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Synthesis, Characterization and Biological Activities of Novel Water-soluble Metalloporphyrins

Zao Ying LI*, Yue Ning ZHANG, Zhen Ting GAO, Yi LIU, Xun Jin ZHU

Department of Chemistry, Wuhan University, Wuhan 430072

Abstract: The synthesis, characterization, and biological activities of a series of metal porphyrins were described. The complexes **3a~3e** were prepared from the reaction of compound **2** with $M(OAc)_2$ in $CHCl_3$, and the treatment of **3a~3f** with pyridine gave corresponding complexes **4a~4f**. These new compounds were identified by absorption spectroscopies, ^1H NMR and elemental analysis. The results of biological activity testing revealed that **4a** and **4c** had stronger inhibiting action for *Escherichia coli* (CCTCC AB91115).

Keywords: Water-soluble metalloporphyrin, synthesis, structure characterization, biological activity testing.

Water-soluble metal porphyrins can sensitize photocatalytic water reduction[1], oxidation[2], and are of potential use in photogalvanic cells[3]. In recent years, porphyrins have been used as photosensitizer for the photodynamic therapy (PDT) of cancers. To constitute a good PDT photosensitizer, a compound must be soluble in the body′s tissue fluids so that it can be injected and carried around the body to the tumour site[4]. Research on the anti-tumor activity of metalloporphyrins has also been reported[5]. It is known that 70% of the human body weight is composed of water. So the synthesis of water-soluble porphyrins is a crucial problem for application of porphyrins in biological systems. Typically, water-soluble porphyrins are derived from *meso*-tetra-4-pyridyl-porphyrin. In this paper, a more versatile synthetic strategy based on *meso*-tetrakis(4-hydroxyphenyl) porphyrin, was described. Here the synthesis and characterization of a series of novel water-soluble metalloporphyrins and their action on the growth metabolism of *Escherichia coli* (CCTCC AB91115) were reported.

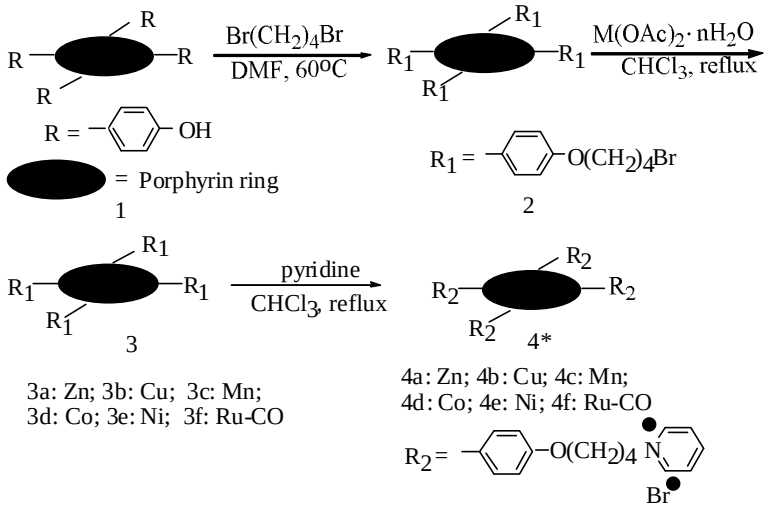
Synthesis and Characterization

*5,10,15,20-Tetrakis(4-hydroxyphenyl)porphyrin **1***

The compound **1** was synthesized with a modification of the literature procedure in ref. 6. Treating 5, 10, 15, 20-tetrakis(4-methoxyphenyl)porphyrin[7] with BBr_3 in CH_2Cl_2 afforded **1**. We use ice-salt bath (–15°C) cooling to replace acetone-dry ice bath (–80°C) cooling[6]. The yield is as high as later′s.

* E-mail: zyliwuc@whu.edu.cn

Scheme 1



4* (M = 2H)**:** 5,10,15,20-Tetrakis[4-(4′-butyloxypyridine bromide)phenyl] porphyrin.

*5,10,15,20-Tetrakis[4-(4′-bromobutyloxy)phenyl]porphyrin **2***

The compound **1** was reacted with 1,4-dibromobutane in the presence of dry K_2CO_3 in DMF at 60~70°C for 3~4 h. Then filtered and all solvent was evaporated under vacuum. The residue was chromatographed on silica gel with chloroform. The product was crystallized from light petroleum to give **2**, in yield of 90%.

*Metal porphyrins **3a~3f***

Treating porphyrin **2** with metal acetate $M(OAc)_2$ (M=Zn, Cu, Mn, Co, Ni) in chloroform at 60°C for 4~5 h gave corresponding metalloporphyrins **3a~3e**, which were isolated in quantitative yields. Compound **3f** was prepared from porphyrin **2** and $Ru_3(CO)_{12}$ in toluene, which were refluxed with stirring under N_2 for 24 h. The residue was chromatographed on a silica gel column using chloroform as eluent, recrystallized from chloroform and light petroleum to give **3f**, in yield of 80%.

*Metalloporphyrin pyridine bromide salts **4a~4f***

The solution of **3a~3f** in excess of pyridine and chloroform was refluxed for 48 h. The precipitate was washed with acetone to obtain **4a~4f**, which are pure enough for biological activities testing.

Results and Discussion

These novel compounds were characterized by absorption spectroscopies and ^{1}H NMR spectra[8-11].

The ^{1}H NMR spectral data of **2** were shown in note 8. All the signals have been well assigned. Its UV-vis spectrum showed five absorption peak at 424 (Soret Band), 519, 556, 602, and 650 nm respectively, which was similar to those of other free base of porphyrins.

The IR spectrum of **4*** showed an absorption peak at 1352 cm^{-1} [H-CN (pyridine)], which indicated that pyridine had been linked to the porphyrin ring, and it also exhibited excellent solubility in water.

The disappearance of the singlet at about –2.9 ppm in the ^{1}H NMR spectra of all the metalloporphyrins indicated that metal atoms had been inserted into the center of the porphyrin ring. Their IR spectra all showed OSMB (Oxidation State Marked Band) at about 1000 cm^{-1}. The 1931 cm^{-1} absorption peak observed in IR spectrum of **4f** indicated the presence of Ru→C≡O.

The LB culture medium (pH = 7.0~7.2) consisted of yeast extract (OXOID), tryptone (OXOID), sodium chloride, and distilled water. An LKB-2277 Thermal Activity Monitor (Thermometric, Järfälla, Sweden) was used to monitor cell metabolic activity in *vitro* to produce thermogenesis curves. The operation of the instrument and details of its construction have been described previously[12].

The growth rate constants k, maximum heat production rates P_{max} and peak times of growth thermogenic curves t_p of *E. coli* in different metalloporphyrins at different concentrations were shown in **Table 1**.

Table 1 Growth rate constants k, maximum heat production rates P_{max} and peak times of growth thermogenic curves t_p of *E. coli* in different metalloporphyrins at 37°C[a]

Concentration (μg/ml)		50	100	200	300	400	500
LB		K = 2.549, P_{max}=687, t_p = 305					
	k/10^{-2}	2.831	1.932	1.814	1.708	1.522	0
4a	P_{max}	670	660	615	510	430	0
	t_p	412.5	425	457.5	512.5	620	
	k/10^{-2}	[b]1.674	[c]1.788	[d]1.992	[e]1.893	[f]1.789	1.55
4b	P_{max}	701	717	705	703	645	505
	t_p	382.5	372.5	349.5	415	420	562.5
	k/10^{-2}	[g]2.049	1.766	1.559	1.309		0.516
4c	P_{max}	660	677	600	542.5		326
	t_p	330	335	380	443		922.5
	k/10^{-2}	2.45	2.41	2.029	1.914	1.879	1.734
4d	P_{max}	590	600	600	655	547	538
	t_p	437.5	440	465	467.5	475	502.5
	k/10^{-2}	1.958	1.978	1.883	1.857	1.521	1.26
4e	P_{max}	685	635	689	680	596	415
	t_p	372.5	330	370	380	442.5	725

[a]1000 μw, 2 V, 0.2 mm/min. k = min^{-1}; P_{max} = μw; t_p = min; [b]41.3 μg/mL; [c]90 μg/ml; [d]180 μg/mL; [e]270 μg/mL; [f]360 μg/mL; [g]20 μg/mL.

From **Table 1**, it can be seen that in general with the increase of the concentration of metalloporphyrins, the inhibiting action on the growth metabolism of *E. coli* was enhanced, the growth rate constants (k) and the maximum heat production rates (P_{max}) got less, and the peak time of growth thermogenic curves (t_p) became longer. The

inhibiting activity was highly dependent on the nature of the central metal atoms of these complexes. Among them, Zn(Por) **4a** and Mn(Por) **4c** have stronger inhibiting activity. Zn(Por) **4a** is the strongest one. At the concentration of 500 ìg/mL, the growth metabolism of *E. coil* is inhibited completely in the presence of **4a**.

In conclusion, this work not only described a convenient method for preparation of water-soluble metalloporphyrins, but also proved their inhibiting activity on *Escherichia coli*. It can be expected that metalloporphyrins might be applied as agent for treatment of malignant diseases. The inhibiting activity dependence on the nature of the central metal atoms and different structures of porphyrin rings is under further investigation.

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8. Spectral data for 2: ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.86 (s, 8H, H_β*); 8.10 (d, 8H, J=9.0 Hz, H_o**); 7.24 (d, 8H, J=9.0 Hz, H_m**); 4.25 (t, 8H, J=6.0 Hz, $ArOCH_2CH_2$); 3.62 (t, 8H, J=6.0 Hz, $ArOCH_2CH_2CH_2CH_2$); 2.28~2.20 (m, 8H, $ArOCH_2CH_2$); 2.16~2.10 (m, 8H, ArO- $CH_2CH_2CH_2CH_2$); -2.75 (s, 2H, N-H); Anal. Calcd. for $C_{60}H_{58}N_4O_4Br_4$: C, 59.13; H, 4.80; N, 4.60. Found: C, 60.31; H, 5.12; N, 4.15.
   *H_β: the protons of pyrrole rings in porphyrin ring;
   **H_o, H_m: the protons in the *ortho* and *meta* positions of the tetraphenyl porphyrin ring.
9. Spectral data for **4*:** ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.27 (d, 8H, J = 6.0 Hz, α-Py. H); 8.85 (s, 8H, H_β); 8.69 (t, 4H, J = 7.5 Hz, γ-Py. H), 8.36 ~ 8.24 (m, 8H, β-Py. H), 8.12 (d, 8H, J = 6.0 Hz, H_o), 7.39 (d, 8H, J = 9.0 Hz, H_m), 4.85 (t, 8H, J = 5 Hz, $ArOCH_2CH_2$); 4.33 (t, 8H, J = 6.0 Hz, $ArOCH_2CH_2CH_2CH_2$); 2.33~2.24 (m, 8H, $ArOCH_2CH_2$); 1.98~1.93 (m, 8H, $ArOCH_2CH_2CH_2CH_2$); -2.90 (s, 2H, N-H); Anal. Calcd. for $C_{80}H_{78}N_8O_4Br_4$: C, 62.59; H, 5.12; N, 7.30. Found: C, 62.14; H, 5.21; N, 7.12.
10. Spectral data for **4a**: ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm), 9.24 (d, 8H, J = 3.0 Hz, α-Py. H); 8.79 (s, 8H, H_β); 8.68 (t, 4H, J = 7.5 Hz, γ-Py. H), 8.27~8.23 (m, 8H, β-Py. H), 8.06 (d, 8H, J=9.0 Hz, H_o), 7.34 (d, 8H, J = 6.0 Hz, H_m), 4.83 (br, 8H, $ArOCH_2CH_2$); 4.32 (br, 8H, $ArOCH_2CH_2CH_2CH_2$); 2.28 (br, 8H, $ArOCH_2CH_2$); 1.95 (br, 8H, $ArOCH_2CH_2CH_2CH_2$).
11. Spectral data for **4f**: ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm), 9.24 (br, 8H, α-Py. H); 8.68 (t, 4H, J = 7.5 Hz, γ-Py. H), 8.58 (s, 8H, H_β); 8.33 ~ 8.23 (m, 8H, β-Py. H), 8.08 (d, 8H, J = 9.0 Hz, H_o), 7.32 (t, 8H, J = 7.5 Hz, H_m), 4.83 (br, 8H, $ArOCH_2CH_2$); 4.31 (br, 8H, $ArOCH_2CH_2CH_2CH_2$); 2.29~2.25 (m, 8H, $ArOCH_2CH_2$); 1.94 (br, 8H, $ArOCH_2CH_2CH_2CH_2$).
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