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Bimodal Complexations of Steroids with Cyclodextrins by a Flexible Docking Algorithm

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Abstract

A flexible docking algorithm was developed for studying the inclusion complexes of cyclodextrins with steroids in aqueous solution by an optimization method and an empirical function. The function is used to estimate the binding free energy including intermolecular interaction energy, the conformational energy change, and the solvation energy. The bimodal complexations of twelve steroids in β- and γ-CD cavities were studied by the algorithm. For the two orientations of the guests in the cavity, the possible binding regions were investigated, and the lowest energies for the inclusion complexes in the binding regions were obtained. The stability constant for each orientation was estimated from the optimized energy components by a quantitative model. Therefore, the preferential orientations of the guests were found out from the results finally.

Introduction

Cyclodextrins (CDs) possess remarkable properties in forming inclusion complexes with a variety of guest molecules [1–2]. Due to the unique characteristics, CDs have been widely applied in many fields [3–5]. Steroid hormones are natural compounds with a great variety of important biological functions, and have been widely used in pharmacy [6]. For example, estradiol and estrone are responsible for the development of female characteristics. However, the lower solubility strongly limits their applications. One of the efficient ways to enhance the solubility of steroid hormones is by complexation with cyclodextrins (CDs) [7–9]. Therefore, the complexation of steroids with CDs in aqueous is an important research topic. A lot of papers that investigated the stability of steroid-CD by measuring their association constants using high-performance liquid chromatography or other experimental techniques have been published [10–15].

On the other hand, the inclusion mode and depth of the steroids into the hydrophobic cavity of CD are also crucial to pharmaceutical purpose. The structural predictions for some steroids with CDs have been provided by NMR studies and other techniques [16–23]. The initial effort to study the binding mechanism of several steroids with α-, β- and γ-CD in a comprehensive fashion was done by Uekama *et al.* [10, 11] and their opinion is that the inclusion occurs primarily at the A-B ring of the steroids. Bednarek *et al.* [18] probed inclusion structures of prednisolone, ethinyloestradiol and estriol with β-CD by an aqueous H NMR technique and molecular dynamics calculation. They concluded that ethinyloestradiol and estriol penetrate deeply into the β-CD cavity and bind strongly, in contrast, prednisolone binds weakly and nonspecifically. Forgo *et al.* [20] studied the inclusion structures of progesterone and hydrocortisone with β-CD using rotating frame *Overhauser* spectroscopy (ROESY) and considered that progesterone is fully immersed in the β-CD, but hydrocortisone is partly included in the cavity of β-CD. Their recent research about inclusion complexes of six ketosteroids with β-cyclodextrin by ROESY also found partial and full immersion phenomena in the cavity [16]. Although the "low resolution" cyclodextrin complexes of some steroids have been studied by NMR and other techniques, the detail structures of the inclusion complexes and the comparison of different binding modes are not yet clear.

In this paper, the goal of our research is to investigate the structures of the inclusion complexes of two main binding modes, and predict the stability constants for each mode. Twelve steroids with β- and γ-CD were examined by our flexible docking algorithm. An empirical function that estimates the binding free energy, including intermolecular interactions, intramolecular flexibility, and the effect of the solvent, was used as an object function to be minimized. The sketch of two main binding modes (bimodal inclusions, A-ring up and A-ring down) is shown in Figure 1. To locate the guest in a reasonable region in the cavity for each binding mode, traversing procedures of the guest in the cavity along the cavity axis were computed. Based on the information of the reasonable binding region, the final complex structures were obtained through docking calculations. For the inclusion complexations of β-CD and γ-CD with the twelve steroids, the stability constants for the two orientations were predicted from the optimized energy components by the nonlinear regression with the experimental values. Comparing

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the stability between each pair, the preferential orientation of each steroidal molecule in the cavity is clear.

Theory and method

Flexible docking

In Figure 1, the XY plane is the mean-plane defined by all the glycosidic oxygens, the Z-axis is perpendicular to the XY plane, through the origin and pointing to the primary side. The geometry center of each steroid molecule was moved to the origin. The position and orientation of the steroid molecule in the cavity are defined by the coordinates of its geometry center and three Eulerian angles, respectively. Therefore, Z-coordinate of the center of the guest denotes the insert depth into the cavity.

All the calculations were implemented using our flexible docking algorithm FDOCK, which is a combination of the global optimization algorithm FAEA [24] with a local optimization algorithm L-BFGS [25]. The energy calculation is based on CFF91 force field [26]. A rapid and efficient implicit solvent model [27] to calculate the solvent effect in aqueous solution was employed in FDOCK.

In rigid docking, only the relative position (T_x, T_y, T_z) and the relative orientation (θ, φ, ψ) of the guest molecule in the cavity of CD are the parameters to be optimized, which can be used in less flexible system [28]. In flexible docking, all the interactive molecules are flexible, therefore, the internal coordinates of each molecule are also needed to be optimized. The flow chart of our flexible docking algorithm FDOCK is showed in Figure 2. At first, the host is fixed at the original point as in Figure 1, and the guest is located at the wide side of the cavity, then, move the guest along the Z-axis step by step. In each step, the relative position (T_x, T_y, T_z) and the relative orientation (θ, φ, ψ) of the guest molecule in the cavity of CD are optimized by FAEA, and the coordinates of the host and guest molecules with a given relative position are optimized with L-BFGS. Furthermore, a random local search procedure was also used to optimize both the relative position and orientation of the guest molecule in the cavity, as well as the atomic coordinates of each molecule.

Usually molecular mechanics methods only search the lowest energy structure, but the corresponding binding site is probably unreasonable, due to not-seeing the big energy barriers during the inclusion procedure. However, in some cases, to explore the possible binding region in the cavity is necessary. In our method, the guest is moved from the wide side of cavity to the narrow side along the Z-axis step by step. In each step, only the range of T_z will be re-calculated. In order to get the local minimum limited in the small range of T_z, the maximum number of iterations for L-BFGS should not be too large (50 was used). Thus, the changing trend of the energy, as a function of the Z-coordinate of the geometry center of the guest, can be obtained, which will give us the information about energy barriers and the possible binding region. Based on the possible binding region, an accurate calculation (maximum number of iterations for L-BFGS =

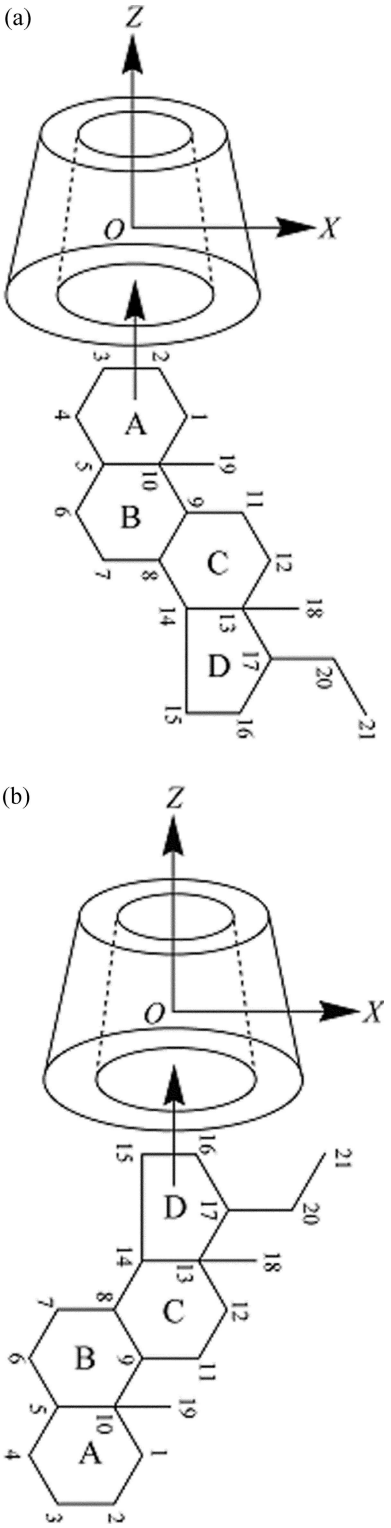


Figure 1. Conventional representation of the steroid ring system (right side), and schematic representation of two orientations of the steroid entering the cavity of CD: (a) A-ring up; (b) D-ring up.

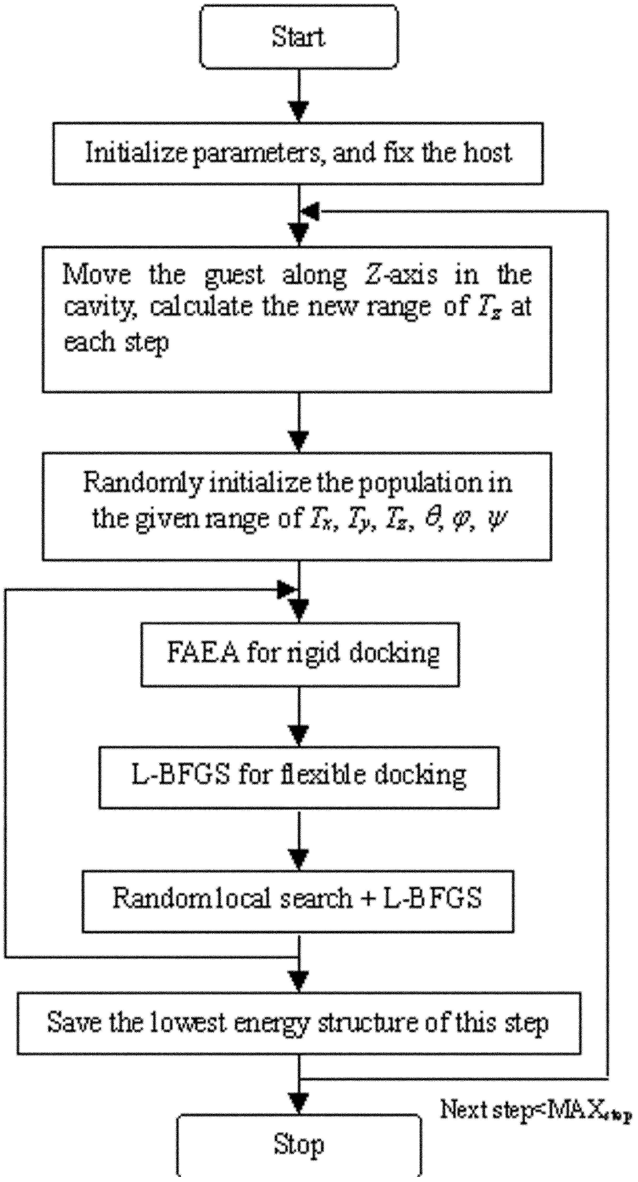


Figure 2. Flowchart of the flexible docking algorithm FDOCK.

200) by FDOCK was employed again to seek the lowest energy structure as the final inclusion complex.

To account for bimodal complexations, two opposite orientations for the steroids A-ring up and D-ring up (see Figure 1) were calculated in two independent runs of FDOCK, by rotating the guest to A or D-ring up as the initial input structure and setting $\theta \in [0, \pi/2]$ to keep the orientation during the moving procedure.

Binding free energy function

The consistent force field (CFF91) [26] was applied to estimate the intramolecular energies of host and guest and the interaction energies between them. According to the basic thermodynamics of the system, the empirical binding free energy gives by:

$$\Delta G_{\text{total}} = E_{\text{inter}} + \Delta E_{\text{intra}}^{\text{host}} + \Delta E_{\text{intra}}^{\text{guest}} + \Delta G_{\text{sol}} \quad (1)$$

The interaction energy E_{inter} is constituted of the van der Waals term E_{vdw} and the electrostatic term E_{elec} between host and guest molecules. $\Delta E_{\text{intra}}^{\text{host}}$, and $\Delta E_{\text{intra}}^{\text{guest}}$ are the changed conformational energy of the host and the guest, respectively, composed of the changed energy of bond stretching, angle bending, torsional energy, out of plane bending, and all the cross terms, as in Equation (2).

$$\begin{aligned}\Delta E_{\text{intra}} \;=\; & \Delta E_{\text{vdw}} + \Delta E_{\text{elec}} + \Delta E_{\text{bond}} + \Delta E_{\text{angle}} \\ & + \Delta E_{\text{tor}} + \Delta E_{\text{out\_of\_plane}} \\ & + \Delta E_{\text{bond\_bond}} + \Delta E_{\text{angle\_angle}} + \Delta E_{\text{bond\_angle}} \\ & + \Delta E_{\text{bond\_dihedral}} \\ & + \Delta E_{\text{angle\_dihedral}} + \Delta E_{\text{angle\_angle\_dihedral}} \\ & + \Delta E_{\text{bond\_bond\_1\_3}}\end{aligned} \quad (2)$$

The distance-dependent dielectric constant $\epsilon = 4r_{ij}$ is used in the calculations. ΔG_{sol} is the solvation free energy, which will be discussed in the next section.

An implicit solvent model

The hydrophobic effect plays an important role in the formation of CD-guest complexes. A way of reducing the computational cost without sacrificing the accuracy of the results is to incorporate the properties of the solvent into the energy function. In this study, an implicit solvent model [27] based on a very efficient analytical evaluation of the solvent accessible surface area (SASA) [29] has been employed in predicting the solvation energy of the complexation. The solvation energy results from:

$$\Delta G_{\text{sol}} = G_{\text{sol}}^{\text{complex}} - G_{\text{sol}}^{\text{free-host}} - G_{\text{sol}}^{\text{free-guest}} = \sum_{i=1}^{N_h+N_g} \sigma_i \Delta A_i \quad (3)$$

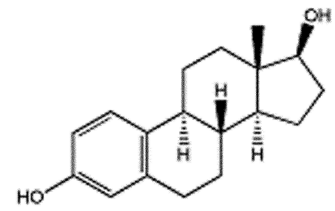
where ΔA_i denotes the difference between the solvent accessible surface area of the ith atom in the complex and the surface area of the same atom in the isolated state. σ_i is the atomic solvation parameter. In this study, the values of the solvation parameters are from Ref. [27]. As the hydrophobic and hydrophilic atoms have positive and negative value of σ, respectively, the solvation energy drives the hydrophobic atom entering the cavity and keeps the hydrophilic atom staying outside.

The exposed solvent-accessible surface area Ai is calculated using the approximate analytical expression of Hasel *et al.* [29]:

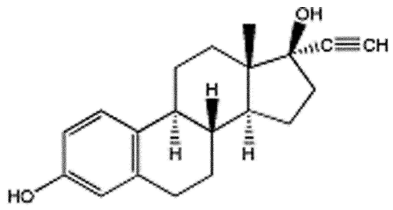
$$A_i = A_i^{\text{tot}} \prod_{i=1}^{N} \left[1 - \frac{p_i p_{ij} b_{ij}(r_{ij})}{A_i^{\text{tot}}} \right] \quad (4)$$

A_i^{tot} is the total solvent-accessible surface area of an isolated atom i with radius R_i as defined with a solvent probe of radius R_{solv} ($R_{\text{solv}} = 0.14$ nm), given by:

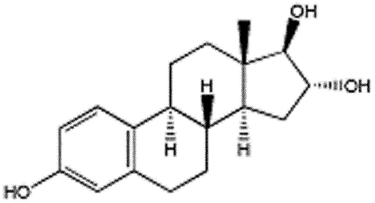
$$A_i^{\text{tot}} = 4\pi (R_i + R_{\text{solv}})^2 \quad (5)$$



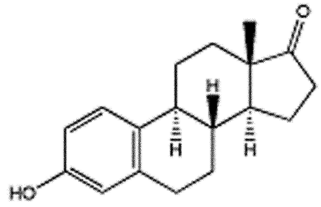
1. Estradiol



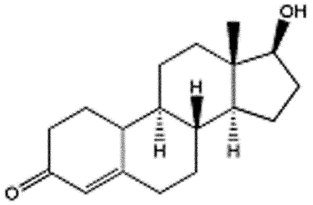
2. Ethinyloestradiol



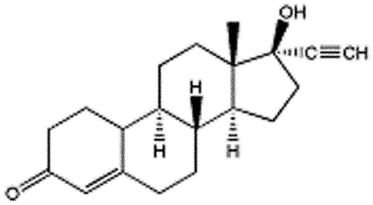
3. Estriol



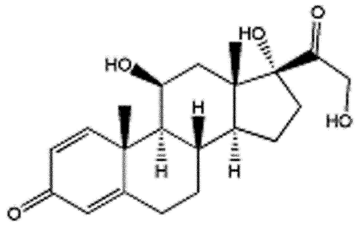
4. Estrone



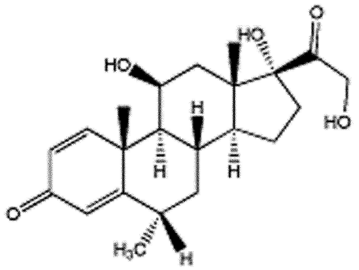
5. Nandrolone



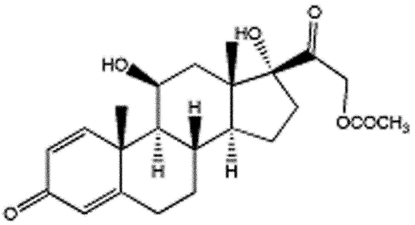
6. Norethisterone



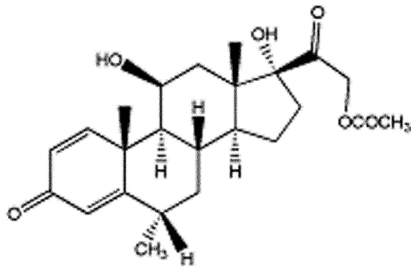
7. Prednisolone



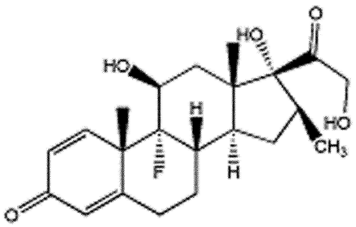
8. Methylprednisolone



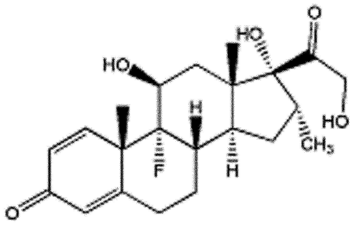
9. Prednisolone_acetate



10. Methylprednisolone_acetate



11. Betamethasone



12. Dexamethasone

Figure 3. Structural formulas of the twelve steroids investigated.

b_{ij} is the amount of surface area removed due to overlap with an atom j with a distance r_{ij} from atom i:

$$b_{ij}(r_{ij} = \begin{cases} 0 \\ \pi[R_i + R_{\text{solv}}][R_i + R_j + 2R_{\text{solv}} - r_{ij}] \\ \quad \times[1 + (R_j - R_i)r_{ij}^{-1}] \\ r_{ij} \geq R_i + R_j + 2R_{\text{solv}} \\ r_{ij} < R_i + R_j + 2R_{\text{solv}} \end{cases} \quad (6)$$

The atom type parameter p_i has been introduced in Equation (4) to reduce empirically the effect of double counting the overlap area when multiple overlaps of the surface of atom i with those of many other atoms j occur. The pair parameter p_{ij} servers as an additional reducing factor that distinguishes between first and next covalently bound neighbour atoms j of atom i. The parameters p_i and p_{ij} ($p_{ij} = 0.8875$ for covalently bound first neighbours and $p_{ij} = 0.3516$ for others) have been optimized by Hasel *et al.* [29]. Their values mapping onto the atom types used in CFF91 force field are listed in Table 1.

In our calculations, all the atoms of guests and hosts are counted in ΔG_{sol}. The change between complex and isolated host and guest drives the hydrophobic guest molecule to enter or partly enter the cavity of CD and the hydrophilic atom keep away from the cavity. To enhance the solvation effect and reduce the intermolecular van der Waals interaction between host and guest, ΔG_{sol} and E_{vdw} between host and guest are weighted by coefficient 2 and 0.5, respectively. ΔG_{total} is used as the object function in energy minimization procedure.

Stability of bimodal complexes

A quantitative model based on each energy term was established to predict the stability constants. In this model, two possible binding positions (A-ring up or D-ring up) are taken into account [30], i.e.,

$$\begin{aligned} \ln K &= \ln(K_{\text{A-up}} + K_{\text{D-up}}) \\ K_{\text{A-up}} &= \exp\Big(-\frac{1}{RT}(a_0 + a_1 E_{\text{vdw}}^{\text{A-up}} + A_2 E_{\text{elec}}^{\text{A-up}} \\ &\quad + a_3 \Delta E_{\text{intra}}^{\text{A-up}} + A_4 \Delta G_{\text{sol}}^{\text{A-up}})\Big) \quad (7) \\ K_{\text{D-up}} &= \exp\Big(-\frac{1}{RT}(a_0 + a_1 E_{\text{vdw}}^{\text{D-up}} + A_2 E_{\text{elec}}^{\text{D-up}} \\ &\quad + a_3 \Delta E_{\text{intra}}^{\text{d-up}} + A_4 \Delta G_{\text{sol}}^{\text{D-up}})\Big) \end{aligned}$$

The coefficients in the above nonlinear equation were estimated by minimizing the error function:

$$\text{err} = \frac{1}{N}\sum_{i=1}^{N}[\ln K_i^{\text{pred}} - \ln K_i^{\text{obs}}]^2 \quad (8)$$

where $\ln K_i^{\text{obs}}$ are given by experimental methods. In our study, the error function was also optimized using the L-BFGS algorithm [25].

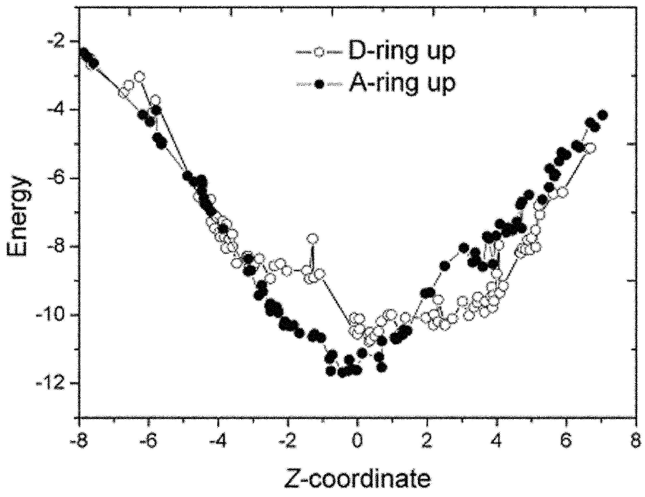


Figure 4. The energy (in kcal mol^{-1}) for the complex of estradiol (**1**) with β-CD, as a function of the Z-coordinate (in Å) of the geometry center of the guest, while the guest moving through the cavity. Solid circle for A-ring first entering from the wide side of CD, hollow circle for D-ring first entering from the wide side of CD.

Results and discussion

The original structures of β-CD and γ-CD were adopted from the literature [31–32] and minimized by InsightII. The initial structures of the steroids were built and also optimized by InsightII. The chemical structures of the twelve investigated steroids are shown in Figure 3.

Investigation of the possible binding region

Figure 4 gives the minima of 100 steps (with solid circle) for molecule **1** with A-ring up moving in the cavity of β-CD from -8 to 8 Å in 100 steps, and another 100 minima (with hollow circle) for D-ring up case. It can be seen that, for this molecule, there is only one stable binding region along the Z-axis without big energy barrier. Furthermore, the bimodal complexations of **1** with β-CD are possible, due to the inclusions of the two orientations are all complete ($Z_{\text{min}} \in [-1, 1]$) and the energy difference is not large. This situation is also found for steroids **3**, **4**, and **5** with β-CD, because for the two entering ways the steric hindrance is not strong.

But for β-CD with the steroids **2**, and **6** in D-ring up orientation, and **7–12** in A-ring orientation, the variation of the 100 minima is completely different. As an example, Figure 5 shows the energy change for **10** with A-ring up (solid circle) during moving in the cavity of β-CD. In Figure 5(a) for A-ring up, two binding regions I (partial inclusion) and **II** (deep inclusion) with lower energy were detected and separated by a sharp peak. Figure 5(b) clearly shows that this peak is caused by the increase of the conformational energy of the complex due to the collision of the substituents of the guest with the narrow cavity wall. When the A-ring of molecule **10** first enters the cavity from $Z = -10$ Å, it will be quickly driven to position **I**, but further movement to position **II** will be hindered by the energy barrier. Therefore, for steroid 10 entering from the secondary side in A-ring up orientation,

Table 1. Atomic types in CFF91 and solvation parameters

Atom type	R_i[a] (Å)	P_i[a]	σ_i[b] (kcal mol^{-1} Å^{-2})	Description
oh	1.52	1.080	−0.060	Oxygen bonded to hydrogen
o'	1.50	0.926	−0.060	Oxygen in carbonyl group
oc, oe	1.70	0.922	−0.060	sp^3 oxygen in ether or ester
c, c*	1.72	1.554	0.012	Generic sp^3 carbon, carbon in carbonyl group, non_amides
c =, c = 1	1.72	1.554	0.012	Non aromatic end doubly bonded carbon, non aromatic next to end doubly bonded carbon
coh	1.72	1.554	0.012	sp^3 carbon in acetals with hydrogen
ct, ct2	1.72	1.554	0.012	sp carbon involved in a triple bond
c1	1.80	1.276	0.012	Aliphatic CH group
c2	1.90	1.045	0.012	Aliphatic CH_2 group
c3	2.00	0.880	0.012	Aliphatic CH_3 group
cp	1.80	1.073	0.012	sp^2 aromatic carbon
h, h*, ho, hc	1.00	0.944	0.000	Hydrogen atom
f	1.47	0.906	0.012	Fluorine atom

[a] Force field parameters R_i and P_i used in Hasel's approximate expression for different atom type [29].
[b] The solvation parameters σ_i is as used in the implicit solvation model [27], the solvation parameters σ_i of fluorine is the same as carbon atom due to its non-polar property.

the deep inclusion complexation at region **II** is impossible, only the partial complexation at region **I** is reasonable, and in good agreement with NMR studies [18, 20]. As a comparison, the energy change for this molecule with D-ring up (hollow circle) was also given in Figure 5, showing that the guest can be easily located in region **III** when D-ring first enters from the wide rim, whereas A-ring first entering from the narrow rim is blocked. From our calculated energy curve, A-ring up or D-ring of some steroids can enter the cavity from the primary side. In Figure 4 if A-ring or D-ring of steroid **1** enters from the primary side ($Z = 10$ Å), it will fall into the same binding region as D-ring or A-ring enters from the secondary side. In Figure 5(a) if D-ring first enters from $Z = 10$ Å (solid circle), i.e., the narrow side, no energy barrier from $Z = 10$ to 1 Å to prevent the guest to go down to position **II**. However, by the experimental research results [6, 18, 20], the steroids like **7–12** enter the cavity from the wide side rather than the narrow side. Therefore, in this study, only two modes that the steroids entering from the secondary side in A-ring up or D-ring up orientations were taken into account.

By investigating the possible binding region in the cavity of β-CD for each steroid in two orientations, the following results can be obtained: (1) for steroids **2** and **6** in D-ring up orientation, the C≡CH group on D-ring locates inside the cavity, without protruding the primary rim; (2) for steroids **7–12** in A-ring up orientation, the partial complexation in the cavity is reasonable; (3) for the other cases, the steroids are deeply included in the cavity. For γ-CD, due to its large cavity size, both for A-ring up and D-ring up orientations, the guests will be deeply immersed in the cavity, forming stable inclusion complexes.

Structures of inclusion complexes

Based on the above investigation, only the complex structure with lowest energy in the reasonable binding region was considered as the optimized result. All the results were listed in Tables 2 and 3 for β-CD and γ-CD system, respectively, including van der Waals energy (E_{vdw}), electrostatic energy (E_{elec}), solvation energy (ΔG_{sol}), the total changed conformational energy of CD and steroid ($\Delta E_{\mathrm{intra}}$) and the Z-coordinate of the geometrical center of steroids in the cavity of CD, reflecting the inclusion depth in the cavity. Larger value of the Z-coordinate means deeper insert in the cavity.

Figure 6 displays some examples in A-ring up orientation. In the complex structure of **1**-β-CD (Figure 6(a)), A-ring penetrates the primary rim of β-CD cavity, and D-ring is located near the β-CD wide rim, whereas the hydrophobic B- and C-rings are completely included in the cavity, and the oxygen atoms connected to 3-C and 17-C are exposed to the solvent.

In the complex structure of **9**-β-CD (Figure 6(b)), the CH_3 group at 10-C forms strong steric hindrance to prevent the A-ring from deeply entering the cavity. The OH group at 11-C increased the hindrance effect, and also the hydrophilic hydroxyl prefers to escape from the cavity, resulting in a partial complexation. Compared to **9**, **10** more shallowly insert in cavity due to the steric hindrance brought by the one more CH_3 group at 6-C shown in Figure 6(c). The insert depth can also be figured out from the Z-coordinate in Tables 2 and 3.

Figure 7(a) shows the inclusion complex for β-CD with **8** in D-ring up orientation, in which D-ring is included in the cavity. The similar complex structures were also found for **10**- and **12**-β-CD in D-ring up orientation. Another two examples in D-ring up orientation are given for the complex structures of **6**-β-CD and **6**-γ-CD in Figures 7(b) and (c). In

Table 2. The component energies obtained by FDOCK for twelve steroids with β-CD[a]

Guest	Orient	ΔG_{total}	E_{vdw}	E_{elec}	ΔG_{sol}	ΔE_{intra}	Z_coord/Å
1	A up	−29.280	−31.340	0.088	1.464	0.508	−0.762
	D up	−28.288	−30.642	0.197	1.478	0.679	0.554
2	A up	−31.549	−33.020	−0.014	1.750	−0.265	−1.242
	D up	−27.011	−29.852	0.083	2.221	0.537	−2.768
3	A up	−29.665	−31.682	−0.017	1.583	0.451	−0.921
	D up	−29.050	−31.346	−0.077	1.900	0.473	0.698
4	A up	−29.158	−31.206	0.029	1.476	0.543	−0.473
	D up	−27.880	−30.344	0.207	1.506	0.751	0.239
5	A up	−28.278	−30.748	0.167	1.423	0.880	0.891
	D up	−28.060	−30.692	0.141	1.516	0.975	0.115
6	A up	−30.063	−32.480	0.163	1.769	0.485	0.894
	D up	−27.390	−30.280	0.060	2.349	0.481	−2.861
7	A up	−28.336	−31.850	−1.095	3.487	1.122	−3.554
	D up	−32.460	−36.560	0.061	3.946	0.093	−2.670
8	A up	−25.679	−29.724	−0.066	3.541	0.570	−4.703
	D up	−31.909	−35.858	−0.044	3.417	0.576	−2.373
9	A up	−29.105	−32.752	−1.134	3.574	1.207	−3.690
	D up	−34.205	−37.768	−0.034	3.441	0.156	−1.954
10	A up	−26.891	−30.900	−0.839	3.701	1.147	−4.492
	D up	−34.442	−38.522	−0.010	3.374	0.716	−1.731
11	A up	−32.065	−35.792	−0.721	3.695	0.753	−4.012
	D up	−34.398	−38.558	−0.206	2.963	1.403	0.287
12	A up	−32.707	−36.848	−0.016	3.733	0.424	−3.947
	D up	−34.669	−38.782	−0.220	2.933	1.400	0.535

[a] The energy unit is kcal mol^{-1}, and $\Delta G_{\text{total}} = E_{\text{vdw}} + E_{\text{elec}} + \Delta G_{\text{sol}} + \Delta E_{\text{intra}}$, $\Delta E_{\text{intra}} = \Delta E_{\text{intra}}^{\text{host}} + \Delta E_{\text{intra}}^{\text{guest}}$.

Table 3. The component energies obtained by FDOCK for twelve steroids with γ-CD[a]

Guest	Orient	ΔG_{total}	E_{vdw}	E_{elec}	ΔG_{sol}	ΔE_{intra}	Z_coord/Å
1	A up	−28.3890	−28.436	0.032	1.135	−1.120	0.291
	D up	−27.7790	−27.496	0.015	0.965	−1.263	0.888
2	A up	−30.2310	−30.250	−0.033	1.174	−1.122	0.563
	D up	−29.4580	−29.532	−0.897	1.731	−0.760	−1.355
3	A up	−28.5230	−28.528	−0.009	1.163	−1.149	−0.466
	D up	−28.1110	−27.898	−0.878	1.446	−0.781	1.390
4	A up	−28.2600	−28.304	−0.007	1.071	−1.020	0.292
	D up	−26.8090	−26.802	−0.019	0.906	−0.894	1.040
5	A up	−25.1960	−26.288	−0.018	1.069	0.041	1.440
	D up	−27.1740	−27.164	−0.002	0.963	−0.971	1.027
6	A up	−30.1520	−30.474	0.039	1.304	−1.021	0.752
	D up	−27.4520	−27.582	−0.017	1.040	−0.893	1.476
7	A up	−30.3800	−31.702	−1.358	2.334	0.346	0.639
	D up	−29.6000	−30.656	−1.573	2.067	0.562	0.966
8	A up	−31.3530	−32.616	−1.044	1.971	0.336	1.098
	D up	−30.6970	−31.554	−1.720	1.921	0.656	1.306
9	A up	−31.7240	−33.834	−0.377	2.144	0.343	0.304
	D up	−31.6900	−32.978	−0.949	2.258	−0.021	0.634
10	A up	−32.6460	−34.006	−0.931	2.138	0.153	0.911
	D up	−32.9370	−34.602	−1.530	2.549	0.646	0.560
11	A up	−32.8650	−34.226	−1.441	2.325	0.477	0.159
	D up	−33.4080	−35.044	−0.737	2.475	−0.102	0.100
12	A up	−32.8210	−33.864	−1.316	2.111	0.248	0.655
	D up	−32.0830	−32.172	−1.727	1.639	0.177	1.789

[a] The energy unit is kcal mol^{-1} $\Delta G_{\text{total}} = E_{\text{vdw}} + E_{\text{elec}} + \Delta G_{\text{sol}} + \Delta E_{\text{intra}}$, $\Delta E_{\text{intra}} = \Delta E_{\text{intra}}^{\text{host}} + \Delta E_{\text{intra}}^{\text{guest}}$.

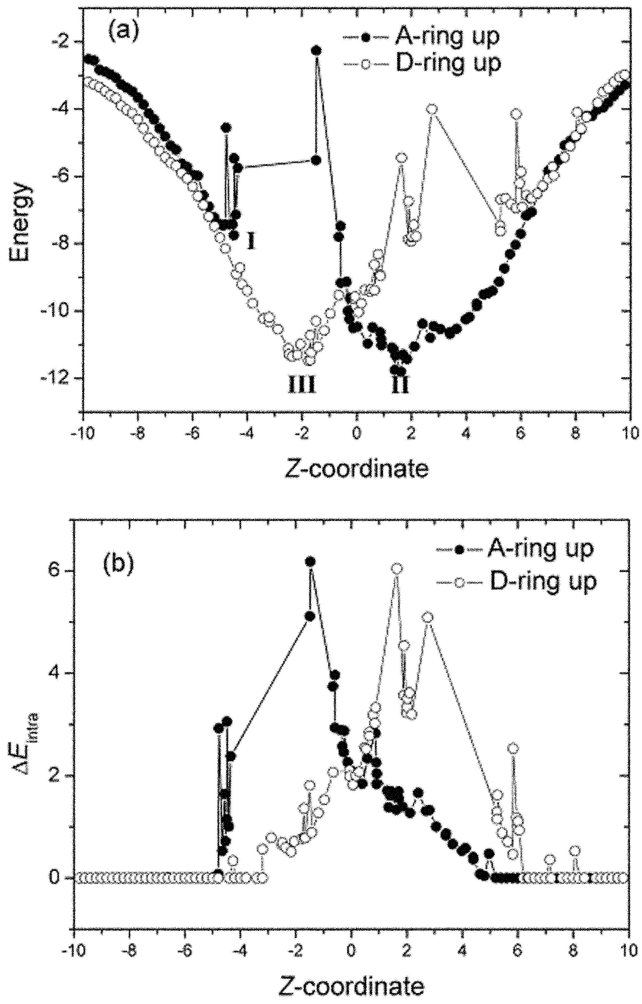


Figure 5. The energy (in kcal mol^{-1}) for the complex of methylprednisolone acetate (**10**) with β-CD, as a function of the Z-coordinate (in Å) of the geometry center of the guest, while A-ring (solid circle) or D-ring (hollow circle) of the guest first entering from the wide side of CD and moving through the cavity: (a) total energy; (b) conformational energy change.

Figure 7(b), due to the steric hindrance, the C≡CH group locates inside the cavity of β-CD, without protruding the primary rim, whereas, for the large cavity of γ-CD (Figure 7(c)), the C≡CH group can protrude outside the narrow rim.

Prediction of the stability constants of complexations and the favourable orientations

The experimental stability constants for the twelve steroids with β-, and γ-CD measured by Sadlej-Sosnowska [13] were adopted (Table 4). This error function in Equation (8) was optimized by the L-BFGS algorithm [27] For β-, and γ-CD, respectively, giving the results of a_0, a_1, a_2, a_3, and a_4. With these coefficients, the predicted ln K, ln K_{A-up} and ln K_{D-up}, were calculated by means of Equation (7), and listed in Table 4. The linear relationships between ln K_{pred} and ln K_{obs} for β-CD were also calculated, respectively, and shown in Figure 8. The correlation coefficients indicated that the predicted stability constants are in good agreement with the experimental data.

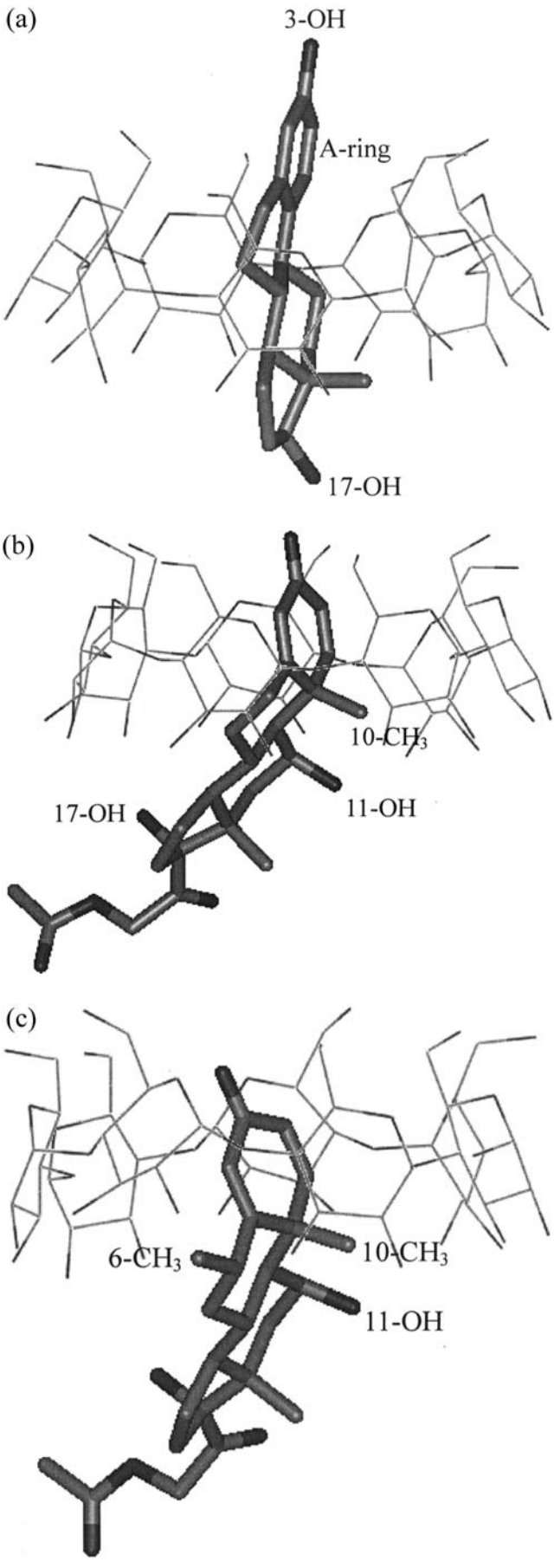


Figure 6. The optimised structures of the inclusion complexes of β-CD with steroids in A-ring up orientation: (a) **1**; (b) **9**; (c) **10**.

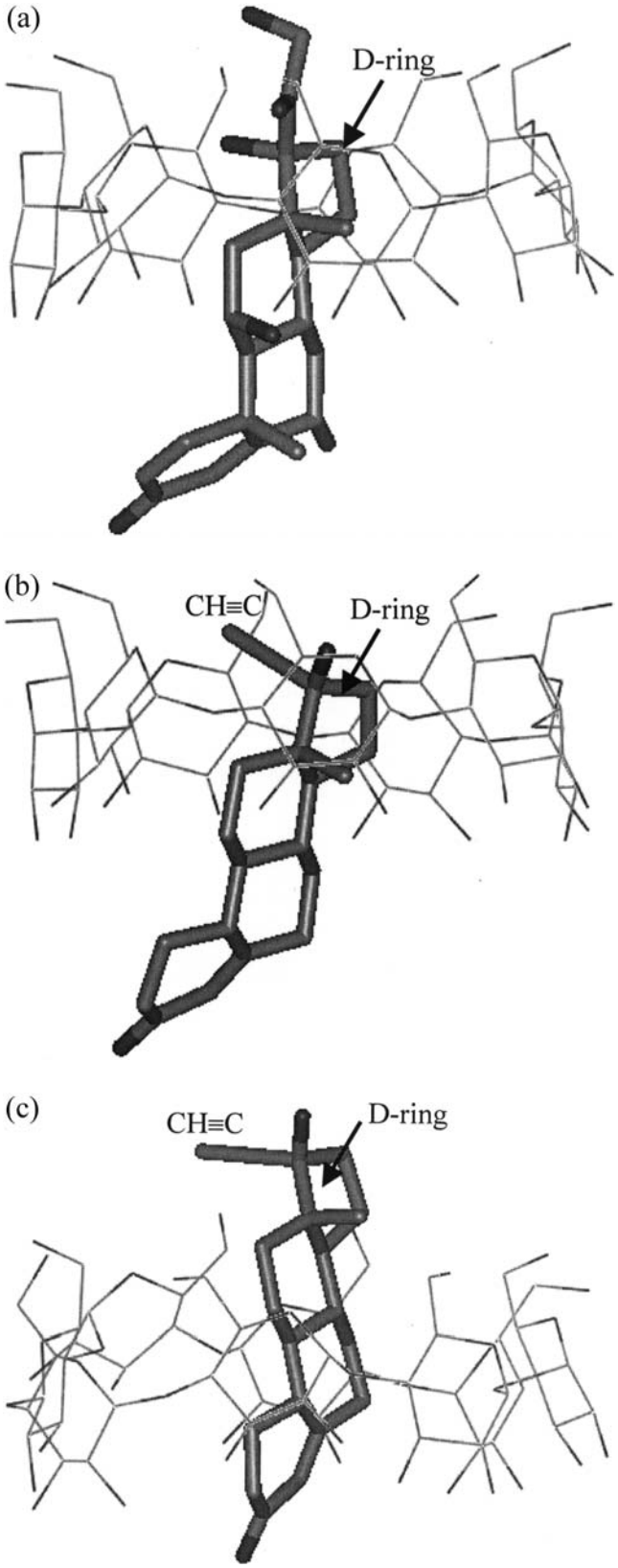


Figure 7. The optimised structures of the inclusion complexes in D-ring up orientation: (a) **8**-β-CD; (b) **6**-β-CD; (c) **6**-γ-CD.

Table 4. Predicted and experimental complex stability constants for the inclusion complexation of twelve steroids with β-CD and γ-CD, and the comparison of stable orientations

Guest	Host	ln K_{A-up} [a]	ln K_{D-up} [a]	ln K_{pred}	ln K_{obs} [b]
1	β-CD	7.745	6.977	8.126	8.829
2	β-CD	8.909	5.634	8.946	8.936
3	β-CD	7.955	7.303	8.375	8.455
4	β-CD	7.807	6.707	8.094	8.039
5	β-CD	6.939	6.666	7.505	7.170
6	β-CD	7.185	5.608	7.373	6.846
7	β-CD	5.982	4.268	6.147	6.328
8	β-CD	3.052	4.903	5.049	5.438
9	β-CD	6.030	5.908	6.664	6.346
10	β-CD	4.473	5.427	5.753	5.438
11	β-CD	5.995	5.984	6.683	6.685
12	β-CD	4.593	6.156	6.346	6.551
1	γ-CD	7.920	8.096	8.705	8.868
2	γ-CD	8.539	7.967	8.987	9.269
3	γ-CD	8.036	7.696	8.574	8.071
4	γ-CD	7.741	7.118	8.171	7.844
5	γ-CD	4.701	7.346	7.415	7.741
6	γ-CD	8.204	7.257	8.532	8.575
7	γ-CD	6.160	5.772	6.678	6.888
8	γ-CD	6.425	6.112	6.974	6.908
9	γ-CD	5.957	7.028	7.322	7.223
10	γ-CD	7.033	6.415	7.464	7.193
11	γ-CD	6.760	7.476	7.874	8.055
12	γ-CD	7.200	7.625	8.128	8.189

[a] ln K_{A-up} (for A-ring up), ln K_{D-up} (for D-ring up), and ln K_{pred} were calculated by Equation (7), where for β-CD $a_0 = -2.085$, $a_1 = 0.159$, $a_2 = 1.720$, $a_3 = 0.806$, $a_4 = 1.316$; and for γ-CD $a_0 = 1.631$, $a_1 = 0.188$, $a_2 = 0.625$, $a_3 = 1.345$, $a_4 = 0.460$.
[b] ln K_{obs} published in Ref. [13].

The binding constants ln K_{A-up} and ln K_{D-up} reflect the stability of the two possible isomeric complexes. By comparing this quantitative information (Table 4), the favourable orientation of the guest molecule can be suggested. For the steroid compounds **1–7** with β-CD, ln K_{A-up} > ln K_{D-up}, indicates that A-ring is more favourable to be included in the β-CD cavity. But for the steroid compounds **8**, **10**, and **12** with β-CD, ln K_{D-up} > ln K_{A-up} means that the D-ring locates in the cavity more unfavourably. It is mainly due to the steric hindrance and the effect of OH preventing A-ring from deeply inserting into the cavity, as we discuss in the previous section. In contrast to β-CD, our calculated results also indicate that the larger cavity of γ-CD was more favorable for the complexation of **8** and **10** than the cavity of β-CD, and the stability constants of two orientations are close. For **9** and **11** with β-CD, ln $K_{D-up} \approx$ ln K_{A-up} hints no obvious tendency between the two orientations.

From Table 4, among the complexes with γ-CD, for most steroids, A-ring up is their preferential orientation, but for the steroid **5**, **9**, **11**, **12**, the D-ring up orientation is prior to the A-ring up orientation. The "steric hindrance" from the substituents may become an advantaged factor for the steroids to tightly contact with the large cavity of γ-CD. Only for steroid **1**, the difference of the calculated stability con-

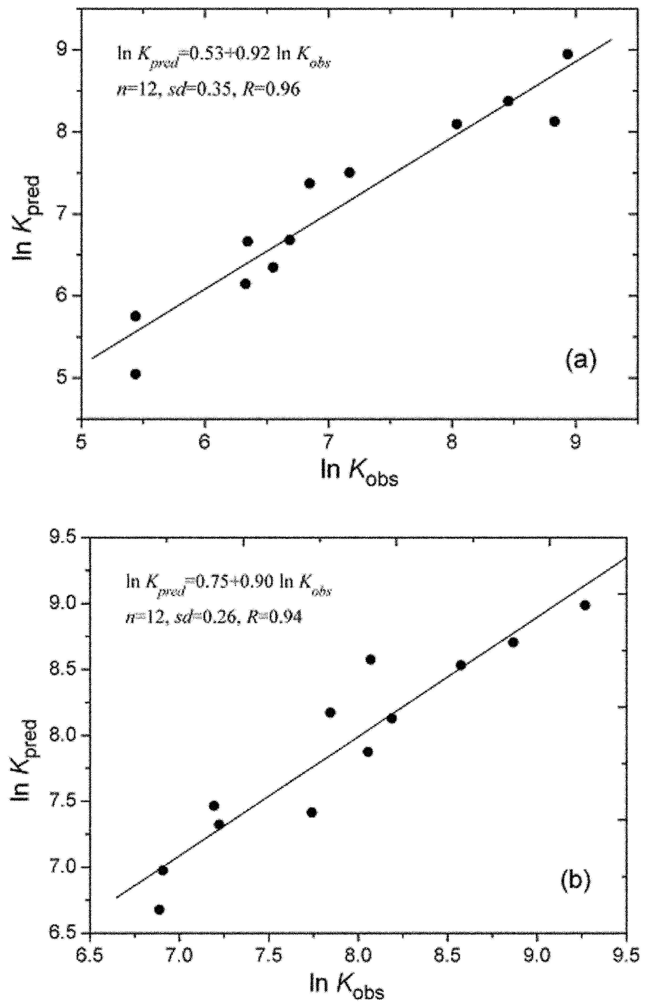


Figure 8. The plot of ln K_{pred} vs. ln K_{obs} for the inclusion of the twelve steroids with (a) β-CD; (b) γ-CD.

stants for the bimodal complexes is very small, indicating no obvious preference between the two orientations.

The only difference between **5** and **6** is the C≡CH group. In A-ring up orientation, **6**-β-CD has larger stability constant than **5**-β-CD, but the former has smaller ln K_{pred}. It is because when **6** inserts into the cavity in D-ring up orientation, the steric hindrance caused by the horizontal C≡CH group destabilize the complexation. It also demonstrates that to take two orientation inclusion modes into the quantitative model, as in Equation (7), to predict the binding constants, is necessary.

Conclusions

The bimodal complexations of twelve steroids with β- and γ-CD were studied in this paper. The empirical binding free energy, including intermolecular interaction energy, the conformational energy change, and the solvation energy, was optimized by a flexible docking algorithm FDOCK. To determine the possible binding region, the energy barrier can not be neglected. The lowest energy structures in the reasonable binding region obtained by FDOCK were taken for the final complex structures for A-ring up and D-ring up orientations, respectively. The stability constants for the two orientations were predicted from the optimized energy components by the non-linear regression with the experimental values. Comparing the stability between each pair, the preferential orientation of each steroidal molecule in the cavity is predicted. For most steroids, A-ring up is their preferential orientation, but for some steroids, e.g., **8**, **10**, **12** with β-CD and **5**, **9**, **11**, **12** with γ-CD, D-ring up is more favourable, mainly due to the steric hindrance.

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