Polyhedron xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Metal complexes of tridentate tripod ligands in medical imaging and therapy

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ARTICLE INFO

Article history:
Received 6 October 2016
Accepted 25 November 2016
Available online xxxx

Keywords:
Scorpionates
Cytotoxic agents
Photosensitizers
CORMs
Radiopharmaceuticals

ABSTRACT

Since their development in the late 1960s, scorpionate ligands have played a great part in the design of novel compounds in the field of coordination chemistry. In this review we focus on the most relevant scorpionate-containing metal complexes that have been explored in the area of medical imaging and therapy. We intend to provide some insight into the chemistry involved in the design of these compounds, and also highlight relevant biological results that have been reported in the literature for scorpionate complexes as cytotoxic drugs, photosensitizers, CO releasing molecules (CORMs) or radiopharmaceuticals.

1. Introduction

Metal-based compounds play an important role in the design of drugs for diagnostic or therapeutic applications, namely anticancer agents, radiopharmaceuticals for nuclear imaging or radionuclide therapy and contrast agents for magnetic resonance imaging (MRI) [1–5]. Compared to purely organic molecules, metal complexes offer several advantages as a result of their structural diversity, varied reactivity pattern and unique photo- and electrochemical properties. To fully profit from these advantages, it is crucial to select good performing chelators with coordinating properties suitable for the proper stabilization of a given metal core. In addition, the chelators play an important role in providing the final complexes with the desired imaging/therapeutic properties and, preferably, with specific and preferential accumulation in the target organ/tissues.

In the past few years, a large variety of acyclic and cyclic chelators have been evaluated for applications in medicinal inorganic chemistry. In this field, poly(amino)carboxylate ligands, like DTPA, DOTA or NOTA derivatives, can be considered the most studied and successful ones [6]. This type of chelators led to important achievements that in some cases were translated into clinics, including

Abbreviations: 2-Tic^{NMe}, tris(N-methylimidazol-2-yl)carbonil; 2-Tip^{iPr2}, tris(1,4-diisopropylimidazol-2-yl)phosphane; 4-Tip^{H}, tris(imidazole-4-yl)phosphane; AO, acridine orange; BBB, blood brain barrier; BBN, bombesin; bdmpza, 3,3-bis(3,5-dimethylpyrazol-1-yl)acetate; bdmpzp, 3,3-bis(3,5-dimethylpyrazol-1-yl)propionate; BFC, bifucntional chelator; BP, bisphophonate; bpea, bis(pyrazolyl)ethylamine; bpza, 3,3-bis(pyrazol-1-yl)acetate; bpzp, 3,3-bis(pyrazol-1-yl)propionate; CAD, coronary artery disease; CORM, CO releasing molecule; CT, computed tomography; CT-DNA, circulating tumor DNA; CuAAC, copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition, "click" addition; cybpamd, N,N-dicyclohexyl-2,2-bis-(3,5-dimethylpyrazol-1-yl)-cetamidinate); *p*-cym, 1-isopropyl-4-methyl-benzene; DAP, 2,3-diamino-propionic acid; DAPTA, 3,7-diacetyl-1,3,7-triazaphosphabicyclo[3.3.1]nonane; DLC, delocalized cations; DMEOP, 3,5-bis(methoxymethyl)pyrazole; DNA, deoxyribonucleic acid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DPA, dipicolylamine; dpq, dipyrido[3,2-d:2′,3′-f]quinoxaline; DTPA, diethylenetriaminepentaacetic acid; GPC, G protein coupled; HEDP, (1-hydroxyethylidene)-diphosphonic acid; HER2, human epidermal growth factor2; HOMO, Highest Occupied Molecular Orbital; IC_{50}, half maximal inhibitory concentration; imzh, imidazole; iNOS, nitric oxide synthase inhibitor; KP1019, trans-[tetrachlorobis(1-H-indazole)ruthenate(III)]; MDR, multidrug resistance; MRI, magnetic resonance imaging; NAMI-A, imidazolium-trans-tetrachloro(dimethylsulfoxide)imidazoleruthenium(III); NIR, near-infrared; NOS, nitric oxide synthase; NOTA, 1,4,7-triazacyclononane-1,4,7-triacetic acid; OTf, trifluoromethanesulfonate; PCN, tris(2-cyanoethyl)phosphine; PCy_3, tricyclohexylphosphine; PDT, photodynamic therapy; PET, positron emission tomography; Ph, phenyl; phen, 1,10-phenanthroline; PSMA, prostate specific membrane antigen; PTA, 1,3,5-triaza-7-phosphaadamantane; Py, pyridine; Pz, pyrazole; pz_4lut, α,α,α,α-tetra(pyrazol-1-yl)-2,6-lutidine; RGD, arginylglycylaspartic acid; SLND, Sentinel Lymph Node Dissection; SPECT, single photon emission computed tomography; SPPS, solid phase peptide synthesis; thp, tris(hydroxymethyl)phosphine; Tim^{Me}, N-methyl-2-mercaptoimidazolyl; TMEOP, 3,4,5-tris(methoxymethyl)pyrazole; Tp, tris(pyrazol-1-yl)borate; Tpa, tris(2-pyridyl)amine; Tpm, tris(pyrazol-1-yl)methane; Tpm^{OH}, 2,2,2-tris(pyrazol-1-yl)ethanol; Tpm^{OMs}, 2,2,2-tris(pyrazol-1-yl)mesyloxymethyl; Tpm^{Py}, 4-{[tris-2,2,2-(pyrazol-1-yl)ethoxy]methyl}pyridine; Tpms, tris(pyrazolyl-1-yl)methanesulfonate; Tpp, tris(pyridine-2-yl)phosphane; TPP, triphenylphosphonium; Tp^{Ph}, tris(3-phenylpyrazolyl)borate; TNBC, triple negative breast cancer; UNICAM-1, [Ru(*p*-cym)(bis(3,5-dimethylpyrazol-1-yl)methane)Cl]Cl.

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http://dx.doi.org/10.1016/j.poly.2016.11.040
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several Gd complexes approved as MRI contrast agents and complexes of trivalent radiometals (e.g. ^{111}In, ^{68}Ga, ^{90}Y or ^{177}Lu) used as radiopharmaceuticals for diagnostic or treatment of cancer [1,3,7,8]. Despite this success, poly(amino)carboxylate chelators do not ensure an adequate stabilization of many medically relevant metals and, in many circumstances, they cannot provide complexes with the physico-chemical properties required for the envisaged medical imaging and/or therapeutic application, such as for example small-sized and lipophilic complexes to be used in cardiac or brain perfusion studies by nuclear imaging techniques.

To foster further advances in medicinal inorganic chemistry and fulfill unmet clinical needs, it is important to explore alternative chelating systems other than the poly(amino)carboxylate ligands that dominate the medical applications of d- and f- coordination complexes. Among the large diversity of chelating systems available, tridentate tripod ligands are particularly suited for a facial coordination in octahedral metal complexes with potential medical relevance. Several classes of compounds fit into this category, such as low-valent tricarbonyl complexes that emerged in recent years as potential radiopharmaceuticals or as CO releasing molecules (CORMs) with possible therapeutic usefulness in different diseases (e.g. inflammation, cardiovascular diseases and cancer) [4,5,9,10].

Tris(pyrazolyl)borates are among the most popular tridentate tripod ligands. These monoanionic and nitrogen donor ligands were discovered in the late 1960's by Stanislaw Trofimenko [11], who suggestively designated these chelators as scorpionates due to their characteristic coordination mode that pictorially can be compared to an embrace of the metal-ion, like the pincers and tail of a scorpion catch and sting its victim. Later on, other poly(azolyl) borates have been introduced, with particular relevance for softer and sulfur donor 2-mercaptoimidazolyl derivatives that are linked to the central boron atom through formation of B–N bonds [12,13], in the same way as the pyrazolyl counterpart. Moreover, congener tris(pyrazolyl)methanes were also devised and synthesized, upon replacement of the central boron atom by a carbon atom [14]. All these possibilities led to a diversified family of homoscorpionates or heteroscopionates, which in the latter case comprised the combination of different azolyl rings or its replacement by other coordinating or non-coordinating functions (e.g. hydride, alkyl or aryl, acetate, etc.) (Fig. 1) [15,16].

Homoscorpionate and heteroscorpionate ligands have been studied for the complexation of a wide variety of d- and f- transition metals. Through the last four decades, these types of ligands have been extensively explored due to their facility to adjust the steric and electronic environment around the metal center. In fact, metal systems supported by scorpionates are widely explored in the field of homogeneous catalysts, magnetic materials and bioinorganic chemistry, namely as biomimetics of metalloenzymes [17–25]. Despite the advances in the coordination chemistry of this type of chelators, systematic studies with scorpionate complexes in the field of biomedical applications are comparatively scarcer.

Our group had a pioneer role in the investigation of scorpionate complexes for biomedical applications. Based on bis- and tris(2-mercaptoimidazolyl)borate M(I) (M = Re, ^{99m}Tc) tricarbonyl complexes, we have proved for the first time that these class of compounds can be obtained under aqueous conditions, being rather resistant under challenging biological conditions [26,27]. Thereafter, we have extended our studies to congener tris(pyrazolyl) methane M(I) (M = Re, ^{99m}Tc) tricarbonyl complexes, and achieved the first examples of scorpionate complexes with evident clinical translational potential, namely as radiopharmaceuticals for cardiac imaging [28]. In this manuscript, we summarize our contribution to the design of metal complexes for nuclear imaging and radionuclide therapy, based essentially on M(I) tricarbonyl complexes (M = Re, Tc) with poly(azolyl)borate and poly(pyrazolyl)methane chelators. Inevitably, there will be some overlap with previous papers where we have reviewed our research work in this field [4,5]. However, the present review will also include the most innovative and promising results from other research groups involved in the evaluation of scorpionate complexes as potential metallodrugs, namely as cytotoxic agents, CORMs and PDT photosensitizers.

Due to their unique chemistry and versatility, heterocycle compounds are present in many of the pharmaceuticals currently marketed, being poised as true cornerstones of medicinal chemistry. In particular, in the last decades research based on pyrazole chem-

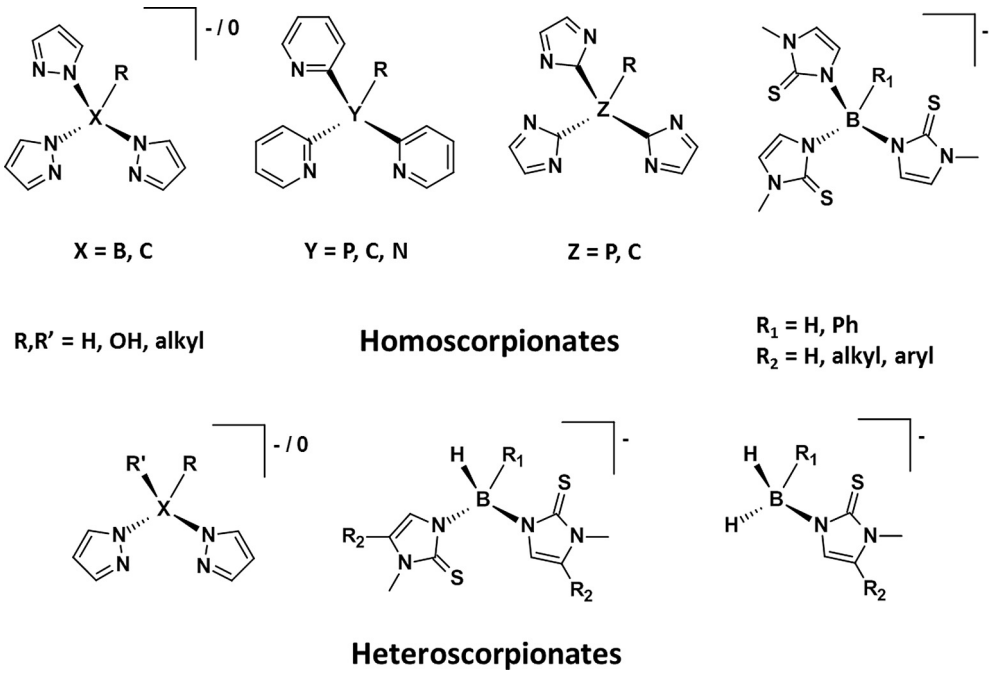

Fig. 1. General structure of borate, methane and phosphine-based scorpionates.

istry have attracted special consideration due to its valuable and extensive range of pharmacological activities, namely as anticancer tools [29–36]. To better contextualize the achievements described for scorpionate complexes, this review we also cover related biologically relevant complexes containing other facially coordinated azolyl- or pyridyl-containing tridentate ligands.

Herein we present a chemical insight into the most demanding challenges that are involved in the design of scorpionate metal complexes for imaging and therapy. This will comprise compounds with a large variety of d- and f-transition elements (see Periodic Table in Fig. 2), but will be more focused on Tc/Re complexes as radioactive probes for nuclear imaging, Mn complexes as CORMs and Co, Cu, Ag, Ru and lanthanide complexes as anticancer drugs. All in all, we have in mind to awake the inorganic chemistry community for the interest of this class of compounds within the biomedical arena.

2. Cytotoxic agents

Platinum compounds, namely cisplatin, carboplatin and oxaliplatin, had a pioneer role in the development of metallodrugs for systemic anticancer therapies. Nowadays, these Pt metallodrugs are used in the clinic as powerful anticancer drugs to treat solid tumors, like lung, bladder, ovarian and testicular cancers. However, there are other neoplasia showing a poorer response to these Pt-based drugs due to the development of cisplatin and cisplatin cross-resistance [37]. Moreover, they can also induce severe side effects. Due to these limitations, there is a need for novel metal-based compounds with more favorable pharmacological profile, i.e. showing the same or higher efficiency with reduced side effects. Therefore, the development of new and more efficient antitumor metallodrugs still remains an important topic of research in biomedical inorganic chemistry. In the past few decades, the research in this field covered a large diversity of organometallic and coordination complexes with metals all along the periodic table, namely Pt, Ru, Os, Au, Cu, Co, Ga to cite a few [1]. Several encouraging preclinical results were reported, particularly for octahedral Ru(III) complexes containing N-donor azoles (NAMI-A and KP1019), which underwent clinical trials [2]. However, so far, none of the tested complexes was approved for clinical use and cisplatin and its derivatives remain the unique inorganic complexes applied in the clinics as anticancer drugs.

As reviewed below, the contribution of tridentate and tripodal chelators, namely hetero- and homoscorpionates, for the development of anticancer metallodrugs has been done essentially based on complexes of Co, Cu, Ag, Ru and lanthanides.

Cobalt complexes have been widely studied for the development of systemic anticancer agents, namely as artificial DNA nuclease or enzyme inhibitors [38]. Taking into account the relevance of Co complexes for biomedical applications, Pombeiro and co-workers have been involved during the past few years on the synthesis and biological evaluation of Co(II) complexes with C-functionalized tris(pyrazolyl)methanes (Tpm's), aiming to demonstrate their interest in the design of anticancer metallodrugs [39]. They have focused on the alcohol derivative Tpm^{OH} (Fig. 3), which represents a more versatile scaffold for further functionalization of this type of ligands, when compared with the simplest member of the series Tpm [40]. For instance, mesylation or O-alkylation reactions of Tpm^{OH} with methanesulfonyl chloride or 4-bromomethyl-pyridine afforded the ligands Tpm^{OMs} and Tpm^{Py}, respectively [40,41,43,44]. Treatment of $CoCl_2$ with Tpm^{OMs} and Tpm^{Py}, in a 1:1 stoichiometric ratio, led to the formation of the respective neutral aquo-dichloro Co(II) complexes (Fig. 3). In the case of Tpm^{OH}, the authors have performed the synthesis using a 1:2 stoichiometric ratio (metal:ligand), which originated a triaquacomplex bearing two Tpm^{OH} molecules coordinated to the metal center and an homoleptic CoL_2^{2+} cation, $[(CoL_2)_2(CoL(H_2O)_3]_2Cl_2 6H_2O$ ($L = Tpm^{OH}$) (Fig. 3). All these complexes are hydrophilic and showed moderate cytotoxicity *in vitro* towards HCT116 colorectal carcinoma and HepG2 hepatocellular carcinoma human cancer cell lines [39]. Spectroscopic studies did not confirm the existence of direct interactions between the complexes and CT-DNA. However, the incubation of all these Co(II) complexes with plasmid DNA induced double strand breaks without the use of any activating agent. The authors reported that the DNA cleavage seems to be mediated by O-centered radical species, namely the hydroxyl radical.

The group of Pombeiro and co-workers have further extended their research on cytotoxic scorpionate-containing complexes with the development of Ag(I) compounds of the '3+1' type bearing the tris(pyrazolyl-1-yl)methanesulfonate (Tpms) ligand and different monodentate phosphines (Fig. 4). These '3+1' complexes, [Ag(Tpms)(L)] ($L = PCy_3$, PPh_3, PTA), were obtained by reaction of the precursor $AgBF_4$ with Li(Tpms) in the presence of the monodentate phosphine [41,42]. X-ray analysis of the PCy_3 complex showed that the Tpms is coordinated to the metal center through N_3-bonding, while for the other two complexes the scorpionate is N_2O-bonded. The authors discussed the coordination versatility of the scorpionate based on the different bending degrees of the coordinated pyrazolyl rings, which appear to be related with the cone angles of the phosphines. Larger cone angles lead to higher $AgNNC_{pz}$ torsion angles to avoid unfavorable interactions between the scorpionate and the phosphine co-ligands. Additionally, the antiproliferative activity of these Ag complexes was studied in A375 human malignant melanoma cancer cell lines. The PCy_3 complex displayed the highest cytotoxic effect, followed by the PPh_3 and then PTA-containing ones. These results are directly in line

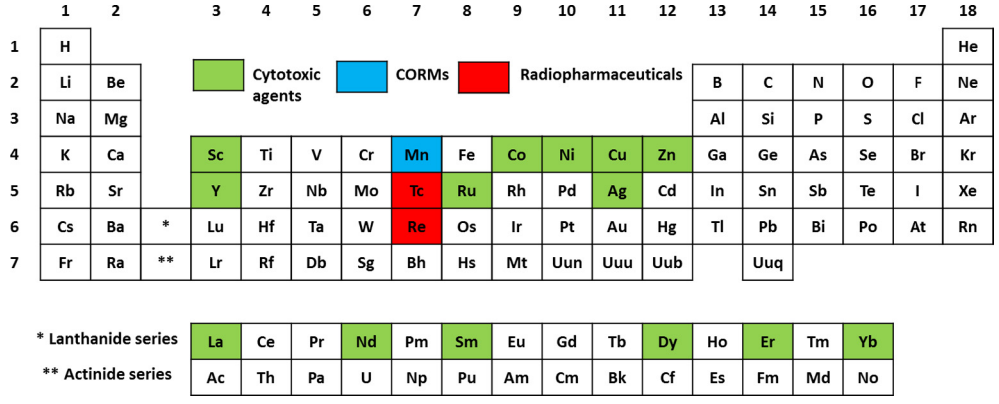

Fig. 2. Periodic table of the scorpionate metal complexes investigated for biomedical applications, as cytotoxic agents, CORMs or radiopharmaceuticals.

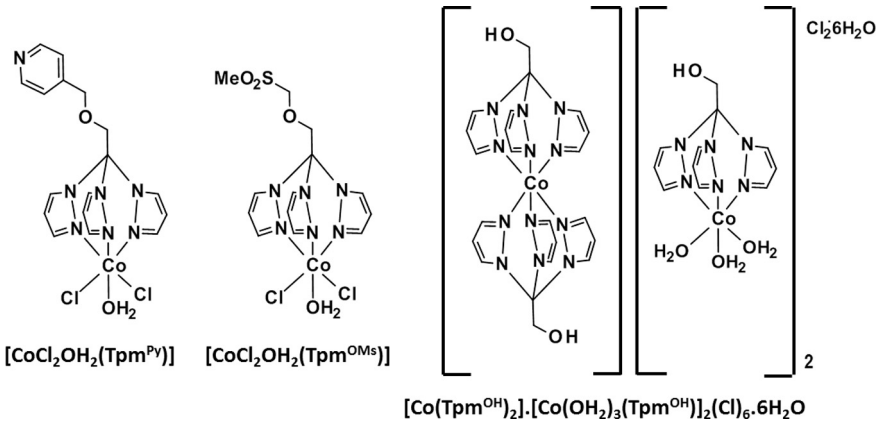

Fig. 3. Co(II) complexes of Tpm^{OH}, Tpm^{Py} and Tpm^{OMs}.

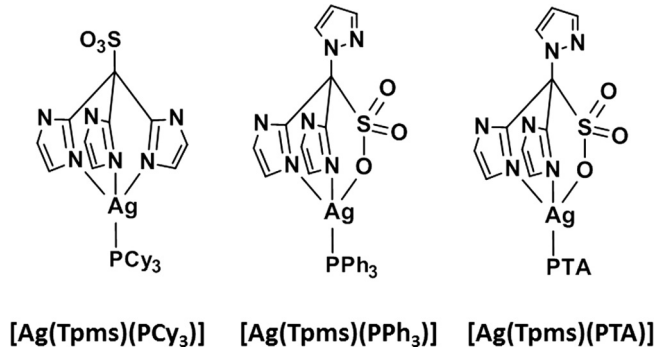

Fig. 4. Ag(I) complexes of the '3+1' type bearing Tpms and a monodentate phosphine.

with the values of the DNA binding constants (K_A) of the compounds; the higher the K_A, the greater the cytotoxicity of the complex.

Tisato and co-workers developed a library of charged and neutral Cu(I) compounds of the '3+1' type with homo- and heteroscorpionates (tris(pyrazolyl/(benzotriazolyl)borate derivatives, tris (pyrazol-1-yl)methane or bis(3,5-dimethylpyrazol-1-yl) acetic acid) and bearing a monodentate phosphine co-ligand (Fig. 5) [43,44]. These '3+1' complexes are synthesized by initially reacting $[Cu(CH_3CN)_4][BF_4]$ with the respective monodentate phosphine followed by reaction with the tripodal ligand. A distorted tetrahedral molecular structure was observed for these complexes, with the coordination sphere comprising the scorpionate ligand, attached as a tripodal chelator through the N,N,N or N,N,O donor atoms, and the monodentate P-donor phosphine. *In vitro* cytotoxicity studies in a variety of cancer cell lines indicated that the charged tris(pyrazol-1-yl)methane Cu(I) complexes exhibit a lower cytotoxic activity, as seen by the significantly higher IC_{50} values relatively to those of the neutral congeners and cisplatin [42]. Another relevant factor that seems to influence the cytotoxic activity of these complexes is their lipophilicity. The compounds containing moderately lipophilic monodentate phosphines (PTA and PCN), with the same tripodal ligand, display a higher antitumor activity than the ones carrying phosphines that are either more lipophilic or hydrophilic. Interestingly, it was also verified that the presence of either electron-donor or electron-withdrawing groups on the pyrazolyl backbones, or replacement of the pyrazoles with benzotriazoles, significantly decreased the antitumor activity of the complexes. Among the Cu(I) complexes bearing unsubstituted tris(pyrazol-1-yl)borate ligands, the derivative [{HB(pz)$_3$}Cu(PCN)] emerged as the most cytotoxic one, with mean IC_{50} values in human tumor cell lines (breast (MCF-7), cervical

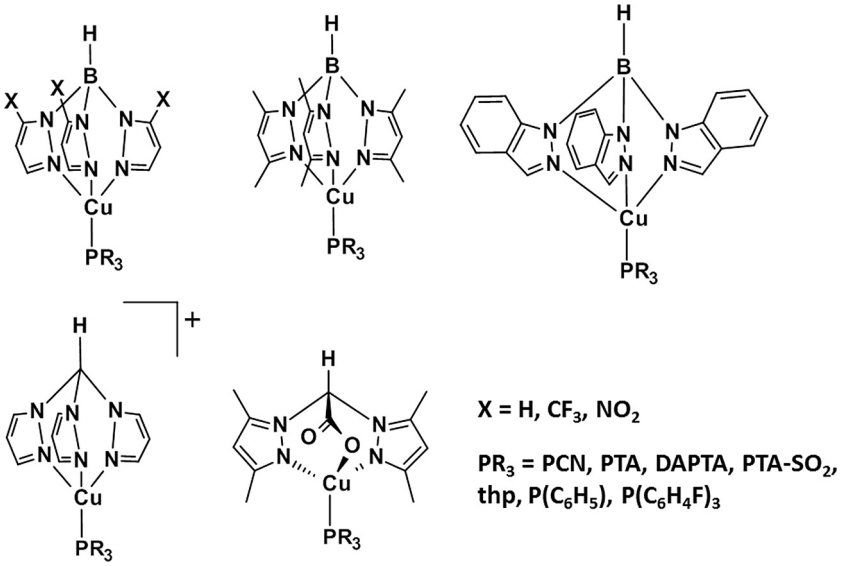

Fig. 5. Cu(I) complexes of the '3+1' type bearing a tripodal ligand and a monodentate phosphine.

(A431), colon (HCT-15), pancreatic (BxPC3), and lung (A549) cancers, neuroblastoma (SHSY5Y), and melanoma (A375)) about one order of magnitude lower than those of cisplatin. *In vivo* studies of [{HB(pz)$_3$}Cu(PCN)] in tumor bearing mice showed that there is a tumor weight loss in the animals treated with this compound similar to those treated with cisplatin; however, no signs of toxicity or total weight loss were observed in the treated mice [42,43].

In the past few years, pronounced strides have been made in the search for ruthenium complexes as new anticancer metallodrugs. Currently, KP1019 (trans-[tetrachlorobis(1-H-indazole)ruthenate (III)]) is on phase I clinical trials, and NAMI-A (imidazolium-trans-tetrachloro(dimethylsulfoxide)imidazoleruthenium(III)) went to phase II trials, but unfortunately it did not go through to phase III. KP1019 is active against primary cancers cells while NAMI-A is active against metastatic tumor cells [45]. Both are a clear example of the potential of ruthenium compounds in the development of cytotoxic agents, giving impetus to proceed with the study of other ruthenium compounds as anticancer drugs, namely scorpionate Ru(II) complexes.

The group of Lastra and co-workers evaluated the DNA binding properties and *in vitro* cytotoxicity of new half-sandwich ruthenium(II) complexes containing 1-hydrotris(pyrazolyl)borate (Tp) and water-soluble phosphines (Fig. 6) [46,47]. Complexes of the type [RuX{k^3(N,N,N)-Tp}(L)(PTA)] (X = Cl, H and L = PMe_2Ph, PMe_3, $P(OMe)_3$ and $P(OPh)_3$) were obtained by reaction of [RuX{k^3(N,N,N)-Tp}(PPh_3)(PTA)] with the corresponding phosphine or phosphite. Treatment of these complexes with $MeCF_3SO_3$ leads to the congeners [RuX{k^3(N,N,N)-Tp}(L)(1-CH_3-PTA)][OTf] bearing the 1-CH_3-PTA phosphine ligand. Additionally, they have also synthesized complexes of the type [Ru(NCMe){k^3(N,N,N)-Tp}(PPh_3)(PTA)][PF_6] through the reaction of [RuCl{k^3(N,N,N)-Tp}(PPh_3)(PTA)] with sodium triflate in an acetonitrile/methanol solution. The DNA binding properties of these complexes were studied by MALDI-TOF mass spectrometry and using the 14-mer single stranded oligonucleotide 5′-ATACATGGTACATA-3′, which indicated the occurrence of coordination of the [Ru{k^3(N,N,N)-Tp}(PTA)] or [Ru{k^3(N,N,N)-Tp}(1-CH_3-PTA)] fragments to a single strand DNA chain. It was observed that the complexes [RuX{k^3(N,N,N)-Tp}(PPh_3)(L)]$^+$ (X = H, L = 1-CH_3-PTA; X = NCMe, L = PTA) and [RuCl{k^3(N,N,N)-Tp}(PPh_3)(1-CH_3-PTA)]$^+$ showed an antitumor activity ($IC_{50} \approx 10^{-6}$ M) similar to that reported for drugs currently used in clinical practice, such as doxorubicin. Furthermore, these compounds were ten times less toxic for normal cells (non-transformed human umbilical vein endothelial cells (HUVEC)) than doxorubicin.

To put into context the work reported for scorpionate Ru(II) compounds as potential anticancer drugs, we also present below relevant examples of complexes anchored by N,N-bidentate pyrazolyl-containing chelators, even though they do not correspond to tridentate tripod ligand. For example, Patra and co-workers developed mononuclear [(*p*-cym)Ru(X)(pz_4lut)]Y (X = Cl, Y = Cl; X = H_2O, Y = $(ClO_4)_2$) and dinuclear[{(*p*-cym)Ru(X)}$_2$(μ-pz_4lut)]Y (X = Cl, Y = Cl_2; X = H_2O, Y = $(ClO_4)_4$) half-sandwich ruthenium(II) complexes comprising a *p*-cym unit (*p*-cym = 1-isopropyl-4-methyl-benzene) and the heteroscorpionate ligand α,α,α′,α′-tetra (pyrazol-1-yl)-2,6-lutidine (pz_4lut) (Fig. 7A) [48]. The compound pz_4lut was reported previously in literature, and its synthesis is based on a $CoCl_2$-catalyzed rearrangement reaction between 2,6-pyridinedicarboxaldehyde and di(pyrazolyl)sulfone [49]. The Ru(II) chloride complexes were obtained by reacting the heteroscorpionate (pz_4lut) with the precursor [(*p*-cym)RuCl(μ-Cl)]$_2$ in 1:1 or 1:2 M ratios, affording mononuclear and dinuclear compounds, respectively. Their aqua congeners were prepared through a Cl/H_2O exchange in the presence of $AgClO_4$. X-ray structures of the dinuclear complexes [{(*p*-cym)Ru(X)}$_2$(μ-pz_4lut)]Y (X = Cl, Y = Cl_2; X = H_2O, Y = $(ClO_4)_4$) reveal that the Ru(II) has a three-legged piano-stool geometry with the heteroscorpionate pz4lut acting as a bridging ligand, coordinated to each metal ion by the two N atoms of the bis(pyrazol-1-yl)methane moieties. The anticancer activities of the complexes have been established in human breast (MCF7), lung (A549) and colon (HCT116) cancer cell lines, being observed a dose dependent suppression of cell viability with only

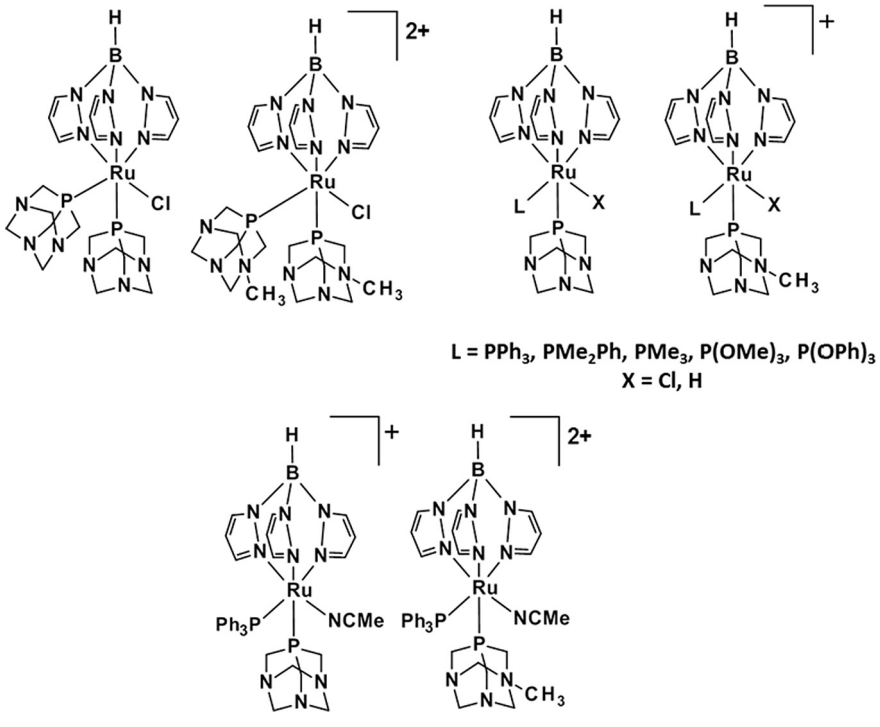

Fig. 6. Ru(II) complexes of Tp with monodentate phosphines.

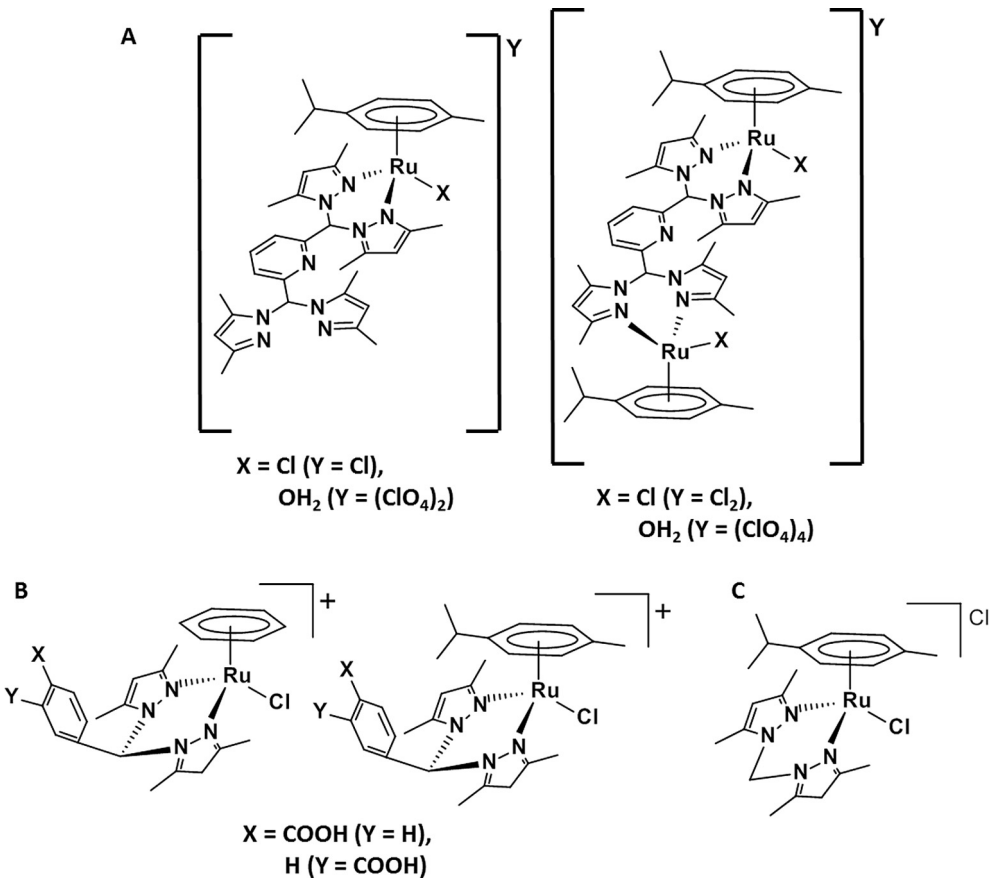

Fig. 7. (A) mononuclear and dinuclear *p*-cym and pz$_4$lut-containing Ru(II) complexes, (B) [(η^6-arene)Ru(II)Cl(L)]$^+$ complexes with L = bis(3,5-dimethylpyrazolyl)metabenzoic acid, bis(3,5-dimethylpyrazolyl)parabenzoic acid and (C) [Ru(*p*-cym)(bis(3,5-dimethylpyrazol-1-yl)methane)Cl]Cl (UNICAM-1).

moderately good IC_{50} values (3.5–92 μM). It was also observed that the mono- and dinuclear aqua derivatives exhibit better cytotoxic effects relatively to the precursor chlorido complexes.

The group of Claudio Pettinari developed Ru(II) complexes, [(η^6-arene)Ru(II)Cl(L)]$^+$ (L = bis(3,5-dimethylpyrazolyl)metabenzoic acid, bis(3,5-dimethylpyrazolyl)parabenzoic acid), having heteroscorpionates that act as bidentate ligands (Fig. 7B) [50]. The synthesis of the ligands was previously described and it is performed by reacting bis(3,5-dimethylpyrazolyl)ketone with 4-carboxybenzaldehyde or 3-carboxybenzaldehyde [51]. These ruthenium complexes were obtained by reaction of the heteroscorpionates with the [(η^6-*p*-cym)$RuCl_2$]$_2$ or [(η^6-benzene)$RuCl_2$]$_2$ precursors. X-ray studies showed that the ruthenium adopts a pseudo-octahedral geometry with the heteroscorpionate ligand coordinated by two N atoms of the bis(pyrazol)methane moiety, and the η^6-arene occupying three facial sites. DNA interaction studies proved that the scorpionate ligand has more influence than the arene on the DNA binding affinity of the complexes. It was observed a higher binding affinity for the bis(3,5-dimethylpyrazolyl)parabenzoic acid-containing complexes compared with the ones with the bis(3,5-dimethylpyrazolyl)metabenzoic acid. However, cytotoxic studies in 15 human cancer cell lines indicated only a moderate activity, even for the complexes with highest DNA affinity.

The Pettinari group has also worked with other half-sandwich (arene)ruthenium(II) containing bis(pyrazolyl)alkane derivatives, and very recently reported on the antitumor capabilities of one of these complexes, [Ru(*p*-cym)(bis(3,5-dimethylpyrazol-1-yl) methane)Cl]Cl (UNICAM-1) (Fig. 7C).[52,53] The compound was obtained by reaction of the bis(3,5-dimethylpyrazol-1-yl)methane with the precursor [{Ru(*p*-cym)Cl_2}$_2$] in a 2:1 M ratio. Cytotoxic evaluation of UNICAM-1 was performed in human MDA-MB-231 and murine A17 cancer cells, which were used as models for triple negative breast cancer (TNBC). TNBC refers to breast cancers that do not display the expression of estrogen receptors, progesterone receptors and HER2 (human epidermal growth factor2), which are some of the most common cellular targets for targeted treatment. UNICAM-1 showed a higher cytotoxic activity compared with NAMI-A, and it was verified that its cell antiproliferative effect is due to apoptosis by activation of the caspase-3. In depth *in vivo* studies of the compound, in tumor-bearing mice, demonstrated that it was capable of suppressing TNBC growth by inhibiting tumor infiltration of regulatory T cells. Additionally, UNICAM-1 also showed low toxicity and favorable clearance properties. The authors have reasoned that the remarkable efficacy of UNICAM-1 appears to be related with its capacity to influence tumor-host interaction, through activation of an immune response specific to malignant cells.

Lanthanides and other trivalent metals have also been explored in the development of antitumor agents stabilized with scorpionate ligands. A series of group 3 and lanthanide (N,N,N)-heteroscorpionate-triflate complexes [M(OTf)(cybpamd)(THF)] (M = Sc, Y, La, Nd, Sm, Dy, Yb; OTf = SO_3CF_3; cybpamd = N,N′-dicyclohexyl-2,2-bis-(3,5-dimethylpyrazol-1-yl)-cetamidinate) (Fig. 8) were described and evaluated by Saturnino and co-workers [54]. The complexes are synthesized by reaction of cybpamd with the respective metallic salt Ln(OTf)$_3$ in THF. On its turn, the synthesis of the (N,N,N)-heteroscorpionate cybpamd is performed by react-

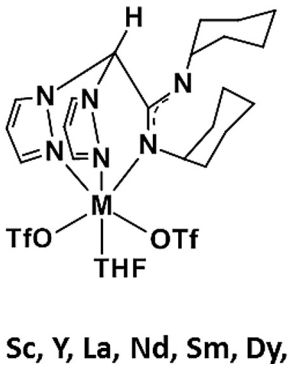

Fig. 8. M(III) (M = Sc, Y, Ln) complexes of cybpamd.

ing bis-(3,5-dimethyl-pyrazol-1-yl)methane with dicyclohexylcarbodiimide in the presence of butyllithium [55]. The molecular structure of the obtained metal compounds displays the anionic ligand cybpamd acting as a tridentate chelator strongly bonded to the metal center, two monodentate OTf groups and a weakly bonded THF. It was found that all the complexes, except that of scandium, show a reasonable activity on the murine macrophage (J774.A1) cell line, but all complexes are poorly active on human adenocarcinoma lung epithelial (A549) and human melanoma (A375) cells. Concerning the cell line HEK-293 only the Nd and Dy complexes show a reasonable activity. On human epithelial cervix adenocarcinoma (HeLa) cells, complexes with Y, Nd and Sm are significantly more active than cisplatin. When studied on human embryonic kidney (HEK-293), epithelial lung adenocarcinoma (A549) and human melanoma (A375) cell lines, only the complex with Nd showed superior activity compared with cisplatin.

3. Photosensitizers

Photodynamic therapy (PDT) is a light based therapy that can be used for both neoplastic and non-neoplastic diseases. The concept of PDT began with studies by Oscar Raab in 1900 on the effects of light and dyes on Paramecia. However, only began to be practiced in the late 1970s with Thomas Dougherty and co-workers. This therapeutic modality involves the activation of a photosensitizing drug by visible light; the interaction between the excited photosensitizer and molecular oxygen produces singlet oxygen ($^1O_2$), as well as other reactive oxygen species (ROS), that induce cell death. Therefore, the photosensitizers play a crucial role in this therapy being porphyrins among the most common ones [56,57]. Metalloporphyrins and a large variety of other metal complexes have also been investigated as photosensitizers, including different M(II) and M(III) scorpionate complexes as reviewed below.

Chakravarty et al. have evaluated the photoinduced DNA-cleavage activity of binary 3d-metal complexes of the '3+2' type with tris(3-phenylpyrazolyl)borate (Tp^{Ph}), $[M(Tp^{Ph})(B)](ClO_4)$ where M is Co(II), Ni(II), Cu(II) and Zn(II) and B is a N,N-donor heterocyclic base, namely 1,10-phenanthroline (phen) and dipyrido[3,2-d:2′,3′-f]quinoxaline (dpq) (Fig. 9) [58,59]. The complexes were synthesized by initial reaction of the metal precursors $[M(H_2O)_6](ClO_4)_2$ with KTp^{Ph}, followed by addition of the heterocyclic base (phen or dpq). In general, the complexes showed only moderate binding affinity to calf thymus DNA and very poor DNA-cleavage activity in the dark, even in the presence of activators like 3-mercaptopropionic acid (MPA) or hydrogen peroxide. The authors attributed these findings to steric factors related with the presence of the Tp^{Ph} protector ligand. Nevertheless, the paramagnetic $[Co(Tp^{Ph})(dpq)]^+$ (d^7) and $[Cu(Tp^{Ph})(dpq)]^+$ (d^9) complexes, bearing the photoactive dpq ligand, show considerable superior DNA-cleavage activity upon UV-A and visible light irradiation. Previous studies with these related binary metal complexes have shown that the photocleavage follows a reductive process generating reactive hydroxyl radicals [60,61]. The authors ascribe this observed enhancement to steric factors related with the Tp^{Ph} ligand. The protection of the photosensitizer by the scorpionate has a relative negative effect in the case of phen complexes but a relatively positive effect in the case of dpq congener complexes.

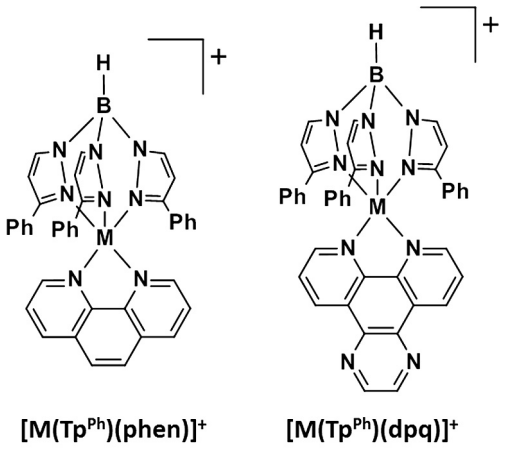

Fig. 9. Metal complexes of the '3+2' type bearing a tris(3-phenyl-pyrazolyl)borate ligand and a N,N-donor heterocyclic base.

4. Carbon monoxide-releasing molecules (CORMs)

Carbon monoxide (CO) is established nowadays as a well-known therapeutic molecule [10,62–64]; it has attracted a lot of attention due to its anti-hypertensive, anti-inflammatory and anti-oxidant effects [65]. Moreover, it can also be explored as a cytoprotective or cytotoxic agent, depending on the concentration of CO. However, in high enough concentrations it is toxic, since it binds to hemoglobin about 200-fold stronger than O_2, and thus the hemoglobin is no longer capable of carrying oxygen in the body [66,67]. In order to overcome this adversity and explore the potential of CO in medical applications, it is crucial to deliver the CO molecules to the target tissues in a controlled manner. To tackle this goal, the pioneer work developed by Motterlini and co-workers was focused on transition-metal carbonyl complexes, which emerged at the time as the first examples of carbon monoxide-releasing molecules (CORMs) [68].

All along the years, many metal carbonyl complexes have been thoroughly explored as prodrugs for the controlled delivery of CO in a biological environment. The release of CO from the carbonyl complexes can be done through different pathways, including ligand exchange with molecules found in the biological medium [69], enzymatic cleavage (ET-CORMs) [70,71], or light induced release of CO (PhotoCORMs) which has been getting a lot of attention recently [72–74]. To better rationalize its use in the design of CORMs, Romão and co-workers [63] have proposed a conceptual model that consists in dividing the metal carbonyl complex in a three part component which includes: (i) a metal center, (ii) an inner coordination sphere, consisting of the CO and ancillary ligands, and (iii) a so called drug sphere, which is obtained by modification of the ancillary ligands at their distal sites (Fig. 10).

In the interaction of CO with the metal center, the Highest Occupied Molecular Orbital (HOMO) of CO donates its electron pair to an empty metal orbital forming a σ bond. Also, occupied metal *d*

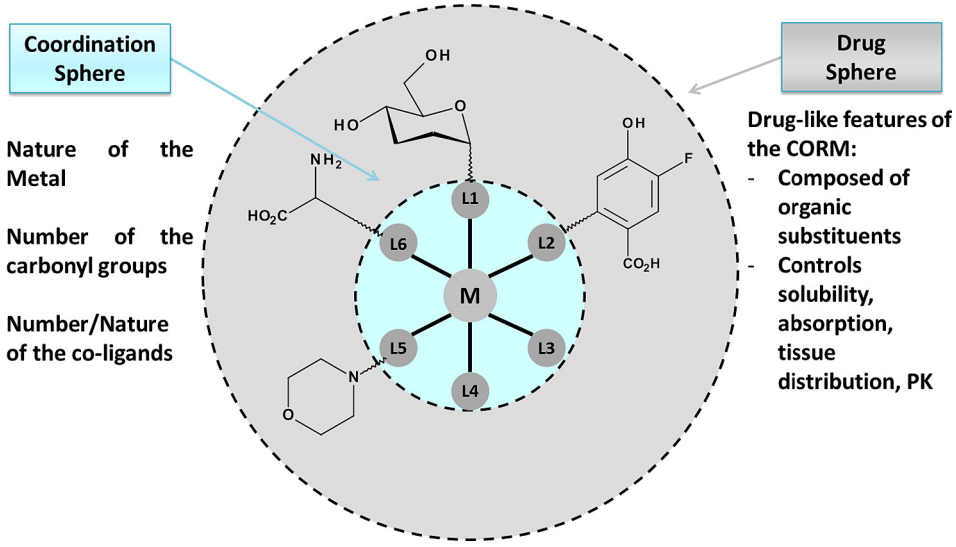

Fig. 10. Conceptual model of CORMs.

orbitals can overlap with the empty low-lying molecular orbitals on the CO, forming a π-acceptor interaction in a synergistic way. This is known as π-backbonding, which is one of the main features responsible for the stability of transition metal carbonyl complexes. Therefore, metals in lower oxidation states and high-energy *d* electrons are more favorable for the metal–carbonyl bonding scheme. Furthermore, it is possible to influence the strength of the metal–carbonyl bond depending on the ancillary ligands used for coordination. By decreasing the *d* electron density of the metal the backbonding is weakened, and thus it will enhance the CO release from the metal coordination sphere [9]. To this day, a great variety of metal-containing CORMs have been developed, which include iron [75,76], molybdenum [77,78], or rhenium [79,80] complexes. Tridentate and tripodal chelators, namely those of the homo- and heteroscorpionate type, are well-suited for a *fac*-coordination in tricarbonyl metal complexes, and are expected to allow a fine tuning of their electronic and steric properties that are crucial to optimize their properties as organometallic CORMs.

4.1. Pyrazole-based CORMs

Schatzschneider et al. described the first study dealing with the evaluation of a scorpionate M(I) tricarbonyl complex as a potential CORM [81]. This study involved the complex $[Mn(CO)_3(Tpm)]^+$ (Tpm = tris(pyrazol-1-yl)methane) (Fig. 11), which was initially reported in the pioneering work where Trofimenko introduced the tris(pyrazolyl)methanes as a new class of chelators [82]. The authors confirmed the solid-state molecular structure of $[Mn(CO)_3(Tpm)]^+$ by X-ray diffraction analysis, showing that the Tpm ligand is facially coordinated to the Mn^{+1} metal ion, within a pseudo-octahedral coordination environment. The complex was shown to undergo photoinduced CO release through irradiation with UV light, acting as a PhotoCORM upon dissociation of two of the CO molecules. Consistently, studies in human colon cancer cells (HT29) proved that the complex only manifest cytotoxic activity when the cells are exposed to UV irradiation. The complex targets the cell nucleus, and accumulates preferentially in the nuclear membrane and nucleoli (Fig. 12) [83]. The promising results obtained for $[Mn(CO)_3(Tpm)]^+$ prompted the study of its alternative delivery to tumor cells based on the conjugation to nanoplatforms, including SiO_2 nanoparticles and nanodiamonds (DND) (Fig. 11) [84,85]. In both cases, the conjugation of the $[Mn(CO)_3(Tpm)]^+$ to the nanoparticle surface was performed via copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC "click" addition). The nanoconstructs have the capability to release CO molecules with irradiation with UV light, similarly to the free complex. So far, the authors did not report any biological assays on these nanoparticles.

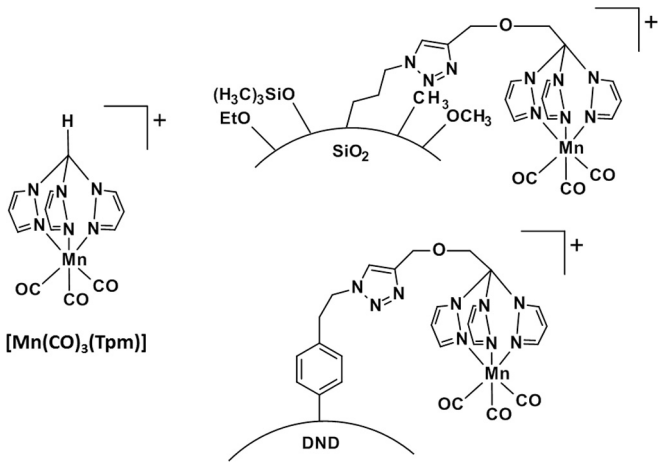

Fig. 11. Tricarbonyl manganese complexes bearing tris(pyrazol-1-yl)methane, free or conjugated to SiO_2 or DND nanoplatforms.

Burzlaff and co-workers have studied tricarbonyl Mn(I) complexes stabilized with pyrazolyl-based (N,N,O) heteroscorpionate ligands containing a central carboxylated coordinating arm of different size: e.g. 3,3-bis(pyrazol-1-yl)acetate/propionate (bpza/bpzp) and 3,3-bis(3,5-dimethylpyrazol-1-yl)acetate/propionate (bdmpza/bdmpzp)) (Fig. 13) [86]. The acetate derivatives, bpza and bdmpza, were synthesized by a classical phase transfer catalyzed reaction of the respective pyrazoles with dibromoacetic acid, while the propionate counterparts (bpzp and bdmpzp) were obtained by reaction of the same pyrazoles with methyl propionate [87,88]. The corresponding tricarbonyl Mn(I) complexes were obtained by reaction of the (N,N,O)-heteroscopionates with $[Br(Mn(CO)_5]$. The complexes with bpza, bdmpza and bdmpzp are stable in aqueous solution in the absence of light, but release about two equivalents of CO when exposed to UV irradiation. This release was determined through the myoglobin (Mb) assay, which is based on the premise that if a complex releases free CO into solution, it will convert deoxy-Mb to Mb-CO. The complex with bpzp on the

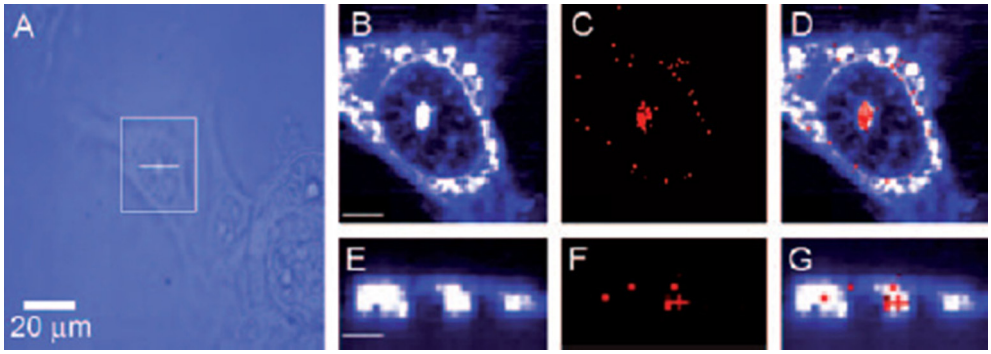

Fig. 12. (A) Optical image of a HT29 human colon cancer cell incubated with an aqueous solution (2 mM) of [Mn(Tpm)(CO)$_3$]Cl for 3 h. (B, C) Raman images reconstructed from integrating the intensities of the C–H and C≡O stretching peaks. The integration range was 2800–3050 (±2) cm^{-1} for (B) and 1945–1965 (±2) cm^{-1} for (C). (D) Overlaid image of panels (B) and (C). (E–G) Cross-section Raman images along the *x*,*z*-direction of the same cell. Scanning positions are indicated by the white bar in the optical image. The scale bar for the Raman images is 6 μm. (Meister K., Niesel J., Schatzschneider U., Nolte-Metzler N., Schmidt D.A., Havenith M.: Label-Free Imaging of Metal-Carbonyl Complexes in Live Cells by Raman Microspectroscopy. Angewandte Chimie. 2010. 49. 3310-3312 .Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

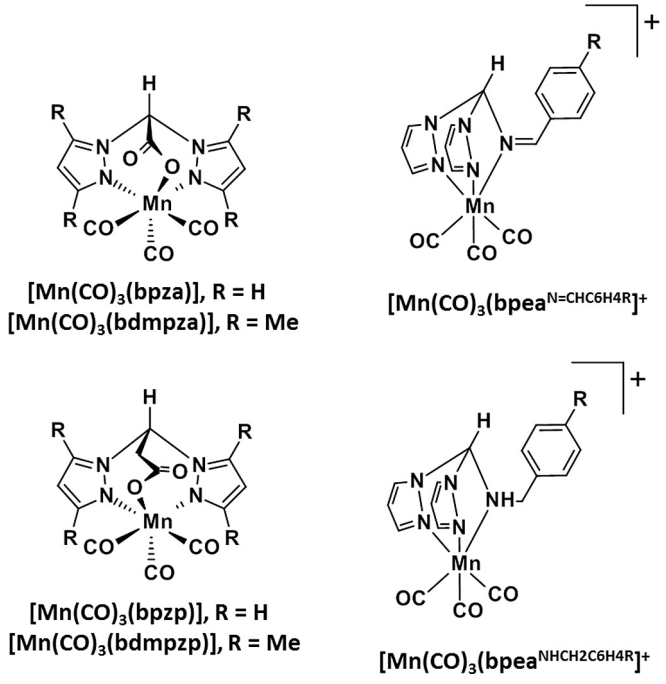

Fig. 13. Tricarbonyl manganese complexes of pyrazolyl-based heteroscorpionates.

other hand is not stable in aqueous medium and tends to release CO even without photoactivation. The authors attributed this difference to the more flexible nature of this ligand compared with the others [86].

Tripodal bis(pyrazolyl)ethylamine (bpea)-based ligands have been introduced by Schatzschneider and co-workers as an alternative to tris(pyrazolyl)methane chelators, expecting to achieve an easier, more flexible and modular attachment of bioactive molecules to the compounds (Fig. 13) [89]. In particular, this can be done based on *para*-substituted benzaldehydes which allow for the conjugation of specific biomolecules to this moiety. The bpea-based ligands are obtained by condensation of 2,2′-bis(pyrazolyl)ethylamine with the benzaldehyde derivatives, and the corresponding amine derivatives are synthesized by reduction of the respective Schiff bases with sodium borohydride. Several bpea-Mn(I) tricarbonyl complexes were synthesized by reacting [BrMn(CO)$_5$] with the desired bpea ligand – either of the Schiff base or amine type. Their potential as CORMs was evaluated using the myoglobin assay, which proved that there is the release of two CO molecules when the complexes are exposed to UV irradiation. The complexes bearing the Schiff bases need a shorter irradiation exposure time to release the CO compared with the amine compounds. The phenyl substituent also influences this parameter, however, not as significantly. The authors have also demonstrated the occurrence of CO release under physiological conditions in the presence of human cells by detection of carbon monoxide using the fluorescent probe COP-1. Incubation of HUVEC cells with [Mn(CO)$_3$(bpea^{NHCH2C6H5}]$^+$ (Fig. 13) led to a significantly augmented response of COP-1 compared with dark controls. However, the fluorescent signal was more pronounced in the supernatant than in the cells (15-fold vs 4-fold increase), indicating that the cellular uptake of the complex is not optimal and would require further improvement.

4.2. Pyridine and imidazole-based CORMs

Tripodal pyridine- and imidazole-based ligands have also been applied in the design of novel CORMs, although in some lower extent than the tris(pyrazolyl)methane congeners. As shown in Fig. 14, this comprised complexes of the type *fac*-[Mn(CO)$_3$L]$^+$ (L = tris(2-pyridyl)amine (Tpa), tris(imidazole-4-yl)phosphane (4-Tip^H), tris(1,4-diisopropylimidazol-2-yl)phosphane (2-Tip^{iPr2}), tris(pyridine-2-yl)phosphane (Tpp) and tris(N-methylimidazol-2-yl)-carbonil (2-Tic^{NMe})) [90–92]. The tricarbonyl complex *fac*-[Mn(CO)$_3$(Tpa)] was initially synthesized by Edwards and co-workers by reaction of the tpa with *fac*-[Mn(CO)$_3$(MeCN)$_3$]ClO$_4$ [92], and latter explored as a PhotoCORM by the group of Mascharak [93]. This Mn(I) complex displays an octahedral geometry that consists of a facially capping tpa ligand and the three CO molecules opposite each to the pyridine rings. The congener Mn(I) tricabonyl complexes with tris(imidazolyl)methane or tris(pyridyl)/tris(imidazolyl) phosphane ligands display a similar molecular structure, as evidenced by their spectroscopic characterization.

Myoglobin assay studies showed that the bulkiness of the imidazolyl ligands with the ^{i}Pr substituents, had a significant influence on the CO-release properties of this family of complexes. The complexes with bulkier ligands undergo the dissociation of a single CO molecule while the other complexes undergo the release of two CO molecules [91]. However, IR studies did not reveal the presence of intermediate decarbonylation products for the different compounds, indicating that even the bulkier ligand-containing Mn(I) complexes lose all three CO molecules upon irradiation. The authors rationalized that most likely there is a significant interac-

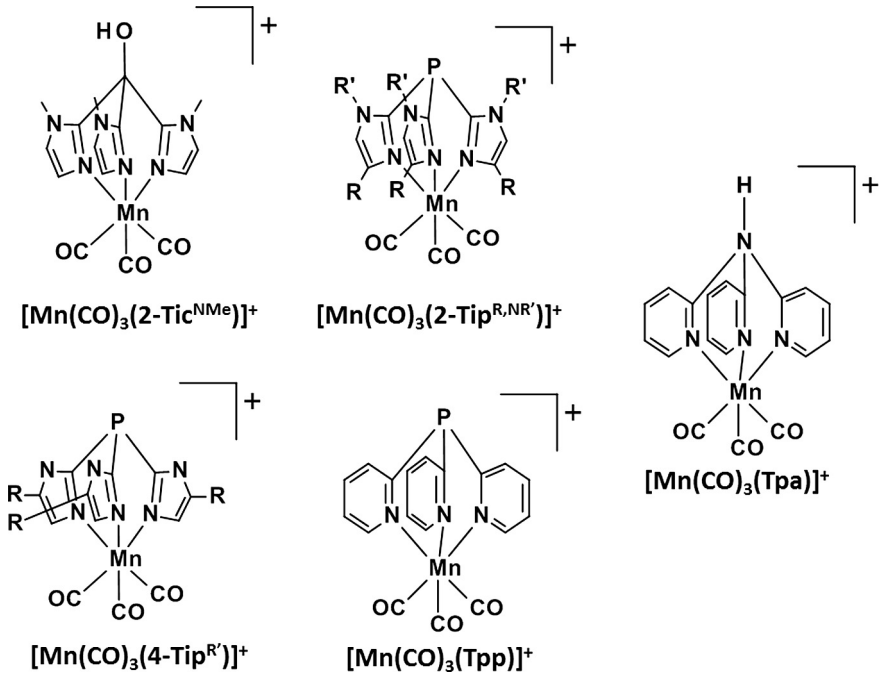

Fig. 14. Tricarbonyl manganese complexes of pyridyl- and imidazolyl-based homoscorpionates.

tion of the complexes with the myoglobin, and a the mechanism of interaction between the complexes and the biological medium should be further investigated [90].

5. Radiopharmaceuticals

Radiopharmaceuticals are drugs containing a radionuclide in their composition and are used in nuclear medicine for diagnosis or therapy. For therapeutic applications, the radiopharmaceuticals must contain radionuclides emitting ionizing particles (α or β^- particles) that selectively target and damage tumor cells. For diagnosis, positron- or gamma-emitting radionuclides are used for positron emission tomography (PET) or single photon emission computed tomography (SPECT), respectively, which are the two available nuclear imaging modalities. Among the radionuclides relevant for medical applications, either for imaging or therapy, radiometals play an important role, as they present very favorable decay and chemical properties for the design of radiopharmaceuticals, namely target-specific ones. The design of target-specific metal-based radiopharmaceuticals is a demanding task requiring the use of bifunctional chelators (BFCs) appropriate to stabilize the metal core and to couple the bioactive molecule, without compromising its biological activity.

To our knowledge, the evaluation of tridentate tripods for the development of radiopharmaceuticals has been dominated by the study of Tc and Re complexes with poly(azolyl)borates, poly(pyrazolyl)methanes and 1,2-diamino-propionic acid derivatives, as reviewed below. However, non-tripodal tridentate pyrazolyl-containing chelators of the pyrazolyl-diamine type had a prominent role as BFCs in the design of a plethora of ^{99m}Tc and ^{188}Re radiopharmaceuticals. For a broader view, the most important achievements obtained for the pyrazolyl-diamine chelators are also summarized in this paper.

5.1. Poly(azolyl)borates

The chemistry of Re and Tc with poly(pyrazolyl)borates has been extensively studied allowing the introduction of a great variety of oxo, carbonyl and polyhydride complexes with the metals in a broad range of oxidation states (I to VII). Despite the rich and versatile coordination chemistry developed for poly(pyrazolyl)borate Re and Tc complexes none of them revealed any potential for the design of radiopharmaceuticals for diagnostic (^{99m}Tc) or therapeutic ($^{186/188}$Re) applications in nuclear medicine. In fact, the majority of the reported complexes was obtained in organic media starting from "conventional" precursors for each oxidation state. Therefore, such compounds hardly could be obtained under the conditions required for the preparation of radiopharmaceuticals due to the lack of adequate synthetic process in aqueous solution using pertechnetate or perrhenate as the starting compounds. ^{99m}Tc and ^{188}Re are always obtained in saline solution in the form of pertechnetate or perrhenate by elution of ^{99}Mo/^{99m}Tc and ^{188}W/^{188}Re generators, respectively [5,94].

The introduction of the *fac*-$[M(CO)_3(OH_2)_3]^+$ (M = ^{99m}Tc, $^{186/188}$Re) precursors by Alberto and collaborators represented an important breakthrough that proved the possibility of obtaining Re and Tc organometallic compounds under aqueous conditions starting from the permetallates MO_4^- (M = ^{99m}Tc, ^{188}Re) eluted from the ^{99}Mo/^{99m}Tc and ^{188}W/^{188}Re generators, respectively [95,96]. The availability of these precursors enabled the use of typically organometallic ligands, like bridging hydrides from poly(mercaptoimidazolyl)borates, cyclopentadienyls and carboranes, in the field of radiopharmaceutical sciences, to stabilize complexes with the *fac*-$[M(CO)_3]^+$ (M = Re, Tc) core [97–100].

The "soft" nature of the poly(mercaptoimidazolyl)borates makes them very adequate to stabilize low valent transition metals, such as Re(I) and Tc(I). Thus, in our group we have studied the coordination behavior of dihydrobis(2-mercaptoimidazolyl)borates and trihydro(2-mercaptoimidazolyl)borates towards the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99}Tc) moiety under aqueous conditions, in collaboration with Roger Alberto's group. These studies led to the first examples of Re and Tc tricarbonyl complexes containing, respectively, one or two coordinated bridging hydrides (Fig. 12) [26,27,100]. The study of the same reactions with sodium or lithium salts of $[RB(Tim^{Me})_3]^-$ (R = H, Me, Ph) led to the formation of related complexes that contain the scorpionate ligand coordinated through the three sulfur atoms (Fig. 15). The coordination

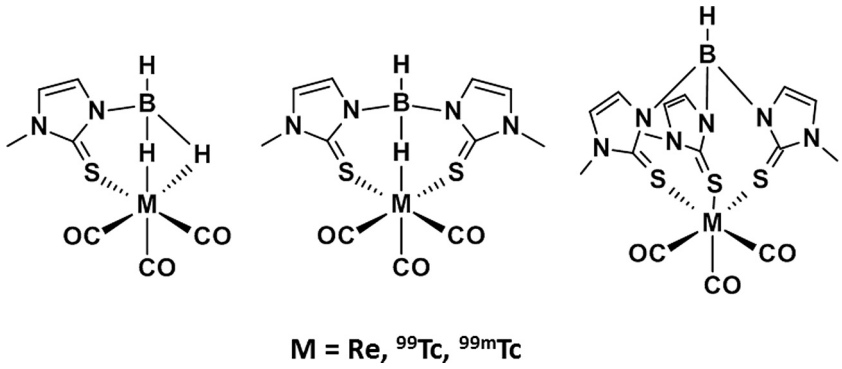

Fig. 15. Tricarbonyl 99mTc complexes of boron-containing ligands of the type κ^3-*S,S,S*, κ^3-*S,S,H* and κ^3-*S,H,H*.

mode of the respective boron-containing ligands ((κ^3-*S,S,H*, κ^3-*S,S,H* or κ^3-*S,H,H*) in each of these complexes was confirmed by X-ray structural analysis, multinuclear NMR and IR spectroscopy. The M-B bond (M = Re, Tc) distance is significantly affected by the number of mercaptoimidazolyl rings present in the ancillary ligands, reflecting the increasing steric requirements upon the successive replacement of the B-H hydrogen atoms by mercaptoimidazolyl rings.

To prove the suitability of poly(mercaptoimidazolyl)borates for radiopharmaceutical chemistry the analogous ^{99m}Tc(I) complexes have been also prepared. Remarkably, the ^{99m}Tc complexes were promptly obtained at room temperature by reacting the aqua-ion *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ with the sodium salts of the corresponding scorpionates, even at low ligand concentrations to obtain the radiocomplexes presented in Fig. 15 with high specific activity. Above all, the resulting ^{99m}Tc complexes are remarkably stable under physiologic conditions showing that the water and the large excess of Cl^- present in solution do not compete with the bridging hydrides. Biodistribution studies in mice have shown that all complexes are able to cross the blood brain barrier (BBB), in agreement with their moderate lipophilicity, small size and neutral charge [101].

The physico-chemical properties (e.g. charge, size and lipophilicity) of dihydrobis(2-mercaptoimidazolyl)borate and trihydrobis(2-mercaptoimidazolyl)borate ^{99m}Tc(I) tricarbonyl complexes and their ability to cross the BBB prompted their evaluation as building blocks to design target-specific complexes for imaging brain receptors, namely the central 5-hydroxytryptamine (5-HT_{1A}) receptors. The design of ^{99m}Tc radioactive probes for *in vivo* imaging of the central 5-hydroxytryptamine (5-HT_{1A}) receptors has received considerable attention, as this subtype of serotonergic receptors are implicated in major neuropsychiatric disorders such as schizophrenia, anxiety and depression [102,103]. Aiming to obtain radiometallated bioconjugates useful as SPECT probes to image central 5-HT_{1A} serotonergic receptors, the authors group proceeded with the functionalization of the mono- and bis(mercaptoimidazolyl)borate Re(I) and Tc(I) building blocks with arylpiperazine pharmacophores having known affinity and selectivity towards the 5-HT_{1A} serotonergic receptors [100,104,101,105]. Different strategies and linkers were used to couple the bioactive fragment to the mercaptoimidazolyl rings. This included the inclusion of two pharmacophores per chelator, within the so-called bivalent approach (Fig. 16). The linker length has a huge influence in the affinity (IC_{50} values) towards the 5-HT_{1A} receptors. Unlike the Re complexes containing a methylenic linker, those containing a butylenic linker for coupling the 2-methoxyphenyl)piperazine fragment to the metal moiety displayed excellent nanomolar or subnanomolar affinity (IC_{50} values) towards the 5-HT_{1A} receptors. Unfortunately, the ^{99m}Tc congeners have shown a relatively poor brain uptake in mice [101].

The coupling of the (2-methoxyphenyl)piperazine pharmacophore to the fac-$[M(CO)_3]^+$ core was also studied using the [2+1] approach, profiting from the reactivity of *fac*-[Re{κ^3-H(μ-H)B(Tim^{Me})$_2$}$(CO)_3$] towards isonitriles, as discussed below. Cleavage of the B-H...Re bond by isonitriles carrying the pharmacophore (2-methoxyphenyl)piperazine led to mixed-ligand complexes, containing methylenic spacers of different length between the pharmacophore and the isonitrile coordinating group (Fig. 16). These mixed-ligand complexes have shown moderate *in vitro* affinity (IC_{50} = 21.9–66.5 nM) for the 5-HT_{1A} receptors. However, at the tracer level, none of these complexes has been described.

Reactivity studies of *fac*-[Re{κ^3-H(μ-H)B(Tim^{Me})$_2$}$(CO)_3$] and *fac*-[Re{κ^3-H(μ-H)$_2$B(Tim^{Me})}$(CO)_3$] with a variety of monodentate and neutral substrates (e.g. imidazole, pyridine, isonitrile and phosphine derivatives) allowed the synthesis of different mixed-ligand complexes of the [2+1] type [106–108]. Solid state structural analysis and NMR studies have shown that the resulting complexes contain mercaptoimidazolyl borates coordinated in a κ^2-*S,S* or κ^2-*S,H* fashion, respectively, as exemplified for the PPh_3 derivatives in Fig. 17. These mixed-ligand complexes present different behavior in solution, depending on the monodentate co-ligand. The isonitrile-containing complexes remain stable in solution in opposition to the other mixed-ligand compounds that tend to slowly release the corresponding monodentate co-ligands (imzH, 4-NMe_2py and PPh_3), regenerating the starting complex *fac*-[Re{κ^3-H(μ-H)B(Tim^{Me})$_2$}$(CO)_3$]. Such difference may be due to the strong π-acceptor character of the isonitrile ligands, which prevents the rebuilding of the B-H...Re bond.

Rhenium tricarbonyl complexes anchored by hybrid poly(azolyl)borates, formed *in situ*, could also be obtained by reacting *fac*-[Re{κ^3-H(μ-H)$_2$B(Tim^{Me})}$(CO)_3$] with pyrazole derivatives (Fig. 18) [109]. The formation of the hybrid poly(azolyl)borates was confirmed by X-ray structural studies of all the isolated Re complexes. At tracer level, reactions of the congener *fac*-[^{99m}Tc{κ^3-H(μ-H)B(Tim^{Me})$_2$}$(CO)_3$] with the same pyrazole derivatives do not yield the corresponding complexes anchored by hybrid poly(azolyl)borates. Probably, this behavior is related with the low concentration of ^{99m}Tc (i.e. 10^{-7}–10^{-9} M), which might render the reactions very slow even under pseudo first-order conditions. Preparation of the complex *fac*-[^{99m}Tc{k^3-H(μ-H)B(Tim^{Me})(3,5-Me_2pz)}$(CO)_3$] has only been achieved in high yield by reacting the precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ with the sodium salt of the corresponding dihydrobis(azolyl)borate [110].

5.2. Neutral scorpionates and related tridentate non-tripodal ligands

Recently, tris(pyrazolyl)methane chelators also started to be foreseen as a class of ligands with potential applications in the design of ^{99m}Tc radiopharmaceuticals. These chelators present higher hydrolytic stability than tris(pyrazolyl)borates and retain

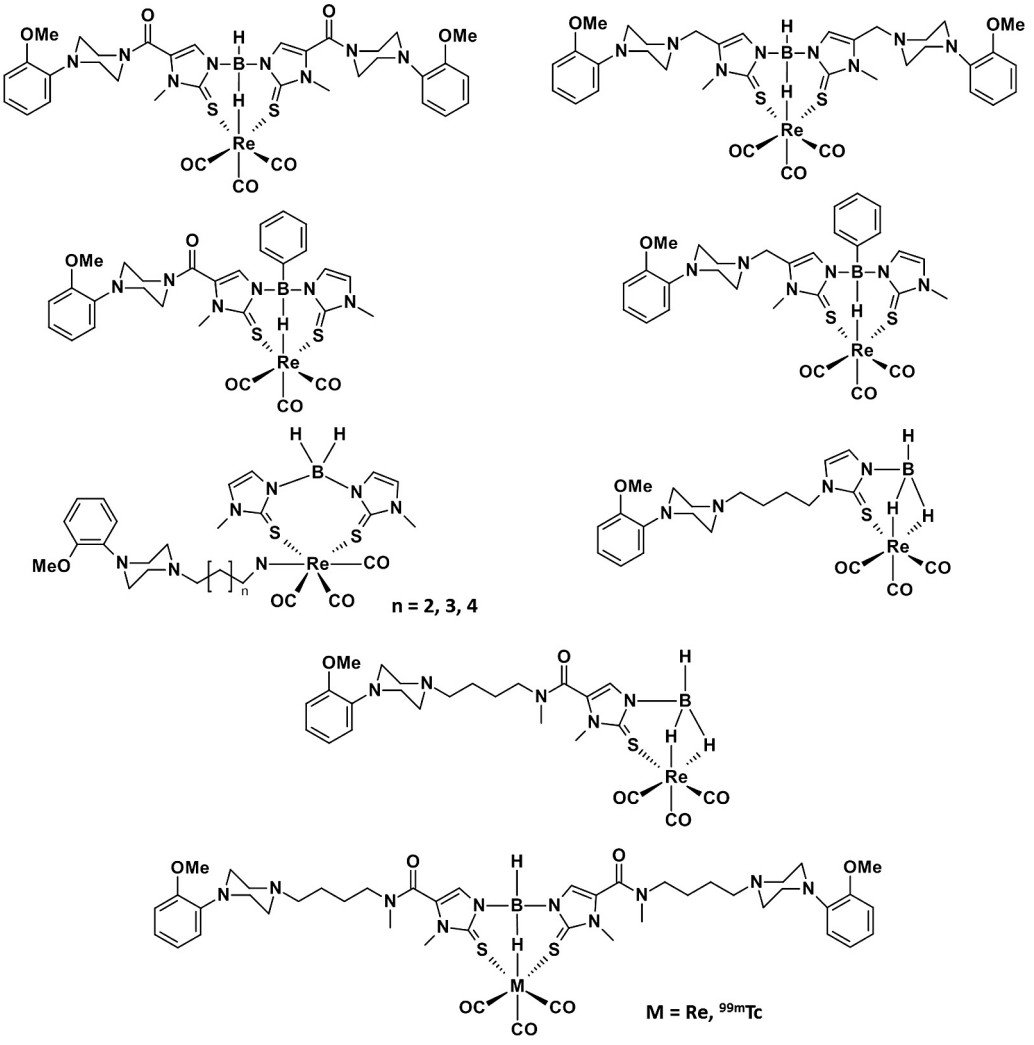

Fig. 16. Scorpionate Re(I) and 99mTc(I) tricarbonyl complexes carrying arylpiperazine pharmacophores.

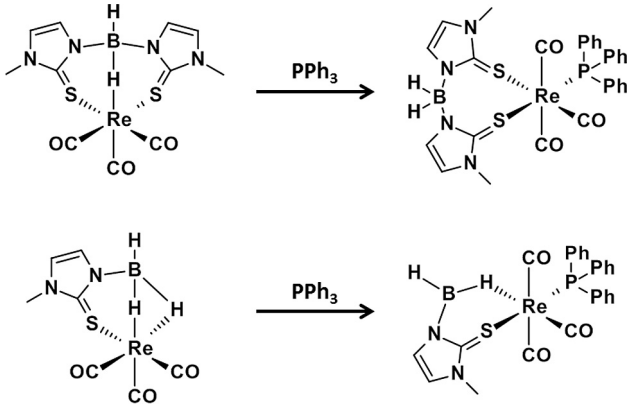

Fig. 17. Reactions of poly(mercaptoimidazolyl)borate Re(I) tricarbonyl complexes with triphenylphosphine [106,108].

a preorganized facial arrangement providing a high stability to the respective Re and Tc complexes. The studies reviewed herein for tris(pyrazolyl)methane ^{99m}Tc complexes have been focused mainly on low-valent complexes with the *fac*-$[M(CO)_3]^+$ moiety for the design of SPECT probes for nuclear cardiac imaging.

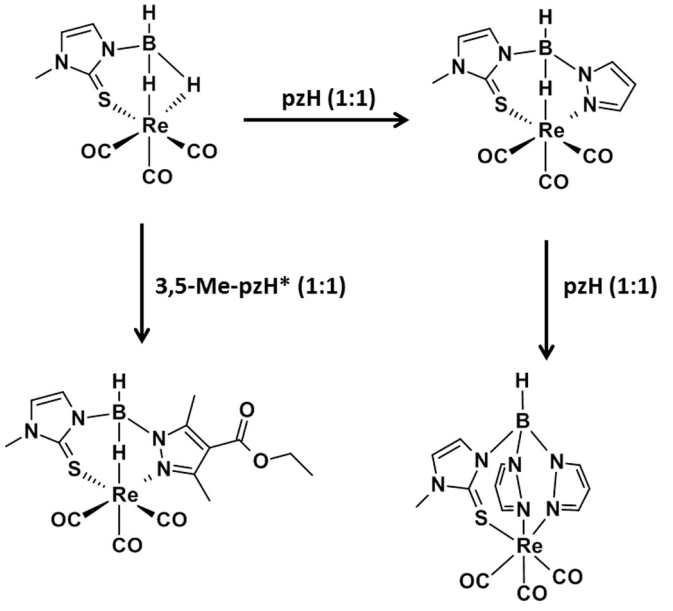

Fig. 18. "*In situ*" formation of Re(I) tricarbonyl complexes with hybrid poly(azolyl) borates [109].

Despite the present interest in target-specific radiopharmaceuticals for nuclear molecular imaging, there is still opportunity for the design of better performing ^{99m}Tc complexes to replace some established perfusion agents in present clinical use. This is particularly relevant for myocardium imaging agents, which are important and non-invasive tools for the clinical evaluation of patients with known or suspected coronary artery disease (CAD), remaining as one of the leading causes of death in western countries. [^{99m}Tc]-Sestamibi and [^{99m}Tc]-Tetrofosmin are lipophilic and cationic SPECT radiopharmaceuticals in clinical use for heart imaging (Fig. 19) [111]. However, both compounds present a relatively low first-pass extraction and a relatively low heart/liver and heart/lung ratios that may interfere in the clinical interpretation of the heart images due to the radioactivity retained in the adjacent non-target organs [111]. Aiming to introduce best performing heart imaging agents, when compared with the radiopharmaceuticals in clinical use, several research groups embarked in the study of new lipophilic and cationic ^{99m}Tc complexes of the Tc(V) nitrido and Tc(I) tricarbonyl type [111,112]. In this field, the authors' group has evaluated bis(pyrazolyl)ethanamine and tris(pyrazolyl) methane derivatives that are neutral tripod chelators, stable in water and offering a versatile functionalization with ether groups that are crucial to improve the biological performance of the respective ^{99m}Tc(I) tricarbonyl complexes [28,113–115].

Among the evaluated tris(pyrazolyl)methane ^{99m}Tc(I) tricarbonyl complexes, *fac*-[^{99m}Tc(CO)$_3${HC[3,5-(MeOCH$_2$)$_2$pz]$_3$}]$^+$ (^{99m}Tc-DMEOP) and *fac*-[^{99m}Tc(CO)$_3${HC[3,4,5-(MeOCH$_2$)$_3$pz]$_3$}]$^+$ (^{99m}Tc-TMEOP) (Fig. 19) showed the most promising biological profile for heart imaging, particularly ^{99m}Tc-TMEOP that carries three methoxymethyl substituents at each azolyl ring [28,113,114]. The synthesis of ^{99m}Tc-TMEOP has been optimized by using the iodide salt of a sodium complex of TMEOP (Fig. 20), which is water-soluble and reacts quantitatively with *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ to afford the desired complex.

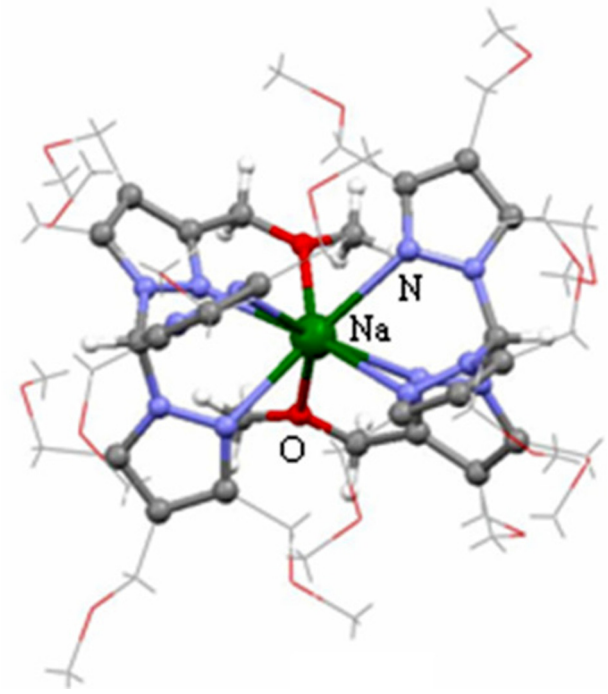

Fig. 20. X-ray diffraction structure of the cation of [Na(TMEOP)$_2$]I.

Most importantly, ^{99m}Tc-TMEOP showed a cardiac uptake comparable to ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin, with a significantly faster liver clearance (Fig. 21), pointing out that these tris (pyrazolyl)methane ^{99m}Tc(I) tricarbonyl complexes may improve the diagnostic accuracy of CAD. As described earlier for ^{99m}Tc-sestamibi, the authors have also shown that ^{99m}Tc-TMEOP and ^{99m}Tc-

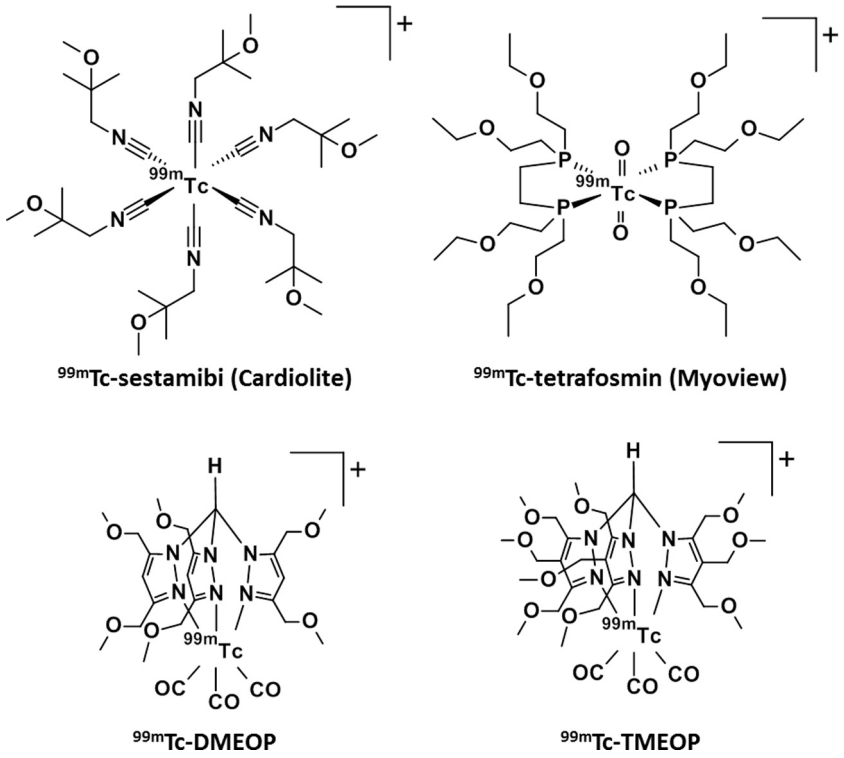

Fig. 19. Molecular structures of 99mTc-Sestamibi and 99mTc-Tetrofosmin, 99mTc-DMEOP and 99mTc-TMEOP.

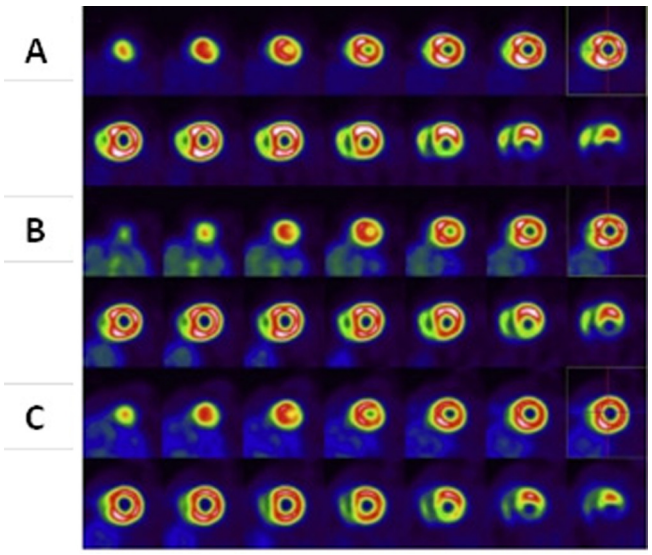

Fig. 21. Representative SPECT image analysis at 40 min after administration: (A) 99mTc-TMEOP; (B) 99mTc-Sestamibi and (C) 99mTc-Tetrofosmin. Reproduced by permission of John Wiley and Sons [5,114].

DMEOP present potential for cancer early detection and non-invasive monitoring of tumor multidrug resistance (MDR) [116].

For a better understanding of the influence of the ether substitution pattern on the physico-chemical (i.e. lipophilicity) and biological properties (i.e. heart uptake, heart/liver and heart/lung ratios, metabolic stability) of this family of tris(pyrazolyl)methane ^{99m}Tc(I) tricarbonyl complexes, the authors studied a large variety of chelators with different ether functions at different positions of the azole rings [117]. This study proved that the functionalization of tris(pyrazolyl)methane chelators with methoxymethyl or ethoxymethyl groups at different position of the azole rings has a marked influence on the biodistribution profile, pharmacokinetics and metabolic stability of the respective ^{99m}Tc(I) tricarbonyl complexes. In fact, complexes containing ether groups exclusively at the 4-position of the azolyl ring, *fac*-[^{99m}Tc(CO)$_3${HC[4-(ROCH$_2$)pz]$_3$}] (R = Me, Et), undergo metabolic transformation to more polar compounds justifying the negligible heart uptake observed for such complexes, in opposition to ^{99m}Tc-TMEOP. Remarkably, the presence of other ether groups at the 3- and 5-positions of the azolyl ring avoids the *in vivo* metabolization of the ethers substituents at the 4-position, as observed for ^{99m}Tc-TMEOP and ^{99m}Tc-DMEOP that do not undergo *in vivo* metabolization.

The authors have made several attempts to identify the metabolites and concluded that the ether functions probably were oxidized to alcohol and carboxylic acid derivatives (Fig. 22). The formation of such type of metabolites was proposed based on a comparative study using *fac*-[^{99m}Tc(CO)$_3${[4-(MeOCH$_2$)pz](CH$_2$)$_2$-NH(CH$_2$)$_2$NH$_2$}], which is stabilized by a congener ether-containing pyrazolyl-diamine chelator [117].

It is worthwhile to mention that pyrazolyl-diamine chelators form M(I) (M = Re, ^{99m}Tc) tricarbonyl complexes with quite favorable complexation kinetics and high thermodynamic stability [118–120]. These favorable features prompted our research group to exhaustively investigate pyrazolyl-diamine derivatives as bifunctional chelators (BFCs) to obtain ^{99m}Tc and ^{188}Re target-specific radiopharmaceuticals for nuclear molecular imaging and targeted therapy. Pyrazolyl-diamine derivatives are quite versatile as BFCs since they allow the introduction of targeting molecules in the azole ring or in the aliphatic chain, namely using pendant arms

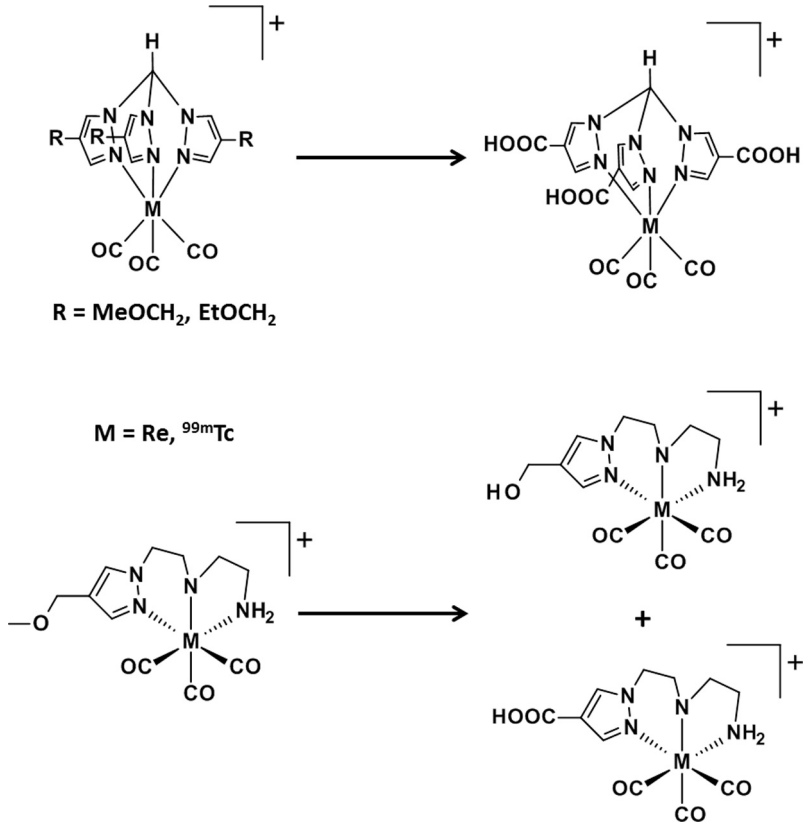

Fig. 22. Tris(pyrazolyl)methane and pyrazolyl-diamine Re/99mTc (I) tricarbonyl complexes bearing ether functions at the 4-position of the pyrazolyl ring and their probable metabolites (acid and alcohol derivatives).

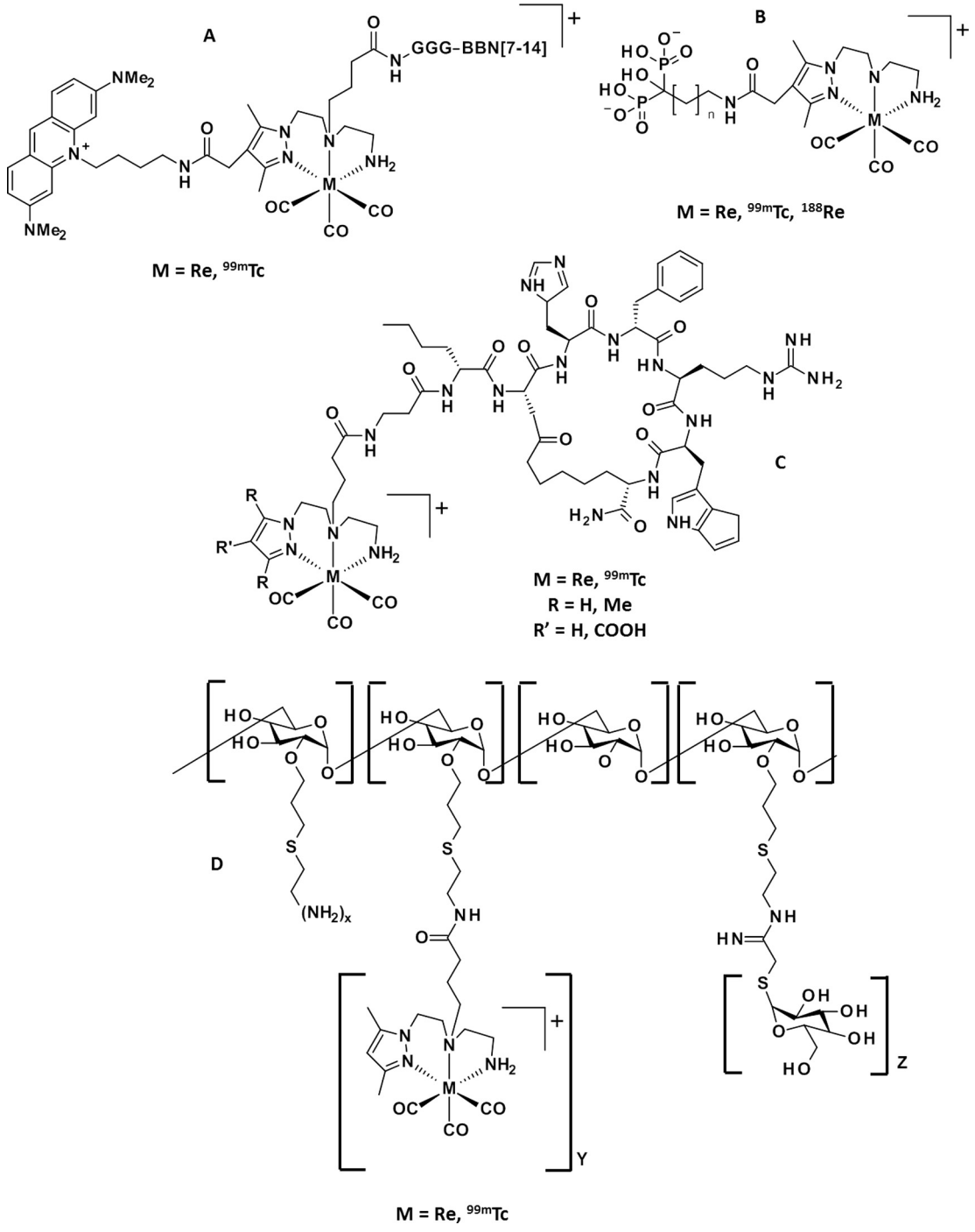

Fig. 23. Pyrazolyl-diamine tricarbonyl complexes: (A) bearing a DNA intercalating acridine orange (AO) moiety and a triglycine linker between a bombesin sequence; (B) with a bisphosphonate moiety; (C) with a cyclic melanocortin analogue and (D) with mannosylated dextran derivatives.

attached at the secondary amine. Moreover, it is possible to introduce different pharmacokinetic modulators at the different positions of the azolyl ring. The pyrazolyl-diamine framework has been used for the labeling of a large variety of targeting molecules with the *fac*-$[^{99m}Tc(CO)_3]^+$ core, which included: (i) bioactive peptides (e.g. RGD, bombesin and melanocortin analogues) targeted at GPC receptors [121–124]; (ii) enzyme inhibitors and substrates (e.g. arginine and quinazoline derivatives) [125,126]; (iii) DNA intercalators [127,128]; (iv) other small biomolecules, like estradiol derivatives [129], delocalized cations (DLC) of the triphenylphosphonium (TPP) type [130], bisphosphonates (BPs) [131,132] and mannose derivatives [133].

For example, we have reported a pyrazolyl-diamine $^{99m}Tc(I)$ complex, bearing a DNA intercalating acridine orange (AO) moiety and a triglycine linker between a bombesin (BBN) sequence and the chelator framework, which presented a remarkably high cellular internalization and nuclear uptake in prostate cancer cell (Fig. 23A) [128]. To the best of our knowledge, this complex remains the first and unique example of a ^{99m}Tc-bioconjugate that combines specific cell targeting with a pronounced nuclear inter-

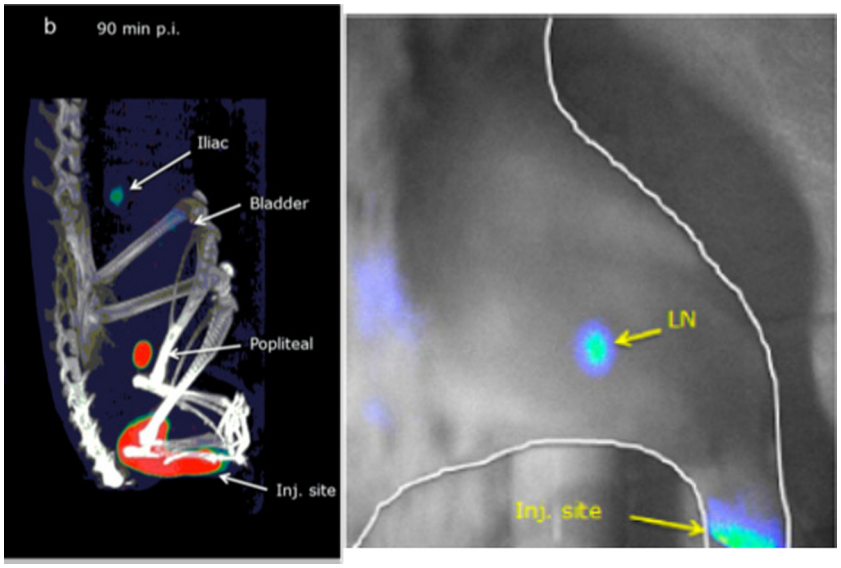

Fig. 24. SPECT/CT image of a Wistar rat injected with a 99mTc(CO)$_3$-mannosylated dextran dual probe at 90 min p.i. (left). NIR optical images of Wistar rat leg injected with (180 min p.i.). The yellow arrows indicate the localization of the bimodal probes in the popliteal lymph node. Reprinted with permission from [147]. Copyright (2014) American Chemical Society.

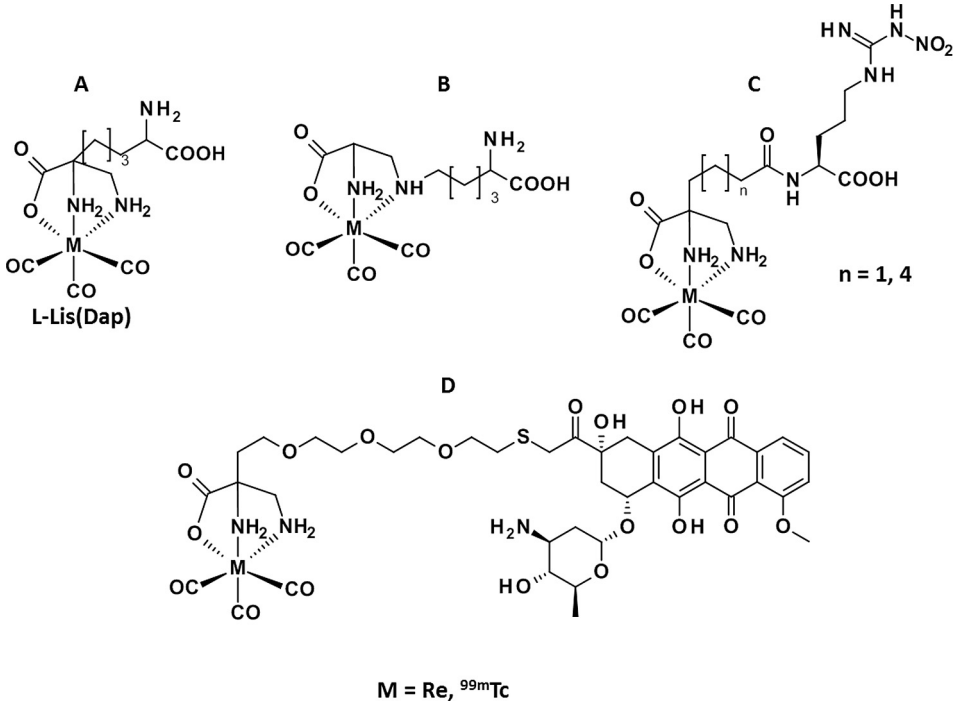

Fig. 25. DAP-M(I)-tricarbonyl complexes bearing pendant small biomolecules: amino acid groups and DNA intercalators.

nalization. The use of pyrazolyl-diamine as BFCs allowed us to obtain other relevant results, including some cases with clinical translational potential, as summarized below.

The labeling of BPs with the *fac*-[^{99m}Tc(CO)$_3$]$^+$ core using pyrazolyl-based chelators afforded BP-containing ^{99m}Tc(I) complexes that, in some cases, have shown a fast blood clearance, high bone uptake and higher bone-to-blood and bone-to-muscle ratios than the imaging agents in clinical use (Fig. 23B) [131,134–136]. The good results obtained at the ^{99m}Tc level encouraged us to prepare the ^{188}Re congeners [136]. Remarkably, the resulting ^{188}Re congener displayed higher bone uptake and enhanced stability towards reoxidation to perrhenate than [^{188}Re-HEDP]. Furthermore, the best performing pyrazolyl-diamine ^{188}Re-BP complex presents a much higher radiocytotoxic effect against tumoral cells, when compared with that elicited by [^{188}ReO$_4$]$^-$ at similar radioactive concentrations. Altogether, these results demonstrate that these ^{99m}Tc(I)/^{188}Re(I) tricarbonyl complexes are attractive candidates for further preclinical evaluation in the diagnostic or systemic radionuclide therapy of bone metastases, respectively.

The design of targeted-radiopharmaceuticals towards melanoma tumors is being intensely investigated using several linear and cyclic small peptides targeting the melanocortin-1 receptor (MC1R) [122,124,137–141] or benzamide derivatives [98,142–144]. We have introduced a cyclic melanocortin analogue labeled

with the *fac*-[^{99m}T(CO)$_3$]$^+$ core using pyrazolyl-diamine BFCs (Fig. 23C) [137]. To improve the biodistribution profile, we studied the effect of different azolyl-ring substitution patterns (carboxylate at the 4-position and/or methyl groups at the 3,5-positions) on the pharmacokinetic profile. Remarkably, the introduction of a free carboxylate at the 4-position of the pyrazolyl ring improved extraordinarily the pharmacokinetic profile leading to a ^{99m}Tc-labeled melanocortin analogue with very high and specific tumor targeting capability and with minimal retention of activity in the hepatobiliary tract [141].

In the past few years, several groups have been involved in the design of nanosized radioprobes for sentinel lymph node detection (SLND). Exploring the ^{99m}Tc-tricarbonyl approach and the superior coordination properties of the pyrazolyl-diamine-based chelators, our group reported fully characterized ^{99m}Tc-mannosylated dextran derivatives with favorable biological features for SLND (Fig. 23D) [133,145,146]. SPECT/CT studies in mice confirmed that the resulting ^{99m}Tc-mannosylated dextran derivatives accumulate in the popliteal lymph node, allowing its clear visualization (Fig. 24). The promising biological results, prompted the design of new nanocompounds containing in the same chemical entity the pyrazolyl-diamine chelator for stabilizing the radiometal ^{99m}Tc and a mannosylated dextran-based nanoconstruct functionalized with a near-infrared (NIR) dye for optical imaging. The multimodal probe allowed a clear visualization of the popliteal node by single-photon emission computed tomography (SPECT/CT), as well as real-time optically guided excision [147]. The ^{99m}Tc(CO)$_3$-mannosylated dextran derivatives containing NIR fluorophores justify further evaluation for pre- and intraoperative SLN mapping.

5.3. Derivatives of 2,3-diamino-propionic acid (DAP)

Besides the classical scorpionates of the poly(azolyl)borate and tris(pyrazolyl)methane type, derivatives of 2,3-diamino-propionic acid (DAP) have also been investigated as tripodal tridentate chelators for the labeling of biomolecules with the [^{99m}Tc(CO)$_3$]$^+$ core. Alberto et al. introduced a complex with the *fac*-[M(CO)$_3$]$^+$ core (M = Re, ^{99m}Tc) stabilized with a DAP chelator previously functionalized with a lysine group at the α-C (Fig. 25A). Additionally, they also synthesized an analogue complex but with the lysine at the terminal amine (Fig. 25B). A distinct behavior was found for both complexes since the compounds functionalized at the terminal amine of the chelator had no affinity for the L-type amino acid transporter (LAT1). On the other hand, the complex functionalized with the lysine at the α-C of the chelator was actively transported and recognize LAT1 [148]. Moreover, it is possible to introduce further functionalities to conjugate a wide variety of biomolecules with the small, hydrophilic, and strong DAP chelator [149–151].

Following their previous work based on pyrazolyl-diamine Re(I) and ^{99m}Tc(I) tricarbonyl complexes for *in vivo* targeting of the indu-

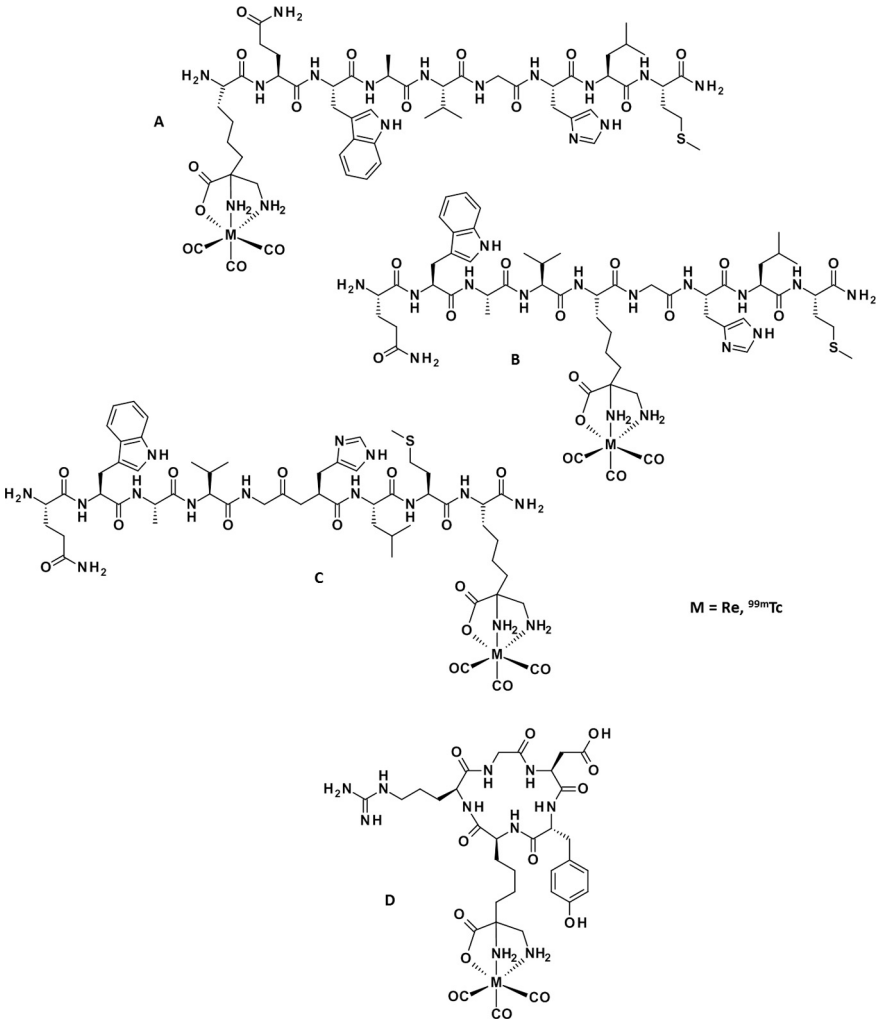

Fig. 26. Structures of the different BBN(7–14) derivatives with the L-Lys(DAP) amino acid: (A) conjugated to the N-Terminus; (B) integrated into the sequence, and (C) bound to the C-Terminus. (D) Complexes bearing the c-RGD peptide analogue cyclo-(Arg-Gly-Asp-D-Tyr-Lys(DAP)).

cible nitric oxide synthase (iNOS) [125,126,152], Correia et al. prepared a new family of rhenium complexes stabilized by a DAP chelator and containing a iNOS-recognizing moiety, based on the arginine amino acid (Fig. 25C) [153]. However, the metallated DAP derivatives bind weaker to iNOS than the previously described pyrazolyl-diamine congeners bearing the same iNOS-recognizing moiety. Using a computational approach, the authors have identified structural determinants that are potentially responsible for the different iNOS-recognizing abilities of the different complexes [153]. Based on these results, it is possible to foresee the design of novel 'MCO_3' complexes (M = Re/^{99m}Tc) that could interact more strongly with the enzyme.

Doxorubicin (ADR) is a widely applied chemotherapeutic known for its accumulation in the nucleus and ability to intercalate into DNA and target hypermitotic cells. Alberto et al. prepared a small array of doxorubicin-metalloconjugates (M = ^{99m}Tc, Re) using different chelators, namely dipicolylamine (DPA) and HS-DAP (Fig. 25D) [154]. In the latter case, the bifunctional chelator contains a biocompatible polyethylene glycol linker to increase the solubility. In this case, the cold rhenium complex retain an appreciable cytotoxicity, strongly bind DNA and effectively act as inhibitor of the human Topoisomerase II enzyme at concentrations well comparable to native ADR [154]. The HS-DAP chelator has also been explored for the [^{99m}Tc(CO)$_3$]-labeling of gold nanoparticles and quantum dots. The nanoparticles were vectorised with a small molecule inhibitor of the prostate specific membrane antigen (PSMA) overexpressed in prostate cancer [155].

Alberto et al. also introduced a DAP derivative with orthogonally protected functional groups to enable its general use in automated SPPS, a very important feature for the development of peptide based radiopharmaceuticals. To demonstrate the strength of this concept the authors conjugated L-Lys(DAP) to three different positions in BBN(7–14) (Fig. 26A-C) and have also replaced the original lysine in the c-RGDyK sequence with L-Lys(DAP) (Fig. 26D) without affecting seriously the binding affinity of the peptide to different integrin subtypes, in particular, to the $\alpha_v\beta_3$ receptor. All the resulting peptides were labeled with the [^{99m}Tc(CO)$_3$]$^+$ core in high yield. Biodistribution studies with the radiolabelled RGD analogue in M21 melanoma bearing nude mice showed a relatively low $\alpha_v\beta_3$-integrin specific tumor uptake [156].

6. Concluding remarks

In this review we have highlighted some of the most relevant metal-based compounds originated from the development of the scorpionate chemistry, with applications as cytotoxic agents, photosensitizers, CORMs and radiopharmaceuticals. In the particular case of radiopharmaceuticals, this research effort led to the introduction of some complexes with unambiguous clinical translational potential, namely tris(pyrazolyl)methane ^{99m}Tc(I) tricarbonyl complexes as perfusion agents for nuclear cardiac imaging.

Although tridentate tripod ligands have had relative success to this date, there is still room for exploration and optimization of these type of ligands to further enhance their potential for biomedical applications. Nowadays, in the development of medically relevant metal-based complexes, it is crucial to design ligands that can be accessibly functionalized with targeting molecules (e.g. peptides, antibodies, proteins) in order to provide higher affinity and specificity to the complexes towards biological or biochemical targets of pathological relevance. We are convinced that scorpionate chelators, either of the poly(azolyl)borate or poly(azolyl)methane type, deserve further evaluation in the design of target-specific metallodrugs for imaging and/or therapy. For this purpose, recent advances on biorthogonal click chemistry proportionate plenty of possibilities to attach the biomolecules, namely through the boron and carbon central atoms of anionic and neutral scorpionates, respectively.

Acknowledgments

The authors thank Fundação para a Ciência e Tecnologia (projects EXCL/QEQ-MED/0233/2012 and ID/Multi/04349/2013).

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Please cite this article in press as: F. Silva et al., Polyhedron (2017), http://dx.doi.org/10.1016/j.poly.2016.11.040