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# Cembrene Diterpenoids: Conformational Studies and Molecular Docking to Tubulin

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**Abstract:** A conformational analysis of the cembrene diterpenoids cembrene, cembrene A, (3*Z*)-cembrene A, isocembrene, casbene, and incensole, has been carried out using density functional theory at the B3LYP/6-31G* level of theory. A molecular docking analysis of these cembrenoids with tubulin has also been performed in order to assess the potential of tubulin binding of these cytotoxic agents. The macrocyclic cembrenoids are conformationally mobile and numerous low-energy conformations were found. Molecular docking reveals that the cembrenoids dock into the colchicine binding site of tubulin with comparable docking energies to colchicine.

**Keywords:** Cembrenes; conformations, tubulin; molecular docking.

## 1. Introduction

Cembrenoids are 14-membered-ring diterpenoid natural products. Cembrene (thunbergene, thumbelene) has been isolated from pine oleoresins [1]. Cembrene A (neocembrene, neocembrene A) is found in a number of higher plants, e.g., *Commiphora* [2] and *Boswellia* spp. [3], but has also been found to be the trail pheromone of the Australian subterranean termite, *Nasutitermes exitiosus* [4], the queen recognition pheromone of the Pharaoh's ant, *Monomorium pharaonis* [5], and it has been isolated from soft corals (*Nephthea* spp.) [6,7] as well as the paracloacal glands of the Chinese alligator (*Alligator sinensis*) [8]. In addition to cembrene A, (3*Z*)-cembrene A has been isolated from the heads of soldier termites, *Cubitermes umbratus* [9]. The macrocyclic cembrenes are conformationally mobile with numerous possible conformations, and there have been a few conformational studies carried out, including cembrene [10,11], and oxygenated derivatives [12,13]. Cembrene A and oxygenated cembrenoids have shown notable *in-vitro* cytotoxic activity on A549,

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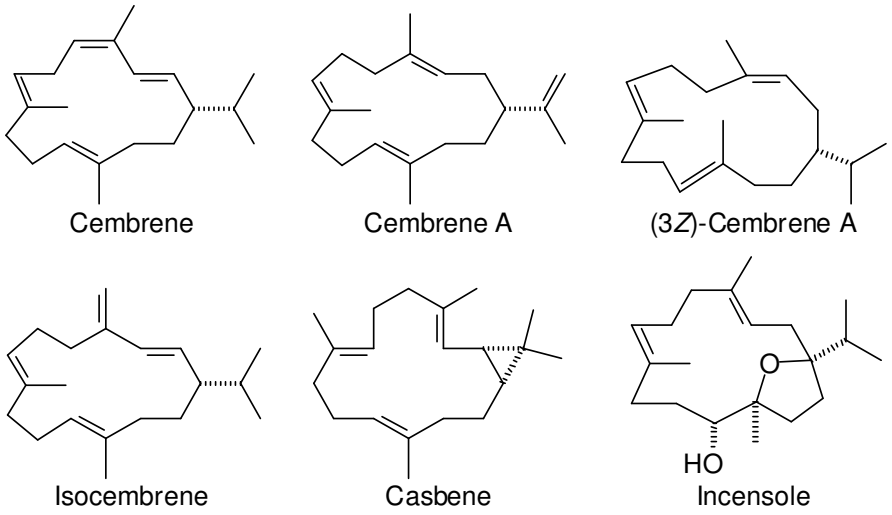
HT-29, KB, and P-388 cell lines [7]. To our knowledge, however, the mechanism of cytotoxic activity has not been determined. In this work, we present conformational analyses of cembrene, cembrene A, (3*Z*)-cembrene A, isocembrene, casbene, and incensole, using molecular mechanics (MMFF) and density functional (B3LYP/6-31G*) methods. In addition, a molecular docking study of these cembrenoids with tubulin has been carried out in order to explore binding to this cancer-relevant protein target as a possible mechanism of cytotoxic activity of cembrenoids.




Microtubules, composed of heterodimers of α-tubulin and β-tubulin, are key components of the cytoskeleton, and are crucial in cell shape and structure. Therefore, interference with tubulin assembly and disassembly, critical to cell division, will disrupt this process and lead to cell death by apoptosis [14-16]. A number of cytotoxic natural products have been shown to bind to tubulin, including the vinca alkaloids vincristine and vinblastine, the combretastatins, the taxanes, and the epothilones [17]. Some promising tubulin binding cytotoxic agents are macrocyclic, including the latrunculins [18], rhizoxin [19,20], epothilones A and B [21-23], and laulimalide [24]. Although the mechanism of cytotoxic activity has not been established for the cembrene diterpenoids, the macrocyclic structures of these compounds prompted us to undertake a molecular docking investigation of cembrenes with tubulin. See [25] for a recent perspective of antitubulin virtual screening.

## 2. Computational Methods

### *2.1. Conformational Analysis*

All calculations were carried out using SPARTAN '08 for Windows [26]. Initial conformational analyses were carried out on each macrocycle using a Monte-Carlo molecular mechanics conformational search using the MMFF force field [27]. For each macrocyclic cembrenoid, all conformations with $E_{rel}$ less than 5 kcal/mol from the MMFF conformational analysis were then modeled using density functional theory (DFT). DFT calculations with the B3LYP functional [28,29] and the 6-31G* basis set [30] were used for the optimization of all stationary points in the gas phase. All enthalpies are zero-point (ZPE) corrected with unscaled frequencies, but with no thermal corrections; they are, therefore, $H_{(0K)}$. Relative energies ($E_{rel}$) were calculated from the $H_{(0K)}$ values.

### *2.2 Molecular Docking*

Protein-ligand docking studies were carried out based on the crystal structures of tubulin crystallized with colchicine (PDB 1sa0) [31], crystallized with podophyllotoxin (PDB 1sa1) [31], with paclitaxel (PDB: 1jff) [32], and crystallized with both colchicine and vinblastine (PDB 1z2b) [33]. All solvent molecules and the co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker v. 4.0 [34,35], with a sphere large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. Different orientations of the ligands were searched and ranked based on their energy scores. The RMSD threshold for multiple cluster poses was set at < 1.00Å. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 30 runs for each ligand.

## 3. Results and Discussion

Conformational analyses were carried out on cembrene, cembrene A, (3*Z*)-cembrene A, isocembrene, casbene, and incensole. Monte Carlo molecular mechanics (MMFF) conformational searches were carried out. All low-energy conformations ($E_{rel} \leq 5$ kcal/mol) from the molecular mechanics analyses were then modeled using density functional theory at the B3LYP/6-31G* level.

**Table 1.** Molegro binding energies (kcal/mol) of best docked poses for cembrene diterpenoids with tubulin.

| Ligand | Tubulin crystal structure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1sa0 | | 1sa1 | | 1jff | 1z2b | | |
| | site B[a] | site D[a] | site B[b] | site D[b] | [c] | site B[a] | site C[d] | site D[a] |
| Colchicine | -22.7 | -25.6 | -18.1 | -24.5 | -16.4 | -28.2 | -20.7 | -24.7 |
| Podophyllotoxin | -28.0 | -27.0 | -29.4 | -28.1 | -19.9 | -26.7 | -25.0 | -24.7 |
| Paclitaxel | -28.1 | -31.5 | -30.9 | -29.2 | -29.3 | -25.7 | -22.0 | -28.0 |
| Vinblastine | -27.8 | -29.1 | -21.1 | -13.5 | -27.7 | -19.0 | -30.6 | -17.5 |
| Cembrene | -21.5 (**G**)[e] | -21.5 (**G**) | -24.0 (**H**) | -22.3 (**G**) | -19.1 (**L**) | -22.3 (**G**) | -18.8 (**L**) | -23.1 (**G**) |
| Cembrene A | -20.9 (**G**) | -21.6 (**C**) | -21.8 (**E**) | -21.5 (**E**) | -18.7 (**H**) | -21.7 (**G**) | -23.1 (**H**) | -21.6 (**G**) |
| (3*Z*)-Cembrene A | -20.5 (**G**) | -20.8 (**F**) | -20.8 (**F**) | -20.7 (**F**) | -17.7 (**D**) | -21.4 (**E**) | -20.0 (**H**) | -21.3 (**F**) |
| Isocembrene | -23.8 (**E**) | -24.0 (**E**) | -21.8 (**H**) | -21.9 (**G**) | -20.4 (**H**) | -23.0 (**G**) | -20.1 (**A**) | -23.4 (**H**) |
| Casbene | -20.6 | -20.7 | -20.9 | -20.8 | -17.8 | -21.8 | -20.1 | -21.4 |
| Incensole | -19.9 (**A**) | -20.2 (**B**) | -19.6 (**A**) | -21.3 (**A**) | -16.3 (**A**) | -21.6 (**B**) | -18.3 (**A**) | -21.8 (**B**) |

[a] Colchicine binding site; colchicine is the co-crystallized ligand.
[b] Colchicine binding site; podophyllotoxin is the co-crystallized ligand.
[c] Paclitaxel binding site; paclitaxel is the co-crystallized ligand.
[d] Vinblastine binding site; vinblastine is the co-crystallized ligand.
[e] The conformation (see below) of the lowest-energy docked form is indicated in parentheses.

A molecular docking analysis of the cembrene diterpenoids was undertaken using the Molegro docking program [34]. The cembrenoids were docked into four different crystal structures of tubulin: PDB 1sa0, which had colchicine as the co-crystallized ligand [31], PDB 1sa1, which had podophyllotoxin as the co-crystallized ligand [31], PDB 1jff, which had paclitaxel as the co-crystallized ligand [32], and PDB 1z2b, which had both colchicine and vinblastine as co-crystallized

ligands [33]. The binding energies for the lowest-energy poses for the cembrene diterpenoids with tubulin are summarized in Table 1.

There are eight low-energy ($E_{rel}$ < 1 kcal/mol) conformations for cembrene (Figure 1). Conformations **E**, **F**, and **B** correspond to structures **A**, **B**, and **C**, respectively, that had been previously reported by Müller and co-workers [11] in a molecular mechanics conformational study of cembrene. One of the conformations adopted by cembrene (conformation **G**) docked into the colchicine binding site of tubulin is only 0.55 kcal/mol higher in energy than the lowest-energy conformation (see Figure 1). Conformation **H**, which corresponds to structure **D** in the study by Müller et al. [11], also docked into the colchicine binding site of tubulin, but this structure is 0.55 kcal/mol higher in energy than conformation **G**. The X-ray crystal structure of cembrene [36] corresponds to conformation **C** and is calculated to be only 0.15 kcal/mol higher in energy than the lowest-energy form. Note that conformations **C** and **B** have the same ring conformation and differ only in orientation of the isopropyl group. Likewise, conformations **D**, **E**, and **J** have the same ring conformation, while **I** has the same ring form as **A**. Conformation **L**, 3.17 kcal/mol higher in energy than **A**, is the conformation adopted by docked cembrene in both the paclitaxel and vinblastine sites of tubulin.

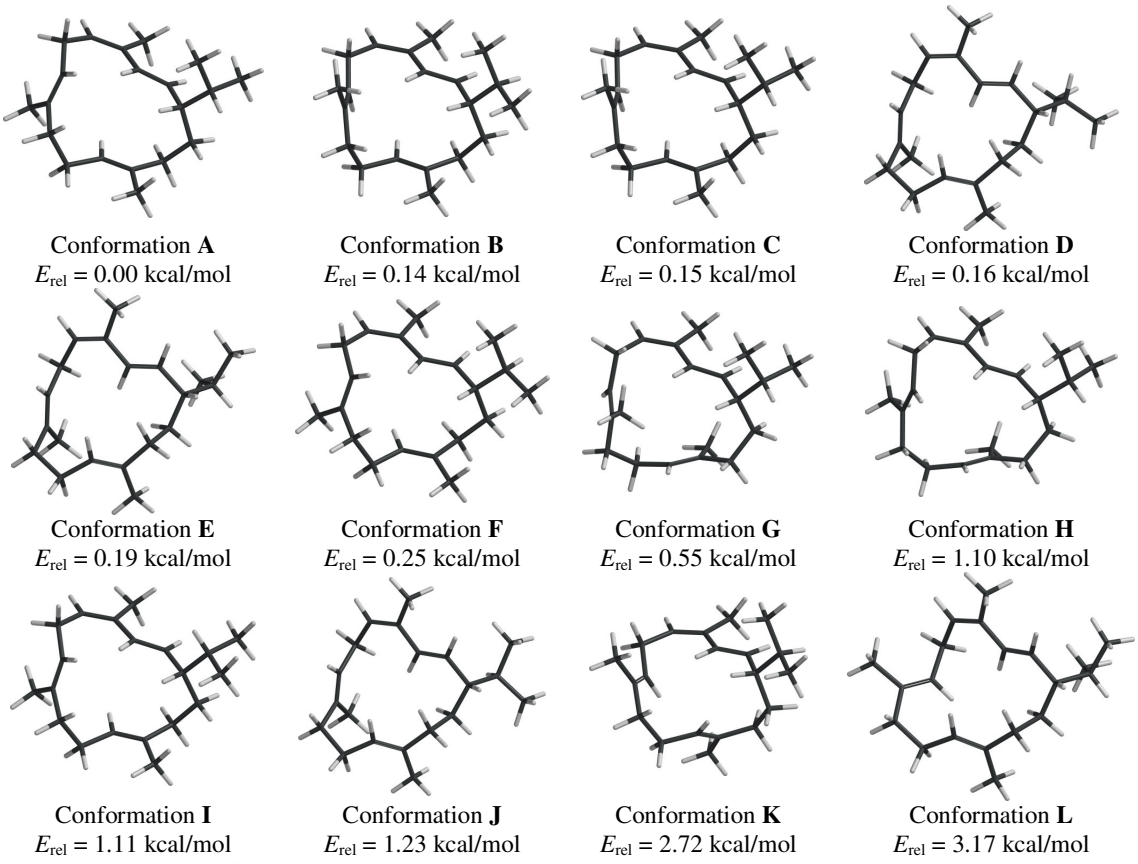



**Figure 1.** Low-energy and docked conformations of cembrene.

Low-energy and other important conformations of cembrene A are shown in Figure 2. Neither of the lowest-energy conformations for cembrene A, conformations **A** or **B**, were found to be the lowest-energy conformations in the molecular docking analysis. Conformation **C**, however, was found to be the lowest-energy docked form of cembrene A in the colchicine binding site of tubulin (PDB 1sa0, site D). Conformation **G** was the lowest-energy docked pose in colchicine site B, however. The lowest-energy docked poses of cembrene A in the colchicine binding site of tubulin

from structure PDB 1z2b were conformations **G** and **H**. Conformation **F** corresponds to the conformation adopted by the cembrenoid ring in the X-ray crystal structure of dihydro epimukulol acetate [12].

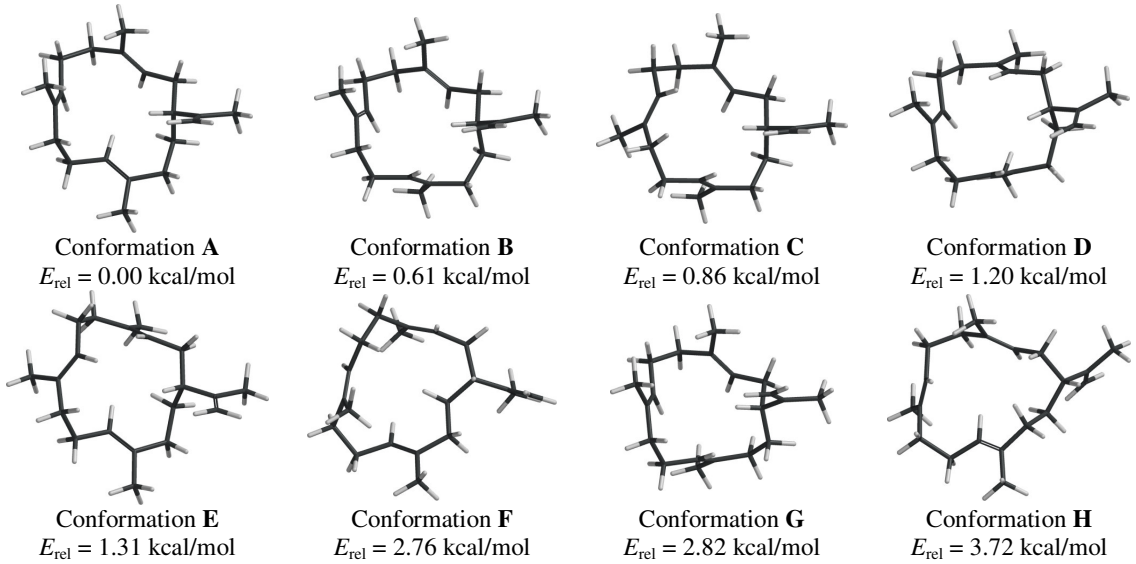



**Figure 2.** Low-energy and docked conformations of cembrene A.

There are four low-energy ($E_{rel}$ < 1 kcal/mol) conformations for (3*Z*)-cembrene A (see Figure 3). Conformation **D** was found docked into the paclitaxel binding site of tubulin (PDB 1jff). The conformations adopted by (3*Z*)-cembrene A in the colchicine binding site of tubulin were **E**, **F**, and **G** (see Table 1) while conformation **H** had preferentially docked into the vinblastine site of tubulin.

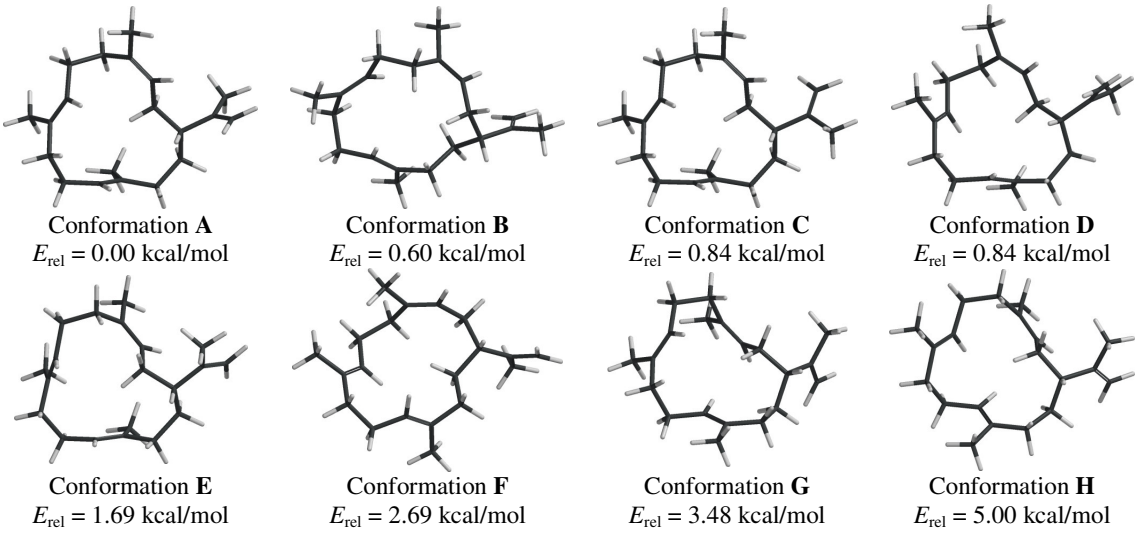



**Figure 3.** Low-energy and docked conformations of (3*Z*)-cembrene A

The lowest-energy conformation of isocembrene, **A**, was the conformation that preferentially docked into the vinblastine site of tubulin (PDB 1z2b). Conformation **E** was the conformation docked

into the colchicine sites of tubulin (PDB 1sa0), whereas conformations **G** and **H** were found docked into the colchicine sites of PDB 1sa1 (podophyllotoxin was the co-crystallized ligand, Table 1), and **H** was also the form docked into the paclitaxel site (PDB 1jff). Conformation **B** has the same ring conformation as **A** with only rotation around the isopropyl group (Figure 4).

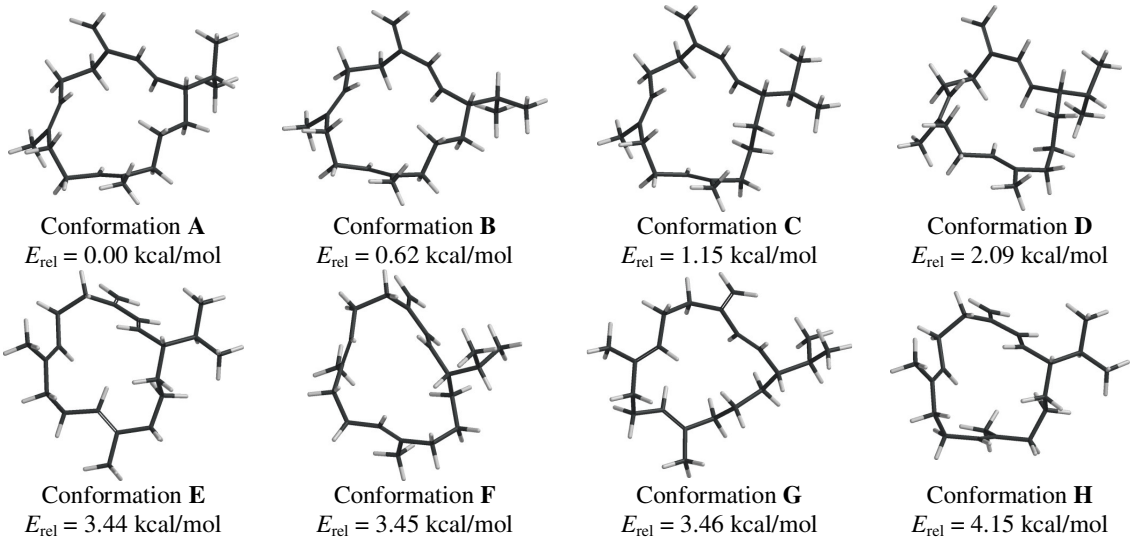



**Figure 4.** Low-energy and docked conformations of isocembrene.

There is only one low-energy conformation for casbene (Figure 5). The only other conformations were 4.4, 5.0, 7.7, and 7.9 kcal/mol higher in energy (not shown). The lowest-energy conformation of casbene was also the only one observed in the molecular docking analysis. Three low-energy conformations were found for incensole (Figure 5). Conformations **A** and **B** (differ only in rotation of the isopropyl group) were found to be the structures docked into the colchicine, paclitaxel, or vinblastine binding sites of tubulin (Table 1).

Colchicine crystallized with tubulin into binding sites best described as hydrophobic. The key interactions between colchicine and tubulin in PDB 1sa0 are hydrophobic interactions with Leu248, Lys254, Leu255, Asn258, and Lys352 [31], and these residues are important hydrophobic contacts with the docked cembrenoids. The hydrophobic nature of the colchicine binding site probably accounts for the relatively strong docking energies of the cembrenoids in this site. Docking energies for cembrenoids are comparable to colchicine in these binding sites (Table 1). The docked structure of isocembrene with the colchicine binding site (PDB 1sa0, site D) is shown in Figure 6.

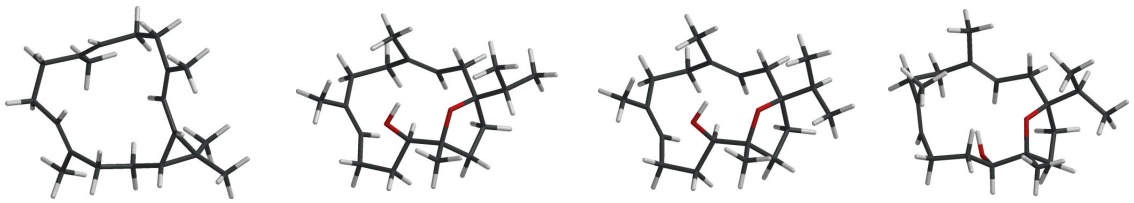



**Figure 5.** Low-energy conformations of casbene and incensole

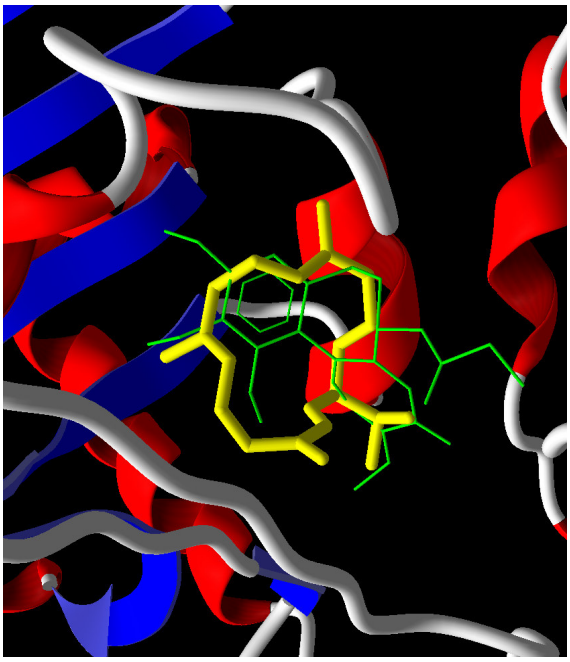


**Figure 6.** Isocembrene (yellow structure) docked into the colchicine binding site of tubulin (PDB 1sa0). The co-crystallized ligand (colchicine) is shown as a green stick figure.

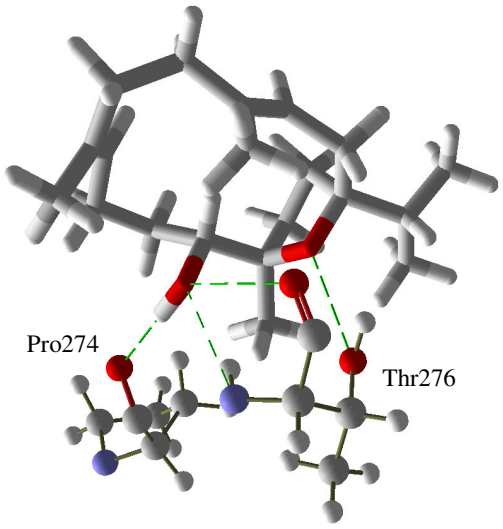



**Figure 7.** Key hydrogen-bonding interactions between incensole docked into the paclitaxel binding site of tubulin (PDB 1jff).

The cembrenoids docked into different locations of the paclitaxel binding site of tubulin (PDB 1jff [32]). Cembrene, isocembrene, and casbene preferentially docked at the hydrophobic site occupied by the 3′-phenyl group of paclitaxel. Important hydrophobic contacts include Pro360, Leu371, Phe272, Gly370, Glu27, and Arg369. Cembrene A and (3*Z*)-cembrene A, on the other hand, preferentially docked at the hydrophobic site occupied by the 2-phenyl group of paclitaxel with important hydrophobic contacts to Leu217, Leu219, His229, Leu230, and Asp226. Incensole occupied the taxane ring site of paclitaxel due to hydrogen-bonding interactions with Thr276 and Pro274 (Figure 7). Docking energies of the cembrenoids ranged from -16 to -20 kcal/mol; much less than the docking energy of paclitaxel (-29 kcal/mol).

Docking of cembrenoid ligands into the vinblastine binding site of tubulin revealed docking energies of around 10 kcal/mol weaker for the cembrenoids than for vinblastine, and so this site appears less important than the colchicine site for cembrene diterpenoid binding. Nevertheless, some key interactions in the docked structures include hydrophobic interactions of cembrene A and (3*Z*)-cembrene A with Tyr224, Gln11, and Cys12; hydrophobic interactions of casbene and cembrene with Val353, Pro325, and Val328 (analogous hydrophobic interactions with the indole ring of vinblastine; and hydrophobic interactions of incensole with Phe351, Asp179, and Thr349.

The cembrene diterpenoids exhibited docking energies comparable to colchicine for the colchicine binding sites of tubulin. Docking energies of the cembrenoids were less than paclitaxel or vinblastine in the paclitaxel and vinblastine binding sites, respectively. We conclude, then, that the cytotoxic activities of cembrene diterpenoids may be due, in part, to interaction with tubulin at the colchicine binding site.

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