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Chloroalkyl piperazine and nitrogen mustard porphyrins: synthesis and anticancer activity

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Abstract—Fifteen new chloroalkyl piperazine and nitrogen mustard porphyrins have been synthesized by the direct condensation of chloroalkyl piperazine, nitrogen mustard benzaldehyde, and pyrrole. Each porphyrin bears 1–4 chloroalkyl piperazine or nitrogen mustard moieties, which have been used as drugs. The Lindsey method was modified to synthesize chloroalkyl piperazine and nitrogen mustard porphyrins. To successfully synthesize chloroalkyl piperazine and nitrogen mustard porphyrins, catalyst acidity was proved to be the key factor, while the ratio of pyrrole to aldehyde had great influence on product yield. The synthetic chloroalkyl piperazine and nitrogen mustard porphyrins were characterized by elementary analysis, MS, ^{1}H NMR, IR, and UV–vis. Their anticancer activity to bel-7404 liver cancer cells was tested by the MTT assay. Most of the synthetic porphyrins had good anticancer activity toward bel-7404 liver cancer cells in the absence of light. These compounds might be potential anticancer medicines.

1. Introduction

Porphyrins were used as sensitizer in photodynamic therapy (PDT) because they tend to accumulate in neoplasm tissue than in the surrounding normal tissue and they might be able to convert dioxygen into singlet state oxygen, which can destroy the cancer cells when irradiated by light.[1–3] Introducing bioactivity groups onto the periphery of porphyrins has profound effect on the PDT properties of porphyrins.[4–6] The synthesis of certain excellent porphyrins potentially useful in PDT has been achieved,[7–12] and the synthesis of porphyrins with aryl or alkyl amine groups having potential applications in cancer chemical diagnosis and therapy has been also realized.[13–15] However, development of new methodologies to functionalize porphyrins and their derivatives at the porphyrin ring is still an active and exciting area due to providing a variety of new compounds that could otherwise only be obtained by total synthesis.[16,17]

Polyhaloalkyl amine compounds, such as nitrogen mustard and halopiperazine, are widely used in pharmaceutical industry.[18] It is possible that chloroalkyl piperazine and nitrogen mustard porphyrins have better bioactivity than chloroalkyl piperazine and chloroalkyl nitrogen mustard, because of the fact that when piperazine compounds are linked to a porphyrin ring, their anticancer activity increases.[15] Chloroalkyl piperazine and nitrogen mustard porphyrins are haloalkyl amino porphyrins. In the synthesis of haloalkyl amino porphyrins, the challenges were to avoid the hydrolysis and aminolysis of halogen–carbon bond of haloalkyl groups.[19] Preparation of haloalkyl amino porphyrins via typical Adler–Longo method[20] was not proved to be successful, while the original conditions of Lindsey method[21] only gave trace amount of products. Herein, we report a modified Lindsey method that is suitable for synthesizing haloalkyl amino porphyrins via one-step condensation of corresponding aldehyde and pyrrole in high yield. Through this method, we have synthesized 15 new haloalkyl amino porphyrins, 12 chloroalkyl nitrogen mustard porphyrins, and 3 chloroalkyl piperazine porphyrins (shown in Fig. 1). The primary anticancer activities of these compounds toward bel-7404 liver cancer cell were investigated in vitro at the absence of light. The results showed that chloroalkyl nitrogen mustard porphyrins and chloroalkyl piperazine

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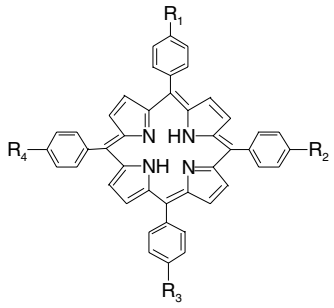

1. $R_1=R_2=R_3=R_4$: $N(CH_2CH_2Cl)_2$
2. $R_1=R_2=R_3=R_4$: $N(CH_2CHClCH_3)_2$
3. $R_1=R_2=R_3=R_4$: $N(CH_2CHClCH_2Cl)_2$
4. $R_1=R_2=R_3=H$, R_4: $N(CH_2CH_2Cl)_2$
5. $R_1=R_2=R_3=H$, R_4: $N(CH_2CHClCH_3)_2$
6. $R_1=R_2=R_3=H$, R_4: $N(CH_2CHClCH_2Cl)_2$
7. $R_1=H$, $R_2=R_3=R_4$: $N(CH_2CH_2Cl)_2$
8. $R_1=H$, $R_2=R_3=R_4$: $N(CH_2CHClCH_3)_2$
9. $R_1=H$, $R_2=R_3=R_4$: $N(CH_2CHClCH_2Cl)_2$
10. $R_1=R_2=R_3=R_4$: $N(CH_2CH_2)_2NCH_2CH_2Cl$
11. $R_1=R_2=R_3=R_4$: $N(CH_2CH_2)_2NCH_2CHClCH_3$
12. $R_1=R_2=R_3=R_4$: $N(CH_2CH_2)_2NCH_2CHClCH_2Cl$
13. $R_1=R_3=H$, $R_2=R_4$: $N(CH_2CH_2Cl)_2$
14. $R_1=R_3=H$, $R_2=R_4$: $N(CH_2CHClCH_3)_2$
15. $R_1=R_3=H$, $R_2=R_4$: $N(CH_2CHClCH_2Cl)_2$

Figure 1. Porphyrins bearing aryl nitrogen mustard derivatives.

porphyrins have anticancer activities. They could be used as potential drugs for treatment of tumors.

2. Results and discussion

2.1. Synthesis of chloroalkyl nitrogen mustard and piperazine porphyrins 1–12

The chloroalkyl nitrogen mustard and piperazine porphyrins **1**–**12** were *meso*-substituted AB_3 and A_4 porphyrins. Up to now, Adler–Longo method and Lindsey method are the best for the synthesis of AB_3 and A_4 porphyrins by the direct condensation of substituted benzaldehyde with pyrrole.[16] Porphyrins **1**–**12** are the porphyrins bearing haloalkyl amino group. Most of the haloalkyl amino-substituted porphyrins cannot be synthesized by the direct condensation of haloalkyl amino-substituted benzaldehyde with pyrrole.[22–24] An attempt was made in our laboratory to synthesize porphyrins **1**–**12** via Adler–Longo method by refluxing reactants in propionic acid, but proved to be unsuccessful. In our synthesis, a series of acids, such as HCOOH, CH_3COOH, and CH_3CH_2COOH were used as refluxing media, zinc acetate was used as template to stabilize the cyclic tetramer structure of intermediates, and the reactions were run for different reaction time, such as 30, 40, and 60 min, but it was found that at all the cases, no corresponding haloalkyl amino porphyrins could be generated by Adler–Longo method. LC/MS analysis showed that all the products were formed from that the nitrogen atom of pyrrole attacks on the halogen–carbon bond of haloalkyl amino benzaldehyde. Therefore, Adler–Longo method is not suitable for the synthesis of chloroalkyl nitrogen mustard and piperazine porphyrins.

Only a small amount of nitrogen mustard porphyrins could be obtained at the original reaction conditions of Lindsey method. The catalyst acidity variation in Lindsey method has great influence on the successful synthesis of polyhaloalkyl amino porphyrins. The influence of different acid catalysts on the yield of nitrogen mustard porphyrin **1** was shown in Table 1.

No porphyrin was found when $BF_3{\cdot}Et_2O$ and CH_3COOH were used as catalyst. The strong acid CF_3COOH gave a poor product yield, while CCl_3COOH gave product yield of 30%. The results indicated that an appropriate catalyst acidity was necessary for the synthesis of chloroalkyl piperazine and nitrogen mustard porphyrins. Our studies showed that the quaternary ammonium was formed by the reaction of nitrogen mustard benzaldehyde with acid when the acidity of the catalysts was too strong.

Nitrogen mustard porphyrins **1**–**3** and chloroalkyl piperazine porphyrins **10**–**12** were synthesized through reaction routes shown in Figure 2.

Table 1. The influence of different acid catalysts on the yield of nitrogen mustard porphyrin **1**

Catalyst	$BF_3{\cdot}Et_2O$	CF_3COOH	CCl_3COOH	$CHCl_2COOH$	CH_3COOH
Yield (%)	0	3.5	30	25	0

Note: Reaction time was 24 h.

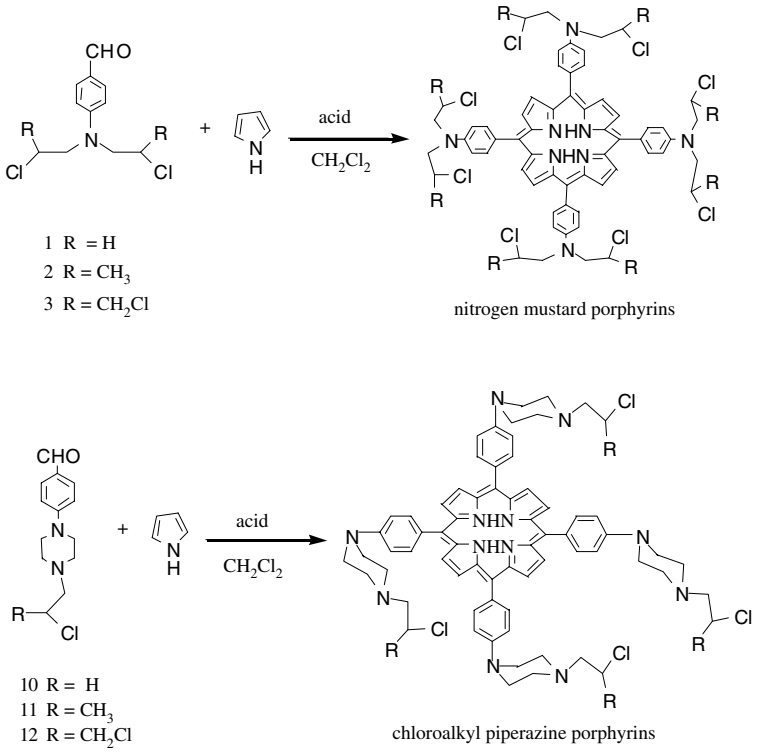

Figure 2. Synthetic routes of nitrogen mustard porphyrins **1**–**3** and chloroalkyl piperazine porphyrins **10**–**12**.

In order to obtain a high yield in Lindsey synthesis, a specific pyrrole to aldehyde ratio is needed for specific pyrrole and aldehyde couple. For example, in the synthesis of the symmetric four substituted porphyrins at *meso*-position by Lindsey method, a high porphyrin yield was obtained only when the ratio of pyrrole to aldehyde was 1:1. However, in the synthesis of the symmetric four nitrogen mustard porphyrins **1**–**3** and chloroalkyl piperazine porphyrins **10**–**12**, high porphyrin yield was obtained only when the ratio of pyrrole to chloroalkyl piperazine or nitrogen mustard benzaldehyde was 2.5:1.

Asymmetric porphyrins **4**–**9** could be synthesized via the same routes as symmetric porphyrins by the condensation of the mixed aldehydes with pyrrole. Besides the catalyst acidity and the ratio of pyrrole to polyhaloalkyl amino benzaldehyde, the ratio of benzaldehyde to polyhaloalkyl amino benzaldehyde was also different from that in the general Lindsey method. The ratio of chloroalkyl piperazine or nitrogen mustard benzaldehyde to benzaldehyde also influenced the yield of asymmetric porphyrins. Experiment results showed that the optimal ratio of chloroalkyl piperazine or nitrogen mustard benzaldehyde to benzaldehyde is 1:1 for nitrogen mustard porphyrins **4**–**6**, while for nitrogen mustard porphyrins **7**–**9**, the ratio is 5:1. Evidently, the ratios of pyrrole to aldehyde and ratios of chloroalkyl piperazine or nitrogen mustard benzaldehyde to benzaldehyde for the synthesis of chloroalkyl piperazine and nitrogen mustard porphyrins does not meet the stoichiometric relationship. It is possible that benzaldehyde is more reactive than polyhaloalkyl amino benzaldehyde during the condensation of pyrrole and aldehyde because the electron-drawing polyhaloalkyl amino group on benzaldehyde does not favor the formation of porphyrins.[20,21]

2.2. Synthesis of polyhaloalkyl amino porphyrins 13–15

The chloroalkyl piperazine porphyrins **13**–**15** were *trans* A_2B_2 porphyrins. It was difficult to synthesize *trans* A_2B_2 porphyrins by mixed aldehyde condensation with pyrrole, since at this case, *cis* A_2B_2 and *trans* A_2B_2 porphyrin mixture will be generated, which was difficult to isolate them by column chromatograph due to the same polarity. In order to obtain *trans* A_2B_2 porphyrins, phenyldipyrromethane was first prepared according to the literature method,[25] then polyhaloalkyl amino benzaldehyde and 2 equiv phenyldipyrromethane were condensed under the catalysis of CCl_3COOH at room temperature for 16 h. This will give *trans* A_2B_2 polyhaloalkyl amino porphyrins **13**–**15** (Fig. 3).

2.3. Anticancer activity

It is well known that substituted tetraphenylporphyrin can be used as sensitizer in PDT,[26] and nitrogenous heterocycle porphyrins have better anticancer activity than the corresponding nitrogenous heterocycles in the absence of light.[15] In order to study whether these synthetic chloroalkyl piperazine and nitrogen mustard porphyrins have anticancer activity to cancer cell, the chloroalkyl piperazine, nitrogen mustard porphyrins **1**–**15**, and two corresponding structure parts, chloroalkyl piperazine, nitrogen mustard, and porphyrin TPP were

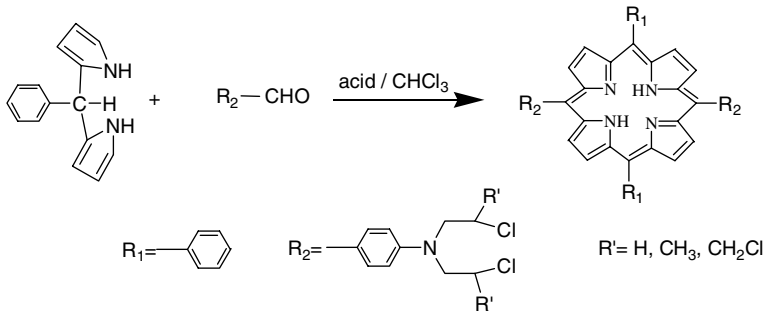

Figure 3. Synthetic route of *trans* A_2B_2 porphyrins **13**–**15**.

tested in vitro against bel-7404 liver cancer cell by MTT method[27] in the absence of light. The ID_{50} values of the bel-7404 liver cancer cell to these compounds were listed in Table 2.

The test results showed that chloroalkyl piperazine and nitrogen mustard porphyrins **1**–**15** have smaller ID_{50} values than that of TPP, the corresponding chloroalkyl piperazine, nitrogen mustard derivatives. This indicates that chloroalkyl piperazine and nitrogen mustard porphyrins have better anticancer activity than that of either structure parts of chloroalkyl piperazine and nitrogen mustard porphyrins. At the same time, it can be found from Table 2 that for similar structure chloroalkyl piperazine and nitrogen mustard porphyrins, such as nitrogen mustard porphyrins **1**–**3**, when substitution group R is on α-carbon atom of the haloalkyl amine (Fig. 2), it decreased the anticancer activity of the porphyrins. For chloroalkyl piperazine and nitrogen mustard porphyrins, the anticancer activity toward different substitution group R follows the order of $H > CH_2Cl > CH_3$. It is interesting that tetraphenylporphine, which is not an anticancer drug, would be a good carrier for toxic moieties to arrive at improved anticancer drugs. The possible reason might be that tetraphenylporphine with anticancer drugs can intercalate into the base pairs of DNA strongly. Further work concerning the issue is in progressing.

Table 2. ID_{50} of porphyrins **1**–**15**

Porphyrin	ID_{50} (μg/mL)
1	6
2	35
3	10
4	23
5	30
6	25
7	13
8	>50
9	15
10	35
11	>50
12	45
13	18
14	25
15	17
TPP	>50
Nitrogen mustard	>50
4-(2-Chloroethyl) phenyl piperazine	>50

In conclusion, we have developed a practical and efficient approach to synthesize polyhaloalkyl amino porphyrins based on Lindsey method. Polyhaloalkyl amino porphyrins showed good anticancer activity toward bel-7404 liver cancer cells in the absence of light. They might be promising agents in chemotherapy and PDT.

3. Experiment

^1H NMR spectra were recorded with tetramethylsilane (TMS) as the internal standard in $CDCl_3$ solvent. All polyhaloalkyl amino benzaldehyde compounds were synthesized according to literature.[15] Pyrrole was distilled before using. Other chemicals were used as received.

3.1. Cell survival assay

The cytotoxic effects of the compounds on bel-7404 liver cancer cell were determined by using the MTT assay.[27] Cells were planted in 100 μL medium at a concentration of 1×10^3 cells per well in 96-well microtiter plates. Plates had been incubated for 24 h at 37 °C under an atmosphere of air containing 5% CO_2. Medium (100 μL) containing the test drugs, which were dissolved in the mixed solvent of H_2O and THF (8:1), was added to quadruplicate wells and incubated for additional 48 h. The medium was then removed from the wells and 200 μL MTT (1 μg/mL in complete medium) was added to each well, and then incubated for another 3 h. The formazan crystals were dissolved in 100 μL dimethylsulfoxide buffered with 25 μL glycine–NaCl solution (0.1 M glycine, 0.1 M NaCl, pH 10.5). The absorbance was measured in an enzyme-linked immunoabsorbent assay plate reader (Bio-Rad) at a wavelength of 570 nm. Based on concentration, 50% of cell death (ID_{50}) was determined for various compounds tested.

3.2. Typical procedure of synthesis of nitrogen mustard porphyrins 1–3

The reactions were performed in a 250 mL three neck round bottom flask fitted with a gas inlet port. The flask was charged with 100 mL of distilled $CHCl_3$, 1 mmol of

substituted benzaldehyde, and 2 mmol of pyrrole. The resulting solution was magnetically stirred at room temperature and purged by nitrogen gas for 20 min. After addition of 2.5 mmol of CCl_3COOH into the solution, the reaction flask was shielded from light for 16–24 h, then 0.8 mmol of *p*-chloranil was added at once. The solution was stirred for another 2–3 min before it was refluxed for 1 h in water bath. After cooling down to room temperature, 2 mmol of Et_3N was added to neutralize the acid. The solvent ($CHCl_3$) was removed by rotary evaporation under vacuum. The crude product was purified by column chromatography with mixture eluent of $CHCl_3$, methanol, and Et_3N. The first band was collected. The solvent was removed under vacuum to obtain the desired products.

3.2.1. 5,10,15,20-Tetra-{4-[*N*,*N*-di(2-chloroethyl)amino]-phenyl}porphyrin (1). Yield: 34%. MS: 1174 (100%), isotope peaks: 1170–1180. Anal. Calcd for $C_{60}H_{58}N_8Cl_8$: C, 61.34; H, 4.98; N, 9.54; Cl, 24.14. Found: C, 60.89; H, 5.08; N, 8.95. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.05–8.89 (8H, pyrrolic), 7.00–7.20 (16H, ArH), 3.94 (16H, Cl–CH_2), 1.01–1.48 (16H, N–CH_2), −2.61 (2H, NH). IR (KBr): 3435 (N–H), 2920, 2852 (CH_2), 1605, 1514, 1469 (C=C), 1349 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 436 (305), 523 (20), 569 (24), 661 (11).

3.2.2. 5,10,15,20-Tetra{4-[*N*,*N*-di(2-chloropropyl)amino]-phenyl}porphyrin (2). Yield: 14%. MS: 1286 (100%), isotope peaks: 1282–1292; Anal. Calcd for $C_{68}H_{74}N_8C_{l8}$: C, 63.46; H, 5.80; N, 8.71; Cl, 22.04. Found: C, 63.02; H, 6.19; N, 8.55. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.05–8.89 (8H, pyrrolic), 7.00–7.20 (16H, ArH), 4.35 (8H, Cl–CH), 2.15–2.33 (16H, N–CH_2), 0.92–1.23 (24H, CH_3), −2.61 (2H, NH). IR (KBr): 3436 (N–H), 2924, 2868 (CH_2), 1605, 1515, 1467 (C=C), 1352 (C–N), 800 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 434 (300), 525 (19), 568 (22), 655 (9.7).

3.2.3. 5,10,15,20-Tetra{4-[*N*,*N*-di(2,3-dichloropropyl)-amino]phenyl}porphyrin (3). Yield: 38%. MS: 1562 (100%), isotope peaks: 1554–1573; Anal. Calcd for $C_{68}H_{66}N_8Cl_{16}$: C, 52.27; H, 4.26; N, 7.17; Cl, 36.30. Found: C, 51.89; H, 4.19; N, 6.61. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.12–8.91 (8H, pyrrolic), 6.78–7.42 (16H, ArH), 4.63 (8H, Cl–CH), 3.97–4.3 (16H, Cl–CH_2), 2.05–2.67 (16H, N–CH_2), −2.90 (2H, NH). IR (KBr): 3431 (N–H), 2922, 2851 (CH_2), 1605, 1513, 1468 (C=C), 1352 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 433 (315), 524 (19), 567 (27), 655 (13).

3.3. Typical synthesis procedure of nitrogen mustard porphyrins 4–9

The synthesis of asymmetric porphyrin is similar to that of symmetric porphyrin excepting the purification process and the ratio of the substituted benzaldehyde to pyrrole or benzaldehyde. Substituted benzaldehyde (3 mmol), 1.0 mmol of benzaldehyde, and 4.0 mmol of pyrrole were mixed in 100 mL of distilled $CHCl_3$. The resulting solution was magnetically stirred at room temperature and purged by nitrogen gas for 20 min. After adding 2.0 mmol of CCl_3COOH or 2.5 mmol of $CHCl_2COOH$ into the solution, the reaction flask was shielded from light for 16 h, then 0.8 mmol of *p*-chloranil was added at once. The solution was stirred for another 2–3 min before it was refluxed for 1 h in a water bath. After cooling down to room temperature, 2.0 mmol of Et_3N was added to neutralize the acid. The solvent ($CHCl_3$) was removed by rotary evaporation under vacuum. The crude products were purified by column chromatography with mixture eluent of $CHCl_3$ and petroleum (3:2). The second band was collected. The solvent was removed under vacuum to obtain the desired products.

3.3.1. 5,10,15-Triphenyl-20-{4-[*N*,*N*-di(2-chloroethyl)-amino]phenyl}porphyrin (4). Yield: 21%. MS: 754 (100%), isotope peaks: 752–758; Anal. Calcd for $C_{48}H_{37}N_5Cl_2$: C, 76.39; H, 4.94; N, 9.28; Cl, 9.39. Found: C, 76.20; H, 4.21; N, 8.69. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.88–8.96 (8H, pyrrolic), 7.12–7.35 (19H, ArH), 3.95–4.09 (4H, Cl–CH_2), 1.25–1.73 (4H, N–CH_2), −2.72 (2H, NH). IR (KBr): 3435 (N–H), 2927, 2849 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 417 (289), 514 (18), 552 (23), 646 (9).

3.3.2. 5,10,15-Triphenyl-20-{4-[*N*,*N*-di(2-chloropropyl)-amino]phenyl}porphyrin (5). Yield: 19%. MS: 782 (100%), isotope peaks: 780–788; Anal. Calcd for $C_{50}H_{41}N_5Cl_2$: C, 76.72; H, 5.28; N, 8.95; Cl, 9.06. Found: C, 76.93; H, 5.83; N, 8.75. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.57–8.95 (8H, pyrrolic), 6.78–7.35 (19H, ArH), 4.23–4.35 (2H, Cl–CH), 2.05–2.11 (4H, N–CH_2), 0.93–1.25 (6H, CH_3), −2.72 (2H, NH). IR (KBr): 3436 (N–H), 2924, 2868 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 800 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 416 (299), 514 (19), 551 (23), 646(9).

3.3.3. 5,10,15-Triphenyl-20-{4-[*N*,*N*-di(2,3-dichloropropyl)amino]phenyl}porphyrin (6). Yield: 27%. MS: 851 (100%), isotope peaks: 849–859; Anal. Calcd for $C_{50}H_{39}N_5Cl_4$: C, 70.51; H, 4.62; N, 8.22; Cl, 16.65. Found: C, 69.95; H, 4.21; N, 7.89. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.89–8.91 (8H, pyrrolic), 6.77–7.35 (19H, ArH), 4.25–4.37 (2H, Cl–CH), 3.97–4.32 (4H, Cl–CH_2), 2.05–2.33 (4H, N–CH_2), −2.74 (2H, NH). IR (KBr): 3435 (N–H), 2923, 2849 (CH_2), 1605, 1513, 1469 (C=C), 1351 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 421 (301), 518 (20), 555 (25), 649 (10).

3.3.4. 5-Phenyl-10,15,20-tri{4-[*N*,*N*-di(2-chloroethyl)-amino]phenyl}porphyrin (7). Yield: 16%. MS: 1034

(100%), isotope peaks: 1031–1039; Anal. Calcd for $C_{56}H_{51}N_7Cl_6$: C, 65.00; H, 4.97; N, 9.48; Cl, 20.56. Found: C, 65.23; H, 4.82; N, 9.33. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.06–8.95 (8H, pyrrolic), 713–7.35 (17H, ArH), 3.97–4.13 (12H, Cl–CH_2), 1.33–1.48 (12H, N–CH_2), −2.72 (2H, NH). IR (KBr): 3434 (N–H), 2921, 2847 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 428 (303), 519 (20), 559 (26), 650 (14).

3.3.5. 5-Phenyl-10,15,20-tri{4-[*N*,*N*-di(2-chloropropyl)-amino]phenyl}porphyrin (8). Yield: 13%. MS: 1118 (100%), isotope peaks: 1115–1123; Anal. Calcd for $C_{62}H_{63}N_7Cl_6$: C, 66.55; H, 5.68; N, 8.76; Cl, 19.01. Found: C, 66.34.23; H, 5.92; N, 8.78. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.76–8.99 (8H, pyrrolic), 6.78–7.39 (17H, ArH), 4.23–4.41 (6H, Cl–CH), 2.08–2.43 (12H, N–CH_2), 0.97–1.34 (18H, CH_3), −2.72 (2H, NH). IR (KBr): 3435 (N–H), 2924, 2867 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 428 (300), 519 (21), 560 (26), 650 (15).

3.3.6. 5-Phenyl-10,15,20-tri{4-[*N*,*N*-di(2,3-dichloropropyl)amino]phenyl}porphyrin (9). Yield: 16%. MS: 1325 (100%), isotope peaks: 1319–1329; Anal. Calcd for $C_{62}H_{57}N_7Cl_{12}$: C, 56.18; H, 4.33; N, 7.40; Cl, 32.09. Found: C, 56.01; H, 4.41; N, 7.41. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.12–8.91 (8H, pyrrolic), 6.98–7.42 (17H, ArH), 4.22–4.63 (6H, Cl–CH), 3.97–4.37 (12H, Cl–CH_2), 2.15–2.67 (12H, N–CH_2), −2.91 (2H, NH). IR (KBr): 3435 (N–H), 2922, 2851 (CH_2), 1605, 1514, 1469 (C=C), 1352 (C–N), 802 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 429 (330), 521 (22), 562 (26), 653 (16).

3.4. Synthesis of chloroalkyl piperazine porphyrins 10–12

Chloroalkyl piperazine porphyrins **10**–**12** was synthesized according to the typical synthesis procedure of nitrogen mustard porphyrins **1**–**3**. The crude products were purified by column chromatography with mixture eluent of $CHCl_3$ and petroleum (3:2). The first band was collected. The solvent was removed under vacuum to obtain the desired products.

3.4.1. 5,10,15,20-Tetra-{4-[4′-(2-chloroethyl)piperazinyl]-phenyl}porphyrin (10). Yield: 17%. MS: 1201 (100%), isotope peaks: 1198–1207; Anal. Calcd for $C_{68}H_{74}N_{12}Cl_4$: C, 67.99; H, 6.21; N, 13.99; Cl, 11.81. Found: C, 67.69; H, 6.08; N, 13.47. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.05–8.97 (8H, pyrrolic), 7.02–7.4 (16H, ArH), 3.94–4.12 (8H, Cl–CH_2), 2.02–2.42 (40H, N–CH_2), −2.67 (2H, NH). IR (KBr): 3435 (N–H), 2922, 2851 (CH_2), 1605, 1512, 1451 (C=C), 1309 (C–N), 802 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 430 (207), 525 (16), 564 (20), 653 (8).

3.4.2. 5,10,15,20-Tetra{4-[4′-(2-chloropropyl)piperazinyl]-phenyl}porphyrin (11). Yield: 13%. MS: 1257 (100%), isotope peaks: 1254–1262; Anal. Calcd for $C_{72}H_{82}N_{12}Cl_4$: C, 68.78; H, 6.57; N, 13.37; Cl, 11.28. Found: C, 69.22; H, 6.18; N, 13.81. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.07–8.97 (8H, pyrrolic), 7.12–7.44 (16H, ArH), 3.94–4.12 (8H, Cl–CH_2), 2.00–2.33 (40H, N–CH_2), 0.97–1.23 (CH_3), −2.68 (2H, NH). IR (KBr): 3435 (N–H), 2924, 2851 (CH_2), 1605, 1513, 1451 (C=C), 1312 (C–N), 802 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 430 (197), 525 (17), 563 (19), 653 (7).

3.4.3. 5,10,15,20-Tetra{4-[4′-(2,3-dichloropropyl)piperazinyl]phenyl}porphyrin (12). Yield: 21%. MS: 1395 (50%), isotope peaks: 1390–1400; Anal. Calcd for $C_{72}H_{78}N_{12}Cl_8$: C, 61.99; H, 5.64; N, 12.05; Cl, 20.33. Found: C, 61.14; H, 6.19; N, 11.31. ^{1}H NMR ($CDCl_3$), δ (ppm): 8.05–8.97 (8H, pyrrolic), 7.02–7.4 (16H, ArH), 3.91–4.22 (8H, Cl–CH_2), 2.05–2.37 (40H, N–CH_2), −2.68 (2H, NH). IR (KBr): 3436 (N–H), 2927, 2856 (CH_2), 1605, 1512, 1452 (C=C), 1309 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 431 (295), 525 (19), 564 (27), 653 (11).

3.5. Typical synthesis procedure of nitrogen mustard porphyrins 13–15

The flask was charged with 100 mL of distilled $CHCl_3$, 1.0 mmol of substituted benzaldehyde, and 3.0 mmol of phenyldipyrromethane that was synthesized according to the literature.[25] The resulting solution was magnetically stirred at room temperature and purged by nitrogen gas for 20 min. After adding 2.0 mmol of CCl_3COOH or 2.5 mmol of $CHCl_2COOH$ to the solution, the reaction flask was shielded from light for 16 h, then 0.8 mmol of *p*-chloranil was added at once. The solution was stirred for another 2–3 min before it was refluxed for 1 h in water bath. After cooling down to room temperature, 2.0 mmol of Et_3N was added to neutralize the acid. The solvent ($CHCl_3$) was removed by rotary evaporation under vacuum. The crude products were purified by column chromatography with mixture eluent of $CHCl_3$ and petroleum (3:2). The dark purple band was further purified for one more time to give the desired products.

3.5.1. 5,15-Diphenyl-10,20-di{4-[*N*,*N*-di(2-chloroethyl)-amino]phenyl}porphyrin (13). Yield: 34%. MS: 894 (100%), isotope peaks: 892–902; Anal. Calcd for $C_{52}H_{44}N_6Cl_4$: C, 69.80; H, 4.96; N, 9.39; Cl, 15.85. Found: C, 70.36; H, 5.10; N, 10.04. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.75–8.95 (8H, pyrrolic), 6.99–7.35 (18H, ArH), 3.9–4.13 (8H, Cl–CH_2), 1.25–1.48 (8H, N–CH_2), −2.69 (2H, NH). IR (KBr): 3435 (N–H), 2921, 2852 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 801 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}$ cm^{-1} mol^{-1} L)] in CH_2Cl_2: 427 (332), 521 (20), 561 (26), 652 (14).

3.5.2. 5,15-Diphenyl-10,20-di{4-[*N*,*N*-di(2-chloropropyl)-amino]phenyl}porphyrin (14). Yield: 33%. MS: 950 (100%), isotope peaks: 948–956; Anal. Calcd for $C_{56}H_{52}N_6Cl_4$: C, 70.74; H, 5.51; N, 8.84; Cl, 14.91. Found: C, 69.98; H, 5.96; N, 9.02. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.77–8.99 (8H, pyrrolic), 6.78–7.35 (18H, ArH), 4.23–4.35 (4H, Cl–CH), 2.05–2.43 (8H, N–CH_2), 0.93–1.25 (12H, CH_3), −2.67 (2H, NH). IR (KBr): 3436 (N–H), 2924, 2868 (CH_2), 1605, 1514, 1467 (C=C), 1354 (C–N), 800 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}\,cm^{-1}\,mol^{-1}\,L$)] in CH_2Cl_2: 422 (337), 519 (20), 559 (28), 651(14).

3.5.3. 5,15-Diphenyl-10,20-di{4-[*N*,*N*-di(2,3-dichloropropyl)amino]phenyl}porphyrin (15). Yield: 41%. MS: 1088 (100%), isotope peaks: 1084–1094; Anal. Calcd for $C_{56}H_{48}N_6Cl_8$: C, 61.78; H, 4.44; N, 7.72; Cl, 26.06. Found: C, 61.23; H, 4.41; N, 7.28. ^{1}H NMR ($CDCl_3$), δ (ppm): 7.99–8.91 (8H, pyrrolic), 7.13–7.42 (18H, ArH), 4.39–4.72 (4H, Cl–CH), 3.77–4.3 (8H, Cl–CH_2), 2.05–2.42 (8H, N–CH_2), −2.73 (2H, NH). IR (KBr): 3436 (N–H), 2922, 2851 (CH_2), 1605, 1513, 1468 (C=C), 1352 (C–N), 799 (C–H) cm^{-1}. UV–vis [λ_{max}, nm ($\varepsilon \times 10^{-3}\,cm^{-1}\,mol^{-1}\,L$)] in CH_2Cl_2: 425 (339), 518 (20), 558 (27), 652 (13).

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