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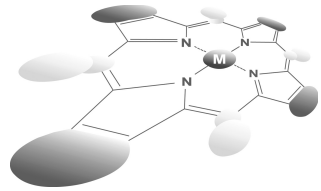

Selective synthesis and biological activity of triazine-porphyrins as potential anti-cancer agents

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ABSTRACT: Ten new triazine-porphyrin derivatives were synthesized using a simple one-pot procedure from the reaction of tetraphenylporphyrin bearing a hydroxyl group with 2,4,6-trichloro-1,3,5-triazine, and then with amines or alcohols. The structures of the products were characterized by ^{1}H NMR, LC/MS, UV-vis and elemental analysis. The cytotoxic activity of the triazine-porphyrin derivatives was evaluated *in vitro* against MCF-7 cell. All new compounds showed similar activity against MCF-7 cells in the absence of light when compared to 5-fluorouracil and hematoporphyrin.

KEYWORDS: triazine, synthesis, biological evaluation.

INTRODUCTION

In the past few decades, the selective affinity of porphyrins for cancer cells, which results in porphyrin and related compounds effectively accumulating within tumor cells and being retained for long periods of time, has attracted much attention [1]. This property of porphyrins provides the possibility for their use as targeting reagents in the design of anti-cancer drugs. Accordingly, the design and synthesis of the potential targeting anti-cancer drugs deriving from porphyrins has currently aroused widespread interest [2].

We are interested in the synthesis of linking a drug moiety to a porphyrin ring and evaluated their cytotoxic activity in the absence of light. From previous studies, porphyrin-bearing drug moieties could make the concentration of the drugs in tumor tissue higher than in surrounding normal tissue due to the tumor-affinity property of porphyrin [3–5]. Thus, it is possible to avoid or mitigate the side effects of drugs at the same dosages of the drugs.

Triazine derivatives have been widely studied due to its broad range of biological activities, such as anti-microbial effects [6], Erm (erythromycin-resistance methylase) methyltransferase inhibition [7], anti-trypanosomal activity [8], VLA-4 (integrin very late antigen-4) antagonism [9], estrogen receptor modulation [10], and cytotoxic activity [11]. Some triazine derivatives such as hexamethylmelamine were developed as an effective agent against breast, lung, and ovarian cancers [12]. More studies based on the triazine scaffold toward anti-tumor activity have been carried out [13–15].

Herein, we report the selective synthesis of the substituted triazine porphyrins using a one-pot procedure from the reaction of tetraphenylporphyrin bearing the hydroxyl group with 2,4,6-trichloro-1,3,5-triazine, and then with amines or alcohols in high yield. Through this method, we have synthesized ten new triazine porphyrins shown in Fig. 1. The primary anti-cancer activities of these compounds toward MCF-7 cancer cells were investigated *in vitro* in the absence of light. The results showed that these triazine porphyrins have anti-cancer activities, and point to their use as potential drugs for treatment of cancerous tumors.

RESULTS AND DISCUSSION

Synthesis of substituted 1,3,5-triazine porphyrin derivatives

The substituted triazine porphyrin **1**–**10** were the *meso*-substituted AB_3 porphyrins. Until now, the Adler-Longo

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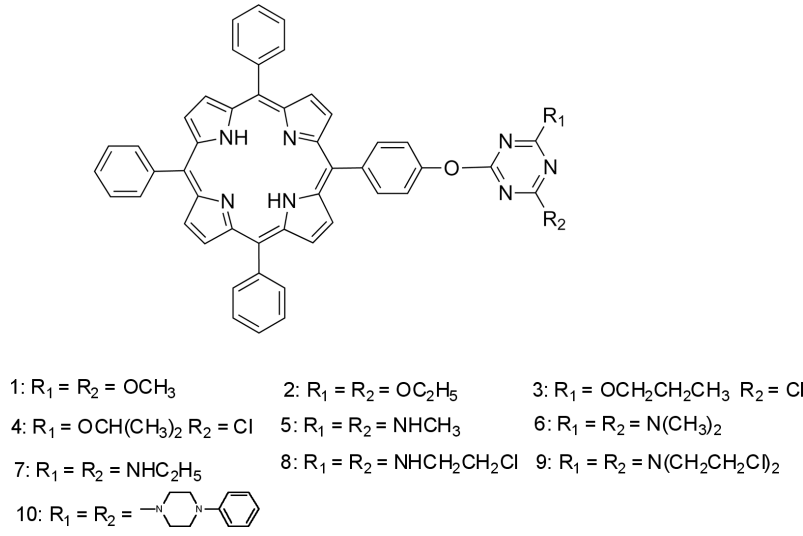


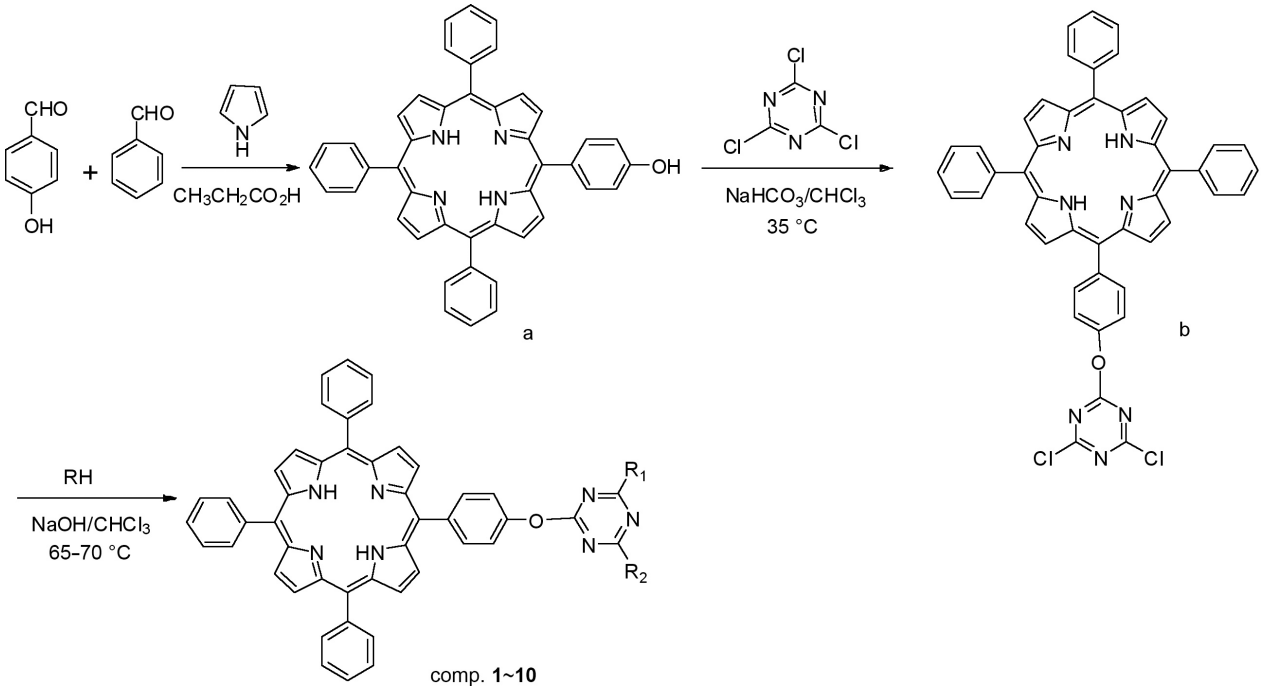
Fig. 1. Structures of ten triazine porphyrins

method and Lindsey methods have been the best for the synthesis of AB_3 by direct condensation of substituted benzaldehyde with pyrrole [16]. The key to the synthesis of the triazine porphyrins **1**–**10** is how to introduce the substituted triazine to the porphyrin ring. An attempt was made in our laboratory to synthesize porphyrins **1**–**10** *via* the Adler-Longo method by refluxing triazine-substituted benzaldehyde and pyrrole in propionic acid, or *via* Lindsey method by the reaction of triazine-substituted benzaldehyde with pyrrole in $BF_3{\cdot}Et_2O/CH_2Cl_2$. Unfortunately, no desired products were obtained. In our synthesis, the reactions were run for different acids, solvents and the reaction temperatures, but it was found that at all cases, no corresponding triazine porphyrins could be generated. All the compounds obtained were the black polymers due to the condensation of pyrrole with triazines. Therefore, the Adler-Longo and Lindsey methods are not suitable for the synthesis of substituted triazine porphyrins.

It is necessary to synthesize the substituted triazine porphyrins. In order to obtain substituted triazine porphyrins **1**–**10**, the synthetic route in Scheme 1 was proposed.

As shown in Scheme 1, 5-(4-hydroxylphenyl)-10,15,20-tri-(4-phenyl) porphyrin **a** was prepared according to procedures described in literature [17]. Compound **a** was first treated with cyanuric trichloride in the presence of $NaHCO_3$ and $CHCl_3$ below 35 °C for 5 hours to generate compound **b** without isolatation. The mixture compound **b** was further converted to the target compounds **1**–**10**, respectively, with the corresponding alcohols or amines in $NaOH/CHCl_3$.

It is well known that three chlorine substituents on the cyanuric trichloride are relatively active and they may be substituted by nucleophiles under suitable conditions [18]. When compound **a** was treated with cyanuric trichloride at 30–35 °C, compound **b** was isolated as the main product with a high yield. However, if the reaction was carried out at > 35 °C, a few side products were generated. This makes it difficult to isolate and purify the desired compound **b**. In addition, the reaction was



Scheme 1. Synthetic route of 1,3,5-triazine porphyrin derivatives

much slower if conducted at < 30 °C. The conversion of compound **a** to **b** was performed under basic conditions. Bases such as NaHCO, Na_2CO, K_2CO, pyridine, and triethylamine have no distinct effect on the reaction.

An attempt to generate a bi-porphyrin derivative by treating compound **b** with compound **a** was not successful even under higher temperature, with longer reaction time, or with a stronger base. However, compound **a** was able to react with small nucleophiles such as alcohols or amines to afford compounds **1**–**10**.

The second and third chlorine sunstituents on the triazine moiety of compound **b** could be substituted by amines to give the corresponding products. For example, compounds **5**–**10** were obtained by treating compound **b** with methylamine, domethylamine, ethylamine, 2-chloroethylamine, *N*,*N*-di(2-chloroethyl)amine and *N*-phenylpiperazine respectively. However, the nucleophilic substituion was much slower when lower amounts of nucleophilic alcohols were employed. For instance, it took 20–24 h to obtain compounds **1**–**2** by reacting compound **b** with methanol or ethanol. Furthermore, when compound was refluxed with *n*-propanol or *i*-propanol for 48 h, only monosubstituted products **3** and **4** were obtained.

The structures of these compounds were confirmed by UV-vis, MS, ^{1}H NMR spectra and elementary analysis. The triazine-porphyrins **1**–**10** have the same wavelength λ_{max} of the Soret and Q bands. However, they have different molar absorptivity ε.

Anti-cancer activity

In order to study whether these synthetic triazine-porphyrins have anti-cancer activity, compounds **1**–**10** and the reference compounds tetraphenylporphyrin, hematoporphyrin and 5-fluorouracil, were evaluated for their cell cytotoxicity using the MTT cytotoxicity assay [19] in the absence of light. The IC_{50} values of the MCF-7 cells to these compounds are listed in Table 1.

The test results showed that all compounds have similar activity against MCF-7 cells when compared to 5-fluorouracil and hematoporphyrin. The tetraphenylporphyrin was significantly less active against tested cancer cell lines in the absence of light. This result is in agreement with previous studies [3, 4] and show that tetraphenylporphyrin has low dark cytotoxicity.

In summary, a series of 1,3,5-triazine porphyrin derivatives were synthesized and evaluated for their *in vitro* cytotoxicity against MCF-7 cells in the absence of light. The results showed these hybrid porphyrins possess good anti-cancer activities. Further biological evaluation and mechanistic studies on this novel class of anti-cancer compounds are currently in progress and will be reported in due course.

Table 1. Cytotoxicity of the target compounds against MCF-7 cells *in vitro*[a,b]

Compound	IC_{50}, μM	Compound	IC_{50}, μM
1	17	**6**	14
2	19	**7**	18.3
3	14.8	**8**	12
4	14.5	**9**	8.5
5	15	**10**	12.5
Tetraphenylporphyrin	245	Hematoporphyrin	22.8
5-fluorouracil	4.7		

[a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation. [b] Values are means of three experiments.

EXPERIMENTAL

Instruments and reagents

^{1}H NMR spectra were recorded on an INOVA-400 spectrometer, and the chemical shifts were reported on the scale relative to TMS. Elemental analyses were obtained on a PerkinElmer 2400 elementary analyzer. Mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer with ESI ionization mode and analyzer. UV-vis absorption spectra were obtained with a PerkinElmer L-17 UV-vis spectrophotometer.

Cell survival assay

The cytotoxic effects of the compounds on the MCF-7 cancer cells were determined by using the MTT assay [20]. Cells were planted in 100 μL of medium at a concentration of 1×10^3 cells per well in 96-well microtiter plates which 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 72 h. The medium was then removed from the wells and 200 μL MTT (1 $\mu g.mL^{-1}$ 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. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Dose-Effect Analysis with Microcomputers".

Typical synthesis procedure of 1,3,5-triazine porphyrin 1–10

A solution of cyanuric trichloride (73.5 mg, 400 μmol) in 5 mL chloroform was added to a chloroform (20 mL) solution of 5-(4-hydroxylphenyl)-10,15,20-tri-(4-phenyl)porphyrin (100 mg, 160 μmol) and sodium bicarbonate

(84 mg, 1 mmol), the reaction mixture was stirred for another 5 hours with the temperature at 35 °C, after which reaction was complete as witnessed by TLC analysis (silica gel, petroleum ether/ethyl acetate = 3:1 (V/V)), showing the disappearance of porphyrin **a** (R_f = 0.21) and the formation of derivative **b** (R_f = 0.87). Product **b** was not isolated but was reacted further with an excess of 3–5 mL corresponding alcohol or amine and sodium hydroxide (40 mg, 1 mmol) and stirred at 45 °C for 1 h and then for another 10 hours at reflux temperature. After removal of the solvent, the residue was partitioned between $CHCl_3$ (15 mL) and water (20 mL × 4). The organic phase was dried on $MgSO_4$ and evaporated, and the crude product was purified by column chromatography (silica gel, petroleum ether/chloroform) to afford corresponding compound with good yields (50–62%).

5-[4-(4,6-dimethoxy-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (1). Yield: 53.2%. Anal. calcd. for $C_{49}H_{35}N_7O_3$: C, 76.45; H, 4.58; N, 12.74. Found: C, 76.23; H, 4.56; N, 12.62. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 419 (213.3), 512 (21.8), 549 (12.56), 630 (11.4). MS (ESI): *m/z* 770 (calcd. for $[M + 1]^+$ 770).

5-[4-(4,6-diethoxy-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (2). Yield: 52.8%. Anal. calcd. for $C_{51}H_{39}N_7O_3$: C, 76.77; H, 4.93; N, 12.99. Found: C, 76.58; H, 5.01; N, 13.20. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 419 (212.6), 513 (27.1), 550 (13.42), 631 (10.46). MS (ESI): *m/z* 798 (calcd. for $[M + 1]^+$ 798).

5-[4-(4-chloro-6-propoxy-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (3). Yield: 50.3%. Anal. calcd. for $C_{50}H_{36}ClN_7O_2$: C, 74.85; H, 4.52; N, 12.22. Found: C, 75.01; H, 4.61; N, 12.06. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 418 (218.5), 510 (27.5), 543 (15.28), 628 (12.1). MS (ESI): *m/z* 802 (calcd. for $[M + 1]^+$ 802).

5-[4-(4-chloro-6-isopropoxy-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (4). Yield: 50.6%. Anal. calcd. for $C_{50}H_{36}ClN_7O_2$: C, 74.85; H, 4.52; N, 12.22. Found: C, 74.98; H, 4.59; N, 12.17. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 418 (217.5), 510 (25.7), 543 (14.28), 628 (11.1). MS (ESI): *m/z* 802 (calcd. for $[M + 1]^+$ 802).

5-[4-(4,6-dimethylamino-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (5). Yield: 60.6%. Anal. calcd. for $C_{49}H_{37}N_9O$: C, 76.64; H, 4.86; N, 16.42. Found: C, 76.69; H, 4.93; N, 16.26. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 419 (215.7), 515 (25.64), 542 (15.78), 632 (12.58). MS (ESI): *m/z* 768 (calcd. for $[M + 1]^+$ 768).

5-[4-(4,6-bis-(dimethylamino)-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (6). Yield: 58.6%. Anal. calcd. for $C_{51}H_{41}N_9O$: C, 76.96; H, 5.91; N, 15.84. Found: C, 76.74; H, 5.93; N, 15.62. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 419.5 (216.8), 518 (28.99), 551 (15.70), 639 (13.87). MS (ESI): *m/z* 796 (calcd. for $[M + 1]^+$ 796).

5-[4-(4,6-diethylamino-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (7). Yield: 56.2%. Anal. calcd. for $C_{51}H_{41}N_9O$: C, 76.96; H, 5.19; N, 15.84. Found: C, 77.24; H, 6.01; N, 15.89. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 419 (215.8), 516 (16.1), 550 (15.87), 637 (13.50). MS (ESI): *m/z* 796 (calcd. for $[M + 1]^+$ 796).

5-[4-(4,6-bis(2-chloroethyl)amino-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (8). Yield: 51.9%. Anal. calcd. for $C_{51}H_{39}Cl_2N_9O$: C, 70.83; H, 4.55; N, 14.58. Found: C, 71.05; H, 4.49; N, 14.63. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 420 (215.5), 516 (26.35), 568 (16.41), 642 (13.67). MS (ESI): *m/z* 863 (calcd. for $[M + 1]^+$ 863).

5-[4-(4,6-bis-(bis-(2-chloroethyl))amino-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (9). Yield: 50.8%. Anal. calcd. for $C_{55}H_{45}Cl_4N_9O$: C, 66.89; H, 4.57; N, 12.67. Found: C, 66.89; H, 4.52; N, 12.87. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 421 (216.5), 517 (25.32), 568 (16.87), 647 (12.8). MS (ESI): *m/z* 988 (calcd. for $[M + 1]^+$ 988).

5-[4-(4,6-di(4-phenylpiperazine)-[1,3,5]triazin-2-yl)phenyl]-10,15,20-tri-(4-phenyl)porphyrin (10). Yield: 52.5%. Anal. calcd. for $C_{67}H_{55}N_{11}O$: C, 78.11; H, 5.38; N, 14.96. Found: C, 78.23; H, 5.35; N, 14.82. UV-vis (CH_2Cl_2): λ_{max}, nm ($\varepsilon \times 10^{-3}$ $M^{-1}.cm^{-1}$) 422 (218.5), 520 (27.97), 570 (16.52), 649 (12.8). MS (ESI): *m/z* 1030 (calcd. for $[M + 1]^+$ 1030).

Acknowledgements

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Supporting information

The ^1H NMR spectra of all the triazine-porphyrins are given in the supplementary material. This material is available free of charge *via* the Internet at http://www.worldscinet.com/jpp/jpp.shtml.

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