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Synthesis and Biological Evaluation of Novel α-Aminophosphonate Derivatives Possessing Thiazole-Piperidine Skeleton as Cytotoxic Agents

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Abstract: A new series of α-aminophosphonate containing thiazole-piperidine skeleton has been synthesized. The fifteen synthesized derivatives were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, mass and HRMS spectrometric methods. The cytotoxicity of these compounds was evaluated against A549, MDA-MB-231, MCF-7, HeLa human tumour cell line. Compound **Xa** showed high potency against MDA-MB-231, MCF-7, and HeLa cell line, while compounds **Xd** and **Xg** were more promising against MDA-MB-231 and HeLa cell line, respectively.

Keywords: α-Aminophosphonate, cytotoxic activities, Thiazole- Piperidine, In vitro.

Introduction

The synthesis of numerous biologically significant compounds possessing thiazole -piperidine skeleton were reported[1-5]. The molecules with thiazole- piperidine skeleton (Fig. 1) such as **I** ((E)-2-(5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl)-1-(4-(4-styrylthiazol-2-yl)piperidi- n-1-yl)ethanone) and **II** ((R)-2-(3,5-bis(difluoromethyl)-1H-pyrazol-1-yl)-1-(4-(4-(5-phenyl-4,5-dihydroisoxazol-3-yl)thiazol-2-yl)piperi -din-1-yl)ethanone) are fungicidal and used in crop protection e.g. for controlling Plasmodiophoro mycetes, Oomycetes, Chytridiomy- cetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deutero- mycetes. The novel agonists **III** (2-(1-(5-ethylpyrimidin-2-yl)piperi- din-4-yl)-4-((4-(trifluoromethylthio) phenoxy)methyl) thiazole) and **IV** (4-((4-(1H-tetrazol-1-yl) phenoxy)methyl)-2-(1-(5-ethylpyrimidin-2-yl) piperidin-4-yl)thiazole) are orally active for the

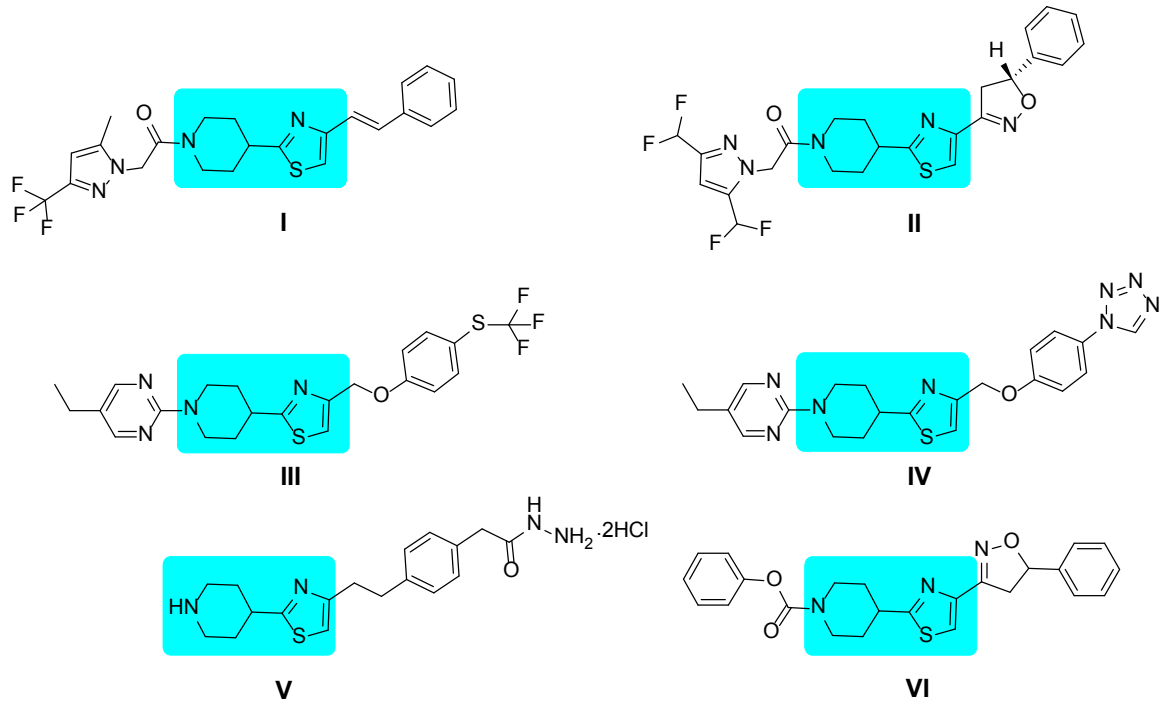


Fig.1. Bioactive molecules with a thiazole-piperidine skeleton.

treatment of, *inter alia*, Type II diabetes. The thiazole derivative **V** (2-(4-(2-(2-(piperidin-4-yl)thiazol-4-yl)ethyl)phenyl)acetohydrazide hydrochloride) is VAP-I inhibitor and medicine for the treatment of a VAP-I associated disease. Furthermore, the compound **VI** (phenyl 4-(4-(5-phenyl-4,5-dihydroisoxazol-3-yl)thiazol-2-yl)piperidine-1-carbo-xylate) was reported as inhibitors of fatty acid amide hydrolase and is useful in the treatment of body pain.

The structure analogy of all molecules **I-VI** (Fig. 1) have shown wide applicability as bioactive compounds. The bioactivity of those molecules is possibly due to the presence of thiazole ring system attached to piperidine moiety and manipulation at both ends with various functionalities.

A literature survey reveals that α-Aminophosphonate have received considerable attention for their significant role in agro, organic and medicinal chemistry since they are structural analogues of natural and synthetic amino acids [6-10]. α-Aminophosphonate derivatives exhibit diverse activities such as antitumor,[11] antibacterial [12], antiviral, antifungal [13], and as inhibitors of phosphatase activity [14]. Jin *et. al.* reported the anti-tumor drugs containing benzothiazole moiety appended with phosphonate groups [15]. Several research groups reported simple and efficient method for the synthesis of α-Aminophosphonates. Some of them are multicomponent synthesis starting from aldehyde, amine, and diethyl phosphite or triethyl phosphite have been reported in presence of Lewis acids [16-25] such as $SnCl_4$, $SnCl_2$, $MgBr_2$, $ZnCl_2$, Cu(OTf) [26], $FeCl_3.8H_2O$ [27] and Brønsted acids [28-29]. Recently, Pawar and his group described ionic liquid promoted synthesis of α- Aminophosphonates and their antiproliferative activity evaluation[30]. Furthermore, Anandan *et. al.* reported the synthesis of α,β-unsaturated hydroxamic acid containing thiazole-piperidine skeleton with enzymatic and antiproliferative activity [31]. In the view of these literature survey our plan is

to develop new cytotoxic agents. In the present study we designed (Fig. 2) and synthesised a series of α-Aminophosphonate with thiazole-piperidine skeleton and further investigated their cytotoxic activity.

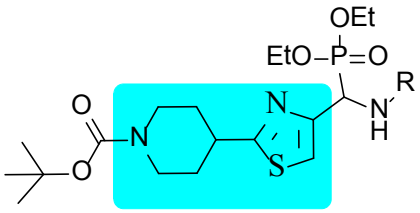


Fig. 2. Design of α-aminophosphonate with a thiazole-piperidine skeleton.

Results and Discussion

Chemistry

To develop a general protocol for the synthesis of α-Aminophosphonate possessing thiazole-piperidine skeleton, we selected three different catalysts reported in the literature such as $ZnCl_2$, $FeCl_3.8H_2O$ and CBr_4 [32]. In a model solvent free reaction 4-(4-formyl-thiazol-2-yl)-piperidine-1-carboxylic acid tert-butyl ester **VII** (1 mmol), 2-aminopyridine **VIIIa** (1 mmol), diethyl phosphite **IX** (1 mmol) and catalyst CBr_4 (5 mol%) were mixed and stirred at room temperature (Scheme 1). The progress of reaction was monitored by thin layer chromatography (1: 1, EtOAc: Hexane). After completion of reaction, aqueous workup and followed by chromatography purification yielded α-Aminophosphonate **Xa** in 82% yield.

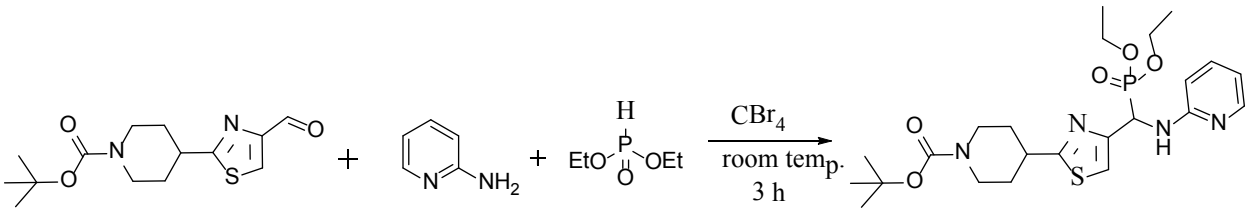
Two other catalysts such as $ZnCl_2$, $FeCl_3.8H_2O$ were tested for this model reaction and the results are presented in the table 1. The yield of compound **Xa** formation while using $ZnCl_2$ and $FeCl_3.8H_2O$ has not remarkable compare to the CBr_4 for 4-(4-formyl-thiazol-2-yl)-piperidine-1-carboxylic acid tert-butyl ester **VII**.

Table 1: Screening of catalyst for the preparation of compound Xa.

Entry	Catalyst	Time/h	Yield %
XI	$ZnCl_2$	5h	71
XII	$FeCl_3.8H_2O$	4.5h	68
XIII	CBr_4	3h	82

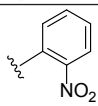
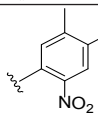
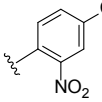
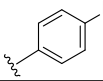
The FT-IR spectrum of compound **Xa** revealed absorption bands at 3774 cm^{-1} for NH stretching vibration and for P=O at 1235 cm^{-1}. In the ^1H NMR spectrum, the POCH proton appears as doublet at δ 5.20 ppm. The ^{13}C NMR spectrum of α-Aminophosphonates showed a signal for the α-carbon near δ 68.5. ^{31}P chemical shift was observed as singlet at δ 20 ppm.

The synthetic utility of developed protocol using CBr_4 catalyst was investigated with structurally diverse aryl amines for the synthesis of α-Aminophosphonates (**Xb-o**), table 2. The aryl amine having functional group such as nitro, methyl, methoxy, fluoro, chloro, bromo and trifluoromethyl were getting good to excellent yields.



Scheme 1. Synthesis of α-Aminophosphonates **4a** having thiazole-piperidine skeleton

Table2. Synthesis of novel α-aminophosphonates **Xa-o** possessing thiazole-piperidine skeleton

Compounds	Amine (R)	Time/h	Yield in %
Xa		3	82
Xb		3	79
Xc		5	84
Xd		5	87
Xe		3	90
Xf		12	82
Xg		12	85
Xh		6	81
Xi		6	78
Xj		4	89
Xk		4	84
Xl		4	75
Xm		5	88
Xn		5	85
Xo		4	81

Biological activity

In vitro cytotoxicity of the novel series of synthesised α-Aminophosphonate compounds (**Xa-o**) were examined on four human tumour cell lines including A549 (human alveolar adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma) and HeLa (human cervical cancer) by the standard MTT assay following literature protocol [33]. The obtained activity results were expressed as IC_{50} in μM and summarized in table 3. The reported IC_{50} values were the average of three different measurements. The cytotoxic activity of the synthesised α-Aminophosphonate compounds tested as compare to standard anticancer agent Doxorubicin.

Table 3. Cytotoxicity evaluation of target compounds, [a] Compounds **Xb, Xe, Xf, Xh, Xi, Xk, Xl, Xm**, and **Xo** showed no activity, [b] NI=No inhibition, [c] The results summarized are the mean values of n=3for IC_{50} values.

	IC_{50} values μM			
Compounds	A549	MDA-MB-231	MCF-7	HeLa
Xa	NI	1.9	4.2	8.8
Xc	32	69.2	NI	83.2
Xd	11.34	3.18	NI	29.7
Xg	31.25	14.85	15.62	7.81
Xj	33.77	31.89	51.99	NI
Xn	58.9	37.7	81.5	NI
Doxorubicin	0.451	0.501	1.05	1.21

According to the cytotoxic activity data reported in table 3, some of the synthesized compounds showed good to moderate activity. Compound **Xa** containing 2-aminopyridine moiety at the α-carbon atom was found to be highly effective against MDA-MB-231, MCF-7 and HeLa cell line with recorded IC_{50} values of 1.9, 4.2 and 8.8 μM, respectively. The compound **Xb** which is structural analog

of **Xa** having 3-aminopyridine moiety at the α-carbon atom showed no cytotoxic activity against the tested cell lines. Among the target compounds **Xc-Xf**, it was found that compound **Xc** having 2-nitrophenyl moiety and **Xd** with 3-nitrophenyl moiety at the α-carbon atom exhibits good to moderate activity against A459, MDA-MB-231 and HeLa cell line. Whereas the compounds **Xe** posseing 4-nitrophenyl moiety and **Xf** with 4,5-dimethyl-2-nitrophenyl at the α-carbon showed no cytotoxic activity. From the series of compounds **Xg-Xi**, **Xg** bearing 4-(trifluoromethyl)-2-nitrophenyl moiety shows cytotoxic activity (IC_{50} 31.25, 14.85, 15.62, 7.81 μM) against all four tested cell line. Furthermore, the compounds **Xh** containing 4-trifluoromethyl phenyl and **Xi** having 3-trifluoromethyl phenyl showed no cytotoxic activity. It is interesting to note that nitro substitution on phenyl ring at 2 (**Xc**) or 3 (**Xd**) position may geometrically permit appropriate fitting of the molecule at the receptor site. It was also found that compound **Xg** having electron withdrawing group such as nitro and trifluoromethyl substitution shows excellent cytotoxic activity compared to the compound **Xc** which having only nitro substitution.

The effects of halogen substituents on the phenyl ring at α-carbon atom were also investigated. Among the compounds **Xj-Xm**, only the compound **Xj** having bromo substitution on phenyl moiety at position 4 exhibits moderate cytotoxic activity (IC_{50} 33.77, 31.89 and 51.99 μM) against A459, MDA-MB-231 and MCF-7 cell line. Whereas the compounds **Xk**, **Xl** and **Xm** containing 3-bromophenyl, 3-chlorophenyl and 4-fluorophenyl respectively showed no cytotoxic activity.

It was also found that compound **Xn** having electron donating methoxy substitution on phenyl ring exhibits cytotoxic activity (IC_{50} 58.9, 37.7 and 81.5) against A459, MDA-MB-231 and MCF-7 cell line. Whereas the compound **Xo** having methyl substitution on phenyl ring showed no cytotoxic activity against tested cell lines.

Conclusion

In summary, we have designed, synthesised and investigated cytotoxic activity of a novel series of α-Aminophosphonate derivatives **Xa-o** possessing thiazole-piperidine skeleton. This study demonstrates that derivative **Xa** exhibited promising cytotoxicity against MDA-MB-231, MCF-7, and HeLa cell line. The derivatives **Xd** and **Xg** are also exhibited significant activity against MDA-MB-231 and HeLa cell line, respectively. The results provided an insight for the development of some more structurally diversified α-Aminophosphonate as promising cytotoxic agents.

Experimental Section

Chemistry

All reagents were purchased from Sigma Aldrich Chemical Co. or Merck and used without further purification. All solvents were received from commercial sources and purified by standard methods. Melting points were determined by open capillary method and were uncorrected on melting point apparatus by Shital Scientific Industries. FT-IR spectra were recorded as KBr pellets on a Perkin Elmer FT-IR 400 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on AVANCE 300 MHz and 75 MHz NMR spectrometers respectively and referenced to TMS. Mass spectrometric data were obtained using the electrospray ionization (ESI-MS) technique on an Agilent Technologies 1100 Series (Agilent Chemistation Software) mass spectrometer. High-resolution mass spectra (HRMS) were obtained by using ESI-Q-TOF mass spectrometry.

General procedure for synthesis of α-Aminophosphonates(Xa-Xo): To a stirred mixture of 4-(4-formyl-thiazol-2-yl)-

piperidine-1-carboxylic acid tert-butyl ester (1 mmol), amine (1 mmol), diethyl phosphite (1 mmol) and carbon tetra bromide (5 mol%) at room temperature with appropriate time. The progress of reaction was monitored by thin layer chromatograph (1: 1 EtOAc / Hexane). After completion of reaction, the reaction mixture was poured in to water (5 mL), and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The obtained crude product was purified by column chromatography using hexane: ethyl acetate as an eluent to afford a pure product. Structures of all synthesized compounds (table 1) were confirmed from spectral data.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (pyridin-2-ylamino) methylphosphonate ***(Xa)***

Brown oil, FT-IR (KBr υ_{max}/cm^{-1}): 3374, 2978, 2857, 2395, 1676, 1366, 1235, 869, 814.; Mass [ESI, 70 eV] m/z: 511 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.26-1.31 (m, 3H, CH_3CH_2), 1.36-1.39 (m, 3H, CH_3CH_2), 1.48 (s, 9H, CH_3), 1.71 (q, 2H, CH_2CH_2), 2.09 (d, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.22 (m, 1H, $CHCH_2$), 4.12-4.20 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.20 (d, 1H, POCH), 5.66 (t, 1H, SCH), 6.69 (d, 1H, PhH), 7.36 (t, 2H, PhH), 7.98 (t, 1H, PhH); ^{13}C NMR (75MHz, $CDCl_3$) δ ppm: 174.44, 154.63, 146.29, 143.3, 132.4, 115.0, 89.9, 79.6, 68.5, 66.4, 63.2, 43.3, 40.3, 32.2, 28.3, 16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 20.0; HRMS (ESI) m/z: calculated for $C_{23}H_{35}O_5N_4PS$: 511.2065, found: 511.2138.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (pyridin-3-ylamino) met -hyl phosphonate ***(Xb)***

Brown oil, FT-IR (KBr υ_{max}/cm^{-1}): 3353, 3108, 2980, 2855, 1692, 1574, 1478, 1238, 1164, 1033, 870, 817; Mass [ESI, 70 eV] *m/z*: $[M+Na]^+$ 523; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.19 (t, 3H, CH_3CH_2), 1.28 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.65-1.74 (m, 2H, CH_2CH_2), 2.06 (t, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.20 (m, 1H, $CHCH_2$), 3.86-4.30 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.05 (d, 1H, POCH), 7.09 (s, 1H, PhH), 7.15 (s, 1H, PhH), 7.74 (d, 1H, PhH), 7.98 (d, 1H, PhH), 8.20 (s, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 173.5, 154.6, 151.1, 142.7, 139.5, 114.9, 111.9, 88.4, 83.7, 58.6, 54.0, 36.3, 32.0, 28.3, 16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.0.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl)(2-nitrophenyl amino) methylphosphonate ***(Xc)***

Brown oil, FT-IR (KBr υ_{max}/cm^{-1}): 3346, 3104, 2953, 1635, 1230,766 cm^{-1} ; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 555; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.25-1.28 (m, 3H, CH_3CH_2), 1.29-1.33 (m, 3H, CH_3CH_2), 1.48 (s, 9H, CH_3), 1.74 (q, 2H, CH_2CH_2), 2.12 (d, 2H, CH_2CH_2), 2.91 (t, 2H, CH_2CH_2), 3.12-3.33 (m, 1H, $CHCH_2$), 3.98-4.32 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.30 (d, 1H, POCH), 6.72 (t, 1H, PhH), 6.91 (t, 1H, PhH), 7.20 (s, 1H, PhH), 7.41 (t, 1 H, PhH), 8.19 (d, 1H, PhH), 8.99(S, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.0, 154.0, 143.5, 135.9, 133.0, 116.6, 115.6, 114.5, 79.5, 76.5, 63.6, 53.0, 51.0, 40.3, 32.1, 29.5, 28.3, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.8; HRMS (ESI) *m/z*: calculated for $C_{25}H_{35}O_7N_4PS$: 555.5960, found: 555.2036.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (3-nitrophenylamino) methyl phosphonate ***(Xd)***

Yellow Solid, m.p. 145-147 °C; FT-IR (KBr υ_{max}/cm^{-1}): 3289, 3106, 2969, 1621, 1464, 1238, 813; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 555; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.20 (t, 3H, CH_3CH_2), 1.30 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.61-1.79 (m, 2H, CH_2CH_2), 2.07 (t, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.219 (m, 1H, $CHCH_2$), 3.82-4.32 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.26 (t, 1H, POCH), 6.98 (d, 1H, PhH), 7.30 (s, 1H, SCH), 7.49 (t, 1H, PhH), 7.55 (d, 1H, PhH), 7.58 (d, 1H, PhH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.9, 154.6, 149.3,

135.9, 133.1, 126.6, 116.6, 115.6, 114.6, 79.6, 56.3, 40.3, 32.03, 28.3,16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.6; HRMS (ESI) *m/z*: calculated for $C_{24}H_{35}O_7N_4NaPS$: 555.5960, found: 555.2036.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (4-nitrophenylamino) methylphosphonate ***(Xe)***

Yellow Solid, m.p. 115-118 ˚C; FT-IR (KBr υ_{max}/cm^{-1}): 3276, 3197, 2973, 1696, 1596, 1237, 1110, 815; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 555; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.18 (t, 3H, CH_3CH_2), 1.28 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.63-1.78 (m, 2H, CH_2CH_2), 2.08 (d, 2H, CH_2CH_2), 2.88 (t, 1H, $CHCH_2$), 3.08-3.21 (m, 1H, $CHCH_2$), 3.82-4.26 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.05 (d, 1H, POCH), 6.83 (d, 1H, PhH), 6.89 (s, 1H, PhH), 6.95 (s, 1H, PhH), 6.97 (s, 1H, SCH), 7.22 (d, 1H, PhH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 175.2, 154.5, 151.0, 149.2, 138.9, 125.9, 112.2, 79.6, 63.5, 53.0, 50.9, 40.4, 32.2, 28.3, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.9; HRMS (ESI) *m/z*: calculated for $C_{24}H_{35}O_7N_4NaPS$: 555.5960, found:555.2036.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (4,5-dimethyl-2-nitro phenyl amino) methylphosphonate ***(Xf)***

Brown oil, FT-IR (KBr υ_{max}/cm^{-1}): 3403, 3108, 2979, 1692, 1477, 1236, 1164, 870; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 583; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.18-1.23 (m, 3H, CH_3CH_2), 1.25-1.33 (m, 3H, CH_3CH_2), 1.42 (s, 9H, CH_3), 1.67 (t, 2H, CH_2CH_2), 1.98-2.07 (m, 2H, CH_2CH_2), 2.83 (d, 1H, $CHCH_2$), 3.05-3.14 (m, 2H, CH_2CH_2), 3.16 (s, 3H, $PhCH_3$), 3.18 (s, 3H, $PhCH_3$), 3.89-4.55 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.10 (d, 1H, POCH), 6.99 (d, 1H, PhH), 7.55 (d, 1H, PhH), 8.43 (s, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 154.6, 146.9, 142.1, 131.0, 126.4, 125.7, 115.1, 79.63, 40.4, 32.2, 28.3, 18.4, 16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 20.9; HRMS (ESI) *m/z*: calculated for $C_{26}H_{40}O_7N_4PS$ = 583.2277 found: 583.2345.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (4-(trifluoromethyl)-2-nitrophenylamino) methylphospho- nate ***(Xg)***

Brown oil, FT-IR (KBr υ_{max}/cm^{-1}): 3306, 2965, 2624, 1694, 1613, 1449, 1317, 1225, 1120, 872, 822; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 623; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.26-1.28 (m, 3H, CH_3CH_2), 1.31-1.36 (m, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.66-1.80 (m, 2H, CH_2CH_2), 2.10 (t, 2H, CH_2CH_2), 2.89 (d, 2H, CH_2CH_2), 3.11-3.20 (m, 1H, $CHCH_2$), 3.85-4.25 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.16 (d, 1H, POCH), 7.05 (d, 1H, PhH), 7.31 (d, 1H, PhH), 7.61 (d, 1H, PhH), 8.49 (s, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.5, 158.4, 150.9, 132.2, 122.3, 114.8, 76.5, 62.9, 54.4, 52.4, 40.3, 32.2, 28.2, 16.1, 16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 15.5.

Diethyl(2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(4-(trifluoromethyl) phenylamino)methyl phosphonate ***(Xh)***

White solid, m.p.105-107 ˚C; FT-IR (KBr υmax/cm^{-1}): 3398, 2979, 1625, 1587, 1235, 1167, 871; Mass [ESI, 70 eV] m/z: $[M+H]^+$ 578; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.18 (t, 3H, CH_3CH_2), 1.29 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.70 (q, 2H, CH_2CH_2), 2.07 (t, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.21 (m, 1H, $CHCH_2$), 3.85-4.25 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 4.98 (d, 1H, POCH), 6.55 (d, 1H, PhH), 7.18 (d, 2H, PhH), 7.21 (s, 1H, PhH), 7.22 (s, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm:174.3, 154.4, 154.6, 143.7, 137.5, 127.3, 124.3, 121.6, 116.1, 89.7, 51.1, 40.4, 28.3, 21.1, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 20.7.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl) (3-(trifluoromethyl) phenyl amino) methylphosphonate ***(Xi)***

Brown oil, FT-IR (KBr υmax/cm^{-1}): 3294, 3179, 1689, 1477, 1227, 1103, 869; Mass [ESI, 70 eV] m/z: $[M+H]^+$ 578; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.20 (t, 3H, CH_3CH_2), 1.29 (t,

3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.63-1.79 (m, 2H, CH_2CH_2), 2.09 (d, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.18 (m, 1H, $CHCH_2$), 3.72-4.29 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.08 (d, 1H, POCH), 6.57 (d, 1H, PhH), 6.68 (t, 1H, PhH), 7.04 (t, 2H, PhH), 7.17 (d, 1H, PhH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm :174.9, 150.2, 148.7, 126.4, 115.6, 113.0, 79.6, 63.2, 53.3, 51.2, 40.5, 32.3, 28.3, 16.4; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 17.8; HRMS (ESI) m/z: calculated for $C_{25}H_{35}F_3N_3O_5PS$: 578.1987, found: 578.2055.

Diethyl (2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(4-bromophenylamino) methyl phosphonate ***(Xj)***

White solid, m.p. 135-138 °C; FT-IR (KBr υmax/cm^{-1}): 3292, 3101, 2974, 1691, 1534, 1229, 1165, 867; Mass [ESI, 70 eV] m/z: $[M+2H]^+$ 590; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.18 (t, 3H, CH_3CH_2), 1.28 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.63-1.79 (m, 2H, CH_2CH_2), 2.06 (t, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.09-3.21(m, 1H, $CHCH_2$), 3.67-4.28 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.05 (d, 1H, POCH), 6.82 (d, 1H, PhH), 6.89 (s, 1 H, SCH), 6.96 (d, 1H, PhH), 7.22 (t, 2H, PhH); ^{13}C NMR (75 MHz ,$CDCl_3$) δ ppm: 174.7, 150.5, 145.3, 131.8, 115.4, 110.3, 79.6, 53.8, 51.8, 40.4, 32.3, 28.3, 16.3, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.4.

Diethyl (2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(3-bromophenylamino)methyl phosphonate ***(Xk)***

Brown solid, m.p. 138-140 °C; FT-IR (KBr υmax/cm^{-1}): 3298, 3102, 2944, 1689, 1465, 1367, 1223, 1056, 850.; Mass [ESI, 70 eV] m/z: $[M+H]^+$ 588; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.19 (t, 3H, CH_3CH_2), 1.29 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.64-1.77 (m, 2H, CH_2CH_2), 2.07 (t, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.08-3.21 (m, 1H, $CHCH_2$), 3.83-4.27 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 4.99 (d, 1H, POCH), 6.58 (d, 1H, PhH), 6.84 (d, 2H, PhH), 6.98(t, 1H, PhH), 7.19 (d, 1H, SCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.7, 154.6, 150.3, 146.6, 116.8, 110.1, 79.6, 53.6, 51.5, 40.5, 32.2, 28.3, 16.4, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.3; HRMS (ESI) m/z: calculated for $C_{24}H_{35}O_5N_3BrPS$: 588.1218, found: 588.1290.

Diethyl (2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(3-chlorophenyl amino) methylphosphonate ***(Xl)***

Brown solid, m.p. 128-130 °C; FT-IR (KBr υ_{max}/cm^{-1}): 3298, 3103, 1596, 1482, 1248, 1018, 816; Mass [ESI, 70 eV] *m/z*: $[M+H]^+$ 544; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.16 (t, 3H, CH_3CH_2), 1.26 (t, 3H, CH_3CH_2) 1.44 (s, 9H, CH_3), 1.68 (q, 2H, CH_2CH_2), 2.05 (t, 2H, CH_2CH_2), 2.86 (t, 2H, CH_2CH_2), 3.08-3.15 (m, 1H, $CHCH_2$), 3.98-4.21 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 4.99 (d, 1H, POCH), 6.09 (s, 1H, PhH), 6.52 (d, 1H, PhH), 6.65 (s, 1H, SCH), 7.01(t, 1H, PhH), 7.91 (s, 1H, PhH); ^{13}C NMR(75 MHz, $CDCl_3$) δ ppm: 174.8, 154.6, 150.3, 130.1, 118.4, 115.5, 112.1, 79.6, 63.4, 63.3, 53.6, 51.5, 40.4, 32.2, 28.3, 16.2, 16.4; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 20.7; HRMS (ESI) *m/z*: calculated for $C_{24}H_{35}ClO_5N_3PS$: 544.1723, found: 544.1797.

Diethyl (2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(4-fluorophenylamino) methylphosphonate ***(Xm)***

Black oil, FT-IR (KBr υmax/cm^{-1}): 3295, 3105, 2999, 1689, 1447, 1233, 1168, 867.16; Mass [ESI, 70 eV] m/z: $[M]^+$ 527; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.19 (t, 3H, CH_3CH_2), 1.29 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.63-1.77 (m, 2H, CH_2CH_2), 2.08 (d, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.06-3.22 (m, 1H, $CHCH_2$), 3.82-4.25 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 4.96 (d, 1H, POCH), 6.62 (d, 1H, PhH), 7.18 (d, 1H, SCH), 7.22 (s,2H, PhH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.4, 154.6, 152.8, 151.2, 140.2, 115.8, 114.2, 79.6, 63.4, 55.0, 32.2, 16.4, 16.2; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 18.8.

Diethyl(2-(1-(tert-butoxycarbonyl)piperidin-4-yl)thiazol-4-yl)(4-methoxyphenylamino) methylphosphonate ***(Xn)***

Brown solid, m.p.100-103 °C; FT-IR (KBr

υmax/cm^{-1}): 3290, 3160, 2975, 1689, 1466, 1100, 846.24; Mass [ESI, 70 eV] m/z: $[M+H]^+$ 540; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.19 (t, 3H, CH_3CH_2), 1.29 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.70 (q, 2H, CH_2CH_2), 2.08 (d, 2H, CH_2CH_2), 2.88 (t, 2H, CH_2CH_2), 3.06-3.22 (m, 1H, $CHCH_2$), 3.71 (s, 3H, OCH_3), 3.74-4.23 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 4.96 (d, 1H, POCH), 6.62 (d,2H, PhH), 6.71 (t, 1H, PhH), 6.75 (d, 1H, SCH), 7.16 (s, 1H, PhH); ^{13}C NMR(75 MHz, $CDCl_3$) δ ppm: 174.7, 154.6, 150.6, 145.2, 131.8, 115.5, 115.4, 110.3, 79.6, 63.5, 63,1, 53.9, 40.5, 32.3, 28.4, 16.4,16.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.2.

Diethyl (2-(1-(tert-butoxycarbonyl) piperidin-4-yl) thiazol-4-yl)(o-tolylamino)methyl phosphonate ***(Xo)*** Brown oil, FT-IR (KBr υmax/cm^{-1}): 3355, 3086, 2959, 1692, 1365, 1170, 838; Mass [ESI, 70 eV] m/z: $[M+H]^+$ 524; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.20 (t, 3H, CH_3CH_2), 1.29 (t, 3H, CH_3CH_2), 1.47 (s, 9H, CH_3), 1.66-1.76 (m, 2H, CH_2CH_2), 2.08 (d, 2H, CH_2CH_2), 2.25 (t, 3H, CH_3), 2.88 (t, 2H, CH_2CH_2), 3.11-3.18 (m, 1H, $CHCH_2$), 3.87-4.24 (m, 7H, 2× $POCH_2$, NH, CH_2CH_2), 5.08 (d, 1H, POCH), 6.11 (s, 1H, PhH), 6.58 (d, 1H, PhH), 7.03 (s, 1H, PhH), 7.04 (s, 1H, SCH), 7.17 (d, 1H, PhH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 174.34, 154.52, 151.0, 144.2, 130.3, 123.0, 118.1, 115.1, 115.0, 112.2, 79.4, 62.9, 61.6, 54.0, 51.9, 43.3, 40.3, 32.2, 28.2, 17.5; ^{31}P NMR (500 MHz, $CDCl_3$) δ ppm: 19.0; HRMS (ESI) m/z: calculated for $C_{25}H_{38}O_5N_3PS$: 524.2269, found: 524.2342.

Biology

The pharmacological evaluations of the products were carried out in the chemical biology laboratory, CSIR-IICT, Hyderabad.

Cytotoxicity Assay

The cytotoxicity of synthesized phosphonate esters were determined on the basis of measurement of in vitro growth inhibition of tumour cell lines in 96 well plates by cell-mediated reduction of a tetrazolium salt to water-insoluble formazan crystals using doxorubicin as a standard. The cytotoxicity of these test compounds was assessed against a panel of four different human tumour cell lines: A549 (ATCC No. CCL-185) derived from human alveolar adenocarcinoma epithelial cells, HeLa (ATCC No. CCL-2) derived from human cervical cancer cells, MDA-MB-231 (ATCC No. HTB-26) and MCF7 (ATCC No. HTB-22) both derived from human breast adenocarcinoma cells using the MTT assay [33]. The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose-response curves. IC_{50} values (in μM) were expressed as the average of three independent experiments.

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