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COMMUNICATION

Towards dual photodynamic and antiangiogenic agents: design and synthesis of a phthalocyanine-chalcone conjugate†

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A phthalocyanine–chalcone conjugate has been designed to combine the vascular disrupting effect of chalcones with the photodynamic effect of phthalocyanines. This potential dual photodynamic and antiangiogenic agent was obtained by the condensation of a tetrahydroxylated non-peripherally substituted Zn(II) phthalocyanine with an amino chalcone converted into the corresponding activated isocyanate. The conjugate was fully characterized.

Multimodal strategies are being developed for the design of third generation photosensitisers for photodynamic therapy.[1] The targeting of the tumour neovasculature aimed at shutting down the tumour feeding is largely investigated *via* different strategies, either exploiting the tumoural Enhanced Permeation and Retention (EPR) effect,[2,3] thus by coupling photosensitisers to nanoobjects: liposomes,[4] nanoparticles,[5,6] or serum albumin[7,8] amongst others, or by vascular endothelial growth factor receptor (VEGFR)-targeting photosensitisers, for example peptide–chlorine conjugates targeting neuropilin.[9,10] This latter approach is also known as anti-vascular photodynamic therapy, and several vascular-targeted photosensitisers coupled with synthetic peptides or antibodies[11,12] have been reported. Phthalocyanines[13,14] are among the photosensitisers exhibiting the required properties for photodynamic therapy.[15] Chalcones are vascular disrupting agents (VDA) which rapidly and specifically destroy tumour neovasculature.[16–18] VDA therapy is emerging as an innovative field in cancer chemotherapy as it is proving more efficient than classical cytotoxic agents.[19,20] The drawback of this therapy lies in the fact that the rim of the tumours, unvascularised, remains untargeted and allows the tumour to recover/regrow.[21–23]

This is likely to be overcome by the photodynamic effect of phthalocyanines. Our approach aims at combining the antiangiogenic and vascular disrupting effect of chalcones with the photodynamic efficiency of phthalocyanines. This original concept is an innovative approach to produce dual agents with complementary properties. In this preliminary work, we present the design, synthesis and complete characterization of the phthalocyanine–chalcone conjugate **1** represented in Fig. 1.

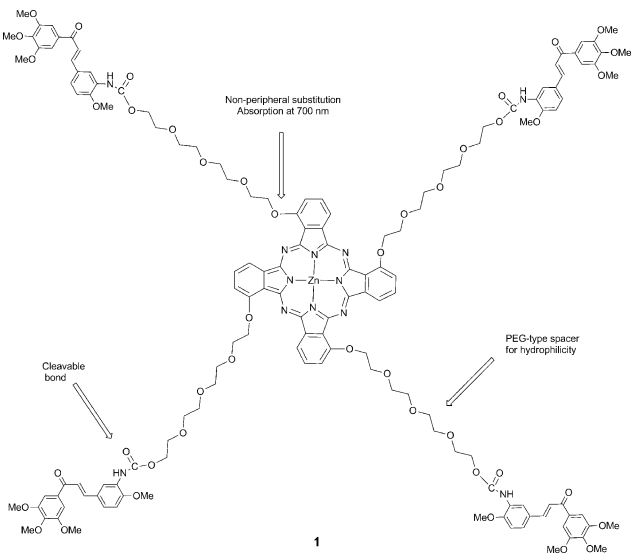

Fig. 1 Structure and design of the phthalocyanine–chalcone conjugate **1** (only the most sterically favoured isomer is shown).

Design and synthetic strategy

Several properties were sought when designing the conjugate: potentially enzyme-cleavable carbamate linkage, suitable maximum absorption wavelength, hydrophilicity if not water-solubility for optimal drug distribution and permeability (Fig. 1). The putative fragility of chalcones in phthalocyanine formation conditions prompted us to graft the chalcone directly onto the phthalocyanine. Phthalocyanine **7** was chosen as it combined several advantages:[24] suitable maximum absorption at 700 nm in a monomeric (non-aggregated) state and functionalization by hydroxyls likely to undergo condensation with a chalcone

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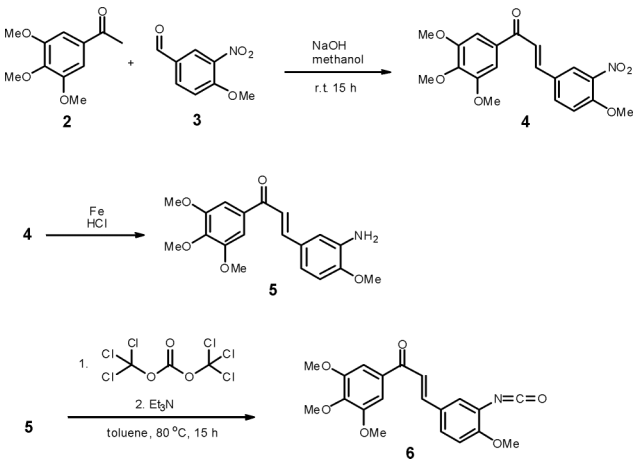
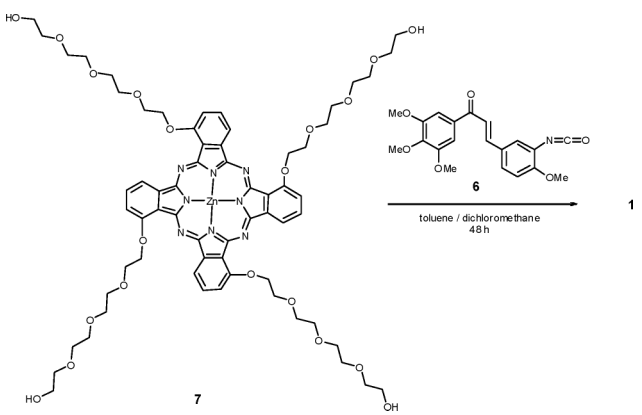
moiety *via* enzymatically cleavable chemical bonds. The isocyanate chalcone derivative **6** was chosen as it is a reactive intermediate, known to easily form carbamate bonds with various types of alcohols. Moreover, this chalcone retains its antivascular effect and the conjugation with **7** should not interfere with the pharmacophore of this class of VDA after expected *in vivo* enzymatic cleavage.

Synthesis and characterization

The synthesis is illustrated in Schemes 1 and 2. The isocyanate chalcone derivative **6** was synthesised in three steps (Scheme 1). Nitrochalcone **4** was obtained quantitatively by a Claisen–Schmidt condensation of 3,4,5-trimethoxyacetophenone (**2**) on 4-methoxy-3-nitrobenzaldehyde (**3**). Subsequent reduction of the nitro compound **3** into the amino chalcone **5** was completed in quantitative yield using iron as the reducing agent. Aminochalcone **5** was easily converted to the corresponding isocyanate chalcone **6** by reacting **5** with triphosgene. The structure of **6** was confirmed by NMR and **6** was used without further purification.



Scheme 1 Preparation of the activated chalcone intermediate **6**.



Scheme 2 Preparation of the conjugate **1**.

In order to obtain the desired tetrasubstituted conjugate **1** and minimize the formation of side-products (mono-, di- and trisubstituted derivatives), a significant excess of the activated chalcone **6** was used (Scheme 2). Phthalocyanine **7** was then added to chalcone **6** dissolved in toluene and dichloromethane and left to stir for 48 h, with the progress of the reaction being monitored by thin layer chromatography. **1** was purified by flash chromatography to afford the desired conjugate **1** in good yield (74%).

Phthalocyanine–chalcone conjugate **1** was characterized by mass spectrometry (MALDI and ESI-HRMS), ATR-IR as well as ^{1}H and ^{13}C NMR spectroscopy performed in deuterated chloroform (see spectra in the ESI†). Its purity was ascertained by HPLC. As tetrasubstituted phthalocyanines are isomeric mixtures, the peaks of the resulting NMR spectra are clustered, rendering their interpretation tedious. In the ^{1}H NMR spectrum, integrals of the polyoxo- area together with the methoxy protons (3.46–5.05 ppm, 112 protons) compared to the aromatic, ethylenic and NH area (6.28–9.14 ppm, 44 protons) are consistent with the proposed structure. The ^{13}C NMR spectrum presents two main areas, an aromatic and an ethylenic one, and a high-shielded aliphatic corresponding to the tetraethylene glycol spacer and the methoxy groups. MALDI analysis gave a single peak fitting the molecular weight (see the ESI†), while the mono- and divalent species were observed in High Resolution ESI-QTOF experiments with a respective precision of 4.0 and 0.7 ppm (Fig. 2a, b and d). The theoretical isotopic distributions of both species fit the experimental data (Fig. 2c and e). Together with the HPLC profile of **1** (Fig. 3), it confirms the high purity of the conjugate.

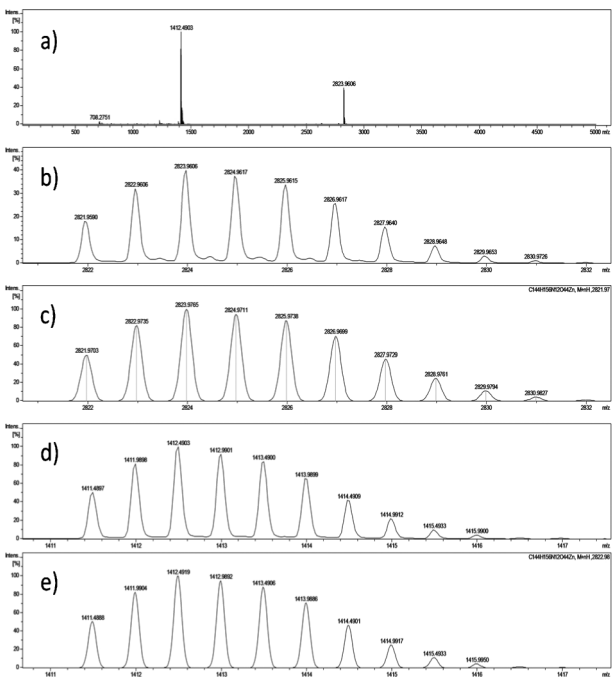


Fig. 2 Full MS spectra of **1** (a), zoom on $[M + H]^+$ (b) and $[M + 2H]^{2+}$ (d) isotopic distributions. The theoretical patterns of $[M + H]^+$ and $[M + 2H]^{2+}$ are respectively (c) and (e).

The electronic absorption of **1** in DMSO, when compared to those of **4**, was modified mainly in the B band area. The chalcone moieties absorbing in the same area[25] enhance the overall absorption of the conjugate **1** compared to phthalocyanine **7** (Table 1 and Fig. 4). The Q band remains unaffected by the

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Table 1 UV-vis absorption data of **1** and **7** in DMSO

	B band λ max, nm (log ε)	Q band λ max, nm (log ε)
1	322 (5.03) 356 (5.10)	704 (5.32)
7	318 (4.61) 376 (4.50)	702 (5.24)

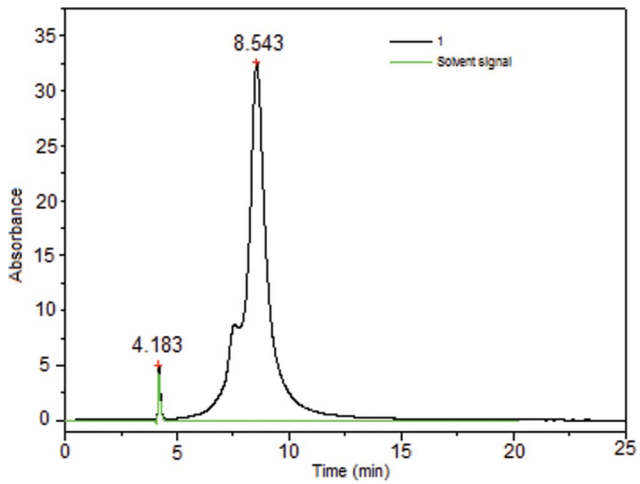

Fig. 3 HPLC profile of **1** (solvent system: 50/50 $CHCl_3$/THF, flow rate: 0.8 ml min^{-1}, injection volume: 10 µL, λ: 695 nm).

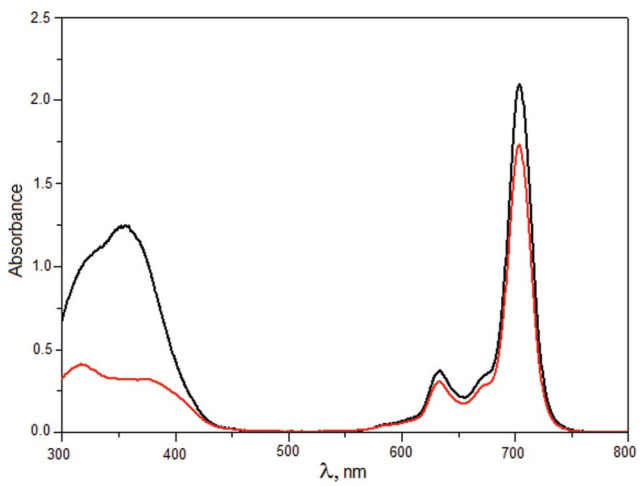

Fig. 4 Electronic absorption data of **1** (black) and **7** (red) in DMSO (10 µM).

conjugation. **1** is monomeric in DMSO as evidenced by the peak sharpness.

The n-octanol/water partition coefficient,[26] a parameter reflecting the amphiphilicity of pharmaceuticals, showed a much more important hydrophobicity of the conjugate **1** compared to the starting phthalocyanine **7**. **1** was found exclusively in the octanol phase. The tetraethylene glycol spacers are not hydrophilic enough to balance the strong hydrophobicity of the phthalocyanine ring and of the aromatic chalcone moieties. Thus, further biological experiments (evaluation of phototoxic and antivascular efficiency) will have to be conducted after formulation of the conjugate, a method widely employed with photosensitising phthalocyanines,[27] or incorporation in delivering nanoparticles.[28–30]

As a conclusion, in order to produce a dual antiangiogenic/photodynamic agent, we rationally designed a phthalocyanine–chalcone conjugate. The coupling was achieved under basic conditions between a tetrahydroxylated Zn(II) phthalocyanine and the isocyanate chalcone. The resulting product was fully characterized. The vascular disrupting effect of the conjugate (with or without cleavage of the carbamate bond), its phototoxicity, cell uptake and dark cytotoxicity are now being investigated. Photophysical and photochemical features are being studied as well to precisely investigate the potential effects of the chalcone on the phthalocyanine properties. *In vivo* behaviour will be examined afterwards. These preliminary results are a key step to a new class of dual agents combining photodynamic toxicity and vascular disrupting properties. Further work will consist of designing more hydrophilic derivatives and exploring other grafting strategies.

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