

Denovo Designing, Virtual Screening and Lead Optimization of Potential Drug Candidate for Herpes Disease

Monica Sharma^{1,2*}, Puneet Rawat² and Ankita Mehta²

¹Department of Biotechnology, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh, India

²Department of Biotechnology, Delhi Technological University, Main Bawana Road, Shahbad Daultapur, Delhi, India

Abstract

Herpes simplex virus (HSV1, HSV 2) is a neurotropic and neuroinvasive virus which becomes latent and causes a lifelong infection. HSV-1 and 2 produce infected cell protein (ICP)-47 against MHC class I antigen presentation pathway by inhibiting the transporter associated with antigen processing (TAP). ICP 47 is also responsible for evasive nature of HSV in human immune system. Currently available antiviral drugs and vaccines only slowdowns the infection but it does not cure the infection. In present study, we have *in silico* designed a potential drug candidate against HSV ICP-47 target through de-novo pathway using eLEA3D. The derived ligand docked with the natural viral receptor ICP-47 showed the binding affinity of -4.07, but it was found toxic in FAF DRUG online ADMET tool, due to presence of high risk imine group. Further manual optimization led to generation of many bioisosteres and final lead structure showed no toxicity and a high binding affinity of -7.53. Our designed lead can act as a potential therapeutic compound against HSV.

Keywords: HSV; ICP-47; eLEA3D; Denovo designing; Bioisosteres

Introduction

Herpes simplex viruses (HSV) 1 and 2, the members of herpes virus family Herpesviridae are ubiquitous, highly contagious agents which infect humans [1,2]. They cause cold sores, herpetic keratitis and genital herpes [3-5]. An estimated 400 million people worldwide are currently infected by HSV-2 and infection of HSV-1 is spreading to developed regions like USA, Western Europe, Australia and New Zealand [WHO Fact Sheet, 2015]. HSV-1 persists in the body in dormant state by escaping the immune system and results in sporadic viral reactivation [6].

HSV evades the immune system by interfering with MHC Class I antigen presentation on the cell surface. Infected cell protein (ICP) 47 caused major histocompatibility complex (MHC) class I proteins retention in the endoplasmic reticulum (ER). MHC presents the antigen to CD⁺8 T cells that are inhibited after expression of ICP 47 in the cell [7]. ICP 47 blocks peptide (viral epitope) transport across the ER membrane by transporter associated with antigen processing (TAP) [8,9] so that MHC class I proteins remain in ER without peptides and cytotoxic T- lymphocytes activation can be stopped. This allows virus to reside for a protracted period in the host.

Due to serious threat to human population a proper treatment and cure of HSV is required. For last two decades HSV glycoprotein D, has been the predominant HSV vaccine candidate [10,11], but the outcome of clinical trials of the vaccine have been really disappointing [12]. Antiviral drugs (valacyclovir, acyclovir, famciclovir, etc.) used against HSV infection, inhibit DNA polymerases and slows down infection by reducing viral replication but does not cure the infection completely [13]. Inappropriate prescribing and wide spread uncontrolled use of antiviral drugs led to emergence of resistance in the viruses. Recently, it has been observed that natural serine protease inhibitor (serpins) like serpinantithrombin III (AT III) inhibit HSV infection during an entry event. Antithrombin III demonstrated a promising result at experimental levels but yet it is far from any therapeutic use [13,14].

Due to inaccessibility of any effective drug and vaccine there is therefore, an urgent need for an effective HSV vaccine or drug that

provides protection against infection and also thwart the virus entering a latent state [15]. Computer aided drug discovery design (CADD) has emerged as a way to significantly decrease compounds screening while retaining same level of lead compound discovery at the same time [16]. In the present manuscript we have designed lead compound by de-novo designing strategy and molecule having highest score was further optimized by virtual screening to increase its affinity to target and reduce toxicity by toxicological analysis by online ADMET profiling tools of FAF DRUG 20 online tool and ORISIS data warrior.

Methodology

Sequence retrieval

DNA sequence of ICP-47 of HSV-2 was retrieved from NCBI and it was blasted to human genomic and transcriptomic database. The structure of target protein ICP-47 was retrieved from rcsb.org in PDB (text) format (PDB I.D. 1QLO).

Denovo drug designing

After exhaustive literature survey and database searching, no ligand was found that bind to this target, so automated de-novo drug design strategy was adopted to get a hit molecule. eLEA3D program was used for denovo generation of ligand, which create new molecules by using a library of molecular fragments and by determining best combinations of molecular fragments that fit user-defined physicochemical properties

***Corresponding authors:** Monica Sharma, Department of Biotechnology, Delhi Technological University, Main Bawana Road, Shahbad Daultapur, Delhi, India, Tel: +91-9717386785; E-mail: monashimla@gmail.com

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18		-3.25
19		-2.43
20		-3.02
21		-3.91
-C=O replacements:		
22		
	(Oxetane)	
23	A. Conformation 1 of oxetane	-3.1
24	B. Conformation 2 of oxetane	-3.28
25	C. Conformation of 3 oxetane	-3.94
26		-3.76
27		-3.7
28		-3.65
29		-3.43
30		-3.82
31		-4.1
32		-3.82

-O-CH₃ Replacement:		
33		-3.35
34		-3.81
35		-3.74
36		-3.08
37		-4.35
38		-4.31
39		-4.34
Replacement of the chain into cyclic compound and other replacements:		
40		-4.43
41		-4.33
42		-3.77

43		-3.41
44		-3.19
45		-3.12
46		-3.84
47		-4.04
48		-3.73
49		-3.07
50		-4.88
51		-4.89

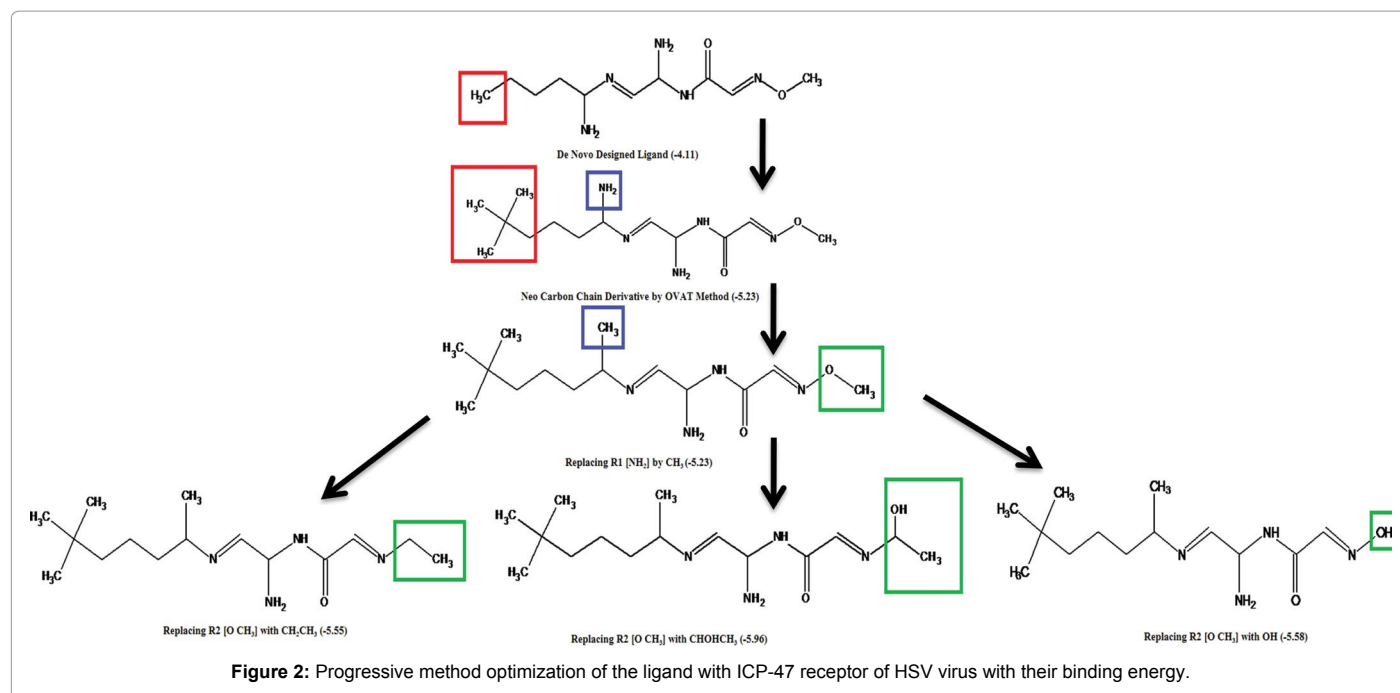
CONH ₂ Replacement:		
52		-2.71
53		-3.76
54		-4.26
55		-3.75
56		-3.06
57		-4.37

Table 1: All the functional group replacements of de-novo designed ligand and their binding affinity with ICP-47.

10 functional group were selected having better value than threshold of -4.11 (Table 1). NH₂ functional group has only one better replacement (-CH₃), so we replaced it in all further structural analysis, for the other 9 passed groups exhibiting binding energy > -4.11 (Figure 2), the OVAT method was utilized and docking with ICP-47 and ADMET profile was simultaneously studied (Table 2). Total 24 iterations were performed which showed promising results with top binding energy of -7.16, -6.6, -6.57 but toxicity still persisted (Table 3).

On analysis it is found that toxicity was being caused by the nitrogen ring replacement, so progressive method was used to generate structure excluding the ring replacements. This gave the best result of -5.96 but the toxicity was still there (Figure 2). To remove toxicity, N atoms of the ligand with the best binding energy (-7.16) were replaced by carbon one at a time and ADMET profiling was done by FAF DRUG 2.0 until any non-toxic ligand was generated. So it would be much close to natural biological compounds and toxicity of imine group and azo group can be removed.

It was further docked in Autodock 4 and finally a non-toxic lead molecule having binding energy of -7.53 (Figures 3 and 4) was obtained. The toxicity of the lead molecule is checked with FAF Drug 2.0 which categorized it non-toxic (Figure 5). Toxicity was also crosschecked with help of ORISIS data warrior which categorized final lead molecule as an irritant. The lead molecule is also checked for binding with any receptor



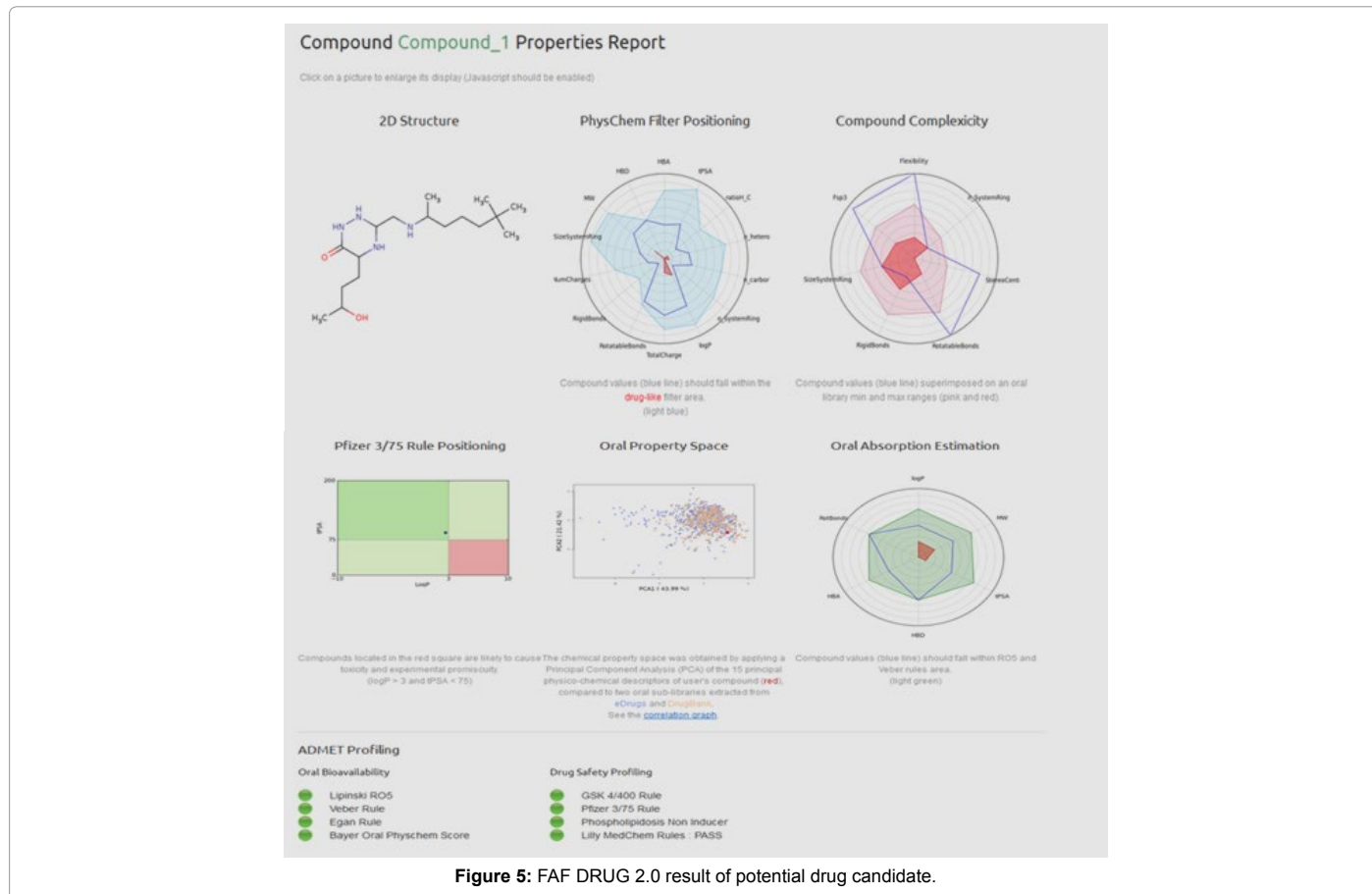
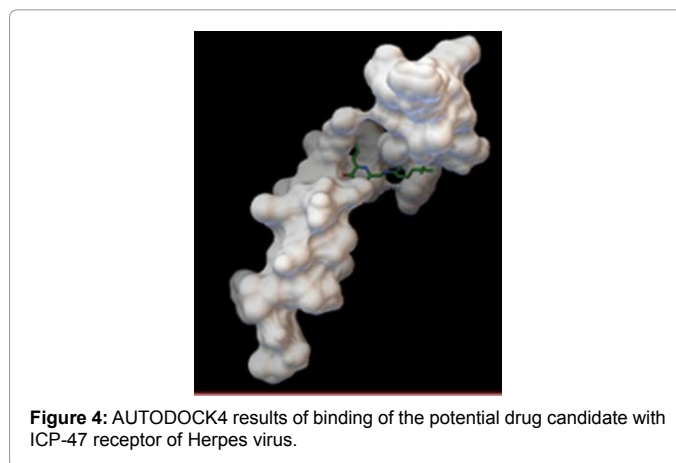
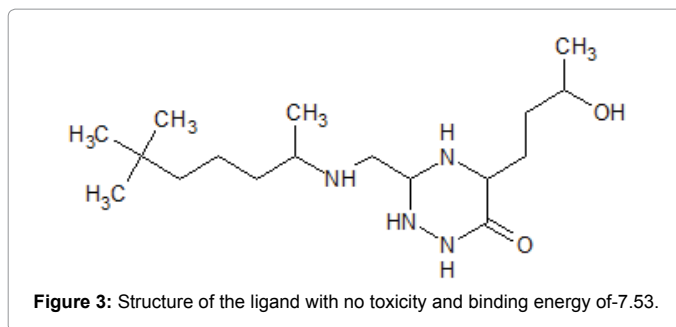
	C-C-C-C chain (X)	O-CH ₃ replacement (Y)	CONH ₂ replacement (Z)
1.		H_3C-CH_3	
2.			
3.		HO—	
4.			

Table 2: All the passed functional group replacements for OVAT analysis.

S.No.	Combination of functional group	Binding energy	S. No.	Combination of functional group	Binding energy
1	X1Y1Z1	-5.82	1.	X2Y1Z1	-5.06
2	X1Y1Z2	-6.6 (2)	2.	X2Y1Z2	-5.52
3	X1Y1Z3	-5.66	3.	X2Y1Z3	-5.15
4	X1Y1Z4	-5.97	4.	X2Y1Z4	-5.07
5	X1Y1Z5	-5.56	5.	X2Y1Z5	-4.89
6	X1Y1Z6	-5.72	6.	X2Y1Z6	-4.92
1	X1Y2Z1	-6.1	1.	X2Y2Z1	-5.7
2	X1Y2Z2	-7.16 (1)	2.	X2Y2Z2	-6.06
3	X1Y2Z3	-6.38	3.	X2Y2Z3	-5.53
4	X1Y2Z4	-6.57 (3)	4.	X2Y2Z4	-5.81
5	X1Y2Z5	-6.36	5.	X2Y2Z5	-4.95
6	X1Y2Z6	-5.8	6.	X2Y2Z6	-5.12
1	X1Y3Z1	-6.49 (5)	1.	X2Y2Z1	-5.69
2	X1Y3Z2	-6.57 (4)	2.	X2Y2Z2	-5.68
3	X1Y3Z3	-5.96	3.	X2Y2Z3	-5.15
4	X1Y3Z4	-6.28	4.	X2Y2Z4	-5.35
5	X1Y3Z5	-5.74	5.	X2Y2Z5	-5.07
6	X1Y3Z6	-5.78	6.	X2Y2Z6	-4.81

Table 3: Binding affinity of the iterations made from the table 2 with ICP-47 receptor (by OVAT method).

present in human or other organism with pharmmapper server [21]. It showed some significant binding with them. Some top results of binding were retinoic acid receptor (PDB ID- 3DZY), Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (PDB ID- 3MDE) and Glutathione S-transferase A1 (PDB ID- 1PL1) with binding energy of -6.39, -5.99, -7.58, respectively.



Discussion

In recent years computer-aided drug design (CADD) approaches have revolutionized the field pharmaceutical research and many powerful standalone tools for CADD were developed, e.g., e-LEA3D, i Drug, iSMART etc. [17,22,23]. In these web servers many techniques were combined (pharmacophore mapping, similarity calculation, scoring, and target identification) to create a web interface which was more consistent and user friendly. Hartenfeller and Schneider have also emphasized that novel biologically active drug like lead molecules can be generated computationally on the basis of rule based fragment assembly [24]. Our initial hit molecule was also generated by fragment assembly method of e-LEA3D. Villoutreix et al. also generated a non enzymatic tyrosine kinase inhibitors lead by virtual screening and its ADMET profiling was done with FAF Drug 2.0. These lead molecules can be used to discover potential Anti-Allergic drugs [25]. Similarly rational drug designing was used to generate many drug like molecules e.g. cimetidine (prototypical H₂-receptor antagonist) [26], enfuvirtide [27], nonbenzodiazepines [28] and raltegravir [29].

Conclusion

The lead molecule generated by the procedure can be checked for its chemical synthesizability and can be checked for its *in vivo* ADMET profiling in clinical trials. Its binding with other receptors can be further analysed to access its pharmaceutical potential in other diseases. Denovo drug design and virtual lead optimization and *in vitro* screening can further lead to decrease the timeline of any drug development and hence holds true potential in modern pharmacology and rational drug design.

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