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REVIEW

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Targeting of peptide-binding receptors on cancer cells with peptide-drug conjugates

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Abstract

Specifically addressing cell surface molecules on cancer cells facilitates targeted cancer therapies that offer the potential to selectively destroy malignant cells, while sparing healthy tissue. Thus, undesired side-effects in tumor patients are highly reduced. Peptide-binding receptors are frequently overexpressed on cancer cells and therefore promising targets for selective tumor therapy. In this review, peptide-binding receptors for anti-cancer drug delivery are summarized with a focus on peptide ligands as delivery agents. In the first part, some of the most studied peptide-binding receptors are presented, and the ghrelin receptor and the Y_1 receptor are introduced as more recent targets for cancer therapy. Furthermore, nonpeptidic small molecules for receptor targeting on cancer cells are outlined. In the second part, peptide conjugates for the delivery of therapeutic cargos in cancer therapy are described. The essential properties of receptor-targeting peptides are specified, and recent developments in the fields of classical peptide-drug conjugates with toxic agents, radiolabeled peptides for radionuclide therapy, and boronated peptides for boron neutron capture therapy are presented.

KEYWORDS

BNCT, G protein-coupled receptor, internalization, peptide drug conjugate, tumor targeting

1 | INTRODUCTION: THE NEED FOR TARGETED ANTI-CANCER DRUG DELIVERY

Cancer is one of the leading causes of death worldwide, only surmounted by cardiovascular diseases.[1,2] In 2012, the number of new cancer cases worldwide was estimated to be 14.1 million and the number of cancer deaths was denoted with 8.2 million.[3] While in women breast cancer is the most frequently occurring form of cancer, lung cancer is the leading cancer subtype in men.[3] Furthermore, the American Cancer Society stated that by 2030 the number of new cancer incidences is projected to rise to 21.7 million and that the number of cancer deaths might rise to 13 million owing to the growth in world population.[2] This estimation clearly indicates that the efforts to develop novel and more efficient treatment modalities for cancer have to be strengthened in the future. State-of-the-art treatment of cancer is accomplished by a combination of surgical procedures, radiation therapy, and chemotherapy. Although the latter proves to be a potent weapon in the fight against cancer in many cases, a closer look at this cancer treatment reveals that further optimization is required. Chemotherapy is still mostly performed by systemic administration of potent cytotoxic drugs, but these compounds lack tumor selectivity and therefore also kill healthy cells in the body. The resulting peripheral toxicity is the cause of severe side-effects in chemotherapy.[4] The described nonspecificity holds true not only for chemotherapeutic agents, but for any therapeutically active molecule that is administered

systemically without bearing an intrinsic cancer cell sensor. However, cancer-sensing properties are needed to achieve the ultimate goal of targeted tumor therapy with drug molecules.[5,6] In a very general view, the idea is to exploit any biochemical characteristics that mark the cancer cells different from healthy cells to achieve a selective therapeutic effect only in the malignant cells. Such characteristics may include dysregulation of translation regulators,[7] changes in epigenetic regulation mechanisms,[8] overproduction of enzymes,[9] or changes in the cellular microenvironment such as lower pH.[10] Furthermore, tumor cells can be addressed by an actively targeted anti-cancer drug delivery owing to the overexpression of a variety of cell surface receptors, which bind to ligands of different nature.[11]

2 | PEPTIDE-BINDING RECEPTORS AS TARGETS FOR ANTI-CANCER DRUG DELIVERY

Cellular receptors that are activated by peptide molecules as ligands are of major interest for targeted tumor therapy.[11,12] These receptors should fulfill two major criteria: first, the receptor should be uniquely or highly overexpressed on cancer cells in comparison to nonmalignant cells. A tumor-to-normal-cell expression ratio of 3:1 or higher is usually desired. Second, the total expression levels of the target receptor on the cancer cells should be sufficiently high to ensure the cellular delivery of appropriate amounts of the drug for obtaining the desired therapeutic effect. Many peptide-binding receptors possess these features and accordingly, their activating peptide ligands (agonists) are promising tumor-selective carriers for use in drug conjugates.[13,14] Additionally, targeting of peptide-binding receptors can be also achieved with chemically designed small molecule binders or antibodies.[15,16] A directed drug delivery is then possible by engineering a modular conjugate system, consisting of the drug compound covalently attached to the receptor-binding molecule (Figure 1). Optimally, the latter should also ensure penetration of the cancer cells for a selective intracellular delivery of the drug. In many cases, a smart linker is additionally applied between the drug and the targeting unit, to facilitate a controlled release of the drug inside the tumor cells.[17]

Apart from the direct conjugation of a drug to the carrier unit, liposomes and nanoparticles are used as delivery platforms.[18–20] In this approach, the particles are loaded with the drug compound and the particle surface is decorated with receptor-targeting (ligand) molecules to enable delivery to the tumor cells. A surface modification of polymeric nanomedicines with receptor-targeting ligands, for example, peptides, can overcome inherent weaknesses of untargeted particles, including low cell selectivity and inefficient cellular uptake, while the polymeric nanomedicine itself offers enhanced stability and the potential to deliver a huge quantity of drug molecules per particle into cells. Peptide-functionalized nanomedicines have been extensively optimized in the past years, which is discussed in various reviews.[20–22] The focus of this review, however, lays on conjugates where the drug is directly conjugated to the receptor-targeting unit, with a major emphasis on peptides as carrier molecules. In the following, some of the most studied peptide-binding receptors that are addressed for targeted tumor therapy with drug conjugates are presented.

2.1 | Integrins

Integrins are a family of transmembrane (TM) receptors that appear as heterodimers of variably and noncovalently associated α and β subunits.[23] They link the extracellular matrix (ECM) to the cytoskeleton, thereby mediating processes such as cell adhesion, migration, and proliferation.[24] Since all of these processes are highly relevant for carcinogenesis, integrin receptors are found to be overexpressed in various cancer subtypes.[25] Among the integrin family, αvβ3 is the most attractive target owing to its importance in tumor angiogenesis and metastasis.[26] Targeting the αvβ3 can be accomplished with the tripeptide arginine-glycine-aspartic acid (RGD) motif, which was found in various proteins of the ECM.[27,28] A cyclic variant c(RGDfK) is thereby used as preferred carrier system for drug conjugation, owing to its improved affinity for the integrin receptors.[29–31] However, the internalization of RGD peptides after integrin binding is still not fully understood and multimeric peptides displayed a higher cell uptake.[32] Furthermore, the cyclic 9-mer iRGD (CRGDKGPDC) is used as smart delivery vehicle to achieve an endocytotic uptake of drugs into cancer cells.[33]

2.2 | Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is part of the ErbB family of receptor tyrosine kinases, consisting of the four members EGFR/HER1 (ErbB-1), HER2/neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4).[34] Overexpression of the EGFR in very high levels was

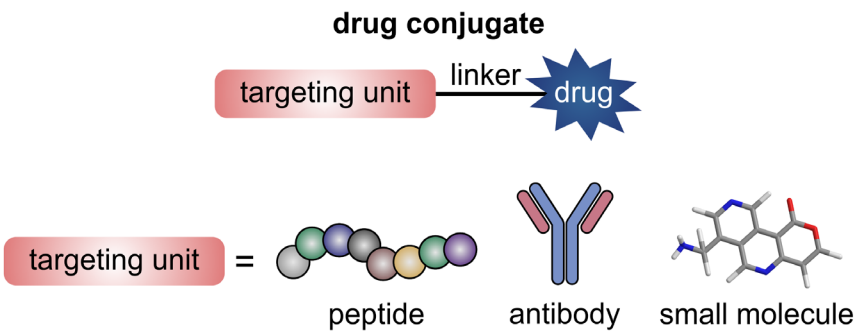


FIGURE 1 Schematic structure of a receptor-targeting drug conjugate

observed in a variety of cancer types and is associated with the strongly enhanced proliferation rate of the cancer cells.[35] Inhibition of the EGFR for achieving an antiproliferative effect is already established in the clinics with monoclonal antibodies or small molecule inhibitors.[36] However, owing to its internalization behavior, the EGFR is also considered as uptake system for drug delivery. Several short peptides with high affinity and selectivity for the EGFR were discovered by phage display libraries and can be used as targeted drug carriers.[37,38]

2.3 | Somatostatin receptors

The class of somatostatin receptors (SSTRs) consists of five members (SSTR1-5), which are widely expressed in different tissues in the body including nervous, pituitary, kidney, lung, and immune cells.[39] Their natural ligand is the neuropeptide somatostatin (SST), which occurs in two active isoforms, the SST-14 and SST-28. In combination with their receptors, both isoforms act as inhibitory hormones.[39] An important physiological function of the SSTR/SST axis is, for example, the inhibition of the release of growth hormones.[39,40] Overexpression of SSTRs and in particular SSTR2 has been found in various neuroendocrine tumors, as well as other tumors such as breast, ovarian, and lung cancer.[41] Targeting of the SSTR2 for drug delivery is accomplished by using stabilized, cyclic somatostatin analogs such as octreotate, octreotide, and lanreotide.[42] These peptides are also directly used in therapy to employ the inhibitory actions mediated by the SSTRs for the treatment of growth hormone-producing tumors.[43]

2.4 | Gonadotropin-releasing hormone receptor

The gonadotropin-releasing hormone receptor (GnRH-R) is primarily expressed on gonadotrope cells in the pituitary but also found in lymphocytes, breast, ovary, and prostate.[44] Activation of the GnRH-R in the pituitary gland by the ligand isoforms GnRH-I and GnRH-III leads to secretion of the two gonadotropins follicle-stimulating hormone and luteinizing hormone.[45] The GnRH-R emerged as highly promising target for targeted therapy, because several human tumor types, including ovarian, prostate, breast, and lung cancer, overexpress or even uniquely express this receptor with respect to the surrounding nonmalignant cells.[46] Targeting of the GnRH-R with activating peptide or small molecule agonists is already applied in cancer therapy.[47] For the generation of drug conjugates, the modified GnRH analogs [D-Lys^6]-GnRH-I and [Lys^4]-GnRH-III are most frequently used.[48,49]

2.5 | Bombesin receptors

The bombesin (Bn) receptor family consists of three members, namely the BB_1, BB_2, and BB_3 receptor. All of the Bn receptors are widely expressed in the central nervous system (CNS), but also in the periphery such as the gastrointestinal tract.[50] They mediate a multitude of physiological functions, including an autocrine growth action on cells and potent CNS effects. The natural peptide ligand for the BB_1 is the neuromedin B and for the BB_2 the gastrin-releasing peptide, while the BB_3 is considered an orphan receptor.[50] However, the 14-mer peptide homolog Bn, originally isolated from the skin of the European fire-bellied toad,[51] is able to bind to all Bn receptors. Upregulation of Bn receptors was found in various cancer subtypes and especially the BB_2 is highly overexpressed in tumors such as breast, prostate, small cell lung, and pancreatic cancer.[52,53] Targeting the Bn receptors for drug delivery can be accomplished with a number of Bn analogs, including for example the peptide [D-Tyr^6,β-Ala^{11},Phe^{13},Nle^{14}]-Bn (6-14) as high affinity agonist for all Bn receptors.[54]

2.6 | Other peptide receptors

Despite these five receptors, a number of other peptide receptors are investigated as potential targets for anti-cancer drug delivery. This includes, for example, the vasoactive intestinal peptide (VIP) receptors 1 and 2, which are overexpressed in various cancers such as colon, breast, and endocrine tumors.[12] The natural ligand VIP and its analogs are investigated for the preparation of drug conjugates.[55,56] The neurotensin receptor 1 (NTSR1) was found to be overexpressed in a number of different cancer subtypes including breast, colon, pancreatic, lung, and prostate cancer.[57] For drug delivery, the short hexapeptide neurotensin(8-13) can be used as carrier with high selectivity and affinity for the NTSR1.[58,59] Of interest is also the cholecystokinin 2 receptor (CCK2R), which is overexpressed in various cancers of the thyroid, lung, pancreas, liver, and the gastrointestinal tract.[60,61] Targeting of this receptor for drug delivery can be accomplished with analogs of its natural peptide ligands cholecystokinin and gastrin.[62,63] The melanocortin receptor 1 (MC_1R) was found to be upregulated in malignant melanoma.[64] Since the success of traditional chemotherapy for treatment of metastatic melanoma is quite limited,[65] targeted therapy addressing the MC_1R is a promising approach. For the generation of drug conjugates for this system, shortened peptide analogs of the natural MC_1R ligand α-MSH, for example, the agonist NAPamide, possess the potential as delivery agents.[66]

3 | TARGETING OF NEW G PROTEIN-COUPLED RECEPTORS

The majority of peptide-binding receptors targeted for drug delivery in cancer therapy belongs to the class of G protein-coupled receptors (GPCRs). Except for the integrins and the EGFR, this holds true for all the receptors introduced so far. GPCRs consist of seven TM helices linked by three intracellular and three extracellular loops, an extracellular N-terminus and an intracellular C-terminus.[67] After ligand binding, these receptors undergo ligand-specific conformational changes,[68] allowing them to activate heterotrimeric G proteins at the intracellular face of the plasma membrane, which then initiates a signaling cascade.[69] Following receptor activation, most GPCRs are

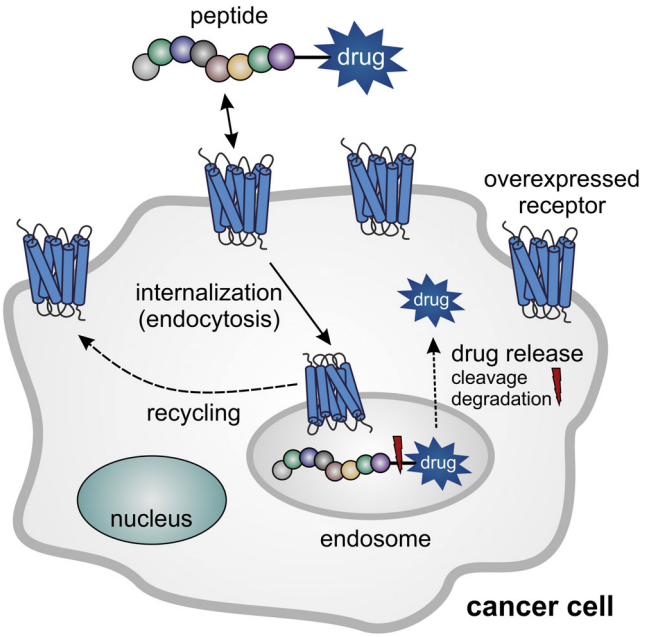


FIGURE 2 Schematic outline of targeting a tumor-expressed G protein-coupled receptor for anti-cancer drug delivery with a peptide-drug conjugate. The drug will be released intracellularly by intentionally using a cleavable linker or just by endo-lysosomal degradation of the peptide-drug conjugate

desensitized by intracellular phosphorylation, recruitment of the adaptor protein arrestin, and finally, internalization by clathrin-mediated endocytosis,[70] which makes them highly interesting for cellular drug delivery (Figure 2). GPCRs form the largest superfamily of cell surface receptors with over 800 members in human and fulfill a myriad of diverse physiological functions. They are the most successful class of pharmaceutical targets and also involved in cancerogenesis.[71] In this review, the focus of investigation was on two GPCRs, which just have recently been considered as target receptors for drug delivery in cancer therapy. Both receptors and their ligands are presented in more detail.

3.1 | Ghrelin receptor

The ghrelin receptor (GhrR), also named growth hormone secretagogue receptor 1a (GHSR1a), is a class A GPCR and was first identified by Smith *et al.* in 1996.[72] It is widely expressed in the brain, especially in the hypothalamus, but also in the hippocampus and the pituitary. Furthermore, the GhrR was found to be expressed in a variety of peripheral tissues, including liver, heart, pancreas, thyroid, ovaries, testis, and more.[73,74] A splice variant of the GhrR, named GHSR1b, is also widely expressed in the human body, however, this subtype is described to be nonfunctional itself.[75] The natural ligand of the GhrR is the peptide hormone ghrelin, which was discovered in 1999 by Kojima *et al.* and is mostly produced in the stomach.[76] Ghrelin is a 28-amino acid peptide and is acylated with *n*-octanoic acid at the Ser^3 residue in its peptide sequence, which is required for activity at the receptor.[77] The ghrelin/GhrR axis plays a role for a multitude of physiological functions such as food intake,[78,79] regulation of energy homeostasis,[80] release of various hormones (e.g., growth hormone, prolactin, adrenocorticotropic hormone)[81] and reward-seeking behavior.[82] An important feature of the GhrR is its ligand-independent constitutive activity.[83] This basal signaling activity is suggested to provide a constant effect on the growth hormone axis, leading to the development of normal stature in humans.[84]

Ligand binding to the GhrR occurs rather deep in the cavity created by the TM helices of the receptor. Following the receptor activation after ligand binding, the classical signaling outcome of the GhrR is the $G\alpha_{q/11}$-mediated signaling pathway.[85] Thereby, activation of the $G\alpha_{q/11}$ protein by the receptor stimulates the phospholipase C, which cleaves the membrane lipid phosphoinositol-4,5-bisphosphate into inositol trisphosphate (IP_3) and diacylglycerol. IP_3 then triggers the release of calcium ions from the endoplasmic reticulum into the cytosol.[86] Besides the $G\alpha_{q/11}$-coupled pathway, initiation of multiple other intracellular signaling cascades after GhrR activation has been described.[85] As for most GPCRs, desensitization of the GhrR occurs by internalization in clathrin-coated pits and was described to peak at around 20 minutes after ligand stimulation. Inside the cell, the GhrR is sorted into endosomes and strongly recycled back to the plasma membrane.[87] Owing to its constitutive activity, internalization of the ghrelin receptor also occurs in a ligand- and arrestin-independent fashion.[88]

The GhrR has been widely investigated as therapeutic target, mostly due to its effects on the growth hormone axis and its impact on appetite stimulation. However, the GhrR might be also considered for cancer therapy, since it was found to be present in a vast number of different cancer subtypes. Expression of the GhrR was described in pituitary adenomas, thyroid, breast, lung, testis, ovarian, prostate, gastric, and colorectal cancer, as well as in astrocytoma.[89,90] Recently, a pan-cancer analysis revealed strong upregulation of the GhrR in pancreatic cancer.[91] Up to now, the exact role of the ghrelin/GhrR axis in promoting or inhibiting cancer progression is still unclear and reported studies partly show deviating results.[91]

Targeting of the GhrR for therapy in general is explored with a variety of different ligand molecules. Due to their induction of food intake and growth hormone release, GhrR agonists are for example considered for the treatment of cancer cachexia or frailty in the elderly. Antagonists are surveyed for the treatment of obesity due to their anorexic effect.[92] In addition, inverse agonists are investigated for obesity treatment, because of their ability to even turn off the basal activity of the GhrR.[93] Many of the developed ligands are peptidic, however, also a large number of peptidomimetic and small molecules were synthesized that bind and act at the GhrR.[94] For the use in targeted anti-cancer drug delivery, short peptide agonists that allow facile synthesis and modification with a drug cargo are a promising choice. They internalize together with the GhrR after activation and therefore allow a cellular uptake of the drug. Furthermore, the described recycling of the GhrR allows multiple rounds of drug

shuttling into receptor-expressing cancer cells. Small peptide agonists for the GhrR have been already described before the endogenous ligand ghrelin was discovered.[95] They are usually classified according to the peptide they originate from, such as shortened and modified versions of ghrelin itself.[96]

Another prominent class of peptidic GhrR agonists are growth hormone secretagogues that are structurally related to Met-enkephalin. This includes, for example, the growth hormone-releasing peptide 2 (GHRP-2), GHRP-6 (Figure 3A), hexarelin, alexamorelin, and ipamorelin.[96] Furthermore, peptide agonists derived from the substance P analog [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]-substance P were described, which itself is an inverse agonist at the GhrR.[97] The C-terminal pentapeptide wFwLL-NH_2 was thereby found to be the minimal sequence capable of binding to the GhrR.[98] N-terminal extension of the pentapeptide with alanine resulted in an agonist[99] as well as substitution of the leucine-leucine motif by isonipecotic acid, with the latter analog exhibiting a signaling bias at the GhrR.[100] Additionally, the hexapeptide KwF-(D-2-Nal)-LL-NH_2 was reported as an agonist with comparable efficacy to the endogenous ligand ghrelin (Figure 3B).[101] Despite the overexpression of the GhrR in a number of different tumor types, there is no report of a GhrR-targeting conjugate for anticancer drug delivery in the literature until now.

3.2 | Human Y_1 receptor and its ligand neuropeptide Y

The human Y_1 receptor (hY_1R) is a class A GPCR from the Y receptor family in human and is predominantly expressed in the CNS, for example, the hypothalamus, but also found in peripheral tissues including heart, lung, or smooth muscle.[102,103] It is involved in various physiological functions such as food intake,[104] anxiety modulation,[105] and vasoconstriction.[106] Besides the hY_1R, three other Y receptors are expressed in human, namely the Y_2 receptor (hY_2R), the Y_4 receptor (hY_4R), and the Y_5 receptor (hY_5R).[107] These receptors are bound and activated by the neuropeptide Y family of peptide hormones, which consists of the neuropeptide Y (NPY), the peptide YY (PYY), and the human pancreatic polypeptide (hPP).[108] The Y receptor/NPY hormone family forms a multi-ligand/multi-receptor system. While NPY and PYY display high affinity for the hY_1R, hY_2R, and hY_5R, hPP has the highest affinity for the hY_4R.[109] Activation of all Y receptors by their natural ligands leads to initiation of the $G\alpha_i$ protein-coupled signaling cascade,[110] resulting in an inhibition of the adenylyl cyclase and hence, a decrease in intracellular levels of the second messenger cAMP. Desensitization of the Y receptors occurs by clathrin-mediated endocytosis in complex with the bound ligand as described before. The hY_1R, hY_2R, and hY_4R internalize rapidly within a few minutes, whereas the internalization process for the hY_5R is significantly slower. Recycling of the hY_1R and hY_2R back to the plasma membrane has been observed via fast and slow endosomal routes.[111,112]

NPY was found to be the most abundant peptide hormone in the mammalian CNS.[113] To achieve a selective therapeutic targeting of the Y receptor family with NPY, there was an early desire for peptide variants that preferentially bind and activate only one of the Y receptors. Endogenous NPY is a 36-amino acid peptide and consists of a flexible N-terminus, a C-terminal amphipathic α-helix, and an amidated C-terminus (Figure 4A).[114] Induction of structural changes in NPY by modification of its backbone sequence facilitated access to the desired Y receptor-preferring peptide variants. Replacement of a large middle part by an aminohexanoic acid spacer yielded in the derivative [Ahx$^{(5\text{-}24)}$]-NPY, a Y_2-receptor-selective ligand.[115] The shortened, C-terminally derived NPY analog [Pro^{30},Nle^{31},Bpa^{32},Leu^{34}]-NPY(28-36) was found to be a Y_1-receptor-selective agonist.[116] Furthermore, two amino acid substitutions in the N-terminal and the very C-terminal part of NPY led to the generation of the hY_1R-preferring, full-length variant [F^{7},P^{34}]-NPY (Figure 4B) with nanomolar potency at the hY_1R and highly reduced affinity for the hY_2R.[117] Recently, the crystal structure of the hY_1R in complex with two different antagonists was solved.[118] Use of this structural data, in combination with molecular docking, NMR, crosslinking, and functional studies, allowed for a more detailed characterization of the NPY binding site at the hY_1R as previously known. This scientific breakthrough might enable further optimization of receptor-selective ligands in the future.

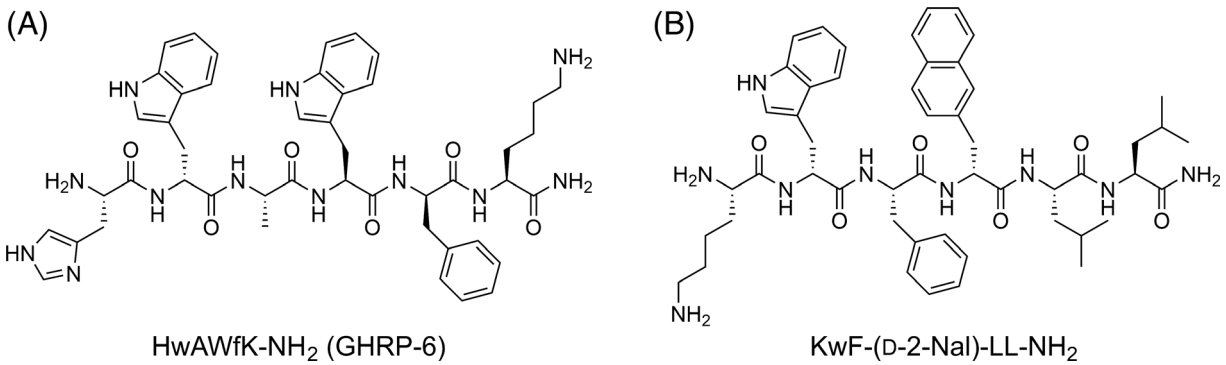


FIGURE 3 Chemical structures of, A, Met-enkephalin derived GHRP-6 and B, substance P-derived KwF-(D-2-Nal)-LL-NH_2 as two examples for short, synthetic ghrelin receptor agonists. GHRP, growth hormone-releasing peptide

FIGURE 4 A, Three-dimensional solution structure of human NPY determined by nuclear magnetic resonance spectroscopy (PDB: 1RON). B, Amino acid sequences of pNPY and hY_1R-preferring [F^7,P^{34}]-NPY. Substituted amino acids are marked in bold

In the past, addressing the hY_1R for therapy was often investigated with antagonists, owing to their potential anorexic effect.[119] However, the presence in certain tumor tissues renders the hY_1R also a highly interesting target for anti-cancer drug delivery. Expression of the hY_1R together with the hY_2R has been described in ovarian sex cord-stromal tumors, nephroblastomas, gastrointestinal stromal tumors, and testicular tumors.[120] Sole expression of the hY_1R was observed in adrenal cortical tumors and renal cell carcinomas, however, the hY_1R is also expressed to a similar extent in the non-neoplastic tissue of origin here.[120] High expression of the hY_1R was also determined in Ewing sarcoma tumors, but the presence in the surrounding normal cells was not investigated.[121] Besides the aforementioned tumor types, an outstanding expression profile of the hY_1R has been identified in breast cancer, rendering this cancer subtype most promising for a hY_1R-targeted therapeutic approach. Reubi *et al.* reported in 2001 that the hY_1R was expressed in very high density in 85% of investigated primary human breast tumors and in 100% of breast cancer-derived metastases.[122] In contrast, in the surrounding non-neoplastic breast tissue expression of the hY_2R was predominantly observed. This switch in the Y receptor expression pattern during neoplastic transformation of breast tissue therefore enables a specific drug shuttling into breast tumors when using a hY_1R-preferring ligand as delivery agent. A full agonist such as [F^7,P^{34}]-NPY is thereby recommended to obtain the complete benefit from the fast internalization and recycling kinetics of the hY_1R. The successful targeting of the hY_1R on breast tumor cells *in vivo* has been already demonstrated. A fluorine-18 (^{18}F)-labeled, fluoroglycosylated [F^7,P^{34}]-NPY analog was prepared and enabled the visualization of a hY_1R-expressing MCF-7 tumor in mice by small-animal positron emission tomography (PET) imaging, thereby indicating an uptake of the peptide conjugate into the tumor cells.[123] Furthermore, [F^7,P^{34}]-NPY was N-terminally labeled with technetium-99m (^{99m}Tc) and administered to four breast tumor patients. Clear visualization of the tumors or metastatic sites in all patients by whole-body scintimammography revealed that the peptide conjugate can be also specifically delivered to breast cancer cells in human.[124] In addition to the use of [F^7,P^{34}]-NPY, Zhang *et al.* reported the short NPY analog [Lys(Pip-^{68}Ga-DOTA)^4,Bip^5]BVD15 (BVD15: [Pro^{30},Tyr^{32},Leu^{34}]-NPY(28-36)), which is able to bind the hY_1R *in vivo*, as demonstrated by high contrast PET images of HEK293T::hY_1R tumor xenografts in mice.[125] Besides the validation of hY_1R binding *in vivo* with imaging agents, [F^7,P^{34}]-NPY was also used in first studies for the generation of drug delivery compounds for targeted tumor therapy. Apart from that, only a few other publications describe a hY_1R-targeted anti-cancer drug design. However, all of these approaches use nanoparticle-based platforms, decorated with hY_1R-binding peptides as targeting units.[126–129]

4 | SMALL MOLECULES FOR TARGETING PEPTIDE-BINDING RECEPTORS ON CANCER CELLS

Nonpeptidic, small molecule compounds for targeting peptide-binding receptors are already established as directly acting therapeutic compounds in cancer treatment. The most intuitive idea is thereby to use antagonists that block the pro-malignant effects of peptide receptor signaling pathways. Inhibition of the EGFR with small-molecule antagonists leads to an antiproliferative effect on those tumor cells that have an activating mutation in the EGFR gene and is used for the treatment of nonsmall cell lung cancer.[36] Several EGFR inhibitors are already approved for this purpose.[130] In addition, blocking the signaling of the GnRH-R on tumor cells with potent antagonists inhibits the release of gonadotropins and hence also results in an antiproliferative effect. Currently, Elagolix is the only nonpeptidic GnRH-R antagonist that is marketed, but several others are in development.[131] Besides that, a large number of small molecule drug conjugates (SMDCs) for targeted tumor therapy has been developed. Virtually all of them address plasma membrane-associated enzymes, transporter proteins or cell surface receptors that are not peptide receptors. The variety of different SMDCs has been described in detail in several review articles.[15,132] One of the best-studied class of SMDCs are compounds that target the folate receptor. As an example, vintafolide consists of folic acid as targeting moiety, connected to the chemotherapeutic agent desacetylvinblastine by a peptide spacer and an intracellularly drug-releasing linker.[133,134] It was investigated for the treatment of ovarian cancer, but eventually failed in a phase 3 study.[135] Until now, only one SMDC that is targeting a peptide receptor was reported. Wayua *et al.* described the generation of a high affinity small molecule ligand for the CCK2R, conjugated to two different microtubule inhibitors as chemotherapeutic agents by a hydrophilic peptide linker. One of the compounds showed high antitumor growth efficacy in CCK2R-positive HEK293 xenografts in mice.[136]

Beyond the investigation as SMDCs, nonpeptidic ligands are also developed as (mostly diagnostic) tools for the imaging of overexpressed peptide-binding receptors on tumor cells. As an example, EGFR inhibitors were labeled with carbon-11 (^{11}C) or ^{18}F and investigated as PET imagers for the detection of EGFR-expressing tumors and tumors with a mutated EGFR gene.[137] Furthermore, a

benzodiazepine-derived CCK2R antagonist, connected to a near-infrared (NIR) dye via a hydrophilic peptide spacer, was reported as potential agent for fluorescence-guided surgery of cancer.[138] The compound (*S*)-6-(4-bromo-2-fluorophenoxy)-3-((1-isopropylpiperidin-3-yl)methyl)-2-methylpyrido[3,2-d]pyrimidin-4(3*H*)-one, its enantiomer and a variant missing the methyl group at the pyrimidine ring were found to be partial agonists with high affinity and selectivity for the GhrR and are therefore considered as suitable parent molecules for radiolabeling.[139] Although the compounds are proposed for visualizing the GhrR in the brain, they might be also used for the detection of GhrR-expressing tumor cells. Recently, the Luyt group also reported GhrR-targeted, fluorine-bearing quinazolinone derivatives as potential PET tracers. They demonstrated successful ^{18}F-radiolabeling of two of their lead compounds with high binding affinity for the GhrR.[140]

5 | RECEPTOR-TARGETING PEPTIDES AS CARRIERS FOR ANTI-CANCER DRUG CARGOS

Peptide ligands are a highly suitable choice for the design of drug conjugates that address peptide-binding receptors (Table 1). They comprise several advantages as carrier molecules for the delivery of therapeutically active moieties to cancer cells. Peptide ligands usually bind with high affinity to their target receptors, which allows the use of low dosages of the peptide-drug conjugate (PDC) to obtain an efficient therapeutic effect. Moreover, peptides are generally considered as safe, since they feature low immunogenicity and produce nontoxic metabolites.[141] Finally, peptides up to 50 amino acids can be readily synthesized by solid-phase peptide synthesis and selectively equipped with complex modifications to generate PDCs with advanced features.[142] A drawback of peptide ligands as drug delivery systems can be their poor *in vivo* stability and short half-life, owing to their fast degradation by proteolytic enzymes in the blood and rapid renal clearance.[143] Hence, the natural peptide hormones of peptide receptors often have to be stabilized. This can be accomplished by backbone- and sequence-modification,[144] which is frequently used for the design of PDCs. Modifications include, for example, cyclization (e.g., c(RGDfK), octreotide), N-methylation, and amino acid substitutions with unnatural or D-amino acids (e.g., [D-Lys^6]-GnRH-I, short GhrR agonists). Another important approach to increase the half-life of peptides for therapy is lipidation.[145] The attached fatty acid moiety is able to bind to human serum albumin in the blood stream, which has a protective effect and leads to a longer circulation time of the peptide conjugate.[146] This concept is impressively demonstrated for the marketed, long-acting glucagon-like peptide-1 (GLP-1) receptor agonists liraglutide (Victoza)[147] and semaglutide (Ozempic),[148] which are used for the treatment of type 2 diabetes. Lipidation can be therefore also useful for the generation of PDCs with enhanced half-life. A longer circulation of PDCs is often required for the treatment of solid

TABLE 1 Overview of peptide-binding receptors studied for anti-cancer drug delivery

Targeted receptors	Peptide ligand	Tumor expression	References
Integrin αvβ3	c(RGDfK)	Glioblastoma, melanoma, breast, prostate cancer	[25,31]
EGFR	GE11 YHWYGYTPQNVI	Glioblastoma, lung, head and neck cancer	[35,37]
SSTR2	Octreotide fc[CFwKTC]T(ol)	NETs, breast, ovarian, cervical cancer	[41,42]
GnRH-R	[D-Lys^6]-GnRH-I pGlu-HWSYkLRPG-NH_2	Ovarian, breast, endometrial, prostate, lung cancer	[46,48]
Bn receptors	[D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-Bn(6-14) yQWAV-βAla-HF-Nle-NH_2	Prostate, breast, small cell lung, pancreatic cancer	[52,149]
VIP receptors	VIP HSDAVFTDNYTRLRKQMAVKKYLNSI LN-NH_2	Endocrine tumors, colon, breast cancer	[12,56]
NTSR1	NT(8-13) RRPYIL	Breast, colon, pancreatic, lung, prostate cancer	[57,58]
CCK2R	Minigastrin 11 eAYGWMDF-NH_2	Gastrointestinal, thyroid, lung, pancreas, liver cancer	[60,61,150]
MC_1R	NAPamide Ac-Nle-DHfRWGK-NH_2	Melanoma	[64,66]
hY_1R	[F^7,P^{34}]-NPY YPSKPDFPGEDAPAEDLARYYS ALRHYINLITRPRY-NH_2	Breast cancer, Ewing sarcoma	[117,120,121]

Note: Exemplary peptide ligands that can be used to address these receptors and the expression of the receptors in human tumors are listed.
Abbreviations: Ac, acetyl; Bn, bombesin; c, cyclic; CCK2R, cholecystokinin 2 receptor; e, D-Glu; EGFR, epidermal growth factor receptor; f, D-Phe; GnRH-R, gonadotropin-releasing hormone receptor; hY_1R, human Y_1 receptor; k, D-Lys; MC_1R, melanocortin receptor 1; NET, neuroendocrine tumor; Nle, norleucine; NPY, neuropeptide Y; NT, neurotensin; NTSR1, neurotensin receptor 1; pGlu, pyroglutamic acid; SSTR2, somatostatin receptor type 2; VIP, vasoactive intestinal peptide; w, D-Trp; y, D-Tyr.

tumors to allow sufficient time for delivery and penetration of the PDC into the malignant tissue. In some cases, however, persistence of PDCs in circulation might cause stronger side effects due to extended exposure of tissues to the toxic agent. Overall, tailoring of the pharmacokinetics of PDCs remains one of the major challenges for clinical translation of these molecules.

Apart from the optimization of the peptide stability, it is also important to minimize any influence of the drug or therapeutically active moiety on the binding affinity and selectivity profile of the peptide. Peptides offer the possibility to incorporate the drug cargo at distinct sites in their sequence via different chemical strategies.[142] In many cases, attachment of the drug compound can be simply performed at the N-terminus of the peptide, as for example, conducted for the somatostatin analog octreotide. However, for several peptides like the short GhrR agonists this is not possible, because the very N-terminus is involved in the binding to the receptor.[101] In this case, the peptide sequence can be screened for lysine, cysteine, glutamate, or serine residues that may allow side-chain modification with drug cargos without loss of receptor activity of the peptide carrier. Furthermore, by amino acid scans, side chains in the peptide sequence that are not essential for receptor binding can be identified. In general, the understanding of the interaction between peptide and receptor allows a rational introduction of a drug cargo. In the best case, the peptide carrier contains multiple conjugation sites for cargo loading, which is one of the advantages of peptides compared to small molecules as targeting units. All of these concepts have been recently realized in [F^7,P^{34}]-NPY, in which in addition to the natural Lys^4, certain other positions were substituted to lysine and modified without any effect on activity and selectivity of the peptide.[151]

For PDCs with chemotherapeutic agents and other therapeutically active cargos, cellular uptake into the receptor-expressing cancer cells is a prerequisite to achieve an intracellular localization of the drug cargo. After binding and activation, the PDC should internalize in complex with the receptor. While the receptor can be recycled back to the plasma membrane, the PDC should maintain in endosomes/lysosomes and the drug or therapeutically active molecule can exert its effect inside the cell. Of major interest are target receptors with fast internalization kinetics and strong recycling/turnover behavior, such as the hY_1R. In combination with a potent PDC, this allows multiple rounds of shuttling into the cancer cells and hence a strong accumulation of drug molecules. The latter is often required to achieve the desired therapeutic efficacy as, for example, chemotherapeutic agents often have a micromolar potency for their intracellular target, while peptide carriers exhibit (sub)nanomolar affinities for their receptors. Increasing the drug cargo loading of the peptide carrier is one approach to reduce the required dose of the PDC, as doses much larger than necessary for full receptor internalization might lead to undesired side effects. Internalization of a designed PDC should be also validated, since modification in general can potentially disturb this property of the peptide ligand. This was observed for hY_4R-targeting hPP analogs that were studied as anti-obesity agents.[152] hPP analogs were lipidated or PEGylated to increase their *in vivo* half-life. While peptides modified with palmitic acid led to a fast internalization of the hY_4R subtype, PEG-modified analogs showed no internalization despite full receptor activation. The internalization of a PDC can be investigated indirectly by tracking fluorescently labeled versions of the target receptors. Specific internalization of the hY_1R by [F^7,P^{34}]-NPY has been demonstrated with cellular systems that were stably or transiently expressing the different hYR subtypes fused to autofluorescent proteins.[153,154] This also illustrates again that the peptide ligand for a PDC has to be chosen with high selectivity for the target receptor in cancer, since peptide-binding receptors frequently consist of different subtypes with varying expression profiles in malignant and nonmalignant tissues.[12] Furthermore, the cellular uptake of PDCs can be directly visualized by additionally tagging the peptide ligand with a small fluorophore. In combination with a labeled receptor, this also enables the investigation of co-localization (Figure 5) and therefore the proof of the receptor-mediated intracellular delivery of the PDC. At last, one of the unknowns of PDC internalization is often the intracellular fate of the drug cargo. The endosomal escape of the drug cargo or PDC remains elusive and insufficient subcellular delivery of the therapeutically active moiety or fast efflux might lead to lower

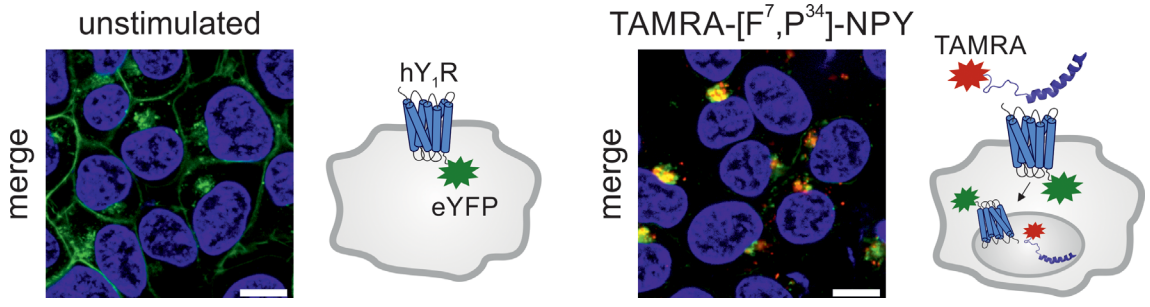


FIGURE 5 Exemplary fluorescence microscopy images demonstrating intracellular delivery of a peptide ligand by receptor-mediated endocytosis. The images show HEK293 cells, stably expressing the human Y_1 receptor (hY_1R), *C*-terminally fused to the enhanced yellow fluorescent protein (eYFP). Cell nuclei are stained with Hoechst 33342 (blue). In the unstimulated state, the fluorescence-tagged hY_1R (green) is predominantly found in the plasma membrane (left image). After stimulation for 1 hour with the fluorescently labeled ligand TAMRA-[F^7,P^{34}]-NPY (red), co-localization of receptor and peptide ligand in intracellular vesicles is observed (yellow overlay fluorescence, right image). TAMRA, 6-carboxytetramethylrhodamine, scale bar = 10 μm

therapeutic efficacy. Further research in this field is highly desired to optimize the effects of PDCs.

6 | VARIATION OF THE DRUG CARGO IN RECEPTOR-TARGETING PEPTIDE-DRUG CONJUGATES

The second major part of a PDC is the drug cargo. In the classical understanding, the term "drug" in peptide-drug conjugate refers to cytotoxic (chemotherapeutic) anti-cancer agents. However, the term "drug" may include broader therapeutically active moieties that can be conjugated to a peptide ligand. Modification of peptides with a radionuclide complex as drug cargo enables the generation of targeted radiopharmaceuticals for the peptide receptor radionuclide therapy. Moreover, boron compounds can be used as cargo to generate PDCs for boron neutron capture therapy.

6.1 | Chemotherapeutic agents

PDCs for targeted chemotherapy usually consist of the peptide carrier, a cleavable linker, and the toxic agent. The toxic agent is chosen to be inactive in its conjugated form, which makes the PDC a prodrug that is activated only in the tumor tissue.[155] The linker must be enzymatically stable during the circulation in the blood to avoid a premature release of the toxic agent, which would result in undesired peripheral toxicity. After reaching the cancer cells, the linker is cleaved intracellularly owing to the change in the biological environment. This releases the toxic agent in a fast and efficient manner, allowing it to exert its desired toxic activity on the cancer cells.

Different linker strategies have been reported for PDCs. One class of linkers are stimuli-responsive, cleavable linkers. They are designed to release the drug from the PDC in the tumor microenvironment or in the altered intracellular microenvironment compared to the extracellular space. As an example, acid-labile bonds such as hydrazone, imine, oxime, acetal or *cis*-aconityl linkages[156] can be cleaved in the lower pH (4.5-6.0) of the endosomes/lysosomes[157] after endocytotic uptake of the PDC. Furthermore, disulfide linkers can be cleaved inside cellular compartments by reducing agents like cysteine and glutathione.[158] Linkers can be also designed to bear an enzymatically cleavable unit. Ester and carbamate bonds can be hydrolyzed by esterases and cytochrome P450 after cellular uptake.[159,160] Succinyl and glutaryl linkers are most commonly used, however, stability of ester linkages in the blood has to be carefully monitored. Peptide linkers that are specifically cleaved by intracellular proteases after internalization have gained significant interest. The peptide bonds in these linkers are stable during circulation,[161] but rapidly hydrolyzed by proteases in endosomes or lysosomes, which makes them optimal structures for the design of PDCs for targeted chemotherapy. Two frequently used peptide sequences are Val-Cit[162] and Gly-Phe-Leu-Gly (GFLG),[163] which are cleaved by the protease cathepsin B. The latter was also found to be overexpressed in some cancer subtypes.[164] A further emerging class of linkers for PDCs are the so-called self-immolative spacers. This type of spacer is used in combination with another cleavable linker and allows the release of the toxic agent from the PDC after simultaneous cascade reactions.[165]

The toxic agent for a PDC can be chosen from the large pool of available and well-established chemotherapeutics. However, the selected drug must comply with certain design principles to serve as suitable compound for the generation of a PDC. It must bear an intrinsic functional group that allows attachment to the cleavable linker structure and hence, the overall conjugation to the carrier peptide. Free hydroxy, carboxy, or amine groups are available in many toxic agents and thus mostly used for conjugation (Figure 6). In the case that the functional group is required for the biological activity of the

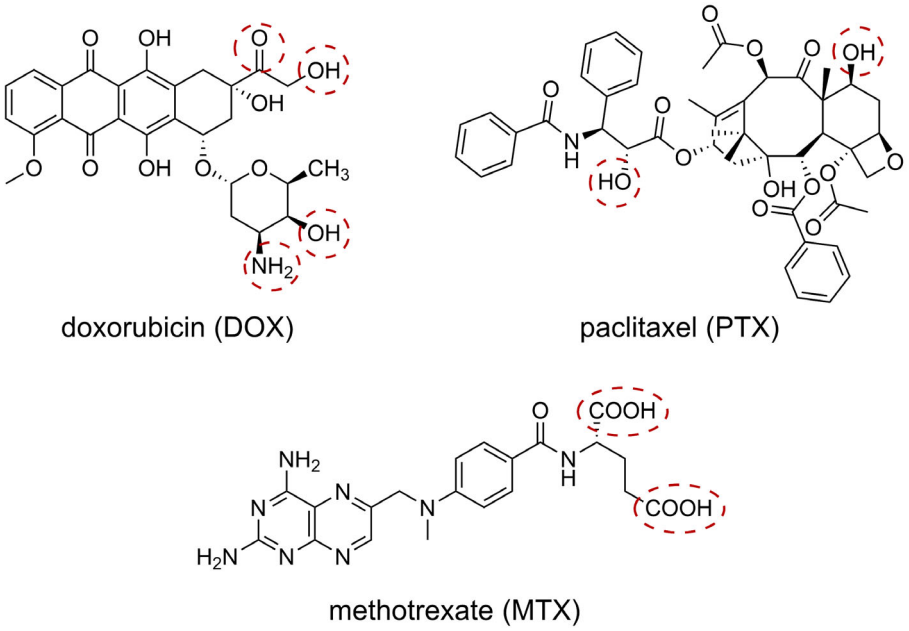


FIGURE 6 Representative examples of suitable toxic agents for the generation of peptide-drug conjugates. Doxorubicin is a DNA intercalator, paclitaxel is an antimitotic agent and methotrexate acts as antifolate. Frequently used conjugation sites in the toxophores are marked with red cycles

toxic agent, the linker has to be able to release the toxophore in a traceless manner.[155] Furthermore, the drug has to be chosen to exhibit sufficient and potent cytotoxicity vs the malignant cells, as drug resistance mechanisms of cancer cells can be an issue.[166] Chemotherapeutic agents for PDCs can be classified according to their general mode of action.[167] Some bind or interact with the cellular DNA or DNA-protein complexes, thereby blocking the transcription and DNA replication or inducing DNA damage. Ultimately, all of these effects lead to apoptosis of the targeted cell. Examples include metal complexes,[168] camptothecin (CPT),[169] and the anthracyclines daunorubicin (DAU) and doxorubicin (DOX).[170] Other toxic agents are antimetabolites that interfere with the DNA biosynthesis, for example, gemcitabine as nucleoside analog of deoxycytidine[171] and methotrexate (MTX) as antifolate that inhibits the enzyme dihydrofolate reductase.[172] Furthermore, anti-mitotic agents that act on microtubules are used as toxic payload in PDCs. This comprises paclitaxel (PTX), which inhibits microtubule depolymerization,[173] and the group of *vinca* alkaloids, which inhibit tubulin polymerization.[174]

A large number of receptor-targeting PDCs with different chemotherapeutic agents as drug cargo has been developed and reported in literature. For targeting the SSTR2, octreotide and its analogs have been conjugated to PTX, DOX, or 2-pyrrolino-DOX by ester linkers and exhibited selective toxicity against receptor-expressing tumors *in vivo*.[175–179] Furthermore, octreotide analogs conjugated to different toxic agents, including CPT, the anti-mitotic agent combretastatin-A4, and the alkylating agent chlorambucil (CLB), via carbamate or amide linkers were developed.[180,181] One CPT conjugate displayed good *in vitro* toxicity and specific uptake into SSTR2-positive pancreatic tumors in a mouse xenograft model.[181] Recently, the integrin-targeting cyclic peptides c(RGDfK) and c(RGDfS) were conjugated to CPT and CLB by a carbamate and ester linkage, respectively. Both PDCs showed growth inhibition in cancer cell lines expressing the integrin αvβ3.[182] Furthermore, a non-RGD, αvβ3-binding cyclic peptide-CPT conjugate with selective cytotoxicity in human melanoma cells has been reported.[183] Targeting of the Bn receptors for selective chemotherapy has been explored with a multitude of Bn analogs that were linked to various toxic agents by different cleavable linkers.[149] As an example, [D-Tyr^6,β-Ala^{11},D-Phe^{13},Nle^{14}]-Bn(6-14) was N-terminally conjugated to CPT via a carbamate linker, thus generating a potent PDC that is cytotoxic for cells overexpressing all Bn receptor subtypes.[184]

A number of PDCs that are targeting the hY$_1$R on cancer cells have been reported. NPY was conjugated to DAU by a cleavable hydrazone and a stable amide linker. In cell viability assays with hY$_1$R-expressing neuroblastoma cells, the hydrazone-linked DAU-NPY conjugate showed a comparable toxicity to free DAU, whereas no toxic effect was observed for the amide-linked DAU-NPY conjugate.[185] To selectively target breast cancer cells, the hY$_1$R-preferring [F^7,P^{34}]-NPY was conjugated at its Lys^4 side-chain to the anti-microtubule agent cytolysine by a disulfide linker.[186] The cytolysine-PDC exerted selective and strong cytotoxicity on hY$_1$R-expressing cell lines, in contrast to the unspecific activity of free cytolysine. In addition, proteomic analysis revealed an identical mode of action of cytolysine delivered by the PDC compared to free cytolysine in the hY$_1$R-expressing breast cancer cell lines MDA-MB-468 and MCF-7. In another study, the toxic agent MTX was coupled to the Lys^4 side-chain of [F^7,P^{34}]-NPY by different linker structures, including an amide, ester, disulfide, and enzymatically cleavable GFLG linkage.[187] The optimal conjugate [K^4(GFLG-MTX),F^7,P^{34}]-NPY displayed high extracellular stability, paired with selective internalization into hY$_1$R-expressing cells and fast intracellular release of the (drug) cargo. Potent toxicity of this PDC against MDA-MB-468 breast cancer cells was observed with no effect on normal HEK293 cells. A follow-up study intended to increase the toxicity of the NPY-based PDC by doubling the amount of toxophores.[188] Therefore, MTX was simultaneously conjugated to the side-chains of Lys^4 and the inserted Lys^{22} in [F^7,P^{34}]-NPY via GFLG-linkers, resulting in the double-modified PDC [K^4(GFLG-MTX),F^7,K^{18}(GFLG-MTX),P^{34}]-NPY. A higher cytotoxic effect of the double-MTX PDC compared to single-MTX PDCs was obtained on various hY$_1$R-expressing breast cancer cell lines.

In the last years, major progress has been made with PDCs based on GnRH agonists for targeting the GnRH-R. The Mező group developed the conjugate [Lys^4(butyryl),Lys^8(DAU = Aoa)]-GnRH-III (Aoa is aminooxyacetal), in which the toxophore DAU is linked to GnRH-III by an oxime linkage.[189] This PDC was found to be highly potent *in vitro* and showed similar or higher *in vivo* antitumor activity than free DAU, without significant toxic side effects on other organs. Until now, the most progressed receptor-targeting PDC for selective chemotherapy is Zoptarelin DOX (also named AN152 or AEZS-108, AEterna Zentaris), which is composed of DOX conjugated to the D-Lys^6 side-chain of [D-Lys^6]-GnRH-I by a glutaryl linker (Figure 7A).[190] In preclinical studies, AEZS-108 showed high antitumor activity against various tumor types with less peripheral toxicity than free DOX.[191] Due to these promising results, AEZS-108 was tested in phase 1 and phase 2 studies starting from 2006. In a phase 2 clinical trial on patients with castration- and taxane-resistant prostate cancer, AEZS-108 displayed significant activity and maintained an acceptable safety profile.[192] Additionally, phase 2 clinical trials on 43 women with GnRH-R-positive, platinum-resistant advanced ovarian cancer, or recurrent endometrial cancer were conducted. Treatment with AEZS-108 resulted in significant antitumor activity and low toxicity in these patients.[193] Recently, a multinational phase 3 study for the treatment of endometrial cancer with AEZS-108 was completed and the results were disclosed in May 2017. Despite the encouraging results obtained before, this trial showed that AEZS-108 did not extend overall survival nor did it improve the safety profile compared to the classical chemotherapy with DOX.[194]

Notably, another PDC named ANG1005 (Angiochem Inc.) has reached phase 3 clinical trials. ANG1005 consists of the blood-brain barrier (BBB)-penetrating peptide angiopep-2 conjugated to three PTX molecules by cleavable ester linkers (Figure 7B).[195] It is able to cross the BBB by receptor-mediated transcytosis after binding to the low-density lipoprotein receptor-related protein 1, with the latter being overexpressed in brain cancer.[196] ANG1005 showed higher brain uptake than free PTX and significant antitumor activity *in vivo* in glioblastoma-bearing mice.[195] In a phase 1 trial on patients with recurrent or progressive malignant glioma, ANG1005 reached

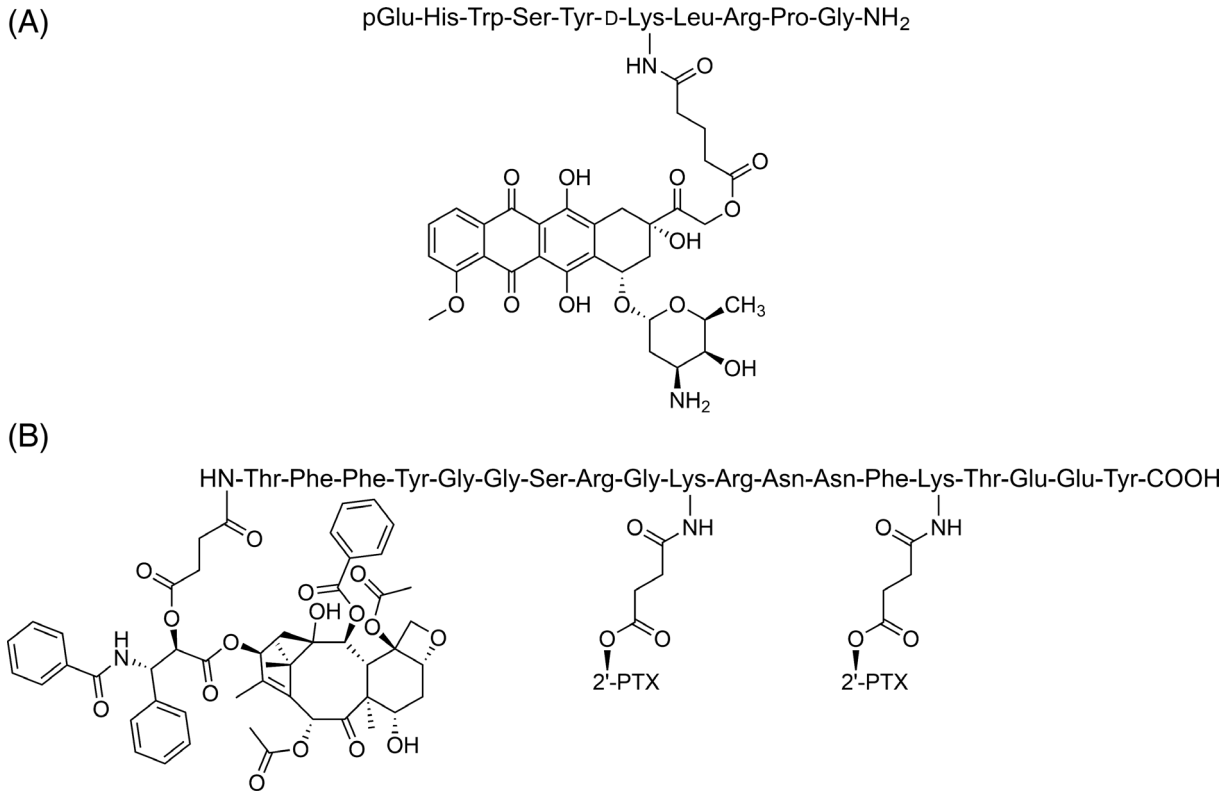


FIGURE 7 Chemical structures of receptor-targeting peptide-drug conjugates that reached phase 3 clinical studies. A, Zoptarelin doxorubicin (AEZS-108, AEterna Zentaris). B, ANG1005 (Angiochem Inc.). PTX, paclitaxel

therapeutic concentrations in the tumor site and was well tolerated.[197] Phase 2 clinical trials on patients with recurrent high-grade gliomas and breast cancer patients with recurrent brain metastases were completed, and results have been recently published for the latter study.[198,199] Symptom improvement and prolonged overall survival compared to historical control was seen in the majority of patients treated with ANG1005, particularly in patients with leptomeningeal carcinomatosis.[199] A phase 3 study to investigate whether ANG1005 can prolong survival of breast cancer patients with newly diagnosed leptomeningeal disease is estimated to start in September 2020.[200]

6.2 | Radionuclides

Radiolabeled peptide ligands in general can be used for two main purposes related to cancer. A major field is their application as diagnostic tools for visualizing the expression of their target receptors in tumor tissue. Peptides can be labeled with positron-emitting radioisotopes such as ^{18}F, copper-64 (^{64}Cu), and gallium-68 (^{68}Ga) to generate PET imaging agents. Furthermore, γ-emitting radioisotopes such as ^{99m}Tc and iodine-123 (^{123}I) are used for the design of peptide-based single-photon emission computed tomography (SPECT) imaging agents. Information about receptor-targeting peptides as molecular imaging agents can be found in several reviews.[201,202] Peptide carriers that are radiolabeled with a therapeutic radionuclide can be also seen as PDCs, in which the radionuclide complex represents the drug cargo. These conjugates hold great promise as targeted radiopharmaceuticals for the peptide receptor radionuclide therapy (PRRT). Currently, an intense discussion regarding the use of antagonists vs agonists for peptide radiopharmaceuticals is taking place. Antagonists can be beneficial, because they do not activate the target receptor, which can reduce undesired side effects. In addition, antagonists were reported to recognize a higher number of receptor-binding sites compared with agonists, which resulted in higher tumor uptake and tumor retention of the radiolabeled antagonists *in vivo*.[203,204] Recently, a SSTR-targeting antagonist was evaluated for PRRT in a pilot study on four cancer patients.[205] The absorbed radiation doses in the tumor and the tumor-to-kidney and tumor-to-bone marrow dose ratios were higher for the antagonistic compound compared with a SSTR agonist. However, clinical trials still have to prove whether antagonists are able to outperform agonists. Furthermore, only the receptor-mediated internalization induced by agonists facilitates the selective accumulation of radioisotopes inside tumor cells, which is highly preferred for certain radioisotopes to achieve an enhanced effect in PRRT.

Radionuclides for PRRT can be divided into three general categories: β^--emitters, Auger electron-emitters and α-emitters.[206,207] A selection of therapeutic radiometals is given in Table 2.

Due to the highly differing characteristics of the emitted radiation types, the choice of the radionuclide for PRRT strongly depends on

TABLE 2 Characteristics of selected therapeutic radiometals for peptide receptor radionuclide therapy

Radionuclide	Half-life	Emission	Maximum particle range in tissue	Source
Copper-67	2.58 days	β^- γ	2–3 mm —	Reactor/cyclotron
Yttrium-90	2.67 days	β^-	11 mm	Generator
Indium-111	2.81 days	Auger e CE γ	10 μm 600 μm —	Cyclotron
Terbium-161	6.90 days	β^- Auger e CE γ	3 mm n.d. n.d. —	Reactor
Lutetium-177	6.65 days	β^- Auger e CE γ	3 mm n.d. n.d. —	Reactor
Rhenium-188	17 hours	β^- γ	11 mm —	Generator
Astatine-211	7.2 hours	α	65 μm (mean)	Accelerator
Bismuth-213	46 minutes	β^- α	n.d. 80-100 μm (mean)	Generator

Abbreviations: CE, conversion electrons, n.d., not determined in the literature.

the size of the targeted tumor or metastases.[208] One of the most commonly used radiometals for peptide radiopharmaceuticals is the pure high-energy β^--emitter yttrium-90 (^{90}Y, maximum energy: 2.27 MeV). The β^- radiation of ^{90}Y has a long tissue penetration range of up to 11 mm and can therefore exert a "cross-fire effect", which is the irradiation of tumor cells to which the ^{90}Y-containing peptide is not directly binding.[209] This can be useful for compensating uptake heterogeneity within large tumors. Another very frequently used radionuclide for PRRT is the medium-energy β^--emitter lutetium-177 (^{177}Lu, maximum energy: 0.5 MeV).[210] Besides the emission of β^- particles with a tissue penetration range of max. 3 mm, ^{177}Lu also emits a certain number of low-energy Auger electrons and conversion electrons (CEs) that deposit their dose over a short distance. Monte Carlo simulations suggest that ^{177}Lu has a higher rate of eradication of small metastases than ^{90}Y,[211] which is supported by an *in vivo* study with radiolabeled antibody conjugates directed against B-cell lymphoma xenografts in mice.[212] Furthermore, ^{177}Lu decays with low abundance of γ radiation, which enables posttherapeutic dosimetry. The radiometal indium-111 (^{111}In) is mostly used for imaging owing to its main emission of γ radiation. However, ^{111}In also emits Auger electrons and CEs, allowing its use as therapeutic radionuclide. Both, a simulation and a biological study, suggested that ^{111}In might outperform ^{177}Lu in the treatment of micrometastases (<100 μm) and small cells.[211–213] A disadvantage of ^{111}In is its high proportion of additional photon emission, which adds to the total body dose. A nuclide that just recently gained interest for use in PRRT is the radiolanthanide terbium-161 (^{161}Tb).[214] ^{161}Tb has a similar medium-energy β^--emission spectrum to ^{177}Lu, but emits a higher number of Auger electrons and CEs, thereby combining advantages of two categories of therapeutic radionuclides. Comparable to ^{111}In, simulation studies suggest that ^{161}Tb is able to deposit higher doses to micrometastases and single cells than ^{177}Lu.[211] Two biological studies reported that ^{161}Tb-labeled anti-L1CAM antibodies and folate conjugates showed a more efficient tumor growth inhibition *in vivo* than their ^{177}Lu-labeled counterparts.[215,216] Moreover, an advantage of ^{161}Tb over ^{111}In is the emission of a much lower number of photons. For radionuclides such as ^{161}Tb with a high emission of short-ranged Auger electrons and CEs in the very low energy-domain (<50 keV), internalization into the tumor cells is desired for an improved therapeutic effect. Additionally, further subcellular delivery to the nucleus would facilitate a maximum dose deposition to the DNA.[217] At last, the use of α-emitters in PRRT is a relatively new approach, but is estimated to further progress in the future. Emission of α-particles produces high linear energy transfer (LET) radiation, leading to very high cytotoxicity on the cellular level and short-range in tissue, which might potentially reduce side effects. Promising α-emitters for PRRT include among others bismuth-213 (^{213}Bi) and astatine-211 (^{211}At).[218]

Labeling of the peptide carrier with a therapeutic radionuclide can be achieved by a direct strategy, in which functional groups in the peptide sequence are used to complex the radiometal. This is predominantly applied for the radionuclide rhenium-188 (^{188}Re).[219] For the majority of radiometals, however, indirect labeling is performed by a bifunctional chelating agent (BFCA).[220,221] BFCAs consist of two functionalities, namely a chelating unit for coordination of the radiometal ion and a functional group that allows covalent attachment to the peptide ligand. Furthermore, two categories of BFCAs exist: the acyclic chelators and the macrocyclic chelators. The most frequently used acyclic BFCA for the generation of peptide radiopharmaceuticals is diethylenetriaminepentaacetic acid

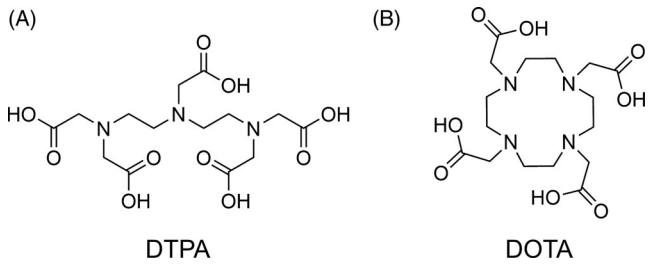


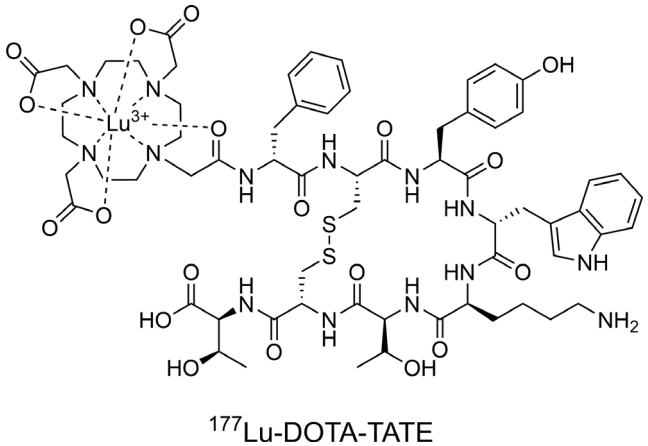
FIGURE 8 Chemical structures of the, A, acyclic chelator diethylenetriaminepentaacetic acid (DTPA) and B, macrocyclic chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)

(DTPA),[220] which can be conjugated to the peptide carrier via its carboxy functions (Figure 8A). DTPA is characterized by rapid metal-binding kinetics, resulting in fast radiolabeling under mild conditions. However, a major disadvantage is that the complexes of DTPA with the most used β^--emitting radiometals possess insufficient *in vivo* stability.[222] From the group of macrocyclic chelators, the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) is the most commonly applied BFCA for peptide conjugation (Figure 8B).[223] DOTA provides eight donor atoms and a suitable cavity size for the complexation of various trivalent radiometals and lanthanides that require a high coordination number. It can be attached to peptides via one of its carboxy groups. Although the labeling kinetics of DOTA are rather slow, this BFCA forms thermodynamically and kinetically stable radiometal complexes that withstand the competing environment in a biological system, rendering it highly suitable for the development of radiolabeled peptides.[222] Introduction of the radionuclide into peptides is usually performed by the postlabeling approach. Here, the BFCA is first covalently attached to the peptide during the solid phase synthesis. For DOTA, this is for example possible by using the protected building block DOTA-tris(tert-butyl ester). Afterwards, radiolabeling is accomplished by simply reacting the purified BFCA-peptide with the radiometal salt solution.

The high interest in PRRT is closely connected to the success of SSTR-targeting radiolabeled somatostatin analogs. ^{111}In-DTPA-octreotide (Octreoscan) was the first approved peptidic radiopharmaceutical for diagnostic imaging of SSTR-positive neuroendocrine tumors (NETs) and also marks the start of PRRT.[224] Administration of ^{111}In-DTPA-octreotide in a high dose to patients with metastasized NETs led to a palliative outcome, but tumor size regression was unsatisfactory.[225] This was attributed to the short-ranged Auger electron emission of ^{111}In, which is not ideal for macroscopic tumors. In the second generation of SSTR-targeting therapeutic radiopharmaceuticals, the peptide carrier, the BFCA and the radionuclide were changed. At first, the SST analog [Tyr^3]-octreotide with higher binding affinity for the SSTR2 was used and N-terminally conjugated to the macrocyclic chelator DOTA.[226] The resulting [DOTA^0,Tyr^3]-octreotide (DOTA-TOC) was radiolabeled with ^{90}Y. Starting from the late 1990s, ^{90}Y-DOTA-TOC was investigated in multiple clinical trials and exhibited a significantly improved therapeutic efficacy in NET patients compared to ^{111}In-DTPA-octreotide.[227,228] Further optimization was performed by exchanging the C-terminal threoninol in DOTA-TOC with threonine, which yielded the peptide conjugate [DOTA^0,Tyr^3]-octreotate (DOTA-TATE) with a ninefold increased affinity to the SSTR2 compared to DOTA-TOC.[229] ^{90}Y-DOTA-TATE was investigated in a phase 2 study in patients with gastroenteropancreatic (GEP) NETs.[230]

The most successful therapeutic peptide radiopharmaceutical so far is ^{177}Lu-DOTA-TATE (Figure 9). PRRT with this conjugate has been extensively investigated in several trials in clinical centers in Europe.[231] Overall, a partial or complete response in around 30% of recruited NET patients and a median progression-free survival of around 36 months have been demonstrated. Furthermore, first results from the NETTER-1 phase 3 study in early 2017 demonstrated that PRRT with ^{177}Lu-DOTA-TATE resulted in longer progression-free survival and a significantly higher response rate than high-dose octreotide long-acting repeatable among patients with advanced midgut NETs.[232] In October 2017, the European Commission approved ^{177}Lu-DOTA-TATE (Lutathera) for the treatment of unresectable or metastatic, progressive SSTR-positive GEP-NETs in adults. FDA approval followed in January 2018.

Besides the success with radiolabeled SST analogs, other receptor-targeting peptides are at various stages of development for PRRT. The dimeric peptide conjugate ^{90}Y-DOTA-E-[c(RGDfK)]$_2$ for integrin targeting was developed and showed tumor growth inhibition in mice with ovarian carcinoma xenografts.[233] However, no improvement in the therapeutic efficacy of this compound by dose fractionation was observed in a follow-up study.[234] Incorporation of PEG linkers in ^{90}Y-DOTA-PEG$_4$-E-[PEG$_4$-c(RGDfK)]$_2$ yielded a conjugate that showed effective growth inhibition of human gliomas in mice paired with low radiotoxicity in the normal organs.[235] For addressing bombesin receptors in PRRT, the peptide ^{177}Lu-DOTA-8-AOC-Bn



^{177}Lu-DOTA-TATE

FIGURE 9 Chemical structure of ^{177}Lu-DOTA-TATE, which is approved in the EU for peptide receptor radionuclide therapy of unresectable or metastatic, progressive SSTR-positive GEP-NETs in adults. GEP-NET, gastroenteropancreatic neuroendocrine tumor; SSTR, somatostatin receptor

(7-14)-NH_2 was investigated in a PC-3 (prostate cancer) xenograft mouse model and displayed significant tumor growth suppression.[236] Additionally, the Bn receptor-binding peptide carrier DO3A-CH_2CO-G-4-aminobenzoyl-QWAVGHLM-NH_2 (AMBA) was developed. Treatment of PC-3 tumor-bearing mice with ^{177}Lu-AMBA prolonged their lifespan and inhibited the tumor growth.[237] The MC_1R-targeting peptide conjugate ^{188}Re-(Arg^{11})CCMSH, with CCMSH being [Cys^{3,4,10},D-Phe^7]α-MSH_{3-13}, was studied for PRRT in a human melanoma-bearing mouse model.[238] Compared to the control group, a significantly reduced tumor size and longer mean survival times were observed in the treated mice. At last, radiolabeled NPY analogs have been claimed for hYR targeting in PRRT. The hY_1R-preferring [F^7,P^{34}]-NPY was conjugated to a DOTA chelator at the side-chain of Lys^4 and radiolabeled with ^{111}In.[239] The resulting conjugate [K^4(DOTA-^{111}In),F^7,P^{34}]-NPY displayed a maintained hY_1R binding affinity *in vitro* and some *in vivo* tumor uptake in MCF-7 tumor-bearing mice. Recently, a multifunctional [F^7,P^{34}]-NPY conjugate, containing an intracellularly releasable DOTA-nuclear localization sequence (NLS) unit, was developed.[234] The conjugate was labeled with native terbium-159 and displayed retained hY_1R activity and selectivity. Furthermore, radiolabeling with ^{111}In as surrogate for radiolanthanides was performed. The radiolabeled peptide specifically internalized into MCF-7 cells and yielded a time-dependent nuclear uptake of ^{111}In. These results demonstrated that the multifunctional NPY conjugate represents a promising concept for the selective nuclear delivery of Auger-electron emitting radiolanthanides such as ^{161}Tb.

6.3 | Boron compounds for BNCT

Receptor-targeting peptides can be also conjugated to special drug cargos for use in next-generation cancer therapies that require novel delivery agents. This is the case for the boron neutron capture therapy (BNCT).

G. L. Locher already described the idea of BNCT back in 1936. First, a boron compound is accumulated in cancer cells, followed by local irradiation of the tumor site with thermal or epithermal neutrons (Figure 10). The ^{10}B isotope, which comprises 19.9% of the naturally occurring boron, has as remarkably high neutron capture cross-section. Neutron capture of ^{10}B results in the fission reaction [^{10}B(^{1}n,α)^{7}Li], thus generating α particles and recoiling lithium-7 (^{7}Li) nuclei with high linear energy transfer (LET). Since these LET particles deposit their ionizing energy over a short distance of 5 to 10 μm, which is in the range of the diameter of a cell, their destructive effect is limited to boron-containing cells.[240,241] In principle, BNCT offers the possibility to combine molecular drug targeting with the regional beam positioning of radiation therapy to achieve a double-selective therapeutic effect.[242] Clinical investigation of BNCT was mainly performed on patients with recurrent head and neck cancer, high-grade gliomas, and advanced melanomas.[243] Despite some promising results, it was not possible to establish BNCT as viable cancer treatment modality in the clinic so far owing to the lack of optimal boron

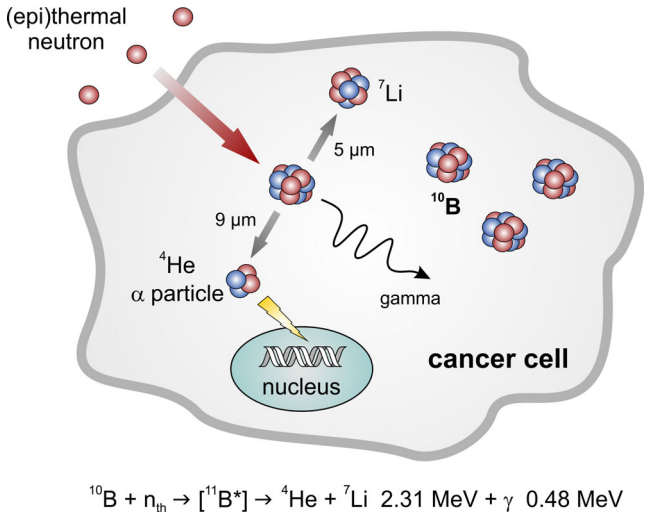


FIGURE 10 Schematic representation of the neutron capture reaction in boron neutron capture therapy (BNCT)

delivery agents for BNCT.[244] The demanding requirements for such compounds are (a) delivery of high amounts of boron to the tumor (20-40 μg ^{10}B/g tumor) to obtain a sufficient generation of destructive LET particles; (b) tumor-selective boron uptake with tumor-to-normal-cell and tumor-to-blood concentration ratios of >3:1 to spare the non-neoplastic tissue; (c) low intrinsic cytotoxicity; and (d) rapid clearance from the blood, but high persistence in the tumor. Preferentially, the BNCT agent should also deliver the required amount of boron into the cancer cells (at least 10^9 ^{10}B atoms per cell) to facilitate a maximum dose deposition to the DNA.[245] Currently, only the two boron-containing drugs L-boronophenylalanine (BPA)[246] and sodium mercaptoundecahydro-*closo*-dodecaborate (BSH)[247] are used in clinical trials (Figure 11A). BPA is thereby mostly applied as BPA-fructose adduct to increase its water solubility.[248] While it is proposed that BPA is taken up into cancer cells through the L-type amino acid transporter,[249] BSH is passively accumulating in the tumor tissue.[250] However, both compounds have several disadvantages. BPA contains only a single boron atom and has to be administered in very high doses to reach the required boron amounts in the tumor. BSH is not able to penetrate the cell membrane owing to its net charge. In addition, both compounds are characterized by rather modest tumor selectivity and hence exhibit a suboptimal BNCT efficiency.[251]

In the search for novel BNCT agents with higher tumor selectivity, a multitude of boronated low molecular weight compounds were developed such as nucleoside, amino acid, sugar, and porphyrin derivatives.[251,252] Furthermore, high molecular weight compounds were designed that additionally allow a much higher boron loading. This includes, for example, polyanionic and polycationic polymer, polyamine, protein, and antibody conjugates and boron-containing liposomes and nanoparticles.[251,252] Receptor targeting in BNCT has been extensively studied for the EGFR in glioblastoma. A heavily boronated polyamidoamine dendrimer (around 1000 boron atoms) was linked to the ligand EGF,[253,254] and the monoclonal antibodies cetuximab[255,256] and L8A4,[257,258] with the latter two targeting the

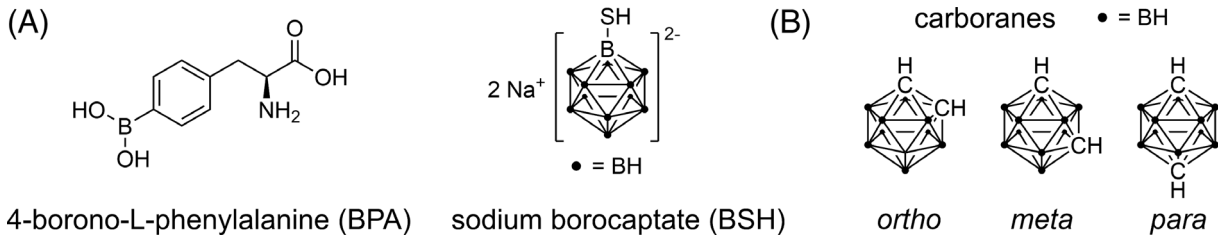


FIGURE 11 A, Structures of L-boronophenylalanine (BPA) and sodium borocaptate (BSH). These two are the only boron-containing drugs that were used for BNCT in clinical trials. B, Chemical structures of *ortho*-, *meta*-, and *para*-carborane isomers

wild-type EGFR or the mutant isoform EGFRvIII, respectively. As *in vivo* model, rats bearing F98 gliomas, transfected with the gene encoding for the human EGFR or EGFRvIII, were used. The bioconjugates were administered by convection-enhanced delivery to the gliomas in the rats and BNCT experiments were performed. A twofold increase in mean survival time (MST) of the rats in comparison with irradiated control animals was observed, when the bioconjugates were combined with intravenous administration of BPA.[259] Furthermore, rats bearing a composite tumor (1:1 $F98_{EGFR}$ + $F98_{EGFRvIII}$ glioma cells) were treated with a mixture of boronated cetuximab and L8A4 or with the antibody conjugates individually before neutron irradiation. A significantly higher MST was found for the rats in the co-administration group, indicating the need for targeting both receptor variants for an optimal therapeutic effect in composite tumors.[259]

For the generation of PDCs as potential boron delivery agents, carboranes are suitable boron compounds. These icosahedral, hydrophobic $C_2B_{10}H_{12}$ clusters have a high boron content and occupy a rather small amount of space, slightly larger than a rotating phenyl ring.[260] Three different isomers exist, which are defined by the position of the carbon atoms in the cluster (Figure 11B). Furthermore, the vertexes of the carborane cluster can be chemically functionalized, thus allowing the facile conjugation to peptides by peptide chemistry. Carboranes also display high biological stability and relatively low cytotoxicity.[261]

The concept of carborane-containing PDCs for BNCT has been described in a few studies. Kimura *et al.* reported the design of a dimeric PDC for integrin targeting.[262] The two carbon atoms of a 1,2-dicarba-*closo*-dodecarborane(12) (*ortho*-carborane) cluster were each linked to the cyclic peptide c(RGDfK) via a butanoic acid linker (Figure 12A). The resulting conjugate, named GPU-201, displayed a high integrin $\alpha v\beta 3$ binding affinity, dose-dependent tumor uptake in squamous cell carcinoma (SCCVII)-bearing mice and longer tumor retention time than BSH. In a follow-up study, ^{10}B-enriched GPU-201 was investigated in BNCT experiments on SCCVII-bearing mice.[263] GPU-201 showed a stronger tumor growth inhibition than BSH after neutron irradiation, however, the PDC was also more toxic than BSH to both proliferating and quiescent cancer cells without irradiation. Schirrmacher *et al.* have first proposed the use of internalizing, GPCR-targeting PDCs for BNCT, which would allow a highly selective delivery of boron into cancer cells.[264] They used SSTR2-addressing [Tyr^3]-octreotate (TATE) and modified it N-terminally with the *ortho*-carborane derivative 5,6-dicarba-*closo*-dodecaboranyl hexynoic acid. In addition, Mier *et al.* reported the conjugation of BSH to N-terminally maleimido-modified TATE by Michael addition.[265] However, for both conjugates, no biological activity was determined.

Betzel *et al.* described the first biological testing of boronated TATE analogs.[266] In their PDCs, the *ortho*-carborane-containing building block 4-(*O*-methylencarboranyl)-benzoic acid (BBB1) was directly coupled to the N-terminus of TATE or attached by glycine-sarcosine spacers with one, three or five sarcosine units. Furthermore, two BBB1 molecules were N-terminally conjugated to TATE by lysine-sarcosine spacers with one or three sarcosine units (Figure 12B). All of the mono-carborane-conjugated PDCs with a spacer showed nanomolar affinity toward the SSTR2 independent of the spacer length. For double-carborane conjugated TATE, the triple-sarcosine spacer was required to obtain a suitable SSTR2 affinity. Further pharmaceutical development of SSTR2-targeting, boronated peptides has not been conducted.

Modification of a novel hexapeptide super-agonist at the GhrR with different carborane monoclusters was evaluated and a *meta*-carborane with a mercaptoacetic acid linker was found to be optimal, owing to its high chemical stability and a suitable GhrR activation efficacy of the conjugate.[267] Introduction of this *meta*-carborane into the known ghrelin receptor ligands GHRP-6 and Ipamorelin yielded boron-rich peptide conjugates with high potency that were specifically shuttled into cells by GhrR-mediated endocytosis.

Boron-modified NPY analogs for hYR targeting in BNCT have been recently described. The *ortho*-carboranyl propionic acid (Cpa)-containing amino acid Fmoc-Lys-N_ε(Cpa)-OH was incorporated at position 4 of NPY, hY_1R-preferring [F^7,P^{34}]-NPY and hY_2R-selective [$Ahx^{(5\text{-}24)}$]-NPY during the solid phase peptide synthesis.[154] Carborane modification only led to a slight loss of hY_1R and/or hY_2R affinity and activity of the NPY conjugates. Additionally, selectivity of [K^4(Cpa),F^7,P^{34}]-NPY and [K^4(Cpa),$Ahx^{(5\text{-}24)}$]-NPY was maintained, which was also demonstrated by induction of hYR subtype-specific internalization. Furthermore, the *ortho*-carborane building block 9-(carboxymethylthio)-1,2-dicarba-*closo*-dodecaborane(12) was attached to the side-chain of lysine residues at different positions in [F^7,P^{34}]-NPY.[151] Conjugates with carborane modification at positions 4, 18, or 22 in the peptide sequence displayed high activity at the hY_1R and these positions were combined to generate a triple-carborane peptide. The resulting boron-rich [F^7,P^{34}]-NPY analog exhibited nanomolar potency at the hY_1R, selectivity against other

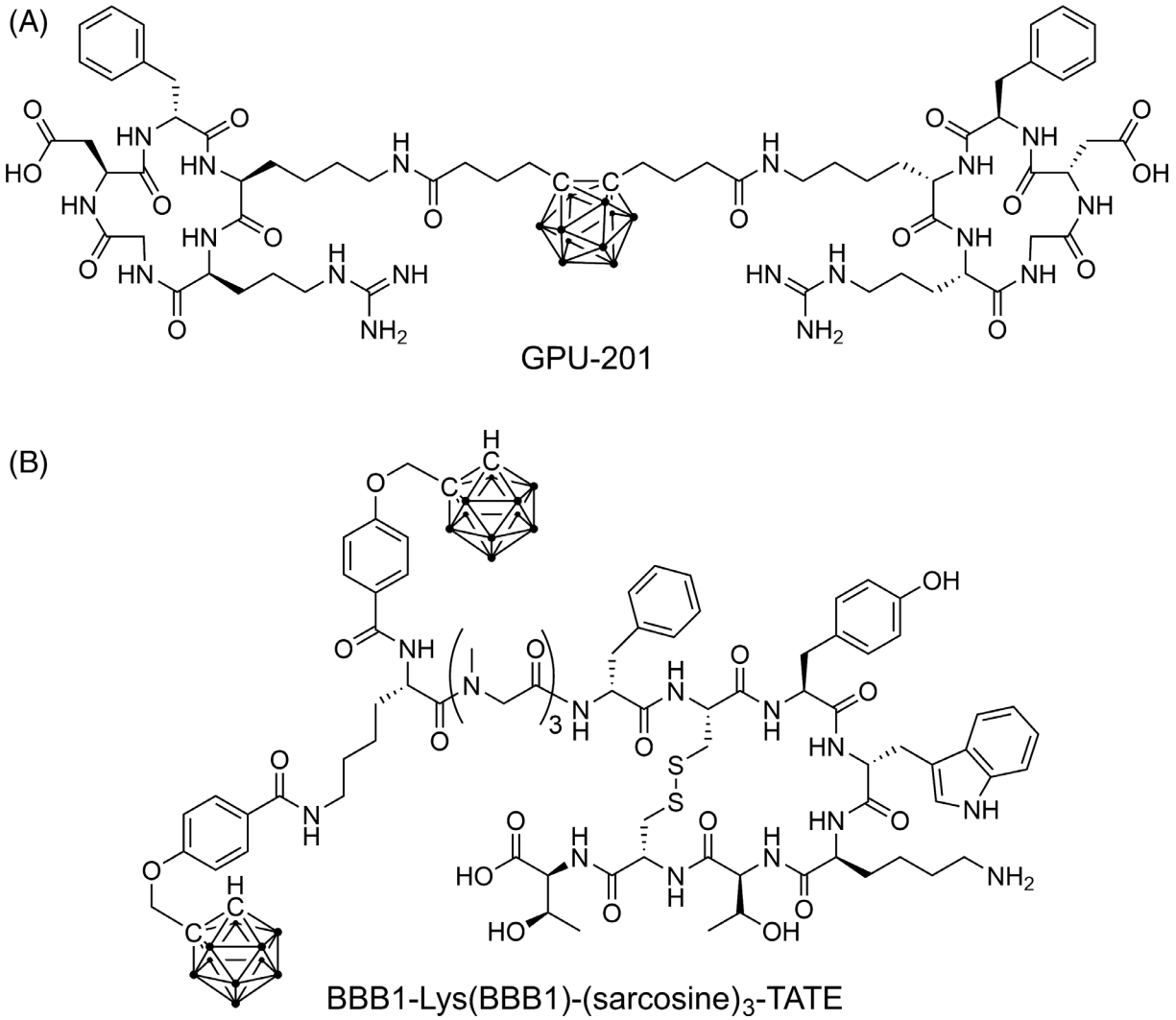


FIGURE 12 Chemical structures of the, A, dimeric carborane-c(RGDfK) conjugate GPU-201 for integrin αvβ3 targeting and B, double-carborane modified [Tyr^3]-octreotate (TATE) for SSTR2 targeting

hYR subtypes and was able to shuttle sufficient amounts of boron for BNCT into hY$_1$R-transfected HEK293 cells. In a third study, a deoxygalactosyl-functionalized, charge-compensated cobalt bis(dicarbollide) building block with enhanced hydrophilicity was conjugated to Lys^4 in [F^7,P^{34}]-NPY and a retained hY$_1$R activity for the conjugate was observed.[268] [F^7,P^{34}]-NPY conjugates with a maximized boron loading were also developed.[269,270] A galactosyl-*meta*-carborane facilitated the generation of a peptide with eight incorporated carborane clusters, that is, 80 boron atoms per peptide molecule. The boron-rich conjugate exhibited high activation and internalization of the hY$_1$R, selectivity against the other hYR subtypes, and low intrinsic cytotoxicity. Substitution of the mono-galactosyl-carboranes with bis-galactosyl-carboranes finally yielded an 80 boron-NPY conjugate with high solubility in aqueous solution and good activity at the hY$_1$R. This novel compound can be considered as promising tumor-selective BNCT agent.[270]

Notably, two studies report the design of boron-modified peptide conjugates that are not targeting a cell-surface receptor. Michiue *et al.* used a poly-arginine (11R) cell-penetrating peptide (CPP) as carrier and linked it to a peptidic dendrimer containing eight BSH molecules.[271] The resulting conjugate 8BSH-11R facilitated the delivery of high amounts of boron (around 5000 ppm/10^6 cells) into U87ΔEGFR glioma cells. In BNCT experiments *in vitro*, treatment with 8BSH-11R before neutron irradiation resulted in significantly stronger growth inhibition of U87ΔEGFR glioma cells compared to treatment with BSH. Furthermore, accumulation of 8BSH-11R in implanted glioma cells in mice with no uptake in the normal brain was observed. In the second study, the same group double-modified an arginine-tripeptide (3R) with BSH and a DOTA chelator.[272] Similar to the previously described 11R conjugate, BSH-3R-DOTA was specifically accumulated in implanted U87ΔEGFR glioma cells in mice and no uptake in the normal brain cells was detected. Additionally, radiolabeling of BSH-3R-DOTA with ^{64}Cu allowed uptake quantification in glioma-bearing mice by PET imaging. For the labeled 3R conjugate, significantly higher tumor-to-normal-brain and tumor-to-blood radioisotope accumulation ratios compared to BSH-DOTA-^{64}Cu were observed.

7 | CONCLUSION AND PERSPECTIVE

High overexpression of peptide-binding receptors in human tumors makes them promising targets for selective anti-cancer drug delivery. Synthetic analogs of natural peptide ligands are thereby of major interest as receptor-targeting units because they possess high target affinity and specificity, fast internalization rates, and low immunogenicity. In addition, facile chemical functionalization with different therapeutic cargos is possible. With ^{177}Lu-DOTA-[Tyr^3]-octreotate, a first receptor-targeting peptide conjugate for cancer therapy has finally reached market approval. However, the translation of peptide-drug conjugates from the laboratory to the clinics remains a big hurdle.

In contrast to antibody-drug conjugates, low plasma stability and short half-lives of PDCs are still considerable problems for their clinical development. Particularly for the treatment of solid tumors, extended circulation of PDCs is required to allow sufficient penetration of the PDC into the malignant tissue. The modern chemical toolbox of peptide modification strategies for stability enhancement and half-life extension, however, has made these weaknesses of PDCs manageable. Tailoring of the pharmacokinetics of PDCs to allow sufficient time for the therapeutic effect to take place while avoiding overexposure of nonmalignant tissues with the drug cargo (toxic agent or radionuclide) is thus of major importance. Another major challenge for clinical development of receptor-targeting PDCs is to achieve the desired selective therapeutic efficacy in the tumor tissue. Absolute selectivity of PDCs is naturally an impossible goal, as most target receptors that are overexpressed on cancer cells are also found in other non-neoplastic tissues. Nonetheless, PDCs can offer a significant improvement of the therapeutic index, which, for example, permits increased dosing to clear metastases. For optimal therapeutic efficacy, PDCs are required to strongly accumulate in the tumor cells, especially when toxic agents and boron compounds for BNCT are used as drug cargos. Peptide carriers with high potency for their target receptor have to be used for PDC design, so that a dosing regimen is possible that allows for maximum internalization of the PDC into receptor-overexpressing tumor cells but prevents toxicity and side effects in nonmalignant tissues. In case of chemotherapeutic agents as drug cargo, the development and use of toxophores with very high potency for their intracellular target are desired to further improve the therapeutic index of such PDCs. Increasing the drug payload of PDCs by chemically functionalizing one peptide carrier with multiple drug molecules is another potential solution to enhance efficacy of PDCs *in vivo*. A last important challenge related to the internalization of PDCs is the intracellular fate of the drug cargo. Current knowledge about the endosomal escape and intracellular sublocalization of internalized PDCs/drug cargos is sparse, but for many therapeutically active moieties optimization of subcellular delivery would lead to a higher therapeutic efficacy (e.g., Auger electron-emitting radionuclides). Furthermore, the extent to which the drug cargo is metabolized inside the cancer cells is often unclear and a strong efflux of the drug cargo bears the risk of exposure to untargeted tissues. New insights into all these aspects of intracellular biochemistry will allow further optimization of PDCs.

Despite the mentioned challenges, the combination of modern chemical and biological expertise has led to the progression of multiple PDCs for established target receptors into clinical studies. Finally, since many receptors such as the ghrelin and Y_1 receptor are still waiting to be explored more extensively as targets in cancer, receptor-targeting peptide-drug conjugates bear the potential to give new impulses to cancer therapies in the future.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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