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Some activities of PorphyChem illustrated by the applications of porphyrinoids in PDT, PIT and PDI

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Photodynamic therapy is an innovative approach to treat diverse cancers and diseases that involves the use of photosensitizing agents along with light of an appropriate wavelength to generate cytotoxic reactive oxygen species. Among the collection of potential dye candidates, porphyrinoids (*i.e.* porphyrins, chlorins, and phthalocyanines) are probably the most promising photosensitizers for PDT applications. This review shows the great potential of these derivatives for their industrial development in the field of health through different applications in photodynamic therapy (PDT), photoimmunotherapy (PIT), ophthalmology, dermatology and photodynamic inactivation (PDI). The purpose of this survey is also to show the new trends and evolutions in these fields.

1. Introduction

Porphyrins and related systems have been involved in a huge number of applications for photodynamic therapy (PDT), photoimmunotherapy (PIT) and photodynamic inactivation (PDI). PorphyChem is involved in the development of these technologies. Due to the evident confidentiality clauses, our company cannot detail the network of our partners and our contracts with private companies. The aim of this paper is to illustrate the various aspects of PorphyChem's activities by choosing some applications already described in the literature in the three techniques mentioned previously, in which we are mainly involved. Moreover, the purpose of this review is not to detail all the applications or potential applications but

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Benoît Habermeyer is the chief executive officer and co-founder of PorphyChem. He received his master's degree in molecular chemistry in 2008 and his PhD in 2011 from the Université de Bourgogne. His PhD research focused on the elaboration of new porphyrinoid models for artificial photosynthesis and multielectronic catalysis. New strategies of synthesis were needed to obtain the targeted trimetallic bisporphyrinic systems. Before the creation of PorphyChem in 2013, he attended a training course in management (2011–2012) for company creation at the "Institut Français de Gestion" in Dijon. During his PhD, he undertook an internship supported by a Global-COE Program grant in the laboratory of Professor Fukuzumi in Osaka, Japan.

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Roger Guilard is the president of the Scientific Advisory Board and co-founder of PorphyChem. He is a professor at the Université de Bourgogne Franche-Comté. Dr Guilard has received the "Grand Prix de l'Académie des Sciences, Prix Gaz de France" in 2001, the "Robert Burns Woodward Achievement Award in Porphyrin Chemistry" in 2010 and the "Grand Prix Emile Jungfleish de l'Académie des Sciences" in 2013. He was elected as a fellow of the European Academy of Sciences in 2011 and of the Academia Europaea in 2015. He is the author of 480 papers and reviews, has edited or co-edited 70 books on the topics of porphyrins and related molecules and has been awarded 25 patents in the area of heterocyclic chemistry and organometallic and coordination chemistry. His major contributions are both in the area of basic research and applications.

to focus on porphyrinoid technologies which illustrate the great potential of these derivatives for their industrial development in the field of health through the use of a photosensitizer (PS).

Also, this survey is based only on a few examples of applications to show their new trends and evolutions. We will mention several recent reviews and books, which give an exhaustive description of all the applications of a given field. Moreover, it is also clear that the classification of applications used in our contribution is guided by our will to identify only the main fields where the porphyrins and related systems play a major role and where significant technologies have been developed.

According to these prerequisites, we have defined five main fields of applications; these are:

- the anti-cancer agents for photodynamic therapy (PDT),
- the anti-cancer agents for photoimmunotherapy (PIT),
- the agents for photodynamic therapy (PDT) in ophthalmology,
- the agents for photodynamic therapy (PDT) in dermatology, and
- the anti-microbial agents for photodynamic inactivation (PDI).

The main macrocycles which are used to develop the diverse technologies are porphyrins, chlorins, phthalocyanines, corroles, BODIPYs and AZABODIPYs (Fig. 1). Before describing the diverse porphyrin applications, we briefly illustrate their technological bases, which allow the performances of the developed technologies.

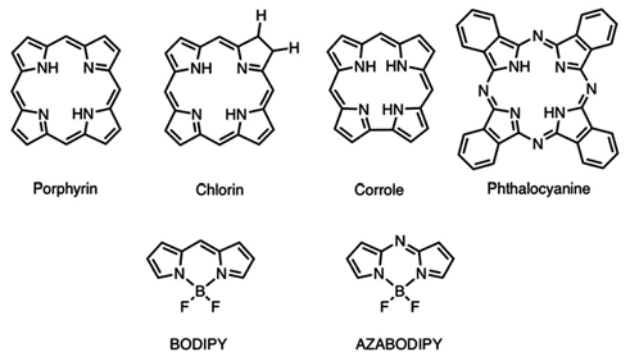


Fig. 1 Structures of porphyrinoids and BODIPYs.

2. Anti-cancer agents for photodynamic therapy

Photodynamic therapy is a technique based on biophysical principles which were proven experimentally at the beginning of the twentieth century.[1,2] For the first time, in 1978, Dougherty applied this technique to gastrointestinal cancer treatment by using hematoporphyrin (HPD).[3] The term "photodynamic therapy (PDT)" refers to tumor phototherapy (or photochemotherapy of cancer), which is a multicomponent anticancer treatment that involves the use of photosensitizing agents (PS) along with light of an appropriate wavelength to generate cytotoxic reactive oxygen species (ROS) able to kill cancer cells. The photochemical reactions in which oxygen is consumed induce the generation of the highly cytotoxic singlet oxygen ($^1O_2$) by energy transfer from the photoexcited sensitizer $^3PS^*$ to the triplet ground state of molecular oxygen ($^3O_2$). This is the so-called type II photochemical reaction. ROS formation from the type I mechanism through electron transfer increases PDT response. Singlet oxygen is responsible for the propagation of redox reactions through the oxidation of intracellular proteins inducing tumor cell killing (Fig. 2).[4] Obviously, a high quantum yield of singlet oxygen generation is needed for efficient tumor destruction but the photosensitizer must possess other properties to be clinically useful and more specifically in terms of pharmacokinetics, pharmacodynamics and toxicity. Another important parameter of the photoreaction is the cross-linking of the latent signal transducing protein STAT-3 in the cytoplasm.[5] In fact, a biomarker to measure the PDT response should correlate the STAT-3 dimerization and the *in vivo* PDT efficiency.

A huge number of reviews have been published on the chemistry, photochemical characteristics and clinical applications of porphyrinoid-based photosensitizers.[6–9] The structures of photosensitizers which are at various stages of clinical trials are shown in Table 1. A long-term follow-up of the clinical studies of these derivatives demonstrates that many para-

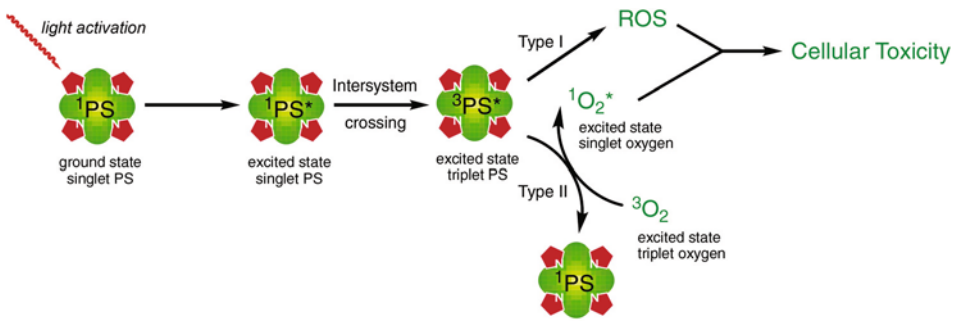


Fig. 2 The PDT reactions inspired by the Jablonski diagram.

Table 1 Photosensitizers used in PDT

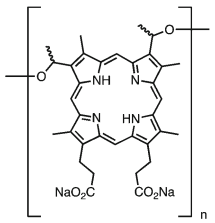
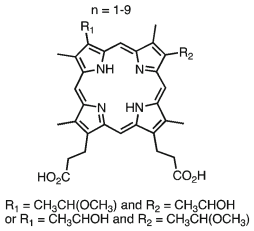
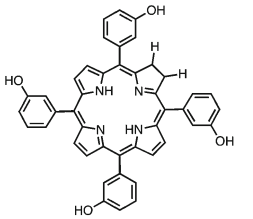
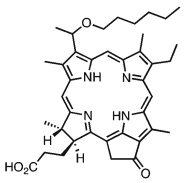
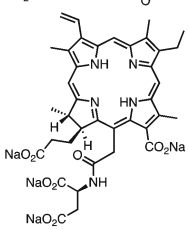
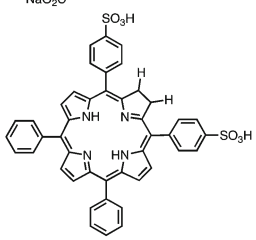
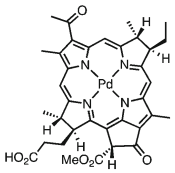
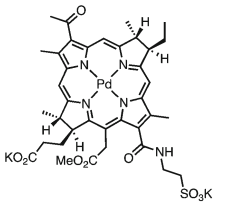
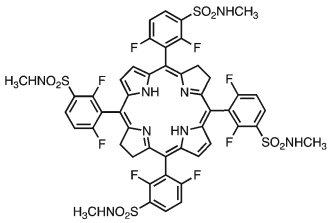
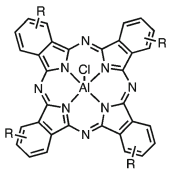
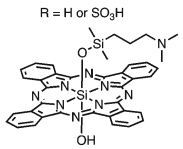
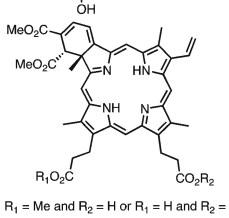
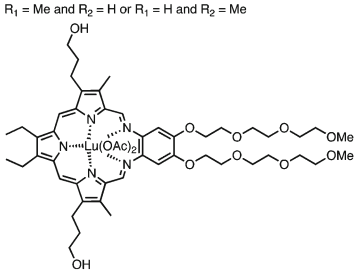
Structure	Photosensitizer	Application(s)	Company
	Porfimer sodium Trademark: Photofrin Class: Porphyrin-based photosensitizers	Approved for clinical use in Canada, Japan and USA Approved for clinical use in Russia and Brazil under the trademark Photogem Approved for clinical use in Europe under the trademark Photosan-3 Approved for bronchial cancer and esophageal cancer	Pinnacle Biologics, Inc.
	Methoxyethyl-hydroxyethyl protoporphyrin IX derivatives Trademark: Deuteporfin Class: Porphyrin-based photosensitizers	Clinical trials for cholangiocarcinoma (phase II) and bladder cancer (phase I/II)	Shanghai Fudan-Zhangjiang Bio-Pharmaceutical
	Temoporfin Trademark: Foscan Class: Chlorin-based photosensitizers	Approved for clinical use in the European Union, Norway and Iceland Approved for advanced head and neck squamous cell carcinoma	Biolitec Pharma Ltd
	3-(1-Hexyloxyethyl)-3-divinylpyropheophorbide *a* (HPPH) Trademark: Photochlor Class: Chlorin-based photosensitizers	Clinical trials for esophageal cancer (phase I/II), non-small cell lung cancer (phase II), basal cell skin cancer (phase I), treating dysplasia, carcinoma of the oral cavity, carcinoma of the oropharynx (phase I) and Barrett's esophagus (phase I/II)	Roswell Park Cancer Institute
	Mono-L-aspartylchlorin-e_6 (Npe$_6$) Trademark: Laserphyrin Class: Chlorin-based photosensitizers	Approved in Japan in 2003 to treat lung cancer Clinical trials for hepatocellular carcinoma (phase III), metastatic colorectal cancer (phase III) and benign prostatic hyperplasia or enlargement of the prostate (phase I/II)	Light Science Oncology
	Disulfonated tetraphenyl chlorin Trademark: Fimaporfin/Amphinex Class: Chlorin-based photosensitizers	Clinical trials for cholangiocarcinomas (phase I/II)	PCI Biotech
	Tin ethyl etiopurpurin Trademark: Purlytin Class: Chlorin-based photosensitizers	Clinical trials for breast adenocarcinoma, basal cell carcinoma and Kaposi's sarcoma (phase I/II)	Pharmacia
	Palladium bacteriopheophorbide *a* (WST-09) Trademark: Tookad Class: Bacteriochlorin-based photosensitizers	Approved for clinical use in the European Union, Norway and Iceland (approved for low-risk prostate cancer)	Steba Biotech

Table 1 *(Contd.)*

Structure	Photosensitizer	Application(s)	Company
	Palladium bacteriochlorin derivatives (WST-11) Trademark: Tookad-Soluble Class: Bacteriochlorin-based photosensitizers	Approved for clinical use against prostate cancer	Steba Biotech
	5,10,15,20-Tetrakis(2,6-difluoro-3-*N*-methylsulfamoylphenyl) bacteriochlorin Trademark: Redaporfin/LUZ11 Class: Bacteriochlorin-based photosensitizers	Clinical trials for head and neck cancer (phase II)	Luzitin SA
	Aluminum phthalocyanine tetrasulfonate Trademark: Photosens Class: Phthalocyanine-based photosensitizers	Approved for clinical use in Russia (stomach, skin, lip, oral cavity, tongue, breast cancer)	NIOPIK
	Silicon phthalocyanine Pc4 Class: Phthalocyanine-based photosensitizers	Clinical trials for cutaneous T-cell non-Hodgkin's lymphoma (phase I/II)	National Cancer Institute
	Benzoporphyrin derivative monoacid ring A (BPD-MA) Trademark: Visudyne/Verteporfin Class: Benzoporphyrin-based photosensitizers	Approved for age-related macular degeneration (AMD) worldwide since 2000	Novartis AG under license
	Motexafin lutetium (LuTex) Trademark: Lutrin/Antrin Class: Texaphyrin-based photosensitizers	Clinical trials for prostate cancer (phase I), age-related macular degeneration, breast cancer, cervical cancer, arterial disease	Pharmacyclics Inc.

meters play a fundamental role in observing a long-term cure. First of all, lipophilicity has a crucial role in cellular uptake and a low toxicity is needed.[10] The PDT efficiency depends also on the chemistry of the PS, the pharmaceutical formulations, the physical localization and the amount of PS in the treated tissues, the time of activation with light, the light doses and the amount of oxygen. Hence, advances in the PDT field are strongly related to the developments in the chemistry of photosensitizers, pharmaceutical formulations, and dosimetry methods and progress in lasers and medical devices such as endoscopes. Chemists devoted many efforts to improving the efficiency and selectivity of the photosensitizers to prevent the side-effects of PDT over the last few decades. The perfect photosensitizers should meet several criteria: (1) pure and stable molecule, (2) no cytotoxicity in the dark, (3) optimal absorption, distribution, metabolism and excretion (ADME) properties, (4) high molar absorption coefficient (ε) in the long wavelength region (650–800 nm) for maximum light penetration through tissue, (5) high $^1O_2$ quantum yield, (6) tumor selectivity and (7) ease of synthesis and a scalable process.[11–17]

Among the collection of potential dye candidates, porphyrinoids (*i.e.* porphyrins, chlorins, and phthalocyanines) were soon investigated as promising photosensitizers for PDT applications since they met several of the required criteria such as strong absorption properties between 650 nm and 700 nm, high triplet state quantum yield, high photostability and often

minimal toxicity. Over the last three decades, the field of PDT has been marked by the development of numerous photosensitizers that are classified as first, second and third generation photosensitizers. The preparation of Photofrin, a well-known first-generation photosensitizer, was reported in the 1980s as a mixture of dimers and oligomers obtained from hematoporphyrin dihydrochloride.[18–21] The major drawbacks of Photofrin were its structural complexity, low extinction coefficient at 630 nm, low selectivity to tumors and bad synthesis reproducibility. The 1990s were prolific in the development of the second generation of photosensitizers aiming to correct the defects of the first generation. Second generation photosensitizers were obtained as one single pure compound either by total synthesis or by hemisynthesis. These photosensitizers include purpurins, chlorins, phthalocyanines and expanded porphyrins which exhibit a high absorption wavelength around 650–800 nm. However, the second generation of photosensitizers are mostly hydrophobic and not suitable to be conjugated with monoclonal antibodies or other small targeting molecules to enhance the specific uptake by tumor cells.

The structures detailed in Table 1 show that the selected derivatives are either used as the free base or metalated derivatives, the insertion of a metal in the macrocycle core being a means to alter drastically the photochemical and photophysical properties. As an example, using a palladium complex of a bacteriochlorin, it is possible to obtain a PS (Tookad, Table 1) which exhibits long wavelength absorption near 763 nm or for the Zn complex of a methyl bacteriopheophorbide-*a* an absorption at 780 nm is obtained.[12] Recently, WST-09 (Tookad) has been replaced with WST-11, Tookad-Soluble, which is currently the photosensitizer approved for clinical use. Disulfonated tetraphenylchlorin (Amphinex, Table 1) has shown promising preclinical results in enhancing and site directing the effects of anticancer drugs in mediating photochemical internalization of bleomycin in patients with advanced or recurrent cutaneous and subcutaneous malignancies.[22] While Zn, Pd, Si and Lu complexes have been mainly used in clinical applications, indium,[23] gallium[24] and aluminum[25] derivatives of porphyrins and chlorins have also been studied. The indium complex (In(III) HPPHMe) presents an increased activity compared to its analogous derivatives due to a higher triplet quantum yield, mitochondrial localization and weak aggregation.[26] Phthalocyanines (Pc) and naphthalocyanines (Npc) have also been used in clinical applications. Their main advantages are a high singlet oxygen producing efficiency and a high absorption band in the range of 650–800 nm. Their hydrophobic character and their tendency to aggregate in solution are a limiting factor for their use in PDT even if the presence of bulky axial ligands prevents their aggregation as their lipophilicity can be altered. It should also be noted that texaphyrins which are another 22-π electron system have been used as PS (Table 1 and Fig. 3).[27–29] The metalation of a texaphyrin by Lu gave a complex exhibiting a strong absorption around 740 nm (ref. 30 and 31) which corresponds to an adequate region for tissue transparency to light and consequently should be particularly efficient for large tumors.[32,33] The drug Lutrin has been evaluated for the treatment of prostate cancer.

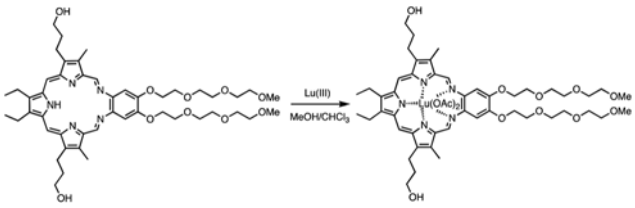


Fig. 3 Metallation of texaphyrin by Lu.

Nowadays, the way to improve the tumor selectivity and specificity of photosensitizers is to create PS conjugates by linking a molecule to the porphyrins which directs the PS to specific tumor targets. Therefore, PSs have been conjugated with various molecules to induce selective accumulation of PSs and to enhance the selectivity of drugs by directing the PSs to the right sites in the cells. These studies contributed to the development of third generation PSs where two levels of targeting can be identified: cellular targeting and subcellular targeting.[34–36] As is well known, carbohydrates play a major role in molecular recognition; the porphyrins conjugated with sugar moieties should present a better hydrophilicity but also exhibit potential membrane interactions.[37,38] Thus tumor specific drugs have been developed by targeting membrane β-galactoside proteins including various galectins. Pandey *et al.* have synthesized a series of β-galactoside conjugate PSs related to purpirinimide and the corresponding carbohydrate analogs.[39,40] These systems can target Galectin-3 which is known for its high expression in various tumors.

Peptide conjugates of PSs can also be used for tumor specific localization.[41–43] The development of compounds for tumor-imaging and therapy – so-called "multifunctional agents" – led to the use of a single agent for tumor-imaging and therapy.[44–52] As an example, 7,8-dihydro-6-hydroxymethylpterin-pyrophospho-kinase (HPPK) has been conjugated to a cyanine dye (Fig. 4). The clinical applications of such a photosensitizer including two modalities for fluorescence imaging and PDT are of major interest.[53]

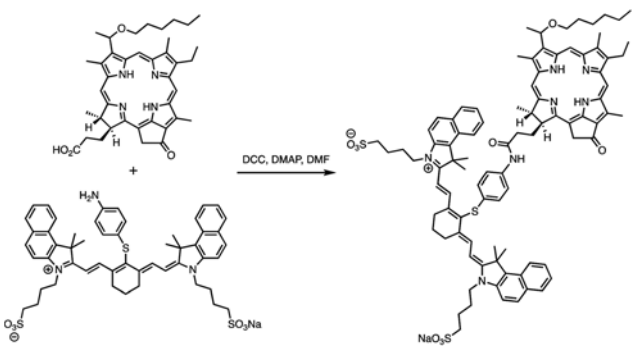


Fig. 4 Conjugation of HPPK to a cyanine dye.

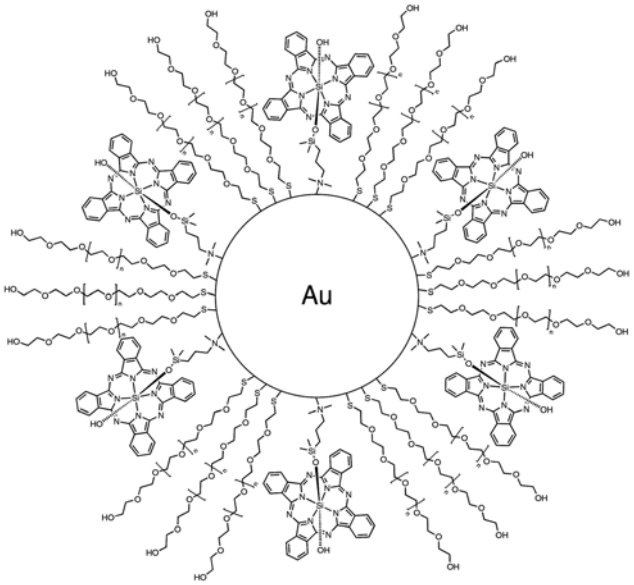


Fig. 5 Representation of the phthalocyanine–nanoparticle conjugates (adapted from ref. 66).

In parallel, conjugates for MR imaging and PDT have been developed as for nuclear imaging (SPECT/PET) and PDT.[54–58] Contrast agents and/or therapeutic drugs can also be loaded in the core of nanoparticles which can be considered as a drug delivery device.[59] Many other examples of nanoparticles were prepared and investigated in the field of PDT as nanocarriers and/or theranostic agents.[60] Nanocrystals and nanoparticles comprised of Ormosil (organically modified silica),[61] polyacrylamide[62,63] and gold[64,65] (Fig. 5) have been used.

As mentioned before, a critical problem in PDT is the depth of light penetration. An alternative technique is to use near infrared (NIR) irradiation up to 1300 nm before to get high water absorption. However, using such wavelengths singlet oxygen will be produced up to *ca.* 860 nm. By excitation of a PS in two-photon absorption processes, the used materials absorb infrared radiation and then produce visible light to excite the PS. As an example related to nanoparticles (NPs), it was needed to design photosensitizers based on photo-upconverting nanoparticles (PUNPs) as shown in Fig. 6.[67] Photon upconverting nanoparticles transform lower-energy light to higher-energy light by excitation with multiple photons. These nanoparticles absorb infrared radiation and emit visible light which further excite the photosensitizer.

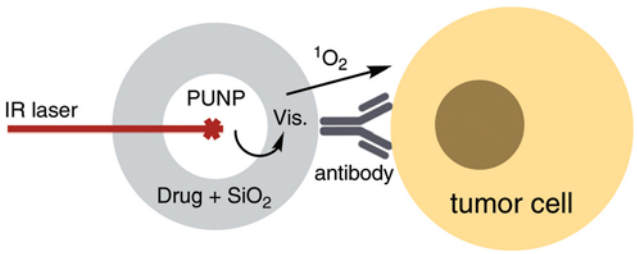


Fig. 6 Design of photon upconverting materials with multiple photons (adapted from ref. 67).

3. Anti-cancer agents for photoimmunotherapy (PIT)

As detailed in the previous section, to minimize the side effects of PDT, molecular-targeted cancer therapies have been developed. As was shown for PDT, drug delivery is a central issue and non-target effects are able to limit the dose which can be safely used. Progress has been made by employing liposomes, nano-micelles and nanoparticles to deliver more drug and make use of the enhanced permeability and retention effect (EPR) to selectively accumulate the drug. Most nanoparticles loaded with PSs can be selectively accumulated in tumors due to the EPR effect.[68] A new type of molecular-targeted therapy, photoimmunotherapy (PIT), uses diverse PSs targeted to monoclonal antibodies (mAbs). This technique allows *a priori* to increase the specificity of PSs for cancerous tissues. The main drawback of the PIT is the efficient conjugation of the photosensitizer to the antibody that is to say to develop a synthesis without the generation of multiple reactive sites or *in situ* reactive intermediates. Several reviews and papers have been dedicated to antibody–photosensitizer conjugates.[9,69–72]

The conjugation of PSs to mAbs leads to active tumor-targeting molecules able to bind antigens or receptors that are overexpressed in tumors. To date, a variety of antigens have been addressed with specific photoimmunoconjugates because several photosensitizers of the first and second generation have been conjugated with carrying antibodies; these are hematoporphyrin derivatives, chlorin e_6, pyropheophorbide, benzoporphyrin derivatives and phthalocyanines (Fig. 7).

According to the nature of the antigens, the conjugates are accumulated externally at the cell membrane or are taken up by the tumor cell. The success of mAbs in PDT thus far has been limited by the fact that normal cells are also targeted and therefore a weak toxicity is observed. The use of photoimmunoconjugates in PIT is a powerful alternative approach as the targeting step is followed by a physical activation step as a second possibility to increase the selectivity. The application of NIR light induces rapid cell death; then the presence of cellular debris activates an immune response which further aids in destroying the cancer. However, these immune cells called T cells, efficient for the destruction of the cancer, are suppressed by other immune cells called regulatory T cells (Tregs); thus it is needed to attach the PS to an antibody targeting Tregs. Finally, activated T cells from the treated tumor can travel to other sites of the tumor and induce significant responses in the tumor. This is the fundamental principle of immunotherapy which helps the immune system of the patients to recognize and eliminate cancer cells. Some companies are currently working with diverse antibodies with a view to treat several types of cancers including head and neck, eso-

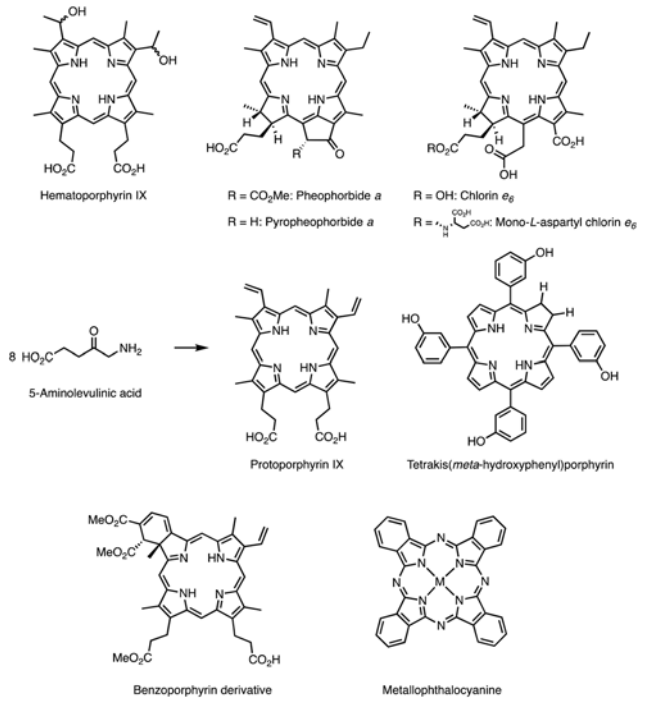


Fig. 7 Structures of PSs used for conjugation to mAbs.

phageal, lung, brain, pancreatic, colorectal, breast, prostate and ovarian and glioblastoma.

Vrouenraets *et al.* described the use of PS/mAbs about twenty years ago.[73] The selection of aluminum(III) phthalocyanine[25] as a photosensitizer was due to its hydrophilicity and inefficiency in PDT in the free form. This PS was obtained from aluminum tetra-sulfonato(phthalocyanine) which was converted into the tetra-glycine derivative. One carboxylic acid group was activated for the direct conjugation of the mAbs leading to the $AlPcS_4$–mAbs conjugate (Fig. 8).

Coupled to the internalizing murine mAbs 425, aluminum phthalocyanine shows a very high phototoxicity in contrast to the unconjugated aluminum phthalocyanine. The group of Kobayashi has compared the efficiency of two monoclonal

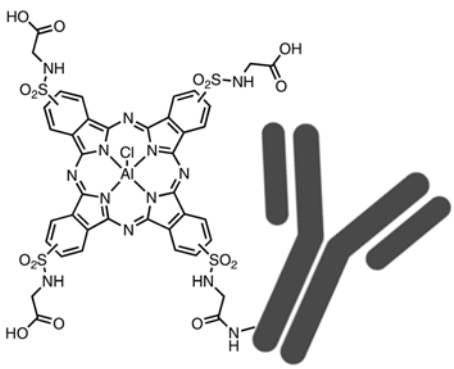


Fig. 8 Aluminum(III) phthalocyanine coupled to the internalizing murine mAbs 425 (adapted from ref. 25).

antibodies targeting the epidermal growth factor receptor (EGFR).[71] The obtained results showed that the therapeutic PIT effects *in vitro* of the IR700-conjugated cetuximab and panitumumab (cet-IR700 and pan-IR700) were identical but pan-IR700 showed superior therapeutic anti-tumor effects *in vivo* in mice models compared to cet-IR700. Thus it clearly appears that the choice of the monoclonal antibody in PS conjugates can critically influence the efficiency of PIT. More recently, Kobayashi *et al.* conjugated the water-soluble silicon phthalocyanine derivative, IRDye700DXNHS ester, with a fully human Ig anti-PSMA monoclonal antibody in a PSMA-expressing PC3 prostate cancer cell line.[74] *In vivo* high tumor accumulation and high tumor–background ratio were observed with anti-PSMA-IR700. Excellent results have been obtained for the treatment of PSMA-expressing tumors which could be translated to humans.

4. Agents for photodynamic therapy (PDT) in ophthalmology

PDT has been considered for a long time as a niche treatment in oncology and often used either as an alternative treatment method or adjuvant therapy next to conventional methods such as chemotherapy, radiotherapy and surgery.[75] Nevertheless, successful photodynamic therapy was applied in ophthalmology as a treatment for age-related macular degeneration (AMD). This technique was developed in the 1990s for the treatment of subfoveal choroidal neovascularization (CNV) in AMD. Three photosensitizing agents were mainly used in clinical trials; these are benzoporphyrin derivatives (verteporfin), tin ethylpurpurin (purlytin) and lutetium texaphyrin (Table 1).[76] These trials showed that verteporfin was the best therapeutic agent due to its absorption spectrum, lipophilic characteristics and short serum half-life.[77,78] Other factors favor the use of this PS: first the liposomal formulation of verteporfin enhances its selectivity for abnormal neovascularization, second the broad absorption spectrum at 689 nm facilitates light penetration through melanin, blood and fibrotic tissues to treat the pigmented or haemorrhagic lesions located in the choroid. The verteporfin-based PDT treatment produces vascular endothelial cell damage leading to platelet aggregation and microvascular occlusion.

The treatment of neovascular AMD rapidly changed in 2006 due to the successful anti-VGEF therapy with ranibizumab for the diverse types of subfoveal CNV lesions.[79–81] Verteporfin therapy in the treatment of neovascular AMD continues to be used for patients not responding to anti-VEGF monotherapy. A study of a verteporfin conjugated to an antibody against vascular endothelial growth factor (VEGF) shows that the conjugation did not induce the loss of photosensitizing properties and the conjugated verteporfin possesses the same efficiency as verteporfin alone to destroy cell targets.[82] A more selective treatment was observed with verteporfin conjugated to the peptide ATWLPPR (alanine–threonine–tryptophan–lysine–proline–proline–arginine) which binds the receptor for the

endothelial growth factor, VEGFR2.[83] The same PDT technique has also been used for the treatment of non-AMD choroidal neovascularization including pathological myopia, angioid streaks, ocular histoplasmosis syndrome and idiopathic CNV. Good results have been obtained for myopic CNV.[84] Today due to the replacement of verteporfin therapy with anti-VEGF therapy for the majority of patients, PDT is mainly viewed as a treatment or a potential treatment for non-AMD causes of subfoveal CNV such as central serous retinopathy[85] and various ocular tumors *i.e.* choroidal haemangioma and capillary hemangiomas.[86] Moreover, PDT offers new perspectives for the treatment of retinoblastoma which is the most common intraocular malignant tumor in childhood.[87,88]

5. Agents for photodynamic therapy (PDT) in dermatology

Compared to other therapies in dermatology, PDT is a non-invasive therapy which allows localized treatment and excellent results have been obtained with rapid recovery periods. This explains why PDT has several applications in dermatology. Although numerous therapy photosensitizers have been used in dermatology, aminolevulinic acid (ALA) and methyl aminolevulinate (MAL) can be viewed as the main photodynamic therapeutic agents in this domain (Fig. 9).[89]

5-Aminolevulinic acid (ALA) is a natural amino acid and a key intermediate in the biosynthesis of protoporphyrin IX (PPIX), which is also an excellent photosensitizer. Most cells of the human body can transform ALA and MAL into porphyrins. This knowledge has stimulated the exploitation of mechanism-based therapeutic approaches to enhance ALA-based modalities. Thus, many encouraging preclinical and clinical results have been obtained.[90] As shown above, significant differences exist in porphyrin accumulation between diverse tissues and cell types. The application of ALA or MAL to human skin leads to the preferential accumulation of porphyrins in sebaceous glands and the epidermis. The main photosensitizers used in dermatology are shown in Table 2. As neoplastic cells accumulate more porphyrins than normal cells, ALA and MAL PDT has been developed for clinical oncology applications and non-oncology applications.

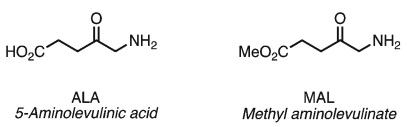


Fig. 9 Structures of ALA and MAL.

PDT has received approval for the treatment of superficial skin cancers and precancerous conditions such as actinic keratoses (AKs), Bowen's disease and superficial basal cell carcinoma (BCC). ALA is marketed as Levulan (DUSA Pharmaceuticals Inc., Toronto, Canada) as a major PDT agent for many clinical indications and ALA-methyl ester (Metvix) is marketed by Photocure ASA (Oslo, Norway) for basal cell carcinoma (BCC) and diverse skin lesions. Other ALA-esters have been registered by Photocure as Benvix (Benzyl-ALA) and Hexvix (Hexyl-ALA). The conversion of Hexyl-ALA into protoporphyrin IX is more efficient than that of Levulan. Metvix is approved in France and in several other countries while Levulan is only approved in the US and Canada.[91–94]

Today ALA and MAL PDT for clinical oncology applications continues to be mainly dedicated to the treatment of actinic keratoses (AKs), superficial basal cell carcinoma (BCC) and Bowen's disease. AK PDT should be used for individual lesions but this technique is also applied on facial areas corresponding to multiple ill-defined AKs. This is also a means to treat subclinical precancerous lesions. For the treatment of basal cell carcinoma (BCC), its long-term efficiency has not been proved. ALA and MAL PDT induces good clinical responses on Bowen's disease. Due to a certain extensive scarring caused by the surgery approach, PDT should be one of the best clinical indications for Bowen's disease.

The potentialities of ALA and MAL PDT have also been confirmed for many non-oncological dermatological diseases such as acne vulgaris, psoriasis, viral warts, localized scleroderma and photoaging. It has to be noted that the protocols of PDT in inflammatory cutaneous conditions differ from those used for tumor treatment. The treatment of tumors is mainly directed towards cellular destruction while the modulation of

Table 2 Photosensitizers used in dermatology

Photosensitizer	Trademark	λ_{max} (nm) (ε_{max} (M^{-1} cm^{-1}))	ϕ_Δ	Application(s)
Porfimer sodium	Photofrin	632 (3000)	0.89	Kaposi's sarcoma
5-Aminolevulinic acid (ALA)	Levulan	632 (5000)	0.56	Actinic keratosis (USA 1999)
Methyl aminolevulinate (MAL)	Metvix	—	—	Actinic keratosis (USA 2004)
Tin ethyl etiopurpurin	Purlytin	664 (30 000)	—	Clinical trials – basal cell carcinoma, Kaposi's sarcoma
3-(1-Hexyloxyethyl)-3-divinylpyropheophorbide *a* (HPPH)	Photochlor	665 (47 000)	—	Clinical trials – basal cell carcinoma
Aluminum phthalocyanine tetrasulfonate	Photosens	676 (200 000)	0.38	Skin (Russia 2001)
Silicon phthalocyanine	—	675 (200 000)	—	Clinical trials – actinic keratosis, Bowen's disease, skin cancer, mycosis fungoides

cellular function is the major objective of PDT in inflammatory dermatosis. For the treatment of acne, the mechanism should proceed through the preferential targeting of sebaceous glands inducing *Propionibacterium acnes* reduction. Photoaging is characterized by the increase of skin elastosis associated with degraded collagen. The mechanism involved in ALA photodynamic therapy for the treatment of photoaging is not well defined but increased collagen synthesis has been proved following ALA PDT. Although this treatment of photoaging is widely used by dermatologists, no follow-up is available.

We have shown the main role of ALA and MAL in PDT treatment in dermatology but many porphyrinoid compounds have received approval from the US FDA and regulatory authorities in other countries.[95,96] First of all, endogenous protoporphyrin IX induced by exogenous ALA (Levulan) was approved by the US FDA for the non-oncologic PDT treatment of actinic keratosis in 1999. Second Photofrin which absorbs light weakly at 632 nm but gives a high singlet oxygen quantum yield (Φ_Δ = 0.89) has been approved for skin treatment.[89,97] At 632 nm, the short effective penetration of light (2–3 mm) allows the limitation of the treatment to surface tumors. The use of this systemic PDT (Porfimer sodium) showed efficiency in the treatment of the superficial and nodular lesions of Kaposi's sarcoma. Other porphyrinic and related derivatives have been studied as potential candidates for PDT treatment in dermatology. *meta*-Tetrakis(hydroxyphenyl)porphyrin (*m*-THPP) causes skin phototoxicity and 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin ($TPPS_4$, Fig. 10) should be a potential candidate for treating basal cell carcinoma.[98]

Purlytin (SnET2, Table 1) has been evaluated in phase I and II trials for the treatment of basal cell carcinoma and Kaposi's sarcoma. Purlytin is activated at 664 nm and has deeper light penetration than Photofrin.[99] Pheophorbide (Photochlor, Table 1) has been approved for use in clinical trials and phase I trials for basal cell skin cancer. Only metallophthalocyanines have been used for PDT treatment because transition metals prevent aggregation due to the presence of axial ligands. Aluminum phthalocyanine tetrasulfonate (Photosens, Table 1) has been used in Russia to treat skin diseases. Silicon phthalocyanine which absorbs at 675 nm has been used for the treatment of actinic keratosis, Bowen's disease and skin cancer. Phase I trials have been completed.

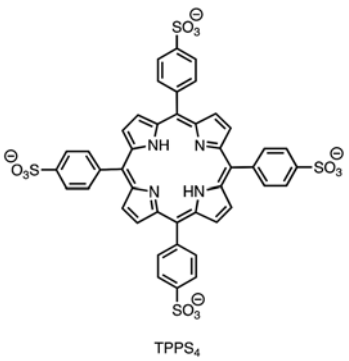


Fig. 10 Structure of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$).

6. Anti-microbial agents for photodynamic inactivation

Today it is well known that bacteria, viruses and parasites present in the hospital environment are liable to provoke nosocomial infections. Moreover, antibiotics have lost some of their efficiency to kill bacteria during the last few years. Several factors can explain the current antibiotic resistance such as the dose and duration of antibiotic treatment, the availability of antibiotics without prescription, the presence of antibiotics in the environment, *etc*. The study of multidrug-resistant (MDR) bacteria strains is a key health challenge and many laboratories around the world have developed research programs. A promising and innovative approach to prevent and treat infections is PDT which combines nontoxic dyes with harmless visible light to produce reactive oxygen species that can selectively kill microbial cells under sublethal PDT conditions.

The two main classes of bacteria (Gram-positive and Gram-negative including *Staphylococcus aureus*) are defined by their response to Gram stain. As an example, the more significant difference in susceptibility to antibacterial photodynamic treatment between Gram-positive and Gram-negative bacteria results from the differences in the organization of their outer membrane structures. The cell wall of Gram-positive bacteria is formed of thick porous layers of peptidoglycan embedded with proteins and lipoteichoic acid which facilitate the binding of cationic agents. By contrast the composition of the cell wall of Gram-negative bacteria gives these species a more pronounced negative charge limiting the penetration of anionic and lipophilic PSs. The addition of a permeabilizing agent is required to improve the efficiency of the photodynamic treatment even if direct photodynamic treatment of Gram-negative bacteria is also possible. In the case of microbial cells growing in biofilms, eradication of bacteria is more difficult due to numerous factors including the genetic diversity and the nature of the biofilm matrix. Many photosensitizers in the porphyrin and phthalocyanine series have been used to develop antimicrobial photodynamic inactivation (PDI) as an alternative to more conventional methods of inactivation of microorganisms.[100]

Consequently, numerous porphyrin-based antimicrobial materials have been studied as potential PDI agents. As already shown for PDT, rational substitution of the macrocycle improves the properties of PSs as the development of tailored drug formulations is needed. The photodynamic antimicrobial activity of numerous porphyrins and related systems against Gram-positive and Gram-negative bacteria has been studied.[101] Two decades ago the bactericide properties of protoporphyrin IX (PPIX) were demonstrated *in vitro* and gallium(III)(PPIX) has the capacity to block genococcal infection in a murine vaginal model. Porphyrin complexes of ytterbium, zirconium, nickel, copper and zinc have also been studied. Hou *et al.*[102,103] have

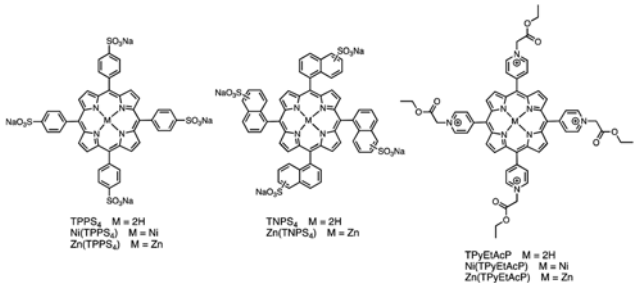


Fig. 11 Structures of diverse water-soluble *meso*-substituted porphyrins and their complexes studied as PSs for PDI.

compared the antibacterial effect of two free bases (H_2TMP: 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin and H_2TTP: 5,10,15,20-tetrakis(4-tolyl)porphyrin) and their corresponding Yb^{3+} porphyrinato complexes against *S. aureus* and *E. coli*. The tested antibacterial activities showed that both Yb^{3+} metalloporphyrins have the highest antibacterial activity at the cellular and sub-cellular levels using microcalorimetry. This result has been attributed to an inhibitory effect of the cationic porphyrin complex compared with the free base porphyrins. Zirconium(IV) porphyrins[104] and Ni(II), Cu(II) and Zn(II) complexes were also tested[105,106] but moderate antibacterial activities were observed.

More recently the design of an efficient PS for PDI treatment has been defined. Zoltan *et al.* have studied a series of water soluble *meso*-substituted porphyrins and their complexes by varying the nature of the metal ions, the charge of the complexes and the type of *meso*-substituent (Fig. 11).[107] 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$) and its Ni and Zn complexes were observed to result in greater production of ROS and showed higher antibacterial activities against *E. coli* among the three series of derivatives shown in Fig. 11. However, as mentioned above, a positive charge on the PS allows it to bind and to penetrate the microbial permeability barrier. This explains why a series of derivatives of 5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphyrin (T_4MPyP) and their analogues bearing one *N*-alkyl substituent with an increasing number of carbon atoms have been studied (Fig. 12).[108] The

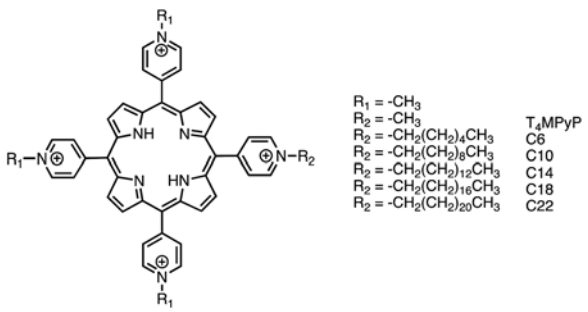


Fig. 12 Structure of a series of derivatives of 5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphyrin (T_4MPyP) and their analogues bearing an *N*-alkyl group with an increasing number of carbon atoms.

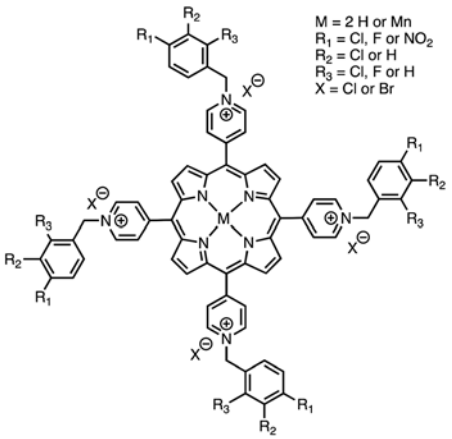


Fig. 13 Structures of a series of quaternary ammonium cationic derivatives of tetrapyridylporphyrin derivatives bearing various electron-withdrawing groups.

substituent linked to the nitrogen atoms is either a methyl group or a hydrocarbon chain ranging from C_6 to C_{22}. The best PDI activity against *S. aureus* and *E coli* was observed for the derivatives where the hydrocarbon chains are C_{14} and C_{18}.

Another series of quaternary ammonium cationic derivatives of tetra(pyridyl)porphyrin derivatives was studied to evaluate the electron-withdrawing effects of the functional groups on their *in vitro* activities against *S. aureus*, *E. coli* and *P. aeruginosa*. It was clearly shown that the antimicrobial activity decreases when the electron-withdrawing strength increases (Fig. 13).[109]

Reddi *et al.* described the antibacterial activity of a water-soluble PS resulting from the conjugation of the anionic 5-(4′-carboxyphenyl)-10,15,20-(triphenyl)porphyrin (cTPP) to the peptide apidaecin (Fig. 14).[110,111] The efficiency of this conjugated PS was proved to be capable of eradicating both Gram-positive and Gram-negative bacteria at very low concentrations. The conjugated PS was clearly more efficient than the corresponding free PS. This work also demonstrated that the conjugation of a non-efficient hydrophobic PS to a cationic peptide gives an efficient PS against Gram-negative bacteria.

Reddi *et al.* have also studied cationic or neutral porphyrins conjugated with other cationic antimicrobial peptides such as

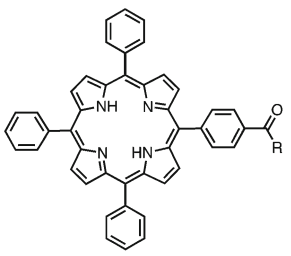


Fig. 14 Structure of 5-(4′-carboxyphenyl)-10,15,20 triphenylporphyrin (cTPP) conjugated to the peptide apidaecin.

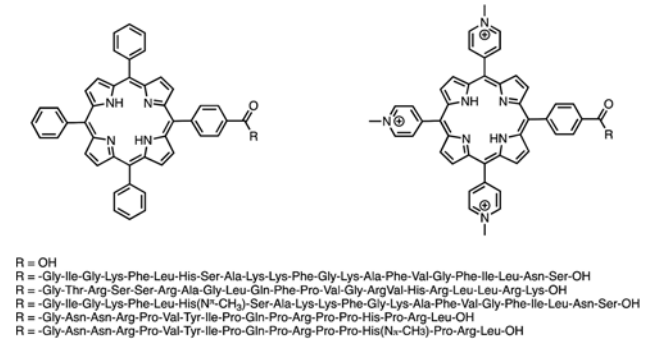


Fig. 15 Structures of porphyrins conjugated to various cationic antimicrobial peptides.

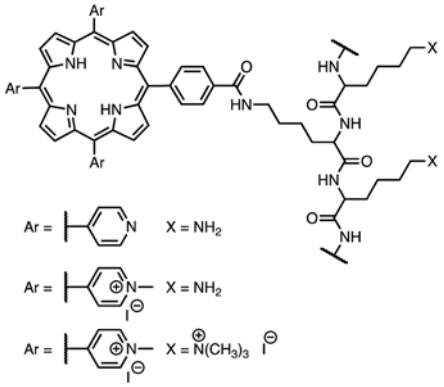


Fig. 16 Structures of *meso*-tetrasubstituted porphyrins conjugated to polylysine.

buforin or magainin and have proved that the phototoxic activity depends critically on the nature of the cationic peptides (Fig. 15).[111] This study also demonstrated that the conjugation allows PSs to be carried inside mammalian cells. Recent work of Sol and Frochot also described the conjugation of polymyxin B to a cationic porphyrin.[112,113] This photobactericidal organic material proved to be efficient against Gram-positive and Gram-negative bacteria.[114]

Phthalocyanines[115] and BODIPY[116] derivatives have also been used as potential PDI agents. PSs with cationic *meso* substituents and water-soluble zinc phthalocyanines are currently used to efficiently eradicate Gram-negative bacteria.[117] Cationic BODIPY dyes have shown eradication effects on *Staphylococcus xylosus* and *E. coli* but BODIPY dyes have the tendency to form aggregates in solution. More studies are needed to obtain efficient PDI treatments using BODIPY dyes. Some remarkable examples of PDI are those described by Lindsey, Hambin *et al.* by using three stable synthetic monosubstituted cationic bacteriochlorins. All three bacteriochlorins bearing cationic quaternary ammonium groups are highly effective against both Gram-positive and Gram-negative bacteria species. The highly active photodynamic inactivation has been attributed to the amphiphilic and cationic features of the bacteriochlorins.[118]

We have shown above that conjugation with peptides of porphyrin compounds is a method which allows the use of non-water-soluble porphyrins for PDI. We have also mentioned that a major problem in PDT is the poor solubility of several PSs and their tendency to aggregate under physiological conditions. Many studies have been devoted to the combination of nanoparticles and PDI for antimicrobial applications.[119–121] The two main objectives are to improve the binding of PSs with microbial cells but also the kinetics of microbial photoinactivation.

As already mentioned above polymer immobilization of PSs proceeds through the functionalization of porphyrins and more specifically of non-water-soluble porphyrin derivatives. As an example, conjugates of poly-*S* lysine with neutral and cationic *meso*-tetrasubstituted porphyrins (Fig. 16) are able to photoinactivate Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*).[122] The results clearly demonstrate that conjugation with polylysine highly increases the activity of the PSs.

Electrospun nanofibers doped with PSs have been used in many biological applications such as wound dressing and water disinfection. Krausz *et al.* were the first to prepare a photobactericidal cotton fabric with grafted *meso*-arylporphyrins (Fig. 17).[123]

Carbon nanotubes (CNTs) after coating with antimicrobial molecules have shown strong antibacterial properties against Gram-positive bacteria.[124] Graphene and graphene oxide which are 2D sheets have also been used to solubilize PSs. Their ability to solubilize PSs such as Photochlor between the sheets is due to π–π stacking.[125] Cellulose which is a natural biopolymer is a remarkable material to prepare innovative biomaterials. Cellulose nanocrystals (CNC) have been covalently attached to a porphyrin (Fig. 18) which has a broad spectrum of antimicrobial activity.

Silica nanoparticles (SiNPs) can also serve as carriers for PDI[126] but the interest in using magnetic nanoparticles (MNPs) to allow the separation of the captured bacteria from the contaminated sites using a magnet should be pointed out. These MNPs result from the conjugation of a PS (5,15-bis-phenyl-10,20-bis(4-methoxycarbonylphenyl)porphyrin platinum, t-PtCP) with Fe_3O_4.[127]

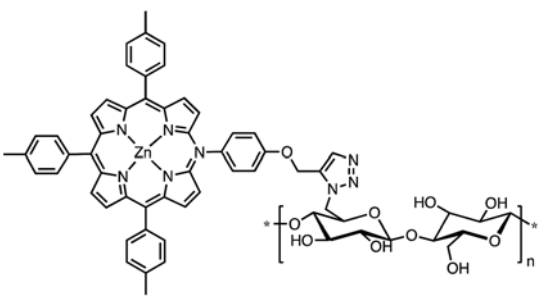


Fig. 17 Photobactericidal cotton fabric with porphyrinic moiety using click chemistry.

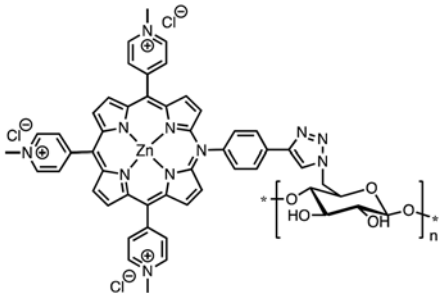


Fig. 18 Structure of porphyrin grafted to cellulose nanocrystals.

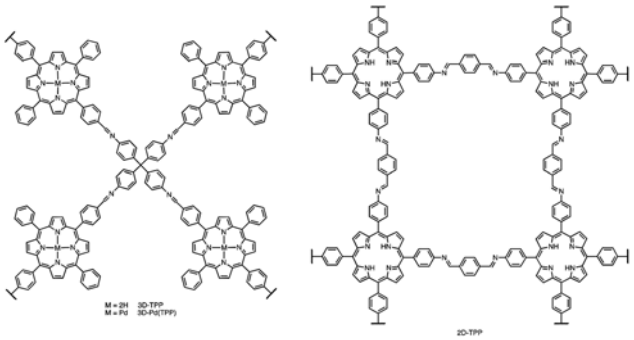


Fig. 19 Porphyrinic covalent organic frameworks for the photoinactivation of bacteria.

The intercalation of porphyrins and phthalocyanines in layered hydroxides leads to hybrid materials where the macrocycle molecules remain in their photoactive form. These are biomaterials which present great potentialities for PDI.[128] Very recently covalent organic frameworks for the photodynamic inactivation of bacteria have been described. These materials with 2D and 3D topology should be adequate for the design of antibacterial coatings for several PDI applications (Fig. 19).[129]

7. Conclusions

This non-exhaustive survey of the applications of porphyrins and related systems in PDT, PIT and PDI, which correspond to 50% of our activity show that new paradigms and strategies should be focused in four main directions.

Currently porphyrinoid sensitizers present several advantages over non-porphyrin sensitizers but further research is required to modify these chromogens in order to adapt their photophysical properties to a given application; it should also be remembered that the ideal photosensitizer must be synthesized according a perfect and reproducible protocol without residual chemicals and compounds from the synthesis, have absorption properties adapted for deeper tissue penetration and of course show efficiency in clinical trials. It is clear that the up-scaling of the synthesis has been achieved for all the promising porphyrinic platforms of cancer-targeting therapy. Most of the precursors are A_4 or A_3B type molecules. The purification and the up-scaling of the corresponding phthalocyanine series were difficult to achieve but recently became much easier due to the use of new techniques of purification and the elaboration of new routes of univocal synthesis. This is a major part of our activity.

The second direction is the PS delivery system which is not only a key parameter for PDT but also for PDI. The drug delivery has already been investigated for a long time but it is only during the last decade that smart targeting and release systems have been described. PS delivery *via* polymer immobilization, membranes, hydrogels, nanofibers, thin films and other supports continues to face challenges and difficulties centered on the stability of the hybrid materials and the incorporated targeting mechanisms.

The third direction is to improve the conjugation reaction of the PS with the support and the development of new drug delivery systems as new molecular targets are identified. As an example, nanofabrication technologies have been very efficient since the size of NPs determines their bio-distribution. PIT enhances delivery of nanosized reagents and should improve therapeutic responses through the increase in vascular permeability.

This article does not detail the key results recently obtained in the corrole series.[130] It has been shown that the conjugation of corroles with targeting molecules leads to remarkable anticancer activity. Biological applications of asymmetric porphycene derivatives are also not described.[131] In PDT the fine-tuning of the porphycene macrocycle leads to the improvement of the solubility and photosensitization of reactive oxygen species as a clear alteration of cellular uptake profiles and subcellular distribution. Their antimicrobial activity is also remarkable when linked to an antibiotic.[132,133] Both series will soon be joint key representatives of the cancer chemotherapeutic arsenal but the up-scaling of the synthesis of these promising derivatives is challenging.

Conflicts of interest

There are no conflicts to declare.

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