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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Anticancer activities of some arylcarbamoylalkyltriphenylphosphonium chlorides

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Abstract A series of novel arylcarbamoylalkyltriphenylphosphonium chlorides were synthesized. The newly synthesized compounds, [R-(p-C_6H_4)-NH-CO-$R_1P^{\oplus}(C_6H_4)_3]Cl^{\theta}$, R = H (**2**), CH_3 (**4**), NO_2 (**6**), R_1 = $–CH_2–$ (**b**), $CH_3CH<$ (**a**), $–CH_2CH_2–$ (**c**), $–CH_2CH_2CH_2$ (**d**), the analogs of an anticancer drug, were characterized by infrared (IR), ^{1}H nuclear magnetic resonance (NMR), ^{13}C-NMR, ^{31}P-NMR, mass spectrometry (MS), thermogravimetry (TG), and conductivity measurements. The anticancer activities of the obtained compounds were measured by MTT. The preliminary results indicated that some compounds showed potent anticancer activities against HCT-8, Bel-7402, A549, and S180.

Keywords Phosphonium · Synthesis · Characterization · Anticancer activity

Introduction

Phosphonium, as a bactericide, has been used in the overseas market (Fu *et al.*, 2003), and its anticancer activity has been studied. Since Dubois and Lin (1978) found that phosphonium bromide showed activity on P-388 of lymphocyte leukemia, there have been many studies of the synthesis and anticancer activity of this kind of compounds. The results of these studies have showed that these compounds demonstrate certain activity against colonic cancer (Patel, *et al.*, 1994; Lou and Zhang, 2000), ovarian cancer (Manetta *et al.*, 1996), liver cancer (Lou and

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Shang, 2000), oropharyngeal cancer (Lou and Shang, 2000), human breast cancer (Delikatny *et al.*, 1996), and so on. The highly negative plasma membrane potentials characteristic of neoplastic cells were believed to account for the selective accumulation and toxicity of phosphonium and other cationic lipophilic compounds against malignant cells (Rideout *et al.*, 1989; Delikatny *et al.*, 2002; Rhys *et al.*, 1995). In order to further study the anticancer activities, to select useful anticancer compounds, and to study their anticancer mechanisms, we synthesized 12 phosphonium compounds based on these facts. Firstly, we chose the compounds containing amino groups as original sources to react with chloroalkyl chlorides, then the products reacted with triphenyl phosphates to produce the following phosphonium compounds: $[R\text{-}(p\text{-}C_6H_4)\text{-}NH\text{-}CO\text{-}R_1P^{\oplus}(C_6H_4)_3]Cl^{\theta}$, R = H (**2**), CH_3 (**4**), NO_2 (**6**),R_1 = $–CH_2–$ (**b**),$CH_3CH<$ (**a**),$–CH_2CH_2–$ (**c**),$–CH_2CH_2CH_2$ (**d**). The anticancer activities of the compounds were studied by MTT. The general synthesis route of the target compounds is shown in Fig. 1.

Results and discussion

Chemistry

^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, ^{31}P NMR, and infrared (IR) analyses were used to characterize all the synthesized arylcarbamoylalkyltriphenylphosphonium chlorides. The formation of the product was further confirmed by mass spectrometry (MS) analyses. Because the target compounds partially decomposed while measuring the melting point, thermogravimetry analyses were conducted (Table 1). The conductivity results are given in Table 2, indicating that all of the products existed as ion in ethanol.

Anticancer activities

The influence of these compounds on the HCT-8, A549, and Bel-7402 cell lines was studied (Table 3). The preliminary bioassays indicated that **4a** and **4d** showed

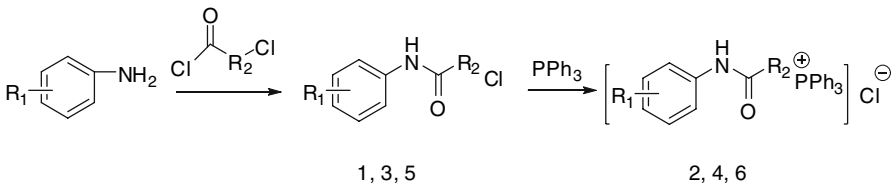

Fig. 1 Synthesis route for the target compounds

Table 1 The thermogravimetry data of target compounds

Compounds	Start losing *T*/°C	Start melting *T*/°C	
2a	225	235	T = 378°C, lost 88%
2b	250	253	T = 34°C , lost 96%
2c	230	224	T = 312°C, lost 99%
2d	287	278	T = 362°C, lost 94%
4a	228	240	T = 340°C, lost 96%
4b	233	234	T = 392°C, lost 90%
4c	230	235	T = 314°C, lost 99%
4d	281	252	T = 377°C, lost 96%
6a	235	223	T = 395°C, lost 89%
6b	224	213	T = 381°C, lost 91%
6c	244	234	T = 396°C, lost 78%
6d	267	224	T = 395°C, lost 89%

Table 2 The molar conductivity of the products dissolved in ethanol

Compounds	Concentration ($mmol \cdot L^{-1}$)	Conductivity ($\times 10^2 \mu s \cdot cm^{-1}$)	Molar conductivity ($s \cdot cm^2 \cdot mol^{-1}$)
2a	4.44	0.72	16.1
2b	4.22	0.80	18.8
2c	4.35	1.00	22.9
2d	4.44	0.94	21.0
4a	4.00	0.68	16.9
4b	4.04	0.83	20.4
4c	4.05	0.88	21.6
4d	4.01	0.82	20.3
6a	4.00	0.55	13.6
6b	4.03	0.62	15.2
6c	4.00	0.77	19.1
6d	4.00	0.80	19.9
Ethanol		0.55 μs/cm	

promising anticancer activity against A549 and Bel-7402, respectively, while **4b** showed promising anticancer activity against HCT-8, A549, and Bel-7402.

We have reported (Lou and Shang, 2000) that benzyltriphenylphosphonium chloride displayed activities against BEL-7420, HCT-8, and KB, at a density of 5 μg/mL, with inhibitions of 57.82%, 19.1%, and 62.8%, respectively. Table 3 indicates that compounds **4a**, **4b,** and **4d** showed higher activities than benzyltriphenylphosphonium chloride.

The inhibition of these compounds on the S180 cell line was measured by MTT, with 5-fluorouracil as a reference (Table 4). The results indicated that the investigated compounds showed certain activities against S180 cell lines at different concentrations, and that most of these compounds displayed better activity

Table 3 Antitumor activities of triphenylphosphonium compounds (5 μg/mL, inhibitory rate)

Compounds	Inhibitory rate (%)		
	HCT-8	Bel-7420	A549
2a	−35.61	25.36	5.31
2b	−18.63	0.33	21.94
2c	−23.95	3.32	19.76
2d	−20.75	−3.54	17.07
4a	−25.53	73.98	12.73
4b	63.34	45.04	70.28
4c	−6.19	−4.39	20.47
4d	43.98	13.14	61.82
6a	14.47	13.94	26.70
6b	−9.03	−1.19	27.84
6c	−2.50	−6.14	20.62
6d	−11.69	−3.64	3.47

Table 4 The inhibitory rate of compounds on S180 [5-fluorouracil (5-Fu) as reference]

Compound	1 mg/ml	100 μg/ml	10 μg/ml	1 μg/ml	Activity range (μg/ml)	^{31}P NMR
5-Fu	72.77%	34.92%	28.3%	21.92%	> 100	
2a	58.80	88.66	89.05	102.24	< 1	26.703
2b	46.20	76.95	74.84	97.45	< 1	21.317
2c	53.69	86.54	89.51	87.27	10	23.289
2d	61.01	87.42	90.57	92.11	< 1	22.841
4a	72.75	103.55	83.14	77.69	100	26.721
4b	61.44	82.62	76.78	84.17	< 1	22.113
4c	46.20	81.04	85.30	143.70	< 1	23.414
4d	35.91	67.21	62.37	43.80	100	22.841
6a	–	57.82	159.51	102.55	10	26.443
6b	81.52	139.20	162.54	176.55	<< 1	21.173
6c	53.44	97.71	80.95	127.69	< 1	23.197
6d	29.10	56.93	69.89	63.82	10	22.608

than 5-fluorouracil at low concentrations. In addition, the inhibition of 5-fluorouracil increased with increasing concentration, while the inhibitory rate of the phosphonium compounds obviously increased with decreasing concentration, suggesting that different mechanisms are involved. The mechanism of 5-fluorouracil is inversion, corresponding to nucleotide and restrained pedantic chest glycoside synzyme, followed by inhibited synthesis of DNA, so the inhibition of 5-Fu increased with increasing concentration. On the other hand, the mechanism of action of the phosphonium compounds is selective accumulation of cationic lipophilic phosphonium salts (CLPS) in mitochondria neoplastic cells and inhibition of mitochondrial function. Thus, the excess charge of mitochondria in tumor cells is

limited. Consequently, the absorptive ability of tumor cells does not increase with increasing concentration. Furthermore, compounds **6a–c** displayed higher activities than the other compounds, while compounds **4a–d** displayed lower activity. Compound **6d**, however, did not display any obvious activity. Thus, the relationship between the structure of the compounds and their activity was analyzed. The effect of compound **6d** disappeared because of the butyl group. Compounds **6a**, **6b**, and **6c** include strong electron-withdrawing groups, and compounds **4a–d** include electron-releasing groups. This clearly shows that the positive charge of the phosphate atom influenced the activity of the compounds. The ^{31}P NMR results also supported this explanation. Although the difference in the structures was small, the difference in the ^{31}P NMR delta values of the compounds was large. Regarding the specific similar structure of the P atom, the positive charge density was higher, and the P spectrum displacement delta value was smaller. Therefore, compounds **6a–d,** including electron-withdrawing nitryl groups, displayed the best antitumor activity, while compounds **4a–d,** including electron-releasing groups, displayed the weakest activity. The order of positive charge density and compound P spectrum displacement were exactly opposite, but were consistent with the rate of tumor cell inhibition, in which both the positive charge density of the compound and the inhibitory rate were higher.

Surface-enhanced Raman spectroscopy

We carried out surface-enhanced Raman spectroscopy (SERS) on the above compounds. The results, shown in Figs. 2–4, indicate that the phosphonium compounds and DNA did not display obviously mutual effects. Figure 4 (SERS of the mixture of DNA and **4d**) may be regarded as the superimposition of Figs. 2 and 3**.** The corresponding Raman displacement and relative strength did not obviously change, so we can predict that the phosphonium compounds have no toxicity

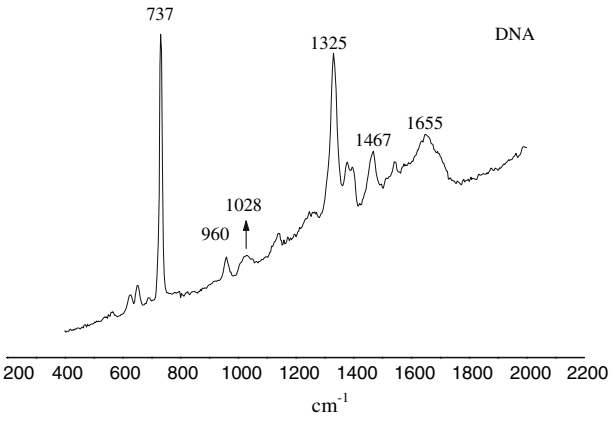

Fig. 2 SERS of DNA

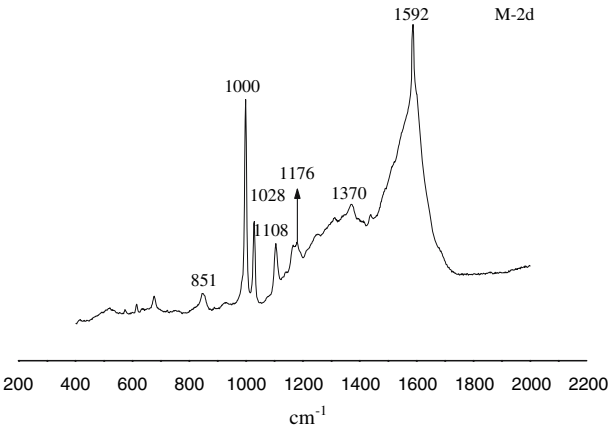

Fig. 3 SERS of compound **4d**

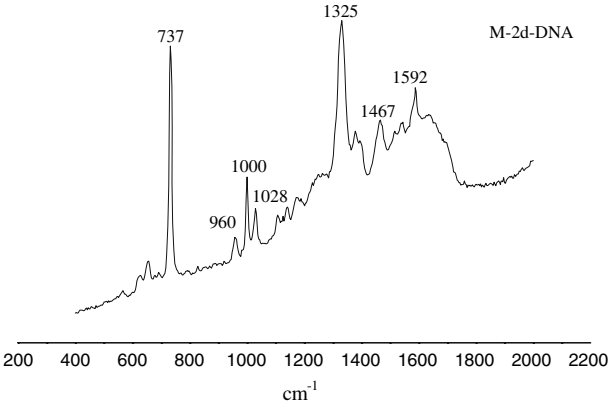

Fig. 4 SERS of a mixture of DNA and compound **4d**

towards DNA. These results prove that the antitumor activity of the phosphonium is mainly concerned with the absorption of tumor cell surface. Fig. 4

Conclusion

In conclusion, arylcarbamoylalkyltriphenylphosphonium chloride derivatives were synthesized and their anticancer activities were evaluated. Compounds **4a**, **4b,** and **4d** showed better activities than benzyltriphenylphosphonium chloride and most of these compounds displayed better activity than 5-fluorouracil at low concentration. The analysis of their anticancer mechanism proved that the antitumor activity of phosphonium is mainly concerned with absorption at the tumor cell surface. Further research in this area is in progress in our laboratory.

Experimental

Chemistry

The solvents and reagents were used as received or were dried prior to use as needed. Melting points were determined with a Tech X-6 micro melting point apparatus made in Beijing. Infrared spectra were recorded on a Perkin-Elmer PE-SPECTRUM ONE apparatus with the υ values recorded in wave numbers (cm^{-1}). Solid compounds were pressed with KBr to form disks. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were obtained on a Varian Unity INOVA-600 MHz spectrometer (600 MHz, ^{1}H; 150 MHz, ^{13}C) in $CDCl_3$ with tetramethylsilane as the internal reference. The mass spectra were obtained on a Finnigen trace mass spectrometer (70 eV). Thermogravimetry was carried out on a Perkin–Elmer TG Thermal System TGS2 in a flow of nitrogen gas and a heating rate of 10°C/min from 20°C to 400°C in a platinum crucible. The conductivities of the products were all measured at the same condition by dissolving them in ethanol. The molar conductivity was calculated by the formula $\lambda = (\kappa_{solu}-\kappa_{solv})/c$.

*Synthesis of N-phenyl-2-chloroacetamide (**1a**)*

To a stirred solution of aniline (9.3 ml, 0.10 mol) in acetone (20 ml), 2-chloroacetyl chloride (4.1 ml, 0.05 mol) at 0–5°C was added dropwise and maintained with an ice bath. The reaction mixture was stirred at room temperature for an additional four hours. Then, HCl (40 ml, 10%) was added to the mixture. A white solid was formed thereafter. The precipitate was separated by filtration and washed successively with HCl (10%) and water. The product was dried under vacuum. Yield, 96.3%; m.p. 89–90°C IR (υ/cm^{-1}): 3260, 1671.

The method for synthesis of **1b–d** was the same as that of **1a**. Data for **1b**: yield, 93.2%; m.p. 140–142°C. IR (υ/cm^{-1}): 3267, 1673. Data for **1c**: yield, 94.8%; m.p. 118–120°C. IR (υ/cm^{-1}): 3302, 1660. Data for **1d**: yield, 93.5%; m.p. 69–71°C . IR (υ/cm^{-1}): 3322, 1669.

*Synthesis of phenylcarbamoyl-methyltriphenylphosphonium chloride (**2a**)*

To N-phenyl-2-chloroacetamide (1.57 g, 9.3 mmol) in benzene (20 ml), triphenyl phosphate (2.44 g, 9.3 mmol) was added and the mixture was refluxed for six hours. The solvent, when cooled to room temperature, was removed from the crystalline reaction product by filtration and the crystals were washed successively with benzene and ethyl acetate to provide 1.5 g of **2a**. The crude product was recrystallized by ethanol. Yield, 37.5%; IR (υ/cm^{-1}): 3394, 1679, 1600, 1553, 1439, 1333, 1111, 738, 689, 509; ^{1}H-NMR δ: 11.89 (1H, s, NH), 7.84 ~ 7.88 (6H, m, ArH), 7.75 ~ 7.78 (3H, m, ArH), 7.60 ~ 7.66 (8H, m, ArH), 7.20 ~ 7.22 (2H, t, ArH), 7.03 ~ 7.05 (1H, t, ArH), 5.26 (2H, d, CH_2); ^{13}C-NMR δ: 160.60, 160.56, 137.77,

134.75, 134.73, 133.85, 133.78, 129.93, 129.84, 128.25, 124.12, 120.01, 118.34, 117; ^{31}P-NMR δ: 26.703; MS (ESI) *m/z*: 396.16 (M^+-Cl).

*Synthesis of 1-(phenylcarbamoyl)-ethyltriphenylphosphonium chloride (**2b**)*

To N-phenyl-2-chloropropanamide (1.51 g, 8.2 mmol) in xylene (20 ml), triphenyl phosphate (2.25 g, 8.6 mmol) was added and the mixture was refluxed for 60 hours. The solvent, when cooled to room temperature, was removed from the crystalline reaction product by filtration and the crystals were washed with ethyl acetate to provide 0.89 g of **2b**. The crude product was dissolved in ethanol and the solution was then subjected to column chromatography (using silica gel), eluting successively with ethyl acetate and ethanol. The elution was analyzed by $AgNO_3$ (5%). Yield, 24.3%; IR (υ/cm^{-1}): 3435, 1678, 1596, 1542, 1495, 1437, 1447, 1109, 749, 778, 757, 724, 697, 686; ^{1}H-NMR δ: 12.03 (1H, s, NH), 7.93 ~ 7.96 (6H, q, ArH), 7.74 ~ 7.76 (3H, t, ArH), 7.62 ~ 7.66 (8H, m, ArH), 7.21 ~ 7.23 (2H, t, ArH), 7.03 ~ 7.05 (1H, t, ArH), 6.59 ~ 6.65 (1H, m, CH_3), 1.76 (3H, q, CH_3); ^{13}C-NMR δ: 166.70, 138.37, 135.25, 135.23, 134.87, 134.80, 130.60, 130.52, 129.03, 124.93, 120.90, 118.63, 118.07, 38.12, 37.78, 15.59; ^{31}P-NMR δ: 21.317; MS (ESI) *m/z*: 410.18 (M^+-Cl).

*Synthesis of 2-(phenylcarbamoyl)-ethyltriphenylphosphonium chloride (**2c**)*

To N-phenyl-3-chloropropanamide (1.56 g, 8.5 mmol) in DMF (15 ml) was added triphenyl phosphate (2.33 g, 8.9 mmol) and the mixture was refluxed for 70 hours. The solvent was evaporated under reduced pressure and the solid obtained was dissolved in ethanol and the solution was then subjected to column chromatography (using silica gel), eluting successively with ethyl acetate and ethanol. The elution was analyzed by $AgNO_3$ (5%). Yield, 51.8%; IR (υ/cm^{-1}): 3430, 1684, 1595, 1543, 1494, 1441, 1112, 756, 743, 722, 698, 690; ^{1}H-NMR δ: 11.31(1H, s, NH), 7.74 ~ 7.80 (11H, m, ArH), 7.67 ~ 7.70 (6H, m, ArH), 7.21 ~ 7.24 (2H, t, ArH), 7.00 ~ 7.02 (1H, t, ArH), 3.85 (2H, m, CH_2), 3.20 (2H, m, CH_2); ^{13}C-NMR δ: 168.13, 168.04, 139.12, 135.44, 135.42, 133.85, 133.78, 133.31, 130.76, 130.67, 130.56, 128.50, 123.59, 120.16, 118.05, 117.47, 29.96, 20.28, 19.91; ^{31}P-NMR δ: 23.289; MS (ESI) *m/z*: 410.26 (M^+-Cl).

*Data for **2d***

Yield, 29.4%; IR (υ/cm^{-1}): 3422, 1675, 1601, 1542, 1439, 1113, 752, 723, 691; ^{1}H-NMR (600 MHz, $CDCl_3$, TMS) δ: 10.91(1H, s, NH), 7.88 ~ 7.89 (3H, d, ArH), 7.68 ~ 7.81(14H, m, ArH), 7.24 ~ 7.27 (2H, t, ArH), 7.02 (1H, s, ArH), 3.68 (2H, s, CH_2), 3.04 (2H, t, CH_2), 2.07 (2H, d, CH_3); ^{13}C-NMR δ: 171.43, 139.61, 135.66, 133.94, 131.04, 128.98, 123.91, 120.57, 118.80, 118.23, 37.42, 20.08; ^{31}P-NMR δ: 22.841; MS (ESI) *m/z*: 424.25 (M^+-Cl).

*Synthesis of N-(p-tolyl)-2-chloroacetamide (**3a**)*

To a stirred solution of p-methylanline (3.42 g, 32 mmol) in toluene (15 ml), a solution of sodium carbonate (2.76 g, 26 mmol) in water (20 ml) was added. To this mixture dropwise 2-chloroacetyl chloride (3.72 ml, 38 mmol) was added at 0 ~ 5°C maintained with an ice bath. The reaction mixture was stirred at room temperature for additional 2.5 h and the precipitate formed was separated by filtration and washed successively with water and toluene. The product was dried under vacuum. The yield was 91.2; %. m.p. 164–165.4°C. IR (υ/cm^{-1}): 3274, 1669.

The method for the synthesis of **3b–d** was the same as that of **3a**. Data for **3b**: yield, 89.9%; m.p.: 124–125.5°C. IR (υ/cm^{-1}): 3252, 1663. Data for **3c**: yield, 85.5%; m.p. 123–124°. IR (υ/cm^{-1}): 3294, 1655. Data for **3d**: yield, 68.9%; m.p. 89.6–92°C. IR (υ/cm^{-1}): 3321, 1661.

*Synthesis of 1-(p-tolylcarbamoyl)-methyltriphenylphosphonium chloride (**4a**)*

Triphenyl phosphate (2.64 g, 10 mmol) was added to N-(p-tolyl)−2-chloroacetamide (1.84 g, 10 mmol) in xylene (20 ml) and the mixture was refluxed for 48 hours. The solvent, when cooled to room temperature, was removed from the crystalline reaction product by filtration and the crystals were washed with ethyl acetate to provide 4.05 g of **4a**. The crude product was dissolved in ethanol and the solution was then subjected to column chromatography (using silica gel), eluting successively with ethyl acetate and ethanol. The elution was analyzed by $AgNO_3$ (5%). Yield, 90.7%; IR (υ/cm^{-1}): 3422, 3031, 1676, 1607, 1542, 1514, 1438, 1111, 820, 747, 727, 689; ^{1}H-NMR δ: 11.76 (1H, s, NH), 7.48 ~ 7.87 (6H, q, ArH), 7.75 ~ 7.77 (3H, t, ArH), 7.63 ~ 7.66 (6H, m, ArH), 7.48 ~ 7.49 (2H, d, ArH), 7.00 ~ 7.02 (2H, d, ArH), 5.24(2H, d, CH_2), 2.25 (3H, s, CH_3); ^{13}C-NMR δ: 160.48, 135.45, 134.79, 133.99, 133.92, 133.72, 130.01, 129.92, 128.85, 120.13, 118.67, 118.08, 33.36, 20.69; ^{31}P-NMR δ: 22.113; MS (ESI) *m/z*: 410.19 (M^+-Cl).

*Data for **4b***

Yield, 35.5%; IR (υ/cm^{-1}): 3421, 1677, 1609, 1544, 1514, 1438, 1110, 821, 752, 726, 690; ^{1}H-NMR δ: 11.92 (1H, s, NH), 7.93 ~ 7.96 (6H, q, ArH), 7.32 ~ 7.56 (3H, t, ArH), 7.64 (6H, s, ArH), 7.50 ~ 7.51 (2H, d, ArH), 7.01 ~ 7.02 (2H, d, ArH), 6.59 (1H, s, CH), 2.25 (3H, s, CH_3), 1.75 (3H, m, CH_3); ^{13}C-NMR δ:166.39, 135.80, 135.19, 134.85, 134.78, 134.41, 130.56, 130.47, 129.48, 120.80, 118.63, 118.07, 38.03, 21.33, 15.56; ^{31}P-NMR δ: 26.721; MS(ESI) *m/z*: 424.22(M^+-Cl).

*Data for **4c***

Yield, 83.7%; IR (υ/cm^{-1}): 3420, 1675, 1605, 1541, 1514, 1438, 1113, 822, 743, 724, 690; ^{1}H-NMR δ: 11.15 (1H, s, NH), 7.75 ~ 7.78 (9H, q, ArH), 7.63 ~ 7.69 (8H,

m, ArH), 7.00 ~ 7.01 (2H, d, ArH), 3.83 (2H, m, CH_2), 3.17 (2H, m, CH_2, 2.26 (3H, s, CH_3); ^{13}C-NMR δ: 168.18, 168.09, 136.85, 135.71, 134.16, 133.30, 130.98, 129.31, 120.52, 120.41, 118.50, 117.93, 30.36, 21.32,20.06; ^{31}P-NMR δ: 23.414; MS (ESI) *m*/*z*: 424.31 (M^+-Cl).

*Synthesis of 3-(p-tolylcarbamoyl)-propyltriphenylphosphonium chloride (**4d**)*

A mixture of N-(p-tolyl)-4-chlorobutyramide (1.81 g, 8.6 mmol) and triphenyl phosphate (2.25 g, 8.6 mmol) was heated to 90? for eight hours. To this mixture ethyl acetate (20 ml) was added and heated to reflux for several minutes. The white solid formed was separated by filtration from the solution and washed with ethyl acetate to provide 2.34g of **4d**. The crude product was dissolved in ethanol and the solution was then subjected to column chromatography (using silica gel), eluting successively with ethyl acetate and ethanol. The elution was analyzed by $AgNO_3$ (5%). Yield, 57.8%; IR (υ/cm^{-1}): 3420, 1674, 1602, 1536, 1514, 1438, 1112, 822, 743, 723, 691; ^{1}H-NMR δ: 10.75 (1H, s, NH), 7.68 ~ 7.81 (17H, m, ArH), 7.05 ~ 7.06 (2H, d, ArH), 3.67 (2H, t, CH_2), 3.01 (2H, t, CH_2), 2.27 (3H, s, CH_3), 2.05 (2H, d, CH_2); ^{13}C-NMR δ: 171.19, 137.03, 135.63, 133.97, 133.32, 131.08, 131.01, 129.47, 120.53, 118.83, 118.26, 37.31, 37.19, 22.29, 21.33, 20.04; ^{31}P-NMR δ: 22.841; MS (ESI) *m*/*z*: 438.24 (M^+-Cl).

*Synthesis of N-(4-nitro-phenyl)-2-chloroacetamide (**5a**)*

To a stirred solution of 4-nitroaniline (1.58 g, 11.4 mmol) in dichloromethane (50 ml), sodium carbonate (0.96 g, 9.1 mmol) was added. To this mixture a solution of 2-chloroacetyl chloride (1.2 ml, 15.1 mmol) in dichloromethane (3 ml) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for additional four hours. Water (15 ml) was added to the mixture. The mixture was separated by separation funnel and the organic layer was dried with anhydrous Na_2SO_4. The organic solvent was evaporated and yellow crystal obtained. The crude product was dissolved in minimum ethanol and deposited by adding water. Yield, 87.2%; m.p. 189–191°C. IR (υ/cm^{-1}): 3278, 1687.

Data for **5b:** yield, 92.3%; m.p. 139–141°C. IR (υ/cm^{-1}): 3278, 1680. Data for **5c:** yield, 89.8%; m.p. 172–174°C. IR (υ/cm^{-1}): 3351, 1705. Data for **5d:** yield, 93.4%; m.p. 102–104°C. IR (υ/cm^{-1}): 3356, 1702.

*Synthesis of 1-(4-nitro-phenylcarbamoyl)-methyl triphenylphosphonium chloride (**6a**)*

A mixture of N-(4-nitro-phenyl)-2-chloroacetamide (0.91 g, 4.2 mmol) and triphenyl phosphate (1.15 g, 4.4 mmol) was heated to 100°C for 11 h. To the mixture was added ethyl acetate (20 ml). The mixture gave 1.85 g yellow solid that was separated by filtration. The crude product was recrystallized with ethanol.

Yield, 91.5%; IR (υ/cm^{-1}): 3422, 1690, 1617, 1596, 1563, 1509, 1334, 1111, 855, 750, 719, 689, 513; ^{1}H-NMR δ: 12.63(1H, s, NH), 8.08 ~ 8.09 (2H, d, ArH), 7.78 ~ 7.87 (11H, m, ArH), 7.65 ~ 7.68 (6H, m, ArH), 5.26 (2H, d, CH_2); ^{13}C-NMR δ: 162.33, 162.30, 144.35, 143.92, 135.55, 134.41, 134.33, 130.63, 130.55, 124.84, 120.05, 118.62, 118.03, 34.07, 33.70; ^{31}P-NMR δ: 21.173; MS (ESI) *m*/*z*: 441.20 (M^+-Cl).

Data for ***6b***

Yield, 57.8%; IR (υ/cm^{-1}): 3433, 1687, 1619, 1595, 1565, 1509, 1437, 1337, 1111, 863, 752, 724, 698; ^{1}H-NMR δ: 12.82 (1H, s, -NH-), 8.06 ~ 8.08 (2H, d, ArH), 7.94 ~ 7.97 (8H, m, ArH), 7.80 ~ 7.83 (3H, t, ArH), 7.69 ~ 7.72 (6H, m, ArH), 6.48 (1H, t, CH), 1.82 (3H, q, CH_3); ^{13}C-NMR δ: 167.34, 167.32, 144.15, 143.62, 135.28, 135.19, 134.42, 134.32, 134.25, 130.45, 130.36, 130.27,124.64, 124.53, 119.98, 119.88, 117.68, 117.11,38.19, 38.13, 37.79, 15.12 ; ^{31}P-NMRδ: 26.443; MS (ESI) *m*/*z*: 455.20 (M^+-Cl).

Data for ***6c***

Yield, 91.5%; IR (υ/cm^{-1}): 3421, 1701, 1614, 1595, 1563, 1499, 1333, 1111, 856, 743, 724, 691; ^{1}H-NMR δ: 12.04 (1H, s, NH), 8.09 ~ 8.11 (2H, d, ArH), 8.00 ~ 8.01 (2H, d, ArH), 7.69 ~ 7.82 (15H, m, ArH), 3.82 (3H, m, CH_2), 3.25 (2H, m, CH_2); ^{13}C-NMR δ: 169.05, 168.97, 145.23, 143.07, 135.63, 135.61, 133.87, 133.80, 130.86, 130.77, 124.65, 119.67, 117.86, 117.28, 30.18, 20.13, 19.78; ^{31}P-NMR δ: 23.197; MS (ESI) *m*/*z*: 455.24 (M^+-Cl).

Data for ***6d***

Yield, 39.8%; IR (υ/cm^{-1}): 3409, 1683, 1612, 1595, 1555, 1507, 1438, 1336, 1112, 856, 744, 723, 690; ^{1}H-NMR δ: 11.72 (1H, s, NH), 8.10 ~ 8.14 (4H, q, ArH), 7.82 ~ 7.83 (3H, q, ArH), 7.72 ~ 7.75 (12H, m, ArH), 3.59 (2H, s, CH_2), 3.09 (2H, s, CH_2), 2.08 (3H, d, CH_2); ^{13}C-NMR δ: 171.53, 145.19, 142.39, 135.10, 133.23, 133.16, 130.44, 130.36, 124.28, 119.19, 117.81, 117.23 36.74, 36.62, 21.92, 18.80; ^{31}P-NMR δ: 22.608; MS (ESI) *m*/*z*: 469.22(M^+-Cl).

Biology

Cell growth inhibition assays

Four human cancer cell lines, HCT-8, A549, Bel-7402, and S180 were used in this study. The cells were added to 96-well plates in 100 μL of growth medium. After treatment with the drug and supplementation with 10% fetal calf serum, the cells

were cultured at 37°C in an atmosphere of 5% CO_2. Then, 12 μL of 5 mg/ml MTT was added to each well. After incubation at 37°C for four hours, the supernatant was removed, and 100 μL DMSO was added to dissolve the water-insoluble phosphonium salt. The absorbancies at OD570 nm was measured with an ELISA micro-plate reader (Bio-Rad, Hercules, CA, USA). Drug resistance was represented as the percentage of viable cells suspension culture after exposure to idarubicin, compared to the untreated control group.

Surface-enhanced Raman spectroscopy (SERS)

The sample was added to a capillary vessel with an inner diameter of 1 mm. An Ar^+ laser with an output of 514.5 nm was used as a light source. The Raman spectrum was collected by the scattering disposition at 90°. SERS of DNA and phosphonium compounds were carried out separately. SERS of a mixture of DNA and phosphonium compounds was tested in 30 min.

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