Structure-Activity Relationship of Taxol Inferring from Docking Taxol Analogues to Microtubule Binding Site

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In order to find the minimal structural requirements to maintain microtubule binding, 12 taxol analogues have been docked to the taxol binding site of tubulin. By comparing the interactions of each analogue with β-tubulin, the structure-activity relationships are summarized as follow: C-2 benzoyl and taxane ring systems are the essential groups for microtubule binding, the improvements of bioactivity and bioavailability are dependent on the substituents at positions C-1, C-4, C-7, C-9, C-10, and C-14, whereas the C-13 side chain mainly provides a specific binding.

Key words: Molecular Docking, Structure-Activity Relationship, Taxol, Tubulin

Introduction

Taxol, a plant diterpenoid widely used as a chemotherapeutic drug, is known to interact with a specific site of β-tubulin (Manfredi *et al.*, 1982; Rao *et al.*, 1992). It binds to microtubules and inhibits their disassembly (Schiff *et al.*, 1979). Cells treated with taxol are arrested in mitosis and eventually undergo death by apoptosis (Rowinsky and Tolcher, 2001). The ability of taxol and taxotere, another taxane (Fig. 1), to kill tumour cells has made them useful chemotherapeutic agents against several types of cancers, including those derived from ovary, breast, head and neck, and lung as well as malignant melanoma (Rowinsky and Tolcher, 2001).

With respect to taxol and its analogues, numerous structure-activity studies over the past two decades have led to several generalizations, and the most crucial one concerns three key side chains of the molecules: the C-2 benzyl, the C-4 acetyl, and the C-13 side chain moieties (Wang *et al.*, 2007). More than that, both atomic constitution and molecular conformation are critical to taxane-tubulin binding and cytotoxicity (Gueritte, 2001; Kingston, 2001). Especially, the side chain at position C-13 and the taxane ring system have been regarded as essential determinants for the activity of taxol and docetaxel. The isolated side chain and the taxane ring system with its other substituents, exemplified by baccatin III, have generally been considered inactive on mammalian microtubules (Andreu and Barasoain, 2001). Interestingly, the interaction of baccatin III with the taxol-binding site of microtubules determined by a homogeneous assay with fluorescent taxoid showed that the interaction of the C-2 and C-4 substituted taxane ring system with the microtubule binding site provides most of the free energy change of taxol binding and is sufficient to activate microtubule stabilization and transmit the antitumour effects of taxol, whereas the C-13 side chain provides a weak specific anchor (Andreu and Barasoain, 2001). The structure-activity relationship of taxol suggested that groups at C-1, C-7, C-9, and C-10 have weaker effects on its bioactivity, while C-2 benzoyl, C-4 acetyl, the D-ring, and the C-13 side chain have stronger effects on it (Tian *et al.*, 2007). This is the basis for our motivation to investigate the structure-activity relationship of taxol analogues by docking them to the taxol binding site of microtubules. Several molecular docking models of taxol binding have recently been presented, which include extensive contacts of the 2-*O*-benzoyl and the taxane ring system with several residues in the structure of β-tubulin. In the present work, analysis of taxol analogues bindings suggests that most of the affinities of taxol analogues are contributed to the interactions of the 2-*O*-benzoyl and taxane ring system with the microtubule binding site, whereas the side chains/groups at positions C-1, C-4, C-7,

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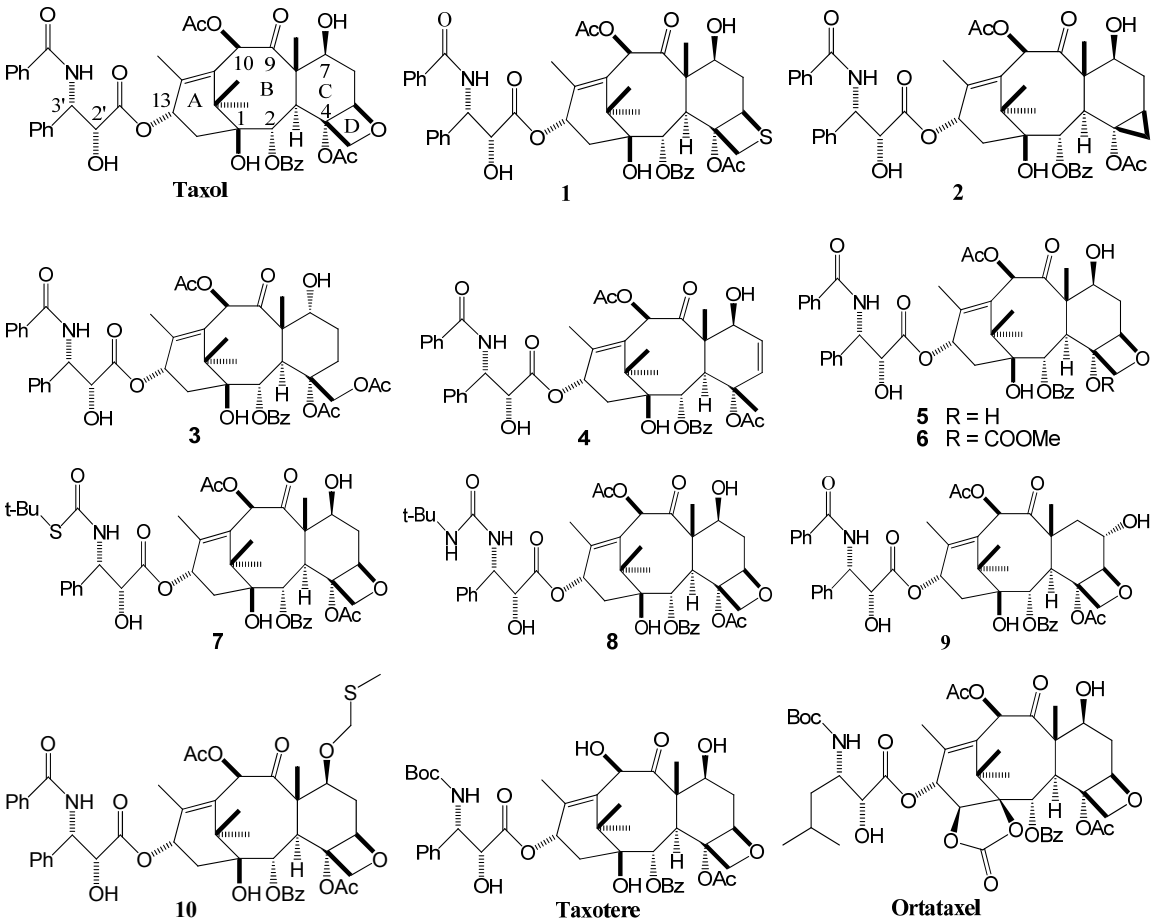

Fig. 1. Chemical structures of taxol and its analogues.

C-9, C-10, and C-13 provide only some specific additional bindings.

Material and Methods

Taxol analogues and tubulin

As shown in Fig. 1, 13 compounds were used in this study: four taxol analogues without a D-ring, two C-4 substituted analogues of taxol, two C-3′ substituted taxol analogues, two taxol analogues are C-7 modified compounds; in addition, taxotere (Gueritte *et al.*, 1991) and ortataxel (Baldelli *et al.*, 2004) were involved.

The 3D structures of all these taxol analogues were generated by VI EWDD (http://rcmd-server.frm.uniroma1.it/rcmd-portal/index.php).

The complex of *αβ*-tubulin with taxol (PDB code: *1jff*) was used as the target with an *α*-tubulin subunit and taxol removed.

The 3D structures minimization of taxol analogues and target protein were conducted by the molecular modeling system UCSF chimera (Pettersen *et al.*, 2004) (http://www.cgl.ucsf.edu/chimera).

Molecular docking

AutoDock4 (Morris *et al.*, 1998) was used for automatic placements of taxol analogues in the taxol binding cavity of the target *β*-tubulin. The target structure was set up using AutoDockTools (Sanner, 1999) for docking by removing all water and ligand atoms, adding polar hydrogen atoms, and assigning AMBER (Ponder and Case, 2003;

Wang *et al.*, 2006) atomic charges and solvation parameters as required by the AutoDock program. In the same way, the chemical structures of taxol analogues were prepared by assigning Gasteiger atomic charges, and rotatable bonds were explicitly defined.

Docking was then carried out using an empirical free energy function and the Lamarckian genetic algorithm. One hundred independent docking runs were performed for each taxol analogue, applying a standard AutoDock protocol, with a grid spacing of 0.375 Å, an initial population of 300 randomly placed individuals, a maximum number of $2.5 \cdot 10^7$ energy evaluations, a maximum number of 1000 generations, a mutation rate of 0.02, and a crossover rate of 0.80.

The post-processing of docking results was conducted by AutoDockTools (Sanner, 1999) and Dockres (http://atlas.physbio.mssm.edu/~mezei/dockres/dockres.html).

Results and Discussion

Validation of docking pose

To validate if all taxol analogues docked to the taxol binding site of β-tubulin, the distribution of target protein residues closest to docked taxol analogues was analyzed (as shown in Fig. 2). For each taxol analogue, only the top 5 poses were considered. It is obvious that all taxol analogues docked to the residue His229, which can be the key residue of the taxol binding site on the microtubule (Rao *et al.*, 1999). In addition, Asp26, Pro274, Thr276, Arg278, Arg284, and Gly370, shown in Fig. 2, were involved in direct interactions with taxol and β-tubulin (Lowe *et al.*, 2001). It is worth to note that less taxol analogues docked to residue Arg284 despite it is the key residue of the taxol binding site (Rao *et al.*, 1999). In fact, unlike taxol, not all taxol analogues can interact with β-tubulin well. In a word, the docked residues shown in Fig. 2 suggest that all taxol analogues fitted into the taxol binding site on the target protein.

The correct docking pose is critical to simulate the interactions of taxol analogues with the microtubule binding site. The complex of β-tubulin with taxol and the docking pose with the lowest docking energy are shown in Fig. 3. The two poses in Fig. 3 are compatible with each other on the whole, and the main difference is the slight rotation of the docking pose. As a simulation model, such a pose is acceptable and can be used to analyze the interaction of taxol with the microtubule binding site. In fact, we are able to generally predict the correct binding mode of ligand and receptor with AutoDock.

Analysis of structure-activity relationship

Compounds **1**–**4** are D-ring modified taxol analogues (Fig. 1). In **1**, the oxygen atom of the D-ring is substituted by a sulfur atom, and the C-13 side chain and the sulfur atom are not involved in the interactions with β-tubulin. In fact, the sulfur derivative **1** was found to be less active than taxol in biological assays (Gunatilaka *et al.*, 1999). Compound **3** is an open D-ring analogue, and is biologically inactive in an *in vitro* cytotoxicity as-

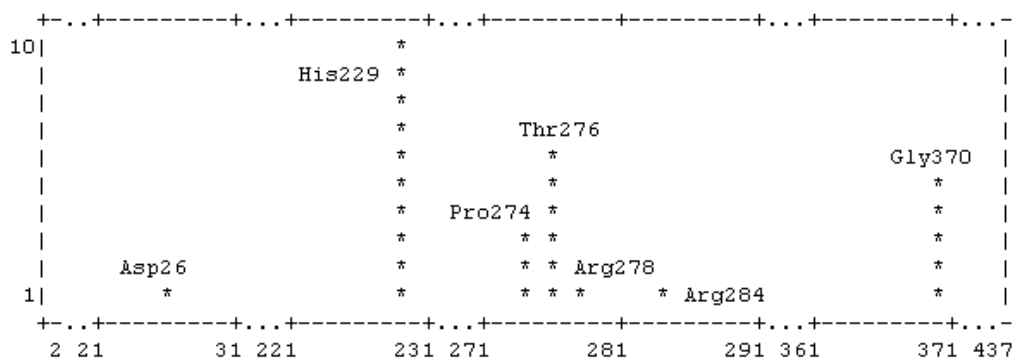

Fig. 2. The distribution of β-tubulin (PDB code: *1jff*) residues closest to docked taxol analogues. The *x*-axis represents the residue number (residue range: 2–437). The height along the *y*-axis is proportional to the number of taxol analogues docked to the residue represented by the *x*-axis.

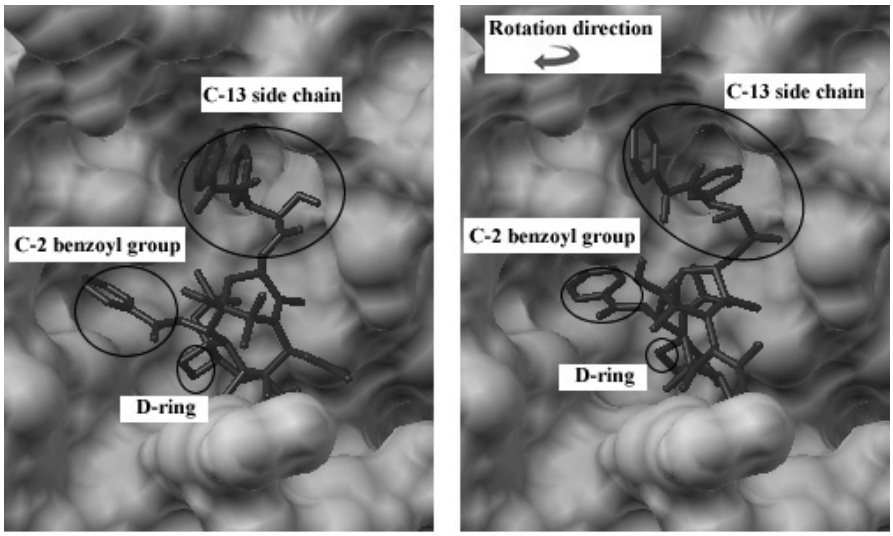

Fig. 3. The location of taxol binding to the β-tubulin site. (A) Complex of β-tubulin with taxol (PDB code: *1jff*). (B) Docking site with the lowest docking energy. The D-ring, C-2 benzoyl group, and C-13 side chain are marked with circles. The rotation direction is marked out, and the rotation of the docking pose contributs to the difference between poses in A and B.

say and a tubulin assembly assay (Barboni *et al.*, 2001). The C-13 side chain (except for two benzene rings) and ring A did not participate in the interactions with the target. Compound **2** cyclized directly without an oxygen atom in the D-ring, and compound **4**, like **3**, is an analogue of taxol with D-ring opening. They had been predicted to be nearly as active as taxol in binding to the binding site of tubulin, but all were biologically inactive in an *in vitro* cytotoxicity assay and a tubulin assembly assay (Barboni *et al.*, 2004; Dubois *et al.*, 2000). Correspondingly, not like **1** and **3**, only the C-1 hydroxy, C-9 carbonyl, C-1′ carbonyl, and C-2′ hydroxy groups in **2** and **4** were not involved in the binding with β-tubulin. The results suggest that: (i) the A-ring and C-13 side chain are important for microtubule binding; (ii) the D-ring is essential for microtubule binding and the oxygen atom in the D-ring plays an important role in the mechanism by which taxol exhibits its anticancer activity. In fact, the D-ring is important in determining the taxane ring system conformation (Tian *et al.*, 2007).

Compounds **5** and **6** are analogues of taxol with the C-4 acetyl group substituted by hydroxy or methoxycarbonyl groups, respectively (Fig. 1). The C-4 methoxycarbonyl group in **6** was involved in binding with β-tubulin, and the C-4 hydroxy group was not. Interestingly, the free binding energies of **5** and **6** (−4.5 and −3.9 kcal/mol, respectively) are lower than that of taxol (−3.6 kcal/mol), which means that derivatives **5** and **6** are as active as taxol in binding to tubulin. The results imply that the C-4 acetyl group may not be as important as the D-ring for microtubule binding.

Compounds **7** and **8** are C-3′ substituted (Fig. 1). Compared to **8**, all C-3′ phenyl, C-2′ hydroxy, and C-1′ carbonyl groups in the C-13 side chain of derivative **7** did not bind to the target site. In other words, the microtubule binding of derivative **7** is not as well as that of **8**. The result was further supported by the free binding energies of **7** and **8** (−3.3 and −4.2 kcal/mol, respectively). Contradictorily, derivative **7** was found to be more potent than taxol in both the tubulin polymerization assay and the *in vitro* cytotoxicity assay, and derivative **8** was inactive in both assays (Xue *et al.*, 2000). The conflicts suggest that the C-13 side chain may provide specific binding in microtubule binding. For the specific binding of the C-13 side chain, the free binding energies of **7** and **8** are nearly similar to that of taxol.

Compound **9** is a derivative with transposition of the C-7 hydroxy group of taxol to the C-6 posi-

tion. Compound **10** is an analogue of taxol with the C-7 hydroxy group substituted by an ether group. As for **9**, C-6 hydroxy, C-9 carbonyl, and C-10 acetyl groups were not involved in microtubule binding, but it was found to be similar to taxol in both the tubulin polymerization assay and the *in vitro* cytotoxicity assay (Wittman *et al.*, 1999). The result suggested that the groups at positions C-7, C-9, and C-10 are not essential groups for microtubule binding. As for **10**, C-1 hydroxy, C-1′ carbonyl, C-4 acetyl, and C-10 acetyl groups did not bind to the target site. Interestingly, the preclinical antitumour activity of **10** was superior to taxol (Altstadt *et al.*, 2001). This suggested that not only the groups at positions C-1, C-4, and C-10 are not essential groups for microtubule binding, but also the proper substitution at these positions, such as C-7, can be helpful to improve the bioactivity of taxol.

In addition, two new-generation taxanes, taxotere and ortataxel, were docked to the taxol binding site of microtubules. Compared to taxol, taxotere was more potent at inhibiting angiogenesis and preferred for clinically treating patients with breast cancer (Crown *et al.*, 2004; Grant *et al.*, 2002). As shown in Fig. 1, taxotere is an analogue of taxol with C-10 acetyl and C-3′ phenylcarbamoyl groups substituted by hydroxy and isobutyl ester groups, respectively. Interestingly, C-1 and C-2′ hydroxy groups were not involved in the microtubule binding. It is to say that the proper substitution at the C-1 and C-13 side chains can improve the bioactivity of taxol. Ortataxel, a taxane derivative with a form of 1,14-carbonate moiety (Fig. 1), exhibits excellent activity against a variety of drug-sensitive and drug-resistant cancer cell lines and is currently in phase II clinical trials (Geney *et al.*, 2005). Especially, it retains encouraging activity and clinical benefit in breast cancer patients who are resistant to taxol or taxotere combinations, and its toxicity, comparable to those of the two other taxanes, was tolerable in heavily treated population (Beer *et al.*, 2008). It is worth to note that C-4 acetyl, C-9 carbonyl, and C-1′ carbonyl groups were not involved in the interactions with ortataxel and β-tubulin. This suggested further that C-4 and C-9 groups are not essential groups for microtubule binding, and the 1,14-carbonate moiety can improve the pharmacological properties such as a better bioavailability.

Finally, we can draw the following conclusions: (1) the C-2 benzoyl group, involved in the interactions with all taxol analogues and β-tubulin, and taxane ring system are essential for microtubule binding; (2) the substituents at positions C-1, C-4, C-7, C-9, C-10, and C-14 can improve the bioavailability and activity spectrum; (3) despite it is important for microtubule binding, the C-13 side chain mainly provides a specific binding.

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