Molecular Mn-Based Contrast Agents

Subjects: Chemistry, Inorganic & Nuclear

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MRI contrast agents are required in clinic to detect some pathologies, such as cancers. Nevertheless, at the moment, only small extracellular and non-specific gadolinium complexes are available for the clinicians. Moreover, safety issues have recently appeared concerning the use of gadolinium complexes, so that alternatives are urgently needed. Manganese-based MRI contrast agents could be one of these alternatives and more and more studies are available in the literature. This work aims at synthesizing all those researches, to highlight all the efforts already made by the scientific community to obtain highly efficient agents, but also evidence the weaknesses of the developed systems.

Keywords: Mn complexes ; magnetic resonance imaging

1. Non-Specific Contrast Agents

Similarly to the Gd-complexes, the efficacy of molecular Mn-based contrast agents is based on the presence of at least one exchanging water molecule in the inner coordination sphere of the metal characterized by a fast exchange rate. With the typical coordination number of Mn(II) complexes in aqueous solution being six, seven, or sometimes eight, this innersphere water molecule is assured if the ligand possesses five or six coordination bonds with the metal. Nevertheless, the thermodynamic stability and the kinetic inertness of Mn complexes is generally lower than that of Gd-complexes because of the lower charge of Mn ions and the lack of ligand-field stabilization energy (high spin d^5 electron configuration). Moreover, the possible oxidation of Mn^{2+} to Mn^{3+}, which is often related to the thermodynamic stability of the Mn(II)-complex, also has to be avoided as it will lead to a loss of efficacy because of the loss of one unpaired electron and of a less favorable electronic relaxation.

Complexes based on linear ligands:

EDTA (ethylene diamine tetraacetic acid, **Figure 1**) is a linear hexadentate ligand able to form very stable complexes with Mn ions and was thus extensively studied. Indeed, previous works on $[Mn(EDTA)(H_2O)]^{2-}$ have shown that its sodium salt is very well tolerated: LD50 is 7.0 mmol/kg in rats following intravenous injection compared with an LD50 of 0.22 mmol/kg for $MnCl_2$ [1]. Moreover, it allows the presence of one fast-exchanging water molecule so that the relaxivities of the complexes $[Mn(EDTA)(H_2O)]^{2-}$ and $[Gd(DTPA)(H_2O)]^{2-}$ are similar (2.9 and 4.1 mM^{-1} s^{-1}, respectively, at 20 MHz, 35 °C) (**Table 1**) [2]. The increased relaxivity of the Gd(III) complex may be due to its larger size and slower tumbling rate. As it is well-known that a decrease in the tumbling rate can boost the efficacy of the contrast agents, several studies have tried to increase the size of the Mn-complex. Other researches were focused on the improvement of the stability of the Mn-complexes based on linear ligands by rigidifying the chelator. Mn-PyC3A [3][4][5][6] can for example be cited for its good thermodynamic (pMn of 8.17 at pH 7.4) and kinetic stability as well as a good relaxivity of 2.1 s^{-1} mM^{-1} at 1.4 T and 37 °C (**Table 1**); it has recently started phase I clinical trials (NCT05413668). Moreover, it has been demonstrated as a potential alternative to gadolinium to characterize acute myocardial infarctions [7]. In the attempts to increase the stability of the complexes and the kinetic inertness toward endogenous ions such as Zn^{2+} ions, the work of Wadepohl et al. [8] opens interesting perspectives. It is based on bispidine derivatives providing rigid and large coordination cavities that perfectly match the size of Mn^{2+} ions.

All those cited complexes often represent difficult synthetic procedures; the study by Stasiuk et al. [9], who proposed a single-pot template reaction to obtain an Mn-based contrast agent endowed with a good kinetic inertness toward zinc transmetallation, as well as an interesting relaxivity of 5.2 s^{-1} mM^{-1} at 1.5 T and 298 K, is thus interesting.

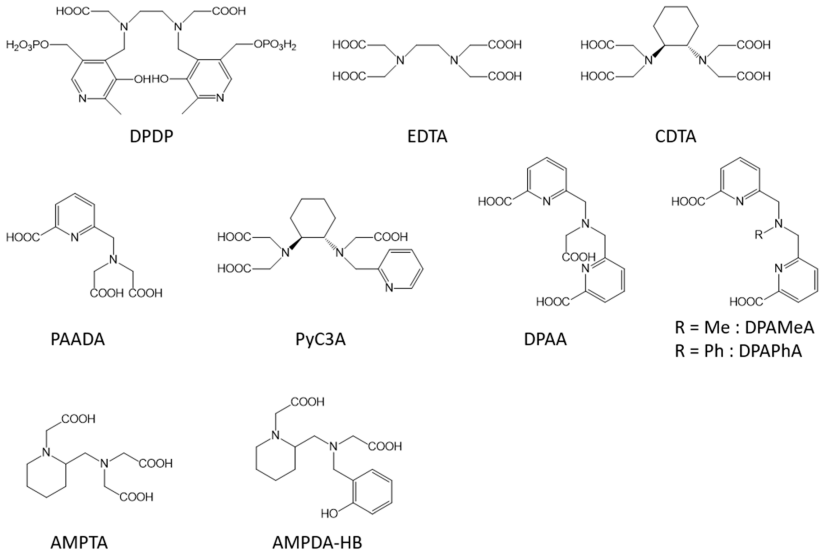

Figure 1. Linear ligands discussed in this work.

Complexes based on macrocyclic ligands:

Globally, complexes based on macrocyclic ligands are more thermodynamically stable than those based on linear ligands, hence, a lot of research is focused on macrocyclic complexes.

The first well-known category of macrocyclic ligands are the triazacyclononane derivatives. Several studies have shown that hexadentate ligands, such as NOTA (**Figure 2**) [10] or its derivatives where one acetate pendant arm is replaced by other donor groups such as a sulfonamide [11], an acetamide [12], or a methylene pyridine group [13], lack the presence of one innersphere water molecule when they are complexed with Mn(II) ions, with consequently very low relaxivities. Rodriguez-Rodriguez et al. [12] took advantage of this absence of any innersphere water molecule to more thoroughly study the effect of the electron spin relaxation at a low field and they have shown that the electronic relaxation is quite insensitive to the nature of the donor atom but depends more on the coordination polyhedron. Nevertheless, if one of the donor groups is replaced by other substituents, pentadentate ligands (1,4,7,-triazacyclononane-1,4-diacetic acid, H_2NO_2A, **Figure 2**, **Table 1**) allowing the presence of one innersphere water molecule when complexed to Mn(II) ions are obtained. Those complexes were extensively studied [13][14][15][16][17] with a special interest in the more recent study [17] on the water exchange rate, which has to be sufficiently high to ensure good relaxivity. The authors have used ^{17}O measurements and DFT calculations to establish that the water exchange rate is greatly influenced by the bulkiness of the substituent at position seven of the triazacyclononane unit.

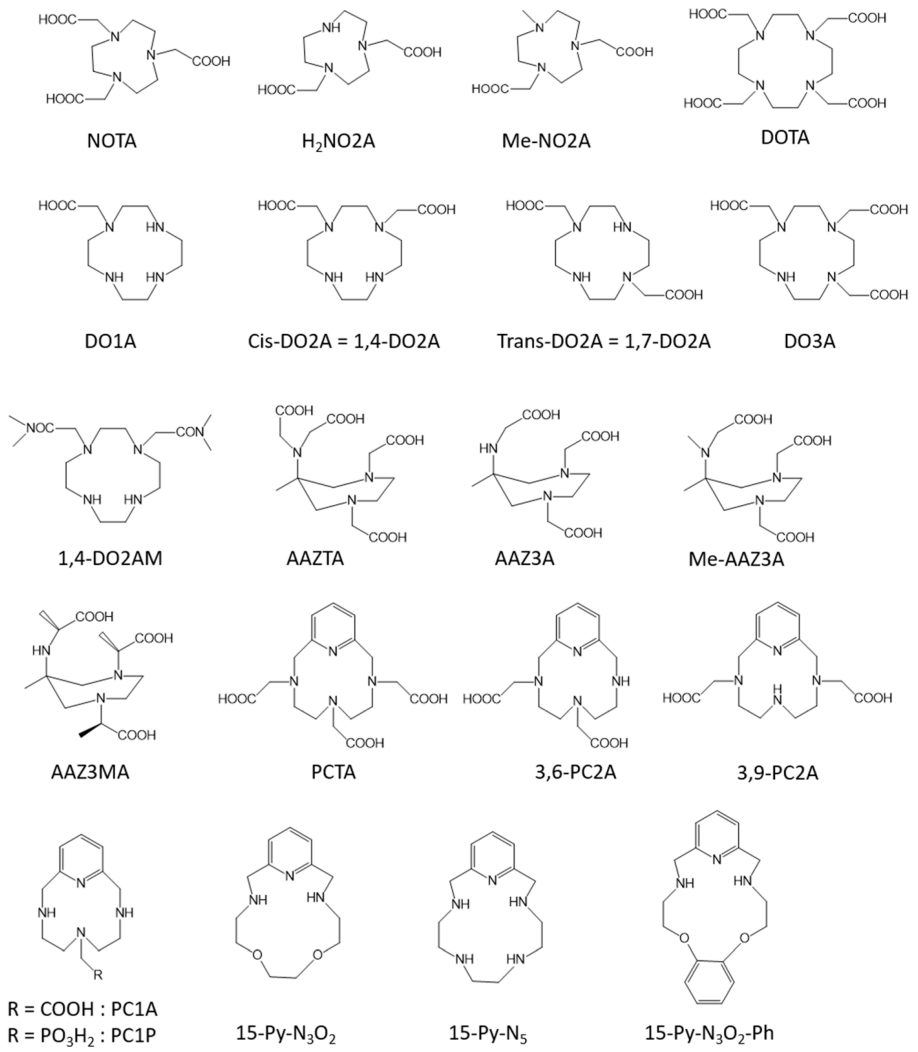

Figure 2. Structure of the macrocyclic ligands discussed in this work.

Similarly to NOTA, the DOTA ligand (**Figure 2**), a well-known tetraazatetradecane ligand, does not allow the presence of one innersphere water molecule when complexed to Mn(II) ions. Nevertheless, as Toth et al. [10] have shown a highest kinetic stability toward zinc transmetallation for the Mn-DOTA complex compared to the Mn-NOTA, derivatives of DOTA allowing the presence of one innersphere water molecules could be interesting to investigate. Therefore, Mn(II) complexes with cyclen-based ligands bearing one, two, and three acetate pendant arms [18][19] (DO1A = 1,4,7,10-tetraazacyclododecane-1-acetic acid, cis- and trans-DO2A (Cis = 1,4,7,10-tetraazacyclododecane-1,4-diacetic acid, Trans = 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid), and DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, **Figure 2**) were studied by ^{1}H and ^{17}O relaxometry. The results were the absence of any innersphere water molecule for Mn-DO3A, as well as for Mn-trans-DO2A, whereas Mn-cis-DO2A and Mn-DO1A complexes contain one innersphere water molecule (**Table 1**). It nevertheless has to be noted that the decreased denticity of the ligand, as expected, results in a decrease in the complex stability. Botta et al. [20] also investigated the replacement of acetate by N,N-dimethylacetamides pendant arms (1,4-DO2AM, **Figure 2**, **Table 1**) and they obtained an increased kinetic inertness. This was confirmed by the study of Garda et al. [21] who replaced the acetate arms by phosphonate arms or mono-, secondary-, or tertiary amides arms and their results point out that phosphonates lead to a decrease in the complex stability whereas tertiary amides afforded encouraging results to increase the stability.

The AAZTA ligand (**Figure 2**) [22] (AAZTA = 6-amino-6-methylperhydro-1,4-diazepine tetraacetic acid) is another macrocyclic chelate able to complex Gd^{3+} and Mn^{2+} ions. Similarly to the case of DOTA, whereas the Gd-AAZTA complex allows the presence of two innersphere water molecules, the Mn-AAZTA complex is characterized by the absence of water co-ligand; hence, its relaxivity is quite low. Botta et al. [23] have thus synthesized three AAZTA derivatives with only three acetate or α-methylacetate arms (Mn-AAZ3A, Mn-MeAAZ3A, and Mn-AAZ3MA, **Figure 2**). Those complexes have one innersphere water molecule and hence a better relaxivity (**Table 1**) but once again to the detriment of the stability (huge decrease in the pMn value for the three derivatives compared to Mn-AAZTA).

Pyclen (3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene) is another interesting 12-membered macrocyclic structure characterized by an *N*-pyridyl donor that rigidifies and pre-organizes the ligand coordinating groups (in particular rendering the four nitrogen atoms coplanar) which could improve the kinetic inertness of the resulting complex. The

pyridine subunit also endows the ligand with an increased degree of lipophilicity that could induce mixed renal and hepatobiliary clearances, another interesting advantage in the context of patients with reduced kidney function. The pyclen [24] PyN4) macrocyclic core is now recognized to form efficient chelators for the Mn^{2+} cation complexation and some studies can be found on the interest of such pyridine-containing (PC) ligands. Garda et al. [21] studied derivatives of PCTA (**Figure 2**) with three pendant arms and studied the influence of the presence of a primary, a secondary, or a tertiary amide instead of the carboxylate functions on the proton relaxometry, the thermodynamic stability, and the kinetic inertness. Similarly to their results on DOTA derivatives, the presence of tertiary amides as pendant arms allows an increase in the stability of the complexes. However, the corresponding Mn(II)-complexes show quite low relaxivities, of less than 2 $s^{-1}{\cdot}mM^{-1}$ at 37 °C and 20 MHz, due to the absence of any innersphere water molecule. To increase the relaxivity, the denticity of the ligand has to be decreased and derivatives of PC2A (with two pendant arms) were developed.

Pyridine-based 15-membered macrocyclic ligands were also developed to complex manganese ions. Drahos et al. [25] developed Mn-15-pyN5 and Mn-15-pyN_3O_2 complexes (**Figure 2**). They obtained a good thermodynamic and kinetic inertness, especially for Mn-15-pyN5, where the two additional nitrogens allowed a higher thermodynamic stability; but, this stability is nevertheless too low for in vivo applications. Moreover, their relaxivities were quite high thanks to the presence of two innersphere water molecules (**Table 1**). Green et al. [26] also developed the same kind of complexes with an additional ortho-phenylene unit (Mn-15-pyN_3O_2-Ph, **Figure 2**) but the stability was rather low so it is unsuitable as an MRI contrast agent. More recently, Drahos et al. [27] added an additional acetate pendant arm to those pyridine-based 15-membered ligands in order to increase the stability and the kinetic inertness as well as the solubility in water. The results show a decrease in the relaxivity compared to Mn-15-pyN5 and Mn-15-pyN_3O_2 complexes since the presence of the additional acetate pendant arm leads to a decrease in the number of innersphere water molecules from two to one. The kinetic inertness is, however, slightly better but remains quite low for in vivo applications.

Recently, Mayilmurugan et al. [28] reported the design of new phenylenediamine-based macrocyclic ligands to complex Mn(II) ions. Their results show good thermodynamic and kinetic inertness as well as interesting relaxivities typical of complexes characterized by one water co-ligand so they could be promising for future use as MRI contrast agents.

Globally, it can be evidenced that the thermodynamic stability and the kinetic inertness of the obtained Mn complexes remains a major issue. Esteban-Gomez et al. [29] tried to analyze this stability using structural descriptors and evidenced some donor groups particularly suited to form stable chelates at physiological pH, such as 2-methylpyridine, secondary and tertiary acetamide or picolinate groups. A lot of efforts are, however, still to be performed to obtain highly stable Mn complexes with good relaxivity, the most promising complexes appearing to be Mn-CDTA and its derivatives, Mn-PyC3A, Mn-PC2A and its derivatives, and Mn-1,4-DO2A and its derivatives.

2. Liver Targeted Contrast Agents

The development of liver-targeted MRI contrast agents has a double objective: first, it can allow the diagnosis of liver diseases such as tumors and secondly, elimination through the liver instead of the kidneys could be safer for patients suffering from a kidney chronic disease. Mn-DPDP (**Figure 3**) [30][31] was the first clinically used Mn complex as liver targeted MRI contrast agent but it is no longer used because of its low relaxivity (2.8 $s^{-1}{\cdot}mM^{-1}$ at 20 MHz and 40 °C) due to the lack of any innersphere water molecule, and toxicity issues due to the low thermodynamic stability of the complex, which releases free Mn ions in vivo. Therefore, the development of those agents remains an important challenge.

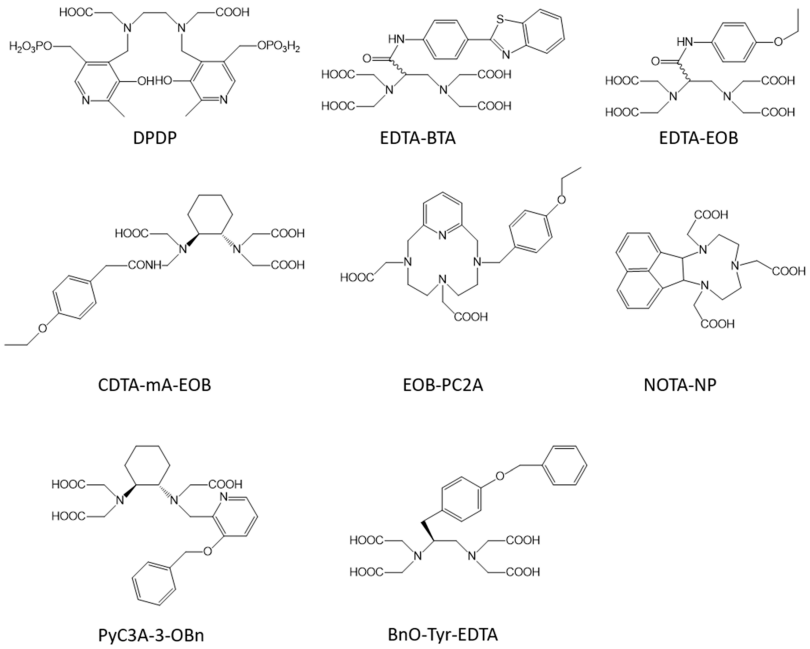

Figure 3. Structure of some ligands used to construct liver-targeted contrast agents.

Human organic anion transporting polypeptides (OATPs), expressed in functioning hepatocytes, can induce the cellular uptake of several amphiphilic organic molecules, such as bile salts, bilirubin, steroid hormones, thyroid hormones, and so on. Therefore, the development of amphiphilic Mn complexes, bearing a lipophilic group on the chelate to mediate an uptake by the liver, is well-studied in the literature. Moreover, it has to be noted that a compromise has to be found between a sufficient lipophilicity to promote an avid hepatobiliary accumulation and a rapid blood clearance to allow a fast diagnosis. Indeed, it has been shown previously that an increased lipophilicity can also promote binding to serum proteins, such as albumin, which will prolong the blood circulation time. The following Mn complexes have shown interesting properties: Mn-EDTA-BTA [32], where the lipophilicity is provided by a benzothiazole aniline grafted on the EDTA coordination cage; complexes incorporating the well-known EOB (ethoxybenzyl) moiety, already used on Gd complexes, on EDTA [33] (Mn-EDTA-EOB), on CDTA [34] (Mn-CDTA-mA-EOB), and on PC2A [35] (Mn-EOB-PC2A); Mn-NOTA-NP [36], where a naphthalene group is grafted on NOTA; Mn-PyC3A-3-OBn [37], where a benzyloxy group is grafted at position 3 of the pyridine group of the chelator PyC3A; or Mn-BnO-TyEDTA [38][39], where the lipophilicity is also provided by a benzyloxy group introduced on the backbone of tyrosine-derived Mn-EDTA (**Figure 3**). All those agents are endowed with a similar relaxivity comprised between 2.5 and 3.5 s^{-1} mM^{-1} at 1.5 T and 298 K (**Table 1**) and undergo partial renal and hepatobiliary excretion.

More specifically, a Mn complex has recently been developed to image liver fibrogenesis. This pathology is accompanied by the upregulation of lysyl oxidase enzymes, which causes the apparition of aldehyde-containing amino acid allysine (Lys^{Ald}) on the extracellular matrix proteins. A series of stable hydrazine-equipped manganese MRI probes able to bind to those modified proteins were thus developed, with promising results [40].

3. Blood Pool Agents

Blood pool agents are characterized by a long vascular circulation time so that they can be used for vascular imaging. MR hardware now enables high-quality vascular images to be recorded with extracellular agents a few seconds after the injection so that the development of blood pool agents appears less important [4]. Nevertheless, they could still be interesting for some specific applications, such as highlighting microvascularization in tumors.

Targeting HSA (human serum albumin), one of the most abundant proteins in the blood plasma, is the most common method in the literature. HSA has two binding sites in its tridimensional structure which are known to bind organic molecules with hydrophobic moieties. Different strategies can thus be evidenced to target HSA. (i) The grafting of different hydrophobic moieties on commonly used chelates. The researchers can cite the grafting on EDTA of one or two benzyloxymethyl (BOM) groups [41], of the same moiety as that used in MS-325 (Mn-LCyPh$_2$) [42] or of deoxycholic acid [43]; the grafting of a biphenyl substituent on the ligand PC2A [44]; the grafting of benzyl groups on the 1,4-DO2AM platform (1,4-BzDO2AM, 1,4-DO2AM-Bz, and DO2AMGly) [45][46]; or the grafting on NOTA of the truncated Evans blue dye [47] (**Figure 4**). (ii) The chelate itself can have hydrophobic moieties able to promote binding to HSA. It is the case for Mn-PyC3A [4][5][6] (**Figure 4**); for the Mn complex developed by Stasiuk et al. [9]; for ligands developed by Platas-Iglesias et al. [48] containing pentadentate 6,6′-((methylazanediyl)bis(methylene)dipicolinic acid binding units able to form mono-

(H_2dpama), di- (mX(H_2dpama)$_2$), and trinuclear (mX(H_2dpama)$_3$) complexes with Mn^{2+} ions (**Figure 4**); or for aza-semi-crown pentadentate ligands rigidified by pyridine and piperidine rings developed by Ai et al. [49]. For all those complexes, a huge increase in the relaxivity is observed in the presence of HSA due to the formation of a non-covalent adduct (**Table 1**).

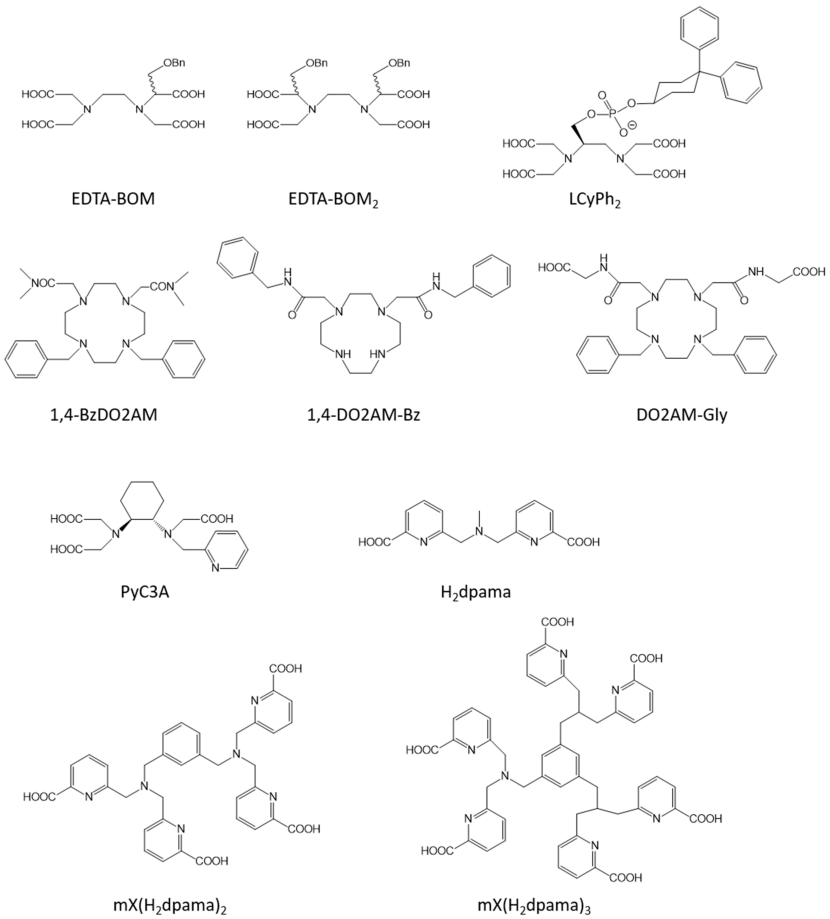

Figure 4. Structures of some of the ligands used for the design of the blood pool agents described in this work.

Another strategy consists of the development of amphiphilic paramagnetic complexes able to form micelles endowed with a high plasmatic half-life and a high relaxivity. Tei et al. [50][51] synthesized six original amphiphilic ligands based on EDTA or on DO2A grafted with aliphatic chains. A strong self-association in micelles was observed, resulting in an enhanced relaxivity. Furthermore, micelles were able to interact with HSA, increasing even more the relaxivity. In another study, PEGylated amphiphilic polymeric Mn complexes were developed and showed an enhanced relaxivity as well as an excellent and relatively long-time-window vascular enhancement effect [52][53].

4. Responsive Contrast Agents

Responsive contrast agents, also called smart or intelligent CAs, are able to report changes in a physiologically relevant parameter, such as pH, redox state, levels of some endogenous ions (Zn^{2+}, Ca^{2+} or Cu^{2+}), etc.

The mapping of tissue pH could allow the diagnosis of tumors at an early stage since their enhanced glucose metabolism induces a decrease in the extracellular pH (Warburg effect) [54]. pH-responsive contrast agents will be able to evidence this pH decrease by a change in their relaxivity induced by a change in the number of coordinated innersphere water molecules. A first example is the Mn-PC2A-EA [55] with an ethylamine pendant arm (**Figure 5**). At acidic pH (between 3.7 and 5.8), the protonation of the amine function allows the presence of one innersphere water molecule, with a relaxivity of 3.5 s^{-1} mM^{-1} at 0.47 T and 25 °C, but when the pH increases, the deprotonation of the amine function allows its coordination to the metal, inducing the loss of the innersphere water molecule and hence a decrease in the relaxivity to 2.1 s^{-1} mM^{-1} (**Table 1**). Other studies have used the interesting protonation transition of sulfonamides groups around the physiological pH to construct pH-responsive contrast agents. Platas-Iglesias et al. [11] developed several complexes characterized by a transition from one innersphere water molecule at basic pH to two innersphere water moleules at acidic pH, with a relaxivity changing from 3.8 s^{-1} mM^{-1} at pH 9 (10 MHz, 25 °C) to 8.9 s^{-1} mM^{-1} at pH 4. In a more recent study involving a sulfonamide group grafted on a triazacyclononane macrocycle, Liang et al. [56] observed a change in relaxivity from 0.9 s^{-1} mM^{-1} at pH 7–9.5 (20 MHz, 25 °C) characteristic of a q = 0 complex to 3.0 s^{-1} mM^{-1} at pH 7–4.5, typical of the presence of one innersphere water molecule.

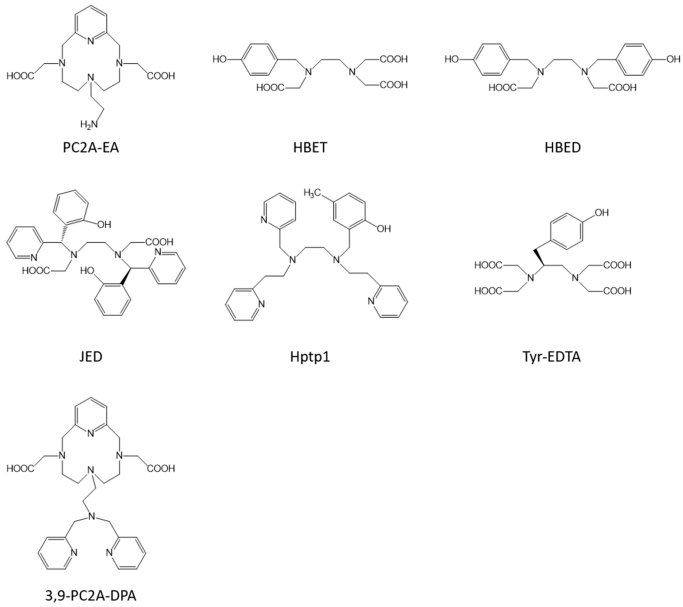

Figure 5. Structures of some of the ligands used to obtain responsive contrast agents.

A modification of the redox status of tissues is a well-known feature of different diseases such as cancers, ischemia, or chronic inflammation. Being able to detect changes in redox activity in vivo could thus be very important in the diagnosis of those pathologies. Toward that aim, using the couple Mn(II)/Mn(III) ions can be an elegant method to monitor redox imbalance. Indeed, Mn(II) complexes are generally characterized by higher relaxivities than their Mn(III) equivalents, as explained earlier. Caravan et al. [57][58][59] largely exploited this way by developing several generations of complexes based on the EDTA core modified with one or several hydroxybenzyl moieties (HBET, HBED, and JED, **Figure 5**) able to form stable complexes with both Mn(II) and Mn(III) ions. The more recent system based on the JED ligand allows a 9-fold enhancement of the relaxivity when Mn(III) is reduced to Mn(II) (**Table 1**).

The detection of oxidative stress is also a major challenge since it is linked to tissue damage in many diseases (Alzheimer, Parkinson, atherosclerosis, etc.). Being accompanied by the production of reactive oxidative species (ROS), Mn complexes able to directly detect ROS have been developed [60][61]. Two generations were elaborated: the first one is based on an original ligand (*N*-(2-hydroxy-5-methylbenzyl)-*N*,*N*′,*N*′-*tris*(2-pyridinylmethyl)-1,2-ethane-diamine, Hptp1, **Figure 5**) able to form a stable complex with Mn(II) ions. Upon reaction with H_2O_2, the complex couples to itself to form a dimer, with a resulting decrease in the relaxivity. However, this strategy has the disadvantage that the production of ROS would be detected by a decrease in the contrast (negative contrast) [60]. A second generation was thus developed where an oxidizable quinol group is grafted on the same type of ligand. This allows to observe an increase in the relaxivity upon the presence of H_2O_2 (**Table 1**) [61].

ROS being produced notably by myeloperoxidase (MPO), a heme protein, another strategy consists of developing contrast agents of which the relaxivity is modified when this enzyme is overexpressed. This is the case for the complex Mn-Tyr-EDTA (**Figure 5**) where a tyrosine derivative is grafted on EDTA and which demonstrates a peroxidase activity-dependent relaxivity by forming oligomers in the presence of the enzyme, inducing an increase in the relaxivity (**Table 1**) [62].

Another example of a responsive Mn-based contrast agent was developed by Tircso et al. [63]. They synthesized a 3,9-PC2A derivative, grafted with a di-(2-picolyl)amine (DPA) moiety as an active arm (**Figure 5**), able to selectively bind Zn^{2+} ions in the co-presence of human serum albumin, with an increased relaxivity (**Table 1**). Moreover, this complex is characterized by a good thermodynamic stability (pMn = 8.79) and a high kinetic inertness toward zinc transmetallation ($t_{1/2}$ at pH 6.0 = 64.5 h).

5. Multimodal Contrast Agents

Multimodal contrast agents are designed to be used in different imaging techniques. One bimodality well developed in the clinical field is the combination of MRI with PET (positron emission tomography) [64] since it allows coupling the high resolution of MRI with the high sensitivity of PET. As MRI images can be recorded without the use of any contrast agents, dual PET/MRI can be performed with single PET probes. The positron-emitting ^{52}Mn having interesting decay properties ($t_{1/2}$ = 5.6 d) for PET imaging, dual probes able to complex radioactive ^{52}Mn and cold ^{55}Mn are thus promising. This allows to overcome the major problem of combining both techniques in the same probe, i.e., the big sensitivity difference

between both techniques which necessitate millimolar concentrations for MRI and nanomolar concentrations for PET. Moreover, it also guarantees that both reporter molecules are chemically identical and are hence endowed with a similar biodistribution. Neumaier et al. [65] described the development of such a probe by grafting different functional groups on the CDTA chelate. They obtained Mn complexes with good thermodynamic and kinetic stabilities as well as interesting relaxivities. Another group has developed dual PET/MRI probes based on a 3,9-PC2A derivative where one of the amine nitrogen was replaced by an etheric oxygen atom, which decreases the basicity of the ligand without affecting its stability when complexed with Mn(II) ions [66].

Another well-developed bimodality is the combination of MRI, characterized by a high resolution, and optical imaging, endowed with a good sensibility. The reporter probes for optical imaging are fluorescent molecules emitting light in the near-infrared (NIR) region to limit the absorption by the tissues. Those fluorescent molecules could be grafted on Mn-based contrast agents and it has been exploited recently by Edwards et al. who grafted hydrophobic functional groups, as chromophores, on EDTA bisamides [67]. Another study by Zhang et al. [68] describes two kinds of terpyridine–Mn(II) complexes (FD–Mn–O_2NO and FD–Mn–FD, **Figure 6**) possessing seven and six coordination modes, respectively, as dual probes for multi-photon fluorescence imaging (MP-FI) and MRI. The second complex FD-Mn-FD is the most promising one, with interesting optical properties (excitation wavelength at 1450 nm (NIR-II)) and relaxometric properties (r_1 = 2.6 s^{-1} mM^{-1} at 20 MHz and 25 °C). Moreover, this complex could also act as a therapeutic agent for the treatment of cancer by photodynamic therapy (PDT). This technique uses a photosensitizer, which, upon activation by light, can kill cancer cells. In that study, FD–Mn–FD generates endogenous $^1O_2$ under irradiation by 808 nm light, thereby enhancing the PDT effect in vitro and in vivo.

Table 1. Relaxometric properties and application area of the molecular Mn complexes discussed in this work.

q	r_1 in Water or Buffer (s^{-1} mM^{-1})	r_1 in the Presence of HSA (s^{-1} mM^{-1})	Application Area	Tested In Vitro and/or In Vivo	
Mn-EDTA	**1**	**2.9 (0.47 T, 35 °C, [2])**		**extracellular**	**no**
Mn-CDTA	**1**	**3.0 (0.47 T, 40 °C, [69])**		**extracellular**	**no**
Mn-PyC3A	**1**	**2.1 (1.4 T, 37 °C, [3])**	**3.5 (1.4 T, 37 °C, [3])**	**extracellular/blood pool**	**yes [5] [6]**
Mn-DPAA	**1**	**2.7 (0.47 T, 37 °C, [70])**		**extracellular**	**no**
Mn-DPAMeA	**2**	**5.1 (0.47 T, 37 °C, [70])**		**extracellular**	**no**
Mn-DPAPhA	**2**	**4.2 (0.47 T, 37 °C, [70])**		**extracellular**	**no**
Mn-PAADA	**2**	**3.3 (0.47 T, 37 °C, [71])**		**extracellular**	**no**
Mn-AMPTA	**1**	**2.6 (0.47 T, 37 °C, [72])**		**extracellular**	**no**
Mn-AMPDA-HB	**1**	**2.7 (0.47 T, 37 °C, [72])**		**extracellular**	**no**
Mn-MeNO2A	**1**	**2.2 (0.47 T, 37 °C, [15])**		**extracellular**	**no**
Mn-DO3A	**0**	**1.3 (0.47 T, 37 °C, [18])**		**extracellular**	**no**
Mn-1,7-DO2A	**0**	**1.3 (0.47 T, 37 °C, [18])**		**extracellular**	**no**
Mn-1,4-DO2A	**1**	**1.7 (0.47 T, 37 °C, [18])**		**extracellular**	**no**
Mn-1,4-DO2AM	**1**	**2.0 (0.47 T, 37 °C, [20])**		**extracellular**	**no**
Mn-AAZTA	**0**	**1.6 (0.47 T, 25 °C, [23])**		**extracellular**	**no**
Mn-AAZ3A	**1**	**2.5 (0.47 T, 25 °C, [23])**		**extracellular**	**no**
Mn-MeAAZ3A	**1**	**2.0 (0.47 T, 25 °C, [23])**		**extracellular**	**no**
Mn-AAZ3MA	**1**	**1.9 (0.47 T, 25 °C, [23])**		**extracellular**	**no**
Mn-3,6-PC2A	**1**	**2.7 (0.47 T, 25 °C, [73])**		**extracellular**	**no**
Mn-3,9-PC2A	**1**	**2.9 (0.47 T, 25 °C, [73])**		**extracellular**	**no**
Mn-15-pyN_5	**2**	**3.1 (0.47 T, 37 °C, [25])**		**extracellular**	**no**

q	r_1 in Water or Buffer (s^{-1} mM^{-1})	r_1 in the Presence of HSA (s^{-1} mM^{-1})	Application Area	Tested In Vitro and/or In Vivo	
Mn-15-pyN_3O_2	2	3.6 (0.47 T, 37 °C, [25])		extracellular	no
Mn-EDTA-BTA	1	3.5 (1.5 T, 24 °C, [33])	15.1 (1.5 T, 24 °C, [33])	liver	yes [32]
Mn-EDTA-EOB	1	2.3 (1.5 T, 24 °C, [33])	6.3 (1.5 T, 24 °C, [33])	liver	yes [33]
Mn-EOB-PC2A	1	2.8 (1.5 T, 25 °C, [35])	5.9 (1.5 T, 25 °C, [35])	liver	yes [35]
Mn-NOTA-NP	1	3.6 (3 T, 25 °C, [36])	9.0 (3 T, 25 °C, [36])	liver	yes [36]
Mn-PyC3A-3-Obn	1	2.6 (1.4 T, 37 °C, [37])	9.0 (1.4 T, 37 °C, [37])	liver	yes [37]
Mn-BnO-TyEDTA	1	4.3 (0.47 T, 32 °C, [38])	15.8 (0.47 T, 32 °C, [38])	liver	yes [38] [39]
Mn-EDTA-BOM	1	3.6 (0.47 T, 25 °C, [41])	55.3 (0.47 T, 25 °C, [41])	blood pool	no
Mn-LCyPh2	1	5.8 (0.47 T, 37 °C, [42])	48.0 (0.47 T, 37 °C, [42])	blood pool	yes [42]
Mn-1,4-BzDO2AM	1	3.8 (0.47 T, 25 °C, [45])	18.5 (0.47 T, 25 °C, [45])	blood pool	no
Mn-1,4-DO2AM-Bz	1	3.5 (0.47 T, 25 °C, [45])	27.4 (0.47 T, 25 °C, [45])	blood pool	no
Mn-DO2AM-Gly	1	4.5 (1 T, 25 °C, [46])	14.0 (1 T, 25 °C, [46])	blood pool	yes [46]
Mn-dpama	2	4.2 (0.47 T, 37 °C, [48])	12.2 (0.47 T, 37 °C, [48])	blood pool	no
mX(Mn-dpama)$_2$	2	6.1 (0.47 T, 37 °C, [48])	39.0 (0.47 T, 37 °C, [48])	blood pool	no
mX(Mn-dpama)$_3$	2	8.3 (0.47 T, 37 °C, [48])	45.2 (0.47 T, 37 °C, [48])	blood pool	no
Mn-PC2A-EA	1	3.5/2.1 (0.47 T, 25 °C, [55])		pH responsive	no
$Mn^{II/III}$-HBET	1	1.0/2.8 (1.4 T, 37 °C, [57])		redox responsive	no
$Mn^{II/III}$-JED	1	0.5/3.3 (1.4 T, 37 °C, [59])		redox responsive	no
Mn-Hptp1	1/2	4.7/5.3 (3 T, 25 °C, [61])		redox responsive	no
Mn-Tyr-EDTA	1	3.3/8.5 (0.47 T, 32 °C, [62])	8.0 (0.47 T, 32 °C, [62])	redox responsive	yes [62]
Mn-3,9-PC2A-DPA	1	3.2 (1.4 T, 37 °C, [63])	12.1 (1.4 T, 37 °C, [63])	Zn responsive	yes [63]

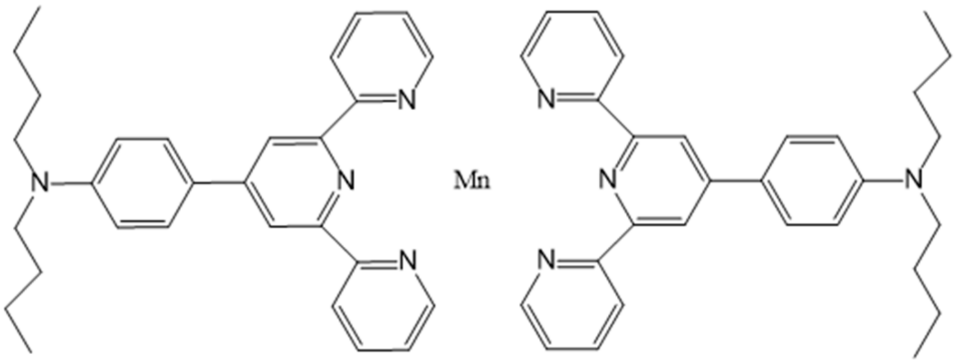

Figure 6. Structure of the theranostic agent FD-Mn-FD.

6. In Vitro/In Vivo Studies and Toxicity Issues

As shown in **Table 1**, only a few Mn complexes were tested in vitro and/or in vivo. Surprisingly, most of those complexes are endowed with an increased lipophilicity, allowing their use as liver-targeting contrast agents or blood pool agents. Biodistribution studies by MRI and ICP show a dual renal and hepatobiliary elimination for all those agents, which is explained by their enhanced lipophilicity compared to small extracellular agents like Gd-DOTA. Mn-PyC3A was also tested in a rat model of renal impairment and the in vivo studies indicate in that case an increased hepatobiliary elimination [6]. Moreover, Mn levels had returned to the baseline within 24 h after injection for all those complexes.

Their efficacy as MRI contrast agents was also tested. Liver-targeted contrast agents were systematically injected into a murine liver tumor model to evaluate their ability to differentiate normal liver and tumor tissue. MRI images show, for most of the complexes, a hypointense signal in tumor tissues compared to normal liver tissues after injection of the Mn-complex. This can be explained by the transport mechanism of the Mn complexes to the liver: they can enter normal hepatocytes through organic anion-transporting polypeptide transporters (OATPs) which are considerably reduced in tumor tissues. The study of Zhu et al. [38] has particularly evidenced the importance of OATPs in the hepatic uptake of Mn complexes by performing images in the presence of an OATP inhibitor as well as cell uptake studies on OATP-transfected and non-transfected cell lines. Nevertheless, the study on Mn-NOTA-NP [36] contradicts the above results since a hyperintense signal is observed in tumor tissues compared to the normal liver. The authors explain this result by decreased MRP2 expression in tumor cells whereas OATP expression is maintained. As the role of MRP2 is to mediate the secretion of the Mn complex from the tumor cells to the lumen, its decreased expression induces an accumulation of the Mn complex in the cytoplasm of tumor cells, which explains the observed hyperintense signal. Thus, it evidences the need for more thorough investigations in the future. A few blood pool agents have also been studied in vivo to evaluate their efficacy. Mn-LCyPh$_2$ was injected into white rabbits at doses of 30 µmol/kg and 10 µmol/kg and good vascular images could be obtained for both doses. The authors were also able to distinguish injured from normal vessels [42]. The study on Mn-DO2AM-Gly was more focused on the ability of the Mn complex to accumulate in a highly vascularized tumor model; interesting results were obtained on subcutaneous breast tumor lesions where a strong contrast enhancement was obtained [46]. The redox responsivity of Mn-Tyr-EDTA was also evaluated in vivo on a murine model with monosodium urate crystal-induced acute gouty arthritis. The contrast enhancement in the inflammation site was higher than that obtained with Gd-DTPA used as a control.

Even if the above studies are encouraging, very few data exist about the possible toxicity of all those agents. Some of the abovementioned studies present cell viability assays [32][33][36][38][46] to evaluate the toxicity of the Mn complexes. The results showed a negligible cytotoxicity toward various cell lines in the concentration range needed for MRI. Nevertheless, it is not sufficient at all to attest to the safe use of those complexes in vivo. Indeed, as for Gd complexes, the release of free Mn^{2+} ions in vivo could be responsible for pathological disorders for patients, such as manganism, a disease with symptoms close to those of Parkinson's disease. It is thus crucial to verify that Mn complexes remain intact when they are injected in vivo, which is nearly never the case. The study by Caravan et al. [42] on Mn-LCyPh$_2$ mentions that the complex should remain intact since no acute cardiac toxicity was evidenced during their study and free Mn^{2+} ions are known to be very toxic for the heart. This is nevertheless indirect proof so that more thorough studies, such as those performed on gadolinium complexes when concerns about NSF and gadolinium retention in the brain start to appear, are needed to attest to the safety of all those Mn complexes. Scientists must take advantage of the knowledge acquired about gadolinium complexes to avoid repeating the same mistakes and develop newer and safer MRI contrast agents.

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