Tetrahedron Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Polyketides from the Mangrove-derived fungal endophyte *Pestalotiopsis clavispora*

Catalina Francis Pérez Hemphill [a], Georgios Daletos [a], Zhen Liu [a], Wenhan Lin [b], Peter Proksch [a,*]

[a] Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University Duesseldorf, Universitaetsstrasse 1, Geb. 26.23, 40225 Duesseldorf, Germany
[b] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, 100191 Beijing, PR China

ARTICLE INFO

Article history:
Received 5 February 2016
Revised 24 March 2016
Accepted 30 March 2016
Available online xxxx

Keywords:
Pestalotiopsis clavispora
Pestalpolyol
Cytotoxic activity
Polyketide biosynthesis

ABSTRACT

Six new polyketide derivatives, including pestalpolyol I (**1**), pestapyrones A (**2**) and B (**3**), (*R*)-periplanetin D (**6**), pestaxanthone (**7**), norpestaphthalide A (**8**), and an isolation artifact pestapyrone C (**4**), were obtained from extracts of the endophytic fungus *Pestalotiopsis clavispora* isolated from the Mangrove plant *Rhizophora harrisonii*. In addition, seven known metabolites, including similanpyrone B (**5**), (*R*,*S*)-5,7-dihydroxy-3-(1-hydroxyethyl)phthalide (**9**), for which we propose the trivial name norpestaphthalide B, pestaphthalides A (**10**) and B (**11**), 2-*epi*-herbarumin II (**12**), and pestalotiollides A (**13**) and B (**14**) were isolated. The structures of the new compounds were unambiguously elucidated on the basis of one- and two-dimensional NMR spectroscopy, as well as by high-resolution mass spectrometry. All compounds were tested for their cytotoxicity against the mouse lymphoma cell line L5178Y. Compound **1** exhibited strong activity with an IC_{50} value of 4.10 μM. All other compounds (**2**–**14**) proved to be inactive (IC_{50} >10 μM) in this assay.

Introduction

According to the WHO cancer is the second leading cause of deaths caused by non-communicable diseases.[1] The WHO's GLOBOCAN 2012 estimated a total of 8.2 million deaths accounted for by cancer worldwide and a total of 14.1 million new cases in 2012, which are expected to rise to 22 million in the next two decades.[2] Corresponding to the vast number of cancer types, the number of chemotherapeutics attacking various different targets is multifold, e.g., the mitotic inhibitor paclitaxel (Taxol®), the topoisomerase inhibitors topotecan (Hycamtin®) and etoposide (Vepesid®), and DNA alkylating agents such as streptozocin (Zanosar®).[3–5] Yet, besides numerous side effects, the limitations of this form of oncological therapy, such as insufficient bioavailability and the lack of sensitivity or the development of resistance, necessitate an ongoing research for the discovery and development of new and improved chemotherapeutic drugs.

One important source for the discovery of new drug leads in cancer chemotherapy is natural products.[6] To this day, a total of 35 naturally derived products are approved by the FDA and are in use in anticancer therapy,[7] which accounts for almost one fifth of all FDA approved antineoplastic agents (1995–2015).[8] Worldwide the number of small molecules that are approved as drugs for oncological therapy and that are derived or inspired from natural products even accounted for almost 50% (1940–2012).[9] More than half of those naturally derived drugs originated from microbial organisms,[7] which can be isolated from different sources.[10] Currently, nine naturally derived products with fungal origin are being evaluated in clinical trials against cancer, of which two are now in a Phase III study.[11a,b] Some of the most interesting fungi with regard to metabolite production with potent anticancer activity are found as endophytes in Mangroves.[12,13] Due to the need of these endophytes to adapt to extreme habitual conditions, their secondary metabolites are structurally diverse and often show biological activity, e.g., against cancer cells.[14,15]

One of the most promising genera of fungi, featuring secondary metabolites with anti-tumor activity, is *Pestalotiopsis*, which among other cytotoxic compounds, was shown to produce the anticancer drug paclitaxel, approved by the FDA, EMA, and TGA for the treatment of ovarian, breast and other cancers.[16–21]

In search of new potential antineoplastic drug leads, *Pestalotiopsis clavispora* was investigated in this study. The crude extract of fungal cultures grown on solid rice medium yielded a total of fourteen compounds (Fig. 1), all of which were submitted to a cytotoxicity assay against the mouse lymphoma L5178Y cell line. Of all compounds tested, **1**[22] with an IC_{50} value of 4.10 μM may be interesting for further investigation due to its strong cytotoxicity in this bioassay.

* Corresponding author. Tel.: +49 211 81 141 63; fax: +49 211 81 119 23.
E-mail address: proksch@uni-duesseldorf.de (P. Proksch).

http://dx.doi.org/10.1016/j.tetlet.2016.03.101
0040-4039/

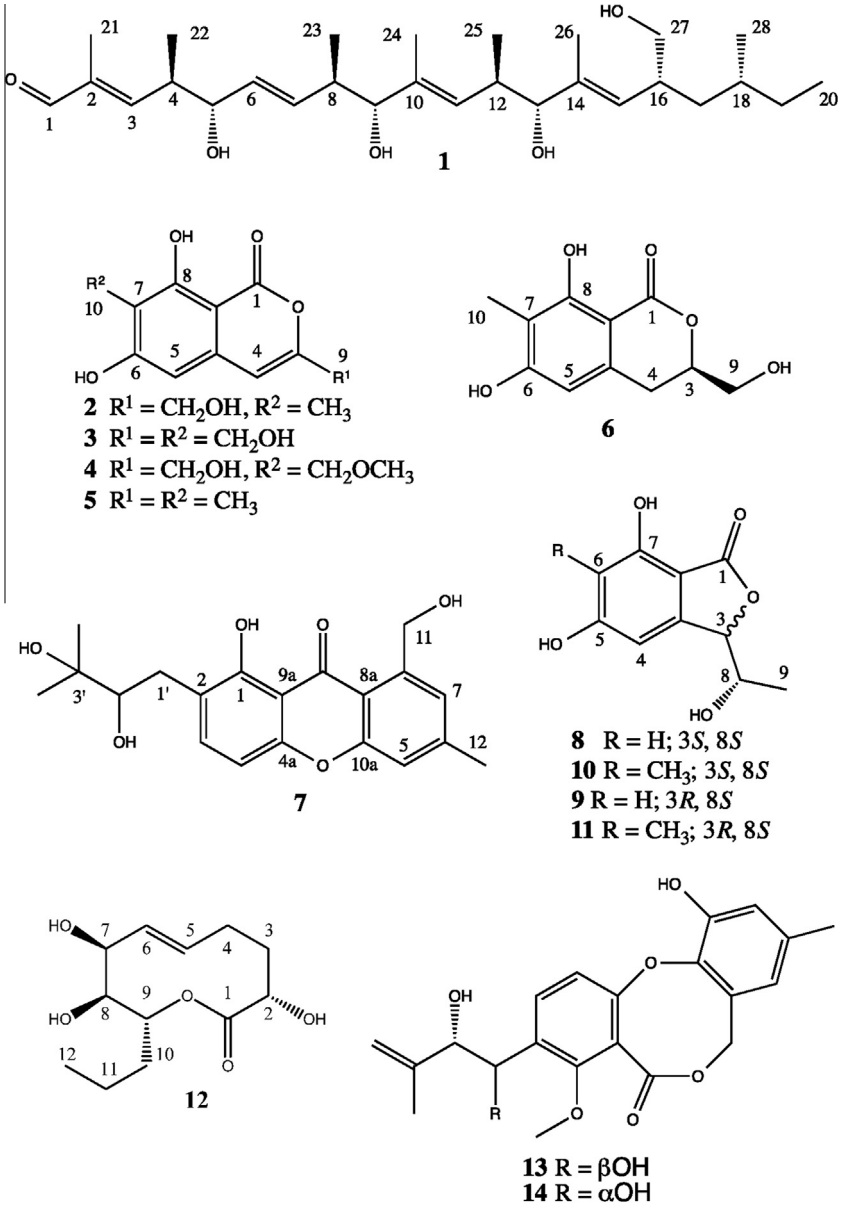

Figure 1. Structures of compounds **1**–**14**.

Results and discussion

P. clavispora was isolated from petioles of the Mangrove *Rhizophora harrisonii*, which was collected in Port Harcourt (Nigeria). The large-scale solid rice culture of *P. clavispora* was extracted with ethyl acetate, and subsequently evaporated to yield the dried extract (4 g). Chromatographic separation yielded fourteen compounds (**1**–**14**), from which pestalpolyol I (**1**), pestapyrones A (**2**) and B (**3**),[23,24] (*R*)-periplanetin D (**6**),[26] pestaxanthone (**7**),[27] and norpestaphthalide A (**8**)[28] proved to be new natural products, whereas pestapyrone C (**4**)[25] is most likely an artifact generated from **3** during extraction and/or isolation (Fig. 1).

Compound **1** was obtained as an amorphous, white powder with a HRESIMS prominent ion peak at *m*/*z* 465.3573 $[M+H]^+$ from which the molecular formula $C_{28}H_{48}O_5$ was established, revealing 5 degrees of unsaturation. It exhibited only one absorption maximum at λ_{max} 232 nm. The ^{1}H NMR spectrum of **1** revealed the presence of a deshielded proton at δ_H 9.39, belonging to an aldehyde function (H-1), five olefinic protons (δ_H 6.56, H-3; δ_H 5.49, H-6; δ_H 5.71, H-7; δ_H 5.27, H-11; and δ_H 5.09, H-15), three oxygenated aliphatic methine protons (δ_H 3.98, H-5; δ_H 3.67, H-9; and δ_H 3.69, H-13), one hydroxymethylene group at δ_H 3.45 and 3.37 (H_2-27), and eight methyl proton signals, including three olefinic (δ_H 1.75, H_3-21; δ_H 1.65, H_3-24; and δ_H 1.69, H_3-26) and five secondary (δ_H 0.87, H_3-20; δ_H 1.06, H_3-22; δ_H 0.85, H_3-23; δ_H 0.79, H_3-25; and δ_H 0.87, H_3-28) methyl groups. In addition, the resonances of four allylic methine protons (δ_H 2.82, H-4; δ_H 2.33, H-8; δ_H 2.65, H-12, and δ_H 2.65, H-16), and a cluster of aliphatic methylene (δ_H 1.22, H_2-17; and δ_H 1.21/1.29, H_2-19) and methine (δ_H 1.31, H-18) signals were observed. Detailed analysis of the COSY spectrum disclosed the presence of a long continuous spin system, which started from the olefinic proton H-3 and sequentially extended until the oxymethine proton H-9, with H-4 and H-8 further correlating with the methyl protons H_3-22 and H_3-23, respectively. Further inspection of the COSY spectrum allowed the assignment of two additional spin systems, corresponding to the fragments CH(11)CH(12)CH(13) and CH(15)CH(16)CH(17)CH(18)CH_2(19)CH_3(20) with H-12, H-16, and H-18 also