FULL PAPER

Synthesis of Novel Porphyrin Derivatives and Their Cytotoxic Activities against A431 Cells

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Three novel porphyrins, including two *Schiff*-bases porphyrins, 5,10,15-triphenyl-20-[4-(2-(4-formyl)phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**1**)), 5,10,15-triphenyl-20-[4-(2-(4-hydroxyimino)phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**2**)) and 5,10,15-triphenyl-20-[4-(2-(4-*m*-hydroxyanilinodeneformyl)phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**3**)), as well as three metalloporphyrins (**CuPp** (**1a**), **ZnPp** (**1b**), and **CoPp** (**1c**)) of porphyrin **H_2Pp**(**1**) were synthesized. Their molecular structures were characterized by ^{1}H-NMR, MS, UV/VIS, and FT-IR spectra. Furthermore, they were evaluated by their cytotoxicities against human epidermal squamous cell carcinoma cell (A431) and normal human horn cells (HaCaT) *in vitro* with MTT assay. Interestingly, these porphyrins and metalloporphyrins, which had a negligible cytotoxicity to HaCaT cells, showed highly cytotoxicity against A431 cells with IC_{50} values in the range of 6.6 – 9.8 μM, and metalloporphyrins exhibited higher cytotoxicity than that of metal-free porphyrins.

1. Introduction. – Human epidermal squamous cell carcinoma cell (A431) is a malignant tumor of the formed epidermis cutin cell [1][2] and is one of the most common human non-melanoma skin malignancies. During the past decades, the incidence rate of this disease has tremendously increased and become the main threat to human health of skin. A variety of treatment modalities have been described to treat human epidermoid carcinoma cells, including surgery, cryotherapy, topical chemotherapy, photodynamic therapy, and immune response modifiers. Surgical intervention remains the only curative treatment and is often the first option in treatment [3][4]. Nevertheless, surgery presents certain limitations: for example, it is poorly tolerated and unsuitable for the patient, and patients show distant metastases at the time of diagnosis, or it is cosmetically unacceptable. Additionally, curative surgery is performed more than just a few small circumscribed lesions [5][6]. Compared with the surgery removal, chemotherapy [7 – 9] is also a main approach in the treatment of tumor for a long time. Unfortunately, the activity of most chemotherapeutic agents attack not only cancer cells, but also other healthy cells of the body, for example blood cells and lining, mouth, stomach, and intestines. Furthermore, they can depress the immune system and cancer cells frequently develop resistance to many anticancer drugs which greatly reduces their therapeutic usefulness [10][11]. Therefore, there is an urgent need to improve and develop more effective therapeutic agents which combat cancer and are able to evade drug resistance and other significant side effects. Recent studies show that porphyrins have become one of the new research targets as therapeutic drugs.

As we all know, porphyrins are a class of naturally occurring macrocyclic compounds, playing a very important role in a diversity of biological processes, including cell respiration, detoxification of xenobiotics, oxygen transport, fatty acid oxidation, and light harvesting [12]. They have been extensively exploited as platforms for the study of synthetic and potential applications in a wide variety of fields such as chemistry, physics, material science, engineering, biology, and medicine [13]. Porphyrins have emerged as hot topics of therapeutic drugs because of their selective accumulation in abnormal or hyperproliferative cells [14 – 19], such as neoplastic tissue [20 – 23]. This selective accumulation can result in effective ablation of the targeted tissue. For this reason, they produce less toxicity than traditional antitumor drugs. *Schiff* bases are a class of organic compounds that contain a C=N bond, such as the imine and azomethine group, which have potential applications as antibacterial, anticancer, and antiviral agents [24][25]. It has been demonstrated that $NH_2OH \cdot HCl$ and *m*-aminophenol are common pharmaceutical intermediates. Herein, they are chosen as peripheral substituents to modify porphyrin through the C=N bond which are expected to be bioactive compounds.

It has been known that metal ions involve in biological processes of life and have been a subject of interest. Among metals, copper, zinc, and cobalt are all essential trace elements presenting in living organisms. They exhibit considerable and biochemical action as a constituent of various exogenous administered compounds in humans. Current interest in metal complexes stem from their potential use as antimicrobial, antitumor agents and enzyme inhibitors [26 – 28]. Especially the transition-metal complexes provide enormously versatile platforms for drug design, for example a number of cisplatin and Cu(II) complexes that exhibit cytotoxic activity through cell

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apoptosis or enzyme inhibition have been reviewed [27 – 29]. These drugs have attracted researchers to synthesize new transition metal compounds which can treat tumours with a selective cytotoxicity to cancer cells. Today, a range of metalloporphyrins due to a part of affinity with the cancer cells, have been investigated as photosensitizers in cancer treatment. For example, the Zn, Ca, Ru, and Co complexes showed encouraging results because of their interaction with biomolecules, mainly proteins and nucleic acids [30 – 34]. In this work, we introduced transition metal Cu^{II}, Zn^{II}, and Co^{II} into the porphyrin molecule to form a metalloporphyrin. Therefore, this is emerging as a promising cytotoxic agent to conventional chemotherapy.

Because *Schiff*-bases are common pharmacophores in the design and development of anticancer chemotherapeutic agents, in this article, we report the synthesis of two novel *Schiff*-bases porphyrins, 5,10,15-triphenyl-20-[4-(2-(4-hydroxyimino)phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**2**)) and 5,10,15-triphenyl-20-[4-(2-(4-*m*-hydroxyanilinodeneformyl)phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**3**); *Fig. 1*), by reacting aldehyde group-substituted tailed porphyrin 5,10,15-triphenyl-20-[4-(2-(4-formyl)-phenoxy)ethoxy]phenyl porphyrin (**H_2Pp**(**1**); *Fig. 1*) with $NH_2OH \cdot HCl$ and *m*-aminophenol respectively. Moreover, three new metalloporphyrins (**CuPp** (**1a**), **ZnPp** (**1b**), and **CoPp** (**1c**); *Fig. 1*) were also synthesized by chelation between the four N-atoms of the center of **H_2Pp**(**1**) ring and transition metal ion Cu^{II}, Zn^{II} and Co^{II}. Their cytotoxic activities against A431 cells and normal skin cells were studied in the dark.

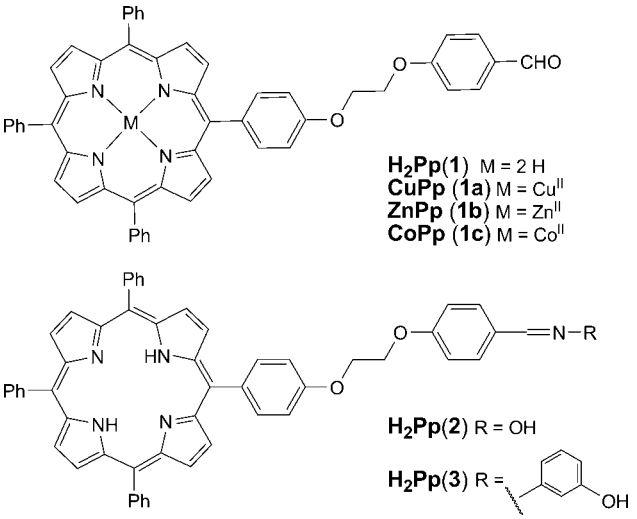
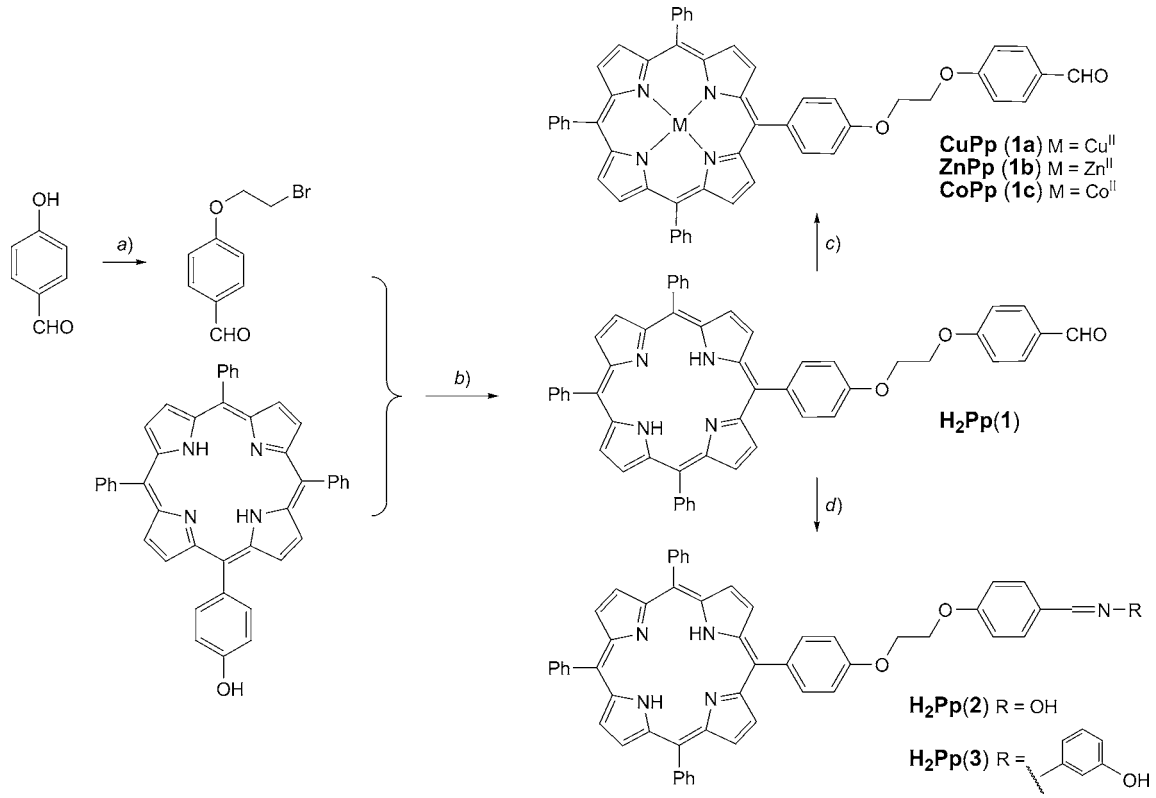


Fig. 1. *Structures of the porphyrins and metalloporphyrins*

2. Results and Discussion. – 2.1. *Synthesis of Porphyrins and Metalloporphyrins.* The synthetic routes to the porphyrins **H_2Pp**(**1**) – **H_2Pp**(**3**) and metalloporphyrins **1a** – **1c** were outlined in the *Scheme*. The porphyrins **H_2Pp**(**1**) was

Scheme. *Synthetic Routes of the Porphyrins and Metalloporphyrins*



a) $Br(CH_2)_2Br$, K_2CO_3, acetone, 56°. *b*) TPPOH, K_2CO_3, DMF, 60°. *c*) $M(Ac)_2$, CH_2Cl_2, r.t. *d*) $NH_2OH \cdot HCl$, Et_3N (for **H_2Pp**(**2**)); *m*-aminophenol, EtOH, CH_2Cl_2, $MgSO_4$, r.t. (for **H_2Pp**(**3**))

synthesized by the reaction of TPPOH and 4-(2-bromoethoxy)benzaldehyde in the presence of K_2CO_3 and DMF at 60° with the yield around 85%.

The *Schiff*-bases porphyrins **H_2Pp(2)** and **H_2Pp(3)** and metalloporphyrins were prepared from **H_2Pp(1)**. The **H_2Pp(2)** and **H_2Pp(3)** were obtained in 65% and 30% yields by treating **H_2Pp(1)** with $NH_2OH \cdot HCl$ and *m*-aminophenol respectively in a mixture of CH_2Cl_2 and EtOH at room temperature. The yields of **H_2Pp(2)** and **H_2Pp(3)** were improved by addition of $MgSO_4$ to absorb the H_2O formed in the formation reaction of C=N bond. Corresponding metalloporphyrins **1a** – **1c** were obtained by reaction of **H_2Pp(1)** with a large excess of $Cu(AcO)_2$, $Zn(AcO)_2$, and $Co(AcO)_2$ at room temperature, respectively. After purification by column chromatography, metalloporphyrins **1a** – **1c** were obtained in 95, 85, and 79% yield, respectively.

2.2. *Characterization of Porphyrins and Metalloporphyrins.* The structures of the porphyrins **H_2Pp(1)** – **H_2Pp(3)** and metalloporphyrins **1a** – **1c** were identified by ^{1}H-NMR spectroscopy, elemental analysis, UV/VIS spectroscopy, FT-IR spectra, and mass spectrometry.

The UV/VIS absorption spectra of the porphyrins **H_2Pp(1)** – **H_2Pp(3)** and metalloporphyrins **1a** – **1c** in CH_2Cl_2 as solvent are shown in *Fig. 2.* The maximum wavelengths and molar absorptivities of the porphyrin compounds were summarized in *Table 1.* An intense *Soret* band situated at 418 nm and four weak Q bands located in the 512 – 646 nm region, characteristic of metal-free porphyrin chromophore were presented in the absorption spectra of **H_2Pp(1)** – **H_2Pp(3)**. Compared to the metal-free porphyrins, a decrease in the number of Q bands on metalloporphyrins was found. The molecular electronic spectra of copper, zinc, and cobalt porphyrin complexes exhibited one or two Q bands, respectively in the 538 – 540 nm and 575 nm spectral ranges. Additionally, a distinct blue shift (3 – 16 nm) of the absorption peak of metalloporphyrins was observed. From the UV/VIS spectra, it can be demonstrated that the peripheral substituents of **H_2Pp(1)** – **H_2Pp(3)** had negligible influence on UV/VIS spectra. Instead, insertion of a metal ion into the core of porphyrin generated an obvious influence.

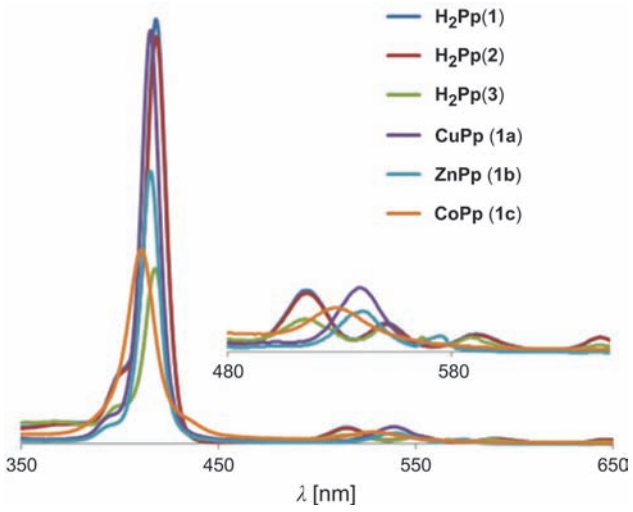


Fig. 2. *UV/VIS Absorption spectra of the porphyrins and metalloporphyrins*

The FT-IR spectra of the porphyrins **H_2Pp(1)** – **H_2Pp(3)**, and metalloporphyrins **1a** – **1c** are shown in *Fig. 3*. A stretching vibration and a bending vibration for the N–H bond appear at 3314 – 3360 cm^{-1} and around 965 cm^{-1} for metal-free porphyrins, respectively. A strong absorption band at 1697 cm^{-1} was assigned to the aldehyde group of stretching vibration peaks for **H_2Pp(1)**. It was shifted to a lower wave number below (1700 cm^{-1}) due to conjugation of the C=O group and the porphyrin ring. In addition, the stretching vibrations for the C=N bonds appeared at 1607 and 1602 cm^{-1} in the FT-IR spectra of the *Schiff*-bases **H_2Pp(2)** and **H_2Pp(3)**, which indicated that the **H_2Pp(2)** and **H_2Pp(3)** were successfully synthesized by condensation of **H_2Pp(1)** with the selected $NH_2OH \cdot HCl$ and *m*-aminophenol, respectively.

In the FT-IR spectra of the metalloporphyrins, a new band appeared around 1004 – 1018 cm^{-1}, and it could be assigned to a metal-dependent in-plane porphyrin deformation mode. Instead, the disappearance of an N–H stretching vibration and a bending vibration in the metal-free porphyrins indicated the coordination of N-atoms of the porphyrinic core to the metal centers. This predicated that the metalloporphyrins **1a** – **1c** were qualitatively prepared.

The ^{1}H-NMR spectra of the porphyrins **H_2Pp(1)** – **H_2Pp(3)** have been recorded in $CDCl_3$. The spectra are consistent with *meso*-substituted porphyrins, showing a single peak around δ(H) – 2.76 ppm due to the H-atoms of the –NH group in porphyrinic core, and the chemical shift of eight β-pyrrolic H-atom resonances at δ(H) 8.84 – 8.88 ppm. The aldehyde H-atom chemical shift is at 9.94 ppm, and as expected, this peak is absent from the spectra of **H_2Pp(2)** and **H_2Pp(3)** in which each a new single peak of H-atom for –OH was detected at 8.08 and

Table 1. *UV/VIS Spectra Data of the Porphyrins and Metalloporphyrins* (25°, in CH_2Cl_2)

Compounds	*Soret* band [nm] (log ε)	Q bands [nm] (log ε)
H_2Pp(1)	417 (5.69)	515 (4.32), 550 (3.92), 590 (3.78), 646 (3.62)
H_2Pp(2)	418 (5.70)	515 (4.33), 550 (3.90), 590 (3.73), 646 (3.62)
H_2Pp(3)	418 (5.65)	512 (4.32), 554 (3.89), 591 (3.70), 645 (3.55)
CuPp (1a)	415 (5.58)	539 (3.87)
ZnPp (1b)	415 (5.61)	540 (3.93), 575 (3.77)
CoPp (1c)	415 (5.67)	538 (3.91)

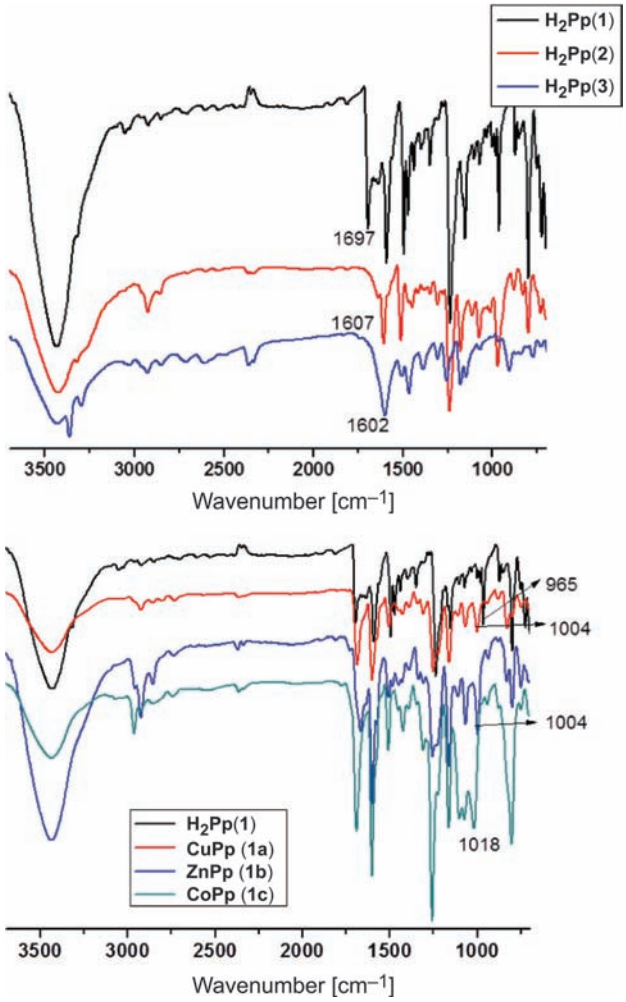


Fig. 3. *IR Spectra of the porphyrins and metalloporphyrins*

8.90 ppm, respectively. The formation of *Schiff*-base porphyrins was confirmed by comparing the ^{1}H-NMR data of **H_2Pp(1)** with those of **H_2Pp(2)** and **H_2Pp(3)**. In addition, all compounds exhibited main peaks (protonated molecule $[M+H]^+$) on high-resolution ESI mass spectroscopy.

2.3. *Cytotoxicity Test.* The cytotoxic activities of all the porphyrins against A431 cells were evaluated in the absence of light after an incubation of 24 h. These porphyrins and metalloporphyrins demonstrated moderate cytotoxicities with comparable values in the cell tests. All IC_{50} values were in the range of 6.6 – 9.8 μM (*Table 2*). Three metalloporphyrins **1a** – **1c** were more effective in stopping cell division and killing the tumor cells than the porphyrins **H_2Pp(1)** – **H_2Pp(3)**, with observed minimum IC_{50} values 6.8 ± 1.2, 6.7 ± 0.5, and 6.6 ± 0.9 μM, respectively. The *Schiff*-base **H_2Pp(3)** was slightly less cytotoxic with the IC_{50} of 7.1 ± 0.8 μM than the metalloporphyrins, and the **H_2Pp(1)** showed the smallest cytotoxicity. These results indicated that introduction of the *Schiff* base to porphyrin molecules enhances cytotoxic activities against A431 cells. In particular, the cytotoxic activities of porphyrin **H_2Pp(3)**, which bears a phenolic group, against A431 cells were of the same order of potency as that of metalloporphyrins.

Table 2. *Cytotoxicity of Porphyrins and Metalloporphyrins to A431 Cancer Cells after 24 h Incubation in the Dark*

Compounds	IC_{50} [μM]
H_2Pp(1)	9.8 ± 0.7
H_2Pp(2)	9.3 ± 0.4
H_2Pp(3)	7.1 ± 0.8
CuPp (1a)	6.8 ± 1.2
ZnPp (1b)	6.7 ± 0.5
CoPp (1c)	6.6 ± 0.9

Comparably, the negligible cytotoxicities were detected in HaCaT cells for all porphyrins with the cell viability of over 80%. The result revealed that these porphyrins could easily and selectively accumulate in tumor cells and have more cytotoxic effects on them.

2.4. *Discussion.* As described, all the compounds exhibit high cytotoxicities against A431 cancer cells, but only negligible cytotoxicities against HaCaT cells, which indicate that these porphyrins have the ability to kill A431 cancer cells selectively. The introduction of *Schiff* base C=N bond in **H_2Pp(2)** and **H_2Pp(3)** increases the cytotoxicity against A431 cancer cells compared with **H_2Pp(1)**, probably due to the intercalation to DNA of cancer cells with more ease [35]. Among porphyrins **H_2Pp(1)** – **H_2Pp(3)**, **H_2Pp(3)** has a better inhibition effect, which might be attributed to the attached phenolic group. The phenolic group is easily oxidized to a benzoquinonyl group in a cellular metabolic pathway [36], which finally causes obvious cell damage. The high inhibition effect of the three metalloporphyrins **1a** – **1c**, may be due to the effect of coordinated metal ions that play a major role in mediating potency of the complexes [26].

3. Conclusion. – In summary, three novel porphyrins **H_2Pp(1)** – **H_2Pp(3)** and Cu^{II} (**1a**), Zn^{II} (**1b**), Co^{II} (**1c**) metalloporphyrins of **H_2Pp(1)** are synthesized and characterized. Their biological activities are evaluated by their cytotoxicities against human epidermal squamous cell carcinoma cell (A431). These compounds exhibit good cytotoxicities towards A431 cancer cells, especially for the metalloporphyrins. The results demonstrate that all the porphyrins can inhibit effectively the A431 cancer cells, with very low toxicities to HaCaT cells. It may provide some valuable information for further deep research.

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Experimental Part

General. 4-Hydroxybenzaldehyde, 4-(2-bromoethoxy)benzaldehyde, and pyrrole were obtained from *Sinopharm Chemical Reagents Company*, MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) were purchased from *Sigma–Aldrich* (Chiba, Japan). The 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin (TPPOH) was

synthesized according to the literature procedures described in [37], and other reagents were obtained from *Beijing Chemical Reagents Company*. Pyrrole was distilled before use. Dimethyl sulfoxide (DMSO) and *N*,*N*-dimethyl formamide (DMF) were dried (with powdered $MgSO_4$) and distilled at normal pressure before use.

UV/VIS Spectra: *Shimadzu UV-1800* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR (4000 – 400 cm^{-1}) Spectra: samples in KBr matrix for the title complexes on *BEQUZNDX-550* series FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR Spectra: *Varian Inova 400* MHZ spectrometer for metal-free porphyrins, in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: carried out on a matrix assisted laser desorption/ionization time of light mass spectrometer (MALDI-TOF MS, *Krato Analytical Company of Shimadzu Biotech*, Manchester, Britain); in *m*/*z*. Elemental analysis (C, H and N): *Vario EL-III CHNOS* instrument.

Synthesis of the Porphyrins **H_2Pp(1) – H_2Pp(3)**. *Synthesis of 5,10,15-Triphenyl-20-[4-(2-(4-formyl)phenoxy)ethoxy]phenyl Porphyrin* (*=4-{2-[4-(10,15,20-Triphenylporphyrin-5-yl)phenoxy]ethoxy}benzaldehyde*; **H_2Pp(1)**). TPPOH (0.126 g, 0.2 mmol) and 4-(2-bromoethoxy)benzaldehyde (0.458 g, 2 mmol) were mixed and dissolved in DMF (10 ml). The soln. was stirred in the presence of K_2CO_3 (0.15 – 0.2 g) for 12 h at 60° in the darkness. The progress of the reaction was monitored by TLC. DMF was removed under reduced pressure after the reaction was finished, and the residue was dissolved in CH_2Cl_2 and purified over a SiO_2 column using CH_2Cl_2 as eluent. The first violet band was collected. After vaporizing and drying under vacuum, **H_2Pp(1)** was obtained. Yield: 85%. M.p. >250°. UV/VIS (CH_2Cl_2): 418 (*Soret* band), 515, 550, 590, 646 (Q bands). IR (KBr): 3419, 2797, 2734, 1687, 1601, 1577, 1507, 1253, 965, 830, 800. ^{1}H-NMR ($CDCl_3$, 400 MHz): 9.94 (*s*, 1 H, aldehyde-H); 8.89 (*s*, 8 β-H of pyrrole); 8.25 (*d*, 6 *o*-H of phenyl); 8.13 – 8.15 (*m*, 2 H of phenyl); 7.77 – 7.84 (*m*, 9 *m*-H and *p-H of* phenyl); 7.13 (*d*, 2 H of substituted phenyl); 5.28 – 5.32 (*m*, 4 H of phenyl); 4.42, 3.61 (2*t*, CH_2CH_2); – 2.76 (*s*, 2 H of imino). MS: 779.71 ($[M+H]^+$) Anal. calc. for $C_{53}H_{38}N_4O_3$ (778.89): C 81.71, H 4.96, N 7.21; found: C 81.73, H 4.92, N 7.19.

Synthesis of 5,10,15-Triphenyl-20-[4-(2-(4-hydroxyimino)phenoxy)-ethoxy]phenyl Porphyrin (= N-*Hydroxy-1-(4-{2-[4-(10,15,20-triphenylporphyrin-5-yl)phenoxy]ethoxy}phenyl)methanimine*; **H_2Pp(2)**). **H_2Pp(1)** (0.078 g, 0.1 mmol) was dissolved in CH_2Cl_2 (20 ml) with anh. $MgSO_4$ (0.5 g, 4 mmol), EtOH soln. (5 ml) with $NH_2OH \cdot HCl$ (0.07 g, 1 mmol), and Et_3N (0.5 ml, 0.004 mmol) were added into the soln. The mixture was stirred for 24 h at r.t. Then, the mixture was filtered off ($MgSO_4$), and the solvent was removed. Further purification of the residue was carried out over a SiO_2 column with CH_2Cl_2 as eluent. The second violet band was collected. After vaporizing and drying under vacuum, **H_2Pp(2)** was obtained. Yield: 65%. M.p. >250°. UV/VIS(CH_2Cl_2): 418 (*Soret* band), 515, 550, 590, 646 (Q bands). IR (KBr): 3427, 2924, 1607, 1512, 1240, 1174, 970, 800. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.84 – 8.86 (*m*, 8 β-H of pyrrole); 8.22 (*d*, 6 *o*-H of phenyl); 8.08 (*s*, 1 H, OH); 7.73 – 7.79 (*m*, 9 *m*-H and *p-H of* phenyl); 7.43 (*d*, 4 H of phenyl); 7.32 (*d*, 2 H of substituted phenyl); 7.07 (*d*, 2 H of substituted phenyl); 6.92 (*s*, 1 H, CH = N); 4.30, 3.64 (2*t*, CH_2CH_2); – 2.77 (*s*, 2 H of imino). MS: 794.67 ($[M+H]^+$). Anal. calc. for $C_{53}H_{39}N_5O_3$ (793.91): C 80.21, H 4.98, N 8.77; found: C 80.18, H 4.95, N 8.82.

*Synthesis of 5,10,15-Triphenyl-20-[4-(2-(4-*m*-hydroxyanilinedene formyl)phenoxy)ethoxy]phenyl Porphyrin* (= 3-*[(4-{2-[4-(10,15,20-Triphenylporphyrin-5-yl)phenoxy]ethoxy}benzylidene)amino]phenol*; **H_2Pp(3)**). $MgSO_4$ (0.5 g, 4 mmol) was added to the soln. of **H_2Pp(1)** (0.078 g, 0.1 mmol) and *m*-aminophenol (0.218 g, 2 mmol) in a mixture of CH_2Cl_2 and EtOH (25 ml). The resulting mixture was stirred magnetically at r.t. for 24 h and monitored by TLC, then, the solvent was removed *in vacuo*. The obtained violet product was dissolved in CH_2Cl_2, and purified by CC (the SiO_2 column was previously treated with alkaline Et_3N to prevent the dissociation of the product) using a binary eluent of CH_2Cl_2/EtOH (10 : 1). The first violet band was collected. After vaporizing and drying under vacuum, **H_2Pp(3)** was obtained. Yield: 30%. M.p. >250°. UV/VIS (CH_2Cl_2): 418 (*Soret* band), 512, 554, 591, 645 (Q bands). IR (KBr): 3361, 2925, 2609, 2362, 1602, 1176, 904, 686. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.85 (*s*, 8 β-H of pyrrole); 8.22 (*d*, 6 *o*-H of phenyl); 8.90 (*s*, 1 H, OH); 7.74 – 7.77 (*m*, 9 *m*-H and *p-H of* phenyl); 7.55(*s*, 4 H of phenyl); 7.03 (*t*, 8 H of substituted phenyl); 6.27 (*s*, 1 H, CH=N); 4.32, 3.74 (2*t*, CH_2CH_2); – 2.77 (*s*, 2 H of imino). MS: 870.88 ($[M+H]^+$). Anal. calc. for $C_{59}H_{43}N_5O_3$ (870.0): C 81.41, H 4.96, N 8.08; found: C 81.45, H 4.98, N 8.05.

General Procedure for Synthesis of **CuPp (1a), ZnPp (1b), *and* CoPp (1c)**. **H_2Pp(1)** (0.078 g, 0.1 mmol) was dissolved in CH_2Cl_2 (20 ml), and $Cu(AcO)_2$ (2 mmol) soln. in EtOH (5 ml) were added, and the mixture was stirred for 24 h at r.t. The progress of the complexation reaction was monitored by TLC. After completion of the reaction, the mixture was filtered, and the solvent was removed under reduced pressure. In order to separate the target compounds, the crude product was dissolved in CH_2Cl_2 and purified by SiO_2 CC, using CH_2Cl_2 as eluent. The obtained violet soln. was concentrated and dried under reduced pressure. **1a** was obtained. The synthetic procedure for **1b** and **1c** was similar to that for **1a** in almost quantitative yield by using $Zn(AcO)_2$ and $Co(AcO)_2$ as the starting material, resp.

Data of Copper(II) 5,10,15-Triphenyl-20-[4-(2-(4-formyl)phenoxy)ethoxy]phenyl Porphyrin (*=[4-{2-[4-(10,15,20-Triphenylporphyrin-5-yl-κ^4N^{21},N^{22},N^{23},N^{24})phenoxy]ethoxy}benzaldehydato(2–)]-copper*; **1a**). Yield: 95%. M.p. >250°. UV/VIS (CH_2Cl_2): 415 (*Soret* band), 539 (Q bands). IR (KBr): 3437, 2925, 2803, 2730, 1685, 1601, 1504, 1251, 1161, 1004, 827. MS: 841.71 ($[M+H]^+$). Anal. calc. for $C_{53}H_{36}CuN_4O_3$ (840.4): C 75.78; H 4.30, N 6.65, Cu 7.54; found: C 75.74, H 4.32, N 6.67, Cu 7.56.

Data of Zinc(II) 5,10,15-Triphenyl-20-[4-(2-(4-formyl)phenoxy)-ethoxy]phenyl Porphyrin (*=[4-{2-[4-(10,15,20-Triphenylporphyrin-5-yl-κ^4N^{21},N^{22},N^{23},N^{24})phenoxy]ethoxy}benzaldehydato(2–)]zinc*; **1b**). Yield: 85%. M.p. >250°. UV/VIS (CH_2Cl_2): 415 (*Soret* band), 540, 575 (Q bands). IR (KBr): 3440, 2853, 2736, 1668, 1598, 1161, 1004, 798. MS: 843.31 ($[M+H]^+$) amu. Anal. calc. for $C_{53}H_{36}N_4O_3Zn$ (842.2): C 75.68; H 4.30, N 6.67, Zn 7.59; found: C 75.58, H 4.31, N 6.65, Zn 7.76.

Data of Cobalt(II) 5,10,15-Triphenyl-20-[4-(2-(4-formyl)phenoxy)ethoxy]phenyl Porphyrin (*=[4-{2-[4-(10,15,20-Triphenylporphyrin-5-yl-κ^4N^{21},N^{22},N^{23},N^{24})phenoxy]ethoxy}benzaldehydato(2–)]-cobalt*; **1c**). Yield: 79%. M.p. >250°. UV/VIS(CH_2Cl_2): *415* (*Soret* band), 538 (Q bands). IR (KBr): 3437, 2839, 2742, 1688, 1600, 1257, 1156, 1018, 802. MS: 836.7 ($[M+H]^+$). Anal. calc. for $C_{53}H_{36}CoN_4O_3$ (835.8): C 75.98; H 4.30, N 6.67, Co 7.12; found: C 76.16, H 4.34, N 6.70, Co 7.05.

4. Cell Culture Conditions. The A431 cells and HaCaT cells were grown in plastic culture flasks at 37° (5% CO_2) by using DMEM/F-12 medium with penicillin (100 UI/ml), streptomycin (100 µg/ml) and 10% heat-activated fetal bovine serum. The medium was replaced with fresh every 48 h. When *ca.* 80% the confluence was reached, cells were washed with 5 ml of phosphate buffer saline (PBS) for two times and treated with trypsin (2 ml) to separate them from the flasks and collected into a centrifuge tube containing 4 ml of the culture medium. The flasks were further washed with 2 ml of PBS to remove the remaining cells and this was transferred into a centrifuge tube. The tube was centrifuged at 1000 rpm at r.t. for 5 min. The pellet was re-suspended in an appropriate culture medium volume and seeded in culture dishes before *in vitro* investigations.

5. Evaluation of Cytotoxic Activity. Cytotoxic effects of metal-free or metal porphyrins were evaluated by MTT method. Cells were seeded at a density of 2×10^4 cells/well in 96-well culture plates containing a 200 µl culture medium per well when cells were in the exponential growth phase. Then cells were incubated for 24 h at 37° to promote their adhesion to the plate. The medium was then removed, replaced with the different metal-free porphyrins and metalloporphyrins and incubated for 24 h in the dark. Every plate had three wells with untreated cells as the control and three wells with cells treated with each compound.

After 24 h incubation, 20 µl MTT (5 mg/ml) was added to each well and cells were incubated for 4 h at 37° and 5% CO_2. The culture medium was removed and the formazan crystal was dissolved by adding 150 µl of DMSO. The absorbance was measured using an enzyme-linked immuneoabsorbent assay plate reader (*Bio-Rad*) at 680 nm. The cell viability was expressed by the absorbance changes, and the survival rate was given as the percentage compared to the untreated cells. The concentration required for 50% inhibition of cell viability (IC_{50}) was determined for the various porphyrins tested. HaCaT cells were treated in the same conditions just for comparision.

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