Synthesis, Crystal Structure and Interaction with BSA of two α-aminophosphonic acids derivatives

Abstract

Two α-amino-phosphonate derivatives (***1*** & ***2***) were synthesized and their compositions and structures were established by EA, FT-IR, ESI-MS, NMR(^{1}H, ^{13}C and ^{31}P) and X-ray crystallography. Compound ***1*** & ***2*** were crystallized in monoclinic system with the space group P2(1)/n and P2(1)/c, respectively. The interaction effects of two α-aminophosphonates derivatives (***1*** & ***2***) with BSA were investigated and the binding constants were 1.07×10^{4} M^{-1}, 1.68×10^{4} M^{-1}, respectively. Besides, the values of n were indicated that 1:1 complex was formed between BSA and **1**&**2**.

Key Words: α-amino-phosphonate derivatives, crystal structure, interaction, BSA

1. Introduction

The formation phosphorus-carbon bond has been received intense interest in recent years because of its exhibit a wide range of biological properties and are able to function as a-aminocarboxylic acid surrogates[1-4]. It is reported that α-aminophosphonates could play as herbicides[5], antibacterial[6], antiviral[7] and antitumor agents[8]. The potential of α-aminophosphonates as enzyme inhibitors pharmacological agents has been established[9-15]. Our previous studies have showed that some α-aminophosphonates could potently inhibit PTP1B and TCPTP with lower cytotoxicity[16]. Do the α-aminophosphonates derivatives inhibit PTPs activity or proteasome activity thus leading to the antiproliferation and apoptosis of tumor cells?

Bovine serum albumin(BSA) is the most important carrier protein in the living body and the most abundant carrier protein in the plasma. Therefore, studying the interaction between drugs and BSA could provide a basis for drugs design and development[17-18]. BSA contains a variety of coordination groups and could bind to many endogenous and exogenous compounds. Therefore, BSA could store and transport some drugs or some other bioactive small molecules. Therefore, target BSA for the design of drug have been paid to special attention.

In this paper, two new α-aminophosphonates derivatives (***1*** & ***2***) with similar structure to the reported compound [16,19,20] are synthesized and characterized (Scheme 1 and Supporting Information). Structural identification of the compound ***1*** & ***2*** were confirmed by IR, EAs ^{1}H-NMR, ^{31}P-NMR, ESI-MS spectroscopy and X-ray single crystal diffraction(Supporting InformationFig. S1-S8). What's more, the interaction with BSA were investigated.

2. Experimental

2.1. Materials and instrumentation

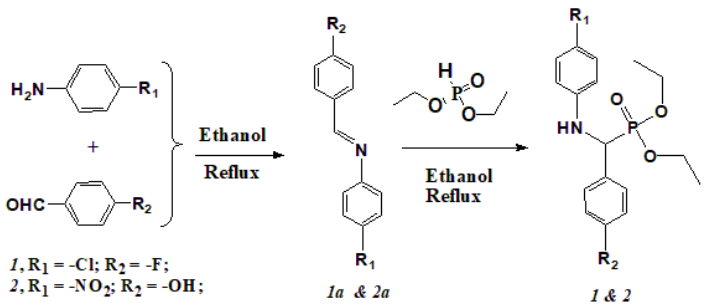
All the chemicals were purchased from commercial and used without further purification. Details information of materials and strumentations were given in the Supplementary Material.

2.2. X-ray crystallographic studies

Single crystals of compound ***1*** & ***2*** were mounted on glass fibers for data collection. Details information were given in the Supplementary Material.

2.3. Synthesis of α-aminophosphonates

From Schemes 1, α-aminophosphonates ***1*** & ***2*** were prepared by the early reported methods with some modified[16, 19,20]: First, 20 mmol of aromatic amine and 20 mmol of aromatic benzaldehyde derivative were added to a 20 mL of C_2H_5OH and reacted for 2h. Schiff Bases were obtained after cooled to room temperature (Yield: ***1*****a** 84.2 %, ***2***a 79.8 %); Second, 22 mmol of diethyl phosphonate diluted in 10 mL of C_2H_5OH and was added into 20 mmol Schiff base compounds which dissoloved in 20 mL of C_2H_5OH. Left the mixture refluxed for another 15-18 h with constant stirring. Yellow solids of ***1*** or ***2*** were obtained. The yellow block crystal (***1*** & ***2***) were collected after a week from C_2H_5OH (***1***) and C_2H_5OH/H_2O(***2***).



Scheme 1. Synthesis of ***1*** & ***2*** in two-step by Pudovik reaction.

***Diethyl (4-chlorophenylamino)(4-fluorophenyl)methylphosphonate* (*1*)**

Yield: 2.92g, 38.4%. Colorless crystals were obtained from ethanol. EAs: calcd/found(%) for $C_{17}H_{20}ClFNO_3P$(***1***): C 54.92/54.88, H 5.42/6.04, N 3.77/3.77. IR(cm^{-1}): 3290 *ν*(O-H, N-H), 2982 ν($C_α$-H), 1221 ν(P=O), 1053 and 1025 ν(P-O-C), 973 (C-P). ^{31}P NMR (D_2O/ethanol, ppm): δ 24.774. ^{1}H NMR(CD_3OD, ppm): δ 0.949-1.116(t, 6H, $2CH_3$), 4.811(m, 2H, -OCH_2-), 5.200(s, 1H, Ar-NHR), 6.518-7.794 (m, 8H, aromatic H). Exact mass for ***1***: 371.0853, ESI-MS: [***1***-H]$^-$ (m/z, 370.0984).

***Diethyl (4-nitrophenylamino)(4-hydroxyphenyl)methylphosphonate* (*2*)**

Yield: 2.24g, 30%. Colorless crystals were obtained from ethanol/H_2O. EAs: calcd/found(%) for $C_{17}H_{21}N_2O_6P$: C 53.69/53.74, H 5.57/5.94, N 7.37/7.37. IR(cm^{-1}): 3422 and 3318 ν(O-H, N-H), 2983 ν($C_α$-H), 1220 ν(P=O), 1056 and 1016 ν(P-O-C), 970 ν(C-P). ^{31}P NMR(D_2O/ethanol, ppm): δ 22.283. ^{1}H-NMR(DMSO, ppm): δ 0.949-1.116 (t, 6H, 2-CH_3), 6.518-7.794 (m, 8H, aromatic H), 5.11(m, 2H, -OCH_2-), 5.11 (s, 1H, NH-), 4.811 (d, 1H, -CH-). Exact mass for ***2***: 380.1137, ESI-MS: [***2***+H]$^+$ (m/z, 381.1121).

3. Results and discussion

3.1. Crystal structure of *1* & *2*

The crystal structures of ***1*** & ***2*** were tried to grow in different organic solvents or mix-solvents and the suitable crystals were obtained. Crystallographic data and hydrogen bonds are listed in Table 1 and Table S1 (showed in supporting information).

Table 1. Crystallographic data for ***1*** & ***2***.

Complex	***1***	***2***		***1***	***2***
Empirical formula	$C_{34}H_{40}Cl_2F_2N_2O_6P_2$	$C_{17}H_{21}N_2O_6P$	*a*(Å)	13.4882(7)	12.2302(5)
CCDC	1405042	1405038	b(Å)	18.1802(8)	8.5754(3)
Formula weight	743.52	380.33	c(Å)	16.1967(8)	17.8559(7)
Temperature	296(2) K	296(2) K	*α*(°)	90	90
Wavelength	0.71073 A	0.71073 A	*β*(°)	98.244(2)	100.193(2)
Crystal system	monoclinic	monoclinic	*γ*(°)	90	90
space group	P2(1)/n	P2(1)/c	V(Å^3)	3930.7(3)	1843.15(12)

Z	4	4	R_{int}	0.0299	0.0297
D_{calc}(g cm^{-3})	1.256	1.371	R_1, wR_2[I>	0.1479	0.0912
F(000)	1552	800	2σ(I)]	0.4145	0.2378
Goodness of fit	1.324	1.111	R_1,wR_2(all	0.1939	0.1093
Completeness (%)	99.1	98.1	data)	0.4610	0.2648
Reflections collect	26371	14955	Reflections unique	6864	4153

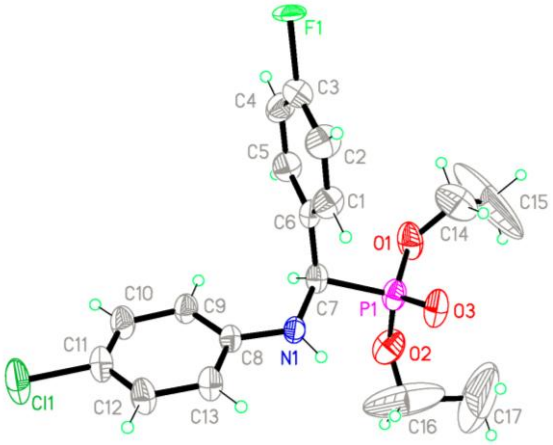


Fig. 1 X-ray structure of the ***1*** with a 30% probability of thermal ellipsoids.

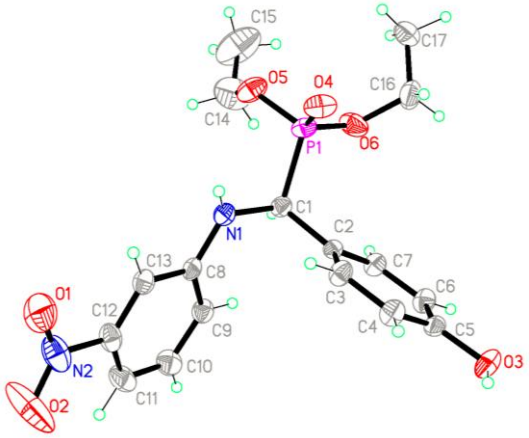


Fig. 2. X-ray structure of the ***2*** with a 30% probability of thermal ellipsoids.

1 & ***2*** were monoclinic system with the space group P 21/n, P2(1)/c, respectively. From Fig.1-2, two -O-CH_2CH_3 groups, one C_α atom, and a double bond O atom to form the tetrahedral geometries of P atoms. Besides, C_α atoms were responsible for the existence of optically activity, it is the same with the early report[16,19,20].

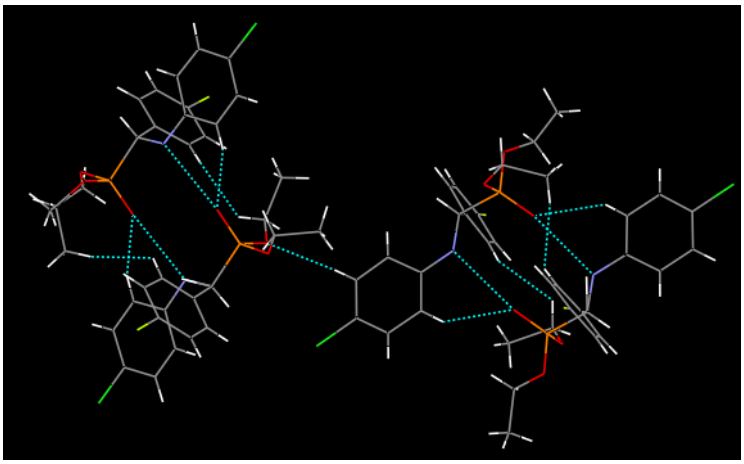


Fig. 3. Hydrogen bonds network for ***1***.

The plane of the arylamine and benzaldehyde derivatives form a dihedral angle of 88.44 (0.19)° and 81.84 (0.10)°, respectively. When the -CH_2CH_3 bonded to P was substituted by hydroxy group, the dihedral angle between the 2-hydroxyphenyl and pyridine rings is 54.9 (1)°[21]. The bond lengths of C_α-P and P=O were almost comparable to the similar structures[16,19,20,22]. There were a lot of weak interactions exited in compound ***1*** & ***2***, such as N-H···O, O-H···O, C-H···O, C-H···π and N-O···π, resulting in stabilization in the structures(shown in Figs. 4-5).

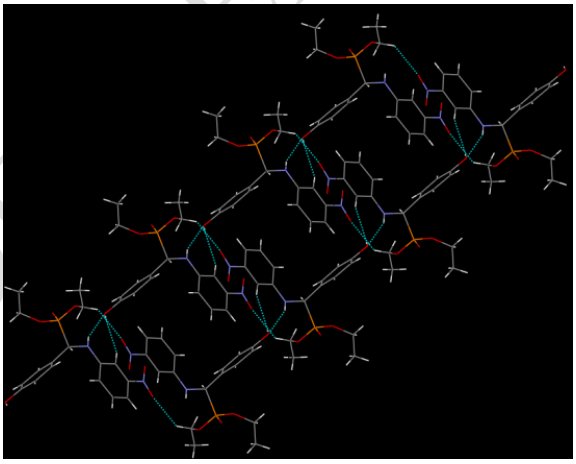


Fig. 4. Hydrogen bonds network for ***2***.

3.2. BSA interaction with compound *1* & *2*

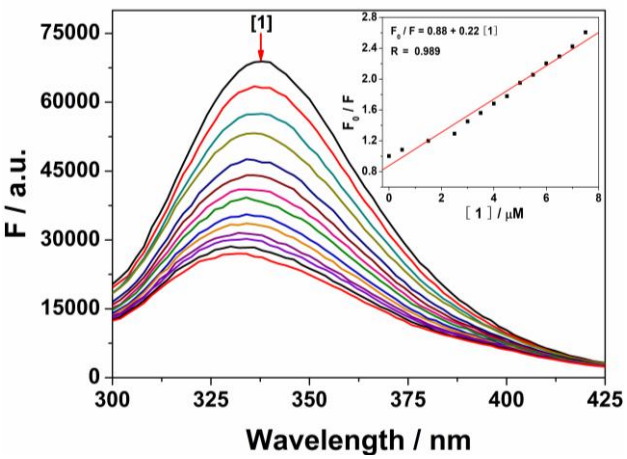


Fig. 5. The Fluorescence spectrum of BSA after difference concentration of compound **1** was added. Inset: Stern-Volmer plots for the concentration of compound **1** with the fluorescence intensity.

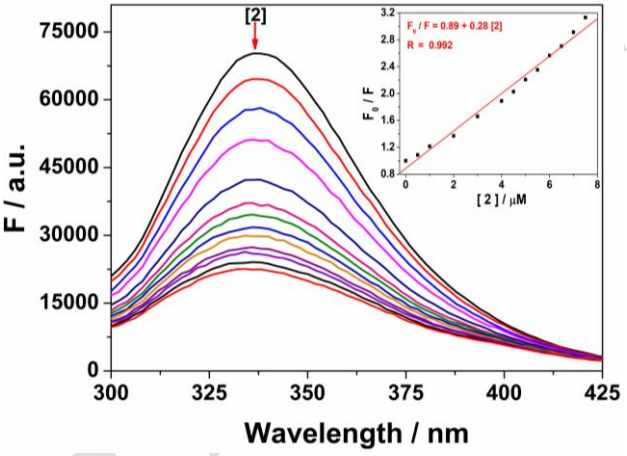


Fig. 6. The Fluorescence spectrum of BSA after difference concentration of compound **2** was added. Inset: Stern-Volmer plots for the concentration of compound **2** with the fluorescence intensity.

As showed in Fig.5-Fig.6, with the amount of conpound **1** and compound **2** increasing, the fluorescence intensity of BSA at 336 nm regularly decreased. The results indicated that compound **1**& **2** could be interacted with BSA result in the fluorescence intensity quenching. Followed by the equation of Stern -Volmer, $F_0/F=1+K_q\tau_0[Q]=1+K_{sv}[Q]$ [23].(where $F_0$and F are on behalf of the fluorescence intensity of BSA and the fluorescence intensities of BSA after difference amount of **1**&**2** were added, respectively. K_q, reaction rate constant, K_{sv}, Kinetic quenching constant, τ_0, about 10^{-8}s), we could find that the values of K_q for **1**&**2** were 2.2×10^{13} L·mol^{-1}·s^{-1} and 2.8×10^{13}L·mol^{-1}·s^{-1}, respectively. The k_q values were higher than the maximum scatter collision-quenching, which told us that the interaction between **1**&**2** and

BSA were static quenching constant, indicating the static quenching mechanism was existed[24].

For static quenching, the binding constant and stoichiometry between BSA and **1**&**2** were calculated by the equation[25] $\lg \frac{F_0 - F}{F} = \lg K_A + n \lg[Q]$

From the slope and the intercept of the line of log(F_0 - F)/F vs. log[Q], the value of n and K_A could be obtained (Fig.7). Table 1 showed that a higher binding constants was found by **2** with BSA than **1**. As the reported results, the values of n were nearly 1, indicated that the formation of 1:1 complex were obtained between BSA and **1**&**2**.

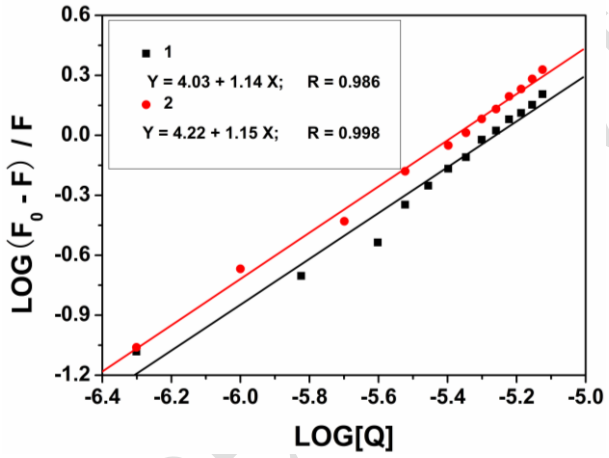


Fig. 7. Plot of log[(F_0-F)/F] vs. log[**Q**] (Q stand for compound **1** and compound **2**)

4. Conclusion

In conclusion, our data indicate that the α-aminophosphonates derivatives(**1**&**2**) can bind to BSA and the fluorescence quenching mechanism of BSA with **1**&**2** were static procedures. The binding constant of BSA and **1**&**2** were 1.07 × 10^4 M^{-1}, 1.68 × 10^4 M^{-1}. Besides, the values of n were manifested that the formation of 1:1 complex were obtained between BSA and **1**&**2**.

Abbreviations:

1, Diethyl (4-chlorophenylamino)(4-fluorophenyl)methylphosphonate ($C_{17}H_{20}ClFNO_3P$)

2, Diethyl (4-nitrophenylamino)(4-hydroxyphenyl)methylphosphonate ($C_{17}H_{21}N_2O_6P$ **)**

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Supporting Information

1. General information and methods

All reagents and solvents were purchased commercially and used without further purification. Deionized water was used for all chemical experiments and double-distilled water was used to prepare buffer solutions as well as biological evaluation. *pH* values of buffer solutions were measured using a PHS-3TC pH meter. All reactions were magnetically stirred. Elemental analyses (EAs) were carried out with a VARI-EL elemental analyzer. IR spectra (4,000–400 cm^{-1}) were recorded using a Shimadzu Fourier transform on NICOLET380 spectrometer in KBr disks. The electronic absorption spectra and K_i were taken on a Hewlett-Packard HP-8453 Chemstation spectrophotometer. ^{1}H NMR spectra were recorded at RT with a DRX 400 instrument at 300 MHz. ^{31}P NMR spectra were recorded with the same spectrometer at 121.49 MHz with H_3PO_4 (85%) as external standard. Electrospray ionization mass spectra (ESI-MS, negative mode) were recorded with a Finnigan LCQ system (Waters, USA) in methanol/water solution. The X-ray data were

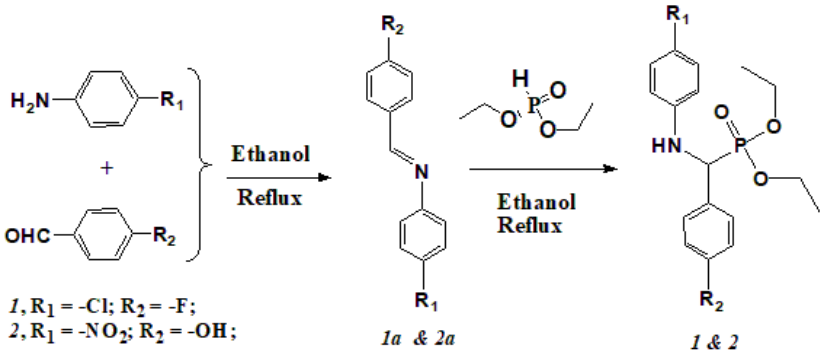
collected using a Bruker SMART APEX 1K CCD diffractometer. Bioactivity assays of the compounds were carried out with a Bio-Rad model 550 micro plate reader for A_{405}.

2. X-ray crystallographic studies

Single crystals of compound ***1*** and ***2*** were mounted on glass fibers for data collection. Data were collected on a Bruker Smart Apex II diffractometer equipped with 1K CCD instrument by using a graphite monochromator utilizing Mo-*Kα* radiation (λ = 0.71073 Å) at room temperature. Cell parameters were determined using SMART software. Data reduction and corrections were performed using SAINTPlus. Absorption corrections were made via SADABS[R1]. The structures were solved by direct methods with the SHELX-97 program and refined on F^2 by full matrix least-squares using the SHELX-97 program package[R2]. The H atoms attached to C atoms were added theoretically and treated as riding on the concerned atoms. Molecular graphics were from Ortep3[R3].

3. Synthesis of α-aminophosphonates

According to Scheme S1, α-aminophosphonates ***1*** & ***2*** were prepared by a two-step procedure following our reported method with some modified[R4]: (a) equimolar amounts (0.02 mol) of amine and benzaldehyde derivative were mixed in a 20 mL of ethanol solution and refluxed for 2h. After the reacting mixture were cooled to room temperature, the generated Schiff bases were crystallized from solution (Yield: ***1*****a** 84.2 %, ***2***a 79.8 %); (b) a 10 mL of ethanol solution containing 22 mmol of diethyl phosphonate was added dropwise into the stirred solution of an equimolar amount of Schiff base compounds in a 20 mL of ethanol. And then, the mixture was refluxed for 15-18 h with constant stirring. The yellow solids of ***1*** or ***2*** were obtained. The yellow solids were dissolved in 10-20 mL of ethanol (***1***) and ethanol/H_2O (***2***), block crystal (***1*** & ***2***) were collected after a week.



Scheme S1. Synthesis of the rigid α-aminophosphonate N-derivatives ***1*** & ***2*** in two-step by Pudovik reaction.

***Diethyl (4-chlorophenylamino)(4-fluorophenyl)methylphosphonate* (*1*)**

Yield: 2.92g, 38.4%. Colorless crystals were obtained from ethanol. EAs: calcd/found(%) for $C_{17}H_{20}ClFNO_3P$(***1***): C 54.92/54.88, H 5.42/6.04, N 3.77/3.77. IR(cm^{-1}): 3290 *ν*(O-H, N-H), 2982 ν($C_α$-H), 1221 ν(P=O), 1053 and 1025 ν(P-O-C), 973 (C-P). ^{31}P NMR (D_2O/ethanol, ppm): δ 24.774. ^{1}H NMR(CD_3OD, ppm): δ 0.949-1.116(t, 6H, $2CH_3$)**,** 4.811(m, 2H, -OCH_2-)**,** 5.200(s, 1H, Ar-NHR)**,** 6.518-7.794 (m, 8H, aromatic H). Exact mass for ***1***: 371.0853, ESI-MS: [***1***-H]$^-$ (m/z, 370.0984).

***Diethyl (4-nitrophenylamino)(4-hydroxyphenyl)methylphosphonate* (*2*)**

Yield: 2.24g, 30%. Colorless crystals were obtained from ethanol/H_2O. EAs: calcd/found(%) for $C_{17}H_{21}N_2O_6P$: C 53.69/53.74, H 5.57/5.94, N 7.37/7.37. IR(cm^{-1}): 3422 and 3318 ν(O-H, N-H), 2983 ν($C_α$-H), 1220 ν(P=O), 1056 and 1016 ν(P-O-C), 970 ν(C-P). ^{31}P NMR(D_2O/ethanol, ppm): δ 22.283. ^{1}H-NMR(DMSO, ppm): δ 0.949-1.116 (t, 6H, 2-CH_3), 6.518-7.794 (m, 8H, aromatic H), 5.11(m, 2H, -OCH_2-), 5.11 (s, 1H, NH-), 4.811 (d, 1H, -CH-). Exact mass for ***2***: 380.1137, ESI-MS: [***2***+H]$^+$ (m/z, 381.1121).

4. Supplemental spectra data

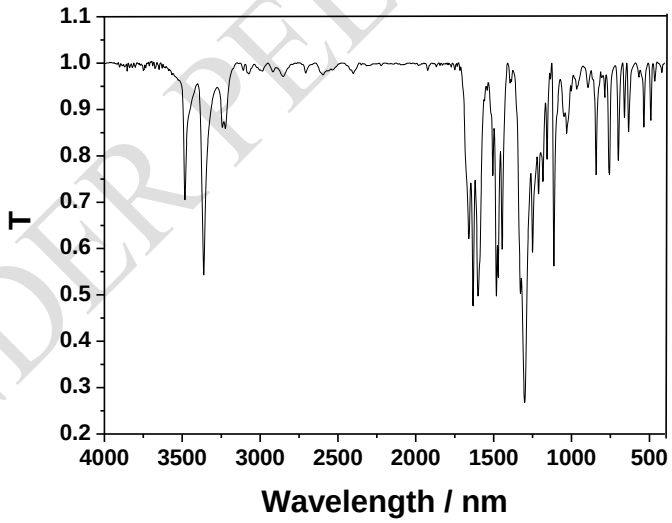
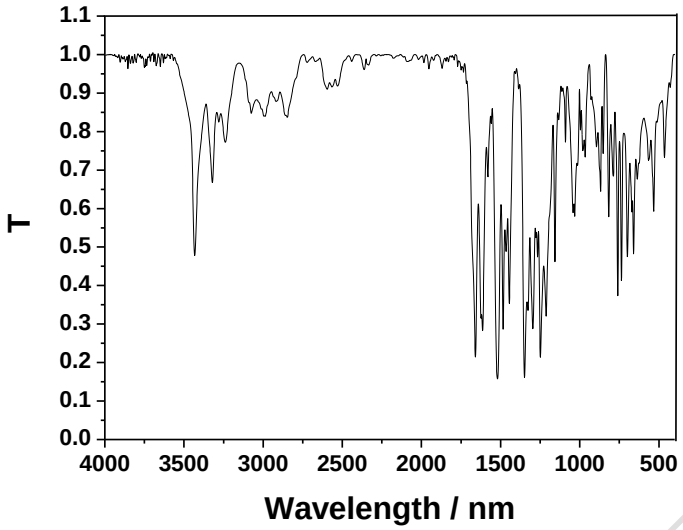


Fig. S1. The FI-IR of compound ***1***



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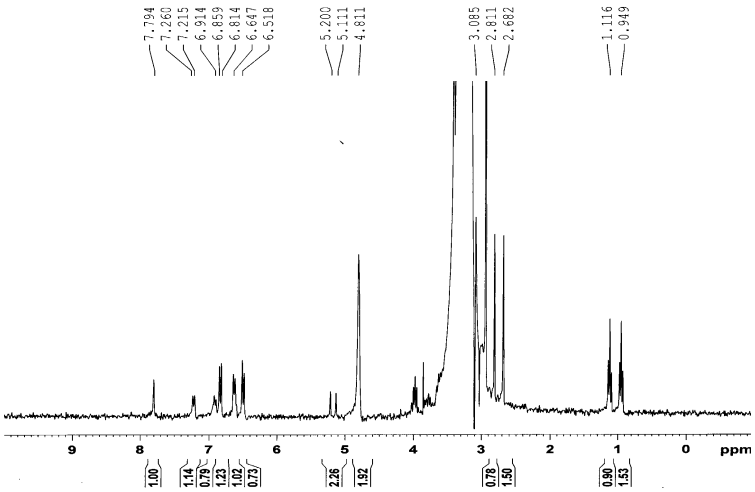
240 **Fig. S2**. The FI-IR of compound ***2***



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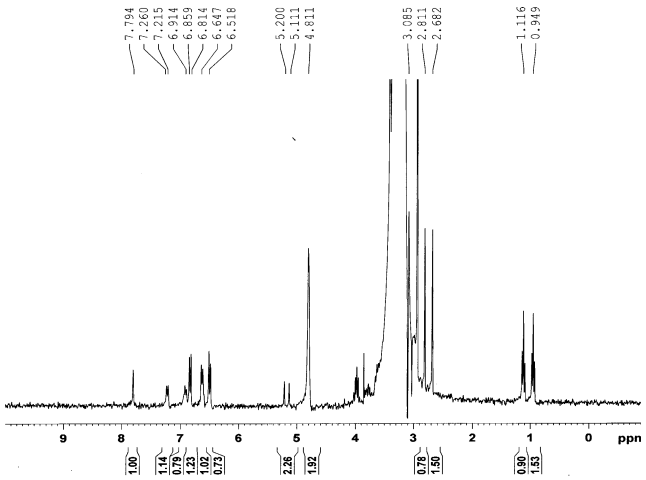
242 **Fig. S3**. The ^{1}H-NMR spectra of compound ***1***



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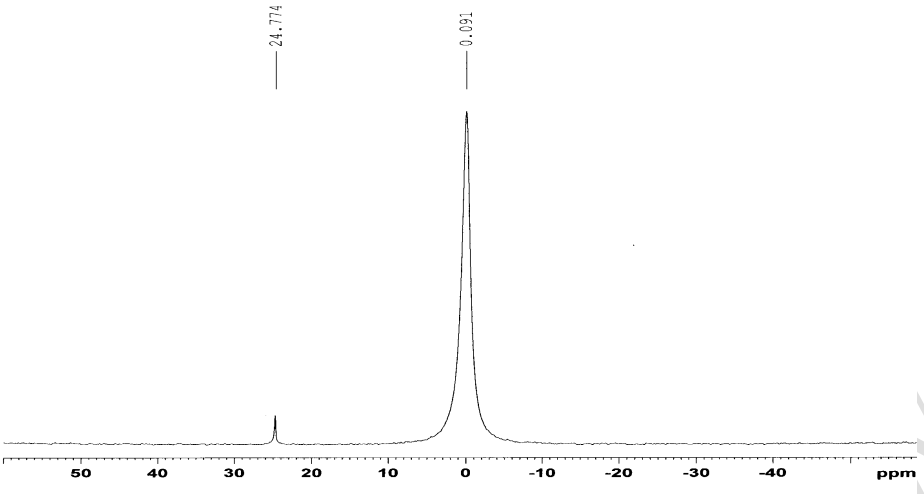
244 **Fig. S4**. The ^{1}H-NMR spectra of compound ***2***



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246 **Fig. S5**. The ^{31}P-NMR spectra of compound ***1***

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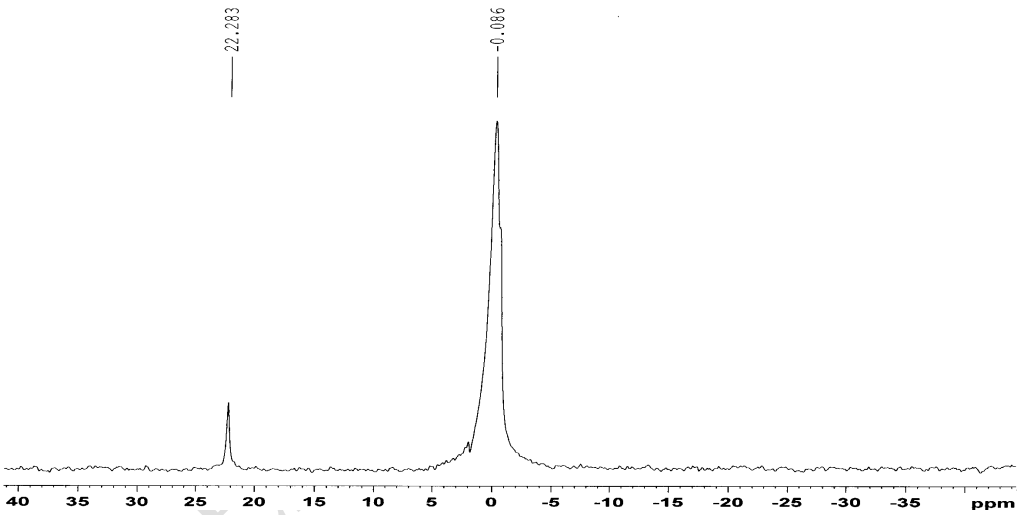
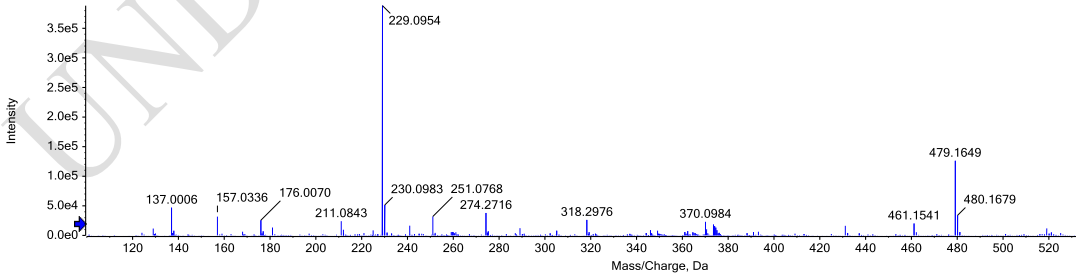


248

249 **Fig. S6**. The ^{31}P-NMR spectra of compound ***2***



250

251 **Fig. S7**.The ESI-MS of spectra compound ***1*** negative ion mode in methanol and water (v/v=1:9)

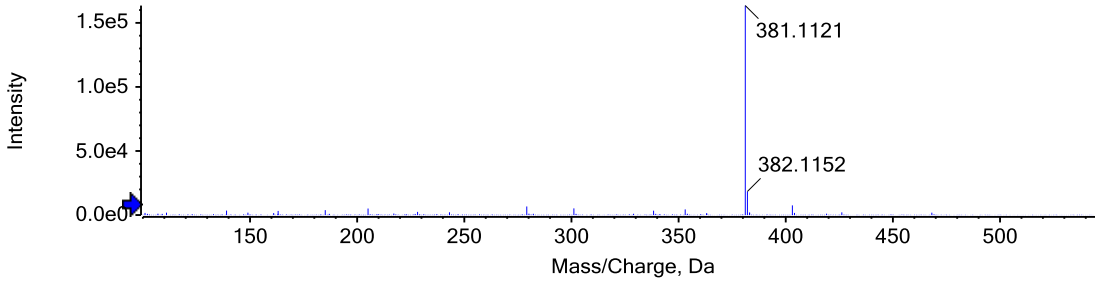


Fig. S8.The ESI-MS of spectra compound ***2*** positive ion mode in methanol and water (v/v=1:9)

Table S1. Hydrogen bonds for ***1***.

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
Compound ***1***				
N(1)-H(1B)...O(5)	0.86	2.28	2.950(6)	134.4
N(2)-H(1A)...O(3)	0.93(7)	2.05(7)	2.943(6)	160(6)
C(13)-H(13)...O(5)	0.93	2.53	3.263(7)	136
C(27)-H(27)...O(2)_$1	0.93	2.55	3.418(9)	156
C(31)-H(31A)...O(5)	0.97	2.55	3.003(10)	109
Symmetry trans formations used to generate equivalent atoms: $1-3/2+x, -1/2-y, -3/2+z				
Compound ***2***				
N(1)-H(1A)...O(3)_$1	0.84(4)	2.48(4)	3.278(4)	158(3)
O(3)-H(3)...O(4)_$2	0.82	2.00	2.749(3)	152.0
C(3)-H(3A)...N(1)	0.93	2.61	2.924(4)	101
C(14)-H(14B)...O(2)_$3	0.97	2.48	3.239(8)	135
Symmetry trans formations used to generate equivalent atoms: $1x,y+1,z; $2 0.5-x, 0.5+y, 0.5-z; $3 3/2-x，3/2+y，0.5-z				

R1 Sheldrick G. M., Correction Software, University of Gotingen, Germany, **1996**.

R2. Sheldrick G. M., Program for the Solution of Crystal Structure, University of Gotingen, Germany, **1997**.

R3. Farrugia L. J., *J. Appl. Cryst.* **1997**, *30*, 565.

R4.Wang Q. M., Zhu M. L., Zhu R. T., Lu L. P., Yuan C. X., Xing S., Fu X. Q., Mei Y. H., Hang Q. W., *Eur. J. Med. Chem.* **2012**, *49*, 354.