Asian Journal of Chemistry; Vol. 23, No. 9 (2011), 4031-4036

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis, Biological Activities and DFT Calculation of α-Aminophosphonate Containing Cyclopropane Moiety

XING-HAI LIU[1,2,*], JIAN-QUAN WENG[1], CHENG-XIA TAN[1], LI PAN[2], BAO-LEI WANG[2] and ZHENG-MING LI[2,*]

[1]College of Chemical Engineering and Materials Science, Zhejiang University of Technology, Hangzhou 310014, P.R. China
[2]State-Key Laboratory of Elemento-Organic Chemistry, National Pesticidal Engineering Centre (Tianjin), Nankai University, Tianjin 300071, P.R. China

*Corresponding authors: E-mail: xhliu@zjut.edu.cn; nkzml@vip.163.com

(*Received*: 20 November 2010; *Accepted*: 16 May 2011) AJC-9962

Five new α-aminophosphonate derivatives containing cyclopropane moiety have been synthesized *via* conventional and microwave irradiation methods under solvent- and catalysis-free condition. The structures of the α-aminophosphonate compounds have been confirmed by ^{1}H NMR, ^{31}P NMR, FT-IR, EI-MS and FTICR-MS studies. Their herbicidal activities were evaluated *in vivo* and *in vitro*. The results showed some compounds were found to exhibit moderate herbicidal activities against *Brassica campestris* at 100 and 10 ppm. Theoretical calculation of **2c** was carried out with B3LYP/6-31G (d,p). The full geometry optimization was carried out using 6-31G(d,p) basis set and the structure-activity relationship was also studied by frontier orbital energy.

Key Words: α-Aminophosphonate, Synthesis, Biological activity, DFT calculation.

INTRODUCTION

Organophosphorus compounds had been focused for many years. They had many applications in industry[1], agriculture[2] and medicinal chemistry[3] because of their multi-properties. α-Aminophosphonates are the analogs of natural amino acid[4] and some of them exhibit broad-spectrum biological activities, such as antitumor[5], fungicidal activities[6], enzyme inhibitors[7], antivirus[8] *etc*. α-Aminophosphonates usually have lower toxicity and residual effects for mammalian. On the other hand, some cyclopropane derivatives exhibited highly bioactivity as insecticidal activity[9], KARI activity[10], fungicidal activities[11], *etc*.

To date, many methods have been described for the preparation of α-aminophosphonate, such as one-pot three-component synthesis[12], multi-step synthesis[13], microwave assistant synthesis[14], ultrasonic assistant synthesis[15], ion liquid[16]. While many catalysts were used in the one-pot three-component synthesis to raise the reacting rate and yield, such as Lewis acids or bases. Lewis acids such as $SnCl_4$, $SnCl_2$, $ZnCl_2$ or $BF_3{\cdot}Et_2O$ have been used as catalysts for such reactions[17].

Microwave-assisted synthesis has shown to be a valuable method in organic synthesis[18,19], since it can often reduce the reaction times dramatically, typically from days or hours to minutes or even seconds.

In view of these facts and also as a part of our work on the development of bioactive compounds, herein we report the synthesis, characterization and biological study of five aminophosphonate containing cyclopropane moiety. The synthesis of these compounds was greatly facilitated by the microwave irradiation. Their structures were confirmed by the ^{1}H NMR, ^{31}P NMR, FT-IR, EI-MS, HRMS. Their herbicidal activities were determined *in vivo* and *in vitro*.

EXPERIMENTAL

Melting points were determined using a Taike X-4 apparatus and were uncorrected. Infrared spectra were recorded on a Nocolet 570 spectrophotometer as KBr tablets. ^{1}H NMR spectra were measured on a Bruker AV-300 instrument (300 MHz) using TMS as an internal standard and $CDCl_3$ as solvent. ^{31}P NMR spectra were measured on a Varain Mercury Plus 400 instrument (400 MHz) using TMS as an internal standard and $CDCl_3$ as solvent. Mass spectra were recorded on a Thermo Finnigan Polaris-Q GC-MS instrument. HRMS data was obtained on a FTICR-MS instrument (Ionspec 7.0T). Microwave reaction was performed on a CEM DiscoverTM focused microwave.

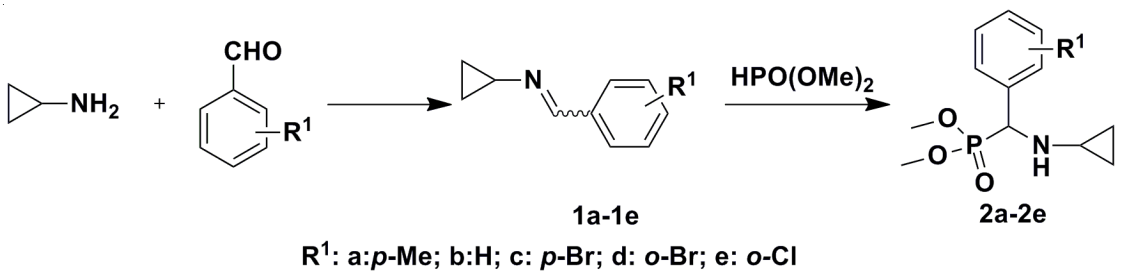
General procedure: Preparation of **2a**: 4-Methyl benzaldehyde (3.6 g, 30 mmol) and cyclopropyl amine was performed at room temperature without solvent. 2 h later, this

was treated with rotary evaporator that eliminated water and superfluous cyclopropyl amine. We checked up the product to be imine by GC-MS. Imine (4.452 g, 0.028 mol) and methylphosphinate (3.08 g, 0.028 mol) was heated under 80 ºC. After 4 h, we could find the final compound by TLC (PE:EA = 4:1). The compound was purified by column chromatography. The final compound is a yellow solid. The reaction is shown as **Scheme-I**. The analytical data are listed Tables 1-3.

N-(4-Methylbenzylidene)cyclopropanamine (1a): Yellow oil, yield 92 %, ^{1}H NMR ($CDCl_3$, δ ppm): 0.89-0.98 (m, 4H, cyclopropane-CH_2), 2.37(s, 3H, CH_3), 3.00-3.01 (m, 1H, cyclopropane-CH), 7.18 (d, *J* = 7.47 Hz, 2H, Ar-H), 7.57 (d, *J* = 7.47 Hz, 2H, Ar-H), 8.41 (s, 1H, HC=); EI-MS: 160.18, 144.27, 131.24, 118.21, 105.25, 78.08.

N-Benzylidenecyclopropanamine (1b): Yellow oil, yield 93 %, ^{1}H NMR (400 M, $CDCl_3$, δ ppm): 0.90-1.01 (m, 4H, cyclopropane-CH_2), 3.00-3.06 (m, 1H, cyclopropane-CH), 7.37-7.39 (d, 3H, Ar-H), 7.66-7.69 (m, 2H, Ar-H), 8.45 (s, 1H, HC=); EI-MS: 146.18, 130.24, 117.31, 90.19, 77.24, 63.29.

N-(4-Bromobenzylidene)cyclopropanamine (1c): White solid, m.p. 78-80 ºC, yield 96 %, ^{1}H NMR ($CDCl_3$, d ppm): 0.93-1.00 (m, 4H, cyclopropane-CH_2), 3.02-3.03 (m, 1H, cyclopropane-CH), 7.50 (d, *J* = 7.96 Hz, 2H, Ar-H), 7.56



Scheme-I: Synthetic route of α-aminophosphonate containing cyclopropane moiety

TABLE-**1**
PHYSICO-CHEMICAL DATA OF TITLE COMPOUNDS

No.	Formula	Appearance	Yield (%)	HR-ESI-MS (Caulc.)
2a	$C_{13}H_{20}NO_3P$	Light yellow ceraceous solid	83	$[M + Na]^+$ 292.1076(292.1073)
2b	$C_{12}H_{18}NO_3P$	Light yellow ceraceous solid	86	$[M + Na]^+$ 278.0923(278.0916)
2c	$C_{12}H_{17}BrNO_3P$	Light yellow ceraceous solid	88	$[M + Na]^+$ 356.0021(356.0022)
2d	$C_{12}H_{17}BrNO_3P$	Light yellow ceraceous solid	81	$[M + Na]^+$ 356.0028(356.0022)
2e	$C_{12}H_{17}ClNO_3P$	Light yellow ceraceous solid	85	$[M + Na]^+$ 312.0531(312.0527)

TABLE-2
^{1}H NMR AND ^{31}P NMR DATA OF TITLE COMPOUNDS

No.	^{1}H NMR (400 M, $CDCl_3$) and ^{31}P NMR (162 M, $CDCl_3$)
2a	^{1}H NMR: 0.30-0.47 (m, 4H, cyclopropane-CH_2), 2.03-2.08 (m, 1H, cyclopropane-CH), 2.35(s, 3H, CH_3), 3.50 (d, *J* = 10.45 Hz, 3H, CH_3), 3.75 (d, *J* = 10.45 Hz, 3H, CH_3), 4.03 (s, 0.5H, CH), 4.09 (s, 0.5H, CH), 7.17 (d, *J* = 7.74 Hz, 2H, Ar-H), 7.30 (d, *J* = 7.74 Hz, 2H, Ar-H); ^{31}P NMR: 27.07.
2b	^{1}H NMR: 0.41-0.49 (m, 4H, cyclopropane-CH_2), 2.72-2.76 (m, 1H, cyclopropane-CH), 3.53 (d, *J* = 10.54 Hz, 3H, CH_3), 3.78(d, *J* = 10.54 Hz, 3H, CH_3), 4.22 (s, 0.5H, CH), 4.28 (s, 0.5H, CH), 7.33-7.54 (m, 5H, Ar-H); ^{31}P NMR: 22.95.
2c	^{1}H NMR: 0.38-0.60 (m, 4H, cyclopropane-CH_2), 2.10-2.19 (m, 1H, cyclopropane-CH), 3.55(d, *J* = 10.57 Hz, 3H, CH_3), 3.76 (d, *J* =10.57 Hz, 3H, CH_3), 4.66 (s, 0.5H, CH), 4.72 (s, 0.5H, CH), 7.38(d, *J* = 7.38 Hz, 2H, Ar-H), 7.50(d, *J* =7.38 Hz, 2H, Ar-H); ^{31}P NMR: 21.38.
2d	^{1}H NMR: 0.63-0.90 (m, 4H, cyclopropane-CH_2), 2.39 (m, 1H, cyclopropane-CH), 3.65(d, *J* =10.67 Hz, 3H, CH_3), 3.90(d, *J* = 10.65 Hz, 3H, CH_3), 5.21 (s, 0.5H, CH), 5.25 (s, 0.5H, CH), 7.49-7.68 (m, 4H, Ar-H); ^{31}P NMR: 20.27.
2e	^{1}H NMR: 0.59-0.86 (m, 4H, cyclopropane-CH_2), 2.31-2.39 (m, 1H, cyclopropane-CH), 3.64 (d, *J* = 10.73 Hz, 3H, CH_3), 3.88 (d, *J* = 10.73 Hz, 3H, CH_3), 5.13 (s, 0.5H, CH), 5.18 (s, 0.5H, CH), 7.31-7.46 (m, 4H, Ar-H); ^{31}P NMR: 21.57.

TABLE-3
FT-IR AND EI-MS DATA OF TITLE COMPOUNDS

No	FT-IR (ν cm^{-1})	EI-MS
2a	3298 (NH), 1612, 1511, 1443 (benzene ring), 1246, 1033 (P=O)	268.12, 254.06, 174.15, 160.14, 144.23, 130.24, 118.23, 105.24, 91.23, 79.17
2b	3283 (NH), 1615, 1451 (benzene ring), 1241, 1030 (P=O)	254.07, 200.08, 160.14, 146.10, 130.20, 117.21, 104.18, 91.21, 79.16
2c	3281 (NH), 1667, 1589, 1486 (benzene ring), 1247, 1033 (P=O)	333.92, 224.07, 208.17, 182.17, 171.14, 144.26, 116.20, 89.19, 79.15, 63.10
2d	3289 (NH), 1674 1588, 1465 (benzene ring), 1246, 1031 (P=O)	333.84, 224.11, 210.14, 199.18, 184.14, 169.11, 144.25, 128.26, 116.20
2e	3293 (NH), 1572, 1470, 1449 (benzene ring), 1259, 1033 (P=O)	289.98, 180.17, 164.21, 144.29, 125.20, 89.24

(d, *J* = 7.96 Hz, 2H, Ar-H), 8.38 (s, 1H, HC=); EI-MS: 224.07, 184.10, 169.11, 144.16, 128.23, 116.14, 89.11, 65.02.

N-(2-Bromobenzylidene)cyclopropanamine (1d): Yellow oil, yield 91 %, ^{1}H NMR ($CDCl_3$, δ ppm): 0.92-1.04 (m, 4H, cyclopropane-CH_2), 3.09-3.14 (m, 1H, cyclopropane-CH), 7.19-7.32 (m, 2H, Ar-H), 7.53-7.55 (d, 1H, Ar-H), 7.93-7.95 (d, 1H, Ar-H), 8.80 (s, 1H, HC=); EI-MS: 224.07, 199.19, 184.18, 171.18, 144.25, 116.21, 89.22.

N-(2-Chlorobenzylidene)cyclopropanamine (1e): Yellow oil, yield 93 %, ^{1}H NMR ($CDCl_3$, δ ppm): 0.94-1.04 (m, 4H, cyclopropane-CH_2), 3.09-3.14 (m, 1H, cyclopropane-CH), 7.25-7.31 (m, 2H, Ar-H), 7.34-7.37 (d, 1H, Ar-H), 7.93-7.96 (d, 1H, Ar-H), 8.87 (s, 1H, HC=); EI-MS: 180.15, 164.21, 151.21, 144.29, 125.17, 116.25, 89.27.

Therotical calculations: An isolated molecule was selected as the initial structure, while DFT-B3LYP/6-31G (d,p)[20,21] methods in Gaussian 03 package[22] were used to optimize the structure of the title compound. Vibration analysis showed that the optimized structures were in accordance with the minimum points on the potential energy surfaces. All the convergent precisions were the system default values and all the calculations were carried out on the Nankai Stars supercomputer at Nankai University.

Biochemistry of KARI

Cloning, expression and purification of rice KARI: The KARI resultant expression plasmid was obtained from the Prof. Ronald G. Duggleby's lab[10a] and was used to transform *Escherichia coli*, BL21(DE3) cells. A single colony of these cells was inoculated into 20 mL of LB medium containing 50 mg/mL kanamycin. The culture was incubated overnight at 37 ºC and was used to inoculate each of two 1000 mL volumes of LB medium containing 50 mg/mL kanamycin; the cultures were incubated at 37 ºC with shaking. When an OD_{600} of 0.6 was reached, expression was induced by adding 1 µL isopropyl β-D-thiogalactoside to each culture. These were then incubated at room temperature (37 ºC) for a further 2 h with shaking and the cells were harvested by centrifugation and were keep in -30 ºC.

The frozen cell pellet was thawed, suspended in ice-cold purification buffer [50 mM *tris*-HCl (pH 7.9)/500 mM NaCl] containing 5 mM imidazole and then treated with lysozyme (10 mg/g of cells for 0.5 h at 0 ºC). The cells were disrupted by sonication, insoluble material was removed by centrifugation and the supernatant was passed through a 0.45 mm filter. The cell extract was applied to a 7 mL column of His·Bind resin (Novagen) that had been charged by using 50 mM $NiSO_4$ then equilibrated with purification buffer containing 5 mM imidazole. The loaded column was washed with 23 mL of the same buffer, followed by 30 mL of purification buffer containing 25 mM imidazole and then KARI was eluted with 30 mL of purification buffer containing 1 M imidazole. Fractions containing the enzyme were pooled, concentrated to 2.5 mL by ultrafltration and exchanged into 20 mM Na-Hepes buffer, pH 8.0 using a Pharmacia PD-10 column. The eluate was snap-frozen in low-temperature refrigerator and stored at -78 ºC.

Enzyme and protein assays (*in vitro*): Gerwick *et al.*[23] reported that the inhibition of *Escherichia coli* KARI is time-dependent. KARI activity was measured by following the decrease in A_{340} at 30 ºC in solutions containing 0.2 mM NADPH, 1 mM $MgCl_2$, substrate 2-acetolactate and inhibitors as required, in 0.1 M phosphate buffer, pH 8.0. Inhibitors was preincubated with enzyme in phosphate buffer at 30 ºC for 10 min before the reaction was started by adding the substrate combining with NADPH and $MgCl_2$. Protein concentrations were estimated using the bicinchoninic acid method[24] and protein purity was assessed by SDS-PAGE[25]. The yield of recombinant rice KARI from a 30 culture was 50 mg with a specific activity (measured with saturating 2-acetolactate) of 1.17 U/mg. The 2-acetolactate was prepared by the authors.

Herbicidal activity tests

Inhibition of the root growth of rape (*Brassica campestris*): The evaluated compounds were dissolved in water and emulsified if necessary. Rape seeds were soaked in distilled water for 4 h before being placed on a filter paper in a 6 cm Petri plate, to which 2 mL of inhibitor solution had been added in advance. Usually, 15 seeds were used on each plate. The plate was placed in a dark room and allowed to germinate for 65 h at 28 ± 1 ºC. The lengths of 10 rape roots selected from each plate were measured and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

Inhibition of the seedling growth of barnyard grass (*Echinochloa crusgalli*): The evaluated compounds were dissolved in water and emulsified if necessary. A total of 10 barnyard grass seeds were placed into a 50 mL cup covered with a layer of glass beads and a piece of filter paper at the bottom, to which 5 mL of inhibitor solution had been added in advance. The cup was placed in a bright room and allowed to germinate for 65 h at 28 ± 1 ºC. The heights of seedlings of above-ground plant parts from each cup were measured and the means were calculated. The check test was carried out in distilled water only. The percentage of the inhibition was calculated.

RESULTS AND DISCUSSION

In the one-pot three-component synthesis, substituent benzaldehyde first reacted with cyclopropyl amine and produced imine, then the reaction of imine and phosphinate yield the α-aminophosphonate compound. The water resulted from first reaction step resulted in the low yield of α-aminophosphonate. But in the stepwise synthesis, water was eliminated after first step and the yield was increased. The microwave irradiation assistant synthesis method was also used in this experiment. When one-pot three-component synthesis under microwave irradiation was carried, the yield was not high. While the imine reacted with phosphinate at 110 ºC under microwave irradiation (MW) about 0.5 h, the yield is higher than that of stepwise synthesis method (Table-4).

^{1}H NMR: In the ^{1}H NMR spectra of title compounds, the NH proton signals of title compounds were not observed, the reason is probably due to the exchange between the NH and $CDCl_3$. The CH proton was appeared around δ 4.00 ppm as two single peak due to the influences of both NH protons and 'P' splitting, which indicated the conversion of -CH=N- group (CH proton signal of intermediates **3** was observed at δ 7.50-8.80 ppm as a singlet) to >CH-NH- group.

TABLE-4
COMPARISON OF YIELDS OF **2b** THROUGH METHODS WITH OR WITHOUT MICROWAVE IRRADIATION

No.	Method	Component	Time (min)	Temperature (°C)	Yield (%)
2b	Heat	One-pot three-component	240	80	20
2b	MW	One-pot three-component	20	80	25
2b	Heat	Imine and phosphinate	240	80	18
2b	MW	Imine and phosphinate	10	80	40
2b	MW	Imine and phosphinate	20	90	47
2b	MW	Imine and phosphinate	30	110	86

^{31}P NMR spectra: All the synthesized title compounds **2a-e** were characterized by the down field ^{31}P NMR signal at δ 21-25 ppm.

Infrared spectra and mass spectra: The infrared spectrum of phosphoramidothioate derivatives **2a-e** showed absorption band at 3298-3281 cm^{-1} for N-H stretching. The characteristic stretching vibrations ν (P=O) appears at 1033-1030 cm^{-1}. The absorption band of benzene is at 1667-1440 cm^{-1}. All the title compounds of mass spectra are molecular ion peak. For example, the Fig. 1 showed the fragmentation route of **2a**.

Frontier orbital energy analysis: According to the frontier molecular orbital theory, HOMO and LUMO are the most important factors that affect the bioactivity. HOMO has the priority to provide electrons, while LUMO can accept electrons firstly[10]. Thus study on the frontier orbital energy can provide useful information about the biological mechanism. From the Fig. 2, the geometry of the frame of **2c** is hardly influenced by the introduction of either phosphonate or benzene ring. The HOMO of the title compound is mainly located on the aromatic ring and cyclopropane ring. While, the LUMO of the title compound is located on the aromatic ring, phosphonate group and cyclopropane ring. The fact that the title compound has strong affinity suggests the importance of the frontier molecular orbital in the π-π stacking or hydrophobic interactions.

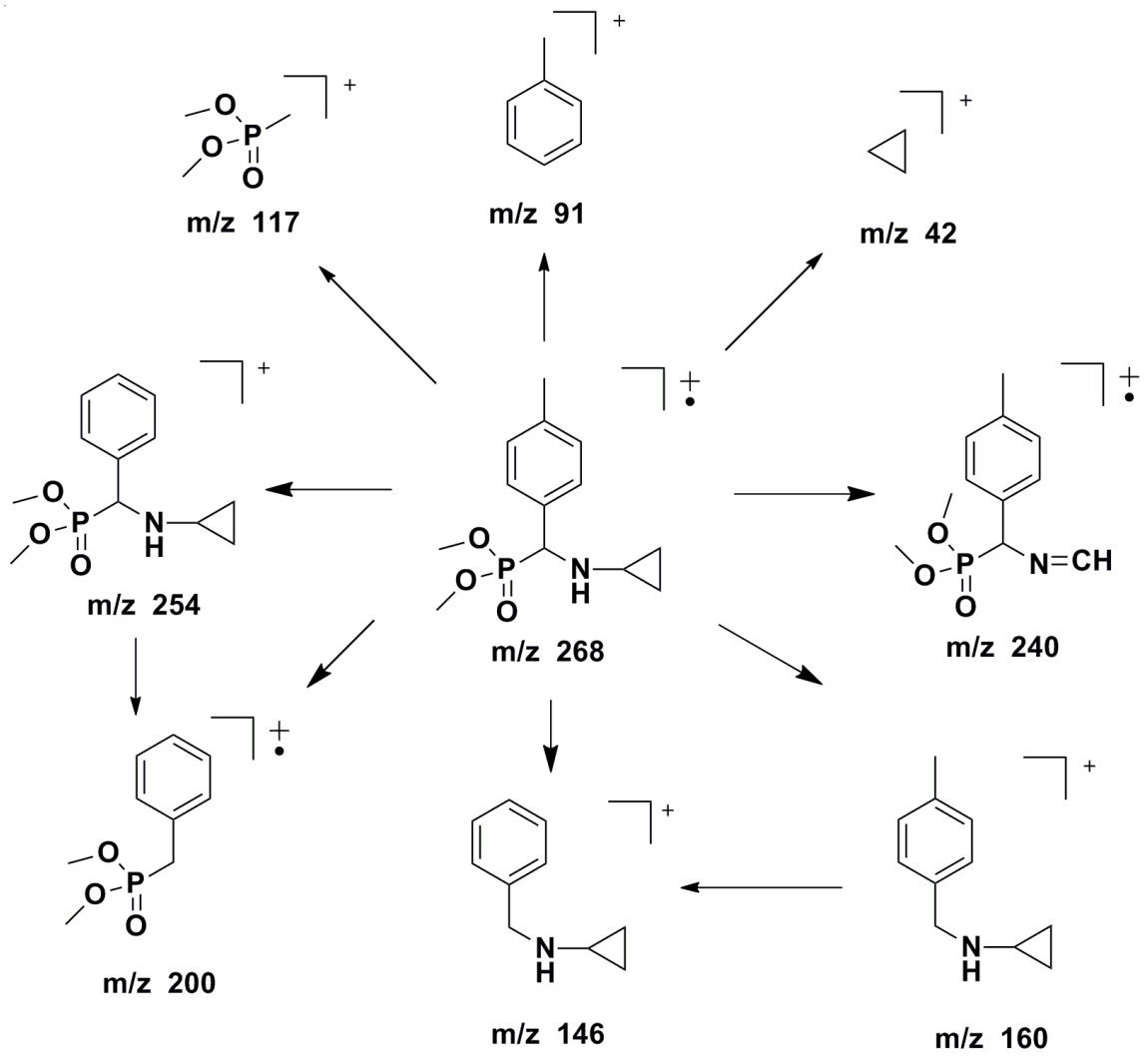
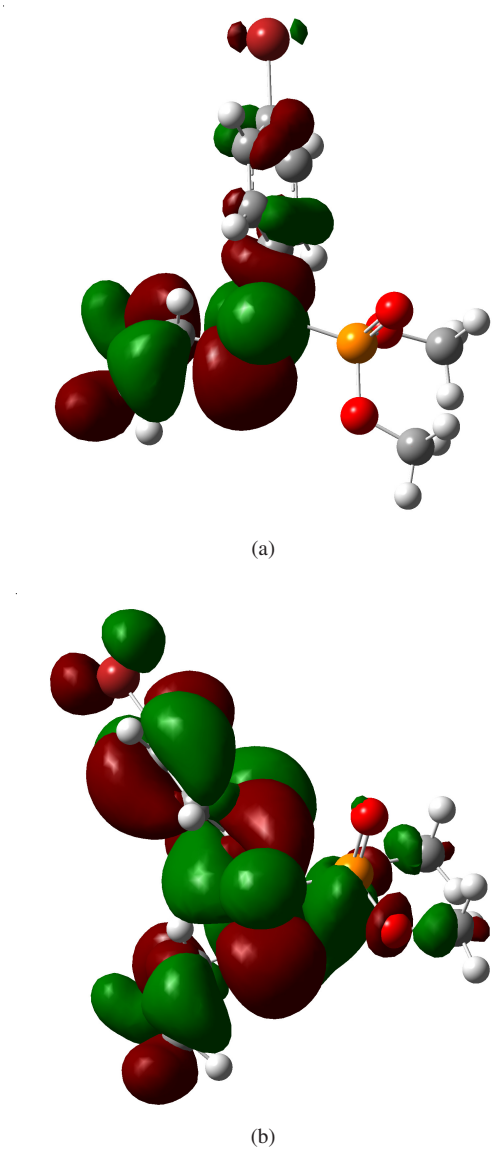


Fig. 1. Fragment route of **2a**

TABLE-5
BIOLOGICAL ACTIVITY OF TITLE COMPOUNDS

No.	KARI 100 (μg/mL)	*Brassica campestris*		*Echinochloa crusgalli*	
		10 (μg/mL)	100 (μg/mL)	10 (μg/mL)	100 (μg/mL)
2a	0	0	21.5	3.0	10.0
2b	18.89	26.9	43.8	15.0	25.0
2c	–	46.5	68.3	5.0	20.0
2d	0	28.4	57.9	5.0	15.0
2e	0.15	0	45.0	8.0	35.0
CPD	100	0	17.2	10.6	27.7

Note: Indicate the compound can not resolve in our test system, so no data obtain.



(a)

(b)

Fig. 2. Frontier molecular orbitals of **2c**: (a) HOMO of **2c**; (b) LUMO of **2c**

Herbicidal activities: The *in vitro* and *in vivo* herbicidal activity results of the title compounds against *Brassica campestris* and *Echinochloa crusgalli* and KARI inhibitory activity were listed in Table-5. Some of the title compounds exhibit good herbicidal activities against *Brassica campestris* at 100 ppm, which was better than that of CPD. Among them, compound **2c**, **2d** and **2e** had good herbicidal activity against *Brassica campestris*. All the present compounds exhibit weak herbicidal activity against *Echinochloa crusgalli*. Meanwhile, all these compounds had no obvious inhibitory against KARI. Because compound **3** can not resolve in present test system, we can not get the data of KARI activity. Some compounds give low activity with KARI.

Conclusion

New α-aminophosphonates were designed and synthesized. Their structures had been confirmed through ^{1}H NMR, ^{31}P NMR, EI-MS and FTICR-MS. Their biological activity tests show that some of these compounds had moderate herbicidal activity against *Brassica campestris*. The structure-activity relationship was also studied by frontier orbital energy.

ACKNOWLEDGEMENTS

This work was funded by National Natural Science Foundation of China (No. 21002090, 30900959) and the Key Laboratory of Pesticide Chemistry and Application, Ministry of Agriculture (MOA), Beijing, China (No. MOAPCA201005) and Specialized Research Fund for the Doctoral Program of Higher Education (No. 20070055044) and Tianjin Natural Science Foundation (No. 08JCYBJC00800); Zhejiang Provincial Natural Science Foundation of China (Y3080096) and Scientific Research Fund of Zhejiang Education Department (Y201018479).

REFERENCES

1. A. Popa, V. Parvulescu, S. Iliescu, N. Plesu, G. Ilia, L. Macarie and A. Pascariu, *Plastic Rubber Compos.*, **37**, 193 (2008).
2. Y.J. Li and H.W. He, *Phosphorus Sulfur Silicon Rel. Elem.*, **183**, 712 (2008).
3. Y.X. Chen, J.T. Du, L.X. Zhou, X.H. Liu, Y.F. Zhao, H. Nakanishi and Y.M. Li, *Chem. Biol.*, **13**, 937 (2006).
4. (a) S.C. Fields, *Tetrahedron*, **55**, 12237 (1999); (b) D. Redmore, *J. Org. Chem.*, **43**, 992 (1978).
5. X.P. Rao, Z.Q. Song and L. He, *Heteroatom Chem.*, **19**, 512 (2008).
6. S. Yang, X.W. Gao, C.L. Diao, B.A. Song, L.H. Jin, G.F. Xu, G.P. Zhang, W. Wang, D.Y. Hu, W. Xue, X. Zhou and P. Lu, *Chin. J. Chem.*, **24**, 1581 (2006).
7. J.A. Steere, P.B. Sampson and J.F. Honek, *Bioorg. Med. Chem. Lett.*, **12**, 457 (2002).
8. B.A. Song, M.G. Jiang, Y.L. Wu, X.Q. He, S. Yang, L.H. Jin, G. Liu and D.Y. Hu, *Chin. J. Org. Chem.*, **23**, 967 (2003).
9. N. Ohno, K. Fujimoto, Y. Mizutani, T. Mizutani, M. Hirano, N. Itaya, T. Honda and H. Yoshioka, *Agric. Biol. Chem.*, **38**, 881 (1974).
10. (a) Y.T. Lee, T.T. Hang and R.G. Duggleby, *Plant Sci.*, **168**, 1035 (2005); (b) X.H. Liu, P.Q. Chen, B.L. Wang, Y.H. Li and Z.M. Li, *Bioorg. Med. Chem. Lett.*, **17**, 3784 (2007); (c) X.H. Liu, P.Q. Chen, F.Q. He, Y.H. Li, S.H. Wang and Z.M. Li, *Struct. Chem.*, **5**, 563 (2007); (d) X.H. Liu, C.Y. Zhang, W.C. Guo, Y.H. Li, P.Q. Chen, T. Wang, W.L. Dong, B.L. Wang, H.W. Sun and Z.M. Li, *J. Enzym. Inhib. Med. Chem.*, **24**, 545 (2009).

11. (a) X.H. Liu, Y.X. Shi, Y. Ma, G.R. He, W.L. Dong, C.Y. Zhang, B.L. Wang, S.H. Wang, B.J. Li and Z.M. Li, *Chem. Biol. Drug Des*., **73**, 320 (2009); (b) X. H. Liu, Y.X. Shi, Y. Ma, C.Y. Zhang, W.L. Dong, P. Li, B.L. Wang, B.J. Li and Z.M. Li, *Eur. J. Med. Chem*., **44**, 2782 (2009).
12. B.C. Ranu and A. Hajra, *Green Chem*., **4**, 551 (2002).
13. L. Pan, X.H. Liu, Y.X. Shi, B.L. Wang, S.H. Wang, B.J. Li and Z.M. Li, *Chem. Res. Chin. Univ*., **26**, 389 (2010).
14. L. Tusek-Bozic, M. Juribasic, M. Komac, V. Cmrecki, I. Zrinski, J. Balzarini and E. De Clercq, *Lett. Org. Chem*., **4**, 332 (2007).
15. B.A. Song, G.P. Zhang, S. Yang, D.Y. Hu and L.H. Jin, *Ultrason. Sonochem*., **13**, 139 (2006).
16. P.P. Sun, X. Yang and Z.X. Hu, *J. Chem. Res*., **4**, 240 (2006).
17. Z. Kudzin, P. Lyzwa, J. Luczak and G. Andrijewski, *Synthesis*, **1**, 44 (1997); (b) J.S. Yadav, B.V.S. Reddy, K. Sarita Raj, K. Bhaskar Reddy and A.R. Prasad, *Synthesis*, **15**, 2277 (2001); (c) S.G. Lee, J.H. Park, J. Kang and J.K. Lee, *Chem. Commun*., **17**, 1698 (2001); (d) D.Y. Hu, B.A. Song, G.P. Zhang, S. Yang, W. He, Y.L. Wu, Y.P. Hong, L.H. Jin and G. Liu, *Chin. J. Org. Chem*., **25**, 854 (2005).
18. X.D. Yang, *J. Chem. Res*., **9**, 489 (2008).
19. W.L. Dong, X.H. Liu, J.Y. Xu and Z.M. Li, *J. Chem. Res*., **9**, 530 (2008).
20. A. Becke, *J. Chem. Phys*., **98**, 5648 (1993).
21. C. Lee, W. Wang and R.G. Parr, *Phys. Rev*., **37B**, 785 (1988).
22. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. AlLaham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez and J.A. Pople, Gaussian 03, Revision C. 01, Gaussian, Inc., Wallingford CT (2004).
23. B.C. Gerwick, L.C. Mireles and R.J. Eilers, *Weed Technol*., **7**, 519 (1993).
24. P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson and D.C. Klenk, *Anal. Biochem.*, **150**, 76 (1985).
25. U.K. Laemmli, *Nature*, **227**, 680 (1970).