LITERATURE REVIEW

Reverse transcriptase (RT) is essential for the life cycle of HIV because it converts the single stranded genomic RNA into the double stranded DNA version which is subsequently integrated into the host chromosome and passed on to all progeny cells (Goff, 1990 and Telesnitsky *et al*., 1997). RT can synthesize DNA using either RNA or DNA template. Because of its pivotal role in the HIV life cycle, HIV RT is a primary target for antiretroviral agents. Two major classes of drugs (or inhibitors) that inhibit the polymerase activities of HIV-1 RT have been identified: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs): such as 3-azido-3-deoxythymidine (AZT, zidovudine), 2,3-dideoxycytidine (ddC, zalcitabine), 2,3-dideoxyinosine (ddI, didanosine), 2,3-didehydro-2,3-dideoxythymidine (d4T, stavudine), 2,3-dideoxy-3_-thiacytidine (3TC, lamivudine), 1592U89 (abacavir), 2,3-dideoxy-5-fluoro-3-thiacytidine (FTC, emtricitabine), and tenofovir [(*R*)-9-(2-phosphonylmethoxypropyl) adenine or PMPA], a nucleotide analog (De Clercq, 1995 and Sarafianos *et al.*, 2004); (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs): i.e. (nevirapine and delavirdine) have been formally licensed for clinical use and several others are in preclinical or clinical development [tivirapine (*TIBO* R-86183), loviride (a-APA R89439), thiocarboxanilide UC-781, HEPT derivative MKC-442, quinoxaline HBY097, DMP266 (efavirenz)], which are highly specific for HIV-1. The NNRTIs are much less toxic than the NRTIs even though the emergence of drug-resistant viral strains has limited the therapeutic efficacy of NNRTIs RT (De Clercq, 1995; De Clercq, 2001; De Clercq, 2002; De Clercq, 2004). The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from the NRTI binding site. NNRTIs are known for rapidly resistance due to mutations of the amino acids surrounding the NNRTI-binding site.

NNRTIs inhibit HIV-1 RT by inducing a conformational change of the enzyme to lock the polymerase active site into an inactive conformation. NNRTIs bind in a pocket approximately 10 Å away from the catalytic site where nucleotides bind (Esnouf *et al.,* 1997) and are generally specific for HIV-1 RT. Moreover, the

intramolecular factors the contribution a common butterfly-like shape conformation of the NNRTIs consisting of two wings. Hydrogen bonds were analysed by geometric and electrostatic criteria. Only the former allowed the elucidation of the relative intensity of hydrogen bonds. The interactions between aromatic rings were contributed to the preferential conformation. It provided a basis for understanding of the nature of the non-nucleoside inhibitor binding and the structure of the binding site as well as the interactions between the bound inhibitors and surrounding amino acid residues. Important differences occurred in the conformation of amino acid residues that form the binding pocket (Ding *et al*., 1995).

Conformational analyses of the potent NNRTIs have been previously studied (Lawtrakul *et al.,* 1999; Lawtrakul *et al* 1999a; Saen-oon *et al*., 1999; Ren *et al*., 1995) and also Hannongbua *et al*. (2001) studied the structure and the conformational behavior of nevirapine by using Gaussian 98 program. They found that the obtained geometrical minimum from semiempirical method, HF/3-21G, HF/6-31G** and B3LYP/6-31G** levels shows an almost identical structure to the geometry of the molecule in the complex structure with HIV-1 reverse transcriptase. The calculated ^{1}H-NMR and ^{13}C-NMR spectra at B3LYP/6-31G** level, using GIAO approximation agree well with the experimental results, which indicated that the geometry of nevirapine in solution is very similar to that of the molecule in the inhibition complex.

Wolinski *et al.* (1990) described the implementation of the gauge independent atomic orbital (GIAO) method for the *ab initio* self-consistent-field (SCF) calculation of nuclear magnetic resonance (NMR) chemical shifts. Using modern techniques borrowed from analytical derivative methods, they were able to improve the efficiency of the GIAO method significantly, results with several basis sets, some of them large, are presented for methane, methyl fluoride, cyclopropene, cyclopropane, oxirane, benzene carbon disulfide, the sulfate and thiosulfate anions, dimethyl sulfide, dimethyl sulfoxide, and dimethyl sulfone. Comparisons are made with the individual gauge for localized orbitals (IGLO) method of Schindler and Kutzelnigg, and with the localized orbital/local origin (LORG) method of Hansen and Bouman. The GIAO

method appears to converge faster than the localized techniques; i.e., it provides the same accuracy with a smaller basis, particularly for the individual tensor components. The computational effort for the *ab initio* calculations of the NMR chemical shifts is only about 2.5 times of the energy calculation. The electronic spin-orbit contribution to nuclear magnetic shielding tensors, which causes the heavy-atom chemical shift of the shielding of light nuclei in the vicinity of heavy elements, as a sum of analytical quadratic response functions was calculated by Vaara *et al*. (1998). They include both the one- and two-electron parts of the spin-orbit Hamiltonian and consider the interaction with both the fermicontact and the spin-dipolar mechanisms. *Ab initio* calculations at the SCF and MCSCF levels were presented for the ^{1}H and ^{13}C shielding tensors in the hydrogen and methyl halides.

Tomasi *et al*. (1999) presented the integral equation formalism (IEF) method to solve the electrostatic solvation problem at the QM level with the aid of apparent surface charge (ASC). They emphasize the good performances of IEF at the lowest level of its potentialities, i.e. for isotropic solvents, as a new approach to compute solvation free energies and properties (dipole hyperpolarizabilities) of molecular solutes, as well as energy gradients for geometry optimization procedures. A new IEF implementation of the nonequilibrium problem for electronic spectra which appears to be decidedly competitive with the previous more standard ASC formulations was proposed. In 2001, the effects of solvents on nuclear magnetic shielding parameters derived from NMR spectroscopy were studied. The study focused on a specific nucleus, nitrogen, in two molecular solutes, acetonitrile and pyridine, immersed in different solvents. Among the solvents, particular attention was devoted to chloroform: its specific characteristics (low polarity and proticity). They exploited a coupling scheme of solute-solvent cluster structures generated through MD simulations and high-level quantum chemical calculations in which a continuum solvation model was also introduced. This scheme permitted the study of the competitive effects due to short-range and highly directional H-bonds and to long-range electrostatic forces and of the way these two effects were taken into account through a discrete, a continuum, or a couple description of the solvent. Natural bond

analysis of computed results had been used to provide insight into the role of solvent-induced modifications of electronic distribution charge in the observed gas-to-solvent shift.(Mennucci *et al*., 2001) Novak *et al*. (2000) also investigated keto-enol tautomeric equilibrium in several β-triketones using NMR spectroscopy and theoretical methods. Structures and stabilities of both long- and short-live tautomeric forms, as well as transition-state structures and barrier heights of enolization processes, were calculated using semiempirical and density functional quantum chemical methods. The study of tetramethylthiourea and thiourea in a wide variety of solvents by high-precision ^{14}N-NMR measurements was done by Witanowski (Witanowski *et al*., 2000). The solvents exhibit a wide range of hydrogen-bonding and polarity/polarisability properties. The observed nitrogen shielding variations of the solutes, due to solvent change, are significant and are attributed to solvent polarity, solute to solvent and solvent to solute hydrogen-bonding effects. The nitrogen NMR shieldings of tetramethylthiourea and thiourea are calculated by the Gauge-Independent Atomic Orbital Coupled Hartree-Fock (CHF-GIAO), *ab initio* molecular orbital procedure using a 6-31++G** basis set. The calculations are for isolated molecules, and their results satisfactorily reproduce the position of the thiourea nitrogen resonance obtained in a dilute solution in cyclohexane with respect to that of urea systems and nitromethane.

Vikić-Topić *et al*. (2000) reported that the ^{13}C and ^{1}H chemical shift values computed at HF, BLYP and B3LYP/6-311G(d,p) levels of theory, for the BLYP/6-31G(d,p) optimized geometries of adamantane and 2,4-methno-2,4-deydroadamantane and compared with the available experimental data. Except for the inverted carbon atoms, the HF values are superior to the DFT ones when the isotropic shifts with respect to TMS are in question. However, in case of the relative shifts computed with respect to the most de-shielded center within the molecule, the DFT methods yield significantly better agreement with the experiment than the HF. The most probable reason for these findings may be the cancellation of errors arising from the inappropriate description of the paramagnetic contributions to the overall shielding tensor within the Kohn-Sham approach when an internal standard (within a

molecule) is chosen, instead of an external one. The CSGT (continuous set of gauge transformations) relative shift values correlate better with the experiment than the GIAO (gauge independent atomic orbitals) ones, the correlations being significantly superior at DFT than at the corresponding HF level of theory. The calculated and experimental ^{13}C-NMR chemical shifts of (1*R*,3*S*,4*S*,8*S*) p-methane-3,9-diol in chloroform solution were reported by Casanovas *et al*. (2001). Theoretical estimations were performed using a combination of molecular dynamics simulations and quantum mechanical calculations.

In 2001, Megiel *et al*. (2001) presented the ^{14}N NMR spectra of liquid pyridine and its mixtures with *n*-heptane which were measured over a full concentration range. The variations of the chemical shifts of the pyridine nitrogen and the value for the pure base were compared with theoretical results, i.e. with the difference in shielding constant for the nitrogen nucleus in the isolated molecule and its associates (calculated by GIAO/CHF Gauge-Independent Atomic Orbital Coupled Hartree-Fock). The geometry of pyridine molecules and of its associates was optimised at RHF/6-31+G** level. On this basis the most probable structures for the associates in liquid pyridine were proposed. The computer simulation of liquid pyridine at a temperature of 300 K, by the molecular dynamics method using the Amber force field, lent validity to the existence of the proposed dimers. Cossi *et al.* (2002) developed the polarizable continuum model for quantum mechanical and classical calculations on molecules in solution in year 2002. The main changes effect the definition of solute cavities, of solvation charges and of the PCM operator added to the molecular Hamiltonian, as well as the calculation of energy gradients, to be used in geometry optimizations. The procedure can be equally applied to quantum mechanical and to classical calculations; as shown also with a number of numerical testes, this PCM formulation is very efficient and reliable. The can also be applied to very large solutes, since all the bottlenecks have been eliminated to obtain a procedure whose time and memory requirements scale linearly with solute size.

Fazaeli *et al.* (2002) predicted the structure and relative energies of the tautomers of cytosine in gas phase and in different solvents using MP2 and density functional theory methods. Solvent induced effect on nitrogen NMR shielding of two dominate tautomers is calculated using density functional theory combined with polarizable continuum model and using the continuous set gauge transformation. Direct and indirect solvent effects on shielding are also calculated. The theoretical ^{1}H, ^{13}C and ^{15}N NMR spectra which were calculated at the Hartree-Fock level by using the polarized double zeta (PDZ) basis set of Hansen and Bouman for the geometries optimized at the same level were proposed by Bednarek (Bednarek *et al*., 2003). The theoretical chemical shifts were calculated by substracting the isotropic shielding calculated for given quinolizine nuclei from that calculated for the reference molecules. In 2003, Paiola (Paiola *et al.*, 2003) performed B3LYP calculation on two thiosemicarbazonic ligands and on their relatives Pd(II) chloro-complexed. A comparison of the structures and experimentally determinated ^{1}H NMR was performed with the correspondent calculated in vacuo and solvent using in order gauge-including atomic orbitals formalism GIAO and PCM GIAO methods. The introduction of the solvent effect described using the polarization continuum model PCM allowed to obtain a good agreement with the experimental data. An additional conformational study on a ligand permitted to confirm that the conformation assumed by the molecule in solution is the same of the solid state.

Mphahlele *et al.* (2004) studied the geometries of 2-arylquinoline-4(1*H*)-thione derivatives in solution, solid and gas states using spectroscopic methods, X-ray crystallography and quantum chemical techniques. The exclusive existence of the NH-4-thiones in solution (NMR and PCM-B3LYP(MP2)/6-31+G(d) calculations) and solid state (FT-IR and X-ray) is also corroborated by comparison of their spectroscopic data with those of the corresponding 2-aryl-1-methyquinoline-4(1*H*)-thione derivatives. The co-existence of the quinoline-4-thione and quinoline-4-thiol(4-mercaptoquinoline) isomers in the gas phase confirmed by mass spectrometry and the preponderance of the 4-thiol is supported by quantum chemical techniques (PM3, MP2 and B3LYP).

Molecular dynamics (MD), principal component analysis (PCA), and binding free energy simulations were employed by Zhou and coworkers (Zhou *et al*., 2005) to explore the dynamics of RT and its interaction with the bound NNRTI nevirapine, for both wild-type and mutant (V106A, Y181C, Y188C) RT. These three mutations commonly arise in the presence of nevirapine and result in resistance to the drug. They showed that a bound NNRTI hinders the motion of almost all RT amino acids. The mutations, located in the non-nucleoside RT inhibitor binding pocket, partially restore RT flexibility. The binding affinities calculated by molecular mechanics/Poisson-Boltzmann surface accessibility (MM-PBSA) show that nevirapine interacts stronger with wild-type RT than with mutant RT. The mutations cause a loss of van der Waals interactions between the drug and the binding pocket. The results from this study suggest that a good inhibitor should efficiently enter and maximally occupy the binding pocket, thereby interacting effectively with the amino acids around the binding pocket.

Three-dimensional computer modeling has shown that the active site of CYP3A4 is especially large, permitting access of nevirapine and carbamazepine. The interactions between nevirapine and aldosterone and between carbamazepine and androstenedione were estimated by theoretical calculations assuming the substrate and steroids to be present in the active site at the same time. It was shown that nevirapine or carbamazepine would be stably fixed close to the oxygen atom at the sixth ligand of heme by interaction with steroids, suggesting that nevirapine and carbamazepine may be hydroxylated more easily due to the interaction with steroids. Estradiol was also expected to interact with nevirapine via a π/π interaction between a benzene ring, in which the nevirapine hydroxylation site is located, and a benzene ring of estradiol, suggested to inhibit the reaction. From these results, interactions between the substrate and endogenous steroids in the active site may change the activity of CYP3A4. (Torimoto *et al*., 2003) Yadav and coworkers (2004) performed *ab initio*. calculations on some selected potent non-nucleosidic reverse transcriptase inhibitors. The results indicate a bent or ‘V’-shaped conformation for each non-nucleosidic inhibitor. The conformational mapping studies identify an amide group in each drug

in an appropriate position to help anchor the drug to the binding site via lysine 101; thus suggesting common binding mode for structurally, chemically diverse non-nucleosidic drugs. The molecular electrostatic potential maps predict a slightly positively charged complementary environment on the receptor.

The energies and physical descriptors for the binding of 20 novel 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)benzimidazole analogues (BPBIs) to HIV-1 reverse transcriptase (RT) have been determined using Monte Carlo (MC) simulations. The crystallographic structure of the lead compound, 1-(2,6-difluorobenzyl)-2-(2,6-difluorophenyl)-4-methylbenzimidazole, was used as a starting point to model the inhibitors in both the bound and the unbound states. The energy terms and physical descriptors obtained from the calculations were correlated with their respective experimental EC_{50} values, resulting in an r^2 value of 0.70 and a root-mean-square deviation (rms) of 0.53 kcal/mol. The terms in the correlation include the change in total Columbic energy and solvent-accessible surface area. Structural analysis of the data files from the BPBI calculations reveals that all of the analogues with good biological activity show the formation of a hydrogen bond between the ligand and the backbone nitrogen atom of lysine 103. By use of the structural results, two novel BPBI inhibitors have been designed and calculations have been carried out. The results show the formation of the desired hydrogen bonds, and the $\Delta G_{binding}$ values predict the compounds to be excellent RT inhibitors. Subsequent synthesis and biological activity testing of these analogues have shown the validity of the predictive calculations. If the BPBIs are modeled in a site constructed from the crystal coordinates of a member of another class of nonnucleoside inhibitors (the 4,5,6,7-tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepine-2(1*H*)-thione and -one (TIBO) compounds), the correlation with the same terms drops slightly, giving an r^2 value of 0.61 with an associated root-mean-square value of 0.53 kcal/mol. Conversely, if the TIBO compounds are modeled in a site constructed from the BPBI complex crystal coordinates, a correlation can be obtained using the drug-protein interaction energy and change in the total number of hydrogen bonds, giving an r^2 value of 0.63. These are the same descriptors that were used for the TIBO compounds modeled in their

own sites, where the r^2 value was 0.72. These data suggest that it may be possible, in some cases, to design novel inhibitors utilizing structural data from related, but not identical, inhibitors. (Smith *et al*., 2003)

A series of targeted molecular dynamics simulations have been carried out in an attempt to assess the effect that the common Lys103Asn mutation in HIV-1 reverse transcriptase (RT) has on the binding of three representative non-nucleoside RT inhibitors (NNRTI), nevirapine, efavirenz, and etravirine. (Rodriguez-Barrios *et al*., 2005) They have shown previously that, in the absence of an incoming inhibitor, creation of the NNRTI binding pocket is hampered due to the existence of a hydrogen bond between the side chains of Asn103 and Tyr188 for which no equivalent exists in the wild-type enzyme. They applied the same methodology to drive the enzyme's conformation from the unbound state to the drug-bound state in the presence of the NNRTI. The location of each drug outside the binding pocket was determined by an automated docking program, and steering into the binding pocket followed a route that is likely to represent the actual entrance pathway. The additional hurdle to inhibitor entry imposed by the extra Asn103-Tyr188 hydrogen bond is seen to affect each NNRTI differently, with the ability to disrupt this interaction increasing in the order etravirine $\gg$ efavirenz $\geq$ nevirapine, in good accord with the experimental findings. This coherent picture strongly suggests that attempts to overcome resistance through structure-based drug design may be considerably more successful if dynamic structural aspects of the type studied here are considered, particularly in cases where binding energy-based structure-activity relationship methods are unable to provide the required information.

Romines (2005) described the benzophenone structure-activity relationships, which culminated in the identification of several compounds with very potent inhibition of both wild type and clinically relevant NNRTI-resistant mutant strains of HIV. These potent inhibitors include **70h** (GW678248), which has in vitro antiviral assay IC_{50} values of 0.5 nM against wild-type HIV, 1 nM against the K103N mutant associated with clinical resistance to efavirenz, and 0.7 nM against the Y181C mutant associated with clinical resistance to nevirapine. Compound **70h** has also

demonstrated relatively low clearance in intravenous pharmacokinetic studies in three species, and it is the active component of a drug candidate which has progressed to phase 2 clinical studies.

ONIOM-GIAO method has been used to accurately predicted ^{13}C NMR chemical shifts for a serious of organic species adsorbed on H-ZSM-5 zeolite (Zheng *et al*., 2005). This is useful for the spectroscopic identification of complicated catalytic systems. The ONIOM-GIAO method was also used for ^{13}C NMR chemical shifts tensors calculations of carboxyl carbon in amino acids (Zheng *et al*., 2004) which are in good agreement with the experimental values. The present investigation has proved that the surrounding lattice environment plays an important role in the calculation of chemical shift tensor of amino acids. Richard and coworkers (2003) also calculated NMR chemical shifts in carbohydrates with ONIOM2 method. A small model system containing the nuclei of interest is described at the MP2-GIAO level of theory, and the rest of the molecule-using the HF-GIAO method. They showed that with an appropriate choice of the model system this construction yields chemical shifts that represent close approximations to the corresponding MP2-GIAO values for the entire molecule, which makes it suitable for post-HF NMR chemical shifts are achieved using the results of the calculations.

Galván and coworkers (Galván *et al*., 2006) compared widely used solvation models by studying triazene molecule in liquid water. They considered (1) a continuum model based on multicentric multipole expansions of the charge distribution, (2) the averaged solvent electrostatic potential from molecular dynamics (ASEP/MD) method, and (3) molecular dynamics simulations using a combined quantum mechanics/molecular mechanics potential (QM/MM/MD). They found that the solvation induced appreciable changes in the geometry and charge distribution of triazene. These changes were only qualitatively reproduced by the dielectric continuum model, which clearly underestimates induced dipole moments and solute-solvent interaction energy. Also Pejov and coworkers (Pejov *et al*., 2005) used MD snapshorts in ab initio and DFT calculations to present the average OH stretching

vibrational frequency for the water molecules in the first hydration shell around a Li^+ ion in a dilute aqueous solution.