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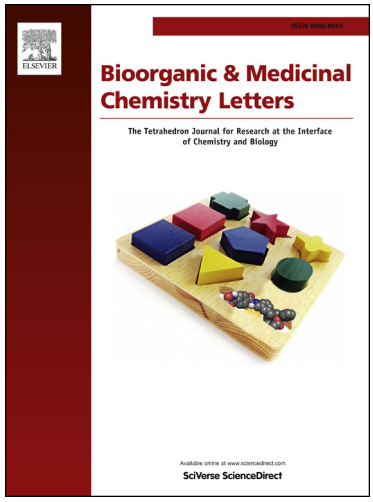

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Graphical Abstract

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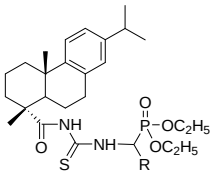

7 (**7a-7q**)

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Synthesis and antitumor activities of novel α-aminophosphonates dehydroabietic acid derivatives

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ABSTRACT

A series of novel α-aminophosphonate derivatives containing DHA structure were designed and synthesized as antitumor agents. *In vitro* antitumor activities of these compounds against the NCI-H460 (human lung cancer cell), A549 (human lung adenocarcinoma cell), HepG2 (human liver cancer cell) and SKOV3 (human ovarian cancer cell) human cancer cell lines were evaluated and compared with commercial anticancer drug 5-fluorouracil (5-FU), employing standard MTT assay. The pharmacological screening results revealed that many compounds exhibited moderate to high levels of antitumor activities against the tested cancer cell lines and that most demonstrated more potent inhibitory activities compared with the commercial anticancer drug 5-FU. The action mechanism of representative compound **7c** was preliminarily investigated by acridine orange/ethidium bromide staining, Hoechst 33258 staining, JC-1 mitochondrial membrane potential staining and flow cytometry, which indicated that the compound can induce cell apoptosis in NCI-H460 cells. Cell cycle analysis showed that compound **7c** mainly arrested NCI-H460 cells in G1 stage.

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Since the success of taxol, vinblastine and their related derivatives as anticancer agents, developing other novel natural compounds and their derivatives with better efficiency have greatly attracted bioorganic chemists' interest and become a central research theme in bioorganic chemistry.[1-5] So natural products have been always a good traditional source of new medicinal leads, and they play a significant role in drug discovery, especially in the area of cancer pharmacology, where the fraction of the drugs derived from natural products amounts to 60%.[6]

Dehydroabietic acid (DHA, **4**) is a natural occurring diterpenic resin acid. Previous studies have revealed that DHA has properties of increasing the inhibition activity of an anticancer drug in various cells, e.g. cervical carcinoma cells, hepatocellular carcinoma cells, and breast cancer cells.[7] So DHA skeleton was then chosen as active pharmacal core to screen for new potential antitumor agents by the introduction of various functional groups on ring B and C, as well as in carboxylic acid group of DHA.[8-11]

Aminophosphonate group, which is considered as potent antitumor agents, is able to effectively improve the antitumor activity by introducing to a pharmacy core.[12] Many aminophosphonates derivatives have shown good inhibition activities against human tumors.[13−16] As natural amino acid analogues, α-aminophosphonate compounds have thus attracted the current interest in organic and medicinal chemistry due to biological and pharmacological properties of these organophosphorus compounds.[17−19]

Owing to the synthetic and biological values of DHA and aminophosphonate groups, it is thus to expect that the condensation of DHA and aminophosphonate groups may contribute to good antitumor activity. In addition, literature survey has proved that compounds containing aminophosphonates with DHA moiety have not been reported so far. Therefore, aminophosphonate group was rationally designed and introduced to the DHA structure. Our present work in this paper is to design and synthesize α-aminophosphonates derivatives containing DHA skeleton, and to evaluate their *in vitro* antitumor activities. Preliminary investigation on the mode of action of representative compound **7c** is also investigated.

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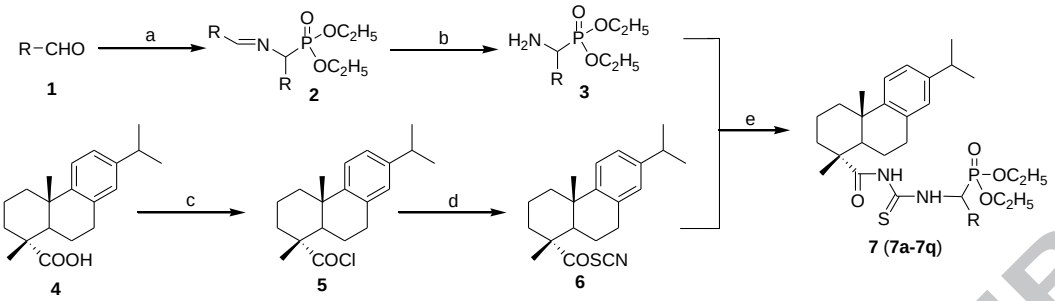
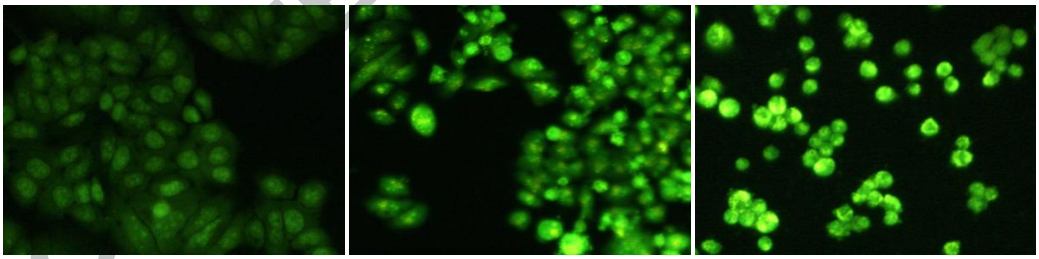


Scheme 1. Synthetic route to aminophosphonate DHA derivatives. Reagents and conditions: (a) NH_4Ac, $HPO(OC_2H_5)_2$, $Al(OTf)_3$; (b) (i) HCl(aq), (ii) NaOH(aq); (c) $(COCl)_2$, CH_2Cl_2, r.t.; (d) $C_6H_5CH_3$, KSCN, 110 °C; (e) CH_2Cl_2, r.t.

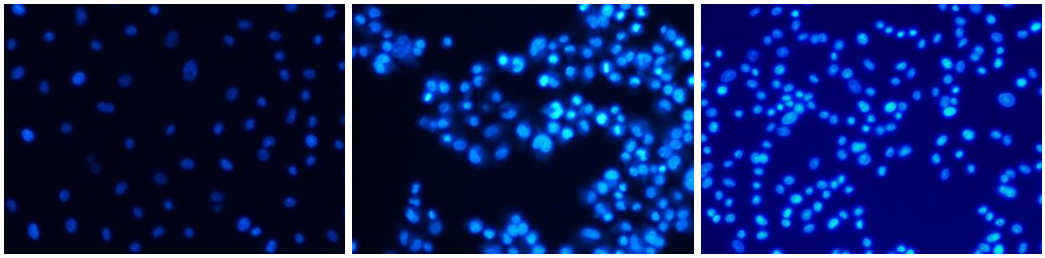
Table 1. Effect of aminophosphonate DHA derivatives **7** against cell viability of different cell lines.

Compounds		IC_{50}(μM)			
	R	NCI-H460	A549	HepG2	SKOV3
7a	Ph	28.05±1.23	24.23±0.78	15.18±0.69	12.14±1.02
7b	p-Ph-OCH_3	25.00±2.12	15.35±1.32	32.08±3.24	21.35±2.01
7c	o-Ph-OCH_3	3.33±0.58	18.37±1.04	7.70±1.06	18.92±2.23
7d	m-Ph-OCH_3	15.83±1.24	29.58±1.46	17.99±2.21	9.96±1.08
7e	p-Ph-CH_3	26.45±1.62	21.95±2.34	29.96±2.46	14.12±1.58
7f	m-Ph-CH_3	17.82±2.09	18.75±2.46	37.30±3.21	16.64±1.68
7g	p-Ph-F	4.39±0.86	23.42±1.86	10.34±1.66	13.50±2.46
7h	o-Ph-F	17.81±2.58	14.03±1.78	11.38±2.46	10.03±1.56
7i	m-Ph-F	27.51±2.67	24.44±3.12	31.63±2.34	13.66±1.54
7j	p-Ph-Cl	19.68±2.53	12.66±1.76	26.46±2.46	14.54±1.34
7k	o-Ph-Cl	30.72±3.26	19.60±2.75	24.87±3.62	15.30±1.89
7l	m-Ph-Cl	36.37±3.48	26.40±2.88	>50	19.69±1.79
7m	p-Ph-Br	18.74±1.83	20.19±2.57	24.03±2.83	13.97±1.73
7n	o-Ph-Br	18.57±2.36	22.19±2.86	27.05±2.67	17.41±1.95
7o	m-Ph-Br	14.84±1.60	19.48±2.14	15.19±1.23	20.67±2.48
7p	1-Naphthalene	32.61±2.85	28.34±3.11	>50	19.09±1.11
7q	2-Naphthalene	22.74±2.19	45.74±3.87	>50	>50
DHA		84.53±1.11	79.46±1.09	85.00±1.16	84.00±1.21
5-FU		44.04±0.54	34.33±0.23	29.98±0.37	24.43±0.41



(a) (b) (c)

Figure 1. AO/EB staining of compound **7c** in NCI-H460 cells. (a) Not treated with the **7c** was used as control for 24h and (b, c) treatment with compound **7c** (5 μM) for 12 h and 24 h, respectively.



(a) (b) (c)

Figure 2. Hoechst 33258 staining of compound **7c** in NCI-H460 cells. (a) Not treated with compound **7c** was used as control for 24h and (b, c) treatment with compound **7c** (5 μM) for 12 h and 24 h, respectively.

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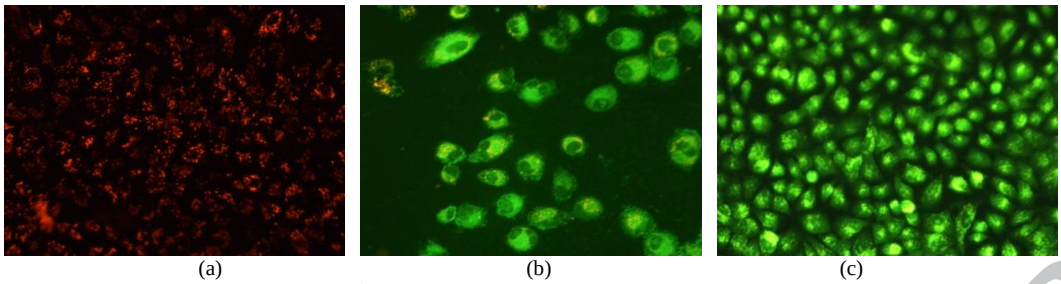
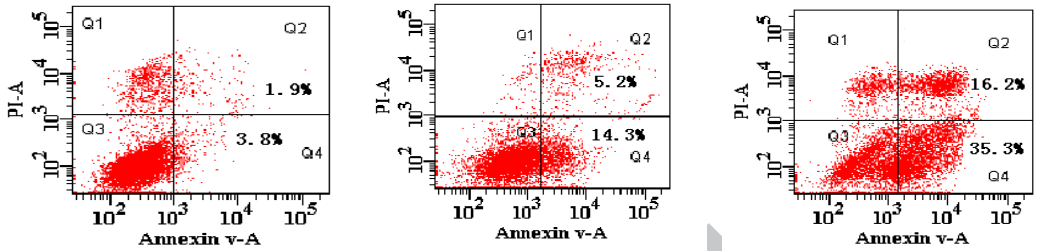


(a) (b) (c)

Figure 3. JC-1 mitochondrial membrane potential staining of compound **7c** in NCI-H460 cells. (a) Not treated with the **7c** was used as control for 24 h and (b, c) treatment with compound **7c** (5 μM) for 12 h and 24 h, respectively .



(a) (b) (c)

Figure 4. Apoptosis ratio detection of compound **7c** by Annexin V/PI assay. (a) Not treated with the **7c** was used as control for 24 h and (b, c) treatment with compound **7c** (5 μM) for 12 h and 24 h, respectively .

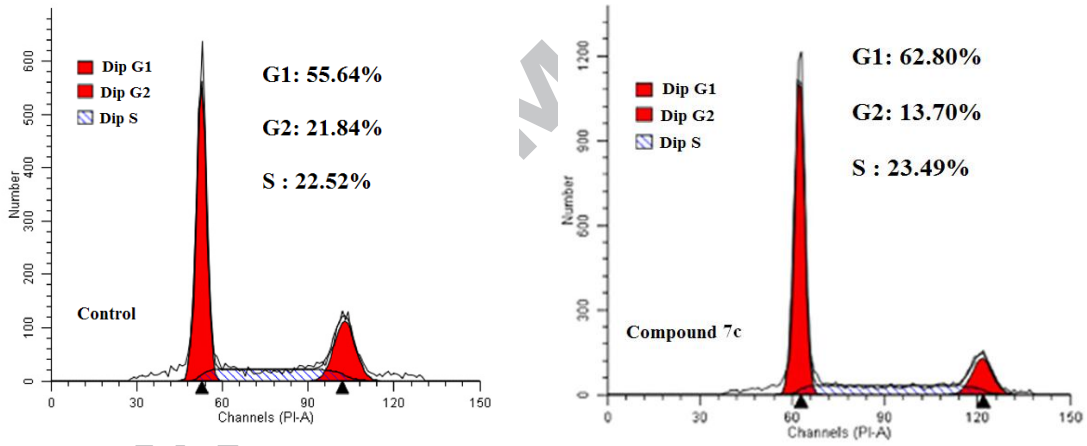


Figure 5. Cell-cycle analysis by flow cytometry of NCI-H460 cells treated with compound **7c** at IC_{50} concentration.

Aminophosphonate DHA derivatives **7** were synthesized as outlined in Scheme 1. Compound **2** was synthesized by the treatment of aromatic aldehydes **1** with the mixture of ammonium acetate and diethyl phosphate in the presence of $Al(OTf)_3$, and it was then treated with HCl (aq) and NaOH (aq), respectively, to offer α-aminophosphonate **3**, according to literature.[20] Dehydroabietic chloride **5** was obtained by the treatment of DHA **4** with oxalyl chloride, and it was then treated with KSCN to offer dehydroabietic isothiocyanate **6**. α-Aminophosphonate DHA derivatives **7** were finally obtained by the condensation of compound **6** with α-aminophosphonate **3** in good yields. The structures of aminophosphonate DHA derivatives **7** were confirmed by ^{1}H NMR, ^{13}CNMR, ^{31}P NMR and high resolution mass spectrum (HRMS).[21]

The *in vitro* antitumor activities of aminophosphonate DHA derivatives **7** were evaluated by MTT assay[22] against NCI-H460, HepG2, A549 and SKOV3 tumor cell lines, using 5-fluorouracil (5-FU) as the positive control. The tested results were shown in Table 1.

As shown in Table 1, all the compounds **7** showed antitumor activities in NCI-H460 assay. It was important to note that in NCI-H460 assay all the compounds **7** exhibited better inhibition than DHA (IC_{50}=84.53 μM), and even better than the commercial anticancer drug 5-FU (IC_{50}=44.04 μM), with IC_{50} in the range of 3.33−36.37 μM, indicating good antitumor activities of these compounds on NCI-H460 cell line. This cytotoxic inhibition screening results showed that the introduction of α-aminophosphonate group on DHA should markedly improve the antitumor activity against NCI-H460 cell line. Among all the compounds, compound **7c** exhibited the best antitumor activity, with IC_{50} of 3.33 μM, while compound **7l** showed the lowest, with IC_{50} of 36.37 μM, respectively. The antitumor activities of tested were found to be in the order of **7c** > **7g** > **7o** > **7d** > **7h** > **7f** > **7n** > **7m** > **7j** > **7q** > **7b** > **7e** > **7i** > **7a** > **7k** > **7p** > **7l** > 5-FU > DHA. On the basis of the above observation, methoxy, methyl, fluoro and bromo groups in benzene group of α-aminophosphonate moiety showed positive influence on antitumor activities in NCI-H460 assay, while the chloro group exhibited negative effect. Besides, the substituent position on

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naphthalene also appeared to have important influences on the antitumor activity in this assay.

In A549 assay, all the compounds **7** exhibited much better inhibition than DHA (IC_{50}=79.46 μM), with IC_{50} in the range of 12.66−45.74 μM. It was worth noting that except **7q** all these compounds **7** also showed further more cytotoxic inhibition than 5-FU (34.33 μM), indicating potent cytotoxicity of these compounds on A549 cell line. This cytotoxicity screening results implied that the introduction of α-aminophosphonate on DHA obviously improve the antitumor activity against A549 cell line. Compound **7j** exhibited the best cytotoxicity among all the compounds, with IC_{50} of 12.66 μM, while compound **7q** showed lowest, with IC_{50} of 45.74 μM. Clearly, the order of antitumor activity of tested in this assay was: **7j** > **7h** > **7b** > **7c** > **7f** > **7o** > **7k** > **7m** > **7e** > **7n** > **7g** > **7a** > **7i** > **7l** > **7p** > **7d** > 5-FU > **7q** > DHA. Based on the above observation, it could be summarized that methoxy, methyl, fluoro and chloro groups at ortho and para position in benzene group of α-aminophosphonate moiety may have important influence on the antitumor activity, while naphthalene exhibited negative effect.

In HepG2 assay, except compounds **7l**, **7p** and **7q**, other compounds displayed better cytotoxicity than DHA (IC_{50}=85.00 μM), with IC_{50} in the range of 7.70−37.30 μM, indicating that the introduction of α-aminophosphonate on DHA should improve the antitumor activity against HepG2 cell line. Moreover, compounds **7a**, **7c, 7d, 7e, 7g, 7h**, **7j**, **7k**, **7m**, **7n** and **7o** even demonstrated better cytotoxic inhibition than 5-FU, implying favorable inhibition activities of these compounds on HepG2 cell line. Compounds **7c** and **7g** showed better inhibition on HepG2 cell line than other compounds, with IC_{50} of 7.70 μM and 10.34 μM, respectively. HepG2 cells inhibiting activities of these tested compounds decreased in the order of **7c**, **7g**, **7h** , **7a** , **7o**, **7d**, **7m**, **7k**, **7j**, **7n**, **7e**, 5-FU, **7i**, **7b**, **7f**, DHA. Evidently, methoxy at ortho, as well as fluoro at ortho and para position in benzene group of α-aminophosphonate moiety may result in the enhancement of antitumor activity, while the presence of methyl, chloro and bromo in benzene lead to the decrease of antitumor activity. In addition, displacement of benzene with naphthalene also appeared to have negative influences on the antitumor activity in this assay.

In SKOV3 assay, all the compounds except **7q** displayed better cytotoxicity than DHA (IC_{50}=84.00 μM), with IC_{50} in the range of 9.96−21.35 μM, exhibiting that the introduction of α-aminophosphonate on DHA should improve the antitumor activity against SKOV3 cell line. It was important to point out that these compounds even exhibited better cytotoxic inhibition than 5-FU, showing good inhibition activities of these compounds on SKOV3 cell line. Among all these compounds, compound **7d** exhibited the best inhibition activity on SKOV3 cells, with IC_{50} of 9.96 μM, while compound **7b** displayed the lowest, with IC_{50} of 21.35 μM. The antitumor activity order in this assay was listed as follow: **7d** > **7h** > **7a** > **7g** > **7i** > **7m** > **7e** > **7j** > **7k** > **7f** > **7n** > **7c** > **7p** > **7l** > **7o** > **7b** > 5-FU > DHA. The order obviously indicated that the benzene group was important to their antitumor activity, and methoxy at meta-position and fluoro at ortho-position in benzene group of α-aminophosphonate moiety were important contributors the antitumor activity.

In the present study, compound **7c**, which exhibited good cytotoxic inhibition in four cell lines and could be used as a good representative of compounds **7**, was selected and its mechanism of growth inhibition of NCI-H460 cells was evaluated by acridine orange(AO)/ethidium bromide(EB) staining[23], Hoechst 33258 staining,[24] JC-1 mitochondrial membrane potential staining[25] and flow cytometry.[26]

Both of AO and EB are important dyes, and AO can stain nuclear DNA across an intact cell membrane, while EB only stains cells that had lost their membrane integrity. Therefore, after synchronous treating with AO and EB, live cells will be evenly stained as green (in the web version) and early apoptotic cells will be thickly stained as green yellow or show green yellow fragments (in the web version), while late apoptotic cells will be densely stained as orange or display orange fragments and necrotic cells will be stained as orange with no condensed chromatin. The cytotoxicity of compound **7c** at the concentration of 5 μM against NCI-H460 cells from 12 to 24 h was assayed by AO/EB staining, and NCI-H460 cells not treated with the **7c** were used as control for 24 h. The results were shown in Fig. 1. As shown in Fig.1, the NCI-H460 cells treated with **7c** from 12 to 24 h had obviously changed. The nuclei markedly stained as yellow green or orange, and the morphology displayed pycnosis, membrane blebbing and cell budding. These phenomena were associated with cell apoptosis. Based on the observation, the cells represented with an apoptotic morphology. These findings indicated that compounds **7c** could induce apoptosis with low cytotoxicity.

Hoechst 33258 is a membrane permeable dye with blue fluorescence. Live cells with uniformly light blue nuclei were obviously detected under fluorescence microscope after treatment with Hoechst 33258, whereas apoptotic cells had bright blue nuclei due to karyopyknosis and chromatin condensation. On the contrary, the nuclei of dead cells could not be stained. NCI-H460 cells treated with compound **7c** at 5 μM from 12 to 24 h were stained with Hoechst 33258. NCI-H460 cells not treated with the **7c** were used as control at for 24 h. The results were given in Fig. 2. As shown in Fig. 2, cells not treated with compound **7c** were normally blue (a). It was worth noting that, for **7c** treatment, the cells displayed strong blue fluorescence and indicated typical apoptotic morphology after 12 (b) and 24 h (c). The observation revealed that compounds **7c** induced apoptosis against NCI-H460 cells, consistent with the results for AO/EB double staining.

Mitochondrial membrane potential changes were also designed and detected to further investigate the apoptosis-inducing effect of target compound **7c**, employing a fluorescent probe JC-1. JC-1 is a lipophilic cationic dye and it can easily pass through the plasma membrane into cells and accumulate in actively respiring mitochondria.[27] NCI-H460 cells treated with compound **7c** at 5 μM from 12 to 24 h were stained with JC-1 and not treated with the compound **7c** were used as control at for 24 h. The results were shown in Fig. 3. The JC-1 monomer and J-aggregates were excited at 514 nm and 585 nm, respectively, and light emissions were collected at 515−545 nm (green) and 570−600 nm (red). For fluorescence microscopy, Fig. 3 displayed that cells not treated with the compound **7c** were normally red (in the web version a), while for **7c** treatment (in the web version b and c), cells exhibited strong green fluorescence as that for 5-FU which is known to induce the apoptosis[26c] (Fig. s1) and indicated typical apoptotic morphology after 12 h and 24 h. Therefore, it could be concluded that compound **7c** induced apoptosis against NCI-H460 cell line. The results were identical with that of previous experiment of AO/EB double staining and Hoechst 33258 staining.

The apoptosis ratios induced by compound **7c** in NCI-H460 tumor cells were then quantitatively determined by flow cytometry. Four quadrant images were observed by flow cytometric analysis: the Q1 area represented damaged cells appearing in the process of cell collection, the Q2 region showed

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necrotic cells and later period apoptotic cells, the Q3 area showed normal cells, and the early apoptotic cells were located in the Q4 area. The results were given in Fig. 4. Fig. 4 revealed that compound **7c** could induce apoptosis in NCI-H460 cells. Apoptosis ratios (including the early and late apoptosis ratios) for compound **7c** were obtained after 12 h of treatment at the concentration of 3 μM and 5 μM, respectively. The apoptosis of NCI-H460 cells treated with compound **7c** increased gradually in a concentration manner. The apoptosis ratios of compound **7c** measured at different concentration points were found to be 19.5% (3 μM) and 51.5% (5 μM), respectively, while that of control was 5.7%. In addition, the apoptosis ratio of 5-FU was assayed to be 23.3% (Fig. s2) under the similar condition. The results evidently illustrated that compound **7c** suppressed cell proliferation by inducing apoptosis. Previous reports[28] have revealed that α-aminophosphonates derivatives may induce apoptosis by affecting associated enzymes in mitochondria-dependent and-independent pathways, it is reasonable to believe that compound **7c** may induce apoptosis in the same mechanism. And the investigation on precise mechanism is in process.

The above studies have proved that compound **7c** can induce apoptosis, therefore the effect of compounds **7c** on the cell cycle of NCI-H460 cell lines was investigated by flow cytometry at its IC_{50} concentrations and showed in Fig. 5. And the effect of compound 5-FU on the cell cycle of NCI-H460 cell was also studied (Fig. s3). As shown in Fig. 5 and Fig. s3, the treatment of NCI-H460 cells with compounds **7c** and 5-FU enhanced cell-cycle arrest at the G1 phase, resulting in concomitant population increase in the G1 phase (62.80% and 71.93%) compared with the control cells (55.64% and 68.60%) and the population of the G2 phase decrease at a certain extent (13.70% and 0) compared with the control cells (21.84% and 8.14%). These results suggested that compound **7c** mainly arrest the cell-cycle of NCI-H460 cells in the G1 stage. It also indicated that 5-FU arrest NCI-H460 cells in the G1 and S stage, consistent with that of it was treated with oral cancer cell.[26c]

In this paper, we have described the design, synthesis and *in vitro* antitumor activities of α-aminophosphonates derivatives, derived from the DHA pharmacy core. The *in vitro* antitumor activities screening showed that almost all the compounds **7** exhibited better inhibition than the commercial anticancer drug 5-FU in both NCI-H460 and A549 assays, and compounds **7a**, **7c, 7d, 7e, 7h, 7j**, **7k**, **7m**, **7n** and **7o** demonstrated better cytotoxic inhibition than 5-FU in HepG2 assay, while all the compounds except **7q** displayed better cytotoxicity than 5-FU in SKOV3 assay, respectively. The apoptosis-inducing activity of representative compound **7c** in NCI-H460 cells was studied and the results revealed that this compound showed clear cell apoptosis inducing effects. Cell cycle analysis indicated that compound **7c** could arrest NCI-H460 cell in G1 stage. The above results demonstrate that the rational design of aminophosphonates DHA derivatives as novel antitumor leads is feasible. The precise mechanism of action is currently under way.

Acknowledgments

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21. (a) General procedure for the preparation of compounds **7**: $Al(OTf)_3$ (0.01 mmol) was added to a stirring mixture of aldehyde (**1**) (2 mmol), NH_4OAc (2 mmol), and diethyl phosphite (2 mmol). The resulting mixture was heated in an oil bath at 100 °C and stirred for 30 min. The reaction mixture was cooled and acidified to pH 1 by HCl (aq). The solution was washed with Et_2O (2 × 10 mL). The aqueous phase was then made alkaline with NaOH (aq), and the product was extracted with Et_2O (2 × 10 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to offer α-aminophosphonates **3**. Compound **4** (1mmol) added to CH_2Cl_2 (15mL) was stirred at 0°C and oxalyl chloride (1.5mmol) was dripped into the mixture and stirred at room temperature for 6 h. After the reaction, the solvent and excess oxalyl chloride was evaporated under reduced pressure. Toluene (15mL) and KSCN (1.2mmol) were added to the mixture and stirred at 110 °C for 12 h. After the reaction, the solvent was evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel eluted with petroleum ether/ethyl acetate (V:V = 10:1) to offer compound **6**. Compound **6** (1mmol) and α-aminophosphonate **3** (1.2mmol) were added to CH_2Cl_2 (15mL) the mixture was stirred at room temperature for 3 h. After the reaction was completed, the solvent was evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel eluted with petroleum ether/ethyl acetate (V:V = 3:1) to offer compounds **7** (**7a**−**7q**); (b) Experimental: NMR spectra were recorded on a BRUKER AVANCE 500 NMR spectrometer in $CDCl_3$. Mass spectra were determined on a FTMS ESI spectrometer. **7a**: Yields 85%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.52 (s, 1H, NH), 8.63 (s, 1H, NH), 7.45 (d, *J* = 6.9 Hz, 2H), 7.40−7.35 (m, 2H), 7.32−7.30 (m, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.01 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.90−6.86 (m, 1H), 6.12−6.05 (m, 1H, N-CH-P), 4.14−3.94 (m, 4H, 2 × O$\underline{CH_2}$$CH_3$), 2.93−2.79 (m, 3H), 2.35 (d, *J* = 13.7 Hz, 1H), 2.13−2.06 (m, 1H), 1.76−1.52 (m, 7H), 1.35 (d, *J* = 3.3 Hz, 3H, CH_3), 1.30−1.26 (m, 3H, CH_3), 1.23 (dd, *J* = 3.5, 1.9 Hz, 6H, 2 × CH_3), 1.23−1.19 (m, 6H, 2 × CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.64, 179.52, 146.26, 146.09, 134.19, 133.69, 128.71, 128.29, 128.25, 128.21, 127.96, 126.93, 124.14, 124.03, 63.52, 63.32, 63.60, 63.57, 63.42, 57.32, 56.11, 48.65, 45.36, 37.69, 37.08, 36.93, 33.49, 29.73, 29.67, 25.10, 25.06, 23.97, 21.45, 18.49, 16.14. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.61. HRMS (m/z) (ESI):

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calcd for $C_{32}H_{45}N_2O_4PS$ [M+H$^+$]: 585.28377; found: 585.28766; **7b**: Yields 86.25%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.61 (s, H, NH), 8.63 (s, 1H, NH), 7.40−7.36 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.03 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.97−6.87 (m, 3H), 6.04−5.98 (m, 1H, N-CH-P), 4.21−3.94 (m, 4H, 2 × O$\underline{CH_2}$CH$_3$), 3.82 (s, 3H, OCH$_3$), 3.04−2.64 (m, 3H), 2.36 (d, *J* = 13.5 Hz, 1H), 2.12−2.18 (m, 1H), 1.86−1.51 (m, 7H), 1.36 (d, *J* = 3.4 Hz, 3H, CH$_3$), 1.31−1.28 (m, 3H, CH$_3$), 1.25 (dd, *J* = 6.5, 4.5 Hz, 9H, 3 × CH$_3$), 1.23 (d, *J* = 5.3 Hz, 3H, CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.16, 179.41, 159.58, 146.29, 146.09, 134.20, 129.56, 126.52, 125.71, 124.14, 124.03, 123.99, 114.19, 63.50, 63.29, 56.66, 55.36, 48.63, 45.34, 37.69, 37.07, 36.96, 36.93, 33.49, 29.70, 25.08, 23.97, 21.44, 18.49, 16.35, 16.14. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.91. HRMS (m/z) (ESI): calcd for $C_{33}H_{47}N_2O_5PS$ [M+H$^+$]: 615.29433; found: 615.29822; **7c**: Yields 88.55%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.81 (s, 1H, NH), 8.59 (s, 1H, NH), 7.33−7.27 (m, 2H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.97−6.95 (m, 1H), 6.94−6.90 (m, 1H), 6.88 (s, 1H), 6.48−6.44 (m, 1H, N-CH-P), 4.16−3.95 (m, 4H, 2 × O$\underline{CH_2}$CH$_3$), 3.92 (s, 3H, OCH$_3$), 2.86−2.80 (m, 3H), 2.33 (d, *J* = 13.2 Hz, 1H), 2.13 (dd, *J* = 12.5, 2.1 Hz, 1H), 1.88−1.63 (m, 7H), 1.34 (d, *J* = 2.1 Hz, 3H, CH$_3$), 1.29−1.28 (m, 3H, CH$_3$), 1.23 (d, *J* = 1.2 Hz, 6H, 2 × CH$_3$), 1.22 (d, *J* = 1.1 Hz, 3H, CH$_3$), 1.20 (dd, *J* = 7.1, 4.4 Hz, 3H, CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.00, 178.87, 157.40, 146.37, 146.04, 134.28, 129.60, 126.92, 124.13, 124.11, 124.03, 120.89, 111.32, 77.30, 77.05, 76.79, 63.20, 55.82, 53.97, 52.74, 52.55, 48.51, 45.19, 37.68, 37.13, 36.58, 33.48, 29.77, 25.12, 23.97, 21.43, 18.51, 16.38, 16.20. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.87. HRMS (m/z) (ESI): calcd for $C_{33}H_{47}N_2O_5PS$ [M+H$^+$]: 615.29433; found: 615.29938; **7d**: Yields 88.25%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.67 (s, 1H, NH), 8.72 (s, 1H, NH), 7.33−7.26 (m, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.08−7.00 (m, 3H), 6.93−6.85 (m, 2H), 6.15−6.06 (m, 1H, N-CH-P), 4.15−4.09 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.81 (s, 3H, OCH$_3$), 2.97−2.79 (m, 3H), 2.35 (d, *J* = 13.1 Hz, 1H), 2.13−2.11 (m, 1H), 1.81−1.50 (m, 7H), 1.36 (d, *J* = 3.5 Hz, 3H, CH$_3$), 1.30−1.29 (m, 3H, CH$_3$), 1.28−1.26 (m, 3H, CH$_3$), 1.24 (dd, *J* = 5.9, 3.4 Hz, 9H, 3×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.60, 179.5, 159.73, 146.29, 146.03, 135.13, 134.18, 129.69, 126.91, 124.13, 124.03, 120.46, 120.41, 113.90, 63.58, 63.46, 60.36, 57.24, 56.03, 55.25, 48.63, 45.29, 37.67, 37.06, 36.86, 33.48, 29.72, 25.09, 23.97, 21.46, 21.02, 18.49, 16.53, 14.20. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.51. HRMS (m/z) (ESI): calcd for $C_{33}H_{47}N_2O_5PS$ [M+H$^+$]: 615.29433; found: 615.29913; **7e**: Yields 86.25%; ^{1}H NMR (500 MHz, $CDCl_3$):δ 11.78 (s, 1H , NH), 8.64 (s, 1H, NH), 7.38−7.33 (m, 2H), 7.23−7.14 (m, 3H), 7.03 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.92−6.87 (m, 1H), 6.07−5.98 (m, 1H, N-CH-P), 4.17−3.94 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 2.94−2.81 (m, 3H), 2.36 (s, 3H, p-$\underline{CH_3}$-C$_6$H$_5$), 2.12−2.10 (m, 1H), 1.88 (dd, *J* = 13.8, 8.0 Hz, 1H), 1.81−1.48 (m, 7H), 1.36 (d, *J* = 3.2 Hz, 3H, CH$_3$), 1.30−1.28 (m, 3H, CH$_3$), 1.26 (dd, *J* = 4.4, 3.0 Hz, 6H, 2×CH$_3$), 1.25−1.22 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.43, 179.46, 146.27, 146.08, 138.11, 134.20, 130.62, 129.44, 128.16, 128.12, 126.93, 124.13, 124.02, 120.01, 63.49, 63.27, 57.05, 55.84, 48.63, 45.35, 37.69, 37.07, 36.93, 33.49, 29.70, 25.08, 23.97, 21.44, 21.20, 18.49, 16.45, 16.02. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.81. HRMS (m/z) (ESI): calcd for $C_{33}H_{47}N_2O_4PS$ [M+H$^+$]: 599.29942; found: 599.30377; **7f**: Yields 87%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.60 (s, 1H, NH), 8.76 (s, 1H, NH), 7.29 (dd, *J* = 5.9, 2.0 Hz, 1H), 7.22−7.15 (m, 2H), 7.04 (dd, *J* = 10.6, 4.3 Hz, 2H), 6.92 (d, *J* = 0.9 Hz, 2H), 6.11−6.02 (m, 1H, N-CH-P), 4.18−3.99 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 2.94−2.87 (m, 3H), 2.40 (s, 3H, m-$\underline{CH_3}$-C$_6$H$_5$), 2.28 (dd, *J* = 12.4, 2.0 Hz, 2H), 2.16 (m, 1H), 1.86−1.75 (m, 7H), 1.39 (d, *J* = 3.6 Hz, 3H, CH$_3$), 1.35−1.31 (m, 3H, CH$_3$), 1.27 (d, *J* = 1.2 Hz, 6H, 2×CH$_3$), 1.25 (d, *J* = 0.9 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.73, 179.44, 146.26, 146.09, 134.21, 129.96, 129.47, 126.92, 124.47, 124.07, 115.77, 115.63, 115.33, 63.58, 51.69, 50.46, 48.64, 45.33, 37.69, 37.01, 33.49, 29.69, 25.10, 25.03, 23.96, 21.45, 18.49, 16.41, 16.02.^{31}P NMR (200 MHz, $CDCl_3$) δ 19.41. HRMS (m/z) (ESI): calcd for $C_{33}H_{47}N_2O_4PS$ [M+H$^+$]: 599.29942; found: 599.30377; **7g**: Yields 83.58%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.66 (s, 1H, NH), 8.65 (s, 1H, NH), 7.48–7.41 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.09 (d, *J* = 8.6, 2.6 Hz, 2H), 7.04 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.93 – 6.89 (m, 1H), 6.06-5.98 (m, 1H, N-CH-P), 4.16–3.98 (m, 4H), 2.97–2.81 (m, 3H), 2.37 (d, *J* = 13.5 Hz, 1H), 2.18–2.08 (m, 1H), 1.78-165 (m, 7H), 1.37 (s, 3H, CH$_3$), 1.31 (d, *J* = 7.0, 3H, CH$_3$), 1.26 (t, *J* = 4.5 Hz, 6H, 2×CH$_3$), 1.24 (d, *J* = 4.4 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.73, 179.63, 163.71, 161.68, 145.89, 133.86, 130.08, 130.03, 130.02, 129.97, 129.65, 126.95, 124.16, 123.98, 63.50, 56.57, 55.35, 48.67, 45.37, 37.69, 37.18, 36.83, 33.48, 29.69, 25.07, 25.03, 23.94, 21.47, 18.48, 16.57, 16.05. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.39. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}FN_2O_4PS$ [M+H$^+$]: 603.27374; found: 603.27887.; **7h**: Yields 89.56%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.36 (s, 1H, NH), 8.66 (s, 1H, NH), 7.41 (t, *J* = 7.5 Hz, 1H), 7.32 (dd, *J* = 10.7, 4.8 Hz, 1H), 7.19 (dd, *J* = 12.8, 5.6 Hz, 2H), 7.11 (d, *J* = 9.2 Hz, 1H), 7.03 (d, *J* = 6.2 Hz, 1H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.39−6.34 (m, 1H, N-CH-P), 4.20−4.01 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.02−2.74 (m, 3H), 2.36 (d, *J* = 13.6 Hz, 1H), 2.18−2.07 (m, 1H), 1.70 (dd, *J* = 90.7, 47.2 Hz, 7H), 1.38−1.35 (m, 3H, CH$_3$), 1.35−1.32 (m, 3H, CH$_3$), 1.27−1.25 (m, 6H, 2×CH$_3$), 1.25−1.22 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.83, 179.38, 146.09, 134.21, 129.96, 129.47, 126.92 , 124.47, 124.13, 124.01, 115.77, 115.63, 115.33, 63.58, 51.69, 50.46, 48.64, 45.35, 45.31, 37.69, 37.07, 36.95, 33.49, 29.69, 25.07, 23.96, 21.49, 21.41, 18.49, 16.41, 16.02. ^{31}P NMR (200 MHz, $CDCl_3$) δ 18.66. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}FN_2O_4PS$ [M+H$^+$]: 603.27374; found: 603.27850; **7i**: Yields 89.46%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.66 (dd, *J* = 14.5, 9.1 Hz, 1H), 8.67 (s, 1H), 7.40−7.33 (m, 1H), 7.26 (d, *J* = 7.7 Hz, 1H), 7.18 (dd, *J* = 8.9, 4.3 Hz, 2H), 7.08−6.99 (m, 2H), 6.91 (d, *J* = 6.1 Hz, 1H), 6.07-5.98 (m, 1H, N-CH-P), 4.17−3.97 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 2.97−2.79 (m, 3H), 2.37 (d, *J* = 13.4 Hz, 1H), 2.13 (m, 1H), 1.84−1.52 (m, 7H), 1.38 (d, *J* = 3.2 Hz, 3H, CH$_3$), 1.33−1.29 (m, 3H, CH$_3$), 1.29−1.26 (m, 6H, 2×CH$_3$), 1.24 (dd, *J* = 6.9, 2.5 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.06, 179.58, 164.08, 161.90, 146.24, 146.12, 136.35, 134.15, 130.20, 126.93, 124.17, 124.04, 124.01, 123.69, 115.36, 115.19, 63.80, 63.11, 56.88, 55.67, 48.70, 45.38, 37.68 , 37.20, 36.56, 33.49, 29.70, 25.08, 23.96, 21.48, 18.48, 16.41, 16.01. ^{31}P NMR (200 MHz, $CDCl_3$) δ19.40. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}FN_2O_4PS$ [M+H$^+$]: 603.27374; found: 603.27887; **7j**: Yields 88.58%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.65 (s, 1H, NH), 8.67 (s, 1H, NH), 7.41−7.39 (m, 2H), 7.37 (dd, *J* = 8.6, 2.5 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.04 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.91 (dd, *J* = 6.0, 1.5 Hz, 1H), 6.04−5.99 (m, 1H, N-CH-P), 4.16−3.99 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.06−2.55 (m, 3H), 2.37 (d, *J* = 13.4 Hz, 1H), 2.12−2.08 (m, 1H), 1.86−1.47 (m, 7H), 1.37 (d, *J* = 3.4 Hz, 3H, CH$_3$), 1.31−1.30 (m, 3H, CH$_3$), 1.29−1.25 (m, 6H, 2×CH$_3$), 1.25−1.23 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.86, 179.67, 146.24, 146.13, 134.29, 134.14, 132.40, 129.61, 129.57, 128.91, 126.93, 124.18, 124.04, 124.00, 123.87, 63.45, 56.68, 55.47, 48.69, 45.36, 37.68, 37.20, 36.57, 33.49, 29.70, 25.08, 23.97, 21.48, 18.48, 16.53, 16.18, 16.13. ^{31}P NMR (200 MHz, $CDCl_3$) δ 19.10. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}ClN_2O_4PS$ [M+H$^+$]: 619.24479; found: 619.24951; **7k**: Yields 88.65%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.74 (s, 1H, NH), 8.66 (s, 1H, NH), 7.43 (dd, *J* = 4.9, 2.7 Hz, 2H), 7.34−7.27 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 7.8 Hz, 1H), 6.90 (dd, *J* = 5.1, 1.5 Hz, 1H), 6.57−6.55 (m, 1H, N-CH-P), 4.23−3.89 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.05−2.71 (m, 3H), 2.36 (d, *J* = 13.5 Hz, 1H), 2.13−2.07 (m, 1H), 1.86−1.45 (m, 7H), 1.37 (d, *J* = 2.4 Hz, 3H, CH$_3$), 1.35 (dd, *J* = 7.1, 1.9 Hz, 3H, CH$_3$), 1.25 (t, *J* = 2.6 Hz, 6H, 2×CH$_3$), 1.24−1.20 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.82, 179.47, 146.18, 145.96, 145.74, 134.21, 132.43, 129.81, 129.33, 129.03, 127.21, 126.92, 124.13, 124.00, 63.82, 63.16, 54.24, 53.02, 48.64, 45.31, 37.69, 37.07, 36.95, 33.49, 29.72, 29.67, 25.08, 23.98, 21.46, 18.49, 16.45, 16.20.. ^{31}P NMR (200 MHz, $CDCl_3$) δ 18.86. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}ClN_2O_4PS$ [M+H$^+$]: 619.24479; found: 619.25000; **7l**: Yields 89.15%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.65 (s, 1H, NH), 8.68 (s, 1H, NH), 7.43 (s, 1H), 7.39−7.30 (m, 3H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.04 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.91 (dd, *J* = 5.4, 1.6 Hz, 1H), 6.08−6.02 (m, 1H, N-CH-P), 4.18−4.03 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.04−2.76 (m, 3H), 2.37 (d, *J* = 13.3 Hz, 1H), 2.13−2.10 (m, 1H), 1.86−1.50 (m, 7H), 1.38 (d, *J* = 3.2 Hz, 3H, CH$_3$), 1.33−1.31 (m, 3H, CH$_3$), 1.30−1.26 (m, 6H, 2×CH$_3$), 1.24 (dd, *J* = 6.9, 2.4 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.96, 179.65, 146.25, 146.21, 135.89, 134.56, 134.15, 129.92, 128.51, 128.27, 126.94, 126.45, 124.17, 124.04, 63.84, 63.42, 56.85, 55.64, 48.70, 45.39, 37.69 , 37.32, 36.77, 33.49, 29.71, 25.09, 23.97, 21.49, 18.48, 16.38, 16.24. ^{31}P NMR (200 MHz, $CDCl_3$) δ 18.91. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}ClN_2O_4PS$ [M+H$^+$]: 619.24479; found: 619.25085; **7m**: Yields 90.25%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.47 (s, 1H, NH), 8.67 (s, 1H, NH), 7.52 (dd, *J* = 8.4, 2.5 Hz, 2H), 7.37−7.32 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.04 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.91 (dd, *J* = 6.2, 1.7 Hz, 1H), 6.05−5.98 (m, 1H, N-CH-P), 4.16−3.97 (m, 4H,

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2×O$\underline{CH_2}$CH$_3$), 3.05−2.73 (m, 3H), 2.37 (d, *J* = 13.5 Hz, 1H), 2.12−2.08 (m, 1H), 1.63−1.48 (m, 7H), 1.37 (d, *J* = 3.3 Hz, 3H, CH$_3$), 1.33−1.29 (m, 3H, CH$_3$), 1.29−1.26 (m, 6H, 2×CH$_3$), 1.24 (dd, *J* = 6.9, 2.9 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ 180.86, 179.67, 146.26, 146.13, 134.14, 131.86, 129.92, 129.88, 126.93, 124.18, 124.04, 124.00, 63.63, 63.52, 56.75, 55.54, 48.69, 45.38, 45.33, 37.71, 37.17, 33.49, 29.73, 29.67, 25.08, 23.97, 21.48, 18.48, 16.41, 16.25. ^{31}P NMR (200 MHz, CDCl$_3$) δ 18.93. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}BrN_2O_4PS$ [M+H$^+$]: 665.19423; found: 665.19678; **7n**: Yields 88.75%; ^{1}H NMR (500 MHz, CDCl$_3$) δ 11.75 (s, 1H, NH), 8.65(s, 1H, NH), 7.61 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.38−7.32 (m, 1H), 7.22−7.13 (m, 2H), 6.90 (d, *J* = 5.0 Hz, 1H), 6.53-6.48 (m, 1H, N-CH-P), 4.25−3.90 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.02−2.74 (m, 3H), 2.36 (d, *J* = 13.2 Hz, 1H), 2.13−2.06 (m, 1H), 1.82−1.47 (m, 7H), 1.37 (d, *J* = 2.5 Hz, 3H, CH$_3$), 1.35 (dd, *J* = 7.1, 1.7 Hz, 3H, CH$_3$), 1.25 (t, *J* = 2.6 Hz, 6H, 2×CH$_3$), 1.24−1.15 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ 180.81, 179.45, 146.27, 146.08, 134.21, 133.15, 129.56, 129.16, 127.82, 126.92, 124.13, 124.03, 120.00, 63.68, 63.55, 56.69, 55.47, 48.64, 45.32, 37.69, 37.07, 36.95, 33.49, 29.72, 29.67, 25.08 , 23.97, 21.46, 18.49, 16.43, 16.27. ^{31}P NMR (200 MHz, CDCl$_3$) δ 18.86. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}BrN_2O_4PS$ [M+H$^+$]: 665.19423; found: 665.19647; **7o**: Yields 88.45%; ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.67(s, 1H, NH), 8.68 (s, 1H, NH), 7.57−7.54 (m, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.24 (dd, *J* = 7.8, 2.5 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.01 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.88 (dd, *J* = 5.0, 1.6 Hz, 1H), 6.06−6.01 (m, 1H, N-CH-P), 4.16−3.98 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.01−2.77 (m, 3H), 2.35 (d, *J* = 13.4 Hz, 1H), 2.16−2.07 (m, 1H), 1.82−1.48 (m, 7H), 1.35 (d, *J* = 3.1 Hz, 3H, CH$_3$), 1.31−1.28 (m, 3H, CH$_3$), 1.28−1.24 (m, 6H, 2×CH$_3$), 1.22 (dd, *J* = 6.9, 2.3 Hz, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) 180.86, 179.65, 146.24, 146.12, 136.11, 134.15, 131.29, 131.14, 130.44, 130.19, 126.93, 124.15, 124.03, 124.00, 122.67, 63.66, 63.51, 56.76, 55.57, 48.69, 45.41, 45.37, 37.68, 37.08, 36.96,,36.42, 33.49, 29.71, 25.09, 23.97, 21.48, 18.48, 16,33, 16.14. ^{31}P NMR (200 MHz, CDCl$_3$) δ 18.92. HRMS (m/z) (ESI): calcd for $C_{33}H_{44}BrN_2O_4PS$ [M+H$^+$]: 665.19423; found: 665.19696; **7p**: Yields 88.85%; ^{1}H NMR (500 MHz, CDCl$_3$): δ11.86 (s, 1H, NH), 8.66 (s, 1H, NH), 8.36 (d, *J* = 8.5 Hz, 1H), 7.87 (dd, *J* = 13.6, 8.4 Hz, 2H), 7.69 (d, *J* = 6.9 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.55−7.50 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 9.7 Hz, 1H), 7.01−6.93 (m, 1H), 6.90−6.88 (m, 1H, N-CH-P), 4.17−3.68 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 3.01−2.80 (m, 3H), 2.36 (d, *J* = 13.2 Hz, 1H), 2.13 (dd, *J* = 11.5, 5.4 Hz, 1H), 1.83−1.51 (m, 7H), 1.37 (d, *J* = 4.0 Hz, 3H, CH$_3$), 1.32 (t, *J* = 7.0 Hz, 3H, CH$_3$), 1.24 (dd, *J* = 7.0, 4.6 Hz, 9H, 3×CH$_3$), 1.01 (t, *J* = 6.8 Hz, 3H, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) δ 180.51, 179.46, 146.26, 146.08, 134.21, 133.81, 130.50, 129.02, 128.72, 126.93, 126.69, 126.08, 126.04, 125.98, 125.32, 124.12, 124.01, 123.75, 63.54, 63.38, 53.38, 52.16, 48.65, 45.36, 37.69, 37.08, 36.94, 33.48, 29.69, 25.08, 23.97, 21.44, 18.49, 16.40, 16.14. ^{31}P NMR (200 MHz, CDCl$_3$) δ 19.86. HRMS (m/z) (ESI): calcd for $C_{36}H_{47}N_2O_4PS$ [M+H$^+$]: 635.29942; found: 635.30341; **7q**: Yields 89.42%; ^{1}H NMR (500 MHz, CDCl$_3$): δ 11.65 (s, 1H, NH), 8.68 (s, 1H, NH), 7.96−7.82 (m, 4H), 7.61 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.51−7.46 (m, 2H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.94−6.85 (m, 1H), 6.30−6.25 (m, 1H, N-CH-P), 4.18−3.96 (m, 4H, 2×O$\underline{CH_2}$CH$_3$), 2.89−2.81 (m, 3H), 2.37 (d, *J* = 11.4 Hz, 1H), 2.15−2.10 (m, 1H), 1.85−1.47 (m, 7H), 1.38 (d, *J* = 3.4 Hz, 3H, CH$_3$), 1.33−1.30 (m, 3H, CH$_3$), 1.27−1.25 (m, 6H, 2×CH$_3$), 1.25−1.21 (m, 6H, 2×CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$) 180.67, 179.58, 146.27, 146.10, 134.20, 133.20, 133.12, 128.54, 128.16, 127.82, 127.69, 127.56, 126.94, 126.38, 126.34, 125.79, 124.15, 124.04, 63.61, 63.39, 57.50, 56.30, 48.68, 45.39, 37.70, 37.09, 36.93, 33.49, 29.75, 29.69, 25.09, 23.98, 21.47, 18.50, 16.56, 16.03. ^{31}P NMR (200 MHz, CDCl$_3$) δ 19.57. HRMS (m/z) (ESI): calcd for $C_{36}H_{47}N_2O_4PS$ [M+H$^+$]: 635.29942; found: 635.30347.

22. (a) Chen, Z. -F.; Gu, Y. -Q.; Song, X. -Y.; Liu, Y. -C.; Peng, Y.; Liang, H. *Eur. J. Med. Chem*. **2013**, *59*, 194; (b) General procedure for cytotoxic evaluation *in vitro:* NCI-H460, A549, HepG2 and SKOV3 cells were seeded into 96-well microculture plates and allowed to adhere for 24 h, respectively. After cells were exposed to compounds at concentrations from 100 to 0.01 μM for 48 h, medium was aspirated and replenished with complete medium. IC_{50} was evaluated by MTT tetrazolium dye assay. Each experiment was performed three times; (c) Statistical analysis: All statistical analysis was performed with SPSS Version 10. Data was analyzed by one-way ANOVA. Mean separations were performed using the least significant difference method. Each experiment was replicated thrice, and all experiments yielded similar results. Measurements from all the replicates were combined, and treatment effects were analyzed.
23. General procedure for AO/EB staining: cells were seeded at a concentration of 5×10^4 cell/ mL in a volume of 2 mL on a sterile cover slip in six-well tissue culture plates. Following incubation, the medium was removed and replaced with fresh medium plus 10% fetal bovine serum and supplemented with compound **7c** (5 μM). After the treatment period, the cover slip with monolayer cells was inverted on a glass slide with 20 μL of AO/EB stain (100 mg/mL). Fluorescence was read on an Nikon ECLIPSETE2000-S fluorescence microscope (OLYMPUS Co., Japan).
24. General procedure for Hoechst 33258 staining: cells grown on a sterile cover slip in six-well tissue culture plates were treated with compounds for a certain range of time. The culture medium containing compounds was removed, and the cells were fixed in 4% paraformaldehyde for 10 min. After being washed twice with PBS, the cells were stained with 0.5 mL of Hoechst 33258 (Beyotime) for 5 min and then again washed twice with PBS. The stained nuclei were observed under an Nikon ECLIPSETE2000-S fluorescence microscope using 350 nm excitation and 460 nm emission.
25. General procedure for mitochondrial membrane potential staining: JC-1 probe was employed to measure mitochondrial depolarization in NCI-H460 cells. Briefly, cells cultured in six-well plates after indicated treatments were incubated with an equal volume of JC-1 staining solution (5μg/ml) at 37^{o}C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using an Nikon ECLIPSETE2000-S fluorescent microscope. Mitochondrial depolarization is indicated by an increase in the green/red fluorescence intensity ratio.
26. (a) General procedure apoptosis ratios: Prepared NCI-H460 cells (1×10^6 cells/mL) were washed twice with cold PBS and then resuspended gently in 500 μL of binding buffer. Thereafter, cells were stained in 5 μL of Annexin V-FITC and shaken well. Finally, the cells were mixed with 5 μL of PI, incubated for 20 min in the dark and subsequently analyzed using FACSCali-bur (Becton Dickinson).; (b) General procedure cell cycle: NCI-H460 cell lines were maintained in Dulbecco's modified Eagle's medium with 10% foetal calf serum in 5% CO_2 at 37 ^{o}C. Cells were harvested by trypsinization and rinsed with PBS. After centrifugation, the pellet (10^5−10^6 cells) was suspended in 1 mL of PBS and kept on ice for 5 min. The cell suspensionwas then fixed by the dropwise addition of 9 mL precooled (4 ^{o}C) 75% ethanol with violent shaking. Fixed samples were kept at 4 ^{o}C until use. For staining, cells were centrifuged, resuspended in PBS, digested with 500 μL of RNase A (250 mg/mL), and treated with 25 μL of propidium iodide (PI) (0.15 mM), then incubated for 30 min at 4 ^{o}C. PI-positive cells were counted with a FACScan Fluorescence-activated cell sorter (FACS). The population of cells in each cell-cycle phase was determined using Cell Modi FIT software (Becton Dickinson); (c) Tong, G.; Poot, M.; Hu, D.; Oda, D. *Oral Oncology*. **2000**, *36(2)*, 236.
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28. (a) Mucha, A.; Drag, M.; Dalton, J. P.; Kafarski, P. *Biochimie*. **2010**, *92*, 1509; (b) Mucha, A.; Kafarski, P.; Berlicki, Ł. *J. Med. Chem.* **2011**, *54*, 5955;(c) Culcasi, M.; Casano, G.; Lucchesi, C.; Mercier, A.; Clement, J. L.; Pique, V.; Michelet, L.; Krieger-Liszkay, A.; Robin, M.; Pietri, S. *J. Med. Chem.* **2013**, *56*, 2487.