Multi-target heteroleptic palladium bisphosphonate complexes

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Supplementary material

Drug screening in MG-63 and A549 tumor cell lines

Cell line and growth conditions: Tissue culture materials were purchased from Corning (Princeton, NJ, USA), Dulbecco's modified Eagle's medium (DMEM) and TrypLE™ were purchased from Gibco (Gaithersburg, MD, USA), and fetal bovine serum (FBS) was purchased from Internegocios (Argentina). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Invitrogen. The MG-63 and A549 cell lines were purchased from ATCC. MG-63 human osteosarcoma cells (CRL-1427™) and A549 human lung carcinoma (CCL-185™) were grown in DMEM containing 10 % FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a 5 % CO_2 atmosphere. Cells were seeded in a 75-cm^2 flask, and when 70–80 % of confluence was reached, cells were subcultured using 1 mL of TrypLE™ per 25-cm^2 flask. For experiments, cells were grown in 96 multiwell plates. When cells reached the desired confluence, the monolayers were washed with DMEM and were incubated with the palladium complexes.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to Mosmann [50]. Briefly, cells were seeded in a 96-well dish, allowed to attach for 24 h, and treated with different concentrations of palladium complexes at 37 °C for 24 h. Afterwards, the medium was changed and the cells were incubated with 0.5 mg/mL MTT under normal culture conditions for 3 h. Cell viability was marked by the conversion of the tetrazolium salt MTT to colored formazan by mitochondrial dehydrogenases. Color development was measured spectrophotometrically with a microplate reader (model 7530, Cambridge Technology, USA) at 570 nm after cell lysis in DMSO (100 µL per well). Cell viability was plotted as the percentage of the control value.

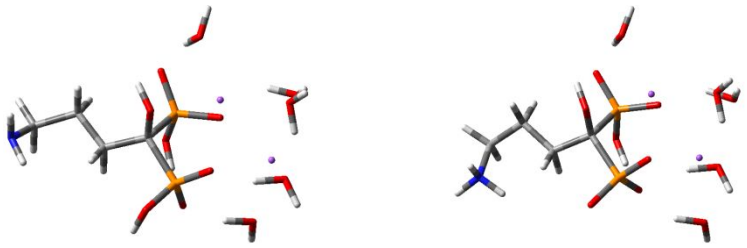

Fig. S1. Molecular models of two isomers of pamidronate (zwitterion form at right) studied at the B3LYP/6-31+G* level of theory (T = 298 K).

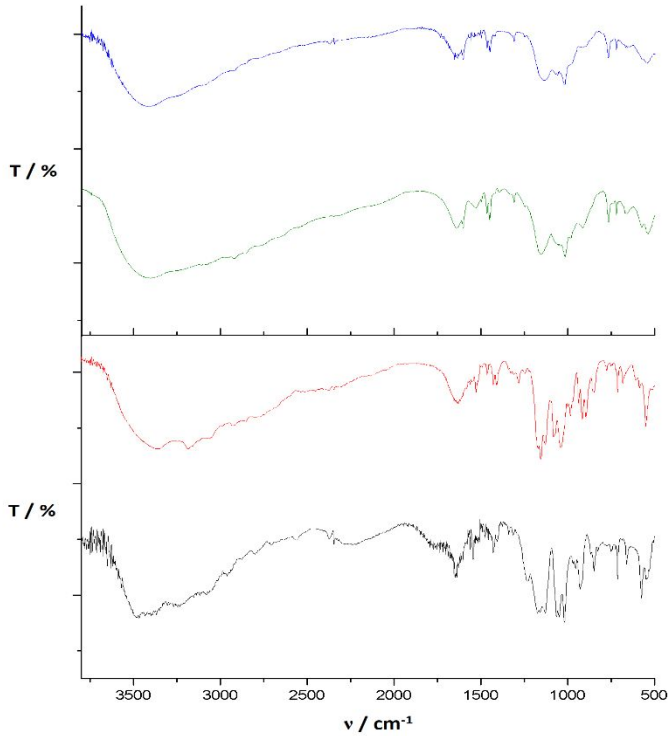

Fig. S2. Experimental FTIR spectra of Pd-pam-bpy (top, blue line) and Pd-ale-bpy (top, green line); Pd-pam-phen (bottom, red line) and Pd-ale-phen (bottom, black line).

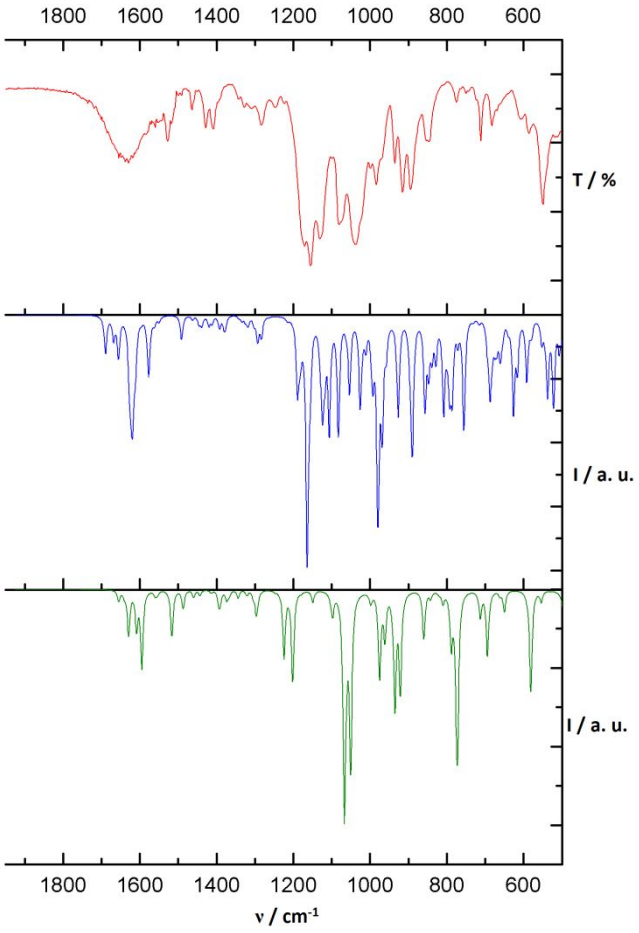

Fig. S3. Vibrational IR spectra of system Pd-pam-phen: experimental result (top, red line), theoretical spectrum of $\{[Pd(phen)(\textit{N}\text{-pam})_2](Na^+)_2(H_2O)_8\}^{2+}$ (middle, blue line), and of $\{[Pd(phen)(\textit{O}\text{-PAM})](H_2O)_4\}^{2+}$ (bottom, green line) as calculated at B3LYP/LANL2DZ/6-31+G* level (T = 298 K). Absorption bands only in the range from 1800 to 500 cm^{-1} are exhibited.

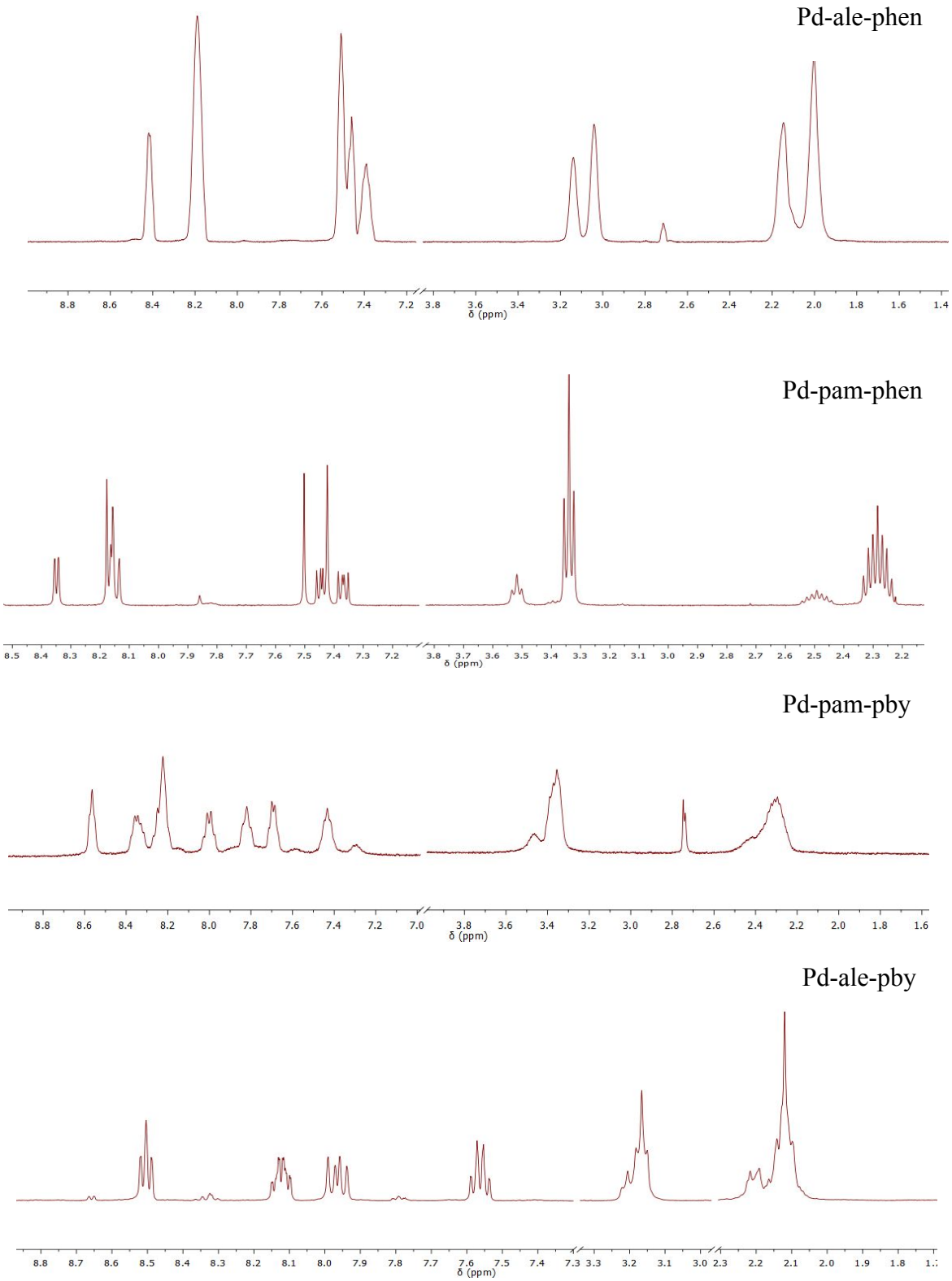

Fig. S4. ^{1}H-RMN spectra in D_2O of the obtained complexes.

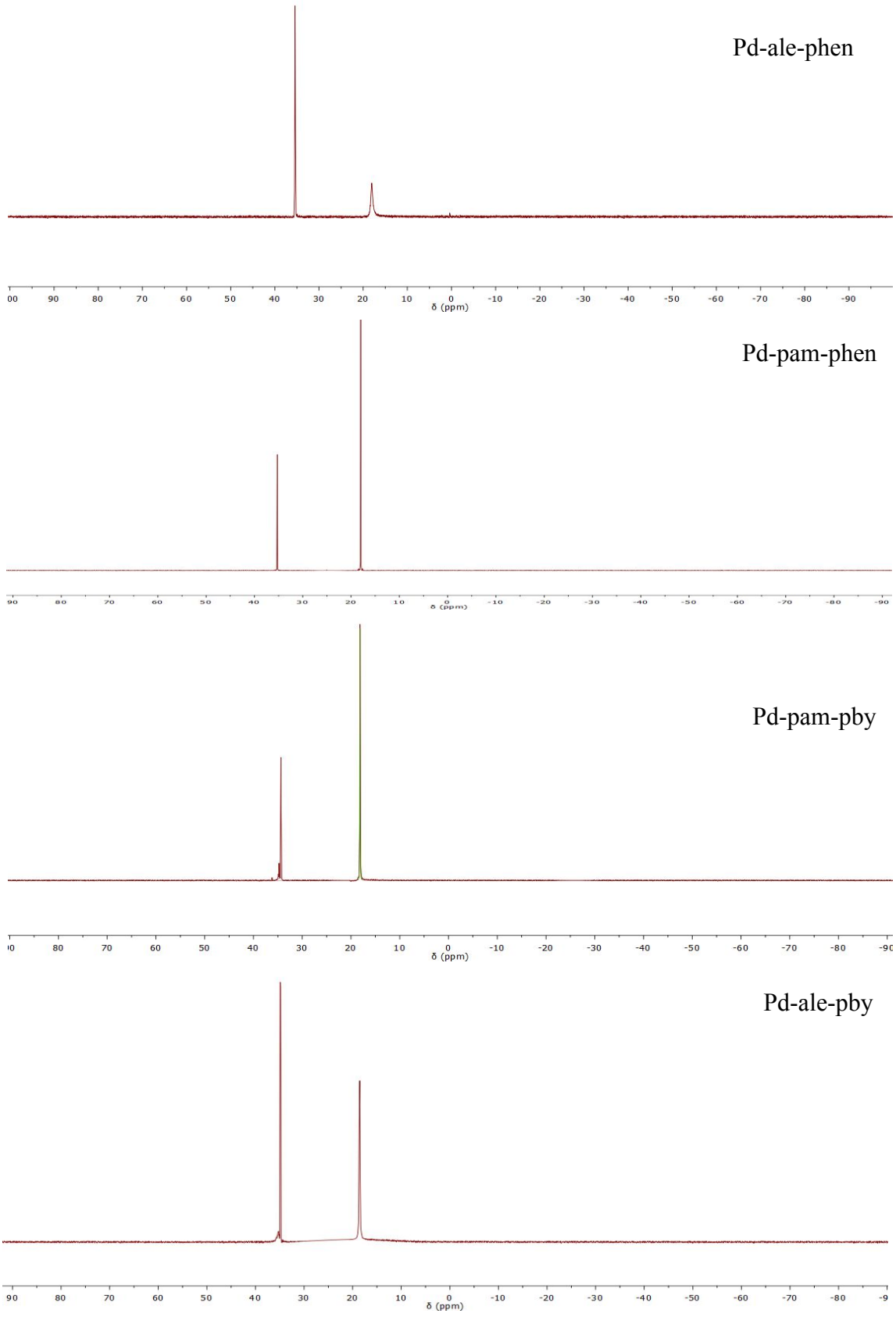

Fig. S5. ^{31}P-RMN spectra in D_2O of the obtained complexes

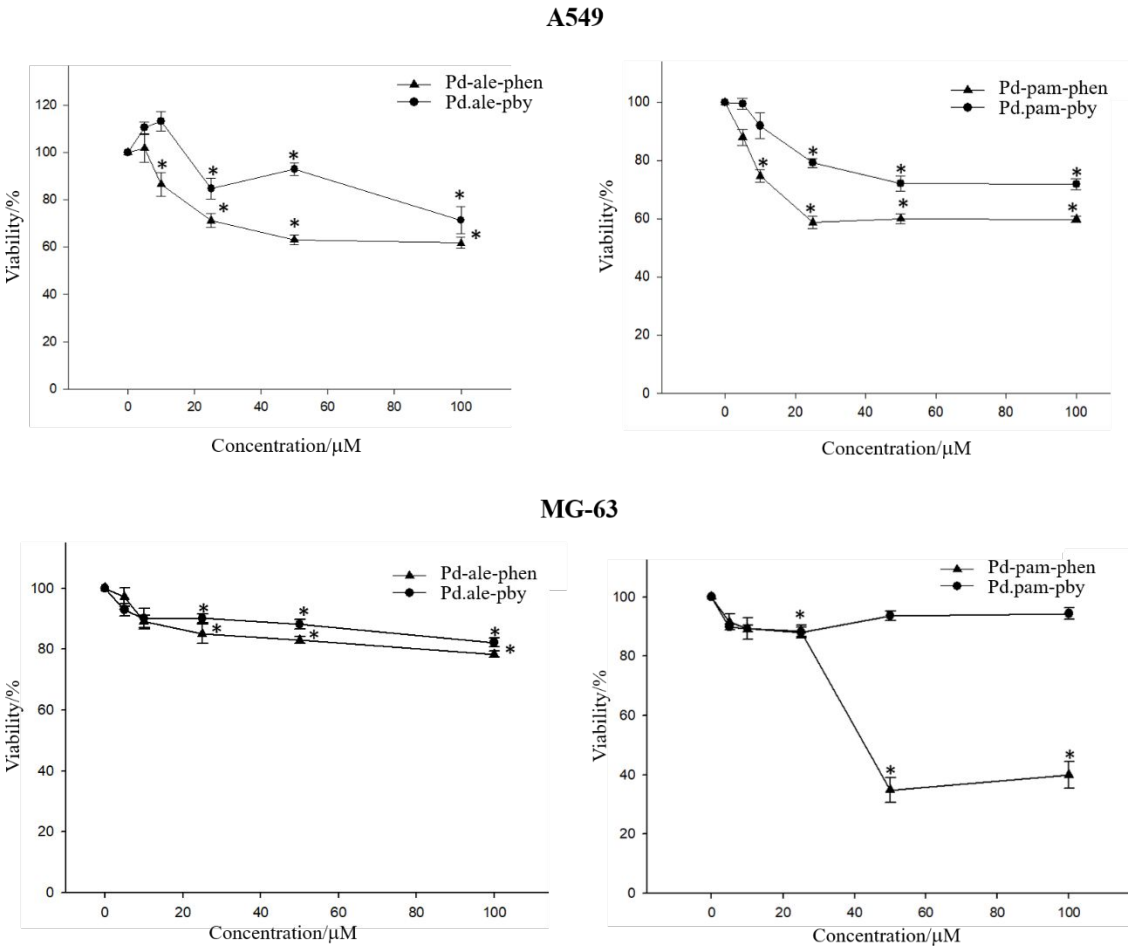

Fig. S6. Evaluation of the mitochondrial succinate dehydrogenase activity by the 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in MG-63 and A549 cells in culture. Osteosarcoma and lung carcinoma cells were incubated with different doses of palladium complexes for 24 h at 37 ° C. After incubation, cell viability was determined by the MTT assay. Results are expressed as the percentage of the basal level and represent the mean ± SEM (n = 18). *p<0.05. In A549 cells a concentration dependent inhibition was observed for all the obtained complexes from 25 to 50 μM. For a given dose, Pd-ale-phen and Pd-pam-phen showed the highest cytotoxic activities. In MG-63 cells, only Pd-pam-phen showed a cytotoxic effect in a concentration-dependent manner up to 50 μM with statistically significant differences versus control conditions (without complex addition) ($p < 0.05$). For this complex, the inhibitory effect showed a plateau in the highest concentrations (50 to 100 μM). On the other hand, Pd-pam-bpy did not show any effects and Pd-ale-phen and Pd-ale-bpy only provoked a slight effect in the cell viability in the 5–100 μM concentration range.

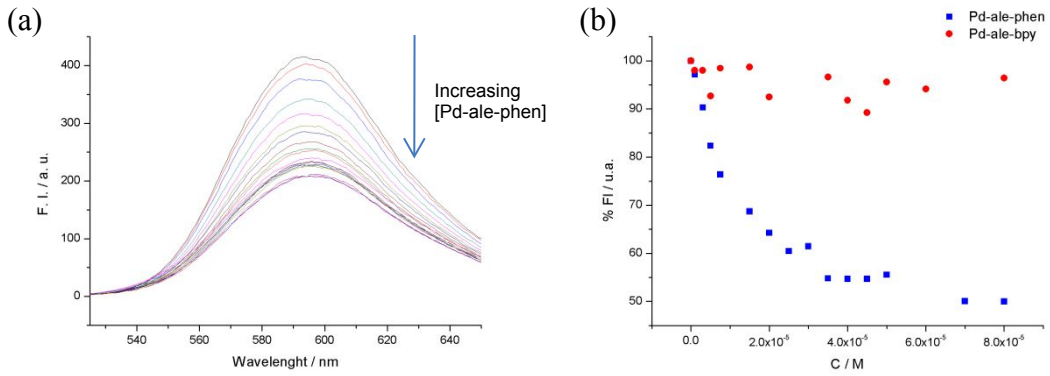

Fig. S7. (a) Fluorescence emission quenching ($\lambda_{exc.}$ = 510 nm) observed upon binding of Pd-ale-phen. (b) Relative fluorescence intensity (%) at $\lambda_{em.}$ = 594 nm with increasing complex concentration obtained for Pd-ale-NN complexes (C_{DNA} = 20 μM, C_{EB} = 10 μM, samples prepared in TRIS-HCl medium, 30 min incubation at 37 °C).

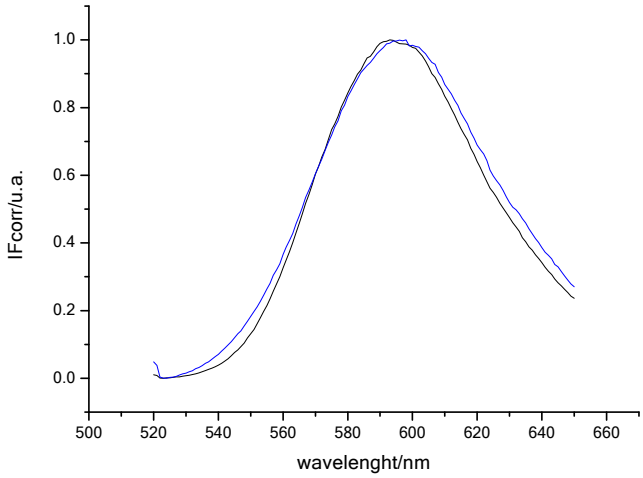

Fig. S8. Fluorescence intensity normalized at $\lambda_{em.}$ = 594 nm of the adduct {DNA-EB} at different concentrations of the complex Pd-ale-phen. Black: 0 μM, Blue: 100 μM

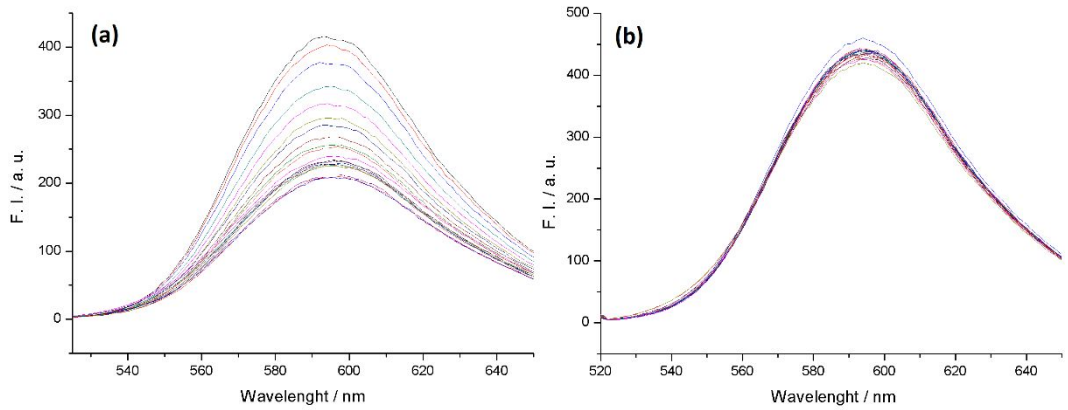

Fig. S9. Fluorescence emission quenching (λexc. = 510 nm) observed upon binding of (a) Pd-pam-phen and (b) Pd-pam-bpy.