Full Paper

Green Synthesis of α-Aminophosphonate Derivatives on a Solid Supported TiO_2–SiO_2 Catalyst and Their Anticancer Activity

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Syntheses of a new series of biologically potent α-aminophosphonates were accomplished by one-pot Kabachnik–Fields reaction using TiO_2–SiO_2 as solid supported catalyst under microwave irradiation conditions. The chemical structures of all the newly synthesized compounds were confirmed by analytical and spectral (IR, ^1H, ^{13}C, ^{31}P NMR, and mass) data. Their anticancer nature was evaluated by screening the *in vitro* activity on two human cancer cell lines, HeLa and SK-BR-3. Compounds **4i** and **4o** showed the best activity on these cancer cells even though the majority of the compounds, and particularly **4l** and **4p**, have good cytotoxic activity against them.

Keywords: α-Aminophosphonates / Anticancer activity / Green synthesis / HeLa and SK-BR-3 human cancer cells / TiO_2–SiO_2

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Introduction

Synthesis and use of phosphonates have received great attention during the last two decades. The α-functionalized phosphonic acid esters serve as valuable intermediates in the preparation of medicinal compounds and as synthetic intermediates [1–6] in phosphonate chemistry for many organic compounds and dyes. They also find use in laser technology and as fluorescent materials for visualization of biomolecules. Because of attractive biological activities of α-aminophosphonates, which are the important α-functionalized phosphonic acid esters, as anti-bacterial, anti-viral, and anti-inflammatory agents [7] they have received much attention and attracted special interest as peptide analogs due to their structural similarity to α-amino acids. The anticancer properties of α-aminophosphonate derivatives have been recently reported [8, 9]. Cancer being the second leading cause of death worldwide, there is a need for the development of more effective anticancer agents.

A number of synthetic methods [10–22] using various Bronsted and Lewis acids, heteropoly acids, heterogeneous catalysts, microwave irradiation (MWI), ultrasound irradiation, catalyst-free, ionic liquid, and nanocatalysts have been reported for α-aminophosphonates. Recently Kabachnik–Fields reaction has been reviewed [23] well about the various reactions conditions. Although significant advances have been made in these syntheses, still some limitations such as use of expensive catalyst, toxic solvents, longer reaction time, elevated temperature, and low product yields are associated with them. Therefore, the search continues for a better synthetic procedure for α-aminophosphonates in terms of operational simplicity, economic viability, and greater selectivity. Green chemical synthetic approaches under solvent-free conditions [24–26] with MWI [27–33] obviously meet this objective.

In heterogeneous solid supported catalysis [34–37], the titania–silica (TiO_2–SiO_2) is an important catalyst for organic reactions due to advantages such as improving the availability of the active sites, ease to handle as a benchtop catalyst, inexpensive, commercial availability, and recyclable due to stability even at a higher temperature. In pursuit of our continued interest in the development of green synthetic methods for the preparation of phosphonate

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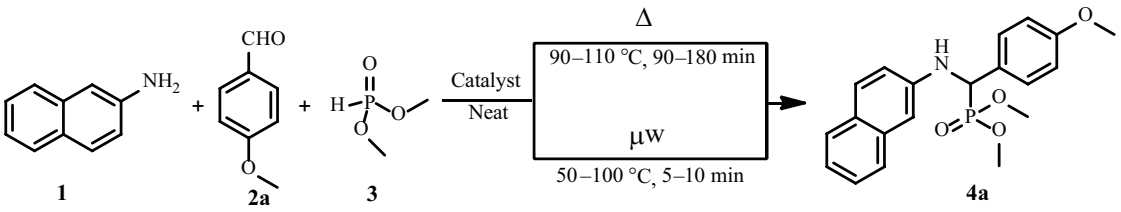


Scheme 1. Preparation of dimethyl ((4-methoxyphenyl)(naphthalen-2-ylamino)methyl)phosphonate (**4a**).

derivatives [38, 39], we accomplished solvent-free synthesis for a new series of α-aminophosphonates via three-component one-pot Kabachnik–Fields reaction using TiO_2–SiO_2 as supported catalyst under MWI (Scheme 1) and studied their cytotoxic activity. We succeeded in identifying these newly synthesized compounds as potential *in vitro* anticancer compounds. Further, this group of compounds with an aminophosphonate pharmacophore may even have broad range of bioactivity against many other bacterial and viral diseases.

Results and discussion

Chemistry

Reaction of naphthalen-2-amine (**1**), 4-methoxy benzaldehyde (**2a**), and dimethyl phosphonate (**3**) to obtain the dimethyl ((4-methoxyphenyl)(naphthalen-2-ylamino)methyl)phosphonate (**4a**) under solvent-free conditions (Scheme 1) was run to standardize the experimental conditions.

In order to establish optimum conditions for Kabachnik–Fields reaction under both conventional and MWI conditions (at 210 W power), the model reaction was run using various catalysts (Table 1) in 10 mol% under solvent-free conditions. All the reactions were carried out at 90–110 and 50–100°C for 90–180 and 5–10 min under conventional and MWI conditions, respectively. When the model reaction was run under conventional conditions, the product yield was very low even after prolonged reaction time. However, the same reaction under MWI conditions afforded high product yields (Table 1, entries **1**–**18**). This observation motivated us to search for a suitable catalyst under MWI conditions. We found that the TiO_2–SiO_2 worked as a better catalyst among all the catalysts used at 70°C (Table 1, entry **18**) under these conditions. We also found that the yields were significantly affected by the amount of TiO_2–SiO_2 loaded. Most excitingly, the reaction progressed very smoothly and gave **4a** in 97% yield when 5 mol% TiO_2–SiO_2 was used (Table 1, entry **19**). Interestingly we could find no drastic change in the product yield when the reaction time was decreased from 10 to 5 min (Table 1, entry **20**) at 5 mol% concentration of the catalyst. But, when loaded with 3 mol% of TiO_2–SiO_2 catalyst, the reaction remained incomplete (Table 1, entry **21**) and use of excess amount of catalyst also did not increase the product yield considerably (Table 1, entry **22**). Therefore, it was established that 5 mol% of TiO_2–SiO_2 and 80 min of reaction time are necessary and sufficient for the total completion of the model reaction to obtain maximum product yield.

We have also examined the TiO_2–SiO_2 catalytic activity for reusability. We reused the catalyst for five consecutive cycles and observed no significant loss of catalytic activity in all

Table 1. Screening of catalyst quantity and reaction time on the synthesis of dimethyl ((4-methoxyphenyl)(naphthalen-2-ylamino)-methyl)phosphonate (**4a**).[a)]

		Conventional			Microwave	
Entry	Catalyst (10 mol%)	Temp (°C)	Time (min)	Yield[b)] (%)	Temp (°C)	Yield[b)] (%)
1	$AlCl_3$	110	140	53	70	65
2	$ZrOCl_2 \cdot 8H_2O$	110	120	58	70	70
3	InF_3	110	110	63	70	76
4	$ZnBr_2$	110	120	45	70	60
5	$ZnCl_2$	110	120	48	70	58
6	ZnO	110	130	52	70	67
7	$ZnCl_2$–SiO_2	110	100	60	70	73
8	$MnCl_2 \cdot 4H_2O$	110	150	49	70	62
9	$Yb(OAc)_3 \cdot H_2O$	110	120	55	70	62
10	CuBr	110	180	50	70	62
11	$NbCl_5$	110	100	56	70	69
12	K-10	110	160	53	70	69
13	Al_2O_3	110	130	61	70	81
14	$NiCl_2 \cdot 6H_2O$	110	120	59	70	82
15	Amberlyst-15	110	150	70	70	84
16	$PS/GaCl_3$	110	100	68	70	81
17	$PS/AlCl_3$	110	100	67	70	80
18	TiO_2–SiO_2	110	90	80	70	97
19	TiO_2–SiO_2 (5 mol%)	90	90	55	70	97
20	TiO_2–SiO_2 (5 mol%)	90	90	75	70	96
21	TiO_2–SiO_2 (3 mol%)	90	90	63	70	83
22	TiO_2–SiO_2 (15 mol%)	90	90	81	70	97
23	TiO_2–SiO_2 (5 mol%)	–	–	–	100	94
24	TiO_2–SiO_2 (5 mol%)	–	–	–	50	64

[a)] Reaction conditions: naphthalen-2-amine (**1**), 4-methoxybenzaldehyde (**2a**), and dimethyl phosphonate (**3**) in 1:1:1 ratio. All the microwave irradiation reactions were run for 10 and 5 min for entries **1**–**19** and **20**–**24**, respectively. All the entries were treated under neat (solvent-free) conditions in both methods.
[b)] Isolated yield.

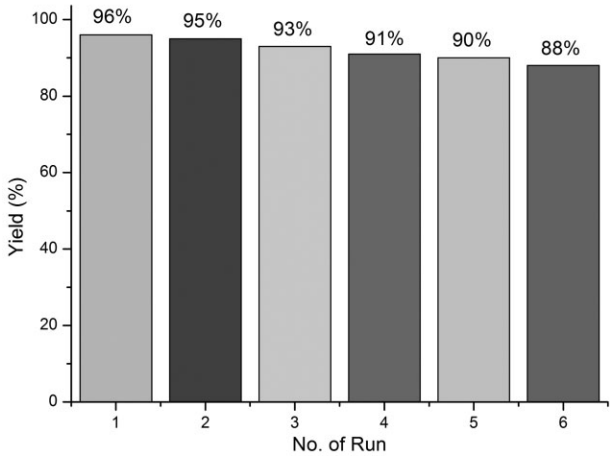


Figure 1. Reusability of TiO_2–SiO_2 catalyst for the model reaction in consecutive cycles.

these reactions; the corresponding product was obtained in 95%, 93%, 91%, 90%, and 88% yields (Fig. 1). These data qualify the catalyst for reusability.

Suitable microwave power required for the model reaction was also studied by using variable MW energy and it was concluded that 210 W MW power output and 70°C were sufficient for the accomplishment of maximum conversion of reactants to the product **4a**. Increasing to 245 W or decreasing to 140 W MW energy, no more appreciable increment in the product yield (Table 1, entries **23** and **24**, respectively) was observed.

The effect of solvent and temperature on this model reaction was investigated for both conventional and MWI conditions with 5 mol% of TiO_2–SiO_2 catalyst using different solvents at varying temperatures (Table 2, entries **1**–**9**) for 90 and 5 min, respectively. The results indicated that the reaction efficiency was also affected by the solvent. In non-polar solvent, lower product yields (Table 2, entries **1**–**3**), and, in polar solvent, better yields were obtained (Table 2, entries **6**–**9**). The polar solvents undergo dipole rotation when exposed to microwaves and generate heat energy, which results in product yield at a faster rate. However, the best result was obtained under solvent-free conditions (Table 2, entry **10**). This motivates us to synthesize the target molecules

Table 2. Effect of the solvent and temperature on the synthesis of dimethyl ((4-methoxyphenyl)(naphthalen-2-ylamino)methyl)phosphonate (**4a**).[a)]

		Conventional		Microwave	
Entry	Solvent	Temp (°C)	Yield[b)] (%)	Temp (°C)	Yield[b)] (%)
1	Toluene	110	57	100	65
2	Carbontetrachloride	75	55	70	61
3	Chloroform	60	51	70	59
4	Dichloromethane	60	52	70	55
5	Tetrahydrofuran	65	56	70	63
6	Ethanol	75	61	70	73
7	Methanol	70	65	70	78
8	Acetonitrile	80	76	100	82
9	Dimethylsulfoxide	150	80	100	86
10	Solvent-free	90	75	70	96

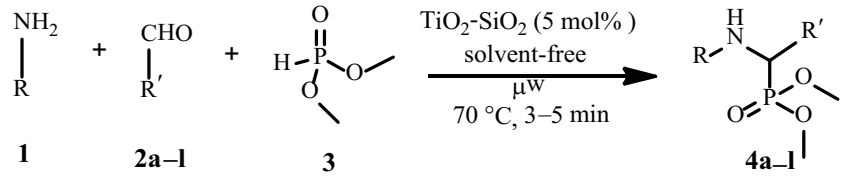
[a)] Reaction conditions: naphthalen-2-amine (**1**), 4-methoxybenzaldehyde (**2a**), and dimethyl phosphonate (**3**) in 1:1:1 ratio. All the reactions under conventional and microwave (at 210 and 245 W) conditions were run at 90 and 5 min reaction time, respectively.
[b)] Isolated yield.

under MWI and solvent-free conditions. We found that the reaction proceeded smoothly in much less reaction time (3–5 min) at lower reaction temperature (70°C) leading to higher product yields (85–97%).

After finding TiO_2–SiO_2 as the best catalyst system for the model reaction, we applied this methodology successfully for a series of aldehydes (**2b**–**l**) and amines (Scheme 2) and achieved excellent yields (Table 3). The aromatic aldehydes (**2a**–**h**) reacted at a faster rate irrespective of the nature of substrates (electron withdrawing or donating) on them. On the other hand, the product yields were lower with aliphatic aldehydes (**2i**–**l**), although the reactions went smoothly.

Cytotoxicity

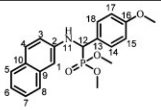
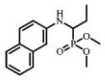
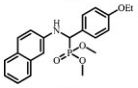
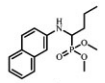
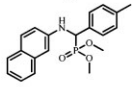
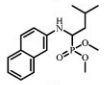
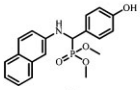
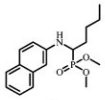
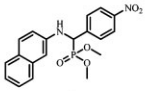
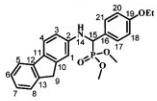
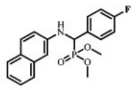
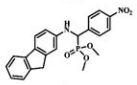
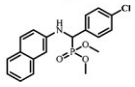
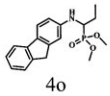
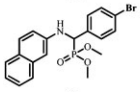
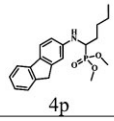
The anticancer activity of test compounds **4a**–**p** was investigated on HeLa (human cervical cancer) and SK-BR-3 (human breast adenocarcinoma) cells. It was determined by measuring the number of live cells after 24 h of treatment (MTT assay); their IC_{50} values are presented [40] in Table 4. The results showed that majority of title compounds (**4a**–**4d**, **4f**,



1 **2a–l** **3** **4a–l**

Scheme 2. Solvent-free microwave synthesis of α-aminophosphonates (**4a–l**) on solid supported TiO_2–SiO_2 catalyst.

Table 3. Synthesis of α-aminophosphonates (**4a–p**) on TiO_2–SiO_2 catalyst by MWI via Scheme 2.[a)]

Entry	R′	Compound	Time (min)	Yield[b] (%)	Entry	R′	Compound	Time (min)	Yield[b] (%)
1	 2a	 4a	3	97	9	 2i	 4i	6	85
2	 2b	 4b	4	96	10	 2j	 4j	5	87
3	 2c	 4c	4	96	11	 2k	 4k	5	85
4	 2d	 4d	3	95	12	 2l	 4l	5	85
5	 2e	 4e	4	94	13	 2b	 4m	4	97
6	 2f	 4f	4	94	14	 2e	 4n	3	96
7	 2g	 4g	4	92	15	 2j	 4o	6	87
8	 2h	 4h	4	92	16	 2l	 4p	5	85

[a)] Reaction conditions: naphthalen-2-amine/2-aminofluorene, various aldehydes, and dimethyl phosphonate on 5 mol% TiO_2–SiO_2 catalyst at 210 W.
[b)] Isolated yields.

4g, **4i–4m**, **4o**, and **4p**) possessed good anti-proliferative activity against the two types of cancer cells. Among them, the compound **4o** has the highest cytotoxicity with the IC_{50} at concentrations of 0.95 ± 0.06 and 1.21 ± 0.09 μg/mL for HeLa and SK-BR-3 cells, respectively. Almost equal levels of activity were observed for **4i**, **4l**, and **4p**. From these observations one may conclude that the dimethyl (1-((9*H*-fluoren-2-yl)amino)butyl)phosphonate (**4o**) is a potential pharmacophore showing higher cytotoxic activity against the two types of cancerous cells.

Microscopic observations

The cytomorphological abnormalities accrued by the effect of test compounds were observed under a phase-contrast and fluorescent microscope for both HeLa and SK-BR-3 cells (Figs. 2 and 3, respectively). The control group that is without test compound showed normal healthy and intact nuclei without any cytological abnormalities (Fig. 2A and C for HeLa cells and Figs. 3A and C for SK-BR-3 cells).

The cells treated with highly active test compound **4o** for 24 h showed obvious morphological changes such as destruction of cellular membrane, chromatin fragmentation, and appearance of apoptotic bodies as granules in the culture media (Fig. 2B). These light microscopy results were consistent with those of fluorescence microscopy using Hoechst 33342 stain for control and treated cells, where most of the treated cells exhibited the symptoms of apoptosis but the damage was severe in some HeLa cells prior to cell membrane blebbing

Table 4. Cytotoxic activity[a)] of test compounds **4a–r** against HeLa and SK-BR-3 cells.

	IC_{50} in μg/mL[b)]	
Compound	**HeLa**	**SK-BR-3**
4a	20.05 ± 1.26	32.26 ± 1.52
4b	22.16 ± 1.38	35.12 ± 1.75
4c	29.70 ± 1.35	32.78 ± 1.81
4d	79.29 ± 3.53	68.37 ± 3.12
4e	>100	>100
4f	13.52 ± 0.89	19.45 ± 0.91
4g	12.76 ± 0.86	11.29 ± 0.81
4h	>100	>100
4i	1.18 ± 0.35	2.52 ± 0.15
4j	6.16 ± 0.19	5.41 ± 0.16
4k	8.34 ± 0.24	7.89 ± 0.22
4l	2.10 ± 0.85	3.37 ± 0.95
4m	26.80 ± 1.15	29.37 ± 1.29
4n	>100	>100
4o	0.95 ± 0.06	1.21 ± 0.09
4p	1.45 ± 0.08	2.79 ± 0.11
Etoposide	13.65 ± 0.55	9.73 ± 0.42
Camptothecin	3.57 ± 0.33	2.83 ± 0.11

[a)] Exponentially growing cells were treated with different concentrations of test compounds for 24 h and cell growth inhibition was analyzed through MTT assay.
[b)] IC_{50} is defined as the concentration that results in a 50% decrease in cell number as compared with that of the control in the absence of an inhibitor. The values represent the mean ± SD of five individual observations.

(inset of Fig. 2B). Hoechst staining revealed typical horseshoe-shaped nuclei of the HeLa cells, which indicates early apoptosis leading to deformed nuclear cytoplasmic regularity followed by margination of chromatin (Fig. 2D).

When observed for the SK-BR-3 cells similar results were obtained but the damage to the cells was less compared to HeLa cells even at the IC_{50} concentration of the ideal compound, **4o**. Interestingly, the SK-BR-3 cells appeared circular with the enlargement of cell membrane in almost all the treated cells (Fig. 3B). This implies that the cell damage is not as quick as in the case of HeLa cells. The bright, condensed and segregated chromatin was identified in the nuclei of the treated cells. The typical characteristic nuclear deformities such as karyorrhexis and picknosis are well evident in the treated SK-BR-3 cells (Fig. 3D).

Structure–activity relationship (SAR)

An organophosphorus compound with a general structure (**I**) is biologically active [41–43]. Its phosphorylating ability on the target molecules depends on the strength of the P–X bond. A weak P–X bond makes it a good phosphorylating agent because of the more electrophilic nature of phosphorus that can facilitate its nucleophilic attack of electron-rich centers of enzymes and proteins. The ease and effectiveness of phosphorus substitution are critically governed by nature, steric size, structure, and configuration of groups attached to phosphorus [44, 45]. Even though, the mechanism of phosphorylation is not clearly proved, this hypothesis opened a new concept for the design of novel organophosphorus compounds that could regulate the normal metabolic biochemical processes in living organisms (Scheme 3).

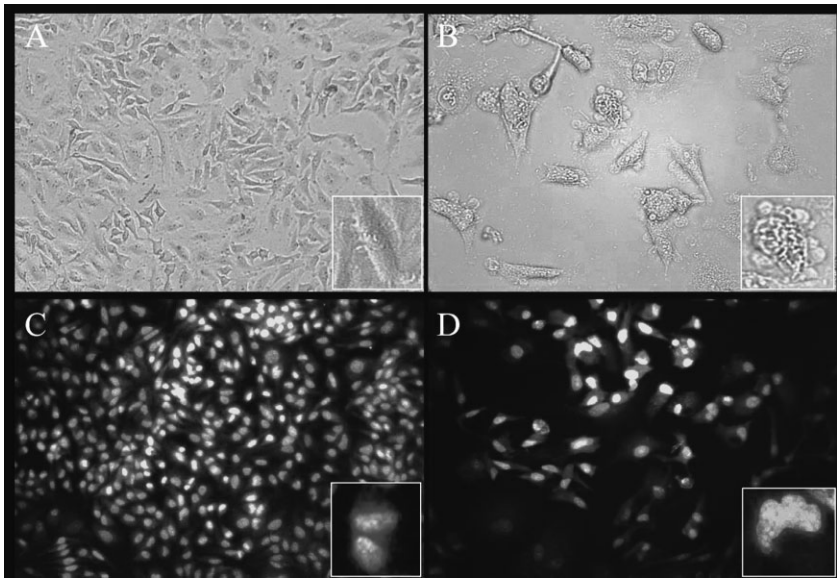


Figure 2. Light and Hoechst stained micrographs of normal and treated HeLa cells. Left-hand panel (A and C) represents the untreated/normal HeLa cells, and the right-hand panel represents the HeLa cells (B and D) treated with IC_{50} concentration (0.95 μg/mL) of the ideal test compound **4o**.

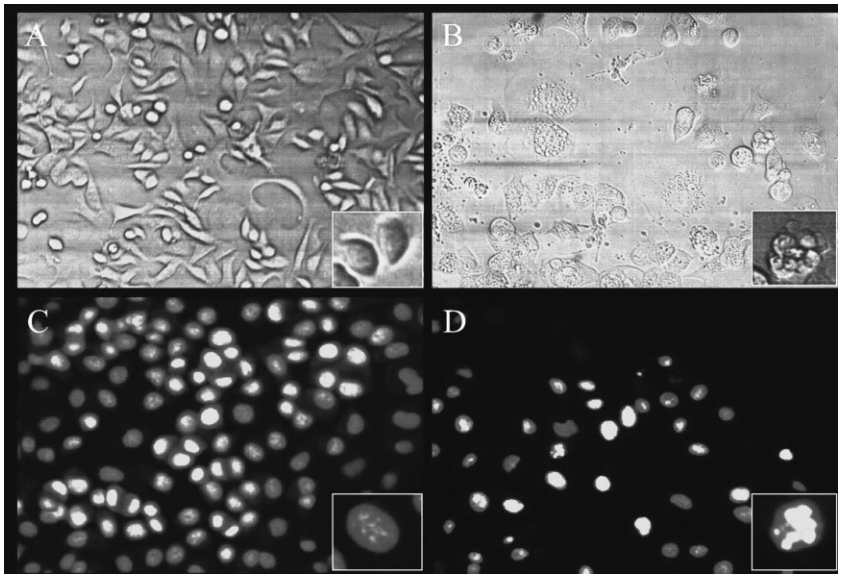
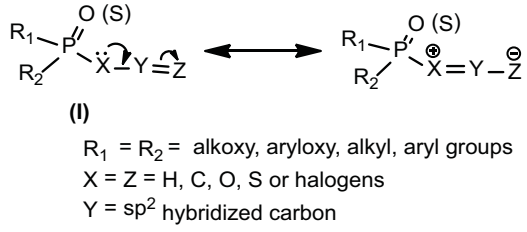


Figure 3. Light and Hoechst stained micrographs of normal and treated SK-BR-3 cells. Left-hand panel (A and C) represents the untreated/normal SK-BR-3 cells, and the right-hand panel represents the SK-BR-3 cells (B and D) treated with IC_{50} concentration (1.21 μg/mL) of the ideal test compound **4o**.

The compounds **4a**–**p** in general are more active than the compound **4o** in particular, hence the necessary steric and electronic configuration in structure (II) that can arrest the abnormal cell metabolic disorders and maintain normal function by phosphorylating cancer cell components (Scheme 4).

Conclusions

We have successfully synthesized a series of new α-aminophosphonates from easily available starting materials under solvent-free conditions using TiO_2–SiO_2 as a solid supported catalyst with MWI in one-pot multi-component Kabachnik–Fields reaction. In this procedure the amount of waste is minimized and atom efficiency is increased in each organic transformation, qualifying it as the best method for the synthesis of α-aminophosphonates. All the newly synthesized α-aminophosphonates were screened for their *in vitro* anticancer activities on HeLa and SK-BR-3 cells and the IC_{50} values obtained were compared against those of standard drugs. Majority of them have good to excellent anticancer activity. The results further signify that these compounds can be developed as potential pharmacological leads to combat cancer.



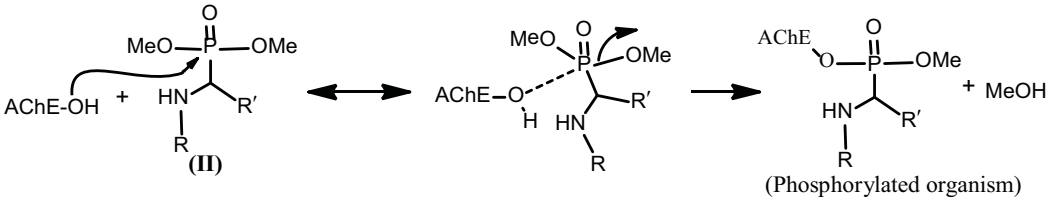
Scheme 3. Biologically active pharmacophore unit of organophosphorus compounds.

Experimental

Chemistry

Materials and methods

All the chemicals purchased from Sigma–Aldrich (Hyderabad, India), Merck (Mumbai, India), and Lancaster Chemical (Mumbai, India) were used as such without further purification. The solvents used for spectroscopic and other physical studies were of analytical grade and were further purified employing the reported methods. All the reactions and purity of products were monitored by thin layer chromatography (TLC) using aluminum



Scheme 4. Hypothetical mechanistic presentation of bioactivity of the title compounds (**4a**–**p**).

plates coated with silica gel (Merck) using 3:7 of ethyl acetate and hexane as mobile phase. Melting points were determined using a calibrated thermometer by Guna Digital melting point apparatus. IR Prestige-21, Fourier transform infrared (FT-IR) spectrometer using KBr optics. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded in $CDCl_3$ on a Varian 400 MHz NMR spectrometer operating at 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, and 161.89 MHz for ^{31}P NMR at Pusan National University, Pusan, Republic of Korea, and referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Mass spectra were recorded at Pukyong National University, Busan, Republic of Korea on a Jeol JMS-700 mass spectrometer. Elemental analyses were performed on a Thermo Finnigan instrument at the University of Hyderabad, Hyderabad, India.

Synthetic procedure for the model reaction

Method 1: Microwave irradiation (MWI) method: A two-necked round bottom (RB) flask, which contains a mixture of naphthalen-2-amine (**1**, 0.716 g, 0.005 mol), 4-methoxybenzaldehyde (**2a**, 0.61 mL, 0.005 mol), dimethyl phosphonate (**3**, 0.46 mL, 0.005 mol), and various catalysts with 10 mol% without any solvent, with an air condenser and a thermo probe was exposed to MWI using a CATA-4R – Scientific Microwave oven at various temperatures shown in Table 1 with 140, 210, and 245 W at ambient pressure. While irradiating with MWI the reaction mixture was stirred continually to maintain the irradiating field homogeneously throughout the reaction mixture. The reaction was stopped as indicated by TLC after 3–10 min. Then the crude products were obtained by separation of the catalyst by filtration followed by evaporation of the filtrate. The crude products were further purified by column chromatography on 60–120 mesh silica gel using ethyl acetate/hexane (1:3) as eluent, and the solvent was evaporated in a rotary evaporator. The residue was recrystallized from ethyl acetate to afford pure **4a** (Table 1; Scheme 1).

Method 2: Conventional (heating) method: The reactants were heated to reflux in an oil bath at 90–110°C for 90–180 min and kept stirring, and the workup of the products was done as in the MWI method.

*Synthesis of α-aminophosphonates (**4a–p**):* As model reaction the amine (**1a**/**1b**, 0.005 mol), various aldehydes (**2b–p**, 0.005 mol), and dimethyl phosphonate (**3**, 0.46 mL, 0.005 mol) were mixed thoroughly with 5 mol% TiO_2–SiO_2 in a RB flask and exposed to MWI at 210 W and 70°C under ambient pressure (Scheme 2). TLC showed the reaction completion in 3–6 min. The product separation and purification were done according to MWI method. Pure products were obtained by recrystallization from ethyl acetate (Table 3). The structures of all the newly synthesized title compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, mass spectral, and elemental analysis.

*Physical and spectral characterization of the title compounds (**4a–p**)*

*Dimethyl ((4-methoxyphenyl)(naphthalen-2-ylamino)methyl)-phosphonate (**4a**):* Brown solid, mp: 162–164°C. IR (KBr) (ν_{max} cm^{-1}): 3304 (N–H), 2955 and 2866 (C–$H_{aromatic}$), 1257 (P=O), 761 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.78 (3H, s, Ar–O<u>CH_3</u>), 3.56 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 3.72 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 6.51–6.54 (1H, dd, $^{2}J_{H-P}$ = 8.4 Hz and $^{3}J_{H-H}$ = 2.0 Hz, P–<u>CH</u>), 6.74 (1H, m, <u>NH</u>), 7.93–6.92 (11H, m, <u>Ar–H</u>). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.5 (C-1), 148.7 (C-2), 119.4 (C-3), 130.8 (C-4), 129.2 (C-5), 122.3 (C-6), 127.1 (C-7), 126.2 (C-8), 134.2 (C-9), 129.2 (C-10), 71.2 (C-12), 131.2 (C-13), 130.1 (C-14 and C-28), 115.6 (C-15 and 17), 160.5 (C-16), 53.6 (P–OCH_3), 53.9 (P–OCH_3), 55.4 (Ar–OCH_3). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 21.07. EI-MS (*m*/*z*, %): 371 ($M^{+\bullet}$). Anal. calcd. for $C_{20}H_{21}NO_4P$: C, 64.68; H, 5.97; N, 3.77; Found: C, 64.63; H, 5.92; N, 3.73.

*Dimethyl ((4-ethoxyphenyl)(naphthalen-2-ylamino)methyl)-phosphonate (**4b**):* Brown solid, mp: 186–188°C. IR (KBr) (ν_{max} cm^{-1}): 3323 (N–H), 2956 and 2848 (C–$H_{aromatic}$), 1221 (P=O), 757 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.45 (2H, q, Ar–O<u>CH_2</u>), δ: 1.56 (3H, t, $^{3}J_{H-P}$ = 10.4 Hz, OCH_2<u>CH_3</u>), 3.55 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 3.75 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 6.54–6.59 (1H, dd, $^{2}J_{H-P}$ = 8.4 Hz, P–<u>CH</u> and $^{3}J_{H-H}$ = 2.0 Hz, NH–<u>CH</u>), 6.58 (1H, m, <u>NH</u>), 7.91–6.90 (11H, m, <u>Ar–H</u>). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.2 (C-1), 148.3 (C-2), 119.5 (C-3), 130.6 (C-4), 129.4 (C-5), 122.7 (C-6), 127.3 (C-7), 126.5 (C-8), 134.6 (C-9), 129.5 (C-10), 71.1 (C-12), 131.6 (C-13), 130.5 (C-14 and C-28), 115.3 (C-15 and 17), 160.6 (C-16), 53.3 (P–OCH_3), 53.5 (P–OCH_3), 65.6 (Ar–O<u>CH_2</u>), 15.2 (OCH_2<u>CH_3</u>). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 21.34. EI-MS (*m*/*z*, %): 385 ($M^{+\bullet}$). Anal. calcd. for $C_{21}H_{24}NO_4P$: C, 65.45; H, 6.28; N, 3.63; Found: C, 65.39; H, 6.22; N, 3.58.

*Dimethyl ((naphthalen-2-ylamino)(p-tolyl)methyl)phosphonate (**4c**):* Yellowish solid, mp: 189–181°C. IR (KBr) (ν_{max} cm^{-1}): 3317 (N–H), 2965 and 2861 (C–$H_{aromatic}$), 1258 (P=O), 752 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (3H, s, Ar–<u>CH_3</u>), 3.52 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 3.74 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 6.54–6.59 (1H, dd, $^{2}J_{H-P}$ = 8.4 Hz and $^{3}J_{H-H}$ = 2.0 Hz, P–<u>CH</u>), 6.12 (1H, m, <u>NH</u>), 7.91–7.16 (11H, m, <u>Ar–H</u>). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.8 (C-1), 148.5 (C-2), 119.5 (C-3), 130.7 (C-4), 129.3 (C-5), 122.6 (C-6), 127.3 (C-7), 126.0 (C-8), 134.0 (C-9), 129.6 (C-10), 71.5 (C-12), 131.8 (C-13), 130.5 (C-14 and C-28), 115.1 (C-15 and 17), 160.6 (C-16), 53.3 (P–OCH_3), 53.5 (P–OCH_3), 25.6 (Ar–<u>CH_3</u>). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 22.13. EI-MS (*m*/*z*, %): 355 ($M^{+\bullet}$). Anal. calcd. for $C_{20}H_{22}NO_3P$: C, 67.60; H, 6.24; N, 3.94; Found: C, 67.54; H, 6.18; N, 3.90.

*Dimethyl ((4-hydroxyphenyl)(naphthalen-2-ylamino)methyl)-phosphonate (**4d**):* Pale yellow solid, mp: 209–211°C. IR (KBr) (ν_{max} cm^{-1}): 3317 (N–H), 2968 and 2878 (C–$H_{aromatic}$), 1239 (P=O), 751 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.27 (3H, s, Ar–<u>OH</u>), 3.54 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 3.72 (3H, d, $^{3}J_{H-P}$ = 10.4 Hz, P–O<u>CH_3</u>), 6.51–6.58 (1H, dd, $^{2}J_{H-P}$ = 8.4 Hz and $^{3}J_{H-H}$ = 2.0 Hz, P–<u>CH</u>), 6.33 (1H, m, <u>NH</u>), 7.90–6.96 (11H, m, <u>Ar–H</u>). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.2 (C-1), 148.6 (C-2), 119.8 (C-3), 130.4 (C-4), 129.5 (C-5), 122.3 (C-6), 127.5 (C-7), 126.6 (C-8), 134.5 (C-9), 129.8 (C-10), 71.3 (C-12), 131.4 (C-13), 130.9 (C-14 and C-28), 115.5 (C-15 and 17), 160.8 (C-16), 53.5 (P–OCH_3), 53.9 (P–OCH_3). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 22.75. Anal. calcd. for $C_{19}H_{20}NO_4P$: C, 63.86; H, 5.64; N, 3.92; Found: C, 63.81; H, 5.58; N, 3.87.

*Dimethyl ((naphthalen-2-ylamino)(4-nitrophenyl)methyl)-phosphonate (**4e**):* Yellow solid, mp: 168–170°C. IR (KBr) (ν_{max} cm^{-1}): 3321 (N–H), 2960 and 2869 (C–$H_{aromatic}$), 1229 (P=O), 1532 and 1347 (N–O), 755 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz,

$CDCl_3$) δ: 3. 28 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.47 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 6.25–6.51 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.53 (1H, m, $\underline{NH}$), 7.84–7.32 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.4 (C-1), 148.7 (C-2), 119.9 (C-3), 129.4 (C-4), 129.6 (C-5), 121.7 (C-6), 127.6 (C-7), 126.5 (C-8), 134.4 (C-9), 129.9 (C-10), 70.8 (C-12), 143.3 (C-13), 130.1 (C-14 and C-28), 125.3 (C-15 and 17), 153.6 (C-16), 53.7 (P–OCH_3), 53.8 (P–OCH_3). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 21.35. EI-MS (*m*/*z*, %): 386 ($M^{+\bullet}$). Anal. calcd. for $C_{19}H_{19}N_2O_5P$: C, 59.07; H, 4.96; N, 7.25; Found: C, 59.01; H, 4.91; N, 7.21.

Dimethyl ((4-fluorophenyl)(naphthalen-2-ylamino)methyl)-phosphonate (4f): Pale yellow solid, mp: 183–185°C. IR (KBr) (ν_{max} cm^{-1}): 3323 (N–H), 2960 and 2872 (C–$H_{aromatic}$), 1235 (P=O), 755 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.56 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.71 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 6.51–6.58 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.42 (1H, m, $\underline{NH}$), 7.95–7.36 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.3 (C-1), 148.3 (C-2), 119.1 (C-3), 130.5 (C-4), 129.6 (C-5), 122.2 (C-6), 127.6 (C-7), 126.3 (C-8), 134.4 (C-9), 129.2 (C-10), 71.9 (C-12), 135.5 (C-13), 130.8 (C-14 and C-28), 119.3 (C-15 and 17), 161.7 (C-16), 53.7 (P–OCH_3), 53.9 (P–OCH_3). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 21.35. EI-MS (*m*/*z*, %): 359 ($M^{+\bullet}$). Anal. calcd. for $C_{19}H_{19}FNO_3P$: C, 63.51; H, 5.33; N, 3.90; Found: C, 63.45; H, 5.28; N, 3.85.

Dimethyl ((4-chlorophenyl)(naphthalen-2-ylamino)methyl)-phosphonate (4g): Pale yellow solid, mp: 173–175°C. IR (KBr) (ν_{max} cm^{-1}): 3334 (N–H), 2969 and 2865 (C–$H_{aromatic}$), 1237 (P=O), 757 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.51 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.68 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 6.53–6.59 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.35 (1H, m, $\underline{NH}$), 7.90–7.32 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.4 (C-1), 148.7 (C-2), 119.6 (C-3), 130.3 (C-4), 130.6 (C-5), 122.8 (C-6), 125.7 (C-7), 126.7 (C-8), 134.7 (C-9), 128.4 (C-10), 70.8 (C-12), 135.1 (C-13), 131.2 (C-14 and C-28), 125.4 (C-15 and 17), 136.5 (C-16), 53.3 (P-OCH_3), 53.7 (P-OCH_3). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.43. EI-MS (*m*/*z*, %): 375 ($M^{+\bullet}$). Anal. calcd. for $C_{19}H_{19}ClNO_3P$: C, 60.73; H, 5.10; N, 3.73; Found: C, 60.68; H, 5.02; N, 3.65.

Dimethyl ((4-bromophenyl)(naphthalen-2-ylamino)methyl)-phosphonate (4h): Brown solid, mp: 206–208°C. IR (KBr) (ν_{max} cm^{-1}): 3325 (N–H), 2968 and 2863 (C–$H_{aromatic}$), 1234 (P=O), 751 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.56 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.62 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 6.55–6.61 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.36 (1H, m, $\underline{NH}$), 7.96–7.30 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.8 (C-1), 148.9 (C-2), 119.2 (C-3), 130.6 (C-4), 130.4 (C-5), 122.2 (C-6), 125.3 (C-7), 126.9 (C-8), 134.2 (C-9), 128.1 (C-10), 70.1 (C-12), 135.9 (C-13), 131.5 (C-14 and C-28), 134.2 (C-15 and 17), 126.5 (C-16), 53.4 (P–OCH_3), 53.8 (P–OCH_3). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.65. EI-MS (*m*/*z*, %): 419 ($M^{+\bullet}$). Anal. calcd. for $C_{19}H_{19}BrNO_3P$: C, 54.30; H, 4.56; N, 3.33; Found: C, 54.23; H, 4.51; N, 3.25.

Dimethyl (1-(naphthalen-2-ylamino)propyl)phosphonate (4i): Pale yellow solid, mp: 145–147°C. IR (KBr) (ν_{max} cm^{-1}): 3312 (N–H), 2954 and 2867 (C–$H_{aromatic}$), 1233 (P=O), 752 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (2H, m, CH$\underline{CH_2}$), 1.11 (3H, t, $CH_2\underline{CH_3}$), 3.53 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.66 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 5.64–5.71 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.31 (1H, m, $\underline{NH}$), 7.52–7.15 (7H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.2 (C-1), 148.3 (C-2), 119.5 (C-3), 130.1 (C-4), 130.3 (C-5), 122.4 (C-6), 125.5 (C-7), 126.3 (C-8), 134.5 (C-9), 128.7 (C-10), 66.7 (C-12), 26.2 (C-13), 16.1 (C-14). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.62. EI-MS (*m*/*z*, %): 293 ($M^{+\bullet}$). Anal. calcd. for $C_{15}H_{20}NO_3P$: C, 61.43; H, 6.87; N, 4.78; Found: C, 61.37; H, 6.81; N, 4.73.

Dimethyl (1-(naphthalen-2-ylamino)butyl)phosphonate (4j): Pale yellow solid, mp: 153–155°C. IR (KBr) (ν_{max} cm^{-1}): 3322 (N–H), 2963 and 2861 (C–$H_{aromatic}$), 1241 (P=O), 751 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38–1.51 (4H, m, 2 × $\underline{CH_2}$), 1.12 (3H, t, $\underline{CH_3}$), 3.55 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.67 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 5.61–5.66 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.36 (1H, m, $\underline{NH}$), 7.68–7.18 (7H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 105.6 (C-1), 146.6 (C-2), 119.8 (C-3), 130.4 (C-4), 130.6 (C-5), 122.2 (C-6), 125.8 (C-7), 126.7 (C-8), 134.8 (C-9), 128.9 (C-10), 66.3 (C-12), 27.3 (C-13), 20.5 (C-14), 14.2 (C-15). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.52. EI-MS (*m*/*z*, %): 307 ($M^{+\bullet}$). Anal. calcd. for $C_{16}H_{22}NO_3P$: C, 62.53; H, 7.22; N, 4.56; Found: C, 62.46; H, 7.15; N, 4.51.

Dimethyl (3-methyl-1-(naphthalen-2-ylamino)butyl)phosphonate (4k): Pale yellow solid, mp: 161–163°C. IR (KBr) (ν_{max} cm^{-1}): 3324 (N–H), 2965 and 2862 (C–$H_{aromatic}$), 1246 (P=O), 754 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28–1.59 (6H, m, 3 × $\underline{CH_2}$), 1.05 (3H, d, $\underline{CH_3}$), 3.55 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.65 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 5.45–5.54 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.43 (1H, m, $\underline{NH}$), 7.61–7.20 (7H, m, $\underline{Ar–H}$). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.54. EI-MS (*m*/*z*, %): 321 ($M^{+\bullet}$). Anal. calcd. for $C_{17}H_{24}NO_3P$: C, 63.54; H, 7.53; N, 4.36; Found: C, 63.45; H, 7.46; N, 4.31.

Dimethyl (1-(naphthalen-2-ylamino)pentyl)phosphonate (4l): Pale yellow solid, mp: 173–175°C. IR (KBr) (ν_{max} cm^{-1}): 3323 (N–H), 2963 and 2859 (C–$H_{aromatic}$), 1251 (P=O), 756 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45–1.53 (2H, m, CH$\underline{CH_2}$), 1.72 (1H, m, $CH_2\underline{CH}$), 1.21 (6H, d, $(\underline{CH_3})_2$), 3.51 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.63 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 5.46–5.58 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz, P–$\underline{CH}$), 6.48 (1H, m, $\underline{NH}$), 7.64–7.19 (7H, m, $\underline{Ar–H}$). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 22.45. EI-MS (*m*/*z*, %): 321 ($M^{+\bullet}$). Anal. calcd. for $C_{17}H_{24}NO_3P$: C, 63.54; H, 7.53; N, 4.36; Found: C, 63.49; H, 7.47; N, 4.30.

Dimethyl (((9H-fluoren-2-yl)amino)(4-ethoxyphenyl)methyl)-phosphonate (4m): Brown solid, mp: 185–187°C. IR (KBr) (ν_{max} cm^{-1}): 3315 (N–H), 2965 and 2865 (C–$H_{aromatic}$), 1255 (P=O), 752 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.82 (3H, q, Ar–O$\underline{CH_2}$), δ: 1.29 (3H, t, $OCH_2\underline{CH_3}$), 3.51 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.81 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.68 (2H, s, Ar–$\underline{CH_2}$–Ar), 6.59–6.65 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz), 6.56 (1H, m, $\underline{NH}$), 7.29–7.73 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 114.5 (C-1), 150.7 (C-2), 108.1 (C-3), 122.7 (C-4), 122.3 (C-5), 128.2 (C-6), 127.7 (C-7), 127.1 (C-8), 36.9 (C-9), 145.7 (C-10), 128.8 (C-11), 137.9 (C-12), 140.1 (C-13), 56.8 (C-15), 130.5 (C-16), 126.5 (C-17 and C-21), 114.7 (C-18 and C-20), 158.9 (C-19), 55.3 (C-23), 52.6 (P–OCH_3), 53.5 (P–OCH_3). ^{31}P NMR (161.89, MHz, $CDCl_3$) δ: 22.07. EI-MS (*m*/*z*, %): 423 ($M^{+\bullet}$). Anal. calcd. for $C_{24}H_{26}NO_4P$: C, 68.07; H, 6.19; N, 3.31; Found: C, 68.01; H, 6.12; N, 3.26.

*Dimethyl (((9H-fluoren-2-yl)amino)(4-nitrophenyl)methyl)-phosphonate (**4n**):* Yellow solid, mp: 186–188°C. IR (KBr) (ν_{max} cm^{-1}): 3332 (N–H), 2964 and 2863 (C–$H_{aromatic}$), 1260 (P=O), 754 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.55 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.74 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.78 (2H, s, Ar–$\underline{CH_2}$–Ar), 6.65–6.72 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz), 6.65 (1H, m, $\underline{NH}$), 7.24–7.74 (11H, m, $\underline{Ar–H}$). ^{13}C NMR (100.56 MHz, $CDCl_3$) δ: 114.7 (C-1), 150.5 (C-2), 108.3 (C-3), 121.6 (C-4), 122.2 (C-5), 127.9 (C-6), 128.4 (C-7), 126.7 (C-8), 37.1 (C-9), 145.1 (C-10), 129.3 (C-11), 138.7 (C-12), 140.8 (C-13), 56.5 (C-15), 138.3 (C-16), 128.2 (C-17 and C-21), 125.7 (C-18 and C-20), 151.7 (C-19), 52.5 (P-OCH_3), 53.6 (P-OCH_3). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.16. EI-MS (*m*/*z*, %): 424 ($M^{+\bullet}$). Anal. calcd. for $C_{22}H_{21}N_2O_5P$: C, 62.26; H, 4.99; N, 6.60; Found: C, 62.20; H, 4.91; N, 6.51.

*Dimethyl (1-((9H-fluoren-2-yl)amino)butyl)phosphonate (**4o**):* Brown solid, mp: 134–136°C. IR (KBr) (ν_{max} cm^{-1}): 3313 (N–H), 2965 and 2865 (C–$H_{aromatic}$), 1255 (P=O), 759 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.54 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.76 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.52 (2H, s, Ar–$\underline{CH_2}$–Ar), 6.63–6.66 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz), 5.79 (1H, m, $\underline{NH}$), 7.23–7.67 (7H, m, $\underline{Ar–H}$), 1.34–1.49 (4H, m, 2 × $\underline{CH_2}$), 1.12 (3H, t, $\underline{CH_3}$). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.62. EI-MS (*m*/*z*, %): 345 ($M^{+\bullet}$). Anal. calcd. for $C_{19}H_{24}NO_3P$: C, 66.07; H, 7.00; N, 4.06; Found: C, 66.00; H, 6.92; N, 4.01.

*Dimethyl (1-((9H-fluoren-2-yl)amino)pentyl)phosphonate (**4p**):* Pale yellow solid, mp: 140–142°C. IR (KBr) (ν_{max} cm^{-1}): 3314 (N–H), 2964 and 2857 (C–$H_{aromatic}$), 1257 (P=O), 755 (P–$C_{aliphatic}$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3. 47 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.70 (3H, d, $^3J_{H-P}$ = 10.4 Hz, P–O$\underline{CH_3}$), 3.51 (2H, s, Ar–$\underline{CH_2}$–Ar), 6.65–6.69 (1H, dd, $^2J_{H-P}$ = 8.4 Hz and $^3J_{H-H}$ = 2.0 Hz), 5.54 (1H, m, $\underline{NH}$), 7.27–7.56 (7H, m, $\underline{Ar–H}$), 1.31–1.54 (6H, m, 3 × $\underline{CH_2}$), 1.06 (3H, t, $\underline{CH_3}$). ^{31}P NMR (161.7, MHz, $CDCl_3$) δ: 21.32. EI-MS (*m*/*z*, %): 359 ($M^{+\bullet}$). Anal. calcd. for $C_{20}H_{26}NO_3P$: C, 66.84; H, 7.29; N, 3.90; Found: C, 66.76; H, 7.21; N, 3.83.

Pharmacology

Cell lines

Human cervical cancer cell line (HeLa) and human breast adenocarcinoma cell line (SK-BR-3) were obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from BioWhittaker®, and Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), and other cell culture materials were purchased from Sigma® (USA). Cells were cultured either in DMEM (HeLa) or in RPMI (SK-BR-3) media supplemented with 10% v/v heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/ mL streptomycin. Cells were maintained in culture at 37°C in an atmosphere of 5% CO_2.

Cell culture

Cell proliferation or viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide) assay. HeLa cells were cultured in T-75 tissue culture flasks (Nunc, Denmark) at 37°C in a 5% CO_2 humidified incubator using appropriate media supplemented with DMEM containing 10% heat-inactivated FBS. Similarly, SK-BR-3 cells were cultured in RPMI media. Cells were seeded in each well containing 100 μL of medium at a final density of 2×10^4 cells/well, in 96-well microtiter plates under identical conditions. After overnight incubation, the cells were treated with different concentrations of test compounds (0.1–100 μg/mL) or DMSO (carrier solvent) in a final volume of 200 μL with three replicates each. After 24 h, 10 μL of MTT (5 mg/ mL) was added to each well and the plate was incubated at 37°C in the dark for 4 h. Then the media along with MTT was removed and the formazan crystals were solubilized by adding DMSO (100 μL/well). Finally, the reduction of MTT was quantified by reading the absorbance at 570 nm using GENios® microplate reader (Tecan Austria GmbH, Austria). The effects of the test compounds on cell viability were evaluated using untreated cells added with DMSO as control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (the concentration at which 50% of the cells are dead) concentrations were calculated using the respective regression equation.

The altered morphology of exposed cells (1×10^4 cells/well) at the respective IC_{50} concentrations of the most potential compound was studied after 24 h using a phase contrast microscope (DMI6000B, Leica Microsystems, Wetzlar, Germany). Subsequently, the cells were Hoechst stained to observe the nuclear/chromosomal condensation that occurred due to the treatment of the highly active test compound. For staining, 96-well cell culture plates were used to culture the cells (1×10^4 cells/well) in three replicates to treat with the best test compound. Then the cells were incubated at 37°C overnight and the media was removed to wash the cells twice with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS for 1 day at 4°C. Further, the cells were stained with 1 μg/mL of the fluorescent DNA-binding dye, Bisbenzimide Hoechst 33342 stain, and incubated for 20 min at room temperature to reveal nuclear condensation/aggregation due to the effect of the test compound. The Hoechst-stained cells were visualized and photographed under fluorescence microscope (CTR 6000; Leica Microsystems).

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