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Structure Based Virtual Screening and Docking Studies of the Replicase Gene of Banana Bunchy Top Virus

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Abstract:

By using the power of informatics, a wealth of genomic data is being generated that can be used to spot and select suitable targets in the discovery of candidate drug models for plant and animal diseases. The project has been planned to design a drug for the replicase gene of the banana bunchy top virus, which causes massive economic losses in the tropical tracts of the globe. The drug is finalized to be "NSC No.: 82221", which is the analogue of Idoxuvidine, an antiviral drug. The

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viral protein is modeled by means of homology modeling, having a similar protein of identical domain regions (5-83 amino acids). The drug has been filtered from thirty ligands, generated from the chemical database based on their energy values and distance from the protein. So, it is hypothetically capable of blocking the replication of banana bunchy top virus that is inside the infected cells by binding with the viral protein of the replicase gene. With reference to the ADME analyses the drug has been found to be safer and so had been authenticated to have no toxic effects on mammals that feed on the treated plants. The molecule needs to be synthesized and tested in infected banana farms to prove its feasibility as an anti-viral spray/pesticide and to evaluate its economic viability in the farmers' holdings. The relevance of bioinformatics methods, in target selection and cheminformatics methods, in the screening of candidate molecules are very constructive in the search of viable and safer drug models for the disease causing proteins (Rajesh et al., 2013).

Key words: Virtual screening, structure based docking, replicase gene, banana bunchy top virus, ADME properties.

Introduction

The necessity for constant development of new drugs for plants and animals needs no special highlighting in light of the current global situation of health and diseases. Customarily, the process of drug development has revolved around a blind screening approach, with no knowledge about the compound or the approach that could act as a drug or a therapy. Such sightless screening method is very time-consuming and arduous. It is projected that a classic drug discovery cycle, from lead identification through to clinical trials, can take 14 years (Song *et al*., 2009) with a cost of 800 million USD. The shortcoming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design" (Meng *et al.* 1992 & Hou and Xu 2004).

Chemogenomics helps in the investigation of target gene families. Small molecule leads, indentified by their ability to

interact with a single member of a gene family, are used in studying the biological role of another gene family member with unknown function. The remaining targets are usually specified by sequence homology (Bredel and Jacob 2004). Chemogenomics aims in the study the biological effect of a large number of ligands on a large number of macromolecular targets (Caron *et al.,* 2001). Rapid developments in the fields of combinatorial chemistry and high-throughput screening (HTS) technologies have made great accessibility for accelerating the process of drug discovery with the advantage of enormous libraries of compounds which are screened very quickly in a relatively shorter period of time (Lavecchia and Di Giovanni 2013).

The virtual screening methods are divided into ligand-based and target-based approaches. These two methods are used to dock either a ligand against a set of proteins or a set of ligands against one specific protein target. The two approaches have been found to be successful and functional in the development of a candidate molecule with the desired bioactivity profile (Keiser *et al*. 2009; Sifert *et al*. 2007). It should be noted that the applicability of *insilico* chemogenomics models depends on the quality and completeness of the training sets that are used for model construction and validation (Carrascosa *et al*. 2011; Rajesh *et al.* 2013).

In the "rational" designing of drugs, the initial necessary step is the identification of a molecular target which is a protein molecule that is critical to a disease or the concerned pathogen. Then the researcher should determine the molecular structure of target. The soundness of "rational" or "structure-based" drug discovery rests exclusively on the accurate target structure of the molecule details that is studied which enables in the choosing of the compounds from huge chemical databases containing millions of molecules which are available publicly. Given all the required information, the "rational" design of drug will be more achievable. So this could help the researchers to

exploit all of the possible approaches to design the most suitable and durable candidates for drug.

Agriculture being the backbone of more than 60 per cent of the rural India, cultivation of economically significant crops and their disease management studies has been very crucial and sensible. B**anana** also known as ***Musa***, of the **Family**, **Musaceae**, is cultivated for its fruit and are closely related to **plantains**. Globally, they rank fourth after **rice**, **wheat** and **maize** in human **consumption** and are grown as a major commercial crop in about 130 countries worldwide. It is one of the most commercial fruit crops in India which fetches a major share of foreign exchange. Its cultivation is subjected to many natural calamities but the viral diseases are a serious issue. In economic terms, viruses are only of importance, if it is likely that they will spread to crops during their commercial lifetime and some estimates put total world damage due to plant viruses as high as US$ 6 x 1010 per year (Dale 1987; Roossink 1999).

Bunchy top of banana is considered as the most serious viral disease and was first recognized in Fiji in 1889. It is always known as a major epidemic and threat to banana cultivation and its export industry (Gowen 1995). Magee (1940) established the viral nature of the disease problem and showed that it is transmitted by the aphid vector, *Pentalonia nigronervosa*. Bunchy top is the most devastating viral disease of bananas world-wide. Caused by banana bunchy top virus (BBTV), this disease is characterized by the 'bunched' appearance of newly emerging leaves and dot-dash flecking of leaves and stem sheaths (Magee 1927). Affected plants do not produce fruit, resulting in significant loss of production on commercial farms (Watanabe *et al.* 2013; Plant Health Australia 2013).

The infected plants become progressively smaller and give the plant an erect, bunchy appearance. Plants infected early in their growth become sterile and never produce fruits resulting in total loss of yield. Plants infected at later stages

produce only deformed fruits of no market value. The plant may eventually die and even if it is alive they remain with the lateral shoots which are sources of infection for further spread. The spread of the disease into new areas can initially remain undetected, complicating timely eradication work and prevention of new outbreaks. Once the disease is present in a region, it is extremely difficult to eradicate.

As for as banana bunchy top virus is concerned, there is no known control measure that could readily stop the virus from infecting the plant and also could stop the viral replication within the host cell. There is no resistant variety for the virus even though some genetically engineered plants with moderate resistance has been culture (Thomas *et al*. 1994; Brunt *et al*. 1996). The objective of this project is to screen and design a model of the most suitable antiviral drug, an inhibitor molecule that could be used to demonstrate its ability to stop the viral replication in the host (banana), by inhibiting the functioning of the primary viral protein, the replicase protein of DNA I.

Materials and Methods

All the computational analyses and the distance measuring and image capturing were carried out using Swiss-PdbViewer – version 3.5, and the SWISS-MODEL, developed within the Swiss Institute of Bioinformatics (SIB), Basel.

Strategies for antiviral therapy

Any of the stages of viral replication can be a target for antiviral intervention. The only requirements are: 1) that the process targeted is essential for virus replication and 2) that the therapeutic agent is active against the virus while having "acceptable toxicity" to the host organism.

Homologue Protein structure

The replicase gene segment of DNA I of the banana bunchy top

virus of isolate V14 was selected from the DNA database of the National Center for Biotechnology Information for the experiment. This locus is AB113660 which is of 1104bp long. The isolate was studied by the Japanese research group of Furuya in 2005 and was directly submitted in the database of NCBI. The sequence was blasted and the best hit was chosen. The chosen protein was analyzed in Pfam to find out the domain category. The chosen protein was used as the template for docking.

Choice of ligand

A variety of antiviral drugs were chosen and they were searched against the Open NCI Database for similar structural compounds of similar biological activity. The general characteristics used for choosing the antiviral drugs are presented in table 1 of the Annexure. The drug analogues were obtained from the NCI enhanced database browser against 250,000 structures. It is based on the chemistry information toolkit CACTVS. All the structures of anticancer and anti-HIV screening data were provided here by NCI's Developmental Program (Ihlenfeldt *et al.* 2002). This data set has been augmented by a large amount of computed data such as calculated log P values, predicted biological activities, systematically determined names and others (Mestres *et al.* 2008).

The molecular topologies for drug designing/docking were generated using the same server. A number of analogues were generated. The selected antiviral drugs and their corresponding analogues are listed here in table 2. Among the selected analogues, molecules will be selected based on energy factor after docking and further based on their distance to the protein. Furthermore, the candidate molecules will be filtered based on their ADME properties using ADME/Tox WEB.

Steps for Drug Designing:

Model Building - Model building was performed using the DeepView/SwissPdbViewer Version 3.5 provided by ExPASy of the Swiss Institute of Bioinformatics.

Model Optimization/Validation - I) Energy minimization was performed using the Swiss-PdbViewer includes a version of the GROMOS 43B1 force field and II) the Active site prediction was done using the CASTp server which uses the weighted Delaunay triangulation and the alpha complex for shape measurements (Binkowski *et al.* 2003). It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules.

Hex Docking

Hex is an interactive protein docking and molecular superimposition program. Currently hex understands protein and DNA structures in PDB format. Up to three PDB files can be loaded into Hex at any one time. These are treated as a receptor, a ligand and a reference complex. The intermolecular axis could be displayed which connects the default centriods of each molecule. The colour scheme selector allows the molecular skeleton to be coloured from a fixed palette of colours. The saved docked file was loaded in the Swiss-PdbViewer and the distance between the protein and ligand was measured.

Adme Calculation

ADME is an **acronym** in **pharmacokinetics** and **pharmacology** for Absorption, Distribution, Metabolization, and Excretion, and describes the nature of a **pharmaceutical compound** within an **organism**. In 1997, **Christopher A. Lipinski** published a series of features commonly found in orally active **drugs**. They are considered to be the 'Lipinski's

rule of five' which are usually referred in drug designing to validate the ADME () analyses of the candidate molecule. The docked structure was applied for the ADME Tox / Web tool in the internet which goes for a step by step retrieval of the ADME properties for each of the selected hits among all the ligands generated.

Results & Discussion

We aimed to design a ligand-based drug, which is docked with the protein of our interest, which is going to be the inhibitor that stops the normal functioning of our concerned protein. The protein is the DNA-1 component of the viral genome that codes for the replicase protein. The sequence of AB113660 of the DNA I of BBTV was retrieved. The protein sequence of the gene in FASTA format was blasted in ASD WU-Blast2 of the EBI services Toolbox. Two potential hits were spotted in Pfam.

The trusted matches were 286 residues long [scoring higher than the gathering threshold (A)] and belonged to the domains Viral Rep (5-83aa; EValue-4.2e44) and Viral Rep C (92-215aa; EValue-2.9e-70). Among the two hits, Viral Rep domain was found to contain the sequence containing the significant alignment. The Chain A of the solution structure of Rsgi Ruh-009, an N-terminal domain of Vtila (*Mus musculus*) which is 102aa long was chosen (Locus- IVCS_A; Score-23.9; EValue-9.0). This protein with the NMR structure in PDB databank was retrieved and was used as the homologue for the replicase gene (Figure 1A). This structure has the UniProtKB ID of 089116. It was deposited in the databank by T. Abe and co-workers in 2004. This structure was used as the template for docking.

The template structure was loaded as a raw model in Swiss-PdbViewer (as pdf file – Figure 1B). Then the raw sequence to model is loaded which opens the sequence of the replicase protein from DNA I component of BBTV genome in

FASTA or SWISSPROT format (Figure 1C). The query and the template sequences were aligned manually to improve the fit. The model was then submitted for model optimization in SWISS-MODEL (Figure 1D). The modeled protein was obtained through email and was displayed in DeepView/Swiss-PdbViewer (Figure 1E).

SwissPdbViewer includes a version of the GROMOS 43B1 force field. This force field allows evaluating the energy of a structure as well as repairing distorted geometries through the manipulation using controls called Torsion and mutate. In this implementation, all computations are done *in-vacuo*, without reaction field. It can repair distorted geometries by moving atoms to release internal constraints (Figure 1F).

By logging on to the CASTp server the modeled protein was submitted to find its active sites. The file containing the coordinate structure of the PDB molecule was received through email. The molecule file was loaded in any visualization software and viewed (Figure 1G). Hex superposes or matches pairs of molecular structures using the same 3D density representation as for docking. Superposition is very much like docking, except now the search is for maximum similarity rather than maximum complementarity (Figures 1H & 1I).

The calculation was arranged so that the intermolecular twist angle search is in the innermost loop of the algorithm. This innermost loop turns out to involve a sum over sines and cosines of the twist angle, which may be accelerated using a one-dimensional FFT. There are several controls which specify the resolution, and in particular the order, N, of the docking correlation. The default settings are for the program to perform an initial Steric Scan at N=16, followed by a Final Search at N=25, just using the steric contribution to the docking energy.

In this mode, about all but the top 20,000 orientations were discarded after the Steric Scan. The Steric Scan may be toggled off, in which case every orientation is evaluated using a steric correlation to order N, as given the Final Search slider.

However, this can significantly increase total docking times. The electrostatic contribution to the docking correlation may be enabled using the electrostatics toggle. Electrostatics is only over calculated in the Final Search phase. The final structure was saved and the distance between the protein and ligand was measured in the Swiss-PdbViewer and. It has to be within the limit of 10 angstrom to corroborate that the drug designed is effective (Figure 1J).

Bioavailability is a measurement of the rate and extent of a therapeutically active drug that reaches the systemic circulation and is available at the site of action. (Shargel & Yu 1999) It is expressed as the letter F. The absolute bioavailability of a drug, when administered by an extravascular route, is usually less than one (i.e. F<1). Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation. Such factors may include, but are not limited to the poor absorption from the gastrointestinal tract or the degradation or metabolism of the drug prior to absorption. Bioavailability, solubility (pure water) and pKa values are all referred to for all the hits selected. As per the selection criteria, the competing molecules from the final big hit, say, the drug molecule, are filtered out, which should have the least energy score at each and every step of the selection process (Figures 2A, 2B & 2C). The energy score refers to the same value arrived at the docking process in the Hex package.

Replicase is primarily responsible for the replication of BBTV in the host cell. So far no drug molecule or pesticidal composition is available that could arrest this replication process in the host cell after the viral infection. The modeling of our protein is done in the Swiss Model, with the help of a homologue of twenty-five percent identity – Chain A, an N – terminal domain of Vtila, which is the template for modeling our protein (target). The quality of the modeled protein is found to be more than 65 percent in the SAV server. This helps with

the next step of generating an array of ligands that are docked with the active site of our protein molecule.

Several analogues (Table 2) were generated as ligands. Nineteen (19) out of thirty (30) ligands were selected based on energy after docking (Table 3). Out of them fourteen (14) were filtered out based on the inter-atomic distances of the docked structures (Table 4). Finally, six (6) molecules were selected based upon their ADME scores (Table 5). The drug that has arrived at last is "NSC No.: 82221" which is an analogue of Idoxuvidine. It is found to have the least energy (-30.14 g / mol) and so should be least toxic to human and cattle that might consume the treated fruits.

Computational and pharmacokinetic models that aid in protein-ligand interactions and the relative molecular interactions with clinical outcomes will afford us worthy clues and so make possible budge in the usual one-drug-one-target drug discovery process to a novel pattern of polypharmacology (Lavecchia and Di Giovanni, 2013). Many case studies have confirmed the successful appliance of this approach in the understanding of the molecular mechanisms of drug side effects.

When a drug is administered **intravenously**, its bioavailability is 100 per cent. But when a drug is administered by mouth its bioavailability decreases. Bioavailability is one of the indispensable tools in pharmacokinetics, since it is considered for the calculation of drug dosages for the non-intravenous routes of administration. The ADME properties of the drug molecule guarantee the transportability of the drug molecule through the bio-membrane into the host cell. This ligand molecule is considered as the appropriate basic molecule for laboratory studies in the development of the final formulation which is the basic ingredient of the anti-viral pesticide. The reliable and useful role of the computational procedures has been accepted by one and all. A careful merging of ligand and structure based methods are anticipated to endow

with worthy inroads in the virtual screening protocols.

Conflict Of Interest

The authors confirm that this article content has no conflicts of interest.

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ANNEXURE

Molecular weight	250 – 740 g/mol
Water solubility	Slightly soluble to molecular solubility
State	Solid
LogP / Hydrophobicity	< 5
Drug type	Approved drug
Protein binding	>90 %
Pharmocology	Nucleotide analogue, suppresses replication by selective inhibition of viral DNA synthesis, selective inhibition of viral DNA polymerase.

Table 1. General characteristics of the anti-viral drugs chosen for the study

Sl. No.	Drug compound	Analogue identified
1.	Acyclovir	a) 177222 b) 187684 c) 187685 d) 309109 e) 366152
2.	Ritonavir	a) 70513 b) 116564 c) 116565 d) 116566 e) 116567
3.	Idoxuvidine	a) 39661 b) 82221 c) 97170 d) 113570 e) 674187
4.	Indinavir	a) 686479

5.	Famciclovir	a) 107183 b) 107402 c) 109515 d) 112853
6.	Glyphosate	a) 17368
7.	Ribavirin	a) 127517 b) 133112 c) 133113 d) 133123 e) 163039 f) 627412 g) 627415 h) 656350 i) 690210

Table 2. List of antiviral (DNA) drugs and their corresponding analogues

S. No.	Drug compound	Analogue identified
1.	Acyclovir	187685 - (-19.33) 309109 - (-29.24) 366152 - (-38.98)
2.	Ritonavir	116564 - (-32.09) 116566 - (-32.09) 116567 - (-26.31)
3.	Idoxuvidine	39661 - (-29.47) 82221 - (-30.14) 92183 - (-25.14) 113570 - (-30.77)
4.	Famciclovir	101160 - (-25.88) 107516 - (-25.33) 112853 - (-26.61)
5.	Ribavirin	225111 - (-25.00) 116284 - (-27.43) 128665 - (-26.61) 131659 - (-22.01) 131664 - (-24.22)

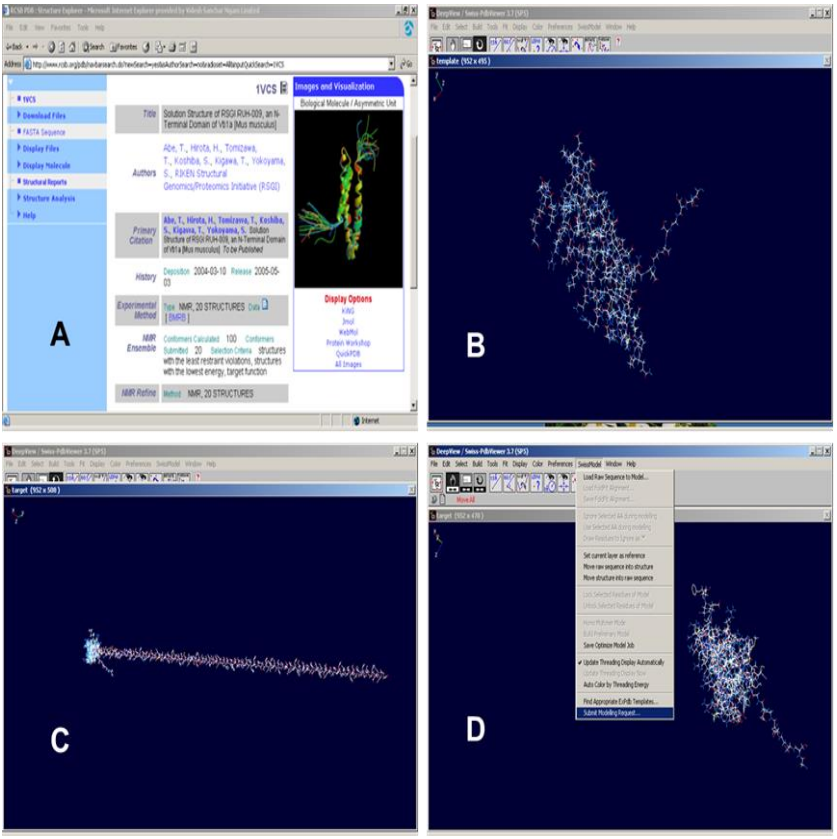
Table 3. List of selected ligands based on energy factor after docking

Sl. No.	Drug Compound	Analogue identified
1.	Acyclovir	366152; 309109
2.	Ritonavir	116564; 116566; 116567
3.	Idoxuvidine	39661; 82221 92183; 113570
4.	Ribavirin	116284; 128665

Table 4. List of selected ligands based on distance between the protein and the ligand

Ligand molecule	Bioavailability (%)	pKa value	Solubility
82221	>30; soluble	Strong acid	Soluble
113570	>30; soluble	Strong acid	Soluble
366152	>30; soluble	Strong base	Soluble
116564	>70; soluble	Strong acid; Strong base	Slightly soluble
116566	>70; soluble	Strong acid	Highly insoluble
116567	>70; soluble	Strong acid	Insoluble

Table 5. List of selected ligands based on ADME properties



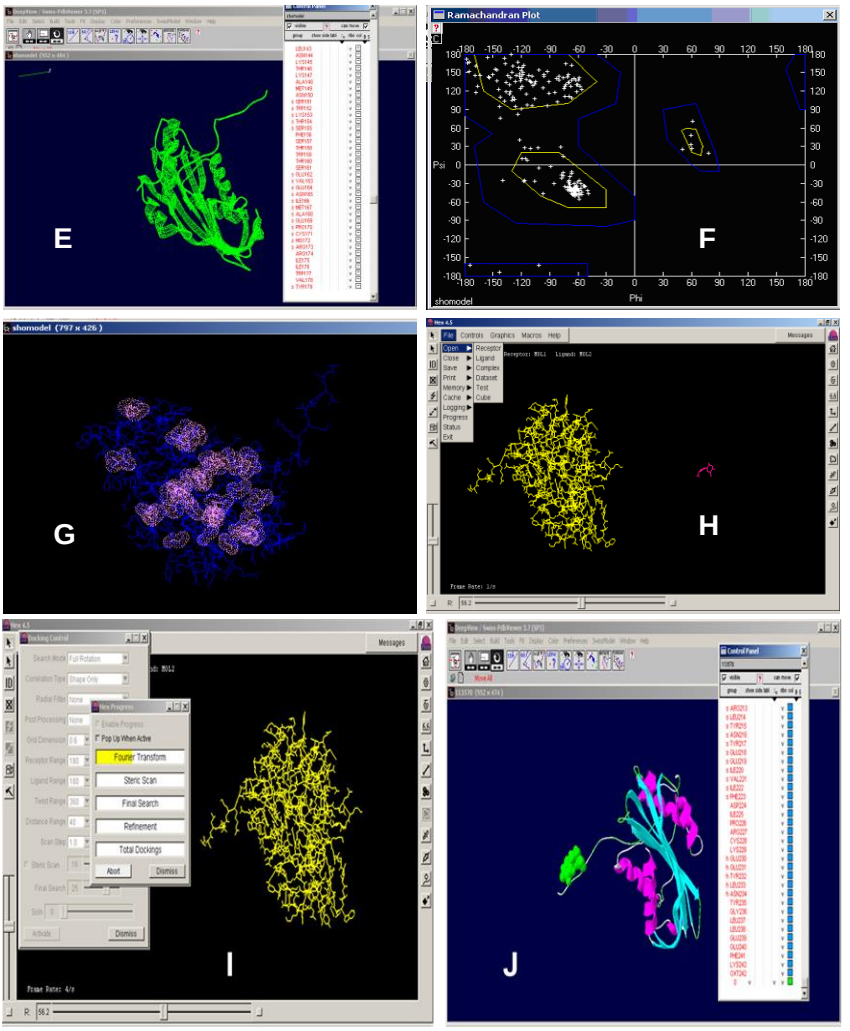


Figure 1. The docking of the modelled protein, a homologue of the replicase gene with selected ligand.

A) The PDB structure of the template (Vtila protein) is displayed. B) The sequence of the replicase gene of DNA I of BBTV is opened in the Swiss-PdbViewer. C) Fitting both the target and the template. D) Submitting the structures to the SWISSS-MODEL after manual fitting. E) Displaying the model from SWISS-MODEL in DeepView. F) Ramachandran plot with no disallowed regions seen. G) Active sites in the model H) Opening the ligand and the receptor for docking in HEX I) Docking in progress J) The final docked structure

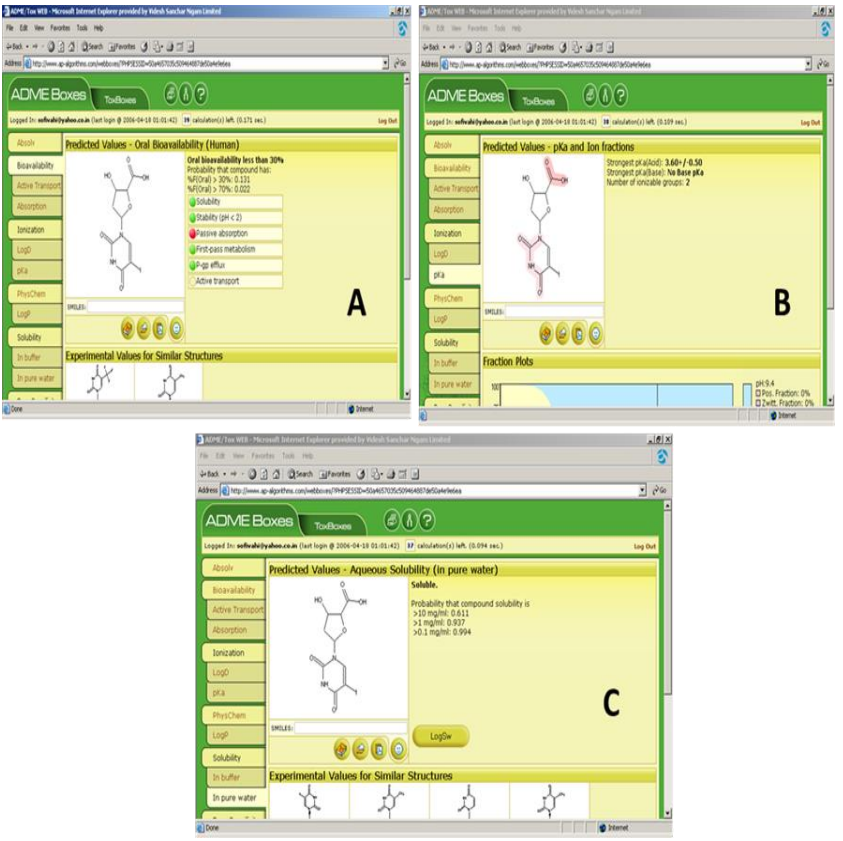


Figure 2. The ADME properties, namely, A) bioavailability B) pKa value and C) solubility value of the chosen analogue NSC No.: 82221 of the viral drug Idoxuvidine.

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