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Designing Novel Analogues for Emtricitabine and Docking Study through In-Silico Approach

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ABSTRACT

Diseases have always been prevalent in human society since ancient civilization. It has always been a curiosity of human being to study diseases and discover their effective and efficient remedies. Human immunodeficiency Virus (HIV) is one such disease causing and most threatening pathogen for human society. HIV destroys T-helper cells of our immune system by entering through CD4 receptor's GP120 lipoprotein layer and kills them through lytic cycle thus rendering a person extremely susceptible to various other diseases. When CD4 cell count gets reduced to less than 200 units per ml of blood and antibody test of blood is found positive HIV, then this condition is known as AIDS (Acquired immuno deficiency syndrome) which eventually leads to painful death.

Keeping in view the havoc and irreparable damage caused by HIV on entire human civilization, an attempt has been made Emtricitabine so as to minimize the side effects and problems caused due its uptake like liver and kidney damage. Here publicly available bioinformatics tools have provided us with quicker, cheaper and less labour intensive methods to work with the above.

This is a novel finding for Emtricitabine, where by two hundred new analogues have been generated, through an open source software Chemsketch. For this work structure of Emtricitabine was downloaded from Drug bank, the interaction between analogues of Emtricitabine and their ligand reverse transcriptase was observed through popular docking software iGEM dock, the degree and binding energy, as well as favourable Lipinsky rule's generation have provided us with alternate and compliment molecules to Emtracitabine. Such kind of study helps in understanding the mechanism of drug action and also designing novel drug candidate for other dreaded diseases and shows a new ray of hope in eliminating human pain and suffering.

Keywords:

INTRODUCTION

T-helper cells in our Immune system recognise antigen, presented by antigen presenting cells (APCs). Antigen(s) presented by APCs in-turn activate immune system cells such as T-helper cells

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that aid our body in eliminating invading pathogen [1].

HIV reduces the cell count of the T-helper cell, thus rendering body susceptible to various other diseases. When CD4 cell count get reduced to less than 200 per ml of blood and antibody test of blood is found positive for HIV, then this condition is known as AIDS (Acquired immune deficiency syndrome) which eventually leads to death [2].

HIV emerged in late 20th Century although predicted to have originated during late 1960's. Its reported spread is stated during 1970's. First case of AIDS was identified in 1981 in Los Angeles (USA) in both homosexual and gay couples. But researchers have identified HIV samples in 1960's, a place in Uganda, nearby central Africa, which has now been believed to be its origin. HIV virus is said to be transferred into humans from gorillas and chimpanzees at the same place [3].

From 1981-2007 there have been 25 million reported death of people due to AIDS World Wide. Currently there is around 45 millon AIDS patient World Wide. AIDS was declared 4th biggest global cause of death in 1999. With 5.8 Million patients, South Africa is world's leading country effected by AIDS, followed by Nigeria and India with 2.7 and 2.6 million patients respectively [3].

The first anti-HIV drugs called Azidothymidine (AZT) or Zidovudine was developed in 1986. Dideoxyinosine (ddI) was the second drug permitted for use in USA in 1991. Since then many efforts have been made for constant development as well as modifications done in the existing for presenting better formulations in the fight against HIV. Various drugs designed to combat HIV are considered to fall in any of the five classes based on mechanism of action.

- Entry and fusion inhibitor [4].
- Integrase inhibitor [4].
- Non nucleoside reverse transcriptase inhibitor (NNRTI) [4].
- Nucleoside reverse trancriptase inhibitor (NRTI) [4].
- Protease inhibitor [4].

These drugs prolong the life span of Aids patient by fighting with HIV. Nucleoside reverse transcriptase inhibitor is one such category that interfere its mechanism when RNA genetic material of HIV virus is converted into DNA inside the host cell through enzyme Reverse transcriptase. Drug get bind to the enzyme Reverse transcriptase, this kind of therapy involving the combination of such drug are commonly known as **HAART** (Highly Active Antiretrovial Therapy) [4]

Emtricitabine is one such antiretroviral medicine of type (NRTI). Emtricitabine is phosphorylated by cellular enzyme to form Emtracitabine 5- tri phosphate, which is responsible for inhibiting of HIV Reverse transcripase. It competes with natural substrate deoxycytidine 5-triphosphate and get incorporated into nascent viral DNA, resulting early chain termination. By inhibiting HIV reverse transcriptase activity this drug lowers replication cycle of HIV in host cell, that directly reduces the virus load in patient's body, which increases the cell count of T-helper cell, hence prolong the life span of HIV patient [4].

Chemical formula of Emtracitabine is C8H10FN3O3S with double ring structure discovered by Dr Dennis C. Liotta, Dr Raymond F. Schinazi and Dr Woo-Baegchoi in year 2003, although effective

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also exhibit side effects like liver problems, build up of acid in the blood [5]. The structure of Emtricitabine is shown in figure 1. Therefore in this manuscript an attempt has been made to design its less toxic analogue using In-Silico approaches. In-silico drug designing is time saving, results are predicted faster than the wet lab and saves the testing cost and time otherwise utilised in downstream processing of the drug.

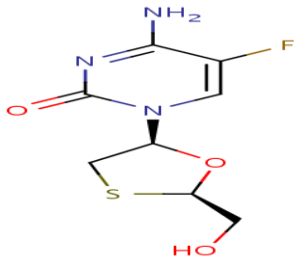

Figure 1: Chemical structure of Emtricitabine [4].

MATERIALS AND METHODS

Materials

AidsInfo – An open source for HIV/AIDS information by National Institute of Health (USA). This source was used to gather information regarding the types of drugs available, their mode of action, their side effects and other related information. By going through this server we selected Emtricitabine as our candidate drug. Its URL is http://aidsinfo.nih.gov/drugs [6].

Drugbank – It is an open database on drug and drug targets. Information like the drug type, its interacting partners (both drugs and chemicals), various structural representations, Pharmacodynamics and metabolism etc. The above said information for Emtricitabine was gathered from this database [4].

Uniprot – It is an open resource of Protein sequence information containing the annotated sequences of proteins and other related information. The fasta sequence for the target Enzyme reverse transcriptase of HIV1 was retrieved from this resource [7].

Swiss model- It is a fully automated protein structure homology-Modeling server. The PDB format of the target enzyme was modelled with the help of this server [8].

ACD Chemsketch- ACD/ChemSketch Freeware is a drawing package that allows one to draw chemical structures including organics, organometallics, polymers, and Markush structures. Here Chemsketch was used to design the monoatomic and diatomic analogues of Emtricitabine [9].

Open babel- This free source was used to convert analogs to their PDB format [10].

Igemdock- It is public domain software for molecular docking and virtual screening. Using iGemDock software the designed analogs were docked with the target molecule and the best docked posed analyzed [11].

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Molinspiration- It is a software for generating lipinski's rule of five. It calculates logP octanol-water partition coefficient, PSA polar surface area, number of nonhydrogen atoms, molecular weight, number of hydrogen-bond acceptors (O and N atoms), number of hydrogen-bond donors (OH and NH groups), number of Rule of 5 violations, number of rotatable bonds, molecular volume of the query molecule. It predicts the molecule's likeliness as a drug candidate. All our analogs were subjected to this prediction software [12].

The drug Emtricitabine was selected for this particular work because no analogues have been designed for this drug so far. The fasta format of the target i.e., reverse transcriptase of HIV1 was submitted to the server Swiss model and its PDB was obtained. Then with the help of ACD Chemsketch, 60 monoatomic and 180 diatomic analogues of Emtricitabine were designed using different combinations of groups linked to the outermost chains of the drug. These analogues were then converted to PDB format with the help of Openbabel. The iGEM docking sever was used to dock the Analogues with the target Enzyme. The analogues showing the best results were further analysed by Molinspiration software for generating parameters of Lipinski's rule for their efficacy as a potential drug candidate for HIV. Lipinski's rule of five proposes following five criteria to be fulfilled by the drug candidate in order to be the potential drug.
The flow chart of the methodology is depicted in figure 2.

B) Methodology Adopted

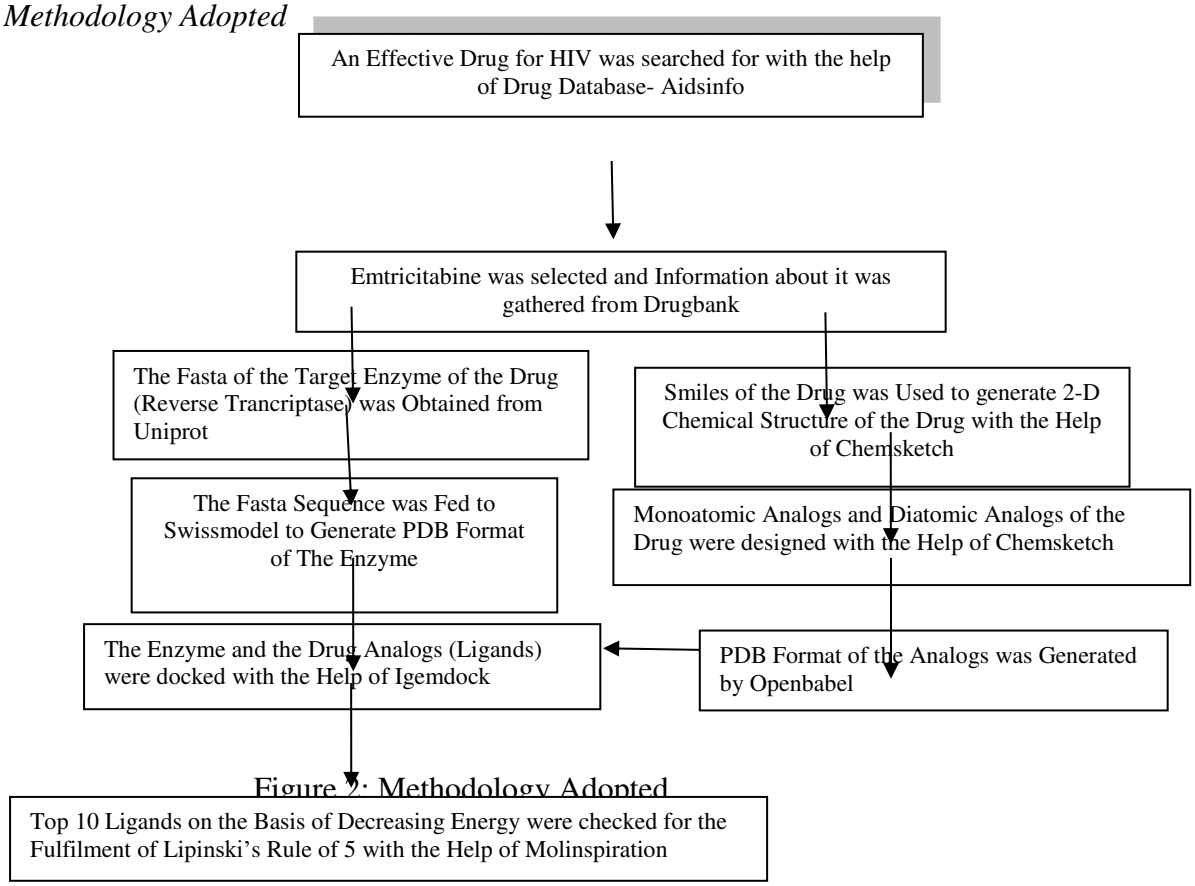

Figure 2: Methodology Adopted

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- Molecular mass less than 500 Dalton [13].
- High lipophilicity (expressed as LogP less than 5) [13].
- Less than 5 hydrogen bond donors [13].
- Less than 10 hydrogen bond acceptors [13].
- Molar refractivity should be between 40-130 [13].

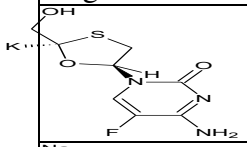
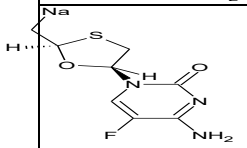
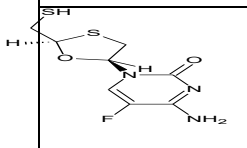
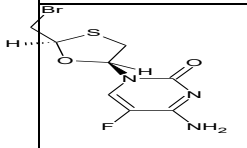
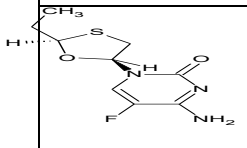
RESULTS AND DISCUSSIONS

According to **aidsinfo** it was observed that there are different categories of drugs available for HIV. Some of them are approved, some are at investigational stages [6].

We came to know various facts about the drug Emtricitabine with the help of DrugBank [4].

The accession number of the enzyme reverse transcriptase which the drug target is Q**5DLN9**. It is located on gene pol of HIV1 genome and is 560 amino acid long. Swiss model provided the ligand-enzyme interaction details. Z-score is -3.141, the molecule is a heterodimer.

TABLE-1
RESULT WITH MONOATOMIC ANALOGS

alogues				ver	gP	SA	W	N	HN	TB
	-101.099	-74.9174	-26.1812	34.5294						
	-97.898	-71.6203	-26.2777	34.625						
	-97.7503	-71.4124	-26.3379	34.8125	281	.154	3.319			
	-97.5568	-72.4892	-25.0675	34.625	697	.154	0.148			
	-97.0574	-72.3541	-24.7033	35.125	339	.154	5.297			

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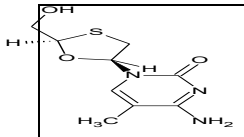
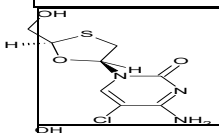
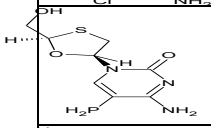
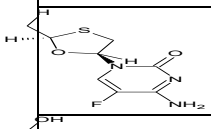
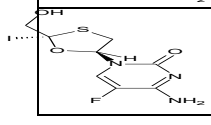
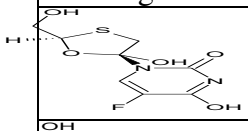
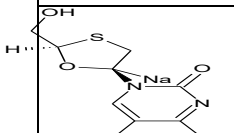
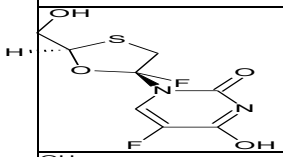
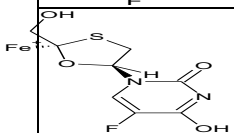
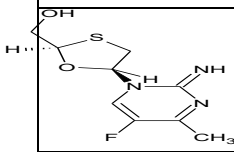
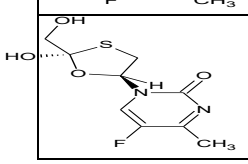
	-97.0285	-70.9537	-26.0747	34.562	387	.382	3.288	6		
	-96.9684	-71.2301	-25.7384	34.437						
	-96.3249	-69.8957	-26.4292	35						
	-93.7885	-69.26	-24.5286	35.0667						
	-91.2708	-74.2407	-17.0301	35.2353						

TABLE-2
RESULTS WITH DIATOMIC ANALOGS

Analogs				ver	gP	SA	W	N	HN	DTB
	-99.6571	-48.5977	-51.0594	31.5294						
	-97.2829	-71.612	-25.6709	31	261	.587	0.217			
	-97.2388	-71.4446	-25.7942	31.2941	019	.587	6.225			
	-92.9453	-76.9129	-16.0324	33.7647						
	-92.755	-75.4296	-17.3254	29.5	157	.154	5.279			
	-92.2553	-73.4833	-18.772	29.3529	631	.587	2.262			

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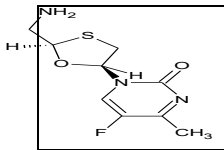
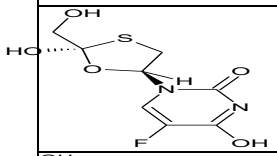
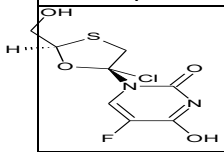
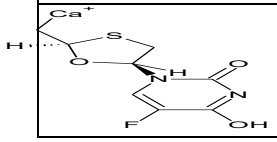
	-91.4799	-73.7675	-17.7124	34.8125	621	.154	5.279			
	-91.4612	-74.1049	-17.3563	29.1765	948	4.815	4.234			
	-91.1866	-69.5868	-21.5998	33.7647	289	.587	2.68			
	-91.0938	-64.9555	-26.1384	31	551	.359	1.306			

Legends: E- Free binding energy, V- VanDer Waal's interaction, H – Hydrogen bond interaction, Aver- aver copair, logP- expression for lipophilicity, TPSA- the top docking hits have lower topological surface area (TPSA) values, MW- Molecular weight, ON- number of hydrogen-bond acceptors (O and N atoms), OHN- number of hydrogen-bond donors (OH and NH groups), ROTB- number of rotatable bonds

CONCLUSION

From the result compiled in table 1, it was observed that the mono analogue which consists of potassium group instead of hydrogen group attached to its pentameric ring exhibits the least free binding energy when docked with target enzyme also it does not violate any of lipinski's rule there by proposed to be an efficient drug candidate. From table 2 we observed that the analogue having hydroxyl group instead of nitrogen group attached to its hexameric ring and hydroxyl group attached to its pentameric ring instead of hydrogen group exhibits the least binding free energy also it do not violates any of Lipinski's rule so it can be an efficient diatomic drug analogue. The results obtained above further need to be tested and verified by wet lab experiments.

Many types of drugs have been designed in-silico and tested for their efficacy. In-silico methods saves clinical trials and are time and cost effective. These methods provide higher precision and better quality of experimental data and instant access to vast sets of experimental data generated by scientific communities. It also leads to faster individual experiments. There is a vast scope and room for further analysis and research in this field which can be done with the help of in-silico techniques.

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13. *(2012) Lipinski's rule Wikipedia*, the free Encyclopaedia. [online]. Available: *http://en.wikipedia.org/wiki/Lipinski's_rule_of_five*