Design and Synthesis of Isoquinolidinobenzodiazepine Dimers, a Novel Class of Antibody—Drug Conjugate Payload

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Supporting Information

ABSTRACT: Antibody—drug conjugates (ADCs) represent an important class of emerging cancer therapeutics. Recent ADC development efforts highlighted the use of pyrrolobenzodiazepine (PBD) dimer payload for the treatment of several cancers. We identified the isoquinolidinobenzodiazepine (IQB) payload (D211), a new class of PBD dimer family of DNA damaging payloads. We have successfully synthesized all three IQB stereoisomers, experimentally showed that the purified (S,S)-D211 isomer is functionally more active than (R,R)-D221 and (S,R)-D231 isomers by >50,000-fold and ~200-fold, respectively. We also synthesized a linker-payload (D212) that uses (S,S)-D211 payload with a cathepsin cleavable linker, a hydrophilic PEG8 spacer, and a thiol reactive maleimide. In addition, homogeneous ADCs generated using D212 linker-payload exhibited ideal physicochemical properties, and anti-CD33 ADC displayed a robust target-specific potency on AML cell lines. These results demonstrate that D212 linker-payload described here can be utilized for developing novel ADC therapeutics for targeted cancer therapy.

KEYWORDS: Antibody—drug conjugates, ADCs, isoquinolidinobenzodiazepine dimers, IQB dimers, CD33, acute myeloid leukemia, AML

Targeted delivery of potent chemotherapeutic drugs in the form of antibody—drug conjugates (ADCs) has been going through an intensive investigation in oncology therapeutics. Current ADC development efforts highlighted the use of pyrrolobenzodiazepine (PBD) dimer payload for the treatment of several cancers. We identified the isoquinolidinobenzodiazepine (IQB) payload (D211), a new class of PBD dimer family of DNA damaging payloads. We have successfully synthesized all three IQB stereoisomers, experimentally showed that the purified (S,S)-D211 isomer is functionally more active than (R,R)-D221 and (S,R)-D231 isomers by >50,000-fold and ~200-fold, respectively. We also synthesized a linker-payload (D212) that uses (S,S)-D211 payload with a cathepsin cleavable linker, a hydrophilic PEG8 spacer, and a thiol reactive maleimide. In addition, homogeneous ADCs generated using D212 linker-payload exhibited ideal physicochemical properties, and anti-CD33 ADC displayed a robust target-specific potency on AML cell lines. These results demonstrate that D212 linker-payload described here can be utilized for developing novel ADC therapeutics for targeted cancer therapy.

KEYWORDS: Antibody—drug conjugates, ADCs, isoquinolidinobenzodiazepine dimers, IQB dimers, CD33, acute myeloid leukemia, AML
binding units (e.g., \((S,S)\)-D241) did not have a particularly dramatic impact on the activity of the compound across an array of cell lines; at the same time, there were no advantages of this modification that were immediately clear. The entire set of IQBs illustrated in Figure 1 can be prepared following a convergent strategy that allows for the dimers to be prepared via a mix-and-match pairing of monomers late in the syntheses. One can get a clear picture of the preparation of this class of IQBs examining the synthesis of \((S,S)\)-D211 (Scheme 1). Its preparation commences with the conversion of benzoic acid 1, via a HATU-mediated amide coupling with methyl \((S)\)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate 2, to amide 3 in a moderate 57% yield. The tetracyclic precursor to \((S,S)\)-D211 was then produced via a zinc-mediated reduction of the nitro group, performed under aqueous conditions, which accomplished the generation of an aniline capable of cyclizing intramolecularly to form the seven-membered ring, characteristic of this class of benzodiazepines, in exceptional yield. The NS of the dilactam can then be protected in a straightforward fashion: treatment with the kinetic base NaH and addition of SEM-Cl nets a compound primed for imine generation at a later stage of the synthesis. The benzyl protecting group is removed rapidly under standard hydrogenolysis conditions to produce the phenolic monomer 5 in nearly quantitative yield. The final target can then be produced in a two-step sequence: generation of the dimer via a double Mitsunobu reaction with 1,3-propanediol is followed by a reduction of the SEM-protected lactam 16 to the \(N_5\)-imine (via treatment with superhydride at low temperature) to smoothly produce the desired IQB, \((S,S)\)-D211. One advantage of this synthetic strategy is that a wide number of variants to \((S,S)\)-D211 can be accessed rapidly through judicious selection of the amine precursor in step one of this sequence and the diol applied during the Mitsunobu step.

As D211 showed comparable in vitro potency to that of PBD payload, we endeavored to prepare a linker-payload construct, suitable for conjugation to an antibody. Not wanting to sacrifice any of the observed cell-killing potency of this new payload, we sought to attach a traceless/cleavable linker to ensure the released payload would be unmodified D211. The use and success of the cathepsin-cleavable linker for ADCs are well-established as phenomena in the clinic; we therefore decided to go with a cathepsin cleavable Val-Ala linker that was attached via \(N_5\) by way of a \(para\)-amino-benzyl carbamate. A PEG8-containing spacer was included in the design to help lower the construct’s overall hydrophobicity. This same strategy, reported recently, was successfully employed with the ADC Rova-T.13

With the earlier convergent syntheses of the free-IQBs (Scheme Figure 2. In vitro potency data of IQB payload in AML2 cell line.

Table 1. *In Vitro* Potency Data of IQB Class of Payloads (IC\textsubscript{50} Values Described Are in Picomolar)\textsuperscript{a}

<table>
<thead>
<tr>
<th>cell line</th>
<th>PBD</th>
<th>D201</th>
<th>D211</th>
<th>D221</th>
<th>D231</th>
<th>D241</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML2</td>
<td>0.22</td>
<td>0.61</td>
<td>0.21</td>
<td>14001</td>
<td>40</td>
<td>0.31</td>
</tr>
<tr>
<td>AML3</td>
<td>0.25</td>
<td>1.14</td>
<td>0.31</td>
<td>13925</td>
<td>49</td>
<td>0.47</td>
</tr>
<tr>
<td>AML5</td>
<td>0.14</td>
<td>0.91</td>
<td>0.18</td>
<td>13925</td>
<td>116</td>
<td>0.85</td>
</tr>
<tr>
<td>HL60</td>
<td>1.58</td>
<td>3.19</td>
<td>1.57</td>
<td>91005</td>
<td>230</td>
<td>2.28</td>
</tr>
<tr>
<td>SHI</td>
<td>0.28</td>
<td>1.25</td>
<td>0.61</td>
<td>150480</td>
<td>685</td>
<td>1.14</td>
</tr>
<tr>
<td>HEPG2</td>
<td>1.21</td>
<td>NA</td>
<td>18.88</td>
<td>NA</td>
<td>NA</td>
<td>11.72</td>
</tr>
<tr>
<td>A704</td>
<td>9.85</td>
<td>NA</td>
<td>2.59</td>
<td>NA</td>
<td>NA</td>
<td>3.67</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Cancer cell lines were incubated with PBD or IQB dimer payload for 72 h. IC\textsubscript{50} values were determined by quantitating viable cells using a CellTiter-Glo luminescent cell viability assay.

Figure 1. Structures of IQB derivatives and SGD-1882, along with ring numbering convention(s).

Scheme 1. Synthesis of IQB Dimer \((S,S)\)-D211\textsuperscript{a}

\textsuperscript{a}Reagents and conditions: (a) HATU, DIEA, 2, DCM, 57%; (b) Zn, NH\textsubscript{4}Cl, THF/H\textsubscript{2}O, 92%; (c) NaH, SEM-Cl, THF, 88%; (d) H\textsubscript{2}, Pd/C, MeOH, 99%; (e) 1,3-propanediol, PPh\textsubscript{3}, DIAD, THF, 37%; (f) superhydride, THF, \(-78^\circ\text{C}\) to rt, 55%.
1), not immediately amenable to the attachment of the linker, we turned our attention to the monofunctionalization approach shown in Scheme 2. The bis-nitro compound 6 is readily available in three steps from vanillic acid. Hydrolysis of the methyl esters was performed, in analogous fashion to the monomers, followed by a HATU-mediated amide coupling with (S)-(1,2,3,4-tetrahydroisoquinolin-3-yl)methanol to produce diol 8 in excellent yield. Using Zn/NH4Cl under the same conditions that were successful for the monomers, a symmetrical bis-aniline can be generated cleanly. It is at this stage in the preparation of D212 that we addressed the need for monofunctionalization, by first reacting the aniline with 1 equiv of allyl chloroformate (Alloc-Cl), followed by treatment with TBS-Cl in DMF, with imidazole as a base to produce the triprotected monoaniline 9. After numerous unsuccessful attempts to attach the linker via trapping of an intermediate isocyanate with a benzyl alcohol, and yet further efforts directed at preparation of a chloroformate-functionalized linker, we were quite pleased to find successful appendage of the linker could be achieved when aniline 9 was mixed in THF, in the presence of the pentafluorocarbonate 10 (see Supporting Information for the synthesis), in the absence of any supplementary base. Initial successes were obtained when the process was performed at 50 °C, but it was soon noted that, at elevated temperature (or in the presence of a base), 10 can decompose to the pentafluorobenzyl ether, a compound also capable of alkylating N5, but found to produce a construct incapable of the facile release of D211 (see Scheme 3).

When the linker attachment was performed at room temperature, the reaction was particularly clean but required 6 days to reach completion. Treatment of 9 under standard desilylation conditions readily produced diol 11 in good yield.

Scheme 2. Synthesis of D212 Linker-Payload

Scheme 3. Generation of Undesired Benzyl Ether/Alkylation Product
The next required hurdle to clear en route to the final payload-linker construct was the sequential oxidation/cyclization transformations that are required to produce the hallmark benzodiazepines. Evaluation of numerous means to accomplish this feat (e.g., PCC mediated-, Swern-,17 Dess Martin,19 and Ley-oxidation) ultimately led to a highly efficient process through the judicious application of IBX20 (2.0 equiv per alcohol functionality for fast reaction yet limited overoxidation side products). With 12 in hand, all that remained was the removal of the Alloc- and Boc-protecting groups under standard conditions, followed by installation of the maleimide spacer via a simple amide coupling to the terminus of the PEGylated linker to produce the D212 linker-payload construct in 15% yield over the last three steps.

Prior to the preparation of an ADC, we thought it appropriate to evaluate the efficiency of payload release via the performance of an in vitro enzymatic cathepsin B cleavage assay. Upon treatment of the D212 linker-payload with bovine spleen derived cathepsin B under standard conditions,21 we were pleased to observe rapid and competent release of the “free-drug” D211. Figure 3 illustrates the time-course and profile of release, both of which were quantified using a RP-HPLC-based method, connected to an inline mass spectrometer. Beginning with the pure D212 linker-payload construct (Figure 3A), the cleavage reaction was determined to be at 59% conversion after 30 min (Figure 3B) and reached over 80% conversion after overnight incubation (Figure 3C).

With the successful preparation of D212 (and the encouraging results of the cathepsin B cleavage assay), we performed its conjugation anti-CD33 with two engineered cysteine residues (S239C) per antibody as described earlier.12 Applying a global reduction (using DTT) and reoxidation (using dhAA) strategy as described earlier,22 the engineered cysteines were unmasked and then reacted with the maleimide-functionalized linker-payload construct to produce anti-CD33-D212 ADC (DAR of 1.7) with both high efficiency and high monomer content, circumventing the need for chromatographic purification. A nonbinding isotype control ADC (IgG1-D212 with a DAR of 1.8) was also produced (see full details within the Supporting Information).

The anti-CD33-D212 ADC and IgG1-D212 ADC were evaluated via incubation with AML cell lines (AML2 and HL60) at 37 °C for 5 days. The cell viability was tested using Promega CellTiter-Glo assay. These data demonstrate excellent target-specific potency (>200−500 fold) of the anti-CD33-ADC compared to a nonbinding control ADC (Figure 4).

In conclusion, D212 exhibits ideal ADC linker-payload characteristics: its conserved mechanism of action of DNA alkylation is similar to the well-known PBD dimer class of payloads (extensively used for several ADC programs that are in clinical development),23 it contains a clinically proven cleavable linker (Val-Ala-PAB),4 inclusion of a PEG8 spacer contributes to the favorable solubility and hydrophilicity properties of the linker-payload, and last, conjugation can be carried out via well-established cysteine-maleimide chemistry. Preliminary in vitro characterization of anti-CD33 ADC using D212 linker-payload suggested that D212 linker-payload exhibited similar potency as a well-characterized PBD linker-payload, as shown earlier.12,13 Thus, D212 could be valuable linker-payload asset to the growing family of diverse linker-payloads that are being used in developing novel ADC therapeutics.

ASSOCIATED CONTENT
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Supporting Information
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Experimental procedures and tabulated and scanned spectra of key compounds, including conjugates (PDF)

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V.J. and J.R.J. conceived the idea. S.W.S., D.B., and S.J.D. established synthesis protocols and synthesized all chemical compounds described in this manuscript. J.Z., J.L., J.H., and Y.-P.J. tested potency of payloads and ADCs. S.W.S, D.B., and J.R.J. wrote the manuscript, and J.R.J. managed and led the program.

Notes
The authors declare the following competing financial interest(s): All authors from TCRS (now Abzena) worked on this project under Cellerant Therapeutics as part of a fee-service agreement. All authors are/ were either full-time employees or contractors of Cellerant Therapeutics or from The Chemistry Research Solutions (now Abzena).

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■ ABBREVIATIONS
ADC, antibody–drug conjugate; AML, acute myeloid leukemia; DCM, dichloromethane; DlEAE, disopropyl ethyl amine; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate; HL, Hodgkin’s lymphoma; PBD, pyrrolobenzodiazepine; IQB, isoquinolindino-benzodiazepine; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran

■ REFERENCES


