LITERATURE REVIEW

Comparative Molecular Field Analysis

For many therapeutic targets of interest, structurebased approaches are not yet applicable because the structure of the target macromolecule is unknown. So, in these cases, QSAR techniques provide the best approach to rational drug design. Traditional (two-dimensional) QSAR methods attempt to correlate biological activity with local features of atoms, whole molecular properties (e.g. charge) and substituent effects (e.g. fragment hydrophobicity indices). The developments in traditional QSAR continue to appear in the literatures. Most interest in this field focuses on three-dimensional QSAR which called 3D-QSAR.

Classical QSAR correlates biological activities of drugs with physicochemical properties or indicator variables which encode certain structural features (Ramsden, 1990; Kubinyi, 1993; Kubinyi, 1995; Hansch and Leo, 1995; Waterbeemd, 1996). In addition to lipophilicity, polarizability, and electronic properties, steric parameters are also frequently used to describe the different size of substituents. In some cases, indicator variables have been attributed to differentiate racemates and active enantiomers (Kubinyi, 1995). However, in general, QSAR analyses consider neither the 3D structures of drugs nor their chirality.

In 1979, Cramer and Milne made a first attempt to compare molecules by aligning them in space and by mapping their molecular fields to a 3D grid (Cramer and Milne, 1979). In the following years, this approach was further developed as the DYLOMMS (dynamic lattice-oriented molecular modelling system) method (Kubinyi, 1993) but was not very well accepted by the scientific community. Several important facts had to work together to allow a broader application of this approach.

In 1986, Svante Wold proposed the use of partial least squares (PLS) analysis, instead of principal component analysis, to correlate the field values with the biological activities.

Especially, in 1988, a key publication appeared in the Journal of the American Chemical Society (Cramer *et al*., 1988) and the method was called comparative molecular field analysis (CoMFA).

Finally, appropriate software became commercially available SYBYL/QSAR, Molecular Modelling Software, Tripos Inc., 1699 S, Hanley Road, St. Louis, MO 63944, USA.

Since 1988, many publications, several reviews and books have appeared on CoMFA subject. This analysis is a useful tool for deriving 3D-QSAR models which related between biological activity and the molecular fields of steric and electrostatic using the Lennerd-Jone and Coulomb potentials, respectively.

CoMFA Applications to Antimalarials

Most successful CoMFA applications on antimalarial compounds are on an artemisinins, quinazolines (Siavoush, 2006; Zhiyong, 1999 and 1997), (Fang, *et al*., 2007; Avery, *et al*., 1997, 2002, 2003; Feng, *et al*., 2002; Jung and Kim, 2001), and alkoxylated-hydroxylated chalcones (Devendra, *et al*., 2005; Xue, *et al*., 2004).

Avery Mitchell A. group designed and synthesized artemisinin derivatives based on a CoMFA model. Next, the new designed artemisinin derivatives was test as part of a program to cinstruct and validate modeling tools for drug design of novel antimalrial agents based on the natural product lead, (+)-artemisinin (Haraldson, *et al*., 1997). Because of the effectiveness of artemisinin in the treatment of drug resistant *Pf* and its rapid clearance of cerebral malaria, development of clinically useful semisynthetic drugs for severe and complicated malaria was prompt. Avery Mitchell A. group still utilized CoMFA and hologram QSAR on artemisinin analogues with known in vitro antimalrial activity for designing the new potent inhibitor for resistant enzymes (Avery, *et al*., 2002 and 2003, Fang, *et al*., 2007; Feng, *et al*., 2002; Jung and Kim, 2001). For the CoMFA application on alkoxylated and hydroxylated

chalcones, were conducted to determine the factors required for the activity of these compounds (Xue, *et al*., 2004, Liu, *et al*., 2003, and Devendra, *et al*., 2005).

There are now a few practical applications of CoMFA in Pyr and Cyc derivatives. For example, recently, Singh, Vineet and Tiwari, Meena (Vineet and Meena, 2007) applied CoMFA method to find the structure activity relationship of some 4,6-diamino-1,2-dihydrotriazine derivatives having good activity against resistant strain of Ala16Val + Ser108Thr *P. falciparum*. Their model developed was shown that the descriptors, ovality (steric descriptor), dipole-dipole energy (thermodynamic descriptor), while stretch bend energy (thermodynamic descriptor) negative to the biological activity. Statistical analysis was shown the model to be fit ($r = 0.924$, $r^2 = 0.853$, F-test = 18.840, t-test = 4.340, stdev = 0.244, variance = 0.051) and predictable ($r^2_{LOO} = 0.693$, $r^2_{pred} = 0.265$). Based on their r^2_{pred}, it can be implied that the model should be re-derived to be the good model for both training set and also test set compounds.

However, the Pyr amd Cyc derivatives have been used as test analogues for the new QSAR techniques, such as, Anton, J. Hopfinger groups used Pyr, Cyc and other antimalarials as a test compounds for their new Free Energy Force Field 3D-QSAR and 4D-QSAR techniques (Duca, *et al*., 2001 and Santos, *et al*., 2001)

In prevoius literatures, there are no CoMFA models of Pyr or Cyc derivatives which active against wild type and quadruple mutant (Asn51Ile, Cys59Arg, Ser108Asn, Ile164Leu) type of *Pf*DHFRs. Therefore, this research we have planed to establish the CoMFA models for Pyr and Cyc derivatives. The obtained CoMFA results have been published as open sources for guiding to develop new and effective antifolate antimalarials against in *Pf*DHFRs.

ONIOM Calculations

Generally, no single theoretical method is able to provide both the accuracy and acceptable computational cost that are required for the investigation of such

chemical processes. Accurate ab initio quantum mechanics (QM) methods either scale nonlinearly with the size of the system, or have large pre-factors that prevent them to be applied to large systems. Low cost molecular mechanics (MM) methods are widely used and scale linearly but have the obvious weakness of the poor description of bond breaking and forming processes. Meanwhile, in many biological systems the actual reaction only localizes in a relatively small region.

A breakthrough came with the realization that it is not necessary to use a single computational method, which resulted in the development and application of a variety of hybrid methods. These methods utilize the localization feature of large reaction systems, and most combine the merit of accuracy of a QM method with the low cost of MM methods. The our Own N-layer Intregrated molecular Orbital molecular Mechanics (ONIOM) method (Dapprich, *et al.*, 1999; Vreven and Morokuma, 1999-2000, Tschumper and Morokuma, 2002; Morokuma, *et al.*, 2006) is a versatile and popular hybrid method. ONIOM divides the system into several onion-like layers, treating the active center with the highest level ab initio QM method, while outer layers can be treated with less expensive methods, such as low-level ab initio QM, semiempirical QM, or MM methods as see their in Figure 8.

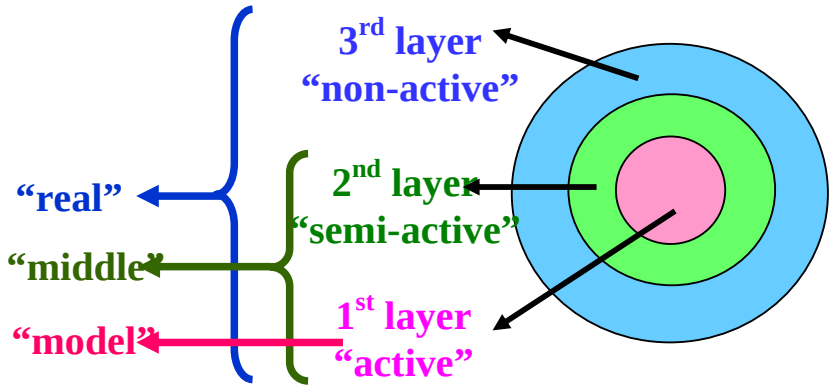

Figure 8 Example of a three-layer partitioning scheme

The three-layer ONIOM(high:medium:low) energy is an approximation to the energy at the high level for the real system, $E_{real\text{-}high}$, referred to as the target, and is

given by where high, medium, and low refer to the high-, medium-, and low-level theoretical methods, respectively, while model, middle, and real refer to the model, middle, and real systems, respectively. The middle system is a part cut from the real system, and the model system is a part cut from the middle system as shown in Figure 9. The model and middle systems are mended by link atoms to satisfy the valencies if covalent bonds are cut. With the term "energy", we typically refer to a relative energy, such as the binding energy or the barrier height (Vreven and Morokuma, 2006, Vreven *et al*. 2006 and Morokuma, 2006).

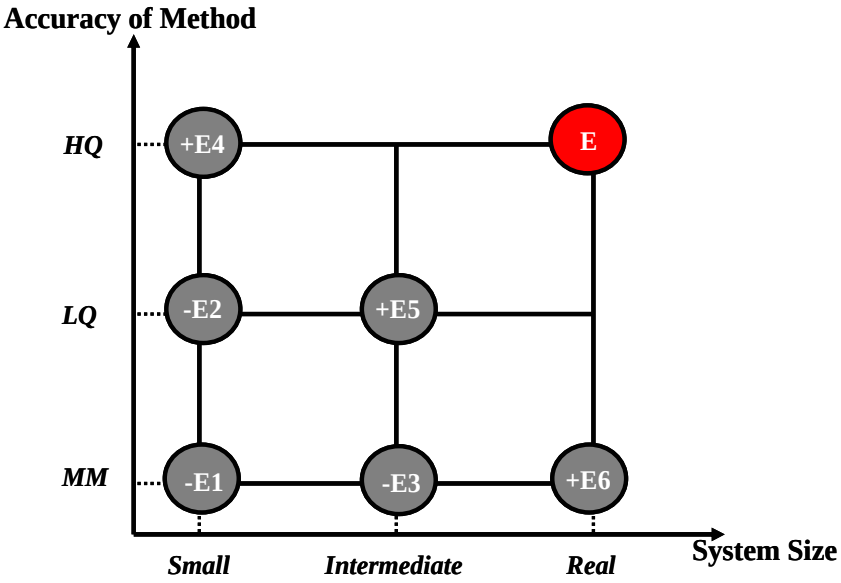

Figure 9 The energy extrapolation scheme of the three-layer ONIOM method

Based on the advantages of ONIOM approach, there are now many applications of ONIOM calculations in the biological systems, especially, to extrapolate the binding energy of ligand-protein interactions.

ONIOM Applications to Ligand-enzyme Binding Energy

Threr are a few applications of ONIOM which applied to the ligand-enzyme biological systems. Mostly, ONIOM calculations on these biological systems came from Hannongbua and co-workers. They applied this method on HIV1-RT enzymes which complexed with many type of ligands. The obtained results clearly demonstrated the different of binding energy between the potent and the poor ligands for wild type and mutant type of HIV1-RT (Kuno, *et al*., 2003 and 2006, Nunrium, *et al*. 2005, Saen-oon, 2005).

Recently, the ONIOM3 approach was also succesful to describe interactions of saquinavir with HIV-1 protease in comparison between the wild-type and mutant types. And the quantum computational chemistry calculations capable of investigating inhibitor–enzyme interactions at the molecular level are employed (Saen-oon, 2007).

To date, the powerful ONIOM techniques study on binding energy of antifolates, Pyr, Cyc and WR99210, are never reports, therefore, the goal of our study is to establish the applicability of the ONIOM method for the antifolate–quadruple mutant *Pf*DHFR enzyme. Gaining insight into the particular interaction energy terms will also give us a better understanding and more detailed information on the interaction between the antifolate inhibitor and the binding pocket of the mutant *Pf*DHFR. Detailed knowledge of the interactions between drug and the binding site of an enzyme can provide a structural explanation for the structure-based drug design of *Pf*DHFR inhibitors and a better understanding of the action of these antifolates, especially with respect to the quadruple mutations.