Photodynamic Therapy and the Development of Metal-Based Photosensitisers

Leanne B. Josefsen and Ross W. Boyle

Department of Chemistry, The University of Hull, Kingston-upon-Hull HU6 7RX, UK

Correspondence should be addressed to Ross W. Boyle, r.w.boyle@hull.ac.uk

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Photodynamic therapy (PDT) is a treatment modality that has been used in the successful treatment of a number of diseases and disorders, including age-related macular degeneration (AMD), psoriasis, and certain cancers. PDT uses a combination of a selectively localised light-sensitive drug (known as a photosensitiser) and light of an appropriate wavelength. The light-activated form of the drug reacts with molecular oxygen to produce reactive oxygen species (ROS) and radicals; in a biological environment these toxic species can interact with cellular constituents causing biochemical disruption to the cell. If the homeostasis of the cell is altered significantly then the cell enters the process of cell death. The first photosensitiser to gain regulatory approval for clinical PDT was Photofrin. Unfortunately, Photofrin has a number of associated disadvantages, particularly pro-longed patient photosensitivity. To try and overcome these disadvantages second and third generation photosensitisers have been developed and investigated. This Review highlights the key photosensitisers investigated, with particular attention paid to the metallated and non-metallated cyclic tetrapyrrolic derivatives that have been studied in vitro and in vivo; those which have entered clinical trials; and those that are currently in use in the clinic for PDT.

1. PHOTODYNAMIC THERAPY: BACKGROUND

The use of light in the treatment of disease has been known for many centuries and can be traced back over 4000 years to the ancient Egyptians [1]. The Egyptian people used a combination of the orally ingested Amni Majus plant and sunlight to successfully manage vitiligo: a skin disorder of unknown cause. The active ingredient of this plant (psoralen, Figure 1) is now successfully employed in the worldwide treatment of psoriasis [1–4].

Photodynamic therapy (PDT) is a treatment modality that has been used in the successful treatment of a number of diseases and disorders, including age-related macular degeneration (AMD), psoriasis, and certain cancers. PDT uses a combination of a selectively localised light-sensitive drug (known as a photosensitiser) and light of an appropriate wavelength. The light-activated form of the drug reacts with molecular oxygen to produce reactive oxygen species (ROS) and radicals; in a biological environment these toxic species can interact with cellular constituents causing biochemical disruption to the cell. If the homeostasis of the cell is altered significantly then the cell enters the process of cell death. The first photosensitiser to gain regulatory approval for clinical PDT was Photofrin. Unfortunately, Photofrin has a number of associated disadvantages, particularly pro-longed patient photosensitivity. To try and overcome these disadvantages second and third generation photosensitisers have been developed and investigated. This Review highlights the key photosensitisers investigated, with particular attention paid to the metallated and non-metallated cyclic tetrapyrrolic derivatives that have been studied in vitro and in vivo; those which have entered clinical trials; and those that are currently in use in the clinic for PDT.

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Figure 1: Structure of psoralen.
2. HISTORY OF PHOTODYNAMIC THERAPY

Reports of contemporary photodynamic therapy came first in the investigations led by Finsen in the late nineteenth century [8]. Finsen successfully demonstrated phototherapy by employing heat-filtered light from a carbon-arc lamp (the “Finsen lamp”) in the treatment of a tubercular condition of the skin known as *lupus vulgaris*, for which he won the Nobel Prize in Physiology or Medicine in 1903 [8]. But it was not until the early twentieth century that reports of photodynamic therapy for the treatment of cancer patients (with solid tumours) were made by von Tappeiner’s group in Munich [2, 4, 6, 9]. In 1913 another German scientist, Meyer-Betz, described the major stumbling block of photodynamic therapy. After injecting himself with haematoporphyrin (Hp, a photosensitiser), he swiftly experienced a general skin sensitivity upon exposure to sunlight—a problem still persistent with many of today’s photosensitisers [2, 3, 7, 18].

Further studies, investigating the accumulation of haematoporphyrin and the purified haematoporphyrin derivative (HpD) in tumours, culminated in the late 1980s with the photosensitiser Photofrin (Figure 3). A photosensitiser which, after further purification, was first given approval in 1993 by the Canadian health agency for use against bladder cancer and later in Japan, USA and parts of Europe for use against certain cancers of the oesophagus and non-small cell lung cancer [3–9, 17, 18, 21, 22].

Photofrin was far from ideal and carried with it the disadvantages of prolonged patient photosensitivity and a weak long-wavelength absorption (630 nm) [6, 7, 21]. This led to the development of improved (second generation) photosensitisers, including Verteporfin (a benzoporphyrin derivative, also known as Visudyne) and more recently, third generation photosensitisers based around targeting strategies, such as antibody-directed photosensitisers [4, 5, 7, 18, 19, 23–25].

3. CYCLIC TETRA PYRROLYLIC CHROMOPHORES AND PHOTOSENSITISERS

Cyclic tetapyrrolic molecules are good examples of fluorophores (see Glossary) and photosensitisers. Photosensitisers are molecules, which, when excited by light energy, can utilise the energy to induce photochemical reactions to
Apoptosis: a form of programmed cell death (often referred to as cell suicide) in response to key physiological cues and intracellular damage. In apoptosis, the orchestrated collapse of a cell is characterised by key morphological and biochemical changes followed by rapid engulfment of cellular remains by neighbouring cells (phagocytosis). These key changes include maintenance of adenosine triphosphate (ATP) levels, caspase activation, membrane blebbing, cell shrinkage, chromatin condensation, DNA fragmentation, and the formation of small intact fragments (apoptotic bodies). Apoptosis can be distinguished from necrotic cell death by the distinct absence of an associated inflammatory response.

Necrosis: an uncontrolled form of cell death. A less ordered process than apoptosis and usually in response to injurious agents such as infection, physical injury, ischemia (deficiency of oxygenated blood), or excessive accumulation of ROS. In contrast to apoptosis, a local inflammatory response is normally observed as a result of cellular debris released directly into surrounding tissue.

Box 1

produce lethal toxic agents. In a cellular environment, these agents (reactive oxygen species (ROS) and radicals) ultimately result in cell death and tissue destruction (Figure 4) [5–9]. Photosensitisers are absorbed into cells all over the body and alone are harmless, that is, in the absence of light, and usually oxygen they have no effect on healthy or abnormal tissue. Ideally, they should be retained by diseased tissue, particularly tumours, for longer periods of time in comparison to healthy tissue; thus it is important to carefully time light exposure and ensure that activation only occurs when the ratio of photosensitiser is greater in diseased tissue than in healthy tissue, thereby minimising unwanted damage to surrounding non-cancerous cells [3, 19].

Photosensitisers also have alternative applications. They have been employed in the sterilisation of blood plasma and water in order to remove blood-borne viruses and microbes and have been considered for agricultural uses, including herbicides and insecticides [5, 9, 26–28].

4. PHOTOCHEMISTRY: PHOTOCHEMICAL PROCESSES

Only when a photosensitiser is in its excited state (3Psen*) can it interact with molecular oxygen (3O2) and produce radicals and activated oxygen species (ROS), crucial to the Type II mechanism which is thought to predominate in PDT (see below). These species include singlet oxygen (1O2), hydroxyl radicals (•OH), and superoxide (O2•−) ions and can interact with cellular components including unsaturated lipids; amino acid residues; and nucleic acids. If sufficient oxidative damage ensues, this will result in target-cell death (only within the immediate area of light illumination).

5. PHOTOCHEMICAL MECHANISMS

When a chromophore, such as a cyclic tetrapyrrolic molecule, absorbs a photon of electromagnetic radiation in the form of light energy, an electron is promoted into a higher-energy molecular orbital, elevating the chromophore from the ground state (S0) into a short-lived, electronically excited state (S*) composed of a number of vibrational sub-levels (S0). The excited chromophore can lose energy by rapidly decaying through these sub-levels via internal conversion (IC) to populate the first excited singlet state (S1), before quickly relaxing back to the ground state (Figure 5).

The decay from the excited singlet state (S1) to the ground state (S0) is via fluorescence (S1 → S0). Singlet state lifetimes of excited fluorophores are very short (τFl = 10−9–10−6 seconds) since transitions between the same spin states (S → S or T → T) conserve the spin multiplicity of the electron and, according to the Spin Selection Rules, are therefore considered “allowed” transitions [3, 6, 8]. Alternatively, an excited singlet state electron (S1) can undergo spin inversion and populate the lower-energy first
Quantum Yields

Photochemical quantum yields (\(\Phi\)) are one of the key measurements made in the photochemical techniques described above. According to the Stark-Einstein law:

“every photon absorbed by a species excites only one molecule of the species.” Later refined to: “one particle is excited for each quanta of radiation absorbed” (Second Law of Photochemistry).

The quantum yield can therefore be described as the number of molecules undergoing the process of interest (formation of product or consumption of reactants) for each quantum of radiation energy absorbed (equation 1):

\[
\Phi = \frac{\text{number of species undergoing process of interest}}{\text{number of photons absorbed}}
\]

Equation 1: Quantum Yields

Quantum yields may also be expressed in terms of the rate of the reaction:

\[
\Phi = \frac{\text{rate of chemical process/event}}{\text{intensity of absorbed light}} = \frac{d[x]/dt}{I_{abs}}
\]

The quantum yield(s) for a species cannot be greater than unity (\(\Phi \leq 1\)) unless a secondary reaction or a series of chain reactions are taking place within the sample, in which case the quantum yields are respectively \(\Phi \leq 2\) and \(\Phi \geq 2\).

Singlet Oxygen Quantum Yields

If the fraction of excited triplet state molecules quenched by molecular oxygen to produce singlet oxygen (\(1\Delta_g\)) is represented as \(S_3\), the quantum yield of singlet oxygen (\(\Phi_{S_3}\)) can be determined (equation 2):

\[
\Phi_{S_3} = \Phi_{S_0} S_3
\]

Equation 2: Singlet Oxygen Quantum Yield

excited triplet state (\(T_1\)) via intersystem crossing (ISC); a spin-forbidden process, since the spin of the electron is no longer conserved [29–34]. The excited electron can then undergo a second spin-forbidden inversion and depopulate the excited triplet state (\(T_1\)) by decaying back to the ground state (\(S_0\)) via phosphorescence \((T_1 \rightarrow S_0)\) [29–34]. Owing to the spin-forbidden triplet to singlet transition, the lifetime of phosphorescence \((\tau_p = 10^{-3} - 1 \text{ second})\) is considerably longer than that of fluorescence.

6. PHOTOSENSITISERS AND PHOTOCHEMISTRY

Tetrapyrrolic photosensitisers in the excited singlet state \((1\text{Psen}^*, S_0)\) are relatively efficient at undergoing intersystem crossing and can consequently have a high triplet-state quantum yield, Box 2 (\(\Phi_T 0.62\) (tetraphenylporphyrin (TPP), methanol)), 0.83 (etiopurpurin, benzene), 0.71 (tetrasulphonated TPP, D2O), and 0.47 (tetrasulphonated zinc phthalocyanine, methanol)) [8, 35, 36]. The longer
lifetime of this species is sufficient to allow the excited triplet state photosensitiser to interact with surrounding biomolecules, including cell membrane constituents [5, 17].

7. PHOTOCHEMICAL REACTIONS

Excited triplet-state photosensitisers can react in two ways defined as Type-I and Type-II processes. Type-I processes can involve the excited singlet or triplet photosensitiser (1Psen*, S1; 3Psen*, T1), however due to the short lifetime of the excited singlet state, the photosensitiser can only react if it is intimately associated with a substrate, in both cases the interaction is with readily oxidisable or reducable substrates. Type-II processes involve the direct interaction of the excited triplet photosensitiser (3Psen*, T1) with molecular oxygen (O2, 3Σg) [5–8, 17, 18, 37].

Type-I processes can be divided into two further mechanisms; Type I(i) and Type I(ii). The first of these mechanisms (i) involves the transfer of an electron (oxidation) from a substrate molecule to the excited state photosensitiser (Psen*), generating a photosensitiser radical anion (Psen*) and a substrate radical cation (Subs+). The majority of the radicals produced from Type-I(i) reactions react instantaneously with oxygen, generating a complex mixture of oxygen intermediates. For example, the photosensitiser radical anion can react instantaneously with molecular oxygen (O2) to generate a superoxide radical anion (O2−•), which can go on to produce the highly reactive hydroxyl radical (OH•, Figure 6), initiating a cascade of cytotoxic free radicals; this process is common in the oxidative damage to biomolecules, including amino acid residues in proteins, such as tryptophan; unsaturated lipids like cholesterol and nucleic acid bases, particularly guanosine and guanine derivatives (Box 3) with the latter base more susceptible to ROS [2, 5, 8, 17, 36, 42–45]. These interactions cause damage and potential destruction to cellular membranes and enzyme deactivation, culminating in cell death [8].

The highly-reactive oxygen species (1O2) produced via the Type-II process act near to their site of generation and within a radius of action of approximately 20 nm, with a typical lifetime of approximately 40 nanoseconds in biological systems [2, 7, 17]. However, it has recently been suggested that (over a 6 microsecond period) singlet oxygen can diffuse up to approximately 300 nm in vivo [39–41]. Singlet oxygen can theoretically only interact with proximal molecules and structures within this radius [17]. ROS are known to initiate a large number of reactions with biomolecules, including amino acid residues in proteins, particularly guanosine and guanine derivatives (Box 3) with the latter base more susceptible to ROS [2, 5, 8, 17, 36, 42–45]. It is highly probable that in the presence of molecular oxygen, and as a direct result of the photoradiation of the photosensitiser molecule, both Type-I and II pathways play a pivotal role in disrupting cellular mechanisms and cellular structure. Nevertheless, there is considerable evidence to suggest that the Type-II photo-oxygenation process predominates in the induction of cell damage, a consequence of the interaction between the irradiated photosensitiser and molecular oxygen [2, 5, 8, 18, 43, 46]. It has been suggested, however, that cells in vivo are partially protected against the effects of photodynamic therapy by the presence of singlet oxygen scavengers (such as histidine) and that certain skin cells are somewhat resistant to photodynamic therapy in the absence of molecular oxygen; further supporting
Typical Singlet Oxygen Reactions

Singlet molecular oxygen is known to react with systems by attacking electron rich double bonds, in an “-ene” type reaction, to form peroxides or hydroperoxides.

\[ h\nu, \text{Psen, } O_2 \rightarrow \text{peroxides or hydroperoxides} \]

\[ h\nu, \text{Psen, } O_2 \rightarrow \text{Guanosine} \]

\[ h\nu, \text{Psen, } O_2 \rightarrow \text{Cholesterol} \]

**Box 3**

![Energy diagram](image)

**Figure 9:** Molecular orbital diagram of oxygen \( (^1\Sigma_g, ^1\Delta_g, \text{and } ^3\Sigma_g) \).

The efficiency of Type-II processes is dependent upon the triplet state lifetime \( \tau_T \) (see Glossary, under luminescence life time) and the triplet quantum yield \( \Phi_T \) of the photosensitiser. Both of these parameters have been implicated in the effectiveness of a photosensitiser in phototherapeutic medicine; further supporting the distinction between Type-I and Type-II mechanisms. However, it is worthy to note that the success of a photosensitiser is not exclusively dependent upon a Type-II process taking place. There are a number of photosensitisers whose excited triplet lifetimes are too short to permit a Type-II process to occur. For example, the copper metallated octaethylbenzochlorin photosensitiser (Figure 10) has a triplet state lifetime of less
than 20 nanoseconds and is still deemed to be an efficient photodynamic agent [13, 43].

8. PHOTΟSENSITISERS—IDEAL PHOTΟSENSITISERS

Although a number of different photosensitising compounds, such as methylene blue (see Glossary), rose bengal, and acridine (Figure 11), are known to be efficient singlet oxygen generators (and therefore potential photodynamic therapy agents), a large number of photosensitisers are cyclic tetrapyroles or structural derivatives of this chromophore; in particular porphyrin, chlorin, bacteriochlorin, expanded porphyrin, and phthalocyanine (PCs) derivatives (Figure 12). This is possibly because cyclic tetrapyrrolic derivatives have an inherent similarity to the naturally occurring porphyrins present in living matter—consequently they have little or no toxicity in the absence of light [2, 5, 17, 18, 36, 44, 49].

Porphyrins are a group of naturally occurring and intensely coloured compounds, whose name is drawn from the Greek word porphura, the Greek word for purple [50, 51]. These molecules are known to be involved in a number of biologically important roles, including oxygen transport and photosynthesis, and have applications in a number of fields, ranging from fluorescence imaging to medicine [2, 5, 17, 42]. Porphyrins are classified as tetrapyrrolic molecules, with the heart of the skeleton a heterocyclic macrocycle, known as a porphine. The fundamental porphine frame consists of four pyrrolic sub-units linked on opposing sides (α-positions, numbered 1, 4, 6, 9, 11, 14, 16, and 19, Figure 13) through four methine (CH) bridges (5, 10, 15, and 20), known as the meso-carbon atoms/positions (Figure 13). The resulting conjugated planar macrocycle may be substituted at the meso- and/or β-positions (2, 3, 7, 8, 12, 13, 17, and 18): if the meso- and β-hydrogens are substituted with non-hydrogen atoms or groups, the resulting compounds are known as porphyrins.

The inner two protons of a free-base porphyrin can be removed by strong bases such as alkoxides, forming a dianionic molecule; conversely, the inner two pyrrolic nitrogens can be protonated with acids such as trifluoroacetic acid affording a dicationic intermediate (Figure 14). The
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9. PORPHYRIN ABSORPTION SPECTROSCOPY

On account of their highly conjugated skeleton, porphyrins have a characteristic ultra-violet visible (UV-VIS) spectrum (Figure 15). The spectrum typically consists of an intense, narrow absorption band ($\epsilon > 200,000 \text{ l mol}^{-1} \text{cm}^{-1}$) at around 400 nm, known as the Soret or B band, followed by four longer wavelength (450–700 nm), weaker absorptions ($\epsilon > 20,000 \text{ l mol}^{-1} \text{cm}^{-1}$) (free-base porphyrins) referred to as the Q bands [6, 17, 50, 53, 54].

The Soret band arises from a strong electronic transition from the (porphyrin) ground state to the second excited singlet state ($S_0 \rightarrow S_2$, Figure 16); whereas the Q band is a result of a weak transition to the first excited singlet state ($S_0 \rightarrow S_1$). The dissipation of energy via internal conversion (IC) is so rapid that fluorescence is only observed from depopulation of the first excited singlet state to the lower-energy ground state ($S_1 \rightarrow S_0$).

10. SECOND-GENERATION PHOTOSENSITISERS

10.1. Ideal photosensitiser properties

The key characteristic of any photodynamic sensitisier is its ability to preferentially accumulate in diseased tissue and, via the generation of cytotoxic species, induce a desired biological effect. In particular, a good photodynamic sensitisier should adhere to the following criteria:

(i) have strong absorption with a high extinction coefficient in the red/near infrared region of the electromagnetic spectrum (600–850 nm)—allows deeper tissue penetration by light [5–7, 17, 36, 43],

(ii) be effective generators of singlet oxygen and other ROS,

(iii) have suitable photophysical characteristics: a high quantum yield of triplet formation ($\Phi_T \geq 0.5$); a high singlet oxygen quantum yield ($\Phi_A \geq 0.5$); a relatively long triplet state lifetime ($\tau_T$, microsecond range); and a high triplet-state energy ($\geq 94 \text{ KJ mol}^{-1}$) [3, 8, 18, 36, 56]. To date the parameters $\Phi_T = 0.83$ and $\Phi_A = 0.65$ (haematoporphyrin); $\Phi_T = 0.83$ and $\Phi_A = 0.72$ (etiopurpurin); and $\Phi_T = 0.96$ and $\Phi_A = 0.82$ (tin etiopurpurin) have been achieved [2, 36],

(iv) have minimum dark toxicity and negligible cytotoxicity in the absence of light,

(v) exhibit greater retention in diseased/target tissue over healthy tissue,

(vi) present rapid clearance from the body,

(vii) be single, well-characterised compounds, with a known and constant composition,

(viii) have a short and high yielding synthetic route (with easy translation into multi-gram scales/reactions),

(ix) have a simple and stable drug formulation,
5-Aminolaevulinic acid (ALA) is a prodrug used in the clinic to treat and image a number of superficial cancers and tumours (see Tables 2 and 3) [5–9, 11, 17, 18]. ALA on its own is not a photosensitiser, but a key precursor in the biosynthesis of the naturally occurring porphyrin, haem (Scheme 1).

Haem is synthesised in every energy-producing cell in the body and is a key structural component of haemoglobin, myoglobin, and other haemproteins. The immediate precursor to haem is protoporphyrin IX (PPIX), an effective photosensitiser. Haem itself is not a photosensitiser, due to the coordination of a paramagnetic ion (iron; see Glossary; see also diamagnetic species) in the centre of the macrocycle, causing significant reduction in excited state lifetimes [5–9, 11].

The haem molecule is synthesised from glycine and succinyl coenzyme A (succinyl CoA). The rate-limiting step in the biosynthesis pathway is controlled by a tight (negative) feedback mechanism in which the concentration of haem regulates the production of ALA. However, this controlled feedback can be by-passed by artificially adding excess exogenous ALA to cells. The cells respond by producing PPIX (photosensitiser) at a faster rate than the ferrochelatase enzyme can convert it to haem [5–9, 11, 17, 18].

ALA, marketed as Levulan (DUSA Pharmaceuticals Incorporated, Toronto, Canada), has shown promise in
photodynamic therapy (tumours) via both intravenous and oral administration, as well as through topical administration in the treatment of malignant and non-malignant dermatological conditions, including psoriasis, Bowen’s disease, and Hirsutism (Phase II/III clinical trials, see Glossary) [5–9, 11, 18].

ALA shows a more rapid accumulation in comparison to other intravenously administered sensitisers [5–9, 11]. Typical peak tumour accumulation levels post-administration for PPIX are usually achieved within several hours; compare this with other (intravenously administered) photosensitisers which may take up to 96 hours to reach peak levels and one of the main advantages of ALA can be clearly seen. ALA is also excreted more rapidly from the body (∼24 hours) than other photosensitisers, minimising patient photosensitivity [5–8, 11].

In an attempt to overcome the poor bioavailability when ALA is applied topically, esterified ALA derivatives with improved pharmacological properties have been examined [5–8, 11]. A methyl ALA ester (Metvix) is now being marketed by Photocure ASA (Oslo, Norway) as a potential photosensitiser for basal cell carcinoma and other skin lesions [5, 6, 9, 17]. Benzyl (Benvix) and hexyl ester (Hexvix) derivatives are also registered by Photocure ASA for the treatment of gastrointestinal cancers and for the diagnosis of bladder cancer [9].

**Verteporfin**

The second generation photosensitiser, benzoporphyrin derivative monoaacid ring A (BPD-MA, Figure 17) has been developed by QLT Phototherapeutics (Vancouver, Canada) under the trade name Visudyne (Verteporfin, for injection) and, in collaboration with Ciba Vision Corporation (Duluth, GA, USA), has undergone Phase III clinical trials (USA) for the photodynamic treatment of wet age-related macular degeneration (AMD, see Glossary) and cutaneous non-melanoma skin cancer [3, 5–7, 9, 57–59]. Verteporfin is currently marketed by Novartis Pharmaceuticals Corporation (NJ, USA).

The chromophore of BPD-MA has a red-shifted and intensified long-wavelength absorption maxima at approximately 690 nm. Tissue penetration by light at this wavelength is 50% greater than that achieved for Photofrin ($\lambda_{\text{max}} = 630$ nm) [5, 60].

Verteporfin has further advantages over the first generation sensitisier Photofrin. It is rapidly absorbed by the tumour (optimal tumour-normal tissue ratio 30–150 minutes post-intravenous injection) and is rapidly cleared from the body, minimising patient photosensitivity (1–2 days) [5, 61].

**Purlytin**

Tin etiopurpurin, a chlorin photosensitiser (Figure 18), is marketed under the trade name Purlytin by Miravant Medical Technologies (Santa Barbara, Calif, USA) [5–9, 62]. Purlytin has also undergone Phase II clinical trials (USA) for cutaneous metastatic breast cancer and Kaposis’s sarcoma in patients with AIDS (acquired immunodeficiency syndrome) [3, 7]. Purlytin has been used successfully to treat the non-malignant conditions psoriasis and restenosis [5].

Chlorins (Figure 12) are distinguished from the parent porphyrins by a reduced exocyclic double bond. The result of the reduced bond is a decrease in the symmetry of the conjugated macrocycle, leading to an increased absorption in the long-wavelength portion of the visible region of the electromagnetic spectrum (650–680 nm). More correctly, Purlytin is a purpurin; a degradation product of chlorophyll [8, 9, 12].

Purlytin has a tin atom chelated in its central cavity which causes a red-shift of approximately 20–30 nm (with respect to Photofrin and non-metallated etiopurpurin, $\lambda_{\text{max}}(\text{SnEt}_2 = 650$ nm) [6, 9, 12]. Purlytin has been reported to localise in skin and produce a photoreaction 7–14 days post-administration [6, 9].

**Foscan**

Tetra($m$-hydroxyphenyl)chlorin ($m$THPC, Figure 19) has been developed and entered into clinical trials (USA and Europe) under the trade name Foscan by Scotia
Pharmaceutics (Guildford, Surrey, UK) and BioLitec Pharma Limited (Dublin, Ireland) [3, 5–9, 11, 18]. Foscan, also known as Temoporfin, has been evaluated as a phototherapeutic agent against head and neck cancers in these trials [5]. It has also been investigated in clinical trials for malignant and non-malignant diseases, including gastric and pancreatic cancers, hyperplasia, field sterilisation after cancer surgery and for the control of antibiotic-resistant bacteria, in the USA, Europe, and the Far East [5, 9, 11].

Foscan has a singlet oxygen quantum yield comparable to other chlorin photosensitisers but the low drug and light doses (approximately 0.1 mg kg$^{-1}$ and as low as 5 J cm$^{-2}$, resp.) required to achieve photodynamic responses (equivalent to Photofrin, 2–5 mg kg$^{-1}$, 100–200 J cm$^{-2}$; therefore Foscan is approximately 100 times more photoactive than Photofrin), potentially make Foscan one of the most potent second generation photosensitisers currently under investigation [5, 7, 9].

Unfortunately, Foscan can render patients photosensitive for up to 20 days after initial illumination [6, 63, 64]. One solution to this problem would be to use lower drug doses.

**Lutex**

Lutetium texaphyrin, marketed under the trade name Lutex and Lutrin (Pharmacyclics, Calif, USA), is a “texas-sized” porphyrin [5–9, 18, 65, 66]. Texaphyrins (first synthesised in 1987 by Sessler and his group) are expanded porphyrins that have a penta-aza core (Figure 20). The result of this macrocyclic modification is a strong absorption in the 730–770 nm region of the electromagnetic spectrum [9, 12]. This region is particularly important since tissue transparency is optimal in this range. As a result, Lutex-based PDT can (potentially) be carried out more effectively at greater depths and on larger tumours [5, 6].

Lutex has entered Phase II clinical trials (USA) for evaluation against breast cancer and malignant melanomas [6, 67].

A Lutex derivative, Antrin, has also undergone Phase I clinical trials (USA) for the prevention of restenosis (see Glossary) of vessels after cardiac angioplasty by photoinactivating foam cells that accumulate within arteriolar plaques [6, 68]. A second Lutex derivative, Optrin, is in Phase I trials for AMD [5].

Texaphyrins are being developed further by Pharmacyclics as radiosensitisers (Xcytrin, see Glossary) and chemosensitisers (see Glossary) [5]. Xcytrin, a gadolinium texaphyrin (motexafin gadolinium), has been evaluated in Phase III clinical trials against brain metastases and Phase I clinical trials (USA) for primary brain tumours [5].

**ATMPn**

9-Acetoxy-2,7,12,17-tetrakis-(β-methoxyethyl)-porphycene (Figure 21) has been evaluated by Glaxo Dermatology (GlaxoWellcome, NC, USA) and Cytopharm (Calif, USA)
as a photodynamic therapy agent for dermatological applications against psoriasis vulgaris and superficial non-melanoma skin cancer [5, 69–72].

**Zinc phthalocyanine CGP55847**

A liposomal formulation of zinc phthalocyanine (CGP55847, Figure 22), developed by QLT Phototherapeutics (Vancouver, Canada) and sponsored by Ciba Geigy (Novartis, Basel, Switzerland), has undergone clinical trials (Phase I/II, Switzerland) against squamous cell carcinomas of the upper aerodigestive tract [5, 18, 73, 74]. Phthalocyanines (PCs) (Figure 12) are related to tetra-aza porphyrins. Instead of four bridging carbon atoms at the meso-positions, as for the porphyrins, PCs have four nitrogen atoms linking the pyrrolic sub-units together. PCs further differ from porphyrins through the presence of an extended conjugate pathway: a benzene ring is fused to the β-positions of each of the four-pyrrolic sub-units. These benzene rings act to strengthen the absorption of the chromophore at longer wavelengths (with respect to porphyrins). The absorption band of PCs is almost two orders of magnitude stronger than the highest Q band of haematoporphyrin [12]. These favourable characteristics, along with the ability to selectively functionalise their peripheral structure, make PCs favourable photosensitiser candidates [10, 75–78].

A sulphonated aluminium PC derivative (Photosense, Figure 23) has also entered clinical trials (Russian Academy of Medical Sciences, and the surgical clinic of the Moscow Medical Academy, Moscow, Russia) against skin, breast, and lung malignancies and cancer of the gastrointestinal tract [5, 18, 79–81]. Sulphonation significantly increases PC solubility in polar solvents including water, circumventing the need for alternative delivery vehicles [9, 12, 18, 82].

A third PC under investigation is a silicon complex, PC4. This photosensitiser is being examined for the sterilisation of blood components at the New York Blood Centre (VI Technologies Incorporated (Vitex), Melville, NY, USA), against human colon, breast, and ovarian cancers and against glioma [5, 83–89].

A shortcoming of many of the metallo-PCs is their tendency to aggregate in aqueous buffer (pH 7.4), resulting in a decrease, or total loss, of their photochemical activity. This behaviour can be minimised in the presence of detergents [12].

Metallated cationic porphyrazines (PZ), including PdPZ+, CuPZ+, CdPZ+, MgPZ+, AlPZ+, and GaPZ+, have been developed and also tested in vitro on V-79 (Chinese hamster lung fibroblast) cells. Results have suggested these photosensitisers are capable of inducing substantial dark toxicity [12].

**Naphthalocyanines**

Naphthalocyanines (NCs, Figure 24) are an extended PC derivative. They have an additional benzene ring attached to each isoindole sub-unit on the periphery of the PC structure. Subsequently, NCs absorb strongly at even longer wavelengths (approximately 740–780 nm) than PCs (670–780 nm), further increasing the depth NC photosensitisers can be effectively used at. This absorption in the near infrared region makes NCs good candidates for photodynamic treatment of highly pigmented tumours, including melanomas, which present significant problems with respect to transmission of visible light.

However, a number of problems are associated with NC photosensitisers. NCs are generally less stable than their PC relatives: they readily decompose in the presence of light and oxygen; and metallo-NCs, which lack axial ligands, have a tendency to form H-aggregates in solution [12, 90]. These aggregates are photoactive, thus compromising the photodynamic efficacy of NCs [12]. The main investigations
into NCs as photodynamic therapy agents have been carried out by Kenney and co-workers, van Lier’s group and the Bulgarian Academy of Sciences (Sofia, Bulgaria) (see below).

Functional groups

Altering the peripheral functionality of porphyrin-type chromophores can also have an effect on photodynamic activity.

Diamino platinum porphyrins show high anti-tumour activity, demonstrating the combined effect of the cytotoxicity of the platinum complex and the photodynamic activity of the porphyrin species [12, 91].

Positively charged PC derivatives have also been investigated [12, 64, 76, 77]. Cationic species are believed to selectively localise in the vital sub-cellular organelle, the mitochondrion. Mitochondria are key to the survival of a cell; being the site of oxidative phosphorylation, and hence are potentially important PDT targets.

Zinc and copper cationic derivatives have been investigated. Although, the positively charged zinc complexed PC was found to be less photodynamically active than its neutral counterpart in vitro against V-79 cells [12].

Water-soluble cationic porphyrins bearing nitrophenyl, aminophenyl, hydroxyphenyl, and/or pyridiniumyl functional groups exhibit varying cytotoxicity to cancer cells in vitro, depending on the nature of the metal ion (Mn, Fe, Zn, Ni), and on the number and type of functional groups [12, 77, 92]. The manganese pyridiniumyl derivative has shown the highest photodynamic activity, while the nickel analogue is photoinactive (Figure 25) [12, 92].

Another metallo-porphyrin complex, the iron chelate, was found to be more photoactive (towards HIV and simian immunodeficiency virus in MT-4 cells) than the manganese complexes; the zinc derivative was found to be photoinactive [12, 93].

The hydrophilic sulphonated porphyrins and PCs (AlPorphyrin and AlPC) compounds were tested for photodynamic activity [94]. The disulphonated analogues (with adjacent substituted sulphonated groups, Figure 26) exhibited greater photodynamic activity than their di-(symmetrical), mono-, tri- and tetra-sulphonated counterparts; tumour activity increased with increasing degree of sulphonation [8, 78].

11. THIRD-GENERATION PHOTOSENSITISERS

The poor solubility of many photosensitisers in aqueous media, particularly at physiological pH, prevents their intravenous delivery directly into the bloodstream. It would be advantageous therefore, if a delivery model could be
conceived which would allow the transportation of these (otherwise potentially useful) photosensitisers to the site of diseased tissue.

Work has recently focused on designing systems to effect greater selectivity and specificity on the photosensitiser in order to enhance cellular uptake [7, 38]. A number of possible delivery strategies have been suggested, ranging from the use of oil-in-water (o/w) emulsions to liposomes and nanoparticles as potential carrier vehicles [3, 7, 18, 36, 95, 96]. There is concern however, that although the use of these systems may increase the therapeutic effect observed as a result of photodynamic therapy, the carrier system may inadvertently decrease the “observed” singlet oxygen quantum yield ($\Phi_\Delta$) of the encapsulated photosensitiser: the singlet oxygen generated by the photosensitiser would have to diffuse out of the carrier system; and since it (singlet oxygen) is believed to have a narrow radius of action, singlet oxygen may not reach the target and elicit its desired effect [18]. It may also be possible that, if the size of the carrier is not sufficiently small or that the carrier system does not fully dissolve in physiological media, the incidence/exciting light may not be appropriately absorbed and light scattering may be significant, thus inadvertently reducing the singlet oxygen yield. An alternative delivery method which would remove this problem is the use of targeting moieties. Typical targeting strategies have included the investigation of photosensitisers directly attached to biologically active molecules such as antibodies [23–25]. These third generation photosensitisers are currently showing promise (in vitro) against colorectal tumour cells [24].

**Metallation**

A wide range of metals have been used to form complexes with photosensitiser macrocycles, with variable photodynamic results. A number of the second generation photosensitisers described earlier contain a chelated central metal ion. The main metals which have been used are transition metals, although a number of photosensitisers co-ordinated to group 13 (Al, AlPcS₄) and group 14 (Si, SiNC, and Sn, SnEt₂) metals have also been synthesised.

There seems to be no consistent observation as to the potential success of metallated photosensitisers. Indeed, a wide range of photosensitisers are metallated, but the metal ion does not confer definite photoactivity on the photosensitiser. Copper (II), cobalt (II), iron (II), and zinc (II) complexes of Hp are all photoinactive in contrast to metal-free porphyrins [12]. Yet the reverse has been observed for texaphyrin and PC photosensitisers; only the metallo-complexes have demonstrated efficient photosensitisation [12].

The presence and nature of the central metal ion, bound by a number of photosensitisers, strongly influences the photophysical properties of the photosensitiser [12, 64, 77]. Chelation of paramagnetic metals to a PC chromophore appears to shorten triplet lifetimes (down to nanosecond range), generating variations in the triplet quantum yield and triplet lifetime of the photoexcited triplet state of the metallated PC (mPC) [12, 64, 77, 97].

Intersystem crossing (ISC) is an important parameter of photosensitisers. The triplet quantum yield and lifetime of a photosensitiser are directly related to the efficiency of singlet oxygen generation; a key component in the success of a photosensitiser [97].

Certain heavy metals are known to enhance ISC. Generally, diamagnetic metals promote ISC and have a long triplet lifetime [64, 77, 97]. In contrast, paramagnetic species deactivate excited states, reducing the excited-state lifetime and preventing photochemical reactions from taking place [97]. However, there are well-known exceptions to this generalisation, including copper octaethylbenzochlorin [13].

For many of the metallated paramagnetic texaphyrin species, triplet-state lifetimes are down in the nanosecond range [97]. These results are also mirrored by metallated PCs. PCs metallated with diamagnetic ions, such as Zn²⁺, Al³⁺, and Ga⁴⁺, generally yield photosensitisers with desirable quantum yields and lifetimes ($\Phi_T$, 0.56, 0.50 and 0.34 and $\tau_T$ 187, 126 and 35 μs, resp.) [12, 97]. The ZnPc photosensitiser (ZnPcS₄) has a singlet oxygen quantum yield of 0.70; nearly twice that of most other mPCs ($\Phi_\Delta$ at least 0.40) [12, 18]. Hence, the latter diamagnetic complexes should be strong candidates for PDT.

Since the heavy metal effect (see Glossary) is known to promote ISC, theoretically, it should be possible to enhance the photophysical properties ($\Phi_T$, $\Phi_\Delta$, and $\tau_T$) of any photosensitiser via metallation. In practice, this is not the case. Only one metallo-porphyrin photosensitiser (copper octaethylbenzochlorin) has shown photodynamic promise, the remaining efficient porphyrin photosensitisers are metal-free [13]. The reverse of this behaviour is observed for PCs and texaphyrins; only the (diamagnetic) metallated complexes have exhibited potential as photosensitisers [10, 12]. The metal-free analogues have shown no promise as photosensitisers [12].

**Expanded metallo-porphyrins**

Expanded porphyrins have a larger central binding cavity, increasing the number of potential metals it can accommodate.

Diamagnetic metallo-texaphyrins have shown encouraging photophysical properties; high triplet quantum yields and efficient generation of singlet oxygen [12, 64, 77]. In particular, the zinc and cadmium derivatives have shown triple quantum yields close to unity [12]. In contrast, the paramagnetic metallo-texaphyrins, Mn-Tex, Sm-Tex, and Eu-Tex, have undetectable triplet quantum yields. This behaviour is parallel with that observed for the corresponding metallo-porphyrins [12].

The cadmium-texaphyrin derivative has shown in vitro photodynamic activity against human leukemia cells and Gram positive (Staphylococcus) and Gram negative (Escherichia coli) bacteria [98–101]. Although follow-up studies have been limited with this photosensitiser due to the toxicity of the complexed cadmium ion.

A zinc-metallated seco-porphyrinazene (Figure 27) has been developed with a high quantum singlet oxygen yield ($\Phi_\Delta$ 0.74) [102]. This expanded porphyrin-like
photosensitiser has shown the best singlet oxygen photosensitising ability of any of the reported seco-porphyrazines. Platinum and palladium derivatives have also been synthesised with singlet oxygen quantum yields of 0.59 and 0.54, respectively, (Figure 27) [102].

Metallochlorins/bacteriochlorins

The tin (IV) purpurins were found to be more active when compared with analogous zinc (II) purpurins, when evaluated against human cancers [5–7, 9, 18, 103, 104].

Sulphonated benzochlorin derivatives have demonstrated a reduced phototherapeutic response against murine leukemia L1210 cells in vitro and transplanted urothelial cell carcinoma in rats, whereas the tin (IV) metallated benzochlorins exhibited an increased photodynamic effect in the same tumour model (Figure 28) [105].

The previously mentioned copper octaethylbenzochlorin (Figure 10) demonstrated an unexpected result. Despite an undetectable triplet state, it appears to be more phototoxic towards leukemia cells in vitro and a rat bladder tumour model [106–108]. Suggestions for this unusual effect have pointed to interactions between the cationic iminium group and biomolecules [109]. Such interactions may allow electron-transfer reactions to take place via the short-lived excited singlet state and lead to the formation of radicals and radical ions. The copper-free derivative exhibited a tumour response with short intervals between drug administration and photodynamic therapy. Increased in vivo activity was observed with the zinc benzochlorin analogue [109].

Metallo-phthalocyanines

The photophysical properties of PCs are strongly influenced by the presence and nature of the central metal ion [12, 18, 64, 77]. Co-ordination of transition metal ions gives metallo-complexes with short triplet lifetimes (nanosecond range), resulting in different triplet quantum yields and lifetimes (with respect to the non-metallated analogues) [12]. The diamagnetic metals, such as zinc, aluminium, and gallium, generate metallo-phthalocyanines (MPC) with high triplet quantum yields (ΦT ≥ 0.4) and short lifetimes (ZnPCS4 τT = 490 Fs and AlPCS4 τT = 400 Fs) and high singlet oxygen quantum yields (ΦΔ ≥ 0.7) [12, 18, 64, 77, 110]. As a result, ZnPC and AlPC have been evaluated as second generation photosensitisers active against certain tumours [12].

Metallo-naphthalocyanines

Wührle and co-workers (Bulgaria) have concentrated their investigations on a zinc NC with various amido substituents. They observed the best phototherapeutic response
Metal-Based Drugs

Figure 29: An aluminium naphthalocyaninesulfobenzoporphyrazine.

Figure 30: A siloxane naphthalocyanine.

(Lewis lung carcinoma in mice) with a tetrabenzamido analogue [112–114]. Kenney’s group in the USA have studied complexes of silicon (IV) NCs (Figure 30) with two axial ligands in anticipation the ligands would minimise aggregation [115]. In particular, they investigated the disubstituted analogues as potential photodynamic agents [116, 117]. Kenney’s results suggested that a siloxane NC substituted with two methoxyethyleneglycol ligands is an efficient photosensitiser against Lewis lung carcinoma in mice and that SiNC(OSi(i-Bu)2-n-C18H37)2 is effective against Balb/c mouse MS-2 fibrosarcoma cells [118, 119]. van Lier and his group in Canada have also extensively investigated siloxane NCs as agents for photodynamic therapy [5, 76]. van Lier’s research on these compounds suggests that they are efficacious photosensitisers against EMT-6 tumours in Balb/c mice also [120, 121]. The ability of certain metallo-NC derivatives (AlNC) to generate singlet oxygen is weaker than the analogous (sulphonated) metallo-PCs (AlPC); reportedly 1.6–3 orders of magnitude less [12].

Table 1: Quantum triplet yields of texaphyrin metallated with paramagnetic and diamagnetic lanthanides (data reproduced from [97]).

<table>
<thead>
<tr>
<th>Paramagnetic MTex</th>
<th>$\Phi_T$</th>
<th>Diamagnetic MTex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu-Tex</td>
<td>0.090</td>
<td>0.563</td>
</tr>
<tr>
<td>Gd-Tex</td>
<td>0.156</td>
<td>0.500</td>
</tr>
<tr>
<td>Yb-Tex</td>
<td>0.126</td>
<td>0.340</td>
</tr>
</tbody>
</table>

It can be seen from the above examples that generalisation(s) between the nature of the parent chromophore; the presence/absence of a central metal ion; and the desirable photophysical properties required for a successful photosensitiser are difficult to make. In the porphyrin systems, the zinc ion appears to hinder the photodynamic activity of the compound; whereas, in the higher/expanded $\pi$-systems, dyes chelated with the same metal ion are observed to form complexes with good to high photophysical/photodynamic properties.

In order to try and address these observations, Sessler and his group undertook an extensive study into the metallated texaphyrins, investigating the “influence of large metal cations on the photophysical properties of texaphyrins.” They particularly studied “the effect of metal cations on the photophysical properties of coordinating ligands.” The group concentrated on the lanthanide (III) metal ions, Y, In, Lu, Cd, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb [97].

Sessler and co-workers observed that when diamagnetic Lu (III) was complexed to texaphyrin, an effective photosensitiser (Lutex) was generated. When they substituted the paramagnetic Gd (III) ion for the Lu metal, photodynamic activity was lost. As a result, the group investigated a range of diamagnetic and paramagnetic ions [97].

Sessler further reported a correlation between the excited-singlet and triplet state lifetimes and the rate of ISC of the diamagnetic texaphyrin complexes, Y(III), In (III), and Lu (III), and the atomic number of the cation [97].

Paramagnetic metallo-texaphyrins were observed to display rapid ISC. Greater effects on the rates of triplet decay were also observed, and the triplet lifetimes were strongly affected by the choice of metal centre [97]. The diamagnetic ions (Y, In, and Lu) were recorded as having triplet lifetimes ranging from 187, 126, and 35 $\mu$s, respectively. Comparable lifetimes for the paramagnetic species (Eu-Tex 6.98 $\mu$s, Gd-Tex 1.11, Tb-Tex < 0.2, Dy-Tex 0.44 $\times$ 10$^{-3}$, Ho-Tex 0.85 $\times$ 10$^{-3}$, Er-Tex 0.76 $\times$ 10$^{-3}$, Tm-Tex 0.12 $\times$ 10$^{-3}$, and Yb-Tex 0.46) were obtained [97].

Sessler and his group were only able to measure the triplet quantum yields for three of the paramagnetic complexes (see Table 1). The results were significantly lower than the diamagnetic metallo-texaphyrins [97].

The data collected from Sessler and co-workers experiments suggests that, in general, singlet oxygen quantum yields closely follow the triplet quantum yields.

Their experimental data leads to the conclusion that various diamagnetic and paramagnetic texaphyrins investigated have independent photophysical behaviour with respect to a complex’s magnetism. The diamagnetic complexes were...
Imaging Agents

Imaging agents enhance the detection of certain tissues and can be used as tools in diagnostic applications including determining the physiology of the body, in managing disease and locating abnormalities in the body. In particular, certain imaging agents such as the fluorescent tetrapyrrolic-type photosensitisers described in this Review can aid in determining (photosensitiser) localisation and degree of photosensitiser uptake by diseased tissue. The main diagnostic imaging techniques include magnetic resonance imaging (MRI), positron emission tomography (PET), scintigraphy (radionucleotides), ultrasound imaging, CT scans and x-rays. MRI, PET, and scintigraphy are well-suited techniques used in medical applications to demonstrate pathological or physiological alterations in living tissue. Unfortunately, the degree of signal enhancement for diseased versus normal tissue is often insufficient and the images formed from these techniques do not adequately show the anatomy or pathology of the desired area. To overcome this problem the images can be enhanced with contrast enhancement agents.

The contrast agents used for MRI imaging are normally paramagnetic complexes, although diamagnetic compounds may also be used. The gadolinium-chelates have shown promise as contrast enhancement agents in pre-clinical trials and clinical use [122]. The gadolinium-enhanced tissue appears brighter on images in comparison to gadolinium-free tissue. Expanded porphyrins are particularly suited to co-ordinating these relatively large cations (Gd ionic radius approximately 1.0 Å, expanded porphyrin ionic radius approximately 2.0 Å) in a stable manner [123, 124]. Forming chelates between a molecule such as an expanded porphyrin and a metal such as gadolinium reduces the toxicity of the free metal ion [122]. The first gadolinium-chelate approved for use as an enhancement agent was Gd-DTPA (gadopentetate dimeglumine, also known as Magnevist [125, 126]. Other chelates such as Gd-DOTA (gadoterate meglumine acid, also known as Dotarem and Gd-Tex have also shown success, with Gd-Tex administered to SMT-F tumour-bearing Balb/c mice [127, 128].

Metallated porphyrins, including manganese (II) tetraarylporphyrins, have also been studied as contrast enhancement agents for MRI [12]. Like the expanded porphyrin example above, metals suited to the technique are generally paramagnetic and include those which are not readily metabolised by the body and which strongly coordinate to the porphyrin macrocycle.

Diamagnetic agents have also shown some success as gastrointestinal imaging agents, while superparamagnetic and ferromagnetic agents have been successful for liver and bowel imaging (Lumirem (Fe²⁺/³⁺, bowel), also known as GastroMark, and Endorem (Fe²⁺/³⁺, liver) [129]. Other paramagnetic agents include LumenHance (Fe²⁺, bowel) [130, 131].

Radiosensitisers and Radiolabels

A number of the metallated photosensitisers described in the Review are also radiosensitisers (see earlier).

The metallo-photosensitisers have shown further applications as radiolabels. Porphyrins and phthalocyanines exhibit tumour retention in animals, potentially making them candidates as carriers of clinically accepted radioisotopes, such as ⁶⁷Ga (III). ⁶⁷Ga (III) coordinated to H₂PCS shows good tumour uptake in C3H mice [12, 132]. They have also been used to image the brain, lymph nodes, melanoma tumours, and as bifunctional chelating agents for antibody labelling. Examples include ¹¹¹In, ⁶⁴Cu, ⁹⁹mTc (HpD labelled), ⁵⁷Co-TPP, ¹⁰⁹Pd-porphyrins, and ⁵⁴Mn-HpD. Vanadium has also been attached to a porphyrin and investigated for PET [12].

PET, also a non-invasive diagnostic imaging technique, produces images of the body’s basic biochemistry. PET can therefore be useful in observing the body’s biochemical changes affected by disease. The imaging agents used in PET contain a radioactive material coupled (labelled) to a compound which is normally present in the body such as water or glucose. The resulting drug (radionucleotide) is administered to the body and physiological images are formed as the body processes the drug. Some of the metals used to label the organic molecules include ⁶⁴Cu and ⁶⁸Ga [133, 134]. The latter metal forms a complex which has been used in scans of the meninges—membrane which surrounds the brain and spinal cord [135].
Table 2: Summary of a collection of different photosensitiser types and their absorption data.

<table>
<thead>
<tr>
<th>CLASS OF PHOTOSENSITISER</th>
<th>LONGEST WAVELENGTH ABSORPTION/nm</th>
<th>EXTINCTION COEFFICIENT/ M\textsuperscript{-1}cm\textsuperscript{-1}</th>
<th>DRUGS IN CLINICAL TRIAL (Phase I–III)</th>
<th>DRUGS APPROVED FOR PDT (PRECLINICAL AND CLINICAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyrins</td>
<td>620–640</td>
<td>3,500</td>
<td>—</td>
<td>Photofrin</td>
</tr>
<tr>
<td>(Expanded Porphyrins)</td>
<td></td>
<td></td>
<td></td>
<td>Levulan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Metvix</td>
</tr>
<tr>
<td>Porphycenes</td>
<td>610–650</td>
<td>50,000</td>
<td>—</td>
<td>ATMPn</td>
</tr>
<tr>
<td>Texaphyrins</td>
<td>730–770</td>
<td>40,000</td>
<td>Lu-Tex</td>
<td>Optrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antrin</td>
<td>Xcytrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Benzvix</td>
<td>Hexvix</td>
</tr>
<tr>
<td>Chlorins</td>
<td>650–690</td>
<td>40,000</td>
<td>Foscan</td>
<td>Visudyne</td>
</tr>
<tr>
<td>Bacteriochlorins</td>
<td>730–800</td>
<td>150,000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phthalocyanines</td>
<td>680–780</td>
<td>200,000</td>
<td>CGP55847</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PC4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Photosense</td>
<td>—</td>
</tr>
<tr>
<td>Naphthalocyanines</td>
<td>740–780</td>
<td>250,000</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3: Summary of a range of photosensitisers and their clinical applications.

<table>
<thead>
<tr>
<th>TRADE NAME</th>
<th>MARKETING COMPANY</th>
<th>PRE-/CLINICAL APPLICATION</th>
<th>COUNTRIES APPROVED IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levulan</td>
<td>DUSA Pharmaceuticals</td>
<td>Actinic keratosis</td>
<td>USA (1999), Sweden (2001), Europe (2001)</td>
</tr>
<tr>
<td>ATMPn</td>
<td>GlaxoWellcome and Cytopharm</td>
<td>Psoriasis and non-melanoma skin cancer</td>
<td>Germany (1997)</td>
</tr>
<tr>
<td>Purlytin</td>
<td>Miravant Medical Technologies</td>
<td>Psoriasis and restenosis</td>
<td>USA (1998)</td>
</tr>
<tr>
<td>Foscan</td>
<td>BioLitec Pharmaceuticals</td>
<td>Head and neck cancers</td>
<td>Europe (2001)</td>
</tr>
</tbody>
</table>
characterised by relatively high fluorescence quantum yields, excited-singlet and triplet lifetimes, and singlet oxygen quantum yields; in distinct contrast to the paramagnetic species investigated [97].

Results suggested that the +2 charged diamagnetic species exhibit a direct relationship between their fluorescence quantum yields, excited state lifetimes, rate of ISC, and the atomic number of the metal ion. The greatest diamagnetic ISC rate was observed for Lu-Tex; a result ascribed to the heavy atom effect. The heavy atom effect also held for the Y-Tex, In-Tex, and Lu-Tex triplet quantum yields and lifetimes. The triplet quantum yields and lifetimes both decreased with increasing atomic number. The singlet oxygen quantum yield correlated with this observation [97].

The photophysical properties displayed by the paramagnetic species were more complex. A simple correlation between the observed data/behaviour and the number of unpaired electrons located on the metal ion could not be made. For example, the ISC rates and the fluorescence lifetimes gradually decreased with increasing atomic number, the Gd-Tex, and Tb-Tex chromophores showed (despite having a larger number of unpaired electrons) slower rates of ISC and longer lifetimes than Ho-Tex or Dy-Tex. Sessler suggested that charge transfer or intermolecular energy transfer is taking place from higher excited states (such as S2) [97].

12. SUMMARY

A variety of second generation photosensitisers have been developed and evaluated against a range of clinical applications (see Tables 2 and 3). The metallation of a number of these chromophores has generated a variety of photosensitisers with improved photophysical properties. The effectiveness of these metallo-photosensitisers depends largely (but not definitively) on the nature of the coordinated central metal ion. Chromophores chelated to diamagnetic transition metals and lanthanide ions have shown the greatest potential as photodynamic agents, a consequence of the heavy metal effect enhancing the rate of ISC. As a result, a number of these metallated tetrpyrrole-based macrocycles are currently photosensitisers of choice, particularly the zinc (II), aluminium (III), and tin (IV) complexes.

GLOSSARY

1. AMD is the leading cause of blindness in humans over the age of 50. AMD is characterised by a rapid growth of abnormal blood vessels under the central retina causing scarring, and an accelerated loss of visual acuity [58].

2. Chemosensitisers are drugs or chemicals which are used to enhance the therapeutic effects of anti-cancer (chemotherapy) drugs. They make the tumour cells more sensitive to the effects of chemotherapy.

3. Diamagnetic species is a species with no unpaired electrons, that is, all electrons are spin-paired.

4. Fluorophore is generally a molecule capable of absorbing light energy when irradiated at a specific wavelength and emitting energy at longer wavelengths.

5. Heavy atom effect enhances coupling between the excited-singlet (S1) and excited-triplet (T1) states. It is the enhancement of a spin-forbidden process by the presence of an atom of high atomic molecular weight. Mechanistically, it responds to a spin-orbit coupling enhancement produced by a heavy atom. Spin-forbidden and spin-allowed processes are highlighted in Section 7.

6. Luminescence lifetime is the average time a molecule spends in an excited state (S_n > 0 or T_n > 0).

7. Methylene blue is used to sterilize/decontaminate freshly frozen plasma units by inactivating extracellularly enveloped viruses (such as HIV), as well as methaemoglobinemia [5, 9].

8. Paramagnetic species is a species with one or more unpaired electrons.

9. Phase I clinical trials are used to determine pharmacokinetic properties (metabolism, elimination, and preferred method of administration) and a safe dosage range, and identify any side effects of a new drug. They are performed on a small number of people (20–80).

10. Phase II clinical trials are performed on a larger group of people (100–300). The drug is further evaluated to test its effectiveness and any side effects.

11. Phase III clinical trials: the drugs effectiveness is confirmed and comparisons are made to more commonly used treatment modalities in a range of 1000–3000 people. Potential side effects are monitored.

12. Phase IV clinical trials are post-marketing observations and evaluations.

13. Photochemotherapy is a combination of a chemical substance and light to treat disease.

14. Phototheraphy is a term used to describe the treatment of disease by a series of (photo-) chemical processes initiated by light.

15. Radiosensitisers are drugs which boost the effect of radiation therapy (radiotherapy) by making the tumour tissue more vulnerable to the applied radiation. They may be used alone or in conjunction with other drugs.

16. Restenosis is the narrowing of a coronary artery after angioplasty or stenting.

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August 2006.


Metal-Based Drugs

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