Synthesis and Cytotoxicity of Nitrogen Mustard/Tripolypyrrole Conjugate

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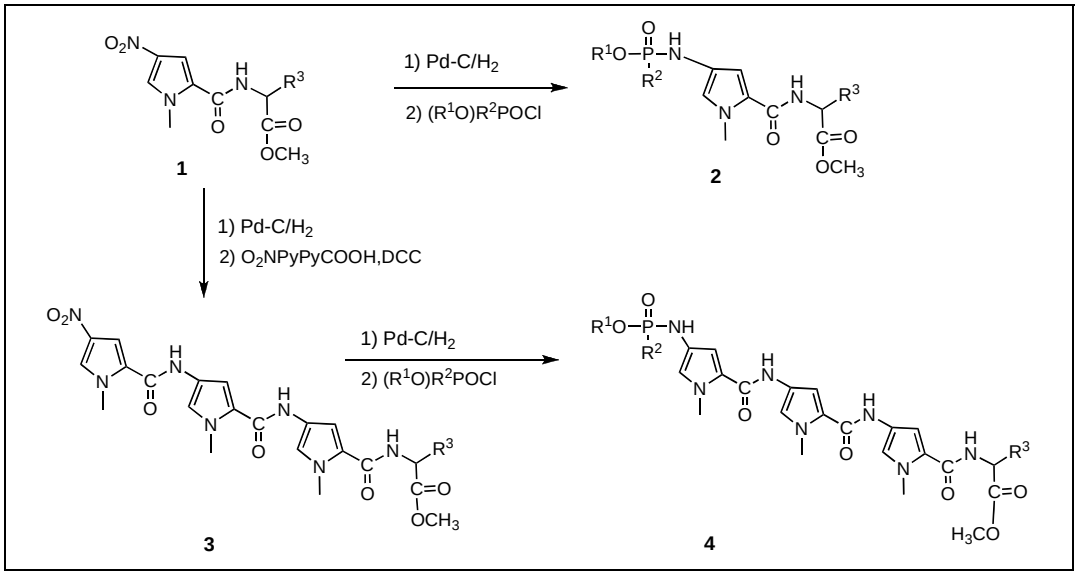

A series of nitrogen mustard derivatives were synthesized by chloroform reaction and coupling reaction using DCC/ HOBT as promoting additives. The structure of compound was confirmed by ^{1}H NMR, ^{31}P NMR, MS and IR. The data show that the nitrogen mustard with tripolypyrrole as the linker inhibits all the four tested cells and has the highest activity.

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INTRODUCTION

The main objective of covalent 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 targetcells, the spacer should be degraded, resulting in the release of the antitumour agent. Recently, the natural product distamycin, netropsin and their analogs have attracted considerable attention on the part of synthetic and biological chemists because they recognize 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 characterized 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].

Nitrogen mustard has been used widely for more than 30 years in the treatment of tumorous and autoimmune diseases [8-9]. However, nitrogen mustards' apparent drawback is its poor specificity, for they are extremely toxic and the use of them is usually accompanied by severe side effects. To improve the specificity of nitrogen mustard derivatives to tumor cells and to reduce the side effects in remote tissue, we synthesized a nitrogen mustard/tripolypyrrole conjugate for further anti-tumor research.

RESULTS AND DISCUSSION

The compounds **2** and **4** are our targeted molecule, which consisted of nitrogen mustard, pyrrole and amino acid methyl ester groups. The synthetic route is shown in Scheme 1. Compounds **1** were synthesized according to Ref [10-12]. The structures of these compounds were confirmed by ^{1}H NMR, MS, IR and HRMS. In the synthesis of compound **3** there is no need of 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.

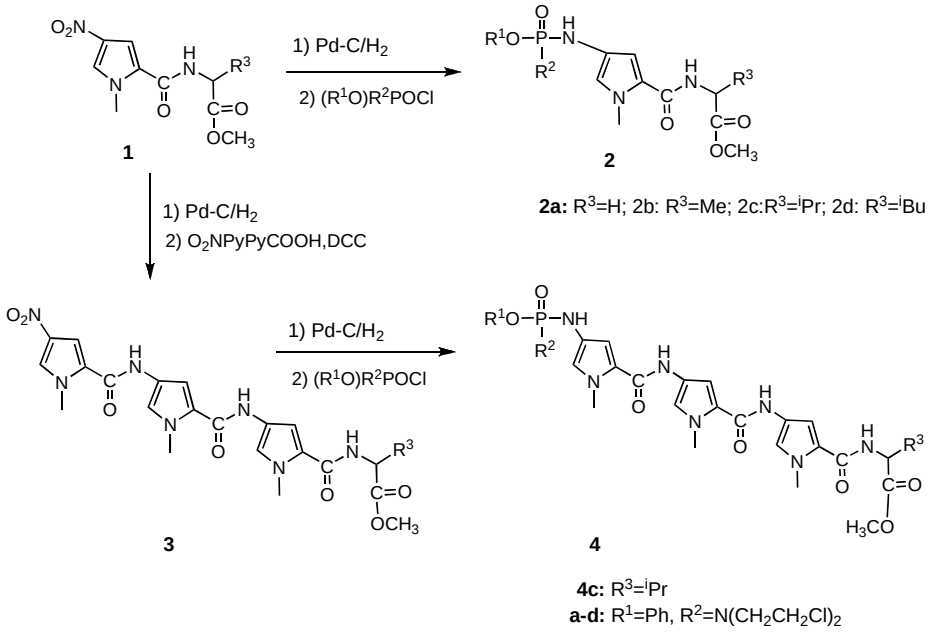
Compound **3** was obtained in good yield (51%). In this step, the qualitative reduction of the nitro group of **1** to an amino group is important. To achieve the optimum result, TLC was employed to monitor the progress of the hydrogenation. In an attempt to achieve a better coupling efficiency, excess of DCC was needed. The nitrogen mustard can be readily transfered to phosphonyl chloride fragments. Similar to the synthesis of **3**, TLC was needed to monitor the progress of the hydrogenation in the syntheisis of **2** and **4**. Compound **4c** can be obtained by coupling **3** to phosphonyl chloride in 42%.

Table 1. The *in vitro* inhibition ratios of compounds **2** and **4** against four cancer cells.

Compds	Inhibition ratio /%			
	KB	HCT-8	Bel-7402	A549
2a	10.33	8.86	-	6.89
2b	14.77	-	1.22	-3.22
2c	-	9.32	5.78	6.27
2d	18.25	10.66	20.45	5.76
4c	50.33	18.22	8.38	14.55

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

Scheme 1



Synthetic route of nitrogen mustard/tripolypyrrole conjugate.

The cytotoxicity was assessed by MTT assay [13]. Human cancer cell, cultured in RPMI-1604 medium supplemented with 10% FBS, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin at 37 ºC in humidified air atmosphere of 5% CO_2, was plated into 96-well plate ($1x10^4$ cells/well). The next day, the compounds diluted in culture medium were added (200μL/well) to the wells. 48 h later 20 μL MTT (0.5 mg/mL MTT in PBS) was added and 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 measuring absorbance at 570 nm with a micro plate reader. As demonstrated by MTT cytotoxicity assay (Table 1), the cytotoxicity of compound **2d** is higher than that of **2a** and **2b**. Compound **2d** and **4c** inhibit all the four tested cells and **4c** has the highest activity.

In conclusion, a convergent synthesis of nitrogen mustard/tripolypyrrole conjugate was achieved *via* coupling reaction with readily accessible materials. The significant feature of this procedure is that there is no need of protecting and deprotecting the 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 the nitrogen mustard with tripolypyrrole as the linker inhibits all the four tested cells and has the highest activity.

EXPERIMENTAL

General. ^{1}H NMR and ^{31}P NMR spectra were measured by using a Bruker AC-P400 spectrometer with TMS and 85% H_3PO_4 as the internal and external reference respectively and with $CDCl_3$ or DMSO as the solvent. IR spectra were recorded

as KBr pellets on a BRUCK spectrometer. Mass spectra was acquired in positive ion mode using a Bruker ESQUIRE-LCTM ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z20000. Solvents were purified and dried by standard procedures. Dipolypyrrole was synthesized according to [11-12].

General Procedure for the synthesis of compound 2. To a solution of compound **1** (0.31 mmol) in 15 mL of CH_2Cl_2 was added Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at room temperature for 15 h. The catalyst was removed by filtration through Celite and the filtrate was added 1 mL Et_3N. The solution was cooled to -10 °C and 0.2 mL $(R^1O)R^2POCl$ was dropped. The result solution was stirred 24h at this temperature. After filtrated and concentrated in vacuo, a yellow oil was obtained. Purification by column chromatography, compound 2 was obtained in oil.

[(4-*O*-phenyl-*N*-(bis-2-chloroethyl)-phosphoramidic-1-methyl-1*H*-pyrrole-2-carbonyl)-amino]-acetic acid methyl ester (2a), this compound was obtained in 50%; ir: 3420 2954 1744 1642 1580 1490 1204 923 770cm^{-1}; ^{1}H nmr (deuteriochloroform): δ7.31(m, 2H) (ph-H), 7.18 (m, 3H) (ph-H), 6.84(t, 1H, J=9.2Hz), (-*NH*C) 6.54 (s, 1H), 6.50(s, 1H), 5.54 (d, 1H, J=10.8Hz)(-*NH*P), 4.08(d, 2H, J=5.5Hz) (-CH_2), 3.81(s, 3H), 3.75(s, 3H), 3.49 ppm (m, 8H) (2-NCH_2, 2-CH_2Cl); ^{31}P nmr: δ7.93 ppm; 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;

[(4-*O*-phenyl-*N*-(bis-2-chloroethyl)-phosphoramidic-1-methyl-1*H*-pyrrole-2-carbonyl)-amino]-propionic acid methyl ester (2b), this compound was obtained in 57%; ir: 3249 2953 1741 1647 1579 1507 1490 1455 1368 1204 923 765cm^{-1}; ^{1}H nmr (deuteriochloroform): δ7.33(m, 2H) (ph-H), 7.19(m, 3H) (ph-H), 6.58(s, 1H), 6.48(d, 1H, J=9.7Hz) (-*NH*C), 6.46(s, 1H), 5.15 (d, 1H, J=10.6Hz)(-*NH*P), 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 ppm (d, 3H, J=7.1Hz)(-CH_3); ^{31}P nmr δ7.74 ppm; ESI-MS: 505.3 [M+H]; HRMS calcd for 527.0994 $C_{20}H_{27}N_4O_5NaPCl_2$ found 527.1000;

[(4-*O*-phenyl-*N*-(bis-2-chloroethyl)-phosphoramidic-1-methyl-1*H*-pyrrole-2-carbonyl)-amino]-3-Methyl-butyric acid methyl ester (2c), this compound was obtained in 62%; ir: 3329 2973 1751 1636 1574 1512 1488 1445 1358 1203 926 766cm^{-1}; ^{1}H nmr (deuteriochloroform): δ7.32(m, 2H) (ph-H), 7.18(m, 3H) (ph-H), 6.57(s, 1H), 6.49(d, 1H, J=9.6Hz) (-*NH*C), 6.47(s, 1H), 5.25 (d, 1H, J=11.5Hz)(-*NH*P), 4.67(m, 1H) (-CH), 3.83 (s, 3H), 3.75 (s, 3H), 3.47(m, 8H)(2-N-CH_2, 2-CH_2-Cl), 2.21 (m, 1H) (-CH), 0.96 ppm (dd, 6H, J_1=10.3Hz, J_2=7.0Hz) (2-CH_3); ^{31}P nmr: δ7.73 ppm; ESI-MS: 533.2 [M+H];

4-Methyl-[2-(4-*O*-phenyl-*N*-(bis-2-chloroethyl)-phosphoramidic-1-methyl-1*H*-y-2-carbonyl)-amino]-pentanoic acid methyl ester (2d), this compound was obtained in 65%; ir: 3268 2957 1739 1649 1579 1490 1439 1204 924 769cm^{-1}; ^{1}H nmr (deuteriochloroform): 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=9.0Hz) (-NHC), 5.38 (dd, 1H, J_1=10.7Hz, J_2=29.3Hz) (-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 ppm (d, 6H, J=6.0Hz) (2-CH_3); ^{31}P nmr: δ7.72 ppm; ESI-MS: 547.3 $[M+H]^+$; HRMS calcd for 569.1463 $C_{23}H_{33}N_4O_5NaPCl_2$ found 569.1473.

Procedure for the synthesis of compound 3. To a solution of NO_2PyPyCOOH (0.513 g, 1.7 mmol) in 18 mL of DMF was added HOBt (0.2254 g, 1.7 mmol) and DCC solution(0.4326 g, 2.1 mmol DCC in 3 mL CH_2Cl_2). The solution was stirred for 4 h at room temperature. After filtrated, the active ester solution was obtained.

Separately, a solution of compound 1 (1.7 mmol) in 20 mL of CH_3OH was mixed with Pd/C catalyst (10%), and the mixture was stirred under a slight positive pressure of H_2 at room temperature for 12 h. The catalyst was removed by filtration through Celite and the filtrate was added to the active ester. Then 1 mL Et_3N was added to the mixture solution, followed by stirring 24 h. The desiccator was removed by filtration, and the filtrate was concentrated *in vacuo*. Column chromatography of the residue (eluant with $CHCl_3$ and MeOH 20:1) provided compound 3 in good yield.

{[1-Methyl-4-({1-methyl-4-[(1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-1*H*-pyrrole-2-carbonyl]-amino}-acetic acid methyl ester (3a), this compound was obtained in 44%; mp: 238-240°C; ^{1}H nmr (DMSO-d_6): δ10.30(s, 1H) (-NH), 9.98(s, 1H) (-NH), 8.43(t, 1H, J=5.9Hz) (-NH), 8.29(s, 1H), 8.17(d, 1H, J=1.72Hz), 7.59(d, 1H, J=1.56Hz), 7.28(d, 1H, J=1.6Hz), 7.25(d, 1H, J=1.56Hz), 7.04(d, 1H, J=1.6Hz), 6.94(d, 1H, J=1.6Hz), 3.96 (s, 3H), 3.92 (d, 2H, J=5.8Hz) (-CH_2), 3.86(s, 3H), 3.80(s, 3H), 3.65 ppm (s, 3H); ir: 3124 2925 1740 1657 1555 1438 1406 1307 810 774 747cm^{-1}; ESI-MS: 484.2$[M-H]^-$.

2-{[1-Methyl-4-({1-methyl-4-[(1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-1*H*-pyrrole-2-carbonyl]-amino}-propionic acid methyl ester (3b), this compound was obtained in 54%; mp: 142-144°C; ^{1}H nmr (deuteriochloroform): δ8.65(s, 1H) (-NH), 8.04(s, 1H) (-NH), 7.58(s, 1H), 7.47(s, 1H) (-NH), 7.28(s, 1H), 7.17(s, 1H), 6.69(s, 2H), 6.46(d, 1H, J=2.8Hz), 4.75(d, 1H, J=8.8Hz)(-CH), 4.03(s, 3H), 3.90(s, 3H), 3.81(s, 3H), 3.75 (s, 3H), 1.52 ppm (d, 3H, J=7.2Hz) (-CH_3); ir: 3398, 3130, 2930, 1738, 1649, 1527, 1400, 1309, 1206, 1112, 812cm^{-1}; ESI-MS: 497.7$[M-H]^-$, 522.3 $[M+Na]^+$; HRMS calcd for 538.1453 $C_{22}H_{25}N_7O_7K$ found 538.1450.

3-Methyl-2-{[1-methyl-4-({1-methyl-4-[(1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-1*H*-pyrrole-2-carbonyl]-amino}-butyric acid methyl ester (3c), yellow power, this compound was obtained in 51%; mp: 141-143°C; ^{1}H nmr (deuteriochloroform): δ 8.69(s, 1H) (-NH) , 7.60(s, 1H) (-NH), 7.52(s, 1H) , 7.42(s, 1H) (-NH), 7.30(s, 1H), 7.17(s, 1H), 6.68(s, 1H), 6.65(s, 1H), 6.38(s, 1H), 4.73(dd, 1H, J_1=8.63Hz, J_2=5.3Hz)(-CH), 4.03(s, 3H), 3.91(s, 3H), 3.81(s, 3H), 3.74(s, 3H), 2.26(m, 1H) (-CH), 1.00(d, 3H, J_1=6.8Hz) (-CH_3), 0.99 ppm (d, 3H, J_1=6.8Hz) (-CH_3) ; ir: 3422 3286 3138 3114 2961 1739 1652 1589 1523 1465 1311cm^{-1}; ESI-MS: 526.3$[M-H]^-$ 550.3 $[M+Na]^+$.

4-Methyl-2-{[1-methyl-4-({1-methyl-4-[(1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl)-amino]-1*H*-pyrrole-2-carbonyl}-amino)-1*H*-pyrrole-2-carbonyl]-amino}-pentanoic acid methyl ester (3d), this compound was obtained in 57%; mp: 135-138°C; ^{1}H nmr (DMSO-d_6): δ10.31(s, 1H) (-NH), 9.98(s, 1H) (-NH), 8.32(s, 1H), 8.20(s, 1H) (-NH), 7.60(d, 1H, J=1.88Hz), 7.28 (d, 1H, J=1.48Hz), 7.22(d, 1H, J=1.6Hz), 7.05(s, 2H), 4.43(m, 1H)(-CH), 3.97(s, 3H), 3.87(s, 3H), 3.79(s, 3H), 3.64(s, 3H), 1.8(m, 1H)(-CH-CH_2), 1.66(m, 1H)(-CH-CH_2),

1.52(m, 1H)(-CH-CH_2), 0.91(d, 3H, J_1=6.5Hz) (-CH_3), 0.87(d, 3H, J_1=6.5Hz) (-CH_3); ir : 3351 3308 3130 2953 1737 1648 1526 1367 1311 1225cm^{-1}; ESI-MS: 540.2[M-H], 542.3$[M+H]^+$, 564.3 $[M+Na]^+$;

Procedure for the synthesis of compound 4c. To a solution of compound **3** (0.31 mmol) in 15 mL of CH_2Cl_2 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 the filtrate was added 1 mL Et_3N. The solution was cooled to -10 °C and 0.2 mL $(R^1O)R^2POCl$ was dropped. The result solution was stirred 24 h at this temperature. After filtration and concentrated *in vacuo*, a yellow oil was obtained. Purification by column chromatography, compound **4c** was obtained in 41% yield. ^{1}H nmr (deuteriochloroform): δ 8.32 (s, 1H), 8.01 (s, 1H), 7.29 (m, 2H), 7.16 (m, 5H), 6.76 (s, 1H), 6.65 (s, 1H), 6.53 (s, 2H), 6.41 (d, 1H, J=8.4Hz) (-NH), 5.62 (d, 1H, J=8.7Hz) (-NH), 4.62 (m, 1H)(-CH), 3.89 (s, 3H), 3.84 (s, 6H), 3.74 (s, 3H), 3.49 (m, 8H) (2-NCH_2, 2-CH_2Cl), 2.20 (m, 1H) (-CH), 0.95 ppm (dd, 6H, J_1=10.2Hz, J_2=7.0Hz) (2-CH_3); ^{31}P nmr: δ 8.07 ppm; ir: 3282 2959 1738 1650 1582 1512 1433 1204 919 772 cm^{-1}; ESI-MS: 799.3 $[M+Na]^+$; HRMS calcd for 799.2267 $C_{34}H_{43}N_8O_7NaPCl_2$ found 799.2263.

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