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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Nano Sb_2O_3 catalyzed green synthesis, cytotoxic activity, and molecular docking study of novel α-aminophosphonates

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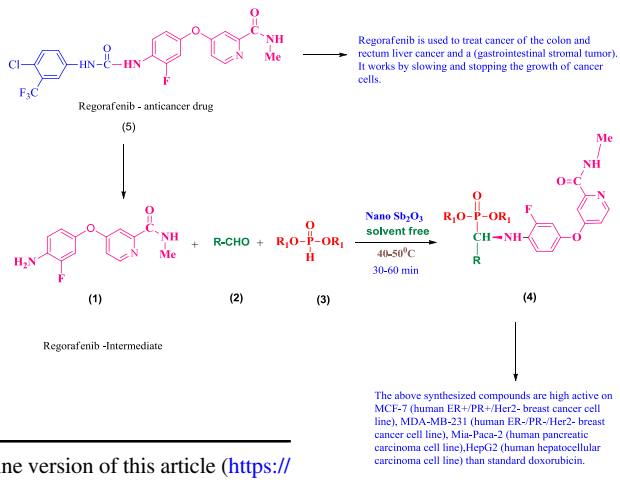
Abstract

Green synthesis of a series of novel dialkyl (aryl substituted)(2-fluoro-4-((2-methylcarbamoyl)pyridine-4-yl)oxy)phenyl) amino)methyl)phosphonates is accomplished by a simple and an efficient one pot three component reaction of 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide with different substituted aromatic aldehydes and dialkyl phosphite in the presence of nano Sb_2O_3 catalyst under solvent free conditions at 40–50 °C to obtain the title compounds. Excellent isolated product yields are obtained (85–95%) with high purity within shorter reaction times (30–60 min). The title compounds are characterized by IR, ^{1}H, ^{13}C, ^{31}P-NMR and mass spectral data. The synthesized compounds are screened for their anticell-proliferation activity on seven cell lines, Control cells–HEK293 (human embryonic kidney), DU-145 (human prostate adenocarcinoma), MCF-7 (human ER+/PR+/Her2− breast cancer), MDA-MB-231 (human ER−/PR−/Her2− breast cancer), Mia-Paca-2 (human pancreatic carcinoma), HeLa (human cervical cancer) cells as well as HepG2 (human hepatocellular carcinoma) cancer cell lines using Sulforhodamine B (SRB) assay method. Docking studies were carried out for all these synthesized compounds against topoisomerase-II by using Auto dock method. Doxorubicin was taken as standard. Compounds **4a**, **4c**, **4d**, **4e**, **4h**, **4i**, **4k**, and **4l** exhibited higher cytotoxic activity than the standard doxorubicin.

Graphical Abstract



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Keywords Novel α-aminophosphonates · Nano Sb_2O_3 · Cytotoxic assay · Docking studies

Introduction

α-aminophosphonates are the structural resemblance of α-amino acids, which has bought them to the center of immense attention in recent years (Moonen et al. 2004), α-aminophosphonates possess wide range of applications in the areas of biological (Smith and Bartlett 1998), medicinal (Yuan et al.1997), and industrial (Dhawan and Redmore 1987) as herbicides (Maier 1990), bactericides (Herczegh et al. 2002), fungicides (Ouimette and Coffey 1989; Yang et al. 2006), antibiotics (Hirschmann et al. 1994; Atherton et al. 1986), antitumor reagents (Bloemink et al. 1999; Jin et al. 2006; Kafarski and Lejczak 2001), antiviral agents (Xie et al. 2017), anti-thrombotic agents (Meyer and Barlett 1998), enzyme inhibitors (Allen et al. 1989), plant growth regulators (Maier and Spoerri 1991), protease inhibitors (Miller et al. 1998), peptide mimetics (Natchev 1988), and glutamine synthetase (Bayer et al. 1972), besides their use in industry as in prevention of metal corrosion (Kuznetsov et al. 2003).

At present, worldwide cancer is the leading cause of death. Despite recent progresses in cancer chemotherapy, high toxicity and low specificity of currently available medications have motivated the scientists to search for safer and more-effective anticancer drugs. Along with the extensive worldwide research, we have also started to study anticancer activity of some new α-aminophosphonates to develop new and safe anticancer drugs. We have synthesized various substituted aminophosphonates (Radha Rani et al. 2013). Present work involves the synthesis of aminophosphonates from the amine 3-(4-Amino-3-fluorobenzyl)-*N*-methylbenzamide having pyridine moiety, which is a part of the structural component obtained from Regorafenib (5) used to treat colon, rectum, and liver cancers. The idea to synthesize aminophosphonates (4) by using the above amine (1), which is phosphorylated to investigate their role on anticancer activity.

Several approaches have been developed for the synthesis of α-aminophosphonates. Two main pathways are: (i) Kabachnik-Fields is a single-pot, three component reaction, in which a carbonyl, an amine, and a di- or tri- alkylphosphite react in the presence of different catalysts (Ranu et al. 1999; Kaboudin and Rahmani 2003; Bhagat and Chakraborty 2007; Heydari and Arefi 2007) and, (ii) Pudovik reaction, where dialkylphosphites are added to imines by using a Lewis acid (Laschat and Kunz 1992; Schlemminger et al. 2001; Manjula et al. 2003; Doye 2004), or Lewis base (Klepacz and Zwierzak 2002; Simoni et al. 1998; Yager et al. 1994). In some reports, these reactions were carried out in a straight-forward one-pot procedures without any catalysts (Bashir et al. 2012; Kobra et al. 2014) whereas in most cases, it was performed using catalysts.

However, many of these reported methodologies are associated with several disadvantages like use of additional reagents, inflammable organic solvents, and longer reaction times. Performing organic reactions under solvent-free conditions is economically viable and environmentally benign. This synthetic protocol is simple, quick, and efficient with easy workup and high yields.

Nano chemistry has received much attention over the last few decades for scientific research and technological applications. This is owing to the exhibition of novel properties by the nanoparticles than bulk materials (Linderoth and Pedersen 1994). Fundamental properties of the nanoparticles depend strongly on their sizes and shapes (Salata 2004). Therefore, researchers have placed much effort in controlling the desired morphologies of these nanostructure materials.

Oxides of antimony (OA) play a key role among all the other metal oxides from V to VI groups (Huang et al. 2001). OA acts as a catalytic agent in organic synthesis. Nano Sb_2O_3 (Zhang et al. 2001) particles possess excellent catalytic performance in polyethylene terephthalate (PET) and organic synthesis industries. In the PET industry, Sb_2O_3 plays the role of a catalyst to produce PET plastic, which is used in the packaging of mineral water and soft drinks (Duh 2002; Spengler et al. 2001; Liu et al. 2001). Nano Sb_2O_3 possess low affinity to side products, easy recovery, insoluble in organic solvents, avoids unwanted color, and acts as a catalytic agent in organic synthesis (Liu and Iwasawa 2002). In the present work, we have carried out the synthesis of α-aminophosphonates in the presence of nano Sb_2O_3. So far, no reports are there on the synthesis of α-aminophosphonates by using the above catalyst.

The present work ensures the synthesis of novel α-aminophosphonates (4) from the amine containing substituted pyridine moiety (1) with different substituted aldehydes (2) and dialkyl phosphite (3) in the presence of nano Sb_2O_3 as catalyst under solvent free conditions at 40–50 °C within 30 min. The synthesized α-aminophosphonates (4) are screened for their anticancer activity on seven cell lines, control cells-HEK293 (human embryonic kidney cell line), DU-145 (human prostate adenocarcinoma cell line), MCF-7 (human ER+/PR+/Her2- breast cancer cell line), MDA-MB-231 (human $ER^-/PR^-/Her2^-$ breast cancer cell line), Mia-Paca-2 (human pancreatic carcinoma cell line), HeLa (human cervical cancer cell line) cells, and HepG2 (human hepatocellular carcinoma cell line) and evaluation of their

cytotoxic activity with respect to the standard doxorubicin as a reference drug. Docking studies were carried out for all the synthesized compounds by Auto dock method. Some of the title compounds were found to be potent than the standard drug.

Materials and methods

Solvents and reagents were procured from Sigma Aldrich, Sd. Fine and Qualigens. All the solvents and reagents were dried and purified by adopting the standard laboratory procedures and techniques. Infrared (IR) spectra were recorded as neat samples on Bruker Alpha-Eco ATR-FTIR [Attenuated Total Reflection–Fourier Transform Infrared] interferometer with single-reflection sampling module equipped with Zn-Se crystal, the data is expressed in reciprocal centimetres (cm^{-1}). NMR spectra were recorded on Jeol Resonance 400 MHz NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C, and 161.8 MHz for ^{31}P-NMR. NMR data were recorded in $CDCl_3$ were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P). Mass spectra were recorded on ESI-MS model instrument in positive mode. Mass spectra were recorded on ESI-MS model instrument in positive mode and elemental analysis was carried out in FLASH EA Thermo Finnigan 1112 instrument.

Results and discussion

Synthesis of a series of novel dialkyl (aryl substituted){2-Fluoro-4-[(2-methylcarbamoyl)pyridine-4-yl)oxy)phenyl) amino)methyl)phosphonates derivatives have been accomplished by one pot three component reaction of 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide with different substituted aromatic aldehydes and dialkyl phosphite in the presence of nano Sb_2O_3 as a reusable catalyst under solvent free conditions at 40–50 °C. Different catalysts were screened in order to optimize the reaction conditions Schemes 1 and 2.

To optimize the reaction conditions, the reaction was run under different solvents like tetrahydrofuran, dimethylformamide, acetonitrile, dichloromethane, toluene, methanol, and water. The yields obtained were low when compared with the solvent-free condition, which was shown in Table 1, presence of solvent decreases the yields of the product by retarding the reaction.

Further to know the effect of catalyst, at first the reaction was run in the presence of different conventional catalysts with different Lewis acids such as $BF_3.SiO_2$, $AlCl_3$, $BiCl_3$, $ZnCl_2$, CuO at 40 °C under neat conditions, which were shown in Table 2. The yields obtained are very low. When the reaction was run under the same conditions in the presence of nano Sb_2O_3 the yields obtained were very high.

Further to optimize the required nano Sb_2O_3 catalyst concentration, the reaction was carried out at different concentrations 1, 2.5, 5, and 7.5 mol (%). It was observed that 5 mol% of catalyst is the optimum quantity, which was sufficient to drive the reaction to completion and good yields were obtained that was shown in Fig. 1.

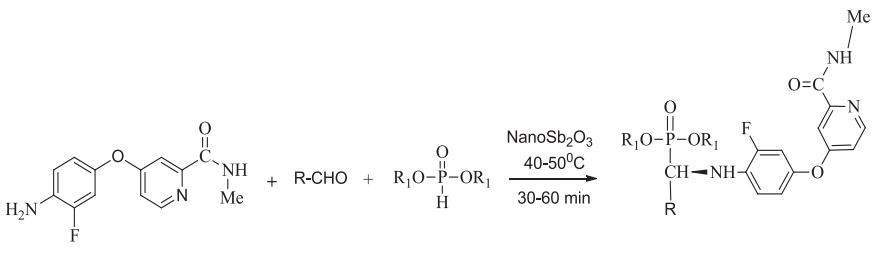
Reusability of the catalyst

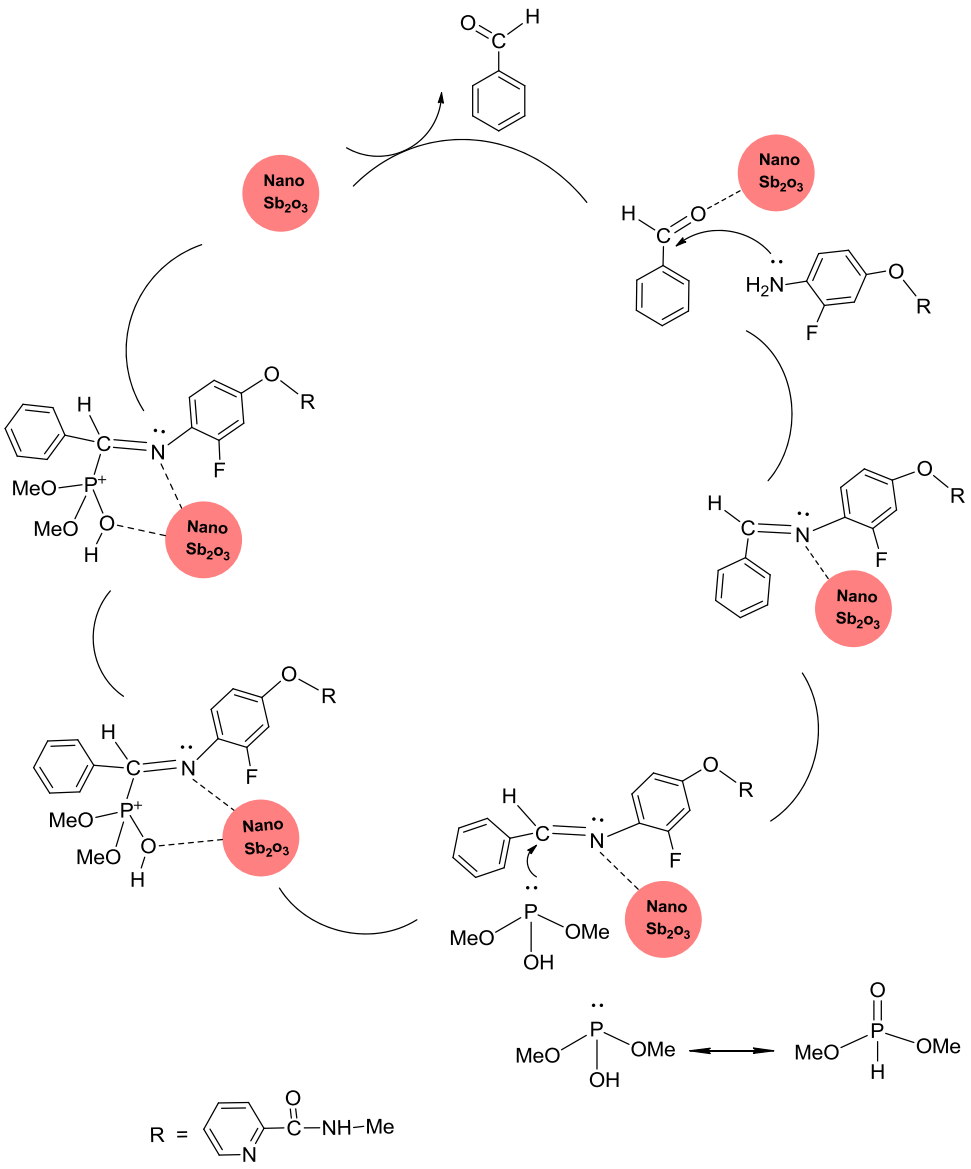
After completion of the reaction, the catalyst nano Sb_2O_3 was recovered by adding dichloromethane to the reaction mixture to separate the insoluble nano Sb_2O_3. It was collected by filtration and washed with dichloromethane and finally dried under vacuum and reused. From the results, it was observed that the efficiency of the recovered catalyst gradually decreases with the number of subsequent usages 92, 90, 88, 85, and 82 as shown in Fig. 2.

Analysis of the results on the synthesis of compounds **4a-l** by the reaction of 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide 1 with different substituted aromatic aldehydes 2 and dialkyl phosphite 3 were presented in Table 3 reveals that the diethyl phosphite reacts faster and offer better yields when compared with dimethyl phosphite.

The reaction mechanism involves the initial formation of the Schiff's base by the nano Sb_2O_3 activation catalysis via a reaction between aldehydes and amines. The nucleophilic addition of dialkyl phosphite to the electrophilic carbon of

Scheme 1 Synthesis of α-aminophosphonate derivatives





Scheme 2 Plausible mechanism for the synthesis of α – aminophosphonates **(4a–l)**

the imine forms an intermediate, which on elimination of the nano Sb_2O_3 catalyst forms the title compounds Fig. 3.

General procedure for the synthesis of (4a)

3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide **1** on reaction with different substituted aldehydes **2** and dialkyl phosphate **3** in the presence of nano Sb_2O_3 under solvent-free conditions at 40 °C for ~ 30 min forms **4**. After completion of the reaction that was monitored by thin-layer chromatography. The catalyst nano Sb_2O_3 was recovered by adding dichloromethane to the reaction mixture to separate the insoluble nano Sb_2O_3. It was collected by filtration and washed with dichloromethane and finally dried under vacuum and reused. The resulting crude was purified by column chromatography and obtained the pure product.

Diethyl{4-[2-(methylcarbamoyl)pyridine-4-yloxy]-2-fluorophenylamino}(3-fluorophenyl) methylphosphonate 4a

Yellow; Yield: 90% Mp: 165–168 °C; IR (KBr) (ν_{max} cm $^{-1}$): 3396(NH), 1237(P=O), 802 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.31 (1H, s, Pyridine-H), 7.96 (1H, s, Pyridine-H), 7.59 (1H, s, N-H) 7.26 (3H, m, Pyridine-H, 2Ar-H), 6.97 (5H, m, Ar-H), 4.94 (1H, s, Ar-NH), 4.73 (1H, d, $J = 21.4$ Hz, P-C-H) 4.14 (2H, m, P-OC$\underline{H_2}$-CH_3), 4.10 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.94 (1H, m, P-OC$\underline{H_2}$-CH_3 2.96 (3H, s, N-CH_3), 1.30 (3H, t, $J = 8.4$ Hz, P-$OCH_2C\underline{H_3}$), 1.19 (3H, t, $J = 8.2$ Hz, $POCH_2C\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 166.58 (C-4), 164.58 (C-26), 152.32 (C-19), 149.69 (C-20), 144.88 (C-24), 138.19 (C-17), 130.42 (C-22), 123.46 (C-6), 116.99 (C-2), 115.24

Table 1 Optimization of solvent conditions for the synthesis of α-aminophosphnates **(4a–l)**[a]

Entry	Solvent	Temp (°C)	Time (min)	[b]Yield (%)
1	Solvent free	rt	40	60
2	Solvent free	40	30	75
3	Solvent free	60	40	65
4	Tetrahydrofuran	40	40	72
5	Dimethylformamide	40	50	70
6	Acetonitrile	40	40	60
7	Dichloromethane	40	40	65
8	Toluene	40	40	70
9	Methanol	40	40	50
10	Water	40	40	68

[a]Reaction of 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide with different substituted aromatic aldehydes and dialkyl phosphite in the presence of nano Sb_2O_3 (5 mol%) catalyst under solvent-free conditions at 40 °C

[b]Isolated yield

Table 2 Optimization of catalyst conditions for the synthesis of α-aminophosphonates **(4a–l)**[a]

Entry	Catalyst (mol%)	Temperature (°C)	Time (hr/min)	Yield[b] (%)
1.	Catalyst free	60	24	60
2.	$BF_3.SiO_2$(5)	40	5	54
3.	$AlCl_3$(7.5)	40	5	65
4.	$BiCl_3$(7.5)	40	3.5	63
5.	$ZnCl_2$(5)	80	4.5	65
6.	CuO(5)	60	6	54
7.	Nano Sb_2O_3(1)	40	30	78
8.	Nano Sb_2O_3(2.5)	40	30	88
9.	Nano Sb_2O_3(5)	40	30	92
10.	Nano Sb_2O_3(7.5)	40	30	86

[a]Reaction of 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide with different substituted aromatic aldehydes and dialkyl phosphite in presence of nanoSb_2O_3 (5 mol%) catalyst under solvent free conditions at 40 °C

[b]Isolated yield

(C-14), 114.92 (C-1), 114.69 (C-15, C-16), 113.99 (C-5), 113.76 (C-3), 109.78 (C-17), 109.78 (C-25), 108.86 (C-27), 63.75 (C-8), 56.62 (C-12, C-13), 26.17 (C-28), 16.52, 16.45 (C-32, C-33); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 21.55; MS m/z(%): 506 $[M+H]^+$; Anal.calcd for $C_{24}H_{26}F_2N_3O_5P$ C, 57.03; H, 5.18; N,8.31; Found: C, 56.87; H, 4.93; N, 8.12.

Diethyl ((3-bromophenyl ((2-fluoro-4-((2-(methyl carbomyl) pyridin-4-yl)oxy)phenyl) amino) methyl) phosphonate 4b

Pale Yellow solid; Yield: 92% Mp: 142–145 °C; IR (KBr) (ν_{max} cm^{-1}): 3284(NH), 1234(P=O), 806 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.42 (1H, s, Pyridine-H), 8.40 (1H, s, Pyridine-H), 7.68 (1H, s, N-H), 7.65–6.46 (8H, m, Pyridine-H 7Ar-H), 4.69 (1H, s, Ar-NH), 4.11 (2H, m, P-O$\underline{CH_2}$-CH_3),) 3.87 (1H, m, P-O$\underline{CH_2}$-CH_3), 3.73 (1H, m, P-O$\underline{CH_2}$-CH_3), 3.01 (1H, d, $J = 21.8$ Hz, P-C-H), 2.07 (3H, s, N-CH_3), 1.34 (3H, t, $J = 8.2$ Hz, P-$OCH_2\underline{CH_3}$), 1.25 (3H, t, $J = 8.0$ Hz, $POCH_2\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 175.60 (C-26), 167.54 (C-19), 163.50 (C-20), 151.16 (C-24), 148.55 (C-17), 131.49 (C-22), 130.41 (C-6), 127.45 (C-1), 126.31 (C-5), 123.67 (C-14) 122.84 (C-3, C-2), 116.89 (C-15), 114.10 (C-14), 113.90 (C-16, C-21), 110.68 (C-18), 108.81 (C-25), 67.74 (C-8), 56.35, 54.84 (C12, C13), 26.39 (C-28), 16.42, 16.22 (C-32, C-33); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ(ppm): 25.43 MS m/z: 566 $[M+H]^+$; Anal.calcd for $C_{24}H_{26}$ $BrFN_3O_5P$ C, 50.90; H, 4.63; N, 7.42; Found: C, 50.75; H, 4.42; N, 7.22.

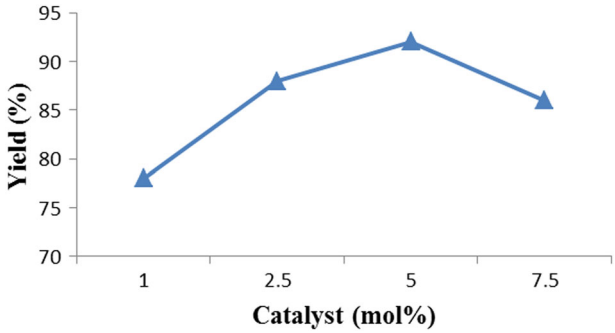


Fig. 1 Optimization of catalyst concentration

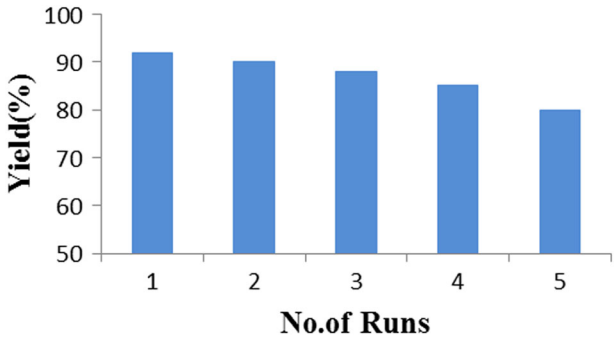
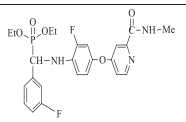
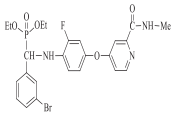
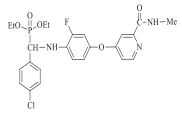
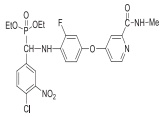
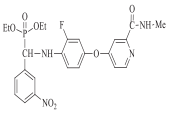
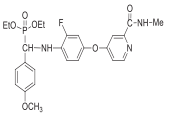
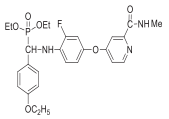
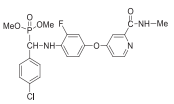
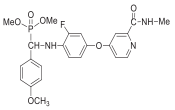
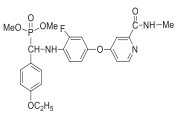
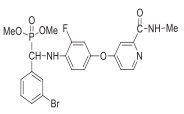
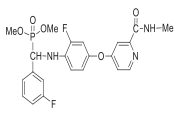


Fig. 2 Reusability of the catalyst

Diethyl((4-chlorophenyl)((2-fluoro-4-((2-methylcarbamoyl) pyridine-4-yl)oxy)phenyl) amino) methyl)phosphonate 4c

White solid; Yield: 93% Mp: 178–181 °C; IR(KBr) (ν_{max} cm^{-1}): 3384(NH), 1238(P=O), 819 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.31 (3H, m, Pyridine-H), 7.57 (1H, s, N-H), 7.35–6.88 (4H, m, Ar-H), 6.74–6.41 (3H, m, Ar-H), 4.71 (1H, s, Ar-NH), 4.09 (2H, m, P-O$\underline{CH_2}$-CH_3),) 3.97 (1H, m, P-O$\underline{CH_2}$-CH_3), 3.79 (1H, m, P-O$\underline{CH_2}$-CH_3), 2.91 (1H, d, $J = 21.1$ Hz, P-C-H), 2.01 (3H, s, N-CH_3), 1.25 (3H, t, $J = 8.4$ Hz, P-$OCH_2\underline{CH_3}$), 1.21 (3H, t, $J = 8.2$ Hz, $POCH_2\underline{CH_3}$);^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 174.87

Table 3 Synthesis of α-aminophosphonate derivatives (**4a–l**)[a]

Compound	R	R_1	Product	[b]Yield(%) /time(min)	Mp(°C)
4a		Et		90/40	165-168
4b		Et		85/50	142-145
4c		Et		95/40	178-181
4d		Et		92/60	184-187
4e		Et		90/45	152-155
4f		Et		88/30	116-119
4g		Et		93/50	138-141
4h		Me		88/60	154-157
4i		Me		90/50	162-165
4j		Me		87/60	193-196
4k		Me		90/40	173-176
4l		Me		91/50	145-148

[a]All reactions were carried out with 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide (0.002 mol) with different substituted aromatic aldehydes (0.002 mol) and dialkyl phosphite (0.0025 mol) in presence of nano Sb_2O_3 (5 mol%) catalyst under solvent-free conditions at 40–50 °C

[b]Isolated yield

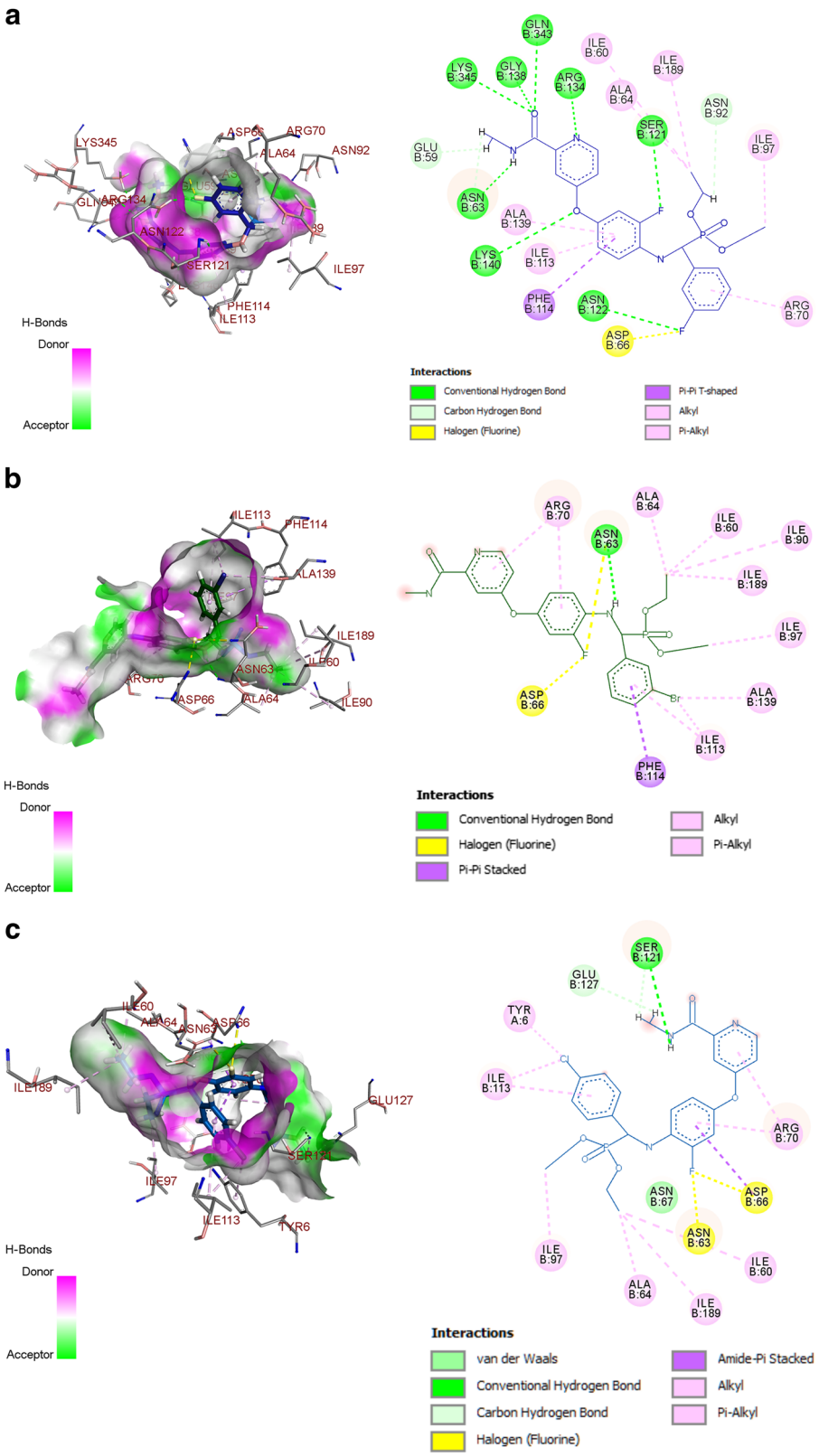
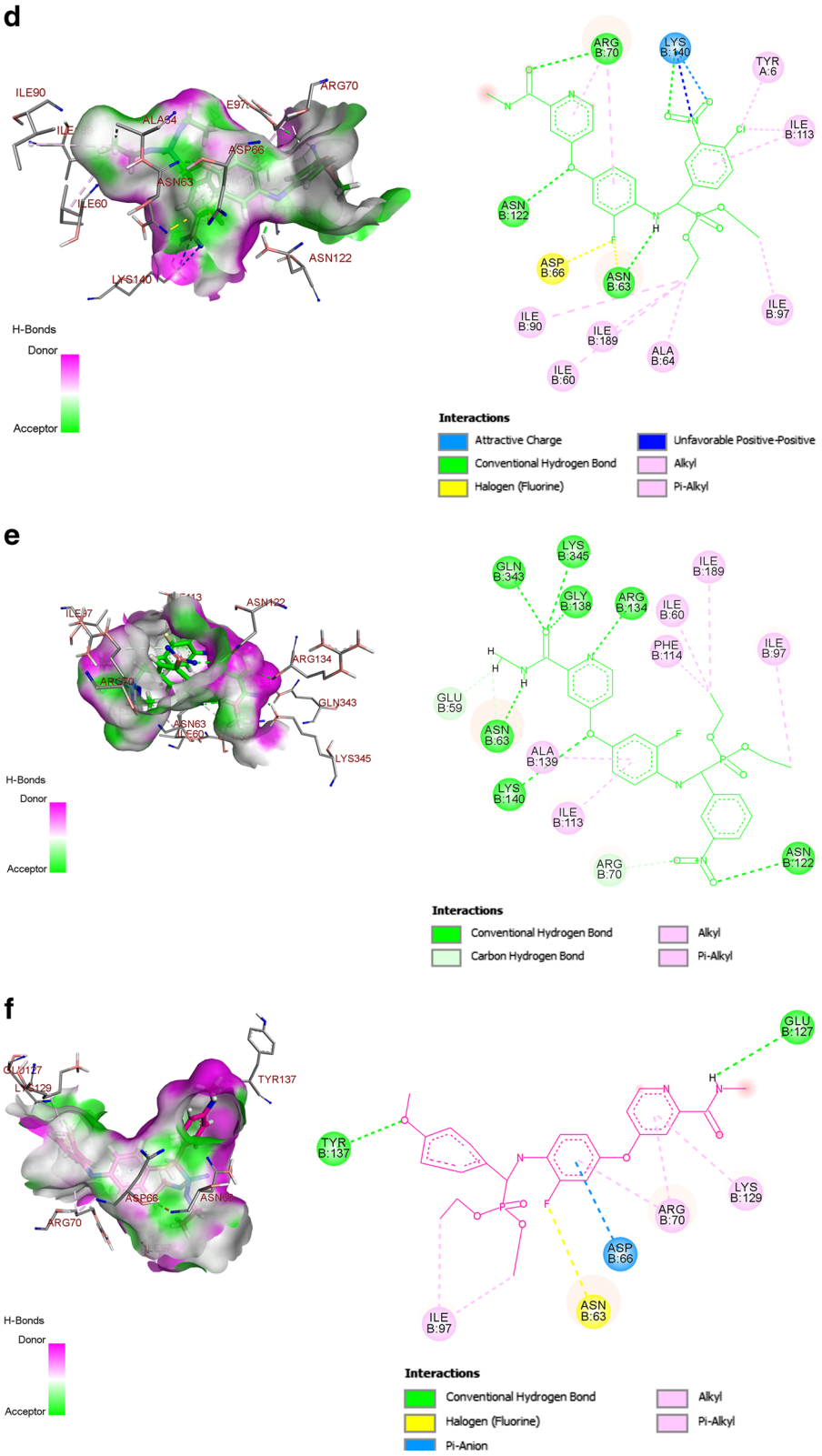


Fig. 3 Docking images of synthesized α-aminophosphonates (**4a–l**) by using 1-Click dock method

(C-19), 167.27 (C-25), 163.76 (C-18), 155.43 (C-23), 148.79 (C-21), 134.14 (C-16), 132.10 (C-6), 131.43 (C-3), 128.65 (C-4, C-2),127.46 (C-1, C-5), 124.43 (C-13), 116.87 (C-14), 115.65 (C-15), 114.04 (C-20), 110.42 (C-10),

Fig. 3 (Continued)



108.97 (C-24), 63.7 (C-7), 56.7, 54.5 (C-11, C-12), 26.3 (C-27), 16.4, 16.2 (C32, C33); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ(ppm): 24.67; MS m/z: 522 $[M+H]^+$; Anal.calcd for $C_{24}H_{26}ClFN_3O_5PC$, 55.23; H, 5.02; N,8.05; Found: C, 54.83; H, 4.84; N, 7.82.

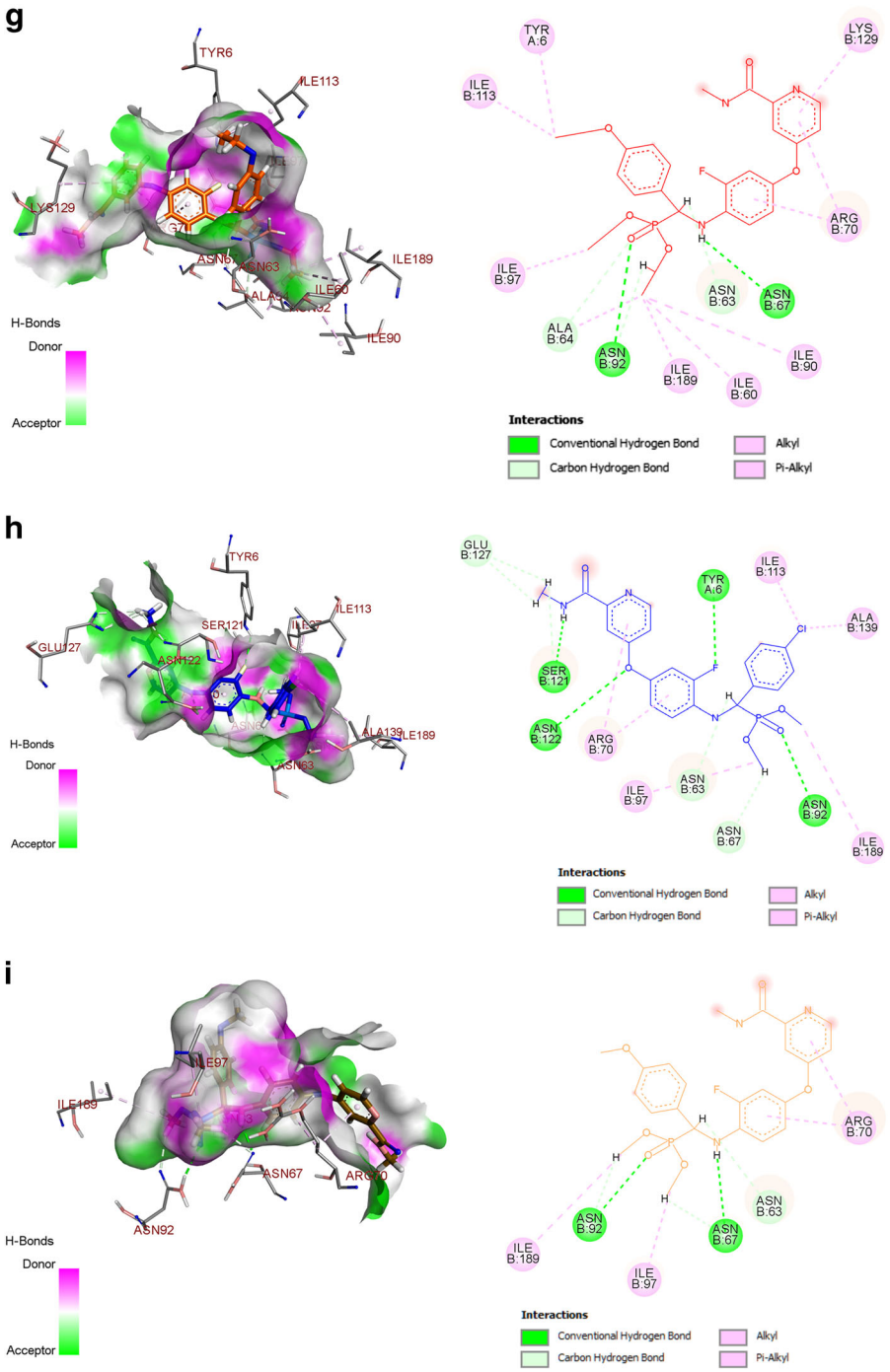


Fig. 3 (Continued)

Diethyl ((4-chloro-3-nitrophenyl)((2-fluoro-4-((2-methylcarbamoylpyridin-4-yl)oxy) phenyl) amino)methyl) phosphonate 4d

Yellow solid; Yield: 96%; Mp: 184–187 °C; IR (KBr) (ν_{max} cm^{-1}): 3324(NH), 1253(P=O), 824 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.29 (1H, s, Pyridine-H), 8.16 (1H, s, Pyridine-H), 8.07 (1H, s, Pyridine-H), 7.63 (1H, s, -NH), 7.42–6.87 (6H, m, Ar-H), 4.76 (1H, s, -NH), 4.08 (2H, m, P-OC$\underline{H_2}$-CH_3),) 3.94 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.91 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.24 (1H, d, $J = 21.8$ Hz, P-C-H), 2.90 (3H, s, N-CH_3), 1.19 (3H, t, $J = 8.4$ Hz, P-$OCH_2C\underline{H_3}$), 1.17 (3H, t, $J = 8.2$ Hz, $POCH_2C\underline{H_3}$); ^{13}C ($CDCl_3$, 100 MHz) δ (ppm): 166.53 (C-19), 164.34 (C-25), 152.92 (C-18), 152.10 (C-23), 149.45 (C-4), 147.92 (C-21), 145.34 (C-16), 136.56 (C-6), 133.45 (C-1) 132.34 (C-2), 126.72 (C-13), 125.43 (C-3), 124.78 (C-5), 117.02 (C-14, C-15), 114.10 (C-20), 113.82 (C-17), 109.26 (C-24), 64.24 (C-7)

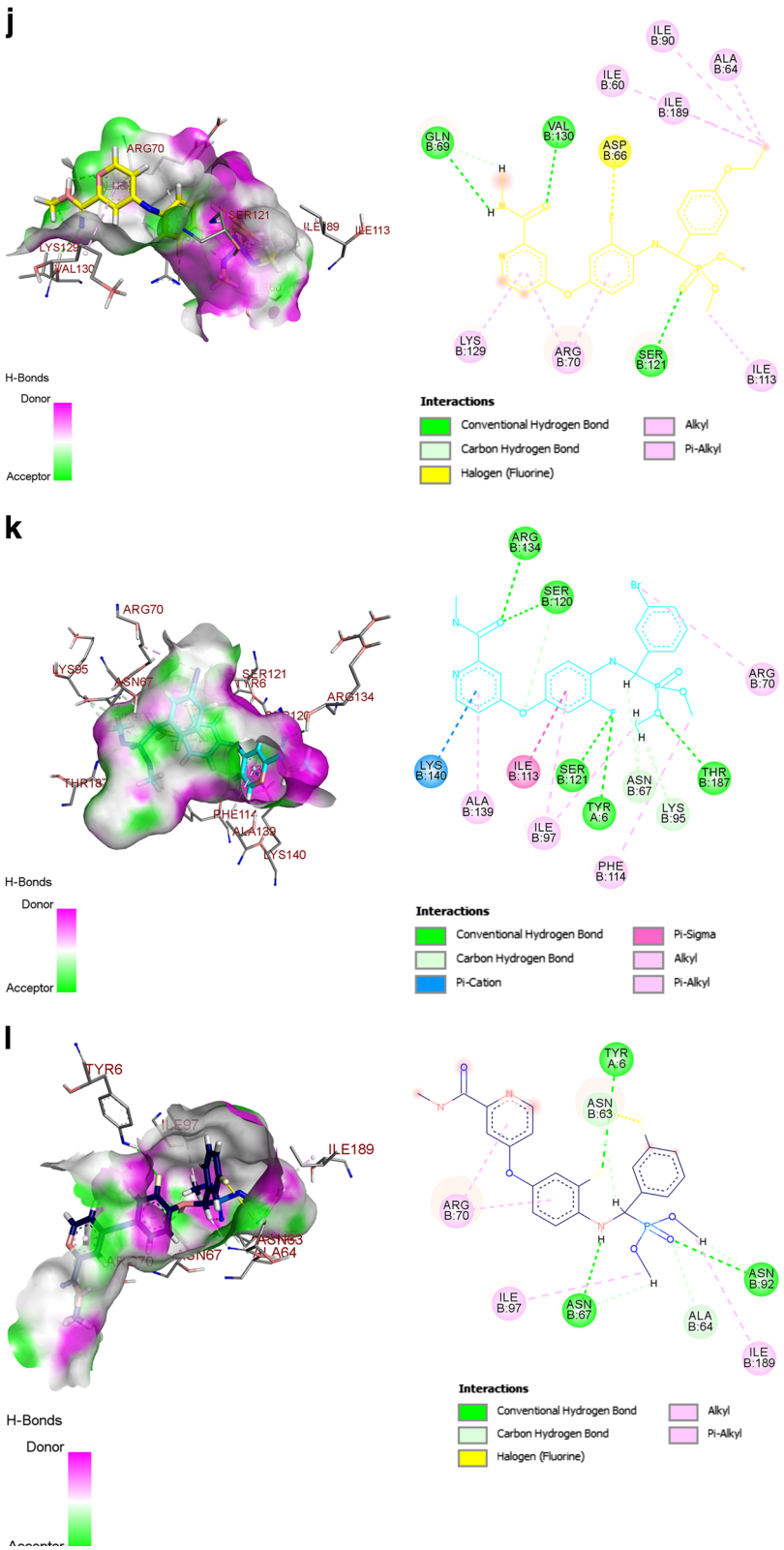


Fig. 3 (Continued)

55.95, 54.47 (C-33, C-34), 26.13 (C-27), 16.34, 16.23 (C-25, C-36); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 22.47; MS m/z: 567 $[M+H]^+$; Anal.calcd for $C_{24}H_{25}ClFN_4O_7P$ C, 50.85; H, 4.44; N, 9.88; Found: C, 50.72; H, 4.28; N, 9.74.

Diethyl(((2-fluoro-4-((2-(methylcarbamoyl)pyridine-4-yl) oxy)phenyl)amino)(3-nitrophenyl) methyl)phosphonate 4e

White solid; Yield: 94%; Mp: 152–155 °C; IR (KBr) (ν_{max} cm^{-1}): 3367 (NH), 1229 (P=O), 815 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.63 (1H, s, Pyridine-H), 8.42 (1H, s, Pyridine-H), 8.03 (1H, s, Pyridine-H), 7.61 (1H, s, -NH), 7.54–6.32 (7H, m, Ar-H), 4.31 (1H, s, Ar-NH), 4.03 (2H, m, P-OC$\underline{H_2}$-CH_3),) 3.96 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.94 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.12 (1H, d, $J = 22.4$ Hz, P-C-H), 2.85 (3H, s, N-CH_3), 1.12 (3H, t, $J = 8.2$ Hz, P-$OCH_2C\underline{H_3}$), 1.05 (3H, t, $J = 8.2$ Hz, $POCH_2C\underline{H_3}$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 167.54 (C-19), 164.43 (C-25), 158.32 (C-18), 151.43 (C-23), 147.52 (C-4), 146.37 (C-21), 145.83 (C-16), 137.24 (C-6), 134.25 (C-1), 129.82 (C-2), 127.64 (C-13), 125.52 (C-5), 122.85 (C-3), 121.31(C-14), 117.24 (C-15), 116.43 (C-20), 110.82 (C-17), 109.64 (C-24), 65.52 (C-7), 53.74 (C-32), 53.6 (C-34), 26.75 (C-27), 16.47, 16.42 (C-33,C-35); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 20.42; MS m/z: 533 $[M+H]^+$; Anal.calcd for $C_{24}H_{26}FN_4O_7P$ C, 54.12; H, 4.90; N,10.50; Found: C, 54.04; H, 4.83; N, 10.44.

Diethyl(((2-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy) phenyl)amino(4-methoxy) phenyl)methyl)phosphonate 4f

White solid; Yield: 92%; Mp: 116–119 °C; IR(KBr) (ν_{max} cm^{-1}): 3354 (NH), 1218(P=O), 823 (P-CAliphatic); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.72 (1H, s, Pyridine-H), 8.42 (1H, s, Pyridine-H), 8.03 (1H, s, Pyridine-H), 7.64 (1H, s, N-H), 7.14 (2H, m, Ar-H), 6.92–6.35 (5H, m, Ar-H), 4.59 (1H, s, Ar-NH), 4.15 (2H, m, P-OC$\underline{H_2}$-CH_3),) 4.12 (1H, m, P-OC$\underline{H_2}$-CH_3), 4.08 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.84 (3H, s, -OCH_3), 3.14 (1H, d, $J = 22.1$ Hz, P-C-H), 2.85 (3H, s, N-CH_3), 1.25 (3H, t, $J = 8.2$ Hz, P-$OCH_2C\underline{H_3}$), 1.23 (3H, t, $J = 8.0$ Hz, $POCH_2C\underline{H_3}$); ^{13}C ($CDCl_3$, 100 MHz) δ (ppm): 168.94 (C-19), 162.37(C-25), 158.52 (C-3), 154.36 (C-18), 153.24 (C-23), 147.63 (C-21), 145.84 (C-16), 129.34 (C-6), 127.53 (C-1, C-5), 126.52 (C-13), 116.77 (C-14), 115.36 (C-15), 114.34 (C-2, C-4), 113.54 (C-20), 110.45 (C-17), 109.71 (C-24), 65.74 (C-7), 58.54 (C-29), 53.43, 53.36 (C-32, C-34), 25.43 (C-27), 16.53, 16.42 (C-33, C-35); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 18.43; MS m/z: 518 $[M+H]^+$; Anal.calcd for $C_{25}H_{29}FN_3O_6P$ C, 58.01; H, 5.64; N, 8.12; Found: C, 57.394; H, 5.54; N, 8.08.

Diethyl((4-ethoxyphenyl)((2-fluoro-4-((2-(methylcarbamoyl) pyridin-4-yl)oxy)phenyl) amino)methyl)phosphonate 4g

Yellow solid; Yield: 90%; Mp: 138–141 °C; IR(KBr) (ν_{max} cm^{-1}): 3329(NH), 1228(P=O), 816 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.47 (1H, s, Pyridine-H), 8.32–8.05 (2H, m, Pyridine-H), 7.65 (1H, m, -NH), 7.47–6.34 (7H, m, Ar-H), 4.52 (1H, s, Ar-NH), 4.24 (2H, m, P-OC$\underline{H_2}$-CH_3), 4.15 (2H, m, -O-$C\underline{H_2}CH_3$), 3.85 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.82 (1H, m, P-OC$\underline{H_2}$-CH_3), 3.14 (1H, d, $J = 21.8$ Hz, P-C-H), 2.82 (3H, s, N-CH_3), 1.54 (3H, t, $J = 7.5$ Hz), 1.39 (3H, t, $J = 8.4$ Hz, P-$OCH_2C\underline{H_3}$), 1.37 (3H, t, $J = 8.4$ Hz, $POCH_2C\underline{H_3}$) ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 168.34 (C-19), 164.57 (C-25), 159.56 (C-3), 157.52 (C-18), 146.32 (C-23), 145.31 (C-21), 144.43 (C-16), 131.57 (C-19), 127.5 (C-1, C-5, C-6), 116.56 (C-14), 115.42 (C-15), 114.65 (C-2, C-4), 113.54 (C-20), 110.84 (C-17), 109.78 (C-24), 66.42 (C-7), 64.31 (C-36), 53.54, 53.42 (C-32, C-34), 26.74 (C-27), 16.53, 16.48 (C-33, C-35), 14.57(C-37); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 18.62; MS m/z: 532 $[M+H]^+$; Anal.calcd for $C_{26}H_{31}FN_3O_6P$ C, 58.74; H, 5.86; N,7.90; Found: C, 58.65; H, 5.74; N, 7.63.

Dimethyl ((4-chlorophenyl)((2-fluoro-4-((-(methylcarbamoyl)pyridine-4-yl)oxy) phenyl) amino) methyl)phosphonate 4h

White solid; Yield: 94%; Mp: 154–157 °C; IR (KBr) (ν_{max} cm^{-1}): 3345(NH), 1243(P=O), 824 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$,400 MHz) δ (ppm): 8.63 (1H, s, Pyridine-H), 8.45–8.08 (2H, m, Pyridine-H), 7.63 (1H, s, Ar-NH) 7.26–7.14 (4H, m, Ar-H), 6.92–6.38 (3H, m, Ar-H) 4.73 (1H, s, Ar-NH), 3.89 (1H, d, $J = 22.4$ Hz, P-C-H), 3.74 (3H, d, $J = 11.2$ Hz P-OCH_3), 3.68 (3H, d, $J = 10.8$ Hz P-OCH_3), 3.18 (3H, s, N-CH_3); ^{13}C ($CDCl_3$, 100 MHz) δ (ppm): 164.34 (C-19), 161.45 (C-25), 155.72 (C-C-18), 151.42 (C-23), 146.63 (C-21), 145.43 (C-17), 134.21 (C-6), 132.63 (C-3), 128.48 (C-2, C-4), 128.21 (C-1, C-5), 126.32 (C-18), 116.56 (C-14), 115.52 (C-15), 113.95 (C-20), 110.78 (C-17), 109.41 (C-24), 68.70 (C-7), 53.87 (C-11, C-12), 26.86 (C-27); ^{31}P-NMR ($CDCl_3$,161.8 MHz) δ (ppm): 19.35; MS m/z: 494 $[M+H]^+$; Anal.calcd for $C_{22}H_{22}ClFN_3O_5P$ C, 53.50; H, 4.49; N, 8.51; Found: C, 53.45; H, 4.43; N, 8.45.

Dimethyl((4-methoxyphenyl)((2-fluoro-4-((2-(methylcarbamoyl)pyridine-4-yl)oxy) phenyl) amino) methyl)phosphonate 4i

Dark Yellow solid; Yield: 95%; Mp: 162–165 °C; IR(KBr) (ν_{max} cm^{-1}): 3384(NH), 1238(P=O), 819 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.65 (1H, s, Pyridine-H), 8.43–8.21 (2H, m, Pyridine-H), 7.64 (1H, s, -NH), 7.43–6.87 (4H, m, Ar-H), 6.74–6.42 (3H, m, Ar-H), 4.53 (1H, s, Ar-NH), 3.98 (1H, d, $J = 22.6.4$ Hz, P-C-H), 3.84 (3H, s, P-OCH_3), 3.68 (3H, d, $J = 11.6$ Hz, P-OCH_3), 3.62 (3H, d, $J = 11.2$ Hz -OCH_3), 2.83 (3H, s, N-CH_3); ^{13}C-NMR ($CDCl_3$,

Table 4 Effect of α-aminophosphonate derivatives against different control cells HEK (human embryonic kidney), DU-145 (human prostate adenocarcinoma), MCF-7 (human ER+/PR+/Her2− breast), MDA-MB-231(human $ER^-/PR^-/Her2^-$ breast), Mia-PaCa-2 (human pancreatic carcinoma), HeLa (human cervical cancer), HepG2 (human hepatocellular carcinoma) cancer cell lines

Compounds IC50/nM ± SD	HEK (control cells)	DU-145	MCF-7	MDA-MB-231	Mia-PaCa-2	HeLa	HepG2
4a	20.02 ± 0.002	68.69 ± 0.004	59.61 ± 0.026	63.78 ± 0.012	43.51 ± 0.028	40.31 ± 0.012	42.76 ± 0.015
4b	45.90 ± 0.025	NA	51.98 ± 0.001	NA	58.93 ± 0.001	NA	NA
4c	NA	65.21 ± 0.024	19.09 ± 0.002	15.86 ± 0.016	35.35 ± 0.008	20.31 ± 0.034	29.45 ± 0.014
4d	18.24 ± 0.003	NA	58.79 ± 0.003	72.69 ± 0.012	49.46 ± 0.014	NA	46.42 ± 0.018
4e	50.27 ± 0.016	72.36 ± 0.045	21.88 ± 0.005	18.88 ± 0.006	42.86 ± 0.012	48.97 ± 0.025	41.87 ± 0.019
4f	58.82 ± 0.006	NA	63.71 ± 0.004	66.28 ± 0.003	59.95 ± 0.023	40.98 ± 0.003	52.48 ± 0.024
4g	60.11 ± 0.002	NA	58.94 ± 0.032	65.19 ± 0.018	47.89 ± 0.001	45.85 ± 0.034	NA
4h	NA	65.43 ± 0.053	11.44 ± 0.012	NA	49.37 ± 0.004	NA	NA
4i	63.5 ± 0.004	NA	66.42 ± 0.003	25.87 ± 0.008	55.56 ± 0.002	54.73 ± 0.004	NA
4j	52.31 ± 0.023	NA	61.78 ± 0.005	68.52 ± 0.001	59.01 ± 0.006	NA	NA
4k	NA	NA	17.04 ± 0.002	37.57 ± 0.034	57.09 ± 0.027	53.82 ± 0.003	41.29 ± 0.043
4l	NA	74.05 ± 0.002	20.08 ± 0.035	72.25 ± 0.008	49.38 ± 0.008	NA	59.59 ± 0.025
Doxorubicin	26.03 ± 0.001	32.16 ± 0.012	24.65 ± 0.004	40.42 ± 0.004	45.27 ± 0.004	38.21 ± 0.006	35.43 ± 0.016

SD, standard deviation; NA, not active

100 MHz) δ (ppm): 165.82 (C-19), 161.35 (C-25), 158.54 (C-3), 155.43 (C-18), 150.61 (C-23), 146.34 (C-21), 145.57 (C-16), 129.62 (C-6), 127.83 (C-1, C-5), 126.45 (C-13), 116.53 (C-14), 115.43 (C-15), 114.54 (C-2, C-4), 113.25 (C-20), 110.14 (C-17), 109.82 (C-24), 67.62 (C-7), 56.43 (C-31), 53.63, 53.54 (C-10, C-11), 26.94 (C-27); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ(ppm): 21.18; MS m/z: 490$[M+H]^+$; Anal.calcd for $C_{23}H_{25}FN_3O_6P$ C, 56.44; H, 5.14; N,8.58 Found: C, 56.26; H, 5.09; N, 8.46.

Dimethyl((4-ethoxyphenyl)((2-fluoro-4-((2-methylcarbamoyl)pyridine-4-yl)oxy) phenyl) amino) methyl)phosphonate 4j

White solid; Yield: 93%; Mp: 193–196 °C; IR (KBr) (ν_{max} cm^{-1}): 3396(NH), 1232(P=O), 802(P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.85 (1H, s, Pyridine-H), 8.42 (2H, m, Pyridine-H), 7.62 (1H, s, -NH) 7.25 (2H, m, Ar-H), 6.91–6.25 (5H, m, Ar-H) 4.35 (1H, s, Ar-NH), 4.24 (2H, m, -OCH_2CH_3), 4.06 (1H, d, $J = 22.4$ Hz, P-C-H), 3.62 (3H, d, $J = 10.6$, P-OCH_3), 3.54 (3H, d, $J = 10.4$ Hz, P-OCH_3), 2.82 (3H, s, N-CH_3), 1.43 (3H, t, $J = 7.8$ Hz, -CH_3); ^{13}C ($CDCl_3$, 100 MHz) δ (ppm): 164.26 (C-19), 161.53 (C-25), 157.72 (C-3), 155.43 (C-18), 151.32 (C-23), 146.48 (C-21), 145.31 (C-16), 128.45 (C-6), 127.72 (C-1, C-5), 126.31 (C-13), 116.32 (C-14), 115.15 (C-15), 114.43 (C-2, C-4), 113.35 (C-20), 110.64 (C-17), 109.35 (C-24), 67.52 (C-7), 64.81 (C-32) 54.74, 54.63 (C-11, C-12), 26.5 (C-27), 14.56 (C-33); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ(ppm): 23.42; MS m/z: 504 $[M+H]^+$; Anal.calcd for $C_{24}H_{27}FN_3O_6P$ C, 57.26; H, 5.41; N,8.35; Found: C, 57.18; H, 5.35; N, 8.24.

Dimethyl ((3-bromophenyl)((2-fluoro-4-((2-(methylcarbamoyl)pyridine-4-yl)oxy) phenyl) methyl) phosphonate 4k

White solid; Yield: 92%; Mp: 178–181 °C; IR (KBr) (ν_{max} cm^{-1}): 3345(NH), 1224(P=O), 812 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.37 (1H, m, Pyridine-H), 8.32–8.16 (2H, m, Pyridine-H), 7.65 (1H, s, Ar-H), 7.63–7.45 (4H, m, Ar-H), 6.94–6.47 (3H, m, Ar-H), 4.98 (1H, s, Ar-NH), 3.92 (1H, d, $J = 21.4$ Hz, P-C-H) 3.84 (3H, d, $J = 10.8$ Hz, P-OCH_3), 3.64 (3H, d, $J = 10.6$ Hz, P-OCH_3), 2.96 (3H, s, N-CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 165.23 (C-20), 161.42 (C-28), 156.43 (C-19), 152.48 (C-24), 146.56 (C-22), 144.32 (C-17), 137.37 (C-6), 131.65 (C-5), 129.42 (C-3), 129.2 (C-2), 128.56 (C-14), 126.45 (C-1), 122.9 (C-4), 116.48 (C-15), 115.43 (C-16), 113.58 (C-21), 110.48 (C-18), 108.46 (C-25), 68.43 (C-8), 54.36, 54.23 (C-12, C-13), 26.17 (C-31); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ(ppm): 20.63; MS m/z: 538 $[M+H]^+$; Anal.calcd for $C_{22}H_{22}BrFN_3O_5P$ C, 49.09; H, 4.12; N, 7.80; Found: C, 49.02; H, 4.02; N, 7.72.

Dimethyl (((2-fluoro-4-((2-methylcarbamoyl)pyridin-4-yl) oxy)phenyl)amino)(3-fluorophenyl) methyl)phosphonate 4l

Yellow solid; Yield: 94% Mp: 145–148 °C IR (KBr) (ν_{max} cm^{-1}): 3303(NH), 1231(P=O), 935 (P-$C_{Aliphatic}$); ^{1}H-NMR ($CDCl_3$,400 MHz) δ (ppm): 8.37 (1H, m, Pyridine-H), 8.12–8.01 (2H, m, Pyridine-H), 7.63 (1H, s, -NH), 7.29–6.87 (4H, m, Ar-H), 6.81–6.42 (3H, m, Ar-H), 4.99 (1H, s, Ar-NH), 3.86 (1H, d, $J = 22.4$ Hz, P-C-H), 3.84 (3H, d, $J =$

Table 5 Docking studies of synthesized α-aminophosphonates by using 1-Click dock method

Compound	Docking score	Active site residues	No.of H-bonds
4a	10.1	Asn B: 63, Ser B: 121, Asn B: 122, Arg B: 134, Gly B: 138, Lys B: 140, Gln B: 343, Lys B: 345	8
4b	9.4	Asn B: 63	1
4c	9.1	Ser B: 121	1
4d	9.7	Asn B: 67, Arg B: 70, Asn B: 122	3
4e	9.5	Asn B: 63, Asn B: 122 Arg B: 134, Gly B: 138, Lys B: 140, Gln B: 343, Lys B: 345	7
4f	9.2	Glu B: 127, Tyr B: 137	2
4g	8.9	Asn B: 67, Asn B: 92	2
4h	9.3	Tyr A: 6, Asn B: 92, Ser B: 121, Asn B: 122	4
4i	9.1	Asn B: 67, Asn B: 92	2
4j	8.8	Gln B: 69, Ser B: 121, Val B: 130	3
4k	10	Tyr A: 6,Ser B: 120, Ser B: 121, Arg B: 134,Thr B: 187	5
4l	9.3	Tyr A: 6, Asn B: 67, Asn B: 92, Asn B: 63	4
Doxorubicin	9.1	Tyr A: 6, Ile B: 60, Thr B: 119, Ser B: 120	4

11.2 Hz, P-OCH_3), 3.64 (3H, d, $J = 10.9$ Hz, P-OCH_3), 2.96 (3H, s, N-CH_3); $^{13}C(CDCl_3$,100 MHz) δ (ppm): 164.34 (C-20), 162.14 (C-4), 160.97 (C-26), 156.74 (C-19), 151.45 (C-24), 147.37 (C-22), 145.53 (C-17), 138.27 (C-6), 130.49 (C-2), 127.35 (C-14), 124.28 (C-2), 116.86 (C-15), 115.61 (C-16), 113.86 (C-5, C-21), 113.43 (C-3), 110.78 (C-18), 109.74 (C-25), 68.85 (C-8), 54.27, 54.13 (C12, C13), 26.4 (C-28); ^{31}P-NMR ($CDCl_3$, 161.8 MHz) δ (ppm): 18.62; MS m/z: 478 $[M+H]^+$; Anal.calcd for $C_{22}H_{22}F_2N_3O_5P$ C, 55.34; H, 4.64; N, 8.80; Found: C, 55.28; H, 4.58; N, 8.74.

Biological activity

Cytotoxic assay–SRB assay

Sulforhodamine B (SRB) is an anionic amino xanthene dye, had been used as an assay for total cell protein, for in vitro screening of cytotoxic assay. This method relies on the property of SRB, which binds stoichiometrically to proteins under mild acidic conditions and then can be extracted using basic condition. The amount of bound dye can be used as a proxy for cell mass, which can then be extrapolated to measure cell proliferation.

The anticancer activity of the synthesized compounds from **4a–l** were evaluated in vitro against *MCF-7* (human $ER^+/PR^+/Her2^-$ breast cancer cell line), *MDA-MB-231* (human $ER^-/PR^-/Her2^-$ breast cancer cell line) and *HepG2* (human hepatocellular carcinoma cell line), *Mia-Paca-2* (human pancreatic carcinoma cell line), *HeLa* (human cervical cancer cell line), *DU-145* (human prostate adenocarcinoma cell line) cells as well as control cells–HEK293 (human embryonic kidney cell line) were seeded on to 96-well plates at a cell density of 5×10^3 cells/well in 100 µl of complete medium and incubated at 37 °C for 24 h. Cells were treated with compounds over a concentration range between 10 and 50 µg/ml for 48 h followed by fixing with 40 µl of 20% trichloracetic acid and incubated at 4 °C for 1 h. Subsequently, plates were washed with deionized water for five times, air dried for 24 h and stained with 0.4% of SRB (40 µl) prepared in 1% acetic acid solution followed by incubation at room temperature in dark for 20 min. Then cells were washed with 1% acetic acid solution thrice after removing SRB and plates were dried for 4 h. Tris base (100 µl) was added to each well to solubilize the bound SRB and absorbance was measured at 510 nm using multimode plate reader (Vichal and Kirtikara K 2006).

IC_{50} values were determined from a graph of cell viability and **4a–l** compounds concentration along with the standard (doxorubicin) for comparison. IC_{50} was determined as a broad range concentration (*X* axis) versus % inhibition (*Y* axis) and then an intersection drawn at 50% inhibition on Y-axis and then it is correlated to the concentration value on X-axis. Finally, that concentration value is considered as IC_{50} in µg/ml.

The results shown in Table 4 revealed that among the synthesized title compounds from **4a–l**, Compound **4a** showed good activity on HEK and Mia-Paca-2 (20.02 ± 0.002, 43.51 ± 0.028) and very less active on DU-145, MCF-7, MDA-MB-231, HeLa, and HepG2 than standard. Compound **4c** showed excellent cytotoxic activity on MCF-7, MDA-MB-231, Mia-PaCa-2, HeLa, and HePG2 cell lines with IC_{50} values (19.09 ± 0.002, 15.86 ± 0.016, 35.35 ± 0.008, 20.31 ± 0.034, 29.45 ± 0.014), it is less active on DU-145 and inactive on HEK cells than standard (24.65 ±

0.004, 40.42 ± 0.004, 45.27 ± 0.004, 38.21 ± 0.006, and 35.43 ± 0.016). Compound **4d** showed good cytotoxic inhibition on HEK (18.24 ± 0.003) less active on MCF-7, MDA-MB-231, Mia-Paca-2, and HepG2 than standard and it is inactive on DU-145 and HeLa. Compound **4e** showed potent cytotoxic inhibition on MCF-7, MDA-MB-231, and Mia-PaCa-2 with IC_{50} values (21.88 ± 0.005, 18.88 ± 0.006, 42.86 ± 0.012) than standard and it is very less active on HEK, DU-145, HeLa, and HepG2. Compound **4h** showed good activity on MCF-7(11.44 ± 0.012) less active on DU-145 and Mia-Paca-2, inactive on HEK, HeLa, and HepG2 than standard. Compound **4i** showed good activity on MDA-MB-231 (25.87 ± 0.008) very less active on HEK, MCF-7, Mia-Paca-2, and HeLa, inactive on DU-145 and HepG2. Compound **4k** showed potent activity on MCF-7 and MDA-MB-231 (17.04 ± 0.002, 37.57 ± 0.034), less active on Mia-Paca-2, HeLa, and HepG2, inactive on HEK and DU-145. Compound **4l** with m-fluoro substitution showed potent activity on MCF-7 (20.08 ± 0.035) very less active on DU-145, MDA-MB-231, Mia-Paca-2, and HepG2, inactive on HEK and HeLa. Compounds **4b**, **4f**, **4g**, and **4j** with bromo, methoxy, ethoxy substitutions showed very less activity on some cell lines and inactive on some cell lines than standard. In summary **4a**, **4c**, **4d**, **4e**, **4h**, **4i**, **4k**, and **4l** showed good anticancer activity than standard. The above results revealed that compounds with electron withdrawing groups as substituents on phenyl ring showed potent anticancer activity than the electron donating groups on phenyl ring. So, the results will be more significant in developing potent and safe anticancer drugs.

Molecular docking studies

Docking study

The anticancer activity of the title compounds were evaluated on the potential cancer target topoisomerase-II, where their binding efficiency was tested through molecular docking studies. Among the various anticancer targets, DNA topoisomerase-II (Topo II) has attracted much attention for cancer treatment and inhibitors include etoposide, doxorubicin, and mitoxantrone. Topoisomerase-II enzyme is located in the nucleus of mammalian cells, which involves in interconversion of topological isomers of DNA by breaking and resealing phosphodiester bonds and also modifies DNA linkages in able to relax the supercoiled form of a circular, closed, double-stranded DNA molecule in the presence of an energetic cofactor such as ATP. These inhibitors interact with DNA and/or topoisomerases to form stable ternary complexes, termed "cleavable complexes," which cause permanent DNA damage and induce apoptosis or other types of cell death.

In order to understand the probable binding sites of the aminophosphonates, molecular interaction studies of the active anticancer test compounds are performed against topoiosmerase-II alpha enzyme, which are targets in cancer treatments. Doxorubicin is also one of the inhibitor of topoisomerase-II protein. The structures of organo- aminophosphonates, **4a–l**, and doxorubicin are constructed and optimized using Chemskecth software. To prepare the topoisomerase-II structure, the crystal structure of topoisomerase was taken from the protein data bank (PDB ID:1ZXM). A and B chains of topoisomerase-II is selected for the docking and hydrogens are added to the enzyme. The molecular docking method is performed using 1-Click docking online server tool, 1-Click docking uses Auto Dock Vina with default parameters and dock multiple ligands into a single target. Default binding site center specification of 1ZXM in 1-Click Docking. Default binding site center X: 9.17 Default binding site center Y: 56.604 Default binding site center Z: 325.1457 https://mcule.com/apps/1-click-docking/). The docked poses and interactions of compounds and protein were analyzed by using Discovery Studio Visualizer V16.1.0.15350.

The docking studies from Table 5 reveal that the docking score correlates with the all docked ligands, which were found to have some interaction between atoms of the ligands and atoms of topoisomerase-II enzyme. Moreover, these docked conformations also formed a H-bonding interaction within the active site of enzyme. Among the title compounds **4a** showed H-bonds with amino-acid residues -NH forms bond with Asn B:63, phenyl ring containing fluorine forms H-bond with Ser B:121, fluoro group substitution on aldehyde containing phenyl ring forms hydrogen bond with Asn B:122, pyridine containing nitrogen forms bond with Arg B:134, linked oxygen (-O-) forms bond with Lys B:140, C=O forms bonds with the three amino acids Gly B:138, Gln B:343, and Lys B:345 with docking score 10.1 much higher than that of standard doxorubicin. Compound **4b** containing -NH bond forms H-bond only with Asn B:63 and its docking score 9.4 higher than standard. Compound **4c** with -NH forms H-bond with Ser B:121 having a docking score 9.1 nearer to standard. Compound **4d** -NH forms H-bond with Asn B:67, C=O group forms one H-bond with Arg B:70, linked oxygen (**-**O-) forms H-bond with Asn B:122 of docking score 9.7 higher than standard. Compound **4e -**NH forms hydrogen bond with Asn B:63, pyridine ring containing nitrogen forms H-bond with Arg B:134, C=O forms Gly B:138, Gln B:343, and Lys B:345, Lys B:140 forms hydrogen bond with -O- and having a docking score 9.5 higher than standard. Compound **4f** -NH group forms H-bond with Glu B:127, oxygen containing -OCH_3 forms H-bond with Tyr B:137 of docking score 9.2 higher than standard. Compound **4g**

containing -NH group forms H-bonds with Asn B:67, C=O group forms H-bond with Asn B:92 of docking score 8.9 less than standard. Compound **4h** Tyr A:6 forms H-bond with -F, C=O group forms H-bond with Asn B:92, -NH forms H-bond with Ser B:121, -O- forms H-bond (-O-) with Asn B:122 and its docking score 9.3 less than the standard. Compound **4i** containing -NH forms H-bond with Asn B:67, C=O group forms hydrogen bond with Asn B:92 and its docking score 9.1 nearer to standard. Compound **4j** -NH forms H-bond with Gln B:69, P=O forms H-bond with Ser B:121, C=O forms H-bond with Val B:130 and its docking score 8.8 less than the standard. Compound **4k** containing C=O forms H-bond with Arg B:134, -NH forms H-bond with Ser B:120, Tyr A:6, Ser B:121, Thr B:187 forms H-bond with -O-P of docking score 10 more than the standard. Compound **4l** with phenyl ring containing -F forms H-bond with Tyr A:6 forms, NH forms H-bond with Asn B:67, P=O forms H-bond with Asn B:92 of docking score 9.3 higher than standard. The docking studies also reveals that the compounds containing electron withdrawing groups as substituents on phenyl ring in the compounds **4a**, **4c**, **4d**, **4e**, **4h**, **4i**, **4k**, and **4l** showed good anticancer activity and their interaction with the enzyme topoisomerase-II on its binding sites is much more higher than the standard.

In summary the docking studies of the title compounds also correlate with the in vitro cytotoxic assay performed on synthesized compounds

Structure activity relationship (SAR studies)

Analysis of structure–bioactivity relationship of the compounds **4a–l** reveals that even though the basic skeleton of aminophosphonate remains same in all compounds, different substituents in the phenyl ring substituted at the α-carbon of the phosphonates show varying percent inhibition on different cancerous cell lines. Further, the same phosphonate is not uniformly active on the different cancerous cells. This shows that not only the basic skeleton structure of compounds, but also its substituents and the type of cancerous cells decides the activity of a particular compound. Compound **4a** ($R = 3\text{-F-}C_6H_4$, R_1=Et) exerts higher percent inhibition on HEK (control cells) and Mia-Paca-2 cancer cell lines than the standard anticancer drug doxorubicin. Interestingly Compound **4b** ($R = 3\text{-Br-}C_6H_4$, R_1=Et) is completely inactive on all cell lines. Compound **4c** ($R = 4\text{-Cl-}C_6H_4$, $R_1 = Et$) exhibits extremely higher percent inhibition on five cell lines, MCF-7, MDA-MB-231, Mia-Paca-2, HeLa, and HepG2. Compound **4d** ($R = 3\text{-Cl,4-}NO_2\text{-}C_6H_4$, R_1=Et) exert higher activity than standard on HEK cells. Compound **4e** ($R = 3\text{-}NO_2\text{-}C_6H_4$, R_1=Et) has better anticancer activity on three cell lines MCF-7, MDA-MB-231 and Mia-Paca-2. Compound **4f** with ($R = 4\text{-}OCH_3\text{-}C_6H_4$, R_1=Et) is totally inactive on all cancerous cell lines. Compound **4g** ($R = 4\text{-}OC_2H_5\text{-}C_6H_4$, R_1=Et) also totally inactive on all cell lines. Compound **4h** ($4\text{-Cl-}C_6H_4$, R_1=Me) has higher activity than the standard on only one cell line MCF-7. Compound **4i** with ($R = 4\text{-}OCH_3\text{-}C_6H_4$, R_1=Me) showed higher activity on only one cell line MDA-MB-231. Compound **4j** ($R = 4\text{-}OC_2H_5\text{-}C_6H_4$, R_1=Me) is also totally inactive on all cancerous cell lines. **4k** ($R = 3\text{-Br-}C_6H_4$, R_1=Me) inhibits MCF-7 and MDA-MB-231 cells only at higher degree than the standard. Similarly, **4l** ($R = 3\text{-F-}C_6H_4$, R_1=Me) has higher activity against only on one cell line MCF-7.

Effect of electronic nature and position of R group on phenyl ring The electronic nature and position of substituent (s) on the phenyl ring at the α-carbon of the phosphonate appears to play a crucial role in inhibiting cancer cell lines. Overall, the compounds substituted with electronegative atoms /electron attracting groups (**4a**, **4c**, **4d**, **4e**, **4h**, **4k**, and **4l**) in the phenyl ring at the α-carbon of the phosphonate are showing higher anticancer activity than the standard doxorubicin. Not only the electronic nature of the substituted atom/group in the phenyl ring but also its position in the phenyl ring seems to play a significant role in showing cytotoxic activity. Overall para substituted compounds with electronegative/electron withdrawing are showing much higher activity than their meta substituted compounds. Perhaps this may be owing to more electron withdrawing effect of these substituents, which renders phosphoryl phosphorous atom more electrophilic, which facilitates its stronger binding with nucleophillic bases present in the cancer cells. This effect appears to be more pronounced in the para substituted compounds than their meta substituted compounds, owing to more effective π-electron delocalization through resonance. The compound 4-ethoxyphenyl compound **4j** stands out as an exception for this generalization.

Further, the nature of alkyl groups on the phosphoryl group of the phosphonate also seems to play complimentary role in inducing cytotoxic activity. The ethyl phosphonates **(4a–g)** are exhibiting more anticancer activity than their methyl counterparts. This disparity may be attributed to their ability to render phosphoryl phosphorous more electrophilic. The less bioactivity of the methyl phosphonates may be due to the decreased electophilicity of the phosphoryl phosphorous atom owing to the increased hyperconjugative effect of the methyl groups.

This assumption derives support from the docking studies data that show that these compounds have higher docking score which is nearer to the standard. Thus, these compounds form a family of novel anticancer active lead molecules and by further fine tuning their structure with respect to bioactivity may offer a new generation potential and safe anticancer compounds.

Conclusion

A simple and efficient green method has been developed for the synthesis of new class of dialkyl(2-fluoro-4-(2-methylcarbamoyl)pyridine-4-yl)oxy)phenyl)amino)-substituted phenyl methyl phosphonates **4a–l** by reacting 3-(4-amino-3-fluorobenzyl)-*N*-methylbenzamide with different substituted aromatic aldehydes and dialkyl phosphite in the presence of nano Sb_2O_3 as a catalyst under solvent-free conditions at 40 °C. The main advantage of this method is it involves green conditions for the reaction to carry out, gives high product yields (85–95%) in less reaction times (30–60 min) and moreover some of the synthesized compounds are potent anticancer activity than standard, which can be used as safe anticancer drugs. Compounds **4a**, **4c**, **4d**, **4e**, **4h**, **4i**, **4k**, and **4l** substituted with F, Cl, OCH_3, and NO_2 moieties exhibited higher cytotoxic activity than the standard doxorubicin. The docking studies also reveal that the docking score correlates with the all docked ligands. The docked conformations also formed a H-bonding interaction within the active site of enzyme. Among the title compounds **4a, 4c**, **4d**, **4e**, **4h**, **4i**, **4k**, and **4l** have shown potent hydrogen bonding interactions with active site of topoisomerase-II enzyme. This work contributed a new family of organophosphorus compounds that serve as lead molecules and help to develop more potential and safe anticancer active compounds by further fine tuning their structure with respect to activity. α-aminophosphonates are the currently available drugs in the market with less toxicity. This new class of drugs is potent in anticancer treatment after their in vivo studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Allen MC, Fuhrer W, Tuck B, Wade R, Wood JM (1989) Renin inhibitors. Synthesis of transition-state analog inhibitors containing phosphorus acid derivatives at the scissile bond. J Med Chem 32:1652–1661

Atherton FR, Hassall CH, Lambert RW (1986) Synthesis and structure-activity relationships of antibacterial phosphonopeptides incorporating (1-aminoethyl)phosphonic acid and (amino methyl) phosphonic acid. J Med Chem 29:29–40

Bashir D, Amrinder S, Akshya S, Praveen P, Arup C, Meena C, Baldev S (2012) Catalyst and solvent-free, ultrasound promoted rapid protocol for the one-pot synthesis of α-aminophosphonates at room temperature. Tetrahedron Lett 53:5497–5502

Bayer E, Gugel K, Hagen H, Jessipow S, Konig W, Zahner H (1972) Metabolites of microorganisms. 98th communication. Phosphinothricin and phosphinothricyl-alanyl-alanine. Helv Chim Acta 55:224–239

Bhagat S, Chakraborty AK (2007) An extremely efficient three-component reaction of aldehydes/ketones, amines, and phosphites (Kabachnik−fields reaction) for the synthesis of α-aminophosphonates catalyzed by magnesium perchlorate. J Org Chem 72:1263–127

Bloemink MJ, Diederen JJH, Dorenbos JP, Heetebrij RJ, Keppler BK, Reedijk J (1999) Calcium ions do accelerate the DNA binding of new antitumor-active platinum aminophosphonate complexes. Eur J Inorg Chem 10:1655–1657

Dhawan B, Redmore D (1987) Optically active 1-aminoalkylphosphonic acids. Phosphorus Sulfur Silicon Relat Elem 32:119–144

Doye S (2004) Development of the Ti-catalyzed intermolecular hydroamination of alkynes. Synlett 1653–1672

Duh B (2002) Effect of antimony catalyst on solid-state polycondensation of poly(ethylene terephthalate). Polymer 43:3147–3154

Herczegh P, Buxton TB, McPherson JC, Kova´cs-Kulyassa A, Brewer PD, Sztaricskai F, Stroebel GG, Plowman KM, Farcasiu D, Hartmann JF (2002) Osteo adsorptive bisphosphonate derivatives of fluoroquinolone antibacterials. J Med Chem 45:2338–2341

Heydari A, Arefi A (2007) One pot three component synthesis of α-aminophosphonates and its derivatives. Catal Commun 8:1023–1026

Hirschmann R, Smith AB, Taylor CM, Benkovic PA, Taylor SD et al. (1994) Synthesis and antifungal activity of novel chiral α-aminophosphonates containing fluorine moiety. Science 265:234–37

Huang MH, Mao S, Feick H, Yan HQ, Wu YY, Kind H, Weber E, Russo R, Yang PD (2001) room-temperature ultraviolet nanowire. Science 292:1897–1899

Jin L, Song B, Zhang G et al. (2006) Synthesis, X-ray crystallographic analysis, and antitumor activity of N-(benzothiazole-2-yl)-1-(fluorophenyl)-O,O-dialkyl-α-aminophosphonates. Bioorg Med Chem Lett 16:1537–1543

Kaboudin B, Rahmani A (2003) Silica-supported ammonium hydrogen carbonate as an efficient reagent for one-pot synthesis of 1-aminophosphonates from aldehydes. Synthesis 17:2705–2708

Kafarski P, Lejczak B (2001) Aminophosphonic acids of potential medical importance. Curr Med Chem Anticancer Agents 1:301–312

Klepacz A, Zwierzak A (2002) An expeditious one-pot synthesis of diethyl N-Boc-1-aminoalkylphosphonates. Tetrahedron Lett 43:1079–1080

Kobra A, Meghdad K, Akbar H (2014) A catalyst-free synthesis of α-aminophosphonates in glycerol. Tetrahedron Lett 55:7236–7239

Kuznetsov YI, Kazanskaya GY, Tsirulnikova NV (2003) Aminophosphonate corrosion inhibitors for steel. Prot Met 39:120–123

Laschat S, Kunz H (1992) Carbohydrates as chiral templates: stereoselective synthesis of (r)- and (s)-α-aminophosphonic acid derivatives. Synthesis 1:90–95

Linderoth S, Pedersen MS (1994) Fe-Al_2O_3 nanocomposites prepared by high-energy ball milling. J Appl Phys 75:5867–5869

Liu H, Imoto H, Shido T, Iwasawa Y (2001) Selective ammoxidation of isobutylene to methacrylonitrile on a new family of crystalline Re–Sb–O catalysts. J Catal 200:69–78

Liu H, Iwasawa Y (2002) Unique performance and characterization of a crystalline $SbRe_2O_6$ catalyst for selective ammoxidation of isobutane. J Phys Chem B 106:2319–2329

Maier L (1990) Organic phosphorus compounds 91.[1] synthesis and properties of 1-amino-2-arylethylphosphonic and-phosphinic acids as well as-phosphine oxides. Phosphorus Sulfur Silicon Relat Elem 53:43–67

Maier L, Spoerri H (1991) Resolution of 1-amino-2-(4-fluorophenyl) ethylphosphonic acid as well as some di- and tripeptides. Phosphorus Sulfur Silicon Relat Elem 61:69–75

Manjula A, Rao V, Neelakanthan P (2003) One-pot synthesis of α-aminophosphonates: an inexpensive approach. Synth Commun 33:2963–2969

Meyer JH, Barlett PA (1998) Macrocyclic inhibitors of penicillopepsin. 1. Design, synthesis, and evaluation of an inhibitor bridged between P1 and P3. J Am Chem Soc 120:4600–4609

Miller DJ, Hammond SM, Anderluzzi D, Bugg TDH (1998) Aminoalkylphosphinate inhibitors of D-Ala-D-Ala adding enzyme. J Chem Soc Perkin Trans 1:131–142

Moonen K, Laureyn I, Stevens CV (2004) Synthetic methods for azaheterocyclic phosphonates and their biological activity. Chem Rev 104:6177–6216

Natchev IA (1988) Synthesis, enzyme-substrate interaction, and herbicidal activity of phosphoryl analogues of lycine. Liebigs Ann Chem 9:861–867

Ouimette D, Coffey M (1989) Comparative antifungal activity of four phosphonate compounds against isolates of nine Phytophthora species. Phytopathology 79:761–767

Radha Rani C, Ira B, Chandra Sekhar Reddy G, Syama Sundar C, Suresh Reddy C (2013) Green synthesis of α-aminophosphonate derivatives on a solid supported TiO2–SiO2 catalyst and their anticancer activity. Arch Pharm Chem Life Sci 346:667–676

Ranu BC, Hajra A, Jana U (1999) General procedure for the synthesis of α-amino phosphonates from aldehydes and ketones using indium (III) chloride as a catalyst. Org Lett 1:1141–1143

Salata OV (2004) Applications of nanoparticles in biology and medicine. J Nanobiotechnol 2:3–8

Schlemminger I, Willecke A, Maison W, Koch R, Lutzen A, Martens J (2001) Diastereoselective Lewis acid mediated hydrophosphonylation of heterocyclic imines: a stereoselective approach towards α-amino phosphonates. J Chem Soc Perkins Trans 1 0:2804–2816

Simoni D, Invidiata FP, Manferdini M, Lampronti I, Rondonin R, Roberti M, Pollini GP (1998) Tetramethylguanidine (TMG)-catalyzed addition of dialkyl phosphites to α, β-unsaturated carbonyl compounds, alkenenitriles, aldehydes, ketones and imines. Tetrahedron Lett 39:7615–7618

Spengler J, Anderle F, Bosch E, Grasselli RK, Pillep B, Behrens P, Lapina OB, Shubin AA, Eberle HJ, Knozinger H (2001) Antimony oxide-modified vanadia-based catalystsphysical characterization and catalytic properties. J Phys Chem B 105:10772–10783

Smith WW, Bartlett PA (1998) Macrocyclic inhibitors of penicillopepsin. 3. Design, synthesis, and evaluation of an inhibitor bridged between P2 and P1. J Am Chem Soc 120:4622–4628

Vichail V, Kirtikara K (2006) Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 1:1112–1116

Xie D, Zhang A, Liu D, Yin, Wan J, Zeng S, Hu D (2017) Synthesis and antiviral activity of novel a-aminophosphonates containing 6-fluorobenzothiazole moiety. Phosphorus Sulfur Silicon Relat Elem 192:1061–1067

Yager KM, Taylor CM, Smith AB (1994) Asymmetric synthesis of α-aminophosphonates via diastereoselective addition of lithium diethyl phosphite to chelating imines. J Am Chem Soc 116:9377–9378

Yang S, Gao XW, Diao CL et al. (2006) Synthesis and antifungal activity of novel chiral α-aminophosphonates containing fluorine moiety. Chin J Chem 24:1581–1588

Yuan CY, Li SS, Li CZ, Chen SJ, Huang WS, Wang GQ, Pan C, Zhang YX (1997) New strategy for the synthesis of functionalized phosphonic acids. Heteroat Chem 8:103–122

Zhang Z, Guo L, Wang W (2001) Synthesis and characterization of antimony oxide nanoparticles. J Mater Res 16:803–805