Electronic Supplementary Information (ESI)

Fluorescence sensing ADP over ATP and PPi in 100% aqueous solution

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1. General Experimentals.

Starting materials were purchased from commercial suppliers and were used without further purification. 9,10-bisaminomethylanthracene (**2**) and 2-Bromomethyl-6-pivalamidopyridine (**3**) were prepared according the previously published methods (see below). All solvents were purified by the most used methods before use. Distilled water was used after passing through a Millipore-Q ultrapurification system. NMR spectra were measured on Varian Mercury 400 and 600 instruments. HR-MS data were obtained with an LC/Q-TOF MS spectrometer. UV-vis spectra and fluorescent spectra were recorded on an Agilent Cary 100 UV-vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. Both UV-vis and fluorescence spectrophotometer are equipped with a temperature controller. Standard quartz cuvettes with a 10 mm lightpath are used for all UV-vis spectra and fluorescent spectra measurements. Cell imaging was performed in an inverted fluorescence microscopy with a 20× objective lens.

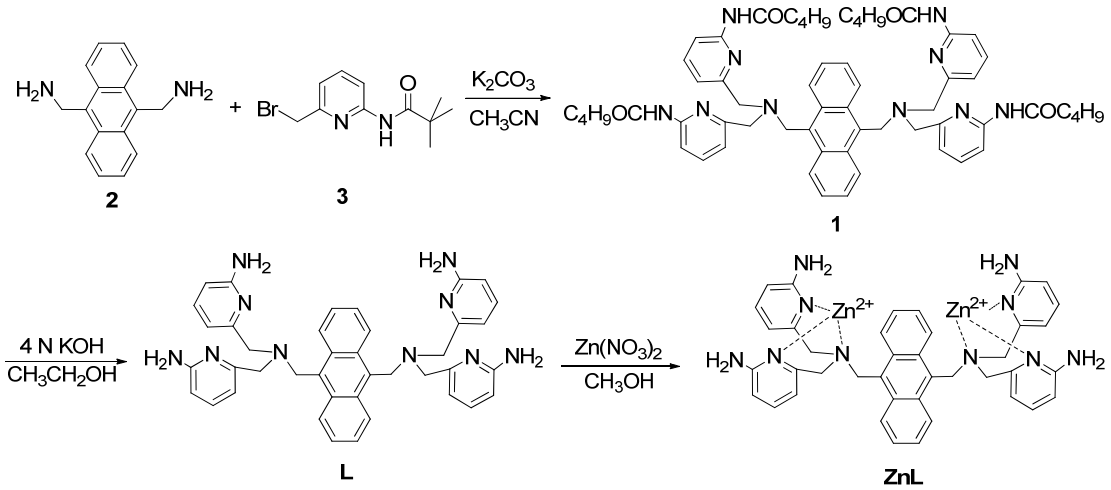
Fluorescent Studies of ZnL upon Addition of Analytes. For a typical optical measurements, 3.0 mL of **ZnL** (5 μM) in a HEPES buffer (10 mM, pH 7.2) was placed in a quartz cell at 25 °C. The fluorescent spectra were then recorded immediately after addition of analytes.

The fluorescence quantum yields of **ZnL**, **ZnL**-2ADP and **ZnL**-2ATP were determined to be Φ = 0.02, 0.99, and 0.50 in HEPES buffer (10 mM, pH 7.2) at 25˚C, using quinine sulfate (Φ_f = 0.58 in 1N H_2SO_4) as standard.

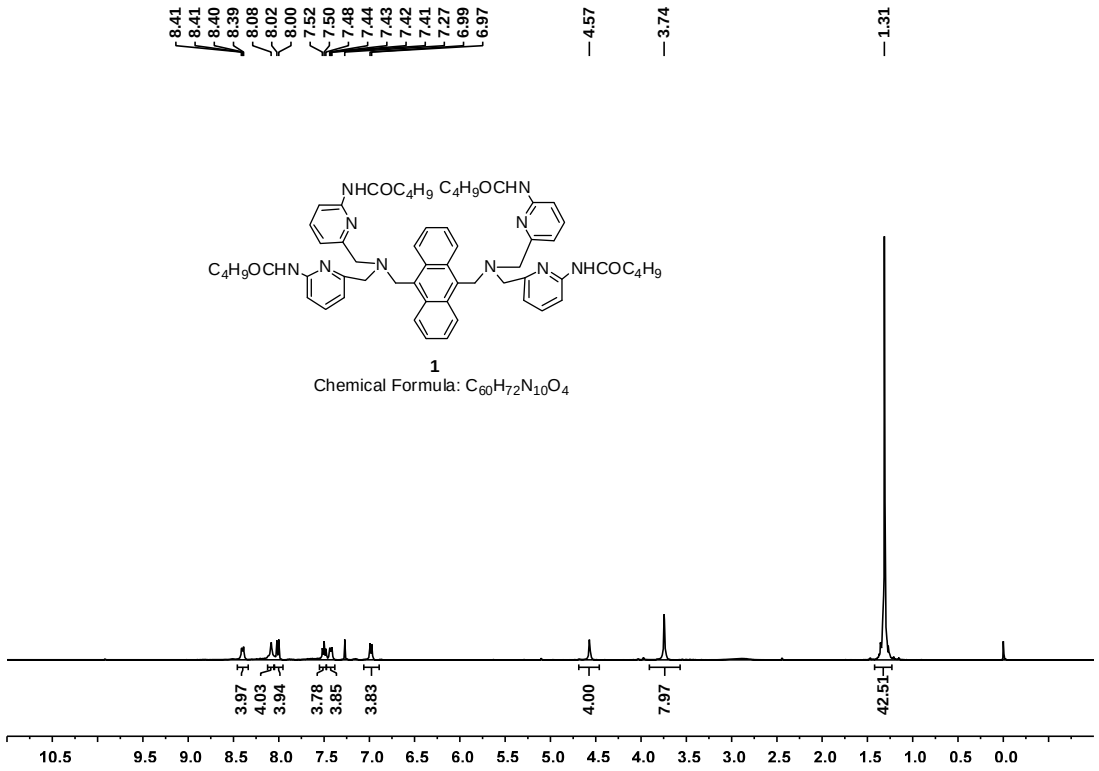
Cell Culture and Imaging. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (fetal bovine serum), 100 mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C. Before cell imaging experiments, HeLa cells were seeded in 12-well culture plate for one night. For living cell imaging experiments, cells were incubated with 25 μM of **ZnL** for 30 min at 37 °C and washed three times with prewarmed PBS buffer, and then imaged immediately. For apyrase treated experiments, HeLa cells were pretreated with 5 units apyrase for 30 min at 37 °C,

washed three times with prewarmed PBS buffer, and then incubated with 25 μM of **ZnL** (or incubated with 125 μM of ADP for 30 min prior to addition of probe **1** for a control experiment) for 30 min at 37 °C. Cell imaging was then carried out after washing cells with prewarmed PBS buffer.

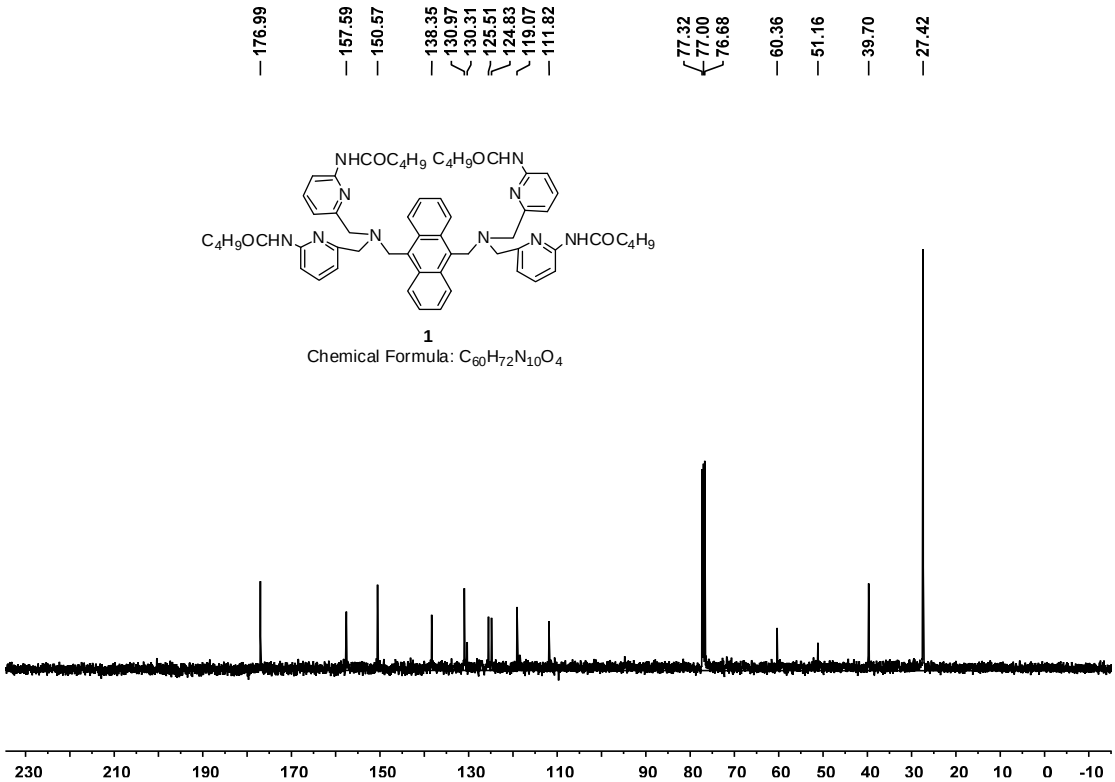
2. Synthesis of ZnL



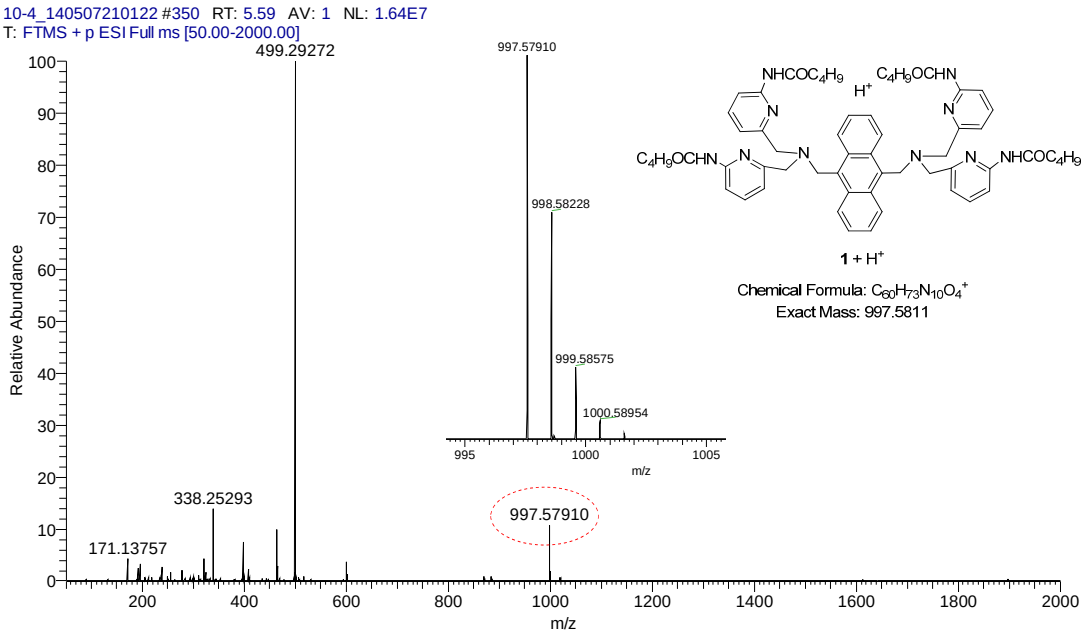
Synthesis of 1: To a solution of **2**[s1] (236 mg, 1.00 mmol) and **3**[s2] (1.14 g, 4.2 mmol) in anhydrous CH_3CN (30 mL) was added K_2CO_3 (621 mg, 4.50 mmol). The reaction mixture was then heated to reflux for 12 hours, and the solvent was evaporated under reduced pressure. To the residue was added dichloromethane (60 mL), and the organic phase was washed with water and brine followed by drying over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by silica gel column (eluent: dichloromethane /methanol = 50:1 (v/v)) to give a yellow powder (800 mg, 80% yield). Mp 116-118 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41-8.39 (dd, J = 6.8, 3.2 Hz, 4H), 8.08 (s, 4H), 8.01 (d, J = 8.0 Hz, 4H), 7.50 (t, J = 8.0 Hz, 4H), 7.43 (dd, J = 6.8, 3.2 Hz, 4H), 6.98 (d, J = 7.6 Hz, 4H), 4.57 (s, 4H), 3.74 (s, 8H), 1.31 (s, 36H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.99, 157.59, 150.57, 138.35, 130.97, 130.31, 125.51, 124.83, 119.07, 111.82, 60.36, 51.16, 39.70, 27.42. IR (KBr) ν_{max} (cm^{-1}): 3437, 2964, 1688, 1578, 1518, 1453, 1306, 1152. HR-MS Calc. for $C_{60}H_{73}N_{10}O_4^+$ ($M + H^+$) 997.5811, found 997.5791.



^{1}H NMR spectrum of **1** in $CDCl_3$.

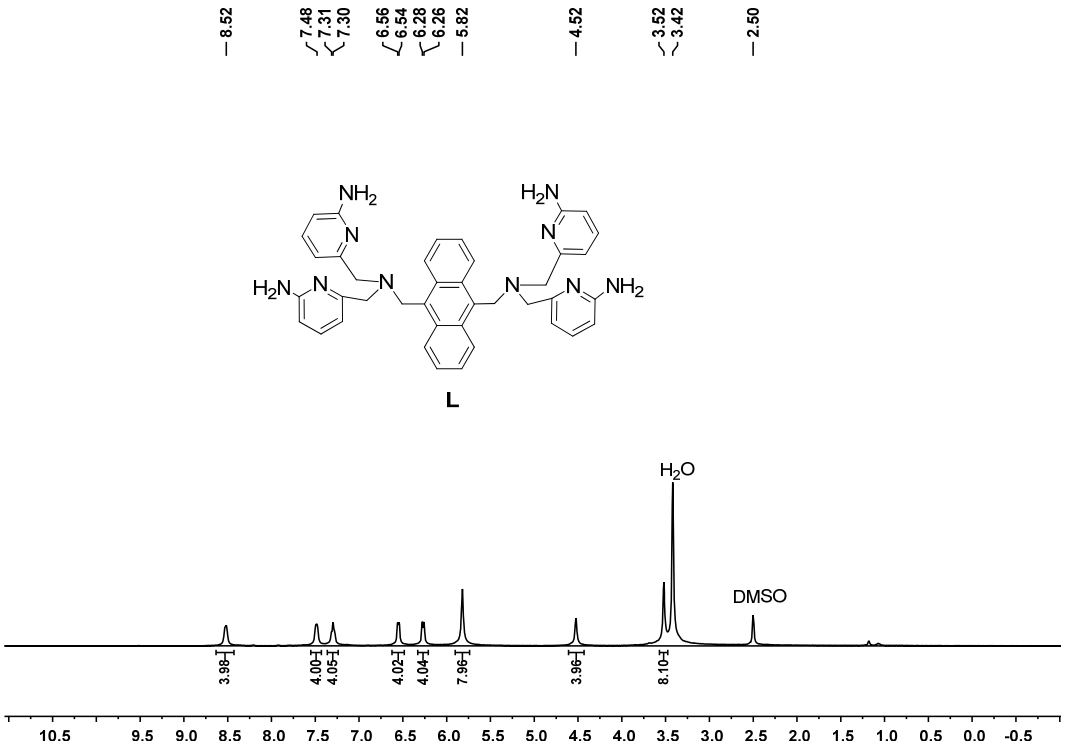


^{13}C NMR spectrum of **1** in $CDCl_3$.

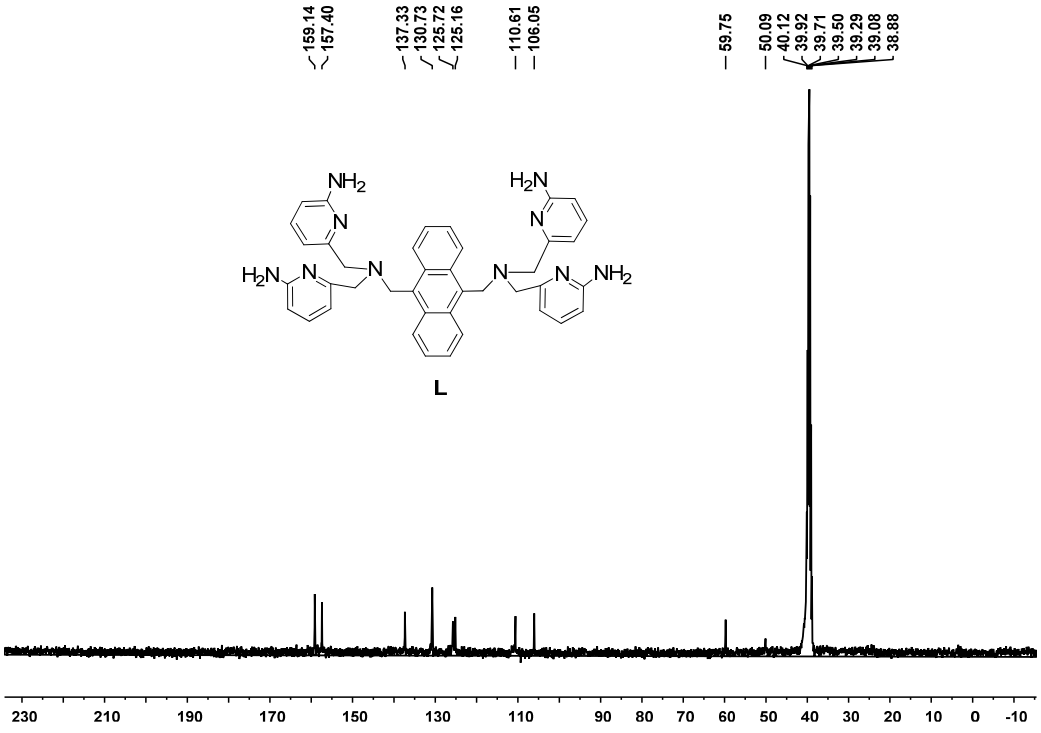


HR-MS spectrum of **1**

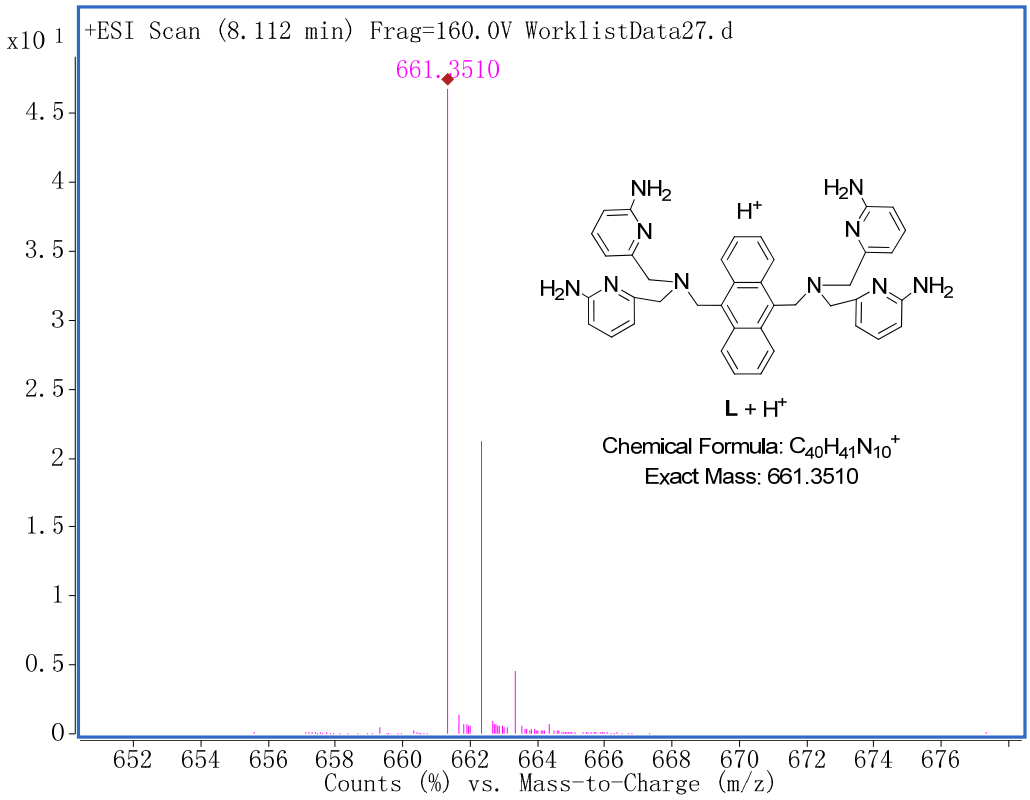
Synthesis of L: To 600 mg of **1** was added 50 ml of 4 M KOH and CH_3CH_2OH 50 mL. The mixture was heated to reflux for 16 hours, and the CH_3CH_2OH was evaporated under reduced pressure. The aqueous solution was then extracted with ethyl acetate (4 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo to yield a brown yellow solid, 330 mg (83%). Mp 254-256ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 4H), 7.48 (s, 4H), 7.30-7.31 (d, J = 4.0 Hz, 4H), 6.54-6.56 (d, J = 8.0 Hz, 4H), 6.26-6.28 (d, J = 8.0 Hz, 4H), 5.82 (s, 8H), 4.52 (s, 4H), 3.52 (s, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.14, 157.40, 137.33, 130.73, 125.72, 125.16, 110.61, 106.05, 59.75, 50.09. IR (KBr) ν_{max} (cm^{-1}): 3446, 3360, 3223, 2929, 1639, 1572, 1484, 1384, 1293, 1170, 1011, 833, 803, 764, 710; ESI-MS: m/z found 661.4 (M + H^+); HR-MS Calc. for $C_{40}H_{41}N_{10}^+$ (M + H^+) 661.351, found 661.351. Elemental analysis calcd for $C_{40}H_{40}N_{10}$: C 72.70, H 6.10, N 21.20; found: C 72.71, H 6.037, N 21.15.



^{1}H NMR spectrum of **L** in DMSO-d_6.

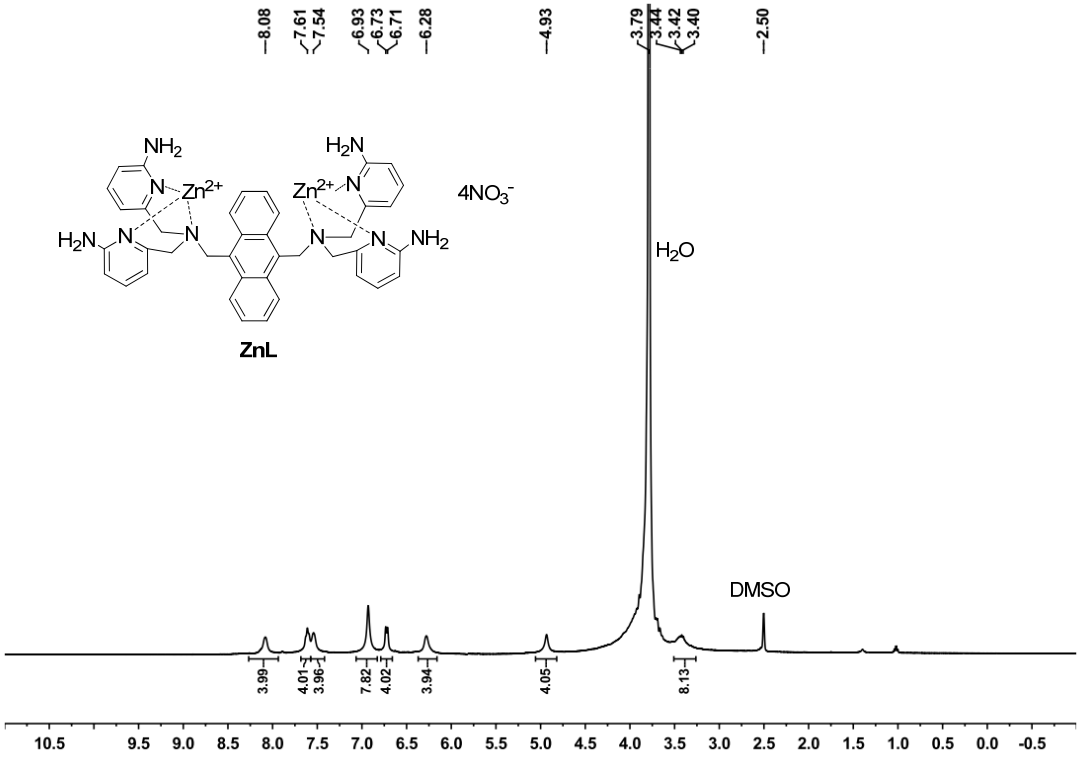


^{13}C NMR spectrum of **L** in DMSO-d_6.

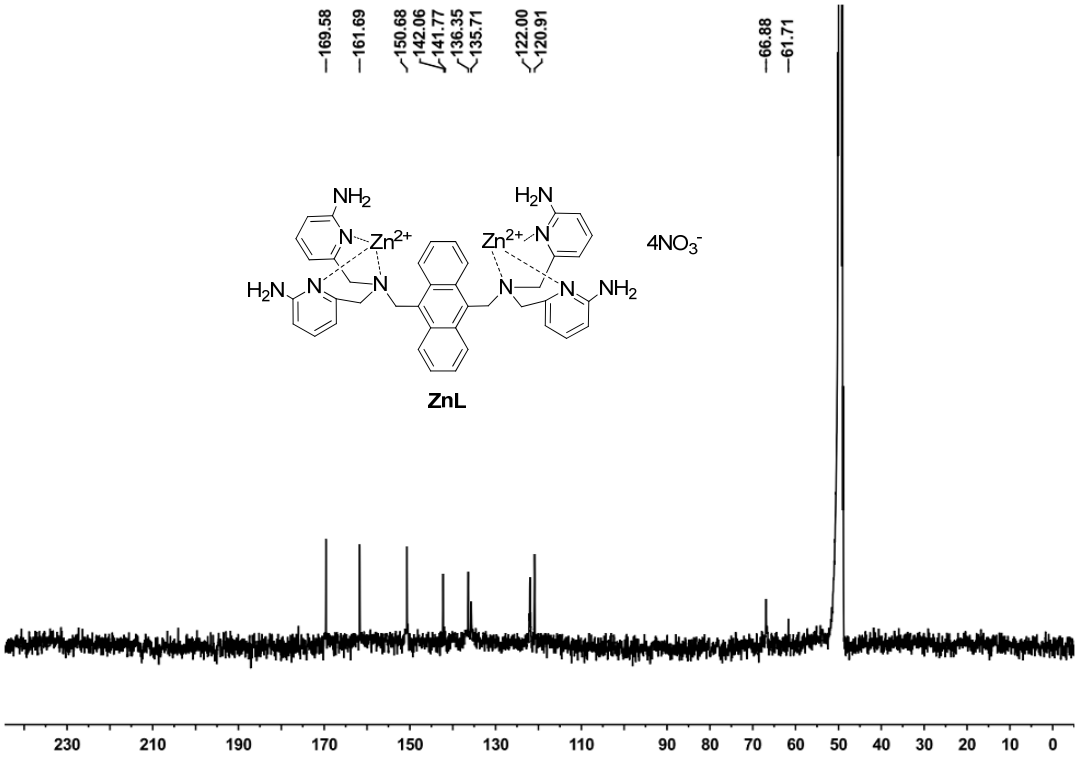


HR-MS spectrum of **L**

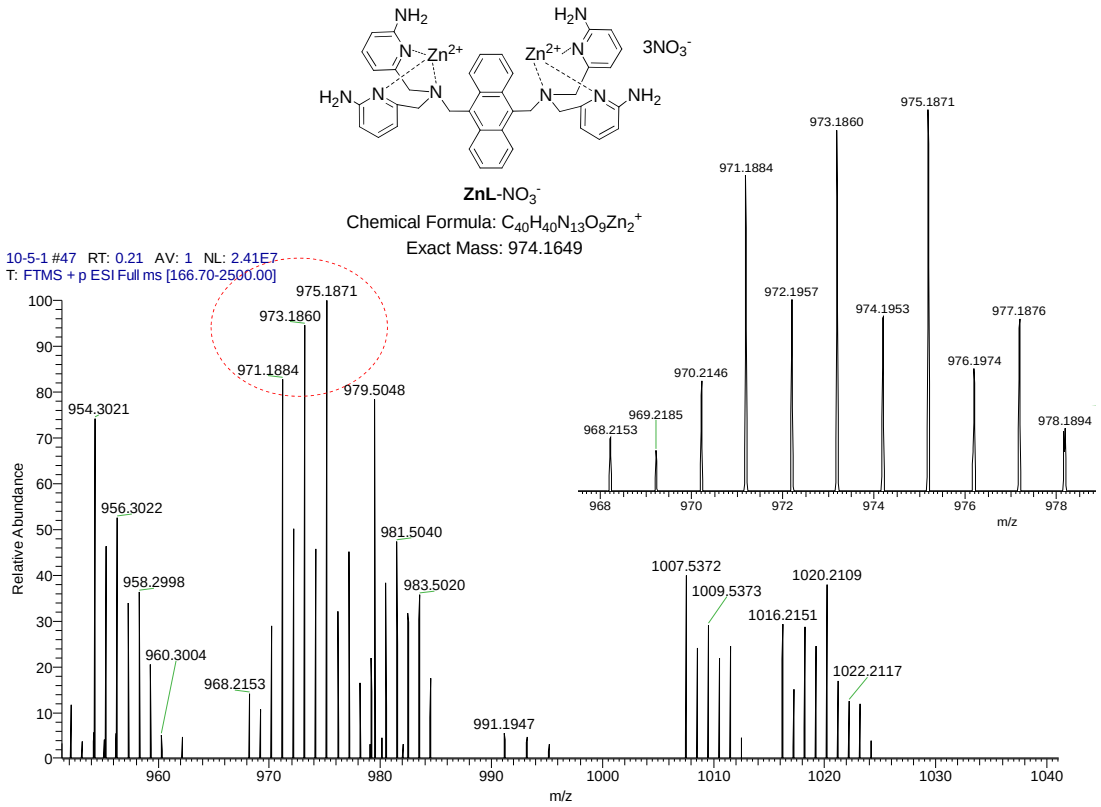
Synthesis of ZnL: To a solution of **L** (33 mg, 0.05 mmol) in 5 mL of MeOH, was added $Zn(NO_3)_2 \cdot 6H_2O$ (33 mg, 0.11 mmol), and the mixture was stirred for 1 h at rt. After concentrating under reduced pressure, the obtained solid was recrystallized from MeOH to afford **ZnL** (41mg, 81%). Mp ＞300 ºC. ^{1}H NMR (400 MHz, DMSO): 8.08(s, 4H), 7.61(s, 4H), 7.54 (s, 4H), 6.93 (s, 8H), 6.72 (d, *J* = 7.8 Hz, 4H), 6.28 (s, 4H), 4.93 (s, 4H), 3.51-3.26 (m, 8H). ^{13}C NMR (100 MHz, DMSO) δ 169.58, 161.69, 150.68, 142.06, 141.77, 136.35, 135.71, 122.00, 120.91, 66.88, 61.71. IR (KBr) ν_{max} (cm^{-1}): 3446, 3360, 1639, 1572, 1484, 1384, 1293, 1170, 1011, 833, 803, 764. HR-MS Calc. for $C_{40}H_{40}N_{13}O_9Zn_2^+$ $[M\text{-}NO_3^-]^+$ 974.1649, found 974.1953. Elemental analysis calcd for $C_{40}H_{40}N_{10}Zn_2(NO_3)_4 \cdot H_2O$: C 45.43, H 4.00, N 18.54; found: C 45.52, H 3.91, N 18.75.



^{1}H NMR spectrum of **ZnL** in DMSO-d_6.



^{13}C NMR spectrum of **ZnL** in DMSO-d_6.



HR-MS spectrum of **ZnL**

(S1) Gassensmith, J. J.; Arunkumar, E.; Barr, L.; Baumes, J. M.; DiVittorio, K. M.; Johnson, J. R.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc*. **2007**, *129*, 15054–15059.

(S2) Livieri, M.; Mancin, F.; Tonellato, U.; Chin, J.; Cowley, A. *Chem. Commun*. **2008**, 392–394.

3. Additional Spectra and Data

Figure S1. Fluorescence colour (under 365 nm light) changes of **ZnL** (5 μM) in 10 mM aqueous HEPES buffer solution (pH = 7.2) with 50 μM of different anions (from left to right: none, ATP, ADP, AMP, PPi, Citrate, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, F^-, Cl^-, Br^-, I^-, ClO_4^-, CO_3^{2-}, HCO_3^-, NO_3^-, NO_2^-, AcO^-, N_3^-, SO_4^{2-}, HSO_4^-, SCN^-, $S_2O_7^{2-}$, S^{2-}, $S_2O_3^{2-}$).

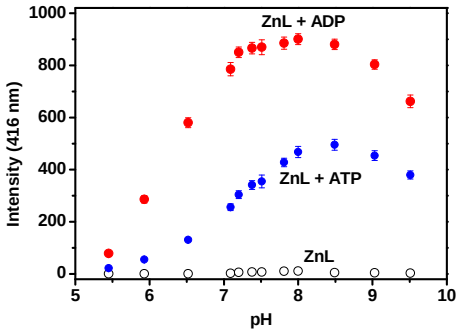


Figure S2. The effect of pH on the fluorescence intensity changes of **ZnL** (5 μM) in the absence and in the presence of ATP (50 μM) or ADP (50 μM) at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

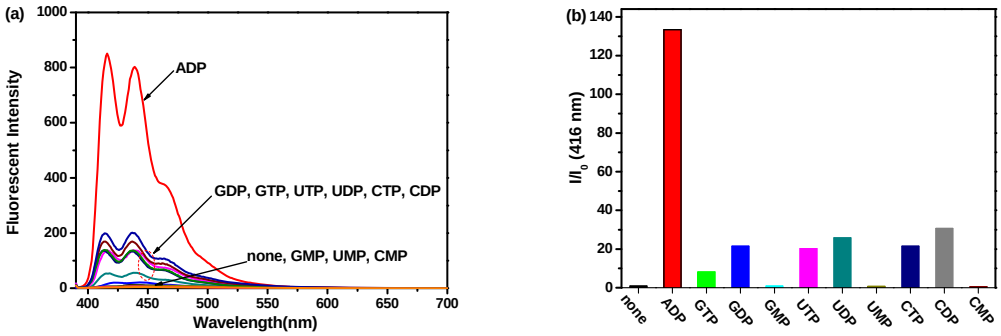


Figure S3. Left: Fluorescence emission spectra of **ZnL**(5 μM) in the presence of various nucleotides (50 μM); Right: A plot of the relative fluorescent intensity (I/I_0) of **ZnL** (5 μM) at 416 nm against 50 μM of different nucleotides (from left to right: none, ADP, GTP, GDP, GMP, UTP, UDP, UMP, CTP, CDP, CMP). λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

Figure S4. Fluorescence colour (under a 365 nm light) changes of **ZnL** (5 μM) in 10 mM aqueous HEPES buffer solution (pH = 7.2) with 50 μM of different anions (from left to right: none, ATP, ADP, AMP, GTP, GDP, GMP, UTP, UDP, UMP, CTP, CDP, CMP).

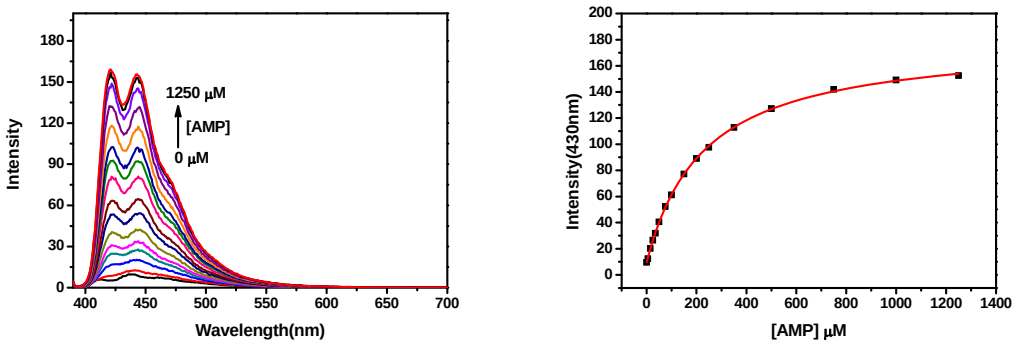


Figure S5. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of AMP (0-1250 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

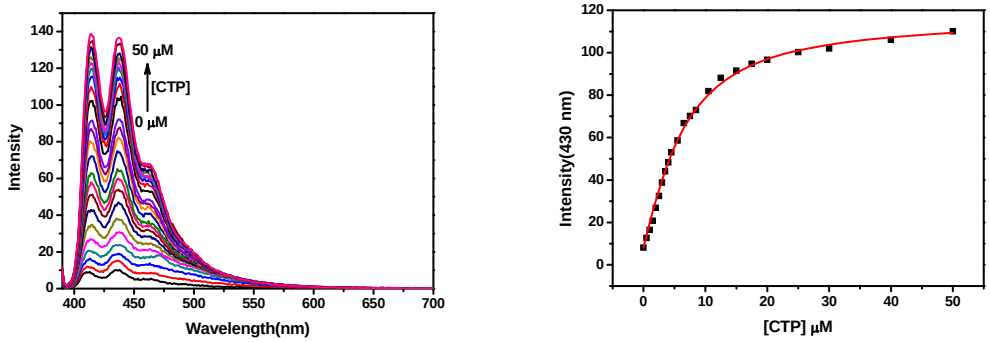


Figure S6. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of CTP (0-50 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

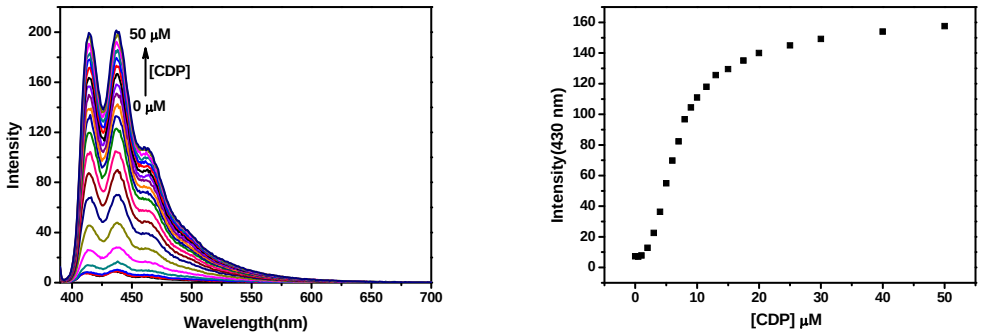


Figure S7. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of CDP (0-50 μM) in pH = 7.2, 10 mM HEPES buffer solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

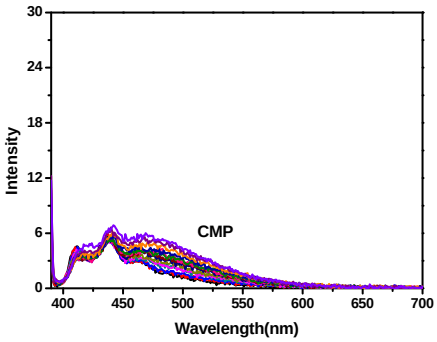


Figure S8. Fluorescent titration of complex **ZnL** (5 μM) upon the addition of CMP (0-150 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

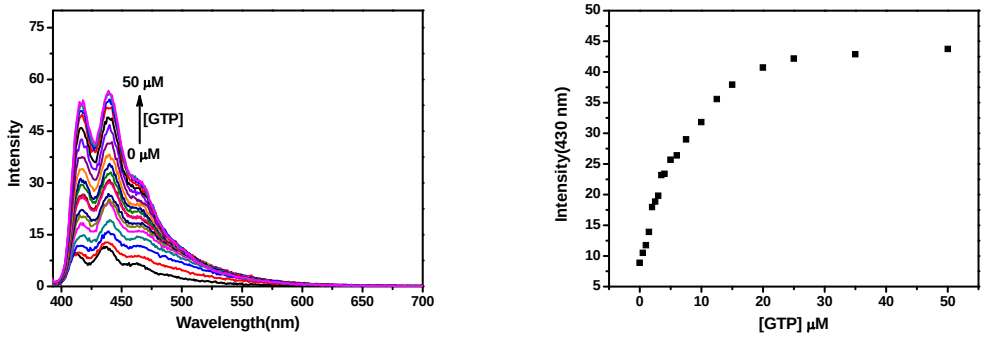


Figure S9. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of GTP (0-50 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

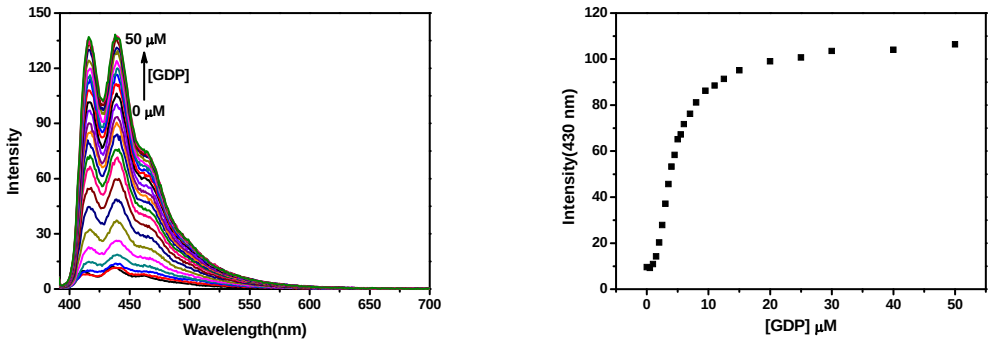


Figure S10. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of GDP (0-50 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

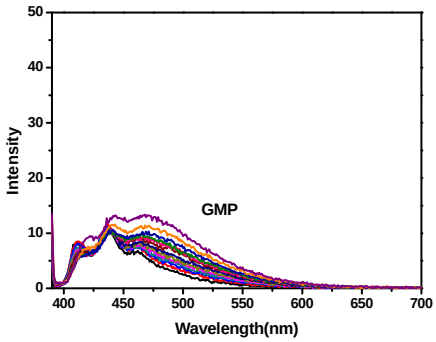


Figure S11. Fluorescent titration of complex **ZnL** (5 μM) upon the addition of GMP (0-250 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

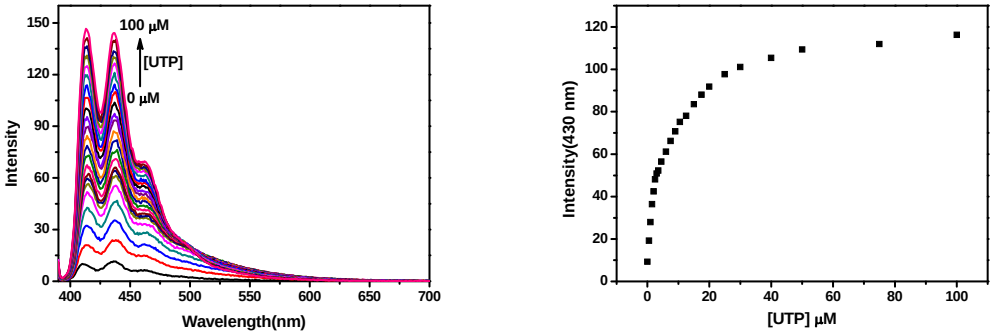


Figure S12. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of UTP (0-100 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

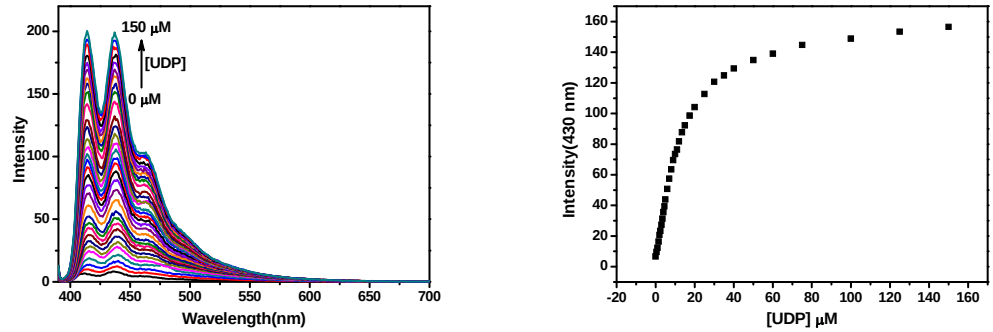


Figure S13. Left: Fluorescent titration of complex **ZnL** (5 μM) upon the addition of UDP (0-150 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C; Right: The saturation curve of fluorescent intensity changes at 430 nm. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

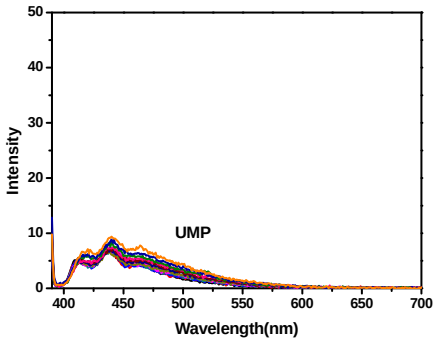


Figure S14. Fluorescent titration of complex **ZnL** (5 μM) upon the addition of UMP (0-250 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

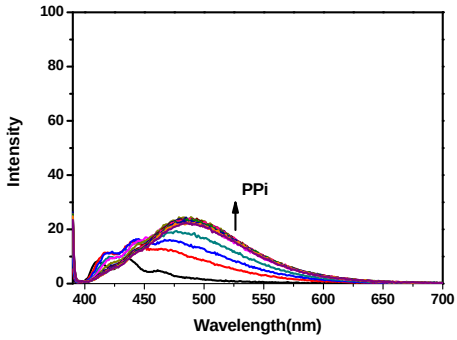
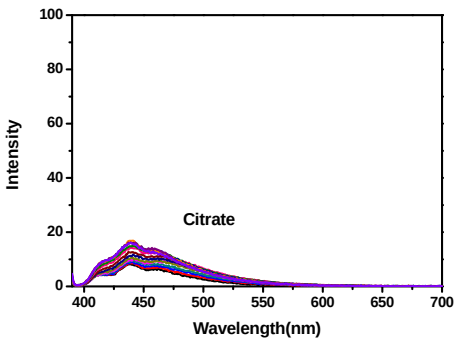
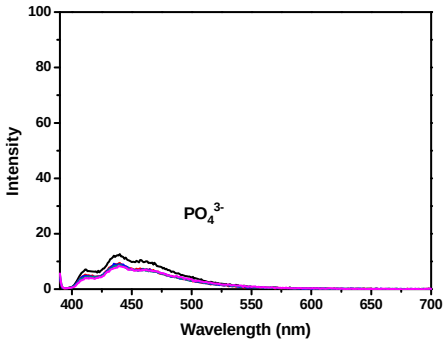


Figure S15. Fluorescent titration of complex **ZnL** (5 μM) upon the addition of PPi (0-25 μM) in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm. This data suggests that PPi induced an anthracene-excimer (λ_{em} ~480 nm) formation of the **ZnL** solution, which is similar to the result of our recent report (see: Huang, F.; Feng, G. *RSC Adv*. **2014**, *4*, 484–487.). However, the fluorescence enhancements induced by PPi are far less than that of ADP.





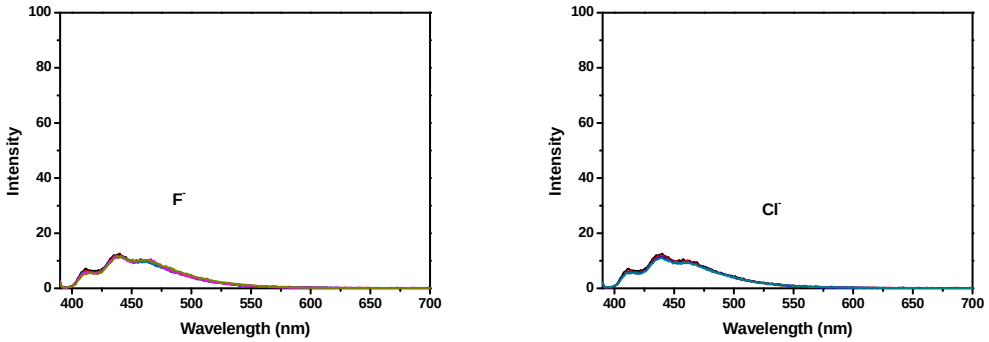


Figure S16. Fluorescent titration of complex **ZnL** (5 μM) upon the addition of citrate, PO_4^{3-}, F^- and Cl^- (0-500 μM) as representative anionic analytes in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. λ_{ex} = 380 nm, d_{em} = d_{ex} = 5 nm.

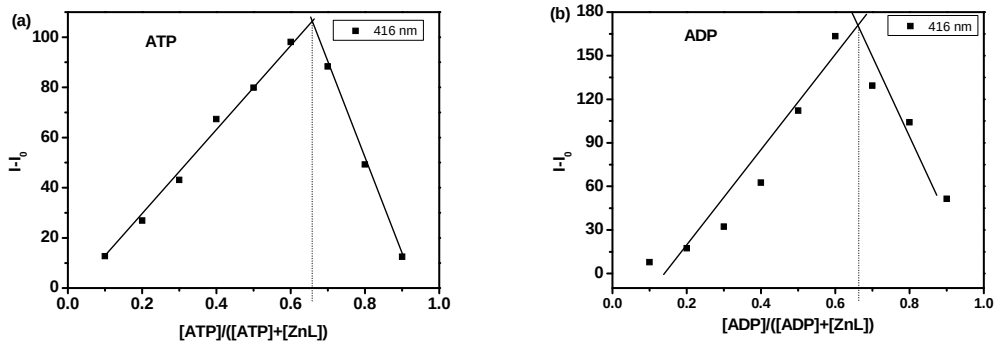


Figure S17. Job's plot examined between (a) **ZnL** and ATP and (b) **ZnL** and ADP in HEPES buffer (10 mM, pH = 7.2) solution at 25˚C. [ATP or ADP] + [**ZnL**] = 5 μM, λ_{ex} = 380 nm, d_{ex} = d_{em} = 5 nm.

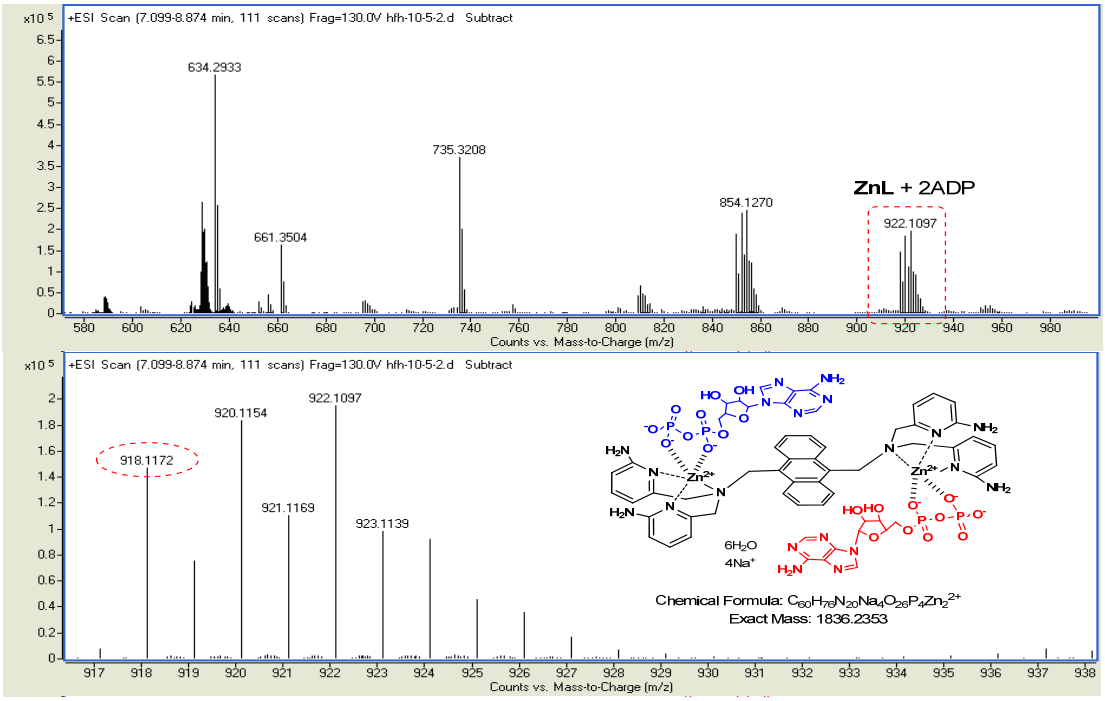


Figure S18. High resolution mass spectrum of a mixture of **ZnL** and ADP. The peak at m/z = 918.1172 corresponds to formula $C_{60}H_{76}N_{20}Na_4O_{26}P_4Zn_2^{2+}$ (calculated mass 1836.2353 = 2 × 918.11765), which can be assigned to **ZnL**$^{4+}$+2ADP^{3-}+4Na^{+}+6H_2O as shown in the mass spectrum.

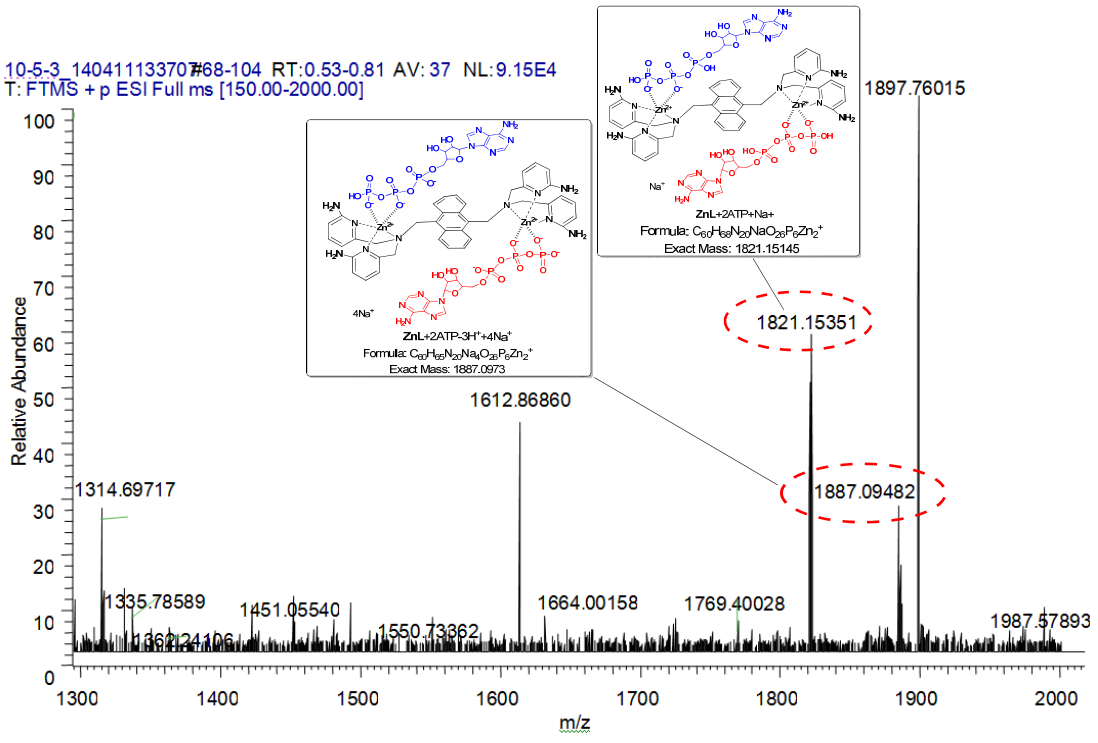


Figure S19. High resolution mass spectrum of a mixture of **ZnL** and ATP. The peak at m/z = 1821.1535 corresponds to formula $C_{60}H_{68}N_{20}NaO_{26}P_6Zn_2^{+}$ (**ZnL**+2ATP+Na^{+}), which was calculated as 1821.15145. The peak at m/z = 1887.09481 corresponds to formula $C_{60}H_{65}N_{20}Na_4O_{26}P_6Zn_2^{+}$ (**ZnL**+2ATP-3H^{+}+4Na^{+}), which was calculated as 1887.09728.

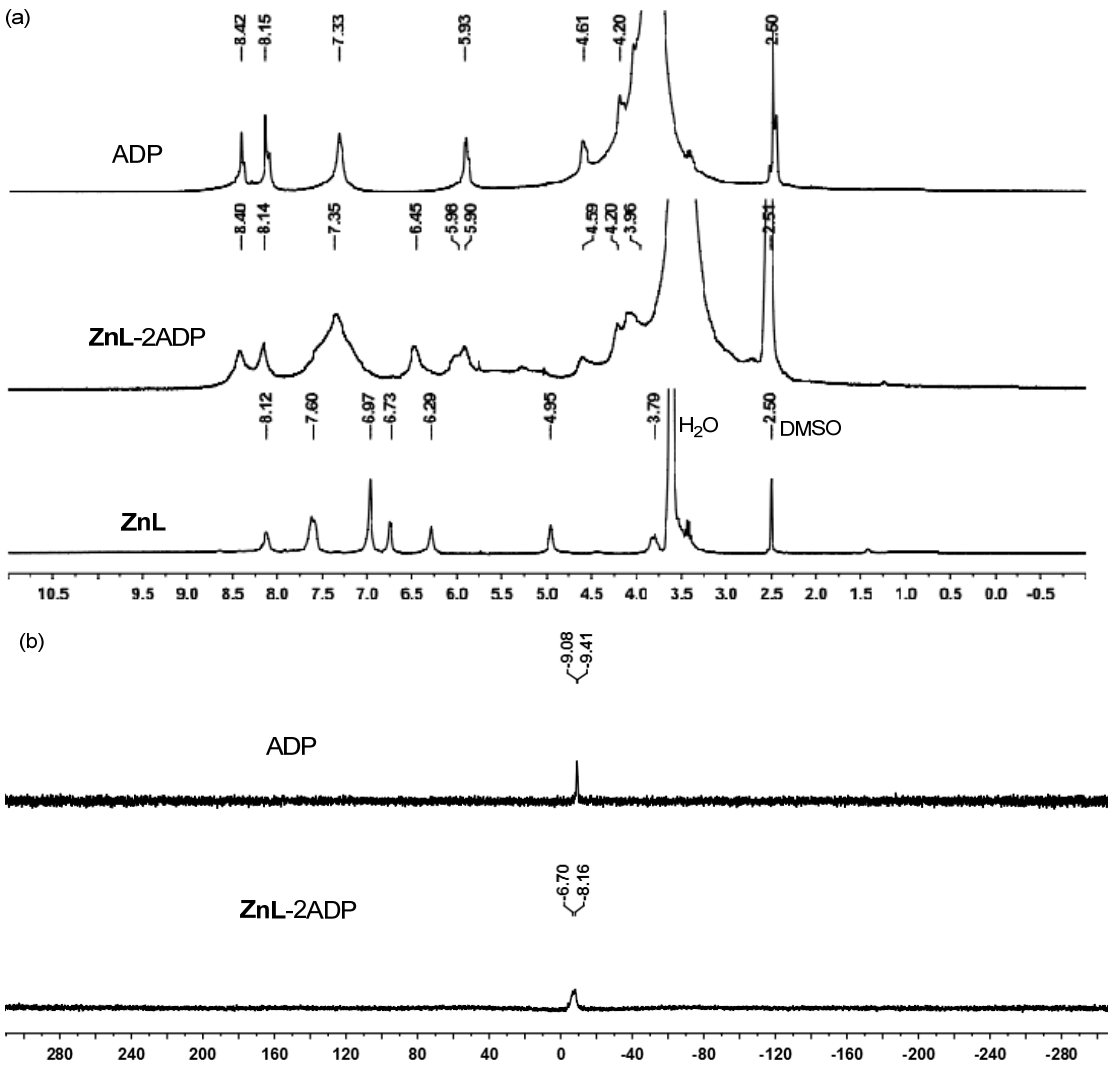


Figure S20. (a) ^{1}H NMR spectra change and (b) ^{31}P NMR spectra change of **ZnL** upon addition of ADP in DMSO-d_6 at 25 °C. [**ZnL**] = 10 mM, [ADP] = 20 mM. Still, we cannot get very good NMR spectra with high resolution due to the reason of low solubility, but it is clear that **ZnL** binds ADP and a new complex was formed.

4. Quantum Mechanical Calculations

Quantum mechanical calculations based on the density functional theory (DFT) were investigated to identify the configuration of **ZnL-2ADP** and **ZnL-2ATP**. The calculations were performed by Gaussian09 suite. Both of the two structures were optimized at the DFT level with the B3LYP functional. The 6-31G(d) basis set was used for C, H, N, O, and P atoms, while the LANL2DZ basis set was used for Zn atom. The charge values were set to 0 for **ZnL-2ADP** and -2 for **ZnL-2ATP**. The optimized configurations of **ZnL-2ADP** and **ZnL-2ATP** are displayed below. For

ZnL-2ADP, the two Zn^{2+} ions link **L** and ADP together via five coordination sites, respectively. The Zn^{2+} forms coordination bonds with three nitrogen atoms (bond lengths are 2.201Å, 2.171Å, and 2.292Å) of **L** and two oxygen atoms (bond lengths are 2.028Å and 1.999Å) of ADP. Different from the configuration of **ZnL-2ADP**, the Zn^{2+} ion in **ZnL-2ATP** join L and ATP together via six coordination sites, including three bonds with nitrogen atoms (bond lengths are 2.312Å, 2.304Å, and 2.332Å) of **L** and other three with oxygen atoms (bond lengths are 2.065Å, 2.042Å, and 2.082Å) of ATP. The results suggest that both **ZnL-2ADP** and **ZnL-2ATP** configurations are stable by forming coordination bonds via Zn^{2+} ions. Furthermore, the adenine structure in ADP have π-π stacking interaction with anthracene of **L** in **ZnL-2ADP**, however, the same interaction does not exist in the configuration of **ZnL-2ATP.**

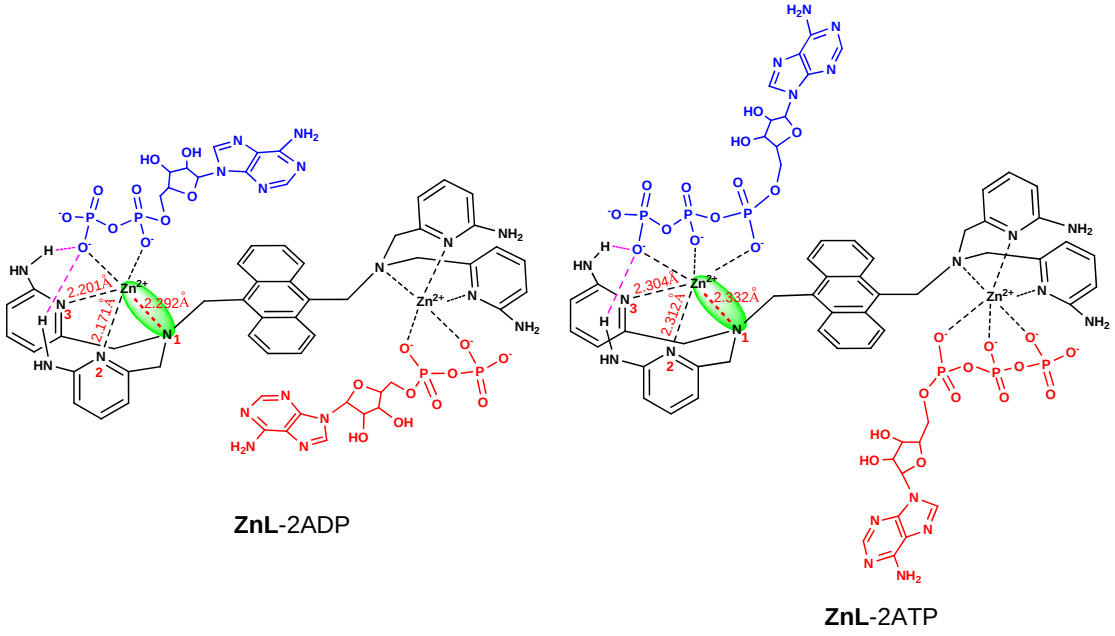


Figure S21. DFT calculated bond lengths between Zn^{2+} and the coordinated three nitrogen atoms for ADP and ATP, respectively.

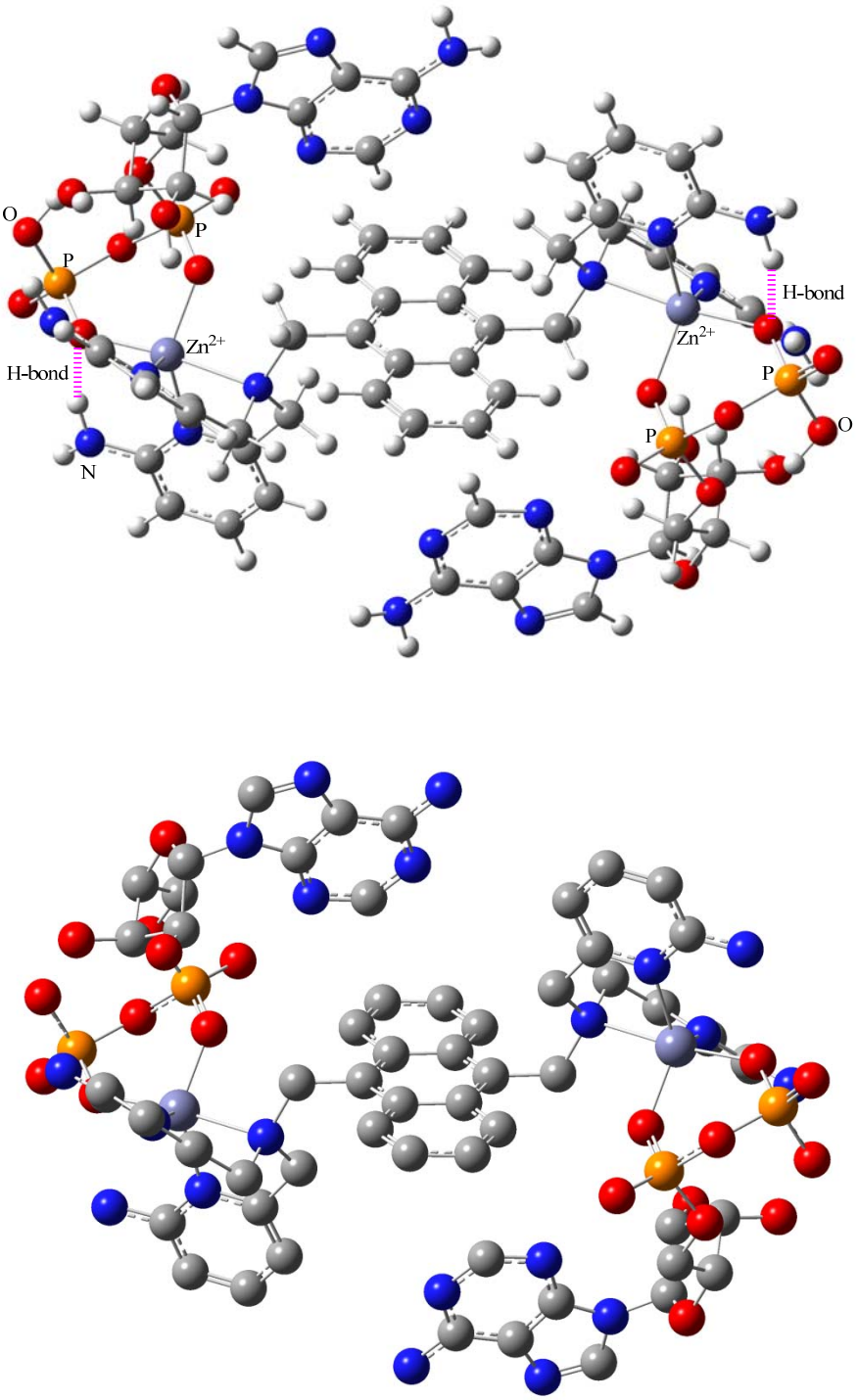


Figure S22. DFT calculated interactions between **ZnL** and ADP. Top: View with hydrogen atoms. Bottom: Hydrogen atoms are omitted for clarity.

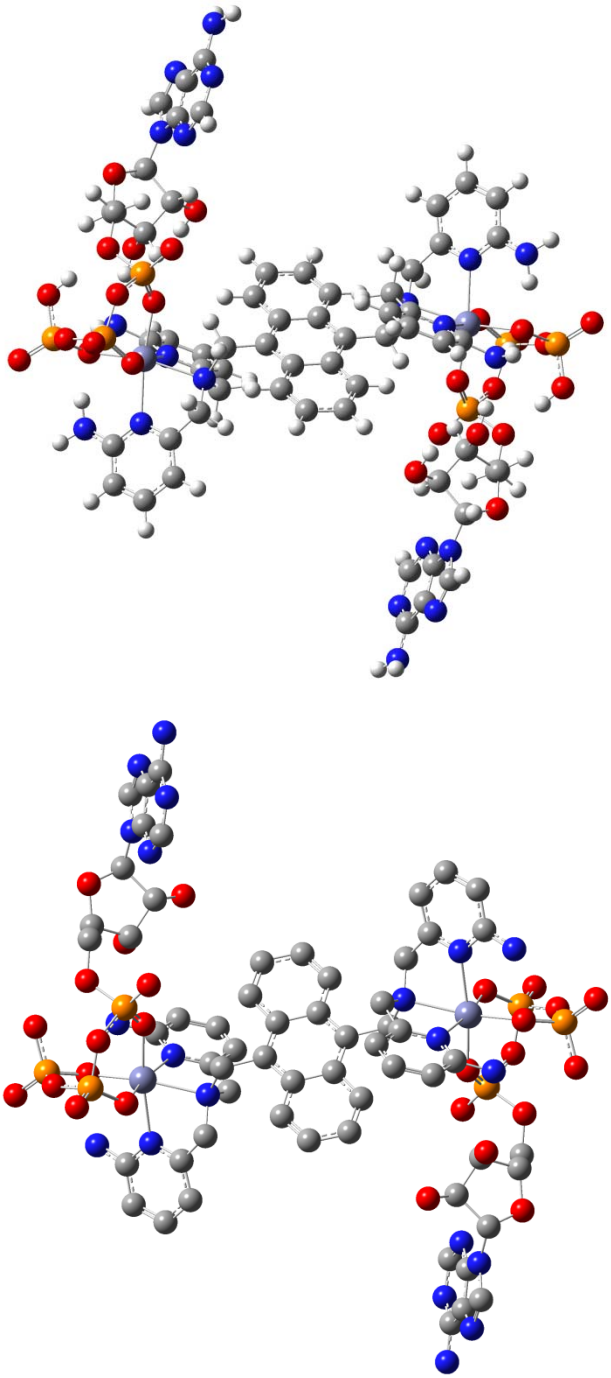


Figure S23. DFT calculated interactions between **ZnL** and ATP. Top: View with hydrogen atoms. Bottom: Hydrogen atoms are omitted for clarity.