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# Molecular docking and High-throughput screening of potent inhibitor to gp46SU involved in ATLL through drug design studies

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**ABSTRACT**

*Adult T-cell Leukemia/Lymphoma, a malignancy of mature activated T-cell is caused by the Human T cell Lymphotropic virus type 1(HTLV-1). HTLV-1 entry into the cell involves the interaction between the surface subunit and cellular receptor of the host. On the basis of studies carried by Kathryn.S and team on the functional domains of the HTLV-1 SU, we carried out computational drug designing and docking studies on HSPG binding domain of gp46SU of env protein. Structure modelling, analysis and validation was done for gp46SU. Scaffold selection was based on the functional properties of HSPG binding domain of the protein. Leads were identified based on several physiochemical properties and we created our library with those new molecules that were generated based on Lipinski's rule of five. We carried out high throughput screening and molecular docking on 446 molecules from scaffolds selected. Screening of molecules was based on the criterions such as, TOPKAT and ADMET properties. Among the ligands used, only three compounds were identified to have interaction within the targeted domain. Eleven molecules were analysed with positive docking results and interactions within the binding site. Pharmacophores were generated and analysed for selected drug candidates. The ranking of ligands helped in the identification of the best inhibitor(SH19 and SH226) that can better target gp46SU in an effective way. To be more specific about our drug candidate we suggest SH19 to be the most potent molecule in terms of physiochemical and docking properties. These results suggest that the identified compounds have the potential to inhibit HSPG binding in ATLL.*

**Keywords**: HTLV-1, gp46SU, modeling, TOPKAT, ADMET, receptor, ligand, docking, pharmacophore

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## INTRODUCTION

Adult T-cell Leukemia/Lymphoma (ATL) is a rare cancer of the immune system's own T-cells. *Human T cell Leukemia/Lymphotropic virus type 1* (HTLV-1) is believed to be the cause of it, in addition to several other diseases.HTLV-1 infects approximately 15to 20 million people worldwide[14,15]. Death is most often caused by infectious complications, uncontrolled hypercalcemia or progressive disease. HTLV-1 also causes HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[16].

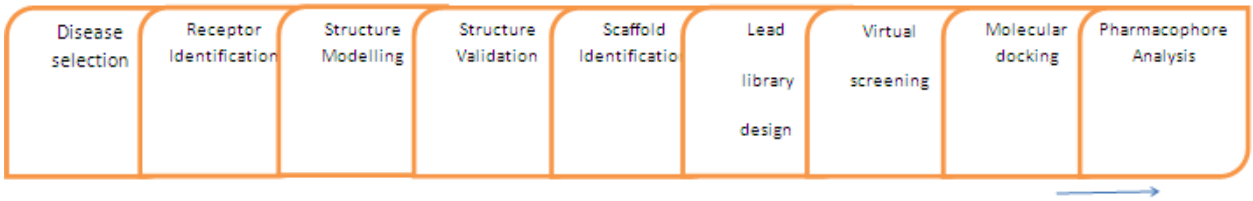
More recent studies demonstrate that HTLV-1 infectivity involves interactions with three different molecules: heparan sulfate proteoglycans (HSPG)[21,22,27], the VEGF-165 receptor Neuropilin 1 (NRP-1)[21,22] and glucose transporter type 1 (GLUT1)[22,23,25].By revisiting studies from different researches we hypothisedthat the HSPGs binding domain[45] of surface subunit in gp46 protein play the key role in the viral attachment to the host cell surface and are often critical determinants of the pathogenic "virulence" and disease-type specificity of the virus.

In the current study we analyse the protein sequence of surface subunit of human T cell leukemia type-1 deposited at GenBank. Taking to the next stage we carried out further studies on HSPGs binding domain(aa215-313) of surface subunit in gp46 protein. Since no structural information are available about this protein in any databases, we

adopted protein models available from different servers and validated them. *In-silico* methods have been applied to pharmacology hypothesis development and testing. These *in-silico* methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, and other molecular modelling, machine learning, data mining, network analysis tools and data analysis tools that use a computer.

During our study, we designed 446 ligands having potential as inhibitor to HSPG binding and inhibit the protein. This inhibitory action of the ligand could minimize the virulence of the virus. All the ligands were screened for Lipinski's Rule of 5[46], toxicity and later on docking was done. These ligands and receptor was also energetically minimized during those process. Majority of our work was performed using Accelrys Discovery studio 2.5.

## EXPERIMENTAL SECTION




***3.1. Target identification and validation*:**
Surface subunit gp46 sequence was retrieved from GenBank (119466) and Uniprot (P23064.1) and analysed for domains using Blastp against Protein Data Bank. Meanwhile sequences of gp46 SU deposited in GenBank at various periods were subjected to multiple sequence alignment to determine the quantity and quality of mutations occurred. The template 1MG1_A having query coverage (17%), having an e-value of 7e-54 was automatically chosen by M4T server and the structure was validated for Ramachandran plot using Procheck and Discovery studio.

***3.2. Scaffold Selection*:**
Searching for drugs having potent inhibitory action on protein with similar function, we analysed drugs from Zinc database for gp70(Env_MMTVG), gp160(Env_HV1H2), gp 160(Env_HV1Y2) and gp160(Env_VILV).Inhibitor for Hemagglutinin against H1N1 Influenza Virus and surface protein of hepatitis B virus were used for lead designing based on various literatures substantiating their biological functionality.

***3.3. Lead library Designing*:**
Lead library was designed based on Lipinski's rule of five. The functional group of all leads were kept unchanged on the course of our designing. Lead design was performed with ChemSketch Freeware. Care was taken not to include heavy atoms or carcinogenic atoms to the molecule.

***3.4. Virtual screening:***
ADMET and TOPKAT protocols were used in Discovery studio to screen ligand molecules. Six models such as NTP Carcinogenicity Call (Male Mouse) (v3.2), FDA Carcinogenicity Female Mouse Single vs. Mult (v3.1), Developmental Toxicity Potential (DTP) (v3.1), Rat Oral LD50 (v3.1), Skin Irritation (v6.1) and Aerobic Biodegradability (v6.1) were deployed for TOPKAT analysis(Table.2).

Molecules screened with TOPKAT were later subjected to ADMET protocol. ADMET - Blood Brain Barrier model predicts blood-brain penetration (blood brain barrier, BBB) after oral administration.(Table.3) This model contains a quantitative linear regression model for the prediction of blood-brain penetration, as well as 95% and 99% confidence ellipses in the ADMET_PSA_2D, ADMET_AlogP98 plane (Egan and Lauri, 2002).

***3.5. Receptor and Ligand Preparation:***
The validated model was then determined for largest binding site using CASTp server and Accelrys Discovery Studio 2.5. The sphere was defined for the binding site; typing was carried out by CHARMm force field (Momany-Rone parital charges methods). Minimization was carried out in Accelrys Discovery Studio 2.5 using 1400 cycles of conjugate gradient; a constant potential energy of -22322.84878 kcal/mol was obtained.

The screened compounds were typed similarly using CHARMm for partial charges set up and minimized by Conjugate Gradient until a constant potential energy was obtained

### *3.6.Receptor-Ligand Docking:*

The minimised receptor and ligand was docked with LibDock, a relatively fast algorithm thatconducts 'HotSpots' matching of ligand conformation and later docked with Hex to obtain a Receptor-Ligand complex. The Receptor-Ligand complex is studied to determine the potentiality of the molecules docked.

The interactions for the pose with low LibDock Score are studied and intermolecular hydrogen bonds and intermolecular bumps were further examined(Table.4).

### *3.7.Pharmacophore Analysis:*

The ligands were analysed for pharmacophore using the common purpose pharmacophore in Pharmacophore protocol available in Discovery studio.Pharmacophore analysis include aromatic group, donor molecule, positive and negative ionizing group ,hydrophobic group and hydrophilic group(Fig.5).

## RESULTS

**Table.1: Molecular properties of molecules used for docking**.

| Mol. Name | ALogP | Molecular Weight | Num_H Acceptors | Num_H Donors | Num_ Rotatable Bonds | Num_ Rings | Num Aromatic Rings | Molecular_ Fractional Polar Surface Area |
|---|---|---|---|---|---|---|---|---|
| SH710 | 4.428 | 364.4376 | 5 | 0 | 6 | 3 | 2 | 0.162 |
| SH19 | 4.678 | 364.4376 | 5 | 0 | 7 | 3 | 2 | 0.165 |
| SH8 | 4.482 | 308.3743 | 4 | 0 | 5 | 3 | 2 | 0.142 |
| SH20 | 4.968 | 322.4009 | 4 | 0 | 5 | 3 | 2 | 0.133 |
| SH226 | 4.833 | 336.3844 | 5 | 0 | 5 | 3 | 2 | 0.182 |
| SH71 | 3.962 | 392.4477 | 6 | 0 | 7 | 3 | 2 | 0.196 |
| SH77 | 2.786 | 324.3306 | 6 | 1 | 5 | 3 | 2 | 0.259 |
| SH78 | 3.028 | 308.3312 | 5 | 0 | 5 | 3 | 2 | 0.203 |
| SH123 | 4.93 | 378.4641 | 5 | 0 | 7 | 3 | 2 | 0.157 |
| SH212 | 4.087 | 364.3945 | 6 | 0 | 6 | 3 | 2 | 0.217 |
| SH121 | 5.134 | 378.4641 | 5 | 0 | 8 | 3 | 2 | 0.158 |

**Inference**:446 molecules were designed and those molecules which obey the Rule of five were selected for further screening and later docking studies

**Table.2: TOPKAT analysis of molecules used for docking**

| Mol. Name | NTP Carcin ogenicity Call (Male Mouse) (v3.2) | FDA Carcin ogenicity Female Mouse Single vs Mult (v3.1) | Develo pmental Toxicity Potential (DTP) (v3.1) | Rat Oral LD50 (v3.1) | Skin Irritation (v6.1) | Aerobic Biodegrad ability (v6.1) |
|---|---|---|---|---|---|---|
| SH710 | -5.95 | 12.051 | 18.161 | 6.4 g/kg | 18.692 | -14.694 |
| SH19 | 12.243 | -32.202 | 11.25 | 1.6 g/kg | -22.653 | -32.85 |
| SH8 | -14.69 | -34.59 | 9.072 | 3.3 g/kg | 6.723 | -22.82 |
| SH20 | -13.717 | -32.847 | 9.053 | 2.4 g/kg | 3 | -32.139 |
| SH226 | -19.118 | -27.603 | 19.752 | 101.1 mg/kg | -6.059 | -30.888 |
| SH71 | -7.615 | -29.621 | 21.168 | 2.6 g/kg | 4.518 | -14.937 |
| SH77 | -7.625 | -21.5 | 9.725 | 6.9 g/kg | 0.508 | -6.773 |
| SH78 | 0.011 | -24.675 | 6.053 | 7.0 g/kg | 3.461 | -10.992 |
| SH123 | -10.882 | -41.49 | 18.573 | 2.3 g/kg | 6.011 | -24.387 |
| SH212 | -10.491 | -29.397 | 15.591 | 112.1 mg/kg | -5.717 | -28.207 |
| SH121 | -10.127 | -36.27 | 5.769 | 1.4 g/kg | -12.328 | -43.518 |

**Inference**: 33 ligand molecules were identified to show positive results. Molecules screened for TOPKAT screening were given flexible criterion on Skin irritation, DTP and Rat oral LD50.

**Fig.1. TOPKAT screening for ligand SH19**

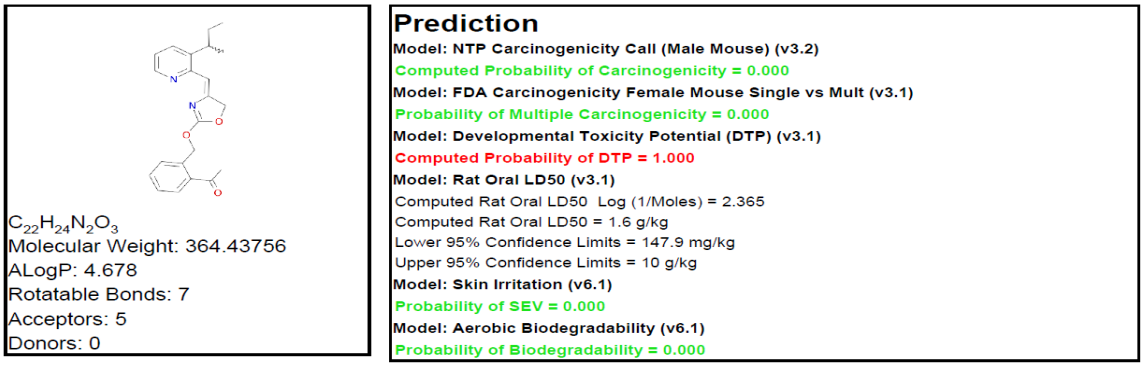
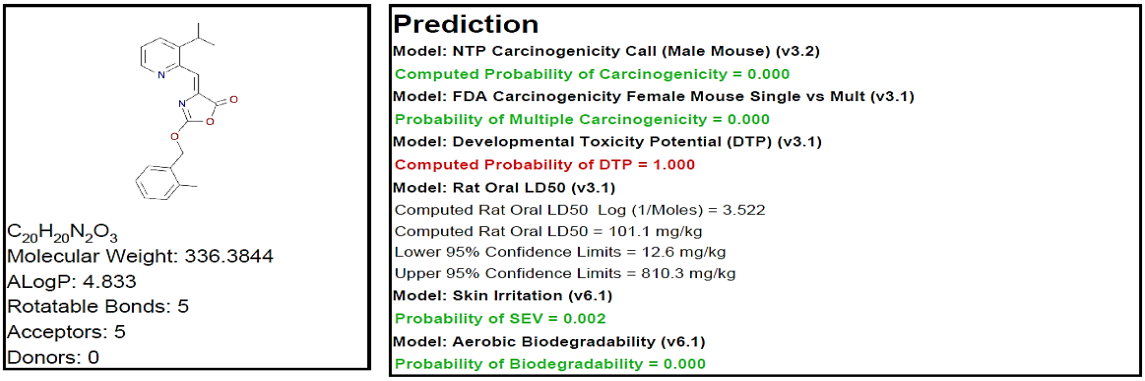



**Fig.2. TOPKAT screening for ligand SH226**




**Inference:** The ligand SH19 and SH226 had good TOPKAT score and are well suited for ADMET screening. Tolerance to skin irritation and DTP were given to these molecule to a certain limit.

**Table.3. ADMET screening for molecules used for docking studies**

| Mol_ Name | BBB | BBB Level | Absorption_ Level | Solubility | Sol Level | Hepatotoxicity | Hepatotoxicity _Probability | CYP 2D6 | CYP2D6 Probability | PPB Level | AlogP 98 | Unknown_ AlogP98 | PSA 2D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SH710 | 0.301 | 1 | 0 | -5.32 | 2 | 0 | 0.476 | 0 | 0.326 | 1 | 4.428 | 0 | 57.745 |
| SH19 | 0.378 | 1 | 0 | -5.341 | 2 | 1 | 0.569 | 0 | 0.396 | 1 | 4.678 | 0 | 57.745 |
| SH8 | -0.45 | 2 | 0 | -4.095 | 2 | 0 | 0.357 | 0 | 0.366 | 0 | 3.055 | 0 | 78.398 |
| SH20 | 0.742 | 0 | 0 | -5.737 | 2 | 0 | 0.357 | 0 | 0.366 | 1 | 4.968 | 0 | 40.444 |
| SH226 | 0.426 | 1 | 0 | -5.707 | 2 | 0 | 0.377 | 0 | 0.316 | 1 | 4.833 | 0 | 57.745 |
| SH71 | -0.117 | 2 | 0 | -4.788 | 2 | 0 | 0.284 | 0 | 0.455 | 1 | 3.962 | 0 | 75.046 |
| SH77 | -0.536 | 3 | 0 | -3.594 | 3 | 0 | 0.337 | 0 | 0.287 | 0 | 2.786 | 0 | 78.56 |
| SH78 | -0.132 | 2 | 0 | -4 | 3 | 1 | 0.536 | 0 | 0.376 | 2 | 3.028 | 0 | 57.745 |
| SH123 | 0.456 | 1 | 0 | -5.592 | 2 | 0 | 0.284 | 1 | 0.504 | 1 | 4.93 | 0 | 57.745 |
| SH212 | -0.078 | 2 | 0 | -4.996 | 2 | 0 | 0.397 | 0 | 0.366 | 1 | 4.087 | 0 | 75.046 |
| SH121 | 0.519 | 1 | 0 | -5.63 | 2 | 1 | 0.562 | 1 | 0.594 | 2 | 5.134 | 0 | 57.745 |

**Inference**: ADMET screening of ligand molecules demonstrated their Blood- Brain Penetration and Hepatotoxicity. Flexible criterions based on probability(<0.6) were given to certain molecule having potential for further analysis.

**Fig.3. ADMET description plot for SH19 (Left) and SH226(Right) ligand molecule.**

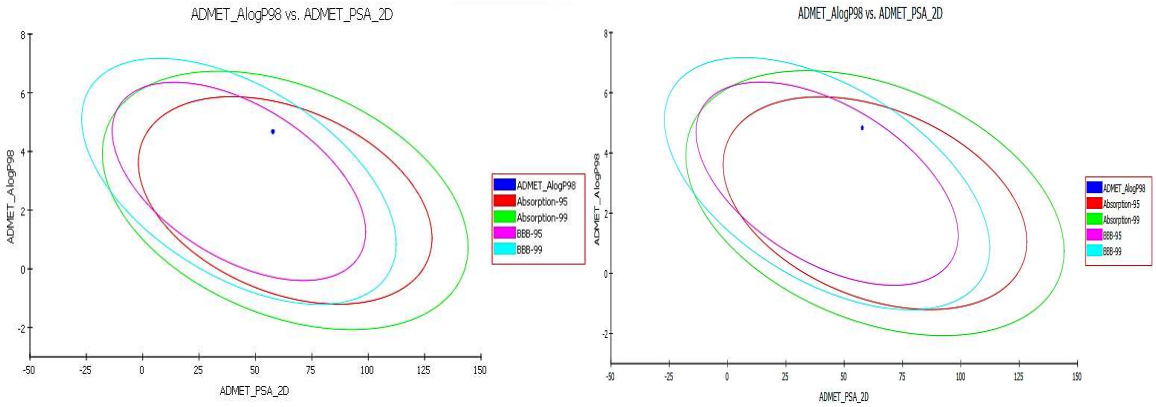
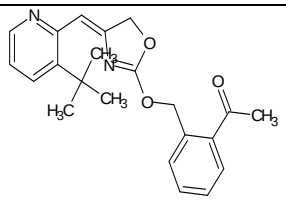
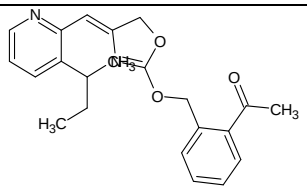
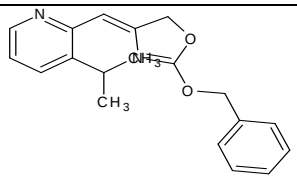
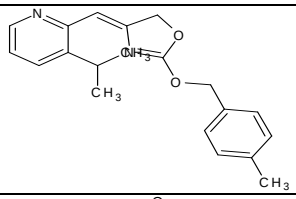
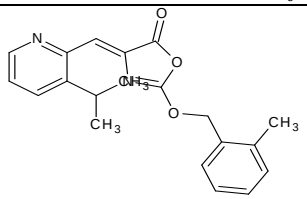
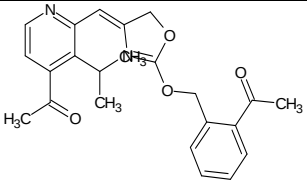
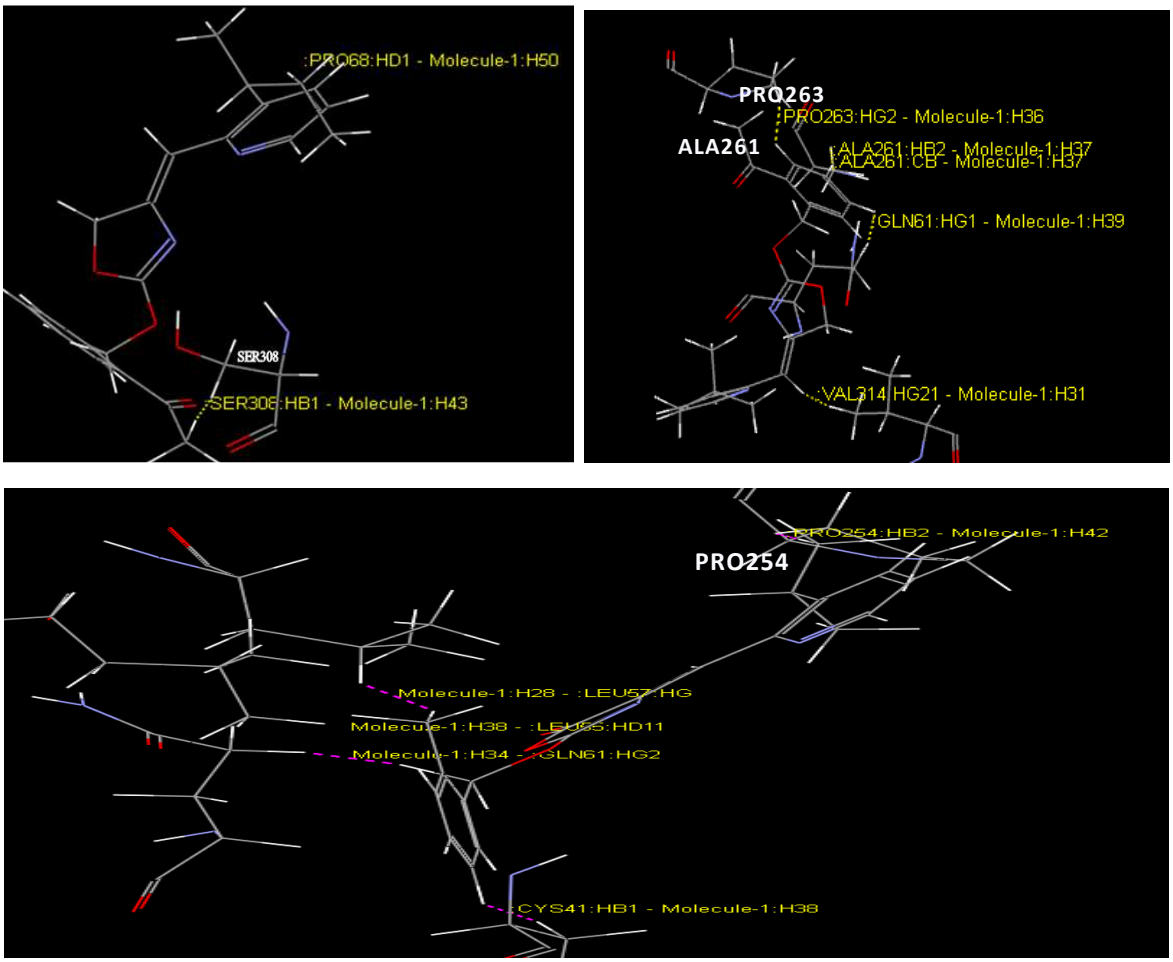



**Table.4. Docking studies on ligand molecules having interactions within the Receptor-Ligand complex.**

| Molecule Name | Mol. Structure | Absolute Energy | Relative Energy | LibDock Score | Amino acids involved in different poses |
|---|---|---|---|---|---|
| SH710 |  | 100.183 | 17.7406 | 99.4365 | GLN61,ALA261, PRO263,VAL314 |
| **SH19** |  | **60.5005** | **3.91126** | **101.672** | PRO68,LEU84, TYR80,SER308, LEU82, ALA78 |
| SH8 |  | 48.3756 | 0.030907 | 106.873 | TYR132,LYS134, ARG147,ASN149, VAL314, LEU57 |
| SH20 |  | 55.4906 | 6.48435 | 109.673 | LYS134,ARG147, TYR132,VAL314, LEU57,CYS41 , LEU55 |
| SH226 |  | 61.2686 | 5.26639 | 113.247 | CYS41,LEU55, LEU57,GLN61, PRO254 |
| SH71 |  | 80.129 | 9.27497 | 114.309 | TVR76 |

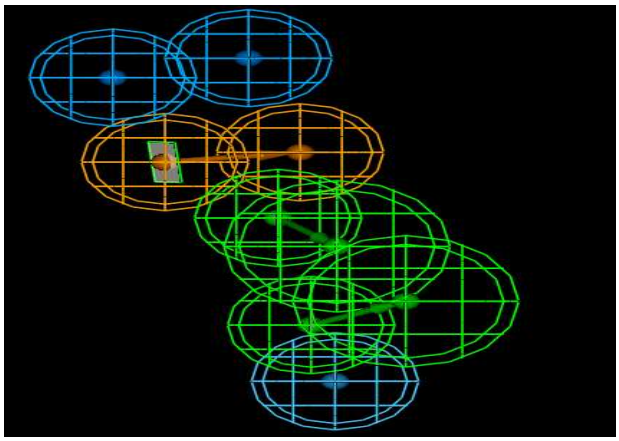
**Inference**: ADMET description of ligand SH19 and SH226 makes it a good candidate for Docking and Pharmacophore studies.

**Fig.4: Intermolecular interactions ligand SH19(left) ,SH710(right), SH226(below)with the receptor.**




**Inference**: Docking studies on selected molecules express a variety of interaction within the Receptor- Ligand complex. Molecules SH710,SH19 and SH226 showed promising interactions within HSPG binding domain(aa215-313). Screening of molecules on the basis of all parameter we used for selection of good ligand candidate, we identified molecule SH19 as a good drug candidate to inhibit ATLL.

**Fig.5: Pharmacophore analysis of SH19 in Discovery Studio 2.5**



**Inference**: The pharmacophore with lowest energy contain one hydrophobic region (cyan), two donors(green), two aromatic groups(orange) and two positive ionizing groups(blue).

## DISCUSSION

The ADMET results showed that SH19 has an positive result as depicted in the Table.3. The probability values for TOPKAT screening with SH19 and SH226 were between 0.0 to 0.30, and are likely to produce a negative response in an experimental assay.The computed rat oral bioavailability was found to be less than 70% and the predicted LD50 values in rat was found to be 1.6 g/kg when administered orally for these molecule. The ADMET Absorption_T2_2D level was between 6.1261 ( 95%) and 6.1261( 99%). The probability of ADMET hepatotoxicity was a bit on the negative side (0.569), but we ignored a fewer chance of dose-dependent liver injuries in SH19. On the basis of several drug parameters the ligand SH19 can be suggested as a good ligand with least toxicity.

A few inhibitors predicted to inhibit the gp46SU are not effective in all forms. The eleven potential inhibitors of the gp46SU were screened by docking and several pharmacological parameters were evaluated. It is clear that the SH19(a novel HSPG domain inhibitor) satisfied almost all properties like drug toxicity value, drug score, lower logP values and Lipinski's rule of five. Thus SH19 can be treated as a potential inhibitor of gp46SU, and can be considered as a good drug candidate for Adult T-cell Leukemia /Lymphoma and suggested for further clinical trials.

## CONCLUSION

The potentiality of our studies are immense in terms of good target and the ligand molecules. The protein of our interest shows the quality and importance of our studies. In our studies all the molecules that we designed qualified the criterions of Lipinski's rule of five like the number of hydrogen donors and the number of hydrogen acceptors, molecular weight, logP values. But surprisingly only a few molecules passed TOPKAT and ADMET. After docking the molecule SH19 had the best LibDock score (101.672) among all of the molecules . Though SH710 showed best binding score it rendered unsatisfactory results on ADMET parameters. While SH226 not only outperformed all physiochemical properties but also had a good binding score though not the best. In order to finalise the best drug candidate we selected SH19 depending on LibDock score and other parameters.

The potentiality of SH710 were immense compared to other drug candidates, but was carcinogenic in nature. To reach a conclusion regarding the best drug candidate from all our ligands we identify SH19 and SH226 as potent inhibitors to HSPG binding domain(aa215-313) of HTLV-1 with narrow exception. To be more specific about the molecule at the best we recommend ligand SH19 having most potentiality among all our ligands designed. Moreover SH19 had the properties or interactions that Jones KS and his team intended for further studies(aa287–311). Further studies are valuable on the basis on our report and the drug can be taken for *in vitro* studies.

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No. 19 ,doi: 10.1128/JVI.78.19.10399–10409.**2004**.
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10.1111/J.1365-2133.**2004**.06274.X.
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