Molecular Theranostic Agents for Photodynamic Therapy (PDT) and Magnetic Resonance Imaging (MRI)
Sébastien Jenni, Angélique Sour

To cite this version:
Sébastien Jenni, Angélique Sour. Molecular Theranostic Agents for Photodynamic Therapy (PDT) and Magnetic Resonance Imaging (MRI). Inorganics, MDPI AG, 2019, 7 (1), pp.10. 10.3390/inorganics7010010. hal-02361916

HAL Id: hal-02361916
https://hal.archives-ouvertes.fr/hal-02361916
Submitted on 8 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Review

Molecular theranostic agents for PDT and MRI

Sébastien Jenni 1 and Angélique Sour 1,*

1 Université de Strasbourg, CNRS/UMR 7177, Institut de Chimie, F-Strasbourg, France; sjenni@unistra.fr
* Correspondence: a.sour@unistra.fr; Tel.: +33-368-851-363

Abstract: MRI (magnetic resonance imaging) is a powerful non-invasive diagnostic tool that can bring important enlightenment for medical treatment monitoring and optimization. PDT (photodynamic therapy), a minimally invasive treatment for various types of tumors, is gaining increasing interest thanks to its temporal and spatial selectivity. The combination of MRI and PDT offers a real-time monitoring of the treatment and can bring significant informations for the drug-uptake and the light-delivery parameters optimization. In this review we will give an overview on molecular theranostic agents that have been designed for potential applications in MRI and PDT.

Keywords: theranostic, MRI, PDT, gadolinium, porphyrin

1. Introduction to the theranostic approach

Theranostics is an innovative medical treatment research field, which gathers the functions of therapy and diagnostic by imaging and paves a way for personalized medicine.[1-4] The emergence of this research field has been made possible by the tremendous progress in the development of instruments for imaging and treatment. Theranostic agents combine an imaging agent and a therapeutic agent within the same scaffold, both agents being thus delivered at the same time and with the same biodistribution. They bring important informations for pre-treatment planning, therapy monitoring, treatment outcome assessment and moreover, for the development of new therapeutic agents. Various imaging and therapeutic modalities can be gathered, with different assembly strategies, to generate theranostic agents. Here, we review on theranostic agents combining MRI (magnetic resonance imaging) and PDT (photodynamic therapy) applications, which have a molecular structure. Theranostic agents based on nanoparticles belong to another promising and active field and have recently been reviewed.[5-7]

1.1. PDT treatment: strength and limitations

PDT is a light activated treatment modality, clinically approved in the treatment of dermatological and ocular disorders and of various cancers. It is a localized treatment with minimal invasiveness and side effects.[8-10] PDT requires the administration of a drug called photosensitizer (PS) that is mainly a tetrpyrrole-based chromophore (Scheme 1). Light of an appropriate wavelength is then applied on the affected tissue and is absorbed by the photosensitizer. The latter is thus activated and reacts with surrounding oxygen and/or with surrounding molecules to generate cytotoxic species, which induce cellular damage, vascular occlusion and/or antitumor immune response.[11]

Most of the clinical PSs are activated with excitation wavelengths between 630 and 690 nm, which have limited tissue penetration depth mainly due to light scattering and absorption by endogenous molecules. The weak tissue penetration of light is a major concern for PDT development. Therefore PSs that can be activated in the optical transparency window of tissues (from 700 nm and up to 1000 nm with a two-photon absorption process) are very appealing.[8] In addition to the wavelength range, strong absorption capacity (characterized by high absorption coefficient value $\varepsilon$ for one-photon absorption and by high two-photon absorption cross-section value $\sigma$) is required for efficient PS delivery at the target site.

Inorganics 2019, 7, x; doi: FOR PEER REVIEW www.mdpi.com/journal/inorganics
for two-photon absorption) increases the production of cytotoxic species and enhances the treatment efficacy. New PSs are designed and studied in order to respond to these criteria. Two porphyrin-based photosensitizers (Scheme 1c and 1d), with strong one-photon absorption in the near infrared, have recently been designed. Padeliporfin (Tookad®WST11), with an excitation wavelength at 763 nm ($\varepsilon = 100 \times 10^3$ M$^{-1}$cm$^{-1}$), has been approved for the treatment of prostate cancer.[12] Redaporfin (LUZ11), that can be activated at 749 nm ($\varepsilon = 140 \times 10^3$ M$^{-1}$cm$^{-1}$) has been approved for biliary tract cancer and is also moving to other clinical trials.[13,14]

![Scheme 1](image)

Some PSs are able to preferentially accumulate in tumor cells, however the mechanisms of this behavior are not fully understood and the tumor selectivity should be improved. There is also a need for precisely identifying the time period of maximum drug accumulation in the tumor. Light irradiation during this time period should generate many reactive oxygen species and lead to best PDT efficiency.[5] Drug accumulation and light delivery is specific to each PS and tumor characteristics. Thus the optimization of the treatment planning is difficult to achieve. With these limitations, it has become evident that there is a need for treatment guidance by imaging that can pilot the pre- and post-treatment evaluation.[6,15-17]

1.2. MRI guidance of PDT

MRI is a non-invasive visualization tool of soft tissues with both high spatial and time resolution.[18] It has already proven to be powerful for therapy monitoring in oncology and for drug development.[19] The MR signal contrast arises from the differences of proton properties such as the relaxation times ($T_1$ and $T_2$) or the density of water molecules.[20] These properties are acquired using appropriate MR pulse sequences; they highlight different proton behaviors and allow tissue discrimination. In some cases, the contrast between healthy and diseased tissue is weak and the use of a contrast agent is necessary. Clinically used contrast agents are small gadolinium-based complexes containing octadentate chelators based on macrocyclic (GdDOTA with DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate) or linear (GdDTPA with DTPA = diethylenetriamine pentaacete) structures (Scheme 2). The efficiency of a contrast agent is measured by its relaxivity that corresponds to the ability to decrease the relaxation time $T_1$ of water protons in presence of a paramagnetic gadolinium complex at a concentration of 1 mM.

The PDT-induced changes in the tumoral area alter the proton behaviors and these changes can also be strongly emphasized by the presence of a contrast agent. The image modification can thus bring essential informations to estimate the PDT effect. It is then possible to establish relations between this effect and the drug and light parameters. The latters can then be finely tuned in order to increase the efficiency of the treatment.[21]
MRI-guided PDT can be realized with repeated contrast agent injection at different time points after the PS injection. Depending on the choice of the MRI methodology, PDT response can be followed at different times after the treatment and give different informations. Contrast-enhanced MRI has shown to be very sensitive to PDT-induced vascular occlusion.[22,23] It was used for the assessment of tumor response to PDT.[24-26] Informations obtained shortly after treatment could also be obtained.[25]

The optimization of the PDT treatment can be further improved using theranostic agents. With this approach, both the imaging and the therapeutic agents will have the same biodistribution and bioelimination behaviors. Gadolinium-based contrast agents linked to a porphyrin-based PS are expected to confer increased tumoral residence time, thus they provide a longer time window to monitor the treatment. They also have increased relaxivities compared to classical MRI contrast agents. Therefore, they can be administered at much lower dose than current clinical contrast agents and offer enhanced safety. The nanoparticle approaches for MRI and PDT applications have attracted increasing attention during the past decade.[5-7,27] In spite of the efficient targeting and high payload of some nanotheranostic agents, clinical translation has not yet been possible.[6,7,27] Molecular theranostic agents for PDT and MRI have been developed to a lesser extent; however, interesting results have been obtained. These small or medium-size molecular agents have their own strengths and advantages (such as high reproducibility, stability, purity and good biocompatibility) and this approach continues to draw attention for cancer treatment.[28]

2. Porphyrin-Gd-complexes conjugates with potential MRI and PDT applications

Several porphyrin analogues have been associated to Gd(III) complexes, their ability to accumulate in cancer cells and/or their relaxivity have been considered without exploring their potential as PDT PS. At an early stage, two compounds, gadophrin-2 and gadophrin-3, composed respectively of a free-base and a copper(II) porphyrin linked to two GdDTPA complexes have been investigated. Studies in mice have shown comparable pharmacological properties and these PSs were found to accumulate in necrotic areas.[29-33] A 5, 10, 15, 20-tetraphenylporphyrin (TPP) core has been linked to one and four GdDTPA complexes through amide bond formation.[34] Increased relaxivity values have been found. Free-base and copper(II) porphyrins linked to one, two and four GdD03A-amide complexes have been developed for potential multimodal MRI/PET (positron emission tomography) applications. Preliminary relaxivity studies indicated promising contrast enhancement.[35,36] Zinc(II) and copper(II) porphyrazine have been linked to two GdD03A-amide complexes. The copper-containing compound showed the highest relaxivity, very good cellular internalization and tumor-bearing mouse images indicated necrotic localization.[37]

3. Molecular theranostic agents with combined PDT and MRI studies

In 1993, a pioneering study has been realized with two bifunctional compounds containing porphyrin derivatives linked to one or two GdDTPA complexes.[38] The compound TPP-Gd$_2$(DTPA)$_4$ consisting of a tetra-p-aminophenylporphyrin core coupled through amide bonds to four DTPA ligands with two of them metalated by Gd(III) ions, was the most promising. The relaxivity value per Gd(III) was found to be twice as high as that of GdDTPA at 20 MHz and the
substantial image contrast enhancement of the tumor compared to adjacent normal tissue in
tumor-bearing mice evidenced the affinity of the compound for tumor tissue. The photoinduced
toxicity studies, realized with irradiation using a multichannel laser beam at 488 and 514 nm on
two cell lines (HT29 and L1210), showed comparable phototoxicities than the one induced by a
commercial HPD PS. This porphyrin-Gd complex conjugate was the first prototype built for MRI
and PDT.

More than a decade later, Pandey and collaborators extensively investigated several theranostic
agents that combine diagnostic imaging (MR and fluorescence imaging) and PDT treatment
properties. They are based on different photosensitizers (pyropheophorbide analogues with
different lipophilic/hydrophilic chains) linked to one, two, three or six GdDTPA complexes.[39-42]
In these compounds, the linkage is realized through the C-functionalization of the
diethylenetriamine backbone and the stability of the GdDTPA core is preserved with the five anionic
carboxylate groups. The theranostic compounds containing one and two Gd complexes required
liposomal formulation to resolve the poor water-solubility problem. With the presence of 3 and 6
GdDTPA units, the water solubility was improved. The compound HPPH-3GdDTPA bearing 3
GdDTPA complexes (Figure 1a) was found to be the best candidate regarding its imaging and
treatment results. It showed remarkable MR contrast enhancement of tumor in mice 24 hours after
injection, with a 10-fold lower dose than Magnevist and preferential uptake in tumor compared to
muscle (Figure 1b). Fluorescence imaging, resulting from the light emission of the HPPH
derivative, also showed maximum intensity 24 hours after injection. Finally this compound also
showed efficient PDT effect after one irradiation at 665 nm (70 J/cm²) 24 hours after injection.

Phthalocyanine and porphyrazine are tetraazaporphyrins known for their intense electronic
absorption in the NIR region, they require addition of peripheric substitution groups to avoid
aggregation and favor water solubilization. They have been linked to GdDO3A-amide complexes
that are GdDOTA derivatives where one carboxylate arm is replaced by an acetamide group. The
amide-bond formation allows rapid access to Gd(III) complex functionalization but it induces
strongly reduced thermodynamic stability while keeping the same kinetic inertness as the GdDOTA
complex.[43] The phthalocyanine-based PS has been linked to one GdDO3A-amide complex
(ZnPht-1Gd, Figure 2a).[44] It exhibited low relaxivity (1.43 mM⁻¹s⁻¹ at 128 MHz) and the authors
proposed that this weak value could be due to the presence of the amide function on the arm that
could block the water access to the metallic center. The compound showed a good ability to produce
cytotoxic singlet oxygen under irradiation with a quantum yield of 0.67 (in DMSO). The
porphyrazine based PS has been linked to one, four and eight GdDO3A-amide complexes to give the
bifunctional compounds ZnPz-nGd (with n = 1, 4, 8, Figure 2b).[45] They showed strong relaxivity
increase (up to 12.8 mM $^{-1}$s$^{-1}$ for ZnPz-8Gd at 60 MHz and 37°C) with the number of Gd-complexes attached to the PS. Cellular uptake was observed only with the compounds bearing one Gd complex (ZnPz-1Gd) and notable phototoxic effect (with 50% cell killing) was assessed after 10 min irradiation with white light (Figure 2c).

Figure 2. Chemical structure of (a) ZnPht-1Gd[44] and (b) ZnPz-1Gd[45]; (c) Phototoxic effect in WI-38 VA13 cells incubated with Zn-Pz-nGd (n = 1, 4, 8; 50 μM, 24 h) and irradiated by white light for 0 or 10 min. The same protocol was realized with Photofrin as a positive control. Adapted with permission from [45]. Copyright 2010 American Chemical Society.

Porphyrin derivatives incorporating a Gd(III) ion are at first sight appealing agents due to their simplified structure and synthesis and to their promising in vitro properties as phosphorescence-based oxygen sensors, PDT photosensitizers and MRI contrast agents. However, their in vivo study have resulted in disparate conclusions. Koenig[46] and Furmanski[47] studied gadolinium-incorporated porphyrins as MRI contrast agents, they observed stability problems with ion dissociation from the porphyrin during their studies in plasma and in mice. Recently, two porphyrazine-based compounds incorporating a Gd(III) ion, GdPz1 and GdPz2, which differ in the nature of the peripheral groups, have been obtained (Figure 3a).[48] A good relaxivity value was obtained at very high magnetic field (4.67 mM$^{-1}$s$^{-1}$ at 9.4 T) for GdPz1 once solubilized in polymer polyimide brushes. Cellular uptake, dual in vivo fluorescence and MR imaging and in vivo PDT activity were studied. Significant in vivo tumor accumulation was demonstrated by fluorescence and MR images for both compounds. PDT activity was assessed in cancerous CT26 cells with light irradiation performed at 615-635 nm (10-20 J/cm$^2$, $10^{-7}$ to $10^{-4}$ M incubation concentration). PDT treatment of CT26 tumor-bearing Balb/c mice (Figure 3b) was realized 3 hours post-injection by irradiation at 593 nm (30 min, 120 J/cm$^2$). Moderate tumor death was observed and this study indicated the need to optimize different parameters such as the drug dose and the light application.
Figure 3. (a) Molecular structure of GdPz1 and GdPz2; (b) Tumor volume variation as a function of time.

Intravenous injection of the theranostic agent and PDT treatment 3 hours after this injection were realized on day 10 after tumor inoculation. Adapted with permission from [48]. Copyright 2017 Elsevier.

A porphyrin-based PS linked to chemotoxic platinum(II) complexes and incorporating a gadolinium(III) ion has been reported for tumor treatment by PDT and chemotherapy and for MR imaging.[49] It has been obtained from 5, 10, 15, 20-tetra(4-pyridyl)-porphyrin (P1) that was coordinated to four Pt(II) complexes (Pt-P1) and to one Gd(III) ion (Gd/Pt-P1, Figure 4a). The compound showed nearly doubled relaxivity at 3 T compared to GdDTPA. A phototoxic effect was observed in C6 cells after 10 min irradiation at 630 nm (Figure 4b). A synergetic chemo-photodynamic antitumor effect was observed in cell and on C6 tumor-bearing mice.

Figure 4. (a) Molecular representation of Gd/Pt-P1; (b) C6 cell viability as a function of three porphyrin (P1, Pt-P1 and Gd/Pt-P1) concentration without irradiation (6 h incubation) and 44 h after irradiation at 630 nm (0.2 W/cm, 10 min). Adapted from Ref. [49] with permission from The Royal Society of Chemistry.

In order to obtain contrast agents with high relaxivity at high magnetic fields, the strategy of increasing the number of coordinated water molecules at the Gd(III) center is particularly appealing as it allows to nearly double the $r_1$ relaxivity value, independently of the magnetic field. In this case, careful design of hepta- or hexadentate ligands is necessary in order to obtain Gd(III) complexes with good thermodynamic stability and kinetic inertness and to avoid ternary complex formation with endogenous molecules. Chen et al. developed a potential theranostic agent (Scheme 3a) consisting of a tetraphenylporphyrin core linked to four GdDTTA complexes.[50] High relaxivity has been measured (14.1 mM$^{-1}$s$^{-1}$ at 0.55 T in Hepes (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) buffer) and this value was doubled in presence of human serum albumin, indicating strong binding of the conjugate to this blood pool protein. The fluorescence of the PS has been evidenced in H1299 lung cancer cells and showed harmless cellular uptake. Singlet oxygen was efficiently produced upon irradiation at 650 nm in deuterated water. These studies showed the potential of this
compound to behave as a contrast agent for multimodal (MR and luminescence) imaging and as PS for PDT.

![Chemical structure of the theranostic compounds](image)

**Scheme 3.** Chemical structure of the theranostic compounds developed by (a) Chen *et al.*[50] and (b) Sour *et al.*[51]

The development of a theranostic agent gathering four GdDTTA complexes and a TPP core has also been realized with a design differing in the nature of the linkers separating the two agents (Scheme 3b). Short and relatively rigid benzyl linkers have been used, they allow to minimize rotational flexibility and thus to optimize the relaxivity gain brought by the increase of the molecular weight.[51] This water-soluble bifunctional system has the highest relaxivity reported for a medium-size system with a maximum of 43.7 mM⁻¹s⁻¹ (per Gd(III) ion at 20 MHz). A 27% relaxivity increase has also been observed in presence of BSA (bovine serum albumin). Phantom images of cell pellets (Figure 5a) obtained at high magnetic field (7 T) evidenced the cellular uptake. ICP-MS measurements showed that the cellular uptake of Gd ions was 60 times more effective with the theranostic agent than with the commercial GdDTPA contrast agent at 10 µM Gd incubation concentration. This result can be explained by the amphiphilic character of the theranostic compound that favors cell internalization. A good PDT effect was observed on HeLa cells (Figure 5b) upon irradiation at 636 nm (1 hour) and was found to increase with light intensity and with the incubation concentration of the theranostic agent.

![Phantom images of cell pellets](image)

**Figure 5.** (a) T₁ maps of HeLa cell pellets at 7 T incubated for 24 h with different concentrations of the theranostic compound; (b) Phototoxicity of the theranostic compound following 1 h irradiation at 636 nm after 24 h incubation at 2.5 µM (dark gray) and 6 µM (light gray). Adapted with permission from [51]. Copyright 2016 American Chemical Society.

The theranostic agents reported so far are activated by one-photon absorption, with excitation wavelengths below 700 nm. The two-photon excitation process allows to use excitation wavelengths in the near infrared.[52,53] This process allows deep treatment and minimal photodamages to healthy tissues. The two-photon irradiation is possible only in a very small area, high spatial
treatment precision can be obtained but the application to bulky tumors is currently limited. The design of new PSs with high two-photon absorption capability requires large π-electronic delocalization. A one- and two-photon activatable PS based on a diketopyrrolopyrrole-zinc-porphyrin component (DPP-ZnP) and linked to a GdDOTA complex as an imaging probe has been studied (Scheme 4).[54] The GdDOTA attachment to the PS has been realized with the use of the commercial DOTAGA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) ligand, that brings local flexibility but keeps the GdDOTA stability intact. Remarkable relaxivity values \( r_1 = 19.9 \text{ mM}^{-1}\text{s}^{-1} \) at 20 MHz for a monohydrated and medium-size system have been obtained. A 20% relaxivity increase in presence of BSA was also observed. Strong one-photon absorption ability has been measured \( \varepsilon_{\text{max}} = 41 \text{ 000 M}^{-1}\text{cm}^{-1} \text{ at 667 nm in water} \). Large two-photon absorption capacity quantified by large \( \sigma_2 \) values has been evidenced in solution over a broad range of wavelength with a maximum of 1000 GM between 910 and 940 nm. High PDT effect evaluated in HeLa cells was observed by one-photon excitation at 660 nm (1 hour, 1 \( \mu \text{M} \) incubation concentration) and moderate two-photon PDT effect was observed at 930 nm (300 scans, 1 \( \mu \text{M} \) incubation concentration).

![Scheme 4. Chemical structure of the theranostic compound DPP-ZnP-GdDOTA developed by Heitz et al.[54]](DPP-ZnP-GdDOTA)

A theranostic agent containing a PS with high one- and two-photon absorption capacity in the near infrared and two stable contrast agents for MR imaging has been studied.[55] The structure of this agent is composed of a Zn-porphyrin dimer (ZnP-ZnP) linked to two GdDOTA complexes (Figure 6a). High relaxivity values have been obtained with a maximum of \( r_1 = 14.4 \text{ mM}^{-1}\text{s}^{-1} \) (at 40 MHz in water containing 2% of pyridine to ensure complete water solubility). Due to the presence of the two Gd(III) complexes, the corresponding molecular relaxivities are doubled, and this trait is important to realize the imaging and therapeutic studies at the same low concentration required for PDT treatment. It is also interesting to note that the relaxivity value was doubled in presence of BSA at 20 MHz. This compound showed strong one-photon absorption capacity in the near infrared (with a maximum at 746 nm with \( \varepsilon = 10^5 \text{ M}^{-1}\text{cm}^{-1} \) in DMSO). It also showed very strong two-photon absorption ability with a maximum between 880 and 930 nm \( (\sigma_2 \approx 8000 \text{ GM in DMSO}) \). Efficient PDT effect was observed in HeLa cells after one-photon irradiation at 740 nm (30 min, 1 \( \mu \text{M} \) incubation concentration). Two-photon PDT effect was observed at 910 nm (300 scans, 2 \( \mu \text{M} \) incubation concentration) as a function of the light power and 100% cell death was observed with an average power of 108 mW at the back pupil of the objective (Figure 6b).
5. Conclusions

The design and study of molecular theranostic agents for potential applications in MR imaging and PDT treatment has been highlighted and discussed. Compared to small commercial contrast agents, the MR imaging properties of these theranostic agents are improved by the presence of the lipophilic PS. Increased cellular uptake and/or tumor accumulation have been observed and together with the increased relaxivity brought by the large size of these compounds, the use of such theranostic agent requires much lower doses than the one used with clinical contrast agents. To further improve the imaging efficiency of the theranostic agent, the same criteria as those for classical contrast agents need to be considered. In particular, the local rigidity and the stability are two important parameters to take into account. In addition to the MR imaging, the fluorescence properties of the tetrapyrrolic core have also been explored in some cases. They bring a second imaging modality with high sensitivity.

Compared to an individual tetrapyrrolic PS, the presence of hydrophilic Gd(III) complexes brings increased water solubility and modulates the in vivo distribution and elimination. A good ratio between the number of Gd(III) complexes and the PS has to be found in order to keep the cellular uptake ability. The design of PDT sensitizers with strong absorption in the biological transparency window is necessary for increased tissue penetration depth and high cytotoxic species production.

Cellular and animal studies ask for strong and long experimentation efforts. Studies showing the influence of the drug concentration and the light application on the PDT efficiency should be developed. Finally, a chemical design that allows better tumoral selectivity will undoubtedly explode the capacity of these theranostic agents.

Acknowledgments: The authors thank the group members and collaborators involved in the work done in the frame of the LSAMM team.

Conflicts of Interest: the authors declare no conflict of interest.

Figure 6. (a) Structure of the zinc porphyrin dimer linked to two GdDOTA complexes; (b) Phototoxicity induced by a two-photon excitation at 910 nm after 24 h incubation in absence (left) or presence (right) of the compound. Scale bar is 100 µm. Adapted with permission from [55]. Copyright 2018 American Chemical Society.
References

1. Yoon, H.Y.; Jeon, S.; You, D.G.; Park, J.H.; Kwon, I.C.; Koo, H.; Kim, K. Inorganic Nanoparticles for Image-Guided Therapy. *Bioconjugate Chem.* **2017**, *28*, 124-134, doi:10.1021/acs.bioconjugchem.6b00512.

2. Crawley, N.; Thompson, M.; Romaschin, A. Theranostics in the Growing Field of Personalized Medicine: An Analytical Chemistry Perspective. *Anal. Chem.* **2014**, *86*, 130-160, doi:10.1021/ac4038812.

3. Terreno, E.; Uggeri, F.; Aime, S. Image guided therapy: The advent of theranostic agents. *J. Controlled Release* **2012**, *161*, 328-337, doi:10.1016/j.jconrel.2012.05.028.

4. Kelkar, S.S.; Reineke, T.M. Theranostics: Combining Imaging and Therapy. *Bioconjugate Chem.* **2011**, *22*, 1879-1903, doi:10.1021/bc200151q.

5. Kunjachan, S.; Ehling, J.; Storm, G.; Kiessling, F.; Lammers, T. Noninvasive Imaging of Nanomedicines and Nanotheranostics: Principles, Progress, and Prospects. *Chem. Rev.* **2015**, *115*, 10907-10937, doi:10.1021/er500314d.

6. Chen, G.; Roy, I.; Yang, C.; Prasad, P.N. Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy. *Chem. Rev.* **2016**, *116*, 2826-2885, doi:10.1021/acs.chemrev.5b00148.

7. Björnmalm, M.; Thurecht, K.J.; Michael, M.; Scott, A.M.; Caruso, F. Bridging Bio–Nano Science and Cancer Nanomedicine. *ACS Nano* **2017**, *11*, 9594-9613, doi:10.1021/acsnano.7b04855.

8. Fan, W.; Huang, P.; Chen, X. Overcoming the Achilles’ heel of photodynamic therapy. *Chem. Soc. Rev.* **2016**, *45*, 6488-6519, doi:10.1039/C6CS00616G.

9. Abrahamse, H.; Hamblin, M.R. New photosensitizers for photodynamic therapy. *Biochem. J.* **2016**, *473*, 347-364, doi:10.1042/BJ20150942.

10. Dąbrowski, J.M.; Arnaut, L.G. Photodynamic therapy (PDT) of cancer: from local to systemic treatment. *Photochem. Photobiol. Sci.* **2015**, *14*, 1765-1780, doi:10.1039/C5PS00132C.

11. Mroz, P.; Yanoskavsky, A.; Kharkwal, G.B.; Hamblin, M.R. Cell Death Pathways in Photodynamic Therapy of Cancer. *Cancers* **2011**, *3*, 2516-2539, doi:10.3390/cancers3022516.

12. Azzouzi, A.-R.; Vincendeau, S.; Barret, E.; Cicco, A.; Kleinlauss, F.; van der Poel, H.G.; Stief, C.G.; Rassweiler, J.; Salomon, G.; Solsona, E., et al. Padeliporfin vascular-targeted photodynamic therapy versus active surveillance in men with low-risk prostate cancer (CLIN1001 PCM301): an open-label, phase 3, randomised controlled trial. *Lancet Oncol.* **2017**, *18*, 181-191, doi:10.1016/S1470-2045(16)30661-1.

13. van Straten, D.; Mashayekhi, V.; de Bruijn, H.; Oliveira, S.; Robinson, D. Oncologic Photodynamic Therapy: Basic Principles, Current Clinical Status and Future Directions. *Cancers* **2017**, *9*, 19, doi:10.3390/cancers9020019.

14. Luz, A.F.S.; Pucelik, B.; Pereira, M.M.; Dąbrowski, J.M.; Arnaut, L.G. Translating phototherapeutic indices from in vitro to in vivo photodynamic therapy with bacteriochlorins. *Lasers Surg. Med.* **2018**, *50*, 451-459, doi:10.1002/lsm.22931.

15. Celli, J.P.; Spring, B.Q.; Rizvi, I.; Evans, C.L.; Samkoe, K.S.; Verma, S.; Pogue, B.W.; Hasan, T. Imaging and Photodynamic Therapy: Mechanisms, Monitoring, and Optimization. *Chem. Rev.* **2010**, *110*, 2795-2836, doi:10.1021/cr900300p.

16. Rai, P.; Mallidi, S.; Zheng, X.; Rahmanzadeh, R.; Mir, Y.; Elrington, S.; Khurshid, A.; Hasan, T. Development and applications of photo-triggered theranostic agents. *Adv. Drug Delivery Rev.* **2010**, *62*, 1094-1124, doi:10.1016/j.addr.2010.09.002.

17. Bhaumik, J.; Mittal, A.K.; Banerjee, A.; Chisti, Y.; Banerjee, U.C. Applications of phototheranostic nanoagents in photodynamic therapy. *Nano Res.* **2015**, *8*, 1373-1394, doi:10.1007/s12274-014-0628-3.
18. Pierre, V.C.; Allen, M.J.; Caravan, P. Contrast agents for MRI: 30+ years and where are we going? *JBC*, 2014, 19, 127-131, doi:10.1007/s00275-013-1074-5.

19. Evelhoch, J.L. In vivo MR in the drug pipeline. *J. Magn. Reson.* 2018, 292, 117-128, doi:10.1016/j.jmr.2018.04.012.

20. De León-Rodríguez, L.M.; Martins, A.F.; Pinho, M.C.; Rofsky, N.M.; Sherry, A.D. Basic MR relaxation mechanisms and contrast agent design: MR Relaxation Mechanisms and Contrast Agents. *Journal of Magnetic Resonance Imaging* 2015, 42, 545-565, doi:10.1002/jmri.24787.

21. Josefsen, L.B.; Boyle, R.W. Unique Diagnostic and Therapeutic Roles of Porphyrins and Phthalocyanines in Photodynamic Therapy, Imaging and Theranostics. *Theranostics* 2012, 2, 916-966, doi:10.7150/thno.4571.

22. Kennedy, S.D.; Szczepaniak, L.S.; Gibson, S.L.; Hilf, R.; Foster, T.H.; Bryant, R.G. Quantitative MRI of Gd-DTPA uptake in tumors: Response to photodynamic therapy. *Magn. Reson. Med.* 1994, 31, 292-301, doi:10.1002/mrm.1910310308.

23. Haider, M.A.; Davidson, S.R.H.; Kale, A.V.; Weersink, R.A.; Evans, A.J.; Toi, A.; Gertner, M.R.; Bogaards, A.; Wilson, B.C.; Chin, J.L., et al. Prostate Gland: MR Imaging Appearance after Vascular Targeted Photodynamic Therapy with Palladium-Bacteriopheophorbide. *Radiology* 2007, 244, 196-204, doi:10.1148/radiol.2441060398.

24. Huang, Z.; Haider, M.A.; Kraft, S.; Chen, Q.; Blanc, D.; Wilson, B.C.; Hetzel, F.W. Magnetic resonance imaging correlated with the histopathological effect of Pd-bacteriopheophorbide (Tookad) photodynamic therapy on the normal canine prostate gland. *Lasers Surg. Med.* 2006, 38, 672-681, doi:10.1002/lsm.20375.

25. Schreurs, T.J.L.; Hectors, S.J.; Jacobs, I.; Grüll, H.; Nicolay, K.; Strijkers, G.J. Quantitative Multi-Parametric Magnetic Resonance Imaging of Tumor Response to Photodynamic Therapy. *PLoS One* 2016, 11, e0165759, doi:10.1371/journal.pone.0165759.

26. Zilberstein, J.; Schreiber, S.; Bloemers, M.C.W.M.; Bendel, P.; Neeman, M.; Schechtman, E.; Kohen, F.; Scherz, A.; Salomon, Y. Antivascular Treatment of Solid Melanoma Tumors with Bacteriochlorophyll-serine-based Photodynamic Therapy. *Photochem. Photobiol.* 2007, 73, 257-266, doi:10.1562/0031-6655(2001)073<0655:ATOSMT2.0.CO;2.

27. Schleich, N.; Danhier, F.; Prêt, V. Iron oxide-loaded nanotheranostics: Major obstacles to in vivo studies and clinical translation. *J. Controlled Release* 2015, 198, 35-54, doi:10.1016/j.jconrel.2014.11.024.

28. Kumar, R.; Shin, W.S.; Sunwoo, K.; Kim, W.Y.; Koo, S.; Bhuniya, S.; Kim, J.S. Small conjugate-based theranostic agents: an encouraging approach for cancer therapy. *Chem. Soc. Rev.* 2015, 44, 6670-6683, doi:10.1039/C5CS00224A.

29. Hofmann, B.; Bogdanov, A.; Marecos, E.; Ebert, W.; Semmler, W.; Weissleder, R. Mechanism of gadophrin-2 accumulation in tumor necrosis. *Journal of Magnetic Resonance Imaging* 1999, 9, 336-341, doi:10.1002/(SICI)1522-2586(199902)9:2<336::AID-JMRI28>3.0.CO;2-3.

30. Barkhausen, J.ö.; Ebert, W.; Debatin, J.ö.F.; Weinmann, H.-J. Imaging of myocardial infarction: comparison of magnevist and gadophrin-3 in rabbits. *J. Am. Coll. Cardiol.* 2002, 39, 1392-1398, doi:10.1016/S0735-1097(02)01777-1.

31. Daldrup-Link, H.; Rudelius, M.; Metz, S.; Piontek, G.; Pichler, B.; Settles, M.; Heinzmann, U.; Schlegel, J.r.; Oostendorp, R.J.; Rummeny, E. Cell tracking with gadophrin-2: a bifunctional contrast agent for MR imaging, optical imaging, and fluorescence microscopy. *Eur. J. Nucl. Med. Mol. Imaging* 2004, 31, doi:10.1007/s00259-004-1484-2.
32. Metz, S.; Daldrup-Link, H.E.; Richter, T.; Räth, C.; Ebert, W.; Settles, M.; Rummeny, E.J.; Link, T.M.; Piert, M. Detection and Quantification of Breast Tumor Necrosis with MR Imaging. *Academic Radiology* 2003, 10, 484-490, doi:10.1016/S1076-6332(03)00056-9.

33. Ni, Y. Metalloporphyrins and Functional Analogues as MRI Contrast Agents. *Curr. Med. Imaging Rev.* 2008, 4, 96-112, doi:10.2174/157340508784356789.

34. Haroon Ur, R.; Umar, M.N.; Khan, K.; Anjum, M.N.; Yaseen, M. Synthesis and relaxivity measurement of porphyrin-based Magnetic Resonance Imaging (MRI) contrast agents. *J. Struct. Chem.* 2014, 55, 910-915, doi:10.1134/S0022476614050163.

35. Gros, C.P.; Eggenspiller, A.; Nonat, A.; Barbe, J.-M.; Denat, F. New potential bimodal imaging contrast agents based on DOTA-like and porphyrin macrorycles. *Med. Chem. Commun.* 2011, 2, 119-125, doi:10.1039/C0MD00205D.

36. Eggenspiller, A.; Michelin, C.; Desbois, N.; Richard, P.; Barbe, J.-M.; Denat, F.; Licona, C.; Gaiddon, C.; Sayeh, A.; Choquet, P., et al. Design of Porphyrin-dota-Like Scaffolds as All-in-One Multimodal Heterometallic Complexes for Medical Imaging: Porphyrin-dota-Like Scaffolds for Medical Imaging. *Eur. J. Org. Chem.* 2013, 2013, 6629-6643, doi:10.1002/ejoc.201300678.

37. Trivedi, E.R.; Ma, Z.; Waters, E.A.; Macrenaris, K.W.; Subramanian, R.; Barrett, A.G.M.; Meade, T.J.; Hoffman, B.M. Synthesis and characterization of a porphyrazine-Gd(III) MRI contrast agent and in vivo imaging of a breast cancer xenograft model: TUMOR IMAGING. *Contrast Media Mol. Imaging* 2014, 9, 313-322, doi:10.1002/cmmi.1577.

38. Hindré, F.; Plouzennec, M.L.; de Certaines, J.D.; Foulquier, M.T.; Patrice, T.; Simonneaux, G. Tetra-p-aminophenylporphyrin conjugated with Gd-DTPA: Tumor-specific contrast agent for MR imaging. *Journal of Magnetic Resonance Imaging* 1993, 3, 59-65, doi:10.1002/jmri.1880030111.

39. Li, G.; Slansky, A.; Dobhal, M.P.; Goswami, L.N.; Graham, A.; Chen, Y.; Kanter, P.; Alberico, R.A.; Spernyak, J.; Morgan, J., et al. Chlorophyll-a Analogues Conjugated with Aminobenzyl-DTPA as Potential Bifunctional Agents for Magnetic Resonance Imaging and Photodynamic Therapy 1. *Bioconjugate Chem.* 2005, 16, 32-42, doi:10.1021/bc049807x.

40. Pandey, R.K.; Goswami, L.N.; Chen, Y.; Gryshuk, A.; Missert, J.R.; Oseroff, A.; Dougherty, T.J. Nature: A rich source for developing multifunctional agents, tumor-imaging and photodynamic therapy. *Lasers Surg. Med.* 2006, 38, 445-467, doi:10.1002/lsm.20352.

41. Sperry, J.R.; Batt, C.; Mazurchuk, R., et al. Hexylether Derivative of Pyropheophorbide-a (HPPH) on Conjugating with 3 Gadolinium(III) Aminobenzyltetraaminopentaacetic Acid Shows Potential for in Vivo Tumor Imaging (MR, Fluorescence) and Photodynamic Therapy. *Bioconjugate Chem.* 2010, 21, 828-835, doi:10.1021/bc9005317.

42. Goswami, L.N.; White, W.H.; Sperry, J.A.; Ethirajan, M.; Patel, N.J.; Goswami, L.; Chen, Y.; Turowski, S.; Missert, J.R.; Batt, C.; Mazurchuk, R., et al. Hexylether Derivative of Pyropheophorbide-a (HPPH) on Conjugating with 3 Gadolinium(III) Aminobenzyltetraaminopentaacetic Acid Shows Potential for in Vivo Tumor Imaging (MR, Fluorescence) and Photodynamic Therapy. *Bioconjugate Chem.* 2010, 21, 828-835, doi:10.1021/bc9005317.

43. Lattuada, L.; Barge, A.; Cravotto, G.; Giovanzana, G.B.; Tei, L. The synthesis and application of polyamino polycarboxylic bifunctional chelating agents. *Chem. Soc. Rev.* 2011, 40, 3019, doi:10.1039/c0cs00199f.

44. Aydin Tekdaş, D.; Garifullin, R.; Şentürk, B.; Zorlu, Y.; Gundogdu, U.; Atalar, E.; Tekinay, A.B.; Chernonosov, A.A.; Yerli, Y.; Dumoulin, F., et al. Design of a Gd-DOTA-Phthalocyanine Conjugate
Combining MRI Contrast Imaging and Photosensitization Properties as a Potential Molecular Theranostic. Photochem. Photobiol. 2014, 90, 1376-1386, doi:10.1111/php.12332.

Song, Y.; Zong, H.; Trivedi, E.R.; Vesper, B.J.; Waters, E.A.; Barrett, A.G.M.; Radosevich, J.A.; Hoffman, B.M.; Meade, T.J. Synthesis and Characterization of New Porphyrin-Gd(III) Conjugates as Multimodal MR Contrast Agents. Bioconjugate Chem. 2014, 90, 1376-1386, doi:10.1111/php.12332.

Song, Y.; Zong, H.; Trivedi, E.R.; Vesper, B.J.; Waters, E.A.; Barrett, A.G.M.; Radosevich, J.A.; Hoffman, B.M.; Meade, T.J. Synthesis and Characterization of New Porphyrin-Gd(III) Conjugates as Multimodal MR Contrast Agents. Bioconjugate Chem. 2014, 90, 1376-1386, doi:10.1111/php.12332.

Hambright, P. Tissue distribution and stability of metalloporphyrin MRI contrast agents. Magn. Reson. Med. 1987, 4, 24-33, doi:10.1002/mrm.1910040104.

Yusheva, D.V.; Lermontova, S.A.; Grigoryev, I.S.; Muravieva, M.S.; Gavrina, A.I.; Shirmanova, M.V.; Balalaev, I.V.; Klapshina, L.G.; Zagaynova, E.V. In vivo multimodal tumor imaging and photodynamic therapy with novel theranostic agents based on the porphyrin framework-chelated gadolinium (III) cation. Biochim. Biophys. Acta, Gen. Subj. 2017, 1861, 3120-3130, doi:10.1016/j.bbagen.2017.09.004.

Wu, B.; Li, X.-Q.; Huang, T.; Lu, S.-T.; Wan, B.; Liao, R.-F.; Li, Y.-S.; Baidya, A.; Long, Q.-Y.; Xu, H.-B. MRI-guided tumor chemophotodynamic therapy with Gd/Pt bifunctionalized porphyrin. Biomater. Sci. 2017, 5, 1746-1750, doi:10.1039/C7BM00431A.

Luo, J.; Chen, L.-F.; Hu, P.; Chen, Z.-N. Tetranuclear Gadolinium(III) Porphyrin Complex as a Theranostic Agent for Multimodal Imaging and Photodynamic Therapy. Inorg. Chem. 2014, 53, 4184-4191, doi:10.1021/ic500238s.

Sour, A.; Jenni, S.; Orti-Suárez, A.; Schmitt, J.; Heitz, V.; Bolze, F.; Loureiro de Sousa, P.; Po, C.; Bonnet, C.S.; Pallier, A., et al. Four Gadolinium(III) Complexes Appended to a Porphyrin: A Water-Soluble Molecular Theranostic Agent with Remarkable Relaxivity Suited for MRI Tracking of the Photosensitizer. Inorg. Chem. 2016, 55, 4545-4554, doi:10.1021/acs.inorgchem.6b00381.

Kolb, F.; Jenni, S.; Sour, A.; Heitz, V. Molecular Photosensitizers for Two-Photon Photodynamic Therapy. Chemical Communications 2017, 53, 12857-12877, doi:10.1039/C7CC06133A.

Sun, Z.; Zhang, L.-P.; Wu, F.; Zhao, Y. Photosensitizers for Two-Photon Excited Photodynamic Therapy. Advanced Functional Materials 2017, 27, 1704079, doi:10.1002/adfm.201704079.

Schmitt, J.; Heitz, V.; Sour, A.; Bolze, F.; Kessler, P.; Flamigni, L.; Ventura, B.; Bonnet, C.S.; Tóth, É. A Theranostic Agent Combining a Two-Photon-Absorbing Photosensitizer for Photodynamic Therapy and a Gadolinium(III) Complex for MRI Detection. Chem. - Eur. J. 2016, 22, 2775-2786, doi:10.1002/chem.201503433.

Schmitt, J.; Jenni, S.; Sour, A.; Heitz, V.; Bolze, F.; Pallier, A.; Bonnet, C.S.; Tóth, É.; Ventura, B. A Porphyrin Dimer–GdDOTA Conjugate as a Theranostic Agent for One- and Two-Photon Photodynamic Therapy and MRI. Bioconjugate Chem. 2018, 29, 3726-3738, doi:10.1021/acs.bioconjchem.8b00634.