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Synthesis and radiopharmacological evaluation of ^{64}Cu-labeled bombesin analogs featuring a bis(2-pyridylmethyl)-1,4,7-triazacyclononane chelator

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ABSTRACT

The bifunctional chelating agent 2-[4,7-bis(2-pyridylmethyl)-1,4,7-triazacyclononan-1-yl]acetic acid, DMPTACN-COOH, has been found to bind strongly to copper(II), resulting in a radiocopper(II)-ligand complex that exhibits high *in vivo* stability. The pendant carboxylic acid group enables this derivative to be conjugated to the N-terminal amino acid residues of peptides. Exploiting this, two stabilized bombesin (BBN) derivatives, βAla-βAla-[Cha^{13},Nle^{14}]BBN(7–14) and β*homo*-Glu-βAla-βAla-[Cha^{13},Nle^{14}]BBN(7–14) have been coupled to DMPTACN-COOH and radiolabeled with the positron emitter copper-64 (**^{64}Cu-1** and **^{64}Cu-3**). The *in vitro* binding characteristics of the [^{64}Cu]Cu-labeled bombesin conjugates in gastrin-releasing peptide receptor (GRPR) over-expressing prostate cancer (PC-3) cells have been evaluated. Biodistribution studies performed in Wistar rats indicate a specific uptake in the GRPR-rich pancreas and rapid renal elimination for both **^{64}Cu-1** and **^{64}Cu-3**. Small animal PET imaging studies performed in NMRI *nu*/*nu* mice bearing the human prostate tumor PC-3 demonstrated a very high degree of tumor accumulation for **^{64}Cu-1** and **^{64}Cu-3**. Incorporation of a single additional glutamic acid residue within the spacer between bombesin and the radiolabeled complex (**^{64}Cu-3**) leads to a higher tumor-to-muscle uptake ratio (amounting to >30 at 100 min post injection) compared to **^{64}Cu-1**.

1. Introduction

The development of radiolabeled receptor-targeting peptides as both radiotracers and radiotherapeutics is currently a major field of research in radiopharmacy and nuclear medicine [1–7]. In particular, tumor diagnosis and therapy derive considerable benefits from the use of radiolabeled peptides. In addition to showing receptor binding affinities that are comparable or even higher than those of antibodies, the small size (~1 kDa) of peptides allows for faster tumor targeting, increased tumor penetration, shorter blood circulation and overall better tumor-to-non-tumor-tissue ratios. Additional advantages of peptide-based radiodiagnostic and therapeutic agents include their relative ease of preparation (via a combination of established solid-phase peptide synthesis, bioconjugation and radiolabeling protocols) and their lack of immunogenicity compared to large polypeptides and antibodies [8]. Peptides often suffer from issues of metabolic instability, however synthetic strategies to improve their circulatory lifetime and other pharmacokinetic properties are well-established [3,9].

Prominent clinical targets for peptide-based radiopharmaceuticals are neuroendocrine tumors, like prostate cancer [10–12]. Primary prostate carcinoma continues to be one of the most commonly diagnosed cancers and one of the leading causes of cancer-related death in men. Despite recent efforts, peptides that can be used for non-invasive detection and therapy of prostate cancer primary lesions and bone metastases remain elusive. This provides impetus to develop new and innovative diagnostic and treatment strategies for prostate cancer sufferers.

The gastrin-releasing peptide receptor (GRPR) has been identified as a molecular target of high clinical relevance in prostate cancer. The GRPR belongs to the bombesin receptor family and,

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thus, can be targeted by bombesin (BBN) derivatives [13]. BBN is the 14-amino acid amphibian peptide analog of the 27-amino acid mammalian regulatory GRP and exhibits a very high affinity for GRPR. Androgen-independent primary prostate carcinoma shows a substantial over-expression of GRPR and other commonly occurring human cancer cell types may express high GRPR levels. BBN and GRP share a homologous 7-amino acid amidated C-terminus, [WAVGHLM-NH_2], which is necessary for receptor binding [14]. Among different GRPR targeting agents, BBN(7–14)NH_2, [QWAVGHLM-NH_2], is of particular interest due to its high affinity for the GRPR and its rather small size which allows for easy synthesis. The majority of published experimental work on radiolabeled BBN derivatives has applied the androgen-independent GRPR-positive human prostate cancer cell line PC-3 as a model. For imaging purposes, mainly ^{18}F, ^{68}Ga and ^{99m}Tc-labeled BBN derivatives have been investigated [15–19].

Recent radiopharmaceutical approaches have focused on the functionalization of BBN derivatives with bifunctional chelating agents (BFCAs) capable of forming stable complexes with copper radionuclides [20–23]. In this regard, the clinically most important copper radionuclides are the positron (β^+)-emitter ^{64}Cu and the β^--emitter ^{67}Cu [24–27]. The use of ^{64}Cu-radiolabeled peptides in positron emission tomography (PET) allows the detection of tumors, non-invasive assessment of receptor expression and/or dosimetry measurements. In this context, ^{64}Cu ($t_{1/2}$ = 12.7 h; β^+_{max} = 0.655 MeV; β^-_{max} = 0.573 MeV) is a positron emitter that can rival ^{18}F (β^+_{max} = 0.633 MeV) in terms of its low maximal β^+-emission energy, an important consideration where PET image resolution is concerned. ^{64}Cu decays by both β^+ and β^- particles, enabling simultaneous diagnostic PET imaging and radiotherapy [28]. The efficacy for radiotherapy can be significantly enhanced using Auger and conversion electrons when ^{64}Cu-labeled compounds are localized in the cell nucleus [29]. In addition, ^{67}Cu meets some criteria of a desirable therapeutic radionuclide, including an appropriate particle emission energy (β^-_{max} = 0.577 MeV) and a suitable physical half-life ($t_{1/2}$ = 62 h). Of importance, both copper radionuclides are amenable to attachment to peptides via the same chemistry.

An important prerequisite from a radiochemical point of view is the use of BFCAs that form radiocopper-ligand complexes exhibiting high kinetic and thermodynamic stability in living organisms. A minimized release of the isotope would limit the background for

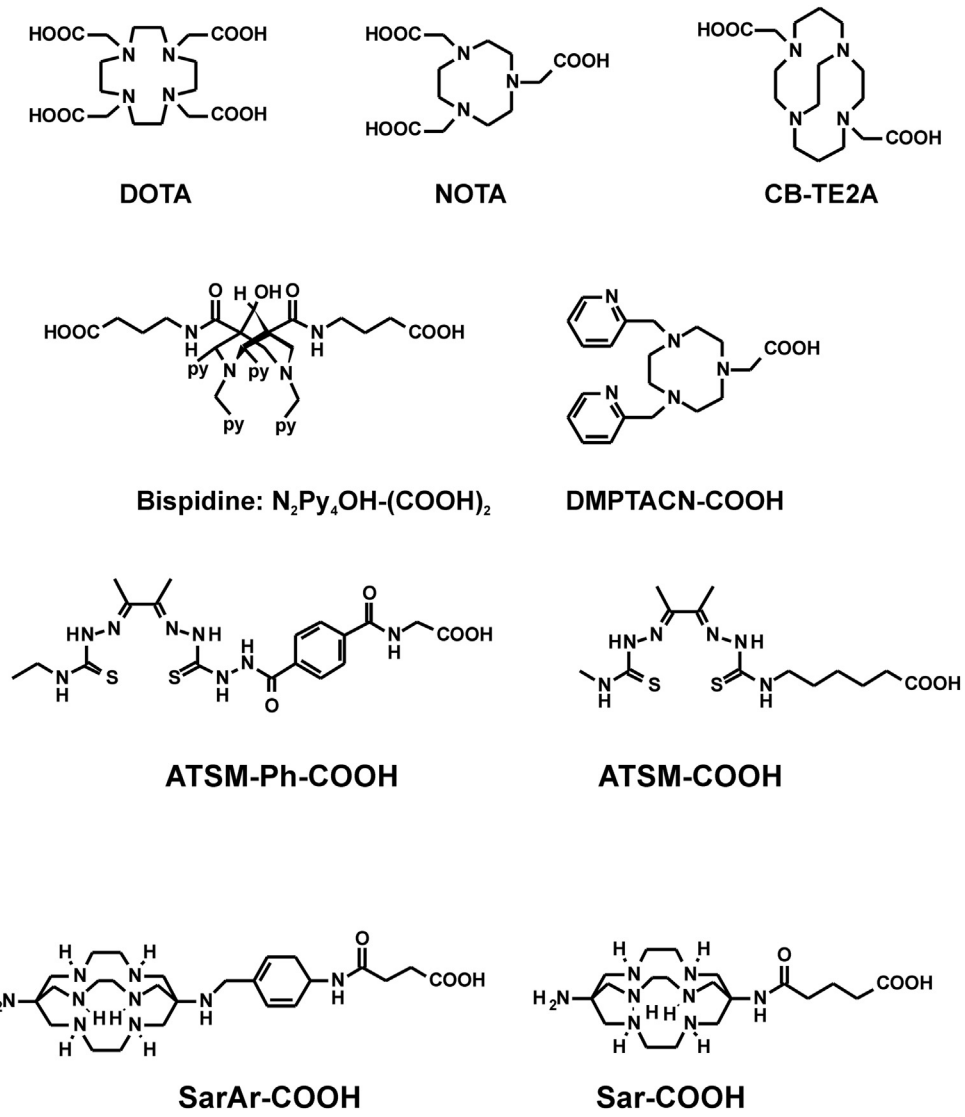

Fig. 1. Structures of BFCAs for complexation of $^{64}Cu^{2+}$ ions and coupling to bombesin derivatives.