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Synthesis, X-ray crystallographic analysis, and antitumor activity of *N*-(benzothiazole-2-yl)-1-(fluorophenyl)-*O*,*O*-dialkyl-α-aminophosphonates

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Abstract—α-Aminophosphonates containing benzothiazole and fluorine moiety, **4a**–**4m**, were synthesized by Mannich-type addition in ionic liquid media with high yield and short reaction time. Their structures were established by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The X-ray crystallographic data of compounds **4j** and **4m** were provided. The newly synthesized compounds were evaluated for their anticancer activities against PC3, A431, A375, and Bcap37 cells in vitro by the MTT method. Compound **4c** is highly effective against PC3 cells and moderate to A431 cells. Hence, further study is necessary to find out the potential antitumor activities.

As isosteres of aminocarboxylic acids, α-aminophosphonate derivatives have high potential bioactivity. They possess a wide range of antitumor, antiviral, and antifungal properties and have been widely used as insecticides and herbicides.[1] Among these compounds, studies are mainly concentrated on those containing heterocycle moieties such as thiophene, furan, pyrole, 1,3,4-thiadiazole, and bezothiazole.[2] For example, the compound containing benzothiazole moiety has been reported as a representative compound because of its excellent fungicidal activity.[2,3] In our previous work, we have synthesized two series of α-aminophosphonates containing fluorine moiety or benzothiazole moiety.[4] α-Aminophosphonates containing benzothiazole moiety have been suggested as antitumor agent,[5] although no data about antitumor activities have been reported. This was the first indication that this kind of compound may possess potential antitumor activity. We put fluorine or trifluoromethyl group at the 2- and 4-positions of the phenyl ring to see if it will enhance the antitumor activity. The aim of the study was to find a new inhibitor to PC3, A431, A375, and Bcap-37 cells.

A typical method for the synthesis of substituted α-aminophosphonates is the one-pot reaction of aldehydes, amine, and dialkyl phosphite by Mannich-type addition, usually in organic solvent system under high temperature. However, the reported method involved poisonous solvent and expensive catalyst, long reaction time, low yields, and complex handling. Recently, ionic liquids, a kind of ion solvent, which combine the advantages of both traditional molecular solvents and melt salts, have been considered as promising new reaction media and have been widely used in catalytic and non-catalytic reactions.[6] As part of our green technology program we would also like to disclose here a more practical green alternative for a new method to synthesize α-aminophosphonate by a three-component condensation of aldehydes, aminobenzothiazole, and dialkyl phosphite at 100–102 °C in ionic liquids. The method was easy going, environment-friendly, and high yielding. The synthetic route is shown in Scheme 1. The results are summarized in Table 3. The structures of the compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and X-ray analysis (Figs. 1 and 2). Preliminary bioassays indicated that compound **4c**

Keywords: α-Aminophosphonate; Benzothiazole moiety; Synthesis; Ionic liquids; Antitumor activity.
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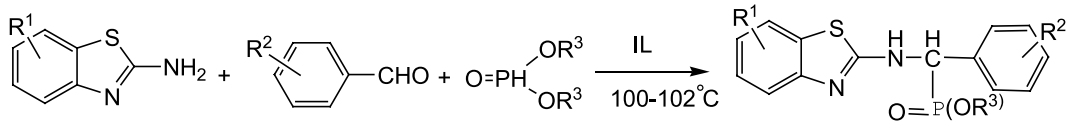

Scheme 1. Synthetic route.

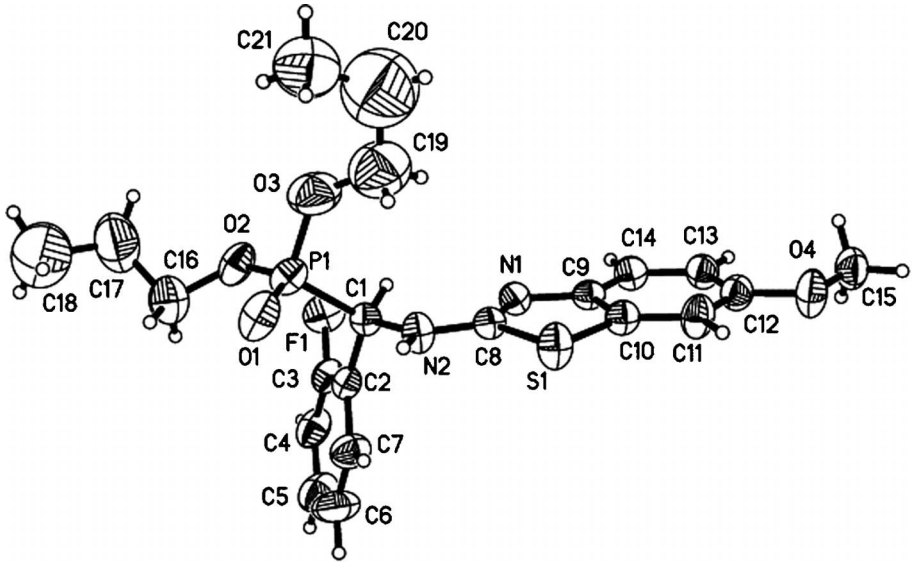

Figure 1. ORTEP drawing of **4j**.

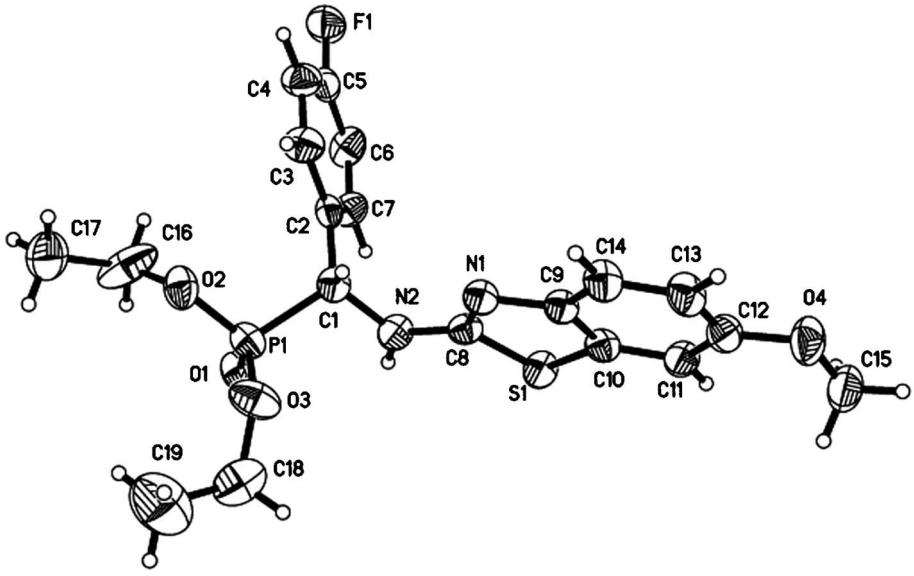

Figure 2. ORTEP drawing of **4m**.

exhibits good antitumor activity against PC3 cell in vitro by the MTT method.

In order to optimize the reaction conditions, the Mannich addition reactions were carried out under several different conditions. First, the reaction technique should be used for four kinds of ionic liquids. We chose a reaction of 1 equiv 2-amino-6-methoxybenzothiazole, 1 equiv diethyl phosphite, and 1 equiv 2-fluorobenzo-aldehyde as a model reaction to test the catalytic activity of these ionic liquids (ILs) and no other solvents were added as initial explorations. The results are shown in Table 1, the former three ILs can catalyze the reaction efficiently (Table 1, entries 1–3) and [bmim][PF_6] shows the highest catalytic activity for the above reaction. [bmim][HSO_4] reacts readily with amine due to its strong acidity, however, it was obtained in low yield (Table 1, entry 4). The result demonstrated that the presence of

Table 1. Results for synthesis of **4m** at different reaction conditions

Entry	Compound	Solvent	Time (h)	Temperature (°C)	Yield (%)
1[a]	**4m**	[bmim][PF_6]	1.5	100–102	90.2
2[a]	**4m**	[bmim][H_2PO_4]	1.5	100–102	74.3
3[a]	**4m**	[bmim][BF_4]	1.5	100–102	79.0
4[a]	**4m**	[bmim][HSO_4]	1.5	100–102	42.2
5[a]	**4m**	[bmim][PF_6]	1.5	20–25	23.4
6[a]	**4m**	[bmim][PF_6]	5	20–25	44.5
7[a]	**4m**	[bmim][PF_6]	15	20–25	68.9
8[a]	**4m**	[bmim][PF_6]	1.5	50–55	66.2
9[a]	**4m**	[bmim][PF_6]	1.5	80–85	70.9
10[a]	**4m**	[bmim][PF_6]	1.5	100–102	91.0
11[a]	**4m**	[bmim][PF_6]	1.5	105–110	92.0
12[b]	**4m**	Toluene	1.5	100–105	45.6
13[b]	**4m**	Toluene	10	100–105	76.2
14[b]	**4m**	—	1.5	100–102	66.0
15[a]	**4m**	[bmim][PF_6]	0.5	100–102	77.8
16[a]	**4m**	[bmim][PF_6]	1.0	100–102	82.1
17[a]	**4m**	[bmim][PF_6]	2.0	100–102	92.4
18[a]	**4m**	[bmim][PF_6]	3.0	100–102	92.0

[a] The reactions were carried out in ionic liquids (4 mL) under stirring.
[b] The reactions were carried out in toluene under stirring.

[bmim][PF_6] can accelerate the Mannich addition reaction. When other solvents were used as solvents instead of [bmim][PF_6] in the present reaction, no remarkable improvement of the yield of the product was observed (Table 1, entries 2 and 3). Second, we examined the influence of reaction temperature and reaction time, as is shown in Table 1. As for the reaction temperature, it could be seen that the yield was relatively lower when the reaction was performed at room temperature (Table 1, entry 5) than at 100–102 °C (Table 1, entry 10). No substantial improvement was observed when the reaction system was heated to 105 °C (Table 1, entry 11). Hence, it's better for the reaction to be proceeded at 100–102 °C instead of a lower or higher temperature. When the reaction time was prolonged from 0.5 to 1.0 h, the yield of **4m** was increased from 77.8% to 82.1% (Table 1, entries 15 and 16). When the reaction time was further prolonged to 2.0 h, a slight improvement of yield (92.4%, Table 1, entry 17) was obtained compared to that of 1.5 h (91.0%, entry 10).

The amount of [bmim][PF_6] was investigated for the synthesis of **4m**. The results are summarized in Table 2. As we can see in Table 2, the best result was obtained when 4 mL [bmim][PF_6] was used (Table 2, entry 2). Increasing the amount reaction of [bmim][PF_6] to 5–8 mL for product **4m**, no remarkable improvement of the yield was observed (Table 2, entries 3–6).

Consequently, we investigated the catalytic activity of recycled [bmim][PF_6] in the reaction of 2-amino-6-methoxybenzothiazole and diethyl phosphite and 2-fluorobenzoaldehyde. As shown in Table 3, [bmim][PF_6] could be reused at least four times without significant loss of activity. So the ionic liquids (ILs) can be reused and provide less pollution as green procedure to the environment.

Table 2. Effect of the amount of [bmim][PF_6] on the synthesis of **4m**

Entry	The amount reaction of [bmim][PF_6] (mL)	Yield (%)
1	2	82.1
2	4	91.0
3	5	90.9
4	6	90.4
5	7	91.2
6	8	89.0

Using the above optimal condition, the compound **4a**–**4m** were prepared by condensation of 2-amino-6-methoxybenzothiazole (or 2-amino-4-methylbenzothi-azole), 2-flurobenzoaldehyde (or 4-fluorobenzo-aldehyde or 4-trifluoromethylbenzoaldehyde) and dialkyl phosphite in ionic liquids as shown in Table 4.[8]

Table 3. Recycling of [bmim][PF_6] in the synthesis of compound **4m** (1.5 h, IL 4.0 mL, 100–102 °C)

Entry	Cycle times	Yield (%)
1	1	90.5
2	2	89.0
3	3	91.4
4	4	88.7

Table 4. Synthesis of α-aminophosphonates with benzothiazole moiety (**4a**–**4m**)[a]

Entry	Compound	R^1	R^2	R^3	Yield[b] (%)
1	**4a**	4-CH_3	2-F	Et	87.0
2	**4b**	4-CH_3	2-F	*n*-Pr	85.6
3	**4c**	4-CH_3	2-F	*n*-Bu	68.2
4	**4d**	4-CH_3	4-CF_3	Me	70.8
5	**4e**	4-CH_3	4-CF_3	Et	84.1
6	**4f**	4-CH_3	4-CF_3	*i*-Pr	76.3
7	**4g**	4-CH_3	4-CF_3	*n*-Bu	72.3
8	**4h**	6-OCH_3	2-F	Me	66.1
9	**4i**	6-OCH_3	2-F	Et	85.6
10	**4j**	6-OCH_3	2-F	*n*-Pr	85.6
11	**4k**	6-OCH_3	2-F	*i*-Pr	84.2
12	**4l**	6-OCH_3	2-F	*n*-Bu	72.1
13	**4m**	6-OCH_3	4-F	Et	84.2

[a] All reactions were carried out at 100–102 °C for 1.5 h in ionic liquids (4 mL).
[b] Yields of isolated products.

Table 5. Inhibition rate (%) of compounds **4a**–**4m** to PC3, A431, A375 and Bcap37 in 72 h at 1, 5, and 10 μM

Compound	PC3 cells			A431 cells			A375 cells			Bcap-37 cells		
	1 μM	5 μM	10 μM	1 μM	5 μM	10 μM	1 μM	5 μM	10 μM	1 μM	5 μM	10 μM
4a	3.51	22.9*	41.9*	17.7*	23.3*	35.3*	−13.9	−6.1	9.2	35.8*	42.6*	58.9*
4b	21.7*	29.4*	43.3*	15.9*	27.3*	31.7*	−0.2	4.8	4.3	−18.9	0.8	21.7
4c	52.1*	86.0*	89.1**	32.1*	49.0*	72.1*	11.2	25.6	32.1*	10.0	29.0*	38.1*
4d	4.0	4.6	8.9	22.1*	26.0*	47.3*	1.8	19.2*	20.8*	−13.2	−0.8	2.5
4e	12.6	13.5	49.3*	0.36	2.06	16.9*	−10.2	1.6	14.2*	−5.2	9.8	16.7*
4f	6.3	15.3	25.9*	17.3	20.5*	29.5*	−1.3	8.6	9.1	−0.7	11.2*	22.7*
4g	5.0	31.6	36.0*	37.7*	40.8*	57.4*	−3.2	21.6*	31.5*	0.1	9.2	20.3
4h	21.4*	26.6	33.3*	1.9	7.9	8.64	4.3	10.1	11.4	11.9	17.8*	25.1*
4i	16.8	22.5	38.9	4.5	12.3	22.1*	7.5	7.9	15.7*	20.5*	34.5*	49.4*
4j	5.0	15.8	28.7*	35.3*	49.2*	58.1*	10.3	13.9	23.8*	21.3*	25.1*	27.1*
4k	−8.5	2.1	18.5*	−7.4	7.5	16.7*	−4.3	1.7	2.8	39.7*	43.2*	50.4*
4l	−6.6	5.3	13.2*	−3.7	5.4	28.8*	−6.2	3.5	17.4*	23.6*	38.2*	44.2*
4m	32.1*	41.0*	49.5*	11.2	20.9*	31.2*	8.0	10.9	34.5*	10.0	39.0*	49.9*

Inhibition rate (%) = $(A_1 - A_2)/A_1 \times 100\%$. A_1, the mean optical densities of untreated cells; A_2, the mean optical densities of drug treated cells.
* $P < 0.05$.
** $P < 0.01$.

The single-crystal structure of **4m** was determined by X-ray crystallography.[9] The molecular structure of **4j** and **4m** are illustrated in Figures 1 and 2. It could be seen from the crystallographic data that the crystal structure of **4j** and **4m** are very similar. Both P atoms exhibit distorted tetrahedral configuration. Take **4m** as example, the bond angles of O(1)–P(1)–O(2) (114.6°) and O(2)–P(1)–O(3) (116.6°) are significantly larger than that of O(2)–P(1)–O(3) (102.5°). The bond length of P(1)–C(1) (1.804 Å) is a little shorter than normal P–C single bond length (1.850 Å)[10] and a benzothiazole ring exists in the atom N(2) which is linked to the α-C so that the free rotation of P(1)–C(1) bond is hindered. The bond length of C(8)–N(2) (1.358 Å) is remarkably shorter than normal C–N (1.47 Å)[11] and close to the C=N double bond distance(1.34 Å),[11] which is indicative of significant double bond character. The bond length of C(8)–N(1) is 1.290(4), nearer to that of typical C=N. The single bond lengths of C(9)–C(10), N(1)–C(9), C(8)–S(1), and C(10)–S(1) are 1.384, 1.396, 1.758, and 1.749 Å, respectively, which are shorter than typical C–C (1.54 Å), and C–S (1.85 Å).[12] There is an intermolecular hydrogen bond which is N(2)–H(2)···O(1) (symmetry code, $-x + 1$, $-y + 1/2$, z), with N(2)–H(2) = 0.860 Å, H(2)···O(1) = 2.053, N(2)···O(1) = 2.814 Å, and N(2)–H(2)···O(1) = 147.2°.

The antitumor activities in vitro for these compounds were evaluated against PC3, A375, A431, and Bcap-37 cells by the MTT method.[13] The result is summarized in Table 5. Usually, when a compound shows a high inhibition rate more than 50% at 1 μM or more than 85% at 10 μM, it will be considered to be strongly effective. According to this standard, it can be found from Table 5 that compound **4c** has strong activity against PC3 cells. The data given in Table 5 indicate that the changes of substituents affected the antitumor activity. Among **4a**–**4m**, compound **4c** with 4-CH_3 (R^1), 2-F (R^2), and *n*-Bu (R^3) has the highest antiproliferation activity on PC3 cells, with an inhibition rate of 52.1% and 86.0% at the concentration of 1 and 5 μM, respectively. The compounds **4a**–**4c** (R^2 = 2-F) have a relatively higher antitumor activity than that of **4d**–**4g** (R^2 = 4-CF_3). The antitumor data indicate that the nature of fluorine and alkyl affects antitumor activity to A431 and PC3 cells. For example, when R^2 was 2-F and R^3 was *n*-Bu, compound **4c** exhibited moderate antitumor activity with the antiproliferation rate of 72.1% against A431 cells at 10 μM. That is to say, the antitumor activity of **4c** to A431 that were substituted by *n*-Bu of phosphonate was higher than the activity of those **4a**, **4b** which were substituted by Et, and *n*-Pr. Disappointingly, most compounds only displayed low to moderate inhibition against A375 cells and Bcap-37 cells.

In summary, we described a practical and efficient procedure for the preparation of α-aminophosphonate with benzothiazole moiety through the three-component reaction of 2-aminobenzothiazole, *O*,*O*-dialkylphosphite and 2- or 4-fluorobenzoaldehyde or 4-trifluorobenzo-aldehyde in ionic liquids for 1.5 h at 100–102 °C. The reaction is, in general, very fast, clean, and atom-economic. One of the title compounds, **4c**, is highly effective against PC3 cells and moderately effective against A431 cells. These identified α-aminophosphonates with benzothiazole moiety can be very useful for further optimization work in antitumor chemotherapy.

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8. General experimental procedure for the synthesis of α-aminophosphonates with benzothiazole moiety. 2-Amino-4-methylbenzothiazole or 2-amino-6-methoxy-benzothiazole (5 mmol), 2- or 4-fluoro or 4-trifluorobenzoaldehyde (5 mmol), dialkyl phosphite (5 mmol) and 4 mL [bmim][PF_6] were added into an oven-dried three-necked 25 mL round-bottomed flask. The mixture was stirred at 100–102 °C for 1.5 h. The resulting mixture was quenched with a few drops of water followed by extraction with Et_2O (3× 6 mL). After removal of the ether solvent in vacuum, the crude solid was recrystallized from ethanol and water (1:1, v/v) to give **4a**–**4m** as a white solid. The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are not corrected. The IR spectra were recorded on a Shimadzu IR prestige-21 spectrometer in KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-INOVA 400 MHz spectrometer in $CDCl_3$ at room temperature using TMS as internal reference. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. The reagents were all analytically or chemically pure. Dialkyl phosphite was prepared according to literature method.[7]

   Compound **4a**: yield 87.0%, mp: 147–148 °C, IR (KBr): 3211.4, 1591.2, 1539.2, 1487.12, 1236.3, 1213.2, 1049.2, 1028.0 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.117–1.344 (m, 6H, $2CCH_3$), 2.523 (s, 3H, Ar-CH_3), 3.796–4.062 (m, 2H, OCH_2), 4.207–4.298 (m, 2H, OCH_2), 5.834 (d, J = 25.4 Hz, 1H, CHP), 6.946–7.376 (m, 7H, Ar-H), 7.623 (t, J = 7.2 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$): 164.56, 130.83, 129.804, 129.34, 129.27, 126.49, 124.40, 123.11, 122.97, 121.76, 118.12, 115.39, 115.18, 63.70, 63.63, 63.56, 49.95, 48.36, 18.15, 16.44, 16.39, 16.14, 16.09. Elemental analysis: C, 55.87, H, 5.43, N, 6.86; calculated from $C_{19}H_{22}FN_2O_3PS$. Observed: C, 55.80, H, 5.20, N, 6.76.

   Compound **4b**: yield 85.6%, mp: 160–162 °C; IR (KBr): 3230.77, 3034.03, 2966.52, 1591.27, 1577.77, 1533.41, 1492.90, 1230.58, 1219.01, 1062.78, 1006.84 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): 0.781–0.929 (m, 6H, $2CH_3$), 1.457–1.733 (m, 4H, $2CH_2Me$), 2.524 (s, 3H, Ar-CH_3), 3.689–3.972 (m, OCH_2), 4.095–4.221 (m, 2H, OCH_2), 5.915 (dd, J = 22.4, 8.0 Hz, 1H, CHP), 6.935–7.395 (m, 7H, Ar-H), 7.666 (t, J = 7.6 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$): 164.54, 164.41, 161.93, 151.00, 130.91, 129.71, 129.63, 129.36, 129.29, 126.40, 124.340, 123.356, 123.217, 121.639, 118.045, 115.298, 115.092, 69.095, 69.018, 68.927, 68.851, 49.629, 48.042, 23.891, 23.830, 23.670, 23.609, 18.130, 9.935, 9.744. Elemental analysis: C, 57.79; H, 6.00; N, 6.42; calculated from $C_{21}H_{26}FN_2O_3$ PS. Observed: C, 57.87; H, 5.83; N, 6.66.

   Compound **4c**: yield 68.2%, mp 107–109 °C, IR (KBr): 3246.2, 3090.7, 2958.8, 1593.20, 1539.2, 1234.4, 1213.2, 1060.8, 1024.2, 10004.9 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): 0.797–0.869 (m, 6H, $2CH_3$), 1.190–1.383 (m, 4H, $2CH_2Me$), 1.408–1.670 (m, 4H, $2CH_2$), 2.524 (s, 3H, Ar-CH_3), 3.724–4.002 (m, 2H, CH_2O), 4.141–4.228 (m, 2H, CH_2O), 5.876–5.932 (d, J = 22.4 Hz,1H, CHP), 6.941–7.368 (m, 7H, Ar-H), 7.638 (t, J = 7.6Hz, 1H, NH). ^{13}C NMR ($CDCl_3$): 164.56, 164.43, 161.94, 161.83, 159.47, 159.41, 150.97, 130.86, 129.72, 129.66, 129.30, 126.43, 124.363, 123.26, 123.12, 124.68, 118.06, 115.34, 115.10, 67.37, 67.29, 67.20, 67.12, 49.71, 48.15,32.51, 32.45, 32.27, 32.21, 18.59, 18.38, 18.14, 13.49, 13.46. Elemental analysis: C, 59.47; H, 6.51; N, 6.03; calculated from $C_{23}H_{30}FN_2O_3PS$. Observed: C, 59.34, H, 6.44, N, 6.13.

   Compound **4d**: yield 70.8%, mp 190–192°C; IR (KBr): 3236.5, 1537.2, 1325.1, 1244.09, 1215.1, 1058.9, 1035.7, 1018.4; ^{1}H NMR ($CDCl_3$, 400 MHz): 2.514 (s, 3H, Ar-CH_3), 3.613–3.860 (m, 6H, $2OCH_3$), 5.610 (dd, J = 22.4, 8.8 Hz, 1H, CHP), 6.873 (t, J = 7.2 Hz, 1H, NH), 6.973–7.725 (m, 7H, Ar-H). ^{13}C NMR ($CDCl_3$): 139.28, 130.76, 129.43, 128.53, 128.48, 126.64, 125.57, 122.09, 118.19, 55.76, 54.23, 54.16, 54.09, 54.04, 53.97, 18.20. Elemental analysis: C, 50.36; H, 4.23; N, 6.53; calculated from $C_{18}H_{18}F_3N_2O_3PS$: Observed: C, 50.42, H, 4.20, N, 6.71.

   Compound **4e**: yield 84.1%, mp 148–150 °C; IR (KBr): 3230.7, 1537.2, 1327.0, 1215.15, 1068.5, 1051.2, 1020.3 cm^{-1}; ^{1}H NMR ($CDCl_3$,400MHz): 1.160–1.327(m, 6H, $2CH_3$), 2.517 (s, 3H, Ar-CH_3), 3.847–4.007 (m, 2H, OCH_2), 4.175–4.246 (m, 2H, OCH_2), 5.538 (d, J = 23.2 Hz, 1H, CHP), 6.9697 (t, J = 15.2 Hz, 1H, NH), 7.079–7.725 (m, 7H, Ar-H). ^{13}C NMR ($CDCl_3$): 139.58, 130.68, 129.32, 128.61, 128.55, 126.64, 125.43, 121.99, 118.19, 109.75, 63.70, 56.39, 54.87, 18.22, 16.45, 16.40, 16.22, 16.16. Elemental analysis: C, 52.40; H, 4.84; N, 6.11; calculated from $C_{20}H_{22}F_3N_2O_3PS$. Observed: C, 52.22; H, 4.69; N, 6.00.

   Compound **4f**: yield 76.3%, mp 168–170 °C; IR (KBr): 3244.1, 1602.0, 1539.2, 1467.0, 1422.1, 1247.0, 1060.0 cm^{-1}; ^{1}H NMR ($CDCl_3$,400 MHz): 0.984–1.222 (m, 6H, $2CH_3$), 1.281–1.332 (m, 6H, $2CH_3$) 2.456 (s, 3H, Ar-CH_3), 4.559–4.846 (m, 2H, 2CHO), 5.815 (dd, J = 28.4, 5.6 Hz, 1H, CHP), 6.923 (t, J = 7.6 Hz, NH), 7.038–7.897(m, 7H, Ar-H). ^{13}C NMR ($CDCl_3$): 142.43, 131.72, 130.12, 130.07, 129.42, 127.13, 125.80, 122.50, 119.01, 72.69, 57.02, 55.48, 30.37, 30.18, 29.99, 29.80, 29.60, 29.41, 29.22, 24.30, 23.97, 23.47, 18.21. Elemental analysis: C, 54.32; H, 5.39; N, 5.76; calculated from $C_{22}H_{26}F_3N_2O_3PS$: C, 54.30; H, 5.28; N, 5.66.

Compound **4g**: yield 72.3%, mp 106–108 °C; IR (KBr): 3241.1, 1600.2, 1542.2, 1480.0, 1458.9, 1247.6, 1047.0 cm^{-1}; ^1H NMR ($CDCl_3$,400 MHz): 0.803–0.843 (m, 6H, $2CH_3$), 1.185–1.359 (m, 4H, $2CH_2Me$), 1.439–1.634 (m, 4H, $2CH_2$), 2.518 (s, 3H, Ar-CH_3), 3.799–4.018 (m, 2H, CH_2O), 4.141–4.209 (m, 2H, CH_2O), 5.687 (d, J = 22.4 Hz,1H, CHP), 6.947 (t, J = 15.2 Hz, 1H, NH), 7.061–7.757 (m, 7H, Ar-H). Elemental analysis: C, 56.02; H, 5.87; N, 5.44; calculated from $C_{24}H_{30}F_3N_2O_3PS$. Observed: C, 56.22, H, 5.34, N, 5.23.

Compound **4h**: yield 68.7%, mp 177–179 °C; IR (KBr): 3244.1, 3045.2, 1604.1, 1546.2, 1510.0, 1240.3, 1213.2, 1051.2, 1029.2 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 3.400 (s, 3H, OCH_3), 3.551 (d, J = 10.4 Hz, 3H, $POCH_3$), 4.011–4.210 (m, 3H, $POCH_3$), 5.561 (dd, J = 21.6 Hz, 9.6 Hz, 1H, CHP), 6.800–7.552 (m, 7H, Ar-H), 8.811 (d, J = 9.6 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$): 163.71, 162.91, 160.50, 154.72, 145.60, 132.21, 131.90, 130.00, 118.83, 113.33, 105.60, 54.56, 52.53. Elemental analysis: C, 51.50; H, 4.58; N, 7.07; calculated from $C_{17}H_{18}FN_2O_4PS$. Observed: C, 51.41; H, 5.27; N, 6.99.

Compound **4i**: yield 66.1%, mp 157–159 °C; IR (KBr): 3253.1, 1604.2, 1541.9, 1229.2, 1058.2 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 1.083 (t, J = 10.0 Hz, 3H, CCH_3), 1.210 (t, J = 9.8 Hz, 3H, CCH_3), 3.40 (s, 3H, OCH_3), 3.55 (d, J = 10.6 Hz, 3H, $POCH_2$), 3.64 (t, J = 7.2 Hz, 3H, $POCH_2$), 5.56 (dd, J = 23.2 Hz, 11.1 Hz, 1H, CHP), 6.80–7.55 (m, 7H, Ar-H), 8.85 (d, J = 8.0 Hz, 1H, NH); ^{13}C NMR ($CDCl_3$): 10.48, 25.68, 38.86, 39.08, 39.28, 39.50, 39.70, 39.91, 40.12, 45.94, 47.52, 53.31, 53.70, 53.76, 55.53, 62.52, 105.60, 113.17, 115.21, 119.03, 123.23, 123.37, 124.69, 129.46, 130.06, 131.92, 145.43, 154.78, 158.24, 158.31, 160.69, 163.58, 163.68. Elemental analysis: C, 53.80; H, 5.22; N, 6.60; calculated from $C_{19}H_{22}FN_2O_4PS$. Observed: C, 53.51; H, 5.08; N, 6.57.

Compound **4j**: yield 85.6%, mp 171–173 °C; IR (KBr): 3253.3, 2951.1, 1681.1, 1541.0, 1259.3, 1215.4, 1064.4 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 0.791 (t, J = 7.1 Hz, 3H, CCH_3), 1.522 (t, J = 7.2 Hz, 3H, CCH_3), 2.472 (t, J = 7.3 Hz, 2H, CH_2Me), 2.494 (t, J = 7.0 Hz, 2H, CH_2Me), 3.351–3.940 (m, 4H, $2OCH_2$), 3.791 (s, 3H,OCH_3), 5.630 (dd, J = 20.2 Hz, J = 9.9 Hz, 1H, PCH), 6.780–7.551 (m, 7H, Ar-H), 8.861 (br, 1H, NH); ^{13}C NMR ($CDCl_3$): 9.98, 9.84, 23.21, 23.27, 23.35, 23.40, 38.87, 39.08, 39.29, 39.50, 39.70, 39.92, 40.12, 54.36, 55.51, 67.64, 67.71, 68.03, 68.10, 105.55, 113.06, 114.98, 115.19, 1118.74, 130.05, 131.85, 132.28, 145.56, 154.62, 163.68, 163.77. Elemental analysis: C, 55.64; H, 5.56; N, 6.00; calculated from $C_{21}H_{26}FN_2O_4PS$. Observed: C, 55.74; H, 5.79; N, 6.19.

Compound **4k**: yield 84.2%, mp 215–216 °C; IR (KBr): 3248.0, 1604.4, 1546.2,1265.5, 1004.1 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 0.921–1.211 (m, 6H, $2CCH_3$), 3.300 (s, 6H, $2CCH_3$), 3.702 (s, 3H, OCH_3), 4.383–4.623 (m, 2H, 2POCH), 5.533 (d, J = 23.1 Hz, 1H, PCH), 6.781–7.542 (m, 7H, Ar-H), 8.853 (br, 1H, NH); ^{13}C NMR ($CDCl_3$): 22.91, 22.96, 23.43, 23.82, 23.85, 23.93, 23.96, 38.87, 39.08, 39.28, 39.50, 39.7, 39.91, 40.12, 46.87, 55.51, 71.22, 71.28, 71.50, 71.57, 105.56, 113.08, 115.01, 115.22, 118.89, 123.69, 123.84, 124.45, 129.55, 129.82, 131.90, 145.50, 154.66, 163.76, 163.86; C, 55.74; H, 5.79; N,6.19. Elemental analysis: C, 55.64; H, 5.56; N, 6.00; calculated from $C_{21}H_{26}N_2FPO_4S$. Observed: C, 55.74; H, 5.94; N, 6.09.

Compound **4l**: yield 72.1%, mp 100–101 °C; IR (KBr): 3251.1, 1606.2, 1541.1, 1267.1, 1217.1, 1022.2, 997.2 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 0.703 (t, J = 7.0 Hz, 6H, $2CCH_3$), 1.080–1.261 (m, 4H, $2CH_2$-Me), 1.311–1.500 (m, 4H, CH_2-C-Me), 3.721 (s, 3H, OCH_3), 3.744–3.882 (m, 2H, $POCH_2$), 3.891–4.032 (m, 2H, $POCH_2$), 5.932 (d, J = 23.1 Hz, 1H, PCH,), 6.792–7.642 (m, 7H, Ar-H), 8.934 (br, 1H, NH); ^{13}C NMR ($CDCl_3$): 13.35, 18.00, 18.14, 31.80, 31.86, 31.99, 32.04, 38.87, 39.08, 39.29, 39.5, 39.7, 39.9, 40.13, 46.29, 47.88, 55.50, 65.89, 65.97, 66.42, 66.48, 105.53, 113.09, 115.08, 115.30, 118.93, 123.44, 123.59, 124.54, 129.99, 131.92, 145.51, 154.70, 158.28, 163.65, 163.74. Elemental analysis: C, 57.49; H, 6.29; N,5.83; calculated from $C_{23}H_{30}FN_2O_4PS$. Observed: C, 57.35; H, 6.37; N, 5.63.

Compound **4m**: yield 84.2%, mp 198–200 °C; IR (KBr): 3242.1, 1604.0, 1544.3, 1469.0, 1436.3, 1236.2, 1222.2, 1049.2, 1018.1 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 1.061 (t, J = 9.8 Hz, 3H, CCH_3), 1.170 (t, J = 9.7 Hz, 3H, CCH_3), 3.493 (t, J = 11.0 Hz, 2H, $POCH_2$), 3.860 (s, 3H, OCH_3), 3.904 (t, J = 10.9 Hz, 2H, $POCH_2$), 5.932 (dd, J = 21.2 Hz, 9.8 Hz, 1H, PCH), 6.791–7.622 (m, 7H, Ar-H), 8.901 (br, 1H, NH); ^{13}C NMR ($CDCl_3$): 52.90, 54.52, 55.53, 62.44, 105.53, 113.03, 115.00, 118.71, 130.02, 132.22, 145.53, 154.60, 160.40, 162.90, 163.81. Elemental analysis: C, 53.80; H, 5.22; N, 6.60; calculated from $C_{19}H_{22}FN_2O_4PS$. Observed: C, 53.67; H, 5.27; N, 6.49.

9. Crystal data of **4m**. $C_{19}H_{22}FN_2O_4PS$, M = 424.42, Tetragonal, a = 21.169(3), b = 21.169(3), c = 18.545(6) Å, β = 90°, V = 8311(3) $Å^3$, T = 296(2) K, space group I4(1)/a, Z = 16, Dc = 1.357 g/cm^3, μ (Mo-Kα) = 0.269 mm^{-1}, F(000) = 3552. 23685 reflections measured, 4265 unique (R_{int} = 0.0556), which were used in all calculation. Fine R_1 = 0.0566, wR (F^2) = 0.1538 (all data). Full crystallographic details of **4m** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 284909. Crystal data of **4j**. $C_{21}H_{26}FN_2O_4PS$, M = 452.47, Triclinic, a = 9.601(3), b = 10.547(4), c = 11.894(4) Å, β = 105.5°, V = 1146.5(7)) $Å^3$, T = 293(2) K, space group P-1, Z = 2, Dc = 1.311 g/cm^3, μ (Mo-Kα) = 0.248 mm^{-1}, F(000) = 476.6029 reflections measured, 4035 unique (R_{int} = 0.0179) which were used in all calculation. Fine R_1 = 0.0710, wR (F^2) = 0.2290 (all data). Full crystallographic details of **4j** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 234280.
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13. MTT assay against cell proliferation: All compounds tested were dissolved in DMSO (1–100 μM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 2×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at a concentration of 1, 5, and 10 μM for 72 h. In parallel, the cells were treated with 0.1% of DMSO as control. An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Roche Molecular Biochemicals, 1 465 007) was performed 30 h later according to the instructions provided by Roche. This assay is based on the cellular cleavage of the tetrazolium salt, MTT, into a formazan that is soluble in cell culture medium and is measured at 550 nm directly in 96-well assay plates. Absorbance is directly proportional to the number of living cells in culture. Four types of cells were used in these studies; PC3 (prostate cancer), A375 (human melanoma), Bcap-37 (breast cancer), and A431 (uterus cancer) cell lines, provided by ATCC and cultivated in F-12 (for A431) or RPMI 1640 (for PC3,

A375 and Bcap-37) supplemented with 10% fetal bovine serum. Tissue culture reagents were obtained from Gibco BRL. Skehan, P.; Storeng, R.; Scadiero, D.; Monks, A.; McMahon, I.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boy, M. R. *Natl. J. Cancer Inst.* **1990**, *82*, 1107.