

## Actinopolysporins A–C and Tubercidin as a Pdc4 Stabilizer from the Halophilic Actinomycete *Actinopolyspora erythraea* YIM 90600

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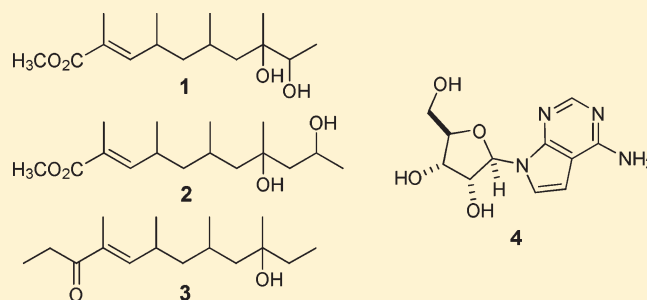
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**S** Supporting Information

**ABSTRACT:** Our current natural product program utilizes new actinomycetes originating from unexplored and underexplored ecological niches, employing cytotoxicity against a selected panel of cancer cell lines as the preliminary screen to identify hit strains for natural product dereplication, followed by mechanism-based assays of the purified natural products to discover potential anticancer drug leads. Three new linear polyketides, actinopolysporins A (1), B (2), and C (3), along with the known antineoplastic antibiotic tubercidin (4), were isolated from the halophilic actinomycete *Actinopolyspora erythraea* YIM 90600, and the structures of the new compounds were elucidated on the basis of spectroscopic data interpretation.

All four compounds were assayed for their ability to stabilize the tumor suppressor programmed cell death protein 4 (Pdc4), which is known to antagonize critical events in oncogenic pathways. Only 4 significantly inhibited proteasomal degradation of a model Pdc4–luciferase fusion protein, with an  $IC_{50}$  of  $0.88 \pm 0.09 \mu M$ , unveiling a novel biological activity for this well-studied natural product.



Many prokaryotic organisms inhabit “extreme environments” where the chemical or physical conditions differ significantly from those found in habitats that support more abundant and varied life forms. An extreme environment is one that humans would consider severe or uninhabitable, through extremes of temperature, pH, salinity, pressure, and even radiation. Such environments are inhabited by diverse populations of microorganisms, most of which are able to live only under the relevant extreme condition.<sup>1,2</sup> The selective pressures of severe pH, temperature, salinity, and pressure have led to organisms apparently adapted to their environment. These extremophiles possess, and presumably benefit from, new enzymes, which thereby enable the biosynthesis of novel molecules.<sup>3</sup> The diversity of proteins and secondary metabolites characteristic of the extremophiles have not been well studied. Indeed, this avenue for natural products discovery remains largely unexplored. Accordingly, we initiated recently a program designed to discover new natural products from new extremophilic actinomycetes originating from unexplored and underexplored ecological niches for

anticancer drug discovery.<sup>4</sup> Both extracts and purified compounds were tested in a high-throughput screening (HTS) assay designed to identify stabilizers of the tumor suppressor programmed cell death protein 4 (Pdc4).<sup>5</sup>

Strain YIM 90600, isolated from the Baicheng salt field in Xinjiang Province, in the northwest of China, has been identified as a new member of the genus *Actinopolyspora* on the basis of polyphasic taxonomy.<sup>6</sup> YIM 90600 is a moderately halophilic actinomycete displaying optimal growth characteristics at 37 °C in pH 7–8 medium containing 10–15% (w/v) NaCl. We previously reported that fermentation of YIM 90600 under these conditions gave rise to new erythromycin analogues.<sup>4</sup> Further investigation of extracts from large-scale fermentation cultures of strain YIM 90600 revealed the presence of additional new metabolites. We report here the isolation, structure elucidation, and preliminary biological activity assays of three new linear

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Table 1. Summary of  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Actinopolysporins A (1), B (2), and C (3) in  $\text{CDCl}_3^a$ 

position	1 (J in Hz)		2 (J in Hz)		3 (J in Hz)	
	$\delta_{\text{H}}$ (mult)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult)	$\delta_{\text{C}}$
1	1.14, d (6.5)	17.9, q	4.22, m	65.8, d	0.94, t (7.0)	8.54, q
2a	3.56, q (6.5)	75.3, d	1.64, dd (14.5, 11.0)	48.3, t	1.58, m	35.6, t
2b			1.41 (overlapped)		1.41, m	
3		75.4, s		74.6, s		73.8, s
4a	1.53, dd (14.5, 6.0)	42.7, t	1.31, dd (14.0, 6.7)	52.5, t	1.36, dd (14.6, 5.8)	49.0, t
4b	1.15 (overlapped)		1.43 (overlapped)		1.46 (overlapped)	
5	1.62 (overlapped)	26.8, d	1.56 (overlapped)	27.0, d	1.57 (overlapped)	27.3, d
6a	1.63 (overlapped)	46.2, t	1.42 (overlapped)	46.7, t	1.55 (overlapped)	46.7, t
6b	1.19, m		1.22, m		1.30, m	
7	2.63, m	31.4, d	2.60, m	31.3, d	2.72, m	31.6, d
8	6.56, brd (10.5)	148.7, d	6.52, brd (10.0)	148.5, d	6.43, brd (9.8)	148.4, d
9		126.4, s		126.5, s		135.7, s
10		169.2, s		169.2, s		203.3, s
11	1.86, d (1.5)	12.8, q	1.86, d (1.5)	12.8, q	1.86, brs	11.8, q
12	0.99, d (6.5)	20.9, q	0.98, d (6.5)	20.8, q	1.06, d (6.5)	20.9, q
13	0.93, d (6.5)	22.4, q	0.94, d (6.5)	22.1, q	0.99, d (6.5)	22.1, q
14	1.16, s	24.2, q	1.28, s	26.9, q	1.19, s	27.0, q
15			1.20, d (6.0)	24.7, q	2.72 (overlapped)	30.7, t
16					1.15, t (7.0)	9.25, q
OMe	3.73, s	52.0, q	3.74, s	52.0, q		

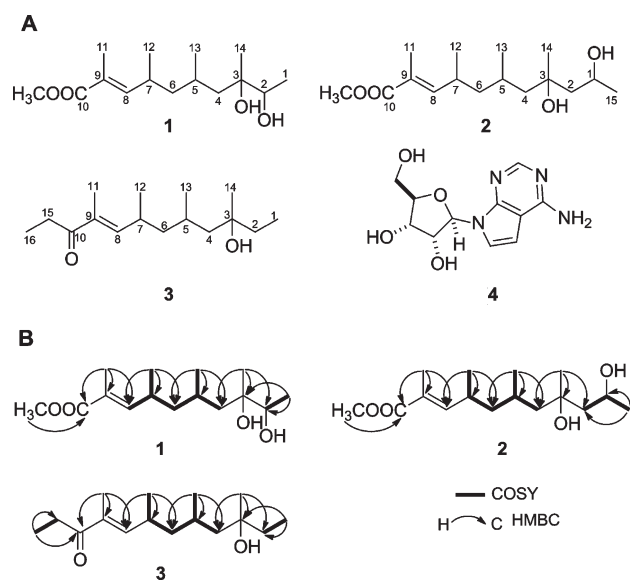
<sup>a</sup> Assignments were made on the basis of  $^1\text{H}$ - $^1\text{H}$  COSY, gHSQC, and gHMBC experiments.

polyketides, actinopolysporins A (1), B (2), and C (3) and the previously known antineoplastic antibiotic tubercidin (4).<sup>7</sup> The carbon backbones of 1–3 are rare among known natural products, and their biosynthesis could be envisioned to proceed through a common polyketide pathway from condensation of an acetate or a propionate starter unit with four or five methyl malonate extender units, followed by varying modifications. Biological activity assays have focused on the ability of these natural products to stabilize Pdc4, a tumor suppressor that has been shown to exert its antitumor function by inhibiting translation.<sup>5</sup>

## RESULTS AND DISCUSSION

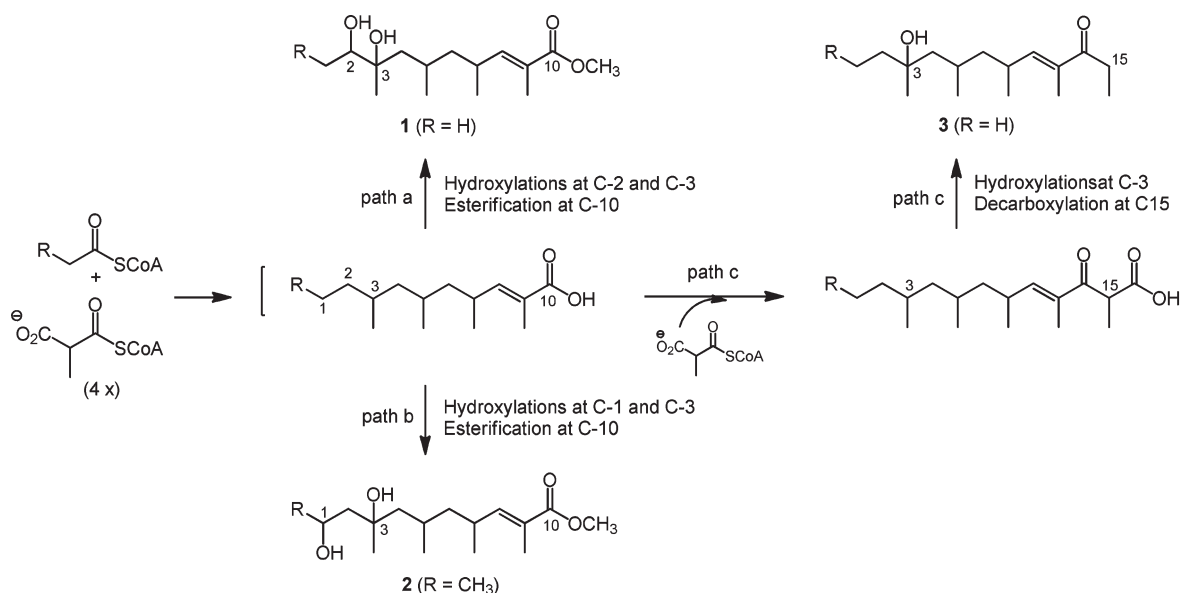
Recent efforts have indicated that YIM 90600 is a novel species of *Actinopolyspora*, for which the name *Actinopolyspora erythraea* sp. nov. has been proposed.<sup>6</sup> This strain was cultivated in a NaCl-based medium for 28 days with vigorous shaking.<sup>4</sup> The fermentation broth was extracted with EtOAc, the mycelia were extracted with acetone, and the combined extracts were subjected to multiple rounds of silica gel, LH-20, and  $\text{C}_{18}$  chromatography to afford pure compounds 1–4.

Compound 1 was isolated as a colorless oil, for which HRMALDIMS established its molecular formula as  $\text{C}_{15}\text{H}_{28}\text{O}_4$ , thereby indicating two degrees of unsaturation. The IR spectrum supported the presence of hydroxy ( $3410\text{ cm}^{-1}$ ), carbonyl ( $1709\text{ cm}^{-1}$ ), and olefinic ( $1620\text{ cm}^{-1}$ ) moieties. Analysis of the NMR spectra (Table 1) revealed chemical shifts indicative of a carboxyl moiety ( $\delta_{\text{C}}$  169.2) conjugated with a double bond, a trisubstituted double bond [ $(\delta_{\text{H}}$  6.56,  $\delta_{\text{C}}$  148.7) and ( $\delta_{\text{C}}$  126.4, s)], a methoxyl ( $\delta_{\text{H}}$  3.73,  $\delta_{\text{C}}$  52.0), one tertiary methyl group ( $\delta_{\text{H}}$  1.16,  $\delta_{\text{C}}$  24.2), four secondary methyl groups [ $(\delta_{\text{H}}$  1.14,  $\delta_{\text{C}}$  17.9), ( $\delta_{\text{H}}$  1.86,  $\delta_{\text{C}}$  12.8), ( $\delta_{\text{H}}$  0.99,  $\delta_{\text{C}}$  20.9), ( $\delta_{\text{H}}$  0.93,  $\delta_{\text{C}}$  22.4)], and a secondary alcohol methine group



**Figure 1.** Actinopolysporins A (1), B (2), C (3), and tubercidin (4) isolated from *Actinopolyspora erythraea* YIM 90600: (A) structures of 1, 2, 3, and 4 and (B) key gCOSY and gHMBC correlations for 1, 2, and 3 supporting their structural assignments.

( $\delta_{\text{H}}$  3.56,  $\delta_{\text{C}}$  75.3). gHSQC NMR experiments supported the presence of six methyls, two methylenes, and four methine carbons, while the remaining quaternary carbons were identified from the gHMBC experiment. Both COSY correlations as well as the MS data were supportive of the linearity of its carbon scaffold. On the basis of the gHMBC correlations from the six methyl groups to the corresponding carbons, the complete



**Figure 2.** A common pathway proposed for actinopolysporins A (1), B (2), and C (3) biosynthesis in *Actinopolyspora erythraea* YIM 90600.

carbon connectivity was established (Figure 1). The absence of any correlation between CH<sub>3</sub>-11 and H-8 in the NOESY spectrum supported the (*E*)-double-bond geometry. This assignment was substantiated by the observed upfield <sup>13</sup>C NMR chemical shift of CH<sub>3</sub>-11 ( $\delta_C$  12.8), resulting from the  $\gamma$ -gauche interaction shielding of the CH<sub>3</sub>-11 by C-7. Compound 1 has been named actinopolysporin A.

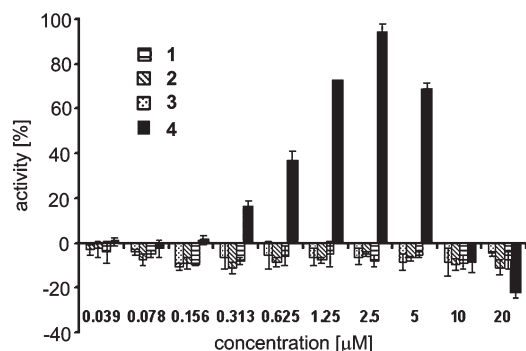
Compound 2 (actinopolysporin B) was also isolated as a colorless oil, with a molecular formula of C<sub>16</sub>H<sub>30</sub>O<sub>4</sub> as determined by HRMALDIMS analysis. Comparison of the spectroscopic data for 2 with those of 1 revealed these compounds to be quite similar except for an additional saturated methylene moiety ( $\delta_C$  48.3, t). gHMBC correlations of CH<sub>3</sub>-14 with this methylene at C-2 and <sup>1</sup>H-<sup>1</sup>H COSY correlations of H<sub>2</sub>-2 with H-1 supported the assignment of C-2 as a methylene moiety (Figure 1).

Compound 3 (actinopolysporin C), a colorless oil, was similarly subjected to HRMALDIMS analysis, establishing its molecular formula as C<sub>16</sub>H<sub>30</sub>O<sub>2</sub>. Comparison of the <sup>1</sup>H NMR data of 3 with those of 1 showed general similarities. An obvious difference found between 3 and 1 was the additional two triplet methyl signals ( $\delta_H$  0.94, t, *J* = 7.0 Hz and 1.15, t, *J* = 7.0 Hz) and the absence of the proton signals of an oxygenated methine and a methoxy group characteristic of 1. In addition, an  $\alpha,\beta$ -unsaturated ketone group ( $\delta_C$  203.3) was evident in the <sup>13</sup>C NMR spectrum of 3. The two triplet methyl signals exhibited obvious gHMBC cross-peaks with those of the C-10 carbonyl and hydroxylated C-3, respectively, hence supporting the final structural assignment of compound 3 (Figure 1).

The linear carbon scaffolds represented by 1–3 are rare among known natural products. We previously reported the isolation and structural elucidation of several analogues of the 14-membered macrolide erythromycin, as well as a linear shunt metabolite for erythromycin biosynthesis from YIM 90600, supporting the processive mechanism for polyketide biosynthesis.<sup>4</sup> While bearing some structural similarity, 1–3 are unlikely to be derived from the erythromycin biosynthesis pathway. Instead, another polyketide biosynthetic process, capable of incorporating multiple starter units and performing a

varying number of chain elongation steps as well as carrying a plethora of tailoring modifications of the nascent polyketide intermediate to imbue additional structural diversity, could be envisaged for 1–3 biosynthesis in YIM 90600; *Streptomyces* species are known to encode multiple polyketide biosynthetic machineries.<sup>8</sup> Thus, a common pathway could be proposed for 1–3 from condensation of an acetate or propionate starter unit with four or five methyl malonate extender units, followed by varying modifications: (i) an acetate starter unit upon four cycles of condensations with the methylmalonyl CoA extender unit followed by hydroxylations at C-2 and C-3 and esterification at C-10 would afford 1 (Figure 2, path a), (ii) a propionate starter unit upon four cycles of condensations with the methylmalonyl CoA extender unit followed by hydroxylations at C-1 and C-3 and esterification at C-10 would yield 2 (Figure 2, path b), and (iii) an acetate starter unit upon five cycles of condensation with the methylmalonyl CoA extender unit followed by hydroxylation at C-3 and decarboxylation at C-15 would give rise to 3 (Figure 2, path c). These features emphasize once again the plasticity and promiscuity of the polyketide biosynthetic machinery, supporting the approach of applying combinatorial biosynthesis strategies and methods to polyketide biosynthetic machinery for producing natural product compound structural diversity.<sup>9</sup>

In addition to the three actinopolysporins, compound 4 was isolated as a white powder, for which its identity as the deazanucleoside tubercidin (Figure 1) was readily established by comparison of the MS and NMR data obtained with those reported in the literature.<sup>10–12</sup> Tubercidin (4) was first isolated from *Streptomyces tubercidicus*;<sup>10</sup> this compound has since been isolated from many other organisms, including the blue–green alga *Tolypothrix byssoidea*<sup>11</sup> and the marine sponge *Caulospongia biflabellata*.<sup>12</sup> *Actinopolyspora erythraea* YIM 90600 now joins a growing list of producers of 4. The significant antineoplastic activity of 4 has been attributed to interaction with a myriad of molecular targets. By virtue of its structural similarity to adenine, 4 is incorporated into DNA and inhibits multiple polymerases, thereby inhibiting DNA replication as well as RNA and protein synthesis.



**Figure 3.** Summary of Pdc4 stabilization data for 1–4. The % activity of the fusion protein values are relative to a DMSO control in which slow proteasomal degradation of the fusion protein parallels that established for intact Pdc4. These data correlate to an  $IC_{50}$  of  $0.88 \pm 0.09 \mu\text{M}$  for Pdc4 stabilization (and protection) by compound 4.

Our current natural product program, aimed at discovering potential anticancer drugs, utilizes new actinomycetes originating from exotic ecological niches, employing cytotoxicity against a selected panel of cancer cell lines as the preliminary screen to identify hit strains for natural product dereplication, followed by mechanism-based assays of the purified natural products to discover potential leads.<sup>13</sup> The combined extracts from YIM 90600 showed potent activity in the preliminary cytotoxicity screen, thereby motivating our subsequent effort on large-scale fermentation and natural product isolation and structural elucidation.<sup>4</sup>

We previously described a luciferase-based reporter construct to monitor Pdc4 stability and developed a new HTS compatible assay for identifying stabilizers of Pdc4, a tumor suppressor that was shown to exert its antitumor function via inhibition of translation.<sup>14</sup> Pdc4 interacts with and inhibits the activity of the RNA helicase eIF4A, a component of the eukaryotic initiation factor 4F (eIF4F) translation–initiation complex.<sup>5</sup> Pdc4-dependent translation inhibition directly correlates with inhibited transformation, as well as compromised cell migration and invasion.<sup>15,16</sup> Furthermore, Pdc4 overexpression was shown to limit tumor formation,<sup>17</sup> while depletion of Pdc4 increased tumorigenesis in vivo.<sup>18</sup> Loss of Pdc4 protein in response to tumor promoters and mitogens has been attributed to phosphorylation-dependent proteasomal degradation. In detail, Pdc4 bears a phosphorylation-dependent degradation (PDD) domain that houses both a p70<sup>S6K1</sup> phosphorylation and a  $\beta$ -transducing repeat containing protein 1 ( $\beta$ -TrCP1)-recognition motif. Phosphorylation within this PDD target domain by p70<sup>S6K1</sup> allows for binding of the E3-ubiquitin ligase  $\beta$ -TrCP and subsequent proteasomal degradation in response to tumor-promoting conditions.<sup>18,19</sup>

We subjected our current natural product collection to this HTS in search for Pdc4 stabilizers. Of the four compounds isolated in this study, 1–3 were found to completely lack Pdc4 stabilizing ability as shown in Figure 3. Hence, luciferase activity was not recovered by compounds 1–3 from 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced down-regulation. The loss of luciferase activity was similar for all three natural products over the range of concentrations spanning from 39 nM to 20  $\mu\text{M}$ , and all were comparable to cells treated with TPA-only controls (Figure 3). In contrast, cotreatment with 4 resulted in a significantly increased luciferase signal in comparison to TPA-only treated cells. Dose response analysis revealed Pdc4 stabilizing activity of 4 with an  $IC_{50}$  of  $0.88 \pm 0.09 \mu\text{M}$  (Figure 3).

Maximal activity reached  $94.2 \pm 3.6\%$  at 2.5  $\mu\text{M}$ . At higher doses ( $>2.5 \mu\text{M}$ ), increasing cell toxicity led to a decreased luciferase signal.<sup>14</sup> These findings highlight 4 as the first example of an adenosine derivative able to stabilize Pdc4 by interfering with its phosphorylation-dependent proteasomal degradation, revealing a potentially significant activity previously unrecognized for 4, a well-established but not yet fully understood anticancer clinical candidate. Importantly, stabilization of Pdc4 under tumorigenic conditions is predicted to result in tumor suppressive effects. On the basis of its eIF4A inhibitory effect, stabilization of Pdc4 by small molecules should selectively inhibit the translation of mRNAs with structured 5'-untranslated regions,<sup>5</sup> which are characteristic of many proto-oncogenes. Consequently, stabilizers of Pdc4 may specifically inhibit tumor-associated changes in translation. Upon the basis of the efficacy of 4 in rescuing Pdc4 from tumor promoter-induced degradation, in combination with the novelty of translation inhibition as a mode of action, we suggest advancing 4 for further development in the treatment and possible prevention of various cancers.

In conclusion, the extremophilic halophile YIM 90600 has been recently classified as a new *Actinopolyspora erythraea* sp. nov. species.<sup>6</sup> In our continued effort to discover novel natural products from new extremophilic actinomycetes, we have previously discovered the new erythromycin analogues, erythronolides H and I,<sup>4</sup> and now report three additional new linear polyketides, actinopolysporins A, B, and C, as well as the known antitneoplastic antibiotic tubercidin (Figure 1). As new targets are discovered and become available in HTS format, libraries of purified natural products present an opportunity for the discovery of specific small molecule probes or potential drug leads. We discovered that 4 is a potent inhibitor of Pdc4 protein degradation and thus a potential lead to develop novel stabilizers of the tumor suppressor Pdc4. These results support the idea that, owing to their unique environments, extremophilic microbes can contribute in a significant way to the array of structural diversity available through secondary metabolism to drug discovery efforts. Extremophilic actinomycetes, in particular, warrant significant attention as producers of biologically active leads with unique modes of action.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured in  $\text{CHCl}_3$  with a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 25 °C with a Varian Unity INOVA 500 instrument operating at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$  nuclei. The chemical shifts were referenced to residual solvent signals:  $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.29 for  $\text{CDCl}_3$ .  $^1\text{H}$ – $^1\text{H}$  gCOSY (mixing time = 80 ms), gHSQC ( $^1J_{\text{CH}}$  = 140 Hz), and gHMBC ( $^2-3J_{\text{XH}}$  = 8.0 Hz) spectra were performed using standard Varian pulse sequences. High-resolution mass spectrometric data were acquired with an IonSpec HiResMALDI FT–mass spectrometer with a 7 T superconducting magnet. Semipreparative HPLC was performed with a Varian liquid chromatograph system equipped with an Apollo C<sub>18</sub> column (5  $\mu\text{m}$ , 10 mm  $\times$  250 mm, Grace Davison Discovery Sciences, Deerfield, IL). Column chromatography was performed either on silica gel (230–400 mesh, Natland International, Research Triangle Park, NC), Lichroprep RP-18 gel (40–63  $\mu\text{m}$ , Merck, Darmstadt, DE), or Sephadex LH-20 (Pharmacia, Kalamazoo, MI).

**Isolation and Phylogenetic Identification of *Actinopolyspora erythraea* YIM 90600.** Strain YIM 90600 was isolated from Baicheng salt field in Xinjiang Province, China, after 3 weeks of incubation at 37 °C on cellulose-casein multisalt medium<sup>9</sup> (microcrystalline cellulose 1%, casein 0.03%,  $\text{KNO}_3$  0.02%,  $\text{K}_2\text{HPO}_4$  0.05%,  $\text{CaCO}_3$  0.002%,  $\text{FeSO}_4$  0.001%, NaCl 10%,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  3%, KCl 2%, agar 1.5%). Multiple salts

were sterilized separately before addition to the medium. The pH of the medium was adjusted to pH 7.5 with 1 N NaOH. The isolate was maintained on ISP 4 agar slants containing 10% (w/v) NaCl at 4 °C and as suspensions of mycelia fragments in 20% (v/v) glycerol. Morphological and physiological studies to identify the genus of the strain were carried out following a standard protocol.<sup>20</sup> Assignment of YIM 90600 as *Actinopolyspora erythraea* sp. nov. on the basis of polyphasic taxonomy has been reported recently.<sup>6</sup>

**Fermentation, Extraction, and Isolation.** The inoculum was prepared by introducing the periphery of 7-day-old Petri dish cultures of *Actinopolyspora erythraea* YIM 90600 into 250 mL baffled flasks containing 50 mL of the broth (soluble starch 0.5%, glucose 2%, peptone 0.2%, yeast extract 0.2%, soybean flour 1%, NaCl 10%, K<sub>2</sub>HPO<sub>4</sub> 0.05%, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05%, CaCO<sub>3</sub> 0.2% in deionized water, pH 7.8, adjusted with 1 N NaOH), followed by shaking at 250 rpm and 28 °C for 5 days. The production fermentation was accomplished by adding the inoculum (50 mL) into 2 L baffled flasks containing 500 mL of the same culture broth and then shaking under the same conditions for 28 days. The fermentation broth (14 L) was then centrifuged at 4000 rpm for 20 min. Mycelia were extracted with acetone (3 × 1 L), with the fermentation broth then extracted with EtOAc (3 × 4 L). Solvent removal in vacuo was followed by combination of the acetone and EtOAc extracts. The oily residue obtained (35 g) was chromatographed on silica gel using CHCl<sub>3</sub>–MeOH (100:0, 50:1, 20:1, 10:1, 5:1, 1:1 and 0:100, 2.5 L each) as the mobile phases, in a stepwise manner, to afford seven fractions, A–G. Fraction B (50:1) (500 mg) was chromatographed further over a Sephadex LH-20 column and eluted with methanol to yield three subfractions, A1–A3. Subfraction A2 was finally purified by semipreparative HPLC to afford **1** (10.4 mg) and **2** (3.2 mg). Fraction C (20:1) (380 mg) was chromatographed over a C<sub>18</sub> column, eluted in a step gradient manner with CH<sub>3</sub>OH–H<sub>2</sub>O, to render **3** (0.9 mg). Finally, **4** (15.0 mg) was obtained from fraction F (1:1) by repeated chromatography on silica gel and Sephadex LH-20 columns.

*Actinopolysporin A* (**1**). Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +20.7 (c 0.15, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  log ( $\epsilon$ ) 240 (3.76) nm; IR  $\nu_{\max}$  3410, 1709, 1620, 1052, 960 cm<sup>-1</sup>; NMR data, see Table 1; HRMALDIMS (positive ion) *m/z* 295.1887 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>4</sub>, 295.1880).

*Actinopolysporin B* (**2**). Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +10.4 (c 0.10, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  log ( $\epsilon$ ) 240 (3.80); NMR data, see Table 1; HRMALDIMS (positive ion) *m/z* 309.2040 [M + Na]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>30</sub>O<sub>4</sub>, 309.2036).

*Actinopolysporin C* (**3**). Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +7.8 (c 0.05, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  log ( $\epsilon$ ) 251 (4.12); NMR data, see Table 1; HRMALDIMS (positive ion) *m/z* 277.2138 [M + Na]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>30</sub>O<sub>2</sub>, 277.2149).

**Pdcd4 Stabilizing Activity Assay.** Stabilization of Pdcd4 was assessed as previously described.<sup>14</sup> In brief, HEK293 cells expressing a fusion protein comprised of a fragment of Pdcd4 containing the regulatory region (amino acids 39–91) and luciferase were plated (1000 cells/well, 40  $\mu$ L/well) in 384-well opaque white plates and allowed to attach overnight (18 h). TPA (final concentration 10 nM) was added, followed (within 15 min) by test samples or controls. Following an 8 h incubation, luciferase activity was measured 10–15 min after the addition of SteadyLite Plus (Perkin-Elmer) reagent. Controls were DMSO only (no TPA), TPA only, and TPA + rapamycin (100 nM final). The activities of compounds were calculated using the following formula: Activity<sub>target</sub> (%) = (RLU<sub>compound+TPA</sub> – RLU<sub>TPA</sub>) / (RLU<sub>DMSO</sub> – RLU<sub>TPA</sub>) × 100.

## ■ ASSOCIATED CONTENT

Supporting Information. <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds **1**–**3**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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