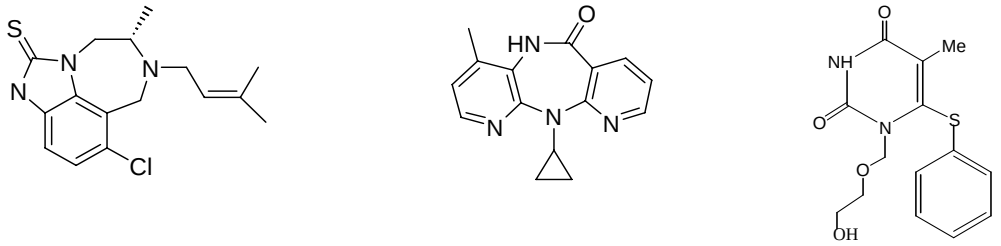
THEORETICAL MULTINUCLEAR NMR AND IR STUDIES USING COMBINED QUANTUM MECHANICS/MOLECULAR DYNAMICS SIMULATIONS OF SOLVATED NEVIRAPINE STRUCTURE

INTRODUCTION

HIV-1 reverse transcriptase (HIV-1 RT) is a major target for anti-acquired immunodeficiency syndrome (AIDS) chemotherapy. HIV-1 RT is an essential enzyme involved in the life cycle of the HIV responsible for virus replication from single-stranded RNA viral genome into a double-stranded proviral DNA, which is then integrated into the host chromosome. During the past decade, several compounds have been identified to resist the activity of HIV-1RT (Arnold *et al*., 1996; De Clercq 1998; Jonckheere *et al*., 2000). Two groups of RT inhibitors have been extensively investigated; nucleoside-analogue inhibitors (NRTI)s and non-nucleoside RT inhibitors (NNRTI)s. (NNRTI)s are chemically diverse, generally hydrophobic and relatively noncytotoxic compounds due to its reported butterfly-like shape binding strongly in a pocket of HIV-1 RT (Ding *et al*., 1995; Ding *et al*., 1995; Terence *et al*., 1995). The crystal structures of HIV-1 RT and complexes with some different (NNRTI)s have been published. (NNRTI)s such as nevirapine (Terence *et al*., 1995), HEPT (Tanaka *et al*., 1992), and TIBO (Pauwels *et al*., 1990) which are pharmacologically active have been studied. It indicated that the majority of HIV-1 RT inhibitors that act on the binding site show a pronounced dependence for their action on seemingly major changes in molecular conformation. (De Clereq 1992) Therefore, the information of a single preferred conformation of a drug molecule in solution to the conformational requirements of the binding site in the inhibition complex is important.

Nevirapine which has already passed pre-clinical and clinical tests and is available on the market shows a rotation of the cyclopropyl ring around the carbon nitrogen single bond (N11-C17) that determines the conformational space of nevirapine. It is found that alpha angle of cyclopropyl ring (C15-N11-C17-C19, α)

plays an important role to determine the structure of nevirapine, where only a limited flexibility of the ring system exists (Hannongbua *et al*., 2001). Accordingly, conformational investigations have been important part of structure-activity relationship studies.



8-Cl *TIBO* : Tivirapine Nevirapine HEPT

Figure 1 Structures of some non-nucleoside reverse transcriptase inhibitors (NNRTIs).

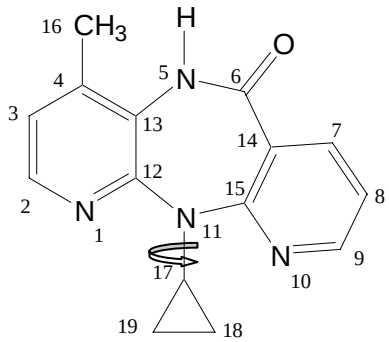
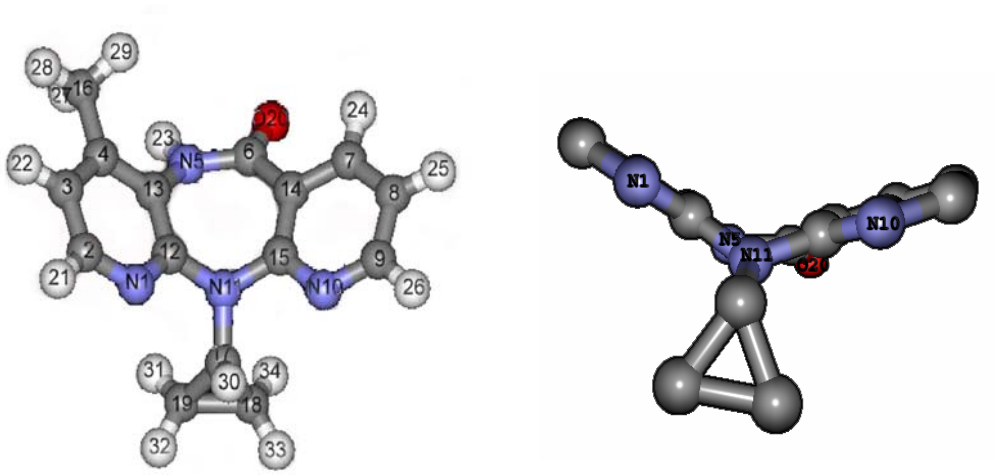


Figure 2 Chemical structure of nevirapine.



(a) (b)

Figure 3 (a) 3-dimensional structure of nevirapine and its atomic numbering (b) butterfly-like-shape

As NMR spectroscopy is an useful technique to investigate conformations of compounds because it can be used to characterize the conformation assumed by the molecule in solution that could be different in respect to the conformation assumed in the solid form, several approaches to overcome the so-called gauge-origin problem have been developed, such as GIAO (gauge including atomic orbitals) (Wolinski *et al*., 1990), IGLO (individual gauge for localized orbitals) (Schindler *et al*., 1982) and LORG (localized orbitals-localized origin).(Hansen *et al*., 1985) Sometimes the interpretation of experimental NMR spectra may be difficult, especially in assigning the correct conformation assumed by the molecule in analysis. The use of computational techniques can help interpret ambiguous experimental NMR spectra. As experimental NMR spectra are most commonly measured from condensed-phase samples (solutions or powders) at ambient temperature, the self-consistent reaction field (SCRF) (Cramer *et al*., 1995) method, using integral equation formalism polarized continuum model (IEF-PCM) (Tomasi *et al*., 1999; Mennucci *et al*., 2001) which solves the electrostatic solvation problem at the QM level with the aid of apparent surface charges (ASC), was expected to improve the calculated NMR chemical shifts in solution.

Nevirapine has four nitrogen atoms which are very sensitive and need high effective nuclear magnetic resonance spectrophotometer to investigate the chemical shifts. Thus predicted ^{15}N-NMR chemical shifts can help to give useful information for structural study. Furthermore, H23 that attached to nitrogen atom of 7-membered can give a broad peak shifted several ppm relative to trimethylsilane (TMS). As this proton is acidic proton, thus, hydrogen bonding can strongly influence on the electronic environment of this proton with oxygen of dimethyl sulfoxide solvent and not possible to predict the acidic proton chemical shift by using only simple static nevirapine model in gas (Hannongbua *et al*., 2001). Therefore, the idea to study dynamic nevirapine is very interesting and might give answers for accurate NMR chemical shift calculations.

Buckingham and coworkers suggested a possible classification in terms of various additive corrections to the shielding arising from (i) the bulk magnetic susceptibility of the solvent, (ii) the magnetic anisotropy of the solvent molecules, (iii) van der Waals interactions and (iv) long-range electrostatic interactions. (Buckingham *et al*., 1960) In the original scheme, strong specific interactions, such as those acting in intermolecular hydrogen bonds, were not dealt with but just mentioned as a possible extreme from of the electrostatic or, more generally, “polar” effect; in the numerous applications which followed Buckingham’s classification, however, this further effect has always been included as a separate contribution. In general, it is possible to correct experimental data for the bulk susceptibility, but there is no way to extract the remaining four effects which, in principle, are included in any measurement. For this reason, they have been the subject of several investigations even if not completely satisfactory rationalizations have been obtained so far. For the theoretical study, the widely believed idea is that short-range interactions can be effectively handle by supermolecule (or discrete) calculations involving a solute surrounded by a number of explicitly treated solvent molecules, while reaction field (or continuum) methods generally provide an effective alternative to describe long-range electrostatic interactions (Mennucci *et al.* 2001).

In this study, semi-empirical, *ab initio*, and density functional theory (DFT) were used to investigate the structure of nevirapine. Moreover, conformation analysis at alpha angle of cyclopropyl ring (C15-N11-C17-C19, α) was performed. ^{1}H ^{13}C and ^{15}N-NMR chemical shifts were calculated by using DFT at all energy minima and compared to the experiment data to determine the possible conformation of nevirapine in solutions and in the binding pocket. To understand the effect of solvent to the chemical shifts calculations, calculating the chemical shifts in IEF-PCM solvation model was performed and compared to gas phase calculations and experimental data. Molecular dynamics simulations (MD) were used to study effect of short-range or hydrogen bond interactions of acidic proton of nevirapine with the DMSO and chloroform solvents. Nevirapine-solvent clusters were obtained from MD and were performed NMR calculations in IEF-PCM solvation model in order to complete short-range and long-range interaction effect to the model of calculations. Furthermore, calculated infrared (IR) frequencies at B3LYP/6-31G* level correcting with a scaling factor were used to confirm the nevirapine structure and compared to the experimental data.

The results obtained are useful for NMR studies, and also gives better understanding of the geometry of nevirapine in DMSO and chloroform solutions and for design of new inhibitors against HIV-1 RT. This MD-GIAO method can be applied to other systems to simulate the electronic environment of solvated molecules.