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Synthesis and antitumor activity of DNA binding cationic porphyrin–platinum(II) complexes

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Abstract—A new series of DNA binding 5,10,15-tri(*N*-methyl-4-pyridiniumyl)porphyrin (TrisMPyP)-platinum(II) conjugates was synthesized, in which different spacer ligands were used for appropriate coordination to platinum(II) complexes. Compound **9b** exhibited in vivo antitumor activity (T/C%, 294) superior to cisplatin (T/C%, 184) against the leukemia L1210 cell line. © 2003 Published by Elsevier Science Ltd.

A variety of strategies and vector systems have been investigated in attempts for selective delivery of anticancer drug to the tumor tissue in order to reduce the dose-related strong side effects.[1] Some porphyrins are known to selectively accumulate in the tumor tissue.[2] In our previous work, water-soluble sulfonatoporphyrin-platinum(II) conjugates exhibited significant tumor-targeting effect (tumor/muscle ratio = 7).[3] It is also known that the effectiveness of platinum(II) antitumor drugs can be improved by linking the reactive platinum functionality to a DNA binding agent such as acridine,[4] anthraquinones[5] and other intercalators.[6]

The cationic porphyrin, 5,10,15,20-tetra(*N*-methyl-4-pyridiniumyl)porphyrin (TMPyP) possesses an appropriate molecular size, a planar chromophore and positive charge, favorable for intercalating into or stacking along DNA.[7] TMPyP exhibits various biological activities against human immunodeficiency virus type 1 (HIV-1)[8] and transmissible spongiform encephalopathies.[9] TMPyP has also been studied recently as an inhibitor of telomerase, a potential target for new antitumor drugs, by interacting with quadruplex DNA.[10]

Such interesting biochemical properties of cationic porphyrin prompted us to use this porphyrin to develop a new generation of platinum(II) antitumor drugs. Usefulness of the cationic porphyrin-platinum(II) conjugates is expected for several reasons: (i) The conjugates may interact with DNA through bifunctional binding modes, (ii) The selective delivery of antitumor platinum(II) moiety may be afforded due to the tumor targeting properties of porphyrin, and (iii) The synergistic or additive effect of the cytotoxicity of platinum(II) moiety and phototoxicity of porphyrin may be achieved in the presence of light.[11] For such reasons, we prepared a series of water-soluble, cationic porphyrin-platinum(II) conjugates in which the porphyrin ligands were based on the tris(*N*-methylpyridiniumyl)porphyrin motif and contain a *meso*-phenyl substituent with a tether terminating with dicarboxylic, mono- or diamine function to allow the coordination of the corresponding platinum(II) moiety.

Cationic porphyrin-platinum(II) conjugates were synthesized according to the procedure depicted in Scheme 1. The starting unsymmetric cationic porphyrin **1** was synthesized by slightly modified Adler–Longo method,[12] and purified by repeated recrystallization and extensive column chromatography on silica (yield, 3%). Porphyrin **1** was reacted with a 2-fold excess of tritylated bromopropylamine or 10-fold excess of diethylbromomalonate in the presence of sodium hydroxide in DMF for 8 h to yield porphyrin **2** or **3**, respectively. Porphyrin **4** was obtained by the reaction of porphyrin **1** with 10-fold excess of spacer **12** in the presence of anhydrous K_2CO_3 in DMF for 8 h. The products were purified by column chromatography on silica and

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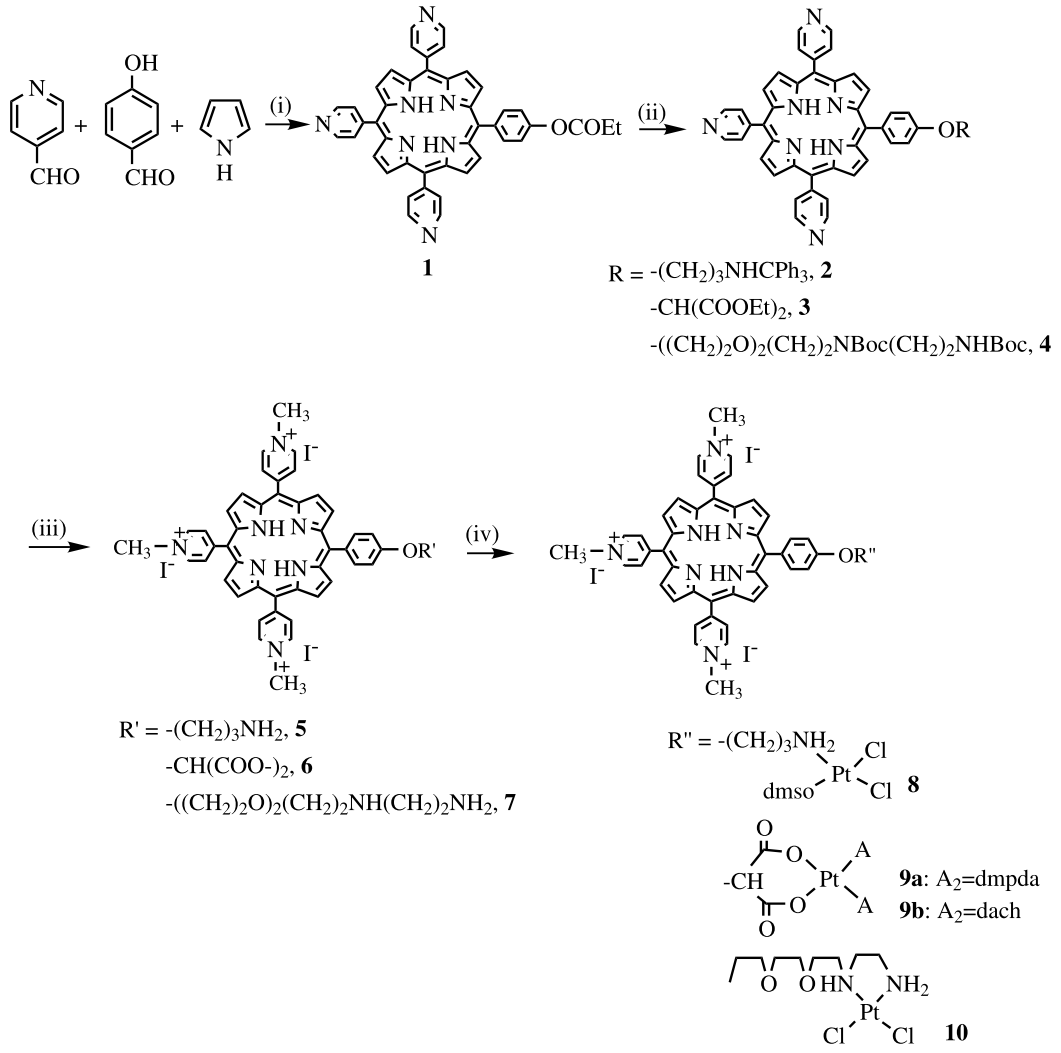
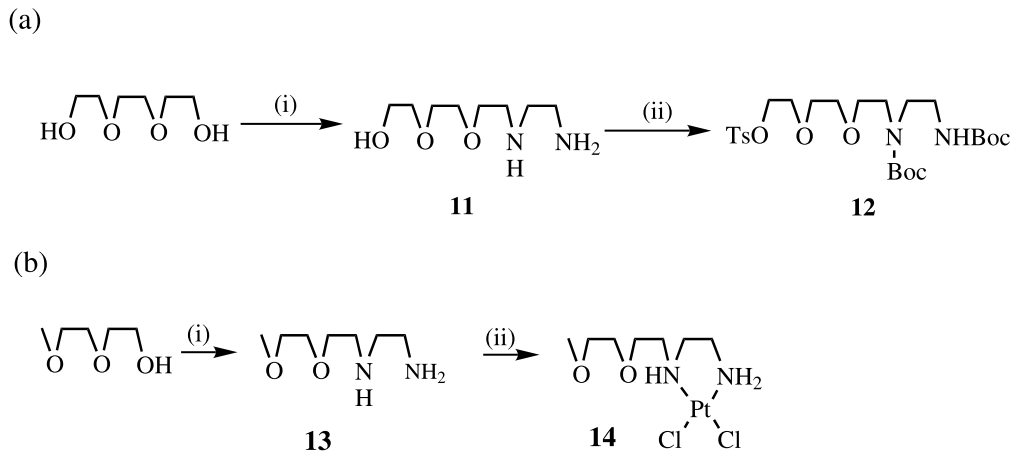


Scheme 1. Synthesis of cationic porphyrin–platinum(II) conjugates. (i) Propionic acid, Ac_2O, 110°C, 3 h; (ii) NaOH, DMF, $Br(CH_2)_3NHCPh_3$ **2**, or $BrCH(COOEt)_2$ **3**, or K_2CO_3, $TsO\{(CH_2)_2O\}_2(CH_2)_2NBoc(CH_2)_2NHBoc$, **4**; (iii) CH_3I/DMF, CF_3COOH/H_2O, rt, 3 h, **5**, **7**, NaOH, MeOH/H_2O, **6**; (iv) $(DMSO)_2PtCl_2$, **8**, (dmpda)$PtSO_4$, **9a**, (dach)$PtSO_4$, **9b**, $(DMSO)_2PtCl_2$, **10**.

reacted with an excess of methyl iodide to yield water-soluble cationic pyridiniumyl derivatives. Deprotection of the tritylated side chain was carried out in a mixture of 50/50 trifluoroacetic acid/water for 1 h at room temperature to give porphyrin ligand **5**. The hydrolysis of the ethyl ester was performed with sodium hydroxide in MeOH/water to yield porphyrin **6** bearing dicarboxylate function. The deprotection of the *t*-Boc group was carried out in a mixture of 50/50 trifluoroacetic acid/water for 3 h at room temperature to yield porphyrin **7**. Porphyrins **5** and **7** were treated with triethylamine to give the porphyrins with tether terminating free amine function. Porphyin–platinum(II) complexes **8** and **10** were then obtained in excellent yields (>90%) by the reaction of the corresponding porphyrin ligands with a slight excess of $(DMSO)_2PtCl_2$ in the solvent mixture of H_2O/EtOH.[13] The reaction of porphyrin ligand **11** with (diamine)Pt(II)sulfate (diamine = 2,2-dimethyl-1.3-propanediamine (dmpda), *trans*-(±)-1,2-diaminocyclohexane (dach)) in water resulted in porphyrin-platinum(II) conjugates **9a** and **9b**, respectively.[14]

To synthesize spacer **12**, triethyleneglycol was reacted with equimolar of TsCl in the presence of triethylamine in CH_2Cl_2 as shown in Scheme 2(a). The mono tosylated derivative was purified by column chromatography and reacted with an excess of ethylenediamine in the presence of anhydrous K_2CO_3 in acetonitrile. Reaction of **11** with Boc_2O in CH_2Cl_2 followed by tosylation of terminal alcohol group gave spacer **12** in the overall yield of 25% through 4 steps. The final product was then purified by flash column chromatography with ethylacetate/hexane (1/2 v/v).[15]

To synthesize spacer-platinum(II) conjugate **14**, diethyleneglycol methyl ether was reacted with TsCl in the presence of triethylamine in CH_2Cl_2 as depicted in Scheme 2(b). The tosylated derivative was purified by column chromatography and reacted with an excess of ethylenediamine in the presence of anhydrous K_2CO_3 in acetonitrile to obtain compound **13**. The final platinum conjugate **14** was obtained by the reaction with $(DMSO)_2PtCl_2$ in the solvent mixture of H_2O/EtOH.[16]



Scheme 2. Syntheses of spacer **12** (a): (i) $TsCl/Et_3N$, ethylenediamine/K_2CO_3; (ii) CH_2Cl_2, Boc_2O, $TsCl/Et_3N$, and spacer–platinum(II) conjugate **14** (b): (i) $TsCl/Et_3N$, ethylenediamine/K_2CO_3; (ii) $(DMSO)_2PtCl_2$.

The antitumor activity of porphyrin-Pt(II) conjugates was assayed in vitro and in vivo against leukemia L1210 cell line according to the previous method.[17] The mean survival time of the drug treated group (T) was compared with that of the untreated control group (C), and the results are listed in Table 1. Compound **9b** bearing the porphyrin moiety as a leaving group showed a remarkable in vivo antitumor activity against leukemia cell line (T/C%, 294 at 50 mg/kg dose), which is superior to carboplatin and cisplatin (T/C%, 168 and 184, respectively). On the other hand, the inactivity of complex **10** seems to be due to the bulkiness of the porphyrin bound to the carrier amine ligand, which is likely to hinder the interaction between platinum atom and its target, DNA. The spacer–Pt(II) complex alone exhibit in vivo activity (T/C%, 166) comparable to carboplatin. In order to test the tumor targeting property of porphyrin–Pt(II) conjugates, the biodistribution study was performed according to the previous method.[5] The Pt-concentrations of compounds **10** in liver, kidney and lung were high, which is probably due to the large number of the LDL receptors in those tissues as well as tumor tissue. The drug concentration in tumor tissue tends to increase with time. The tumor/muscle ratios of complex **10** are 1.3 at 2 h after injection and 2.4 at 24 h after injection whereas those of carboplatin after 2 and 24 h are 1.3 and 1.2, respectively.

Table 1. In vitro and in vivo activity against leukemia L1210 cell line

Compound	In vitro	In vivo	
	ED_{50}	Dosage (mg/kg)	T/C(%)
8	>40		
9a	8.9		
9b	3.4	50	294
		25	201
10	>40	100	100
14	8.4	40	166
		20	137
Carboplatin	3.8	40	168
Cisplatin	0.3	4	184

In conclusion, a new class of porphyrin–platinum(II) conjugates synthesized in this study exhibited high antitumor activity superior to cisplatin and elevated tumor-localizing effect (tumor/muscle ratio>2) compared to carboplatin. The DNA binding property of the present bifunctional conjugates is under investigation.

Acknowledgements

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13. Compopund **8**: ^{1}H NMR (DMSO-d_6, δ): 9.4 (d, 6H, 2,6-pyridiniumyl, $J=6.2$ Hz), 9.2 (m, 8H, β-pyrrol), 8.9 (d, 6H, 3,5-pyridiniumyl, $J=6.3$ Hz), 8.0 (d, 2H, 2,6-phenyl, $J=8.5$ Hz), 7.3 (d, 2H, 3,5-phenyl, $J=8.5$ Hz), 4.7 (s, 9H, CH_3), 4.4 (t, 2H, OCH_2), 3.6 (t, 2H, CH_2N), 3.50 (s, 6H, DMSO), 2.5 (m. 2H, CH_2).), −2.9 (s, 2H, pyrrole-NH). Compound **10**: ^{1}H NMR (DMSO-d_6, δ): 9.5 (d, 6H, 2.6-pyridiniumyl, $J=6.4$ Hz), 9.2–9.1 (m, 8H, β-pyrrole), 9.0 (d, 6H, 3,5-pyridiniumyl, $J=6.4$ Hz), 8.1 (d, 2H, 2,6-phenyl, $J=8.5$ Hz), 7.5 (d, 2H, 3,5-phenyl, $J=8.5$ Hz), 4.7 (s, 9H, CH_3), 4.3 (m, 2H, CH_2), 4.0 (m, 2H, CH_2), 3.8–3.3 (m, 12H, CH_2), −2.9 (s, 2H, pyrrole-NH). ESI/MS: m/z 713.8 [(M−2Cl)$^{2+}$], 650.7 [(M−2Cl–I–H$^+$)$^{2+}$], 433.8 [(M−2Cl–I)$^{3+}$].
14. Compound **9a**: ^{1}H NMR (DMSO-d_6, δ): 9.4 (d, 6H, 2,6-pyridiniumyl, $J=6.4$ Hz), 9.3 (m, 8H, β-pyrrol), 8.9 (d, 6H, 3,5-pyridiniumyl, $J=6.3$ Hz), 8.0 (d, 2H, 2,6-phenyl, $J=8.3$ Hz), 7.3 (d, 2H, 3,5-phenyl, $J=8.3$ Hz), 4.9 (s, 9H, CH_3), 3.4–3.3 (m, 4H, CH_2), 2.3 (s, 4H, dmpda-CH_2), 0.8 (s, 6H, dmpda-CH_3), -3.0 (s, 2H, NH pyrrol). Compound **9b**: ^{1}H NMR (DMSO-d_6, δ): 9.4 (d, 6H, 2,6-pyridiniumyl, $J=6.2$ Hz), 9.3 (m, 8H, β-pyrrol), 8.9 (d, 6H, 3,5-pyridiniumyl, $J=6.2$ Hz), 8.0 (d, 2H, 2,6-phenyl, $J=8.2$ Hz), 7.3 (d, 2H, 3,5-phenyl, $J=8.2$ Hz), 4.9 (s, 9H, CH_3), 3.4–3.3 (m, 4H, CH_2), 2.3 (br, 2H, dach-CH_2), 1.9 (br, 2H, dach-CH_2), 1.4 (br, 2H, dach-CH_2), 1.2 (br, 2H, dach-CH_2), 1.1 (br, 2H, dach-CH_2), -3.1 (s, 2H, NH pyrrol).
15. Compound **12**: ^{1}H NMR ($CDCl_3$, δ): 7.8 (d, 2H, $J=8.3$ Hz, tosyl ArH), 7.3 (d, 2H, $J=8.3$ Hz, tosyl ArH), 5.2 (s, br, 1H, NHBoc), 4.2–4.1 (m, 2H, CH_2), 3.7 (m, 2H, CH_2), 3.6–3.5 (m, 6H, CH_2), 3.4–3.3 (m, 4H, CH_2), 3.3–3.2 (m, 2H, CH_2), 2.4 (s, 3H, tosyl CH_3), 1.4 (m, 18H, Boc-CH_3).
16. Compound **14**: ^{1}H NMR (D_2O, δ): 3.9 (m, 2H, CH_2), 3.8–3.6 (m, 8H, CH_2), 3.3 (s, 5H, CH_2, CH_3); EI–MS m/z: 423 (MH$^+$).
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