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Full Length Research Paper

Diketopiperazine alkaloids produced by the endophytic fungus *Penicillium citrinum* and evaluation of their antileishmanial activity

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Chromatographic fractionation of the antileishmanial extract obtained from fermentation of the endophytic fungus *Penicillium citrinum*, isolated from leaves of *Ageratum myriadenia*, yielded three diketopiperazine alkaloids; *cyclo*(L-Pro-L-Leu) (1), *cyclo*-(L-Pro-L-Phe) (2) and tryprostatin B (3). The structures of these compounds were established on the basis of spectroscopic methods and comparison with the literature. Compounds 1 and 2 were active against both amastigote-*like* forms of *Leishmania* (*Leishmania*) *amazonensis* and intracelular amastigotes of *L.* (*Leishmania*) *infantum* with approximately 50% of parasite growth inhibition at 100 µM. None of the compounds were considered toxic against human leukemia monocyte cell line (THP-1) at 100 µM. It is the first report about isolation of these diketopiperazines from *P. citrinum* and their antileishmanial potential against *L.* (*L.*) *infantum*.

Key words: Microfungi, natural products, *in vitro*, *Leishmania*, amphotericin B, cytotoxic activity.

INTRODUCTION

Penicillium is cosmopolitan genera and comprises pathogens, saprobes, opportunists or endophytes taxa, which are able to produce a large number of secondary metabolites being reported 1338 exometabolites (Frisvad, 2015). *Penicillium citrinum* is assigned to section Citrina with a worldwide distribution, occurring commonly in soils; they are known as producers of the mycotoxins citrinin and citreoviridin (Houbraken et al., 2011).

Penicitrinine A has been claimed as antitumor agents in patents (Zheng et al., 2015; Ying et al., 2015). Other secondary metabolites isolated from these species such as penicillanthranin A and chrysophanol, which are anthraquinone-citrinin derivatives, have exhibited moderate antibacterial activity against *Staphylococcus*

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aureus, both with MIC values of 16 μg/ml (Khamthong et al., 2012).

In previous study, it was shown that the crude extract from the fungus *Penicillium* citrinum UFMGCB 579, isolated from leaves of bioactive plant species *Ageratum myriadenia*, had antileishmanial activity (Rosa et al., 2010) in the assays using amastigotes-*like* forms of *Leishmania* (*Leishmania*) *amazonensis*. Chemical investigation of the ethyl acetate extracts from the broth and mycelia of *P. citrinum* UFMGCB 579 led to the isolation of three metabolites. In this study, the occurrence of three diketopiperazine alkaloids in addition to the evaluation of their antileishmanial activity is reported.

MATERIALS AND METHODS

General experimental procedures

Thin-layer chromatography (TLC) analyses were conducted on silica gel G-60/F_{254} (0.25 mm, Merck) and the spots were visualized under visible light after heating the plate sprayed with a mixture (1:1) of ethanolic solutions of vanillin (1% w/v) and sulfuric acid (10% v/v). Gel Permeation Chromatography (GPC) was carried out by using two coupled glass columns (Büchi column n° 17980) filled with Sephadex LH-20 TM (GE Healthcare, U.S.A.) gel. Semi-preparative HPLC purifications were carried out by a Shimadzu chromatographic system (Shimadzu, Kyoto, Japan), equipped with a LC6AD pump and a dual wavelength detector (SPD10A). The mass spectra were acquired in a maXis ETD high-resolution ESI-QTOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The optical rotations were determined on a Modular Circular Polarimeter MCP 300 (Anton Paar, Ashland, Virginia, USA). 1D and 2D nuclear magnetic resonance (NMR) experiments were run on a Bruker Avance 400 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany).

Fungal isolation

The endophytic fungus *P. citrinum* was isolated from the plant *Ageratum myriadenia* (DC) R.M. King & H. Rob (Asteraceae) that was deposited in the herbarium of the "Departamento de Botânica da Universidade Federal de Minas Gerais" under the code BHCB 5816. A fungal sample was deposited at "Coleção de Microrganismos e Células da Universidade Federal de Minas Gerais" under the code UFMGCB 579 and at GenBank by accession number FJ466725 (Rosa et al., 2010).

Fermentation and extraction

The fungus was cultured in a bioreactor containing 10 L of MEC medium (2% malt extract, 0.1% peptone, 1.5% glucose) for fourteen days at 28°C and 150 rpm. Both filtrate and biomass were extracted with ethyl acetate (EtOAc). The organic solvent was removed using a rotary evaporator to afforded 3.3 g of EtOAc extract.

Isolation of secondary metabolites from the endophytic fungi

All extract was subjected to GPC using EtOH as mobile phase at flow rate of 120 ml/h to produce 16 fractions after TLC analyses. Fraction 6 (210 mg) was purified on semi-preparative HPLC, using a Shim-pack® C18 column (5 μm, 250 x 20 mm i.d.), detection at λ 220 and 254 nm, flow rate of 7 ml/min and eluted with mixture of MeOH:H_2O (MeOH, 10–100% in 60 min and 100% for 10 min) to obtain **1** (12 mg). Fraction 8 (220 mg) was also purified as same conditions and yielded compounds **2** (5 mg) and **3** (2.8 mg), respectively.

Cyclo(L-Pro-L-Leu) ((3S,8aS)-3-(2-methylpropyl)-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione)(**1**) is a colorless amorphous solid (8.0 mg); $[\alpha]_D^{25}$ –117.0 (*c* 0.40, MeOH). The molecular formula is $C_{11}H_{18}N_2O_2$, as determined by HR-ESI-MS (*m/z* 211.1419, $[M+H]^+$; calc. for $C_{11}H_{19}N_2O_2$: 211.1441). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.96 (d, *J*= 6.58 Hz, H-12b), 1.0 (d, *J*= 6.58 Hz, H-12a), 1.76 (m, H-11), 2.02 and 1.90 (m, H-4b and a), 2.35 and 2.13 (m, H-5b and a), 2.07 and 1.53 (m, H-10b and a), 3.57 (m, H-3), 4.02 (dd, *J*=9.38, 3.47 Hz, H-9), 4.12 (t, *J*=8.14 Hz, H-6), 6.07 (br s, NH).^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.2 (CH_3, C-12b), 22.8 (CH_2, C-4), 23.3 (CH_3, C-12a), 24.7 (CH, C-11), 28.1 (CH_2, C-5), 38.7 (CH_2, C-10), 45.5 (CH_2, C-3), 53.4 (CH, C-9), 59.0 (CH, C-6), 166.2 (C=O, C-1), 170.3 (C=O, C-7).

Cyclo-(L-Pro-L-Phe) ((3S,8aS)-3-benzyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione) (**2**) is a colorless amorphous solid (5.0 mg); $[\alpha]_D^{25}$ –71.5 (*c* 0.25, MeOH). The molecular formula is $C_{14}H_{16}N_2O_2$, as determined by HR-ESI-MS (*m/z* 245.1255, $[M+H]^+$; calc. for $C_{14}H_{17}N_2O_2$: 245.1285).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.80 (m, H-4), 2.09 and 1.24 (m, H-5), 3.16 (d, *J*=5.01 Hz, H-10), 3.36 and 3.54 (m, H-3), 4.06 (ddd, *J*= 10.75, 6.44, 1.67 Hz, H-6), 4.43 (tt, *J*= 5.01, 5.01, 0.99 Hz, H-9), 7.23 (br m., H-2'/6' and H-4'), 7.27 (br m, H-3'/5').^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 21.4 (CH_2, C-4), 28.0 (CH_2, C-5), 36.8 (CH_2, C-10), 44.6 (CH_2, C-3), 56.3 (CH, C-9), 58.7 (CH, C-6), 126.7 (CH, C-4'), 129.6 (CH, C-2'/-6'), 128.1 (CH, C-3'/-5'), 136.0 (C, C-1'), 165.5 (C=O, C-1), 169.5 (C=O, C-7).

Tryprostatin B ((3S,8aS)-3-[[2-(3-methylbut-2-enyl)-1H-indol-3-yl]methyl]-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione) (**3**) is a pale yellow amorphous solid (2.8 mg); $[\alpha]_D^{25}$ –29.5 (*c* 0.14, MeOH). The molecular formula was $C_{21}H_{25}N_3O_2$, as determined by HR-ESI-MS (*m/z* 352.2019, $[M+H]^+$; calc. for $C_{21}H_{26}N_3O_2$: 352.2020). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.65 and 1.38 (m, H-14), 1.75 (br s, H-21), 1.76 (br s, H-22), 1.95 and 0.93 (m, H-13), 3.20 (dd, J= 14.80, 6.90 Hz H-8b), 3.24 (dd, *J*= 11.3, 3.9 Hz, H-15b), 3.34 (d *J*= 4.60 Hz, overlapped, H-8a), 3.49 (m, H-15a) 3.50 (m, H-18), 3.97 (ddd, *J*=10.87, 6.51, 1.44 Hz, H-12), 4.37 (ddd, *J*=6.80, 4.6, 1.8 Hz, H-9), 5.35 (tt, *J*= 7.21, 7.21, 1.34 Hz, H-19), 6.96 (td, *J*= 7.52, 7.52, 1.15 Hz, H-5), 7.02 (td, *J*= 7.52, 7.52, 1.15 Hz, H-6), 7.27 (d, *J*= 7.94 Hz, H-7), 7.46 (d, *J*= 7.81 Hz, H-4), 8.52 (s, NH-1). ^{13}C NMR (101 MHz, CD_3OD-d_4) δ ppm 18.0 (CH_3, C-22), 22.5 (CH_2, C-14), 25.9 (CH_3, C-21), 26.2 (CH_2, C-18), 28.6 (CH_2, C-8), 29.0 (CH_2, C-13), 46.0 (CH_2, C-15), 57.2 (CH, C-9), 60.1 (CH, C-12), 104.5 (C, C-3), 111.7 (CH, C-7), 119.1 (CH, C-4), 119.8 (CH, C-5), 121.9 (CH, C-6), 122.1 (CH, C-19), 129.5 (C, C-3a and C-2), 134.7 (C, C-20), 137.3 (C, C-7a), 167.5 (C, C-17), 170.4 (C, C-11).

Assays of anti-leishmanial activity

Stationary phase promastigotes of *L.* (*Leishmania*) *amazonensis* (strain IFLA/BR/67/PH-8) were stimulated to differentiate into amastigote-*like* forms by rising the incubation temperature to 32°C and lowering the pH of the medium to 6.0. The parasites were seeded in 96-well plates using 90 μl of parasite suspension at 16 × 10^8 parasites per milliliter, followed by 10 μl of the sample-tests to give a final concentration of 100 μM. Amphotericin B at 0.5 μM was used as positive control. The plates were incubated at 32°C for 72 h and the number of parasites was estimated using the methyl thiazolyl tetrazolium (MTT). The results were calculated from the absorbance measurements using the percentage of parasite death

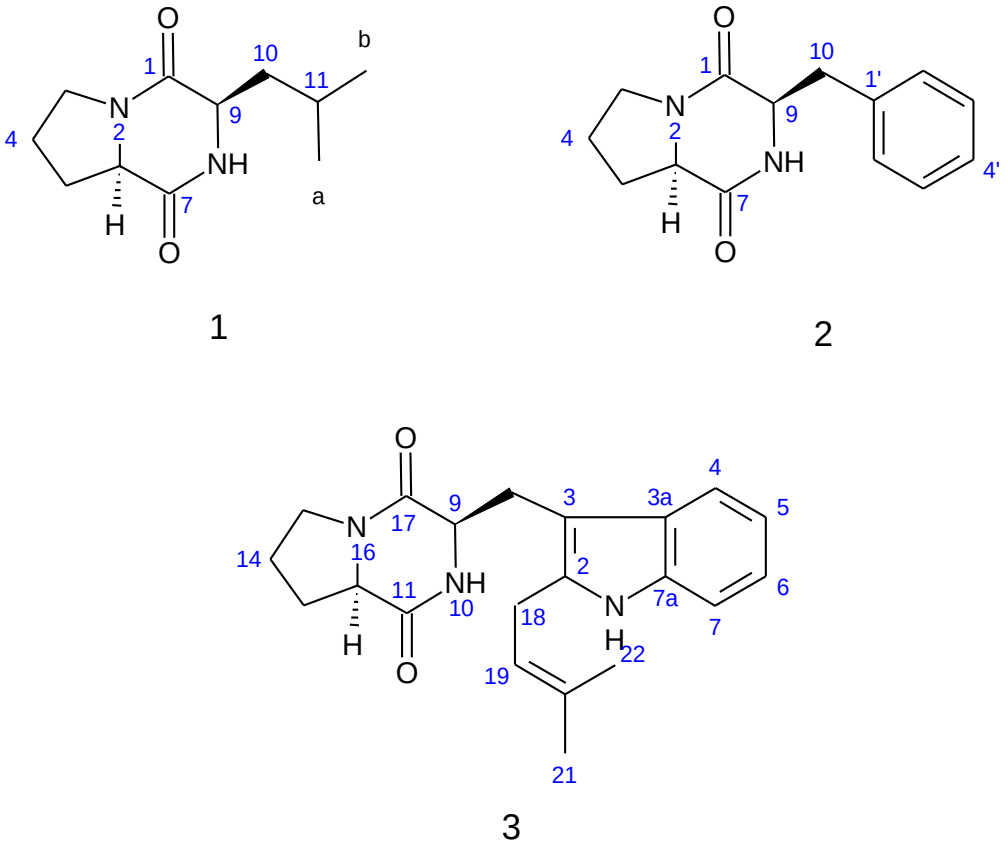

Figure 1. Structure of *cyclo*-(L-Pro-L-Leu) (**1**), *cyclo*-(L-Pro-L-Phe) (**2**) and tryprostatin B (**3**) isolated from *Penicillium citrinum.*

in relation to the controls without drug (Teixeira et al., 2002).

THP-1, maintained in RPMI 1640 medium supplemented with 10 % FBS cells and differentiated in the presence of 20 ng/ml phorbol myristate acetate (PMA) for 72 h at 37°C, were infected at a parasite/macrophage ratio of 10:1 for 3 h with *L.* (*Leishmania*) *infantum* (strain MHOM/MA/67/ITMAP-263) promastigotes expressing the firefly luciferase as reporter gene. Non-internalized parasites were removed by five washes with HEPES/NaCl buffer (20 mM HEPES, 0.15 M NaCl, 10 mM glucose, pH 7.2). The infected cells were then treated with 100 µM of the sample-tests and of amphotericin B at 0.5 µM. After 72 h, RPMI was aspirated and the luciferase activity was assessed by adding 20 µl of reconstituted One-Glo™ Luciferase Assay System solution as enzyme substrate (Promega, Madison, WI, USA). Luciferase activity was measured in a luminometer SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA) using 1 s integration/well (Roy et al., 2000).

Cell viability assays

Non-infected THP-1 macrophages were used as signal background while non-treated infected THP-1 cells were used as control for growth comparison (Garcia et al., 2013). THP-1 macrophages were seeded in 96-well plates at a density of 2×10^5 cells per well. After 72 h treatment with compounds at a final concentration of 100 µM, the cell death was estimated using the MTT. The results were calculated from the absorbance measurements using the percentage of cell death in relation to the controls of untreated cells.

Statistical analysis

Statistical analyses were performed using Graph Pad Prism software 5.03 (GraphPad Software, Inc., San Diego, CA, USA). Differences were assessed by analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

In an ongoing survey of the bioactive potential of microorganisms present in the Brazilian ecosystems, the EtOAc extract of an endophytic fungus *P. citrinum* UFMGCB 579 was able to inhibit the growth of amastigotes-*like* forms of *L. (L.) amazonensis* by 86% at 20 µg/ml and showed an IC_{50} value of 4.6 µg/ml (Rosa et al., 2010). The crude EtOAc extract was subjected to preparative GPC by medium pressure liquid chromatography to afford a series of fractions. HPLC purification yielded the known compounds *cyclo*-(L-Pro-D-Leu) (**1**), *cyclo*-(L-Pro-L-Phe) (Campbell et al., 2009) (**2**) and tryprostatin B (**3**) (Cui et al., 1996) (Figure 1).

These compounds were elucidated by comparing their spectral data (MS, and 1D and 2D NMR data) and specific rotation values with those in the literature values.

The *cyclo*-(L-Pro-Leu) (**1**) was reported to be isolated

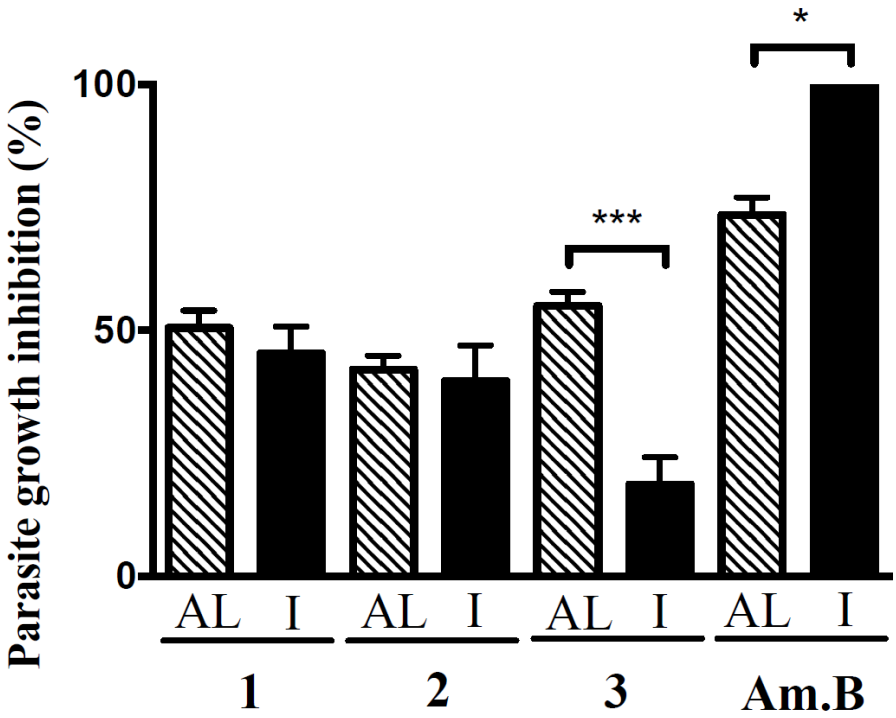

Figure 2. Antileishmanial effects of compounds **1**, **2** and **3** at 100 µM and of amphotericin B (Am.B) at 0.5 µM. Compounds were assayed against intracelular amastigotes (I) of *Leishmania infantum* and amastigote *like*-forms (AL) of *Leishmania amazonensis*. The results showed here are representative of at least 3 experiments in triplicates. Compounds **1** and **2** showed similar activities against intracelular amastigotes and amastigote-*like* forms of the parasite. Compound **3** was more active against amastigote-*like* forms. Statistical differences were calculated using One- way ANOVA with multiple comparisons: * $p < 0.05$; *** $p < 0.0001$.

from the EtOAc extract of the culture broth of the marine fungus *Penicillium chrysogenum*, which was obtained from the North China Sea (Wang et al., 2014) and it was obtained together with *cyclo*-(L-Pro-L-Phe) (**2**) from a neomycin resistant mutant of the marine-derived fungus *Penicillium purpurogenum* G59 (Wang et al., 2016).

Compounds **1** and **2** have antibacterial activity against gram positive and gram negative bacteria (Kumar et al., 2012; Mangamuri et al., 2016) and tryprostatin B (**3**) is a microtubule inhibitor on the cell cycle progression in the M phase of mouse tsFT210 cells (Cui et al., 1996). Compound **3** has also immunosuppressive activity against mouse splenic lymphocytes stimulated with lipopolysaccharide (IC_{50} 3 µg/ml) (Fujimoto et al., 2000) and cytotoxic activity against human leukemia cancer cell line K562 (IC_{50} 21.1 µM) (Wollinsky et al., 2012).

Compounds **1** and **2** were active against both intracellular amastigotes and amastigote-like forms of *Leishmania* parasites, showing approximately 50% of parasite growth inhibition at 100 µM (Figure 2). Compound **3** was more active in the amastigote-*like* model than in the intracellular amastigote form of the parasite ($p < 0.001$), showing a reduction of parasite growth of 55 and 19%, respectively. In addition, treatment with amphotericin B reduced more intracellular amastigote form of the parasite than amastigote-*like* form ($p < 0.05$). This could be explained by the different susceptibility of different species of *Leishmania* or by an activation of the compound by the macrophage. The variation of susceptibility to drugs in different species and strains of *Leishmania* is described in clinical isolates and reference strains, reinforcing the difficulty to find a single drug that can treat all forms of leishmaniasis (Fernández et al., 2012). The intracellular amastigote model is the best to represent human infection and it is important in the drug discovery process because it is the only *in vitro* model that allows the discovery of pro-drugs that need to be metabolized by macrophages in order to become active, such as antimony, and of drugs that activates the immune response of the macrophages in order to eliminate the parasites (Siqueira-Neto et al., 2010).

None of the compounds (**1-3**) were toxic for THP-1 cells when they were tested up to 100 µM ($p < 0.0001$, Figure 3). They reduced the viability of cells less than 10% at 100 µM (**1** = 1%; **2** = 0%; **3** = 8%). Amphotericin B, although more active than the compounds against the parasites, is also more toxic, showing 95% of cell death at the same concentration.

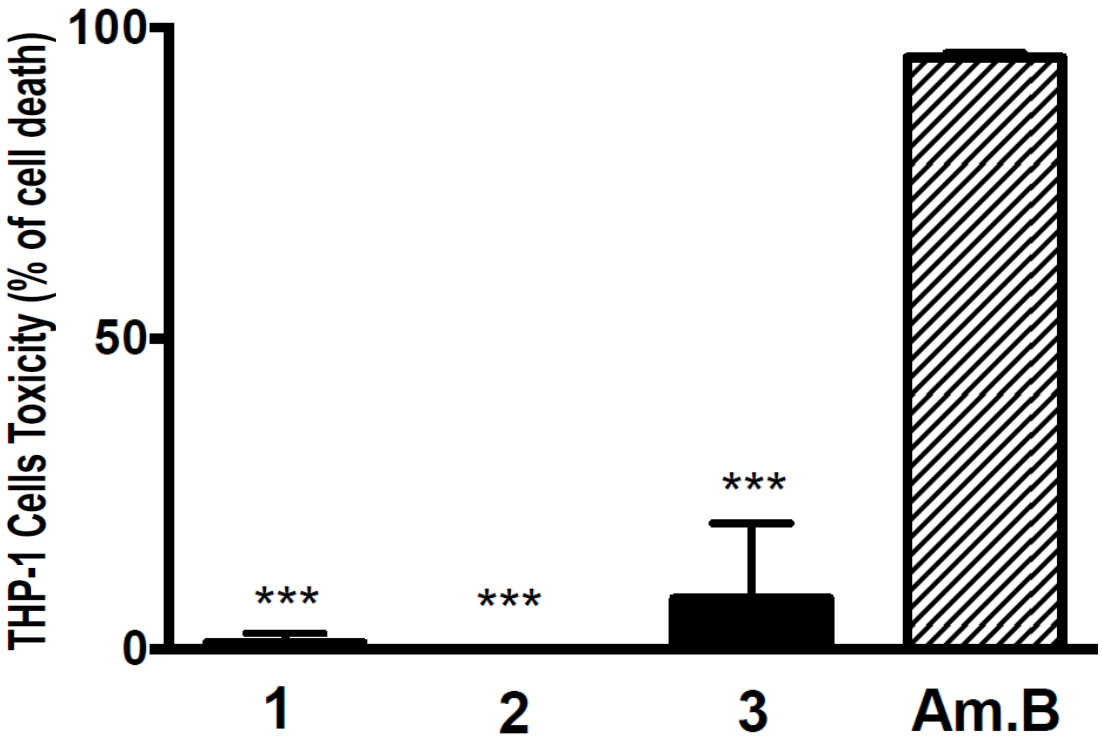

Figure 3. Cytotoxic effects of compounds (**1**-**3**) and, amphotericin B at 100 µM against human monocyte-derived THP-1 macrophages. The results showed here are representative of at least 3 experiments in triplicates. None of the compounds (**1**-**3**) were considered toxic. Statistical differences were calculated using One- way ANOVA with multiple comparisons: *** $p < 0.0001$.

Conclusion

This study demonstrated that the endophytic fungus *P. citrinum* is a source of the diketopiperazine alkaloids. To the researchers' knowledge, this is the first report on the occurrence of tryprostatin B in *Penicillium* species and of the diketopiperazines (**1-2**) in the *P. citrinum.* The biological evaluation of these compounds showed that they are not toxic for THP-1 although they have shown weak antiparasitic potential against two *Leishmania* species in comparison with amphotericin B.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Campbell J, Lin Q, Geske GD, Blackwell HE (2009). New and unexpected insights into the modulation of LuxR-type quorum sensing by cyclic dipeptides. ACS Chemical Biology 4(12):1051-1059.

Cui CB, Kakeya H, Osada H (1996). Novel mammalian cell cycle inhibitors, tryprostatins A, B and other diketopiperazines produced by *Aspergillus fumigatus*. II. Physico-chemical properties and structures. The Journal of Antibiotics 49(6):534-540.

Fernández O, Diaz-Toro Y, Valderrama L, Ovalle C, Valderrama M, Castillo H, Perez M, Saravia NG (2012). Novel approach to *in vitro* drug susceptibility assessment of clinical strains of *Leishmania* spp. Journal of Clinical Microbiology 50(7):2207-2211.

Frisvad JC (2015). Taxonomy, chemodiversity,and chemoconsistency of *Aspergillus*, *Penicillium*, and *Talaromyces* species. Frontiers in Microbiology 5:773.

Fujimoto H, Fujimaki T, Okuyama E, Yamazaki M (2000). Immunosuppressive constituents from an ascomycetes, *Sordaria gondaensis*. Japanese Society of Mycotoxicology 50(2):93-99.

Garcia I, Pouzet C, Brulas M, Bauza E, Botto JM, Domloge N (2013). Evaluation of THP-1 cell line as an in vitro model for long-term safety assessment of new molecules. International Journal of Cosmetic Science 35(6):568-574.

Houbraken J, Frisvad JC, Samson RA (2011). Taxonomy of *Penicillium* section *Citrina*. Studies in Mycology 70(1):153-138.

Khamthong N, Rukachaisirikul V, Phongpaichit S, Preedanon S, Sakayaroj J (2012). Bioactive polyketides from the sea fan-derived fungus *Penicillium citrinum* PSU-F51. Tetrahedron 68(39):8245-8250.

Kumar SN, Siji JV, Nambisan B, Mohandas C (2012). Activity and synergistic antimicrobial activity between diketopiperazines against bacteria in vitro. Applied Biochemistry and Biotechnology 168(8): 2285-2296.

Mangamuri UK, Muvva V, Poda S, Chitturi B, Yenamandra V (2016). Bioactive natural products from *Pseudonocardia endophytica* VUK-10. Journal of Genetic Engineering and Biotechnology 14(2):261-267.

Rosa LH, Gonçalves VN, Caligiorne RB, Alves TMA, Rabello A, Sales PA, Romanha AJ, Sobral MEG, Rosa CA, Zani CL (2010). Leishmanicidal, trypanocidal, and cytotoxic activities of endophytic fungi associated with bioactive plants in Brazil. Brazilian Journal of Microbiology 41(2):420-430.

Roy G, Dumas C, Sereno D, Wu Y, Singh AK, Tremblay MJ, Ouellette M, Olivier M, Papadopoulou B (2000). Episomal and stable expression of the luciferase reporter gene for quantifying *Leishmania* spp. infections in macrophages and in animal models. Molecular Biochemical Parasitology 110(2):195-206.

Siqueira-Neto JL, Song OR, Oh H, Sohn JH, Yang G, Nam J, Jang J, Cechetto J, Lee CB, Moon S, Genovesio A, Chatelain E, Christophe T, Freitas-Junior LH (2010). Antileishmanial high-throughput drug screening reveals drug candidates with new scaffolds. PLoS Neglected Tropical Diseases 4(5):e675.

Teixeira MC, De Jesus SR, Sampaio RB, Pontes-De-Carvalho L, Dos Santos WL (2002). A simple and reproducible method to obtain large numbers of axenic amastigotes of different *Leishmania* species. Parasitology Research 88(11):963-968.

Wang J, Zhao Y, Men L, Zhang Y, Liu Z, Sun T, Geng Y, Yu Z (2014). Secondary metabolites of the marine fungus *Penicillium chrysogenum*. Chemistry of Natural Compounds 50(3):405-407.

Wang N, Cui CB, Li CW (2016). A new cyclic dipeptide penicimutide: the activated production of cyclic dipeptides by introduction of neomycin-resistance in the marine-derived fungus *Penicillium purpurogenum* G59. Archives of Pharmacal Research 39(6):762-770.

Wollinsky B, Ludwig L, Hamacher A, Yu X, Kassack MU, Li SM (2012). Prenylation at the indole ring leads to a significant increase of cytotoxicity of tryptophan-containing cyclic dipeptides. Bioorganic & Medicinal Chemistry Letters 22(12):3866-3869.

Ying M, Liu Q, Chen L, Zheng Q, Zhou T (2015). Penicitrinine A derived from *Penicillium citrinum*, and application thereof in preparation of medicines for treatment of human colorectal cancer. Faming Zhuanli Shenqing CN 105061444 A 20151118.

Zheng Q, Liu Q, Chen Li, Ying M, Zhou Tong (2015). Penicitrinine A derived from *Penicillium citrinum*, and application thereof in preparation of medicines for treatment of nasopharyngeal carcinoma. Faming Zhuanli Shenqing CN 105061446 A 20151118.