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REGULAR ARTICLE

DFT Study of Binding Energies between Acetohydroxyacid Synthase and its Sulfonylurea Inhibitors: An Application of Quantum Pseudoreceptor Model

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Abstract: The quantum mechanical interaction energy between the Acetohydroxyacid synthase (AHAS) and its sulfonylurea inhibitors were calculated with an efficient density functional theory (DFT) and a pseudoreceptor model composed of the amino acids surrounding the ligands. The results show that the calculated quantum mechanical interaction energies correlate well with experimental free energies with the correlation coefficients of 0.92 for six sulfonylurea inhibitors and the standard deviation of 0.83kcal/mol. In comparison with the force field method, the binding free energies were estimated by AutoDock 4.2 program with the correlation coefficient of 0.76 and the standard deviation of 1.40kcal/mol. It indicates that the binding between the AHAS and herbicides can be well characterized by quantum pseudoreceptor model. Based on the quantum mechanical interaction energies, some AHAS inhibitors with high binding affinity were designed by introducing a hydroxyl group at the *para* position of aromatic ring and on the sulfonylurea bridge respectively.

AMS subject classifications: 92E10, 92C05

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Key words: Acetohydroxyacid synthase, Sulfonylurea herbicides, DFT, AutoDock, Binding energy

1 Introduction

Acetohydroxyacid synthase (AHAS) is a key enzyme in the biosynthetic pathway of the branched-chain amino acids, such as valine, leucine and isoleucine in plants and microorganisms [1, 2]. It catalyzes the condensation of two molecules of pyruvate into 2-acetolactate or one molecule of pyruvate and one molecule of 2-ketobutyrate into 2-aceto-2-hydroxybutyrate as the precursors in valine, leucine and isoleucine biosynthesis [3-5]. Inhibition of AHAS may lead to the starvation of microorganisms and plants due to lack of branched-chain amino acids [6] . As a result, AHAS becomes an important target for inhibitors to be used as herbicides, and several class of effective herbicides were discovered [7,8]. AHAS herbicides fall into five families: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidines (TP), pyrimidinylbenzoates (PB), and sulfonylamino carbonyltriazolinones (SCT) [9,10]. The typical sulfonylurea herbicides are effective ultralow dosage agrochemicals that are non-toxic to animals. The general structure is a central bridge with an o-substituted aromatic ring attached to the sulfur atom and a heterocyclic ring disubstituted in both meta positions and attached to the distal nitrogen atom of the sulfonylurea bridge as shown in **Figure 1** [11]. The heterocyclic ring can be either a pyrimidine as in chlorimuron ethyl (CE) or a triazine as in metsulfuron methyl (MM) shown in **Table 1**. With the wide use of the sulfonylureas, resistant weeds began to emerge, to overcome the herbicidal resistance, it is imperative to develop new and high effective AHAS inhibitors [13,14]. Recently, Duggleby and coworkers reported the crystal structure of Arabidopsis thaliana AHAS (*At*AHAS) in complex with chlorimuron ethyl [15], thus it is possible to design some novel AHAS inhibitors with the aid of molecular modeling techniques.

In the computational aided drug design, the biggest challenge is accurate estimation of the binding affinity between protein and inhibitors [16]. Among a variety of methods for calculating the binding energy between inhibitor candidates and their biological targets, Molecular mechanics (MM) is generally applicable to study biological systems with thousands of atoms, but it is hard to describe the charge transfer and explicit polarization between the protein and the ligands [17-18]. Quantum mechanical (QM) method can fully take into account the electronic charge transfer and polarization, but most of QM approaches are limited to small systems with less than one hundred atoms [19,20]. Quantum mechanics

approach can be used to estimate the interaction between receptor and ligands by simplifying system and lowering accuracy. Semi-empirical QM-based scoring function was first used by Merz to estimate the binding energies of protein-ligand complexes [21]. Molecular fraction with a conjugate caps method (MFCC) and the fragment molecular orbital method (FMO) were proposed by Zhang and Fukuzawa, where a large system is divided into smaller parts to perform quantum mechanical calculations one by one [22-25]. Wang studied the interaction energies between CDK2, H1N1, FKBP12 and its inhibitors in the combination quantum receptor model with density functional theory [26,27]. In addition, the hybrid QM/MM approaches provide a useful alternative where the most important parts are treated quantum mechanical, and the other parts are molecular mechanically [28-31]. In the QM/MM methods, parameters for novel ligands are still required.

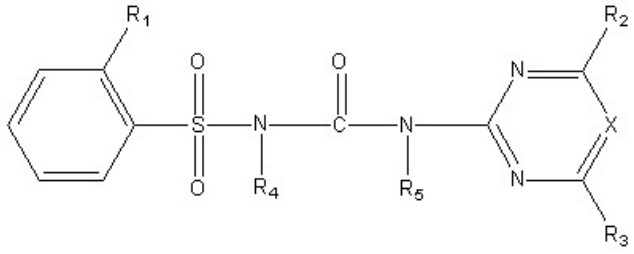
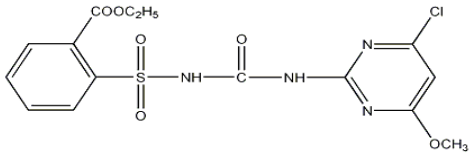
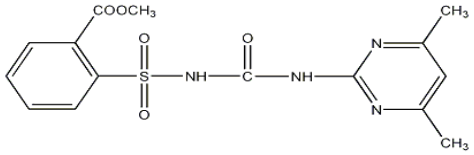
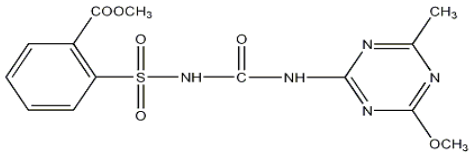
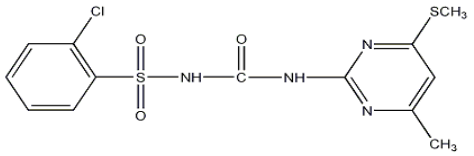
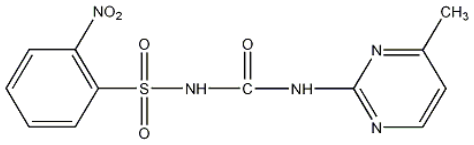
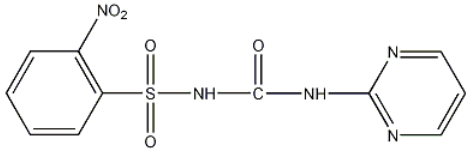


Figure 1: The general structure of sulfonylurea inhibitor of *At*AHAS.

In this paper, the binding energies between *At*AHAS and its six sulfonylurea inhibitors are estimated by combining DFT approach with a protein model. The calculated results show a good correlation between the quantum interaction energies and experimental binding free energies with the correlation coefficients of 0.92. In comparing results from Autodock4.2 with the correlation coefficient R=0.76, it was indicated that the quantum interaction energy gives a better performance in rank-ordering the binding affinity between *At*AHAS and its inhibitors. Finally, a few new inhibitors were designed based on the quantum interaction energy.

Table 1: Structures and experimental K_i of the six sulfonylurea inhibitors, $\Delta G_{\exp} = RT \ln K_i \ (T = 298K)$, the experimental data taken from [12].

Ligand	Structure	K_i (μM)	ΔG_{exp} (kcal/mol)
CE		0.0108	-10.860
SM		0.0255	-10.350
MM		0.0362	-10.150
A		7.0100	-7.031
B		0.2450	-9.019
C		32.7000	-6.119

2 Methods of calculation

2.1. Preparation of quantum pseudoreceptor model

To perform protein-ligand interaction energy fully quantum mechanically, the whole protein is simplified to a pseudoreceptor model composed of the amino residues only close to the ligands [26,27]. In general, the residues close enough to the ligand has a great effect on the binding energy and the residues far from the binding site may have little contributions to the

interaction energy. The interaction energy between the protein and ligands thus could be approximated by the interaction energy between the smaller binding pocket residues and ligands. The pseudoreceptor model has been successfully applied to the H1N1, FKBP12 and CDK2 systems. In the quantum calculation of the interaction between *At*AHAS and its inhibitors, the homodimer of wild-type *At*AHAS was firstly constructed by the symmetry operations in Pymol software based on the crystal structure of *At*AHAS-CE (1YBH) [15]. The quantum pseudoreceptor model was then built by selecting the CE inhibitor and 37 amino residues within 6.5Å of the inhibitor chlorimuron ethyl as shown in **Figure 2**. The water molecules were removed during the construction of protein model and the dangling bonds were capped with hydrogen atoms. All ionized residues were assigned protonation states according to the pdb2pqr package at neutral pH [32].

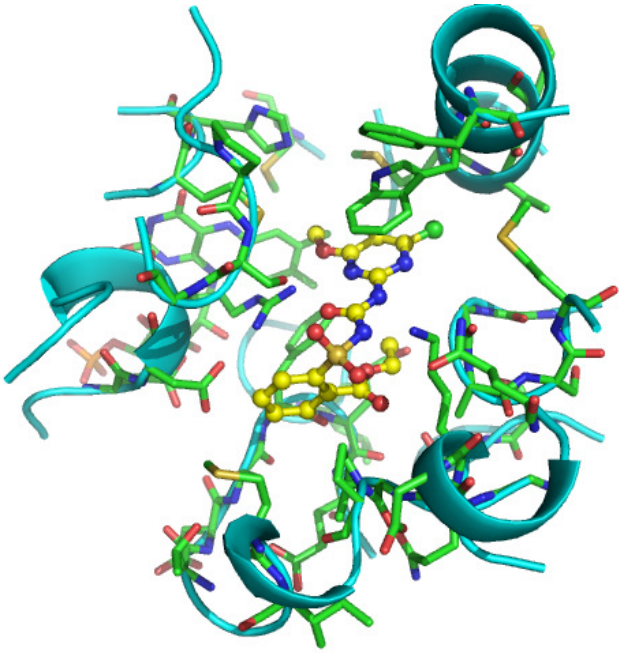


Figure 2: The structure of the quantum pseudoreceptor model. The 37 amino acids within 6.5Å of ligand and FAD are displayed in sticks; ligand CE is shown in ball-and-stick representation, *At*AHAS is displayed in cartoon.

2.2. Minimization and calculation of binding energies

Density functional theory (DFT) has emerged as a QM method that is both sufficiently rigorous and efficient to be used for accurately describing biologically relevant molecular

systems at a reasonable computational cost [33]. SIESTA (Spanish Initiative for Electronic Simulations with Thousands of Atoms) is an original method and a software that uses density functional theory, it can be used to perform geometry minimization and calculate interaction energy [34]. To reduce the computational cost, the smaller minimal single zeta (SZ) basis set was used for the *At*AHAS protein model, the larger double zeta (DZ) basis set was used for the carbon atoms and the double zeta polarization (DZP) basis was for other atoms in the ligands. During the optimizations of the quantum pseudoreceptor model, the heavy atoms were fixed at the X-ray positions, the hydrogen atoms were relaxed by 100 steps of conjugate gradient minimization implemented in the Siesta package. Then, the structure of each ligand was fully optimized in the pocket of the fixed pseudoreceptor model. For each geometry optimization of the protein-ligand complexes, the conjugate gradient method was implemented until the maximum atomic force is less than 0.04eV/Å. The initial coordinates of compound CE is obtained from PDB entry 1YBH, the starting structure of other ligands was determined by superposing with ligand CE. The binding energy was estimated by:

$$\Delta E_{QM} \approx E_{complex} - E_{model} - E_{ligand}, \quad (1)$$

where $E_{complex}$, E_{model} and E_{ligand} are the energy of the receptor-ligand complex, pseudoreceptor model and the isolated ligand respectively.

2.3. Autodock 4.2 Method

To compare with molecular mechanics force fields (MM) methods, the AutoDock4.2 programs were performed [32-37]. AutoGrid4.2 was used to calculate the grid maps representing the protein in the actual docking process. The grid dimensions were selected to be 45^3, with a spacing of 0.375 Å between the grid points. As the location of CE in the complex was known, the grid box was centered on the binding site of the ligand. Docking was performed with AutoDock4.2 program, using the Lamarckian genetic algorithm (LGA) [17]. Docking parameters includes an initial population of random individuals with a population size of 150 individuals, a maximum number of 25 million energy evaluations, a maximum of 27,000 generations, an elitism value of 1, a mutation rate of 0.02, and a crossover rate of 0.80. For each ligand, 20 independent docking runs were carried out. The docking results were clustered by positional root-mean-square deviation (RMSD) of 2.0 Å, only conformations with this RMSD deviation or less will be placed in the same cluster and ranked by increasing energy. The best docked conformations were those with the lowest binding energy. During the docking process, all the cofactors such as FAD and Mg^{2+} in the

complex structure of *At*AHAS are considered.

3 Results and discussion

Based on the energy-minimized structure, the quantum mechanical interaction energies between *At*AHAS and ligands are estimated following Eq. (1). The used chemical structures and apparent inhibition constants $K_i(\mu M)$ of six sulfonylurea inhibitors [12] are listed in **Table 1**, and the chlorimuron ethyl (CE), sulfometuron methyl (SM) and metsulfuron methyl (MM) are commercial sulfonylurea herbicides. In the calculations, Arabidopsis thaliana AHAS was modeled by quantum pseudoreceptor model composed of 37 amino acids within 6.5Å surrounding the ligand CE as shown in **Figure 2**. The calculated quantum mechanical interaction energies are summarized in **Table 2**. As illustrated in **Figure 3**, the interaction energies were well correlated with the experimental binding energies at correlation coefficients of R=0.92 and standard deviation of 0.83 kcal/mol. However the QM interaction energies are much larger than the experimental ones due to negligence of solvent effects.

Table 2: Calculated quantum interaction energies between six sulfonylurea inhibitors and *At*AHAS based on the pseudoreceptor model and $E_{model} = -77334.1921\text{eV}$.

Ligand	$E_{complex}(\text{eV})$	$E_{ligand}(\text{eV})$	$\Delta E_{QM}(kcal/mol)$
CE	-84273.3729	-6932.9048	-144.6620
SM	-83982.7802	-6642.6602	-132.7520
MM	-83449.2214	-6109.2700	-136.6380
A	-82906.0981	-5567.6023	-99.2003
B	-83171.4176	-5832.8199	-101.5490
C	-82983.8161	-5645.6682	-91.1812

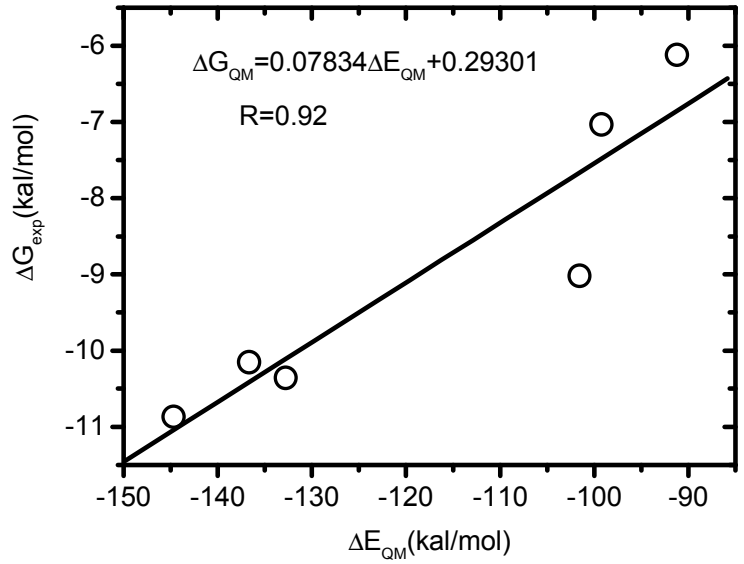


Figure 3: Relationship between calculated quantum mechanical interaction energies and the experimental binding energy $\Delta G_{\exp}$ calculated as $RT \ln K_i$. Calculations were performed using quantum pseudoreceptor model.

The binding process between receptor and its ligands occurs in solution; therefore the solvation effects play an important role in binding affinity. To consider the solvation effects on the binding between *At*AHAS and its ligands, the solvation free energies of ligands were estimated by combining the density functional theory and Possion-Boltzmann equation [19]. The differences of solvation free energies between CE and other ligands were calculated and are summarized in **Table 3** following the equation,

$$\Delta G_{ligand}^{sol} = G_{ligand}^{sol} - G_{CE}^{sol}. \quad (2)$$

As shown in **Table 3**, the difference of solvation free energies ΔG_{ligand}^{sol} is less than 3.6kcal/mol. As the cancellation of solvation free energies would occur among the complex $\Delta G_{complex}^{sol}$, $\Delta G_{receptor}^{sol}$ and ΔG_{ligand}^{sol}, the solvent effect on the relative binding energy is expected to have fewer orders of magnitude than that of binding interaction energies. For similar ligands, the solvent and entropy effects could be assumed similar, although the solvent and entropy effects are ignored, the calculated binding interaction energies nevertheless show an excellent correlation with the experimental binding energies.

Table 3: Solvation free energy of six sulfonylurea ligands and the relative solvation free energies were calculated using $\Delta G_{Ligand}^{Sol} = G_{Ligand}^{Sol} - G_{CE}^{sol}$.

Ligand	$G_{Ligand}^{Sol}(kcal/mol)$	$\Delta G_{Ligand}^{Sol}(kcal/mol)$
CE	-43.884	0
SM	-43.865	0.019
MM	-44.671	-0.787
A	-40.308	3.576
B	-41.153	2.731
C	-41.802	2.082

To compare with the calculation of the molecular mechanics force field, AutoDock4.2 program was carried out for the same set of ligands. The Autodock binding energies are summarized in **Table 4**. The relationship between the binding energies estimated from Autodock4.2 with experimental results is plotted in **Figure 4**, where the correlation coefficient R=0.76 and standard deviation of 1.40 kcal/mol were obtained. In comparison with the results from quantum receptor model, it indicates that the QM interaction energies show a much better performance than those of AutoDock4.2.

Table 4: Binding energies between *At*AHAS and its six sulfonylurea ligands calculated from Autodock4.2 program, $\Delta G_{\exp} = RT \ln K_i (T = 298K)$ taken from [12].

Ligand	$\Delta G_{\exp}(kcal/mol)$	$\Delta G_{MM}(kcal/mol)$
CE	-10.86	-10.93
SM	-10.35	-9.55
MM	-10.15	-9.65
A	-7.03	-9.30
B	-9.01	-8.95
C	-6.11	-8.57

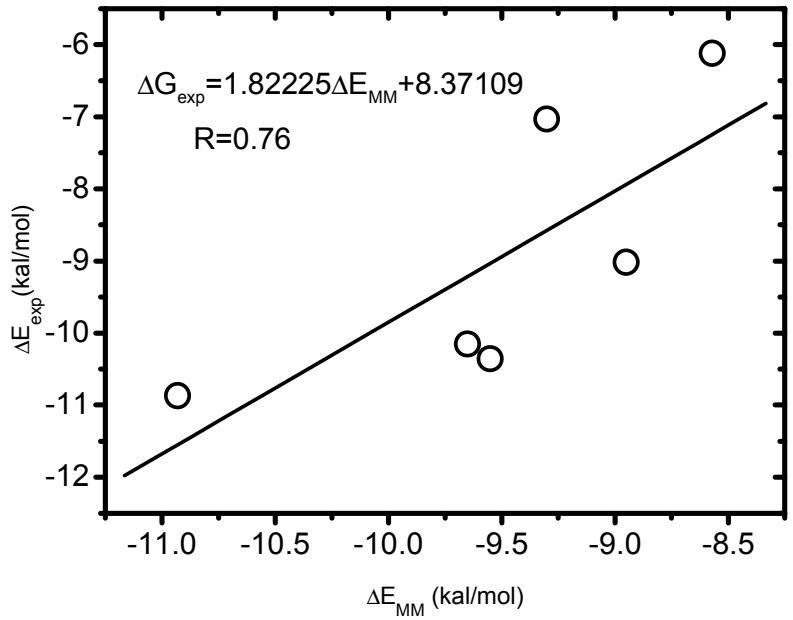


Figure 4: Plot of experimental binding energy $\Delta G_{\exp}$ calculated as $RT \ln K_i$ versus the binding energy ΔG_{MM} calculated by AutoDock4.2.

The binding between sulfonylurea herbicide chlorimuron ethyl and the target *At*AHAS has been well determined by X-ray diffraction experiment [15], the structural modifications on the ligand CE were guided by the QM interaction energies. Six new sulfonylurea inhibitors were obtained, whereby the structure and QM interaction energies were summarized in **Table 5**, in which the K_i values are predicted based on the linear relationship between the calculated QM interaction energies and the experimental binding energy in **Figure 3**. It was indicated that some of them exhibit much improved binding affinities to the target *At*AHAS in comparison with CE. In particular, by adding a hydroxyl group at the *para* position of aromatic ring and substituting methoxy group for chlorine in the CE structure, the compound 6 is obtained, which is predicted to possess high binding affinity. The important hydrogen bond interactions between the sulfonylurea inhibitor 6 and key residues of *At*AHAS are shown in **Figure 5**. There exist four strong hydrogen bonds between the ligand 6 and the binding pocket. It indicates that a strong hydrogen bond forms between the hydroxyl group at the *para* position of aromatic ring and the carboxyl group of the residue Asp376 with the hydrogen bond length of 1.63 Å, could effectively improve the binding affinity. In addition, there are two hydrogen bond forms between Arg377 and methoxy group as well as C=O group of the sulfonylurea bridge of the compound 6 with the hydrogen bond distance of 2.21 Å and 1.83 Å. It also appears that there is a typical hydrogen

bond interaction between SO_2 group of the sulfonylurea bridge and Lys256 with the hydrogen bond length of 1.90 Å. Some new inhibitor with much improved binding affinities can be obtained by introducing a hydroxyl group at the *para* position of aromatic ring and on the sulfonylurea bridge respectively, these results could be helpful to find new sulfonylurea inhibitors for experimentalists in the future.

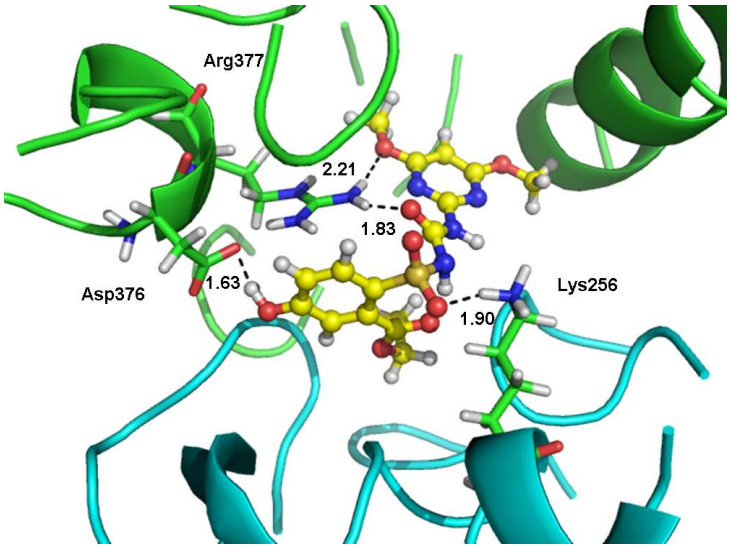
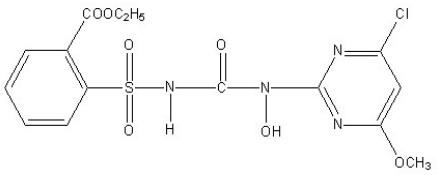
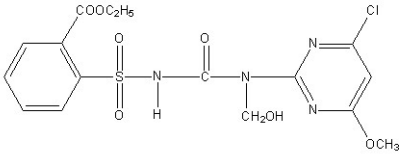
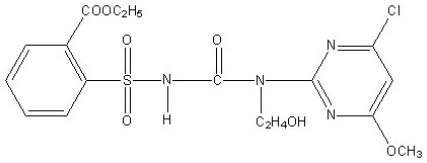
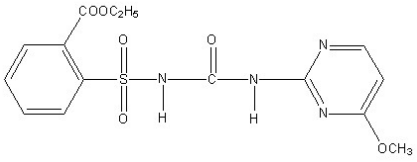
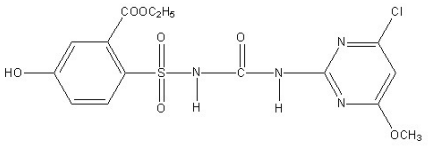
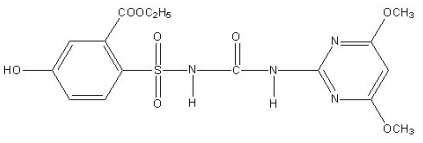


Figure 5: The important hydrogen bond interactions with H-bond length (unit Å) between the sulfonylurea inhibitor 6 and the key residues of *At*AHAS. The inhibitor is represented by ball-and-stick, the key residue is shown in sticks, *At*AHAS is displayed in cartoon.

Table 5: The structure, QM interaction energies and calculated inhibition constants of the six designed inhibitors. All energies are in kcal/mol and the K_i values are predicted based on the linear relationship between the calculated QM interaction energies and the experimental binding energy in **Figure 3**.

Designed Inhibitors	Structure	ΔE_{QM} $(kcal/mol)$	Calculated $K_i(\mu M)$
1		-137.5186	0.020804
2		-156.1177	0.001779
3		-172.5730	0.000202
4		-149.7213	0.004144
5		-150.8784	0.003556
6		-184.2894	0.000043

4. Conclusions

The quantum mechanical interaction energy between the *At*AHAS and its sulfonylurea inhibitors were calculated with an efficient density functional theory (DFT) and a pseudoreceptor model composed of the amino acids surrounding the ligands. The results show that the calculated quantum mechanical interaction energies correlate well with experimental free energies at the correlation coefficients of 0.92 and the standard deviation of 0.83kcal/mol for six sulfonylurea inhibitors. To compare with the force field method, the MM binding energies were obtained by AutoDock 4.2 program with the correlation coefficient of 0.76 and the standard deviation of 1.40kcal/mol. It indicates that the binding between the protein and herbicides can be well characterized by quantum pseudoreceptor model. Based on the quantum pseudoreceptor model, new *At*AHAS inhibitors with high binding affinity were designed, which can be helpful for experimentalists to find new sulfonylurea inhibitors.

Acknowledgements

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