Supplementary data

Design, synthesis and pharmacological evaluation of new 2-oxo-quinoline derivatives containing α-aminophosphonates as potential antitumor agents

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Part 1. Selected relevant parameters of compounds $4c_5$, $4d_2$ and 5c

Table S1 Crystallographic data and refinements of compounds **$4c_5$**, **$4d_2$** and **5c**.

Formula	$C_{21}H_{24}N_3O_7P$(**$4c_5$**)	$C_{22}H_{24}NO_7P$ C_2H_3N(**$4d_2$**)	$C_{22}H_{26}N_2O_5P$(**5c**)
Mr	461.40	486.45	427.42
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	P-1	*P*21/*c*	*P*21/*n*
a/Å	8.0905 (8)	12.598 (13)	8.405 (2)
b/Å	11.3328 (17)	15.985 (17)	16.660 (3)
c/Å	11.780 (4)	12.834 (13)	16.156 (3)
α/°	91.967 (17)	90	90
β/°	90.537 (14)	93.364 (18)	99.29(2)°
γ/°	99.464 (10)	90	90
V/Å^3	1064.6 (4)	2580 (5)	2232.5 (9)
T/K	293(2)	293(2)	293(2)
Z	2	4	4
Dc/g.cm^{-3}	1.439	1.439	1.272
θ/°	3.64 to 25.2°	1.6 to 26.4	3.5 to 26.4
F (000)	484	1024	904
μ (Mo, Kα)(mm^{-1})	0.18	0.15	0.16
Total no. reflns	4332	23246	4055
No. indep. reflns	2256	5264	3201
R_{int}	0.060	0.0206	0.033
R1 [I＞2σ (I)]	0.074	0.072	0.073
ωR2(all data)	0.200	0.198	0.220
S	1.04	0.93	1.02

Table S2 Selected bond lengths (Å) and angles (°) for compound **4c$_5$**.

Bond names	Bond length(Å)	Bond angle	Angle(°)
P1—O1	1.455 (3)	O1—P1—O2	117.21 (16)
P1—O2	1.575 (3)	O1—P1—O3	114.53 (18)
P1—O3	1.568 (3)	O1—P1—C15	115.07 (17)
P1—C15	1.824 (4)	O2—P1—C15	100.79 (17)
O2—C1	1.450 (6)	O3—P1—O2	102.59 (16)
O3—C3	1.455 (5)	O3—P1—C15	104.77 (17)
O4—C5	1.427 (5)	C1—O2—P1	123.2 (3)
O4—C6	1.370 (4)	C3—O3—P1	122.8 (3)
O5—C11	1.248 (4)	C6—O4—C5	118.6 (3)
O6—N3	1.219 (4)	O6—N3—O7	122.1 (3)
O7—N3	1.241 (4)	O6—N3—C19	119.4 (4)
N1—C11	1.347 (5)	O7—N3—C19	118.5 (4)
N2—C15	1.438 (4)	O2—C1—C2	109.4 (5)
N2—C16	1.369 (4)	O3—C3—C4	108.6 (4)
N3—C19	1.436 (5)	O4—C6—C14	123.2 (4)

Table S3 Selected bond lengths (Å) and angles (°) for compound **4d$_2$**.

Bond names	Bond length(Å)	Bond angle	Angle(°)
P1—O5	1.481 (3)	O5—P1—O6	116.01 (17)
P1—O6	1.581 (3)	O5—P1—O7	110.32 (19)
P1—O7	1.582 (3)	O5—P1—C18	113.91 (18)
P1—C18	1.812 (4)	O6—P1—O7	106.10 (18)
O1—C1	1.433 (6)	O6—P1—C18	103.4 (2)
O1—C2	1.396 (5)	O7—P1—C18	106.34 (18)
O2—C1	1.427 (6)	C2—O1—C1	105.6 (4)
O2—C10	1.386 (5)	C10—O2—C1	106.3 (4)
O3—C7	1.260 (5)	C11—O4—C18	120.8 (3)
O4—C11	1.429 (5)	C14—C13—C12	122.7 (4)
O4—C18	1.470 (5)	C13—C14—C15	123.6 (5)
O6—C19	1.445 (6)	C13—C14—C16	116.4 (5)
O7—C21	1.451 (6)	C16—C14—C15	120.0 (5)
O6—C19	1.445 (6)	C19—O6—P1	125.2 (3)
O7—C21	1.451 (6)	C21—O7—P1	123.9 (3)
N1—C7	1.358 (5)	C7—N1—C8	126.2 (3)

Table S4 Selected bond lengths (Å) and angles (°) for compound **5c**.

Bond names	Bond length(Å)	Bond angle	Angle(°)
P1—O3	1.463 (4)	O3—P1—O4	114.8 (3)
P1—O4	1.580 (5)	O3—P1—O5	115.9 (3)
P1—O5	1.581 (5)	O3—P1—C19	115.8 (3)
C9—C10	1.419 (7)	O4—P1—O5	104.1 (3)
C10—C11	1.414 (7)	O4—P1—C19	101.6 (3)
P1—C19	1.767 (6)	O5—P1—C19	102.8 (3)
O1—C1	1.440 (6)	C2—O1—C1	116.2 (5)
O1—C2	1.367 (6)	C20—O4—P1	123.1 (6)
O2—C7	1.266 (5)	C22—O5—P1	124.8 (5)
O4—C20	1.431 (11)	C12—N1—C13	119.7 (5)
O5—C22	1.345 (7)	C14—C13—N1	125.5 (5)
N1—C12	1.261 (6)	C14—C13—C18	117.7 (6)
N1—C13	1.409 (7)	C18—C13—N1	116.7 (5)
C13—C14	1.383 (7)	C15—C14—C13	120.7 (5)
C13—C18	1.391 (7)	C14—C15—C16	121.8 (6)
C14—C15	1.369 (8)	C15—C16—C19	121.2 (6)

Part 2. Experimental methods

2.1. *In vitro cytotoxicity*

HepG2 human liver hepatocellular carcinoma cells, SK-OV-3 human ovarian carcinoma cells, NCI-H460 human large cell lung carcinoma cell, HL 7702 human liver hepatocellular cells were all obtained from the Institute of Biochemistry and Cell Biology, China Academy of Sciences. They were cultured in a humidified, 5% CO_2 atmosphere at 37 ℃ and maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin and 100 mg/mL penicillin. Chemosensitivity was assessed with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, exponentially growing cells were seeded into 96-well plates and treated with the indicated concentrations of compounds **4-5** for 48 h, and then 10 mL of MTT (10 mg/mL) was added. After incubation for 4 h at 37^{o}C, the purple formazan crystals (a reduced form of MTT) generated in viable cells were dissolved by adding 100 μL DMSO to each well. The plates were swirled gently for 10 min to dissolve the precipitate, and quantified by measuring the optical density of the plates at 490 nm using a plate reader (TECAN infinite M1000). Each concentration was repeated in three wells and the same experimental conditions were maintained for all testing procedures. The MTT assays were repeated three times for each cell line.

2.2. Mitochondrial Membrane Potential Staining

Mitochondrial depolarization was assayed in T24 cells using a JC-1 probe. Briefly, cells cultured in six-well plates after the indicated treatment were incubated with an equal volume of JC-1 staining solution (5 μg/mL) at 37^{o}C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using a Nikon ECLIPSETE2000-S fluorescent microscope. Mitochondrial depolarization was indicated by an increase in the green/red fluorescence intensity ratio.

2.3. AO/EB Staining

Cells were seeded at a concentration of 5×10^{4} cell/mL in a volume of 2 mL on sterile cover slips in six-well tissue culture plates. Following incubation, the medium was removed and replaced with fresh medium plus 10% FBS and supplemented with compound **3d**. After treatment, cover slips with cell monolayers were inverted on a glass slide with 20 μL of AO/EB stain (100 mg/mL). Fluorescence was read on a Nikon ECLIPSETE2000-S fluorescence microscope (OLYMPUS Co., Japan).

2.4. Hoechst 333258 Staining

Cells grown on a sterile cover slips in six-well tissue culture plates were treated with test compounds for the indicated time. The culture medium containing the compounds was removed, and the cells were fixed in 4%

paraformaldehyde for 10 min. After washing twice with phosphate buffered saline (PBS), the cells were stained with 0.5 mL of Hoechst 33258 (Beyotime) for 5 min and again washed twice with PBS. Nuclear staining was observed with a Nikon ECLIPSETE2000-S fluorescence microscope at 350 nm excitation and 460 nm emission wavelengths.

2.5. Apoptosis Analysis

Apoptosis was assayed by annexin V-FITC and PI. Cells were seeded at 2×10^6/well in 10% FBS–DMEM into six-well plates and treated with test compounds for 24 h. The cells were then washed twice with cold PBS and resuspended in 1×binding buffer (0.1 M pH 7.4 Hepes/NaOH, 1.4 M NaCl, 25 mM $CaCl_2$) at a concentration of 1×10^6 cells/mL. A 100 μL volume of the solution (1×10^5 cells) was transferred to a 5 mL culture tube; 5 μL of FITC Annexin V (BD, Pharmingen) and 5 μL PI were added to each tube. The cell suspension was gently vortexed and incubated for 30 minutes at room temperature (25°C) in the dark, and then 200 μl PBS was added to each tube. The apoptosis assay was carried out by flow cytometry (FACSVerse, BD, USA) at 488 nm excitation. The lower left quadrant included viable cells (annexin V^-PI^-); lower right quadrant included early apoptotic cells (annexin V^+/PI^-); upper right quadrant included late apoptotic cells (annexin V^+/PI^+); and the upper left quadrant included necrotic cells (annexin V^-/PI^+). The percentage of PI^+ and/or Annexin V-$FITC^+$ cells inside the quadrants was reported.

2.6. Cell Cycle Analysis

Cell cultures were treated with the indicated concentrations of compound **3d** and after 48 h incubation, the cells were washed twice with ice-cold PBS, fixed and permeabilized with ice-cold 70% ethanol at −20°C overnight. The cells were treated with 100 μg/mL RNase A at 37°C for 30 min after washing with ice-cold PBS, and finally stained with 1 mg/mL PI in the dark at 4°C for 30 min. Cell cycle analysis was performed by flow cytometry (FACSVerse, BD, USA) at an excitation of 488 nm and an emission of 620 nm.

2.7. ROS Assay

T24 cells were seeded into six-well plates, and following treatment, were incubated with 10 mM DCFH-DA (Beyotime, Haimen, China) dissolved in cell-free medium for 30 min at 37°C and in the dark. They were then washed three times with PBS. Cellular fluorescence was measured with a Nikon ECLIPSETE2000-S fluorescence microscope at 485 nm excitation and 538 nm emission.

2.8. Calcium Analysis

To monitor the effect of compounds **5b** on calcium release, T24 cells were seeded into six-well plates, and loaded with 5 mM of the membrane-permeable calcium indicator Fluo-3 acetoxymethyl ester (Beyotime, Haimen, China) in PBS buffer for 40 min at 37°C. After loading with the Fluo-3 dye, cells were washed with PBS and suspended in Ca-free PBS containing 5 mM EGTA. Fluo-3 was excited by argon laser light at 488 nm; fluorescence was measured

at 515 nm, and quantified with a Nikon ECLIPSETE2000-S fluorescence microscope.

2.9. Western Blot Assay

HepG2 cells were collected after treatment with compound **5b** (10 μM) for 12 h and then lysed in ice-cold lysis buffer (1% sodium dodecyl sulfate in 25 mM pH 7.5 Tris–HCl, 4 mM EDTA, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mg/mL leupeptin and 10 mg/mL soybean trypsin inhibitor). Whole-cell lysates were centrifuged at 12,000×*g* for 5 min. Thereafter, the protein concentration was determined with a bicinchoninic acid protein assay kit (Beyotime Co, China). An aliquot of cell lysate (40–50 μg) was fractionated by SDS-PAGE on 12% polyacrylamide gels for 2 h and transferred to polyvinylidene difluoride membranes. After blocking with 5% non-fat dry milk in PBS-t for 1 h at room temperature, the membranes were incubated with β-actin, cytochrome c, caspase-9, caspase-3, Bax or Bcl-2 antibodies (Bioworld Technology Inc, USA) overnight at 4^{o}C, washed with tris-buffered saline and Tween 20, and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Proteins were detected by electrochemiluminescence, Thermo Fisher Scientific, USA) and analysed by Image J software.

2. 10. Statistical Analysis

Data are expressed as mean ± SD for three different determinations. Statistical significance was analyzed by one-way ANOVA. Mean separations were performed using the least significant difference method. $P<0.05$was defined as statistically significant.

Part 3. ^{1}H NMR, ^{13}CNMR and HRMS of compounds $4a_1$−$4d_7$ and 5a−5d.

$4a_1$: Yield54.29%, ^{1}H NMR (500 MHz, DMSO) δ 11.87 (s, 1H), 8.05 (d, *J* = 3.7 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 4.48 (d, *J* = 21.9 Hz, 1H), 4.14 – 4.05 (m, 2H), 3.96 – 3.84 (m, 2H), 2.40 (ddd, *J* = 25.7, 13.6, 7.0 Hz, 2H), 1.36 (dt, *J* = 14.0, 7.0 Hz, 2H), 1.26 (ddd, *J* = 16.7, 10.7, 5.3 Hz, 5H), 1.08 (t, *J* = 7.0 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.98, 161.93, 138.40, 137.70, 137.64, 130.60, 129.88, 128.18, 122.42, 119.55, 119.53, 115.37, 62.92, 62.87, 62.42, 62.36, 53.20, 51.96, 47.67, 47.55, 40.48, 40.31, 40.23, 40.14, 39.98, 39.81, 39.64, 39.48, 31.86, 20.17, 16.79, 16.74, 16.63, 16.58, 14.26.ESI-HRMS *m/z* Calc for $C_{18}H_{27}N_2O_4P$ $[M+H]^+$:367.1787; found: 367.1782.

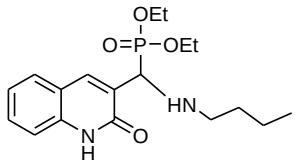


Fig. 1. Chemical structure of compound $4a_1$

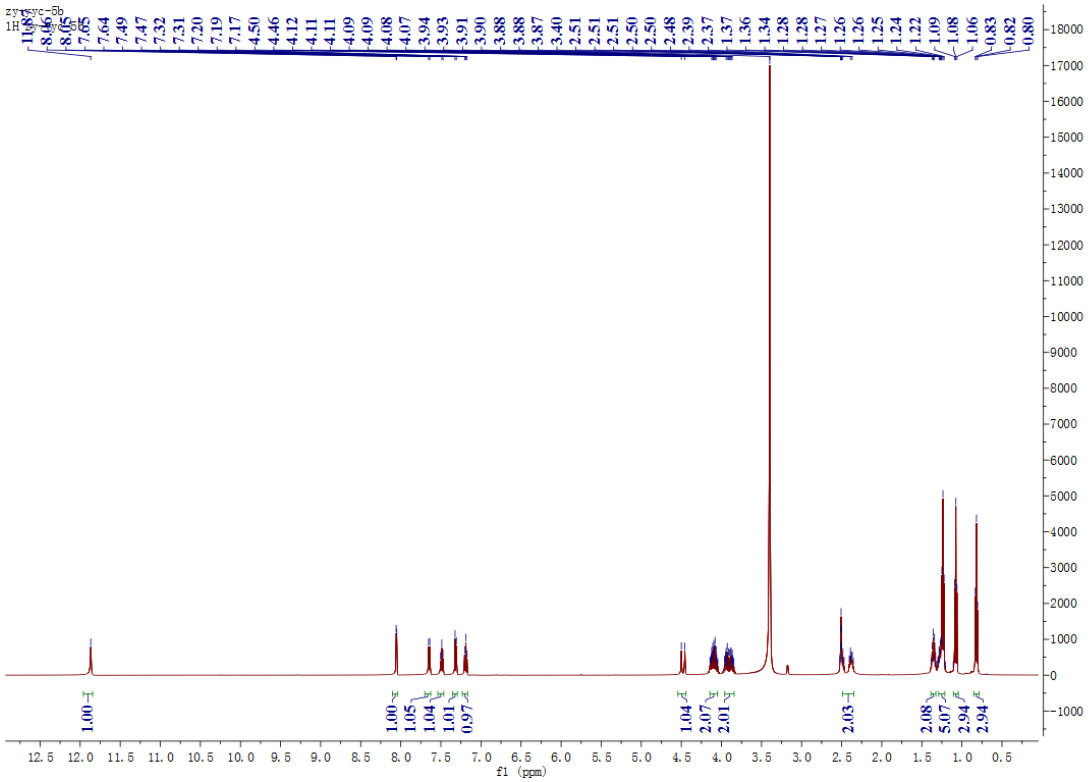


Fig. 2.^{1}H NMR of compound $4a_1$

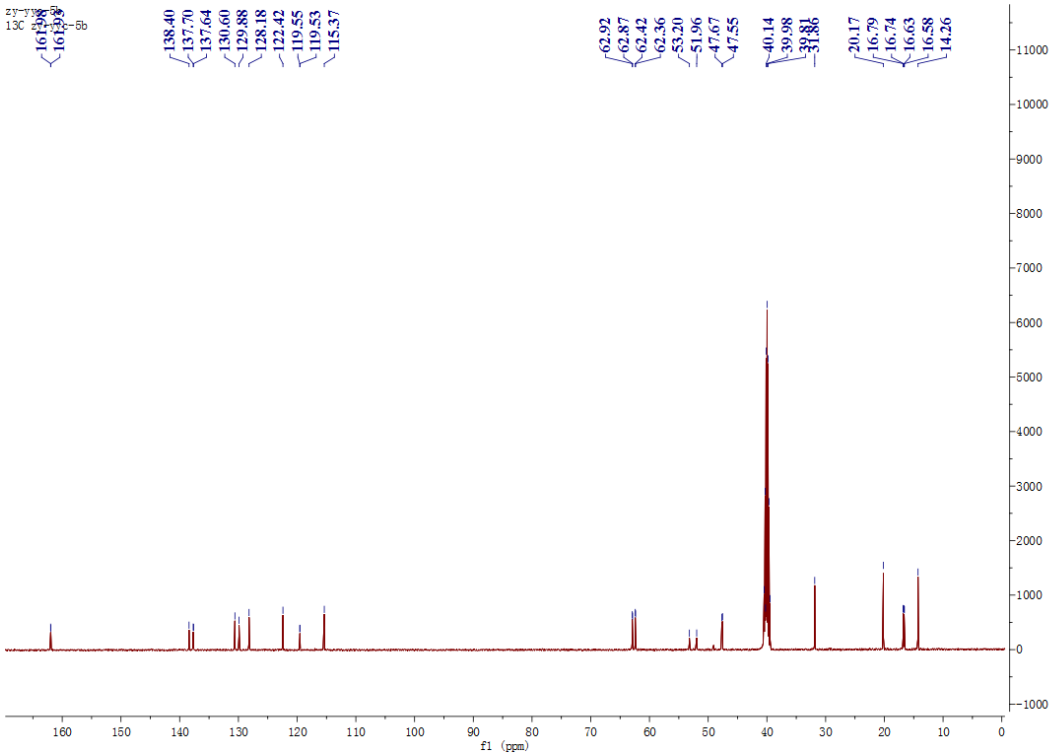


Fig. 3.^{13}C NMR of compound 4a$_1$

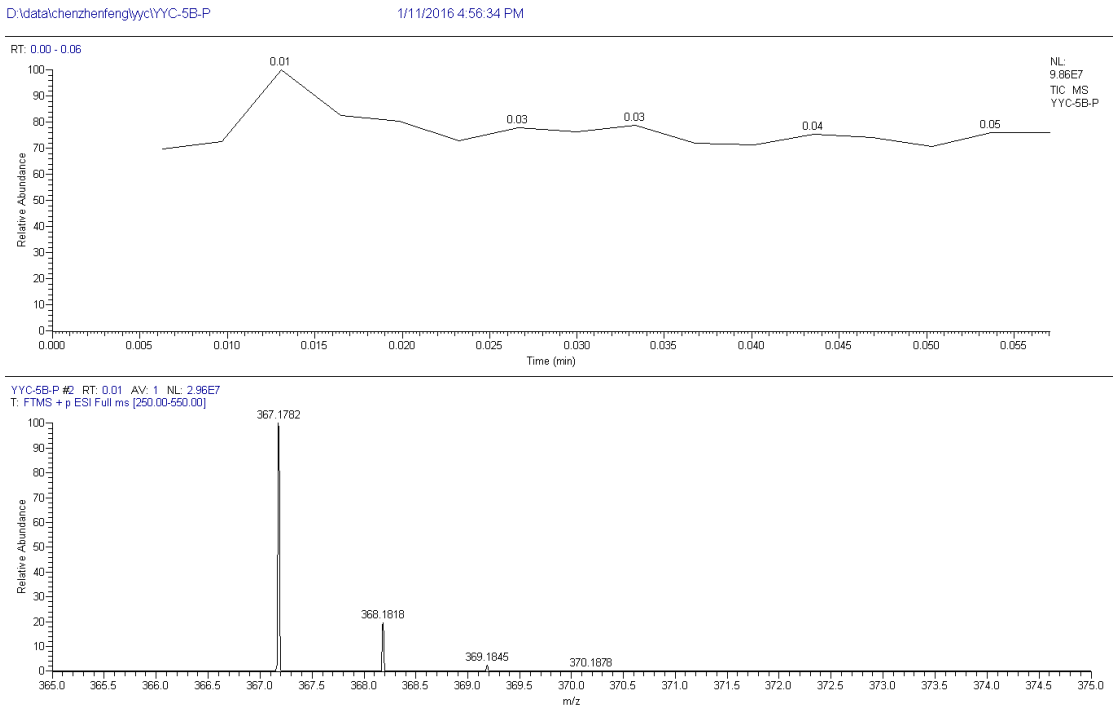


Fig. 4.ESI-HRMS **of compound 4a$_1$**

4a$_2$: Yield75.82%, ^{1}H NMR (500 MHz, DMSO) δ 11.96 (s, 1H), 8.08 (d, *J* = 3.7 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.19 – 7.14 (m, 1H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.62 (d, *J* = 8.5 Hz, 2H), 6.11 (dd, *J* = 10.4, 6.2 Hz, 1H), 5.26 (dd, *J* = 24.5, 10.4 Hz, 1H), 4.11 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.02 – 3.87 (m, 2H), 2.09 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.68, 161.64, 145.11, 144.99, 138.47, 137.65, 137.60, 130.80, 130.13, 129.78, 128.12, 126.32, 122.56, 119.40, 119.38, 115.50, 113.86, 63.22, 63.16, 62.92, 62.86, 48.28, 47.05, 40.50, 40.43, 40.34, 40.26, 40.17, 40.00, 39.84, 39.67, 39.50, 20.45, 16.77, 16.73, 16.58, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{25}N_2O_4P$ $[M+Na]^+$:423.1450; found: 423.1447.

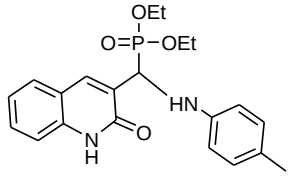


Fig. 5. Chemical structure of compound4a$_2$

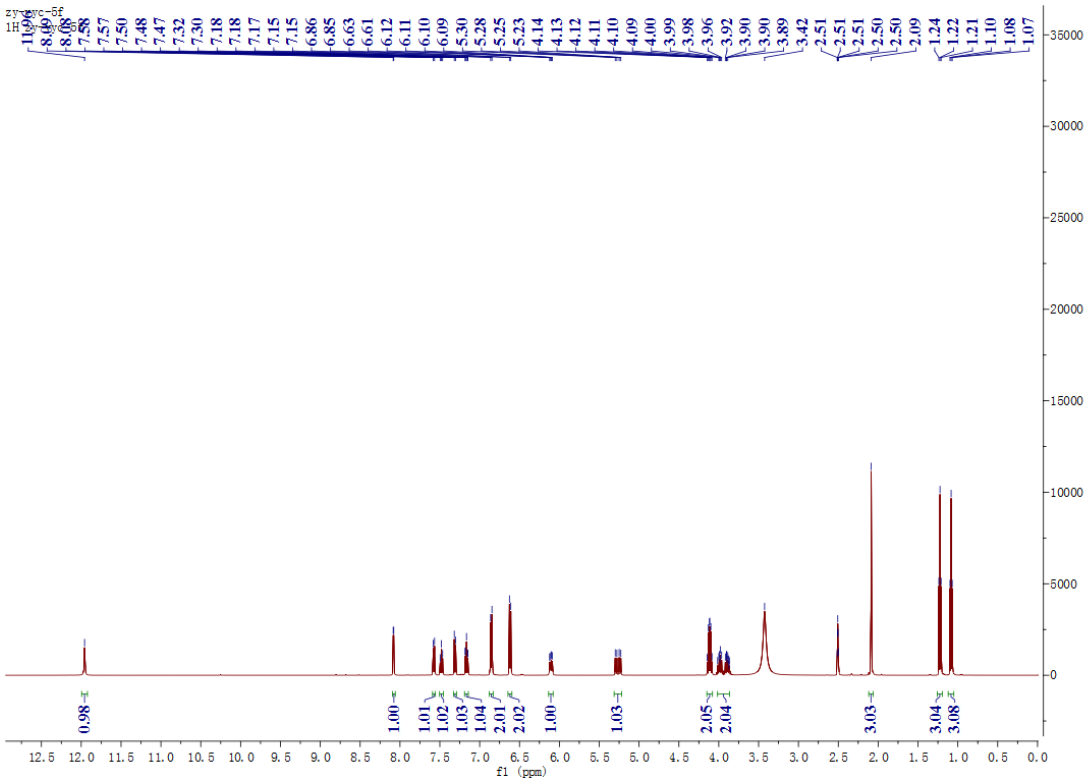


Fig. 6.^{1}H NMR of compound 4a$_2$

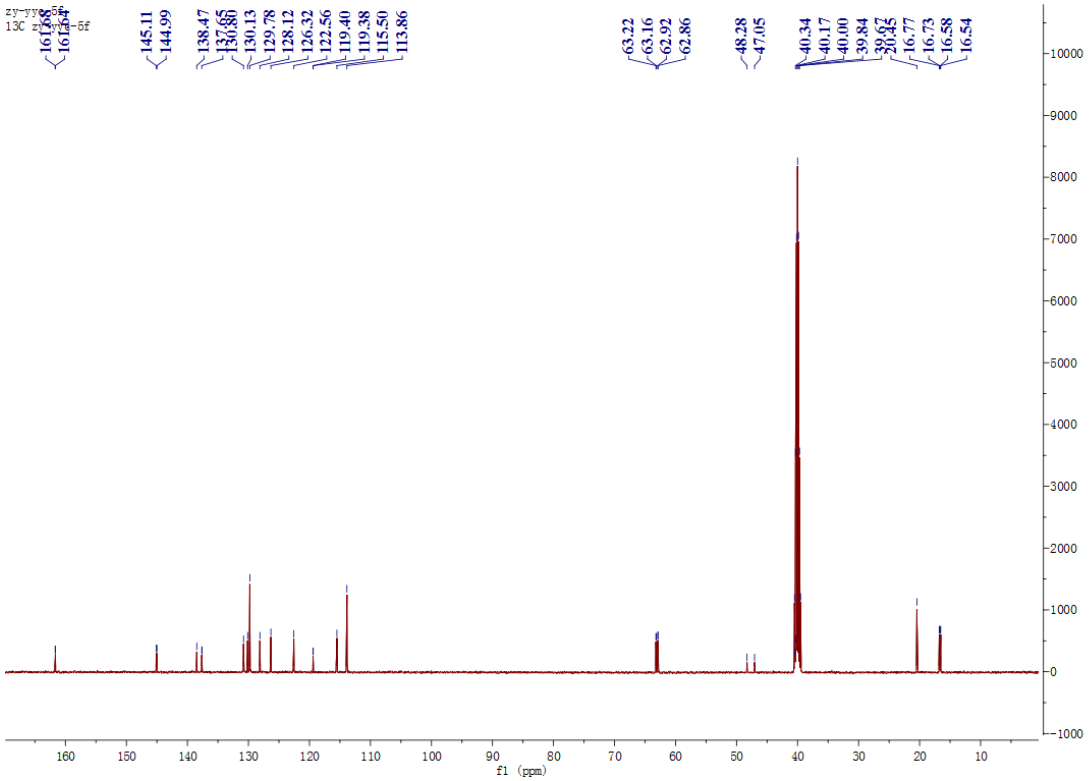


Fig. 7.^{13}C NMR of compound 4a$_2$

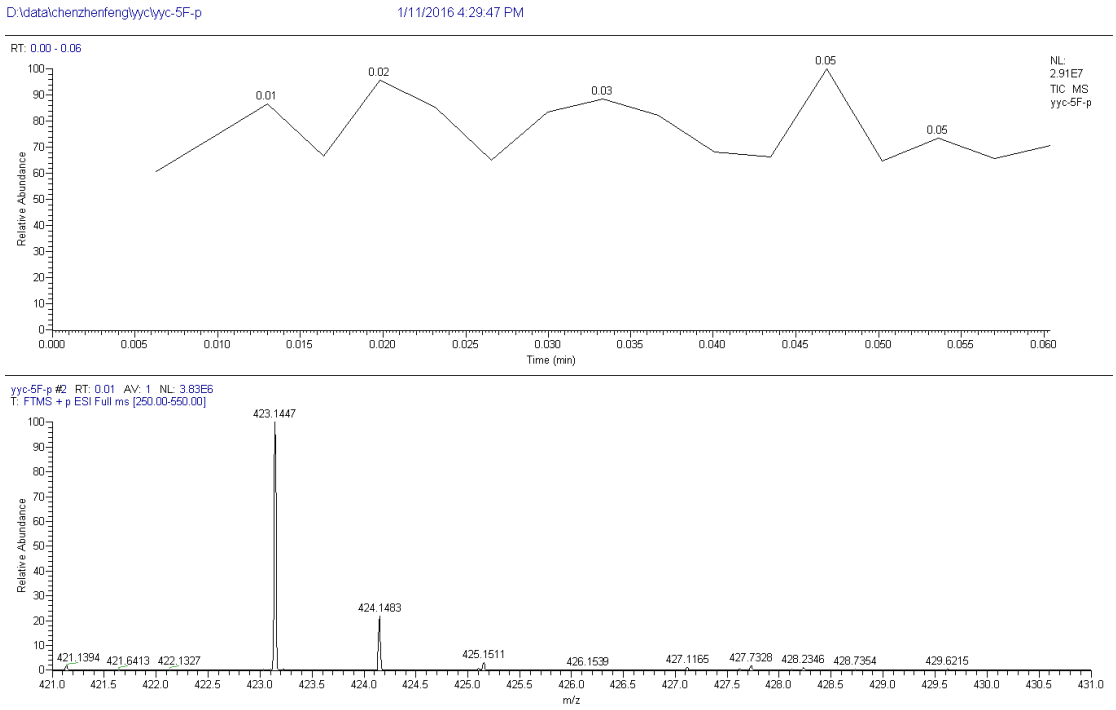


Fig. 8.ESI-HRMS **of compound 4a$_2$**

4a$_3$: Yield70.24%, ^{1}H NMR (400 MHz, DMSO) δ 12.00 (s, 1H), 8.10 (d, *J* = 3.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 6.93 (t, *J* = 7.8 Hz, 1H), 6.57 (s, 1H), 6.50 (d, *J* = 8.1 Hz, 1H), 6.39 (d, *J* = 7.4 Hz, 1H), 6.25 (dd, *J* = 10.2, 6.2 Hz, 1H), 5.30 (dd, *J* = 24.4, 10.3 Hz, 1H), 4.12 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.04 – 3.85 (m, 2H), 2.13 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.66, 161.61, 147.46, 147.32, 138.46, 138.35, 137.73, 137.67, 130.86, 130.14, 129.24, 128.16, 122.60, 119.39, 119.36, 118.69, 115.51, 114.43, 110.74, 63.26, 63.19, 62.97, 62.90, 48.02, 46.47, 40.57, 40.36, 40.15, 39.95, 39.74, 39.53, 39.32, 21.78, 16.79, 16.73, 16.60, 16.54. ESI-HRMS *m/z* Calc for $C_{21}H_{25}N_2O_4P$ $[M-H]^-$:399.1474; found: 399.1493.

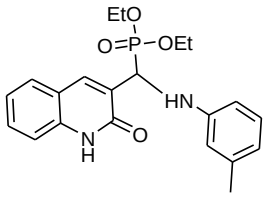


Fig. 9. Chemical structure of compound4a$_3$

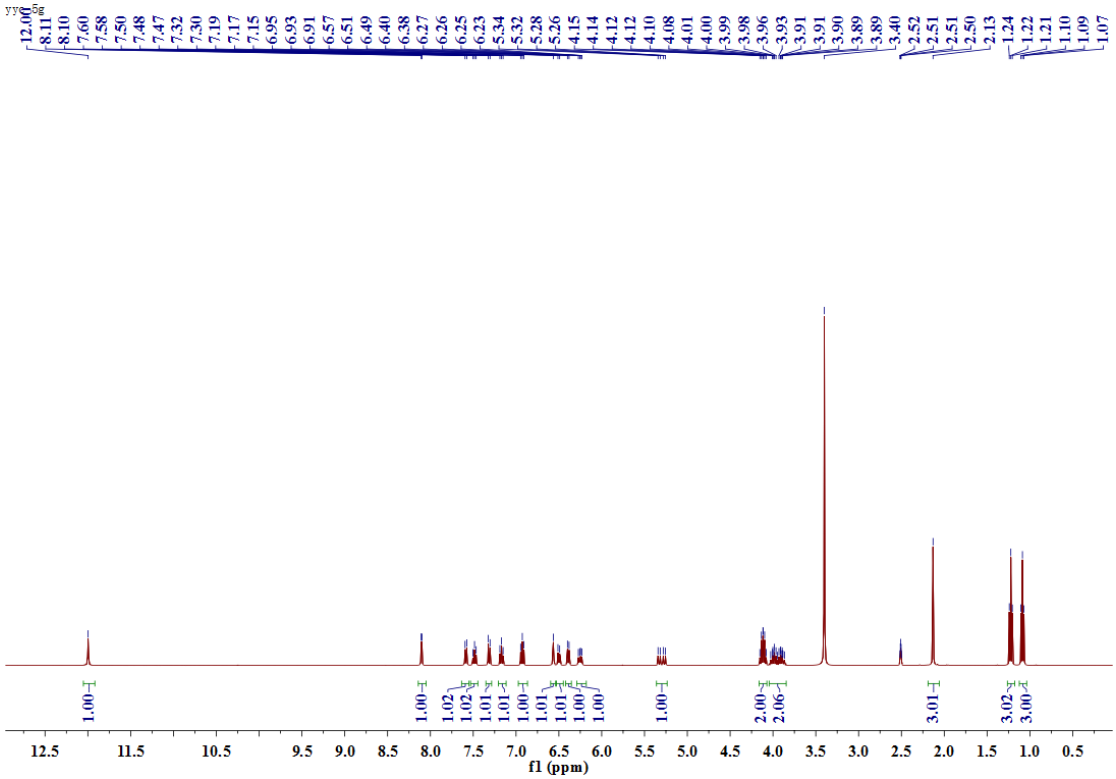


Fig. 10.^{1}H NMR of compound 4a$_3$

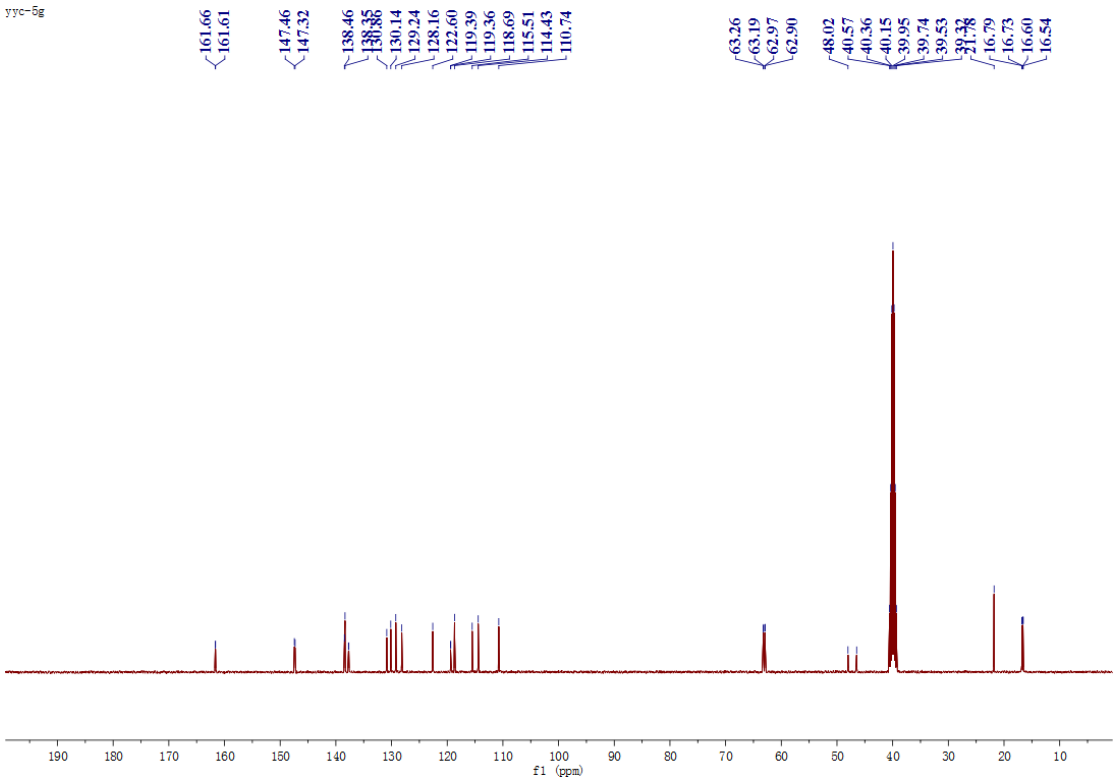


Fig. 11.^{13}C NMR of compound 4a$_3$

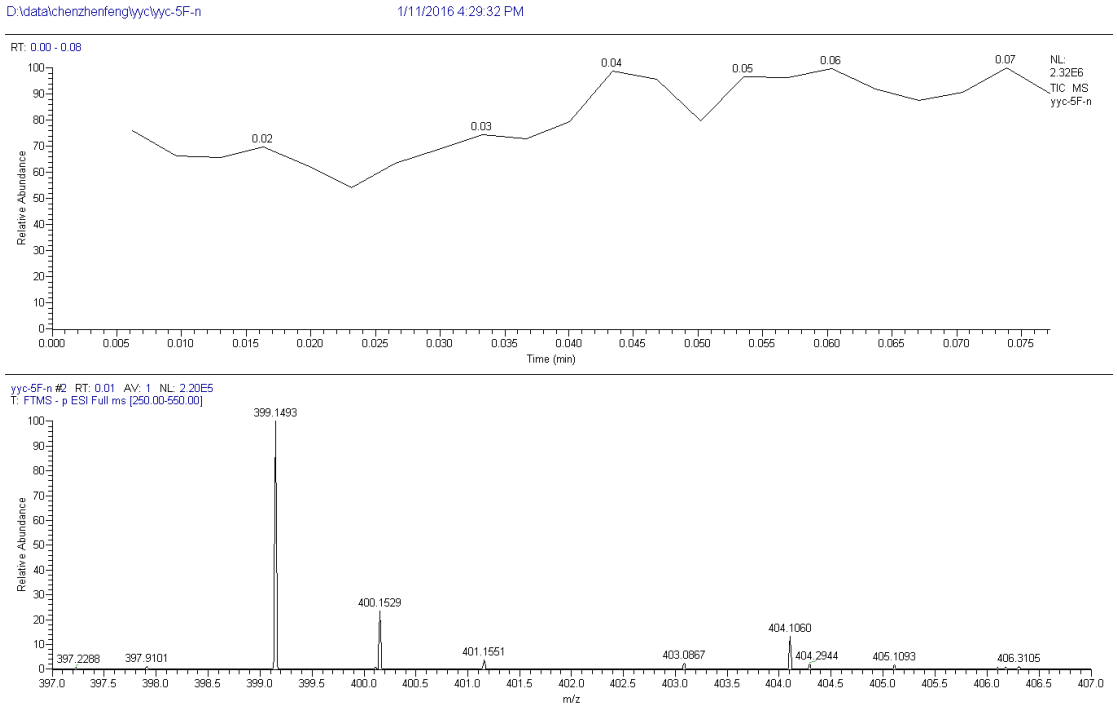


Fig. 12.ESI-HRMS **of compound 4a$_2$**

4a$_4$: Yield63.62%, ^{1}H NMR (500 MHz, DMSO) δ 11.87 (s, 1H), 8.05 (d, J = 3.7 Hz, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.31 (d, J = 8.2 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 4.48 (d, J = 21.9 Hz, 1H), 4.14 – 4.05 (m, 2H), 3.96 – 3.84 (m, 2H), 2.40 (ddd, J = 25.7, 13.6, 7.0 Hz, 2H), 1.36 (dt, J = 14.0, 7.0 Hz, 2H), 1.26 (ddd, J = 16.7, 10.7, 5.3 Hz, 5H), 1.08 (t, J = 7.0 Hz, 3H), 0.82 (t, J = 7.3 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.66, 161.62, 147.44, 147.33, 138.48, 137.76, 137.71, 130.87, 129.99, 129.36, 128.16, 122.60, 119.39, 119.36, 117.79, 115.53, 113.65, 63.26, 63.21, 62.99, 62.93, 47.97, 46.73, 40.47, 40.30, 40.14, 39.97, 39.80, 39.63, 39.47, 16.76, 16.72, 16.58, 16.53.ESI-HRMS *m/z* Calc for $C_{20}H_{23}N_2O_4P$ $[M+Na]^+$:409.1293; found: 409.1293.

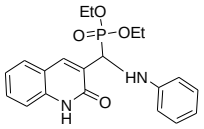


Fig. 13. Chemical structure of compound4a$_4$

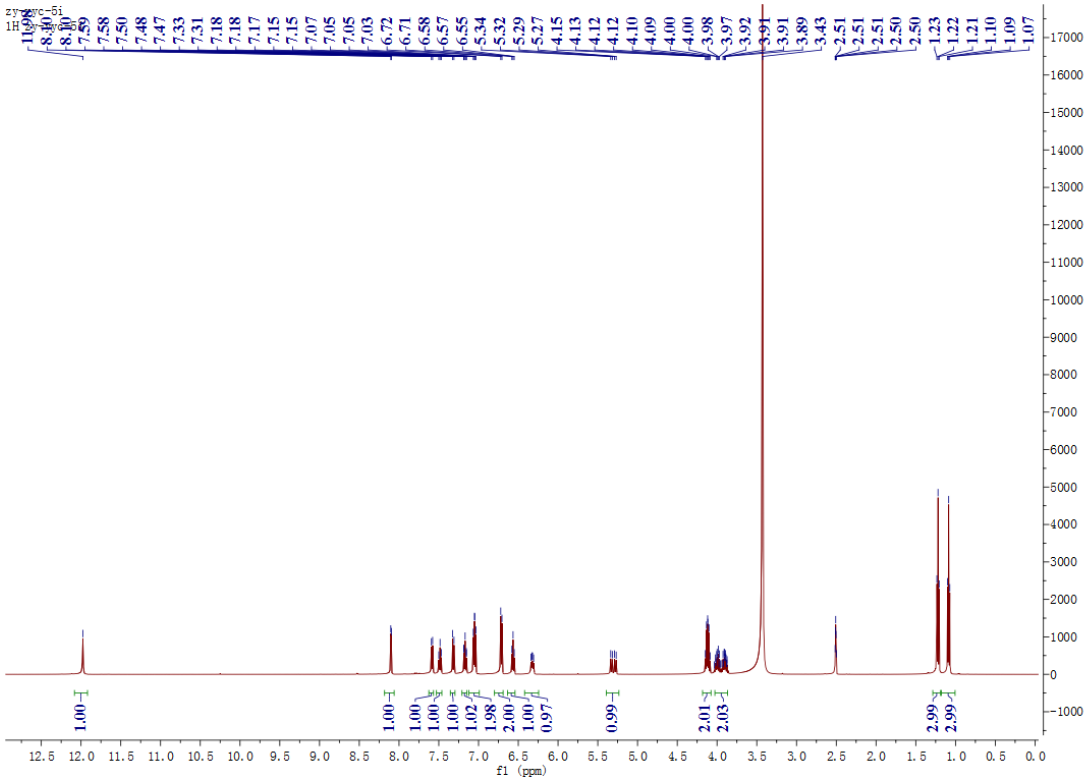


Fig. 14.^{1}H NMR of compound 4a$_4$

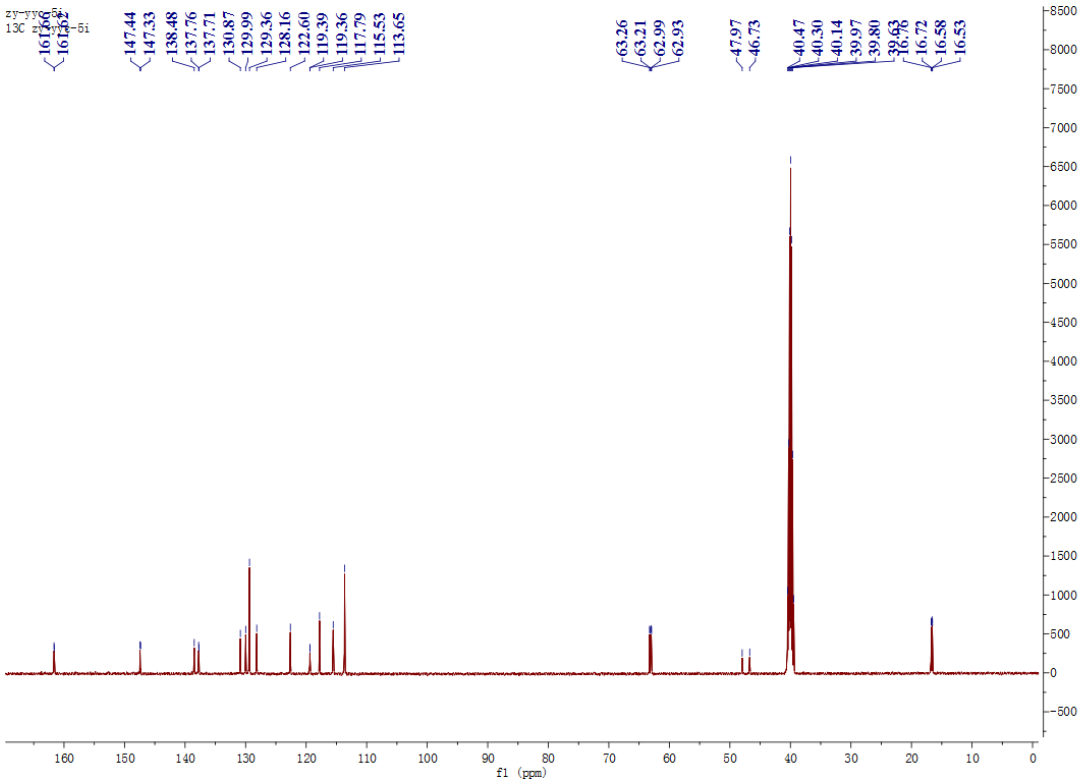


Fig. 15.^{13}C NMR of compound 4a$_4$

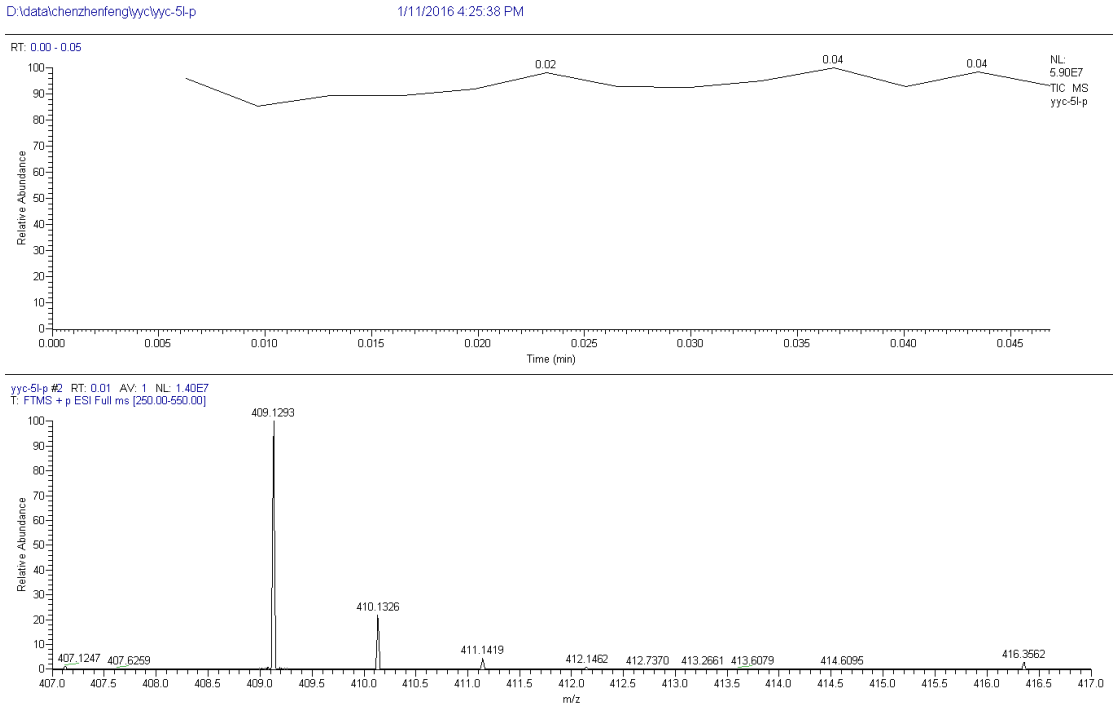


Fig. 16.ESI-HRMS **of compound 4a$_4$**

4a$_5$: Yield73.01%, ^{1}H NMR (400 MHz, DMSO) δ 12.13 (s, 1H), 8.13 (d, J = 3.4 Hz, 1H), 8.03 (d, J = 9.3 Hz, 2H), 7.95 (dd, J = 9.4, 5.6 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.25 – 7.17 (m, 1H), 6.86 (d, J = 9.3 Hz, 2H), 5.47 (dd, J = 22.6, 9.4 Hz, 1H), 4.21 – 3.88 (m, 4H), 1.21 (t, J = 7.0 Hz, 3H), 1.11 (t, J = 7.0 Hz, 3H)^{13}C NMR (101 MHz, DMSO) δ 161.37, 161.31, 153.69, 153.58, 138.63, 138.45, 138.39, 137.66, 131.28, 128.59, 128.41, 126.45, 122.77, 119.18, 119.15, 115.65, 112.43, 63.46, 63.39, 63.36, 63.29, 47.73, 46.17, 40.60, 40.39, 40.18, 39.98, 39.77, 39.56, 39.35, 16.78, 16.72, 16.59, 16.54. ESI-HRMS *m/z* Calc for $C_{20}H_{22}N_3O_6P$ $[M+Na]^+$:454.1144; found: 454.1163.

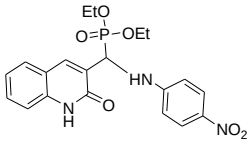


Fig. 17. Chemical structure of compound4a$_5$

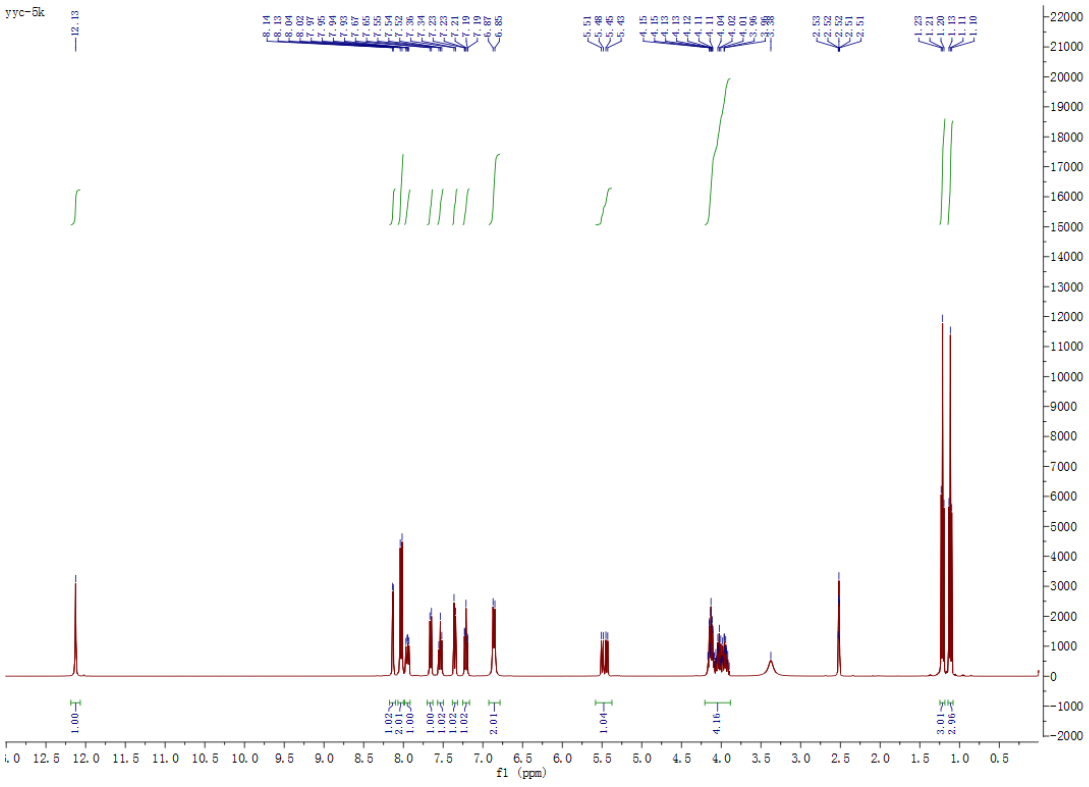


Fig. 18.^{1}H NMR of compound 4a$_5$

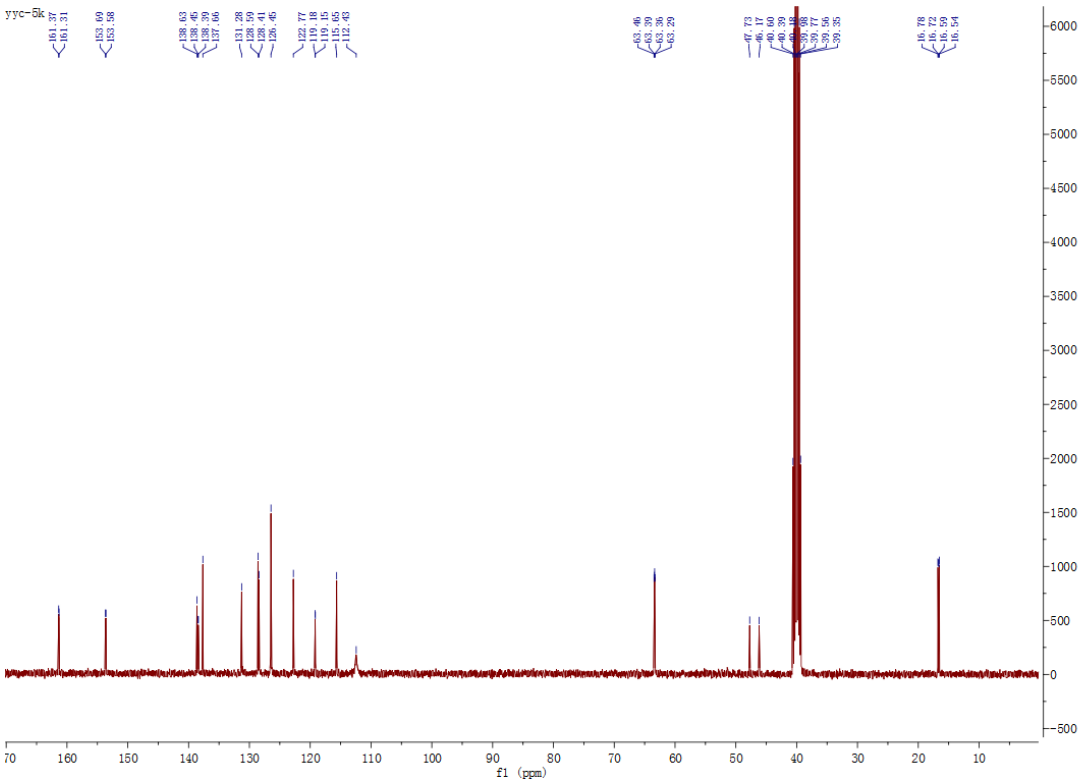


Fig. 19.^{13}C NMR of compound 4a$_5$

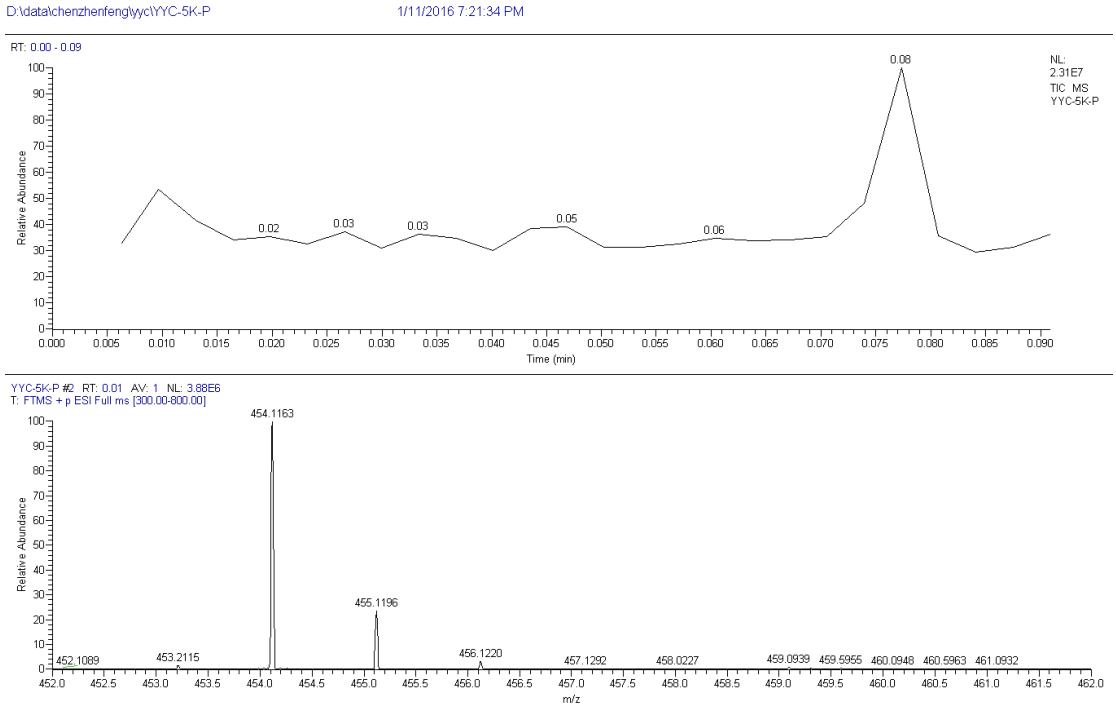


Fig. 20.ESI-HRMS of compound 4a$_5$

$4a_6$: Yield79.13%, ^{1}H NMR (400 MHz, DMSO) δ 12.00 (s, 1H), 8.10 (d, *J* = 3.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.22 – 7.14 (m, 1H), 6.96 – 6.85 (m, 2H), 6.71 (ddd, *J* = 6.8, 5.2, 2.8 Hz, 2H), 6.37 (dd, *J* = 10.3, 6.3 Hz, 1H), 5.25 (dd, *J* = 24.4, 10.3 Hz, 1H), 4.13 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.05 – 3.84 (m, 2H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.09 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.68, 161.62, 156.67, 154.36, 144.10, 143.95, 138.49, 137.78, 137.71, 130.92, 129.82, 128.19, 122.62, 119.35, 119.31, 115.88, 115.66, 115.54, 114.60, 114.53, 63.27, 63.20, 62.99, 62.92, 48.53, 46.98, 40.60, 40.39, 40.19, 39.98, 39.77, 39.56, 39.35, 16.79, 16.74, 16.59, 16.54.ESI-HRMS *m/z* Calc for $C_{20}H_{22}FN_2O_4P$ $[M+Na]^+$:427.1199; found: 427.1204.

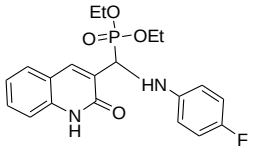


Fig. 21. Chemical structure of compound $4a_6$

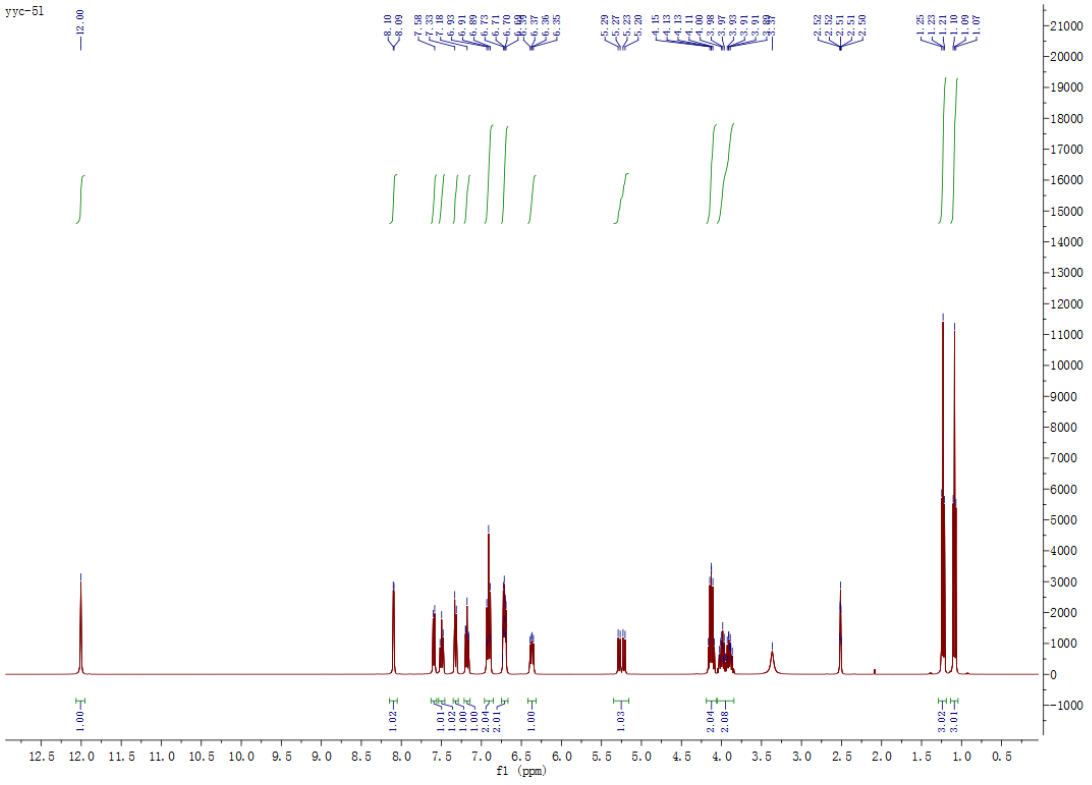


Fig. 22.^{1}H NMR of compound $4a_6$

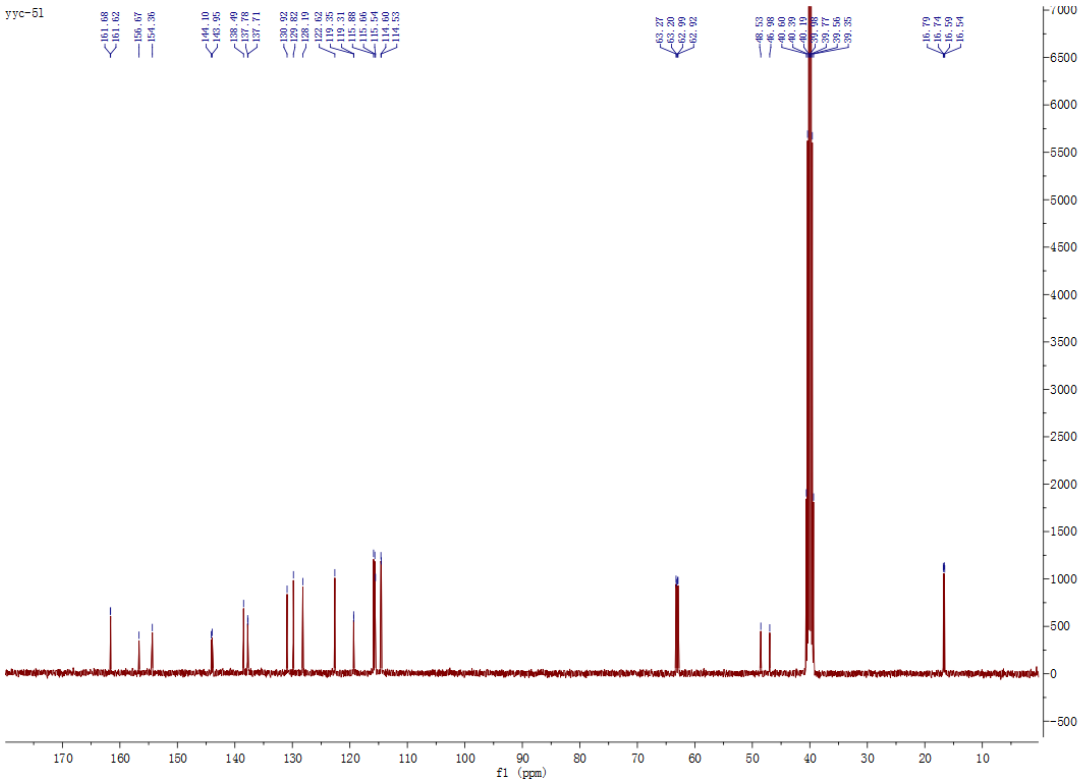


Fig. 23.^{13}C NMR of compound 4a$_6$

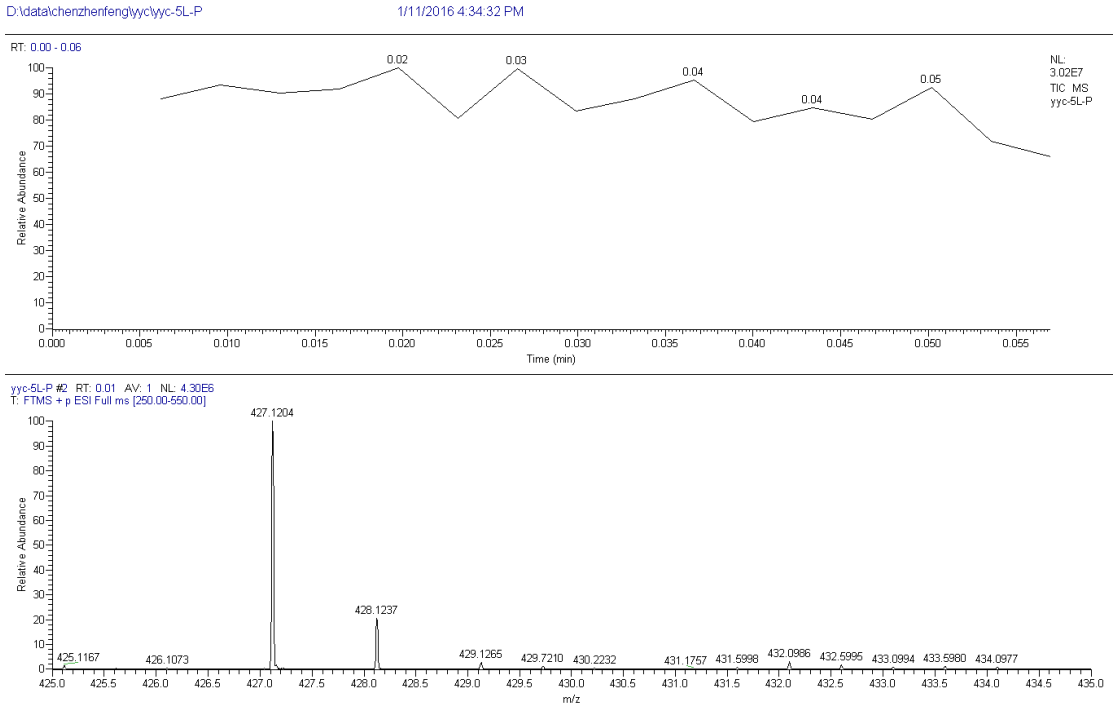


Fig. 24.ESI-HRMS of compound 4a$_6$

4a$_7$: Yield80.64%, ^{1}H NMR (400 MHz, DMSO) δ 12.13 (s, 1H), 8.13 (d, *J* = 3.4 Hz, 1H), 8.03 (d, *J* = 9.3 Hz, 2H), 7.95 (dd, *J* = 9.4, 5.6 Hz, 1H), 7.66 (d, *J* = 7.7 Hz, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.25 – 7.17 (m, 1H), 6.86 (d, *J* = 9.3 Hz, 2H), 5.47 (dd, *J* = 22.6, 9.4 Hz, 1H), 4.21 – 3.88 (m, 4H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.52, 161.47, 150.77, 150.64, 138.56, 138.03, 137.97, 131.07, 129.29, 128.28, 126.88, 126.75, 126.71, 124.19, 122.68, 119.27, 119.24, 117.62, 117.30, 115.59, 112.99, 99.99, 63.35, 63.29, 63.16, 63.09, 47.66, 46.10, 40.60, 40.39, 40.18, 39.97, 39.76, 39.56, 39.35, 16.76, 16.71, 16.59, 16.53.ESI-HRMS *m/z* Calc for $C_{21}H_{22}F_3N_2O_4P$ $[M+Na]^+$: 477.1167; found: 477.1165.

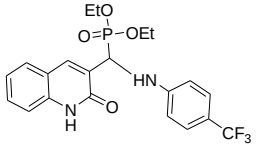


Fig. 25. Chemical structure of compound 4a$_7$

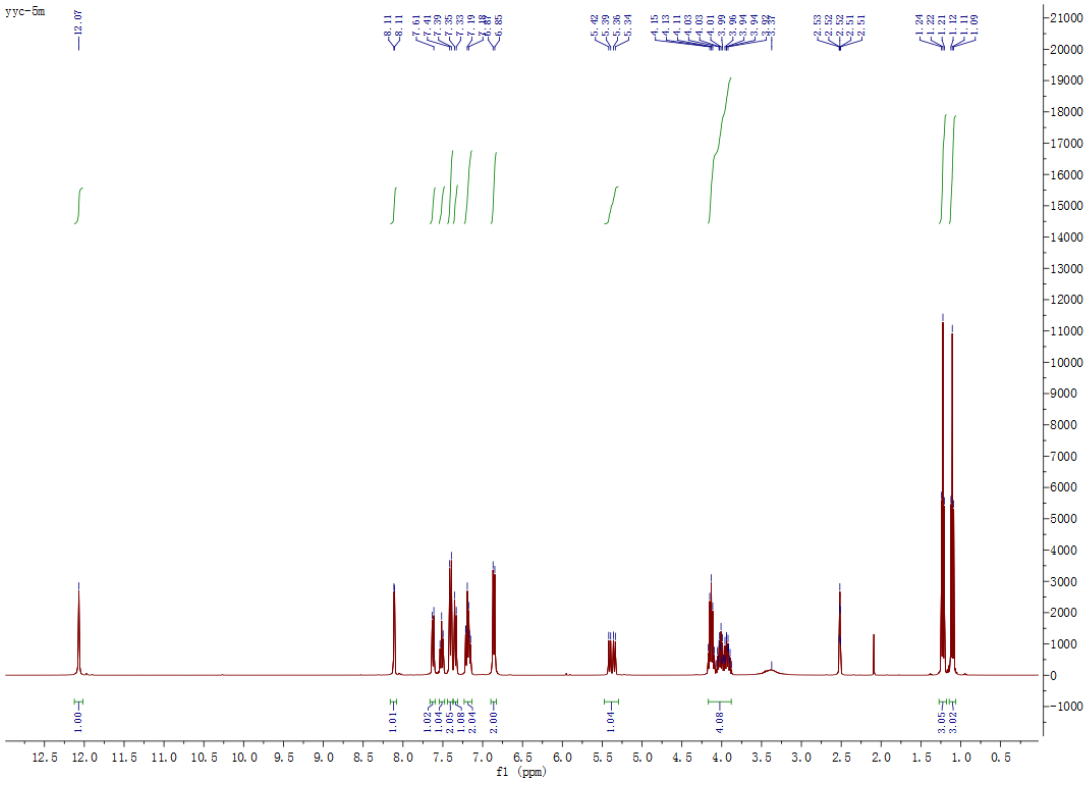


Fig. 26.^{1}H NMR of compound 4a$_7$

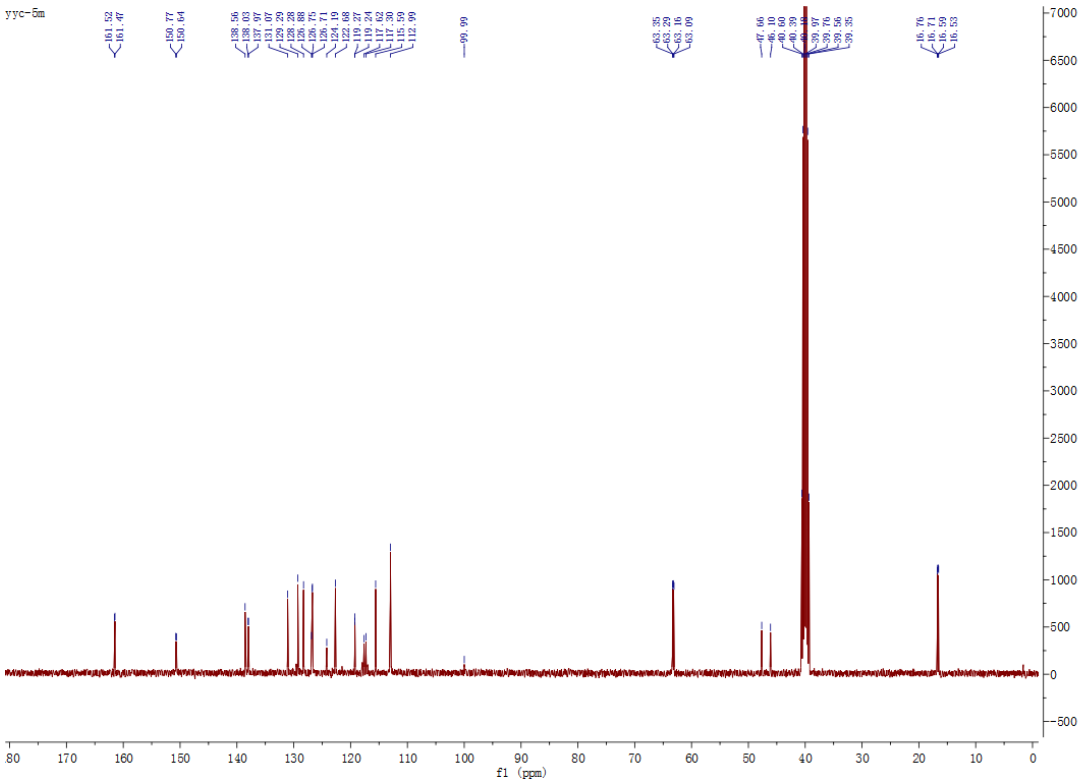


Fig. 27.^{13}C NMR of compound 4a$_7$

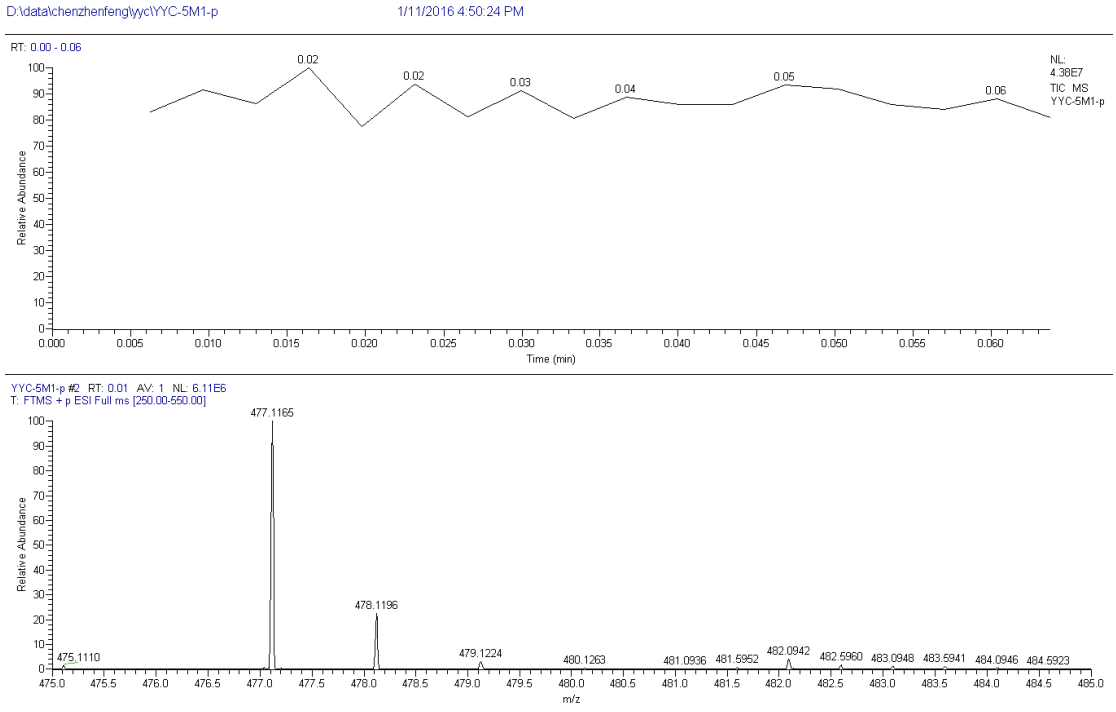


Fig. 28.ESI-HRMS of compound 4a$_6$

4b$_1$: Yield38.61%, ^{1}H NMR (500 MHz, DMSO) δ 11.87 (s, 1H), 8.05 (d, *J* = 3.7 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 4.48 (d, *J* = 21.9 Hz, 1H), 4.14 – 4.05 (m, 2H), 3.96 – 3.84 (m, 2H), 2.40 (ddd, *J* = 25.7, 13.6, 7.0 Hz, 2H), 1.36 (dt, *J* = 14.0, 7.0 Hz, 2H), 1.26 (ddd, *J* = 16.7, 10.7, 5.3 Hz, 5H), 1.08 (t, *J* = 7.0 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.86, 161.82, 137.44, 137.39, 136.41, 131.83, 131.39, 129.77, 127.65, 119.50, 119.47, 115.29, 62.87, 62.82, 62.39, 62.34, 53.21, 51.97, 47.65, 47.53, 40.52, 40.35, 40.18, 40.02, 39.85, 39.68, 39.52, 31.85, 20.83, 20.18, 16.80, 16.76, 16.64, 16.60, 14.28.ESI-HRMS *m/z* Calc for $C_{19}H_{29}N_2O_4P$ $[M+H]^+$: 381.1943; found: 381.1953.

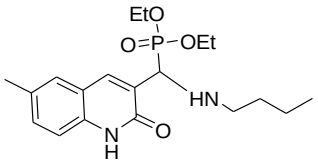


Fig. 29. Chemical structure of compound 4b$_1$

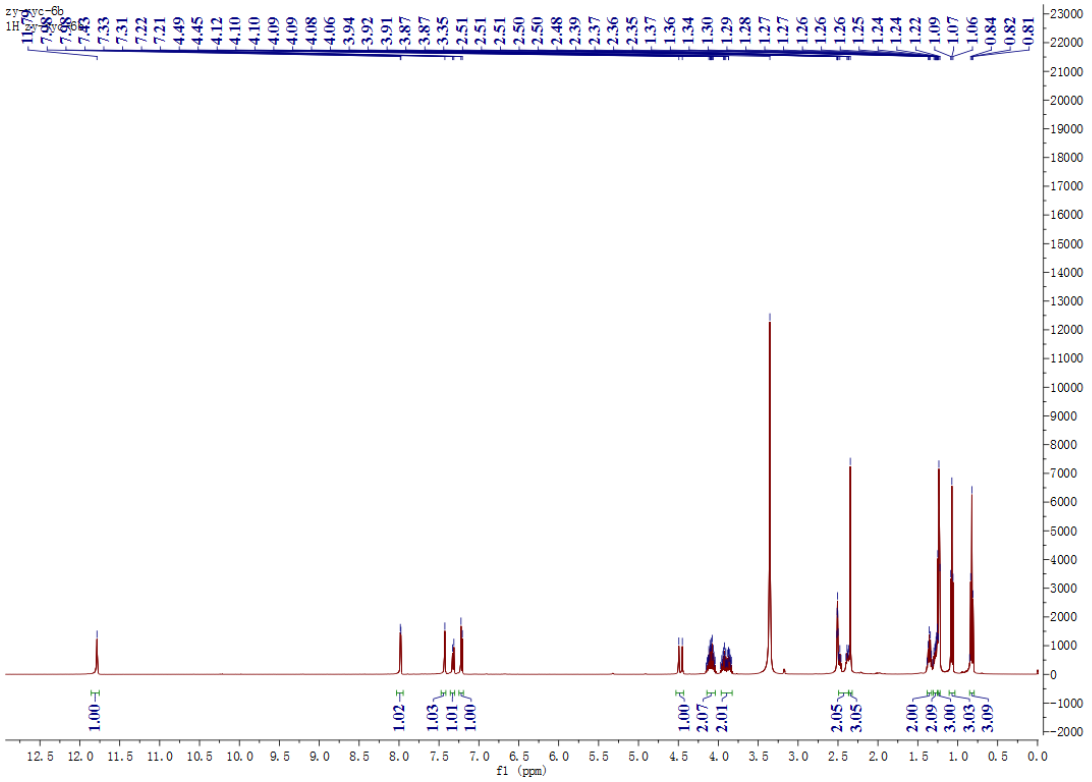


Fig. 30.^{1}H NMR of compound 4b$_1$

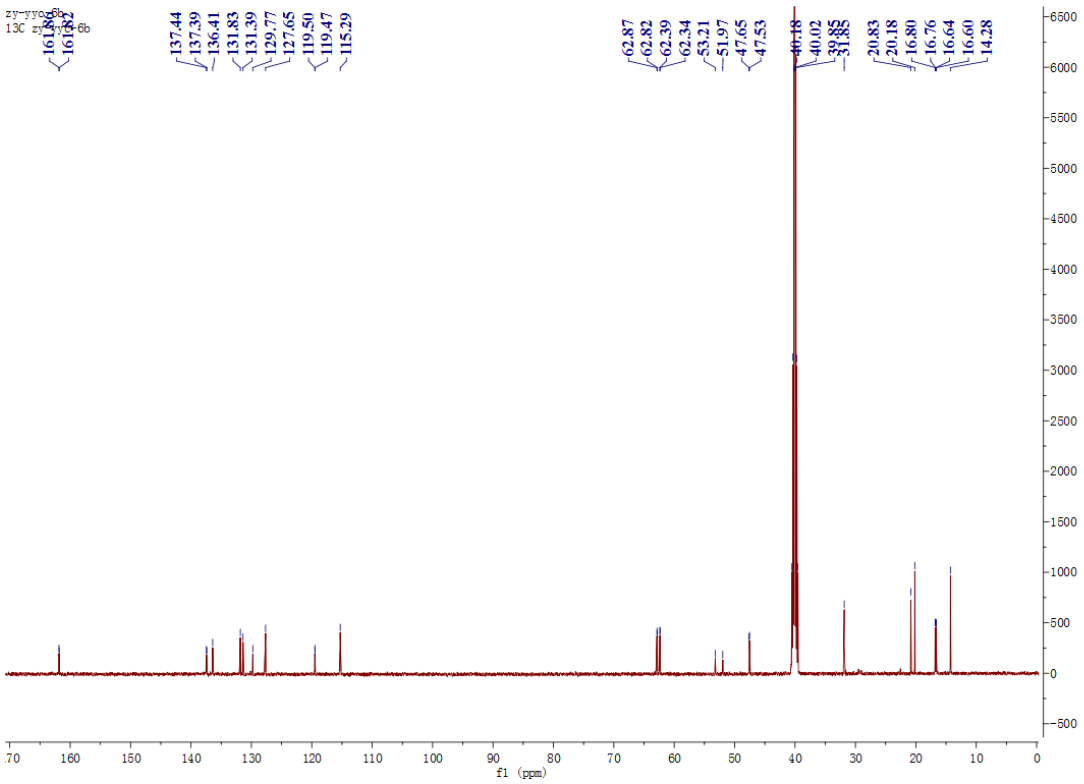


Fig. 31.^{13}C NMR of compound 4b$_1$

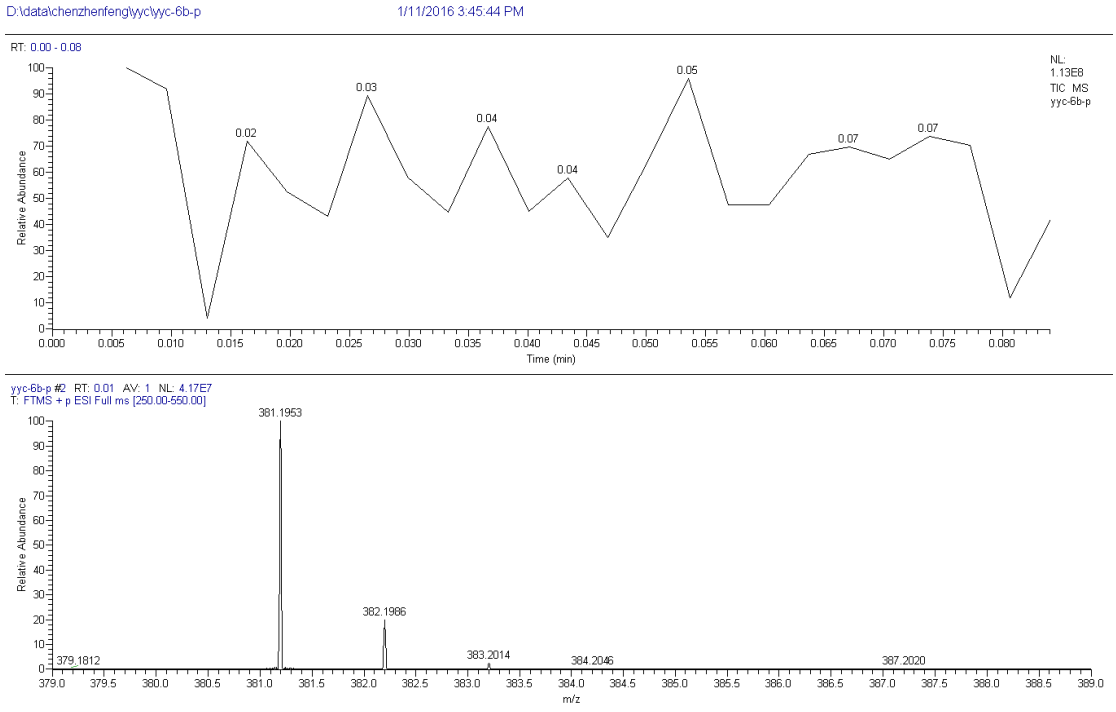


Fig. 32.ESI-HRMS of compound 4b$_1$

4b$_2$: Yield75.87%, ^{1}H NMR (400 MHz, DMSO) δ 11.88 (s, 1H), 8.01 (d, *J* = 3.6 Hz, 1H), 7.38 – 7.27 (m, 2H), 7.21 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.61 (d, *J* = 8.3 Hz, 2H), 6.12 (dd, *J* = 10.4, 6.2 Hz, 1H), 5.26 (dd, *J* = 24.5, 10.5 Hz, 1H), 4.11 (p, *J* = 7.2 Hz, 2H), 4.02 – 3.82 (m, 2H), 2.31 (s, 3H), 2.08 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 161.58, 161.53, 145.14, 144.99, 137.44, 137.37, 136.46, 132.05, 131.60, 129.99, 129.76, 127.57, 126.27, 119.35, 119.32, 115.39, 113.87, 63.20, 63.13, 62.93, 62.86, 48.37, 46.82, 40.59, 40.38, 40.17, 39.96, 39.75, 39.54, 39.34, 20.78, 20.46, 16.79, 16.74, 16.60, 16.54.ESI-HRMS *m/z* Calc for $C_{22}H_{27}N_2O_4P$ $[M+Na]^+$:437.1606; found: 437.1616.

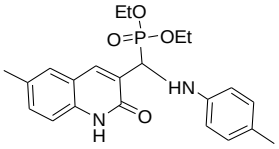


Fig. 33. Chemical structure of compound 4b$_2$

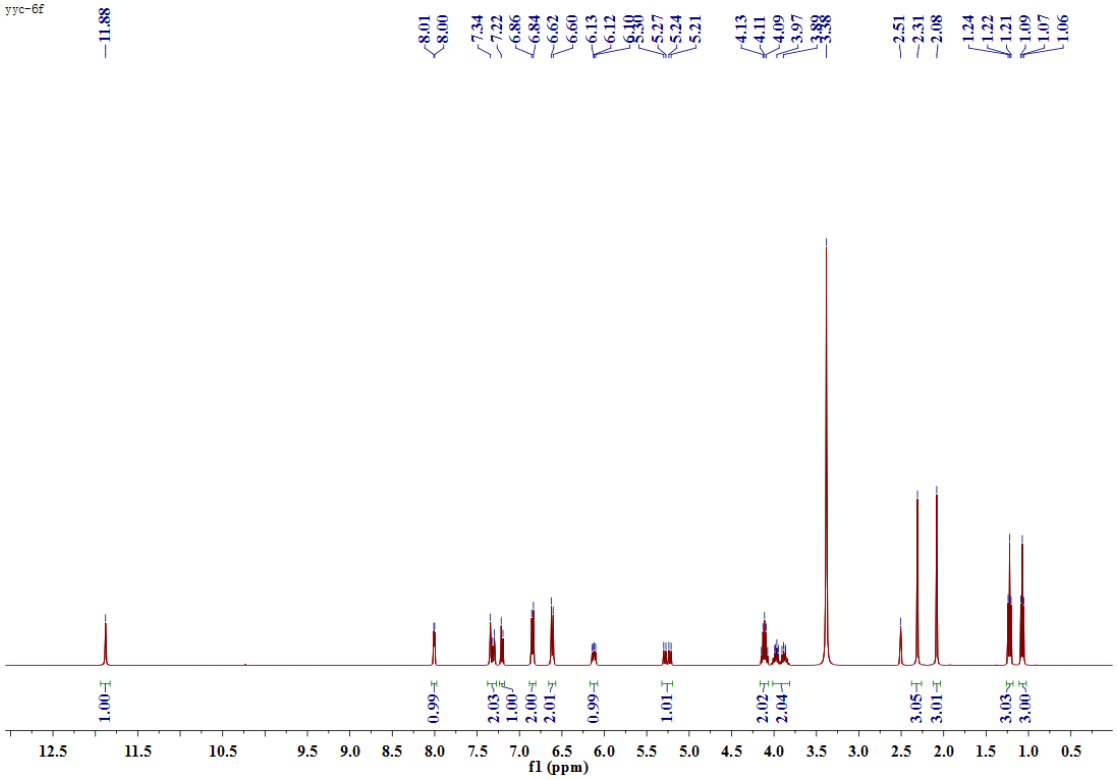


Fig. 34.^{1}H NMR of compound 4b$_2$

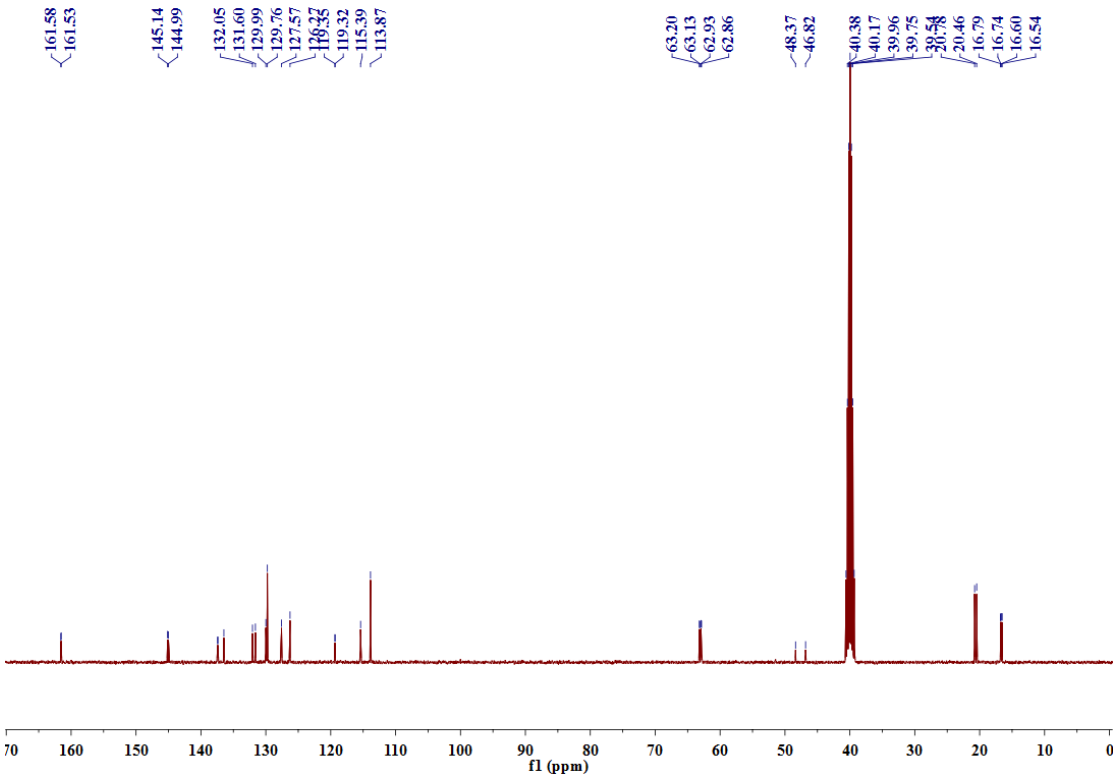


Fig. 35.^{13}C NMR of compound 4b$_2$

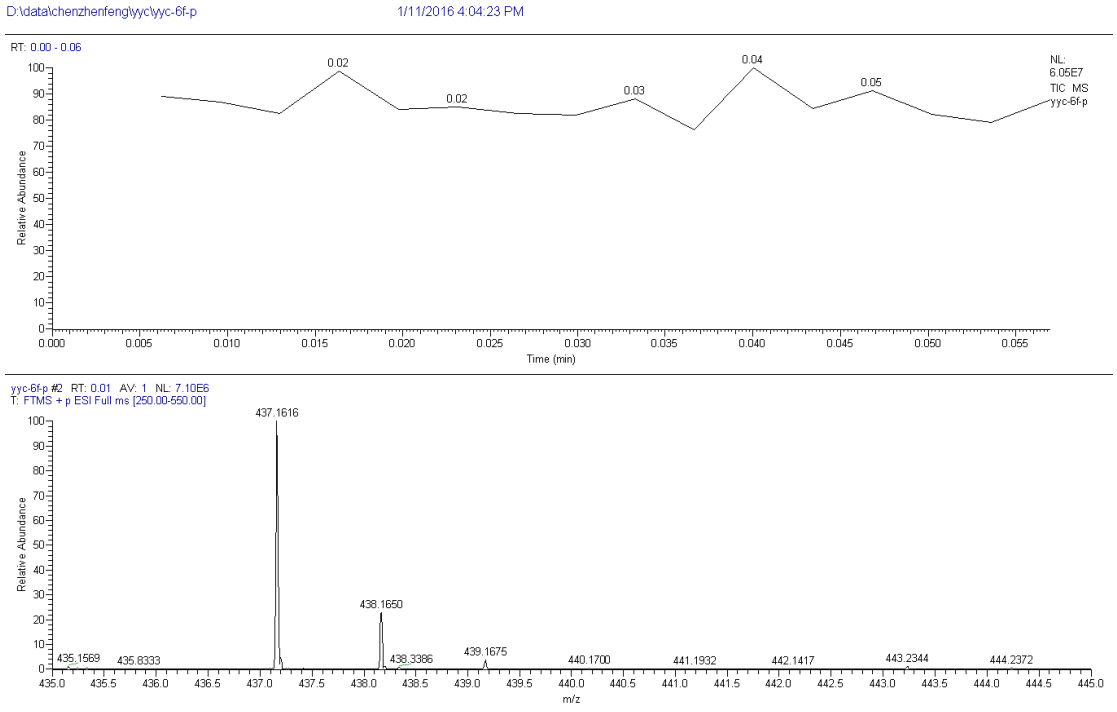


Fig. 36.ESI-HRMS of compound 4b$_2$

$4b_3$: Yield71.80%, ^{1}H NMR (500 MHz, DMSO) δ 11.89 (s, 1H), 8.02 (d, *J* = 3.7 Hz, 1H), 7.36 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.92 (t, *J* = 7.8 Hz, 1H), 6.56 (s, 1H), 6.49 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.39 (d, *J* = 7.4 Hz, 1H), 6.21 (dd, *J* = 10.3, 6.2 Hz, 1H), 5.29 (dd, *J* = 24.4, 10.3 Hz, 1H), 4.12 – 3.86 (m, 4H), 2.31 (s, 3H), 2.13 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 1H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.55, 161.51, 147.46, 147.35, 138.32, 137.49, 137.44, 136.47, 132.06, 131.62, 130.00, 129.20, 127.60, 119.36, 119.34, 118.66, 115.41, 114.48, 110.79, 63.19, 63.14, 62.94, 62.89, 48.00, 46.76, 40.49, 40.42, 40.32, 40.25, 40.16, 39.99, 39.82, 39.66, 39.49, 21.77, 20.77, 16.77, 16.73, 16.59, 16.54.ESI-HRMS *m/z* Calc for $C_{22}H_{27}N_2O_4P$ $[M-H]^-$:413.1681; found: 413.1681.

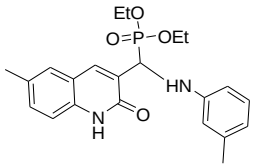


Fig. 37. Chemical structure of compound $4b_3$

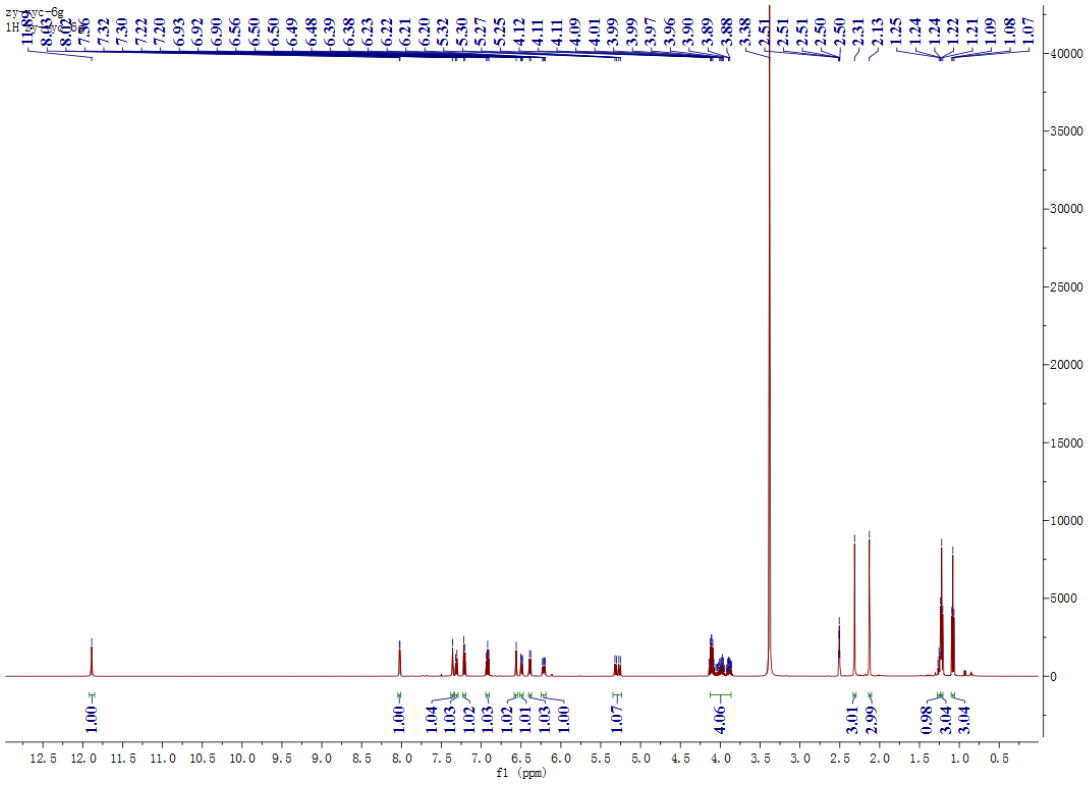


Fig. 38.^{1}H NMR of compound $4b_3$

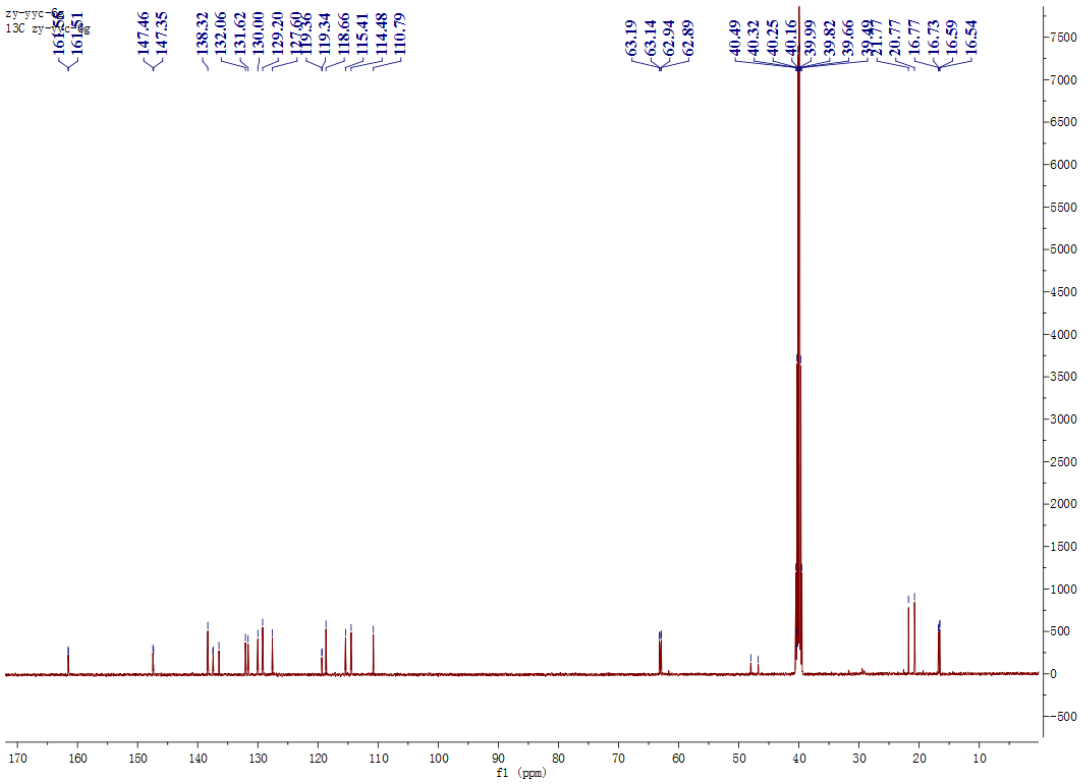


Fig. 39.^{13}C NMR of compound 4b$_3$

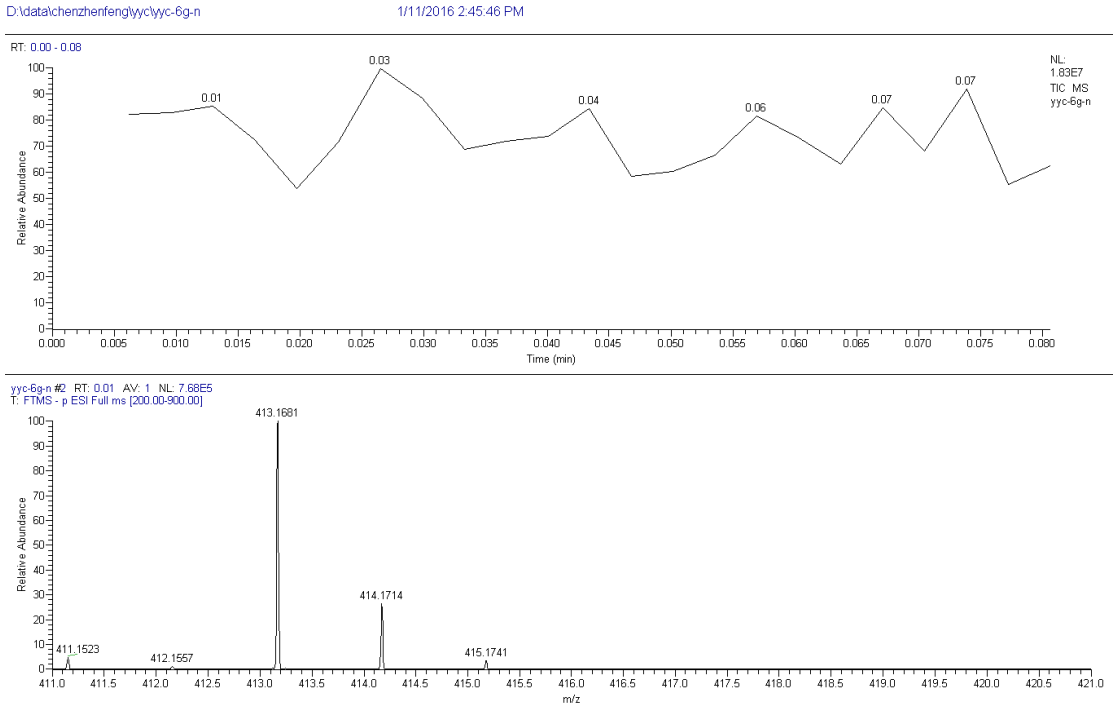


Fig. 40.ESI-HRMS of compound 4b$_2$

4b$_4$: Yield71.72%, ^{1}H NMR (500 MHz, DMSO) δ 11.89 (s, 1H), 8.03 (d, *J* = 3.7 Hz, 1H), 7.35 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 8.4, 7.4 Hz, 2H), 6.71 (d, *J* = 7.8 Hz, 2H), 6.56 (t, *J* = 7.3 Hz, 1H), 6.32 (dd, *J* = 10.2, 6.3 Hz, 1H), 5.30 (dd, *J* = 24.4, 10.2 Hz, 1H), 4.13 – 3.86 (m, 4H), 2.31 (s, 3H), 1.27 – 1.24 (m, 1H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.56, 161.52, 147.45, 147.34, 137.53, 137.48, 136.47, 132.10, 131.65, 129.85, 129.34, 127.61, 119.35, 119.32, 117.76, 115.43, 113.67, 63.23, 63.18, 62.99, 62.93, 47.98, 46.73, 40.46, 40.38, 40.29, 40.21, 40.12, 40.05, 39.96, 39.79, 39.62, 39.46, 20.76, 16.76, 16.72, 16.57, 16.53.ESI-HRMS *m/z* Calc for $C_{21}H_{25}N_2O_4P$ $[M+Na]^+$:423.1450; found: 423.1451.

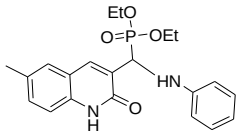


Fig. 41. Chemical structure of compound 4b$_4$

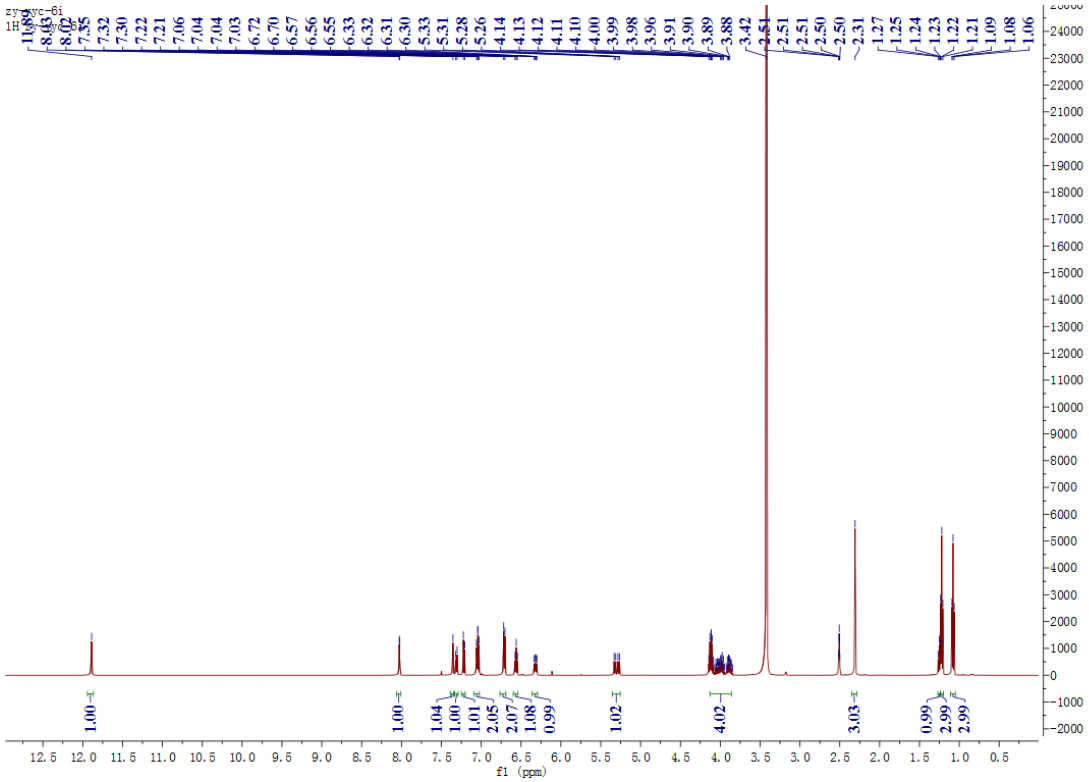


Fig. 42.^{1}H NMR of compound 4b$_4$

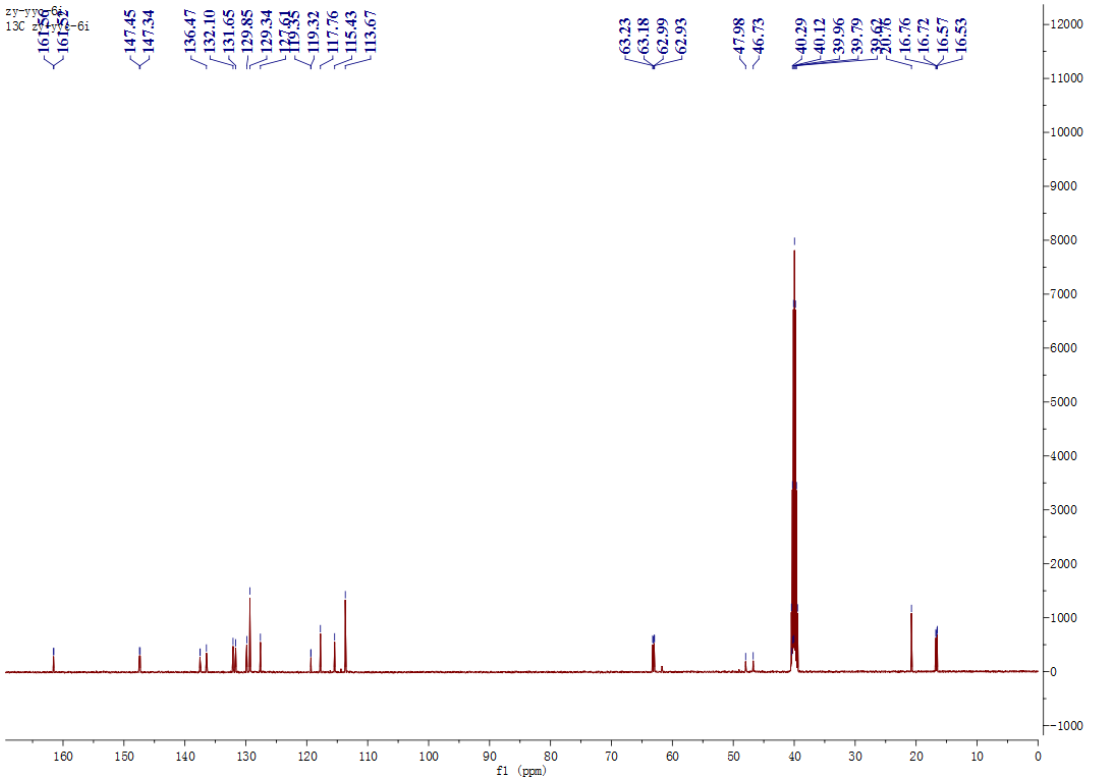


Fig. 43.^{13}C NMR of compound 4b$_4$

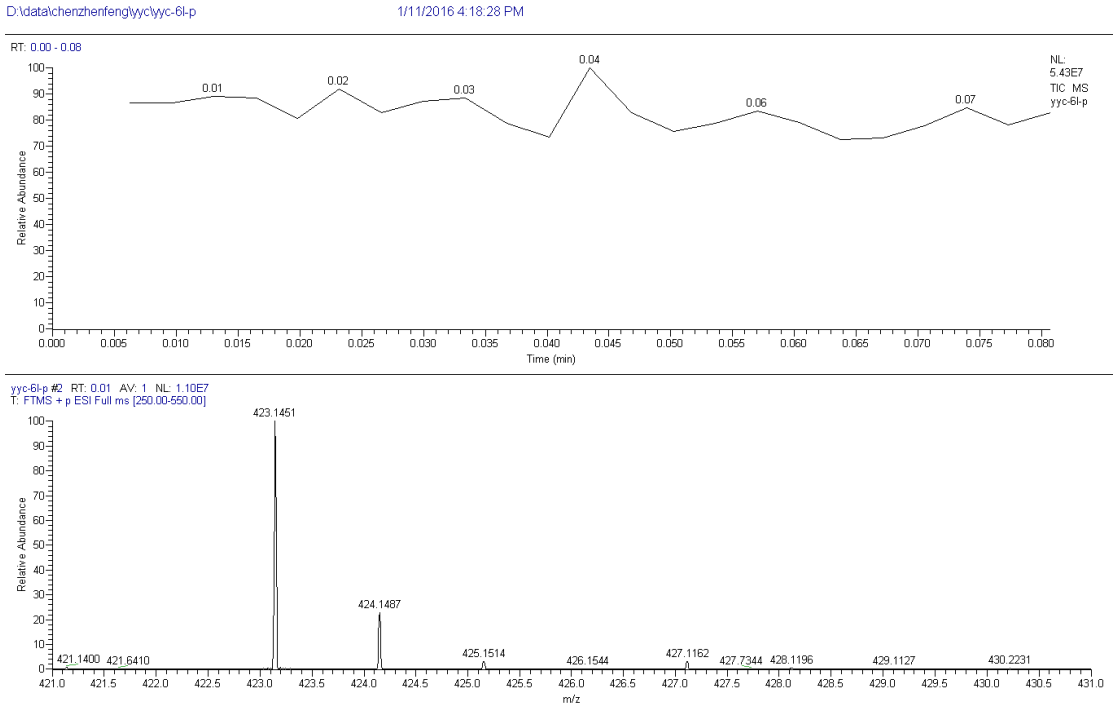
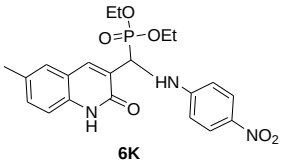


Fig. 44.ESI-HRMS of compound 4b$_4$

4b$_5$: Yield76.32%, ^{1}H NMR (400 MHz, DMSO) δ 12.05 (s, 1H), 8.06 (d, *J* = 3.4 Hz, 1H), 8.02 (d, *J* = 9.3 Hz, 2H), 7.96 (dd, *J* = 9.3, 5.6 Hz, 1H), 7.43 (s, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 9.3 Hz, 2H), 5.47 (dd, *J* = 22.6, 9.4 Hz, 1H), 4.16 – 3.90 (m, 4H), 2.34 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.27, 161.21, 153.72, 153.61, 138.23, 138.17, 137.63, 136.63, 132.53, 131.85, 128.47, 127.81, 126.43, 119.14, 119.12, 115.55, 112.42, 63.42, 63.35, 63.29, 47.73, 46.18, 40.60, 40.39, 40.18, 39.97, 39.76, 39.56, 39.35, 20.77, 16.78, 16.73, 16.59, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{24}N_3O_6P$ $[M-H]^-$:444.1344; found: 444.1357.



6K

Fig. 45. Chemical structure of compound 4b$_5$

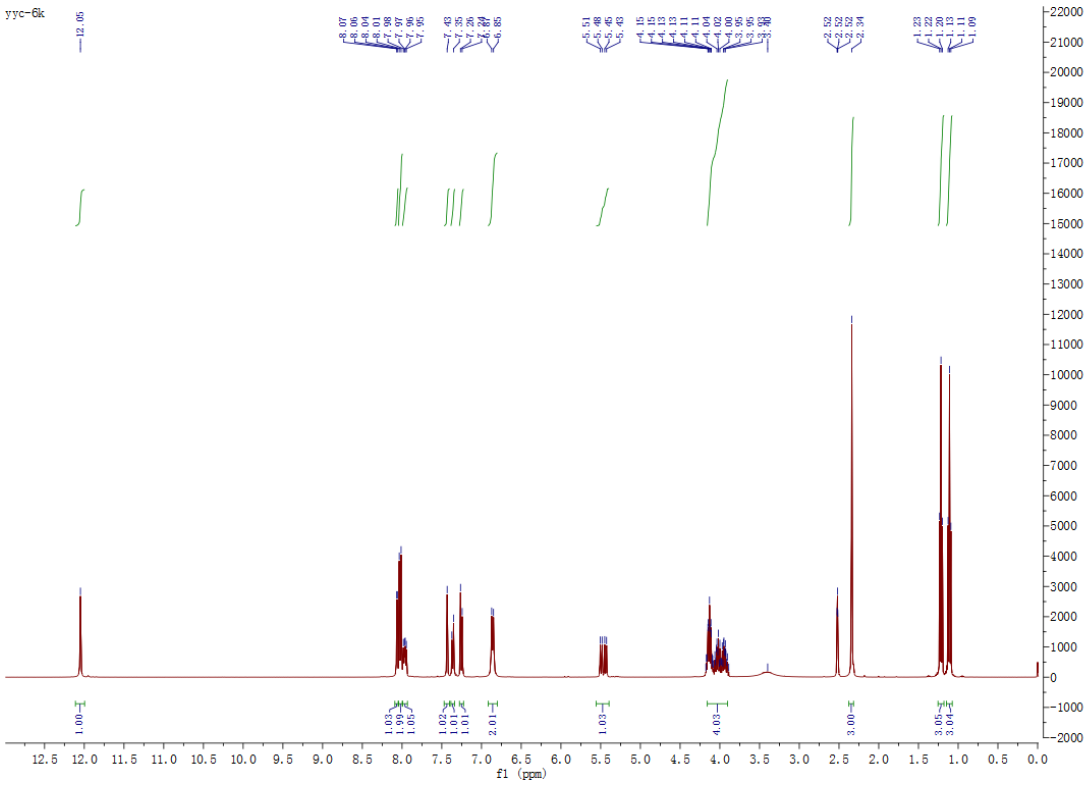


Fig. 42.^{1}H NMR of compound 4b$_5$

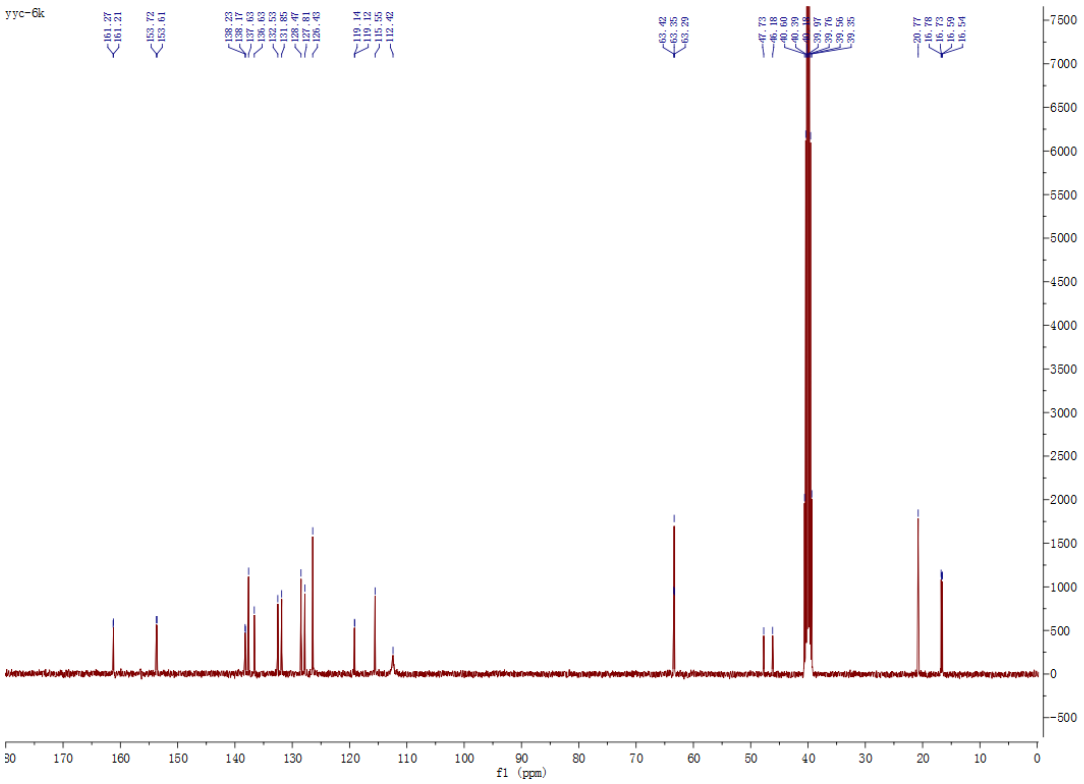


Fig. 43.^{13}C NMR of compound 4b$_5$

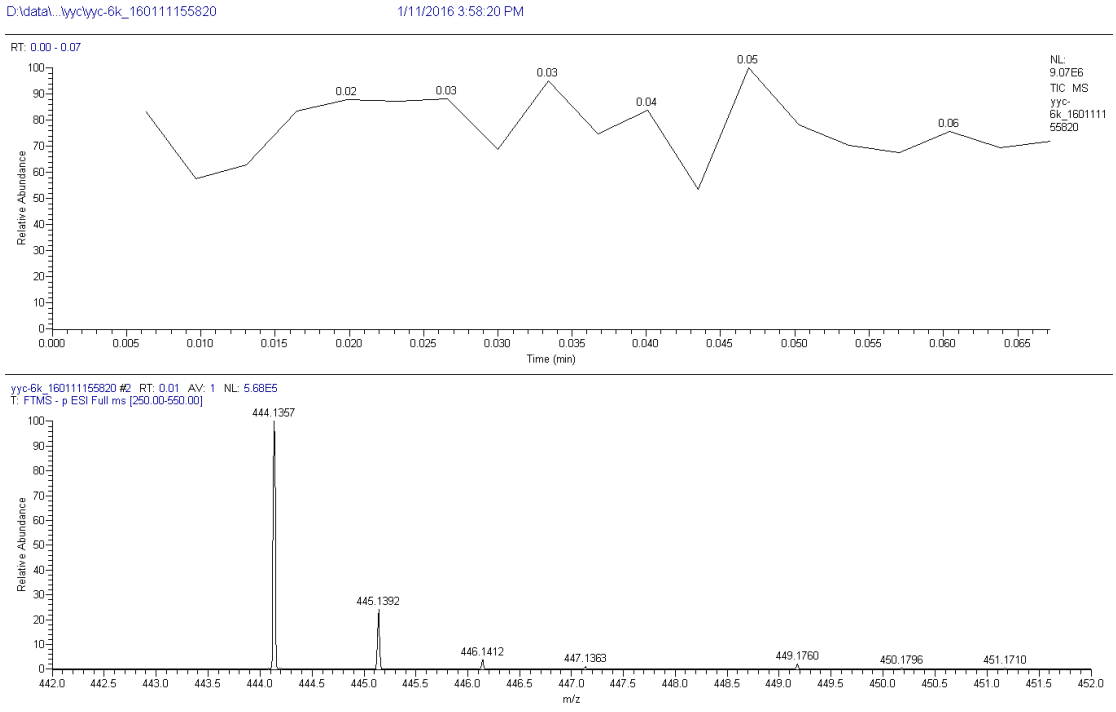


Fig. 44.ESI-HRMS of compound 4b$_5$

4b$_6$: Yield84.63%, ^{1}H NMR (400 MHz, DMSO) δ 11.98 (s, 1H), 8.03 (d, *J* = 3.5 Hz, 1H), 7.42 – 7.37 (m, 3H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.17 (dd, *J* = 9.7, 6.1 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.37 (dd, *J* = 23.7, 9.7 Hz, 1H), 4.16 – 3.88 (m, 4H), 2.33 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.10 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.42, 161.37, 150.79, 150.66, 137.80, 137.74, 136.55, 132.32, 131.74, 129.16, 127.70, 126.88, 126.73, 126.69, 124.20, 119.23, 119.20, 117.58, 117.27, 116.95, 115.49, 113.00, 63.31, 63.25, 63.16, 63.09, 47.66, 46.11, 40.60, 40.39, 40.18, 39.98, 39.77, 39.56, 39.35, 20.77, 16.77, 16.72, 16.59, 16.53.ESI-HRMS *m/z* Calc for $C_{21}H_{24}FN_2O_4P$ $[M+Na]^+$:441.1355; found: 441.1368.

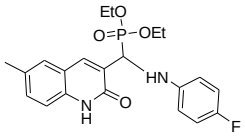


Fig. 45. Chemical structure of compound 4b$_6$

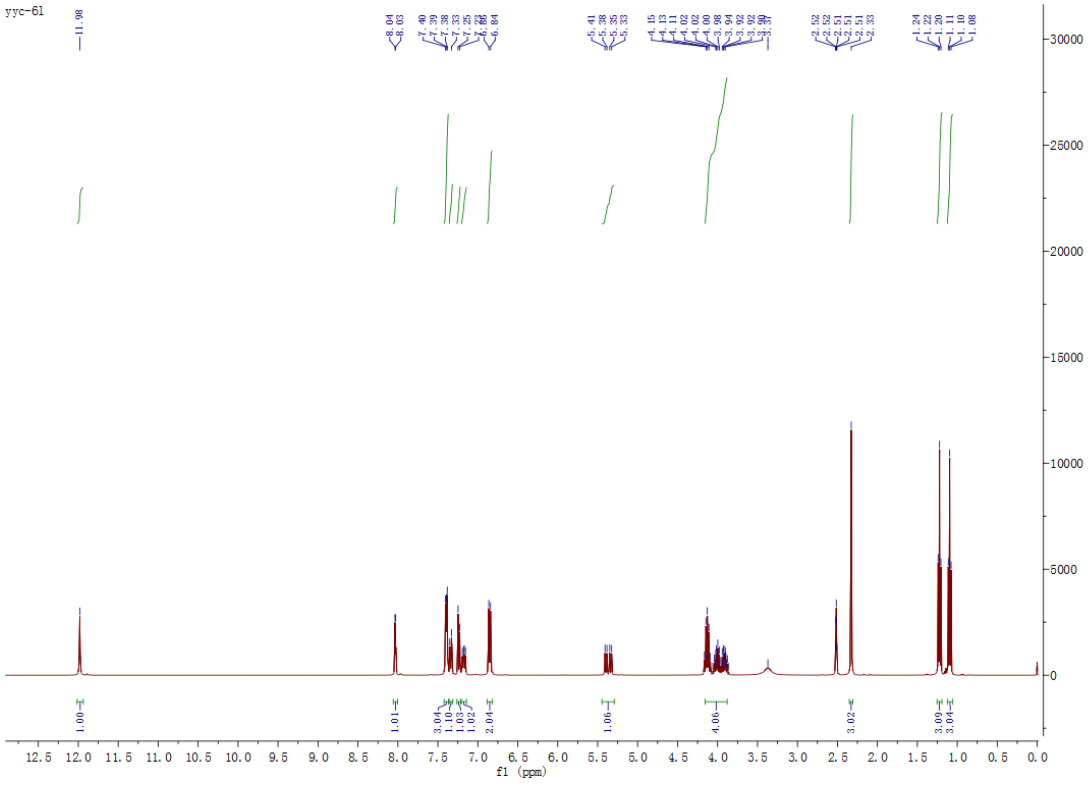


Fig. 46.^{1}H NMR of compound 4b$_6$

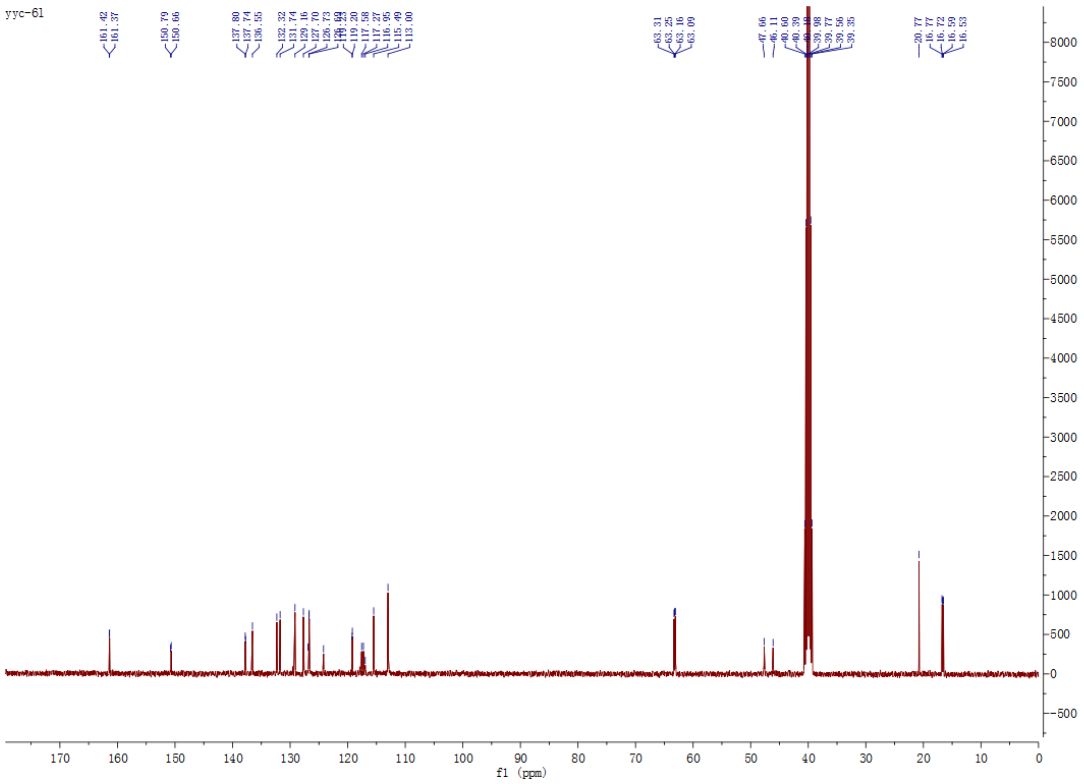


Fig. 47.^{13}C NMR of compound 4b$_6$

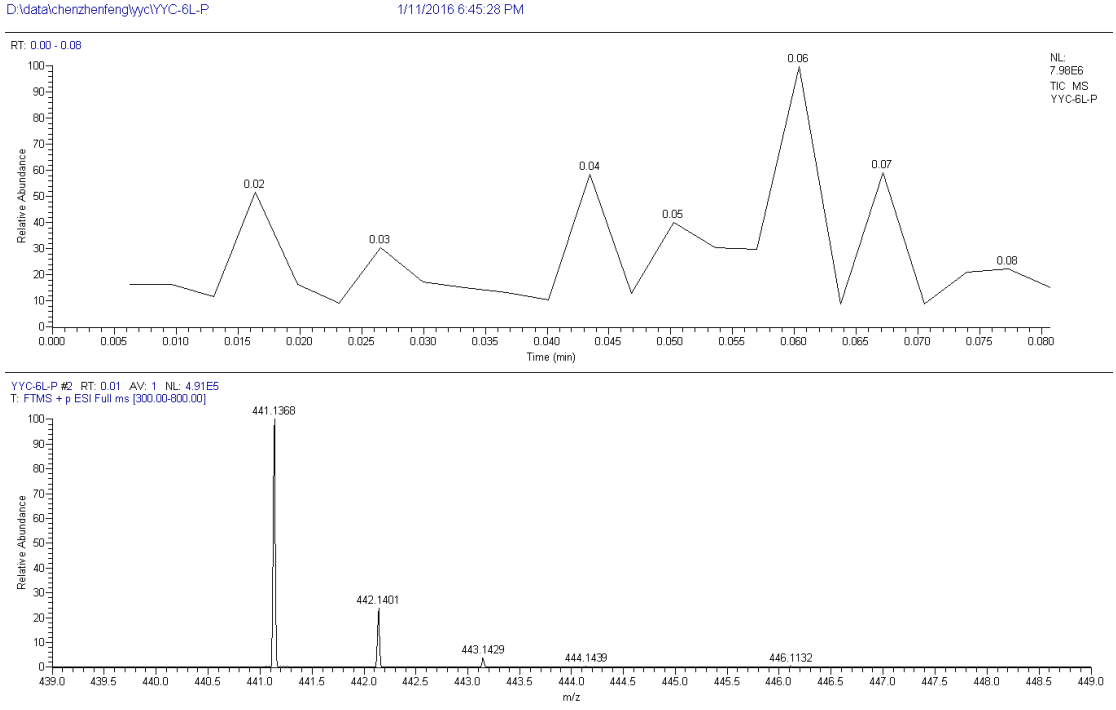


Fig. 48.ESI-HRMS of compound 4b$_6$

4b$_7$: Yield59.59%, ^{1}H NMR (500 MHz, DMSO) δ 11.76 (s, 1H), 8.00 (d, *J* = 3.6 Hz, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.18 (d, *J* = 2.5 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.6 Hz, 1H), 4.48 (d, *J* = 21.8 Hz, 1H), 4.13 – 4.05 (m, 2H), 3.94 – 3.85 (m, 2H), 3.79 (s, 3H), 2.49 – 2.31 (m, 2H), 1.35 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.30 – 1.22 (m, 8H), 1.08 (t, *J* = 7.0 Hz, 3H), 0.83 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.52, 161.47, 154.73, 137.33, 137.28, 132.91, 130.20, 120.11, 119.90, 116.69, 109.52, 62.91, 62.85, 62.44, 62.38, 55.93, 53.21, 51.97, 47.66, 47.54, 40.48, 40.41, 40.31, 40.24, 40.15, 39.98, 39.81, 39.65, 39.48, 31.85, 20.17, 16.79, 16.75, 16.64, 16.60, 14.27, 8.41.ESI-HRMS *m/z* Calc for $C_{22}H_{24}F_3N_2O_4P$ $[M+Na]^+$:491.1323; found: 491.1328.

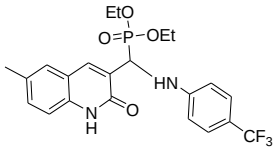


Fig. 49. Chemical structure of compound 4b$_7$

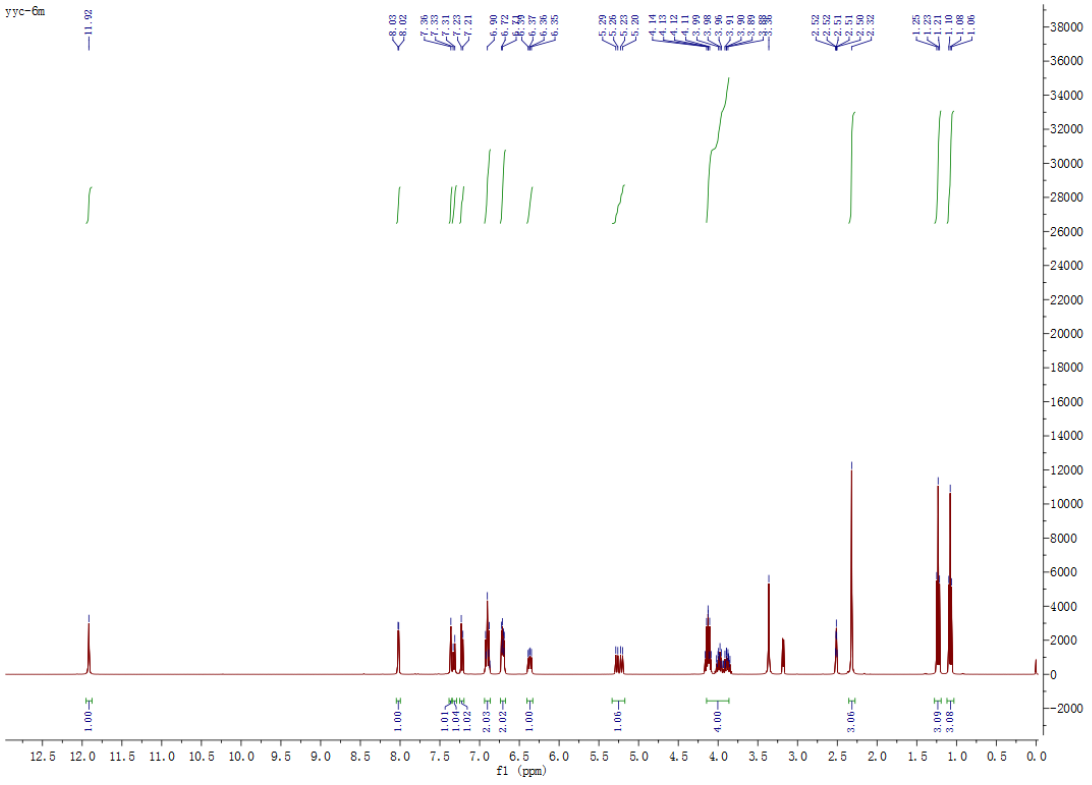


Fig. 50.^{1}H NMR of compound 4b$_7$

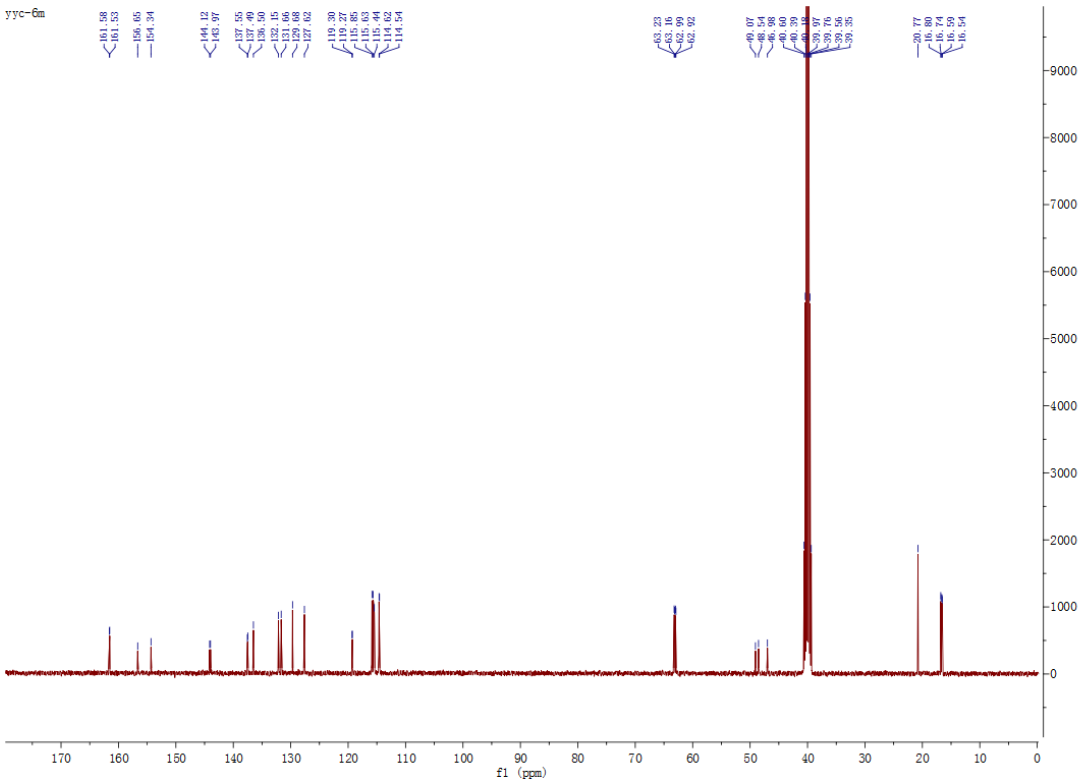


Fig. 51.^{13}C NMR of compound 4b$_7$

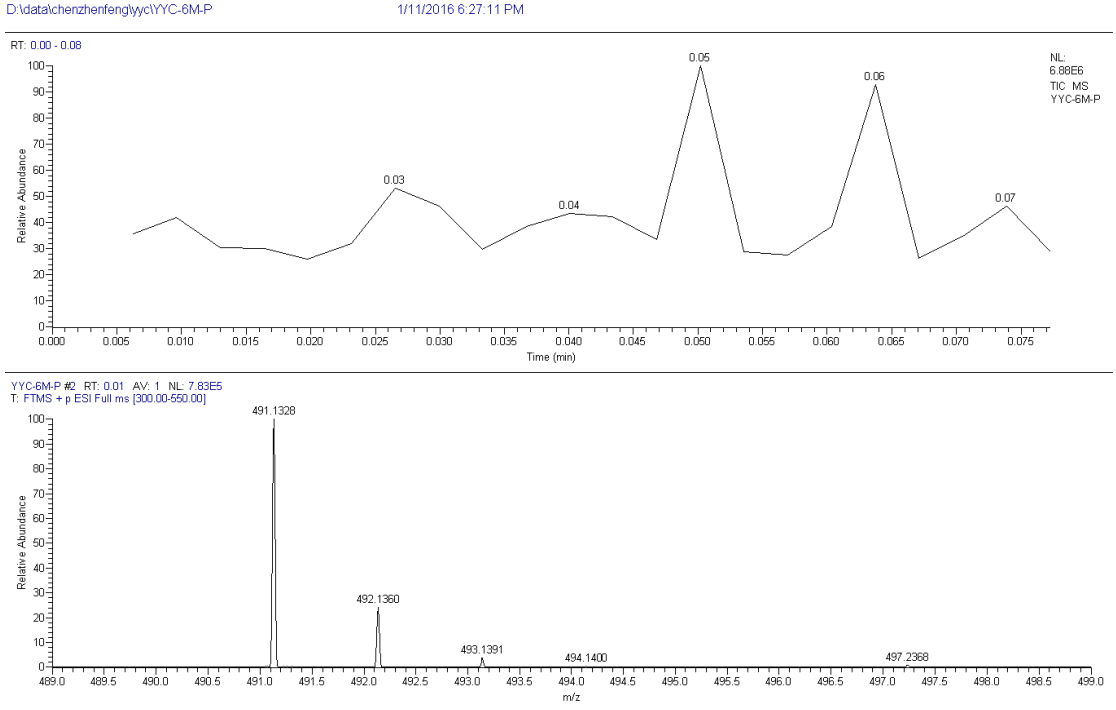


Fig. 52.ESI-HRMS of compound 4b$_7$

4c$_1$: Yield59.59%, ^{1}H NMR (500 MHz, DMSO) δ 11.76 (s, 1H), 8.00 (d, *J* = 3.6 Hz, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.18 (d, *J* = 2.5 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.6 Hz, 1H), 4.48 (d, *J* = 21.8 Hz, 1H), 4.13 – 4.05 (m, 2H), 3.94 – 3.85 (m, 2H), 3.79 (s, 3H), 2.49 – 2.31 (m, 2H), 1.35 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.30 – 1.22 (m, 8H), 1.08 (t, *J* = 7.0 Hz, 3H), 0.83 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.52, 161.47, 154.73, 137.33, 137.28, 132.91, 130.20, 120.11, 119.90, 116.69, 109.52, 62.91, 62.85, 62.44, 62.38, 55.93, 53.21, 51.97, 47.66, 47.54, 40.48, 40.41, 40.31, 40.24, 40.15, 39.98, 39.81, 39.65, 39.48, 31.85, 20.17, 16.79, 16.75, 16.64, 16.60, 14.27, 8.41.ESI-HRMS *m/z* Calc for $C_{19}H_{29}N_2O_5P$ $[M+H]^+$:397.1892; found: 397.1908.

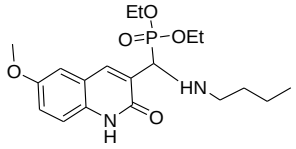


Fig. 53. Chemical structure of compound 4c$_1$

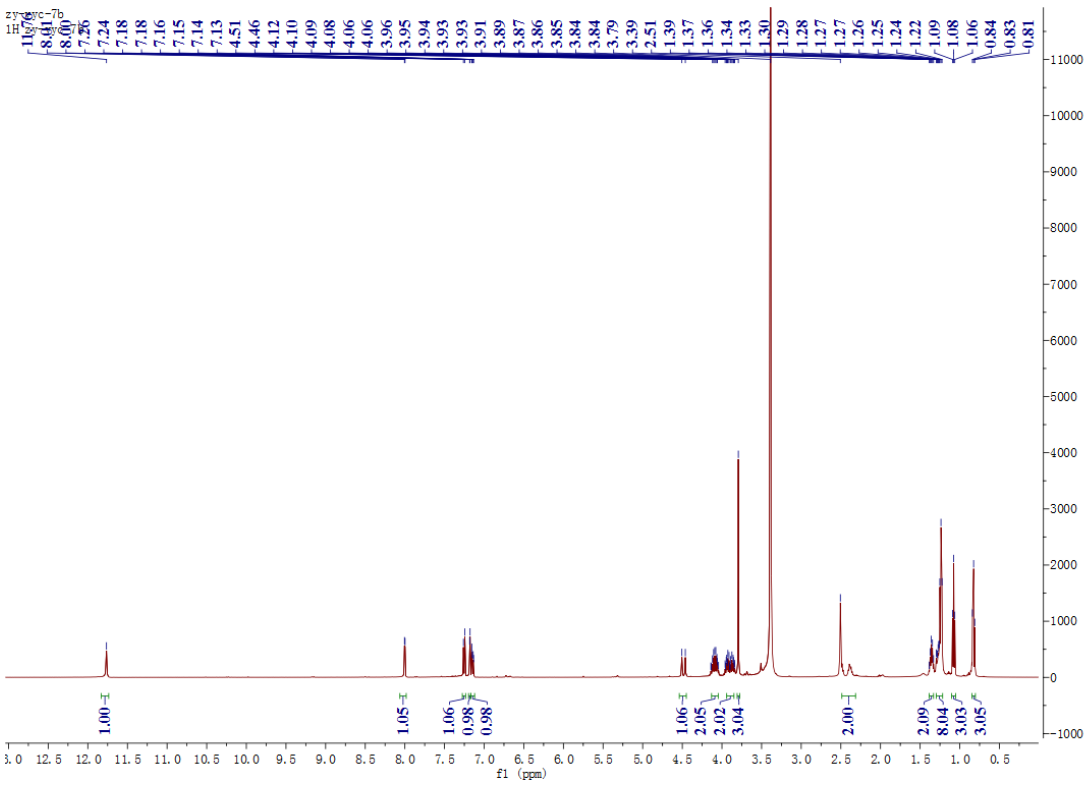


Fig. 54.^{1}H NMR of compound 4c$_1$

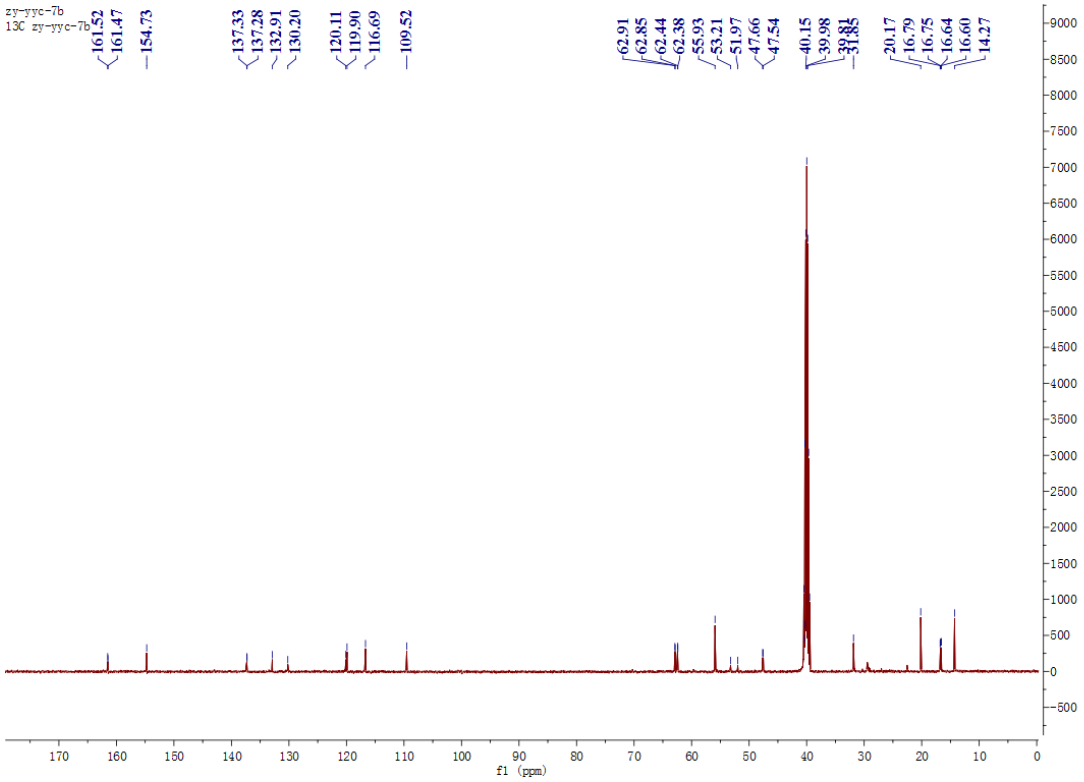


Fig. 55.^{13}C NMR of compound 4c$_1$

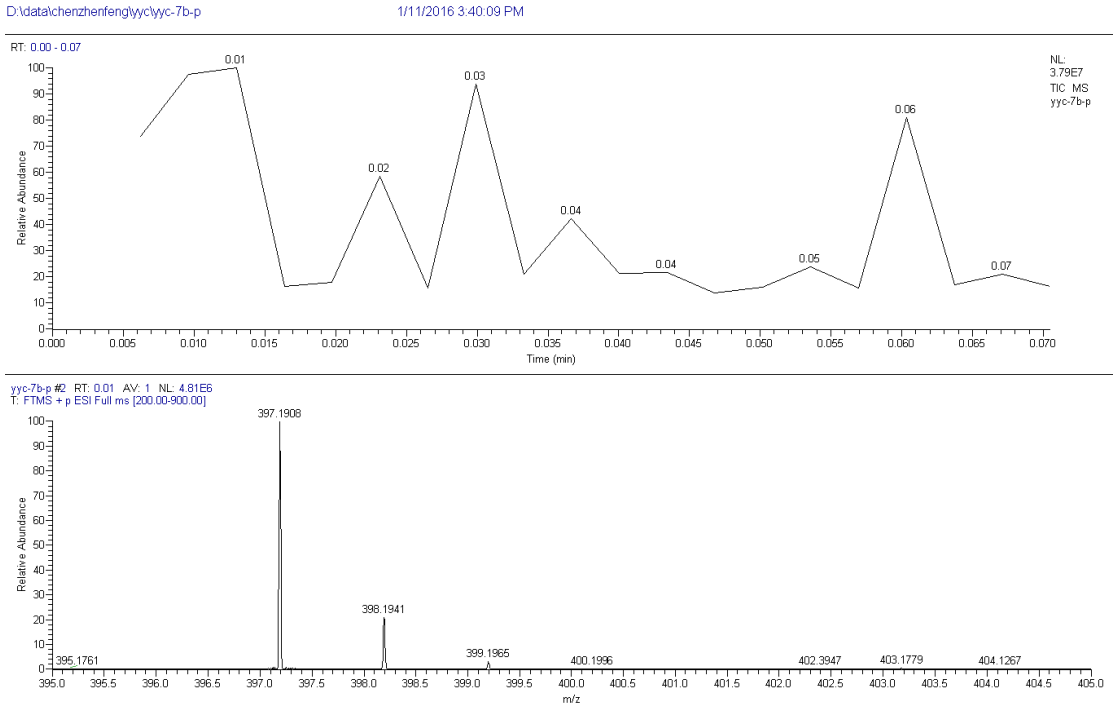


Fig. 56.ESI-HRMS of compound 4c$_1$

4c$_2$: Yield79.27%, ^{1}H NMR (500 MHz, DMSO) δ 11.86 (s, 1H), 8.02 (d, *J* = 3.7 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.13 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.07 (d, *J* = 2.7 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.61 (d, *J* = 8.5 Hz, 2H), 6.06 (dd, *J* = 10.1, 6.6 Hz, 1H), 5.26 (dd, *J* = 24.6, 10.2 Hz, 1H), 4.12 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.01 – 3.86 (m, 2H), 3.76 (s, 3H), 2.09 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.22, 161.18, 154.78, 145.06, 144.94, 137.19, 137.14, 132.97, 130.46, 129.77, 126.28, 120.16, 119.96, 119.94, 116.83, 113.81, 109.33, 63.19, 63.14, 62.95, 62.90, 55.90, 48.33, 47.09, 40.50, 40.42, 40.33, 40.26, 40.17, 40.00, 39.83, 39.67, 39.50, 20.45, 16.77, 16.73, 16.59, 16.55.ESI-HRMS *m/z* Calc for $C_{22}H_{27}N_2O_5P$ $[M+H]^+$:431.1736; found: 431.1742.

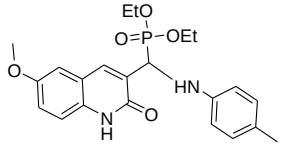


Fig. 57. Chemical structure of compound 4c$_2$

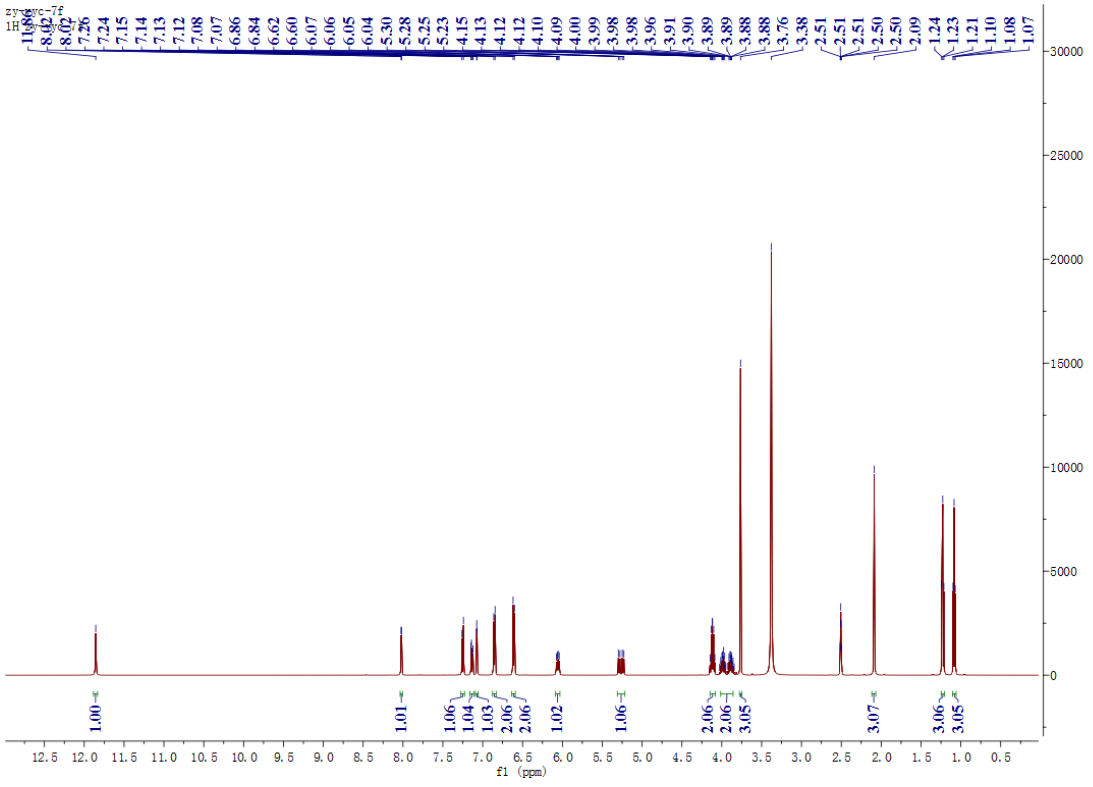


Fig. 58.^{1}H NMR of compound 4c$_2$

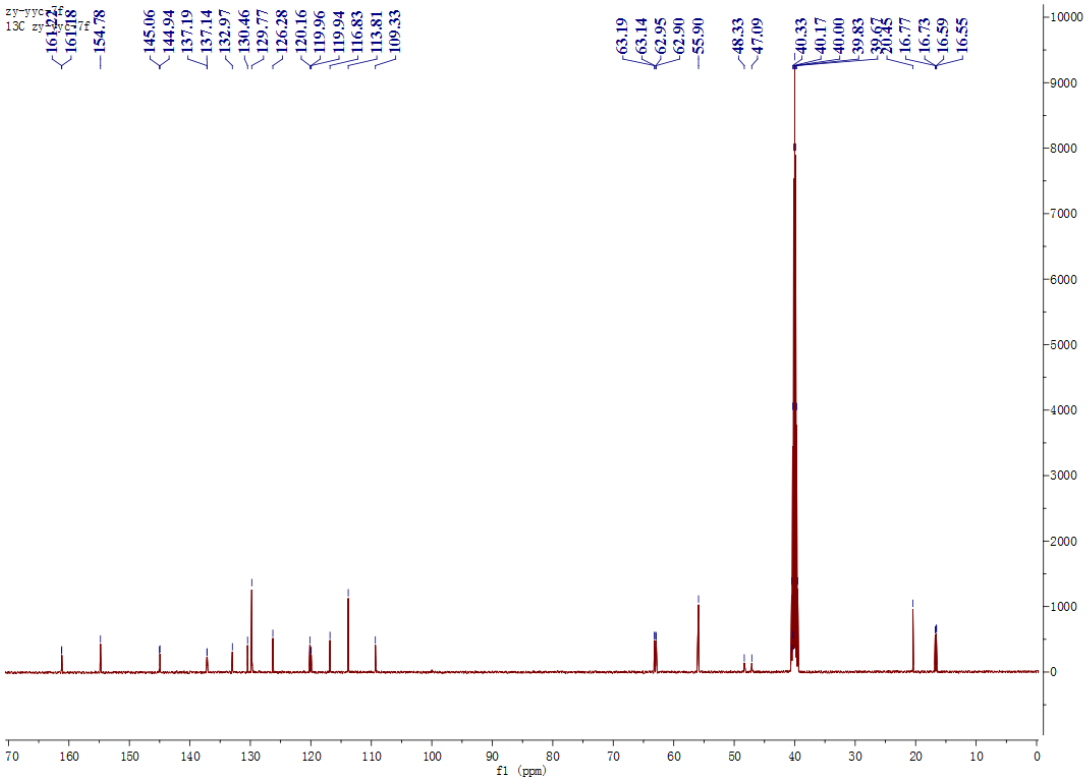


Fig. 59.^{13}C NMR of compound 4c$_2$

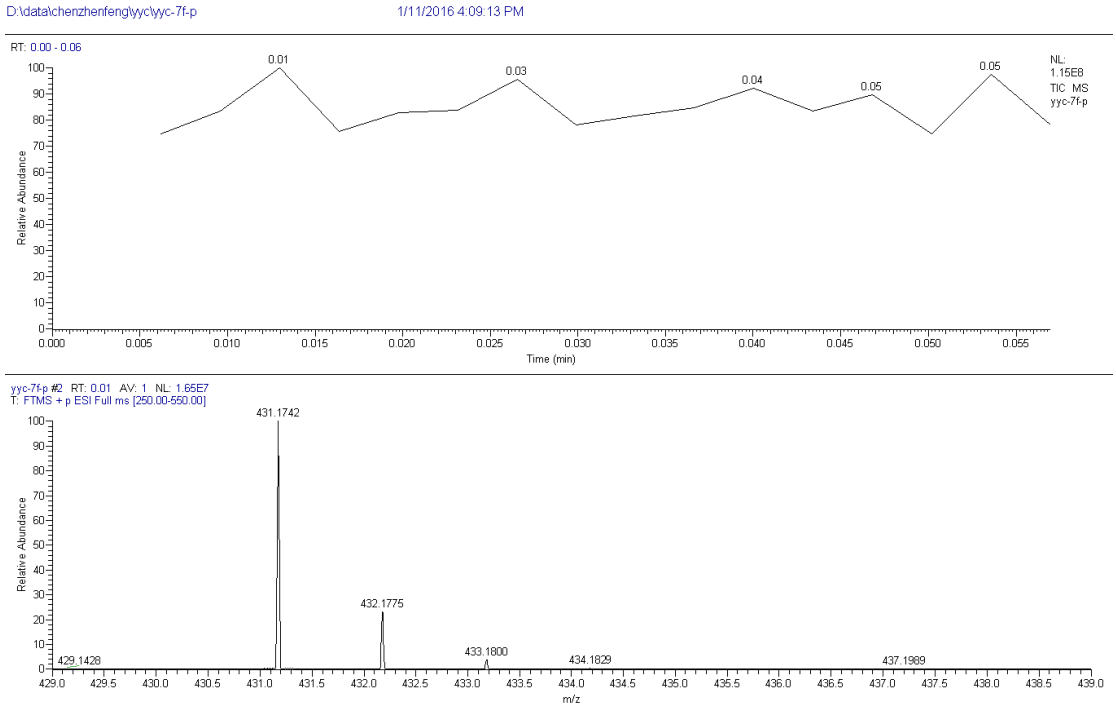


Fig. 60.ESI-HRMS of compound 4c$_2$

4c$_3$: Yield74.36%, ^{1}H NMR (500 MHz, DMSO) δ 11.87 (s, 1H), 8.04 (d, *J* = 3.6 Hz, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.09 (d, *J* = 2.7 Hz, 1H), 6.92 (t, *J* = 7.8 Hz, 1H), 6.56 (s, 1H), 6.48 (d, *J* = 8.1 Hz, 1H), 6.39 (d, *J* = 7.4 Hz, 1H), 6.17 (dd, *J* = 10.0, 6.5 Hz, 1H), 5.29 (dd, *J* = 24.5, 10.0 Hz, 1H), 4.11 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.01 – 3.87 (m, 2H), 3.77 (s, 3H), 2.13 (s, 3H), 1.24 (dt, *J* = 14.1, 7.0 Hz, 4H), 1.09 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.20, 161.16, 154.80, 147.39, 147.27, 138.35, 137.25, 137.20, 132.99, 130.46, 129.23, 120.20, 119.97, 118.67, 116.85, 114.44, 110.70, 109.36, 63.21, 63.16, 62.99, 62.93, 55.91, 48.03, 46.79, 40.48, 40.41, 40.32, 40.24, 40.15, 39.98, 39.82, 39.65, 39.48, 21.77, 16.77, 16.72, 16.59, 16.55.ESI-HRMS *m/z* Calc for $C_{22}H_{27}N_2O_5P$ $[M+Na]^+$:453.1555; found: 453.1574.

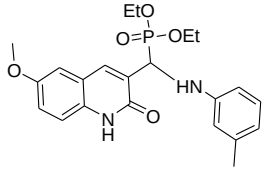


Fig. 61. Chemical structure of compound 4c$_3$

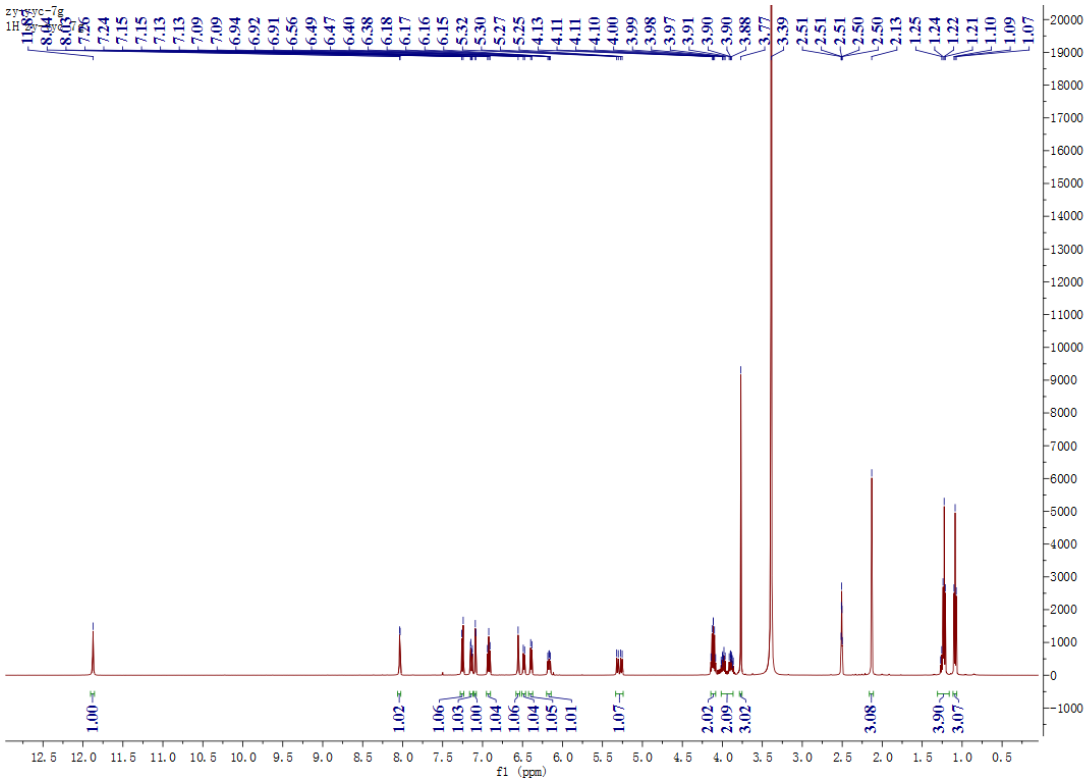


Fig. 62.^{1}H NMR of compound 4c$_3$

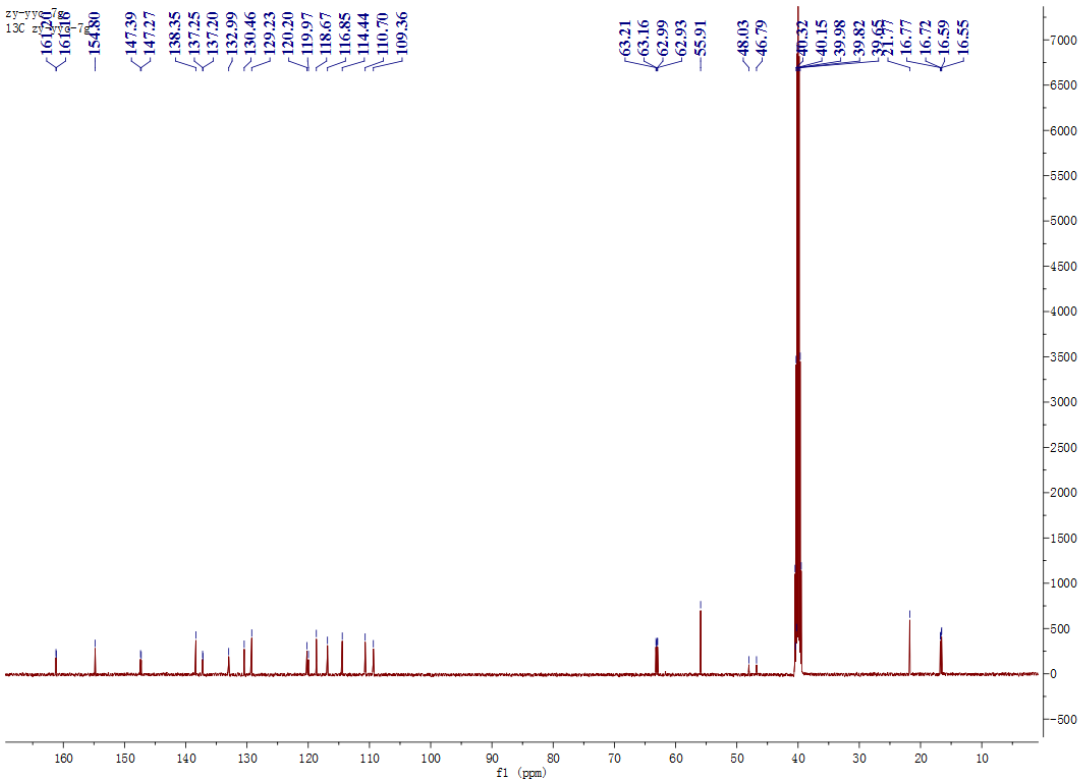


Fig. 63.^{13}C NMR of compound 4c$_3$

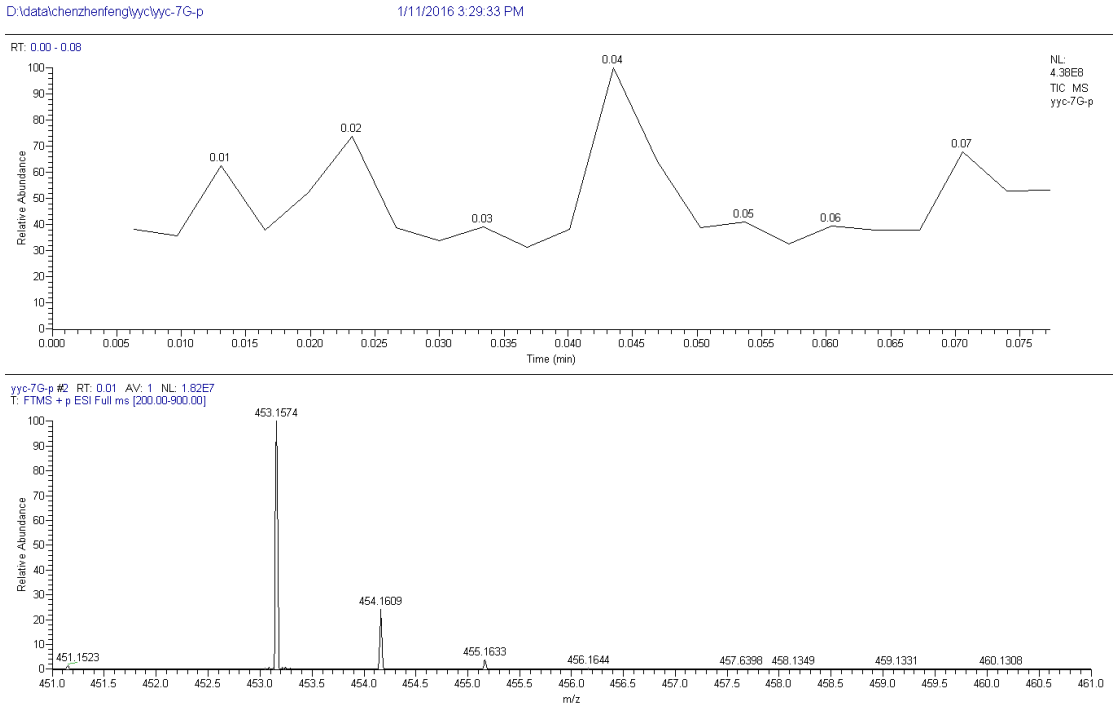
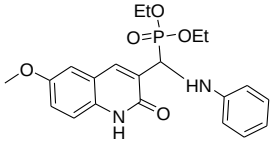


Fig. 64.ESI-HRMS of compound 4c$_3$

4c$_4$: Yield77%, ^{1}H NMR (500 MHz, DMSO) δ 11.88 (s, 1H), 8.04 (d, *J* = 3.6 Hz, 1H), 7.26 (d, *J* = 8.9 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.09 (d, *J* = 2.7 Hz, 1H), 7.05 (dd, *J* = 8.3, 7.5 Hz, 2H), 6.70 (d, *J* = 8.1 Hz, 2H), 6.57 (t, *J* = 7.3 Hz, 1H), 6.28 (dd, *J* = 9.9, 6.6 Hz, 1H), 5.30 (dd, *J* = 24.5, 10.0 Hz, 1H), 4.15 – 4.09 (m, 2H), 4.02 – 3.88 (m, 2H), 3.76 (s, 3H), 1.27 – 1.24 (m, 1H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.09 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 161.21, 161.17, 154.81, 147.38, 147.27, 137.31, 137.26, 132.97, 130.30, 129.36, 120.24, 119.96, 119.94, 117.78, 116.88, 113.62, 109.36, 63.26, 63.20, 63.04, 62.98, 55.90, 48.03, 46.79, 40.44, 40.27, 40.11, 39.94, 39.77, 39.60, 39.44, 16.75, 16.71, 16.58, 16.53.ESI-HRMS *m/z* Calc for $C_{21}H_{25}N_2O_5P$ $[M+Na]^+$:439.1399; found: 439.1403.



7l

Fig. 65. Chemical structure of compound 4c$_4$

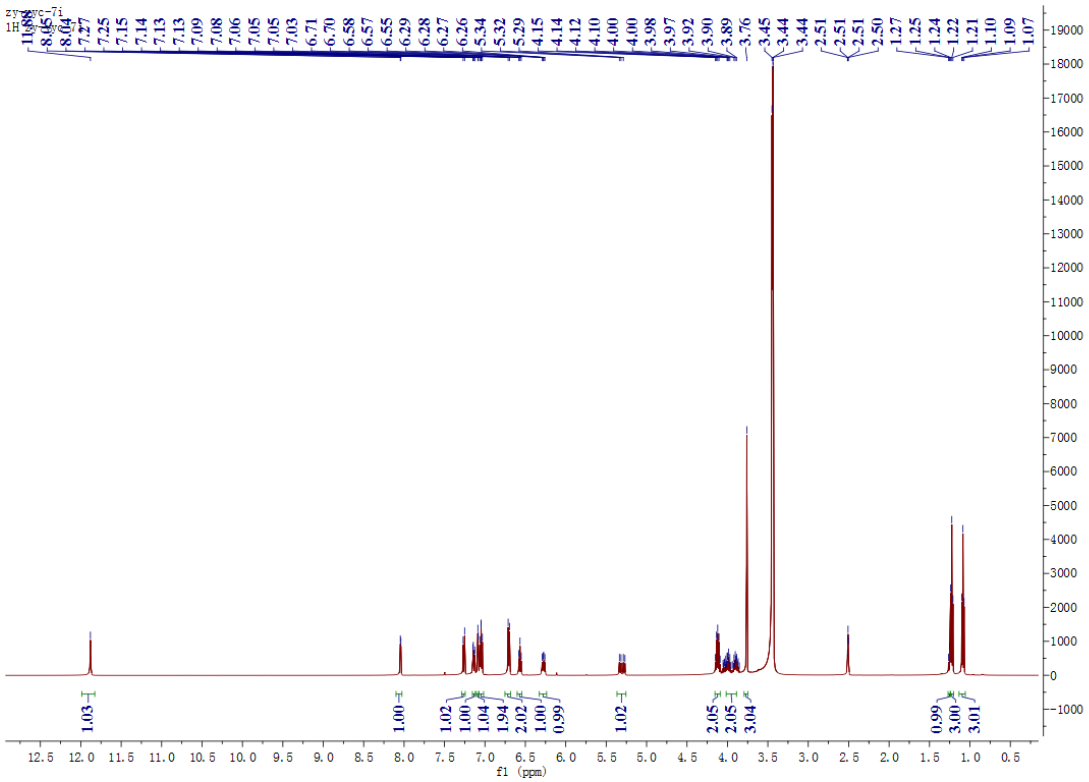


Fig. 66.^{1}H NMR of compound 4c$_4$

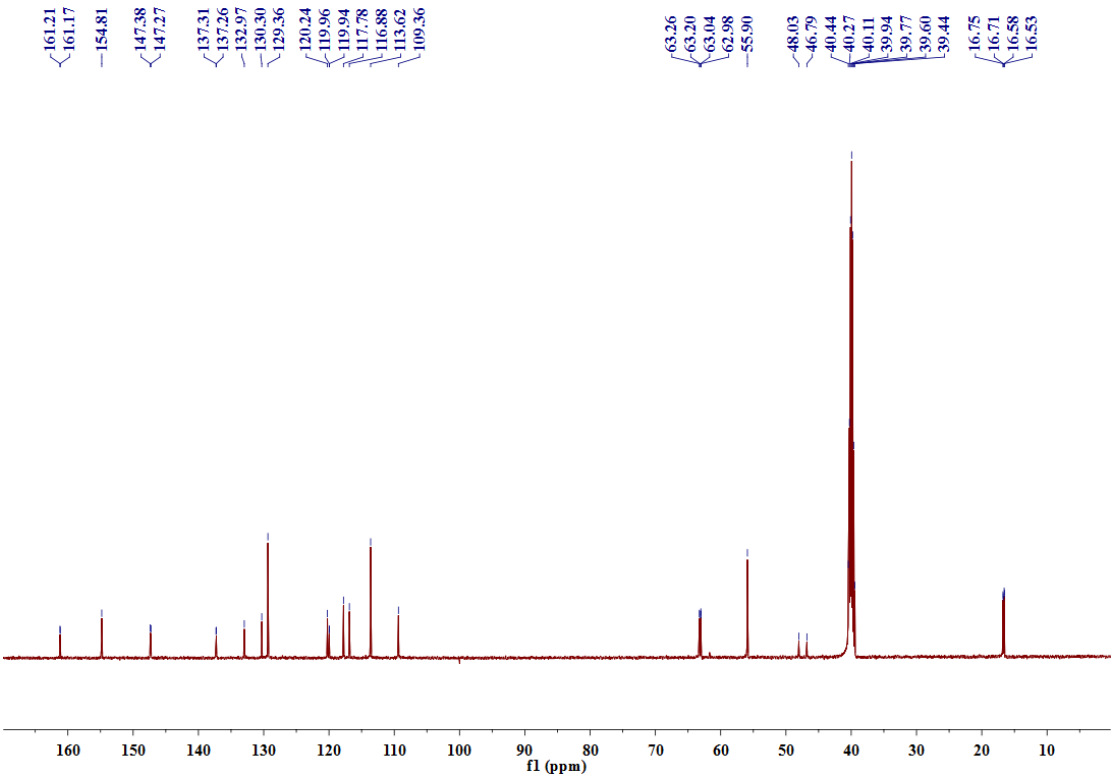


Fig. 67.^{13}C NMR of compound 4c$_4$

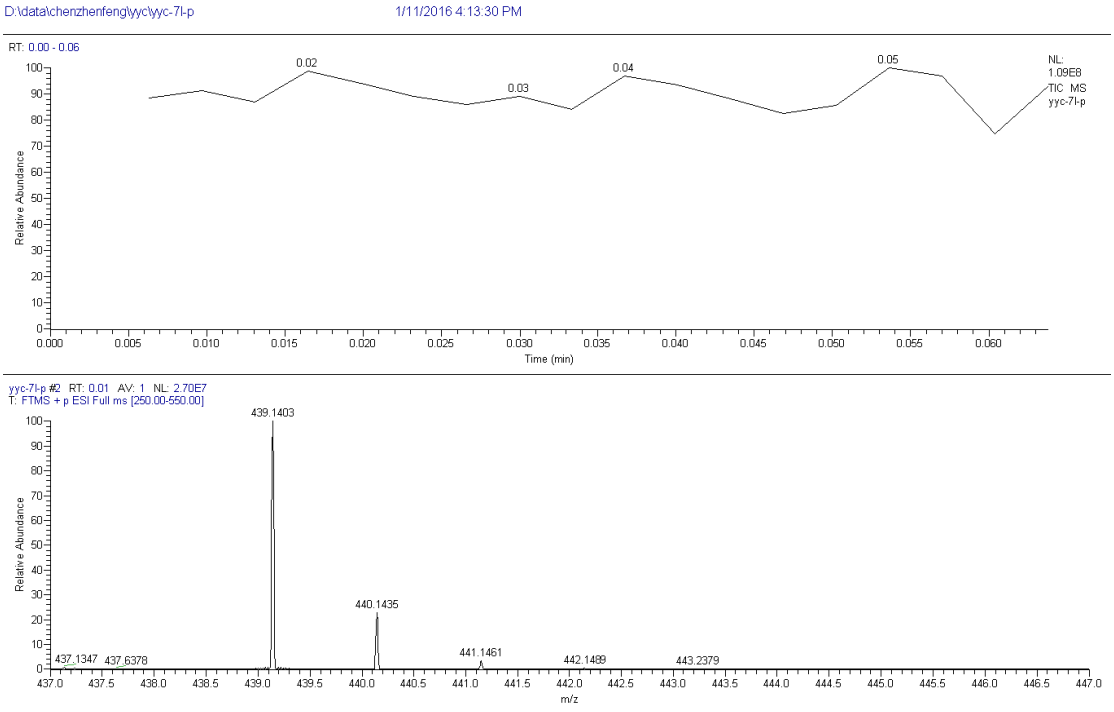


Fig. 68.ESI-HRMS of compound 4c$_4$

4c$_5$: Yield80.08%, ^{1}H NMR (400 MHz, DMSO) δ 12.03 (s, 1H), 8.07 (d, *J* = 3.4 Hz, 1H), 8.02 (d, *J* = 9.3 Hz, 2H), 7.94 (dd, *J* = 9.1, 6.1 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.18 (dt, *J* = 8.1, 2.6 Hz, 2H), 6.85 (d, *J* = 9.2 Hz, 2H), 5.46 (dd, *J* = 22.7, 9.1 Hz, 1H), 4.18 – 3.93 (m, 4H), 3.79 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 160.91, 160.85, 154.89, 153.69, 153.58, 137.98, 137.93, 137.63, 133.13, 128.91, 126.43, 120.71, 119.76, 119.73, 117.00, 109.47, 63.43, 63.38, 63.31, 55.95, 47.82, 46.27, 40.60, 40.39, 40.18, 39.97, 39.77, 39.56, 39.35, 16.78, 16.72, 16.60, 16.55.ESI-HRMS *m/z* Calc for $C_{21}H_{24}N_3O_7P$ $[M+Na]^+$:484.1250; found: 484.1273.

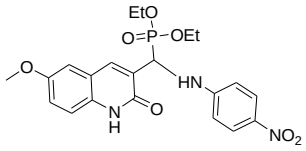


Fig. 69. Chemical structure of compound 4c$_5$

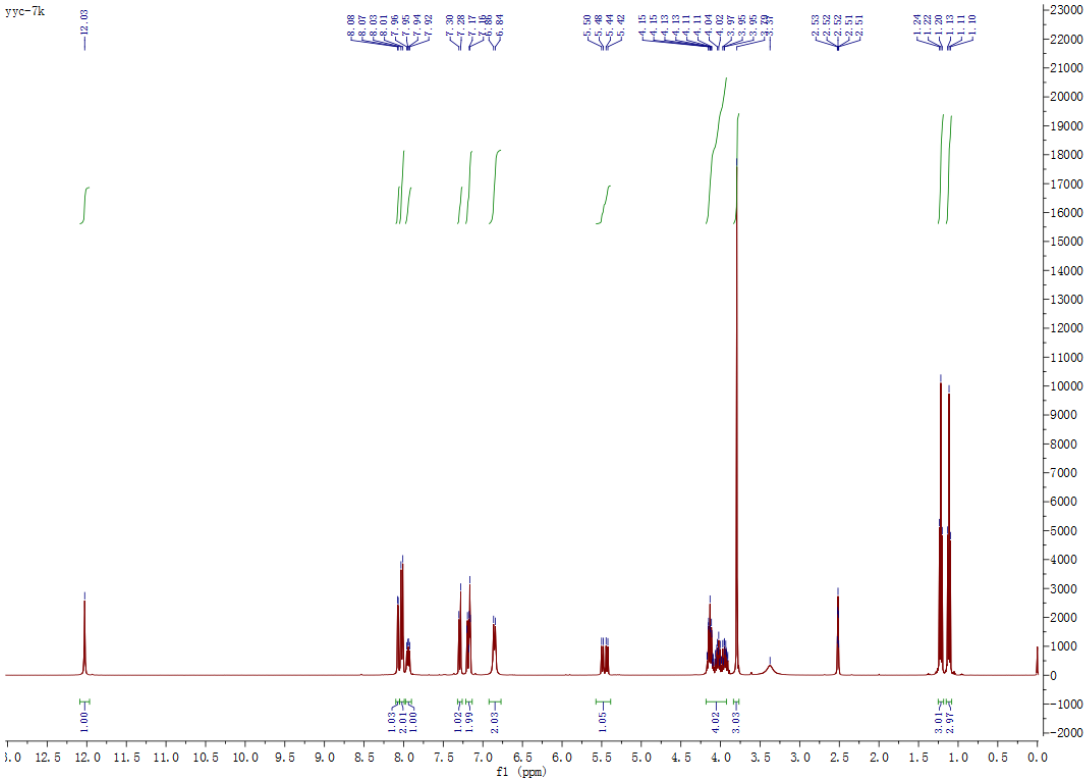


Fig. 70.^{1}H NMR of compound 4c$_5$

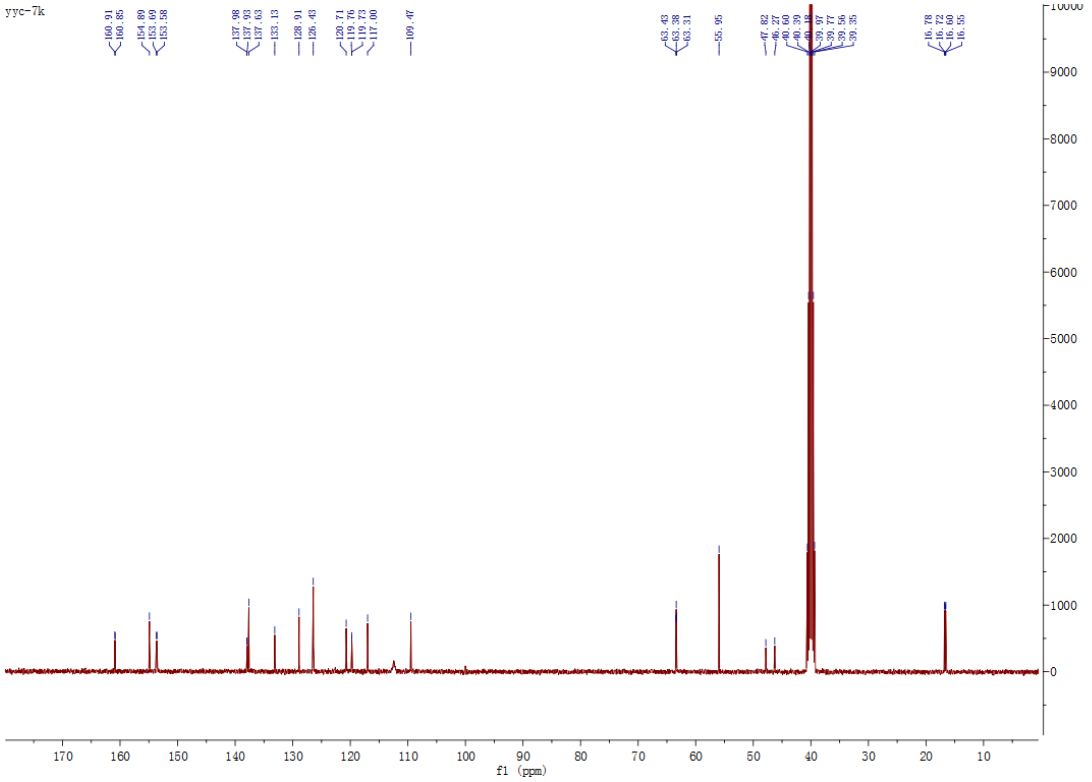


Fig. 71.^{13}C NMR of compound 4c$_5$

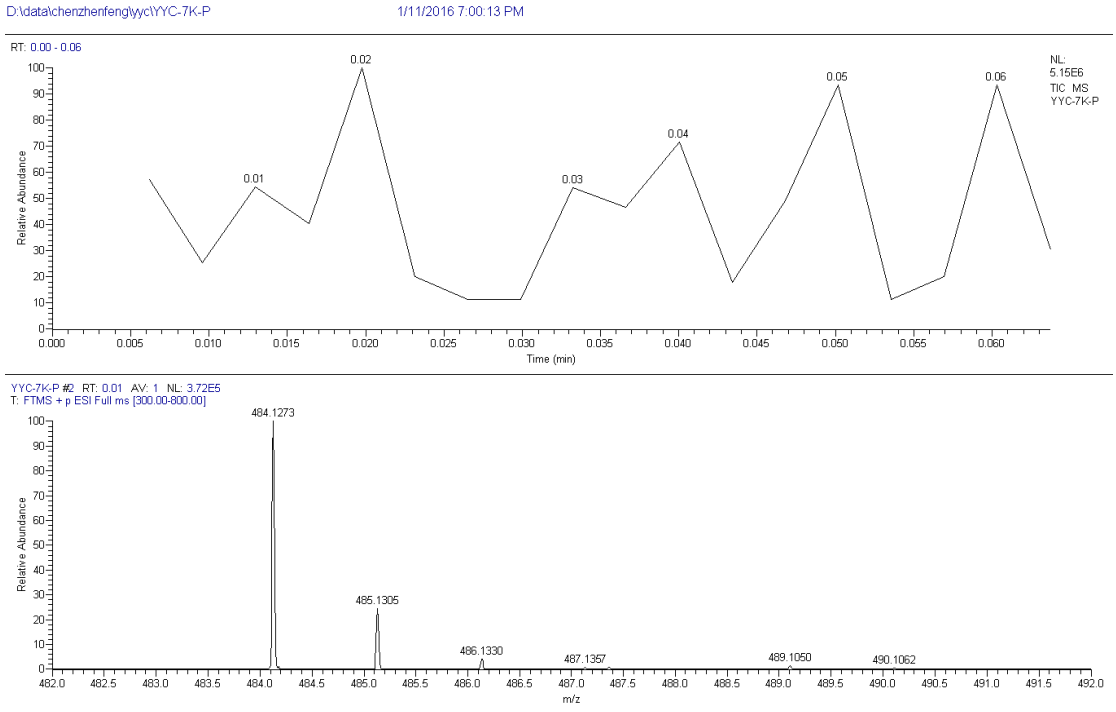


Fig. 72.ESI-HRMS of compound 4c$_5$

$4c_6$: Yield86.00%, ^{1}H NMR (400 MHz, DMSO) δ 11.90 (s, 1H), 8.03 (d, *J* = 3.6 Hz, 1H), 7.26 (d, *J* = 9.0 Hz, 1H), 7.15 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.09 (d, *J* = 2.6 Hz, 1H), 6.90 (t, *J* = 8.9 Hz, 2H), 6.74 – 6.66 (m, 2H), 6.33 (dd, *J* = 9.9, 6.7 Hz, 1H), 5.24 (dd, *J* = 24.4, 10.0 Hz, 1H), 4.15 – 3.84 (m, 4H), 3.77 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.21, 161.16, 156.64, 154.79, 154.33, 144.05, 143.90, 137.31, 137.25, 132.99, 130.14, 120.31, 119.90, 119.87, 116.88, 115.87, 115.65, 115.43, 115.06, 114.99, 114.53, 114.45, 109.28, 63.23, 63.17, 63.02, 62.95, 55.90, 48.57, 47.02, 40.61, 40.40, 40.19, 39.98, 39.77, 39.56, 39.35, 16.79, 16.74, 16.60, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{24}FN_2O_5P$ $[M+Na]^+$:457.1305; found: 457.1323.

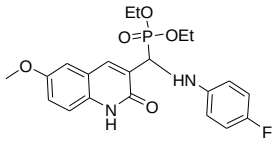


Fig. 73. Chemical structure of compound $4c_6$

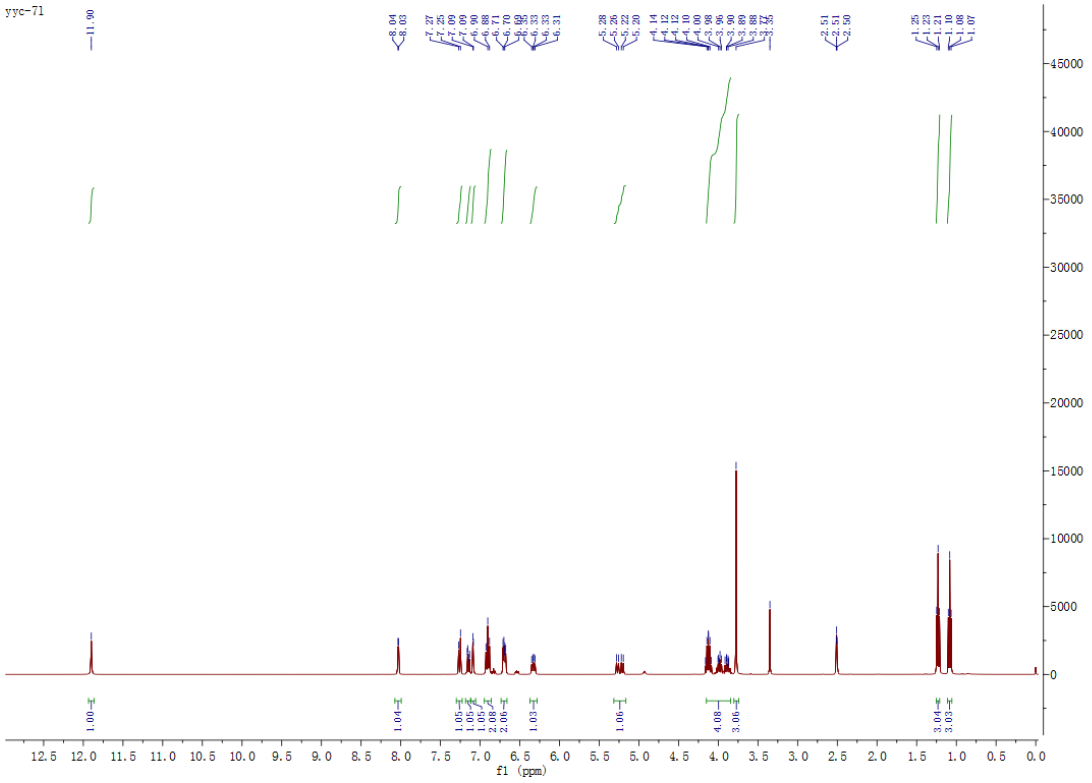


Fig. 74.^{1}H NMR of compound $4c_6$

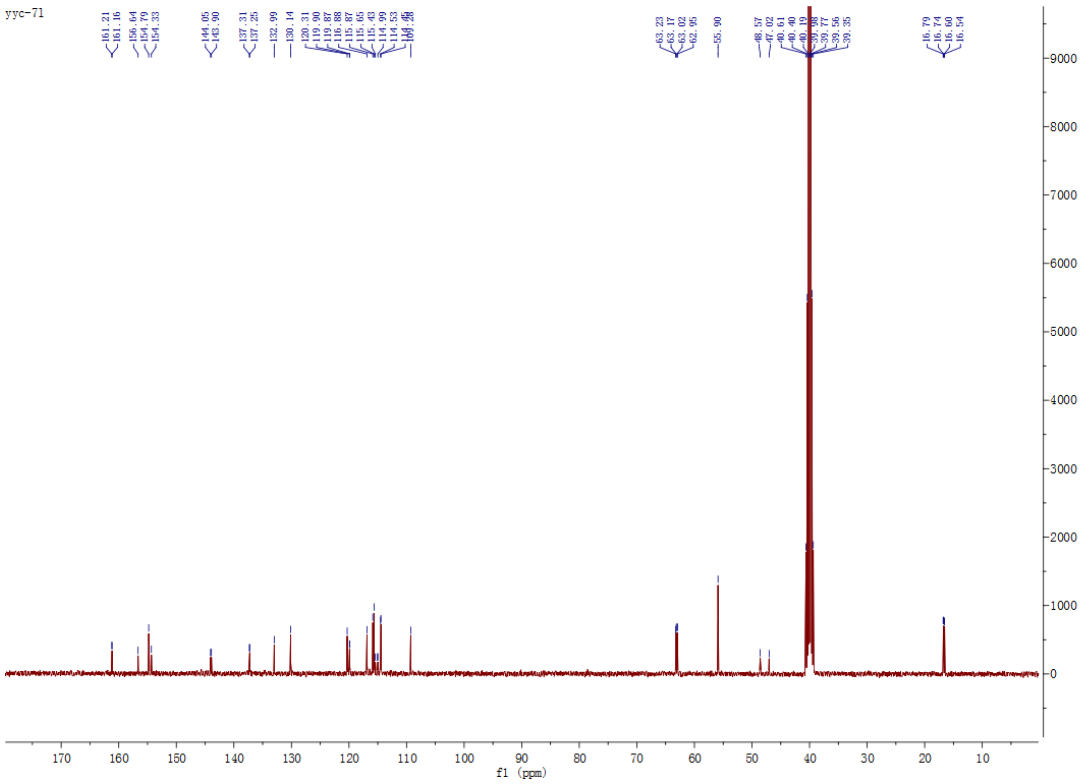


Fig. 75.^{13}C NMR of compound 4c$_6$

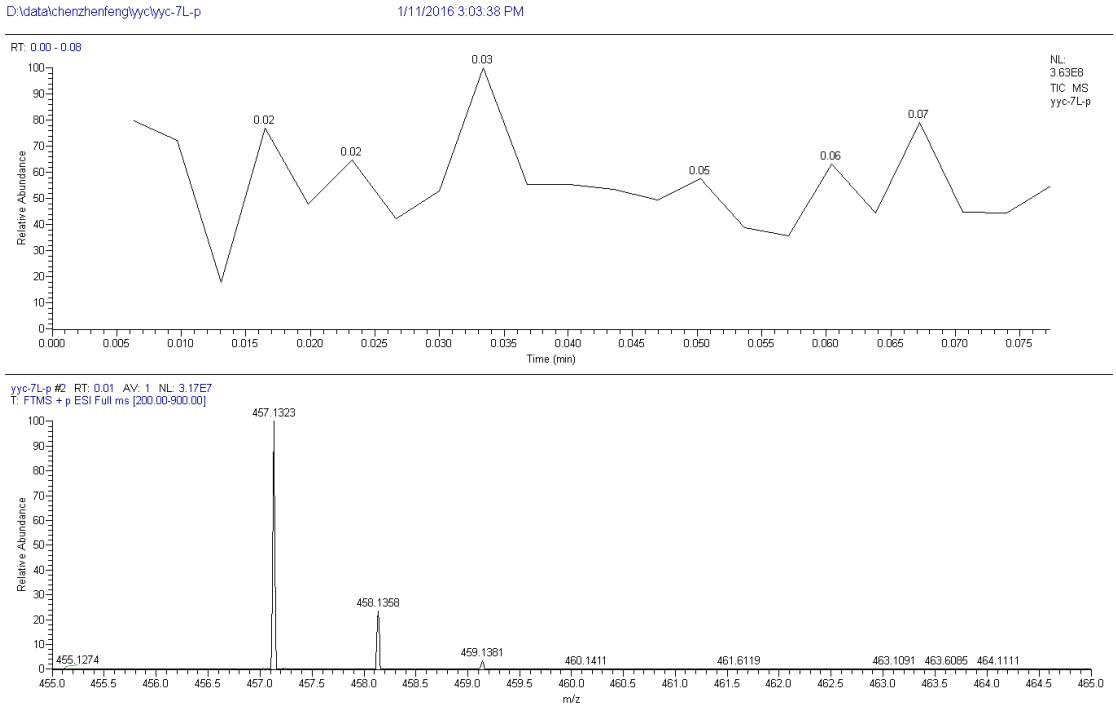


Fig. 76.ESI-HRMS of compound 4c$_6$

4c$_7$: Yield74.08%, ^{1}H NMR (400 MHz, DMSO) δ 11.96 (s, 1H), 8.04 (d, *J* = 3.6 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.27 (d, *J* = 8.9 Hz, 1H), 7.15 (ddd, *J* = 13.0, 7.7, 2.6 Hz, 3H), 6.84 (d, *J* = 8.6 Hz, 2H), 5.36 (dd, *J* = 23.8, 9.4 Hz, 1H), 4.15 – 3.89 (m, 4H), 3.78 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.10 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.06, 161.01, 154.84, 150.74, 150.61, 137.55, 137.49, 133.05, 129.59, 126.89, 126.70, 124.20, 120.49, 119.83, 119.80, 117.57, 117.25, 116.93, 112.96, 109.36, 63.32, 63.26, 63.19, 63.12, 55.91, 47.73, 46.18, 40.60, 40.39, 40.18, 39.97, 39.76, 39.56, 39.35, 16.77, 16.72, 16.60, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{24}F_3N_2O_5P$ $[M+Na]^+$:507.1273; found: 507.1252.

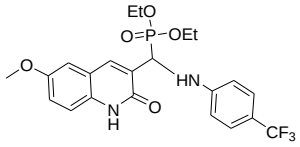


Fig. 77. Chemical structure of compound 4c$_7$

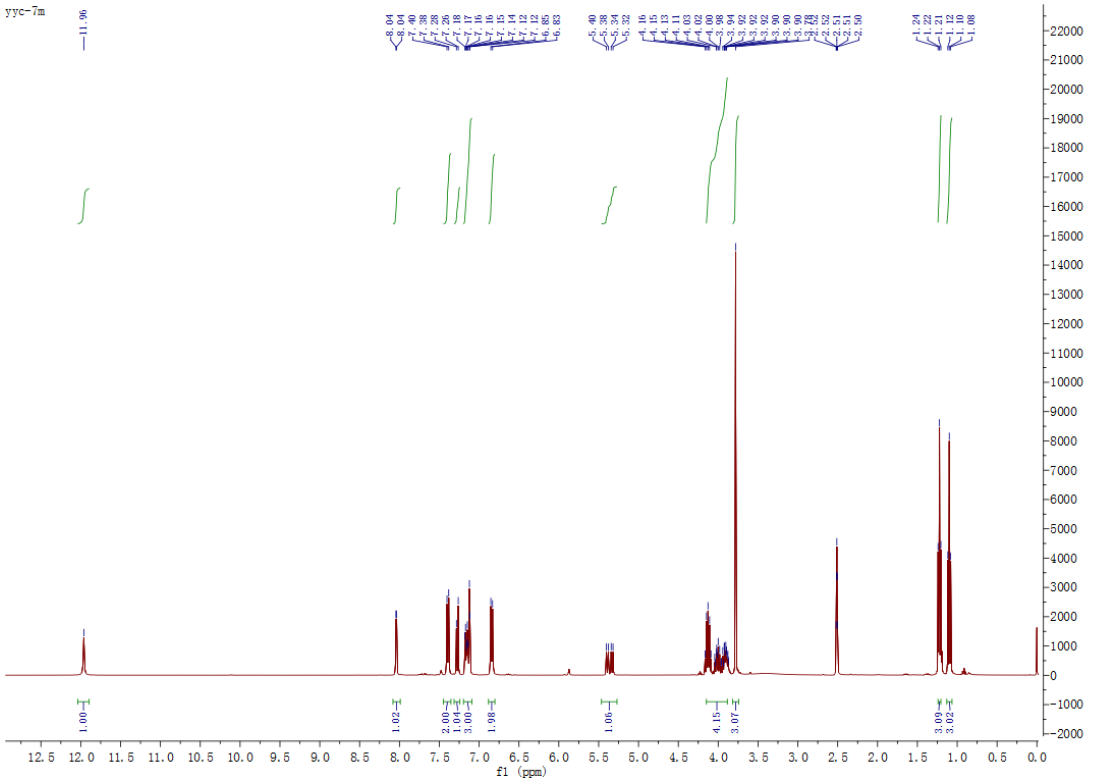


Fig. 78.^{1}H NMR of compound 4c$_7$

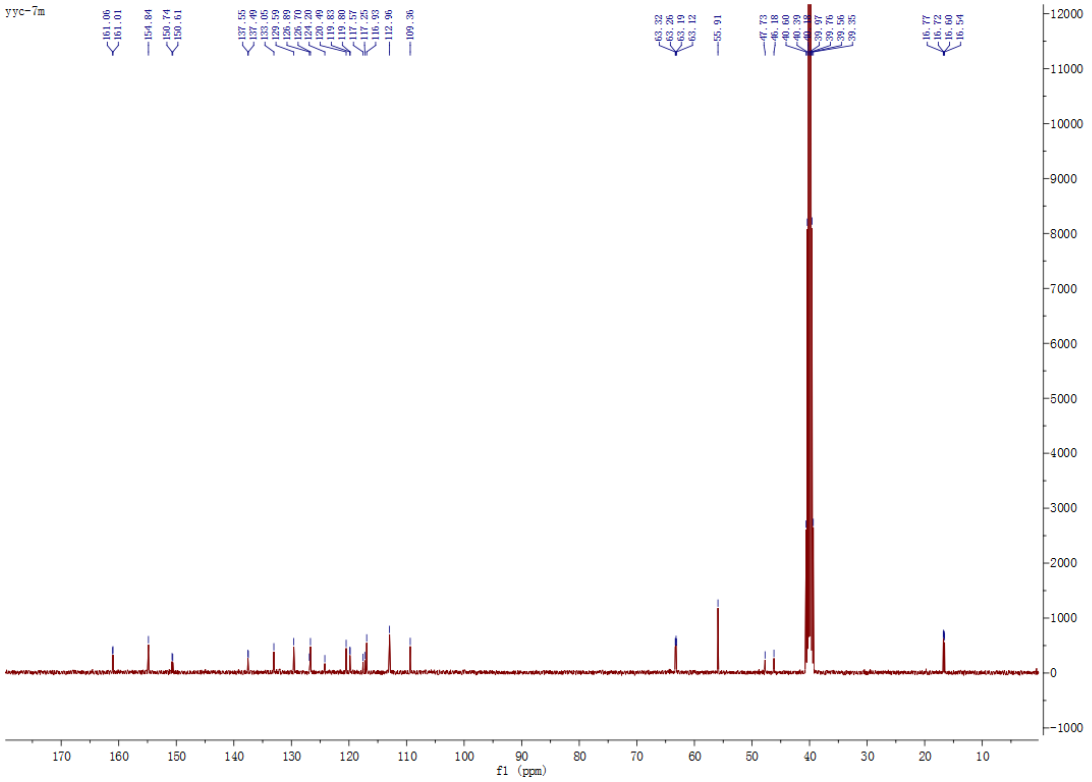


Fig. 79.^{13}C NMR of compound 4c$_7$

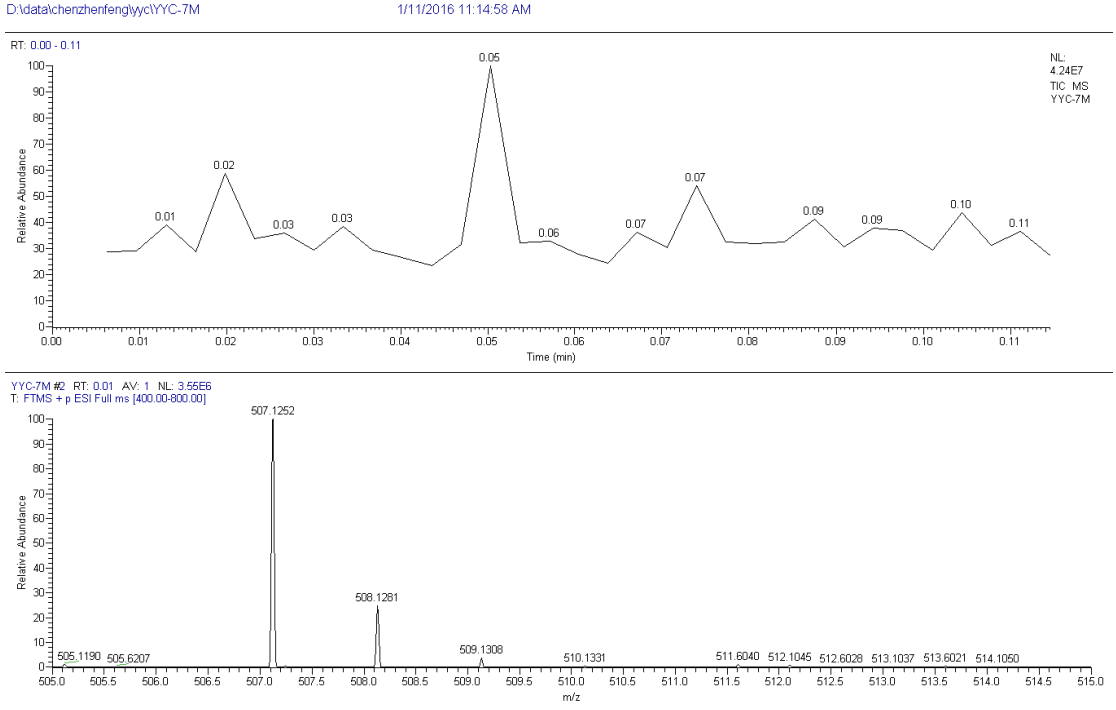


Fig. 80.ESI-HRMS of compound 4c$_7$

4d$_1$: Yield43.26%, ^{1}H NMR (400 MHz, DMSO) δ 11.80 (s, 1H), 7.92 (d, *J* = 3.4 Hz, 1H), 7.17 (s, 1H), 6.83 (s, 1H), 6.10 (s, 2H), 4.44 (d, *J* = 21.4 Hz, 1H), 4.08 (ddd, *J* = 10.2, 8.7, 5.3 Hz, 2H), 3.97 – 3.82 (m, 2H), 2.50 – 2.34 (m, 2H), 1.40 – 1.22 (m, 10H), 1.08 (t, *J* = 7.0 Hz, 3H), 0.83 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 174.78, 161.75, 161.69, 150.38, 143.78, 137.65, 137.58, 135.35, 130.11, 126.35, 113.81, 113.78, 105.58, 102.20, 95.38, 62.85, 62.78, 62.41, 62.34, 53.17, 51.62, 47.63, 47.47, 31.78, 30.84, 29.56, 29.51, 29.46, 29.31, 29.18, 29.06, 27.02, 25.59, 22.58, 20.18, 16.82, 16.77, 16.66, 16.61, 14.42, 14.30.ESI-HRMS *m/z* Calc for $C_{19}H_{27}N_2O_6P$ $[M+H]^+$:411.1585; found: 411.1519.

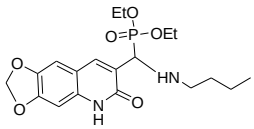


Fig. 81. Chemical structure of compound 4d$_1$

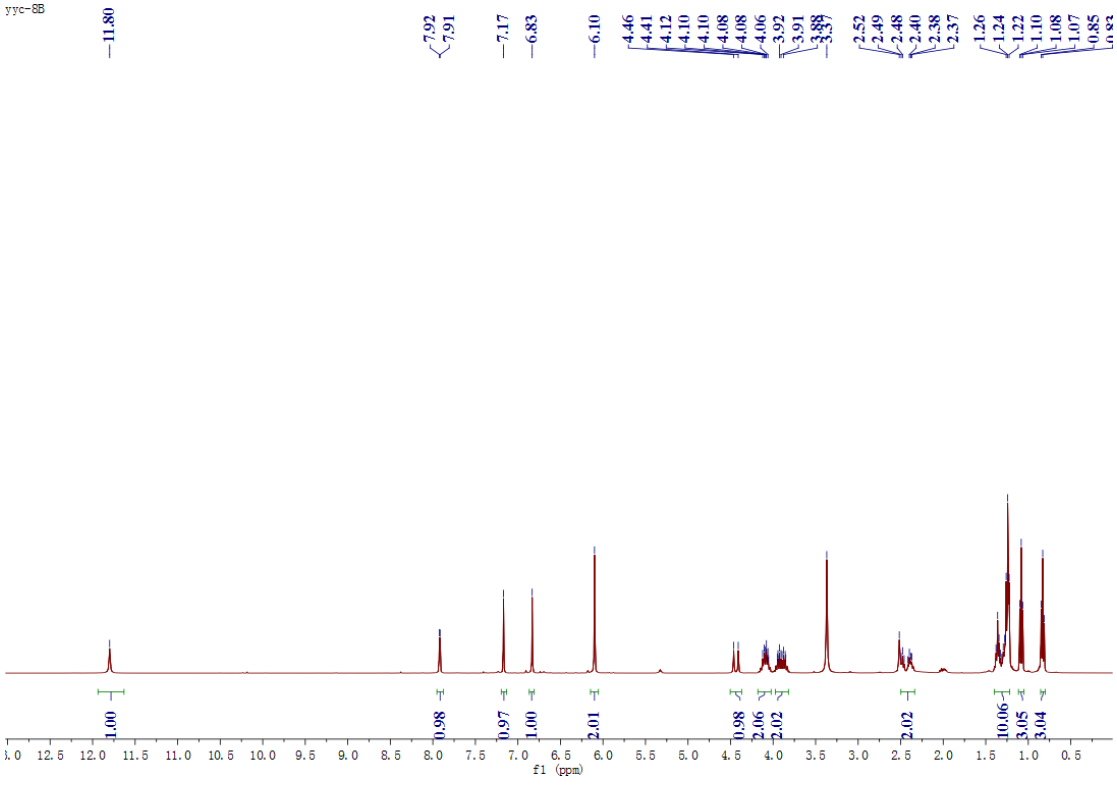


Fig. 82.^{1}H NMR of compound 4d$_1$

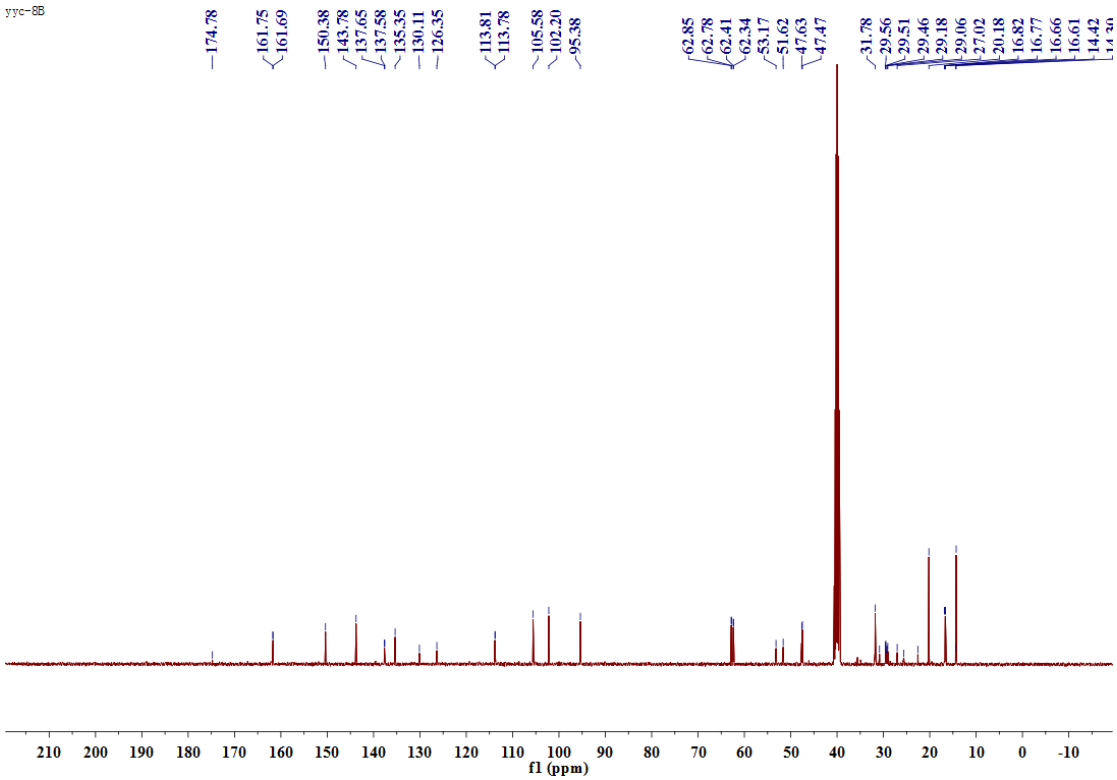


Fig. 83.^{13}C NMR of compound 4d$_1$

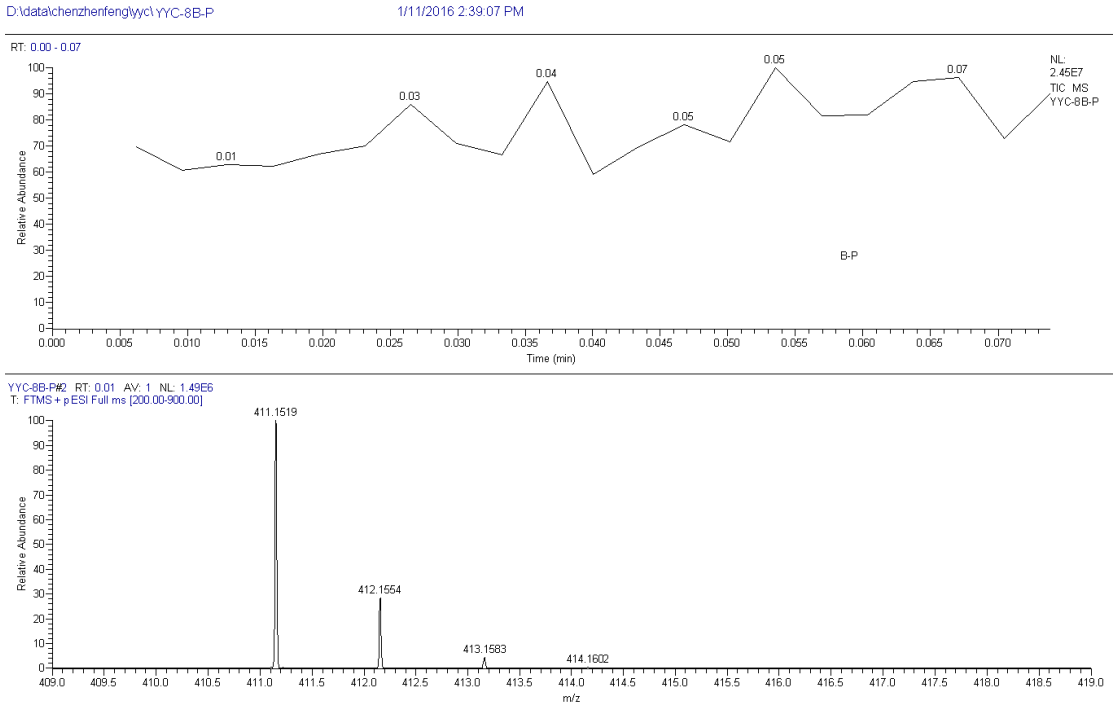


Fig. 84.ESI-HRMS of compound 4d$_1$

4d$_2$: Yield66.81%, ^{1}H NMR (500 MHz, DMSO) δ 11.85 (s, 1H), 7.92 (d, *J* = 3.5 Hz, 1H), 7.08 (s, 1H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.82 (s, 1H), 6.60 (d, *J* = 8.5 Hz, 2H), 6.07 (s, 2H), 6.02 (dd, *J* = 10.1, 6.5 Hz, 1H), 5.20 (dd, *J* = 24.3, 10.1 Hz, 1H), 4.10 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.01 – 3.83 (m, 2H), 2.09 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.44, 161.40, 150.49, 145.13, 145.01, 143.88, 137.47, 137.42, 135.47, 129.74, 126.68, 126.18, 113.79, 113.70, 113.68, 105.44, 102.22, 95.45, 63.10, 63.05, 62.89, 62.83, 48.14, 46.90, 40.49, 40.42, 40.33, 40.25, 40.16, 39.99, 39.83, 39.66, 39.49, 20.45, 16.77, 16.72, 16.59, 16.54.ESI-HRMS *m/z* Calc for $C_{22}H_{25}N_2O_6P$ $[M+Na]^+$:467.1348; found: 467.1381.

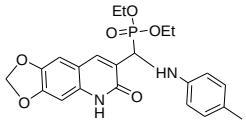


Fig. 85. Chemical structure of compound4d$_2$

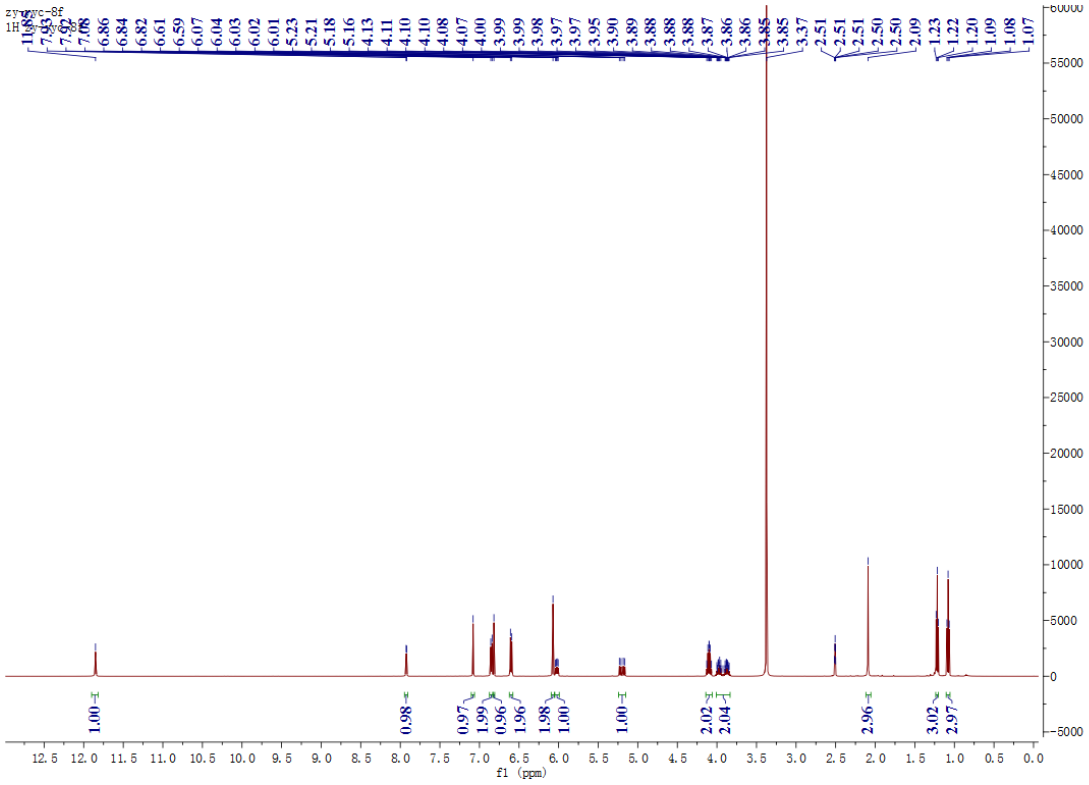


Fig. 86.^{1}H NMR of compound 4d$_2$

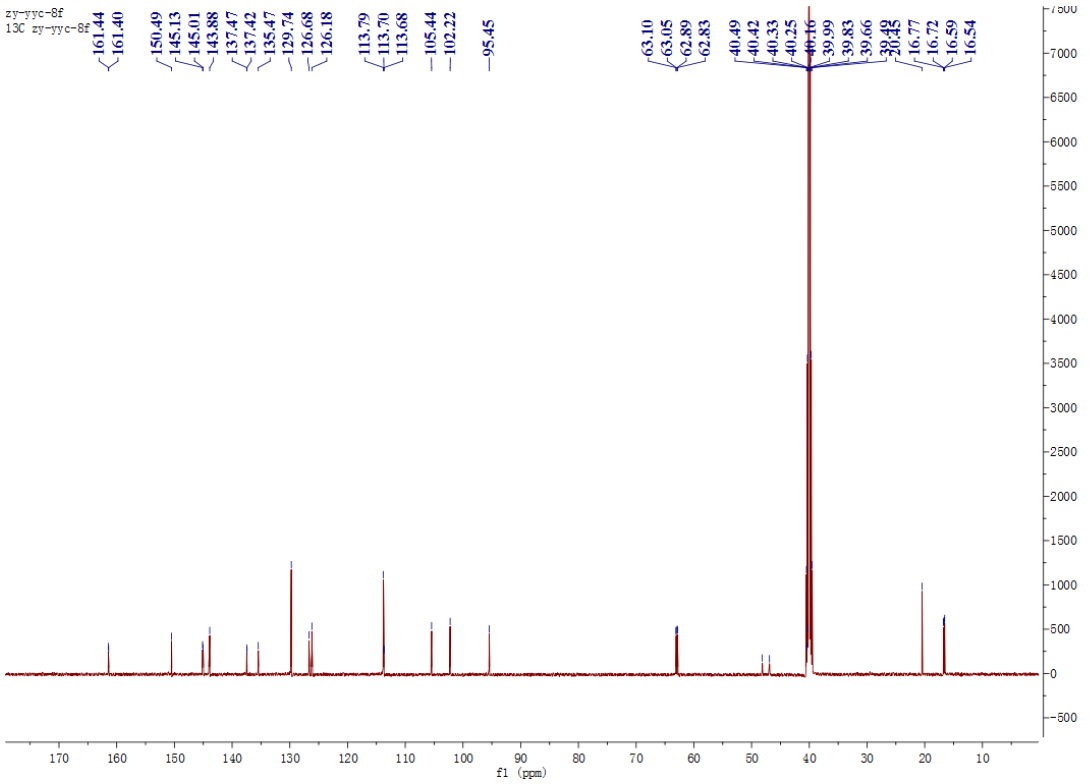


Fig. 87.^{13}C NMR of compound $4d_2$

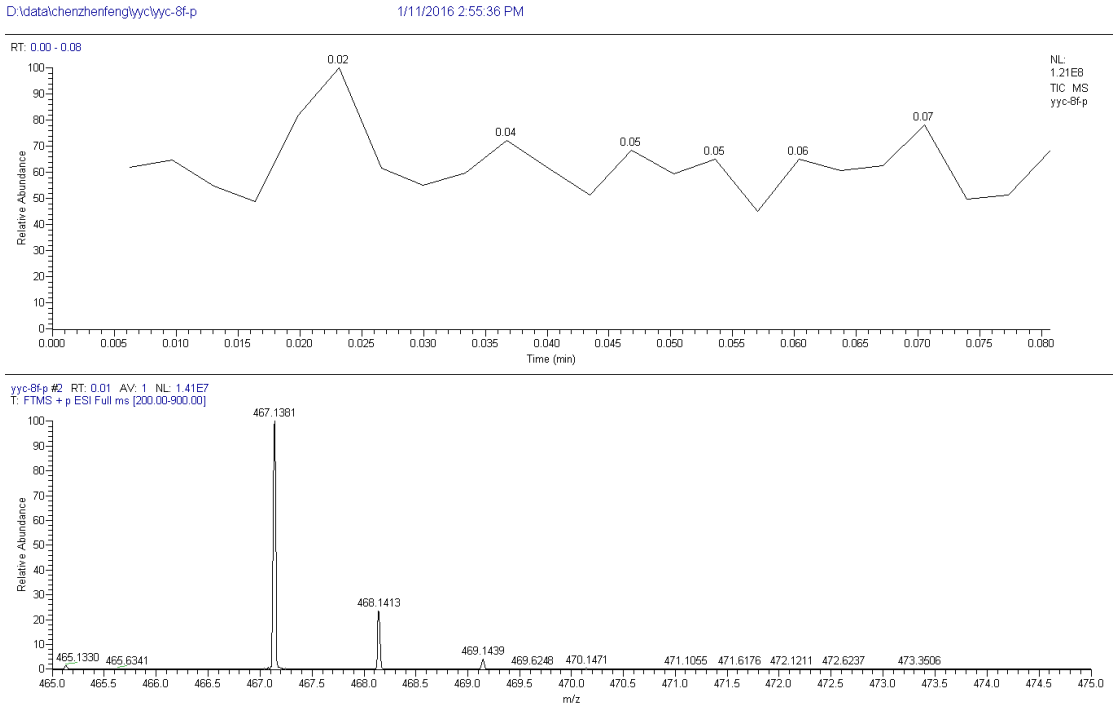


Fig. 88.ESI-HRMS of compound $4d_2$

4d$_3$: Yield77.29%, ^{1}H NMR (400 MHz, DMSO) δ 11.94 (s, 1H), 7.95 (d, *J* = 3.3 Hz, 1H), 7.10 (s, 1H), 6.89 (dd, *J* = 15.8, 7.0 Hz, 3H), 6.70 (dd, *J* = 8.9, 4.5 Hz, 2H), 6.30 (dd, *J* = 9.8, 6.6 Hz, 1H), 6.08 (s, 2H), 5.19 (dd, *J* = 24.2, 10.0 Hz, 1H), 4.16 – 4.06 (m, 2H), 3.94 (ddt, *J* = 25.1, 10.0, 7.5 Hz, 2H), 3.06 (d, *J* = 7.2 Hz, 1H), 1.21 (q, *J* = 7.2 Hz, 6H), 1.09 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.46, 161.41, 156.62, 154.31, 150.58, 144.14, 143.98, 143.94, 137.63, 137.57, 135.54, 130.11, 126.31, 115.84, 115.63, 114.53, 114.46, 113.69, 113.66, 105.47, 102.28, 95.51, 63.18, 63.12, 62.99, 62.92, 48.40, 46.84, 45.82, 40.56, 40.35, 40.14, 39.93, 39.73, 39.52, 39.31, 35.59, 31.75, 30.84, 29.45, 29.17, 29.04, 27.01, 25.60, 22.57, 16.78, 16.73, 16.60, 16.54, 14.41, 8.89.ESI-HRMS *m/z* Calc for $C_{22}H_{25}N_2O_6P$ $[M-H]^-$:443.1372; found: 443.1727.

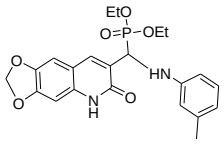


Fig. 89. Chemical structure of compound4d$_3$

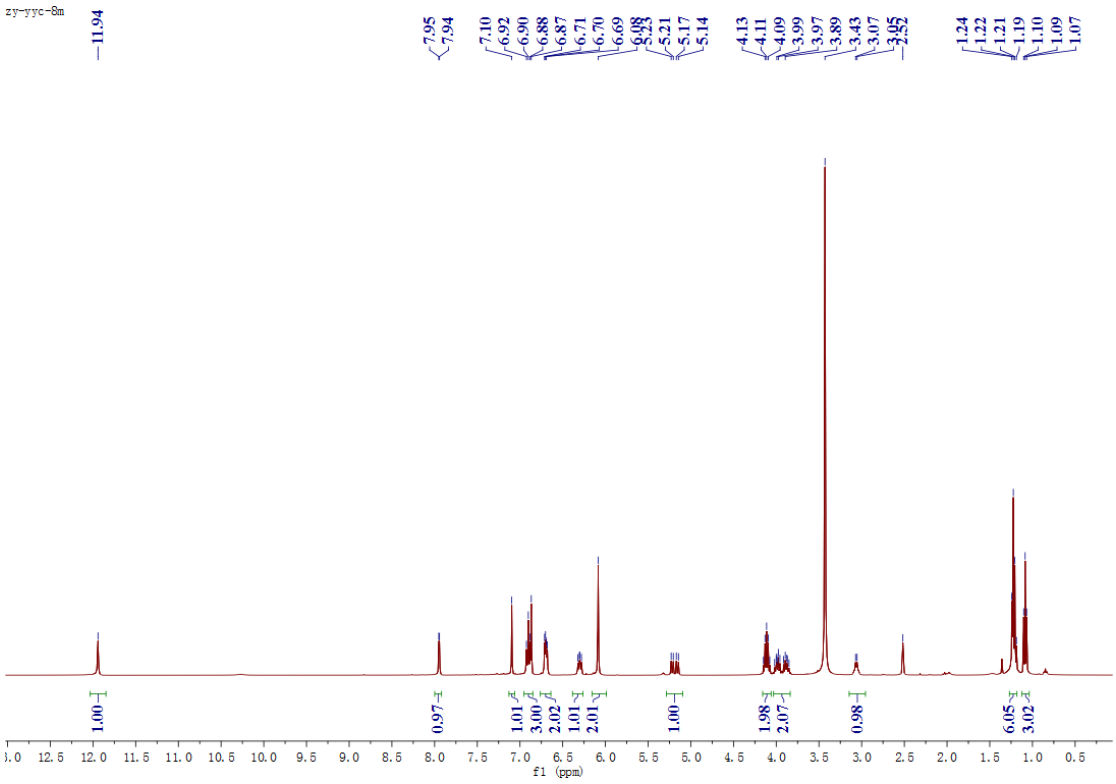


Fig. 90.^{1}H NMR of compound 4d$_3$

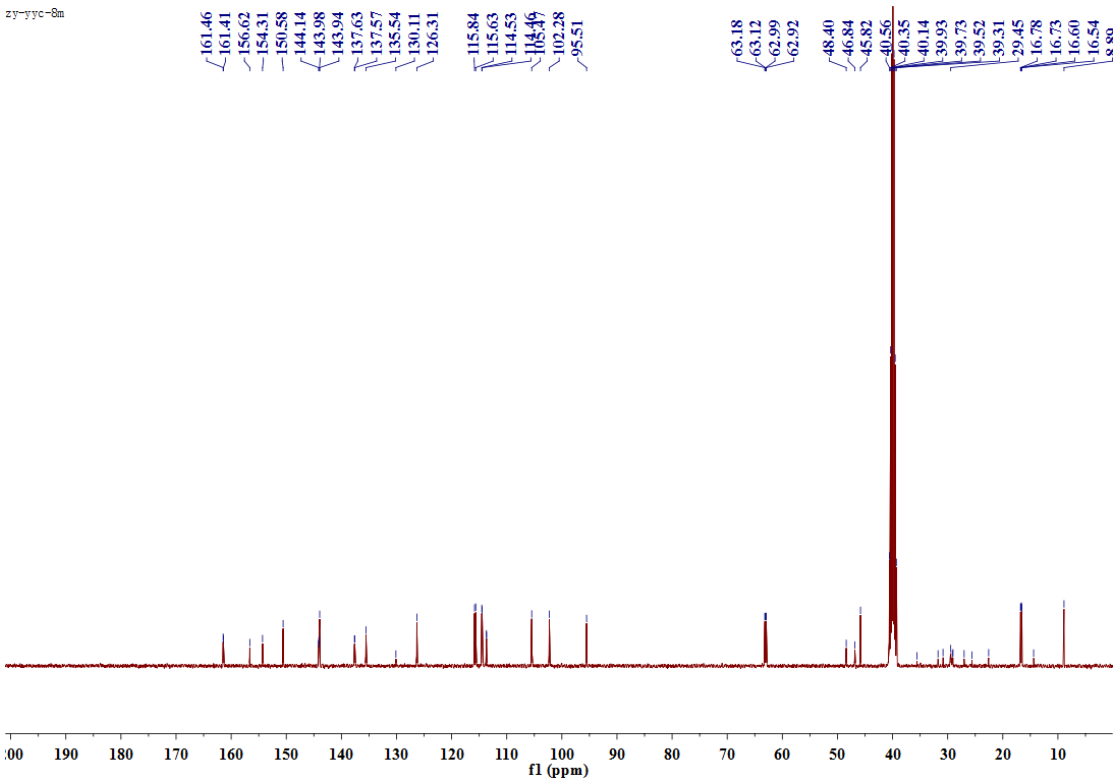


Fig. 91.^{13}C NMR of compound 4d$_3$

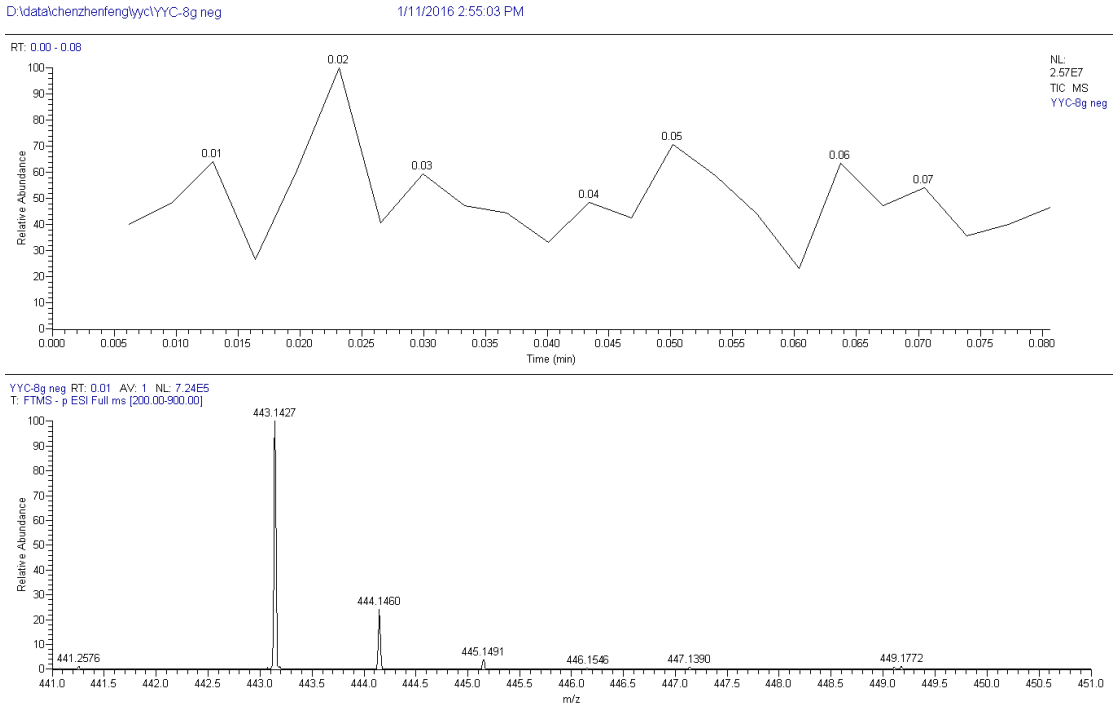
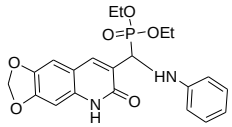


Fig. 92.ESI-HRMS of compound 4d$_3$

4d$_4$: Yield77.32%, ^{1}HNMR (500 MHz, DMSO) δ 11.87 (s, 1H), 7.94 (d, *J* = 3.4 Hz, 1H), 7.09 (s, 1H), 7.04 (dd, *J* = 8.3, 7.5 Hz, 2H), 6.82 (s, 1H), 6.70 (d, *J* = 7.8 Hz, 2H), 6.56 (t, *J* = 7.3 Hz, 1H), 6.25 (dd, *J* = 9.9, 6.6 Hz, 1H), 6.07 (s, 2H), 5.23 (dd, *J* = 24.2, 9.9 Hz, 1H), 4.10 (dq, *J* = 14.2, 7.1 Hz, 2H), 4.02 – 3.84 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 161.43, 161.38, 150.54, 147.49, 147.38, 143.92, 137.56, 137.52, 135.52, 129.32, 126.57, 117.65, 113.70, 113.68, 113.59, 105.48, 102.25, 95.48, 63.13, 63.08, 62.95, 62.89, 47.83, 46.58, 40.49, 40.42, 40.33, 40.25, 40.16, 39.99, 39.82, 39.66, 39.49, 16.76, 16.72, 16.59, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{23}N_2O_6P$ $[M+Na]^+$:453.1191; found: 453.1169.



8l

Fig. 93. Chemical structure of compound4d$_4$

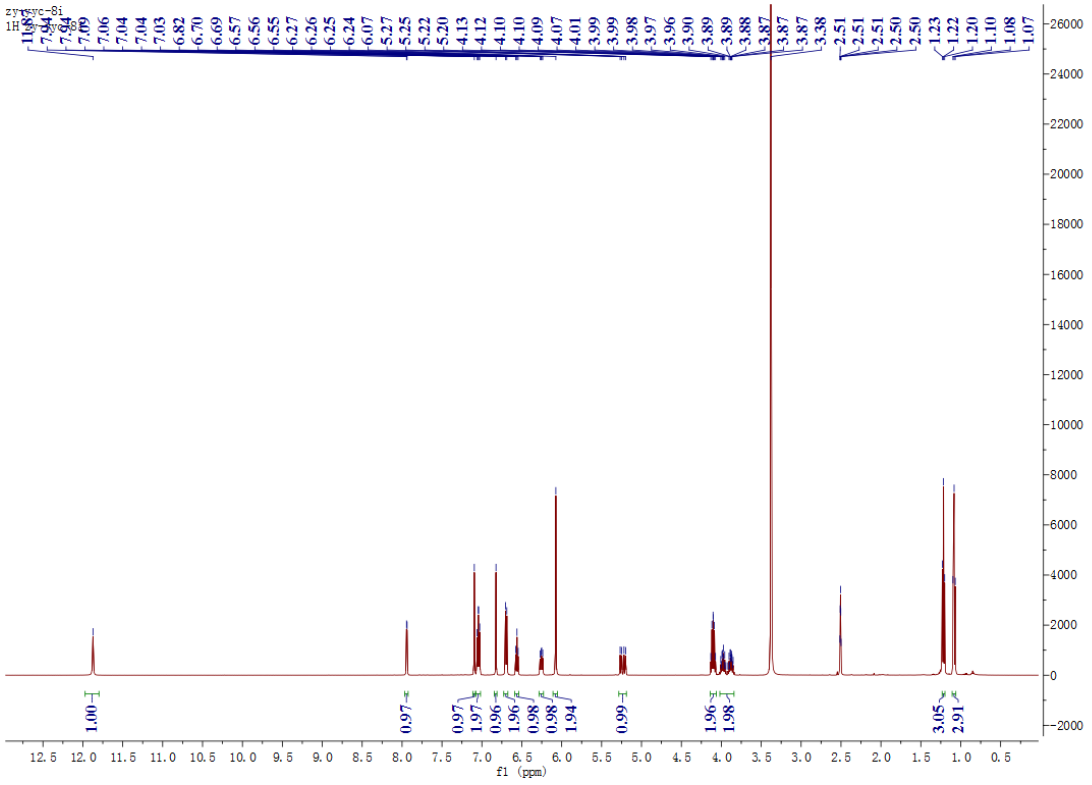


Fig. 94.^{1}H NMR of compound 4d$_4$

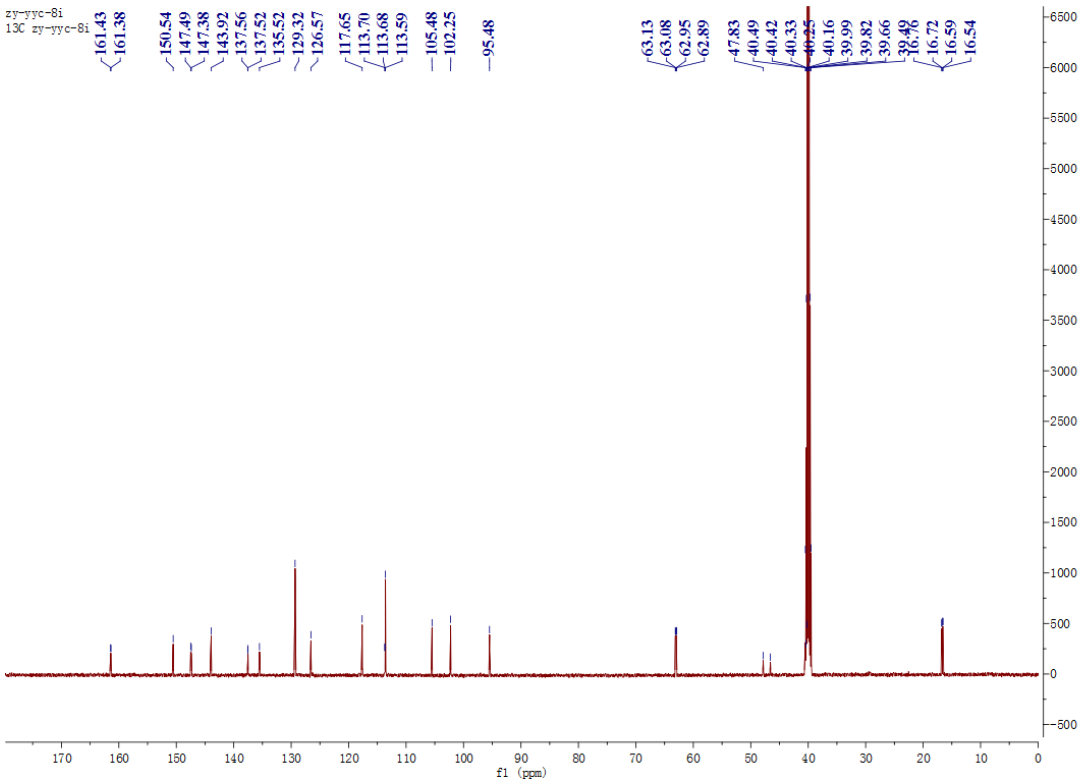


Fig. 95.^{13}C NMR of compound 4d$_4$

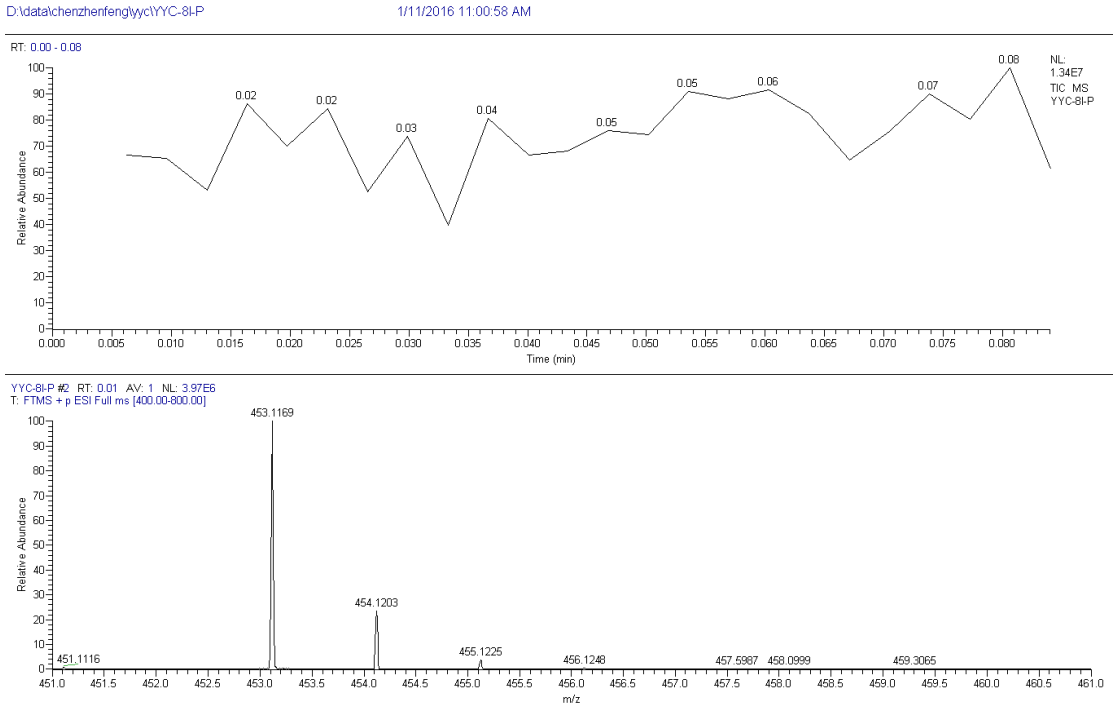


Fig. 96.ESI-HRMS of compound 4d$_4$

4d$_5$: Yield73.71%, ^{1}H NMR (400 MHz, DMSO) δ 12.02 (s, 1H), 8.08 – 7.93 (m, 4H), 7.18 (s, 1H), 6.84 (s, 3H), 6.10 (s, 2H), 5.40 (dd, *J* = 22.4, 9.2 Hz, 1H), 4.13 – 3.91 (m, 4H), 1.20 (t, *J* = 7.0 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 161.16, 161.10, 153.75, 153.65, 150.89, 144.09, 138.24, 138.18, 137.54, 136.93, 135.78, 126.44, 125.10, 113.55, 113.52, 112.29, 105.67, 102.38, 95.51, 63.34, 63.32, 63.28, 63.25, 47.62, 46.06, 40.59, 40.38, 40.17, 39.97, 39.76, 39.55, 39.34, 16.78, 16.72, 16.60, 16.54, -14.99.ESI-HRMS *m/z* Calc for $C_{21}H_{22}N_3O_8P$ $[M+Na]^+$:498.1042; found: 498.1064.

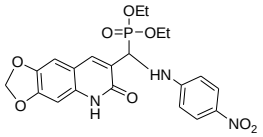


Fig. 97. Chemical structure of compound4d$_5$

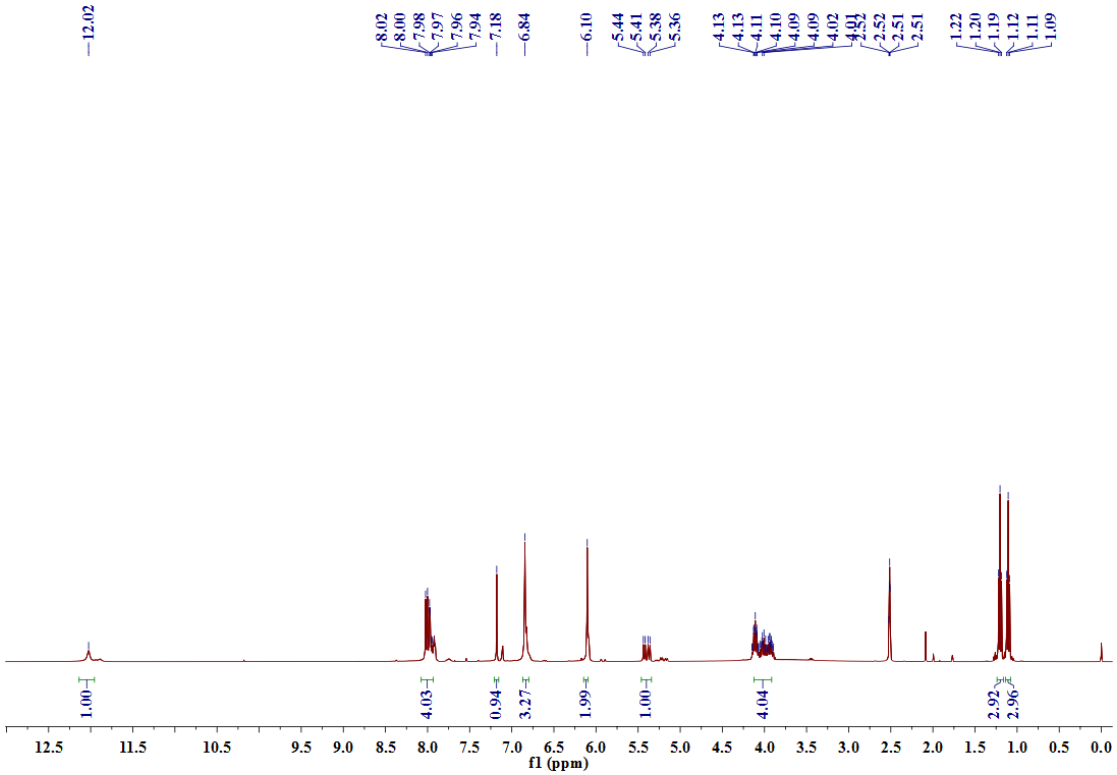


Fig. 98.^{1}H NMR of compound 4d$_5$

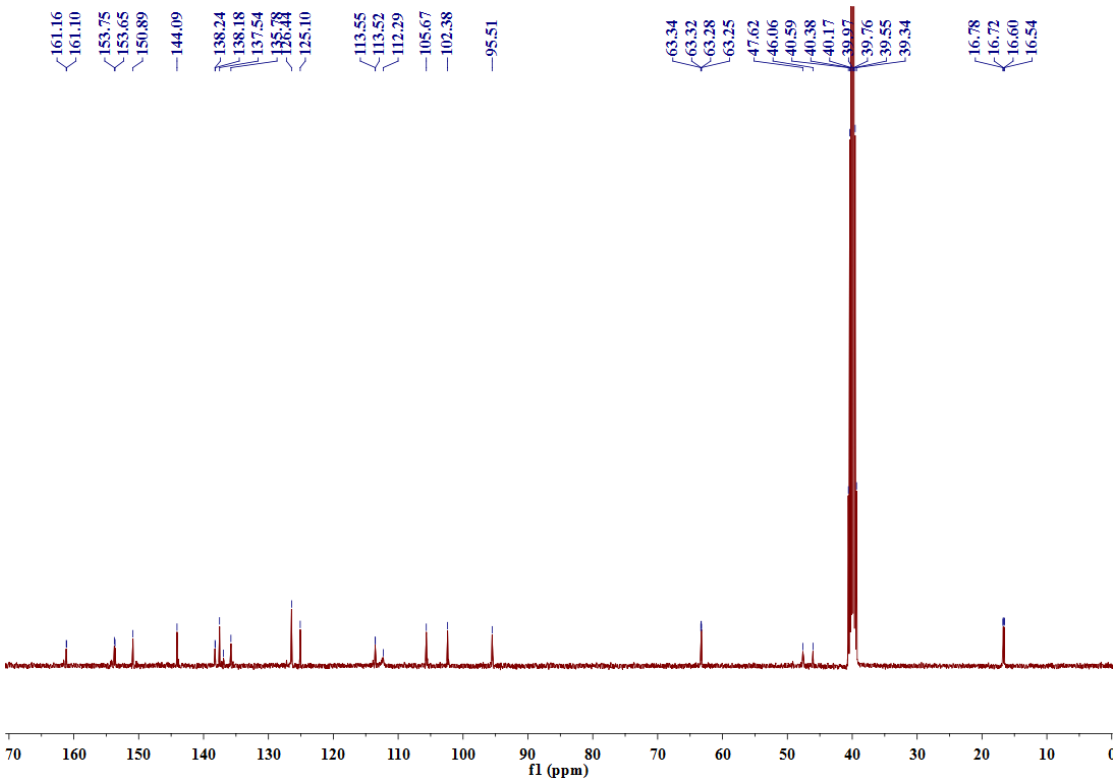


Fig. 99.^{13}C NMR of compound 4d$_5$

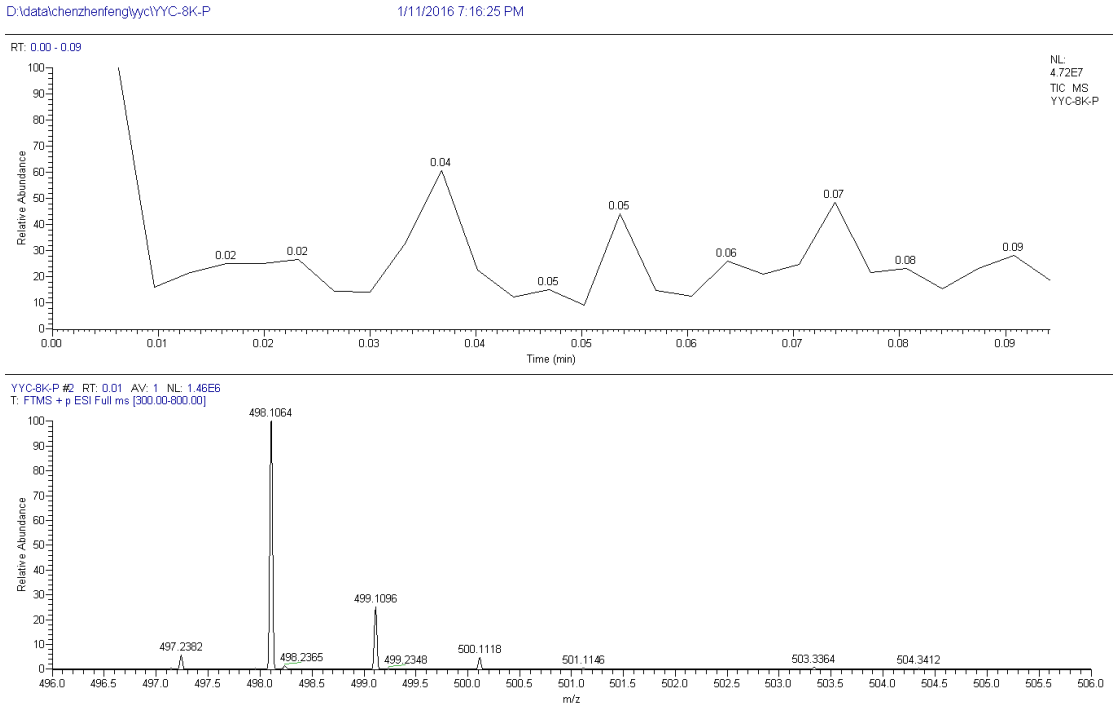


Fig. 100. ESI-HRMS of compound 4d$_5$

4d$_6$: Yield85.70%, ^{1}H NMR (400 MHz, DMSO) δ 11.98 (s,1H), 7.94 (d, *J* = 3.2 Hz,1H), 7.38 (d, *J* = 8.6 Hz,2H), 7.15 – 7.06 (m,2H), 6.88 – 6.79 (m,3H), 6.08 (s,2H), 5.29 (dd, *J* = 23.5, 9.4 Hz,1H), 4.13 – 4.07 (m,2H), 4.02 – 3.86 (m,3H), 1.26 – 1.18 (m,6H), 1.09 (t, *J* = 7.0 Hz,3H).^{13}C NMR (101 MHz, DMSO) δ 161.32, 150.72, 144.01, 137.81, 135.65, 126.71, 125.77, 113.63, 112.94, 105.56, 102.32, 95.52, 63.27, 63.20, 63.15, 63.08, 47.54, 45.97, 40.54, 40.33, 40.12, 39.91, 39.70, 39.50, 39.29, 29.02, 22.56, 16.76, 16.71, 16.59, 16.53.ESI-HRMS *m/z* Calc for $C_{21}H_{22}FN_2O_6P$ $[M+Na]^+$:471.1097; found: 471.1118.

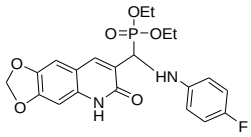


Fig. 101. Chemical structure of compound4d$_6$

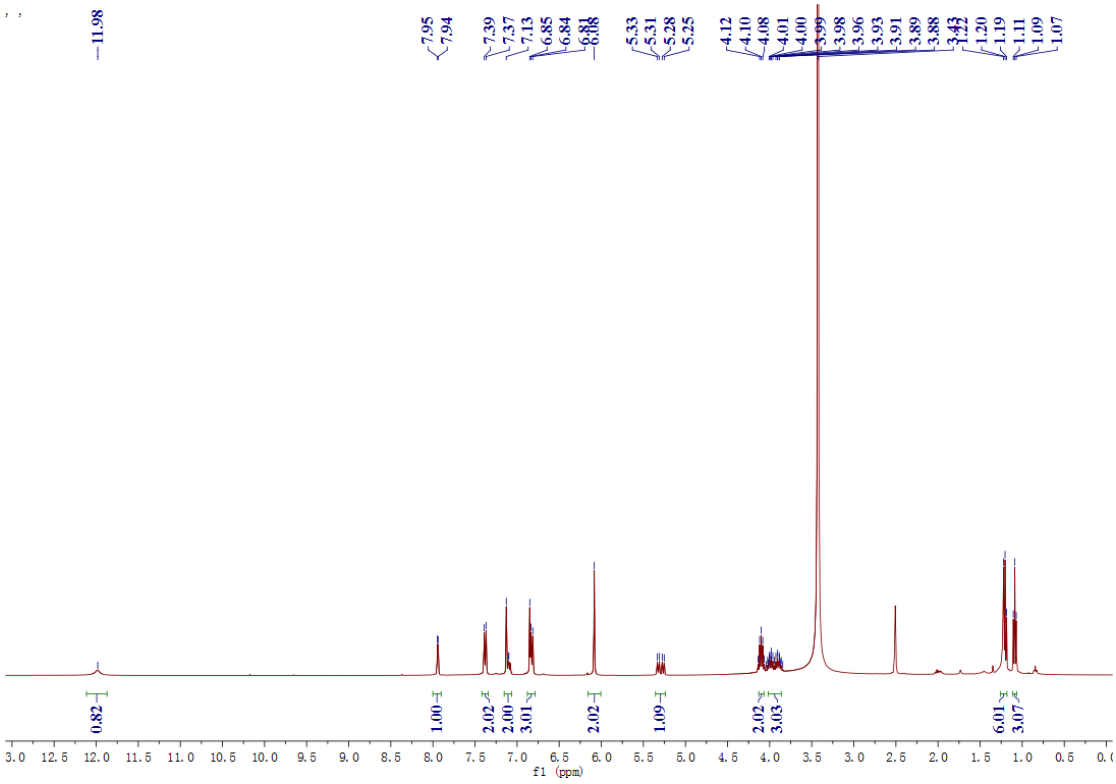


Fig. 102.^{1}H NMR of compound 4d$_6$

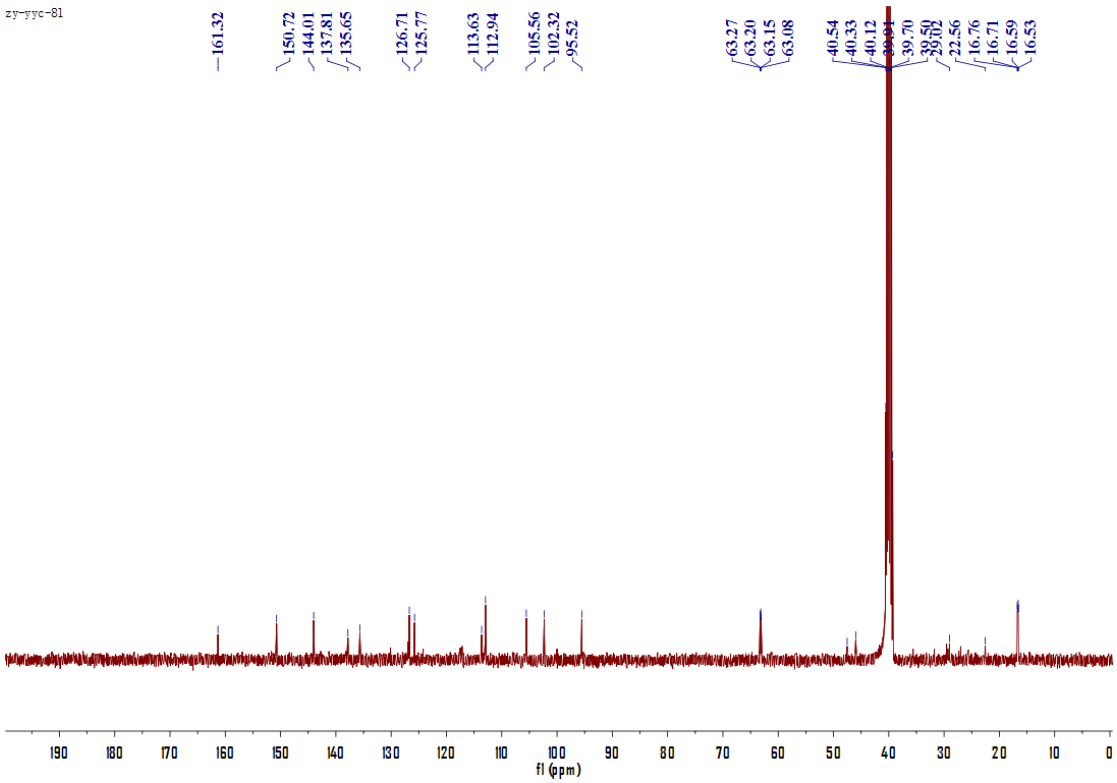


Fig. 103.^{13}C NMR of compound 4d$_6$

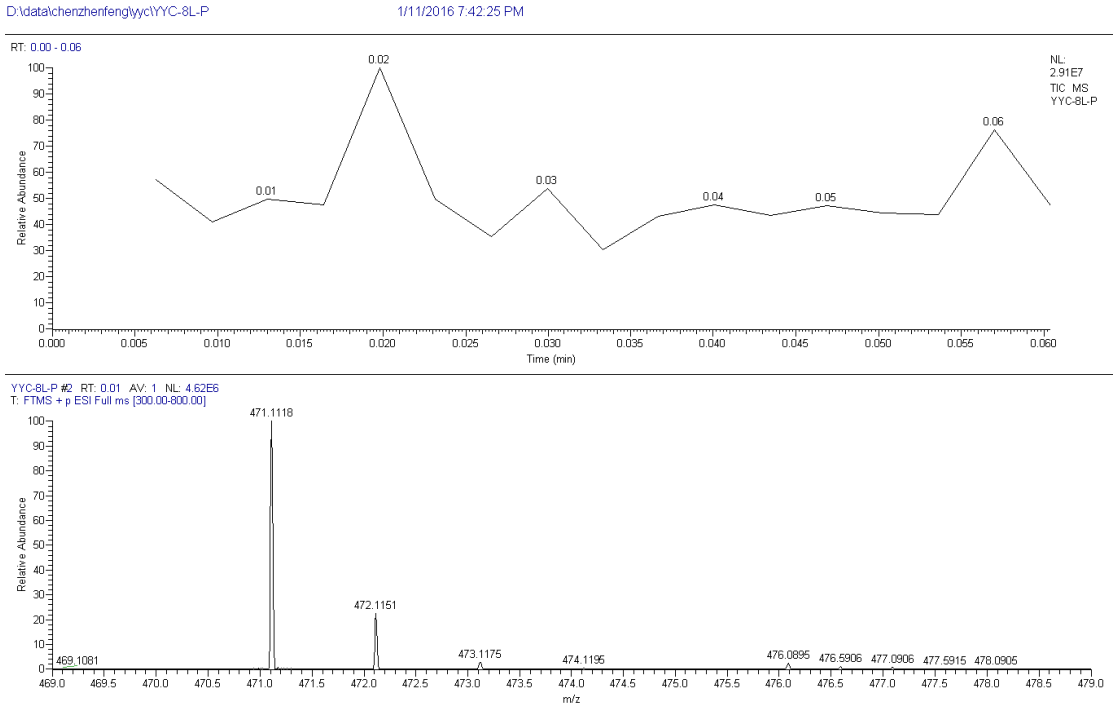


Fig. 104.ESI-HRMS of compound 4d$_6$

4d$_7$: Yield75.49%, ^{1}H NMR (400 MHz, DMSO) δ 11.94 (s, 1H), 7.95 (d, *J* = 3.3 Hz, 1H), 7.10 (s, 1H), 6.89 (dd, *J* = 15.8, 7.0 Hz, 3H), 6.70 (dd, *J* = 8.9, 4.5 Hz, 2H), 6.30 (dd, *J* = 9.8, 6.6 Hz, 1H), 6.08 (s, 2H), 5.19 (dd, *J* = 24.2, 10.0 Hz, 1H), 4.17 – 4.06 (m, 2H), 4.03 – 3.83 (m, 2H), 3.06 (d, *J* = 7.2 Hz, 1H), 1.21 (q, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 161.03 (d, *J* = 5.4 Hz), 154.84 (s), 150.68 (d, *J* = 12.7 Hz), 137.52 (d, *J* = 6.0 Hz), 133.05 (s), 129.59 (s), 126.79 (d, *J* = 19.3 Hz), 120.49 (s), 119.82 (d, *J* = 3.1 Hz), 117.57 (s), 117.25 (s), 116.93 (s), 112.96 (s), 109.36 (s), 63.22 (dd, *J* = 14.0, 6.9 Hz), 55.91 (s), 47.73 (s), 46.18 (s), 40.60 (s), 40.39 (s), 40.18 (s), 39.97 (s), 39.76 (s), 39.56 (s), 39.35 (s), 16.66 (dd, *J* = 17.6, 5.4 Hz).ESI-HRMS *m/z* Calc for $C_{22}H_{22}F_3N_2O_6P$ $[M+Na]^+$:521.1065; found: 521.1089.

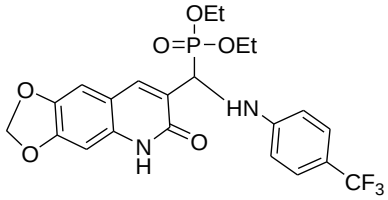


Fig. 101. Chemical structure of compound4d$_7$

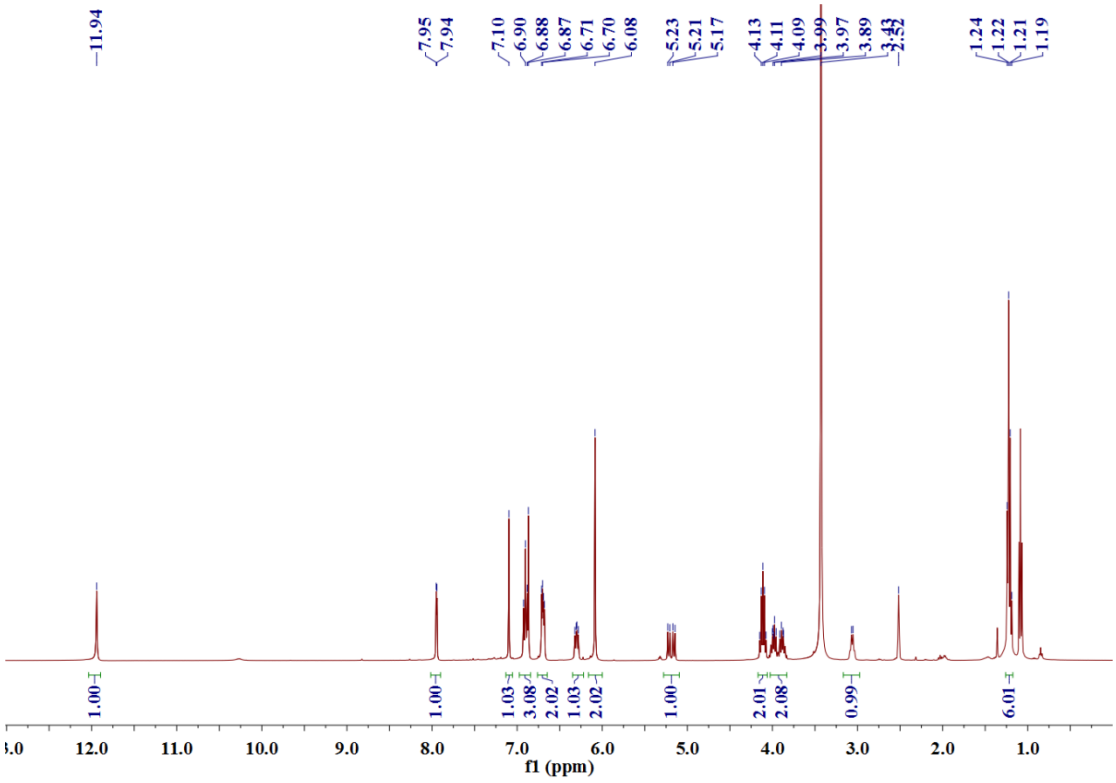


Fig. 102.^{1}H NMR of compound 4d$_7$

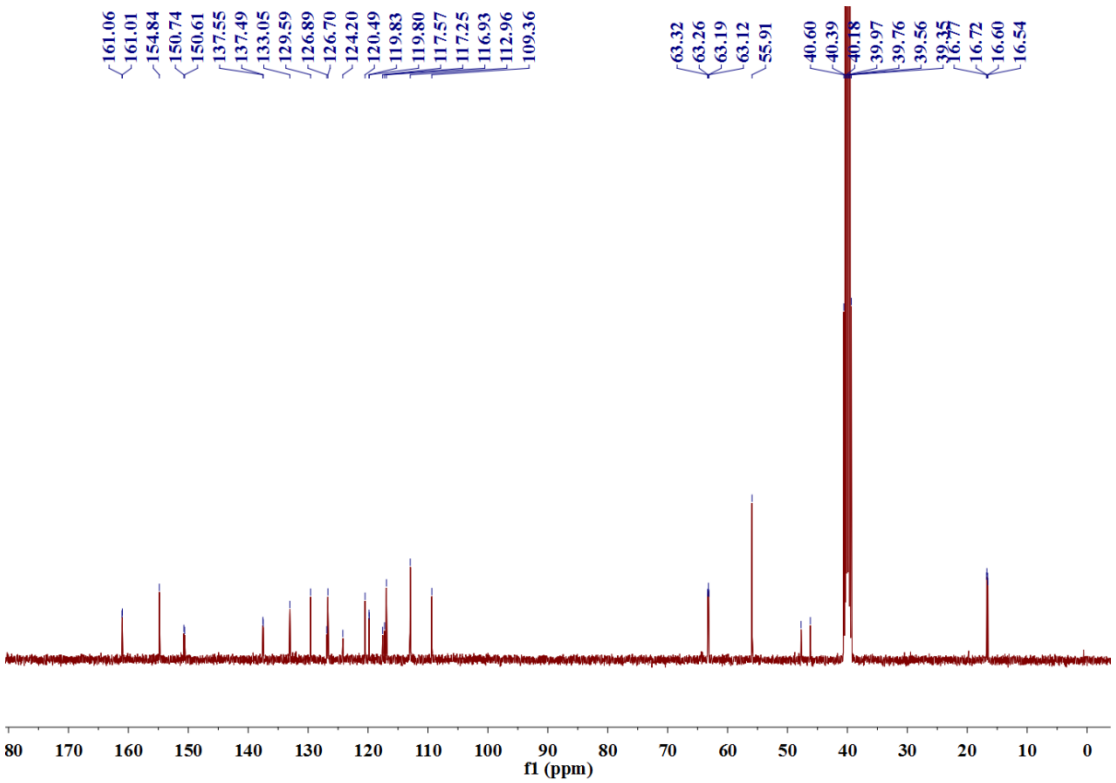


Fig. 103.^{13}C NMR of compound 4d$_7$

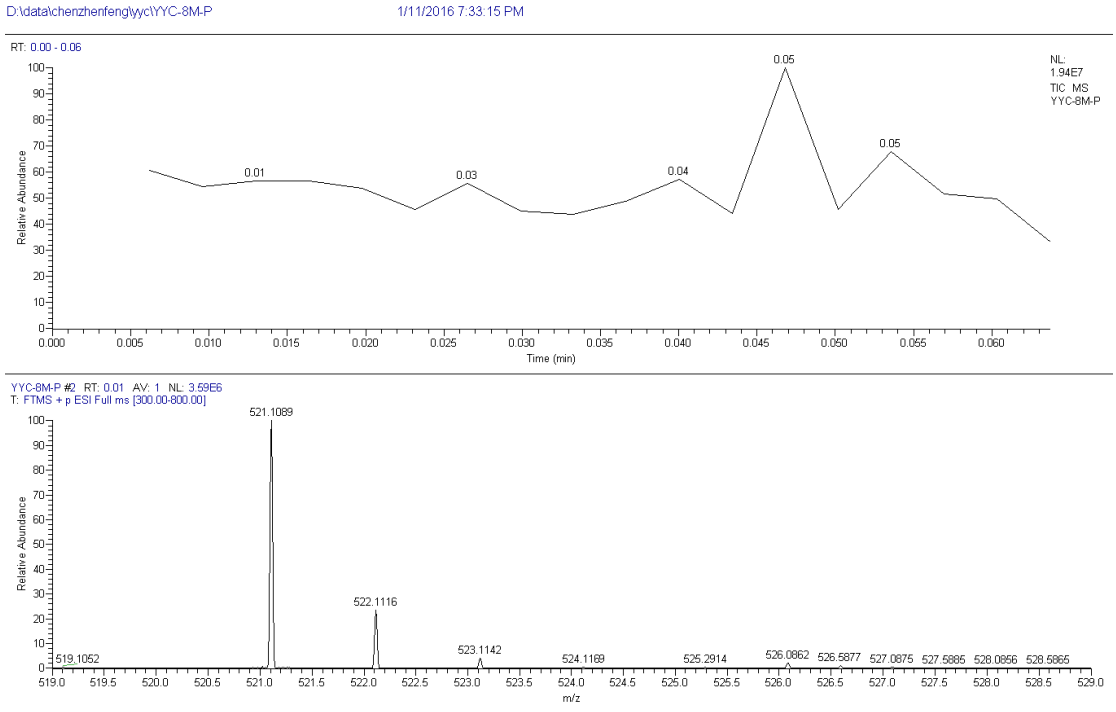


Fig. 104.ESI-HRMS of compound 4d$_7$

5a: Yield70.81%, ^{1}H NMR (400 MHz, DMSO) δ 11.68 (s, 1H), 7.72 (d, J = 2.6 Hz, 1H), 6.86 (s, 1H), 6.69 (t, J = 7.7 Hz, 1H), 6.60 (s, 1H), 6.33 (s, 1H), 6.26 (d, J = 8.0 Hz, 1H), 6.16 (d, J = 7.3 Hz, 1H), 5.98 – 5.90 (m, 1H), 5.85 (s, 2H), 5.01 (dd, J = 24.2, 10.0 Hz, 1H), 3.93 – 3.82 (m, 2H), 3.79 – 3.62 (m, 2H), 1.91 (s, 3H), 0.99 (t, J = 7.0 Hz, 3H), 0.86 (t, J = 7.0 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 161.44, 161.38, 150.52, 147.49, 147.35, 143.91, 138.31, 137.56, 137.49, 135.49, 129.20, 126.69, 118.57, 114.40, 113.71, 113.68, 110.68, 105.46, 102.25, 95.46, 63.14, 63.08, 62.94, 62.87, 47.92, 46.36, 46.03, 21.78, 16.78, 16.73, 16.60, 16.54.ESI-HRMS *m/z* Calc for $C_{21}H_{23}N_2O_4P$ $[M+H]^+$:399.1474; found: 339.1476.

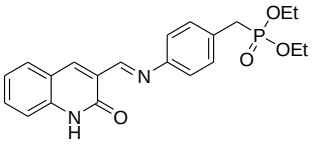


Fig. 105. Chemical structure of compound5a

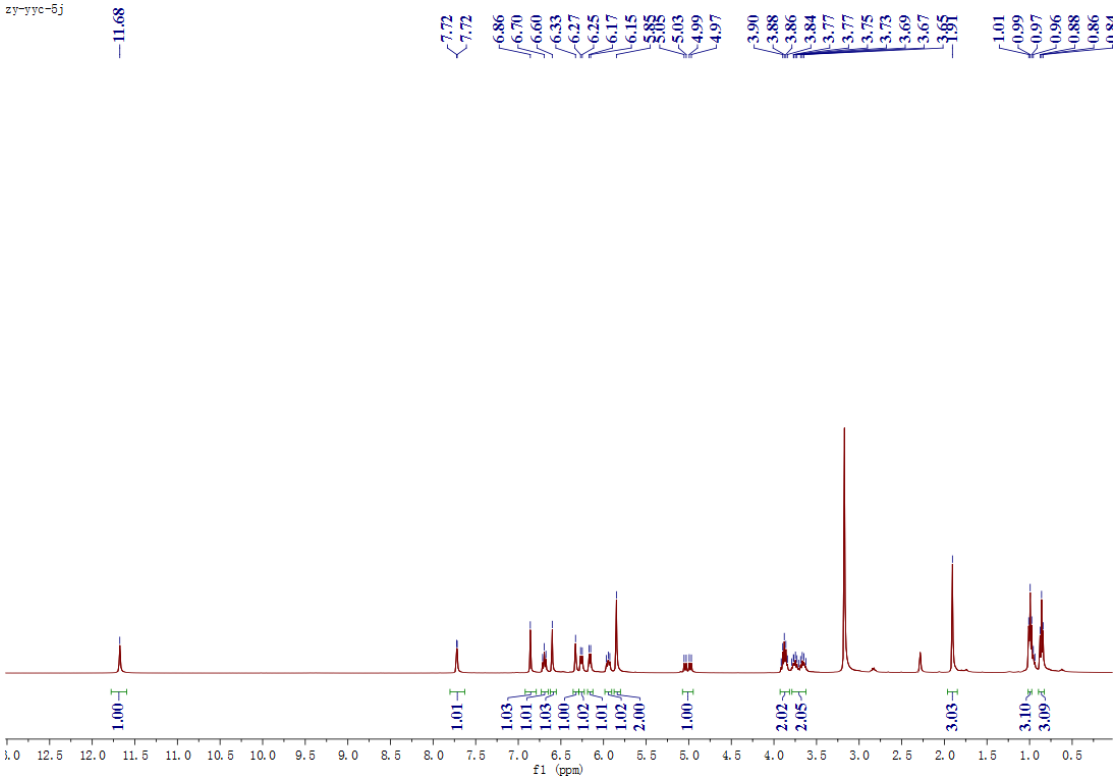


Fig. 106.^{1}H NMR of compound 5a

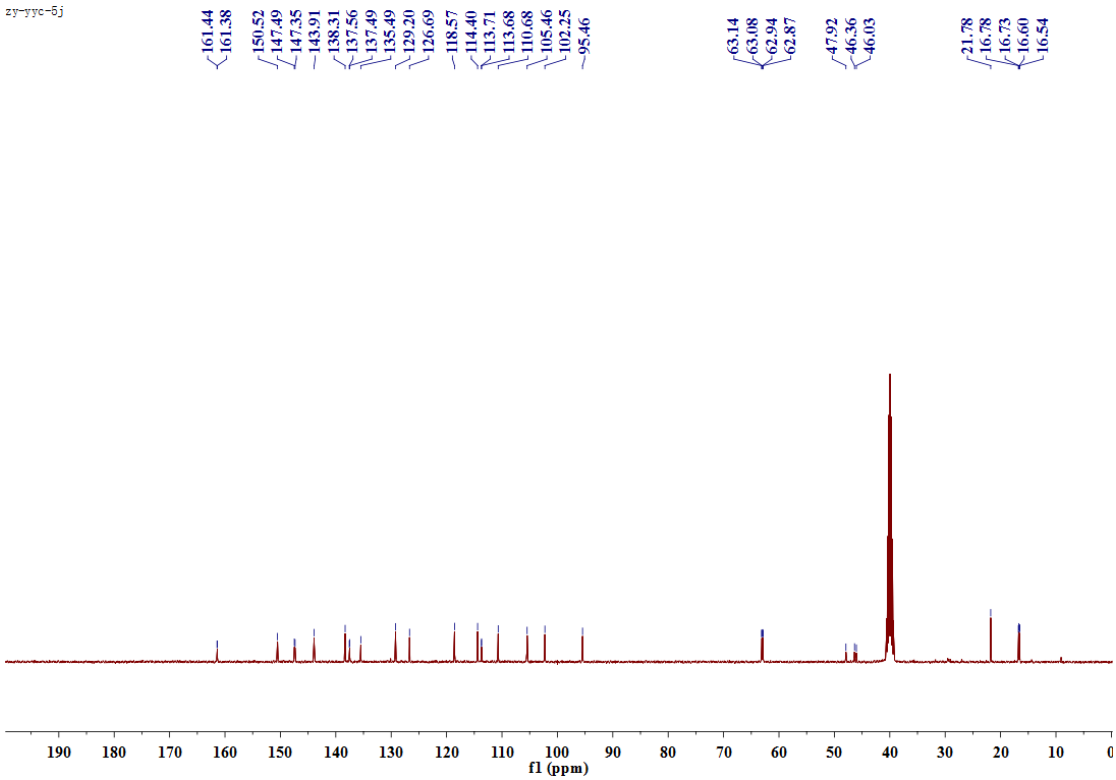


Fig. 107.^{13}C NMR of compound 5a

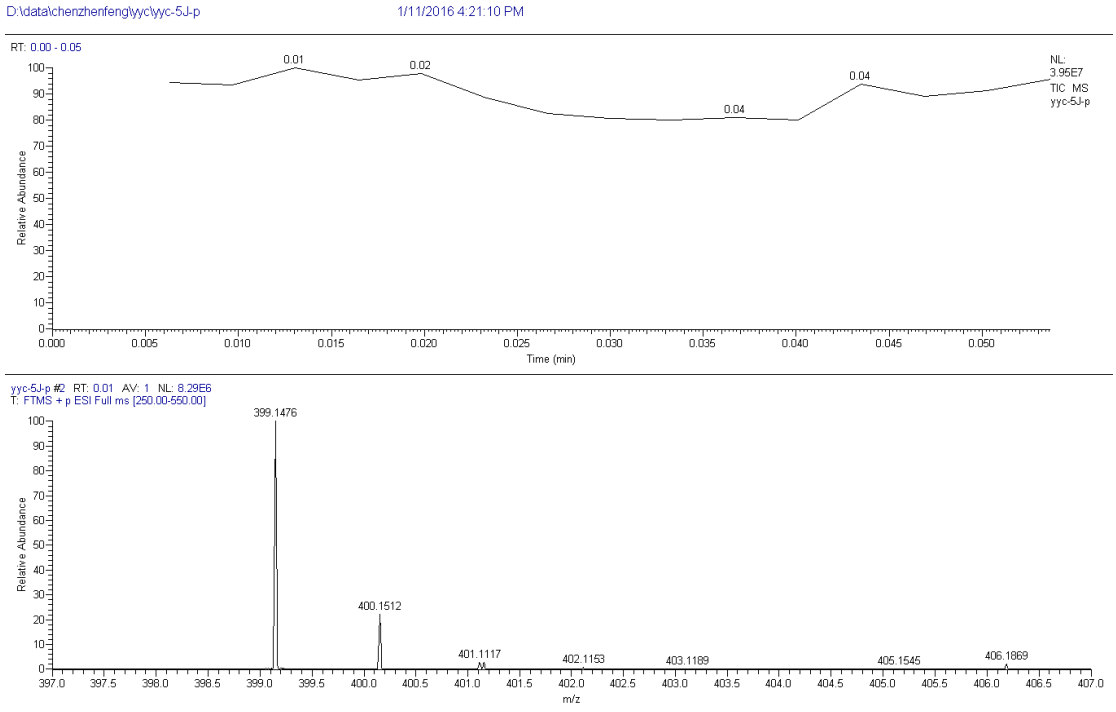


Fig. 108.ESI-HRMS of compound 5a

5b: Yield63.13%, ^{1}H NMR (400 MHz, DMSO) δ 12.10 (s, 1H), 8.79 (s, 1H), 8.59 (s, 1H), 7.67 (s, 1H), 7.42 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.34 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.25 (dd, *J* = 12.0, 8.3 Hz, 3H), 3.97 (dq, *J* = 14.2, 7.1 Hz, 4H), 3.27 (d, *J* = 21.5 Hz, 2H), 2.36 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 6H).^{13}C NMR (101 MHz, DMSO) δ 161.91, 155.52, 150.44, 150.40, 138.36, 137.77, 133.82, 131.91, 131.19, 131.13, 130.97, 130.88, 129.41, 126.80, 121.47, 121.44, 119.29, 115.60, 61.90, 61.84, 40.60, 40.40, 40.19, 39.98, 39.77, 39.56, 39.35, 32.93, 31.59, 20.87, 16.72, 16.66. ESI-HRMS *m/z* Calc for $C_{22}H_{25}N_2O_4P$ [M-H]$^-$: 411.1474; found: 411.1549.

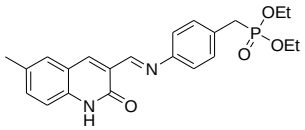


Fig. 109. Chemical structure of compound5b

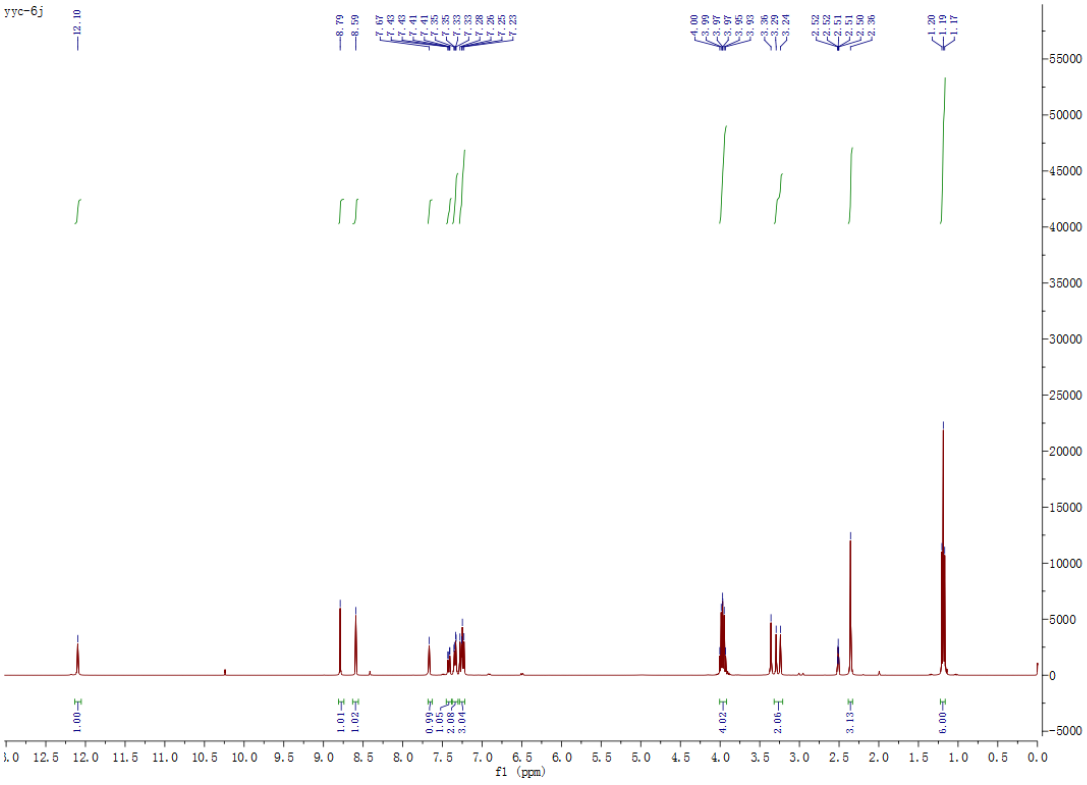


Fig. 110.^{1}H NMR of compound 5b

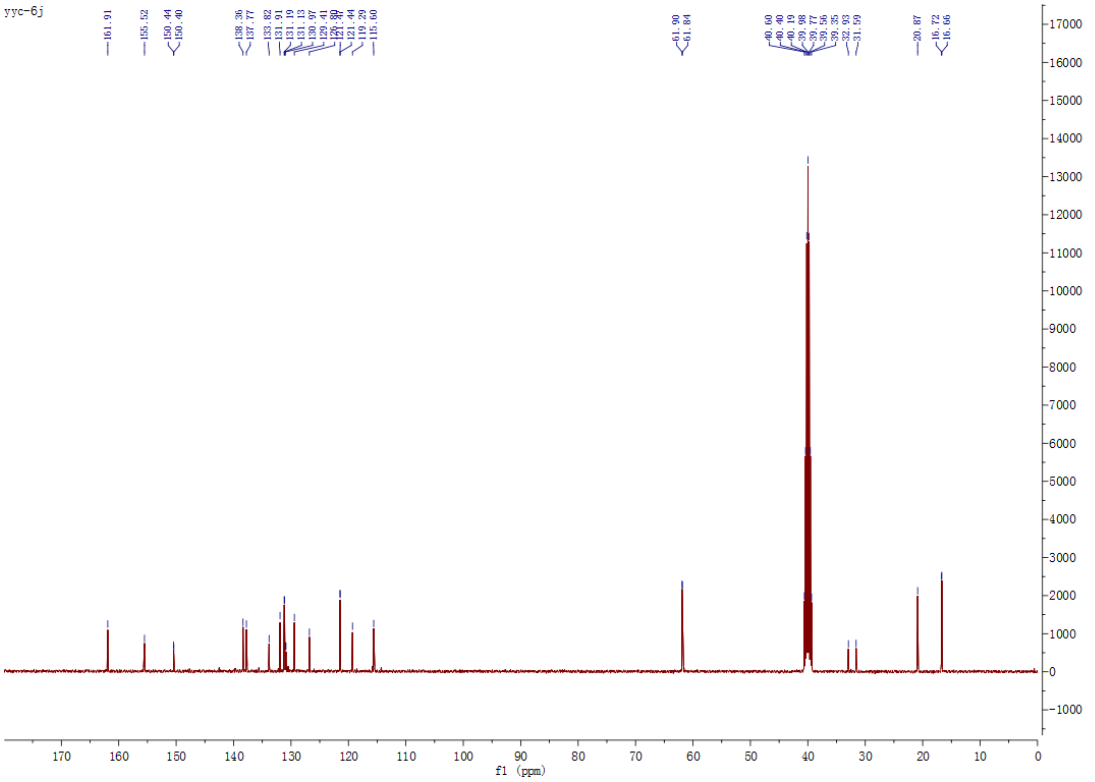


Fig. 111.^{13}C NMR of compound 5b

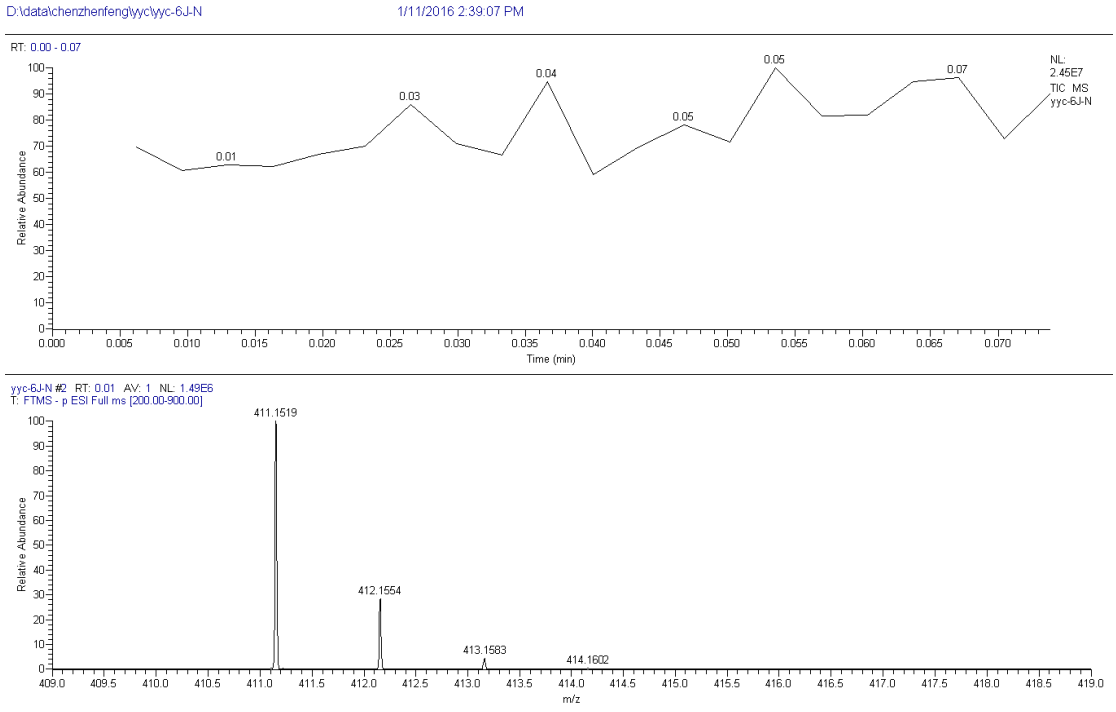


Fig. 112.ESI-HRMS of compound 5b

5c: Yield64.65%, ^{1}H NMR (500 MHz, DMSO) δ 12.06 (s, 1H), 8.80 (s, 1H), 8.64 (s, 1H), 7.46 (d, *J* = 2.7 Hz, 1H), 7.34 (dd, *J* = 8.4, 2.3 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 1H), 7.24 (dd, *J* = 8.9, 2.8 Hz, 3H), 3.96 (dd, *J* = 15.1, 7.1 Hz, 4H), 3.26 (d, *J* = 21.5 Hz, 2H), 1.18 (t, *J* = 7.0 Hz, 6H).^{13}C NMR (126 MHz, DMSO) δ 190.33, 161.58, 155.45, 154.95, 150.38, 150.35, 137.62, 135.01, 131.19, 131.14, 131.02, 130.94, 127.13, 122.03, 121.45, 121.43, 119.94, 116.98, 110.97, 61.90, 61.85, 55.99, 40.52, 40.45, 40.36, 40.28, 40.19, 40.02, 39.85, 39.69, 39.52, 32.85, 31.78, 16.70, 16.66, 1.60.ESI-HRMS *m/z* Calc for $C_{22}H_{25}N_2O_5P$ $[M+Na]^+$: 451.1399; found: 451.1404.

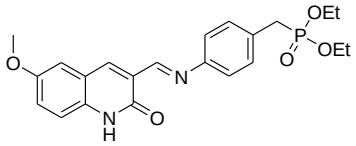


Fig. 113. Chemical structure of compound5c

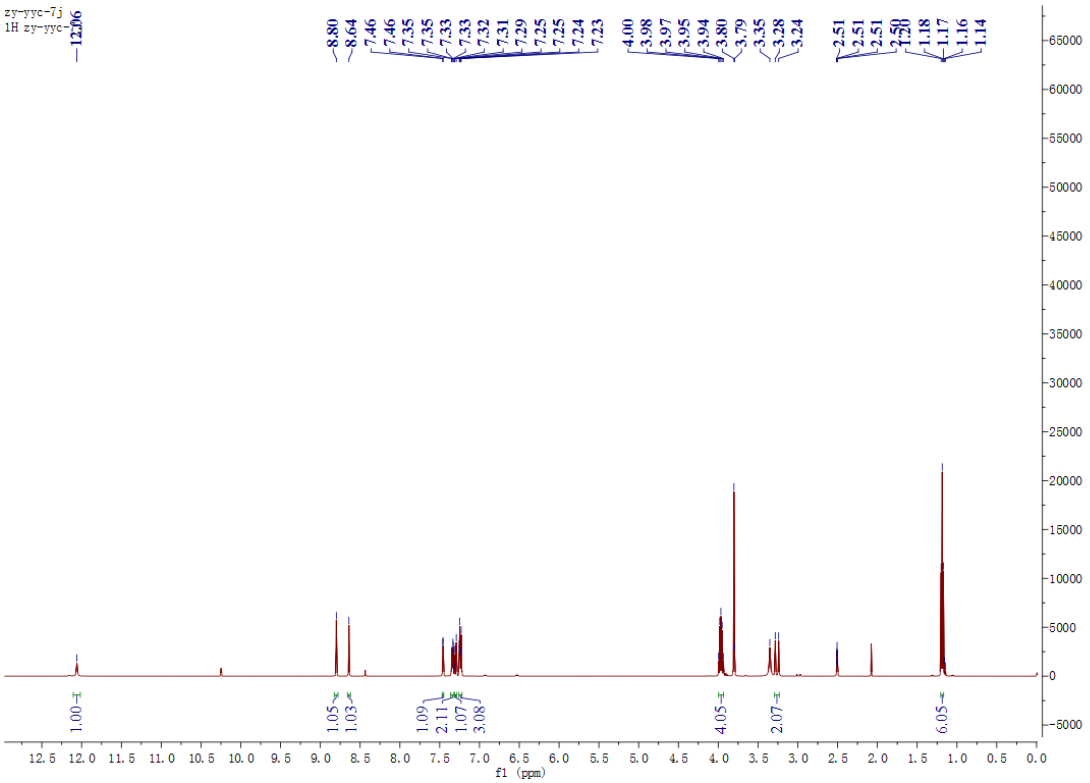


Fig. 114.^{1}H NMR of compound 5c

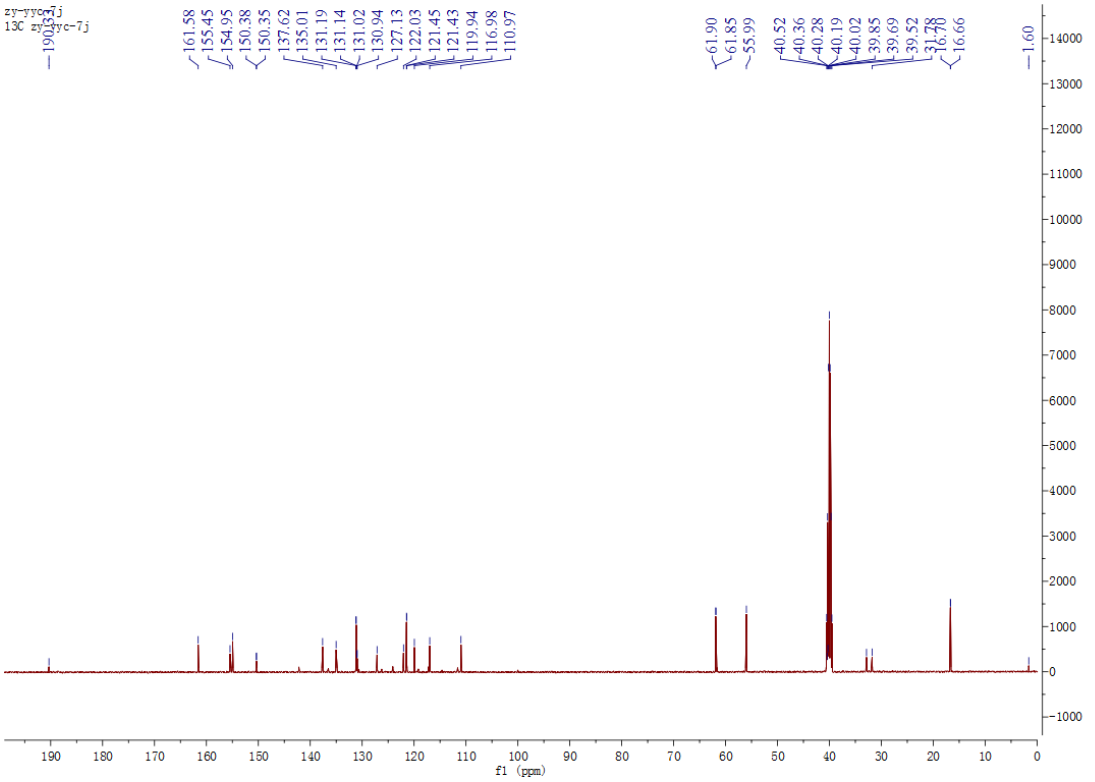


Fig. 115.^{13}C NMR of compound 5c

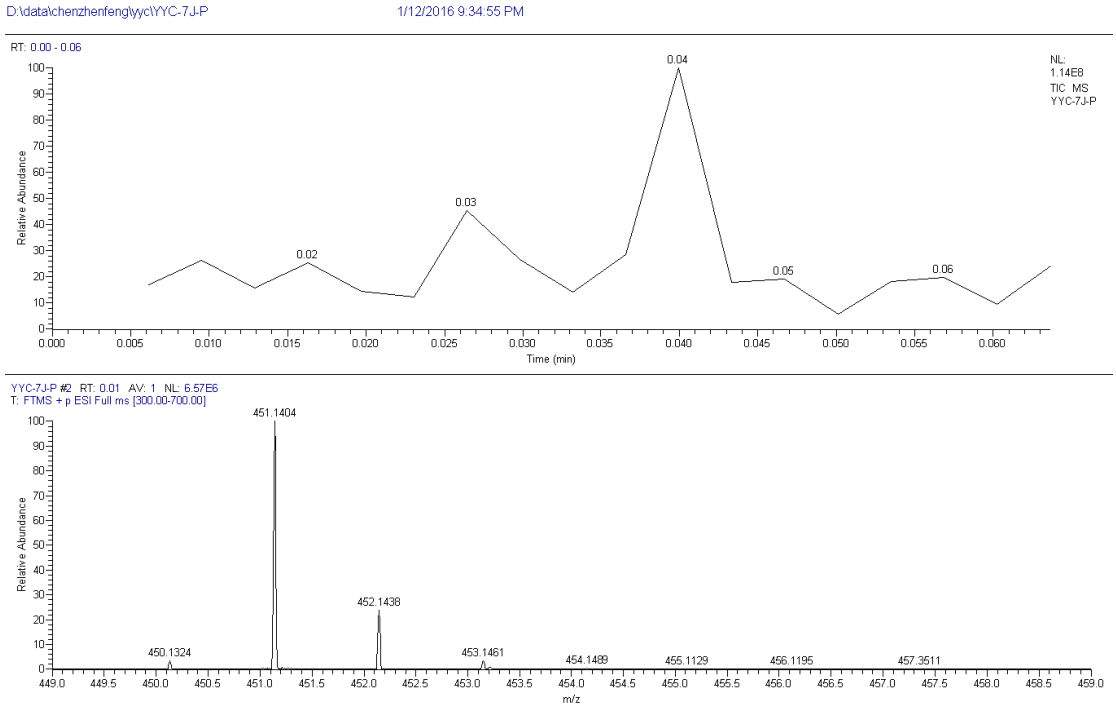


Fig. 116.ESI-HRMS of compound 5c

5d: Yield70.98%, ^{1}H NMR (400 MHz, *d*-DMSO) δ 12.08 (s, 1H), 8.74 (s, 1H), 8.56 (s, 1H), 7.38 (d, J = 8.7 Hz, 2H), 7.32 (dd, J = 8.4, 2.3 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.15 (d, J = 8.2 Hz, 2H), 3.97 (dd, J = 8.0, 7.2 Hz, 4H), 3.26 (d, J = 21.5 Hz, 2H), 1.19 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 189.85, 161.90, 155.37, 153.66, 152.08, 144.53, 142.07, 140.40, 131.17, 122.90, 121.34, 113.87, 113.23, 107.29, 106.73, 103.01, 102.65, 95.37, 61.83, 32.81, 31.44, 16.71.ESI-HRMS *m/z* Calc for $C_{22}H_{23}N_2O_6P$ $[M+H]^+$: 443.1372; found: 443.1391.

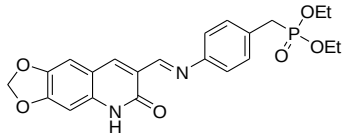


Fig. 113. Chemical structure of compound5d

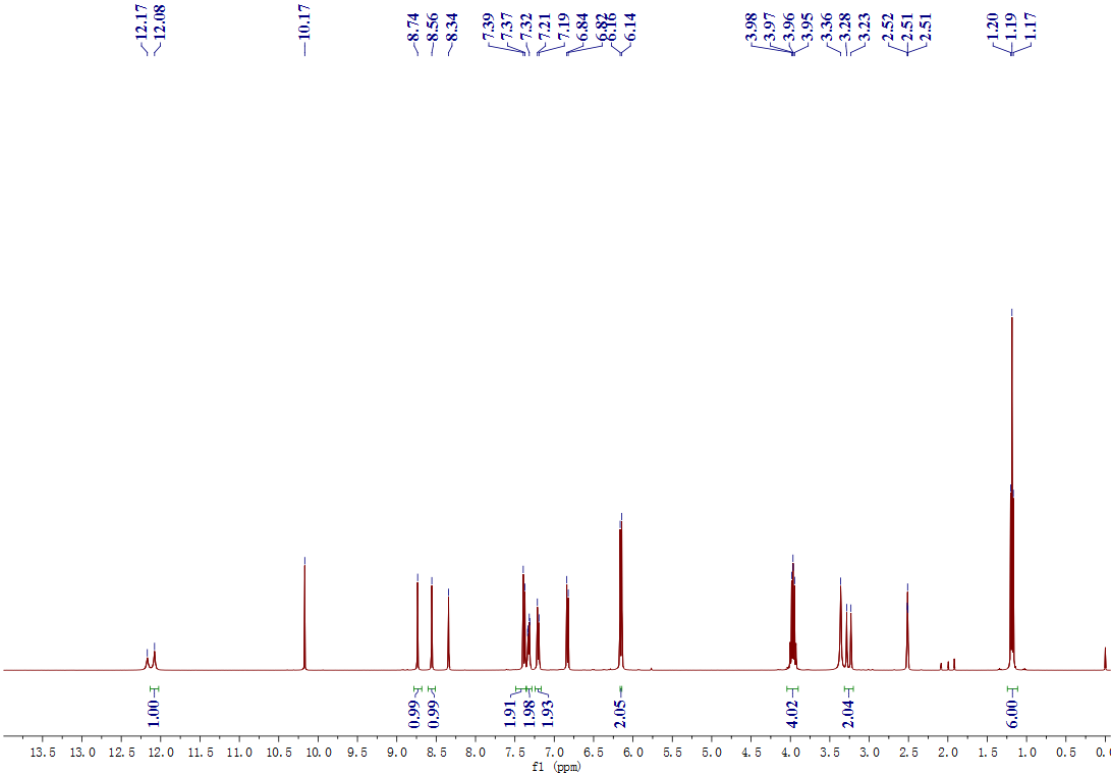


Fig. 114.^{1}H NMR of compound 5d

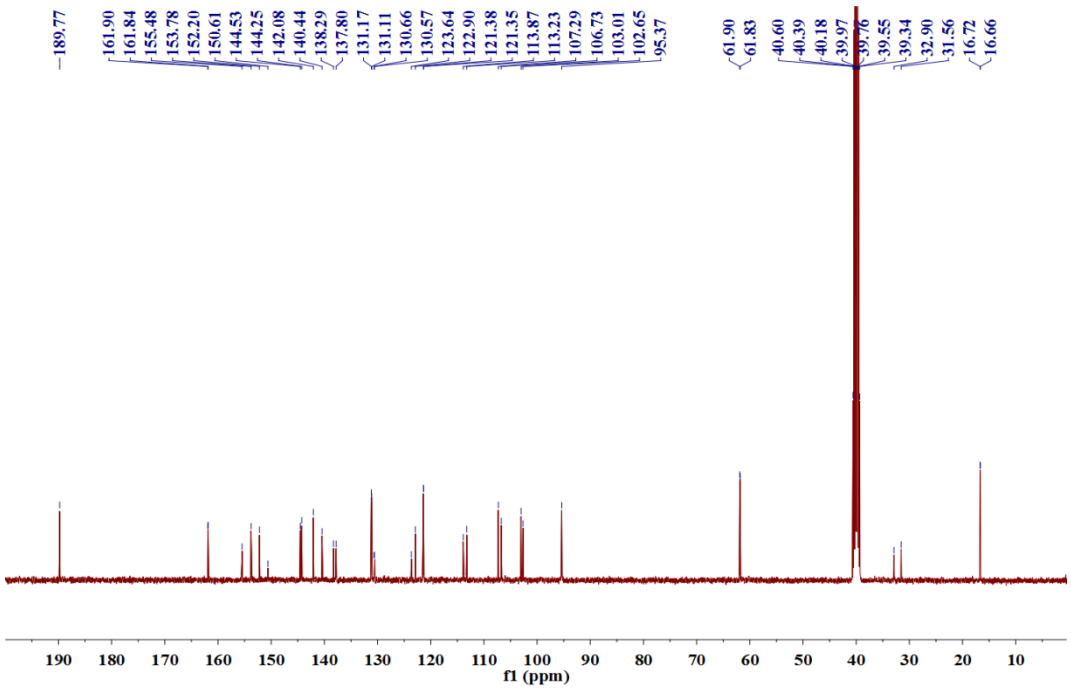


Fig. 115.^{13}C NMR of compound 5d

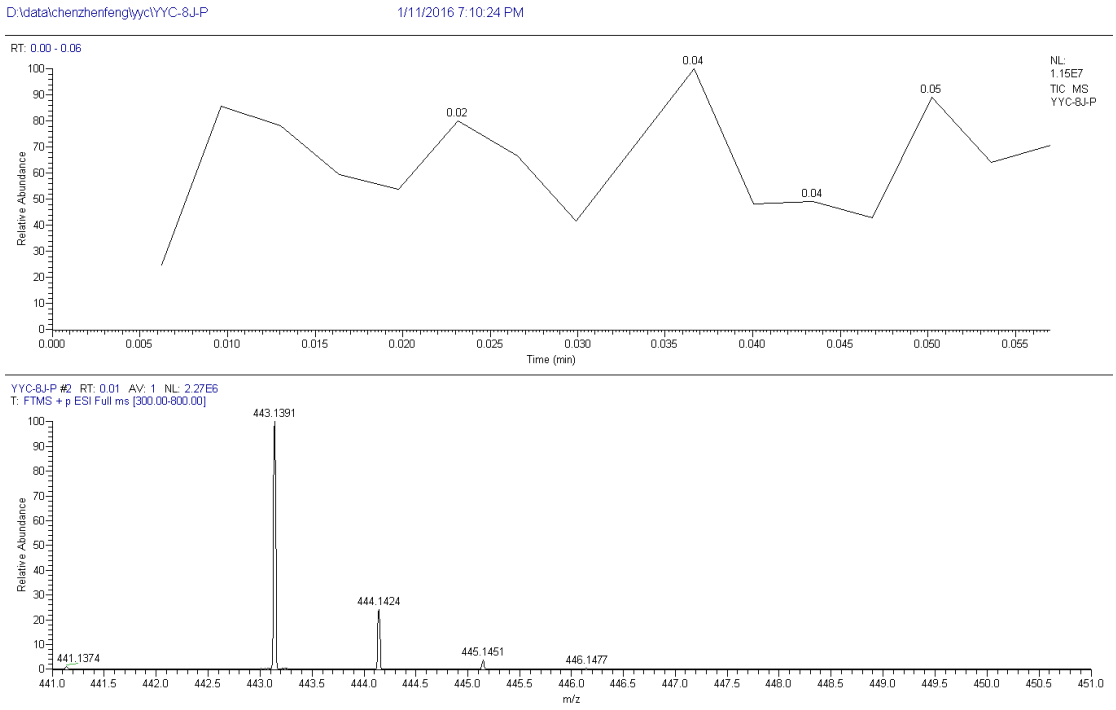


Fig. 116.ESI-HRMS of compound 5d