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Synthesis and photodynamic activity of some tetraazoporphyrin derivatives

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Summary — Photodynamic therapy (PDT) is a treatment modality for a number of human neoplasms. The drug preparation most widely used in current clinical trials is photofrin. Photofrin lacks definite structure and as a result the interpretation of its pharmacokinetic data is difficult. Thus the development of new sensitizers of known structure such as the phthalocyanines and naphthalocyanines has been proposed. In our approach, we have studied the use of tetraazaporphyrins (porphyrazines) as sensitizers. This class of sensitizers has received very little attention. A number of porphyrazines were synthesized via the cyclization of tricyanovinyl amines and dinitrile amines in pentanol solution of magnesium oxide. These porphyrazines were converted to their respective zinc chelates and tested for their stability in acidic media and for their ability to photoinactivate cells in culture. Results indicate that these porphyrazines show promise and must be investigated further for applications in PDT.

photodynamic therapy / tetraazoporphyrin / sensitizer / photofrin / singlet oxygen quantum yield / photoinactivate

Introduction

Porphyrins and phthalocyanines have been widely evaluated during the past few years for their use in detection and treatment of neoplasms [1]. These applications are based on the principle that preferential retention of certain porphyrinic compounds happens in rapidly proliferating tissues, such as neoplastic tissues, and the delivery of light of appropriate wavelength to malignant tissues after injection of the dye results in the selective destruction of tumors with minimal damage to normal tissue [2, 3].

The drug preparation most widely used in current clinical trials is photofrin, a composition which shows a number of well-documented disadvantages such as chemical purity and prolonged skin photosensitivity in patients receiving therapy. However, photofrin has also produced results which suggest that PDT is a very promising treatment modality for the treatment of selected neoplasms [3]. For example, it has been reported by Kato et al that 84% of early stage lung cancers are curable by PDT if the peripheral tumor is less than 2 cm in size and it has not metastasized. For early stage, central type lung cancer, curability increases to 100% [4].

Photofrin is a mixture of monomers and oligomers of ester- or ether-linked hematoporphyrins. The composition of photofrin seems to change with time (specifically the ratio of ester/ether). It thus has to be kept at low temperature and be protected from sunlight [5]. Also its low absorption (600 nm, $\varepsilon = 6000$) limits its use to the treatment of superficial tumors, since light at 600 nm does not have sufficient energy to penetrate tissue [6]. There is thus need for compounds which strongly absorb red light (700–800 nm), which would be of greater effectiveness in PDT. Tetraazaporphyrins or porphyrazines (Pz) **3** mimic porphyrins **1** and phthalocyanines **2** (fig 1), have an important

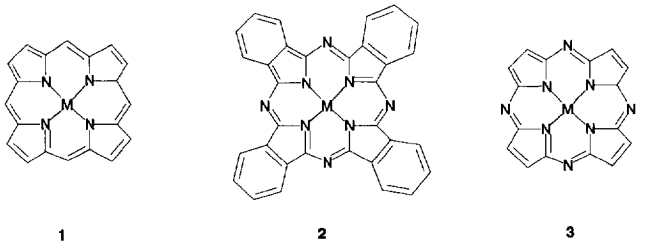

Fig 1. Structure of a porphyrin **1**, phthalocyanine **2** and a porphyrazine **3**.

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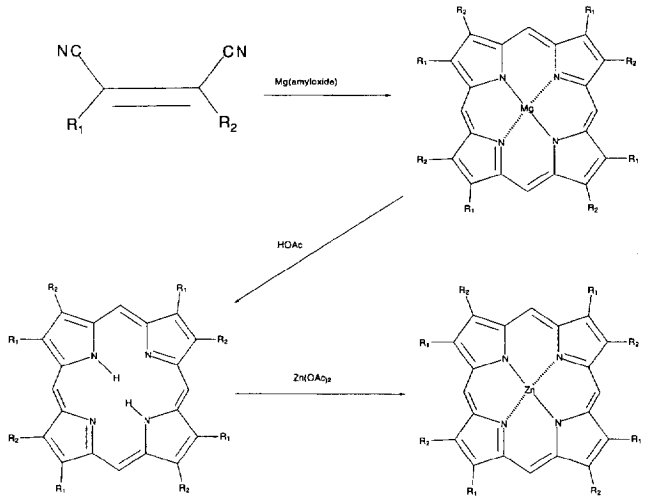
absorption maximum between 250 and 350 nm (Soret), like photofrin in many respects, but in addition they also exhibit strong absorption bands between 710 and 800 nm. Porphyrazines are heterocyclic compounds consisting of four pyrrole nuclei fused via nitrogen bridges and form stable chelates with metal cations. The first tetraazaporphyrin was prepared as the product of the bromination of 3-ethyl-4-methylpyrrole in the presence of ammonia [7–12]. Tetraazaporphyrin itself was synthesized using a method analogous to that used in the synthesis of phthalocyanines, ie, treatment of maleonitrile with magnesium *n*-propyloxide to afford the magnesium tetraazaporphyrin complex in up to 15% yield. Conversion to the free base was achieved by treatment of the metal complex with glacial acetic acid. Magnesium tetraazaporphyrin is also formed in low yield from succinimidine by heating with magnesium formate [7–12].



Scheme 1. Synthesis of cyano and cyano-free, and metallo-Pz's (R_1 = CN,H, R_2 = amino).

Chemistry

In order to verify experimental conditions, tetra (*t*-butylamino)tetracyano porphyrazine **9** was prepared according to the procedure reported previously by Kopranenkov [13]. Reaction of *t*-butylaminotricyanovinyl **1** with magnesium pentyloxide in pentyl alcohol resulted in the formation of **9** (as magnesium chelate) in a mixture of three isomers. Silica-gel chromatography allowed separation of the major isomer, which by statistical considerations is thought to be the symmetric isomer. Subsequent treatement with glacial acetic acid resulted in isolation of the metal-free compound **15** (scheme 1). The zinc complex **16** was prepared by treating **15** with zinc acetate in DMF (scheme 1). Using the same procedure, porphyrazines **14**, **18** and **20** were prepared as well. A number of cyano-free Pz's (**21–23**) were also prepared via the dinitrile reaction with pentanol solution of magnesium pentyloxide. The visible spectrum is indicative of the formation of the macrocycle and this is confirmed by FAB mass spectrometry.

The stability of both the cyano and cyano-free porphyrazines in sulfuric acid was examined. Khelevina [14] and coworkers previously reported that the tetra(tetramethylene)tetraazaporphyrin (TTMTAP) in sulfuric acid has a K_{eff} of 1.59×10^5 s^{-1} at 298 K with an energy of activation (E_a) of 81 J/mol and an entropy of activation of ($\Delta S^{\#}$) –72 ± 2 J/mol·deg. In the present study, K_{eff} was calculated from the change in the optical density of the starting solution from the equation (figs 2 and 3, table I):

$$K_{eff} = (1/\tau) \ln C_0/C$$
$$= (1/\tau) \ln A_0 - A/A_\tau - A$$

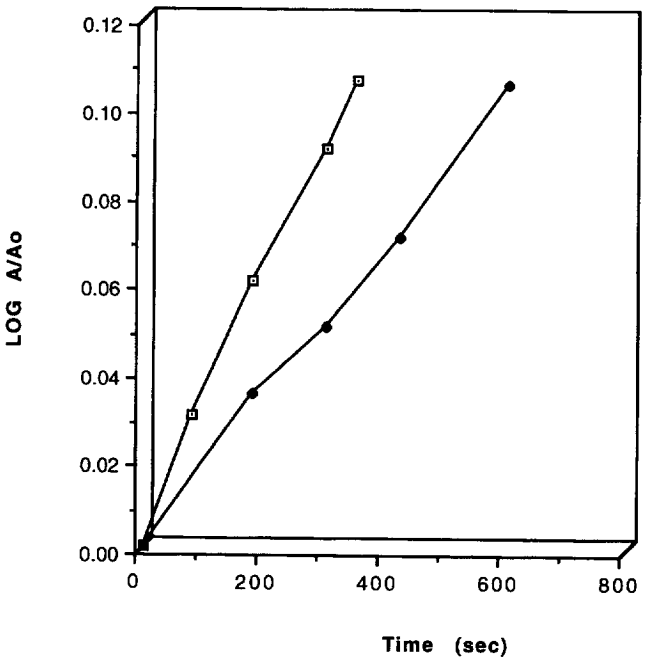


Fig 2. Acid stability curve of Zn(cyano)porphyrazine at 298 K (♦) and 308 K (⊡).

C_0 and C are the initial and instantaneous concentrations of the porphyrazines, and A_0, A_τ, and A are the initial, instantaneous, and final optical densities.

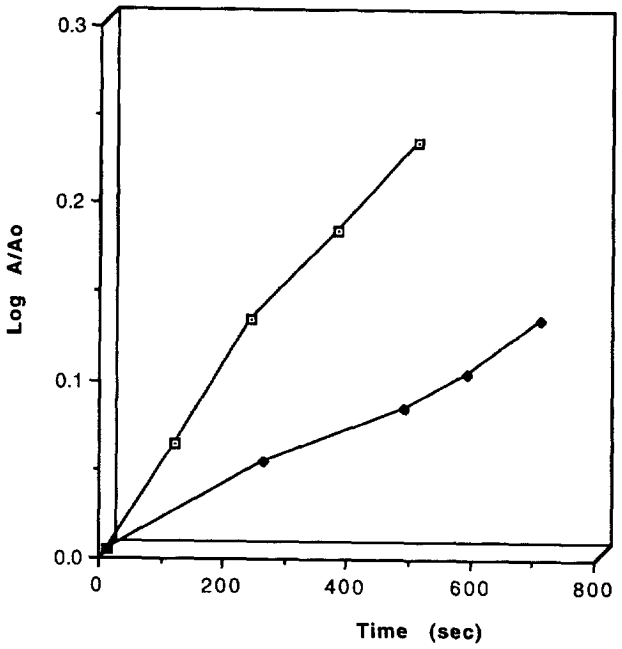


Fig 3. Acid stability curve of a Mg(cyano-free)porphyrazine at 298 K (♦) and 308 K (⊡).

Biology

Cells were incubated for 1 h with the porphyrazine in question at different concentrations. After washing the cells with buffer to remove residual drug, the cells were exposed to red light for 4 min at which time cells were incubated for 6 days for colony formation. The results were expressed as the LD_{90} which is the required concentration needed to produce 90% kill (table II). The light source was a tungsten/halogen lamp adjusted to produce 25 mW/cm^2. The plates containing the drugs were irradiated for 4 min resulting in 6 J/cm^2 total. A red filter was used to remove light at other wavelengths as well as a water filter in order to remove IR radiation. The plates were kept at 37 °C during irradiation. Appropriate standards were used. Each data point corresponds to three plates and each experiment was done three times. All drugs tested were delivered in an emulsion of polypropylene glycol and cremophore EL. The concentration of the drug in the emulsion was recalculated in order to eliminate error. This was done as follows: 1, 5, 10, 20, and 50 mL of the emulsion were placed in five separate 1 mL volumetric flasks and brought to volume using DMF (in order to eliminate aggregation and as a result error). Each solution was read using a spectrophotometer and the epsilon (ε) values and Beer's law

Table I. Acid stability of cyano and cyano-free porphyrazines

Compound	K_{eff} *(s^{-1}, 298 K/308 K)*	E_a *(J/mol)*	$\Delta S^{\#}$ *(J/mol·deg)*
Cyano Pz **19**	$7 \times 10^{-5}/2.4 \times 10^{-5}$	−81.68 (+1)	−81.36 (+2)
Cyano-free Pz **23**	$1.878 \times 10^{-4}/3.103 \times 10^{-4}$	38.309	−70.4797
TTMTAP	1.59×10^{5}	N/A	N/A

K_{eff} = decomposition constant; E_a = energy of activation; $\Delta S^{\#}$ = entropy of activation.

Table II. Photoinactivation of V-79 Chinese hamster cells

Compound	R_1	R_2	*M*	λ_{max} *(nm)*	LD_{90} *(μM, μg/mL)*
9[a]	CN	NBu^t	Mg^{2+}	750	2–4, 1.4–2.9
18[a]	CN	NEt_2	Zn^{2+}	754	1, 0.76
20[b]	CN	$Npentyl^n$	Zn^{2+}	715	>70, >57
16[a]	CN	NBu^t	Zn^{2+}	755	2–2.5, 1.5–1.9
14[b]	CN	Piperidine	Zn^{2+}	764	>75, >61
10[b]	H	Piperidine	Mg^{2+}	710	>70, >43.4
22[b]	H	NBu^t	Mg^{2+}	705	>70, >43.4
23[b]	H	NEt_2	Mg^{2+}	703	>70, >44.4

LD_{90} = lethal dose that will kill 90% of the cells; [a]no toxicity was observed in the dark at > 5 mg/mL; [b]no toxicity was observed in the dark at > 60 mg/mL

were used to calculate the concentrations. Appropriate controls were used such as irradiation of cells in the absense of the drug and incubation of the cells with the drug in the dark. A number of statistical techniques were employed in order to test the validity and accuracy of experimental data. The Q test was applied in deciding if a value was to be rejected or retained.

Results and discussion

The absence of the electron-withdrawing cyano group has a marked influence on the visible spectrum as shown by the blue shift of the Q-band. Also broadening of the longwave band is observed on account of their succeptibility to aggregation. Attempts to demetalate these tetrasubstituted porphyrazines resulted in the decomposition of the macrocycle. This observation suggests that the removal of the cyano electron- withdrawing group results in an increase of the K_b of the peripheral amine functionality. This leads to more rapid protonation of the peripheral nitrogens which subsequently results to the decomposition of the macrocycle. It has been observed that, in phthalocyanines, protonation of peripheral nitrogens followed by protonation of the *meso* nitrogens also leads to the decomposition of the ring. The use of the slope from the plot $\log A_0/A$ vs time, allows for the calculation of K_{eff} (figs 2 and 3; table I). The data obtained for magnesium tetra(*t*-butylamino)porphyrazine **22** and zinc tetra(*t*-butylamino)tetracyano porphyrazine **16** are shown below. As can be seen, K_{eff} differs by almost an order of magnitute between the two compounds (**22**, 1.878×10^{-4} s^{-1}; **16**, 7×10^{-5} s^{-1}). It appears that the presence of a nitrogen at the periphery of the macrocycle dramatically increases its sensitivity to mineral acids, and as result its decomposition. The difference between **16** and **22** can be attributed to the change in electron density at the amine nitrogen due to the presense or absence of the cyano groups. Successive protonation of the peripheral nitrogen occurs before the *meso* nitrogens or internal nitrogens are protonated. As expected therefore, the peripheral nitrogens have a lower pK_b than the *meso* nitrogens.

All the cyano and cyano-free Pz's were tested in vitro for their ability to photoinactivate V-79 Chinese hamster fibroblasts. Among all the derivatives tested the *N,N*-diethyl amine **18** displayed the highest photosensitivity in vitro in Chinese hamster cells (1 μM, 0.76 μg/mL).

Apparently the incorporation of zinc (Zn^{2+}) in the central cavity results in no change in biological activity, but long aliphatic chains or cyclic amines in the periphery of the cyano-porphyrazines cause for a loss in activity (table II). Also the removal of the cyano moiety has a detrimental effect on the efficacy of these compounds. It is possible that in the absence of the cyano group the ability of the amino moiety, to quench $^1O_2$ (singlet oxygen) or other active oxygen species, is highly increased. Preliminary studies suggested that low singlet oxygen quantum yields for the cyano-free porphyrazines (table III). Apparently the singlet oxygen produced is probably quenched by the porphyrazine itself, as mentioned above, but further studies are necessary to confirm this preliminary observation.

Table III. Quantum yields of $^1O_2$ for the cyano and cyano-free porphyrazines.

Compound	I_a	*Slope*	Φ_Δ
Phenazine	0.61	12.31	0.86 (reference)
16	0.67	2.55	0.16 (±0.01)
18	0.61	5.18	0.36 (±0.009)
14	0.61	4.12	0.29 (±0.02)
20	0.60	2.76	0.19 (±0.017)
10	0.66	2.47	0.15 (±0.007)
22	0.68	2.50	0.17 (±0.009)
23	0.68	2.60	0.17 (±0.006)

All done in CH_3CN.

Experimental protocols

General

^1H and ^{13}C nuclear magnetic resonance spectra were recorded on a Varian VXR-400 spectrophotometer. Chemical shifts are expressed in parts per million (δ scale). All spectra were recorded using $CDCl_3$ as solvent unless otherwise stated. Visible spectra were recorded with a Hewlett Packard 8452A diode array spectrophotometer or a Bausch & Lamp Spectronic 2000 spectrophotometer system. Absorptions are given in nanometers (solutions in CH_2Cl_2 unless otherwise stated). GC-MS spectra were recorded on a Hewlett Packard 5890 equipped with a 12 m metal silicon capilary column. Low and high resolution mass spectra (fast atom bombardment) were recorded by a Jeol HX110 mass spectophotometer using a Xe ionization source at Michigan State University Mass Spectrometry facility. FTIR spectra were recorded (CH_2Cl_2 solution) using a Nicolet machine equipped with a Hewlett Packard 7470A plotter. HPLC spectra were recorded using a Perkin Elmer 410 series LC pump along with an LC-95 UV/vis spectrophotometer detector and a 3 × 3CR C^{18} silica column (reverse phase). All singlet oxygen quantum yields were obtained by the relative method using a Nd/YAG laser operating at the third (355 nm) harmonic.

Kodak silica-gel 13181 precoated sheets (0.2 mm) were used for performing analytical thin-layer chromatography (TLC) and silica gel 230–400 mesh supplied by American Scientific Products were used for column chromatography. Basic alumina

was also used (supplied by Kodak). Preparative TLC was carried out using a Harrison Research Chromatotron 7924.

The irradiation system used as a source of red light consisted of a projector fitted with a 400 W halogen lamp. Light of wavelengths shorter than 590 nm was eliminated by means of a red filter. The cells were irradiated in the Petri dish positioned 30 cm from the lamp source. This corresponded to 14 mW/cm^2. The energy flux rate was 8.3 J/cm^2 s^2 based on a 10 min irradiation time. The temperature of the Petri dish was kept around 37 °C.

Chemistry

General procedure for the preparation of N-tricyanovinylamines
To a solution of 20 mL DMF and amine was added tetracyanoethylene in small portions under stirring. Once the addition was complete the mixture was allowed to stand for 3 h. At this time the mixture was added to an equal volume of water and extracted three times with diethylether with subsequent chromatography using silica gel as the absorbent and choroform as the eluting solvent.

N-(t-Butylamino)tricyanovinyl ***1***. ^{1}H NMR, δ: 1.5475 (s, CH_3); ^{13}C NMR, δ: 140.170 (C^1), 111.700 (C^3), 111.431 (C^5), 109.806 (C^4), 63.8113 (C^2), 57.5484 (aliphatic), 29.8243 (aliphatic); IR spectrum, cm^{-1}: 1594 (C=C), 2219 (CN), 3094 (NH); mass spectrum: *m/e* = 175 (M + H); mp = 170–172 °C.

N-Piperidinotricyanovinyl ***2***. ^{1}H NMR, δ: 3.8955 (t, CH_2), 1.8127 (quintet, CH_2); ^{13}C NMR, δ: 139.491 (C^1), 114.220 (C^3), 113.129 (C^5), 110.447 (C^4), 59.8999 (C^2), 26.2958, 23.0526 (aliphatic); IR spectrum, cm^{-1}: 2218 (CN), 1578 (C=C); mass spectrum: *m/e* = 187 (M + H).

N-(N,N-Diethylamino)tricyanovinyl ***3***. ^{1}H NMR, δ: 1.4057 (t, CH_3), 3.7739 (quartet, CH_2); ^{13}C NMR, δ: 139.314 (C^1), 59.2111 (C^2), 114.250 (C^3), 113.025 (C^5), 110.487 (C^4), 13.9205 (CH_3), 45.2717 (CH_2); IR spectrum, cm^{-1}: 2218 (CN), 1578 (C=C); mass spectrum: *m/e* = 174 (M$^+$).

N-(n-Pentylamine)tricyanovinyl ***4***. ^{1}H NMR, δ: 3.5367 (t, CH_2), 0.8971 (t, CH_3), 1.3292 (h, CH_2), 1.6608 (quintet, CH_2); ^{13}C NMR, δ: 142.866 (C^1), 48.7849 (C^2), 112.424 (C^3), 112.383 (C^5), 109.129 (C^4), 13.7947, 22.09115 (aliphatics); IR spectrum, cm^{-1}: 2218 (CN), 1597 (C=C); mass spectrum: *m/e* = 188 (M$^+$).

Product 103b. ^{13}C NMR, δ: 162.784 (C^1), 59.9493 (C^2), 127.512 (C^3), 28.36, 31.5106 (aliphatic); mass spectrum: *m/e* = 246 (M$^+$).

Preparation of monochloromaleonitrile ***5***

Method A. A mixture of succinonitrile (40 g, 0.5 mol) and phosphorus pentachloride (200 g, 1 mol) in chlorobenzene was refluxed for 3 h (liberated HCl gas was neutralized with NaOH. The resulting brown solution was distilled at 120 °C and 0.05 mmHg to give a white liquid identified as the product. The product was purified by vaccum distillation at 45 °C to give both *cis* and *trans* isomers.

Method B. To a mixture of NaCl and MnO_2 was added to concentrated H_2SO_4 slowly under gentle heating. The chlorine gas produced was bubbled into a solution of succinonitrile in chlorobenzene while heating at the boil. The resulting 1,1-dichlorosuccinonitrile (95% yield) was converted to chloromaleonitrile upon heating (90% yield). ^{1}H NMR, δ: 6.3994 (s, olefinic, *trans*), 6.3148 (s, olefinic, *cis*); ^{13}C NMR, δ: 123.282 (C^1), 62.0431 (C^2), 116.251 (C^3), 114.321 (C^4); mass spectrum: 113 (M$^+$); bp = 45–48 °C, 0.05 mmHg.

General preparation of amino-substituted maleonitriles
A mixture of 20.6 mmol of compound **5** and 20.6 mmol of an amine were reacted neat at 0 °C. The resulting solid was chromatographed on silica gel with chloroform as the eluting solvent. The ratio of *cis* and *trans* isomers was 1:1.3 by NMR.

N,N-Diethylmaleonitrile ***6***. ^{1}H NMR, δ: 4.3155 (s, olefinic, *cis*), 5.2322 (s, olefinic, *trans*), 1.2295 (t, CH_3), 3.5364 (quartet, CH_2); ^{13}C NMR, δ: 133 (C^1), 114 (C^4), 116 (C^3), 54 (C^2), 30, 28, (aliphatic); mass spectrum: *m/e* = 149 (M$^+$); mp = 78.5–80 °C.

Piperidinomaleonitrile ***7***. ^{1}H NMR, δ: 4.5015 (s, olefinic, *cis*), 5.225 (s, olefinic, *trans*), 1.6229 (bs, CH_2), 3.2948 (bs, CH_2); ^{13}C NMR, δ: 136.343 (C^1), 117.828 (C^3), 111.238 (C^4), 49.2567, 24.230 (aliphatic); IR spectrum, cm^{-1}: 2219 (CN), 1593 (C=C); mass spectrum: *m/e* = 147 (M$^+$); mp = 62.5–64.5 °C.

t-Butylaminomaleonitrile ***8***. ^{1}H NMR, δ: 4.5430 (s, olefinic, *cis*), 4.6118 (s, olefinic, *trans*), 1.3425 (s, CH_3, *cis*), 1.4342 (s, CH_3, *trans*); IR spectrum, cm^{-1}: 2281 (CN), 1594 (C=C); mass spectrum: *m/e* = 149 (M$^+$); mp = 97–98.5 °C.

General procedure for the preparation of cyano-substituted magnesium porphyrazines
A mixture of 1 g of tricyanovinylamine and magnesium amyloxide obtained from 0.13 g of magnesium in 10 mL of amyl alcohol was refluxed for 4 h. The colored reaction mass was filtered by suction filtration while washing with aqueous alcohol (1:1, ethanol/water) until the filtrate was clear. Removal of the solvent from the filtrate and subsequent chromatography on silica gel using a MeOH/H_2O mixture as the eluting solvent yielded between and 20 and 35% of a magnesium porphyrazine.

Magnesium tetra(t-butylamino)tetracyano porphyrazine ***9***. IR spectrum, cm^{-1}: 2306 (CN), 1600 (C=C); UV/vis, λ (nm) (mixture of three isomers): 752 (4.1), 731 (4.22), 656 (3.73), 3.42 (4.34, Soret); (major isomer): 732 (4.24),656 (3.8), 342 (4.3, Soret); HPLC, RT (min): 1.268, 2.241, 4.320 (mixture), 2.230 (major); mass spectrum, calculated for $C_{36}H_{40}N_{16}Mg$, *m/e* = 721.1242 (M$^+$), found, *m/e* = 721.3539.

General procedure for the preparation of metal-free cyano-substituted porphyrazines
A mixture of the magnesium porphyrazine and DMF was refluxed for 30 min at which time the reaction mixture was added to an equal volume of water and neutralized with aqueous sodium bicarbonate. The neutral solution was extracted with dichloromethane three times and dried over anhydrous sodium sulfate for 1 h to yield between 75 and 95% metal-free porphyrazine. To prepare the zinc derivatives, the porphyrazine was dissolved in DMF and 5 equiv of zinc acetate was added. The resulting mixture was refluxed for 2 h and then poured into water. The mixture was extracted with dichloromethane and the organic layer was collected and evaporated to dryness under reduced vacuum. The following porphyrazines were prepared in this way.

Tetrapiperidinotetracyano porphyrazine ***13***. UV/vis, λ (nm): 816, 753, 710, 660 (s), 521, 371.5 (Soret); mass spectrum, calc for $C_{40}H_{42}N_{16}$, *m/e* = 746.8790 (M$^+$), found, *m/e* = 746.3809.

Zinc derivative ***14***. UV/vis, λ (nm): 758 (4.25), 686 (3.43), 346 (4.36, Soret); mass spectrum, calc for $C_{40}H_{40}N_{16}Zn$, *m/e* = 809.8472 (MH^+), found, *m/e* = 809.2972.

Tetra(t-butylamino)tetracyano porphyrazine ***15***. UV/vis, λ (nm): 774.7, 748.3, 719.6, 683.5, 444.7 403.4, 335 (Soret); mass spectrum, calc for $C_{36}H_{42}N_{16}$, *m/e* = 698.8350 (M^+), found, *m/e* = 699.3866

Zinc derivative ***16***. UV/vis, λ (nm): (mixture), 752, 730, 656, 342 (Soret); (major), 730 (4.25), 656 (3.8), 342 (4.3, Soret); (minor), 734, 660, 344 (Soret); mass spectrum, calc for $C_{36}H_{40}N_{16}Zn$, *m/e* = 762.1992 (M^+), found, *m/e* = 761.2983; IR spectrum, cm^{-1}: 2259 (CN), 1438 (C=C).

Tetra(N,N-diethylamino)tetracyano porphyrazine ***17***. UV/vis, λ (nm): 802, 742.5, 705, 670, 437.5 (vb), 425, 343 (Soret); mass spectrum, calc for $C_{36}H_{42}N_{16}$, *m/e* = 698.8350 (M^+), found, *m/e* = 698.3756.

Zinc derivative ***18***. UV/vis, λ (nm): 745 (4.5), 662 (3.8), 343 (4.3, Soret); mass spectrum, calc for $C_{36}H_{40}N_{16}Zn$, *m/e* = 761.8428 (MH^+), found, *m/e* = 761.30061.

Tetra(n-pentylamino)tetracyano porphyrazine ***19***. UV/vis, λ (nm): 754, 670, 460, 406, 328 (Soret); mass spectrum, calc for $C_{40}H_{50}N_{16}$, *m/e* = 754.9422 (M^+), found, *m/e* = 755.4497.

Zinc derivative ***20***. UV/vis, λ (nm): 728 (4.20), 338, 332 (4.5, Soret); mass spectrum, calc for $C_{40}H_{48}N_{16}Zn$, *m/e* = 818.3064 (MH^+), found, *m/e* = 817.3625.

General procedure for the preparation of tetra-substituted porphyrazines

A mixture of 1 g of the aminomaleo(fumaro)nitrile and magnesium amyloxide obtained from 0.13 g of magnesium in 10 mL amyl alcohol was refluxed for 4 h. The colored reaction mass was filtered by suction filtration while washing with aqueous alcohol (1:1, ethanol/water) until the filtrate was clear. Removal of the solvent from the filtrate and subsequent chromatography on basic alumina using $MeOH/H_2O$ mixture as the eluting solvent yielded 20–35% of a magnesium porphyrazine.

Magnesium tetrapiperidino porphyrazine ***21***. UV/vis, λ (nm): 705 (4.28), 346 (4.60, Soret); mass spectrum, calc for $C_{36}H_{44}N_{12}Mg$, *m/e* = 669.1290 (M^+), found, *m/e* = 669.3776.

Magnesium tetra(t-butylamino)porphyrazine ***22***. UV/vis, λ (nm): 708 (4.29), 336 (4.59, Soret); mass spectrum, calc for $C_{32}H_{44}N_{12}Mg$, *m/e* = 621.0850 (M^+), found, *m/e* = 621.3704.

Magnesium tetra(N,N-diethylamino)porphyrazine ***23***. UV/vis, λ (nm): 714 (4.3), 338 (4.5, Soret); mass spectrum, calc for $C_{32}H_{48}N_{12}Mg$, *m/e* = 625.0900 (M^+), found, *m/e* = 625.4100.

Photophysical properties

In the type II mechanism, the sensitizer can transfer its excitation energy to a ground state oxygen molecule ($^3\Sigma$) [15]. Since spin is conserved in this process and the ground state of oxygen is itself a triplet, singlet molecular oxygen ($^1\Delta g$) is produced.

Singlet oxygen ($^1\Delta g$) quantum yields were obtained by direct detection of its luminescence at 1270 nm. A dilute solution (with an absorbance of 0.450) of the sensitizer in CH_3CN was excited, at 355 nm, using a Nd/Yag laser. The plot of the initial $^1O_2$ luminescence intensity (t = 0) vs percentage laser power gives a straight line and from the absorption of the sensitizer and the slope, the quantum yield (Φ_Δ) of singlet oxygen can be calculated (table III).

Photocytotoxicity assay

Comparative studies on cell survival were conducted with V-79 cells derived from lung fibroblasts of the Chinese hamster. The cells were maintained in plastic culture flasks using growth medium (E-MEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% vitamins. Survival experiments were conducted using the following protocol which is frequently used in studies of porphyrin photosensitization.

The appropriate number of cells were plated in growth medium, in 60 mm culture dishes, to yield 200 colonies after treatment. Dishes were held in a CO_2 incubator at 37 °C overnight to allow cell attachment. The cells were then rinsed with serum-free medium, and incubated in the dark for 1 h at 37 °C in the presence of 1 mL of the appropriate dye solution (1% serum). After removal of the test substance, cells were again rinsed with serum-free medium and exposed at room temperature to red light. After treatment cells were re-fed with growth medium and incubated at 37 °C for 6–7 days. Colonies were fixed, stained with crystal violet and counted. Plating efficiency of cells in the absence of drug were determined for each experiment.

After an incubation of 1 h with the highest drug concentration, cells were rinsed once and immediately re-fed with growth medium and incubated at 37 °C for colony formation.

Drug/emulsion solution

The drug (200–300 μg) was placed in a 20 mL glass graduated tube and 0.5 mL of cremophore EL and 1 mL of propylene glycol were added and the mixture was sonicated until all the drug was dissolved. This emulsion was diluted to 5 mL using saline solution and then centrifuged and filtered through a 0.20 μm filter in order to remove any microscopic particles. Small aliquots of this solution were removed and diluted (with DMF) 5, 10, 20, and 50 times and their absorptions were obtained on a spectrophotometer. The concentrations were calculated using a standard curve and the epsilon values.

References

1 Leznoff CC, Lever ABP (1989) In: *Phthalocyanines*, VCH Publishers Inc, 1-3
2 Dougherty TJ (1974) *J Natl Cancer Inst* 52, 1333–1334
3 Dougherty TJ, Grindley GB, Fiel R, Weishaupt K, Boyle DG (1975) *J Natl Cancer Inst* 55, 115–116
4 Dougherty TJ (1990) *SPIE* 6, 1–4
5 Dougherty TJ, Potter WR, Weishaupt KR (1984) In: *Porphyrin Localization and Treatment of Tumors* Alan R Liss Inc, New York, 301–304
6 Lipson MS, Gray MJ, Baldes EJ (1966) *Proc 9th Intl Cancer Congr* 393–394
7 Dent CE, Linstead RP (1934) *J Chem Soc* 1027–1029
8 Barrett PA, Dent CE, Linstead RP (1936) *J Chem Soc* 1719–1720
9 Byrne GT, Linstead RP, Lowe AR (1934) *J Chem Soc* 1017–1020
10 Linstead RP, Whalley M (1952) *J Chem Soc* 4839–4840
11 Elvidge JA, Linstead RP (1955) *J Chem Soc* 3536–3538
12 Ficken GE, Linstead RP (1952) *J Chem Soc* 4846–4847
13 Kopranenkov VN, Goncharova LS, Luk'yanets EA (1977) *Zhurnal Obshechei Khimii* 479, 2143–2148
14 Kheleniva S, Mihalenko SA, Derkacheva VM, Luk'yanets EA (1981) *Zhurnal Obshechei Khimii (J Gen Chem USSR)* 51, 1650–1652
15 Foote CS (1984) *Mechanisms of Photosensitization* Alan R Liss Inc, 3–18
16 Foote CS (1979) In: *Singlet Oxygen* (Wasserman HH, Murray RW, eds) Academic Press, New York, 139–145