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Synthesis, biological evaluation and radiochemical labeling of a dansylhydrazone derivative as a potential imaging agent for apoptosis

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ABSTRACT

To develop a small molecule-based tracer for in vivo apoptosis imaging, dansylhydrazone (DFNSH) was synthesized in 93% yield in less than 30 min. The biological evaluation showed that DFNSH selectively binds to paclitaxel-induced apoptotic cancer cells. The high magnification fluorescent images demonstrate that DFNSH is localized within the cytoplasm of cells that bound Alexa® 488 labeled annexin V on the plasma membrane. [^{18}F]-DFNSH ([^{18}F]-**3**) was synthesized and isolated in 50–60% radiochemical yields, based on [K/K$_{222}$]^{18}F, with a synthesis time of 50 min (EOB). The straightforward preparation of fluorine-18 labeled **3** makes it a promising tracer for PET imaging of apoptosis.

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Apoptosis is an important process involved in the etiology, pathogenesis, and response to therapy of a variety of diseases. Imaging apoptosis in vivo is a potentially powerful tool for the early diagnoses of strokes,[1–3] myocardial infarctions,[4] and a range of neurodegenerative disorders.[5,6] In addition, imaging apoptosis in patients could also be valuable for the early evaluation of organ transplant rejection[7,8] and cancer treatment response.[9,10] Applications of annexin V, a 35.8-kDa protein, to imaging apoptosis have a long history.[11–15] These studies generally involve the incorporation of a probe, either a fluorochrome for fluorescence detection or a radiolabeled linker for nuclear imaging studies. Annexin V binds to externalized phosphatidylserine (PS) on the outer membrane of apoptotic cells.[16] However, this cell surface binding is not completely specific, as annexin V also binds to a small percentage of normal cells.[17] In addition, annexin V accumulates in human healthy organs such as the kidney, bladder, liver, and spleen, which limits its application in imaging studies focused on diseases in the abdominal.[18] The non-specific biodistribution profile, poor target/background contrast ratio, slow clearance from the blood, along with its cost, hinder the use of annexin V derivatives as imaging agents for apoptosis in the clinic. Because of this, a number of small molecules,[19–23] peptides,[24,25] and nanoparticles[17] have been examined as alternatives to annexin V. Among the small molecules investigated, compounds containing a fluorescent dansyl core[21,22] have demonstrated great promise.

In vitro and ex vivo studies have demonstrated that dansyl compounds bearing amino acid moieties [*N*′,*N*′-didansyl-L-cystine (DDC), **1** and 5-(dimethylamino)-1-naphthalene-sulfonyl-α-ethyl-fluoroalanine (NST-732), **2**] are selectively bound to apoptotic cells.[19–22] Confocal fluorescent imaging studies suggest that the dansyl compounds accumulate within the cytoplasm of the apoptotic cell. Generally speaking, intracellular uptake results in improved target to background contrast ratios. The preparation of fluorine-18 labeled NST-732 was recently reported[26] for potential use in positron emission tomographic (PET)[27] studies. However, the poor radiolabeling efficiency limits its clinical application. Herein we report the preparation and preliminary evaluation of a dansylhydrazone (DFNSH, **3**) that demonstrates great selectivity toward apoptotic cells. 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 (Fig. 1).

Compound **3** was prepared by condensation of dansylhydrazine with 4-fluorobenzaldehyde (Scheme 1). After a detailed evaluation of reaction parameters (solvent and temperature), it was found that **3** can be isolated in 93% yield in less than 30 min by carrying out the reaction in refluxing methanol. These reaction conditions are quite amenable to the preparation of the fluorine-18 labeled analogue, [^{18}F]-**3**.

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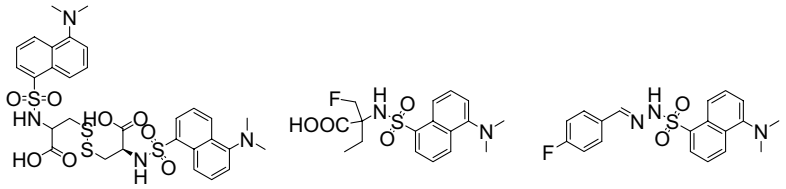
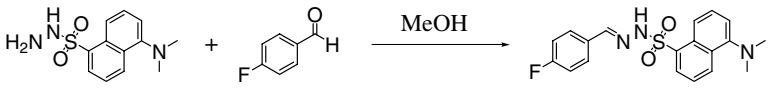


N,N'-didansyl-L-cystine, **1** NST-732, **2** DFNSH, **3**

Figure 1. Structure of *N*′,*N*′-didansyl-L-cystine (DDC), NST-732, and **3**.



4 **5** **3**

Scheme 1. Synthesis of compound DFNSH (**3**).

A series of experiments were performed to evaluate the efficacy of compound **3** in detecting apoptosis in breast cancer MCF-7 cells by comparing **3** to Alexa® 488 labeled annexin V. Images were obtained using Applied Spectral Imaging (ASI, Migdal Ha'Emek, Israel) microscopy. The apoptosis of MCF-7 cells was induced by exposing the cells to 50 nM paclitaxel at 37 °C for 16 h. The paclitaxel-treated and untreated MCF-7 cells were dually stained with Alexa® 488 labeled annexin V and compound **3** (50 μM in HEPES buffer, 2.5 mM $CaCl_2$, pH 7.4) using a standard protocol.[22] Actively growing (approximately 80% confluent) MCF7 cells were not stained by compound **3** and Alexa® 488 labeled annexin V (Fig. 2). Paclitaxel treatment (Fig. 3A), induced approximately 30–40% of the cells to adopt a rounded morphology, which was indicative of the induction of apoptosis [arrows **a** and **b** identify apoptotic cells, and **c** and **d** identify actively growing (spreading and dendritic morphology) cells]. The apoptotic MCF-7 cells, which were dually stained with Alexa® 488 labeled annexin V and compound **3**, were observed using Applied Spectral Imaging microscope. The corresponding filters with excitation at 300–400 nm and emission at 445–480 nm were employed for the detection of compound 3 (Fig. 3B), where the filters with excitation at 480–505 nm and emission at 510–550 nm were used for Alexa® 488 detection (Fig. 3C). Figure 3B illustrates that compound **3** was taken up and accumulated in the cytoplasm of the apoptotic cells. By comparison with Figure 3C obtained using Alexa® 488 labeled annexin V, it was confirmed that the cells detected by compound **3** were apoptotic cancer cells. The minimal nonspecific-binding of annexin V can be seen in Figure 3C, where a few normal cells were also stained (arrow d). Colocalization,of the two probes, performed using AutoDeblur® (Silver Spring, MD), occurred on the periphery of the apoptotic cells (Fig. 3D). Staining of apoptotic cells by compound **3** was stable and was not reduced by additional washing, suggesting that the uptake is irreversible.

The high magnification images demonstrate that compound **3** was taken up by the cytoplasm (Fig. 4B and D) while annexin V bound to the outside membrane of the apoptotic cells (Fig. 4C and D). The three dimensional imaging analysis (Fig. 5) confirmed the intracellular uptake of compound **3**. Dansylhydrazone analogues **7**–**10** (Fig. 6) were also synthesized and subjected to evaluation. It was found that these dansylhydrazone derivatives were also taken up by the cytoplasm in apoptotic cells. There is an argument that annexin V is not specific for apoptosis since it images PS externalized on cells undergoing necrosis, autophagy, and apoptosis,[18] suggesting that dansyl derivatives may also detect these modes of cell death. Furthermore, these dansylhydrazones can also detect imatinib-induced cell death in K562 leukemia cells and HT-29 colon carcinomas (unpublished data). The ability to detect multiple pathways leading to cell death may be advantageous in the evaluation of cancer treatment response since necrosis, autophagy and apoptosis are all indicative of a positive therapeutic outcome.[18]

Results from the biological evaluation encouraged us to prepare the F-18 labeled analogue of **3** ([^{18}F]-**3**) for the potential use in PET imaging. The radiosynthetic approach to [^{18}F]-**3** is outlined in Scheme 2. Fluorine-18 labeled fluorobenzaldehyde ([^{18}F]-**5**) was prepared from 4-formyl-*N*,*N*,*N*-trimethylanilinium triflate as previ-

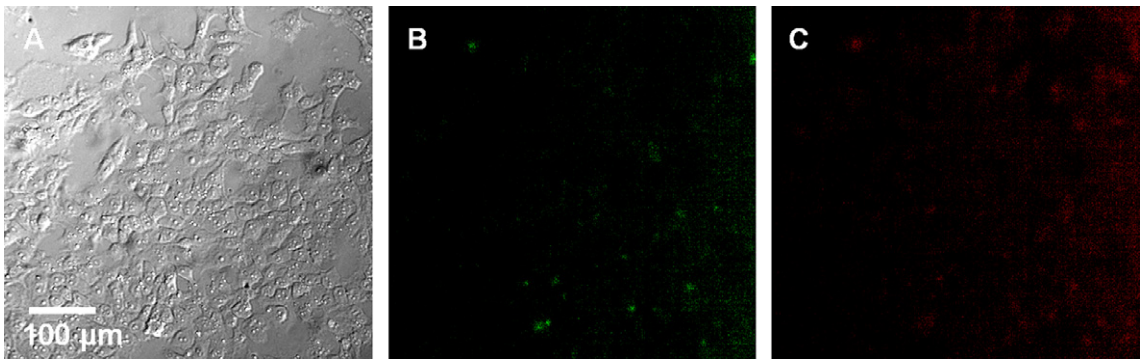


Figure 2. Images in the same field of view of untreated MCF-7 cells dually stained with annexin V labeled with Alexa® 488, and DFNSH. (A) DIC image; (B) DFNSH in green; (C) Alexa® 488 labeled annexin V in red. Total magnification (100×).

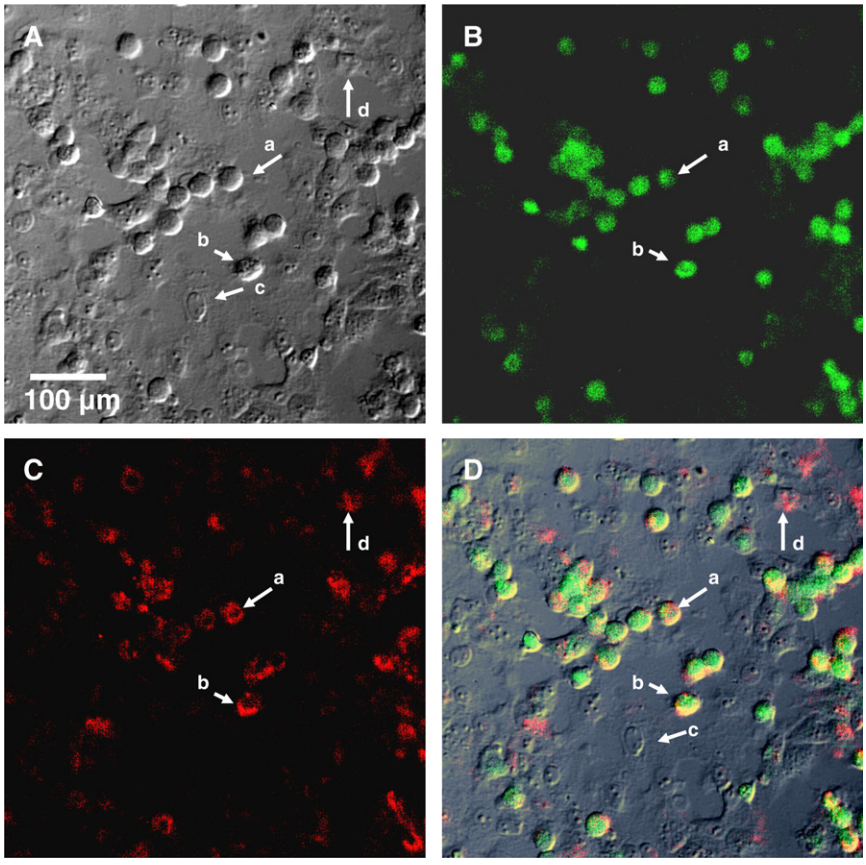


Figure 3. Images in the same field of view of MCF-7 cells dually stained with annexin V labeled with Alexa® 488, and DFNSH. (A) DIC image; (B) DFNSH in green; (C) Alexa® 488 labeled Annexin V in red; (D) composite image of (A), (B), and (C). Total magnification (100×).

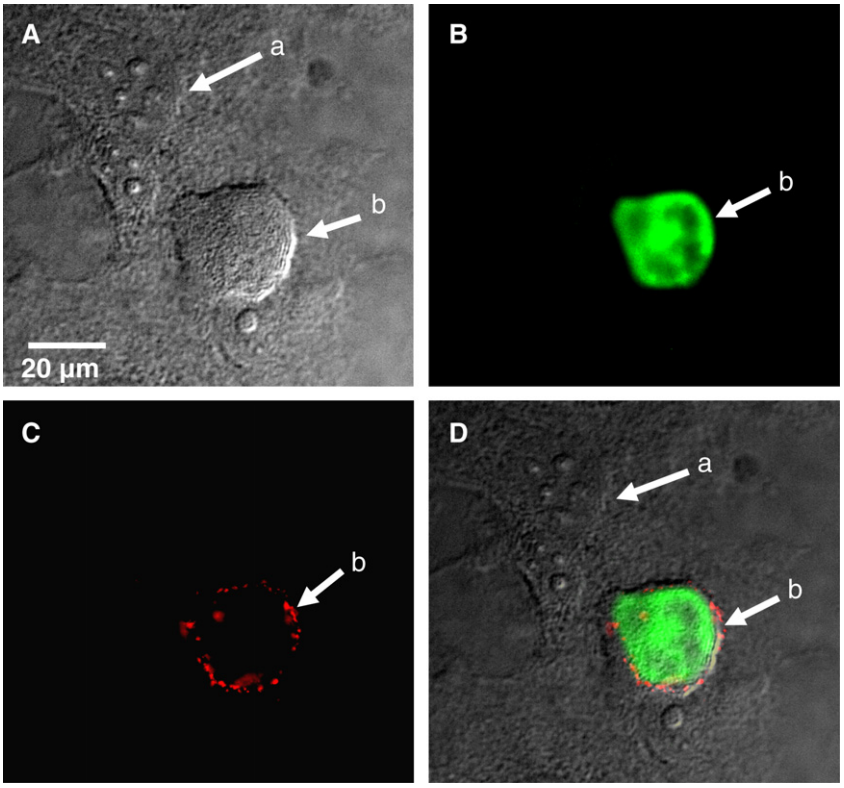


Figure 4. High magnification images in the same field of view of MCF-7 cell dually stained with annexin V labeled with Alexa® 488, and DFNSH. (A) DIC image showed a round-shaped apoptotic cell (arrow b) beside a normal cell (arrow a); (B) the apoptotic cell was specifically stained by DFNSH in green; (C) the apoptotic cell was also stained by Alexa® 488 labeled annexin V in red; (D) composite image of (A), (B), and (C). Total magnification (600×).

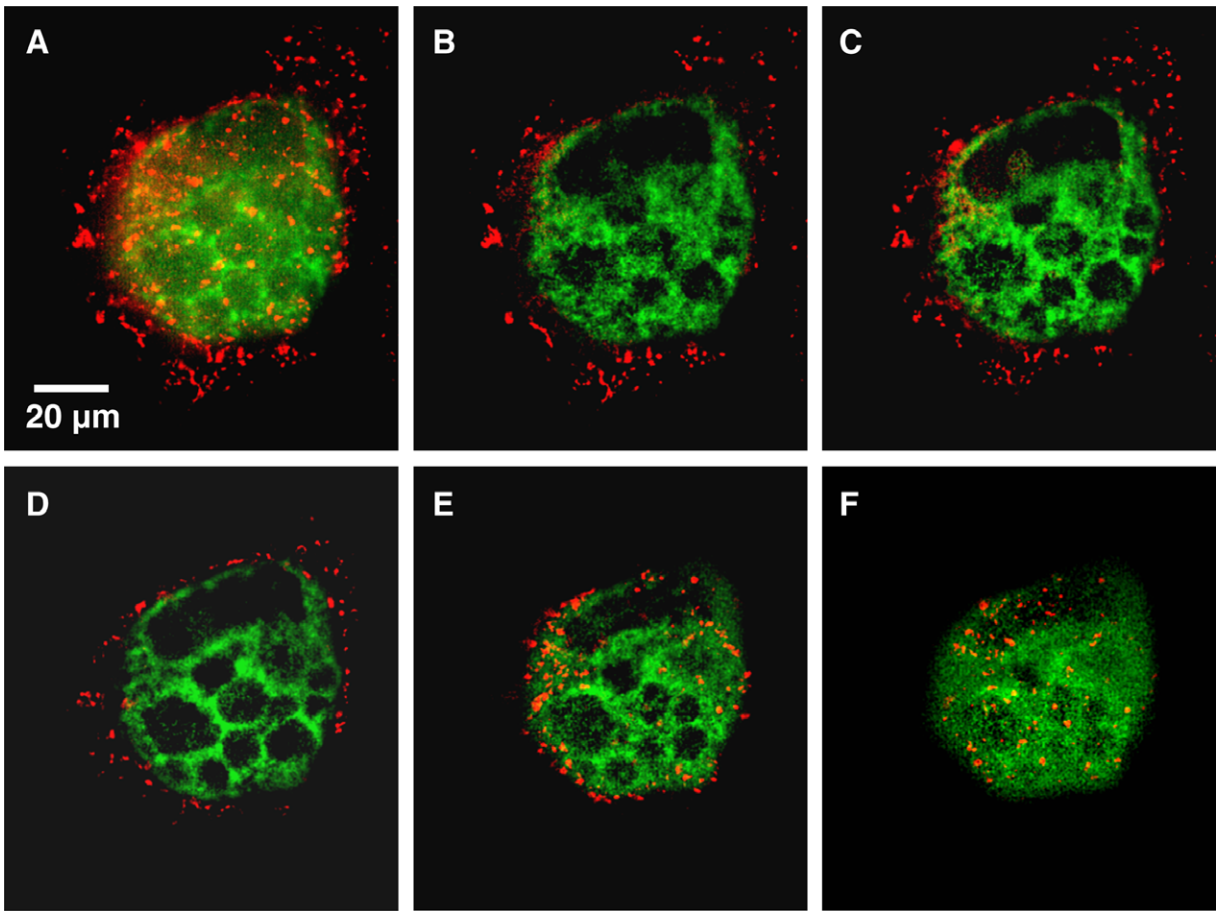


Figure 5. 3D representation of 4.8 mm thick MCF-7 cell dually stained with annexin V labeled with Alexa® 488, and DFNSH, comprising five optical slices captured at 1.2 mm intervals. (A) Composite image of all slices; (B) bottom slice at 0 mm; (C) slice at 1.2 mm; (D) slice at 2.4 mm; (E) slice at 3.6 mm; (F) top slice at 4.8 mm. Total magnification (600×).

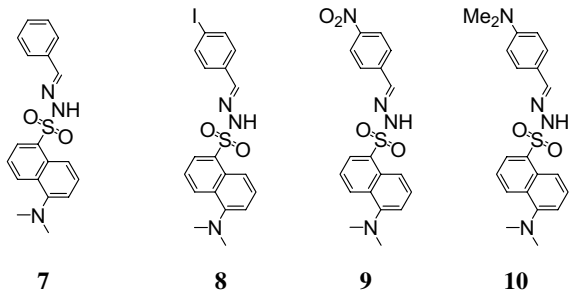
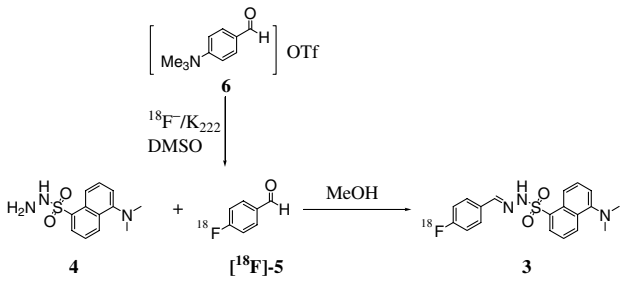


Figure 6. Other dansylhydrazone derivatives synthesized for biological evaluation.



Scheme 2. The synthesis of [^{18}F]-**3**.

ously reported.[28] After purification using a semi preparative silica Sep-Pak, compound [^{18}F]-**5** was allowed to react with dansylhydrazine at 100 °C for 30 min. [^{18}F]-DFNSH ([^{18}F]-**3**) was isolated in 50–60% radiochemical yields, based on [K/K$_{222}$]^{18}F, with a synthesis time of 50 min (EOB). The [^{18}F]-DFNSH was purified by HPLC in a radiochemical purity greater than 99%. PET imaging studies of tumor apoptosis in rodent models are currently underway.

In summary, we have found that dansylhydrazone derivatives selectively bind to paclitaxel-induced apoptotic cancer cells. 3D image analysis demonstrates that compound **3** exhibits intracellular uptake and accumulation in apoptotic cells. The straightforward preparation of fluorine-18 labeled **3** makes it a promising tracer for PET imaging of apoptosis. The evaluation of related dansyl derivatives is currently underway.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.002.

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