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Synthesis and characterization of $\alpha_v\beta_3$-targeting peptidomimetic chelate conjugates for PET and SPECT imaging

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ABSTRACT

There is growing interest in small peptidomimetic $\alpha_v\beta_3$ integrin antagonists that are readily synthesized and characterized and can be easily handled using physiological conditions. Peptidomimetic 4-[2-(3,4,5,6-tetrahydropyrimidine-2-ylamino)ethyloxy]benzoyl-2-[*N*-(3-amino-neopenta-1-carbamyl)]-aminoethylsulfonyl-amino-β-alanine (**IAC**) was successfully conjugated to 1-(1-carboxy-3-carbo-*t*-butoxypropyl)-4,7-(carbo-*tert*-butoxymethyl)-1,4,7-triazacyclononane (NODA-GA(tBu)$_3$) and 1-(1-carboxy-3-carbo*tert*butoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA-GA(tBu)$_4$) and radiolabeled with ^{111}In, ^{67}Ga and ^{203}Pb. Results of a radioimmunoassay demonstrated binding to purified $\alpha_v\beta_3$ integrin when 1–4 equiv of integrin were added to the reaction. Based on this promising result, investigations are moving forward to evaluate the NODA-GA-**IAC** and DOTA-GA-**IAC** conjugates for targeting tumor associated angiogenesis and $\alpha_v\beta_3$ integrin positive tumors to define their PET and SPECT imaging qualities as well as their potential for delivery of therapeutic radionuclides.

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Integrins are a family of transmembrane glycoproteins with associated α and β subunits forming 25 unique heterodimers that facilitate adhesion and migration of cells on the extracellular matrix proteins found in intercellular spaces and basement membranes.[1] One of these integrins, $\alpha_v\beta_3$ integrin, interacts with vitronectin, fibronectin, fibrinogen, thrombospondin, collagen, laminin and von Willebrand factor. This integrin is over-expressed in tumor induced angiogenic vessels and in various human tumors, but is found at low levels on epithelial and endothelial cells. It is therefore a widely recognized target for the development of molecular probes for imaging angiogenesis and cancer therapy. Towards this end, the tumor imaging capability of several RGD peptides that act as $\alpha_v\beta_3$ integrin antagonists has been demonstrated by several research groups. Additionally, several of these peptides have been shown to inhibit tumor angiogenesis and interrupt metastasis in many models.[2–4]

There is growing interest in peptidomimetic $\alpha_v\beta_3$ integrin antagonists composed of a stable core scaffold with basic and acidic groups that mimic the guanidine and carboxylate pharmacophore of RGD peptides. Peptidomimetics tend to have higher activity, specificity and longer duration of action compared to the peptides. One such peptidomimetic $\alpha_v\beta_3$ integrin antagonist, 4-[2-(3,4,5,6-tetrahydropyrimidine-2-ylamino)ethyloxy]benzoyl-2-aminoethylsulfonyl-amino-β-alanine (**IA**) was synthesized by Hood et al.[5] Subsequent, modification of **IA** to the corresponding carbamate derivatives by the Danthi group resulted in 4-[2-(3,4,5,6-tetrahydropyrimidine-2-ylamino)ethyloxy]benzoyl-2-[*N*-(3-amino-neopenta-1-carbamyl)]-aminoethylsulfonyl-amino-β-alanine (**IAC**), with a binding affinity 20 times greater than that of **IA**.[6] A SPECT (single photon emission computed tomography) imaging study with ^{111}In-DOTA-Bz-SCN-**IAC** was also performed and tumor was clearly visualized at 4 h p.i.[7] ^{111}In-DOTA-Bz-SCN-**IAC** was prepared using the bifunctional chelate DOTA-Bz-SCN which differs from the DOTA-GA described in this study.

Clinically, SPECT and PET (positron emission tomography) play significant roles allowing noninvasive imaging of internal physiological and biochemical function and pathologies in vivo. While PET is more expensive, it has significant advantages over SPECT with respect to its ability to better quantify images. Of metallic radionuclides currently being investigated for PET applications, gallium-68 (^{68}Ga) has grown in popularity.[8,9] The popularity of ^{68}Ga stems from the ease of on site production from a long lived generator system (^{68}Ge/^{68}Ga) rather than a cyclotron, and automation for incorporation into radiolabeled compounds.[10] The 67.7 min half-life of ^{68}Ga is an appropriate match to the biological

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half-lives of peptides. Gallium(III) typically binds with chelates possessing multiple anionic oxygen donors preferring a coordination number of six in an octahedral geometry. Fitting these preferences, the macrocyclic ligand, 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), is well established as forming very stable complexes with a wide variety of metals, Ga(III) being one of them.[11] Several NOTA derivatives have been reported (Fig. 1) for use in the radiolabeling of proteins and peptides. Recently, Knetsch et al. reported a ^{68}Ga-labeled NODA-GA-conjugated RGD peptide ([^{68}Ga]NODA-GA-RGD) that showed better tumor to blood ratio in vivo than the corresponding [^{68}Ga]DOTA-RGD derivative.[12]

While interest in ^{68}Ga for PET imaging is currently significant, there are Pb(II) isotopes that have also been of interest for biomedical applications. Specifically, ^{203}Pb and ^{212}Pb are radiometals possessing favorable properties for use in nuclear medicine for potential diagnostic and therapeutic applications, respectively.[13–21] ^{203}Lead ($t_{1/2}$ = 51.9 h) emits a γ-ray (279 keV) that is ideal for single photon emission computed tomography (SPECT) imaging and is suitable for pharmacokinetic and pharmacodynamic tracer studies. In addition, ^{203}Pb can serve as one half of a potential matched-pair of radioisotopes when combined with ^{212}Pb for therapeutic applications. ^{212}Lead ($t_{1/2}$ = 10.6 h) has been studied as an 'in vivo generator' of ^{212}Bi ($t_{1/2}$ = 60 min) to overcome the short half-life of that daughter isotope. The macrocyclic polyaminocarboxylate chelate DOTA (1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid) labeled with ^{212}Pb provides a complex that is adequately stable in vivo to sequester the radionuclide.[17]

The Mäcke group in Switzerland have synthesized a DOTA derivative analogous to NOTA-GA, 1-(1-carboxy-3-carbo*tert*butoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA-GA(tBu)$_4$).[22] The DOTA-GA(tBu)$_4$ affords four intact carboxylic acid functional groups with a free carboxylate side chain ready for conjugation to the N-terminus of peptides which makes it useful for biomedical applications.

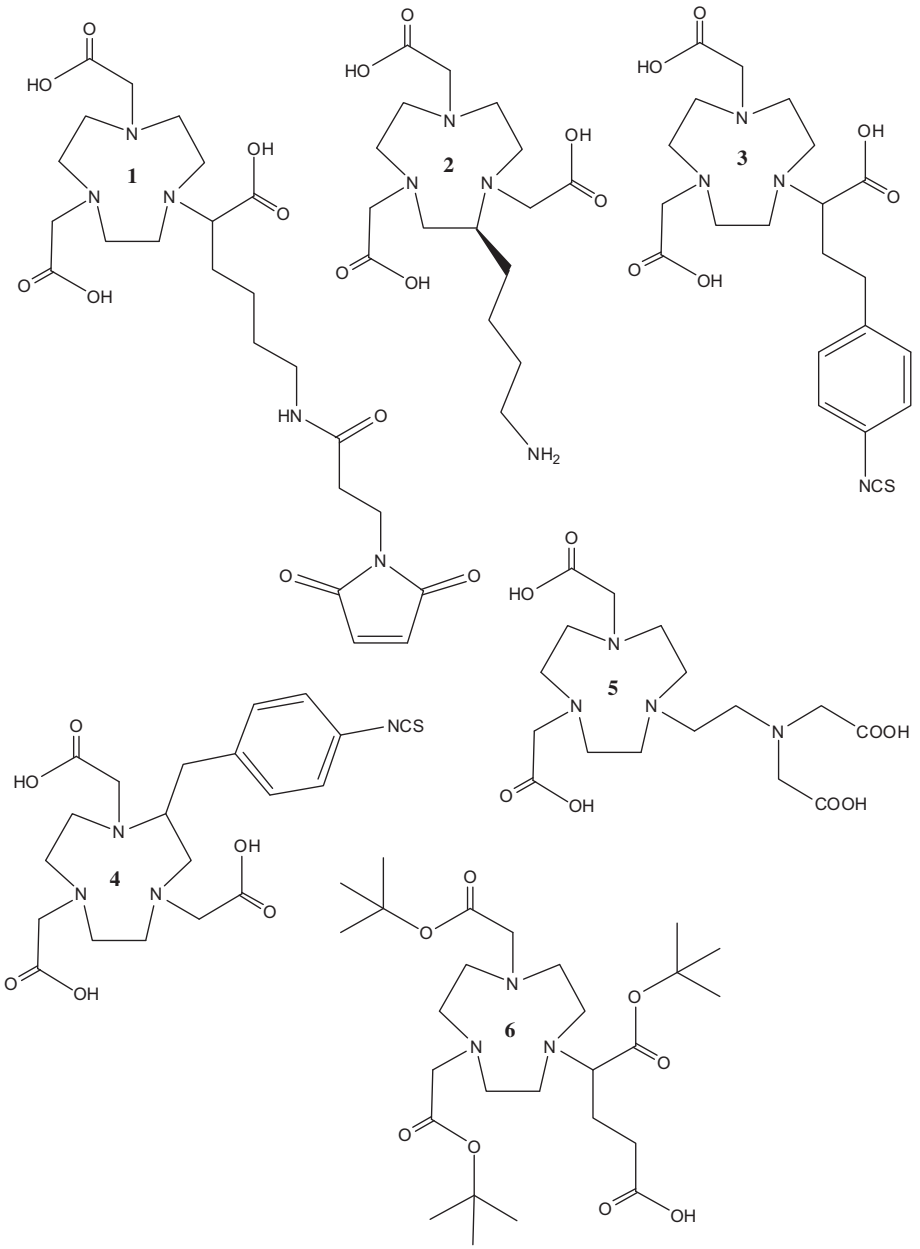

Figure 1. Structures of NOTA derivatives. 7-(5-Maleimido-1-ethoxycarbonylphenyl)-1,4,7-triazacyclononane-1,4-diylacetic acid (**1**), 2-(4-aminobutyl)-1,4,7-triazacyclononane-1,4,7-triyltriacetic acid (**2**), nNOTA (**3**), *p*-SCN-Bn-NOTA (**4**), NETA (**5**), and NODA-GA(tBu)$_3$ (**6**).