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Synthesis and biological evaluation of a novel ^{99m}Tc labeled 2-nitroimidazole derivative as a potential agent for imaging tumor hypoxia

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ABSTRACT

Tumor hypoxia plays a major role in reducing the efficacy of therapeutic modalities like chemotherapy and radiation therapy in combating cancer. In order to target hypoxic tissues, a tripeptide ligand having a 2-nitroimidazole moiety, as a bioreductive species, was synthesized. The latter was radiolabeled with ^{99m}Tc for imaging hypoxic regions of tumors and was characterized by means of its rhenium analogue. The biodistribution and scintigraphic image of the corresponding ^{99m}Tc-complex showed accumulation in tumor and these results suggest that it could be a marker for imaging tumor hypoxia.

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Hypoxia is characterized by an imbalance between the rate of cellular oxygen consumption and oxygen supply in cells. Hypoxic tissues are a hallmark of advanced solid tumors. The distribution of hypoxic tissue in tumors is heterogeneous and variable over time. It has been shown at the beginning of the twentieth century that hypoxia plays a role in increased radio resistance.[1] Indeed, oxygen has a crucial role in the response to radiotherapy. Being a potent radiosensitizer, it enhances the effect of ionizing radiation leading to a lethal effect on tumor cells by breaking the double-stranded DNA. More recently it has been shown that hypoxia was a predictor for drug resistance.[2] The decrease in the response to radiotherapy of hypoxic cells then leads to an increased risk of metastasis and poor prognosis for the patient.[3] It is therefore of major interest to have a precise knowledge of the level of oxygen in the tissues in order to understand the formation mechanism of this hypoxic state, and develop strategies to address this imbalance. This requires quantifying the level of oxygenation of tissues with good spatial and temporal resolution.

We can distinguish two classes of markers of hypoxia: the nitroimidazole ligands and non-nitroimidazoles ligands. Nitroimidazole derivatives are known for their ability to be selectively retained in hypoxic tissues. Nakamura has first demonstrated in 1955 that, the 2-nitroimidazole showed activity against particular types of infections associated with a deficiency of oxygen.[4] Therefore nitroimidazole analogues were synthesized and proved to be effective against bacteria and protozoa that proliferate in hypoxic cells.[5] This particular behavior of nitroimidazole derivatives in oxygen-deficient environment led to their study in the detection of hypoxia.[6,7]

Most clinically used tracers are ^{18}F-labeled tracers in order to perform a PET imaging but this radiochemistry needs a cyclotron and a GMP laboratory. Thus, owing to limited availability, high cost of production and short half-life of cyclotron isotopes, a ^{99m}Tc-based agent could be a better alternative, especially as all nuclear medicine centers possess gamma cameras whereas only few has one PET-CT camera. So, many radio pharmaceuticals[8–10] (Fig. 1) having a nitroimidazole moiety and radiolabeled with technetium-99m for SPECT imaging of hypoxic tissues were developed. However, due to their high background levels in normal tissues, quantification of hypoxia may be difficult.[11] So, there is a crucial need to develop new technetium-99m candidates.

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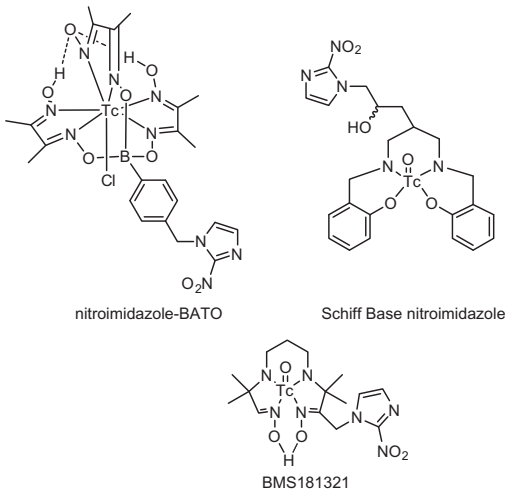
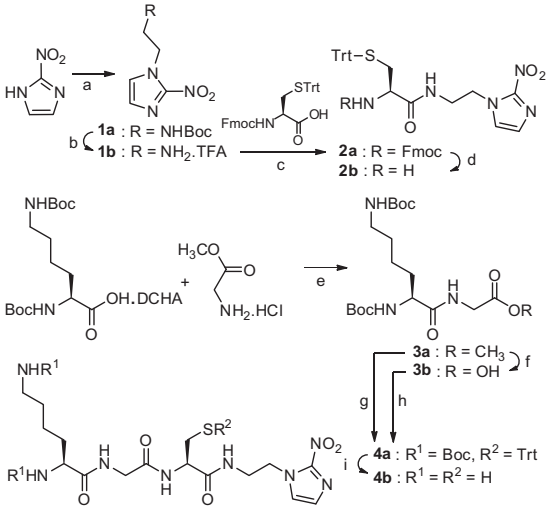


Figure 1. Structures of selected technetium-containing nitroimidazoles tested for imaging.

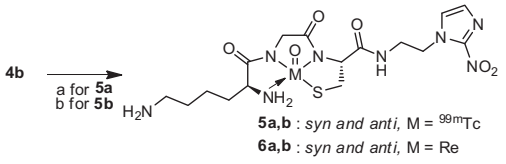
There are many examples in the literature[12] reporting the use of peptides acting as chelating agent of ^{99m}Tc (N_xS_{4-x}). But to date there are only few examples of hypoxia radiotracers using such ligands to complex ^{99m}Tc. Indeed, only Ballinger and co-workers.[13] report the use of sequences glycine–serine–cysteine and glycine–cysteine–glycine to chelate ^{99m}Tc.

In this study, a cysteine–glycine–lysine sequence with a bioreducible moiety, 2-nitroimidazole, was synthesized and labeled with ^{99m}Tc. We chose to use a 2-nitroimidazole moiety as a vector since it has a better response to hypoxia than 4- and 5-nitroimidazoles isomers.[14] The latter was then evaluated as a tumor hypoxia marker.

The chelating agent **4b** was synthesized through a multi-step reaction using 2-nitroimidazole as a starting material. The synthesis procedure is outlined in Scheme 1. On the one hand, according to a procedure reported in the literature by Hay et al.,[15] 2-nitroimidazole was reacted with *tert*-butyl 2-bromoethylcarbamate to give compound **1a**. Then, the Boc protecting group was cleaved with trifluoroacetic acid to lead to the expected derivative **1b**. The so-obtained amine was reacted with commercially available Fmoc-L-Cys(Trt)-OH using propylphosphonic anhydride T3P® to afford the desired protected nitroimidazole derivative **2a**. The subsequent Fmoc-deprotection was achieved by classical reaction with piperidine, leading to amine **2b**. On the other hand, a peptidic coupling reaction with available glycine methyl ester hydrochloride and Boc-L-Lys(Boc)-OH, DCHA gave the protected dipeptide **3a**. The ester group of **3a** was also converted into an acid moiety to give **3b**. Then, the required protected tripeptide **4a** was prepared from ester **3a** following a classical hydrolysis with LiOH and coupling with **2b** in the presence of the cationic coupling agent HBTU. Moreover, it should be noted that the protected tripeptide **4a** was also synthesized starting from **3b** and T3P®. Finally, the cleavage of both NH-Boc and *S*-Trityl protecting groups was conducted by means of TFA in the presence of triethylsilane and phenol as scavengers to give the desired tripeptide **4b**. Optimization of the radiolabeling procedure of **4b** was then conducted (Scheme 2, Table 1). So, peptide **4b** (10 μg) was radiolabeled with sodium tartrate (20 mg in 0.5 mL, pH 7), $^{99m}TcO_4^-$ (740 MBq) and tin(II) chloride (40 μg in 0.1 mL). The resulting solution was analyzed by radio-HPLC. The reaction was first studied at 25 °C and a 95% radiochemical yield was obtained when the reaction time was changed from 10 to 60 min (Table 1, entries 2 and 3). We then decided to perform the reaction at 100 °C (Table 1, entry 4). These conditions have enabled us to considerably reduce the reaction time to 10 min (Table 1, entry 4). Using this labeling procedure, the complex was obtained with a radiochemical purity of 95% as a *syn* and *anti*-diastereoisomers mixture **5a**,**b** (Fig. 2). It has been demonstrated that the presence of two radiometric peaks is due to the resolution of diasteroisomers resulting from the chiral centers on the peptide backbone and the chiral technetium.[12b,16] It is difficult to determine directly the formation of the ^{99m}Tc-complex because of the extremely small amount of the compound present. However, the formation of ^{99m}Tc-complex **5a**,**b** could be indirectly determined by comparison with **6a**,**b** which can be easily prepared. Re and Tc belongs to the same group of the periodic table and are similar in size. In our case, the analogue rhenium complex **6a**,**b** was



Scheme 1. Reagents and conditions: (a) *tert*-Butyl 2-bromoethylcarbamate, K_2CO_3, DMF, 110 °C, 5 h (57%); (b) TFA, 25 °C, 10 min (99%); (c) T3P®, DIEA, CH_2Cl_2, 25 °C, 12 h (86%); (d) piperidine (20% mol), DMF, 25 °C, 16 h (53%); (e) T3P®, DIEA, CH_2Cl_2, 25 °C, 12 h (86%); (f) LiOH, H_2O, 25 °C, 12 h (85%); (g) LiOH, H_2O, DMF, CH_2Cl_2 then **2b**, HBTU, DIEA, DMF, 25 °C, 12 h (43%); (h) **2b**, T3P®, DIEA, CH_2Cl_2, 25 °C, 12 h (53%); (i) TFA, H_2O, TES, phenol, 0 °C, 1 h (52%).



Scheme 2. Reagents and conditions. (a) $SnCl_2$, $^{99m}TcO_4^-$, sodium tartrate pH 7.0, 100 °C, 10 min (radiochemical yield 95%); (b) $ReOCl_3(PPh_3)_2$, NaOAc, MeOH, 70 °C, 4 h.

Table 1
Labeling conditions of ^{99m}Tc-complex **5a**,**b** with sodium pertechnetate and tin(II) chloride

Entry	**4b** (μg)	Sodium tartrate (mg)	Final pH	Temperature (°C)	Time (min)	RCY (%)
1	100	—	5	25	10	76
2	10	20	5	25	10	89
3	10	20	5	25	60	95
4	10	20	5	100	10	95
5	10	20	9[a]	100	10	38
6	10	20	4[b]	100	10	38

[a] Phosphate buffer 0.2 M pH 9, 0.5 mL.
[b] Citric acid buffer, 20 mM pH 4, 0.5 mL.