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Receptor-mediated tumor targeting based on peptide hormones

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Importance of the field: Tumor targeting with peptides is based on the discovery that receptors for many regulatory peptides are overexpressed in tumor cells, compared with their expression in normal tissues. Consequently, these peptides and their analogues can be used as carriers/targeting moieties for the preparation of diagnostic and therapeutic agents that have increased selectivity and decreased peripheral toxicity.
Areas covered in this review: Here an overview is given of the most relevant gonadotropin-releasing hormone (GnRH) and somatostatin derivatives, as well as of their applications in cancer diagnosis and therapy. For this purpose, recently published data in these areas (mostly articles published from 2000 to 2009) were reviewed.
What the reader will gain: In contrast to other regulatory peptides that stimulate the tumor growth, GnRH and somatostatin derivatives have inhibitory effect; therefore, they were used primarily for the preparation of various conjugates to be used in targeted chemotherapy, targeted radiotherapy, photodynamic therapy, boron neutron capture therapy and cancer diagnosis. Some of these conjugates have already found clinical applications, whereas others are now in preclinical and clinical trials.
Take home message: Tumor targeting with hormone peptides provides a basis for the development of new diagnostic and therapeutic approaches for cancer.

Keywords: cancer diagnosis and therapy, drug delivery systems, gonadotropin-releasing hormone, receptor-mediated tumor targeting, somatostatin

1. Introduction

Cancer is a leading cause of death and a major public health problem worldwide. According to the statistics of the World Health Organization, in 2007 cancer accounted for 7.9 million deaths (~ 13% of all deaths) all over the world. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030 [1] if no breakthrough in the therapeutic approaches occurs.

Since the middle of the last century, chemotherapy has been the main procedure for the treatment of advanced or metastatic cancer [2]. However, the application of free anticancer drugs has several drawbacks, such as the lack of selectivity, toxicity to normal cells, fast elimination from the blood circulation, and the acquired or intrinsic multi-drug resistance of cancer cells [3]. In the last two decades, cancer research has turned to a more selective, targeted approach, focused on the development of anticancer therapies with improved efficacy and reduced peripheral toxicity [4]. One of the most promising therapeutic strategies is based on the peptide receptors that are overexpressed in tumor cells, in comparision with their expression in normal tissues [3,5]. Many regulatory peptides (e.g., somatostatin,

Article highlights.

- Specific tumor targeting with peptides is based on the discovery that receptors for many regulatory peptides are overexpressed in tumor cells, compared with their expression in normal tissues.
- Gonadotropin-releasing hormone (GnRH) receptors are important molecular targets for cancer diagnosis and therapy.
- Cytotoxic GnRH derivatives are developed as drug delivery systems for targeted cancer chemotherapy.
- The application of radiolabeled GnRH derivatives is limited.
- High-affinity somatostatin receptors are expressed on the plasma membrane of tumor cells.
- Cytotoxic somatostatin derivatives are available for targeted cancer chemotherapy.
- Somatostatin-based boron neutron capture therapy is still in the *in vitro* testing phase.
- Radiolabeled somatostatin derivatives are used in targeted radiotherapy and tumor imaging.
- Tumor targeting with hormone peptides provides a basis for the development of new diagnostic and therapeutic approaches for cancer.
- GnRH and somatostatin analogues are promising tools to target radionuclides and antineoplastic agents to tumor cells.

This box summarises key points contained in the article.

gonadotropin-releasing hormone (GnRH), bombesin, gastrin-releasing peptide (GRP), neurotensin, substance P, vasoactive intenstinal peptide (VIP) and cholecystokinin (CCK)) have membrane-bound receptors on different types of tumor [5]. The receptors recognized by these hormone/neurotransmitter peptides are G-protein-coupled receptors; therefore, they can internalize into the cells after binding of their ligands. Consequently, a cytotoxic agent attached to the peptide can enter the tumor cell by a receptor-mediated way (**Figure 1**). The advantages of small peptides as carriers are their excellent tissue permeability (except crossing the blood–brain barrier), the lack of immunogenicity, high affinity to the receptors, minimal side effects, rapid clearance from the body, easy synthesis and chemical modifications as well as detailed and precise characterization. The only disadvantage is their rapid proteolytic degradation, which can be partially prevented by insertion of D-amino acids, reduced peptide bonds, *N*-methyl- or non-native amino acids, and so on. However, it has to be taken into account that regulatory peptides, which control and modulate the function of almost all key organs and metabolic processes, may also play prominent roles in cancer progression. Thus, peptide derivatives (agonists or antagonists) selected for drug delivery should have an inhibitory but not stimulatory effect on tumor growth [5,6]. Somatostatin and GnRH analogues have an inhibitory effect on tumor growth, whereas bombesin, GRP, VIP, CCK, and so on, stimulate it. Considering these aspects, the application of antagonistic derivaties as carriers in the case of the latest ones is suggested by several authors [5,6]. However, others prefer agonists (e.g., in the case of bombesin) as drug targeting moieties owing to the observation that agonists induce rapid receptor–ligand internalization by tumor cells, which might be important in the case of targeted cancer chemotherapy [7,8].

Tumor targeting with peptides has found both diagnostic and therapeutic applications. In the case of cancer diagnosis and radiotherapy, a radioligand is usually attached to the regulatory peptide carrier by the aid of a chelator. A wide variety of chelating agents (e.g., diethylenetriaminepentaacetic acid [DTPA] and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid [DOTA]) have been developed for convenient radiolabeling of peptides. Radionuclides such as ^{99m}Tc, ^{111}In, ^{68}Ga, ^{123}I, ^{64}Cu and ^{18}F can be used for diagnosis and ^{90}Y, ^{177}Lu and ^{111}In for therapeutic purposes. Radionuclides used in cancer radiotherapy emit high energy β-rays or Auger electrons and have longer half-life (> 60 h) in comparision with the γ-ray or positron emitter radionuclides used as diagnostic tools [5,9].

Another therapeutic application of peptide-based tumor targeting is targeted cancer chemotherapy. In this case, cytotoxic drugs are attached to hormone/neurotransmitter peptides with the aim of increasing their selectivity and decreasing the peripheral toxicity (**Figure 1**).

The main difference between the conjugates containing radionuclides or drugs is that the antineoplastic agent should be released from the conjugate in order to obtain an efficient tumor growth inhibition, whereas in the case of radiopharmaceuticals this process is not necessary. Therefore, the insertion of pH-sensitive or enzymatic cleavable linkers between the drug and the peptide might be required for the antitumor activity of the cytotoxic conjugates [10,11].

The selection of the peptide to be used as a targeting moiety is based on the type of cancer to be treated. An important aspect of targeted cancer chemotherapy is that the receptor incidence and density must be different on tumoral tissues compared with normal ones. Therefore, VIP peptides seem not to be efficient for drug delivery compared with the others [5].

Here an overview is given of GnRH and somatostatin analogues that are widely used as carriers for radionuclides and antineoplastic agents.

2. GnRH receptors as molecular targets for cancer diagnosis and therapy

Type I and type II GnRH receptors (GnRH-IR and GnRH-IIR) have been identified among vertebrates, including mammals. GnRH-IR protein consists of 329 amino acids and it has no C-terminal cytoplasmic tail [12]. Therefore, GnRH-IRs do not desensitize rapidly and they internalize slowly [13]. The full-length GnRH-IIR have been found in marmoset and rhesus monkeys but not in humans [14,15]. The

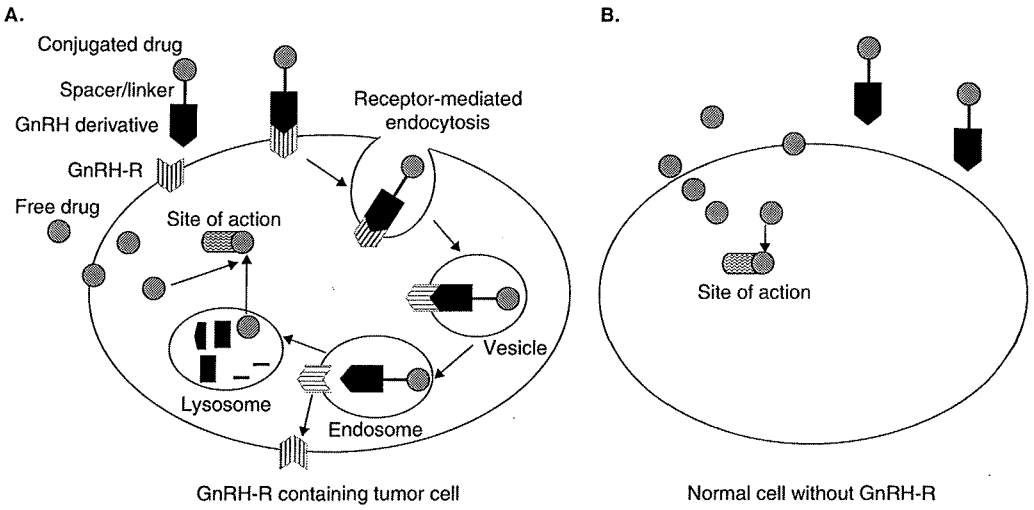


Figure 1. Cellular uptake of a free and conjugated drug into GnRH-receptor containing tumor cells (A) and into normal cells without GnRH receptors (B).

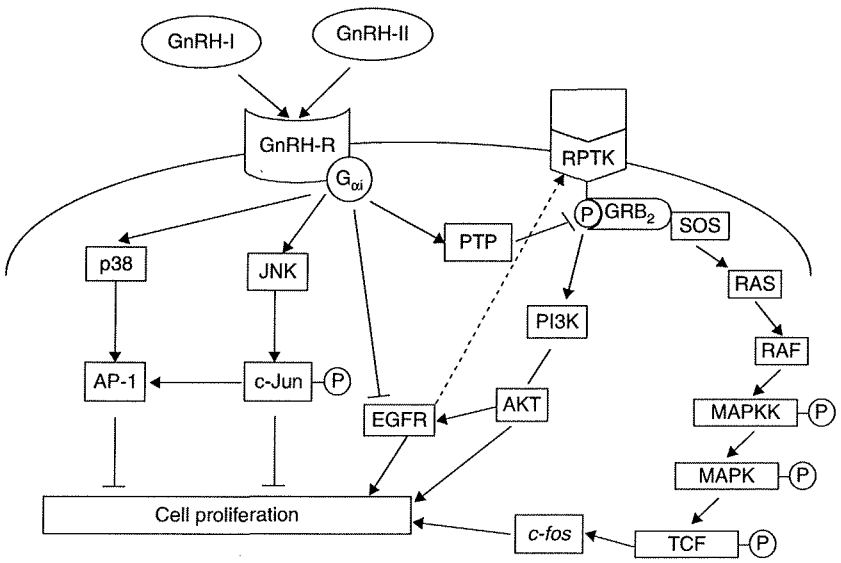


Figure 2. Schematic representation of signaling pathways of GnRH-I,II in cancer cells.

AP-1: Activator protein-1; EGFR: Epidermal growth factor receptor; $G_{\alpha i}$: G-protein αi; GRB2: Growth factor receptor bound protein; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MAPKK: MAPK kinase; PI3K: Phosphoinositide 3 kinase; PTP: Phosphotyrosine phosphatase; RPTK: Receptor protein tyrosine kinase; SOS: Son of Sevenless; TCP: Transcription factor.

GnRH-IIR has a cytoplasmic tail and undergoes rapid arrestin-dependent internalization and desensitization [16]. The mRNA expression of this full-length GnRH-IIR has also been shown by real-time polymerase chain reaction (RT-PCR) in human cancer cell lines [17]. However, it became evident that GnRH-IIRs have a frameshift and a premature stop codon and thus they are not functional. Therefore, it is believed that the GnRH-II signaling might also occur through the GnRH-IR (Figure 2) [18]. By contrast, after knockout of GnRH-IR expression in endometrial and ovarian cancer cells, the antiproliferative effect of GnRH agonist Triptorelin ([D-Trp^6]-GnRH) was abrogated, whereas the tumor growth-inhibitory effect of GnRH-II persisted [19]. Recent data of extensive research have shown that functional GnRH-IIR might occur on human cancer cells [20]. However, an intensive study of the role of GnRH-IIRs on tumor cells

Table 1. *In vivo* and *in vitro* antitumor effect of AN-152 and AN-207 conjugates.

Tumor type	Receptor expression in specimens	Cell types that express GnRH-R	AN-152	AN-207
Breast	> 50	MCF-7	AN-152 > Dox*	AN-207 >> Dox
		MDA-MB-231	n.d.	XXXX‡
		MDA-MB-435	n.d.	XXX
		MX-1	XXX	XXXXX
		T47-D	AN-152 > Dox	n.d.
		HCC-70	AN-152 > Dox	n.d.
		ZR-75-1	AN-152 < Dox	n.d.
		MXT (mouse)	XXXX	XXXX
Ovarian	> 70	OV-1063 OVCAR-3	XXXX	XXX
		ES-2	XXXXX	n.d.
		EFO-21	XX	XXX
		EFO-27	AN-152 > Dox	n.d.
			AN-152 < Dox	n.d.
Endometrial	> 75	Ishikawa	AN-152 > Dox	n.d.
		HEC-1A	XXX	XXX
		HEC-1B	XXXXX	n.d.
		RC-95-2	n.d.	XXX
Prostate	> 85	LNCaP	XXXX	n.d.
		MDA-PCa-26	XXX	n.d.
		MDA-PCa-2b	XXX	XXX
		DU-145	n.d.	XXXX
		C4-2	XXX (PSA inh.)§	n.d.
		PC-82	n.d.	XXX
		LuCaP-35	n.d.	XXXX
		R-3327-H (rat)	XXX	XXX
		R-3327-AT-1 (rat)	XX	n.d.
Colon	n.d.	HT-29	XX	XXX
		HCT-116	X	XXX
		HCT-15	XXXX	XXXX
		LoVo	XXX	XXX
		Colo-320DM	XXXX	XX
Renal	80	A-498	n.d.	XXXX
		ACHN	n.d.	XXXX
		786-0	n.d.	XXX
Melanoma	100	MRI-H255	n.d.	XXXX
		MRI-H187	n.d.	XXX
Lung	n.d.	NCI-H720	AN-152 > Dox	n.d.
Non-Hodgkin's lymphoma	100	RL	n.d.	XXXX
		HT	n.d.	XXXX
Hepatocellular	n.d.	SK-Hep-1	n.d.	XXXX
Oral	n.d.	KB	AN-152 > Dox	n.d.
Laryngeal	n.d.	HEp-2	AN-152 > Dox	n.d.

*AN-152 > Dox represents the comparison of the *in vitro* antitumor effect of free drug and its GnRH derivative conjugate.
‡*In vivo* studies: XXXXX: regression; XXXX: > 70% inhibition; XXX: 50 – 70% inhibition; XX: 25 – 50% inhibition; n.d.: not determined.
§PSA inh.: not the tumor volume, but PSA level was determined as a measure of the inhibitory effect.
The effects are not absolutely comparable because of different doses and treatment schedules used.

and of the mechanism of action of GnRH-II should be carried out. In addition to GnRH-IR, GnRH-IIR might be a good target for tumor therapy.

GnRH-R expression was identified on different tumors (breast, ovarian, endometrial, prostate, renal, brain, pancreatic, melanomas, non-Hodgkin's lymphomas, etc.) (Table 1) [21-23]. The limited number of GnRH-Rs in normal tissues provided a basis for the development of diagnostic and therapeutic approaches of cancer (Figure 1).

3. Cytotoxic GnRH derivatives

Cytotoxic GnRH derivatives were initially developed by Schally's group after the mid-1980s. The first series of

conjugates contained agonistic (e.g., [D-Lys^6]-GnRH; Glp-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2) or antagonistic (e.g., [Ac-D-Nal(2)^1,D-Phe(pCl)^2,D-Pal^3,Arg^5,D-Lys^6, D-Ala^{10}]-GnRH) GnRH derivatives as carriers and cytotoxic moieties such as alkylating agents cisplatin and melphalane, antimetabolite methotrexate, DNA intercalating agents doxorubicin (Dox) and (2-hydroxymethyl)anthraquinone [21,24,25]. Based on the results of *in vitro* and *in vivo* studies using these compounds, more efficient conjugates were developed in which Dox or its analogue (2-pyrrolino-Dox; AN-201, which is 500 – 1000 times more potent than Dox) were attached to [D-Lys^6]-GnRH [26]. In these compounds AN-152 (1) (**Figure 3A**) and AN-207 (2) (**Figure 3B**), the cytotoxic agents connected to glutaric acid by means of an ester bond (Dox-14-*O*-hemiglutarate), were conjugated to the ε-amino group of D-Lys^6 of GnRH. The ester bond could be cleaved by carboxylesterases (CE), leading to the release of the free cytotoxic radicals. Cellular uptake studies showed that the conjugates were internalized in a receptor-mediated way by GnRH-R-positive cells (**Figure 1A**) but not by GnRH-R-negative cells (**Figure 1B**). The receptor-mediated internalization was also confimed by blocking the receptors with the superagonist Triptorelin [27].

The antitumor action of AN-152 and AN-207 was studied intensively on numeouros tumor types (**Table 1**). Their efficiency was tumor type-dependent, but in almost all cases AN-207 had higher antitumor activity. Significant *in vivo* tumor growth inhibition and the regression of several tumor types (e.g., ovarian, endometrial, breast and prostate cancers) were observed. These results have been reviewed extensively [3,21,28,29]. In the last few years, the strong inhibitory activity of the conjugates has been shown on more tumor types, such as renal, non-Hodgkin's lymphomas, melanomas, colorectal and hepatocellular carcinoma cell lines that expressed GnRH-Rs [22,23,30-32]. In most cases, the intravenous-administered conjugates were far less toxic and inhibited the growth of GnRH receptor-positive tumors better than the equimolar doses of free doxorubicin. Furthermore, AN-152 induces apoptosis in GnRH receptor-positive human ovarian and endometrial cancer cell lines without activating the MDR-1 (multi-drug resistance-1) efflux pump system [33].

Although the tumor growth inhibition effect of AN-207 was higher, AN-152 was the first cytotoxic GnRH derivative investigated in preclinical and clinical studies. In the Phase I clinical trial for ovarian and endometrial cancers (Æterna Zentaris, AEZS-108), AN-152 was well tolerated using escalated doses of 160 and 267 mg/m^2. Even at the highest dose, the hematological toxicity of AN-152 was low and the other non-hematological side effects were mild [34]. The Phase II clinical trial started in January 2008.

It is worth mentioning that the side effects of the conjugates were mainly related to the easy drug release by CE. In an early study, it was demonstrated that the half-life of AN-152 in mouse serum was ~ 20 min, whereas in human serum it lasted a longer time, with a half-life of 2 h [35]. This difference could be explained by the CE levels in mouse and human sera (the CE level in mouse serum is ~ 10 times higher than in human serum). Therefore, to reduce the toxicity of the conjugate in many *in vivo* experiments, diisopropyl-fluorophosphate was used as a CE inhibitor. In this way the half-life of AN-152 in mouse serum was enhanced to ~ 70 min and the maximal tolerated dose (MTD) was increased from 200 to 2 × 400 nM. The hydrolyzed Dox and especially 2-pyrrolino-Dox had a non-receptor specific cytotoxic effect on rapidly proliferating cells, the main toxic side effect being the myelotoxicity. Another side effect might originate from the presence of GnRH-Rs on normal reproductive cells and gonadotroph cells. The temporary damage of gonadotrophs using AN-207 was recovered ~ 1 week after cessation of the treatment [36]. In the case of hormone-dependent tumors patients are usually hypophysectomized, thus this side effect of the cytotoxic GnRH derivatives is not harmful [37].

A natural isoform of GnRH, GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2), which is a weak GnRH agonist, has recently been used as a carrier in the authors' laboratories. This hormone peptide, isolated from sea lamprey (*Petromyzon marinus*) [38], specifically binds to the GnRH-Rs on cancer cells [39]. GnRH-III has antiproliferative activity on several tumor cell lines and 500 – 1000 times less potency in LH and FSH secretion both *in vitro* and *in vivo*, indicating its selective antitumor activity [40]. The modification of the side chain of Lys^8 did not result in the loss of the antiproliferative activity of GnRH-III [41]. Furthermore, the absence of the free ε-amino group of lysine decreased the endocrine effect of the compounds [42]. Therefore, Lys^8 might be used as a conjugation site for the attachment of cytotoxic drugs. Daunorubicin (Dau), an anthracycline derivative that differs from doxorubicin in the substitution at the C-14-position (H instead of OH) and has lower cardiotoxicity than Dox, was conjugated to GnRH-III via oxime (3) (**Figure 3C**) or hydrazone (4) bonds (**Figure 3D**). As the oxime bond is relatively stable under physiological conditions, a GFLG tetrapeptide spacer known to be cleaved by lysosomal enzymes, especially by Cathepsin B which is overexpressed in tumor cells [41,43,44], was inserted between the drug and the hormone peptide. Ester bond-containing Dox-GnRH-III conjugates were also prepared. *In vitro* cytostatic effect of the conjugates was determined by MTT assay on MCF-7 human breast cancer and HT-29 human and C26 murine colon carcinoma cell lines [25,41]. The results were compared with the IC_{50} values of the free drugs and AN-152. All compounds were more potent on breast cancer cell lines; however, there were no significant differences between their antitumor effects on colon cancer cells. The ester bond-containing conjugates had similar antitumor activity as the free drugs and one order of magnitude higher activity than that of the conjugates containing hydrazone or oxime bonds.

The *in vivo* toxicity study on BDF-1 mice indicated high tolerability of oxime bond-linked GnRH-III(Dau=Aoa-GFLG) conjugate. This conjugate had an MTD > 54 μmol

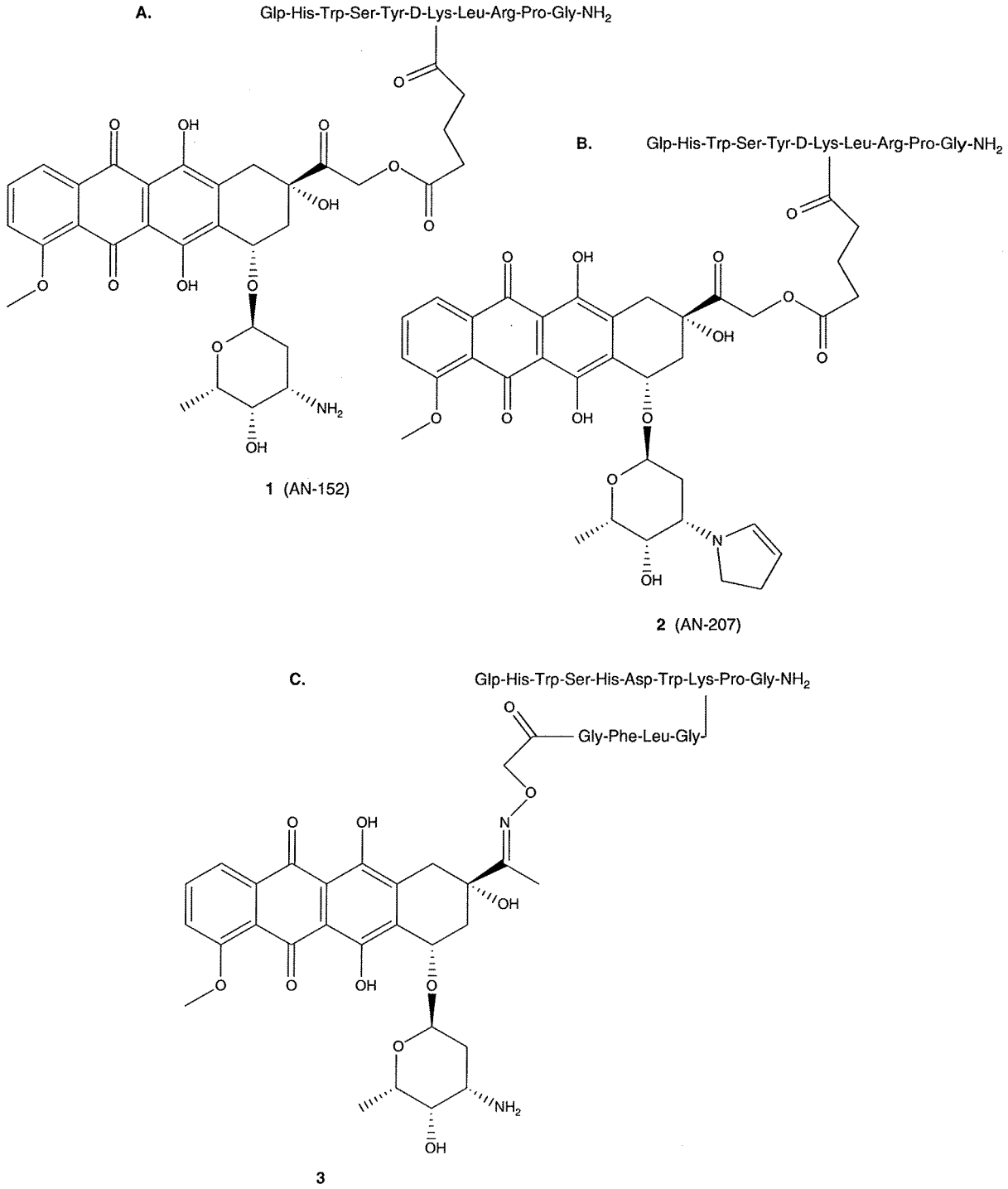
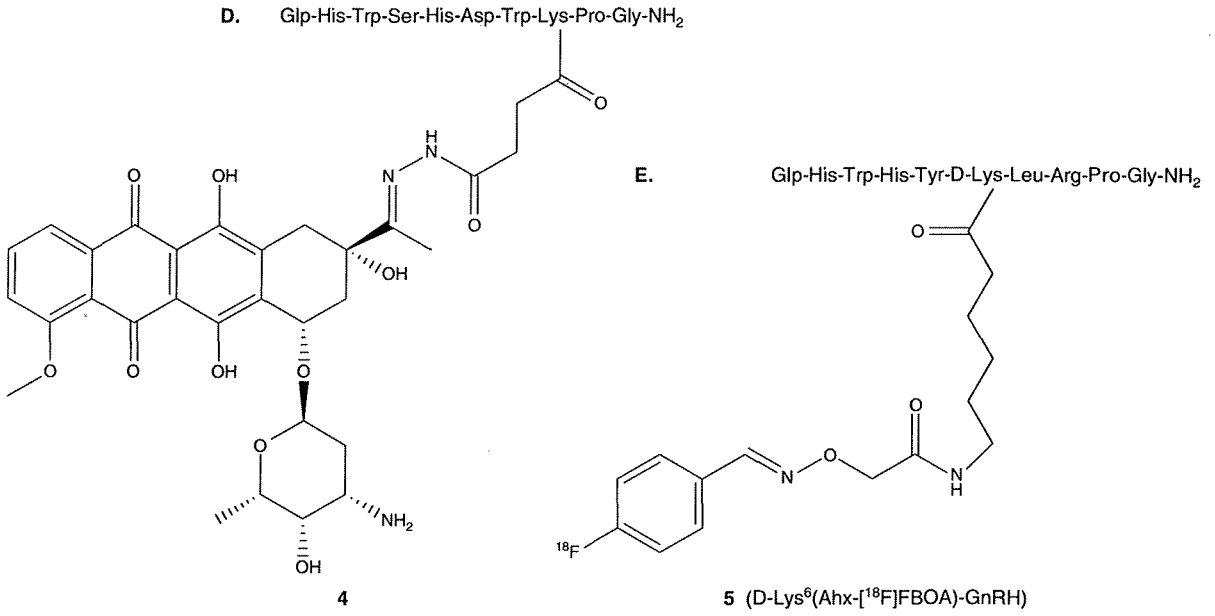


Figure 3. Chemical structures of cytotoxic GnRH derivatives: ester bond-linked doxorubicin-[D-Lys^6]-Gn RH (A) and 2-pyrrolino-doxorubicin-[D-Lys^6]-GnRH (B), oxime bond containing daunorubicin-GnRH-III (C), hydrazone bond-linked daunorubicin-GnRH-III (D) and of a radiolabeled GnRH derivative, D-Lys^6(Ahx-[^{18}F]FBOA)-GnRH (E).

Ahx: ε-Aminohexanoic acid; FBOA: *p*-Fluorobenzyloxime acetyl.



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Ahx: ε-Aminohexanoic acid; FBOA: *p*-Fluorobenzyloxime acetyl.

Dau-content in the conjugate per kilogram using intraperitoneal administration. The *in vivo* antitumor activity of the conjugate was evaluated on C26 colon cancer-bearing mice and the tumor growth inhibition was 20 – 46% depending on the dose and on the time schedule. Similar results were obtained after the administration of the free Dau. However, the survival of the animals treated with the conjugate increased by 15 – 40% compared to the controls, whereas the animals treated with the free drugs died earlier [41].

Rahimipour *et al.* applied [D-Lys^6]-GnRH and [D-Glp^1,D-Phe^2,D-Trp^3,D-Lys^6]-GnRH (an antagonistic peptide) to increase the selectivity of protoporphyrin IX (PpIX) as a photosensitizer for photodinamic therapy [45]. In both cases, PpIX was conjugated to D-Lys^6 by means of amide bonds. The conjugates bound to the GnRH-Rs with lower affinity than the parent peptides, but their selective and efficient phototoxicity compared with the unconjugated PpIX could be demonstrated.

Drug delivery systems based on GnRH targeting moieties were developed by Dharap *et al.* Camptothecin (CPT) was connected to Glp-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-EA (EA: ethylamide) through a polyethylene glycol (PEG) chain. CPT was attached to the cysteine by means of an ester bond and then Cys-CPT was conjugated to NHS-PEG-VS (NHS: *N*-hydroxysuccinimide; VS: vinylsulfone) by a thioether bond. After that, the GnRH derivative was attached to the PEG conjugate by means of an amide bond. PEG increased the solubility and bioavailibility of the conjugate. The GnRH-PEG-CPT conjugate showed higher cytotoxicity and apoptosis induction than the free CPT or PEG-CPT [46]. These results indicated the efficiency of receptor-mediated endocytosis for selective drug targeting over the simple diffusion or direct endocytosis [47]. Moreover, *in vivo* experiments did not show any disturbance of the reproductive functions of female mice. GnRH-PEG-CPT conjugate accumulated in tumor cells but, similarly to the free CPT and PEG-CPT, caused overexpression of *BCL-2* and *BCL-XL* genes and thereby increased the cellular antiapoptotic defense. To overcome this drawback, a multifunctional targeted proapoptotic drug delivery system was designed. This new conjugate contained a BH3 antiapoptotic peptide (MGQVGRQLAIIGDDINRRY) beside the GnRH derivative, CPT and PEG. The conjugation of the three components to PEG was performed by inserting a multifunctional citric acid spacer that allowed simultaneous binding of several copies of different active components to one polymeric carrier [48,49]. It is important to note that BH3 peptide could not enter the cells without any targeting moiety [50]. The conjugate containing all four components induced the greatest supression of the antiapoptotic gene expression and the highest cytotoxic and apoptotic effects in A2780 human ovarian carcinoma cell line as compared with the conjugates CPT-PEG-BH3 and GnRH-PEG-CPT or their physical mixture. The conjugate also showed antitumor activity on A2780/AD multi-drug resistant human ovarian carcinoma cell line, but the effect was lower than in case of non-resistant cell lines.

4. Radiolabeled GnRH derivatives

Except radioiodinated compounds, only a few data have been published so far about the development of other radiolabeled GnRH derivatives. Barda *et al.* prepared a ^{99m}Tc-labeled GnRH derivative as a potential single photon emission computed tomography (SPECT) tracer [51]. However, the insertion of the radiometal via backbone cyclization of the peptide led to a dramatic loss of the binding affinity. Schottelius *et al.* investigated ^{68}Ga- and ^{18}F-labeled GnRH analogues [52]. Ga was incorporated by the aid of DOTA, while for the insertion of F, *p*-fluorobenzyloxime acetyl (FBOA) moiety was applied. DOTA was attached directly to the ε-amino group of D-Lys in [D-Lys^6]-GnRH, whereas in case of the fluor-containing derivative, either β-alanine or ε-aminohexanoic acid (Ahx) were inserted between the peptide and the radioligand. In the *in vitro* binding experiments, radioiodinated Triptorelin (^{125}I-[D-Trp^6]-GnRH) was also used. The compound radiolabeled with ^{68}Ga had very low binding capacity, whereas the binding of fluorinated derivatives was four to six times lower than the radiolabeled Triptorelin. Compared with Triptorelin, the internalization of D-Lys^6(Ahx-[^{18}F]FBOA)-GnRH (5) (**Figure 3E**) into EFO-27 ovarian carcinoma cells was 86 ± 16%, whereas the β-Ala-derivative showed only 42 ± 3%. There was no significant internalization observed in case of the Ga-labeled GnRH derivative. These data suggested that the lipophilicity of the compound that modified the lysine side chain and the distance between the peptide backbone and the radiolabeled moiety had significant influence on the binding capacity of the conjugate to the receptor and on its internalization properties. However, the biodistribution of the conjugates in OVCAR-3 ovarian tumor-bearing mice indicated no significant tumor localization (~ 0.5%) of radiolabeled compounds. The results showed a very low level of GnRH-R expression on tumor cell surfaces, suggesting that the GnRH-R system is not a suitable target for peptide-receptor imaging using radiolabeled GnRH analogues. This is in contrast to the effective tumor growth inhibition by cytotoxic GnRH derivatives. The contradiction might be explained by the observations that GnRH-R protein is inefficiently processed by the cells under physiological conditions and it is retained at the site of production [53]. A proportion of intracellular receptors might traffic to the cell surface during stimulation, thus providing a rationale for the successful application of GnRH analogues for suppressing the tumor growth [54].

Considering these data, one can conclude that GnRH-Rs represent a highly interesting target for pharmacological interventions; however, many open questions have to be answered in the future.

5. Somatostatin receptors as molecular targets for cancer diagnosis and therapy

It has been shown that somatostatin and its analogues can inhibit tumor growth. Their action is mediated by specific, high-affinity somatostatin receptors located on the plasma membrane of the tumor cells. Up to now, five human somatostatin receptor subtypes (sst_1 – sst_5) have been cloned and partially characterized [5,9]. The most frequent receptor subtypes on tumor cells are sst_2 and sst_5. On ligand binding, sst_3 and sst_2 internalize more efficiently than sst_1 or sst_5. Somatostatin receptors were detected in a low amount in human brain and in many peripheral tissues such as pancreas, gut, thyroid, adrenal and kidney. Therefore, during the application of somatostatin analogues for targeted therapy, the unwanted side effects should be carefully investigated. As both somatostatin-14 and somatostatine-28 bound with high affinity to all receptor subtypes, more selective derivatives to sst_2 and sst_5 were developed for tumor therapy, one of the most efficient compounds being octreotide (OCT, Sandostatin®, Sandoz), a cyclic octapeptide alcohol (D-Phe-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-ol) [55].

6. Cytotoxic somatostatin derivatives

In the mid-1980s, similarly to GnRH derivatives, intensive studies of new somatostatin analogues were carried out by Schally's group. An analogue of octreotide (H-D-Phe-c(Cys-Tyr-D-Trp-Lys-Val-Cys)-Thr-NH_2, RC-121) [28,56] was found to be efficient, especially for drug targeting. An initial study of a conjugate in which methotrexate was linked to the N terminus of RC-121 indicated the tolerance of the carrier for the amino terminal modifications. Later on, Dox and pyrrolino-Dox (AN-201) were conjugated to RC-121, resulting in AN-162 (6) (**Figure 4A**) and AN-238 (7) (**Figure 4B**) derivatives [57]. The conjugates that fully retained the cytotoxicity of the drugs and the receptor binding affinity of the peptide carrier showed strong growth inhibition of various tumors (**Table 2**) that express sst_2, sst_3 and sst_5 (breast, renal, ovarian, lung, colorectal, pancreatic tumors) [3,6,58,59]. AN-238, similarly to the AN-207 GnRH analogue, was very effective intravenously at ~ 200 nmol/kg doses corresponding to 0.4 – 0.45 mg/kg. The estimated human doses would be ~ 22.5 mg/50 kg. In mice, AN-238 showed no or low toxicity up to a dose of 400 nmol/kg. The toxic side effects might be related to the release of AN-201 resulting from the cleavage of the ester bond by carboxylesterases. The toxicity occurred mostly on the fast dividing cells, such as gastrointestinal or myeloid cells, resulting in a transient fall in the white blood cell (WBC) count and body weight. As the esterase activity is much lower in humans than in mice, the hematotoxicity of AN-238 is expected to be reduced in patients, as demonstrated in case of the GnRH derivative. In addition, no significant nephrotoxicity was observed under the treatment with AN-238.

In the past few years, it has been shown that AN-238 is efficient on human endometrial carcinomas that have not responded well to chemotherapy [60]. Chemoresistance mediated by membrane transporters, such as multi-drug resistance (MDR-I) glycoprotein, can be avoided by targeted therapy. AN-238 not only induced tumor growth inhibition

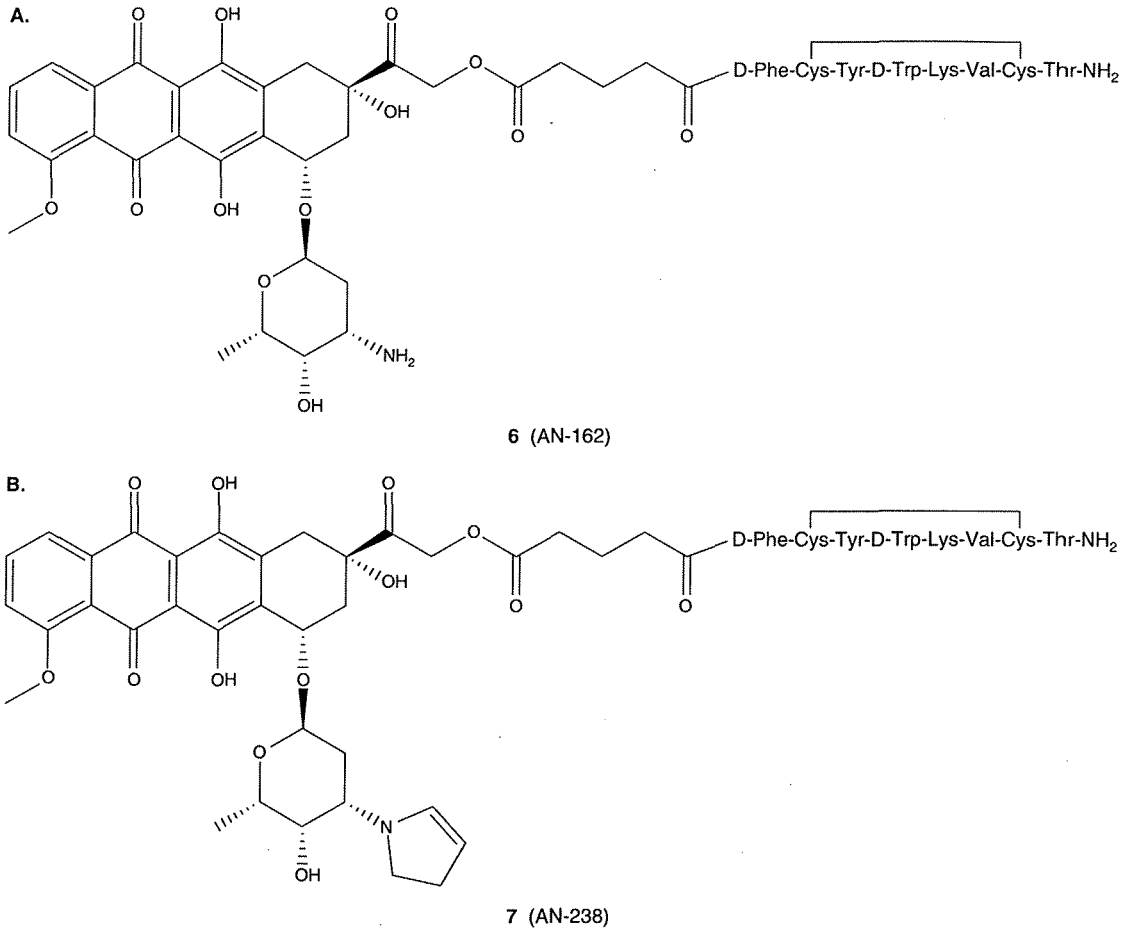

Figure 4. Chemical structures of cytotoxic somatostatin derivatives in which doxorubicin (A) and 2-pyrrolino-doxorubicin (B) were attached via ester bond to an octreotide analogue; ester bond-linked paclitaxel-octreotide (C) and carbamate containing camptothecin-somatostatin derivative conjugate (D).

(50 – 65%) of endometrial carcinomas, but also the expression of MDR-I protein during the treatment was much lower than in case of the treatment with AN-201.

Lung cancer is one of the tumor types that can be treated better by targeted therapy based on somatostatin derivative carriers than by using cytotoxic GnRH derivatives. About 80% of all lung cancers are classified as non-small cell lung cancer (NSCLC). Therefore, the presence of somatostatin receptors in NSCLCs to a greater extent than in normal cells makes them good targets for cytotoxic somatostatin derivatives [61,62]. AN-238 was found to be effective on H69 (SCLC) and H157, H838 (NSCLCs) [63,64]. However, large-scale synthesis of an AN-238 is still in progress. Therefore, AN-162 (Æterna Zentaris, AEZS-124) was used for preclinical studies, and it has recently been investigated for the inhibition of NSCLCs [65]. All studied NSCLCs (A549, H460, H838 and H1299) expressed sst_1, sst_2 and sst_4. Strong expression of mRNA for sst_5 was found in A549 and H460 and it was marginal in H838, whereas sst_3 was present in H460 and H1299. A549, H460 and H1299 are resistant to doxorubicin, thus AN-162 was more effective than the free drug on proliferation inhibition *in vitro*. The best results (~ 17% cell viability at 50 μM) were obtained in the case of H460 cells that express all the receptor subtypes. A less significant difference between Dox and AN-162 was observed in the case of H1299 cells, which do not express sst_5. In Dox-sensitive H838 cells, both Dox and AN-162 showed significant proliferation inhibition at much lower concentrations. *In vivo* studies showed ~ 70% tumor growth inhibition on H460 xenografted nude mice, and ~ 55% on H1299 xenografted ones. Upregulation of apoptotic genes by the treatment with AN-162 was also indicated. Similar results were obtained on tumor growth inhibition of the MDA-MB-231 estrogen-independent human breast cancer cell line xenografted into nude mice [66].

The treatment of hepatic cancer cells with a combination of two or three cytotoxic conjugates (AN-207 and AN-238 with

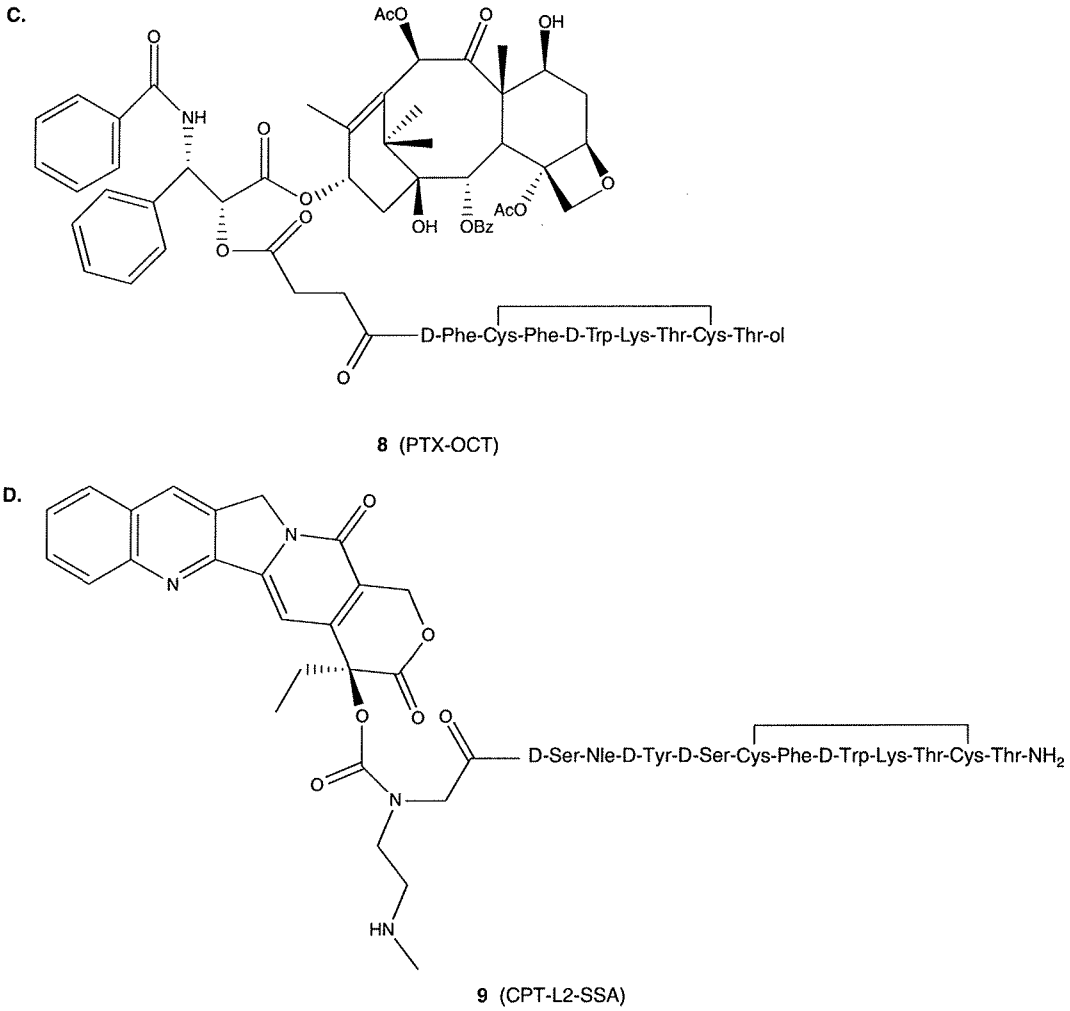

Figure 4. Chemical structures of cytotoxic somatostatin derivatives in which doxorubicin (A) and 2-pyrrolino-doxorubicin (B) were attached via ester bond to an octreotide analogue; ester bond-linked paclitaxel-octreotide (C) and carbamate containing camptothecin-somatostatin derivative conjugate (D). (Continued)

an extra bombesin-Dox conjugate; AN-215) resulted in higher *in vitro* tumor growth inhibition in comparision with their separate administration [32].

It has been shown that Paclitaxel (PTX) has high antitumor activity owing to its ability to promote tubulin assembly into microtubules, but it has a low cell specificity. Therefore, it was modified by succinic acid on its 2′-OH functional group and attached by means of an amide bond to the N terminus of octreotide [67]. This linkage allows the drug release by carboxylesterases, regenerating the free 2′-OH, which appears essential for tubulin binding [68]. MCF-7 human breast cancer cell lines expressing sst_1, sst_2, sst_4 and sst_5 but not sst_3, as well as CHO (Chinese hamster ovary) cells that have a very low level of somatostatin receptor (196 fmol/mg) were used to study the biological activity of the PTX-OCT (8) conjugate (Figure 4C). In the presence of PTX or PTX-OCT (10^{-6}, 10^{-5} and 10^{-4} M), the percentage of MCF-7 cells containing tubulin bundles was similar (67, 83 and 92% or 65, 81 and 93%, respectively). However, on CHO cells, only PTX was significantly active. A large amount of octreotide could antagonize the cellular uptake of the conjugate but not of PTX, suggesting the difference between the cellular uptake mechanisms of the compounds (receptor-mediated versus passive diffusion). The viability of the MCF-7 cells incubated for 24 h with the conjugate or with the free PTX was similar (46 ± 2.7 and 41 ± 3.3%, respectively). These results indicate that PTX-OCT conjugate is highly selective to the cells containing somatostatin receptors in comparision with the cells that express low levels of the receptors.

Recently, two PTX molecules were attached to the Lys-modified octreotide (2PTX-OCT) and the conjugate was tested on human NSCLC tumors [69,70]. Higher *in vitro*

Table 2. *In vivo* and *in vitro* antitumor effect of AN-238 and other cytotoxic somatostatin derivative-conjugates.

Tumor type	Receptor expression in specimens	Cell types that express SSTR	AN-238	Others
Breast	sst2, sst5	MX-1	XXXXX*	
	sst2, sst5	MDA-MB-231	XXX	XXXX (AN-162)
	sst2, sst5	MCF-7-MIII	XXX	PTX-OCT ~ PTX
	sst2a, sst4, sst1, sst5	MCF-7	n.d.	
Ovarian	sst2a, sst3, sst5	UCI-107	XXX	
		OVCAR-3	XXX	
		ES-2	XX	
Endometrial	sst2a, sst5	HEC-1A	XXX	
	sst2a, sst5	RL-95-2	XXX	
	sst2a, sst5	AN3CA	XXX	
Prostate		MDA-PCa-2b	XXX (PSA)§	
	sst2a, sst5	C4-2	XXX (PSA)	
	sst2a, sst5	DU-145	XXXX	
	sst2a, sst5	PC-3	XXXX	
		R-3327-AT-1	XXXX	
Colon	sst2, sst5	HCT-15	XXXX	
	sst5	HT-29	XXXX	
	sst2, sst5, sst3	HCT-116	XXXX	
Renal	sst2a	SW-839	XXX	
	sst5	786-0	XXXX	
Melanoma	sst1-sst5	MRI-H255	XXX	
		MRI-H187	XXX	
Lung	sst2, (sst5)	H838 (NSCLC)	XXX	
	sst2a, (sst5)	H157 (NSCLC)	XXXX	XXX (2Ptx-OCT)
	sst5, sst3, sst1, (sst2)	H727	AN-238 < AN-201	AN-162 < Dox‡
	(sst2a)	H720	AN-238 < AN-201	CPT-L1(L2)-SSA
	sst1, sst2a, sst3, (sst5)	KRJ-I	AN-238 = AN-201	AN-162 < Dox
	sst2a, sst4, sst1, sst5	A549 (NSCLC)	n.d.	CPT-L1(L2)-SSA
	sst2a, (sst5)	H69 (SCLC)	XXX	XXX (AN-162)
	sst2	H345 (SCLC)	AN-238 < AN-201	XXX (AN-162)
	sst2a, sst5, sst3, sst1	H460 (NSCLC)	n.d.	
	sst2a, sst4, sst1, sst3	H1299 (NSCLC)	n.d.	
Gastric	sst2a, sst5, sst3	AGS	XXX	
	sst2a, sst5, (sst3)	Hs 746T	XXXX	AN-162 < Dox
	sst2a, sst5, (sst3	NCI-N87	XXX	
		MKN-45	AN-238 < AN-201	
Pancreatic	sst3, sst5	SW-1990	XXXX	
	sst3, (sst2), (sst5)	Panc-1	XXXX	
	sst3, (sst2), (sst5)	Capan-1	XXXX	
	sst3, sst2, (sst5)	Capan-2	XXX	
	sst2, sst5	CFPAC-1	XXXX	
	sst3, sst2, (sst5)	Mia-PaCa-2	XX	
Non-Hodgkin's lymphoma	sst1-sst5	RL	XXXX	
	sst1-sst5	NL	XXXX	
Hepatocellular	sst2, sst3, sst5	SK-Hep-1	XXXX	
	sst2, sst3, sst5	HepG2	AN-238 > AN-201	
	sst2, sst3, sst5	Hep3B	AN-238 > AN-201	
Brain	sst2	IMR-32 neuroblastoma	n.d.	CPT-L1(L2)-SSA¶
	sst2	U-118MG glioblastoma	XX	< XX (AN-162)
	sst2	U87-MG glioblastoma	XXXXX	

**In vivo* studies: XXXXX: regression; XXXX: > 70% inhibition; XXX: 50 – 70% inhibition; XX: 25 – 50% inhibition; n.d.: not determined.

‡AN-162 > Dox represents the comparison of the *in vitro* antitumor effect of free drug and its GnRH derivative conjugate.

§PSA inh.: not the tumor volume, but PSA level was determined as a measure of the inhibitory effect.

¶CPT was more active than CPT-L1-SSA and CPT-L2-SSA.

The effects are not absolutely comparable because of different doses and treatment schedules used.

and *in vivo* antitumor activity of 2PTX-OCT than that of PTX-OCT and free PTX was clearly demonstrated. Furthermore, its toxicity indicated by the white blood cell count was significantly lower than that of the free PTX. The antitumor effect of 2PTX-OCT (3 × 150 nmol/kg) resulted in the same inhibition on H838 xenografted nude mice (60.7%) as observed by Schally's group using AN-238 (~ 60%) [64].

Fuselier *et al.* conjugated camptothecin as a cytotoxic agent to a somatostatin derivative D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH_2. The N-terminal linking motif allowed the attachment of large groups without significant loss of receptor recognition of the cyclic somatostatin analogue [71]. CPT, which has antitumor activity by inhibiting the topoisomerase I, contains a hydroxyl group as a conjugation site, similarly to Dox and PTX. However, in this study a carbamate was applied as a linker because of its high stability in plasma compared with the commonly used ester-type linkage. The idea was that the cytotoxic agent should be masked until the internalization of the conjugate. Basic conditions or enzymatic attack result in the nucleophile-assisted release of the free camptothecin. Carbamates are metabolized by P450 enzyme that is upregulated in human cancer cells [72,73]. The half-life of the prepared compounds was between 18 and 106 h in rat serum, depending on the type of carbamate linker. The highest cytotoxic activity on human neuroblastoma IMR-32 cells was obtained in the case of CPT-*N*-methyl-aminoethyl-Gly-D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH_2 (CPT-L1-SSA) (9) (**Figure 4D**) (IC_{50} = 54.2 ± 6 nM). Furthermore, the antitumor activity of the conjugate was evaluated on small cell lung cancer cells [74]. The conjugate bound to the NCI-H69 homogenate one order of magnitude better than its non-methylated version (CPT-aminoethyl-Gly-D-Ser-Nle-D-Tyr-D-Ser-c(Cys-Phe-D-Trp-Lys-Thr-Cys)-Thr-NH_2 [CPT-L2-SSA]). Significant *in vitro* cytotoxicity of the conjugate at 0.3 – 1 µM concentration was obtained on NCI-H69 and NCI-H345 cell lines that have mRNA for sst_2 receptors.

CPT was also attached to the same somatostatin analogue via a cleavable carbamate group but using a different peptide linker sequence (D-Lys-D-Tyr-Lys-D-Tyr-D-Lys) [75]. This conjugate retained the potent biological activity and had comparatively low toxicity [76]. Its efficiency against PC-3 prostate cancer cell invasion was also demonstrated [77]. The CPT-somatostatin vector conjugate inhibited the expression of MMP-2 and MMP-9, but not the expression of MMP-3 and MMP-10 (MMP: matrix metalloproteinase) in PC-3 tumor cells. MMP-2 and MMP-9 have higher expression levels in tumor cells than in normal tissues and their expression correlates with the metastatic potential of the tumor cells [78]. CPT and the conjugate, but not the somatostatin analogue, inhibited the expression of $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins at cell surfaces, tumor progression and metastasis, leading to the conclusion that CPT was responsible for this effect.

All these data show that the conjugation of CPT to somatostatin analogues increases the water solubility of CPT, lowers the toxicity and increases the selectivity. The specific antitumor and antiangiogenic effects depend on the tumor cells that overexpressed somatostatin receptors.

7. Somatostatin-based boron neutron capture therapy

Boron neutron capture therapy (BNCT) is based on the nuclear capture and fission reactions that occur when non-radioactive ^{10}B is irradiated with low energy (0.025 eV) thermal neutrons to produce ^{11}B in an unstable form, which undergoes instantaneous nuclear fission to produce α-particles and recoiling ^{7}Li nuclei. These high linear energy transfer particles have a range of 5 – 9 µm, thereby restricting their destructive effects to only those cells containing ^{10}B. If ^{10}B could be accumulated selectively in tumor tissues, which subsequently could be irradiated with thermal neutrons, the nuclear reaction products would specifically destroy the tumor DNA. The presence of 10^9 (1.67×10^{-15} mole) boron atoms per cell is generally required to achieve an antitumor effect. The target cells that efficiently bind the somatostatin conjugates contain several hundred femtomols (~ 10^{-9} – 10^{-10} mole) of receptor per milligram membrane protein (~ 10^7 cells) that might allow the uptake of the required amount of *closo*-boran cluster derivative for efficient BNCT. For this purpose, [Tyr^3]-octreotate (D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr-OH) coupled with a borane cluster was prepared and functionally studied [79,80]. [Tyr^3]-octreotate is an octreotide analogue with carboxyl group at the C terminus and Tyr in position 3 instead of Phe, which shows higher binding affinity and internalization rate [5,81]. A correlation was found between the binding affinity and the length of the inserted spacer between the borane cluster (10 boron atoms) and the octreotate [80]. However, the attachment of a higher number of *closo*-borane clusters (20 boron atoms altogether) in the conjugate had a negative influence on the affinity of the peptide. The binding affinity of the best compound (10) (**Figure 5A**) was 2 – 3 nM (IC_{50}) on sst_2, but also some binding was observed on sst_3, sst_4 and sst_5 receptor subtypes. This range was similar to that observed in the case of radioligand-labeled somatostatin derivatives. Results of *in vivo* studies have not been published yet; however, the promising *in vitro* binding affinity of the conjugates to the somatostatin receptor-containing cells (Chinese hamster ovary carcinoma cells [CHO-K1] and Chinese hamster fibroblasts [CCL39]) may open a new direction for BNCT.

8. Radiolabeled somatostatin derivatives

Targeted radiotherapy of tumors overexpressing somatostatin receptors was shown to be a promising cancer therapeutic approach [5,9]. Although ^{111}In-DTPA-octreotide (OctreoScan®, Mallinckrodt Medical) has become the most widely used tracer for somatostatin receptor scintigraphy [82,83], in the case of radiotherapy ^{90}Y-DOTA-Tyr^3-octreotide (^{90}Y-DOTATOC, 11) (**Figure 5B**) has been the most frequently

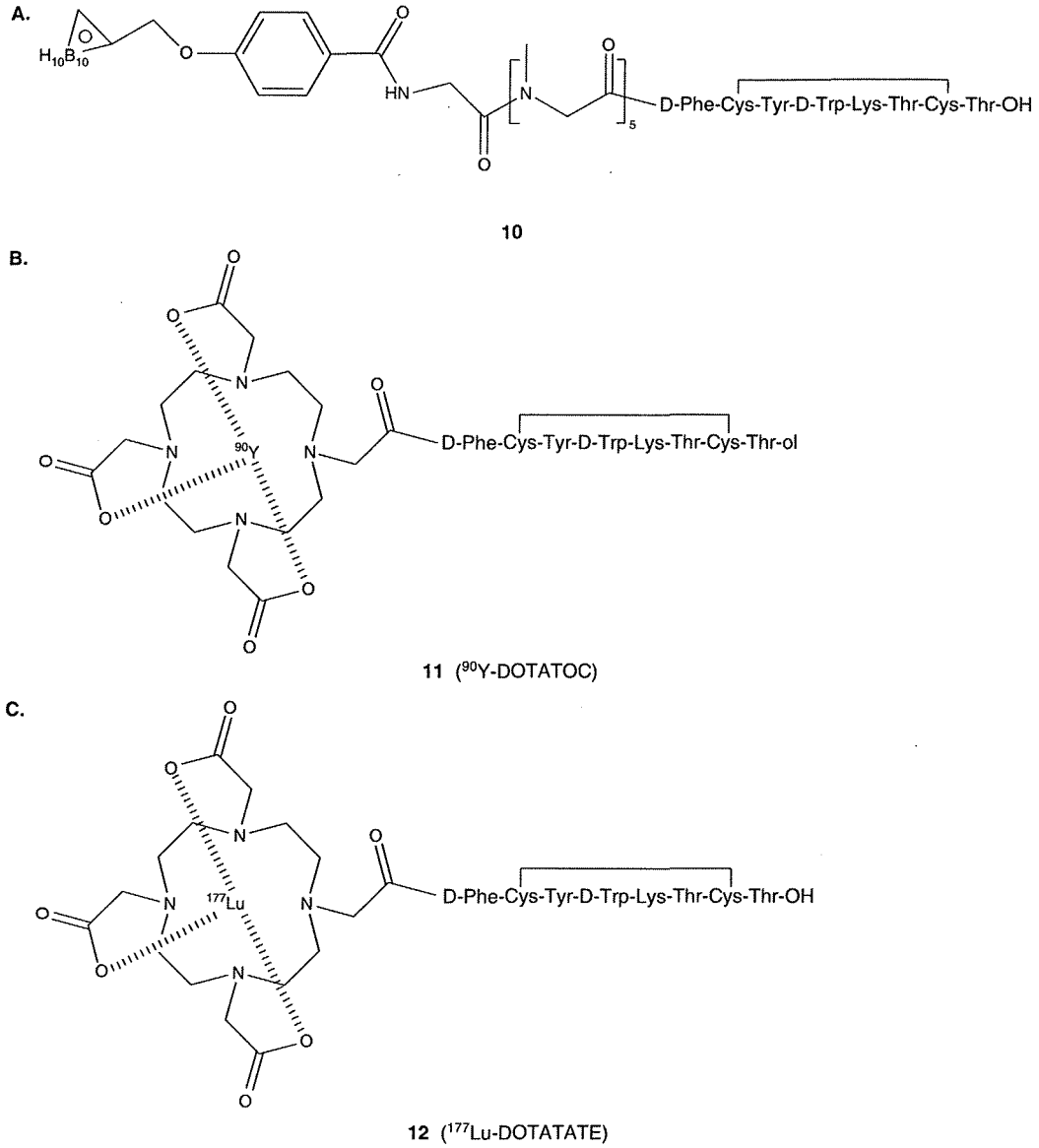

Figure 5. Chemical structures of a somatostatin conjugate for boron neutron capture therapy (A) and of radiolabeled somatostatin derivatives: ^{90}Y-DOTA-Tyr^3-octreotide (B) and ^{177}Lu-DOTA-Tyr^3-octreotate (C).

used clinically for > 15 years [84,85]. In the last decade, numerous compounds were developed in order to increase the receptor selectivity and/or bioactivity of the radiolabeled somatostatin derivatives. The modifications were carried out on the peptide sequence or by changing the radionuclides and/or chelator. Insertion of Ga into DOTATOC markedly improved the sst_2 binding affinity and the *in vivo* tumor imaging [86-88]. Recent data also showed the high efficiency of ^{99m}Tc-EDDA/HYNIC-TOC for radioimaging (EDDA: ethylenediamine-*N,N'*-diacetic acid, HYNIC: 6-hydrazinopyridine-3-carboxylic acid) [89-91]. The treatment of neuroendocrine gastroenteropancreatic tumors with ^{177}Lu-DOTA-Tyr^3-octreotate (^{177}Lu-DOTATATE, 12) (Figure 5C), which had high affinity to sst_2, resulted in complete or partial tumor remission in 30% of patients in comparision with 10 – 30% in the case of ^{90}Y-DOTATOC treatment [92]. ^{177}Lu-DOTATATE also showed lower and mostly transient side effects (e.g., nephrotoxicity) and milder bone marrow supression than ^{90}Y-DOTATOC. However, owing to the higher adsorbed dose but the lower tissue penetration range of ^{177}Lu than of ^{90}Y, the ^{177}Lu-DOTATATE may be more effective for smaller tumors. The combination of

the two compounds might be also beneficial [92,93]. Incorporation of multiple DOTA chelators into the [Tyr^3]-octreotate was described as a possible way to increase the efficacy [94]. Recently, it has been shown that the gemcitabine pretreatment upregulated the somatostatin receptor expression and acted as a radiosensitizer. The uptake of ^{177}Lu-DOTATOC was 1.5 – 3 times greater than that of untreated control cells [95]. Furthermore, the replacement of β-emitting ^{177}Lu (E_{max} = 0.5 MeV) in DOTATOC by α-emitting ^{213}Bi (E_{max} = 5.87 MeV, half-life 45.6 min) increased the effectivity 3.4 times [96]. The short-distance energy deposition of α-ray (~ 0.1 mm) might be very active for inhibiting the tumor growth without side effects in the case of efficient tumor localization. Moreover, this high energy radiation depresses enzymatic DNA repair mechanisms.

The usefulness of somatostatin antagonists for tumor targeting has been demonstrated in the last few years. Somatostatin receptor-selective antagonists did not trigger receptor internalization, yet they were excellent tumor markers [97,98]. Antagonists are more hydrophobic and chemically more stable than agonists, resulting in longer duration of action and possible stabilization in the lipid-rich environment of the receptors. The strong receptor–ligand interaction and the slow internalization in the case of antagonists may provide a longer-lasting accumulation of the radioligand-labeled antagonist conjugate on tumor cells. A series of highly sst_2-selective somatostatin antagonists for radiotargeting were prepared [99]. The two most promising compounds were DOTA-*p*NO_2Phe-c[D-Cys-Tyr-D-Aph(Cbm)-Lys-Thr-Cys]-D-Tyr-NH_2 and DOTA-Cpa-c[D-Cys-Aph(Hor)-D-Aph (Cbm)-Lys-Thr-Cys]-D-Tyr-NH_2 (Cpa: 4-Cl-phenylalanine; Aph: 4-aminophenylalanine; Cbm: carbamoyl; Hor: L-hydroorotyl) with high binding affinities (IC_{50} < 1 nM). These results suggest that somatostatin antagonists as targeting moieties are suitable for radiotherapy.

9. Conclusion

Tumor targeting with hormone peptides provides a basis for the development of various cancer diagnostic and therapeutic approaches (targeted chemotherapy, targeted radiotherapy, photodynamic therapy and BNCT). The increased selectivity and decreased peripheral toxicity of such systems have already been demonstrated on different types of tumor. Moreover, increased solubility and bioavailability can be obtained by the application of these conjugates. Both somatostatin- and GnRH-containing conjugates are effective for targeting antineoplastic agents; however, the advantages of somatostatin analogues in targeted radiotherapy have been presented clearly. The first most active anticancer drug–peptide hormone conjugates are now in preclinical or clinical trials. However, it has to be taken into account that the side effects are mostly related to the low stability in serum of peptide drug conjugates, which leads to early drug release and consequently to 'nonspecific' toxicity. Furthermore, the presence of hormone receptors (especially somatostatin receptors) on normal cells might prevent the clinical application of some compounds. Therefore, the application of targeting moieties with increased specificity for the peptide receptors expressed on cancer cells and the application of linkers/spacers between the peptide and the drug that are stable in serum might improve the selectivity and efficacy of hormone–drug conjugates in targeted chemotherapy.

10. Expert opinion

Targeted chemotherapy represents a promising approach for the treatment of cancer. One possibility to enhance the selectivity and to decrease the peripheral toxicity of the well-known antineoplastic agents as well as radionuclides is the attachment to hormone peptides that bind to their receptors overexpressed on tumor cells. However, only a few of these hormone peptides (GnRH and somatostatin) inhibit the tumor growth by receptor-mediated signal transduction pathway, the others stimulate the cell proliferation. Although the hormone peptide–drug conjugates are used at concentrations lower than the hormone dose required for tumor growth inhibition, the peptides with inhibitory effect should be preferentially used as targeting moieties for the preparation of drug delivery systems. Furthermore, the application of agonist derivatives instead of antagonists (preferred in the case of stimulatory peptides) might have a further benefit of faster receptor–ligand internalization. The internalization may be more important in the case of delivery of antineoplastic agents that have an intracellular site of action, whereas the intracellular delivery of radionuclides might not be necessary, especially in the case of β-emitters. However, the internalization of somatostatin agonist-bound radionuclids may lead to longer residence times of radionuclids in the tumor cells.

There are many promising data that confirm the efficiency of targeted therapy based on hormone peptides as targeting moieties. However, their receptors on cancer cells are limited, and their distribution on the cell surface might be heterogeneous. Futhermore, some receptors desensitize under continuous exposure to the hormone conjugate treatment. Research in the future should be focused on increasing the efficacy of the targeted therapeutic approaches. Several strategies to be pursued are the following.

(1) The combination of conjugates containing different targeting moieties. In this case different hormone receptors can be targeted. The same or different anticancer drugs can be attached to the selected hormone peptides. The latter combination might have the benefit that the drugs have different sites of action, resulting in high toxic effects on tumor cells.

(2) The development of conjugates in which two antineoplastic agents are coupled to a single hormone peptide. In this case, there should be an appropriate distance between the drugs and the targeting peptide in order to avoid the significant decrease of receptor binding affinity of the

hormone. The number of drug molecules in a conjugate could be increased by the aid of multifunctional linkers.

(3) The combination of radionuclides and anticancer drugs in one conjugate or physical mixing of two different conjugates with the aim of combining radiotherapy and chemotherapy.

(4) The development of targeted nanoparticles containing encapsuled drugs by modifying their surface with hormone peptides.

It has been shown that the most efficient hormone–drug conjugates prepared so far have fairly low stability in serum. Therefore, the linkage and/or spacer between the components should be optimized further. The improvement of the serum stability of the conjugates as well as the efficient drug release might increase the therapeutic window and the efficacy of the drug targeting.

Further development of carriers, especially in the case of GnRH, is required in order to prepare more potent compounds. Peptide derivatives with low endocrine but high antitumor activity are good candidates.

As intensive research is conducted in this field, more and more compounds as drug candidates might reach preclinical and clinical trials in the near future. Although in this review only GnRH- and somatostatin-based targeting approaches have been discussed in detail, other types of peptides as targeting moieties should be taken into account for further studies.

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Declaration of interest

The manuscript represents original work and has not been published previously or simultaneously submitted elsewhere for publication. The manuscript has been read and approved by both authors. The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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