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Rational design of novel diketoacid-containing ferrocene inhibitors of HIV-1 integrase

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Abstract

Molecular interaction field, density functional, and docking studies of novel potential ferrocene inhibitors of HIV-1 integrase (IN) are reported. The high docking scores, analysis of the ligand–receptor interactions in the active site as well as the molecular interaction potential calculations at the binding site of the receptor indicate important features for novel HIV-1 IN inhibitors. We also confirm in this work a novel binding trench in HIV-1 integrase, recently reported in a theoretical work by other authors. This observation may be interesting since the lack of detailed structural information about IN–ligand interactions has hampered the design of IN inhibitors. Our proposed ligands are open to experimental synthesis and testing.
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1. Introduction

Enzymes essential for the replication cycle of HIV-1, such as reverse transcriptase (RT), protease (PR), and integrase (IN), are important targets for the development of

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anti-AIDS drugs. Effective inhibitors have been developed and marketed as anti-AIDS. However, viral genome integration is essential for the replication of the retroviruses and IN, unlike PR and RT, has no known functional analog in human cells. The development of drugs which can inhibit one of the various steps involved in integration could be important in HIV-1 therapy. However, although HIV-1 IN is an attractive and unexploited target for HIV therapy, the design of inhibitors has been hampered by the lack of more detailed structural information regarding the IN–ligand interaction [1–6]. The crystal structure of IN complexed with an inhibitor has been recently reported [5]. However, the information provided by the crystal structure is still unclear and not precisely defined. Recently, Schames et al. [6] reported an excellent theoretical work involving docking of the 5CITEP to HIV-1 IN. We have also used in this work computational simulation techniques to propose novel potential HIV-1 IN inhibitors.

The HIV-1 IN enzyme, which belongs to the superfamily of polynucleotidyltransferases, inserts via separate reactions a double-stranded DNA copy of the viral RNA genome in the chromosomes of an infected cell through two separate reactions. In the first step of the 3′-end processing, two nucleotides are cleaved from each 3′ end of the viral DNA to form the DNA substrate for integration. For DNA strand transfer, in the next step, the 3′ hydroxyls at the ends of the viral DNA attack in the target DNA a pair of phosphodiester bonds. On the two target DNA strands, the sites of attack are separated, in the case of HIV-1 integrase, by five nucleotides. The integration process is completed when the two unpaired nucleotides of the viral DNA are removed. The single strand gaps between viral and target DNA are filled [6,7].

Several integrase inhibitors (hydrazides, catechols, DNA binders, and nucleotide-based inhibitors) have been reported, most of which function in extracellular oligonucleotide assays but often lack inhibitory potency or fail to show antiviral effects against HIV-infected cells. L-731988 and aryl diketoacids (ADKs) such as 5CITEP represent a class of compounds that are both selective HIV-1 integrase inhibitors and antiviral agents. Members of the ADK family, in particular, inhibit, by blocking viral replication, the second step of the integrase reaction, which is the strand transfer step. During HIV-1 infection, selective inhibition of strand transfer allows the viral DNA to become accessible to metabolism by cellular recombination and repair enzymes leading to the irreversible blocking of viral replication due to unstable integration. ADKs, which are comprised of three structural components, have good structure–activity relationships and have been investigated, including replacement of the left aryl portion of various nitrogen, sulfur- and oxygen-containing heterocycles. The right acid functionality has been replaced with a variety of groups including triazoles. In this work, we design and introduce novel aryl diketoacid compounds based on the ferrocene framework, which has conformational flexibility in the binding trench regions of the HIV-1 IN active site.

The actual binding site of IN is not understood; only key residues are known. Ten years of research on HIV-1 integrase has resulted in establishing ADK derivatives as bona fide inhibitors of integrase. As examples, we have S-1360, a diketo analog, which has progressed into phase II clinical trials and L-870810, a modified 1,3-propa-

dione which is in phase I clinical trials. Recent work has attempted to rationally target divalent metal ions on the active site of IN which may have potential implications for the design of a second generation of diketoacid-containing class of inhibitors.

We have designed novel potential ferrocene HIV-1 IN inhibitors and made molecular interaction field analysis, docking studies, density functional calculations, analysis of hydrogen bonding, electrostatic and hydrophobic interactions, conformations, trenches, comparison with 5CITEP, and binding to metal ions at the active sites. These studies indicate that at least one of our novel ferrocene inhibitors, with the highest fitting score, good superposition with 5CITEP, binding with close distance to Mg, and able to accommodate at both binding trenches, is a good potential inhibitor of HIV-1 IN. Our novel design and docking suggest new potential inhibitors for HIV-1 therapies.

2. Docking of 5CITEP

We have used the GOLD 2.1.2 [8] and InsightII [9] software, described in our previous work on glycosidase inhibitors for AIDS chemotherapy [4], to obtain the structure of 5CITEP with the top ranking gold solution. The NBO partial atomic charges obtained for this ligand from the density functional geometry optimization were used in the docking procedure, whereas calculations were performed inside a sphere with a radius of 20 Å, centered in the magnesium ion. Atomic charges for the receptor atoms were obtained using an all atom force field CVFF. Docking parameters here used have been optimized for single docking calculations, and the program fully validated against 221 diverse and extensively checked protein–ligand complexes from the PDB [8]. GOLD uses genetic algorithm to perform flexible docking and each docking result is slightly different from the other. Flexible docking was parameterized for 0 ligand bumps, a population size of 100, 5 islands, 100,000 operations, 95 mutations, and 95 crossovers, adjusted for 10 dockings. The superpositions of the top three solutions (ligand orientations) are within 1.5 Å mean square root deviation (RMSD). The fitness function (GoldScore) is evaluated in six stages: (1) a conformation of the ligand and protein active site is generated; (2) the ligand is placed within the active site using a least squares fitting procedure; (3) a hydrogen bonding energy is obtained for the complex; (4) as well as a steric energy of interaction between the protein and the ligand; (5) molecular mechanics expressions are used to generate a measure of the internal energy of the ligand; and (6) The energy terms are summed to give a final fitness score.

We give in Fig. 1 the GOLD solution (using the 1QS4 PDB file [4]) for the receptor after removing the inhibitor, and the fully optimized crystallographic ligand using density functional theory at the B3LYP/6-31G* level from the Gaussian 03 software [10]. The crystallographic inhibitor is also shown for comparison. There is good agreement between the localization of the inhibitor from docking and from the crystal structure, i.e., having both primary interactions with the same residues. In addition, our docking introduces an interesting result by indicating an additional interaction to a recently reported novel binding trench in HIV-1 integrase [6].

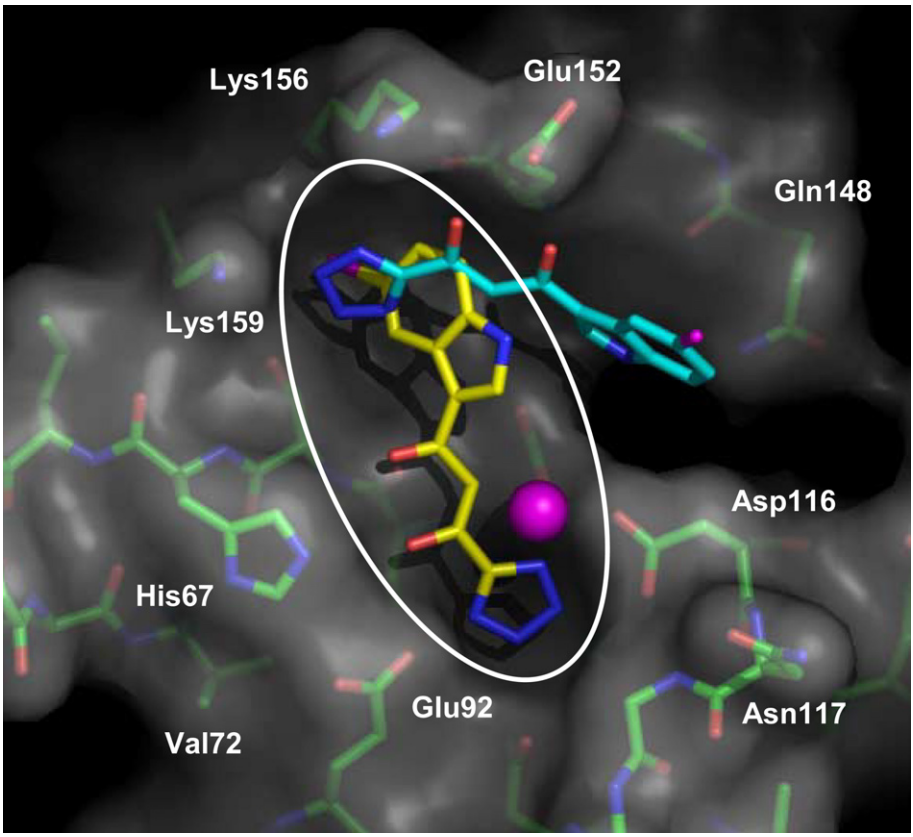

Fig. 1. Superposition of the top-ranked GOLD solution (carbon atoms in yellow) and the crystal orientation (carbon atoms in cyan) of 5CITEP binding with the receptor (PDB code 1QS4). Magnesium ion is represented as a magenta sphere. Selected residues which have contact with the ligand are shown. A novel binding trench is selected by a white circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

3. Docking of the potential HIV-1 IN inhibitors

A series of 'ferrocene ligands' (Fig. 2) were fully optimized, using the density functional method, at the B3LYP/6-31G* level, with the Gaussian software [10]. The complexes of HIV-1 IN (PDB code 1QS4) with our potential inhibitors were modeled as described in the previous section, using NBO atomic charges.

Our docking results indicate that, in general, our potential inhibitors occupy a similar region occupied by the 5CITEP molecule in the 1QS4 complex (Fig. 3). We also note that the ferrocene rings, in general, occupy the same binding site as that of the two terminal rings of the 5CITEP (indole and tetrazole). The major interactions at the tetrazole ring binding site are hydrophobic, due mainly to the Ile151 side chain as well as the hydrocarbon moieties of both Thr66 and Asn155 side chains. In our

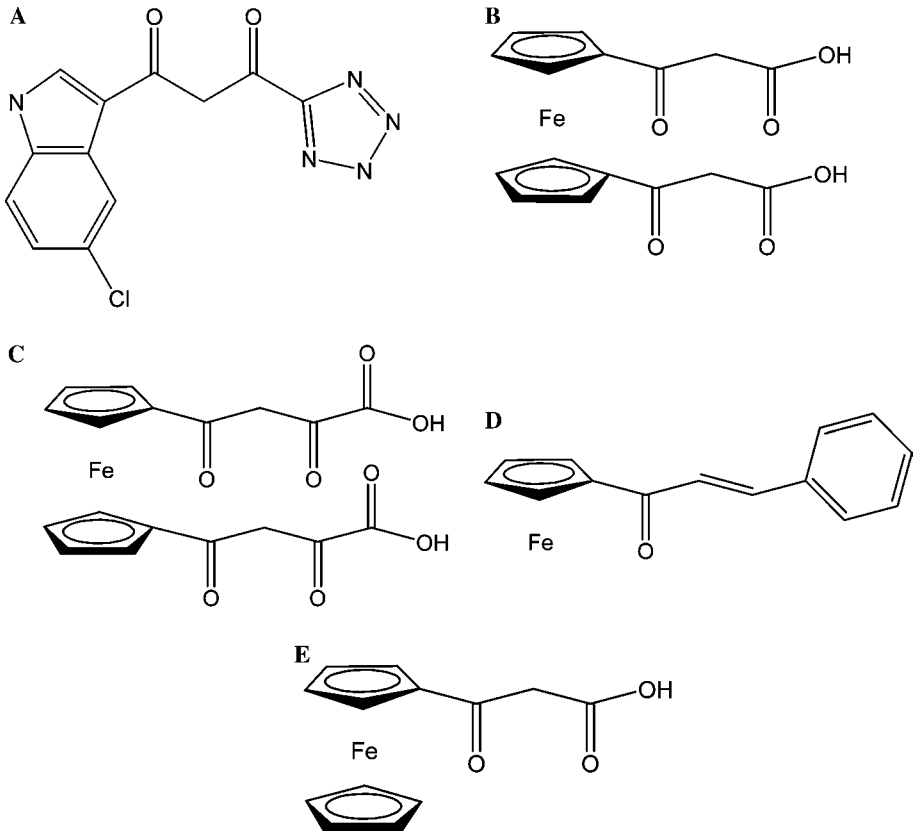

Fig. 2. Structures of 5CITEP (A) and our four novel ferrocene potential inhibitors of HIV-1 integrase; (B) compound 6; (C) compound 8; (D) compound 10; and (E) compound 3.

docking calculations, we have also considered the crystallographic magnesium ion. The dominant interactions between this divalent ion and the carboxyl or hydroxyl groups are electrostatic. The ligand 3 (Fig. 2E) yields the best GOLD score (40.5) and is closer to the magnesium ion, i.e., with distances of 2.26 and 1.77 Å between the carboxyl atoms and the magnesium ion (Fig. 4). The top-ranked solution obtained for 5CITEP is 39.5 whereas this ligand is at a larger distance of 4.98 Å from the magnesium ion. The ligand 6 (Fig. 2B) has two keto groups close to the magnesium ion, i.e., at distances of 2.33 and 1.58 Å. Not withstanding, the geometric position of ligand 6 with respect to the magnesium ion of the receptor is not as favorable as the position observed for ligand 6 . Ligand 10 indicates a distance of 2.27 Å from the magnesium ion, emphasizing the importance of this cation for the binding of substrates as well as ligands. Effectively, it is generally accepted that Mg^{2+} is a reasonable cofactor for integration [7].

The dihedrals in the tetrazole/keto-enol as well as the cyclopentadienyl rings in the 5CITEP and ferrocene inhibitors, respectively, were allowed to rotate yielding flexi-

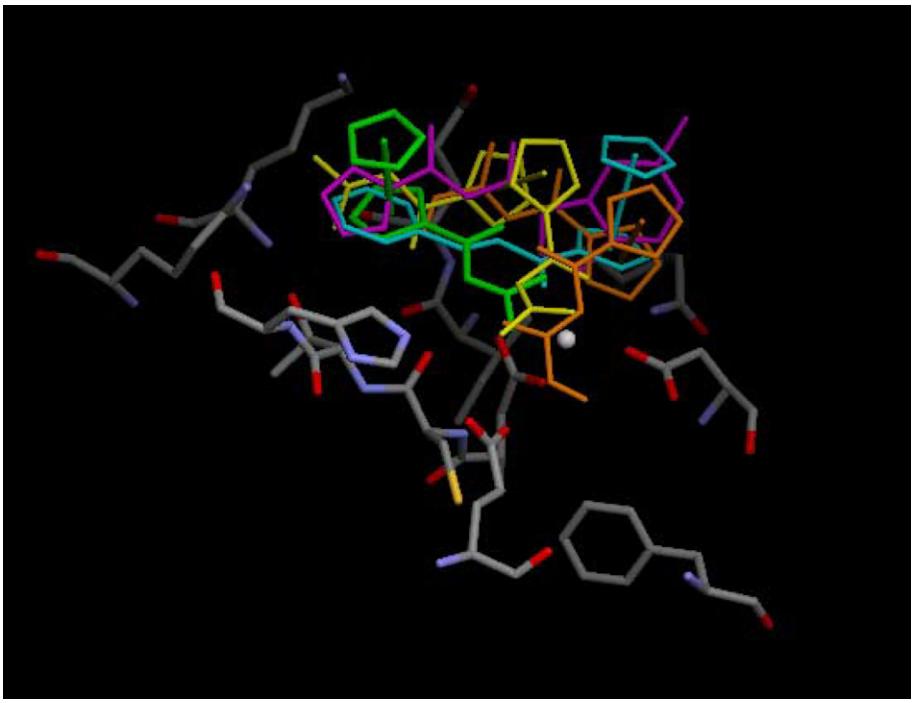

Fig. 3. Superposition of the top-ranked solutions of the four novel potential inhibitors of HIV-1 integrase with the crystal structure of the HIV-1 IN in complex with 5CITEP (in magenta). Ligands 3, 6, 8, and 10 are colored by green, yellow, orange, and blue, respectively. Selected residues of the active site are shown. Magnesium ion is represented as a white sphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

ble dihedrals and rings, which can rotate in our ligands (Fig. 2). We observe in Fig. 1 (circled) the novel binding trench in HIV-1 IN, recently reported [6]. Our GOLD top-ranked solutions for 5CITEP and a moiety of ligand 6 (Figs. 2B and 3) occupy the trench region.

4. Molecular interaction field studies

Molecular interaction field (MIF) calculations were performed with the classical molecular interaction potential (cMIP) program [11] to quantify the ability of the HIV-1 IN binding site to interact with ligands. For this purpose, the interaction energies between the enzyme and two typical probes were computed. The probes used were: water and methyl, placed in a 0.5 Å spacing grid covering the overall binding site, with grid dimensions of 40 Å, centered in the magnesium ion. This region includes all the residues that define both the crystallographic 5CITEP binding site and the novel binding trench of HIV-1 IN, recently reported [6], such

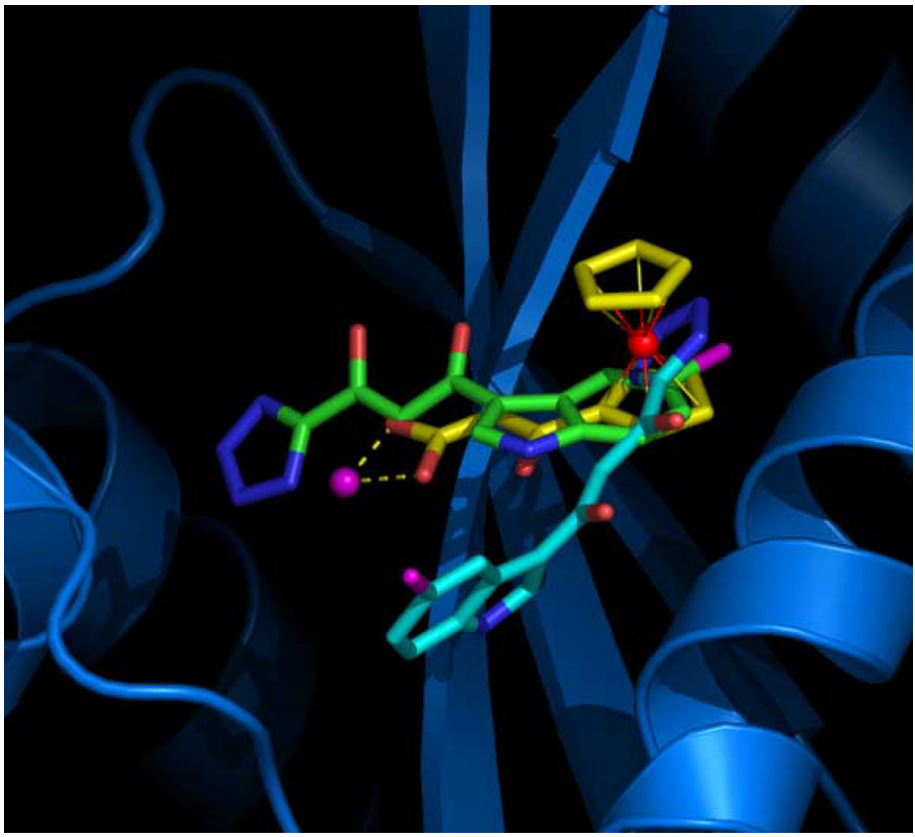

Fig. 4. Superposition of the top-ranked GOLD solution of the ligand 3 with the best score among all the novel ferrocene potential inhibitors (carbon atoms in yellow) and both the crystal orientation (carbon atoms in cyan), and Gold solution (carbon atoms in green) of 5CITEP binding with the receptor (PDB code 1QS4). Magnesium ion is represented as a magenta sphere. The dashed lines represent the electrostatic interaction between the carboxylate group of the ligand 3 and the magnesium ion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

as illustrated in Fig. 1. These two probes can explore the main interactions between the residues of HIV-1 IN and all the ligands investigated, including hydrophobic interactions (with the methyl probe) as well as hydrogen bonds between the receptor and ligands. Then the water probe can also explore the hydroxyl groups binding sites due to the enolic tautomers of the ligands. The total interaction energy was determined as the sum of the electrostatic and the van der Waals interactions.

The electrostatic contribution was calculated from the solvent-screened potential determined with standard procedures [12]. To capture the effect of the whole protein and solvent on the electrostatic potential at the binding site, a focusing strategy was used [12]. Our results indicate that there are two major hydrophobic

regions. One region can be attributed to the indole binding site of the 5-CITEP in the complex crystal structure (PDB code 1QS4), which is defined mainly by the Gln148 residue. The other region, with stronger interaction energy, and defined by the hydrocarbonic chains of the Thr66 and His67 residues, coincides with the cyclopentadienyl ring binding site proposed by our docking results with the ferrocene compounds (Fig. 5). Our results also suggest that there is a strong polar region close to the magnesium ion (energy contoured at −9.0 kcal/mol) as well as the Asp64 and Asn155 residues, indicating that novel HIV-1 inhibitors should preferentially have polar groups that bind the magnesium ion as well as these polar residues (Fig. 6).

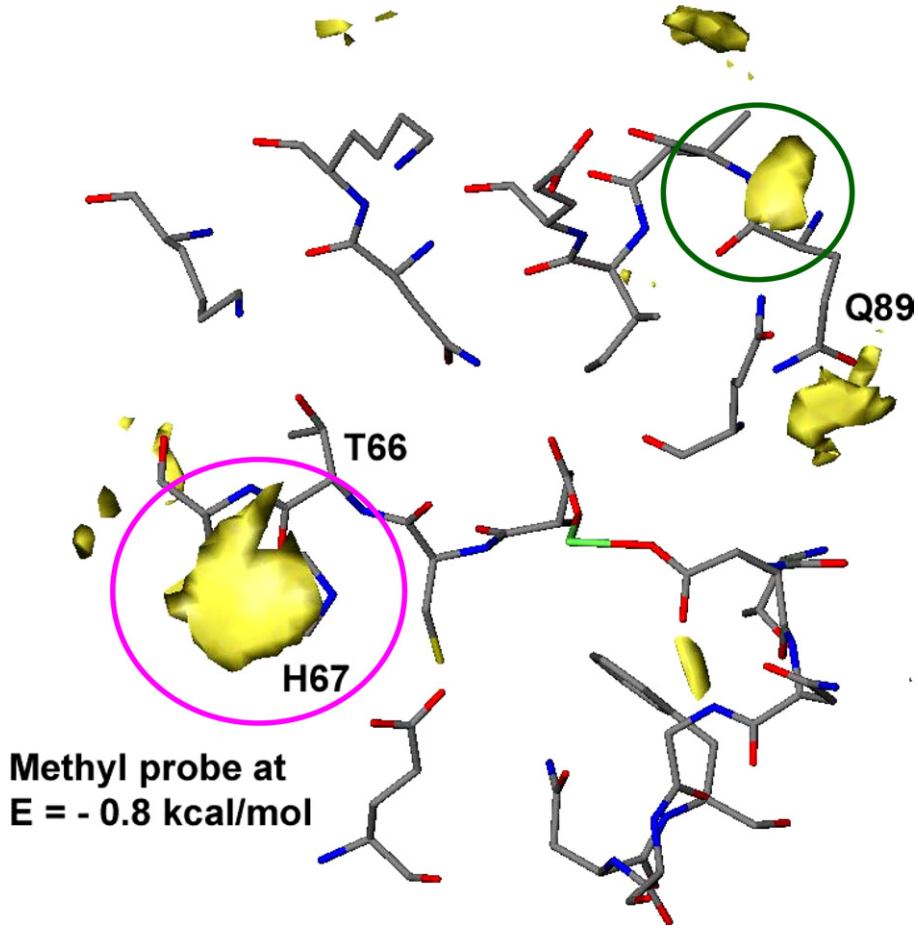

Fig. 5. Molecular interaction fields calculated for the HIV-1 IN active site, using a hydrophobic probe (methyl). Energy is contoured at −0.8 kcal/mol. Regions selected by green and magenta circles represent the indole (of 5CITEP) and the cyclopentadienyl (of ferrocene derivatives) binding sites, respectively. These contours can be attributed mostly to the labeled residues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

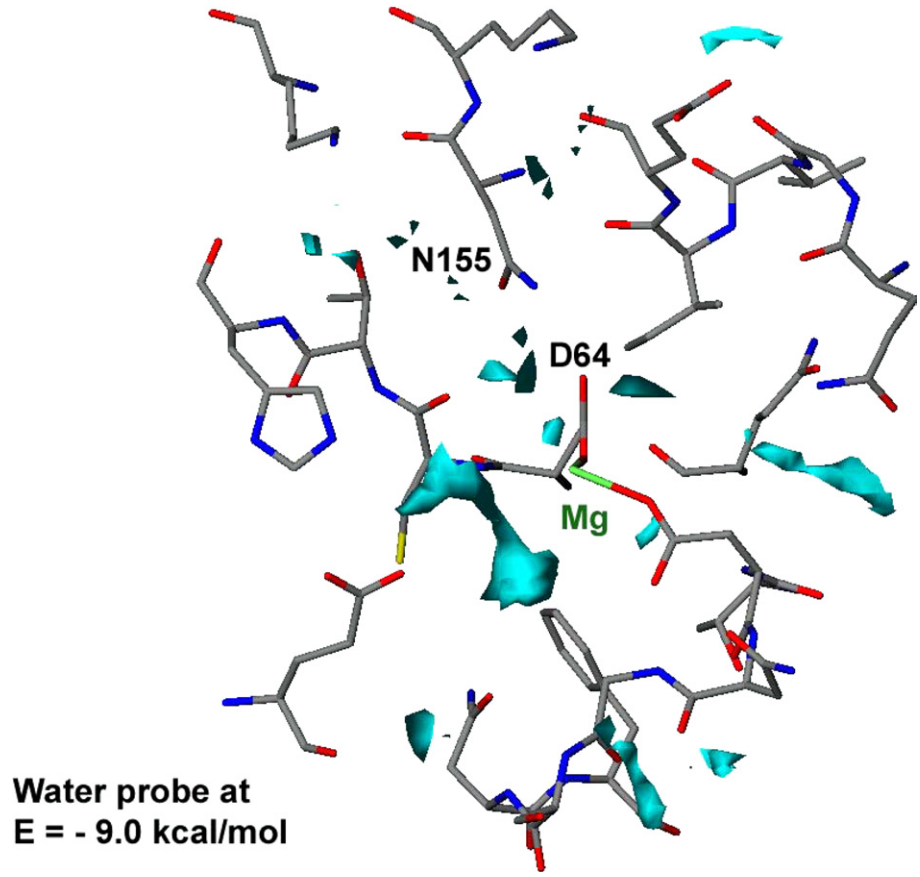

Fig. 6. Molecular interaction fields calculated for the HIV-1 IN active site, using a water probe. Energy is contoured at −9.0 kcal/mol. These contours can be attributed mostly to the labeled residues.

5. Discussion

We have proposed novel potential HIV-1 IN with high GOLD scores, which are open to experimental testing. For the structure of HIV-1 IN our results sheds some additional light on the ligand–receptor interactions in the vicinity of the active site region. Our results support the existence of a novel binding trench [6] in HIV-1 IN which can bind both the 5CITEP as well as of ferrocene potential inhibitors. Notably, ferrocene inhibitors introduce transition metals, rotatable rings, and flexible dihedrals which can accommodate in both regions of the active site, resulting in interesting features to design future potent and selective HIV-1 inhibitors. The existence of a ferrocene derivative as HIV-1 IN inhibitor has been recently reported [13], however only one of the rings is substituted. Our proposal contains one and two substituted rings. The importance of the two cyclopentadienyl substituted rings is to adjust the inhibitor to the two binding trenchs. This consideration is very interesting since in ferrocene

derivatives the cyclopentadienyl rings are able to rotate freely in solution [14]. We propose that novel HIV-1 IN inhibitors should have polar groups that bind to the magnesium ion and the Asp64 and Asn155 residues at one end, and large hydrophobic groups that bind to the active site close to Thr66, at the other end. One interesting feature of our ferrocene derivatives is the flexibility of the two cyclopentadienyl substitutions which can allow their accommodation at the two binding trenches of the active site. We hope that our proposed new ferrocene will contribute to the necessary development of a new generation of pharmaceuticals for AIDS treatment. Since the emergence of multidrug resistant viral strains infected patients can complicate the response of the AIDS treatment. The addition of novel IN inhibitors to existing components of combination regime could improve the outcome of therapy.

Acknowledgments

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