Pharmacophore modeling, atom based 3D-QSAR and Molecular docking approaches to screen C-X-C chemokine receptor type 4 antagonists as microbicides for human immunodeficiency virus-1

Radhika Ramachandran¹ · Shanmughavel Piramanyagam¹

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Abstract The C-X-C chemokine receptor type 4 receptor CXCR4 which acts as a co-receptor for human immunodeficiency virus-1, expressed in the later stages of infection is considered as an attractive and new target for drug design. Microbicides acting as co-receptor blockers are highly significant as these drugs block HIV lifecycle at early stage itself. The urgent need for a safe and effective microbicide urges to explore new CXCR4 antagonists which could be developed as microbicides. The pharmacophore based 3D-QSAR models and docking models were developed using PHASE and GLIDE modules of Schrodinger software. Three-dimensional quantitative structure–activity relationships (3D-QSAR) studies and pharmacophore modelling was carried out on a dataset of 114 CXCR4 antagonists with the intention of exploring entry inhibitors with better therapeutic potential. A training set of 43 compounds was used to create 3D-QSAR models and they were validated using a test set of 28 compounds. CXCR4 antagonists with good inhibitory activity could be designed and structurally modified based upon the QSAR model developed with necessary pharmacophore features. The results revealed that the common pharmacophore hypothesis ADHPR.1 was used for 3D-QSAR model development and the most active compound, CXCR4 antagonist no.44 which is a imidazopyridine-tetrahydro-8-quinolinamine derivative interacted with the CXCR4

receptor residue ASP 97 by the formation of a hydrogen bond. Also, the docking studies were carried out for the dataset for analyzing the binding conformation of CXCR4 and 114 antagonists. The results obtained from the 3D-QSAR studies and docking simulation can be used for designing new and potent CXCR4 antagonists. The compound identified from this study can be taken up further for validation by in vitro/in vivo studies.

Keywords Pharmacophore · 3D-QSAR · CXCR4 antagonists · Microbicides · Docking · HIV-1

Introduction

Microbicides are chemical agents applied to vaginal or rectal mucosal surfaces with the goal of preventing, or at least significantly reducing, the transmission of sexually transmitted infections (STI's) including Human Immunodeficiency Virus -1 (HIV-1). These are given much importance in the society as it proves to be a HIV-prevention tool for women to safe guard their health and their families [10]. HIV is a lentivirus, a member of the retrovirus family, which causes acquired immunodeficiency syndrome (AIDS) [9]. Mostly, these products follow the mechanism of action as antiretroviral drugs which interfere with the viral replication process within the cell. The highly active antiretroviral therapy (HAART) consists of a combination of three or more HIV-1 Protease and Reverse transcriptase inhibitors. Apart from this, the discovery of entry inhibitors such as chemokine antagonists as promising leads for the development of microbicides; very much effective against AIDS. These agents block HIV replication by interfering entry mechanism and slow down the progression of HIV infection towards AIDS [13]. Hence, these

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✉ Shanmughavel Piramanyagam
shanmughavel@buc.edu.in

¹ Department of Bioinformatics, Bharathiar University, Coimbatore, Tamilnadu 641 046, India

molecules are proposed as lead compounds suitable for microbicide development.

Several factors like poor adherence to HAART regimen and long term toxicities leading to drug resistance has arisen the need for new treatment methods. Since the discovery of chemokine receptors such as CXCR4 (C-X-C chemokine receptor type 4) and CCR5 (C-C chemokine receptor type 5) as co-receptors for HIV, much importance has been given to prevent HIV infection by blocking the entry mechanism. CXCR4 and CCR5 belong to the seven transmembrane G-protein coupled receptor (GPCR) superfamily. The CCR5 (R5) utilizing strains or M-tropic are generally associated with the initial infection and asymptomatic phase. As the disease progresses towards AIDS, dual tropic virus emerge that can utilize both CCR5 and CXCR4 chemokine receptors or solely utilize the CXCR4 receptor to gain entry into the host cells. Most of the research was focused on CCR5 antagonists as they are the commonly transmitted HIV-1 strains [5, 9]. However, CXCR4 (R4) utilizing or T tropic strains of HIV is associated with a decrease in the number of T-cells and accelerated disease progression [11, 13]. The X4 variants have been observed in a minority of infected persons. Unlike the other chemokine receptors, CXCR4 has only one endogenous natural ligand, stromal-cell-derived factor (SDF-1) [7]. In-vitro studies have proven that addition of the natural CXCR4 ligand SDF-1 or small molecule antagonists can block HIV infection [13, 30]. So, microbicides acting as CXCR4 antagonists comprising both peptides as well as small molecules can be developed to overcome this problem. A number of small-molecule CXCR4 antagonists such as AMD 3100, AMD 070 were tested in different phases of clinical trials to be developed as microbicides [1, 5].

The challenges encountered in this research are high adsorption rate, systemic toxicity and development of drug resistance. Specifically, hindrances in conducting clinical trials are limited availability of trial sites, difficulties in dose selection and safety monitoring and a lack of adherence to the objectives. Once a product has been found to be safe and effective, there arises a problem of regulatory approval and for success launch of products by using appropriate methods for implementing in women.

Computer-aided drug design (CADD) is an in silico simulation technique to screen for novel compounds by their structure and bioactivity from database. The structure-based drug design and ligand-based drug design are two major application areas of CADD. Structure-based drug design relies on the knowledge of the three-dimensional structure of the biomolecular target and ligand based drug design relies on knowledge of other molecules that bind to the biological target of interest. In the ligand based drug design, the molecules may be used to derive a pharmacophore which

defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target [6, 11]. In this study, computational approaches like ligand and structure based drug design are undertaken to explore CXCR4 antagonists with enhanced inhibitory activity and reveal the structural characteristics responsible for its activity. These in silico approaches help us to design newer drugs in an easy manner based upon the properties of the compounds selected for study. It has the advantage of overcoming the many obstacles and practical challenges in developing a microbicide. Therefore, in the present study, an attempt has been made to find out effective CXCR4 antagonists that could be promoted as entry inhibitor based microbicides.

Materials and methods

Data set

All the computational analysis was carried out by Maestro modeling package from Schrodinger software [28].

The structures of a dataset of 114 chemically diverse CXCR4 antagonists with known experimental inhibitory concentration (IC_{50}) values were generated using structure drawing programs such as Chemschetch (<http://www.acdlabs.com>) and Chemdraw (<http://www.cambridgesoft.com/software/chemdraw>) from published literature (ESM Appendix) and were saved in .mol file format. The CXCR4 antagonists numbered 1–28 [11, 13], 29–49 [2, 7], 50–61 [22, 30], 62–89 [1, 19], 90–114 [5, 9] were retrieved. The biological data, inhibitory concentration (IC_{50}) of these antagonists were determined by the inhibition of binding of SDF-1 α to CXCR4 in SDF-1 α binding assay, derived from the above cited literatures. The microbicides such as AMD 3100 and AMD 070 were taken as reference molecules for comparison with the CXCR4 antagonists taken for this study [6, 16].

Protein preparation and receptor grid generation

The three dimensional structure of CXCR4 receptor was obtained from Protein Data Bank (PDB) (PDB ID: 3ODU, A chain) (<http://www.acdlabs.com>, <http://www.cambridgesoft.com/software/chemdraw>). The protein preparation was carried out using the protein preparation wizard available at Maestro of Schrodinger Suite, 2012 using default parameters (<http://www.acdlabs.com>) [15].

The receptor grid was generated from the receptor grid generation panel of grid based ligand docking with energetics (glide) module by default settings. The electrostatic and Vander Waals potentials of the binding pocket was calculated using grid based method by considering the active site residues. The active site residues were found to be ARG 183, ILE 185, CYS 186, TRP 102, ASP 97, ASP

187, TRP 94, VAL 112, TYR 116 and GLU 288. The grid was centered on the active site residues of the receptor. The CXCR4 receptor grid size was set to be 12, 14 and 6 which was saved in the (.grd) format.

Ligand preparation

Ligands were prepared and were assigned an appropriate bond order using Ligprep module of Schrodinger suite (<http://www.cambridgesoft.com/software/chemdraw>) [17]. Ligprep converted 2D to 3D structure by including tautomeric, stereo chemical and ionization variations as well as energy minimization and flexible filters to generate fully customized ligand libraries that are optimized for computational analysis. Compounds with atom types that were not recognized by Ligprep were eliminated. For each processed input structure, it produced a low-energy 3D structure with correct chiralities, unwanted molecules removed and added hydrogen's.

Molecular docking

Molecular docking was carried out using glide (grid based ligand docking with energetics) [2, 24]. It generated the conformations and orientations of ligands inside the binding pocket in the presence of grid potentials. The molecular docking between CXCR4 antagonists and its receptor was carried out using previously calculated grid in the receptor grid generation step. The library of small molecules used for docking was initially screened using standard precision (SP) docking followed by extra precision (XP) docking and the XP descriptor information for the results files were saved. The XP method weeds out false positives and provides better correlation between good poses and good scores. Since extra-precision mode is a refinement tool designed for use only on ligand poses, large sets of ligands are screened by standard precision (SP) mode. Ultimately, optimization of the ligand structures in the field of the receptor was done and then the ligands were scored mainly in terms of glide score, H bond score and the number of hydrogen bonds formed.

Pharmacophore modelling

The pharmacophore modelling for CXCR4 antagonists was carried out using the PHASE module of Schrodinger molecular modelling package [22, 23]. The property of ligands, pIC_{50} -Exp was selected as the experimental activity variable in order to build model. Inhibitory concentration (IC_{50}) was converted to predicted inhibitory concentration (pIC_{50}) using the formula $pIC_{50} = -\log IC_{50}$ where pIC_{50} is the dependent variable that characterizes the biological parameter for the developed model.

The CPH was identified by dividing the dataset into active and inactive sets based upon their activity threshold (pIC_{50}) values. The maximum and minimum pIC_{50} values found to be 3.222 and -4.150 and the activity threshold value determined the division of molecules into active and inactive data set. The active threshold value pIC_{50} above -1.0 were assigned as active and below -2.5 as inactive. Active set comprised molecules responsible for generated pharmacophore models whereas inactive set constituted of molecules which did not contribute for pharmacophore model development.

A common 5-point or 5 site pharmacophore features in the ligand conformations used for hypothesis generation are hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), positively ionisable (P), negatively ionisable (N), and aromatic rings (R) defined by a set of chemical structure patterns. The pharmacophores of active ligands that contained identical sets of features with very similar spatial arrangements were grouped together to give rise to a CPH. Once, the various CPH's were generated, they were scored and ranked in order to identify the best hypothesis for QSAR model generation. This was done using a scoring algorithm which included contributions from the alignment of site points and vectors, volume overlap, selectivity, number of ligands matched and relative conformational energy. All these parameters were combined together to obtain the final scoring function called as survival score. The quality of each alignment was measured by (1) the *alignment score*, which is the root-mean-squared deviation (RMSD) in the site-point positions; (2) the *vector score*, which is the average cosine of the angles formed by corresponding pairs of vector features (acceptors, donors, and aromatic rings) in the aligned structures; and (3) a *volume score* based on the overlap of vander Waals models of the non-hydrogen atoms in each pair of structures.

In the CPH, the most active ligand was considered as the reference ligand with highest activity and fitness score 3. The inactive/non-modelled molecules in the dataset were aligned, based on the matching of at least three of the pharmacophore features out of the maximum five features. A common pharmacophore model ADHPR.1 for CXCR4 antagonists was generated after the creation and identification of pharmacophoric sites in all the molecules of the dataset.

3D QSAR studies

The 3D QSAR modelling for CXCR4 antagonists was carried out using the PHASE module of Schrodinger package [22, 23]. PHASE QSAR models were based on partial least squares (PLS) regression. These dataset of ligands were separated into proper training and test sets for generating hypotheses. Therefore, in a random manner,

60% was considered as training set and 40% as test set i.e., about 43 training set molecules and 28 test set molecules for QSAR model development. The training set molecules were used for developing QSAR models and the test set molecules were used for externally validating the obtained QSAR models. The atom-based QSAR models were generated using the atom classes: (1) D: hydrogen-bond donor; (2) H: hydrophobic or non-polar; (3) W; electron-withdrawing (hydrogen bond acceptors) (4) P: Positively ionizable; (5) R: Aromatic rings. The Atom-based QSAR models were built by setting default parameters and maximum PLS factors to 3. The QSAR models were visualized as a combined effect of the atom classes used for building QSAR models; to find out the favourable and unfavourable regions of the structure contributing to increase or decrease in its activity respectively. Here, the hypothesis ADHPR. 1 for CXCR4 antagonists was used for QSAR model generation.

Results and discussion

Molecular docking

Before the availability of crystal structures of CXCR4 and CCR5 (co-receptors), homology models of the sequences from Uniprot were built using rhodopsin templates for ligand based drug design and structure based drug design [18, 25]. The 2.5 Å-resolution crystal structure of human CXCR4 bound to the small molecule antagonist IT1t (PDB ID: 3ODU) was reported recently. The IT1t ligand occupied part of the pocket defined by side chains from helices I, II, III and VII, but made no contact with helices IV, V and VI, in contrast to ligands in previous GPCR structures. This reported structure revealed a ligand-binding site that was distinct from the proposed major recognition sites for chemokines and gp120, providing insights into the mechanism of the allosteric inhibition of chemokine signaling and HIV entry. The active site residues of CXCR4 receptor were found to be ARG 183, ILE 185, CYS 186, TRP 102, ASP 97, ASP 187, TRP 94, VAL 112, TYR 116 and GLU 288 [27, 28]. In our results, molecular interaction of the CXCR4 antagonists was found to interact with residues present in the active site. The antagonists were also found to interact with a residue LYS 110. The results of molecular interaction of CXCR4 antagonists with its receptor are given in Table 1. This high-resolution crystal structure of CXCR4 has been used for discovering drugs for the treatment of HIV-1 infection [3].

In an earlier study involving integrated computational tools for identification of CCR5 antagonists as HIV-1 entry inhibitors, a receptor grid box was defined around the active site residues, namely PHE 85, TRP 89, LEU 104,

TYR 108, ILE 198, TYR 251 and GLU 283 based upon the crystal structure of CXCR4(PDB ID: 3ODU) and majority of the compounds occupied the spaces between the residues TYR 89, TRP 94, GLU 283, LEU 104, THR 195, TYR 251, PHE 109, ILE 198 and TRP 248 [12, 27].

The microbicide compounds selected for this study were found to show inhibitory activity against CXCR4. The microbicides such as AMD 3100 showed glide score of -4.214 and interacted with residues GLU 32, HIS 281 and CYS 186; AMD 070 showed glide score of -5.881 and interacted with residues GLU 288, ASP 97 and CYS 186 with the formation of hydrogen bonds (Table 2). These compounds were found to interact with the binding pockets of CXCR4 receptor. Out of the docking of the 114 CXCR4 antagonists into the receptor active site, most of the molecules had glide score below -5.0 . Glide scores were determined to be in the range -4.0 to -3.0 for the antagonists given in Table 1. The docked conformation having lowest docking energy was selected as the most probable binding conformation [4]. The CXCR4 antagonist 49 was found to be the top scoring docked pose where CXCR4 antagonist 49 is a imidazopyridine-tetrahydro-8-quinolinamine derivatives, 51 and 50 are indole based derivatives; compound 18 is obtained by amine substitution at *N*-(1*H*-benzimidazol-2-ylmethyl)-5,6,7,8-tetrahydro-8-quinolinamines and the compound 94 is an isoquinoline CXCR4 antagonist. In our docking results, the best interaction of antagonist 49 with CXCR4 receptor occurred mainly with residues LYS110, PHE189, PRO191 and ASN192.

Regarding the other docked antagonists, their binding mode was not analyzed in detail as their glide scores were below -3.0 . The docking results also disclosed that each of the compound formed one or two hydrogen bonds interactions. However, the compound responsible for CPH, CXCR4 antagonist 44 showed glide score of -3.21 and interacted with residue ASP 97 by the formation of a hydrogen bond. The glide scores for the CXCR4 antagonists were compared to microbicide compounds selected for this study and they were found to be good and satisfactory. Thus, the CXCR4 antagonists can be proposed as compounds to be developed as microbicides.

According to another study, allosteric modulators of cellular receptor CXCR4-pepducins (ATI-2341) acting as CXCR4 agonists and small molecules (AMD11070, GSK812397) acting as CXCR4 antagonists were used for analyzing the binding interactions between the CXCR4 receptor and the allosteric modulators. Thus the above cited literatures provided knowledge about the key residues of CXCR4 receptor which showed interaction with agonists and antagonists. The findings of the above mentioned studies have important implications in the development and evaluation of CXCR4-selective small molecule antagonists for therapeutic use [15, 16].

Table 1 Dock results of top five CXCR4 antagonists

Antagonist name	Glide score	Number of hydrogen bonds	Interacting residues	H bond distance (Å)
CXCR4_Antagonist 49	−4.10	4	LYS110, PHE189, PRO191, ASN192	2.3, 2.4 & 1.8, 2.3, 2.3
CXCR4_Antagonist 51	−3.54	4	LYS110, ILE169, PHE172, VAL177	1.9, 2.0, 2.3, 2.3
CXCR4_Antagonist 94	−3.41	3	LYS110, ILE173, ALA175	2.4, 2.1, 2.0
CXCR4_Antagonist 50	−3.38	4	LYS110, PHE172, TYR190, PRO191	2.4, 2.0, 2.2, 2.0
CXCR4_Antagonist 18	−3.38	1	LYS110, PHE172	2.5, 2.2, 2.1

Tabular representation of the glide score, hydrogen bond score, interacting residues, number of hydrogen bonds and their distance of top five CXCR4 antagonists obtained out of 114 docked antagonists

Table 2 Dock results of Microbicides acting as CXCR4 antagonists

Ligand	Glide score	H bond score	Number of hydrogen bonds	Interacting residues	H bond distance (Å)
AMD 070	−5.8809	−1.25	3	GLU288, ASP97, CYS186	1.981, 1.903, 1.964
AMD 3100	−4.2138	−1.44	3	GLU32, HIS281, CYS186	2.042, 2.198, 1.759

This table represents the glide score, hydrogen bond score, interacting residues, number of hydrogen bonds and their distance for the selected microbicides acting against CXCR4. The results indicate that microbicides show very good CXCR4 antagonist activity against their receptor

Table 3 Atom based 3D QSAR model statistical parameters of five point pharmacophore hypotheses

Hypothesis	Survival score	R ²	F	Q ²
ADHPR.1	3.446	0.664	81	0.501
ADPRR.25	3.361	0.6042	62.6	0.5249
DHPRR.2	3.344	0.6027	60.7	0.4849

Survival score denotes the survival rate of hypothesis; R², correlation regression coefficient. R-squared is always between 0 and 1

F, variance ratio; Q², cross-validated R² for the predicted activities

Pharmacophore and atom based 3D-QSAR studies

The best five point common pharmacophore hypothesis (CPH) ADHPR.1 was generated for the active data set (pIC₅₀ > 1.932) with the pharmacophore features—1 hydrogen bond acceptor (A2), 1 hydrogen bond donor (D3), 1 hydrophobic (H4), 1 positively ionisable (P6) and 1 aromatic ring (R9) which is represented in Fig. 1. In an earlier study [8, 17], a six point pharmacophore hypothesis AADHHR.47 produced a significant 3D-QSAR model with R² value 0.803. The results of the present study were found to be in good agreement with this study.

The variant ADHPR.1 with the Survival Score of 3.446, correlation regression coefficient R² 0.664 and cross validated regression coefficient Q² 0.501 was selected to be the five point common pharmacophore hypothesis. The best five pharmacophore hypothesis whose survival score is above 3 are represented in Table 3. The most active compound of the CPH i.e., CXCR4 antagonist 44, a imidazopyridine-tetrahydro-8-quinolinamine derivative is

shown in Fig. 1a along with pharmacophore features and all active molecules/modeled molecules aligned to it are shown in Fig. 1b respectively. The overlaid molecules on the most active molecule of CPH included CXCR4 antagonists 45, 46, 47, 48 and 49. The training set molecules overlaid on the pharmacophore model ADHPR.1 very well producing a very good alignment was used for building QSAR model. The distances and angles between various predicted pharmacophore features for hypothesis ADHPR.1 are also represented in Fig. 2a, b, respectively. Their site score was found to be 0.8, vector score as 0.985 and volume score as 0.665.

The best CPH generated for the selected CXCR4 antagonists was subjected to QSAR model development and its validation using PHASE statistical analysis. The inactive/nonmodeled molecules in the dataset were aligned, based on the matching of at least three of the pharmacophore features. The hypotheses were sorted by survival score. The reference ligand, CXCR4 antagonist no. 44 which had a fitness score 3.0 was considered as the maximum possible score with the selected scoring options. The greater the fitness score, the greater the activity prediction of the compound. The fitness value determines feature mapping as well as it contains a distance term for measuring the distance that separates the feature on the molecule from the centroid of the hypothesis feature [8, 17]. The dataset was randomly divided into 60% of the molecules in the training set and 40% of the molecules in the test set. The variant ADHPR.1 with highest survival score 3.446 was chosen to be the common pharmacophore hypothesis for CXCR4 antagonists.

Fig. 1 The best five point common pharmacophore hypothesis ADHPR.1. **a** The most active data set compound, CXCR4 antagonist 44 of the CPH ADHPR.1 which contains the pharmacophore features—hydrogen bond acceptor (A2), hydrogen bond donor (D3),

hydrophobic (H4), positively ionisable (P6) and aromatic ring (R9), **b** overlay of all active molecules on the most active ligand conformation of the five point CPH ADHPR.1

Fig. 2 Distance and angles of the best common pharmacophore hypothesis ADHPR.1. **a** Distance calculations between the sites of 5 point pharmacophore hypothesis ADHPR.1 which determines the

alignment score which in turn determines survival score, **b** angle calculations between the sites of 5 point pharmacophore hypothesis ADHPR.1 for vector score leading to survival score

In a earlier study, a QSAR model was built by maximum number of PLS factor 3 and by using tree-based partitioning algorithm, 72 three-featured and 24 four featured probable CPHs were generated from the list of variants [20, 24]. As per this previous study, we also built a QSAR model for 3 PLS factors based on tree-based partitioning algorithm and five featured CPHs were generated from the list of variants. In a previous study [21, 23], survival score was kept as a criterion to ascertain the quality of alignment. Apart from the survival score analysis, the QSAR model was validated by predicted activities of training set molecules. The models having a high correlation value R^2 between observed and predicted activity for training set molecules were considered and it was found to be 0.664. It was externally validated by cross validated correlation

value between experimental and predicted activity for test set molecules. The correlation graph denoting regression coefficient value for training set molecules is represented in Fig. 3a. The PHASE statistical analysis using cross validated correlation coefficient for CXCR4 test set molecules is shown in Fig. 3b. The validity of each of the models was predicted from the calculated correlation coefficient for the randomly chosen test set comprising diverse structures. The squared correlation value Q^2 determines the effective predictability of the final QSAR model for the test set and the Q^2 value was found to be 0.501. For a reliable model, the squared predictive correlation coefficient should exceed 0.60 [26, 31].

Based upon the atom-based PHASE 3D-QSAR, the volume occlusion maps represented by color codes were

Fig. 3 QSAR scatter plot of experimental versus predicted activities. **a** Scatter plot diagram representing the correlation coefficient ($R^2 = 0.664$) between the observed and predicted activities of the training set molecules of the phase hypothesis ADHPR.1. The higher R^2 value indicates good correlation, thereby prediction of good 3D

QSAR models. **b** Validation of predicted 3D QSAR models by cross correlation coefficient ($Q^2 = 0.501$) between the observed and predicted activities of the test set molecules of the phase hypothesis ADHPR.1

generated. The colour maps for CXCR4 receptor are represented in Fig. 4. These maps denote the regions of favorable and unfavorable interactions. Based upon the sign of the coefficient values, colour coding was represented. The default colour code was blue for positive coefficients and red for negative coefficients. Positive coefficients indicated an increase in its activity whereas negative coefficients indicated a decrease in its activity. Here, blue cubes were present in all the pharmacophore features of the CPH denoting that the substitution of groups can be made for favorable interactions.

A study provided the structural details about the interactions between the inhibitors (AMD11070 derivatives, AMD3100 and AMD3465) and CXCR4 (PDB ID: 1ltt), which is useful for rational drug design of CXCR4. The results illustrated that the binding was affected by two crucial residues Asp97 and Glu288 and the butyl amine moiety of AMD11070 contributed to its high antiretroviral activity [3, 29]. The above mentioned study was very much useful for interpreting the results obtained from the current study. In our results, it was found that CXCR4 antagonist 44 showed interaction with the active site residue ASP 97 with the formation of a hydrogen bond in accordance with the obtained 3D QSAR model which is represented in Fig. 5. The model showed a hydrogen bond donor present at the N15 position of ring attached to R9 pharmacophore feature, aromatic ring interacting with the amino acid residue present in CXCR4. The presence of blue cubes

Fig. 4 The QSAR model showing the favorable regions of CPH for the best hypothesis ADHPR.1. Representation of 3D QSAR model of the most active compound, CXCR4 antagonist 44 and its aligned compounds in terms of cubes; the blue cubes appearing on the pharmacophore features indicate favorable regions for substitution of similar groups having more property that could lead to increase in their antagonist activity

indicate a positive correlation coefficient indicating the substitution of groups enhancing donor activity that could lead to the increase in activity of the molecule.

Since the discovery of CXCR4 as a co-receptor for HIV-1, many structurally diverse small molecule CXCR4

Fig. 5 Representation of the molecular interaction between the CXCR4 receptor and the most active ligand, CXCR4 antagonist 44 of the CPH ADHPR.1. *Purple color* represents ligand, *grey color* represents active site residues and the *red color dotted line* indicates hydrogen bond interaction between CXCR4 antagonist and its receptor (color figure online)

antagonists have been discovered. The present study deals with computational approaches like pharmacophore and 3D-QSAR modelling as well as docking studies on CXCR4 antagonists acting as HIV-1 entry inhibitors. 3D-QSAR based pharmacophore modelling was utilized to study the dataset of 114 CXCR4 antagonists to derive the structural or chemical characteristics necessary for enhancing pharmacological activity. Thus, the five point pharmacophore model obtained based upon the survival score was used for 3D-QSAR model development. The pharmacophore hypothesis ADHPR.1 consisting of 1 acceptor, 1 donor, 1 hydrophobic feature, one positive feature and an aromatic ring was used for QSAR model. The R^2 and Q^2 values obtained values thus obtained indicated that the generated model has good correlation and good predictability. The molecular interaction of the most active compound of the CPH ADHPR.1, i.e. CXCR4 antagonist 44 was analyzed. Moreover, the potential binding mode of the top scoring compounds from a dataset of 114 CXCR4 antagonists with its receptor was also studied. It revealed the active site residues involved in the molecular interaction between the target protein and its allosteric modulators.

The study was successful in screening a CXCR4 antagonist out of a dataset of 114 molecules belonging to chemically diverse groups. The screened compound has the potential to be developed as a microbicide for HIV following entry inhibition mechanism. Thus the results obtained by structure based drug design and ligand based drug design would be helpful for designing new leads with improved activity. The study reported here will be useful for designing more effective HIV-1entry inhibitors.

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