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Exploration of the influence of spiro-dienone moiety on biological activity of the cytotoxic marine alkaloid discorhabdin P

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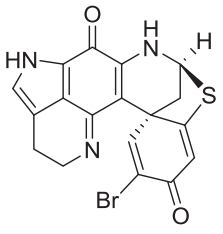
ABSTRACT

In order to clarify the importance of the C-3 carbonyl group to the cytotoxicity observed for discorhabdin marine alkaloids a number of semi-synthetic analogues of discorhabdin P were prepared. C-3 Reduction and acetylation typically resulted in a 4- to 10-fold reduction in cytotoxic potency (P388 cell line) compared to the corresponding keto parent compound. X-ray crystallography of a C-3 dienol derivative of discorhabdin P (**6**) allowed assignment of (3*r*, 6*r*) pseudoasymmetric configuration to the natural product 3-dihydrodiscorhabdin C (**5**). Analogues incorporating increasingly bulky substitution at C-3 only retained cytotoxicity if they bore (3*s*, 6*s*)-configuration at the spiro-dienol moiety. A variety of fluorophore-labelled probes were prepared of which only a dansyl analogue (**14**) exhibited (modest) cytotoxicity.

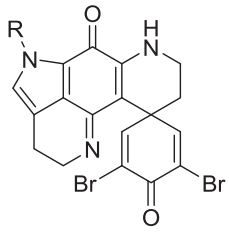
1. Introduction

A diverse array of over forty members of the discorhabdin/prianosin/epinardin family of natural products have been reported from marine sponges.[1,2] These pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline-skeletoned alkaloids, which include discorhabdins B (**1**), C (**2**) and P (**3**), typically exhibit potent cytotoxicity,[3–8] anti-bacterial,[9–11] enzyme inhibitory[11,12] and anti-malarial activities.[10] More recently, several members of the discorhabdin family have been reported to be moderate inhibitors of protein-protein interaction, interrupting the interaction between hypoxia-inducible factor 1α (HIF-1α) and its transcriptional coactivator p300.[13]

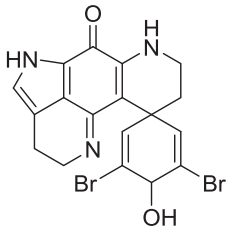
Structure-cytotoxicity relationship studies of discorhabdins have suggested a model for bioactivity with the combination of both the iminoquinone and spiro-enone fragments being essential for cytotoxity.[2,14] In the specific case of discorhabdin B (**1**), we have more recently demonstrated support for this model, showing that the relative electrophilic reactivity of the marine natural product and analogues correlates with cytotoxicity.[15]



1 (+)-discorhabdin B



2 R = H discorhabdin C
3 R = CH_3 discorhabdin P



4 3-dihydrodiscorhabdin C

Somewhat contrary to this SAR proposal are the biological activities observed for 3-dihydrodiscorhabdin C (**4**). While the original report of semi-synthetically-derived 3-dihydrodiscorhabdin C

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noted it to be less cytotoxic towards the murine leukemia cell line P388 (IC_{50} 0.91 μM)[16] compared to spiro-dienone discorhabdin C (IC_{50} 0.09 μM),[16] subsequent reports of naturally occurring **4**[5,10,11] noted that the two alkaloids exhibited similar levels of cytotoxicity towards the K562 human myeloleukemia[11] and Vero monkey kidney non-malignant cell lines.[10]

In an effort to clarify this ambiguity in potency of biological activity, we have prepared a series of C-3 modified analogues of a discorhabdin alkaloid, each bearing variation in substituent steric bulk and stereochemical configuration at C-3. The series of analogues also included fluorescent derivatives that could be utilized in mechanism of action studies or to help define specific cellular targets of the discorhabdins. Herein we report the semi-synthesis and *in vitro* anti-tumour evaluation of a library of C-3 modified analogues of discorhabdin P (*N*-13 methyl discorhabdin C), **3**. During the course of this study, the relative configuration of naturally occurring 3-dihydrodiscorhabdin C was secured by single crystal X-ray analysis of a derivative.

2. Results and discussion

While our original interest was directed towards preparing C-3 derivatives of discorhabdin C (**2**), given the previously noted nucleophilic reactivity of pyrrolic *N*-13,[17] we opted to study the *N*-13 methyl analogue, discorhabdin P (**3**).[12,18] The semi-synthetic preparation of 3-dihydrodiscorhabdin P can be achieved from discorhabdin C via two routes: (i) reduction at C-3, followed by *N*-13-methylation or (ii) the reverse sequence i.e. methylation at N-13 followed by C-3 reduction (Scheme 1). Following the first route, reduction of discorhabdin C with $NaBH_4$ in methanol afforded two dienol products **4** and **5** in 2:1 ratio, that were separable by C_{18} reversed-phase flash column chromatography. Direct comparison of ^1H NMR data observed for both products with data reported for naturally occurring 3-dihydrodiscorhabdin C[5,10,11,13,16] identified that the lower yielding isomer **5** was identical to the natural product.

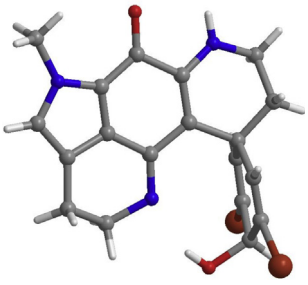
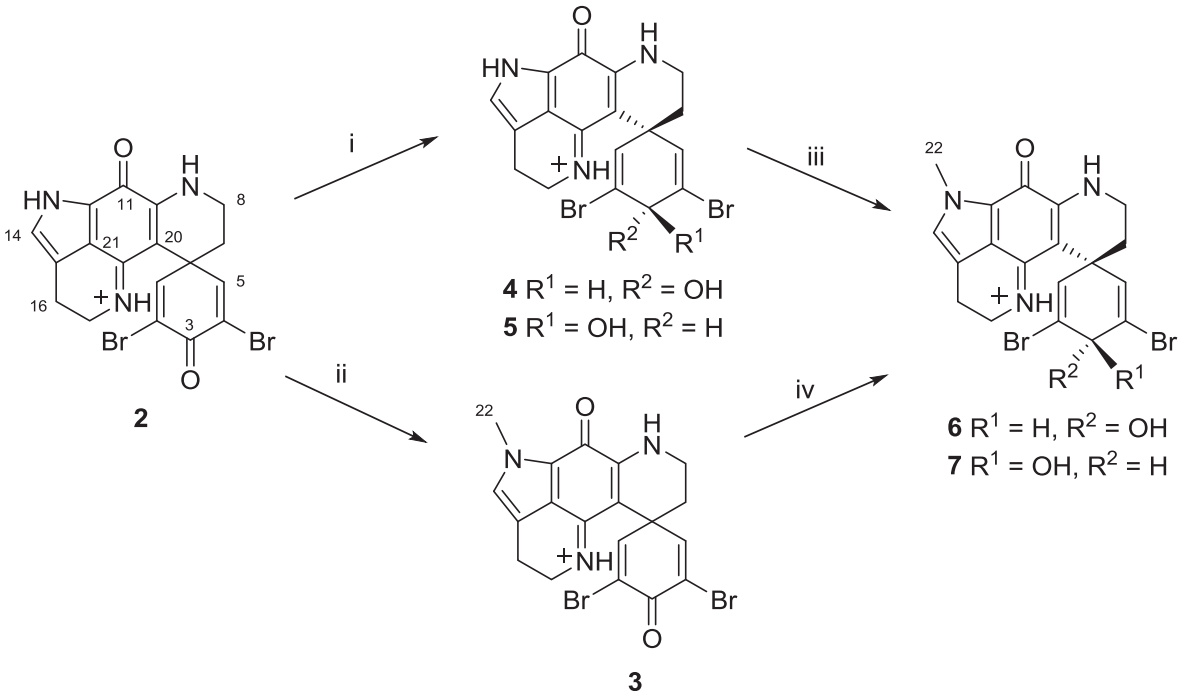


Fig. 1. X-Ray crystallographic structure of (3*s*, 6*s*)-3-dihydrodiscorhabdin P (**6**) free base (CCDC 928777).

Both dienol isomers were then converted to their respective *N*-13 methyl derivatives **6** and **7** by reaction with methyl iodide in acetone with yields of 80% and 90%, respectively (Scheme 1). Using the second route (Scheme 1), methylation of discorhabdin C gave discorhabdin P **3**[18] (96% yield), which followed by reduction with $NaBH_4$ in methanol gave **6** (59%) and **7** (32% yield). Spectroscopic data for these products were identical to those observed for samples prepared via the first route. Crystallization of the free base of **6** from $CH_3OH - CH_2Cl_2$ resulted in red crystals that yielded an X-ray crystal structure, allowing the assignment of the pseudoasymmetric configuration[19] as 3*s*, 6*s* (Fig. 1). This result allowed indirect assignment of (3*r*, 6*r*) configuration to naturally occurring 3-dihydrodiscorhabdin C (**5**). It is interesting to note that while the major product of hydride reduction at C-3 (**4**) is due to approach of reductant from the 'outside' face of the spiro-dienone, the naturally occurring C-3 dienol derivative of discorhabdin C (**5**) is the result of hydride delivery to the more sterically-crowded 'inner' face of the dienone ring.

With stereochemically-defined dienols **6** and **7** in hand, our attention turned to derivatization of the secondary alcohol at C-3. Successful acetylation of dienol **6**, to afford acetate **8** in 79% yield



Scheme 1. *Reagents and conditions*: (i) $NaBH_4$, MeOH, r.t., 5 min, **4** 46% and **5** 36%; (ii) CH_3I, K_2CO_3, acetone, 70 °C, 1 h, 96%; (iii) CH_3I, K_2CO_3, acetone, 70 °C, 1 h, 80% for **6** and 90% for **7**; (iv) $NaBH_4$, MeOH, r.t., 5 min, **6** 59% and **7** 32%.