Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 2 February 2019

Research Article

In Silico Exploration of HIV Entry Co-receptor Antagonists: A combination of Molecular Modeling, Docking and Molecular Dynamics Simulations

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Received: January 16, 2019; **Published:** January 22, 2019

Abstract

Screening multi-functional ligands is a recent strategy by which multiple targets can be inhibited by a single entity. A series of compounds with Anthracene, Phenanthrene and Naphthalene derivatives have been used as anti-HIV agents in this study. Homology modeling, molecular docking and molecular dynamics simulation studies were performed to explore the structural features and binding mechanism of some inhibitors of HIV entry co-receptors CCR5, CXCR4 and CCR3. Since CCR3 lacks a perfect 3-dimensional structure, homology modeling procedure was employed using Prime module of Schrodinger to construct the 3D model of CCR3. Pharmacokinetic and physiochemical properties of 10 synthetic and 5 natural compounds were studied to assess their drug ability using Qik Prop. The screened compounds were docked to the active sites of the three receptors using GLIDE and the 3D pictures of inhibitor-protein complex provided precious data regarding the binding orientation of each antagonist into the active site of these receptors. Molecular dynamics simulation at 1ns was done on the initial structures of protein-ligand complex resulted from the previous docking calculations using macro model module of schrodinger.

Keywords: Co-Receptors; Ligands; Homology Modeling; Molecular Docking; HIV; Drug Ability; Molecular Dynamics Simulation; Pharmacokinetic

Introduction

The successful treatment of Acquired Immune Deficiency Syndrome (AIDS) has been due to the introduction of Highly Active Antiretroviral therapy (HAART). Two classes of HIV drugs (e.g. proteases and reverse transcriptases) are used in HAART to delay or stop the progression of AIDS. However, because of side effects, development of resistance, and challenges with patient compliance there is a continuing demand for new generations of viral inhibitors. Ideally, other classes of drugs interfering with viral replication by new mechanisms could complement existing therapies [1]. One such new class, the entry inhibitors, prevents HIV entry into the target cell.

To enter a target cell, HIV-1 requires CD4 [2] and other co-receptors [3]. Among the latter, the chemokine receptors CXCR4, CCR5 and CCR3, which belong to the family of G-protein coupled receptors with seven membrane-spanning domains, have been identified as the principal HIV-1 co-receptors [4]. Antagonists inhibiting or altering the interaction of HIV with these coreceptors prevent viral fusion with the cell membrane, which ultimately results in viral load reduction when administered to humans [5]. While CCR5 is used as a coreceptor in the early stages of infection, the CXCR4 coreceptor using virus is linked to significant disease progression leading to AIDS [6].

HIV entry receptors

Chemokine receptors belong to the seven transmembrane G-protein coupled receptor (GPCR) family. The G-protein-coupled receptor (GPCR) superfamily is one of the most important classes of transmembrane (TM) proteins being involved in cell communication processes and in mediating such senses as vision, smell, taste, and pain. Specifically, chemokines in particular are involved in cell growth and HIV infection [7]. The word ''chemokine'' is derived from their function as chemotactic cytokines, resulting in activation and migration of leukocytes toward sites of inflammation. This process is mediated by the interaction of chemokines with their respective chemokine receptor [8]. HIV replication first requires

the virus to enter an uninfected host cell such as CD4+ cells or macrophages. Macrophages carry the CCR5 co-receptor; hence HIV strains requiring the CCR5 co-receptor for entry are also referred to as 'macrophage-tropic' although they also infect lymphocytes [9].

Chemokine receptors CCR5 and CXCR4 remain the two major co-receptors of HIV [10]. Of the two major HIV co-receptors, CCR5 plays an essential role in HIV transmission and pathogenesis. CCR5 is the principal entry coreceptor for macrophage-tropic Human Immunodeficiency Virus type 1 (HIV-1) strains, which predominate at the early stages of the HIV-infection [11]. HIV-1 gp120 interacts with CCR5 following its binding to CD4, and such an interaction is thought to involve the V3 region of gp120 and the N-terminus and extracellular loops of CCR5 [12]. The second extracellular loop (ECL2) of GPCRs is known to play a critical role in ligand binding and ensuing signal transduction. The ECL2 of CCR5 is also thought to play an important role in CCR5 interactions with HIV-1 envelope [13].

Some strains of HIV-1 utilize CCR5 exclusively, while for other strains inhibiting either CCR3 (CC-Chemokine Receptor 3) or CCR5 alone decreased HIV-1 replication. These data suggest that for some strains of HIV-1, including primary isolates from both the CNS and the periphery, CCR3 may be an important partner co-receptor that is used together with CCR5 in HIV-1 entry into macrophages and brain microglia [14]. Hence, therapeutic strategies targeting CCR3, CCR5 and CXCR4 may thus be helpful in inhibiting HIV type I.

From the literature survey, only few natural compounds viz. 10-methoxydihydrofuscin, fuscinarin, and fuscin isolated from the soil fungus *Oidiodendron griseum* [15] and Betulinic acid, a naturally occurring pentacyclic triterpenoid [16] were identified as CCR5 antagonists. So, these compounds and some synthetic compounds having anti-viral activity were used in this study to access their antagonist activity for HIV entry receptors.

Materials and Methods

Homology Modeling

The target sequence CCR3 with Uniprot ID: P51677 was retrieved from the Uniprot database [17]. The homologs of the query sequence were searched using BLAST. Multiple templates with PDB ID: viz., 4MBS, 5T1A, 5LWE were structurally aligned and imported to model the three dimensional structure of the target CCR3 protein. The three dimensional structure of the target protein was modeled using MODELLER v9.18, a powerful and complete tool for generating accurate receptor models for structure-based drug design [18]. The Loop modeling procedure was carried out using SwissPDB viewer [19].

The parameters including the covalent bond distances and angles, steriochemical validation and atom nomenclature were validated using RAMPAGE server [20]. Backbone conformations were evaluated by the inspection of Phi/Psi Ramachandran Plot obtained from PROCHECK analysis. The statistics of non-bonded interactions between different atom types were detected and value of the error function was analyzed by ERRAT [21].

Structure Retrieval of target proteins and Ligands

The three dimensional crystal structure of CCR5 (PDB ID: 2QAD) and CXCR4 (PDB ID: 3OE6) were downloaded from the Protein databank. Five natural compounds 10-Methoxy-dihydrofuscin, Fuscinarin, Fuscin, Dihydrofuscin, Betulinic acid and 10 Synthetic compounds viz. 1,7dihydroxy-9H-xanthen-9-one, Euxanthone, Ugaxanthone, 1-Methoxyxanthone, 3-Methoxyxanthone, Hydroxy-7-Methoxy-3-methylxanthone, flavones, Apigenin, Luteolin, Anibamine were drawn using Maestro 9.0 build panel.

Pharmacokinetic studies

The pharmacokinetic properties of the compounds screened through docking were calculated using Qikprop version 3.5 of Schrodinger, a quick, accurate and easy-to-use absorption, distribution, metabolism and excretion (ADME) prediction program designed by Professor William L. Jorgensen. Using QikProp the widest variety of pharmaceutically relevant properties, namely octanol/water and water/gas log P, log S, log BB, overall CNS activity, CaCo2 and MDCK cell permeability's, human oral absorption, log Khsa for human serum albumin binding and log IC50 for HERG K+ Channel blockage were predicted for the above filtered molecules.

Molecular Docking

The hetero atoms and water molecules were removed from the protein and the chemistry of the proteins were corrected for the missing atoms. The target proteins and the drawn compounds were prepared using protein preparation wizard and Ligprep respectively. Site map was used to detect the active sites and docking procedure was performed using GLIDE 5.7 (Grid based Ligand Docking with Energetics) [22] based on conformational search algorithm, where the shape and properties of the receptor are represented on a grid by several different sets of fields that provides progressively more accurate scoring of the ligand pose. During the docking process, initially Glide performs a complete systematic search of the conformational, orientational, and positional space of the docked ligand and eliminating unwanted conformations using scoring and followed by energy optimization.

Molecular dynamics Simulation

Molecular dynamics simulation of the modeled protein was done using the Macromodel module of Schrodinger, which has the ability to perform molecular dynamics simulations to model systems at finite temperatures using stochastic dynamics and mixed Monte Carlo algorithms. An NVT (Canonical) ensemble was adopted at constant temperature of 298.15 k and with temperature relaxation time of 0.01 ps for 100 molecular dynamics steps.

Results and Discussion

Prediction of 3D structure of CCR3

The primary sequence of the CC-Chemokine receptor 3 (CCR3) having 355 amino acid residues, retrieved from uniprot was analyzed for homology. Multiple templates were searched for the target protein CCR3 using BLAST. The templates 4MBS, 5T1A, 5LWE were found to have maximum identity. The domain identified in the protein sequence was positioned at 51 to 301 residues. The three dimensional structure of the target protein was modelled based on multiple templates identified using BLAST search. For majority of homology model building cases, the alignment between model and template sequence contain gaps. These are addressed by simply omitting residues from the template, thus creating a hole in the model. Gaps in the template sequences are treated by inserting the missing residues into the continuous backbone. Since most of the residues in the modeled protein were found to be in the disallowed region, Loop modeling was carried out in order to increase the accuracy of the structure by modeling the loops. The modeled three dimensional structure of CCR3 was depicted in Figure 1A.

Modeled Protein	Residues in favoured region	Residues in allowed region	Residues in outlier region
CCR3	95.2%	4.8%	0.0%

Table 1: Validation results for Modeled CCR3 protein.

The stereo chemical quality of the predicted model was evaluated after the refinement process using Ramachandran Map calculations computed with the PROCHECK program. In Ramachandran plot analysis, the residues were classified according to its region in the quadrangle. The red regions in the graph indicate the most allowed regions whereas the yellow regions represent allowed regions.

The results revealed that the modeled structure has residues in allowed region. The distribution of main chain bond lengths and bond angles were found to be within the limits. The Φ and Ψ distributions of the Ramachandran plots are summarized in Figure 1B. 95.2% of the residues were found to be in the most favorable region. Errat analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of each residue.

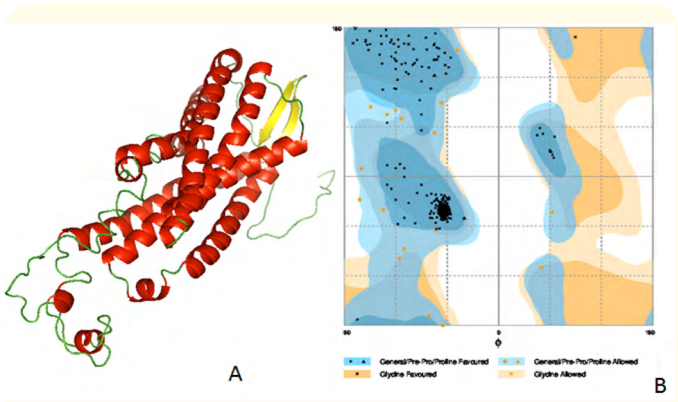

Figure 1: A) Modeled three dimensional structure of CCR3. Helices, sheets and coils are represented in red, yellow and green respectively. B) Ramachandran Plot for the modeled protein CCR3. Glycine is represented by triangles and other residues are represented by squares.

The overall quality factor was obtained as 81.99 which is very much satisfactory. The validation of the model was done using Ramachandran Plot calculations computed using Rampage. Verify-3D determines the compatibility of an atomic model with its own amino acid sequence assigned by a structural class based on its location and environment (alpha, beta, loop, non-polar etc.) and comparing the results to good structures. The modeled structure was refined using the prime refinement panel. The modeled structure was submitted to the Protein Model Database (PMDB). The structure of the protein can be downloaded by general pubic using the provided ID 'PM0079223'.

ADME/T Properties Prediction

The ADME properties of these compounds were analyzed using QikProp tool of Schrodinger software. The ADMET properties such as permeability through MDCK cells (QPlog MDCK), logKp (Skin permeability), qikProp predicted log IC50 value for blockage of K+ channels (QPlogHERG), percentage of human oral absorption of compounds were reported in Table 2. QikProp also predicted the physiochemical properties of the compounds such as, their molecular weights, hydrogen bond donars, hydrogen bond acceptors and solubility and these properties were well within acceptable range of the Lipinski rule. The pharmacokinetic properties such as octanol/water partition coefficient, cell permeability of Caco-2 cells and blood/brain partition coefficient were also evaluated and reported in Table 2.

Molecular Docking

Molecular docking is the most widespread method for the calculation of protein-ligand interactions. It is an efficient technique to predict the potential ligand binding sites on the whole protein target. The screened compounds from ADMET studies were used for docking analysis.

S. No	Compounds	Molecular weight (130-725)	Hb donar (0-6.0)	Hb acceptor (2-20)	QP Log S (-6.5-0.5)	QP Log BB (-3.0-1.2)	Log IC_{50} for HERG K+ Channel Blockage (below -5)	QP Log Kp (Kp in cm/hr)	QP Log P for oct/wat (-2.0/6.5)	Solute Ionization potential (7.9/10.5)	Lipinski Rule of 5 Violations (maximum 4)	QPPCao < 25 poor, > 500 great	Human Oral absorption (< 25 % is poor, > 80% is high)
1	Ugaxanthone	328.321	3.00	4.5	-3.830	-1.720	-4.766	-4.182	2.110	9.092	0	3920	75
2	Euxanthone	288.204	1.00	3.00	-2.659	-0.722	-4.597	-2.900	1.755	8.926	0	468	85
3	1-Methoxy xanthone	226.231	0.00	3.25	-2.792	0.142	-4.872	-1.112	2.602	9.032	0	3920	100
4	3-Methoxy xanthone	226.231	0.00	3.25	-2.704	0.110	-4.761	-1.223	2.502	9.162	0	3573	100
5	1,7dihydroxy-9H-xanthen-9-one	228.204	1.00	3.00	-2.662	-0.725	-4.599	-2.907	1.754	8.858	0	464	85
6	Fuscinarin	264.277	2.00	5.25	-2.877	-0.720	-3.352	-3.811	1.248	9.184	0	468	82
7	Dihydrofuscin	278.304	2.00	5.25	-3.229	-0.822	-3.481	-3.990	1.455	8.922	0	480	82
8	10-Methoxy-dihydrofuscin	308.83	2.00	6.95	-3.251	-0.680	-3.495	-3.448	1.383	9.016	0	643	85
9	Flavone	238.242	0.00	2.25	-3.730	-0.319	-5.349	-1.846	3.243	9.138	0	1266	100
10	Luteolin	286.236	1.00	3.00	-2.362	-0.310	-3.654	-1.324	1.365	8.365	0	583	100
11	Betulinic acid	456.700	0.00	4.25	-4.65	-0.655	-3.521	-2.932	1.523	8.262	0	656	87
12	Fuscin	276.284	1.00	3.00	-2.51	-0.613	-4.321	-3.459	2.361	9.325	0	732	100
13	Apigenin	270.23	5.00	3.00	-2.64	-0.264	-4.368	-4.658	2.598	8.214	0	621	85
14	Hydroxy-7-methoxy-3-methyl xanthone	288.256	1.00	4.5	-3.239	-0.913	-4.629	-3.036	2.000	8.580	0	491	87

Table 2: Qikprop Results for the Ligands.

S. No	Compound	CCR5		CCR3		CXCR4	
		G-Score	G-Energy	G-Score	G-Energy	G-Score	G-Energy
Drug							
1	Maraviroc	-4.02	-32.60	-4.20	-37.91	-5.55	-33.22
Synthetic Compounds							
2	Euxanthone	-5.32	-39.01	-4.79	-32.82	-6.35	-33.23
3	1,7-dihydroxy-9H-xanthen-9-one	-5.32	-39.01	-0.52	-0.14	-6.11	-31.50
4	Flavone	-4.92	-30.066	-3.41	-28.37	-5.67	-29.55
5	Luteolin	-4.87	-35.16	-4.73	-38.79	-6.08	-41.61
6	3-Methoxy xanthone	-4.66	-26.43	-3.99	-28.37	-5.22	-28.49
7	Apigenin	-4.55	-31.63	-4.67	-35.42	-6.12	-43.91
8	Ugaxanthone	-4.511	-37.27	-4.88	-40.53	-6.13	-43.91
9	1-methoxy xanthone	-4.49	-24.43	-4.32	-28.82	-5.20	-28.67
10	Hydroxy-7-methoxy-3-methyl xanthone	-4.33	-31.63	-5.23	-36.64	-4.80	-35.26
Natural Compounds							
11	Dihydrofuscin	-5.98	-59.88	-5.56	-35.71	-5.55	-33.22
12	Fuscinarin	-5.81	-39.82	-5.28	-38.03	-5.77	-30.75
13	10-Methoxy hydrofuscin	-4.85	-28.18	-5.32	-39.18	-5.36	-32.24
14	Betulinic acid	-3.67	-26.86	-3.59	-35.29	-4.68	-39.66
15	Anibamine	-2.95	-24.25	-0.76	-28.39	-4.55	-31.09
16	Fuscin	-	-	-4.78	-33.11	-5.99	-34.24

Table 3: Docking results for CCR5, CCR3 and CXCR4.

Docking results for CCR5

The Glide score, which distinguishes molecules based on interacting ability, was calculated for all ligands. A more negative the glide score indicates better fitting to the receptor active sites [23]. The best ligand binding pose with the least Glide score or energy was chosen. The binding site of CCR5 is hydrophobic in nature due to multiple aromatic residues present in pocket. This property imparted tight binding to the inhibitors [24]. Although all CCR5 antagonists predicted were bind to the same hydrophobic pocket, previous studies indicated that ligands may occupy different subcavities also [25]. The active site residues of CCR5 were found to be Lys50, Ile76, Glu77, Ser79, Asp80, Thr81, and Lys166.

Among the screened synthetic compounds Euxanthone and 1,7-dihydroxy-9H-xanthen-9-one shows good interactions with CCR5 having the least Glide score of -5.32, -5.32 and Glide energy of -39.01, -39.01 respectively which has least glide score than the drug Maraviroc. Hydrogen bonding plays a major role in determining the specificity of intermolecular interactions. It also plays an important role in determining the three-dimensional structures adopted by proteins and nucleic acids. Euxanthone forms four hydrogen bond with CCR5 involving the residues Tyr82, Pro48, Asn78 and Asp80 and is shown in Figure 2A.

All compounds with least Glide score clearly showed the ability to make a maximum number of hydrogen bonds with the active site residues [24]. Of the 6 Natural compounds selected from literature, only two compounds Dihydrofuscin and Fuscinarin showed the Glide score value of -5.99, -5.81 and Glide energy of -39.99, -39.01 respectively. Nitrogen atom of Lys50 forms hydrogen bond with dihydrofuscin by donating a hydrogen atom with a bond distance of 2.091Å. Asp80 also formed hydrogen bond with dihydrofuscin.

Docking results for CXCR4

The extracellular interface of CXCR4 consists of 34 N-terminal residues, extracellular Loop 1 (ECL1, residues 100–104) linking helices II and III, ECL2 (residues 174–192) linking helices IV and V, and ECL3 (residues 267–273) linking helices VI and VII. The intracellular side of CXCR4 contains intracellular loop 1 (ICL1, residues 65–71) linking helices I and II, ICL2 (residues 140–149) linking helices III and IV, and ICL3 (residues 225–230) linking helices V and VI, and the C-terminus [26]. Asp97, Trp94, His113, Tyr116, Thr117, Asp187, Arg188, Phe189, Tyr190, Pro191, Phe199, Asn192, Tyr254, Glu272, Gly277, His281, Ile284, Ile185 and Glu288 were found to be the active site residues in CXCR4. From this study most of the active site residues were found to be on the transmembrane domain.

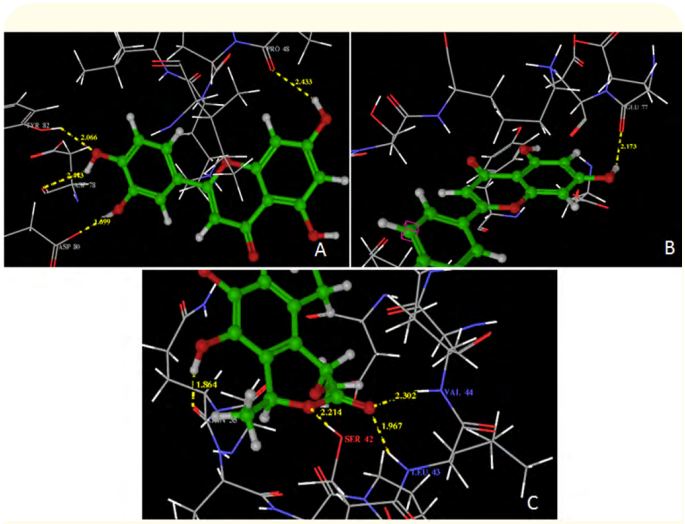

Figure 2: Interaction of A) Euxanthone with CCR5. B) Euxanthone with CXCR4 and C) 10-Methoxy hydrofuscin with CCR3. Interatomic distances between H-bonded atoms are indicated in yellow. All distances are in Angstroms.

The synthetic compounds Euxanthone, 1,7-dihydroxy-9H-xanthen-9-one, Flavone, Luteolin, 3-Methoxy xanthone, Apigenin, Ugaxanthone and 1-methoxy-xanthone showed good interaction with CXCR4 with a Glide score of -6.35, -6.11, -5.67, -6.08, -5.22, -6.12, -6.13 and -5.20 respectively. The natural compounds Dihydrofuscin, Fuscinarin and Fuscin shows better interaction having Glide score of -5.55, -5.77 and -5.99 respectively. Euxanthone interacted with CXCR4 forming two hydrogen bonds as shown in figure 2B.

Docking results for CCR3

The residues D171 (TM 4), H203 (TM 5), D262 (TM 6), and E288 (TM 7) are important in antagonist binding [27]. Docking based virtual screening on CXCR4 inhibitors form a hydrogen bond to one of the Glu288, Asp171, or Asp262 [28]. In this study also most of the ligands showed hydrogen bond interaction with the oxygen atom of E288 of TM7 and N97 of TM1. Active site residues of CCR3 includes Ala27, asp28, arg30, Ala31, Ala34, Gln35, Phe36, Val37, Ser42, Ala77, Ile78, Asp80, Gly48, Thr69 and Ile67. Based on this study, Asp80 and Ser42 forms hydrogen bond interaction with almost all the inhibitors. Among the natural compounds Dihydrofuscin, 10-Methoxy hydrofuscin and Fuscinarin shows good interaction of Glide scores -5.57, -5.32 and -5.28 respectively. 10-Methoxy hydrofuscin showed Glide energy of -39.18 showing hydrogen bonds with residues Val44, Leu43, Gln35.

By docking analysis the compounds dihydrofuscin, 1,7-9H-xanthen-9-one was found to inhibit both CCR5 and CXCR4 and the

compounds dihydrofuscin, fuscinarin and 10-methoxy hydrofuscin inhibits both CCR3 and CXCR4. Similarly, the compounds dihydrofuscin and fuscinarin had inhibitory activity against CCR5 and CCR3. The expression and function of CCR5 in other human immune diseases, including multiple sclerosis, rheumatoid arthritis, and renal allograft rejection, suggest that blocking CCR5 function might be beneficial in these diseases in addition to HIV [29,30]. Hence the newly identified CCR5 inhibitors may be considered as a lead compounds for multi diseases. CCR3 may be an important partner co-receptor that is used together with CCR5 in HIV-1 entry into macrophages and brain microglia. Therapeutic strategies targeting both CCR3 and CCR5 may thus be helpful in inhibiting HIV-1 infection [31]. This study suggests that Dihydrofuscin, Hydroxy-7-methoxy-3-methyl xanthone and Fuscinarin may act as a drug for treatments targeting both CCR5 and CCR3.

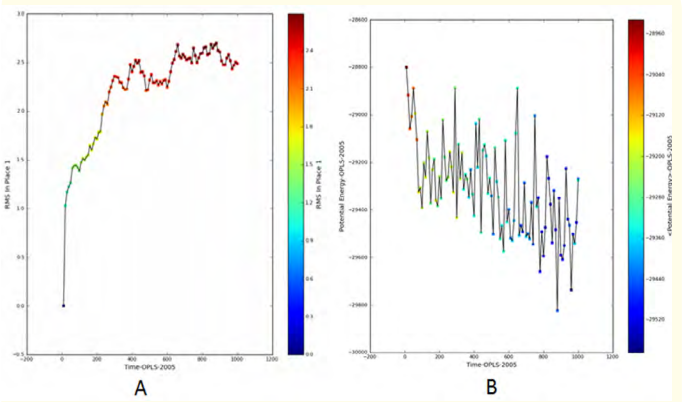

Figure 3: Molecular Dynamics simulation of CCR5 (A) RMSD verses simulation time (B) potential energy of the system versus time.

MD Simulation of Complexes

The obtained docking results of HIV coreceptors (CCR5, CCR3 and CXCR4) antagonists allowed us to propose a general binding mode of these ligands and to determine residues involved in the ligand recognition. Molecular dynamics simulation on the ligand-protein complex was performed for the investigation of binding modes of ligands and to explain the effects of ligand binding on the conformation of protein. Hence 18 ligand- receptor complex was selected as a representative for MD simulation. This complex (18 ligand-CCR5, 18 ligand-CCR3 and 18 ligand-CXCR4) were used as the starting conformation for MD run. The aim of MD run was to get more precise ligand-receptor model in a state close to the natural conditions and to explore the binding modes of the ligands further.

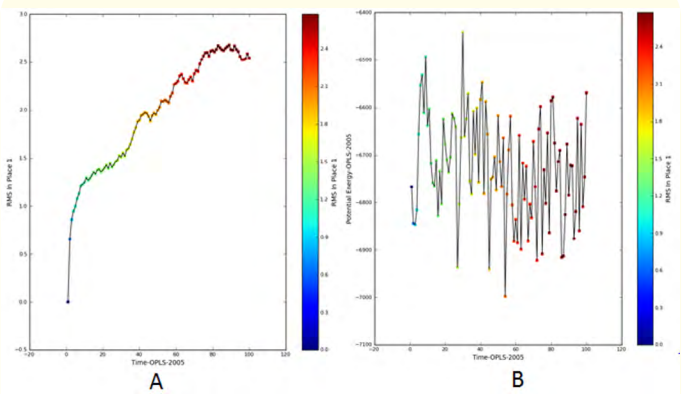

Figure 4: Molecular Dynamics simulation of CXCR4 (A) RMSD verses simulation time (B) potential energy of the system versus time.

Trajectories were set up to 1000 which corresponds to 1 nano seconds total equilibrium simulation time. Protein stability maintained for CCR5 after 856th trajectories which has Potential Energy in run -29464.90353E model value and RMSD 1.509365A which is depicted in figure 3. From 857th trajectories it started stability OPLS force field deviation till 1000th sample. Temperature kept between 300 K with non-SHAKE with Stochastic dynamics held up to its fluctuation of Heavy atom profile. But the temperature deviated between 295-305 K in the protein Solvent Surfaced Area. For CXCR4 the protein stability is maintained after 86th trajectories which have Potential Energy in run -29134.71378E model value and RMSD 2.5013 as in figure 4. For CCR3 the protein stability is maintained after 874th trajectories which has Potential Energy in run -69530.70379E model value and RMSD 2.5013 as in figure 5. From 875th trajectories it started stability OPLS force field deviation till 1000th sample.

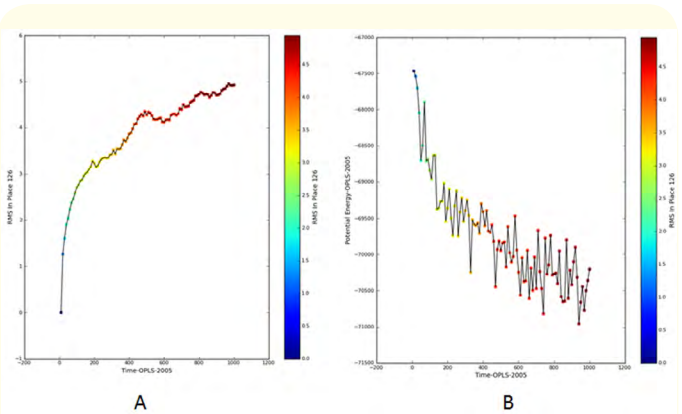

Figure 5: Molecular Dynamics simulation run for CCR3 (A) RMSD verses simulation time (B) potential energy of the system versus time.

Significance of Obtained Molecular Simulation complex:

After one third of the total simulation, the three proteins CCR5, CCR3 and CXCR4 have got stabilized SASA volume with its ligand interaction. It doesn't show any denaturation within the volume surface or getting away from the interacted place which shows the complex of 18 with ligand has highest binding affinity and Molecular Mechanics property to make protein stabilize when its interacted.

Conclusion

Most of the residues of the modeled CCR3 protein were found to be in the allowed regions which are found to be satisfactory. Highly active inhibitors of HIV reverse transcriptase and protease have made it possible to dramatically reduce virus load in HIV-positive individuals. However, the presence of viral reservoirs, the emergence of drug-resistant HIV variants and the side effects of these compounds call for research into new drugs that target different stages of the viral life cycle. One attractive target is the first step in HIV replication: entry of virus into cells. CCR5, CCR3 and CXCR4 were found to be the coreceptors for HIV entry.

Based on our analysis, the compounds dihydrofuscin, 1,7-9H-xanthen-9-one, fuscinarin, and 10-methoxy hydrofuscin has inhibitory activity on multiple targets namely CCR3, CCR5 and/or CXCR4. Pharmacokinetic studies of these compounds revealed that they have druggable property and may be used as an Anti-HIV drug in future. The obtained docking results of HIV entry coreceptors antagonists allowed to propose a general binding mode of these ligands to the three co-receptors (CCR5, CCR3 and CXCR4) and to determine residues involved in the ligand recognition. For further investigation of binding modes of ligands and to explain the effects of ligand binding on protein conformation, MD simulation was performed. At the end of the MD simulation, a change in the position and orientation of the ligand in binding site was observed. This important observation indicated that the application of MD simulation after docking of ligands was useful.

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