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Enzyme transformation to modulate the ligand-receptor interactions between small molecules

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Abstract

Enzymatic transformation is a fundamental process to control ligand-receptor interactions among proteins for signal transduction in cells. Here we report the first example of enzymatic transformation regulated ligand-receptor interactions of small molecules, in which enzymatic reaction changes the stoichiometry of the ligand-receptor binding from 1:1 to 1:2. We also show that this unique integration of enzymatic transformation and ligand-receptor interactions of small molecules is able to affect the fate of cells.

This communication describes the use of enzymatic reaction to modulate the binding between small molecules. On the cell surface, many receptors carry out signalling functions upon enzymatic transformation. For example, protein kinases and phosphatases catalyze phosphorylation and dephosphorylation of proteins as a key mechanism of immune responses.[1] This ubiquitous type of enzyme-associated ligand-receptor interactions of cells illustrates a fundamental feature of live system. Thus it would be of great importance to use small molecules to mimic the essence of this process for controlling the fate of cells. Despite its profound implication, this approach receives little attention and has yet to be explored, largely due to the lack of a proper ligand-receptor system of small molecules.

One of well-established ligand-receptor pair of small molecules is vancomycin (Van) and D-Ala-D-Ala. As an important antibiotic to treat methicillin-resistant Gram-positive infections, the ligand-receptor interaction between Van and D-Ala-D-Ala has received extensive investigation, especially in the works of Williams,[2, 3] Whitesides,[4] as well as other groups.[5] Specifically, Van binds with D-Ala-D-Ala via five hydrogen bonds (Fig 1A, upper panel) and can achieve binding in micromolar concentrations. Since the proper N-terminal functionalization confers additional features to D-Ala-D-Ala without seriously compromising the hydrogen bonding between Van and D-Ala-D-Ala, this relatively simple ligand-receptor pair offers a versatile model system to mimic and to control fundamental biological processes. For example, we recently found that Van binds with two Fmoc-D-Ala-D-Ala molecules to catalyze the formation of the aggregates of Fmoc-D-Ala-D-Ala that inhibit cell proliferation.[6] Besides offering a reproducible way to generate aggregates of small molecules, this ligand-receptor catalysed aggregation offers a unique opportunity to

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combine enzymatic transformation, an emerging, powerful approach to control spatiotemporal self-assembly of small molecules,[7] with ligand-receptor interactions for achieving specific biological functions.[8–10]

Based on the above rationale, we design molecule **1a** (Fmoc-$_p$-Tyr-Gly-Gly-D-Ala-D-Ala) to incorporate an enzyme trigger, tyrosine phosphate, into peptide Fmoc-Gly-Gly-D-Ala-D-Ala. Phosphatase converts **1a** to **1b** by catalytic dephosphorylation. Isothermal titration calorimetry (ITC) reveals that Van binds with one molecule of **1a**, but two molecules of **1b**. Moreover, light scattering results show that the use of enzymatic catalysis regulates the ligand-receptor interactions between Van, **1a**, and **1b** by dephosphorylating **1a** to form **1b** that binds Van in 2:1 ratio, leading to the formation of the aggregates of Van and **1b** (Fig. 1). Moreover, the aggregates generated are able to inhibit cell proliferation. As the first example of enzymatic transformation regulated the ligand-receptor interactions of small molecules, this work illustrates a new approach to mimic the essence of living systems.

To investigate how Van binds with the D-Ala-D-Ala derivatives (i. e., **1a** and **1b**), we use isothermal titration calorimetry (ITC) to examine heating profile during titration. Figure 2A shows the heating flow of each injection during the titration of Van (8.0 mM) into solution of **1a** (0.8 mM). After correcting the background dilution and fitting with the independent model, we obtain the dissociation constant (K_d) to be 291 μM and the binding ratio of Van and **1a** at 1:1. This result not only agrees with the high affinity between Van and D-Ala-D-Ala,[3] but also suggests that one Van binds with one molecule of **1a**. ITC indicates that the dissociation constant (K_d) between **1b** and Van is 75 μM, and one Van binds with two molecules of **1b**. This result implies that the binding affinity between Van and the D-Ala-D-Ala derivatives would increase about four times and the binding stoichiometry would change from 1:1 to 1:2 upon enzymatic dephosphorylation. In other words, these results confirm that enzymatic transformation modulates the ligand-receptor interaction between Van and the D-Ala-D-Ala derivatives. Moreover, such binding likely originates from hydrogen bonding and intermolecular aromatic-aromatic interactions between the fluorene group in **1b**, which induces the aggregation of **1b** (a plausible mode of interaction in Fig. S1).

To evaluate the aggregation of **1b** regulated by ligand-receptor interaction and enzyme catalysis, we use light scattering to monitor the change of light scattering signal before and after initiating the enzymatic dephosphorylation of **1a** in the presence of Van. As shown in Figure 3, the light scattering signal of the solution containing **1a** and Van increases gradually in one hour after the addition of alkaline phosphatase (ALP, 0.5 U/mL), then reaches the maximum around 3h, which suggests that, after being produced by the dephosphorylation of **1a, 1b** binds with Van to form aggregates. The signal starts decreasing after 3h, accompanied by the observation of micron size aggregates (over 5 μm) (Fig S2). This result agrees with that the large aggregates accumulate to form precipitates, which is consistent with that ALP continues catalysing the transformation of **1a** to **1b**. As a negative control, we also measure the light scattering signal of the solution containing only **1a** and Van (**1a**&Van) without the addition of ALP. As shown in Figure 3, the solution exhibits negligible signal for over 9h, indicating no detectable aggregates form without the enzymatic formation of **1b**. In addition, we also found the solution of **1b** even at 500 μM

exhibits negligible light scattering signal after 24 h (Shown in Fig. S4), suggesting **1b** itself hardly aggregates. As a positive control, we directly mix **1b** and Van (**1b**&Van), the light scattering intensity of the mixture starts with a large signal, continually decreases, and reaches the minimum after 4h, accompanied by the formation of precipitates. This result suggests that mixing **1b** and Van immediately results in aggregates, which further cluster to precipitate to the bottom of light-scattering tubes (Inset of Fig. 3). Collectively, these results, unambiguously, demonstrate that enzymatic dephosphorylation of **1a** and the subsequent binding between **1b** and Van result in the formation of the aggregates. Such difference in light scattering signals before and after the addition of the enzyme further confirms that enzymatic transformation is able to regulate the binding mode between Van and D-Ala-D-Ala derivatives.

To explore the biological function of aggregates of Van and D-Ala-D-Ala derivatives formed by enzymatic transformation, we use MTT assay to examine the viability of HeLa cells upon treatment with **1a** (or **1b**), with or without Van. As shown in Figure 4, **1a, 1b**, and Van alone is cell compatible even the concentration as high as 500 μM. After mixing with Van at same molar ratio, both **1a** and **1b** are able to inhibit the cell proliferation, but **1a** exhibits higher cytotoxicity than **1b**, with IC_{50} values of 276 μM and 372 μM for **1a** and **1b**, respectively. Presumably, such differences are associated with the enzymatic transformation of **1a** to **1b** and subsequent aggregation processes.[9] The resulting aggregates **1b**&Van likely adhere to cell surface to cause cell death, which is consistent with our recent discovery that the aggregates catalysed by ligand-receptor interaction are able to inhibit the proliferation of cells.[6] The lower IC_{50} of **1a**&Van than that of **1b**&Van may result from the self-assembly of certain amounts of **1b** on cell surface due to the dephosphorylation of **1a**.[8, 9, 11] In another experiment, after the incubation of HeLa cells with **1a** for 12h, the addition of Van at equal molar ratio inhibits HeLa cells proliferation, but IC_{50} value is slightly higher (385 μM (Fig.S5)) and is comparable to that of the mixture of **1b**&Van. Since HeLa cells overexpress ectophosphatases,[12] this outcome agrees with that **1b**, produced via dephosphorylation of **1a** by endogenous phosphatases from the HeLa cells, interacts with Van to form the aggregates that inhibit cell proliferation. These results, thus, indicate that enzyme catalysis and ligand-receptor interaction generate the aggregates for inhibiting the proliferation of the HeLa cells.[6]

Conclusions

In summary, this work demonstrates the integration of enzymatic transformation and ligand-receptor interaction of small molecules to control the fate of cells. The spatiotemporal control provided by enzymatic reaction[8, 9] and the specificity offered by ligand-receptor interaction should lead a new way to mimic essential functions of living systems for tailoring the formation and the properties of small molecule aggregates[13] that are emerging as a new class of biofunctional entities.[14]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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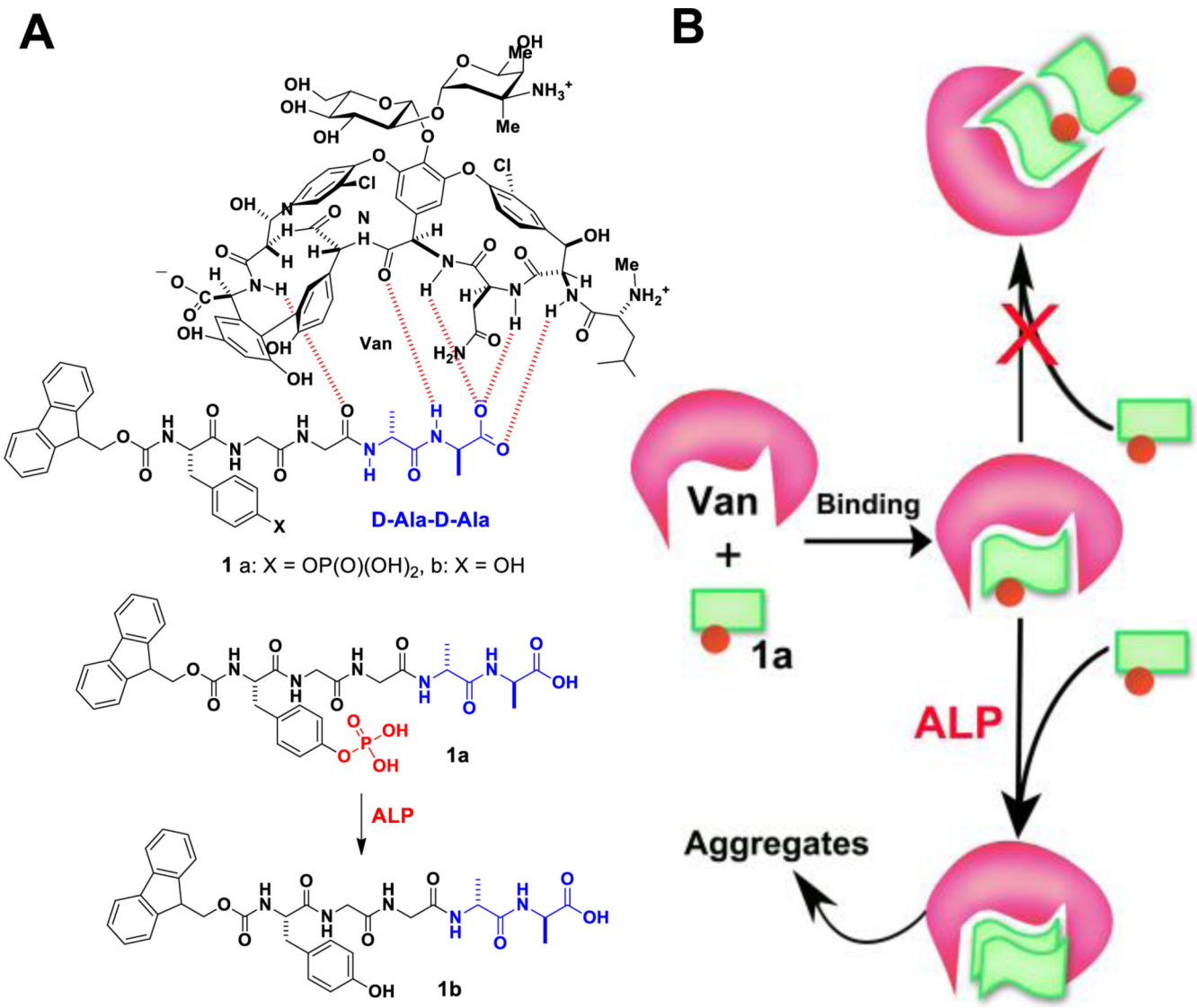

Figure 1.

(A) The chemical structures of vancomycin and its receptors, derivatives of D-Ala-D-Ala (**1a** and **1b**), **1a** is a substrate of phosphatase. (B) Illustration of enzyme transformation to modulate the mode of ligand-receptor binding, to induce the dimerization of the receptors, and to initiate aggregation.

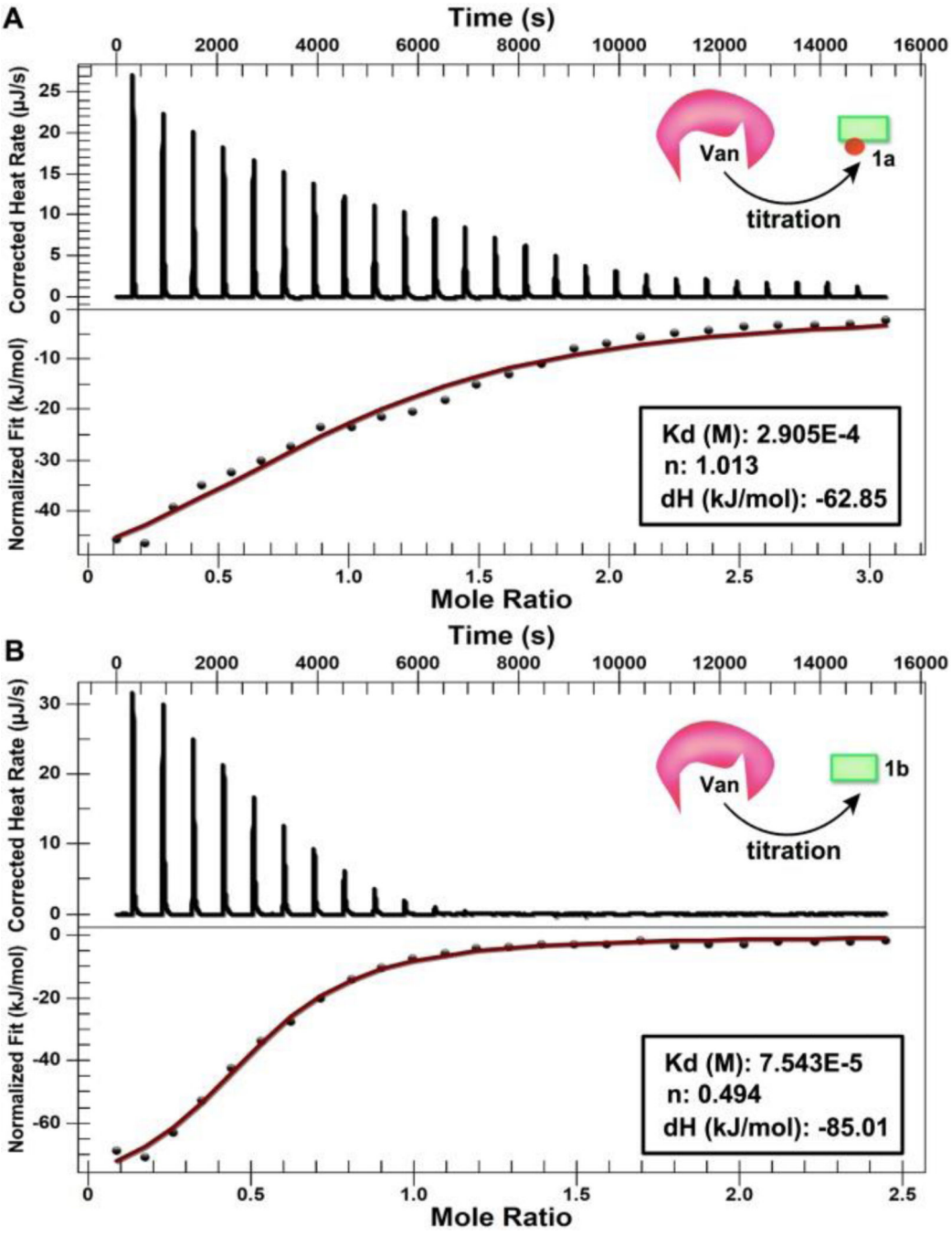

Figure 2.

Isothermal titrations of a) **1a**, and b) **1b** with Van at 25 °C for the determination of dissociation constant (K_d) and stoichiometry (n).

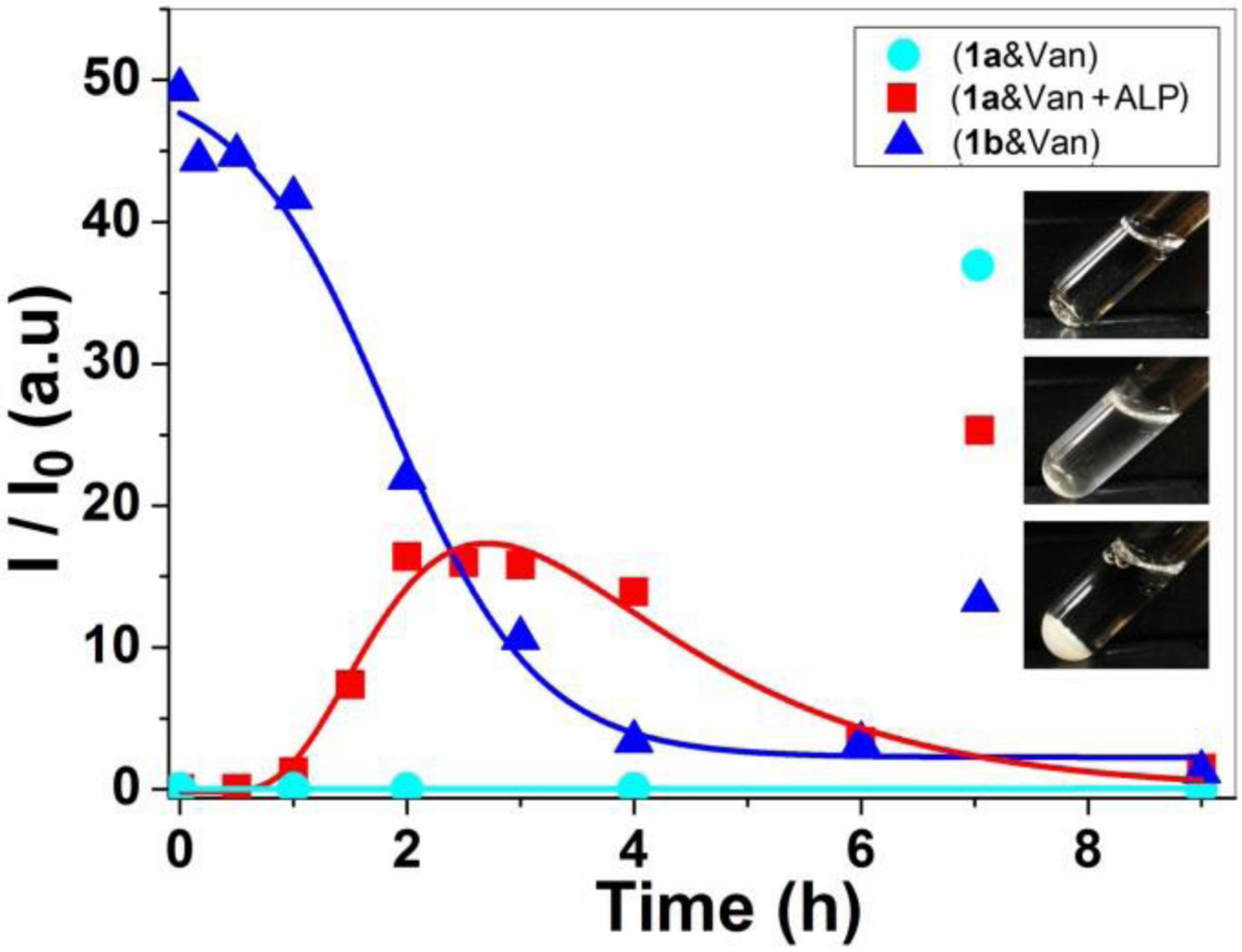

Figure 3.

Light scattering intensity (I/I_0) as a function of time for the mixtures of **1a** and Van (**1a**&Van), of **1a**, Van, and ALP (**1a**&Van+ALP), and of **1b** and Van (**1b**&Van). Insets are their corresponding optical images after 24 h. $[\mathbf{1a}]_0 = [\mathbf{1b}]_0 = [\text{Van}]_0 = 300$ μM, ALP is 0.5 U/mL.

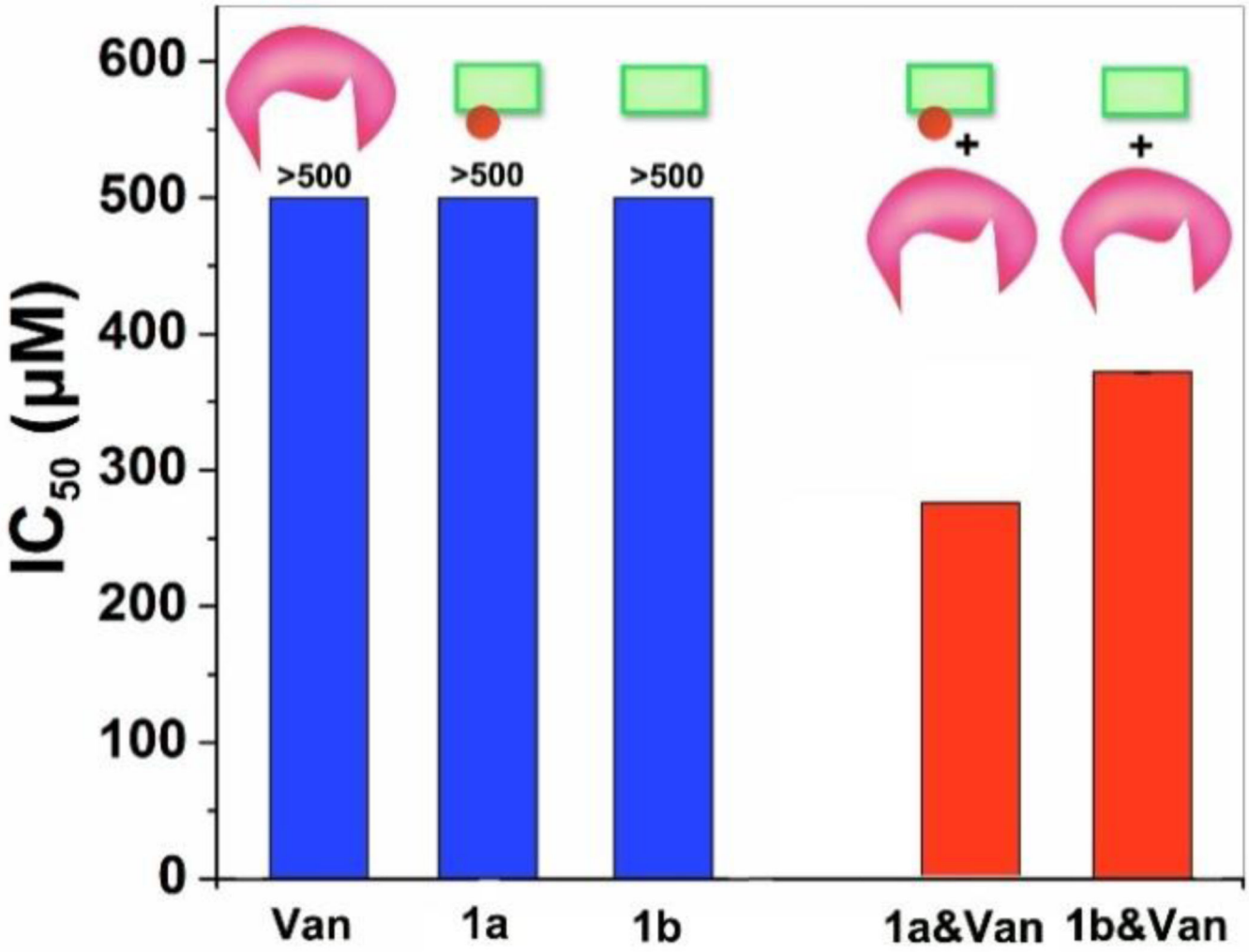

Figure 4.

IC_{50} values of **1a, 1b**, Van and their mixtures against HeLa cell. **1a** (or **1b**) mixes with Van at same molar ratio, then treated cells immediately. The concentration herein indicated the concentration of individual component.