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Purpurinimides as Photosensitizers: Effect of the Presence and position of the Substituents in the In Vivo Photodynamic Efficacy

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Abstract—This study presents a novel approach for the regioselective synthesis of a series of alkyl ether analogues of purpurin-18-*N*-alkylimide. In the purpurinimide series, this is the first example which demonstrates that the presence and position of the substituents in the macrocycle makes a remarkable difference in the in vivo PDT efficacy. © 2000 Elsevier Science Ltd. All rights reserved.

For quite some time one of the main objectives of our laboratory has been to establish structure–activity relationships in a variety of photosensitizers.[1] This study is aimed to establish a generic structural requirement(s) for effective photosensitizing agents. The ultimate goal is to increase the tumor selectivity of a drug, so that the undesirable side-effects associated with most of the photosensitizers could be eliminated or reduced. In such effort, we have previously reported our results obtained from QSAR studies of a series of alkyl ether analogues of pyropheophorbide *a*.[2] These data indicated that overall lipophilicity of the molecule made a significant difference in PDT efficacy. We then extended this approach to the purpurinimide series in which the lipophilic characteristic of the molecule can be varied either by altering the length of the imide-*N*-alkyl group or the alkyl ether substituent regioselectively introduced at position-3 of the macrocycle.[3] Among the compounds evaluated so far, the 3-devinyl-3(l′-heptyloxy ethyl)-purpurin-18-*N*-hexylimide **1** was found to be quite effective (100% tumor cure at day 30 at a dose of 1.0 μmol/kg, treated with light (135J/cm^2), 24 h post injection of the drug). In the same series, we have also shown that among the compounds with similar lipophilicity, the photosensitizers containing the methyl ester functionalities are much more effective than those possessing amide substituents.[4]

Our next step was to investigate the effect of substituent regiochemistry on PDT efficacy. In this effort, purpurinimides **2**–**5** were synthesized (Scheme 1) and their in vivo efficacy under similar treatment conditions was compared with compound **1**. Compounds **2** and **4**, in which the (1′-heptyloxyethyl) group was regioselectively introduced at positions-8 and -20 of the macrocycle, were prepared to investigate the effect of the same substituent present at various positions of the ring system. In order to investigate the effect of a primary versus secondary alkyl ether group in PDT efficacy, the (1′-heptyloxyethyl) substituent present at position-3 of purpurinimide **1** was replaced with an octyloxymethyl group (compound **3**) without altering the overall lipophilicity. Finally, in our attempt to determine the importance of the secondary heptyloxyethyl group in PDT efficacy, compound **5** lacking alkyl ether substituent at position **3**, but, possessing lipophilicity similar to purpurinimide **1** was also prepared.

Results and Discussion

Purpurinimides **1**–**5** were synthesized by following the reaction sequence shown in Scheme 1. In brief, methylpheophorbide-a **6** was extracted from the algae *Spirulina pacifica* by following the literature procedure.[5] The five member isocyclic ring present in **6** was then converted into a fused six member cyclic anhydride system **7**, known as purpurin-18 methyl ester by following the methodology developed in our laboratory.[6] Reaction of purpurin-18 with *n*-hexylamine produced **8** as a mixture of two isomers, which were not separated into individual

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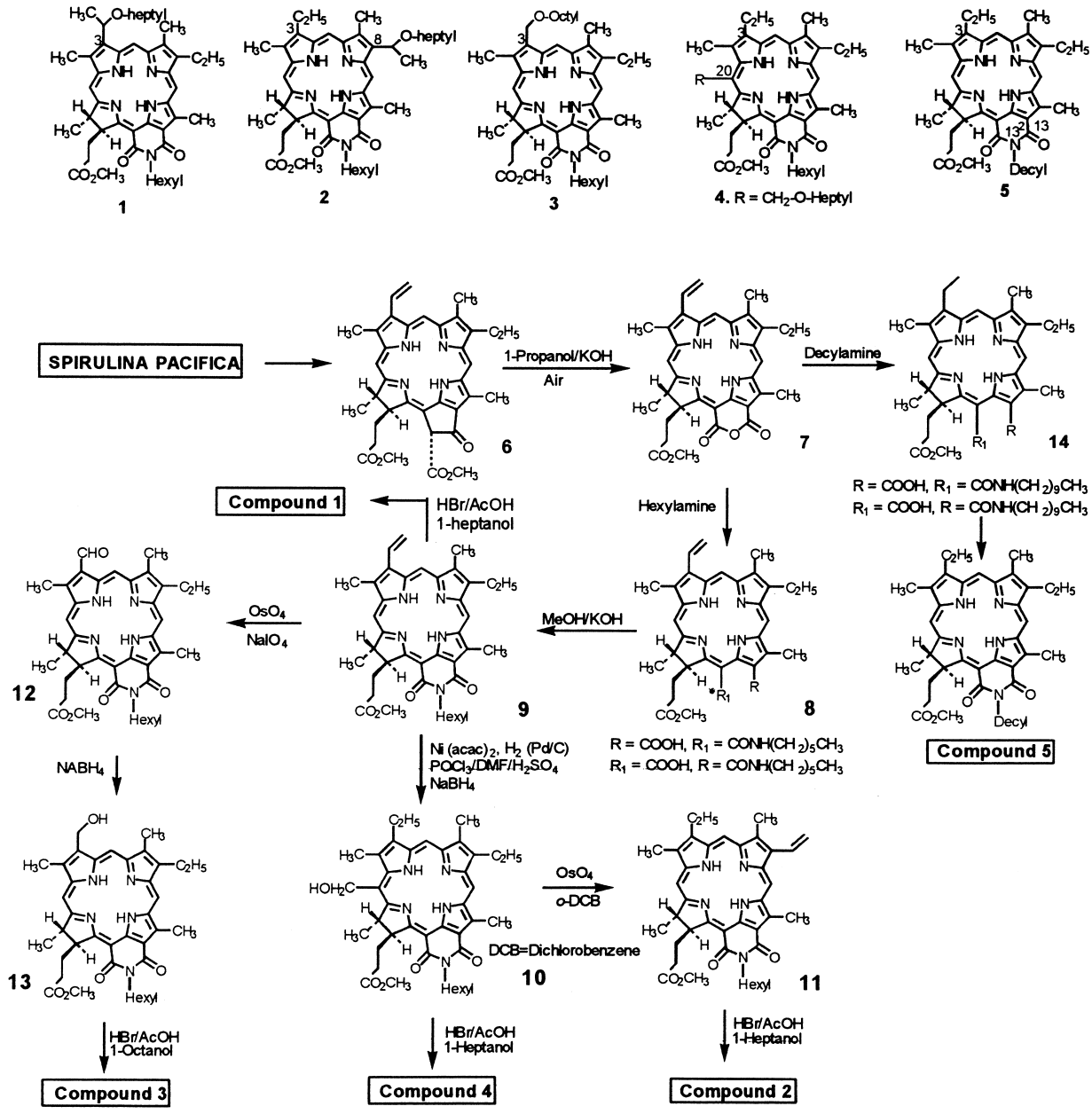

Scheme 1. Synthesis of various purpurinimide analogues.

isomers and via intramolecular base catalyzed cyclization afforded purpurin-18-*N*-hexylimide **9** in 70% yield (based on compound **7**) exhibiting a long wavelength absorption near 705 nm. Reaction of **9** with HBr/acetic acid and then with 1-heptanol produced purpurinimide **1** (*m*/*z* calcd for $C_{47}H_{63}N_5O_5$: 777.48; found: 778.2 (M+1)) in 72% yield. For the preparation of purpurinimide **2**, the 3-vinyl group in imide **9** was first converted into an ethyl substituent on hydrogenation using Pd/C as a catalyst to produce compound **10**, which on reacting with osmium tetraoxide/*o*-dichlorobenzene[7] generated 8-vinyl analogue **11** (*m*/*z* calcd for $C_{40}H_{48}N_5O_5$: 677.37; found: 678.86 (M+1)). At the final step, reaction of **11** with 30% HBr/acetic acid and 1-heptanol gave the desired purpurinimide **2** in 60% yield (*m*/*z* calcd for C_{47} $H_{63}N_5O_5$: 777.48; found: 778.50 (M+1)).

In order to investigate the effect of a primary versus secondary alkyl ether group in PDT efficacy, the 3-vinyl-*N*-hexyl purpurinimide **9** was converted into the corresponding formyl analogue **12** by reacting with sodium periodate and osmium tetroxide as a catalyst in 70% yield. The completion of the reaction was monitored by UV–visible spectroscopy (disappearance of a peak at 705 nm and appearance of a new peak at 735 nm). Reaction of **12** with sodium borohydride ($NaBH_4$) in tetrahydrofuran (THF) produced the 3-devinyl-3-hydroxymethyl-purpurin-18-*N*-hexyl-imide **13** in 78% yield with long wavelength absorption at 700 nm (*m*/*z* calcd for $C_{39}H_{47}N_5O_5$: 665.35; found: 666.50 (M+1)). Further reaction of **13** with HBr/acetic acid and subsequent treatment with 1-octanol produced the desired 3-(1-octyloxyethyl) derivative **3** in 60% yield (*m*/*z* calcd

for $C_{47}H_{63}N_5O_5$: 778.06; found: 779.2). The synthesis of compound **4** was achieved in a sequence of reaction from purpurinimide **9** in good yield (*m*/*z* calcd for $C_{48}H_{65}O_5N$; 792.08; found: 793.4 (M + 1)).

The structures **1**–**4** were further confirmed by NMR spectrometry. Some important features observed in the NMR spectra are shown in Figure 1. In all the alkyl ether analogues, the vinyl(−CH = CH_2) resonances generally observed at δ 7.84 (dd), δ 6.27(d) and 6.16(d) were absent (Figs. 1A–D). In both 3- and 8-(l-heptyloxymethyl) analogues **1** and **4**, the presence of a quartet at δ 5.80 ppm for −C*H*(O-heptyl), integrating for one proton, confirmed the presence of the desired products (Figs 1A and B). As expected, in purpurin imides containing either a hydoxymethyl group or a hexyloxymethyl substituent at position-3 the macrocycle showed singlet at δ 5.82 ppm for CH_2-OR (R = H or −octyl) integrating for two protons (Fig. 1c). In the NMR spectrum **4**, the presence of only two downfield singlets at the *meso*-region (9.12 and 9.35 ppm) indicated that the position adjacent to the reduced ring is occupied with a heptyl oxymethyl group (the methylene resonances appeared as doublets instead of a singlet as observed for the 3-octyloxy-methyl analogue **3**) at δ 5.5 ppm and 5.2 ppm (Fig. 1D) due to the interaction of the -CH_2O-heptyl protons with the 18-H proton of ring D (*trans*-reduced)). In order to prepare purpurinimide **5**, lacking an alkyl ether substituent, but, having lipophilicity similar to photosensitizer **1** (log P 10.83), mesopurpurin-18 methyl ester **7** was reacted with *N*-decylamine. The intermediate amide **14** was converted into the desired *N*-decylimide **5** in 60% yield in the same manner as discussed for the preparation of the related imide analogue **8**. The reaction sequence followed for the preparation of the desired purpurinimide is illustrated in Scheme 3. The structure was confirmed by NMR (which clearly indicated the absence of the vinyl resonances and presence of an additional ethyl group), and mass spectrometry analyses (*m*/*z* calcd for $C_{44}H_{57}O_4N_5$: 720.00; found: 721.10).

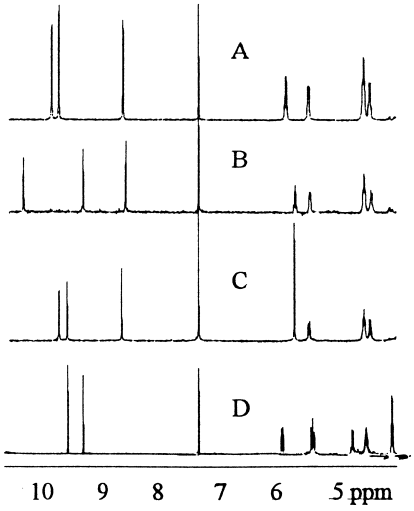

Figure 1.

In Vivo Photosensitizing Efficacy

The photosensitizers at various doses were injected intravenously to 5–7 weeks old mice (6 mice/group, transplanted subcutaneously with RIF tumor into the axilla). Mice were restrained in aluminum holders and each tumor was illuminated with 135J/cm^2 light from a laser tuned at the longest wavelength absorption maximum of the photosensitizers. The percentage of the tumor regrowth was recorded daily.

In an initial experiment, groups of mice bearing RIF tumors were treated with laser light (75 mw/cm^2, 135J/cm^2, 702 nm) after the administration of purpurinimide **1** at three variable doses (4.0, 1.0 and 0.4 μmol/kg) and the best tumor response (100% tumor cure at day 30) was obtained at a dose of 1.0 μmol/kg. The treatment wavelength (702 nm) was selected by in vivo reflectance spectroscopy.[8] The sensitizing efficacy of other photosenitizers was then compared at the same dose and treatment conditions. The biological results are summarized in Table 1. As can be seen, among the compounds tested, the 3-devinyl-3 (1-heptyloxymethyl)-purpurin-18-*N*-hexylimide **1** appeared to be most effective, and produced 100% tumor cure at day 30. To our surprise, the positional isomer **2**, in which the heptyloxyethyl group was substituted at position-8 of the macrocycle, under similar treatment conditions did not produce any PDT activity (day 1–2: tumor response 0%). Replacement of the secondary heptyl ether side-chain at position-3 of purpurinimide **1** with a primary alkyl group (purpurinimide **3**) produced a remarkable decrease in antitumor activity. Photosensitizers **4**, containing an alkyl ether side-chain at the *meso*-position was found to be toxic at a dose of 1 μmol/kg (3 out of 5 mice died after treating with light but the surviving mice were tumor free at day

Table 1. Preliminary in vivo antitumor activity of certain purpurinimides[a]

Compound	Log *P* value	Drug dose μmol/kg	Light dose (J/cm^2)	In vivo absorption	Tumor response (% tumor cure)				
					Day 1–2	Day 7	Day 14	Day 21	Day 30
1	10.83	1.0	135	702 nm	100	100	100	100	100
2	10.83	1.0	135	702 nm			No response		
3	10.80	1.0	135	702 nm			No response		
4[b]	10.78	1.0	135	702 nm	50	50	50	50	50
5	10.62	1.0	135	702 nm	100		Tumour regrowth on day 7		

[a]4–6 mm diameter RIF tumors (6 mice/group) were exposed to 75 MW/cm^2 for 30 mm from a tunable dye laser tuned to the maximun red absorption peak at 24 h post injection.
[b]At a dose of 1.0 mmol/kg, there was 50% mortality after light exposure. Further studies at lower doses are currently in progress.

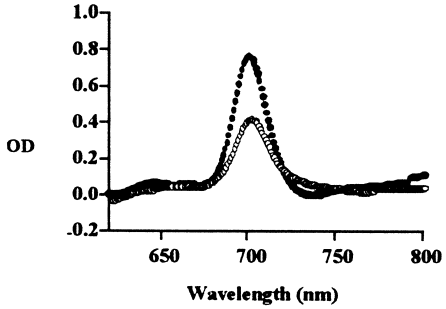

Figure 2. Open circles are for skin and closed circles are for tumor uptake (mice transplanted with RIF tumor) of isomer **1**, at a dose of 5.0 μmol/kg, 24 h post injection.

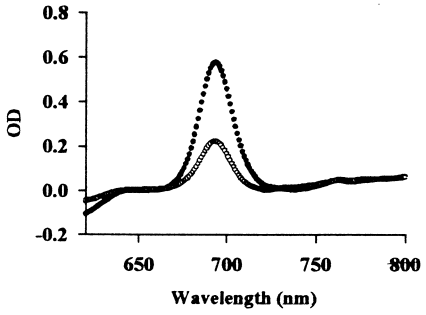

Figure 3. Open circles are for skin and closed circles are for tumor uptake (mice transplanted with RIF tumor) of isomer **2**, at a dose of 5.0 μmol/kg, 24 h post injection.

30). These results suggest that at this particular dose, either the purpurinimide **4** is too toxic or is quite potent. Further studies at lower drug doses are currently in progress. Compared to imide **1**, photosensitizer **5**, in which the alkyl ether group at position-3 was replaced by an ethyl substituent, and the *N*-hexyl chain is substituted with a 10-unit saturated carbon chain to keep a similar lipophilicity produced limited PDT efficacy.

The tumor versus muscle uptake of the purpurinimide analogues and their in vivo shifts were determined by in vivo reflectance spectroscopy. These compounds produced higher tumor uptake than the surrounding muscle, and were retained in tumors for at least 24 h post injection. However, among all the analogues, purpurinimide **1** containing a secondary heptyloxyethyl group at position-3 was found to be most effective (100% tumor cure on day 30). Purpurinimide **2**, containing a (1′-heptyloxyethyl) group at position-8 (Fig. 2) and the corresponding 3-(1′-heptyloxyethyl) analogue **1** (Fig. 3) had similar tumor uptake, but produced a remarkable difference in PDT efficacy (Table 1). These results indicate that high tumor uptake of the photosensitizer is not the only criteria for its efficacy. For a compound to be effective it is likely that it binds or localizes into the more sensitive site(s) of the tumor cells and these studies are under investigation.

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