*molbank*

MDPI

*Short Note*

# (*E*)-*N*-(3-(5-(3-Acetamidopropyl)-3,6-dioxopiperazin-2-yl)propyl)-5-hydroxy-3-methylpent-2-enamide

**Gagan Preet [1], Rishi Vachaspathy Astakala [1], Joy Ebenezer Rajakulendran [2], Emmanuel T. Oluwabusola [1], Rainer Ebel [1] and Marcel Jaspars [1,*]**

[1] Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Old Aberdeen AB24 3UE, UK; s01gp3@abdn.ac.uk (G.P.); r01ra19@abdn.ac.uk (R.V.A.); emmanuel.oluwabusola2@abdn.ac.uk (E.T.O.); r.ebel@abdn.ac.uk (R.E.)
[2] Department of Chemistry, Eastern University, Chenkalady 30350, Sri Lanka; joye@esn.ac.lk
* Correspondence: m.jaspars@abdn.ac.uk

**Abstract:** The Atacama Desert is an unexplored habitat with interesting possibilities for natural product chemistry due to the adaptations employed by microorganisms to survive the extreme salinity and high UV radiation present. Several soil samples were collected over the course of a few years in locations across the desert from which microorganisms were isolated. This paper reports on the isolation and structural characterisation, using LC-MS, and 1D and 2D NMR, of a new diketopiperazine that came from one of the fungi isolated from the Atacama Desert.

**Keywords:** diketopiperazine; fungal natural products; secondary metabolites; HR-ESI-LC-MS; nuclear magnetic resonance; Atacama Desert

## 1. Introduction

Secondary metabolites in the form of scaffolds and pharmaceutically active compounds have been a rich source of novel chemistry for several decades, and perhaps even for a couple of centuries, starting with the isolation of morphine by Serturner from poppy seeds. These metabolites form the frontline drugs against a variety of diseases including cancer and bacterial and viral infections. Examples of such drugs include doxorubicin, an extremely potent anticancer drug produced by the bacterium *Streptomyces peucetius* [1]; lovastatin, which is a cholesterol-reducing drug isolated from *Aspergillus terreus* [2]; and the cephalosporin class of antibiotics isolated from the fungal genus *Acremonium*, which are known to act against a wide spectrum of bacterial infections. However, due to technical issues such as a high rate of rediscovery and the low availability of materials for clinical trials, their usage has dropped in the past few decades [3]. Certain extreme environments such as the Atacama Desert in the north of Chile continue to attract the interest of natural product chemists from around the world due to its hostile elements, such as high salinity and UV radiation, which lead to interesting adaptations [4], and thus, secondary metabolites [5]. Several interesting classes of potential therapeutics have been isolated from microorganisms found in the desert. These include lasso-peptides, chaxamycins, and aminoheptosyl glycosides [6], among other classes. This paper reports on the isolation and structural characterisation of albrnazine PS (**1**, see Figure 1), a new diketopiperazine from the fungal strain GP-3F- which was isolated from the Salar Tati region of the Atacama Desert.

check for updates

**Citation:** Preet, G.; Astakala, R.V.; Rajakulendran, J.E.; Oluwabusola, E.T.; Ebel, R.; Jaspars, M. (*E*)-*N*-(3-(5-(3-Acetamidopropyl)-3,6-dioxopiperazin-2-yl)propyl)-5-hydroxy-3-methylpent-2-enamide. *Molbank* **2023**, *2023*, M1680. https://doi.org/10.3390/M1680

Received: 6 June 2023
Revised: 27 June 2023
Accepted: 29 June 2023
Published: 1 July 2023

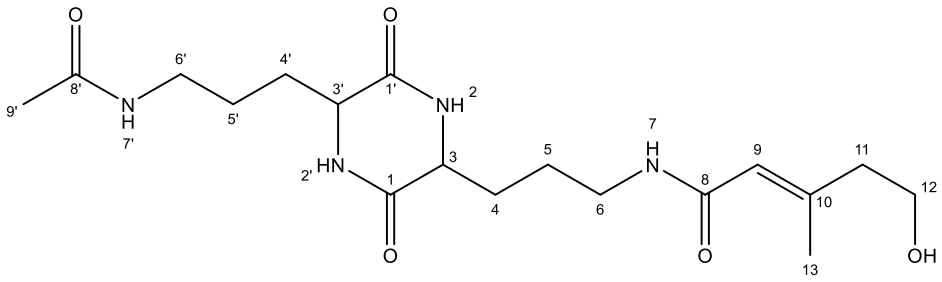


**Figure 1.** Structure of albrnazine PS (**1**).

## 2. Results and Discussion

Albrnazine PS (**1**) was isolated as a colourless amorphous powder from the fungal strain GP-3F- (identified as being closely related to Chrysosporium merdarium and Geomyces pannorum var. pannorum based on sequence analysis), which was isolated from an Atacama soil sediment. The molecular formula of this compound was established as $C_{18}H_{30}N_4O_5$, requiring six degrees of unsaturation based on the positive-mode Q-TOF data, which indicated a $[M+H]^+$ with an $m/z$ of 383.2289 (Δ 1.7 ppm). The NMR spectra of **1** (full data in Table 1) revealed the presence of a near-symmetrical compound, displaying the characteristic chemical shifts of two CONH groups ($\delta_C$ 168.3) and two α-methine residues ($\delta_H$ 3.80)(full spectrum in Figure S1) of a diketopiperazine ring. In total, from the $^{13}C$ NMR (full spectrum in Figure S2) and HSQC (Figure S4) spectra, eighteen carbon signals were identified, including five quaternary carbons, three tertiary carbons, eight secondary carbons, and two primary carbons.

**Table 1.** NMR data of albrnazine PS (1) in DMSO-$d_6$ (400/100 MHz).

| Position [a] | $\delta_C$ (ppm) | $\delta_H$ (ppm), Multiplicity (*J* in Hz) | COSY [a] | HMBC [a] (H→C) |
|---|---|---|---|---|
| 1/1′ | 168.3 C | - | - | - |
| 2/2′ | - | 8.13 s | 3/3′ | 1/1′, 3′/3 |
| 3/3′ | 54.1 CH | 3.80 t (5.1) | 2/2′, 4/4′ | 1′/1, 4/4′, 5/5′ |
| 4/4′ | 31.0 $CH_2$ | 1.65 m (2H) | 3/3′, 5/5′ | 3/3′ |
| 5/5′ | 25.0 $CH_2$ | 1.43 m (2H) | 4/4′, 6/6′ | 4/4′ |
| 6 | 38.3 $CH_2$ | 3.04 m (2H) | 5, 7 | 4, 5, 8 |
| 7 | - | 7.75 t (5.2) | 6 | 8 |
| 8 | 166.5 C | - | - | - |
| 9 | 120.3 CH | 5.62 s | 11, 13 | 8, 10, 11, 13 |
| 10 | 149.8 C | - | - | - |
| 11 | 43.8 $CH_2$ | 2.17 t (6.8, 2H) | 12 | 9, 10, 12, 13 |
| 12 | 59.3 $CH_2$ | 3.51 t (6.8, 2H) | 12-OH, 11 | 10 |
| 12-OH | - | 4.54 s | 12 | - |
| 13 | 17.9 $CH_3$ | 2.07 s (3H) | 9 | 8, 9, 10, 11 |
| 6′ | 38.3 $CH_2$ | 2.99 m (2H) | 5′, 7′ | 4′, 5′, 8′ |
| 7′ | - | 7.81 t (5.1) | 6′ | 8′ |
| 8′ | 169.5 C | - | - | - |
| 9′ | 22.8 $CH_3$ | 1.78 s (3H) | - | 8′ |

[a] Overlapping signals related by pseudosymmetry that are separated by "/".

The COSY spectrum (see Figure 2 and Figure S3) revealed two near-identical major spin systems comprising H-2 (δ 8.13), H-3 (δ 3.80), $H_2$-4 (δ 1.65), $H_2$-5 (δ 1.43), $H_2$-6 (δ 3.04), and 7-NH (δ 7.75), and analogously, H-2′ (δ 8.13), H-3′ (δ 3.80), $H_2$-4′ (δ 1.65), $H_2$-5′ (δ 1.43), $H_2$-6′ (δ 2.99), and 7′-NH (δ 7.81). Furthermore, a third spin system comprising H-9 (δ 5.62), $H_2$-11 (δ 2.17), $H_3$-13 (δ 2.07), $H_2$-11 (δ 2.17), $H_2$-12 (δ 3.51), and 12-OH (δ 4.54) was observed.

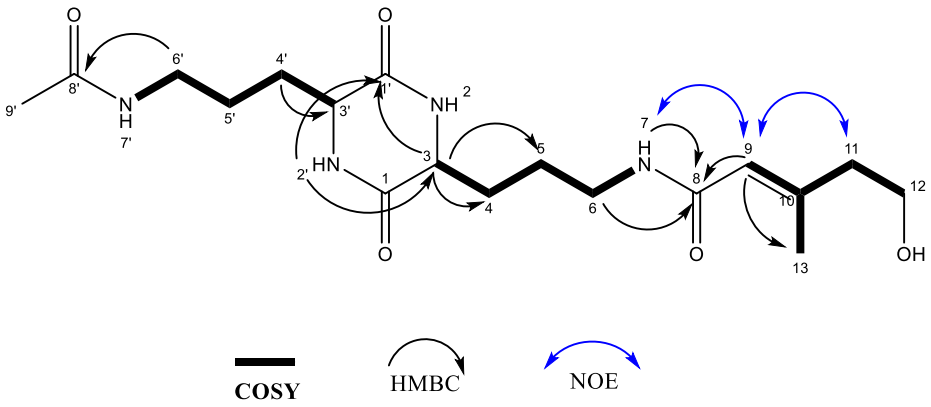


**Figure 2.** Key COSY (**bold**), HMBC, and NOE correlations (arrows, from H- to C) for albrnanazine PS.

The HMBC spectrum (see Figure 2 and Figure S5) displayed key correlations between H-3 and C-1′, C-4, and C-5; $H_2$-6 and C-8; NH-7 and C-8; H-9 and C-8, C-10, C-11, and C-13; $H_2$-11 and C-9, C-10, C-12, and C-13; and $H_3$-13 and C-9, C-10, and C-11. The stereochemistry of the double bond was established as E based on 2D NOE data (Figure S6) which displayed a strong through-space correlation between protons H-9 and $H_2$-11.

The specific rotation, $[\alpha]_D^{21}$, was measured to be −331.4°.

The FTIR spectrum, as seen in Figure 3 (full report in Figure S7), of the compound in methanol revealed a broad peak between 3600 and 3000 $cm^{-1}$, signifying the solvent and perhaps occluding the −OH, −NH (secondary amine), and −CH (alkene) stretching modes. A strong peak at 1660 $cm^{-1}$ indicates the C=O stretching mode of the amide groups and C=C stretching of the triply substituted alkene. Another strong peak at 1023 $cm^{-1}$ indicates the C−O stretching mode of the primary alcohol at position 12.

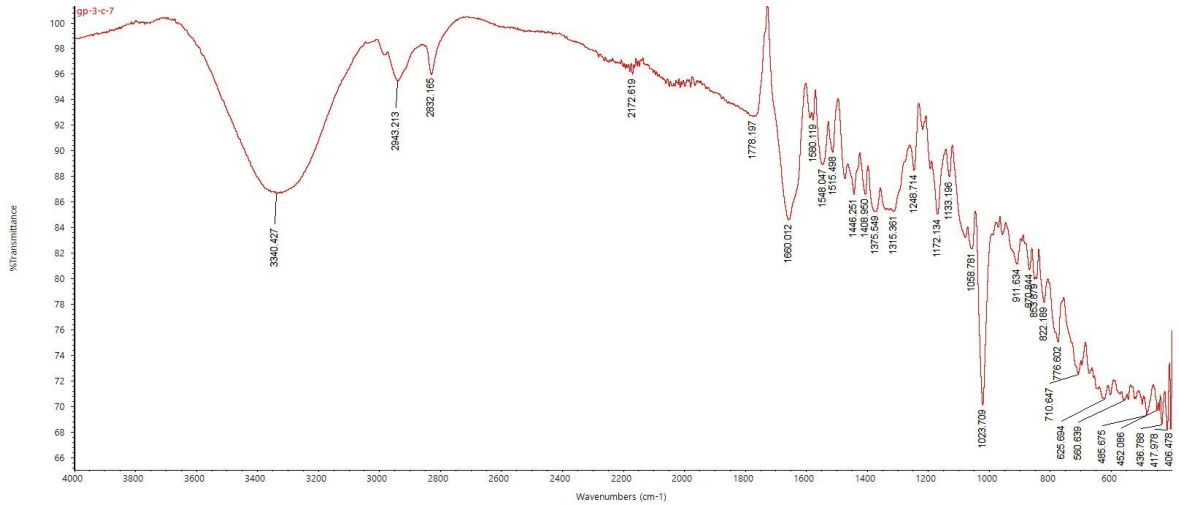


**Figure 3.** The FTIR spectrum of compound **1**.

Based on the above spectroscopic analysis, the planar structure of 1 was established as depicted, representing a new 2,5-diketopiperazine for which we suggest the name albrnazine PS.

## 3. Materials and Methods

### *3.1. Isolation and Cultivation of the Fungus*

The fungus was isolated from a sample of soil collected at 23.00.010 S, 67.15.822 W at an altitude of 4339 m, as described previously [7]. The isolated fungus (strain # GP-3F) was

submitted to NCIMB (Aberdeen, UK) for identification (GenBank accession #NC022575_GP-3F-), but did not give a sufficiently high species level match in the MicroSeq database. Therefore, the sequence was searched against the non-validated EMBL public database, with the top matches Chrysosporium merdarium and Geomyces pannorum var. pannorum each displaying a 99.65% similarity. The strain was stored on ISP2 plates at 4 °C and in 25% glycerol stock solutions at −80 °C. The fungus was cultivated in two 1 L Erlenmeyer flasks, each containing 100 g of rice and 100 mL of water, autoclaved at 121 °C for 20 min, and sealed off using cotton wool and aluminium foil at room temperature for 4 weeks.

### *3.2. Isolation and Structural Characterisation of Albrnazine PS (**1**)*

After cultivation for 4 weeks, the fungal culture was extracted using 200 mL of methanol 3 times per flask, which was followed by using 200 mL of ethyl acetate 2 times per flask, after which the combined extracts were dried under vacuum. The dried extract was redissolved in methanol and subjected to flash chromatography using a Buchi Reveleris X2 flash chromatography system with a C-18 column (Flashpure eco-flex 80 g; particle size: 50 μm; pore size: 92–108 Å) for the stationary phase, whereas the mobile phase utilized a starting gradient of water and methanol, which yielded four fractions. The third fraction was subjected to semi-preparative HPLC using an Agilent 1100 series binary pump and 1100 series DAD (diode array detector) with a Sunfire column (10 μm, 10 mm × 250 mm) at 2 mL/min, resulting in 3.2 mg of albrnazine PS (**1**) being isolated. LC-MS was performed using an Agilent 1290 infinity UHPLC and a Phenomenex Kinetex XB-C18 (2.6 μM, 100 × 2.1 $mm^2$) column with mobile phases of 5% acetonitrile + 0.1% formic acid and 94.9% water, and 100% acetonitrile + 0.1% formic acid. MS spectra were obtained using a Bruker Maxis Q-tof II (Coventry, UK) where the mass range was set from 100–2000, the capillary voltage was set to 4.5 kV, the nebuliser gas was set to 4 bar, the dry gas was set to 9 L/min, and the dry temperature was set to 220 °C. The MS/MS experiments were conducted under Auto MS/MS scan mode with a step collision energy from 80 to 200%. A Bruker Avance III HD 400 MHz system (Coventry, UK) was used to acquire NMR spectra at 25 °C. The IR spectrum of the compound was measured using a Nicolet summit OA FTIR spectrometer, which was fitted with a DTGS-KBR detector at room temperature.

## 4. Conclusions

This paper reports the structural characterisation, using LC-MS, 1D and 2D NMR spectroscopy, of a new 2,5 diketopiperazine isolated from a fungus obtained from a soil sample from the Atacama Desert. The name albrnazine PS has been proposed for this compound.

**Supplementary Materials:** Figure S1: $^{1}$H NMR spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 400 MHz; Figure S2: $^{13}$C NMR spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 100 MHz; Figure S3: COSY spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 400 MHz; Figure S4: HSQC spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 400 MHz; Figure S5: HMBC spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 600 MHz; Figure S6: NOE spectrum of albrnazine PS (**1**) in DMSO-$d_6$ at 400 MHz: Figure S7: Full report of the FTIR spectrum of compound **1**.

**Author Contributions:** Conceptualisation, G.P. and R.V.A.; methodology, G.P., J.E.R. and E.T.O.; software, G.P.; validation, R.E. and M.J.; formal analysis, G.P., R.E. and M.J.; investigation, G.P., R.E. and M.J.; resources, G.P.; data curation, G.P.; writing—original draft preparation, R.V.A.; writing—review and editing, G.P, R.E. and M.J.; visualisation, G.P. and R.V.A.; supervision, R.E. and M.J.; project administration, R.E. and M.J.; funding acquisition, M.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** The collection of materials was carried out with financial support from the UK Newton Project for UK–Chile Collaboration (JIC CA 586) to Professor Mervyn Bibb, John Inness Centre, Norwich, UK.

**Data Availability Statement:** The spectroscopic data presented in this study are available as Supplementary Materials.

**Acknowledgments:** The authors would like to thank Albrn Care India, Nidhan Singh Oberoi, and P.S. Oberoi, I.C.A.R-National Dairy Research Institute, India, as well as Aparna Koride for their continued support. They would also like to thank Russell Gray for his support in running the NMR experiments. Data collection was carried out in collaboration with Michael Goodfellow, University of Newcastle, and Juan Asenjo and Barbara Andrews, University of Chile.

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

## References

1. Lomovskaya, N.; Otten, S.L.; Doi-Katayama, Y.; Fonstein, L.; Liu, X.C.; Takatsu, T.; Inventi-Solari, A.; Filippini, S.; Torti, F.; Colombo, A.L.; et al. Doxorubicin Overproduction in *Streptomyces peucetius*: Cloning and Characterization of the dnrU Ketoreductase and dnrV Genes and the doxA Cytochrome P-450 Hydroxylase Gene. *J. Bacteriol.* **1999**, *181*, 305–318. [CrossRef] [PubMed]
2. De Oliveira, M.C.L.; Paulo, A.J.; de Albuquerque Lima, C.; de Lima Filho, J.L.; Souza-Motta, C.M.; Vidal, E.E.; Nascimento, T.P.; de Araújo Viana Marques, D.; Porto, A.L.F. Lovastatin Producing by Wild Strain of *Aspergillus terreus* Isolated from Brazil. *Prep. Biochem. Biotechnol.* **2021**, *51*, 164–172. [CrossRef] [PubMed]
3. Huang, M.; Lu, J.J.; Ding, J. Natural Products in Cancer Therapy: Past, Present and Future. *Nat. Prod. Bioprospect.* **2021**, *11*, 5–13. [CrossRef] [PubMed]
4. Gómez-Silva, B.; Rainey, F.A.; Warren-Rhodes, K.A.; McKay, C.P.; Navarro-González, R. Atacama Desert Soil Microbiology. In *Microbiology of Extreme Soils*; Dion, P., Nautiyal, C.S., Eds.; Soil Biology; Springer: Berlin/Heidelberg, Germany, 2008; Volume 13. [CrossRef]
5. Azua-Bustos, A.; González-Silva, C.; Corsini, G. The Hyperarid Core of the Atacama Desert, an Extremely Dry and Carbon Deprived Habitat of Potential Interest for the Field of Carbon Science. *Front. Microbiol.* **2017**, *8*, 993. [CrossRef] [PubMed]
6. Rateb, M.E.; Ebel, R.; Jaspars, M. Natural Product Diversity of Actinobacteria in the Atacama Desert. *Antonie Van Leeuwenhoek* **2018**, *111*, 1467–1477. [CrossRef] [PubMed]
7. Carro, L.; Razmilic, V.; Nouioui, I.; Richardson, L.; Pan, C.; Golinska, P.; Asenjo, J.A.; Bull, A.T.; Klenk, H.P.; Goodfellow, M. Hunting for Cultivable *Micromonospora* Strains in Soils of the Atacama Desert. *Antonie Van Leeuwenhoek* **2018**, *111*, 1375–1387. [CrossRef] [PubMed]