Bioorganic & Medicinal Chemistry Letters 20 (2010) 853–856

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of matrix metalloproteases selective and activated peptide–doxorubicin prodrugs as anti-tumor agents ☆

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ARTICLE INFO

Article history:
Received 3 November 2009
Revised 21 December 2009
Accepted 22 December 2009
Available online 4 January 2010

Keywords:
Doxorubicin
Matrix metalloproteases
Prodrug

ABSTRACT

To selectively target doxorubicin (Dox) to tumor tissue and thereby improve the therapeutic index and/or efficacy of Dox, matrix metalloproteinases (MMP) activated peptide–Dox prodrugs were designed and synthesized by coupling MMP-cleavable peptides to Dox. Preferred conjugates were good substrates for MMPs, poor substrates for neprilysin, an off-target proteinase, and stable in blood ex vivo. When administered to mice with HT1080 xenografts, conjugates, such as **19**, preferentially released Dox in tumor relative to heart tissue and prevented tumor growth with less marrow toxicity than Dox.

Doxorubicin (Dox) is an anthracycline natural product that is widely used to treat tumors such as breast cancer, liver cancer, soft-tissue sarcomas, and non-Hodgkin's lymphoma. Dox has a complex mechanism of action with some of its activity arising from inhibition of nucleic acid synthesis within cancer cells.[1] Like other cytotoxic drugs, the therapeutic efficacy of Dox is limited by unwanted toxicity to non-tumor tissues, most notably myelosuppression.[2] In addition to these typical chemotherapeutic toxicities, Dox also causes cardiomyopathy which depends on the cumulative dose of drug.

There have been several attempts to develop Dox prodrugs that increase its therapeutic index.[3–5] For example, investigators used the prostate-specific antigen to activate Dox conjugates in mice leading to increased efficacy with reduced toxicity in mouse xenografts.[6] Unfortunately, these results did not effectively target Dox to tumors in humans.[7] It was hoped that this approach could deliver an efficacious concentration of Dox at tumor sites with limited systemic exposure, and hence would significantly increase its therapeutic index.

Matrix metalloproteases (MMPs) are a family of structurally related zinc-containing proteases containing more than 20 members.[8] Under normal conditions, these enzymes play an important role in the maintenance and remodeling of connective tissues. These enzymes are also implicated in several critical events in tumor evolution including tumorigenesis, tumor growth, angiogenesis, generation of reactive stroma and tumor cell metastasis. In fact, elevated level of MMP expression in human tumors was frequently found to correlate with disease progression.[9]

We reasoned that a MMP-activated prodrug of Dox might selectively release Dox at the tumor sites and thereby reduce side effects. We chose MMP-2, -9 and -14 to guide our in vitro structure–activity relationship (SAR) effort because of good expression of these enzymes in tumors.[9]

The desired conjugates of Dox should possess several properties. In particular, they should be good substrates for MMP enzymes to allow efficient activation in the tumor. Conjugates should be poor substrates for other enzymes, including enzymes typically found in the plasma compartment. Of particular concern for MMP-activated prodrugs was neprilysin since this cell-surface protease is expressed outside tumor tissue and may therefore lead to non-tumor activation of the prodrugs.[10] In addition, the prodrugs should not be cytotoxic prior to activation[11] and should have aqueous solubility compatible with intra venous administration. When properly designed, the resulting prodrugs have the potential to efficiently and preferentially deposit Dox in tumor tissue relative to non-tumor tissue leading to an improved therapeutic index and improved tumor growth inhibition.

Based on these considerations, our medicinal chemistry approach was to design and link an MMP peptide substrate, $\cdots P_3P_2P_1\text{–}P'_1P'_2P'_3\cdots$, to Dox to form a peptide–Dox conjugate. The COOH terminus of a peptide was conjugated by an amide bond

☆ Presented in part at the 231st National Meeting of the American Chemical Society, Atlanta, GA, March 2006, MEDI-271, 272.

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doi:10.1016/j.bmcl.2009.12.084

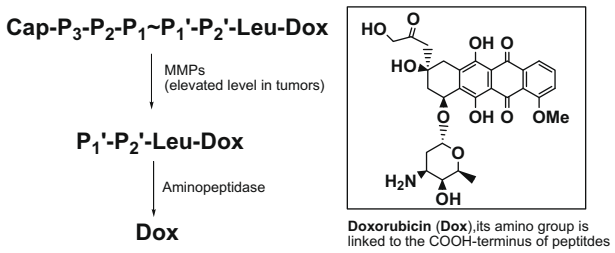
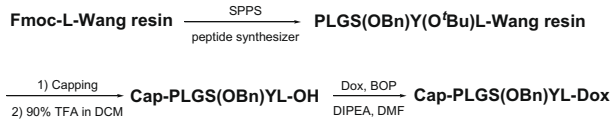


Figure 1. Activation of peptide–Dox prodrug.



Scheme 1. Synthesis of peptide–Dox conjugates.

with the amino group of Dox. In our design of the peptide sequence, Glycine (Gly, G) was chosen as the P_1 group because it was found to be optimal for MMP cleavage from our initial findings and also a literature report.[12] Leucine (Leu, L) was chosen as the COOH terminal residue linked directly to Dox because L-Dox was reported to be more efficiently converted to Dox than other conjugates.[13–15] The N-termini of the conjugates were capped to prevent aminopeptidase degradation before MMP cleavage. Based on these considerations, N-terminus capped peptide conjugates with Gly as P_1 and Leu linked to Dox as shown in Figure 1 were designed and optimized for MMP cleavage and selectivity. Incorporation of polar groups either within the N-terminal caps or within the side chains of amino acid residues was used to improve solubility of the conjugates. A target solubility of 1 mg/mL was chosen to guide compound design.

The synthesis of an example of the peptide–Dox conjugates is outlined in Scheme 1. Compound preparation was performed on a peptide synthesizer following a standard Fmoc solid phase protocol starting from Fmoc-Leu–Wang resin using HBTU as the coupling reagent.[16] The N-terminus of the completed peptide on resin was capped and the peptide was then cleaved from the resin with 90% trifluoroacetic acid in dichloromethane. The resulting peptide was then coupled to Dox to form the conjugate using the BOP coupling reagent.

The initial SAR was based on simple collagen-like peptide conjugates containing the sequence PLG ~ L, which is cleaved by most MMPs.[14] We first determined the preferred peptide length. As we previously reported[17] and show in the set of conjugates **1**–**6** in Table 1, the optimized conjugate length was a hexapeptide with

Table 1
In vitro profiles of peptide–Dox conjugates

No.	Conjugate	Enzyme cleavage k_{cat}/K_m (mM^{-1} s^{-1})				Stability[a] (%)	Solubility[b] (mg/mL)
		MMP-2	MMP-9	MMP-14	Neprilysin		
1	Ac-PLG–L-Dox	<1	<1	—	—	—	—
2	Ac-PLG–LL-Dox	18	>120	4	22	—	—
3	Ac-LG–LL-Dox	<1	<1	<1	5	—	—
4	Ac-LG–LYL-Dox	6	1	24	8	—	—
5	Ac-PLG–LYL-Dox	88	390	>120	22	—	—
6	Ac-PLG–LYAL-Dox	>120	>120	>120	>120	—	—
7	Ac-PLG–S(OMe)[c]YL-Dox	24	79	69	2.1	31	0.04
8	Ac-PLG–S(OBn)[d]YL-Dox	7	34	25	<1	—	—
9	Ac-PLG–Hof[e]AL-Dox	11	<1	19	1	—	—
10	Ac-PLG–HofYL-Dox	>120	34	>120	<1	47	0.01
11	Ac-PLG–HofHoy[f]L-Dox	116	>120	>120	<1	13	0.001
12	Ac-PLG-Hoa[g]YL-dox	21	43	58	<1	59	0.38
13	Ac-PLG–HofGmp[h]L-Dox	55	73	>120	<1	100	0.13
14	Ac-PLG–HofK(NMe_2)L-Dox	31	43	62	<1	90	1.27
15	Cap1[i]-PLG–S(OBn)YL-Dox	20	52	89	<1	100	1.4
16	Cap2[j]-PCit[k] G–S(OBn)YL-Dox	25	38	56	<1	29	>2.3
17	Ac-γE-PQG–S(OBn)YL-Dox	29	120	72	8	70	1.9
18	Ac-γE-PCitG–S(OBn)YL-Dox	21	64	48	<1	83	2.1
19	Ac-γE-PLG–S(OBn)YL-Dox	31	55	83	<1	88	>2.6
20	Ac-γE-PLG–C(SBn)YL-Dox	40	107	97	<1	90	>2.2
21	Ac-γE-PLG–HoyYL-Dox	>120	>120	>120	<1	100	1.5
22	Ac-βD-PLG–S(OBn)YL-Dox	69	>120	>120	<1	95	>3.9
23	Cap3[l]-PQG–S(OBn)YL-Dox	47	68	97	<1	81	>2.2
24	Cap3-PLG–S(Bn)YL-Dox	30	85	76	<1	87	1.8
25	Cap2-PLG–S(OBn)YL-Dox	37	25	111	<1	90	>2.4(2.9)[n]
26	Cap3-PCitG-T(OBn)YL-Dox	59	68	>120	<1	90	(2.9)
27	Cap4[m]-PSG-T(OBn)YL-Dox	114	79	>120	<1	100	(2.5)

[a] % Remaining after 6 h in blood.
[b] In pH 7.4 buffer solution.
[c] *O*-Methylserine.
[d] *O*-Benzylserine.
[e] Homophenylalanine.
[f] Homotyrosine.
[g] 2-Amino-4-(pyridine-4-yl)butanoic acid.
[h] *N*-Methylpiperazinepropylglycine.
[i] Cap1: succinic acid.
[j] Cap2:2-sulfoacetic acid.
[k] Citrulline.
[l] Cap3:3-sulfobenzoic acid.
[m] Cap4: 3,5-disulfobenzoic acid.
[n] Value in bracket was the solubility in 5% dextrose solution.