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IN-SILICO ANALYSIS OF QUINOXALINE LIGAND CONFORMATIONS ON 2BB9: HIV-1 PROTEASE

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Abstract- HIV-1 protease (HIV PR) is an essential enzyme needed in the proper assembly and maturation of infectious virions. HIV PR catalyses proteolytic cleavage of the polypeptide precursors into mature enzymes and structural proteins which is a critical step in the life cycle of HIV. The necessity of this enzyme in the virus life cycle makes it a promising target for therapy of the HIV infection. In order to discover potential inhibitors of HIV PR, structure based virtual screening was performed using crystal structure of the protein obtained from PDB (2BB9). 46 Quinoxaline analogues were docked with 2BB9 and the highly interacting compounds based on their binding energies are reported here.

Keywords- protease, virion, HIV PR, precursor, quinoxaline

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Introduction

The human immunodeficiency virus (HIV), a retrovirus causes the disease complex called acquired immunodeficiency syndrome (AIDS) which affects the immune system [1,2]. The disease, to develop takes a long incubation period. First it presents as a common flu or fever or diarrhea or weight loss or fatigue without any a known cause. The infection may remain without symptoms for long period before its emergence as a full-fledged syndrome. Factors that hastens its presentations and severity of infection are; the immune response, age, co-infections and the behavioral and psychological factors of the individual.

The birth of HIV is still a mystery to all; scientists believe that they were transferred to the humans through the African chimpanzees during the early 19th century [3,4]. During early days the symptoms were confused with other illness like common flu or diarrhea. The virus was first isolated in 1983 by Dr. Luc Montagnier and was named as Lymphadenopathy associated virus [5]. Later in 1984 Dr. Robert Gallo published a paper on a retrovirus, which showed similar feature, and he named it as Human T cell leukemia virus III (HTLV III) [6]. He explained that it was this virus that caused AIDS. In 1985, a blood test, ELISA became available to measure the antibodies to HIV. This still remains the golden tool for the detection of HIV.

HIV-1 protease, a retroviral aspartyl protease is the essential enzyme needed by the virus for its replication in our body [7]. Antiretroviral treatment for HIV is based on the inhibition of this protease [8]. For the treatment of retroviral infections, multiple drug therapies for a longer time are required. The term Highly Active Antiretroviral Therapy (HAART) is used to describe a combination of three or more anti-HIV drugs and has come to be a common regimen among those infected with HIV [9].

According to UNAIDS, more than 40 million people in the worldwide are having HIV/AIDS, among them 18,500,000 (44%) of whom are women, and 3,000,000 (7.1%) of whom are children. The most heavily affected area of the world is sub-Saharan Africa, with almost 30 million people infected with HIV. The spreading of AIDS are increasing in China, India, and Eastern Europe, due to high rates of parenteral drug use, cumulative occurrence of other sexually transmitted diseases, and public health management systems that are inadequately prepared to prevent the spread of HIV [10,11]. There were 2,400,000 deaths from AIDS in 2001 and some 14,000,000 children orphaned by the disease are living in the world.

Drug design is the innovative or rational process of finding new drug candidate based on the knowledge of available information of the corresponding receptors or known actives. The drug is any organic or organometallic small molecule that activates or inhibits the function of a bio-molecule such as a protein, which in turn results in therapeutic benefit to the patient. Ligand based drug design depends on the knowledge of the molecules that bind to the biolog-

ical target, where as structure based drug design relies on the knowledge of the three dimensional structure of the biological target [12-16]. In contrast to traditional or classical methods of drug discovery, depends on random synthesis of the chemical substances in a large scale and there by testing them for biological activity on cell cultures and/or animals. Computer aided drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value. Computer-aided drug design uses molecular modeling to find out novel compound (lead identification) and also optimize (lead optimization) or study the existing biologically active molecules and analyse the for discovering novel compounds. The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly.

Materials and Methods

Large conformational changes in proteins play an important role in cellular signaling. HIV-1 protease (HIV PR) is an essential enzyme needed in the proper assembly and maturation of infectious virions. Understanding the chemical mechanism of this enzyme has been a basic requirement in the development of efficient inhibitors. HIV PR catalyses proteolytic cleavage of the polypeptide precursors into mature enzymes and structural proteins which is a critical step in the life cycle of HIV. The necessity of this enzyme in the virus life cycle makes it a promising target for therapy of the HIV infection. In order to discover potential inhibitors of HIV PR, structure based virtual screening was performed using crystal structure of the protein obtained from PDB (2BB9). A common strategy in the life cycle of viruses is the utilization of polycistronic messenger RNAs that can be translated into precursor polyproteins which subsequently are processed enzymatically into mature, functional proteins during virus assembly [17,18]. Processing enzymes may be either cellular or viral encoded and offer novel targets for intervention. Virus-encoded proteases afford particularly attractive therapeutic targets for the design of antiviral agents that are highly specific and non-toxic to their host cells [19].

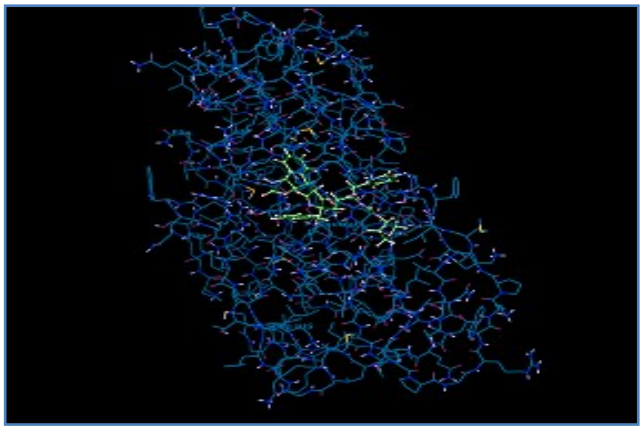


Fig. 1- 3D Structure of 2BB9

The receptor protein was downloaded from Protein Data Bank [PDB]. The 3D structure of 2BB9 is shown in [Fig-1]. The X-ray coordinates of the protein obtained from PDB is missing with various structural information and hence cannot be considered directly for structure based drug design without refinement. Any PDB structure file contains heavy atoms and may include a ligand or substrate, water molecules, metal ions, and cofactors only. Some PDB files existing in multimeric, and hence required reduce them to a single unit is therefore needed to prepare proteins in a form that is suitable for modeling calculations The protein preparation wizard of Schrodinger suite 2012 is used for the purpose [21,22]. The prep-wizard refines the protein by fixing structures by adding hydrogen, assigning bond order and valency, and modifying the structure by deleting undesirable chains and waters, then fixing or deleting hetero groups, and later generating proper hydrogen bond network and minimizing the structure to relieve steric clashes.

Quinoxaline and its derivatives are an important class of benzoheterocycles displaying a broad spectrum of biological activities which have made them privileged structures in pharmacologically active compounds. Quinoxaline, also called benzopyrazine has been considered as a wonder nucleus which possess broad spectrum of biological activities. The wide range of biological response profile has attracted the attention of many researchers to explore this skeleton due to its multiple potential against several activities. They are clinically effective in anti-bacterial, anti-fungal, anti-inflammatory, anti-cancer, anti-tubercular and anti-neoplastic agents, interestingly, it also shows anti-HIV properties [20]. Modification in their structure has offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity.

The quinoxaline ligands, 46 in numbers obtained from literature was prepared in dot mol format. The structure of a typical quinoxaline is shown in [Fig-2]. The ligands were refined using ligand preparation wizard. The docking experiments provide with structure which can bind the protein with least energy. Such a structure is considered as lead drug. In this case all docking calculations were carried out with Schrodinger Glide 2012 [21-23]. This program performs a hierarchical search of ligand conformations undergoing a filtering procedure and finally minimizes in the field of the receptor using the OPLS-AA force fields in conjunction with a distance-dependent dielectric model. The lowest-energy poses obtained in this fashion were subjected to a Monte Carlo procedure to obtain the final set of docking solutions. Glide uses two concentric boxes to generate the potential grids and define the binding site. The grids are computed within the space defined by the “outer box”, which encompasses all the ligand atoms. The “inner box” is defined as containing all acceptable positions for the ligand center upon docking.

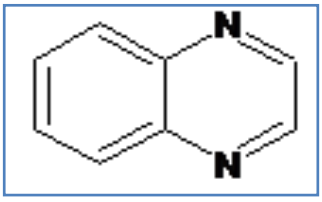


Fig. 2- Structure of Quinoxaline

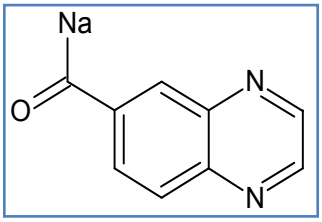


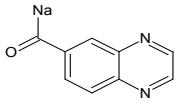
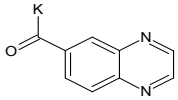
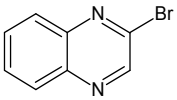
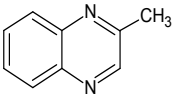
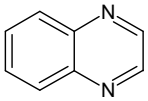
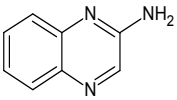
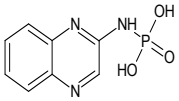
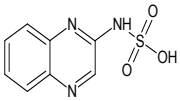
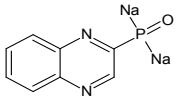
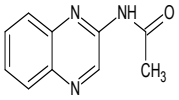
Fig. 3- Structure of q45 quinoxalin-6-ylcarbonyl sodium

Default input parameters were used in all docking computations.All compounds were docked and scored using the Glide standard-precision (SP) mode. Upon completion of each docking calculation, 30 poses per ligand were saved. The best-docked structures were ranked using a model energy score (Emodel) derived from a combination of Glide Score, Coulombic, and van der Waals energies, and the strain energy of the ligands. The top- ranked compounds obtained in this way were docked and scored again with the Glide extra-precision (XP) mode, and the best of 10 XP-docked structures were finally selected as final docking solution.

Results and Discussions

The following results were found in the docking process. The 10 moieties showed the maximum docking score are given below in the descending order.

Table 1- Docking score table of 10 moieties with highest docking score

Compound Name	Structures	GScore	Lipophilic EvdW	PhobEn	Hbond	Electro
q45		-5.45	-2.07	-1	-1.18	-0.3
q46		-5.45	-2.07	-1	-1.18	-0.3
q10		-5.33	-2.61	-1.5	-0.54	-0.18
q2		-5.3	-2.57	-1.5	-0.55	-0.18
q1		-5.27	-2.54	-1.5	-0.55	-0.18
q14		-5.25	-2.61	-1.5	-0.55	-0.18
q19		-5.5	-2.94	0	-1.31	-0.9
q18		-5.22	-2.74	0	-0.66	-1.24
q31		-4.97	-2.75	0	-1.08	-0.64
q22		-4.95	-3.35	0	-0.7	-0.3

From docking scores in [Table-1], the following conclusions can be drawn. Quinoxalin-6-ylcarbonyl) sodium (q45) show the maximum glide score value of -5.45. The structure of q45 is shown in [Fig-3] and its best fit with the inhibitor is shown in [Fig-4]. Quinoxalin 6-ylcarbonyl) potassium (q46) also gave the same value of -5.45. q45 gives the Lipophillic van der Waal's energy of -2.07. Hydrophobic enclosure reward is the cumulative hydrophobic interaction between the ligand and the receptor atom [24]. The ligand q45 gives the value of the hydrophobic enclosure reward (-1). Glide score and the other docking values mentioned in the [Table-1] revealed that Quinoxalin-6yl-carbonyl sodium (q45) is the best candidate drug ligand and it is well placed in the pocket of receptor atom also. This best fit is depicted in [Fig-5].

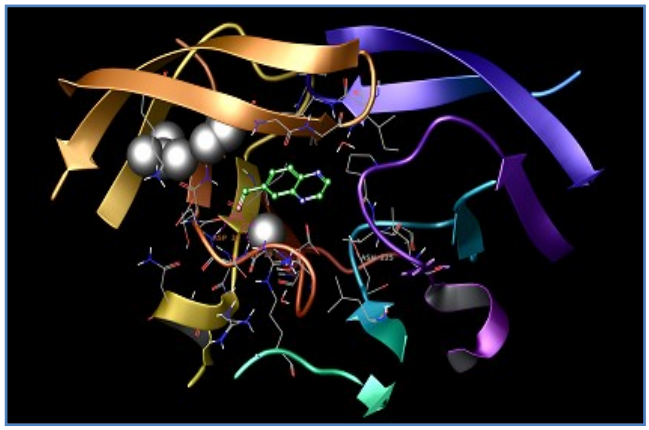


Fig. 4- The Optimal docking of q45 with 2BB9

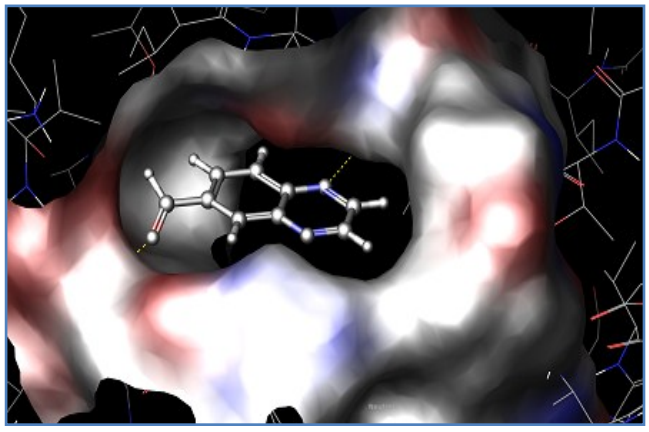


Fig. 5- Display showing 2BB9-q45 best fit

In the case of hydrogen bond energy released, q45 releases the highest value of -1.18 compared to all the other 44 ligands. The electrostatic energy of the ligand q45 is -0.3. So from the highest dock score and the other docking values we can conclude that the ligand q45 i.e. Quinoxalin-6yl-carbonyl sodium is the best ligand among other 46 ligands docked against the protein, 2BB9. q45 and q46 are of same value. It demonstrates that the atomic value of the alkaline ion has no effect on the docking property. Similar is the effect of ionization value of carboxylic acid salt of the alkali metals.

Conclusion

Quinoxalin-6yl-carbonyl sodium (q45) binds effectively to the active site of 2BB9 with binding energy -5.45 (Kcal/mol). There is no extensive study carried out in the ligand Quinoxalin-6ylcarbonyl sodium. So this result got from *in silico* studies reveal that the molecule is potential lead, which invites wet lab trials. Further the compound should be optimized to have better bioavailability, optimum metabolic half-life and with minimal side effects etc before making it a safe and efficacious drug.

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