Life Sciences 136 (2015) 163–167

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Fluorescent kapakahines serve as non-toxic probes for live cell Golgi imaging

Danilo D. Rocha [a], Vinson R. Espejo [b], Jon D. Rainier [b,*], James J. La Clair [c,*], Letícia V. Costa-Lotufo [a,d,*]

[a] Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil
[b] Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112, USA
[c] Xenobe Research Institute, P.O. Box 3052, San Diego, CA 92163-1052, USA
[d] Departamento de Farmacologia, Universidade de São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 14 May 2015
Accepted 9 June 2015
Available online 2 July 2015

Keywords:
Fluorescent probes
Golgi apparatus
Chemical biology
Natural products
Kapakahine

ABSTRACT

Aims: There is an ongoing need for fluorescent probes that specifically-target select organelles within mammalian cells. This study describes the development of probes for the selective labeling of the Golgi apparatus and offers applications for live cell and fixed cell imaging.
Main methods: The kapakahines, characterized by a common *C*(3)-*N*(1′) dimeric tryptophan linkage, comprise a unique family of bioactive marine depsipeptide natural products. We describe the uptake and subcellular localization of fluorescently-labeled analogs of kapakahine E. Using confocal microscopy, we identify a rapid and selective localization within the Golgi apparatus. Comparison with commercial Golgi stains indicates a unique localization pattern, which differs from currently available materials, therein offering a new tool to monitor the Golgi in live cells without toxic side effects.
Key findings: This study identifies a fluorescent analog of kapakahine E that is rapidly uptaken in cells and localizes within the Golgi apparatus.
Significance: The advance of microscopic methods is reliant on the parallel discovery of next generation molecular probes. This study describes the advance of stable and viable probe for staining the Golgi apparatus.
© 2015 Elsevier Inc. All rights reserved.

1. Introduction

An increasing demand has developed for next-generation fluorescent probes [4,5,13] given the recent advances in methods and associated instruments for cellular microscopy [24,25,29]. As recognized by the 2014 Nobel Prize in Chemistry [16], high-resolution instrumentation now exists for evaluating processes in live cells or tissues [23,27,28]. These methods rely on the use of fluorescent probes whose uptake and localization, in part, define the scope and applications of its associated instrumentation. As part of an ongoing effort to elucidate the mode of action (MOA) of a diversity of natural products [14,33–35], we have searched for materials that display modest or weak cytotoxicity yet deliver a rapid and potent cellular localization. Here, we describe a set of fluorescent probes, advanced from a family of marine depsipeptides, that selectively stain the Golgi apparatus in mammalian cells and suggest an expanded arsenal for the study of Golgi biogenesis [36], Golgi function [11,22] and cell cycle events [3,32].

In 1995, Scheuer reported the isolation of kapakahine B (**2**, Fig. 1), a first member of a unique family of cyclic peptides bearing a common *C*(3)-*N*(1′) linkage, from the marine sponge *Cribrochalina olemada* [19]. Subsequent efforts have led to the discovery of six additional congeners with modifications in the peptidyl ring and oxidation state (Fig. 1) [18,37]. Preliminary biological analyses on these materials indicated only a modest cytotoxic activity for kapakahines A, B, C and E with IC_{50} values of ~5.0 μg/mL in the P388 murine leukemia cell line [18,19,37]. While cytotoxicity data has been reported, there have been no detailed biological studies conducted on this family of compounds.

Recently, a solution to the supply problem arose through chemical synthesis [6,15,20,40]. In 2009, Baran reported the synthesis of kapakahines B (**2**) and F through adaption of their oxidative coupling methods [40]. The elegance of this route was exemplified by their ability to access materials at gram scales [20]. Complementary efforts in the Rainier laboratory provided access to kapakahines E and F by the use of a bromopyrroloindoline heterodimerization approach [6]. We now report on the translation of materials from the latter studies into molecular probes.

* Corresponding authors.
E-mail addresses: rainier@chem.utah.edu (J.D. Rainier), i@xenobe.org (J.J. La Clair), costalotufo@gmail.com (L.V. Costa-Lotufo).

http://dx.doi.org/10.1016/j.lfs.2015.06.014
0024-3205/© 2015 Elsevier Inc. All rights reserved.

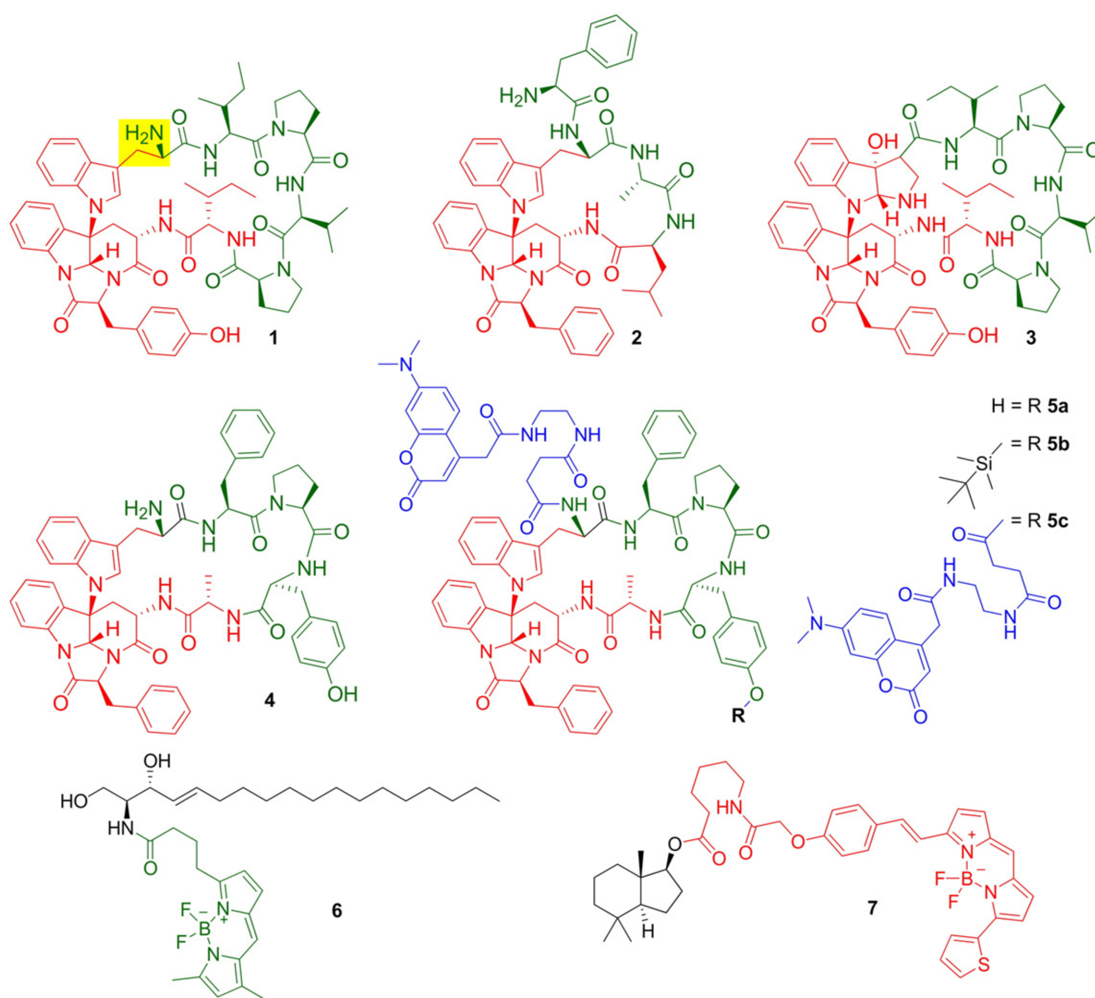

Fig. 1. Structures of kapakahines A (**1**), B (**2**), C (**3**) and E (**4**), probes **5a**–**5c**, and Golgi stains BODIPY FL-C5-ceramide (**6**) and BODIPY 630/650-labeled norrisolide probe (**7**). Colors denote a fixed unit (red), a variable unit (green), and the immunoaffinity fluorescent (IAF) tag (blue). (two column figure).

2. Materials and methods

2.1. Cytotoxicity assays

Cytotoxicity of kapakahine E (**4**) and probes **5a**–**5c** was evaluated in three tumor cell lines including: HCT-116 (adenocarcinoma colon), MALME-3M (metastatic melanoma), and PC-3M (metastatic prostate carcinoma) using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay [17,31]. All cell lines were obtained from the National Cancer Institute (Bethesda, MD, USA). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C under a 5% CO_2 atmosphere. All compounds were tested in concentrations ranging from 0.01 μM to 5 μM over 72 h.

2.2. Time course imaging studies

Cell uptake and localization studies were conducted by treating PC-3M cells at a density of 10^5 cells/cm^2 in a 35 mm glass-bottom dish from MatTek Corporation (Ashland, MA, USA). Each compound was added as a 10× stock in media such that the net DMSO content remained under 0.5% (v/v). Cells were incubated at 37 °C under a 5% CO_2 atmosphere with 5 μM probes **4**, **5a**, **5b** or **5c**. Confocal studies were conducted on a LSM 710 inverted confocal microscope (Zeiss, Oberkochen, Germany) containing a scanning module with three detection channels, a Plan-Apochromat 63 × 1.4 na objective, and multiple lasers including a diode laser at CW pulsed (405 nm, 30 mW), an Ar-laser (458 nm, 488 nm, 514 nm, each at 25 mW), and a HeNe-laser (543 nm, 1 mW) and HeNe-laser (633 nm, 5 mW). The probes were imaged using the following settings: **5a**–**5c** (λ_{ex} 405 nM (5%), MBS 405 beam splitter and λ_{em} 410–480 nM); **6** (λ_{ex} 488 nM (5%), MBS 488 beam splitter and λ_{em} 492–541 nM); and **7** (λ_{ex} 543 nM (5%), MBS 458/543 beam splitter and λ_{em} 547–680 nM). For live cell imaging, the plates of cells were removed from the incubator and imaged within 15 min. Individual plates were used for each time point with multiple plates (repetitions) being examined at each time point. Images were collected after 1 h and 24 h incubation. Co-staining was conducted using BODIPY FL-C5-ceramide (**6**) obtained from Life Technologies (Carlsbad, CA, USA) and red-fluorescently labeled norrisolide (**7**) obtained from the Xenobe Research Institute (San Diego, CA, USA). Each experiment was repeated 2–4 times and select images are provided in Figs. 2 and 3. Live cell imaging was conducted as the procedures used for fixation such as those typical for immunostaining led to significant loss of the fluorescence from **5a**–**5c**.

3. Results and discussion

Our studies began by applying our recently developed immunoaffinity fluorescent (IAF) system to prepare active kapakahine probes [10,12,30,38,39]. We installed an IAF tag containing a fluorescent 7-(dimethylamino)coumarin-4-acetic acid reporter at the amino group of the tryptophan residue. As highlighted in yellow in Fig. 1,

Download English Version:

https://daneshyari.com/en/article/2550883

Download Persian Version:

https://daneshyari.com/article/2550883

Daneshyari.com