# Supplementary Information

## Substituent-Dependent Fluorescent Sensors for Zinc Ions Based on Carboxamidoquinoline

Yu Zhang,[a] Xiangfeng Guo,*[a] Lihua Jia,[a] Shicheng Xu,[a] Zhihui Xu,[a] Libo Zheng[a] and Xuhong Qian*[b]

[a]*College of Heilongjiang Province Key Laboratory of Fine Chemicals, Qiqihar University, Qiqihar, 161006, China, and* [b]*Shanghai Key Laboratory of Chemical Biology, East China University of Science and Technology, Shanghai, 200237, China*

*Corresponding author: xfguo@163.com; xhqian@ecust.edu.cn

**Materials and methods**

All of solvents were of analytic grade. All photophysical studies of carboxamidoquinoline were operated at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES). The stock solution of carboxamidoquinoline was prepared in methanol (1 mM). Cationic stock solutions were prepared from $NaClO_4$, $KClO_4$, $Mg(ClO_4)_2$, $Ca(NO_3)_2$, $Fe_2(SO_4)_3$, $CoSO_4$, $NiSO_4$, $Cu(NO_3)_2$, $ZnSO_4$, $AgNO_3$, $CdSO_4$, $HgCl_2$, or $Pb(NO_3)_2$ in distilled water with a concentration of 0.05 or 2.5 M, respectively. The added volume of cationic stock solutions was less than 100 μL to remain the concentration of sensors unchanged. All spectra were recorded at 25 °C. Fluorescence spectra of sensors were obtained by excitation at the absorption maximum of each ligand. The fluorescence quantum yield was determined using that of quinine bisulphate ($\Phi$ = 0.55) in 100 mM $H_2SO_4$ as a standard.[1]

**Determination of the apparent dissociation constants of $Zn^{2+}$ complexes.** The 100 mM HEPES buffer (methanol/water = 1:9 (*v*/*v*), pH 7.2, *I* = 0.1 (KCl), 10 mM *N*-(2-aminoethyl)ethane-1,2-diamine, 0-5.0 mM $ZnSO_4$) were prepared.

The stability constants for $Zn^{2+}$ complexes of *N*-(2-aminoethyl)ethane-1,2-diamine were taken from ref. 2.

For *N*-(2-aminoethyl)ethane-1,2-diamine (25 °C, *I* = 0.1 (KCl)),

$pK_{a1}$ = 9.94, $pK_{a2}$ = 9.13, $pK_{a3}$ = 4.34, log*K*(ZnL) = 8.90

Protonation constants must be corrected upward by 0.11 when working at 0.1 M ionic strength. Using these values, concentrations of free $Zn^{2+}$ were calculated through the method described in ref. 3. Thus,

$[Zn^{2+}]_{free} = [Zn^{2+}]_{total}/\beta' a_M[L]_{free}$ (Eq. 1)

$\beta' = K(ZnL)/(a_M a_L)$

$a_L = 1 + 10^{(pKa1-pH)} + 10^{(pKa1+ pKa2-2pH)} + 10^{(pKa1+ pKa2+ pKa3-3pH)}$

$[L]_{free} \approx [L]_{total} - [Zn^{2+}]_{total}$

$[L]_{total}$ was set at 10 mM, and $[Zn^{2+}]_{total}$ was varied from 0-5.0 mM.

The values of $[Zn^{2+}]_{free}$ obtained from equation (1):

$[Zn^{2+}]_{total}$ (mM): 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.5, 2.8, 3.2, 3.6, 4.0, 4.5, 5.0

$[Zn^{2+}]_{free}$ (μM): 2.02, 4.12, 6.30, 8.59, 10.98, 13.47, 16.08, 18.82, 21.68, 24.70, 27.86, 32.93, 38.42, 46.49, 55.57, 65.86, 80.82, 98.79

The stock solution of carboxamidoquinoline in methanol (1 mM) was diluted to the final concentration of 10 μM with 100 mM HEPES buffer (methanol/water = 1:9 (*v*/*v*), pH 7.2, $I$ = 0.1 (KCl), 10 mM *N*-(2-aminoethyl)ethane-1,2-diamine, 0-5.0 mM $ZnSO_4$). The new absorption band produced by $Zn^{2+}$ at longer wavelength was measured, and fitted to the following equation (Eq. 2).[4]

$$A = (A_{min}K_d + A_{max}[Zn^{2+}]_{free})/(K_d + [Zn^{2+}]_{free}) \quad \text{(Eq. 2)}$$

In the above equation, $A$ is absorbance, $A_{max}$ is the maximum absorbance, $A_{min}$ is absorbance in the absence of $Zn^{2+}$, $[Zn^{2+}]_{free}$ is the free $Zn^{2+}$ concentration, and $K_d$ is the apparent dissociation constants.

**Cell studies.** Yeast (*Saccharomyces cerevisiae*) was obtained from Zhuhai Ziying Biotechnology Co. Ltd. and used as a cell model. Before use, it was dispersed in the YPD medium (1.0% yeast extract, 4.0% peptone, 2.0% glucose, 0.2% $(NH_4)_2SO_4$) and incubated for 12 h at 37 °C. For yeast staining, some colonies were selected and incubated with 10 μM **AQZ4MP** at pH 7.2 (DMSO/water = 1:9 (*v*/*v*), $I$ = 0.1 (KCl), 100 mM HEPES) for 1 h at 37 °C. The sensor solution was then removed and the cells were washed twice with 10 mM PBS buffer (pH 7.4) to remove free **AQZ4MP**. **AQZ4MP-**pretreated cells were then diluted upon addition of 40 μM $ZnSO_4$ and incubated for different time at 37 °C. The cells for experiments were dropped on glass slides and covered with a cover glass.

**Synthesis of compound 6-7**

**The preparation of compound 6.** Stannous chloride crystal (10 equiv.) was added to a stirred solution of nitroquinoline **5** (200 mg) and hydrochloric acid (5 mL) in ethanol (20 mL). After stirred 0.5 h at rt, the reaction mixture was heated to 40-50 °C for 2 h. After cooling to rt, the mixture was basified with aqueous ammonia and extracted with chloroform (3 × 15 mL). The combined extract was dried over magnesium sulfate and evaporated to afford a yellow solid, which was purified by column chromatography over silica gel (100-200 Mesh) using the chloroform/ethyl acetate mixture as the eluent. Yields and spectral details of each product are given below.

**8-Amino-4-chloroquinoline (6a)**: Yield: 65% (111 mg), mp: 86.7-87.8 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.60 (d, $J$ = 4.4 Hz, 1H), 7.52 (d, $J$ = 8.0 Hz, 1H), 7.46 (d, $J$ = 4.8 Hz, 1H), 7.42 (t, $J$ = 8.0 Hz, 1H), 6.98 (d, $J$ = 7.6 Hz, 1H), 5.17 ($NH_2$, s, 2H) ppm. MS (ESI): *m*/*z* 179.0 $(M + H)^+$.

**8-Amino-4-methoxyquinoline (6b)**: Yield: 72% (122 mg), mp: 95.7-96.4 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.64 (d, $J$ = 5.6 Hz, 1H), 7.51 (d, $J$ = 7.6 Hz, 1H), 7.34 (d, $J$ = 7.6 Hz, 1H), 6.97 (t, $J$ = 8.0 Hz, 1H), 6.78 (d, $J$ = 4.8 Hz, 1H), 5.12 ($NH_2$, s, 2H), 4.07 ($OCH_3$, s, 3H) ppm. MS (ESI): *m*/*z* 175.1 $(M + H)^+$.

**8-Amino-4-morpholinoquinoline (6c)**: Yield: 59% (105 mg), mp: 172.0-173.3 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.64 (d, $J$ = 4.8 Hz, 1H), 7.33 (m, 2H), 6.92 (d, $J$ = 5.6 Hz, 1H), 6.87 (d, $J$ = 4.8 Hz, 1H), 5.02 ($NH_2$, s, 2H), 4.00 ($O(CH_2CH_2)_2N$, t, $J$ = 4.6 Hz, 4H), 3.25 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H) ppm. MS (ESI): *m*/*z* 203.1 $(M + H)^+$.

**8-Amino-4-(piperidin-1-yl)quinoline (6d)**: Yield: 76% (134 mg), mp: 143.9-144.9 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.56 (d, $J$ = 4.8 Hz, 1H), 7.32 (d, $J$ = 9.6 Hz, 1H), 7.26 (t, $J$ = 3.6 Hz, 1H), 6.87 (d, $J$ = 8.8 Hz, 1H), 6.81 (d, $J$ = 5.2

Hz, 1H), 4.96 ($NH_2$, s, 2H), 3.17 ($N(CH_2CH_2)_2CH_2$, t, $J$ = 4.8 Hz, 4H), 1.83 ($N(CH_2CH_2)_2CH_2$, m, 4H), 1.69 ($N(CH_2CH_2)_2CH_2$, m, 2H) ppm. MS (ESI): $m/z$ 228.1 $(M + H)^+$.

**8-Amino-2-morpholinoquinoline (6e)**: Yield: 82% (146 mg), mp: 171.6-172.6 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.61 (d, $J$ = 4.8 Hz, 1H), 7.30 (t, $J$ = 6.2 Hz, 1H), 7.26 (d, $J$ = 8.0 Hz, 1H), 6.89 (d, $J$ = 8.4 Hz, 1H), 6.84 (d, $J$ = 4.8 Hz, 1H), 5.00 ($NH_2$, s, 2H), 3.98 ($O(CH_2CH_2)_2N$, t, $J$ = 4.6 Hz, 4H), 3.23 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H) ppm. MS (ESI): $m/z$ 203.1 $(M + H)^+$.

**8-Amino-5-morpholinoquinoline (6f)**: Yield: 83% (148 mg), mp: 128.3-130.0 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 8.78 (d, $J$ = 4.0 Hz, 1H), 8.54 (d, $J$ = 10.0 Hz, 1H), 7.41 (m, 1H), 7.06 (d, $J$ = 8.0 Hz, 1H), 7.08 (d, $J$ = 10.0 Hz, 1H), 4.92 ($NH_2$, s, 2H), 3.94 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H), 2.99 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H) ppm. MS (ESI): $m/z$ 203.1 $(M + H)^+$.

**Preparation of compound 7.** 2-Chloroacetyl chloride (1.5 equiv.) was dissolved in chloroform (5 mL), then added dropwise to a cooled, stirred solution of amionquinoline **6** (100 mg) and pyridine (2.0 equiv.) in chloroform (10 mL) within 1 h, after stirred 2 h at rt, the mixture was evaporated to afford a white solid, which was purified by column chromatography over silica gel (100-200 Mesh) using the chloroform/ethyl acetate mixture as the eluent. Yields and spectral details of each product are given below.

**2-Chloro-*N*-(4-chloroquinolin-8-yl)acetamide (7a)**: Yield: 93% (133 mg), mp: 149.2-150.3 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.90 (NHCO, s, 1H), 8.83 (d, $J$ = 7.6 Hz, 1H), 8.72 (d, $J$ = 4.4 Hz, 1H), 7.96 (d, $J$ = 9.6 Hz, 1H), 7.66 (t, $J$ = 8.2 Hz, 1H), 7.58 (d, $J$ = 4.4 Hz, 1H), 4.31 ($COCH_2$, s, 2H) ppm. MS (ESI): $m/z$ 255.0 $(M + H)^+$.

**2-Chloro-*N*-(4-methoxyquinolin-8-yl)acetamide (7b)**: Yield: 93% (134 mg), mp: 142.3-143.1 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.91 (NHCO, s, 1H), 8.74 (d, $J$ = 8.0 Hz, 1H), 8.70 (d, $J$ = 5.2 Hz, 1H), 7.92 (d, $J$ = 7.6 Hz, 1H), 7.50 (t, $J$ = 8.0 Hz, 1H), 6.82 (d, $J$ = 4.8 Hz, 1H), 4.30 ($COCH_2$, s, 2H), 4.07 ($OCH_3$, s, 3H) ppm. MS (ESI): $m/z$ 251.1 $(M + H)^+$.

**2-Chloro-*N*-(4-morpholinoquinolin-8-yl)acetamide (7c)**: Yield: 75% (100 mg), mp: 168.5-169.7 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.99 (NHCO, s, 1H), 8.70 (m, 2H), 8.73 (d, $J$ = 8.4 Hz, 1H), 7.49 (t, $J$ = 8.0 Hz, 1H), 6.92 (d, $J$ = 4.8 Hz, 1H), 4.30 ($COCH_2$, s, 2H), 3.99 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H), 3.26 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H) ppm. MS (ESI): $m/z$ 306.1 $(M + H)^+$.

**2-Chloro-*N*-(4-(piperidin-1-yl)quinolin-8-yl)acetamide (7d)**: Yield: 77% (103 mg), mp: 118.8-119.7 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.99 (NH, s, 1H), 8.67 (d, $J$ = 8.8 Hz, 1H), 8.62 (d, $J$ = 4.8 Hz, 1H), 7.71 (d, $J$ = 9.6 Hz, 1H), 7.45 (t, $J$ = 8.2 Hz, 1H), 6.87 (d, $J$ = 5.2 Hz, 1H), 4.29 ($COCH_2$, s, 2H), 3.21 ($N(CH_2CH_2)_2CH_2$, t, $J$ = 5.0 Hz, 4H), 1.85 ($N(CH_2CH_2)_2CH_2$, m, 4H), 1.70 ($N(CH_2CH_2)_2CH_2$, m, 2H) ppm. MS (ESI): $m/z$ 304.1 $(M + H)^+$.

**2-Chloro-*N*-(2-morpholinoquinolin-8-yl)acetamide (7e)**: Yield: 75% (100 mg), mp: 170.8-172.4 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.99 (NHCO, s, 1H), 8.70 (m, 2H), 7.43 (d, $J$ = 8.4 Hz, 1H), 7.49 (t, $J$ = 8.2 Hz, 1H), 6.92 (d, $J$ = 5.2 Hz, 1H), 4.00 ($COCH_2$, s, 2H), 3.97 ($O(CH_2CH_2)_2N$, t, $J$ = 4.4 Hz, 4H), 3.26 ($O(CH_2CH_2)_2N$, t, $J$ = 4.2 Hz, 4H) ppm. MS (ESI): $m/z$ 306.1 $(M + H)^+$.

**2-Chloro-*N*-(5-morpholinoquinolin-8-yl)acetamide (7f)**: Yield: 98% (130 mg), mp: 150.2-150.8 °C. $^1$H-NMR ($CDCl_3$, 400MHz): $\delta$ 10.80 (NHCO, s, 1H), 8.87 (d, $J$ = 4.0 Hz, 1H), 8.68 (d, $J$ = 8.4 Hz, 1H), 8.56 (d, $J$ = 8.4 Hz, 1H),

7.49 (m, 1H), 7.16 (d, $J$ = 8.4 Hz, 1H), 4.30 ($COCH_2$, s, 2H), 3.97 ($O(CH_2CH_2)_2N$, t, $J$ = 4.0 Hz, 4H), 3.08 ($O(CH_2CH_2)_2N$, t, $J$ = 4.0 Hz, 4H) ppm. MS (ESI): $m/z$ 306.1 $(M + H)^+$.

**Additional spectra**

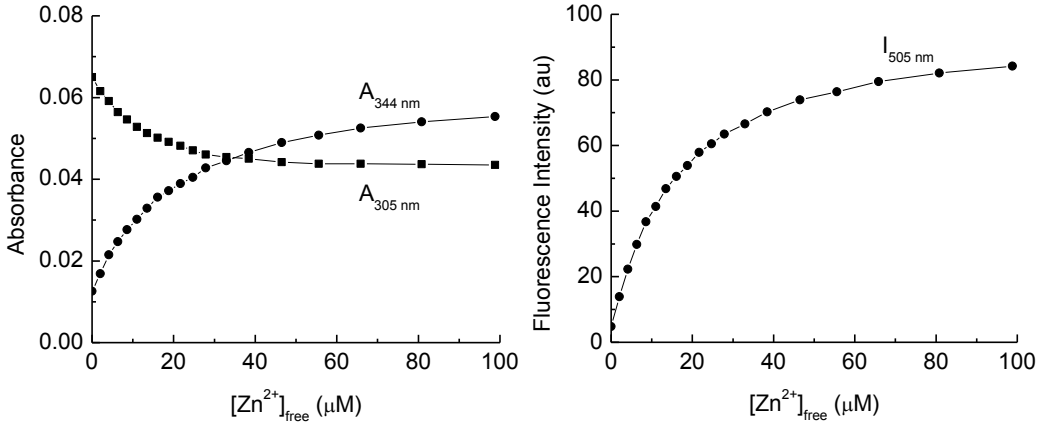


**Fig. S1** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 ($v/v$), $I$ = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 305 nm).

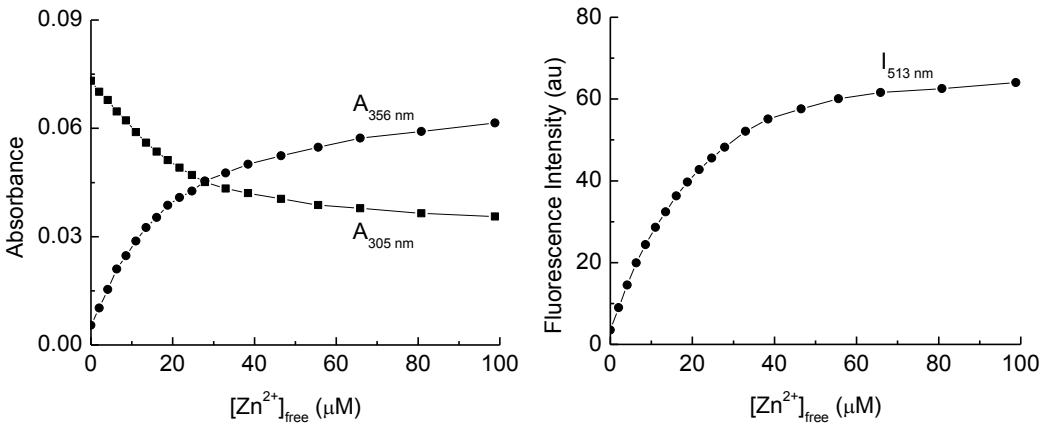


**Fig. S2** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ4Cl** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 ($v/v$), $I$ = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 305 nm).

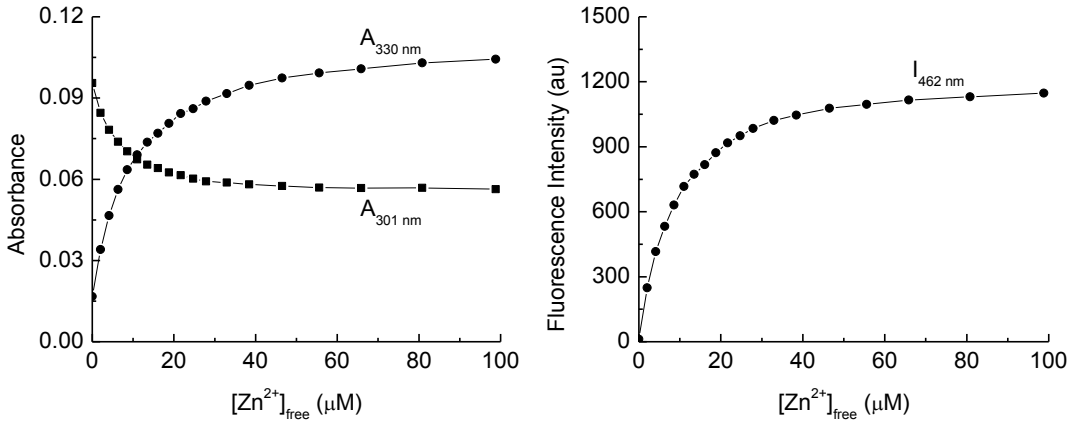


**Fig. S3** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ4MO** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 ($v/v$), $I$ = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 301 nm).

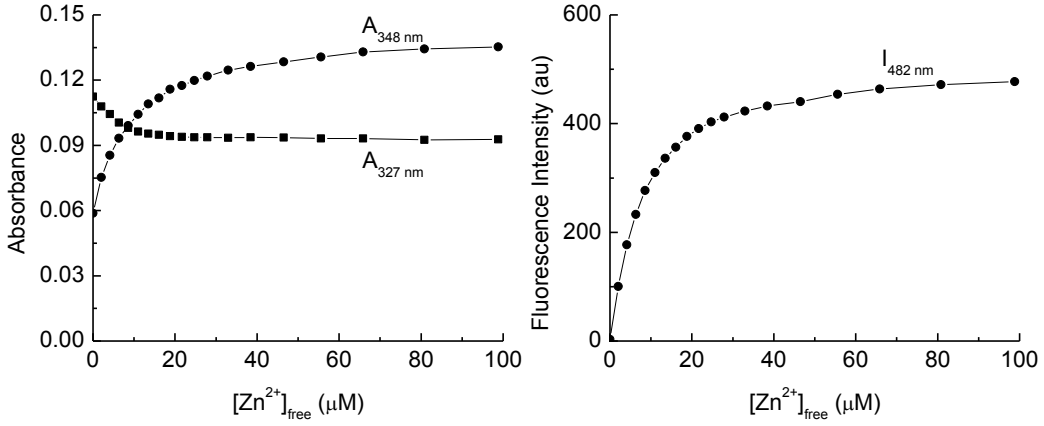


**Fig. S4** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ4MP** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 327 nm).

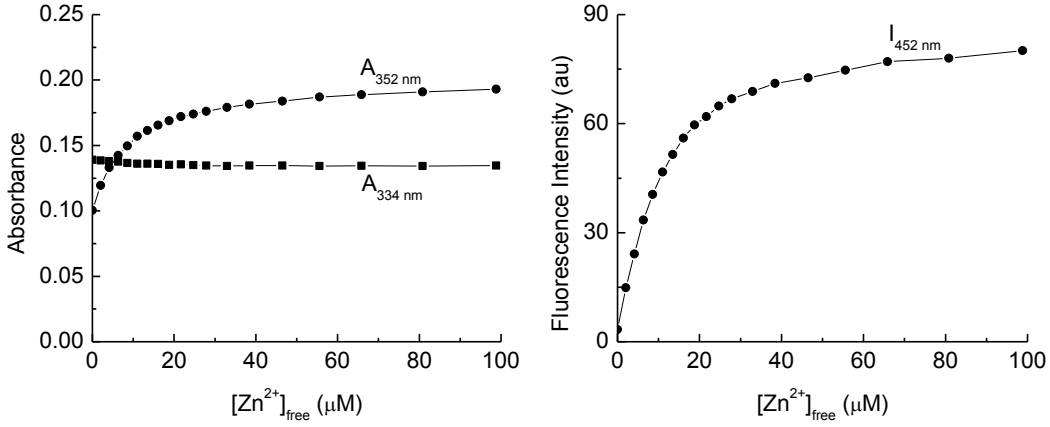


**Fig. S5** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ4PP** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 334 nm).

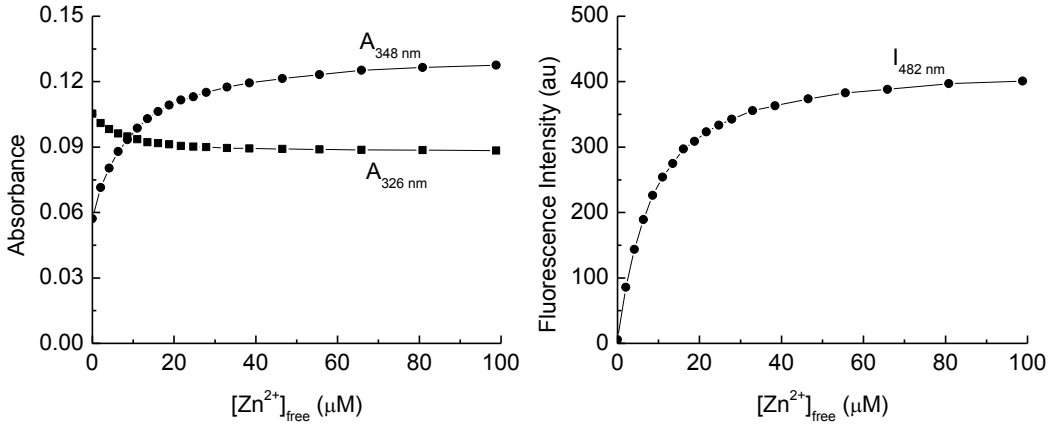


**Fig. S6** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ2MP** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 326 nm).

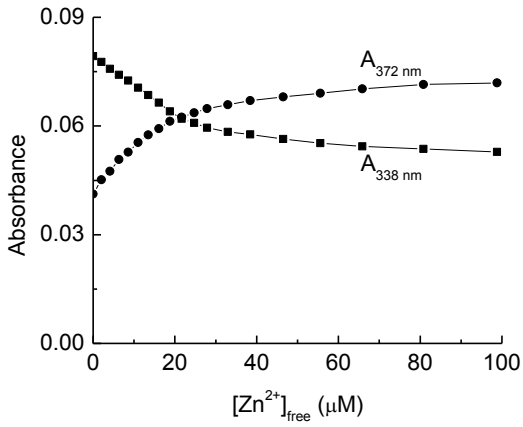


**Fig. S7** Absorbance of 10 μM **AQZ5MP** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES).

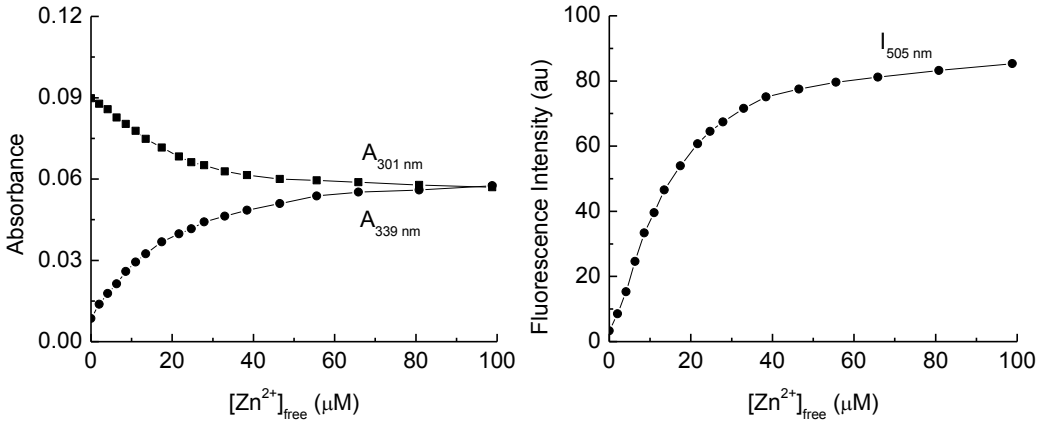


**Fig. S8** Absorbance (left) and fluorescence intensity (right) of 10 μM **AQZ8BA** as a function of added different concentrations of free $Zn^{2+}$ at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES, $\lambda_{ex}$ = 301 nm).

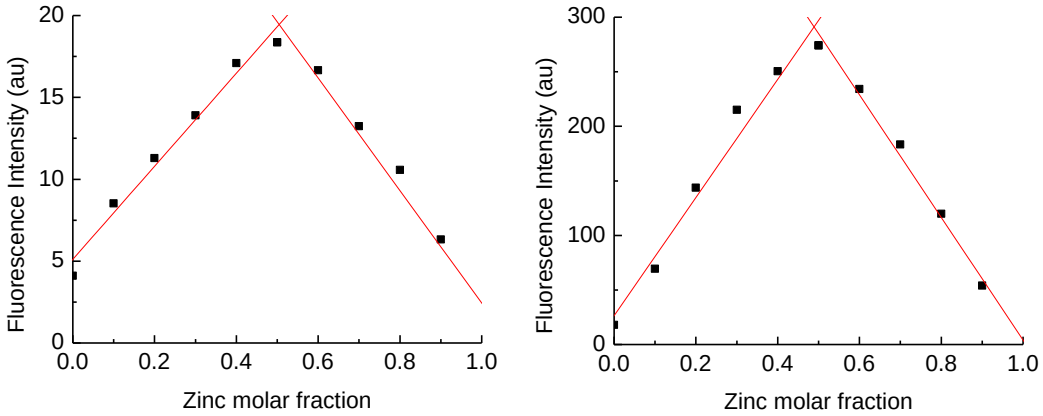


**Fig. S9** Job's plot for **AQZ4Cl** (left) and **AQZ4MO** (right) at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES). The total [carboxamidoquinoline] + [$ZnSO_4$] = 10 μM.

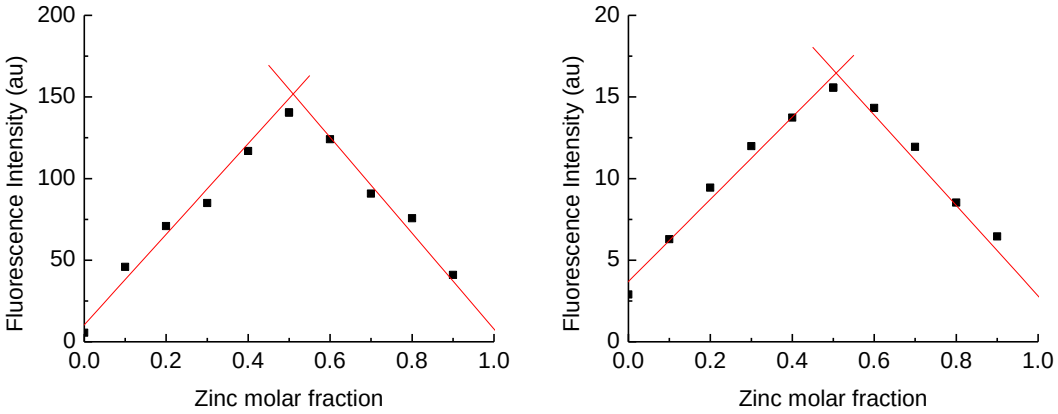


**Fig. S10** Job's plot for **AQZ4MP** (left) and **AQZ4PP** (right) at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES). The total [carboxamidoquinoline] + [$ZnSO_4$] = 10 μM.

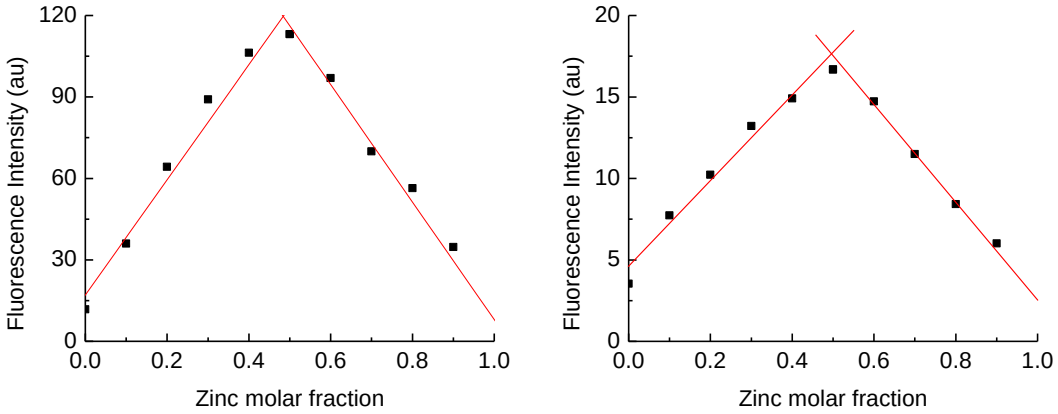


**Fig. S11** Job's plot for **AQZ2MP** (left) and **AQZ8BA** (right) at pH 7.2 (methanol/water = 1:9 (*v*/*v*), *I* = 0.1 (KCl), 100 mM HEPES). The total [carboxamidoquinoline] + [$ZnSO_4$] = 10 μM.

**NMR and MS copies**

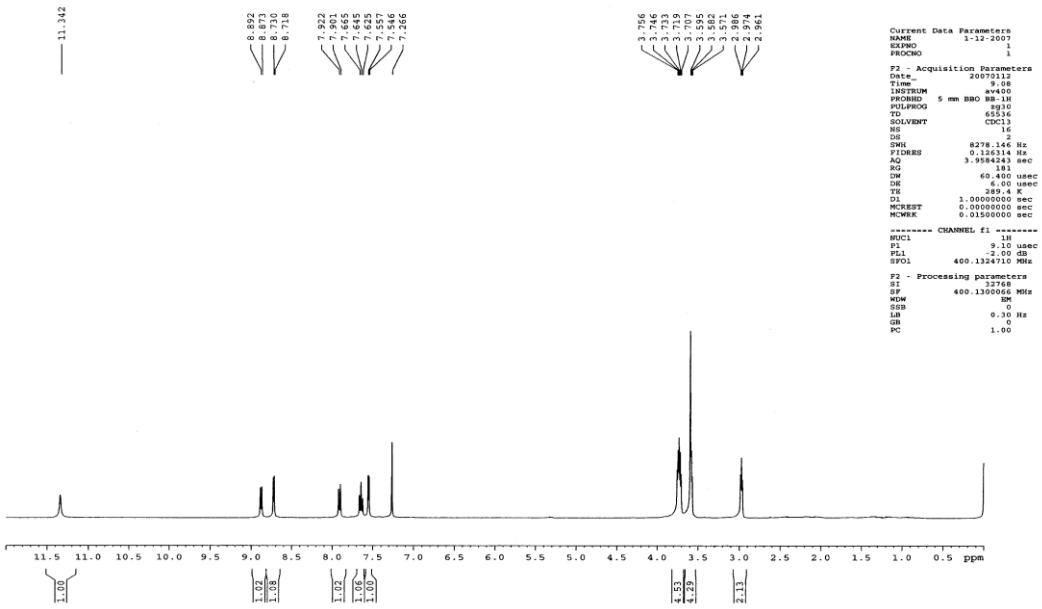

**Fig. S12** The $^1$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ4Cl**.

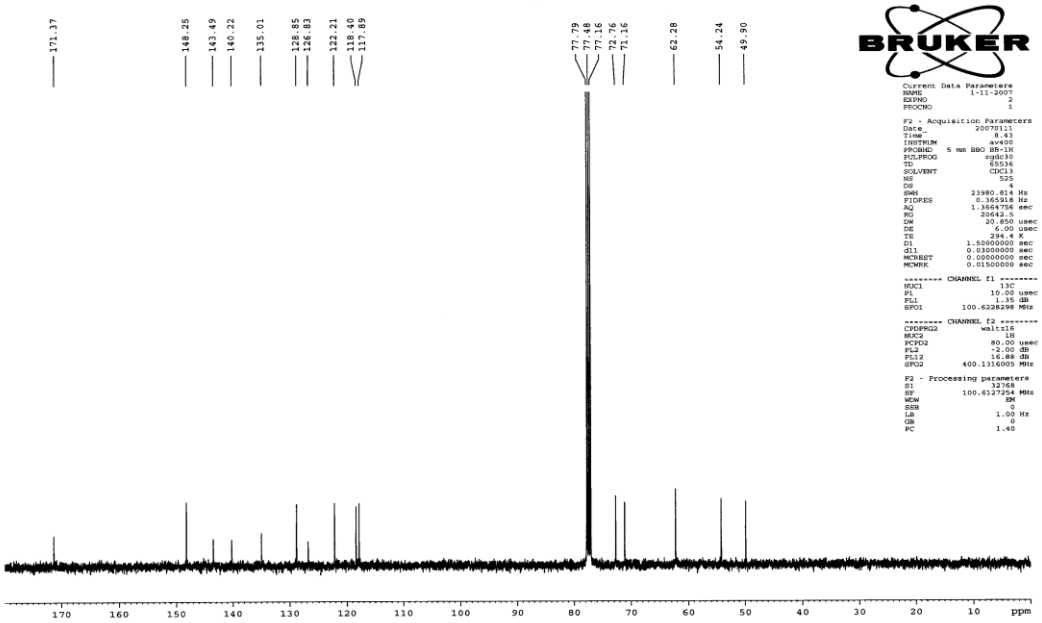


**Fig. S13** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ4Cl**.

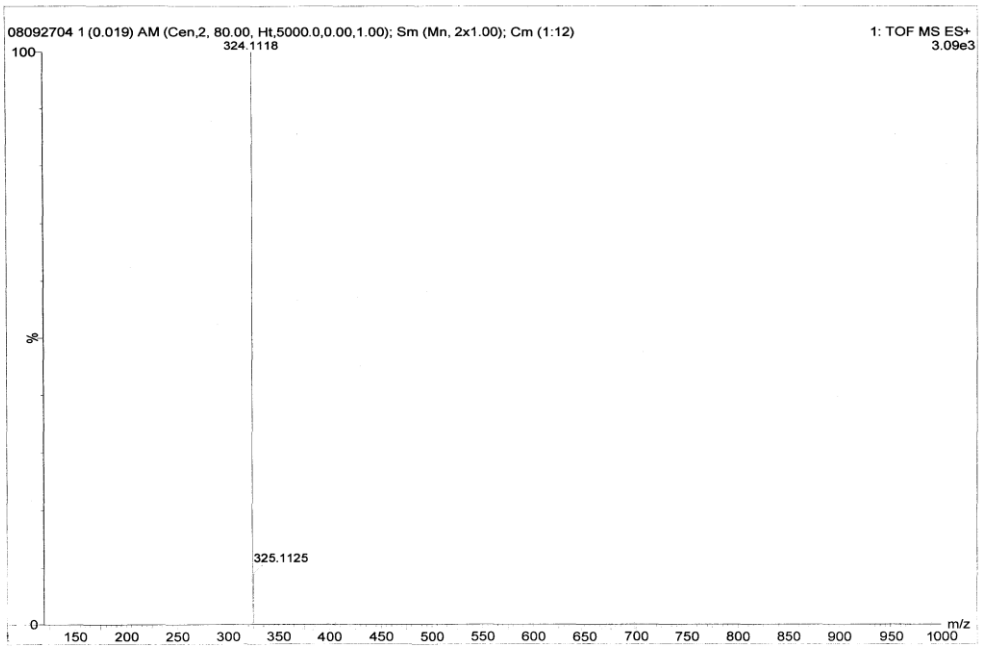


**Fig. S14** The MS spectrum of compound **AQZ4Cl**.

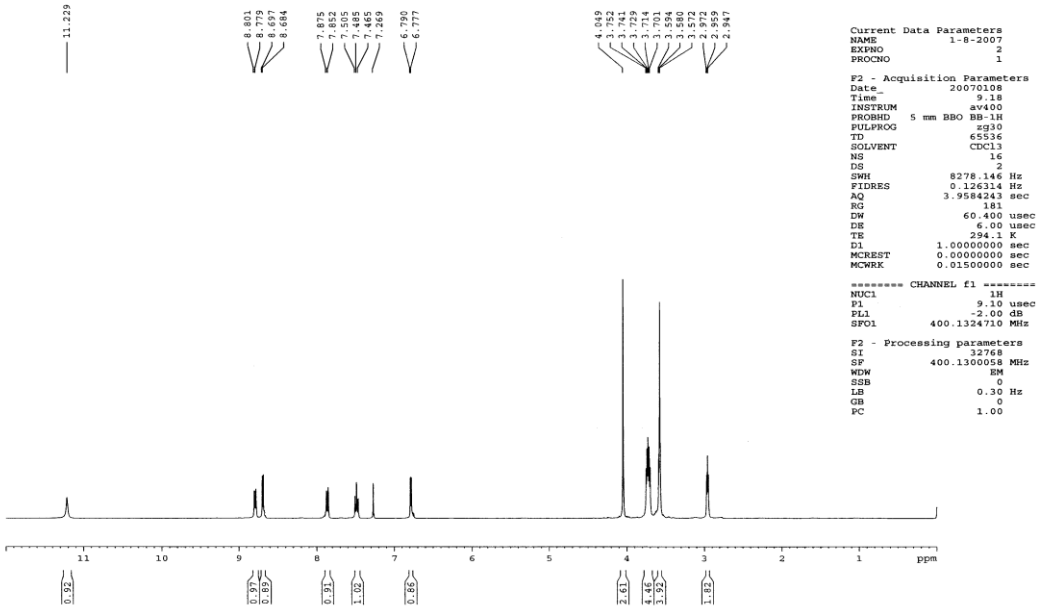


**Fig. S15** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ4MO**.

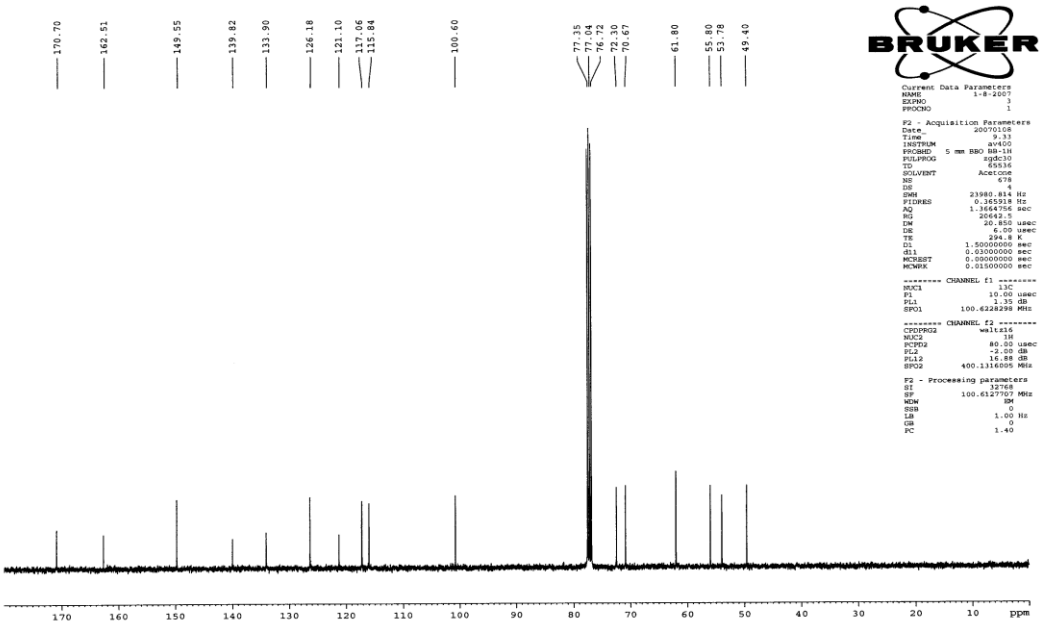


**Fig. S16** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ4MO**.

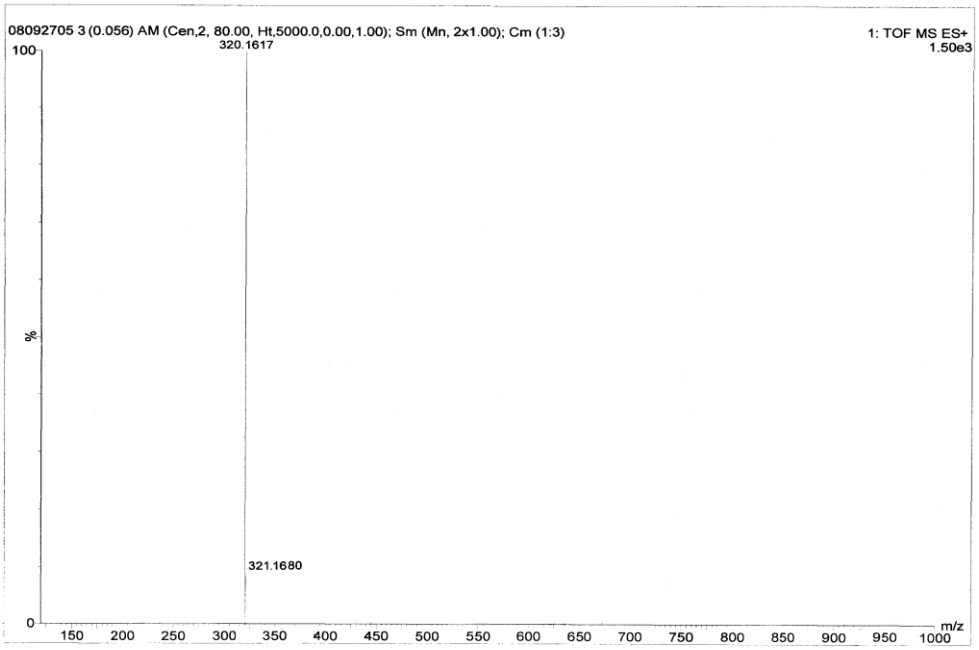


**Fig. S17** The MS spectrum of compound **AQZ4MO**.

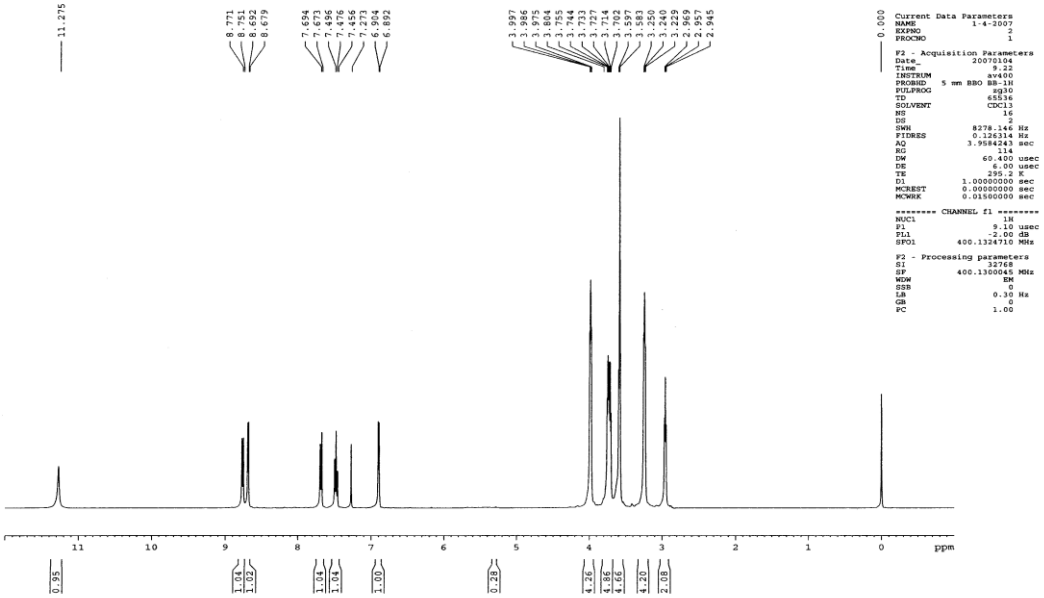


**Fig. S18** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ4MP**.

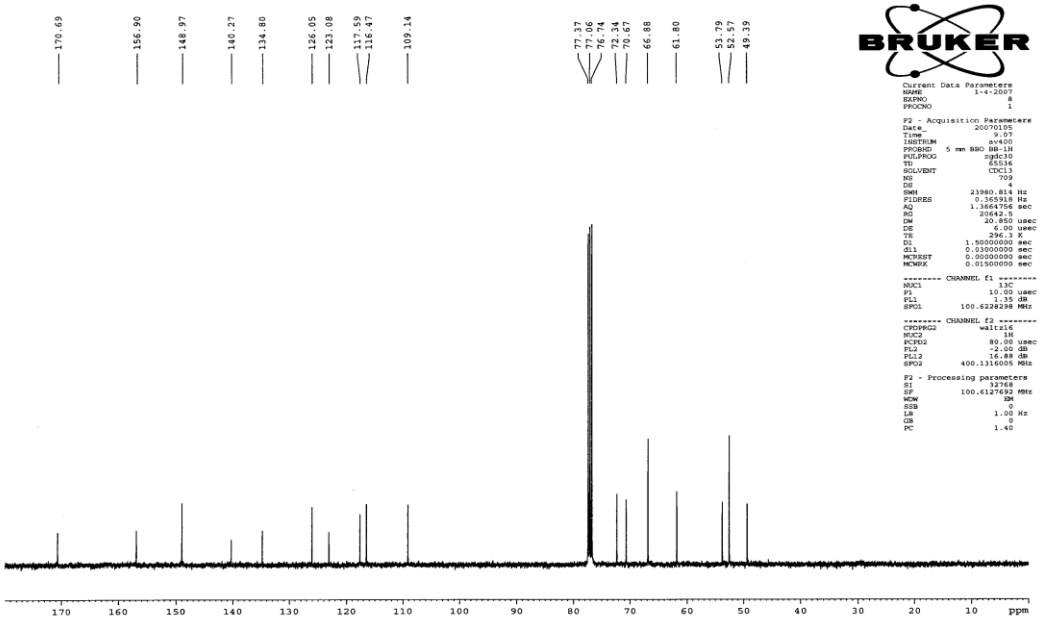


**Fig. S19** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ4MP**.

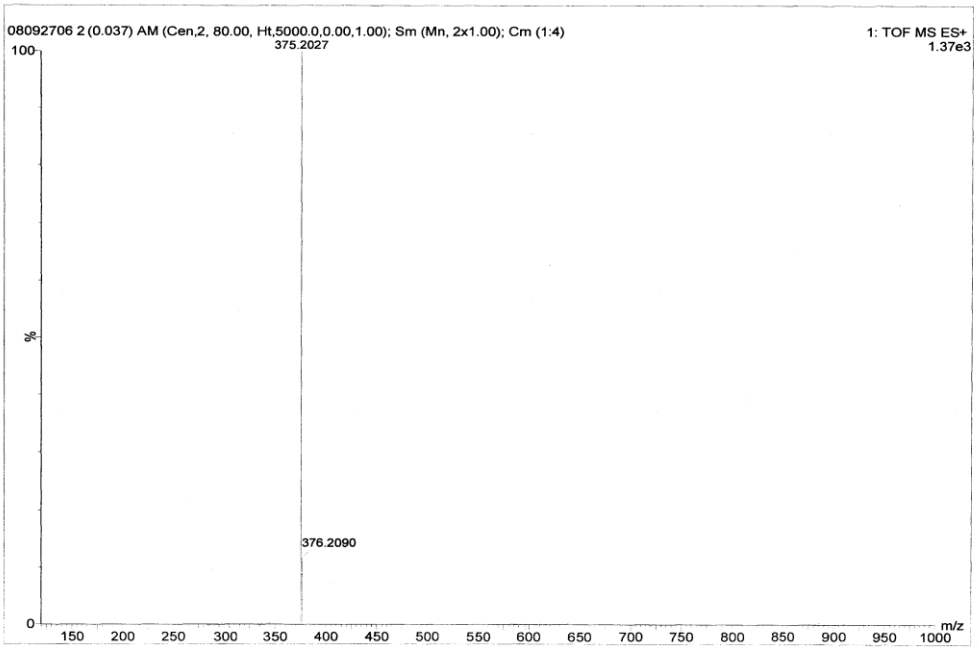


**Fig. S20** The MS spectrum of compound **AQZ4MP**.

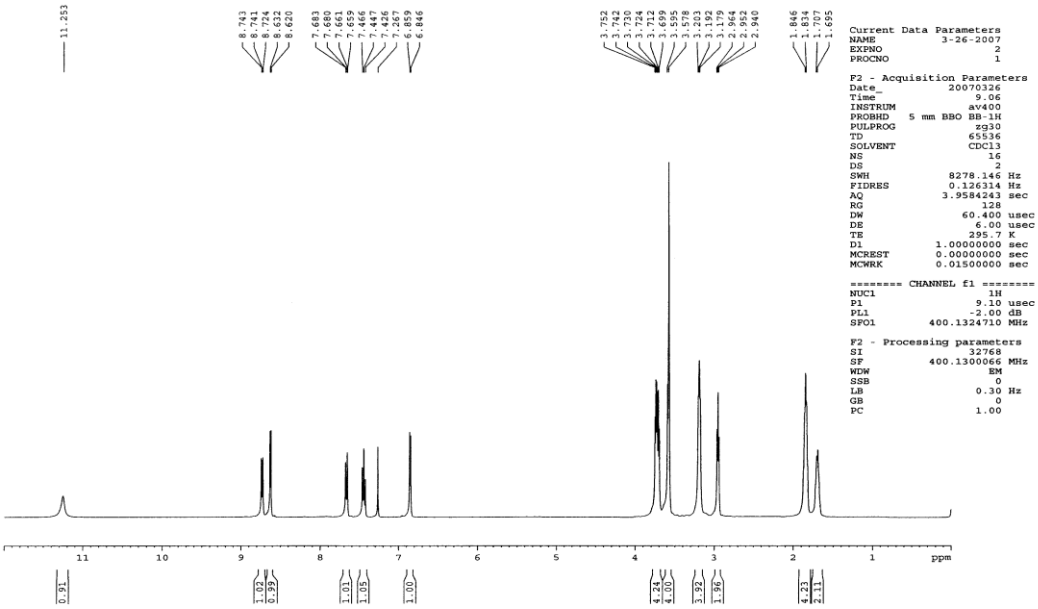


**Fig. S21** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ4PP**.

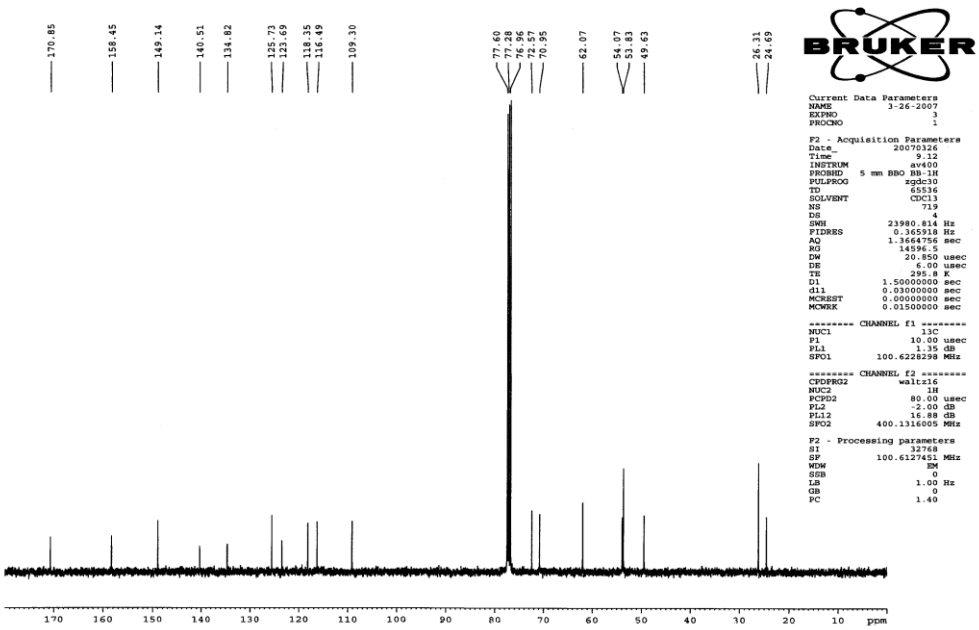


**Fig. S22** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ4PP**.

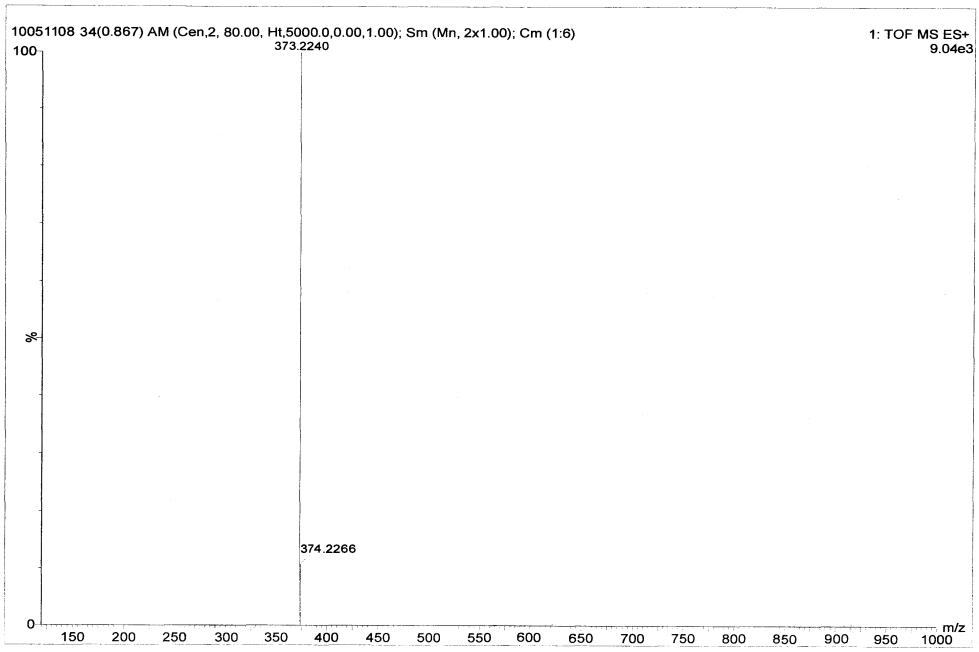


**Fig. S23** The MS spectrum of compound **AQZ4PP**.

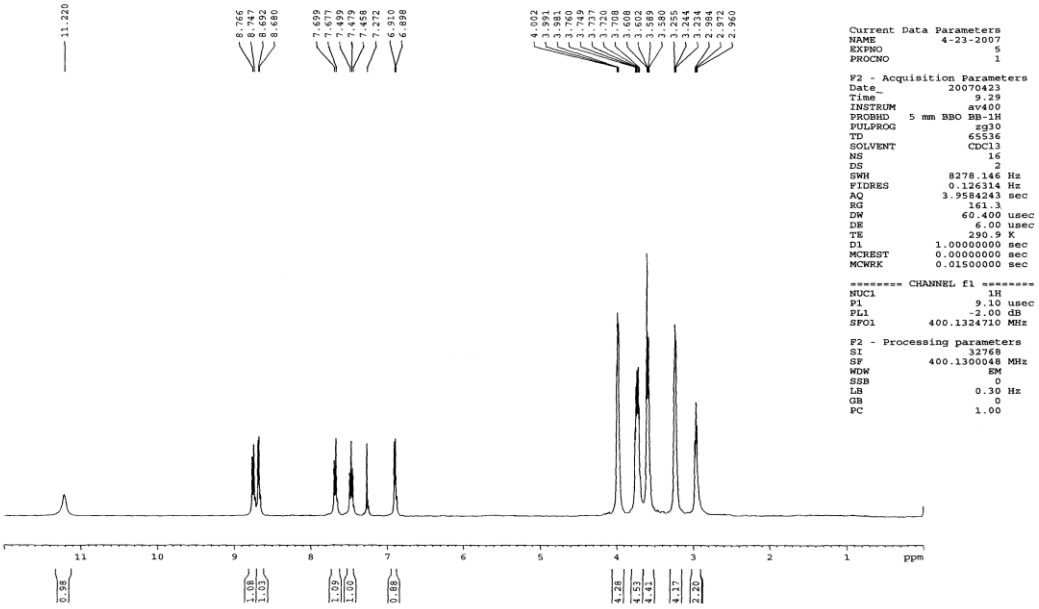


**Fig. S24** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ2MP**.

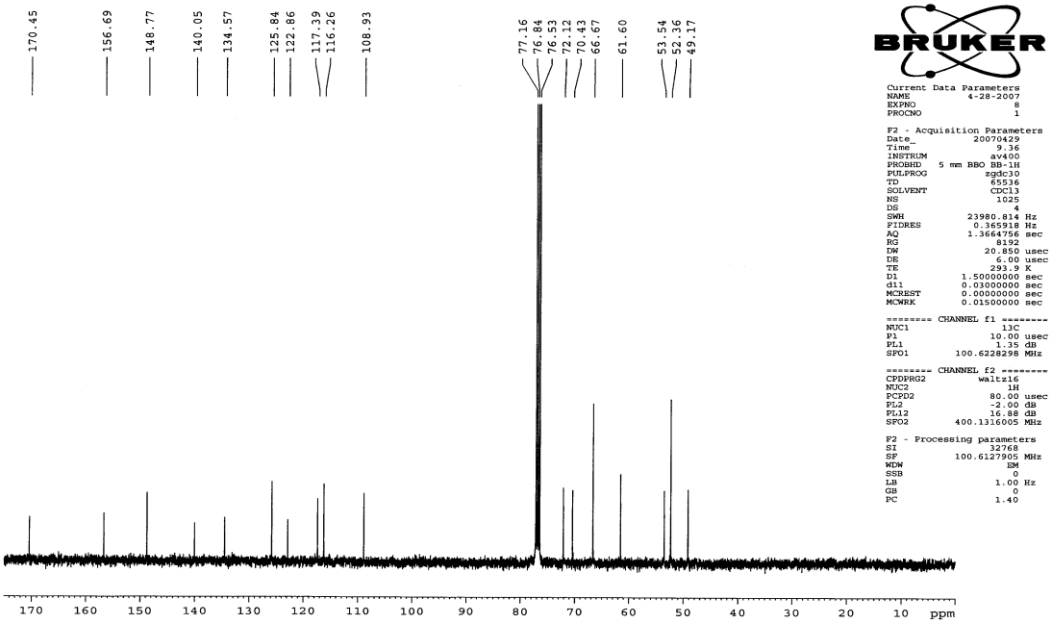


**Fig. S25** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ2MP**.

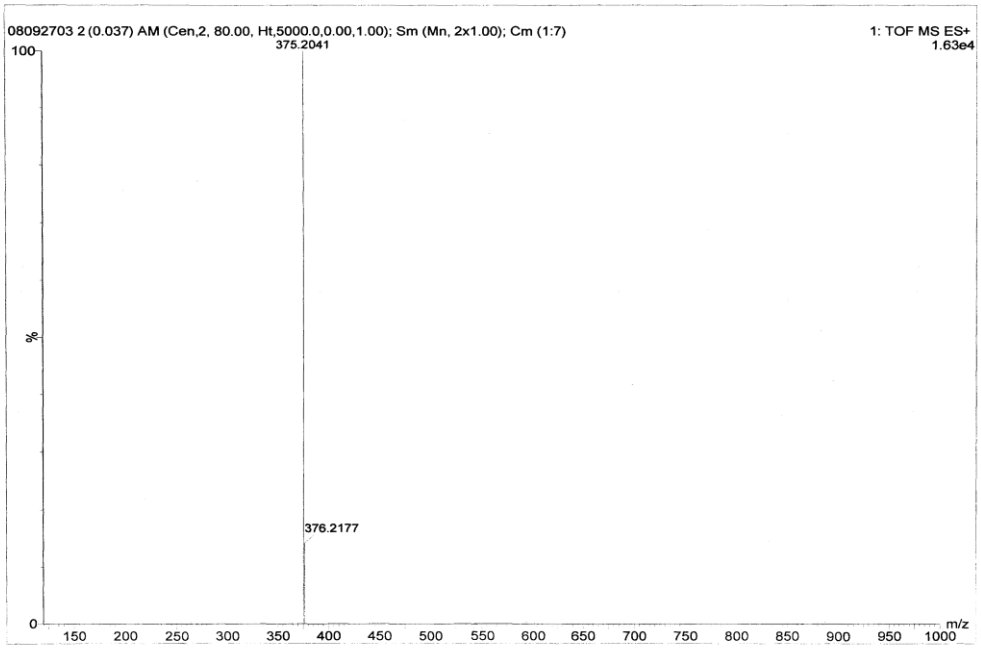


**Fig. S26** The MS spectrum of compound **AQZ2MP**.

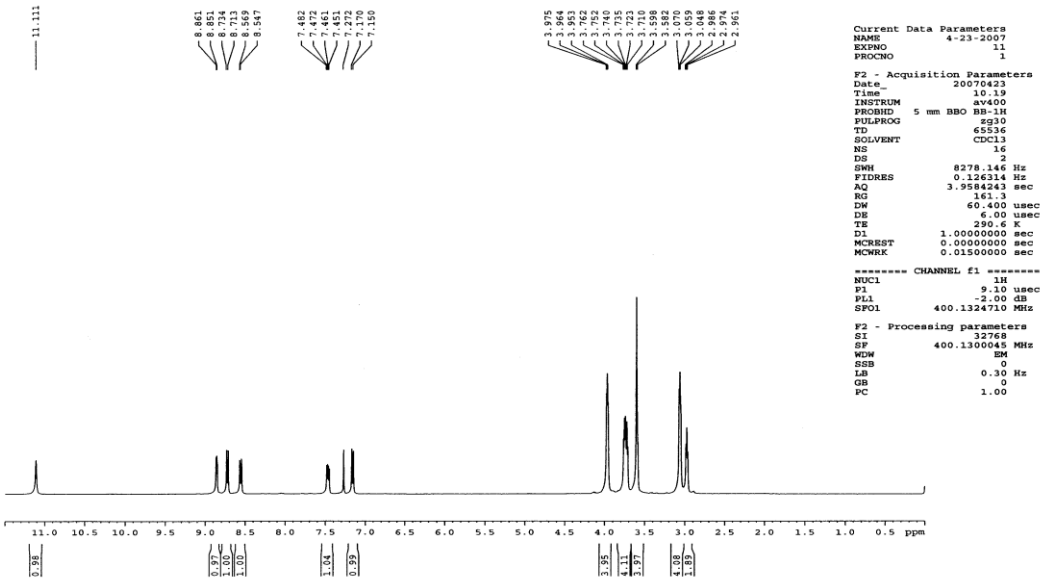


**Fig. S27** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ5MP**.

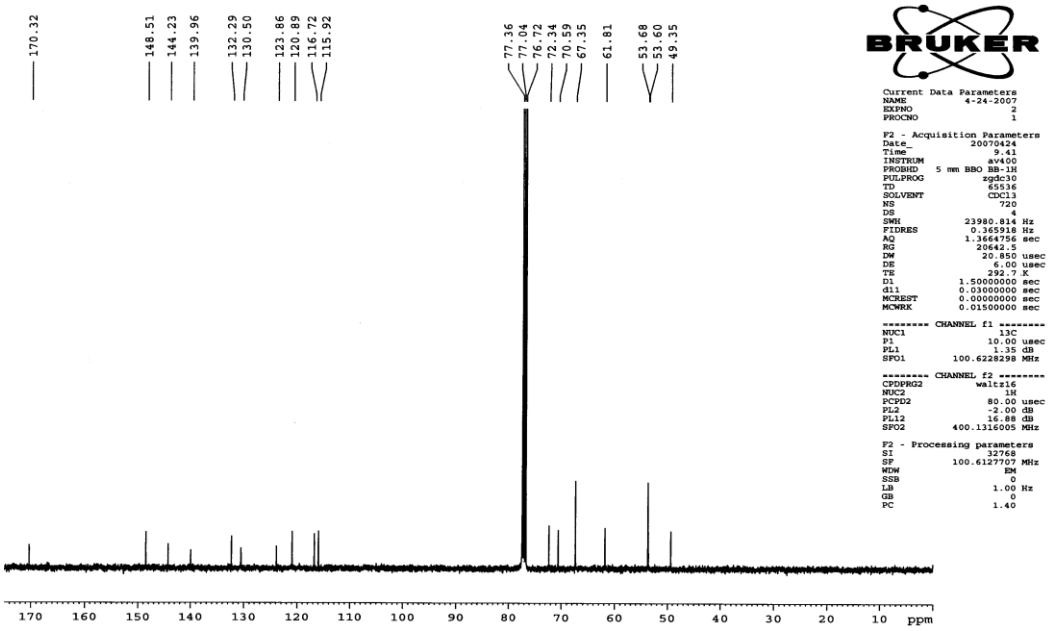


**Fig. S28** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ5MP**.

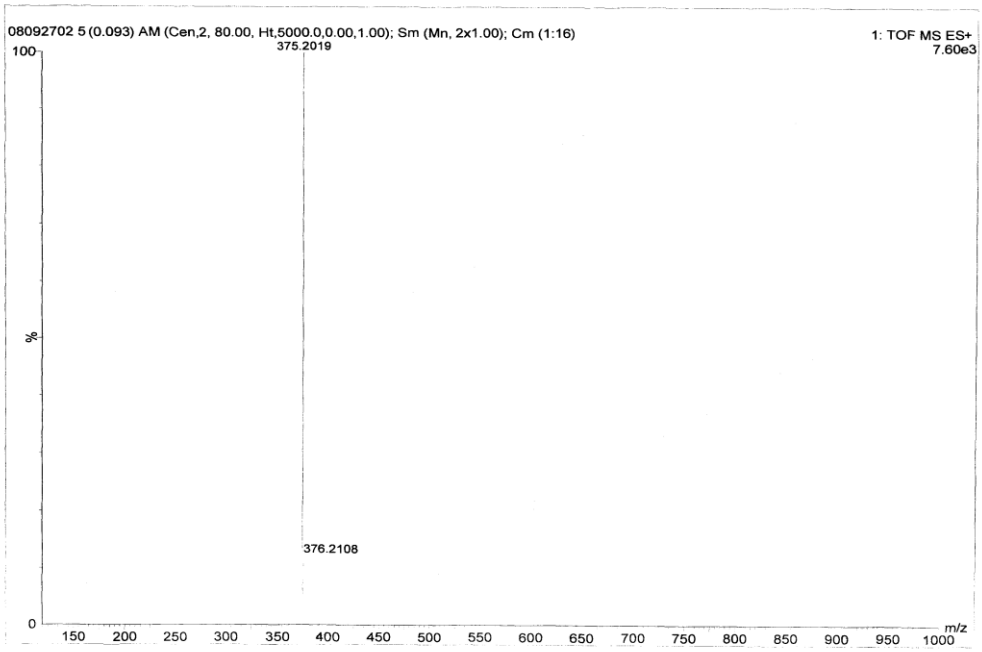


**Fig. S29** The MS spectrum of compound **AQZ5MP**.

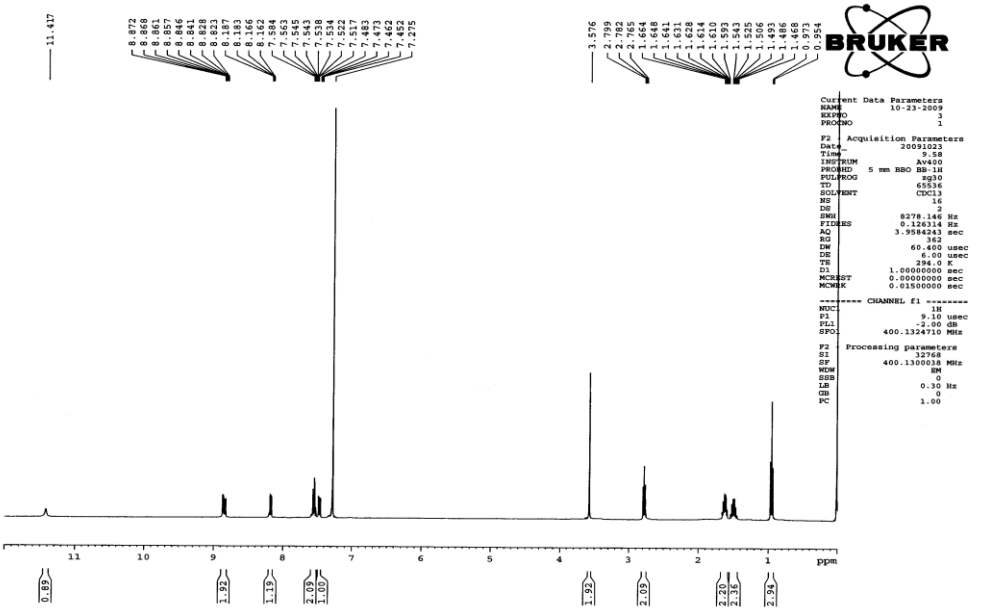


**Fig. S30** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ8BA**.

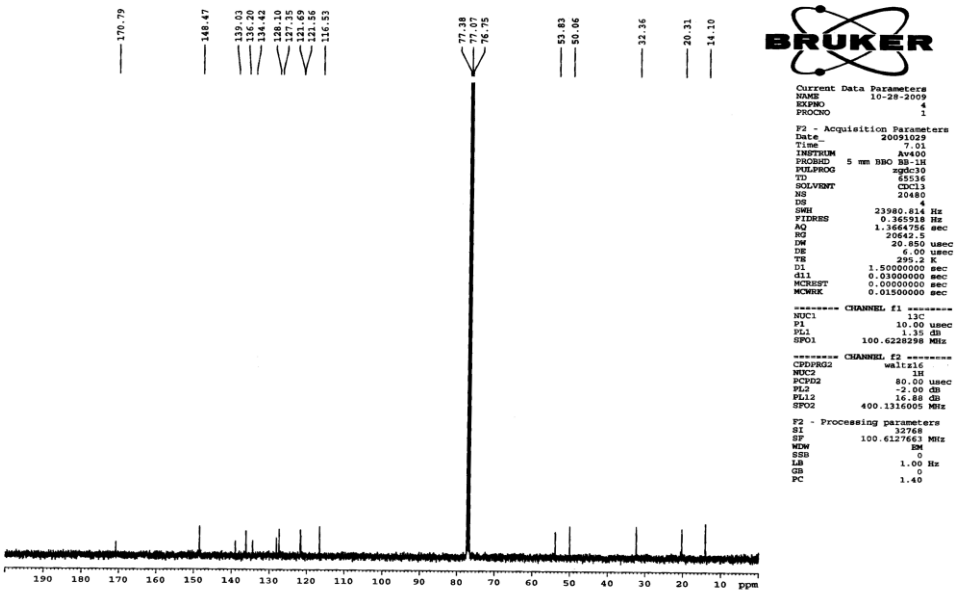


**Fig. S31** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ8BA**.

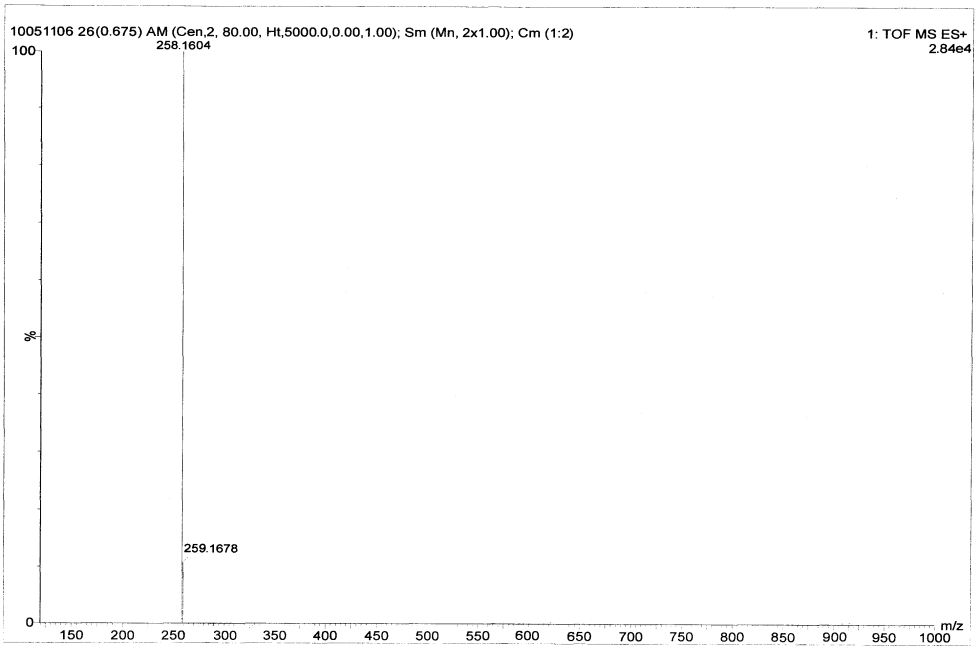


**Fig. S32** The MS spectrum of compound **AQZ8BA**.

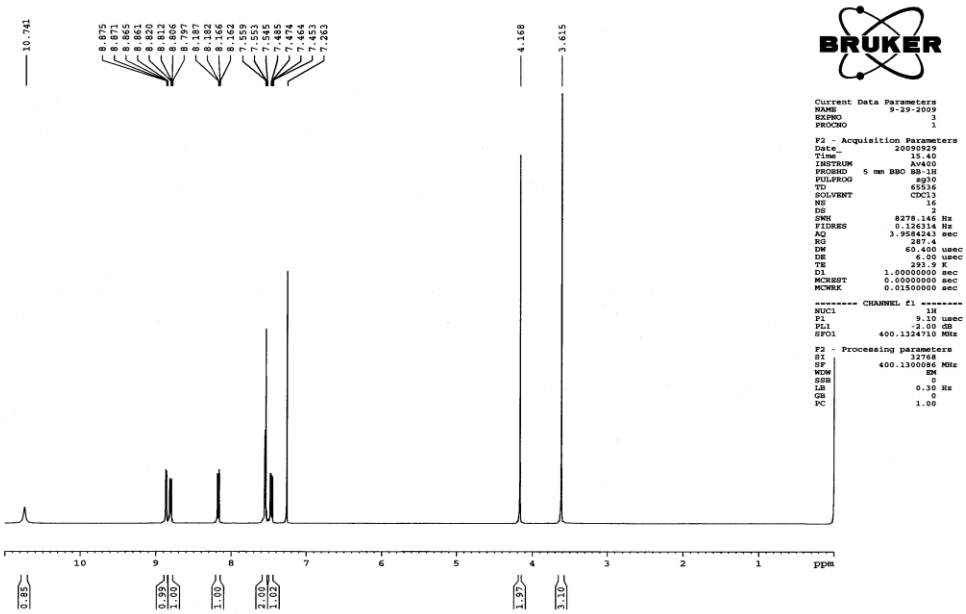


**Fig. S33** The $^{1}$H-NMR ($CDCl_3$, 400 MHz) spectrum of compound **AQZ8MO**.

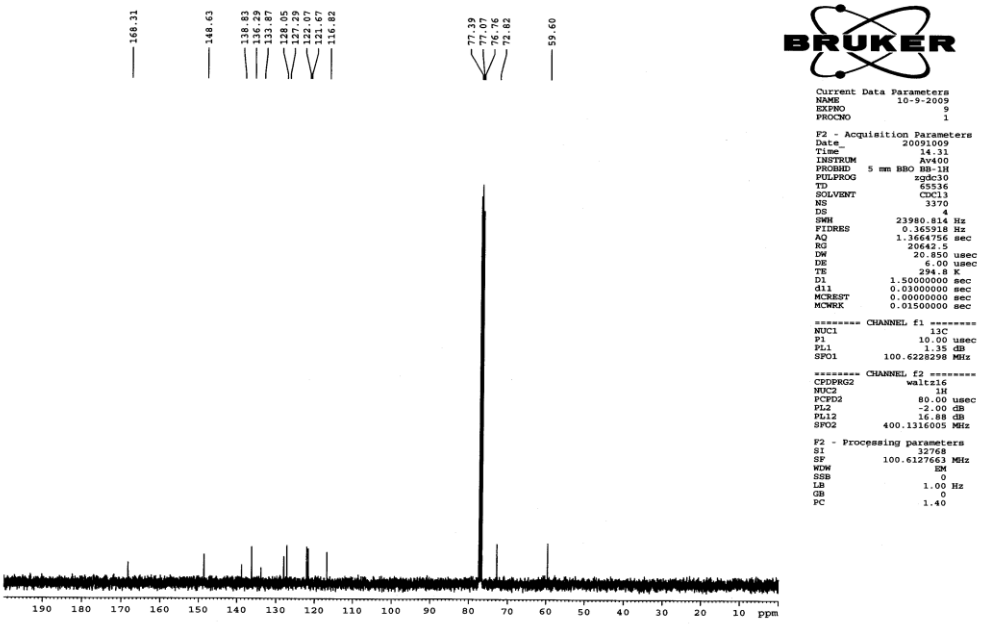


**Fig. S34** The $^{13}$C-NMR ($CDCl_3$, 100 MHz) spectrum of compound **AQZ8MO**.

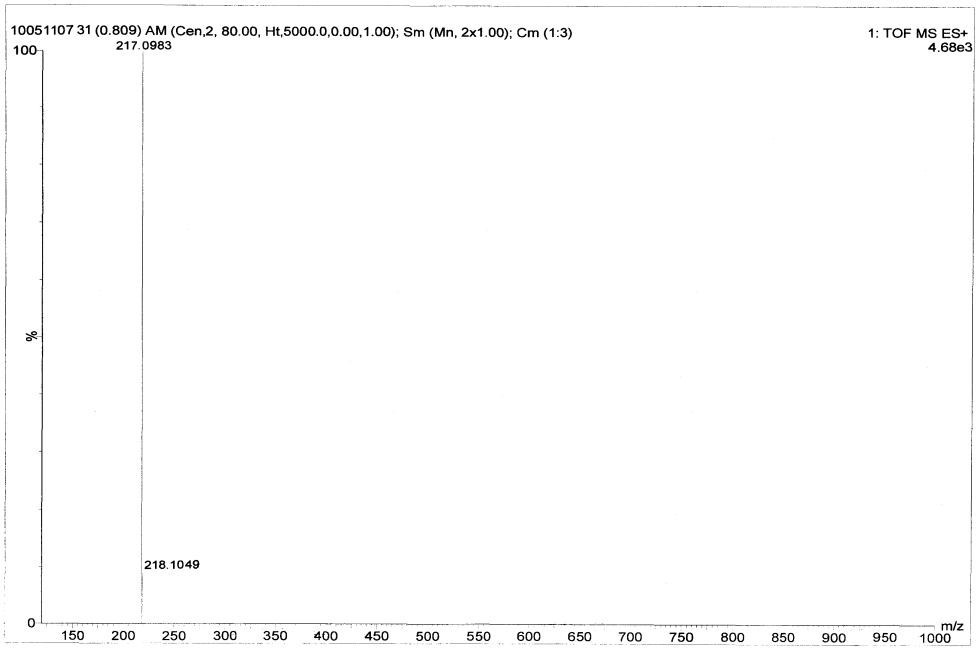


**Fig. S35** The MS spectrum of compound **AQZ8MO**.

## Reference

(1) (a) C. A. Parker and W. T. Rees, *Analyst*, 1960, **85**, 587; (b) H. Sunahara, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 5597.

(2) S. Zhang, F. Tang and T. Zhang, *The Handbooks of Modern Chemical Reagent: Chemical Analysis Reagent*, 1st ed.; Beijing: Chemical Industry Press, 1987.

(3) C. J. Fahrni and T. V. O'Halloran, *J. Am. Chem. Soc.*, 1999, **121**, 11448.

(4) (a) D. Atar, P. H. Backx, M. M. Appel, W. D. Gao and E. Marban, *J. Biol. Chem.*, 1995, **270**, 2473; (b) T. Hirano, K. Kikuchi, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2002, **124**, 6555.