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A novel natural phenyl alkene with cytotoxic activity

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ABSTRACT

A novel phenyl alkene (**1**) was isolated from a mixture of three Florida sponges, *Smenospongia aurea*, *Smenospongia cerebriformis*, and *Verongula rigida*. Unlike terpenoids or amino acid derivatives, which are commonly known classes of secondary metabolites from these genera, the chemical structure of **1** showed an unprecedented linear phenyl alkene skeleton. Through comprehensive analyses of NMR and MS data, the gross structure of **1** was determined to be (*E*)-10-benzyl-5,7-dimethylundeca-1,5,10-trien-4-ol. The absolute configuration at C-4 was established as *R* by a modified Mosher's method. Based on the relative configuration between C-4 and C-7, the absolute configuration at C-7 was assigned as *S*. Compound **1** showed in vitro cytotoxic activity against HL-60 human leukemia cancer cells with an IC_{50} value of 8.1 μM. Molecular docking study suggests that the structure of compound **1** matches the pharmacophore of eribulin required to display cytotoxic activity through the inhibition of microtubule activity.

Marine sponges are regarded as a rich source of secondary metabolites with chemically diverse structures and potential biological benefits.[1] While investigation of secondary metabolites from marine sponges *Smenospongia* (order Dictyoceratida, family Thorectidae) and *Verongula* (order Verongida, family Aplysinidae) has received great attention, only a few chemical groups that belong to alkaloids and terpenoids have been isolated from these marine invertebrates.[2,3] Of these, sesquiterpene quinones and hydroquinones are the best known classes of secondary metabolites, accounting for more than 170 compounds published from these sponges.[4] Our previous study on the isolation of antidepressant compounds from three Florida sponges, *Verongula rigida* (Esper, 1794), *Smenospongia aurea* (Hyatt, 1875), and *Smenospongia cerebriformis* (Duchassaing and Michelotti, 1864),[3c] showed that the same brominated alkaloids, as well as sesquiterpene quinones and hydroquinones, were found in both *V. rigida* and *S. aurea* despite their apparent taxonomic differences. We thus proposed that similar metabolites found in these distinct species of two different genera provide evidence for a microbial origin of the metabolites.[3c] Due to the similar metabolite profile of these three sponges (as shown by our previous study and LC–MS analysis), we have decided to combine these in order to be able to isolate and characterize compounds present even in minute quantities and to provide larger amounts of brominated metabolites for further animal testing. Comparing to individual isolation of secondary metabolites from each species, we could effectively obtain substantial amounts of brominated indole alkaloids and sesquiterpene quinones from the combined extracts of the three sponges. Recently, our interest in the same sponge materials has focused on the fact that the mixed extracts displayed significant cytotoxicity against HL-60 human leukemia cancer cells. In the course of our continuing research on the isolation of cytotoxic metabolites from the extracts, we found that trace amounts of a novel cytotoxic compound containing an unprecedentedly linear phenyl alkene skeleton existed in each sponge based on LC–MS analyses (Fig. S15). Up to now, to the best of our knowledge, no phenyl alkene type molecule has been identified in these genera. Herein, we describe the isolation and structure elucidation of the new phenyl alkene, and evaluation of cytotoxic activities of the isolate against HL-60 human leukemia cancer cells and MCF-7 human breast cancer cells.

Three sponges[5] were obtained during our large-scale collection in October 2008 and mixed together prior to extraction due to a similar profile of secondary metabolites, especially halogenated indole alkaloids with interesting antidepressant activity in behavioral in vivo tests, revealed in our earlier studies.[3c] The dried ethanol extract (3.6 kg) was subjected to silica gel vacuum liquid chromatography (VLC) and eluted with a stepwise gradient of hexane/acetone/methanol/water to give 13 fractions (Fr. 1–13). Fr. 10

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Table 1
NMR spectroscopic data of compound **1** in $CDCl_3$

Position	δ_C, mult.[a]	δ_H, mult. (*J* in Hz)[b]	COSY	HMBC	NOE
1	117.7, CH_2	5.04, d (10.2); 5.08, br d (17.4)	H-2		
2	135.0, CH	5.72, m	H-1, 3		
3	40.1, CH_2	2.26, m	H-2, 4	C-1, 2	H-13
4	76.8, CH	3.99, t (6.6)	H-3	C-2	H-6
5	135.9, C				
6	132.8, CH	5.12, d (9.6)	H-7		H-4
7	32.3, CH	2.31, m	H-6, 8, 13		H-14
8	34.8, CH_2	1.24, m; 1.39, m	H-7, 9	C-6, 10	
9	28.4, CH_2	2.05, t (8.4)	H-8	C-8, 10	
10	142.5, C				
11	41.3, CH_2	3.33, s		C-9, 10, 12, 1′, 2′	
12	114.0, CH_2	5.79, s		C-9, 10	
13	21.0, CH_3	0.92, d (6.6)	H-7	C-6, 7, 8	H-3
14	11.9, CH_3	1.57, d (1.2)		C-4, 5, 6	H-7
1'	138.4, C				
2'	129.1, 2CH	7.13, d (7.5)		C-11	
3'	128.6, 2CH	7.27, dd (7.5, 7.5)			
4'	126.7, CH	7.20, dd (7.5, 7.5)			

[a] Assignment based on HSQC and HMBC NMR data (150 MHz).
[b] Assignment based on COSY and HMBC NMR data (600 MHz).

(39.3 g) was further divided into 9 fractions (Fr. 10-1 to 10-9) using silica gel VLC with a gradient order of the same four solvents. Fr. 10-6 (2.7 g) was applied to C_{18} MPLC (15.5 × 4 cm) with an isocratic solvent system of methanol/water (85:15) to yield 7 subfractions (Fr. 10-6-1 to 10-6-7). Compound **1** (1.9 mg) was obtained from Fr. 10-6-1 (95.1 mg) over C_{18} HPLC (250 × 21.2 mm, 10 μm) eluted with isocratic methanol/water (78:22). Repeated purification was carried out on Fr. 10-6-2 (457.8 mg) by C_{18} HPLC (250 × 20.0 mm, 5 μm) with isocratic methanol/water (78:22) to yield **1** (4.4 mg; total yield 6.3 mg, 0.000175% dry weight).

Compound **1**[6] was isolated as colorless oil. The HRFABMS showed a molecular ion peak at *m*/*z* 285.2215 in positive mode with the molecular formula $C_{20}H_{29}O$ $[M+H]^+$ (calcd 285.2218) with seven degrees of unsaturation. The ^1H and ^{13}C NMR data in $CDCl_3$ indicated that **1** has a mono-substituted benzene moiety [δ_H 7.27 (2H, dd, *J* = 7.5, 7.5 Hz), 7.20 (1H, dd, *J* = 7.5, 7.5 Hz), 7.13 (2H, d, *J* = 7.5 Hz) and δ_C 138.39 (C-1′), 129.09 (C-2′), 128.60 (C-3′), 126.68 (C-4′)] and six olefinic protons and carbons of three double bonds [δ_H 5.79 (2H, s), 5.72 (1H, m), 5.12 (1H, d, *J* = 9.6 Hz), 5.08 (1H, br d, *J* = 17.4 Hz), 5.04 (1H, d, *J* = 10.2 Hz) and δ_C 142.46 (C-10), 135.86 (C-5), 135.00 (C-2), 132.78 (C-6), 117.67 (C-1), 113.96 (C-12)], which accounted for four and three degrees of unsaturation, respectively (Table 1). An oxygenated methine [δ_H 3.99 (1H, t, *J* = 6.6 Hz) and δ_C 76.84 (C-4)], a benzylic methylene [δ_H 3.33 (2H, s) and δ_C 41.33 (C-11)], an allylic methylene [δ_H 2.05 (2H, t, *J* = 8.4 Hz) and δ_C 28.36 (C-9)], an olefinic methyl [δ_H 1.57 (3H, d, *J* = 1.2 Hz) and δ_C 11.87 (C-14)], and a secondary methyl [δ_H 0.92 (3H, d, *J* = 6.6 Hz) and δ_C 21.02 (C-13)] were distinctively observed in the ^1H and ^{13}C NMR spectra as well (Table 1). As shown in Figure 1, the detailed analysis of the ^1H–^1H COSY, HSQC, HMBC, NOESY, ROESY, and HRFABMS data suggested that the structure of **1** should be (*E*)-10-benzyl-5,7-dimethylundeca-1,5,10-trien-4-ol. The HMBC correlations from the benzylic protons at δ_H 3.33 (H_2-11) to two olefinic carbons at δ_C 142.46 (C-10) and 113.96 (C-12), as well as the allylic methylene carbon at δ_C 28.36 (C-9), indicated the (2-methylenealkyl)-benzene framework (Fig. 1). The consecutive analyses of the ^1H–^1H COSY correlations from the allylic protons at δ_H 2.05 (H-9) to the olefinic proton at δ_H 5.12 (H-6) and the secondary methyl protons at δ_H 0.92 (H-13) showed that an isopentane unit comprised the alkyl chain, in agreement with the analysis of HMBC data where the cross-peaks from H-13 to the olefinic carbon at δ_C 132.78 (C-6), a methylene carbon at δ_C 34.76 (C-8), and a methine carbon at δ_C 32.31 (C-7) were detected (Fig. 1). The next partial structure was deduced by the HMBC correlations from the olefinic methyl protons at δ_H 1.57 (H-14) to the olefinic carbons at δ_C 135.86 (C-5) and 132.78 (C-6), and oxygenated methine carbon at δ_C 76.84 (C-4), presenting a connection to the isopentane group at C-6 (Fig. 1). The analyses of successive ^1H–^1H COSY correlations from the oxygenated methine proton at δ_H 3.99 (H-4) to the olefinic protons at δ_H 5.08 and 5.04 (H_2-1) established the last partial structure, which turned out to be a secondary alcohol, joining the existing part of the structure at C-4. Corresponding analysis of the HMBC data verified the arrangement in which the cross-peaks from the H-4 to the olefinic carbon at δ_C 135.00 (C-2), as well as from methylene protons at δ_H 2.26 (H-3) to the olefinic carbons at δ_C 117.67 (C-1) and C-2 were observed (Fig. 1). The *E*-geometry of the C-5/C-6 olefin was supported by ^{13}C NMR chemical shift of C-14 at δ_C 11.87[7] (vs δ_C 20.5 for *Z*-geometry)[7] and confirmed by the NOE correlation between H-7 and H-14 (and not between H-6 and H-14 which would be seen in the case of *Z*-geometry).

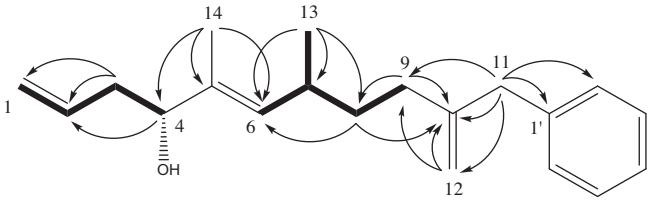

Figure 1. ^1H–^1H COSY (▬) and key HMBC (→) correlations of compound **1**.

The relative configurations of the two stereogenic centers at C-4 and C-7 were initially proposed by comparing ^1H NMR data with synthetic diastereomers.[8] In common partial structures from C-3 to C-8 including C-13 and C-14, the most distinguishable feature of ^1H NMR data between the two diastereomers is a coupling constant (*J*) of H-4. While H-4 has two *J* values as 3.5 and 8.9 Hz which appeared to be a doublet of doublets in a *syn* conformation, *J* is 6.0 Hz as a triplet in an *anti* conformation which corresponds to compound **1** (Fig. S1).[8] Also, every possible rotamer was carefully considered by virtue of the $^3J_{H,H}$ value, as well as the 1D and 2D NOE correlations (Fig. 2). The large ^3J(H-6, H-7) value (9.6 Hz) showed the *anti* orientation of H-6/H-7,[9] which was in agreement with the rotamers A-1 and B-1, in addition to the NOE correlations of H-7/H-14 and H-4/H-6. However, cross-peaks between H-3 and H-13 in the NOESY and ROESY data (Fig. 2, Figs. S6 and S7), which were consistent with the 1D NOE analysis (Fig. S8), demonstrated rotamer A-1 in which the relative configurations of C-4 and C-7 were R^* and S^*, respectively.

The absolute configuration of **1** was assigned by high-field FT NMR application of Mosher's method.[10] The *R*-(−) and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (5 μL) were added to two portions (each 0.8 mg) of **1** in dry pyridine (200 μL) under N_2 gas stream and respectively afforded the (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA) esters at

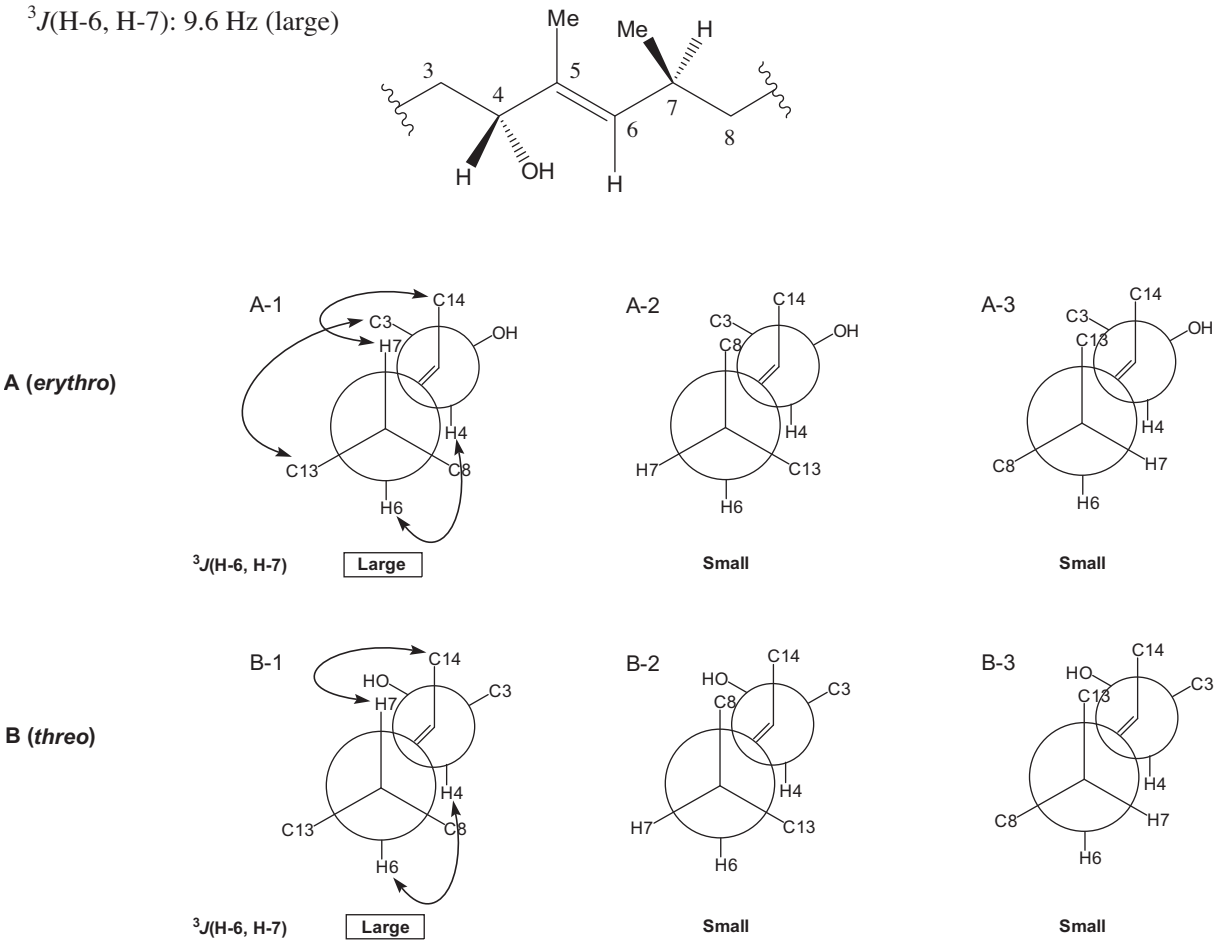

Figure 2. Newman projections of all possible rotamers for C-4–C-7 are shown. Predicted coupling constant values are labeled below the projections, and corresponding values with the observation are box highlighted. The NOE correlations are depicted as double-headed arrows (↔).

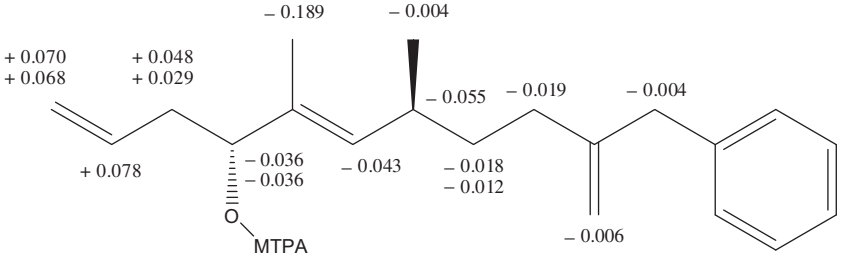

Figure 3. $\Delta\delta_{S-R}$ values in ppm for *S*- and *R*-MTPA esters of compound **1** in $CDCl_3$

room temperature after 48 h. The ^1H NMR chemical shifts were assigned by the analysis of the ^1H–^1H COSY NMR data for each MTPA ester (Figs. S11 and S12).[11,12] The calculated $\Delta\delta_{S-R}$ values were positive for the C-1–C-3 segment but negative for the C-4–C-14 segment (Fig. 3), which implied the absolute configuration of C-4 was *R*. Considering the relative configuration of C-4 and C-7, the absolute configuration of C-7 was assigned to be *S*. Consequently, the chemical structure of compound **1** was elucidated as (4*R*, 7*S*, *E*)-10-benzyl-5,7-dimethylundeca-1,5,10-trien-4-ol.

Due to limited quantities, compound **1** was only evaluated for cytotoxic activity against the cancer cell lines HL-60 and MCF-7 in the MTT (3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyl-2H-tetrazolium hydrobromide) assay. Compound **1** was cytotoxic toward HL-60 cancer cells with an IC_{50} value of 8.1 μM. No significant cytotoxicity was observed against MCF-7 cancer cells.

Eribulin is a tubulin-inhibiting chemotherapeutic drug effective on multiple cancer cell types including leukemia, breast, and colon cancers.[13] Eribulin is a structurally simplified synthetic analogue of halichondrin B, which was initially isolated from the marine sponge *Halichondria okadai*[14] and has also been isolated from three other sponge species.[15] To test whether compound **1** shares the cytotoxic pharmacophore of eribulin, conformational searches and consecutive flexible alignment analyses were conducted employing the MacroModel and Phase programs (Schrödinger LLC)(Tables Figs. S1, S2 and S16).[16] The conformers of compound **1** which demonstrated partial structural similarity with eribulin

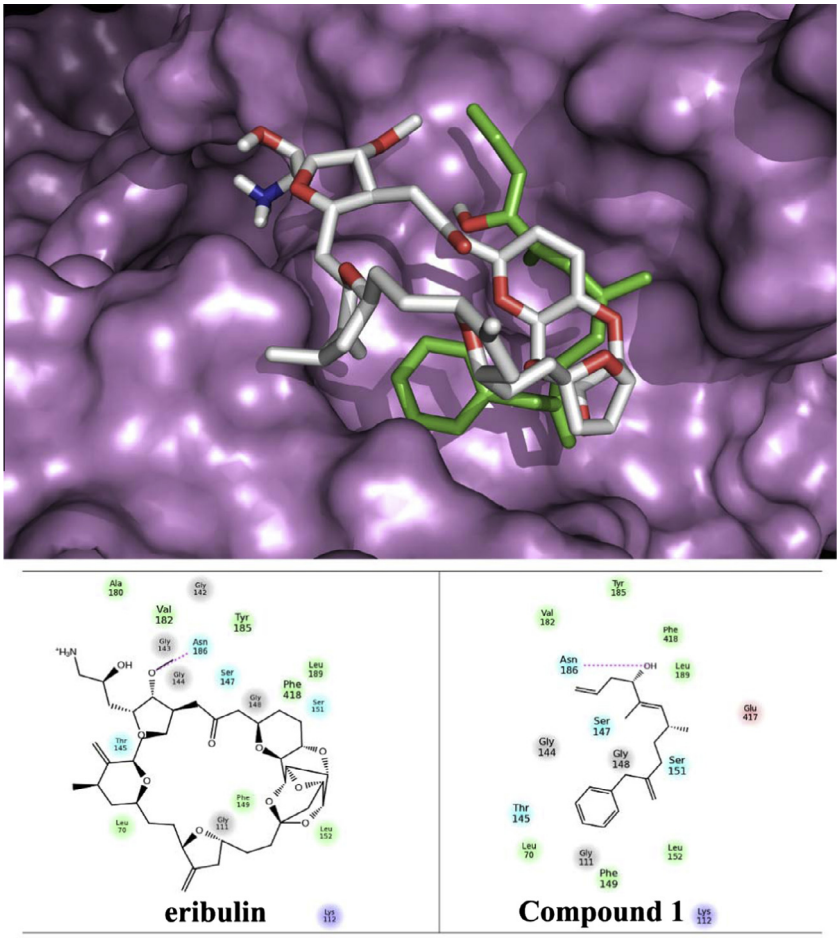

Figure 4. Overlaid image of compound **1** and eribulin docked to the β-tubulin protein (top) and ligand interaction diagrams (bottom). Colors stand for different types of interactions: gray (glycine interactions), green (hydrophobic interactions), cyan (polar interactions), purple (positively-charged interactions), and pink dots (hydrogen-bonding interaction with a side chain of a residue).

were further docked to the β-tubulin protein following the previously reported approach to eribulin docking.[17] Compound **1** and eribulin exhibited a stereoelectronic fit to the glycine-rich helix H4 based on the observed hydrophobic interactions with Gly144 and Gly148 (Fig. 4 and Ref. [17a]). According to the proposed docking pose, stabilizing factors for eribulin such as the hydrophobic side chains of Val182 and Leu189 in helix H5 and Leu70 from T2-loop were detected in the docking pose of compound **1** as well (Fig. 4 and Ref. [17a]). This suggests that the two ligands pack into a similar binding pocket generated by the hydrophobic residues of helixes H4 and H5, and T2-loop (Fig. 4 and Ref. [17a]). In addition to these lipophilic interactions, a favorable hydrogen bonding interaction of Asn186 to the methoxy group of eribulin and to the hydroxy group of compound **1** further stabilized the docking poses (Fig. 4, bottom). Even though eribulin bears more oxygen atoms than its counterpart, only one oxygen atom of each ligand participates in hydrogen bonding in the proposed docking poses. Based on the similar interactions of the two ligands with the above-mentioned residues of the β-tubulin protein, compound **1** seems to match the pharmacophore of eribulin required to exert cytotoxic activity through inhibition of microtubule activity.

The synthetic approach will be undertaken to explore the anticancer potential and mechanism of action for the compound.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.05.032.

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5. Sponge material: *S. aurea*, *S. cerebriformis*, and *V. rigida* were collected from the Key Largo, FL in October 2008. The sponges were collected from a shallow coral reef habitat between 3 and 24 m depth.
6. Compound **1**: colorless oil; $[\alpha]_D^{25}$ − 0.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 205 nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 1; HRFABMS *m*/*z* 285.2215 [M+H]+ (calcd for $C_{20}H_{29}O$: 285.2218).
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11. S-MTPA ester of **1**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.79 (2H, s), 5.63 (1H, m), 5.39 (1H, dd, *J* = 7.5, 6.0 Hz), 5.25 (1H, br d), 5.07 (1H, br d, *J* = 17.0 Hz), 5.01 (1H, br d, *J* = 10.5 Hz), 3.32 (2H, s), 2.47 (1H, m), 2.32 (1H, m), 2.25 (1H, m), 2.01 (2H, m), 1.373 (3H, d, *J* = 1.5 Hz), 1.372 (1H, m), 1.22 (1H, m), 0.91 (3H, d, *J* = 6.5 Hz) (Fig. S9).
12. R-MTPA ester of **1**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.80 (2H, s), 5.55 (1H, m), 5.43 (1H, dd, *J* = 7.5, 6.5 Hz), 5.30 (1H, br d), 5.00 (1H, br d, *J* = 17.0 Hz), 4.94 (1H, br d, *J* = 10.5 Hz), 3.32 (2H, s), 2.42 (1H, m), 2.30 (1H, m), 2.29 (1H, m), 2.03 (2H, m), 1.56 (3H, d, *J* = 1.5 Hz), 1.39 (1H, m), 1.23 (1H, m), 0.92 (3H, d, *J* = 6.5 Hz) (Fig. S10).
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16. All conformational searches were conducted using the MACROMODEL program included in the Schrödinger software package (Schrödinger LLC). The searches were executed in water using the OPLS2005 force field, a 10 kcal/mol upper energy limit, and 0.001 convergence threshold. Alignment of eribulin with the generated conformers of compound **1** was conducted using the Flexible Alignment algorithm of the Phase program, Schrödinger LLC, but restricting the two aligned structures to be strictly rigid.
17. (a) Bai, R.; Nguyen, T. L.; Burnett, J. C.; Atasoylu, O.; Munro, M. H. G.; Pettit, G. R.; Smith, A. B.; Gussio, R.; Hamel, E. *J. Chem. Inf. Model.* **2011**, *51*, 1393–1404; (b) The conformers having shape similarity scores >0.3 were chosen for a docking study. 1JFF (http://www.pdb.org) was prepared and minimized using the protein preparation wizard (Schrödinger LLC). Two loops (residues 94–119 and 399–414) were removed to accommodate the binding of eribulin and the apo-protein structure was subjected to a grid generation process using the GLIDE program (Schrödinger LLC). The docking site was the centroid of the three active residues reported previously (Ref. [17a]) (Trp103, Leu189 and Tyr408). Given that Trp103 and Tyr408 are located in the removed loops, before their removal the *xyz*-coordinates of the active residues were selected and used to fashion a grid for further Glide docking experiments. The conformations of the ligands were retained during all docking procedures.