Electronic supplementary information

Highly Efficient Complexation of Sanguinarine Alkaloid by Carboxylatopillar[6]arene: pK_a Shift, Increased Solubility and Enhanced Antibacterial Activity

Guchuan Ping, Yiliang Wang, Lingyun Shen, Yuting Wang, Xiaoshi Hu, Junyi Chen, Bingwen Hu, Lei Cui, Qingbin Meng, and Chunju Li

1. Materials and methods.

CP5A and **CP6A** were synthesized and purified according to our previously reported procedure.[S1] Sanguinarine chloride hydrate was purchased from Shanghai Aladdin Bio-Chem Technology Co.,LTD. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV500 instrument. 2D NOESY spectra were recorded on a Varian 700MHz instrument. The fluorescence emission spectra were determined by PERKINELMER LS-55, and the absorption spectra were recorded on Shimadzu UV2501-PC. Deuterated phosphate buffer solutions (20 mM) of pD 7.4 for ^{1}H NMR experiments were prepared by mixing K_2DPO_4 Deuterium oxide solution (20 mM) and KD_2PO_4 Deuterium oxide solution (20 mM) according to the calculated volume ratios. Phosphate buffer solutions (20 mM) with different pH values for the fluorescence and UV-vis experiments were prepared by mixing Na_2HPO_4 water solution (20 mM) and NaH_2PO_4 water solution (20 mM) according to the calculated volume ratios. The pH/pD values of the buffer solutions were verified on a pH-meter calibrated with two standard buffer solutions. To measure the solubility of **SA** in the presence of **CP6A**, excess amount of **SA** (solid) was added into **CP6A**'s solutions with known concentrations in deuterated phosphate buffer (pD = 7.4). The mixtures were stirred for 24h to achieve the equilibrium. During this period, the solution pDs were monitored every 6h and adjusted back to 7.4 if they changed. During the first 6h, the pD values of the solutions were sometimes decreased by 0.1 unit. Then the insoluble **SA** was removed by centrifugation and the concentration of soluble **SA** was determined by ^{1}H NMR integration of **SA**'s resonances versus **CP6A**'s.

2. Copies of ^{1}H NMR and ^{13}C NMR spectra of CP6A.

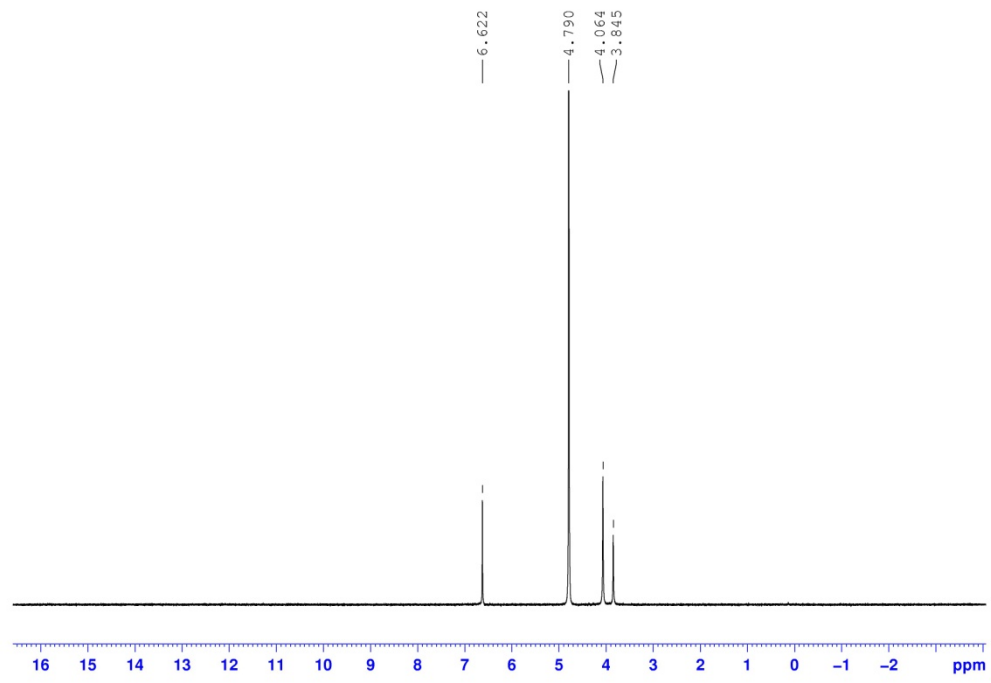


Fig. S1 ^{1}H NMR spectrum (500 MHz) of **CP6A** in D_2O

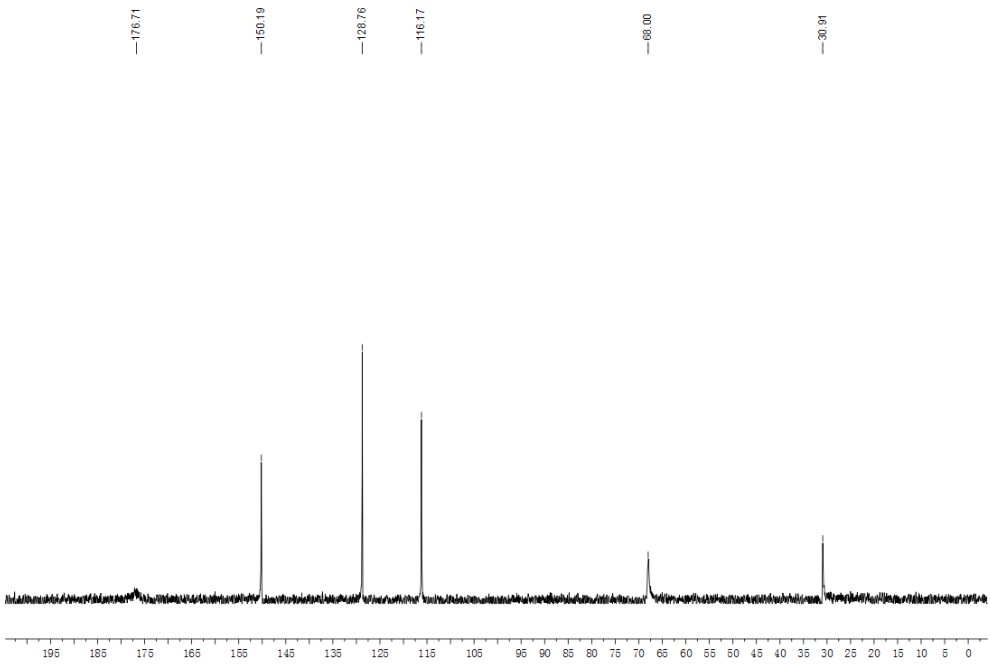


Fig. S2 ^{13}C NMR spectrum (125MHz) of **CP6A** in D_2O.

3. ^{1}H NMR spectra of SA in the absence and presence of CP5A.

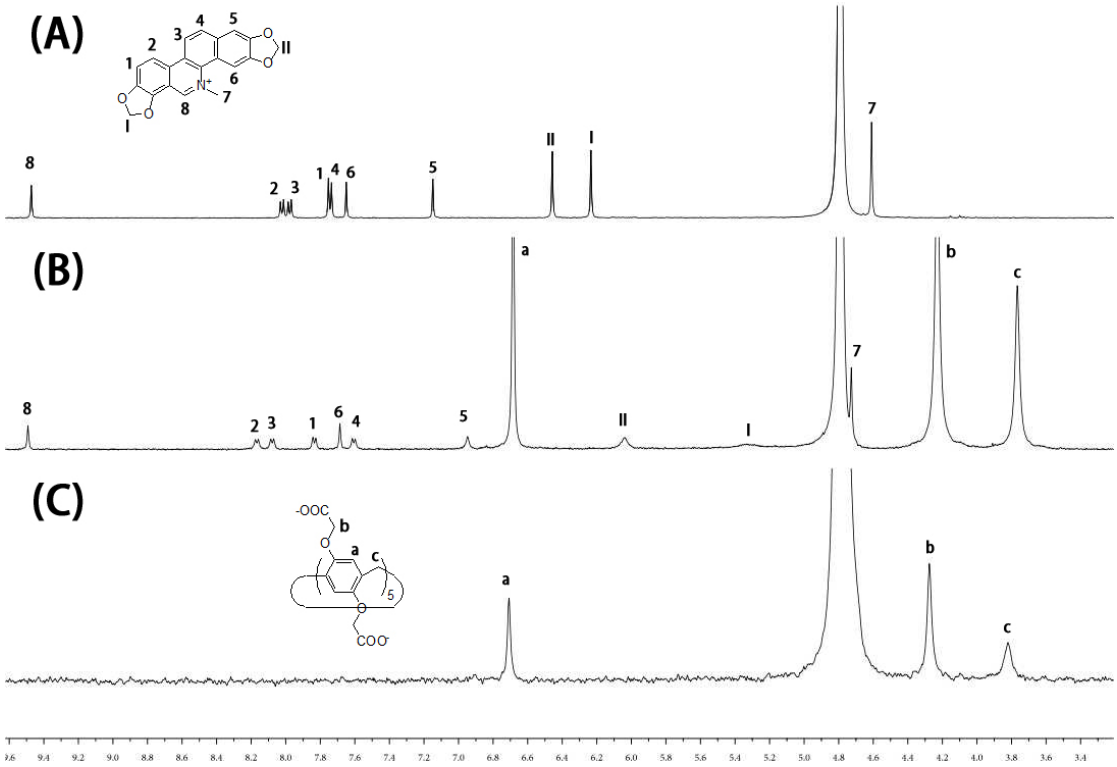


Fig. S3 ^{1}H NMR spectra (500 MHz, 293 K) of (A) **SA**, (B) **SA** + **CP5A** and (C) **CP5A** in deuterated phosphate buffer (pD = 7.4).

4. 2D NOESY spectrum of SA⊂CP6A.

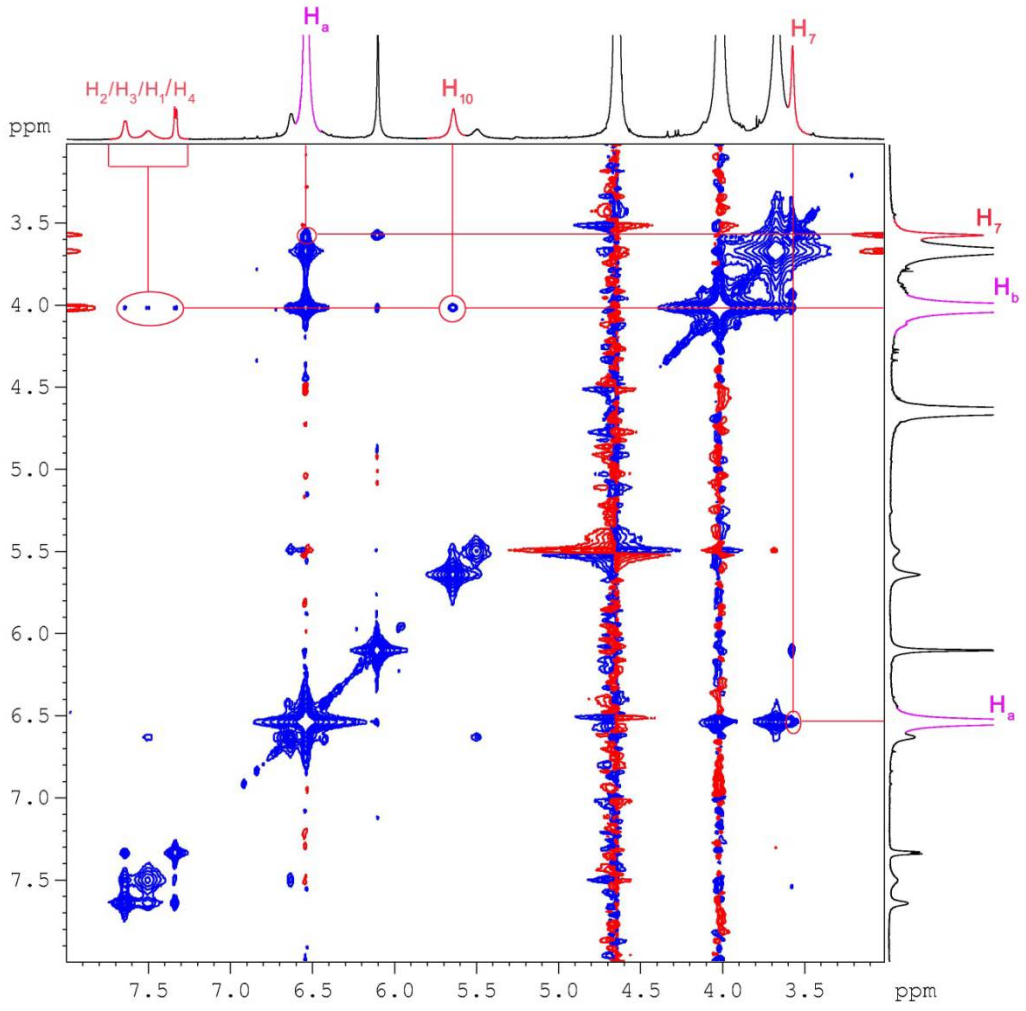


Fig. S4 2D NOESY analysis (700 MHz, 298 K) of **SA** with **CP6A** in deuterated phosphate buffer (pD = 7.4) with a mixing time of 400 ms. The concentrations of host and guest are 3.0 and 2.0 mM, respectively).

5. UV-vis spectra of SA in the absence and presence of CP6A.

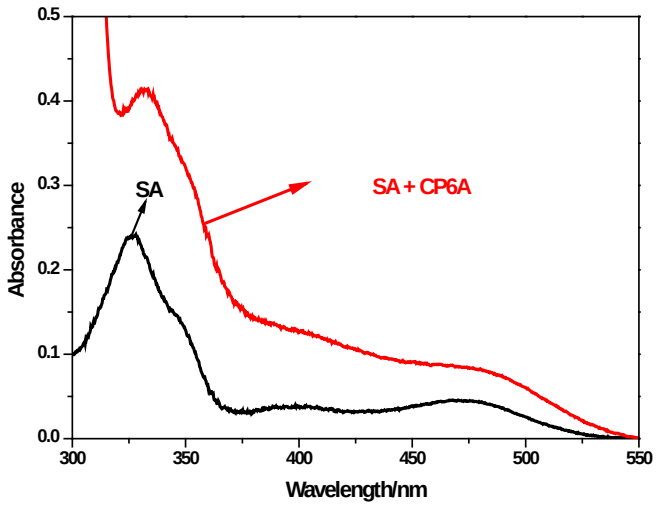


Fig. S5 The UV-vis spectra of **SA** in the absence (black line) and presence of 50 equiv. of **CP6A** (red line).

6. Job plot.

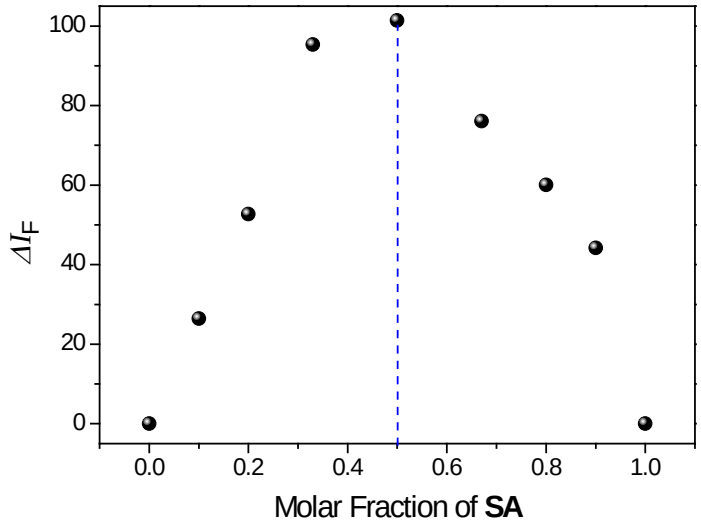


Fig. S6 Job plot showing the 1:1 stoichiometry of the complex between **SA** and **CP6A** in pH=7.4 buffer by plotting the ΔI_F values at λ = 580 nm against the mole fraction of **SA** ([**SA**] + [**CP6A**] = 1.0×10^{-5} M).

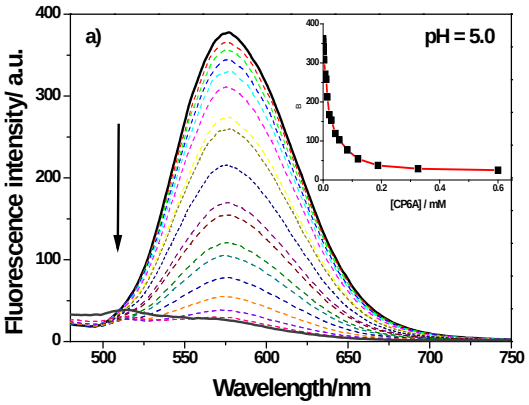
7. Determination of the association constants

The present of **CP6A** could quenched **SA**'s fluorescence, so the association constants (K_a) could be calculated by analyzing the fluorescence emission changes of **SA** that occurred with changes in host concentration. Using the nonlinear curve-fitting method, the association constant was obtained for host-guest combination from the following equation:

$$I=I_0-(0.5\alpha((G_0/2+[CP6A]+(1/K_S))-(sqrt((G_0/2+[CP6A]+(1/K_S))(G_0/2+[CP6A]+(1/K_S))-4G_0/2X))))$$

Where I is the fluorescence intensity of the system, I_0 is the intensity of sanguiranine in the absence of **CP6A**, [**CP6A**] is the initial concentration of host.

For each guest examined, the plot of *I* as a function of [**CP6A**] gave an excellent fit ($R > 0.98$), verifying the validity of the 1:1 inclusion complexation stoichiometry assumed.



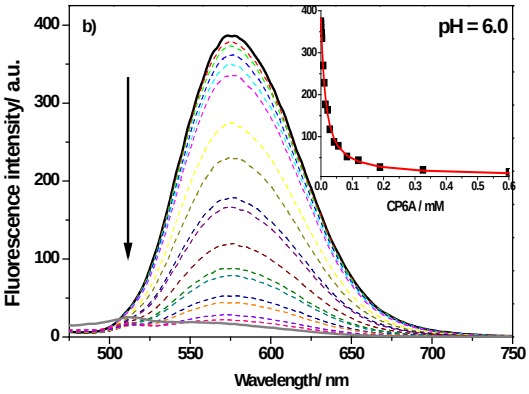


Fig. S7 Fluorescence spectra of **SA** in the absence and presence of **CP6A** in aqueous phosphate buffer solution at pH 5.0 (a) and pH 6.0 (b) at 298 K. The excitation wavelength is at 440 nm. Inset: the nonlinear least-squares analysis to calculate the association constant.

8. Antibacterial assay.

The MICs of compounds were determined by the broth microdilution method. Briefly, sterile 96-well plates (Corning, Inc., Corning, NY) were filled with 100 μL of sterile broth (pH 7.4) containing 3 g of Lab-Lemco Powder (Oxoid), 10 g of peptone (Oxoid), and 5 g of NaCl in deionized water to a final volume of 1 L. Then, portions (100 μL) of samples dissolved in saline at a concentration of 1000 μg/mL were added to the first line of plates, each sample repeated in triplicate, and two-fold serial dilutions resulting in final concentrations ranging from 0.25 to 160 μg/mL were prepared with sterile broth. Diluted (1:1000) bacterial suspensions (10 μL; approximately 1.0 X 10^6 CFU/mL) were then added to the plates and incubated with two-fold dilutions of samples at 37 ^oC for 16 h with continuous shaking. The MIC was defined as the lowest concentration of compound required to completely inhibit bacterial growth.

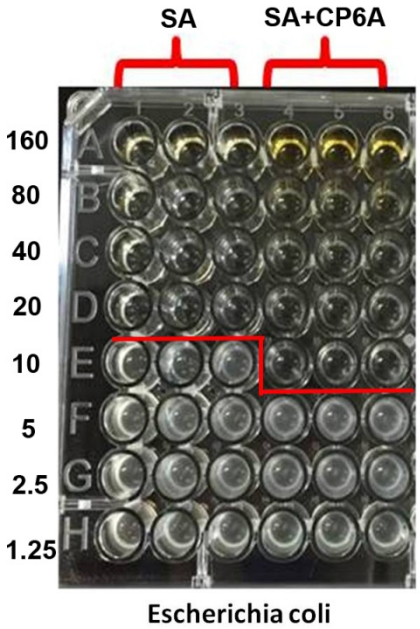


Fig. S8 Antibacterial assay (E. coli) of **SA** and 1:1 (molar ratio) **SA** + **CP6A.** The

concentration range of **SA** is changed from 160 μg/mL to 1.25 μg/mL.

[S1] Chunju Li, Xiaoyan Shu, Jian Li, Songhui Chen, Kang Han, Min Xu, Bingjie Hu, Yihua Yu and Xueshun Jia, *J. Org. Chem.*, 2011, **76**, 8458–8465.