported ligand showed efficacy against HBV by targeting HBeAg through computational analyses. Extensive *in silico* analyses of HBeAg showed higher efficacy and probability based on used parameters and least binding energy. The potential interacting residues (Asp-51, Phe-53, Val-56, Arg-57, Met-95, Ala-98, Asn-103, Arg-111, Asp-112, Val-115, Val-118 and Asn-119) identified by molecular docking analyses may be significant for site-directed mutagenesis.

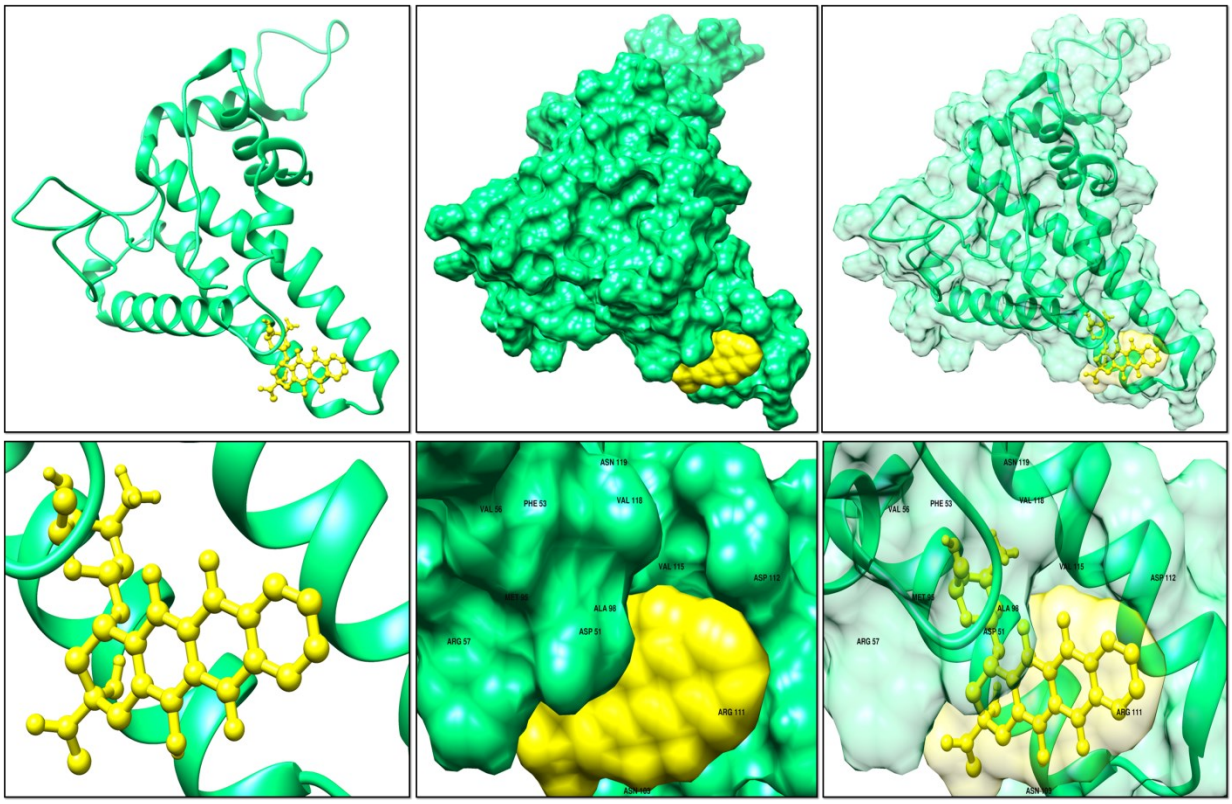

Fig. 3: The observed binding interactions of the reported ligand with the receptor protein. The 3D structural in sight information of the target protein.

Acknowledgment

Authors are thankful to Muhammadan Laboratory of Computational Biology, Faisalabad, Pakistan for support and guidance.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Tsukuda S, Watashi K. Hepatitis B virus biology and life cycle. Antiviral Res 2020;182:104925.

[2] Tsai KN, Kuo CF, Ou JJ. Mechanisms of Hepatitis B Virus Persistence. Trends Microbiol 2018;26(1):33-42.

[3] Shih C, Yang CC, Choijilsuren G, Chang CH, Liou AT. Hepatitis B Virus. Trends Microbiol 2018;26(4):386-387.

[4] UniProt C. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res 2023;51(D1):D523-D531.

[5] Kramvis A. The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. Rev Med Virol 2016;26(4):285-303.

[6] Waqas M, Haider A, Sufyan M, Siraj S, Sehgal SA. Determine the Potential Epitope Based Peptide Vaccine Against Novel SARS-CoV-2 Targeting Structural Proteins Using Immunoinformatics Approaches. Front Mol Biosci 2020;7:227.

[7] Tahir RA, Wu H, Rizwan MA, Jafar TH, Saleem S, Sehgal SA. Immunoinformatics and molecular docking studies reveal potential epitope-based peptide vaccine against DENV-NS3 protein. J Theor Biol 2018;459:162-170.

[8] Tahir RA, Bashir A, Yousaf MN, Ahmed A, Dali Y, Khan S, Sehgal SA. In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1. PLoS One 2020;15(2):e0228265.

[9] Sajid M, Marriam S, Mukhtar H, Sohail S, Sajid M, Sehgal SA. Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2. PLoS One 2022;17(3):e0264700.

[10] Waqas M, Haider A, Rehman A, Qasim M, Umar A, Sufyan M, Akram HN, Mir A, Razzaq R, Rasool D, Tahir RA, Sehgal SA. Immunoinformatics and Molecular Docking Studies Predicted Potential Multiepitope-Based Peptide Vaccine and Novel Compounds against Novel SARS-CoV-2 through Virtual Screening. Biomed Res Int 2021;2021:1596834.

[11] Tahir RA, Hassan F, Kareem A, Iftikhar U, Sehgal SA. Ligand-Based Pharmacophore Modeling and Virtual Screening to Discover Novel CYP1A1 Inhibitors. Curr Top Med Chem 2019;19(30):2782-2794.

[12] Tahir RA, Mughal S, Nazir A, Noureen A, Jawad A, Waqas M, Sehgal SA. The Life Cycle and in silico Elucidation of Non-structural Replicating Proteins of HCV Through a Pharmacoinformatics Approach. Comb Chem High Throughput Screen 2022;25(4):689-701.

[13] Sehgal SA, Kanwal S, Tahir RA, Khalid Z, Hammad MA. In silico elucidation of potential drug target sites of the Thumb Index Fold Protein, Wnt-8b. Tropical Journal of Pharmaceutical Research 2018;17(3):491.
[14] Sehgal SA, Khattak NA, Mir A. Structural, phylogenetic and docking studies of D-amino acid oxidase activator (DAOA), a candidate schizophrenia gene. Theor Biol Med Model 2013;10:3.
[15] Sehgal SA, Mannan S, Ali S. Pharmacoinformatic and molecular docking studies reveal potential novel antidepressants against neurodegenerative disorders by targeting HSPB8. Drug Des Devel Ther 2016;10:1605-18.
[16] Tahir RA, Wu H, Javed N, Khalique A, Khan SAF, Mir A, Ahmed MS, Barreto GE, Qing H, Ashraf GM, Sehgal SA. Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6. J Cell Physiol 2019;234(8):13263-13276.
[17] Kanwal S, Jamil F, Ali A, Sehgal SA. Comparative Modeling, Molecular Docking, and Revealing of Potential Binding Pockets of RASSF2; a Candidate Cancer Gene. Interdiscip Sci 2017;9(2):214-223.
[18] Sehgal SA, Mannan S, Kanwal S, Naveed I, Mir A. Adaptive evolution and elucidating the potential inhibitor against schizophrenia to target DAOA (G72) isoforms. Drug Des Devel Ther 2015;9:3471-80.
[19] Sehgal SA, Hassan M, Rashid S. Pharmacoinformatics elucidation of potential drug targets against migraine to target ion channel protein KCNK18. Drug Des Devel Ther 2014;8:571-81.
[20] Sehgal SA. Pharmacoinformatics, Adaptive Evolution, and Elucidation of Six Novel Compounds for Schizophrenia Treatment by Targeting DAOA (G72) Isoforms. Biomed Res Int 2017;2017:5925714.
[21] Tahir RA, Sehgal SA. Pharmacoinformatics and Molecular Docking Studies Reveal Potential Novel Compounds Against Schizophrenia by Target SYN II. Comb Chem High Throughput Screen 2018;21(3):175-181.
[22] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000;28(1):235-42.
[23] Eswar N, Eramian D, Webb B, Shen MY, Sali A. Protein structure modeling with MODELLER. Methods Mol Biol 2008;426:145-59.
[24] Sehgal SA, Mirza AH, Tahir RA, Mir A. Quick guideline for computational drug design. Bentham Science Publishers 2018.
[25] Sehgal SA, Tahir RA, Waqas M. Quick Guideline for Computational Drug Design (Revised Edition) 2021.
[26] Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2(9):1511-9.
[27] Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography 1993;26(2):283-291.
[28] Lovell SC, Davis IW, Arendall WB, 3rd, de Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins 2003;50(3):437-50.
[29] Melo F, Devos D, Depiereux E, Feytmans E. ANOLEA: a www server to assess protein structures. Proc Int Conf Intell Syst Mol Biol 1997;5:187-90.
[30] Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 2010;66(Pt 1):12-21.
[31] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31(2):455-61.
[32] Bolton YW, Paul A. PubChem: Integrated Platform of Small Molecules and Biological Activities. 2008: 217-241.
[33] Leeson P. Drug discovery: Chemical beauty contest. Nature 2012;481(7382):455-6.
[34] Zaka M, Sehgal SA, Shafique S, Abbasi BH. Comparative in silico analyses of Cannabis sativa, Prunella vulgaris and Withania somnifera compounds elucidating the medicinal properties against rheumatoid arthritis. J Mol Graph Model 2017;74:296-304.
[35] Sehgal SA, Adnan Tahir R. Molecular Modeling and Docking Analysis of CYP1A1 Associated with Head and Neck Cancer to Explore its Binding Regions. Journal of Theoretical and Computational Science 2014;1(3):6.