Tetrahedron 72 (2016) 2964–2971

Contents lists available at ScienceDirect

Tetrahedron

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

Antifungal bromopyrrole alkaloids from the South China Sea sponge *Agelas* sp.

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ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form 5 April 2016
Accepted 6 April 2016
Available online 8 April 2016

Keywords:
Marine sponges
Bromopyrrole alkaloids
Enantiomers
Antifungal
Nematodes

ABSTRACT

Six new bromopyrrole alkaloids, longamides D–F (**1**–**3**), 3-oxethyl-4-[1-(4,5-dibromopyrrole-2-yl)-formamido]-butanoic acid methyl ester (**4**), 2-oxethyl-3-[1-(4,5-dibromopyrrole-2-yl)-formamido]-methyl propionate (**5**), and 9-oxethyl-mukanadin F (**6**), along with two known metabolites, hanishin (**7**) and longamide B methyl ester (**8**), were isolated from the South China Sea sponge *Agelas* sp. The racemic mixtures **1**–**5**, **7** and **8** were resolved into seven pairs of enantiomers **1a/1b**–**5a/5b**, **7a/7b**, and **8a/8b** through HPLC using a Chiralcel OJ-RH column. The structures were elucidated on the basis of spectroscopic data. The absolute configurations of each enantiomer of (±)-**1** and (±)-**2** were established through the quantum mechanical calculations of the corresponding electronic circular dichroism (ECD) spectra and using the CD exciton chirality method, respectively, whereas the absolute configurations of **3a** and **3b** were confirmed by comparing the experimental CD spectra with that of (−)-*S*-longamide B methyl ester. Individual enantiomers (+)-(9*S*, 10*R*)-**1a**, (−)-*S*-**2b**, (+)-*R*-**3a**, (+)-*R*-**7a**, and (+)-*R*-**8a** exhibited effective antifungal activity against *Candida albicans* in a *Caenorhabditis elegans* candidiasis model.

1. Introduction

Candida albicans is the most common fungal pathogen, causing diseases ranging from superficial mucosal infections to lethal systemic disorders, particularly in immunocompromised individuals.[1–3] Bloodstream infections with *C. albicans* are associated with a >30% mortality rate.[1,3–5] Moreover, the number of available antifungal agents remains limited, and drug resistance is a significant challenge.[2,6] This situation prompted us to search for new antifungal substances.

The biodiversity of the marine environment and the associated chemical diversity provide a practically unlimited source of new active substances for the field of bioactive products development.[7] Among marine organisms, sponges are rich sources of natural products and have provided a seemingly inexhaustible supply of bioactive metabolites.[8–10] Among these natural products, bromopyrrole alkaloids constitute a family of exclusively marine alkaloids, and more than 140 derivatives with different structures and biological activities have been isolated from more than 20 different types of sponges, e.g., *Hymeniacidon* sp., *Homaxinella* sp., *Polymistia* sp., *Axinella* sp., *Stylotella* sp., *Pseudaxynissa* sp., *Stylissa* sp., *Pseudoceratina* sp., *Phakellia* sp., and *Agelas* sp.[11] Many of these derivatives feature a 4-bromo- or 4,5-dibromopyrrole-2-carboxylic acid moiety, and they have shown interesting bioactivities, such as activating actomyosin ATPase,[12] serving as an antagonist of serotonergic receptors,[13] and inhibiting kinase activity,[14] as well as antitumor,[15] antibacterial,[16] antihistamine,[17] and antifungal activities.[18,19] Their unique structural features and interesting biological activities have attracted much attention as synthetic targets from the synthetic community, and a series of bromopyrrole alkaloids have been synthesized already.[20–22]

In our screening program to discover new marine antifungal agents, we focused our efforts on the antifungal activity of the crude extracts of the marine sponge *Agelas* sp. collected off the Xisha Islands in the South China Sea. Bioassay-guided fractionation led to the isolation of five pairs of bromopyrrole alkaloids enantiomers (±)-**1**–**5** and one monomeric compound 9-oxethyl-

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http://dx.doi.org/10.1016/j.tet.2016.04.020
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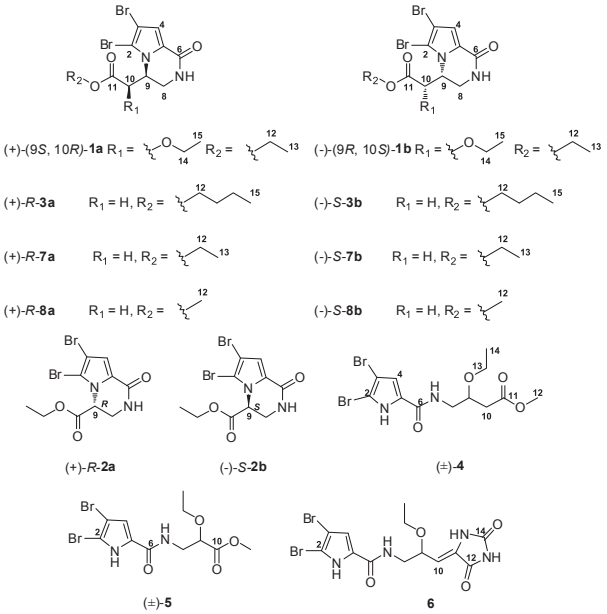
mukanadin F (**6**), along with two pairs of known bromopyrrole alkaloids enantiomers (±)-hanishin (**7**) and (±)-longamide B methyl ester (**8**),[23,24] which were evaluated for their antifungal activity using a *Caenorhabditis elegans* candidiasis model. Herein, we report the isolation, structure elucidation, and antifungal activity of these compounds.



2. Results and discussion

Longamide D (**1**) was obtained as a yellow amorphous solid. Its ESIMS showed an $[M+Na]^+$ ion cluster at *m/z* 445/447/449 with a ratio of 1:2:1, indicating the presence of two bromine atoms. The molecular formula of **1** was established as $C_{13}H_{16}N_2O_4Br_2$ on the basis of HR-ESIMS and ^{13}C NMR data, implying six degrees of unsaturation. The IR spectrum of **1** exhibited absorption bands at 1737 and 1666 cm^{-1}, indicative of ester and amide carbonyl groups, respectively.[25] The UV absorption at 282 nm was attributed to a substituted pyrrole chromophore.[26] The typical ^{13}C NMR resonances at δ 125.5 (qC), 115.8 (CH), 108.0 (qC), and 101.3 (qC) indicated the presence of a 2-carbonyl-4,5-dibromopyrrole moiety.[18,27] Analysis of the ^1H NMR and HSQC data revealed the presence of an olefinic methine (δ_H 7.00/δ_C 115.8), an amide proton (δ_H 5.83, br s), two aliphatic methines (δ_H 4.54/δ_C 54.8 and δ_H 4.08/δ_C 76.3), two oxygenated methylenes (H_2-12, δ_H 4.15, 4.02/δ_C 62.0 and H_2-14, δ_H 3.67, 3.38/δ_C 67.2), a methylene (δ_H 3.85/δ_C 40.1), and two methyls (δ_H 1.18/δ_C 14.9 and δ_H 1.16/δ_C 13.7). The COSY cross-peaks of H_2-12/H_3-13 (δ 1.16, t) and H_2-14/H_3-15 (δ 1.18, t) indicated the presence of two *O*-ethyl groups (Fig. 1). Furthermore, the COSY correlations of NH-7/H-8, H-8/H-9, and H-9/H-10 delineated another spin system N_7–C_8–C_9–C_{10} (Fig. 1). HMBC cross-peaks from both H-10 (δ 4.08) and H_2-12 (δ 4.15, 4.02) to C-11 (δ 169.7) clearly indicated that the *O*-ethyl group (O–C_{12}–C_{13}) was connected to C_{10} via an ester carbonyl (Fig. 1). The observed HMBC correlation of H_2-14/C-10 (δ 76.3) placed the other *O*-ethyl group O–C_{14}–C_{15} at C-10 (Fig. 1). Another HMBC correlation from H_2-8 (δ_H 3.85) and NH-7 (δ_H 5.83) to the carbonyl carbon resonance at δ_C 158.5 (C-6) indicated that the NH-7 group must be part of the previously identified amide function. With all carbon connectivities accounted for, the remaining degree of unsaturation was assigned to form a pyrroloketopiperazine nucleus due to the downfield methine proton resonance at δ_H 4.54 (1H, m, H-9).[28] Thus, **1** was identified as shown and named as longamide D. Because EtOH was used for the extractions, it is probable that compound **1** is an artifact.

Because the rotation value of **1** was zero, the compound is presumably a racemate. This hypothesis was confirmed by chiral HPLC analysis, in which **1** was separated into two peaks with a ratio of 1: 1 [peak A, (−)-**1b**: $[\alpha]_D^{24}$ −1.4 (*c* 0.15, MeOH); peak B, (+)-**1a**: $[\alpha]_D^{24}$ +1.2 (*c* 0.14, MeOH)]. The CD spectra of (+)-**1a** and (−)-**1b** were mirror images (Fig. 2).

In order to determine the relative and absolute configurations of compounds (+)-**1a** and (−)-**1b**, two structures [(9*R*, 10*R*)-longamide D, **A**; (9*R*, 10*S*)-longamide D, **B**; C10-epimers] were initially submitted to Merck Molecular Force Field (MMFF) conformational analyses. The resultant 27 conformers for **A** and 6 conformers for **B** were re-optimized at the B3LYP/6-31G (d,p) level in vacuo. The re-optimization in vacuo produced 14 conformers above 1% population for **A** and 6 conformers for **B**, which were then used in the GIAO NMR shielding constants calculations at the same level in vacuum. Table S1 in the Supplementary data shows a summary of how the computed ^{13}C NMR data of the structures **A** and **B** compare to those experimental data for (±)-**1** (see Fig. 1 for structures). It is clear that the two diastereomers are similar, with the largest deviations between their computed shifts and the experimental shifts for compound (±)-**1** being only 1.9 ppm, however, on the basis of these CMADs (**A** 1.9 vs **B** 1.7), and the deviations for outliers (**A** 4.5 vs **B** 4.3), structure **B** displays better overall agreement with the experimental data (see Supplementary data). The DP4 probability analyses,[29,30] using *t* distribution and DP4-database 2, also identified structure **B** as more likely, with a probability of 75.0% (the remaining 25.0% probability was assigned to structure **A**). Since we only chose 11 ^{13}C NMR chemical shifts and eliminate the two Br-substituted carbons in our comparison and these two diastereomers are similar, the relative low probability to distinguish these two structures is reasonable. With all four functionals (B3LYP, CAM-B3LYP, BH&HLYP, and PBE0) combined with the TZVP basis set in CH_3OH (PCM), the ECD spectrum, using B3LYP functional, of **B** provides the best agreement with the experimental ECD spectrum

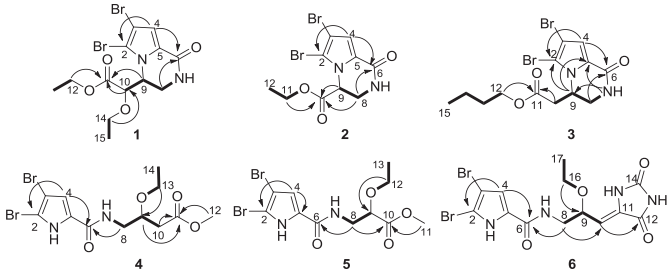


Fig. 1. Selected HMBC (→) and COSY (▬) correlations of compounds **1**–**6**.

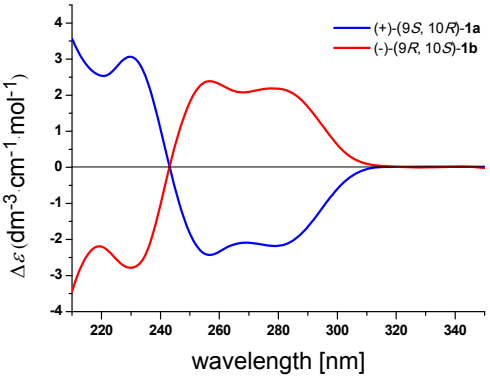


Fig. 2. CD spectra of (+)-(9*S*, 10*R*)-**1a** and (−)-(9*R*, 10*S*)-**1b** in MeOH.