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Potent inhibition of checkpoint kinase activity by a hymenialdisine-derived indoloazepine ☆

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Abstract—The marine sponge metabolite hymenialdisine is a potent inhibitor of a variety of kinases including MEK-1, GSK-3β, and CK1. In addition, hymenialdisine and debromohymenialdisine exhibit inhibition of the G_2 cell cycle checkpoint at micromolar concentrations. We report herein the potent inhibition of cell cycle kinase Chk2 by the indolic-hymenialdisine indoloazepine **1** ($IC_{50} = 8$ nM).

1. Introduction

DNA replication is a process that requires great accuracy and relies on surveillance mechanisms, which monitor DNA damage and initiate DNA repair.[1] The inability to carry out DNA repair often leads to the transformation of normal cells into malignancies.[2] Upon DNA damage, cell cycle checkpoints get activated, which delay the cell cycle progression and allow for DNA repair. A multi-faceted involvement of these checkpoint pathways regulate DNA repair,[1–3] telomere length,[4] and the induction of apoptotic cell death.[1,5]

Protein kinases regulate a host of cellular processes such as growth and differentiation, cell proliferation, and apoptosis.[6] DNA damage caused by radiation or chemotherapy triggers the DNA damage-responsive protein kinases ATM and ATR, which activate Chk1 and Chk2. Chk1 and Chk2 in turn phosphorylate Cdc25 and prevent Cdc2 activation, resulting in cell cycle arrest.[7] Hence, small molecules that can inhibit the checkpoints may enhance the efficacy of DNA damaging chemotherapeutics or radiation therapy.[8]

The marine sponge metabolite hymenialdisine (Fig. 1) was originally isolated from the sponges *Axinella verrucosa* and *Acantella aurantiaca* and its structure was established on the basis of X-ray crystallography.[9] Hymenialdisine and other oroidin compounds have been established to be potent anti-proliferative, anti-neurodegenerative, and anti-inflammatory agents in various cell lines and animal models.[10–14] Hymenialdisine was found to be a potent kinase inhibitor of several related CDKs.[10] In addition, hymenialdisine was found to be a potent inhibitor of the nuclear transcription factor NF-κB[11] and acts as a competitive nanomolar inhibitor of the cyclin-dependent kinases, GSK-3β and CK1 with IC_{50} values of 10 and 35 nM, respectively.[12] Crystallographic data portrayed the binding of the kinase inhibitor in the ATP binding pocket of the kinases.[12] In addition, Ireland and co-workers have identified hymenialdisine as a very potent mitogen-activated protein kinase kinase-1 (MEK-1) inhibitor with low nanomolar

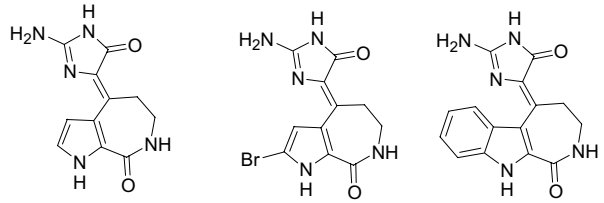

Figure 1. Structures of debromohymenialdisine, hymenialdisine, and hymenialdisine derivative **1**.

Keywords: Hymenialdisine; Kinase; Checkpoint; Chk2; Apoptosis.
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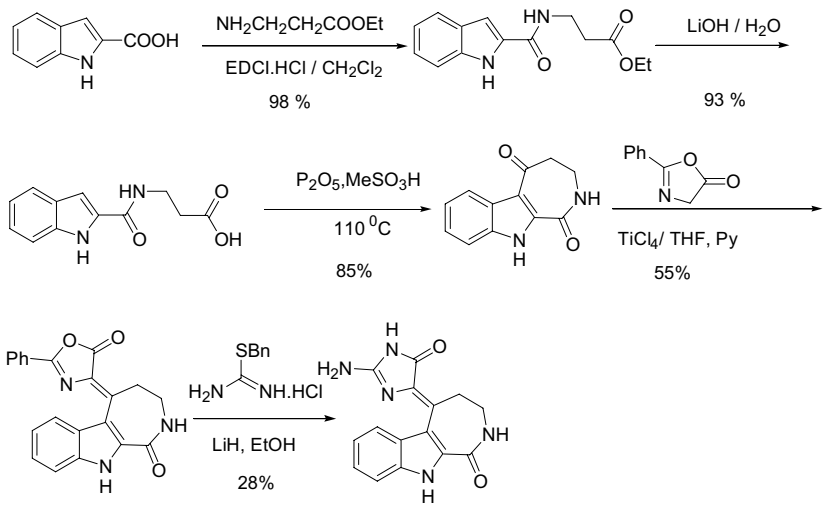

Scheme 1. Synthesis of indolic-hymenialdisine derivative **1**.

Table 1. IC_{50} values for kinase inhibition by compound **1**, hymenialdisine, and debromohymenialdisine

Kinase	IC_{50} (nM) compound **1**	Hymenialdisine	Debromohymenialdisine
CK1δ(h)	1352	35[10,12]	NA
CK2(h)	>10,000	7000[10,12]	NA
MEK1(h)	89	6[13]	824[13]
PKCα(h)	2539	700[10,12]	NA
PKCβII(h)	3381	1200[10,12]	NA
Chk1	237	1950	725
Chk2	8	42	183

NA: value not available.

IC_{50} values ($IC_{50} = 6$ nM).[13] In these studies we describe the synthesis and kinase activity of the indole-derivative of hymenialdisine, **1**.

2. Chemistry and biology

Roberge and co-workers have shown the inhibition of the G_2 DNA damage checkpoint by debromohymenialdisine as well as hymenialdisine in cells.[7] Inhibition of Chk1 and Chk2 by debromohymenialdisine was reported to correspond with IC_{50} values of 3 and 3.5 μM, respectively. Both debromohymenialdisine and hymenialdisine showed comparable IC_{50} values for inhibition of the G_2 checkpoint at 8 and 6 μM, respectively. This indicates that the bromide at the C2 position of hymenialdisine did not significantly influence the inhibition of the G_2 checkpoint.[7]

As a part of a program to develop analogs of the oroidin family of natural products, we recently synthesized **1** from the indole carboxylic acid in five steps and overall yield of 12% as shown in Scheme 1.[14] The indole-based hymenialdisine derivative **1** was found to contain potent anti-inflammatory activity as well as anti-proliferative activity.[14] Concurrently with our studies, the inhibition of purified GSK-3β, CDK5/p25, and CDK1/cyclin B by several hymenialdisine derivatives was investigated.[10] These studies indicated that the indole analogs exhibited kinase inhibition profiles similar to those of the pyrrole-based compounds.

Here, we present the potent inhibitory activity of the indoloazepine **1** against several purified kinases including the checkpoint kinases Chk1 and Chk2.[15] Table 1 illustrates the comparison between the in vitro activity of indoloazepine **1**, hymenialdisine, and debromohymenialdisine. Compound **1** exhibits very potent inhibition of Chk2 activity in the low nanomolar range ($IC_{50} = 8$ nM). Interestingly, unlike the natural product hymenialdisine, compound **1** exhibits an increased selectivity for the checkpoint kinases. Compound **1** exhibited a significant increase in potency for Chk 1 and Chk2 inhibition when compared to hymenialdisine. However, a significant drop in activity was found for all other kinases tested.

These kinase inhibition studies suggest that analogs of hymenialdisine may improve its overall kinase profile and significantly affect its kinase selectivity. Further in vivo studies would help in validating the therapeutic potential of hymenialdisine analogs for cancer therapy and neurodegenerative disorders.

Acknowledgements

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15. In vitro kinase assay: Compound **1**, hymenialdisine and debromohymenialdisine were tested in vitro against the kinases indicated by Upstate, UK using a Kinase Profiler Assay according to the manufacturer's protocol. Briefly, in a final volume of 25 μL, the kinase was incubated with the desired buffer and the required polypeptide substrate, in presence of 10 mM magnesium acetate and γ-^{33}P-ATP (10 μM). After incubation for 40 min at room temperature, the reaction was stopped by the addition of 3% H_3PO_4 (5 μL). A 10 μL of the reaction was then spotted on a P30 filtermat and washed 3× in 75 mM H_3PO_4 and finally in methanol. Samples were then dried and signals counted on a scintillation counter.