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# 3D QSAR studies of the interaction between β-tubulin and microtubule stabilizing antimitotic agents (MSAA). A combined pharmacophore generation and pseudoreceptor modeling approach applied to taxanes and epothilones

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**Abstract**

Based on the conformer of paclitaxel extracted from the experimental tubulin structure, a pharmacophoric model has been generated and used to find the chemical features common to the taxane and epothilone classes of compounds. This original alignment has been translated into the experimental tubulin binding site obtaining an assembly subsequently submitted to the pseudoreceptor modeling approach. As a result, an original 3D QSAR model, able to evaluate, at a quantitative level, the relationships between the molecular structures and biological data of the studied compounds, has been obtained.


*Keywords:* Pseudoreceptor modeling; 3D QSAR; Tubulin; Taxanes; Epothilones

## 1. Introduction

Paclitaxel and docetaxel (Taxol® (**1**) and Taxotere® (**9**), Scheme 1, Table 1) are the most prominent microtubule-stabilizing antimitotic agents (MSAA) currently applied in the clinical treatment of ovarian, breast, and lung carcinomas [1]. However, the dose-limiting toxicities of peripheral neuropathy and neutropenia of paclitaxel [2], the vascular leak problems of docetaxel [3], and the limited solubility of both the drugs have generated additional impetus to discover new classes of potential anticancer agents acting by a similar mechanism of action.

In addition to discodermolide and eleutherobins, epothilones, isolated from the myxobacterium *Solangium cellulosum*, have been demonstrated to stabilize tubulin polymers [4] blocking disassembly of microtubules during cell division. Interestingly, some epothilone derivatives have been shown to be more water-soluble analogues, less toxic, and have an improved therapeutic index [5] with respect to the taxanes and the prototypical epothilone B (**16**) which is currently in clinical trials [5,6]. Moreover, many phase II clinical trials with epothilones have been reported, especially in treatment of ovarian cancer, metastatic colorectal cancer, and leukemia [7–11], and different clinical protocols have been approved in the treatment of renal cell carcinoma, refractory neoplasms, and pediatric refractory solid tumors [12].

Epothilones are also characterized by a molecular structure less complex with respect to taxanes (Scheme 1, Table 1), that allows for relatively easy total synthesis approaches and analogue design.

The refined electron crystallography structure of the β-tubulin binding site with embedded paclitaxel as an inhibitor has been recently reported [13], and several modeling studies on taxanes and other MSAA have been described including pharmacophoric alignments and pseudoreceptor models [14–20]. All of them could be used to facilitate the rational design of new antimitotic agents.

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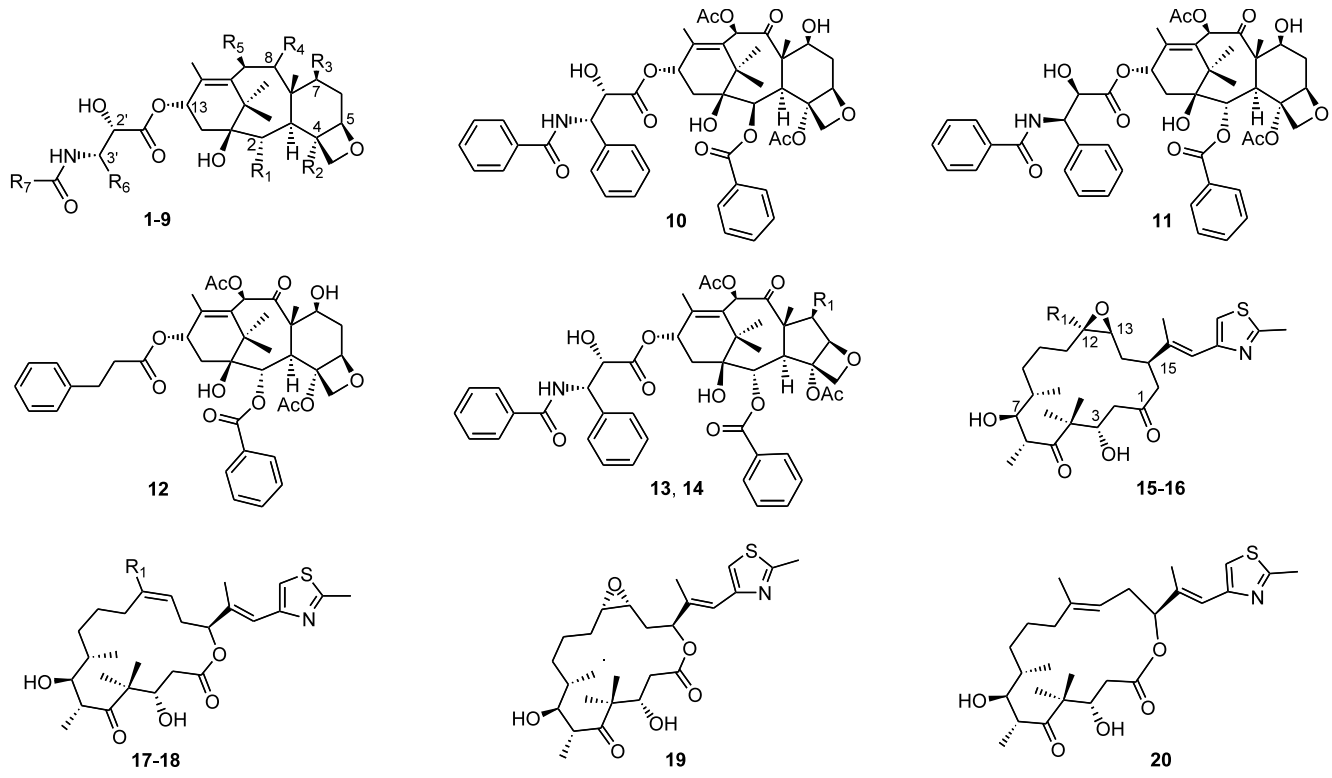


Scheme 1.

## 2. Modeling studies

The computational approach presented in this paper is based on two steps. The first one is aimed at generating a pharmacophore model for both epothilones and taxanes, to be translated into a pseudoreceptor model of the tubulin binding site with the purpose of building a 3D QSAR model able to estimate or predict (second step), at a quantitative level, the biological data of the studied compounds.

Table 1
Structural and biological properties of taxanes (**1**–**14**) and epothilones (**15**–**20**) used in this study

| Comp. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | $\Delta G_{exp}$ | $\Delta G_{calc}$ | $\Delta\Delta G$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | PhCOO | AcO | OH | =O | AcO | Ph | Ph | −6.464 | −6.628 | −0.164 |
| **2** | H | AcO | OH | =O | AcO | Ph | Ph | −3.145 | −4.443 | −1.298 |
| **3** | *p*-$CF_3$PhCOO | AcO | OH | =O | AcO | Ph | Ph | −5.421 | −6.448 | −1.027 |
| **4** | PhCOO | OH | OH | =O | AcO | Ph | Ph | −3.381 | −3.405 | −0.024 |
| **5** [a] | PhCOO | AcO | $NH_2CH_2COO$ | =O | $NH_2CH_2COO$ | Ph | *t*-BuO | −6.358 | −4.468 | 1.890 |
| **6** | PhCOO | AcO | OH | OH | AcO | Ph | Ph | −6.635 | −7.282 | −0.647 |
| **7** [a] | PhCOO | AcO | OH | =O | H | Ph | Ph | −6.856 | −6.342 | 0.514 |
| **8** [a] | PhCOO | AcO | OH | =O | AcO | $C_6H_{11}$ | Ph | −7.184 | −5.574 | 1.610 |
| **9** | PhCOO | AcO | OH | =O | OH | Ph | *t*-BuO | −6.900 | −6.157 | 0.743 |
| **10** | | | | | | | | −3.145 | −3.500 | −0.355 |
| **11** [a] | | | | | | | | −5.657 | −4.924 | −0.733 |
| **12** [a] | | | | | | | | −4.815 | −5.074 | −0.259 |
| **13** [a] | OH | | | | | | | −5.232 | −5.376 | −0.144 |
| **14** | AcO | | | | | | | −4.220 | −4.893 | −0.673 |
| **15** [a] | H | | | | | | | −6.504 | −5.134 | 1.370 |
| **16** | Me | | | | | | | −7.233 | −5.813 | 1.420 |
| **17** | H | | | | | | | −6.167 | −6.290 | −0.123 |
| **18** [a] | Me | | | | | | | −7.345 | −7.365 | −0.020 |
| **19** | | | | | | | | −6.240 | −5.028 | 1.212 |
| **20** | | | | | | | | −5.908 | −4.974 | 0.934 |

$\Delta G_{calc}$, experimental value; $\Delta G_{exp}$, calculated value; $\Delta\Delta G$, difference between $\Delta G_{exp}$ and $\Delta G_{calc}$.
[a] Compounds belonging to the test set. All the remaining compounds constitute the training set.

With the purpose of collecting a large number of antimitotic agents, 14 taxanes and six epothilones were taken from the literature under the assumption that all substances are acting at the paclitaxel binding site on tubulin. Biological data were converted into the corresponding free energy of binding ($\Delta G$) for the purpose of a 3D QSAR correlation.

The first step of our computational work has been addressed by means of a ligand-based drug design (pharmacophore development) approach. In detail, the conformer of paclitaxel extracted from the experimental complex with tubulin [13] was transformed into a four-point pharmacophoric hypothesis by using the software Catalyst [21]. Three hydrogen bond acceptor groups (corresponding to the oxetane oxygen, the C1′-carbonyl group, and the C2′-hydroxy substituent) and an aromatic function accommodating the phenyl ring at the C3′-position were chosen as the features of the pharmacophoric model.

Despite the huge amount of work in this field, the role of the oxetane ring is still unclear. In fact, it has been reported [22–27] that the four-membered ring contributes to rigidify the A–C ring system of paclitaxel and interacts by hydrogen bond with the tubulin. On the other hand, the oxetane ring has been suggested as unnecessary for bioactivity, compounds with no D ring being as active as paclitaxel and docetaxel [22,23,28]. Moreover, inspection of the paclitaxel–tubulin complex showed the oxetane oxygen atom at a hydrogen bond distance from the backbone NH of Thr276, prompting us to choose this oxygen as a hydrogen bond acceptor group. Similarly, location of the 1′-carbonyl group and 2′-hydroxy group of paclitaxel, both of them at a close distance from the backbone NH of Gly370 [13], in conjunction with the literature reporting their essential role for paclitaxel activity [23] led us to choose the 1′-carbonyl and 2′-OH functions as additional hydrogen bond acceptor features. Finally, considering that an aryl (or hydrophobic) moiety at the 3′-position is also a crucial element for activity of taxane derivatives [23], the phenyl ring of paclitaxel has been allowed to represent the aromatic moiety of the proposed pharmacophoric model.

The pharmacophoric model was then used as a three-dimensional template to superpose epothilones to paclitaxel by finding the common chemical features shared by both compounds. In particular, the BestFit option of Catalyst was used to find, among all the conformers of each epothilone structure, the conformation able to better satisfy the spatial constraints imposed by the pharmacophoric model. As a result, an original alignment hypothesis of all epothilones into taxanes has been obtained. In particular, the oxetane oxygen atom corresponded to the hydroxy group at the 7-position of epothilone B, while the C1′-carbonyl group and C2′-hydroxy substituent of paclitaxel were matched by the C3-hydroxy and C1-carbonyl groups of epothilone, respectively. Finally, the C3′-phenyl ring of paclitaxel was superposed to the thiazole moiety of epothilone.

All the remaining taxanes were added to the pharmacophoric model by superposition to the template conformation of paclitaxel.

Next, the pharmacophore model (to be intended as taxanes and epothilones superposed each other) was imported into the PrGen software [29] to perform the second step of our computational protocol.

In detail, starting from the cocrystallized complex between paclitaxel and tubulin (1JFF entry in the Brookhaven protein data bank) [13], a shell of 32 amino acid residues in close contact with the inhibitor was selected as the pseudoreceptor model and used to accommodate taxanes and epothilones aligned as described above. The pseudoreceptor model with embedded inhibitors was submitted to a calibration procedure using a standard protocol consisting in an iterative equilibration and minimization of amino acid residues followed by minimization of inhibitors [30]. As a result, the 3D QSAR model obtained [31] was able to correlate the structural properties of the training set compounds with their biological data. Differences between calculated and experimentally determined $\Delta G$ for compounds of the training set (and test set) are reported in Table 1.

## 3. Results and discussion

Analysis of the fitting model of inhibitors into the final pseudoreceptor can be summarized as follows: both the oxetane oxygen atom of paclitaxel and the 7-hydroxy group of epothilone B interact by hydrogen bonds with Thr276 (Fig. 1A), a residue demonstrated as a crucial key in determining activity of epothilones [16]. In fact, a dramatic loss in activity found for epothilone in epothilone-resistant cell lines, characterized by the Thr276Ile mutation. Moreover, lack of activity due to chirality change or substitution of 7-hydroxy group of epothilone is accounted by the model with the interaction with Thr276. The C1′-carbonyl moiety of epothilone and the C2′-hydroxy group of paclitaxel are involved in a hydrogen bond interaction with the backbone NH of Gly370 (Fig. 1B), reported at close contact with inhibitors [13]. The thiazole ring of epothilone is accommodated in the hydrophobic pocket (mainly defined by Ala233, Ser236, and Phe272) where the C3′-phenyl ring of paclitaxel lies (Fig. 1C), in agreement with what suggested by site-directed mutagenesis experiments [16]. Substituent at C13 of epothilones lies between the C2-benzoyl and C4-acetate groups of paclitaxel, in a region of space mainly defined by hydrophobic residues such as His229 and Leu217 (Fig. 1D). It is important to note that this orientation

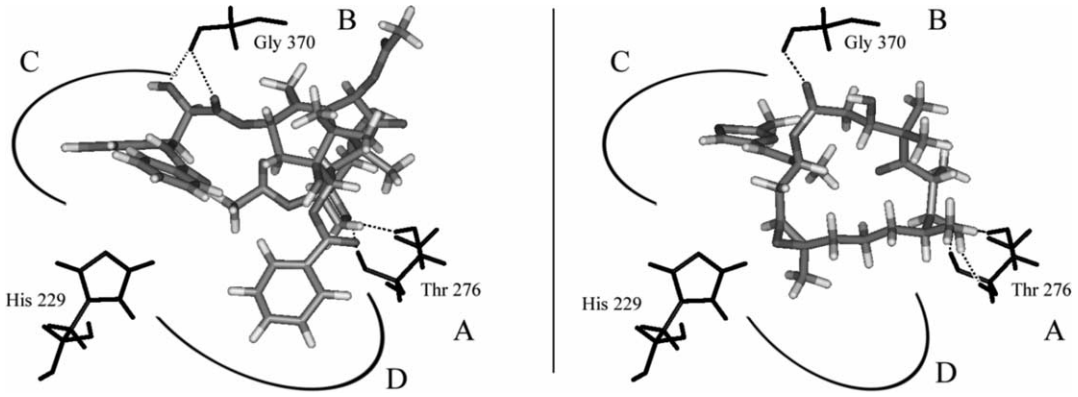


Fig. 1. Paclitaxel (left) and epothilone B (right) fitted into the pseudoreceptor model of β-tubulin. (A) The oxetane oxygen atom of paclitaxel and the 7-OH group of epothilone bound by hydrogen bonds to both the backbone NH groups and side-chain OH groups of Thr276. An additional hydrogen bond interaction is found between the 7-OH group of epothilone and the carbonyl moiety of Thr276. (B) The 2′-OH group of paclitaxel and the 1-carbonyl group of epothilone interact by hydrogen bond to Gly370. (C) The phenyl ring at the 3′-position of paclitaxel and the thiazole ring of epothilone are located inside a hydrophobic cleft mainly defined by Ala233, Ser236, and Phe272. (D) C2-benzoyl group fits in the same hydrophobic pocket where the C12-methyl substituent of epothilones lies.

accounts for SARs of both taxanes and epothilones. In fact, it has been reported that the C2-phenyl ring of paclitaxel can be replaced by a cyclohexyl moiety as well as a long hydrophobic moiety can be inserted in place of the C4-acetate [27]. Moreover, epothilone B, bearing a methyl group at the C13-position, is more active than the corresponding desmethyl analogue (epothilone A), while bulkier groups such as acetyl or cyclohexyl are tolerated instead of the methyl one [32–34].

With the aim of validating the 3D QSAR model, a test set of eight compounds was built and activity data were predicted by the model in good agreement with respect to the experimentally determined values (Table 1).

In summary, the proposed alignment of epothilones and taxanes within the tubulin binding site as well as the application of the pseudoreceptor modeling approach to the experimental structure of tubulin represent the elements of novelty in the field of MSAA interacting with tubulin.

Considering that the search for compounds with improved activity is always a crucial step in anticancer therapy, this model could be useful for predicting binding affinity of new compounds targeting tubulin. Moreover, it can be anticipated that the improvement of this model is ongoing by the incorporation of paclitaxel mimics into the training set to develop a common pharmacophoric model for all the classes of compounds proved to be microtubule-stabilizing agents.

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