J|A|C|S
COMMUNICATIONS

Published on Web 03/09/2006

Chemical Analysis of Norrisolide-Induced Golgi Vesiculation

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Isolated from the nudibranch *Chromodoris norrisi*,[1] norrisolide (**1**) has defined a new class of rearranged diterpenes that have an unusual structure, unexplored biology, and potent cytotoxicity.[1,2] The chemical motif of **1** is distinguished by the attachment of a perhydroindane core to a side chain containing an acetylated γ-lactol-γ-lactone ring system.[3] This peculiar side chain is also found in other biogenetically related natural products, such as macfarlandin C (**2**)[4] and dendrillolide A (**3**)[5] (Figure 1).

We have recently shown that norrisolide is the only compound known to fragment irreversibly the Golgi apparatus.[6] The Golgi complex, composed of stacks of flattened cisternae, is a central organelle of the secretory pathway, and its structure and organization is maintained by a controlled balance of membrane input and output. Transport into, across, and out of the Golgi complex is mediated by small vesicles. Additionally, the Golgi complex undergoes complete vesiculation during mitosis. Norrisolide is therefore a useful tool to characterize the process of Golgi fragmentation (vesiculation).[8] Here we report our studies aiming to define the chemical origins of the norrisolide-induced Golgi vesiculation.

To assess the biological activity of norrisolide as a function of its structure, we studied the effect of fluorescent probes **4**−**6** on the Golgi complex (Figure 2).[9,10] Normal rat kidney (NRK) cells plated on coverslips in complete growth medium were incubated with these probes (80 μM/DMSO) for 60 min, then fixed and processed for immunofluorescence microscopy. While **5** had no effect on the Golgi apparatus (data not shown), compound **4** was found to induce extensive Golgi fragmentation (Figure 3b). However, in contrast to **1**, this fragmentation was reversed upon washing (Figure 3c). The intracellular localization of **4** was evaluated in fixed cells. After fixation, the cells were first treated with Golgi-specific antibodies (Figure 3d) and then incubated with **4**. Under these conditions, probe **4** was shown to localize on the Golgi complex (Figure 3e,f). On the other hand, probe **5** did not show any specific localization.

To evaluate whether compound **4** and norrisolide bind to the same receptor, we performed a competition experiment (Figure 3g−i). We found that the natural product can displace **4** from the Golgi complex, as shown by the loss of green color, which is due to staining by **4**, but without perturbing the overall Golgi organization, as shown by the red coloring due to antibody staining. These studies indicate that norrisolide binds to the Golgi complex. A similar localization to **4** was obtained using probe **6** containing the entire framework of the natural product. Competition experiments showed again that **1** could displace **6** from its target (see Supporting Information). These results indicate that norrisolide induces phenotypic changes in cells by binding to the Golgi complex and suggest that the perhydroindane core of norrisolide is essential and necessary for such a binding. In the absence of the C19 acetoxy group of **1**, this binding does not resist washout and induces a reversible Golgi vesiculation.[6]

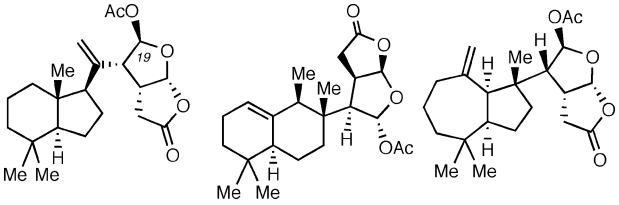

Figure 1. Representative structures of spongiane diterpenes.

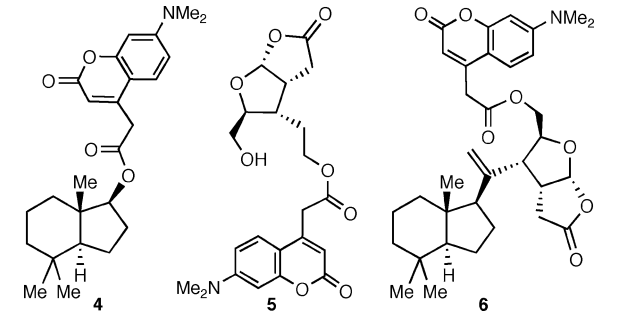

Figure 2. Structures of norrisolide-based fluorescent probes.

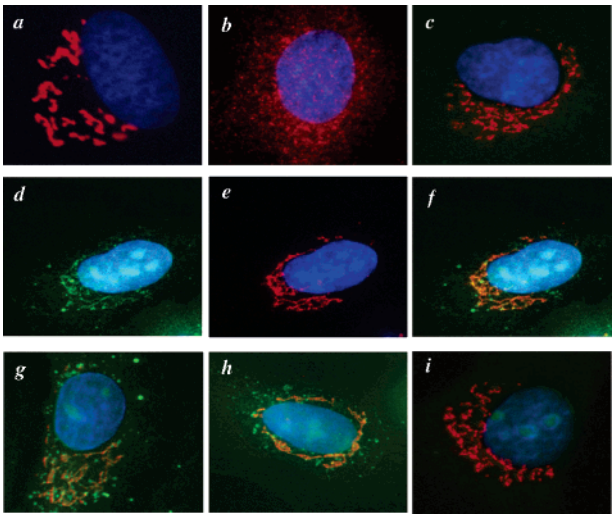

Figure 3. Activity and intracellular localization of compound **4**. (a−c) NRK cells treated with (a) DMSO for 60 min; (b) **4** for 60 min; and then (c) washed and allowed to recover for 60 min. Golgi is shown in red, nuclei in blue. (d−f) Fixed cells stained with (d) Golgi-specific antibody; and then (e) incubated with **4**. In (f) is shown the co-localization between the Golgi antibody and **4** (yellow color). (g−i) Competition experiment. Fixed cells preincubated with **4** were treated with PBS (g), DMSO (h), and norrisolide (100 μM) (i). The green coloring is due to compound **4**.

The above findings suggest that the C19 acetoxy group of **1** plays an essential role in the irreversibility of the fragmentation either

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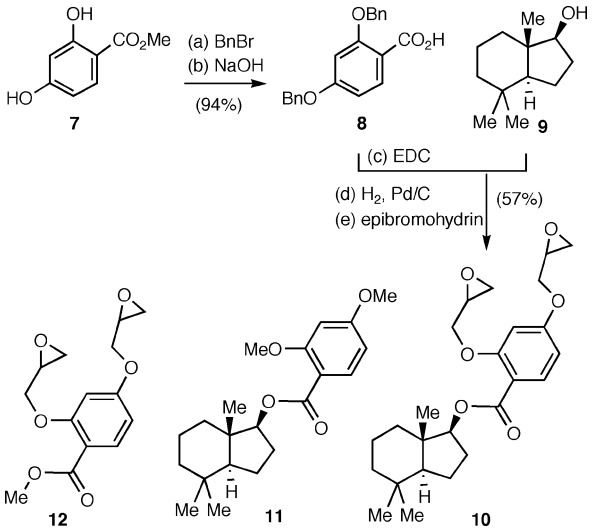
10.1021/ja058259a CCC: $33.50

Scheme 1 [a]



[a] Reagents and conditions: (a) BnBr (2.5 equiv), K_2CO_3 (4.0 equiv), acetone, reflux, 6 h, 95%; (b) NaOH (1 N), THF, 50 °C, 4 h, 99%; (c) **8** (1.7 equiv), **9** (1.0 equiv), EDC (1.7 equiv), DMAP (1.7 equiv), CH_2Cl_2, 25 °C, 24 h, 92%; (d) 10% Pd/C (0.1 equiv), H_2 (1 atm), EtOAc, 25 °C, 6 h, 92%; (e) *epi*-bromohydrin (4.0 equiv), $CsCO_3$ (4.0 equiv), DMF, 25 °C, 12 h, 75%.

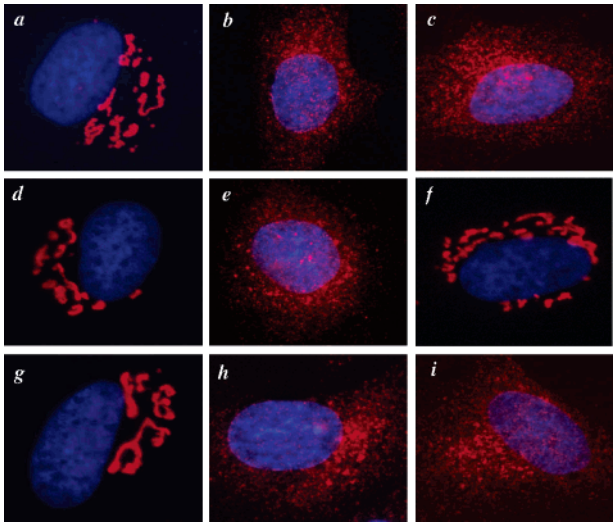


Figure 4. Effect of norrisolide and analogues on Golgi membranes. NRK cells (a, d, and g) were incubated with 40 μM of **1** (b), of **11** (e), and of **10** (h) for 60 min, then washed out and incubated in fresh medium for 60 min (c, f, and i). The cells were fixed and processed for immunofluorescence. The Golgi is shown in red (AlexaFluor 594), and the nuclei in blue (Hoechst).

by stabilizing the binding or by creating a covalent bond with its target protein.[11] Inspired by a study that identified epoxides as suitable functionalities for protein labeling,[12] we replaced the entire side chain of **1** with a bisepoxide motif to form adduct **10**. The synthesis of **10** is summarized in Scheme 1.[9] Compounds **11** and **12** were synthesized in a similar manner and used as controls.

The effect of norrisolide on the Golgi membranes is shown in Figure 4a−c. Cells treated with **1** underwent extensive Golgi vesiculation (Figure 4b) that persisted even after washing (Figure 4c). Analogue **11**, containing the core fragment of the natural product, induced a similar vesiculation (Figure 4e) that was, however, reversible upon washing (Figure 4f). In contrast, compound **10**, in which the perhydroindane core was attached to a bisepoxide scaffold, induced an irreversible vesiculation of the Golgi membranes and reproduced the cellular phenotype of the natural product (Figure 4g−i). On the other hand, compound **12**, lacking the perhydroindane motif, had no effect on Golgi membranes (data not shown), attesting to the importance of the norrisolide core in Golgi localization and structure.

In conclusion, we have dissected the chemical origins of the cellular phenotype of norrisolide using a variety of synthetic analogues. We found that this natural product induces an irreversible vesiculation of the Golgi complex in intact cells by binding to the Golgi membranes. This binding is attributed exclusively to the presence of the perhydroindane core that acts as the recognition element. Attachment of this core to a γ-lactone-γ-lactol side chain or similar motifs, such as in **11**, leads to a reversible Golgi vesiculation. This vesiculation becomes irreversible when the side chain is armed with electrophilic functionalities, such as the C19 acetyl group of **1** or the bisepoxide motif of **10**. The latter modification attests to the potential of the epoxide group for the development of affinity probes for protein labeling.[12,13] Moreover, compound **10** induces an identical phenotype to that of norrisolide, suggesting that it may be used to isolate the biological target of this natural product.

Acknowledgment. Dedicated to Professor K. C. Nicolaou on the occasion of his 60th birthday. Financial support by the NIH (CA 086079 to E.A.T., and GM 46224 and GM 53747 to V.M.) is gratefully acknowledged.

Supporting Information Available: Procedures for the synthesis, characterization and cellular evaluation, and immunofluorescence processing of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA058259A