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Original article

Diversity-oriented synthesis of α-aminophosphonates: A new class of potential anticancer agents

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Dedicated to Prof. Dr. Richard R. Schmidt on the occasion of his 78th birthday.

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ABSTRACT

A small library of structurally diverse α-aminophosphonates has been synthesized by reacting alkyl/aryl aldehydes, alkyl/aryl amines and alkyl/aryl phosphites in one-pot catalyzed by Amberlite-IR 120 resin (acidic). All the synthesized α-aminophosphonates were assayed for their *in vitro* cytotoxic activities against a panel of five human cancer cell lines including A-549, NCI-H23 (Lung), Colo 320DM (Colon), MG-63 (Bone marrow) and Jurkat (Blood T lymphocytes). Compound **4n** having (*R*)-1-phenylethanamine was found to be the most active amongst all the synthesized α-aminophosphonates against all the five cancer cell lines, most prominent being against Jurkat cell line with an IC_{50} value of 4 μM. Surprisingly, compound **4o** having (*S*)-1-phenylethanamine was found to be devoid of any cytotoxicity. Our finding suggests that these chemical entities could further serve as interesting template for the design of potential anticancer agents.

1. Introduction

Diversity-oriented synthesis (DOS) has garnered much attention in recent times as a tool to generate structurally diverse and complex set of small molecules. The basic intention of DOS is to furnish collections of small molecules having diversified scaffolds to spearhead drug discovery programme [1]. α-Aminophosphonates are considered to be structural analogs of the corresponding α-amino acids and transition-state mimics of peptide hydrolysis. α-Aminophosphonates have been reported to exert several pharmacological activities such as peptide mimics [2], haptens of catalytic antibodies [3], antibiotics and pharmacological agents [4] and herbicides [5]. Additionally, phosphinic acid and phosphinic amino acid analogs have been reported to be enzyme inhibitors [6]. We have been intrigued by the biological properties of aminophosphonates and have further explored amino-phosphonates as novel cysteine protease inhibitors [7a,b] and its anti-HIV activities [7c]. In literature, several synthetic methods have been reported for the synthesis of α-aminophosphonates, but the most preferred methods is the nucleophilic addition reaction of phosphites with imines, which is generally catalyzed by an alkali metal alkoxide, *e.g.*, NaOEt or Lewis acids [8] such as $SnCl_4$, $SnCl_2$, $ZnCl_2$, $MgBr_2$ and $BF_3{\cdot}Et_2O$ [9,10]. During the formation of imine, water is formed which could either deactivate or decompose the Lewis acid catalyst thereby limiting the scope of carrying out this reaction in one-pot [11]. Recently, we found [12] that bismuth nitrate pentahydrate to be an efficient Lewis acid to catalyze synthesis of α-aminophosphonates in one-pot due to its ability to tolerate the water generated during the course of the reaction. This result suggests that there is further scope to develop convenient and general approach utilizing an environmentally benign and recyclable catalyst for the synthesis of α-aminophosphonates.

The advantages associated with the use of solid acidic catalysts in organic synthesis *e.g.*, non-toxicity, low cost, operational simplicity, reusability, and ease of isolation after completion of the reaction has rendered them as the most preferred heterogeneous catalysts. Our search for efficient, cost effective, reusable and environmentally benign catalyst converged on Amberlite-IR 120 resin. As an efficient heterogeneous catalyst, Amberlite-IR 120 has been reported to catalyze various chemical transformations

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[13–15]. The application of microwave irradiation in organic synthesis is nowadays a widely employed procedure allowing fast product formation circumventing some of the harsh reaction conditions and also reactions could be performed under solvent-free conditions [16–20].

Cancer, which is a broad group of diverse diseases and characterized by uncontrolled growth of abnormal cells is a major health problem worldwide. Though, through these years medicinal chemistry research has afforded a number of new and effective remedies, the drugs currently being used for treatments have some limitations. Hence, there is an all out effort in the discovery of new scaffolds to find potent, safe and selective anticancer drugs. Barring some preliminary reports [21], α-aminophosphonate moiety has not been explored for its cytotoxic activities. Therefore, we opined that detailed investigation of α-aminophosphonates related to its cytotoxicity shall be of importance from the view point of medicinal chemistry since phosphonates have been known to be stable at physiological conditions and have good cell permeability [22].

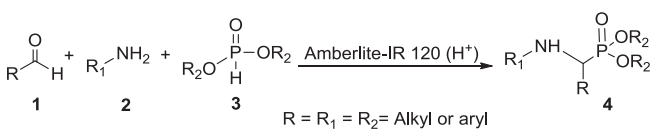
2. Results and discussion

2.1. Chemistry

In this full Article [preliminary report: Ref. [23]], we report Amberlite-IR 120 as an heterogeneous acid catalyst for the synthesis of α-aminophosphonates in one-pot under solvent-free reaction conditions using microwave irradiation (Scheme 1). In our preliminary communication [23], we have reported reaction of various aromatic/aliphatic aldehyde or ketone, amine and diethyl phosphite in one-pot to furnish α-aminophosphonates in good to excellent yields (Scheme 1). Taking a clue from this reaction and in order to generate a small library of diverse α-aminophosphonates, we have selectively reacted diverse alkyl/aryl aldehydes, alkyl/aryl amines and alkyl/aryl phosphites to generate structurally diverse α-aminophosphonates in good to excellent yields (Table 1). The three-component reaction in one-pot proceeded smoothly in all cases to furnish the corresponding α-aminophosphonates. The reaction of aromatic aldehydes resulted in excellent yields of the products due to their higher reactivity. But the product was formed in low yield in case of conjugated aldehyde (Table 1, entry 26). It is noteworthy to mention that the present method was found to be tolerant toward various functional groups present in the substrates *i.e.* substrates bearing methylenedioxy, methoxy, ethers, halides, olefinic and hydroxy groups. It is pertinent to mention here that the catalyst, Amberlite-IR 120 (acidic) can be reused after completion of reaction by filtration, washing and drying it without affecting the yield of the desired product and reaction time thereby rendering it environmentally benign.

2.2. Biological activity

All the synthesized compounds were assayed for their *in vitro* cytotoxicity [24] against a panel of five human cancer cell lines including A-549, NCI-H23 (Lung), Colo 320DM (Colon), MG-63 (Bone marrow) and Jurkat (Blood T lymphocytes). IC_{50} values were based on dose–response curves. The MTT assay for each test compound was performed thrice in triplicates. Each test compound displayed a concentration-dependent cytotoxic profile in all five cell lines. The synthesized α-aminophosphonates were evaluated against five human cancer cell lines for their cytotoxic profiles and the results of active compounds are summarized in Table 2. Compound **4c**, **4d**, **4j**, **4l**, **4r**, **4x**, **4y** and **4aa** were found to be active against four cell lines A-549, NCI-H23, Colo 320DM and MG-63, respectively. In general, compounds having piperonal moiety showed cytotoxicity against studied cell lines. Compounds (**4x**, **4y** and **4aa**) having aniline and diethyl phosphite moieties showed cytotoxic activities against A-549, NCI-H23, Colo 320DM and MG-63 cell lines with IC_{50} values in the range of 31.9–74.1 μM. The product **4p** containing piperonal, 3-fluoroaniline and diphenyl phosphite moiety was found to be selectively active against NCI-H23 cell line having IC_{50} value of 48.2 μM. Of all the compounds synthesized, compound **4n** having piperonal, dibutyl phosphite and (*R*)-1-phenylethanamine was found to be the most active with IC_{50} values of 43.4, 28.6, 15.8, 13.6 and 4 μM against all the five cancer cell lines, A-549, NCI-H23, Colo 320DM and MG-63 and Jurkat, respectively. However, it is interesting that compound **4o** having piperonal, dibutyl phosphite and (*S*)-1-phenylethanamine was devoid of any cytotoxicity against all the cell lines. It could not reach up to the IC_{50} at the significant concentration. The change in concentration of **4o** with change in cell line was adopted with the relative concentration of other compounds.



1 2 3 4

Scheme 1. One-pot synthesis of α-aminophosphonates.

3. Conclusions

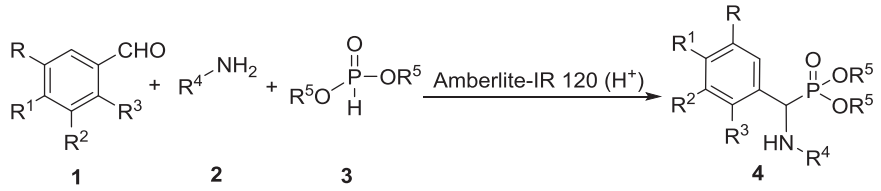
In conclusion, Amberlite-IR 120 catalyzed one-pot multicomponent reaction of alkyl/aryl aldehydes, alkyl/aryl amines, and alkyl/aryl phosphite to afford corresponding α-aminophosphonates in good to excellent yields has been established. The utilities of the present developed synthetic protocol are solvent-free reaction conditions, reusable catalyst and easy reaction work-up procedure. The efficacy of all synthesized α-aminophosphonate derivatives were assayed for their *in vitro* cytotoxic activities against a panel of five human cancer cell lines including A-549, NCI-H23 (Lung), Colo 320DM (Colon), MG-63 (Bone marrow) and Jurkat (Blood T lymphocytes). Compound **4n** having (*R*)-1-phenylethanamine, was amongst all the synthesized α-aminophosphonates found to be the most active against all the five cancer cell lines, most prominent being against Jurkat cell line with an IC_{50} value of 4 μM whereas compound **4o** having opposite stereocenter *i.e.* (*S*)-1-phenylethanamine was totally inactive against all the cell lines. Due to the fact that phosphonates are known to be stable at physiological conditions and have good cell permeability, it is presumed that results from these studies will provide an approach to design and synthesize new α-aminophosphonate derivatives for further structure optimization and development to obtain new leads for the treatment of cancer.

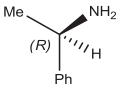
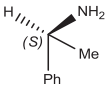
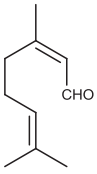
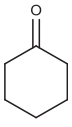
4. Experimental section

4.1. General

Melting points were recorded in open capillaries and are uncorrected. The FT-IR spectra were recorded on an FT-IR-8300 Shimadzu spectrometer and microanalyses were carried out on a Carlo-Erba instrument. NMR spectra were recorded on Bruker ACF 200 and AV200 (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR), AV400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and AV500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR), using $CDCl_3$ as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR, respectively. Chemical shifts are expressed in parts per million (ppm). Mass spectra were recorded on LC–MS/MS-TOF API QSTAR

Table 1
Amberlite-IR 120 (H^+) catalyzed one-pot synthesis of α-aminophosphonates.



Entry	Aldehyde (**1**)	Amine (**2**)	Phosphite (**3**)	Product (**4**)	Time (min)	Yield[a] (%)
1	$R = R^1 = R^2 = R^3 = H$	R^4 = Bn	R^5 = Et	**4a**	1	87
2	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 4-OHC_6H_4	R^5 = Et	**4b**	3	98
3	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2-OHC_6H_4	R^5 = Et	**4c**	2	79
4	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2,6-diMeC_6H_3	R^5 = Et	**4d**	3	91
5	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3,4-$OCH_2CH_2OC_6H_3$	R^5 = Et	**4e**	2	96
6	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = *n*-C_3H_7	R^5 = Et	**4f**	3	88
7	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3,4-$OCH_2OC_6H_3$	R^5 = ^{n}Bu	**4g**	3	90
8	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2-OHC_6H_4	R^5 = ^{n}Bu	**4h**	1	96
9	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2,6-diMeC_6H_3	R^5 = ^{n}Bu	**4i**	4	91
10	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3-FC_6H_4	R^5 = ^{n}Bu	**4j**	2	90
11	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2-MeC_6H_4	R^5 = ^{n}Bu	**4k**	2	93
12	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3-ClC_6H_4	R^5 = ^{n}Bu	**4l**	2	92
13	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = Bn	R^5 = ^{n}Bu	**4m**	3	90
14	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$		R^5 = ^{n}Bu	**4n**	3	92
15	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$		R^5 = ^{n}Bu	**4o**	3	90
16	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3-FC_6H_4	R^5 = Ph	**4p**	3	88
17	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3-ClC_6H_4	R^5 = Ph	**4q**	2	90
18	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = Ph	R^5 = Et	**4r**	2	92
19	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = Ph	R^5 = Allyl	**4s**	2	95
20	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 3,4-$OCH_2OC_6H_3$	R^5 = Allyl	**4t**	1	98
21	$R,R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = Bn	R^5 = Allyl	**4u**	1	94
22	$R = R^1 = R^2 = H$, R^3 = OMe	R^4 = Ph	R^5 = Et	**4v**	1	87
23	$R = R^2 = R^3 = H$, R^1 = F	R^4 = Ph	R^5 = Et	**4w**	1	87
24	$R = R^3 = H$, $R^1 = R^2$ = OMe	R^4 = Ph	R^5 = Et	**4x**	2	90
25	$R = R^3 = H$,R^1 = OMe, R^2 = OH	R^4 = Ph	R^5 = Et	**4y**	2	87
26		R^4 = Ph	R^5 = Et	**4z**	1	11
27	$R = R^2 = R^3 = H$, $R^1 = NO_2$	R^4 = Ph	R^5 = Et	**4aa**	1	95
28	$R = R^1 = R^3 = H$, $R^2 = NO_2$	R^4 = Ph	R^5 = Et	**4ab**	2.5	88
29	$R = R^1 = OCH_2O$, $R^2 = R^3 = H$	R^4 = 2-MeC_6H_4	R^5 = Et	**4ac**	3	90
30		R^4 = Bn	R^5 = Et	**4ad**	2	84
31	$R = R^2 = R^3 = H$, R^1 = Me	R^4 = Ph	R^5 = Et	**4ae**	1	89
32	$R = R^2 = R^3 = H$, R^1 = Me	R^4 = 2-$OMeC_6H_4$	R^5 = Et	**4af**	2	90
33	$R = R^2 = R^3 = H$, R^1 = Me	R^4 = Bn	R^5 = Et	**4ag**	1	93
34	$R = R^2 = R^3 = H$, R^1 = Me	R^4 = 3-FC_6H_4	R^5 = Et	**4ah**	1	91
35	$R = R^2 = R^3 = H$, $R^1 = NO_2$	R^4 = 2-$OMeC_6H_4$	R^5 = Et	**4ai**	1	94
36	$R = R^1 = R^3 = H$, $R^2 = NO_2$	R^4 = Bn	R^5 = Et	**4aj**	2	90
37	$R = R^2 = R^3 = H$, $R^1 = NO_2$	R^4 = Bn	R^5 = Et	**4ak**	1	93
38	$R = R^2 = R^3 = H$, R^1 = OMe	R^4 = Ph	R^5 = Et	**4al**	5	81

[a] Yields refer to those of pure isolated products fully characterized by spectral data (refer to Experimental section and Supporting information).

Table 2
IC_{50} values of synthesized α-aminophosphonates against human cancer cell lines of different tissues.

Compound	IC_{50} (μM)				
	Lung cancer		Colon cancer	Bone marrow cancer	Blood T lymphocytes
	A-549	NCI-H23	Colo 320DM	MG-63	Jurkat
4c	277.8 (±1.36)	190.4 (±0.95)	79.4 (±0.79)	114.8 (±0.66)	>500 (±2.74)
4d	>500 (±2.15)	248.0 (±1.24)	105.3 (±2.01)	114.3 (±0.73)	>500 (±2.16)
4g	191.9 (±1.54)	NA[a]	66.5 (±0.79)	NA[a]	80.0 (±1.47)
4j	363.8 (±2.42)	95.8 (±0.47)	80.3 (±0.81)	121.7 (±0.91)	>500 (±1.99)
4k	NA[a]	NA[a]	NA[a]	NA[a]	>500 (±2.65)
4l	263.3 (±1.87)	77.2 (±0.38)	93.0 (±0.98)	108.3 (±0.88)	>500 (±2.54)
4n	43.4 (±0.39)	28.6 (±0.14)	15.8 (±0.12)	13.6 (±0.12)	4.0 (±0.08)
4o	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]
4p	NA[a]	48.2 (±0.24)	NA[a]	NA[a]	490.0 (±3.01)
4r	134.4 (±0.94)	65.8 (±0.32)	92.0 (±1.37)	68.8 (±1.32)	>500 (±3.45)
4w	NA[a]	NA[a]	160.0 (±2.01)	NA[a]	NA[a]
4x	74.1 (±0.51)	39.5 (±0.19)	36.4 (±0.98)	34.0 (±0.89)	NA[a]
4y	62.4 (±0.49)	39.7 (±0.23)	47.9 (±1.31)	37.5 (±0.91)	57.0 (±1.14)
4aa	57.5 (±0.40)	37.4 (±0.18)	42.1 (±0.87)	31.9 (±0.45)	NA[a]
4al	NA[a]	53.8 (±0.26)	NA[a]	105.8 (±1.06)	NA[a]
Doxorubicin	4.0 (±0.02)	NT[b]	NT[b]	3.17 (±0.01)	NT[b]
Mytomycin	NT[b]	2.0 (±0.11)	NT[b]	NT[b]	NT[b]
5-Fluorouracil	NT[b]	NT[b]	2.0 (±1.98)	NT[b]	NT[b]
Tamoxifen	NT[b]	NT[b]	NT[b]	NT[b]	8.3 (±0.16)

(±) Standard deviation; $n = 3$.
[a] NA: Not active up to significant concentration.
[b] NT: Not tested.

PULSAR spectrometer, samples introduced by infusion method using Electrospray Ionization Technique (ESI). Flash chromatography was performed using CombiFlash Companion, Isco Teledyne Inc., USA. All other chemicals were of analytical grade.

4.2. General experimental procedure for the synthesis of α-aminophosphonates

The corresponding alkyl/aryl aldehyde (1 mmol), alkyl/aryl amine (1 mmol), alky/aryl phosphite (1 mmol) and Amberlite-IR 120 (100 mg) were taken in a Pyrex test tube and exposed to microwave irradiation (Samsung Model No. C103FL; 2450 MHz, 900 W) for the appropriate time (see Table 1). After completion of the reaction (as assessed by TLC), the reaction mixture was cooled, and dichloromethane (25 mL) was added. The catalyst was filtered from the reaction mixture and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography (CombiFlash Companion, RediSep® flash silica gel column, 12 g) eluting with petroleum ether/ethyl acetate (15–30%, gradient) to afford the corresponding pure α-aminophosphonates. All the products were characterized from their spectral data. The spectral data of all new compounds are given.

4.3. Diethyl (benzo[d][1,3]dioxol-5-yl((2,6-dimethylphenyl)amino) methyl)phosphonate (**4d**)

Semisolid; $R_f = 0.25$ (EtOAc/PE, 3:7); IR ($CHCl_3$): ν_{max} 3351, 2910, 2850, 1463, 1377, 1033, 722; ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ_H 7.00–6.73 (m, 6H), 5.94 (s, 2H), 4.44 (d, $^1J_{PH} = 25.0$ Hz, 1H), 4.15 (m, 3H), 3.96 (m, 1H), 3.69 (m, 1H), 2.25 (s, 6H), 1.30 (t, $J = 10.0$ Hz, 3H), 1.10 (t, $J = 10.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ_C 147.6, 147.3, 144.1, 130.8, 129.0, 128.7, 121.9, 121.8, 108.7, 108.6, 108.1, 101.1, 63.0 (d, $^2J_{PC} = 6.6$ Hz, $-OCH_2CH_3$), 62.9 (d, $^2J_{PC} = 7.3$ Hz, $-OCH_2CH_3$), 58.7 (d, $^1J_{PC} = 149.7$ Hz, $-CHP$), 18.9, 16.4 (d, $^3J_{PC} = 6.6$ Hz, $-OCH_2CH_3$), 16.2 (d, $^3J_{PC} = 5.9$ Hz, $-OCH_2CH_3$); MS (ESI): m/z 392.2505 $[M + H]^+$, 414.2394 $[M + Na]^+$, 430.1762 $[M + K]^+$; Found: C, 61.40; H, 6.74; N, 3.51. Calcd for $C_{20}H_{26}NO_5P$: C, 61.37; H, 6.70; N, 3.58%.

4.4. Diethyl (benzo[d][1,3]dioxol-5-yl((2,3-dihydrobenzo[b][1,4] dioxin-6-yl)amino)methyl) phosphonate (**4e**)

Colorless solid; mp 120–21 °C; $R_f = 0.60$ (EtOAc/PE, 3:2); IR (Nujol): ν_{max} 3318, 2900, 2830, 1463, 1376, 1169, 1022, 722 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.95–6.61 (m, 4H), 6.17–6.10 (m, 2H), 5.93 (s, 2H), 4.56 (d, $^1J_{PH} = 24.0$ Hz, 1H), 4.20–3.74 (m, 8H), 1.29 (t, $J = 8.0$ Hz, 3H), 1.17 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.0, 147.5, 143.9, 141.2, 140.9, 136.3, 129.8, 121.5, 117.6, 108.4, 107.7, 102.7, 101.1, 63.4 (d, $^2J_{PC} = 7.0$ Hz, $-OCH_2CH_3$), 63.3 (d, $^2J_{PC} = 7.0$ Hz, $-OCH_2CH_3$), 56.4 (d, $^1J_{PC} = 150.0$ Hz, $-CHP$), 16.5 (d, $^3J_{PC} = 5.8$ Hz, $-OCH_2CH_3$), 16.3 (d, $^3J_{PC} = 5.8$ Hz, $-OCH_2CH_3$); MS (ESI): m/z 422.0933 $[M + H]^+$, 444.0867 $[M + Na]^+$; Found: C, 57.05; H, 5.78; N, 3.24. Calcd for $C_{20}H_{24}NO_7P$: C, 57.01; H, 5.74; N, 3.32%.

4.5. Diethyl (benzo[d][1,3]dioxol-5-yl(propylamino)methyl) phosphonate (**4f**)

Semisolid; $R_f = 0.66$ (EtOAc/PE, 3:2); IR (Neat): ν_{max} 3461, 3316, 2931, 2875, 1609, 1504, 1487, 1441, 1246, 1032, 965, 808, 752, 629 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.97–6.75 (m, 3H), 5.95 (s, 2H), 4.17–3.87 (m, 5H), 2.53–2.33 (m, 3H), 1.48 (m, 2H), 1.30 (t, $J = 8.0$ Hz, 3H), 1.20 (t, $J = 8.0$ Hz, 3H), 0.87 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.8, 147.2, 129.9, 122.1, 108.6, 108.0, 101.0, 62.9 (d, $^2J_{PC} = 7.0$ Hz, $-OCH_2CH_3$), 62.8 (d, $^2J_{PC} = 7.0$ Hz, $-OCH_2CH_3$), 60.7 (d, $^1J_{PC} = 154.1$ Hz, $-CHP$), 49.5, 22.9, 16.5 (d, $^3J_{PC} = 5.5$ Hz, $-OCH_2CH_3$), 16.3 (d, $^3J_{PC} = 5.8$ Hz, $-OCH_2CH_3$), 11.6; MS (ESI): m/z 330.1139 $[M + H]^+$, 352.0248 $[M + Na]^+$; Found: C, 54.68; H, 7.30; N, 4.32. Calcd for $C_{15}H_{24}NO_5P$: C, 54.71; H, 7.35; N, 4.25%.

4.6. Dibutyl (benzo[d][1,3]dioxol-5-yl(benzo[d][1,3]dioxol-5-yl-amino)methyl)phosphonate (**4g**)

Colorless solid; mp 76–77 °C; $R_f = 0.46$ (EtOAc/PE, 2:3); IR ($CHCl_3$): ν_{max} 3325, 2934, 2876, 1466, 1364, 1216, 1040, 931, 757 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.94–6.55 (m, 3H), 6.21 (d, $J = 4.0$ Hz, 1H), 6.03–5.98 (m, 2H), 5.93 (s, 2H) 5.81 (s, 2H),

4.56 (d, $^1J_{PH}$ = 24.0 Hz, 1H), 4.11–3.70 (m, 4H), 1.69–1.21 (m, 8H), 0.94–0.82 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.2, 148.0, 142.0, 140.3, 129.8, 121.4, 108.5, 105.8, 101.2, 100.7, 97.0, 67.0 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 66.9 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 56.6 (d, $^1J_{PC}$ = 152.2 Hz, $-CHP$), 32.6 (d, $^3J_{PC}$ = 7.7 Hz, $-OCH_2CH_2CH_2CH_3$), 32.5 (d, $^3J_{PC}$ = 7.7 Hz, $-OCH_2CH_2CH_2CH_3$), 18.7, 13.6; MS (ESI): *m/z* 464.3811 $[M + H]^+$; Found: C, 59.55; H, 6.48; N, 3.12. Calcd for $C_{23}H_{30}NO_7P$: C, 59.61; H, 6.52; N, 3.02%.

4.7. Dibutyl (benzo[d][1,3]dioxol-5-yl((2-hydroxyphenyl)amino) methyl)phosphonate (**4h**)

Semisolid; R_f = 0.66 (EtOAc/PE, 3:2); IR (Nujol): ν_{max} 3400, 3305, 2954, 2854, 1611, 1505, 1459, 1376, 1242, 1215, 1037, 762, 736, 627, 540 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.96–6.46 (m, 7H), 5.87 (s, 2H), 4.79 (d, $^1J_{PH}$ = 26.0 Hz, 1H), 4.23–3.64 (m, 4H), 1.65–1.22 (m, 8H), 0.89–0.80 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.9, 145.3, 135.0, 129.6, 121.6, 119.8, 118.2, 114.3, 111.8, 108.6, 101.1, 68.0 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CH_2CH_2CH_3$), 67.2 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CH_2CH_2CH_3$), 55.6 (d, $^1J_{PC}$ = 155.9 Hz, $-CHP$), 32.5 (d, $^3J_{PC}$ = 8.4 Hz, $-OCH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{PC}$ = 8.4 Hz, $-OCH_2CH_2CH_2CH_3$), 18.6, 13.6; MS (ESI): *m/z* 436.2792 $[M + H]^+$, 458.2665 $[M + Na]^+$; Found: C, 60.63; H, 6.88; N, 3.33. Calcd for $C_{22}H_{30}NO_6P$: C, 60.68; H, 6.94; N, 3.22%.

4.8. Dibutyl (benzo[d][1,3]dioxol-5-yl((2,6-dimethylphenyl)amino) methyl)phosphonate (**4i**)

Colorless solid; mp 70–71 °C; R_f = 0.70 (EtOAc/PE, 3:2); IR (Neat): ν_{max} 3390, 3018, 2963, 1594, 1504, 1487, 1443, 1250, 1215, 1099, 1040, 989, 934, 756, 668 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.98–6.70 (m, 6H), 5.93 (s, 2H), 4.41 (d, $^1J_{PH}$ = 22.0 Hz, 1H), 4.10–3.84 (m, 4H), 3.21 (m, 1H), 2.23 (s, 6H), 1.60–1.21 (m, 8H), 0.93–0.77 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.6, 147.3, 144.2, 131.0, 130.0, 128.6, 121.9, 121.8, 108.8, 108.1, 101.1, 66.6 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 66.5 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 58.6 (d, $^1J_{PC}$ = 149.3 Hz, $-CHP$), 32.5 (d, $^3J_{PC}$ = 9.5 Hz, $-OCH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{PC}$ = 9.5 Hz, $-OCH_2CH_2CH_2CH_3$), 19.0, 18.6, 13.6; MS (ESI): *m/z* 448.2405 $[M + H]^+$, 470.2261 $[M + Na]^+$; Found: C, 64.37; H, 7.61; N, 3.22. Calcd for $C_{24}H_{34}NO_5P$: C, 64.41; H, 7.66; N, 3.13%.

4.9. Dibutyl (benzo[d][1,3]dioxol-5-yl((3-fluorophenyl)amino) methyl)phosphonate (**4j**)

Colorless solid; mp 69–70 °C; R_f = 0.50 (EtOAc/PE, 3:2); IR (Neat): ν_{max} 3298, 3018, 2963, 2965, 1619, 1594, 1489, 1443, 1240, 1216, 1040, 758, 668, 628 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.06–6.75 (m, 4H), 6.42–6.23 (m, 3H), 5.94 (s, 2H), 4.61 (d, $^1J_{PH}$ = 24.0 Hz, 1H), 4.11–3.85 (m, 3H), 3.72–3.63 (m, 1H), 1.69–1.20 (m, 8H), 0.94–0.82 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 166.3, 161.4, 148.1, 130.4, 129.4, 121.4, 109.7, 108.4, 101.5, 100.9, 100.4, 67.1 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 67.0 (d, $^2J_{PC}$ = 7.1 Hz, $-OCH_2CH_2CH_2CH_3$), 55.7 (d, $^1J_{PC}$ = 152.6 Hz, $-CHP$), 32.6 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 18.7, 13.6; MS (ESI): *m/z* 438.4244 $[M + H]^+$, 460.3364 $[M + Na]^+$, 476.3144 $[M + K]^+$; Found: C, 60.44; H, 6.72; N, 3.12. Calcd for $C_{22}H_{29}FNO_5P$: C, 60.40; H, 6.68; N, 3.20%.

4.10. Dibutyl (benzo[d][1,3]dioxol-5-yl(o-tolylamino)methyl) phosphonate (**4k**)

Semisolid; R_f = 0.70 (EtOAc/PE, 3:2); IR (Neat): ν_{max} 3392, 3016, 2960, 2870, 1590, 1510, 1486, 1430, 1245, 1225, 1102, 1045, 986, 930, 760, 668 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.10–6.38 (m, 7H), 5.93 (s, 2H), 4.69 (d, $^1J_{PH}$ = 22.0 Hz, 1H), 4.10–3.69 (m, 4H), 2.26 (s, 3H), 1.65–1.22 (m, 8H), 0.93–0.82 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.0, 147.3, 144.2, 130.2, 129.9, 127.0, 122.9, 121.2, 118.1, 111.2, 108.3, 101.1, 67.0 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CH_2CH_2CH_3$), 66.9 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CH_2CH_2CH_3$), 55.8 (d, $^1J_{PC}$ = 151.5 Hz, $-CHP$), 32.6 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 32.5 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 18.7, 17.6, 13.6; MS (ESI): *m/z* 434.2427 $[M + H]^+$, 456.2332 $[M + Na]^+$; Found: C, 63.78; H, 7.48; N, 3.14. Calcd for $C_{23}H_{32}NO_5P$: C, 63.73; H, 7.44; N, 3.23%.

4.11. Dibutyl (benzo[d][1,3]dioxol-5-yl((3-chlorophenyl) amino) methyl)phosphonate (**4l**)

Semisolid; R_f = 0.46 (EtOAc/PE, 2:3); IR (Neat): ν_{max} 3415, 3296, 3018, 2963, 2875, 1598, 1504, 1487, 1250, 1216, 1040, 990, 933, 757, 668 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.05–6.44 (m, 7H), 5.94 (s, 2H), 4.61 (d, $^1J_{PH}$ = 22.0 Hz, 1H), 4.11–3.63 (m, 4H), 1.68–1.17 (m, 8H), 0.93–0.82 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.1, 147.7, 134.9, 130.1, 129.3, 121.4, 118.3, 113.7, 112.0, 108.4, 108.2, 101.2, 67.1 (d, $^2J_{PC}$ = 8.0 Hz, $-OCH_2CH_2CH_2CH_3$), 67.0 (d, $^2J_{PC}$ = 8.0 Hz, $-OCH_2CH_2CH_2CH_3$), 55.5 (d, $^1J_{PC}$ = 152.0 Hz, $-CHP$), 32.6 (d, $^3J_{PC}$ = 6.1 Hz, $-OCH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{PC}$ = 6.1 Hz, $-OCH_2CH_2CH_2CH_3$), 18.7, 13.6; MS (ESI): *m/z* 454.2110 $[M + H]^+$, 476.2034 $[M + Na]^+$; Found: C, 58.17; H, 6.40; N, 3.17. Calcd for $C_{22}H_{29}ClNO_5P$: C, 58.21; H, 6.44; N, 3.09%.

4.12. Dibutyl (benzo[d][1,3]dioxol-5-yl(benzylamino)methyl) phosphonate (**4m**)

Semisolid; R_f = 0.39 (EtOAc/PE, 2:3); IR ($CHCl_3$): ν_{max} 3452, 3307, 2960, 2873, 2932, 1607, 1504, 1487, 1442, 1361, 1246, 1101, 984, 810, 732, 699, 629, cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.31–7.25 (m, 5H), 6.98–6.76 (m, 3H), 5.97 (s, 2H), 4.08–3.76 (m, 6H), 3.52 (d, $^1J_{PH}$ = 14.0 Hz, 1H), 1.65–1.24 (m, 8H), 0.95–0.82 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.9, 147.4, 139.3, 129.5, 128.4, 127.1, 122.4, 108.8, 108.2, 101.1, 66.7 (d, $^2J_{PC}$ = 8.8 Hz, $-OCH_2CH_2CH_2CH_3$), 66.5 (d, $^2J_{PC}$ = 8.8 Hz, $-OCH_2CH_2CH_2CH_3$), 59.1 (d, $^1J_{PC}$ = 155.2 Hz, $-CHP$), 32.6 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 32.5 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_2CH_2CH_3$), 18.6, 13.6; MS (ESI): *m/z* 434.2477 $[M + H]^+$, 456.2236 $[M + Na]^+$; Found: C, 63.67; H, 7.48; N, 3.25. Calcd for $C_{23}H_{32}NO_5P$: C, 63.73; H, 7.44; N, 3.23%.

4.13. Dibutyl (benzo[d][1,3]dioxol-5-yl(((R)-1-phenylethyl)amino) methyl)phosphonate (**4n**)

Semisolid; R_f = 0.60 (EtOAc/PE, 3:2); IR ($CHCl_3$): ν_{max} 3448, 3293, 2961, 2874, 1604, 1488, 1442, 1369, 1241, 1064, 984, 759, 630 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.32–7.20 (m, 5H), 6.89–6.71 (m, 3H), 5.94 (s, 2H), 4.14–3.59 (m, 6H), 1.70–1.60 (m, 3H), 1.50–1.12 (m, 8H), 0.98–0.78 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.8, 147.2, 145.3, 144.2, 130.5, 128.5, 126.8, 122.0, 108.7, 108.1, 101.1, 66.7 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 66.6 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 57.8 (d, $^1J_{PC}$ = 153.0 Hz, $-CHP$), 32.7 (d, $^3J_{PC}$ = 6.0 Hz, $-OCH_2CH_2CH_2CH_3$), 32.5 (d, $^3J_{PC}$ = 6.0 Hz, $-OCH_2CH_2CH_2CH_3$), 24.9, 22.4, 18.8, 13.7; MS (ESI): *m/z* 448.3595 $[M + H]^+$, 470.3450 $[M + Na]^+$; Found: C, 64.36; H, 7.62; N, 3.22. Calcd for $C_{24}H_{34}NO_5P$: C, 64.41; H, 7.66; N, 3.13%.

4.14. Dibutyl (benzo[d][1,3]dioxol-5-yl(((S)-1-phenylethyl)amino) methyl)phosphonate (**4o**)

Colorless syrup; R_f = 0.66 (EtOAc/PE, 3:2); IR (Neat): ν_{max} 3329, 3018, 2964, 1608, 1504, 1487, 1443, 1248, 1216, 1063, 989, 934, 668, 628 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.27–7.19 (m, 5H),

6.90–6.70 (m, 3H), 5.93 (s, 2H), 4.14–3.59 (m, 6H), 1.71–1.60 (m, 3H), 1.49–1.14 (m, 8H), 0.98–0.78 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 147.9, 147.3, 145.3, 144.2, 130.5, 128.5, 126.7, 122.3, 121.9, 108.7, 108.1, 101.0, 66.6 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 66.3 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_2CH_2CH_3$), 57.8 (d, $^1J_{PC}$ = 152.5 Hz, $-CHP$), 32.7 (d, $^3J_{PC}$ = 5.5 Hz, $-OCH_2CH_2CH_2CH_3$), 32.4 (d, $^3J_{PC}$ = 5.5 Hz, $-OCH_2CH_2CH_2CH_3$), 24.9, 22.3, 18.8, 13.7; MS (ESI): *m/z* 448.7261 $[M + H]^+$, 470.7327 $[M + Na]^+$; Found: C, 64.45; H, 7.70; N, 3.05. Calcd for $C_{24}H_{34}NO_5P$: C, 64.41; H, 7.66; N, 3.13%.

4.15. Diphenyl (benzo[d][1,3]dioxol-5-yl((3-fluorophenyl)amino) methyl)phosphonate (**4p**)

Semisolid; R_f = 0.56 (EtOAc/PE, 3:2); IR ($CHCl_3$): ν_{max} 3294, 3056, 3018, 2926, 2855, 1730, 1615, 1594, 1537, 1443, 1288, 1193, 1040, 934, 819, 731, 594, cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.32–6.39 (m, 17H), 5.92 (s, 2H), 5.01 (d, $^1J_{PH}$ = 22.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 166.3, 161.5, 155.1, 150.3, 150.2, 148.3, 147.9, 147.7, 130.5, 129.8, 128.0, 125.6, 120.7, 120.3, 109.9, 108.6, 108.4, 101.3, 100.7, 55.6 (d, $^1J_{PC}$ = 155.0 Hz, $-CHP$); MS (ESI): *m/z* 500.2444 $[M + Na]^+$; Found: C, 65.45; H, 4.35; N, 2.97. Calcd for $C_{26}H_{21}FNO_5P$: C, 65.41; H, 4.43; N, 2.93%.

4.16. Diphenyl (benzo[d][1,3]dioxol-5-yl((3-chlorophenyl)amino) methyl)phosphonate (**4q**)

Semisolid; R_f = 0.65 (EtOAc/PE, 3:2); IR ($CHCl_3$): ν_{max} 3350, 2778, 2489, 2243, 1602, 1468, 1398, 1122, 975, 824, 679 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.32–6.50 (m, 17H), 5.92 (s, 2H), 5.01 (d, $^1J_{PH}$ = 22.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 150.3, 148.3, 147.2, 146.9, 135.0, 130.3, 129.8, 128.0, 125.6, 122.0, 120.7, 120.2, 118.8, 113.9, 112.2, 108.5, 101.3, 55.5 (d, $^1J_{PC}$ = 155.0 Hz, $-CHP$); MS (ESI): *m/z* 516.2692 $[M + Na]^+$, 532.2478 $[M + K]^+$; Found: C, 63.27; H, 4.21; N, 2.87. Calcd for $C_{26}H_{21}ClNO_5P$: C, 63.23; H, 4.29; N, 2.84%.

4.17. Diethyl (benzo[d][1,3]dioxol-5-yl(phenylamino)methyl) phosphonate (**4r**)

Semisolid; R_f = 0.71 (EtOAc/PE, 1:1); IR ($CHCl_3$): ν_{max} 3348, 2905, 2848, 1455, 1365, 1027, 723; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.15–6.57 (m. 8H), 5.92 (s, 2H), 4.66 (d, $^1J_{PH}$ = 24 Hz, 1H), 4.18–3.79 (m, 4H), 1.33–1.14 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.0, 147.4, 146.4, 129.7, 129.2, 124.5, 118.5, 113.9, 108.4, 108.2, 101.2, 63.3 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_3$), 63.2 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CH_3$), 55.7 (d, $^1J_{PC}$ = 152.2 Hz, $-CHP$), 16.5 (d, $^3J_{PC}$ = 8.1 Hz, $-OCH_2CH_3$), 16.4 (d, $^3J_{PC}$ = 8.1 Hz, $-OCH_2CH_3$); MS (ESI): *m/z* 364.7069 $[M + H]^+$, 386.7274 $[M + Na]^+$, 402.7225 $[M + K]^+$; Found: C, 59.56; H, 6.14; N, 3.76. Calcd for $C_{18}H_{22}NO_5P$: C, 59.50; H, 6.10; N, 3.85%.

4.18. Diallyl (benzo[d][1,3]dioxol-5-yl(phenylamino)methyl) phosphonate (**4s**)

Semisolid; R_f = 0.58 (EtOAc/PE, 2:3); IR (Neat): ν_{max} 3307, 3062, 2960, 2873, 2932, 2901, 1718, 1628, 1607, 1504, 1487, 1442, 1361, 1246, 1101, 984, 930, 810, 732, 699, 629, 574 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.15–6.55 (m, 8H), 6.00–5.68 (m, 4H), 5.37–5.13 (m, 4H), 4.72 (d, $^1J_{PH}$ = 22.6 Hz, 1H), 4.60–4.43 (m, 3H), 4.25–4.05 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.3, 147.5, 141.8, 141.5, 140.4, 132.8, 132.6, 129.5, 121.5, 118.4, 108.5, 105.9, 101.2, 97.1, 67.6 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CHCH_2$), 67.5 (d, $^2J_{PC}$ = 7.3 Hz, $-OCH_2CHCH_2$), 56.9 (d, $^1J_{PC}$ = 152.6 Hz, $-CHP$); MS (ESI): *m/z* 388.3389 $[M + H]^+$, 410.3642 $[M + Na]^+$, 426.3478 $[M + K]^+$; Found: C, 62.05; H, 5.65; N, 3.65. Calcd for $C_{20}H_{22}NO_5P$: C, 62.01; H, 5.72; N, 3.62%.

4.19. Diallyl (benzo[d][1,3]dioxol-5-yl(benzo[d][1,3]dioxol-5-ylamino)methyl)phosphonate (**4t**)

Colorless syrup; R_f = 0.56 (EtOAc/PE, 1:1); IR (Neat): ν_{max} 3310, 3019, 2912, 2896, 1720, 1634, 1611, 1504, 1488, 1440, 1366, 1215, 1041, 758, 669 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 6.96–6.55 (m, 4H), 6.23–5.76 (m, 6H), 5.37–5.14 (m, 4H), 4.68–4.05 (m, 7H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.2, 147.5, 141.9, 140.4, 132.8, 132.6, 129.4, 121.5, 118.4, 108.5, 108.3, 105.9, 101.2, 100.7, 97.1, 67.5 (d, $^2J_{PC}$ = 7.5 Hz, $-OCH_2CHCH_2$), 67.4 (d, $^2J_{PC}$ = 7.5 Hz, $-OCH_2CHCH_2$), 56.9 (d, $^1J_{PC}$ = 152.0 Hz, $-CHP$); MS (ESI): *m/z* 432.7462 $[M + H]^+$, 454.7790 $[M + Na]^+$, 470.7651 $[M + K]^+$; Found: C, 58.41; H, 5.23; N, 3.22. Calcd for $C_{21}H_{22}NO_7P$: C, 58.47; H, 5.14; N, 3.25%.

4.20. Diallyl (benzo[d][1,3]dioxol-5-yl(benzylamino)methyl) phosphonate (**4u**)

Semisolid; R_f = 0.60 (EtOAc/PE, 1:1); IR ($CHCl_3$): ν_{max} 3300, 3055, 2955, 2862, 2928, 2910, 1705, 1610, 1509, 1460, 1370, 1242, 1105, 981, 729, 631, 550 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ_H 7.34–7.25 (m, 5H), 7.00 (s, 1H), 6.87–6.81 (m, 2H), 5.99 (s, 2H), 5.94–5.60 (m, 2H), 5.32 (d, $^1J_{PH}$ = 24 Hz, 1H), 5.25–5.15 (m, 3H) 4.58–4.31 (m, 4H), 4.00 (d, *J* = 20.0 Hz, 1H), 3.83 (d, *J* = 16.0 Hz, 1H), 3.56 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.0, 147.4, 139.21, 133.1, 132.9, 129.2, 128.4, 127.2, 122.4, 117.8, 108.8, 108.2, 101.1, 67.3 (d, $^2J_{PC}$ = 7.0 Hz, $-OCH_2CHCH_2$), 67.1 (d, $^2J_{PC}$ = 6.5 Hz, $-OCH_2CHCH_2$), 59.3 (d, $^1J_{PC}$ = 154.5 Hz, $-CHP$), 51.2; MS (ESI): *m/z* 402.3432 $[M + H]^+$, 424.2536 $[M + Na]^+$; Found: C, 62.76; H, 6.08; N, 3.53. Calcd for $C_{21}H_{24}NO_5P$: C, 62.84; H, 6.03; N, 3.49%.

4.21. Diethyl (benzo[d][1,3]dioxol-5-yl(o-tolylamino)methyl) phosphonate (**4ac**)

Colorless solid; mp 106–07 °C; R_f = 0.22 (EtOAc/PE, 3:7); IR ($CHCl_3$): ν_{max} 3428, 3007, 2927, 1606, 1587, 1505, 1488, 1241, 1216, 1041, 972, 754, 667, 624 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ_H 7.06–6.90 (m, 4H), 6.78–6.62 (m, 2H), 6.41 (d, *J* = 8.0 Hz, 1H), 5.93 (s, 2H), 4.69 (d, $^1J_{PH}$ = 24.0 Hz, 1H), 4.16–3.95 (m, 3H), 3.82–3.78 (m, 1H), 2.26 (s, 3H), 1.29 (t, *J* = 8.0 Hz, 3H), 1.19 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ_C 148.0, 147.5, 144.5, 144.2, 130.2, 129.9, 127.0, 123.0, 121.2, 118.1, 111.2, 108.3, 101.1, 67.0, 57.6, 54.3, 18.3, 16.3 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_3$), 16.1 (d, $^3J_{PC}$ = 6.2 Hz, $-OCH_2CH_3$); MS (ESI): *m/z* 378.1438 $[M + H]^+$, 400.2078 $[M + Na]^+$, 416.0956 $[M + K]^+$; Found: C, 60.44; H, 6.38; N, 3.77. Calcd for $C_{19}H_{24}NO_5P$: C, 60.47; H, 6.41; N, 3.71%.

4.22. Biological assays

In vitro cytotoxicity of synthesized compounds against human cancer cell lines: Cell lines MG-63 (Human osteosarcoma) was maintained in MEM + Na pyruvate + 10% FBS + 1% ampicillin whereas NCI-H23 (alveolar adenocarcinoma); Jurkat (T cell human leukemia) and Colo 320DM (colorectal human adenocarcinoma) were maintained in RPMI 1640 + 10% FBS + 1% ampicillin; and A549 (alveolar adenocarcinoma) was maintained in 90% Dulbecco's MEM + 10% FBS. Briefly, cell lines were inoculated into a series of 96-well microtiter plates, seeding densities depending on the growth characteristics of particular cell lines. Following a 24-h compound-free incubation, test compounds were added routinely at 2-fold dilutions till sixth concentration with a maximum concentration of 50×10^{-4} M. After 48 h of treatment MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] at concentration of 10 mg/ml in PBS was added to evaluate the mitochondrial function (cell viability) with incubation at 37 °C for 4 h.

Thereafter, the plates were centrifuged for 10 min at 1500 g. The media was removed from each well and calculated amount of DMSO was added to each well. The absorbance was checked at 570 nm using ELISA plate reader [24]. For each test compound, varied concentrations were used *e.g.*, **4c** (200 μM–500 μM); **4d** (75 μM–500 μM); **4g** (10 μM–250 μM); **4j** (50 μM–500 μM); **4k** (100 μM–500 μM); **4l** (50 μM–500 μM); **4n** (1 μM–100 μM); **4o** (100 μM–500 μM); **4p** (10 μM–500 μM); **4r** (25 μM–500 μM); **4w** (50 μM–500 μM); **4x** (10 μM–100 μM); **4y** (10 μM–100 μM); **4aa** (10 μM–100 μM) and **4al** (10 μM–200 μM).

Conflict of interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.05.036.

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