Bioorganic & Medicinal Chemistry 21 (2013) 7735–7741

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

A novel nitro-substituted benzothiadiazole as fluorescent probe for tumor cells under hypoxic condition

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ARTICLE INFO

Article history:
Received 19 July 2013
Revised 14 October 2013
Accepted 16 October 2013
Available online 1 November 2013

Keywords:
Benzothiadiazole
Hypoxic
Fluorescent probe
Nitroreductase

ABSTRACT

Most of solid tumor cells are hypoxic and hard to trace and measure. A new compound, 4,7-bis(4-dodecylthiophen-2-yl)-5,6-dinitrobenzo[*c*][1,2,5]thiadiazole (**BTTD-NO_2**), was synthesized for labeling the hypoxic cells specially in this paper. **BTTD-NO_2** showed no cytotoxicity to MG63 cells by MTT method. When MG63 cells were cultured with **BTTD-NO_2** under hypoxic condition for 24 h, strong red fluorescence distribution in cytoplasm was observed. Flow cytometry results showed that 65% of MG63 cells were labeled with strong red fluorescence in hypoxic condition while only 2.4% in oxic condition. Furthermore, Real time RT-PCR proved that **BTTD-NO_2** could stimulate high gene expression of the nitroreductase in the cells which could improve the conversion rate of **BTTD-NO_2** to **BTTD-NH_2** in turn. It proved that the fluorescence of **BTTD-NO_2** was quenched by its two nitro groups, however, strong red fluorescence could emit in the cytoplasm after the reduction of its nitro groups to amino groups in the tumor cells under hypoxic condition. These results suggested that **BTTD-NO_2** had the potential as a superior fluorescent probe for tumor detection.

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1. Introduction

Cancer is one of the diseases which are the most pressing public health now. The great challenges for future personalized oncology are to explore improved methodology for the early detection of localized and disseminated tumor cells in patients, which is very critical to the success of cancer therapy and improvement of patients' survival rates. Compared to other technologies of tumor detection, such as radioisotope labeling, magnetic resonance imaging (MRI), electron spin resonance (ESR) spectroscopy, and electrochemical detection, fluorescence imaging has many advantages, for example, it enables highly sensitive, less-invasive and safe detection using readily available instruments. Another advantage of fluorescence imaging is that the fluorescence signal of a molecule can be drastically modulated, so that probes relying on activation, not just accumulation, can be utilized.[1]

Many works had carried out on the fluorescence imaging by using luminescence probes for tumor cells in vitro.[2–6] Intravital microscopic imaging approaches, based on the use of fluorescence imaging, had been shown to allow the study of cell morphology and cell–cell interaction with high (single-cell) resolution in living organisms.[7] However, distinguishing of tumor cells compared with normal cells should be considered. With fast growth in the tumor of cancer, hypoxic condition would aroused in the center of the tumor. Hypoxic cells in some human solid tumors are thought to limit the effectiveness of radiotherapy and chemotherapy, and in some cases may contribute to failure to control the disease,[8] as most of solid tumor cells are hypoxic. Therefore, both before and during therapy, tracing and measurement of the hypoxic cell fraction in tumor would be considerable important in clinical treatment. Yin et al.[9] had designed a series of aliphatic N-oxide of naphthalimides and fluorescence image analysis showed that one of compounds had 17 times intensity of fluorescence in V79 cells under hypoxic condition compared with oxic environment. Many other researching works indicated that fluorescent detection for tumor cells could be effective by distinguishing those tumor cells under hypoxic condition.[10–12]

In the past few years, we had developed a few families of benzothiadiazole fluorophores which were optically stable and had easily tunable properties.[13,14] Due to the electron-withdrawing properties and high fluorescence quantum yields both in solution and in the solid state, benzo[*c*][1,2,5]thiadiazole (BTD) had been used for developing optoelectronic materials, liquid crystal displays (LCDs), organic semiconductors and light technology.[15] Previous researches showed that benzothiadiazole could selective label the mitochondria of the cells,[16,17] hence, we attempted to induce the stem of benzothiadiazole and synthesize a new derivative as a fluorescent probe for tumor cells.

After the new benzothiadiazole derivative was synthesized, we hypothesized that this compound could be transmitted into cells and emitted red fluorescence in the cells after bio-reduction under

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http://dx.doi.org/10.1016/j.bmc.2013.10.019

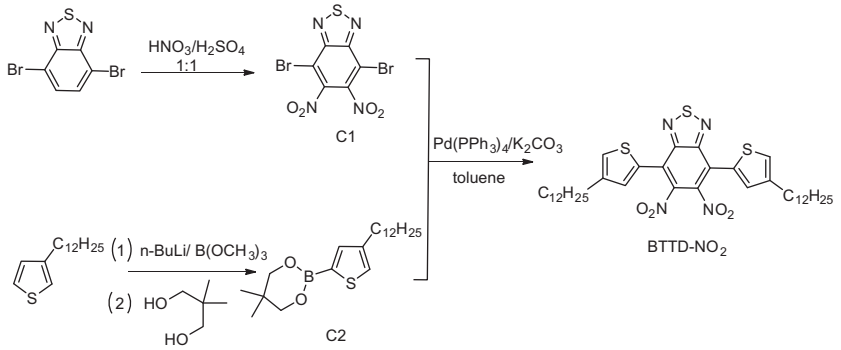

Figure 1. Synthesis of target compound, **BTTD-NO$_2$**.

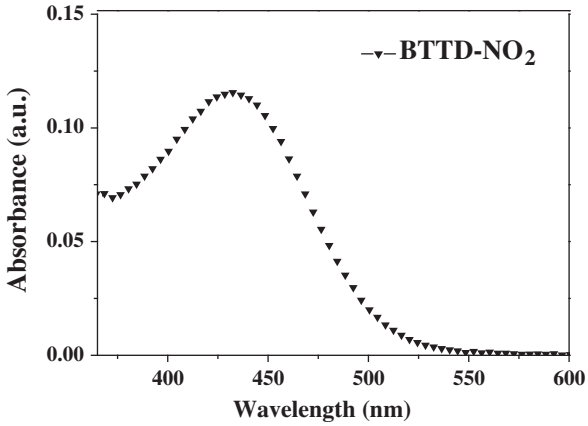

Figure 2. Absorption spectra of the target compound **BTTD-NO$_2$** (10^{-5} mol/L methanol).

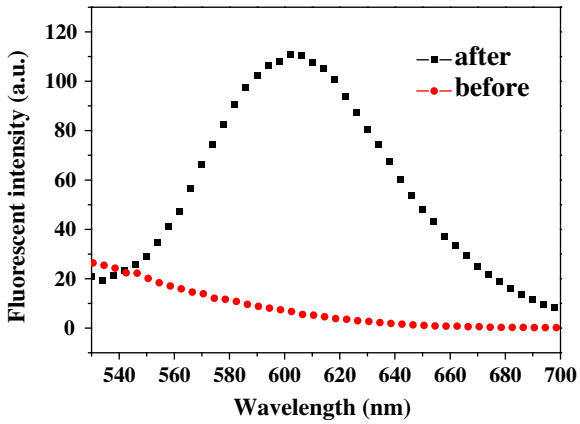

Figure 4. Fluorescence intensity of the target compound **BTTD-NO$_2$** before and after chemical reduction (10^{-6} mol/L methanol), excited at 480 nm.

hypoxic condition. To testify the hypothesis, the osteosarcoma cell line, MG63, was employed to the following biological experiments.

2. Results and discussion

2.1. Synthesis and spectra analysis

2.1.1. Synthesis

The new benzothiadiazole derivative, 4,7-bis(4-dodecylthiophen-2-yl)-5,6-dinitrobenzo[*c*][1,2,5]thiadiazole (**BTTD-NO$_2$**), was synthesized from 4,7-dibromobenzo[*c*][1,2,5]thiadiazole and 3-dodecylthiophene as shown in Figure 1. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR.

2.1.2. Absorption spectra of BTTD-NO$_2$

The absorption spectra of **BTTD-NO$_2$** were shown in Figure 2. In methanol solution, its maximal absorption band lie at 432 nm, arising from π–π^* transitions ($\log\varepsilon = 4.06$).

2.1.3. Fluorescence spectra of BTTD-NO$_2$

To study the fluorescent features, the **BTTD-NO$_2$** was reduced firstly according to chemical reduction which was illustrated in Figure 3. The results of fluorescence intensity of the **BTTD-NO$_2$** before and after reduction were shown in Figure 4, no fluorescence was detectable before chemical reduction of **BTTD-NO$_2$**, whereas **BTTD-NH$_2$** (chemical reduction from **BTTD-NO$_2$**) had broad and bright red luminescence with the peak at 600 nm after it was excited at 480 nm. Previous reports had proved that emission wavelength at 600 nm could greatly reduce the background fluorescence and improve the resolution.[18] The fluorescent features of **BTTD-NO$_2$** had reached our initial expectation.

2.1.4. Biological reduction of BTTD-NO$_2$

To further testify whether **BTTD-NO$_2$** could be reduced by nitroreductase, liver microsomes provided as nitroreductase (P450 reductase) in this experiment, the possible reaction principle was shown in Figure 5. After different concentration of **BTTD-NO$_2$** was treated by liver microsomes for 4 h, the fluorescence spectra

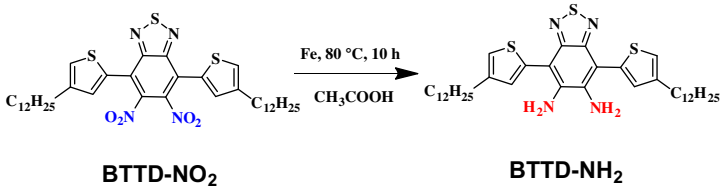

Figure 3. Chemical reduction of target compound **BTTD-NO$_2$**.

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