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Synthesis, Biodistribution and Antitumor Activity of Hematoporphyrin–Platinum(II) Conjugates

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Abstract—A new series of platinum(II) complexes of pegylated hematoporphyrin derivatives with controlled hydrophobic/hydrophilic balance were synthesized by introducing different kinds of poly(ethylene glycol) and amine ligands to the porphyrin ring. The antitumor activity of the porphyrin–platinum(II) conjugates was assayed in vitro and in vivo against leukemia L1210 cell line and various human tumor cell lines. The present complexes exhibited high antitumor activity and improved water solubility as well as considerable lipophilicity. In particular, complex **16** showed not only higher in vivo activity (T/C% = 258) than cisplatin (T/C% = 184) and carboplatin (T/C% = 168), but also excellent solubility in water and organic solvent. The antitumor activity of complex **20** was superior to that of carboplatin against all human tumor cell lines tested. Moreover, some amphiphilic complexes (**7** and **12**) exhibited elevated tumor-localizing effect (tumor/muscle ratio > 2).

Introduction

Cisplatin is most effective for the treatment of ovarian, testicular, head and neck cancers,[1–3] but its severe side effects such as nephotoxicity and ototoxicity remain as one of the major problems in the clinical use of the drug.[4–5] These dose-related strong side effects are in great part due to the lack of selectivity of the drug to tumor tissue and may be reduced by more selective enrichment of cisplatin in the tumor tissue compared to the normal tissue. A variety of strategies and vector systems have been investigated in attempts for selective delivery of anticancer drugs to the tumor tissue.[6–8]

One rational approach to design tumor-targeting platinum(II) complexes is to introduce a suitable carrier ligand which tends to accumulate in the tumor tissue. Some porphyrins are known to selectively accumulate in the tumor tissue.[9–11] In our previous work, sulfonatoporphyrin–platinum(II) conjugates exhibited significant tumor-targeting effect (tumor/muscle ratio = 7).[12] Porphyrins play important roles in photodynamic therapy and fluorescence imaging, which are based on the preferential uptake and retention of the porphyrins by tumor tissue.[13–21] Although the exact mechanism of porphyrin uptake by tumor tissue is still unknown, it is most likely that the porphyrins are carried by plasma low density lipoproteins (LDL) and incorporated into the tumor cell via receptor mediated endocytosis of LDL, since cancer cells express elevated levels of LDL receptors.[22] Brunner et al. reported recently the antitumor activity of a series of platinum(II) complexes bearing porphyrin ligands that revealed the additive cytotoxic and photodynamic effects. [23–25]

The tumor-targeting properties of porphyrins are known to be dependant on their hydrophobicity and hydrophilicity balance.[26] In general, insolubility of most porphyrin derivatives in aqueous solution causes serious problems in biological applications, but some amphiphilic porphyrins are known to selectively accumulate in tumor tissues.[27] A systematic variation of the amphiphilic properties requires a regiochemical arrangement of hydrophobic and hydrophilic substituents in the structure.[16,28] In this work, the poly(ethylene glycol) (PEG) side chains with different lengths were attached covalently to hematoporphyrin for effective improvement in water solubility and modulation of hydrophilicity/hydrophobicity balance, and then antitumor

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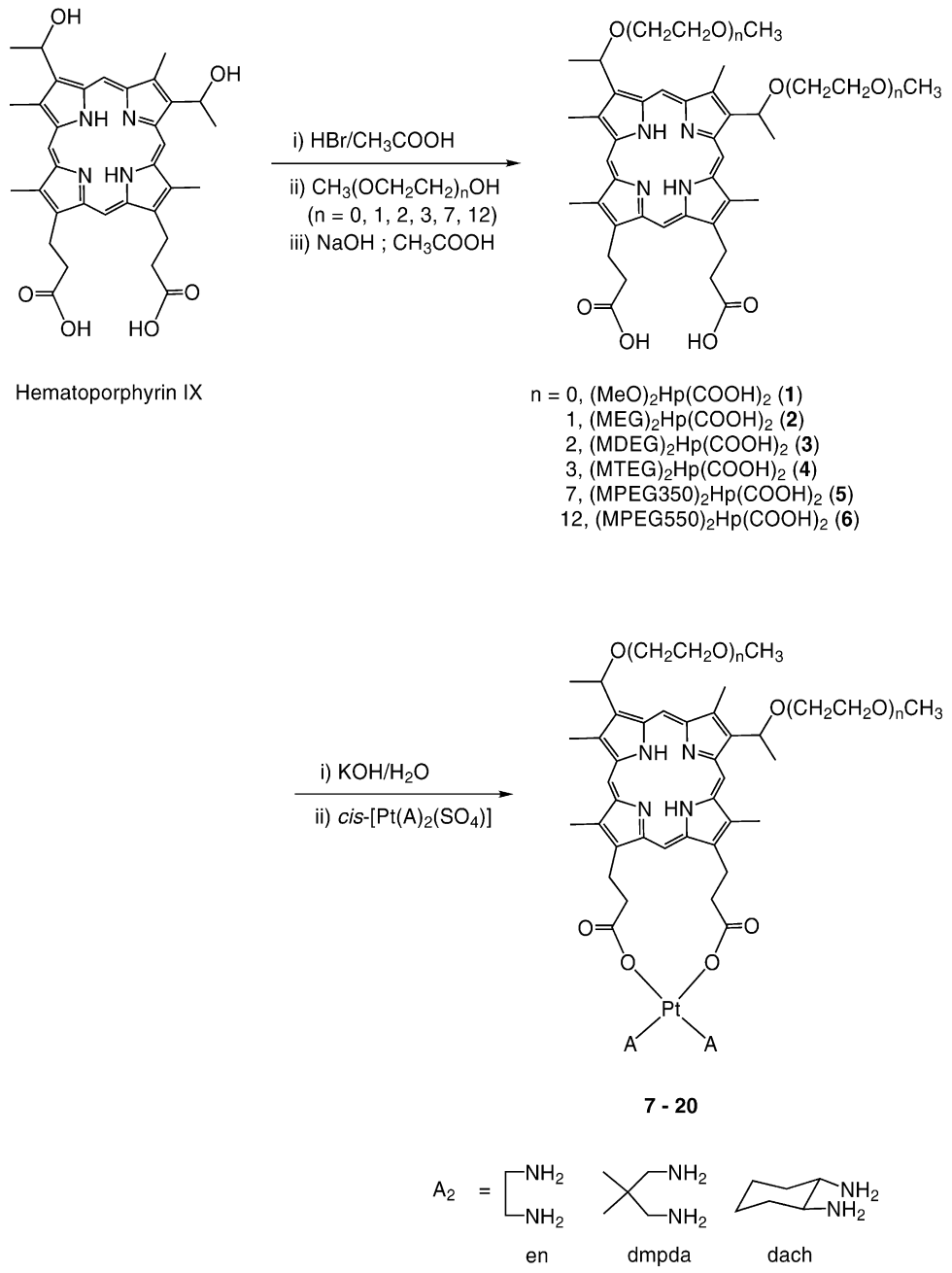

Scheme 1. The synthetic route to hematoporphyrin derivatives and their Pt(II) conjugates.

(diamine)Pt(II) moiety was chelated by the carboxylate groups of the porphyrin. Such chemical modification of the drugs with PEG is expected to improve their internalization efficiency, to resist protein adsorption, and to increase their circulation time.[29–31] We report here synthesis, biodistribution and antitumor activity of hematoporphyrin–platinum(II) conjugates.

Results and Discussion

Synthesis and characterization

PEG conjugated porphyrin ligands were prepared by the literature method (Scheme 1).[10,23] The ^{1}H NMR spectra of porphyrin diacid ligands showed the resonance of two internal pyrrol protons at −3.9 ppm as a singlet. The resonance of the *meso* protons appeared at 10.4–10.0 ppm as four separated peaks with the ratios of 1:1:1:1, which implies high asymmetry of the porphyrin macrocycle. The resonances of CH protons and adjacent methyl protons on the side chains appeared at 6.2 ppm as multiplet and 2.1 ppm as doublet, respectively. On the other hand, the resonance of methyl protons on the porphyrin was shown down field (3.7 ppm) due to the well known anisotropic effect resulting from the ring current of the porphyrin macrocycle.[32] All porphyrin ligands exhibited strong C=O stretching vibrations in the range of 1722–1705 cm^{-1} and pyrrole NH stretching bands at near 3313 cm^{-1}.

Porphyrin–Pt(II) conjugates were synthesized by the reaction of potassium salt of the porphyrin ligand and

(diamine)platinum(II) sulfate in water (Scheme 1). A 14-membered metallacycle was formed from this reaction. The ^{1}H NMR spectra of a series of porphyrin-(dach)Pt(II) conjugates showed the resonance of (dach) protons at a slightly higher field than the unconjugated (dach)Pt(II) complexes (0.7–1.9 ppm compared to 1.0–2.2 ppm). The anisotropic effect seems to be not strongly involved because the platinum moiety is conjugated through the propionic acid chain far from the porphyrin macrocycle. Likewise, the resonance of the methyl and methylene protons of the dmpda ligand of the porphyrin-(dmpda)Pt conjugates appeared at 0.5 and 1.9 ppm, respectively. The resonance of the *meso* protons appeared at 10.8–10.3 ppm as four separated peaks showing the high asymmetry of the porphyrin macrocycle, which were resultant from coordination of the platinum(II) moiety to one side of the porphyrin. All complexes exhibited strong C=O stretching vibration in the range of 1618–1606 cm^{-1}, which is characteristic of coordinated carboxylate ligand. The ^{195}Pt NMR spectra of complexes **14** and **15** showed a single ^{195}Pt resonance at −1821.9 and −1747.4 ppm, respectively, compared to −1550 ppm for unconjugated (diamine)platinum(II) sulfate complex.

Hydrophobicity is one of the most important factors related to drug absorption through the biological membrane. In the photodynamic field, the most demanding criterion is the solution properties, rather than photophysical chemistry.[18] The partition coefficient of the representative conjugates was determined in water/*n*-octanol system and the results are shown in Table 1. The conjugation of the platinum moiety to the porphyrin ligands increased the hydrophobicity by a factor of about 5. In the case of compounds **16**–**18**, the difference of the amine ligand of the platinum moiety did not significantly affect the hydrophobicity. Complexes **16**–**20** are presumed to be amphiphilic, since they are water soluble as well as considerably lipophilic.

Table 1. Partition coefficients of the conjugates

Compd.	Octanol/water partition ratio	Partition coefficient (log *P*)
4	5.58	0.75
7	8.69	0.94
15	7.56	0.88
16	2.45	0.39
17	1.70	0.23
18	2.52	0.40
20	1.10	0.04

Antitumor activity

The antitumor activity of porphyrin–Pt(II) conjugates were assayed in vitro and in vivo against leukemia L1210 cell line, and the results are listed in Table 2. The porphyrin ligands were cytotoxic in vitro but inactive in vivo. Therefore, it is presumed that the in vivo activity of the conjugates comes from the conjugated antitumor platinum moiety. The in vivo activities of all complexes except porphyrin–(en)Pt(II) conjugates are comparable or superior to those of carboplatin. In particular, complex **16** exhibited not only higher in vivo activity (T/C% = 258) than cisplatin and carboplatin, but also excellent solubility in water and organic solvent. The antitumor activity of the present conjugates is shown to be dependent on the structure of the amine ligand and the PEG side chains. Comparing the series of the porphyrin–Pt(II) conjugates bearing the same ethylene oxide units, the (dach)Pt(II) and (dmpda)Pt(II) complexes are comparable to each other but more active than the (en)Pt(II) complexes in in vivo activity. Such results are unexpected because most platinum(II) complexes having (dach) ligand are known to be more active than those bearing other amine ligand such as dmpda and en.[33] Inactivity of complexes **7** and **8** seems to be due to their low solubility in aqueous solution.

Table 2. In vitro and in vivo activities of hematoporphyrin derivatives and their platinum(II) conjugates against leukemia L1210 cell line

Compd	Chemical formula	In vitro	In vivo	
		ED_{50} (μg/mL)	Dosage (mg/kg)	T/C (%)
Cisplatin	$(NH_3)_2PtCl_2$	0.3	4	184
Carboplatin	$(NH_3)_2Pt(CBDC)$	3.8	40	168
2	$(MEG)_2Hp(COOH)_2$	2.6	60/30	97/107
3	$(MDEG)_2Hp(COOH)_2$	3.7		
4	$(MTEG)_2Hp(COOH)_2$	6.6	60/30	102/106
5	$(MPEG350)_2Hp(COOH)_2$	>40.0		
7	$(MeO)_2Hp(COO)_2Pt(dach)$	>40.0	100	107
8	$(MeO)_2Hp(COO)_2Pt(dmpda)$	>40.0		
9	$(MEG)_2Hp(COO)_2Pt(dach)$	>40.0	100	132.6
10	$(MEG)_2Hp(COO)_2Pt(dmpda)$	4.3		
11	$(MDEG)_2Hp(COO)_2Pt(dach)$	8.1	40/20	166/158
12	$(MDEG)_2Hp(COO)_2Pt(dmpda)$	2.7	40/20	193/151
13	$(MDEG)_2Hp(COO)_2Pt(en)$	15.7	20	99
14	$(MTEG)_2Hp(COO)_2Pt(dach)$	23.1	60/30	120/118
15	$(MTEG)_2Hp(COO)_2Pt(dmpda)$	6.3	60/30	115/113
16	$(MPEG350)_2Hp(COO)_2Pt(dach)$	21.8	60/30	258/215
17	$(MPEG350)_2Hp(COO)_2Pt(dmpda)$	2.8	60/30	174/144
18	$(MPEG350)_2Hp(COO)_2Pt(en)$	15.5	60/30	111/105
19	$(MPEG550)_2Hp(COO)_2Pt(dach)$	22.4	60/30	137/137
20	$(MPEG550)_2Hp(COO)_2Pt(dmpda)$	2.4	60/30	205/206

Table 3. In vitro activity (ED_{50}, μM) against human cancer cell lines of lung (A 549), ovarian (SK-OV-3), melanoma (SK-MEL-2), brain (XF 498) and colon (HCT 15)

No.	Compd	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
16	$(MPEG350)_2HpPt(dach)$	2.08	7.59	7.03	>19.35	11.15
20	$(MPEG550)_2HpPt(dmpda)$	0.76	1.38	1.77	1.47	0.63
	Cisplatin	1.29	2.94	2.81	2.36	1.80
	Carboplatin	21.73	52.86	54.10	29.73	37.78

Compounds **16** and **20** exhibiting high antitumor activity against the murine leukemia cell line were subjected to further investigation of their cytotoxicity toward various human tumor cell lines, and the results are listed in Table 3. Enhanced cytotoxicity was observed for both compounds relative to carboplatin and cisplatin. In particular, compound **20** exhibited the antitumor activity superior to carboplatin and cisplatin against all human cancer cell lines tested. The ED_{50} values of compound **20** were 1.38, 1.77 and 0.63 μM for the SK-OV-3, SK-MEL-2 and XF498 cell lines, respectively, which represent about 50 times higher activity as compared with carboplatin (52.86, 54.10 and 37.78 μM, respectively). The antitumor activity of the present pegylated porphyrin–Pt(II) conjugates was significantly improved probably due to the excellent solubility and facility of intracellular internalization of the antitumor platinum moiety.[34]

Biodistribution

The biodistribution study of representative porphyrin–Pt(II) conjugates was performed using tumor bearing mouse, and analysis of the complexes in each organ was carried out using ICP-MS (Model ELAN5000, Perkin Elmer, Norwalk, CT).[21] The time dependent biodistribution pattern of complexes, **7**, **12**, **18** and carboplatin at 2 and 24 h after injection is illustrated in Fig. 1. The Pt-concentrations of all the compounds in liver, kidney and lung were high, which is probably due to the large number of LDL receptors in those tissues. The biodistribution pattern of relatively hydrophobic conjugates **7** and **12** is similar to that of the porphyrin compounds reported in the literature.[21,35] Especially, the tropism for liver appears to be shared with others of the porphyrin family.[36,37] On the other hand, the distribution pattern of hydrophilic compound **18** is more similar to that of carboplatin showing highest concentration in kidney at the time interval tested. The tumor/muscle ratios of the complexes **7**, **12** and **18** are 1.5, 1.9 and 2.2, respectively at 2 h after injection and 2.1, 2.5 and 1.6 at 24 h after injection, respectively. In the case of complexes **7** and **12**, the drug concentration in tumor tissue tends to increase with time whereas that of complex **18** decreases like carboplatin. The tumor/muscle ratio of carboplatin after 2 and 24 h are 1.3 and 1.2, respectively, showing no affinity to the tumor tissue. The total amount of platinum in the case of complex **7** recovered from the biological samples was 69.8 mg/kg at 2 h and 55.2 mg/kg at 24 h, whereas the total amount of platinum recovered in the case of complex **18** was 7.7 mg/kg at 2 h and 4.2 mg/kg at 24 h. The amounts of complex **18** in the body decrease very rapidly probably due to its high hydrophilicity and exhibited high concentration in kidney like carboplatin. Complex **12** exhibited the intermediate biodistribution pattern, that is, high concentration in both liver and kidney, and the clearance rate is in the middle of those of complexes **7** and **18**. In this study to afford balanced water solubility and hydrophobicity of the porphyrin–platinum(II) conjugates, complexes **7** and **12** exhibited elevated tumor-localizing effect (tumor/muscle ratio > 2) as compared to carboplatin, but the very hydrophilic complex **18** did not exhibit any tumor selectivity, which is consistent with the results in the literature.[27]

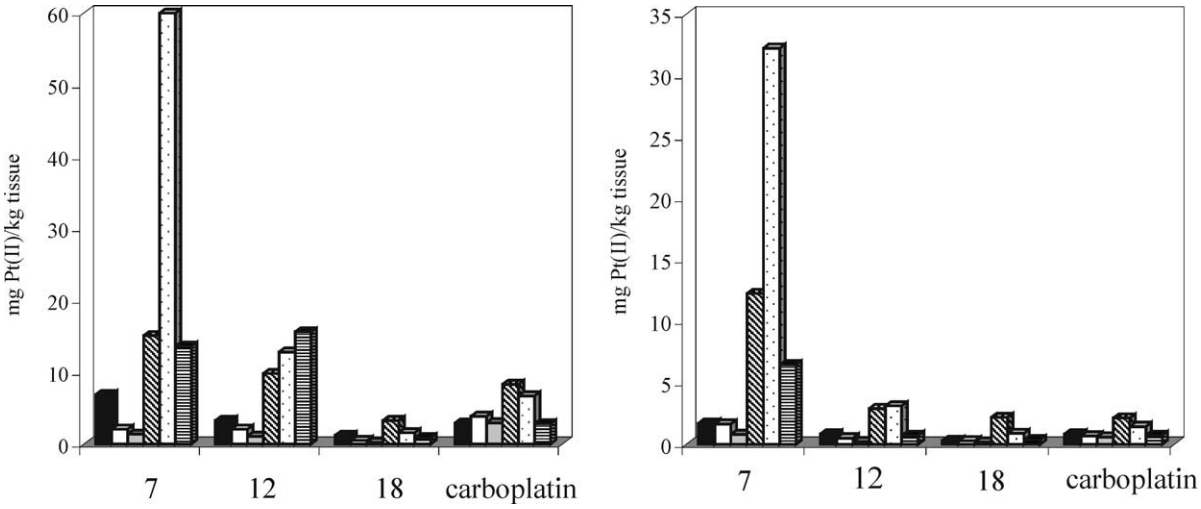

Figure 1. The biodistribution pattern of porphyrin–Pt(II) conjugates and carboplatin in tumor bearing mouse. Blood (■); tumor (□); muscle (▒); kidney (▨); liver (▫); lung (▤).

Conclusions

A new series of platinum(II) complexes of pegylated hematoporphyrin derivatives with controlled hydrophobic/hydrophilic balance were synthesized, by introducing different kinds of poly(ethylene glycol) and amine ligands to the porphyrin ring. Pegylated hematoporphyrin–platinum(II) conjugates revealed enhanced antitumor activity with improved water solubility over carboplatin. In particular complex **16** exhibited higher activity than carboplatin against murine leukemia cell line, and the antitumor activity of complex **20** was superior to that of carboplatin against all human tumor cell lines tested. Moreover, some amphiphilic complexes (**7** and **12**) exhibited elevated tumor-localizing effect (tumor/muscle ratio > 2).

Experimental

Materials and measurements

Potassium tetrachloroplatinate(II) purchased from Kojima, hematoporphyrin IX, *trans*-(±)-1,2-diaminocyclohexane (dach), 2,2-dimethyl-1.3-propanediamine (dmpda), 1,2-diaminoethane (en), diethyleneglycol monomethylether (MDEG) and ethyleneglycol monomethylether (MEG) from Aldrich, MPEG550, MPEG350, triethyleneglycol monomethylether (MTEG) and 33% HBr-acetic acid solution from Fluka were used without further purification. The porphyrin ligands, $(MeO)_2Hp(COOH)_2$ (**1**), $(MDEG)_2Hp(COOH)_2$ (**3**), $(MTEG)_2Hp(COOH)_2$ (**4**) and $(MPEG550)_2Hp(COOH)_2$ (**6**) were prepared according to procedures reported in the literature.[10,23] Elemental analyses were performed by the Advanced Analysis Center at KIST. UV–vis spectra were recorded on a Hewlett-Packard 8453 spectrometer. Mass spectra were recorded at Korea Basic Science Institute. IR spectra were recorded as KBr pellets on a Perkin Elmer 16F PC FT-IR spectrometer. ^{1}H NMR spectra were measured with a Bruker 250 MHz spectrometer. ^{195}Pt NMR spectra were measured with a Varian Gemini-300 NMR spectrometer using K_2PtCl_4 in D_2O (^{195}Pt, −1628 ppm) as the external standard. The partition coefficient of the platinum complexes was determined in a water/*n*-octanol system. The combined phases were shaken by Vortex for 10 min, centrifuged for 20 min at 4000 t/min, and each phase was analyzed by spectrophotometry.

Synthesis of hematoporphyrin derivatives, $R_2Hp(COOH)_2$

The hematoporphyrin derivatives **1**–**6** were prepared by a similar method reported in the literature.[10,23] Briefly, hematoporphyrin IX (5 g, 8.35 mmol) was stirred for 4 h in 33% HBr-acetic acid solution (200 mL) at room temperature, and then the solution was evaporated to dryness under reduced pressure. The resultant HBr adduct was dissolved in corresponding alcohol (30–40 mL), and stirred for 24 h at 60 °C. The reaction mixture was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain a purple oil. The oil was dissolved in methanol (100 mL) and NaOH (3.2 g, 80 mmol) was added. The solution was stirred overnight at room temperature and methanol was evaporated under reduced pressure. The residual oil was dissolved in water (50 mL), and the aqueous solution was acidified to pH 4 by addition of acetic acid and extracted with chloroform. The solid product was purified by flash column chomatography (silica gel (Merck, 70–230 mesh, 60 Å), CH_2Cl_2/methanol = 10:1, v/v).

$(MeO)_2Hp(COOH)_2$ (1). Yield, 80%. ^{1}H NMR (DMSO-d_6, δ): 10.6–10.2 (m, 4H, meso-*H*), 6.1 (m, 2H, C*H*CH$_3$), 4.3 (m, 4H, CH_2CH$_2$COO), 3.7–3.5 (m, 18H, OCH_3, CH_3), 3.16 (m, 4H, CH_2COO), 2.1 (d, CHCH_3), −3.9 (s, 2H, pyrrole-H).

$(MEG)_2Hp(COOH)_2$ (2). Yield, 75%. Anal. calcd ($C_{40}H_{50}N_4O_8$): C, 67.21; H, 7.05; N, 7.87. Found: C, 67.0; H, 6.97; N, 7.70. ^{1}H NMR (DMSO-d_6, δ): 10.6–10.2 (m, 4H, meso-*H*), 6.1 (m, 2H, C*H*CH3), 4.3 (m, 4H, CH_2CH$_2$COO), 3.9–3.1 (m, 30H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.1 (d, 6H, 6.3 Hz, CHCH_3), −3.9 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3310 w, 1720 s, 1448 m, 1374 m, 1198 m, 1094 s. MALDI-TOF/MS: 715.7 (M + H).

$(MDEG)_2Hp(COOH)_2$ (3). Yield, 75%. ^{1}H NMR (DMSO-d_6, δ): 10.6–10.2 (m, 4H, meso-*H*), 6.3 (m, 2H, C*H*CH$_3$), 4.3 (m, 4H, CH_2CH$_2$COO), 3.9–3.1 (m, 38H, CH_2COO, CH_2CH_2O, OCH_3, CH_3), 2.1 (d, 6H, 6.5 Hz, CHCH_3), −3.9 (s, 2H, pyrrole-*H*).

$(MTEG)_2Hp(COOH)_2$ (4). Yield, 73%. ^{1}H NMR (DMSO-d_6, δ): 10.6–10.2 (m, 4H, meso-*H*), 6.3 (m, 2H, C*H*CH$_3$), 4.3 (m, 4H, CH_2CH$_2$COO), 3.9–3.1 (m, 46H, CH_2COO, CH_2CH_2O, OCH_3, CH_3), 2.1 (d, 6H, 6.5 Hz, CHCH_3), −3.9 (s, 2H, pyrrole-*H*). MALDI-TOF/MS: 892.1 (M + H).

$(MPEG350)_2Hp(COOH)_2$ (5). This compound was used in situ for the next step. Yield, 40%. ^{1}H NMR (DMSO-d_6, δ): 10.7–10.2 (m, 4H, meso-*H*), 6.3 (m, 2H, C*H*CH$_3$), 4.3 (m, 4H, CH_2CH$_2$COO), 3.9–3.1 (m, 78H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 6.5 Hz, CHCH_3), −3.9 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3312 w, 1722 s, 1562 m, 1456 m, 1230 m, 1100 m. MALDI-TOF/MS: 1200.9 (M + H − CH_2CH_2O), 1244.8 (M + H), 1288.9 (M + H + CH_2CH_2O).

$(MPEG550)_2Hp(COOH)_2$ (6). This compound was used in situ for next step. Yield, 35%. ^{1}H NMR (DMSO-d_6, δ): 10.7–10.2 (m, 4H, meso-*H*), 6.2 (m, 2H, C*H*CH$_3$), 4.3 (m, 4H, CH_2CH$_2$COO), 3.9–3.1 (m, 118H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.1 (d, 6H, 6.5 Hz, CHCH_3), −3.9 (s, 2H, pyrrole-*H*).

Synthesis of porphyrin–Pt(II) conjugates, $[R_2Hp(COO)_2Pt(A)_2]$

$(MeO)_2Hp(COO)_2Pt(dach)$ (7). **1** (0.28 g, 0.3 mmol) and KOH (34 mg, 0.6 mmol) were dissolved in water (20 mL) and the solution was stirred for 30 min. To the solution was added *cis*-[Pt(A)$_2$(SO)$_4$] (0.12 g, 0.3 mmol)

in water (15 mL) and the reaction mixture was stirred overnight at room temperature. It was extracted with chloroform and was purified by flash column chomatography (silica gel (Merck, 70-230 mesh, 60 Å), CH_2Cl_2/methanol = 10:1, v/v). Yield, 80%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.4–6.2 (m, 4H, C*H*CH$_3$, N*H*), 4.8–4.6 (br, s, 2H, N*H*), 4.3 (m, 4H, CH_2CH$_2$COO), 3.8–3.2 (m, 22H, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 6.45 Hz, CHCH_3), 1.9 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.2 (m, 2H, dach-C*H*), 1.1 (m, 2H, dach-C*H*), 0.7 (m, 2H, dach-C*H*), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 1606 s, 1448 m, 1376 s, 1112 m, 1086 m. MALDI-TOF/MS: 935.9 (MH$^+$). Anal. calcd ($C_{42}H_{54}N_6O_6Pt$): C, 54.01; H, 5.83; N, 9.00. Found: C, 53.6; H, 5.81; N, 8.83.

Compounds **8–20** were prepared by the same method.

(MeO)$_2$Hp(COO)$_2$Pt(dmpda) (8). Yield, 35%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.5–6.3 (m, 4H, C*H*CH$_3$, N*H*), 4.5–4.3 (m, 6H, CH_2CH$_2$COO, N*H*), 4.0–3.1 (m, 22H, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 6.09 Hz, CHCH_3), 1.9 (m, 4H, dmpda-CH_2), 0.5 (m, 6H, dmpda-CH_3), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 1604 s, 1443 m, 1376 s, 1230 m, 1110 m, 1086 m. MALDI-TOF/MS: 923.9 (MH$^+$). Anal. calcd ($C_{41}H_{54}N_6O_6Pt$): C, 53.41; H, 5.90; N, 9.12. Found: C, 53.1; H, 6.02; N, 8.96.

(MEG)$_2$Hp(COO)$_2$Pt(dach) (9). Yield, 83%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.4–6.2 (m, 4H, C*H*CH$_3$, N*H*), 4.8–4.6 (br, s, 2H, N*H*), 4.3 (m, 4H, CH_2CH$_2$COO), 4.0–3.1 (m, 30H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 5.88 Hz, CHCH_3), 1.9 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.2–1.0 (m, 4H, dach-C*H*), 0.7 (m, 2H, dach-C*H*), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 1606 s, 1448 m, 1374 s, 1313 m, 1098 s. MALDI-TOF/MS: 1023.6 (MH$^+$). Anal. calcd ($C_{46}H_{62}N_6O_8Pt$): C, 54.05; H, 6.11; N, 8.22. Found: C, 53.72; H, 6.02; N, 8.13.

(MEG)$_2$Hp(COO)$_2$Pt(dmpda) (10). Yield, 45%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.5–6.3 (m, 4H, C*H*CH$_3$, N*H*), 4.4 (m, 6H, CH_2CH$_2$COO, N*H*), 4.0–3.1 (m, 30H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 6.09 Hz, CHCH_3), 1.9 (m, 4H, dmpda-CH_2), 0.5 (m, 6H, dmpda-CH_3), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 1603 s, 1458 m, 1376 s, 1230 m, 1098 s. MALDI-TOF/MS: 1010.8 (MH$^+$). Anal. calcd ($C_{45}H_{62}N_6O_8PtH_2O$): C, 52.57; H, 6.27; N, 8.17. Found: C, 52.4; H, 6.20; N, 8.02.

(MDEG)$_2$Hp(COO)$_2$Pt(dach) (11). Yield, 55%. ^{1}H NMR (DMF-d_7): 10.8–10.3 (m, 4H, meso), 6.20 (m, 2H, C*H*CH$_3$), 6.1 (br, 2H, NH_2), 4.4 (s, br, 2H, NH$_2$), 4.2 (m, 4H, CH_2COO), 3.9–3.1 (m, 26H, CH_2CH$_2$COO, CH_2CH_2OCH_3, CH_3), 2.1 (d, 6H, CH_3CH), 1.8 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.1 (m, 4H, dach-C*H*), 0.6 (m, 2H, dach-C*H*),-3.7 (m, 2H, pyrrol). IR (KBr, cm^{-1}): 1603s, 1376s, 1098s. MS (MALDI-TOF): 1111.9 (MH$^+$). Anal. calcd ($C_{50}H_{70}N_6O_{10}Pt$): C, 54.09; H, 6.36; N, 7.57. Found: C, 55.0; H, 6.28; N, 7.52.

(MDEG)$_2$Hp(COO)$_2$Pt(dmpda) (12). Yield, 55%. ^{1}H NMR (DMF-d_7): 10.8–10.3 (m, 4H, meso), 6.20 (m, 2H, C*H*CH$_3$), 6.1 (br, 2H, NH_2), 4.4 (s, br, 2H, NH$_2$), 4.2 (m, 4H, CH_2COO), 3.9–3.1 (m, 26H, CH_2CH$_2$COO, CH_2CH_2OCH_3, CH_3), 2.1 (d, 6H, CH_3CH), 1.6 (m, 4H, dmpda-CH_2), 0.4 (s, 6H, dmpda-CH_3), −3.7 (m, 2H, pyrrol). MS (MALDI-TOF): 1109.9 (MH$^+$). Anal. calcd ($C_{49}H_{70}N_6O_{10}Pt$): C, 53.59; H, 6.42; N, 7.65. Found: C, 53.51; H, 6.35; N, 7.66.

(MDEG)$_2$Hp(COO)$_2$Pt(en) (13). Yield, 55%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso), 6.20 (m, 2H, C*H*CH$_3$), 6.1 (br, 2H, NH$_2$), 4.4 (s, br, 2H, NH$_2$), 4.2 (m, 4H, CH$_2$Por), 3.9–3.1 (m, 26H, CH_2CH$_2$COO, CH_2CH_2OCH_3, CH_3), 2.5 (s, 4H, *en*-CH_2), 2.1 (d, 6H, CH$_3$CH), −3.7 (m, 2H, pyrrol). IR (KBr, cm^{-1}): 1603s, 1376s, 1098s. Anal. calcd ($C_{46}H_{64}N_6O_{10}Pt$): C, 52.31; H, 6.11; N, 7.96. Found: C, 52.39; H, 6.04; N, 8.03.

(MTEG)$_2$Hp(COO)$_2$Pt(dach) (14). Yield, 65%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.4-6.2 (m, 4H, C*H*CH$_3$, N*H*), 4.8–4.7 (br, s, 2H, N*H*), 4.4–4.3 (m, 4H, CH_2COO), 4.0–3.1(m, 42H, CH_2CH$_2$COO, CH_2CH_2O, OCH_3, CH_3), 2.2 (d, 6H, 3.70 Hz, CHCH_3), 1.9 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.2–1.0 (m, 4H, dach-C*H*), 0.7 (m, 2H, dach-C*H*), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 3310 w, 3200 w, 1608 s, 1448 m, 1368 s, 1312 m, 1102 s. FAB/MS: 1198.6 (MH$^+$). Anal. calcd ($C_{54}H_{78}N_6O_{12}Pt$): C, 54.12; H, 6.56; N, 7.01. Found: C, 53.8; H, 6.52; N, 6.89.

(MTEG)$_2$Hp(COO)$_2$Pt(dmpda) (15). Yield, 45%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.5–6.3 (m, 4H, C*H*CH$_3$, N*H*), 4.4 (m, 6H, CH_2CH$_2$COO, N*H*), 4.0–3.1 (m, 46H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, 5.67 Hz, CHCH_3), 1.9 (m, 4H, dmpda-CH_2), 0.5 (m, 6H, dmpda-CH_3), −3.7 (s, 2H, pyrrole-*H*). IR (KBr, cm^{-1}): 1608 s, 1454 m, 1378 s. MALDI-TOF/MS: 1186.6 (MH$^+$). Anal. calcd ($C_{53}H_{78}N_6O_{12}Pt$): C, 53.66; H, 6.63; N, 7.08. Found: C, 53.3; H, 6.75; N, 6.97.

(MPEG350)$_2$Hp(COO)$_2$Pt(dach) (16). Yield, 75%. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.4 (m, 2H, C*H*CH$_3$), 6.2 (br, s, 2H, NH_2). 4.7 (br, s, 2H, NH_2), 4.4 (m, 4H, CH_2CH$_2$COO), 4.0–3.1 (m, 78H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, CH_3CH), 1.9 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.2 (m, 2H, dach-C*H*), 1.1 (m, 2H, dach-C*H*), 0.7 (m, 2H, dach-C*H*), −3.7 (s, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 1610 s, 1450 m, 1384 m. Anal. calcd ($C_{70}H_{110}N_6O_{20}$P-t2H$_2$O): C, 52.99; H, 7.24; N, 5.30. Found: C, 52.6; H, 7.07; N, 5.19.

(MPEG350)$_2$Hp(COO)$_2$Pt(dmpda) (17). Yield, 55%. Anal. calcd ($C_{69}H_{110}N_6O_{20}Pt3H_2O$): C, 52.03; H, 7.34; N, 5.28. Found: C, 51.7; H, 7.30; N, 5.19. ^{1}H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso-*H*), 6.4 (m, 2H, C*H*CH$_3$), 6.2 (br, s, 2H, N*H2*). 4.7 (br, s, 2H, NH_2), 4.4 (m, 4H, CH_2CH$_2$COO), 4.0–3.1 (m, 78H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, CH_3CH), 1.6 (s. 4H, dmpda-CH_2), 0.4 (s, 6H, dmpda-CH_3), −3.7 (m, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 1618 s, 1446 m, 1376 s, 1174 m, 1102 s.

$(MPEG350)_2Hp(COO)_2Pt(en)$ (18). Yield, 70%. ^1H NMR (DMF-d_7, δ): 10.8–10.3 (m, 4H, meso), 6.4 (m, 2H, C*H*CH_3), 6.2 (br, s, 2H, NH_2). 4.7 (br, s, 2H, NH_2), 4.4 (m, 4H, CH_2CH_2COO), 4.0–3.1 (m, 78H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, CH_3CH), 1.5 (s, 4H, en-CH_2), −3.7 (m, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 1616 s, 1458 m, 1378 m. Anal. calcd ($C_{66}H_{104}N_6O_{20}Pt2H_2O$): C, 51.72; H, 7.10; N, 5.48. Found: C, 51.54; H, 6.98; N, 5.38.

$(MPEG550)_2Hp(COO)_2Pt(dach)$ (19). Yield, 75%. ^1H NMR (DMF-d_7, δ): 10.8–10.2 (m, 4H, meso-*H*), 6.4 (m, 2H, C*H*CH_3), 6.2 (br, s, 2H, NH_2). 4.7 (br, s, 2H, NH_2), 4.4 (m, 4H, CH_2CH_2COO), 4.0–3.1 (m, 118H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, CH_3CH), 1.9 (m, 2H, dach-C*H*), 1.7 (m, 2H, dach-C*H*), 1.2 (m, 2H, dach-C*H*), 1.1 (m, 2H, dach-C*H*), 0.7 (m, 2H, dach-C*H*), −3.7 (m, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 1618 s, 1458 m, 1376 m, 1302. Anal. calcd ($C_{90}H_{150}N_6O_{30}Pt3H_2O$): C, 52.85; H, 7.69; N, 4.11. Found: C, 52.6; H, 7.66; N, 4.03.

$(MPEG550)_2Hp(COO)_2Pt(dmpda)$ (20). Yield, 55%. ^1H NMR (DMF-d_7, δ): 10.8–10.2 (m, 4H, meso-*H*), 6.4 (m, 2H, C*H*CH_3), 6.2 (br, s, 2H, NH_2). 4.7 (br, s, 2H, NH_2), 4.4 (m, 4H, CH_2CH_2COO), 4.0–3.1 (m, 118H, CH_2CH_2O, CH_2COO, OCH_3, CH_3), 2.2 (d, 6H, CH_3CH), 1.6 (s. 4H, dmpda-CH_2), 0.4 (s, 6H, dmpda-CH_3), −3.7 (m, 2H, pyrrol-*H*). IR (KBr, cm^{-1}): 1618s, 1562 s, 1386 m. Anal. calcd ($C_{89}H_{150}N_6O_{30}PtH_2O$): C, 53.52; H, 7.67; N, 4.21. Found: C, 53.2; H, 7.65; N, 4.15.

Bioassay and Biodistribution

Assay of antitumor activity. The antitumor activity of these compounds was assayed in vitro and in vivo against the leukemia L1210 cell line at the Korea Research Institute of Chemical Technology as described in the literature.[38] Human cancer cell lines of lung (A 549), ovarian (SK-OV-3), melanoma (SK-MEL-2), brain (XF 498) and colon (HCT 15) were used for in vitro cytotoxicity test using Sulforhodamine B (SRB) assay. They were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cell cultures were transferred once or twice weekly using trypsin-EDTA to detach the cells from their culture flasks. The rapidly growing cells were harvested, counted, and incubated at the appropriate concentration ($1–2\times10^4$ cells/well) in 96 well microplates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate and incubated for 48 h at 37 °C under 5% CO_2 atmosphere. The cultures were fixed with cold trichloroacetic acid and stained with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound stain with 10 mL of unbuffered tris base solution (pH 10.5) using gyratory shaker, the absorbance at 520 nm was measured with a microplate reader. Cytotoxic activity was evaluated by measuring the concentration of a compound which was required to inhibit the protein synthesis by 50% (ED_{50}) as compared with that of carboplatin and cisplatin.

Biodistribution experiment. Male C57 BL/6N mice (8–9 weeks old, 25–27 g) were adopted for 4 days in the dark and light at intervals of 12 h, and then inoculated subcutaneously with the B16F10 melanoma cells (1×10^6 cells suspended in PBS) in the back region. After 2 weeks, when the tumor was grown up to 10 mm in diameter, the drugs dissolved in saline (20 mg/kg) were injected in a tail vein. The animals were sacrificed at 2 and 24 h after drug administration. Blood samples were collected by heart puncture with a syringe. Tumor, muscle, liver, lung and kidney were removed from animals, and stored at −80 °C for analysis. The analysis of the drugs in the biological samples was based on the measurement of the Pt(II) as reported in the literature.[18] After the samples were treated with c-H_2SO_4, c-HNO_3 and finally *aqua regia*, platinum content was measured by ICP-MS (Model ELAN5000, Perkin Elmer, Norwalk, CT).

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