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New Steroids with a Rearranged Skeleton as (h)P300 Inhibitors from the Sponge *Theonella swinhoei*

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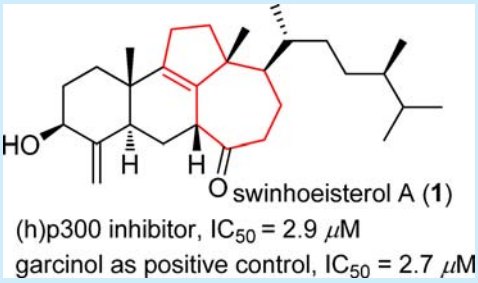
Supporting Information

ABSTRACT: Swinhoeisterols A (**1**) and B (**2**), two novel sterols with an unprecedented 6/6/5/7 ring system, were isolated from the sponge *Theonella swinhoei*. The structures and absolute configurations were elucidated by spectroscopic analysis, X-ray single-crystal diffraction, modified Mosher's method, and TDDFT/ECD calculations. The cytotoxicity of these compounds toward A549 and MG-63 cells encourages studies on their potential target using an inverse virtual screening approach. The predicted inhibitor of h(p300) was corroborated by an in vitro biological test.



Marine sponges have been proven to be rich sources of diverse and biogenetically unprecedented steroids.[1] These compounds are sometimes very complex with extensive modifications to both the side-chain and the nucleus, many without a terrestrial counterpart. The unconventional features in the nucleus of steroids isolated from sponges include additional oxygenation, alkylation, degradation, sulfate esterification, *cis* fused A/B ring junctions, aromatization or contraction in the A ring, unsaturation in the D ring, or secostructures with one of the rings oxidatively cleaved.[2−4]

As part of our ongoing search for secondary metabolites from marine organisms of the South China Sea,[5−8] we collected the marine sponge *Theonella swinhoei* (Order Lithisida, Class Demospongia) off the coast of Xisha island. *T. swinhoei* has been found to be a chemically prolific species, and more than 100 metabolites have been reported up to date,[9] including nonribonsomal polypeptides, polyketide macrolides, polyene derivatives, and uncommon steroids. In this regard, steroids with a 4-methylenyl group are proposed as taxonomic markers for the genus.[10,11] Our current investigation on the nonpolar extract of *T. swinhoei* led to the isolation of two novel sterols, swinhoeisterols A (**1**) and B (**2**), which feature an unprecedented 6/6/5/7 ring system (Figure 1). At the same time, two known sterols, conicasterol (**3**)[12] and swinhosterol B (**4**),[13,14] were isolated as well. We report here the isolation, structural elucidation, and biological activity of these two new compounds.

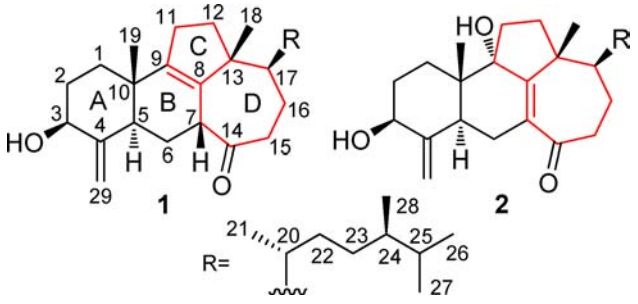


Figure 1. Structures of **1** and **2**.

The sponge *T. swinhoei* (3.6 kg) was extracted with acetone (6 × 1.5 L), and the combined extracts were concentrated and partitioned between 90% MeOH and petroleum ether. The petroleum ether layer (13.6 g) was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and preparative HPLC to give compounds **1** (6.0 mg), **2** (1.5 mg), **3** (123.8 mg), and **4** (28.9 mg).

Swinhoeisterol A (**1**), an optically active, off-white amorphous powder, showed a molecular formula of $C_{29}H_{46}O_2$ as determined by HRESIMS at *m*/*z* 449.3398 $[M + Na]^+$ (calcd 449.3396), requiring seven double bond equivalents. The IR displayed absorptions for hydroxy (3392 cm^{-1}) and carbonyl

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(1703 cm^{-1}) functionalities. The ^{13}C and DEPT NMR spectra revealed the presence of 5 sp^2 carbon atoms (1 × C═O, 1 × C═C, 1 × CH_2═C) and 24 sp^3 carbon atoms (6 × CH_3, 9 × CH_2, 6 × CH, 1 × OCH, 2 × C), accounting for three double bond equivalents (Table S1, Supporting Information). The remaining unsaturations were attributed to four rings in the molecule. The ^1H NMR spectrum of **1** showed signals characteristic of a 4-methylene-24-methyl steroidal system: two methyl singlets (δ_H 0.78, 1.00), four methyl doublets [δ_H 0.78 (d, J = 6.8 Hz), 0.80 (d, J = 7.1 Hz), 0.85 (d, J = 6.8 Hz), 0.92 (d, J = 6.8 Hz)], one oxymethine proton (δ_H 4.03, m), and two exomethylene protons (δ_H 4.68, s; 5.09, s).

Analysis of the COSY spectrum readily delineated four isolated proton spin system: H_2-1 to H-3, H-5 to H-7, H_2-11 to H_2-12, and H_2-15 to H_3-26/27 (Figure 2). The connection of

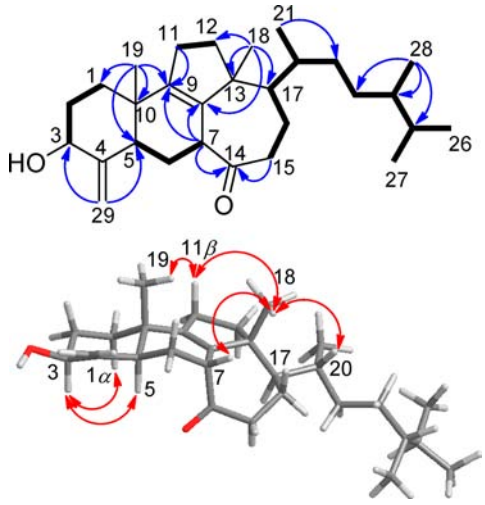

Figure 2. Key $^1H-^1H$ COSY (bold), HMBC (blue arrow), and NOE (red arrow) correlations of compound **1**.

H-3 and H-5 was established by the allylic correlations between H_2-29 and H-3 and H-5 and by HMBC correlations from H_2-29 to C-3 and C-5. The obvious downfield-shifted signals of H-7 (δ_H 3.14) and H_2-11 (δ_H 2.21 and 2.27) suggested that both were allylic hydrogens, inferring the presence of a Δ^8 double bond. This was further supported by HMBC correlations from H-7 to C-8 and C-9 and from H_2-11 to C-9. HMBC correlations from H_3-19 to C-1, C-5, C-9, and C-10 secured the AB ring system. The HMBC correlations from both H-7 and H_2-15 to C-14 (δ_C 213.7) provided the connection from C-7 to C-15 through the carbonyl group at C-14. The HMBC correlations from the angular methyl group H_3-18 to C-8, C-12, C-13, and C-17 allowed the formation of rings C and D through the C-13 quaternary carbon atom. The 24-methyl side chain attached at C-17 was easily deduced from the COSY and HMBC spectra.

The distinct NOE cross-peaks of H-3 with both H-1α and H-5 indicated a 1,3,5-triaxial arrangement for these protons (Figure 2). A β orientation of H_3-19, H_3-18, and H-7 was deduced from the NOE correlations of H-11β with H_3-18 and H_3-19, and H_3-18 with H-7. The NOE cross peaks of H_3-18 with H-20 suggested a β configuration of the side chain and an α configuration of H-17, which was consistent with the absence of NOE correlation between H_3-18 and H-17. Therefore, the relative configuration of the core structure of **1** was tentatively assigned as (3S*,5R*,7R*,10S*,13R*,17R*).

The (3*S*) absolute configuration of **1** was determined by the modified Mosher's method by measuring the differences in the chemical shifts ($\Delta\delta^{R-S}$) of its (*R*)- and (*S*)-MPA esters (Figure S1, Supporting Information), leading to the determination of the absolute configuration of the core structure.[15] The (*R*) configuration at C-24 was determined by comparison of the ^{13}C NMR chemical shift difference of C-26 and C-27 (2.1 ppm) with those of epimeric steroidal side chains (1.8 ppm for 24*R* and 2.8 ppm for 24*S*).[16] Although it is widely accepted that the naturally occurring sterols possess a common (20*R*) configuration,[3] this was still investigated by comparison of the recorded specific rotation ($[\alpha]_D$) values with those of calculated ones for (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*)-**1** and its (20*S*)-epimer. The $[\alpha]_D$ values were calculated using the density functional theory (DFT) framework at B3LYP/6-31+G(d,p) level using polarizable continuum model (PCM) for $CHCl_3$ (Table S11, Supporting Information). The calculated value for (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*)-**1** (+117.9) is in good agreement with the experimental $[\alpha]_D$ value in $CHCl_3$ (+96.0) and greatly differs from that of its (20*S*) epimer (+198.9).

The absolute configuration of **1** was further confirmed by measurement of the electronic circular dichroism (ECD) spectrum and comparison with calculated ECD data. The initial CVFF conformational search of (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*)-**1** resulted in three conformers, the DFT reoptimization of which gave a dominant conformer with 99.9% population. The lowest energy conformer was submitted to ECD calculation by the time-dependent DFT (TDDFT) method with the same basis set within the PCM solvent model for CH_3CN. The measured ECD curve exhibited a positive Cotton effect (CE) at 242 nm and a negative CE at 295 nm, matching well with the calculated ECD curve for (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*)-**1** (Figure 3; Figures S2 and S3 and Tables S3−S6, Supporting Information). Thus, the absolute configuration of **1** was unambiguously established to be (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*).

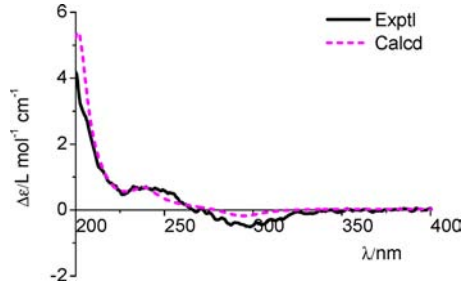

Figure 3. Comparison between the calculated ECD spectrum (in red) and experimental ECD spectrum (in black) of (3*S*,5*R*,7*R*,10*S*,13*R*,17*R*,20*R*,24*R*)-**1**.

Swinhoeisterol B (**2**) was isolated as a colorless crystal and showed a molecular formula $C_{29}H_{46}O_3$ as determined by HRESIMS at m/z 465.3343 $[M + Na]^+$ (calcd 465.3345). The ^1H and ^{13}C NMR data of **2** (Table S1, Supporting Information) greatly resembled those of **1**, with the exception of resonances from C-7 to C-9. The tetrasubstituted double bond (δ_C 132.3 and 157.5) was assigned to be Δ^7 on the basis of diagnostic HMBC correlations from H_2-6 to C-7 and C-8 and from H_3-18 to C-8. The location of Δ^7 was in agreement with the observation of UV absorption at 252 nm, showing the appearance of an enone functionality in the molecule. An

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additional oxygen-bearing quaternary carbon (δ_C 84.5) was located at C-9 by HMBC correlations from H_2-11 and H_3-19 to C-9. An α configuration for the hydroxy group at C-9 was indicated by pyridine-induced solvent deshielding shifts of H-1α and H-5 due to the 1,3-diaxial relationship of these protons to the 9-OH (Table S2, Supporting Information).[17] The absolute configuration of the side chain in **2** remained the same as indicated by comparison of its ^{13}C NMR data with those of **1** and of the epimeric steroidal side chain.[16] Thus, the relative configuration of the nucleus in **2** was determined as (3S*,5S*,9S*,10S*,13R*,17R*,20R*). The structure and relative configuration of **2** were confirmed by a single-crystal X-ray diffraction analysis using Cu Kα radiation with Flack parameter of −0.3(3) (Figure 4; Table S12, Supporting Information).[18]

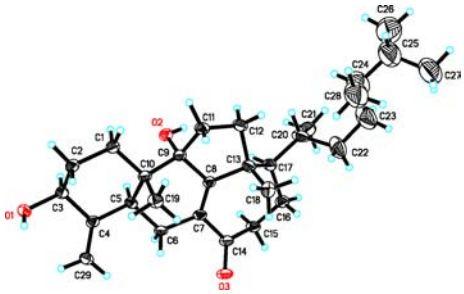


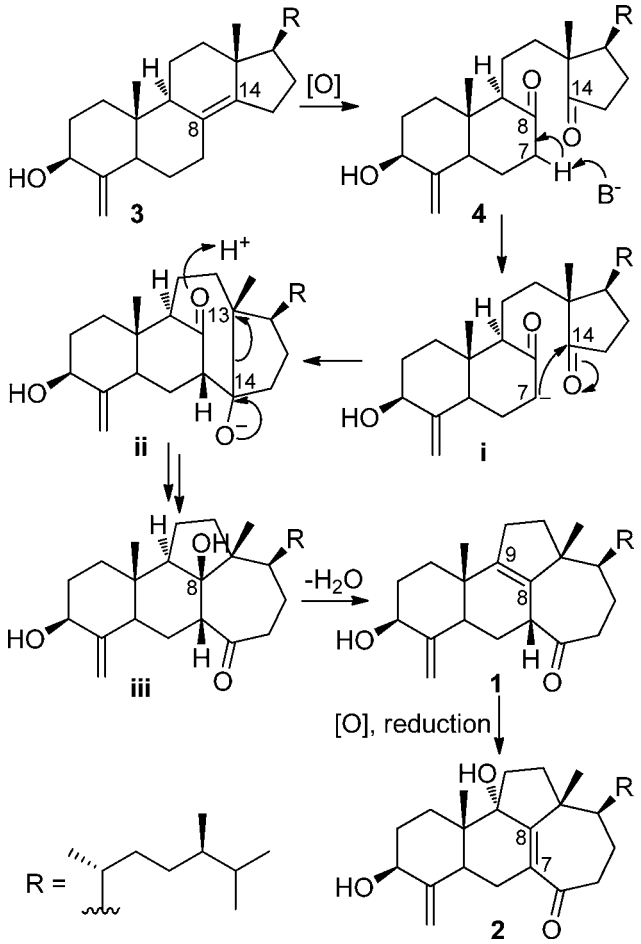
Figure 4. Single-crystal X-ray structure of **2** (ORTEP drawing).

The absolute configuration of **2** was established by comparisons of ECD and $[\alpha]_D$ experimental values with theoretical data. The measured ECD curve of **2** showed CEs at λ_{max} ($\Delta\varepsilon$) 250 (+6.25) and 324 (−1.58) nm, which were in good agreement with the calculated spectra. The (20*R*) configuration was also confirmed by comparison of the experimental $[\alpha]_D$ (+55.0) of **2** with theoretical data (+50.1) and that of its (20*S*) epimer (+28.0) (Tables S7−S11 and Figures S4 and S5, Supporting Information).

The biogenetic origin of **1** and **2** can plausibly be tracked back to compound **3**. As shown in Scheme 1, oxidative cleavage of the $\Delta^{8(14)}$ in **3** yields compound **4**. Deprotonation of H-7 with base generates a nucleophile intermediate **i**, which initiates an intermolecular aldol condensation to form aldol product **ii**. An enzyme-catalyzed cleavage of the C13−C14 bond and subsequent rearrangement yields intermediate **iii** with the unprecedented 6/6/5/7 ring system present in metabolites **1** and **2**. Compound **iii** may undergo dehydration via *trans*-elimination to give the unsaturated carbonyl compound **1** with $\Delta^{8(9)}$. Compound **2** may be derived from **1** by simple allylic oxidation involving base abstraction of the 7β proton, formation of $\Delta^{7(8)}$, addition of molecular oxygen to C-9, and peroxidase-catalyzed reduction to form the 9α hydroxy group.

Swinhoeisterols A (**1**) and B (**2**) were evaluated in vitro for their tumor cell growth inhibitory activity toward A549 (human lung adenocarinoma) and MG-63 (human osteosarcoma cell) cells using adriamycin as a positive control (IC_{50} = 5.2 and 5.9 μM, respectively). These compounds exhibited cytotoxicity against A549 (IC_{50} = 8.6 and 14.6 μM, respectively) and MG-63 (IC_{50} = 10.3 and 20.0 μM, respectively) cells. An in silico study was subsequently used to explore the possible protein targets of compound **1**, using the recently introduced inverse virtual screening approach.[19,20] Inverse virtual screening represents a fast tool for the selection of the most promising targets of bioactive compounds. As previously reported,[20] the

Scheme 1. Plausible Biosynthetic Pathway for 1 and 2



reliability of this approach mainly derives from the specific scoring function of the software employed. We screened **1** against 211 targets involved in cancer processes by means of molecular docking calculations. After the normalization process, a ranking was obtained (Table S13, Supporting Information). The most favorable computational models between **1** and the first 10 targets were then analyzed and the results were corroborated by in vitro biological assays. Among the tested targets, **1** showed a remarkable inhibitory activity against (h)p300, a histone acetyltransferase associated with the manifestation of cancer,[21] with an IC_{50} value of 2.9 μM (garcinol as positive control, IC_{50} = 2.7 μM).

Docking models of the **1**-(h)p300 (PDB code 3BIY) complex gave potential insights into the molecular basis for the observed inhibitory activity. As shown in parts a−c of Figure 5, the compound is well accommodated in (h)p300, occupying a large part of the ligand binding site (LBD) and possessing both polar and hydrophobic interactions. More precisely, O3 on ring D is hydrogen bonded with Q1455, while polar interactions are observable between ring A of **1** and S1400, H1402, R1410, and K1456. The remaining core part of the molecule establishes several hydrophobic contacts (P1440, P1458, L1463, W1466) as well as the side chain, interacting with I1395, Y1397, L1398, W1436, A1437, C1438, P1439, Y1446, Y1467 (Figure 5d, e). Since h(p300) was identified as the best second target for **2** (Table S14, Supporting Information), we decided to evaluate its putative inhibitory activity. The docking model of the **2**-h(p300) complex

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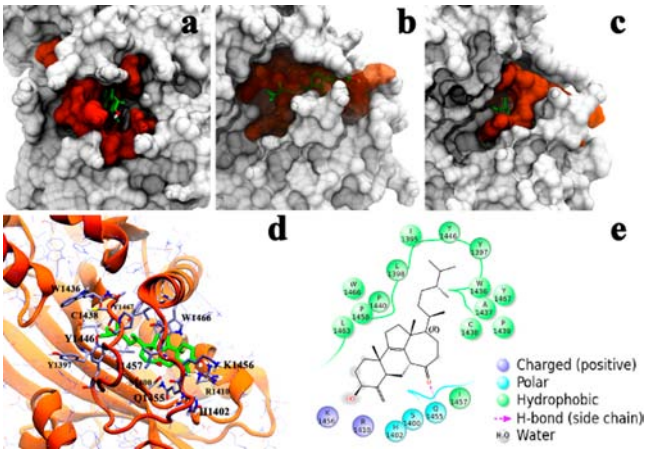

Figure 5. Three different views (a–c) of the docking predicted model of **1** (represented in licorice and colored by atom type: C, green; H, white; O, red) in the ligand binding site of h(p300) (molecular surface represented in light gray, ligand binding site highlighted in orange); (d) 3D docking model of **1** (represented in licorice and colored by atom type: C, green; H, white; O, red) in the ligand binding site of h(p300); (e) 2D panel representing the interactions between **1** and the residues of the receptor counterpart.

confirmed the most of the interactions previously found for **1** (Figure S6, Supporting Information). However, biological tests showed a poor inhibitory activity of **2** against (h)p300 with an IC_{50} value of 240 μM. In this regard, future perspectives on inverse virtual screening will concern the combination of different docking softwares (consensus docking) or the refinement of the results by means of molecular dynamics, in order to increase the sampling phase (ensemble docking) and the accuracy of binding energy estimation.

Two novel steroids with an unprecedented 6/6/5/7 ring system were isolated and characterized from the sponge *T. swinhoei*, expanding the family of steroids with a new rearranged carbon skeleton. This discovery gives additional insight into the productivity and biosynthetic capacity of this sponge. The potent cytotoxity of these compounds encourages further studies on their potential target using the inverse virtual screening approach. The predicted inhibitor of h(p300) was corroborated by an in vitro biological test. H(p300) is the most thoroughly studied protein among histone acetyltransferases (HATs) family, a group of enzymes that play a significant role in the regulation of gene expression in eukaryotes as well as all viral DNA that integrates into the human genome.[22] Dysfunction of h(p300) has been implicated in several diseases, predominantly cancer. Several small-molecule p300 inhibitors, such as Lys-CoA, garcinol, C646, anacardic acid, curcumin, plumbagin, and isothiazolones, have been reported to date.[23,24] These observations lead to the proposal that swinhoeisterols may serve as a new structural type of h(p300) inhibitor. This research gives a new paradigm for drug discovery by integration of natural products science with computation chemistry.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, text, tables, figures giving HRMS and NMR spectra for **1** and **2**, crystallographic data for **2** (CDDD 983568), low energy conformers for **1** and **2**, atom coordinates, and absolute energies of the computed structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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