Letters

Synthesis of Indoleamine 2,3-Dioxygenase Inhibitory Analogues of the Sponge Alkaloid Exiguamine A

Gavin Carr,[†] Marco K. W. Chung,[‡] A. Grant Mauk,*[,‡] and Raymond J. Andersen*[,†]

Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada, and Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

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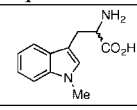
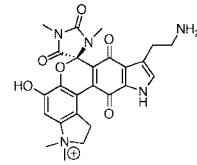
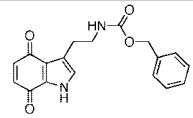
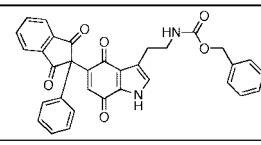
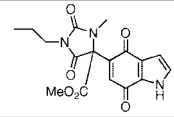
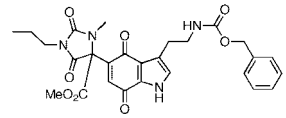
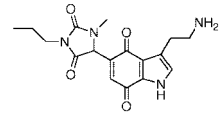
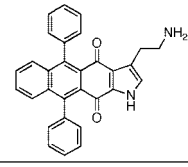
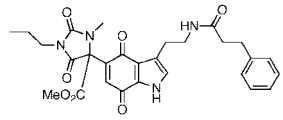
Abstract: Synthetic analogues of the sponge natural product exiguamine A (**3**) have been prepared and evaluated for their ability to inhibit indoleamine 2,3-dioxygenase in vitro.

Indoleamine 2,3-dioxygenase (IDO[a]) catalyzes the conversion of tryptophan into *N*-formylkynurenine in the first and rate limiting step in the catabolism of this essential amino acid.[1] A link between IDO and immune tolerance was first established by Munn et al. when they showed that treatment of pregnant mice with the IDO inhibitor 1-methyltryptophan (**1**) removed the toleragenic state protecting fetal tissue from the maternal immune system.[2] In environments where tryptophan concentration has been depleted by IDO, killer T cells cannot be activated by antigens, and they undergo G1 cell cycle arrest leading to apoptosis and immunosuppression.[3] One of the hallmarks of solid tumor cancers is immune escape, and it has been proposed that IDO contributes to this process by depleting local concentrations of tryptophan, much as it does in protecting fetal tissue.[4] Consistent with this hypothesis is the observation that IDO is overexpressed in many tumor cell types[4c] and that increased expression of IDO in tumor cells is correlated with poor prognosis for survival in patients with serious ovarian and colorectal cancers.[5,6]

Recently, there has been considerable interest in evaluating the potential of IDO inhibitors to mobilize the body's immune system against solid tumors. Muller et al. found that the IDO inhibitor **1** used in combination with a cytotoxic agent led to regression of tumors in mouse models that showed no response to the cytotoxin administered alone.[7] A similarly positive in vivo response has been obtained by using siRNA to silence the IDO gene in B16F10 tumor-bearing mice.[8]

Most of the experimental studies looking at small molecule IDO inhibitors have focused on the properties of **1**, which is not very water soluble or potent ($K_i \approx 62\ \mu$M, Table 1). Despite its limitations, **1** is in preclinical development at NCI as part of their RAID program.[9] In an attempt to find better IDO inhibitors that could be used as experimental tools and drug leads, we have screened a library of marine natural product extracts against recombinant human IDO in vitro. One of the first compounds discovered using the in vitro screen was the hydroid metabolite annulin C (**2**), which has a K_i of $\approx$ 140 nM, making it much more potent than **1**.[10] Exiguamine A (**3**), an even more potent IDO inhibitor having a K_i of 41 $\pm$ 3 nM, was subsequently obtained from the marine sponge *Neopetrosia exigua*.[11]

Table 1. In Vitro Inhibition of IDO

compound	K_i (nM)
1 	62,000 ± 7,000
3 	41 ± 3
9 	1490 ± 70
13 	10,900 ± 600
18 	Not active at ≤ 500,000
19 	190 ± 20
20 	420 ± 30
21 	260 ± 30
22 	200 ± 10
24 	Not active at ≤ 500,000
25 	260 ± 10

* To whom correspondence should be addressed. Phone: 604 822 4511. Fax: 604 822 6091. E-mail: randersn@interchange.ubc.ca.
[†] Chemistry and EOS.
[‡] Biochemistry and Molecular Biology.

[a] Abbreviations: IDO, indoleamine 2,3-dioxygenase; Cbz, carbobenzyloxy; DDQ, dichlorodicyanoquinone; siRNA, small interfering ribonucleic acid.

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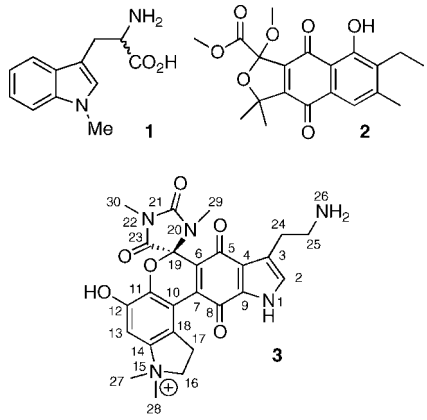


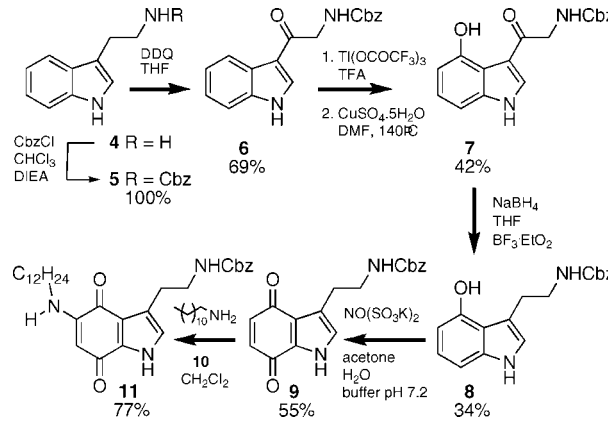
Exiguamine A (**3**), which is the most potent IDO inhibitor reported to date and combines structural features found in **1** (tryptamine) and annulin C (**2**) (quinone), is a particularly appealing lead structure for the development of synthetic IDO inhibitors. We envisioned that the tryptamine quinone substructure comprised the core IDO inhibitory pharmacophore in exiguamine A (**3**). However, it seemed unlikely that an unsubstituted tryptamine quinone would be stable. In exiguamine A (**3**), the hydantoin substituent at C-6 and the phenyl substituent at C-7 presumably stabilize the core pharmacophore by acting as steric impediments to the intermolecular Michael addition and intramolecular iminoquinone-forming reactions expected to take place with an unsubstituted tryptamine quinone.[12] At the outset, we also assumed that the positively charged quaternary ammonium ion in exiguamine A might prevent the molecule from crossing cell membranes. On the basis of the above analysis, we set out to prepare uncharged derivatives of tryptamine quinone that were substituted with hydantoin and various other moieties in an attempt to find easily accessible stable analogues of **3** having potent IDO inhibitory properties against the pure enzyme.

Our synthesis of the tryptamine quinone fragment followed an elegant sequential oxidative strategy developed by Tatsuta et al. for the preparation of indole quinone intermediates used in the synthesis of lymphostin.[13] Tryptamine **4** was first protected as its Cbz derivative **5** and then oxidized to the ketone **6** in good yield with DDQ (Scheme 1). Regioselective thallation of **6** at C-4 by reaction with $Tl(OCOCF_3)_3$ followed by treatment with $CuSO_4 \cdot 5H_2O$ gave phenol **7**. Ketophenol **7** was deoxygenated with $NaBH_4$ in the presence of BF_3 etherate to give **8**, which was oxidized with Fremy's salt [$NO(SO_3K)_2$] to give Cbz-protected tryptamine quinone **9** in good yield.

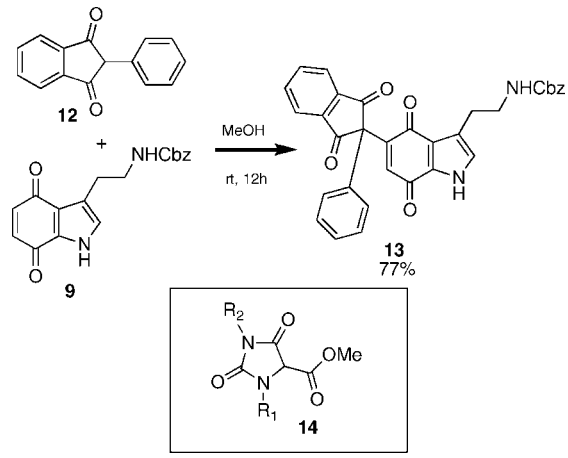
Reaction of quinone **9** with dodecylamine (**10**) at rt gave the Michael addition product **11**. This was consistent with our observation that deprotection of **9** by hydrogenolysis failed to give any isolatable products. The deprotected tryptamine quinone presumably polymerizes by intermolecular nucleophilic attack of the primary C-2′ amine at C-5.

A literature report that the readily deprotonated 2-phenyl-1,3-indanedione **12** adds to electrochemically generated 2,3-dimethylparaquinone in the presence of sodium acetate served as a model for our approach to adding a hydantoin substituent to the quinone moiety in **9**.[14] We found that **12** reacted smoothly with the protected tryptamine quinone **9** to give the C-5 Michael adduct **13** in excellent yield (Scheme 2). The ability of **12** to act as an effective carbanion nucleophile in a Michael addition to **9** suggested that a readily enolizabile hydantoin of the general structure **14** might serve as a synthon for adding the desired hydantoin substituent to C-5 of indole quinones.

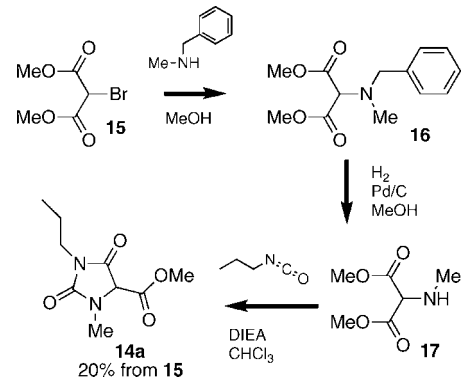
Scheme 1



Scheme 2

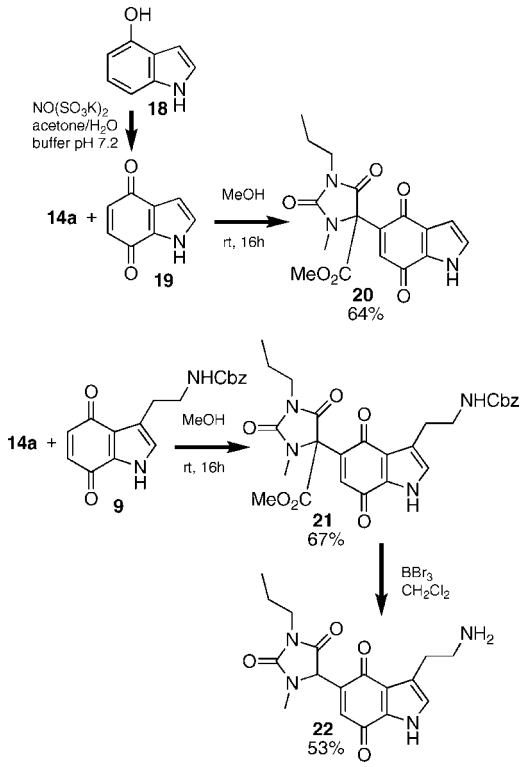


Scheme 3

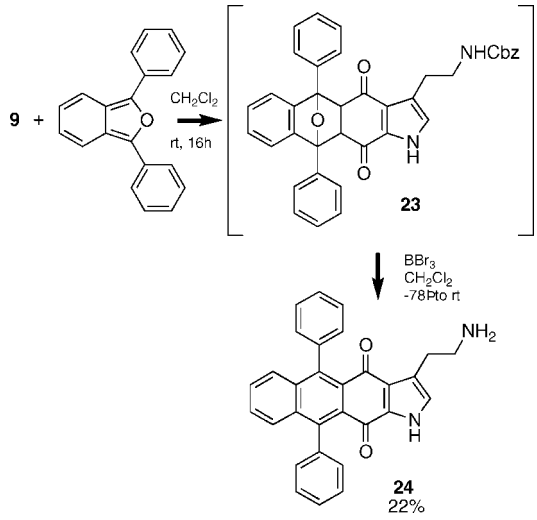


Hydantoin **14a** was routinely prepared in good yield from 2-(*N*-methylamino)dimethylmalonate (**17**) and *n*-propyl isocyanate using a modification of a literature procedure as shown in Scheme 3.[15] *n*-Propyl isocyanate was used instead of methyl isocyanate for ease of handling. Reaction of hydantoin **14a** and indolequinone **19**, prepared by Fremy's salt oxidation of the commercially available 4-hydroxyindole (**18**), gave the Michael adduct **20** in good yield (Scheme 4). Similarly, reaction of **14a** with the Cbz-protected tryptamine quinone **9** gave the Michael adduct **21**. Deprotection of **21** with BBr_3 removed the Cbz group and the methyl ester resulting in spontaneous decarboxylation to give the desired C-5-substituted tryptamine quinone **22**. Compound **22** was stable to normal organic chemistry manipulations, indicating that the hydantoin substituent at C-5 was

Scheme 4



Scheme 5



sufficient to block further Michael additions at C-6 and cyclic iminoquinone formation.

As an alternate approach to blocking the potential Michael addition and intramolecular iminoquinone-forming reactions of tryptamine quinone, compound **24** was prepared (Scheme 5). Reaction of quinone **9** with 2,5-diphenylisobenzofuran gave the Diels−Alder adduct **23**,[16] which was not isolated. Treatment of **23** with BBr_3 converted it to the desired product **24**, which was also stable to routine chemical manipulations.

The ability of the compounds prepared in this study to inhibit purified recombinant human IDO was evaluated with a steady-state spectrophotometric assay[17] using microtiter plates and a plate reader (Tecan Infinite M200). All assays were performed in triplicate, and the resulting double-reciprocal plots were analyzed by weighted linear-least-squares fits to the data. The results of these assays are provided in Table 1 and include the

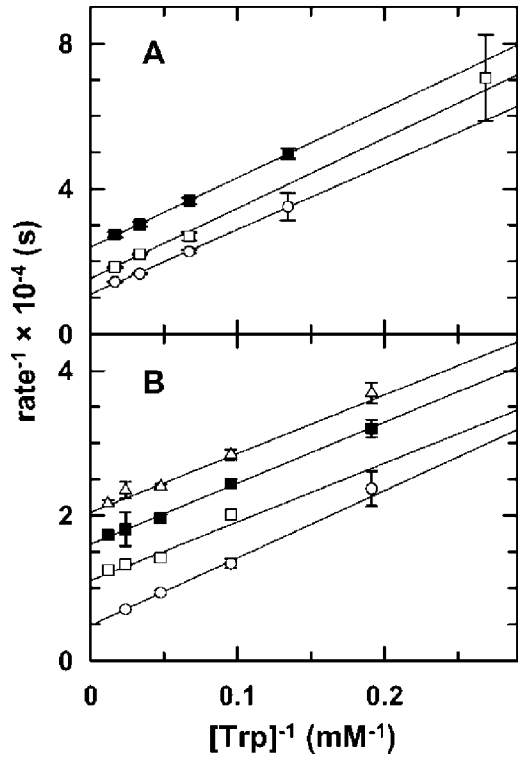


Figure 1. Steady-state kinetic analysis of recombinant human IDO by (A) **20** (inhibitor concentrations: open circle, no inhibitor; open square, 0.16 μM; closed square, 0.65 μM) and (B) **25** (inhibitor concentrations: open circle, no inhibitor; open square, 0.2 μM; closed square, 0.4 μM; triangle, 0.6 μM.) Error bars represent the standard deviation of each measurement for those cases where they exceed the dimensions of the symbols.

K_i determined for inhibition by **1**, the natural product exiguamine A (**3**), and the synthetic analogues of the putative pharmacophore of exiguamine A. It should be noted that the K_i of 41 nM reported here for exiguamine A (**3**) is lower than the value of ≈200 nM reported when the natural product structure was first described.[11] The difference in K_is for exiguamine A (**3**) results from the use of slight modifications to the data acquisition and analysis protocol in the two studies. In the current work, the K_i for **1** was redetermined with the revised protocol as a form of validation, and the result is comparable to the values reported previously (34 μM[7] and 62 μM[18]). Representative double-reciprocal kinetics plots (for **20** and **25**, Figure 1) are consistent with uncompetitive inhibition of IDO. Similar, uncompetitive inhibitory kinetics were observed for the other compounds included in Table 1.

The data in Table 1 shows that unsubstituted indole quinone (**19**), with a K_i of 190 nM, is a very good inhibitor of IDO in vitro and is roughly 300-fold more potent than **1**. In contrast, 4-hydroxyindole (**18**) is completely inactive, demonstrating that the quinone functionality in **19** is critical for activity. The reactivity of indole quinones (i.e., **9**, Scheme 1) toward Michael addition of amines at C-5 means that, even though indolequinone (**19**) is a potent inhibitor of IDO in vitro, it would be expected to have significant off target toxicity in vivo and it is not a good candidate for use in animal models. Simply blocking C-5 with a hydantoin substituent to give **20** should prevent Michael additions, but it results in a 2-fold reduction in potency. Adding a Cbz-protected ethylamine side chain at C-3 of indolequinone to give **9** reduced the potency 8-fold, and further substitution at C-5 with the 2-phenyl-1,3-indanedione moiety to give **13** led

to another 7-fold reduction in potency compared with **9**. Nevertheless, compound **13** is still more potent than **1**.

Combining the C-5 hydantoin substituent in **20** with a Cbz-protected ethylamine side chain at C-3 to give compound **21** produced an analogue that was only slightly less active than indole quinone **19**. Replacing the Cbz group in **21** with a phenylpropionamide residue to give **25** produced no change in potency, as exected. Removing the Cbz-protecting group in **21** to liberate a C-3 ethylamine side chain and at the same time removing the methyl ester fragment from the hydantoin moiety by decarboxylation to give **22**, gave an analogue that recaptured the potency of indolequinone **19**. The Diels−Alder adduct **24**, which has the C-5 and C-6 positions blocked with a fused aromatic ring, was completely inactive. It is noteworthy that a combination of the hydantoin and tryptaminequinone substructures present in the natural product exiguamine A (**3**) was required to give the potent synthetic analogue **22**.

In summary, starting from the lead structure of the sponge natural product exiguamine A (**3**), we have prepared the simpler synthetic analogue **22** that is a potent uncompetitive in vitro inhibitor of IDO. Although **22** is somewhat less active than the natural product **3**, it is readily accessible via synthesis and is roughly 300-fold more potent than **1**, which is currently undergoing development for clinical evaluation.[9] Compound **22** should be a useful new tool to study IDO in vitro and also for whole cell and animal model evaluations of the potential of IDO as a drug target in cancer and other human diseases.

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Supporting Information Available: Synthetic procedures and ^{1}H NMR spectra for compounds **9**, **13**, **19**, **20**, **21**, **22**, **24**, and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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