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Optimization of an Albumin-Binding Prodrug of Doxorubicin That Is Cleaved by Prostate-Specific Antigen

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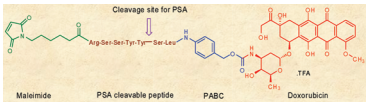
ABSTRACT We have developed a novel albumin-binding prodrug of doxorubicin that incorporates *p*-aminobenzyloxycarbonyl (PABC) as a 1,6 self-immolative spacer in addition to the heptapeptide, Arg-Ser-Ser-Tyr-Tyr-Ser-Leu, as a substrate for the prostate-specific antigen (PSA) that is overexpressed in prostate carcinoma and represents a molecular target for selectively releasing an anticancer agent from a prodrug formulation. The prodrug exhibited good water solubility and was bound rapidly to the cysteine-34 position of human serum albumin. Incubation studies with PSA demonstrated that the albumin-bound form of the prodrug was cleaved rapidly at the P_1-P_1' scissile bond, releasing H-Ser-Leu-PABC-DOXO, which was further degraded to release doxorubicin as a final cleavage product within a few hours in prostate tumor tissue homogenates as well as in PSA-positive LNCaP LN cell lysates. Moreover, our prodrug exhibited antiproliferative activity in a low micromolar range against a PSA-expressing prostate cancer cell line (LNCaP).

KEYWORDS Doxorubicin, albumin, prodrug, drug delivery, prostate cancer, prostate-specific antigen



Prostate cancer is the most common nondermatological male malignancy and is the second most common cause of male cancer-related death.[1] Prostate cancer progression is characterized by metastases primarily in the bone and lymph nodes, and to date, there is no effective therapy for treating patients with metastatic disease if they no longer respond to hormone therapy.[2,3] The benefit of therapy with conventional chemotherapy is limited due to the systemic toxicity and lack of tumor selectivity of antineoplastic agents. Thus, there is an urgent medical need to design drug delivery systems that transport the anticancer agent to the primary tumor and metastases.

Besides the development of suitable galenic formulations such as liposomes or micelles, several promising carrier-linked prodrug approaches have been developed over the last decades that rely on attaching chemical groups or carriers to the drug through a predetermined cleavage point that is specifically cleaved to release the drug in the tumor cell or tumor tissue, either extra- or intracellularly.[4] A drug carrier that is playing an increasing role in the clinical setting is human serum albumin (HSA) (66.5 kDa).[5] Uptake of albumin in tumors is mediated by the enhanced permeability and retention (EPR) effect, that is, EPR of macromolecules in relation to passive tumor targeting (reviewed in ref 6). Over the past years, we have investigated a targeting strategy that is based on two features:[5,7,8] (1) in situ binding of a thiol-binding prodrug to the cysteine-34 position of circulating albumin after intravenous administration with subsequent accumulation of the drug−albumin conjugate in the tumor due to passive targeting and (2) release of the albumin-bound drug at the tumor site due to the incorporation of a cleavable bond between the drug and the carrier.

The first and most advanced prototype of these types of prodrugs is the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH, now INNO-206), an acid-sensitive prodrug of doxorubicin that is under phase II clinical development[9] (see http://www.cytrx.com). Inspired by the translational research with DOXO-EMCH, a broad spectrum of albumin-binding prodrugs has been developed by Kratz and co-workers that incorporates an enzymatically cleavable peptide linker. Examples include doxorubicin prodrugs that are cleaved by matrix metalloproteases 2 and 9,[8] cathepsin B,[10] urokinase,[11] or prostate-specific antigen (PSA),[12,13] methotrexate prodrugs that are cleaved by cathepsin B and plasmin,[14] and camptothecin prodrugs that are cleaved by cathepsin B.[10]

Among these proteases, the PSA is especially attractive as a target protease because it is almost exclusively expressed

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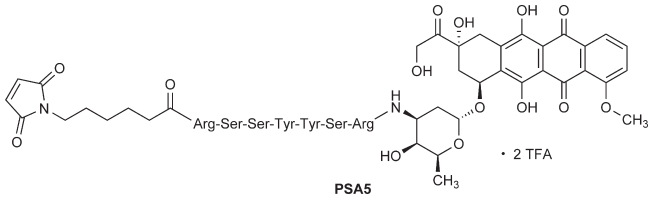

Figure 1. Structure of the PSA-cleavable prodrug PSA5.[13]

in prostate tissue and prostate carcinoma. PSA is a serine protease that belongs to the kallikrein gene family with chymotrypsin-like activity that is involved in the hydrolytic processing of semenogelins (cleavage of the seminal fluid proteins semenogelin I and II), which is required for liquefaction of seminal fluids.[15] Overexpression of PSA has primarily been demonstrated in prostate carcinoma and at low levels in breast cancer.[16−18] PSA levels in human primary prostate tumors can be very high depending on the stage of the disease (up to 1600−2100 nM), and 80−90% of the secreted PSA is active in the tumor environment.[17]

PSA is secreted in an active form in prostate cancer but forms two stable complexes with α_1-antichymotrypsin and α_2-macroglobulin in the blood. PSA complexed to α_1-antichymotrypsin is the predominant fraction of PSA. Only a minor fraction of serum PSA is not associated with proteinase inhibitors, and it is unknown whether this free fraction still has enzymatic activity.[18] In addition, it has recently been shown that PSA in mice bearing LNCaP tumors forms complexes similar to those in man, but the major immunoreactive complex contains α_1-antitrypsin rather than α_1-antichymotrypsin.[19]

Consequently, we and others have investigated prodrug strategies that exploit PSA as a molecular target for releasing an anticancer drug in prostate tumors.[12,13,19−21] Recently, a PSA-cleavable albumin-binding prodrug of doxorubicin EMC-Arg-Ser-Ser-Tyr-Tyr-Ser-Arg-DOXO (abbreviated PSA5; EMC = ε-maleimidocaproic acid) was developed in our group and is depicted in Figure 1.[13]

PSA5 was rapidly bound to circulating albumin and was superior over doxorubicin in an orthotopic PSA-positive model (LNCaP) with respect to antitumor efficacy and tolerability but did not induce tumor remissions.[13] Cleavage studies of the albumin-bound form of PSA5 (HSA-PSA5) with PSA demonstrated an efficient cleavage between Tyr and Ser, releasing the doxorubicin dipeptide H-Ser-Arg-DOXO as a final cleavage product within 24 h. This dipeptide, however, was cleaved slowly to H-Arg-DOXO in LNCaP tumor homogenates, and only minute amounts of doxorubicin were released. These results revealed that the full potential of the prodrug had not been exploited considering that only small amounts of the active agent doxorubicin were liberated in PSA-positive prostate carcinoma tissue.[13]

As a consequence and as a main goal of the present work, we set out to optimize the release characteristics of PSA-cleavable prodrugs with albumin-binding properties that would rapidly liberate doxorubicin as the final cleavage product. Initially, we aimed to synthesize and subsequently investigate the in vitro cytotoxicity of a new doxorubicin dipeptide by incorporating a self-eliminating linker between Arg and doxorubicin. Arg was initially chosen in this position because our previous studies with doxorubicin amino acid derivatives had shown that Arg-DOXO was cleaved more efficiently in tumor homogentates than other doxorubicin amino acid derivatives.[11] As a self-eliminating linker, we selected *p*-aminobenzyloxycarbonyl (PABC), which was initially introduced by Carl et al.[22] and proved to reduce a possible steric hindrance of bulky drugs during the enzymatic cleavage process.[23] Upon deacylation, the PABC spacer decomposes in a 1,6-benzyl elimination and spontaneously releases the free drug.[22]

Unfortunately, our synthetic efforts to obtain H-Ser-Arg-PABC-DOXO failed as a result of the chemical incompatibility of the Arg moiety in this position. Alternatively, we synthesized the doxorubicin dipeptide H-Ser-Leu-PABC-DOXO (**6**). Leucine is known to occupy this position in some natural PSA peptide substrates[24] and has also been used by the Merck group in the development of their low molecular weight doxorubicin prodrug L-377,202 Mu-His-Ser-Ser-Lys-Leu-Gln-Leu-DOXO (Mu = morpholinocarbonyl).[20]

Compound **6** could be synthesized in good yields, and as a comparison, we also synthesized the doxorubicin-dipeptide H-Ser-Leu-DOXO (**8**) (see the Supporting Information) and subsequently compared their cleavage profiles. Compound **6** showed a clearly improved cleavage profile over **8** after incubation with LNCaP tumor homogenates (Figure 2a) as well as in PSA-positive LNCaP cell lysates (Figure 2b), releasing doxorubicin as the final cleavage product within a few hours. In contrast, **8** was degraded slowly in the LNCaP tumor homogenates (Figure 3a) as well as in the PSA-positive LNCaP cell lysates (Figure 3b), being cleaved to the doxorubicin monopeptide H-Leu-DOXO (**7**) that slowly liberated a minimal amount of doxorubicin over 20 h.

In a similar way, H-Ser-Arg-DOXO was reported to release the doxorubicin monopeptide H-Arg-DOXO as a final cleavage product, which liberated a small percent of doxorubicin over 24 h in the LNCaP tumor homogenates.[13] These results underline the pivotal role of the PABC spacer for ensuring effective degradation of suitable doxorubicin dipeptide derivatives to doxorubicin, which is apparently catalyzed by unspecified proteases in tumor homogenates. In subsequent cell culture experiments using the PSA-expressing prostate cancer cell line LNCaP [transduced with a luciferase-neomycin (LN) resistance fusion gene cassette, which allows for sensitive quantification of viable cells with a wide dynamic range], the cytotoxicity of the different doxorubicin dipeptides, **6**, **8**, and H-Ser-Arg-DOXO correlated well with their cleavage rates (see Table 1).

Compound **6**, which rapidly produced doxorubicin in LNCaP cell lysates, exhibited an IC_{50} value in the low micromolar range of 0.15 μM that was quite comparable to the cytotoxicity of doxorubicin, which had an IC_{50} value of 0.11 μM (Table 1). On the other hand, both of the doxorubicin dipeptides lacking the PABC self-eliminating linker were either inactive against the PSA-positive LNCaP LN cells as in the case of H-Ser-Arg-DOXO with an IC_{50} value of >50 μM or proved to be approximately 20-fold less active (IC_{50} value of 3.09 μM for **8**).

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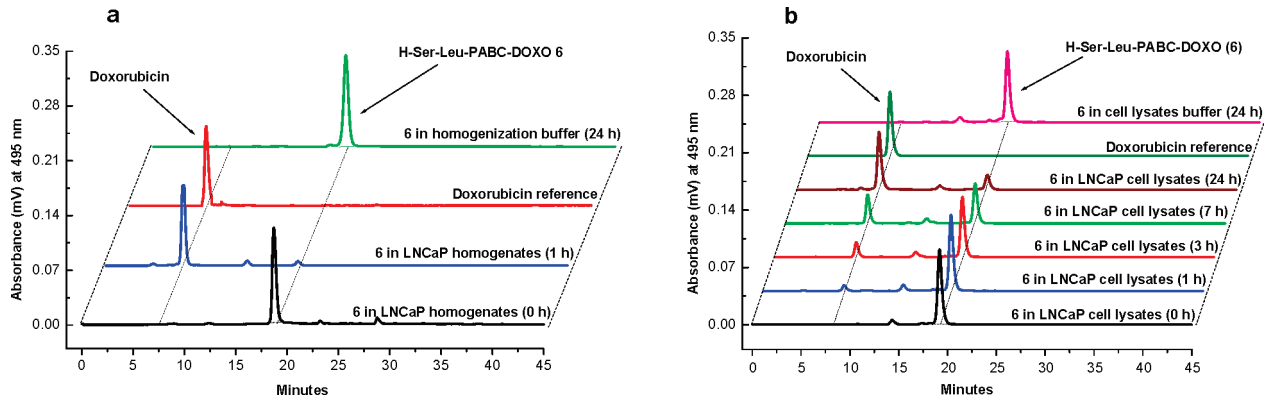

Figure 2. Chromatograms of incubation studies of **6** (50 μM) in the presence of (a) LNCaP tumor tissue homogenates and (b) LNCaP cell lysates at 37 °C.

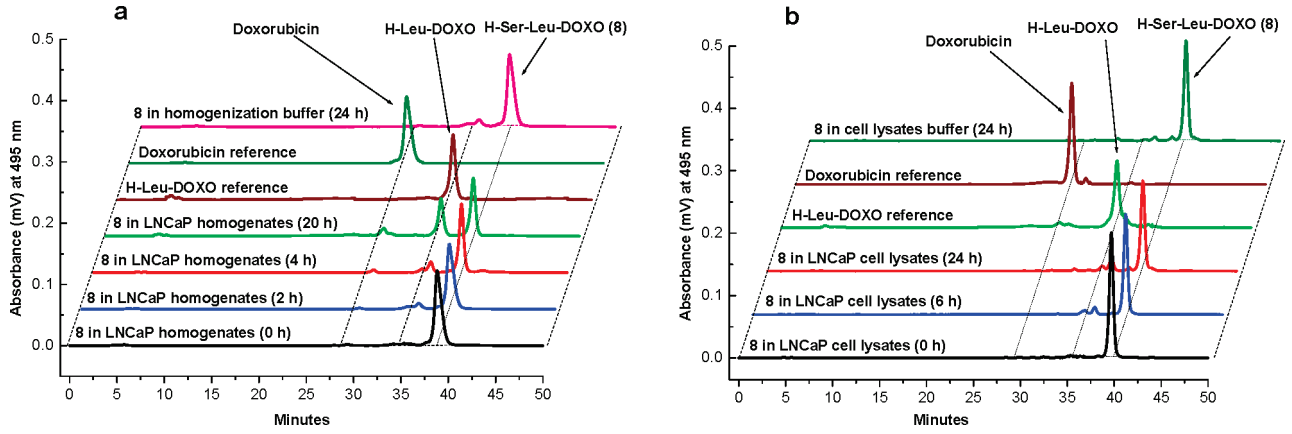

Figure 3. Chromatograms of incubation studies of **8** (50 μM) in the presence of (a) LNCaP tumor tissue homogenates and (b) LNCaP cell lysates at 37 °C.

These encouraging results prompted us to synthesize a novel albumin-binding prodrug of doxorubicin that incorporates the PABC self-immolative spacer between the doxorubicin and the peptide substrate. The new prodrug EMC-Arg-Ser-Ser-Tyr-Tyr-Ser-Leu-PABC-DOXO (**9**) was obtained through coupling of **6** with EMC-Arg-Ser-Ser-Tyr-Tyr-OH in anhydrous dimethyl formamide (DMF) in the presence of 1-hydroxybenzotriazole hydrate (HOBt), 4-methylmorpholine, and *N*,*N*′-diisopropylcarbodiimide (DIPC) (see Supporting Information — Scheme 1S).

The prodrug **9** was purified on a C-18 reverse-phase column. The lyophilized red product was characterized by mass spectrometry, and its purity was determined by HPLC (see Supporting Information). Compound **9** exhibited good water solubility (~4 mg/mL in isotonic 5% glucose solution) and was bound rapidly within a few minutes to the cysteine-34 position of endogenous albumin in a Michael addition in accordance to our previous work on the albumin binding properties of maleimide-bearing prodrugs (see the Supporting Information).

In addition, the prodrug showed a good stability in human plasma as well as in the PSA cleavage buffer (Tris buffer, pH 7.8) over 24 h (Figure 4a). Incubation studies with PSA demonstrated that the albumin-bound form of the prodrug (HSA-**9**) was rapidly cleaved at the P_1-P_1' scissile bond, releasing **6** as the cleavage product within 3 h (Figure 4a).

Table 1. IC_{50} Values of Doxorubicin, **6**, **8**, HSA-**9**, and HSA-PSA-**5** in the LNCaP LN Cell Line and the *p* Values of These IC_{50} Values in Correlation to That of Doxorubicin

compounds	IC_{50} value (μM)	*p* values
doxorubicin	0.11 ± 0.008	
HSA-PSA-**5**	33.78 ± 12	0.0073
HSA-**9**	1.30 ± 0.15	0.0002
6	0.15 ± 0.03	0.7600
8	3.09 ± 0.86	0.0035
H-Ser-Arg-DOXO	> 50.0	ND[a]

[a] ND, not determined.

Compound **6** was also observed as an intermediate cleavage product in an incubation study of HSA-**9** over 6 h in the LNCaP tumor homogenate as detected by HPLC, but free doxorubicin was the predominant final cleavage product (Figure 4b). The overall reactions that are involved in the cleavage process of HSA-**9** are depicted in Scheme 1. Incubation studies of HSA-**9** with PSA showed complete cleavage to **6** after 3 h under the experimental conditions chosen (20 μg/mL PSA; Figure 4a), and complete cleavage to

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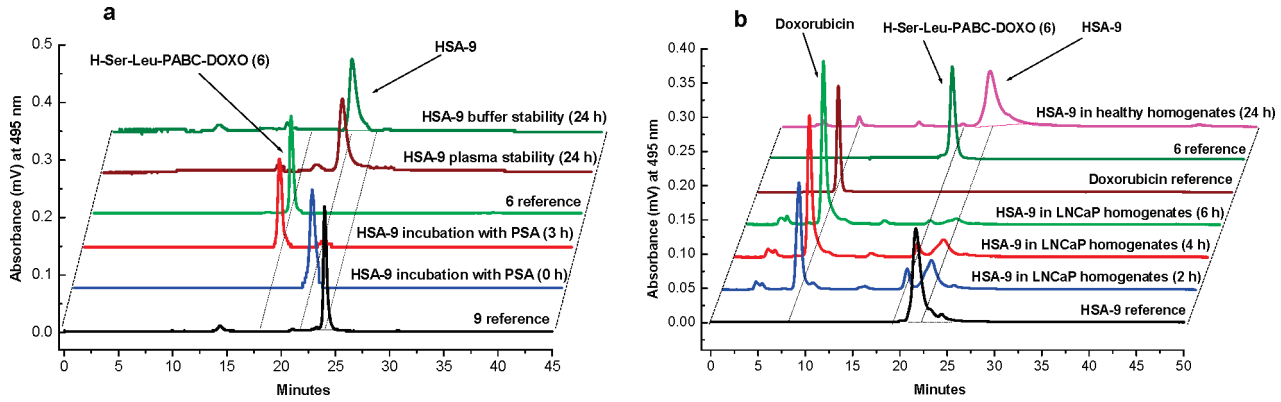
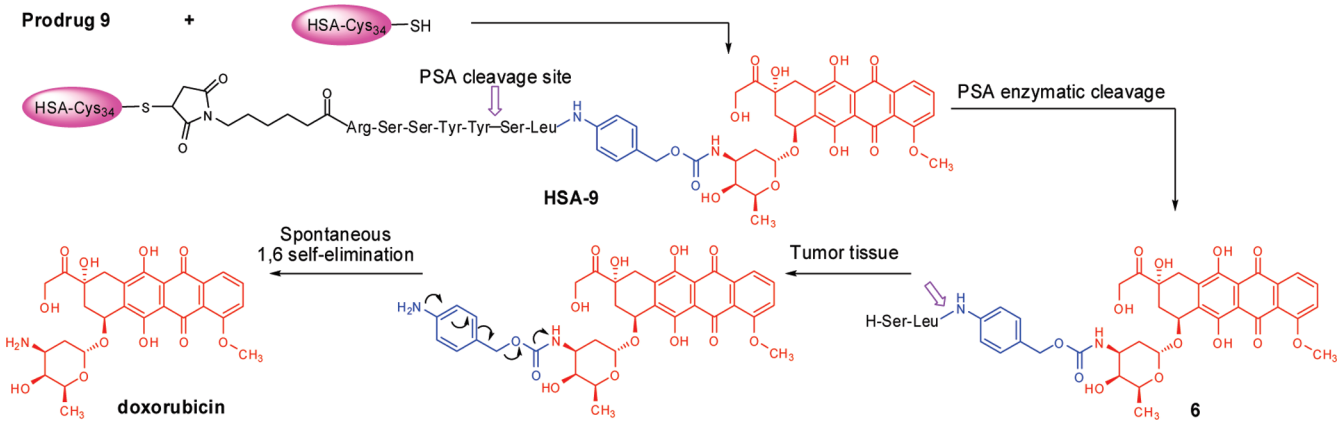


Figure 4. Chromatograms of incubation studies of HSA-**9** in the presence of (a) enzymatically active human PSA (20 μg/mL) and (b) LNCaP tumor tissue homogenates at 37 °C.

Scheme 1. Schematic Illustration of the Binding of Prodrug **9** to the Cysteine-34 Position of Albumin and Subsequent Enzymatic Cleavage by PSA of the Formed Albumin Conjugate and Degradation of **6** to Doxorubicin by Further Proteases



doxorubicin was observed after incubation of HSA-**9** with a homogenate from a LNCaP tumor after 6 h (see Figure 4b). This cleavage pattern can be judged as a significant improvement over the former HSA-PSA5, which only yielded small amounts of Arg-DOXO and doxorubicin when incubated with LNCaP tumor homogenates.[13]

To clarify the role of PSA is the cleavage of HSA-**9**, the chromatogram in Figure 4b in the background shows a cleavage study of HSA-**9** with a homogenate of a healthy murine prostate over 24 h. There is no indication of the appearance of **6**, and only small amounts of doxorubicin are liberated over this long time period. Furthermore, we have performed cleavage studies of HSA-**9** with a PSA-negative prostate tumor homogenate (xenograft DU145). The homogenate was prepared identically to the LNCaP tumor (200 mg of tumor in 800 μL of Tris buffer, pH 7.8). The chromatograms over 24 h are shown in the Supporting Information (Figure 3S). Only marginal cleavage to **6** and doxorubicin was observed after 16−24 h. These comparative results clearly indicate that PSA is involved in the cleavage of HSA-**9** because incubation studies with homogenates from LNCaP tumor homogenates showed **6** as an intermediate and complete cleavage to doxorubicin already after 6 h.

In the cell culture experiments, HSA-**9** exhibited antiproliferative activity in the low micromolar range with an IC_{50} value of 1.3 μM against LNCaP cells (see Table 1). Consistent with our earlier results with PSA-cleavable doxorubicin prodrugs,[12,13] HSA-**9** possesses a significantly higher IC_{50} value than doxorubicin, presumably due to the low PSA levels secreted in the cell culture medium during 96 h (26.9−312 ng/mL PSA; see the Supporting Information). Of note is that HSA-**9** was approximately 26-fold more active than HSA-PSA5 against the PSA-positive LNCaP LN cells, which can be explained by a more efficient cleavage of HSA-**9** by PSA with concomitant liberation of doxorubicin, which is far more pronounced than for HSA-PSA5.

In conclusion, by introducing the self-eliminating spacer PABC, we have optimized the cleavage profile as well as the release characteristics of a PSA-cleavable prodrug with albumin-binding properties that rapidly liberate doxorubicin as the final cleavage product. In addition, in stability studies of HSA-**9** with murine serum from the experiment in the LNCAP model that contains a high PSA level from a control tumor-bearing animal (PSA ~ 150 ng/mL) as well as with human serum from a patient with prostate cancer that showed a very high level of circulating PSA in his serum (PSA ~ 718 ng/mL), only marginal cleavage of doxorubicin or other byproduct was observed over 24 h at 37 °C (data not shown), making it a worthy candidate for further preclinical studies.

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We have recently completed an in vivo study with the new PSA-cleavable prodrug **9** in the orthotopic LNCaP model, and **9** demonstrated a distinct superiority over doxorubicin and the former doxorubicin prodrug lacking the PABC spacer (PSA5), especially regarding the antitumor effects on lung and bone metastases. We will report on these results in a separate communication.[25]

SUPPORTING INFORMATION AVAILABLE

Procedures for the preparation of the key compounds **6**, **8**, and **9** and their mass spectra as well as the methods and information on binding, cleavage, and cytotoxicity studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Damber, J. E.; Aus, G. Prostate cancer. *Lancet* **2008,** *371*, 1710–1721.
(2) de Wit, R. Chemotherapy in hormone-refractory prostate cancer. *BJU Int.* **2008,** *101* (Suppl. 2), 11–15.
(3) Kumar, S. K.; Williams, S. A.; Isaacs, J. T.; Denmeade, S. R.; Khan, S. R. Modulating paclitaxel bioavailability for targeting prostate cancer. *Bioorg. Med. Chem.* **2007,** *15*, 4973–4984.
(4) Kratz, F.; Muller, I. A.; Ryppa, C.; Warnecke, A. Prodrug strategies in anticancer chemotherapy. *ChemMedChem* **2008,** *3*, 20–53.
(5) Kratz, F. Albumin as a drug carrier: Design of prodrugs, drug conjugates and nanoparticles. *J. Controlled Release* **2008,** *132*, 171–183.
(6) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *J. Controlled Release* **2000,** *65*, 271–284.
(7) Kratz, F.; Warnecke, A.; Scheuermann, K.; Stockmar, C.; Schwab, J.; Lazar, P.; Drückes, P.; Esser, N.; Drevs, J.; Rognan, D.; Bissantz, C.; Hinderling, C.; Folkers, G.; Fichtner, I.; Unger, C. Probing the cysteine-34 position of endogenous serum albumin with thiol-binding doxorubicin derivatives. Improved efficacy of an acid-sensitive doxorubicin derivative with specific albumin-binding properties compared to that of the parent compound. *J. Med. Chem.* **2002,** *45*, 5523–5533.
(8) Mansour, A. M.; Drevs, J.; Esser, N.; Hamada, F. M.; Badary, O. A.; Unger, C.; Fichtner, I.; Kratz, F. A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2. *Cancer Res.* **2003,** *63*, 4062–4066.
(9) Kratz, F. DOXO-EMCH (INNO-206): The first albumin-binding prodrug of doxorubicin to enter clinical trials. *Expert Opin. Invest. Drugs* **2007,** *16*, 855–866.
(10) Schmid, B.; Chung, D. E.; Warnecke, A.; Fichtner, I.; Kratz, F. Albumin-binding prodrugs of camptothecin and doxorubicin with an Ala-Leu-Ala-Leu-linker that are cleaved by cathepsin B: Synthesis and antitumor efficacy. *Bioconjugate Chem.* **2007,** *18*, 702–716.
(11) Chung, D. E.; Kratz, F. Development of a novel albumin-binding prodrug that is cleaved by urokinase-type-plasminogen activator (uPA). *Bioorg. Med. Chem. Lett.* **2006,** *16*, 5157–5163.
(12) Kratz, F.; Mansour, A.; Soltau, J.; Warnecke, A.; Fichtner, I.; Unger, C.; Drevs, J. Development of albumin-binding doxorubicin prodrugs that are cleaved by prostate-specific antigen (PSA). *Arch. Pharm.* **2005,** *338*, 462–472.
(13) Graeser, R.; Chung, D. E.; Esser, N.; Moor, S.; Schachtele, C.; Unger, C.; Kratz, F. Synthesis and biological evaluation of an albumin-binding prodrug of doxorubicin that is cleaved by prostate-specific antigen (PSA) in a PSA-positive orthotopic prostate carcinoma model (LNCaP). *Int. J. Cancer* **2008,** *122*, 1145–1154.
(14) Warnecke, A.; Fichtner, I.; Sass, G.; Kratz, F. Synthesis, cleavage profile, and antitumor efficacy of an albumin-binding prodrug of methotrexate that is cleaved by plasmin and cathepsin B. *Arch. Pharm. (Weinheim)* **2007,** *340*, 389–395.
(15) Akiyama, K.; Nakamura, T.; Iwanaga, S.; Hara, M. The chymotrypsin-like activity of human prostate-specific antigen, gamma-seminoprotein. *FEBS Lett.* **1987,** *225*, 168–172.
(16) Diamandis, E. P. New diagnostic applications and physiological functions of prostate specific antigen. *Scand. J. Clin. Lab. Invest. Suppl.* **1995,** *221*, 105–112.
(17) Denmeade, S. R.; Sokoll, L. J.; Chan, D. W.; Khan, S. R.; Isaacs, J. T. Concentration of enzymatically active prostate-specific antigen (PSA) in the extracellular fluid of primary human prostate cancers and human prostate cancer xenograft models. *Prostate* **2001,** *48*, 1–6.
(18) Ravery, V.; Boccon-Gibod, L. Free/total prostate-specific antigen ratio—Hope and controversies. *Eur. Urol.* **1997,** *31*, 385–388.
(19) Denmeade, S. R.; Isaacs, J. T. Enzymatic activation of prodrugs by prostate-specific antigen: Targeted therapy for metastatic prostate cancer. *Cancer J. Sci. Am.* **1998,** *4* (Suppl. 1), S15–S21.
(20) DeFeo-Jones, D.; Garsky, V. M.; Wong, B. K.; Feng, D. M.; Bolyar, T.; Haskell, K.; Kiefer, D. M.; Leander, K.; McAvoy, E.; Lumma, P.; Wai, J.; Senderak, E. T.; Motzel, S. L.; Keenan, K.; Van Zwieten, M.; Lin, J. H.; Freidinger, R.; Huff, J.; Oliff, A.; Jones, R. E. A peptide-doxorubicin 'prodrug' activated by prostate-specific antigen selectively kills prostate tumor cells positive for prostate-specific antigen in vivo. *Nat. Med.* **2000,** *6*, 1248–1252.
(21) Khan, S. R.; Denmeade, S. R. In vivo activity of a PSA-activated doxorubicin prodrug against PSA-producing human prostate cancer xenografts. *Prostate* **2000,** *45*, 80–83.
(22) Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. A novel connector linkage applicable in prodrug design. *J. Med. Chem.* **1981,** *24*, 479–480.
(23) Dubowchik, G. M.; Firestone, R. A. Cathepsin B-sensitive dipeptide prodrugs. 1. A model study of structural requirements for efficient release of doxorubicin. *Bioorg. Med. Chem. Lett.* **1998,** *8*, 3341–3346.
(24) Malm, J.; Hellman, J.; Hogg, P.; Lilja, H. Enzymatic action of prostate-specific antigen (PSA or hK3): Substrate specificity and regulation by Zn(2+), a tight-binding inhibitor. *Prostate* **2000,** *45*, 132–139.
(25) Elsadek, B.; Graeser, R.; Esser, N.; Schäfer-Obodozie, C.; Tsurumi, C. A novel albumin-binding prodrug of doxorubicin with improved efficacy against prostate cancer metastases. *Int. J. Cancer* **2010,** submitted for publication.

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