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PAPER

Synthesis and antitumor activity of α-aminophosphonates containing thiazole[5,4-*b*]pyridine moiety†

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A new procedure is developed for the synthesis of α-aminophosphonates containing thiazole[5,4-*b*]-pyridine moiety from conveniently available starting materials. The target compounds were characterized by infrared, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, mass spectrometry and elemental analysis. The newly synthesized compounds were evaluated for their anticancer activities against PC-3, Bcap-37, H460 cells *in vitro* by the MTT method. Compounds **3b** and **3f** are highly effective against PC-3, Bcap-37 cells and good to H460 cells. Further study is necessary to find out the potential antitumor activities.

Introduction

α-Aminophosphonic acids and their corresponding α-aminophosphonates have received much interest in organic and medicinal chemistry because they are analogous to both natural and unnatural amino acids.[1] Some of them have been used as potent enzyme inhibitors, antimicrobial, antitumor and antiviral agents.[2–6] Among these compounds, studies are mainly concentrated on those containing heterocycle moieties.

Fused systems containing a pyridine ring, in particular thiazolopyridines, are widely used as base structures in the design of various biologically active compounds.[7,8] There are six isomeric thiazolopyridine systems reported in the literature.[9] Thiazole[5,4-*b*]-pyridine belong to an important class of compounds which show a wide range of biological activities such as anticancer, antiviral, antibacterial.[10]

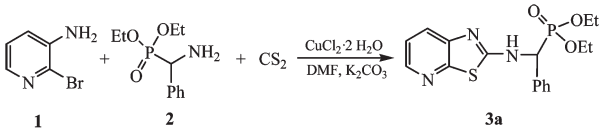
In view of this research and our desire to develop new anticancer agents of high potency, we fused biological thiazole[5,4-*b*]-pyridine and aminophosphonate structures to obtain compounds possessing better biological activities. In this paper, we have designed and synthesized a series of new α-aminophosphonates containing thiazole[5,4-*b*]pyridine moiety.

Results and discussion

Chemistry

The standard procedures for the syntheses of α-aminophosphonates containing heterocycle moieties mainly rely on the Kabachnik–Fields reaction. The Kabachnik–Fields reaction is a three-component reaction of a dialkyl phosphonate, a carbonyl and an amino compound.[11] The investigation was initiated by using the reaction of diethyl phosphate, benzaldehyde and thiazolo[5,4-*b*]pyridin-2-yl amine. The desired product *O*,*O*′-diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(phenylmethyl) phosphonate **3a** was obtained in a moderate yield (57%) under solvent-catalyst free condition. Although the reaction is easy to operate, the starting materials thiazolo[5,4-*b*]pyridin-2-ylamine is not readily available.

Recently, Ma and his group have developed a copper-catalyzed three-component synthesis of 2-*N*-substituted benzothiazoles.[12] According to the structure of *O*,*O*′-dialkyl-α-(thiazolo-[5,4-*b*]pyridin-2-yl)amino(substituted phenylmethyl) phosphonate **3**, we envisioned that 3-amino-2-bromopyridine **1** may be used to react with primary 1-aminophosphonate **2** under the catalysis of $CuCl_2{\cdot}2H_2O$ in presence of carbon disulfide, K_2CO_3 and *N*,*N*-dimethylformamide (DMF) to synthesize such products. Performing the reaction using the conditions similar to those reported by Ma and coworkers, the desired product was obtained in 86% yield Scheme 1.



1 2 3a

Scheme 1 Synthesis of α-aminophosphonate derivative **3a**.

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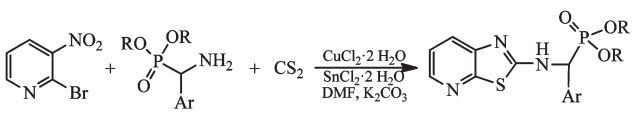
Encouraged by the result, we replaced 3-amino-2-bromopyridine **1** with 3-nitro-2-bromopyridine **4** and carried out the reaction using the following reaction conditions: 3-nitro-2-bromopyridine (1 equiv.), CS_2 (1.5 equiv.), primary 1-aminophosphonate (1.4 equiv.), K_2CO_3 (3 equiv.), $CuCl_2{\cdot}2H_2O$ (1 equiv.) and $SnCl_2{\cdot}2H_2O$ (3 equiv.) in DMF. To our delight, the desired product was isolated with 72% yield after reacting for 8 h at 105 °C. Then, we briefly examined the effect of different temperatures and ratio of **4**/$SnCl_2{\cdot}2H_2O$. The results showed that at 105 °C, the reaction preceded smoothly in high yield. To further evaluate the influence of the ratio of **4**/$SnCl_2{\cdot}2H_2O$, the reaction was carried out in DMF at 105 °C using a 1 : 1 to 1 : 5 ratio of **4**/$SnCl_2{\cdot}2H_2O$, leading to **3a** in 31%, 46%, 72%, 82%, and 78% yields, respectively. We concluded the best ratio of **4**/$SnCl_2{\cdot}2H_2O$ was 1 : 4.

With the optimized conditions in hand, we then performed the reaction of a variety of primary 1-aminophosphonates **5** and 3-nitro-2-bromopyridine **4** (Scheme 2). The results are summarized in Table 1.

As shown in Table 1, we were pleased to find that the method was applicable to a broad substrate scope on both substituted primary 1-aminophosphonates **2** and 3-nitro-2-bromopyridine **4**. It can be seen that this protocol can be applied to the primary 1-aminophosphonates with electron-withdrawing groups or electron-donating groups. Meanwhile, it was particularly noteworthy that the effects of different 3-nitro-2-bromopyridine **4** were also investigated. 4-Substituted 3-nitro-2-bromopyridin **6** can also delivered the desired product **7** (Scheme 3) in high yields (83%).

It was found that 3-nitro-2-bromobenzene **8** could undergo the reaction. The synthetic sequence was depicted in Scheme 4. The reaction of 3-nitro-2-bromobenzene **8** with primary

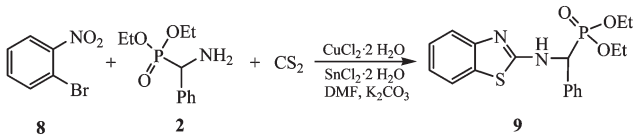


Scheme 2 Synthesis of α-aminophosphonate derivatives **3**.

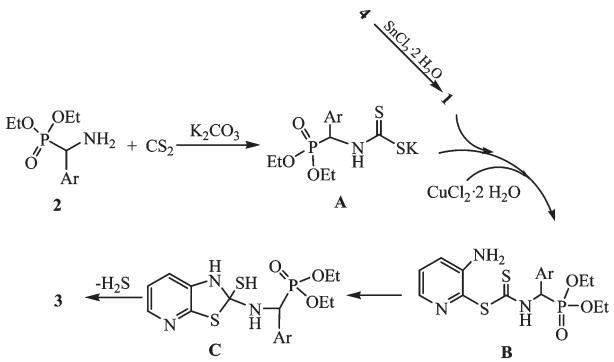
Table 1 Substrate scope of primary 1-aminophosphonates

Entry	Ar	R	Product	Yield (%)
1	Ph	Et	**3a**	82
2	*o*-F-C_6H_4	Et	**3b**	80
3	*p*-Me-C_6H_4	Et	**3c**	83
4	*p*-Cl-C_6H_4	Et	**3d**	85
5	*p*-MeO-C_6H_4	Et	**3e**	81
6	*p*-F-C_6H_4	Et	**3f**	80
7	Ph	Me	**3g**	83

Scheme 3 Synthesis of α-aminophosphonate derivative **7**.



Scheme 4 Synthesis of α-aminophosphonate derivative **9**.



Scheme 5 Proposed reaction mechanism.

1-aminophosphonates **2** gave *O*,*O*′-diethyl-α-(benzothiazole-2-yl) amino(phenylmethyl) phosphonate **9** in 80% yield.

A plausible mechanism for the formation of the target compounds was illustrated in Scheme 5. The reaction of primary 1-aminophosphonate **2** with carbon disulfide in the presence of K_2CO_3 to give dithiocarbamate salts **A**, and 3-nitro-2-bromopyridine **4** was reduced by tin(II) chloride to 3-amino-2-bromopyridine **1**. Under the catalysis of $CuCl_2{\cdot}2H_2O$, dithiocarbamate salts **A** can react with 3-amino-2-bromopyridine **1** to provide dithiocarbamates **B**. The amine group in **B** could in turn attack the C–S double bond to give cyclization products **C** which would deliver the desired products **3** upon elimination of hydrogen sulfide under the action of $CuCl_2{\cdot}2H_2O$.

The structure of α-aminophosphonate derivatives **3a–g** and **7** was thoroughly characterized with spectral and elemental analyses. The infrared spectrum of compound **3a** displayed bands at 3213 cm^{-1}, 1241 cm^{-1} and 1011 cm^{-1} due to N–H, P═O, and P–O stretching frequencies, respectively. The ^1H NMR spectrum of **3a** exhibited a multiplet appeared at δ 7.31–7.42, which accounts for the aromatic protons of the phenyl ring; a doublet appeared at δ 5.77, which accounts for CH proton; a multiplet appeared at δ 4.05–4.27, which accounts for the CH_2 proton. The two ethoxy groups of the phosphonates are magnetically nonequivalent, which is caused by the different shielding effects of α-benzene on the two ethoxy groups. The mass spectrum displayed the molecular ion $[M]^+$ peak at *m*/*z*: 377. All of the nonequivalent carbon atoms were identified in ^{13}C NMR and the total number of protons calculated from the integration curve accorded (in ^1H NMR) with the assigned structures.

Biological activities

The antitumor activities were determined by the standard MTT assay[13] against three cancer cell lines PC-3, Bcap-37 and H460. The screening results expressed as IC_{50} are summarized in

Table 2 The antitumor activities of the target compounds

	IC_{50} (μM)		
	PC-3	Bcap-37	H460
Compd. **3a**	6.13	8.49	12.55
Compd. **3b**	0.84	1.72	3.19
Compd. **3c**	12.02	17.75	9.26
Compd. **3d**	2.97	6.62	5.13
Compd. **3e**	25.18	33.71	23.10
Compd. **3f**	1.04	0.81	2.23
Compd. **3g**	8.91	0.96	14.64
Compd. **7**	5.65	8.41	11.69
Compd. **9**	24.77	22.83	27.21

Table 2. The IC_{50} values were the average of three independent experiments. According to the antitumor activity data, some of the target compounds exhibited potential anticancer activities. Compound **3f** bearing a fluorine atom at 4-position of phenyl ring showed good activity against the tested cancer cell lines, removal of the fluorine atom of **3f** to give **3a** caused the inhibition to decrease more than 5-fold, and replacement of the fluorine atom with methyl or methoxyl substituent in the 4-position of phenyl ring had a deactivating effect. Compound **7** with methyl substituent in thiazole[5,4-*b*]pyridine moiety retained micromolar activity against PC-3 and Bcap-37 cells. Interestingly, the compound containing thiazole[5,4-*b*]pyridine moiety was significantly more active than their benzothiazole-containing analogue (**3a** *vs.* **9**, Table 2), which suggested that the thiazole[5,4-*b*]pyridine moiety was crucial for the potency enhancements.

From the above results, some interesting structure–activity relationships can be disclosed that: (i) the substituted position of the phenyl group has no evident effect on the antitumor activity. (ii) The introduction of fluoro groups was crucial for improving their activity.

In conclusion, we have developed a new and efficient synthetic route to α-aminophosphonates containing thiazole[5,4-*b*]-pyridine moiety *via* a cascade three-component reaction from conveniently available starting materials. The antitumor activities of the target compounds were evaluated against three human cancer cell lines (PC-3, Bcap-37 and H460). Most of the target compounds displayed from moderate to excellent antitumor activities against one or two cancer cell lines. Compounds **3b** and **3f** were the most efficient, revealing a potential therapeutic application of antitumor. Influence of subtle structural modification and steric parameters on structure activity relationships for identifying lead bioactive compound would be taken up in our future course of investigation.

Experimental

Synthesis

Materials and equipment. Reagents were obtained commercially and used as received. Solvents were purified and dried by standard methods. Thiazolo[5,4-*b*]pyridin-2-yl amine was synthesized according to a modified literature procedure.[12] primary 1-aminophosphonates **2** was synthesized according to the method described in the literature.[13] The melting points were determined on an XT-4 micro melting point apparatus and uncorrected. IR spectra were recorded on an EQUINOX-55 spectrometer on a KBr matrix. NMR spectra were recorded on an INOVA-400 NMR instrument at room temperature using TMS as internal standard. Coupling constants (J) were measured in Hz. Chemical shift values (δ) are given in ppm. Elemental analyses were performed on a Vario EL III CHNS analyzer. Electrospray mass spectra were obtained with an MALDI-TOF Mass spectrometer. 200–300 Mesh silica gel was used for column chromatography.

Synthesis of target compound 3a (solvent-catalyst free condition). A reaction mixture of benzaldehyde (3 mmol), thiazolo-[5,4-*b*]pyridin-2-yl amine (3 mmol) and diethyl phosphate (3 mmol) was stirred in silicone oil bath at 100 °C for 2 h. The resultant viscous liquid was dissolved in ether and then washed, first with saturated aqueous $NaHCO_3$ and next with distilled water. The organic layer was separated, followed by extraction of the aqueous layer with ethyl acetate (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to yield the crude product. The crude product was purified by flash chromatography with ethyl acetate/petroleum ether as eluent on silica gel to afford the desired product.

Typical procedure for the synthesis of target compounds (cascade reaction condition). A reaction mixture of 3-nitro-2-bromopyridine **4** (1 mmol), CS_2 (1.5 mmol), primary 1-aminophosphonate **5** (1.4 mmol), K_2CO_3 (3 mmol), $CuCl_2{\cdot}2H_2O$ (1 mmol) and $SnCl_2{\cdot}2H_2O$ (4 mmol) in DMF (3.5 mL) was stirred at 105 °C for 8 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield the crude product. The crude product was purified by flash chromatography with ethyl acetate/petroleum ether as eluent on silica gel to afford the desired product.

O,O′-*Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(phenylmethyl)-phosphonate (**3a**)*. White powder, m.p. 114–116 °C; IR (KBr) ν: 3213, 2929, 2203, 1241 and 1011 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.42 (dd, J = 5.2 Hz, J = 1.2 Hz, 1 H), 8.11 (dd, J = 7.2 Hz, J = 0.8 Hz, 1 H), 7.81 (dd, J = 6.8 Hz, J = 2.4 Hz, 1 H), 7.31–7.42 (m, 5 H), 5.77 (d, J = 20.8 Hz, 1 H), 4.67 (bs, 1H), 4.05–4.27 (m, 2 H), 3.83–3.92 (m, 2 H), 1.31 (t, J = 7.2 Hz, 3 H), 1.20 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.3, 160.1, 142.7, 138.4, 128.5, 128.3, 126.1, 126.0, 125.6, 123.2, 122.8, 121.2, 65.4 (d, J = 3.0 Hz), 59.0, 58.8, 16.6 16.1; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 19.7. MALDI-TOF MS: m/z = 377 $(M)^+$. Anal. Calcd for $C_{17}H_{20}N_3O_3PS$: C 54.10, H 5.34, N 11.13, S 8.50; found: C 54.13, H 5.38, N 11.07, S 8.46.

O,O′-*Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(2-fluorophenyl-methyl)phosphonate (**3b**)*. White powder, m.p. 103–105 °C; IR (KBr) ν: 3234, 2981, 2208, 1233 and 1013 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.31 (dd, J = 4.4 Hz, J = 4.4 Hz, 1 H), 8.17 (dd, J = 6.0 Hz, J = 4.8 Hz, 1 H), 7.95 (dd, J = 2.0 Hz, J = 1.6 Hz, 1 H), 7.30–7.59 (m, 4 H), 5.82 (d, J = 22.8 Hz, 1 H), 4.59 (bs, 1H), 4.26–4.30 (m, 2 H), 3.98–4.17 (m, 2 H), 1.34 (t, J = 6.4 Hz, 3 H), 1.26 (t, J = 6.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.8, 157.9, 140.7, 135.0, 129.5, 128.5, 128.1,

127.1, 126.6, 125.8, 125.1, 121.8, 68.6 (d, J = 3.6 Hz), 57.7, 56.1, 16.2, 16.1; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 20.3. MALDI-TOF MS: m/z = 396 $[M + H]^+$. Anal. Calcd for $C_{17}H_{19}FN_3O_3PS$: C 51.64, H 4.84, N 10.68, S 8.11; found: C 51.67, H 4.88, N 10.71, S 8.13.

*O,O′-Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(4-methylphenylmethyl)phosphonate (**3c**).* White powder, m.p. 111–112 °C; IR (KBr) v: 3285, 2988, 2202, 1231 and 1027 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.28 (dd, J = 2.4 Hz, J = 0.4 Hz, 1 H), 8.16 (dd, J = 1.2 Hz, J = 2.4 Hz, 1 H), 7.96 (dd, J = 1.2 Hz, J = 6.4 Hz, 1 H), 7.20 (d, J = 2.0 Hz, 2 H), 7.18 (d, J = 2.0 Hz, 2 H), 5.28 (d, J = 22.0 Hz, 1 H), 4.90 (bs, 1H), 3.77–4.20 (m, 4 H), 2.33 (s, 3 H), 1.29 (t, J = 7.2 Hz, 3 H), 1.16 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.8, 161.3, 139.7, 139.1, 129.5, 129.0, 128.4, 128.1, 127.7, 127.2, 126.3, 67.2 (d, J = 3.0 Hz), 59.7, 58.6, 20.8, 14.6, 14.4; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 21.5. MALDI-TOF MS: m/z = 391 $(M)^+$. Anal. Calcd for $C_{18}H_{22}N_3O_3PS$: C 55.23, H 5.67, N 10.74, S 8.19; found: C 55.22, H 5.70, N 10.71, S 8.21.

*O,O′-Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(4-chloro phenylmethyl)phosphonate (**3d**).* White powder, m.p. 121–123 °C; IR (KBr) v: 3233, 2931, 2211, 1239 and 1038 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.29 (dd, J = 4.8 Hz, J = 2.4 Hz, 1 H), 8.17 (dd, J = 4.0 Hz, J = 1.2 Hz, 1 H), 7.92 (dd, J = 5.6 Hz, J = 0.8 Hz, 1 H), 7.47 (d, J = 2.4 Hz, 2 H), 7.32 (d, J = 2.4 Hz, 2 H), 5.66 (d, J = 21.6 Hz, 1 H), 4.63 (bs, 1H), 3.87–4.23 (m, 4 H), 1.44 (t, J = 5.6 Hz, 3 H), 1.39 (t, J = 5.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 162.6, 158.7, 143.2, 140.1, 130.5, 129.1, 128.6, 128.0, 127.3, 126.9, 126.1, 63.2 (d, J = 3.0 Hz), 60.4, 59.2, 14.8, 14.0; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 19.9. MALDI-TOF MS: m/z = 434 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{19}ClN_3O_3PS$: C 49.58, H 4.65, N 10.20, S 7.79; found: C 49.61, H 4.58, N 10.27, S 7.82.

*O,O′-Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(4-methoxyl phenylmethyl)phosphonate (**3e**).* White powder, m.p. 107–109 °C; IR (KBr) v: 3224, 2979, 2201, 1232 and 1026 cm^{-1}. δ 8.06–8.30 (m, 3 H), 7.30 (d, J = 8.4 Hz, 2 H), 6.90 (d, J = 8.4 Hz, 2 H), 5.49 (d, J = 22.0 Hz, 1 H), 4.81 (bs, 1H), 3.79–4.23 (m, 7 H), 1.32 (t, J = 7.2 Hz, 3 H), 1.19 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.4, 159.1, 142.5, 140.2, 131.6, 130.3, 129.8, 128.2, 127.7, 127.1, 126.7, 65.9 (d, J = 3.75 Hz), 58.7, 57.6, 55.3, 16.4, 16.2; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 20.9. MALDI-TOF MS: m/z = 430 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{22}N_3O_4PS$: C 55.23, H 5.67, N 10.74, S 8.19; found: C 53.06, H 5.44, N 10.31, S 7.87.

*O,O′-Diethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(4-fluoro phenylmethyl)phosphonate (**3f**).* White powder, m.p. 119–121 °C; IR (KBr) v: 3221, 2911, 2209, 1251 and 1026 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.24 (dd, J = 2.4 Hz, J = 1.2 Hz, 1 H), 8.11 (dd, J = 2.0 Hz, J = 0.8 Hz, 1 H), 7.92 (dd, J = 2.4 Hz, J = 1.2 Hz, 1 H), 7.59 (d, J = 2.0 Hz, 2 H), 7.48 (d, J = 2.0 Hz, 2 H), 5.57 (d, J = 22.4 Hz, 1 H), 4.81 (bs, 1H), 3.83–4.24 (m, 4 H), 1.32 (t, J = 7.2 Hz, 3 H), 1.18 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.7, 159.2, 140.8, 139.9, 130.2, 129.6, 129.0, 128.3, 127.5, 126.2, 125.7, 65.1 (d, J = 7.4 Hz), 59.7, 58.9, 14.4, 14.1; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 21.9. MALDI-TOF MS: m/z = 418 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{19}FN_3O_3PS$: C 51.64, H 4.84, N 10.63, S 8.11; found: C 51.71, H 4.90, N 10.66, S 8.12.

*O,O′-Dimethyl-α-(thiazolo[5,4-*b*]pyridin-2-yl)amino(phenylmethyl)-phosphornate (**3g**).* White powder, m.p. 102–104 °C; IR (KBr) v: 3219, 2941, 2217, 1252 and 1026 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.25 (dd, J = 6.4 Hz, J = 1.6 Hz, 1 H), 8.18 (dd, J = 6.8 Hz, J = 1.2 Hz, 1 H), 8.09 (dd, J = 1.2 Hz, J = 2.4 Hz, 1 H), 7.27–7.42 (m, 5 H), 5.63 (d, J = 22.0 Hz, 1 H), 4.72 (bs, 1H), 3.83 (d, J = 10.8 Hz, 3 H), 3.63 (d, J = 10.8 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.5, 159.4, 142.7, 140.1, 129.1, 128.3, 127.7, 127.1, 126.3, 123.4, 122.1, 121.7, 63.8 (d, J = 8.7 Hz), 55.7, 53.2; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 20.4. MALDI-TOF MS: m/z = 349 $(M)^+$. Anal. Calcd for $C_{15}H_{16}N_3O_3PS$: C 51.57, H 4.62, N 12.03, S 9.18; found: C 51.62, H 4.66, N 12.00, S 9.12.

*O,O′-Diethyl-α-(4-methylthiazolo[5,4-*b*]pyridin-2-yl)amino(phenylmethyl)phosphonate (7).* White powder, m.p. 107–109 °C; IR (KBr) v: 3233, 2923, 2206, 1252 and 1019 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.45 (d, J = 2.8 Hz, 1 H), 8.33 (d, J = 2.8 Hz, 1 H), 7.37–7.08 (m, 5 H), 5.65 (d, J = 22.4 Hz, 1 H), 4.54 (bs, 1H), 3.76–4.24 (m, 4 H), 2.22 (s, 3 H), 1.32 (t, J = 7.2 Hz, 3 H), 1.23 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.1, 162.3, 152.2, 141.2, 138.4, 130.0, 129.1, 128.1, 125.6, 123.4, 122.6, 121.2, 66.7 (d, J = 3.8 Hz), 59.1, 58.0, 21.3, 16.2 16.0; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 21.4. MALDI-TOF MS: m/z = 414 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{22}N_3O_3PS$: C 55.23, H 5.67, N 10.74, S 8.19; found: C 55.34, H 5.74, N 10.81, S 8.10.

O,O′-Diethyl-α-(benzothiazole-2-yl)amino(phenylmethyl)phosphonate (9). White powder, m.p. 112–114 °C (lit.[14] m.p. 112 °C); IR (KBr) v: 3210, 2907, 1256 and 1031 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54 (dd, J = 7.2 Hz, J = 7.2 Hz, 4 H), 7.06–7.39 (m, 5 H), 5.54 (d, J = 22.4 Hz, 1 H), 4.84 (bs, 1H), 3.98–4.22 (m, 4 H), 1.30 (t, J = 7.2 Hz, 3 H), 1.15 (t, J = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 151.9, 135.0, 131.0, 128.5, 128.2, 128.1, 125.8, 121.8, 120.7, 119.3, 63.6 (d, J = 10.7 Hz), 56.7, 55.1, 16.4, 16.2; ^{31}P NMR ($CDCl_3$, 200 MHz): δ 22.7. MALDI-TOF MS: m/z = 399 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{21}N_2O_3PS$: C 57.44, H 5.62, N 7.44, S 8.52; found: C 57.56, H 5.69, N 7.41, S 8.49.

Antitumor activities assay *in vitro*

The antitumor activities of the target compounds (**3a–3g**, **7**) were evaluated with human prostate cancer cell line (PC-3), human breast cancer cell line (Bcap-37), and human large cell lung cancer cell line (H460). PC-3, Bcap-37 and H460 were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 and maintained in a Thermo incubator (Waltham, MA) with a humidified air containing of 5% CO_2, 95% air. All culture media contained 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin solution (10 000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl). The cancer cell lines were cultured in minimum essential medium (MEM). Four-thousand cells (per well) suspended in MEM, were plated onto each well of a 96-well plate and incubated for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at the terminal concentration of 5 μg mL^{-1} and incubated

with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured using the ELISA reader. All of the compounds were tested in triplicate in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three measurements and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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