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SUPPLEMENTARY MATERIAL TO

DNA/bovine serum albumin interaction studies and immunomodulatory effects of dinuclear platinum(II) complex with aromatic 1,5-naphthyridine bridging ligand

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EXPERIMENTAL

Chemicals and methods

Commercially pure chemicals such as potassium tetrachloroplatinate(II) ($K_2[PtCl_4]$), 1,5-naphthyridine (1,5-nphe), calf thymus DNA(CT-DNA), ethidium bromide (EB), bisBenzimide (Hoechst 33258, Hoe), bovine serum albumin (BSA), phosphate-buffered saline (PBS, 10 mM, pH 7.40), deuterium oxide (D_2O), 1-octanol were purchased from the Sigma-Aldrich Chemical Co and used as received. All the other common chemicals were of reagent grade and used without purification. The platinum(II) complex, [*cis*-{$PtCl(NH_3)_2$(μ-1,5-nphe)](ClO_4)$_2$, was synthesized starting from the mononuclear *cis*-[$PtCl_2(NH_3)_2$] complex and corresponding aromatic nitrogen-containing heterocyclic compound by following the literature reported methods.[1,2] The stability of the platinum(II) complex in water and phosphate-buffered saline (PBS) was evaluated by monitoring changes in its UV-Vis absorption spectra over a 48 h at room temperature. Spectra were recorded in the 220–420 nm range using a PerkinElmer Lambda 35 spectrophotometer equipped with a thermostated 1.00 cm quartz cuvette. No changes were observed in the UV–Vis spectra of the studied complex (Fig. S1). Based on this, we concluded that the investigated Pt(II) complex remains stable in both water and phosphate-buffered saline (PBS) throughout the duration of the experiment. After dissolving the dinuclear platinum(II) complex in water, the UV-Vis spectra were acquired over the wavelength range of 200-600 nm using a Shimadzu double-beam spectrophotometer fitted with thermostated 1.00 cm quartz Suprasil cells. The Pt(II) complexes had a concentration of 50 μM. Fluorescence

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measurements were performed using an RF-1501 PC spectrofluorometer (Shimadzu, Japan). The excitation and emission bandwidths were each 10 nm.

Interactions of dinuclear platinum(II) complex with CT–DNA

UV–Vis spectroscopy was used to study the interaction between the [*cis*-{PtCl(NH_3)$_2$}$_2$(μ-1,5-nphe)]$^{2+}$ complex and CT–DNA. Based on these results, the Pt(II) complex's intrinsic binding constant (K_b) to CT–DNA was calculated. The UV–Vis measurements were performed using solutions prepared with a 10 mM phosphate buffer solution (pH = 7.4) and at 37 °C. An absorbance ratio of 1.8–1.9 at 260 and 280 nm (A_{260}/A_{280}) indicates that the CT-DNA was released from the protein part. The CT–DNA content was estimated using the UV absorbance at 260 nm and the extinction value ε = 6600 $M^{-1}cm^{-1}$.[3] The concentration of Pt(II) complex in Pt(II)/CT–DNA solution remained constant at 50.4 μM, whereas CT–DNA concentrations ranged from 10.1–80.7 μM. UV–Vis measurement was performed from 200 to 500 nm. The internal binding constants (K_b) were calculated using the equation:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b}(\varepsilon_b - \varepsilon_f) \quad \text{(S-1)}$$

where [DNA] are the concentration of CT–DNA, while ε_a and ε_b are the extinction coefficients of the free and bound complexes, respectively. The extinction coefficient ε_f is computed from a calibration curve that measures the absorption of the free complex at various concentrations. The results are visually represented as a dependence of [DNK]/(ε_b- ε_f) on [DNK]. The slope of the resulting line is 1/(ε_b-ε_f), and the intercept on the y axis is 1/K_b (ε_b - ε_f). The Gibbs energy (ΔG) of the Pt(II)/CT–DNA complex was estimated using the equation: $\Delta G = -RT\ln K_b$.

Emission fluorescence spectroscopy was used to study the interaction between the Pt(II) complex and CT–DNA in the presence of ethidium bromide (EB) or bisBenzimide (Hoe). A solution of Pt(II) complex was added to the solution generated by mixing EB (or Hoe) CT–DNA in a 1:1 molar ratio, resulting in a concentration ratio of Pt(II) complex and CT–DNA ranging from 0.0 to 0.9. The above solutions were prepared using a 10 mM phosphate buffer solution (pH = 7.4). Emission spectra were recorded in the range of 550–750 nm for EB, with excitation at 527 nm and fluorescence emission observed at 612 nm.[4] For Hoechst 33258 (Hoe), spectra were recorded from 360–600 nm, with excitation at 346 nm. The Stern-Volmer constant (K_{sv}) was calculated using the following equation:

$$\frac{F_0}{F} = 1 + K_{SV} \cdot [Pt(II)] \quad \text{(S-2)}$$

where F_0 and F are the fluorescence intensities before and after adding the Pt(II) complex to the EB/CT-DNA solution, respectively. The obtained results can be shown visually as F_0/F versus [Pt(II)]. The Scatchard equation was used to determine the stability constant (K_a) and the number of binding sites (n).

$$log\frac{(F_0 - F)}{F} = logK_a + n \cdot \log[Pt(II)] \quad \text{(S-3)}$$

The obtained results are shown graphically as the dependence of log(F_0–F)/F on log[Pt(II)].

Interactions of dinuclear platinum(II) complex with bovine serum albumin

UV-Vis spectroscopy was employed to study the interactions between [Pt(II) complexes and BSA. The absorption spectra of BSA- Pt(II) complex were recorded in the 220-450 nm region, where the BSA concentration was constant (8 μM) and the Pt(II) complex concentration ranged from 0 to 120 μM. The binding constant (K_{app}) was determined using the Benesi-Hildebrand equation.[5]

$$\frac{(A_\infty - A_0)}{(A_x - A_0)} = 1 + \frac{1}{K_{app}}[Pt(II)] \quad \text{(S-4)}$$

where A_0 is the absorbance of BSA at 278 nm, A_x is the absorbance of BSA after addition of the complex at the same wavelength, A_∞ is the absorbance of BSA fully bound to the Pt(II) complex.

Emission fluorescence spectroscopy was used to study the interactions between the Pt(II) complex and serum albumin obtained from bovine blood (BSA). Emission spectra were recorded in the 300–500 nm range, with extinction at 295 nm.[6] The binding effect of the tested complex to BSA was observed based on the decrease in the emission intensity of albumin (8 μM in 10 mM PBS) at 366 nm after the addition of the Pt(II) complex. The emission spectrum was collected under identical experimental conditions. The Stern-Volmer constant (K_{sv}) was determined employing the equation:

$$\frac{F_0}{F} = 1 + K_{SV} \cdot [Pt(II)] = 1 + k_q \cdot \tau_0 \cdot [Pt(II)] \quad \text{(S-5)}$$

where F_0 is the initial fluorescence intensity of tryptophan in BSA, F is the fluorescence intensity of tryptophan in BSA after addition of the Pt(II) complex into the protein solution, k_q is the fluorescence quenching constant, τ_0 is the average fluorescence time of albumin in the absence of the complex and [Pt(II)] is the complex concentration. The binding constant (K_a) and the number of binding sites (n) were determined using equation S3.

Lipid–water partition coefficient (logP)

The lipophilicity of the investigated dinuclear platinum(II) complex was evaluated by determining the partition coefficient using the shake-flask method. The biphasic system consisted of 1-octanol and a 10 mM phosphate buffer (pH 7.4; 137 mM NaCl and 2.7 mM KCl). The absorbance of the platinum(II) complex in the aqueous phase was measured spectrophotometrically at his absorption maxima (A_{max} 309 nm). The concentration of each complex was calculated based on its previously determined molar extinction coefficient at the absorption maximum.

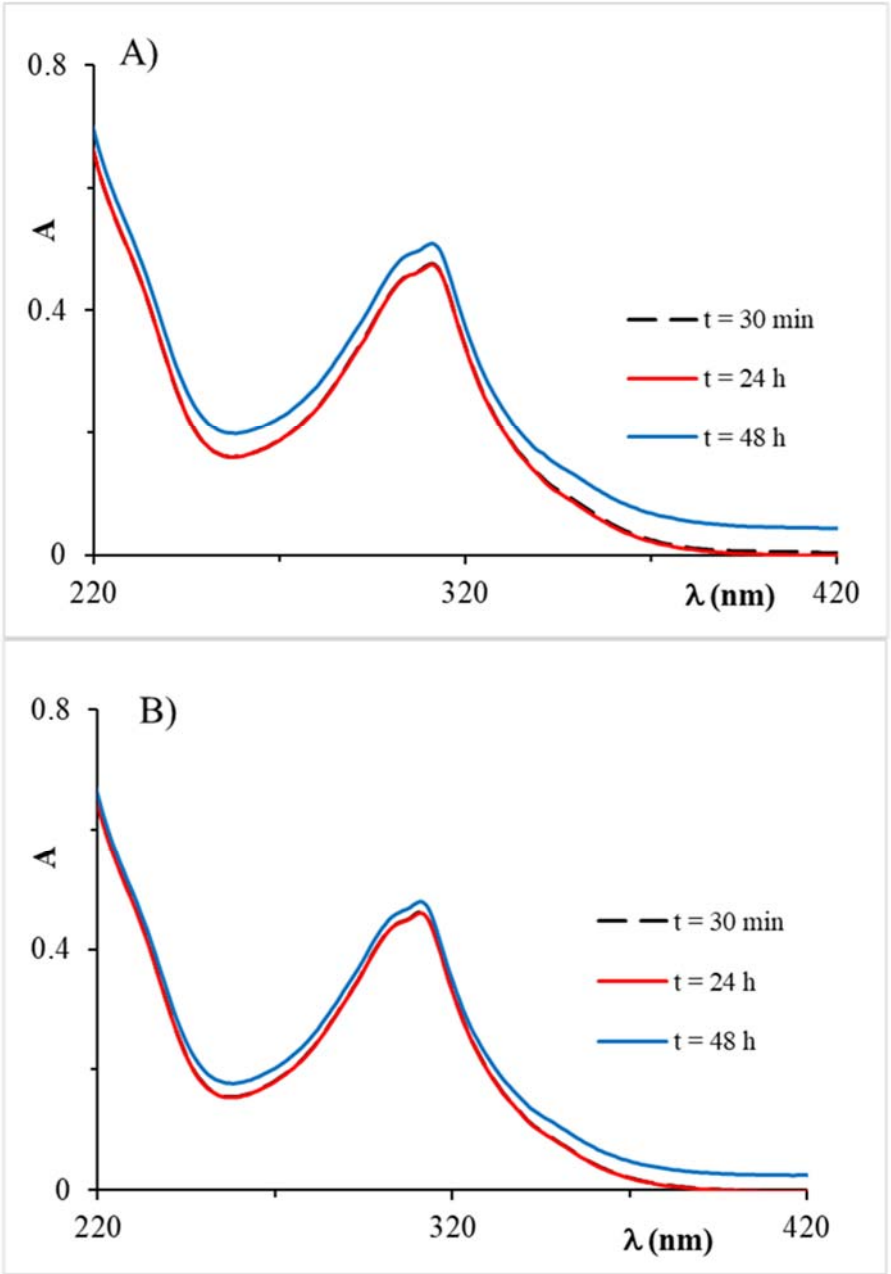

Fig. S-1. A) Stability of [*cis*-{PtCl(NH_3)$_2$(μ-1,5-nphe)]$^{2+}$ complexes followed by UV-Vis spectrophotometry in 0.01 M PBS at pH 7.40; B) Stability of [*cis*-{PtCl(NH_3)$_2$(μ-1,5-nphe)]$^{2+}$ complexes followed by UV-Vis spectrophotometry in H_2O. All measurements were performed at different time intervals and room temperature.

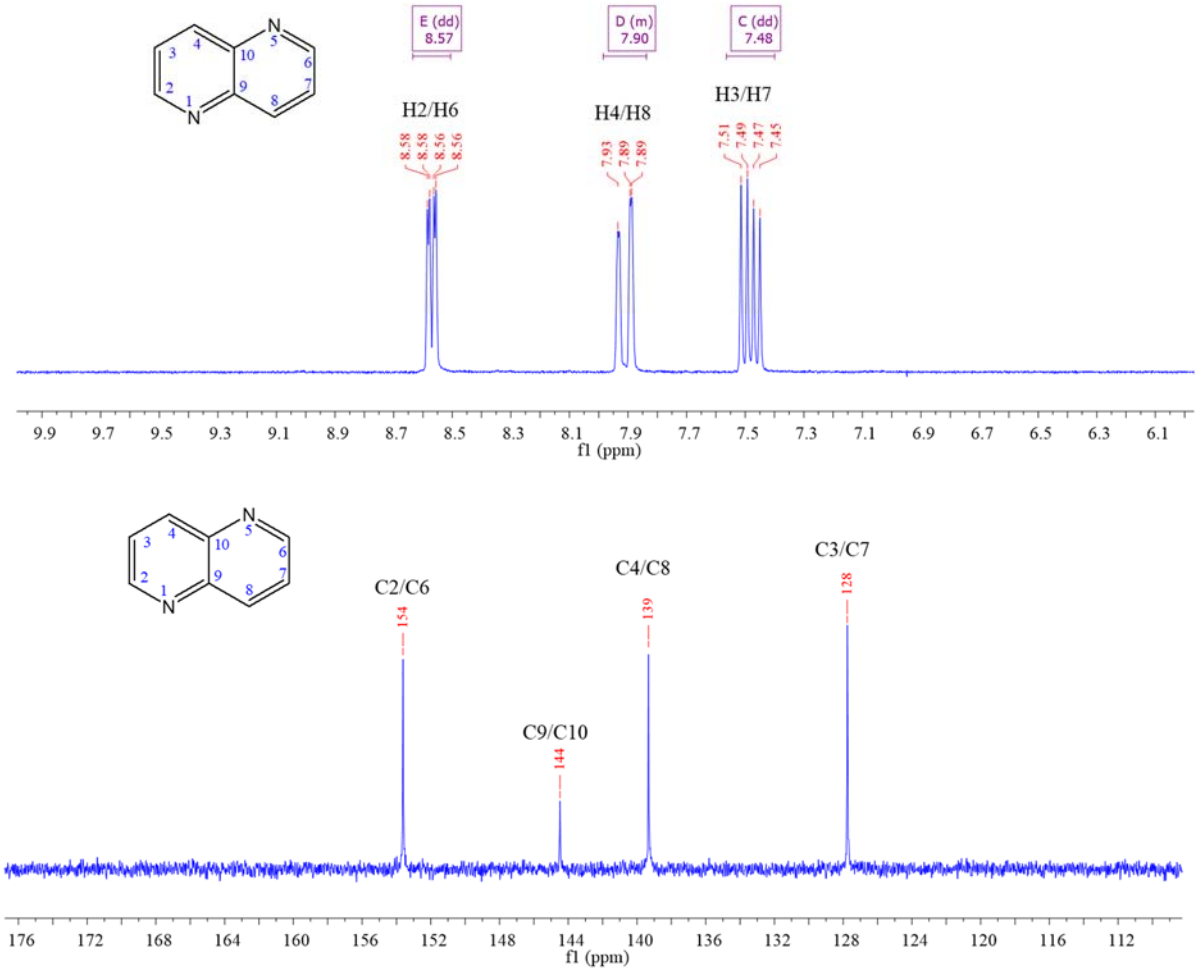

Fig. S-2. ^{1}H and ^{13}C NMR spectrum of 1,5-naphthyridine (200 MHz (^{1}H), 50 MHz (^{13}C), D_2O, 298 K).

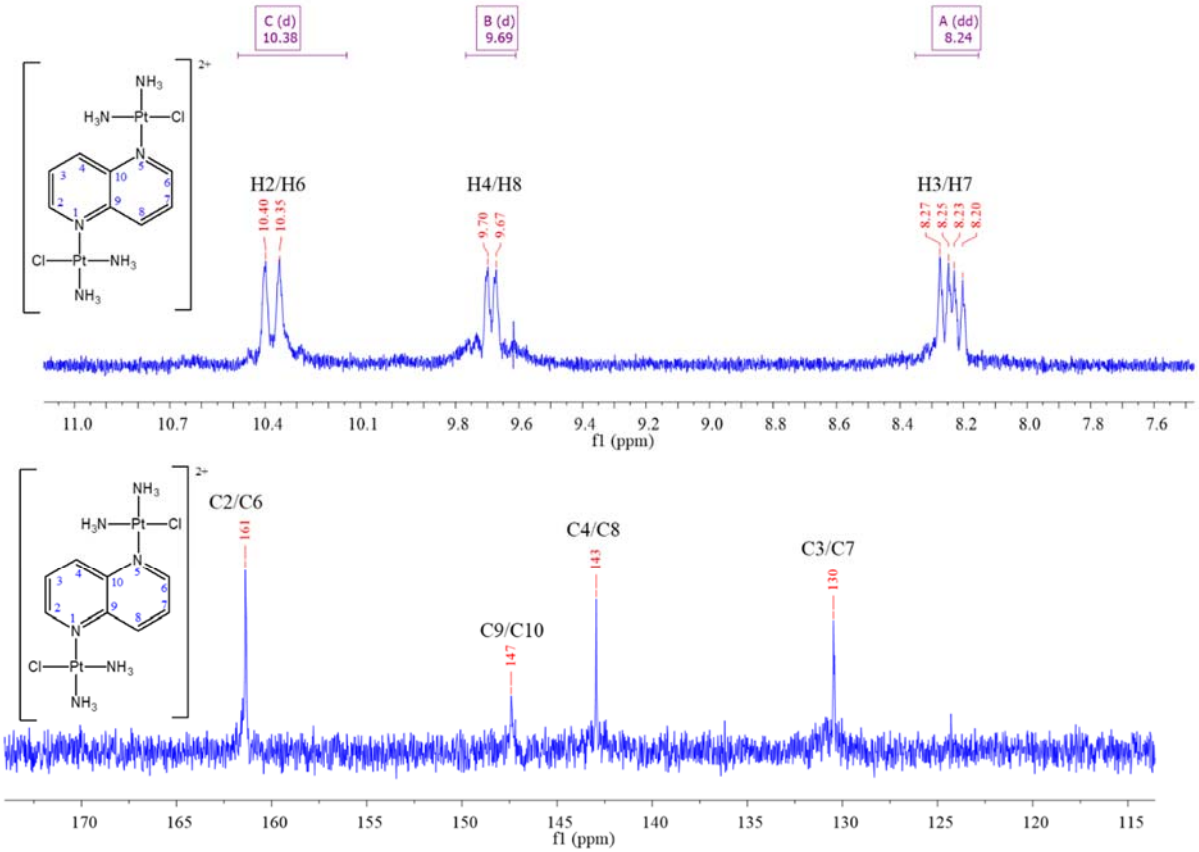

Fig. S-3. ^{1}H and ^{13}C NMR spectrum of [*cis*-{$PtCl(NH_3)_2$(μ-1,5-nphe)}]$^{2+}$ complex (200 MHz (^{1}H), 50 MHz (^{13}C), D_2O, 298 K).

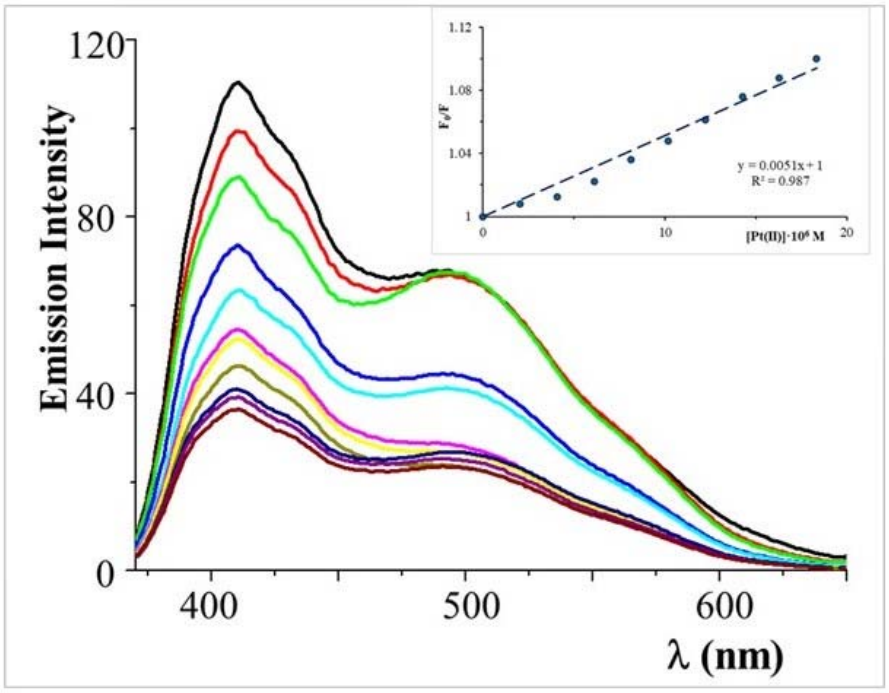

Fig. S-4. Emission spectra of CT-DNA/Hoe in the presence of Pt(II) complex (The inserted graph represents the Stern–Volmer plot of the F_0/F versus [Pt(II)])

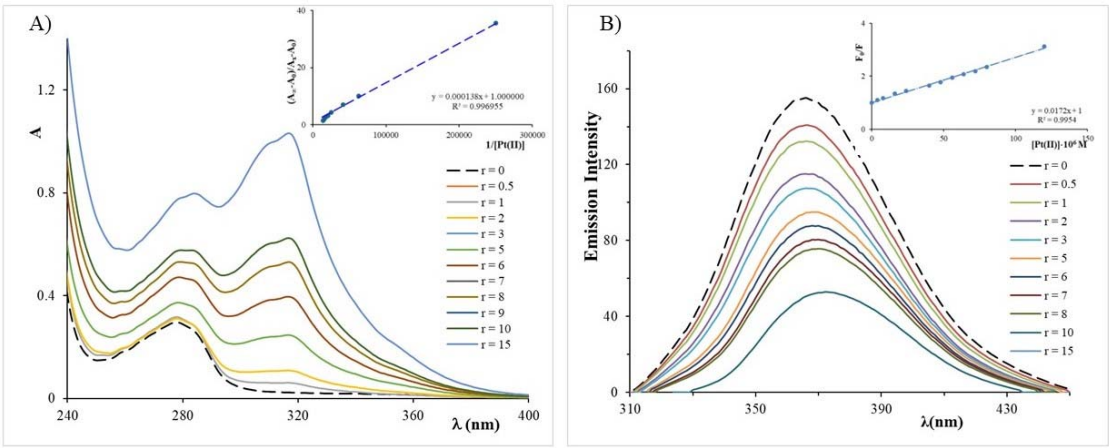

Fig. S-5. A) UV-Vis absorption of BSA in the presence of increasing concentrations of Pt(II) complex (Inset graph: Plot of $(A_\infty-A_0)/(A_x-A_0)$ versus 1/[Pt(II)]); B) Emission spectra of BSA in the presence of Pt(II) complex (Inset graph: Plot of F_0/F versus [Pt(II)]]).

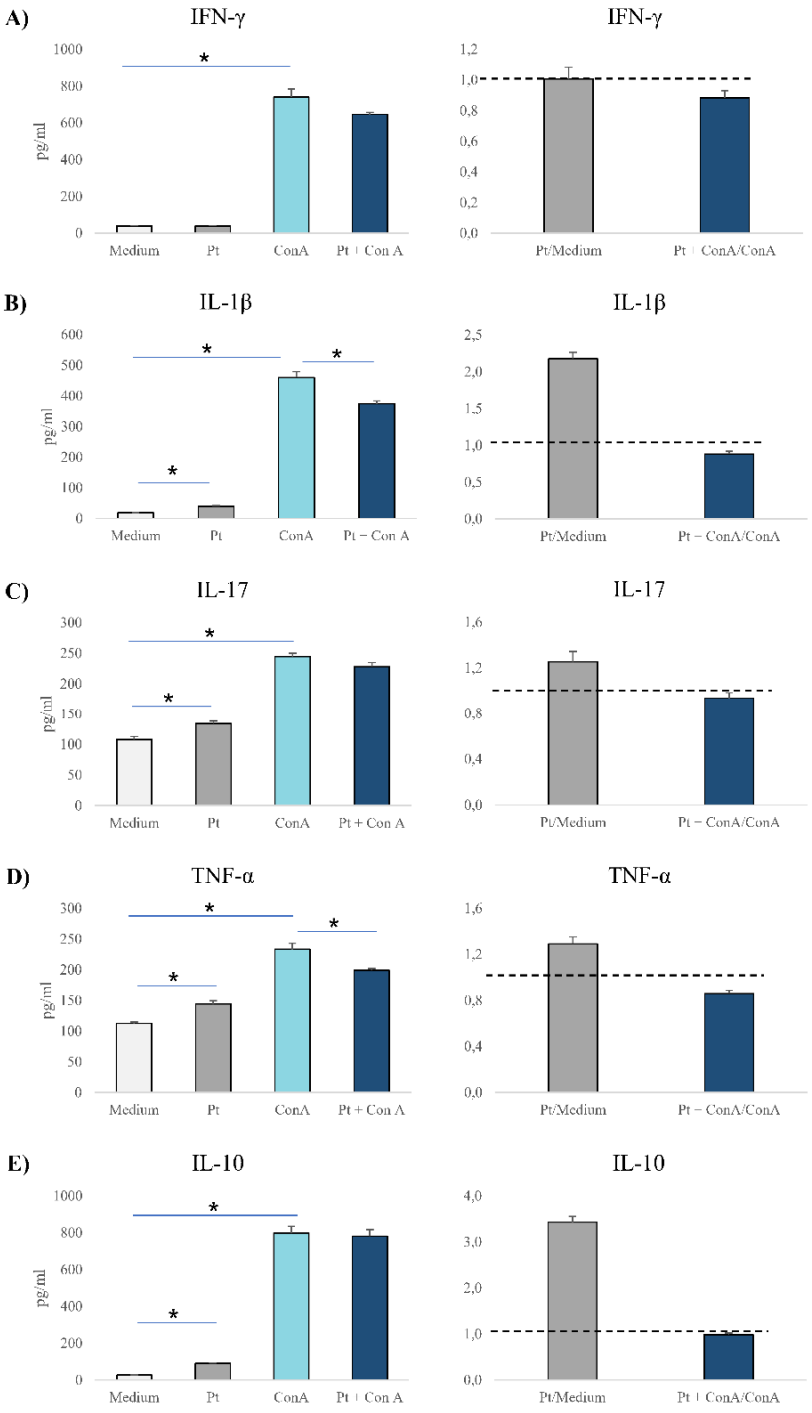

Fig. S-6. Impact of Pt(II) complex on cytokine production by splenocytes. The graphs show the concentration of IFN-γ (A), IL-1β (B), IL-17 (C), TNF-α (D) and IL-10 (E) determined by ELISA in the supernatants of splenocytes derived from healthy BALB/C mice after 24-hours of incubation with DMEM medium only, Pt(II) complex, ConA, or a combination of Pt(II) complex and ConA. Cytokine production ratios in splenocytes -Pt(II) complex/medium and treatment with Pt(II) complex + ConA/ConA (A-E). The data are shown as mean ± SEM. Statistical significance was determined by Student's t-test and Mann-Whitney U test, where appropriate (*$p<0.05$).

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