Synthesis and Antiproliferative Activity of Novel α-Aminophosphonates

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A novel series of carbazole-based α-aminophosphonates were synthesized by three component coupling of 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde, amine and diethyl phosphite using polyethylene glycol (PEG-400) as a green reaction media. The antiproliferative activity of these molecules was evaluated against three cancer cell lines. Of these, compounds 4c, 4e and 4m were found to exhibit good antiproliferative activity against three cancer cells, A549, MCF-7, and NCI-N87.

Key words α-aminophosphonate; carbazole; antiproliferative activity; cancer cell

Organophosphorus compounds have been found a wide variety of applications in the areas of agricultural, industrial, and medicinal chemistry due to their biological and physical properties as well as their utility as synthetic intermediates.[1–5] In recent years, a significant attention has been focused on the synthesis and biological evaluation of α-aminophosphonic acids and their phosphonate derivatives. Since α-aminophosphonate derivatives are structural mimics of α-amino acids, some of these compounds exhibit very high potency in inhibiting the enzymes that are involved in the metabolism of the corresponding amino acids. Generally, low mammalian toxicity of these compounds makes them attractive for use in agriculture and medicine.[6–9] Numerous of them possess antifungal, pesticidal, herbicidal and plant growth regulatory activity and are of particular interest for agrochemistry.[8–12] α-Aminophosphonates have also been found to act as enzyme inhibitors, haptens of catalytic antibiotics, inhibitors of serine hydrolases, uridine 5′-diphosphate (UDP)-galactopyranose mutase, human immunodeficiency virus (HIV) protease, and antitumor agents.[13–20] On the other hand, carbazole derivatives exhibit diverse biological activities such as antimalarial, antibacterial, anti-tuberculosis (TB), anti-HIV, anti-inflammatory, antihistaminic, and antitumor activities.[21–24] Furthermore, carbazole-based oligomers or polymers have also attracted much interest in materials science because of their interesting electrical and optical properties.[25–27]

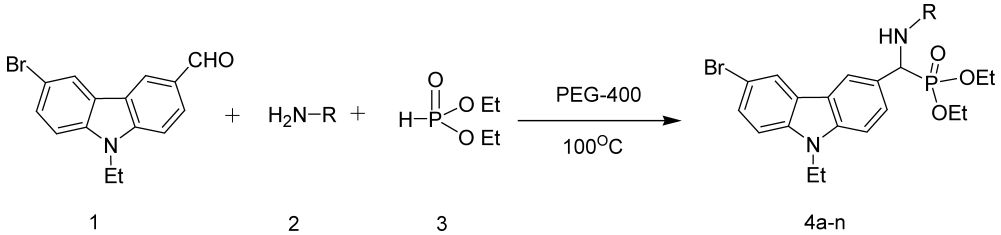
Owing to their synthetic and biological importance, the chemistry of aminophosphonates and carbazole derivatives has stimulated an increasing interest and the synthesis of carbazole-based aminophosphonates remains a great interest in the field of medicinal chemistry.

As part of our continued interest in developing new biologically important organophosphorus compounds,[28–31] we herein report the synthesis of a newly designed series of carbazole-based α-aminophosphonates. The antiproliferative activity of these molecules was evaluated against three cancer cell lines A549 (human lung cancer), MCF-7 (human breast cancer), and NCI-N87 (human stomach cancer).

Results and Discussion

On the basis of our previous results[28] polyethylene glycol (PEG-400) was used for the one pot three component synthesis of α-aminophosphonates. The carbazole-based α-aminophosphonates were synthesized by the coupling of 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde, amine, and diethyl phosphite in PEG-400. The reaction was complete in 6–7 h at 100°C temperature (Chart 1). After completion of the reaction, cold water was added to the reaction mixture and the resulting precipitate was filtered and washed with water. The solid product was then dried and purified by column chromatography to give the pure α-aminophosphonate.

In this PEG mediated reaction, different kinds of substituted anilines carrying either electron donating or electron withdrawing substituents and also benzylamine reacted well to give the desired products in good yields and the results are summarized in Table 1. In this reaction PEG-400 not only acts as the solvent but also accelerates the imine formation and nucleophilic addition of phosphite to the imine by increasing its electrophilicity through hydrogen bonding by its hydroxyl group with the imine nitrogen. The structures of all the compounds were confirmed by IR, ^{1}H-, ^{13}C-, and ^{31}P-NMR and mass spectroscopy. The IR spectra of compounds **4a**–**n** showed the expected absorption bands at 3378–3269, 1234–1231, and 973–963 cm^{-1}, which are attributed to N–H,



1 2 3 4a-n

Chart 1. Synthesis of Carbazole-Based α-Aminophosphonates

The authors declare no conflict of interest.

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Table 1. Synthesis of Carbazole-Based α-Aminophosphonates Using PEG-400

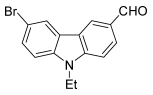
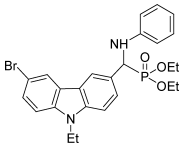
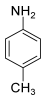
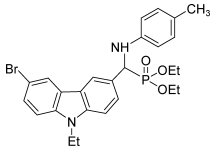
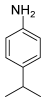
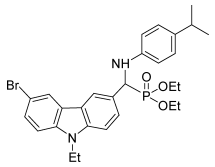
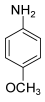
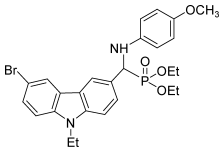
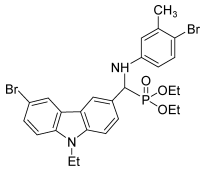
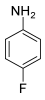
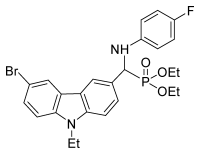
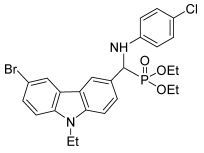
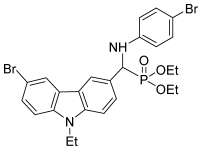
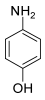
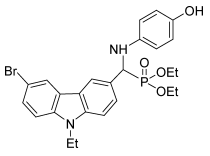
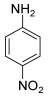
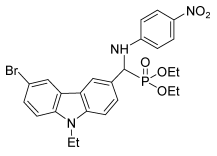
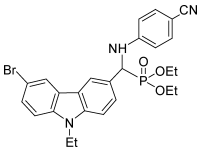
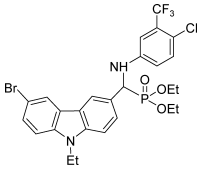
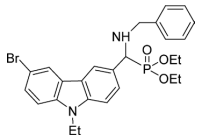
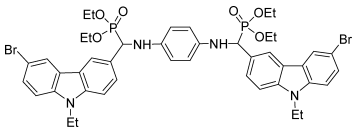
Entry	Aldehyde	Amine	Product	Yield (%)[a]
a				88
b				90
c				92
d				90
e				86
f				89
g				90
h				89
i				84
j				82

Table 1. Synthesis of Carbazole-Based α-Aminophosphonates Using PEG-400

Entry	Aldehyde	Amine	Product	Yield (%)[a]
k				78
l				81
m				79
n				75

a) Isolated yields.

Table 2. Maximum Percentage of Inhibition for Compounds **4a**–**n** at Doses of 5 and 10 μM

Compound	Inhibition (%) of cell lines (mean±S.E.M.)					
	A549		MCF-7		NCI-N87	
	5 μM	10 μM	5 μM	10 μM	5 μM	10 μM
4a	42.19±0.21	36.85±0.32	0[a]	0[a]	27.17±0.31	35.72±0.11
4b	21.09±0.29	36.91±0.05	0[a]	0[a]	5.23±0.54	25.5±1.72
4c	63.76±0.02	68.64±0.24	26.15±2.62	31.78±2.12	44.13±0.22	55.84±0.98
4d	50.45±0.21	51.65±0.56	0[a]	0[a]	27.91±0.19	46.66±0.59
4e	56±0.05	56.72±0.42	39.04±1.01	48.92±3.01	76.67±0.14	85.93±0.14
4f	15.71±0.77	22.35±0.22	0[a]	0[a]	21.32±0.93	26.61±0.77
4g	8.61±0.05	16.99±4.54	0[a]	0[a]	0[a]	8.27±0.57
4h	4.69±1.20	17.65±0.50	0[a]	0[a]	0[a]	13.25±1.11
4i	4.69±0.85	4.34±0.13	0[a]	0[a]	29.94±0.75	40.72±0.74
4j	10.35±0.37	11.23±1.17	0[a]	0[a]	0[a]	13.88±1.01
4k	1.31±0.56	8.4±0.51	0[a]	0[a]	0[a]	5.09±0.34
4l	29.71±0.16	38.64±0.50	0.85±0.73	3.46±0.57	2.31±0.63	16.49±0.54
4m	71.47±0.32	84.05±0.56	25.58±3.10	61.81±0.97	53.23±0.45	89.86±1.39
4n	1.29±0.31	61.36±0.46	0[a]	0[a]	0[a]	0[a]

a) The score zero shows no inhibition of cell proliferation.

P=O, P–O stretching vibrations, respectively. In ^{1}H-NMR spectra, doublet or doublet of doublet in the region of 4.74–4.99 ppm indicates the presence of methine proton. The remaining proton signals are observed in the expected regions. In ^{13}C-NMR spectra, doublet in the region of 55.3–59.5 ppm ($^1J_{P-C}$=150.2–154.8 Hz) confirms the presence of methine carbon which is directly attached to phosphorus. In ^{31}P-NMR spectra of all α-aminophosphonates **4a**–**n** showed a signal in the region of 22.83–25.11 ppm. The high-resolution mass spectral data for compounds **4a**–**n** are provided in Experimental.

Antiproliferative Activity The antiproliferative activity of the synthesized compounds **4a**–**n** was evaluated *in vitro* against three different cancer cell lines A549 (human lung cancer), MCF-7 (human breast cancer) and NCI-N87 (human stomach cancer) by WST-1 cell proliferation assay. The results are summarized in Table 2. As evident from the results obtained, all the aminophosphonates (**4a**–**n**) exhibited good to moderate antiproliferative activity on A549 cells compare to MCF-7 and NCI-N87 cells. Especially, compounds **4c**, **4d**, **4e**, and **4m** showed good antiproliferative activity on A549 cells. It was observed that the compounds **4a**–**n** displayed lower activity against MCF-7 cells. Except **4c**, **4e**, and **4m**, the remaining compounds showed zero or negligible antiproliferative activity on MCF-7 cells. In the case of NCI-N87 cells, compounds **4c**, **4e**, and **4m** showed good antiproliferative activity, and other compounds showed negligible antiproliferative

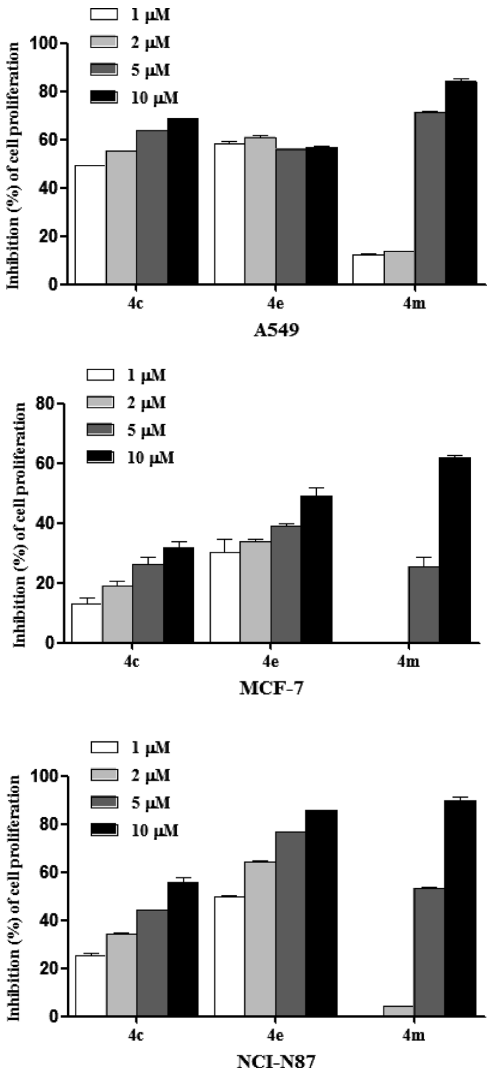


Fig. 1. Dose-Dependent Antiproliferative Effect of **4c**, **4e**, and **4m** on Cancer Cells

Three cancer cells were treated with each compound at concentrations of 1, 2, 5, and 10 μM for 24 h, and measured the cell proliferation by WST-1 assay, as described in Experimental. Results are represented as inhibition percentage of the cell proliferation with the mean±S.E.M. of three independent experiments.

activity. Among all the aminophosphonates, compounds **4c**, **4e**, and **4m** were found to exhibit good antiproliferative activity against all three cell lines A549, MCF-7, and NCI-N87. The antiproliferative activity of these compounds may perhaps be attributed to the presence of relatively high electron density on the exocyclic amine nitrogen atom which facilitates the binding ability of these compounds with the enzymes of cancer cells thereby preventing the proliferation.

Furthermore, for compounds **4c**, **4e**, and **4m**, the cell proliferation assay was conducted in a concentration-dependent (1–10 μM) manner (Fig. 1). The inhibition percentage of these compounds at concentrations of 1, 2, 5, and 10 μM is presented in Table 3. It has been observed that the compounds **4c** and **4e** active against A549 cells and compound **4e** active against NCI-N87 cells even at concentration of 2 μM. Compound **4m** exhibited the strong antiproliferative activity compared to **4c** and **4e** against all cancer cells at higher concentration (10 μM).

Conclusion

In summary, we have synthesized a novel series of carbazole-based α-amino phosphonates and subsequently evaluated their antiproliferative activity. Among all the compounds, **4c**, **4e** and **4m** were found to exhibit good anti-proliferative activity against all three cancer cells A549, MCF-7, and NCI-N87. In this reaction, PEG-400 acts as an efficient "green" promoter. The advantages of this method are simple and mild experimental conditions, avoiding hazardous solvents and toxic organic reagents.

Experimental

The 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde, was prepared by the ethylation[32] followed by formylation[33] and bromination of carbazole.[34] Melting points were determined in an open capillaries using Electrothermal (IA 9100) digital melting point apparatus and are uncorrected. IR spectra were recorded on Bruker (Tensor 37) FT-IR spectrometer using KBr pellets. ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were recorded on a VARIAN 200 MHz instrument. Mass spectral data were obtained from the Korea Basic Science Institute (Daegu) on JEOL JMS-700 high resolution mass spectrometer.

General Experimental Procedure A mixture of 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde (1 mmol), amine (1 mmol), and diethyl phosphite (1.3 mmol) was added to PEG-400 (1 g) and the mixture was heated to 100°C and stirred for 6–7 h. After completion of the reaction as monitored by TLC, the mixture was diluted with cold water and the resulting precipitate was filtered and washed with water. The residue was then dried and purified by column

Table 3. Maximum Percentage of Inhibition for **4c**, **4e**, and **4m** at Doses of 1, 2, 5, and 10 μM

Cancer cell	Compound	Inhibition (%) of cell proliferation (mean±S.E.M.)			
		1 μM	2 μM	5 μM	10 μM
A549	**4c**	49.15±0.24	55.25±0.26	63.76±0.02	68.64±0.24
	4e	58.32±0.88	61.04±0.77	56.00±0.05	56.72±0.42
	4m	12.43±0.53	13.71±0.05	71.47±0.32	84.05±0.56
MCF-7	**4c**	13.09±1.87	18.89±1.95	26.15±2.62	31.78±2.12
	4e	30.23±4.32	33.74±0.97	39.04±1.01	48.92±3.01
	4m	0[a]	0[a]	25.58±3.10	61.81±0.97
NCI-N87	**4c**	28.38±1.14	34.30±0.56	44.13±0.22	55.84±0.98
	4e	49.68±0.42	64.22±0.82	76.67±0.14	85.93±0.14
	4m	0[a]	4.21±0.37	53.23±0.45	89.86±1.39

a) The score zero shows no inhibition of cell proliferation.

chromatography over silica gel (ethyl acetate–hexane) to give the pure α-aminophosphonate. In the case of **4m**, the product was gummy therefore it was extracted with ethyl acetate and then, washed with water, and dried over sodium sulfate. Removal of the solvent followed by purification on column chromatography over silica gel afforded the pure α-aminophosphonate.

Entry **4a**: White solid; mp 175–176°C; IR (KBr) cm^{-1}: 3307, 2976, 1601, 1234, 1020, 964, 754; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.0 Hz, 3H), 1.30 (t, J=7.0 Hz, 3H), 1.40 (t, J=7.0 Hz, 3H), 3.57–3.73 (m, 1H), 3.83–3.98 (m, 1H), 4.09–4.17 (m, 2H), 4.31 (q, J=7.0 Hz, 2H), 4.93 (d, J=23.6 Hz, 1H), 6.63–6.70 (m, 3H), 7.05–7.13 (m, 2H), 7.23–7.28 (m, 2H), 7.36 (d, J=8.6 Hz, 1H), 7.50–7.64 (m, 2H), 8.12–8.19 (m, 2H);^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 37.7, 56.1 (d, J_{P-C}=150.2 Hz), 63.1, 108.9, 109.9, 111.6, 113.9, 118.3, 119.9, 123.2, 124.3, 126.1, 126.5, 128.3, 129.1, 138.8, 139.9, 146.3, 146.6; ^{31}P-NMR ($CDCl_3$) δ: 24.20; high resolution (HR)-MS (electron ionization (EI)) *m/z*: Calcd for $C_{25}H_{28}BrN_2O_3P$: 514.1021 (M^+), Found: 514.1020.

Entry **4b**: White solid; mp 115–116°C; IR (KBr) cm^{-1}: 3296, 2978, 1616, 1232, 1024, 965, 797; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.0 Hz, 3H), 1.30 (t, J=7.0 Hz, 3H), 1.39 (t, J=7.2 Hz, 3H), 2.15 (s, 3H), 3.58–3.75 (m, 1H), 3.83–3.99 (m, 1H), 4.09–4.17 (m, 2H), 4.29 (q, J=7.2 Hz, 2H), 4.91 (d, J=23.8 Hz, 1H), 6.57 (d, J=8.4 Hz, 2H), 6.90 (d, J=8.6 Hz, 2H), 7.22–7.26 (m, 1H), 7.35 (d, J=8.4 Hz, 1H), 7.49–7.63 (m, 2H), 8.12–8.18 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 20.3, 37.7, 56.4 (d, J_{P-C}=151.7 Hz), 63.1, 108.8, 109.8, 111.6, 114.0, 119.9, 122.0, 123.2, 124.3, 126.1, 126.7, 127.5, 128.3, 129.6, 138.8, 139.9, 143.9, 144.2; ^{31}P-NMR ($CDCl_3$) δ: 24.36; HR-MS (EI) *m/z*: Calcd for $C_{26}H_{30}BrN_2O_3P$: 528.1177 (M^+), Found: 528.1181.

Entry **4c**: Pale yellow solid; mp 182–183°C; IR (KBr) cm^{-1}: 3297, 2958, 1615, 1233, 1025, 966, 796; ^{1}H-NMR ($CDCl_3$) δ: 1.04–1.14 (m, 9H), 1.29 (t, J=7.2 Hz, 3H), 1.40 (t, J=7.2 Hz, 3H), 2.62–2.83 (m, 1H), 3.54–3.73 (m, 1H), 3.82–3.98 (m, 1H), 4.07–4.13 (m, 2H), 4.31 (q, J=7.2 Hz, 2H), 4.90 (d, J=23.6 Hz, 1H), 6.59 (d, J=8.4 Hz, 2H), 6.96 (d, J=8.4 Hz, 2H), 7.23–7.27 (m, 1H), 7.36 (d, J=8.4 Hz, 1H), 7.52 (dd, J=8.4, 2.0 Hz, 1H), 7.59–7.65 (m, 1H), 8.12–8.19 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.4, 24.1, 33.0, 37.7, 56.4 (d, J_{P-C}=150.2 Hz), 63.1, 108.8, 109.9, 111.6, 113.8, 119.9, 122.0, 123.2, 124.4, 126.2, 127.0, 128.3, 138.7, 139.9, 144.3, 144.6; ^{31}P-NMR ($CDCl_3$) δ: 24.30; HR-MS (EI) *m/z*: Calcd for $C_{28}H_{34}BrN_2O_3P$: 556.1490 (M^+), Found: 556.1489.

Entry **4d**: Pale yellow solid; mp 155–156°C; IR (KBr) cm^{-1}: 3298, 2979, 1511, 1233, 1025, 967, 795; ^{1}H-NMR ($CDCl_3$) δ: 1.09 (t, J=7.0 Hz, 3H), 1.30 (t, J=7.0 Hz, 3H), 1.40 (t, J=7.2 Hz, 3H), 3.56–3.76 (m, 4H), 3.83–3.99 (m, 1H), 4.06–4.21 (m, 2H), 4.30 (q, J=7.2 Hz, 2H), 4.86 (d, J=23.4 Hz, 1H), 6.58–6.70 (m, 4H), 7.23–7.27 (m, 1H), 7.36 (d, J=8.4 Hz, 1H), 7.52 (dd, J=8.6, 2.0 Hz, 1H), 7.57–7.63 (m, 1H), 8.10–8.19 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 37.7, 55.5, 57.0 (d, J_{P-C}=150.2 Hz), 63.1, 108.8, 109.9, 111.6, 114.7, 115.2, 119.9, 122.0, 123.2, 124.3, 126.2, 126.7, 128.3, 138.8, 139.9, 140.3, 140.6, 152.6; ^{31}P-NMR ($CDCl_3$) δ: 24.36; HR-MS (EI) *m/z*: Calcd for $C_{26}H_{30}BrN_2O_4P$: 544.1127 (M^+), Found: 544.1123.

Entry **4e**: Pale yellow solid; mp 167–168°C; IR (KBr) cm^{-1}: 3292, 2977, 1600, 1233, 1024, 966, 794; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.0 Hz, 3H), 1.31 (t, J=7.0 Hz, 3H), 1.40 (t, J=7.4 Hz, 3H), 2.23 (s, 3H), 3.53–3.73 (m, 1H), 3.85–3.98 (m, 1H), 4.05–4.21 (m, 2H), 4.31 (q, J=7.2 Hz, 2H), 4.87 (d, J=23.8 Hz, 1H), 6.34 (dd, J=8.6, 2.8 Hz, 1H), 6.57 (d, J=3.0 Hz, 1H), 7.17 (d, J=8.6 Hz, 1H), 7.23–7.27 (m, 1H), 7.36 (d, J=8.6 Hz, 1H), 7.50–7.60 (m, 2H), 8.09–8.17 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3 (d, J_{P-C}=6.0 Hz), 23.0, 37.7, 56.1 (d, J_{P-C}=151.7 Hz), 63.2, 108.9, 109.9, 111.6, 112.8, 116.4, 119.8, 119.9, 122.1, 123.2, 124.3, 126.1, 128.4, 132.5, 138.2, 138.8, 140.0, 145.6, 145.9; ^{31}P-NMR ($CDCl_3$) δ: 23.92; HR-MS (EI) *m/z*: Calcd for $C_{26}H_{29}Br_2N_2O_3P$: 606.0283 (M^+), Found: 606.0281

Entry **4f**: Pale yellow solid; mp 147–148°C; IR (KBr) cm^{-1}: 3293, 2979, 1509, 1232, 1025, 969, 793; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.0 Hz, 3H), 1.31 (t, J=7.2 Hz, 3H), 1.40 (t, J=7.2 Hz, 3H), 3.54–3.74 (m, 1H), 3.82–3.98 (m, 1H), 4.06–4.20 (m, 2H), 4.31 (q, J=7.2 Hz, 2H), 4.85 (d, J=23.4 Hz, 1H), 6.54–6.61 (m, 2H), 6.74–6.83 (m, 2H), 7.22–7.27 (m, 1H), 7.36 (d, J=8.6 Hz, 1H), 7.50–7.61 (m, 2H), 8.10–8.18 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 37.7, 56.8 (d, J_{P-C}=151.7 Hz), 63.2, 108.9, 109.9, 111.6, 114.8, 115.3, 115.8, 119.9, 122.0, 123.2, 124.3, 126.1, 128.4, 138.8, 140.0, 142.6, 142.9, 153.8, 158.5; ^{31}P-NMR ($CDCl_3$) δ: 24.09; HR-MS (EI) *m/z*: Calcd for $C_{25}H_{27}BrFN_2O_3P$: 532.0927 (M^+), Found: 532.0927.

Entry **4g**: White solid; mp 138–139°C; IR (KBr) cm^{-1}: 3292, 2978, 1598, 1232, 1024, 967, 795; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.2 Hz, 3H), 1.31 (t, J=7.0 Hz, 3H), 1.40 (t, J=7.0 Hz, 3H), 3.53–3.72 (m, 1H), 3.82–3.98 (m, 1H), 4.06–4.22 (m, 2H), 4.30 (q, J=7.2 Hz, 2H), 4.87 (d, J=23.6 Hz, 1H), 6.53–6.61 (m, 2H), 6.99–7.06 (m, 2H), 7.23–7.27 (m, 1H), 7.36 (d, J=8.6 Hz, 1H), 7.50–7.60 (m, 2H), 8.01–8.16 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.4, 37.7, 56.2 (d, J_{P-C}=151.7 Hz), 63.2, 108.9, 109.9, 115.0, 115.6, 119.8, 120.0, 122.0, 122.8, 123.2, 124.2, 126.1, 128.4, 128.9, 138.7, 139.9, 144.9, 145.2; ^{31}P-NMR ($CDCl_3$) δ: 23.87; HR-MS (EI) *m/z*: Calcd for $C_{25}H_{27}BrClN_2O_3P$: 548.0631 (M^+), Found: 548.0631.

Entry **4h**: Light grey solid; mp 137–139°C; IR (KBr) cm^{-1}: 3292, 2978, 1594, 1232, 1024, 969, 796; ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, J=7.0 Hz, 3H), 1.31 (t, J=7.0 Hz, 3H), 1.40 (t, J=7.2 Hz, 3H), 3.52–3.72 (m, 1H), 3.82–4.01 (m, 1H), 4.07–4.21 (m, 2H), 4.31 (q, J=7.0 Hz, 2H), 4.87 (d, J=23.4 Hz, 1H), 6.53 (d, J=7.6 Hz, 2H), 7.16 (t, J=7.4 Hz, 2H), 7.24–7.28 (m, 1H), 7.37 (d, J=8.4 Hz, 1H), 7.50–7.60 (m, 2H), 8.10–8.18 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 37.7, 56.1 (d, J_{P-C}=151.7 Hz), 63.3, 108.9, 109.9, 111.6, 115.5, 119.8, 122.1, 123.2, 124.2, 126.0, 128.4, 131.8, 138.8, 140.0, 145.3, 145.6; ^{31}P-NMR ($CDCl_3$) δ: 23.83; HR-MS (EI) *m/z*: Calcd for $C_{25}H_{27}Br_2N_2O_3P$: 592.0126 (M^+), Found: 592.0125.

Entry **4i**: Light brown solid; mp 206–207°C; IR (KBr) cm^{-1}: 3378, 3197, 2979, 1515, 1231, 1026, 963, 796; ^{1}H-NMR (DMSO-d_6) δ: 0.99 (t, J=7.0 Hz, 3H), 1.18–1.30 (m, 6H), 3.56–3.69 (m, 1H), 3.78–3.90 (m, 1H), 4.01–4.15 (m, 2H), 4.39 (q, J=7.0 Hz, 2H), 4.98 (dd, J=24.1, 10.8 Hz, 1H), 5.67–5.76 (m, 1H), 6.44 (d, J=8.8 Hz, 2H), 6.67 (d, J=8.8 Hz, 2H), 7.53–7.66 (m, 4H), 8.25–8.30 (m, 2H), 8.45 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ: 13.6, 16.2 (d, J_{P-C}=9.1 Hz), 37.1, 55.3 (d, J_{P-C}=153.2 Hz), 62.2 (dd, J_{P-C}=15.1, 6.0 Hz), 108.9, 110.7, 111.2, 115.1, 115.3, 120.7, 122.5, 123.8, 127.0, 127.9, 138.4, 139.3, 139.5, 139.8, 149.1; ^{31}P-NMR ($CDCl_3$) δ: 24.39; HR-MS (EI) *m/z*: Calcd for $C_{25}H_{28}BrN_2O_4P$: 530.0970 (M^+), Found: 530.0973.

Entry **4j**: Yellow solid; mp 148–149°C; IR (KBr) cm^{-1}: 3269, 2978, 1597, 1231, 1023, 973, 796; ^{1}H-NMR ($CDCl_3$) δ: 1.09 (t, J=7.0 Hz, 3H), 1.22–1.43 (m, 6H), 3.51–3.71 (m, 1H), 3.83–3.99 (m, 1H), 4.07–4.22 (m, 2H), 4.30 (q, J=7.2 Hz, 2H), 4.99 (dd, J=23.5, 7.2 Hz, 1H), 6.06 (t, J=8.2 Hz, 1H),

6.61–6.68 (m, 2H), 7.20–7.25 (m, 1H), 7.39 (d, J=8.4 Hz, 1H), 7.48–7.60 (m, 2H), 7.96–8.04 (m, 3H), 8.13 (t, J=2.2 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.4, 37.7, 55.5 (d, J_{P-C}=154.8 Hz), 63.6 (dd, J_{P-C}=20.5, 6.1 Hz), 109.0, 109.8, 111.6, 112. 3, 119.7, 122.2, 123.0, 124.0, 125.1, 126.0, 128.4, 138.7, 140.0, 152.1, 152.4; ^{31}P-NMR ($CDCl_3$) δ: 22.83; HR-MS (EI) m/z: Calcd for $C_{25}H_{27}BrN_3O_5P$: 559.0872 (M^+), Found: 559.0870.

Entry **4k**: Pale yellow solid; mp 116–117°C; IR (KBr) cm^{-1}: 3277, 2979, 2213, 1606, 1233, 1023, 969, 797; ^{1}H-NMR ($CDCl_3$) δ: 1.09 (t, J=7.2 Hz, 3H), 1.25–1.43 (m, 6H), 3.51–3.71 (m, 1H), 3.83–3.98 (m, 1H), 4.09–4.21 (m, 2H), 4.31 (q, J=7.2 Hz, 2H), 4.94 (dd, J=23.7, 7.0 Hz, 1H), 5.77 (t, J=7.8 Hz, 1H), 6.65 (d, J=9.0 Hz, 2H), 7.22 (d, J=8.6 Hz, 1H), 7.29–7.40 (m, 3H), 7.48–7.59 (m, 2H), 8.04 (d, J=1.8 Hz, 1H), 8.12 (d, J=1.8 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3 (d, J_{P-C}=4.5 Hz), 37.6, 55.3 (d, J_{P-C}=151.7 Hz), 63.4 (dd, J_{P-C}=14.4, 6.0 Hz), 99.6, 108.9, 109.7, 111.4, 113.4, 119.7, 120.1, 122.1, 123.0, 124.0, 125.3, 126.0, 128.2, 133.4, 138.6, 139.9, 150.0, 150.3; ^{31}P-NMR ($CDCl_3$) δ: 23.13; HR-MS (EI) m/z: Calcd for $C_{26}H_{27}BrN_3O_3P$: 539.0973 (M^+), Found: 539.0976.

Entry **4l**: White solid; mp 185–186°C; IR (KBr) cm^{-1}: 3286, 2980, 1611, 1232, 1026, 969, 796; ^{1}H-NMR ($CDCl_3$) δ: 1.10 (t, J=7.0 Hz, 3H), 1.26–1.43 (m, 6H), 3.54–3.74 (m, 1H), 3.84–4.00 (m, 1H), 4.10–4.34 (m, 4H), 4.89 (d, J=23.8 Hz, 1H), 6.66 (dd, J=8.7, 2.8 Hz, 1H), 7.04–7.23 (m, 3H), 7.37 (d, J=8.4 Hz, 1H), 7.46–7.59 (m, 2H), 7.99–8.14 (m, 2H), ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.3, 37.7, 55.9 (d, J_{P-C}=153.2 Hz), 63.4, 108.9, 109.6, 111.4, 113.4, 116.6, 119.7, 122.2, 123.0, 124.1, 125.5, 126.2, 128.2, 131.8, 138.7, 140.0, 145.3. 145.6; ^{31}P-NMR ($CDCl_3$) δ: 23.39; HR-MS (EI) m/z: Calcd for $C_{26}H_{26}BrClF_3N_2O_3P$: 616.0505 ($M^+$), Found: 616.0504.

Entry **4m**: Yellow solid; mp 92–93°C; IR (KBr) cm^{-1}: 3272, 2977, 1597, 1233, 1029, 964, 792; ^{1}H-NMR ($CDCl_3$) δ: 1.05 (t, J=7.2 Hz, 3H), 1.22 (t, J=7.0 Hz, 3H), 1.37 (t, J=7.0 Hz, 3H), 3.51 (d, J=13.4 Hz, 1H), 3.67–3.84 (m, 2H), 3.87–4.17 (m, 4H), 4.28 (q, J=7.2 Hz, 2H), 7.18–7.26 (m, 6H), 7.33 (d, J=8.4 Hz, 1H), 7.50–7.54 (m, 2H), 8.01 (t, J=1.8 Hz, 1H), 8.14 (d, J=2.0 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 13.7, 16.4, 37.8, 51.1 (d, J_{P-C}=16.7 Hz), 59.5 (d, J_{P-C}=153.2 Hz), 62.8, 108.7, 109.9, 111.6, 120.8, 120.9, 121.9, 123.2, 124.5, 126.3, 127.1, 128.3, 138.8, 139.4, 140.1; ^{31}P-NMR ($CDCl_3$) δ: 25.11; HR-MS (EI) m/z: Calcd for $C_{26}H_{30}BrN_2O_3P$: 528.1177 (M^+), Found: 528.1180.

Entry **4n**: Light brown solid; mp 205–206°C; IR (KBr) cm^{-1}: 3297, 2977, 1605, 1233, 1024, 968, 799; ^{1}H-NMR ($CDCl_3$) δ: 1.04 (t, J=7.0 Hz, 6H), 1.23 (t, J=7.0 Hz, 6H), 1.31–1.41 (m, 6H), 3.51–3.70 (m, 2H), 3.78–3.93 (m, 2H), 3.99–4.14 (m, 4H), 4.20–4.33 (m, 4H), 4.74 (dd, J=23.3, 3.6 Hz, 2H), 6.44–6.45 (m, 4H), 7.18–7.32 (m, 4H), 7.46–7.55 (m, 4H), 8.04–8.05 (m, 2H), 8.13 (d, J=2.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ: 13.4, 16.0, 37.4, 56.7 (d, J_{P-C}=150.2 Hz), 62.8, 108.5, 109.6, 111.2, 115.2, 119.7, 121.6, 122.8, 124.0, 126.0, 126.6, 128.0, 138.4, 139.1, 139.6; ^{31}P-NMR ($CDCl_3$) δ: 24.36; HR-MS (FAB) m/z: Calcd for $C_{44}H_{50}Br_2N_4O_6P_2$: 950.1572 ($M^+$), Found: 950.1575.

Biological Assay. Cell Lines and Cell Culture The A549 (human lung cancer cell line), MCF-7 (human breast cancer cell line), and NCI-N87 (human stomach cancer cell line) were purchased from ATCC (American Type Culture Collection, VA, U.S.A.), and cultured in RPMI-1640 (Gibco BRL, Grand Island, NY, U.S.A.) or Eagle's minimal essential medium (EMEM, Gibco BRL) supplemented with 10–20% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37°C in a humidified incubator with an atmosphere containing 5% CO_2.

Cell Proliferation Assay The antiproliferative activity of all compounds was measured by the WST-1 Cell Proliferation Assay Kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). The cells were seeded at 1×10^5 cells/well in 24-well flat-bottom culture plates, and incubated with compounds at various concentrations for 24 h. The WST-1 reagent (10 μL) was added to each well and further incubated for 2 h at 37°C in a CO_2 incubator. The plate was then gently mixed on an orbital shaker for 1 min to ensure homogenous distribution of color. The absorbance was read at 450 nm using an automated microplate reader (SpectraMAX 340; Molecular Devices, Sunnyvale, CA, U.S.A.).

Statistical Analysis All statistical analyses were performed using GraphPad Prism 5.0 for the Macintosh (GraphPad Software, San Diego, CA, U.S.A.). Data signify the means plus or minus the standard error of mean (means± S.E.M.) of three samples and are representative of three independent experiments.

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