Supplementary Information

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Enhancement of the physicochemical properties of [Pt(dien)(nucleobase)]$^{2+}$ for HIVNCp7 Targeting.

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1. Synthesis and Characterization

Materials and reagents

The complex [PtCl(dien)]Cl (dien = diethylenetriamine) was prepared by literature methods. [1] Purity was confirmed by ^{1}H and ^{195}Pt NMR Spectroscopy, and Elemental Analysis (performed by QTI Laboratory, USA). All reagents were purchased from Sigma Aldrich, USA and used without further purification. The NCp7 C-terminal peptide sequence (KGCWKCGKQEHQMKDCTER) was purchased from GenScript Corporation. The "full" 1-55bp HIVNCp7 peptide was a gracious gift of R.J. Gorelick, National Institutes of Health

NMR Spectroscopy. ^{1}H and ^{195}Pt NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer. ^{1}H NMR spectra are reported with reference to solvent

resonances of D_2O at δ 4.79 and then adjusted for TMS = 0.00 ppm. The $^{195}Pt\{^1H\}$ NMR spectra were obtained in D_2O with 40 mM solutions, at an operating frequency of 85.80 MHz, range of -1900 to -3500 ppm and 5000 scans. The spectra were referenced externally using standards of Na_2PtCl_6 in D_2O (δ = 0 ppm).

Fluorescence spectroscopy. Fluorescence studies were recorded on a Varian Cary Eclipse fluorometer with a single-cell Peltier accessory. Samples were irradiated with 280 nm light and spectra were recorded from 300 to 450 nm with a scan rate of 600 nm/min at 25°C. The experiments were carried out in 20 mM Tris buffer with 50 mM NaCl at pH 7.4. 5 μM N-acetyl tryptophan or zinc finger was titrated with $[M(dien)L]^{n+}$ at molar ratios of drug from 10 to 100 for all complexes. The absorbance for all compounds at 100 molar equivalents was < 0.05; therefore, the inner filter effect was disregarded. The emission maximum (362 nm) was measured after each titration. The Ka was determined from Eadie-Hofstee plots from an average of 3 trials using the equation: $\Delta F = (K_a)^{-1} * \Delta F/[quencher] + \Delta F_c$.

Circular Dichroism*:* Methods were adapted from those previously published.[2]

Synthesis of PtN_4 complexes

[Pt(dien)(9-EtGua)](NO$_3$)$_2$ - [PtCl(dien)]Cl was dissolved in H_2O and 9-Ethylguanine (1 mol eq.) and $AgNO_3$ (1.98 mol eq.) were added. The solution was heated at 50°C in the dark for 24 hours. The solution was cooled to room temperature and filtered through celite to remove the precipitated AgCl. The solution was evaporated to dryness and acetone was added to precipitate the final product. The product may be recrystallized from H_2O/acetone. The solid was dried *in vacuo*. Anal. Calcd for $C_{11}H_{22}N_{10}O_7Pt$: C 21.97; H, 3.69; N, 23.20. Found: C, 21.19; H, 3.39; N, 22.86. ^1H NMR (D_2O): 1.44 (3H, t), 3.00 (8H, m), 4.14 (2H, q), 8.19 (1H, s). ^{195}Pt NMR: -2850 ppm.

[Pt(dien)(Guo)]NO$_3$ Cl 0.8H$_2$O – [Pt(dien)(Guo)]NO_3 Cl was synthesized in a similar manner as [Pt(dien)(9EtG)](NO$_3$)$_2$. Briefly, [PtCl(dien)]Cl was dissolved in H_2O and 9-ethylguanine (1 mol eq.) and $AgNO_3$ (0.98 mol eq.) were added. The solution was heated at 50°C in the dark for 24 hours. The solution was cooled to room temperature and filtered through celite to remove the precipitated AgCl. The solution was evaporated to dryness and acetone was added to precipitate the final product. The solid was dried *in vacuo*. Anal. Calcd for $C_{14}H_{27.6}N_9ClO_{8.8}Pt$: C 24.25; H, 4.01; N, 18.18. Found: C, 24.62; H, 3.99; N, 17.80. ^1H NMR (D_2O): 3.00 (8H, m), 3.78 (2H, m), 4.26 (1H, t), 4.39 (2H, m), 5.95 (1H, d), 8.42 (1H, s). ^{195}Pt NMR: -2857 ppm.

[Pt(dien)(Ino)](NO$_3$)$_2$ - [Pt(dien)(Ino)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)](NO$_3$)$_2$. Anal. Calcd for $C_{14}H_{24}N_9O_{11}Pt$: C 24.38; H, 3.48; N, 18.28.

Found: C, 24.65; H, 3.47; N, 18.35. ^{1}H NMR (D_2O): 3.00 (8H, m), 3.90 (2H, m), 4.41 (1H, t), 4.73 (2H, m), 6.14 (1H, s), 8.32 (1H, s), 8.81 (1H, s). ^{195}Pt NMR: -2854 ppm.

[Pt(dien)(Xan)]$(NO_3)_2$ 2H_2O- [Pt(dien)(Xan)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{14}H_{29}N_9O_{14}Pt$: C 22.65; H, 3.94; N, 16.98. Found: C, 22.35; H, 3.24; N, 16.73. ^{1}H NMR (D_2O): 3.00 (8H, m), 3.93 (2H, m), 4.37 (2H, m), 4.56 (2H, m), 5.93 (1H, s), 8.43 (1H, s). ^{195}Pt NMR: -2849 ppm.

[Pt(dien)(7-MeGua)]$(NO_3)_2$ - [Pt(dien)(7-MeGua)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9EtG)]$(NO_3)_2$. Anal. Calcd for $C_{14.18}H_{25.36}N_9O_{11.06}Pt$: C 24.54; H, 3.68; N, 18.17. Found: C, 24.65; H, 3.47; N, 17.35. ^{1}H NMR (D_2O): 3.00 (8H, m), 3.99 (3H, s), 8.12 (1H, s). ^{195}Pt NMR: -2890 ppm.

[PtCl(N-Medien)]Cl 0.5H_2O- [Pt(NMedien)Cl]Cl was prepared in the same manner as [PtCl(dien)]Cl [1]. Anal. Calcd for $C_5H_{15}N_3Cl_2Pt$: C 15.31; H, 4.11; N, 10.71. Found: C, 15.49; H, 4.00; N, 10.19. ^{1}H NMR (D_2O): 3.03 (11H, m). ^{195}Pt NMR: -2598 ppm.

[Pt(N-Medien)(9-EtGua)]$(NO_3)_2$ - [Pt(N-Medien)(9-EtGua)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(-9EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{12}H_{24}N_{10}O_7Pt$: C 23.42; H, 3.93; N, 22.76. Found: C, 23.16; H, 3.35; N, 22.20. ^{1}H NMR (D_2O): 1.44 (3H, t), 3.03 (11H, m), 4.14 (2H, q), 8.18 (1H, s). ^{195}Pt NMR: -2725 ppm.

[Pt(N-Medien)(Guo)]$(NO_3)_2$. 0.75H_2O– [Pt(N-Medien)(Guo)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{15}H_{29.5}N_{10}O_{11.75}Pt$: C 24.58; H, 4.06; N, 19.11. Found: C, 24.11; H, 3.48; N, 19.04. ^{1}H NMR (D_2O): 3.03 (11H, m), 3.89 (2H, m), 4.26 (1H, t), 4.40 (2H, t), 4.72 (1H, t), 5.97 (1H, d), 8.42 (1H, s). ^{195}Pt NMR: -2729 ppm.

[Pt(N-Medien)(Xan)]$(NO_3)_2$. 2.5H_2O- [Pt(N-Medien)(Xan)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{15}H_{325}N_9O_{14.5}Pt$: C 23.53; H, 4.21 N, 16.47. Found: C, 23.44; H, 4.20; N, 15.99. ^{1}H NMR (D_2O): 3.03 (11H, m), 3.93 (2H, m), 4.37 (2H, m), 4.56 (2H, m), 5.93 (1H, s), 8.43 (1H, s).

[PtCl(N,N'-Me_2dien)]Cl.0.5H_2O - [Pt(N,N'-Me_2dien)Cl]Cl was prepared in the same manner as [PtCl(dien)]Cl.[1] Anal. Calcd for $C_6H_{17}N_3Cl_2Pt$: C 18.14; H, 4.31; N, 10.58. Found: C, 18.33; H, 3.84; N, 10.41. ^{1}H NMR (D_2O): 2.88 (14H, m). ^{195}Pt NMR: -2824 ppm.

[Pt(N,N'-Me_2dien)(9EtG)]$(NO_3)_2$.2H_2O - [Pt(N,N'-Me_2dien)(9EtG)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{13}H_{30}N_{10}O_9Pt$: C 23.46; H, 4.54; N, 21.05. Found: C, 23.07; H, 4.21; N, 20.73. ^{1}H NMR (D_2O): 1.50 (3H, t), 2.88 (14H, m), 4.21 (2H, q), 8.70 (1H, broad s). ^{195}Pt NMR: -2876, -2907 ppm.

[Pt(N,N'-Me_2dien)(Guo)]$(NO_3)_2$ 2.5H_2O– [Pt(N,N'-Me_2dien)(Guo)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{16}H_{35}N_{10}O_{13.5}Pt$: C 24.68; H, 4.49; N, 17.99. Found: C, 24.27; H, 4.83; N, 17.71. ^{1}H

NMR (D_2O): 3.03 (14H, m), 3.90 (2H, m), 4.28 (2H, t), 4.43 (2H, t), 6.00 (1H, d), 8.88 (1H, d).^{195}Pt NMR: -2880, -2912.

[Pt(N,N'-Me$_2$dien)(Xan)](NO$_3$)$_2$. 2.5H$_2$O- [Pt(N,N'-Me_2dien)(Xan)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9EtG)]$(NO_3)_2$. Anal. Calcd for $C_{16}H_{34}N_9O_{14.5}Pt$: C 24.65; H, 4.37; N, 16.17. Found: C, 24.36; H, 4.77; N, 15.71. ^{1}H NMR (D_2O): 3.03 (14H, m), 3.98 (2H, m), 4.43 (2H, m), 4.65 (2H, t), 6.02 (1H, s), 8.66 (1H, broad s). ^{195}Pt NMR -2900 ppm (br).

[PtCl(N,N'-Me$_4$dien)]Cl - [Pt(N,N'-Me_4dien)Cl]Cl was prepared in the same manner as [PtCl(dien)]Cl [1]. Briefly, $[PtCl_2(DMSO)_2]$ was suspended in acetone, N,N'-Me_4dien (1 mol. eq.) was added and the solution was refluxed for 3 hrs. The solution was cooled to room temperature and the solvent was reduced to near dryness. Ether was added to precipitate the final product and the solid was dried *in vacuo*. ^{1}H NMR (D_2O): 2.90 (20H, m). ^{195}Pt NMR: -2704 ppm.

[Pt(N,N'-Me$_4$dien)(9-EtGua)](NO$_3$)$_{2.4}$ 3H$_2$O- [Pt(N,N'-Me_4dien)(9-EtGua)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{15}H_{36}N_{10}O_{10}Pt$: C 25.31; H, 5.06; N, 19.69. Found: C, 25.12; H, 4.83; N, 19.12. ^{1}H NMR (D_2O): 1.51 (3H, t), 2.90 (20H, m), 4.22 (2H, q), 8.69 (1H, d). ^{195}Pt NMR: -2758, -2773 ppm.

[Pt(N,N'-Me$_4$dien)(Guo)](NO$_3$)$_2$ 3H$_2$O – [Pt(N,N'-Me_4dien)(Guo)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{18}H_{40.4}N_{10.4}O_{15.2}Pt$: C 26.50; H, 4.90; N, 17.17. Found: C, 25.94; H, 4.36; N, 16.83. ^{1}H NMR (D_2O): 2.90 (20H, m), 3.92 (2H, m), 4.31 (2H, t), 4.46 (2H, t), 6.03 (1H, d), 8.90 (1H, d). ^{195}Pt NMR: -2763, -2778 ppm.

[Pt(N,N'-Me$_4$dien)(Xan)](NO$_3$)$_2$ 3H$_2$O - [Pt(N,N'-Me_4dien)(Xan)]$(NO_3)_2$ was synthesized in a similar manner as [Pt(dien)(9-EtGua)]$(NO_3)_2$. Anal. Calcd for $C_{16}H_{39}N_9O_{15}$ Pt: C 26.47; H, 4.78; N, 15.44. Found: C, 26.19; H, 5.19; N, 15.11. ^{1}H NMR (D_2O): 2.90 (20H, m), 4.00 (2H, m), 4.45 (2H, m), 4.66 (2H, t), 6.03 (1H, s), 8.97, 9.00 (1H, s). ^{195}Pt NMR: - 2750 ppm (br).

Cytotoxicity and Cellular Accumulation

Overall, procedures followed those published from our group (See Ref. 3) Four million cells were incubated in 10 mL of RMPI media (10% FBS, 1%Pen-Strep) with 50 μM drug for 3 or 6 hours. The solutions were centrifuged at 1500 rpm at 4oC for 5 minutes, the media was removed and the cell pellet was washed with 2x10mL cold PBS. To digest samples for ICP-MS analysis, 1 mL of conc. HNO3 was added to the pellet and left to digest for 72 hours. Two mL of water were added, the solutions were filtered through a 0.45 _M GHP filter, and run on the ICP-MS to determine the concentration of platinum in each sample.

Cytotoxicity CCRF-CEM or Jurkat cells were seeded at a concentration of 2.5 x10^4 cells/100 μL. Drugs 1a, 1b, 2a, and 3a were added at varying concentrations (100, 50, 25,

12.5, 6.25, 3.125, 1.6125 μM) and the cells were incubated at 37°C for 72 hours. WST-1 (10 μL of 100 commerical solution) was added and incubated for 4 hours. The absorbance at 405 nm was recorded and the IC_{50} was determined.

Molecular Docking

HIV1 NCp7 structure was retrieved from PDB (entry 1MFS). The first model from a set of 30 deposited NMR structures was used for docking with CLC Drug Discovery Workbench 2.4. Protein preparation: missing hydrogens were added and Glu (OE2) and His (ND1) protonation states were selected. Two different Cys protonation states were also evaluates, with no major differences observed on the final docking scores and poses. 10 poses were returned and manually inspected for each compound. No binding pockets were used as guides.

Theoretical Methods

Geometry optimizations of small models were performed using Gaussian 09[4] with the B97-D semi-local *generalized gradient approximation* (GGA) DFT functional, a re-parameterization of Becke's B97 functional with a semi-empirical dispersion correction. B97-D performs very well for non-bonded interactions and adequately models interactions found in biological systems. The D95V basis set with polarization functions was used for C, H, N and O. The Hay and Wadt effective core potential basis sets for S and Pt were augmented with polarization functions. Larger models were optimized using the ONIOM method and sub-dividing the model into semi-empirical (SE) and a quantum mechanical (QM, DFT(B-97D) regions. The SE region was constrained to the solution structure to preserve the steric effects of the surrounding protein, while the QM region was allowed to freely optimize. The SE regions was modeled with the PM6 method, which was parameterized to emphasize biochemical systems.[5] Charge decomposition analysis (CDA), as implemented in the Multiwfn program,[6] was used to quantify charge relocation between fragments in the π-stacked complex.[7] The charge donation from the occupied (occ) orbitals of the donor to the virtual (vir) orbitals of acceptor is denoted by d (Equation 1) where i and η are the index and occupation number of the molecular orbitals (MO) of the complex, $C_{m,i}$ is the coefficient of fragment orbital (FO) m in MO i of the complex, and $S_{m,n}$ is the overlap integral between m and n.[7]

$$d_i = \sum_{m \in A}^{occ} \sum_{n \in B}^{vir} \eta_i C_{m,i} C_{n,i} S_{m,n} \qquad (1)^7$$

$$b_i = \sum_{m \in A}^{vir} \sum_{n \in B}^{occ} \eta_i C_{m,i} C_{n,i} S_{m,n} \qquad (2)^{7}$$

Back donation from the occupied orbitals of the acceptor to the virtual orbitals of the donor is denoted by b (Equation 2).[7] The net electrons transferred is the difference of these values (d-b). Donor-acceptor energies ($\Delta E_{d \rightarrow a}$), Wiberg bond indices (WBIs)[8](Equation 3) and the natural population analysis (NPA) charges on all atoms were calculated with Natural Bond Order (NBO) Version 3.1.[9,10] WBIs measure the bonding interactions between two atoms[11] and were calculated as the sum of the squares of the off-diagonal elements of the density matrix P between atom pairs.[8]

$$\overset{Wiberg}{AB} = \sum_{s \in A} \sum_{t \in B} P_{st}^2 \qquad (3)^{8}$$

Figures

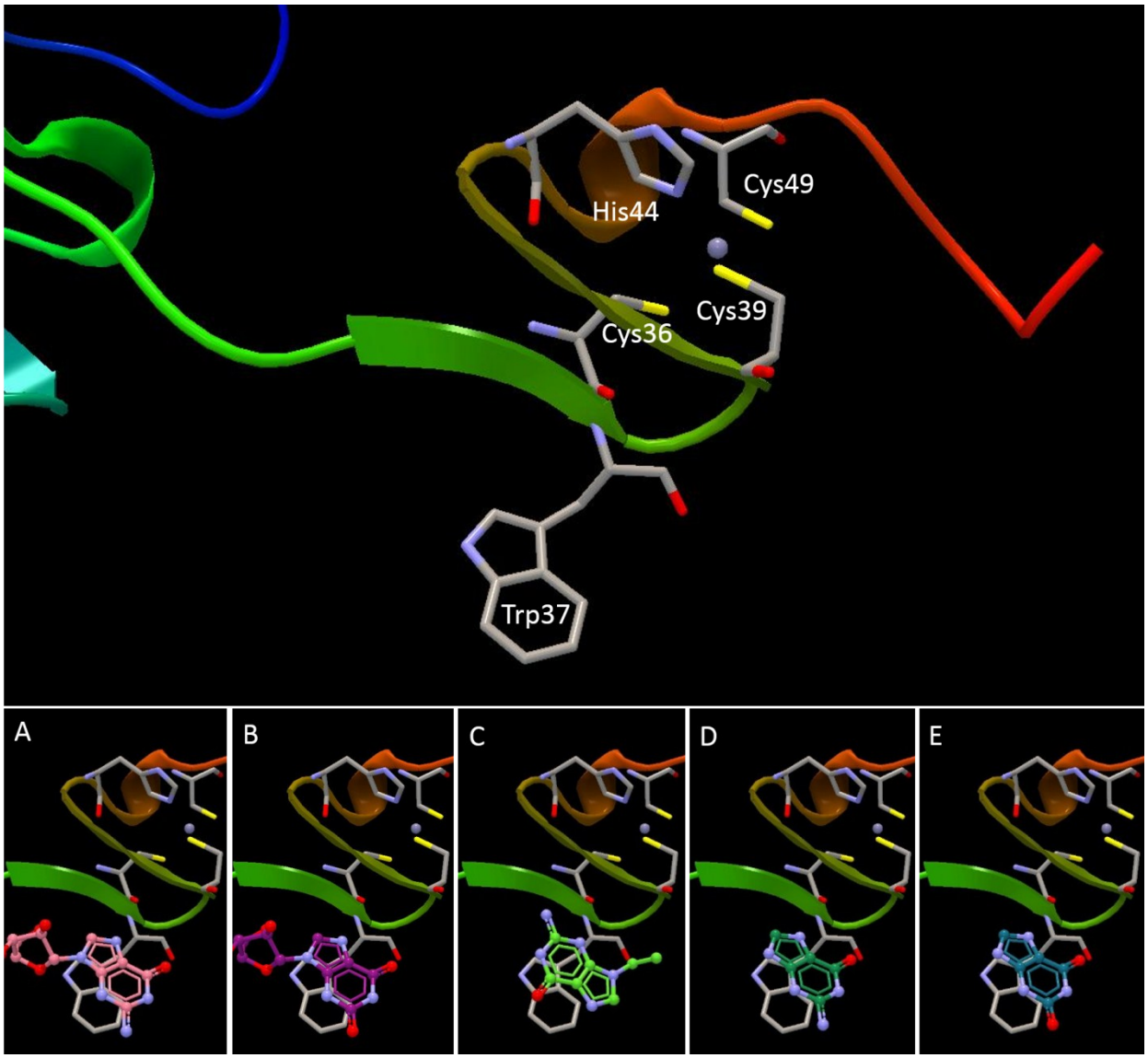

Figure S1. Best-scored molecular poses returned for docking of (A) Guanosine, (B) Xanthosine, (C) 9-EtGua, (D) Guanine and (E) Xanthine in the structure of HIV-1 C-terminal ZF from NCp7 (retrieved from PDB entry 1MFS).

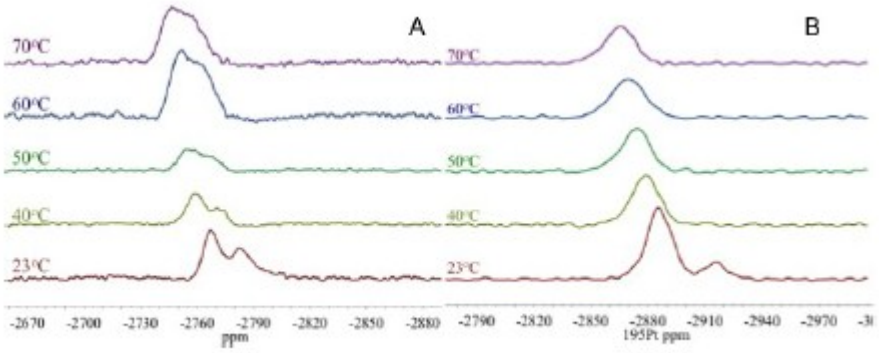

Figure S2. Temperature dependence of the ^{195}Pt NMR spectra of A – [Pt(N,N”-Me_4dien)(9-EtGua)]$^{2+}$ and B- [Pt(N,N”-Me_2dien)(9-EtGua)]$^{2+}$.

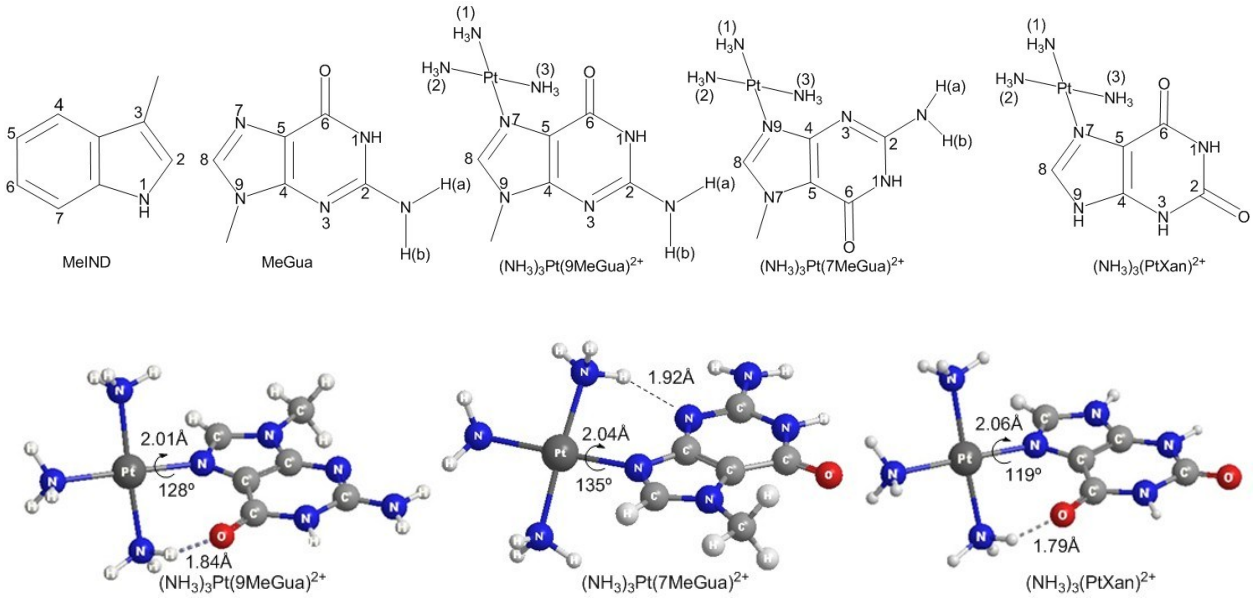

Figure S3. top) Numbering scheme for MeInd, MeGua, $[Pt(NH_3)_3(9MeGua)]^{2+}$, $[Pt(NH_3)_3(7MeGua)]^{2+}$ and $(NH_3)_3Pt(Xan)]^{2+}$.
(bottom) Optimized structures for $[Pt(NH_3)_3(9MeGua)]^{2+}$, $[Pt(NH_3)_3(7MeGua)]^{2+}$]and $[Pt(NH_3)_3Xan]^{2+}$

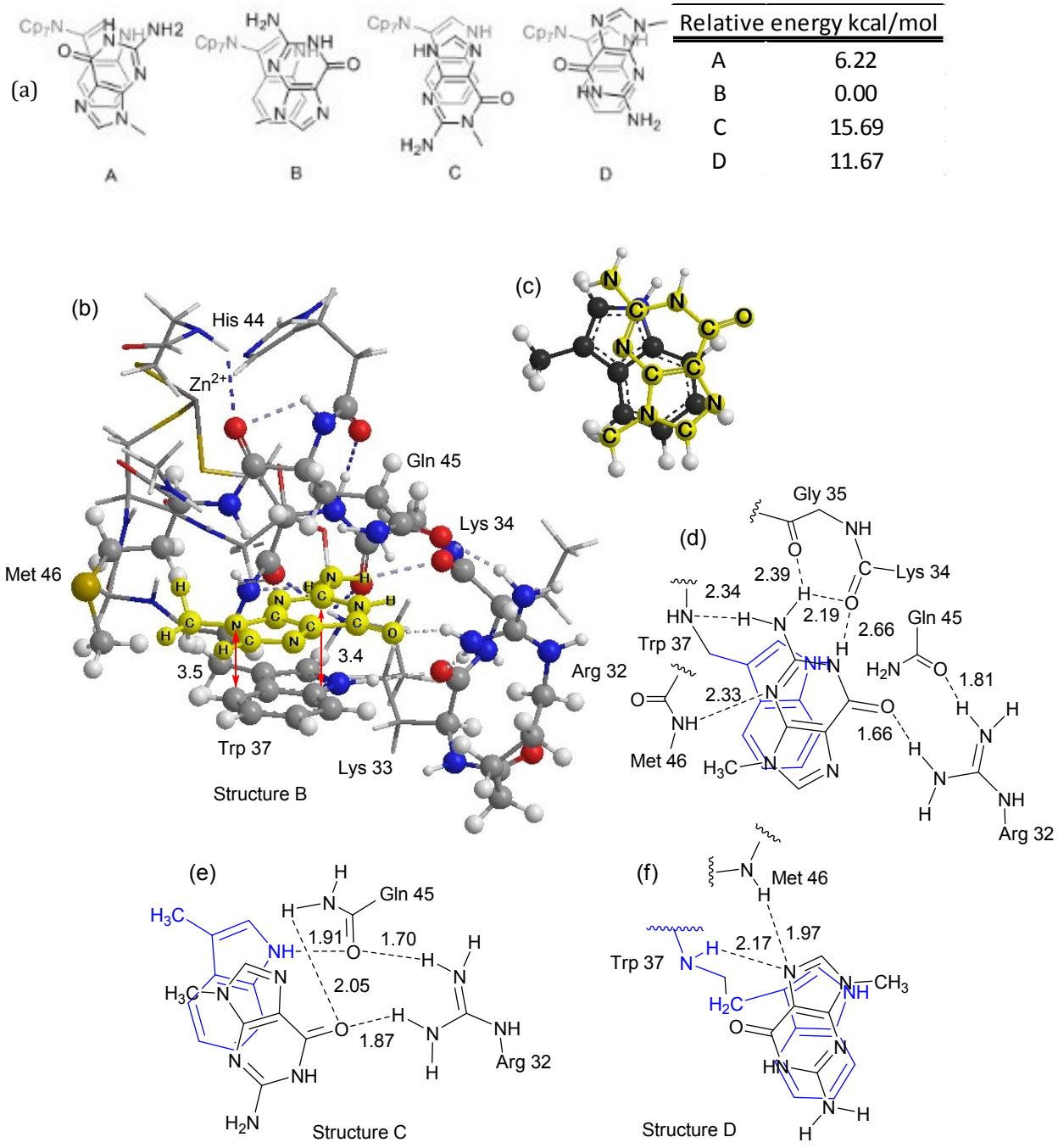

Figure S4.(a) Orientations and relative energies of conformations A-D of MeGua in the binding site of NCp7. (b) Truncated optimized model B. (b) Top view of the π-stacking interaction in B. Key hydrogen-bonding interactions in conformations (d) B, (e) C, and (f) D.

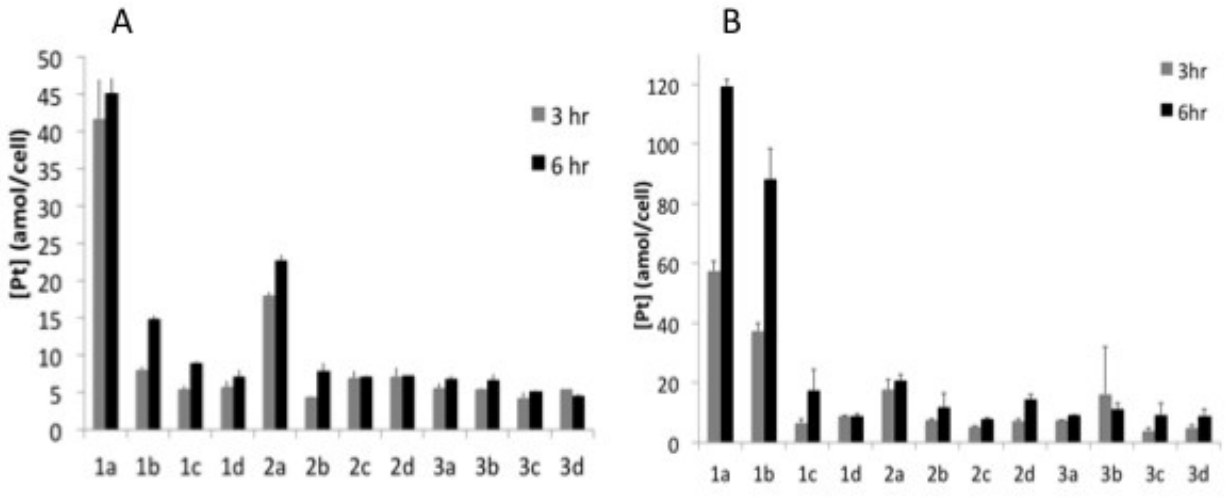

Figure S5. Cellular accumulation of platinum-nucleobase compounds in (A) Jurkat and (B) CCRF-CEM cells.

Table S1. Docking scores summary for free nucleobases (guanine and xanthine), alkyl-functionalized nucleobase (9-EtGua) and nucleosides (Gu0 and Xan).

Nucleobase derivate	C-terminal finger			N-terminal finger		
	Score	H-bonding	Steric interaction	Score	H-bonding	Steric interaction
Guanine	-26.93	-2.00	-24.93	-27.57	-12.72	-14.85
9-EtGua	-34.62	-6.94	-28.28	-30.17	-6.00	-24.77
Guanosine	-43.00	-9.60	-34.42	-40.13	-9.39	-31.6
Xanthine	-26.83	-2.00	-24.83	-26.95	-9.74	-17.21
Xanthosine	-43.02	-9.57	-34.47	-40.18	-9.55	-31.48

Docking calculations also corroborate the fluorescence-based experiments regarding Trp tracking. In Figure 4, it is clear that Trp37 acts as a molecular recognition factor, with all final poses for the docked compounds (Figure 4) appearing in close proximity of Trp37, even though no ligand pockets were used as guides for docking.

Table S2. ONIOM(B97-D:PM6) results for orientations **A-D** of MeGua in the binding site of the NCp7 model. NPA charges of hydrogen bond donors (q_D) and acceptors (q_A), distances and WBI values for the hydrogen bonded pairs

Donor	q_D (e)	Acceptor	q_A (e)	d, Å	$\Delta E_{d\rightarrow a}$ (kcal/mol	WBI
Native **A**						
MeGau C=O	-0.697	M46 BB N-H	0.436	1.91	7.19	0.040
MeGau C=O	-0.697	W37 BB N-H	0.417	2.28	1.80	0.009
K34 BB C=O	-0.722	MeGua N-H	0.459	2.12	2.81	0.021
K34 BB C=O	-0.722	MeGau NH_2-Ha	0.431	1.94	9.69	0.035
Q45 SC C=O	-0.686	MeGau NH_2-Hb	0.452	1.86	14.32	0.057
MeGau NH_2	-0.925	R32 SC N-H	0.433	1.82	21.92	0.080
MeGau N_3	-0.660	R32 SC N-H	0.439	1.96	12.07	0.057
B						
MeGau C=O	-0.685	R32 SC N-H	0.434	1.66	31.76	0.110
K34 BB C=O	-0.689	MeGau N_1-H	0.447	2.66	0.55	0.004
K34 BB C=O	-0.689	MeGau NH_2-Ha	0.429	2.19	2.24	0.018
W37 BB N	-0.705	MeGau NH_2-Hb	0.428	2.34	2.77	0.013
G35 BB C=O	-0.693	MeGau NH_2-Ha	0.429	2.39	0.22	0.004
MeGau N_3	-0.651	M46 BB N-H	0.416	2.33	2.34	0.017
C						
MeGau C=O	-0.731	Q45 SC N-H	0.431	2.05	7.95	0.029
MeGau C=O	-0.731	R32 SC N-H	0.437	1.87	14.37	0.048
D						
MeGau N_7	-0.592	M46 BB N-H	0.415	1.97	8.70	0.026
MeGau N_7	-0.592	W37 BB N-H	0.419	2.17	5.05	0.012

Table S3 Cellular Accumulation (A) and Cytotoxicity (B) of selected platinum-purine and nucleoside complexes

A

Compound	**Log($P_{oct/water}$)**
1a	-1.59 +/- 0.28
1b	-0.36 +/- 0.06
1c	0.22 +/- 0.09
1d	-0.36 +/- 0.04

B

	IC50 (μM)	
Compound	**CCRF-CEM**	**Jurkat**
1a	>100	>100
1b	>100	>100
2a	>100	>100
3a	>100	>100

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