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The cytotoxic styryl lactone goniothalamin is an inhibitor of nucleocytoplasmic transport

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ABSTRACT

An in vivo nuclear export assay (immunostaining of Rio2 in HeLa cells) demonstrated that (*R*)-goniothalamin is an inhibitor of nucleocytoplasmic transport above 500 nM, which was rationalized also by molecular modeling. The cytotoxic styryl lactone natural product was prepared via an enantioselective Cr(III) catalyzed hetero Diels–Alder reaction and a Sonogashira coupling. A series of analogs was synthesized and only the oxidized goniothalamin derivative featuring an alkyne spacer was found active. Unsaturated lactones of natural origin other than leptomycin (LMB) are thus suggested to operate via a similar mechanism targeting the CRM1 nuclear receptor.

Goniothalamin (**1** Fig. 1) is the prototypical example of many styryl lactones that have been discovered in phytochemical studies of plants of the genus *Goniothalamus* (Annonaceae).[1] For this compound, a broad manifold of biological activities along the general theme of cytotoxicity has been described in the literature, ranging from antifungal,[2,3] antimicrobial,[3] insecticidal[4] to anticancer properties.[5] In the context of antiproliferative activity, Xiao and co-workers reported selectivity of goniothalamin on both drug-resistant and transformed hepatocytes when compared to a primary cell line.[6] Pilli and co-workers prepared analogs of goniothalamin and evaluated them in a panel of eight cancer cell lines leading to potent derivatives against kidney cancer cells and adriamycin resistant breast cancer cells.[7] Goniothalamin has been reported to selectively trigger caspase induced apoptosis in cancer cells, with little activity on normal cell lines.[8] Interestingly, up-regulation of p53 has been observed in goniothalamin treated rats.[9] In all these studies, the exact mechanism of action and the target of goniothalamin (**1**) remained unclear.

The polyketide lactone leptomycin (**2**, LMB) has been demonstrated to be a potent inhibitor of the CRM1-mediated nuclear export of proteins,[10] and hundreds of studies document its usefulness as tool compound in cell biology.[11]

Both leptomycin and the closely related derivative callystatin have been evaluated in clinical trials against cancer, but have failed in phase II due to unfavorable toxicity issues.[12] Recent studies suggested these toxic effects to be the result of off-target effects, and second generation analogs display promising results in cancer xenograft models.[13] In the context of our synthetic and biological studies on the anguinomycins,[14] it has been discovered that the truncated analog **3** retained almost the full biological potency of the parent compound. The structural similarity of this leptomycin analog **3** to goniothalamin (**1**) thus led to the hypothesis that goniothalamin (**1**) and similar naturally occurring lactones would

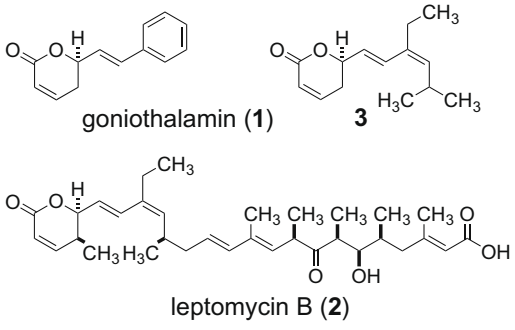

Figure 1. Goniothalamin (**1**), lemptomycin B (**2**, LMB) and the truncated analog **3**.

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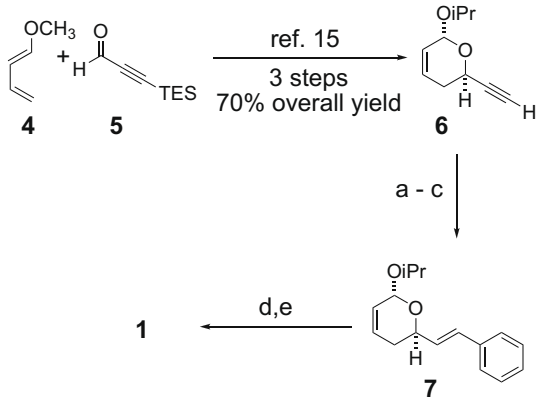
also constitute inhibitors of nucleocytoplasmic transport leading to potent antiproliferative effects. In this study, we present a new synthetic route to goniothalamin (**1**) and analogs, and analyze their role as inhibitors of nucleocytoplasmic transport in an in vivo transport assay in order to evaluate the above-mentioned hypothesis.

The Cr(III) catalyzed asymmetric hetero Diels–Alder reaction developed by Jacobsen and co-workers[15] provides a rapid and high-yielding synthetic entry into α,β-unsaturated lactones. In addition, the resulting intermediates can be rapidly functionalized by cross-coupling to provide a range of derivatives. We chose thus to utilize this synthetic approach for the preparation of goniothalamin (**1**) and analogs.

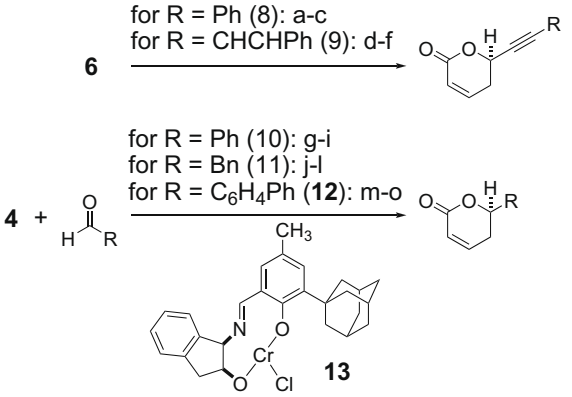
The key intermediate **6** was obtained by the reaction of methoxybutadiene (**4**) with TES protected propargylic aldehyde (**5**), followed by transacetalization and deprotection in 70% overall yield over three steps (Scheme 1).[14,15] The dihydropyran was then converted to the goniothalamin lactol ether **7** by hydrozirconation, transmetallation and Negishi cross-coupling.[16] Deprotection and oxidation furnished synthetic goniothalamin (**1**), the spectral properties of which were identical to those reported for the natural product.[17]

Analogs **8** and **9** were accessed via Sonogashira cross-coupling of intermediate **6** with bromobenzene or bromostyrene followed by deprotection/oxidation in 53% and 47% overall yield, respectively, over three steps. Finally, analogs **10**–**12** were directly obtained via the Cr-catalyzed hetero Diels–Alder reaction involving 1-methoxy-1,3-butadiene **4** and different aromatic aldehydes (phenylacetaldehyde, benzaldehyde or 4-biphenylcarboxaldehyde) followed by the deprotection/oxidation sequence (Scheme 2).

Overall, goniothalamin (**1**) and series of different lactones have been prepared, which allow for the evaluation of the distance of the aromatic ring to the lactone. Whereas in the truncated goniothalamin analogs, the double bond was omitted (**10**) or replaced by a CH_2 group (**11**), extended analogs containing an additional aromatic spacer (**12**) or an alkyne (**9**) were obtained. Lastly, in the alkyne **8**, the geometry of the side chain has changed by converting the bent alkene to the linear alkyne. The natural product **1** and all these analogs **8**–**12** have been tested for their ability to block CRM1-mediated nuclear export of the protein kinase Rio2 in HeLa cells (Fig. 2).



Scheme 1. Synthesis of goniothalamin (**1**). Reagents and conditions: (a) Cp2ZrHCl, 0 °C to room temperature, THF, 1 h; (b) $ZnCl_2$, THF, room temperature, 30 min; (c) iodobenzene, $Pd(PPh_3)_4$ (5 mol %), DIBAH (1.0 M in hexane), room temperature to 40 °C, 12 h, 85% over three steps; (d) PPTS, acetone/water (3:1), room temperature, 22 h; (e) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 61% over two steps.



Scheme 2. Synthesis of goniothalamin analogs **8**–**12**. Reagents and conditions: (a) iodobenzene, $Pd(PPh_3)_4$ (5 mol %), CuI, $EtN(iPr)_2$, THF, room temperature, 1 h; (b) PPTS, acetone/water (3:1), room temperature, 22 h; (c) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 53% over three steps; (d) bromostyrene, $Pd(PPh_3)_4$ (5 mol %), CuI, $EtN(iPr)_2$, THF, room temperature, 1 h; (e) PPTS, acetone/water (3:1), room temperature, 22 h; (f) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 47% over three steps; (g) **4**, benzaldehyde, **13** (5 mol %), 4 Å MS, room temperature, 14 h; (h) PPTS, acetone/water (3:1), room temperature, 22 h; (i) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 70% over three steps; (j) **4**, phenylacetaldehyde, **13** (5 mol %), 4 Å MS, room temperature, 14 h; (k) PPTS, acetone/water (3:1), room temperature, 22 h; (l) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 61% over three steps; (m) **4**, biphenyl-4-carboxaldehyde, **13** (5 mol %), 4 Å MS, room temperature, 14 h; (n) PPTS, acetone/water (3:1), room temperature, 22 h; (o) PCC, 4 Å MS, AcOH, CH_2Cl_2, room temperature, 3 h, 59% over three steps.

Goniothalamin **(1)** exhibited a strong inhibition of CRM1-dependent nuclear export at higher concentrations (Fig. 2). The compound fully blocked export of human Rio2 protein at 1 μM, with partial inhibition starting between 200 and 500 nM. Of all the other derivatives **8–12** tested, only **8** showed activity similar to goniothalamin (Fig. 2). All other compounds, namely **9–12**, did not show any significant inhibition of nuclear export up to a concentration of 2 μM (data not shown).

These biological data can be rationalized by inspecting the published model[14b] of LMB-mediated inhibition of CRM1, based on the recently published X-ray crystal structures of this exportin.[18] Superposition of goniothalamin (**1**) to LMB reveals a complementary fit of this natural product into the hydrophobic binding grove of the export receptor (Fig. 3). Goniothalamin is expected to be covalently bound to CRM1 and the styryl group mimics the hydrophobic diene part of LMB. Consequently, several both smaller and larger analogs of goniothalamin lead to negative steric interactions with this binding grove and thus to much lower (or no) activity towards CRM1. The exception is the alkyne **8**, which can still be accommodated by the substrate binding grove of the exportin.

Goniothalamin (**1**) has been reported to display cytotoxic activity in breast cancer cells with an IC_{50} value of about 1.46 μM, and to induce cell cycle arrest and apoptosis.[5] Based on our results, we hypothesize that these effects, and therefore the cytotoxicity, might be due to a block of CRM1-mediated nuclear export by goniothalamin at concentrations above 1 μM. This correlation is supported by certain described phenotypes of goniothalamin exposed cells, as for example up-regulation of p53 has been reported.[9]

In conclusion, we report a new route to goniothalamin (**1**) based on stereoselective hetero Diels–Alder and cross-coupling reactions. The versatility of the dihydropyran platform **6** allowed for the generation of several analogs probing the distance of the aromatic group to the lactone ring. Biological evaluation revealed that goniothalamin (**1**) is an inhibitor of

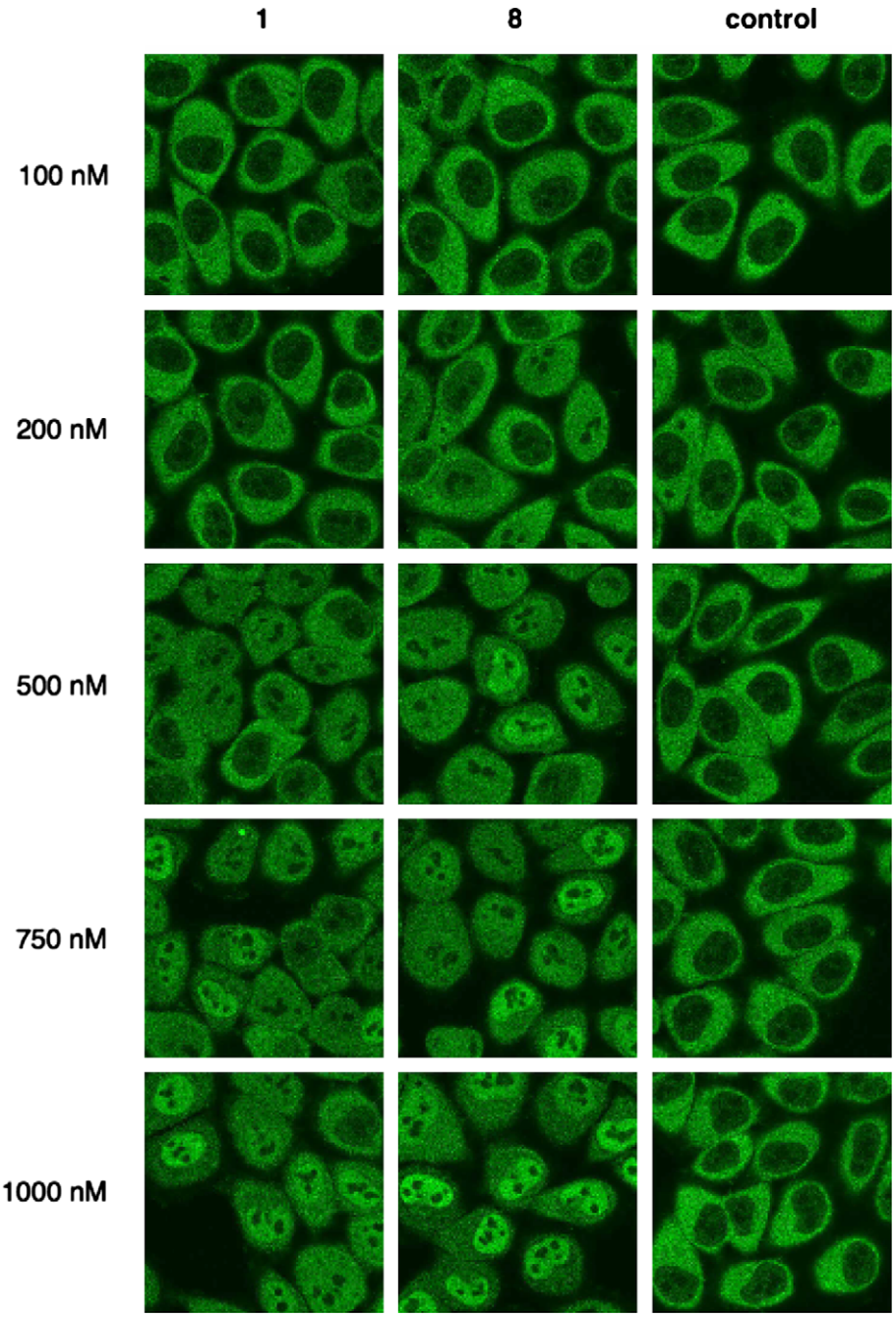


Figure 2. Goniothalamin (**1**) and the alkyne analog **8** inhibit CRM1-dependent nuclear export of Rio2 in HeLa cells.

nucleocytoplasmic transport, and structure–activity relationships established the molecular requirements for binding, which could also be rationalized by an atomistic description of the goniothalamin/CRM1 interaction.

The results documented in this study thus support the hypothesis that a target of goniothalamin (**1**) is the exportin CRM1 and that the antiproliferative activity of **1** might be due to a block of CRM1-mediated nuclear export. With a mechanism of action of this natural product revealed, the search for structurally simpler LMB analogs useful for anticancer purposes might be stimulated.

In addition, the arsenal of natural products capable of interfering with nuclear transport receptors is thus expanded by goniothalamin (**1**). It is reasonable to assume that other natural products, or even endogenous compounds featuring similar structural elements such as α,β-unsaturated lactones with hydrophobic appendages, might constitute inhibitors of CRM1-mediated nuclear export. Current efforts in our laboratories are directed towards their identification.

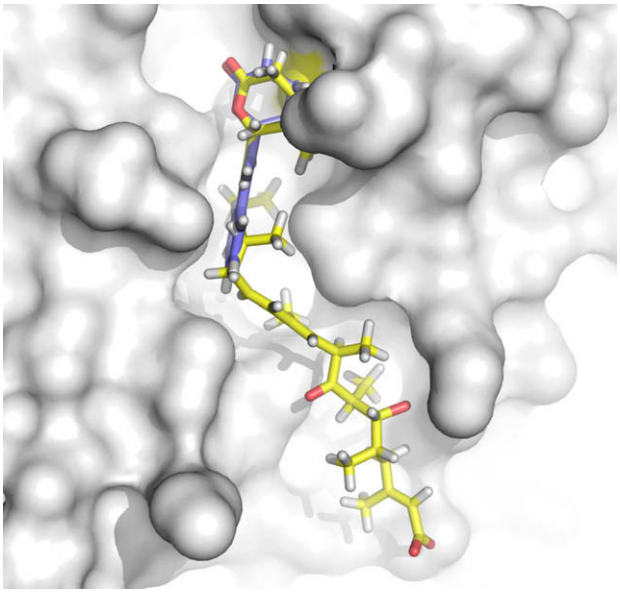


Figure 3. Superposition of goniothalamin (**1**, blue) and the model of leptomycin B (**2**, LMB, yellow) binding to CRM1[14b] based on the X-ray structures.[18]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.049.

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