Multivalent Antibiotics via Metal Complexes: Potent Divalent Vancomycins against Vancomycin-Resistant Enterococci

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Dimers of vancomycin (Van), linked by a rigid metal complex, \([\text{Pt(en)}(\text{H}_2\text{O})_2]^2+\), exhibit potent activities (MIC ~0.8 \mu g/mL, ~720 times more potent than that of Van itself) against vancomycin-resistant enterococci (VRE). The result suggests that combining metal complexation and receptor/ligand interaction offers a useful method to construct multivalent inhibitors.

**Introduction**

Drug resistance of bacteria poses a serious public health threat and demands effective counter measures. Among promising approaches, multicentricity—multiple simultaneous binding of two or more ligands and receptors—is beginning to be explored systematically. In the research of multivalency, the ligand—receptor pair of vancomycin (Van)-D-Ala-D-Ala has attracted a great deal of research attention because it relates to vancomycin-resistant enterococci (VRE).

Walsh and colleagues have deciphered the mechanism of vancomycin resistance: VRE mutates its terminal peptides from D-Ala-D-Ala to D-Ala-D-Lac (i.e., $\text{D-Ala-D}-\text{Lac}$), which has substantially lowered its affinity to Van. Though Van self-associates to form homodimers upon binding to D-Ala-D-Ala, as elucidated by Williams et al., this noncovalent dimerization of Van alone is insufficient to act against VRE. Griffin et al., Nicolaou et al., and an Eli Lilly group have used organic linkers to synthesize dimers of Van, and demonstrated that covalently linked dimeric Vans exhibit enhanced potency against VRE. It was, however, suggested that the flexibility of the organic linker limited the avidity of the multivalent binding due to the loss of conformational entropy upon binding. We believe that the combination of receptor/ligand interaction and a metal complex, which has special geometry, structural rigidity, and stability, can serve as an alternative approach to minimize the loss of conformational entropy.

To test this strategy, we used a derivative of cisplatin, \([\text{Pt}(\text{en})(\text{H}_2\text{O})_2]^2+\), a rigid, square planar metal complex—to form dimeric Vans, and evaluated their activities against VRE. These rigidly linked dimeric Vans exhibit enhanced activities against VRE and are up to ~720 times more potent against VRE than Van itself in the best case (MIC: 0.8 \mu g/mL). Our results suggest that combining metal coordination and receptor/ligand interactions offers a useful method to construct multivalent receptors.

**Results and Discussions**

We chose \([\text{Pt}(\text{en})]^2+\) as the rigid linker to form dimeric Van via complexation due to its extensive developed chemistry, well understood properties, and well preserved planar rigidity. Moreover, the ionic nature of the \([\text{Pt}(\text{en})]^2+\) retains—if not increases—the solubility of Van in aqueous media, which is necessary for in vitro study. As shown in Scheme 1, commercially available vancomycin (1) reacted with 3-picolyamine, 4-picolyamine, 3-(2-aminoethyl)pyridine, or 1-(3-aminopropyl)midiadazole to give the vancomycin carboxamide derivatives (VanCONH-L) in good yields (>65%), respectively. Compounds 2a–d were purified using reversed-phase HPLC according to modified literature procedure and characterized by high field $^1\text{H}$ NMR spectroscopy and mass spectrometry (MS). The attachment of the ligands to the C-terminal of Van hardly changes conformation of Van, as indicated by $^1\text{H}$ NMR—the chemical shifts of the protons belonging to Van on 2a–d remain essentially the same as that in 1, suggesting that the binding pockets of 2a–d are undisturbed and should function similarly as that of 1. [\text{Pt}(\text{en})(\text{H}_2\text{O})_2]^2+ (3a) coordinates with 2a–d to give dimeric Vans 4a–d, and \([\text{Pt}(\text{en})(\text{H}_2\text{O})_2](\text{N-pyr})_2\text{methylacetamide})^2+ (3b)$ binds to 2a,b to afford monomeric Vans 5a,b.

As shown in Table 1, 2a–d are inactive against VRE, and 3a or 3b alone is inactive against both the Van-sensitive strain and VREs (MIC > 128 \mu g/mL). A simple mixture of Van and \([\text{Pt}(\text{en})(\text{H}_2\text{O})_2](\text{NO}_3)_2\) (1+3a) or the monovalent complexes of Van and \([\text{Pt}(\text{en})]^2+\) behave similarly to the corresponding monomeric Vans. These results exclude the possibility that enhanced activities of dimeric Vans against VRE originate from some unrelated synergistic effects between monomeric Van and \([\text{Pt}(\text{en})]^2+\). 4a–d exhibits enhanced activity against VRE in comparison to Van. In fact, 4a is ~10^3 times more potent against VRE (genotype VanA) than Van itself. In an attempt to form the tetraivalent compound, \([\text{Zn}(\text{en})]^2+\), the activity of the mixture of 2d and Zn-(OAc)$_2$ (4:1, at pH = 7) against VRE is the same as that...
of 2d, suggesting that there is no multivalent Van formed by metal complexation when 2d and Zn(OAc)\(_2\) react at the condition of the in vitro experiment. We also did not observe the formation of \([\text{Zn}(2d)_{\_n}]^{2n+2+}\) (\(n = 1\)–\(4\)) by ESI-MS, which is consistent with the observed activity.

Figure 1 illustrates the plausible divalent interaction between 4a and the terminal peptides of peptidoglycan precursors. According to this binding mode, both the configuration and rigidity of the dimeric Vans determine their activities.\(^{34}\) To further understand the structural-activity relationship, we performed semiquantitative entropy analysis according to the reported methods.\(^{36}\)

$$\Delta S_{\text{conf}}$$ of 6 would be the sum of torsional entropies of two C–C bonds and an S–S bond (\(-\Delta S_{\text{conf}} = 7.3 \times 2 + 3.5 = 18.1\) J/mol K).\(^{36}\) If we consider only the contribution of \(\Delta S_{\text{conf}}\) to the \(\Delta G\) and assume that the binding occurs at room temperature, the ratios of binding constants \(K_{\text{dil}}/K_n\) of 4a–d, 6, and 7 can be calculated. In the case of VanA strains (Table 2), the ratios of binding constants \(K_{\text{dil}}/K_n\) agree qualitatively with the MIC values (except 4b). For example, 4a is \(\sim 5\) times more potent than 6,\(^{17}\) which agrees with their \(\Delta S_{\text{conf}}\) values. 4c resembles 4a structurally except that it has an extra CH\(_2\) increasing the flexibility its linker; therefore, 4c exhibits lower activity against VRE. Similarly, additional flexibility in 4d or

### Table 1. Antibacterial Activity (MIC: \(\mu\text{g/mL}\)) of Vancomycin Derivatives and Dimeric Vans

<table>
<thead>
<tr>
<th>compound</th>
<th>ATCC29212 (sensitive)</th>
<th>E. gall (VanC)</th>
<th>E. faecium (VanB)</th>
<th>E. faecalis (VanA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>8</td>
<td>102</td>
<td>576</td>
</tr>
<tr>
<td>2a</td>
<td>2</td>
<td>4</td>
<td>76.8</td>
<td>(&gt;128)</td>
</tr>
<tr>
<td>2b</td>
<td>2</td>
<td>4</td>
<td>70.4</td>
<td>(&gt;128)</td>
</tr>
<tr>
<td>2c</td>
<td>2</td>
<td>2</td>
<td>19</td>
<td>104</td>
</tr>
<tr>
<td>2d</td>
<td>2</td>
<td>2</td>
<td>22</td>
<td>64</td>
</tr>
<tr>
<td>3a</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
</tr>
<tr>
<td>3b</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
<td>(&gt;128)</td>
</tr>
<tr>
<td>1+3a</td>
<td>4</td>
<td>8</td>
<td>64</td>
<td>160</td>
</tr>
<tr>
<td>4a</td>
<td>1</td>
<td>0.03</td>
<td>0.28</td>
<td>0.8</td>
</tr>
<tr>
<td>4b</td>
<td>1</td>
<td>0.05</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>4c</td>
<td>1</td>
<td>1</td>
<td>1.2</td>
<td>3.3</td>
</tr>
<tr>
<td>4d</td>
<td>1</td>
<td>1</td>
<td>4.8</td>
<td>38</td>
</tr>
<tr>
<td>5a</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>84</td>
</tr>
<tr>
<td>5b</td>
<td>1</td>
<td>2</td>
<td>32</td>
<td>88</td>
</tr>
<tr>
<td>2d+Zn(_2)</td>
<td>1</td>
<td>2</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1.6</td>
<td>5.5</td>
</tr>
</tbody>
</table>
7 results in the loss of $\Delta S_{\text{conf}}$ upon divalent binding and the decrease of its activity. Similar qualitative agreement also exhibits between $\Delta S_{\text{conf}}$ and the MIC values in the case of VanB strains. The discrepancy between $K_{d4a}/K_{d4b}$ and the MIC of 4b apparently originates from the configuration of 4b, which contributes to the changes of enthalpy. Though other mechanisms cannot be ruled out at this moment, the above analysis supports the hypothesis of the roles of rigidity in divalency for the case of VanA and VanB strains. $\Delta S_{\text{conf}}$ correlates, however, to little enhancement of the activity of dimeric Vans against vancomycin-sensitive strains and the less resistant VRE (VanC), which has been observed in other systems of divalent Vans,16,17,34 suggesting the enhancement of the activity of dimeric Vans may depend on the composition of peptidoglycan precursors produced by the strains.43

Conclusion

In summary, we have demonstrated that a metal complex can be used as a new platform to construct multivalent inhibitors, which are as effective as other rigid linkers44–46 used for multivalency. One of the concerns on platinum-based complexes is its cytotoxicity. Our preliminary study has shown that these cis-platin-based divalent Vans are not toxic toward mammalian cells (the detailed work will be published elsewhere). Our future work will examine other metal complex linkers, which may help further elucidate the structural basis of vancomycin resistance,43,47 as well as the mechanism of multivalent Vans binding to vancomycin-sensitive strains,48,49 which has yet to be established.

Experimental Section

General. Chemical reagents and solvents were used as received from commercial sources. Dimethyl sulfoxide (DMSO) was dried over 4 Å molecular sieves and dimethylformamide (DMF) was dried over silica gel. $^1$H NMR spectra were obtained on a 500 MHz Varian XL-500 in $d_{6}$-acetone (CDCl$ _3$) or $d_{6}$-acetone (CDCl$ _3$) with Me$_2$Si as internal reference. $^1$H and $^13$C NMR spectra were acquired on a 400 MHz Varian Unity spectrometer. HR-ToF-MS was measured with a Micromass TOF system. UV data were recorded on a Shimadzu UV-2101 spectrometer. HPLC was carried out with Waters 600 Controller and 4a, 2.5 mg of [Pt(en)-(H$_2$O)$_2$](NO$_3$)$_2$ (0.0060 mmol, 1.0 equiv) was added to a solution of 2c (20 mg, 0.0129 mmol, 2.15 equiv) in 1 mL of DMSO. The mixture was stirred for 20 h at room temperature in dark. During the whole procedure, RP-HPLC was used to monitor the reaction. In the first 3 h, a vancomycin-3-ethylenic-pyridine-carboxamide peak exists at the elution time of 24 min. With increased time, a new peak at 18 min was found and the peak intensity of the starting material at 24 min was decreased. After 14 h, RP-HPLC indicated that almost all vancomycin-3-ethylenic-pyridine-carboxamide was consumed completely. Then by quenching the reaction with 10 mL of acetone, a white solid was precipitated. This crude product was filtered and washed three times with acetone and dried under vacuum before it was redissolved in H$_2$O and separated by RP-HPLC. After purification by HPLC, 15.6 mg of pure product was obtained (yield: 75.8%): $^1$H NMR (500 MHz, DMSO-$ d_{6}$) $\delta$ 9.18 (v br s), 8.77 (v br s), 8.47 (d, 5.5 Hz), 8.67 (br s), 8.54 (s), 8.30 (s), 8.09 (d, 7.8 Hz), 7.98 (s), 7.75 (overlapped), 7.73 (d, 7.0 Hz), 7.59 (d, 7.8 Hz), 7.57 (s), 7.45 (d, 8.6 Hz), 7.33 (s), 7.31 (d, 8.6 Hz), 7.15 (br s), 7.08 (d, 10.1 Hz), 6.89 (d, 8.5 Hz), 6.82 (d, 8.5 Hz), 6.80 (br s), 6.64 (v br s), 6.47 (s), 6.34 (s), 6.09 (br s), 5.87 (d, 7.8 Hz), 5.69 (s), 5.57 (s), 5.38 (s), 5.37 (s), 5.35 (s), 5.31 (br s), 5.04 (br m), 4.79 (d, 6.2 Hz), 4.57 (d, 3.1 Hz), 4.43 (br q, 5.5 Hz), 4.34 (d, 10.1 Hz), 4.04 (d, 11.0 Hz), 3.37 (s), 3.29 (s), 2.96 (m), 2.75 (s), 2.65 (d, 4.7 Hz), 2.52 (s), 2.27 (dd, 16.4 Hz, 6.5 Hz), 2.01 (br d, 9.4 Hz), 1.85 (br d, 10.6 Hz), 1.79 (non 7.0 Hz), 1.69 (q, 7.0 Hz), 1.66 (q, 7.0 Hz), 1.42 (s), 1.18 (d, 6.2 Hz), 1.01 (d, 6.2 Hz), 0.96 (d, 6.2 Hz). ESI-MS: The peaks at m/z 1212.3, 1680.4, 1158.6, and 1737.5 correspond to M$ ^+$, M$ ^{+2}$, (M + TFA)$ ^{+3}$, and (M + TFA)$ ^{+4}$, respectively.

Synthesis of dimeric Van 4a: Similar to the synthesis of 4c, 2.5 mg of [Pt(en)-(H$_2$O)$_2$](NO$_3$)$_2$ (0.0060 mmol, 1.0 equiv) was added to a solution of 2a (20 mg, 0.0130 mmol, 2.16 equiv) in 1 mL of DMSO. After being purified by HPLC, 14.0 mg of pure product was obtained (yield: 69.6%): $^1$H NMR (500 MHz, DMSO-$ d_{6}$) $\delta$ 9.18 (v br s), 9.09 (v br s), 8.81 (d, 4.9 Hz), 8.76 (s), 8.72 (s), 8.69 (br s), 8.54 (v br s), 8.09 (d, 8.6 Hz), 7.95 (s), 7.76-
Synthesis of dimeric Van 4b: 2.5 mg of \([\text{Pt}(\text{en})(\text{H}_2\text{O})]_2\) \((\text{NO}_3)_2\) \((0.0060 \text{ mmol}, 1.0 \text{ equiv})\) was added to a solution of 2b \((20 \text{ mg}, 0.0130 \text{ mmol}, 2.16 \text{ equiv})\) in 1 mL of DMSO. After being purified by HPLC, 15.5 mg of pure product was obtained (yield: 76.1\%): 

**ESI-MS:** The peaks at m/z 667.3, 833.7, 1111.5, 689.6, 862.2, and 1149.6 correspond to M\(^{+}\), M\(^{+}\), M\(^{+}\), (M + TFA)\(^{+}\), and (M + TFA)\(^{+}\), respectively.

Synthesis of dimeric Van 4c: Again, similar to the synthesis of 4c, 2.5 mg of \([\text{Pt}(\text{en})(\text{H}_2\text{O})]_2\) \((\text{NO}_3)_2\) \((0.0060 \text{ mmol}, 1.0 \text{ equiv})\) was added to a solution of 2d \((20 \text{ mg}, 0.0128 \text{ mmol}, 2.15 \text{ equiv})\) in 1 mL of DMSO. After being purified by HPLC, 16.0 mg of pure product was obtained (yield: 78.8\%): 

**ESI-MS:** The peaks at m/z 834.16, 1111.15, and 1666.25 correspond to M\(^{+}\), M\(^{+}\), and M\(^{+}\), respectively.

Synthesis of dimeric Van 4d: Following to the same procedure as for 2a, 6.8 mg of cystamide dihydrochloride \((30 \text{ mmol}, 1.0 \text{ equiv})\) was added to a solution of vancomycin hydrochloride \((100 \text{ mg}, 67 \text{ mmol}, 2.2 \text{ equiv})\) in 1 mL of dry DMSO. The mixture was cooled to 0°C and HBTU \((90 \text{ mmol}, 3 \text{ equiv})\) in 1 mL of DMF was added, followed by DIEA \((0.057 \text{ mL}, 328 \text{ mmol}, 4.88 \text{ equiv})\). The reaction was allowed to rise to room temperature and stirred for overnight. At this time, analytical RP-HPLC showed that a vancomycin peak still existed. Further addition of HBTU \((10 \text{ mg}, 0.026 \text{ mmol}, 0.39 \text{ equiv})\) and DIEA \((0.024 \text{ mL}, 0.164 \text{ mmol}, 0.24 \text{ equiv})\) was made. After another 24 h, the reaction was monitored with HPLC again and almost all vancomycin was found to have been consumed. To quench the reaction, the reaction mixture was added dropwise into 15 mL of acetone by using syringe. A white solid was precipitated out and filtered, and 5 mL of acetone was used to wash the solid once. The white solid was purified by reversed-phase HPLC (RP-HPLC). The percentage yield is 52\%: 

**ESI-MS:** The peaks at m/z 579.5, 833.7, 1111.5, and 1588.1 correspond to M\(^{+}\), M\(^{+}\), M\(^{+}\), and M\(^{+}\), respectively.

### Multivalent Antibiotics via Metal Complexes

The crude product was purified by flash column chromatography (silica gel, Ethocel, 1:4) and yielded 412 mg (22\%) to give white solid. **ESI-MS** (CDI-MS, m/z 7.26 ppm) of 37,486.3, J = 2.2 Hz, 1H, CH), 8.40 (d, J = 8.2 Hz, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). **NMR** (CDI-MS, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). The crude product was purified by flash column chromatography (silica gel, Ethocel, 1:4) and yielded 412 mg (22\%) to give white solid. **ESI-MS** (CDI-MS, m/z 7.26 ppm) of 37,486.3, J = 2.2 Hz, 1H, CH), 8.40 (d, J = 8.2 Hz, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). **NMR** (CDI-MS, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). The crude product was purified by flash column chromatography (silica gel, Ethocel, 1:4) and yielded 412 mg (22\%) to give white solid. **ESI-MS** (CDI-MS, m/z 7.26 ppm) of 37,486.3, J = 2.2 Hz, 1H, CH), 8.40 (d, J = 8.2 Hz, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). **NMR** (CDI-MS, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). The crude product was purified by flash column chromatography (silica gel, Ethocel, 1:4) and yielded 412 mg (22\%) to give white solid. **ESI-MS** (CDI-MS, m/z 7.26 ppm) of 37,486.3, J = 2.2 Hz, 1H, CH), 8.40 (d, J = 8.2 Hz, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). **NMR** (CDI-MS, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). The crude product was purified by flash column chromatography (silica gel, Ethocel, 1:4) and yielded 412 mg (22\%) to give white solid. **ESI-MS** (CDI-MS, m/z 7.26 ppm) of 37,486.3, J = 2.2 Hz, 1H, CH), 8.40 (d, J = 8.2 Hz, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)). **NMR** (CDI-MS, 1H, CH), 7.86 (dd, J = 8.2 Hz, 2.2 Hz, 1H, CH), 4.54 (s, 2H, CH\(_2\)).
5.5'-Bis(azidomethyl)-2,2'-bipyridine (7b). NaN₃ (42 mg, 0.66 mmol, 2.2 equiv) was added to a solution of 5,5'-bis(bromomethyl)-2,2'-bipyridine (100 mg, 0.29 mmol, 1 equiv) in dry DMF (3 mL). The solution was heated for 17 h at 90 °C and then concentrated under vacuum to yield a mixture, which was treated with CH₂Cl₂. The solid (NaBr) was filtered off, and the extract was concentrated in a vacuum to yield the product (white solid), which was purified by flash column chromatography (silica gel, EtOAc/hexane, 40/60) and yielded 69 mg (90%) of pure white solid. ¹H NMR (CDCl₃, δ 7.26 ppm) δ 8.64 (d, J = 1.8 Hz, 1H, CH), 8.45 (d, J = 8.2 Hz, 1H, CH), 7.81 (dd, J = 8.2 Hz, 2.3 Hz, 1H, CH), 4.44 (s, 2H, NH), 3.45 ppm. [2C NMR (CDCl₃, δ 77.7 ppm) δ 156.35, 149.44, 137.41, 131.99, 121.81, 52.68. ESI-MS: The peak at m/z 267 corresponds to (M + 1)⁺.

5.5'-Bis(aminomethyl)-2,2'-bipyridine (7c). 10% Pd on activated carbon (15 mg) was dissolved in 1 mL of dry CH₂Cl₂ in a closed round-bottom flask with vigorous stirring. 6M HCl was added to another round-bottom flask that contained zinc powder in order to generate hydrogen gas. These two round-bottom flasks were connected by a rubber pipe. Make sure that there is no leakage. An outlet was introduced in the round-bottom flask containing 10% Pd on activated carbon. 5.5'-bis(azidomethyl)-2,2'-bipyridine (60 mg, 0.23 mmol, 1 equiv) in 2 mL of dry CH₂Cl₂ was added to the round-bottom flask containing 10% Pd on activated carbon until the outlet was introduced. The reaction was completed within 4 h. 10% Pd on activated carbon was filtered by Celite and CH₂Cl₂ was removed. The reaction mixture was treated with 10% Pd on activated carbon 3 min after the outlet was added. The reaction was filtered by Celite and CH₂Cl₂ was removed. 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