

Characterization of *Streptomyces* sp. LS462 with high productivity of echinomycin, a potent antituberculosis and synergistic antifungal antibiotic

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Abstract: A biologically active microbial strain, designated as “LS462,” was isolated from a soil sample collected from Yaoli Virgin Forest of Jiangxi Province, China. The strain was able to produce a high yield of echinomycin (172 mg/l) even under nonoptimized culture conditions and is proposed to serve as a promising source of echinomycin. In this study, echinomycin exhibited strong anti-*Mycobacterium tuberculosis* H37Rv activity and synergistic antifungal effect with a greatly reduced dosage of posaconazole on *Candida albicans* SC5314. The strain belongs to the genus *Streptomyces* according to its morphological and 16S rDNA phylogenetic analysis. The 16S rDNA was found to have the highest sequence identity with *Streptomyces fuscichromogenes* (99.37% similarity). Extensive nuclear magnetic resonance and mass spectroscopic data were used to determine the structure of echinomycin. The strain *S. fuscichromogenes* has not been previously reported to produce echinomycin. Strain LS462 may be exploited as a new potential source for the commercial production of echinomycin. Also, this work is the first to report the new synergistic antifungal activity of echinomycin and further study of the synergistic mechanism will be helpful to guide the development of antifungal agents.

Keywords: Keywords *Streptomyces fuscichromogenes*, Echinomycin, Antituberculosis, Synergistic antifungal activity, Posaconazole

Introduction

Infections by pathogenic—including multidrug-resistant—microorganisms are responsible for causing significant morbidity and mortality in health-care facilities. Invasive fungal and *Mycobacterium tuberculosis* (MTB) infections have emerged as leading causes of death in immunocompromised patients, including those with human immunodeficiency virus (HIV) infection, cancer, and bone marrow or solid organ transplant recipients (TB Alliance, 2006; Torres et al., 2005). The increasing numbers of immunocompromised patients have caused an increased number of patients who need antifungal and antituberculosis drugs. This increased drug usage has led to increasing frequencies of drug-resistant strains and new drugs or new therapeutic ways are urgently required for the battle against pathogenic infections. To meet the desired goals for discovering better drugs and improve the yield of bioactive metabolites, biodiversity- and taxonomy-guided natural product library construction (Ashforth et al., 2010), high-throughput screening (HTS) (Liu et al., 2010), and genetic engineering projects (Zhang et al., 2005; Zhuo et al., 2010) were performed as effective tools. Based on these efforts, much attention has been paid to the screening and discovery of compounds with antituberculosis and antifungal activities.

A natural product library based screening program for bioactive metabolites against *Mycobacterium bovis* BCG (BCG) gave a hit

rate of 1.2%. The BCG strain used here was transformed with green fluorescent protein (GFP) constitutive expression plasmid pUV3583 with direct readout of fluorescence for measure of bacterial growth (Cowley and Av-Gay, 2001). An extract of LS462 exhibited strong activity against BCG and was then analyzed by liquid chromatography–diode array detection–mass spectrometry. One of the main peaks showed similar UV characteristics and molecular weight (MW) to those of echinomycin. Nevertheless, the strain still attracted our attention because it showed a high ability to produce echinomycin. Further study led to the isolation of echinomycin and the confirmation of its high productivity. The pure compound was tested on MTB H37Rv antifungal and synergistic antifungal screening models. Echinomycin exhibited strong antituberculosis activity and the combination of echinomycin and posaconazole showed a potent synergistic antifungal effect on *Candida albicans* SC5314.

Echinomycin, which was first discovered from *Streptomyces echinatus* in 1957, is a cyclic depsipeptide antibiotic and has broad activities against bacteria, fungi, viruses, and tumor cells (Corbaz et al., 1957). It was reported to cause DNA damage (Waring and Wakelin 1974; Wu et al., 2018), inhibition of hypoxia-inducible factor 1 (Kong et al., 2005; Plastino et al., 2021; Vlaminck et al., 2007; Wang et al., 2020), potential FKBP12 binding effect (Singh et al., 2014), inhibition of bacterial RNA synthesis (Sato et al., 1967),

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inhibition of HIV-1 Tat transactivation (IC₅₀ 1.5 nM) (Jayasuriya et al., 2005), cell apoptosis, and cell signaling inhibition (Kim, Ahn, et al., 1996; Kim, Yang, et al., 1996). Echinomycin showed excellent antitumor activity and tolerable toxicity in preclinical and phase I trials, propelling it into phase II clinical trial for the treatment of solid cancers such as colon cancer and ovarian cancer (Chang et al., 1998; Foster et al., 1985).

In this article, we describe the *Streptomyces* sp. LS462, which produces a high yield of echinomycin, under nonoptimized culture conditions, with potent antituberculosis and synergistic antifungal activities. The characterization and the taxonomical status of the strain are also reported.

Materials and Methods

Microbial Strains

The strain LS462 was isolated from a soil sample collected from Yaoli Virgin Forest of Jiangxi Province, China. The strain was grown on a Gauze-asparagine (GA) agar slant consisting of soluble starch 2.0%, L-asparagine 0.05%, KNO₃ 0.1%, K₂HPO₄·H₂O 0.05%, NaCl 0.05%, and MgSO₄·7H₂O 0.05% (pH 7.5) at 28°C. LS462 was deposited at the China General Microbiological Culture Collection Center (CGMCC) with the accession number CGMCC no. 5453.

Mycobacterium phlei ATCC 11758, used as an indicator organism during the product isolation process, was grown on an agar plate consisting of peptone 1.0%, malt extract 0.5%, yeast extract 0.5%, casein acid hydrolysate 0.5%, beef extract 0.2%, glycerol 0.2%, Tween 80 0.005%, MgSO₄·7H₂O 0.1%, and agar 1.2%, with pH 7.2 at 37°C.

Mycobacterium bovis BCG (Pasteur 1173P2) and *M. tuberculosis* H37Rv (ATCC 27294) were used here for bioactivity screening and assays.

Candida albicans SC5314 (ATCC MYA-2876) grown on a YPD agar plate (yeast extract 1.0%, peptone 2.0%, glucose 2.0%, and agar 2.0%) at 35°C was used for antifungal and synergistic antifungal assays.

Compounds for Bioassays

Posaconazole (Selleck Chemicals Co. Ltd. Houston, TX, USA), ketoconazole (Sigma Chemical Co., St. Louis, MO, USA), fluconazole (Sigma Chemical Co., St. Louis, MO, USA), econazole (Sigma Chemical Co., St. Louis, MO, USA), miconazole (Sigma Chemical Co., St. Louis, MO, USA), itroconazole (Sigma Chemical Co., St. Louis, MO, USA), clotrimazole (Sigma Chemical Co., St. Louis, MO, USA), voriconazole (Sigma Chemical Co., St. Louis, MO, USA), amphotericin B (Sigma Chemical Co., St. Louis, MO, USA), caspofungin (Merck Research Laboratories, Rahway, NJ, USA), terbinafine (Sigma Chemical Co., St. Louis, MO, USA), and micafungin (Merck Research Laboratories, Rahway, NJ, USA) were obtained commercially.

Cultural and Morphological Properties of Strain LS462

A set of cultural and phenotypic characteristics was examined using media and the International *Streptomyces* Project (ISP) procedures recommended by Shirling and Gottlieb (1966). Mature aerial mycelium and substrate mycelium pigmentation was recorded on GA agar medium following incubation at 28°C for 20 days. Spore chain morphology and spore surface features of the isolate were observed by examining the gold-coated dehydrated specimens with a scanning electron microscope (Quanta 200).

16S rRNA Gene Sequence and Phylogenetic Analysis

Genomic DNA of LS462 was extracted as described by the TINAamp Bacteria DNA Kit (GENELib). Universal primers (27f: 5'-GAGAGTTTGATCCTGGCTCAG-3'; 1492r: 5'-CTACGGCTACCTTGTACGA-3') were used to amplify the 16S rDNA. Polymerase chain reaction (PCR) amplification (25 µl final volume: 0.4 µl, 20 µM of each primer, 2.5 µl 10× buffer [TaKaRa], 2.5 µl 2.5 nM deoxynucleotide (dNTP) (TakaRa Bio USA, Inc.), 2 U rTaq polymerase (TakaRa Bio USA, Inc.), and 1 µl DNA template) of the 16S rDNA was performed on a TaKaRa PCR Thermal Cycler with the initial denaturation at 94°C for 5 min, 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min) and elongation (72°C, 1 min, 15 s), and a final elongation at 72°C for 10 min. Multiple alignments with sequences of most closely related *Streptomyces* and calculations of levels of sequence similarity were carried out using CLUSTAL W (Thompson et al., 1994). The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) by MEGA 4.0 (Tamura et al., 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method with 1,000 replicates (Felsenstein, 1985).

Nucleotide Sequence Accession Number

The nucleotide sequence of the 16S rRNA gene reported in this article was assigned to the GenBank accession number JN621239.

Fermentation Conditions

A stock culture of the strain was grown and maintained on a GA agar slant. The stock culture was transferred into 250-ml Erlenmeyer flasks containing 40 ml of the seed medium with the same components as the agar slant medium. The culture was incubated on a rotary shaker (220 rpm) at 28°C for 96 hr. Ten milliliters of the seed culture was transferred into 1000-ml Erlenmeyer flasks containing 250 ml of the producing medium, which consisted of glucose 1.0%, millet meal 2.0%, cottonseed protein powder 2.0%, and 3-(N-morpholino)-propanesulfonic acid 2.0% (pH 7.0). The cultures were incubated at 28°C for 8 days on a rotary shaker at 220 rpm. The antibiotic activity of the fermentation broth was determined by a conventional paper-disk agar diffusion assay (Raahave, 1974) using *M. phlei* and the zones of inhibition were measured after 18 hr incubation at 37°C.

Production Analysis by HPLC

After incubation for 3 days, aliquots (2 ml) of the cultures were collected every 48 hr and prepared for high performance liquid chromatograph (HPLC) analysis as follows. After centrifugation (8000 rpm, 3 min), the mycelia were extracted 3 times with acetone to give the organic fraction. The supernatants were mixed with 0.5 ml HP-20 resins, rocked for 4 hr on a shaker, and filtrated, and the resins were washed 3 times with acetone. The acetone fractions from the mycelium and the supernatants were combined and dried to give crude extracts. Each crude extract was then redissolved in 2 ml acetone, filtered through a 0.45-µm membrane filter, and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) equipped with an Agilent XDB-C8, 5 µm column (4.6 mm × 150 mm) and 70% methanol in water as the mobile phase. The external standard method was used in the quantitative analysis with echinomycin (Alexis) as the standard and 243 nm as the detection wavelength.

Purification and Characterization of Echinomycin

The fermentation broth (4 l) was fractionated by centrifugation. The mycelium was extracted with acetone and filtered. The filtrate was diluted to 10 times volume with water and then combined with the supernatant. The mixture was charged on a column of HP-20 resins, which was washed with distilled water and then eluted with 40%, 60%, and 80% acetone, respectively. The active fraction eluted with 60% acetone was concentrated under reduced pressure. The residue was then resuspended in acetone and gave a pure active constituent (compound **1**) using preparative RP-HPLC equipped with an Eclipse XDB-C18 5 μm column (9.4 mm \times 250 mm, Agilent) and 70% MeOH in water as the mobile phase. Bioactive fractions were tracked by a paper-disk agar diffusion assay using *M. phlei*.

Compound **1** was characterized using spectroscopic analyses. UV data were detected on a Mariner System 5304 instrument. Electrospray ionization mass spectra (ESI-MS) were recorded on a Bruker Esquire 3000 plus spectrometer and various NMR spectra were recorded on a Varian Inova 500 MHz spectrometer and the solvent was CDCl_3 .

Anti-BCG and MTB Assays

The *in vitro* activities of compounds against BCG and MTB H37Rv were determined in 96-well plate format as previously described (Wang et al., 2010). BCG and MTB H37Rv were grown at 37°C to midlog phase in Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) enrichment (Becton Dickinson), 0.05% Tween 80, and 0.2% glycerol. The culture was then diluted with culture medium to a bacterial suspension with OD_{600} values of 0.025. For primary screening, aliquots (80 μl) of the bacterial suspension were added to each well of the clear flat-bottom 96-well microplates, followed by adding 2 μl of sample solutions (1 mg/ml). Isoniazid was used as the positive control and dimethyl sulfoxide (DMSO) as the negative control. The plates incubated at 37°C for 3 days for *M. bovis* BCG or 10 days for *M. tuberculosis*. GFP fluorescence was measured with a multilabel plate reader (Perkin-Elmer Envision 2103) using the bottom read mode, with excitation at 485 nm and emission at 535 nm. Hits were defined as compounds that could inhibit more than 99% of bacterial growth compared with the blank control at a concentration of 100 $\mu\text{g/ml}$ or less.

For determination of the minimum inhibitory concentrations (MICs) of the compounds, bacterial suspensions were prepared and dispensed as described earlier, and compounds were serially diluted twofold from 25 to 0.19 $\mu\text{g/ml}$ in each column. Isoniazid as a positive control (800–6.25 ng/ml) and DMSO as a negative control were also included in each plate. The plate was bottom read to record the GFP fluorescence after incubation at 37°C. MIC is defined as the minimum concentration of drug that inhibits more than 99% of bacteria growth reflected by fluorescence values. Anti-BCG and anti-MTB H37Rv assays were performed in biosafety level (BSL) 2 and 3 settings, respectively.

Antifungal and Synergistic Antifungal Assays

Candida albicans SC5314 grown on a YPD plate overnight at 35°C was picked for preparing the strain suspension with fresh RPMI 1640 medium at the concentration of 1×10^4 cells/ml.

For the antifungal assay, 2 μl of the twofold diluted samples were added to the test wells in 96-well plates, followed by an additional 80 μl of the strain suspension. The test plates were incubated at 35°C for 18 hr. The antifungal positive control was ketoconazole and antifungal MICs were determined by measuring

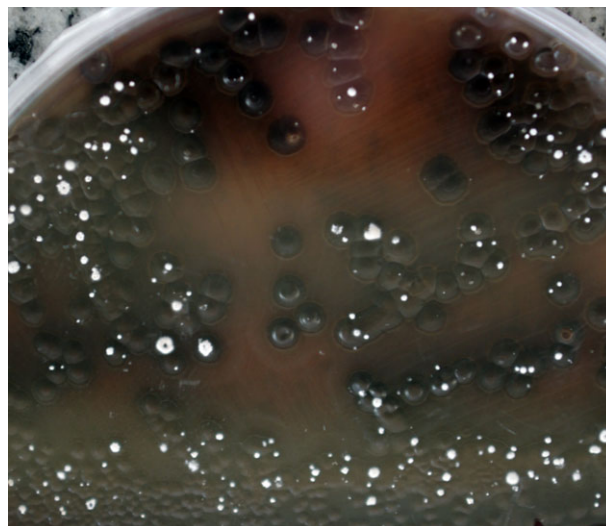


Fig. 1 Colony characteristics of LS462 grown on Gauze-asparagine (GA) agar at 28°C for 20 days.

and comparing the optical densities of the blank control and test wells.

A synergistic antifungal assay by the checkerboard method of drug combinations against *C. albicans* SC5314 was performed according to a previously described method (Orhan et al., 2005). Combinations of echinomycin and other known antifungal azole compounds (posaconazole, ketoconazole, fluconazole, econazole, miconazole, itroconazole, clotrimazole, and voriconazole), amphotericin B, caspofungin, terbinafine, and micafungin were tested, respectively, in this assay. The compounds were prepared as stock solutions in DMSO. To determine the MICs, serial twofold dilutions of echinomycin and posaconazole were prepared according to a modified protocol from the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) M-38A and M-27A2 methods. *C. albicans* SC5314 cells ($\sim 1 \times 10^4$ cells/ml) were inoculated in a final volume of 80 μl mixture of RPMI 1640 medium, and 2 μl drugs in each well of flat-bottom, 96-well microplates. The first drug of the combination, posaconazole, was serially diluted along the ordinate, while the second drug echinomycin was diluted along the abscissa. The plates were incubated at 35°C for 18 hr. MICs were determined by measuring and comparing the optical densities of the blank control (DMSO only) and tested wells. The combination of beauvericin and ketoconazole was the positive control for the synergistic antifungal assay (Zhang et al., 2007). All the experiments were tested in triplicate.

To identify whether the drug interaction was synergistic, additive, or antagonistic for the combination of echinomycin and posaconazole, the fractional inhibitory concentration index (FICI) was used: $\text{FICI} = (\text{MIC}_{\text{drug A in combination}}/\text{MIC}_{\text{drug A alone}}) + (\text{MIC}_{\text{drug B in combination}}/\text{MIC}_{\text{drug B alone}})$ (Zhang et al., 2007). The interaction was defined as synergistic if the FICI was ≤ 0.50 , additive if the FICI was 0.50–4.0, and antagonistic if the FICI was ≥ 4.0 .

Results

Characterization and Identification of the Isolated Strain LS462

The crude extract of strain LS462 with strong anti-BCG activity and high productivity of echinomycin led to further investigation of the strain. The colonial and morphological properties (Fig. 1) suggested that LS462 is a member of the genus *Strepto-*



Fig. 2 Scanning electron micrograph of LS462 grown on Gauze-asparagine (GA) agar at 28°C for 20 days. Bar, 5 μ m.

myces. Sporulation occurred on inorganic salt-starch agar such as GA agar after 15 days at 28°C. LS462 formed a long, straight substrate mycelium and aerial hyphae with few branches. Also, brown pigments were produced on GA agar. The straight or curving spore chains that occurred on GA agar were composed of oval- to cylindrical-like spores (0.5–0.7 μ m \times 1.1–1.3 μ m) with spinous surfaces (Fig. 2). The determination of the 16S rRNA gene sequence (1447 nt) of strain LS462 and phylogenetic analysis revealed that it was most closely related to *Streptomyces fuscichromogenes* KC771428.1 with 99.37% 16S rRNA gene similarities (Fig. 3).

Production of Echinomycin

The production of echinomycin reached maximum (172 mg/L) after 7 days of fermentation and declined afterward (Fig. 4). The mycelium contains a large quantity of the total echinomycin production.

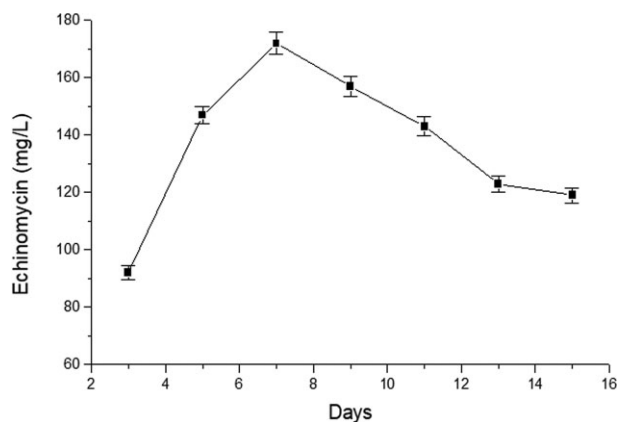


Fig. 4 Time course production of echinomycin by strain LS462 cultured in producing medium. Results represent the means \pm standard errors of the means (SEMs) of three independent experiments.

Purification and Characterization of Echinomycin

The 60% acetone fraction (Fig. 5) from the HP-20 resin column showed strong activity against *M. phlei* by paper-disk agar diffusion assay. The fraction was selected for further studies, and led to the discovery of compound **1**. The structure of compound **1** (Fig. 6) was elucidated by UV, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and MS analysis as well as by comparison with previously reported data of echinomycin (Li, Zhang, et al., 2004; Shi et al., 1999). Compound **1** showed a typical UV spectrum (Fig. 5) with maximal absorbance at 243 and 323 nm, similar to that of echinomycin. ESI-MS (Supplementary Fig. S1) exhibited quasi-molecular ion peaks at m/z 1101.3 $[\text{M} + \text{H}]^+$ and 1123.3 $[\text{M} + \text{Na}]^+$ for compound **1**, indicating an MW of 1100, which is consistent with the MW of echinomycin. The $^{13}\text{C-NMR}$ spectrum of **1** revealed the presence of 51 carbon atoms (Supplementary Table S1). The sp^2 carbon region contained 10 carbonyls and 16 aromatic carbon atoms, 8 of which connected to nitrogen atoms. The aliphatic region contained 6 methyls, 2 methylenes, 8 methines, 4 methyls, and 1 methylene connected to nitrogen atoms, 1 methyl and 1 methylene connected to a sulfur atom, and 1 methine connected to 2 sulfur atoms. All the physical and spectral data, including $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and MS, were in agreement with the respective published data (Dell et al., 1975;

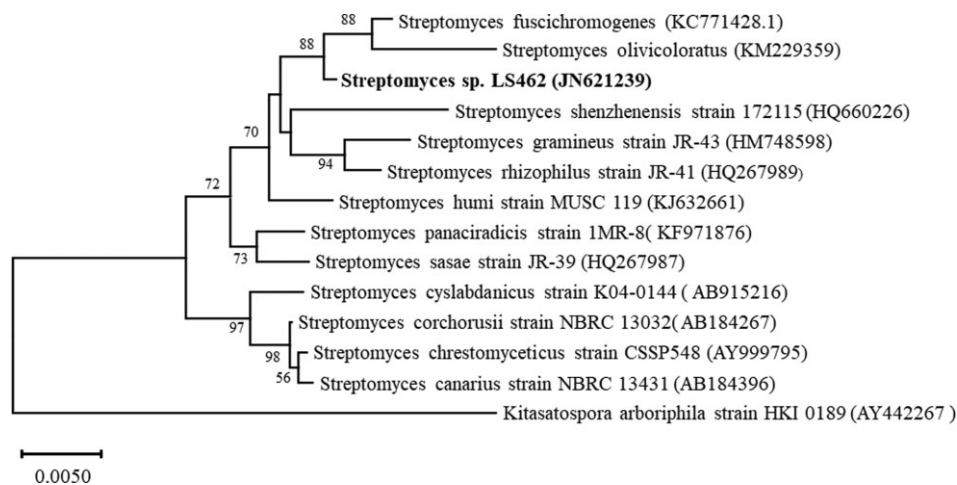


Fig. 3 Neighbor-joining phylogenetic tree of strain LS462 based on 16S rRNA gene sequence generated using Mega4.0. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbor-joining analysis of 1,000 resampled datasets; only values $>50\%$ are given. National Center for Biotechnology Information (NCBI) accession numbers are given in parentheses. Bar, 0.005 nucleotide substitutions per site. *Kitasatospora arboriphila* HK1 0189^T (AY442267) was chosen as the outgroup strain.

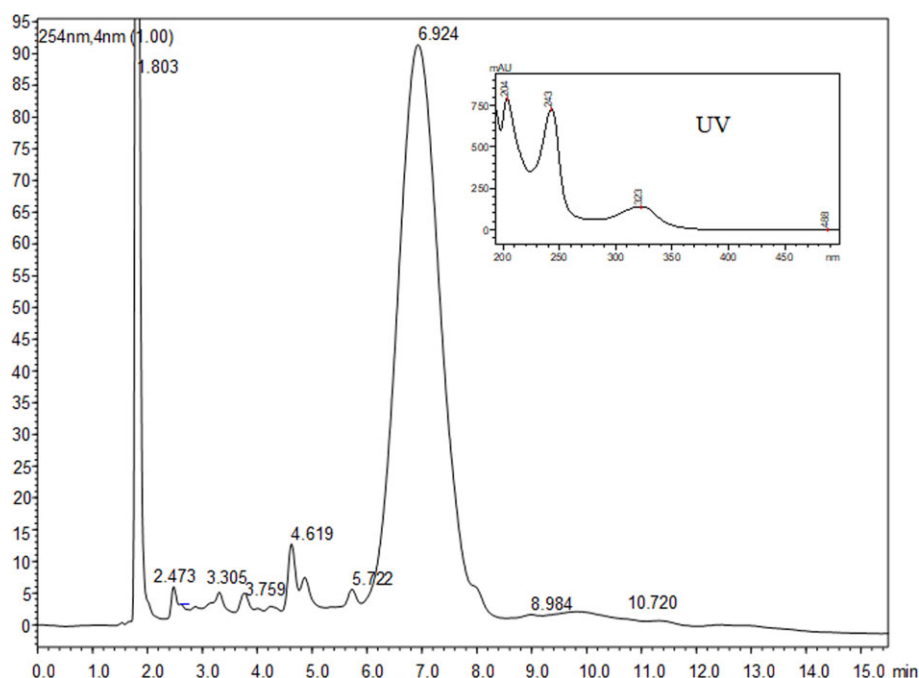


Fig. 5 HPLC analysis of crude extracts of LS462 cultured in MPG medium. Extracts were analyzed using Shimadzu 20A HPLC with an Agilent XDB-C8 column (5 μ m, 4.6 mm \times 150 mm), 70% methanol as the mobile phase, 1.0 ml/min as the flow rate, 254 nm as the detection wavelength.

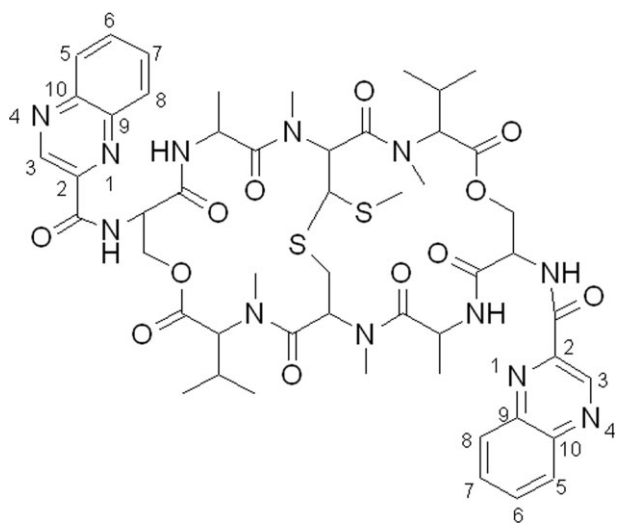


Fig. 6 The structure of compound 1.

Li, Zhang, et al., 2004; Shi et al., 1999), confirming that compound 1 is echinomycin.

Anti-BCG and Anti-TB Activity of Echinomycin

Compound 1 exhibited potent activities against BCG and MTB H37Rv, with MIC values of 0.1 and 0.5 μ g/ml, respectively.

Antifungal and Synergistic Antifungal Activity of Echinomycin

A batch of pure compounds was screened for finding synergistic antifungal agents. Echinomycin was included in this assay and exhibited antifungal activity (6.25 μ g/ml) and synergistic antifungal activity with a greatly reduced dosage (to 60-fold) of posaconazole on *C. albicans* 5314. Table 1 shows the respective MIC values for the synergistic effect of the combination of posaconazole and echinomycin. Each combination caused 100% inhibition of *C. albicans* 5304 cells.

Table 1 Synergistic Antifungal Screening Results

Posaconazole	MIC values for synergistic activity (μ g/ml)	
	Echinomycin	FICI
0.008	0	—
0.004	0.003125	0.5005
0.002	0.025	0.2540
0.001	0.05	0.1330
0.0005	0.1	0.0785
0.00025	0.1	0.0473
0.000125	0.2	0.0476
0	6.25	—

Note. FICI = fractional inhibitory concentration index. $FICI = (MIC_{\text{drug A in combination}}/MIC_{\text{drug A alone}}) + (MIC_{\text{drug B in combination}}/MIC_{\text{drug B alone}})$.

When the concentrations of posaconazole and echinomycin were 0.00025 and 0.10 μ g/ml, respectively, the combination of the two compounds exhibited the strongest synergistic activity (FICI 0.0473, far less than 0.5) on *C. albicans* 5304.

Interestingly, the synergistic activity of echinomycin was selective. For the combinations of echinomycin and the other compounds tested, no synergistic activity was observed.

Discussion

Echinomycin contains quinoxaline chromophores in its structure and acts as a bis-intercalator (Watanabe et al., 2006). It can bind to DNA by inserting two planar chromophores between the base pair of duplex DNA and place its cyclic depsipeptide backbone in the minor groove (Foster et al., 1985; Waring and Wakelin, 1974).

Echinomycin showed distinct activities against a variety of Gram-positive bacteria, including vancomycin-resistant *Enterococci* and methicillin-resistant *Staphylococcus aureus* (Kim et al., 2004; Park et al., 2008), Gram-negative bacteria (*Shigella dysenteriae*), viruses (poliomyelitis virus, HIV, and bacteriophage) (Fox et al., 1980; Jayasuriya et al., 2005; Minor and Dimmock 1977),

malarial parasite (*Plasmodium falciparum*) (Castillo et al., 2003), and thrombosis (Lee et al., 2007). It displayed excellent protection in mice against acute peritoneal infections caused by both methicillin-sensitive and methicillin-resistant *S. aureus* strains, and may be superior to vancomycin (Park et al., 2008). Our results of echinomycin against BCG and TB added a new prospect to its extensive activities and further proved that it deserves more exploration on its action mechanism.

As a bis-intercalator, echinomycin has low affinity for single-stranded RNA or DNA. It is used as a novel electrochemical redox indicator (Karadeniz et al., 2006) and a DNA fluorescent probe (Chen and Zhao, 2006). In light of its unique structural and biological properties, echinomycin has been looked at as an important tool for research in clinical, molecular, and cellular biology. A few echinomycin-producing microorganisms are known, such as *S. echinatus* (Corbaz et al., 1957) and *Actinomadura* sp. INA654 (Galatenko et al., 2006), but the productivity of these organisms is uncertain. Two *Streptomyces* sp. strains were reported to afford 2.5 mg/L (Jayasuriya et al., 2005) and 10 mg/L (Park et al., 1998) of echinomycin, respectively. The first total chemical synthesis of echinomycin was accomplished by Kojima et al. (2020). As its chemical synthesis is low yield, process complicated, environmentally unfriendly, and has a lot of byproducts that are difficult to separate, new natural sources of echinomycin would have latent applied value. To our knowledge, this is the first report of echinomycin produced by a new source and obtained with a high yield, and it is easy to isolate and purify from the fermentation broth.

The exciting potential of echinomycin has stimulated a great deal of research into its physical, chemical, and biological nature, such as the biosynthesis pathway (Hirose et al., 2011; Watanabe et al., 2006), mechanism of action, and structure–activity relationship (Socha et al., 2009). As an effective antitumor agent, echinomycin has been evaluated by phase II clinical trial from the 1990s (Brown et al., 1991; Chang et al. 1998; Gradishar et al., 1995; Hakes et al., 1990; Marshall et al., 1993; Muss et al., 1990, 1993; Schilsky et al., 1991; Shevrin et al., 1994; Taylor et al., 1990; Wadler et al., 1994). However, the *in vivo* antitumor efficacy was limited due to its high hydrophobicity and low bioavailability (Alshaer et al., 2019). A recent study suggested that echinomycin is a potent inhibitor of pancreatic cancer that targets the stem cells by inhibiting Notch signaling proteins (Ponnurangam et al., 2016). It can selectively kill the leukemia-initiating cell in relapsed acute myeloid leukemia (AML) without normal stem cell toxicity (Wang et al., 2014). Oncolmmune, a clinical-stage biopharmaceutical company, is working to develop new echinomycin formulations such as nanoliposomal echinomycin to improve its half-life and therapeutic index for treating patients with hematological malignancies, such as triple-negative breast cancer (Bailey et al., 2020). Echinomycin has received orphan drug status for AML in 2015 and for graft-versus-host disease (GVHD) in 2017 in the United States. As an inhibitor of HIF-1 DNA-binding activity, echinomycin has also been looked at as a possible therapeutic agent in ischemic diseases (Plastino et al., 2021). Studies on echinomycin in biomedical and medicinal aspects are ongoing and remain active. New natural sources of echinomycin would be valuable for industrial production of the antibiotic to fulfill the requirements of medicinal application and biological studies.

Posaconazole is a potent, novel, broad-spectrum triazole drug and is clinically used to treat invasive infections by *Candida* species (Li, Brown, et al., 2004), *Mucor*, and *Aspergillus* species (Cacciapuoti et al., 2000) in severely immunocompromised patients. It was considered to be superior to other triazoles, such as

fluconazole and itraconazole, in the prevention of invasive fungal infections (Cacciapuoti et al., 2000) and was suggested to be the most effective treatment for both chronic and acute Chagas disease, showing much better efficacy than benznidazole (Pinazo et al., 2010). As an effective chemotherapeutic drug, posaconazole is very expensive. Moreover, more and more posaconazole cross-resistance with fluconazole, itraconazole, or both, was found in some *Candida* isolates and *Aspergillus fumigatus* isolates (Torres et al., 2005). So, the promising combination of posaconazole and echinomycin may not only lighten patients' financial burden, but also reduce opportunities for the emergence of drug resistance and lower the side effects of posaconazole. This new finding will lead to further study of the selectively synergistic mechanism, which will be helpful to find new actions of echinomycin and guide the development of antifungal agents.

Supplementary Material

Supplementary material is available online at JIMB (www.academic.oup.com/jimb).

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Conflict of Interest

The authors declare no conflict of interest.

References

- Alshaer, W., Zraikat, M., Amer, A., Nsairat, H., Lafi, Z., Alqudah, D. A., Qadi, E. A., Alsheleh, T., Odeh, F., Alkaraki, A., Zihlif, M., Bustanji, Y., Fattal, E., & Awidi, A. (2019). Encapsulation of echinomycin in cyclodextrin inclusion complexes into liposomes: *in vitro* anti-proliferative and anti-invasive activity in glioblastoma. *RSC Advances*, 9, 30976–30988. <https://doi.org/10.1039/c9ra05636j>.
- Ashforth, E. J., Fu, C. Z., Liu, X. Y., Dai, H. Q., Song, F. H., Guo, H., & Zhang, L. X. (2010). Bioprospecting for antituberculosis leads from microbial metabolites. *Natural Product Reports*, 27, 1709–1719. <https://doi.org/10.1039/c0np00008f>.
- Bailey, C. M., Liu, Y., Peng, G., Zhang, H., He, M., Sun, D., Zheng, P., Liu, Y., & Wang, Y. (2020). Liposomal formulation of HIF-1 α inhibitor echinomycin eliminates established metastases of triple-negative breast cancer. *Nanomedicine: Nanotechnology, Biology and Medicine*, 29, 102278. <https://doi.org/10.1016/j.nano.2020.102278>.

- Brown, T. D., Goodman, P. J., Fleming, T. R., Taylor, S. A., & Macdonald, J. S. (1991). Phase II trial of echinomycin in advanced colorectal cancer. A Southwest Oncology Group study. *Investigational New Drugs*, 9, 113–114. <https://doi.org/10.1007/BF00194561>.
- Cacciapuoti, A., Loebenberg, D., Corcoran, E., Menzel, F., Moss, E. L., Norris, C., Michalski, M., Raynor, K., Halpern, J., Mendrick, C., Arnold, B., Antonacci, B., Parmegiani, R., Yarosh-Tomaine, T., Miller, G. H., & Hare, P. S. (2000). In vitro and in vivo activities of SCH 56592 (posaconazole), a new triazole antifungal agent, against *Aspergillus* and *Candida*. *Antimicrobial Agents and Chemotherapy*, 44, 2017–2022. <https://doi.org/10.1128/AAC.44.8.2017-2022.2000>.
- Castillo, U., Harper, J. K., Strobel, G. A., Sears, J., Alesi, K., Ford, E., Lin, J., Hunter, M., Maranta, M., Ge, H., Yaver, D., Jensen, J. B., Porter, H., Robison, R., Millar, D., Hess, W. M., Condrón, M., & Teplow, D. (2003). Kakadumycins, novel antibiotics from *Streptomyces* sp. NRRL 30566, and endophyte of *Grevillea pteridifolia*. *FEMS Microbiology Letters*, 224, 183–190. [https://doi.org/10.1016/S0378-1097\(03\)00426-9](https://doi.org/10.1016/S0378-1097(03)00426-9).
- Chang, A. Y., Kim, K., Boucher, H., Bonomi, P., Stewart, J. A., Karp, D. D., & Blum, R. H. (1998). A randomized phase II trial of echinomycin, trimetrexate, and cisplatin plus etoposide in patients with metastatic nonsmall cell lung carcinoma: An Eastern Cooperative Oncology Group study (E1587). *Cancer*, 82, 292–300. [https://doi.org/10.1002/\(SICI\)1097-0142\(19980115\)82:2<301::AID-CNCR8>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1097-0142(19980115)82:2<301::AID-CNCR8>3.0.CO;2-T).
- Chen, N. & Zhao, S. J. (2006). Research progress of quinoxalines as antitumor antibiotics. *Chinese Journal of Practical Medicine*, 22, 2569–2570.
- Corbaz, R., Ettinger, L., Gaumann, E., Keeller-Schierlein, W., Kradolfer, F., Neipp, L., Prelog, V., Reusser, P., & Zahner, H. (1957). Metabolic products of actinomycetes. VII. Echinomycin. *Helvetica Chimica Acta*, 40, 199–204.
- Cowley, S. C. & Av-Gay, Y. (2001). Monitoring promoter activity and protein localization in *Mycobacterium* spp. using green fluorescent protein. *Gene*, 264, 225–231. [https://doi.org/10.1016/S0378-1119\(01\)00336-5](https://doi.org/10.1016/S0378-1119(01)00336-5).
- Dell, A., Williams, D. H., Morris, H. R., Smith, G. A., Feeney, J., & Roberts, G. C. K. (1975). Structure revision of the antibiotic echinomycin. *Journal of the American Chemical Society*, 97, 2497–2502. <https://doi.org/10.1021/ja00842a029>.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>.
- Foster, B. J., Clagett-Carr, K., Shoemaker, D. D., Suffness, M., Plowman, J., Trissel, L. A., Grieshaber, C. K., & Leyland-Jones, B. (1985). Echinomycin: The first bifunctional intercalating agent in clinical trials. *Investigational New Drugs*, 3, 403–410. <https://doi.org/10.1007/BF00170766>.
- Fox, K. R., Gauvreau, D., Goodwin, D. C., & Waring, M. J. (1980). Binding of quinoline analogues of echinomycin to deoxyribonucleic acid. Role of the chromophores. *Biochemical Journal*, 191, 729–742. <https://doi.org/10.1042/bj1910729>.
- Galatenko, O. A., Terekhova, L. P., Li, L. V., Malkina, N. D., Boikova, V., Zenkova, V. A., & Katrukha, G. S. (2006). Echinomycin production by *actinomadura* sp. INA654. *Antibiotiki I Khimioterapii*, 51, 3–7.
- Gradishar, W. J., Vogelzang, N. J., Kilton, L. J., Leibach, S. J., Rademaker, A. W., French, S., & Benson, A. B., 3rd. (1995). A phase II clinical trial of echinomycin in metastatic soft tissue sarcoma. An Illinois Cancer Study. *Investigational New Drugs*, 13, 171–174. <https://doi.org/10.1007/BF00872868>.
- Hakes, T., Markman, M., & Phillips, M. (1990). A phase II trial of echinomycin in metastatic cervix carcinoma. *Investigational New Drugs*, 8(3), 311–312. <https://doi.org/10.1007/BF00171844>.
- Hirose, Y., Watanabe, K., Minami, A., Nakamura, T., Oguri, H., & Oikawa, H. (2011). Involvement of common intermediate 3-hydroxy-L-kynurenine in chromophore biosynthesis of quinomycin family antibiotics. *The Journal of Antibiotics*, 64, 117–122. <https://doi.org/10.1038/ja.2010.142>.
- Jayasuriya, H., Zink, D. L., Polishook, J. D., Bills, G. F., Dombrowski, A. W., Genilloud, O., Pelaez, F. F., Herranz, L., Quamina, D., Lingham, R. B., Danzeisen, R., Graham, P. L., Tomassini, J. E., & Singh, S. B. (2005). Identification of diverse microbial metabolites as potent inhibitors of HIV-1 Tat transactivation. *Chemistry & Biodiversity*, 2, 112–122. <https://doi.org/10.1002/cbdv.200490162>.
- Karadeniz, H., Gulmez, B., Erdem, A., Jelen, F., Ozsoz, M., & Palecek, E. (2006). Echinomycin and cobalt-phenanthroline as redox indicators of DNA hybridization at gold electrodes. *Frontiers in Bioscience*, 11, 1870–1877. <https://doi.org/10.2741/1930>.
- Kong, D., Park, E. J., Stephen, A. G., Calvani, M., Cardellina, J. H., Monks, A., Fisher, R. J., Shoemaker, R. H., & Melillo, G. (2005). Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Research*, 65, 9047–9055. <https://doi.org/10.1158/0008-5472.CAN-05-1235>.
- Kim, J. B., Lee, G. S., Kim, Y. B., Kim, S. K., & Kim, Y. H. (2004). In vitro antibacterial activity of echinomycin and a novel analogue, YK2000, against vancomycin-resistant enterococci. *International Journal of Antimicrobial Agents*, 24, 613–615. <https://doi.org/10.1016/j.ijantimicag.2004.03.018>.
- Kim, S. K., Ahn, C. M., Kim, T. U., Choi, S. J., Park, Y. S., Shin, W. S., & Koh, C. M. (1996). In vitro chemosensitivity test of SK-302B on human colon carcinoma cell lines. *Archives of Pharmacal Research*, 19, 261–263. <https://doi.org/10.1007/BF02976237>.
- Kim, T. U., Yang, S. H., & Kim, S. K. (1996). Cytotoxic and dependence of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-apoptotic affects of echinomycin on murine leukemia cells. *Journal of Biochemistry and Molecular Biology*, 29, 489–492.
- Kojima, K., Yakushiji, F., Katsuyama, A., & Ichikawa, S. (2020). Total synthesis of echinomycin and its analogues. *Organic Letters*, 22, 4217–4221. <https://doi.org/10.1021/acs.orglett.0c01268>.
- Lee, Y. K., Park, J. H., Moon, T. H., Lee, D. Y., Yun, J. H., & Byun, Y. (2007). The short-term effects on restenosis and thrombosis of echinomycin-eluting stents topcoated with a hydrophobic heparin-containing polymer. *Biomaterials*, 28, 1523–1530. <https://doi.org/10.1016/j.biomaterials.2006.11.020>.
- Li, G. K., Zhang, Q., Jiang, Y., Zhu, B. Q., & Hu, H. F. (2004). Studies on compounds SIPI-1140A and B with inhibitory activity against antibiotic-resistant microbes from microorganisms. *Chinese Journal of Natural Medicines*, 2, 189–192.
- Li, X., Brown, N., Chau, A. S., Lopez-Ribot, J. L., Ruesga, M. T., Quindos, G., Mendrick, C. A., Hare, R. S., Loebenberg, D. L., DiDomenico, B., & McNicholas, P. M. (2004). Changes in susceptibility to posaconazole in clinical isolates of *Candida albicans*. *Journal of Antimicrobial Chemotherapy*, 53, 74–80. <https://doi.org/10.1093/jac/dkh027>.
- Liu, X. Y., Ashforth, E., Ren, B., Song, F. H., Dai, H. Q., Liu, M., Wang, J., Xie, Q., & Zhang, L. X. (2010). Bioprospecting microbial natural product libraries from the marine environment for drug discovery. *The Journal of Antibiotics*, 63, 415–422. <https://doi.org/10.1038/ja.2010.56>.
- Marshall, M. E., Wolf, M. K., Crawford, E. D., Taylor, S., Blumenstein, B., Flanigan, R., Meyers, F. J., Hynes, H. E., Barlogie, B., & Eisenberger, M. (1993). Phase II trial of echinomycin for the treatment of advanced renal cell carcinoma. A Southwest Oncology Group study. *Investigational New Drugs*, 11, 207–209. <https://doi.org/10.1007/BF00874157>.
- Minor, P. D. & Dimmock, N. J. (1977). Selective inhibition of influenza virus protein synthesis by inhibitors of DNA function. *Virology*, 78, 393–406. [https://doi.org/10.1016/0042-6822\(77\)90116-7](https://doi.org/10.1016/0042-6822(77)90116-7).

- Muss, H. B., Blessing, J. A., Baker, V. V., Barnhill, D. R., & Adelson, M. D. (1990). Echinomycin (NSC 526417) in advanced ovarian cancer. A phase II trial of the Gynecologic Oncology Group. *American Journal of Clinical Oncology*, 13, 299–301. <https://doi.org/10.1097/0000421-199008000-00006>.
- Muss, H. B., Blessing, J. A., & DuBeshter, B. (1993). Echinomycin in recurrent and metastatic endometrial carcinoma. A phase II trial of the Gynecologic Oncology Group. *American Journal of Clinical Oncology*, 16, 492–493. <https://doi.org/10.1097/0000421-199312000-00006>.
- Orhan, G., Bayram, A., Zer, Y., & Balci, I. (2005). Synergy tests by E test and checkerboard methods of antimicrobial combinations against *Brucella melitensis*. *Journal of Clinical Microbiology*, 43, 140–143. <https://doi.org/10.1128/JCM.43.1.140-143.2005>.
- Park, Y. S., Kim, Y. H., Kim, S. K., & Choi, S. J. (1998). A new antitumor agent: methyl sulfonium perchlorate of echinomycin. *Bioorganic & Medicinal Chemistry Letters*, 8, 731–734. [https://doi.org/10.1016/S0960-894X\(98\)00113-9](https://doi.org/10.1016/S0960-894X(98)00113-9).
- Park, Y. S., Shin, W. S., & Kim, S. K. (2008). *In vitro* and *in vivo* activities of echinomycin against clinical isolates of *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 61, 163–168. <https://doi.org/10.1093/jac/dkm421>.
- Pinazo, M. J., Espinosa, G., Gallego, M., Lopez-Chejade, P. L., Urbina, J. A., & Gascon, J. (2010). Case report: Successful treatment with posaconazole of a patient with chronic Chagas disease and systemic lupus erythematosus. *The American Journal of Tropical Medicine and Hygiene*, 82, 583–587. <https://doi.org/10.4269/ajtmh.2010.09-0620>.
- Plastino, F., Santana-Garrido, A., Pesce, N. A., Aronsson, M., Lardner, E., Mate, A., Kvant, A., Vazquez, C. M., & Andre, H. (2021). Echinomycin mitigates ocular angiogenesis by transcriptional inhibition of the hypoxia-inducible factor-1. *Experimental Eye Research*, 206, 108518. <https://doi.org/10.1016/j.exer.2021.108518>.
- Ponnurangam, S., Dandawate, P. R., Animesh, D., Tawfik, O. W., Parab, R. R., Mishra, P. D., Ranadive, P., Sharma, R., Mahajan, G., Umar, S., Weir, S. J., Sugumar, A., Jensen, R. A., Padhye, S. B., Anant, S., & Subramaniam, D. (2016). Quinomycin A targets Notch signaling pathway in pancreatic cancer stem cells. *Oncotarget*, 7, 3217–3232. doi.org/10.18632/oncotarget.6560.
- Raahave, D. (1974). Paper disk-agar diffusion assay of penicillin in the presence of streptomycin. *Antimicrobial Agents and Chemotherapy*, 6, 603–605. <https://doi.org/10.1128/AAC.6.5.603>.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
- Sato, K., Yoshida, T., & Katagiri, K. (1967). Inhibition of RNA synthesis in *E. coli* protoplasts by quinomycins A and C. *Journal of Antibiotics*, 20, 188–189.
- Schilsky, R. L., Faraggi, D., Korzun, A., Vogelzang, N., Ellerton, J., Wood, W., & Henderson, I. C. (1991). Phase II study of echinomycin in patients with advanced breast cancer: A report of cancer and leukemia group B protocol 8641. *Investigational New Drugs*, 9, 269–272. <https://doi.org/10.1007/BF00176982>.
- Shevrin, D. H., Lad, T. E., Guinan, P., Kilton, L. J., Greenburg, A., Johnson, P., Blough, R. R., & Hoyer, H. (1994). Phase II trial of echinomycin in advanced hormone-resistant prostate cancer. An Illinois Cancer Council study. *Investigational New Drugs*, 12, 65–66. <https://doi.org/10.1007/BF00873239>.
- Shi, J. J., Qi, C. Q., & Chen, W. J. (1999). The revision of ^1H , ^{13}C -NMR assignments of quinomycin A and C. *Chinese Journal of Antibiotics*, 24, 258–261.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16, 313–340.
- Singh, V., Nand, A., Chen, C., Li, Z., Li, S., Wang, S., Yang, M., Merino, A., Zhang, L., & Zhu, J. (2014). Echinomycin, a potential binder of FKBP12, shows minor effect on calcineurin activity. *Journal of Biomolecular Screening*, 19, 1275–1281. <https://doi.org/10.1177/1087057114544742>.
- Socha, A. M., Laplante, K. L., Russell, D. J., & Rowley, D. C. (2009). Structure-activity studies of echinomycin antibiotics against drug-resistant and biofilm-forming *Staphylococcus aureus* and *enterococcus faecalis*. *Bioorganic & Medicinal Chemistry Letters*, 19, 1504–1507. <https://doi.org/10.1016/j.bmcl.2009.01.010>.
- Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599. <https://doi.org/10.1093/molbev/msm092>.
- Taylor, S. A., Metch, B., Balcerzak, S. P., & Hanson, K. H. (1990). Phase II trial of echinomycin in advanced soft tissue sarcomas. A Southwest Oncology Group study. *Investigational New Drugs*, 8, 381–382. <https://doi.org/10.1007/BF00198595>.
- TB Alliance. (2006). Drug resistant TB is a growing, deadly threat. <https://www.tballiance.org/news/drug-resistant-tb-growing-deadly-threat>.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>.
- Torres, H. A., Hachem, R. Y., Chemaly, R. F., Kontoyiannis, D. P., & Raad, I. I. (2005). Posaconazole: A broad-spectrum triazole antifungal. *The Lancet Infectious Diseases*, 5, 775–785. [https://doi.org/10.1016/S1473-3099\(05\)70297-8](https://doi.org/10.1016/S1473-3099(05)70297-8).
- Vlamincik, B., Toffoli, S., Ghislain, B., Demazy, C., Raes, M., & Michiels, C. (2007). Dual effect of echinomycin on hypoxia-inducible factor-1 activity under normoxic and hypoxic conditions. *FEBS Journal*, 274, 5533–5542. <https://doi.org/10.1111/j.1742-4658.2007.06072.x>.
- Wadler, S., Tenteromano, L., Cazenave, L., Sparano, J. A., Greenwald, E. S., Rozenblit, A., Kaleya, R., & Wiernik, P. H. (1994). Phase II trial of echinomycin in patients with advanced or recurrent colorectal cancer. *Cancer Chemotherapy and Pharmacology*, 34, 266–269. <https://doi.org/10.1007/BF00685088>.
- Wang, J. F., Dai, H. Q., Wei, Y. L., Zhu, H. J., Yan, Y. M., Wang, Y. H., Long, C. L., Zhong, H. M., & Zhang, L. X. (2010). Antituberculosis agents and an inhibitor of the para-aminobenzoic acid biosynthetic pathway from *Hydnocarpus anthelmintica* seeds. *Chemistry & Biodiversity*, 7, 2046–2053. <https://doi.org/10.1002/cbdv.201000072>.
- Wang, Y., Liu, Y., Tang, F., Bernot, K. M., Schore, R., Marcucci, G., Caligiuri, M. A., Zheng, P., & Liu, Y. (2014). Echinomycin protects mice against relapsed acute myeloid leukemia without adverse effect on hematopoietic stem cells. *Blood*, 124, 1127–1135. <https://doi.org/10.1182/blood-2013-12-544221>.
- Wang, Y., Liu, Y., Bailey, C., Zhang, H., He, M., Sun, D., Zhang, P., Parkin, B., Baer, M. R., Zheng, P., Malek, S. N., & Liu, Y. (2020). Therapeutic targeting of TP53-mutated acute myeloid leukemia by inhibiting HIF-1 alpha with echinomycin. *Oncogene*, 39, 3015–3027. <https://doi.org/10.1038/s41388-020-1201-z>.
- Waring, M. J. & Wakelin, L. P. G. (1974). Echinomycin: A bifunctional intercalating antibiotic. *Nature*, 252, 653–657. <https://doi.org/10.1038/252653a0>.
- Watanabe, K., Hotta, K., Praseuth, A. P., Koketsu, K., Migita, A., Boddy, C. N., Wang, C. C. C., Oguri, H., & Oikawa, H. (2006). Total

- biosynthesis of antitumor nonribosomal peptides in *Escherichia coli*. *Nature Chemical Biology*, 2, 423–428. <https://doi.org/10.1038/nchembio803>.
- Wu, P. C., Tzeng, S. L., Chang, C. K., Kao, Y. F., Waring, M. J., & Hou, M. H. (2018). Cooperative recognition of T: T mismatch by echinomycin causes structural distortions in DNA duplex. *Nucleic Acids Research*, 46, 7396–7404. <https://doi.org/10.1093/nar/gky345>.
- Zhang, L. X., An, R., Wang, J. P., Sun, N., Zhang, S., Hu, J. C., & Kuai, J. (2005). Exploring novel bioactive compounds from marine microbes. *Current Opinion in Microbiology*, 8, 276–281. <https://doi.org/10.1016/j.mib.2005.04.008>.
- Zhang, L. X., Yan, K. Z., Zhang, Y., Huang, R., Bian, J., Zheng, C. S., Sun, H. X., Chen, Z. H., Sun, N., An, R., Min, F. G., Zhao, W. B., Zhuo, Y., You, J. L., Song, Y. J., Yu, Z. Y., Liu, Z. H., Yang, K. Q., & Gao, H., Chen, X. P. (2007). High-throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. *Proceedings of the National Academy of Sciences*, 104, 4606–4611. <https://doi.org/10.1073/pnas.0609370104>.
- Zhuo, Y., Zhang, W. Q., Chen, D. F., Gao, H., Tao, J., Liu, M., Gou, Z. X., Zhou, X. L., Wang, Q., Ye, B., Zhang, Q., Zhang, S., & Zhang, L. X. (2010). Reverse biological engineering of hrdB to enhance the production of avermectins in an industrial strain of *Streptomyces avermitilis*. *Proceedings of the National Academy of Sciences*, 107, 11250–11254. <https://doi.org/10.1073/pnas.1006085107>.