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In-vitro Evaluation and Molecular docking calculation of Tricyclic Phthalimide Quinoxaline Analogues as Novel inhibitors of HIV-1 Integrase using GLIDE and GOLD

S. Balasubramanian[1], P. Sasikumar[1*], D. Velmurugan[2]

[1]*Department of Bio-Medical Engineering, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India*
[2]*Department of Crystallography and Biophysics, University of Madras, Chennai, Tamil Nadu, India*

ABSTRACT
As quinoxaline analogues have been computationally shown to be competent with other commercial antiviral drugs in terms of size and efficacy, their lack of utility is exemplified in the case of HIV integrase. The ability of molecular docking methods to locate selective inhibitors reinforces our view of structure-based drug discovery as a valuable strategy, not only for identifying lead compounds, but also for addressing receptor specificity. This study focuses on series of ligands that are screened for a successful candidate drug using rational drug design. In the present work, we proposed and evaluated the interaction of quinoxaline analogues along with HIV integrase (1QS4) as target by using the docking program GOLD and GLIDE. To study the molecular basis of interaction and binding affinity of quinoxaline analogues, these compounds were docked into active site of receptor using GLIDE. The best 10 compounds were screened out using high throughput virtual screening. These 10 compounds were further subjected to Induced Fit Docking. Based on overall studies, we can conclude that quinoxaline compounds were found to be more potent inhibitors based on glide score, glide energy and interaction with residues in the active site of the HIV integrase (1QS4). In future, these ten compounds (CHEMBL35109, CHEMBL369834, CHEMBL177311, CHEMBL177547, CHEMBL177515, CHEMBL177405, CHEMBL177705, CHEMBL174851, CHEMBL367104, CHEMBL369841 and CHEMBL424782) can be considered as effective candidates for the second generation drug discovery.

Keywords: HIV, Quinoxaline analogues, GLIDE, GOLD, Docking.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) has been identified as the etiologic agent causing the Acquired Immuno Deficiency Syndrome (AIDS). The currently used drugs for the treatment of HIV infection mainly target two important viral enzymes or inhibit viral fusion. The nucleoside analogs prematurely terminate the transcription of the viral RNA into dsDNA by reverse transcriptase. The non-nucleoside inhibitors constitute the second class of reverse transcriptase inhibitors. HIV protease, a homodimeric enzyme, is susceptible to inhibition by peptide-like structures. The process of entry of the HIV into the target cell can be divided into an attachment step and a fusion step. While only one inhibitor of the fusion step, Enfuvirtide, has entered the market up to now, several small-molecule inhibitors are currently in advanced development. Important efforts are being directed at the development of vaccines that can protect against HIV infection. Continued efforts are being directed at the discovery of therapies which target other essential features of the virus' life cycle, that is, the viral enzyme integrase, the viral maturation process, and the viral infectivity factor.

***Corresponding author: Mr. P. Sasikumar,**
Department of Bio-Medical Engineering, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India;
E-mail: sasibiology@gmail.com

MATERIALS AND METHODS

Databases and software's tools used

1. Pubchem database

PubChem database is an online database contains validated chemical depiction information provided to describe substances in PubChem substance. Structures stored within PubChem compounds are pre-clustered and cross-referenced by identity and similarity groups.

2. GOLD

System requirements for GOLD

- Internet Explorer 6 (SP1)
- Netscape 7.1 and above
- Opera 7.53
- Mozilla Firefox 0.9.2 and above

- JavaScript must be enabled
- Per-session browser cookies must be enabled/permitted
- A suitable JAVA Run-Time Environment (version 1.4.X) must be correctly installed and configured in order to use the AstexViewer JAVA plug-in. The JAVA RTE can be downloaded from Sun's JAVA website.
- Security settings must be set at a suitable level to allow the AstexViewer plug-in to be run within your browser.

3. GLIDE

System requirements for GLIDE

- LINUX
- Pentium or better
- Linux kernel 2.4 (Red Hat 7.3) or later
- 256 MB memory

4. PYMOL visualizing tool

System requirements for PYMOL

- Windows 2000 or XP.
- A late-model 3D OpenGL compatible graphics accelerator card from nVidia, ATI, 3Dlabs or similar.
- 512 MB RAM (768 MB or 1 GB preferred).
- 3 GHz Pentium 4 processor or similar.

5. Argus lab

System requirements for Argus Lab

- Memory: At least 1GB of RAM (2 GB for Vista/Win 7);
- Processor: 1.5 GHz processor (or faster);
- Display: VGA 1024 × 768 colour monitor or better;
- Windows operating systems XP, Vista, Windows 7.

6. Swiss pdb viewer

System requirements for Swiss pdb viewer

Windows Intel 486 or Pentium processor with 1.1 Mb hard-hard spaces for minimum installation, 8.1 Mb available for full installation; OpenGL

7. Chemsketch

System requirements for Chemsketch

- Pentium class processor with a clock rate of no less than 1 GHz.
- Graphics adapter with a resolution of no less than 800 by 600 with 256 colors.
- Disk space requirements can range from 10 to 1200 MB depending on the modules purchased.
- A Microsoft® mouse or fully compatible pointing device.
- Windows® 2000 SP4, or XP Professional SP2 with 128 MB or more of RAM.

TARGET

The protein molecule chosen for the docking studies is HIV-1 Integrase. There are different forms of HIV-1 Integrase. The crystal structure of HIV-1 Integrase, complexed with Mg^{++} and 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl) - propenone (5CITEP), was used as target complex structure in current study. It was obtained from RCSB Protein Data Bank with the PDB ID: 1QS4.

LIGANDS

Over 190 compounds pertaining to various structurally diverse classes of integrase inhibitors including tricyclic phthalimide analogues, N-Methyl pyrimidones, coumarines, quinones, hydrazides etc. were screened and subsequently used for docking studies which were selected using literature studies. The canonical structure or PDB files of the compounds were used for docking.

Methods using Gold

Preparation of Protein Molecule

The PDB code of the protein was 1QS4. The protein molecules were prepared mainly by using the software Swiss-pdb viewer. Active site residues within a range of 3.5 Å were selected and saved in pdb format. Later, the active site residues werex minimized in Argus lab after adding hydrogen bonds. The list of atoms in active site, were saved separately as a list file in text document format, which will be used as an input for GOLD.

Preparation of Ligands

The ligand structures were drawn using Chemsketch and saved in mol format. The saved ligand compounds were later imported and minimized in Argus lab after adding hydrogen bonds. The molecules thus obtained were saved in pdb format.

Setting up GOLD Parameter

The protein molecule was imported into GOLD. The ligands were also imported. GOLD was run in a particular way such that a particular atom number was given from the identified active site. The GOLD was setup to run at an active site radius of 3.5 Å. The output folder was also specified. All the other fitness function parameters and the genetic algorithm parameters were kept in default mode. Pymol is used to view the GOLD output.

Screening Criteria

The output was produced as GOLD Fitness scores and different energy functions. The fitness scores were mainly considered for the results and the screening. The ligands with scores of 60 (GOLD Fitness score for co-crystallized ligand) and above were only considered. The output of these protein-ligand complexes were exported as PDB files using Gold. These complexes were then analysed using a good molecular graphics viewer like Pymol. The output were analysed for the properties such as disulphide bonds, interactions between hydrophobic residues, ionic interactions, hydrogen bonds, aromatic-aromatic interactions, aromatic-sulphur interactions, Cation-pi interactions and electrostatic interactions.

Docking Method – GLIDE (Grid Based Ligand Docking with Energetics)

Glide searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule e.g. a protein and a cofactor. GLIDE can be run in rigid or flexible docking modes; the later automatically generates conformation for each input ligand. The combination of positions and orientation of the ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The ligand poses that GLIDE generates pass through a series of hierarchical filters that evaluate the ligand interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complimentarity of ligand-receptor interactions using the GRID based method patterned after the empirical ChemScore function.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non-bonded ligand-

receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE Score multi ligand scoring function is used to score the poses. If Glide Score was selected as the scoring function, a composite E-model score is then used to rank the poses of each ligand and to select the poses to report to the user. E-model combines Glide Score non-bonded interaction energy, and for flexible docking, the excess internal energy of the generated energy conformation.

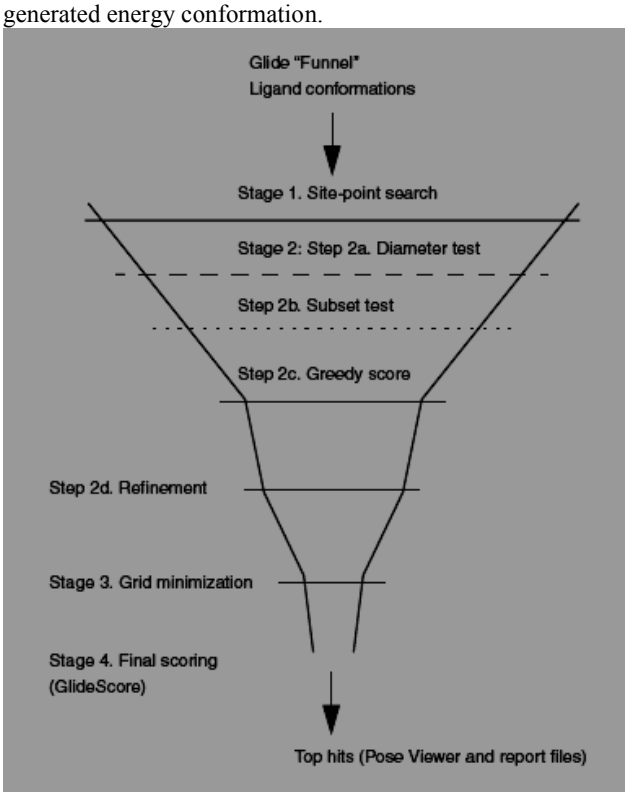


Fig. 1: Glide Hierarchical Docking Strategy

Glide Docking Hierarchy

Two types of Docking Algorithm used for our studies are:

- High throughput virtual screening
- Induced fit docking

High Throughput Virtual Screening using Glide (htvs)

1. The grid files produced by a single receptor grid generation task can be used for any number of jobs docking ligands to that receptor.
2. After correcting formal charges and bond orders in the ligand, set up and start the automated preparation and refinement portions of the protein preparation procedure using the Protein Preparation panel.
3. Ensure that the ligands to be docked are in the right form.
4. With the prepared receptor-ligand complex in the workspace, use the receptor grid generation panel to specify settings, and start the receptor grid generation job.
5. Specify the base name for the receptor grid files you want to use in the ligand docking panel, and use the other settings and options in the panel to set up and start a ligand docking job. As many docking jobs as you want can be set up in this panel, using the current receptor grids or specifying a different set of grids to use.

Glide docking uses the assumption of a rigid receptor, although scaling of van der Waals radii of non-polar atoms, which decreases penalties for close contacts, can be used to model a slight "give" in the receptor and/or ligand.

Grid generation

Choose receptor grid generation from the Glide submenu of the applications menu. The receptor grid generation panel has three tabbed folders, to specify settings for the receptor grid generation job:

- Receptor
- Site
- Constraints

Specifying the Receptor Grid

To specify the receptor grid for the docking job, click browse in the receptor grid section of the settings folder to open a file selector and choose a grid file (.grd). The file name, without the .grd extension, is displayed in the receptor grid base text box. You can also enter the base name directly into the text box.

High Throughput Virtual Screening (HTVS) - it is intended for the rapid screening of very large numbers of ligands. HTVS has much more restricted conformational sampling than SP docking (Shape and Physicochemical docking), and cannot be used with constraints, score-in-place, or rigid docking. Advanced settings are not available for HTVS, but are fixed at predetermined values.

Setting Docking Options

Under options in the docking section of the settings folder, you can choose whether ligands are docked flexibly, rigidly, or not at all (score in place), and set options for conformation generation. These options are described below:

Flexible Docking: This is the default option, and directs Glide to generate conformations internally during the docking process; this procedure is known as *flexible docking*. At present, conformation generation is limited to variation around acyclic torsion bonds, generation of conformations of non-aromatic five and six-membered rings, and generation of pyramidalizations at certain trigonal nitrogen centers, such as in sulfonamides. You can control whether ring conformations are generated or with the option. Allow flips of 5- and 6-member rings. This option is selected by default.

Energy Minimization Settings

The energy minimization stage of the docking algorithm minimizes the energy of poses that are passed through the selection of initial poses scoring phase. The energy minimization section of the settings- Advanced settings dialog box contains two options: Distance-dependent dielectric constant Glide uses a distance-dependent dielectric model in which the effective dielectric "constant" is the supplied constant multiplied by the distance between the interacting pair of atoms. The default setting is 2.0, and Glide's sampling algorithms are optimized for this value. Although this text box allows you to set the dielectric constant to any real value greater than or equal to 1.0, changing this setting is not recommended.

Glide does not allow for receptor flexibility in docking, but scaling of van der Waals radii of non-polar atoms, which decreases penalties for close contacts, can be used to model a slight "give" in the receptor and the ligand. This may not be sufficient to treat systems where ligand binding induces substantial conformation changes in the receptor ("induced fit."). Schrödinger has developed a procedure for such cases which uses Prime and Glide to perform induced fit docking.

Induced Fit Docking Using Glide

The induced fit docking allows the receptor to alter its binding sites, so that it more closely conforms to the shape and binding mode of the ligand. The ability to model induced fit docking has two main applications:

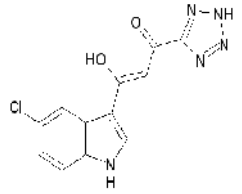
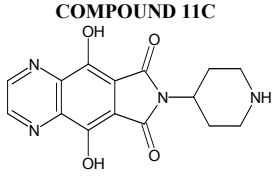
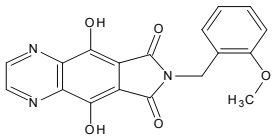
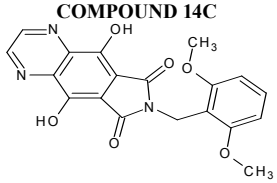
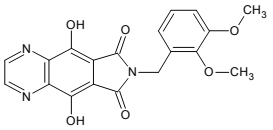
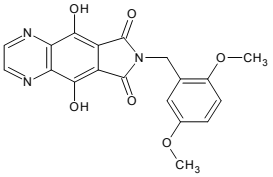
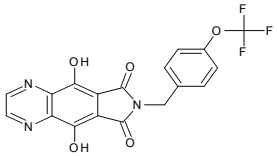
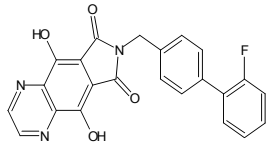
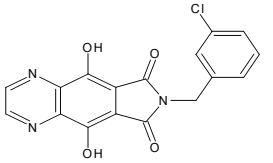
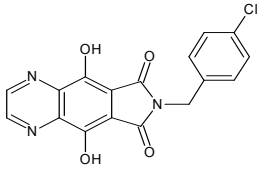
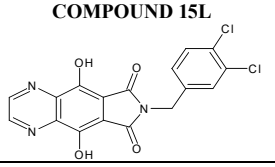
1. Generation of an accurate complex structure for a ligand known to be active but that cannot be docked in an existing (rigid) structure of the receptor.
2. Rescue of false negatives (poorly scored true binders) in virtual screening experiments, where instead of screening against a single conformation of the receptor, additional conformations obtained with the induced fit protocol are used.
3. The prepared protein is docked using induced fit protocol using the following system.

Induced Fit Docking Protocol

1. Constrained minimization of the receptor (Glide protein preparation, refinement only) with an RMSD cutoff of 0.0018 Å.
2. Initial Glide docking of each ligand using a softened potential (Van der Waals radii scaling). By default, a maximum 20 poses per ligand are retained, and by default poses to be retained must have a Coulombic-vdW score less than 100 and an H-bond scoreless than –0.05.
3. One round of Prime side-chain prediction for each protein/ligand complex, on residues within a given distance of any ligand poses (default 5).
4. Prime minimization of the same set of residues and the ligand for each protein/ligand complex pose. The receptor structure in each pose now reflects an induced fit to the ligand structure and conformation.
5. Glide re-docking of each protein/ligand complex structure within a specified energy of the lowest-energy structure (default 30 kcal/mol). The ligand is now rigorously docked, using default Glide settings, into the induced-fit receptor structure.
6. Minimization of the re-docked ligand within the protein.
7. Estimation of the binding energy (Glide Energy) for each output pose.

Schrödinger has developed a Python script that automates the induced fit docking process. This Python script has an interface in Maestro, in which you can specify the structures and enter settings for various options, and then the job running. The script then completes the protocol without further intervention. The structures used for induced fit docking must be prepared in the same manner as for Glide. The protein and ligand preparation must precede the use of the protocol.

Table 1: List of selected compounds, its structure and IUPAC name

S. No.	Compounds	IUPAC Names
	Co-crystal ligand	
1.		**1-(5-chloroindol-3-yl)-3-hydroxy-3-(2H-tetrazol-5-yl)-propenone** **ChemSpider ID:** 4449801 **CHEMBL35109**
2	**COMPOUND 11C** 	**5,9-dihydroxy-7-(piperidin-4-yl)-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136192 **CHEMBL369834**
3	**COMPOUND14A** 	**5,9-dihydroxy-7-(2-methoxybenzyl)-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136200 **CHEMBL177311**
4	**COMPOUND 14C** 	**7-(2,6-dimethoxybenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136202 **CHEMBL177547**
5	**COMPOUND 14D** 	**7-(2,3-dimethoxybenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136203 **CHEMBL177515**
6	**COMPOUND 14F** 	**7-(2,5-dimethoxybenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136205 **CHEMBL177405**
7	**COMPOUND 15B** 	**5,9-dihydroxy-7-[4-(trifluoromethoxy)benzyl]-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136207 **CHEMBL177705**
8	**COMPOUND 17D** 	**7-[(2'-fluorobiphenyl-4-yl)methyl]-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136219 **CHEMBL174851**
9	**COMPOUND 15J** 	**7-(3-chlorobenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136211 **CHEMBL367104**
10	**COMPOUND 15K** 	**7-(4-chlorobenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136212 **CHEMBL369841**
11	**COMPOUND 15L** 	**7-(3,4-dichlorobenzyl)-5,9-dihydroxy-6H-pyrrolo[3,4-g]quinoxaline-6,8(7H)-dione** **ChemSpider ID:** 20136213 **CHEMBL424782**

RESULTS

Protein: HIV-1 integrase
Work: Comparison studies using GLIDE and GOLD
PDB id: 1QS4
Compounds taken: Tricyclic phthalimide quinoxaline compounds

Table 2: Ten best compounds results (GOLD)

S. No	Compounds	Interaction (D-H…A)	Bond distance between Donor & Acceptor(Å)	GOLD score
1	CO-CRYSTAL	GLU152(O…NH) GLU152(O…NH)	2.493 2.695	31.9032
2	COMPOUND 11c	LYS156(N-H…N) LYS156(N-H…N)	2.548 2.733	28.3968
3	COMPOUND 14a	LYS159(N-H…O)	2.325	20.4971
4	COMPOUND 14c	LYS156(NH…O) ASN115(NH…O)	2.663 2.625	22.5124
5	COMPOUND 14d	LYS156(N-H…N) LYS156(N-H…N) LYS156(N-H…N)	2.735 2.556 2.497	20.6701
6	COMPOUND 14f	(OH…O)GLU152 (OH…O)GLU152 LYS156(N-H…N) LYS156(N-H…N)	2.611 2.430 2.46 0 2.610	21.3399
7	COMPOUND 15b	LYS156(N-H…N) LYS156(N-H…N) (O-H…O)ASP64	2.572 2.568 2.413	26.5166
8	COMPOUND 17d	LYS156(N-H…O)	2.610	24.0365
9	COMPOUND 15j	LYS156(N-H…N) LYS156(N-H…N) (OH…O)GLU152	2.319 2.342 2.333	25.8973
10	COMPOUND 15k	LYS156(N-H…N) LYS156(N-H…N) LYS156(N-H…N)	2.651 2.480 2.377	26.2175
11	COMPOUND 15l	LYS159(N-H…N) LYS156(N-H…N) LYS156(N-H…N)	2.339 2.550 2.623	26.4807

Table 3: Details of the active site residues of 1QS4 using PYMOL

PDB	Interaction D-H…A	Distance between donor and acceptor (Å)
1QS4	THR66(N-H...N)	2.75
	GLU152(O-H...O)	2.80
	GLU152(O-H...O)	2.58
	LYS156(N-H...O)	3.27

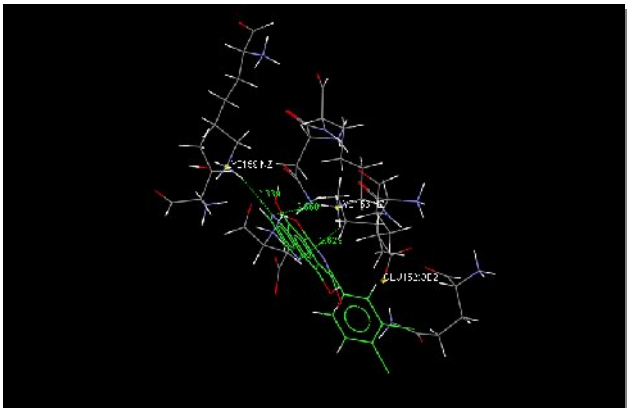


Fig. 2: Print view result of interaction between compound 15L and HIV-1 integrase protein (GOLD)

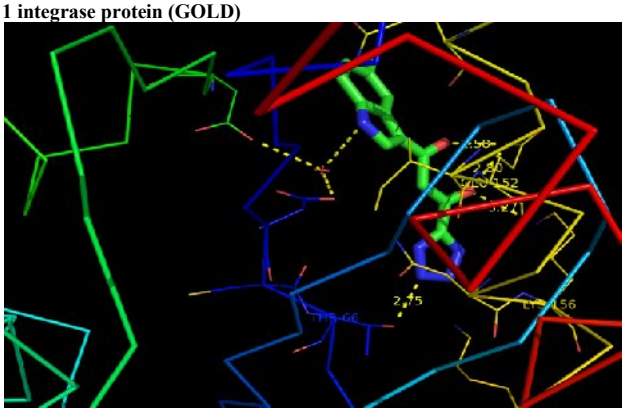


Fig. 3: PYMOL view of active site residues in 1QS4

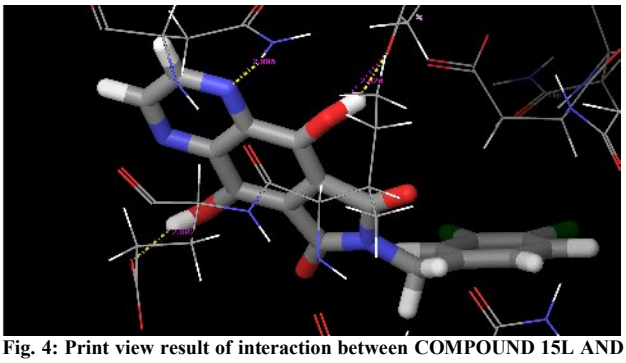


Fig. 4: Print view result of interaction between COMPOUND 15L AND HIV-1 Integrase (Induced Fit Docking)

The GOLD Fitness score for compound 15l was **26.4807**. The interaction of the ligand with the receptor molecule was viewed using Silver. From the output of the silver, the resulting interaction was analyzed. The ligand has shown good interaction with the residues **LYS159 (N-H…N), LYS156 (N-H…N), LYS156 (N-H…N), by** forming the hydrogen bond lengths of **2.339 Å, 2.550 Å, and 2.623 Å respectively**.

DISCUSSION

As tricyclic phthalimide analogues have been computationally shown to be competent with other commercial antiviral drugs in terms of size and efficacy, their lack of utility is exemplified in the case of HIV. A plethora of polymorphic resistance mutations have almost instantly arisen in response to both raltegravir and the purported second-generation IN inhibitor, elvitegravir. It is clear to see that, the virus is capable of eventually avoiding interaction with many a once potent inhibitor, and attempts at recreating these original interactions will most likely fall victim to the same mode of viral escape. Although some pharmacokinetic properties may be optimized through phthalimide analogues development research, and some profitable drugs may be cleared for marketing, the long term efficacy of most of these drugs will likely be susceptible to the ever present mutational ultra-competence of HIV.

The ability of molecular docking methods to locate selective inhibitors reinforces our view of structure-based drug discovery as a valuable strategy, not only for identifying lead compounds, but also for addressing receptor specificity. This study focuses on series of ligands that are further screened for a successful candidate drug using rational drug design.

In the present work, we proposed and evaluated the interaction of tricyclic quinoxaline phthalimide analogues and various antiviral drugs along with 1QS4 as target by using the docking program GOLD and GLIDE.

To study the molecular basis of interaction and binding affinity of tricyclic quinoxaline phthalimide analogues, these compounds were docked into active site of receptor using GLIDE. The best 10 compounds were screened out using high throughput virtual screening. These 10 compounds were further subjected to Induced Fit Docking.

Based on overall studies, we can conclude that, tricyclic quinoxaline phthalimide compounds 11c, 15b, 15l, 15k, 15j, 17d, 14c, 14f, 14d, 14a were found to be more potent inhibitors based on glide score, glide energy and interaction with residues in the active site of the 1QS4. In future, this can be taken as an effective drug candidate for the second – generation drug discovery.

Table 4: Induced fit docking results of best compounds (GLIDE)

S. No.	Compounds	GLIDE score	GLIDE energy (Kcal/mol)	Interaction D-H…A	Distance Between Donor and Acceptor (Å)
1	Co-Crystal	-4.779	-36.028	ASN155(NH...O)	3.082
				(N-H…O)GLN148	2.775
				(O-H...O)GLU152	2.638
2	11c	-5.716	-45.633	HIS67(N-H...O)	3.100
				ASN155(N-H...O)	3.056
				LYS159(N-H...O)	3.092
3	14a	-4.953	-45.170	ASN155(N-H...N)	3.254
				(O-H...O)ASP64	2.707
				GLN148(N-H...N)	3.105
4	14c	-4.313	-36.899	(O-H...O)ASP64	2.749
				ASN155(N-H...O)	2.962
				(O-H...O)GLU152	2.931
5	14d	-5.096	-44.370	HIS67(N-H...O)	2.954
				LYS159(N-H...O)	2.888
				LYS159(N-H...N)	3.239
6	14f	-5.145	-44.357	HIS67(N-H...O)	3.248
				ASN155(N-H...N)	2.300
				(O-H...O)ASP64	3.080
7	15b	-5.369	-46.078	HIS67(N-H...O)	3.238
				HIS67(N-H...N)	2.917
				LYS159(N-H...O)	3.110
				THR66(O-H...O)	3.189
8	17d	-4.687	-42.170	(O-H...O)ASP64	2.796
				HIS67(N-H...O)	2.887
				LYS159(N-H...O)	3.300
				(O-H...O)CYS65	3.004
9	15j	-5.197	-39.4043	(O-H...O)ASP64	2.735
				HIS67(N-H...O)	3.202
				ASN155(N-H...N)	3.115
10	15k	-5.318	-42.846	(O-H...O)ASP64	2.992
				HIS67(N-H...O)	2.734
				ASN155(N-H...N)	3.157
11	15l	-5.240	-37.857	ASN155(N-H...O)	2.892
				(O-H...O)GLU152	2.995
				(O-H...O)ASP64	2.626

Table 5: High throughput virtual screening results (GLIDE)

S. No.	Compound	GLIDE score	GLIDE energy (Kcal/mol)	Interaction D-H…A	Distance Between Donor and Acceptor (Å)
1	Co-Crystal	-3.5451	-26.3129	HIS67(N-H…O)	2.716
				LYS156(N-H…N)	3.314
2	11c	-5.7195	-48.1053	HIS67(N-H…O)	2.924
				(O-H…O)ASP64	2.702
3	14a	-5.2928	-48.1559	(O-H…O)ASP64	2.786
4	14c	-5.2632	-47.5958	HIS67(N-H…O)	2.881
				(O-H…O)ASP64	2.530
5	14d	-5.5347	-50.5713	HIS67(N-H…O)	2.829
				(O-H…O)ASP64	2.593
6	14f	-5.3254	-48.7508	HIS67(N-H…O)	2.853
				(O-H…O)ASP64	2.608
				(O-H…O)CYS65	2.751
7	15b	-4.8569	-52.3498	(O-H…O)CYS65	2.955
				SER119(N-H…F)	2.886
8	17d	-4.1890	-43.4448	HIS67(N-H…O)	2.973
				(O-H…O)ASP64	2.708
9	15j	-4.8693	-45.1935	(O-H…O)ASP64	2.641
10	15k	-4.9228	-43.0124	HIS67(N-H…O)	2.847
				(O-H…O)ASP64	2.615
11	15l	-4.7448	-43.-0910	HIS67(N-H…O)	2.833
				(O-H…O)ASP64	2.594

Table 6: Interpretation of observations:

Compounds	GOLD score	HTVS GLIDE score and energy		Induced fit docking GLIDE score and energy	
11c	28.3968	-5.7195	-48.1053	-5.716	-45.633
15b	26.5166	-4.8569	-52.3498	-5.369	-46.078
15l	26.4807	-4.7448	-43.0910	-5.240	-37.857
15k	26.2175	-4.9228	-43.0124	-5.318	-42.846
15j	25.8973	-4.8693	-45.1935	-5.197	-39.404
17d	24.0365	-4.1890	-43.4448	-4.687	-42.170
14c	22.5124	-5.2632	-47.5958	-4.313	-36.899
14f	21.3399	-5.3254	-48.7508	-5.145	-44.357
14d	20.6701	-5.5347	-50.5713	-5.096	-44.370
14a	20.4971	-5.2928	-48.1559	-4.953	-45.170

Binding affinity of tricyclic quinoxaline phthalimide and its analogues were docked into active site of 1QS4 receptor using GOLD. Among the compounds which were docked, 11c, 15b, 15l, 15k has given higher fitness score compared to other compounds.

The type of interaction it exhibits and the residues with which it interacts convey that they are good inhibitors of HIV-1 Integrase as they exhibit drug like activity. Instead of taking Raltegravir which is approved by U.S. Food and Drug Administration (FDA) for the treatment of HIV infection in adults and children 2 years of age and older that causes serious, life-threatening side effects. These include skin reactions, allergic reactions, and liver problems. The tricyclic quinoxaline phthalimide compounds herewith proposed are showing orientation close to active site and these compounds

can be used as a lead for designing future pharmaceuticals that may be used as inhibitors of HIV-1 Integrase. HIV drug resistance is due to any or combination of factors like HIV diversity, HIV replication, and anti-HIV drug selection pressure. Since the type and occurrence of drug resistance in HIV strains is very least known or proven scientifically or clinically, the compounds used in this study have produced better results for identification of most effective drugs and to solve the drug resistance problems in future. In the past, these new antiretroviral drugs against novel targets like Integrase have yielded a range of new therapeutic options for multiclass drug-resistant HIV infection and produced excellent efficacy results.

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REFERENCES

1. Ansari-Lari MA. Donehower LA, Gibbs RA. Analysis of human immunodeficiency virus type 1 integrase mutants. Virology 1995; 211:332-335.
2. Barreca ML, Lee KW, Chimirri A, Briggs JM. Molecular dynamics studies of the wild-type and double mutant HIV-1 integrase complexed with the 5CITEP inhibitor: Mechanism for inhibition and drug resistance. Biophys. J. 2003; 84:1450-1463.
3. Beale K, Robinson WE, Jr. Combinations of reverse transcriptase, protease, and integrase inhibitors can be synergistic in vitro against drug-sensitive and RT inhibitor-resistant molecular clones of HIV-1. Antivir. Res. 2000; 46:223-232.
4. Bischerour J, Leh H, Deprez E, Brochon JC, and Mouscadet JF. Disulfide-linked oligomers involving C280 residues are formed in vitro and in vivo but are not essential for human immunodeficiency virus replication. J. Virol. 2003; 77:135-141.
5. Buolamwini, JK, Assefa H. CoMFA and CoMSIA 3D QSAR and docking studies on conformationally-restrained cinnamoyl HIV-1 integrase inhibitors: exploration of a binding mode at the active site. J. Med. Chem. 2002; 45:841-852.
6. DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, Elion R, Farthing C, Zhong L, Cheng AK, McColl D, Kearney BP, for the 183-0101 Study Team: Antiviral activity, pharmacokinetics, and dose response of the HIV integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. J. Acquir. Immune Defic. Syndr. 2006; 43:1-5.
7. Di Santo R, Costi R, Roux A, Artico M, Lavecchia A, Marinelli L, Novellino E, Palmisano L, Andreotti M, Amici R, Galluzzo CM, Nencioni L, Palamara AT, Pommier Y, Marchand C. Novel bifunctional quinolonyl diketo acid derivatives as HIV-1 integrase inhibitors: design, synthesis, biological activities, and mechanism of action. J. Med. Chem. 2006; 49:1939-1945.
8. Espeseth AS, Felock P, Wolfe A, Witmer M, Grobler J, Anthony N, Egbertson M, Melamed JY, Young S, Hamill T, Cole JL, Hazuda DJ. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. Proc. Natl. Acad. Sci. USA 2000; 21:11244-11249.
9. Farnet CM, Bushman FD. HIV-1 cDNA integration: requirement of HMGI(Y) protein for function of preintegration complexes in vitro. Cell .1997; 88:483–492.
10. Fesen MR, Kohn KW, Leteurtre F, Pommier Y. Inhibitors of human immunodeficiency virus integrase. Proc. Natl. Acad. Sci. USA. 1993; Mar 15; 90(6):2399–2403.
11. Goldgur Y, Craigie R, Cohen GH, Fujiwara T, Yoshinaga T, Fujishita T, Sugimoto H, Endo T, Murai H, Davies DR. Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. Proc. Natl. Acad. Sci. USA 1999; 23:13040-13043.
12. Leitner, T, Foley B, Hahn B, Marx P, McCutchan F, Mellors J, Wolinsky S, Korber B. HIV Sequence Compendium. Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM. 2004.
13. Lipford JR, Worland ST, Farnet CM. Nucleotide binding by the HIV-1 integrase protein in vitro, J. Acquir. Immune Defic Syndr. 1994; 7(12):1215–1223.
14. Mazumder A, Gupta M, Perrin DM, Sigman DS, Rabinovitz M, Pommier Y. Inhibition of human immunodeficiency virus type 1 integrase by a hydrophobic cation: the phenanthroline-cuprous complex. AIDS Res. Hum. Retroviruses. 1995; 11(1):115–125.
15. Shaw-Reid CA, Munshi V, Graham P, Wolfe A, Witmer M, Danzeisen R, Olsen DB, Carroll SS, Embrey M, Wai JS, Miller MD, Cole JL, Hazuda DJ. Inhibition of HIV-1 ribonuclease H by a novel diketo acid, 4-[5-(benzoylamino) thien-2-yl]-2,4-dioxobutanoic acid. J. Biol. Chem. 2003; 5:2777-2780.
16. Swanson P, Devare SG, Hackett J. Molecular characterization of 39 HIV isolates representing group M (subtypes A-G) and group O: Sequence analysis of gag p24, pol integrase, and env gp41. AIDS Res Hum Retroviruses. 2003; 19:625-629.
17. Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR. The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem. Biophys. Res. Commun. 1986; 141:956-962.