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Preparation and characterization of a novel Al^{18}F–NOTA–BZA conjugate for melanin-targeted imaging of malignant melanoma

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ABSTRACT

Melanin is an attractive target for the diagnosis and treatment of malignant melanoma. Previous studies have demonstrated the specific binding ability of benzamide moiety to melanin. In this study, we developed a novel ^{18}F-labeled NOTA–benzamide conjugate, Al^{18}F–NOTA–BZA, which can be synthesized in 30 min with a radiochemical yield of 20–35% and a radiochemical purity of >95%. Al^{18}F–NOTA–BZA is highly hydrophilic ($\log P = -1.96$) and shows good in vitro stability. Intravenous administration of Al^{18}F–NOTA–BZA in two melanoma-bearing mouse models revealed highly specific uptake in B16F0 melanotic melanoma (6.67 ± 0.91 and 1.50 ± 0.26% ID/g at 15 and 120 min p.i., respectively), but not in A375 amelanotic melanoma (0.87 ± 0.21 and 0.24 ± 0.09% ID/g at 15 and 120 min p.i., respectively). The clearance from most normal tissues was fast. A microPET scan of Al^{18}F–NOTA–BZA-injected mice also displayed high-contrast tumor images as compared with normal organs. Owing to the favorable in vivo distribution of Al^{18}F–NOTA–BZA after intravenous administration, the estimated absorption dose was low in all normal organs and tissues. The melanin-specific binding ability, sustained tumor retention, fast normal tissues clearance and the low projected human dosimetry supported that Al^{18}F–NOTA–BZA is a very promising melanin-specific PET probe for melanin-positive melanoma.

Melanoma is one of the most lethal forms of skin cancer.[1] It ranks fifth in men and seventh in women as the most common cancer in the United States.[2] The incidence of melanoma has increased significantly over the past 3 decades, doubling almost every decade.[3] The prognosis and the patient's survival depend on the clinical stage of the disease at the time of diagnosis.[4]

A key feature of melanoma is the extensive melanin expression in most tumor cells. The entirely nonpigmented form is very uncommon in melanoma. Melanotic tumors represent over 90% of all malignant melanomas.[5] Melanotic melanoma develops from genetically altered neoplastic melanocytes that contain extensive melanin, thus making it a very attractive target for diagnostic imaging and therapy.[6] Melanin is a group of biopolymers that contain indole units with carboxyl functionalities and phenolic hydroxyl groups,[7] with the capability of binding many different types of compounds.[8]

Several melanin-targeted PET and single-photon emission computed tomography (SPECT) probes containing benzamide or nicotinamide moiety have been successfully developed (Fig. 1 and Table 1), such as *N*-(2-diethylaminoethyl)-4-^{123}I-iodobenzamide (^{123}I-IBZA),[9,10] *N*-(2-diethylaminoethyl)-4-^{18}F-fluorobenzamide (^{18}F-FBZA),[11] *N*-(2-diethylaminoethyl)-4-(4-fluorobenzamido)-5-^{123}I-iodo-2-methoxybenzamide (^{123}I-MIP-1145),[12] ^{18}F-6-fluoro-*N*-((2-(diethylamino)ethyl)pyridine-3-carboxamide (^{18}F-MEL050),[13,14] ^{123}I-*N*-(2-(diethylamino)ethyl)-5-iodonicotinamide (^{123}I-MEL008),[15] $^{123/131}$I-6-chloro-*N*-(4-((2-(diethylamino)ethyl)carbamoyl)-2-iodo-5-methoxyphenyl)nicotinamide ($^{123/131}$I-Iochlonicotinamide),[16] ^{68}Ga-labeled *N*-(2-diethylaminoethyl)benzamide derivative (^{68}Ga-SCN–NOTA–BZA),[17] ^{68}Ga-labeled DOTA–benzamide derivative (^{68}Ga-SCN–DOTA–PCA)[18] and the radioiodinated phenylacetamide and its homologue ($^{123/131}$I-IHPA/IHPP).[19] However, most benzamide/nicotinamide-based probes show relatively high accumulation in some normal organs, particularly the liver.

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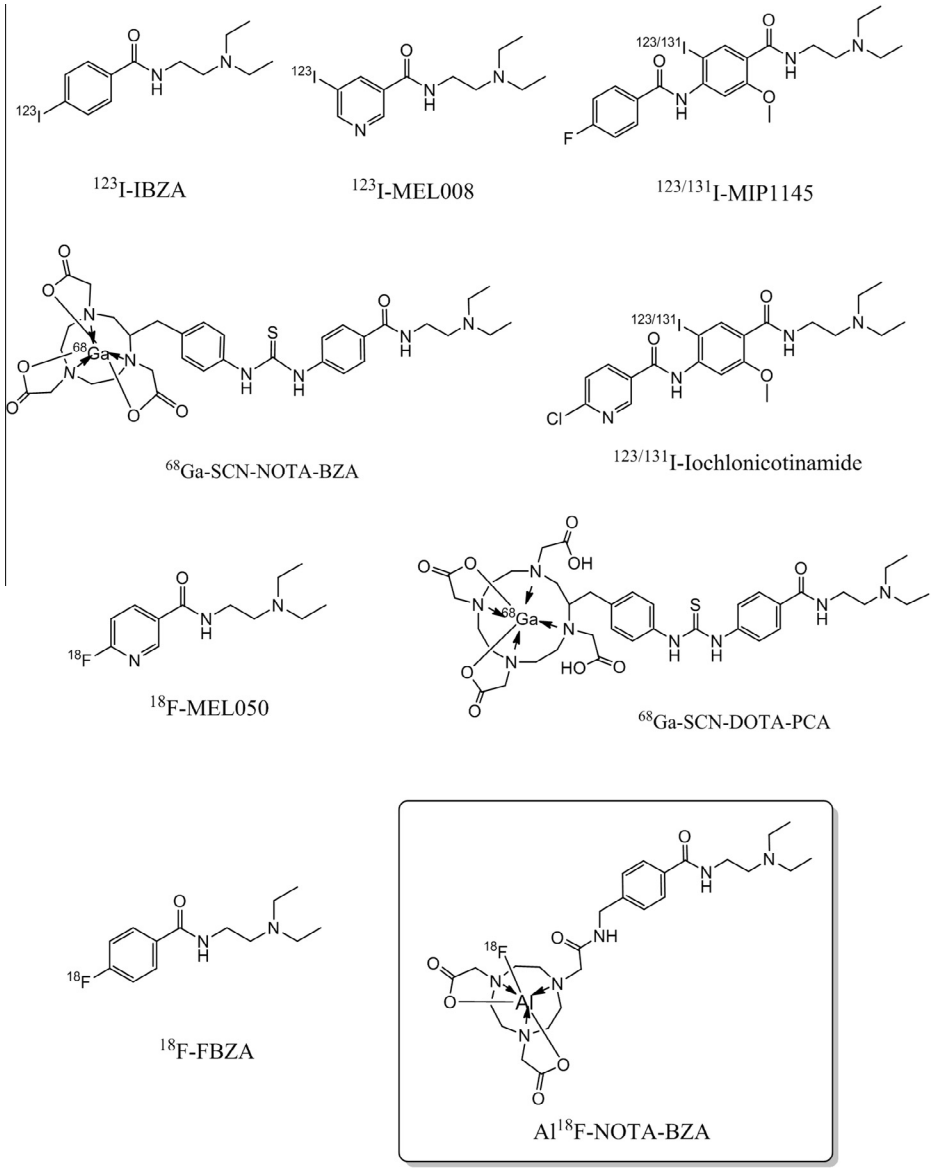

Figure 1. Chemical structure of melanin-targeting melanoma probes developed for imaging and/or targeted radionuclide therapy of melanoma and Al^{18}F–NOTA–BZA.

Among various positron-emitting isotopes, ^{18}F (half-life, 110 min) has nearly ideal properties for PET, such as low positron energy, lack of side emissions, and a suitable half-life. The routinely used nucleophilic ^{18}F fluorination requires multistep procedures including repeated azeotropic distillation, radiolabeling at high temperature in an organic solvent containing base catalyst, and the evaporation of organic solvent.[20] For example, the synthesis of ^{18}F-FBZA is usually conducted via conjugation of ^{18}F-*N*-succinimidyl-4-fluorobenzoate (^{18}F-SFB) to the primary amino group of *N*,*N*-diethylethylenediamine (DEDA), which requires a multistep synthesis and is time-consuming and laborious.[4]

Recently, several pioneering studies on one-step ^{18}F-labeling methods have been reported.[20–22] Fluoride binds strongly to aluminum, which can then form a stable chelate with NOTA in water.[23] A one-step radiofluorination via Al–^{18}F complex may greatly reduce the total synthesis time and enable the kit-production of ^{18}F-labeled complex. The water-compatible reaction is ideal for the incorporation of ^{18}F-fluoride into biologically active ligands, especially those biomolecules that are soluble only in water. A validated lyophilized kit that enables rapid and reliable labeling of a ligand by a simple addition of $^{18}F^{-}$ in saline, then a brief heating step, followed by a rapid and simple purification process, would make the Al–^{18}F radiofluorination of molecules more compatible with the already established good manufacturing practices (GMP) applied for the preparation of $^{99m}T_c$-agents.[24]

In this study, we developed a novel NOTA-benzamide derivative, Al^{18}F–NOTA–BZA, by forming Al–F complex (Fig. 1), and we performed the biological characterizations in two melanoma mouse models to demonstrate it as a promising PET probe for targeted melanin imaging. All the procedures of chemical syntheses (compounds **2**, **3**, **4**, **5**, **6** and **7a**), the Al–^{18}F radiofluorination (**7b**), and the chemical and biological characterizations are detailed in Supplementary materials.

The authentic standard **7a** and radiotracer **7b** were prepared from NOTA-conjugated precursor NOTA–BZA (**6**), which was synthesized from 4-(aminomethyl)benzoic acid **1** through a five-step procedure (Scheme 1). Compound **2** was produced by the reaction of **1** with $(Boc)_2O$ in 1 N NaOH at room temperature for 8 h, followed by further purification by silica gel column chromatography