CHAPTER VI
CONCLUSION

6.1 3D-QSAR

A single 3D-QSAR model describing the structural properties and biological activities of 11 and 10 diverse classes for HIV-1 IN inhibitors against 3'-processing and ST mechanism, respectively was successfully derived. The obtained 3D-QSAR models yielded good statistical results. For 3'-processing activity, the CoMFA gave the highest r^2_{cv} of 0.698 and r^2_{pred} of 0.704 while the highest r^2_{cv} of 0.724 and r^2_{pred} of 0.524 were obtained from CoMSIA. For ST activity, the CoMFA yielded the highest r^2_{cv} of 0.720 and r^2_{pred} of 0.570 and the highest r^2_{cv} of 0.656 and r^2_{pred} of 0.461 were obtained from CoMSIA.

Compounds with activities against 3'-processing and ST mechanism generally shared somewhat similar 3D-QSAR contours. An analysis of contour maps revealed how steric, electrostatic, hydrogen bond donor and hydrogen bond acceptor influence the different inhibitory potency of HIV-1 IN inhibitors. CoMFA steric contours suggested that major sterically favorable regions were observed near a plane of indole ring of 5CITEP. This steric bulk of ligand might interact with the hydrophobic amino acids such as Phe121, Phe139, Ile141, Pro142 and Tyr143 via hydrophobic interaction. In addition, small bulky groups close to the tetrazole ring of 5CITEP are required to enhance the inhibitory potency of ligands. This is possibly because the pocket of enzyme is very narrow, therefore, large substituents in this region might decrease the activity. CoMFA electrostatic contour of the two activities suggested that the high electron density group near tetrazole ring of 5CITEP is required to better interact with the positive charge amino acids such as Lys156 and Lys159. Moreover in the case of 3'-processing, a negatively charged group close to CH adjacent to the carbonyl carbon and the hydroxyl group carbon of 5CITEP was also preferred where as this structural requirement was not observed in ST activities. This is in agreement well with a hypothesis that the 3'-processing reaction should be carried out in a region of the active site where a divalent metal ion is located [20, 135]. Because the ST subsite is located quite far from Mg^{2+}, no negative charge group was found in the region near to this catalytic metal ion.

An analysis of CoMSIA indicated that hydrogen bond donor and acceptor fields can be mapped back to the HIV-1 IN active site and they are consistent with the experimentally observed hydrogen bond between ligand and amino acids such as Asp64, Glu152, Asn155, Lys156 and Lys159. Hydrogen bond donor favorable region close to Asp64 was noticed indicating that aspartate side chain of this residue may act as an acceptor to establish hydrogen bond with the donor group of ligand. Hydrogen bond acceptor favorable was observed near the keto-enol moiety of 5CITEP. Glu152 located close to this region may act as a donor to form hydrogen bond with the acceptor group of ligand. Basically, the information gained from 3D-QSAR analysis provides the understanding of structural requirement of HIV-1 IN inhibitors and this should guide the design of high potent and high selectivity of HIV-1 IN inhibitors.

6.2 Classical and hybrid QM/MM MD simulations

This work intended to investigate the structural and dynamical properties of HIV-1 IN-inhibitor complexes. The promising diketo acid compounds (5CITEP and its analogue, DKA) were studied. However, to check whether classical MD is sufficient and suitable for the investigated systems, the MD simulations based on force field were conducted for systems of HIV-1 IN-5CITEP and HIV-1 IN-DKA. Comparison of the results of protein-ligand binding in terms of protein structure, ligand mobility, metal coordination and salt link interaction between the two levels of calculation showed a significant difference. Since the classical MD simulation cannot explain the difference of the inhibitory potency of 5CITEP and DKA, QM/MM was further used to compare structure and dynamical properties between both inhibitors to gain insight into protein-ligand binding.

Structural analysis of the two complexes showed substantial differences at (i) residues 60-68, (ii) residues 116-119, and (iii) residues 140-149. The noticed differences at the former two regions are probably because the side chains orientation of both Asp64 and Asp116 of IN-5CITEP are different from that of IN-DKA. However, the difference of the loop residues 140-149 was not surprising owing to its flexible in nature. Protein-ligand binding between the two systems in terms of for example, salt-link interaction, residue contribution, binding free energy, atomic charge polarization, and electron

density difference, were compared. In summary, this study provides insights into the detailed mechanism of action of 5CITEP and DKA and supports the higher inhibitory potency of DKA than 5CITEP. Information gained from this study leads to a better understanding of the HIV-1 IN inhibition and should be used as guidance for future developing HIV-1 IN inhibitors.

6.3 Suggestions for future works

For HIV-1 IN, although much progress has been made over last five years and lead candidates are beginning to enter clinical trials with promising outcomes, the studying and searching for HIV-1 IN inhibitors are still required. The results of this study could be useful in making progress in the HIV-1 IN system, however, there are many open questions concerning this particular system and it would very interesting to:

A) Extend MD simulations for longer time period to see whether dynamical behaviors of protein-ligand complexes are significantly different from that reported in this work.

B) Perform MD simulations or molecular docking studies of HIV-1 IN inhibitors which show high potency against 3'-processing mechanism but exhibit low potency against ST mechanism or vice versa. The information might used to describe the difference of mechanism of action of HIV-1 IN inhibitors against these two processes and to explain why some compounds inhibit 3'-processing/ ST while some compounds do not.

C) Simulate the mutated HIV-1 INs. This would be useful to describe drug resistance.

D) Investigate the enzyme's activities. This would help to gain better understanding of the molecular mechanism of HIV-1 IN and its association.