Biological activities of phthalocyanines – XVI. Tetrahydroxy- and tetraalkylhydroxy zinc phthalocyanines. Effect of alkyl chain length on in vitro and in vivo photodynamic activities

R.W. Boyle¹, C.C. Leznoff² & J.E. van Lier³

¹MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Quebec, Canada J1H 5N4; ²Department of Chemistry, York University, North York, Ontario, Canada M3J 1P3.

Summary

Zinc phthalocyanine substituted with four hydroxyl groups attached to the macrocycle, either directly or via spacer chains of three or six carbon atoms, were tested for their photodynamic ability to inactivate Chinese hamster lung fibroblasts (line V-79) in vitro, and to induce regression of EMT-6 tumours grown subcutaneously in Balb/c mice. Their potential to inflict direct cell killing during photodynamic therapy was investigated by examining vascular stasis immediately following photoirradiation using fluorescein as a marker, and increased photodynamic activity were developed to provide an alternative to P-II. Phthalocyanines (Pc’s) have been proposed as promising potential PDT agents (for recent reviews see van Lier, 1990; Rosenthal, 1991) primarily because of the high molar absorptivity of these compounds in the red region of the visible spectrum (ε ≈ 10⁵ M⁻¹ cm⁻¹ at 670–680 nm when fully monomerised compared to ε = 10⁴ M⁻¹ cm⁻¹ at 630 nm for P-II) where tissue transmission by visible light is more efficient. Their photodynamic activities in vitro largely depend on pharmacokinetics and intratissular distribution pattern and only limited structure-activity relationships have been put forth (Paquette & van Lier, 1992). Degree of sulfonation of Pc’s is the most studied structural variable, however, many alternative substituents are possible and remain unexplored. An approach towards the synthesis of novel Pc’s on the basis of known structural data, and good photodynamic properties of related compounds, could be potentially rewarding, and this is the rationale for the current study.

Berenbaum et al. (1986) tested the ortho, meta and para isomers of meso-tetra(hydroxyphenyl)porphine (THPP) for their in vitro photodynamic activity and skin photosensitisation. Although the m- and o-THPP have very similar photophysical properties and similar absorbances at the wavelength used for PDT (Bonnet et al., 1988), the m-THPP is twice as effective as o-THPP in sensitising tumours, while o-THPP is four times as potent as m-THPP in sensitising skin. Doses of p-THPP and m-THPP that produce equal tumour necrosis are of the ratio 5:1, while their molar absorbances at the illuminating wavelengths are of the ratio 2:1. These data suggest that m-THPP has considerably greater ability than the para and ortho isomers to localise at the appropriate site, indicating that the relative special orientation of the hydroxy groups around the macrocycle is of crucial importance. On such accounts we synthesised zinc phthalocyanines (ZnPc) substituted with hydroxyl groups fused directly, and rigidly, to the Pc skeleton (i.e. tetrahydroxy zinc phthalocyanine, ZnPc(OH)₄), and with hydroxy groups attached to the Pc by aliphatic chains of three and six carbons respectively (tetrapropylhydroxy zinc phthalocyanine, ZnPc(prOH)₄ and tetrahexylhydroxy zinc phthalocyanine, ZnPc(hxOH)₄) (Figure 1). Reddi et al. (1990) showed that unsubstituted ZnPc is photodynamically active when incorporated in liposomes or low density lipoprotein. The present series of ZnPc derivatives allowed us to study the effect of increasing distances and freedom of movement for the four hydroxyl groups on photodynamic activities both under in vitro and in vivo conditions. Certain aspects of the mechanism of action were also investigated using an in vivo/in vitro assay, and the assessment of vascular occlusion immediately following PDT.

Materials and methods

Photosensitisers

Synthesis of ZnPc(OH)₄ has been described in detail elsewhere (Rosenthal et al., 1987). The ZnPc(prOH)₄ and ZnPc(hxOH)₄ were prepared from 4-iodophthalonitrile (Maruccio et al., 1985) by palladium catalysed alkylation, followed by catalytic hydrogenation over 10% palladium on charcoal to give 4-(propylhydroxy)phthalonitrile and 4-(hexylhydroxy)phthalonitrile, both of which condensed cleanly with zinc metal at 170°C to give ZnPc(prOH)₄ and ZnPc(hxOH)₄ respectively. The final products were characterised by their absorbance and mass spectra. ZnPc(prOH)₄: λₐₕₐₓ (log ε) = 675 nm (5.48); FAB-MS, M⁺ ( %) = 810 (100). ZnPc(hxOH)₄: λₐₕₐₓ (log ε) = 673 nm (5.4); FABS-MS, M⁺ ( %) = 978 (100). Full details of these syntheses will be published elsewhere. Photofrin II™ was obtained from Quadrilogic Technologies Inc., Vancouver, BC, Canada. Tetrasulphonated zinc phthalocyanine (ZnPcS₄) was prepared by the condensation method, as previously described (Ali et al., 1988).

Correspondence: J.E. van Lier.
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Animal experiments

Animal experiments were conducted following the recommendations of the Canadian Council on Animal Care and of an in-house ethics committee. The animals were allowed free access to water and food throughout the experiments. Female Balb/c mice had one tumour transplanted in to the right hind thigh by intradermal injection of 2 × 10⁶ EMT-6 mouse mammary tumours (obtained from Dr C.-W. Lin, Massachusetts General Hospital, Boston) suspended in 0.05 ml of Waymouth's medium (Gibco) (Brasseur et al., 1988). Mice were used 6–7 days post-inoculation when tumours had reached a diameter of 3–5 mm. Mice were injected, via the tail vein, with Pc and P-II in a solution of Cremophore EL (Sigma), propane-1,2-diol, and saline (10:3:87). Sulphonated Pc have been shown to reach optimal concentrations in various rodent tumours at 24–48 h post-injection (Tralau et al., 1987; Rousseau et al., 1990) and we assumed similar biodistribution pattern for the novel Pc derivatives. After 24 h the tumour was irradiated with 650–700 nm light (400 J cm⁻² at a fluence rate of 180 mW cm⁻²) delivered by a 1000 W Xenon lamp fitted with 10 cm water filter, and LS-700 (Corion) and 2-58 (Corning) filters. In the case of P-II a band of 600–650 nm was used at the same fluence, and fluence rate, using LS-600 (Corion) and 650-FLOT-50 (Ealing) filters. Light was focused on the tumour with lenses to give a final beam of 8 mm in diameter. The fluence at the surface of the tumour was measured (Brasseur et al., 1987) and rose to 35°C externally and 32°C internally after 10 min, in both cases the temperature remained constant for the remainder of the irradiation time. Tumour response was assessed qualitatively and followed from initial necrosis (within 24 h), to cure, and for a follow-up period of 30 days. Tumour cure was defined as necrosis of the tumour within 48 h, followed by regrowth of normal tissue in the treatment area and no recurrence of the neoplasm up to 30 days post irradiation. Nine mice were used to confirm the minimal dose of dye needed to reach the cure. No spontaneous regression of the tumour was noted in a control group of nine mice over the time course of the experiment.

In vitro photocytotoxicity assay

Cell survival of Chinese hamster lung fibroblasts (line V-79) was determined by a colony forming assay (Brasseur et al., 1985). Plated cells were incubated with 1 ml of dye in medium containing 1% serum for 1 h in the dark at 37°C in 5% CO₂. After removal of the dye solution and washing with PBS, cells were refed with growth medium and exposed for 4 min to red light from two 500 W tungsten/halogen lamps (Sylvania FCL) fitted with a refrigerated filter containing aqueous Rhodamine (OD₅₆₀ = 1.25) and a red filter (26–4390, Ealing). The fluence over the absorption peaks of the photoactive monomeric dyes was 2.4 J cm⁻². The cells were then incubated at 37°C in 5% CO₂ for 6–7 days. The dye concentration in μM required for 90% cell mortality, i.e. the extracellular LD₉₀, was used to quantify the activity of each dye preparation. Experiments were run at least three times with three dishes for each concentration point.

In vivo/in vitro assay

The procedure is essentially as described by Henderson (1990). Balb/c mice were implanted with two EMT-6 tumours in the hind thighs. When the tumours reached a diameter of 3–5 mm (6–7 days) mice were injected with 10 μmol kg⁻¹ of Pc or 10 mg kg⁻¹ of P-II in saline containing 10% Cremophore EL and 3% propane-1,2-diol. Twenty-four hours post-injection of drug animals were sacrificed and the tumours were excised, minced, and enzymatically digested (30 min in CaCl₂ 10 mM, proteinase K (Sigma) 6.5 U, micococcal (Sigma) 3 U, collagenase (Sigma) 17 U, in 10 ml Hank's buffer saline solution). The digested preparation was then filtered through a 200 mesh sieve and centrifuged at 600 g for 5 min. Two hundred cells were placed in 6 cm Petri dishes and incubated for 3 h at 37°C in 5% CO₂ in Waymouth's culture medium to allow adhesion to the support. Cells were illuminated as described under in vitro photocytotoxicity assay. Fluences were calculated from the area under the absorbance peaks of the monomeric dye in methanol solution. It is evident that during the cell isolation procedure loosely bound dye may detach from the cells resulting in an underestimation of the potential of the dye to inflict direct cell killing during PDT. However, a positive effect in this assay strongly indicates the involvement of a direct photodynamic action on the tumour cells as part of the overall PDT response.

Fluorescein exclusion assay

Animals were prepared as for PDT, but in this case two tumours were grown, one on each hind thigh. The right tumour was irradiated while the left (control) tumour was shielded from light. Immediately following irradiation mice were injected via the tail vein with 2 mg sodium fluorescein in 0.2 ml phosphate buffered saline. After 2 min mice were sacrificed and placed under a longwave UV lamp to visualise areas penetrated by the dye. Any exclusion of fluorescein from the irradiated area of tumour and surrounding tissue, relative to the control, was noted and photographed.
Results

The novel compounds ZnPc(OH)₄, ZnPc(prOH)₄, and ZnPc(hxOH)₄ were initially tested for their capacity to photo-inactivate V-79 cells under in vitro conditions. As can be seen from the survival curves in Figure 2, the ZnPc(prOH)₄ is greater than two orders of magnitude more active than the ZnPc substituted with four hydroxy (ZnPc(hxOH)₄) or sulphonate (ZnPcS₄) groups. The derivative substituted with hydroxyl groups without spacer chains shows little activity under our experimental conditions, even at the maximum concentration used (150 μM).

The minimum injected dye doses required for 100% cure of the EMT-6 tumours grown in Balb/c mice are shown in Figure 3. Data for P-II and ZnPcS₄ are included for comparison and dye doses are expressed in terms of mg kg⁻¹ animal body weight to allow for comparison with P-II for which the molecular weight is unknown. ZnPc(OH)₄ clearly shows poor photodynamic activity in this system as, even at the highest injected doses (10 μmol kg⁻¹; 6.4 mg kg⁻¹) no tumour cure could be obtained. ZnPc(prOH)₄ induced 100% tumour cure at doses as low as 0.5 μmol kg⁻¹ (0.4 mg kg⁻¹) representing an improvement of ten times over ZnPcS₄ and 25 times over P-II. PDT with ZnPc(hxOH)₄ required twice the injected dose of ZnPc(prOH)₄ in order to induce the same photodynamic tumour response, i.e. 1 μmol kg⁻¹ (1.0 mg kg⁻¹). With both compounds ZnPc(prOH)₄ and ZnPc(hxOH)₄ administered at the minimum dose levels, fluorescein injected immediately after PDT was excluded from the irradiated area, indicating that tumour necrosis is preceded by vascular stasis. Unirradiated control tumours on the same animals showed strong fluorescence. Failure to observe significant photosensitised EMT-6 cell killing in vitro after in vivo administration of the novel dyes, suggests that a relatively small amount of dye is retained by the tumour cells (Figure 4). Combined with the vascular shut-down observed immediately after PDT these data strongly suggest that tumour necrosis with these dyes results from an initial vascular effect rather than direct tumour cell killing. In contrast, ZnPcS₄ scored in the in vivo/in vitro assay an LD₅₀ of less than 20 J cm⁻² light dose, indicative of a good potential to inflict direct cell killing during PDT.

Discussion

If advantage is to be taken of the favourable absorption characteristics of Pc type molecules in PDT it is important that structure-activity relationships for this class of compound be defined. To date, research conducted in this area has mainly addressed the effect of the degree of sulphonation of Pcs's on their biodistribution and photodynamic activity (for a recent review see Faquette and van Lier, 1992). Commercially available aluminium phthalocyanine sulphonate (AlPcs) has most widely been used for biological studies (Tralau et al., 1987; Matthews & Cui, 1990; Bedwell et al., 1991), but consists of a mixture of tetra, tri, and monosulphonated AlPc with an average sulphonation level of approx-
The amphiphilic AlPcS₄a (featuring sulphonate groups on adjacent benzo rings of the Pc macrocycle) localise in tumour cells whereas the tetrasulphonated analogue is mainly detected in the stroma of the tumour (Paquette et al., 1988; Peng et al., 1990; 1991). This suggests an overall tumour retention of the tri- and tetrasulphonated derivatives as compared to the mono- and disulphonated analogues (Rousseau et al., 1990; Chan et al., 1990), the latter exhibit better photodynamic properties due to their advantageous intratissular tumour distribution and cell penetration. These different activities and localisation pattern also correlate well with the action mechanisms. In the case of the AlPcS₄a light and electron microscopy showed that there was a direct, extensive, photo-damaging action on human tumour cells (LOX xenographs) grown in athymic nude mice whereas treatment of AlPcS₄a and light resulted initially in a functional vasogenic response and ultimate damage to the vascular structure of the tumour (Peng et al., 1990). Simple procedures to prepare AlPcS₄ enriched in the active disulphonated derivatives have been reported (Ali et al., 1988; Ambroz et al., 1991) and their in vivo pattern of photosensitisation have been studied in detail (Nuutinen et al., 1991; Lob et al., 1992). Addition of hydrophobic tertary butyl (Paquette et al., 1991), phthalimidomethyl (Boyle et al., 1992) or benzene groups (Margaron et al., 1992) on the non-substituted, adjacent benzo rings of disulphonated Pcs further enhances the amphiphilic properties of these drugs resulting in increased potential for direct cell killing during PDT. Differently sulphonated tetraphenylporphins follow similar biodistribution pattern as the analogus sulphpthalocyanines, with the derivative featuring two adjacent sulfonates showing the highest photocytotoxicity (Kessel et al., 1987).

The work of Bonnett et al. (1987) with the meso-tetra-(hydroxyphenyl)porphins (THPP) yielded structure-activity relationships of particular interest for the development of Pc based photosensitisers. Whereas the o-, m- and p-isomers of THPP exhibited similar photophysical properties (Bonnett et al., 1988) large differences were noted in their biological activities, with the m-isomer showing the highest potency in sensitising tumours in vivo (Berenbaum et al., 1986). Unlike the disulphonated phthalocyanines and tetraphenylporphins, the THPP isomers are symmetric, lipophilic molecules lacking the characteristic amphiphilic properties of the disulphonated photosensitisers. They distribute in tumours similarly to the main components of P-II with membranes as the major target and some localisation in the cytoplasm (Peng et al., 1991). These data suggest that an array of four hydroxyl groups arranged judiciously around a rigid photosensitising structure could impart interesting biological activities to our Pcs. As it was difficult to ascertain the exact orientation of the hydroxy groups in m-THPP, due to the rotational flexibility of the single bond linking the hydroxypben groups to the porphine macrocycle, we synthesised three Pc molecules with distinct differences in the distances between, and flexibility of the hydroxyl groups (Figure 1). Our tumour response results indicated that the spatial arrangement and conformational flexibility of the hydroxyl groups is essential for PDT efficacy, as ZnPc(OH)₄ was found to be completely photodynamically inactive, even at 10 μmol kg⁻¹ injected dose (twice the dose at which 100% tumour eradication can be achieved using ZnPcS₄). In contrast, tumour cure with ZnPc(hxOH)₄ and ZnPc(prOH)₄ was reached at doses 5 and 10 times lower than for ZnPcS₄ and up to 25 times lower than for P-II. These differences in biological activities are further accentuated under in vitro conditions using a population of rapidly dividing V-79 cells, a pattern previously noted with isomeric disulphonated Pcs (Brasseur et al., 1988). The relative photoactivities between the propylhydroxy and hexylhydroxy ZnPc derivatives changes from a factor 2 in vivo to a factor 10² under in vitro conditions. The ZnPc(OH)₄ shows little activity in both our in vivo and in vitro model and similar pattern similar to the differences in activity between o-, m- and p-THPP we see that in both cases the compounds with hydroxy groups locked rigidly, relative to one another and the macrocycle (ZnPc(OH)₄ and p-THPP) are the least active, and that allowing some degree of movement to the hydroxy groups increases this activity. Such correlations are relevant to recent theoretical considerations put forth by Winkelman et al. (1993) concerning stereochemical requirements for photosensitisers to facilitate transport and binding in biological systems. These authors compared geometrical features of a number of substituted porphyrins and phthalocyanines with published biological activities and suggested that there exists a critical distance of about 1.2 nm between oxygens (sulphonate, carboxyl or hydroxyl substituents) that characterises the biologically active structures for PDT. The flexibility of both our alkylhydroxy ZnPcs will allow conformations to match such a geometry and particularly the tetrapropylhydroxy derivative has a high number of low energy conformers in which two adjacent OH groups are separated by 1.1–1.2 nm.

Vascular occlusion of the EMT-6 tumours immediately after PDT with both the ZnPc(prOH)₄ and ZnPc(hxOH)₄ indicates that tumour regression mainly results from indirect photodynamic effects. The absence of photodynamic cell killing in the in vivo/in vitro assay is in line with such a mechanism and suggest that only low concentrations of these dyes are retained by the tumour cells. This does however not exclude the possible presence of labile-bound dye in vivo, providing direct photodynamic cytotoxicity, as seems to be the case with m-THPP mediated PDT response (Peng et al., 1991). Although early PDT-induced hypoxia can severely limit the potential for direct cell killing of a dye (Henderson & Fingar, 1989), the ultimate tumour eradication likely depends upon the effective destruction of the microvasculature in the tumour and the surrounding normal tissue (Finagor & Henderson, 1987). Intratissular localisation pattern of m-THPP in human melanoma xenografts in nude mice resembles that of P-II (Peng et al., 1991), whereas the alkylhydroxy ZnPc's gave similar response pattern at P-II in our current assays. This suggests that P-II, m-THPP, and our novel ZnPc(prOH)₄ and ZnPc(hxOH)₄ share the capacity to induce similar photodynamic action mechanisms, with the extent of their potential for direct photocytotoxicity in vivo depending on the actual PDT conditions used.

In conclusion, we have shown that it is possible to extrapolate structure-activity relationships determined for porphyrin based photosensitisers to other photoactive species with better absorption characteristics. This could allow the considerable data available on structure-activity relationships for porphyrins to be applied of phthalocyanine based photosensitisers and permit a focus of synthetic efforts for the design of a viable Pc based PDT agent.

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Abbreviations: Pc, phthalocyanine; PDT, photodynamic therapy; ZnPc, zinc phthalocyanine; ZnPcS₄, tetrasulphonated zinc phthalocyanine; ZnPc(OH)₄, tetrahexylhydroxy zinc phthalocyanine; ZnPc(hxOH)₄, tetrapropylhydroxy zinc phthalocyanine; ZnPc(prOH)₄, tetrahexylydroxy zinc phthalocyanine; P-II, Photofrin II™.
References

ALI, H., LANGEIS, R., WAGNER, J.R., BRASSEUR, N., PAQUETTE, B. & VAN LIER, J.E. (1988). Biological activities of phthalocyanines - X. Synthesis and analyses of sulphonated phthalocyanines. Photochem. Photobiol., 47, 713-717.

AMBROZ, M., BERRY, A., MACROBERT, A.J., SIMPSON, M.S.C., SVENSEN, R.K. & PHILLIPS, D. (1991). Preparation, analysis, and fluorescence spectroscopic studies of sulphonated aluminium phthalocyanine photosensitizers. J. Photochem. Photobiol. B. Biol., 9, 87-95.

BEDWELL, J.H., CHATLANI, P.T., MACROBERT, A.J., ROBERTS, J.E., BARR, H., DILLON, J. & BOWN, S.G. (1991). Enhanced tumour selectivity of photodynamic therapy in the rat colon using a radioprotection agent. Photochem. Photobiol., 53, 753-756.

BERENAUM, M.C., ARANDE, S.L., BONNETT, R., KAU, H., IOANNOS, S., WILKINSON, R.D. & WINEFIELD, U.J. (1986). Mesotetra-(hydroxypyphenyl)phorpyrin, a new class of potent tumour photosensitisers with favourable selectivity. Br. J. Cancer, 54, 717-725.

BONNETT, R., IOANNOS, S., WHITE, R.D., WINEFIELD, U.J. & BERENAUM, M.C. (1987). Mesotetra-(hydroxypyphenyl)porphyrins as tumour photosensitisers: chemical and photographic aspects. In From Photosciences to Photobiology, Favre, A., Tyrrell, R. & Cadet, J. (eds), Biophotochem. Photobiophysics, Suppl. pp. 45-46. Elsevier: Amsterdam.

BONNETT, R., MCNEAVE, D.J., HARRIMAN, A., LAND, E.J., TRUSCOTT, T.G. & WINEFIELD, U.J. (1988). Photophysical properties of meso-tetraphenylporphin and some meso-tetra-(hydroxypyphenyl) porphyrins. Photochem. Photobiol., 48, 271-276.

BOYLE, R.W., PAQUETTE, B. & VAN LIER, J.E. (1992). Biological activities of phthalocyanines. XIV. Effect of hydrophobic phthali- midometyl groups on the in vivo phototoxicity and mechanism of photodynamic action of sulphonated aluminium phthalocyanines. Br. J. Cancer, 65, 813-817.

BRASSEUR, N., ALI, H., AUTENRIETH, D., LANGLOIS, R. & VAN LIER, J.E. (1985). Phototherapy of malignant tumours in mice with sulphonated phthalocyanines. Photochem. Photobiol., 45, 581-586.

CHAN, W.S., MARSHALL, J.F., SVENSEN, R., BEDWELL, J. & HART, J.R. (1995). Sulphonation of sulphonated phthalocyanines on the cell and tumour distribution of the photosensitizer aluminium phthalocyanine. Cancer Res., 50, 4533-4538.

CHATLANI, P.T., BEDWELL, J., MACROBERTS, A.J., BARR, H., BOULOS, P., KRASNER, N., PHILLIPS, D. & BOWN, S.G. (1991). Comparative study of the biological effects of dis- and tetra-sulphonated aluminium phthalocyanines in normal rat colon. Photochem. Photobiol., 53, 745-751.

DOUGHERTY, T.J. (1987). Photosensitizers: therapy and detection of malignant tumors. Photochem. Photobiol., 45, 897-899.

FINGER, V.H. & HENDERSON, B.W. (1987). Drug and light dose dependence of photodynamic therapy: a study of tumour and normal tissue response. Photochem. Photobiol., 46, 837-841.

HENDERSON, B.W. (1990). Probing the effects of photodynamic therapy through the use in vitro in vitro methods. In Photodynamic Therapy of Neoplastic Disease, Vol. 1, Kessel, D. (ed.), pp. 169-188, CRC Press: Boca Raton, FL.

HENDERSON, B.W. & DOUGHERTY, T.J. (1992). How does photo- dynamic therapy work? Photochem. Photobiol., 55, 145-157.

HENDERSON, B.W. & FINGER, V.H. (1989). Oxygen limitation of direct tumor cell kill during photodynamic treatment of a murine tumor model. Photochem. Photobiol., 49, 299-304.

KESSEL, D., THOMPSON, P., SAATIO, K. & NANTWJ, K.D. (1987). Tumor localisation and photosensitisation by sulphonated derivatives of tetraphenylporphin. Photochem. Photobiol., 45, 787-790.

LOH, C.S., BEDWELL, J., MACROBERT, A.J., KRASNER, N., PHILLIPS, D. & BOWN, S.G. (1992). Photodynamic therapy of the normal rat stomach: a comparative study between di-sulphonated aluminium phthalocyanine and 5-aminolevulinic acid. Br. J. Cancer, 66, 452-462.

MARCUCCIO, S.M., SIRSKAYA, P.I., GREENBERG, S., LEVER, A.B.P. & LEZNOFF, C.C. (1985). Binuclear phthalocyanines covalently linked through two and four atom bridges. Can. J. Chem., 63, 623-631.

MARGARON, P., LANGLOIS, R., VAN LIER, J.E. & GASPARD, S. (1992). Photodynamic properties of naphthalosulphonazopyrano- zines, novel asymmetric, amphiphilic phthalocyanine derivatives. J. Photochem. Photobiol. B. Biol., 14, 187-199.

MAY, E.K. & CUI, Z.J. (1990). Photodynamic action of sulphonated aluminum phthalocyanines (SALPC) on AR4-2J cells, a carcinoma cell line of rat exocrine pancreas. Br. J. Cancer, 61, 695-701.

MOAN, J. & BERG, K. (1992). Phototherapy of cancer: experimental research. Photochem. Photobiol., 55, 913-948.

NUUTINEN, P.J.O., CHATLANI, P.T., BEDWELL, J., MACROBERT, A.J., PHILLIPS, D. & BOWN, S.G. (1991). Distribution and photodynamic effect of di-sulphonated aluminium phthalocyanine in the pancreas and adjacent tissues in the Syrian golden hamster. J. Cancer, 63, 1108-1113.

PAQUETTE, B., ALI, H., LANGLOIS, R. & VAN LIER, J.E. (1988). Biological activities of phthalocyanines. VIII. Cellular distribution in V-79 Chinese hamster cells and phototoxicity of selectively sulphonated aluminium phthalocyanines. Photochem. Photobiol., 48, 215-220.

PAQUETTE, B., ALI, H. & VAN LIER, J.E. (1991). Phthalocyanines for photodynamic therapy of cancer: the effects of substituents tertio-butylyl on the accumulation cellular and the photodynamic activity of phthalocyanines sulfonated de gallium. J. Chrom. Phys., 86, 1113-1123.

PAQUETTE, B. & VAN LIER, J.E. (1992). Phthalocyanines and related compounds: structure-activity relationships. In Photodynamic Therapy: Basic Principles and Clinical Aspects, Henderson, B.W. & DOUGHERTY, T.J. (eds), pp. 145-156. Marcel Dekker: New York, NY.

PENG, Q., MOAN, J., FARRANTS, G., DANIELSEN, M.E. & RIMINGTON, C. (1991). Localization of potent photosensitizers in human tumor LOX, by means of laser scanning microscopy. Cancer Res., 51, 17-27.

PENG, Q., MOAN, J., NELSAND, J.M. & RIMINGTON, C. (1990). Aluminum phthalocyanines with asymmetrical lower sulphonation and with symmetrical higher sulphonation: a comparison of localizing and photosensitizing mechanism in human tumor LOX xenografts. Int. J. Cancer, 46, 719-726.

REDDY, E., ZHOU, C., BIOLO, R., MENEALDO, E. & JORI, G. (1990). Liposome- or LDL-administered Zn(II)-phthalocyanine as a photodynamic agent for tumours. J. Pharmacokinetic properties and phototherapeutic efficacy. Br. J. Cancer, 61, 407-411.

ROSENTHAL, I. (1991). Photodynamic agents as photodynamic sensitizers. Photochem. Photobiol., 53, 859-870.

ROSENTHAL, I., BEN-HUR, E., GREENBERG, S., CONCEPTION-LAM, A., DREW, D.M. & LEZNOFF, C.C. (1987). The effect of substituents on phthalocyanine phototoxicity. Photochem. Photobiol., 46, 959-963.

ROUSSEAU, J., LANGLOIS, R., ALI, H. & VAN LIER, J.E. (1990). Biological activities of phthalocyanines. XII. Synthesis, tumor uptake and biodistribution of 14C-labeled di- and trisulfonated gallium phthalocyanine in C57H mice. J. Photochem. Photobiol. B. Biol., 6, 121-132.

TRALAU, C.J., BARR, H., SANDEMAN, D.R., BARTON, T., LEWIN, M.R. & BOWN, S.G. (1987). Aluminium sulphonated phthalocyanine distribution in rodent tumours of the colon, brain and pancreas. Photochem. Photobiol., 46, 777-781.

VAN LIER, J.E. (1990). Phthalocyanines as sensitizers for PDT of cancer. In Photodynamic Therapy of Neoplastic Disease, Vol. I, Kessel, D. (ed.), pp. 279-290. CRC Press: Boca Raton, FL.

WINCKELMAN, J.W., ARAD, D. & KIMEL, S. (1993). Stereochemical factors in the transport and binding of photosensitizers in biological systems and in photodynamic therapy. J. Photochem. Photobiol. B. Biol. (in press).