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SYNTHESIS AND PHOTOTOXICITY OF A SERIES OF HAEMATOPORPHYRIN ANALOGUES

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Abstract: The synthesis of new haematoporphyrin analogues is described. These porphyrins have been assayed for phototoxicity and cellular localization and show promise for use in photodynamic therapy of cancer.

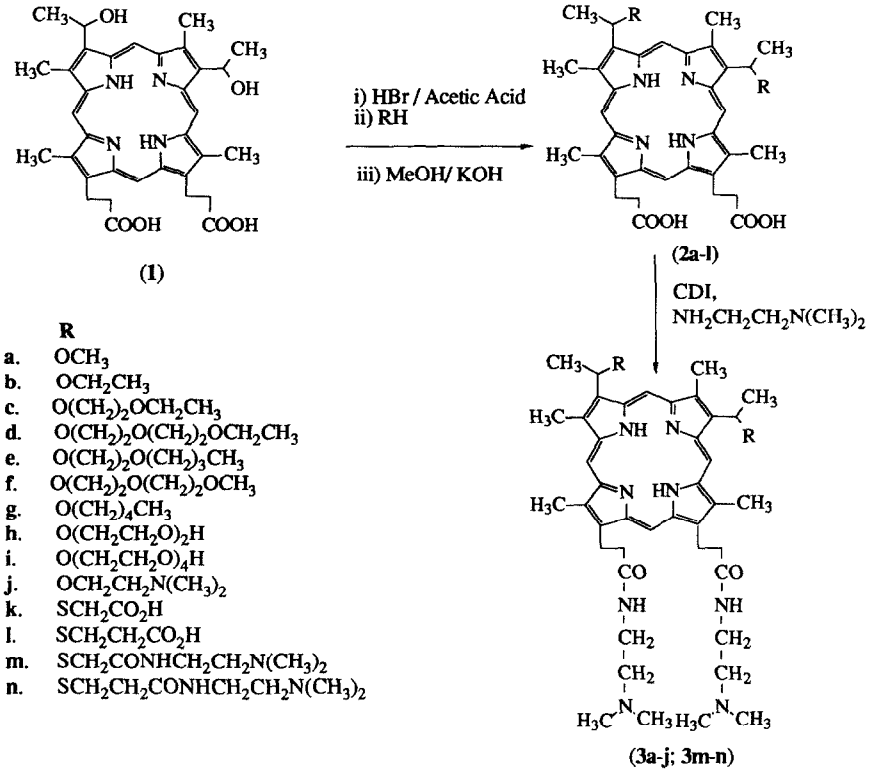
Photodynamic therapy (PDT) utilizes the selective retention of certain porphyrins by tumours and the photosensitization properties of these compounds to produce an encouraging modality of treatment.[1,2] Although haematoporphyrin derivative (HpD) is the most frequently used photosensitizer it is a complex mixture and the constituents responsible for localization and photosensitization have yet to be fully elucidated.[3] In an effort to establish structure-activity relationships involved in PDT, we report the synthesis of discrete and well-defined porphyrins, their characterization and the evaluation of some biological properties.

Haematoporphyrin (Hp, **1**) was used as the starting material for all the porphyrins synthesized and reported in this paper. All procedures were carried out in subdued light. Typically, Hp (1 g) was stirred with 45% w/v hydrogen bromide in glacial acetic acid (40 ml) for 1 h.[4,5] The solvent was removed *in vacuo*, to yield the HBr adduct. The alcohol or thiol (5 ml) was added to the HBr adduct and stirred for 8 h at 50° to yield diether/diesters (from reaction with alcohols) and tetra carboxylic acids **2k-l** (from reaction with thiols). The thiol solutions were evaporated *in vacuo*, diluted with water, adjusted to pH 3.5 with 25% glacial acetic acid, centrifuged, washed with water (3x) and then dried to give **2k** and **2l**. The diether/diester solutions were hydrolyzed in methanolic KOH for 24 h and the lower boiling alcohols were removed *in vacuo* . The pH was brought to pH 3.5 with 25% glacial acetic acid, the precipitate centrifuged or extracted with dichloromethane, washed with water, then dried to give the dicarboxylic acids **2a-j**.

The porphyrin dicarboxylic acids (**2a-l**) (100 mg) were dissolved in DMF (10 ml) and treated with CDI (50 mg) under N_2 for 20 min. The amine (0.5 ml) was added to the activated porphyrin and the mixture stirred for 16 h at RT. The DMF was evaporated *in vacuo* and the porphyrin precipitated with either 25% glacial acetic acid or 5% NaOH, centrifuged, washed three times with water and dried *in vacuo*. The carboxamides **3a-j** and **3m-n** were obtained in 80-95% yields. Diode array analytical C-18 HPLC was used to confirm purity and no impurities were detected. Structures were confirmed using IR, ^1H and ^{13}C NMR and FAB mass spectrometry. The NMR spectral assignments of porphyrins **2d**, **3b** and **3n** were made by performing H-C correlations.

We have assayed and reported the cellular localization and phototoxicity properties of some of these porphyrins.[6-8] Briefly, the *in vitro* subcellular localization sites were determined using confocal laser scanning microscopy on C6 glioma and V79 cells.[6] The porphyrins exhibited localization in either mitochondria, lysosomes or throughout the cytoplasm. The distribution patterns were related to pendant side chains in terms of hydrophobicity and charge, structure-distribution patterns were established.[6] A significant correlation was observed between subcellular localization sites and phototoxicities.[7] *In vivo* localization capacities were

calculated at 6 h and 24 h post injection in a mouse C6 intracerebral glioma model.[8] The tumour:normal brain tissue ratios for porphyrins **2b**, **3b**, **2c**, **3c** and **2k** were 17.5, 2.4, 37.7, 15.2 and 23 respectively at 6 h post injection and 8.7, 6.4, 25.1, 8.0 and 28.2 respectively for the 24 h time period. Although HpD exhibits higher concentration ratios (53.7 at 6 h and 34.4 at 24 h), it fails to discern between subcellular localization sites to the extent that these studied porphyrins do. However, the *in vitro* results suggest that subcellular distribution patterns play a more significant role in PDT than mere concentration effects. *In vivo* phototoxicity experiments are currently underway to clarify and ascertain the effect of subcellular distribution on photodynamic effectiveness to ultimately enable PDT to be a more potent technique for the treatment of localized tumours.



(1)

(2a-l)

(3a-j; 3m-n)

R	
a.	OCH_3
b.	OCH_2CH_3
c.	$O(CH_2)_2OCH_2CH_3$
d.	$O(CH_2)_2O(CH_2)_2OCH_2CH_3$
e.	$O(CH_2)_2O(CH_2)_3CH_3$
f.	$O(CH_2)_2O(CH_2)_2OCH_3$
g.	$O(CH_2)_4CH_3$
h.	$O(CH_2CH_2O)_2H$
i.	$O(CH_2CH_2O)_4H$
j.	$OCH_2CH_2N(CH_3)_2$
k.	SCH_2CO_2H
l.	$SCH_2CH_2CO_2H$
m.	$SCH_2CONHCH_2CH_2N(CH_3)_2$
n.	$SCH_2CH_2CONHCH_2CH_2N(CH_3)_2$

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