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S-Adenosylmethionine Reverses Ilimaquinone's Vesiculation of the Golgi Apparatus: A Fluorescence Study on the Cellular Interactions of Ilimaquinone

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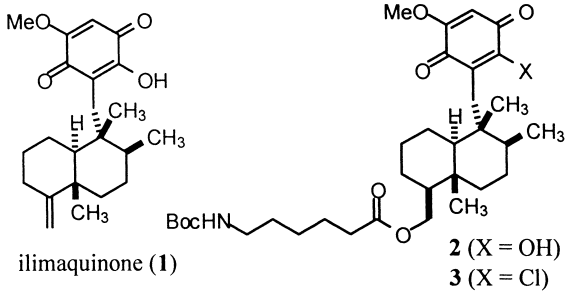
Abstract—The marine sponge metabolite ilimaquinone has a wide range of biological activities, including vesiculation of the Golgi apparatus and interference with intracellular protein trafficking. Some of these activities may arise from ilimaquinone's influence on the activated methyl cycle. To visualize the morphological effects of ilimaquinone on the Golgi apparatus, NRK (normal rat kidney) cells were labeled with fluorescent wheat germ agglutinin and treated with ilimaquinone in the presence and absence of the methylating agent *S*-adenosylmethionine (SAMe). While ilimaquinone alone fragments the Golgi apparatus, the organelle remains intact when SAMe is included in the incubation mixture. This observation supports ilimaquinone's interaction with methylation enzymes as the cause of Golgi vesiculation. The examination of a fluorescently labeled ilimaquinone analogue in NRK cells suggests that the cellular interactions of ilimaquinone are not localized to the Golgi apparatus. © 2001 Published by Elsevier Science Ltd.

The marine sponge metabolite ilimaquinone[1] (**1**, Fig. 1) has biological activities that include anti-HIV,[2] anti-inflammatory, antimicrobial, and antimitotic activities,[3] as well as protecting the cell against the toxic influence of ricin and diphtheria toxin.[4] The natural product **1** has also been shown to impede protein trafficking events by reversibly breaking the Golgi apparatus into small vesicles.[5] We recently identified *S*-adenosylhomocysteine hydrolase (AdoHcy hydrolase) as a cellular target of **1**.[6] When AdoHcy hydrolase is inhibited by **1**, a build-up of *S*-adenosylhomocysteine causes a feedback inhibition of methyltransferases and thereby disrupts cellular methylations. A better understanding of how **1** and related compounds interact with AdoHcy hydrolase and inhibit cellular methylation could impact various diseases related to cellular methylation such as cancer,[7] pathogenic entry into cells (including HIV[8] and Ebola[9]),[10] metabolic deficiencies,[11] and neurodegenerative diseases (e.g., Alzheimer's disease).[12]

Our study had shown that when the cellular methylating agent, *S*-adenosylmethionine (SAMe), is added to cells displaying hindered protein secretion due to the action of ilimaquinone, secretion is recovered.[13] The question that remained was whether SAMe reverses the reported vesiculation of the Golgi apparatus caused by ilimaquinone. NRK (normal rat kidney) cells were incubated with various concentrations of ilimaquinone and SAMe for 30 min, fixed, permeabilized, and stained with TRITC-labeled wheat germ agglutinin, a known Golgi-specific label.[14] As shown in Figure 2a, the Golgi apparatus of normal NRK cells are juxtanuclear and bright. Of the 200 cells counted, 96% had an intact Golgi apparatus. When 50 μM ilimaquinone is incubated with the cells (Fig. 2c), staining is more punctate and distributed farther from the nucleus, leaving only 5% of cells with an intact Golgi apparatus. However, when 50 μM SAMe is also included in the incubation (Fig. 2d), the staining more closely resembles the cells without ilimaquinone (i.e., Fig. 2a), and 72% of the cells counted had an intact Golgi apparatus. This indicates that



ilimaquinone (**1**)

2 (X = OH)
3 (X = Cl)

Figure 1. Ilimaquinone and biologically active analogues.[6,15]

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vesiculation of the Golgi apparatus by ilimaquinone is hindered in the presence of exogenous SAMe. When SAMe is added to NRK cells without ilimaquinone (Fig. 2b), staining is indistinguishable from the control cells (97% intact). This shows that SAMe alone does not change Golgi morphology, but does cause the Golgi apparatus that has been disrupted by ilimaquinone to reassociate. Taxol®, another known Golgi antagonist,[16] displays a staining pattern of Golgi dissociation even in the presence of SAMe (100%) (Fig. 2e–f). This is as expected since Taxol®, unlike ilimaquinone, vesiculates the Golgi via interactions with microtubules rather than with methylation enzymes.

A second question addressed concerns the localization preference of ilimaquinone. Since AdoHcy hydrolase is cytosolic,[17] its interaction with ilimaquinone should not be organelle-specific. To identify the cellular location of ilimaquinone, a fluorescent analogue **5** was synthesized from the biologically active analogue **3** (Scheme 1). Formic acid deprotection of the amine and subsequent addition of the BODIPY R6G succinimidyl ester[18] (**4**) in pyridine provides fluorescent analogue **5** in 79% yield after Sephadex LH-20 chromatography (4:3 chloroform:methanol).[19] Fluorescence measurements of compound **5** reveal a substantial emission peak at 547 nm, indicating that the fluorescence of the BODIPY group

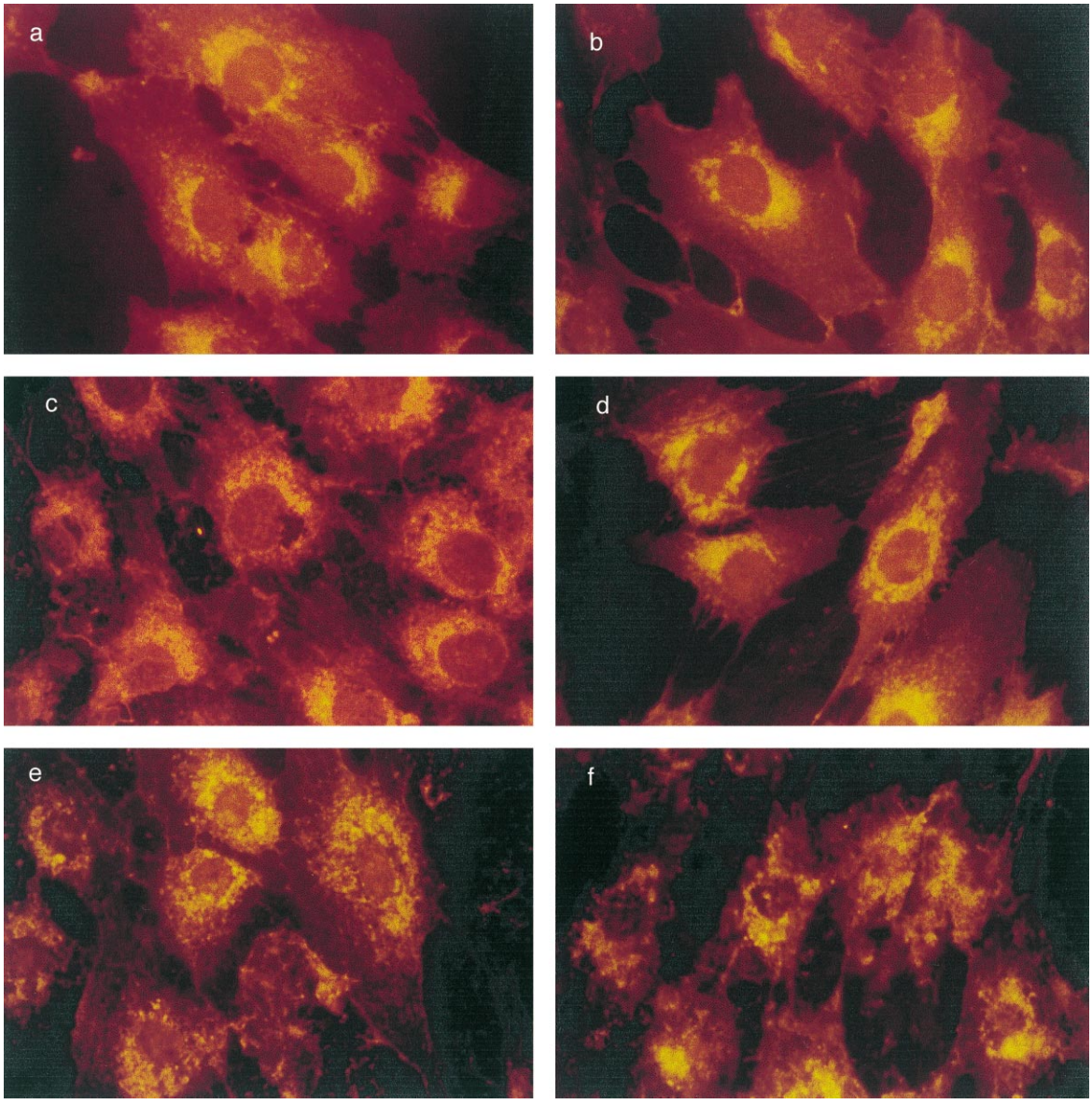
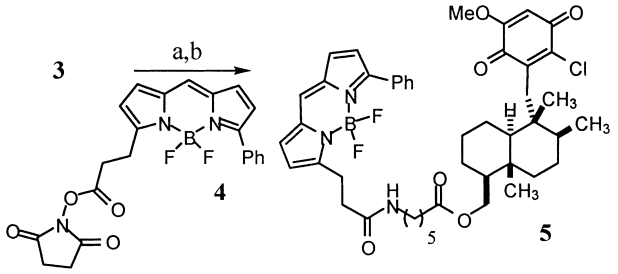


Figure 2. NRK cells (ATCC) were grown on glass coverslips in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal bovine serum and penicillin/streptomycin. Cells were 60% confluent on the day of assay. The coverslips were washed twice with DMEM and incubated with various concentrations of SAMe, ilimaquinone, or Taxol® in DMEM for 30 min (3 h for cells treated with Taxol®) at 37 °C, 5% CO_2, 100% humidity. The cells were washed three times with phosphate buffered saline (PBS), fixed with 0.5% glutaraldehyde in PBS (15 min, 23 °C), washed twice, permeabilized (30 min, 23 °C) with 0.05% IGEPAL CA-630 (Sigma) in PBS, washed twice, and labeled (30 min, 23 °C) with 100 μg/mL TRITC-labeled wheat germ agglutunin (Sigma) in PBS supplemented with 2.7 mM each of $MgCl_2$ and $CaCl_2$. Coverslips were washed thoroughly and mounted on slides in 25% glycerol in PBS. Slides were viewed on an Olympus AX70 Provis upright microscope, 100× oil objective, using a rhodamine filter set, and photographed with an Olympus PM-VB-3 camera using Kodak Elitechrome 400 film: (a) normal cells; (b) 50 μM SAMe; (c) 50 μM ilimaquinone; (d) 50 μM ilimaquinone and 50 μM SAMe; (e) 10 μM Taxol®; (f) 10 μM Taxol® and 50 μM SAMe.

is not quenched by the presence of the quinone functionality.

Incubation of NRK cells with 10 μM fluorescent analogue **5** for 30 min affords a staining pattern that is less localized than the TRITC-wheat germ agglutinin labeling in the previous study (Fig. 3). Since the Golgi apparatus is mainly intact under these conditions,[20] the results suggest that analogue **5** is cytosolic and does not localize specifically to the Golgi apparatus. When 50 μM **1** is included in the incubation, the magnitude of the fluorescence diminishes. While the higher concentration of ilimaquinone (**1**) vesiculates the Golgi apparatus more completely, causing any Golgi-specific stain to dissipate, it is more likely that the excess **1** competes with the fluorescent analogue **5** for general cytosolic targets (AdoHcy hydrolase), such that most of the fluorescent label is washed away after incubation. Together, these data indicate that the interaction of ilimaquinone analogue **5** with its specific target (AdoHcy hydrolase) in NRK cells is not localized to a single organelle, but rather is distributed throughout the cytosol.

In summary, as predicted by secretion assays, the morphological changes to the Golgi apparatus of NRK cells by ilimaquinone (**1**) can be reversed by inclusion of exogenous SAMe. This supports ilimaquinone's interaction with the activated methyl cycle, specifically AdoHcy hydrolase, as the cause of its vesiculation of the Golgi apparatus. Incubation of NRK cells with a fluorescent ilimaquinone analogue **5** indicates that the natural product is cytosolic, but not necessarily accumulating in the region of the Golgi apparatus. Further studies are necessary to detail the specific methylation events involved in vesicular trafficking of the Golgi apparatus.



Scheme 1. Reagents: (a) formic acid; (b) **4**, pyridine, 79%.

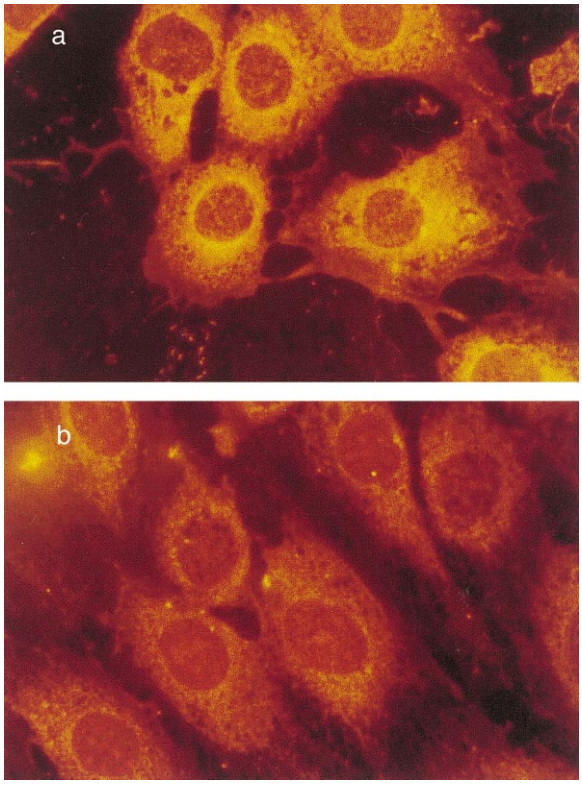


Figure 3. Compound **5** (10 μM) in NRK cells (a) alone, and (b) with 50 μM ilimaquinone as excess nonfluorescent competitor. After 30 min incubation, cells were washed, fixed, mounted, and viewed as in the previous study.

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19. Formic acid was added to starting material **3** at 0 °C, and the mixture was stirred 1.5 h (0 °C→23 °C). Formic acid was removed under vacuum. Label **4** was dissolved in pyridine and added to the reaction at 23 °C. After 1.5 h, the pyridine was removed under vacuum. The residue was taken up in 4:3 chloroform:methanol and purified on a Sephadex LH20 column[1a] to give compound **5** in 79% yield. **5** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (2H, dd, J = 6.0, 1.6 Hz), 7.47 (3H, m), 7.23 (1H, s), 7.11 (1H, d, J = 3.6 Hz), 7.03 (1H, d, J = 4.0 Hz), 6.64 (1H, d, J = 4.0 Hz), 6.41 (1H, d, J = 4.0 Hz), 6.03 (1H, s), 5.70 (1H, br s), 4.18 (1H, dd, J = 6.4, 4.0 Hz), 3.84 (3H, s), 3.80 (1H, t, J = 9.6 Hz), 3.68 (1H, t, J = 11.2 Hz), 3.28 (2H, t, J = 7.2 Hz), 3.16 (2H, q, J = 6.8), 2.75 (2H, dd, J = 14.4, 12.8 Hz), 2.60 (2H, t, J = 7.2 Hz), 2.21 (2H, t, J = 7.6 Hz), 1.78–0.88 (18H, m), 0.84 (3H, s), 0.82 (3H, s), 0.81 (3H, s); ^{13}C NMR (500 MHz, $CDCI_3$) δ 180.3, 179.2, 174.0, 171.5, 162.2, 159.6, 145.2, 142.7, 136.4, 135.2, 132.6, 131.0, 130.5, 129.9, 129.5, 128.5, 128.2, 120.4, 117.7, 109.0, 106.8, 65.6, 57.0, 54.2, 50.0, 45.5, 39.8, 39.6, 39.4, 38.6, 37.8, 36.0, 34.4, 29.9, 29.3, 27.9, 26.8, *26.5,* 25.8, 25.4, 24.7, 23.9, 19.0, 16.8, 15.1, 1.2. HRMS: calcd for $C_{46}H_{56}N_3O_6C1BF_2$, 830.3920; found, 830.3919.
20. Control experiments indicate the Golgi apparatus is approximately 80% intact in the presence of 10 μM ilimaquinone.