Electronic Supplementary Information

Palladium (II) complexes with thiosemicarbazones derived from pyrene as topoisomerase IB inhibitors

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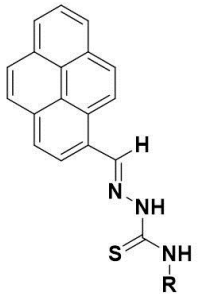
Supporting Information

Preparation of HPrEt and HPrCh

The ligands were synthesized by refluxing a solution of 1-pyrenecarboxaldehyde (1 mmol) and the desired thiosemicarbazide (1 mmol) in methanol (15 mL) with the addition of 3 drops of HCl for 1.5 h at 60 ˚C. The yellow precipitate formed was collected by filtration, washed with diethyl ether, and recrystallized from dichloromethane/acetonitrile. Crystals suitable for X-ray diffraction of HPrEt were obtained by slow evaporation of a dichloromethane/acetonitrile solution of the compound at ambient temperature.



R = Et (ethyl) (HPrEt)
R = Ch (cyclohexyl) (HPrCh)

Data for HPrEt (331.43 g/mol). Yield: 235 mg (71%). M.P.: 253 − 255 °C. IR (ν_{max}/cm^{-1}): 3358, 3151 ν(N-H), 1591, 1537, 1519 ν(C=N) + ν(C=C), 840 δ(C−H)$_{Pr}$, 817 ν(C=S). ^{1}H NMR(DMSO-d_6): δ =1,21 (t, ^3J = 6.0 Hz, 3H, −CH$_2$CH$_3$), 3,67 (dq, $^3J_1$ = $^3J_2$= 6.0 Hz, 2H, −NHCH$_2$CH$_3$), 8.11 (t, ^3J = 8 Hz, 1H, Pr−H), 8.21 – 8.26 (m, 2H, Pr−H), 8.36 – 8.32 (m, 4H, Pr−H), 8.46 (d, ^3J = 8 Hz, 1H, Pr−H), 8.75 (t, ^3J = 6.0 Hz, 1H, −NHCH$_2$CH$_3$), 8.89 (d, ^3J = 8 Hz, 1H, Pr−H), 9.28 (s, 1H, HC=N), 11.54 (s, 1H, NH−C=S). ^{13}C RMN (DMSO-d_6): δ 15.12 (CH$_3$), 38.93 (CH$_2$), 121.97, 124.30, 124.39, 124.54, 125.56, 126.14, 126.47, 127.02, 127.46, 127.90, 128.64, 129.08, 129.16, 130.60, 131.33, 132.20 (CH−Pr), 140.12 (C=N), 177.05 (C=S). Anal. Calcd for $C_{20}H_{17}N_3S\cdot\frac{1}{2}H_2O$: C, 70.56; H, 5.33; N, 12.34 %. Found: C, 70.88; H, 5.12; N, 12.68 %. MS (ESI+): *m/z* for $C_{20}H_{17}N_3S$ $[M + Na]^+$: calcd 354.1035, found 354.1045. UV–Vis, DMSO solution concentration: 6.0 x 10^{-6} M [λ_{max} (Ɛ, L mol^{-1} cm^{-1})]: 409.00 nm (49 500), 387.00 nm (60 666), 280.00 (44 833). HPLC: R_t = 31.6 min (97.1% at 254 nm).

Data for HPrCh (385.52 g/mol). Yield: 350 mg (92%). M.P.: 251 − 253 °C. IR (ν_{max}/cm^{-1}): 3329, 3134 ν(N-H), 1591, 1541, 1519 ν(C=N) + ν(C=C), 844 δ(C−H)$_{Pr}$, 823 ν(C=S). ^{1}H NMR (DMSO-d_6): δ 1.17 – 1.22 (m, 1H, Ch−H), 1.29 – 1.36 (m, 2H, Ch−H), 1.48 – 1.56 (m, 2H, Ch−H), 1.62 – 1.65 (m, 1H, Ch−H), 1.75 – 1.77 (m, 2H, Ch−H), 1.92 – 1.95 (m, 2H, Ch−H), 4.22 – 4.30 (m, 1H, Ch-CH), 8.11 (t, ^3J = 8 Hz, 1H, Pr−H), 8.21 – 8.26 (m, 3H, Pr−H), 8.32 – 8.36 (m, 4H, Pr−H), 8.47 (d, ^3J = 8 Hz, 1H, Pr−H), 8.87 (d, ^3J = 8 Hz, 1H,

−NHCh), 9.28 (s, 1H, N=CH), 11.53 (s, 1H, NH−C=S). ^{13}C RMN (DMSO-d_6): δ 25.42, 25.62, 32.29, 53.29 (CH−Ch), 121.98, 124.28, 124.52, 124.55, 125.57, 126.15, 126.47, 127.01, 127.32, 127.89, 128.66, 129.07, 129.19, 130.58, 131.31, 132.23 (CH−Pr), 140.54 (C=N), 176.08 (C=S). Anal. Calcd for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90 %. Found: C, 75.21; H, 6.16; N, 10.90 %. MS (ESI+): *m/z* for $C_{24}H_{23}N_3S$ $[M + Na]^+$: calcd 408.1505, found 408.1502. UV−Vis, DMSO solution concentration: 7.3 x 10^{-6} M [λ_{max} (Ɛ, L mol^{-1} cm^{-1})]: 407.00 nm (51 377), 382.00 nm (60 192), 282.00 (43 801). HPLC: R_t = 40.6 min (97.1% at 254 nm).

Table S1. Selected bond lengths (Å) and angles (deg) for [PdCl(PPh_3)(PrCh)] (**1**) and [PdCl(PPh_3)(PrEt)].

	1	**2**
Bond length (Å)		
Pd(1)−N(1)	2.088(2)	2.101(2)
Pd(1)−S(1)	2.2421(11)	2.2341(7)
Pd(1)−P(1)	2.2387(8)	2.2535(6)
Pd(1)−Cl(1)	2.3376(12)	2.3539(6)
S(1)−C(2)	1.747(3)	1.753(3)
N(2)−C(2)	1.305(4)	1.302(3)
Angle (°)		
N(1)− Pd−S(1)	83.11(7)	83.41(6)
N(1)− Pd−P(1)	177.07(7)	174.70(6)
S(1)− Pd−P(1)	94.05(4)	91.48(2)
N(1)− Pd−Cl(1)	93.27(7)	95.85(6)
S(1)− Pd−Cl(1)	173.04(3)	176.34(3)
P(1)− Pd−Cl(1)	89.63(4)	89.34(2)

Table S2. Selected bond lengths (Å) and angles (deg) for HPrCh and HPrEt.

	HPrCh	**HPrEt***
Bond length (Å)		
N(1)-C(1)	1.277(4)	1.262(3) / 1.275(3)
N(1)-N(2)	1.366(4)	1.373(3) / 1.375(3)
S(1)-C(2)	1.691(4)	1.678(3) / 1.685(3)
N(2)-C(2)	1.350(4)	1.351(3) / 1.353(4)
N(3)-C(2)	1.323(5)	1.322(4) / 1.326(4)
Angle (°)		
N(3)-C(2)-N(2)	116.5(3)	115.4(3) / 116.1(3)
N(3)-C(2)-S(1)	125.0(2)	125.0(2) / 125.4(2)
C(1)-N(1)-N(2)	115.5(3)	116.6(3) / 116.1(2)
C(2)-N(2)-N(1)	121.1(3)	120.5(3) / 119.9(3)
N(1)-C(1)-C(3)	121.8(3)	122.7(3) / 121.4(3)

* Values for two crystallographically independent molecules.

Table S3. Crystallographic data for HPrCh, HPrEt, [PdCl(PPh_3)(PrCh)] (**1**) and [PdCl(PPh_3)(PrEt)](**2**).

	HPrCh	**HPrEt**	**1**	**2**
Formula	$C_{24}H_{23}N_3S$	$C_{40}H_{34}N_6S_2$	$C_{42}H_{37}ClN_3PPdS$	$C_{38}H_{31}ClN3PPdS$
MW	385.51	662.85	788.62	734.54
Temperature / K	296(2)	296(2)	296(2)	296(2)
λ/ Å	0.71073	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic
Space group	*C2/c*	$P\bar{1}$	*C2/c*	$P2_1/c$
a (Å)	23.6353(6)	7.8769(3)	31.1556(6)	14.2704(11)
b (Å)	9.8015(3)	8.6861 (3)	10.4263(2)	12.0045(8)
c (Å)	21.9884(5)	26.3559(10)	26.4258(5)	21.2465(16)
α (˚)	90	94.574(2)	90	90
β (˚)	118.1150(10)	92.802(2)	120.4580(10)	109.283(2)
γ (˚)	90	112.551(2)	90	90
V (Å^3)	4492.8(2)	1653.88(11)	7399.5(2)	3435.5(4)
Z	8	2	8	4
Density (calculated)	1.140 (Mg.m^{-3})	1.331 (Mg.m^{-3})	1.416 (Mg.m^{-3})	1.420 (Mg.m^{-3})
Absorption coefficient	0.157 (mm^{-1})	0.201 (mm^{-1})	0.708 (mm^{-1})	0.756 (mm^{-1})
Crystal size/ mm^3	0.260 x 0.180 x 0.130	0.600 x 0.280 x 0.080	0.600 x 0.300 x 0.230	0.590 x 0.540 x 0.510
2Θ range for data collection	1.954 to 25.475°	1.556 to 25.078°	1.658 to 25.099°	1.512 to 26.420°
Index ranges	-28<=h<=28, -11<=k<=11, -26<=l<=26	-9<=h<=9,-10<=k<=10 -10<=k<=10, -31<=l<=31	-32<=h<=37, -12<=k<=12, -31<=l<=31	-17<=h<=17, -14<=k<=14, -26<=l<=26
Reflections collected	14823	20426	21535	73175
Independent reflections	4163 [R(int) = 0.0260]	5829 [R(int) = 0.0343]	6521 [R(int) = 0.0194]	7061 [R(int) = 0.0298]

Abs. Corr.	Multi-scan	Multi-scan	Multi-scan	Multi-scan
Final R indices [I>2σ(I)]	R_1 = 0.0699, wR_2 = 0.2137	R_1 = 0.0514, wR_2 = 0.1296	R_1 = 0.0231, wR_2 = 0.0561	R_1 = 0.0283, wR_2 = 0.0731
R indices (all data)	R_1 = 0.0982, wR_2 = 0.2423	R_1 = 0.0924, wR_2 = 0.1521	R_1 = 0.0284, wR_2 = 0.0598	R_1 = 0.0319, wR_2 = 0.0766
GOF	1.064	1.043	1.051	1.106

Table S4. Flow cytometry analysis of cell cycle of A2780 cells exposed to 7.5 μM and 0.75 μM concentrations of complexes **1** and **2**, respectively.

Compounds	G1 (%)	S (%)	G2/M (%)
Negative Control	73.6 ± 0.5	18.6 ± 0.3	7.9 ± 0.8
Complex 1	47 ± 3	24± 1	30 ± 1
Complex 2	44.8 ± 0.2	38± 2	17± 3

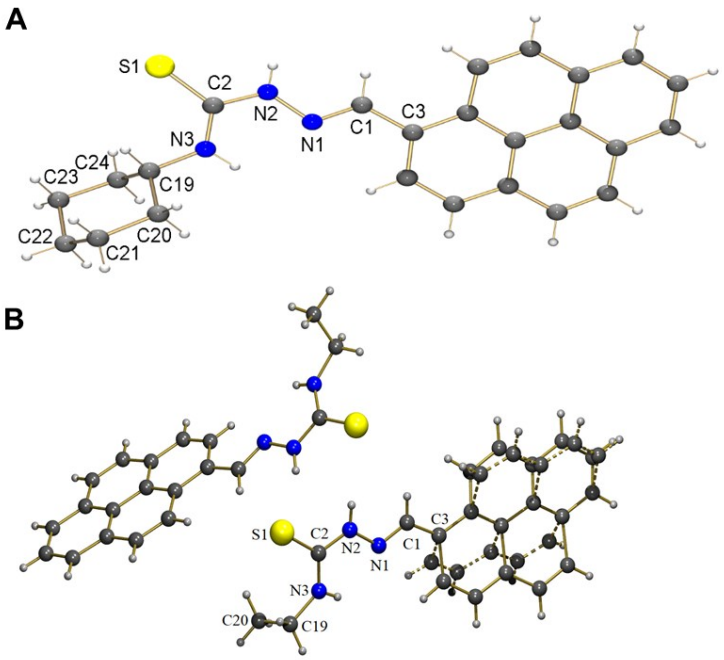


Figure S1. Crystalline and molecular structure of **A**) HPrCh and **B**) Molecules in the asymmetric unit of HPrEt (a disorder in the pyrene fragment is found in one of the molecules).

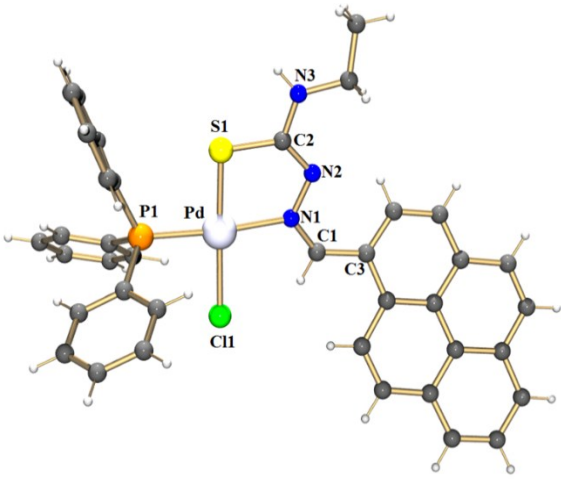


Figure S2. Crystalline and molecular structure of [PdCl(PPh_3)(PrEt)].

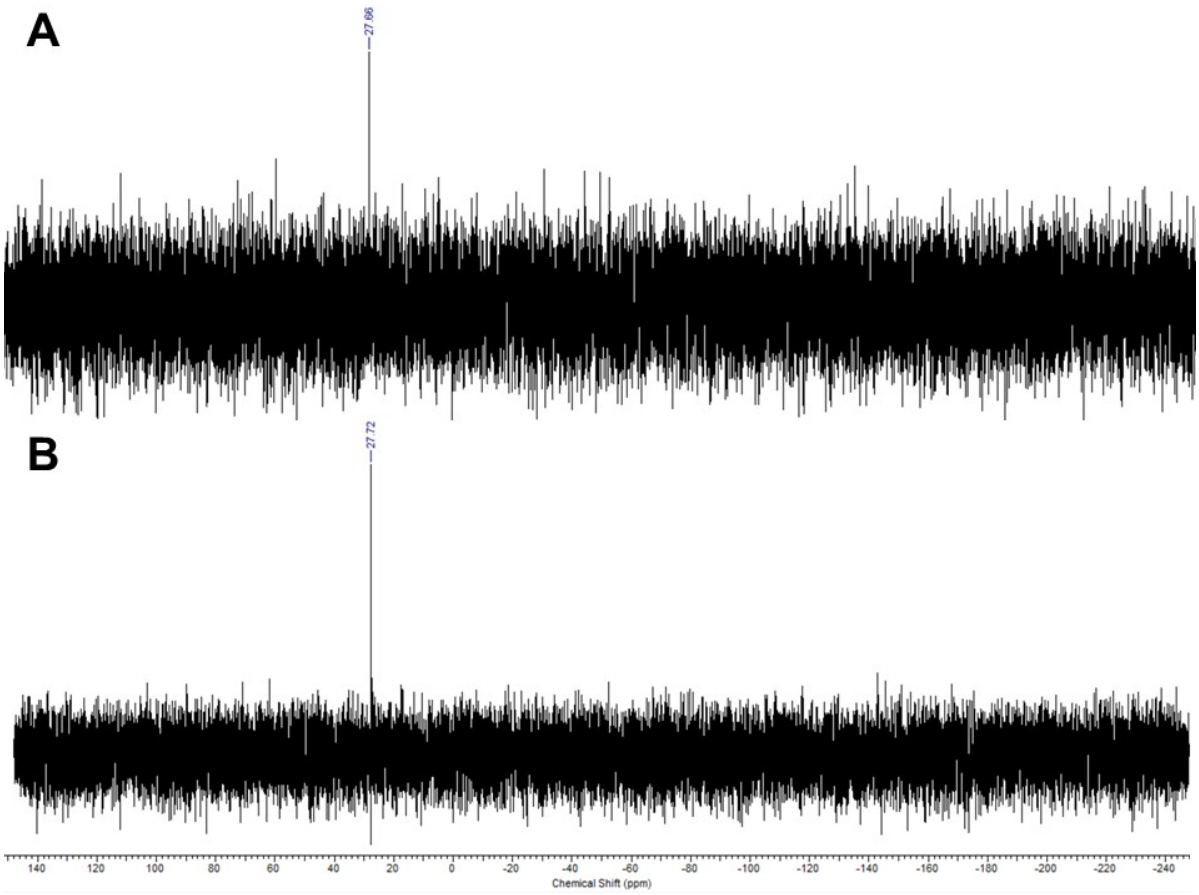


Figure S3. ^{31}P NMR spectra A) carried out with fresh solution of the complex 1 in DMSO-d_6 containing 20% D_2O and B) spectrum of the same solution performed 24 hours later.

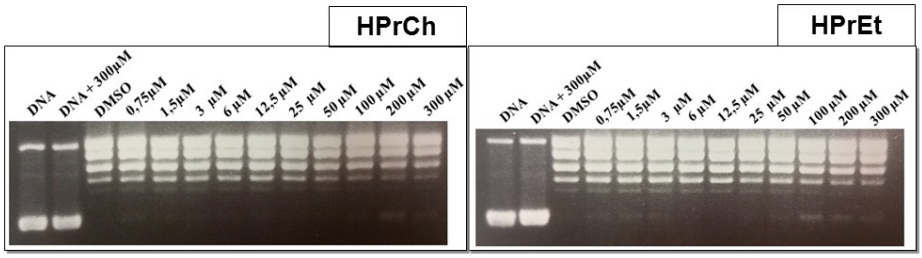


Figure S4. Relaxation of negative supercoiled plasmid DNA by TopIB in presence of increasing concentrations of the free ligands HPrCh and HPrEt. Lane 1, no protein added; lane 2, control reaction with DNA and the maximum compound concentration (300 μM); lane 3, positive control reaction with DNA and enzyme in absence of the compounds. The reaction products were resolved on an agarose gel and visualized with ethidium bromide. SC, supercoiled plasmid DNA.

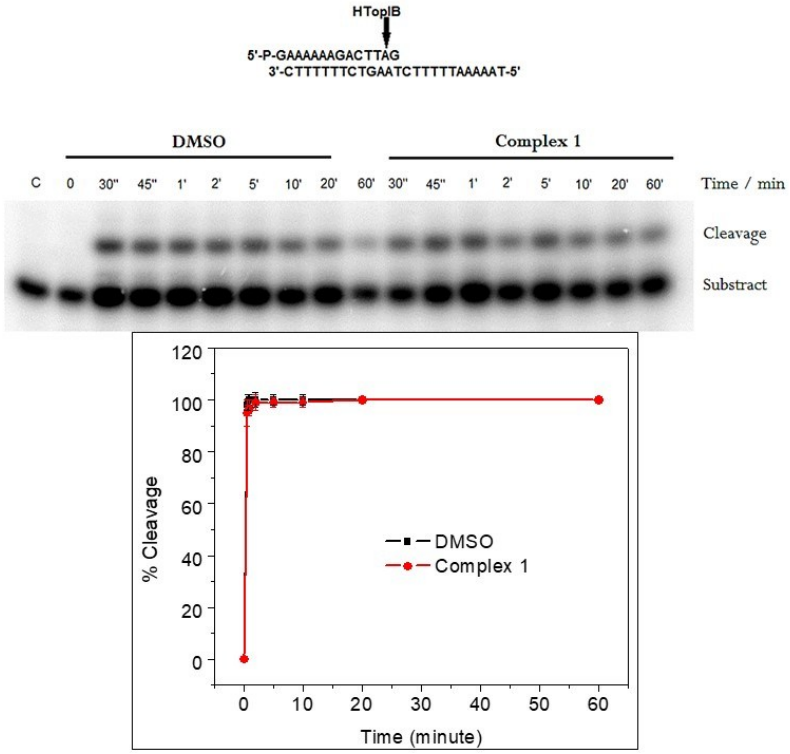


Figure S5. Upper panel: The CL14/CP25 substrate used to measure the cleavage kinetics of the TopIB, with the preferential cleavage site, indicated by an arrow. CL1 represents the DNA fragment cleaved. Lower panel: Gel analysis of the cleavage kinetics and percentage of the cleavage product plotted at different times for topoisomerase IB.

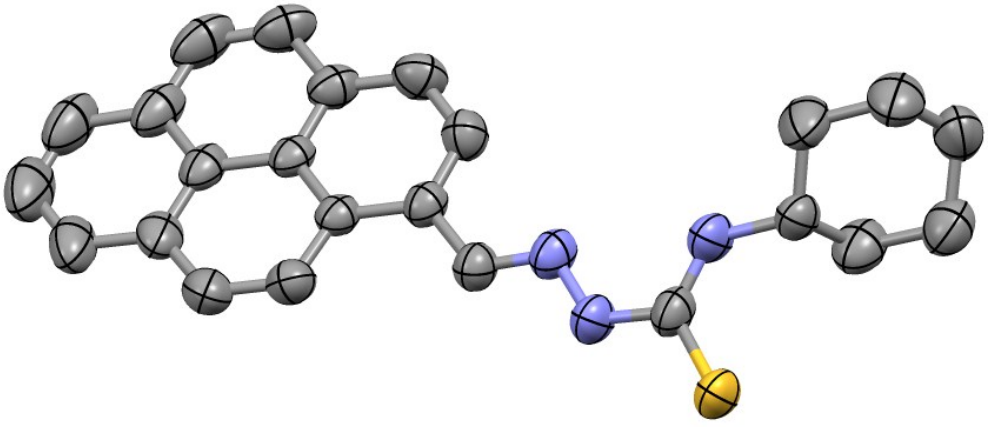


Figure S6. ORTEP for HPrCh at the 50% probability level. The hydrogen atoms are omitted for clarity.

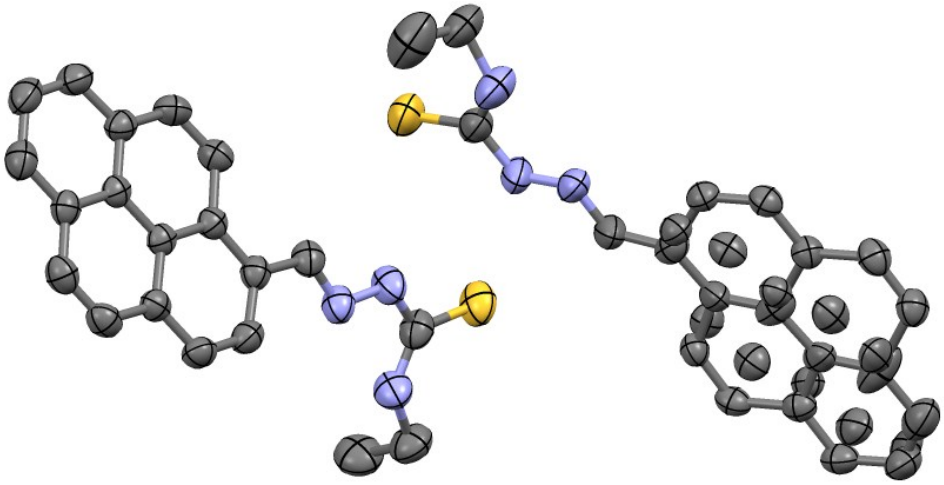


Figure S7. ORTEP for HPrEt at the 50% probability level. The hydrogen atoms are omitted for clarity.

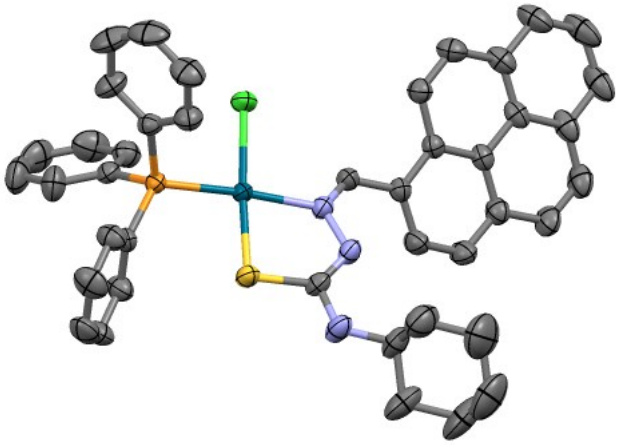


Figure S8. ORTEP for **1** at the 50% probability level. The hydrogen atoms are omitted for clarity.

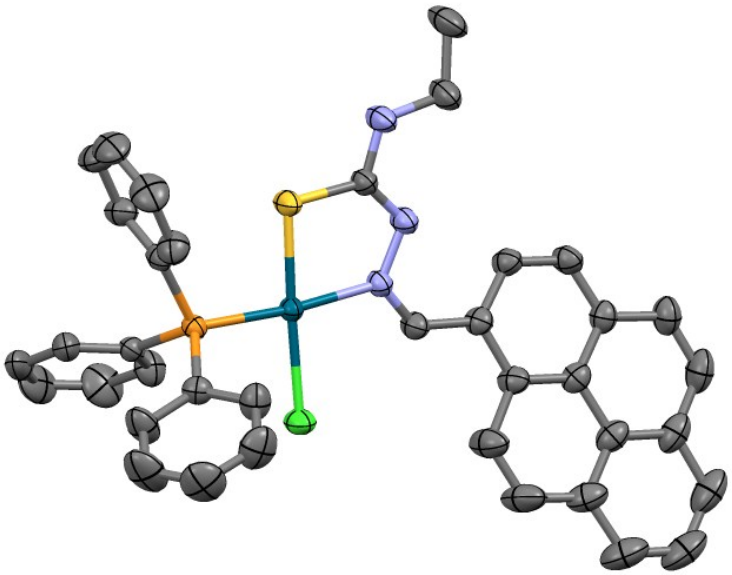


Figure S9. ORTEP for **2** at the 50% probability level. The hydrogen atoms are omitted for clarity.