Electronic Supplementary Information

Inhibition of human amylin fibril formation by insulin-mimic vanadium complexes

*Lei He, Xuesong Wang, Cong Zhao, Dengsen Zhu, Weihong Du**

Department of Chemistry, Renmin University of China, Beijing 100872, China

Synthesis of vanadium complexes

Synthesis of ammonium (2,6-pyridnedicaroxylic)dioxovanadate, $NH_4[V(O_2)(di–pic)]$ **(1)** was completed according to the previous literature. Quantities of 2.9 g of NH_4VO_3 and 4.2 g of pyridine-2,6-dicarboxylic acid (dipic) in 25 mL of water were heated to 80 ^oC until a yellow solution resulted which was cooled to 0 ^oC. Nearly colorless crystals of the desired product precipitated which were filtered off, followed by washing with ethanol and ether (yield 67%). IR (cm^{-1}, KBr disk) 1697, 1424($\nu_{C=O}$ and $\nu_{C=C}$); 943($\nu_{V=O}$).

Synthesis of Bis(maltolato)oxovanadium, BMOV **(2)** was carried out referring to earlier report. $VOSO_4 \cdot 3H_2O$ (12.5 g) dissolved in 12.5 mL of hot water was added slowly to a solution of maltol (14.8 g) dissolved in 65 mL of hot water. In order to bring the pH of the solution to ～8.5, KOH (6.5 g) in 5 mL of water was slowly added (dropwise over 30 min). The resulting mixture was refluxed overnight, and, upon cooling to room temperature, a birefringent purple/green solid was collected by vacuum filtration. The soild was washed with 3 portions (40 mL) of cold water and dried overnight in vacuo (yield 75%). IR (cm^{-1}, KBr disk) 1610, 1550, 1485 ($\nu_{C=O}$ and $\nu_{C=C}$); 995 ($\nu_{V=O}$).

Synthesis of Bis(N',N'-dimethylbiguanidato)oxovanadium, $VO(metf)_2 \cdot H_2O$ **(3)**. $VOSO_4 \cdot 3H_2O$ (1.0 g) was dissolved in 5 mL water and added slowly, with stirring and bubbling with Ar, to a solution of 2 equivalents metformin (1.5 g) in 5 mL H_2O. NaOH (2 M) was added dropwise to bring the pH to ～12. Initial addition of base resulted in brown hydroxide complex formation; however, upon complete addition, a light green solid eventually precipitated. The solution was stirred for 2-3 h and the solid was collected by vacuum filtration, washed with cold water followed by ether, and dried overnight in vacuo (yield 45%). IR (cm^{-1}, KBr disk) 929 ($\nu_{V=O}$).

Synthesis of potassium oxalato-oxodiperoxovanadate, $K_3[VO(O_2)_2(ox)] \cdot 2H_2O$ **(4)**. Vanadium pentoxide (0.9 g) and potassium hydroxide (1.9 g) were dissolved in water (20 mL) and to this solution oxalic acid (1.26 g), dissolved in water (10 mL) and hydrogen peroxide (30%, 20 mL), was added. Precipitation was initiated by ethanol as

above, the precipitate redissolved, and the reaction mixture set aside to crystallize in a cool place. Orange crystals obtained were filtered off and dried on the filter paper (yield 86%). IR (cm^{-1}, KBr disk) 930 ($\nu_{V=O}$), 877, 852 ($\nu_{1\ O\text{-}O}$); 631 ($\nu_{3\ MO2}$); 585 ($\nu_{2\ MO2}$).

Synthesis of ammonium (2,2'-bipyridine)oxodiperoxovanadate, $(NH_4)[VO(O_2)_2$-(bipy)] $\cdot 4H_2O$ **(5)**. Ammonium trioxovanadate (0.59 g) was dissolved under cooling in hydrogen peroxide (20%, 20 mL) and 2,2'-bipyridine (0.8 g), dissolved in ethanol (10 mL), was added with stirring. After 2-3 min, more ethanol (20 mL) was added and the reaction mixture set aside to crystallize at 4 ^oC. The yellow crystals obtained were filtered off, washed once with ethanol, and dried in air (yield 58%). IR (cm^{-1}, KBr disk) 930 ($\nu_{V=O}$), 870, 858 ($\nu_{1\ O\text{-}O}$); 622 ($\nu_{3\ MO2}$); 585 ($\nu_{2\ MO2}$).

Synthesis of ammonium (1,10-phenanthroline)oxodiperoxovanadate, $(NH_4)[VO$-$(O_2)_2(phen)]\cdot 2H_2O$ **(6)**. It was prepared similarly, but the amounts of water and hydrogen peroxide were reduced to half the above quantities because of the solubility properties of this complex (yield 79%). IR (cm^{-1}, KBr disk) 930 ($\nu_{V=O}$), 870, 850 ($\nu_{1\ O\text{-}O}$); 636 ($\nu_{3\ MO2}$); 588 ($\nu_{2\ MO2}$).

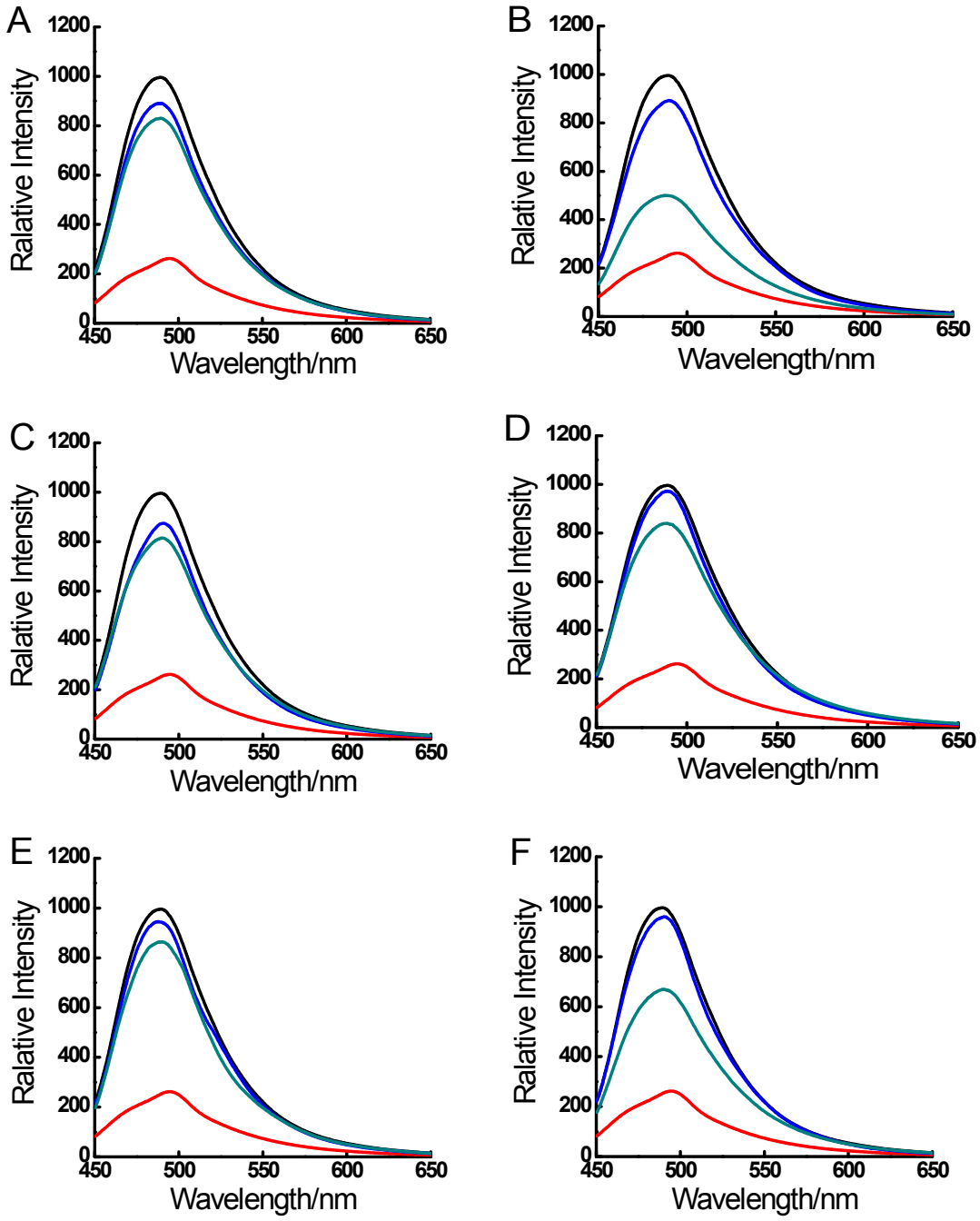

Fig. S1 Evaluation of the ability of V complexes and their corresponding ligands to inhibit hIAPP aggregation as measured by ThT fluorescence (pH = 7.5). (A) **1**; (B) **2**; (C) **3**; (D) **4**; (E) **5** and (F) **6**. Black line represented hIAPP alone, blue line represented the incubation of hIAPP with the corresponding ligands, green line represented the incubation of hIAPP with V complexes, and the red line represented the ThT intensity as comparison.

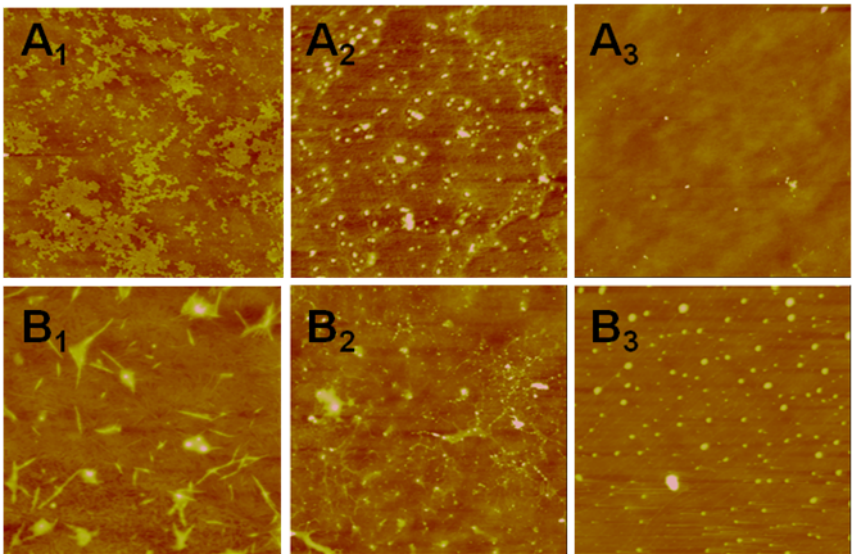

Fig. S2 AFM images for the inhibitory effects of V complexes on hIAPP (5 μM) fibrils formation (pH = 7.5). The concentrations of V complexes were 5, 15 and 25 μM respectively (from left to right) for **2** (A_1-A_3) and **6** (B_1-B_3). The scale bar is 100 nm.

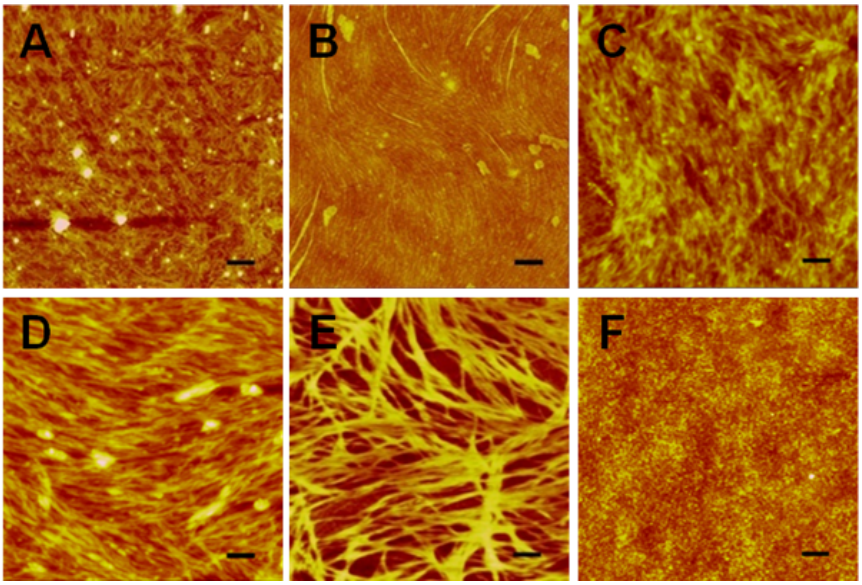

Fig. S3 AFM images for the inhibitory effects of the corresponding ligands (50 μM) on hIAPP (5 μM) fibrils formation (pH = 7.5). (A) pyridine-2,6-dicarboxylic acid; (B) maltol; (C) metformin; (D) oxalic acid; (E) 2,2'-bipyridine; and (F) 1,10-Phenanthroline monohydrate. The scale bar is 100 nm.

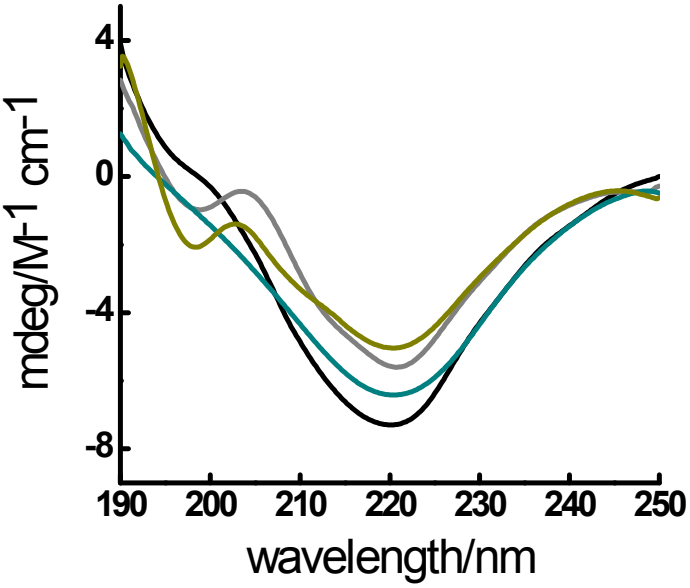

Fig. S4 CD spectra of hIAPP in the absence (black) and presence of **1** (grey line), **3** (green line) and **5** (yellow line) (pH = 7.5). The spectra are smoothed by OriginPro software after subtracting the absorption of V complex.

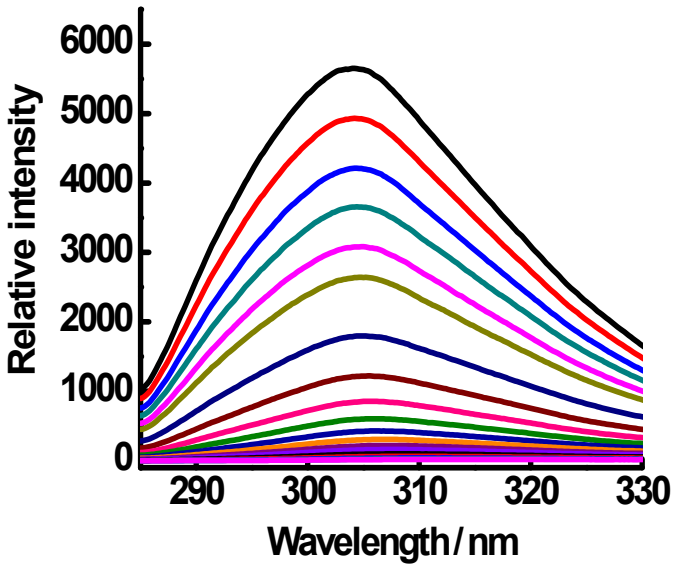

Fig. S5 The intrinsic fluorescence spectra of 10 μM hIAPP by titration of complex **6** (pH = 7.5). The concentration of complex **6** was 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 and 100 μM, respectively (from *top* to *bottom*).

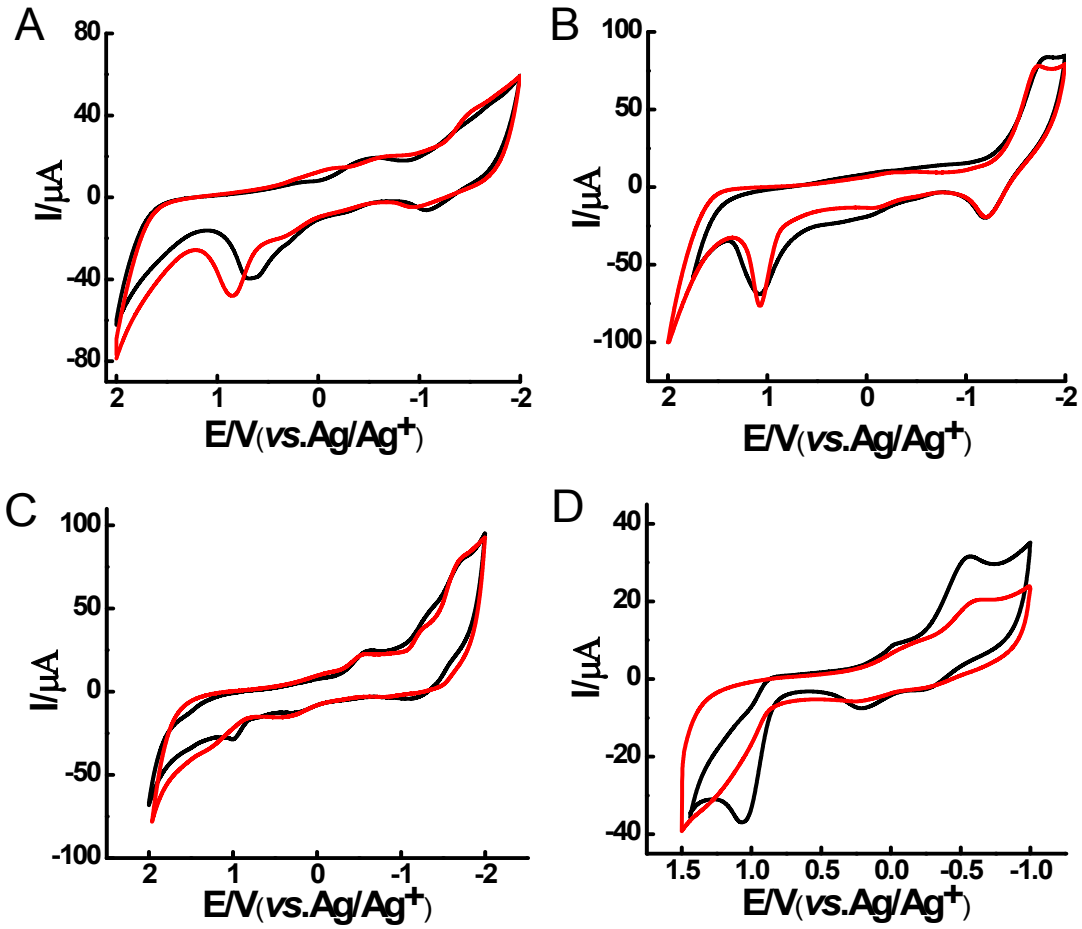

Fig. S6 Cyclic voltammograms of V complexes in the absence (black line) and presence (red line) of hIAPP obtained at glassy carbon electrode. (A) **1**; (B) **3**; (C) **5**; (D) **6** (pH = 7.5).

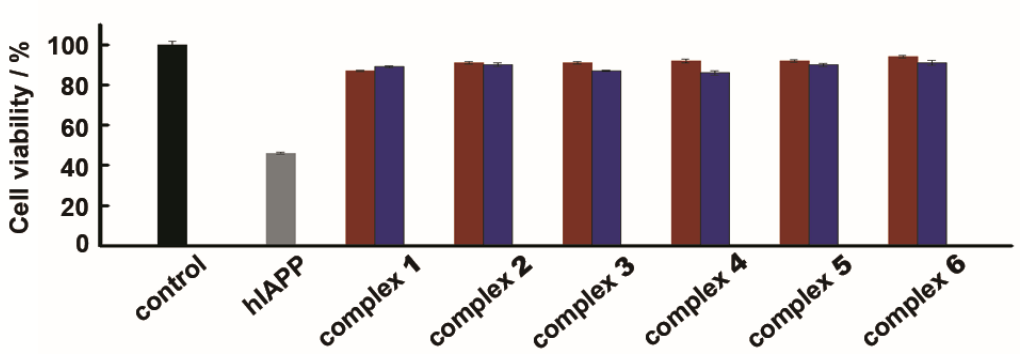

Fig. S7 Cell viability was monitored using the MTT assay. INS-1 cells were treated with different concentrations of V complexes at 1.5 μM (red column) and 15 μM (blue column) to detect the cytotoxicity of V complex independently. The cytotoxicity test of treating with hIAPP alone (grey column) was used for comparison. The data are shown as means ± SD.