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# Synthesis and in vitro and in vivo evaluation of three radioiodinated nitroimidazole analogues as tumor hypoxia markers

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**Abstract**

Three novel nitroimidazole-based thioflavin-T derivatives, *N*-[4-(benzothiazol-2-yl)phenyl]-3-(4-nitroimidazole-1-yl)propanamide, *N*-[4-(benzothiazol-2-yl) phenyl]-3-(4-nitroimidazole-1-yl)-*N*-methylpropanamide and *N*-[4-(benzothiazol-2-yl)phenyl]-3-(2-nitroimidazole-1-yl) propanamide were synthesized and radiolabeled with iodine-131. Three $^{131}$I-labeled compounds continuously accumulated in hypoxic murine sarcoma S180 cells in vitro but not in aerobic cells. Biodistribution results in mice bearing S180 tumor indicated that the tracers could localize in the tumor and eliminate from it slowly. In contrast, the uptake in other organs (stomach excluded) was little and the clearance was quick. The tumor-to-tissue ratios of three compounds all increased with time.


*Keywords:* Nitroimidazole; Iodine 131; Hypoxia; Tumor; Biodistribution

## 1. Introduction

In the development of tumor hypoxia-imaging agents, nitroimidazole-containing compounds have received most attention [1–12]. The nitro group undergoes an enzyme-mediated one-electron reduction in viable cells to a radical anion. In normoxic tissues, the radical anion is rapidly back-oxidized to the original compound and eventually diffuses out of the cells and the tissues. However, under hypoxic condition, this radical anion is further reduced to some products, which are trapped within the cells by binding to cellular components [13,14]. Thus, these compounds could selectively accumulate in tumor hypoxia and be used as tumor hypoxia markers if they are radiolabeled [15–18].

In addition, the lipophilicity of these compounds, measured by the octanol/water partition coefficient ($P_{O/W}$), appears to play an important role [3,19–21]. A higher $P_{O/W}$ is often associated with a higher and longer retention in background tissues, including blood. However, a drug with an extremely low $P_{O/W}$ has the poor penetration across the membrane [19,20]. $^{99m}$Tc-BMS181321 and $^{99m}$Tc-BRU59-21 (previously known as BMS194796) have not evolved the marketed radiopharmaceuticals, despite their preferential accumulation in hypoxic tissues. It is probably due to their high lipophilicity, which leads to their slow plasma clearance and high hepatic uptake [4,5]. Recently, Klunk and others synthesized a series of thioflavin-T (ThT) derivatives with appropriate $P_{O/W}$ value and good penetration across the intact blood–brain barrier [22–27]. Therefore, we conjugated 2- or 4-nitroimidazole with ThT derivatives, 2-(4′-aminophenyl)benzothiazole (BTA) or 2-(4′-methylaminophenyl)benzothiazole (BTA-1); thus, these compounds could accumulate in tumor hypoxia and quickly clear from blood and other organs and may be possibly used as brain hypoxia markers.

In this article, we report the synthesis of three nitroimidazole-containing ThT derivatives, their in vitro binding to viable hypoxic or aerobic tumor cells and their in vivo biodistribution in tumor-bearing mice.

## 2. Materials and methods

2-Nitroimidazole (98%), 4-nitroimidazole (98%) and *N*,*N*-diisopropylethylamine (DIEA, >98%) were purchased from ACROS Organics and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 99%) was from GL Biochem, Dulbecco's modified Eagle medium (DMEM) was obtained from Gibco BRL Life Technologies and fetal bovine serum was from Beijing

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Xinjingke Biotechnology. All other reagents were of AR grade. BTA and BTA-1 were synthesized according to methods of Klunk and others [22,23]. No-carrier-added (NCA) $Na^{131}I$ (aqueous solution) was obtained from China Institute of Atomic Energy. Murine sarcoma S180 cell line was kindly provided by the College of Life Sciences, Peking University. Kunming mice (20–25 g, male) were supplied by Breeding Center of the Institute of Zoology, Chinese Academy of Sciences.

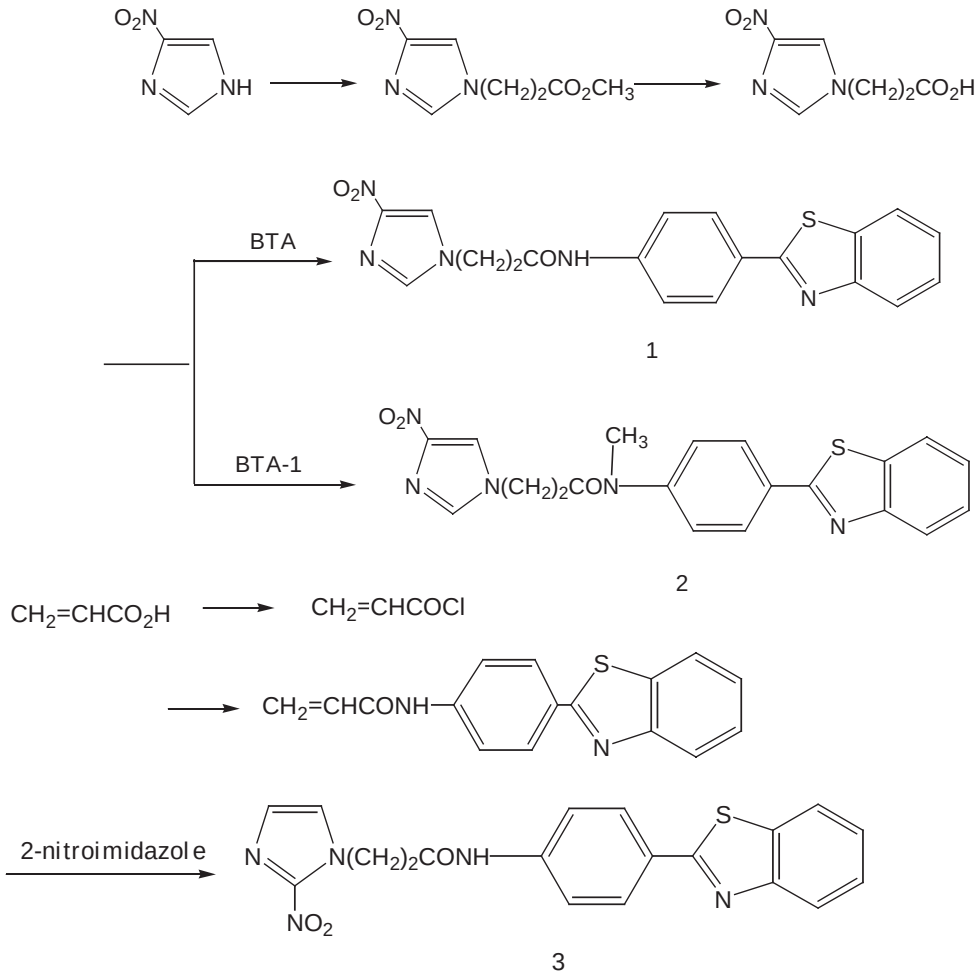
NMR spectra were recorded on a Bruker ARX-400 (400 MHz) spectrometer with deuterated dimethyl sulfoxide (DMSO-$d_6$) as a solvent and with tetramethylsilane as an internal standard. Elements analyses were carried out on the Elementar Vario EL (Germany). Electron impact mass spectra (EI-MS) were obtained with a ZAB-HS mass spectrometer (Micromass). Radioactivity in organs or tissues of mice was assayed using a Cobra II series Auto-Gamma Counting System (Packard). The FT-603 γ well counter was obtained from Beijing Nuclear Instrument Factory and the JPSJ-605 dissolved oxygen meter was from REX Instrument Factory of Shanghai Precision and Scientific Instrument.

### 2.1. Synthesis

#### 2.1.1. N-[4-(Benzothiazol-2-yl)phenyl]-3-(4-nitroimidazole-1-yl)propanamide (4NPBTA) (**1**)

Methyl acrylate (9.0 ml, 0.1 mol) was added dropwise to a solution of 4-nitroimidazole (1.13 g, 0.01 mol) in triethylamine (9.0 ml), and the mixture was refluxed for 5 h (Scheme 1). The solvent was removed under vacuum. The residue was washed with water, filtered off and air-dried. 1.60 g (90% yield) of white sheets of methyl 3-(4-nitroimidazole-1-yl)propanoate was produced. mp 142–143°C (lit. [28] yellow solid, mp 116–117°C). $^1$H NMR $\delta$ (DMSO-$d_6$): 8.43 (s, 1H, imi-H), 7.87 (s, 1H, imi-H), 4.30 (t, 2H), 3.60 (s, 3H), 2.97 (t, 2H).

One gram of methyl 3-(4-nitroimidazole-1-yl) propanoate was added into NaOH solution and the solution was stirred for 5 min. Then its pH was brought to 1 with 6 mol/l HCl. Standing for 12 h gave 0.83 g of needle-like crystals of 3-(4-nitroimidazole-1-yl)propanoic acid. mp 176–178°C, yield 90%. $^1$H NMR $\delta$ (DMSO-$d_6$): 12.55 (s, 1H), 8.42 (d, 1H, imi-H), 7.87 (d, 1H, imi-H), 4.27 (t, 2H), 2.87 (t, 2H).




Scheme 1. Synthesis of compounds (**1**), (**2**), and (**3**).