Bioorganic & Medicinal Chemistry Letters 23 (2013) 47–54

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Indoleamine 2,3-dioxygenase inhibitory activity of derivatives of marine alkaloid tsitsikammamine A

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ARTICLE INFO

Article history:
Received 28 September 2012
Revised 7 November 2012
Accepted 9 November 2012
Available online 22 November 2012

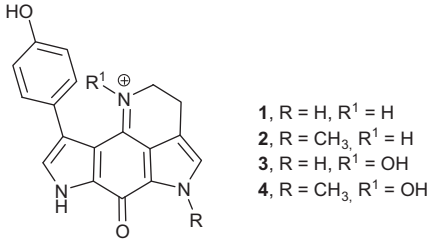
Keywords:
Pyrroloiminoquinones
Tsitsikammamines
Indoleamine 2,3-dioxygenase
Cancer immunology
Molecular modeling

ABSTRACT

Tsitsikammamines are marine alkaloids whose structure is based on the pyrroloiminoquinone scaffold. These and related compounds have attracted attention due to various interesting biological properties, including cytotoxicity, topoisomerase inhibition, antimicrobial, antifungal and antimalarial activity.

Indoleamine 2,3-dioxygenase (IDO1) is a well-established therapeutic target as an important factor in the tumor immune evasion mechanism. In this preliminary communication, we report the inhibitory activity of tsitsikammamine derivatives against IDO1. Tsitsikammamine A analogue **11b** displays submicromolar potency in an enzymatic assay. A number of derivatives are also active in a cellular assay while showing little or no activity towards tryptophan 2,3-dioxygenase (TDO), a functionally related enzyme. This IDO1 inhibitory activity is rationalized by molecular modeling studies. An interest is thus established in this class of compounds as a potential source of lead compounds for the development of new pharmaceutically useful IDO1 inhibitors.

Many alkaloids based on the pyrroloiminoquinone scaffold have been isolated and characterized from marine and other natural sources in recent years. These natural products of unique structures have attracted attention due to various interesting biological properties, such as cytotoxicity, inhibition of topoisomerases, antimicrobial, antifungal and antimalarial activity.[1–8] A subfamily of this class of compounds, the bispyrroloiminoquinones tsitsikammamine A (**1**) and B (**2**, Fig. 1),were first isolated and characterized in 1996 from a sponge of the *Latrunculiidae* family in the Tsitsikamma Marine Reserve, South Africa.[9] Further search for pyrroloiminoquinone metabolites as chemotaxonomic markers yielded two oxime derivatives **3** and **4** isolated from *Triceratium Favus* (Fig. 1).[10] Promising anticancer activities of these compounds were reported because of their abilities to intercalate into DNA and topoisomerase I inhibition.[7,9–13] More recently, the biosynthesis of tsitsikammamines in the latrunculid sponge *T. favus* has been suggested to originate from the microbial community associated with the species.[14]



1, R = H, R^1 = H
2, R = CH_3, R^1 = H
3, R = H, R^1 = OH
4, R = CH_3, R^1 = OH

Figure 1. Structures of tsitsikammamines **1**–**4**.

These results prompted studies on the synthetic accessibility of this class of compounds[15–17] aiming at an improved therapeutic value.[7,18–20] In this context, our group described a cycloaddition-based synthesis of aza-analogues of tsitsikammamines (wherein either of the pyrrole rings was replaced with a pyrazole) and evaluated their topoisomerase I and II inhibition as well as their in vitro

Abbreviations: IDO1, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; NHDF, normal fibroblast cell lines; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EDTA, ethylenediaminetetraacetic acid; IMAC, immobilized metal ion affinity chromatography; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; HBSS, Hanks balanced salt solution; SAR, structure–activity relationships.

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http://dx.doi.org/10.1016/j.bmcl.2012.11.036

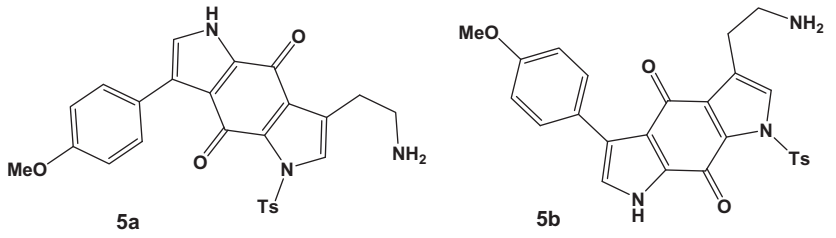
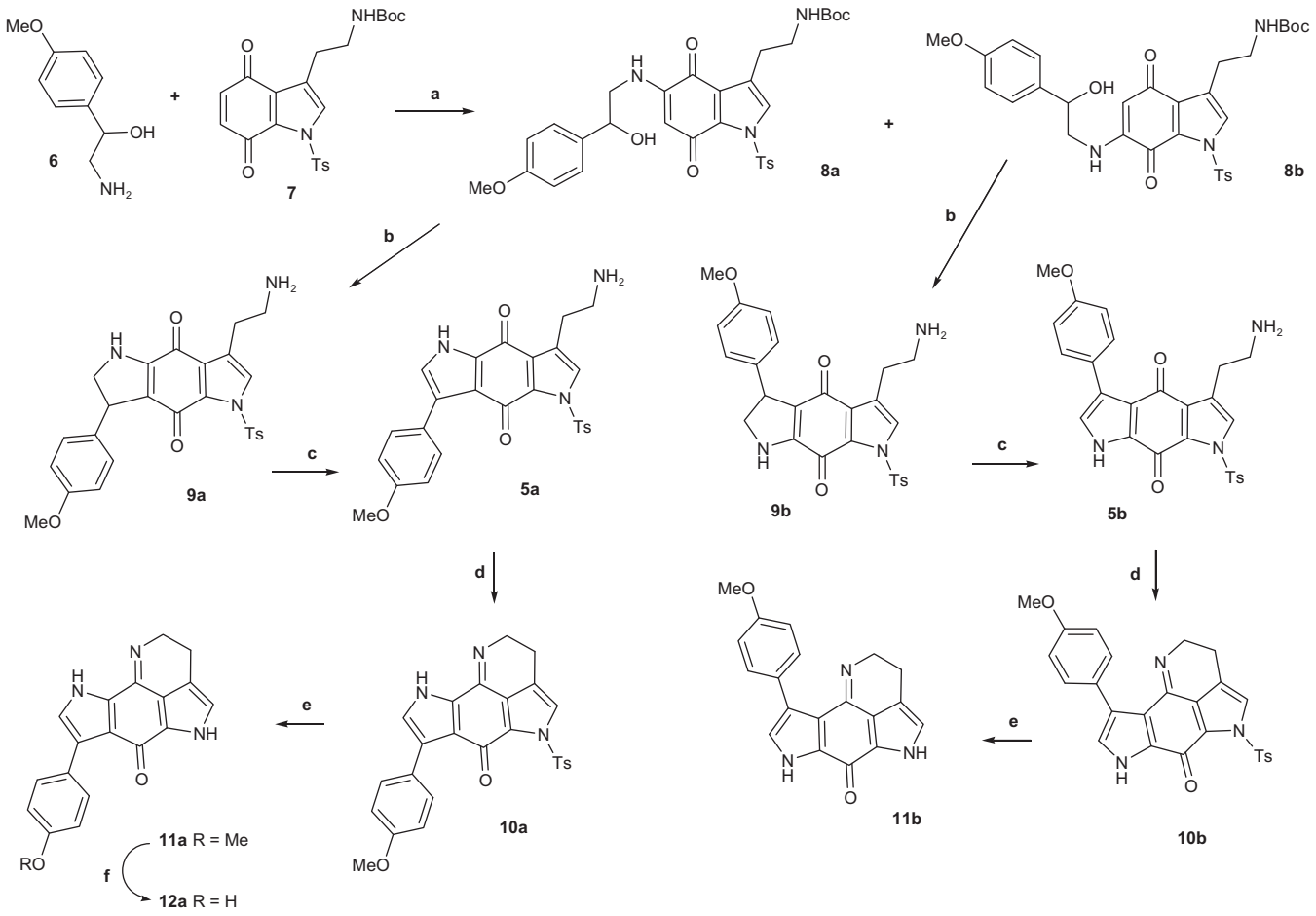


Figure 2. Structures of potential anticancer compounds **5a** and **5b**; Ts = *p*-$CH_3C_6H_4SO_2$–.



Scheme 1. Synthesis of compounds **8**–**12**. (a) abs EtOH, rt, 2 h; (b) TFA, CH_2Cl_2, rt, 4 h; (c) MnO_2, CH_2Cl_2, rt, overnight; (d) abs EtOH, 4 Å mol sieves, reflux, 3 h; (e) 1 M NaOH, dioxane, rt, overnight; (f) BBr_3, CH_2Cl_2, N_2, 4h.

antiproliferative activity.[21,22] Interestingly, some of these compounds showed an antiproliferative effect while being devoid of any topoisomerase activity, thus suggesting another mechanism of cell toxicity for this series. More recently, two regioisomeric series of tsitsikammamine analogues along with the corresponding synthetic intermediates were evaluated in an in vitro antiproliferative assay against the U373 glioblastoma, A549 non-small-cell-lung cancer and PC-3 prostate cancer cell lines as well as two human normal fibroblast cell lines (NHDF and Wi38).[23] This work allowed identifying the cytotoxic synthetic intermediate **5b** (Fig. 2) and its regioisomer **5a**. Using the MTT colorimetric assay, compound **5b** showed potent cytotoxicity towards all the cell lines in this study. Isomer **5a** displayed somewhat higher IC_{50} values in this test but also relative selectivity towards some cell lines. It was suggested that this effect could be associated with a specific antiproliferative activity. The conclusion was that compound **5a** and its derivatives represent interesting novel anti-cancer agents with an unknown mechanism of action.

Cancer immunotherapy, a strategy for cancer treatment consisting of stimulating the immune system to attack and destroy tumor cells, has so far shown limited efficacy in vivo, the main reason being the ability of tumors to escape an immune response. Among the various factors accounting for this phenomenon[24–27] are the tryptophan-catabolizing enzymes indoleamine 2,3-dioxygenase (IDO1; EC 1.13.11.52)[28–34] and tryptophan 2,3-dioxygenase (TDO; EC 1.13.11.11).[35] IDO1 is a monomeric extrahepatic cytosolic enzyme while TDO is homotetrameric and normally expressed at a high level in the liver and at low level in the brain.[36,37] Both enzymes are heme dioxygenases catalyzing the oxidation of the indole ring of L-tryptophan (Trp) to produce *N*-formylkynurenine. This reaction is the first and rate-limiting step of the kynurenine pathway of tryptophan catabolism. Another indoleamine 2,3-dioxygenase isoform, IDO2,