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RESEARCH ARTICLE

Design Space Filling Model, Synthesis and Evaluation of Novel 2-Indolinone HIV-1 Inhibitors

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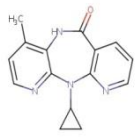
Abstract

Weed out HIV from all of its hiding places within the body is difficult. The approach can be used in bioinformatics practices, including complex binding energy estimation in novel HIV-1 inhibitors. Here we show how a space filling model-based approach used infection HIV-1 inhibitors and role of target compound located insight into the host cell machinery. Process technology by parallel synthesizer major was used for the purposes of synthesized 2- Indolinones potential derivatives. In vitro anti-HIV-1 activity of target compound 8-methoxy-5-(morpholin-4-ylmethyl)-4,4a,5,9b-tetrahydro-1H-pyrazino[2,3-*b*]indole-2,3 dicarbonitrile (1b) and studies have suggested that above is a novel reservoir has very low cytotoxicity (CC_{50}>1mM) and it has been displays 15.5 ug/ml to cell lines, TZM-bl. Also it displayed potent anti-HIV-1 activity and found 3.96 µg/ml against laboratory adapted strains UG070,7 th PID.

INTRODUCTION

Among currently available HIV-1 inhibitors hamper the polymerase activity of HIV-1 through allosterically binding to the binding pocket but geometry of the DNA and its cycle emergence mutations (especially K103N,Y181C) rapidly develop resistance and potency, efficacy is an another effort to address the structural feature of novel smaller building blocks **(Li X et al., 2014)**.Specific recognition of ligands by HIV -1 protein is at the crucial biological functions so that binding affinities characterizes the strength of such recognition. Space filling model hinges and binding energies on a better way to interatomic interactions and its vital for to build interaction in specific, precise more authoritative. In those research advancements in computing, prediction of the binding affinity based on principles of molecular interaction has come to the forefront of active research and has been the subject potentially offers accurate prediction of binding energies of ligands to protein. Currently drugs used to treat HIV AIDS (**De Clercq E. et al., 2009**)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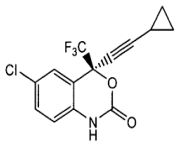
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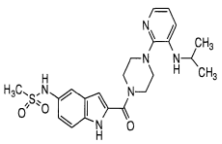
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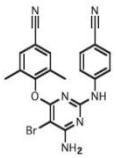
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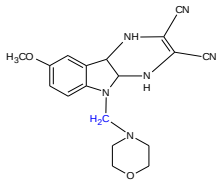

Target Molecule

Ethics statement

The study was conducted in accordance with basic principles of the Biosafety Laboratory II and III National AIDS Research Institute (ICMR) Pune. This study and the informed consent process were approved by the Indian Council of Medical Research New Delhi.

Compounds and reagents

All chemicals used were procured from Sigma-Aldrich, Invitrogen company USA. The purity of chemicals were checked before use and purified. Melting points were recorded by open capillary tube method and uncorrected.

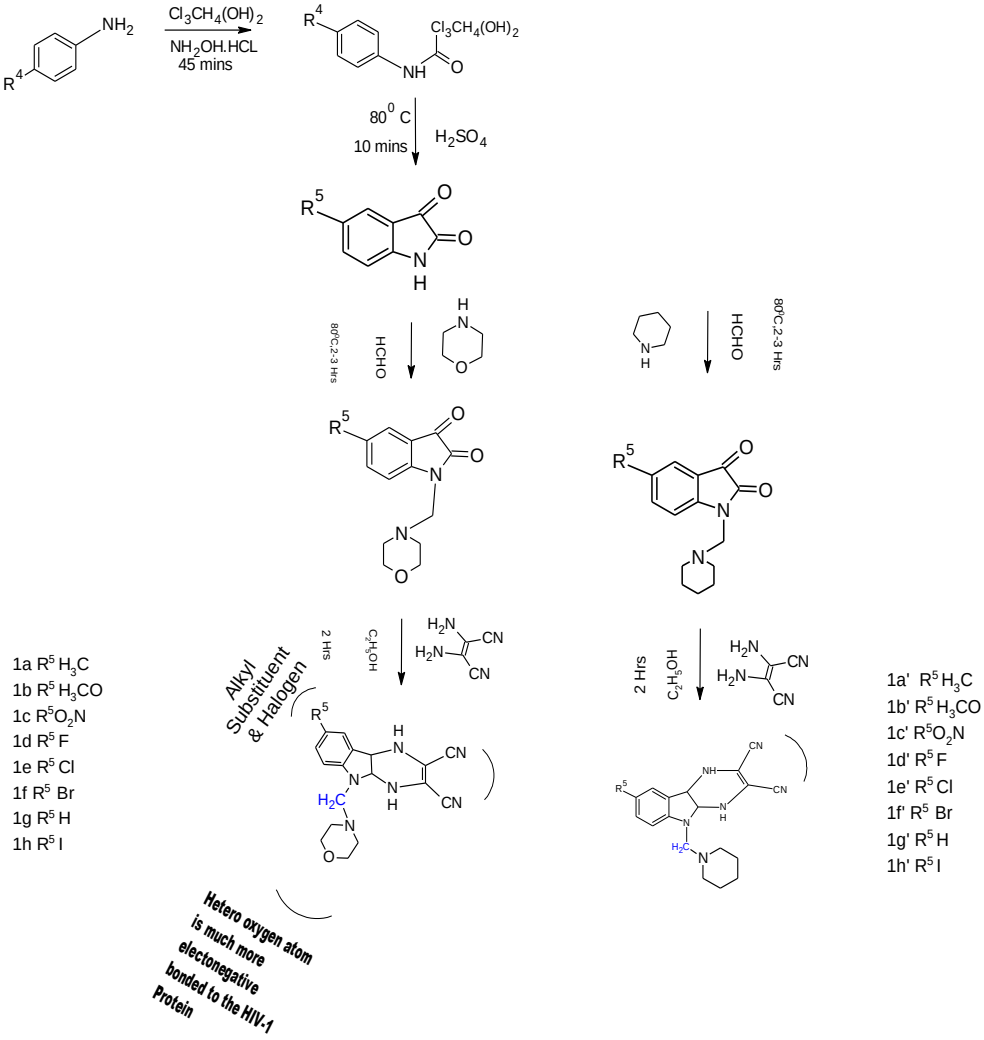
Cells and viruses

TZM-bl indicator cell line was kindly provided by the TZM-bl NIH AIDS Reagent Program USA. The cells were further optimized and laboratory adapted strains, including HIV-1UG070, 7 th PID was obtained from the department of molecular virology BSL-II laboratory National AIDS Research Institute (ICMR) Pune.

Experimental

Process technology and methodology to synthesized potential 2- Indolinones, total 16 derivatives using N-methyl morpholine-N-methyl piperidine, diaminomaleonitrile, halogens and alkyl group as substituent. Synthesized compounds were recrystallized by using ethanol-chloroform (9:1) mixture. Purity and characterization of synthesized compounds were done by chromatographic methods (TLC), infrared spectroscopy, NMR. Synthesized compounds 2-Indolinones and its derivatives screen for their Anti-HIV-1 activity.

Scheme



Molecular docking

Studies were performed using VLife MDS^{R} 4.3 program (Crystal structure of mutant HIV-1 (PDB code:4I2Q) was downloaded from the Protein Data Bank (PDB) at the Research Collaboration for Structural Bioinformatics (**http://www.rcsb.org/pdb/home/home.do**).The bound ligand and all water interfaces were removed from the protein. Gasteiger charges were assigned to both protein and ligands. A grid with spacing of 0.375 Å and 60×60×60 points in the x, y, and z axes was built and centered on the center of mass of the bound ligand in the crystal structure.

Energy grid maps for all possible ligand atom types were calculated before performing docking. Lamarckian genetic algorithm (LGA) to search the conformational and orientational space of the ligands. For each small molecule, 100 separate docking calculations were performed with the following settings of parameters: population size of individuals at 350, a maximum number of 25 million energy evaluations, a maximum number of generations of at 27,000, a mutation rate of 0.02, a crossover rate of 0.8, and an elitism value of 1.Cluster analysis was performed on the results with the root-mean-square (RMS) deviations less than1.0 Å. The best-docked conformation was then selected as the lowest energy pose in the most populated cluster. Thus, the lowest binding energy complex of more populated cluster more specific and highly reliable for efficacy.

Result and Discussion

A model-based approach is proposed here as a tool for the designed, synthesized and evaluated 1, 2, 3, 5-substituted-2, 3-dione derivatives which showed nanomolar activity against HIV-1.Taste compounds 1a, 1b, 1c, 1d, 1e, 1f, 1g and 1h showed HIV-1 inhibitory activity. Target compound 8-methoxy-5-(morpholin-4-ylmethyl)-4,4a,5,9b-tetrahydro-1*H*-pyrazino[2,3-*b*]indole-2,3-dicarbonitrile (1b) displays IC_{50} at micromolar level and low cytotoxicity (CC_{50}s > 1mM) against HIV-1 UG070 7 th PID. Simulation scenarios were used to complexity of ligand and HIV-1 protein more tactically and response by binding energy. We developed and examined selected new series of resistance mutation (refence protein 4I2Q PDB) and noted changes in interaction between compound and HIV-1 strain. Developed and validated results as shown in supplemental figures 1 and 2. The analysis reveals a role of space filling substitution in compound and binding energy could be related to IC50, changes noted more negative of the binding energy results in the formation of stronger complexes rightly assigned, therefore when a complexity have a low binding energy its affinity towards the target enzyme is bigger and highlights the role of space filling substitution in compound which is high degree to cover all hiding region in IBP. Also it has been clearly indicates the impact of different exposure levels as well as the effect of varying patterns of compounds on strain and its levels results show that inadequate as well as poor adherence to binding energy. In this study we found reported determined binding energy value of refence protein and reference ligand complexes shows -11.43 kcal/mol and average of experimental binding energy of newly ligands and refence protein complexes were shows -12.02 kcal/mol .Target compound [1b] has been shown binding energy -13.59 kcal/mol, which was show its affinity towards the protein 4I2Q enzyme is bigger in comparison with other lead ligands binding energy also. IC 50 of target compound 8-methoxy-5-(morpholin-4-ylmethyl)-4,4a,5,9b-tetrahydro-1*H*-pyrazino[2,3-*b*]indole-2,3 dicarbonitrile (1b) was found to be 3.96 µg/ml against laboratory adapted strains UG070,7 th PID. In this work we also examined the ability of docking and G scoring methods to predict the binding of compound to a HIV-1 strain, somewhat surprisingly, the binding area observed in docking does not always overlap with the binding of the high-affinity ligand. The comparatively higher interaction scores of lead compounds compared to Rilpivirine when docked with HIV-1 mutant protein at the active site suggest these novel leads would potentially bind more strongly to the pockets of HIV -1 4I2Q proteins. Further, the leads are docked with prone to mutation Lys101, Val106, Leu234 and His235 residue to predict their binding efficiencies with HIV-1 protein. All the sixteen designed 1,2,3 5-substituted-2,3-dione and its analogs with chemical substitutions at the R^1,R^2,R^3,R^5 position showing better G Scores respectively than reference ligand (G Score = -65.57), as a result of docking of among sixteen analogs eight lead compounds 1a,1b,1c,1d,1e,1f,1g and 1h were shows G score -81.69,-88.28, -80.98, -85.92, -85.35, -74.04, -67.87,-78.17. The target compound 1b, when docked with 4I2Q, have shown better dock scores, interaction score -88.28 compared to reference ligand and have suggested that above is a new highly functional novel derivative has very low cytotoxicity (CC_{50}>1mM) and it has been displays 15.5 ug/ml to cell lines, TZM-bl.

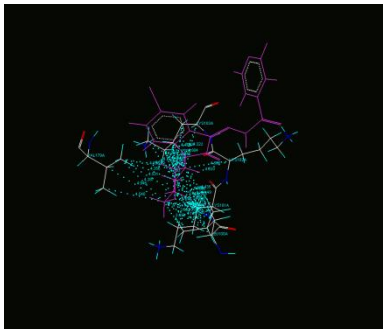


Fig 1

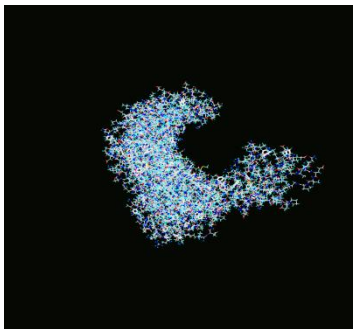


Fig 2

3D of target compound, 8-methoxy-5-(morpholin-4-ylmethyl)-4,4a,5,9b-tetrahydro-1*H* pyrazino[2,3-*b*]indole-2,3-dicarbonitrile (1b) into K103N/Y181C mutant HIV-1 strain, (PDB code:4I2Q), showed interaction of target compound in purple colour and 4I2Q in green and blue colour. Specified fig. 1 showed hydrophobic interaction, pi-pi interaction between target compound and HIV protein along with with hydrogen bonds formed between isolated oxygen atom of target compound and K101 were maintained which is crucial for drug resistance and fig. 2 merged molecule creation in inhibitors binding pocket cavity endorsed lock form in open position.

Acknowledgments

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We declare no competing interests.

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