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Design, synthesis, nuclear localization, and biological activity of a fluorescent duocarmycin analog, HxTfA

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ABSTRACT

HxTfA **4** is a fluorescent analog of a potent cytotoxic and antimalarial agent, TfA **3**, which is currently being investigated for the development of an antimalarial vaccine, PlasProtect®. HxTfA contains a p-anisylbenzimidazole or Hx moiety, which is endowed with a blue emission upon excitation at 318 nm; thus enabling it to be used as a surrogate for probing the cellular fate of TfA using confocal microscopy, and addressing the question of nuclear localization. HxTfA exhibits similar selectivity to TfA for A-tract sequences of DNA, alkylating adenine-N3, albeit at 10-fold higher concentrations. It also possesses in vitro cytotoxicity against A549 human lung carcinoma cells and *Plasmodium falciparum*. Confocal microscopy studies showed for the first time that HxTfA, and by inference TfA, entered A549 cells and localized in the nucleus to exert its biological activity. At biologically relevant concentrations, HxTfA elicits DNA damage response as evidenced by a marked increase in the levels of γH2AX observed by confocal microscopy and immunoblotting studies, and ultimately induces apoptosis.

Duocarmycin SA **1** (Fig. 1),[1] yatakemycin,[2] and CC-1065[3] are the parent members of a potent class of antitumor natural products that contain two components: the cyclopyrroloindolone (CPI) DNA alkylating moiety and a non-covalently active portion, such as the 5,6,7-trimethoxyindole (TMI). The crescent-shaped molecules fit snugly in the minor groove of AT-rich sequences of DNA. Upon binding, the reactivity of the cyclopropane moiety, as in **1**, is enhanced for covalent bonding with adenine-N3,[4] leading to the killing of cancer cells through apoptosis.[5] Due to clinical toxicity observed with the initial group of CC-1065 and duocarmycin analogs, such as adozelesin, bizelesin, carzelesin, and KW2189, a major effort has been underway for over two decades to develop analogs that are more targeted, more selectively active, and less systemically toxic.[6] As a result, numerous duocarmycin analogs have been developed. Examples include: *seco*-cyclopropylbenzoindoline-TMI (**2**, Fig. 1), a stable, sequence specific, and highly cytotoxic agent[7]; centanamycin, (achiral *seco*-amino-CBI-TMI),[8] an orally active anticancer agent against human cancer xenografts[9]; and *seco*-iso-cyclopropylfuranoindoline-trimethoxyindole (*seco*-iso-CFI-TMI or tafuramycin A or TfA **3**),[10] a potent cytotoxic agent against cancer cells. Recently, both centanamyin[11] and TfA[12] were reported to exhibit potent cytotoxic activity against *Plasmodium falciparum*. *Seco*-compounds, such as **2** and **3**, Fig. 1, eliminate HCl in cells to form the ultimate cyclopropane drugs, e.g., **3a**, which bind and covalently bond with adenine-N3 on DNA. TfA is a critical component in the development of the antimalarial vaccine, PlasProtecT®, which is currently being tested clinically.[12] Another duocarmycin analog, DUBA, is currently undergoing clinical evaluation and is part of a HER2-targeting antibody-drug conjugate SYD985, which is being developed clinically for HER2-expressing breast cancers.[13]

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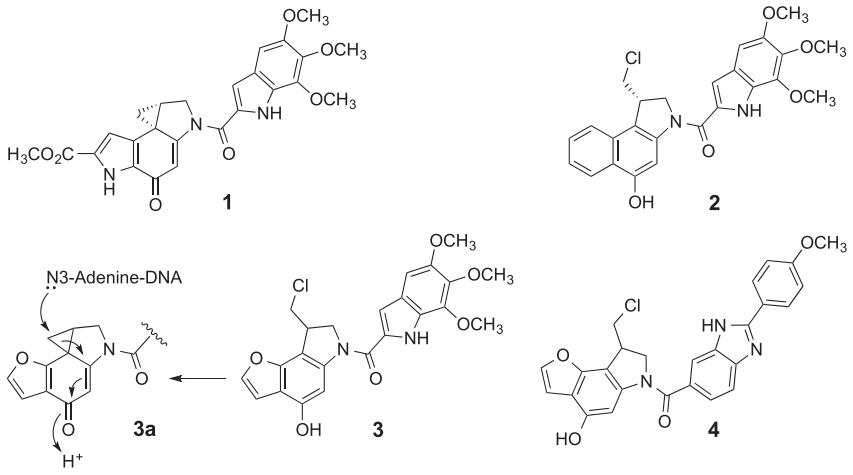

Fig. 1. Structure of duocarmycin SA **1**; *seco*-CBI-TMI **2**; *seco*-iso-CFI-TMI or tafuramycin A or TfA **3**; **3a** is the putative active cyclopropane drug of tafuramycin A, and its reaction with adenine-N3; and *seco*-iso-CFI-Hx or HxTfA **4**.

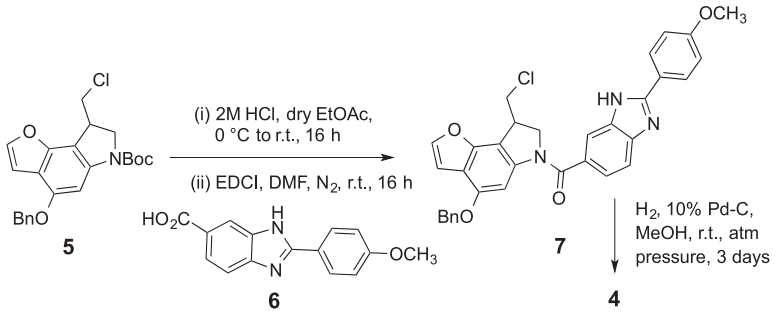

Fig. 2. Synthesis of HxTfA **4**.

Despite the major progress made so far in the duocarmycins and CC-1065 field for the development of novel anticancer and antiparasitic agents, one important question that needs to be answered is evidence of nuclear localization of these compounds. Since duocarmycins have been shown to bind and alkylate naked and genomic DNA extracted from cells,[1,8b,14] it is often assumed that these compounds interact with nuclear DNA. Recently, Tietze's group reported the design and synthesis of a number of coumarin-based, fluorescent duocarmycin analogs for the purpose of studying the molecular fate of the compounds in live cancer cells using confocal microscopy.[15] Even though the compounds displayed strong cytotoxicity against the growth of cancer cells, surprisingly, they penetrated the cellular membrane and accumulated in the mitochondria, inducing apoptosis. Another effort led by Tercel's group involved the development of terminal-alkyne-containing duocarmycin analogs; upon binding to DNA, the alkyne moiety could be "clicked" with a reporter fluorophone-azide to determine nucleus localization.[16] The authors cautioned that "care must be exercised in interpreting sites of intracellular probe molecule localization on the basis of a click fluorescent read-out, especially where multiple diverse targets are possible, and even more so when some of these targets may be within the nucleus."[16] Since our group has been actively developing inherently fluorescent, DNA sequence selective, Hx-containing pyrrole-imidazole polyamides, or Hx-amides,[17] we envisioned that the Hx-moiety would be an appropriate replacement of the TMI unit in duocarmycins. Hx would retain the AT sequence and minor groove selectivity, and provide an anchor needed to "twist" and activate the cyclopropane toward nucleophilic reaction with adenine-N3. It would also come with the added benefit of the inherent fluorescent property afforded by the Hx moiety. Accordingly, we report herein

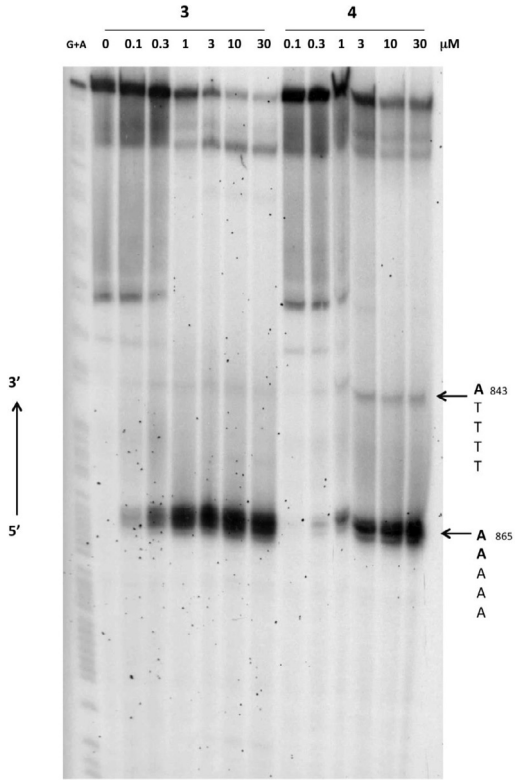

Fig. 3. Autoradiogram of a thermal cleavage gel showing purine–N3 lesions on the bottom strand of a 5′-^{32}P-labeled 208-bp fragmemt of pUC18. G + A lane; 0, control; TfA **3**: 0.1, 0.3, 1, 3, 10, and 30 μM; and HxTfA **4**: 0.1, 0.3, 1, 3, 10, and 30 μM.