Oxazinin A, a Pseudodimeric Natural Product of Mixed Biosynthetic Origin from a Filamentous Fungus

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Supporting Information

ABSTRACT: A racemic, prenylated polyketide dimer, oxazinin A (1), was isolated from a novel filamentous fungus in the class Eurotiomycetes, and its structure was elucidated spectroscopically. The pentacyclic structure of oxazinin A (1) is a unique combination of benzoxazine, isoquinoline, and a pyran ring. Oxazinin A (1) exhibited antitycobacterial activity and modestly antagonized transient receptor potential (TRP) channels.

Marine fungal isolates have attracted attention as an important resource for novel natural products. Most natural product-synthesizing marine fungi were isolated from marine sediment, sponges, and algae. Only 3% were isolated from ascidians,† marine animals that are rich sources of structurally elegant, pharmaceutically potent secondary metabolites.‡ It has been demonstrated in some cases that symbiotic bacteria produce the compounds isolated from ascidians.‡ However, in one collection we identified a novel filamentous fungus, Eurotiomycetes strain 110162, the crude extract of which exhibited antibacterial activity against Mycobacterium tuberculosis.

Strain 110162 was isolated from the ascidian Lissochlinum patella collected in Papua New Guinea (10.0370785 S 145.767741 E). Strain analysis using 18S rRNA and internal transcribed spacer (ITS) gene sequences indicated that strain 110162 represents a member of the class Eurotiomycetes (Figure S1S, Supporting Information). Using a variety of molecular markers, strain 110162 forms a branch point at the root of order Oosyneciales,† such that it either falls within this order as a relatively novel strain or it may form a related, new order. Gene sequences have been deposited in GenBank, accession nos. KM054976 and KM054977.

Bioassay-guided fractionation led to the isolation of oxazinin A (1) as the compound primarily responsible for antimycobacterial activity. The molecular formula C₈₈H₆₂N₂O₁₆ was assigned to 1 on the basis of ESI-FT-ICR MS analysis (m/z 947.4460 [M + H]⁺). The structure of 1 was elucidated using a combination of NMR experiments observed in CD₃CN, including 1H, 13C, HSQC, HMBC, COSY, NOESY, and 1H−15N-HMBC. Analysis of the 1H, 13C, and HSQC spectra (Table S1, Supporting Information, and Figure 1) suggested the presence of 10 aromatic protons and 8 double-bond protons, along with 34 olefinic or aromatic carbons and 4 carboxyls. Detailed interpretation of the HMBC and COSY correlations indicated the presence of two 4-chromanone moieties, two prenyl groups, and two anthranilate moieties, suggesting 1 has a dimeric structure.

A 4-chromanone moiety was deduced by the HMBC correlations from the aromatic singlet proton at δH 7.41 (H-6') to the two nonpronated aromatic carbons (C-8' and C-10') and the ketone carbonyl at δC 194.4 (C-4'), along with HMBC correlations from H-3' to C-4' and the oxygenated quaternary carbon C-2'. An upfield chemical shift of C-4' indicated conjugation with the phenol ring. The chemical shift of C-2' (δC 84.6) and C-10' (δC 157.2) indicated that the phenol ether bond of the chromanone moiety links C-2' to C-10'. A hydroxyl group at C-3' was deduced by the chemical shifts of H-3' and C-3', and by the COSY correlation between H-3' and 3'-OH observed in DMSO-d₆ (Supporting Information). The HMBC correlations from the protons of two methyl groups (H-1'/17') to C-2' and C-3' indicated a dimethyl substitution at C-2'. Furthermore, the HMBC correlations

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correlation between H-25′ and H-24′ confirmed the connectivity between the 4-chromanone moiety and the anthranilate moiety through the C24′-N25′ bond. A 1H−15N HMBC experiment further reinforced the connectivity: correlations were observed from H-24′ and H-27′ to N-25′.

On the second half of the dimer structure of I, prenylated 4-chromanone and anthranilate moieties were similarly confirmed by 2D NMR correlations (Table S1, Supporting Information, and Figure 1). Both halves of the dimer showed similar chemical shifts, with three notable exceptions. (1) No exchangeable NH proton was observed for the second anthranilate moiety, indicating a trisubstituted N atom. The chemical shift of the carbonyl (δC 166.1, C-32) was shifted upfield by 4 ppm in comparison to that of C-32′, suggesting an ester bond at C-32 rather than a free carboxylic acid. (2) A methine group (δH 6.51, H-24, δC 80.8, C-24) was connected to C-9 of the second 4-chromanone moiety, as shown by the HMBC correlations from H-24 to C-8, C-9, and C-10. The methine proton H-24 also showed a HMBC correlation to C-32. The methine proton H-24 has an unusual downfield chemical shift of δH 6.51, which suggested that C-24 was substituted by both oxygen and nitrogen. 1H−15N HMBC correlations from H-24 to N-25 and from H-27 to N-25 suggested that the second anthranilate moiety and the methine group at C-24 were cyclized to form a hydro-1,3-benzoxazine-4-one substructure (Figure 1). (3) The presence of two more methine carbon signals (δC 46.0, C-11 and δC 59.1, C-12) indicated the pentadienyl group at C-8′. The pentadienyl group thus established was shown to be connected to the nitrogen atom of an anthranilate unit. The COSY correlations of H-27′/28′/29′/30′ and the HMBC correlations from H-30′ to a carbonylic carbonyl (C-32′), and from NH proton H-25′ to C-27′ and C-31′ established an anthranilate moiety. The chemical shift of C-32′ at δC 170.9, along with the observation of an exchangeable proton at δH 10.34 indicated a carbonylic acid at C-32′. Furthermore, the HMBC correlations from H-25′ to C-9′ of the 4-chromanone moiety, and from H-24′ to C-8′ and C-10′, as well as a COSY correlation between H-25′ and H-24′ confirmed the connectivity between the 4-chromanone moiety and the anthranilate moiety through the C24′-N25′ bond. A 1H−15N HMBC experiment further reinforced the connectivity: correlations were observed from H-24′ and H-27′ to N-25′.

Figure 1. Key HMBC, 1H−15N HMBC, and COSY correlations of compound 1.

Figure 2. Key ROESY correlations for compound 1. (A) ROESY correlations in red and blue determine the relative configurations of centers C-3, C-24, C-12 and C-3′, C-24′, respectively. (B) Along with a 10 Hz coupling constant between H-24′ and H-11, the ROESY correlations (black arrows) define the relative configuration of the molecule except at position C-11, for which two possibilities remained. Presented are not true Newman projections, but they clearly indicate the relative orientations of the labeled groups.
The ROESY correlations H-24/H-13 and H-24/H$_3$-17 indicated that these protons are in the β-orientation (Figure 2A). The ROESY correlations between H-3/H$_2$-16 and between H$_2$-17/H-24 indicated that the 3-OH is also in the β-orientation, establishing stereocenters 3$^R$,11$^R$,24$^S$. A ROESY correlation between H-3$'$ and H$_2$-16$'$ indicated that these protons are on the same face (Figure 2A). Moreover, the strong ROESY correlations between H$_2$-16$'$/H$_2$-18 and H$_2$-17$'$/H$_2$-25 are only possible if the molecule adopts a preferred conformation orienting the pyrone portion of the 4-chromanone moiety anti (or nearly anti) to H-24$'$. This allowed the relative configuration between C-24 and C-3$'$ to be assigned as 3$^S$,24$^R$. What remained was to define the relative configuration between C-12/C-11/C-24$'$.

From modeling with Chem3D and molecular dynamics simulation (MD) using AMBER 14, it was clear that rotation about the bonds C-11/C-24$'$/C-9$'$ was hindered due to steric constraints, and the molecule should adopt a single conformation. The large coupling constant (10.0 Hz) between H-11 and NH-24$'$ suggested either a nearly 180° or a 0° dihedral angle between H-11 and H-11$'$ (Figure 2B). The ROESY correlations between H-11/H-25$'$ and H$_2$-18/NH-25$'$ were only likely if H-11 was in the eclipsed conformation with H-24$'$ in the 11$^R$,24$^S$ configuration or if the bond was in the anti conformation with the 11$^R$,24$^R$ configuration (Figure 2B).

With that information, the four remaining possible configurations of the molecule were 3$^S$,125$^S$,24$^S$ with the remaining centers: (A) 11$^R$,3$^R$,24$^S$ (eclipsed C-11/C-24$'$); (B) 11$^S$,3$^S$,24$^R$ (eclipsed C-11/C-24$'$); (C) 11$^R$,3$^S$,24$^S$ (anti C-11/C-24$'$); and (D) 11$'^S$,3$'^R$,24$'^S$ (anti C-11/C-24$'$). Possibilities A and D could be readily ruled out because, with these isomers, H$_2$-16$'$/H$_2$-18 are very distantly located and therefore could not lead to the observed ROESY correlation. Finally, molecular modeling was used to determine the configuration of C-11 (Figure 3).
unique combination of benzoaxazine, isoquinoline, and a pyran ring.

Oxazinin A (1) exhibits many structurally novel features, and it comes from a taxonomic group of fungi for which little chemical data is available. The strain groups most closely with the order Onygenales, a group from which few compounds have been described. However, it may not lie within that order, but instead may represent a fairly novel fungal group. Further work will be needed to clarify the phylogenetic relationships among these strains. Further work is also required to determine whether or not strain 110162 is a true associate of the ascidian from which it was cultivated, as well as whether it is a true marine fungus.

**ASSOCIATED CONTENT**

Supporting Information

Full NMR data and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**


