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Antibody-Mediated Delivery of Chimeric Protein Degraders Which Target Estrogen Receptor Alpha (ERα)

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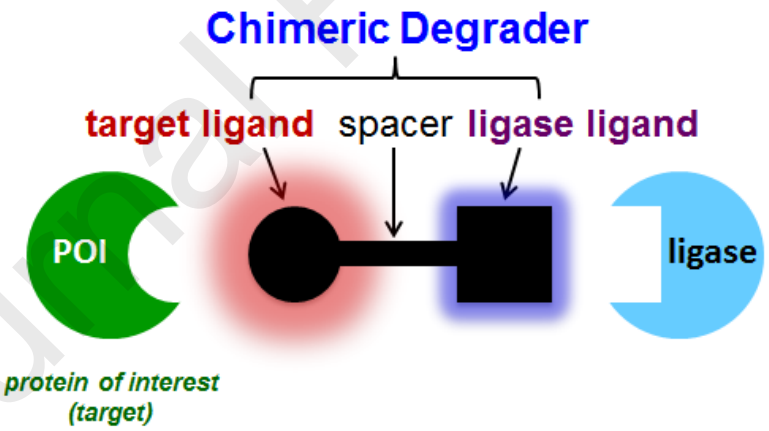
Abstract

Chimeric molecules which effect intracellular degradation of target proteins via E3 ligase-mediated ubiquitination (e.g., PROTACs) are currently of high interest in medicinal chemistry. However, these entities are relatively large compounds that often possess molecular characteristics which may compromise oral bioavailability, solubility, and/or *in vivo* pharmacokinetic properties. Accordingly, we explored whether conjugation of chimeric degraders to monoclonal antibodies using technologies originally developed for cytotoxic payloads might provide alternate delivery options for these novel agents. In this report we describe the construction of several degrader-antibody conjugates comprised of two distinct ERα-targeting degrader entities and three independent ADC linker modalities. We subsequently demonstrate the antigen-dependent delivery to MCF7-neo/HER2 cells of the degrader payloads that are incorporated into these conjugates. We also provide evidence for efficient intracellular degrader release from one of the employed linkers. In addition, preliminary data are described which suggest that reasonably favorable *in vivo* stability properties are associated with the linkers utilized to construct the degrader conjugates.

The use of synthetic molecules to induce the intracellular degradation of targeted proteins is a rapidly expanding area of medicinal chemistry research.[1] These entities often function by forming a ternary complex between a protein of interest and an E3 ubiquitin ligase which results in ubiquitination of the target and its subsequent destruction via trafficking to the proteasome.[2] Degraders which operate in this manner are typically comprised of (1) a moiety which binds to the target of interest, (2) an E3 ligase recognition element, and (3) a spacer group which connects the first two components (Figure 1).[2] These entities have been previously named PROTACs (proteolysis targeting chimeras),[3] SNIPERS (specific and nongenetic IAP-dependent protein erasers),[4] and degronimers in the chemical literature. However, for consistency and simplicity purposes, the term "chimeric degrader" will be used to describe such molecules throughout the course of this work.

Figure 1. General description of chimeric degrader construction and biological interactions.

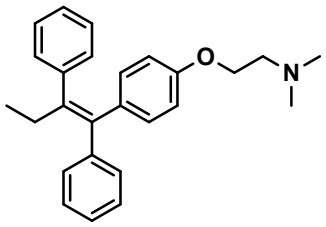


Biologically active chimeric degraders that utilize a variety of distinct E3 ligases have been reported to effectively degrade many different target proteins.[1,2,5] These results suggest that the degradation approach to protein modulation may have broad application in the biology and medicinal chemistry fields. Given their mechanisms of action, chimeric degraders offer several advantages over conventional small-molecule inhibitors including the

potential to operate catalytically,[6] the ablation of target protein scaffolding properties,[6] and the ability to function by binding to virtually any site on a target protein.[1,2] However, such degraders are also relatively large entities that often possess molecular characteristics which may compromise oral bioavailability, solubility, and/or *in vivo* pharmacokinetic properties. The majority of *in vivo* experiments conducted with chimeric degraders to date employ subcutaneous or intraperitoneal dosing regimens,[7] although reports of intravenously-delivered[8] and orally bioavailable[9] entities are now beginning to emerge.[10] Accordingly, we wished to explore whether conjugation of chimeric degraderss to monoclonal antibodies might provide alternate delivery options for the degrader molecules.[11] In this report, we broaden our initial studies involving BET-targeting chimeric degrader antibody-conjugates[12] by successfully expanding the conjugation concept to entities which degrade a different intracellular protein target.[13]

Modulation of the estrogen receptor (ER) is known to have beneficial effects in patients with ER-positive breast cancers, and the selective estrogen receptor modulator (SERM) tamoxifen (**1**) is widely prescribed to such individuals as an adjuvant therapy following surgery (Figure 2).[14] More recently, the selective ER down-regulator (SERD) fulvestrant (**2**), which strongly induces degradation of the ER protein, was introduced as an improved treatment for ER-positive breast cancers in patients with disease progression following traditional anti-estrogen therapy.[15] The clinical success of fulvestrant has spurred the identification of additional ER degraders, and multiple examples of similarly functioning small molecules have recently appeared in the literature.[15] Several chimeric degraders which target the alpha-isoform of the ER protein (ERα) have also been described, and representative examples of these entities are shown in Figure 2 (**3** and **4**).[4a,16] Importantly, these molecules illustrate the diversity with respect to ERα binding elements and ligase ligands (**3** = VHL, **4** = XIAP) that can be tolerated in these types of chimeric ERα degraders.

Figure 2. Molecules which modulate estrogen receptor activity and/or protein levels.



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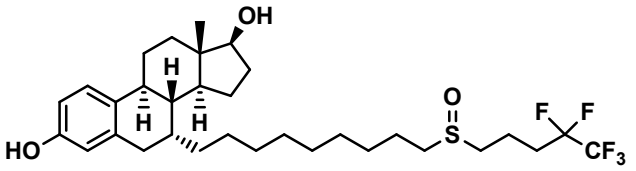

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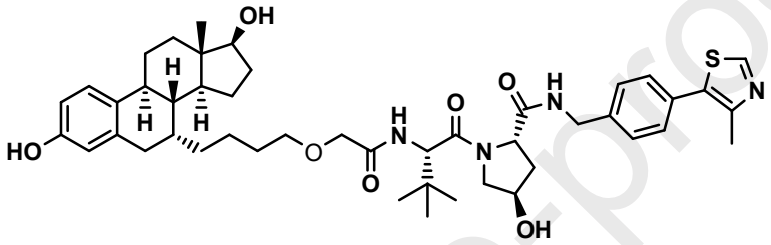

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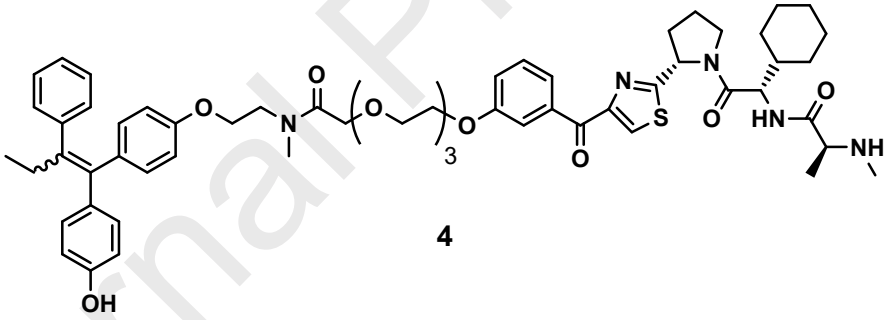


4

As part of our own exploration of ER-modulating compounds, we profiled the ability of chimeric degraders **5** and **6** to degrade the ERα protein in cells (Figure 3). Like compound **4** above, these molecules both contain the known tamoxifen metabolite endoxifen (**9**) that was prepared as a roughly 1:1 mixture of *E* and *Z* olefin isomers.[17] In addition, compound **5** incorporates a ligase binding element that recognizes the baculoviral IAP repeat 2 (BIR2) domain of the XIAP protein.[18] In contrast, compound **6** contains a peptidomimetic moiety

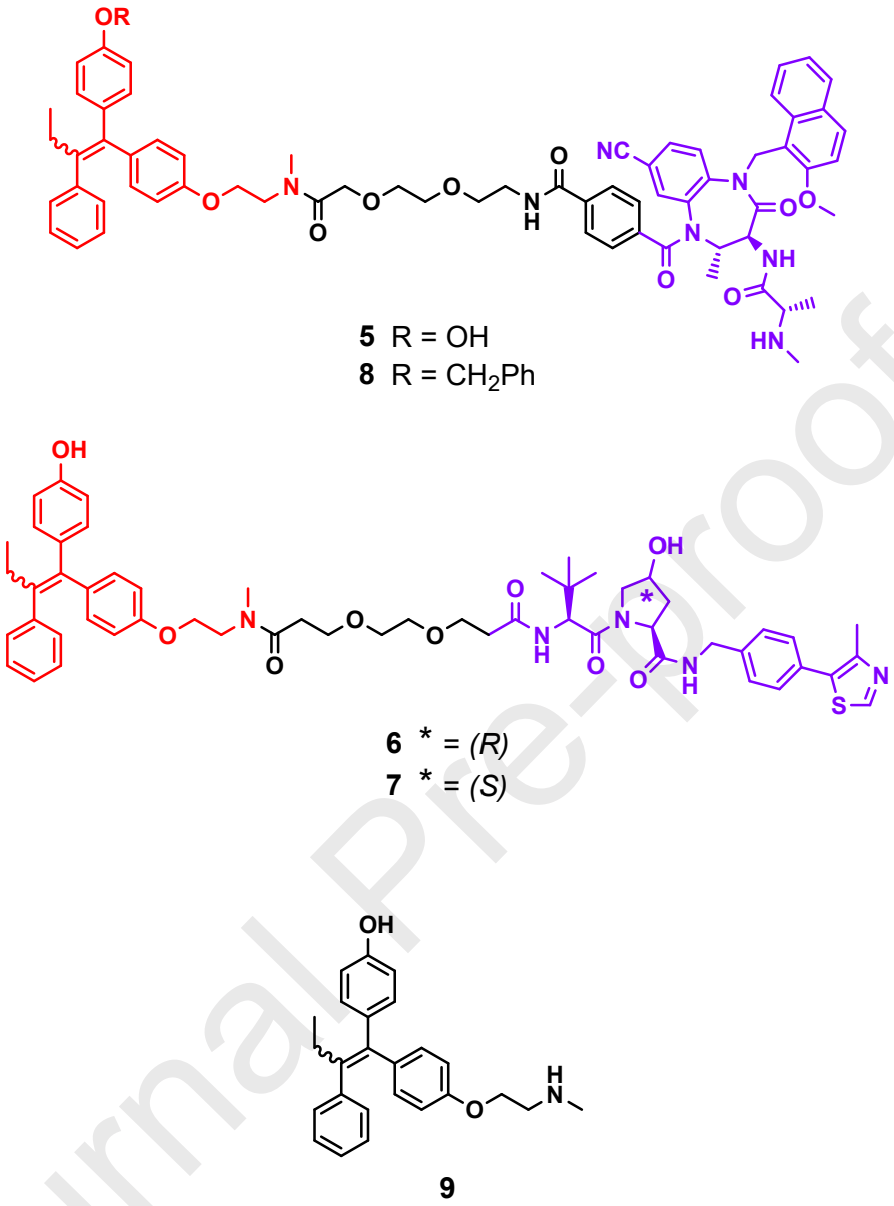
known to potently associate with an alternate E3 ligase: the von Hippel-Lindau tumor suppressor protein (VHL).[19,20] As shown in Table 1 and Figure S1, compounds **5** and **6** induced potent and near-complete degradation of the ERα protein when assessed via an immunofluorescence (IF) readout in parental MCF7 breast cancer cells as well as in MCF7 cells that had been engineered to express high levels of the HER2 cell-surface receptor.[21] A control compound which incorporated an epimer of the hydroxy-proline moiety present in **6** (compound **7**) exhibited attenuated but measurable ERα degradation activity relative to the latter molecule (Table 1 and Figure S1, note differences in Max Degradation outcomes). This result was consistent with proper recognition of VHL by degraders such as **6** being a requirement for potent and extensive degradation effects. The observed degradation properties exhibited by **7** likely resulted from binary complex formation with ERα due to the endoxifen ligand contained within its chemical structure. Consistent with this hypothesis, endoxifen itself (compound **9**) displayed ERα degradation effects in the IF assessment that were comparable to those exhibited by compound **7** (Max Degradation = approximately 50%; Table 1 and Figure S1).

Table 1. Degradation of the estrogen receptor alpha by SERDs **2**, **5**, and **6** and associated control compounds in MCF7-neo/HER2 and parental MCF7 cell lines. Time point = 4 h.

	MCF7-neo/HER2		MCF7 Parental	
Cmpd	ERα DC_{50} (nM)[a]	ERα % Max Degradation[b]	ERα DC_{50} (nM)[a]	ERα % Max Degradation[b]
2	0.38 ± 0.07	84	0.45 ± 0.01	87
5	1.6 ± 0.12	85	0.68 ± 0.13	87
6	4.9 ± 0.57	92	2.7 ± 0.71	92
7	7.9 ± 0.71	57	5.2 ± 0.01	54
8	305 ± 103	76	131 ± 2.1	75
9	1.1 ± 0.37	48	1.6 ± 0.01	49

[a]DC_{50} indicates the concentration achieving 50% degradation of ERα.
[b]Maximum reduction of ERα protein levels relative to DMSO-treated controls.

Figure 3. Structures of chimeric degraders **5-8**.



Legend. The red portions depict the ERα-binding regions of the compounds. The purple sections indicate the fragments that recognize an E3 ligase (**5** and **8** = XIAP, **6** and **7** = VHL). The structure of endoxifen (**9**) is also shown.

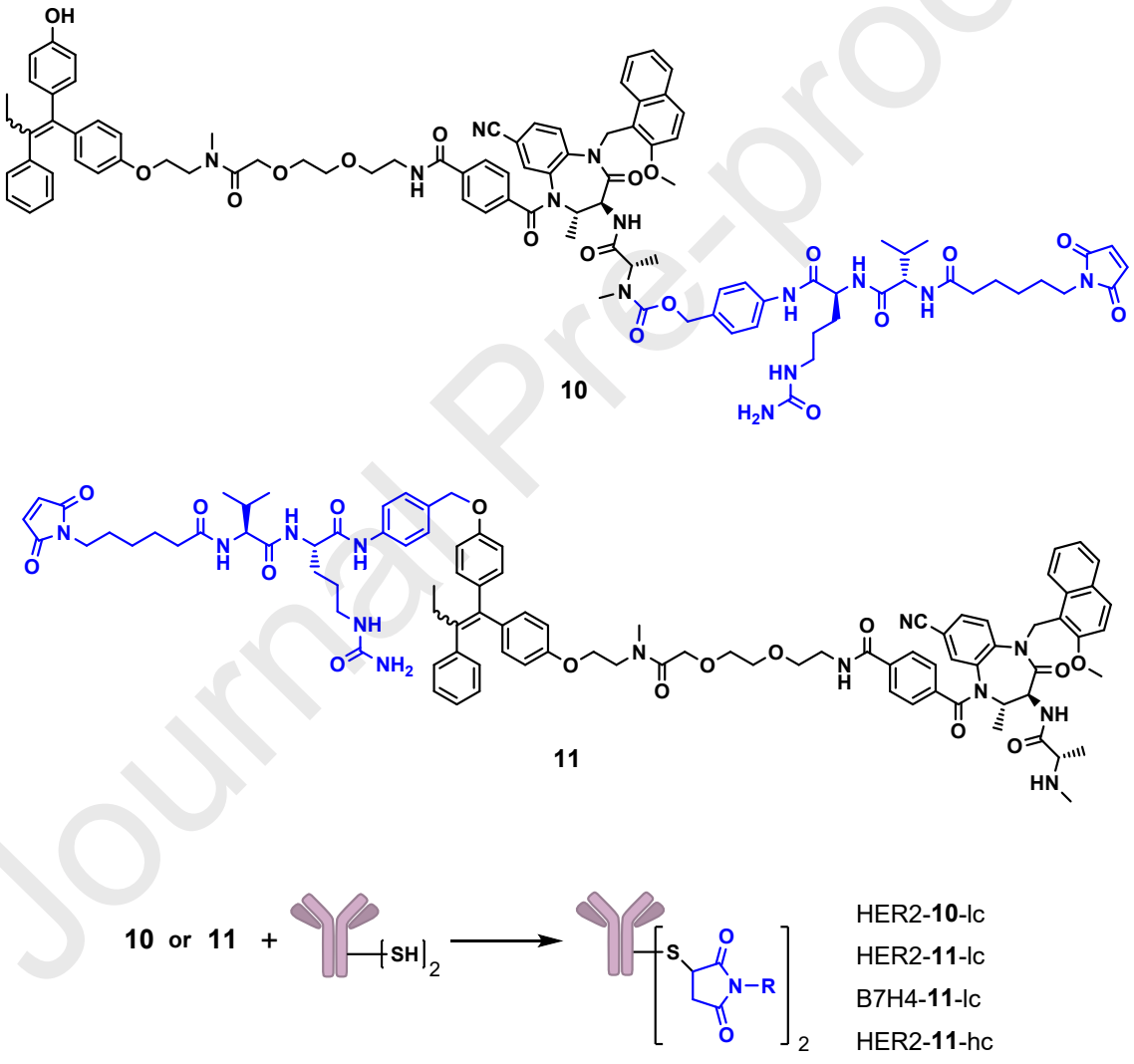
The concentration-dependent ERα degradation activity of compound **6** was also confirmed via western blot analysis in MCF7-neo/HER2 cells (Figure S3). However, the ERα-degradation effects observed via IF for compounds **7** and **9** were not apparent in the western blot assessments (Figure S3). Related discrepancies between IF and western ERα-

degradation outcomes were previously observed for relatively weak and/or incomplete degraders (e.g., tamoxifen).[22] These differences may result from ERα conformational and/or subcellular localization changes induced by the weak ligands that limit/prevent access of the ERα detection antibody in the intracellular environment during IF assessments (related access problems would not exist for western blot analysis since the cells are lysed and the ERα protein is fully denatured).[22] A second control compound which contained a benzylated phenol moiety in the ERα-binding region exhibited significantly weaker DC_{50} values relative to the parent molecule and thereby illustrated the importance of the free phenol in maintaining biological potency (compare compound **8** with **5**; Table 1).

As a prelude to preparing antibody conjugates capable of delivering compounds **5** and **6**, we first assessed their stability toward human liver lysosomes to ensure that they were robust enough to survive exposure to the intracellular lysosomal environment following antibody-mediated delivery.[23] As shown in Table S1, both molecules displayed acceptable stability properties in these *in vitro* assessments ($T_{1/2}$ >24 h), although some hydrolysis of an amide linkage present in **6** was observed. We then transformed chimeric degrader **5** into the linker-drug molecule **10** in preparation for synthesizing the desired conjugates (Figure 4). Compound **10** contains a Valine-Citrulline-*para*-amino-benzyloxy moiety (Val-Cit-PAB) linker that can undergo protease-mediated cleavage in lysosomes following antibody-directed intracellular delivery to efficiently release a variety of attached payloads (Scheme S1).[24] In addition, the maleimide present in linker-drug **10** rapidly reacts with engineered Cys residues on the surface of monoclonal antibodies to afford homogeneous conjugation products (Figure 4).[25,26] Accordingly, we first attempted to prepare a conjugate derived from **10** and a HER2-targeting antibody in which engineered Cys residues were introduced at the LC-K149 site [target drug-antibody ratio (DAR) = 2.0].[27] This mAb location affords maleimide-derived ADCs that are highly stable *in vivo* (i.e., do not appreciably undergo retro-Michael-related

deconjugation).[25,28] However, although the desired conjugate could be synthesized, it exhibited extensive self-aggregation behavior that precluded its isolation in quantities and purities sufficient for subsequent biological profiling. We suspected that such undesired properties resulted from the highly hydrophobic nature of linker-drug **10** that was not well-tolerated on the surface of the antibody.[29]

Figure 4. Structures of linker-drugs **10** and **11** derived from chimeric degrader **5** and construction of associated ADCs.



Legend. Blue fragments depict the linker portions of the molecules employed for mAb attachment.

Table 2. Degradation of the alpha estrogen receptor by unconjugated HER2 mAb, HER2 antibody-conjugates, and various control antibody-conjugates in MCF7-neo/HER2 and parental MCF7 cells. Time point = 72 h.

Conjugate[a]	DAR[b]	Site[c]	MCF7-neo/HER2		MCF7 Parental	
			ERα DC_{50} (μg/mL)[d]	ERα Max Degrad. (%)[e]	ERα DC_{50} (μg/mL)[d]	ERα Max Degrad. (%)[e]
HER2-**10**-lc[f]	NA[g]	LC-K149	ND	ND	ND	ND
HER2-**11**-lc	2.0	LC-K149	0.11 ± 0.001	81	22 ± 3.4	71
B7H4-**11**-lc	2.3	LC-K149	50 ± 23	40	43 ± 16	62
HER2-**12**	5.9	multi	0.04 ± 0.007	99	0.23 ± 0.07	95
CD22-**12**	5.7	multi	0.51 ± 0.094	90	0.48 ± 0.16	93
HER2-**13**	5.9	multi	0.05 ± 0.016	87	0.70 ± 0.068	51
HER2-**14**	5.6	multi	0.03 ± 0.002	94	0.09 ± 0.013	95
CD22-**14**	5.9	multi	4.2 ± 0.078	70	1.6 ± 0.035	91
HER2-mAb	NA	NA	0.04	43	>100	9

[a]Unless otherwise indicated, all HER2 conjugates were prepared using the 4D5 mAb variant that recognizes HER2 extracellular domain region IV.

[b]Drug-antibody ratio.

[c]DAR2 ADCs were prepared via conjugation to Cys engineered at the indicated mAb location (LC = light chain, HC = heavy chain); DAR6 conjugates were all prepared via simultaneous derivatization of engineered Cys at the LC-K149, HC-L174, and HC-Y373 mAb locations.

[d]DC_{50} indicates the concentration achieving 50% degradation of ERα.

[e]Maximum reduction of ERα protein levels relative to DMSO-treated controls.

[f]Conjugate was prepared using the 7C2 mAb variant that recognizes HER2 extra cellular domain region I. This alternate mAb variant is not anticipated to significantly alter aggregation outcomes relative to the 4D5 format.

[g]Significant aggregation was observed during the conjugation process (28%). The aggregated material could not be separated from the monomer conjugate via various purification techniques.

NA = not applicable.

ND = not determined.

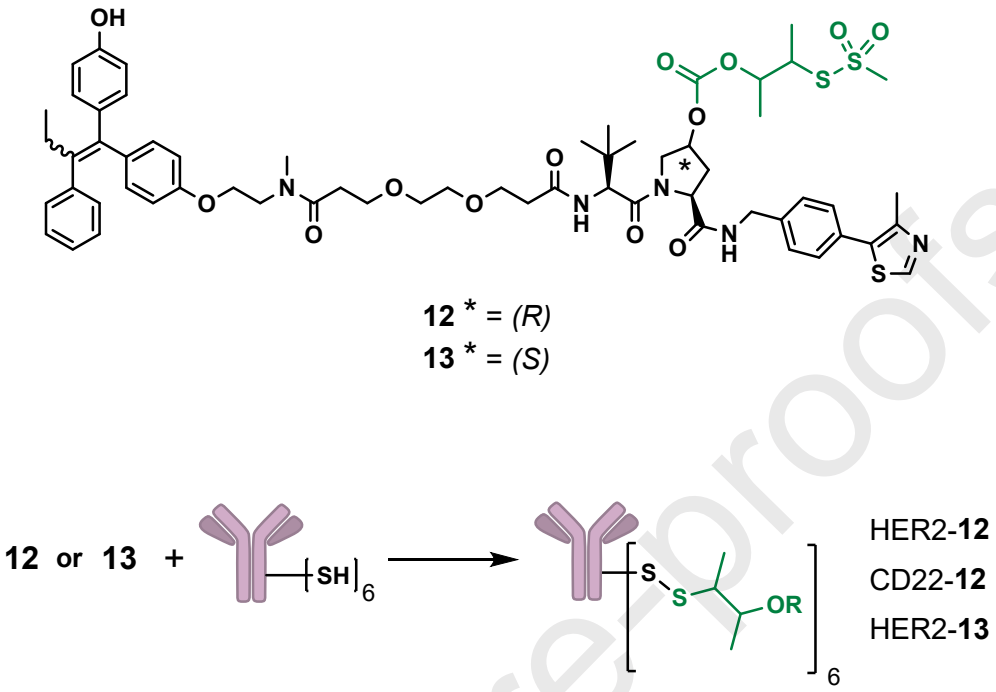
As one solution to this aggregation challenge, we prepared compound **11** in which the Val-Cit-PAB moiety was attached to compound **5** via an ether linkage to the phenol that was present in the ERα-binding portion of the chimeric degrader. The electron withdrawing substituents located *para* to this phenol moiety (olefin substituted with additional aromatic rings) should appropriately polarize that functional group such that efficient release of **5** would follow protease-mediated cleavage of the Val-Cit-PAB linker present in **11** (Scheme S2).[30] In addition, the alternate linker connection approach employed with compound **11** did not modify the basic amine present in **5** (as was the case with compound **10**). Accordingly, protonation of this moiety at physiological pH should enhance the aqueous solubility of **11** relative to **10** and thereby reduce the aggregation potential of conjugates prepared using the former linker-drug molecule.

With the above strategies in mind, a HER2-targeting DAR2 conjugate was successfully prepared from compound **11** that displayed manageable aggregation properties (Table 2, HER2-**11**-lc). Encouragingly, the conjugate exhibited potent reduction in ERα protein levels when assessed using MCF7-neo/HER2 cells, and this activity was well-separated from that displayed by both a B7H4-targeting control conjugate prepared from the same linker-drug (B7H4-**11**-lc) and an unconjugated HER2-targeting mAb (Table 2, Figure S2A).[31] The HER2-**11**-lc ADC also exhibited attenuated ERα degradation activity when tested using parental MCF7 cells that did not strongly express the HER2 receptor (Table 2, Figure S2B). Collectively, these outcomes were consistent with the antibody-mediated delivery of compound **5** via the HER2-targeting conjugate to the MCF7-neo/HER2 cells. The data also supported efficient intracellular release of **5** by the employed cleavable linker, since catabolites containing *O*-benzyl-substituted endoxifen moieties should exhibit significantly attenuated ERα degradation properties (c.f., compound **8**, Table 1).

In preparation for possible *in vivo* pharmacodynamic and/or efficacy studies utilizing the HER2-**11**-lc ADC, we first assessed its stability in a mouse pharmacokinetic experiment. Unfortunately, as a result of unexpected biotransformation of the attached chimeric degrader, the conjugate was surprisingly unstable during *in vivo* circulation in mouse (Figure S4). Specifically, a critical amide linkage present in linker-drug **11** was observed to undergo near-complete biotransformation after 4 days following initial intravenous administration of the HER2-**11**-lc ADC to mice (Figures S4). Although we could not confirm the structure(s) of the biotransformation products with certainty (Figure S5), we suspected that the described modifications were likely to significantly impair the ability of the corresponding degrader to interact with the intended E3 ligase. Accordingly, we were concerned that the noted instability would complicate the interpretation of subsequent *in vivo* pharmacodynamic and/or efficacy experiments conducted with conjugates such as HER2-**11**-lc, and we therefore shifted our studies to the exploration of ADCs that did not incorporate **5** in some form.

With these new goals in mind, we prepared linker-drugs **12** and **13** respectively from compound **6** and its corresponding hydroxy-proline epimer **7** (Figure 5). These molecules contain a methanethiosulfonyl (MTS) disulfide moiety that efficiently reacts with Cys residues on the surface of engineered monoclonal antibodies and thereby connects the linker-drugs to the biologics via new disulfide bonds (Figure 5).[32] The resulting disulfide-based conjugates should release the attached degrader payloads following internalization into targeted cells, disulfide reduction, and subsequent linker self-immolation (Scheme S3).[33] In addition, linker-drugs **12** and **13** utilize *carbonate* functional groups to connect the disulfide linkers to the associated payloads as opposed to the *carbamate* moieties that are frequently employed in ADC linker-drug designs.[34] Accordingly, a methyl group was incorporated adjacent to the carbonate groups of **12** and **13** in order to protect those moieties from unwanted hydrolysis during circulation *in vivo*.

Figure 5. Structures of linker-drugs **12** and **13** derived from chimeric degrader **6** and epimer **7** and construction of associated ADCs.



Legend. **Green fragments** depict the linker portions of the molecules employed for mAb attachment.

As shown in Table 2 and Figure 5, several antibody-drug conjugates were successfully prepared using linker-drugs **12** and **13**. Since the latter molecules were significantly less hydrophobic than linker-drugs **10** and **11** described above, **12** and **13** could be conjugated to mAbs bearing six engineered surface Cys residues without encountering significant aggregation issues [target drug-antibody ratio (DAR) = 6.0]. The higher drug loading of the new conjugates relative to the entities described earlier in this work would likely afford increased intracellular concentrations of the corresponding chimeric degraders following ADC-mediated delivery.[35] As with the DAR2 conjugates described earlier in this work, the locations of the introduced Cys residues (LC-K149, HC-L174, and HC-Y373)[27] all afforded disulfide-linked conjugates that exhibited relatively good *in vivo* stability properties.[28]

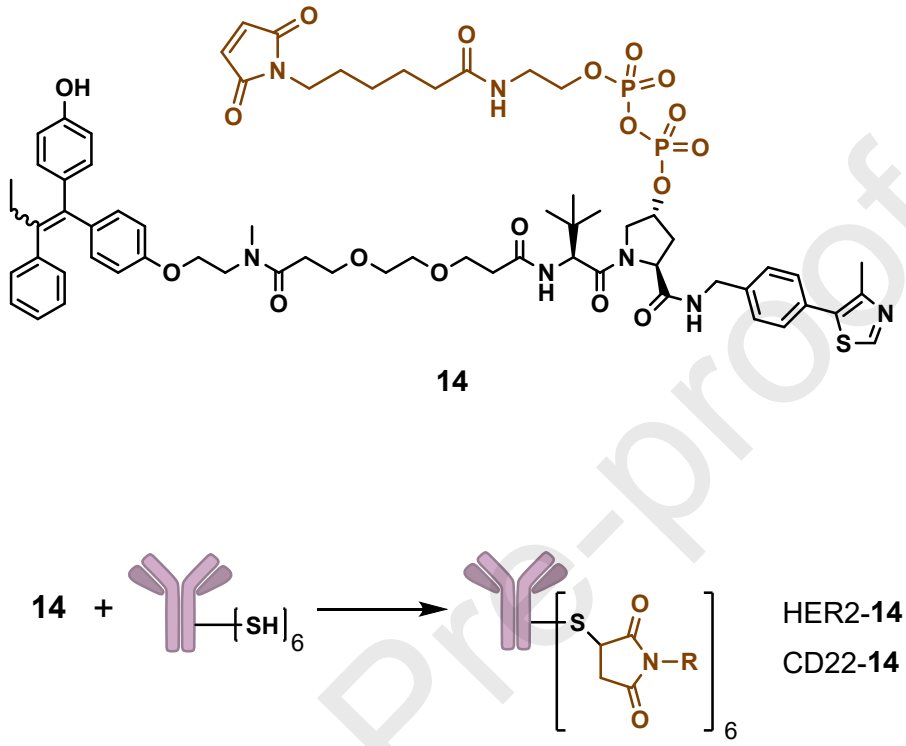
The HER2-targeting conjugate derived from linker-drug **12** exhibited strong ERα degradation activity when assessed using the MCF7-neo/HER2 cell line that was much more extensive than effects observed for the corresponding unconjugated HER2 mAb (Table 2, compare HER2-**12** with HER2-mAb, Figure S2A). Weaker ERα degradation was noted for a related control conjugate (CD22-**12**, Table 2, Figure S2A), although the potency difference relative to the HER2-**12** ADC was not as dramatic as that observed previously for conjugate pairs derived from linker-drug **11** (c.f., HER2-**11**-lc and B7H4-**11**-lc, Table 2, Figure S2A). These IF outcomes observed for HER2-**12**, CD22-**12**, and the unconjugated HER2 mAb in the MCF7-neo/HER2 cell line were qualitatively reproduced in separate western blot experiments (Figure S3). A relatively small activity difference was also detected when the HER2-**12** conjugate was tested using MCF7 parental cells as compared to the MCF7-neo/HER2 outcomes (Table 2, Figure S2B). The degradation data obtained with HER2-**12** and CD22-**12** suggested that the disulfide linker present in the conjugates underwent partial cleavage in the cell-culture media during the course of the *in vitro* experiments. Similar *in vitro* instability was previously noted for conjugates derived from related disulfide-containing linker-drugs.[21] Importantly however, these legacy ADCs exhibited reasonably good *in vivo* stability properties and also displayed large separations between targeted and non-targeted pharmacodynamic effects in xenograft tumor models.[21] We were therefore encouraged by (and not concerned with) the *in vitro* activity profiles displayed by HER2-**12** and CD22-**12**.

Somewhat surprisingly, control conjugate HER2-**13** displayed ERα degradation properties in MCF7-neo/HER2 cells that were similar to those exhibited by the HER2-**12** ADC (Table 2, Figure S2A; these results were also observed qualitatively via western blot, Figure S3). In contrast, greater activity differences between the two conjugates were noted when they were tested in MCF7 cells that did not express high levels of the HER2 receptor (Table 2, Figure S2B). The latter results were consistent with (1) a nominal level of ERα

degradation activity associated with HER2-**13** in MCF7 cells due to the presence of the endoxifen ERα binding fragment in the released degrader **7** (compare compounds **7** and **9** in Table 1 and Figure S1B) and (2) enhanced activity of HER2-**12** relative to HER2-**13** due to additional VHL-mediated ERα degradation effects (compare compounds **6** and **7** in Table 1 and Figure S1B). The stronger ERα degradation activity observed for HER2-**13** in the MCF7-neo/HER2 experiments relative to parental MCF7 cells presumably results from the combination of endoxifen-derived activity and the (relatively small) HER2-mAb ERα alterations that are exclusively observed in the former line (c.f., Table 1, Figure S2A). Due to these combined effects, it was difficult to assess the efficiency of intracellular release of degrader **6** from HER2-**12** in the MCF7-neo/HER2 experiments.

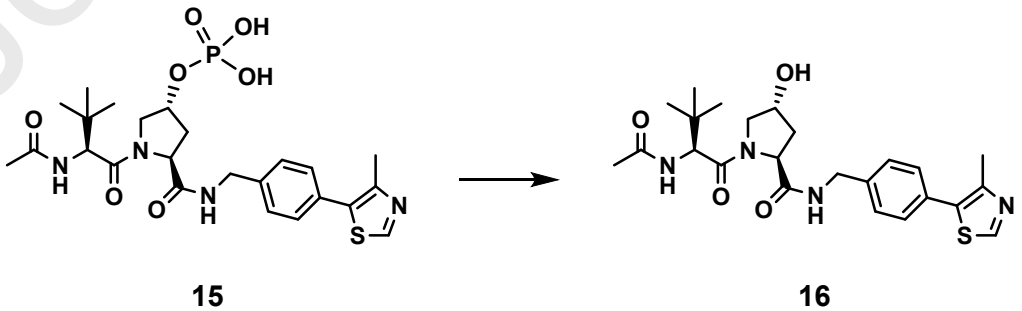
We also explored an alternate method to attach **6** to various antibodies that did not involve disulfide-based linkers. As shown in Figure 6 below, we utilized a pyrophosphate di-ester moiety to derivatize the secondary hydroxyl group present in **6** and thereby connect the chimeric degrader molecule with a maleimide that could be employed for conjugation purposes. The corresponding antibody-drug conjugates should undergo sequential phosphodiesterase and phosphatase-mediated hydrolysis following lysosomal antibody catabolism to release **6** (Scheme S4). Related pyrophosphate di-esters were described by others and were utilized to construct ADCs bearing glucocorticoid payloads.[36] However, one of these earlier reports also described the failure of phosphatase enzymes to efficiently hydrolyze a secondary glucocorticoid phosphate ester that was generated following initial pyrophosphate di-ester cleavage.[36a] Accordingly, prior to synthesizing linker-drug **14**, we first confirmed via assessment of a simple model system that the corresponding secondary phosphate moiety present in the expected intracellular catabolite was cleaved in a simulated lysosomal environment (transformation of **15** to **16**, Figure 7, see Table S2 and Supporting Information for additional details).

Figure 6. Structure of alternate linker-drug **14** derived from chimeric degrader **6** and construction of associated ADCs.



Legend. **Brown fragments** depict the linker portion of compound **14** employed for mAb attachment.

Figure 7. Conversion of model compound **15** to **16** via phosphate ester hydrolysis.



Encouragingly, a DAR6 HER2-targeting conjugate prepared using linker-drug **14** displayed strong ERα degradation activity in MCF7-neo/HER2 cells that was clearly separated from the activities of the corresponding anti-CD22 ADC and the unconjugated HER2 mAb (Table 2, Figure S2A, compare HER2-**14** with CD22-**14** and HER2-mAb). These results were consistent with the antigen-selective delivery of compound **6** to the engineered MCF7 cells via the HER2-targeting conjugate. The data also suggested that efficient intracellular release of **6** occurred via the anticipated phosphodiesterase cleavage mechanism (c.f., Scheme S4). However, since we did not prepare and test a pyrophosphate di-ester control conjugate derived from compound **7**, we cannot rule out that the ERα degradation activity observed for HER2-**14** results from a combination of endoxifen ligand and HER2-mAb effects. Somewhat surprisingly, the HER2-**14** conjugate exhibited relatively strong ERα degradation activity when assessed using parental MCF7 cells that did not over-express the surface receptor (Table 2, Figure S2B). The origin of this biological activity is not known with certainty, but it may involve intrinsic low-level HER2 expression on the parental cells whose effects are enhanced with the DAR6 ADC relative to the previously tested DAR2 conjugate (compare HER2-**11**-lc with HER2-**14**, Table 2).

Similar to our characterization of HER2-**11**-lc, we conducted preliminary *in vivo* assessments of the HER2-**12** and HER2-**14** conjugates in mice to evaluate their stability and pharmacokinetic properties. As shown in Figure S6, both conjugates were moderately stable in mice with each retaining approximately 80% of the original DAR value after 72 h in circulation. For each conjugate, the majority of DAR loss was associated with deconjugation of the corresponding linker-drugs: disulfide exchange in the case of HER2-**12** and retro-Michael transformation of HER2-**14**. Importantly, cleavage of the carbonate moiety present in HER2-**12** was not observed to a significant extent. This favorable outcome suggested that the 1,2-dimethyl-containing linker present in HER2-**12** accomplished its intended purpose of

protecting the carbonate from unwanted hydrolysis during circulation *in vivo*. Related cleavage of the HER2-**14** pyrophosphate moiety was also not appreciably observed in accordance with prior literature reports.[36]

Sparse (two-point) pharmacokinetic data were also obtained for HER2-**12** and HER2-**14** in these mouse experiments and the results are depicted in Figure S7. These limited data were consistent with lower exposures (especially at 72 h) being associated with the two conjugates relative to the unconjugated HER2 mAb. Related exposure differences between other conjugates bearing multiple copies of hydrophobic payloads and the corresponding unconjugated antibodies have been described in the literature.[37] Additional (more extensive) pharmacokinetic data are required to fully characterize the clearance and distribution properties of conjugates such as HER2-**12** and HER2-**14**.

The prospect of modulating intracellular proteins levels via rationally designed chimeric degrader molecules is rapidly transforming the biological and medicinal chemistry fields. Such entities offer multiple advantages over traditional small-molecule inhibitors (e.g., the ablation of target protein scaffolding properties), that may enhance their ability to more strongly impact biological pathways. Given their typical modular composition in which independently optimized target and ligase recognition elements are attached to each other via a chemical spacer, chimeric degraders frequently exhibit molecular and/or physiochemical properties well outside parameters currently associated with orally bioavailable compouds.[10a,38] These shortcomings may also compromise other *in vivo* delivery approaches employed with such compounds. For example, as shown in Table S4, both compounds **5** and **6** exhibited extremely poor *in vitro* DMPK properties (e.g., solubility, permeability, and metabolic stability). Consistent with these observations, both molecules also displayed poor *in vivo* performance when administered to mice orally or intravenously (Table S4, Figure

S8).[39] These unfavorable DMPK outcomes influenced our desire to explore alternate chimeric degrader delivery options including the conjugation of **5** and **6** to antibodies.

In this report, we describe a new application of antibody-drug conjugate technologies to effectively deliver chimeric ERα degrader molecules to targeted cells that expands on our related work involving BET-degrading entities.[12] Highlights of this disclosure include: (1) the antigen-dependent delivery to MCF7-neo/HER2 cells of two distinct chimeric degrader entities (compounds **5** and **6**) using three independent ADC linker modalities, (2) strong evidence for efficient intracellular degrader release from the benzyl-ether linker contained in HER2-**11**-lc following antibody-mediated delivery of the conjugate, and (3) preliminary data suggesting that the described linkers can afford *in vivo* stability properties that may enable their use in pharmacodynamic and/or efficacy experiments. Based on these outcomes, it is our expectation that this disclosure will drive interest in exploring the potential of degrader-antibody-conjugates to broadly impact medicinal chemistry and biological research activities.

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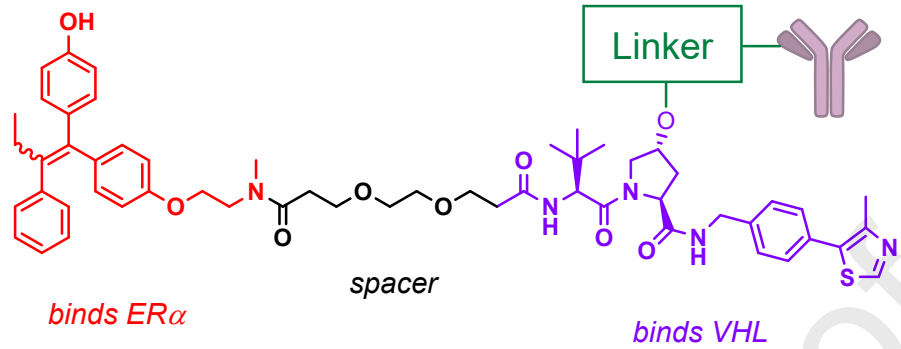
39. We did not explore alternate formulations and/or delivery approaches (IP/SC) that might improve the *in vivo* DMPK outcomes described for compounds **5** and **6**.

Graphical Abstract.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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