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Photophysical properties and photodynamic activity in vivo of some tetrapyrroles

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Pheophorbide *a*; Phthalocyanine; Naphthalocyanine; Singlet oxygen generation; Photosensitization; Photodynamic therapy

Some of the photophysical properties (stationary absorbance and fluorescence, fluorescence decay times and singlet oxygen quantum yields) of pheophorbide *a*, metal-free, ClAl-, Cu- and Mg-*t*-butyl-substituted phthalocyanines, metal-free, ClAl- and Cu-*t*-butyl-substituted naphthalocyanines and of a number of tetraphenylporphyrins (5,10,15,20-tetraphenylporphyrin, 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin, 5,10,15,20-tetra(*p*-hydroxyphenyl)porphyrin) have been studied in comparison with hematoporphyrin IX in order to select potent photosensitizers for the photodynamic treatment of cancer. The photodynamic activity of these compounds was investigated using Lewis lung carcinoma in mice. As a consequence of the photophysical parameters (relatively short singlet state lifetimes, and high singlet oxygen quantum yields) the photodynamic activities of pheophorbide *a*, *t*-butyl-substituted ClAl-phthalocyanine and ClAl-naphthalocyanine were selected for study in greater detail. Under the conditions employed in the present study, pheophorbide *a* was found to be the most effective sensitizer, as judged from its strong absorption at the excitation wavelength as compared with the hematoporphyrin derivative and greater singlet oxygen quantum yield relative to the phthalocyanines and naphthalocyanines. The photodynamic activity was observed to be strongly dependent on the photophysical parameters of the compounds. The primary mechanism underlying the photodynamic activity of these sensitizers probably consists of energy transfer from the lowest triplet state of the dyes to molecular oxygen, resulting in the formation of singlet oxygen (type II of photosensitization).

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Abbreviations: HP, hematoporphyrin IX; HPD, hematoporphyrin derivative; Pheo, pheophorbide *a*; PC, metal-free *t*-butyl-substituted phthalocyanine; ClAl-, Mg-, Cu-PC, *t*-butyl-substituted phthalocyanines containing the metals shown; NP, metal-free *t*-butyl-substituted naphthalocyanine; ClAl-, Cu-NP, *t*-butyl-substituted naphthalocyanines containing the metals shown; TPP, 5,10,15,20-tetraphenylporphyrin; TPPS, 5,10,15,20-tetra(*p*-sulfophenyl)porphyrin; m-TPP, 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin; p-TPP, 5,10,15,20-tetra(*p*-hydroxyphenyl)porphyrin; PDT, photodynamic therapy; DCM, 4-dicyanomethylene-2-methyl-6-*p*-2-dimethylaminostyryl-4H-pyran; $\Delta\phi$, singlet oxygen quantum yield; LLC, Lewis lung carcinoma; i.t., intratumoral; MST, mean survival time.

1. Introduction

It is generally accepted that photodynamic therapy (PDT) in the presence of porphyrins activated by red laser light is an effective treatment for various types of cancer [1,2]. The most commonly used sensitizer in experimental and clinical therapy is a complex mixture of porphyrins, derived from HP and termed HPD [3,4]. A detailed investigation of the photophysical and photochemical properties of HP and HPD has been reported [5].

HPD is far from ideal and its use entails the disadvantage of compositional variability between

batches, low tumor selectivity and weak absorption in the red region of the spectrum where tissue penetration is extensive. Berenbaum et al. [6] have described the photosensitizing efficacy of a number of TPPs. Subsequent work demonstrated the efficacy of TPPS in a human colon adenocarcinoma cell line [7], as shown in a preliminary clinical study with PDT by topical TPPS administration in neoplastic skin lesions [8]. Nevertheless, these compounds display a low value of the molar absorption coefficient in the red area of the spectrum.

On the basis of the reasons given above, the present study was undertaken with the goal of obtaining more detailed information on other groups of tetrapyrroles – viz., phorbides, phthalocyanines and naphthalocyanines – that exhibit strongly absorption in the red and near-infrared regions.

As shown previously [9–11], pheophorbide *a* (Pheo) is an excellent sensitizer which accumulates selectively in tumor tissue [12] and displays more intense absorption than does HPD in the red region. In a recent review of phthalocyanines [13], it was suggested that some members of this group could be potentially valuable sensitizers by virtue of their superior optical properties compared with HPD, as well as because of their stability and chemical purity. Several authors have reported that metallophthalocyanines and their sulfonated derivatives exhibit photosensitizing properties both in vitro [14–17] and in vivo [18–23]. Various phthalocyanines are known to photosensitize the killing of mammalian cell lines in culture [17]. Additionally, a number of phthalocyanines have been demonstrated to be selectively retained in malignant tissue [24]. The most commonly used and investigated sensitizers of this chemical class are sulfonated phthalocyanines.

Firey et al. [25] investigated the photoproperties of a silicon naphthalocyanine in order to assess its potential for use as a photosensitizer for PDT, however, no information concerning the biological activity of the compounds was provided.

In accordance with the established primary mechanisms of sensitization [26,27], we investigated some of the photophysical parameters (stationary absorption and fluorescence spectra, fluorescence decay, and singlet oxygen quantum yield) of pheophorbide (**2**), several tetraphenylporphyrins (**5a–5c**), *t*-butyl-substituted phthalocyanines (**3a–3d**) and naphthalocyanines (**4a–4c**) in comparison with HP. Relative to their unsubstituted derivatives, compounds belonging to groups **3** and **4** exhibit excellent solubility in common organic solvents, especially nonpolar media. The most interesting compounds were used for in vivo studies and their photodynamic activity investigated in a comparative analysis relative to HPD.

2. Materials and methods

2.1. Chemicals

Pheo was extracted from young leaves of *Urtica urens* according to previously reported procedures [28,29]. HP was obtained from Serva and used without further purification. HPD was kindly provided by Dr. Hausdorf (Institute of Biochemistry, Humboldt University). TPP and TPPS were supplied by Professor A.F. Mironov (Lomonosov Institute of Fine Chemical Technology, Moscow) and the hydroxylated TPPs were obtained from Dr. Grummt (Friedrich Schiller University, Jena).

The phthalocyanines and naphthalocyanines were prepared from the corresponding dinitriles as detailed in ref. 30. These compounds were purified by chromatography over Al_2O_3 columns in the dark. Compounds **3a–3c** (see fig. 2) were purified further via sublimation under a high vacuum.

Ethanol of spectroscopic grade and CCl_4 from VEB Laborchemie Apolda (G.D.R.) were used, whereas additional purification and drying of CCl_4 were carried out according to standard methods. Distillation was performed under an atmosphere of N_2.

2.2. Methods

2.2.1. Spectroscopy

Absorption spectra were recorded on a Beckman UV 5270 spectrophotometer at room temperature. For studying the stationary fluorescence and singlet oxygen luminescence at 1270 nm, an

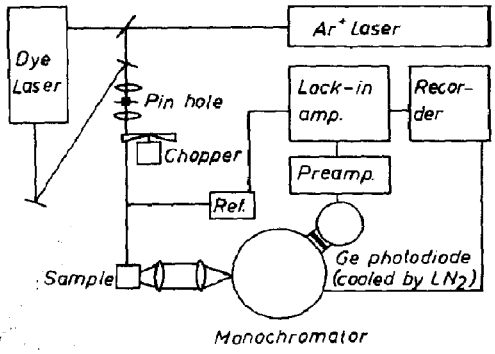


Fig. 1. Scheme representing the apparatus used for stationary fluorescence measurements and detection of singlet oxygen luminescence.

Ar^+ laser (ILA-120, VEB Carl Zeiss, G.D.R.) or Ar^+ laser pumped dye laser (FSL 100, ZWG, G.D.R.) with DCM as the active dye were used as excitation sources. The detector was a liquid N_2-cooled Ge diode (Judson Infrared). The experimental set-up is shown in fig. 1. The absolute singlet oxygen quantum yields were determined using the same solvent and identical values for the absorbance at the excitation wavelength of the standard and the compound investigated. The standard, in CCl_4 as solvent, was TPP ($\Delta\phi = 0.7 \pm 0.05$ [31]) at a concentration of 10^{-6} mol/l, corresponding to $A_{652\,nm} = 0.02$ for 5 cm optical path length. For investigations on NPs a 1000-fold lower absorbance value was used. The excitation wavelength for both PCs (3) and NPs (4) was 652 nm (intensity: 0.1 W/cm^2). For investigations in ethanol the standard TPPS was used ($\Delta\phi = 0.7 \pm 0.05$ [31]). Excitation at 514.5 nm was employed for compounds (1, 2, 5), the absorbance of the dye solutions at this wavelength being 0.2 (1 cm optical path length), which corresponds to concentrations of $(1-3) \times 10^{-5}$ mol/l. The optical path length in all experiments was 1 cm.

Fluorescence decay profiles were measured using a high-sensitivity pulse fluorometer described by Naether [32]. Decay times were calculated using a deconvolution procedure. Fluorescence excitation of the samples was provided by a mode-locked Ar^+ laser (ILA-120; $\lambda = 514.5$ nm; pulse duration, 100 ps; repetition rate, 125 MHz) or by a synchronously pumped Ar^+ dye laser (FSL 100; DCM; $\lambda = 630-670$ nm; pulse duration, < 10 ps; repetition rate, 125 MHz). Reabsorption and reemission effects on the decay curves were shown to be negligible at the highest concentrations of the compounds ($c < 10^{-7}$ mol/l).

2.2.2. In vivo studies

Female $(C57/Bl/6 \times DBA/2)F_2$ Shoe hybrid mice were used for all in vivo experiments. At the beginning of each experiment, the animals were 6 weeks old and weighed 22–24 g. Mice were kept under germ-poor and standard conditions. The tumor system was the Lewis lung carcinoma (LLC). Tumors were transplanted into the right hind footpads of mice on day 0, by injection of about 10^5 LLC cells suspended in 0.04 ml Hank's balanced salt solution. Each experiment involved three groups of six animals each: (group 1) control, consisting of LLC mice without PDT (treated with a saline injection of 0.04 ml on days 4 and 6); (group 2) animals treated only with the sensitizer; and (group 3) PDT mice.

The photosensitizers were stored at 280 K in the dark. Dyes freshly dissolved in ethanol were used throughout. HPD was dissolved in saline. The sensitizer solutions were injected intratumorally (0.02 ml per animal) on days 4 and 6.

The photodynamic treatment consisted of localized exposure of the tumors to 630 nm (HPD) or 670 nm (all other sensitizers) laser light, 30 min after the intratumoral administration of sensitizer. The excitation source was an Ar^+ dye-pumped laser with DCM as the active dye. The laser light was split into two equal beams to permit simultaneous irradiation of two mice. In each case, the beam was directed onto the whole tumors which had a diameter of 1–2 mm on day 4. The tumors were irradiated at 100 mW for 30 min (180 J per tumor) on days 4 and 6. During irradiation, animals were anesthetized with an intraperitoneal injection (40 mg/kg) of Radenarkon (Etomitat, VEB Arzneimittelwerk, Dresden, G.D.R.). Mice of groups 1 and 2 were anesthetized 30 min after saline or sensitizer injection at the same dosage.

Mice were observed until death occurred due to growth of foot pad tumors and metastases in the lung. Cured animals were included in calculations

of mean survival time (MST), a value of 60 days being employed. The Mann-Whitney U-test was used to evaluate the significance of duration of survival for different experimental groups ($p < 0.05$).

3. Results and discussion

3.1. Spectroscopic investigations

We firstly recorded the absorption and fluorescence spectra of all test compounds (structural formulae given in fig. 2). The results are summarized in table 1. As is well known, porphyrins have Q-bands of very low intensity, near 600 nm ($\epsilon \approx 10^4$ l/mol per cm). Also, TPPs absorb only weakly at wavelengths around 600 nm and, accordingly, PDT should benefit greatly from the use of new sensitizers that are strong absorbers of red light. In the case of phorbides [33,34], the Q-band is more intense ($\epsilon \approx 5 \times 10^4$ l/mol per cm) and shifted toward the red region of the spectrum up to 670 nm (see table 1).

The absorption spectra of PCs (**3a–3c**) in very dilute CCl_4 solutions exhibit the expected Q-band with the most intense Q(0,0) transition occurring between 680 and 700 nm with the value $\epsilon = 2 \times 10^5$ l/mol per cm (table 1). In the case of metal-free PC (**3a**), the symmetry of the ring is reduced by the two pyrrole hydrogen atoms and the Q-band is split into two peaks of almost equal intensity [35]. The absorption spectra of the *t*-butyl-substituted PCs (**3**) differ only to an insignificant extent from the unsubstituted compounds [36].

The absorption behavior of the NPs (**4a–4d**) is different from that of the corresponding PCs (**3a–3c**). The absorption maxima of the Q(0,0) transition of **4a–4c** occur at 770–790 nm. The bathochromic shift of the Q-band of about 100 nm is attributed to the acen annelation [37]. Significantly, the macrocycles **4a–4c** aggregate more readily than the PCs (**3**) in CCl_4. Even at concentrations lower than 10^{-7} mol/l, evidence for the existence of aggregates of **4b** and **4c** is found in the absorption spectra. At such a concentration, NPs exist entirely as monomers. With the exception of **3c** and **4c**, all PCIs and NPs investigated resulted in fluorescence at room temperature that could be recorded in CCl_4 (table 1). At approx. 10^{-7} mol/l, the observed fluorescence can be attributed to the monomeric form. The Stokes shifts for the PCs, **3a**, **3b** and **3d**, as well as those of the acen annelated compounds, **4a** and **4b**, are small

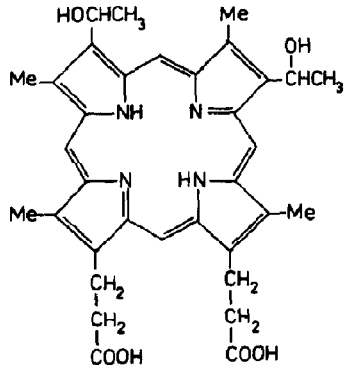
Table 1

Absorption maxima and their relative intensities, and fluorescence maxima and decay times of the investigated tetrapyrroles in CCl_4 ($c = 10^{-7}$ mol/l) at $T = 293$ K

Positions of absorbance and fluorescence maxima are expressed in nm; sh, shoulder.

Compound	Absorption maxima (relative intensity)	Fluorescence decay time (ns; ±0.5 ns)	Fluorescence maxima
1 [a]	398 (1), 501 (0.11), 533 (0.07), 571 (0.05), 616 (0.03)	12.0 [b]	628, 690
2 [a]	409 (1), 507 (0.14), 537 (0.13), 608 (0.12), 667 (0.48)	6.2	675, 720
3a	702 (1), 662 (0.74), 645 (0.33), 598 (0.20), 585 (0.14)	7.1	708, 738, 784
3b	693 (1), 659 (0.15), 624 (0.15), 610 (0.05)	4.1	705, 734, 778
3c	677 (1), 646 (0.17), 608 (0.17), 590^{sh} (0.05)		
3d	679 (1), 650^{sh} (0.12), 615 (0.11)	5.9	705, 738, 778
4a	784 (1), 770 (0.83), 745 (0.39), 720 (0.37), 700 (0.32), 670^{sh} (0.13)	4.6	794, 833, 873, 897
4b	785 (1), 773^{sh} (0.74), 750^{sh} (0.25), 695 (0.21), 665^{sh} (0.08)	3.6	795, 875
4c	771 (1), 733 (0.38), 700 (0.29), 670 (0.17)		
5a	419 (1), 515 (0.05), 550 (0.02), 592 (0.01), 652 (0.01)	10.5	655, 721
5b [a]	416 (1), 512 (0.05), 545 (0.03), 588 (0.02), 648 (0.02)	8.5	652, 716
5c [a]	420 (1), 517 (0.05), 554 (0.04), 594 (0.02), 650 (0.03)	8.8	659, 725

[a] Dissolved in ethanol.
[b] From ref. 33.



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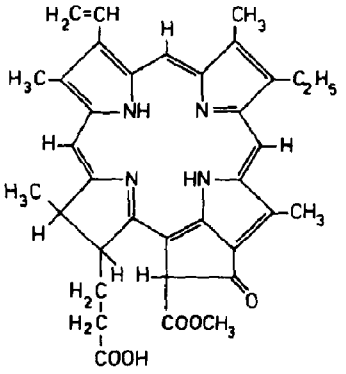

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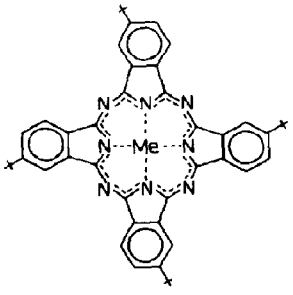
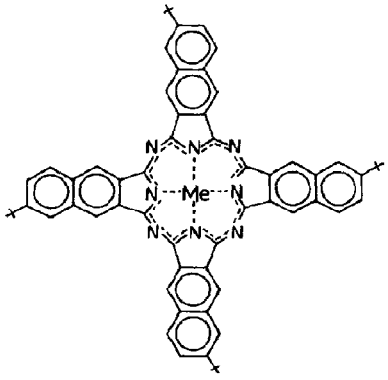
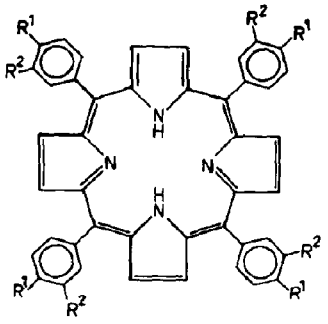


No.	3a	3b	3c	3d
Me	H_2	AlCl	Cu	Mg



No.	4a	4b	4c
Me	H_2	AlCl	Cu



No.	R^1	R^2
5a	H	H
5b	H	OH
5c	OH	H

Fig. 2. Structural formulae of the tetrapyrroles investigated here: (**1**) hematoporphyrin IX, (**2**) pheophorbide *a*, (**3a**) *t*-butyl-substituted metal-free phthalocyanine, (**3b**) ClAl-, (**3c**) Cu-, (**3d**) Mg-*t*-butyl-substituted phthalocyanine, (**4a**) *t*-butyl-substituted metal-free naphthalocyanine, (**4b**) ClAl-, (**4c**) Cu-*t*-butyl-substituted naphthalocyanine, (**5a**) 5,10,15,20-tetraphenylporphyrin, (**5b**) 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin, (**5c**) 5,10,15,20-tetra(*p*-hydroxyphenyl)porphyrin.

relative to the Q-band absorption maximum (figs. 3 and 4).

Table 1 lists the fluorescence decay times of all compounds studied here. The longest decay times were measured for HP monomers (**1**, 12 ns) and the TPPs (**5**, 8.5–10.5 ns) followed by the metal-free PC (**3a**, 7.1 ns). A further decrease in decay time was observed on measuring the NPs (**4a**, 5.6 ns). In both groups of compounds (**3** and **4**), we observed that the central ion exerted an influence on the fluorescence decay time. The shortest decay times in these chemical groups were detected for ClAl-PC (**3b**, 4.3 ns) and ClAl-NP (**4b**, 3.6 ns). For every compound at below 10^{-7} mol/l, single-exponential decay behavior was observed.

The singlet oxygen quantum yields of the dyes were determined in ethanol (fig. 5) or CCl_4 (fig. 6) as shown in table 2. We found that the TPPs (**5**), HP (**1**) and Pheo (**2**) possess high quantum yields of the same order of magnitude (about 0.5–0.6). With the exception of ClAl-PC (**3b**, $\Delta\phi = 0.31$) the PCs show only low quantum yields of about 0.13 (**3a**, **3c**, **3d**). For the NPs the same behavior occurs but singlet oxygen luminescence was detectable

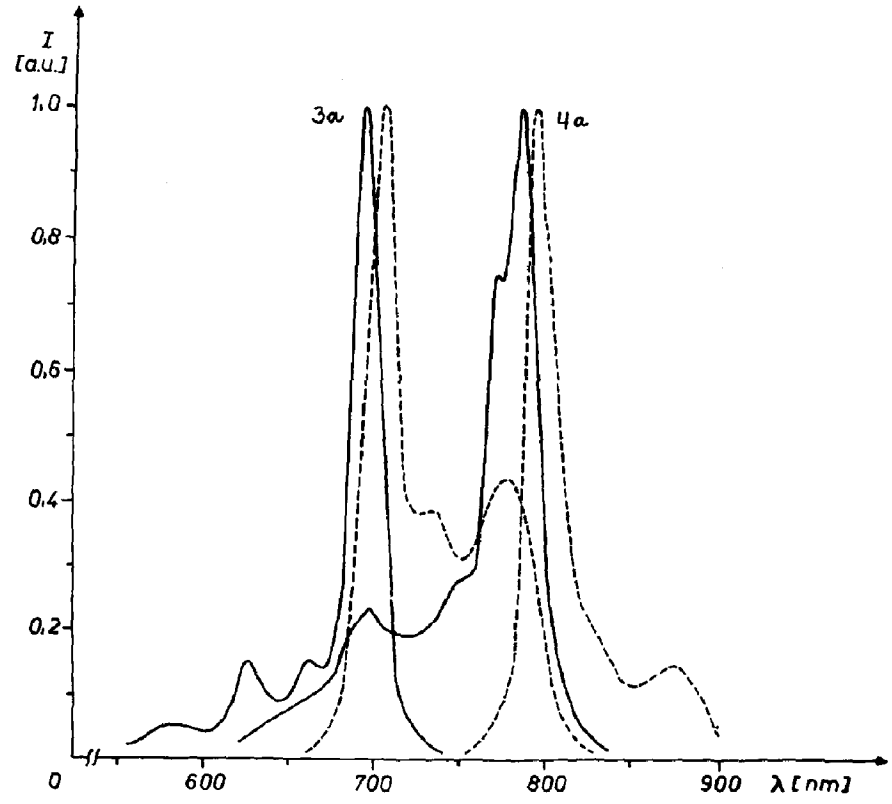


Fig. 3. Normalized absorption (———) and fluorescence (— — —) spectra of metal-free *t*-butyl-substituted phthalocyanine (**3a**) and naphthalocyanine (**4a**) in CCl_4 ($c = 10^{-7}$ mol/l).

only at concentrations of about 10^{-10} mol/l due to the very efficient self-quenching of these compounds. Only in the case of ClAl-NP (**4b**, $\Delta\phi = 0.42$) was an appreciable level of singlet oxygen generation observed while for the NP (**4a**, 0.17) and Cu-NP the quantum yields were significantly lower. Therefore, in both groups (**3** and **4**) the ClAl compounds represent the most promising candidates for use in PDT on the basis of their low singlet state lifetime and high singlet oxygen quantum yield.

Wagner et al. [38] calculated a value for the singlet oxygen quantum yield of sulfonated GaCl-phthalocyanine of 0.5 from indirect measurements (via the concentration of photoproducts). This relatively high value was probably obtained as a consequence of the high triplet quantum yield and triplet lifetime [39,40] of this phthalocyanine. Rosenthal et al. [41] determined the singlet oxygen quantum yields of sulfonated ClAl-phthalocyanine and sulfonated metal-free phthalocyanine as 0.34 and 0.14, respectively, and found the corresponding values to be negligible for those containing paramagnetic ions. In this context, it should be noted that sulfonated phthalocyanines containing paramagnetic ions display little, if any, photodynamic activity [16] and have very short triplet state lifetimes [42]. Otherwise, the sulfonated ClAl-phthalocyanine was shown to be a very effective photosensitizer both in vitro and in vivo [15,17,19,22].

The singlet oxygen quantum yields (table 2) determined for PC (**3a**) and ClAl-PC (**3b**) are in agreement with the results reported by Rosenthal

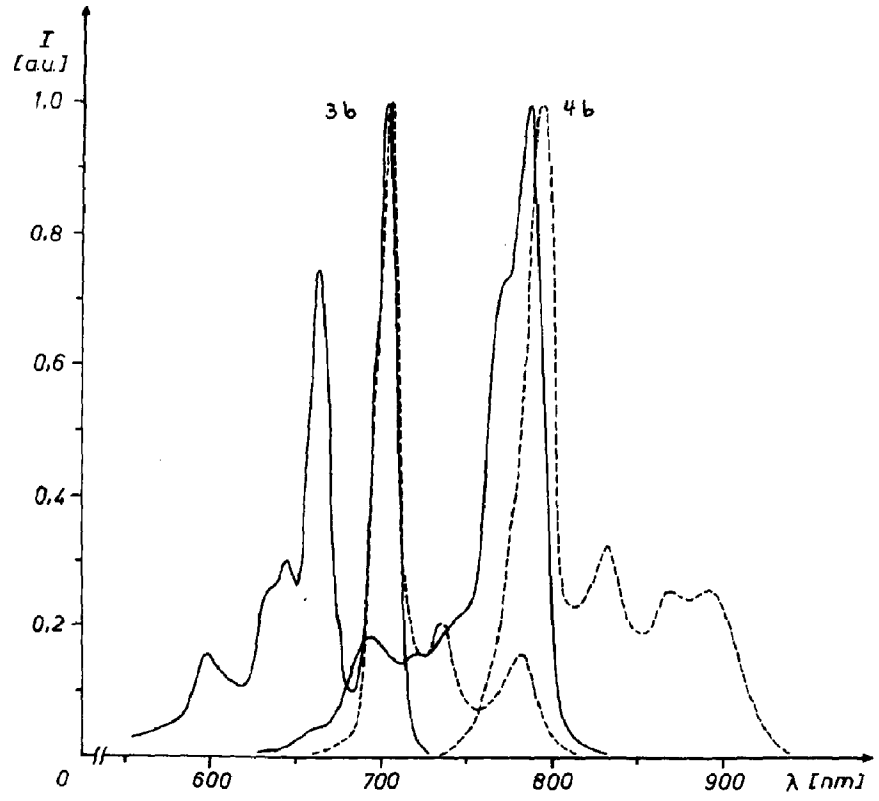


Fig. 4. Normalized absorption (———) and fluorescence (— — —) spectra of ClAl-*t*-butyl-substituted phthalocyanine (**3b**) and naphthalocyanine (**4b**) in CCl_4 ($c = 10^{-7}$ mol/l).

et al. [41] for sulfonated phthalocyanines, thus signifying that *t*-butyl substitution or sulfonation has no effect or merely an equal influence on the singlet oxygen generation of these compounds.

3.2. In vivo studies

In the present study, we have assessed the photodynamic efficiency of a number of tetrapyrroles selected from our photophysical studies in comparison with HPD. The sensitizers were administered intratumorally, as proposed by others [43–45]. We prefer this form of administration, since the retention of systemically administered sensitizer is not limited to malignant tissue alone. Furthermore, normal organs and skin can accumulate high levels of sensitizer [46–48]. Apart from this aspect, the pharmacokinetics of the various compounds investigated in this work have not been studied in detail. We suppose that, as a result of administration locally and the short interval (30 min) between sensitizer injection and laser exposure, the pharmacodynamic effects were of relatively small magnitude in our experiments.

Using the tumor model described we were able to confirm the level of photodynamic activity of m-TPP and p-TPP (results not shown) reported by Berenbaum et al. [6]. Besides HPD we selected three new effective sensitizers: Pheo (**2**), ClAl-PC (**3b**), and ClAl-NP (**4b**).

The tumor response of control animals (group 1) from five experiments (30 animals) with and without laser irradiation was monitored. Laser irradiation at a dose of 180 J per tumor on days 4

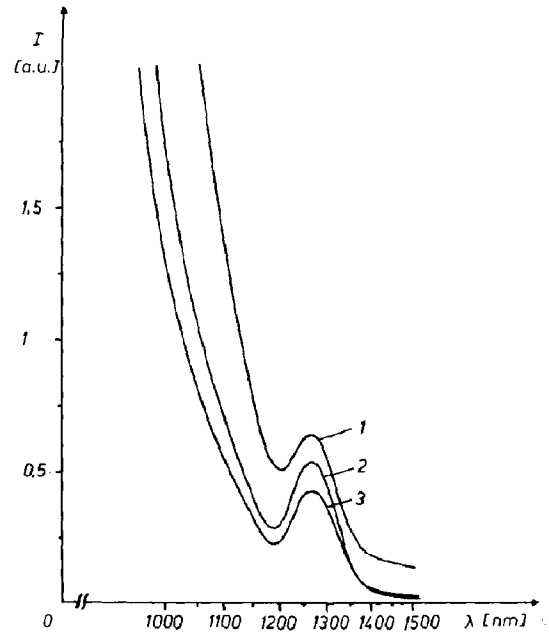


Fig. 5. Singlet oxygen luminescence at 293 K of (1) hematoporphyrin IX, (2) pheophorbide *a* and (3) 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin in ethanol ($A^{1\,cm}_{514\,nm} = 0.2$).

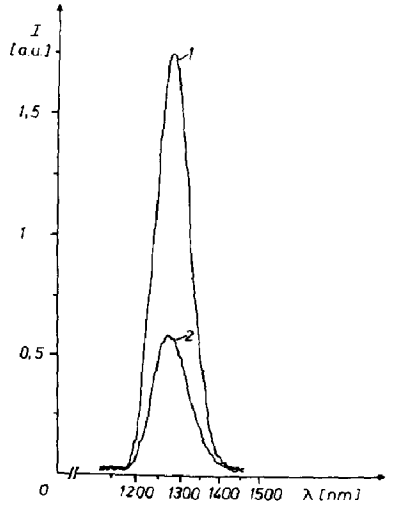


Fig. 6. Singlet oxygen luminescence at 293 K of (1) 5,10,15,20-tetraphenylporphyrin and (2) ClAl-*t*-butyl-substituted phthalocyanine in CCl_4 ($A^{1\,cm}_{652\,nm} = 0.005$).

Table 2

Absolute singlet oxygen quantum yields (mean values of 5 measurements)

Luminescence standards: 5,10,15,20-tetraphenylporphyrin (TPP) in CCl_4; 5,10,15,20-tetra(*p*-sulfophenyl)porphyrin (TPPS) in ethanol

Compound	No	Singlet oxygen quantum yield	
		In C_2H_5OH	In CCl_4
TPPS	–	0.70	
HP	(1)	0.60 ± 0.05	
Pheo	(2)	0.51 ± 0.07	
m-TPP	(5b)	0.50 ± 0.06	
p-TPP	(5c)	0.58 ± 0.06	
TPP	(5a)		0.70
PC	(3a)		0.16 ± 0.02
ClAl-PC	(3b)		0.31 ± 0.02
Cu-PC	(3c)		0.13 ± 0.02
Mg-PC	(3d)		0.07 ± 0.06
NP	(4a)		0.17 ± 0.03
ClAl-NP	(4b)		0.42 ± 0.04
Cu-NP	(4c)		not detectable

and 6 showed no effect on the survival time of mice and was therefore selected as the dosage for use in all experiments. Mice injected with the investigated sensitizers without subsequent exposure to light showed no apparent toxic reactions.

Fig. 7 demonstrates the tumor response of HPD-treated mice summarized from three experiments. 31% of the animals survived 60 days after PDT. The difference between the mean survival

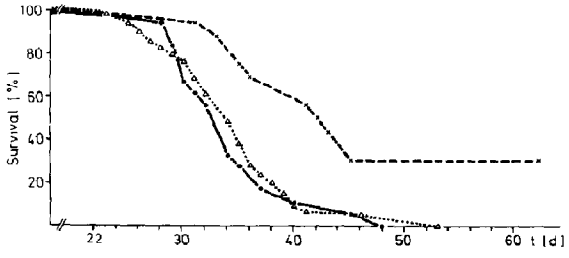


Fig. 7. Survival of Lewis lung carcinoma mice photosensitized by hematoporphyrin derivative (HPD); dose level, 10 mg/kg. (●———●) HPD intratumoral, $n = 18$, MST = 34.3 ± 5.5 days; (×——×) HPD intratumoral + irradiation, $n = 16$, MST = 45.4 ± 10.9 days; (△———△) control, $n = 18$, MST = 32.2 ± 7.7 days.

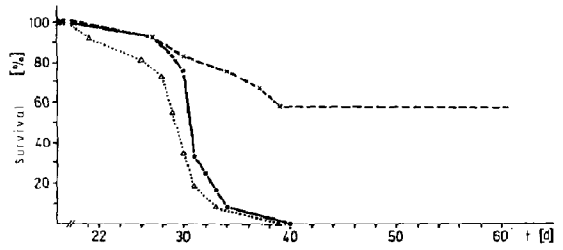


Fig. 8. Survival of Lewis lung carcinoma mice photosensitized by pheophorbide *a* (Pheo); dose level, 10 mg/kg. (●———●) Pheo, intratumoral, $n = 12$, MST = 31.7 ± 3.2 days; (×——— ×) Pheo + irradiation, $n = 12$, MST = 49.0 ± 13.9 days; (△———△) control, $n = 11$, MST = 29.7 ± 4.4 days.

times of PDT and control mice is statistically significant.

In fig. 8 the effectiveness of Pheo (**2**), summarized from two experiments, is shown. The mean survival time of PDT groups shows a significant increase and 58% of animals were cured.

The tumor response on ClAl-PC (**3b**) treatment (results not shown) was within the same range as for HPD treatment, the results being in agreement with those reported by others [17,19,22] for the photodynamic efficacy of sulfonated ClAl-phthalocyanine. In the case of the metal-free phthalocyanine (**3a**) we observed no significant photodynamic effects.

The tumor response on ClAl-NP (**4b**) treatment with and without light exposure is shown in fig. 9.

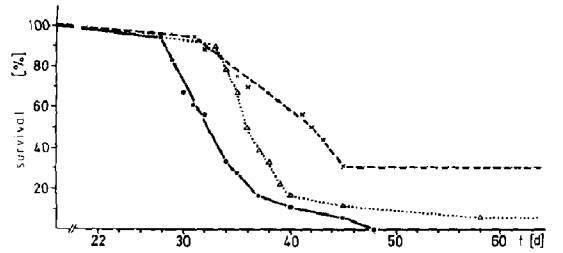


Fig. 9. Survival of Lewis lung carcinoma mice photosensitized by ClAl-*t*-butyl-substituted naphthalocyanine (ClAl-NP); dose level, 10 mg/kg. (●———●) ClAl-NP, intratumoral, $n = 18$, MST = 39.0 ± 8.2 days; (×——— ×) ClAl-NP + irradiation, $n = 18$, MST = 43.9 ± 11.6 days; (△———△) control, $n = 18$, MST = 36.0 ± 9.0 days.

The curves represent the means from three experiments. However, only in two experiments did the PDT cause a significant increase in survival time compared to the saline-treated controls. Therefore, the difference between the curves shown in fig. 9 is not significant. In the PDT groups, five out of 18 animals (28%) were cured, as well as two out of 18 mice (11%) treated only with ClAl-NP. In one of the three experiments one control mouse (6%) survived 60 days. This results in a relatively high standard deviation of the mean survival time (9.00). We suppose that the low rate of cure may be caused by the light absorption of ClAl-NP (**4b**) at 670 nm (cf. fig. 5) being less than optimal. Again, for the metal-free compound NP (**4a**), no significant photodynamic activity could be observed.

4. Conclusions

The photosensitizers we selected on the basis of photophysical investigations exhibit a good photodynamic activity in vivo. Under the present experimental conditions, Pheo was the best sensitizer due to its stronger absorptivity in the red region of the spectrum as compared with HPD and its higher singlet oxygen quantum yield relative to the PCs and NPs. In our experiments, the NP were not effectively excited by laser irradiation, so that photodynamic activity, especially that of ClAl-NP (**4b**) which has a singlet oxygen quantum yield of about 0.42, requires further investigation. Moreover, the efficient self-quenching of singlet oxygen by these compounds as a result of the very low energy gap between singlet oxygen and the lowest triplet state of the sensitizer could be the process responsible for the lower efficiency of ClAl-NP compared with Pheo.

In our experiments we observed a good correlation between singlet oxygen generation and photodynamic efficacy of the dyes. The primary mechanism of photodynamic activity of these compounds probably consists of energy transfer from the triplet state of the dyes to molecular oxygen, resulting in the generation of singlet oxygen (type II mechanism of photosensitization).

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