Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(2):1200-1206

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Discovery of Some Potential HIV Inhibitors as Anti -Dengue Drugs: an Insilco Approach

Prangya Ranjan Rout[1], Raghunath Satpathy[2], Gyana Ranjan Satpathy[3]

[1]Department of Biotechnology, National Institute of Technology, Warangal, Andhra Pradesh, India
[2]MIRC Laboratory, Department of Biotechnology, MITS Engineering College, Rayagada, Odisha, India
[3]Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha, India

---

ABSTARCT

The present work is a computational approach which predicts about some potential HIV protease inhibitors that acts as anti dengue drugs by inhibiting NS3 protease of Dengue virus. In order to study the activity of these selected potential drug molecules, taking two best docked complexes Molecular Dynamic Simulation was performed considering water as solvent and at 1 nanosecond time scale to investigate the structure, dynamics, thermodynamics and stability of the complex in the targeted environment. Further pharmacophore modelling and finally QSAR analysis was performed by taking some of the photochemical and structural descriptors. The effect was calculated for each type of descriptors by taking Andrews affinity as a dependent variable. The results obtained with these models suggest, for this particular drug molecule photochemical descriptors play a major role in controlling the activity which is consistent with the result obtained from filmmaker modelling.

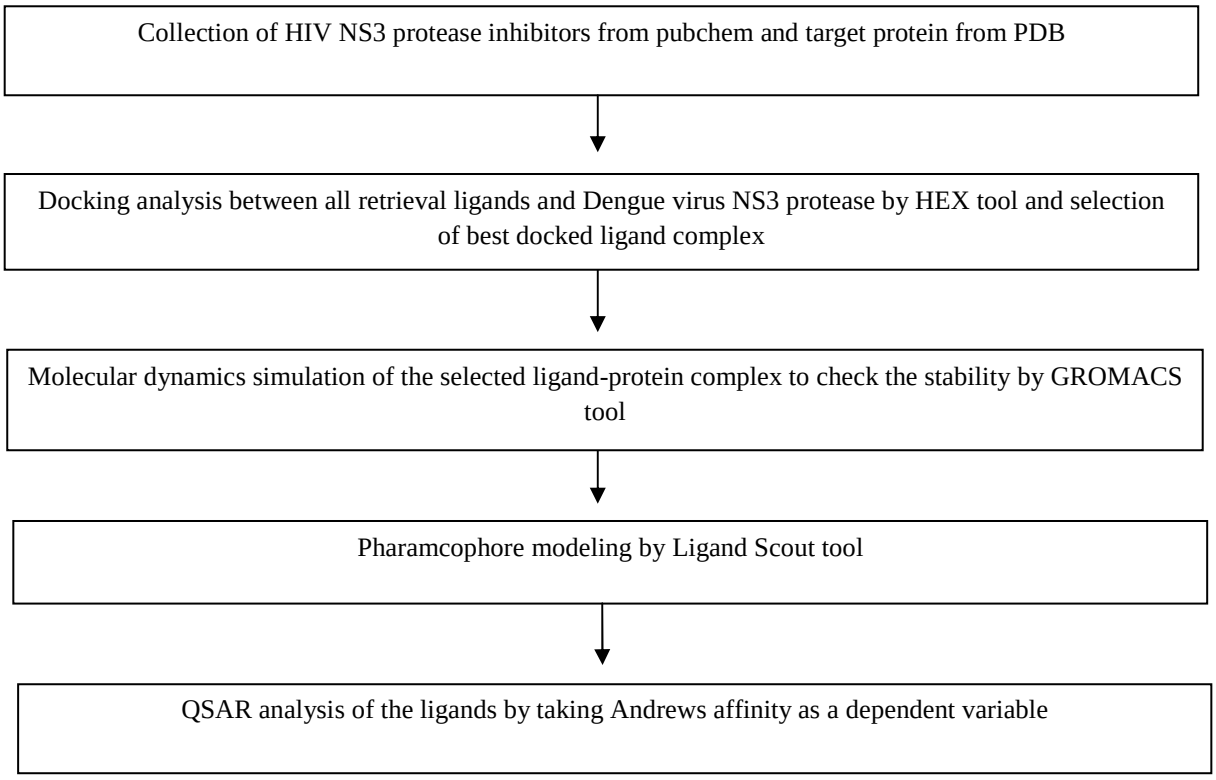
Key words: NS3 protease inhibitor, Docking study, QSAR analysis, Pharmacophore, Molecular Dynamic Simulation, Multiple Regression.

INTRODUCTION

Dengue virus (DENV) is one of the most global pathogens and may represent a global pandemic. Approximately 2.5 billion people worldwide at risk for dengue infection. About 100 million cases of Dengue Fever (DF) and 50,0000 cases of Dengue Hemorrhagic Fever (DHF) have been reported globally annually [1-2].There are four serotypes of dengue virus, DEN1, DEN2, DEN3 and DEN4, based on antigenic property or sequence similarity. The most prevalent of the four dengue serotypes is Dengue virus type 2 (DEN 2), which contains a single-stranded RNA of positive polarity [3].Till date little success has been achieved in antiviral therapy against Dengue virus however NS3 viral protease, required for virus replication is a potential target for antiviral drugs [4].Targets currently being investigated include viral entry, viral RNA polymerase/ methyltransferase, nucleotide synthesis, viral helicase/ NTPase, viral serine protease, R-glucosidases, and kinases [5].The non-structural 3 (NS3) protease is one of the most promising targets for drug development against flaviviridae infections because it is responsible for cleavage of the viral polyprotein precursor and plays a pivotal role in viral replication [6].For NS3 protease activity a ~40 residue hydrophilic domain from NS2B is required as co-factor [7]. Recently also two inhibitors of the closely related hepatitis C virus protease are under late-stage development [8]. The goal of this study is to identify novel dengue virus (type 2) NS3 protease inhibitors for eventual development as effective anti-flaviviral drugs. Various methods and tools for docking, molecular dynamics simulation, pharmacophore modeling and QSAR analysis has been used computationally to screen and evaluate the effectiveness and bioactivity of drug molecules [9].

EXPERIMENTAL SECTION

The molecular structure of HIV NS3 protease inhibitors were collected from PubChem database available in the NCBI server (http://pubchem.ncbi.nlm.nih.gov/).The structure were drawn by Marvin sketch 5.0 tool (http://www.chemaxon.com/marvin/sketch/index.jsp) and corresponding 3D structure were obtained. The molecules were then energy minimized by PRODRG server [10]. Prodrg is an online tool where the energy minimization of the molecule was performed by using Gromos 96 force field. Further in the docking study the receptor was chosen as dengue NS3 protease from Protein Data Bank (PDB ID 2FOM). The protein was further processed to remove the ligands and crystal water molecules. Docking was performed between the retrieved ligands and the receptor by HEX 5.0 tool. Hex is a reliable tool for rigid docking [11].To check the reliability of the binding of selected drug molecules to the receptor, molecular dynamics study and pharamcophore modelling analysis were performed. QSAR analysis was performed after calculation of various descriptors. Preadmet tool (www.bmdrc.org/04_product/01_preadme.asp) was used to calculate structural and physiochemical descriptors .The different combinations of the above two types of descriptors were subjected to multiple regression analysis by MINITAB 14 software to find out the effect. Detail stepwise methods of the present work are given below.



RESULTS AND DISCUSSION

Docking and molecular dynamics simulation study

The docking of all total 19 HIV protease selected inhibitors with the dengue ns3 protease was performed by HEX tool. After docking the ligands were carefully observed for its conformation and docking energy. The ligands having PubChem database ID CID 482206 and CID 484561 found to be having highest binding affinity as -400.08 (Table 1). Again to check the reliability of the ligand–protein complex, molecular dynamics simulation was carried out by GROMACS tool by using GROMOS 43 a1 force field. The two best selected docked complex obtained from docking results were used as the starting material for molecular dynamics simulations. Each of the protein-ligand complexes was analyzed independently by solvated in a cubic box with water molecules. As the system was neutral no counter ions were added for further neutralization purpose. 0.8 nm gap was maintained between the protein atoms and edge of the solvated box. The system was initially subjected to energy minimization for 200 steps by steepest descent. The minimized system was equilibrated for 50 ps at a temperature of 300 K. Then the system in equilibrium was subjected to molecular dynamics simulations for 1 nanosecond. Periodic boundary condition was used with constant number of particles in the systems, constant pressure, and constant temperature simulation criteria (NPT). For all simulation the computing power was utilized as High performance cluster for Biological Applications which is based on Intel Xeon Dual Quad core as processor, Gcluster HPC 1.3 X86-64 bit edition, total 16 nodes each having 4GB of memory [12].From molecular dynamics simulation the features for the complexes were obtained

give in Fig.(1).The RMSD (root mean square deviation) across C alpha - C alpha back bone, RMSF (root mean square fluctuation) which represents individual fluctuation of amino acid during simulation and energy profile (kinetic, potential and total) were computed Fig. (2). The RMSF calculation indicates first 50 residues after binding in complex 1 shows fluctuations but the rest of the residue fluctuations are same and within a tolerable range. The RMSD in case of both the complex is constant which indicates that after binding to the ligands the complexes remains stable during the molecular dynamics simulation.

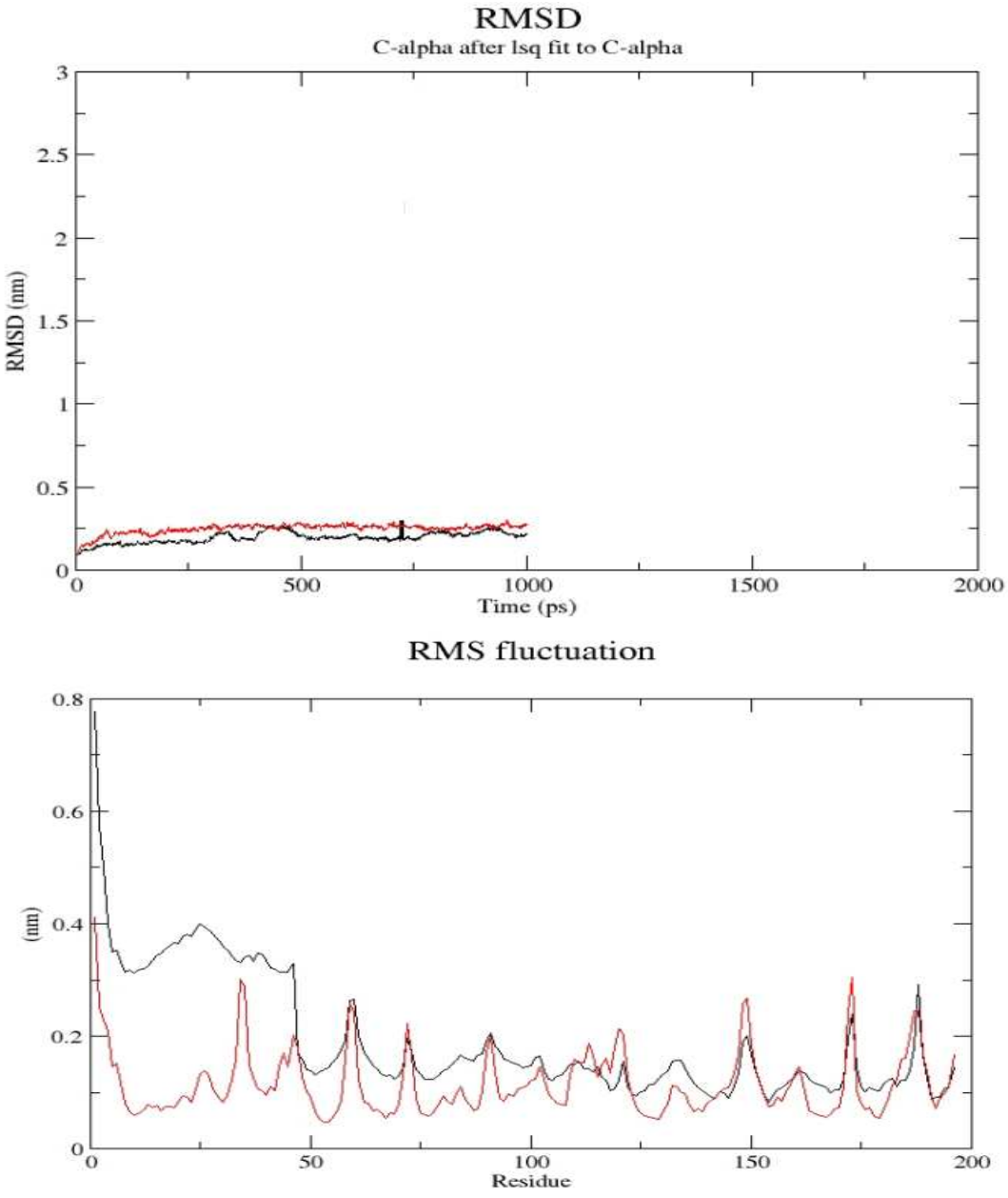


Figure 1: Showing the RMSD, RMS fluctuation of residues of the complex 1 (———) and complex 2 (———) during molecular dynamics simulation

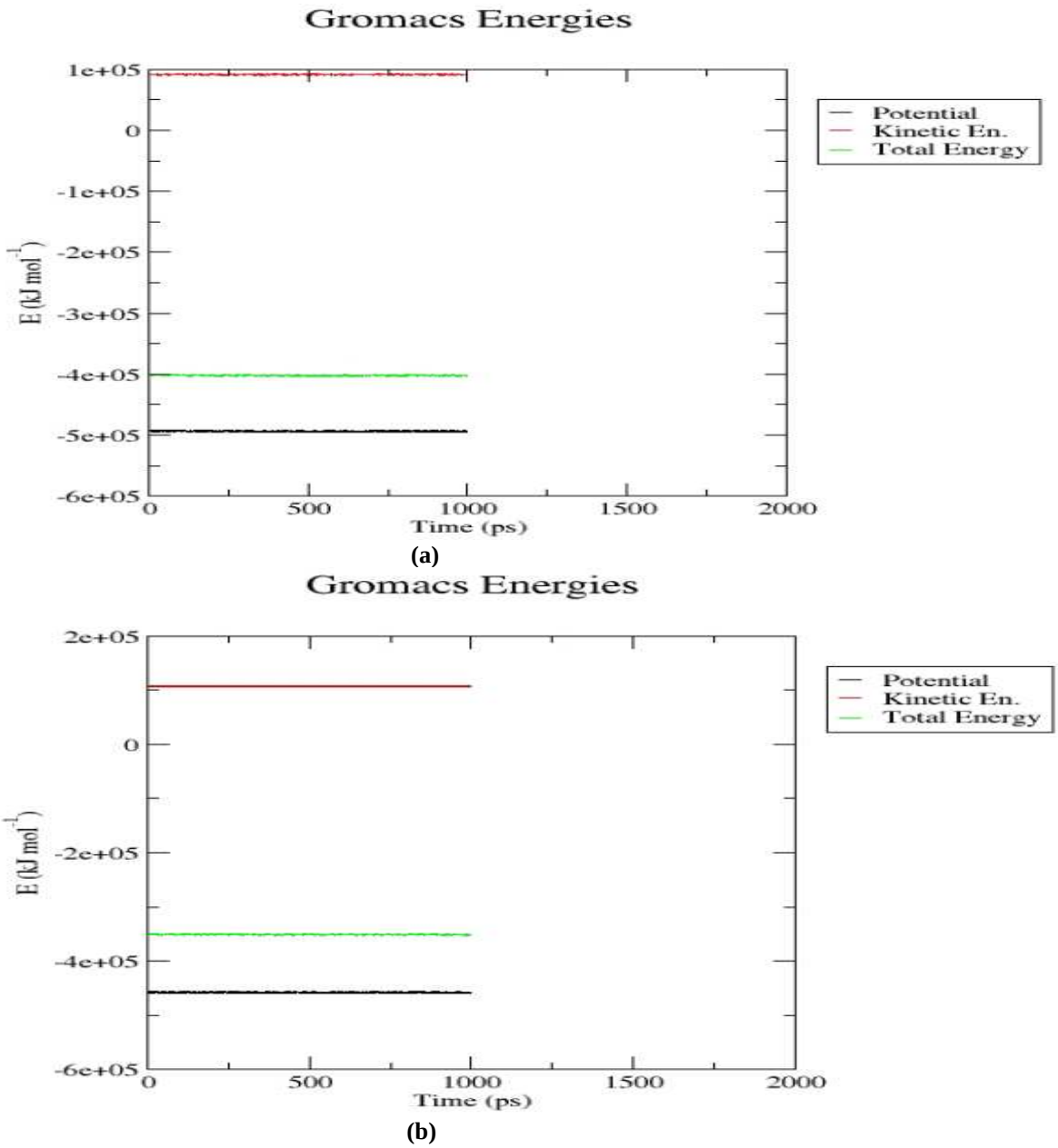


Figure 2: Energy profile of complex 1 and complex 2 computed from Gromacs tool

Pharmacophore modelling analysis

Pharmacophore models are collection of features that are essential for optimal interaction with specific biological targets and to trigger its function. Pharmacophore modelling is playing a key role in the identification of ligand features for the particular targets [13][14]. By using the Ligand Scout tool various pharamcophoric features were calculated [15]. It was observed that the features like number of hydrogen bond donor and number of hydrogen acceptor were showing consistency for all the ligands (Table 1). The binding residues for two considered ligand were found to consist of GLU and LYS in their active site despite of their binding position in receptor protein. The distance between the HBA and HBD was calculated as 8.6 angstrom during functional orientation of the best drug molecule after docking Fig. (3).

Table 1: Results showing the docking energy of all ligands with the receptor and computed pharmacophoric features

Pubchem Ligand ID	Molecular weight (g/mol)	Docking Energy	POCKET Binding residues	Number of hydrogen bond donor	Number of Hydrogen bond acceptor
CID 482206	936.058100	-400.88	GLY62,GLN 93	4	2
CID 482207	**923.062640**	-355.44	**GLU 86, LYS 84, GLU86**	**1**	**3**
CID 482209	830.942520	-358.94	THR 120, ASN 119	2	3
CID 482210	693.872600	-346.37	LYS 28, GLY 62, GLN 93,	4	2
CID 482211	913.001540	-310.67	ARG 54	2	10
CID 484561	**797.914140**	-400.88	**LYS 104, GLU 90, GLU 101**	**2**	**2**
CID 484563	439.482700	-222.51	LYS 28	4	2
CID 484564	897.045200	-313.19	VAL 146, TRP 83	2	3
CID 484565	781.957800	-292.62	ASN 167	2	3
CID 484566	652.843820	-273.89	LYS 73, ASN 152, LYS 74, GLY 87, TRP 89	3	3
CID 484567	423.526360	-229.06	VAL 147, LEU 76, ALA 164, VAL 154	2	4
CID 484568	780.842320	-251.07	LYS 28, GLN 93	4	2
CID 484570	824.894880	-344.75	ALA 166, ASN 167, LEU 76, MET 149, THR 118, ASN 119	4	6
CID 484571	820.927780	-318.63	LYS 28, GLY 62, GLN 93	4	2
CID 484572	912.059840	-342.25	LYS 28, GLY 2	4	2
CID 484573	864.938820	-314.63	LYS 28, GLY 62, GLN 93	4	2
CID 484574	760.852680	-324.20	LYS 28, GLY GLN 93,	4	2
CID 302867	393.478700	- 226.72	LYS 28, GLY 62, GLN 93	4	2

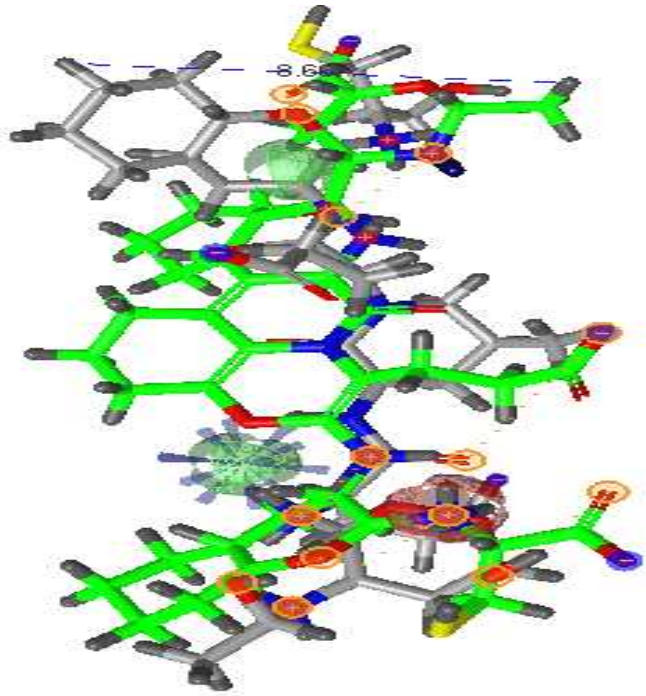


Figure 3: Pharmacophore model of ligand 1 (gray) and ligand 2 (green)

QSAR study

Two types of descriptors sets were calculated by the PREADMET server as given in Table 2. From the QSAR analysis. The different combinations of the above two types of descriptors were subjected to multiple regression analysis by MINITAB 14 software.

Table 2: Considered descriptors for the present study

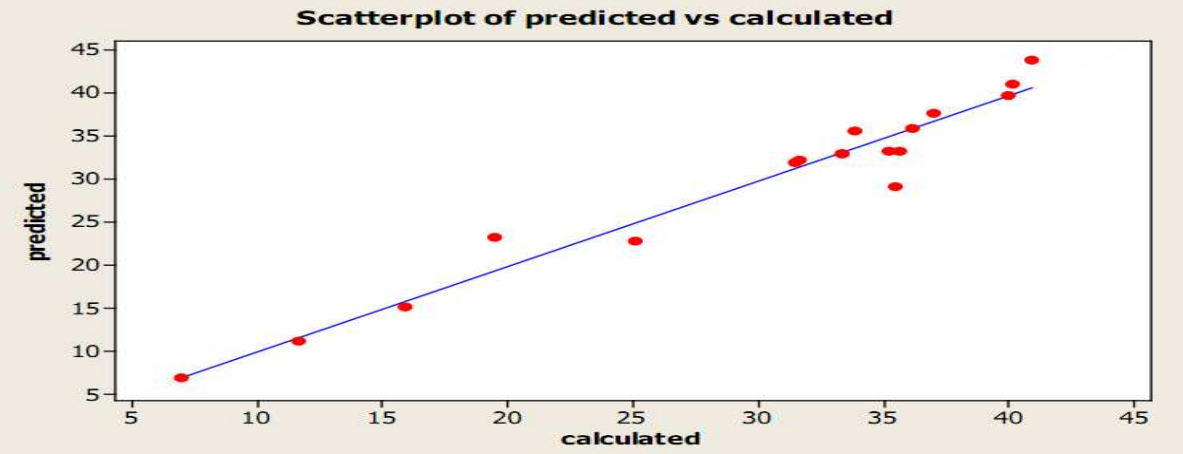
Serial No.	Physiochemical Descriptors	Structural descriptors
1	Partition co-efficient (Log P)	Wiener index (WI)
2	Water salvation free energy (WSE)	Hydrophobic surface area (HSA)
3	Water solubility in buffer system (WSBS)	Polar surface area (PSA)
4	SK Log P	Vander walls volume (VV)

For the best model selection various parameters like high F value, R-Sq and P value was considered. The Andrews affinity was chosen as dependent variable as the Andrews affinity parameter is calculated based on the drug receptor binding affinity, hence suitably can be used as a parameter for calculating the effect [16][17]. From the regression analysis the equations were derived predicted effect or as below.

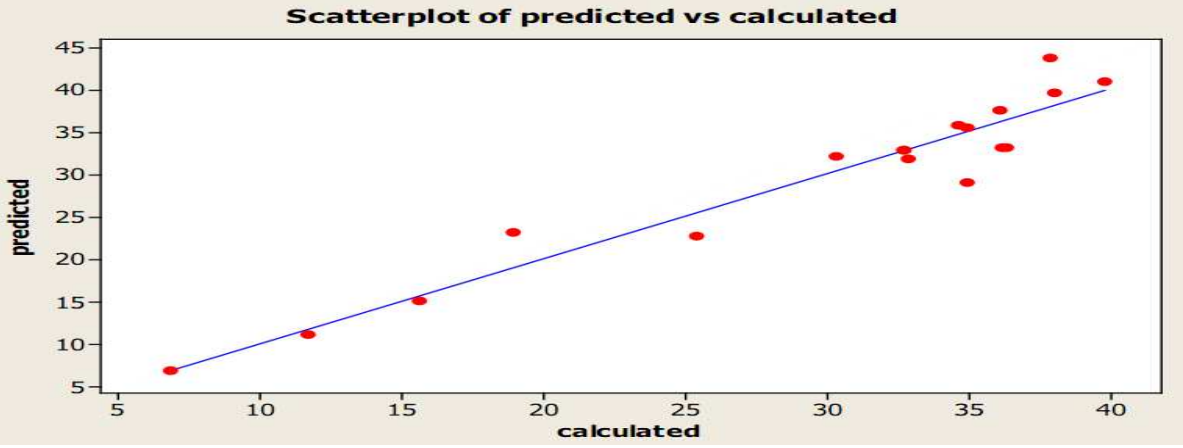
Andrews coefficient (physiochemical) = - 15.9 - 1.49 sk log d - 0.613 WSE - 0.000000 WSBS + 2.53 SK log p

Andrews coefficient (structural) = - 14.4 - 0.0885 HSA + 0.032 PSA + 0.118 VV - 0.000044 WI

The statistical parameters obtained as S = 2.47372 R-Sq = 95.5% R-Sq (adj) = 94.2% for physiological and S = 3.11250 R-Sq = 92.9% R-Sq (adj) = 90.8% for topological descriptors. From the equations the predicted and experimental Andrew's affinity value were compared by plotting scatter plots Fig. (4).



(a)



(b)

Figure 4: The predicted and calculated affinity relationship in case of physiochemical (a) and structural descriptors (b)

The binding energy analysis can lead to a conclusion that some HIV protease inhibitors can bind to the dengue NS3 protease almost with equal efficiency as they can bind with the HIV protease itself. The amino acid LYS and GLU are observed most frequently in the binding sites of the target protein. In order to prove the hypothesis the two best docked ligands were taken and subjected to MD Simulation and analysis of RMSD, RMSF, and Energy graphs lead to the prediction of stability of the ligand-receptor complex [18]**.** Further by pharmacophore modelling method computes the features like hydrogen bond donor and acceptors in case of all ligands**.** From the QSAR Analysis the best models were chosen by observing R-Sq value, F-value and residual errors[19]. The results obtained with these models suggest, for this particular drug physiochemical descriptors are mainly depends strongly on the activity as consistent with the pharmacophoric features [20]. The hydrogen bonding is an important phenomena in case of ligand binding which is also mostly reflects the biochemical features of ligand- receptor complex formation [21][22]. The results obtained from the study provide the fact about some HIV protease inhibitors which also could be used against Dengue virus as anti dengue drugs. However this work is an Insilco based study; hence experimental verification of this result would be more useful.

Acknowledgment
We are thankful to Chief executive, Majhighariani Institute of Technology & Science, Rayagada for his encouragement and for providing us MIRC lab for computing facility.

REFERENCES

[1] http://www.who.int/mediacentre/factsheets/fs117/en/
[2] I Umareddy**;** O Pluquet**;** QY Wang**;** SG Vasudevan**;** E Chevet**;** F Gu**.** *Virology Journal*, **2007**, 4:91 doi: 10.1186/1743-422X-4-91
[3] RF Qi; ZW Song; CW Chi. *Acta Biochimica eta Biophysica Sinica*. **2005**, 37, 283–292.
[4] JF Bazan; RJ Fletterick. *Virology Journal* **1989**,171, 637–639.
[5] A Cahour; B Falgout; CJ Lai. *J. Virol*. **1992**, 66, 1535– 1542.
[6] H Li; S Clum; S You; KE Ebner; R Padmanabhan. *J. Virol*. **1999**, 73, 3108–3116.
[7] R Yusof; S Clum; M Wetzel; HM Murthy; R Padmanabhan. *J. Biol. Chem*., **2000**, 275, 9963-9969.
[8] C Yon; T Teramoto; N Mueller; J Phelan; VK Ganesh; KH M.Murthy; R Padmanabhan. *J. Biol. Chem*. **2005**, 280, 27412–27419.
[9]. SM Tomlinson; RD Malmstrom; A Russo; N Mueller; YP Pang; SJ Watowich.*Antiviral Res*. **2009**,82(3),110-4.
[10]. AW Schuettelkopf; DMF VanAalten. *Acta Crystallographica*, **2004**, D60: 1355-1363.
[11]. DW Ritchie. *PROTEINS. Structure, Function and Genetics*. 52, 98-106, **2003**.
[12]. R Satpathy; RK Guru; R Behera; A Priyadarshini. *JCSB*, **2010**, 3(3), 059-061.
[13]. EB Mustapha; L Ismaili; I Tomassoli; L Nicod; M Pudlo. *International Journal of Medicinal Chemistry* **2011** (2011), Article ID 592879, 10 pages. doi: 10.1155/2011/592879
[14]. PP Kapupara; SP Dholakia; VP Patel; BN Suhagia. *J. Chem. Pharm. Res.*, **2010**, 2(3):287-294
[15].TM Steindl; D Schuster; G Wolber; C Laggner; T Langer. *J. Comp. Aided Mol. Design*, **2006**,20:703-715.
[16]. R Satpathy; RK Guru; R Behera. *J. Chem. Pharm. Res*., **2010**, 2(6):344-350
[17]. AB Padavala; VV Prasanth; SA Jayanthi; A Vadlamani; S Chitti. *J. Chem. Pharm. Res.*, **2010**, 2(2): 147-162.
[18]. J Zuegg; JE Gready . *J. Biochemistry* **1999**, 38, 13862 13876
[19]. LVB Hoelz; BAC Horta; JQ Araújo; MG Albuquerque; RB Alencastro; JFM Silva. *J. Chem. Pharm. Res.*, **2010**, 2(5):291-306
[20].LK Sukhbir; A Husain; JS Jain. *Der Pharmacia Sinica*, **2011**, 2 (4): 241-252
[21].N Suresh; NS Vasanthi. *J Proteomics Bioinformatics*3: 020028.doi:10.4172/jpb. 1000117.
[22]. AJM Reddy; MR Barik; GL Muli; Parthasarathy.T. *J. Chem. Pharm. Res.*, **2012**, 4(1):265-271