RESEARCH LETTER

Genomic sequence-based discovery of novel angucyclinone antibiotics from marine Streptomyces sp. W007

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genome sequence; polyketide; antimicrobial activity; cytotoxicity; angucyclinone antibiotics.

Abstract

A large number of novel bioactive compounds were discovered from microbial secondary metabolites based on the traditional bioactivity screenings. Recent fermentation studies indicated that the crude extract of marine Streptomyces sp. W007 possessed great potential in agricultural fungal disease control against Phomopsis asparagi, Polystigma deformans, Cladosporium cucumerinum, Moniliinia fructicola, and Colletotrichum lagenarium. To further evaluate the biosynthetic potential of secondary metabolites, we sequenced the genome of Streptomyces sp. W007 and analyzed the identifiable secondary metabolite gene clusters. Moreover, one gene cluster with type II PKS implied the possibility of Streptomyces sp. W007 to produce aromatic polyketide of angucyclinone antibiotics. Therefore, two novel compounds, 3-hydroxy-1-keto-3-methyl-8-methoxy-1,2,3,4-tetrahydro-benz[a]anthracene and kiamycin with potent cytotoxicities against human cancer cell lines, were isolated from the culture broth of Streptomyces sp. W007. In addition, other four known angucyclinone antibiotics were obtained. The gene cluster for these angucyclinone antibiotics could be assigned to 20 genes. This work provides powerful evidence for the interplay between genomic analysis and traditional natural product isolation research.

Introduction

Microbial natural products are an important source of new drugs (Solanki et al., 2008). Among the producers of commercially important metabolites, actinomycetes have proven to be a prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds. Secondary metabolites produced by actinomycetes possess a wide range of biological activities (Moore et al., 1999; Li et al., 2005; Sujatha et al., 2005; Miller et al., 2007), and the vast majority of novel bioactive compounds are derived from the genus Streptomyces (Bérdy, 2005; Dharmaraj, 2010). However, the discovery ratio of novel compounds from Streptomyces has decreased in recent years owing to the failure to dereplicate known compounds. Therefore, new and improved approaches for screening systems are required to discover novel natural products.

Actinomycete secondary metabolites such as polyketides and nonribosomal peptides are often biosynthesized by large and multifunctional synthases that sequentially assemble small carboxylic acid and amino acid building blocks into their products in an assembly line, so genomics is particularly useful for microbial natural products studies (Fischbach & Walsh, 2006). Some unanticipated biosynthetic gene clusters have been revealed in the genome sequences of Streptomyces coelicolor and Streptomyces avermitilis, which suggested that the strains have the potential to yield new metabolites, and the genomic information has been used to predict the chemical structures of previously unobserved metabolites (Omura et al., 2001; Bentley et al., 2002; Udwary et al., 2011).
Moreover, some significant and new natural products have been discovered based on genome scanning. (Zazopoulos et al., 2003; McAlpine et al., 2005).

Polyketides are structurally diverse secondary metabolites with various biological activities (Monaghan & Tkacz, 1990; Komaki et al., 2009), which are biosynthesized from acyl CoA precursors by polyketide synthases (PKSs) and three types of PKSs are known to date (Shen, 2003). Type II PKSs are multienzyme complexes that carry a core part (minimal PKS) consisting of two ketosynthase (KSα and KSβ) subunits and one acyl carrier protein (ACP). KSα and KSβ catalyze the condensation of acyl-thioesters to form a carbon skeleton. KSβ also works as a chain length-determining factor, while ACP acts as an anchor for the growing polyketide chain during the condensation and subsequent modification steps (Shen et al., 2001; Staunton & Wilkinson, 2001). Until now, many molecular approaches such as genome scanning have successfully revealed the presence of large numbers of cryptic or novel PKS genes that have the possibilities to produce novel polyketides (Metsä-Ketelä et al., 2002; Ikeda et al., 2003; Zazopoulos et al., 2003; Ayuso et al., 2005; McAlpine et al., 2005; Banskota et al., 2006; Ohnishi et al., 2008). For example, two novel angucyclines were discovered by way of the novel PKS genes analysis from a rubromycin producer (Metsä-Ketelä et al., 2004). With the development of sequencing technology, it is expediently to get the draft or complete genome sequences of streptomycetes that provide the abundant PKSs genes information to elucidate the probable metabolic pathways.

Streptomycetes are also distributed widely in marine habitat, and marine-derived streptomycetes have become a focus in the search for novel secondary metabolites (Fenical & Jensen, 2006). In the previous study, we presented the draft genome sequence of marine Streptomyces sp. W007 because of its potential in agricultural fungal disease control (Qin et al., 2012). Genome analysis revealed the most diverse assemblage of polyketide biosynthetic modules involved in producing type II polyketides. In this study, based on the genome sequence, we discussed the possible functions of the putative PKS genes and isolated the novel polyketide compounds from the culture broth of Streptomyces sp. W007.

Materials and methods

Strains description and antifungal activities of crude extract

Marine Streptomyces sp. W007 was isolated from Jiaozhou Bay, China. Phomopsis asparagi, Polystigma deformans, Cladosporium cucumerinum, Monilinia fructicola, and Colletotrichum lagenarium were collected from Qingdao Agricultural University (Shandong, China).

Agar diffusion assay was carried out according to the method previously described (Zhang et al., 2011) with slight modification: the crude extract of Streptomyces sp. W007 was dissolved in MeOH/CH3Cl2 (1 : 1) at concentrations of 50 μg μL−1. Twenty microliters of MeOH/CH3Cl2 (1 : 1) were pipetted onto a sterile filter disk for the blank groups.

Genome sequence analysis

The genomic DNA of Streptomyces sp. W007 was extracted using a TIANamp Bacteria DNA kit [Tiangen Biotech (Beijing) Co., Ltd, China] after treatment with lysozyme. The whole genome shotgun project of Streptomyces sp. W007 has been deposited at DDBJ/EMBL/GenBank under the accession no. AGSW00000000 (Qin et al., 2012). Functional annotation was based on BLASTP with NCBI nr database.

Angucyclinone antibiotics isolation

Methods and materials of chromatography have been reported (Zhang et al., 2011). In the isolation procedure of the crude extract of Streptomyces sp. W007, we obtained the fractions 1–5, C1, C2, and compound 1 (Zhang et al., 2011). Fraction 1 was further separated into A1-A5 by Sephadex LH-20. A1 was crystallized with methanol into brown needle crystal (c. 1 g), structural elucidation as compound 2. C1 and C2 were purified by HPLC (CH3OH/20% H2O), and compounds 3 and 4 were obtained, respectively. Compound 6 was purified from fraction 3 by Sephadex LH-20 and reverse column. Fraction 4 was further separated into D1–D3 by Sephadex LH-20, and the colorless crystal D3 was elucidated to compound 5. Crystal data were determined on Bruker Smart APEX-II DUO.

Cancer cell lines and cytotoxicity assay

Preliminary screening of cytotoxicities was carried out using the human cancer cell lines of lung cancer A549, gastric cancer BGC-823, and breast cancer MCF7 according to the Methyl-Thiazol-Tetrazolium (MTT) method previously described by Wang et al. (2008, 2011).

Human cancer cell lines A549, BGC-823, and MCF7 were cultured in RPMI-1640 media supplemented with 10% fetal calf serum as described before (Wang et al., 2008). The viability of the cells after treatment with various chemicals was evaluated using MTT assay as reported previously (Wang et al., 2011). Briefly, cells were seeded into 96-well plates and then treated with the tested
articles at the desired concentration for 72 h. MTT solution was added into the wells and incubated for 2 h. After the medium was removed, DMSO was added to each well. The plates were gently agitated until the color reaction was uniform, and the OD$_{570}$ was determined using a microplate reader (Wellscan MK3; Labsystems Dragon). Media-only treated cells with DMSO served as the indicator of 100% cell viability.

**Results**

**Antifungal activities of the crude extract of marine Streptomyces sp. W007**

Antifungal activity tests showed that inhibition zone diameters of the crude extract against Phomopsis asparagi, Polystigma deformans, Cladosporium cucumerinum, Moniliinia fructicola, and Colletotrichum lagenarium were 15, 25, 20, 15, and 20 mm, respectively; however, the inhibition zone diameters of blank groups were only 6 mm, which implied great potential of *Streptomyces* sp. W007 in agricultural fungal disease control.

**Sequence analysis of biosynthetic gene clusters**

Genome sequence of *Streptomyces* sp. W007 revealed the presence of 149 open reading frames (ORFs) in the contig 151. A homology search showed that some ORFs were homologous to angucyclinone derivatives biosynthesis genes reported previously. Based on their positions and deduced functions, we identified 20 ORFs (from ORF 4216 to 4235, named ang 1 to ang 20) probably involved in the biosynthesis of angucyclinone antibiotics (Fig. 1). The putative functions of ORFs and the closest homologues are shown in Table 1.

According to BLASTP results with NCBI nr database, ang 2 shows high percent identity (93%) to SAM-dependent methyltransferase from *Streptomyces griseus* IFO 13350 (Ohnishi et al., 2008), which can regulate spor development and antibiotic synthesis (Bao et al., 2010). Ang 4 is identified as hydrolase (Ohnishi et al., 2008). Ang 7 and ang 5 are in accordance with short-chain dehydrogenase/reductase (SDR) and 3-oxoacyl-(acyl carrier protein) reductase, which catalyze the reduction in ketone group. Ang 10 shares 56% amino acid identity with O-methyltransferase of *Streptomyces* sp. 2238-SVT4 (Kawasaki et al., 2010). Ang 11 is a FAD-binding hydroxylase similar to the type II polyketide gene cluster from *Streptomyces fradiae* (Decker & Haag, 1995). Ang 12 has high similarity of 89% to cyclase in *Streptomyces* sp. SCC2136 (Basnet et al., 2006).

In the gene cluster of angucyclinone antibiotics, the mini PKS is found to be composed of ang 13, 14, and 15 that present the functions of ketoacyl synthase (KS), chain length factor (KSβ), and ACP, respectively. Ang 16 shows similarity to ketone group reductase of urdamycin A biosynthesis and can be assigned to reduce C-9 (Decker & Haag, 1995). Ang 17 and 20 show high percent identities to aromatase from *Streptomyces* sp. SCC 2136 (Basnet et al., 2006) and acetyl-coenzyme A carboxyl transferase alpha chain in *Streptomyces venezuelae* ATCC 10712 (Pullan et al., 2011), respectively. Ang 18 and 19 show sequence similarities to oxygenase reductase and ketoacyl reductase from *Streptomyces* sp. 2238-SVT4 (Kawasaki et al., 2010).

Ang 1 is predicted to be an ATP-binding cassette (ABC) transporter-related protein that belongs to a ubiquitous superfamily of integral membrane proteins, which are responsible for the ATP powered translocation of many substrates across membranes, including small molecules, amino acid, secondary metabolites, and protein (Rees et al., 2009). Ang 9 shows similarity to the drug resistance transporter, EmrB/QacA subfamily, possibly involved in secretion of secondary metabolites. Therefore, ang 1 and 9 could be responsible for the excretion of angucyclinone antibiotics out of the cell.

Ang 6 shows similarity of 52% to the LuxR family transcriptional regulator that is a widespread and functionally diverse transcription factor and belongs to TetR protein superfamily. It could both activate and inhibit the expressions of many genes contingent on the contexts and thereby is involved in many crucial physiological events, such as virulence factors production, quorum sensing (QS), biosynthesis, metabolism, and ecological competition (Zeng & Xie, 2011). Ang 8 is identified as the TetR family transcriptional regulator, which consists of two domains: a DNA-binding domain with a helix-turn-helix motif and a regulatory domain as signal recognition function via ligand binding. This protein family is mainly as repressors or regulator for the biosynthesis of
Table 1. Putative functions of the ORFs in the angucyclinone antibiotics biosynthesis gene cluster

<table>
<thead>
<tr>
<th>ORFs (aa)</th>
<th>Name</th>
<th>Homologous protein</th>
<th>Accession No.</th>
<th>Identity (%)</th>
<th>Similarity (%)</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4216 (249)</td>
<td>ang 1</td>
<td>ABC transporter-related protein</td>
<td>ZP_08807043</td>
<td>56</td>
<td>71</td>
<td>Streptomyces zircinesistens K42</td>
<td>Lin et al. (2011)</td>
</tr>
<tr>
<td>4217 (279)</td>
<td>ang 2</td>
<td>SAM-dependent methyltransferase</td>
<td>YP_001824878</td>
<td>93</td>
<td>95</td>
<td>Streptomyces griseus IFO 13350</td>
<td>Ohnishi et al. (2008)</td>
</tr>
<tr>
<td>4218 (146)</td>
<td>ang 3</td>
<td>Hypothetical protein</td>
<td>YP_001824877</td>
<td>86</td>
<td>90</td>
<td>Streptomyces griseus IFO 13350</td>
<td>Ohnishi et al. (2008)</td>
</tr>
<tr>
<td>4219 (268)</td>
<td>ang 4</td>
<td>Hydrolase</td>
<td>YP_001824876</td>
<td>94</td>
<td>97</td>
<td>Streptomyces griseus IFO 13350</td>
<td>Ohnishi et al. (2008)</td>
</tr>
<tr>
<td>4220 (260)</td>
<td>ang 5</td>
<td>3-oxoacyl-(acyl carrier protein) reductase</td>
<td>YP_004806473</td>
<td>50</td>
<td>63</td>
<td>Streptomyces sp.</td>
<td>DOE JGI</td>
</tr>
<tr>
<td>4221 (221)</td>
<td>ang 6</td>
<td>LuxR family transcriptional regulator</td>
<td>YP_004015262</td>
<td>35</td>
<td>51</td>
<td>SirexAA-E</td>
<td>DOE JGI</td>
</tr>
<tr>
<td>4222 (235)</td>
<td>ang 7</td>
<td>Short-chain dehydrogenase/reductase (SDR)</td>
<td>YP_004924949</td>
<td>66</td>
<td>76</td>
<td>Streptomyces flavogriseus ATCC 33331</td>
<td>DOE JGI</td>
</tr>
<tr>
<td>4223 (222)</td>
<td>ang 8</td>
<td>TetR family transcriptional regulator</td>
<td>YP_004814969</td>
<td>60</td>
<td>72</td>
<td>Streptomyces violaceusniger Tu 4113</td>
<td>DOE JGI</td>
</tr>
<tr>
<td>4224 (470)</td>
<td>ang 9</td>
<td>Drug resistance transporter, EmrB/QacA subfamily</td>
<td>ZP_06416632</td>
<td>50</td>
<td>68</td>
<td>Frankia sp. EUN1f</td>
<td>DOE JGI</td>
</tr>
<tr>
<td>4225 (311)</td>
<td>ang 10</td>
<td>O-methyltransferase</td>
<td>BA107860</td>
<td>56</td>
<td>69</td>
<td>Streptomyces sp. 2238-SVT4</td>
<td>Kawasaki et al. (2010)</td>
</tr>
<tr>
<td>4226 (500)</td>
<td>ang 11</td>
<td>Oxygenase, hydroxylase</td>
<td>CAA60567</td>
<td>70</td>
<td>80</td>
<td>Streptomyces fradiae</td>
<td>Decker &amp; Haag (1995)</td>
</tr>
<tr>
<td>4227 (99)</td>
<td>ang 12</td>
<td>Cyclase</td>
<td>CAH10118</td>
<td>77</td>
<td>89</td>
<td>Streptomyces sp. SCC 2136</td>
<td>Basnet et al. (2006)</td>
</tr>
<tr>
<td>4228 (426)</td>
<td>ang 13</td>
<td>Ketoacyl synthase</td>
<td>CAA60569</td>
<td>84</td>
<td>92</td>
<td>Streptomyces fradiae</td>
<td>Decker &amp; Haag (1995)</td>
</tr>
<tr>
<td>4229 (406)</td>
<td>ang 14</td>
<td>Ketoacyl synthase beta</td>
<td>CAG14966</td>
<td>75</td>
<td>85</td>
<td>Streptomyces antibioticus ATCC 11891</td>
<td>Lombó et al. (2004)</td>
</tr>
<tr>
<td>4230 (89)</td>
<td>ang 15</td>
<td>Acyl carrier protein</td>
<td>AAO65348</td>
<td>74</td>
<td>83</td>
<td>Streptomyces murayamaensis</td>
<td>Gould et al. (1998)</td>
</tr>
<tr>
<td>4231 (256)</td>
<td>ang 16</td>
<td>Keto reductase</td>
<td>CAA60572</td>
<td>82</td>
<td>88</td>
<td>Streptomyces fradiae</td>
<td>Decker &amp; Haag (1995)</td>
</tr>
<tr>
<td>4232 (323)</td>
<td>ang 17</td>
<td>Aromatase</td>
<td>CAH10113</td>
<td>75</td>
<td>84</td>
<td>Streptomyces sp. SCC 2136</td>
<td>Basnet et al. (2006)</td>
</tr>
<tr>
<td>4233 (656)</td>
<td>ang 18</td>
<td>Oxygenase reductase</td>
<td>BA107846</td>
<td>65</td>
<td>74</td>
<td>Streptomyces sp. 2238-SVT4</td>
<td>Kawasaki et al. (2010)</td>
</tr>
<tr>
<td>4234 (254)</td>
<td>ang 19</td>
<td>Keto reductase</td>
<td>BA107858</td>
<td>67</td>
<td>78</td>
<td>Streptomyces sp. 2238-SVT4</td>
<td>Kawasaki et al. (2010)</td>
</tr>
<tr>
<td>4235 (525)</td>
<td>ang 20</td>
<td>Acetyl-coenzyme A carboxyl transferase alpha chain</td>
<td>CCA59277</td>
<td>84</td>
<td>91</td>
<td>Streptomyces venezuelae ATCC 10712</td>
<td>Pullan et al. (2011)</td>
</tr>
</tbody>
</table>

antibiotics, drug-efflux pumps, and other proteins (Ramos et al., 2005). Therefore, the gene cluster analysis implies that Streptomyces sp. W007 has potential to produce angucyclinone antibiotic analogs. Based on the sequence data, novel angucyclinone antibiotics are isolated from the crude extract of Streptomyces sp. W007.

Structure elucidation of angucyclinone antibiotics

Compounds 2, 3, 4, 5, and 6 (Fig. 2) were separated followed by compound 1. Based on 1H, 13C-NMR, and ESI-MS spectra, compounds 2, 3, 4, 5, and 6 were proved to be X-14881E (Maehr et al., 1982), 6-deoxy-8-O-methylrabelomycin (Shigihara et al., 1988; Gilpin et al., 1989), 8-O-methylrabelomycin (Shigihara et al., 1988), kiamycin (Xie et al., 2012), and 7-acefylchrysophanol (Delle Monache et al., 1991), respectively. Besides, relative configuration of compound 1 has been reported (Zhang et al., 2011). However, to further test the absolute configuration of compound 1, X-ray ORTEP was conducted (Fig. 3). In the structure of compound 1, ring A, C, and D show the same structure as found in known compounds 2, 3, and 4. However, ring B is not quinoid and shows novel reduction state at C-7 and C-12, and no keto or hydroxy groups at C-7 and C-12.

Surprisingly, without using any staining reagent, partial compound 3 (brilliant yellow) changed into 2 (orange) quickly after exposing the TLC plate in air for only 5 minutes. The transformation is possibly due to H+-catalysis, and this process could be catalyzed by aromatase (ang 17) and reductase (ang 5 and 7) in the biotransformation.

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Cytotoxicities of compounds 2, 5, and 6

In the cytotoxicity tests, compound 2 exhibited a potent selective inhibition activity against lung cancer cells (A549) with inhibition value of 71.8% at concentration of 10 μM, but had no cytotoxic activities against gastric cancer cells (BGC-823) and breast cancer cells (MCF7). Compound 6 showed a moderate activity against gastric cancer cells (BGC-823) with inhibition value of 48% at concentration of 1 μM and had weaker cytotoxic activity against lung cancer cells (A549). Kiamycin (compound 5) exhibited weaker inhibition activity against gastric cancer cells (BGC-823) and had no cytotoxic activities against lung cancer cells (A549) and breast cancer cells (MCF7).

The results indicated that the compounds 2 and 6 might have potential selective target against the cancer cells, as shown in Table 2.

**Discussion**

In this study, the draft genome sequence of *Streptomyces* sp. W007 contained an intact biosynthetic gene cluster for angucyclinone antibiotics, which provided insight into the biosynthesis of angucyclinone antibiotics. Meanwhile, two novel and four known angucyclinone antibiotics were isolated from the culture broth of marine *Streptomyces* sp.

**Table 2.** Cytotoxicities of compounds 2, 5, 6, and adriamycin against human lung cancer cell line (A549), gastric cancer cell line (BGC-823), and breast cancer cell line (MCF7) at concentration of 10, 1, and 0.1 μM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μM)</th>
<th>A549 (%)</th>
<th>BGC-823 (%)</th>
<th>MCF7 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 2</td>
<td>10</td>
<td>71.8 ± 1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30.8 ± 2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>5.5 ± 1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound 5</td>
<td>10</td>
<td>0</td>
<td>29.0 ± 2.1</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>22.2 ± 1.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0</td>
<td>11.9 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td>Compound 6</td>
<td>10</td>
<td>24.2 ± 1.9</td>
<td>48.9 ± 2.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20.0 ± 2.1</td>
<td>48.0 ± 1.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>13.9 ± 1.3</td>
<td>40.8 ± 1.8</td>
<td>0</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>10</td>
<td>76.7 ± 2.2</td>
<td>83.4 ± 1.8</td>
<td>74.9 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>64.0 ± 1.4</td>
<td>56.2 ± 2.1</td>
<td>29.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>34.6 ± 1.5</td>
<td>21.2 ± 1.1</td>
<td>15.2 ± 0.8</td>
</tr>
</tbody>
</table>

Fig. 2. Structures of compounds 1–6.

Fig. 3. Structure of the 3(R)-hydroxy-1-keto-3(R)-methyl-8-methoxy-1,2,3, 4-tetrahydro-benz[a]anthracene as determined by X-ray crystallography with ORTEP.

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W007. We have already defined the chemical structure and cytotoxicities of these angucyclinone antibiotics, but the biosynthetic pathways remain unclear. We focus research on biosynthetic pathways of the two new compounds and elucidate 22-kb DNA fragment containing type II PKS genes involved in the biosynthesis of compound 1 and kiamycin. Two primary transporters (ABC transporter-related protein and EmrB/QacA family drug resistance transporter) and two regulators (LuxR family transcriptional regulator and TetR family transcriptional regulator) existed in the gene cluster of aromatic polyketide and might have important roles on the synthesis, regulation, and release of secondary metabolites.

The detection of some genes with sequence similarity to the biosynthetic gene clusters of the angucycline antibiotics urdamycin A (Decker & Haag, 1995), jadomycin B (Han et al., 1994), simocyclinone (Galm et al., 2002), hatomarubigin (Kawasaki et al., 2010), ovedomycin (Lombo et al., 2004), sch47554, and sch47555 (Basnet et al., 2006) strongly suggested that the identified DNA sequence indeed represented the compound 1 biosynthetic gene cluster. However, it is characteristic of compound 1 to contain methoxyl group at C-8 and no keto or hydroxy groups at C-7 and C-12, which was in accordance with analysis of the biosynthesis gene of angucyclinone antibiotic. There is O-methyltransferase gene (ang 10) in the cluster with high percent identity to related gene in Streptomyces sp. 2238-SVT4 (Kawasaki et al., 2010). This O-methyltransferase catalyzed the methoxyla-

Ang 5, ang 7, and ang 18 are oxygenase reductases and should catalyze the 6, 7, 8-hydroxylation and dehydration reaction to generate compound 1. In this case, marine Streptomyces sp. W007 has the potential to produce novel angucyclinone antibiotics, which are specific and in accordance with the structure of 3-hydroxy-1-keto-3-methyl-8-methoxy-1,2,3,4-tetrahydro-benzo[\(\text{a}\) anthracene (Zhang et al., 2011) (Fig. 4). Compared with other angucyclinone antibiotics mentioned previously, kiamycin has two distinctive characteristics, 6a-OH and epoxy moiety. A plausible pathway was that oxidoreductases (ang 5 and ang 18) were in charge of synthesis of 6a-OH and epoxy structure, respectively (Fig. 4).

In our study, we have used a genome scanning method to discover metabolic loci. The basis of this approach is that the genes required for secondary metabolites biosynthesis are typically clustered together in a streptomycete chromosome (Martin & Liras, 1989; Zazopoulos et al., 2003). Genomic sequence analysis reveals the most diverse assemblage of biosynthetic modules involved in producing polyketides and nonribosomal peptides in the Streptomyces. This work provides powerful evidence for discovering cryptic metabolic potential and directing traditional natural product research based on genome sequence.

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Authors’ contribution

H. Zhang and H. Wang contributed equally to this work.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**File S1. NMR data and ESI-MS of compound 1.**

**File S2. NMR data and ESI-MS of compound 2.**

**File S3. NMR data and ESI-MS of compound 3.**

**File S4. NMR data and ESI-MS of compound 4.**

**File S5. NMR data and ESI-MS of compound 5.**

**File S6. NMR data and ESI-MS of compound 6.**

**File S7. Antifungal activities of the crude extract of Marine *Streptomyces* sp. W007.**

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