

# HIV capsid inhibitor capacity of different isomers of di-pyridine benzene and its alkyl and halide derivatives

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HIV-1 Capsid-A inhibitor capacity of different isomers of di-pyridine benzene and its alkyl and halide derivatives are studied systematically. It is found that p-di-pyridine o-ethyl benzene has very good inhibition constant. It is a small, cost effective organic compound and very promising as a HIV drug. Different important properties of this compound like  $IC_{50}$ ,  $CC_{50}$ , Log P, metabolism etc., which are required before pre-clinical studies are also computed and reported here.

## 1 Introduction

There are more than 25 FDA-approved HIV-1 drugs for seven classes; nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), co-receptor inhibitors (CRIs) and integrase inhibitors (INIs), that target one of the three viral enzymes require to complete its (HIV virus) life cycle in the host body (human body). There are three key steps involved in viral attack; attachment, replication and assembling. HIV virus after entering into the blood circulation system of human body, tries to get attached to CCR5 receptor of CD4 cells, which is one of the two T-cell types lymphocytes of white blood cell. CD4 cells which control and direct the immune response and coordinate to other immune cells, act as a virus manufacturing machine after HIV infection. In spite of many available drugs, lifelong therapy is required for sustained viral suppression [3,4]. Due to very fast mutation of HIV-1, drug resistance is acquired within very short period of time. Moreover, cross-resistance within mechanistic classes underscoring the need to discover new class of HIV-1 inhibitor.

HIV-1 capsid (CA) protein plays essential roles in the viral replication process which is an attractive new therapeutic target for antiretroviral treatment. During assembling and budding the proteolytic cleavage of 55-kDa Gag polyprotein yields CA protein which provides key protein-protein interactions among immature

virion proteins to assemble into the cone-shaped central capsid that surrounds the viral RNA genome, reverse transcriptase (RT) and integrase (IN). CA mutations impair the assembly and inhibit viral replication. N-terminal domain of CA (CANTD) includes 1 to 146 residues and C-terminal domain (CACTD) is composed of 151 to 231 residues. These two highly helical domains are connected through a very short (only four residues) flexible linker. High-resolution structures of CA hexagons and pentagons are reported by Pornillos et al. There are three independent inhibitor binding sites on CA; at the junction of helices 1, 2, 4 and 7, a conserved hydrophobic cleft within the CACTD four-helix bundle and between helix 4 and helix 7 of the CANTD [18, 19]. CA binding inhibitors of several classes have been reported and providing evidence that CA may be a viable drug target. Christopher et al. studied two different classes of capsid inhibitors and found that mutations conferring resistance to both classes of compound had two different types of effects. Substitutions within the inhibitor binding site decrease the inhibitor binding affinity, but at the same time viral fitness also decreases. Thus, though due to mutation inhibitor binding probability decreases, viral replication processes do not enhance. This suggests that the development of drug resistance against capsid assembly inhibitors due to mutation is not possible for the HIV virus. Christopher et al. also shown that due to mutation, stability of inhibitor-enzyme complex increases. Thus, possibility of the development of drug resistance against capsid assembly inhibitors is very less for those inhibitors whose protein binding free energies are highly negative.

In spite of enormous effort, highly efficient HIV Capsid-A inhibitor drug is not market available. Thus, we model very small organic molecules which should be synthetically cost effective and at the same time highly active. For this purpose we systematically study HIV capsid inhibitor capacity of different isomers of di-pyridinyl benzene and its alkyl and halide derivatives. For geometry optimization we use Density Functional Theory (DFT)

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calculation with help of GAUSSIAN 09 package. Molecular docking method is used for binding affinity calculation using docking server.

## 2 Computational details

All geometry optimizations of our used compounds are done using Gaussian 09 package. We optimize structures of all compounds mentioned here without any symmetry constraints and confirmed the minimum energy structures by the harmonic vibrational frequency calculation with all positive mode of vibrational frequency. The convergence thresholds we set to 0.000015 Hartree/Bohr for the forces, 0.00006 Å for the displacement and  $10^6$  Hartree for the energy change. Calculations of organic molecules are performed with the density functional theory (DFT) with unrestricted Becke's three parameter hybrid exchange functional combined with Lee-Yang-Parr non-local correlation function, abbreviated as B3LYP.

Docking calculations were carried out using Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotative bonds were defined. Docking calculations were carried out on di-py-ben protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of  $xx$  Å grid points and 0.375 Å spacing were generated using the Auto grid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotative torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

## 3 Results and discussions

We know that all DNA and RNA bases are benzene derivative thus we chose working with benzene derivative as our starting ligand. We chose pyridine derivative of benzene for the same reason - in guanine, pyridine structure exists. We computed binding affinity of this ligand (from here and onwards, it would be termed as ligand-1) with p24 HIV Capsid-A protein. Then we systematically substitute methyl, ethyl, iodine, chlorine and hydroxyl group to our ligand to search for improvement in binding affinity.

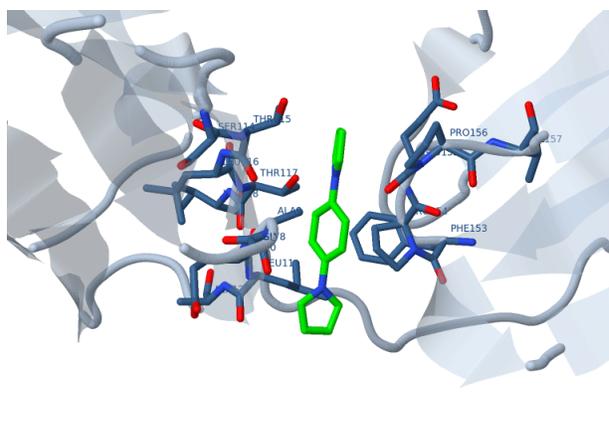
### 3.1 p-di-pyridine benzene

Docked structure of ligand-1 with HIV-1 capsid-A protein is presented at Figure 1. It is observed that as our ligand is small it easily goes to the binding site. But, it is too small to tightly fit to the binding cavity. As a result binding energy is very small, only -4.9 kcal/mol. There is only one hydrogen bonding formation is observed which is presented in Figure 2. This hydrogen bonding is made by a glutamate residue which is 155th residue.

**Table 1** docking parameters used

tstep	0.2
qstep	5.0
dstep	5.0
rmstol	2.0
ga-pop-size	150
ga-num-evals	250000
ga-num-generations	540000
ga-run	10

**Fig. 1** Docked structure of ligand-1 with p24 HIV protein



All other interactions are Van der Waals interaction. Though its binding energy is small, its binding frequency is very high, as high as 30.0%. It is because its size is very small and its charge density is very high. It has three interaction zone which is shown in HB plot presented in 3. Estimated inhibition constant for this ligand is 250  $\mu$  mole.

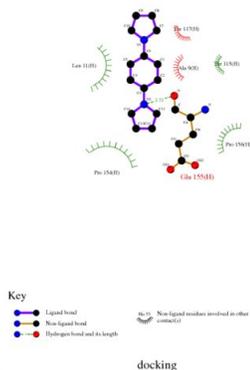
### 3.2 p-di-pyridine o-methyl benzene

We observe that ligand-1 is quite good as a HIV-1 capsid inhibitor. Thus to obtain improved ligand we substitute with methyl at the ortho position of benzene ring (ligand-2). Docked structure of ligand-2 is presented in Figure 4 and interaction is plot is presented in Figure 5. Binding affinity of ligand-2 is -5.13 kcal/mol and binding frequency 50.0% which is very high. Thus, ligand-2 is a improved inhibitor. It is observed that ligand-2 is attached to one side of the cavity. Thus its binding frequency is very high. Inhibition constant of this ligand is 174.9  $\mu$  mole.

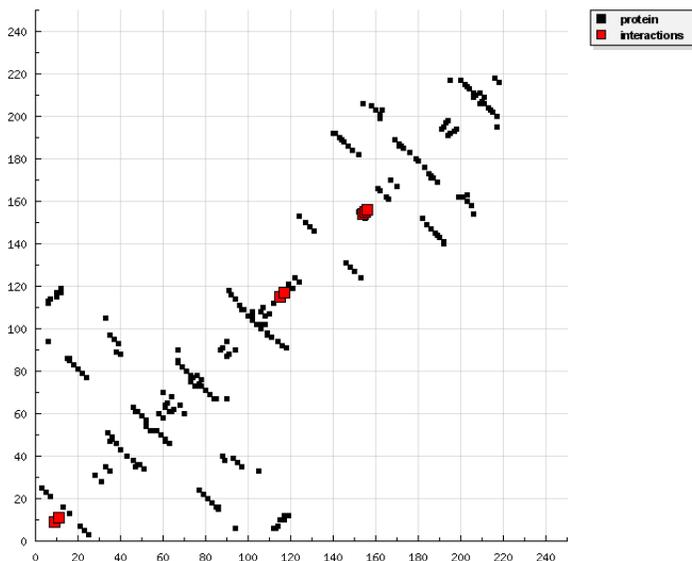
### 3.3 p-di-pyridine o-ethyl benzene

We observe that ligand-2 is a improved ligand as a HIV-1 capsid inhibitor compare to ligand-1. Thus for further improvement we substitute with ethyl at the ortho position of benzene ring (ligand-3). Docked structure of ligand-3 is presented in Figure 6 and interaction is plotted in Figure 7. Binding affinity of ligand-

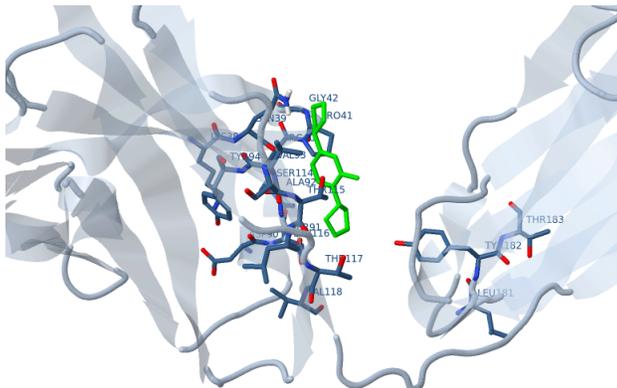
**Fig. 2** Interacting residues of p24 HIV protein when docked with ligand-1



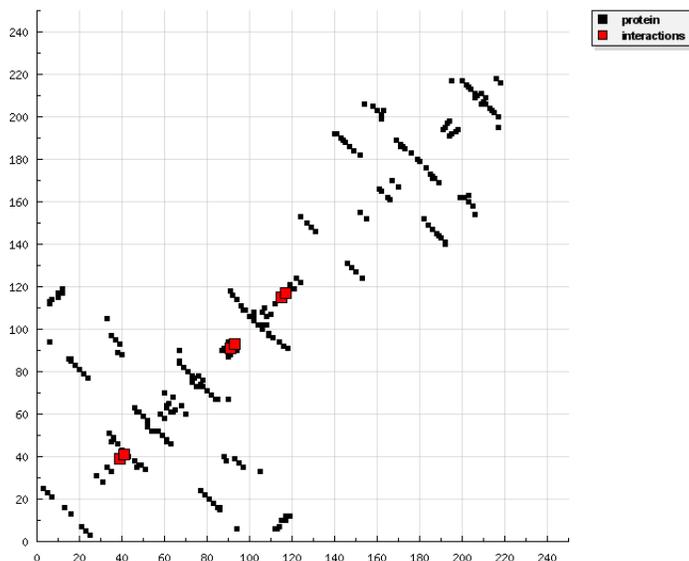
**Fig. 3** HB plot of ligand-1 with p24 HIV protein



**Fig. 4** Docked structure of ligand-2 with p24 HIV protein



**Fig. 5** HB plot of ligand-2 with p24 HIV protein



3 is  $-6.17$  kcal/mol and binding frequency 20.0% which is not high as its methyl counter part but it is acceptable. Its binding frequency is less due to its larger size. It is observed that ligand-3 is attached to the center of the cavity. Inhibition constant of ligand-3 is only  $29.78 \mu$  mole, which is excellent and comparable to market available drugs. Thus, this ligand may be a new drug for HIV-1 capsid-A inhibitor. But for that this has to be passed biological tests.

## 4 Conclusion

In this research work small organic molecules are modeled and tested for HIV-1 Capsid-A inhibitor capacity. It is observed that p-di-pyridine o-ethyl benzene has very good inhibition constant. Since it is a very small organic compound it may be cost effective as a drug. Thus, biological test is required for its demand for a HIV drug.

## 5 acknowledgement

A. Bag acknowledge the computational support from IISER Kolkata.

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