Synthesis and cytotoxicity of pyrrole-amino acid dipeptides containing phosphonyl group

Ruo-yu Liu[a], Yong Ye[a, b]*, Jun-liang Yang[a], Cun-jiang Liu[a] and Yu-Fen Zhao[a, b]*

[a]*Key Laboratory of Chemical Biology and Organic Chemistry, Department of Chemistry, Zhengzhou University, Zhengzhou 450052, P.R. China*

[b]*Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, P.R. China*

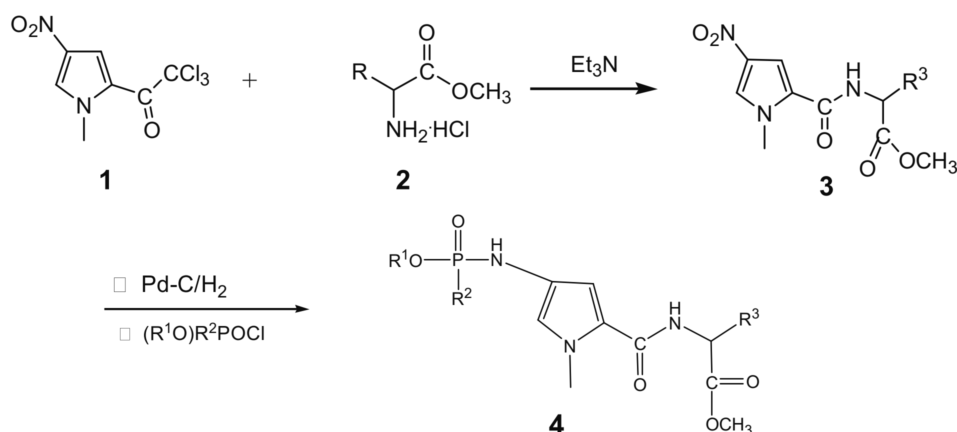
A series of pyrrole-amino acid dipeptide were synthesised by chloroform reaction and coupling reaction using DCC/HOBT as promoting additives. The structure of compound was confirmed by ^{1}H NMR, ^{31}P NMR, MS and IR. The biological activity data showed that the dipeptide containing nitrogen mustard group inhibited all four cells that were examined.

Keywords: pyrrole-amino acid dipeptide, nitrogen mustard, biological activity

The main objective of covalently linking a conventional antitumour agent to a macromolecular carrier is to improve the therapeutic index of the parent drug. A site-specific drug release can be controlled by selecting a proper drug–polymer linkage. During circulation in the bloodstream the linkage should be stable, but in or near the target cells, the spacer should be degraded, resulting in the release of the antitumour agent. Recently, the natural products distamycin, netropsin and their analogues have attracted considerable attention on the part of synthetic and biological chemists because they recognise and bind in the minor groove of predetermined DNA sequences with high affinity and specificity.[1-3] Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression.[4] Therefore, these polyamides are one of the most widely studied class of agents characterised by their high level of sequence specificity and they are still an interesting class of DNA ligands which demonstrate a wide spectrum of biological activity.[5-7]

Our group has found that some phosphonyl amino acids had good biological activity.[8] In order to obtain high biological activity compounds, a series of polyamides containing phosphonyl amino acid methyl esters were synthesised. The synthetic route is shown in Scheme 1. Compounds **1** and **2** were synthesised according to refs 9 and 10, and 11 and 12 respectively. The structures of title compounds were confirmed by ^{1}H NMR, ^{31}P NMR, MS, IR and HRMS. In the synthesis of compound **3,** there was no need for protecting and deprotecting the amino group. The coupling of the dipolypyrrole to **1** was accomplished in MeOH at room temperature using DCC/HOBT as promoting additives. **3** was obtained in 83–87%. In this step, the qualitative reduction of the nitro group of **1** to an amino group is important. To achieve the optimum result, TLC was employed to monitor the progress of the hydrogenation. In an attempt to achieve a better coupling efficiency, excess DCC was needed. Although the hydrogenation proceeded almost quantitatively, the amino products were rather unstable. After the reduction of the nitro group of **3**, title compound **4** can be readily obtained by coupling the amino products of **3** to the phosphonyl chloride. Similar to the synthesis of **3**, TLC was needed to monitor the progress of the hydrogenation in the synthesis of **4**.

The cytotoxicity was assessed by the MTT assay.[13] Human cancer cells, cultured in a RPMI-1604 medium supplemented with 10% FBS, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin at 37 °C in a humidified air atmosphere of 5% CO_2, were plated into a 96-well plate (1×10^4 cells/well). The next day, the compounds, diluted in culture medium were added (200μl/well) to the wells. 48 hours later 20 μl MTT (0.5 mg/ml MTT in PBS) was added and the cells were incubated for a further 4 h. DMSO (0.2 ml) was added to each well to dissolve the reduced MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by



Scheme 1 Synthetic route of pyrrole-amino acid dipeptides.

* Correspondent. E-mail: yeyong03@tsinghua.org.cn

Table 1 The *in vitro* inhibition ratios of compounds **4** against four cancer cells

Compds	Inhibition ratio/%			
	KB	HCT-8	Bel-7402	A549
4a	10.33	8.86	–	6.89
4b	14.77	–	1.22	–3.22
4c	18.25	10.66	20.45	5.76

KB: human oral epitheliocellalar carcinoma; Bel-7402: human hepatocellalar carcinoma; HCT-8: human colocellalar carcinoma. A549: Human lung carcinoma.

measuring absorbance at 570 nm with a micro plate reader. As demonstrated by MTT cytotoxicity assay (Table 1), the cytotoxicity of compound **4c** was higher than that of **4a** and **4b**. Compound **4c** inhibited all four cells that were tested. The cytotoxicity of **4d–f** also was checked, but they had lower biolog activity.

In conclusion, a synthesis of pyrrole-amino acid dipeptide was achieved via a coupling reaction with readily accessible materials. The significant feature of this procedure is that there was no need for protecting and deprotecting amino group. The reactions are convenient and efficient. The *in vitro* anti-tumor activities of these compounds were tested preliminarily on KB, Bel-7402, HCT-8, and A549 cells. The data show that **4c** inhibited all the four cells and had good activity.

Experimental

General

Melting points were determined using YRT-3 melting point apparatus and were uncorrected. Optical rotations were measured with Perkin–Elmer, model 341 Polarimeter at 20 °C in AcOEt or $CHCl_3$. ^1H NMR and ^{31}P NMR spectra were measured using a Bruker AC-P400 spectrometer with TMS and 85% H_3PO_4 as the internal and external reference respectively and with $CDCl_3$ as the solvent. IR spectra was recorded as KBr pellets on a Bruker spectrometer. Mass spectra was acquired in positive ion mode using a Bruker ESQUIRE-LCTM ion trap spectrometer equipped with a gas nebuliser probe, capable of analysing ions up to *m/z* 20000. Solvents were purified and dried by standard procedures.

Synthesis of (**3**)

To a solution of compound **2** (5.0 mmol) in CH_2Cl_2 20 ml was added Et_3N (0.8 ml). After being stirred for half an hour, and filtered, the active amino acid solution was obtained. Separately, a solution of compound 1 (4.8 mmol) in CH_2Cl_2 5 ml was dropped into the active amino acid solution, followed by stirring for 24 h. Then the mixture was concentrated *in vacuo*. Column chromatography of the residue (eluant $CHCl_3$:MeOH 40 : 1) provided a light yellow power of compound **3**.

3a: R^3 = H, yield:84%; m.p. 153–154 °C; ^1H NMR: 7.58 (d, 1H, J = 1.7 Hz), 7.18 (d, 1H, J = 1.9 Hz), 6.49 (br, 1H,) (–NH), 4.17 (d, 2H, J = 5.3 Hz) (–CH_2), 3.98 (S, 3H), 3.82 (S, 3H); IR υ/cm^{-1}: 3399, 3135, 2958, 1724, 1665, 1520, 1324, 1231, 755; ESI-MS: 263.3 $[M + Na]^+$; HRMS calcd for 241.0699 $C_9H_{11}N_3O_5$, found 241.0704.

3b: R^3 = CH_3, yield: 83%; m.p. 147–148 °C; $[\alpha]_D^{20}$ – 2.5 (c 0.008, $CHCl_3$); ^1H NMR:7.56 (d, 1H, J = 1.4 Hz), 7.16 (d, 1H, J = 1.7 Hz), 6.48 (d, 1H, J = 6.4 Hz) (–NH), 4.68 (m, 1H) (–CH), 3.98 (s, 3H), 3.80 (s, 3H), 1.50 (d, 3H, J = 7.1H) (–CH_3); IR υ/cm^{-1}: 3354, 3125, 2966, 1720, 1665, 1526, 1373, 1314, 1212, 750; ESI-MS: 255.8 $[M + H]^{+,}$ 277.8 $[M + Na]^+$; HRMS calcd for 255.0855 $C_{10}H_{13}N_3O_5$, found 255.0849.

3c: R^3 = ^iBu, yield:87%; m.p. 99–100 °C; $[\alpha]_D^{20}$ + 22.8 (c 0.0404, $CHCl_3$); ^1H NMR: 7.56 (d, 1H, J = 1.6 Hz), 7.16 (d, 1H, J = 1.8 Hz), 6.41 (d, 1H, J = 8.1 Hz) (–NH), 4.68 (m, 1H) (–CH), 3.96 (S, 3H), 3.78 (S, 3H), 1.97 (m, 1H), 1.48 (m, 1H), 1.26(m, 1H), 0.96(m, 6H, 2-CH_3); IR υ/cm^{-1}:3312, 3130, 2953, 1742, 1642, 1527, 1313, 1207, 754; ESI-MS: 298.6 $[M + H]^+$320.6 $[M + Na]^+$; HRMS calcd for 297.1325 $C_{13}H_{19}N_3O_5$, found 297.1331.

Synthesis of compound (**4**)

To a solution of compound **3** (0.31 mmol) in CH_2Cl_2 15 ml was added Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at room temperature for 18 h. The catalyst was removed by filtration through Celite and Et_3N (1 ml) was added to the filtrate. The solution was cooled to –10 °C and $(R^1O)R^2POCl$ 0.2 ml was added. The solution was stirred for 24 h at this temperature. After filtration and concentration *in vacuo*, a yellow oil was obtained. Purification by column chromatography ($CHCl_3$:CHOH = 20 : 1, compound **4** was obtained in oil.

4a: Yield: 50%; ^1H NMR: δ7.31(m, 2H) (ph-H), 7.18 (m, 3H) (ph-H), 6.84(t, 1H, J = 9.2 Hz), (–NHC) 6.54 (s, 1H), 6.50(s, 1H), 5.54 (d, 1H, J = 10.8 Hz)(–NHP), 4.08(d, 2H, J = 5.5 Hz) (–CH_2), 3.81(s, 3H), 3.75(s, 3H), 3.49 (m, 8H) (2-NCH_2, 2-CH_2Cl); ^{31}P NMR: δ7.93 ppm; IR: 3420, 2954, 1744, 1642 1580 1490 1204 923 770 cm^{-1}; ESI-MS: m/z 491.2 $[M + H]^+$, 513.2$[M + Na]^+$; HRMS calcd for 513.0837 $C_{19}H_{25}O_5NaPCl_2$, found 513.0834.

4b: Yield: 57%; $[\alpha]_D^{20}$ –1.5 (c 0.0438, $CHCl_3$); ^1H NMR: δ7.33(m, 2H) (ph-H), 7.19(m, 3H) (ph-H), 6.58(s, 1H), 6.48(d, 1H, J = 9.7 Hz) (–NHC), 6.46(s, 1H), 5.15 (d, 1H, J = 10.6 Hz)(–NHP), 4.66(m, 1H) (–CH), 3.83 (s, 3H), 3.76 (s, 3H), 3.50(m, 8H)(2-N–CH_2, 2-CH_2–Cl), 1.45(d, 3H, J = 7.1 Hz)(–CH_3); ^{31}P NMR: 7.74; IR: 3249, 2953, 1741, 1647, 1579, 1507, 1490, 1455, 1368, 1204, 923, 765 cm^{-1}; ESI-MS: 505.3 [M + H]; HRMS calcd for 527.0994 $C_{20}H_{27}N_4O_5NaPCl_2$ found 527.1000.

4c: Yield: 65%; $[\alpha]_D^{20}$ + 5.6 (c 0.0536, $CHCl_3$); ^1H NMR: δ7.31(m, 2H) (ph-H), 7.18 (m, 3H) (ph-H), 6.55 (s, 1H), 6.47 (s, 1H), 6.45 (t, 1H, J = 9.0 Hz) (–NHC), 5.38 (dd, 1H, J_1 = 10.7 Hz, J_2 = 29.3 Hz) (–NHP), 4.69 (m, 1H) (–CH), 3.81(s, 3H), 3.74 (s, 3H), 3.45(m, 8H)(2-N–CH_2, 2-CH_2–Cl), 1.67(m, 3H) (–CH–CH_2), 0.95 (d, 6H, J = 6.0 Hz) (2-CH_3); ^{31}P NMR: 7.72 ppm; IR: 3268, 2957, 1739, 1649, 1579, 1490, 1439, 1204, 924, 769 cm^{-1}; ESI-MS: 547.3 $[M + H]^+$; HRMS calcd for 569.1463 $C_{23}H_{33}N_4O_5NaPCl_2$ found 569.1473.

4d: Yield 78%; ^1H NMR: 7.21(t, 1H, J = 7.8 Hz), 6.88(m, 1H), 6.59(dd, 2H, J_1 = 12.4 Hz, J_2 = 2.0 Hz), 5.16(d, 1H, J = 10.0 Hz) (–NH), 4.08 (m, 4H)(2-OCH_2), 3.84(s, 3H), 3.79(s, 3H), 1.65(m, 2H) (–CH_2), 1.36(m, 5H)(–CH_2–CH_3), 0.90(t, 3H, J = 7.4 Hz)(–CH_3); ^{31}P NMR: 3.88; IR (KBr) υ: 3419, 3196, 2959, 1708, 1450, 1259, 1022, 776 cm^{-1}; HRMS calcd for 375.1559 $C_{15}H_{26}N_3O_6P$ found 375.1552.

4e: Yield 62%; $[\alpha]_D^{20}$ –0.2 (c 0.0948, AcOEt); ^1H NMR: 6.50(s, 1H), 6.35(s, 1H), 6.34 (d, 1H), 5.13(d, 1H, J = 10.4 Hz) 4.67 (m, 1H) (–CH), 4.08(m, 4H) (2-OCH_2), 3.84(s, 3H), 3.75(s, 3H), 1.66(m, 2H) (–CH_2), 1.47 (d, 3H, J = 7.2 Hz) (–CH_3), 1.37(m, 5H) (–CH_2–CH_3), 0.92 (t, 3H, J = 7.4 Hz) (–CH_3); ^{31}P NMR: 3.96; IR υ/cm^{-1}: 3272, 2959, 1743, 1648, 1526, 1457, 1206, 1024; ESI-MS: 390.3 $[M + H]^{+,}$ 412.3 [M + Na], HRMS calcd for 389.1716 $C_{16}H_{28}N_3O_6P$, found 389.1722.

4f: Yield 70%; $[\alpha]_D^{20}$ + 2.1 (c 0.1381, $CHCl_3$); ^1H NMR: 6.50 (s, 1H), 6.34(s, 1H), 6.12(d, 1H, J = 8.4 Hz) (–NH), 4.83 (d, 1H, J = 7.4 Hz), 4.71(m, 1H) (–CH), 4.12(m, 4H) (2-OCH_2), 3.84(s, 3H), 3.75(s, 3H), 1.67(m, 6H) (3-CH_2), 1.37(m, 5H) (–CH_2–CH_3), 0.94(m, 9H) (3-CH_3); ^{31}P NMR: 3.98; IR υ/cm^{-1}: 3423, 2959, 1741, 1652, 1581, 1526, 1440, 1204, 1025; ESI-MS: 432.4 $[M + H]^+$, HRMS calcd for 431.2185 $C_{19}H_{34}N_3O_6P$, found 431.2179.

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