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# Antimitotic activity of two macrocyclic bis(bibenzyls), isoplagiochins A and B from the Liverwort *Plagiochila fruticosa*

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ABSTRACT

Two bis(bibenzyls), isoplagiochins A (**1**) and B (**2**) have been isolated by the guidance of inhibitory effect of tubulin polymerization from the liverwort *Plagiochila fruticosa* (Plagiochilaceae). Isoplagiochins A and B inhibited the polymerization of tubulin at $IC_{50}$ 50 and 25 μM, respectively. Furthermore structure–activity relationship based on their conformations was discussed. The presence of two aromatic rings which can be connected through two atoms bridge spacer of double bond may serve to maintain the backbone conformation.

Antimitotic agents that target tubulin and microtuble formation and inhibit the mitotic arrest of eucaryotic cells are a class of antitumor agents, which are important components of current anticancer therapy, and can be classified into a several classes such as microtubule stabilizing agents, and vinca and colchicine site binding agents.[1] Recently much effort has been directed to the isolation and synthesis of new antimitotic drugs that target the tubulin/microtubule system and display efficacy against drug-refractory carcinomas.[2]

During our search for bioactive compounds targeting the tubulin/microtubules from medicinal plants,[3] we found that the extract from the liverwort *Plagiochila fruticosa* remarkably inhibited the polymerization of tubulin. Liverworts are rich sources of both terpenoids and aromatic compounds with various biological activities.[4] Our efforts on identifying new agents that target tubulin resulted in the isolation of two known bis(bibenzyls), isoplagiochins A (**1**) and B (**2**).[5] This paper describes effects of two bis(bibenzyls) on tubulin assembly as well as structure–activity relationship based on conformational analysis carried out by NMR spectra and computational chemistry.

The MeOH extract of fresh material of *P. fruticosa* was subjected repeatedly to column chromatography using Sephadex LH-20 ($CHCl_3$:MeOH = 1:1) and silica gel ($CHCl_3$—MeOH) by the guidance of inhibition of polymerization of tubulin to afford isoplagiochins A (**1**) and B (**2**).

Generally antimitotic agents such as colchicines and vinblastine bind to either the colchicine binding site or the vinca alkaloid binding site.[1] On the other hand, paclitaxel promotes the polymerization of tubulin by binding to and stabilizing the resulting microtubule polymer, which differs from those of colchicines, podophyllotoxin, and the vinca alkaloids.[1] In this study, it was found that isoplagiochins A (**1**) and B (**2**) remarkably inhibited the polymerization of tubulin (Table 1). Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. Inhibitory effects of isoplagiochin B (**2**) to tubulin polymerization are shown in Fig. 1, in which tubulin polymerization was inhibited in a concentration-dependent manner. On the other hand, dihydroisoplagiochins A (**3**) and B (**4**), which were derived by hydrogenation of **1** and **2** in the presence of $PtO_2$, respectively, were found to be less potent ($IC_{50}$, >100 μM, respectively) than **1** and **2**, indicating that the restricted biaryl ring system of bis(bibenzyls) may be favorable for tubulin binding.

The above evidence and our interest in cyclic formation led to elucidate the three-dimensional structures of isoplagiochins and dihydroisoplagiochins. The flexibility of cyclic molecules in solution is somewhat difficult to determine the conformation, although

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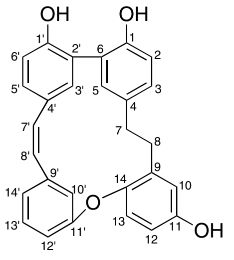
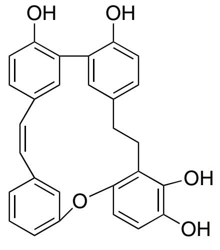
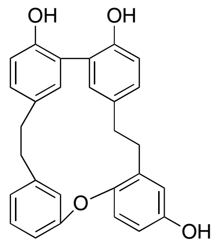


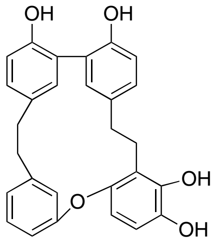
isoplagiochin A (**1**)



isoplagiochin B (**2**)



dihydroisoplagiochin A (**3**)



dihydroisoplagiochin B (**4**)

**Table 1**
Inhibitory effects of isoplagiochins A (**1**) and B (**2**), their dihydroderivatives (**3** and **4**), and vinblastine to the polymerization of tubulin

| Compounds | **1** | **2** | **3** | **4** | Vinblastine |
|---|---|---|---|---|---|
| $IC_{50}$(μM) | 50 | 25 | >100 | >100 | 2.0 |

the NMR provides the most reliable information about the structure in solution. To elucidate the solution conformation of isoplagiochin B (**2**) and dihydroisoplagiochin B (**4**), complete assignments of the $^{1}H$ and $^{13}C$ signals were made by 2D NMR measurements (Table 2).

NMR spectra of **2** and **4** showed only one set of signals and did not provide any evidence for the existence of different disastereomers within the NMR time scale. The NMR experiments thus hinted at the existence of either only one stable stereoisomer or at the presence of the mixture of rapidly interconverting diastereomers.

To analyze the conformational features of **2** and **4**, a series of computational search was conducted by Monte Carlo (MC) search.[6] A total of 3000 MC steps were performed to confirm the reproducibility of calculation results. After the MC conformational search, each of the resulting conformations was subjected to the energy-minimization calculation by MMFF force field.[7] Each of the lowest energy conformers belonging to two separate clusters are represented as **2a** (330.58 kJ/mol) and **2b** (346.83 kJ/mol) for isoplagiochin B, and three clusters **4a** (360.80 kJ/mol), **4b** (358.16 kJ/mol), and **4c** (368.27 kJ/mol) for dihydroisoplagiochin B (Fig. 2). For isoplagiochin B, **2a** was abundant, whereas energy levels for **4a** and **4b** for dihydroisoplagiochin B were within a range of 1 kcal/mol. The presence of

**Table 2**
$^{1}H$ [$\delta_H$ (J, Hz)] and $^{13}C$ [$\delta_C$] NMR Data of isoplagiochin B (**2**) and dihydroisoplagiochin B (**4**) in $CD_3OD$ at 300 K

| Position | **2** | **4** | **2** | **4** |
|---|---|---|---|---|
| | $\delta_H$ | | $\delta_C$ | |
| 1 | | | 150.9 | 150.9 |
| 2 | 6.76(1H, d, 8.1) | 6.74 (1H, d, 8.4) | 115.5 | 115.3 |
| 3 | 7.02 (1H, dd, 2.1, 8.1) | 6.99 (1H, dd, 1.9,8.4) | 127.7 | 127.5 |
| 4 | | | 135.9 | 135.7 |
| 5 | 6.53 (1H, d, 2.1) | 6.49 (1H, brs) | 132.9 | 133.1 |
| 6 | | | 126.5 | 127.0 |
| 7 | 2.70 (2H, s) | 2.70 (2H, m) | 34.2 | 34.3 |
| 8 | 2.70 (2H, s) | 2.76 (2H, m) | 29.0 | 28.8 |
| 9 | | | 123.1 | 123.3 |
| 10 | | | 144.6 | 144.4 |
| 11 | | | 142.4 | 142.2 |
| 12 | 6.73 (1H, d, 8.6) | 6.71 (1H, d, 8.5) | 112.5 | 112.5 |
| 13 | 6.47 (1H, d, 8.6) | 6.44 (1H, d, 8.5) | 112.6 | 112.6 |
| 14 | | | 144.8 | 145.0 |
| 1′ | | | 152.7 | 151.4 |
| 2′ | | | 126.0 | 125.8 |
| 3′ | 7.36 (1H, d, 2.0) | 6.51 (1H, d, 1.9) | 132.2 | 132.8 |
| 4′ | | | 129.1 | 133.4 |
| 5′ | 7.15 (1H, dd, 2.0, 8.2) | 7.08 (1H, d, 8.2) | 130.2 | 128.6 |
| 6′ | 6.88 (1H, d, 8.2) | 6.83 (1H, d, 8.2) | 116.3 | 116.1 |
| 7′ | 6.58 (1H, s) | 3.00 (2H, m) | 129.9 | 35.4 |
| 8′ | 6.61 (1H, s) | 2.89 (2H, d, 5.2) | 128.0 | 37.5 |
| 9′ | | | 141.0 | 143.8 |
| 10′ | 7.29 (1H, brs) | 7.01 (1H, m) | 115.3 | 117.3 |
| 11′ | | | 160.2 | 159.8 |
| 12′ | 6.37 (1H, dd, 2.3, 8.0) | 6.35 (1H, dd, 2.1, 8.0) | 110.8 | 109.7 |
| 13′ | 7.13 (1H, t, 8.0) | 7.07 (1H, m) | 129.4 | 129.0 |
| 14′ | 6.69 (1H, d, 7.5) | 6.68 (1H, d, 7.1) | 121.8 | 122.1 |

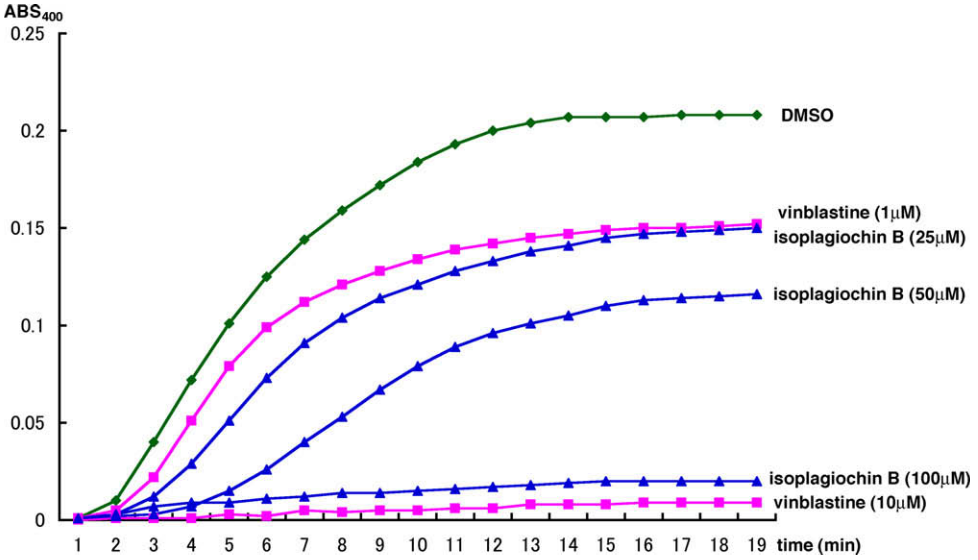
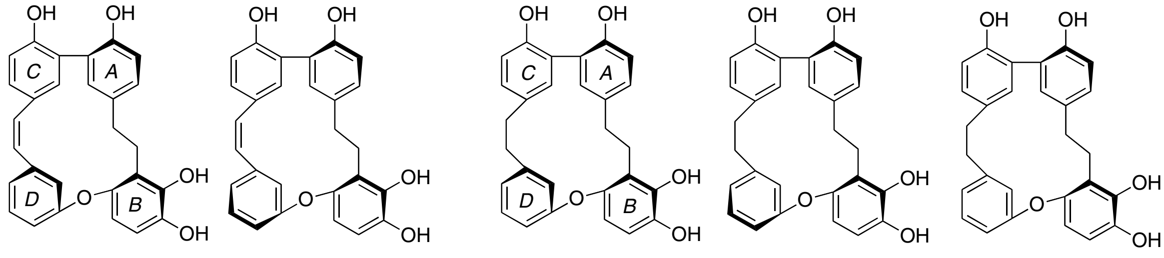


**Figure 1.** Inhibitory effects of isoplagiochin B (**2**) and vinblastine to the polymerization of tubulin protein. Various concentrations of **2** were mixed with tubulin protein (1.5 mg/mL) at 0° and incubated at 37°. The absorbance at 400 nm was measured.



**2a**: 330.58kJ/mol **2b**: 346.83kJ/mol **4a**: 360.80kJ/mol **4b**: 358.16kJ/mol **4c**: 368.27kJ/mol

**Figure 2.** Two representative stable conformers (**2a** and **2b**) of isoplagiochin B (**2**) and three conformers (**4a**, **4b**, and **4c**) of dihydroisoplagiochin B (**4**) analyzed by Monte Carlo simulation followed by minimization and clustering analysis.

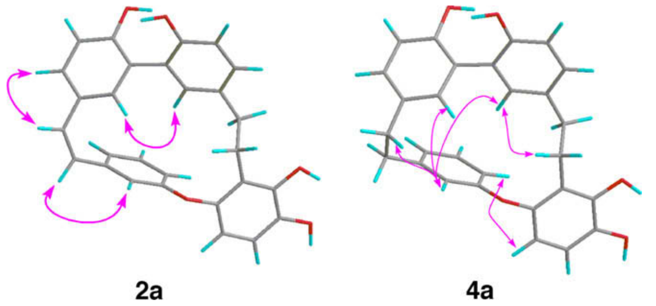
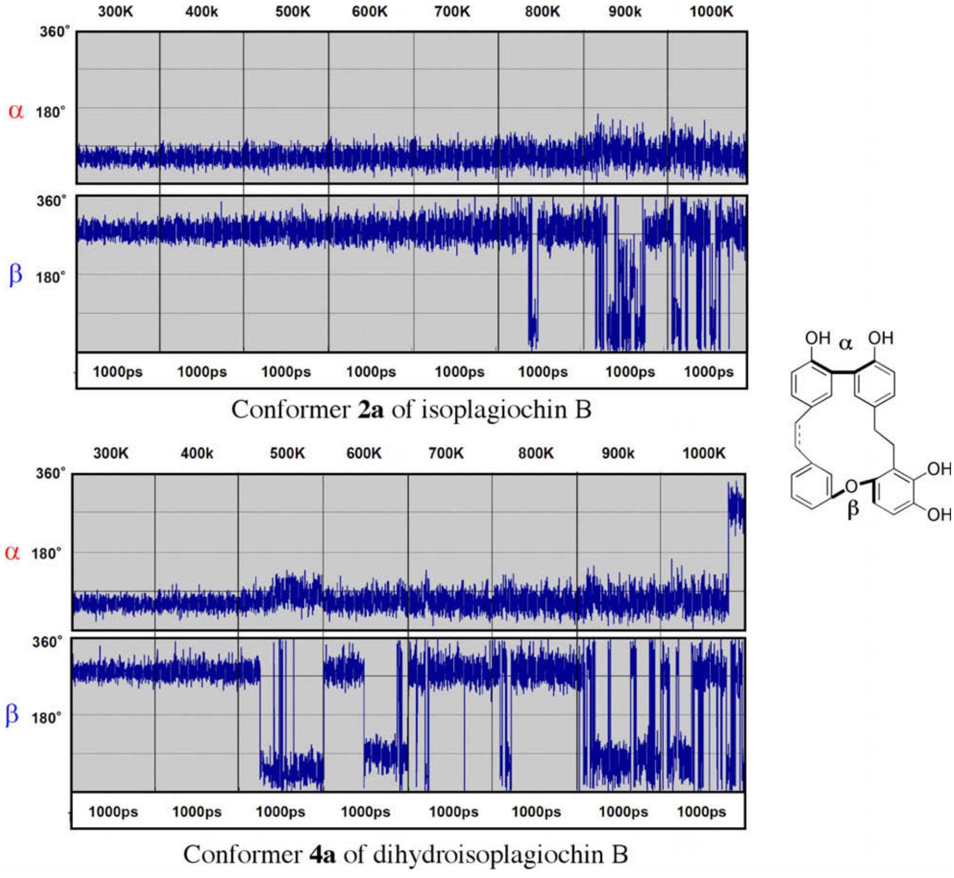


**Figure 3.** NOESY correlations (arrows) for two stable conformers (**2a** and **4a**) at 300 K and relative configurations.

conformations **2a** and **4a** was consistent with the NOESY data as shown in Figure 3. These conformational characteristics of **2** were compatible with those observed in the crystal conformation of isoplagiochin B triacetate by X-ray analysis.[5]

The conformational change among these conformers was examined by molecular dynamics calculation.[8] MD simulations were performed from **2a** and **4a**. No hydrogen bonding and dihedral angle restraints were taken into consideration. Each system was equilibrated for duration of 1000 ps with a thermal bath from 300 K to 1000 K. During 100 ps, starting conformer **4a** was converted into **4b** and then **4a** again at 500 K. On the other hand, the stable conformer of **2a** was not converted into the other conformers until 800 K. (see Fig. 4).

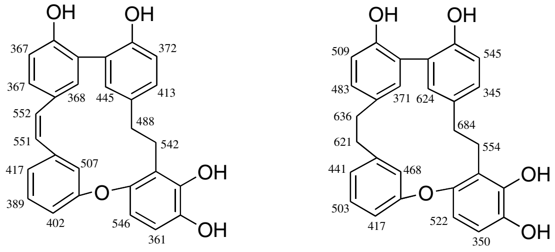
Information about the structural flexibility of this compound can be experimentally obtained from the $T1$ relaxation times of the carbon resonances. The $NT1$ values ($N$ = number of attached protons, $T1$ = longitudinal relaxation time) correlate directly with the molecular mobility.[9] The experimental data provide us with information about the rotational motion of the backbone (Fig. 5).



Conformer **2a** of isoplagiochin B

Conformer **4a** of dihydroisoplagiochin B

**Figure 4.** MD simulation for Isoplagiochin B (**2a**) and Dohydroisoplagiochon B (**4a**). Analysis of the dihedral angles α and β during the MD simulation.



**2**

**4**

**Figure 5.** *NT*1 values in milliseconds of the carbon atoms in isoplagiochin B (**2**) and dihydroisoplagiochin B (**4**) (*N* = number of attached protons, *T*1 = longitudinal relaxation time).

The *NT*1 value of the aromatic rings *A* and *C* in **4** increased compared with those in **2**, indicating that the mobility was increased when the double bond was reduced. In this respect, the two consecutive methylene carbons at C-7 and C-8 of **4** have also large NT1 values. There was no difference between **2** and **4** about the NT1 values of the aromatic rings *B* and *D*. This supports the finding that reduction of the double bond at C-7 caused increase in the mobility, which agrees with the results of MC and MD simulations as described above.

The global minimum structure of **2a** for isoplagiochin B obtained from MC conformational search gave the good agreement with that expected from the NOE relationship, whereas for dihydroisoplagiochin B, it was suggested that **4a** and **4b** have exchangeable mobility in solution. The presence of two aromatic rings which can be connected through the double bond may serve to maintain the backbone conformation. Efforts are currently underway to determine the precise backbone conformation and its relationship to biological activity of a series of bis(bibenzyls), such as Marchantins as antimitotic agents.[4]

## Acknowledgements

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