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Exiguamine A, an Indoleamine-2,3-dioxygenase (IDO) Inhibitor Isolated from the Marine Sponge *Neopetrosia exigua*

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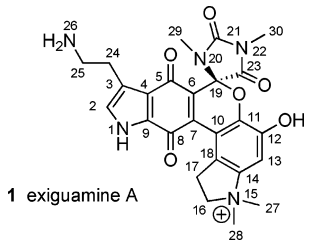
Indoleamine-2,3-dioxygenase (IDO; EC 1.13.11.42) catalyzes the oxidation of tryptophan to *N*-formylkynurenine in the first and rate-limiting step in the metabolic degradation of this essential amino acid.[1] IDO is thought to be responsible for providing immune protection to developing mammalian fetal tissue during gestation by decreasing local tryptophan availability and thereby suppressing the maternal T-cell based immune response.[2] T-cells are exquisitely sensitive to tryptophan depletion, which prevents them from undergoing antigen dependent activation, causes them to arrest in G_1, and leads to apoptosis and immunosuppression.[3] In addition, IDO is overexpressed in most tumors, so a role for the enzyme in the immune escape exhibited by solid tumors has been proposed that parallels the role postulated for IDO in the placenta.[4,5] Marshalling the immune system against solid tumors is an attractive approach to treating cancer, which makes IDO an appealing drug target.[4] Muller et al. have reported significant regression of established tumors in a mouse model system by combining even a relatively poor IDO inhibitor (e.g., 1-methyltrptophan) with cytotoxic agents such as paclitaxel, many of which were ineffective when used alone.[6] These in vivo experiments have provided proof of principle demonstration for the potential value of IDO inhibitors in cancer treatment.

Most known IDO inhibitors are tryptophan analogues that are active only at concentrations of $\geq 10\ \mu$M and, therefore, are marginal drug candidates.[4b] As part of a program designed to find more potent IDO inhibitors belonging to new structural classes,[7] we have screened a library of marine invertebrate extracts for their ability to inhibit purified recombinant human IDO in vitro. A MeOH extract of the sponge *Neopetrosia exigua* collected in Papua New Guinea showed potent activity in the assay. Bioassay guided fractionation revealed that the novel alkaloid exiguamine A (**1**) was the major IDO inhibitory component of the crude extract. Details of the isolation, structure elucidation, and biological activity of exiguamine A are presented below.

Neopetrosia exigua (140 g wet wt) was collected by hand using SCUBA in Milne Bay, Papua New Guinea and frozen on site. Freshly thawed specimens were extracted exhaustively with MeOH, which was evaporated in vacuo to give a brown gum (5.6 g). The gum was partitioned between water and *n*-butanol, and the bioactive *n*-butanol soluble material was fractionated via several rounds of reversed-phase HPLC (Supporting Information) to give pure exiguamine A (**1**: 20 mg).



1 exiguamine A

Exiguamine A (**1**) was isolated as deep-red optically-inactive crystals that gave a $[M]^+$ ion at *m*/*z* 492.1882 in the HRESIMS consistent with an elemental composition of $C_{25}H_{26}N_5O_6$ (calcd 492.1883). The ^{13}C NMR spectrum showed 25 well-resolved resonances in agreement with the HRMS analysis, and the HMQC/DEPT data indicated that 22 of the 26 hydrogen atoms were attached to carbon (C × 15; CH × 2, CH_2 × 4, CH_3 × 4). A LRESIMS measurement made in MeOH gave a $[M]^+$ ion at *m*/*z* 492.2, and the same experiment using CD_3OD gave a $[M]^+$ ion at *m*/*z* 496.2, confirming that the four remaining hydrogen atoms were exchangeable. ^{1}H,^{15}N gs-HMQC correlations identified five distinct nitrogen resonances (δ −349, N-26; −310, N-15; −275, N-20; −248, N-22; −218, N-1), also in agreement with the HRMS data.

Fragments of exiguamine A (**1**) could be assembled from the 2D NMR data (Figure 1). A complex multiplet at δ 2.99 (H_2-25: HMQC to 38.3) showed COSY correlations to a second multiplet at 2.92 (H_2-24: HMQC to δ 23.3) and to a broad singlet at 7.82 (NH_3-26). The H_2-24 resonance and the NH-26 resonance showed HMQC correlations to a nitrogen resonance at δ −349 (N-26) in agreement with the presence of an ethylamine moiety. A methine at δ 7.30 (d, J = 2.2 Hz, H-2) showed a HMBC correlation to the C-24 resonance at δ 23.3, and H_2-24 (δ 2.92) showed HMBC correlations to carbon resonances at δ 120.7 (C-3), 121.3 (C-4), and 126.5 (C-2) indicating that the ethylamine moiety was attached to a trisubstituted double bond. The methine at δ 7.30 (H-2) showed a COSY correlation to an exchangeable resonance at δ 13.10 (NH-1) and a HMQC correlation to the nitrogen resonance at δ −218 (N-1), which demonstrated that C-2 was bonded to an NH. A pair of carbon resonances at δ 173.4 (C-5 or C-8) and 179.7 (C-5 or C-8) were assigned to quinone carbonyls. Weak HMBC correlations observed between H-2 (δ 7.30) and both of the quinone carbonyl resonances, assigned to W coupling, and between NH-1 (δ 13.10) and carbon resonances at δ 179.7 (C-5 or C-8), 173.4 (C-5 or C-8), 120.7 (C-3), 126.5 (C-2), and 131.6 (C-9), were consistent with fragment **I** shown in Figure 1.

An isolated ^{1}H spin system composed of two sets of adjacent methylene protons (δ 3.22, H-17; 3.73, H-17′; HMQC to δ 28.5: 3.84, H-16; 4.17, H-16′; HMQC to 67.4) was identified from the COSY, HMQC, and HMBC data. A pair of methyl resonances at

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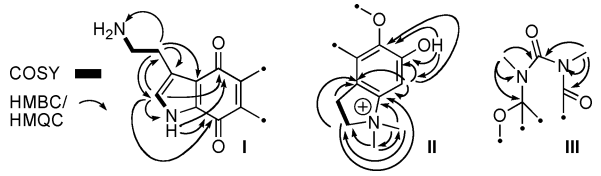

Figure 1. Fragments of exiguamine A (**1**) identified from NMR data.

δ 3.43 (Me-27: HMQC to δ 54.3, HMBC to 53.2) and 3.51 (Me-28: HMQC to δ 53.2, HMBC to 54.3) each showed HMBC correlations to the carbon correlated to the other methyl resonance in the HMQC spectrum, indicating that the methyls were geminal, and their carbon chemical shifts suggested they were attached to nitrogen. The Me-27 and Me-28 resonances (δ 3.43 and 3.51) both showed HMBC correlations to the C-16 resonance at δ 67.4 and both the H-16 and H-16′ resonances (δ 3.84 and 4.17) showed HMBC correlations to Me-27 (δ 54.3) demonstrating that C-16 was attached to the same nitrogen (N-15). H-16′ (δ 4.17), H-17′ (3.73), Me-27 (3.43), and Me-28 (3.51) all showed HMBC correlations to a nonprotonated carbon resonance at δ 142 (C-14), and H-16′, H-17′, and H-17 (3.22) all showed HMBC correlations to a nonprotonated carbon resonance at δ 122.8 (C-18), which together demonstrated that N-15 and C-17 were linked via a tetrasubstituted double bond to form an *N*,*N*-dimethyldihydropyrrole.

Strong HMBC correlations were observed from a singlet methine at δ 7.52 (H-13: HMQC to 108.7) to carbon resonances at δ 122.8 (C-18), 142 (C-14 and C-12), and 146.5 (C-11), and a weak correlation was observed to 114.7 (C-10). Additional HMBC correlations were observed between an exchangeable proton (OH-12; δ 10.42) and carbon resonances at δ 108.7 (C-13), 142 (C-12), and 146.5 (C-11). The H-13 (δ 7.52), Me-27 (3.43), Me-28 (3.51), and H-17 (3.22) resonances all showed HMQC correlations to a nitrogen resonance at δ −310 (N-15). A NOESY correlation was observed between δ 7.52 (H-13) and 3.51 (Me-28). All of the above data were consistent with the fragment **II** (Figure 1).

A methyl resonance at δ 3.07 (Me-30: HMQC to δ 25.2) showed HMBC correlations to carbon resonances at δ 154.5 (C-21) and 168.6 (C-23) and a HMQC correlation to a nitrogen resonance at δ −248 (N-22), while a second methyl resonance at δ 2.44 (Me-29: HMQC to δ 26.0) showed HMBC correlations to carbon resonances at δ 154.5 (C-21) and 85.4 (C-19) and a HMQC correlation to a nitrogen resonance at δ −275 (N-20). These data were assigned to fragment **III** (Figure 1).

Although fragments **I**−**III** accounted for all of the atoms in exiguamine A (**1**), there was insufficient information in the NMR data to assign a constitution to the entire molecule. Therefore, exiguamine A was subjected to single-crystal X-ray diffraction analysis (Figure 2), which confirmed the presence of fragments **I**−**III** and revealed their connectivity as represented in **1**. Exiguamine A (**1**) crystallized in space group *C*2/*c*. The mirror symmetry relationship produced by the *c*-glide plane in this space group requires that both enantiomers must be present in the unit cell in equal amounts. This means exiguamine A occurs as a natural racemate in agreement with its lack of optical activity.

Exiguamine A (**1**) has a complex hexacyclic alkaloid skeleton without precedent among known natural products. Figure 3 shows a proposed biogenesis for the new skeleton starting from DOPA, tryptophan, and *N*,*N*-dimethylhydantoin. The generation of enantiomeric atropisomers during formation of the C-7/C-10 bond could account for the occurrence of **1** as a racemate.

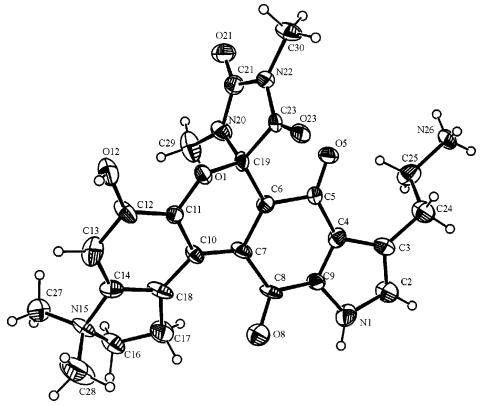

Figure 2. ORTEP diagram (33% ellipsoids) for exiguamine A (**1**).

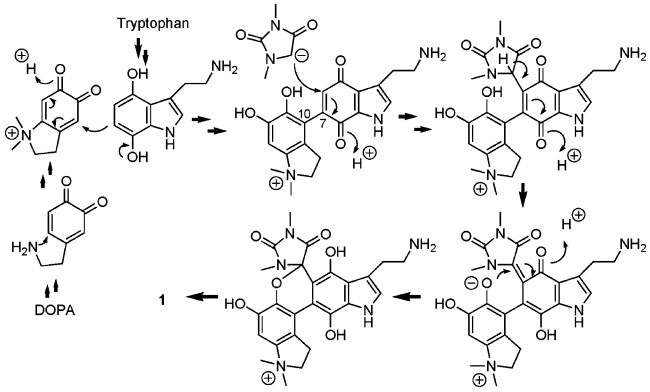

Figure 3. Proposed biogenesis for exiguamine A (**1**).

Exiguamine A (**1**) has a K_i of 210 nM for inhibition of IDO in vitro, making it one of the most potent IDO inhibitors known to date. It represents a novel natural product template that can be used to guide the design of synthetic inhibitors needed for the further validation of IDO as a drug target for treating cancer.

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Supporting Information Available: Experimental details and X-ray diffraction data; NMR data and spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Shimizu, T.; Nomiyama, S.; Hirata, F.; Hayaishi, O. *J. Biol. Chem.* **1978**, *253*, 4700−4706.
(2) Munn, D. H.; Zhou, M.; Attwood, J. T. *Science* **1998**, *281*, 1191−1193.
(3) Munn, D. H.; Shafizadeh, E.; Attwood, J. T.; Bondarev, I.; Pashine, A.; Mellor, A. L. *J. Exp. Med.* **1999**, *189*, 1363−1372.
(4) (a) Muller, A. J.; Prendergast, G. C. *Cancer Res.* **2005**, *65*, 8065−8068. (b) Muller, A. J.; Malachhowski, W. P.; Prendergast, G. C. *Expert Opin. Ther. Targets* **2005**, *9*, 831−849.
(5) Uyttenhove, C.; Pilotte, L.; Theate, I.; Stroobant, V.; Colau, D.; Parmentier, N.; Boon, T.; van den Eynde, B. *Nat. Med.* **2003**, *9*, 1269−1274.
(6) Muller, A. J.; Duhadaway, J. B.; Donover, P. S.; Sutanto-Ward, E.; Prendergast, G. C. *Nat. Med.* **2005**, *11*, 312−319.
(7) (a) Pereira, A.; Vottero, E.; Roberge, M.; Mauk, A. G.; Andersen, R. J. *J. Nat. Prod.* **2006**, *69*, 1496−1499. (b) Vottero, E.; Balgi, A.; Woods, K.; Tugendreich, S.; Melese, T.; Andersen, R. J.; Mauk, A. G.; Roberge, M. *Biotechnol. J.* **2006**, *1*, 282−288.

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