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Synthesis and biological evaluation of novel naphthoquinone fused cyclic aminoalkylphosphonates and aminoalkylphosphonic monoester

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Summary. A series of novel naphthoquinone fused cyclic α-aminophosphonates, 2-alkoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **3**–**17** and naphthoquinone fused cyclic α-aminophosphonic monoester **18** were synthesized for the first time. These cyclic α-aminophosphonates were evaluated for antitumor activity on four human tumor cell lines, and three of them showed significant cytotoxicity (IC_{50}: 0.019–5.15 μM) comparable to that of the reference drug doxorubicin. Furthermore, inhibition assays for topoisomerase II-mediated relaxation of supercoiled DNA indicated that the naphthoquinone fused cyclic aminophosphonates were catalytic inhibitors of topoisomerase II.

Keywords: Amino acid – Aminoalkylphosphonic acid – Topoisomerase II inhibitors – Mannich-type reaction – Phosphorus heterocycles

Introduction

It is well known that α-aminophosphonic acids are an important class of compounds that have received much attention due to their potential biological activities as well as their ability to mimic their carboxylic counterparts (Kafarski and Lejczak, 2000). The replacement of carboxylic group by phosphonic acid moiety has a number of important consequences deriving from their differences in shape (tetrahedral phosphonic versus flat carboxylic one), acidity (with phosphonic acid being significantly more acidic) and steric bulk (phosphorus atom has significantly bigger radius than carbon atom).

Heterocyclic quinone compounds represent an important class of natural (Thompson, 1997) and synthetic drugs (Gutierrez, 1989; Powis, 1989) for the treatment of human cancer. In recent years, combination chemotherapy employing antineoplastic drugs with different functional mechanisms is one of the methods that is being adopted to cure cancer. As a consequence, a great number of pharmaceuticals and biologically active quinone derivatives bearing amino carboxylic acids, amino sulphonic acids or peptides, have been synthesized (Bade Shrestha-Dawadi et al., 1996; Bittner et al., 2000; Bittner and Alnabari, 2001; Gorohovsky and Bittner, 2001; Bittner et al., 2002; Gorohovsky et al., 2003). It is worth mentioning that the attachment of several free or blocked amino acids to quinone moeties are considerably important factors to affect their redox properties and cytotoxic potential. Surprisingly, the quinone fused cyclic α-aminophosphonic acids and their derivatives were not explored in the literature. The therapeutic potential for modified quinones with improved pharmacokinetic properties, potency or spectrum, and lower side effects, prompted us to start a synthetic program to explore new quinone-aminoalkylphosphonate or quinone-aminoalkylphosphonic acid derivatives. In this paper we would like to present the synthesis and biological evaluation of the naphthoquinone fused cyclic α-aminophosphonates, 2-alkoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxides **3**–**17**, and their quninone-aminoalkyphosphonic monoester **18**.

Materials and methods

Instruments and materials

All melting points were determined on a Yanaco apparatus and uncorrected. NMR spectra were measured on a Bruker AVANCE 300 NMR instrument in $CDCl_3$ and chemical shifts are expressed as δ. Coupling constants *J* are given in Hz. Tetramethylsilane was used as an internal standard for

^{1}H NMR, and 85% H_3PO_4 as an external standard for ^{31}P NMR spectroscopy. MS were recorded on a Polaris-Q instrument of Thermofinnigan. Elemental analysis was carried out on a Yanaco CHNCORDER MT-3 Analyzer. X-Ray analysis was done on a Bruker SMART 1000 CCD diffractometer with MoKα radiation (λ = 0.71073 Å). Column chromatograghy was performed using silica gel H (10–40 μm, Haiyang chemical Factory of Qingdao). THF was dried with sodium and redistilled. Alkyl phosphorodichloridites were prepared according to the document (Martin and Pizzolato, 1950).

Synthesis

General procedure for synthesis of 2-alkoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxides **3**–**17**

2-Amino-3-hydroxy-1,4-naphthoquinone (0.5 g, 2.6 mmol) and alkyl phosphorodichloridite (2.6 mmol) were dissolved in anhydrous THF (25 mL) with stirring at 0 °C. After 15 min, the ketone (2.6 mmol) was added. The reaction mixture was allowed to warm to room temperature and was continuously stirred for 24 h. The resulting mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel and eluted with EtOAc/hexane (1:1, v/v) to afford the analytically pure products.

2-Methoxy-3,3-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **3**

Orange solid, yield 95%; mp 182–184 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.57 (d, 3H, $^3J_{PH}$ = 11.9 Hz, CH_3), 1.62 (d, 3H, $^3J_{PH}$ = 11.9 Hz, CH_3), 3.93 (d, 3H, $^3J_{PH}$ = 11.3 Hz, OCH_3), 5.30–5.38 (br, 1H, NH), 7.65–8.15 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.67. Anal. Calcd. for $C_{14}H_{14}NO_5P$: C, 54.73; H, 4.59; N, 4.56. Found: C, 54.98; H, 4.61; N, 4.80. MS *m*/*z* 307 (M^+).

3,3-Diethyl-2-methoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **4**

Orange solid, yield 79%; mp 215–217 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.12 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.75–2.18 (m, 4H, $2CH_2CH_3$), 3.92 (d, 3H, $^3J_{PH}$ = 11.3 Hz, OCH_3), 5.29–5.36 (br, 1H, NH), 7.62–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.09. Anal. Calcd. for $C_{16}H_{18}NO_5P$: C, 57.31; H, 5.41; N, 4.18. Found: C, 57.41; H, 5.61; N, 4.29. MS *m*/*z* 335 (M^+).

2-Methoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclopentane}2-oxide **5**

Orange solid, yield 75%; mp 205–207 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26–1.88 (m, 8H, $4CH_2$), 3.91 (d, 3H, $^3J_{PH}$ = 10.7 Hz, OCH_3), 5.41–5.48 (br, 1H, NH), 7.64–8.15 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.33. Anal. Calcd. for $C_{16}H_{16}NO_5P$: C, 57.66; H, 4.84; N, 4.20. Found: C, 57.49; H, 4.80; N, 4.21. MS *m*/*z* 333 (M^+).

2-Methoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclohexane}2-oxide **6**

Orange solid, yield 76%; mp 218–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.79–1.79 (m, 10H, $5CH_2$), 3.84 (d, 3H, $^3J_{PH}$ = 12.8 Hz, OCH_3), 5.53–5.61 (br, 1H, NH), 7.55–8.08 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 13.59. Anal. Calcd. for $C_{17}H_{18}NO_5P$: C, 58.79; H, 5.22; N, 4.03. Found: C, 58.79; H, 5.20; N, 4.01. MS *m*/*z* 347 (M^+).

2-Methoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cycloheptane}2-oxide **7**

Orange solid, yield 65%; mp 187–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26–1.92 (m, 12H, $6CH_2$), 3.91 (d, 3H, $^3J_{PH}$ = 11.3 Hz, OCH_3), 5.46–5.53 (br, 1H, NH), 7.61–8.12 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.93. Anal. Calcd. for $C_{18}H_{20}NO_5P$: C, 59.83; H, 5.58; N, 3.88. Found: C, 59.91; H, 5.60; N, 3.78. MS *m*/*z* 361 (M^+).

2-Ethoxy-3,3-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **8**

Orange solid, yield 78%; mp 213–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (t, 3H, *J* = 7.72 Hz, OCH_2CH_3), 1.56 (d, 3H, $^3J_{PH}$ = 11.1 Hz, CH_3), 1.61 (d, 3H, $^3J_{PH}$ = 11.6 Hz, CH_3), 4.30–4.38 (m, 2H, OCH_2CH_3), 5.25–5.34 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 13.40. Anal. Calcd. for $C_{15}H_{16}NO_5P$: C, 56.08; H, 5.02; N, 4.36. Found: C, 56.00; H, 5.00; N, 4.28. MS *m*/*z* 321 (M^+).

2-Ethoxy-3,3-diethyl-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **9**

Orange solid, yield 80%; mp 245–247 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.01 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.12 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.38 (t, 3H, *J* = 7.70 Hz, OCH_2CH_3), 1.75–2.18 (m, 4H, $2CH_2CH_3$), 4.10–4.23 (m, 2H, OCH_2CH_3), 5.29–5.36 (br, 1H, NH), 7.62–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 13.36. Anal. Calcd. for $C_{17}H_{20}NO_5P$: C, 58.45; H, 5.77; N, 4.01. Found: C, 58.55; H, 5.79; N, 3.97. MS *m*/*z* 349 (M^+).

2-Ethoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclopentane}2-oxide **10**

Orange solid, yield 78%; mp 239–241 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.35 (t, 3H, *J* = 7.12 Hz, OCH_2CH_3), 1.67–2.10 (m, 8H, $4CH_2$), 4.31–4.36 (m, 2H, OCH_2CH_3), 5.62–5.69 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 15.71 Anal. Calcd. for $C_{17}H_{18}NO_5P$: C, 58.79; H, 5.22; N, 4.03. Found: C, 58.83; H, 5.25; N, 3.95. MS *m*/*z* 347 (M^+).

2-Ethoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclohexane}2-oxide **11**

Orange solid, yield 81%; mp 247–249 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.35 (t, 3H, *J* = 6.97 Hz, OCH_2CH_3), 1.45–2.11 (m, 10H, $5CH_2$), 4.28–4.36 (m, 2H, OCH_2CH_3), 5.61–5.68 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 12.35 Anal. Calcd. for $C_{18}H_{20}NO_5P$: C, 59.83; H, 5.58; N, 3.88. Found: C, 59.85; H, 5.60; N, 3.90. MS *m*/*z* 361 (M^+).

2-Ethoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cycloheptane}2-oxide **12**

Orange solid, yield 72%; mp 244–246 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (t, 3H, *J* = 6.97 Hz, OCH_2CH_3), 1.45–2.13 (m, 12H, $6CH_2$), 4.28–4.36 (m, 2H, OCH_2CH_3), 5.61–5.69 (br, 1H, NH), 7.64–8.15 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 13.12 Anal. Calcd. for $C_{19}H_{22}NO_5P$: C, 60.80; H, 5.91; N, 3.73. Found: C, 60.85; H, 5.79; N, 3.74. MS *m*/*z* 375 (M^+).

3,3-Dimethyl-2-propoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **13**

Orange solid, yield 71%; mp 213–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.15 (t, 3H, *J* = 7.72 Hz, $O(CH_2)_2CH_3$), 1.37–1.51 (m, 2H, $OCH_2CH_2CH_3$), 1.56 (d, 3H, $^3J_{PH}$ = 11.1 Hz, CH_3), 1.61 (d, 3H, $^3J_{PH}$ = 11.6 Hz, CH_3), 4.30–4.38 (m, 2H, $OCH_2CH_2CH_3$), 5.25–5.34 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.10. Anal. Calcd. for $C_{16}H_{18}NO_5P$: C, 57.31; H, 5.41; N, 4.18. Found: C, 57.38; H, 5.52; N, 4.20. MS *m*/*z* 335 (M^+).

3,3-Diethyl-2-propoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2] oxazaphosphinane-5,10-dione 2-oxide **14**

Orange solid, yield 60%; mp 267–269 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.01 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.12 (t, 3H, *J* = 7.35 Hz, CH_2CH_3), 1.38 (t, 3H, *J* = 7.70 Hz, $OCH_2CH_2CH_3$), 1.43–1.54 (m, 2H, $OCH_2CH_2CH_3$), 1.75–2.18 (m, 4H, $2CH_2CH_3$), 4.10–4.23 (m, 2H, $OCH_2CH_2CH_3$), 5.29–5.36 (br, 1H, NH), 7.62–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 14.14. Anal. Calcd. for $C_{18}H_{22}NO_5P$: C, 59.50; H, 6.10; N, 3.85. Found: C, 59.37; H, 6.15; N, 3.95. MS *m*/*z* 363 (M^+).

2-Propoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclopentane}2-oxide **15**

Orange solid, yield 67%; mp 276–278 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, *J* = 7.12 Hz, $O(CH_2)_2CH_3$), 1.43–1.55 (m, 2H, $OCH_2CH_2CH_3$), 1.67–2.11 (m, 8H, $4CH_2$), 4.31–4.36 (m, 2H, $OCH_2CH_2CH_3$), 5.62–5.69 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 15.71 Anal. Calcd. for $C_{18}H_{20}NO_5P$: C, 59.83; H, 5.58; N, 3.88. Found: C, 59.92; H, 5.62; N, 3.78. MS *m*/*z* 361 (M^+).

2-Propoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cyclohexane}2-oxide **16**

Orange solid, yield 62%; mp 245–247 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, 3H, *J* = 7.12 Hz, $O(CH_2)_2CH_3$), 1.43–1.54 (m, 2H, $OCH_2CH_2CH_3$), 1.67–2.10 (m, 10H, $5CH_2$), 4.31–4.36 (m, 2H, $OCH_2CH_2CH_3$), 5.62–5.69 (br, 1H, NH), 7.64–8.14 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 15.71 Anal. Calcd. for $C_{19}H_{22}NO_5P$: C, 60.80; H, 5.91; N, 3.73. Found: C, 60.92; H, 5.73; N, 3.78. MS *m*/*z* 375 (M^+).

2-Propoxy-4*H*-spiro{naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione,1′-cycloheptane}2-oxide **17**

Orange solid, yield 68%; mp 261–263 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (t, 3H, *J* = 7.12 Hz, $O(CH_2)_2CH_3$), 1.43–1.50 (m, 2H, $OCH_2CH_2CH_3$), 1.67–2.12 (m, 12H, $6CH_2$), 4.31–4.36 (m, 2H, $OCH_2CH_2CH_3$), 5.64–5.70 (br, 1H, NH), 7.64–8.12 (m, 4H, C_6H_4). ^{31}P NMR ($CDCl_3$): δ = 15.71 Anal. Calcd. for $C_{20}H_{24}NO_5P$: C, 61.69; H, 6.21; N, 3.60. Found: C, 61.72; H, 6.21; N, 3.78. MS *m*/*z* 389 (M^+).

Synthesis of naphthoquinone fused cyclic aminoalkylphosphonic monoester **18**

2-Ethoxy-3,3-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **8** (0.5 g, 1.56 mmol) was dissolved in anhydrous $CHCl_3$ (15 mL) with stirring at 0 °C. Trimethylsilyl bromide (5 mL) was added dropwise over 30 min to above solution. The reaction mixture was allowed to warm to room temperature and was continuously stirred for 24 h. The resulting mixture was concentrated in vacuo. The residue was dissolved in methanol (5 mL) and stored at low temperature for one day until a red solid precipitated. The solid was filtered and recrystallized from water-ethanol (1:1) to afford orange crystal (0.35 g).

3,3-Dimethyl-2-hydroxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2] oxazaphosphinane-5,10-dione 2-oxide **18**

Orange crystal, yield 76%; mp 278–280 °C. ^{1}H NMR (300 MHz, *d*-DMSO): δ = 1.40 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 4.35–4.53 (br, 1H, NH), 7.71–8.01 (m, 4H, C_6H_4). ^{31}P NMR (*d*-DMSO): δ = 11.56. Anal. Calcd. for $C_{13}H_{12}NO_5P$: C, 53.25; H, 4.13; N, 4.78. Found: C, 53.20; H, 4.20; N, 4.81. MS *m*/*z* 295 (M^+).

Cytotoxicity studies

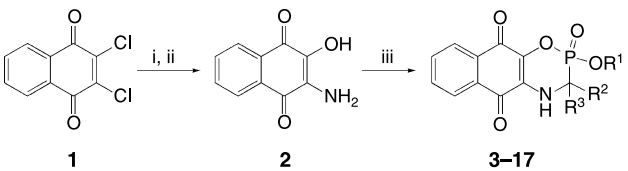
Compounds **3**–**18** dissolved in dimethyl sulfoxide (DMSO) were subjected to cytotoxic evaluation against A549 (human lung carcinoma), HeLa (human cervical carcinoma), HEp2 (human laryngeal carcinoma) and LoVo (human colon carcinoma) cell lines employing the colorimetric method (Carmichael et al., 1987). Doxorubicin was used as the reference drug. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was dissolved in saline to make a concentration of 5 mg/mL as a stock solution. Cancer cells (3×10^3 cells) suspended in 100 μg/well of MEM medium containing 10% fetal calf serum were seeded onto a 96-well culture plate. After 24 h of preincubation at 37 °C in a humidified atmosphere of 5% CO_2/95% air to allow cell attachment, various concentrations of test solution were added and then incubated for 72 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added to each well and then incubated at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected by a Microplate reader (Bio-Rad, Benchmark Microplate reader) at 570 nm. Each determination represents the mean of four replicates. The 50% inhibition concentration (IC_{50}) was determined by curve fitting.

Topoisomerase II assays

The topoisomerase relaxation reactions were carried out with 0.2 μg of supercoiled pBR322 DNA and 2 units of topoisomerase II in 20 *u*L reaction system (50 mM Tris–HCl, Ph 8.0, containing 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, and 0.5 mM dithioreitol, 30 μg/mL nuclease free BSA). Doxorubicin and variable amounts of compound 3 were added to some reactions. The assay incubation was carried out at 37 °C for 30 min and was terminated by the addition of 3 μL of solution (30 mM EDTA, 36% glycerol, 0.05% xylene cyanol FF, 0.05% bromophenol blue). Samples were loaded on 0.8% agarose gel and run at 50 V for 3 h. Then the gel was stained with ethidium bromide for 40 min. Results were recorded by UV transilluminator.

Results and discussion

Our approach was based on the three-component Mannich-type condensation comprising amines, phosphorus and ketone. This reaction, introduced by Moedritzer and Irani, was a very useful procedure for the preparation of the linear N-substituted aminomethanephosphonic acids



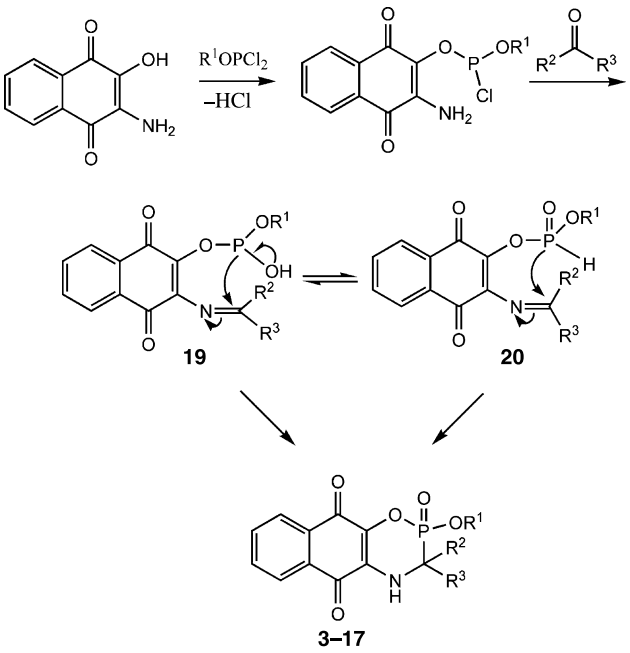
1 **2** **3–17**

	R^1	R^2	R^3		R^1	R^2	R^3
3	Me	Me	Me	**11**	Et	$(CH_2)_5$	
4	Me	Et	Et	**12**	Et	$(CH_2)_6$	
5	Me	$(CH_2)_4$		**13**	n-Pr	Me	Me
6	Me	$(CH_2)_5$		**14**	n-Pr	Et	Et
7	Me	$(CH_2)_6$		**15**	n-Pr	$(CH_2)_4$	
8	Et	Me	Me	**16**	n-Pr	$(CH_2)_5$	
9	Et	Et	Et	**17**	n-Pr	$(CH_2)_6$	
10	Et	$(CH_2)_4$					

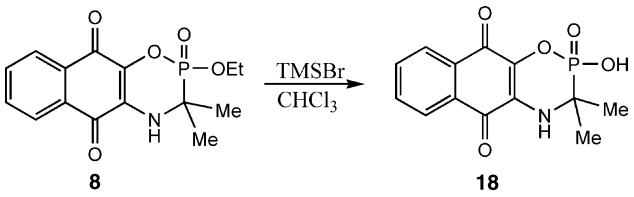
Scheme 1. Reagents and conditions: (i) $NaNO_2$, HCl, CH_3OH, H_2O, 80 °C, 3 h; (ii) $Na_2S_2O_4$, CH_3CH_2OH, H_2O, rt, 30 min, 78% for two steps; (iii) R^1OPCl_2, $R^2(CO)R^3$, THF, 0 °C to rt, 24 h

(Moedritzer and Irani, 1966). Very recently, this methodology was successfully applied to the synthesis of cyclic α-aminoalkanephosphonates. (Zhou et al., 1999; Wang et al., 2006, 2007). As shown in Scheme 1, the intermediate 2-amino-3-hydroxy-1,4-naphthoquinone **2** was synthesized from 2,3-dichloro-1,4-naphthoquinone **1** in 78% yield according to the published procedure (Podrebarac and Cheng, 1970). The naphthoquinone fused cyclic α-aminoalkanephosphonates **3**–**17** could be easily obtained via a three-component reaction of R^1OPCl_2, intermediate **2**, and ketones.

A possible mechanism for this ring-closure reaction is presented in Scheme 2. Reaction of 2-amino-3-hydroxy-1,4-naphthoquinone with alkyl phosphorodichloridite and ketones would lead to the formation of two intermediates **19** and **20**, which could further undergo a ring-closure to provide the titled products via the Mannich-type cycliza-



Scheme 2. Possible mechanism for the synthesis of the naphthoquinone fused cyclic α-aminophosphonates



Scheme 3. Synthesis of the naphthoquinone fused aminoalkylphosphonic monoester **18**

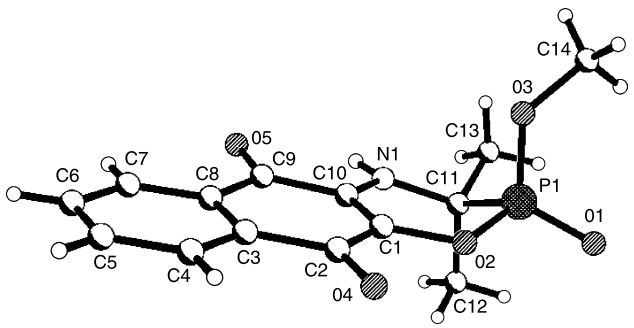


Fig. 1. The X-ray structure of compound **3**

Table 1. Cytotoxic activity of compounds **3**–**17**

Compound	Substituents			Cancer cell lines (IC_{50}, μM)			
	R^1	R^2	R^3	A549	HeLa	HEp2	LoVo
3	Me	Me	Me	0.64 ± 0.03	0.18 ± 0.06	0.95 ± 0.13	0.019 ± 0.004
4	Me	Et	Et	5.15 ± 0.52	2.79 ± 0.31	1.88 ± 0.25	0.54 ± 0.07
5	Me	$(CH_2)_4$		14.87 ± 4.19	67.27 ± 5.12	36.37 ± 3.33	>108
6	Me	$(CH_2)_5$		inactive	>150	49.86 ± 7.02	67.32 ± 5.31
7	Me	$(CH_2)_6$		>130	66.10	81.27 ± 11.9	inactive
8	Et	Me	Me	2.61 ± 0.21	0.64 ± 0.29	5.12 ± 0.00	0.81 ± 0.08
9	Et	Et	Et	11.89 ± 0.17	48.19 ± 3.21	16.56 ± 1.11	52.01 ± 0.39
10	Et		$(CH_2)_4$	74.83 ± 4.02	77.47 ± 6.37	>137	86.37 ± 3.13
11	Et		$(CH_2)_5$	inactive	>100	49.86	77.32 ± 8.21
12	Et		$(CH_2)_6$	>343	96.10 ± 8.78	>410	inactive
13	n-Pr	Me	Me	8.41 ± 1.12	16.10 ± 0.69	17.45 ± 2.54	12.62 ± 1.17
14	n-Pr	Et	Et	50.69 ± 5.02	73.12 ± 6.10	33.38 ± 1.97	49.02 ± 4.17
15	n-Pr		$(CH_2)_4$	80.03 ± 6.79	92.54 ± 6.80	>440	inactive
16	n-Pr		$(CH_2)_5$	>502	>346	inactive	inactive
17	n-Pr		$(CH_2)_6$	inactive	inactive	inactive	>100
doxorubicin				0.020	0.834	1.002	0.011

tion. The naphthoquinone fused aminoalkylphosphonic monoester **18** can also be prepared from compound **8** via a facile method, as depicted in Scheme 3. The structures of **3**–**18** were confirmed by ^{1}H NMR, ^{31}P NMR, MS and microanalysis. Compound **3** was recrystallized from ether and hexane (2:1). An orange single crystal with approximate dimensions of 0.12 mm × 0.08 mm × 0.06 mm was selected for X-ray crystallographic analysis (Fig. 1).

The naphthoquinone fused cyclic α-aminophosphonates were examined for antiproliferative activity against A549 (human lung carcinoma), HeLa (human cervical carcinoma), HEp2 (human laryngeal carcinoma) and LoVo (human colon carcinoma) employing the MTT colorimetric method (Carmichael et al., 1987). As listed in Table 1, the results are expressed as IC_{50} values, that is, as the micromolar concentration of a compound that achieves 50% cellular growth reduction after 72 h of drug exposure. For comparison purposes, the activities of doxorubicin are also included. From the cytotoxicity data presented in Table 1, several features are evident from the investigation of the side chains. First, a comparison of various alkoxy groups (R^1) at 2 position suggests that the methoxy-substituted naphthoquinone fused cyclic α-aminophosphonates such as compounds **3**–**7** demonstrate potent cytotoxicity relative to that of ethoxy-substituted analogues **8**–**12** and propoxy-substituted analogues **13**–**17**. Compound **3** with 2-methoxyl group displayed a very potent cytotoxic activity on four cell lines, with IC_{50} values of 0.64, 0.18, 0.95 and 0.019 μM, respectively, similar to that of doxorubicin, but ethoxy-substituted analogue **8** (median = 3.87 μM) and propoxy-substituted analogue **13** (median = 14.4 μM) were less potent than compound **3** (median = 0.41 μM).

Moreover, the 3,3-dimethyl substituted naphthoquinone fused cyclic α-aminophosphonate **3** was more potent than the 3,3-diethyl analogue **4**. For the ethoxy- and propoxy-substituted heterocycles (**8**, **9**, **13** and **14**), a similar pattern was observed, which 3,3-dimethyl substituted compounds were more potent than their 3,3-diethyl analogues. The results also reveal that compounds (**5**, **10** and **15**) containing a spiro-ring at C-3 of phosphorus heterocycle demonstrate poor cytotoxicity relative to that of compounds (**4**, **9** and **14**) with linear alkyl substituents of equal carbons at the position. In general, it is clear that cytotoxicity is greatly improved with the utilization of shorter side chains. We then prepared the phosphonic derivative of compound **3** (Scheme 3) based on the generalization that "a shorter chain length is better" for cytotoxicity. Unfortunately, the obtained naphthoquinone fused aminoalkylphosphonic monoester **18** does not show any cytotoxic activity on four cell lines. Presumably, the hydrophobicity from the methoxyl group in the heterocycle structure could be responsible for cytotoxicity of **18**, and replacement of the OCH_3 with a hydroxyl group will sharply reduce its hydrophobicity.

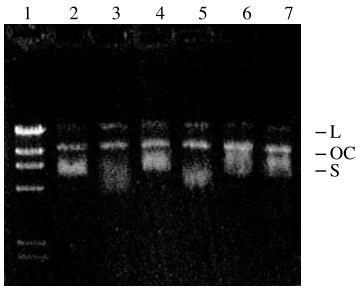


Fig. 2. Effects of compound **3** and doxorubicin on the DNA relaxing activity of human topoisomerase II. Supercoiled plasmid pBR322 was incubated with purified human topo II in the presence or absence of the tested agents: (lane 1) λ-*Hin*dIII digest, DNA marker; (lane 2) no enzyme control; (lane 3) enzyme control; (lane 4) 5 μM of doxorubicin; (lane 5–7) 5, 25, 80 μM of compound **3**. S: supercoiled form; OC: open circular form; L: linear form

Topoisomerases are nature's solution to the topological problems associated with manipulating double-stranded, helical DNA during essential cellular processes. The main classification of topoisomerases is dependent upon the enzyme's ability to create a single-stranded (type I) or double-stranded (type II) nick in DNA to alleviate torsional strain(Wang, 1996, 2002; Nitiss, 1998; Champoux, 2001). Several quinone-containing antitumor drugs, such as doxorubicin (Tewey et al., 1984) and anthracenedione (Burckhardt et al., 1988) are topoisomerase II inhibitors. For studying one possible mechanism of the cytotoxic activity of 2-alkoxy-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2] oxazaphosphinane-5,10-dione 2-oxides, we evaluated the topoisomerase II inhibitory activity of the most active compound **3** by electrophoresis method (Sullivan et al., 1986). As shown in Fig. 2, compound **3** displayed topoisomerase II catalytic activity in a dose-dependent manner (25–80 μM), while it demonstrated poor topoisomerase II inhibition relative to doxorubicin at low concentration (5 μM).

Conclusion

We have developed a convenient method for preparing naphthquinone fused cyclic α-aminophosphonates and aminoalkylphosphonic monoester. The structure of compound **3** has been confirmed by X-ray diffraction analysis. The cytotoxicities of the prepared compounds were evaluated by using a MTT assay versus doxorubicin. Compounds **3**, **4**, and **8** proved to be potent cytotoxic

agents against all tumor cell lines. In particular, the cytotoxic activity of 2-methoxy-3,3-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*e*][1,4,2]oxazaphosphinane-5,10-dione 2-oxide **3** against human cervical carcinoma (HeLa) was 4–5 times higher than that of doxorubicin. It was found that compound **3** inhibited topoisomerase II, which suggested a possible cytotoxicity mechanism.

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