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# Acredinones A and B, Voltage-Dependent Potassium Channel Inhibitors from the Sponge-Derived Fungus *Acremonium* sp. F9A015

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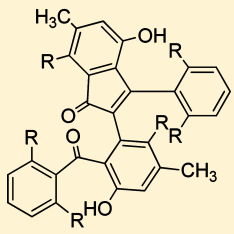
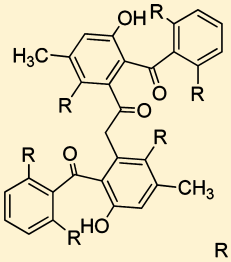
Supporting Information

**ABSTRACT:** Two new benzophenones, acredinones A (**1**) and B (**2**), were isolated from a marine-sponge-associated *Acremonium* sp. fungus. Their chemical structures were elucidated on the interpretation of spectroscopic data. The structure of **1** was confirmed by palladium-catalyzed hydrogenation, followed by spectroscopic data analysis. Acredinones A (**1**) and B (**2**) inhibited the outward $K^+$ currents of the insulin secreting cell line INS-1 with $IC_{50}$ values of 0.59 and 1.0 $\mu$M, respectively.




Acredinone A (**1**)




Acredinone B (**2**)

Metabolic diseases associated with obesity, such as diabetes, are an emerging threat to public health. Type II diabetes mellitus (T2DM) is characterized by high levels of glucose in the blood as a consequence of insulin resistance.[1] T2DM is treated by several approaches: insulin secretagogues (e.g., sulfonylureas), insulin-sensitizing agents (e.g., metformin and thiazolidinediones), and $\alpha$-glucosidase inhibitors, among others.[2] In particular the sulfonylureas, the $K_{ATP}$ channel inhibitors of the pancreatic $\beta$-cells, which help the release of insulin, have been widely used as orally available antidiabetics. The $K_{ATP}$ channel plays a major role in controlling the $\beta$-cell membrane potential. However, the use of $K_{ATP}$ channel blockers causes a high risk of hypoglycemic events.[3] Recently, it was discovered that the modulation of voltage-gated potassium channels may be an alternative in treating antidiabetic indications.[4]

The voltage-dependent $K^+$ (Kv) channel Kv2.1 is a major contributor to repolarization of insulin-secreting cells and regulates insulin secretion in the pancreas.[5] Above all, the inhibition of $\beta$-cell Kv2.1 currents prolongs the action potentials, sustains the opening of voltage-dependent $Ca^{2+}$ channels, and thereby enhances glucose-induced insulin release without causing risky hypoglycemia. Thus, the $\beta$-cell Kv2.1 channel is an attractive therapeutic target for the treatment of T2DM.

As a part of our program to discover voltage-gated potassium channel inhibitors, including the Kv2.1 channel, the secondary metabolites of a marine-derived *Acremonium* sp. fungus, isolated from the inner tissue of the marine sponge *Suberites japonicas*, were investigated. Bioactivity-guided isolation of the organic extract of *Acremonium* sp. has yielded acredinones A (**1**, 4.8 mg) and B (**2**, 3.1 mg). Herein, we describe the isolation and structure elucidation of acredinones A and B as well as their inhibitory activity against the outward $K^+$ currents in INS-1 cells.

## RESULTS AND DISCUSSION

The molecular formula of acredinone A (**1**) was determined to be $C_{36}H_{34}O_{10}$ by analysis of its molecular ion in the HRFABMS spectrum, requiring 20 degrees of unsaturation. Interpretation of the $^1H$, $^{13}C$, HSQC, and HMBC spectroscopic data of **1** revealed the presence of four independent aromatic spin-systems; two of them were 1,3-dimethoxy-1,2,3-trisubstituted benzene groups, and the other two were pentasubstituted benzene moieties with one hydroxy, one methyl, and one methoxy group. The four-bond correlations in the HMBC

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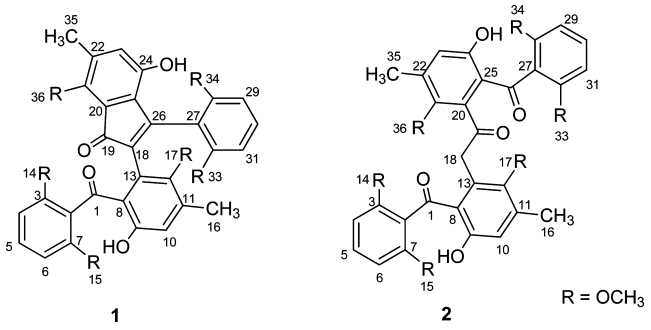



spectrum of acredinone A (**1**) from H-4 to C-1, H-10 to C-1, 9-OH to C-11, and 24-OH to C-23 were very helpful in establishing the partial structures shown in Figure 1A (a–c). The HMBC correlations from the methoxy and hydroxyl protons clearly established the placements of these groups (Figure 1A).

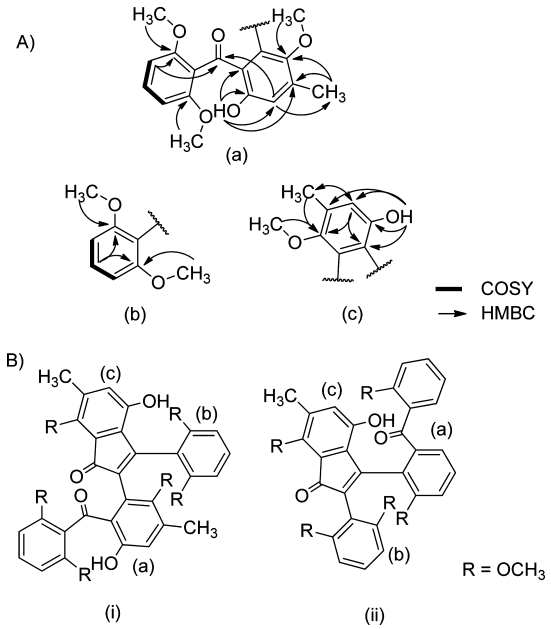


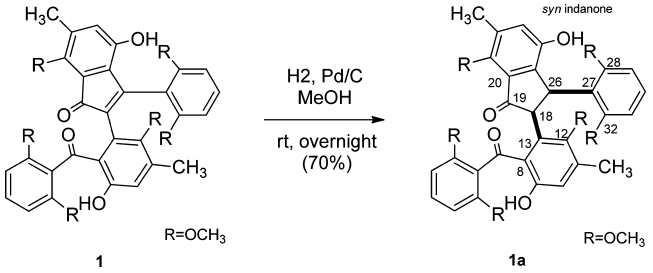

**Figure 1.** (A) Partial structures a–c for **1** and (B) proposed structures (i and ii) for **1** from the partial structures.

Three unassigned carbon signals, one carbonyl and two fully substituted $sp^2$ carbons, remained. However, establishing the connectivity for the three substructures and the three carbons was difficult due to the lack of HMBC correlations.

To obtain four- and five-bond HMBC correlations, we undertook modified HMBC experiments optimizing for 2, 4, 6, and 8 Hz $^nJ_{H-C}$ coupling constants; however, we did not observe any long-range HMBC correlations. Thus, we carefully considered chemical shifts of the three carbons C-18 ($\delta_C$ 134.5), C-26 ($\delta_C$ 150.0), and C-19 ($\delta_C$ 190.2) and the required degree of unsaturation for **1**. From these considerations, the most plausible structure would incorporate these carbons into a five-membered ring with an $\alpha,\beta$-unsaturated ketone moiety fused to substructure (c). Although this proposed core structure for **1** corresponded to the unsaturation number and was consistent with the chemical shifts for the three carbons, two structures (i and ii) were still possible, as shown in Figure 1B.

To establish the structure of **1**, we analyzed a chemically modified acredinone. Reduction of the C-18/C-26 double bond in acredinone **1** by heterogeneous catalytic hydrogenation (Pd/C, $H_2$) yielded the *syn* product indanone **1a** (Scheme 1).[6]

**Scheme 1. Preparation of 1a from Acredinone A (1)**




HMBC correlations from the new methine proton H-18 to C-12/C-13/C-19/C-20/C-26 and from H-26 to C-18/C-20/C-25/C-27/C-28 provided the connectivity between C-18 ($\delta_C$ 56.1) and C-13 ($\delta_C$ 125.6) of the partial structure (a) and between C-26 ($\delta_C$ 42.8) and C-27 ($\delta_C$ 119.5) of the partial structure (b), respectively (Figure 2 and Supporting

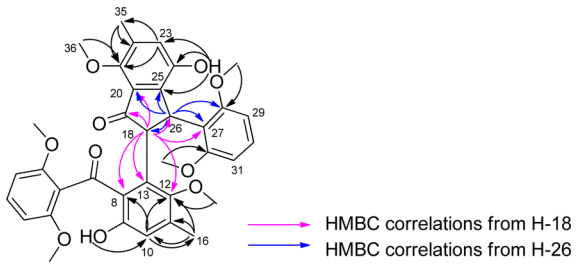



**Figure 2.** Key HMBC correlations of **1a**.

Information S14), securing the final structure for acredinone A (**1**). Restricted rotation within these biaryl ring systems could result in an optically active compound, but the measured $[a]^{25}_D$ was zero.

The molecular formula of acredinone B (**2**) was determined as $C_{36}H_{36}O_{11}$ (19 degrees of unsaturation) by analysis of its sodium adduct ion by HRFABMS and NMR spectroscopic data (Table 1). The $^1$H NMR spectrum of acredinone B was almost identical to that of acredinone A (**1**) except for the presence of one additional methylene group and an extra ketone carbonyl. Analysis of the 2D NMR spectroscopic data revealed two sets of partial structure (a) possessed by acredinone A (**1**), as shown in Figure 1A. HMBC correlations from the methylene protons $H_2$-18 to carbons C-8, C-12, C-13, C-19, and C-20 provided a connectivity of C-13/C-18/C-19 for the two sets of partial structure (a), which allowed the completion of the structure assignment for acredinone B (**2**).

We hypothesized that these dimeric secondary metabolites, acredinones A (**1**) and B (**2**), were biosynthesized from a monomer possessing a similar structure to that of substructure (a) in Figure 1A. We therefore carefully reinvestigated the extracts of the culture broth of this strain to isolate monomeric secondary metabolites. LC-MS analysis of the extract revealed an *m/z* 374 peak, almost half the *m/z* value of the acredinones. This secondary metabolite was isolated, and the structure was determined by interpretation of NMR spectroscopic and X-ray crystallographic data (Figure 3). This secondary metabolite,


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Table 1. NMR Spectroscopic Data for Acredinones A (1)[a] and B (2)[b]

| | acredinone A[a] | | | acredinone B[b] | |
|---|---|---|---|---|---|
| position | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | HMBC | $\delta_C$, type | $\delta_H$ ($J$ in Hz) |
| 1 | 201.4, C | | | 197.8, C | |
| 2 | 119.6, C | | | 119.7, C | |
| 3 | 157.3, C | | | 158.1, C | |
| 4 | 104.4, CH | 6.20, d (8.4) | 2, 6 | 104.4, CH | 6.55, d (8.4) |
| 5 | 131.3, CH | 7.02, t (8.4) | 3, 7 | 132.1, CH | 7.30, t (8.4) |
| 6 | 104.4, CH | 6.20, d (8.4) | 2, 4 | 104.4, CH | 6.55, d (8.4) |
| 7 | 157.3, C | | | 158.1, C | |
| 8 | 125.0, C | | | 122.2, C | |
| 9 | 155.8, C | | | 158.0, C | |
| 10 | 119.2, CH | 6.65, s | 8, 9, 12 | 118.2, CH | 6.50, s |
| 11 | 138.5, C | | | 138.6, C | |
| 12 | 150.1, C | | | 150.7, C | |
| 13 | 126.9, C | | | 128.0, C | |
| 14 | 55.6, $CH_3$ | 3.46, s | 3 | 55.4, $CH_3$ | 3.53, s |
| 15 | 55.6, $CH_3$ | 3.46, s | 7 | 55.4, $CH_3$ | 3.53, s |
| 16 | 17.3, $CH_3$ | 2.09, s | 10, 11, 12 | 16.1, $CH_3$ | 1.98, s |
| 17 | 61.0, $CH_3$ | 3.32, s | 12 | 59.9, $CH_3$ | 3.23, s |
| 18 | 134.5, C | | | 42.0, $CH_2$ | 3.37, s |
| 19 | 190.2, C | | | 197.6, C | |
| 20 | 120.0, C | | | 136.9, C | |
| 21 | 150.4, C | | | 147.7, C | |
| 22 | 135.0, C | | | 140.7, C | |
| 23 | 124.7, CH | 6.53, s | 21, 22, 25, 35 | 120.0, CH | 6.64, s |
| 24 | 145.4, C | | | 157.7, C | |
| 25 | 127.0, C | | | 118.6, C | |
| 26 | 150.0, C | | | 197.0, C | |
| 27 | 110.3, C | | | 118.4, C | |
| 28 | 157.9, C | | | 158.0, C | |
| 29 | 103.8, CH | 6.40, d (8.4) | 27, 31 | 104.0, CH | 6.36, d (8.5) |
| 30 | 131.5, CH | 7.18, t (8.4) | 28, 32 | 132.7, CH | 7.20, t (8.4) |
| 31 | 104.3, CH | 6.44, d (8.4) | 27, 29 | 104.0, CH | 6.36, d (8.5) |
| 32 | 158.1, C | | | 158.0, C | |
| 33 | 54.7, $CH_3$ | 3.57, s | 32 | 55.3, $CH_3$ | 3.53, s |
| 34 | 56.3, $CH_3$ | 3.66, s | 28 | 55.3, $CH_3$ | 3.53, s |
| 35 | 16.4, $CH_3$ | 2.12, s | 21, 22, 23 | 15.6, $CH_3$ | 1.97, s |
| 36 | 61.6, $CH_3$ | 3.82, s | 21 | 61.8, $CH_3$ | 3.10, s |
| 9-OH | | 10.7, s | 8, 9, 10 | | 11.6, s |
| 24-OH | | 4.82, s | 23, 24, 25 | | 11.5, s |

[a]600 MHz for $^1$H NMR and 150 MHz $^{13}$C NMR in $CDCl_3$. [b]700 MHz for $^1$H NMR and 175 MHz $^{13}$C NMR in acetone-$d_6$.

preacredinone A (3), represents a new monomeric benzophenone natural product.

Biogenetically, two units of 3 or a closely related monomer could combine to form acredinone B (2). An intramolecular aldol reaction between the enolate-ion nucleophile and the electrophilic C-26 carbonyl can then occur (Figure 4). The $\beta$-hydroxy ketone, compound **2b**, can be easily dehydrated to yield the conjugated enone product, acredinone A (1).

Acredinones A (1) and B (2) and preacredinone A (3) were assayed for inhibition of the outward $K^+$ currents in INS-1. Acredinones A (1) and B (2) showed inhibitory activity against

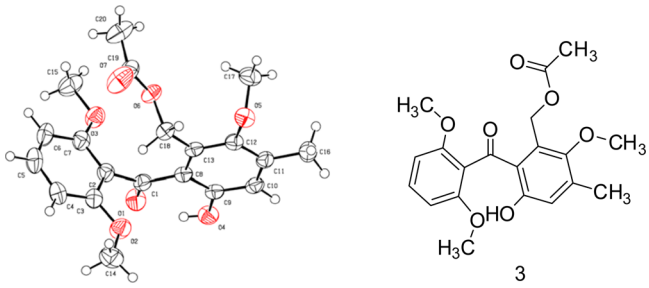


Figure 3. X-ray crystal and planar structure of preacredinone A (3).

the voltage-gated potassium channel in INS-1 cells with $IC_{50}$ values of 0.59 and 1.0 $\mu$M, respectively, while 3 did not display any inhibitory activity. The major component of voltage-gated potassium channels in INS-1 cells is known to be Kv2.1.[5] Most known natural product inhibitors of the Kv2.1 channel are peptide toxins such as hanatoxin (HaTx), *Scodra griseipes* toxin 1 (SGTx1), heteropodatoxin 2 (HpTx2), and phrixotoxin (PaTx), all isolated from the venom of spiders. These polypeptides consist of 25−40 amino acids and bind to the voltage sensor domain of the Kv2.1 channel in the open and resting states.[7] Acredinones A and B are the first nonpeptidic natural products that display inhibitory activities against the voltage-gated potassium channel. The selective activities for various voltage-gated potassium channels of acredinones A and B are under investigation.

## CONCLUSIONS

Three new benzophenone class natural products, acredinones A (1) and B (2) and preacredinone A (3), were isolated from a sponge-derived *Acremonium* fungus. Although the structures of the new compounds have a low H/C ratio (1: 0.94 and 2: 1.00), which suggests that the structure elucidation using NMR methods would be very challenging based on Crews's rule,[8] we elucidated the planar structures using extensive NMR experiments and chemical derivatization. Acredinones A (1) and B (2) are the first nonpeptidic natural products showing significant inhibition of the outward $K^+$ currents in INS-1 cells.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The melting point was recorded on an Electrothermal IA9100 apparatus (Bibby Scientific Limited, Staffordshire, UK). Optical rotations were measured in MeOH using a 1.0 cm cell on a Rudolph Research Autopol III, #A7214. UV spectra were also recorded in MeOH on a Scinco UVS-2100. IR spectra were recorded on KBr plates on a Thermo Electron Corp. Nicolet 5700. All NMR spectra were recorded on Bruker Advance DPX-600 and DPX-700 spectrometers with chloroform-*d* or acetone-$d_6$. Mass spectrometric data were obtained on a JEOL, JMS-AX505WA instrument. Solvents used in partitioning were first grade products of Dae-Jeong & Metals Co., Korea. HPLC grade solvents from Burdick & Jackson were used for TLC and HPLC. A Waters 1525 binary HPLC pump and a Waters 2489 UV/visible detector were used for purifying compounds. NMR solvents were purchased from Cambridge Isotope Laboratories (CIL), Inc.

**Isolation and Cultivation of the Fungal Strain.** *Acremonium* sp. F9A015 was isolated as a sponge-derived fungus from a marine sponge collected in August 2010 from Ga-geo Island near the southwest sea of Korea. The sponge was identified as *Suberites japonicus* #3674 (registration no. QMG331989) by Dr. M. Ekins of the Queensland Museum, PO Box 3300, South Brisbane, Queensland, 4101, Australia. Under sterile conditions, the sponge was rinsed in filtered seawater followed by rounds of successive surface sterilization in 70% EtOH. A piece of tissue from the inner part of the surface-sterilized sponge was


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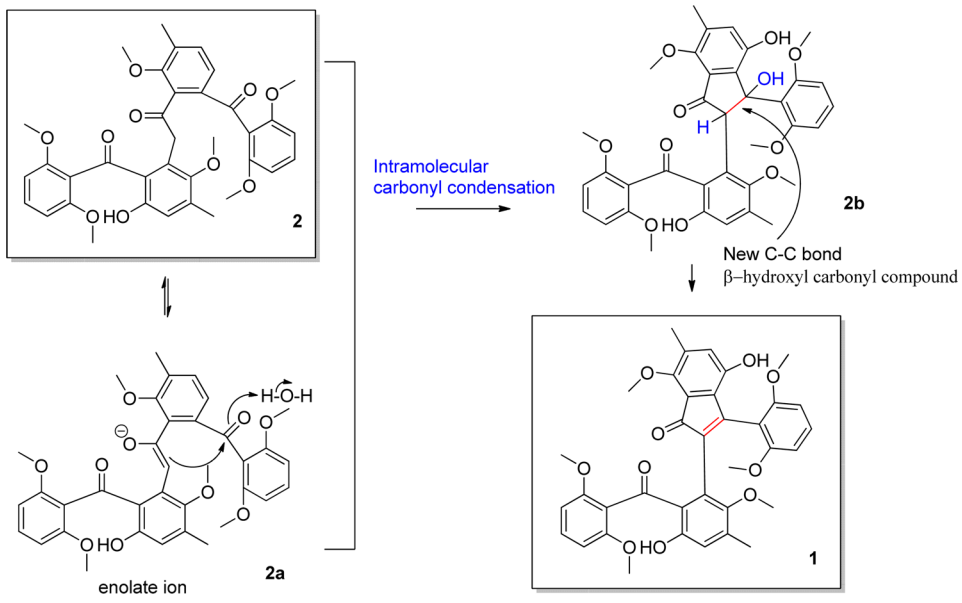


**Figure 4.** Proposed formation of acredinones A (**1**) and B (**2**).

cut and inoculated onto a potato dextrose agar (PDA) plate consisting of 15 g/L agar and filtered seawater. The agar plate was incubated at 25 °C, and a pure fungal culture was isolated from the plate after repeated inoculation on fresh PDA plates. The isolated fungus was identified as an *Acremonium* sp. based on microscopic examination. It was also identified by internal transcribed spacer (ITS) sequencing. The sequence of ITS 1 and 4 of the endophytic fungus has been deposited in GenBank (http://www.ncbi.nlm.nih.gov) with accession number KP239988. Although visually identified as an *Acremonium* sp., the ITS sequence of strain F9A015 (Supporting Information S36) showed the highest identity in a BLAST search to an unidentified fungus within the Sordariomycetes class (94%), indicating that classification of this strain to the genus level requires additional work.

The fungus was cultured in potato dextrose broth (PDB, Difco) using filtered natural seawater instead of distilled water. The fungus was inoculated with cultured seed agar blocks into 40 × 500 mL plastic culture flasks, each containing 50 mL of PDB, and incubated under static conditions at 25 °C for 8 weeks.

**Isolation of the Secondary Metabolites.** Fungal cells were separated from the broth (2 L) by filtration. The filtered culture broth was partitioned with EtOAc. The EtOAc layer was evaporated in vacuo. The previously filtered fungal cells were separately extracted with MeOH and acetone (1:1) and filtered. The filtrate from the fungal cells was evaporated and partitioned with EtOAc. After evaporating the latter EtOAc, the two EtOAc extracts were combined (1.62 g). The extract was subjected to step-gradient silica gel column chromatography with a solvent system consisting of 0−100% $CHCl_3$/MeOH to yield nine fractions. Further purification of fractions 2 and 4 by RP-HPLC (Phenomenex Luna $C_{18}$ (2), 5 μm, 100 Å, 250 × 100 mm, 2.0 mL/min, UV = 210 nm), eluting with $H_2O/CH_3CN$ (40:60), yielded **1** (4.8 mg), **2** (3.1 mg), and **3** (10.2 mg).

*Acredinone A (**1**):* orange-red, amorphous solid; $[\alpha]^{25}_D$ 0 (*c* 0.002, $CHCl_3$); UV/vis (MeOH) $\lambda_{max}$ (log $\varepsilon$) 218 (4.60), 252 (4.06), 334 (3.68), 487 (3.06) nm; IR (KBr) $\nu_{max}$ 3417, 2933, 1693, 1595, 1473, 1254, 1112 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, Table 1.; LRFABMS *m/z* 627 [M + H]$^+$; HRFABMS *m/z* 627.2236 [M + H]$^+$ (calcd for $C_{36}H_{35}O_{10}$, 627.2230).

*Acredinone B (**2**):* yellow, amorphous solid; $[\alpha]^{25}_D$ 0 (*c* 0.002, $CHCl_3$); UV/vis (MeOH) $\lambda_{max}$(log $\varepsilon$) 214 (4.30), 267 (3.52), 333 (3.29) nm; IR (KBr) $\nu_{max}$ 3446, 2935, 1622, 1598, 1475, 1256, 1115 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, Table 1.; LRFABMS *m/z* 667 [M + Na]$^+$; HRFABMS *m/z* 667.2161 [M + Na]$^+$ (calcd for $C_{36}H_{36}O_{11}Na$, 667.2155).

*Preacredinone A (**3**):* yellowish crystals; mp 140−145 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 210 (4.65), 271 (3.86), 340 (3.62) nm; IR (KBr) $\nu_{max}$ 3446, 2935, 1710, 1598, 1476, 1256, 1115 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, Supporting Information S31; LRFABMS *m/z* 374 [M + H]$^+$; HRFABMS *m/z* 374.1363 [M + H]$^+$ (calcd for $C_{20}H_{22}O_7$, 374.1365).

**X-ray Crystallographic Analyses of Preacredinone A (3).** Crystals of preacredinone A were grown in a mixture of MeOH and $CHCl_3$ (1:1) at room temperature (rt). The single-crystal X-ray diffraction data of preacredinone A (**3**) were collected on a Bruker SMART APEX CCD detector employing graphite-monochromated Mo K$\alpha$ radiation ($\lambda$ = 0.710 73 Å) at 200 K. Data collection and integration were performed with SMART[9] and SAINT-Plus.[10] The structure was solved by direct methods and refined by full-matrix least-squares on $F^2$ using SHELXTL.[11] All the non-hydrogen atoms were refined anisotropically, and hydrogen atoms were added to their geometrically ideal positions. Crystallographic data for **3** have been deposited at the Cambridge Crystallographic Data Centre. Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html.

*Crystal Data of **3**.* $C_{20}H_{22}O_7$, $M_r$ = 374.38, monoclinic, *a* = 28.5983(12) Å, *b* = 8.3343(4) Å, *c* = 7.7428(3) Å, $\alpha$ = 90°, $\beta$ = 90°, $\gamma$ = 90°, *V* = 1845.47(14) Å$^3$, space group *Pca*2$_1$, *Z* = 4, $D_x$ = 1.1.347 mg/m$^3$, $\mu$(Cu K$\alpha$) = 0.102 mm$^{-1}$, and *F*(000) = 792. Crystal dimensions: 0.32 × 0.23 × 0.20 mm$^3$. Independent reflections: 4386 [*R*(int) = 0.0501]. The final R1 values were 0. 0474, wR2 = 0.1038 ($I > 2\sigma(I)$). CCDC number: 1022434.

**Reduction of Acredinone A (1) to Compound 1a.** A solution of **1** (3 mg, 0.0047 mmol) in MeOH (4 mL) was treated with 10% Pd/C (10 mg, 0.0094 mmol) and stirred under an atmosphere of hydrogen for 10 h. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure. The crude mixture was purified by semipreparative HPLC (250 × 10.00 mm 5 μm Phenomenex Lunar $C_{18}$ (2), $CH_3CN/H_2O$/TFA, 65:34.99:0.01, gradient) to yield compound A (**1a**, 2.1 mg) (70%) as a brown, amorphous solid.

**1a:** brown, amorphous solid; $[\alpha]^{25}_D$ −45 (*c* 0.002, $CHCl_3$); UV (MeOH) $\lambda_{max}$(log $\varepsilon$) 212 (4.59), 262 (4.13), 332 (3.86) nm; IR (KBr) $\nu_{max}$ 3370, 2922, 1754, 1733, 1464, 1203 $cm^{-1}$; $^1H$ and $^{13}C$ data, Supporting Information S15; LRFABMS *m/z* 629 [M + H]$^+$; HRFABMS *m/z* 629.2393 [M + H]$^+$ (calcd for $C_{36}H_{37}O_{10}$, 626.2387).

***In Vitro* Assay.** The insulin-secreting cell line INS-1 was used in the present study. INS-1 cells (passages 20−50) were cultured on poly-L-lysine-coated coverslips in RPMI-1640 medium containing 10%


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FBS and 11 mM D-glucose. Electrophysiological recordings from INS-1 cells were performed in the whole cell configuration of the patch clamp technique at rt (20−22 °C). The bath solution contained (in mmol/L) 143 NaCl, 5.4 KCl, 5 HEPES, 1.8 $CaCl_2$, and 0.5 $MgCl_2$. The patch electrodes had resistances of 2−4 MΩ when filled with an electrode solution composed of (in mmol/L) 110 K-aspartate, 30 KCl, 10 HEPES, 5 MgATP, 0.1 NaGTP, and 1 $MGCl_2$. The cells were placed on a 12 mm wide round coverslip in a recording chamber and were continuously superfused at a rate of 2−5 mL/min. Acredinones A and B were added to solutions containing bovine serum albumin (0.1 wt %:vol). DMSO was used as a control. The $IC_{50}$ for GxTX-1E (a spider toxin, Kv2.1 channel blocker) inhibition of $K^+$ currents was about 3 nM.[12]

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1H$ and $^{13}C$ NMR spectra, X-ray data (CIF), and ITS DNA sequence. 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.

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## ■ DEDICATION

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