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Short communication

Synthesis and cytotoxicity of *O*,*O*′-dialkyl {[2-(substituted phenoxy) acetamido](substituted phenyl)methyl}phosphonates

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ABSTRACT

A series of *O*,*O*′-dialkyl {[2-(substituted phenoxy)acetamido](substituted phenyl)methyl}phosphonates was synthesized and their cytotoxic activities were tested against various human tumor cell lines. Some compounds (**5q**, **5r**, **5s**, **5w**, **5x** and **5y**) showed relatively high cytotoxicity. Especially, compounds **5x** and **5q** exhibited the best cytotoxicity against KB and CNE2 cells with IC_{50} 7.1 and 11.4 μM, respectively. Their inhibitory activities against KB and CNE2 cells were even higher than that of fluorouracil.

1. Introduction

Organophosphorus compounds have attracted intense interest owing to their wide applications in the areas of industrial, agricultural, and medicinal chemistry and their potential biological and physical properties [1–4]. As mimics of α-amino acids, the α-aminophosphonic acids and their derivatives are an important class of compounds that exhibited intriguing biological activities [5–14]. Indeed a number of potent antibiotics [5,6], enzyme inhibitors [7], antiviral, and antitumor agents [8,9] are α-aminophosphonic acids or peptide analogues thereof. On the other hand, a variety of the reports regarding synthetic studies of the amide derivatives have been presented because they can serve not only as agrochemicals such as fungicide, insecticide, and plant virucide, but also as medicines such as antitumor agents [15–19]. Especially some amide derivatives containing α-aminophosphonate moiety have been recently described as potent antifungal and antiviral agents [20].

In our previous work, some α-(substituted phenoxyacetoxy) alkylphosphonate derivatives were synthesized and shown to be endowed with notable herbicidal activities [21]. To extend our research work of developing novel herbicides, some α-aminophosphonate derivatives were designed and synthesized. It is very interesting that all of these compounds have poor herbicidal activities, however, some of them showed potential cytotoxicity against two human tumor cells. Here we would like to describe the synthesis and cytotoxicity of *O*,*O*′-dialkyl {[2-(substituted phenoxy) acetamido](substituted phenyl)methyl}phosphonates **5** from easily accessible *O*,*O*′-dialkyl α-aminophosphonates **4**.

2. Results and discussion

2.1. Chemistry

The synthesis of *O*,*O*′-dialkyl {[2-(substituted phenoxy)acetamido](substituted phenyl)methyl}phosphonates **5a**–**z** is outlined in Scheme 1.

O,*O*′-dialkyl *N*-(phenylmethylene)-α-amino-α-(substituted phenyl)methylphosphonates **2**, obtained by reaction of substituted benzaldehyde **1** with ammonia and *O*,*O*′-dialkyl phosphite, were converted easily to *O*,*O*′-dialkyl α-amino-α-(substituted phenyl)methylphosphonate **4** via hydrolysis with *p*-toluenesulfonic acid and neutralization of the sulfonate salt [22]. The α-aminophosphonates **4** reacted with substituted phenoxyacetic chloride to give *O*,*O*′-dialkyl {[2-(substituted phenoxy)acetamido](substituted phenyl)methyl}phosphonates **5** in satisfactory yields. The results are listed in Table 1.

All the compounds in the series **5** were obtained as white solids or yellowish oils after chromatography on silica gel with ethyl acetate/petroleum ether. Their structures were fully characterized by IR, ^{1}H NMR, EI-MS and elemental analysis. For example, the IR

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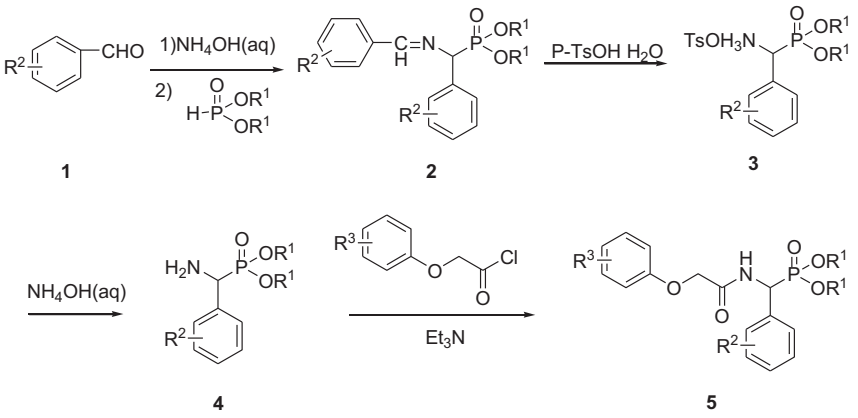

Scheme 1. General synthetic route for compound **5a**–**z**.

spectrum of **5a** revealed absorption bands at 1693 (C═O), 3067 (C_6H_5), and 3224 (NH) cm^{-1}. The corresponding ^1H NMR spectrum showed the two methoxy groups attached with phosphorus appears as two doublet at 3.57 and 3.74, respectively. The chemical shifts of the two methyl hydrogens differentiate due to the low rate of environmental exchange caused by the slow rotation of the P–C bond, and the magnetic nucleus phosphorus makes the signal of both methyls split into a doublet. The signal corresponding to the methylene group ($-CH_2-$) flanked by the phenoxy group and carbonyl group appears as a quartet, the outside lines smaller in size, which belongs to the AB system with the difference in chemical shift between the two mutually coupled protons A and B, at 4.52 and 4.54 in the former series and at 4.59 and 4.62 in the latter series, respectively. The other signals resonated at d(H) 5.57 (dd, 1H, PCH), 6.81–7.45 (m, 8H, Ar-H), 7.72 (d, 1H, NH). The structures of **5a** and the other analogs were further confirmed on the basis of elemental analysis.

2.2. Cytotoxicity

The cytotoxicity results of all of the compounds against KB and CNE2 tumor cell lines are listed in Table 1. As shown in Table 1, some compounds (**5q**, **5r**, **5s**, **5w**, **5x** and **5y**) exhibited good to moderate cytotoxicity. Especially, compounds **5x** exhibited the best cytotoxicity against KB cell with IC_{50} 7.1 μM, which is even higher than that of fluorouracil. However, replacement of R^1 *n*-butyl group in phosphonate moiety of compound **5x** with methyl, resulted in the compound **5d** with complete loss in activity ($IC_{50} > 50$ μM), and the other compounds with methyl or ethyl as R^1 substituent (**5a**–**j**) also showed no inhibitory activity. It could be concluded that *n*-butyl as R^1 is favorable to the cytotoxicity. As for CNE2, in the series of compounds **5p**–**5z** substituted at the R^1 position with *n*-butyl derivatives, compound **5q** showed the best inhibitory activity with IC_{50} 11.4 μM. The compounds **5q**, **5r**, **5w**, and **5y** with chlorine at 2-position in benzene ring (R^3) showed better inhibitory activity against CNE2 compared to the compounds **5p**, **5s**, **5x** with 2-methyl or 2-unsubstituted **5t**, **5v** and **5z**, which indicated that the chlorine introduced was beneficial to cytotoxic activity.

3. Conclusion

We synthesized a new series of *O*,*O*′-dialkyl {[2-(substituted phenoxy)acetamido](substituted phenyl)methyl}phosphonates via reaction of functionalized α-aminophosphonates with various substituted phenoxyacetic chloride. Their cytotoxic activities were tested against various human tumor cell lines. Some compounds (**5q**, **5r**, **5s**, **5w**, **5x** and **5y**) showed relatively high cytotoxicity. Especially, compounds **5x** and **5q** exhibited the best cytotoxicity against KB and CNE2 cells with IC_{50} 7.1 and 11.4 μM, respectively. Their inhibitory activity against KB and CNE2 cells were even higher than that of fluorouracil.

4. Experimental

4.1. Chemistry

^1H NMR spectra were recorded at 600 MHz, in $CDCl_3$ on a Varian Mercury-Plus 600 spectrometer and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. MS spectra were determined using a TraceMS 2000 organic mass spectrometry, and signals were given in *m*/*z*. Melting points were determined using a X-4 apparatus and were uncorrected. Elemental analysis (EA) was measured on a Vario ELIII CHNSO elemental analyzer. Element analyses (C, H, N) indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. All commercially available solvents and reagents were used as supplied by Acros Organics unless otherwise stated. The silica gel (200–300 meshes) for flash column chromatography was from Qingdao Marine Chemical Factory in China.

4.2. General procedure for the preparation of *O*,*O*′-dialkyl {[2-(substituted phenoxy)acetamido](substituted phenyl)methyl} phosphonates *5*

A suspension of aromatic aldehyde (10 mmol) and ammonium hydroxide (30%, 10 mL) was stirred for 5 h at reflux. The reaction mixture was filtered to give the white precipitate **2**, to which the dialkyl phosphite (4 mmol) was added and the resulting solution was stirred for 2–5 h at 70 °C *p*-Toluenesulfonic acid (4 mmol) in 30 mL THF was added to the reaction mixture, which was stirred for 2 h at 0 °C to give the precipitate **3**. The precipitate was filtered and washed with THF (15 mL), and then it was added to 10 mL aqueous ammonium hydroxide (30%) and stirred for 30 min at room temperature. Extraction with ethyl acetate (3 × 30 mL) and evaporation of the solvent gave the oils of **4**, which was purified by column chromatography on silica gel with ethyl acetate. To a mixture of **4** and pyridine in CH_2Cl_2 (10 mL), the appropriate substituted phenoxyacetic chloride was added at 2–5 °C and the mixture was stirred at ambient temperature for another 3 h, then washed with saturated sodium hydrogen carbonate solution and brine, dried with anhydrous sodium sulfate and evaporated. The residue was purified by column chromatography on silica gel with

Table 1
Yields and in vitro cytotoxicity (IC_{50},[a]μM) of *O,O′*-dialkyl {[2-(substituted phenoxy) acetamido](substituted phenyl)methyl}phosphonates **5**.

Compd	R^1	R^2	R^3	Yield[b] (%)	Cytotoxicity against KB[c]	Cytotoxicity against CNE2[c]
5a	Me	H	2,4-diCl	78	>50	>50
5b	Me	4–CH_3	2,4-diCl	82	>50	>50
5c	Me	4–CH_3	3–CH_3–4–Cl	73	>50	>50
5d	Me	4–OCH_3	2,3-diCH_3	63	>50	>50
5e	Me	4–OCH_3	2,4-diCl	80	>50	>50
5f	Et	4–OCH_3	2,4-diCl	75	>50	>50
5g	Et	4–OCH_3	4-Cl	77	>50	>50
5h	Et	4–OCH_3	3–CF_3	88	>50	>50
5i	Et	4–OCH_3	4–NO_2	80	>50	>50
5j	Et	4–OCH_3	2–CH_3–4–Cl	95	>50	>50
5k	*n*-Pr	H	2,4-diCl	58	16.0 ± 0.9	26.0 ± 1.4
5l	*n*-Pr	H	4-Cl	75	23.0 ± 1.1	42.9 ± 2.1
5m	*n*-Pr	H	3–CF_3	61	21.6 ± 1.1	41.8 ± 2.0
5n	*n*-Pr	4–OCH_3	2,4-diCl	74	17.4 ± 0.8	27.2 ± 1.4
5o	*n*-Pr	4–OCH_3	3–CF_3	60	19.8 ± 1.0	38.5 ± 2.0
5p	*n*-Bu	H	2,3-diCH_3	93	18.2 ± 0.9	25.1 ± 1.3
5q	*n*-Bu	H	2-Cl	78	15.8 ± 0.8	11.4 ± 0.6
5r	*n*-Bu	H	2,4-diCl	64	17.8 ± 0.9	13.8 ± 0.7
5s	*n*-Bu	H	2–CH_3–4–Cl	80	12.3 ± 0.6	19.9 ± 1.0
5t	*n*-Bu	H	3–CH_3–4–Cl	75	18.1 ± 0.9	43.0 ± 2.2
5u	*n*-Bu	H	2,4,5-triCl	65	15.9 ± 0.8	>50
5v	*n*-Bu	H	4–OCH_3	78	25.5 ± 1.3	41.2 ± 2.0
5w	*n*-Bu	H	2-Cl-4-F	66	12.3 ± 0.6	13.1 ± 0.6
5x	*n*-Bu	4–OCH_3	2,3-diCH_3	68	7.1 ± 0.4	20.7 ± 1.0
5y	*n*-Bu	4–OCH_3	2-Cl	60	14.5 ± 0.7	13.4 ± 0.7
5z	*n*-Bu	4–OCH_3	4–$C(CH_3)_3$	92	13.3 ± 0.6	31.1 ± 1.5
Fluorouracil					9.0 ± 0.6	13.5 ± 1.0

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.
[b] Isolated yields based on α-aminophosphonates **4**.
[c] KB cells were the drug sensitive human oral carcinoma cells and CNE2 cells were the nasopharyngeal carcinoma cells.

petroleum ether/ethyl acetate (3:1) to give the pure title compounds **5** as white solids or yellowishish oils.

*4.2.1. O,O′-dimethyl {[2-(2,4-dichlorophenoxy)acetamido](phenyl) methyl}phosphonate(**5a**)*

White solid. m.p. 150–152 °C. IR(KBr) *ν*: 3224, 3067, 1693(C=O), 1557, 1458, 1228 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 3.57 (d, *J* = 10.8 Hz, 3H, OCH_3), 3.74 (d, *J* = 10.8 Hz, 3H, OCH_3), 4.52–4.62 (q, AB system, 2H, OCH_2CO), 5.57 (dd, *J* = 9.6, 20.4 Hz, 1H, PCH), 6.82 (d, *J* = 9.0 Hz 1H, Ar-H), 7.20–7.45 (m, 7H, Ar-H), 7.72 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 49.1 (d, *J* = 159.2 Hz, PCH), 53.5 (d, *J* = 60.0 Hz, $POCH_3$), 67.9 (OCH_2), 114.5, 123.7, 127.3, 127.6, 127.7, 128.3, 128.7, 130.0, 133.9, 151.2, 166.3 (CO). MS (70 ev) *m/z* (%): 417 (M^+). Anal. $C_{17}H_{18}Cl_2NO_5P$ (C, H, N).

*4.2.2. O,O′-dimethyl {[2-(2,4-dichlorophenoxy)acetamido](4-methylphenyl)methyl}phosphonate(**5b**)*

White solid. m.p. 165–166 °C. IR(KBr) *ν*: 3224, 3054, 1697(C=O), 1552, 1481, 1225 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 2.34 (s, 3H, Ar-CH_3), 3.57 (d, *J* = 7.8 Hz, 3H, OCH_3), 3.74 (d, *J* = 7.8 Hz, 3H, OCH_3), 4.51–4.61 (q, AB system, 2H, OCH_2CO), 5.53 (dd, 1H, PCH, *J* = 8.4, 21.6 Hz), 6.81–7.44 (m, 7H, Ar-H), 7.66 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 21.2, 49.1(d, *J* = 154.4 Hz, PCH), 53.7 (d, *J* = 60.0 Hz, $POCH_3$), 68.2 (OCH_2), 114.7, 123.9, 127.5, 127.7, 128.0, 129.6, 130.2, 131.1, 138.4, 151.5, 166.4 (CO). MS (70 ev) *m/z* (%): 431 (M^+). Anal. $C_{18}H_{20}Cl_2NO_5P$ (C, H, N).

*4.2.3. O,O′-Dimethyl {[2-(3-methyl-4-chlorophenoxy)acetamido](4-methylphenyl)methyl}phosphonate(**5c**)*

White solid. m.p. 91–92 °C. IR(KBr) *ν*: 3255, 3062, 1689(C=O), 1554, 1480, 1234 cm^{-1} ^1H NMR (400 MHz $CDCl_3$) *δ*(ppm): 2.34 (s, 6H, 2Ar-CH_3), 3.51 (d, *J* = 11.2 Hz, 3H, OCH_3), 3.73 (d, *J* = 10.8 Hz, 3H, OCH_3), 4.44–4.56 (q, AB system, 2H, OCH_2CO), 5.54 (dd, *J* = 10.0, 20.8 Hz, 1H, PCH), 6.81 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.71 (dd, *J* = 2.4 Hz, 1H, Ar-H), 7.16–7.29 (m, 5H, Ar-H), 7.34 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 20.3, 21.2, 49.0 (d, *J* = 157.8 Hz, PCH), 53.7 (d, *J* = 83.3 Hz, $POCH_3$), 67.5 (OCH_2), 113.4, 117.4, 127.5127.9, 129.6, 129.9, 131.1, 137.5, 138.4, 155.5, 167.4 (CO). MS (70 ev) *m/z* (%): 411 (M^+). Anal. $C_{19}H_{20}ClNO_5P$ (C, H, N).

*4.2.4. O,O′-Dimethyl {[2-(2,3-dimethylphenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5d**)*

White solid. m.p. 141–142 °C. IR(KBr) *ν*: 3225, 3055, 1696(C=O), 1554, 1471, 1249 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 2.27 (s, 3H, Ar-CH_3), 2.31 (s, 3H, Ar-CH_3) 3.55 (d, *J* = 10.8 Hz, 3H, OCH_3), 3.73 (d, *J* = 10.2 Hz, 3H, OCH_3), 4.48–4.57 (q, AB system, 2H, OCH_2CO), 5.52 (dd, 1H, PCH, *J* = 9.6, 20.4 Hz), 6.64(d, 1H, *J* = 7.8 Hz, Ar-H), 6.86–7.30 (m, 6H, Ar-H), 7.48 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 11.9, 20.1, 48.6 (d, *J* = 157.8 Hz, PCH), 53.7 (d, *J* = 69.8 Hz, $POCH_3$), 55.3, 67.7 (OCH_2), 109.5, 114.3, 123.8, 125.1, 126.1, 126.3, 129.0, 138.4, 155.1, 159.6, 167.8 (CO). MS (70 ev) *m/z* (%): 407 (M^+). Anal. $C_{20}H_{26}NO_6P$ (C, H, N).

*4.2.5. O,O′-Dimethyl {[2-(2,4-dichlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5e**)*

White solid. m.p. 122–123 °C. IR(KBr) *ν*: 3222, 3053, 1698(C=O), 1552, 1482, 1240 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 3.58 (d, *J* = 10.8 Hz, 3H, OCH_3), 3.75 (d, *J* = 10.8 Hz, 3H, OCH_3), 3.80 (s, 3H, Ar-OCH_3), 4.50–4.61 (q, AB system, 2H, OCH_2CO), 5.51 (dd, *J* = 9.0, 20.4 Hz, 1H, PCH), 6.82 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.81–7.44 (m, 6H, Ar-H), 7.63 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 48.7 (d, *J* = 156.7 Hz, PCH), 53.7 (d, *J* = 66.0 Hz, $POCH_3$), 55.3, 68.2 (OCH_2), 114.3, 114.8, 123.9, 126.1, 127.5, 127.9, 129.1, 130.2, 151.5, 159.6, 166.4 (CO). MS (70 ev) *m/z* (%): 448 (M^+). Anal. $C_{18}H_{20}Cl_2NO_6P$ (C, H, N).

*4.2.6. O,O′-Diethyl {[2-(2,4-dichlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5f**)*

White solid. m.p. 101–102 °C IR(KBr) *ν*: 3443, 3224, 1695(C=O), 1610, 1565, 1473, 1318 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 1.16–1.30 (m, 6H, $2CH_3$), 3.80 (s, 3H, OCH_3), 3.83–4.12 (m, 4H, OCH_2), 4.50–4.60 (q, AB system, 2H, OCH_2CO), 5.47 (dd, *J* = 9.6, 19.8 Hz, 1H, PCH), 6.81–7.44 (m, 7H, Ar-H), 7.65 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 16.4, 49.1 (d, *J* = 153.0 Hz, PCH), 55.2, 63.1 (d, *J* = 52.7 Hz, $POCH_2$), 68.1 (OCH_2), 114.1, 114.7, 123.8, 126.5, 127.3, 127.9, 129.0, 130.1, 151.5, 159.5, 166.3 (CO). MS (70 ev) *m/z* (%): 477 (M^+). Anal. $C_{20}H_{24}Cl_2NO_6P$ (C, H, N).

*4.2.7. O,O′-Diethyl {[2-(4-chlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5g**)*

White solid. m.p. 119–120 °C. IR(KBr) *ν*: 3227, 3060, 1694(C=O), 1553, 1490, 1218 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 1.11–1.29 (m, 6H, $2CH_3$), 3.80 (s, 3H, OCH_3), 3.72–4.10 (m, 4H, OCH_2), 4.46–4.55 (q, AB system, 2H, OCH_2CO), 5.46 (dd, *J* = 9.6, 20.4 Hz, 1H, PCH), 6.86–7.33 (m, 8H, Ar-H, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) *δ*(ppm): 16.3, 49.0 (d, *J* = 153.0 Hz, PCH), 55.2, 63.2 (d, *J* = 65.0 Hz, $POCH_2$), 67.5 (OCH_2), 114.0, 116.1, 126.6, 126.9, 129.2, 129.5, 155.8, 159.5, 167.2 (CO). MS (70 ev) *m/z* (%): 441 (M^+). Anal. $C_{20}H_{25}ClNO_6P$ (C, H, N).

*4.2.8. O,O′-Diethyl {[2-(3-trifluoromethylphenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5h**)*

White solid. m.p. 103–104 °C. IR(KBr) *ν*: 3212, 3051, 1692(C=O), 1550, 1460, 1222 cm^{-1} ^1H NMR (600 MHz $CDCl_3$) *δ*(ppm): 1.11–1.28 (m, 6H, $2CH_3$), 3.80 (s, 3H, OCH_3), 3.75–4.10 (m, 4H, OCH_2), 4.53–4.61 (q, AB system, 2H, OCH_2CO), 5.47 (dd, *J* = 9.6, 20.4 Hz, 1H, PCH), 6.87–7.44 (m, 8H, Ar-H), 7.45 (m, 1H, NH). ^{13}C NMR (150 MHz

$CDCl_3$) δ(ppm): 16.0, 48.8 (d, J = 154.2 Hz, PCH), 54.9, 63.0 (d, J = 62.4 Hz, $POCH_2$), 67.0 (OCH_2), 111.4, 113.8, 117.9, 118.4, 126.3, 129.1, 130.0, 131.7 (d, J = 32.0 Hz, CF_3), 131.6, 131.8, 157.2, 159.3, 166.7 (CO). MS (70 ev) *m/z* (%): 475 (M^+). Anal. $C_{21}H_{25}F_3NO_6P$ (C, H, N).

*4.2.9. O,O′-Diethyl {[2-(4-nitrophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5i**)*

White solid. m.p. 115–116 °C. IR(KBr) ν: 3223, 3134, 1696(C=O), 1535, 1490, 1222 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 1.22–1.31 (m, 6H, $2CH_3$), 3.80 (s, 3H, OCH_3), 3.97–4.11 (m, 4H, OCH_2), 4.61–4.73 (q, AB system, 2H, OCH_2CO), 5.55 (dd, J = 9.6, 19.8 Hz, 1H, PCH), 6.90–8.09 (m, 8H, Ar-H, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 16.3, 49.0 (d, J = 153.0 Hz, PCH), 55.4, 63.1 (d, J = 65.0 Hz, $POCH_2$), 67.7 (OCH_2), 114.0, 116.1, 126.6, 126.9, 129.2, 140.7, 155.8, 159.5, 167.8 (CO). MS (70 ev) *m/z* (%): 452 (M^+). Anal. $C_{20}H_{25}N_2O_8P$ (C, H, N).

*4.2.10. O,O′-Diethyl {[2-(2-methyl-4-chlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5j**)*

White solid. m.p. 113–114 °C. IR(KBr) ν: 3229, 3058, 1699(C=O), 1556, 1490, 1223 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 1.12–1.30 (m, 6H, $2CH_3$), 2.34 (s, 3H, Ar-CH_3), 3.80 (s, 3H, OCH_3), 3.76–4.10 (m, 4H, OCH_2), 4.47–4.54 (q, AB system, 2H, OCH_2CO), 5.44 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.67 (d, J = 8.4 Hz, 1H, Ar-H), 6.88 (d, J = 8.4 Hz, 2H, Ar-H), 7.11 (d, J = 8.4 Hz, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 7.32 (d, J = 7.8 Hz, 2H, Ar-H), 7.41–7.43 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm):16.2, 16.3, 17.3, 49.1(d, J = 155.5 Hz, PCH), 55.2 (CH_3OAr), 63.2 (d, J = 62.4 Hz, $POCH_2$), 67.5 (OCH_2), 112.6, 114.1, 123.8, 126.5, 126.6, 128.5, 129.0, 130.8, 153.9, 159.5, 167.2 (CO). MS (70 ev) *m/z* (%): 455 (M^+). Anal. $C_{21}H_{27}ClNO_6P$ (C, H, N).

*4.2.11. O,O′-Dipropyl {[2-(2,4-dichlorophenoxy)acetamido](phenyl)methyl}phosphonate(**5k**)*

White solid. m.p. 111–112 °C. IR(KBr) ν: 3230, 2972, 1701(C=O), 1556, 1478, 1218 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.80–0.92 (m, 6H, $2CH_3$), 1.51–1.68 (m, 4H, CH_2), 3.70–4.01 (m, 4H, OCH_2), 4.51–4.60(q, AB system, 2H, OCH_2CO), 5.52–5.57 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.82 (d, J = 9.0 Hz, 1H, Ar-H), 7.20–7.44 (m, 7H, Ar-H), 7.71–7.73 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 9.9, 11.0, 23.8, 49.8 (d, J = 154.2 Hz, PCH), 68.3 (d, J = 50.3 Hz, $POCH_2$), 68.7 (OCH_2), 114.7, 123.9, 127.4, 127.8, 127.9, 128.2, 128.7, 130.1, 134.6, 151.5, 166.4 (CO). MS (70 ev) *m/z* (%): 473 (M^+). Anal. $C_{21}H_{26}Cl_2NO_6P$ (C, H, N).

*4.2.12. O,O′-Dipropyl {[2-(4-chlorophenoxy)acetamido](phenyl)methyl}phosphonate(**5l**)*

White solid. m.p. 102–103 °C. IR(KBr) ν: 3215, 3052, 1694(C=O), 1550, 1484, 1221 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.78–0.92 (m, 6H, $2CH_3$), 1.46–1.65 (m, 4H, CH_2), 3.59–4.00 (m, 4H, OCH_2), 4.48–4.55 (q, AB system, 2H, OCH_2CO), 5.51–5.56 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.82 (d, J = 8.4 Hz, 2H, Ar-H), 7.27–7.43 (m, 7H, Ar-H, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 9.9, 11.0, 23.6, 23.8, 49.7 (d, J = 155.4 Hz, PCH), 67.5(OCH_2), 68.6(d, J = 50.1 Hz, $POCH_2$), 116.1, 127.0, 128.0, 128.2, 128.6, 129.5, 134.7, 155.8, 167.2 (CO). MS (70 ev) *m/z* (%): 439 (M^+). Anal. $C_{21}H_{27}ClNO_6P$ (C, H, N).

*4.2.13. O,O′-Dipropyl {[2-(3-trifluoromethylphenoxy)acetamido](phenyl)methyl}phosphonate(**5m**)*

White solid. m.p. 63–64 °C. IR(KBr) ν: 3229, 3066, 1695(C=O), 1552, 1492, 1218 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.78–0.92 (m, 6H, $2CH_3$), 1.47–1.64 (m, 4H, CH_2), 3.61–4.00 (m, 4H, OCH_2), 4.54–4.61 (q, AB system, 2H, OCH_2CO), 5.54 (dd, J = 9.6, 21.0 Hz, 1H, PCH), 7.12 (d, J = 8.4 Hz, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.30–7.45 (m, 7H, Ar-H, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 9.8, 23.6, 23.8, 49.8 (d, J = 155.4 Hz, PCH), 67.4 (OCH_2), 68.7 (d, J = 58.8 Hz, $POCH_2$), 111.9, 118.0, 118.8, 128.0, 128.3, 128.6, 130.3, 132.1 (d, J = 31.8 Hz, CF_3), 134.6, 157.3, 166.9 (CO). MS (70 ev) *m/z* (%): 473 (M^+). Anal. $C_{22}H_{27}F_3NO_6P$ (C, H, N).

*4.2.14. O,O′-Dipropyl {[2-(2,4-dichlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5n**)*

White solid. m.p. 101–102 °C. IR(KBr) ν: 3226, 3070, 1697(C=O), 1554, 1477, 1222 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.82–0.92 (m, 6H, $2CH_3$), 1.52–1.67 (m, 4H, CH_2), 3.80 (s, 3H, OCH_3), 3.71–4.00 (m, 4H, OCH_2), 4.50–4.59 (q, AB system, 2H, OCH_2CO), 5.49 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.82 (d, J = 9.0 Hz, 1H, Ar-H), 6.89 (d, J = 8.4 Hz, 2H, Ar-H), 7.20–7.44 (m, 4H, Ar-H), 7.65 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 9.9, 10.9, 23.8, 49.0 (d, J = 155.3 Hz, PCH), 55.2, 68.2 (d, J = 40.4 Hz, $POCH_2$), 68.6 (OCH_2), 114.0, 114.7, 123.8, 126.6, 127.3, 127.9, 129.0, 130.0, 151.5, 159.5, 166.3 (CO). MS (70 ev) *m/z* (%): 504 (M^+). Anal. $C_{22}H_{28}Cl_2NO_6P$ (C, H, N).

*4.2.15. O,O′-Dipropyl {[2-(3-trifluoromethylphenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5o**)*

White solid. m.p. 59–60 °C. IR(KBr) ν: 3216, 3055, 1690(C=O), 1553, 1459, 1223 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.80–0.93 (m, 6H, $2CH_3$), 1.48–1.66 (m, 4H, CH_2), 3.80 (s, 3H, OCH_3), 3.64–4.00 (m, 4H, OCH_2), 4.53–4.60(q, AB system, 2H, OCH_2CO), 5.49 (dd, J = 9.6 Hz, 1H, PCH), 6.87 (d, J = 9.0 Hz, 1H, Ar-H), 7.11 (d, J = 7.8 Hz, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.30–7.37 (m, 4H, Ar-H), 7.43–7.45 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 9.8, 23.8, 49.0 (d, J = 157.9 Hz, PCH), 55.1, 67.3 (OCH_2), 68.6 (d, J = 58.8 Hz, $POCH_2$), 111.8, 114.0, 118.0, 118.7, 126.6, 129.2, 130.3, 132.0 (d, J = 33.0 Hz, CF_3), 157.3, 159.5, 166.9 (CO).MS (70 ev) *m/z* (%): 503 (M^+). Anal. $C_{23}H_{29}F_3NO_6P$ (C, H, N).

*4.2.16. O,O′-Dibutyl {[2-(2,3-dimethylphenoxy)acetamido](phenyl)methyl}phosphonate(**5p**)*

Yellowish oil. IR(KBr) ν: 3225, 3061, 1694(C=O), 1550, 1471, 1222 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.82–0.90 (m, 6H, $2CH_3$), 1.20–1.37 (m, 4H, $2CH_2$), 1.43–1.61 (m, 4H, $2CH_2$), 2.29 (s, 3H, Ar-CH_3), 2.32 (s, 3H, Ar-CH_3), 3.68–4.04 (m, 4H, $2CH_2$), 4.49–4.56 (q, AB system, 2H, OCH_2CO), 5.53 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.65 (d, J = 9.0 Hz, 1H, Ar-H), 6.87 (d, J = 8.4 Hz, 1H, Ar-H), 7.04–7.41 (m, 6H, Ar-H), 7.58 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 11.8, 13.4, 18.4, 18.5, 19.9, 32.4, 49.8 (d, J = 155.4 Hz, PCH), 66.7 (d, J = 35.4 Hz, $POCH_2$), 67.6 (OCH_2), 109.3, 123.5, 124.9, 126.0, 127.8, 128.0, 128.5, 134.9, 138.1, 155.2, 167.7 (CO). MS (70 ev) *m/z* (%): 461 (M^+). Anal. $C_{25}H_{36}NO_5P$ (C, H, N).

*4.2.17. O,O′-Dibutyl {[2-(2-chlorophenoxy)acetamido](phenyl)methyl}phosphonate(**5q**)*

Yellowish oil. IR(KBr) ν: 3215, 3052, 1694(C=O), 1550, 1484, 1221 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.83–0.90 (m, 6H, $2CH_3$), 1.21–1.37 (m, 4H, $2CH_2$), 1.47–1.62 (m, 4H, $2CH_2$), 3.77–4.05 (m, 4H, $2CH_2$), 4.05–4.63 (q, AB system, 2H, OCH_2CO), 5.56 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.90 (d, 1H, Ar-H, J = 8.4 Hz), 6.99–7.45 (m, 8H, Ar-H), 7.82 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 18.5, 32.4, 49.8 (d, J = 154.2 Hz, PCH), 66.9 (d, J = 35.4 Hz, $POCH_2$), 68.0 (OCH_2), 113.9, 123.0, 123.1, 127.8, 128.0, 128.2, 128.7, 130.4, 134.6, 152.7, 166.8 (CO). MS (70 ev) *m/z* (%): 467 (M^+). Anal. $C_{18}H_{20}Cl_2NO_5P$ (C, H, N).

*4.2.18. O,O′-Dibutyl {[2-(2,4-dichlorophenoxy)acetamido](phenyl)methyl}phosphonate(**5r**)*

Yellowish oil. IR(KBr) ν: 3222, 3065, 1694(C=O), 1558, 1480, 1228 cm^{-1} ^{1}H NMR (400 MHz $CDCl_3$) δ(ppm): 0.82–0.90 (m, 6H, $2CH_3$), 1.21–1.37 (m, 4H, $2CH_2$), 1.44–1.63 (m, 4H, $2CH_2$), 3.73–4.06 (m, 4H, $2CH_2$), 4.50–4.61 (q, AB system, 2H, OCH_2CO), 5.53 (dd, J = 9.6, 20.8 Hz, 1H, PCH), 6.82 (d, J = 8.8 Hz, 1H, Ar-H), 7.20–7.45

(m, 7H, Ar-H), 7.70–7.73 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.4, 18.5, 32.4, 49.8 (d, J = 154.2 Hz, PCH), 66.8 (d, J = 33.0 Hz, $POCH_2$), 68.1 (OCH_2), 114.7, 123.8, 127.2, 127.8, 128.1, 128.6, 130.0, 134.6, 151.6, 166.4 (CO). MS (70 ev) m/z (%): 502 (M^+). Anal. $C_{23}H_{30}Cl_2NO_5P$ (C, H, N).

4.2.19. O,O′-Dibutyl {[2-(2-methyl-4-chlorophenoxy) acetamido](phenyl)methyl}phosphonate(**5s**)

Yellowish oil. IR(KBr) ν: 3221, 3061, 1691(C═O), 1555, 1491, 1227 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.81–0.90 (m, 6H, $2CH_3$), 1.20–1.36 (m, 4H, $2CH_2$), 1.42–1.63 (m, 4H, $2CH_2$), 2.34 (s, 3H, Ar-CH_3), 3.65–4.02 (m, 4H, $2CH_2$), 4.48–4.54 (q, AB system, 2H, OCH_2CO), 5.49 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.67 (d, J = 8.4 Hz, 1H, Ar-H), 7.11 (d, J = 8.4 Hz, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.31–7.40 (m, 5H, Ar-H), 7.48 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 17.3, 18.6, 32.4, 49.7 (d, J = 153.0 Hz, PCH), 66.9 (d, J = 58.8 Hz, $POCH_2$) 67.5 (OCH_2), 113.9, 123.0, 123.1, 127.8, 128.0, 128.2, 128.7, 130.4, 134.6, 152.7, 167.2 (CO).MS (70 ev) m/z (%): 481 (M^+). Anal. $C_{24}H_{33}ClNO_5P$ (C, H, N).

4.2.20. O,O′-Dibutyl {[2-(3-methyl-4-chlorophenoxy) acetamido](phenyl)methyl}phosphonate(**5t**)

Yellowish oil. IR(KBr) ν: 3223, 3065, 1696(C═O), 1555, 1479, 1224 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.81–0.91 (m, 6H, $2CH_3$), 1.20–1.36 (m, 4H, $2CH_2$), 1.40–1.61 (m, 4H, $2CH_2$), 2.33 (s, 3H, Ar-CH_3), 3.64–4.03 (m, 4H, $2CH_2$), 4.46–4.54 (q, AB system, 2H, OCH_2CO), 5.51 (dd, J = 9.6, 20.8 Hz, 1H, PCH), 6.70–6.72 (q, 1H, Ar-H), 6.82 (d, J = 3.0 Hz, 1H, Ar-H), 7.25 (d, J = 9.0 Hz, 1H, Ar-H), 7.30–7.39 (m, 5H, Ar-H), 7.42–7.44 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 18.6, 20.2, 32.2, 49.7 (d, J = 151.8 Hz, PCH), 66.8 (d, J = 51.5 Hz, $POCH_2$), 67.5 (OCH_2), 113.3, 117.3, 127.2, 128.0, 128.1, 128.5, 129.7, 134.7, 137.3, 154.4, 167.4 (CO). MS (70 ev) m/z (%): 481 (M^+). Anal. $C_{24}H_{33}ClNO_5P$ (C, H, N).

4.2.21. O,O′-Dibutyl {[2-(2,4,5-trichlorophenoxy) acetamido](phenyl)methyl}phosphonate(**5u**)

Yellowish oil. IR(KBr) ν: 3235, 3067, 1691(C═O), 1555, 1477, 1227 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.82–0.91 (m, 6H, $2CH_3$), 1.22–1.37 (m, 4H, $2CH_2$), 1.44–1.63 (m, 4H, $2CH_2$), 3.72–4.06 (m, 4H, $2CH_2$), 4.51–4.60 (q, AB system, 2H, OCH_2CO), 5.53 (dd, J = 9.6, 21.0 Hz, 1H, PCH), 6.99 (s, 1H, Ar-H), 7.31–7.54 (m, 6H, Ar-H), 7.65 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 18.5, 32.4, 49.7 (d, J = 155.2 Hz, PCH), 66.8 (d, J = 55.5 Hz, $POCH_2$), 67.5 (OCH_2), 114.8, 115.8, 127.9, 128.2, 128.6, 134.7, 151.3, 154.8, 167.9 (CO). MS (70 ev) m/z (%): 536 (M^+). Anal. $C_{23}H_{29}Cl_3NO_5P$ (C, H, N).

4.2.22. O,O′-Dibutyl {[2-(4-methoxyphenoxy)acetamido](phenyl) methyl}phosphonate(**5v**)

Yellowish oil. IR(KBr) ν: 3228, 3066, 1694(C═O), 1554, 1506, 1227 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.82–0.91 (m, 6H, $2CH_3$), 1.20–1.37 (m, 4H, $2CH_2$), 1.42–1.65 (m, 4H, $2CH_2$), 3.67–4.02 (m, 4H, $2CH_2$), 3.78 (s, 3H, OCH_3), 4.46–4.52 (q, AB system, 2H, OCH_2CO), 5.53 (dd, J = 9.6, 21.0 Hz, 1H, PCH), 6.84–7.47 (m, 9H, Ar-H), 7.49 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 18.5, 32.4, 49.7 (d, J = 156.3 Hz, PCH), 55.6, 66.8 (d, J = 47.7 Hz, $POCH_2$), 68.2 (OCH_2), 114.8, 115.8, 127.9, 128.2, 128.6, 134.7, 151.3, 154.8, 167.9 (CO). MS (70 ev) m/z (%): 463 (M^+). Anal. $C_{24}H_{34}NO_6P$ (C, H, N).

4.2.23. O,O′-Dibutyl {[2-(2-chloro-4-fluorophenoxy) acetamido](phenyl)methyl}phosphonate(**5w**)

Yellowish oil. IR(KBr) ν: 3224, 3063, 1693(C═O), 1556, 1492, 1229 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.82–0.90 (m, 6H, $2CH_3$), 1.23–1.37 (m, 4H, $2CH_2$), 1.46–1.61 (m, 4H, $2CH_2$), 3.74–4.04 (m, 4H, $2CH_2$), 4.50–4.59 (q, AB system, 2H, OCH_2CO), 5.55 (dd, J = 9.6, 21.0 Hz, 1H, PCH), 6.85 (d, J = 4.8 Hz, 1H, Ar-H), 6.87 (d, J = 4.8 Hz, 1H, Ar-H), 6.95–7.45 (m, 6H, Ar-H), 7.75 (m, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.4, 18.5, 32.3, 49.8 (d, J = 158.7 Hz, PCH), 66.8 (d, J = 59.7 Hz, $POCH_2$), 68.5 (OCH_2), 114.6, 117.8, 123.8, 127.7, 128.2, 128.6, 134.5, 149.3, 156.5, 158.1, 166.6 (CO). MS (70 ev) m/z (%): 485 (M^+). Anal. $C_{23}H_{30}ClFNO_5P$ (C, H, N).

4.2.24. O,O′-Dibutyl {[2-(2,3-dimethylphenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5x**)

Yellowish oil. IR(KBr) ν: 3220, 3048, 1695(C═O), 1552, 1482, 1246 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.83–0.90 (m, 6H, $2CH_3$), 1.23–1.36 (m, 4H, $2CH_2$), 1.44–1.61 (m, 4H, $2CH_2$), 2.27–2.31 (d, 3H, Ar-CH_3), 3.70–4.02 (m, 4H, $2CH_2$), 3.80 (s, 3H, OCH_3), 4.48–4.54 (q, AB system, 2H, OCH_2CO), 5.48 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.64 (d, J = 7.8 Hz, 1H, Ar-H), 6.88 (d, J = 7.8 Hz, 3H, Ar-H), 7.04–7.06 (t, 1H, Ar-H), 7.33 (d, J = 7.8 Hz, 1H, Ar-H), 7.51 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 11.8, 13.6, 18.5, 19.0, 32.4, 49.5 (d, J = 155.4 Hz, PCH), 55.2 (CH_3OAr), 66.7 (d, J = 50.6 Hz, $POCH_2$), 67.5 (OCH_2), 109.3, 114.1, 123.7, 125.0, 126.1, 126.7, 128.9, 138.4, 155.1, 159.4, 167.7 (CO). MS (70 ev) m/z (%): 532 (M^+). Anal. $C_{26}H_{38}NO_6P$ (C, H, N).

4.2.25. O,O′-Dibutyl {[2-(2-chlorophenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5y**)

Yellowish oil. IR(KBr) ν: 3234, 3075, 1698(C═O), 1567, 1513, 1246 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.84–0.90 (m, 6H, $2CH_3$), 1.26–1.37 (m, 4H, $2CH_2$), 1.49–1.62 (m, 4H, $2CH_2$), 3.80 (s, 3H, OCH_3), 3.80–4.03 (m, 4H, $2CH_2$), 4.53–4.61 (q, AB system, 2H, OCH_2CO), 5.50 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.88–7.44 (m, 8H, Ar-H), 7.74 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.6, 18.6, 32.4, 49.2 (d, J = 158.7 Hz, PCH), 55.1, 66.9 (d, J = 50.1 Hz, $POCH_2$), 67.4 (OCH_2), 113.9, 114.1, 122.9, 123.0, 126.6, 128.1, 129.0, 130.4, 152.6, 159.4, 166.7 (CO). MS (70 ev) m/z (%): 566 (M^+). Anal. $C_{24}H_{33}ClNO_6P$ (C, H, N).

4.2.26. O,O′-Dibutyl {[2-(4-t-butylphenoxy)acetamido](4-methoxyphenyl)methyl}phosphonate(**5z**)

Yellowish oil. IR(KBr) ν: 3216, 3046, 1691(C═O), 1552, 1410, 1243 cm^{-1} ^{1}H NMR (600 MHz $CDCl_3$) δ(ppm): 0.83–0.91 (m, 6H, $2CH_3$), 1.22–1.61 (m, 8H, $4CH_2$), 1.30 (s, 9H, Ar-C(CH_3)$_3$), 3.70–4.03 (m, 4H, $2CH_2$), 3.79 (s, 3H, OCH_3), 4.48–4.54 (q, AB system, 2H, OCH_2CO), 5.49 (dd, J = 9.6, 20.4 Hz, 1H, PCH), 6.85–7.33 (m, 8H, Ar-H), 7.41 (d, 1H, NH). ^{13}C NMR (150 MHz $CDCl_3$) δ(ppm): 13.5, 18.5, 31.4, 31.5, 31.9, 32.4, 34.1, 49.0 (d, J = 155.6 Hz, PCH), 55.1, 66.8 (d, J = 50.1 Hz, $POCH_2$), 67.4 (OCH_2), 114.0, 114.3, 126.4, 126.7, 129.2, 144.8, 154.9, 159.4, 167.8 (CO). MS (70 ev) m/z (%): 493 (M^+). Anal. $C_{28}H_{43}NO_6P$ (C, H, N).

4.3. Cytotoxicity assay

Cytotoxic activities were evaluated by using standard MTT assay [17] after exposure of cells to the tested compounds for 72 h. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below 10%.

The in vitro cytotoxicity of the synthesized compounds against different cancer cell lines was performed with the MTT assay according to the Mosmann's method [23]. The MTT assay is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blueepurple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2Y′ 104/mL) were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator. After 12 h, the test sample (2 mL) was added to the cells (2Y′ 104/mL) in 96-well plates and cultured at 37 °C for 3 days. The cultured cells were mixed with 20 mL of MTT solution and incubated for 4 h at 37 °C. The supernatant was carefully removed from each well and 100 mL of DMSO was

added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below 10%.

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