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Stereocontrolled synthesis of a novel pharmacophore of the tubulin-depolymerizing marine natural product spongistatin

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Abstract

We report here the synthesis and X-ray structure of a novel spiroketal pyran, SPIKET-P1, as a pharmocophore for the tubulin-depolymerizing marine natural product spongistatin 1. Following its retro-synthetic analysis, SPIKET-P1 was prepared using a versatile 11-step synthetic scheme in a stereocontrolled fashion.

Spongistatins are macrocyclic lactone compounds containing six pyran type rings and four of the six pyran rings are incorporated into two spiro[5,5]ketal moieties.[1] Spongistatin 1 (SP) (R=Cl, Fig. 1) is a potent tubulin-depolymerizing natural product which has been isolated from an Eastern Indian Ocean sponge in the genus Spongia.[1,2] SP exhibited potent cytotoxicity with subnanomolar IC_{50} values against the members of the NCI panel of 60 human cancer cell lines.[1] We examined the electron crystallographic structure of tubulin[3] using graphics programs including Grasp and InsightII to identify a possible binding site for SP which would have suitable dimensions to accommodate the large molecular volumn of SP and contain a cluster of hydrophobic residues near the protein surface. The search resulted in the discovery of a unique candidate binding pocket on the tubulin surface which is large enough to accommodate SP and can also provide a highly hydrophobic environment for extensive molecular interactions.[4] The SP molecule was docked into this candidate binding site using the affinity module within the InsightII program. The docking simulation results indicated that the putative SP binding pocket which is located on the surface of tubulin is approximately 8 Å wide×18 Å long×11 Å deep. The pocket consists of an unusual cluster of 10 aromatic residues situated in close proximity that includes Y108, W103, Y185, W407, Y408, F399, F404, F395, F418 and H406 (Fig. 1B).

Advanced modeling studies of the interactions of SP with this putative SP binding pocket indicated that the two spiroketal groups of SP are in close contact with protein residues lining the binding pocket and may therefore serve as the critical binding components of SP. The identification of the spiroketal subunits as the likely tubulin binding elements of SP prompted the hypothesis that simple synthetic spiroketal pyrans ('SPIKET-P compounds') representing these subunits, such as compound SPIKET-P1

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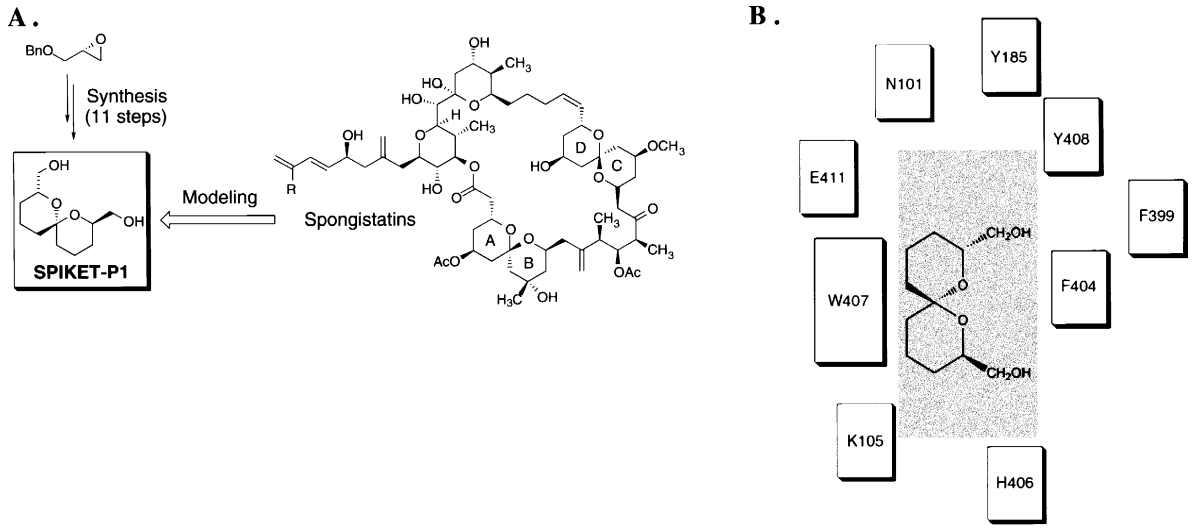
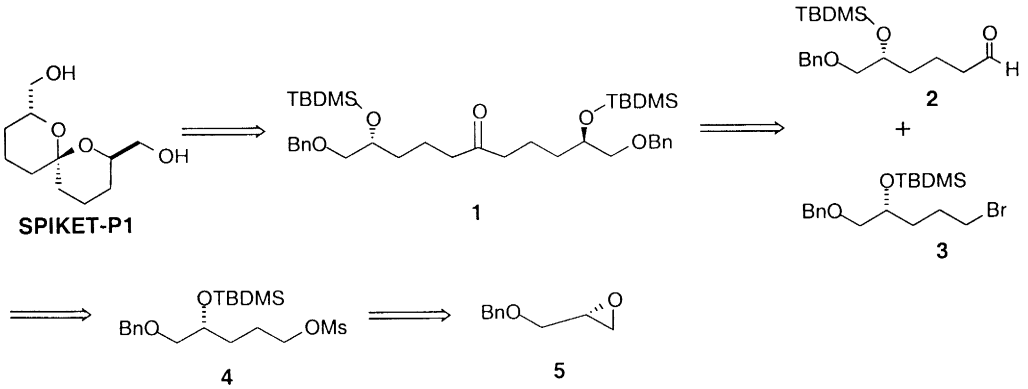


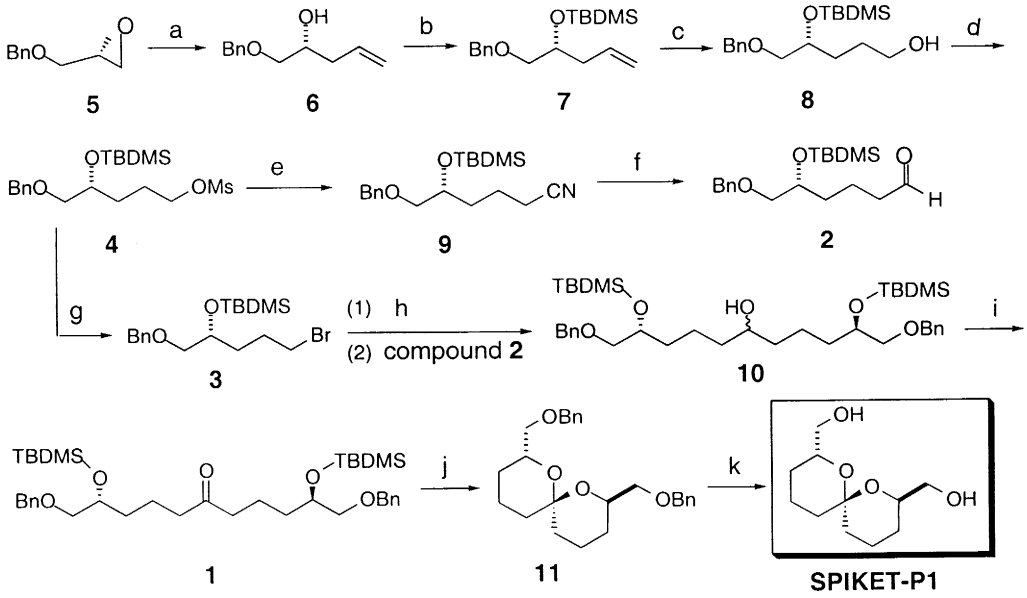
Fig. 1. Modeling design of SPIKET-P as a new pharmocophore of tubulin-depolymerizing agents. **A**: Structures of spongistatin and SPIKET-P1. **B**: SPIKET-P1 as a spongistatin pharmacophore. The spiroketal ring is sandwiched between phenylalanine 404 and tryptophan 407 and close to histidine 406. The spiroketal ring of the SPIKET-P1 is in close contact with the surrounding hydrophobic residues

(Fig. 1A), could serve as SP pharmacophores.[4] Docking studies indicated that, when bound to tubulin, the spiroketal ring of SPIKET-P1 would be sandwiched between aromatic residues F404 and W407 in the binding pocket and would provide favorable hydrophobic interactions and van der Waals contacts with these residues (Fig. 1B). The spiroketal group of SPIKET-P1 has a molecular surface of 218 Å,^{2} 75% of which would be covered by the aforementioned two aromatic rings. Therefore, SPIKET-P1 was selected as our first synthetic target.

Retro-synthetic analysis (Scheme 1) was started by converting the spiroketal group in SPIKET-P1 to the carbonyl and hydroxyl groups in **1**, which was further converted to two segments **2** and **3**. Both **2** and **3** could be derived from the commercially available benzyl (*R*)-(−)-glycidyl ether **5**. The synthesis was initiated by opening the commercially available epoxide **5** using vinylmagnesium bromide to obtain the alcohol **6** which was protected as a *tert*-butyldimethylsilyl ether to form **7** (Scheme 2). Hydroboration of the terminal olefin in **7** yielded the primary alcohol **8**, which was then converted into the mesylate **4**. The mesylate group in **4** was substituted by bromide to form **3**. Also the mesylate in **4** was substituted by cyanide to form **9** which was further converted to aldehyde **2** by DIBAL reduction followed by acid-



Scheme 1. Retro-synthetic analysis



Scheme 2. Stereocontrolled synthesis and structural characterization of the SP pharmacophore SPIKET-P1:[6] (a) vinylMgBr, CuBr, 2 h, 0°C; 81%. (b) TBDMSCl, imidazole, DMAP, 1 h at 0°C, 3 h at rt; 98%. (c) (1) BH_3–THF, (2) H_2O_2, NaOH; 70%. (d) MsCl, Et_3N, 2 h, 0°C; 94%. (e) NaCN, DMSO, 15-crown-5, 40°C, overnight; 95%. (f) DIBAL-H, −78°C, 3 h, 10% tartaric acid; 93%. (g) LiBr, acetone, reflux, 0.5 h; 80%. (h) Mg/THF; 78%. (i) oxalyl chloride, DMSO, Et_3N, −78°C; 83%. (j) 5% HF/CH_3CN, 0.5 h, rt; 100%. (k) LDBB, 0°C; 50%

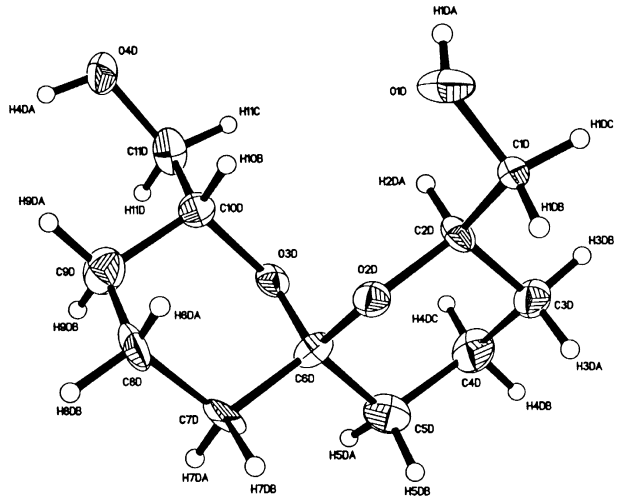


Fig. 2. X-Ray crystal structure of SPIKET-P1, molecule D (30% probability ellipsoids, T=22°C, solvent=chloroform/ether). SPIKET-P1: Space group: $P2_1$, unit cell: a=11.4510(15) Å, b=8.9628(11) Å, c=22.942(3) Å, α=90°, β=93.396(3)°, γ=90°, volume=2350.5(5) Å^3, Z=8, θ range for data collection=1.78 to 28.32° (λ=0.71073 Å), total reflections collected=12806, independent reflections=9044, data/restraints/parameters=9044/1/542, R1 [I>2σ(I)]=0.139, wR2=0.187, goodness-of-fit on F^2=1.009

catalyzed hydrolysis in a one pot reaction. Compound **3** was coupled with **2** by first converting **5** to a Grignard reagent and then reacting it with aldehyde **2** to form **10** (Scheme 2). Swern oxidation converted **10** to **1**. Deprotection of the two *tert*-butyldimethylsilyl protected hydroxyl groups in **1** followed by acid-catalyzed acetal formation to give **11**, the immediate benzyl-protected precursor of SPIKET-P1, was carried out in a one pot reaction by treating **1** with 5% HF in acetonitrile at room temperature

for 30 minutes. After the unsuccessful attempt to remove the two protecting benzylic groups in **11** by platinum-catalyzed hydrogenation, SPIKET-P1 was obtained by treating **11** with lithium 4,4′-di-*tert*-butylbiphenylide (LDBB).[5]

The structure of SPIKET-P1 was confirmed by X-ray crystallography (Fig. 2). SPIKET-P1 caused tubulin-depolymerization and exhibited potent cytotoxicity against taxol-resistant and vincristine-resistant human cancer cells at nanomolar concentrations.[7]

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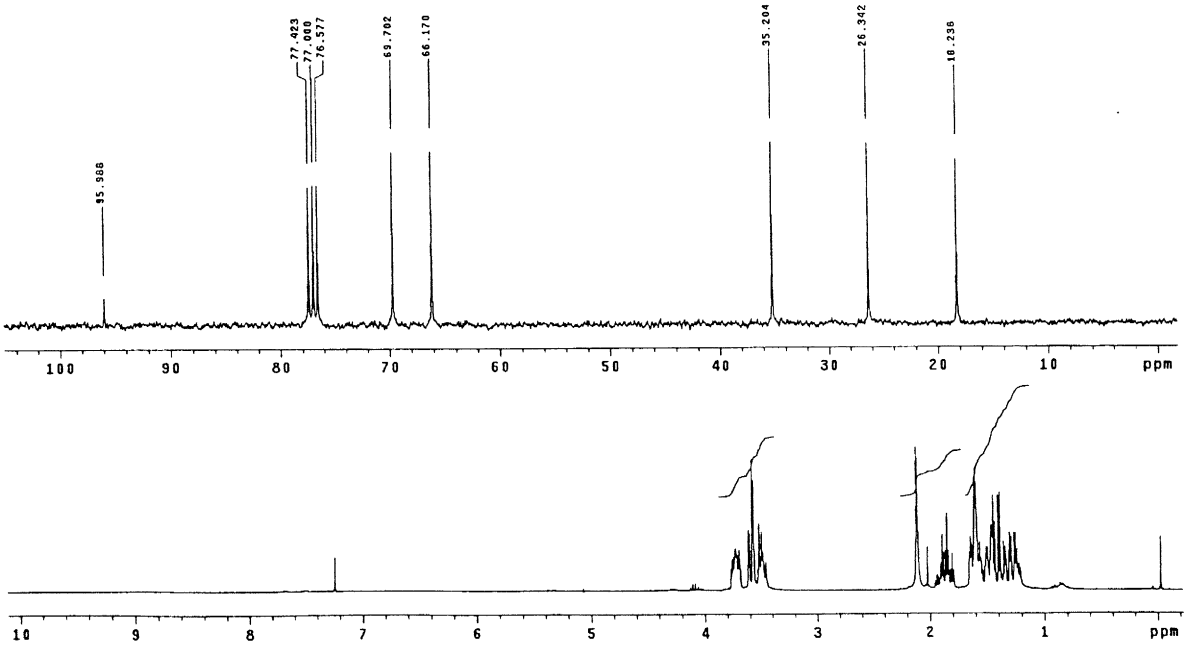
6. Characterization data: [(2*R*,8*R*)-8-(hydroxymethyl)-1,7-dioxaspiro[5,5]undec-2-yl]methan-1-ol (SPIKET-P1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26–1.53 (m, 6H), 1.63 (m, 4H), 1.88 (m, 2H), 2.14 (bs, 2H), 3.52 (dd, J=11.0, 7.0 Hz, 2H), 3.62 (dd, J=11.0, 3.5 Hz, 2H), 3.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 96.0, 69.7, 66.2, 35.2, 26.3, 18.2; GC/MS *m/z* 216 (M^+); IR (neat): 3373, 2939, 2871 cm^{-1}; $[\alpha]_D^{22}$ −59.4 (c 0.7, $CHCl_3$).



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