

Supporting Information

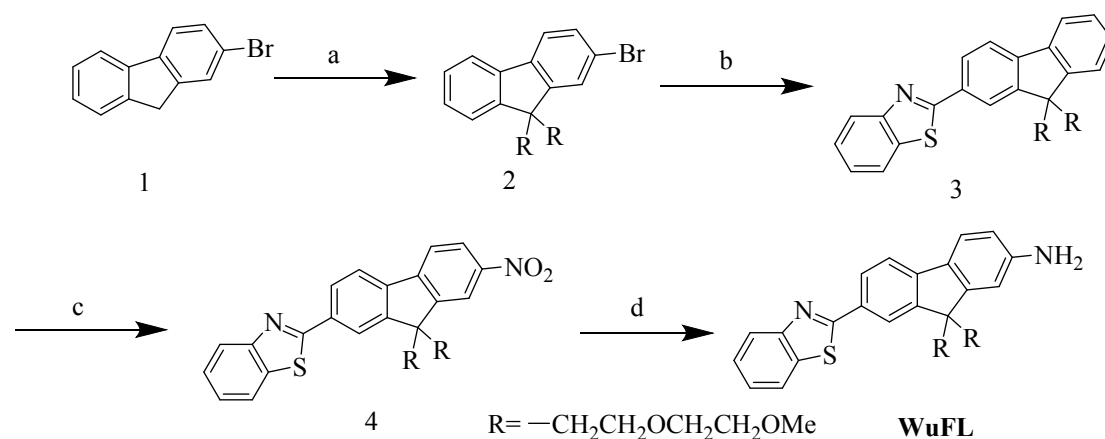
Xueyan Zhang, Xiangzhu Chen, Yuanyuan Zhang, Gui Gao, Xiaoqian Huang, Shicong Hou*,
and Xiaodong Ma*

College of Science, China Agricultural University, Beijing 100193, P.R. China

*Corresponding author

E-mail: houshc@cau.edu.cn (Shicong Hou); dongxm@cau.edu.cn (Xiaodong Ma)

Synthesis of WuFL



Reagents and conditions: (a) $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OTs}$, KOH, anhyd DMSO, RT, overnight, 73%. (b) Benzothiazole, Cs_2CO_3 , $\text{Pd}(\text{OAc})_2$, CuI, $t\text{-Bu}_3\text{P}$, anhyd DMF, 145°C , overnight, 74%. (c) Concentrated HNO_3 , H_2SO_4 $\text{Ac}_2\text{O}/\text{AcOH}$ (1:1), RT, 2 h, 75%. (d) Fe, NH_4Cl , $\text{EtOH}/\text{H}_2\text{O}$, 85°C , 2 h, 79%.

Compound 2. 2-bromofluorene (5.21 g, 21.27 mol) was added to a stirred solution of diethyleneglycol monomethyl ether tosylate (13.4 g, 48.9 mmol) in anhydrous DMSO (80 mL), the mixture was then purged with dry nitrogen by repeated filling and deflating of a nitrogen balloon. To this was added anhydrous powdered KOH (2.98 g, 54.4 mmol). The reaction mixture was stirred for overnight under nitrogen atmosphere at RT. Then poured the mixture into a break containing 150 mL 1N HCl and 100ml EA, stirred for 5 min. The organic layer was separated, washed by saturated aq. Na_2CO_3 solution (50 mL), brine (50 mL), dried

(Na₂SO₄), and filtered. Evaporation of the solvent under reduced pressure gave the crude product which was subjected to silica gel column chromatography (10% ethyl acetate in petroleum ether) to get compound 3 (7.0 g, 73.0%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.67 – 7.62 (m, 1H), 7.55 (dd, *J* = 7.1, 4.9 Hz, 2H), 7.45 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.36 – 7.30 (m, 2H), 3.32 – 3.25 (m, 10H), 3.22 – 3.13 (m, 4H), 2.80 – 2.68 (m, 4H), 2.46 – 2.35 (m, 4H).

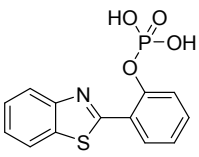
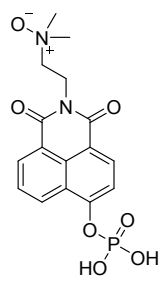
Compound 3. Benzothiazole (2.2 ml, 20 mmol) was added to a stirred solution of compound 2 (6 g, 15.5 mmol) in anhydrous DMF (60 ml). Purged the mixture with dry nitrogen by repeated filling and deflating of a nitrogen balloon. To this were added successively Cs₂CO₃ (6.6 g, 20.22 mmol), Pd(OAc)₂ (0.448 g, 1.98 mmol), CuI (381 mg, 1.98 mmol), and t-Bu₃P (6 mL, 10wt% in hexanes, 1.98 mmol). The reaction mixture was stirred for overnight under nitrogen atmosphere at 145 °C. Then filtered the mixture and washed residue by ethyl acetate (20 ml), then concentrated the combined filtrate under reduced pressure to get a black residue. Dissolved the residue with 80 ml EA, washed by 1N HCl (50 mL), brine (50 mL), dried (Na₂SO₄), and filtered. Solvent was removed under reduced pressure and silica gel column chromatography (30% ethyl acetate in petroleum ether) of the crude product gave a solid which was recrystallized from ethyl acetate-hexane to get compound 3 (5 g, 73.0%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 8.19 (d, *J* = 1.4 Hz, 1H), 8.10 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.81 – 7.72 (m, 2H), 7.50 (ddd, *J* = 8.2, 7.0, 2.5 Hz, 2H), 7.39 (ddd, *J* = 6.8, 4.2, 1.5 Hz, 3H), 3.30 – 3.24 (m, 10H), 3.18 (dd, *J* = 5.6, 3.3 Hz, 4H), 2.80 (ddd, *J* = 8.8, 6.0, 3.4 Hz, 4H), 2.61 – 2.42 (m, 4H).

Compound 4. A stirred solution of compound 3 (5 g, 0.01 mol) in AcOH (25 mL) and Ac₂O (25 mL) was added to 65% HNO₃ (2.7 ml, 20 mmol) and 1 ml 98% H₂SO₄ dropwise for 10 min at RT. After react 2 h, it was then poured into a beaker containing 100 mL water. Ethyl acetate (100 mL) was added to it and stirred for 5 min. separate the organic layer, washed the mixture by water (50 mL), brine (50 mL), dried (Na₂SO₄), and filtered. Solvent was removed under reduced pressure and silica gel column chromatography (30% ethyl acetate in petroleum ether) of the crude product gave a solid which was recrystallized from ethyl acetate-hexane to get compound 4 (4.11g, 75.0%). ¹H NMR (300 MHz, CDCl₃) δ 8.35 (d, *J* = 1.7 Hz, 1H), 8.30 (dd, *J* = 8.3, 2.1 Hz, 1H), 8.24 (d, *J* = 1.0 Hz, 1H), 8.15 (dd, *J* = 8.0, 1.6 Hz,

1H), 8.11 (dd, $J = 7.6, 0.6$ Hz, 1H), 7.95 (dd, $J = 8.0, 0.7$ Hz, 1H), 7.91 – 7.82 (m, 2H), 7.56 – 7.49 (m, 1H), 7.46 – 7.39 (m, 1H), 3.27 – 3.19 (m, 10H), 3.19 – 3.08 (m, 4H), 2.98 – 2.75 (m, 4H), 2.55 (t, $J = 7.1$ Hz, 4H).

Compound **WuFL**. A stirred solution of compound 4 (4 g, 7.3 mmol) and NH_4Cl (1.32 g, 25 mmol) in EtOH (75 mL) and H_2O (25 mL) was purged with dry nitrogen by repeated filling and deflating of a nitrogen balloon, to it was added Fe (2.3 g, 41 mmol) powder. The mixture was then refluxed for 4h under nitrogen atmosphere at 90 °C. Then filtered reaction mixture through celite and the residue was washed by ethyl acetate (75 mL). The combined filtrate was concentrated under reduced pressure to get a yellow residue which was redissolved in ethyl acetate (100 mL) and H_2O (50 mL). Separated the organic layer, then washed the mixture by water (50 mL), brine (50 mL), dried (Na_2SO_4), and filtered. Solvent was removed under reduced pressure and silica gel column chromatography (50% ethyl acetate in petroleum ether) of the crude product gave a solid which was recrystallized from ethyl acetate-hexane to get compound **WuFL** (3 g, 79.0%). ^1H NMR (300 MHz, CDCl_3) δ 8.11 (d, $J = 1.1$ Hz, 1H), 8.09 – 8.05 (m, 1H), 8.02 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.90 (ddd, $J = 7.9, 1.2, 0.6$ Hz, 1H), 7.60 (dd, $J = 8.0, 0.4$ Hz, 1H), 7.49 (ddd, $J = 8.2, 5.1, 1.4$ Hz, 2H), 7.41 – 7.34 (m, 1H), 6.77 (d, $J = 1.9$ Hz, 1H), 6.69 (dd, $J = 8.1, 2.1$ Hz, 1H), 3.33 – 3.28 (m, 4H), 3.28 – 3.17 (m, 11H), 2.86 – 2.76 (m, 4H), 2.57 – 2.44 (m, 2H), 2.43 – 2.30 (m, 2H).

Table S1.

Probe	Detection limit	Linear range	Response time	Wavelength (E_m) (nm)	Literature
 HPBI	1.3 U L^{-1}	0-60 U L^{-1}	40 min (100 U L^{-1})	363/430	1
	0.38 U L^{-1}	20-140 U L^{-1}	20 min (50 U L^{-1})	468/554	2

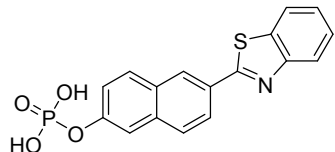
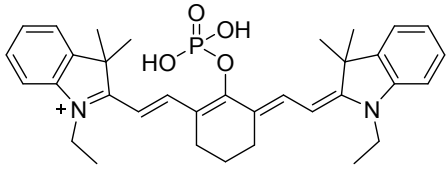
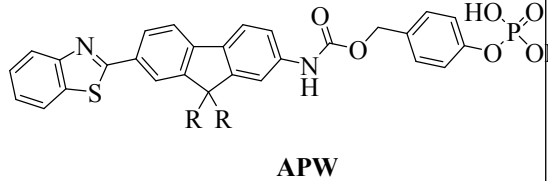
	2.3 U L ⁻¹	20- 180U L ⁻¹	30 min	428/508	3
 Cy-OP	0.37 U L ⁻¹	0-13U L ⁻¹	20 min (400 U L ⁻¹)	766/616	4
 APW	0.46 U L ⁻¹	80- 225U L ⁻¹	15 min (250 U L ⁻¹)	450/550	This work

Table S2. Photophysical data for **FLM** and **FL** in buffer^[a].

Compound	$\lambda_{\text{abs}}^{\text{[b]}}$	$\lambda_{\text{fl}}^{\text{[c]}}$	$\Delta\lambda^{\text{[d]}}$	$\Phi^{\text{[e]}}$
APW	360	450	11111	0.24
WuFL	370	550	5555	0.60

[a] All data were measured in Tris-HCl (10 mM, pH 8) buffer solution. [b] λ_{max} of absorption spectra in nm. [c] λ_{max} of fluorescence spectra in nm. [d] Stokes shift in cm^{-1} . [e] Relative fluorescence quantum yield with quinine sulfate in 0.1 M H_2SO_4 (0.60) as a reference.

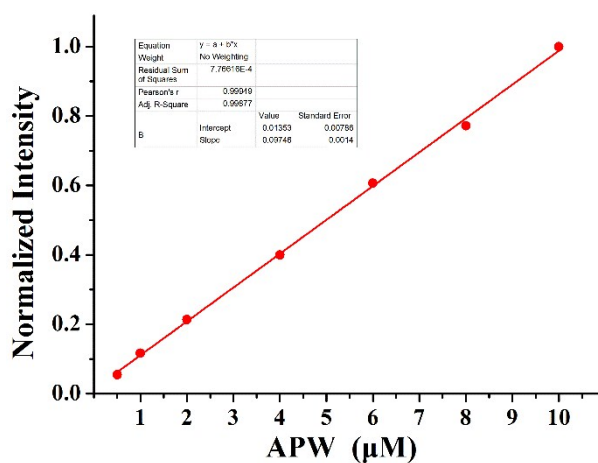


Fig. S1. Plot of fluorescence intensity against the concentration of APW (0 to 10 μM) in Tris-HCl (10 mM, 0.5% DMSO, pH 8.0).

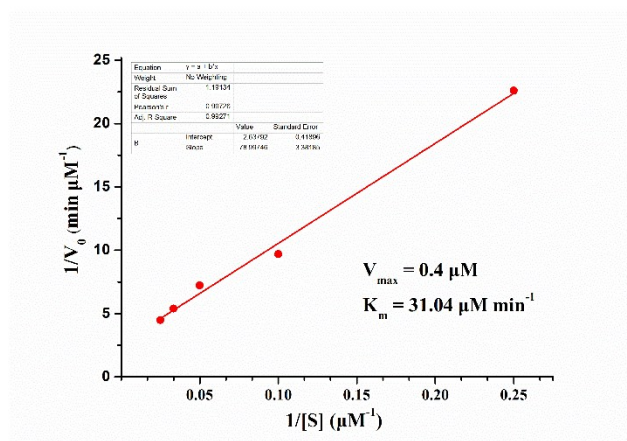


Fig. S2. Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V_0 = V_{max} \times [S]/(K_m + [S])$, where V_0 is the reaction rate, V_{max} is the maximum reaction rate, $[S]$ is the probe concentration, and K_m is the Michaelis constant.

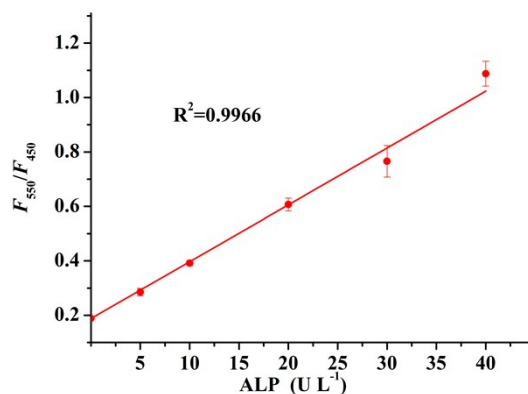


Fig. S3. Plot of fluorescence intensity against the concentration of ALP (0 to 40 U L⁻¹) in Tris-HCl (10 mM, 0.5% DMSO, pH 8.0).

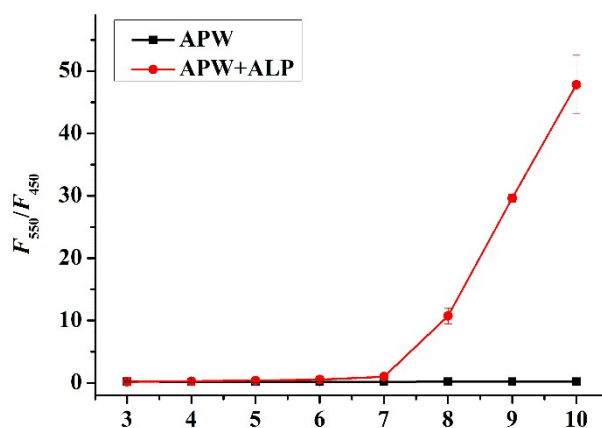


Fig. S4. Reaction of APW and ALP (250 U L⁻¹) under different pH conditions, at 37 °C in Tris-HCl (10 mM, 0.5% DMSO, pH 8.0) recorded within 15 min.

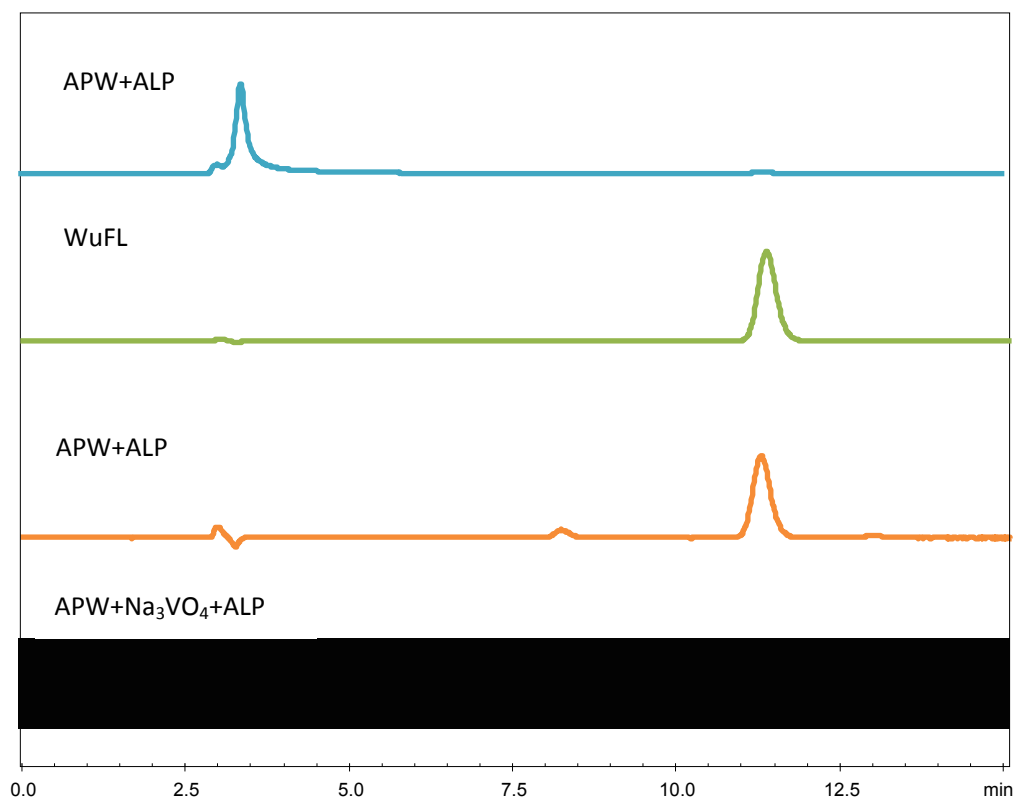


Fig. S5. HPLC chromatograms of APW (50 μM), WuFL (50 μM), APW (50 μM) reacted with ALP (250 U L^{-1}) and the reaction of APW with ALP (250 U L^{-1}) in the presence of an inhibitor (Na_3VO_4 0.1 mM). The HPLC mobile phase was as follows: solvent A (H_2O), solvent B (CH_3OH), A/B=2/8 (v/v).

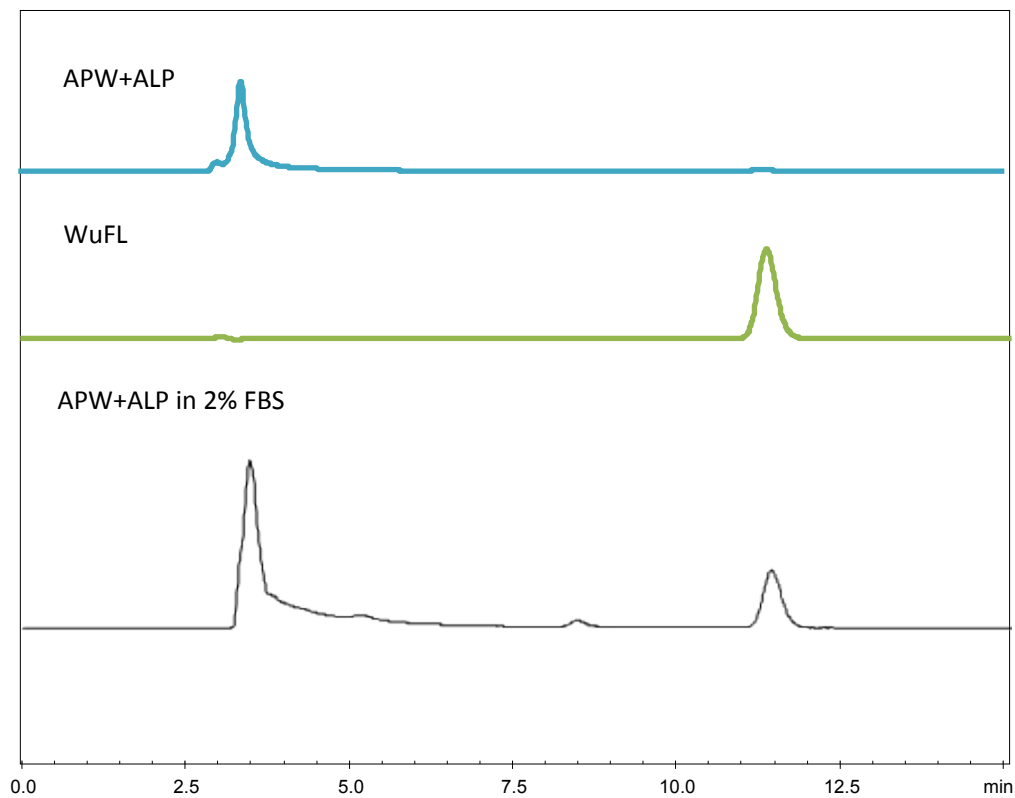


Fig. S6. HPLC chromatograms of WuFL (50 μM), APW (50 μM) reacted with ALP (250 U L^{-1}) and the reaction of APW with ALP (250 U L^{-1}) in Tris-HCl (10 mM , pH 8.0) buffer solution containing 2% FBS. The HPLC mobile phase was as follows: solvent A (H_2O), solvent B (CH_3OH), A/B=2/8 (v/v).

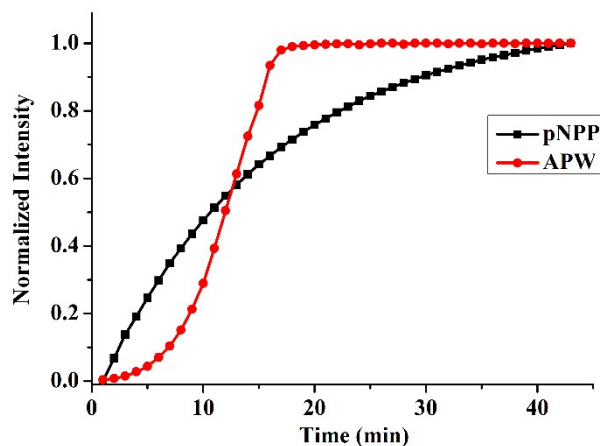


Fig. S7. Comparison of APW (5 μM) and Gold Standard Law (pNPP 0.1 mM), ALP (250 U L^{-1}) in Tris-HCl (10 mM , 0.5% DMSO, pH 8.0).

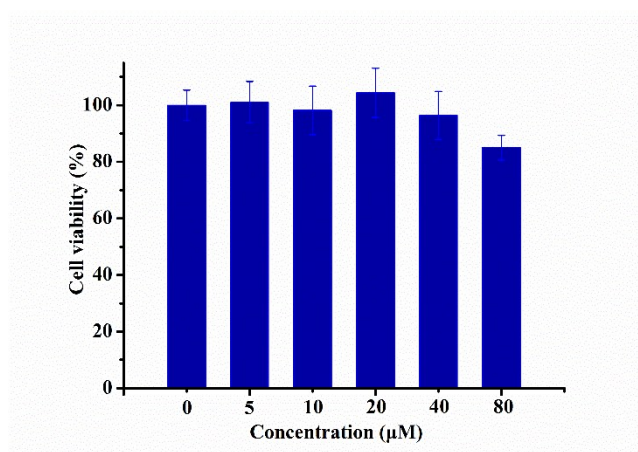


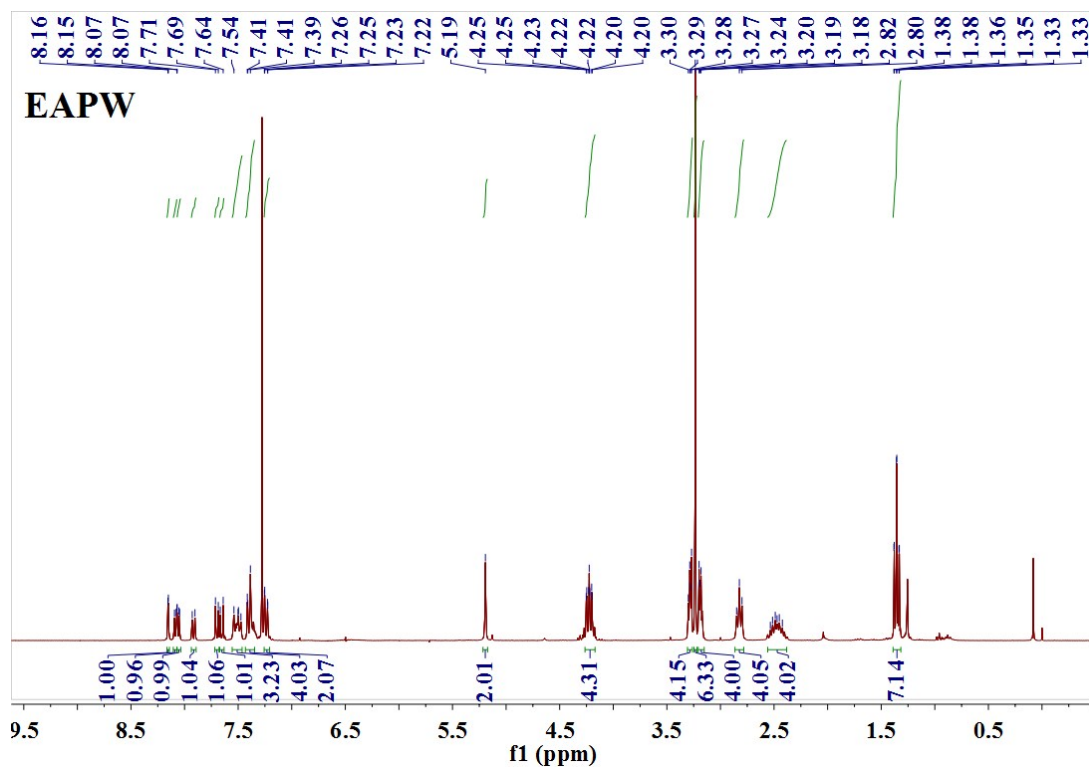
Fig. S8. Viability of MDBK cells in the presence of different concentrations of APW.

Calculation of detection limit

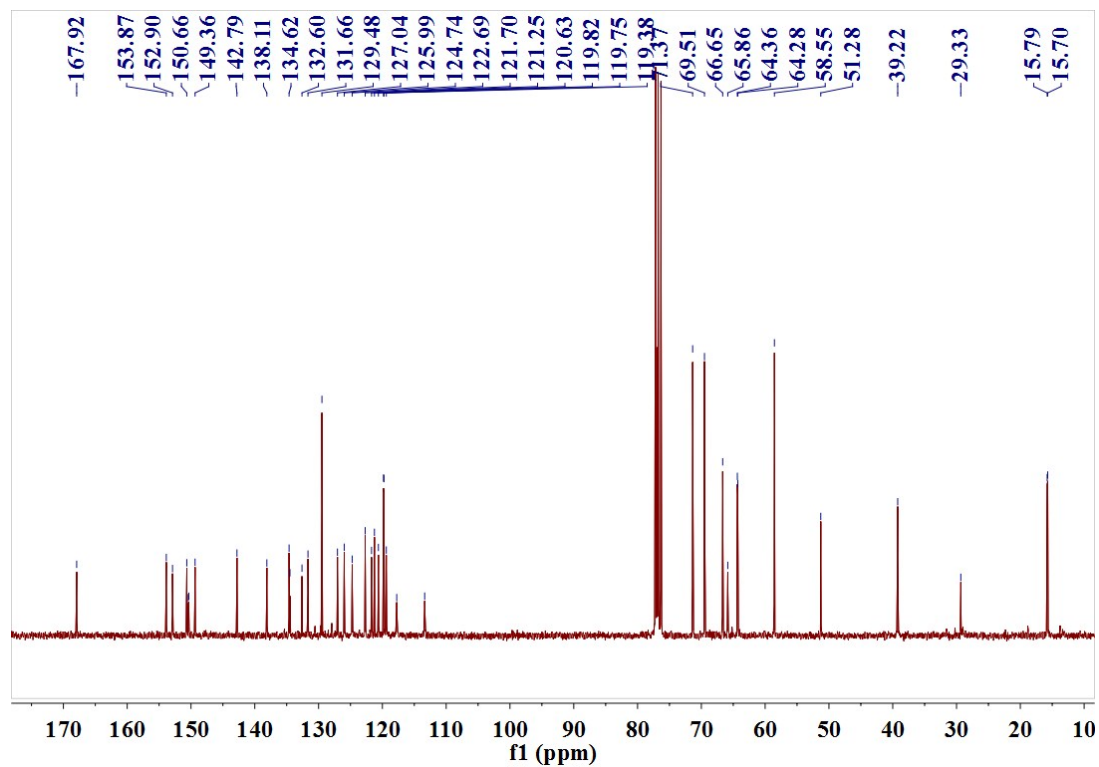
The detection limit = $3\sigma/S$, where σ is the standard deviation of blank measurements, $n = 11$, and S is the slope of the linear equation.

The limit of quantification (LQD) = $10\sigma/S$, where σ is the standard deviation of blank measurements, $n = 11$, and S is the slope of the linear equation. LQD = 1.5320 U L^{-1}

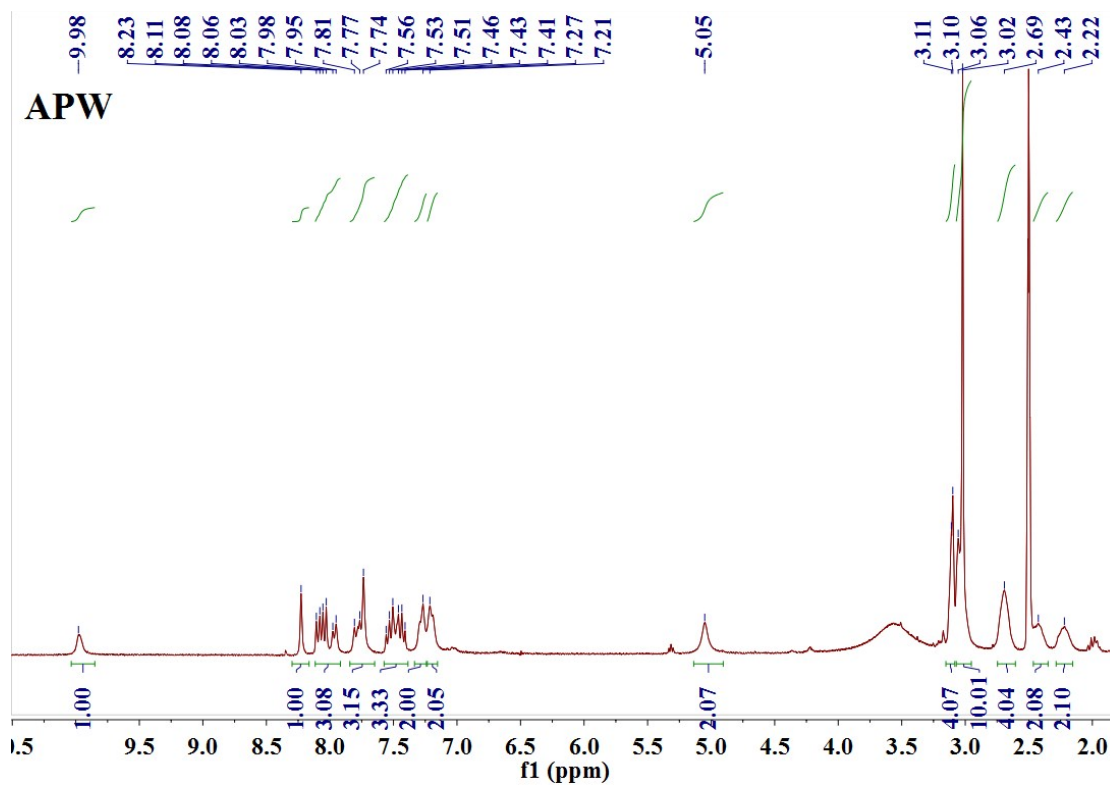
¹H NMR and ¹³C NMR data



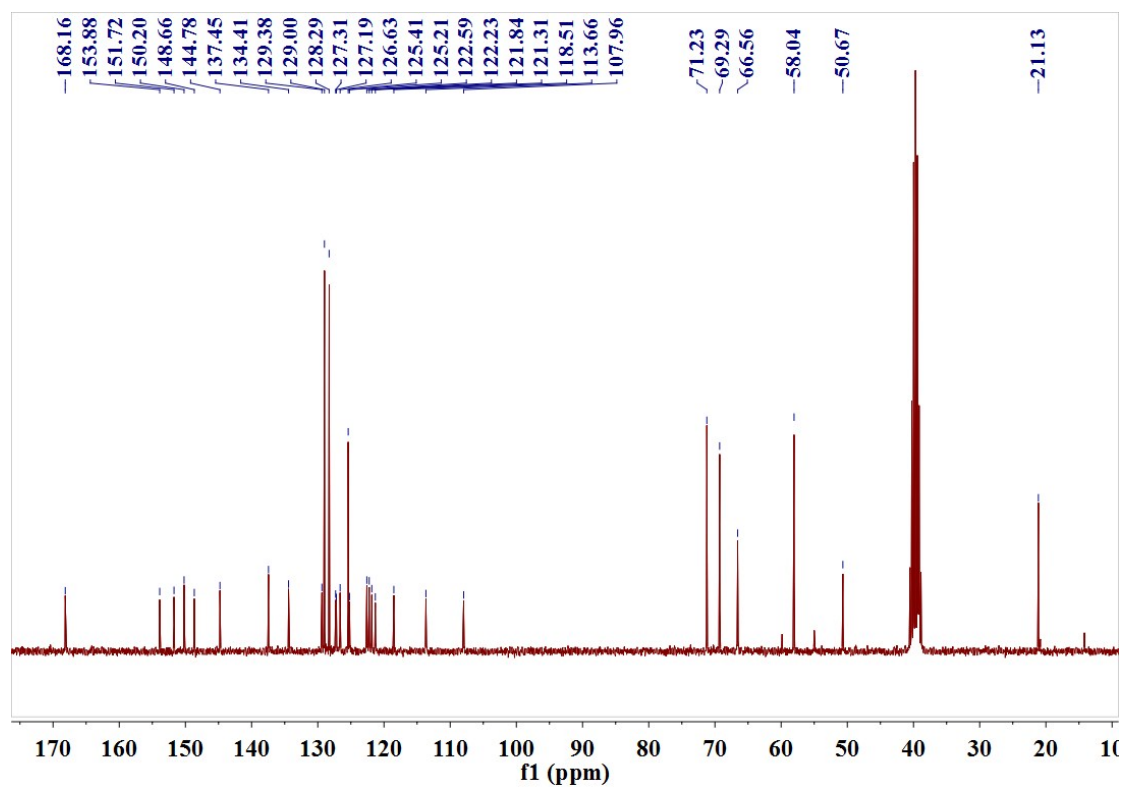
¹H NMR spectrum of the compound **EAPW**.



¹³C NMR spectrum of the compound **EAPW**.



^1H NMR spectrum of the compound APW.



^{13}C NMR spectrum of the compound APW.

1. Fan, C.; Luo, S.; Qi, H., A ratiometric fluorescent probe for alkaline phosphatase via regulation of excited-state intramolecular proton transfer. *Luminescence* **2016**, *31* (2), 423-7.
2. Hou, X.; Yu, Q.; Zeng, F.; Ye, J.; Wu, S., A ratiometric fluorescent probe for in vivo tracking of alkaline phosphatase level variation resulting from drug-induced organ damage. *Journal of Materials Chemistry B* **2015**, *3* (6), 1042-1048.
3. Zhou, X.; Jiang, Y.; Zhao, X.; Zhu, Y., A New Two-Photon Ratiometric Fluorescent Probe for Detecting Alkaline Phosphatase in Living Cells. *Molecules* **2016**, *21* (12).
4. Zhang, Q.; Li, S.; Fu, C.; Xiao, Y.; Zhang, P.; Ding, C., Near-infrared mito-specific fluorescent probe for ratiometric detection and imaging of alkaline phosphatase activity with high sensitivity. *Journal of Materials Chemistry B* **2019**, *7* (3), 443-450.