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New ^{68}Ga-PhenA bisphosphonates as potential bone imaging agents

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ABSTRACT

Introduction: In vivo positron emission tomography (PET) imaging of the bone using [^{68}Ga]bisphosphonates may be a valuable tool for cancer diagnosis and monitoring therapeutic treatment. We have developed new [^{68}Ga] bisphosphonates based on the chelating group, AAZTA (6-[bis(hydroxycarbonyl-methyl)amino]-1,4-bis(hydroxycarbonyl methyl)-6-methylperhydro-1,4-diazepine).
Method: Phenoxy derivative of AAZTA (2,2′-(6-(bis(carboxymethyl)amino)-6-((4-(2-carboxyethyl)phenoxy) methyl)-1,4-diazepane-1,4-diyl)diacetic acid), PhenA, **2**, containing a bisphosphonate group (PhenA-BPAMD, **3**, and PhenA-HBP, **4**) was prepared. Labeling of these chelating agents with ^{68}Ga was evaluated.
Results: The ligands reacted rapidly in a sodium acetate buffer with [^{68}Ga]$GaCl_3$ eluted from a commercially available ^{68}Ge/^{68}Ga generator (pH 4, >95% labeling at room temperature in 5 min) to form [^{68}Ga]PhenA-BPAMD, **3**, and [^{68}Ga]PhenA-HBP, **4**. The improved labeling condition negates the need for further purification. The ^{68}Ga bisphosphonate biodistribution and autoradiography of bone sections in normal mice after an iv injection showed excellent bone uptake.
Conclusion: New ^{68}Ga labeled bisphosphonates may be useful as in vivo bone imaging agents in conjunction with positron emission tomography (PET).

1. Introduction

It is well recognized that there are many unique physical characteristics for using a ^{68}Ge/^{68}Ga generator for PET (positron emission tomography) imaging. The long-lived parent isotope, germanium-68 (^{68}Ge) ($t_{1/2}$ 271 d), allows for easy and worldwide generator distribution [24–26]. The physical decay properties of the short-lived daughter ^{68}Ga ($t_{1/2}$ 68 min, 89% β^+, 1.92 MeV max energy) are suitable for PET imaging. Thus, ^{68}Ga provides a convenient alternative source for position emitting isotopes without the need for a nearby cyclotron. In the past few years, ^{68}Ga labeled radiopharmaceuticals, such as DOTATOC, DOTATATE, and DOTANOC, used mainly for detecting over-expression of somatostatin receptors (SSTRs) associated with neuroendocrine tumors, have successfully demonstrated clinical utility and attracted significant attention for the use of PET imaging in the diagnosis of various diseases [27–31]. For example, ^{68}Ge/^{68}Ga generators are now commercially available for routine clinical use, thereby making ^{68}Ga agents attractive for routine PET imaging studies.

Due to the relatively short physical half-life of ^{68}Ga ($t_{1/2}$ = 68 min) there are several practical requirements for developing new ^{68}Ga radiopharmaceuticals: (a) high in vitro and in vivo stability of the desired ^{68}Ga complexes; (b) fast kinetics in the formation of ^{68}Ga complexes; (c) ability to form bifunctional molecules for targeting, preconjugation, to biologically active molecules; and (d) capacity to display in vivo stability in blood circulation [1,24,32]. In the past two decades, many Ga complexes have been analogs of macrocyclic ligands of

Abbreviations: PET, positron emission tomography; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; NET, neuroendocrine tumor; DTPA, diethylenetriaminepentaacetic acid [1]; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid [2]; NOTA, 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid [3, 4]; NODAGA, 1-(1,3-carboxypropyl)-4,7-carboxymethyl-1,4,7-triazacyclononane [1, 5–9]; TRAP, 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonic) acid [4]; DEDPA, 1,2-[[6-(carboxy)-pyridin-2-yl]-methylamino]ethane [1, 10]; HBED-CC, *N*,*N*′-bis[2-hydroxy-5-(carboxyethyl)-benzyl]ethylenediamine-*N*,*N*′-diacetic acid [11] [12]; AAZTA, 6-[bis(hydroxycarbonyl-methyl)amino]-1,4-bis(hydroxycarbonyl methyl)-6-methyl perhydro-1,4-diazepine [13, 14]; EDTMP, (ethylene-diamino-*N*,*N*,*N*′,*N*′-tetrakis-methylene-phosphoric acid) [15]; BPAMD, (4-{[(bis-phosphonomethyl) carbomoyl]methyl}-7,10-bis-(carboxy-methyl)-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid [16–18]; BPAPD, (4-{[(bis–phosphonopropyl) carbomoyl]methyl}-7,10-bis-(carboxymethyl)-1,4,7,10-tetra azacyclododec-1-yl)-acetic acid [18]; BPPED, (DO3ABP): tetraethyl-10-{[(2,2-bis-phosphonoethyl)-hydroxyl phosphoryl]methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid [18]; NOTA-BP, 2-[4,7-di(carboxymethyl)-1,4,7-triazonan-1-yl]-5-[(4-hydroxy-4,4-diphosphonobutyl)-amino]-5-oxopentanoic acid [11, 19] [20]; NOTAMBP, 4-{[(bis–phosphonopropyl) carbomoyl]methyl}-1,4,7-triaza cyclonone-1,4-diacetic acid [21] ; TRAP (NOTP), 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris[(bis-phosphonopropyl) carbomoyl] methyl-methylenephosphonic acid [4]; TRAP(MDP)$_3$, 1,4,7-triazacyclononane-1,4,7-tri(methylene phosphinic acid) [22,23]; MDP, methylene disphosphonate; EDTMP, ethylenediamine-tetra(methylene phosphonic acid, phosphinic acid functionalized polyazacycloalkane chelators [4].

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polyaza carboxylic acids; these are often metal-chelating ligands designed to form gadolinium (Gd) complexes for use as magnetic resonance imaging (MRI) contrast agents. A large number of cyclic polyaza carboxylic acids, such as DTPA, DOTA, NOTA, and related derivatives (Fig. 1) are commonly employed to chelate radioactive metal ions. This includes isotopes for SPECT imaging – ^{67}Ga, ^{99m}Tc, and ^{111}In, as well as isotopes for PET imaging – ^{64}Cu, ^{86}Y, ^{89}Zr, ^{68}Ga, and ^{44}Sr [1,2,33,34] (Chart 1).

Literature reports on extensive studies of polyaza carboxylic acids, such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and related ligands [1,2], suggested that they form Gd(III) complexes with high thermodynamic stabilities. However, the complexation of no-carrier-added (n.c.a.) ^{68}Ga with DOTA derivatives is less efficient, often requiring heating (80–100 °C). Formation of DOTA ligands with Ga(III) is more sensitive to experimental conditions than that of NOTA analogs (NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid) [18,21]. It appears that the smaller cavity created by the NOTA derivatives is a better fit to the ionic radius of Ga(III). Derivatives of NOTA, especially NODAGA [6], proved to be more suitable for chelating the Ga(III) ion than those of DOTA. The Ga(III)NODAGA complexes exhibited much higher thermodynamic stability as well as rapid complex kinetics. As Ga(III) is a small ion and mostly requires an octahedral coordination sphere, Ga(III)NODAGA analogs provide optimal in vitro and in vivo stability [21]. There are several reports in which Ga(III)NODAGA was preferentially chosen as the chelating group in making bifunctional imaging agents [1,7,8,35]. Using DOTA and NOTA derivatives, many ^{68}Ga labeled bisphosphonates were prepared and tested for bone imaging [16,18,20,21,36].

Originally, AAZTA was reported as an excellent chelating group for Ga(III), through which many useful MRI contrast enhancing agents were developed [37,38]. There are several advantages to using AAZTA, a conformationally constrained macrocyclic polyaza carboxylic acid, for chelating Ga(III); the ligand displayed high binding affinity, excellent in vitro stability, fast kinetics in complex formation at room temperature, and stability in plasma [1]. Recently, a few reports appeared that employed 6-[bis(hydroxycarbonyl-methyl)amino]-1,4-bis(hydroxy carbonylmethyl)-6-methylperhydro-1,4-diazepine, AAZTA, for chelating Ga(III) [14,39–41]. Stability constants, (log K) for Ga(III)-DOTA [42], Ga(III)-NOTA [43], and Ga(III)AAZTA [41] complexes were reported (21.33, 30.98, and 22.18, respectively). It was also reported that the "cold" Ga(III)AAZTA was stable after incubation in serum transferrin at room temperature [41]. Preliminary results of the ^{68}Ga-AAZTA-conjugated RGD peptide displayed excellent uptake and retention in tumors compared to that of ^{68}Ga-DOTA-conjugated RGD peptides [2].

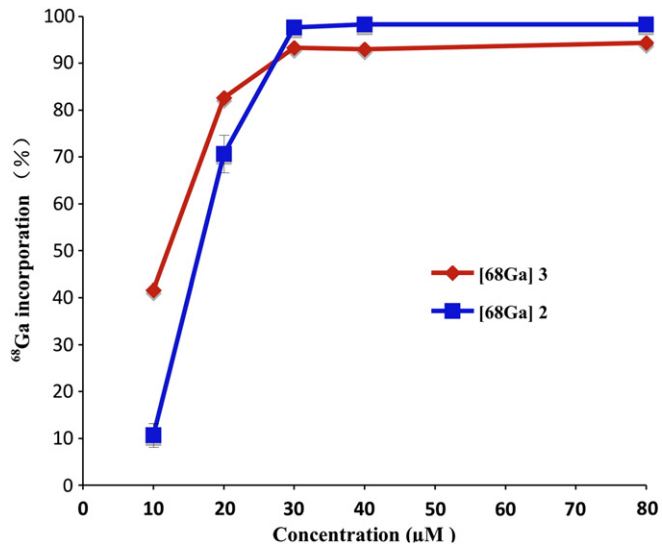

Fig. 1. Formation (% incorporation) of [^{68}Ga]PhenA, **2**, and [^{68}Ga]PhenA-BPAMD, **3** (at room temperature, 5 min, pH 4.10), as a function of ligand concentration (n = 3).

Bisphosphonates are a group of drugs [44] that are commonly prescribed for treatment of osteoporosis — a bone disease that shows an imbalance between osteoclastic (bone dissolving) and osteoblastic (bone forming) activities. The disease leads to a net loss of bone mass due to either the excessive bone-resorbing activity of osteoclasts or the impaired bone-forming activity of osteoblasts, such that osteoblasts do not optimally replenish the lost bone [45]. In the past few decades, [^{99m}Tc]-methylene diphosphonate (MDP), a radiolabeled bisphosphonate, was used extensively in planar or single-photon emission computerized tomography (SPECT) bone imaging scans. Tc-MDP-SPECT is one of the most commonly performed nuclear medicine procedures in the evaluation of bone disorders, such as infection (osteomyelitis), noninfectious inflammation (arthritis), trauma, metabolic bone disease, benign and malignant neoplasms, and metastasis [46,47]. Recently, [^{18}F]NaF in conjunction with PET was approved for the clinical evaluation of patients with known or suspected bone metastases [46,47].

By taking advantage of AAZTA's use as an effective chelator for radioactive ^{68}Ga, we have prepared a derivative containing a phenol carboxylic acid, designated herein as PhenA, **2**, through which a bisphosphonate group was selectively attached. These ^{68}Ga labeled PhenA bisphosphonates, PhenA-BPAMD, **3**, and PhenA-HBP, **4**, are designed such that the bisphosphonates bind to hydroxyapatite on active bone surfaces while the AAZTA-chelating group forms a stable complex with [^{68}Ga]Ga(III). They may be useful as bone imaging agents for detecting tumor metastases. Reported herein are the synthesis, radiolabeling, and in vivo biodistribution studies. Two known bone imaging agents, [^{68}Ga]DOTA-BPAMD, **1**, and [^{18}F]NaF, were used as positive controls [46,48] while the core structure [^{68}Ga]PhenA, **2**, containing no bisphosphonate group, was the negative control. [^{68}Ga]PhenA-BPAMD, **3**, and [^{68}Ga]PhenA-HBP, **4** (Chart 2) are potential new bone imaging agents.

2. Materials and methods

2.1. General

All reagents and solvents were purchased commercially (Aldrich, Acros, or Alfa Inc.) and were used without further purification, unless otherwise indicated. Solvents were dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.). ^{1}H spectra and ^{13}C NMR were recorded on a Bruker AVANCE II spectrometer at 400 MHz and 100 MHz respectively, and referenced to NMR solvents as indicated. Chemical shifts are reported in ppm (δ), with a coupling constant, *J*, in Hz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). High-resolution mass spectrometry (HRMS) data were obtained with an Agilent (Santa Clara, CA) G3250AA LC/MSD TOF system. Thin-layer chromatography (TLC) analyses were performed using Merck (Darmstadt, Germany) silica gel 60F_{254} plates. Generally, crude compounds were purified by flash column chromatography (FC) packed with silica gel (Aldrich). High performance liquid chromatography (HPLC) was performed on an Agilent 1200 series system. Reactions of non-radioactive chemical compounds were monitored by thin-layer chromatography (TLC) analysis with pre-coated plates of silica gel 60F_{254}. [^{68}Ga]$GaCl_3$ was eluted with 0.05 N hydrochloric acid from a ^{68}Ge/^{68}Ga generator (ITG GmbH) or with 0.6 N HCl from a ^{68}Ge/^{68}Ga generator (iThemba LABS, South Africa).

2.2. Chemistry

Synthesis of target compounds, PhenA, **2**, PhenA-BPAMD, **3**, and PhenA-HBP, **4**, were prepared by reactions as described in Schemes 1 and 2.