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ORIGINAL PAPER

Exploring the binding of HIV-1 integrase inhibitors by comparative residue interaction analysis (CoRIA)

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Abstract Since the recognition of HIV-1 integrase as a novel and rational target for HIV therapeutics, remarkable progress has been made in the development of integrase inhibitors. Computational techniques have played a critical role in accelerating research in this area. However, most previous computational studies were based solely on ligand information. In the present work, we describe the application of one of our recently developed receptor-based 3D-quantitative structure activity relationships (QSAR) methods, i.e. comparative residue interaction analysis (CoRIA), in exploring the events involved in ligand-integrase binding. In this methodology, the non-bonded interaction energies (van der Waals and Coulombic) of the inhibitors with individual active site residues of the integrase enzyme are calculated and, along with other thermodynamic descriptors, are correlated with biological activity using chemometric methods. Different combinations of descriptors were used to develop three types of QSAR models, all of which were found to be statistically significant by internal and external validation. This is the first report of such a dedicated receptor-based 3D-QSAR approach being applied to comprehend the integrase–inhibitor recognition process. In addition, the study was performed on 13-different series of inhibitors, thereby exploring the most structurally diverse data set ever used in understanding the inhibition of HIV-1 integrase. The major advantage of this technique is that it can quantitatively extract crucial residues and identify the nature of interactions between the ligand and receptor that modulate activity. The models suggest that Asp64, Thr66, Val77, Asp116, Glu152 and Lys159 are the key residues influencing the binding of ligands with the integrase enzyme, and the majority of these results are in line with earlier studies. The approach facilitates easy lead-to-hit conversion and design of novel inhibitors by optimisation of the interaction of ligands with these specific residues of the integrase enzyme.

Keywords CoRIA · Docking · G/PLS · HIV Integrase · QSAR

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Introduction

Human immunodeficiency virus type 1 (HIV-1) is the primary cause of acquired immuno-deficiency syndrome (AIDS)—a slow, progressive and degenerative disease of the human immune system that has been one of the world's most serious health problems since 1981 [1–3]. The estimated number of persons living with HIV worldwide in 2007 was 33.2 million and about 2.1 million people died due to AIDS in the same year [4]. Despite the gravity of the situation, only a few anti-HIV drugs have been approved by the FDA and are currently available for clinical use. The therapy currently employed for HIV infection is a combination of inhibitors of the reverse transcriptase and protease enzymes, known as triple therapy or highly active antiretroviral therapy (HAART). Although this therapy has drastically decreased viral spread and led to significant improvements in the quality of life of AIDS patients [5], problems like tolerability, tapered antiviral spectrum, long-

term toxicity, high cost, lack of efficacy against latent virus, complexity of the drug regimen, development of resistance, high mutation rate and poor patient compliance are some of the reasons for the unabated search for new drugs in this area with novel structures or mode of action [6–8].

Three enzymes that play significant roles in the replication of HIV are reverse transcriptase, protease and integrase. HIV reverse transcriptase catalyses the conversion of the single-stranded viral RNA genome into the double-stranded proviral DNA, which is subsequently integrated into cellular DNA. HIV protease cleaves a key polypeptide that is essential for the successful assembly of infectious daughter virions. HIV integrase catalyses the integration of viral DNA into the host DNA in two steps: 3′-processing and strand transfer. Amongst the various targets that have been identified for the development of anti-HIV agents, these three enzymes are considered the most promising [9]. Reverse transcriptase and protease have been the primary focus of research against HIV/AIDS over the last two decades. HIV-1 integrase has now been recognised as another crucial and rational target for inhibiting viral replication, mainly because, in addition to being obligatory in the HIV lifecycle, it has no known direct cellular counterparts in the host cell, thereby allowing design of specific and non-toxic inhibitors [9–12]. Remarkable progress has been made since integrase was recognised as a rational therapeutic target for the treatment of HIV infection. Recombinant integrase can be readily produced and used for high-throughput and molecular pharmacology assays. Several atomic structures of the integrase domains are also now available for docking studies [13]. Recently, an integrase inhibitor from Merck, Raltegravir (MK-0518), was approved by the United States FDA for use in combination antiretroviral therapy for the treatment of HIV-1 [14, 15].

Various efforts have been made by researchers to develop potential integrase inhibitors and to throw more light on the ligand–receptor recognition process. Most of these endeavours include computational studies such as pharmacophore modelling and 3D-database searching [16–27], classical and multi-dimensional quantitative structure activity relationships (QSARs) [28–50], docking and de novo drug design [37, 51–63], and molecular dynamics (MD) simulations [64–72]. Recently, comprehensive reviews summarising the use of such computational techniques in the development of HIV-1 integrase inhibitors have been published [73, 74]. Except for a few studies [26, 41, 54, 58] that have hypothesised some residues of the receptor to be vital for ligand binding, none have commented on the important residue types and the nature of interactions involved in ligand–receptor binding. In this paper, we report an application of one of the newer 3D-QSAR methodologies developed in our laboratory—comparative residue interaction analysis (CoRIA) [75–77]—to a data set of 81 molecules belonging to a comprehensive set of 13 structurally different classes of HIV-1 integrase inhibitors, in order to glean critical information regarding the interactions of the inhibitors with residues in the active site of the receptor. This methodology, which is based on the thermodynamics of the ligand–receptor binding process, explicitly takes into consideration the wealth of information contained in the available ligand–receptor complexes to uncover both qualitative as well as quantitative facets of binding. CoRIA involves calculation of the interaction energies (usually non-bonded, i.e. van der Waals and Coulombic) of every ligand with each individual residue in the active site of the receptor, which, along with other thermodynamic descriptors, are used as independent variables that can be correlated to the biological activity/affinity by chemometric methods. The approach is capable of extracting crucial residues of the receptor that are involved in a specific type of interaction (van der Waals and Coulombic) with the ligand. Such information can profitably be utilised by medicinal chemists in designing new compounds or in optimising existing leads. Although some related methodologies like COMBINE (comparative binding energy) [78] and AFMoC (adaptation of fields for molecular comparison; a reverse variant of CoMFA) [79] do exist, this is the first time that this kind of approach has been applied to study the inhibition of HIV-1 integrase enzyme. Also, the data set employed in the study is the most diverse (covering 13 different series of HIV-1 integrase inhibitors) ever used in this type of application.

Methods

Biological data

The biological data used in this study comprises 81 molecules belonging to 13 structurally different classes of HIV-1 integrase inhibitors that were selected from the literature so as to maintain the spread of biological activity and structural diversity within and between the series. These molecules are derivatives of the following classes: arylamide and naphthalene [80], geminal disulfones [81], coumarins [82], salicylhydrazines [83] or hydrazides [84], indole β-diketo acids [61], thiazolothiazepines [85], quinolinone-3-carboxamides [86], curcumins [87], mercaptobenzenesulfonamides [41], sulfonamides [16], tyrphostins [88], diarylsulfones [89], depsides and depsidones [18]. The inhibitory activities of all these molecules were measured on a wild-type integrase enzyme by the same protocol and are reported as IC_{50} values. The IC_{50} values were converted to negative logarithmic values (i.e. pIC_{50}), which range from 3.18 to 6.43 units. Table 1 lists the molecules used in

Table 1 Molecules used in this study, and their experimental pIC_{50} values

Molecule	pIC_{50}	Set	Molecule	pIC_{50}	Set
Arylamides and naphthalenes			Curcumins		
01	4.48	Training	45	3.92	Training
02	3.76	Test	46	3.82	Training
03	4.27	Training	47	5.22	Test
04	6.01	Training	48	4.74	Training
Geminal disulfones			49	5.05	Training
05	4.15	Training	Mercaptobenzenesulfonamides		
06	5.39	Test	50	4.78	Training
07	4.30	Training	51	5.44	Training
08	4.52	Training	52	5.09	Training
09	4.10	Training	53	4.29	Training
Coumarins			54	4.10	Training
10	5.82	Test	55	4.22	Training
11	4.09	Test	56	4.12	Test
12	4.26	Training	Sulfonamides		
13	4.24	Training	57	4.62	Training
14	4.46	Test	58	5.08	Training
15	5.02	Training	59	4.12	Test
16	4.76	Training	60	4.31	Training
17	6.43	Training	61	3.89	Training
Salicylhydrazines and hydrazides			62	3.71	Training
18	3.54	Training	63	3.61	Test
19	4.14	Training	Tyrphostins		
20	6.22	Training	64	5.52	Training
21	5.85	Test	65	5.48	Training
22	5.17	Training	66	6.35	Test
23	5.63	Training	67	6.00	Training
24	4.13	Training	68	5.33	Training
Indole-β-diketo acids			Diarylsulfones		
25	4.30	Training	69	3.53	Test
26	4.96	Test	70	3.81	Training
27	4.79	Test	71	4.79	Training
28	4.60	Test	72	3.93	Training
29	4.00	Training	73	4.49	Training
30	4.34	Training	74	4.72	Test
31	4.92	Training	75	4.23	Training
Thiazolothiazepines			Depsides and depsidones		
32	3.18	Training	76	5.33	Training
33	3.67	Training	77	5.26	Training
34	3.62	Training	78	5.31	Training
35	3.69	Training	79	4.28	Training
36	4.55	Test	80	4.21	Test
37	4.07	Training	81	5.43	Test
Quinolinone-3-carboxamides					
38	5.39	Training			
39	4.30	Training			
40	4.08	Training			
41	4.82	Training			
42	4.14	Training			
43	4.09	Test			
44	4.00	Training			

this study along with their experimental pIC_{50} values. The molecules were divided into a training set consisting of 61 molecules and a test set of 20 molecules (as indicated in Table 1) based on the Tanimoto coefficient using the 'select diverse' utility in Cerius2 (v 4.6) [90]. The structures of the molecules used in this study can be found in the electronic supplementary material (ESM).

Molecular modelling

Molecules were built with the builder module in Sybyl v 7.1 [91] running on a Pentium IV computer under the Linux RedHat Enterprise 2.1 OS. The ligand geometries were optimised by energy minimisation using the Powel gradient method with the MMFF94 charges [92], until a gradient of 0.01 kcal/mol/Å was reached. To date, only one crystal structure of HIV-1 integrase complexed with the molecule 5-CITEP [93] (PDB code 1QS4) is available in the Protein Data Bank [94], and this was used for the modelling studies. The errors in the crystal structure were rectified and hydrogens were added equivalent to pH 8.0 using the Biopolymer module in Sybyl 7.1 [95], resulting in a +1 charge for arginines and lysines, and a −1 charge for aspartates and glutamates. Since histidines are non-ionised at this pH, they were used per se in this study. The ligand–receptor complex was energy minimised using steepest descents and conjugate gradients to a maximum derivative of 0.01 kcal/mol/Å. During minimisation, the ligand atoms were allowed free movement but the enzyme backbone atoms were tethered with a force constant of 100 kcal/mol/$Å^2$. The same protocol was followed for all the ligands.

For ligand docking, residues within a 10 Å radius from 5-CITEP were defined as the active site. The docking studies were carried out with the program GOLD (v 3.1) [96], which uses a genetic algorithm (GA) procedure to identify the best binding configuration. The program was run for 20 GA cycles, which was found optimal to reproduce the X-ray-derived position of 5-CITEP in the integrase enzyme, with an acceptable root mean squares derivative (rmsd) value of less than 1.0. Most of the other GA parameters, like population size, as well as the genetic operators were kept at their default values. The putative binding (bioactive) conformations of the inhibitors in the integrase active site were determined on the basis of Gold score and visual analysis. The enzyme–inhibitor complexes thus obtained were used for the computation of the non-bonded interaction energies. The other descriptors (vide infra) were calculated for ligands extracted from this bound conformation.

Computation of descriptors for CoRIA approach

Ligand–receptor binding is governed by thermodynamic events like interaction, solvation and entropy changes, all of

which are accounted for in the CoRIA approaches described below.

Interaction energies

Since most biological processes are determined by specific non-covalent (non-bonded) interactions between ligands and receptors, these are accounted for in CoRIA methodology. This completely enthalpic contribution is equal to the difference between the total energy of the complex and the energy of the free protein and free ligand. The key components of the non-bonded interaction energy are van der Waals (E_{vdw}) and electrostatic (E_{ele}) interactions between the ligand and the receptor, which are functionally computed as follows:

$$E_{vdw} = \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}$$

$$E_{ele} = \frac{q_i q_j}{\varepsilon r_{ij}}$$

where A_{ij} and B_{ij} are the repulsive and attractive terms between atoms i and j, respectively; r_{ij} is the interatomic distance between atoms i and j; q_i and q_j are the atomic charges of interacting atoms i and j; respectively; and ε is the dielectric constant. The van der Waals and Coulombic interaction energies were computed using the CFF91 force field [97] in the Discover module of the program InsightII [98].

A total of 72 interacting residues within a radius of 10 Å from the ligand are accounted for in the CoRIA approach. Thus, for each molecule, there are 72 entries each (i.e. columns) in the QSAR table, corresponding to the van der Waals, Coulombic and total non-bonded (TNB: van der Waals plus Coulombic) interactions.

Solvation free energy

The solvation free energy (SFE) of the ligand under physiological conditions is the hydration free energy, and is equal to the difference between the free (e.g. cellular) and the bound state. The SFE refers to the amount of energy needed to strip the solvent molecules off the ligand when shifting from aqueous surroundings to a hydrophobic receptor cavity. The electrostatic contribution to the SFE of the ligands was computed using the method developed and validated by Still et al. [99] and implemented in the Prepare module of the program QUASAR [100].

Strain energy

Another important term in binding that is taken into account in the CoRIA approach is the conformational change in the ligand that occurs during the binding process. During ligand–receptor binding, the conformational change in the ligand, which is much more pronounced compared than that of the receptor, can be described by the strain energy (SE) upon binding. This can be calculated with a molecular mechanics potential function as the energy related to changes in bond lengths, angles, torsions and non-covalent interactions. The ligands were extracted from their complexes and subsequently minimised using several steps of steepest descents, conjugate gradients and Newton Raphson (BFGS) methods, until a maximum energy derivative of 0.001 kcal/mol/Å was reached. SE was finally computed as the difference in the energy of the ligands in their docked conformations and the conformations minimised in vacuo.

Entropy loss

Entropy loss (EL) corresponds to the loss of torsional, vibrational, rotational and translational free energies of the ligand upon binding with the receptor. This loss of entropy, which results from abridged conformational flexibility upon receptor binding, is estimated based on the method of Searle and Williams [101] by assigning an amount of 0.7 kcal/mol to every freely rotatable (i.e. single) bond, excluding the terminal $–CH_3$ groups, and was calculated with the Prepare module in the program QUASAR [100].

Solvent accessible surface area

Solvent accessible surface area (SASA) corresponds to the residual surface of the ligand that is still accessible to the solvent after it has bound to the receptor. SASA is usually associated with the tightness and roughly with the depth, strength and number of binding interactions with the receptor active site. SASA was also estimated with the Prepare module in the program QUASAR [100].

Statistical analysis

All QSAR models were generated with the G/PLS chemometric method as implemented in the Cerius2 program (v 4.6) [90], which brings together the paramount features of the genetic function approximation (GFA) [102] and the partial least squares (PLS) [103] approaches. Pretreatment of the data based on a correlation matrix was avoided in view of the fact that interaction energies are not absolutely orthogonal, i.e. they tend to be partially correlated to each other numerically but in terms

Table 2 Statistical analysis of the comparative residue interaction analysis (CoRIA) models

Model	r^2	r^2 (BS)	r^2 (random)	q^2 by LOO	q^2 by LGO	r^2_{pred}
1	0.76	0.71	0.07	0.37	0.35	0.63
2	0.79	0.79	0.11	0.30	0.32	0.64
3	0.80	0.80	0.11	0.35	0.34	0.65

The number of molecules in the training and test sets are 61 and 20 respectively. r^2 correlation coefficient, r^2 *(BS)* mean values of r^2 from bootstrap analysis; r^2 *(random)* mean value of r^2 after randomisation at 99% confidence interval, q^2 *by LOO* cross-validation correlation coefficient by leave-one-out, q^2 *by LGO* cross-validation correlation coefficient by leave-group-out (group of 5), r^2_{pred} predictive correlation coefficient of test set

of interpretation they may convey different information. All descriptors in the dataset were scaled to zero mean and unit standard deviation, by subtracting each value in a given column from the column mean and then dividing it by the standard deviation of that column. The sole purpose of this scaling was to allocate equal weight to all the descriptors and place them on the same platform for meaningful statistical analysis. Only linear terms were used to develop the QSAR models, and the optimal number of components selected was six, at which the crossvalidated r^2 (i.e. q^2) was found to be the maximum. In order to facilitate simple interpretation and easy use of the models in designing new ligands, the length of the equations was confined to seven terms (including the constant) for which the r^2 and PRESS (predictive residual sum of squares) values were found to be optimum. The number of generations was set to 10,000 and population size to 500. Crossover and mutation probabilities of 50% (default settings) were employed, with a smoothness parameter of 1.0 (the smoothness function penalises the equations on their size and thus controls for bias in the scoring factor between equations with different numbers of terms). The models developed with a training set of 61 molecules were internally validated using randomisation at 99% confidence interval, leave-one-out (LOO), leave-group-out (LGO, group of 5) and by boot-strapping techniques [104]. Externally, the models were assessed for their predictive power on a test set of 20 molecules.

Results and discussion

Three different QSAR models were developed using various combinations of the descriptors. In Model 1, Coulombic (C) and van der Waals (V) interaction energies between the ligands and residues in the receptor active site were considered for the construction of the CoRIA equations (i.e. C+V). Model 2 included different events leading to ligand–receptor binding, i.e. SE, SFE, EL, and SASA, in addition to the Coulombic and van der Waals terms (i.e. C+V+SE+SFE+EL+SASA). Model 3 incorporated additionally TNB interaction energies (TNB = C+V), besides the terms engaged in Model 2 (i.e. C+V+TNB+SE+SFE+EL+SASA).

The statistical analysis and the best QSAR equations of the models developed are reported in Tables 2 and 3, respectively. The models generated by the CoRIA approach are statistically significant, with correlation coefficients (r^2) varying from 0.76 to 0.80. After randomisation of the activity data, the r^2 values decrease to smaller numbers, indicating that the correlations developed are not a result of chance. Cross-validation by LOO and leave-five-out techniques resulted in statistically acceptable q^2 values. The bootstrapping results further supported the sturdiness of the models. The predictive r^2 of all the models on the test set of 20 molecules is also more than 0.6, indicating a good extrapolative power of the models for molecules not covered in the training set.

Table 3 Best quantitative structure activity relationships (QSAR) models developed by the CoRIA approach

Model	Best QSAR equation
1	pIC_{50}=4.59–0.20 (V_Asp64) - 0.55 (C_Thr66) + 0.49 (V_Lys159) - 0.22 (V_Asp116) + 0.60 (C_Val77) + 0.15 (V_Glu152)
2	pIC_{50}=4.62 - 0.21 (V_Asp64) - 0.19 (V_Asp116) + 0.45 (V_Lys159) - 0.51 (C_Thr66) - 0.10 (V_Leu68) + 0.48 (C_Val77)
3	pIC_{50}=4.53 + 0.20 (TNB_Val176) - 0.07 (V_Asp116) - 0.33 (C_Thr66) + 0.23 (V_Lys159) - 2.45 (C_Gly149) - 0.04 (V_Asp64)

C Coulombic interactions, *V* van der Waals interactions, *V_Asp64* van der Waals interaction of the ligand with the receptor residue Asp64, *C_Thr66* Coulombic interaction of the ligand with the receptor residue Thr66, *TNB_Val176* total non-bonded (TNB) interaction of the ligand with the receptor residue Val176

The plots of experimental vs predicted pIC_{50} values of the molecules in the training and test sets for all the models are shown in Fig. 1. The ten best equations of each model were examined for the frequency with which a particular term appears in the population of equations. The plots of the most repeatedly occurring descriptors for different models are shown in Fig. 2. The frequency of occurrence of different descriptors is shown on the *x*-axis, whereas the signs of the terms in the equations are shown on the *y*-axis. Terms with positive coefficients in the equations are displayed as positive frequency values, whereas those with negative coefficients appear with negative frequencies.

A comprehensive analysis of the CoRIA models is described below. However, while interpreting the results, one should bear in mind that the more negative the value of the van der Waals and Coulombic interaction energies, the stronger the interaction between ligand and receptor. This means that positive values of these interaction energies entail weaker ligand–receptor interactions and vice versa. However, it is the sign of the coefficient of these descriptors/terms in the QSAR equations that will ultimately decide whether to strengthen/increase (i.e. make the interaction energy more negative) or weaken/decrease (i.e. make the interaction energy relatively more positive) the interaction, in order to improve binding.

CoRIA analysis

In the CoRIA approach, the van der Waals and Coulombic interaction energies of the ligand with individual active site residues of the receptor are calculated, and correlated with biological activity along with other important descriptors.

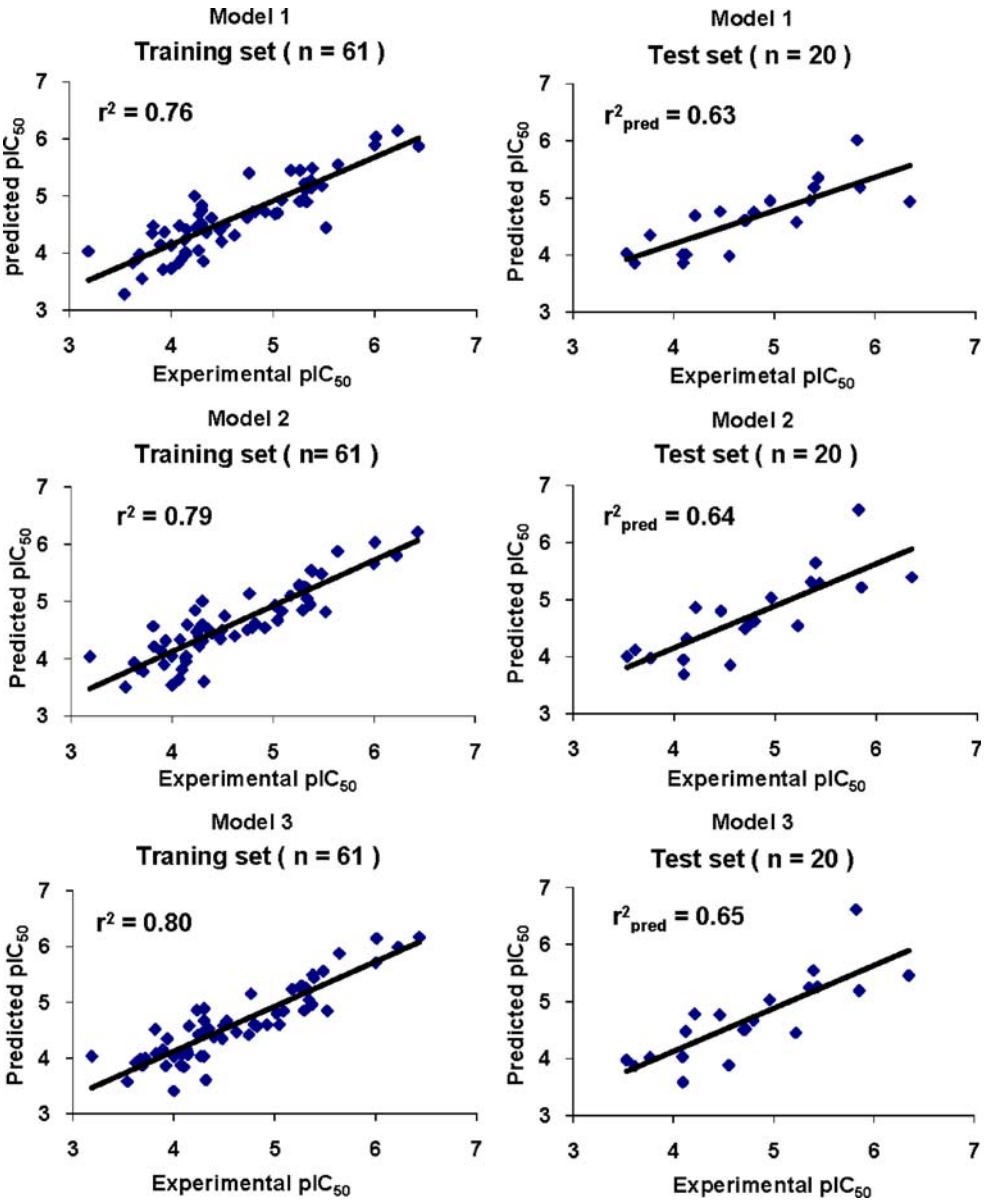


Fig. 1 Plots of experimental vs predicted pIC_{50} values of the integrase inhibitors in the training and test sets for the best comparative residue interaction analysis (CoRIA) models

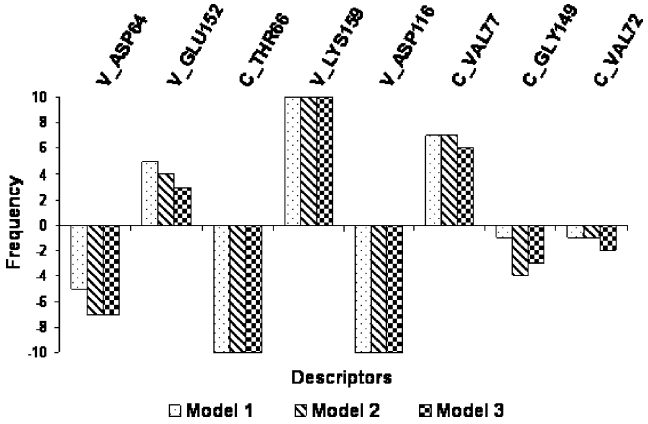


Fig. 2 Frequency plots of descriptors appearing in the equations of the CoRIA models. *C* Coulombic interactions, *V* van der Waals interactions, *V_Asp64* van der Waals interaction of the ligand with the receptor residue Asp64, *C_Thr66* Coulombic interaction of the ligand with the receptor residue Thr66

An assessment of all the CoRIA models (Table 3) reveals that almost the same set of amino acids appear in all the CoRIA models, indicating that these residues are significant in the interactions. The models suggest that Asp64, Thr66, Val77, Asp116, Glu152 and Lys159 are the major residues influencing the binding of ligands with the receptor. The van der Waals interactions of the ligand with receptor residues Asp64 and Asp116 appear with negative coefficients in the equations (Fig. 2). This indicates that the biological activity can be amplified by strengthening the van der Waals interactions of the ligands with these residues of the integrase enzyme. Similarly, enhancing the vigour of the Coulombic interaction of the ligand with Thr66 will increase the binding affinity, due to the negative coefficient of this interaction in the equations (Fig. 2). On the other hand, because of the positive coefficient of the Coulombic interaction of the ligand with residue Val77 (Fig. 2), an overall positive value of the Coulombic interaction energy of the ligand with this particular amino acid will favour binding. Interaction of the ligand with receptor residues Glu152 and Lys159 appears to be very sensitive, thereby recommending cautious modifications to optimise activity.

It is worth mentioning that most of the residues in the integrase enzymes that are designated as imperative for ligand binding by CoRIA models, have also been considered vital by previous studies including X-ray crystallography, computational and mutation studies [54, 58, 64, 93, 105–108]. For example, using docking, Sotriffer et al. [54] have suggested that Lys159 is one of the anchor points for tight binding of inhibitors. Binding of ligands is further complemented by favourable interaction with Thr66. Based on docking and molecular interaction field analysis, da Silva and co-workers [58] have proposed that inhibitors should possess polar groups that can interact via electrostatic interactions with Asp64 and Thr66. Lins et al. [64] used MD to demonstrate the role of Asp64, Asp116 and Glu152 in enzyme stability and ligand binding. Several residues near the integrase active site (e.g. Lys159) have been recognised by site-directed mutagenesis and photocrosslinking studies to be crucial for binding the substrate—viral DNA—and also the inhibitor [93]. In the present study, favourable Coulombic interactions of the ligand with receptor residues Gly149 and Val72 also appear in the QSAR equations (Fig. 2), but their frequency of occurrence is too low to be utilised profitably for improving the activity. Besides the above-mentioned terms, a few other residues, such as Leu68, Val74, and Lys160, have also been exposed by CoRIA to be crucial in regulating activity. Though no earlier study or point mutation data have yet been reported showing the significance of these residues in integrase inhibition, according to CoRIA the interactions of inhibitors with these amino acids are critical for the ligand–receptor recognition process.

The final set of CoRIA models did not contain any of the thermodynamic descriptors like solvation, entropy, strain energy, etc, despite assigning equal weight to all the descriptors and avoiding any bias in their selection during model development. However, they do appear briefly during the evolution process of the genetic algorithm but then unfortunately die down as the function converges. It seems that, for the present dataset, these terms may not be the dominant factors in overall ligand–receptor binding, and the interaction of the molecules with specific active site residues of the integrase enzyme alone are sufficient to explain the disparity in biological activity. Also, the quality and applicability of the CoRIA models can be significantly augmented by improving various aspects like solvation of the entire ligand–receptor complexes and sampling important configurations using simulation methods (e.g. MD or Monte Carlo simulations), calculation of interaction energies using more accurate and reliable techniques (e.g. ab initio or DFT calculations), and incorporating other types of important interactions (e.g. hydrogen-bonding, hydrophobicity, etc) into model development.

Rationalisation of CoRIA approach

It is evident from the CoRIA equations that Coulombic interaction of the ligands with receptor residue Thr66, and van der Waals interaction with residues Asp64 and Asp116 needs to be maximised to improve activity. Figure 3 shows molecule 38 (Table 1) surrounded by important active site residues as revealed by the CoRIA equations. The crucial descriptors that appear in the CoRIA models, along with their values for some selected molecules, are shown in Table 4. The table demonstrates how the values of these

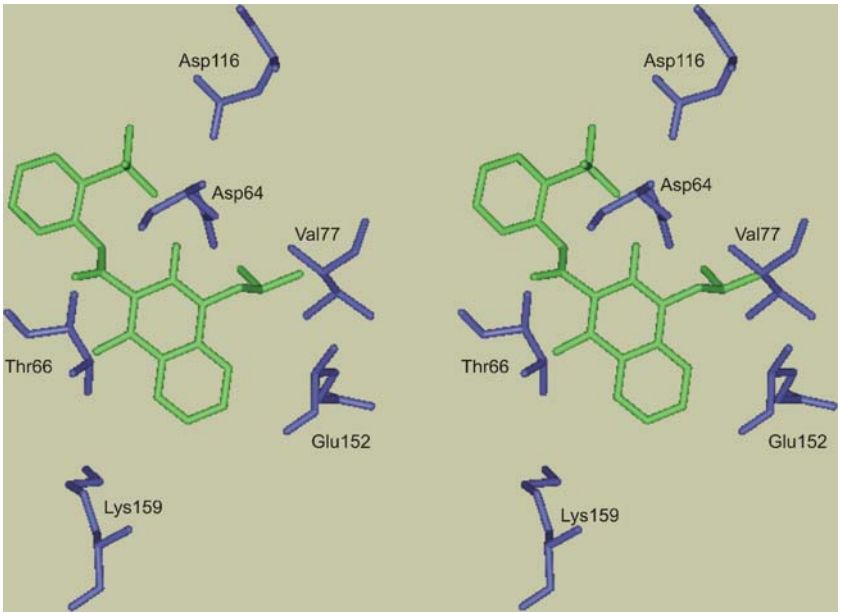


Fig. 3 An InsightII [98]-generated stereoview of the active site of HIV-1 integrase enzyme showing molecule 38 (*green*, heavy atoms only) with the important active site residues (*blue*, heavy atoms only) that are highlighted by the CoRIA equations

descriptors (interaction energies) commensurate with the significant differences in the activities of pairs of molecules belonging to the same chemical class. For example, in the salicylhydrazines/hydrazides series, molecule 20 (Table 1) is more active than molecule 22 because of its increased (comparatively more negative) Coulombic interaction with residue Thr66 as well as its strong (comparatively more negative) van der Waals interactions with receptor residues Asp64 and Asp116. Similarly, modification of $–CH_3$ (molecule 25, Table 1) to $–CH_2CH_3$ (molecule 27, Table 1) in the indole β-diketo acids class causes an increase in activity by half a log unit. This boost in activity is also due to an increase in the strength of its Coulombic interaction with Thr66 and an increase in its van der Waals interactions with Asp64 and Asp116. It is apparent from this table that the interaction of the molecules with some specific residues of the integrase enzyme (as revealed by the CoRIA models), are clear reflections of their biological activities, and thereby firmly justifies the CoRIA equations that emerge from this study.

The predictive ability of the CoRIA models was validated by an attempt to predict the activity of 5-CITEP, whose complex with HIV-1 integrase is known [93] (PDB code 1QS4, this molecule was not considered in the CoRIA analysis). The interaction energies of 5-CITEP with the important active site residues revealed by the CoRIA models were calculated, and its activity was predicted by substituting the interaction energies into the best QSAR equations of the three CoRIA models (Table 3). The activity of the 5-CITEP molecule as predicted by the CoRIA models is shown in Table 5.

The suggestions of the CoRIA models were also used as guidelines to rationally modify the inhibitors so as to improve their activity. For example molecule 31 (Table 1),

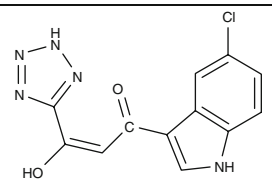
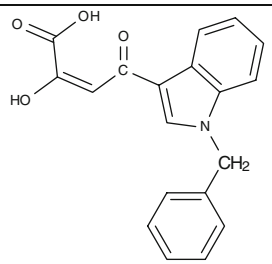
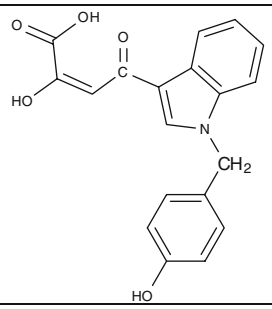
Table 4 Crucial CoRIA descriptors and their values for some selected molecules

Molecule	pIC_{50}	C_Thr66 (-)[a]	V_Asp64 (-)	V_Asp116 (-)	V_Glu152 (+)	V_Lys159 (+)
20	6.22	−1.26	−1.03	−0.18	−0.60	1.23
22	5.17	−0.74	−0.77	0.11	−0.82	1.45
25	4.30	−0.29	−0.24	−0.28	0.78	0.42
27	4.79	−0.33	−0.36	−0.34	0.75	0.42
38	4.39	0.24	−0.18	−0.24	−0.76	0.15
44	4.00	0.63	0.18	−0.09	−0.72	0.17
46	3.82	−0.01	−0.45	−0.07	−0.71	0.15
49	5.05	−0.29	−0.43	−0.06	−0.36	0.15
77	5.26	−1.21	−0.56	−0.12	−0.40	0.03
79	4.28	−0.98	−0.57	0.45	−0.32	0.25

C Coulombic interactions, *V* van der Waals interactions, *V_Asp64* van der Waals interaction of the ligand with the receptor residue Asp64, *C_Thr66* Coulombic interaction of the ligand with the receptor residue Thr66

[a] + and − signs in parenthesis refer to the signs of the coefficients of the respective descriptor in the QSAR equations

Table 5 Activity values of 5-CITEP, molecule 31 and the newly designed molecule

Activity (pIC_{50})	CoRIA Model			Structure
	1	2	3	
5-CITEP				
Predicted Activity	5.28	5.36	5.25	
Experimental Activity	5.65			
Molecule 31				
Predicted Activity	4.85	4.90	4.88	
Experimental Activity	4.92			
Designed Molecule				
Activity predicted from the best QSAR model	5.22	5.18	4.45	
Activity predicted from the regression line	5.40	5.33	4.45	

an indole β-diketo acid derivative, was structurally modified based on the results of CoRIA, by substituting a hydroxyl group at the *para* position of the benzyl group. The new molecule was minimised, docked into the active site and its interaction energies with important residues (recommended by CoRIA models) calculated according to the protocols described earlier. The activity of the designed molecule was then predicted by substituting its interaction energy values into the best QSAR equations of the three models (Table 3). In addition, the probable experimental activity of the designed molecule was obtained from the regression lines (Fig. 1) of the three CoRIA models. Table 5 shows the predicted and (probable) experimental activities of the designed molecule along with those of molecule 31. The higher activity of the newly designed molecule over its parent is attributed to its improved interactions with the key residues of HIV-1 integrase enzyme, as explained by the CoRIA approach. Thus, CoRIA methodology can be applied convincingly to design more potent inhibitors of HIV-1 integrase enzyme.

Conclusions

In recent years, the structure–activity relationships of many HIV-1 integrase inhibitors have been studied but most of these earlier QSAR studies were performed independently of integrase structure. Comparative residue interaction analysis (CoRIA) [75–77] is a receptor-based QSAR formalism that makes use of the 3D structures of small molecules as well as their macromolecular targets to dig out both the type as well as the nature of important interactions between ligands and receptors. In the present work, this approach was applied to an extremely diverse set of integrase inhibitors, in order to explore events that are significant in the ligand–integrase recognition process. The CoRIA methodology has efficiently extracted crucial residues (as well as type of interactions) in the integrase enzyme that have already been claimed by X-ray crystallography and site-directed mutagenesis studies to be essential in ligand–receptor binding. As indicated by our study, the van der Waals interaction of residues Asp64 and

Asp116 in the enzyme, and the Columbic interaction of residue Thr66 with the ligands should be fortified to enhance binding affinity. On the other hand, reducing the strength of Columbic interaction with Val77 will favour the overall binding of integrase inhibitors. Interaction of the inhibitors with receptor residues Glu152 and Lys159 is quite sensitive and will require careful exploitation in order to control activity. In addition to these interactions, CoRIA analysis also highlighted other residues, like Gly149 and Val72, that have not yet been signalled by earlier studies but that may play a hidden role in providing additional stabilisation of a ligand's explicit receptor binding. In a nutshell, careful optimisation of the interaction of ligands with the specific integrase residues revealed by the CoRIA methodology can assist not only in improving the binding affinity of existing molecules but also in designing novel, more potent, HIV-1 integrase inhibitors.

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