CONCLUSIONS

For an inhibitor, the interactions with the amino acids of the HIV-1 RT allosteric binding pocket are highly dependent on its conformation. Therefore, a conformational analysis of nevirapine was performed, in particular with regard to the cyclopropyl group which is able to rotate, varying the angle α (C15-N11-C17-C19). *Ab initio* and DFT procedures were applied and for all methods three conformational minima were found with α ≈ 100°, α ≈ 220° and α ≈ 340°. Energy minimization of these geometries by B3LYP/6-31G** showed that the global minimum structure occurs at an angle α = 217.4°. This compares favourably with the experimentally determined angle α = 208.5° for nevirapine when complexed with the HIV-1 RT enzyme.

The three optimized local minima structures of nevirapine in DMSO and chloroform IEF-PCM gas-phase solvation models were used to calculate ^{1}H, ^{13}C and ^{15}N-NMR chemical shifts. The models with dihedral angle α = 217.4° were in a good agreement with experimentally measured NMR spectra. Moreover, the results showed that the chemical shifts of atoms in the cyclopropyl ring and the nitrogen atom in the diazepine ring are dependent on the alpha angle and, hence the geometry of nevirapine in solution is very similar to that of the molecule in the inhibition complex. This was the first time the calculated ^{15}N-NMR chemical shifts of nevirapine were compared to experimental data. Since NMR shifts are affected by molecular geometries in solution, a comparison between experimentally measured spectra and calculated ones information on the quality of a theoretical modelling method and the accuracy of predicted molecular energies. Taking into account the influence of the solvent in calculating NMR chemical shifts leads to an improvement over previous results, as shown by the linear regressions of experimental chemical shifts against calculated isotropic shielding constants for a series of nitrogen-containing heterocycles.

Using the IEF-PCM solvation model in the GAUSSIAN03 program can predict ^{1}H, ^{13}C and ^{15}N-NMR chemical shifts of nevirapine in solution, and it gives

better results compared to gas phase calculations and IEF-PCM solvation model in the GAUSSIAN98 program. Various NMR calculations for the molecule in gas phase and interacting with the solvents DMSO and $CHCl_3$ have been performed. Comparing experimental and calculated chemical shifts, no significant improvement in prediction could be seen for the solvent models, except for the acidic proton located at the nitrogen atom (H23) which was better simulated with the newest model.

A simple nevirapine model in gas-phase only or with IEF-PCM solvation model cannot predict the ^{1}H-NMR chemical shift of H23 which is an acidic proton on the nitrogen atom of the 7-membered ring and can form a hydrogen bond with a polar solvent. Therefore, a MD-QM approach was used. Using ONIOM2 method for optimisation and NMR calculations, first discrete nevirapine-DMSO models were generated by MD combined with the ONIOM2 method. These were subsequently used to study the effects of hydrogen bonding on the calculations of ^{1}H-NMR chemical shifts of nevirapine in DMSO. The results showed that the chemical shift of the acidic proton H23 is especially sensitive to the bound DMSO molecule and intermolecular hydrogen bonding. This approach successfully reproduced the experimental results, and is an improvement over previous modelling with only a simple nevirapine model. Thus the combined MD-ONIOM2 method can be applied to include solvent effects in the outer low-level layer. Then the same approach was applied to generate nevirapine-$CHCl_3$ models and the chemical shift. As chloroform is an apolar solvent, the short range interaction is less significant to the calculations of the acidic proton of nevirapine. The predicted shift of H23 was the same as the simple nevirapine calculations with $CHCl_3$ IEF-PCM model. The results obtained are useful for NMR studies, and also give better understanding of the geometry of nevirapine in DMSO and chloroform solutions. From the IR frequency calculations, it was confirmed that the acidic proton formed a pronounced hydrogen bond with a DMSO molecule, and thus nevirapine in DMSO solution has a different structure compared to a chloroform solution.

Generally, the MD-ONIOM2 approach can also be useful to simulate the electronic environment of solvated molecules of other systems and to simulate other

properties such as IR spectra. Further development of the presented MD-ONIOM2 approach can be a promising task for the modelling of solute-solvent interactions.