Abstract: An extract prepared from the culture of a marine-derived actinomycete Streptomyces sp. ZZ338 was found to have significant antimicrobial and antiproliferative activities. A chemical investigation of this active extract resulted in the isolation of three known bioactive actinomycins (1–3) and two new metabolites (4 and 5). The structures of the isolated compounds were identified as actinomycins D (1), V (2), X_0β (3), 2-acetylamino-3-hydroxyl-4-methyl-benzoic acid methyl ester (4), and N-1S-(4-methylaminophenylmethyl)-2-oxo-propyl acetamide (5) based on their nuclear magnetic resonance (NMR) and high resolution electrospray ionization mass spectroscopy (HRESIMS) data as well as their optical rotation. This class of new compound 5 had never before been found from a natural resource. Three known actinomycins showed activities in inhibiting the proliferation of glioma cells and the growth of methicillin-resistant Staphylococcus aureus, Escherichia coli, and Candida albicans and are responsible for the activity of the crude extract. Actinomycin D (1) was also found to downregulate several glioma metabolic enzymes of glycolysis, glutaminolysis, and lipogenesis, suggesting that targeting multiple tumor metabolic regulators might be a new anti-glioma mechanism of actinomycin D. This is the first report of such a possible mechanism for the class of actinomycins.

Keywords: marine Streptomyces sp. ZZ338; new metabolites; actinomycins; antimicrobial activity; antiproliferative activity; glioma cells; glioma metabolic enzymes

1. Introduction

Gliomas are the most aggressive and frequently diagnosed human brain tumors. Despite advances in therapies including chemotherapy, radiotherapy, and surgical resection, the prognosis is still very poor [1]. While chemotherapy has played an important role in the treatment and prevention of cancer, very few drugs have been approved for treating gliomas including temozolomide (TMZ), carmustine, lomustine, and bevacizumab [2]. Furthermore, only TMZ has been independently used for the treatment of gliomas and the efficacy of TMZ remains unsatisfactory [2]. Therefore, there is an urgent need to discover lead compounds for the development of novel anti-glioma drugs.

Many anti-cancer bioactive compounds, originally isolated from marine invertebrates are actually produced by marine microorganisms [3,4]. For example, several anti-cancer metabolites from marine sponges that have progressed to preclinical or clinical trial phases, such as discodermolide, halichondrin B, bryostatin1, and phorboxazole A, may actually be products derived from their microbiotic consortia [5–7]. Therefore, bioactive natural products produced by marine microorganisms are promising sources for the discovery and development of novel anti-glioma drugs or drug leads [7–9].

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During the course of our ongoing project to discover novel antglioma agents from marine sources [10–15], a marine-derived actinomycete strain, ZZ338, was isolated from sea squirts grown on coastal rocks. A crude extract prepared from the culture of this isolated strain ZZ338 in Gause’s liquid medium showed antimicrobial and antiproliferative activities. A chemical investigation of this bioactive extract resulted in the isolation of three known actinomycins (1–3) and new compound 4. In order to obtain more bioactive metabolites, a second medium of BMPM was also used to culture this isolated strain ZZ338, which produced actinomycin 2 and new compound 5. In this study, we report the isolation and culture of strain ZZ338, the structural elucidation of the new metabolites, and the bioactive evaluation of the isolated compounds against the proliferation of glioma cells and the growth of methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

2. Results and Discussion

The isolated strain, ZZ338, was assigned as *Streptomyces* sp. ZZ338 based on its 16S rDNA gene sequence, which completely (99% identity for a 1396 bp stretch of sequence) matched those of several *Streptomyces* strains (Supplementary Materials, Figure S1 and Table S1) in the GenBank database. Two different liquid media of Gause and Bristol Myers Production Medium (BMPM) were used to culture this actinomycete, which produced three known actinomycins (1–3) and two new metabolites (4 and 5) (Figure 1).

![Figure 1. The structures of compounds 1–5.](image-url)

The known compounds were proved to be actinomycin D (1) [16–18], actinomycin V (2) [17,19], and actinomycin X₀β (3) [17,20] based on their nuclear magnetic resonance (NMR) and high resolution electrospray ionization mass spectroscopy (HRESIMS) (Figures S2–S70) data as well as a comparison of published data. The $^{13}$C and $^1$H NMR data of the three actinomycins are summarized in the Tables S2–S4 (Supplementary Materials).

Compound 4 has a molecular formula of C$_{11}$H$_{13}$NO$_4$ deduced from its HRESIMS at m/z [M – H]$^-$ 222.0779 (calcd for C$_{11}$H$_{12}$NO$_4$, 222.0766) and $^{13}$C NMR data. The $^{13}$C NMR spectrum of 1 exhibited 11 carbons including two carboxyls (δ 170.2, 166.6), six aromatic carbons (δ 149.9, 131.1, 127.4, 124.9, 124.3, 120.7), one methoxy (δ 51.8), and two methyls (δ 23.1, 16.6). In the $^1$H NMR spectrum, two aromatic proton signals appeared as doublets at δ 7.19 (7.9 Hz) and 7.07 (7.9 Hz) and were attributed to H-6 and H-5, three were singlets at δ 3.71, 2.21, 2.07 and easily assigned to one methoxy and two methyls. In addition, a singlet at δ 9.68 indicated the presence of an NH unit. HMBC correlations
(Table 1 and Figure 2) of both H-6 (δ 7.19) and H-11 (δ 3.71) with C-7 (δ 166.6) indicated the methoxy at C-7 position. Similarly, HMBC correlations of H-8 (δ 2.21) with C-3 (δ 149.9), C-4 (δ 131.1), and C-5 (δ 127.4) determined the position of a methyl at C-4, while the other methyl related to the acetylamino unit was deduced from a HMBC correlation of H-10 (δ 2.07) with C-9 (δ 170.2). The downfield chemical shift at δ 149.9 (C-3), in combination of the HRESIMS data, suggested a hydroxyl at C-3. Based on the above NMR and MS data analyses, the structure of 4 was elucidated as 2-acetylamino-3-hydroxy-4-methyl-benzoic acid methyl ester, a new compound. The 13C and 1H NMR data of 4 are listed in the Table 1. NMR and MS spectra of 4 see Figures S71–S81.

Table 1. 13C and 1H NMR data of compounds 4 and 5.

| No. | 13C, Type | 1H (J = Hz) | HMBC (H → C) | 13C, Type | 1H (J = Hz) | HMBC (H → C) |
|-----|-----------|-------------|-------------|-----------|-------------|-------------|
| 1   | 124.3, C  | –           | –           | 124.5, C  | –           | –           |
| 2   | 124.9, C  | –           | –           | 129.9, CH | 6.92, d (8.2)| C-4, 6.7 |
| 3   | 149.9, C  | –           | –           | 112.1, CH | 6.43, d (8.2)| C-1, 4.5 |
| 4   | 131.1, C  | –           | –           | 148.8, C  | –           | –           |
| 5   | 127.4, CH | 7.07, d (7.9) | C-1, 3    | 112.1, CH | 6.43, d (8.2)| C-1, 4.5 |
| 6   | 120.7, CH | 7.19, d (7.9) | C-2, 4.7  | 129.9, CH | 6.92, d (8.2)| C-2, 4.7 |
| 7   | 166.6, C  | –           | –           | 35.0, CH2 | 2.56, dd (14.0, 9.0); 2.80, dd (14.0, 5.4)| C-1, 2.6, 8.9 |
| 8   | 16.6, CH3 | 2.21, s     | C-3, 4.5   | 61.0, CH  | 4.27, m    | C-1.7, 9.12 |
| 9   | 170.2, C  | –           | –           | 208.5, C  | –           | –           |
| 10  | 23.1, CH3 | 2.07, s     | C-9        | 27.5, CH3 | 2.02, s    | C-8, 9     |
| 11  | 51.8, CH3 | 3.71, s     | C-7        | 30.2, CH3 | 2.61, s    | C-4        |
| 12  |           |             |            | 170.4, C  | –           | –           |
| 13  |           |             |            | 22.5, CH3 | 1.80, s    | C-12       |
| NH  |           |             |            | 9.68, s   | 8.21, d (6.8)| C-7, 8, 12 |

Figure 2. 1H–1H COSY and key HMBC correlations of compounds 4 and 5.

Compound 5 was isolated as a yellow oily and has UV absorbance at 252 and 303 nm. Its HRESIMS showed [M + H]+ ion at m/z 235.1443 and [M + Na]+ ion at 257.1263, supporting a molecular formula of C13H16N2O2. The 13C and 1H NMR spectra of 5 showed one ketone (δC 208.5), one carbonyl (δC 170.4), six aromatic carbons with four protons (δC 148.8, 129.9, 129.9, 124.5, 112.1, 112.1; δH 6.92, 2H, d, 6.43, 2H, d), one nitrogenated methine (δC 61.0; δH 4.27), one methylene (δC 35.0; δH 2.56, 2.80), and three methyls (δC 30.2, 27.5, 22.5; δH 2.61, 2.02, 1.80). The 1H NMR spectrum also exhibited a doublet at δ 8.21 (1H, d, 6.8 Hz), which was attributed to the NH unit at C-8 position. Two doublets at δH 6.92 (2H, d, 8.2 Hz) and δH 6.43 (2H, d, 8.2 Hz) correlating with the carbon signals at δC 129.9 (CH) and δC 112.1(CH) observed in the HSQC spectrum, together with the COSY correlation of H-5 (δ 6.43) and H-6 (δ 6.92), indicated that the benzene ring had a symmetrical structure. 1H–1H COSY correlations (Figure 2) of H-7 (δ 2.56, 2.80) with H-8 (δ 4.27) and H-8 with NH-8 (δ 8.21) confirmed the connection.
Accordingly, the stereochemistry of C-8 in compound 5 (an antifungal drug) were used as positive control. The results (Table 2) showed that all three compounds had potent activity against the proliferation of the three tested tumor cell lines with IC\(_{50}\) values from 1.01 to 10.06 nM for actinomycin D (1), 0.42 to 1.80 nM for actinomycin V (2), 3.26 to 25.18 nM for actinomycin X\(_{0\beta}\) (3), while the control DOX showed activity with IC\(_{50}\) values in a range from 0.70 to 9.61 \(\mu\)M. Unfortunately, new compounds 4 and 5 at concentration of 100 \(\mu\)M had no activity against the three tested pathogens.

Table 2. Antimicrobial activity of actinomycins D (1), V (2), and X\(_{0\beta}\) (3) (MIC; \(\mu\)M).

| Microbes     | Actinomycin D (1) | Actinomycin V (2) | Actinomycin X\(_{0\beta}\) (3) | Gentamicin | Amphotericin |
|--------------|-------------------|-------------------|-------------------------------|------------|--------------|
| S. aureus    | 0.08              | 0.08              | 0.61                          | 0.26       | -            |
| E. coli      | 0.12              | 0.12              | 0.61                          | 0.51       | -            |
| C. albicans  | 9.96              | 9.85              | 9.83                          | -          | 0.05         |

Compounds 1–5 were also tested for their activity in inhibiting the proliferation of human glioma U251 and SHG44 cells and rat glioma C\(_6\) cells by an SRB assay, a method that measures total cellular protein content. Doxorubicin (DOX) [23] was used as a positive control. Glioma cells were treated with tested compounds for 72 h at different concentrations. The data (Table 3) obtained from this study indicated that three actinomycins had potent activity against the proliferation of the three tested tumor cell lines with IC\(_{50}\) values from 1.01 to 10.06 nM for actinomycin D (1), 0.42 to 1.80 nM for actinomycin V (2), 3.26 to 25.18 nM for actinomycin X\(_{0\beta}\) (3), while the control DOX showed activity with IC\(_{50}\) values in a range from 0.70 to 9.61 \(\mu\)M. Unfortunately, new compounds 4 and 5 were inactive.

Table 3. Antiproliferative activity of actinomycins D (1), V (2), and X\(_{0\beta}\) (3) against glioma cells (IC\(_{50}\)).

| Glioma Cells | Actinomycin D (1, nM) | Actinomycin A\(_5\) (2, nM) | Actinomycin X\(_{0\beta}\) (3, nM) | DOX (\(\mu\)M) |
|--------------|-----------------------|-----------------------------|--------------------------------|---------------|
| U251         | 10.06 ± 0.68          | 1.80 ± 0.19                 | 8.71 ± 0.66                    | 9.61 ± 1.25   |
| SHG44        | 3.31 ± 0.25           | 1.37 ± 0.07                 | 3.26 ± 0.32                    | 2.54 ± 0.23   |
| C\(_6\)      | 1.01 ± 0.05           | 0.42 ± 0.23                 | 25.18 ± 0.47                   | 0.70 ± 0.01   |

Actinomycin D (1) was further investigated for its effects on several important tumor metabolic enzymes from different metabolic pathways including HK2 [24,25] and PKM2 [25,26] from glycolysis, GLS [27,28] from glutaminolysis, and FASN [28,29] from lipogenesis. These metabolic regulators have
been demonstrated to be upregulated in the glioma cells and are preferentially used by cancer cells, making them promising targets for the discovery of novel anticancer drugs [24–29]. U87-MG cells were treated by actinomycin D (1, 0.01 nM) for 48 h. Protein prepared from the actinomycin D-treated U87-MG cells was subjected to a Western blot analysis. The results showed that actinomycin D (1) significantly reduced the expression levels of HK2, GLS, and FASN (Figure 3), suggesting that targeting multiple metabolic enzymes may be one of actinomycin D’s anti-glioma mechanisms.

![Figure 3](image-url)

**Figure 3.** Effects of actinomycin D (1) on the expression levels of HK2, PKM2, GLS, and FASN in U87-MG cells. U87-MG cells were treated with actinomycin D (1, 0.01 nM) for 48 h. Protein extracted from cells was subjected to a Western blot analysis (HK2: hexokinase 2; PKM2: pyruvate kinase M2; GLS: glutaminase; FASN: fatty acid synthase; β-actin: internal control).

Actinomycins are a family of chromopeptide lactone antibiotics, among which actinomycin D is one of the older anticancer drugs and has been studied extensively and widely used clinically for the treatment of several types of malignant tumors, such as Wilms’ tumor and childhood rhabdomyosarcoma [20]. Despite their initial discovery more than 70 years ago, actinomycins continue to be a focus of many research areas, especially in their biological activity and medicinal use. For example, actinomycin D has been reported to have potent activities against HIV and tuberculosis [20,30,31]. It is known that the antitumor mechanism of actinomycin D is to inhibit transcription by binding DNA at the transcription initiation complex and preventing elongation of the RNA chain by RNA polymerase [32]. In this study, actinomycin D was found to significantly downregulate the expression levels of several glioma metabolic enzymes, suggesting that targeting multiple metabolic regulators might be a new anti-glioma mechanism of actinomycin D.

3. Materials and Methods

3.1. General Experimental Procedures

NMR spectra were recorded on a Bruker 500 spectrometer (Fällanden, Switzerland) using standard pulse programs and acquisition parameters and chemical shifts were reported in δ (ppm) referencing to the NMR solvent used. Octadecyl-functionalized silica gel (Octadecylsilyl (ODS), Cosmosil 75C3-Prep, Nacalai Tesque Inc., Kyoto, Japan) was used for open column chromatography. HPLC separation was performed on an Agilent 1260 HPLC system with a Diode Array Detector (DAD) detector using a Zorbax SB-C18 column (250 × 9.4 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA). All solvents used for this study were purchased from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Human glioma U251, HSG44, and U87-MG cells and rat glioma C6 cells were purchased from the Cell Bank of the Chinese Academy of Sciences. Methicillin-resistant S. aureus ATCC 43300, E. coli ATCC 25922, and C. albicans were gifts from Zhongjun Ma, Pinmei Wang, and Bin Wu, respectively. Doxorubicin (DOX, >98.0%) was ordered from Sigma-Aldrich, gentamicin (99.6%) and amphotericin B (>95.0%) from Meilune Biotechnology Co. Ltd. (Dalian, China). Nutrient Broth (NB), Mueller-Hinton Broth (MHB), and Gause’s agar were purchased from Hangzhou Microbial Reagent...
Co. Ltd. (Hangzhou, China). BMPM liquid medium (glucose 20 g, glycerol 20 g, soy flour 10 g, cotton seed embryo meal 10 g, (NH₄)₂SO₄ 1 g, CaCO₃ 10 g, 1.0 L water) was made in our laboratory.

3.2. Isolation and Identification of Strain ZZ338

The strain ZZ338 was isolated from sea squirts collected in January 2016 off the coastal rocks of Dongji Island, close to the East China Sea. The sea squirts were grinded with sterile water in a mortar and then diluted to 1 × 10⁻³ g/mL. The diluted liquid was coated on the surface of Gause’s agar medium. After incubation at 28 °C for 7 days, the ZZ338 colonies were grown and then transferred to a Gause’s agar plate by using a sterile needle. After being cultured at 28 °C for 3 days, the single colony (ZZ338) grew well was transferred onto Gause’s agar slants and stored at 4 °C for later use.

The taxonomic identity of strain ZZ338 was determined by 16S rDNA analysis, which was conducted by Majorbio (Shanghai, China). The top sequence using BLAST (nucleotide sequence comparison) was compared to the GenBank database. A voucher strain of *Streptomyces* sp. ZZ338 was preserved at the Laboratory of Institute of Marine Biology, Ocean College, Zhejiang University, China.

3.3. Fermentation of Strain ZZ338

Two different liquid media of Gause and BMPM were applied to culture the strain ZZ338. First, the single colony (ZZ338) was transferred into 250 mL of Gause’s liquid media in a 500 mL Erlenmeyer flask and then incubated at 28 °C for 4 days on a rotary shaker (180 rpm) to prepare the seed broth. Secondly, the seed broth (4 mL) was inoculated into 250 mL of Gause’s liquid media and BMPM’s liquid media in 500 mL Erlenmeyer flasks and then incubated at 28 °C for 8 days on a rotary shaker (180 rpm). A total of 20.0 L of Gause’s fermentation broth and 0.25 L of BMPM’s culture were made for this study.

3.4. Isolation of Compounds 1–5

The Gause’s fermentation broth of strain ZZ338 (20.0 L) was filtered to harvest mycelia and filtrate. The mycelia were extracted with MeOH three times to give a MeOH extract and the filtrate was extracted with ethyl acetate three times to afford an EtOAc extract. The two extracts were combined and then fractionated on a column of ODS (600 g) eluting with 40%, 70%, and 100% MeOH to give three fractions of 40 M, 70 M, and 100 M. Fraction 100 M was separated by HPLC using a Zorbax SB-C₁₈ column (250 × 9.4 mm, 5 μm; mobile phase 90% MeOH; flow rate 1.0 mL/min; detection wavelength: 445 nm) to give compounds 1 (22.1 mg, tᵣ 19.0 min), 2 (96.10 mg, tᵣ 16.9 min), and 3 (5.40 mg, tᵣ 15.5 min). Compound 4 (3.60 mg, tᵣ 20.0 min) was obtained from fraction 70 M by HPLC purification (mobile phase: 70% MeOH; detection wavelength: 250 nm).

The BMPM’s fermentation broth (0.25 L) was extracted with MeOH three times. The concentrated MeOH extract was separated by an ODS column eluting with 30%, 50%, 70% and 100% MeOH to give Frs. 30 M, 50 M, 70 M, and 100 M. Compound 5 (26.20 mg, tᵣ 19.0 min) was obtained from fraction 70 M by HPLC purification (mobile phase: 50% MeOH; flow rate: 1.0 mL/min; detection wavelength: 252 nm). Compound 2 was also found in the fraction 100 M by HPLC analysis.

Actinomycin D (1): Red amorphous powder; molecular formula C₆₂H₈₆N₁₂O₁₆; mp 242–245 °C (lit. 244–246 °C) [18]; UV λ_max 240, 445 nm; ¹³C NMR data (125 MHz, in CDCl₃), see Table S2, ¹H NMR data (500 MHz, in CDCl₃) see Table S3; HRESIMS m/z [M + H]⁺ 1255.6426 (calcd for C₆₂H₇₇N₁₂O₁₆, 1255.6363).

Actinomycin V (2): Red amorphous powder; molecular formula C₆₂H₈₄N₁₂O₁₇; UV λ_max 242, 445 nm; ¹³C NMR data (125 MHz, in CDCl₃), see Table S2, ¹H NMR data (500 MHz, in CDCl₃) see Table S3; HRESIMS m/z [M + H]⁺ 1269.6131 (calcd for C₆₂H₈₅N₁₂O₁₇, 1269.6156), [M + Na]⁺ 1291.5945 (calcd for C₆₂H₈₄Na₁₂O₁₇, 1291.5975), [M – H]⁻ 1267.5967 (calcd for C₆₂H₈₃N₁₂O₁₇, 1267.5999).

Actinomycin X₁₈ (3): Red amorphous powder; molecular formula C₆₂H₈₆N₁₂O₁₇; UV λ_max 239, 445 nm; ¹H NMR data (500 MHz, in CDCl₃) see Table S4; HRESIMS m/z [M + H]⁺ 1271.6309 (calcd for C₆₂H₈₇N₁₂O₁₇, 1271.6312), [M – H]⁻ 1269.6110 (calcd for C₆₂H₈₅N₁₂O₁₇, 1269.6156).
Compound 4: faint yellow amorphous powder; molecular formula C_{11}H_{13}NO_{4}; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 252 (3.21), 304 (2.95) nm; \(^{13}C\) NMR data (125 MHz, in DMSO-\(d_6\)) and \(^1H\) NMR data (500 MHz, in DMSO-\(d_6\)) see Table 1; HRESIMS m/z [M – H]– 222.0779 (calcd for C_{11}H_{12}NO_{4}, 222.0766).

Compound 5: yellow oily; molecular formula C_{13}H_{18}N_{2}O_{2}; UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 208 (4.41), 252 (4.13), 303 (3.32) nm; [\( \alpha \)]_{D}^{25} + 25.6 (c 0.10, MeOH); \(^{13}C\) NMR data (125 MHz, in DMSO-\(d_6\)) and \(^1H\) NMR data (500 MHz, in DMSO-\(d_6\)) see Table 1; HRESIMS m/z [M + H]\(^+\) 235.1443 (calcd for C_{13}H_{19}N_{2}O_{2}, 235.1447), [M + Na]\(^+\) 257.1263 (calcd for C_{13}H_{18}N_{2}NaO_{2}, 257.1266), [2M + Na]\(^+\) 491.2626 (calcd for C_{13}H_{18}N_{2}NaO_{2}, 491.2634).

3.5. Antimicrobial Assay

The micro broth dilution method, as described in the previous study \cite{14}, was used to determine the antimicrobial activity of the isolated compounds against the growth of methicillin-resistant \textit{Staphylococcus aureus} ATCC 43300, \textit{Escherichia coli} ATCC 25922, and \textit{Candida albicans}. Gentamicin (an antibiotic against both Gram-positive and Gram-negative bacteria) and amphotericin B (an antifungal drug) were used as positive control.

3.6. Tumor Cell Culture

Human glioma U251 and rat glioma C6 cells were cultured in DMEM (Dulbecco’s Modified Eagle Medium, Gibco, Grand Island, NY, USA) with 10% FBS (Fetal Bovine Serum, PAA Laboratories Inc., Toronto, ON, Canada), human glioma SHG44 in RPMI-1640 (Roswell Park Memorial Institution 1640, Gibco, Grand Island, NY, USA), and human glioma U87-MG cells in MEM (Minimum Essential Medium, Gibco, Grand Island, NY, USA). All cells were incubated at 37 °C in a humidified incubator with 5% CO\(_2\). Cells were used for experiment after the third generation.

3.7. Sulforhodamine B (SRB) Assay

The previously described SRB assay \cite{10,11,15} was applied to evaluate the activity of isolated compounds to inhibit the proliferation of different glioma cell lines. Doxorubicin was used as a positive control. Briefly, glioma cells were treated with different concentrations of tested compounds after cells adhesion for 24 h. After 72 h of the treatment, compound-treated cells were fixed with 50 \( \mu\)L of 10% cold TCA solution at 4 °C for 1 h, washed with distilled water five times, and then dried at room temperature. The dried cells were stained with 50 \( \mu\)L of 0.4% SRB for ten minutes and rinsed with 1% acetic acid solution five times. After being dried, dye was dissolved in 10 mM Tris buffer and measured at 515 nm on a microplate reader (Bio-Tech, Winooski, VT, USA).

3.8. Western Blot Analysis

A Western blot was used to determine the expression levels of tumor metabolic regulators. The detailed procedures, including protein sample preparation, determination of protein concentration, and a Western blot analysis was referred to a previous publication \cite{12}.

4. Conclusions

The bioactive metabolite-produced \textit{Streptomyces} sp. strain, ZZ338, was isolated from sea squirts. This isolated actinomycete, cultured in two different media, produced known actinomycins D (1), V (2), X\(_{0B}\) (3) and new compounds of 2-acetylamino-3-hydroxyl-4-methyl-benzoic acid methyl ester (4) and N-15S-(4-methylaminophenylmethyl)-2-oxo-propyl acetamide (5). The three actinomycins significantly inhibited the growth of both bacteria and fungi and the proliferation of different glioma cell lines; and are the components responsible for the activity of the crude extract of strain ZZ338. Interestingly, actinomycins D (1) was found to downregulate several glioma metabolic enzymes of different metabolic pathways (glycolysis, glutaminolysis, and lipogenesis), suggesting that targeting multiple glioma metabolic regulators might be a new antitumor mechanism of actinomycin D. To the
best of our knowledge, such a possible mechanism for this class of actinomycins is the first report herein. Additionally, for the first time, the class of new compound 5 is found from natural resources.

**Supplementary Materials:** The following are available online at www.mdpi.com/1660-3397/14/10/181/s1, NMR and HRESIMS spectra (Figures S1–S95) of compounds 1–5 as well as other supporting data.

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**Author Contributions:** X.Z. performed the isolation and culture of strain ZZ338 and the isolation and structural elucidation of compounds; W.C. and X.Y. conducted the bioactive assay; X.Y.L. and Z.Z. designed the experiments and wrote the paper.

**Conflicts of Interest:** The authors declare no conflict of interest.

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