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Synthesis and Antitumor Activity of Novel α-Aminophosphonates from Diterpenic Dehydroabietylamine

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ABSTRACT: *A series of novel α-aminophosphonates were synthesized from diterpenic dehydroabietylamine, and their structures were characterized by IR, ^{1}H NMR, and ^{31}P NMR spectroscopy. Their antitumor activities against SMMC7721 liver cancer cells were evaluated by the MTT method. Compounds* **4** *and* **6** *exhibited higher activities even at very low concentrations, and the inhibition ratios reached 75% and 79% at 0.1 μM, respectively. The inhibition ratio of compound* **9** *reached 99% after 72-h incubation. α-Aminophosphonates with a fluorine atom and a nitro group fused to the benzene ring exhibited higher activities.* © 2008 Wiley Periodicals, Inc. Heteroatom Chem 19:512–516, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20471

INTRODUCTION

Natural products still play an important role in drug discovery. About 30% of new chemical entities approved as drugs by the U.S. Food and Drug Administrator (FDA) from 1981 to 2002 were natural or natural product derived molecules [1]. Cancer is the primary cause of death in most of the countries, and as a result there is a need to search for cancer-effective compounds [2]. During the past decade, many natural antitumor compounds such as taxol and topotecan were isolated from natural sources and were clinically used [3]. However, the low content of active principles extracted from natural sources and complex extract procedures compelled scientists to search for other naturally abundant resources.

Abietane diterpenoids are widely distributed natural products, which exhibited a variety of industrial uses [4]. In particular, the tricyclic diterpenoid abietic acid is a highly interesting compound for its special structure and properties. It is the main component of rosin, which has the yield of about 1.2 million tonnes per year all over the world. Dehydroabietylamine is an abietane diterpenic amine derived from abietic acid, which can be isolated from an industrial product of Amine D. Its derivatives exhibited a wide range of biological properties including antibacterial, antifungal, and antipenetrant activities [5,6]. Recently, antiinflammatory activities for derivatives of dehydroabietylamine at N position were also evaluated [7,8]. Encouraged by these research results, dehydroabietylamine was chosen as the raw material in screening for new potential antitumor compounds.

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As phosphorus analogs of α-amino acids and their esters, α-aminophosphonates are widely studied as biologically active substances. They exhibit antibacterial, anticancer, insecticidal, hypoglycemic, herbicidal properties and are investigated as starting materials for the synthesis of phosphonopeptides [9–12]. α-Functionalized organic phosphonates are valuable medical compounds and synthetic intermediates. However, the biological activities of tricyclic diterpenic modified phosphonates have seldom been studied.

In this report, we describe the synthesis and antitumor activity of synthesized diterpenic dehydroabietylamine modified α-aminophosphonates against SMMC7721 liver cancer cell, and preliminary structure–activity relationships of these compounds were also investigated.

RESULTS AND DISCUSSION

Synthesis

Novel α-aminophosphonates were synthesized from diterpenic dehydroabietylamine by two steps of reactions through imines intermediates (Fig. 1). Although high steric hindrance of tricycle structure to amine group, dehydroabietylamine and substituted benzaldehyde afforded good yields of imines without catalysts [13]. The addition of phosphite to imines was proved to be very difficult because of high steric hindrance from two reaction components. Although we obtained the diterpenic α-aminophosphonates easily in the toluene solution, the yields were relatively low to 20%–30% for benzyl phosphonates, ethyl phosphonates give higher yields due to less steric hindrance than benzene [14].

1: $C_{39}H_{46}NO_3P$; mp 229.4°C, yield 22.8%; IR (cm^{-1}): 3429 (—NH); 2926 (—CH_3'—CH_2); 1220 (P=O); 1096 (C—N); 832(Ar-H); ^{1}H NMR (DAc (CH_3COOD); δ/ppm, 400 MHz): 7.69–6.82 (18H, Ph-H); 4.82 (1H, N—CH—); 3.38 (2H, N—CH_2—); 2.88 (1H, —$CH(Me)_2$); 2.28–1.72 (10H, —CH_2—);1.70 (1H, >CH—); 1.22–1.09 (12H, —CH_3); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 5.46.

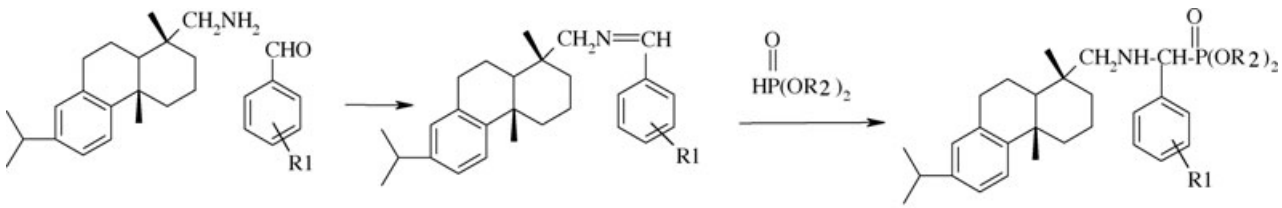
2: $C_{40}H_{48}NO_4P$; mp 223.6°C; yield 27.5%; IR (cm^{-1}): 3434 (—NH); 2930 (—CH_3'—CH_2); 1219 (P=O); 1094 (C—N); 913(Ar-H); ^{1}H NMR (DAc; δ/ppm, 400 MHz): 7.18–6.84 (17H, Ph-H); 4.72 (1H, N—CH—); 3.84 (3H, —OCH_3); 3.38 (2H, N—CH_2–); 2.82 (1H, —$CH(Me)_2$); 2.28–2.06 (10H, —CH_2–); 1.70 (1H, >CH—); 1.22–0.98 (12H, —CH_3); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 5.88.

3: $C_{39}H_{45}ClNO_3P$; mp 230.5°C, yield 30.7%; IR (cm^{-1}): 3458 (—NH); 2929 (—CH_3'—CH_2); 1218 (P=O); 1093 (C—N); 910 (Ar-H); ^{1}H NMR (DAc; δ/ppm, 400 MHz): 7.48–6.86 (17H, Ph-H); 4.80 (1H, N—CH—); 3.40 (2H, N—CH_2—); 2.82 (1H, —$CH(Me)_2$); 2.29–2.06 (10H, —CH_2—); 1.71 (1H, >CH—); 1.20–1.00 (12H, —CH_3); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 4.92.

4: $C_{39}H_{45}FNO_3P$; mp 225.6°C, yield 18.9%; IR (cm^{-1}): 3437 (—NH); 2931 (—CH_3'—CH_2); 1220 (P=O); 1094 (C—N); 910 (Ar-H); ^{1}H NMR (DAc; δ/ppm, 400 MHz): 7.07–6.83 (17H, Ph-H); 4.85 (1H, N—CH—); 3.40 (2H, N—CH_2—); 2.89 (1H, —$CH(Me)_2$); 2.84–2.06 (10H, —CH_2—); 1.76 (1H, >CH—); 1.71–0.99 (12H, —CH_3); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 5.22.

5: $C_{39}H_{45}FNO_3P$; mp 234.6°C, yield 19.7%; IR(cm^{-1}); 3421 (—NH); 2932 (—CH_3'—CH_2); 1223 (P=O); 1097 (C—N); 913 (Ar-H); ^{1}H NMR (DAc; δ/ppm, 400 MHz): 7.51–6.84 (17H, Ph-H); 4.87(1H, N—CH—); 3.47 (2H, N—CH_2—); 2.90 (1H, —$CH(Me)_2$); 2.84–2.06 (10H, —CH_2—); 1.63 (1H, >CH—); 1.58–1.01 (12H, —CH_3); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 4.63.

6: $C_{40}H_{45}F_3NO_3P$; mp 237.8°C, yield 20.5%; IR (cm^{-1}): 3428 (—NH); 2931 (—CH_3'—CH_2); 1217 (P=O); 1094 (C—N); 911 (Ar-H); ^{1}H NMR (DAc; δ/ppm, 400 MHz): 7.87–6.84 (17H, Ph-H); 4.99 (1H, N—CH—); 3.52 (2H, N—CH_2—); 2.90 (1H, —$CH(Me)_2$); 2.84–2.06



Compds	1	2	3	4	5	6	7	8	9	10
R1	H	*p*-OMe	*p*-Cl	*p*-F	*m*-F	*p*-CF3	*o*-OH, *p*-NO2	*o*-OH, *p*-Cl	*o*-OH, *p*-NO2	*o*-OH, *p*-Cl
R2	Ph	Ph	Ph	Ph	Ph	Ph	Ph	Ph	Et	Et

FIGURE 1 Synthetic scheme of α-aminophosphonates.

(10H, $-CH_2-$); 1.73 (1H, >CH—); 1.20–1.01 (12H, $-CH_3$); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 4.29.

7: $C_{39}H_{45}N_2O_6P$; mp 271.5°C, yield 22.3%; IR (cm^{-1}): 3408 (—NH); 2956 ($-CH_3'-CH_2$); 1261 (P=O); 1095 (C—N); 916 (Ar-H); ^1H NMR (DAc; δ/ppm, 400 MHz): 8.59–6.92 (17H, Ph-H); 5.02 (1H, N—CH—); 3.54 (2H, $N-CH_2-$); 2.95 (1H, $-CH(Me)_2$); 2.83–2.06 (10H, $-CH_2-$); 1.84 (1H, >CH—); 1.57–1.07 (12H, $-CH_3$); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 9.56.

8: $C_{39}H_{45}ClNO_4P$; mp 248.8°C, yield 20.3%; IR (cm^{-1}): 3428 (—NH); 2931 ($-CH_3'-CH_2$); 1217 (P=O); 1094 (C—N); 911 (Ar-H); ^1H NMR (DAc; δ/ppm, 400 MHz): 7.67–6.84 (17H, Ph-H); 4.86 (1H, N—CH—); 3.41 (2H, $N-CH_2-$); 2.89 (1H, $-CH(Me)_2$); 2.82–2.06 (10H, $-CH_2-$); 1.84 (1H, >CH—); 1.57–1.00 (12H, $-CH_3$); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 4.93.

9: $C_{31}H_{45}N_2O_6P$; mp 244.4°C, yield 66.2%; IR (cm^{-1}): 3394 (—NH); 2948 ($-CH_3'-CH_2$); 1234 (P=O); 1060 (C—N); 827 (Ar-H); ^1H NMR (DAc; δ/ppm, 400 MHz): 8.49–6.89 (17H, Ph-H); 5.08 (1H, N—CH—); 3.82 (2H, $N-CH_2-$); 2.89 (1H, $-CH(Me)_2$); 2.85–2.06 (10H, $-CH_2-$); 1.78 (1H, >CH—); 1.76–1.05 (12H, $-CH_3$); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 8.58.

10: $C_{35}H_{45}ClNO_4P$; mp 235.2°C, yield 63.5%; IR (cm^{-1}): 3398 (—NH); 2944 ($-CH_3'-CH_2$); 1237 (P=O); 1064 (C—N); 822 (Ar-H); ^1H NMR (DAc; δ/ppm, 400 MHz): 7.52–6.89 (17H, Ph-H); 4.83 (1H, N—CH—); 3.85 (2H, $N-CH_2-$); 2.89 (1H, $-CH(Me)_2$); 2.82–2.06 (10H, $-CH_2-$); 1.84 (1H, >CH—); 1.57–1.00 (12H, $-CH_3$); ^{31}P NMR (DAc; δ/ppm, 400 MHz): 9.26.

Biological Activity

Compounds **4** and **6** were treated with SMMC7721 liver cancer cells at different concentrations from 0.1 to 100 μM. The data of inhibition ratios are listed in Table 1. Usually, when the concentration of the compound solution is 1 μmol/L, the inhibition ratio of the compound to cancer growth is more than 50%, the compound is considered as strongly effective. It has been found from Table 1 that the growth of SMMC7721 liver cancer cells was strongly inhibited by compounds **4** and **6**, even at very low concentration of 0.1 μM, after the 48-h incubation, the inhibition ratios of compounds **4** and **6** reached 75% and 79%, respectively. The dose-dependent response of these two compounds to the tumor cells was also monitored; it was found that when the concentration of the drugs was changed, the inhibition ratios were nearly 75% at different concentrations, and the increase in the concentration has no obvious effects on the inhibition ratios after the 48-h incubation.

TABLE 1 Inhibition Ratios of Compounds **4** and **6** against SMMC7721 Liver Cancer Cells at Different Concentrations

Compounds	*Concentration (μM)*	*Optical Density*	*Inhibition Ratio(%)*
Control		1.74 ± 0.05	0
4	0.1	0.44 ± 0.07	75.00
	1.0	0.42 ± 0.04	76.00
	10	0.43 ± 0.05	76.00
	100	0.37 ± 0.05	77.00
6	0.1	0.40 ± 0.06	79.00
	1.0	0.41 ± 0.08	76.00
	10	0.47 ± 0.08	73.00
	100	0.38 ± 0.03	78.00

TABLE 2 Inhibition Ratios of Compounds against SMMC7721 Liver Cancer Cells at Different Concentrations

Compounds	*Concentration (μM)*	*Inhibition Ratio (%) 24 h*	*Inhibition Ratio (%) 72 h*
Control		0	0
1	10	6.79	2.12
	100	15.08	29.49
2	10	5.70	38.03
	100	12.26	40.38
3	10	0.00	0.00
	100	10.94	19.04
5	10	6.25	40.19
	100	41.40	40.19
6	10	33.07	17.50
	100	5.45	40.38
7	10	10.70	9.81
	100	55.21	66.46
8	10	20.30	46.79
	100	57.81	75.00
9	10	5.53	38.03
	100	41.41	99.00
10	10	3.67	2.76
	100	25.26	29.70

The inhibition ratios of other compounds against SMMC7721 liver cancer cells were evaluated at the concentrations of 10 and 100 μM at 24 and 72 h, respectively; the data of inhibition ratios are listed in Table 2. It was found that compounds **7, 8**, and **9** exhibited high activities when at 100 μM after the 72-h incubation, and the inhibition ratios reached 66.46%, 75.00%, and 99.00%, respectively. Most compounds exhibited relatively low activity after the 24-h incubation. The dose-dependent response indicated that most of the compounds exhibited higher activity when at higher concentrations; however, compound **6** is the exception. Compared with the data of inhibition ratios of compound **6** at 24, 48, and 72 h, it exhibited highest activity when incubated for 48 h.

On the basis of the screening results, we made the preliminary conclusions. Some

α-aminophosphonates from diterpenic dehydroabietylamine exhibited high activity against SMMC7721 liver cancer cells. They exhibited time- and dose-dependent activities. The compounds containing a fluorine atom and a nitro group in the benzene ring exhibited high activities; this kind of compounds exhibited higher activity at 48 h of bioassay. The different substituted group has prodigious diversity of antitumor activity; changing the group is possible to improve their activity. The title compound is a kind of lead compounds of antitumor agent that warrants further investigation.

EXPERIMENTAL

General

Melting points were determined with XT5 melting point apparatus, and the temperature was uncorrected. Infrared spectra were obtained by using the KBr method on a Bio-Rad FTS-185 IR spectrophotometer. ^{1}H NMR and ^{31}P NMR spectra were recorded on DPX a Bruker AVANCE spectrometer ($CDCl_3$ as the solvent of imines and DAc as the solvent of α-aminophosphonates).

Synthesis

Synthetic Procedure for Imine Intermediates. Dehydroabietylamine (10 mmol) was dissolved in 20 mL ethanol, 10 mmol of substituted benzaldehyde was added to the mixture and heated to reflux for 2 h, and the resulted solids were crystallized from ethanol. The crystals were collected and dried under vacuum.

Synthesis of α-Aminophosphonates. The synthesized imines (10 mmol) and 10 mmol of diphenyl phosphite were added to the three-necked bottle, and 20 mL toluene was added. The reaction mixture was heated to 120°C and was maintained for 6 h. Then the mixture was cooled to room temperature, and the solids were crystallized from acetic acid. The crystals were collected and dried under vacuum. Diethyl phosphonates were synthesized at the same conditions without using solvents.

MTT Assay

SMMC7721 liver cancer cells were propagated continuously in culture and grown in RPMI l640 medium with 10% inactivated fetal calf serum and antibiotics. The cells harvested from exponential phase were seeded equivalently into 96 well plates and incubated for 8 h; then the studied compounds were added in the concentration gradient. The final concentrations were maintained at l00, l0, l, and 0.1 μM, respectively. Control was added the same amount of solvent. The plates were maintained at 37° in a humidified 5% CO_2, and incubated for 48 h, then 20 μL MTT solution was added, and the plate was incubated for additional 4 h. Supernatant from each well was taken out carefully, and l50 mL of DMSO was added to each well and was subjected to vibration on an ELISA spectrophotometer at 570 nm. Inhibition ratios were calculated by using the following equation:

$$\text{Inhibiton ratio} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0 represents total absorbance of the tumor cell control and A_1 represents the absorbance of the drug treatment group.

CONCLUSIONS

Novel α-aminophosphonates were synthesized from natural product of diterpenic dehydroabietylamine. Their antitumor activities against SMMC7721 liver cancer cells were evaluated by the MTT method. They exhibited time- and dose-dependent activities to SMMC7721 liver cancer cells. Compounds **4** and **6** exhibited high activities even at very low concentrations. The inhibition ratio of compound **9** reached 99% after the 72-h incubation. α-Aminophosphonates with a fluorine atom and a nitro group fused to the benzene ring exhibited high activities. Further investigations will be required for a more detailed biological evaluation of these agents.

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