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Texaphyrins and water-soluble zinc(II) ionophores: development, mechanism of anticancer activity, and synergistic effects

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Abstract

Texaphyrins, first prepared by Sessler and coworkers in the 1980s, represent early examples of expanded porphyrins. This class of pentaaza, oligopyrrolic macrocycles demonstrates excellent tumor localization and metal-chelating properties. In biological milieus, texaphyrins act as redox mediators and are able to produce reactive oxygen species. Furthermore, texaphyrins have been shown to upregulate zinc *in vivo*, an important feature that inspired us to develop new zinc ionophores that might allow the same function to be elicited but via a simpler chemical means. In this review, the basic properties of texaphyrins and the zinc ionophores they helped spawn will be discussed in the cadre of developing an understanding that could lead to the preparation of new, redox-active anticancer agents.

Keywords

expanded porphyrins; MRI contrast agents; porphyrins; radiation sensitizers; reactive oxygen species; zinc ionophores

Introduction

Texaphyrins are pentaaza, Schiff-base expanded porphyrins that were first reported by Sessler et al. in 1988 (Sessler and Seidel, 2003). They were then studied extensively as

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ligands useful for stabilizing 1:1 complexes of larger metal cations, particularly those of the trivalent lanthanide series. Among the texaphyrins prepared to date, the water-solubilized Gd(III) species known as motexafin gadolinium (MGd; Figure 1) has received the most attention, having been studied in considerable detail by Pharmacyclics, Inc., a publicly traded company cofounded by Richard A. Miller and Jonathan L. Sessler in 1991 (Miller et al., 1999; Rosenthal et al., 1999; Young et al., 1999; Sessler and Miller, 2000; Carde et al., 2001; Mody et al., 2001; Mehta et al., 2002, 2003).

One of the more striking characteristics of water-soluble texaphyrins is their ability to accumulate selectively in cancer cells (Carde et al., 2001; Mody et al., 2001; Mehta et al., 2002, 2003). In the case of the Gd(III) derivative, MGd, this property allows for the selective visualization of the compound within tumorous lesions via magnetic resonance imaging (MRI). The paramagnetic nature of the centrally coordinated gadolinium(III) cation and the presence of two exchangeable axial coordination sites makes MGd an effective T_1 contrast agent (Young et al., 1994). MGd was also found to exhibit moderate cytotoxic properties on its own. The likely determinants for this latter feature and some of the studies it engendered are discussed in detail below.

To date, MGd has been the focus of a large number of clinical trials, including ones involving advanced head and neck cancer, multiple myeloma, chronic lymphocytic leukemia, non-Hodgkins lymphoma, and recurrent glioma. Unfortunately, however, efforts to obtain regulatory approval for MGd as an adjuvant for the whole-brain radiation-based treatment of metastatic non-small cell lung cancer failed. Although the most advanced trial, a Phase III study involving this proposed indication, revealed what could be taken as signs of efficacy, the pre-negotiated statistical endpoints were not met. The drug was thus ultimately rejected for this indication by the Food and Drug Administration (FDA) in December 2007 (Jungbauer, 2007). However, the ability of metallotexaphyrins to localize in neoplastic lesions, coupled with their unique mode of action, is currently making texaphyrins of interest as potential enhancers of other cancer chemotherapeutics and as carriers for agents, such as the platinum drugs, whose localization and toxicity profiles could benefit from improvement. This provides an ongoing incentive to understand the mode of action of MGd and its implications. Efforts along these lines are summarized in this review.

Localization of texaphyrins in tumorous tissues

Tissue and subcellular localization studies conducted with MGd and other texaphyrin species revealed that texaphyrins are incorporated into cells via an active transport process (Woodburn, 2001; Byrne et al., 2003). MGd has been shown to be taken up selectively by non-small cell lung cancer, breast cancer tissues, chronic lymphocytic leukemia, non-Hodgkins lymphoma, and myeloma cells (Evens et al., 2005). On the basis of fluorescence-based colocalization studies, texaphyrins were found to be located within lysosomes, endosomes, and mitochondria. However, no significant incorporation into the nucleus was observed over the full multihour time scale associated with these studies. Taken in concert, these findings provide support for the notion that the primary biological function of MGd is not the direct modification of DNA. Rather, as detailed below, alternative modes of action,

including perturbation of cytoplasmic redox processes, are believed to be operative (Magda et al., 2001).

In early animal studies, the ratio of MGd in tumor cells to that in surrounding normal cells was reported to be 9:1 (Miller et al., 1999). A subsequent report, based on initial clinical data, led to the conclusion that this ratio increases to 50:1 in the case of metastatic brain tumors (cf. Figure 2) (Mehta et al., 2002). This latter finding is best interpreted in terms of an ability to accumulate and persist in brain metastases. Interestingly, it was found that either the gadolinium texaphyrin or the gadolinium(III) cation, originally coordinated in the texaphyrin core, remains in the cancerous milieu of patients for several months. This selective accumulation in brain tumors stimulated investigation of MGd in the context of stereotactic radiosurgery (McHaffee et al., 2011).

Complexation ability and photophysical properties of texaphyrins

As mentioned briefly above, one prominent feature of texaphyrins is their ability to complex larger cations, specifically those that cannot be readily accommodated in a 1:1 fashion by more traditional ligands such as, e.g., the porphyrins. This ability is rationalized in terms of the texaphyrins containing five, rather than four, inward-pointing nitrogen atoms; the texaphyrins also possess an inner coordinating core that is roughly 20% larger than that of the porphyrins. Of particular note is that texaphyrins are able to form stable 1:1 complexes with a variety of metal cations, including all members of the lanthanide(III) series with the exception of promethium, which does not have any stable (or even long-lived) isotopes. The highly versatile metal complexation features of the texaphyrins are highlighted in Figure 3 (Preihs et al., 2013).

The UV-Vis spectrum of MGd **1** is dominated by two absorption bands (cf. Figure 4). The higher-energy absorption centered at 474 nm is analogous to the ~400-nm Soret band of porphyrins and is characteristic of the absorption features seen for other vividly pigmented porphyrin-type moieties. The Soret-like band is flanked by N- and Q-like bands at higher and lower energies, respectively, with the lowest-energy Q band for MGd being seen at 740 nm.

Interestingly, there is a steady shift in the Q-like band from red to the blue ($\lambda = 15$ nm) as the Ln(III) ion in question is changed from lanthanum to lutetium. This shift in the Q-like bands appears to follow the contraction of the metal ions as the lanthanide series is traversed. A plot of the wavelength (in nm) of the Q-like band versus the ionic radius of the Ln(III) ion gives a linear relationship (cf. Figure 5) (Hemmi, 1992).

MGd cytotoxicity and proposed mode of action

One remarkable feature of the lanthanide(III) complexes of texaphyrin is exhibited in their redox behavior. Although not easy to be oxidized, these complexes are reduced readily in the presence of electron-rich species, such as ascorbate (cf. Scheme 1) (Mikheev and Kamenskaya, 1991; Sessler et al., 1994, 1999). This is clearly another sharp contrast to what is true for metalated and unmetalated porphyrins. It is also an important feature that led to explorations of texaphyrins as possible radiation sensitizers for use in anticancer therapy. In

the context of the latter investigations, particular emphasis was placed on trying to enhance the efficacy of standard radiation therapy treatments by overwhelming the natural defense mechanisms against so-called reactive oxygen species (ROS).

Prior to the initial clinical studies of MGd, it was known that several classes of anticancer drugs, e.g., quinone- based agents, were able to promote the generation of ROS at tumor sites (Mikheev and Kamenskaya, 1991). It was also known that a variety of enzymes, including superoxide dismutase (SOD), glutathione peroxidase, and catalase, serve to regulate ROS levels. It was also appreciated that other endogenous species, such as glutathione (GSH), thioredoxin/thioredoxin reductase, ascorbate (vitamin C), and α -tocopherol (vitamin E), act to regulate the cellular redox environment. Many of these latter agents can serve as ROS scavengers. Compounds that either compromise these defense mechanisms or are able to produce ROS are thus attractive as potential anticancer therapeutic agents. In this light, MGd, as a cancer-localizing agent capable of enhancing ROS levels, was considered worthy of detailed investigation. The electrochemical potential (vs. Ag/AgCl in dimethylformamide, 0.1 M tetrabutyl ammonium perchlorate, 200 mV/s) for the cyclic voltammetric reduction of MGd was reported to be -263 mV (reduction I $E_{1/2}$) and -757 mV (reduction II $E_{1/2}$) (Sessler et al., 1999).

Within the intracellular environment, MGd is able to accept an electron from an endogenous electron-rich species, such as ascorbate, reduced nicotinamide-adenine dinucleotide phosphate (NADPH), thioredoxin reductase, GSH, or dihydrolipoate. MGd catalyzes the oxidation of these reducing metabolites (see Scheme 1) (Magda et al., 2002; Evens et al., 2005). Important to note is the fact that the unfavorably high reduction potential for the gadolinium(III)/(II) couple leads us to suggest that the reduction occurs on the texaphyrin ligand rather than at the metal center (Mikheev and Kamenskaya, 1991; Sessler et al., 1999). This reduced texaphyrin radical then in turn reacts with oxygen to form first superoxide. This latter species then disproportionates to form hydrogen peroxide, a process that within cells is likely mediated through the action of SOD (Guldi et al., 2000). The underlying electron transfer processes are fast on the laboratory time scale and lead to the regeneration of MGd, which is thus available to recommence the process. As implied in Scheme 1, superoxide and hydrogen peroxide are produced *in vitro*, and presumably *in vivo*. Peroxide is known to function as apoptosis trigger (Koch and Biaglow, 1978; Sessler et al., 2001).

Because MGd is able to accumulate selectively in neoplastic tissues, selective cancer cell death is expected. In fact, diminished levels of cell proliferation across a number of cell lines are seen with MGd. These effects are enhanced in the presence of added ascorbate. On this basis, the antiproliferative effect produced by MGd is ascribed in large measure to the production of ROS. An increase of oxidative stress is also observed in cell studies. This is presumably due to an MGd-engendered decrease in the concentration of reducing metabolites, such as GSH and ascorbate, species that under normal circumstances serve to mitigate the effects of oxidative stress. The key reactions of MGd and reducing metabolites to form ROS are outlined in Scheme 2.

Prior to understanding that MGd would react with reducing metabolites it was thought that MGd would mediate a radiation-enhancing effect by acting as an 'electron sponge' (Adams

et al., 1976). Specifically, it was believed that MGd would be able to trap solvated electrons produced when ionizing radiation (such as X-ray radiation) interacts with water molecules. This would increase the concentration of reactive hydroxyl radicals, which, in turn, could then lead to DNA damaging and ultimately cell death (Bump and Brown, 1990).

More detailed subcellular localization studies revealed that MGd is unable to enter the nucleus (*vide supra*), thus making this mode of action rather unlikely. In fact, pulse radiolysis experiments designed to confirm that MGd would trap solvated electrons revealed that after electron capture, the one-electron reduced form of MGd would react with molecular dioxygen in a fast, reversible manner to produce superoxide. The rate constant for the nonenzymatic disproportionation of superoxide is reported to be $1 \times 10^5 \text{ m}^{-1} \text{ s}^{-1}$ at physiological pH (Buettner, 1993). In biological milieus, superoxide is rapidly converted into hydrogen peroxide, as noted above.

Because superoxide and hydrogen peroxide are known cytotoxic species that are capable of inducing apoptosis (programmed cell death) and potentiating the effects of ionizing radiation (Koch and Biaglow, 1978; Biaglow et al., 1992; Magda et al., 2002), it was believed that these redox processes would play a key role in mediating the biological activity of MGd. More recently, it has been proposed that the ability of MGd to produce ROS could provide a synergetic benefit when used in conjunction with other treatment modalities, including magnetic hyperthermia (Yoo et al., 2012).

As detailed earlier in this review, the basic texaphyrin ligand is able to form stable complexes with a variety of lanthanide(III) metals. The biological activity of these metal complexes could differ from that of MGd. The reason for this supposition is that the atomic radius of the lanthanide cation contracts as the series is traversed from left to right in the periodic table (cf. Figure 3). Thus, the corresponding texaphyrin complexes exhibit a gradual increase in lipophilicity, ease of reduction, and stability as the series is traversed. This means that, e.g., the dysprosium(III) analogue of MGd displays a slightly different redox potential than the corresponding gadolinium(III) complex. In fact, the lutetium(III) analog of MGd, MLu, has been found to be effective in terms of oxidizing NADPH; however, it is not particularly reactive with regard to ascorbate (Sessler et al., 1999). This could lead to a more refined biological response for MLu than MGd. Unfortunately, MLu suffers significantly from aggregation in aqueous media. Other problems, including solubility, plague other analogues of MGd. Thus, detailed comparisons of structure and function across the lanthanide series have yet to be completed.

MGd and interactions with thiol groups

As mentioned previously, in addition to the electron-rich species NADPH and ascorbate, MGd is also able to react with the thiol groups present in a variety of biologically important molecules. In general, peptides and proteins contain cysteine as one or more of their building blocks, and generally these species are redox active. Typically, the sulfur-bearing functional sites are found as either part of a disulfide linkage or in the corresponding reduced, sulfhydryl (thiol) form. In the presence of MGd and oxygen, the thiol forms can trigger generation of ROS. In fact, vicinal thiols have proved to be particularly reactive;

these species, when combined with MGd and dioxygen, serve to generate superoxide and hydrogen peroxide (via a combination of disproportionation and direct reaction with the substrate) as shown in Scheme 3 (Magda et al., 2005a).

It is well appreciated that sulfhydryl groups are able to bind a variety of metal cations. This is particularly important in the case of the metallothioneins (Kagi and Schaffer, 1988). Among other metals, this family of cysteine-rich, low molecular weight proteins is believed to be a major site of intracellular zinc storage and transport (Vallee, 1991; Cherian et al., 2003). Therefore, Zn-metallothioneins play a key role in maintaining zinc homeostasis. Upon oxidation of metallothionein, a transformation that can occur coincident with MGd reduction, free zinc, or so-called non-chelatable zinc, is released inside the cell. This has important biological consequences.

Maintaining zinc homeostasis is important for normal cell function. When perturbations occur, including when an excess of intracellular free zinc is present, a series of biochemical responses is induced. These include the production of zinc transporter 1, a plasma membrane-bound protein able to transport zinc to the cell exterior (Langmade et al., 2000; Andrews, 2001; Giedroc et al., 2001; Lichtlen and Schaffner, 2001; Lichtlen et al., 2001; Cousins et al., 2003; Hasumi et al., 2003). Furthermore, expression of more thionein is induced, presumably to overcome directly the effect of excess free zinc. If the oxidation of metallothionein through MGd-mediated processes occurs, and does so at a level that is able to overwhelm these natural responses, changes at the cellular level due to the effects of excess free zinc would be expected to be manifest. These effects include the triggering of various apoptosis mechanisms, as shown in Scheme 4 and discussed further below.

In general, an increased concentration of free zinc has been proposed to stabilize hypoxia-inducible factor-1 (HIF-1) and thus influence processes such as glycolysis, angiogenesis, and apoptosis (Semenza, 2003; Schofield and Ratcliffe, 2004; Hirsila et al., 2005; Lecane et al., 2005). Moreover, as can be inferred from an inspection of Scheme 4, the enzyme thioredoxin reductase, which is responsible for the reduction of disulfide bonds to sulfhydryl functional groups in metal-free thionein and a key mediator in the cellular response to oxidative stress, is also deactivated in the presence of free zinc. It is also deactivated by MGd. This latter finding was rationalized in terms of the redox features of MGd, specifically its acting as a catalytic conduit for the transfer of electrons present in metallothionein to dioxygen and the release of free zinc, as noted above. Thus, MGd was expected to act as a potent disruptor of zinc homeostasis in tumor cells.

On the basis of detailed experiments involving the zinc-specific fluorescent probe, FluoZinTM-3, MGd was reported to effect the release of free zinc in A549 human lung cancer, PC3 prostate cancer, and Ramos B-cell lymphoma cells (Schofield and Ratcliffe, 2004). Additional support for the suggestion that MGd serves to disrupt zinc homeostasis as the result of specific, compound-mediated redox reactions came from Northern blotting and lipoate reduction inhibition measurements, together with flow cytometry (Biaglow et al., 2000; Gee et al., 2002).

The upregulation of zinc by MGd is of particular interest in cancer therapy because of the role intracellular free and non-protein-bound zinc plays in regulating cellular functions. In fact, the finding that MGd serves as a potent agent for the disruption of zinc homeostasis, coupled with its ability to produce ROS in a tumor-selective manner, was put forward as an explanation for the suggestions of efficacy inferred from various clinical trials (Mehta et al., 2003).

Rationale for developing new zinc ionophores

To study further the effect of zinc in the determinants of activity in the case of MGd, a number of additional experiments were conducted. Among the more informative of these were *in vitro* assays that were designed to measure the bioreductive activity of MGd as a function of modulated zinc levels. Toward this end, studies were carried out where MGd was assayed in the presence of the zinc ionophore, zinc hydroxypyridinethione (ZnHPT; complex **2**, Figure 6) (Magda et al., 2005b). The latter complex is used widely as a bactericide and fungicide (Doose et al., 2004). In accord with expectations, synergy was observed.

ZnHPT is of intrinsic interest in the context of drug development. It has been shown to be able to increase intracellular zinc *in vitro*. It has also been administered to laboratory animals for toxicity testing and to probe the effects of zinc homeostasis *in vivo*. In these tests, the complex demonstrated high potency (Jeffcoat et al., 1980; Gibson et al., 1982; Jasim and Tjalve, 1986a,b). However, the compound lacks solubility in water and is poorly bioavailable (Jasim and Tjalve, 1986b).

Appreciating the potential role that zinc delivery might play in mediating a direct anticancer effect and in an effort to overcome the deficiencies associated with ZnHPT, new water-soluble hydroxypyridinethione ligands were developed (complexes **3–5**; Figure 6). These new systems bear short polyethylene glycol chains, a feature that was expected to increase both water solubility and bioavailability (Magda et al., 2008).

Using complexes **3–5**, it was found that ionophore treatment led to cell death in non-cycling A549 (adenocarcinomic human alveolar basal epithelial) cell lines at higher concentrations. Antiproliferative effects are observed even at low concentrations and in the absence of supplementary zinc. However, these effects were enhanced in the presence of supplementary zinc (cf. Figure 7) (Magda et al., 2008).

It has been calculated that a 1 $\mu\text{mol/l}$ solution of ZnHPT **2** at pH 7 is actually composed of 43% unbound zinc, 43% zinc bound by one ligand, and merely 14% intact complex (Doose et al., 2004). To the extent that similar speciation holds for **3–5**, it could account for why a greater effect is seen upon co-incubation with additional zinc (as zinc acetate present in the supplemented medium), particularly in exponential and plateau phase cultures. As the effective concentrations of the complex tend to be higher in plateau phase cultures where specific zinc-dependent behavior was seen, one can assume that the biologically active species is the intact zinc complex. This observation is supported by the fact that cell death in plateau phase cultures correlates with increased intracellular free zinc levels. Furthermore,

the modified and water-soluble zinc ionophores **4** and **5** are also able to suppress tumor growth in nude mice xenografted with A549 or PC3 (human prostate cancer) tumors.

In contrast to the above, the partial dissociation of the ionophore leads us to suggest that the antiproliferative activity of ZnHPT (or its analogues) observed in non-plateau phase cells is due in part to the presence of unbound ligands. Presumably, the zinc-free species are able to interfere with other cellular processes, perhaps by binding a variety of biologically important metal cations. To the extent this is true, it would mean that the ligands giving rise to **2–5** and perhaps newer analogues could show direct antiproliferative effects that are not zinc dependent.

Because MGd **1** is able to upregulate zinc and therefore potentiate the effects of zinc *in vitro* (cf. Figure 8) (Magda et al., 2005b) the synergistic effect of MGd in combination with zinc ionophore **5** was also examined. No appreciable A549 tumor growth inhibition could be observed using MGd alone. However, in combination with complex **5**, significant tumor growth inhibition was observed.

In the PC3 model, tumor growth was inhibited in a significant manner using zinc ionophore **4** in mice at a dose level of 100 $\mu\text{mol/kg}$ (300 $\mu\text{mol/m}^2$) (cf. Figure 9) after four doses, but not after two doses. These findings lead us to suggest that even more effective tumor control could be obtained by using zinc ionophores in combination with MGd, particularly in the context of an optimized and pro-longed treatment regimen. A further attractive feature is that complexes **3–5** showed no acute signs of toxicity. For instance, complexes **4** and **5** were found to be well tolerated at the highest intravenous dose (120 mmol/kg, 360 $\mu\text{mol/m}^2$) (Magda et al., 2008).

The above findings are intriguing; this is particularly so for those involving the PC3 (prostate) cell line. Prostate cancer is the second leading cause of death from cancer among men in the USA (Altekruse et al., 2010; American Cancer Society, 2012). Because current treatment options often involve aggressive surgery, with the choice of modality often being uncertain for many patients, new treatment options are much needed. This is underscored by the fact that of the 250,000 men diagnosed with prostate cancer this year, approximately 28,000 patients will fail to respond to treatment and will die (Altekruse et al., 2010; American Cancer Society, 2012). As opposed to zinc concentrations in normal prostate tissue or benign prostatic hypertrophy, zinc levels in tissues from fully developed neoplastic disease are extremely low (Costello and Franklin, 2006). Therefore, it has been proposed that zinc is playing a critical role in the underlying transformations in the progression of prostate disease (Feng et al., 2003; Franklin et al., 2005; Costello and Franklin, 2006; Franklin and Costello, 2009; Johnson et al., 2010; Costello and Franklin, 2011; Trock, 2011).

In 2006, Costello and Franklin suggested that the restoration of high zinc levels in premalignant/malignant prostate cells will arrest and/or abort prostate malignancy (Costello and Franklin, 2006). In their 2006 study and in more recent reports water-soluble zinc salts were used for zinc-cancer correlation experiments (Costello and Franklin, 2006; Dubi et al., 2008; Feng et al., 2008; Golovine et al., 2008; Wong and Abubakar, 2008; Lin et al., 2009;

Yang et al., 2009). Unfortunately, these salts are not suitable as drugs due to their hydrophilicity and inability to cross cell membranes easily. Further, they are likely to be excreted rapidly if administered *in vivo*. Therefore, we believe that more biocompatible approaches to synthetic ion transport will be required if treatment protocols based on increasing cellular zinc are to advance toward clinical testing. On this basis, we propose that our approach to upregulate zinc in tumor cells using efficient and well-tolerated zinc ionophores may have a role to play in the context of developing novel prostate cancer protocols. Used alone or in combination with MGd, systems such as **3–5** could thus represent a new and potentially beneficial approach to anticancer treatment. Further tests of this appealing postulate are currently ongoing in our laboratory.

Conclusions

In this review we have traced the redox-based mode of action put forward for a lead texaphyrin complex, MGd. On the basis of the underlying analyses, it was determined that MGd mediates its effect in part through the frank involvement of zinc-related homeostasis-related processes. This, in turn, has led to a new approach to possible anticancer drug development, wherein biocompatible zinc ionophores are used to mediate changes in zinc concentrations *in vivo*. The combination of these ionophores with other agents, including MGd, that serve to induce changes to the cellular redox states, is viewed as a particularly attractive approach to generating new cancer chemotherapies. Such putative therapies could prove particularly useful in the case of prostate cancer where a combination of redox and changes in zinc concentration accompanies the onset of disease.

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Figure 1.
Structure of motexafin gadolinium (MGd) 1.

Figure 2.

T₁ weighted noncontrast brain MRI before (A) and after (B) administration of MGD. Figure reprinted with permission from (Carde et al., 2001). Copyright 2013 by the American Society of Clinical Oncology.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rd
Fr	Ra	Ac															
Lanthanides		Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
Actinides		Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr		

Figure 3.

Stable texaphyrin complexes with all metals shown in green are known. Figure reprinted with permission from (Preihs et al., 2013). Copyright 2013 by The American Chemical Society.

Figure 4.
UV-Vis spectrum of MGd 1.25 μm in methanol.

Figure 5.
Plot of the wavelength (in nm) of the Q-like band versus the ionic radius of several Ln(III) ions (Hemmi, 1992).

Figure 6.
ZnHPT and water-soluble ZnHPT analogues.

Figure 7. Cell proliferation assay of A549 cells with zinc ionophore 4 upon addition of supplemented zinc.

Figure 8. (A) Effect of MGd and zinc treatment [as $\text{Zn}(\text{OAc})_2$] on proliferation of A549 cells. (B) Effect of MGd and zinc treatment [as $\text{Zn}(\text{OAc})_2$] on proliferation of PC3 cells. For both (A) and (B), exponential growth phase cultures were treated with MGd (0, 6.25, and 25 μM) and metal cation (0–100 μM) for 24 h, medium replaced, and proliferation relative to control assessed by colorimetric assay after 72 h. Figure reprinted with permission from (Magda et al., 2005b). Copyright 2013 by The American Society for Cancer Research.

Figure 9.

Preliminary *in vivo* results with zinc ionophore 4 (PC3 xenograft model) in mice at a dose level of 100 $\mu\text{mol/kg}$ (300 $\mu\text{mol/m}^2$) (Magda et al., 2008).

Scheme 1.
Interaction of MGd in the production of ROS.

Scheme 2.

Key redox reactions of MGd and reducing metabolites.

Scheme 3.

Redox reactions involving a representative vicinal thiol.