Inhibitory Effects of Diketopiperazines from Marine-Derived Streptomyces puniceus on the Isocitrate Lyase of Candida albicans

Heegyu Kim 1, Ji-Yeon Hwang 2, Jongheon Shin 2,* and Ki-Bong Oh 1,*

1 Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea; hqhqeori@snu.ac.kr
2 Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Korea; yahyah7@snu.ac.kr
* Correspondence: shinj@snu.ac.kr (J.S.); ohkibong@snu.ac.kr (K.-B.O.);
Tel.: +82-2-880-2484 (J.S.); +82-2-880-4646 (K.-B.O.)

Academic Editor: Raphaël E. Duval
Received: 16 May 2019; Accepted: 3 June 2019; Published: 4 June 2019

Abstract: The glyoxylate cycle is a sequence of anaplerotic reactions catalyzed by the key enzymes isocitrate lyase (ICL) and malate synthase, and plays an important role in the pathogenesis of microorganisms during infection. An icl-deletion mutant of Candida albicans exhibited reduced virulence in mice compared with the wild type. Five diketopiperazines, which are small and stable cyclic peptides, isolated from the marine-derived Streptomyces puniceus Act1085, were evaluated for their inhibitory effects on C. albicans ICL. The structures of these compounds were elucidated based on spectroscopic data and comparisons with previously reported data. Cyclo(L-Phe-L-Val) was identified as a potent ICL inhibitor, with a half maximal inhibitory concentration of 27 µg/mL. Based on the growth phenotype of the icl-deletion mutants and icl expression analyses, we demonstrated that cyclo(L-Phe-L-Val) inhibits the gene transcription of ICL in C. albicans under C2-carbon-utilizing conditions.

Keywords: diketopiperazine; marine actinomycete; Streptomyces puniceus; Candida albicans; isocitrate lyase

1. Introduction

The glyoxylate cycle exists in a wide range of organisms, such as archaea, bacteria, fungi, plants, and nematodes. This cycle plays roles in cell metabolic processes as an anaplerotic pathway of the tricarboxylic acid cycle catalyzed by isocitrate lyase (ICL) and malate synthase, which is a key component of the glyoxylate cycle [1]. In microorganisms this cycle is essential for the uptake and utilization of non-fermentable carbon sources, such as ethanol, acetate, and fatty acids [2]. During host infection, pathogenic microorganisms including Mycobacterium tuberculosis and C. albicans cause up-regulation of the glyoxylate cycle [3,4]. In particular, the icl gene of Candida albicans is strongly activated when cells are exposed to macrophages. In mice, an icl-deletion mutant of C. albicans was unable to utilize C2 carbon substrates and had diminished virulence compared with the wild-type strain [2,5–8]. As this cycle does not exist in mammalian cells, ICL appears to be a prospective target for the development of antifungal drugs. Numerous studies have been conducted to find a potent ICL inhibitor [9–11].

Diketopiperazines are simple cyclic peptides composed of two amino acids with conformationally constrained heterocyclic structures, which are stable to proteolysis [12]. These compounds are a relatively unexplored class of bioactive peptides. The naturally occurring bioactive diketopiperazines...
are produced by a variety of organisms, such as bacteria (Bacillus sp. and Streptomyces sp.), fungi (Alternaria alternata and Penicillium sp.), and marine sponges (e.g., Dysidea fragilis) [13]. Recently, the interest in these compounds has increased due to their various characteristics. Diketopiperazines possess a number of diverse biological properties, such as antimicrobial activity [12], antitumor activity [14], antiviral activity [15], inhibition of quorum-sensing signaling [16], plant-growth promotion [17], inhibition of carbonic anhydrases [18], and inhibition of aflatoxin production [19].

As part of our ongoing search for inhibitors of ICL, we encountered Streptomyces puniceus Act1085, isolated from marine sediment from Jeju Island, Republic of Korea [20]. In this study, an organic extract of the culture broth demonstrated inhibitory activity toward C. albicans ICL. The subsequent activity-guided separation of the organic extract led to the isolation of five diketopiperazines. Although these diketopiperazines have been reported to possess biological properties, such as antimicrobial, antitumor, anti-inflammatory, and neuroprotective activities [21–23], the inhibitory activity of the isolated compounds toward ICL has not yet been explored. Thus, we investigated the potential use of these compounds as inhibitors of C. albicans ICL.

2. Results

2.1. Isolation and Structural Elucidation of Diketopiperazines

The Act1085 strain was cultured for seven days in MTYB medium and fractionated using equal volumes of n-hexane, ethylacetate (EtOAc), and n-butanol. Based on the results of the C. albicans ICL activity assay, the EtOAc fraction was separated by reverse-phase C18 vacuum flash chromatography and preparative high-performance liquid chromatography (HPLC) to yield five compounds. Using combined spectroscopic analyses, including ¹H, ¹³C nuclear magnetic resonance (NMR), 2D NMR spectral analyses (COSY, HMQC, and HMBC), and UV data, the isolated compounds were identified as diketopiperazines: cyclo(L-Phe-L-Pro) [24], cyclo(L-Pro-L-Leu) [25], cyclo(L-Pro-L-Tyr) [24], cyclo(L-Pro-L-Val) [25], and cyclo(L-Phe-L-Val) [26] (Figure 1). The spectroscopic data obtained from the isolated compounds are consistent with previously reported values.

![Figure 1. Structures of diketopiperazines isolated from S. puniceus Act1085.](image)

2.2. ICL Inhibitory Activity and Antifungal Activity of Diketopiperazines

Isolated diketopiperazines were tested for ICL inhibitory activity according to methods reported previously [27]. The inhibitory concentrations (IC₅₀) values of the isolated compounds are shown in Table 1. Of the isolated diketopiperazines, cyclo(L-Pro-L-Leu) and cyclo(L-Pro-L-Val) demonstrated weak inhibitory activity toward the ICL enzyme, with IC₅₀ values of 533.79 µM and 516.28 µM, respectively (Table 1). Cyclo(L-Phe-L-Val) exhibited the strongest inhibitory activity of the test compounds but demonstrated inhibitory potency that was less than that of 3-nitropropionate, with IC₅₀ values of 109.50 µM and 15.95 µM, respectively (Figure 2a). The others exhibited no inhibitory
activity. To determine the type of inhibition, kinetic analysis was performed with cyclo(L-Phe-L-Val) (inhibitor) and phenylhydrazine (substrate). The inhibitor constant (Ki) was calculated from the Dixon plot. The results showed that cyclo(L-Phe-L-Val) behaved as a mixed inhibitor, with a K_i value of 64.86 µM (Figure 2b). Fungal growth inhibition tests indicated that diketopiperazines at a concentration of 256 µg/mL did not exhibit inhibitory effects on SC5314 cultured in glucose (Table 1).

Table 1. Inhibitory activity of isolated diketopiperazines toward the ICL enzyme and growth of C. albicans SC5314. 3-Nitropropionate was used as a reference inhibitor of ICL. Amphotericin B was used as a standard antifungal drug.

| Compound             | ICL IC_{50}, µM (µg/mL) | MIC (µg/mL) | Glucose |
|----------------------|--------------------------|-------------|---------|
| Cyclo(L-Phe-L-Val)   | 109.50 ± 4.17 (27.74 ± 2.24) | >256       |
| Cyclo(L-Pro-L-Val)   | 516.28 ± 9.18 (101.32 ± 4.22) | >256       |
| Cyclo(L-Pro-L-Leu)   | 533.79 ± 3.12 (112.24 ± 1.94) | >256       |
| Cyclo(L-Phe-L-Pro)   | >1048.75 (>256)           | >256       |
| Cyclo(L-Pro-L-Tyr)   | >984.16 (>256)            | >256       |
| 3-Nitropropionate    | 15.94 ± 2.13 (1.90 ± 1.57) | >256       |
| Amphotericin B       | ND 1                     | 1          |

1 ND means not determined.

Figure 2. (A) Comparison of the dose-dependent curves of the cyclo(L-Phe-L-Val) (test compound) and 3-nitropropionate (positive control) inhibitory activity toward the ICL enzyme from C. albicans ATCC10231. The data were analyzed using non-linear regression curve fitting in GraphPad software ver. 8.0 (Prism). The vertical bars indicate the standard errors (n = 3). (B) Lineweaver-Burk plot for ICL inhibition in the presence of cyclo(L-Phe-L-Val). S and V represent the substrate concentration (mM) and reaction velocity (ΔA_{324nm}/min), respectively. Each data point represents the mean of three experiments.
2.3. Inhibition of C₂ Substrate Utilization

The glyoxylate cycle is necessary for virulence in C. albicans, which can survive in macrophages under abundant carbon sources, such as fatty acids or their breakdown products [5,8]. When C. albicans is phagocytosed by a macrophage, the shift in metabolism from glycolysis to the glyoxylate cycle is activated so that the cells can utilize C₂ carbon sources. It was expected that ICL inhibitors would reduce the nutrient uptake capacity and impede survival of the pathogen in the macrophage. To determine whether cyclo(L-Phe-L-Val) affects C₂ substrate use, C. albicans strains SC5314, ATCC10231, ATCC10259, ATCC11006, and ATCC18804 were grown in YNB liquid broth containing either 2% glucose or 2% acetate as the sole carbon source. Cyclo(L-Phe-L-Val) exhibited a potent inhibitory effect on C. albicans in acetate (minimum inhibitory concentration of 32–64 µg/mL) but no inhibitory effect on C. albicans in glucose (Table 2). These results demonstrate that cyclo(L-Phe-L-Val) affects ICL-mediated proliferation of the fungus under C₂-carbon-utilizing conditions.

Table 2. Effect of cyclo(L-Phe-L-Val) on C. albicans strains grown in glucose or acetate as sole carbon source. C. albicans cells (1 × 10⁴ cells/mL) were incubated with varying concentrations of cyclo(L-Phe-L-Val) for 72 h at 28 °C in YNB medium containing 2% glucose or 2% potassium acetate.

| Strain    | Glucose | Acetate |
|-----------|---------|---------|
|           | Cyclo(L-Phe-L-Val) | Amph B ¹ | Cyclo(L-Phe-L-Val) | Amph B ¹ |
| SC5314    | >256    | 1       | 32               | 0.5      |
| ATCC10231 | >256    | 1       | 32               | 0.5      |
| ATCC10259 | >256    | 1       | 64               | 0.5      |
| ATCC11006 | >256    | 0.5     | 32               | 0.5      |
| ATCC18804 | >256    | 1       | 64               | 0.5      |

¹ Amphotericin B (Amph B) was used as a standard antifungal drug.

2.4. Effects of Cyclo(L-Phe-L-Val) on Growth Phenotype and icl Expression

To determine whether the cell phenotype of the icl-deletion mutant is affected by the presence of cyclo(L-Phe-L-Val), a growth assay was conducted using C. albicans SC5314 (wild type) and two icl-deletion mutants (MRC10 and MRC11). After pre-culture, these strains were streaked onto YNB agar containing 2% glucose or 2% potassium acetate with or without 32 µg/mL cyclo(L-Phe-L-Val). All strains grew normally on both the plates with glucose and with glucose plus the compound. However, MRC10 did not grow when acetate was the sole carbon source. Furthermore, none of the tested strains exhibited growth on the YNB agar plate with cyclo(L-Phe-L-Val) (Figure 3a). We further conducted semi-quantitative reverse-transcription (RT)-PCR to assess the effects of cyclo(L-Phe-L-Val) on ICL expression. No ICL-specific PCR product was detected in the cultures when SC5314 and MRC11 were grown in YNB liquid broth containing glucose. However, icl was strongly induced when these cells were cultured in YNB broth containing acetate. The intensity of the PCR band corresponding to the icl product decreased with increasing cyclo(L-Phe-L-Val) concentrations in the cells grown under icl expression conditions (Figure 3b). GPDH expression was detected in all treatments regardless of cyclo(L-Phe-L-Val) exposure. These results indicate that cyclo(L-Phe-L-Val) inhibits icl expression in C. albicans under C₂-carbon-utilizing conditions.
The glyoxylate cycle, which is an anaplerotic pathway of the tricarboxylic acid cycle, is well documented in prokaryotes and eukaryotes. Diverse pathogenic fungi utilize the glyoxylate cycle during host infection. The function of this cycle has been confirmed by the analysis of mutant pathogenic microorganisms deficient in ICL and malate synthase, which are key components of the glyoxylate cycle. Research on candidiasis in mice has revealed that C. albicans, the most serious pathogenic fungus in humans, requires ICL to be fully virulent. As the glyoxylate cycle does not exist in mammalian cells, ICL is a promising target for antimicrobial agents. Therefore, we are interested in identifying an ICL inhibitor derived from natural products. In this study, five diketopiperazine compounds were isolated from the marine-derived S. puniceus, and their structures and inhibitory activities toward the C. albicans ICL enzyme were evaluated. Our work concluded that cyclo(L-Phe-L-Val) possesses ICL inhibitory activity. Analyses of the phenotype of icl-deletion mutants by growth assays and semi-quantitative RT-PCR demonstrated that this compound inhibits ICL expression in C. albicans under C2-carbon-utilizing conditions.

Figure 3. Analysis of growth phenotypes and icl mRNA expression. (A) C. albicans SC5314 (wild-type), MRC10 (Δicl), and MRC11 (Δicl + ICL) were cultured on YNB agar plates containing the indicated carbon source (2% glucose or 2% potassium acetate) with or without 32 µg/mL cyclo(L-Phe-L-Val) for 2 days at 28 °C. (B) Strains were grown until the mid-log phase in minimal YNB liquid medium containing 2% glucose. Cells were collected by centrifugation and transferred to the same medium containing 2% glucose (Glu), 2% potassium acetate (Ace), or 2% potassium acetate (Ace) plus cyclo(L-Phe-L-Val) (C1: 8 µg/mL; C2: 16 µg/mL; and C3: 32 µg/mL) and cultured for 4 h at 28 °C. Total RNA was prepared from these cells, and icl mRNA expression was analyzed by semi-quantitative RT-PCR. The GPDH housekeeping gene was evaluated as a loading control.

3. Discussion

The glyoxylate cycle, which is an anaplerotic pathway of the tricarboxylic acid cycle, is well documented in prokaryotes and eukaryotes. Diverse pathogenic fungi utilize the glyoxylate cycle during host infection. The function of this cycle has been confirmed by the analysis of mutant pathogenic microorganisms deficient in ICL and malate synthase, which are key components of the glyoxylate cycle. Research on candidiasis in mice has revealed that C. albicans, the most serious pathogenic fungus in humans, requires ICL to be fully virulent. As the glyoxylate cycle does not exist in mammalian cells, ICL is a promising target for antimicrobial agents. Therefore, we are interested in identifying an ICL inhibitor derived from natural products. In this study, five diketopiperazine compounds were isolated from the marine-derived S. puniceus Act1085, and their structures and inhibitory activities toward the C. albicans ICL enzyme were evaluated. Our work concluded that cyclo(L-Phe-L-Val) possesses ICL inhibitory activity. Analyses of the phenotype of icl-deletion mutants by growth assays and semi-quantitative RT-PCR demonstrated that this compound inhibits ICL expression in C. albicans under C2-carbon-utilizing conditions.
Diketopiperazines possess various biological properties [12,14–19]. Many research groups have focused on the pharmacological potential of diketopiperazines due to their advantages in medical chemistry, such as stability against proteolysis, mimicry of peptidic pharmacophoric groups, conformational rigidity, substituent group stereochemistry, and the existence of donor and acceptor groups for hydrogen bonding (which favor interactions with targets) [28]. Furthermore, diketopiperazines can be easily synthesized and isolated from natural products. In this study, five diketopiperazines, cyclo(L-Phe-L-Pro), cyclo(L-Pro-L-Leu), cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val), and cyclo(L-Phe-L-Val), were obtained from a marine actinomycete, *S. puniceus*, using activity-guided separation processes. Among these diketopiperazines, cyclo(L-Pro-L-Leu), cyclo(L-Pro-L-Val), and cyclo(L-Phe-L-Val) exhibited ICL inhibitory activity, with IC$_{50}$ values in the range of 27–112 µg/mL (Table 1). The structures of the isolated diketopiperazines exhibiting ICL inhibition activity contained an isopropyl moiety, which suggests that the isopropyl moiety plays a role in the inhibition of ICL activity.

In *M. tuberculosis*, a bacterium, and *C. albicans*, a fungus, virulence led to expression of *icl*, which encodes a component of the glyoxylate cycle during persistent infection of macrophages [5,8]. These findings indicate that inhibitors of the glyoxylate cycle may reduce nutrient uptake capacity, leading to the death of these pathogens in macrophages. Thus, the effect of cyclo(L-Phe-L-Val) on *C. albicans* ICL was investigated by analyzing the growth phenotype and *icl* transcript levels in *C. albicans* SC5314 (wild-type) and *icl*-deletion mutants (MRC10 and MRC11) [8]. The growth assay revealed that cyclo(L-Phe-L-Val) specifically inhibits the ICL enzyme, because no growth of SC5314 or MRC11 was observed on the YNB agar plates containing acetate plus 32 µg/mL of the compound. Moreover, *icl* transcript levels declined as a result of treatment with cyclo(L-Phe-L-Val). Overall, this research uncovered a compound of the diketopiperazine class as a promising antifungal agent that acts by suppressing *C. albicans* pathogenicity.

4. Materials and Methods

4.1. General Experimental Procedure

The NMR instruments and mass spectrometry equipment have been described in a previous report [29]. HPLC analysis was conducted using the Shimadzu SCL-10A (Shimadzu, Tokyo, Japan) control system connected to the RID-10A refractive index detector (Shimadzu) and UV-Vis SPD-10A detector (Shimadzu). All organic solvents were of analytical reagent grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

4.2. Bacterial and Fungal Strains

*S. puniceus* Act1085 was used as the diketopiperazine-producing strain. *C. albicans* SC5314 (ATCC MYA-2876) (wild-type), MRC10 (∆*icl*) (icl-deletion mutant), MRC11 (∆*icl* + ICL) (icl-deletion mutant), ATCC10261, ATCC18804, and ATCC11006 were used for the growth assay. *C. albicans* ATCC10231 was used to obtain recombinant ICL. Prof. Michael C. Lorenz (University of Texas Health Science Center, Houston, TX, USA) kindly provided the ICL mutants [8].

4.3. Fermentation and Isolation of Diketopiperazines

*S. puniceus* Act1085 was streaked onto GTYB agar plates and incubated at 28 °C for 5 days. The composition of the GTYB medium was 2 g/L tryptone, 1 g/L yeast extract, 1 g/L beef extract, 16 g/L agar, and 10 g/L glucose. The colonies were brown in color and produced white spores. Spores of Act1085 were cultivated in 2 L flasks containing 300 mL MTRYB broth for fermentation. The MTRYB medium composition was 2 g/L tryptone, 1 g/L yeast extract, 1 g/L beef extract, and 10 g/L mannitol. After cultivation for 1 week under static conditions at 28 °C, the culture broth (40 L) was filtered through filter paper to remove mycelia, and the filtered culture broth was sequentially fractionated using equal volumes (80 L) of n-hexane, EtOAc, and n-butanol to obtain 146 mg of n-hexane extract, 1.7 g of EtOAc extract, and 3.9 g of n-butanol extract. Based on the results of the ICL activity assay...
(IC₅₀ values of n-hexane, EtOAc, and n-butanol extracts were >256 µg/mL, 47 µg/mL, and >256 µg/mL, respectively), the EtOAc extract (1.7 g) was selected for further separation experiments. The EtOAc extract was subjected to reverse-phase flash column chromatography using a column packed with YMC Gel ODS-A (S-75 µm) and eluted with MeOH-H₂O (gradient from 0:100 to 100:0), acetone, and EtOAc to produce seven fractions. Based on the bioactivity analyses, the H₂O-MeOH (60:40, 325 mg) fraction was isolated and purified by semi-preparative reverse-phase HPLC (Agilient C₁₈ column, 10.0 x 250 mm; 2 mL/min flow rate; UV 254, 365 nm detection; 40:60 H₂O-MeOH in 50 min) to yield five diketopiperazines: cyclo(L-Phe-L-Pro) (tᵣ = 8.3 min, 7.2 mg), cyclo(L-Pro-L-Val) (tᵣ = 10.7 min, 5.2 mg), cyclo(L-Pro-L-Leu) (tᵣ = 21.3 min, 10.3 mg), cyclo(L-Pro-L-Tyr) (tᵣ = 28.5 min, 7.4 mg), and cyclo(L-Phe-L-Val) (tᵣ = 37.2 min, 6.4 mg).

4.4. ICL Inhibition Assay

Before evaluation of the ICL inhibitory activity of the test compounds, the recombinant ICL from C. albicans was prepared according to methods described in a previous report [27]. The ICL inhibitory activity of the test compounds was determined according to a previously described method [30,31]. The ICL inhibition assay is based on the principle that glyoxylate phenylhydrazone forms in the reaction reagent after treatment with isocitrate and phenylhydrazine. Each test compound was dissolved in DMSO. The reaction mixture consisted of 20 mM sodium phosphate buffer (pH 7.0), 1.27 mM threo-DL(+)-isocitrate, 3.75 mM MgCl₂, 4.1 mM phenylhydrazine, and 2.5 µg/mL purified ICL and was incubated with or without the test compounds at a concentration range of 1–128 µg/mL at 37 °C for 30 min. The increase in intensity of absorbance resulting from the formation of glyoxylate phenylhydrazone was observed using a spectrophotometer (Shimadzu) at a wavelength of 324 nm. The inhibitory activity of the test compound was calculated relative to that of the DMSO control (n = 3).

The methods for determination of the IC₅₀ value and ICL enzyme concentrations have been described in previous reports [29]. A known ICL inhibitor, 3-nitropropionate, was used as a reference control [32].

4.5. In Vitro Growth Assay

C. albicans strains were cultured in YNB broth containing 2% glucose at 28 °C for 24 h, centrifuged at 15,000 × g for 1 min, and washed twice with sterile distilled water. Each test compound was dissolved in DMSO and diluted with YNB broth containing 2% glucose or 2% potassium acetate at concentrations ranging from 1 to 256 µg/mL. Additional DMSO was added to the medium at a final concentration of 0.5%. The fungal strain culture (20 µL) was poured into a 96-well assay plate at a final concentration of 1 x 10⁴ cells/mL and total volume of 100 µL. The culture plates were incubated at 28 °C for 3 days. The positive control was amphotericin B, which is a known antifungal compound.

4.6. Growth Phenotype and Icl Expression Analysis

C. albicans SC5314 (wild type) and icl-deletion mutants (MRC10 and MRC11) were cultured in YNB broth containing 2% glucose at 28 °C on a rotary shaker for 24 h. Cells were collected and washed as described above. The harvested cells were streaked on YNB agar plates containing 2% glucose, 2% potassium acetate, or 2% potassium acetate plus 32 µg/mL cyclo(L-Phe-L-Val) and incubated at 28 °C for 2 days. For icl expression analysis, the harvested cells were added to YNB liquid broth containing 2% glucose, 2% potassium acetate, or 2% potassium acetate plus cyclo(L-Phe-L-Val) (8, 16, or 32 µg/mL) and incubated at 28 °C for 4 h. RNA extraction, cDNA synthesis, and semi-quantitative RT-PCR were performed using previously described methods [33]. GPDH was used as the loading control.

Author Contributions: H.K. and J.-Y.H. carried out the isolation and structural elucidation; H.K. carried out the ICL inhibition assays and gene expression analysis; J.S. and K.-B.O. supervised the research work and prepared the manuscript.

Funding: This research was supported by the Basic Science Research Program through the National Research Foundation (NRF-2018R1D1A1B07043375) of Korea funded by the Ministry of Education, Science and Technology.
Acknowledgments: We are grateful to the Basic Science Research Institute in Daegu, Korea, for providing the mass data and to the National Center for Inter-University Research Facilities, Seoul National University for providing the NMR data.

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

References
1. Vanni, P.; Giachetti, E.; Pinzauti, G.; McFadden, B.A. Comparative structure, function and regulation of isocitrate lyase, an important assimilatory enzyme. Comp. Biochem. Physiol. Part B Comp. Biochem. 1990, 95, 431–458. [CrossRef] 
2. Dunn, M.F.; Ramirez-Trujillo, J.A.; Hernández-Lucas, I.; Dunn, M. Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. Microbiology 2009, 155, 3166–3175. [CrossRef] [PubMed]
3. Kunze, M.; Pracharoenwattana, I.; Smith, S.M.; Hartig, A. A central role for the peroxisomal membrane in glyoxylate cycle function. Biochim. Biophys. Acta (BBA) Bioenerg. 2006, 1763, 1441–1452. [CrossRef] [PubMed]
4. Strijbis, K.; Distel, B. Intracellular Acetyl Unit Transport in Fungal Carbon Metabolism. Eukaryot. Cell 2010, 9, 1809–1815. [CrossRef] [PubMed]
5. McKinney, J.D.; Zu Bentrup, K.H.; Muñoz-Elíbas, E.J.; Miczak, A.; Chen, B.; Chan, W.-T.; Swenson, D.; Sacchettini, J.C.; Jacobs, W.R.; Russell, D.G.; et al. Persistence of Mycobacterium tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 2000, 406, 735–738. [CrossRef] [PubMed]
6. Muñoz-Elíbas, E.J.; McKinney, J.D. Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. Nat. Med. 2005, 11, 638–644. [CrossRef]
7. Lorenz, M.C.; Fink, G.R. The glyoxylate cycle is required for fungal virulence. Nature 2001, 412, 83–86. [CrossRef]
8. Ramírez, M.A.; Lorenz, M.C. Mutations in alternative carbon utilization pathways in Candida albicans attenuate virulence and confer pleiotropic phenotypes. Eukaryot. Cell 2007, 6, 280–290. [CrossRef]
9. Kratky, M.; Vinsova, J. Advances in Mycobacterial Isocitrate Lyase Targeting and Inhibitors. Curr. Med. Chem. 2012, 19, 6126–6137. [CrossRef]
10. Greene, J.; Sheu, S.-S.; Gross, R.; Greenamyre, J.T. 3-Nitropipionic acid exacerbates N-methyl-d-aspartate toxicity in striatal culture by multiple mechanisms. Neuroscience 1998, 84, 503–510. [CrossRef]
11. Ko, Y.H.; Smith, B.L.; Wang, Y.; Pomper, M.G.; Rini, D.A.; Torbenson, M.S.; Hullihen, J.; Pedersen, P.L. Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem. Biophys. Res. Commun. 2004, 324, 269–275. [CrossRef]
12. Borthwick, A.D. 2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products. Chem. Rev. 2012, 112, 3641–3716. [CrossRef]
13. Huang, R.; Zhou, X.; Xu, T.; Yang, X.; Liu, Y. Diketopiperazines from Marine Organisms. Chem. Biodivers. 2010, 7, 2809–2829. [CrossRef]
14. McCleland, K.; Frost, C.; Van De Venter, M.; Du Plessis, J.; Dyason, K.; Milne, P.; Lucierto, F.; Brauns, S. An investigation into the biological activity of the selected histidine-containing diketopiperazines cyclo(His-Phe) and cyclo(His-Tyr). J. Pharm. Pharmacol. 2004, 56, 1143–1153. [CrossRef]
15. Mas, V.; Falco, A.; Brocal, I.; Perez, L.; Coll, J.; Estepa, A. Identification of selective inhibitors of VHSV from biased combinatorial libraries of N,N’-disubstituted 2,5-piperazinediones. Antivir. Res. 2006, 72, 107–115. [CrossRef]
16. Park, D.-K.; Lee, K.-E.; Baek, C.-H.; Kim, I.H.; Kwon, J.-H.; Lee, W.K.; Lee, K.-H.; Kim, B.-S.; Choi, S.-H.; Kim, K.-S. Cyclo(Phe-Pro) Modulates the Expression of ompU in Vibrio spp. J. Bacteriol. 2006, 188, 2214–2221. [CrossRef]
17. Degrazia, G.; Aguilar, C.; Bosco, M.; Zahariiev, S.; Pongor, S.; Venturi, V. Plant Growth-Promoting Pseudomonas putida WCS358 Produces and Secretes Four Cyclic Dipeptides: Cross-Talk with Quorum Sensing Bacterial Sensors. Curr. Microbiol. 2002, 45, 250–254. [CrossRef] [PubMed]
18. Mollica, A.; Macedonio, G.; Stefanucci, A.; Carradori, S.; Akdemir, A.; Angeli, A.; Supuran, C.T. Five- and Six-Membered Nitrogen-Containing Compounds as Selective Carbonic Anhydrase Activators. Molecules 2017, 22, 2178. [CrossRef]
19. Yan, P.-S.; Song, Y.; Sakuno, E.; Nakajima, H.; Nakagawa, H.; Yabe, K. Cyclo(l-Leucyl-l-Prolyl) Produced by Achromobacter xylosoxidans Inhibits Aflatoxin Production by Aspergillus parasiticus. *Appl. Environ. Microbiol.* **2004**, *70*, 7466–7473. [CrossRef]

20. Park, W.; Woo, J.-K.; Shin, J.; Oh, K.-B. nonG, a constituent of the nonactin biosynthetic gene cluster, regulates nocardamine synthesis in Streptomyces albus J1074. *Biochem. Biophys. Res. Commun.* **2017**, *490*, 664–669. [CrossRef]

21. Wang, Y.; Wang, P.; Ma, H.; Zhu, W. Developments around the bioactive diketopiperazines: a patent review. *Expert Opin. Ther. Patents* **2013**, *23*, 1415–1433. [CrossRef]

22. Minelli, A.; Grottelli, S.; Mierla, A.; Pinnen, F.; Cacciatore, I.; Bellezza, I. Cyclo(His-Pro) exerts anti-inflammatory effects by modulating NF-κB and Nrf2 signalling. *Int. J. Biochem. Cell Biol.* **2012**, *44*, 525–535. [CrossRef]

23. Faden, A.; Movsesyan, V.; Knoblach, S.; Ahmed, F.; Cernak, I. Neuroprotective effects of novel small peptides in vitro and after brain injury. *Neuropharmacology* **2005**, *49*, 410–424. [CrossRef]

24. Barrow, C.J.; Sun, H.H. Spiroquinazoline, a Novel Substance P Inhibitor with a New Carbon Skeleton, Isolated from Aspergillus flavipes. *J. Nat. Prod.* **1994**, *57*, 471–476. [CrossRef]

25. Huang, Q.; Tezuka, Y.; Hatanaka, Y.; Kikuchi, T.; Nishi, A.; Tubaki, K. Studies on metabolites of mycoparasitic fungi. III. New sesquiterpene alcohol from Trichoderma koningii. *Chem. Pharm. Bull.* **1995**, *43*, 1035–1038. [CrossRef]

26. Schmidtz, F.J.; Vanderah, D.J.; Hollenbeak, K.H.; Enwall, C.E.L.; Gopichand, Y.; Sengupta, P.K.; Hossain, M.B.; Van Der Helm, D. Metabolites from the marine sponge Tedania ignis. A new atisanediol and several known diketopiperazines. *J. Org. Chem.* **1983**, *48*, 3941–3945. [CrossRef]

27. Shin, D.S.; Kim, S.; Yang, H.C.; Oh, K.B. Cloning and expression of isocitrate lyase, a key enzyme of the glyoxylate cycle, of Candida albicans for development of antifungal drugs. *J. Microbiol. Biotechnol.* **2005**, *15*, 652–655. [CrossRef]

28. Martins, M.B.; Carvalho, I. Diketopiperazines: Biological Activity and Synthesis. *Tetrahedron* **2007**, *63*, 9923–9932. [CrossRef]

29. Lee, S.-H.; Won, T.H.; Kim, H.; Ahn, C.-H.; Shin, J.; Oh, K.-B.; Fusetani, N. Suvanine Sesterterpenes from a Tropical Sponge Coscinoderma sp. Inhibit Isocitrate Lyase in the Glyoxylate Cycle. *Mar. Drugs* **2014**, *12*, 5148–5159. [CrossRef]

30. Dixon, G.H.; Kornberg, H.L. Assay methods for key enzymes of the glyoxylate assay. *Biochem. J.* **1959**, *72*, 3P.

31. Hautzel, R.; Anke, H.; Sheldrick, W.S. Mycenon, a new metabolite from a Mycena species TA 87202 (basidiomycetes) as an inhibitor of isocitrate lyase. *J. Antibiot.* **1990**, *43*, 1240–1244. [CrossRef] [PubMed]

32. Sharma, V.; Sharma, S.; Zu Bentrup, K.H.; McKinney, J.D.; Russell, D.G.; Jacobs, W.R.; Sacchettini, J.C. Structure of isocitrate lyase, a persistence factor of Mycobacterium tuberculosis. *Nat. Genet.* **2000**, *7*, 663–668. [CrossRef] [PubMed]

33. Ahn, C.-H.; Won, T.H.; Kim, H.; Shin, J.; Oh, K.-B. Inhibition of Candida albicans isocitrate lyase activity by cadiolides and synoilides from the ascidian Synoicum sp. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 4099–4101. [CrossRef] [PubMed]

**Sample Availability:** Samples of the compounds isolated are available from the authors.

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).