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Synthesis and biological activity of novel platinum(II) complexes of glutamate tethered to hydrophilic hematoporphyrin derivatives

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Abstract—A new series of hematoporphyrin–platinum(II) conjugates was prepared by platination of the glutamate ligand tethered to hydrophilic hematoporphyrin derivatives, in which different numbers of ethylene oxide unit were introduced to modulate the hydrophobic/hydrophilic balance of the conjugates. The antitumor activity of the hematoporphyrin–platinum(II) conjugates was assayed in vitro and in vivo against the leukemia L1210 cell line. Among the complexes, compound **11** exhibited not only higher in vivo activity (T/C% = 192) than cisplatin (T/C% = 184) and carboplatin (T/C% = 168), but also elevated tumor-localizing effect (tumor/muscle ratio >3).

Cisplatin (*cis*-diamminedichloroplatinum(II)) and carboplatin (*cis*-diammine(cyclobutane-1,1-dicarboxylato)-platinum(II)) are currently used as one of the most widely used antitumor agents in cancer chemotherapy.[1,2] However, its toxic side effects such as kidney toxicity and neurotoxicity limit the clinical utility of these drugs.[3–5] Various strategies using vector systems such as antibodies and small or polymeric molecules recognizing specific receptors or enzymes over-expressed in tumor cells have been extensively investigated in attempts for selective delivery of anticancer drug to the tumor tissue in order to reduce the strong systemic side effects.[6–8] For example, certain porphyrins are known to accumulate selectively in tumor tissue via an unknown mechanism, probably involving the low-density lipoprotein (LDL) receptor-mediated endocytosis.[9,10] Our previous studies on the selective delivery of platinum(II) complexes to tumor cells involved the use of water-soluble sulfonatoporphyrin–platinum(II) conjugates that exhibited a significant tumor-targeting effect (tumor/muscle ratio = 7).[11]

Among many different types of platinum(II) complexes, the chelates of aminodicarboxylates such as aminomalonic acid (Amal), L-aspartic acid (Asp), and L-glutamic acid (Glu) have attracted attention by several groups.[12–15] For example, Gandolfi et al. reported a series of aminomalonatoplatinum(II) complexes with a general formula of *cis*-[Pt(diamine)(O,O-Amal)].[12] Recently we have reported a new class of antitumor platinum(II) complexes of aminomalonate, glutamate, and aspartate, in which the amine function was protected by a steric substituent to avoid undesirable (O,N)-linkage isomerization.[15] Such interesting chemical/biological properties of hematoporphyrin derivatives and glutamatoplatinum(II) complexes prompted us to conjugate these two moieties to develop a new generation of platinum(II) antitumor drugs. This paper describes synthesis, biodistribution, and antitumor activity of a new family of Pt(II) complexes of hydrophilic porphyrin derivatives bearing the glutamate ligand to chelate the antitumor (diamine)Pt(II) moiety.

The synthetic route to the present porphyrin–Pt(II) conjugates is depicted in Scheme 1. The macrocyclic porphyrin ring bears a hydrophilic group of ethylene oxide chain for the appropriate solubility and the (diamine)platinum(II) moiety for the antitumor activity. Hematoporphyrin diacid derivatives, **1**–**4**, were prepared from hematoporphyrin IX by the literature method (Scheme 1),[16] and then reacted with glutamic acid

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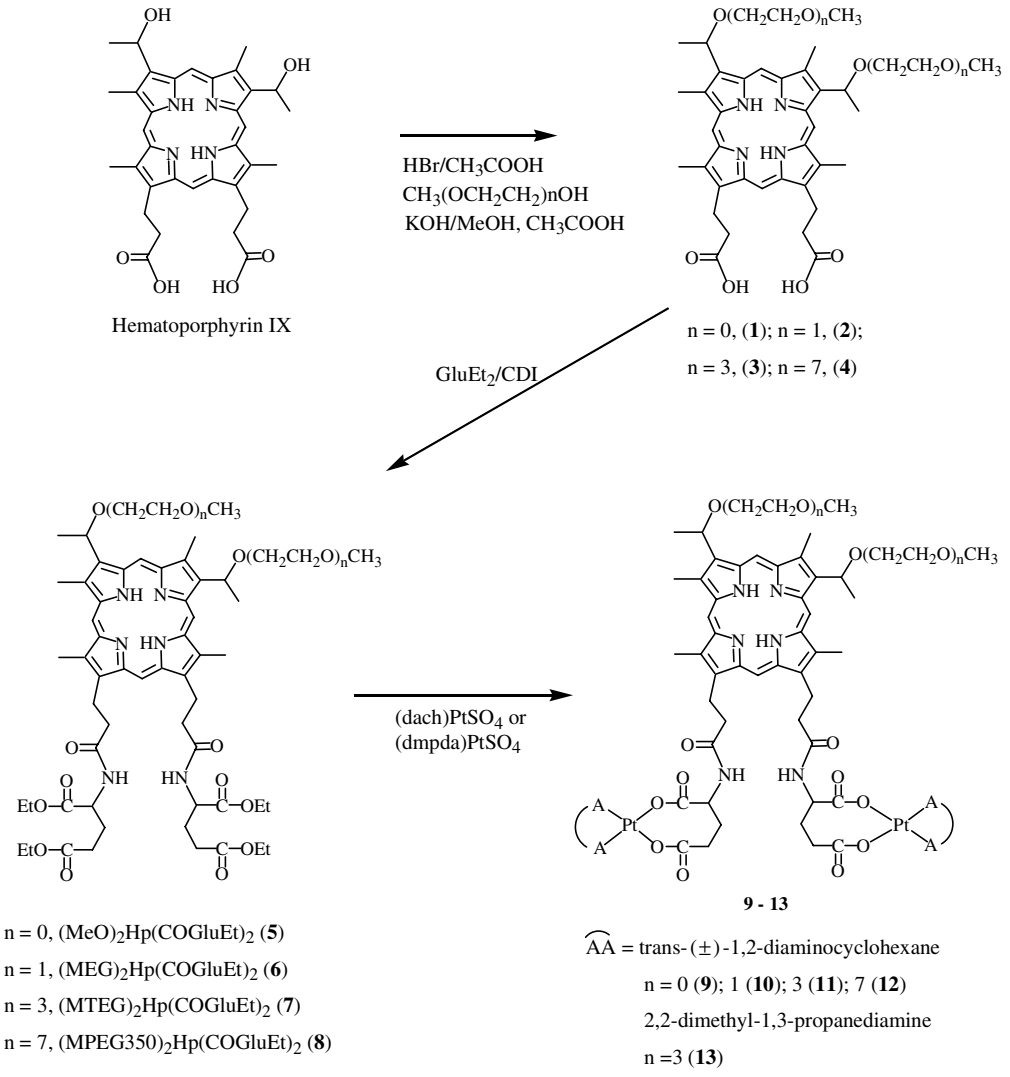

Scheme 1. The synthetic scheme for hematoporphyrin–platinum(II) conjugates.

diethyl ester in anhydrous DMF solution using 1,1′-carbonyldiimidazole (CDI) as a coupling agent, producing the porphyrin derivatives bearing the glutamate functional group (**5**–**8**) in relatively high yield (>80%).[17] All these porphyrin derivatives were clearly identified by the elemental analysis, ^{1}H NMR, IR, and MALDI-TOF mass spectroscopy. The ^{1}H NMR spectra of porphyrin derivatives in $CDCl_3$ showed the resonance of the amide NH proton and methyl protons of $GluEt_2$ moiety at 7.1–7.2 ppm as a broad singlet and 0.9–0.8 ppm as a multiplet, respectively. The slight shift to the downfield of the terminal methyl group of the glutamate seems to be due to the influence by the ring current of the adjacent porphyrin macrocyclic ring. The resonances of the internal pyrrole and *meso* protons appeared at −3.7 to −3.9 ppm as a singlet and 10.6–10.2 ppm as a multiplet, respectively.

The final porphyrin–Pt(II) conjugates (**9**–**12**) were synthesized by the reaction of (diamine)platinum(II) sulfate with the potassium salt of the corresponding porphyrin derivatives obtained by hydrolysis of $R_2Hp(COOH)_2$–$GluEt_2$ (**5**–**8**) using KOH in methanol.[18] These porphyrin–platinum(II) conjugates (**9**–**13**) were characterized by means of elemental analyses, IR, ^{1}H NMR, and MS spectroscopies. The molecular ion peak of the present complexes in the ESI-MS was not observed. Instead, the characteristic pattern of the fragmentation due to the loss of (diamine)platinum(II) moiety and one methyl group of the porphyrin ligand was observed in all spectra of the porphyrin–platinum(II) conjugates. A strong C=O stretching vibration appeared in the range of 1610–1580 cm^{-1} in their IR spectra, which is characteristic of a coordinated carboxylate ligand, and also strong amide C=O stretching vibrations appeared near at 1630 cm^{-1}.

The antitumor activity of the present porphyrin–Pt(II) conjugates (**9**–**13**) along with carboplatin as a reference was assayed in vitro and in vivo against leukemia L1210 cell line,[19] and the results are listed in Tables 1 and 2, respectively. The cytotoxicity of metal-free ligands **2** and **3** was also included as reference in the tables. The cytotoxicity of conjugate **13** is higher than that of carboplatin and tends to increase with increasing solubility. Porphyrin ligands **2** and **3** are cytotoxic in vitro but

Table 1. Cytotoxicity of conjugates against the leukemia L1210 cell line

Compd	IC_{50} (μM)
$(MEG)_2Hp(COOH)_2$ (**2**)	2.60
$(MTEG)_2Hp(COOH)_2$ (**3**)	6.60
$(MeO)_2Hp[COGluPt(dach)]_2$ (**9**)	>26.7
$(MEG)_2Hp[COGluPt(dach)]_2$ (**10**)	>23.0
$(MTEG)_2Hp[COGluPt(dach)]_2$ (**11**)	19.6
$(MPEG\text{-}350)_2Hp[COGluPt(dach)]_2$ (**12**)	4.40
$(MTEG)_2Hp[COGluPt(dmpda)]_2$ (**13**)	3.05
Carboplatin	9.92

Table 2. In vivo activity of selected conjugates against the leukemia L1210 cell line

Compd	Dosage (mg/kg)	T/C (%)
$(MEG)_2Hp(COOH)_2$ (**2**)	60/30	97/107
$(MTEG)_2Hp(COOH)_2$ (**3**)	60/30	102/106
$(MEG)_2Hp[COGluPt(dach)]_2$ (**10**)	100	139
$(MTEG)_2Hp[COGluPt(dach)]_2$ (**11**)	100	192
$(MTEG)_2Hp[COGluPt(dmpda)]_2$ (**13**)	60	186
	30	146
Carboplatin	40	168
Cisplatin	4	184

inactive in vivo. Therefore, it may be presumed that their in vitro cytotoxic effect could be associated with phototoxicity of the hematoporphyrin moiety. The in vivo activities of conjugates **11** and **13** are also better than those of cisplatin and carboplatin. In particular, conjugate **11** exhibited not only higher in vivo activity (T/C% = 192) than cisplatin (T/C% = 184) and carboplatin (T/C% = 168), but also good solubility in both water and organic solvent. The antitumor activity of the present conjugates is dependent in great part on the length of the ethylene oxide units of the porphyrin ligand. Such a result implies that the solubility of the compound plays an important role for the biological activity. Comparing two porphyrin–Pt(II) conjugates, **11** and **13**, bearing the same ethylene oxide units, their in vivo antitumor activity is not so different. Inactivity of conjugate **10** seems to be due to its low solubility in aqueous solution.

The biodistribution study of the representative conjugate **11** was performed using tumor bearing mouse, and analysis of the complexes in each organ was carried out using ICP-MS (Table 3).[20] The tropism for liver appears to be shared with other porphyrin family (data not shown).[21] The tumor/muscle ratio for conjugate **11** is 2.27 at 2 h after injection and 3.03 at 24 h after injection. In the case of conjugate **11**, the drug concentration in tumor tissue retains nearly constant during the experimental period of time (1.53 and 1.21 mg/kg at 2 and 24 h, respectively), whereas that of carboplatin decreases rapidly (3.87 and 0.67 mg/kg at 2 and 24 h, respectively) and the tumor/muscle ratios for carboplatin after 2 and 24 h are 1.46 and 1.16, respectively, showing no selectivity to the tumor tissue. In summary, among the present complexes designed to have appropriate amphiphilic character and tumor targeting property,

Table 3. Biodistribution of conjugate **11** and carboplatin at 2 and 24 h post injection (p.i.)[a]

Organs	Pt concentration in tissue (mg/kg tissue)			
	2 h p.i.		24 h p.i.	
	11	Carboplatin	**11**	Carboplatin
Blood	3.10 ± 0.51[b]	2.90 ± 0.86	1.11 ± 0.35	0.83 ± 0.28
Tumor	1.53 ± 0.20	3.87 ± 0.30	1.21 ± 0.27	0.67 ± 0.04
Muscle	0.74 ± 0.22	2.98 ± 0.98	0.40 ± 0.03	0.58 ± 0.05
TTR[c]	2.27 ± 0.86	1.46 ± 0.57	3.03 ± 0.83	1.16 ± 0.13

[a] Conjugate **11** and carboplatin were administered 20 and 10 mg/kg, respectively, by iv in a tail vein.

[b] The values are mean ± standard error of the mean (three measurements for each time point at each tissue).

[c] TTR means the ratio of the Pt(II) concentration in tumor tissue to that in muscle.

conjugate **11** exhibited not only higher activity than carboplatin and cisplatin but also clearly elevated tumor-localizing effect (tumor/muscle ratio >3) compared with carboplatin.

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17. Synthesis of compounds **5**–**8**: CDI (1.46 g, 9 mmol) was added to a solution of **1**–**4** (2.24 mmol) in anhydrous DMF (40 mL), which was stirred for 2 h at room temperature. To this solution was added a solution of $GluEt_2$·HCl (2.68 g, 11.2 mol) and triethylamine (1.5 g, 15 mmol) in anhydrous DMF (40 mL) and the solution mixture was stirred for 36 h at room temperature. After filtering, the filtrate was evaporated to dryness. The residual solid was dissolved in CH_2Cl_2 and the solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to obtain a purple solid. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2:methanol = 20:1, v/v). Data for **5**. Yield: 80%. ^{1}H NMR ($CDCl_3$, ppm): 10.55, 10.50, 10.22, 10.12 (m, 4H, *meso-H*), 7.17 (br, 2H, amide N*H*), 6.05 (m, 2H, C*H*CH_3), 5.17 (m, 2H, Glu–C*H*), 4.48 (m, 4H, C$H_2$$CH_2$COO), 3.70 (m, 8H, Glu–C$H_2$$CH_3$), 3.65 (m, 12H, C$H_3$) 3.58 (s, 6H, OC$H_3$), 3.24 (m, 4H, C$H_2$COO), 2.40 (m, 4H, Glu–C$H_2$), 2.29 (d, 6H, CHC$H_3$), 1.75 (m, 4H, Glu–C$H_2$), 0.90 (m, 12H, Glu–$CH_2CH_3$), −3.9 (s, 2H, pyrrole-*H*). MALDI-TOF/MS: 997.4 [M^++H]. Data for **6**. Yield: 78%. ^{1}H NMR ($CDCl_3$, ppm): 10.64, 10.62, 10.25, 10.14 (m, 4H, *meso-H*), 7.21 (br, 2H, amide N*H*), 6.20 (m, 2H, C*H*CH_3), 5.15 (m, 2H, Glu–C*H*), 4.48 (m, 4H, C$H_2$$CH_2$COO), 3.91 (m, 8H, Glu–C$H_2$$CH_3$), 3.70 (m, 20H, C$H_2CH_2$O, CH_3), 3.44 (s, 6H, OCH_3), 3.22 (m, 4H, CH_2COO), 2.39 (m, 4H, Glu–CH_2), 2.30 (d, 6H, CHCH_3), 1.71 (m, 4H, Glu–CH_2), 0.90 (m, 12H, Glu–CH_2CH_3), −3.72 (s, 2H, pyrrole-*H*). MALDI-TOF/MS: 1086.3 [M^++H]. Data for **7**. Yield: 80%. ^{1}H NMR ($CDCl_3$, ppm): 10.60, 10.58, 10.26, 10.13 (m, 4H, *meso-H*), 7.21 (br, 2H, amide N*H*), 6.20 (m, 2H, C*H*CH_3), 5.15 (m, 2H, Glu–C*H*), 4.48 (m, 4H, C$H_2$$CH_2$COO), 3.91 (m, 8H, Glu–C$H_2$$CH_3$), 3.70 (m, 36H, C$H_2CH_2$O, CH_3) 3.28 (s, 6H, OCH_3), 3.22 (m, 4H, CH_2COO), 2.39 (m, 4H, Glu–CH_2), 2.30 (d, 6H, CHCH_3), 1.90 (m, 4H, Glu–CH_2), 0.80 (m, 12H, Glu–CH_2CH_3), −3.74 (s, 2H, pyrrole-*H*). MALDI-TOF/MS: 1262.7 [M^++H]. Data for **8**. Yield: 60%. ^{1}H NMR ($CDCl_3$, ppm): 10.62, 10.55, 10.20, 10.17 (m, 4H, *meso-H*), 7.25 (br, 2H, amide N*H*), 6.28 (m, 2H, C*H*CH_3), 5.19 (m, 2H, Glu–C*H*), 4.41 (m, 4H, C$H_2$$CH_2$COO), 3.87 (m, 8H, Glu–C$H_2$$CH_3$), 3.65 (m, 68H, C$H_2CH_2$O, CH_3), 3.30 (s, 6H, OCH_3), 3.25 (m, 4H, CH_2COO), 2.36 (m, 4H, Glu–CH_2), 2.29 (d, 6H, CHCH_3), 1.91 (m, 4H, Glu–CH_2), 0.82 (m, 12H, Glu–CH_2CH_3), −3.76 (s, 2H, pyrrole-*H*).
18. Synthesis of compounds **9**–**13**: A solution of 1 M KOH (1.8 mL) was added to a solution of **5**–**8** (0.4 mmol) in methanol (50 mL), which was stirred for 12 h at room temperature. The solution was evaporated to dryness under reduced pressure and the residual solid was dissolved in water (15 mL). To this solution was added *cis*-Pt(dach)(SO_4)(H_2O) (0.38 g, 0.9 mmol) or *cis*-Pt(dmpda)(SO_4)(H_2O) (0.37 g, 0.9 mmol) in water (15 mL) and the reaction solution was further reacted for 5 h at room temperature. The brown precipitate was filtered, washed with methanol and ether, and dried under vacuum. Data for **9**. Yield: 80%. ^{1}H NMR (DMF-d_7, ppm): 10.75, 10.37 (m, 4H, *meso-H*), 6.40 (m, 2H, C*H*CH_3), 5.15 (br, 2H, Glu–C*H*), 4.43 (br, 4H, C$H_2$$CH_2$COO), 3.91–3.12 (m, 22H, CH_3, OCH_3, CH_2COO), 2.39–2.0 (br, 14H, Glu–CH_2, CHCH_3, dach–C*H*), 1.80–1.0 (m, 20H, Glu–CH_2, dach–C*H*), −3.65 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3310, 3222, 1632, 1604, 1544, 1446, 1374. Anal. Calcd ($C_{58}H_{80}N_{10}O_{12}Pt_2$·$5H_2O$): C, 43.83; H, 5.67; N, 8.16. Found: C, 43.69; H, 5.43; N, 8.44. ESI/MS: 1522 [M+Na], 1229 [M-Pt(dach)+K], 1214 [M-Pt(dach)-CH_3+K]. Data for **10**. Yield: 80%. ^{1}H NMR (DMF-d_7, ppm): 10.72, 10.34 (m, 4H, *meso-H*), 6.37 (m, 2H, C*H*CH_3), 5.19 (br, 2H, Glu–C*H*), 4.40 (br, 4H, C$H_2$$CH_2$COO), 3.92–3.10 (m, 30H, CH_3, CH_2CH_2O, OCH_3, CH_2COO), 2.45–1.96 (br, 14H, Glu–CH_2, CHCH_3, dach–C*H*), 1.77–0.99 (m, 20H, Glu–CH_2, dach–C*H*), −3.66 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3312, 3230, 1636, 1610, 1544, 1446, 1374. Anal. Calcd ($C_{62}H_{88}N_{10}O_{14}Pt_2$·$3H_2O$): C, 45.37; H, 5.73; N, 8.54. Found: C, 45.40; H, 5.57; N, 8.19. ESI/MS: 1633 [M+2Na], 1332 [M-Pt(dach)-CH_3+3Na]. Data for **11**. Yield: 75%. ^{1}H NMR (DMF-d_7, ppm): 10.75, 10.37 (m, 4H, *meso-H*), 6.40 (m, 2H, C*H*CH_3), 5.15 (br, 2H, Glu–C*H*), 4.43 (br, 4H, C$H_2$$CH_2$COO), 3.91–3.12 (m, 46H, CH_3, CH_2CH_2O, OCH_3, CH_2COO), 2.39–2.0 (br, 14H, Glu–CH_2, CHCH_3, dach–C*H*), 1.80–1.0 (m, 20H, Glu–CH_2, dach–C*H*), −3.65 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3304, 3225, 1628, 1602, 1446, 1378. Anal. Calcd ($C_{70}H_{104}N_{10}O_{18}Pt_2$): C, 47.67; H, 5.90; N, 7.95. Found: C, 47.8; H, 6.00; N, 7.82. ESI/MS: 1199 [M-2Pt(dach)-CH_3+3Na]. Data for **12**. Yield: 70%. ^{1}H NMR (DMF-d_7, ppm): 10.75, 10.37 (m, 4H, *meso-H*), 6.40 (m, 2H, C*H*CH_3), 5.15 (br, 2H, Glu–C*H*), 4.43 (br, 4H, C$H_2$$CH_2$COO), 3.91–3.12 (m, 78H, CH_3, CH_2CH_2O, OCH_3, CH_2COO), 2.39–2.0 (br, 14H, Glu–CH_2, CHCH_3, dach–C*H*), 1.80–1.0 (m, 20H, Glu–CH_2, dach–C*H*), −3.65 (s, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 3290, 3186, 1624, 1580, 1440. Anal. Calcd ($C_{86}H_{136}N_{10}O_{26}Pt_2$·$10H_2O$): C, 44.99; H, 6.80; N, 6.10. Found: C, 43.22; H, 6.51; N, 6.23. Data for **13**. Yield: 78%. ^{1}H NMR (DMF-d_7, ppm): 10.75, 10.37 (m, 4H, *meso-H*), 6.40 (m, 2H, C*H*CH_3), 5.15 (br, 2H, Glu–C*H*), 4.43 (br, 4H, C$H_2$$CH_2$COO), 3.91–3.12 (m, 46H, CH_3, CH_2CH_2O, OCH_3, CH_2COO), 2.39–2.0 (br, 14H, Glu–CH_2, CHCH_3, dmpda-CH_2), 1.80–1.0 (m, 20H, Glu–CH_2, dmpda-CH_3), −3.65 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3300, 3198, 1634, 1590, 1448. Anal. Calcd ($C_{68}H_{104}N_{10}O_{18}Pt_2$·$3H_2O$): C, 45.54; H, 6.14; N, 7.81. Found: C, 45.80; H, 5.65; N, 7.73. ESI/MS: 1779 [M+K], 1506 [M-Pt(dmpda)-CH_3+2K].

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