Res Chem Intermed (2017) 43:7087–7103
DOI 10.1007/s11164-017-3060-y

One-pot green synthesis and cytotoxicity of new α-aminophosphonates

Kandula Madhu Kumar Reddy[1] · Shaik Mahammad Sadik[1] · Nagaripati Saichaithanya[1] · Kotha Peddanna[2] · Nemallapudi Bakthavatchala Reddy[1,3] · Gundala Sravya[1,3] · Zyryanov Grigory V[3,4] · Cirandur Suresh Reddy[1]

Received: 16 February 2017 / Accepted: 6 July 2017 / Published online: 21 July 2017

Abstract A novel series of α-aminophosphonates containing the trifluoromethyl aniline moiety were obtained in high yields by condensation of 2-methyl-3-trifluoromethyl aniline, aryl/heteroaryl aldehydes and dimethylphosphite in the presence of chitosan as a catalyst. The molecular modeling studies revealed their important structural features of binding affinities towards the target enzyme. The cytotoxicity of these compounds was evaluated against PC-3(prostate cancer), MCF-7 (breast cancer), HeLa(cCervix Cancer), U973, K562 and HL60 human lLeukemia cell lines. Compound **4k** with a pyrene moiety showed high potency against a breast cancer cell line, while compounds **4g** and **4k** exhibited more promising cytotoxicity against U973, K562 and HL60 cell lines.

Keywords Kabachnik-fields reaction · Chitosan · α-aminophosphonate · Molecular docking studies · Cytotoxic activity · In vitro

Electronic supplementary material The online version of this article (doi:10.1007/s11164-017-3060-y) contains supplementary material, which is available to authorized users.

✉ Cirandur Suresh Reddy
csrsvu@gmail.com

[1] Department of Chemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh 517 502, India

[2] Department of Bio-Chemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh 517 502, India

[3] Department of Organic and Biomolecular Chemistry, Chemical Engineering Institute, Ural Federal University, 19 Mira Street, Yekaterinburg, Russian Federation 620002

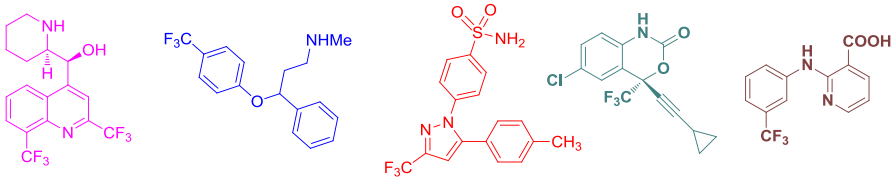
[4] Ural Division of the Russian Academy of Sciences, I. Ya. Postovskiy Institute of Organic Synthesis, 22 S. Kovalevskoy Street, Yekaterinburg, Russian Federation 620219

Introduction

Trifluoromethyl anilines are important constituents of many bio-active synthetic organic compounds, and this background triggered increasing interest in the chemistry of fluorine-containing compounds (Fig. 1) which have unique properties such as high thermal stability and enhanced lipophilicity compared to non-fluorinated counterparts. The significance of the fluorine substitution in organic compounds is well illustrated by way of their use as antimalarial (1), anti-depressant (2), COX-2 inhibitor (3), HIV protease inhibitors (4) and other important bio-activities, including analgesic and anti-inflammatory (5) [1–5].

On the other hand, the role of α-aminophosphonates in the biological system stimulated the researchers to develop various methods to synthesize novel bioactive α-aminophosphonates [6]. Many natural and synthetic α-aminophosphonates and their derivatives have potential applications as anticancer and antibiotic agents [7, 8]. Furthermore, they are used in agriculture as fungicides, herbicides, and plant growth regulators [9–11]. They have been synthesized by using acid catalysts, such as, Lewis (like, $SnCl_4$ [12], $InCl_3$ [13], $In(OTf)_3$ [14], $Yb(PfO)_3$ [15], SmI_2 [16] and $TaCl_5$-SiO_2 [17]) and Bronsted (like, sulfamic acid [18] and oxalic acid [19]) acids, solid acids $H_3PMo_{12}O_{40}$ [20], silica sulfuric acid [21], and base catalysts (like, $CaCl_2$ [22], PPh_3 [23]) and Amberlyst-15 [24]. Other catalysts such as $NbCl_5$ [25], Nano-TiO_2 [26], β-cyclodextrine [27] and quaternary ammonium salts [28] have also promoted this reaction. However, these methods have many disadvantages. They require long reaction times, moisture sensitive toxic catalysts, require stoichiometric amounts of catalysts, offer poor product yields and generate large amounts of waste.

In recent years, green chemical synthesis has received extensive attention [29–31]. In this context, heterogeneous catalysis has emerged as a useful tool. Since it drives the reaction in eco-friendly conditions, it produces relatively pure products without waste. In this connection, chitosan, which is a biodegradable, optically active polymer with a strong affinity for transition metals has been used as a solid support in the form of colloids, flakes, gel beads, fibers or as an immobilized form on inorganic material supports (like, alumina, silica, or other metal oxides) [32]. The ease with which it can be modified physically and chemically, opens up avenues for manufacturing a wide range of catalysts from it for applications in the fields of hydrogenation, oxidation, and fine chemical synthesis.



(1) (2) (3) (4) (5)

Fig. 1 CF_3 containing drugs

Therefore, in the present work we report green one-pot synthesis of a new series of α-aminophosphonates (**4a-o**) containing a trifluoromethyl aniline moiety with the hope of developing new anticancer agents. Their synthesis was accomplished by reacting different aldehydes, dialkyl phosphites under microwave irradiation conditions using chitosan as an efficient catalyst. The newly synthesized compounds were screened for their in vitro anticancer activities against PC-3(prostate cancer), MCF-7(breast cancer), HeLa(cervix cancer), U-973, K-562 and HL-60 (leukemia) cell lines.

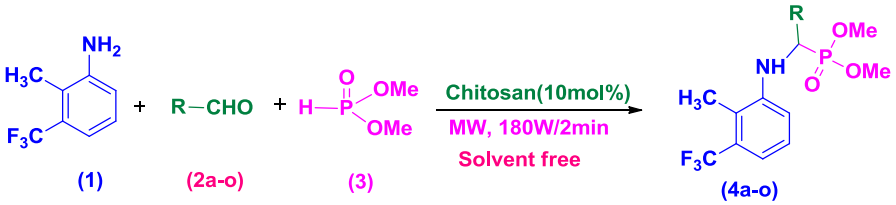
Results and discussion

Chemistry

We reinvestigated the one-pot Kabachnik–Fields reaction of 2-Methyl-3-(trifluoromethyl) aniline (**1**), Aryl/heteroaryl aldehydes (**2a–o**), and dimethylphosphite (**3a**) under different experimental conditions to obtain corresponding phosphonate (**4a–o**) (Scheme 1). First, the optimum experimental conditions for this reaction such as different heating techniques, with or without different solvents, catalysts, optimum time and temperature are determined (Table 1).

We have evaluated different common Lewis acids as catalysts, which promote this three-component Kabachnik–Fields reaction. Lewis acids such as $ZnCl_2$, Tween-20, $AlCl_3$, $Incl_3$, PTSA, PEG-SO_3H, TMG, $NanoTiO_2$ and Nano ZnO afforded the desired product but only in moderate yield (Table 1). However, with chitosan as catalyst, the reaction goes to completion rapidly and afforded the desired products in pure form with high yields.

The same reaction under neat conditions with conventional heating resulted in poor yields even after prolonged reaction time (Table 1, entry 1). The reaction in any solvent medium with conventional heating turned out to be ineffective as the product separation and purification was found to be difficult and have lower product yields that resulted even after over 4 h reaction time (Table 1, entries 3 and 4). The same reaction, when run by using Tween 20 and $AlCl_3$ as catalysts at 10 mol% concentration in different solvents and without solvent by both conventional heating and microwave irradiation (MWI), had the product yields improved, but the reaction times were long (Table 1, entries 5–8). The reaction was also tried with other $NbCl_5$ and $InCl_3$ catalysts under solvent-free conditions. The reaction went smoothly, but



Scheme 1 Synthesis of dimethyl (2-methyl-3-(trifluoromethyl)phenyl amino) (aryl/heteroaryl) methylphosphonates

Table 1 Optimization of reaction conditions for the synthesis of dimethyl (((2-methyl-3-(trifluoro methyl)phenyl)amino)(4-nitrophenyl)methyl)phosphonate (**4a**)

Entry	Catalyst (10 mol%)	Solvent	Conventional			Microwave		
			Temp. (°C)	Time min	Yield[b] (%)	Temp. (°C)	Time min	Yield[b] (%)
1.	–	–	120	300	52	70	15	75
2.	–	Toluene	110	360	70	60	12	75
3.	$ZnCl_2$	Acetonitrile	85	270	58	80	8	72
4.	$ZnCl_2$	–	85	240	60	70	5	67
5.	Tween-20	Ethanol	75	240	64	70	8	72
6.	Tween-20	–	75	210	68	60	4	74
7.	$AlCl_3$	THF	70	310	65	50	5	75
8.	$AlCl_3$	–	70	300	70	60	6	78
9.	$NbCl_5$	–	75	210	70	60	3	85
10.	$InCl_3$	–	75	180	75	60	2	93
11.	PTSA	–	75	180	72	60	2	90
12.	PEG-SO_3H	–	75	210	80	60	3	94
13.	TMG	–	75	240	78	60	2	90
14.	NanoTiO_2	–	75	180	82	60	2	93
15.	NanoZnO	–	75	180	76	60	3	89
16.	Chitosan (10 mol %)	–	75	180	85	60	2	98
17.	Chitosan (5 mol %)	–	75	180	82	60	3	96
18.	Chitosan (15 mol %)	–	75	180	80	60	2	95
19.	Chitosan (20 mol %)	–	75	180	80	60	2	95

[a] Reaction conditions: 2-methyl-3-(trifluoromethyl)aniline (**1**), 4-Nitrobenzaldehyde (**2a**) and dimethyl phosphite (**3**) in 1:1:1 ratio. All the microwave reactions run at 180 W

[b] Isolated yield

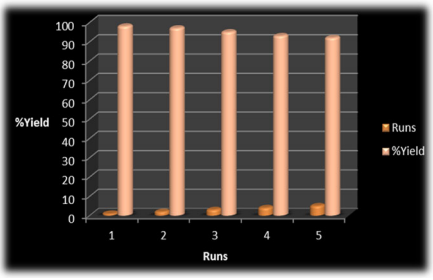
results were not better (Table 1, entries 9 and 10). The reported advantages of catalysts such as PTSA, PEG-SO_3H, TMG, Nano TiO_2 and Nano ZnO were not found (Table 1, entries 11–15). But almost quantitative yields were obtained with chitosan as catalyst (Table 1, entry 16). In addition, we found that the MWI of substrates with chitosan catalyst at 60 °C for 2 min without solvent was the optimum condition for the formation of compound **4a** in high yield. The quantity of the catalyst required for this reaction was optimized by studying the effect of different amounts of catalyst (Table 1, entries 17–19). We found that 10 mol% of chitosan was sufficient to drive the reaction to completion in 2 min, giving 98% product yield. Less amounts of catalyst gave lower yields even after prolonged reaction time and higher mol% quantities could not increase the product yield and decreased the reaction time. The reusability of the chitosan catalyst was also

examined for this reaction. After each run, the catalyst was filtered, washed with petroleum ether and reused. In each subsequent reuse over five cycles of the chitosan catalyst, the yield of α-aminophosphonate (**4a**) was 98, 97 95, 93, and 92%, respectively, and the results are summarized in Fig. 2. These results indicate that the chitosan catalyst is a reusable one.

It is observed that the Kabachnik–Fields reaction is sensitive to temperature. Even though high wattage of microwaves enhance the reaction rate, they trigges side reactions and decompose the formed products to the corresponding aldehyde and amine. Thus, it was found that irradiation of reaction substrates with 180 W of microwaves for 2 min is sufficient to drive the reaction to completion. The generality and scope of this method were examined. Several substituted aryl/heteroaryl aldehydes, 2-methyl-3-(trifluoromethyl) aniline, and dimethyl phosphite using chitosan under neat conditions offered the desired products (Table 1). In all cases, aromatic aldehydes substituted with either electron-donating or electron-withdrawing groups reacted smoothly and afforded corresponding products in good yields. However, the aldehydes bearing electron-withdrawing groups required shorter reaction time and gave higher yields (Table 2).

2-Methyl-3-(trifluoromethyl)aniline (**1**) reacted with 4-Nitrobenzaldehydes (**2a**) and dimethylphosphite (**3**) in the presence of 10 mol% of chitosan catalyst under microwave irradiation at 180 W for 2 min. The progress of the reaction was monitored by thin layer chromatography. The reaction proceeded smoothly and was completed in 2 min to afford the corresponding α-aminophosphonates in high yield (98%). This showed that chitosan acts as an effective catalyst in this reaction. A probable reaction mechanism for the three-component reaction of thev benzaldehyde, amine and dimethylphosphite scaffold catalyzed by chitosan catalyst is shown in Scheme 2. The free amino group in chitosan distributed on the surface of chitosan activates the carbonyl group of benzaldehyde through nucleophilic attack to produce the corresponding intermediate. Further, amine reacts with this intermediate and produces imine, then chitosan catalyst may be free, which again participates in the mechanism, then imine reacts with dimethylphosphite to afford the target product. An important feature is that the chitosan can be easily recovered from the reaction mixture after its completion and can be reused.

The chemical structures of all the new compounds were confirmed by, IR, ^{1}H-, ^{13}C- and ^{31}P-, ^{19}F-NMR and Mass spectra. Compounds (**4a–o)** exhibited



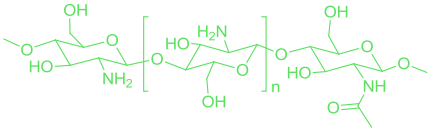
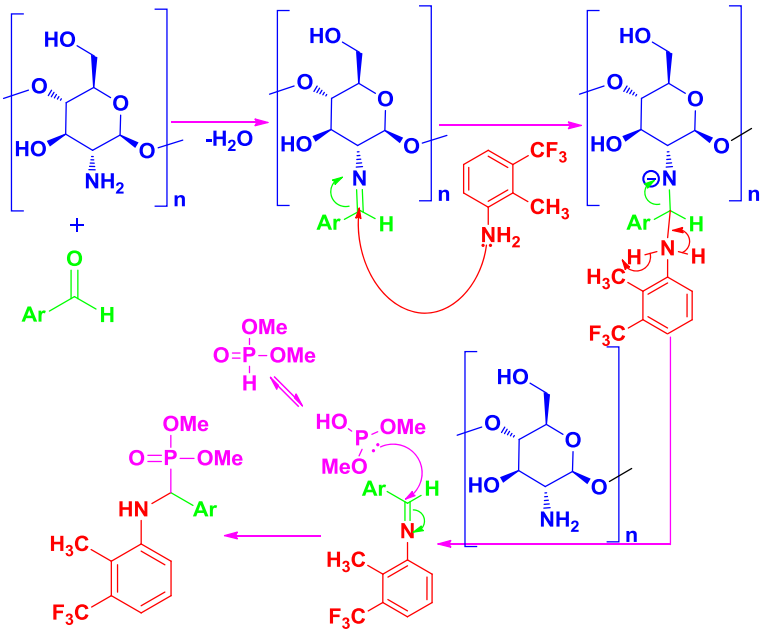


Fig. 2 Recyclability and structure of chitosan catalyst

Table 2 Synthesis of α-aminophosphonates (**4a–o**) on Chitosan catalyst by MWI

Entry	Product	R	Time (min)	Yield (%)
1	**4a**	4-$NO_2C_6H_4$	2	98
2	**4b**	4-Cl C_6H_4	3	96
3	**4c**	3-F C_6H_4	5	97
4	**4d**	2-SH C_6H_4	2	95
5	**4e**	3,4-OMe C_6H_3	4	93
6	**4f**	3,4-dioxol C_6H_3	3	94
7	**4g**	2-SH,5-NO_2 C_6H_3	2	96
8	**4h**	6-Me,1-py C_5H_4	6	91
9	**4i**	$C_5H_{10}N$	3	90
10	**4j**	4-N,N-CH_3 C_6H_4	2	95
11	**4k**	$C_{16}H_9$	2	97
12	**4l**	4-OH C_6H_4	4	94
13	**4m**	C_8H_6N	3	95
14	**4n**	4-OMe C_6H_4	2	92
15	**4o**	3-NO_2 C_6H_4	2	96



Scheme 2 Probable mechanistic pathway by chitosan catalyst

characteristic IR stretching frequencies in the regions 3320–3490, 1210–1250, 980–1030 cm^{-1} for N–H, P=O, and C–F, respectively. The aromatic protons of the benzene rings of the α-aminophosphonates **(4a–o)** showed a complex multiplet at δ 6.81–8.36. The P–C–H proton signal appeared as a doublet at δ 5.04–5.89 due to its

coupling with both phosphorus and the N–H proton. The N–H proton signal appeared at δ 5.70–5.99 as a singlet. The methoxy group protons of the dimethylphosphite moiety resonated as two distinct doublets in the range of δ 3.54–3.57 and δ 3.73–3.76, indicating their non-equivalence. The carbon chemical shifts for aliphatic and aromatic carbons in the title compounds were observed at δ 13.0–160.0, respectively. The ^{31}P-NMR chemical shifts appeared in the region δ 20.30–27.80 and ^{19}F-NMR chemical shifts appeared in the region δ −59.13 to −59.93 for these compounds.

Pharmacology

Molecular docking studies

The docking conformations of compounds in topoisomerase-II are shown in Fig. 3. Among the different docking conformations of compounds, the compounds **4a**, **4b**, **4g**, **4k** and **4n** are shown as the highest docking scores −9.8, −9.0, −9.9, −9.8 and −9.4, respectively, due to the fluorine interaction, and π-Alkyl interactions in addition to conventional hydrogen bonding interactions with active pocket of topoisomerase-II enzyme. These compounds have the highest docking scores when compared to standard anticancer drug adriamycin, which has a docking score of −9.6 only. Adriamycin has only π-Alkyl interactions and conventional hydrogen bonding interactions with an active pocket of topoisomerase-II enzyme. Among the test compounds, **4a**, **4g** and **4k** have higher docking scores than the standard adriamycin because of stronger molecular interactions with topoisomerase-II enzyme and have less docking scores. The types of molecular interactions, interacting atoms of proteins, ligands, and docking scores are shown in Table 3.

Fluorine in α-aminophosphonate compounds (**4a–o**) was actively binding to topoisomerase protein by the conventional hydrogen bonding and halogen interactions. They were also showing π-alkyl and conventional hydrogen bonding interactions. Because of these molecular interactions of these compounds (**4a–o**) were strongly inhibiting the topoisomerase-II enzyme of cancer cells.

Cytotoxicity

The cytotoxicity of **4a–o** was evaluated in vitro against U-973, K-562 & HL-60 (human leukaemia), PC-3(prostate cancer), MCF-7(breast cancer), HeLa(cervical cancer) cells after 24 h exposure and their GI_{50} values were determined from a graph of cell capability measured over a range of concentrations between 0.1 mM–0.1 μM. Each data point was the average of four determinations that in all cases differed by 10–20% or less. These results are summarized in Table 4. Initially, the GI_{50} was determined by a broad range of concentrations, specifically, 0.1, 1, 10 and 0.1 mM of the title compounds against the cancer cell lines. From the data it is revealed that all compounds exhibited a different range of significant cytotoxic activities varying from 0.1 mM–0.1 μM due to structural differences. As evident from the cytotoxicity data, compound **4k** with a pyrene moiety at the α-carbon atom

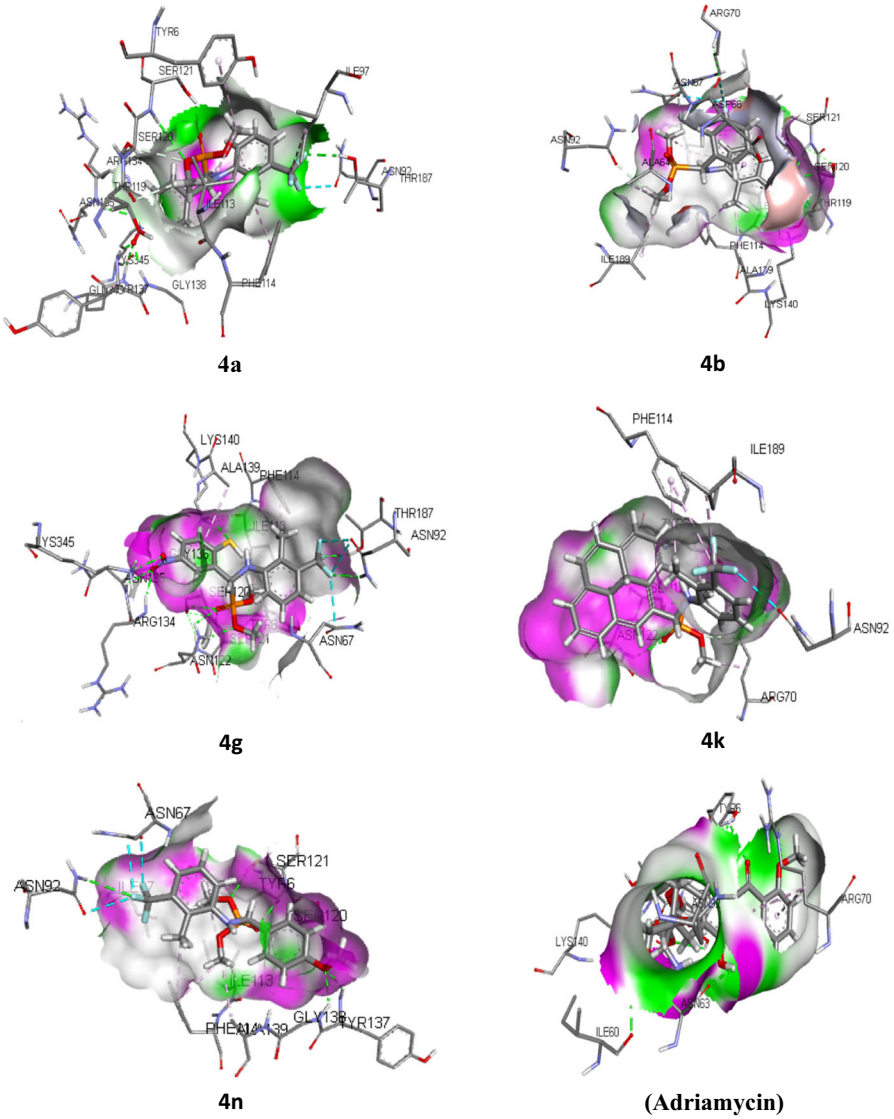


Fig. 3 Docking interactions of active cytotoxic compounds of α-aminophosphonates **4a, 4b, 4g, 4k** and **4n**

showed the highest activity against the MCF-7 breast cancer cell lines at the concentration of 0.1 mM, and it has shown a very low GI_{50} value (-136.50 ± 5.90) when compared to positive control drug Adriamycin (-121.2 ± 1.68). Similarly, the same compound **4k** exhibit high cytotoxicity against HeLa, PC-3, U937 & K562 cell lines at the concentration of 0.1 mM and their GI_{50} values -119.0 ± 7.34, -96.9 ± 1.03, -52.4 ± 13.63 and 71.5 ± 10.44, respectively. The compounds **4a**, **4b**, **4g** and **4n** were shown to have significant cytotoxicity activity against cancer

Table 3 Docking results of active cytotoxicity compounds

Compound	Amino acids interacted with ligands	Docking score
4a	ASN67, SER121, ASN122, SER120, LYS345, ASN135, ARG134, TYR137, GLY138, GLN343	−9.8
4b	ILE113, THR119, ILE97, LYS140, SER120, SER121	−9.0
4g	ASN67, ASN92, ARG134, ASN135, GLY136, LYS345, LYS140, SER120, SER121, ASN122, ASN92, THR187, TYR6, ILE113	−9.9
4k	ASN92, PHE114, ASN122, SER121, ILE189, TYR6	−9.8
4n	ASN67, ASN92, PHE114, TRY6, ASN92, SER121, TYR137, GLY138	−9.4
Adriamycin	ARG70, SER120, THR119, ILE60, ASN63, TYR6, ASN63	−9.6

cell lines at the concentration of 0.1 mM and which have low GI_{50} data in Table 4. There was moderate cytotoxicity at the concentration of 0.1 mM of **4c**, **4h** and **4i** compounds. The cytotoxicity activity of **4d**, **4e**, **4i**, **4j**, **4l**, **4m** and **4o** showed a low percentage of inhibition of growth of cancer cell lines at all the concentrations and higher GI_{50} values when compared to the **4k** compound. The results of percentage of growth inhibition (GI_{50}) of compounds when compared with the positive control drug have shown significant cytotoxicity against cancer cell lines. The data of GI_{50} values of compounds and Adriamycin are shown in Table 4. The percentage inhibition of growth in human leukemia cells (K-562), human prostate cancer (PC-3), human breast cancer cells (MCF-7) and human cervix cancer (HeLa) cells following treatment with the title compounds (**4a–o**). They were measured at 0.1 mM and 0.1 μM concentrations. They were the mean values determined from three independent experiments run in triplicate. This study thus discovered a new family of dimethyl (2-methyl-3-(trifluoromethyl) phenyl amino) (aryl/heteroaryl) methyl phosphonate (**4a–o**) that have significant target specific cytotoxicity on some cancer cells (Table 4).

Experimental

Analysis and instruments

Reagents were purchased from common commercial sources. All solvents were purified and dried by standard procedures. All the reactions were monitored by thin-layer chromatography (TLC) on silica gel GF254 plates from Qingdao Haiyang Chemical Co. Ltd (China), visualized in an iodine chamber or with an UV lamp (254 nm). Column chromatography was performed using silica gel (100–200 mesh) purchased from Qingdao Haiyang Chemical Co.Ltd (China). The melting points of the products were determined on a Guna Digital melting point apparatus (China) and are uncorrected. The IR spectra were recorded on a Bruker Alpha ECO-ATR FTIR (Attenuated total reflection–Fourier transform infrared) interferometer with a single reflection sampling module equipped with ZnSe crystal. Elemental analysis

Table 4 Cytotoxic activity of α–aminophosphonates(**4a–o**)

Compound	$GI_{50}(\mu M)$[a]						
	R	PC-3	MCF-7	HeLa	U-973	K-562	HL-60
4a	4-NO_2	75.1 ± 2.64	81.8 ± 1.65	62.1 ± 3.20	69.6 ± 7.91	71.6 ± 8.23	84.1 ± 16.29
4b	4-Cl	66.2 ± 2.35	83.3 ± 2.45	67.4 ± 34.22	59.4 ± 9.72	75.7 ± 5.53	94.8 ± 13.39
4c	3-F	74.4 ± 4.37	77.8 ± 3.12	171.2 ± 3.85	164.4 ± 1.72	97.5 ± 2.47	72.2 ± 16.62
4d	2-SH	122.8 ± 1.96	63.1 ± 5.29	125.2 ± 6.08	135.6 ± 6.74	95.6 ± 15.34	71.6 ± 12.42
4e	3,4-OMe	98.6 ± 3.01	68.8 ± 3.39	135.8 ± 2.92	159.2 ± 3.97	102.4 ± 5.95	108.4 ± 5.90
4f	1,3-Dioxolane	69.9 ± 3.22	79.5 ± 5.15	189.6 ± 1.64	166.4 ± 10.07	79.8 ± 16.69	117.6 ± 12.54
4g	2-SH 5-NO_2	30.4 ± 4.22	27.6 ± 4.82	43.5 ± 3.580	41.4 ± 7.31	63.1 ± 8.546	36.9 ± 11.90
4h	6-Me 2-Py	145.4 ± 4.09	75.1 ± 2.94	111.2 ± 6.78	87.8 ± 2.75	145.4 ± 11.27	69.7 ± 06.88
4i	1-piperidine	153.2 ± 2.005	102.0 ± 8.78	182.6 ± 6.59	166.8 ± 1.17	153.2 ± 5.62	138.8 ± 12.56
4j	4-$N(Me)_2$	159.8 ± 2.15	106.2 ± 5.77	184.8 ± 7.24	157.8 ± 6.22	159.8 ± 6.86	146.0 ± 14.34
4k	pyrane	−96.9 ± 1.03	−136.4 ± 5.90	−119.0 ± 7.34	−52.4 ± 13.63	71.5 ± 10.44	49.2 ± 11.40
4l	4-OH	126.0 ± 6.79	78.6 ± 4.76	134.6 ± 8.65	100.6 ± 4.79	126.0 ± 14.52	77.8 ± 13.24
4m	Benz imidazole	134.0 ± 6.76	93.2 ± 3.48	166.0 ± 8.26	142.4 ± 9.60	134.2 ± 2.97	124.0 ± 09.37
4n	4-OMe	59.0 ± 7.21	90.3 ± 4.65	59.0 ± 3.91	73.5 ± 8.10	69.4 ± 7.54	56.6 ± 04.01
4o	3-NO_2	134.2 ± 2.60	80.5 ± 2.33	129.8 ± 3.43	126.0 ± 1.25	134.2 ± 14.42	100.0 ± 08.23
ADR	–	−120.4 ± 1.32	−121.2 ± 1.68	−129.0 ± 0.47	−62.6 ± 3.48	−40.2 ± 2.79	40.4 ± 03.52

[a] Each data represents mean ± S.D. from three different experiments performed in triplicate

was performed on an ElementarVario-III CHN analyzer. NMR spectra were recorded on a Bruker Alpha instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C, 125 MHz for ^{31}P, and 470 MHz for ^{19}F) using $CDCl_3$ and DMSO d_6 as solvent. TMS ($\delta = 0$) served as an internal standard for ^{1}H-NMR, $CDCl_3$ ($\delta = 77.0$) was used as an internal standard for ^{13}C-NMR, H_3PO_4 ($\delta = 0$) was used as an external standard for ^{31}PNMR, CF_3COOD was used as an external standard ($\delta = -76.5$) for ^{19}F-NMR. Mass spectra were recorded on a LC–MS/MS-TOF API QSTAR PULSAR spectrometer, samples were introduced by the infusion method using the Electrospray Ionization Technique (ESI). All other chemicals were of analytical grade.

General procedure for synthesis of α-aminophosphonates (4a–o)

An equimolar mixture of 2-methyl-3-(trifluoromethyl)aniline (0.351 g, 0.002 mol), corresponding aldehyde (0.002 mol), dimethyl phosphite (0.18 ml, 0.002 mol) and chitosan catalyst(10 mol%) were taken in a reaction glass tube, degassed for 10 min and microwave irradiated at 180 W for 2 min at 60 °C. The progress of the reaction was monitored by TLC using petroleum ether and ethylacetate (3:7) as solvent. After completion of the reaction, the mixture was diluted with ethyl acetate, washed with water (2 × 15 ml) followed by brine (1 × 10 ml), dried over Na_2SO_4 and evaporated to dryness. The crude mass was purified by column chromatography on silicagel (100–200 mesh) by using a 7:3 mixture of ethylacetate in hexane to afford the pure α-aminophosphonates.

*Dimethyl (2-methyl-3-(trifluoromethyl) phenylamino)(4-nitrophenyl)methylphosphonate (**4a**)* Yellow solid; Yield: 98%. M.p.132–134 °C. IR (cm^{-1}): *ν* 3420 (NH), 2851 (C–H), 1000 (C–F), 1232 (P=O).^{1}H-NMR (DMSO-d_6): *δ* 8.23(d, $J = 8.8$ Hz, 2H, Ar–H), 7.86–7.89 (m, 2H, Ar–H), 7.11 (t, $J = 16.0$ Hz, 1H, Ar–H), 7.00(d, $J = 8.0$ Hz, 1H, Ar–H), 6.81 (d, $J = 8.0$ Hz, 1H, Ar–H); 5.92 (s, 1H, NH), 5.58 (d, $J = 23.2$ Hz, 1H, P–C–H), 3.76 (d, $J = 10.8$ Hz, 3H, P–OCH_3), 3.57 (d, $J = 10.4$ Hz, 3H, P–OCH_3), 2.35 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): *δ* 147.82(C_6), 143.90(C_{15}), 143.06(C_{11}), 128.47(C_{17} & C_{13}), 126.50(C_4), 123.61(C_{14} & C_{16}), 121.36(C_{18}), 116.21(C_1), 114.54(C_5), 56.44(C_9), 54.77(C_{23}), 53.94(C_{25}), 12.32(C_7). ^{31}P-NMR ($CDCl_3$): *δ* 25.24. ^{19}F-NMR ($CDCl_3$): *δ* −59.86. MS (ESI): *m/z* 419$[M + H]^+$, 441$[M + Na]^+$ Anal.Calcd.for $C_{17}H_{18}F_3N_2O_5P$: C, 48.81; H, 4.34; N, 6.70. Found C, 48.03; H, 4.74; N, 6.48.

*Dimethyl (4-chlorophenyl)(2-methyl-3-(trifluoromethyl)phenylamino)methylphosphonate (**4b**)* White solid; Yield: 96%. M.p. 125–127 °C. IR (cm^{-1}): *ν* 3415 (NH), 2845 (C–H), 1007 (C–F), 1228 (P=O).^{1}H-NMR (DMSO-d_6): *δ* 7.69 (d, $J = 8.6$ Hz, 2H, Ar–H), 7.29–7.31 (m, 2H, Ar–H), 7.09 (t, $J = 7.8$ Hz, 1H, Ar–H), 6.95 (d, $J = 8.0$ Hz, 1H, Ar–H), 6.81 (d, $J = 8.2$ Hz, 1H, Ar–H); 5.90 (s, 1H, NH), 5.53 (d, $J = 22.6$ Hz, 1H, P–C–H), 3.73(d, 3H, $J = 10.2$ Hz, OCH_3), 3.54 (d, $J = 9.8$ Hz, 3H, P–O–CH_3), 2.35 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): *δ* 147.80(C_6), 136.20(C_{11}), 135.40(C_{15}), 129.03($C_{14\ \&\ 16}$), 128.70(C_2), 127.32($C_{13\&17}$), 124.86(C_4), 123.70(C_{18}), 116.05(C_1), 114.50(C_3), 56.73(C_9), 54.93(C_{23}),

53.98(C_{25}), 13.40(C_7). ^{31}P-NMR($CDCl_3$): δ 25.16. ^{19}F-NMR ($CDCl_3$): δ −59.56. MS (ESI): m/z (%) 408[M + H]$^+$, 430[M + Na]$^+$. Anal.Calcd.for $C_{17}H_{18}ClF_3NO_3P$: C, 50.08; H, 4.45; N, 3.44. Found C, 50.12; H, 4.41; N, 3.47.

*Dimethyl (3-fluorophenyl) (2-methyl-3-(trifluoromethyl) phenylamino) methylphosphonate (**4c**)* Orange solid; Yield: 97%. M.p.128–130 °C. IR (cm^{-1}): 3426 (NH), 2841 (C–H), 1003 (C–F), 1230 (P=O). ^{1}H-NMR (DMSO-d6): δ 7.33(m, 1H, Ar–H), 7.09(d, J = 7.70 Hz, 1H, Ar–H), 6.97 (m, 2H,Ar–H), 6.82 (m, 2H, Ar–H), 6.77 (s, 1H, Ar–H), 6.65 (d, J = 8.0 Hz, 1H, Ar–H); 5.94 (s, 1H, NH), 5.55 (d, J = 23.4 Hz, 1H, P–C–H), 3.77(d, J = 10.6 Hz,3H, OCH_3), 3.52 (d, J = 9.6 Hz, 3H, P–O–CH_3), 2.37 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ 163.05(C_{16}), 145.89(C_6), 137.20(C_{11}), 129.80(C_{14}), 128.56(C_2), 126.78(C_4), 123.70(C_{18}), 115.97(C_1), 114.45(C_5), 113.89(C_{17}), 56.93(C_9), 54.39(C_{23}), 53.86(C_{25}), 12.43(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.13. ^{19}F-NMR ($CDCl_3$): δ −59.93. MS (ESI): *m/z* (%) 392[M + H]$^+$, 414[M + Na]$^+$. Anal. Calcd. for $C_{17}H_{18}F_4NO_3P$: C, 52.18; H, 4.64; N, 3.58. Found C, 52.14; H, 4.59; N, 3.62.

*Dimethyl((2-mercaptophenyl)((2-methyl-3-(trifluoromethyl)phenyl)amino)methyl) phosphonate (**4d**)* Yellow solid: Yield: 95%. M.p.136–138 °C. IR (cm^{-1}): *v* 3445 (NH), 2874 (C–H), 1009 (C–F), 1228 (P=O).^{1}H-NMR (DMSO-d_6): δ 7.65(d, J = 8.2 Hz, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.47(d, J = 7.8 Hz,1H, Ar–H), 7.45(d J = 7.2 Hz, 1H, Ar–H), 7.28 (d, J = 7.0 Hz, 1H, Ar–H), 7.18 (d, J = 7.3 Hz, 1H, Ar–H), 7.01 (d, J = 7.0 Hz, 1H, Ar–H),; 5.90 (s, 1H, NH), 5.15 (d, J = 22.4 Hz, 1H, P–C–H),3.75(d, J = 10.4 Hz, 3H, OCH_3), 3.68 (d, J = 10.6 Hz, 3H, P–O–CH_3), 3.59 (s, 1H, SH), 3.17 (s, 1H, SH), 2.27 (s, 3H, CH_3).^{13}C-NMR ($CDCl_3$): δ 145.06(C_6), 144.93(C_{11}), 138.87(C_{17}), 130.06(C_{16}), 129.78(C_2), 128.73(C_{13}), 127.41(C_{15}), 126.51(C_{14}), 126.01(C_{19}), 123.28(C_1), 121.73 116.30(C_5), 77.45(C_9), 54.34(C_{24}), 52.63(C_{26}), 12.92(C_7).^{31}P-NMR ($CDCl_3$): δ 25.28. ^{19}F-NMR ($CDCl_3$): δ −59.73. MS (ESI): *m/z* (%) 406[M + H]$^+$, 428[M + Na]$^+$. Anal.Calcd.for $C_{17}H_{19}F_3NO_3PS$: C, 50.37; H, 4.72; N, 3.46. Found C, 50.98; H, 4.38; N, 3.06.

*Dimethyl ((3,4-dimethoxyphenyl)((2-methyl-3-(trifluoromethyl)phenyl)amino)methyl) phosphonate (**4e**)* Orange solid: Yield: 93%. M.p.133–135 °C. IR (cm^{-1}): *v* 3415 (NH), 2834 (C–H), 1003 (C–F), 1220 (P=O). ^{1}H-NMR (DMSO-d_6): δ 7.54(s, 1H, Ar–H), 7.22 (d, J = 7.7 Hz, 2H, Ar–H), 7.13(t, J = 6.8 Hz, 1H, Ar–H), 7.09(d, J = 8.2 Hz, 1H, Ar–H), 6.98 (d, J = 8.0 Hz, 1H, Ar–H), 6.92 (d, J = 7.8 Hz, 1H, Ar–H); 5.94 (s, 1H, NH), 5.17 (d, J = 9.6 Hz, 1H, P–C–H),3.75(d, J = 8.6 Hz, 6H, OCH_3),3.72(d, J = 9.6 Hz, 3H, OCH_3), 3.49 (d, J = 9.1 Hz, 3H, OCH_3), 2.32 (s, 3H, CH_3).^{13}C-NMR ($CDCl_3$): δ 149.39(C_{16}), 149.09(C_{15}), 145.36(C_6), 129.90(C_{11}), 128.74(C_2), 127.24(C_4), 126.42(C_{19}), 123.30(C_{13}), 121.25(C_{17}), 119.97(C_1), 115.72(C_3), 114.86(C_5), 111.36(C_{14}), 77.46(C_9), 56.36(C_{28}), 55.83(C_{30}), 54.84(C_{26}), 53.92(C_{24}), 12.97(C_7). ^{31}P-NMR ($CDCl_3$): δ 24.89.^{19}F-NMR ($CDCl_3$): δ −59.31. MS (ESI): m/z (%) 434[M + H]$^+$, 456[M + Na]$^+$. Anal. Calcd.for $C_{19}H_{23}F_3NO_5P$: C, 52.66; H, 5.35; N, 3.23. Found C, 52.64; H, 5.43; N, 3.20.

*Dimethyl(benzo[d] [1, 3] dioxol-5-yl((2-methyl-3-(trifluoromethyl)phenyl)amino) methyl)phosphonate (**4f**)* Red solid: Yield: 94%. M.p.110–112 °C. IR (cm^{-1}): *v*

3441 (NH), 2878 (C–H), 1003 (C–F), 1232 (P=O); ^{1}H-NMR (DMSO-d_6): δ 6.97(t, $J = 8.2$ Hz, 1H, Ar–H), 6.91 (s, 1H, Ar–H), 6.82(d, $J = 7.2$ Hz, 1H, Ar–H), 6.76(d, $J = 6.8$ Hz, 1H, Ar–H), 6.67 (d, $J = 7.4$ Hz, 1H, Ar–H),6.01 (s, 2H, CH_2),; 5.91 (s, 1H, NH), 5.13 (d, $J = 22.6$ Hz, 1H, P–C–H), 3.71(d, 3H, $J = 10.6$ Hz, OCH_3),3.63(d, $J = 10.2$ Hz, 3H, OCH_3), 2.29 (s, 3H, CH_3).^{13}C-NMR ($CDCl_3$): δ 148.60(C_{14}), 146.28($C_{15\&6}$), 129.94(C_{11}), 128.40(C_2), 127.20(C_4), 123.78(C_{21}), 120.74(C_{17}), 116.87(C_1), 114.30(C_3), 113.89(C_5), 112.80($C_{13\&16}$), 102.70(C_{19}), 69.52(C_9), 52.98(C_{26}), 52.83(C_{28}), 12.34(C_7). ^{31}P-NMR ($CDCl_3$): δ 26.32. ^{19}F-NMR ($CDCl_3$): δ −59.03. MS (ESI): *m/z* (%) 418[M + H]$^+$, 440[M + Na]$^+$. Anal. Calcd. for $C_{18}H_{19}F_3NO_5P$: C, 51.81; H, 4.59; N, 3.36. Found C, 51.36; H, 4.83; N, 3.09.

*Dimethyl((2-mercapto-5-nitrophenyl)((2-methyl-3(trifluoromethyl)phenyl)amino) methyl) phosphonate (**4g**)* Orange solid: Yield: 96%. M.p.141–143 °C. IR (cm^{-1}): *v* max 3398 (NH), 2842 (C–H), 1002 (C–F), 1226 (P=O). ^{1}H-NMR (DMSO-d_6): δ 8.03(s, 1H, Ar–H), 7.98 (d, $J = 7.7$ Hz, 1H, Ar–H), 7.59(d, $J = 7.2$ Hz, 1H, Ar–H), 6.97(t, $J = 8.0$ Hz, 1H, Ar–H), 6.89 (d, $J = 7.8$ Hz, 1H, Ar–H), 6.68 (d, $J = 6.8$ Hz, 1H, Ar–H); 5.95 (s, 1H, NH), 5.53(d, $J = 23.4$ Hz, 1H, P–C–H), 3.73(d, $J = 10.6$ Hz, 3H, P–OCH_3), 3.54(d, $J = 9.8$ Hz, 3H, P–OCH_3), 2.25 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ 146.78(C_6), 144.40(C_{14}), 137.90(C_{17}), 132.70(C_{16}), 127.30(C_2), 125.90(C_4), 123.40(C_{18}), 121.80(C_{15}), 118.06(C_1), 113.60(C_5), 63.08(C_9), 53.90(C_{23}), 53.80(C_{25}), 12.90(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.31. ^{19}F-NMR ($CDCl_3$): δ −59.76. MS (ESI): *m/z* (%) 451[M + H]$^+$, 473[M + Na]$^+$. Anal.Calcd.for $C_{17}H_{18}F_3N_2O_5PS$: C, 45.34; H, 4.03; N, 6.22. Found C, 45.81; H, 4.34; N, 6.07.

*Dimethyl(((2-methyl-3-(trifluoromethyl)phenyl)amino)(6-methylpyridin-2-yl)methyl) phosphonate (**4h**)* Brown solid: Yield: 91%. M.p. 136–138 °C. IR (cm^{-1}): *v* 3423 (NH), 2864 (C–H), 1006 (C–F), 1218 (P=O) ^{1}H-NMR (DMSO-d_6): δ 7.68(d, $J = 7.8$ Hz, 2H, Ar–H), 7.45 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.67(m, 3H, Ar–H), 7.08(t, $J = 7.4$ Hz, 1H, Ar–H), 6.82 (d, $J = 7.2$ Hz, 2H, Ar–H), 6.70 (d, $J = 7.6$ Hz, 2H, Ar–H); 5.81 (s, 1H, NH), 5.48 (d, $J = 22.8$ Hz, 1H, P–C–H), 3.73(d, $J = 9.8$ Hz, 3H, OCH3), 3.52(d, $J = 9.6$ Hz, 3H, P–O–CH3), 2.61 (s, 3H, CH3), 2.16 (s, 3H, CH3).^{13}C-NMR ($CDCl_3$): δ 160.52(C_{11}), 157.94(C_{16}), 147.80(C_6), 137.48(C_{14}), 129.20(C_2), 126.82(C_4), 123.40(C_{18}), 123.20($C_{15\&13}$), 115.91(C_1), 115.80(C_3), 113.89(C_5), 57.34(C_9), 54.80(C_{23}), 54.45(C_{25}), 24.90(C_{26}), 13.70(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.68. ^{19}F-NMR ($CDCl_3$): δ −59.81. MS (ESI): *m/z* (%) 389[M + H]$^+$, 411[M + Na]$^+$. Anal.Calcd.for$C_{17}H_{20}F_3N_2O_3P$: C, 52.58; H, 5.19; N, 7.21. Found C, 52.17; H, 5.38; N, 7.29.

*Dimethyl(((2-methyl–3-(trifluoromethyl)phenyl)amino)(piperidin-1-yl)methyl)phosphonate (**4i**)* Orange solid: Yield: 90%. M.p. 118–116 °C.IR (cm^{-1}): *v* 3436 (NH), 2834 (C–H), 1012 (C–F), 1232 (P=O); ^{1}H-NMR (DMSO-d_6): δ 7.12(t, $J = 8.0$ Hz, 1H, Ar–H), 7.05 (d, $J = 7.7$ Hz, 1H, Ar–H), 7.68(d, 1H, $J = 7.2$ Hz, Ar–H); 5.79 (s, 1H, NH), 5.43 (d, $J = 21.8$ Hz, 1H, P–C–H), 3.72(d, 3H, $J = 10.4$ Hz, POCH3), 3.52 (d, $J = 10.6$ Hz, 3H, P–O–CH3), 2.53 (m, 4H, CH_2), 2.19 (s, 3H, CH_3), 1.65 (m, 2H, CH_2), 1.56 (m, 4H, CH_2).^{13}C-NMR ($CDCl_3$): δ 146.98(C_6), 128.80(C_2),

127.08(C_4), 124.45(C_{13}), 116.17(C_1), 114.30(C_3), 94.34(C_9), 54.80(C_{25}), 53.84(C_{21}), 53.70($C_{20\&18}$), 26.40(C_{24}), 25.94(C_{22}), 25.60(C_{23}), 13.70(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.02. ^{19}F-NMR ($CDCl_3$): δ −59.43. MS (ESI): *m/z* (%) 381$[M + H]^+$ 403$[M + Na]^+$. Anal.Calcd.for $C_{16}H_{24}F_3N_2O_3P$: C, 50.55; H, 6.36; N, 7.37. Found C, 50.46; H, 6.38; N, 7.29.

*Dimethyl((4-(dimethylamino)phenyl)((2-methyl-3-(trifluoromethyl)phenyl)amino) methyl) phosphonate (**4j**)* Orange solid: Yield: 95%. M.p. 126–128 °C. IR (cm^{-1}): *v* 3438 (NH), 2854 (C–H), 1004 (C–F), 1227 (P=O); ^{1}H-NMR (DMSO-d_6): δ 7.36(d, $J = 7.6$ Hz, 2H, Ar–H), 7.10 (d, $J = 8.7$ Hz, 2H, Ar–H), 6.94 (t, $J = 7.4$ Hz, 1H, Ar–H), 6.89(d, $J = 7.2$ Hz, 1H, Ar–H), 6.69 (d, $J = 6.8$ Hz, 1H, Ar–H); 5.84 (s, 1H, NH), 5.10 (d, $J = 22.8$ Hz, 1H, P–C–H), 3.77 (d, $J = 9.8$ Hz, 3H, P–OCH3), 3.69 (d, $J = 9.6$ Hz, 3H, P–OCH3), 2.90 (s, 6H, CH3), 2.29 (s, 3H, CH3).^{13}C-NMR ($CDCl_3$): δ 147.02(C_{22}), 143.78(C_6), 128.43(C_2), 126.05(C_4), 123.56(C_{25}), 121.26(C_{12}), 116.12(C_1), 114.43(C_3), 56.34(C_9), 54.76($C_{17\&19}$), 53.92($C_{27\&28}$), 41.33($C_{29\&30}$), 12.33(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.34. ^{19}F-NMR ($CDCl_3$): δ −59.78. MS (ESI): *m/z* (%) 417$[M + H]^+$, 439$[M + Na]^+$. Anal.Calcd.for $C_{19}H_{24}F_3N_2O_3P$: C, 54.81; H, 5.81; N, 6.73. Found C, 54.98; H, 5.83; N, 6.40.

*Dimethyl(2-methyl-3-(trifluoromethyl)phenylamino)(pyren-1-yl)methyl phosphonate (**4k**)* Brown solid: Yield: 97%. M.p. 138–140 °C.IR (cm^{-1}): *v* 3445 (NH), 2874 (C–H), 1009 (C–F), 1228 (P=O) ^{1}H-NMR (DMSO-d_6): δ 8.12(d, $J = 7.8$ Hz, 1H, Ar–H), 7.94 (d, $J = 7.0$ Hz, 1H, Ar–H), 7.89(m, 1H, Ar–H),7.82 (d, $J = 6.7$ Hz, 1H, Ar–H), 7.87 (m, $J = 7.2$ Hz, 4H, Ar–H), 7.78 (d, $J = 7.6$ Hz, 1H, Ar–H), 6.98(d, $J = 7.0$ Hz, 1H, Ar–H), 6.89 (d, $J = 6.6$ Hz, 1H, Ar–H), 6.73(d, $J = 6.8$ Hz, 1H, Ar–H),; 5.87 (s, 1H, NH), 5.57 (d, $J = 23.1$ Hz, 1H, P–C–H), 3.78(d, $J = 10.2$ Hz, 3H, OCH3), 3.59 (d, $J = 10.4$ Hz, 3H, P–O–CH3), 2.19 (t, 3H, CH3). ^{13}C-NMR ($CDCl_3$): δ 147.28(C_6), 140.52(C_{29}), 133.90(C_{30}), 134.45(C_{26}), 128.83($C_{2\&35}$), 126.84(C_4), 126.48(C_{21}), 126.28($C_{31,32\&34}$), 125.92($C_{23\&27}$), 124.94(C_{28}), 123.70(C_{24}), 123.48(C_{25}), 118.74(C_1), 115.87(C_3), 57.34(C_9), 53.48(C_{17}), 53.26(C_{19}), 12.84(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.28. ^{19}F-NMR ($CDCl_3$): δ −59.73. MS (ESI): *m/z* (%) 498$[M + H]^+$, 520$[M + Na]^+$. Anal.Calcd.for $C_{27}H_{23}F_3NO_3P$: C, 65.19; H, 4.66; N, 2.82. Found C, 64.98; H, 4.38; N, 2.90.

*Dimethyl((4-hydroxyphenyl)((2-methyl-3-(trifluoromethyl)phenyl)amino)methyl) phosphonate (**4l**)* White solid; Yield: 94%. M.p. 113–115 °C. IR (cm^{-1}): *v* 3418 (NH), 2839 (C–H), 1000 (C–F), 1223 (P=O).^{1}H-NMR (DMSO-d_6): δ 9.46 (s, 1H, Ar–H), 7.13 (d, $J = 7.70$ Hz, 2H, Ar–H), 7.09 (m, 1H, Ar–H), 6.89 (d, $J = 8.0$ Hz, 1H, Ar–H), 6.78 (d, $J = 7.8$ Hz, 1H, Ar–H), 6.63 (d, $J = 6.6$ Hz, 2H, Ar–H); 5.84 (s, 1H, NH), 5.53 (d, $J = 22.0$ Hz, 1H, P–C–H), 3.67 (d, $J = 10.2$ Hz, 3H, OCH_3), 3.54 (d, $J = 10.0$ Hz, 3H, P–O–CH_3), 2.31 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ 157.45(C_{22}), 144.85(C_6), 130.40(C_{24}), 129.40(C_{21}), 126.10(C_4), 123.90(C_2), 122.83(C_{12}), 116.80(C_1), 115.80(C_{23}), 115.70(C_{20}), 113.50(C_5), 61.24(C_9), 53.80(C_{17}), 53.70(C_{19}), 12.90(C_7). ^{31}P-NMR ($CDCl_3$): δ 26.12. ^{19}F-NMR ($CDCl_3$):

δ −59.18. MS (ESI): *m/z* (%) 390$[M + H]^+$, 412$[M + Na]^+$. Anal.Calcd.for $C_{17}H_{19}ClF_3NO_4P$: C, 52.08; H, 4.86; N, 3.64. Found C, 52.12; H, 4.81; N, 3.67.

*Dimethyl((1H-indol-3-yl)((2-methyl-3-(trifluoromethyl)phenyl)amino)methyl)phosphonate(**4m**)* Red solid: Yield: 95%. M.p.122–124 °C. IR (cm^{-1}): *v* 3446 (NH), 2883 (C–H), 1006 (C–F), 1236 (P=O) ^{1}H-NMR (DMSO-d_6): δ 10.91 (s, 1H, NH), 7.93(d, $J = 8.2$ Hz, 1H, Ar–H), 7.39(d, $J = 7.8$ Hz, 1H, Ar–H), 7.38 (s, 1H, CH), 7.19 (m, 2H, Ar–H), 7.12(t, $J = 7.2$ Hz,1H, Ar–H), 6.85(d, $J = 7.6$ Hz, 1H, Ar–H), 6.69 (d, $J = 7.4$ Hz, 1H, Ar–H), 5.89 (s, 1H, NH), 5.18 (d, $J = 21.4$ Hz, 1H, P–C–H), 3.74(d, $J = 10.3$ Hz, 3H, OCH_3), 3.64(d, $J = 10.6$ Hz, 3H, OCH_3), 2.31 (s, 3H, CH_3).^{13}C-NMR ($CDCl_3$): δ 146.82(C_6), 136.70(C_{29}),136(C_{25}), 128.52(C_2), 126.68($C_{21\&24}$), 125.70($C_{20\&23}$), 124.48(C_{30}), 122.83(C_{22}), 121.34($C_{32\&34}$), 119.98(C_{33}), 118.74(C_{26}), 116.42(C_1), 115.30(C_3), 114.09(C_5), 112.80(C_{31}), 68.52(C_9), 54.94(C_{17}), 54.60(C_{19}), 13.34(C_7). ^{31}P-NMR ($CDCl_3$): δ 25.92. ^{19}F-NMR ($CDCl_3$): δ −59.13. MS (ESI): m/z (%) 413$[M + H]^+$, 435$[M + Na]^+$. Anal.Calcd.for $C_{19}H_{20}F_3N_2O_3P$: C, 55.34; H, 4.89; N, 6.79. Found C, 55.36; H, 4.83; N, 6.90.

*Dimethyl((4-methoxyphenyl)((2-methyl-3-(trifluoromethyl)phenyl)amino)methyl) phosphonate (**4n**)* Orange solid: Yield: 92% M.p. 130–132 °C.IR (cm^{-1}): *v* 3412 (NH), 2823 (C–H), 1005 (C–F), 1213 (P=O). ^{1}H-NMR (DMSO-d_6): δ 7.22 (d, $J = 7.4$ Hz, 2H, Ar–H), 7.03(t, $J = 7.2$ Hz, 1H, Ar–H), 6.94(d, $J = 8.0$ Hz, 2H, Ar–H), 6.88 (d, $J = 7.8$ Hz, 1H, Ar–H), 6.76 (d, $J = 7.4$ Hz, 1H, Ar–H); 5.94 (s, 1H, NH), 5.19 (d, $J = 22.5$ Hz, 1H, P–C–H),3.85(s, 3H, OCH_3),3.72(d, $J = 9.6$ Hz, 3H, OCH_3), 3.64 (d, $J = 9.1$ Hz, 3H, OCH_3), 2.32 (s, 3H, CH_3).^{13}C-NMR ($CDCl_3$): δ 158.39(C_{22}), 149.36(C_6), 129.60(C_2), 128.40(C_{25}), 126.82(C_4), 123.30(C_{12}), 118.72(C_1), 114.86(C_3), 113.56($C_{20\&23}$) 111.36(C_5), 61.82(C_9), 56.32(C_{27}), 5484(C_{17}), 53.92(C_{19}), 12.79(C_7). ^{31}P-NMR ($CDCl_3$): δ 24.78.^{19}F-NMR ($CDCl_3$): δ −59.23. MS (ESI): m/z (%) 404$[M + H]^+$, 426$[M + Na]^+$. Anal. Calcd. for $C_{18}H_{21}F_3NO_4P$: C, 53.60; H, 5.25; N, 3.47. Found C, 53.24; H, 5.34; N, 3.35.

*Dimethyl (2-methyl-3-(trifluoromethyl) phenylamino) (3-nitrophenyl) methylphosphonate (**4o**)* Yellow solid; Yield: 96%. M.p. 128–130 °C. IR (cm^{-1}): *v* max 3412 (NH), 2814 (C–H), 1000 (C–F), 1224 (P=O). ^{1}H-NMR (DMSO-d_6): δ 8.18(s, 1H, Ar–H), 8.09(d, $J = 8.2$ Hz, 2H, Ar–H), 7.75 (d, $J = 7.7$ Hz, 1H, Ar–H), 7.61(m, 1H, Ar–H), 7.05(t, $J = 7.8$ Hz, 1H, Ar–H), 6.83 (d, $J = 8.0$ Hz, 1H, Ar–H), 6.75(d, $J = 7.4$ Hz, 1H, Ar–H); 5.90 (s, 1H, NH), 5.35 (d, $J = 22.4$ Hz, 1H, P–C–H), 3.76(d, $J = 2.6$ Hz, 3H, P–OCH_3), 3.57 (d, $J = 2.6$ Hz, 3H, P–OCH_3), 2.35 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ 148.02(C_{20}), 146.90(C_6), 138.06(C_{25}), 135.47(C_{24}), 129.43(C_{23}), 128.50(C_2), 126.61(C_4), 121.36(C_{12}), 121.08(C_{21}), 116.21(C_1), 114.54(C_3), 56.44(C_9), 54.77(C_{17}), 53.94(C_{19}), 12.36(C_7). ^{19}F-NMR ($CDCl_3$): δ −59.68. MS (ESI): m/z (%) 419$[M + H]^+$, 441$[M + Na]^+$. Anal.Calcd.for $C_{17}H_{18}F_3N_2O_5P$: C, 48.81; H, 4.34; N, 6.70. Found C, 48.68; H, 4.51; N, 6.64.

Conclusion

An efficient and environmentally benign method for the synthesis of new α-aminophosphonates from 2-methyl-3-(trifluoromethyl)aniline, aryl/heteroaryl aldehydes, and dimethyl phosphite using 10 mol% chitosan by microwave irradiation under neat conditions has been developed. The advantages of this protocol are the use of inexpensive, reusable catalysts, short reaction times at low temperature, easy work-up and high product yields. The efficacy of all synthesized α-aminophosphonate derivatives was assayed for their in vitro cytotoxic activities against a panel of six human cancer cell lines including PC-3(prostate cancer), MCF-7(breast cancer), HeLa(cervical cancer), U973, K562, HL60 (human leukemia). Compound **4k** with a pyrene moiety showed higher cytotoxic potency against a breast cancer cell line. But compounds **4g** and **4k** have exhibited promising cytotoxicity against U973, K562 and HL60 cancer cell lines.

Acknowledgements The authors express thanks to Prof. C. Devendranath Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati, for his helpful discussions, acknowledge the DST-SERB, New Delhi, India for providing financial support through the Project File No: SB/S1/OC-96/2013, dated: 05-11-2014) and the Anti-Cancer Drug screening facility in ACTREC, Tata Memorial Centre, Navi Mumbai for the in vitro SRB assay of compounds for anti-cancer activity.

References

1. R.W. Steketee, J.J. Wirima, L. Slutsker, C.O. Khoromana, D.L. Heymann, J.G. Breman, Am. J. Trop. Med. Hyg. **55**, 50 (1996)
2. D.T. Wong, F.P. Bymaster, E.A. Engleman, Life Sci. **57**, 411 (1995)
3. S. Hayashi, N. Ueno, A. Murase, Y. Nakagawa, J. Takada, Eur. J. Med. Chem. **50**, 179 (2012)
4. B. Stowasser, K.H. Budt, L. Jain-Qi, A. Peyman, D. Ruppert, Tetrahedron Lett. **33**, 6625 (1992)
5. C. Isanbor, D.O. Hagan, J. Fluor. Chem. **127**, 303 (2006)
6. M.S. Wu, Q.Q. Feng, G.N. Lia, D.H. Wana, Acta Cryst. **C69**, 1070–1072 (2013)
7. A.K. Bhattacharya, S.R. Dnyaneshwar, C.R. Kalpeshkumar, K.P. Innaiah, M. Sajid Khan, I. Sana, Eur. J. Med. Chem. **66**, 146 (2013)
8. F.R. Atherton, C.H. Hassall, R.W. Lambert, J. Med. Chem. **29**, 29 (1986)
9. L. Maier, H. Sporri, Phosphorus, Sulfur Silicon Relat. Elem. **61**, 69 (1991)
10. L. Maier, Phosphorus, Sulfur Silicon Relat. Elem. **47**, 43 (1990)
11. P. Kafarski, B. Lejczak, R. Tyka, L. Koba, E. Pliszczak, P. Wiecczorek, J. Plant Growth Regul. **14**, 199 (1995)
12. S. Laschat, H. Kunz, Synthesis **1992**, 90 (1992)
13. B.C. Ranu, A. Hajra, U. Jana, Org. Lett. **1**, 1141 (1999)
14. R. Ghosh, S. Maiti, A. Chakraborty, D.K. Maiti, J. Mol. Catal. A: Chem. **210**, 53 (2004)
15. J. Tang, L. Wanga, W. Wang, L. Zhang, S. Wu, D. Mao, J. Fluor. Chem. **132**, 102 (2011)
16. F. Xu, Y.Q. Luo, M.Y. Deng, Q. Shen, Eur. J. Org. Chem. **2003**, 4728 (2003)
17. S.C. Sekhar, S.J. Prakash, V. Jagadeshwar, C. Narsihmulu, Tetrahedron Lett. **42**, 5561 (2001)
18. S.D. Mitragotri, D.M. Pore, U.V. Desai, P.P. Wadgaonkar, Catal. Commun. **9**, 1822 (2008)
19. S.M. Vahdat, R. Baharfar, M. Tajbakhsh, A. Heydari, S.M. Baghbanian, S. Haksar, Tetrahedron Lett. **49**, 6501 (2008)
20. P. Sreekanth Reddy, P. VasuGovardhana Reddy, S. Mallikarjun Reddy, Tetrahedron Lett. **55**, 3336 (2014)
21. J.J. Yang, N. Dang, Y.W. Chang, Lett. Org. Chem. **6**, 470 (2009)
22. S.S. Sudha, C.S. Sundar, N.B. Reddy, K.U.M. Rao, S.H.J. Prakash, C.S. Reddy, Phosphorus, Sulfur Silicon Relat. Elem. **188**, 1402 (2013)

23. B. Kaboudin, H. Zahedi, Chem. Lett. **37**, 540 (2008)
24. Y.P. Tian, F. Xu, Y. Wang, J.J. Tang, H. Li, J. Chem. Res. **2009**, 78 (2009)
25. J.T. Hou, J.W. Gao, Z.H. Zhang, Appl. Organomet. Chem. **25**, 47 (2011)
26. C. Syama Sundar, N. Bakthavatchala Reddy, S. Sivaprasad, K. Uma Maheswara Rao, S.H. Jaya Prakash, C. Suresh Reddy, Phosphorus, Sulfur Silicon Relat. Elem. **189**, 551 (2014)
27. B. Kaboudin, M. Sorbiun, Tetrahedron Lett. **48**, 9015 (2007)
28. Y.Q. Yu, D.Z. Xu, Synthesis **47**, 1869–1876 (2015)
29. P. Kafarski, M.G. Gorniak, I. Andrasiak, Curr. Green Chem. **2**, 218 (2015)
30. G. Keglevich, A. Szekrenyi, Lett. Org. Chem. **5**, 616 (2008)
31. G. Keglevich, E. Balint, Molecules **17**, 12821 (2012)
32. D. Pradip Kumar, D. Joydeep, V.S. Tripathi, JSIR **63**, 20 (2004)