LITERATURE REVIEW

Non-nucleoside reversetranscriptase inhibitors (NNRTIs)

Despite the advances in the available regimen of drugs to treat HIV-1 infection, there remains vital need to improve patient compliance, cost-of-goods and resistance profiles of each of the classes of anti-retroviral drugs (Hopkins *et al.*, 2004). One of the four main classes of licensed antiretroviral drugs are the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the other three classes being nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and cell fusion inhibitors. NNRTIs represent a class of structurally diverse, potent and highly-selective anti-HIV agents that were first discovered in the early nineties as a result of large scale compound library screening in antiviral and/or biochemical assays, followed by extensive chemical lead optimization. Because NNRTIs that are used the low concentrations and less toxic, they are considered as the preferential candidate compounds to be included in a double, triple, or quadruple drug combination therapy with NRTIs and protease inhibitors.

The first-generation NNRTIs consist of nevirapine, 9-Cl TIBO, loviride, and delavirdine. The important interaction for first generation NNRTIs are interaction with Y181 and Y188. Unfortunately, resistance to these NNRTIs arises rapidly upon drug treatment. All of the mutations associated with NNRTI resistance are located in and around the NNIBP. Common NNRTI resistance mutations include K103N, Y181C, L100I, V106A, Y188L and G190A (Sarafianos, 2004). The clinically most important mutations of HIV-1 RT occur in amino acids K103, Y181, and Y188 (Pata *et al.*, 2004). To improve the efficiency of the first generation NNRTIs, the second-generation NNRTIs (i.e. UC-781, HBY-097 (nowadays replaced by GW867), efavirenz, PETT-4, and S-1153 (capravirine) are proposed. They are used at lower concentrations than the first-generation NNRTIs and improve resistance profiles. They are also likely better drug candidates to be part of future combination treatment modalities than the first-generation NNRTIs. Three NNRTIs, nevirapine

(Viramune®), delavirdine (Rescriptor®) and efavirenz (Sustiva®, Stocrin) were approved for the treatment of HIV infection in 1996, 1997 and 1998, respectively.

Because of the fast drug resistance from the mutation, new drugs are still needed for the aid treatment. New drugs are expected to show enhanced activity against at least parts of the current mutants. Therefore, new NNRTI discovery approach should include: searching for new and/or further evolved chemical scaffolds that allow more flexible and multiple drug-binding modes; better 'quality' binding through aiming for multiple drug interactions, in particular, with more conservative and/or critical residues; and multiple interactions with the main chain of the amino acids that line-up the NNRTI binding site (Pauwel, 2004). Some new NNRTIs have been proposed to improve the problems about the mutations, e.g. PNU-142721 (Wishka *et al.*, 1998), 8-aryloxymethyl- and 8-arylthiomethyldipyridodiazepinones derivatives (Cywin *et al.*, 1998), urea-PETT derivatives (Högberg *et al.*, 1999), MKC-442 (emivirine) analogues (Hopkins *et al.*, 1999), 2,3-diaryl-1,3-thiazolidin-4-ones derivatives (Barreca *et al.*, 2002), indolyl aryl sulfones derivatives (Silvestri *et al.*, 2003), benzophenones derivatives (GW4511, GW4751, and GW3011) (Chan *et al.*, 2004), sulfonylindolecarboxamide derivatives (L-737,126) (Silvestri *et al.*, 2004), conjugation of D4T with 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, Emivirine) (Peterson *et al.*, 2005), pyrazinone derivatives (Heeres *et al.*, 2005), diarylpyrimidine (DAPY) derivatives (Guillemont *et al.*, 2005), pyridinone derivatives (R157208, R165481, and R221239) (Himmel *et al.*, 2005). Furthermore, Ren and Stammers (2005) proposed a new NNRTI, as called CP94707, active against commonly observed mutation, i.e. K103N, Y181I and Y188L. The structure of CP94707/HIV-1 RT revealed that CP94707 binds to the binding pocket of HIV-1 RT in a novel mode with reduced contact with Y181 and Y188. The less contact with Y181 and Y188 may improve the resistance of CP94707. The derivatives of 4-benzyl and 4-benzoyl-3-dimethylaminopyridin-2(1H)-ones are proposed by Benjahad *et al.* (2005). Some compounds of these derivatives are found that they are active against eleven single (L100I, K101E, V106A, K103N, E138K, V179E, Y181C, Y188L, G190A/S, and F227C) and four double HIV mutant strains (L100I + K103N, K101E + K103N, K103N + Y181C, and F227L + V106A).

Computer-aided design is a powerful method that was used to study and design novel potent NNRTIs. There are many research groups working on computer-aided design with ligand or structure-based drug design to develop new NNRTIs active against mutants. 3D-QSAR analysis by Ragno *et al.* (2006) led to the discovery of exceptionally potent indolyl aryl sulfones (IASs) characterized by the presence of either a pyrrolidyn-2-one nucleus at the indole-2-carboxamide or some substituents at the indole-2-carbohydrazide. Molecular docking methods were undertaken using a number of published crystal structures of HIV-1 reverse transcriptase complexes with various non-nucleoside inhibitors to study and design new NNRTIs (Titmuss *et al.*, 1999). To date, a variety of structure-based drug design approaches are used to improve the activity of some NNRTIs derivatives, e.g. pyrrolyl aryl sulfones (PASs) (Artico *et al.*, 2000), 2-Alkylthio-6-[1-(2,6-difluorophenyl)alkyl]-3,4-dihydro-5-alkyl-pyrimidin-4(3H)-ones (Mai *et al.*, 2001), neotripterifordin (Zhou *et al.*, 2002), Phenethylthiazolylthiourea derivatives (Ranise *et al.*, 2003, 2005), 2-Alkylamino-6-[1-(2,6-difluorophenyl)alkyl]-3,4-dihydro-5-alkylpyrimidin-4(3*H*)-ones (Ragno *et al.*, 2004), TMC125-R165335 (Etravirine) (Das *et al.*, 2004), quinolone derivatives (Hopkins *et al.*, 2004; Freeman *et al.*, 2004), pyrrolobenzoxazepinones (PBOs) (Fattorusso *et al.*, 2005), 4-[[4-[[4-[(1*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile (R278474, Rilpivirine) (Janssen *et al.*, 2005), The combinations of ligand and structure-based drug design are also an alternative approach to develop NNRTIs. The examples are shown as following.

Mao *et al.* (2000) rationally designed NNI compounds HI-236, HI-240, HI-244, HI-253, HI-443, and HI-445 combine these three features and outperform other anti-HIV agents examined by using structure-based drug design approach. They suggested that the following features of NNRTIs to be active against RT mutants, such as the Y181C RT mutant should be required: (a) the inhibitor should be highly potent against wild-type RT and therefore capable of tolerating a considerable activity loss against RT mutants (i.e. a picomolar-level inhibitor against wild-type RT may still be effective against RT mutants at nanomolar concentrations), (b) the inhibitor should maximize the occupancy in the Wing 2 region of the NNI binding site of RT, and (c) the inhibitor should contain functional groups that provide favorable chemical

interactions with Wing 2 residues of wild-type as well as mutant RT, as shown in Figure 7. Zhou and Madura (2004) studied 3D-QSAR analysis of TIBO derivatives based on docking conformation and alignment. The flexible docking using Autodock version 3.0 program was used on determination of active conformation and molecular alignment. Barreca *et al.* (2005) used 3D-pharmacophore models of TBZ derivatives and molecular docking method in searching for new lead as NNRTIs. Griffith *et al.* (2005) performed 3D-pharmacophore database searching. Their pharmacophore models are constructed by using the important interaction between NNRTIs and HIV-1 RT. Hydrogen bond acceptor on HIV-1 RT residue is also included in pharmacophore models. In the work of Ragno *et al.* (2005), three-dimensional quantitative structure-activity relationship (3-D QSAR) studies and docking simulations were developed on indolyl aryl sulfones (IASs), a class of novel NNRTIs highly active against wild type and some clinically relevant resistant strains (Y181C, the double mutant K103N-Y181C, and the K103R-V179D-P225H strain, highly resistant to efavirenz). The synthesis of six designed derivatives (prediction set) allowed disclosure of new IASs endowed with high anti-HIV-1 activities. An efficiency approach by using hierarchical database screenings was presented by Wang *et al.* (2005). This approach combines a pharmacophore model, multiple-conformation rigid docking, solvation docking, and molecular mechanics-Poisson-Boltzmann/surface area (MM-PB/SA) sequentially. The structural information of varied HIV-1 RT/NNRTIs complexes are used in the study. Jorgensen *et al.* (2006) used automated lead generation with the growing program BOMB, Monte Carlo simulations with free-energy perturbation theory for lead optimization, and property analysis with QikProp to design new NNRTIs. They tried to design with simultaneous goals of enhanced performance against common RT mutants, high bioavailability, and facile synthesis. An initial 30 μM lead has been optimized rapidly to the 10 nM level.

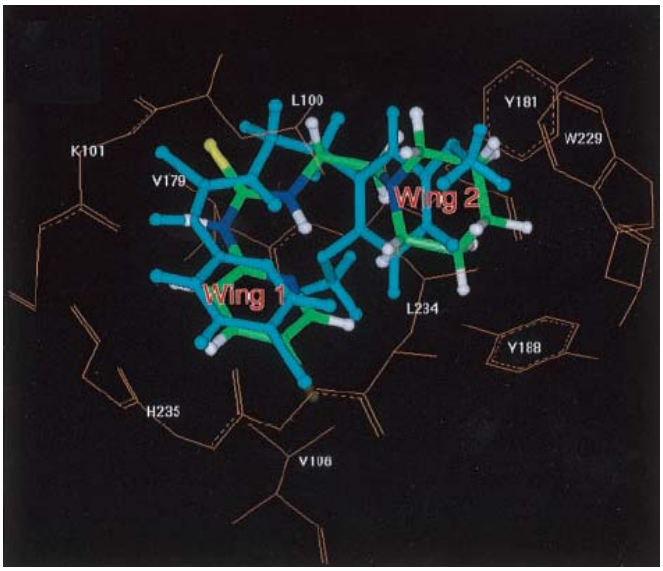

Figure 7 Models of the compound *N*-[2-(1-piperidinoethyl)]-*N*'-[2-(5-bromopyridyl)]-thiourea (color coded by atom type) and compound *N*-[2-(2,5-dimethoxyphenylethyl)]-*N*'-[2-(5-bromopyridyl)]-thiourea (in blue) (HI-236) in the NNI binding site of HIV RT, positioned by a docking procedure. Wing 1 and Wing 2 represent two different regions of the NNI binding site.

Source: Mao *et al.* (2000).

Structure-based drug design

In the 1970s, the development of structural biology and the growing availability of atomic structures lead to the method to identify new medicines by first solving structure of the potential drug target at the atomic level and then using this information to design small molecules that have the required effect (Böhm and Schneider, 2000). In recent years, with the increase of protein structures structure-based drug design is most powerful when it is a part of an entire drug lead discovery process (Anderson, 2003). Structure-based drug design can save time and money by reducing the number of compounds to be experimentally tested, also improving the drug discovery success rate by identifying more-potent and specific binders (Orry *et al.*, 2006).

With structure-based idea, the goal of structure-based drug design is to assemble collections of molecules that are potential ligands of a given target. It can be separated into two approaches. The first is to use ligand design tools to generate new molecules within the boundaries of a binding site, as called de novo design. De novo design comprised methods that attempt to generate compounds starting from an empty binding site and with few or no restrictions of the chemical space to be searched. Fragments of molecules, usually small functional groups, are docked into the binding site and linked together. This method can give the new compounds. The examples of de novo design programs are GrowMol, SMoG, Builder, LUDI, etc. Another approach is to use docking method to select them from larger libraries, as called virtual screening. The examples of docking program are GOLD, DOCK, AutoDock, FlexX, etc.

The general process of structure-based drug design is described as follows. The binding site of the protein is defined. The protein structures can be taken from X-ray crystallography, NMR and homology modeling methods. Then, using computer algorithm, compounds or fragments of compounds from database are positioned into the defined binding pocket, by using docking or de novo algorithms. These compounds are scored and ranked based on their steric and electrostatic interactions

with the target site, by using the selected scoring function. The best compounds are tested with biochemical assays.

In recent years, there are many successes in structure-based drug design (Lyne, 2002). Two of the first drugs to reach the market for the treatment of AIDS by using structure-based drug design approach are amprenavir (agenerase) and nelfinavir (viracept) (Kaldor *et al.*, 1997). They are nonpeptidic inhibitors of HIV-1 protease and are identified by using a combination of structure-based design, an analysis of oral pharmacokinetics, and antiviral activity. Zanamivir (relenza) was developed against neuraminidase (Varghese, 1999). Tomudex was developed against thymidylate synthase (Rutenber and Stroud, 1996). Imatinib mesylate (Gleevec) inhibits Abelson tyrosine kinase that is the enzyme causes chronic myelogenous leukemia (Schindler *et al.*, 2000).

To date, with the development of structure-based drug design, it can be used to design new ligands with increased binding affinity. The examples are shown as follows. A series of acylphenylalanine derivatives was found to be a competitive inhibitor of IL-2/IL-2Rα binding with a midmicromolar IC_{50}, based on a combination of structural information obtained by X-ray crystallographic and NMR studies5 of IL-2 (Tilley *et al.*, 1997). Wiesmann *et al.* (1998) used structure-based drug design approach to design small molecule vascular endothelial growth factor (VEGF) antagonist for the treatment of cancer. The design depends on detailed structural and functional characterization of VEGF-receptor interactions. Structure-based computational database screening revealed the ability to discover new compound inhibiting the target of TAR-RNA of HIV-1 (Lind *et al.*, 2002). From the screening, it was found eleven compounds that can inhibit the Tat-TAR interaction (between 0.1 and 1 μM). Ding *et al.* (2005) revealed a successful structure-based design of a novel class of potent, non-peptide small-molecule MDM2 inhibitors based upon the spiro-oxindole core structure to target the p53-MDM2 interaction. Rastelli *et al.* (2005) studied the effects of displacing some of four buried water molecules during the binding between 7-carbamate and the N-terminal domain of heat shock protein 90 (Hsp90), by using a structure-based drug design approach. Hsp90 is important to the

survival of cancer cells. Modeling of Hsp90–ligand interactions suggested the suitable substituents to the design of 7-carbamate derivatives. Structure-based drug design and optimization from the starting point of diarylurea, reported as a moderate CDK1,2,4,6 inhibitor led to the design of a novel series of cyclin-dependent kinase (CDK) inhibitors containing a macrocyclic quinoxaline-2-one (Kawanishi *et al.*, 2006). Kuno *et al.*, (2006) developed a non-nucleoside adenosine deaminase inhibitor, FR234938, by using rational structure-based drug design. The results indicated that FR234938 has potential anti-inflammatory activity.