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Phospho sulfonic acid: an efficient and recyclable solid acid catalyst for the solvent-free synthesis of α-hydroxyphosphonates and their anticancer properties†

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As a means of developing biologically active compounds, a series of α-hydroxyphosphonates, $ArC(OH)PO(OR)_2$ (Ar = 5-G-2-OHPh; R = Me, Et, iPr, Bu; G = H, Br, Cl, NO_2), have been synthesized by reacting diversely substituted salicylaldehydes with dialkyl phosphites by employing the Pudovik reaction in the presence of phospho sulfonic acid under neat conditions. The cytotoxicity of the compounds was tested in two human cancer cell lines by using MTT assay. Compounds bearing R = Et; G = Cl, R = Bu; G = Cl, and R = Et; G = NO_2 showed good anticancer activity.

1. Introduction

Cancer is a class of diseases involving rapid cell growth with possible spreading to other parts of the body. The most common types of cancer in males are lung, prostate, colorectal, and stomach cancers, whereas breast, colorectal, lung, and cervical cancers are the most common cancer types in females. In addition, approximately 22% of cancers are caused by tobacco use[1,2] while an additional 10% are associated with obesity, poor diet, lack of physical activity, and alcohol consumption. Other factors associated with cancer include certain infections, exposure to ionizing radiation, and environmental pollutants. Furthermore, in the developing world, nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C, and human papillomavirus.

α-Hydroxyphosphonates (HPPs) are an important class of organophosphorus compounds (OPCs)[3] because of their wide range of biological activities, including anticancer, antibacterial, antiviral, and anti-oxidant activities.[4,5] Additionally, HPPs are structural analogs of α-hydroxyphosphonic acids[6–8] and can act as enzyme inhibitors for farnesyl protein transferase (FPT),[9] human protein tyrosine phosphatase (PTP),[10–12] purine nucleoside phosphorylase (PNP),[13] 5-enolpyruvylshikimate-3-phosphate synthase (EPSP),[14] and human rennin.[15,16] They also serve as useful precursors in the synthesis of other biologically important phosphonates such as α-amino,[17] α-diketo,[18] α-keto,[19] α-halo,[20] and α-acetoxy phosphonates.[21]

HPPs are primarily synthesized by the Pudovik reaction, in which the C–P bond is formed by the addition of a dialkyl phosphite to an unsaturated system, and the Abramov reaction, in which the C–P bond is formed by the addition of a trialkyl phosphite (TAP) to an unsaturated system. Carbonyl compound hydrophosphylation is usually mediated by a base-catalyzed reaction.[22] Numerous bases can mediate HPP formation, including ethyl magnesium bromide,[23] quinine,[24] potassium fluoride (KF)–alumina,[25] and magnesium oxide (MgO).[26] Furthermore HPPs are also synthesized by Brønsted acid catalysts such as Amberlyst-15,[27] HCl,[28] oxalic acid,[29] and guanidine–HCl.[30] Nevertheless, Brønsted acids are moisture sensitive; thus, the reaction requires high catalyst loading, a long reaction time, high temperature, and ultrasonication or a microwave to proceed. Hence, the development of an inexpensive protocol for α-hydroxyphosphonate synthesis with easy accessibility, a low toxicity solid acid catalyst, and the ability to proceed under neat conditions is highly preferred for the synthesis of HPPs.

In recent years, the use of recyclable solid acid catalysts has received significant attention in organic synthesis as a result of their profitable and environmental benefits. These types of reagents not only make simpler purification processes but also help in reducing the liberation of toxic reaction residues into the environment. The solid acid catalysts are simply removed from the reaction mixture by filtration or centrifugation without the need for neutralization, thereby enabling more eco-friendly processes. Phospho sulfonic acid (PSA) is a non-corrosive, non-volatile, recyclable, and eco-friendly solid acid catalyst. It has been used as a promising solid acid catalyst in various organic transformations.[31,32]

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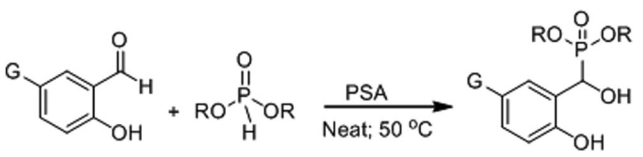

Compound	1	2	3	4	5	6	7	8
G	H	H	H	H	Br	Br	Br	Br
R	Me	Et	^{i}Pr	Bu	Me	Et	^{i}Pr	Bu

Compound	9	10	11	12	13	14	15	16
G	Cl	Cl	Cl	Cl	NO_2	NO_2	NO_2	NO_2
R	Me	Et	^{i}Pr	Bu	Me	Et	^{i}Pr	Bu

Scheme 1 Synthesis of a series of α-hydroxyphosphonates (**1–16**).

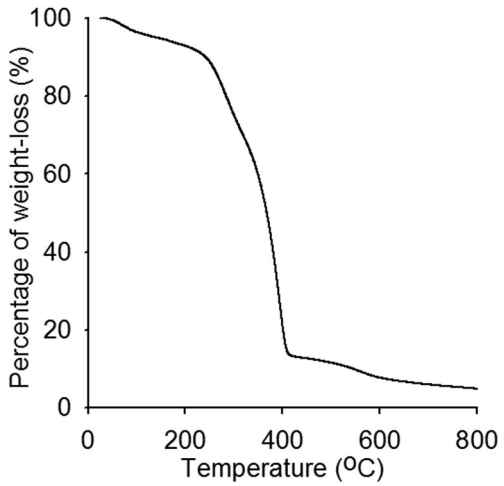

Fig. 1 TGA thermogram of PSA.

Regardless of an extensive survey there have been no reports on the anticancer properties of the synthesised HPPs. Thus, our aim was to develop an environmentally friendly reaction methodology to obtain novel HPPs with anticancer activity. Herein, we report an eco-friendly one-pot synthesis of HPPs, in which labile, P–H-containing dialkyl phosphite (DAP) underwent nucleophilic addition to salicylaldehyde (SA) in the presence of PSA under neat conditions (Scheme 1).

2. Results and discussion

In this study, a diversity of biologically potent HPPs have been synthesized by using the ecofriendly PSA catalyst under neat conditions. This method offers several advantages over the existing methodologies in terms of operational procedure, yield of the product, stability of the catalyst and recyclability. In addition we studied the anticancer properties of our synthesized compounds for the possible cancer cell lines. The synthesized PSA is fully characterized by infrared spectroscopy, X-ray powder diffraction (XRD), and scanning electron microscopy-energy-dispersive X-ray spectroscopy (SEM-EDX).[32] Furthermore we also studied the stability of PSA using thermogravimetric analysis (TGA) (Fig. 1). The complete degradation occurs between 244 and 413 °C, indicating that PSA is stable up to around 200 °C.

2.1. Chemistry

Our lab has focused on the development of eco-friendly syntheses to reduce waste and establish operationally simple synthetic methodologies.[32–34] In continuation with these studies, we describe the efficacy of PSA for the synthesis of novel HPPs using a two component reaction between various SAs and different dialkyl phosphites at ambient temperatures.

In general the Pudovik reaction requires longer reaction times, higher reaction temperatures, and higher catalyst loading than the Abramov reaction, because DAPs are less reactive than trialkyl phosphites (TAPs). The Pudovik reaction of SA (1 mmol) with DAP (1 mmol) was first performed with a variety of catalysts at 50 °C by using a solvent-free method (Table 1). Primary acidic

Table 1 Effect of various catalysts on the synthesis of compound **1** under neat conditions at 50 °C[a]

Entry	Catalyst	Catalyst amount (mol%)	Time (min)	Yield[b] (%)
1	$ZnCl_2$	10	180	50
2	$ZrOCl_2$	10	210	55
3	$BF_3{\cdot}SiO_2$	10	180	45
4	$SiO_2{\cdot}SO_3H$	10	240	58
5	Amberlyst-15	10	180	75
6	$PEG{\cdot}SO_3H$	10	300	67
7	PSA	0.1	48	82
8	PSA	0.3	35	86
9	PSA	0.5	32	94
10	PSA	1.0	30	94

[a] Reaction conditions: salicylaldehyde = 1 mmol, dialkyl phosphite = 1 mmol, and PSA = 0.5 mol%. [b] Isolated yield measured gravimetrically.

species such as zinc chloride ($ZnCl_2$), zirconyl chloride ($ZrOCl_2$) silica-supported boron trifluoride (BF_3–SiO_2) and silica–sulfonic acid (10 mol%) were examined at room temperature over a 3–4 h reaction period. Compound **1** was obtained with yields of 50%, 55%, 45%, and 30%, respectively (entries 1–4). We then examined the polymer-based catalysts Amberlyst-15 and polyethylene glycol-supported sulfonic acid (PEG–SO_3H), which yielded little products (entries 5 and 6). Finally, we examined solid acid catalyst PSA (entry 7), which is an efficient catalyst for producing the desired product **1** in good yields.

In order to compare the efficiency of the neat conditions with solvent-based conditions, the model reaction was re-examined with PSA in different solvents (Table 2). When the reaction was performed in protic solvents, such as methanol (MeOH), ethanol (EtOH), or water (H_2O), the reaction proceeded slowly and recorded reduced product yields (Table 2, entries 2–4). However, when using aprotic solvents such as chloroform ($CHCl_3$), methyl cyanide (CH_3CN), and dichloromethane (CH_2Cl_2), the reactions proceeded faster and resulted in higher product yields.

To determine the optimal molar catalyst loading of PSA, the model reaction was carried out by using 0.1, 0.3, 0.5, and 1 mol% of PSA under neat conditions at room temperature (rt), yielding

Table 2 Effects of various solvents on the synthesis of compound **1**[a]

Entry	Solvent	Time (min)	Yield (%)
1	Solvent-free	34	94
2	MeOH	60	65
3	EtOH	80	60
4	H_2O	90	55
5	CH_3Cl	50	85
6	CH_3CN	45	88
7	CH_2Cl_2	35	90

[a] Reaction conditions: salicylaldehyde = 1 mmol, dialkyl phosphite = 1 mmol, and PSA = 0.5 mol%.

82, 86, 94, and 94% product, respectively. Increasing the amount of catalyst (0.5 mol%) had no additional effects on the reaction progress. Having determined the optimal conditions (entry 9, Table 1), we next established the generality of this methodology by using various substituted SAs with different DAPs. All reactions were performed with 0.5 mol% of PSA under solvent-free conditions. The results are summarized in the Experimental section. All reactions undergo easily and products are obtained in good yields. SAs containing halogens and nitro substituents form the corresponding products, as summarized in the Experimental section, in good product yields.

The reusability of PSA was also examined for the preparation of HPPs 1, formed from the reaction of SA with DMP. The catalyst was easily recovered by adding ethanol to the reaction mixture; the insoluble PSA could be separated by simple filtration, washed twice with ethanol (30 mL), and finally dried under vacuum. The catalyst displayed good reusability after 5 runs (Fig. 2).

The structures of all the compounds were confirmed by infrared (IR) spectroscopy, ^{1}H, ^{13}C, and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. The IR spectra of compounds **1–16** showed the expected absorption bands at 3376–3268, 1238–1221, and 983–863 cm^{-1}, which are attributed to O–H, P═O, and P–C stretching vibrations, respectively.[35] In the ^{1}H NMR spectrum, the P–CH proton signal appeared as a doublet or a multiplet in the region of 4.17–3.87 ppm, due to coupling with phosphorus and ethyleneoxy protons.[36] The remaining proton signals were observed in the expected regions. In the ^{13}C NMR spectra, the doublet at 70.4–69.6 ppm ($^1J_{P-C}$ = 162.0–140.2 Hz) confirms the presence of a methine carbon, which is directly attached to a phosphorus atom. In the ^{31}P NMR spectra of all synthesized compounds (**1–16**), a signal was observed in the region of 22.8–18.3 ppm. Detailed descriptions of the spectral data for all compounds (**1–16**) are given in the Experimental section.

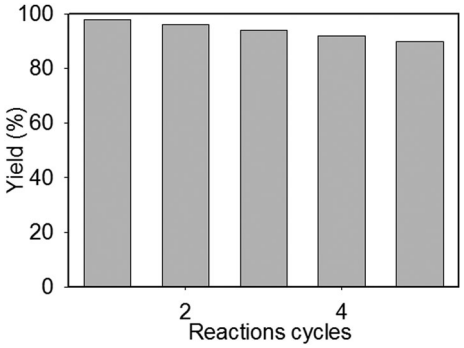

Fig. 2 Effect of recycling of PSA on the yield of compound **1**.

2.2. Cytotoxicity

The cell lines used in the present study were the human alveolar basal epithelial adeno carcinoma cell line (A549) and the epidermal cancer cell line (KB). The growth inhibitory effects of the HPP derivatives were evaluated by using the MTT. As shown in Fig. 3a and b, the compounds (**1–16**) showed no cytotoxicity towards the two cell lines at concentrations less than 2 μM. Most of the compounds were non-cytotoxic at 40 μM. However, compound **12**

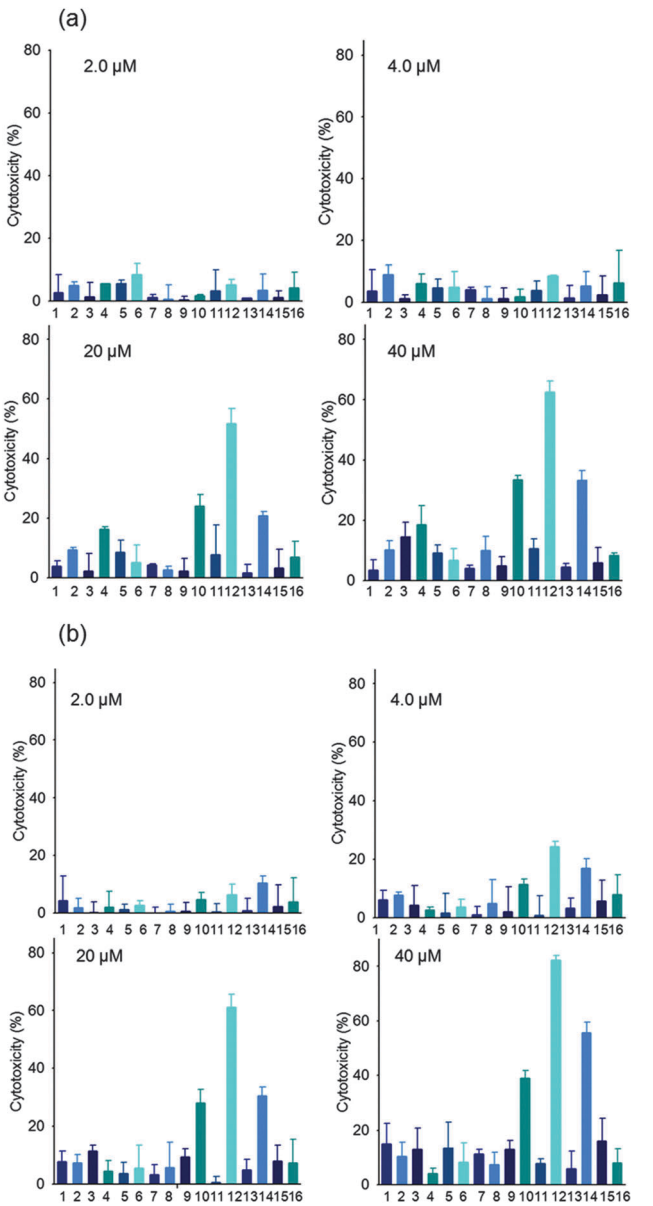

Fig. 3 The cytotoxicity of the synthesized compounds (**1–16**) against (a) A549 and (b) KB cancer cells at concentrations of 2.0, 4.0, 20, and 40 μM for 24 h.

showed some cytotoxicity in A549 cells, while two other compounds (**10** and **14**) exhibited a noticeable increase in cytotoxicity and showed significant dose-dependent effects on the proliferation of both cell lines. At a concentration of 20 μM, compounds **10**, **12**, and **14** were cytotoxic (24%, 52%, and 21% in A549 cells and 28%, 61%, and 30% in KB cells, respectively). Similarly, at a concentration of 40 μM, compounds **10**, **12**, and **14** were also cytotoxic (34%, 62%, and 33% in A549 cells and 39%, 82%, and 55% in KB cells, respectively). These data indicate that compounds **10**, **12**, and **14** are potentially cytotoxic, and in particular, compound **12** would be a promising candidate for anticancer therapy.

2.3. Structure activity relationship (SAR) study

A SAR study revealed that the different-substituted HPPs induced anticancer effects in a variety of ways on the two human cancer cell lines. The synthesized compounds (**1–16**) consisted of different substitutions on the benzene ring and different alkoxy-linkages on the phosphorus atom. In these two cell lines, the chloro-substituent on the benzene moiety showed enhanced cytotoxicity as compared to the bromo and no substitution. Interestingly, the nitro-substituted HPPs also showed good activity. The alkoxy substitution on the phosphorus atom played a key role in cytotoxicity. As shown previously, ethoxy and butoxy groups on the phosphorous atom improved activity as compared to isopropoxy and methoxy groups,[37] and the ethoxy substitution on the phosphorus atom showed greater activity. We studied four different alkoxy groups on the phosphorus atom, of which the ethoxy and butoxy substitutions showed incredible activity.

3. Conclusions

We have described here the use of PSA as a highly efficient catalyst for the synthesis of α-hydroxyphosphonates under neat conditions at 50 °C. The readily synthesized phospho sulfonic acid showed many advantages in the synthesis of α-hydroxyphosphonates by employing the Pudovik reaction like operational simplicity, mild reaction conditions, good yield, easy work-up, and recyclability, making it an eco-friendly alternative to currently existing protocols. The anticancer properties of the synthesized compounds (**1–16**) were evaluated, and all the compounds showed moderate activity at 20 and 40 μM, whereas compounds **10**, **12**, and **14** showed remarkable activity against the two tested cancer cell lines (A549 and KB).

4. Experimental

4.1. General

The substituted 2-hydroxybenzaldehydes (SAs), diethyl phosphite (DEP) (Sigma-Aldrich Co.), dibutylphosphite (DBP), (Fisher Scientific UK Ltd), diisopropylphosphite (DIP) (Alfa-Aesar), and the tetrazolium dye (MTT) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Fetal bovine serum (FBS), antibiotics, and Dulbecco's modified Eagle's medium (DMEM) were purchased from Hycolne Co. All experiments were carried out under neat conditions. Pre-coated silica gel plates (Merck Chem., Germany) were developed by using iodine in analytical thin layer chromatography (TLC). Melting points were uncorrected and determined on a digital Stuart SMP3 apparatus (Bibby Scientific Limited, Staffordshire, UK). The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{31}P NMR (161.9 MHz) spectra were recorded on a Varian INOVA 400 NMR spectrometer at room temperature. Chemical shift values were relative to tetramethylsilane (TMS, Me_4Si). The data are presented as follows: a chemical shift (ppm), a multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, brs = broad singlet), and a coupling constant J (Hz). The Fourier transform infrared (FT-IR) spectra were recorded on a Shimadzu IR Prestige 21 spectrometer at room temperature. The samples were analyzed as KBr discs in the range 3500–500 cm^{-1}. The XRD analysis was performed by using an automatic Philips powder diffractometer with nickel-filtered Cu Kα radiation. The diffraction pattern was collected in the 2 range of 0–80° in steps of 0.02° and counting times of 2 s $step^{-1}$. The microstructures of the samples and energy dispersive X-ray spectroscopy (EDX) were investigated using an S-3000 scanning electron microscope (SEM; Hitachi, Japan) and thermogravimetric analysis was carried out by using a TGA N-1000 (Scinco, Seoul, and Republic of Korea).

4.2. Synthesis of PSA

The PSA is synthesized by using the earlier reported method.[32]

The isolated yield is 97% and the mp is 128–130 °C.

4.3. General procedure for the preparation of HPPs 1–16

PSA (0.5 mol%) was added to a mixture of SA and diethylphosphite (1 mmol each) under neat conditions at 50 °C with stirring. The progress of the reaction was monitored by TLC. After completion of the reaction, the product was extracted with 20 mL of ethanol and the solvent was evaporated under reduced pressure. The crude product was purified by recrystallization from ethanol solution. To perform recrystallization, the crude product was saturated in boiling ethanol and the solution mixture was filtered when it was hot in order to remove the undissolved solid. The hot filtrate was cooled to room temperature to obtain the pure compounds. A similar experimental procedure was adopted for the synthesis of all HPPs.

4.4. Spectral characterization

4.4.1. Dimethyl (hydroxy(2-hydroxyphenyl)methyl)phosphonate (1). 90% yield after 33 min of reaction; solid, mp 91–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.54–6.86 (m, 4H, Ar–H), 4.80 (s, 2H, –OH), 3.87 (d, 1H, J = 16.4 Hz, –P–CH), 3.45 (d, 6H, J = 6.8 Hz, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 159.3, 130.7, 127.6, 125.3, 120.1, 116.1, 69.2 (d, $^{1}J_{CP}$ = 148.4 Hz), 53.2, 25.6; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 20.2; FTIR (KBr): ν = 3431, 3415, 2768, 2164, 1650, 1248, 1034, and 961 cm^{-1}.

4.4.2. Diethyl (hydroxy(2-hydroxyphenyl)methyl)phosphonate (2). 91% yield after 35 min of reaction; solid, mp 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.42–6.72 (m, 4H, Ar–H), 4.82 (s, 2H, –OH), 4.14–4.01 (m, 4H, $-OCH_2$), 3.92 (d, 1H, J = 17.2 Hz, –P–CH), 0.92 (t, 6H, J = 7.4 Hz, $-CH_3$); ^{13}C NMR

(100 MHz, $CDCl_3$): δ (ppm) 156.3, 134.8, 130.8, 128.1, 126.3, 120.6, 115.2, 66.0 (d, $^1J_{CP}$ = 148.4 Hz), 62.2, 18.5, 13.0; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 19.8; FTIR (KBr): ν = 3345, 3160, 2892, 2182, 1641, 1490, 1241, 1032, and 965 cm^{-1}.

4.4.3. Diisopropyl (hydroxy(2-hydroxyphenyl)methyl)phosphonate (3). 94% yield after 30 min of reaction; solid, mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.36–6.91 (m, 4H, Ar–H), 4.94 (s, 2H, –OH), 4.71–4.64 (m, 1H, –OCH), 4.45–4.37 (m, 1H, –OCH), 3.78 (d, 1H, J = 16.0 Hz, –P–CH), 1.84–1.01 (m, 12H, $-(CH_3)_4$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 155.2, 129.7, 128.8, 122.5, 120.3, 118.8, 72.6, 70.5 (d, $^1J_{CP}$ = 162.0 Hz), 24.0; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 19.3; FTIR (KBr): ν = 3339, 3172, 2964, 2174, 1644, 1492, 1320, 1020, and 970 cm^{-1}.

4.4.4. Dibutyl (hydroxy(2-hydroxyphenyl)methyl)phosphonate (4). 92% yield after 35 min of reaction; solid, mp 97–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37–6.95 (m, 4H, Ar–H), 5.01 (s, 2H, –OH), 4.14–3.80 (m, 5H, $-OCH_2$ & P–CH), 1.68–1.13 (m, 8H, $-(CH_2)_4$), 0.97 (t, 6H, J = 6.8 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 155.5, 129.4, 128.5, 122.0, 119.9, 117.9, 70.1 (d, $^1J_{CP}$ = 161.2 Hz), 67.4, 67.1, 32.3, 22.5, 18.6, 13.4; ^{31}P NMR (161.9 MHz, $CDCl_3$):δ (ppm) 24.2; FTIR (KBr): ν = 3335, 3150, 1656, 1474, 1248, 1034, and 876 cm^{-1}.

4.4.5. Dimethyl((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (5). 91% yield after 40 min of reaction; solid, mp 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.47–6.84 (m, 3H, Ar–H), 4.84 (s, 2H, –OH), 4.12 (d, 1H, J = 18.8 Hz, –P–CH), 3.56 (d, 3H, J = 6.8 Hz, OCH_3), 3.45 (d, 3H, J = 6.8 Hz, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 161.3, 133.9, 132.4, 124.2, 120.0, 118.2, 65.6 (d, $^1J_{CP}$ = 161.7 Hz), 52.3; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 20.1; FTIR (KBr): ν = 3322, 3134, 2982, 2864, 1652, 1034, and 871 cm^{-1}.

4.4.6. Diethyl((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (6). 93% yield after 43 min of reaction; solid, mp 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.44 (t, 1H, J = 2.4 Hz, Ar–H), 7.34 (t, 1H, J = 2.0 Hz, Ar–H), 6.72 (d, 1H, J = 8.8 Hz, Ar–H), 4.82 (s, 2H, –OH), 4.12–3.98 (m, 4H, $-OCH_2$), 3.88 (d, 1H, J = 19.2 Hz, –P–CH), 0.96 (t, 3H, J = 7.4 Hz, $-CH_3$), 0.89 (t, 3H, J = 7.4 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 158.3, 132.8, 129.7, 125.5, 120.4, 117.2, 66.9 (d, $^1J_{CP}$ = 160.2 Hz), 64.5, 13.5; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 20.4; FTIR (KBr): ν = 3332, 3128, 2959, 2139, 1462, 1249, 1045, and 878 cm^{-1}.

4.4.7. Diisopropyl((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (7). 92% yield after 36 min of reaction; solid, mp 98–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.25 (t, 1H, J = 2.4 Hz, Ar–H), 7.16–6.12 (m, 2H, Ar–H), 5.06 (s, 2H, –OH), 4.70–4.58 (m, 2H, –OCH), 3.95 (d, 1H, J = 17.2 Hz, –P–CH), 1.35–1.26 (m, 12H, $-(CH_3)_4$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 158.8, 134.1, 130.6, 128.1, 121.0, 116.3, 71.8, 69.4 (d, $^1J_{CP}$ = 146.5 Hz), 24.5; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 22.6; FTIR (KBr): ν = 3356, 3135, 2975, 1465, 1296, 1046, and 876 cm^{-1}.

4.4.8. Dibutyl((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (8). 94% yield after 40 min of reaction; solid, mp 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.24 (d, 1H, J = 2.2 Hz, Ar–H), 7.16 (d, 1H, J = 3.8 Hz, Ar–H), 6.52 (d, 1H, J = 6.6 Hz, Ar–H), 4.97 (s, 2H, –OH), 4.18–3.99 (m, 4H, $-OCH_2$), 3.89 (d, 1H, J = 17.2 Hz, –P–CH), 1.81–1.48 (m, 8H, $-(CH_2)_4$), 0.90 (t, 6H, J = 7.2 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 156.7, 135.6, 132.8, 124.1, 120.2, 116.3, 67.1 (d, $^1J_{CP}$ = 148.4 Hz), 65.2, 18.6, 13.5; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 18.7; FTIR (KBr): ν = 3326, 3125, 2925, 2188, 1748, 1276, 1056, and 866 cm^{-1}.

4.4.9. Dimethyl((5-chloro-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (9). 93% yield after 20 min of reaction; solid, mp 98–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.33 (d, 1H, J = 4.2 Hz, Ar–H), 7.23 (d, 1H, J = 3.2 Hz, Ar–H), 6.94 (d, 1H, J = 5.2 Hz, Ar–H), 4.87 (s, 2H, –OH), 3.98 (d, 1H, J = 14.2 Hz, –P–CH), 3.82 (d, 3H, J = 7.0 Hz, OCH_3), 3.72 (d, 3H, J = 7.0 Hz, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 155.4, 129.8, 128.5, 121.5, 120.3, 118.0, 69.8, (d, $^1J_{CP}$ = 162.0 Hz), 54.3 (d, J = 7.3 Hz), 53.9 (d, J = 7.3 Hz); ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 20.9; FTIR (KBr): ν = 3385, 3168, 2974, 2154, 1632, 1492, 1265, 1044, and 892 cm^{-1}.

4.4.10. Diethyl((5-chloro-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (10). 92% yield after 40 min of reaction; solid, mp 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.42 (t, 1H, J = 2.4 Hz, Ar–H), 7.24 (t, 1H, J = 2.0 Hz, Ar–H), 6.62 (d, 1H, J = 8.8 Hz, Ar–H), 4.72 (s, 2H, –OH), 4.16–3.99 (m, 4H, $-OCH_2$), 3.90 (d, 1H, J = 15.2 Hz, –P–CH), 0.92 (t, 3H, J = 7.4 Hz, $-CH_3$), 0.84 (t, 3H, J = 7.4 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 153.4, 136.5, 128.7, 126.2, 120.3, 117.0, 66.9 (d, $^1J_{CP}$ = 160.2 Hz), 64.5, 13.5; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 20.4; FTIR (KBr): ν = 3330, 3123, 2956, 2133, 1468, 1240, 1035, and 898 cm^{-1}.

4.4.11. Diisopropyl((5-chloro-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (11). 92% yield after 45 min of reaction; solid, mp 92–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.26–7.22 (m, 2H, –ArH), 6.72–6.59 (m, 1H, –ArH), 4.71 (s, 2H, –OH), 4.40–4.30 (m, 2H, –OCH), 4.13 (d, 1H, J = 17.4 Hz, –P–CH), 1.31–1.04 (m, 12H, $-(CH_3)_4$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 157.1, 133.5, 131.1, 122.5, 118.6, 110.2, 72.0, 64.5 (d, $^1J_{CP}$ = 167.0 Hz), 23.9; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 23.4; FTIR (KBr): ν = 3340, 3161, 2982, 2063, 1691, 1450, 1283, 1041, and 932 cm^{-1}.

4.4.12. Dibutyl((5-chloro-2-hydroxyphenyl)(hydroxy)methyl)phosphonate (12). 94% yield after 30 min of reaction; solid, mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.72–6.17 (m, 3H, Ar–H), 4.81 (s, 2H, –OH), 4.12–3.77 (m, 5H, $-OCH_2$ & P–CH), 1.74–1.26 (m, 8H, $-(CH_2)_4$), 0.94 (t, 6H, J = 7.2 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 157.3, 133.3, 133.2, 133.0, 118.3, 109.8, 66.9 (d, $^1J_{CP}$ = 146.0 Hz), 60.6 (d, $^1J_{CP}$ = 6.4 Hz), 27.0, 20.5, 16.7, and 13.9; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 23.6; FTIR (KBr): ν = 3362, 3129, 2896, 2085, 1694, 1492, 1286, 1040, and 958 cm^{-1}.

4.4.13. Dimethyl (hydroxy(2-hydroxy-5-nitrophenyl)methyl)phosphonate (13). 91% yield after 30 min of reaction; solid, mp 108–110 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.20 (s, 1H, –OH), 9.54 (s, 1H, –OH), 7.42 (d, 1H, J = 3.2 Hz, Ar–H), 7.08–6.97 (m, 1H, Ar–H), 6.84–6.71 (m, 1H, Ar–H), 5.39 (dd, 1H, J = 14.2 Hz, 10.5 Hz –P–CH), 3.61 (d, 3H, J = 12.0 Hz, OCH_3), 3.41 (d, 3H, J = 12.0 Hz, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 154.6, 136.4, 132.1, 124.9, 119.2, 109.9, 62.6 (d, $^1J_{CP}$ = 164.9 Hz), 53.5; ^{31}P NMR (161.9 MHz, DMSO-d_6): δ (ppm) 21.4; IR (KBr): ν = 3385, 3168, 2974, 2154, 1632, 1492, 1265, 1044, and 892 cm^{-1}.

4.4.14. Diethyl (hydroxy(2-hydroxy-5-nitrophenyl)methyl)-phosphonate (14). 89% yield after 35 min of reaction; solid, mp 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.25 (s, 1H, Ar–H), 7.18 (d, 1H, J = 7.6 Hz, Ar–H), 7.06 (t, 1H, J = 7.8 Hz, Ar–H), 4.78 (s, 2H, –OH), 4.15–3.82 (m, 4H, $-OCH_2$), 3.03 (s, 1H, –P–CH), 1.33 (t, 3H, J = 7.2 Hz, $-CH_3$), 1.13 (t, 3H, J = 7.2 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 161.2, 148.7, 137.3, 127.8, 126.5, 124.5, 119.1, 117.1, 63.1 (d, $^1J_{CP}$ = 148.9 Hz), 60.8, 36.6, 13.4; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 21.8; FTIR (KBr): ν = 3342, 3162, 2985, 2068, 1692, 1458, 1285, 1040, and 939 cm^{-1}.

4.4.15. Diisopropyl (hydroxy(2-hydroxy-5-nitrophenyl)methyl)-phosphonate (15). 93% yield after 40 min of reaction; solid, mp 112–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.48 (d, 1H, J = 3.4 Hz, Ar–H), 8.30 (s, 2H, –OH), 8.06 (d, 1H, J = 2.4 Hz, Ar–H), 6.90 (d, 1H, J = 4.4 Hz, Ar–H), 4.65 (d, 1H, J = 18.4 Hz, –P–CH), 4.37–4.21 (m, 2H, –OCH), 1.29–0.71 (m, 12H, $-(CH_3)_4$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.9, 138.9, 127.4, 125.5, 121.8, 115.9, 71.3, 54.1 (d, $^1J_{CP}$ = 167.0 Hz), 24.5; ^{31}P NMR (161.9 MHz, DMSO-d_6): δ (ppm) 31.1; FTIR (KBr): ν = 3340, 3161, 2982, 2063, 1691, 1450, 1283, 1041, and 932 cm^{-1}.

4.4.16. Dibutyl (hydroxy(2-hydroxy-5-nitrophenyl)methyl)-phosphonate (16). 94% yield after 35 min of reaction; solid, mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.25–7.27 (m, 2H, Ar–H), 6.96 (d, 1H, J = 6.8 Hz, Ar–H), 4.81 (s, 2H, –OH), 4.12–3.77 (m, 5H, $-OCH_2$ & P–CH), 1.74–1.26 (m, 8H, $-(CH_2)_4$), 0.94 (t, 6H, J = 7.2 Hz, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 157.3, 133.3, 133.2, 133.0, 118.3, 109.8, 63.9 (d, $^1J_{CP}$ = 146.0 Hz), 60.6 (d, $^1J_{CP}$ = 6.4 Hz), 27.0, 20.5, 16.7, 13.9; ^{31}P NMR (161.9 MHz, $CDCl_3$): δ (ppm) 23.6; FTIR (KBr): ν = 3362, 3129, 2896, 2085, 1694, 1492, 1286, 1040, and 958 cm^{-1}.

4.5. Evaluation of cytotoxicity

The two cancer cell lines (A549 and KB) were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and antibiotics (100 IU mL^{-1} of penicillin G sodium and 100 μg mL^{-1} of streptomycin sulfate). The cells were maintained in an incubator supplied with 5% CO_2 air in a humidified atmosphere at 37 °C. The cells were transferred to a 96 well plate at 7 × 104 cells per well. The next day, the medium was exchanged for fresh medium. When the cells reached a confluence of 80%, the compounds were added at different concentrations (2.0, 4.0, 20, and 40 μM). The cells were then incubated for 24 h. The cytotoxicity was assayed using MTT and a micro plate-reader.

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