Tetrahedron Letters 49 (2008) 6429–6432

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Design, synthesis, and biological evaluation of a dansyled amino acid derivative as an imaging agent for apoptosis

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ARTICLE INFO

Article history:
Received 1 August 2008
Revised 23 August 2008
Accepted 25 August 2008
Available online 29 August 2008

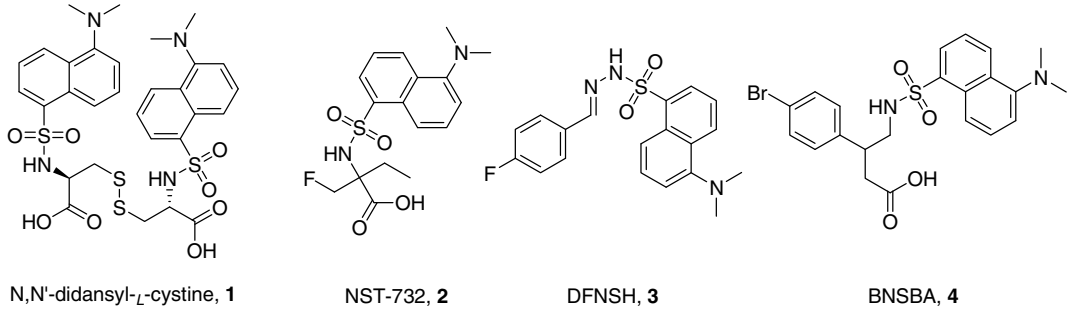
Keywords:
Amino acid
Imaging
Biomarker
Apoptosis

ABSTRACT

To develop a small molecule-based biomarker for in vivo apoptosis imaging, a dansyled amino acid derivative (BNSBA) was designed and synthesized in good yield. The biological evaluation demonstrated that BNSBA selectively binds to apoptotic cancer cells and is localized within the cytoplasm of cells that bound annexin V on the plasma membrane.

Apoptosis, or programmed cell death, is an essential biological process for all living organisms. It is an active process of cellular self-destruction that plays an important role in a large number of disorders, including organ rejection after transplantation, strokes,[1] myocardial infarct,[2] and neurodegenerative diseases.[3] Apoptosis also plays a major role in cancer therapy.[4] For this reason, considerable efforts have been made in developing and validating methods to image apoptosis.

An often used agent for imaging apoptosis involves the protein annexin V, which binds to extracellular phosphatidylserine, an early marker of apoptosis.[5] However, the non-specific biodistribution profile, poor target/background contrast ratio, slow clearance from the blood, along with the cost, inhibit the use of annexin V derivatives as imaging agents for apoptosis in the clinic. Therefore, a number of small molecules,[6] nanoparticles,[7] and peptides[8] have been examined as alternatives to annexin V. Among the small molecules investigated, *N,N′*-didansyl-L-cystine (DCC, **1**)[9] and 5-(dimethylamino)-1-naphthalene-sulfonyl-a-ethylfluoroalanine (NST-732, **2**)[10] have demonstrated great promise (Scheme 1). Though NST-732 has potential use in positron emission



Scheme 1. Dansyl derivatives for imaging apoptosis.

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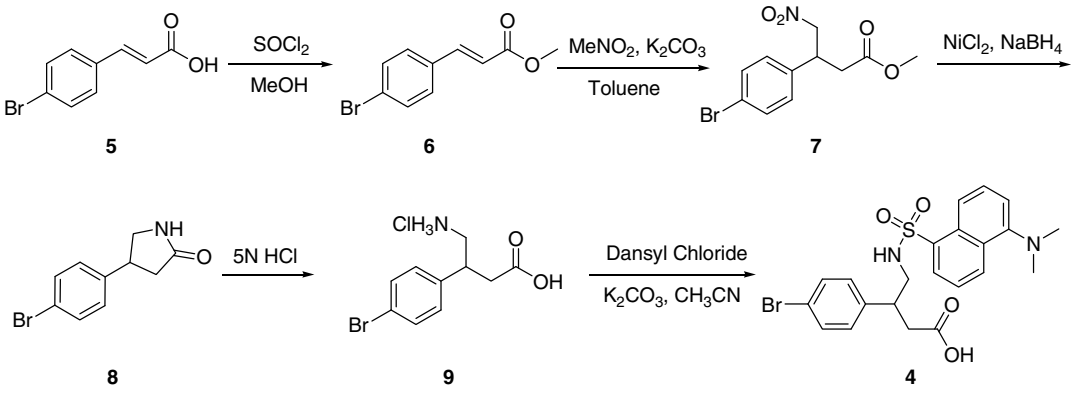
doi:10.1016/j.tetlet.2008.08.091

tomographic (PET)[11] studies, poor radiolabeling efficiency limits the clinical application. We have been exploring small molecule-based biomarkers for use as imaging agents for apoptosis. Recently, we reported the preparation and preliminary evaluation of a dansylhydrazone (DFNSH, **3**) which demonstrates great selectivity for apoptotic cells.[12] The straightforward, highly efficient synthesis of the fluorine-18 analog of **3** makes it potentially valuable for PET imaging studies when compared to NST-732. Bromine-76 is also useful as a radiolabel for PET imaging.[13] In previous work,[14] we developed a rapid and high yield synthesis of high specific activity bromine-76 labeled molecules from the corresponding organotrifluoroborates. In the light of the interest in developing a biomarker with better in vivo features such as in vivo affinity, metabolic stability, and pharmacokinetics, we investigated the synthesis of a brominated dansyl derivative (4-bromophenyl)-4-(5-(dimethylamino)naphthalene-1-sulfonamido)butanoic acid (BNSBA, **4**). In this Letter, we report both the facile preparation of BNSBA and the in vitro evaluation as an imaging agent for apoptosis.

The synthesis of dansyled γ-amino acid **4** is outlined in Scheme 2. Methyl 4-bromocinnamate, prepared in 92% yield by the reaction of 4-bromocinnamic acid (**5**) with methanol and thionyl chloride, was allowed to react overnight with a 10-fold excess of nitromethane, using K_2CO_3/toluene and benzyltriethylammonium, instead of 1,1,3,3-tetramethylguanidine/THF for four days, to produce nitro ester **7** in 75% yield. Ester **7** was reduced with nickel



Scheme 2. Synthesis of DNSBA (**4**).

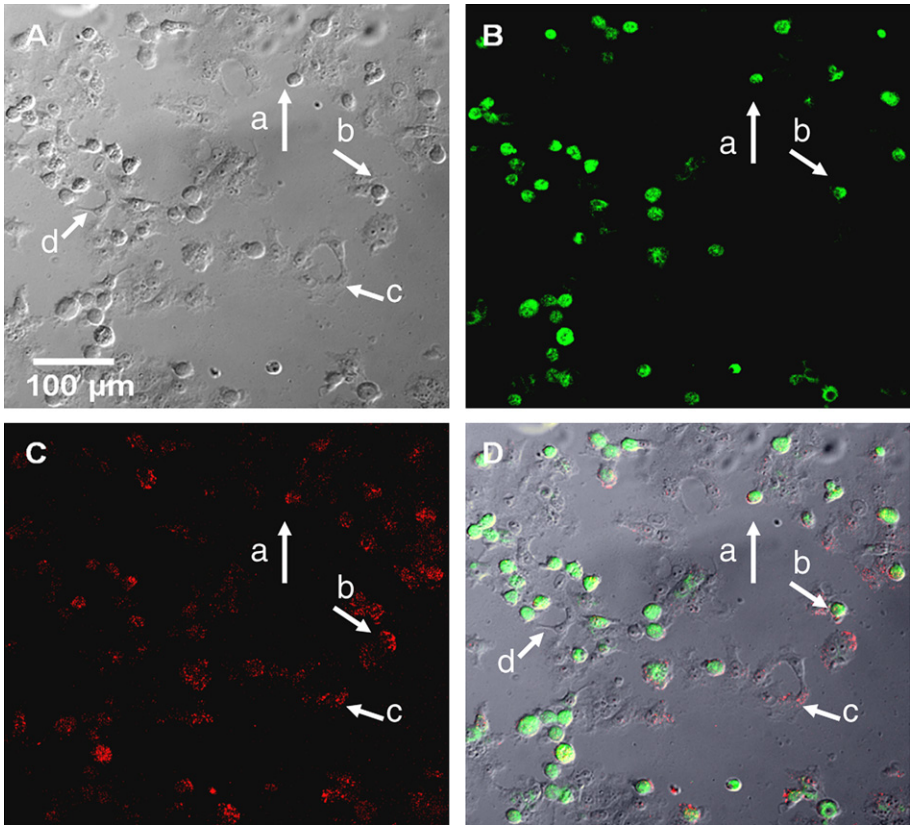


Figure 1. Images in the same field of view of MCF-7 cells dually stained with annexin V labeled with Alexa®488 and **4**. (A) DIC image; (B) **4** in green; (C) Alexa®488 in red; (D) composite image of A, B, and C. 100× total magnification.

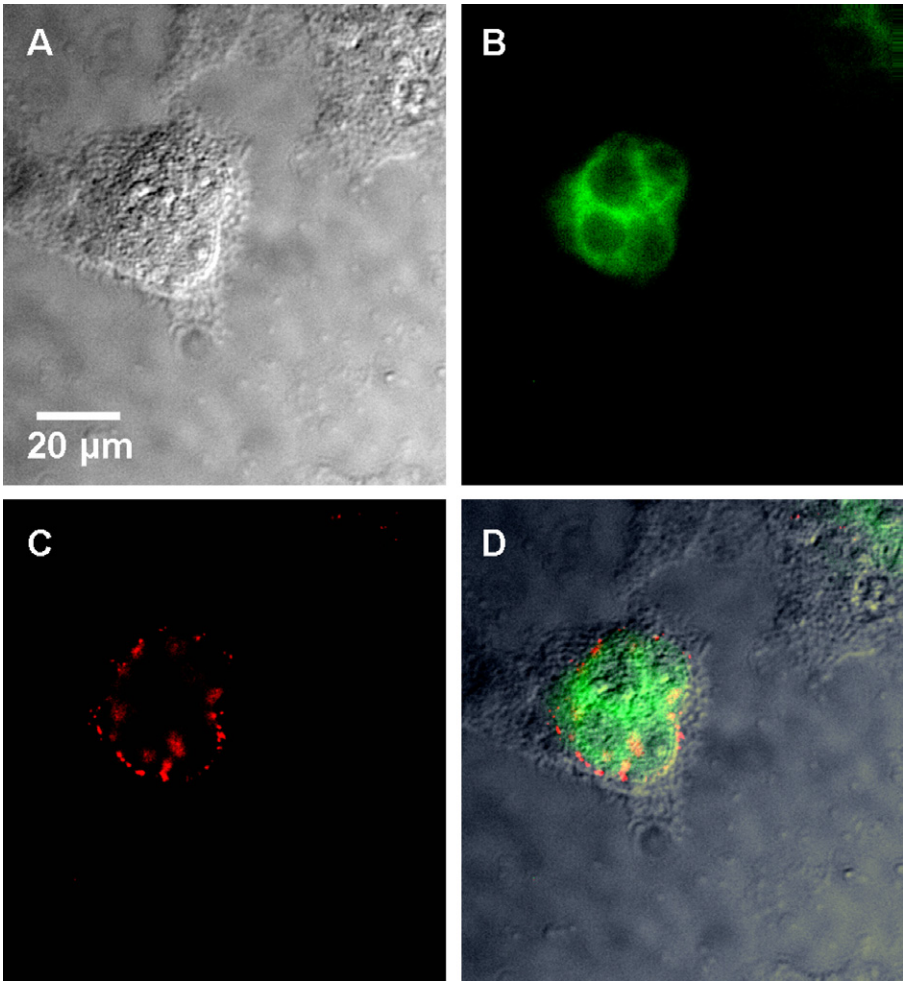


Figure 2. Images in the same field of view of MCF-7 cell dually stained with annexin V labeled with Alexa®488 and **4**. (A) DIC image; (B) **4** in green; (C) Alexa®488 in red; (D) composite image of A, B, and C. 600× total magnification.

boride[15] to give the lactam **8** in 85% yield. Hydrochloride salt **9** was obtained by hydrolysis of lactam **8** with aqueous 5 N HCl. The reaction of **9** with dansyl chloride was carried out in acetonitrile to give the desired biomarker **4** in 84% yield.

A series of experiments were performed to evaluate the efficacy of compound **4** in detecting apoptosis in MCF-7 breast cancer cells by comparing **4** to Alexa®488 labeled annexin V. Images were obtained using spectral imaging microscopy. The apoptosis of MCF-7 cells was induced by exposing the cells to 50 nM paclitaxel at 37 °C for 16 h. The apoptotic MCF-7 cells were dually stained with Alexa®488 labeled annexin V and compound **4** (50 μM in HEPES buffer, 2.5 mM $CaCl_2$, pH 7.4) using a standard protocol.[10] Excitation at 300–400 nm and emission at 445–480 nm were employed for the detection of compound **4**, whereas excitation at 480–505 nm and emission at 510–550 nm were used for Alexa®488 detection. Actively growing (approximately 80% confluent) MCF7 cells were not stained by **4** and Alexa®488 labeled annexin V (Fig. 1A). Approximately 30–40% of the cells were rounded, which was indicative of the induction of apoptosis [arrows **a** and **b** identify apoptotic cells, and **c** and **d** identify actively growing (spreading and dentritic morphology) cells]. The data in Figure 1B illustrate that compound **4** undergoes exclusive uptake and accumulation in the cytoplasm of the apoptotic cells**.** By comparison with Figure 2C obtained using Alexa®488 labeled annexin V, it was confirmed that the cells detected by compound **4** were apoptotic cancer cells. A co-localization technique (Fig. 1D) was employed to compare the degree of overlap between two images. The result showed that co-localization of the two dyes occurred on the periphery of the apoptotic cells (Fig. 1D). In addition, the non-specific binding of annexin V can be seen in Figure 1C, where the normal cells were also stained (arrow **c**), while compound **4** specifically binds to the apoptotic cancer cells. Moreover, it was found that the staining of apoptotic cells by compound **4** was stable and was not reduced by additional washing, suggesting that the uptake is irreversible.

The high magnification images demonstrate that compound **4** was taken up by the cytoplasm (Fig. 2B and D), while annexin V bound to the outside membrane of the apoptotic cells (Fig. 2C and D).

In summary, λ-amino acid **4** was synthesized in good yield and found to selectively bind to paclitaxel-induced apoptotic cancer cells. The in vitro image analysis demonstrates that compound **4** exhibits intracellular uptake and accumulation in apoptotic cells. The radiochemical production of [^{76}Br]-**4** and PET imaging studies of tumor apoptosis in rodent models are currently underway.

Acknowledgments

We wish to recognize the contribution of Dr. Meixiang Yu in the early stages of this study. This work was supported by a grant from Physician Medical Education and Research Foundation. We also wish to thank the Robert H. Cole Foundation for support.

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