Dalton Transactions

Dynamic Article Links

Cite this: *Dalton Trans.*, 2011, **40**, 5377

www.rsc.org/dalton

PERSPECTIVE

Metalloprobes for functional monitoring of tumour multidrug resistance by nuclear imaging

Filipa Mendes, António Paulo and Isabel Santos*

Received 22nd September 2010, Accepted 11th January 2011
DOI: 10.1039/c0dt01275k

Cancer chemotherapy has been used since the early 1950s and still remains one the major therapeutic options for many malignant tumours. A major obstacle to successful cancer chemotherapy is drug resistance. Frequently resistance is intrinsic to the cancer, but as therapy becomes more effective, acquired resistance has also become more frequent. One form of resistance, named multidrug resistance (MDR), is responsible for the failure of tumours to respond to a wide spectrum of chemotherapeutic agents. The *in vivo* monitoring of MDR could assist in the selection of patients for therapy and can avoid ineffective and potentially toxic treatments. Therefore, methods for functionally interrogating MDR transport activity have been sought, namely single photon emission computed tomography (SPECT) and positron emission tomography (PET). Cationic radiotracers originally developed as SPECT myocardial imaging agents, such as $[^{99m}Tc(MIBI)_6]^+$ and $[^{99m}Tc(tetrofosmin)_2O_2]^+$, are used for both early cancer detection and non-invasive monitoring of the tumour MDR transport function. With the ultimate goal of obtaining better performing radioprobes for MDR imaging, other metal-based complexes and/or small molecules have also been synthesized and biologically evaluated. In this perspective we will report on the chemical efforts made to find metalloprobes for *in vivo* monitoring of MDR by nuclear imaging techniques. The current knowledge on the biological mechanisms and proteins involved in tumour MDR will be also briefly presented, as its understanding is invaluable for the rational design and biological evaluation of new radioprobes.

Introduction

Cancer is a leading cause of death worldwide. The World Health Organization reported that cancer accounted for 7.4 million deaths

Unidade de Ciências Químicas e Radiofarmacêuticas, Instituto Tecnológico e Nuclear, Estrada Nacional 10, Sacavém 2686–953, Portugal; Fax: 351-219946185; Tel: 351-219946201. E-mail: isantos@itn.pt

Filipa Mendes

Filipa Mendes has been a research scientist at the Radiopharmaceutical Sciences Group at ITN since late 2008. She completed her B.Sc. in Molecular Biology and Genetics in 1999, and PhD in Cellular Biology in 2004 at the University of Lisboa. From 2005 to 2008, she was a post-doctoral fellow at the Centre of Human Genetics, National Institute of Health, Lisboa, where she focused on the ABC transporter CFTR. Presently, her scientific activity is focused on molecular imaging and radiopharmaceutical sciences with emphasis on the biological mechanisms and cellular/molecular interactions of radioactive compounds potentially useful as probes for in vivo *molecular imaging and/or targeted radiotherapy.*

António Paulo

António Paulo was born in Bencatel, Portugal, in 1962. He obtained a degree in Chemical Engineering at the Technical University of Lisbon in 1986, and then took a PhD in Chemistry at the same University in 1998. Since 1986 he has worked at the Instituto Tecnológico e Nuclear, where he was appointed as Principal Researcher in 2006. His current research interests are focused on the design of novel tools for diagnostic and/or therapeutic applications in Nuclear Medicine, particularly based on radioactive complexes of d-transition metals. He is author or co-author of numerous papers, published in renowned journals of inorganic and organometallic chemistry.

(around 13% of all deaths) in 2004.[1] In the EU and the USA, it is only surpassed by heart disease, and according to the American National Institutes of Health the estimated overall cost of cancer in the USA in 2010 will be $263.8 billion.[2] Better prevention, early detection, and advances in treatment have helped developed nations to reduce the incidence and mortality rates for certain cancers but, in most parts of the world, cancer is still a growing problem.[2]

To a large extent these achievements have been supported by the increasing knowledge on the aetiology and physiology of cancer, which allowed the identification of biomarkers potentially useful for diagnosis and treatment.[3] Nuclear medicine and radiopharmaceutical sciences have explored some of these biomarkers as potential targets for *in vivo* molecular imaging and targeted radionuclide therapy of cancer.[4–6] Despite the great wealth of information available, the development of specific tools is not an easy task, and the successful application of these new targeted strategies is still limited.

As a consequence, chemotherapy still remains an important therapy in many malignant tumours and is used extensively, but resistance to chemotherapeutic agents is a major obstacle in the successful treatment of cancer patients. Many tumours are intrinsically resistant to chemotherapy (*e.g.* kidney, pancreas, liver, and colon), whereas others initially respond to treatment, but acquire resistance to a broad spectrum of cytotoxic drugs during chemotherapy.[7,8] Numerous mechanisms have been proposed to mediate intrinsic or acquired multidrug resistance (MDR) in cancer cells.[9] The most common is the ejection of anticancer drugs by one or more energy-dependent transporters expressed in cells, mainly P-glycoprotein (Pgp) but also multidrug-resistant protein 1 (MRP1) and homologues (MRP2 to 6), and breast cancer resistance protein (BCRP).[9]

Measurement of MDR is potentially important in planning systemic therapy, as accurate selection of chemo-sensitive patients would result not only in their effective treatment and avoidance of potentially toxic side effects but also in significant cost savings for health care providers without a significant loss of life expectancy for patients.[10,11] To assess Pgp expression, determination of mRNA and protein levels are important methodologies, but have poor sensitivity and specificity and fail to provide functional information.[10] So, non-invasive *in vivo* detection of a MDR phenotype in tumours is of great interest and significant efforts have been directed towards the non-invasive detection of transporter-mediated resistance using radiopharmaceuticals characterized as transport substrates for Pgp and other MDR proteins.

Two noninvasive imaging techniques – single-photon emission computed tomography (SPECT) and positron emission tomography (PET) – can be used to visualize and measure MDR *in vivo*. With the availability of specific radioprobes, these nuclear techniques would be able to evaluate *in vivo* the function of Pgp or other MDR proteins, based on the uptake of the radioprobes in a target tissue expressing such proteins.[12] Table 1 shows gamma- and positron-emitting radionuclides which have been explored in the search for radioprobes for MDR imaging by SPECT or PET, respectively.

From the radionuclides shown in Table 1, the positron emitters ^{11}C and ^{18}F have been used to label small molecules such as MDR cytotoxic drugs or classic modulator/substrate analogs. Some examples of such labeled compounds are ^{11}C/^{18}F-paclitaxel,[13] ^{11}C-colchicine,[14] ^{11}C-daunorubicin,[15] ^{11}C-verapamil,[16] and ^{11}C-*N*-desmethyl-loperamide[17] (Fig. 1).

Some promising *in vitro* and *in vivo* preliminary data have been reported for these small labeled molecules, but they present several limitations, such as modest radiochemical yields and fast *in vivo* metabolization. Moreover, due to the short half-life of ^{11}C and ^{18}F, their preparation needs a cyclotron nearby, limiting their access and widespread distribution.

On the contrary, some radiometals, namely ^{99m}Tc, $^{67/68}$Ga and ^{64}Cu, due to their half-life, chemistry, cost, availability and metabolic stability of their complexes, present advantages compared to ^{11}C and ^{18}F and have been significantly explored for MDR imaging. Despite an incomplete understanding of the transport mechanisms, several lipophilic cationic complexes of these transition and post-transition elements, originally explored for SPECT/PET myocardial perfusion imaging, were also identified as potentially useful for MDR evaluation. This class of compounds accumulates in tumour cells due to the increased negative mitochondrial potentials, acting as substrates for Pgp and/or MRP1.[18–21] So far, from all these cationic metal complexes, only $[^{99m}Tc(MIBI)_6]^+$ and $[^{99m}Tc(tetrofosmin)_2O_2]^+$ are in clinical use. However, the diagnostic and prognostic values are often limited, due to their high uptake in the liver, which makes it difficult to detect small lesions in the chest and abdominal regions.

Thus, there is an unmet medical need for radiotracers that are able to monitor noninvasively the MDR transport function in tumours. Such need has fostered research on chemistry and biology with the ultimate goal of identifying a good performing radiopharmaceutical for MDR functional assessment.

Herein, we will present an overview of the chemical efforts made to find technetium, gallium and copper-based probes for *in vivo* monitoring of MDR by nuclear imaging techniques. The *in vitro* and *in vivo* evaluation of these metal complexes will also be presented, together with a brief review of the current knowledge of the biological mechanisms and proteins involved in tumour MDR. Such biological context is invaluable for a rational design

Isabel R. Santos

Isabel R. Santos graduated in Chemical Engineering at the Technical University of Lisbon, Portugal, where she also received her PhD and Professor Degree in Chemistry. She works at the Instituto Tecnológico e Nuclear, where she was appointed as Principal Researcher in 1999 and leader of the Radiopharmaceutical Sciences Group in 2000. Her research is in inorganic and organometallic chemistry, and on the radiochemistry of elements for Nuclear Medicine. Isabel is author or co-author of more than 130 scientific papers, several invited reviews, and co-inventor of some patents. She has supervised several Masters and PhD students, and Post-doctoral fellows. She teaches several courses at the University of Lisbon.

Table 1 Physical properties of radionuclides used in SPECT and PET

Radionuclides	Physical half life t $\frac{1}{2}$ (h)	Production method	Imaging modality
^{99m}Tc	6.01	^{99}Mo/^{99m}Tc generator	SPECT
^{67}Ga	78.26	^{68}Zn (p,2n) -^{67}Ga/cyclotron	SPECT
^{94m}Tc	0.9	^{94}Mo(p,n) -^{94m}Tc/cyclotron	PET
^{68}Ga	1.13	^{68}Ge/^{68}Ga generator	PET
^{64}Cu	12.7	^{64}Ni(p,n) -^{64}Cu/cyclotron	PET
^{11}C	0.33	^{14}N(p,a) -^{11}C/cyclotron	PET
^{18}F	1.83	^{18}O(p,n) -^{18}F/cyclotron	PET

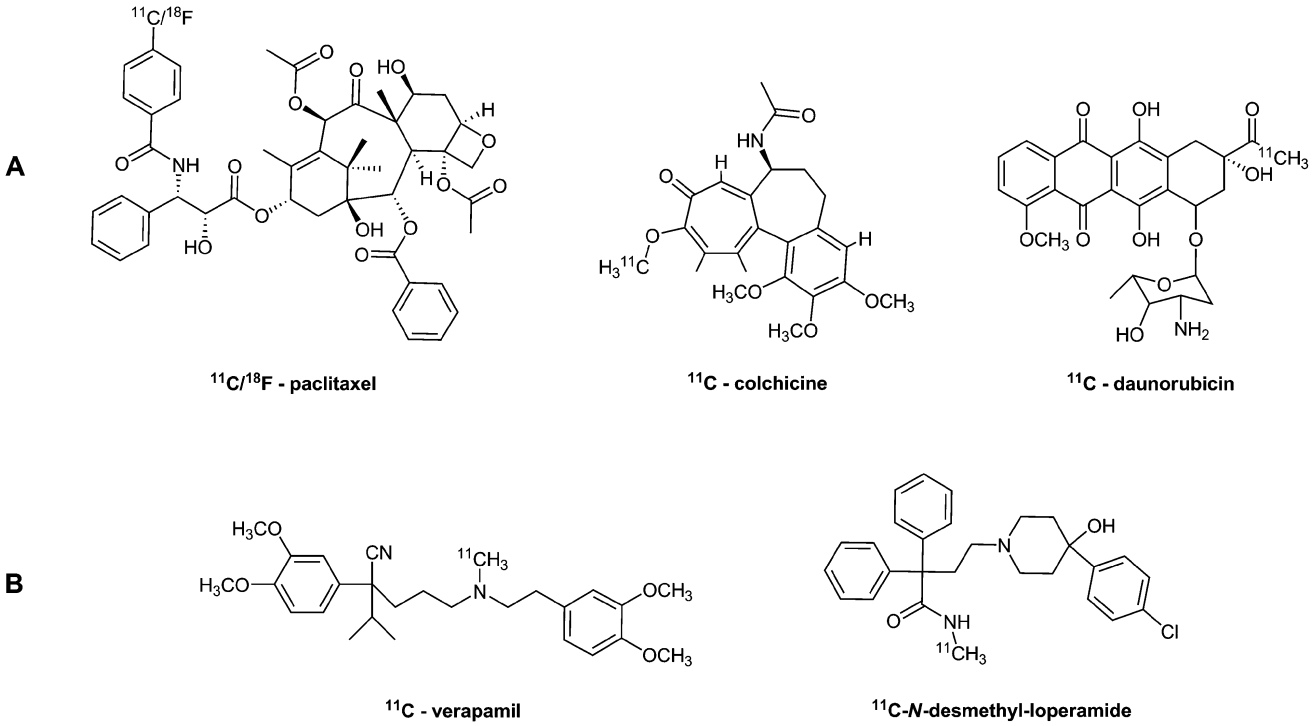


Fig. 1 Radiolabelled MDR cytotoxic drugs (A) or modulator/substrate analogs (B).

and evaluation of new complexes for SPECT or PET MDR imaging. This contribution intends to update previous reviews. However, to provide some context to the current manuscript, some overlap with its predecessors is unavoidable.[10,22–24]

Multidrug resistance

Cancer multidrug resistance is defined as the cross-resistance or insensitivity of cancer cells to the cytotoxic action of various anticancer drugs which are structurally or functionally unrelated and have different molecular targets.[7] These agents include conventional cytotoxic natural products, alkylating agents, platinum-containing compounds, antimetabolites and nucleoside/nucleotide analogs. Moreover, the efficacy of recently developed targeted therapeutic agents, such as imatinib mesylate, appears to be also limited by resistance.[25]

Non-cellular and cellular based mechanisms have been proposed to mediate MDR in cancer cells. Non-cellular mechanisms involve factors such as limited vascular accessibility or cell growth environment, and are typically associated with solid tumours which exhibit unique physiological properties and show inherent or natural resistance to chemotherapy at the initial exposure to the drug. Poor vascularization hinders the accessibility of drugs to regions within the solid tumours and thus protects tumour cells from cytotoxicity. Additionally, the physiological properties of solid tumours also result in tumour regions that are deficient in nutrients and oxygen, inducing additional resistance mechanisms.

The cellular mechanisms include modification of the drug target, changes in ability to repair DNA, disruptions in apoptotic signalling pathways and changes in the expression of enzymes associated with tumour resistance and cellular metabolism.[8] To date, the most widely studied cellular mechanisms of tumour resistance are those associated with drug efflux, involving members of the adenosine triphosphate (ATP)-binding cassette membrane transporter family, typically designated by ABC transporters. Fig. 2 schematically represents the cellular-based mechanisms that mediate MDR.

ABC Transporters

ABC transporters form one of the largest protein families, and its members have been found in each kind of organism examined so far.[26] The human genome contains at least 48 ABC genes; 16 of these have a known function, 14 are associated with a defined human disease and 8 have identified drug substrates.[27,28] Thus far,

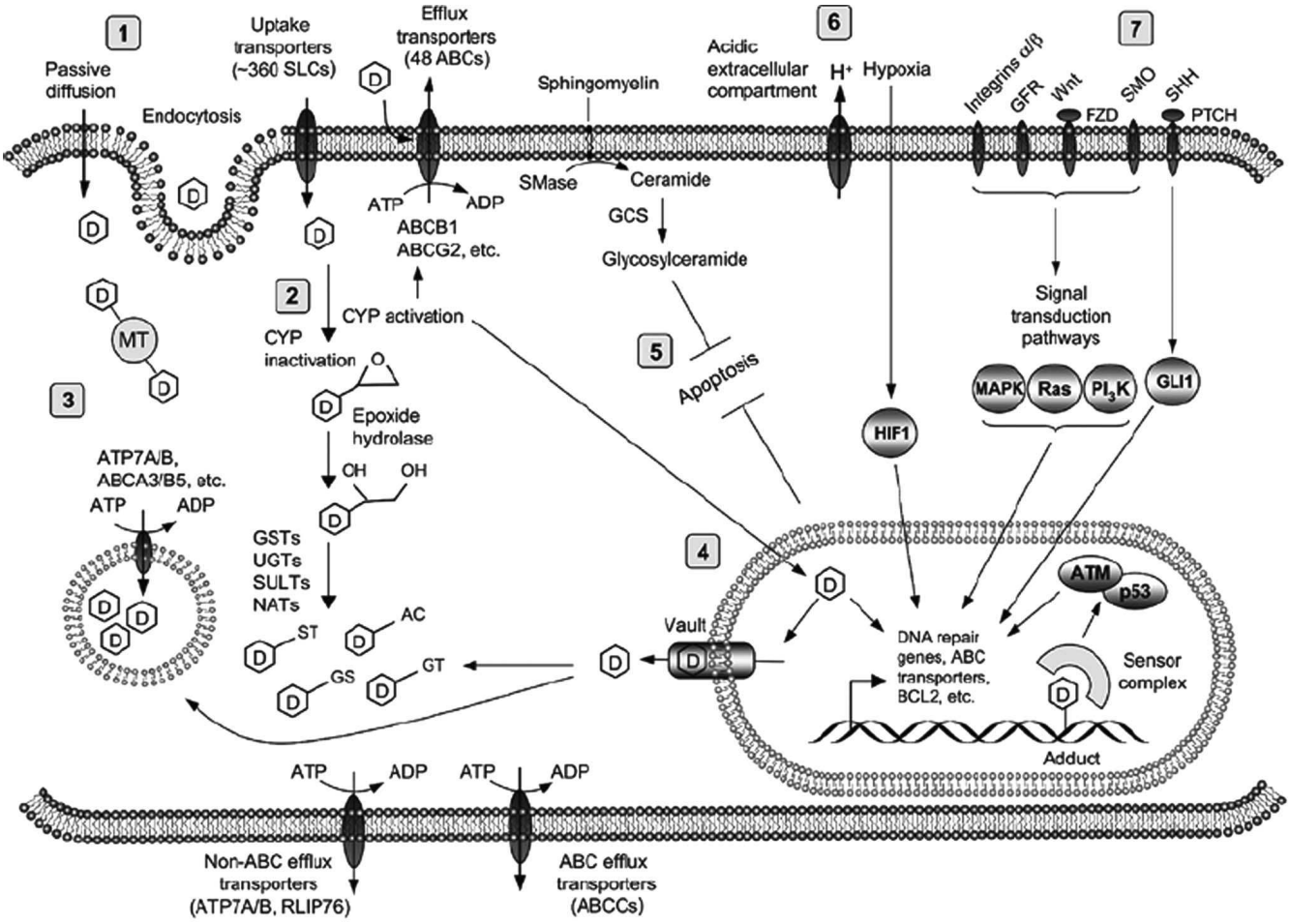


Fig. 2 Pleotropic mechanisms of multidrug resistance. (1) Drug (D) entry – passive diffusion, endocytosis, or facilitated transport (uptake transporters). Uptake can be significantly reduced by ABC transporters and alterations in the ceramide pathway, usually found in MDR cells. (2) Drug metabolism – Phase I is mediated mainly by cytochrome P450 enzymes (CYPs) and epoxide hydrolases. Drug species are metabolized and converted into highly mutagenic aromatic metabolites that are conjugated by phase II enzymes, GSTs, UGTs, SULTs, and NATS. These conjugated metabolites are then effluxed by transporters. (3) Drug sequestration – Drug species can be trapped in subcellular organelles as lysosomes and endosomes by ATP7A/B, ABCA3, or ABCB5 and then expelled from the cell. "Scavenger" metallothioneins ensnare metal ions and reactive oxygen species, leading to resistance to metal-based therapy and radiation. (4) Nuclear mechanisms – Drug species can be effluxed from the nucleus *via* vault proteins into the cytoplasm and be either sequestered in intracellular vesicles or effluxed from the cell *via* ATP-dependent transport. (5) Evasion of apoptosis – Blockage of apoptosis can result from the inhibitory effect of glycosylceramide and other pathways. (6) Microenvironment – Hypoxia upregulates the expression of numerous MDR-linked genes such as ABC transporters, Bcl2 family genes, glutathione, MT, *etc.*, through the activation of the transcription factor HIF1. (7) Signal transduction pathways – Cancer cells have altered signal transduction pathways, governed *via* integrin receptors, growth factor receptors, frizzled receptors, and smoothened-patched receptors. These altered pathways can lead to the blockage of apoptosis and expression of MDR-linked genes involved in DNA repair and drug-efflux pumps. Cancer cells often display chromosomal abnormalities that can lead to the overexpression of antiapoptotic genes. SLC – solute carriers, ABCs – ATP-binding cassette transporters, SMase – sphingomyelinase, GFR – growth factor receptor, Wnt – wingless, FZD – frizzled, Smo – smoothened, SHH – sonic hedgehog, PTCH – patched, MT – metallothionein, GSTs – glutathione-S-transferases, UGTs UDP – glucuronosyltransferases, SULTs – sulfotransferases, NATs – arylamine *N*-acetyltransferases, GCS – glucosylceramide synthase. With kind permission from Springer Science+Business Media: Multi-Drug Resistance in Cancer, J. Zhou (ed.), *Methods in Molecular Biology*, vol. 596, 2010, p. 65: Mechanisms of Multidrug Resistance in Cancer, J. P. Gillet and M. M. Gottesman, Fig. 4.3.[8]

the ABC transporters clearly related to MDR are Pgp, MRP1 and homologues (MRP2 to 6) and BCRP. A list of ABC transporters related to MDR, respective substrates and their localization is given in Table 2.

An ABC transporter is typically composed of two similar halves, each consisting of a transmembrane domain (TMD) which participates in substrate binding and forms the pathway through which substrate crosses the membrane, and a nucleotide-binding domain (NBD), which couples the energy of ATP hydrolysis to transport. The ABC proteins bind ATP and use the energy to drive the transport of various molecules across the plasma membrane as well as across intracellular membranes of the endoplasmic reticulum, peroxisomes, and mitochondria. These transporters are found in normal cells, where their role includes the transport of lipids, bile salts, peptides for antigen presentation and clearance and protection against excessive extracellular and intracellular concentrations of xenobiotics and toxins. Tumour cells expressing ABC transporters present a reduced ability to accumulate certain

Table 2 ABC transporters with known drug substrates

Protein	Gene	Substrates	Localization
Pgp, MDR1	*ABC B1*	Neutral and cationic organic compounds	Intestine, liver, kidney, blood-brain barrier
MRP1	*ABC C1*	Glutathione conjugates, organic anions	Widespread
MRP2, cMOAT	*ABC C2*	Glutathione conjugates, organic anions	Liver, kidney, intestine
MRP3, MOAT-D	*ABC C3*	Glutathione conjugates, anti-folates, bile acids, etoposide	Pancreas, kidney, intestine, liver, adrenal glands
MRP4, MOAT-B	*ABC C4*	Nucleoside analogs, methotrexate	Prostate, testes, ovary, intestine, pancreas, lung
MRP5, MOAT-C	*ABC C5*	Nucleoside analogs,cyclic nucleosides, organic anions	Widespread
MRP6, MOAT-E	*ABC C6*	Anionic cyclic pentapeptide	Liver, kidney
BCRP, MXR	*ABC G2*	Anthracyclines, mitoxantrone	Placenta, intestine, breast, liver

cytotoxic agents intracellularly, resulting in ineffective cellular levels that fail to induce cell death.[7,9]

P-glycoprotein (Pgp)

The first ABC transporter gene discovered was *ABC B1* in 1976. The transporter, called Pgp, was found to be expressed in Chinese hamster ovary cells selected for colchicine resistance. Importantly, the authors found that these cells also displayed resistance to a variety of structurally and mechanistically unrelated drugs.[29]

Human Pgp, the product of the *MDR1* or *ABC B1* gene, was subsequently shown to confer MDR on drug-sensitive cells.[30] This protein is localized in the plasma membrane, on the apical (or luminal) surface of polarized epithelial cells. These include the brush border membrane of intestinal cells, the biliary canalicular membrane of hepatocytes, and the luminal membrane in proximal tubules of kidney. It is also present at the pharmacological barriers of the body, *e.g.* at the blood–brain barrier and at the choroid plexus. Based on its localization and ability of transport, it has been proposed that the physiological function of Pgp is the protection of the cells and organism against toxic compounds.

Recently, a major breakthrough was achieved with the publication of the crystal structure of mouse Pgp, a protein that presents 87% sequence identity with the human Pgp (Fig. 3).[31]

This crystal structure revealed two 'portals' up to 9 Å, opening up into an internal drug binding cavity of ~6000 $Å^3$, which is able to accommodate more than one molecule. The internal cavity has an arrangement of inward facing residues capable of distinct spatial intermolecular bonding modes, allowing cross-recognition of small molecules irrespective of the spatial distribution of non-covalent bonding partners.[31] Drug substrate extrusion occurs *via* a drug-binding site accessible from the lipid bilayer, and competition assays with substrates have showed the existence of multiple distinct drug binding sites, probably spatially separate but overlapping areas of a large contiguous drug recognition site.[32] By building drug extrusion into the lipid bilayer, Pgp acts as a membrane vacuum cleaner, intercepting drugs before they reach high affinity targets within the cell, thereby precluding their intracellular accumulation.[32] A number of models have been proposed for the mechanistic steps of drug efflux. A plausible hypothesis is that when ATP binds to an ATP-binding domain, the two domains are brought together, initiating ATP hydrolysis and the release of ADP and P_i. The hydrolysis of ATP likely disrupts NBD dimerization and resets the system back to its inward facing state, reinitiating the transport cycle and enabling substrate efflux.[31]

Multidrug resistance-associated proteins (MRPs)

After the discovery of Pgp and the demonstration of its widespread expression in many human cancers, it was found that some MDR cancers, such as lung cancers, rarely express Pgp. Using a multidrug-resistant lung cancer cell line as a model, Deeley, Cole and colleagues cloned another ABC family member, known as MRP1 (multidrug resistance associated protein 1) and showed that it had a broad spectrum of anticancer drug transport activity.[33] Initially, the substrate specificity of MRP1 looked similar to that of Pgp, but vesicular transport experiments established that MRP1 is in fact a versatile glutathione *S*-conjugate export pump (GS-X pump). It transports a variety of drugs conjugated to glutathione, to sulfate or to glucuronate, as well as anionic drugs and dyes, but also neutral/basic amphipathic drugs, and even oxyanions.[28] MRP1 is widely expressed, with high levels reported in the lung, testes, kidney, skeletal and cardiac muscles, and the placenta. Notably, MRP1 is barely detectable in adult human liver, but in proliferating hepatocytes and liver cancer cell lines, such as HepG2, expression is considerably higher. MRP1 typically localizes predominantly to the plasma membrane and traffics selectively to the basolateral component in polarized cells. This contrasts with the apical membrane localization of other efflux pumps such as Pgp, BCRP, and MRP2 (see the following paragraphs).

The discovery of MRP1 led to a search for other members of this family (Table 2), resulting in the discovery of a total of 9 or 10 MRP genes, at least 6 of which have been characterized enough to indicate that they transport anticancer and antiviral compounds.[25] In 1996, the gene *cMOAT* (now MRP2) was cloned;[34] MRP3–5 soon followed when 21 potential human ABC transporters were identified.[35] Recent work has added four more members to this MRP family: MRP6, MRP7, and MRP8 and 9.[25] This probably completes the family, as there are no other putative MRP genes among the 48 human ABC transporter genes. Many of these appear to transport drugs that are potentially important for the treatment of cancer, and their role in conferring drug resistance on cancer cells is under active investigation.

Breast cancer resistance protein (BCRP)

Although the increased level of Pgp and MRP1 account for the drug resistance of most cell lines, the resistance of a few cell lines remained unexplained. These lines were characterized by high mitoxantrone resistance and lower resistance to anthracyclines and camptothecins. Resistance was a result of decreased drug accumulation, suggesting the presence of a new drug transporter.

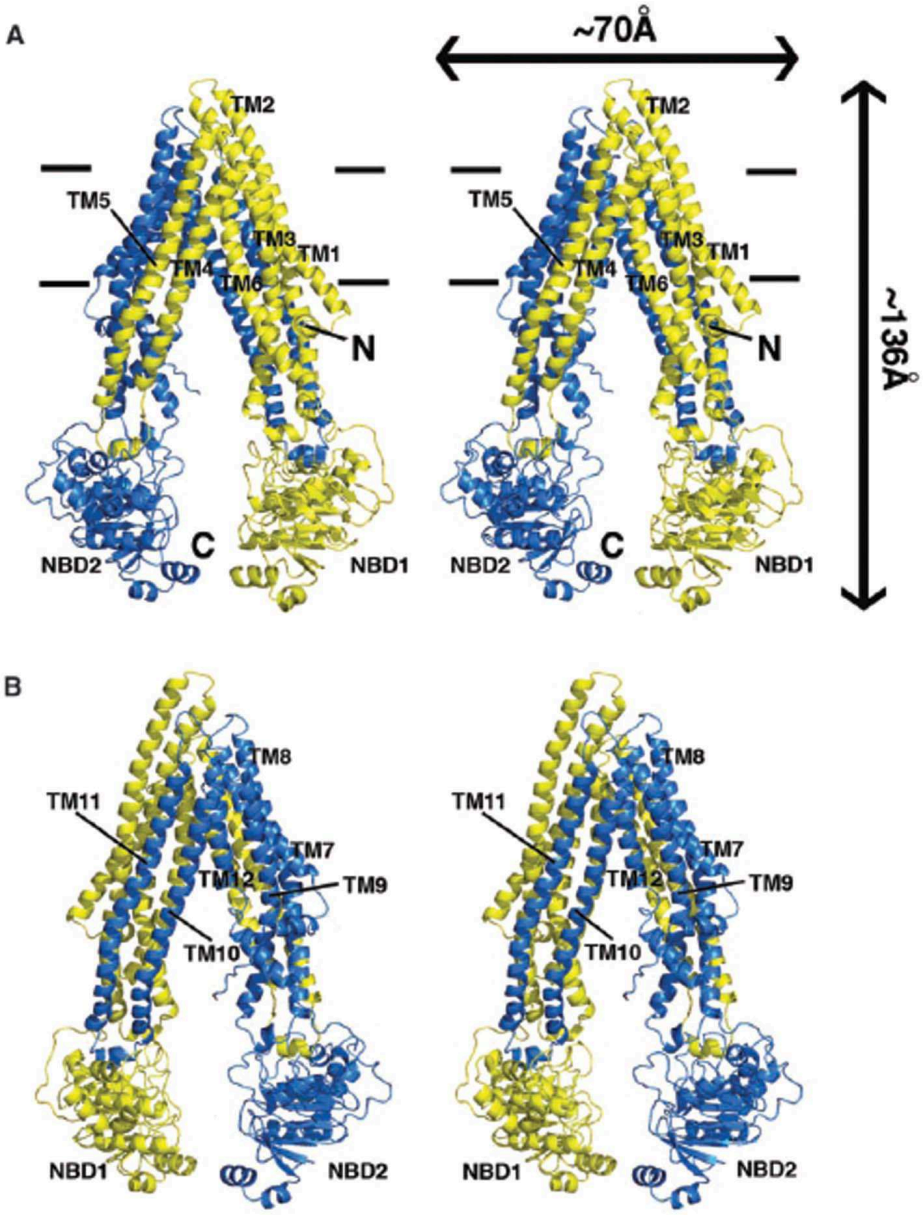


Fig. 3 Structure of mouse Pgp. (A) Front and (B) back stereo views of Pgp, representing a nucleotide-free inward-facing conformation arranged as two "halves" with pseudo two-fold molecular symmetry spanning ~136 Å perpendicular to and ~70 Å in the plane of the bilayer. The nucleotide-binding domains (NBDs) are separated by ~30 Å. The inward-facing conformation, formed from two bundles of six transmembrane helices (TMs 1 to 3, 6, 10, 11 and TMs 4, 5, 7 to 9, 12), results in a large internal cavity open to both the cytoplasm and the inner leaflet. The N- and C-terminal half of the molecule is colored yellow and blue, respectively. Horizontal bars represent the approximate positioning of the lipid bilayer. The N- and C-termini are labeled in (A). TM domains and NBDs are also labeled. From S. G. Aller *et al.*, *Science*, 2009, **323**, 1718.[31] Reprinted with permission from AAAS.

This transporter was finally identified by Doyle *et al.* as the BCRP.[36] The range of drugs to which BCRP can confer resistance is less broad than the one found for Pgp. In addition to mitoxantrone, topotecan derivatives, and anthracyclines, these drugs include bisantrene, etoposide, prazosin, and flavopiridol. Like Pgp, BCRP does not require GSH for the transport of electroneutral amphipathic drugs.[37] As referred in Table 2, BCRP is present in the plasma membrane of cultured cells, and in polarized cells it traffics to the apical membrane. ABCG2 RNA is detectable in many tissues, with the highest levels in the placenta.[38] The role of BCRP in clinical drug resistance is yet to be fully asserted.

Metal complexes for MDR imaging

General comments

Over the past few years, different radioisotopes of technetium, gallium and copper (see Table 1) have been used in the design of SPECT or PET probes for functional imaging of MDR. To design such probes the quite different coordination chemistry of these elements must be taken into consideration. Moreover, their chemistry has to be developed in aqueous solutions.

^{99m}Tc is the workhorse of nuclear medicine due to its ideal nuclear properties, low-cost and availability from commercial

^{99}Mo/^{99m}Tc generators, being the most used radionuclide in the development of metalloprobes for nuclear imaging by SPECT. The preparation of technetium radiopharmaceuticals is done in aqueous solution starting from the Tc(VII) permetallate anion (TcO_4^-) that needs to be reduced, prior to its complexation by adequate ligands. A variety of oxidation states (from (–I) to (VII)) are available for technetium. Until recently, Tc(V) was the most explored in the design of perfusion or specific radiopharmaceuticals, based essentially on complexes with the $[^{99m}Tc(O)]^{3+}$ core stabilized by tetradentate chelators. However, the diverse and rich chemistry of this radiometal allowed the introduction of innovative and alternative methodologies into the chemistry of other metal cores and/or oxidation states (Fig. 4). Among the most remarkable are the nitrido ($[^{99m}Tc(N)]^{2+}$) and the tricarbonyl (*fac*-$[^{99m}Tc(CO)_3]^+$) cores.[39]

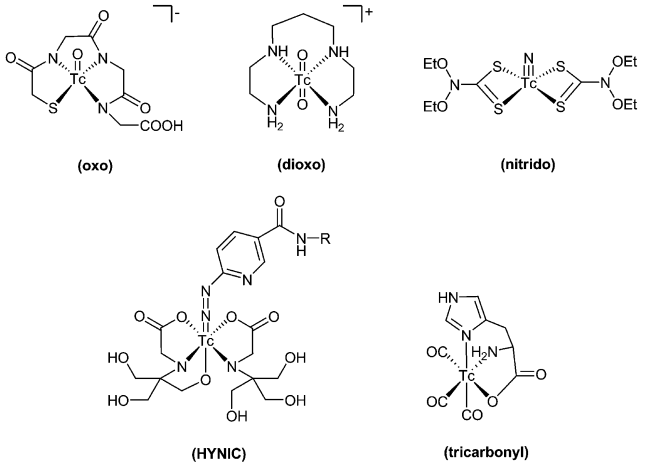


Fig. 4 Complexes with different technetium cores explored for radiopharmaceutical applications.

In particular, the so-called tricarbonyl approach has gained considerable attention, following the introduction by Alberto and co-workers of a convenient and fully aqueous-based kit preparation of the organometallic precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$.[40–42] The easy preparation of *fac*-$[M(CO)_3(H_2O)_3]^+$ (M = Re, Tc) directly from $[MO_4]^-$, the chemical robustness of the *fac*-$[M(CO)_3]^+$ fragment and the lability of the three water molecules, which are exchangeable with a large variety of ligands, offer a great number of advantages for the design of radiopharmaceuticals. For biological applications, tridentate chelators are the most suitable, independent of their charge or type of donor atoms (Fig. 5).[43,44]

Gallium is a post-transition element presenting radionuclides suitable for SPECT (^{67}Ga) or PET (^{68}Ga) imaging (see Table 1), and can be envisaged as an alternative to ^{99m}Tc.[45–47] Gallium-67 is a cyclotron produced gamma emitter obtained at reasonable cost and deliverable to different users over relatively large distances. Gallium-68 is a positron emitter readily accessible from the ^{68}Ge/^{68}Ga generator, offering the possibility to obtain on-site a PET radionuclide without needing the presence of a nearby cyclotron. Unlike technetium, the chemistry of gallium in aqueous media is exclusively limited to the oxidation state +III, which is unique in the design of radiopharmaceuticals. In aqueous solution, the Ga(III) ion has a marked tendency to undergo hydrolysis, being stable only under acidic conditions, in the absence of stabilizing ligands. Therefore, in the design of radiopharmaceuticals it is

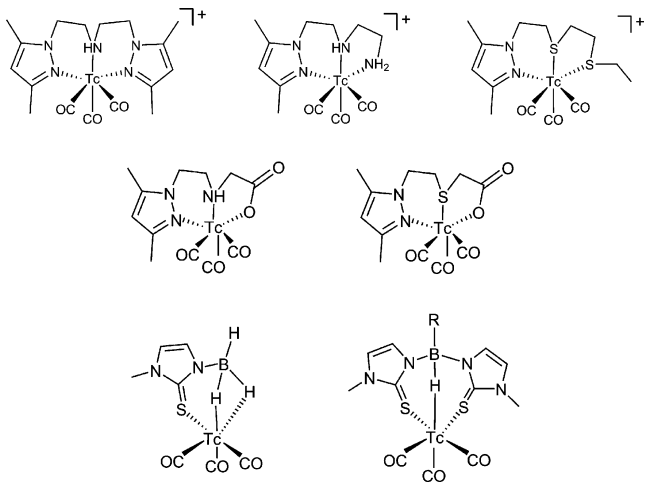


Fig. 5 Examples of cationic and neutral Tc(I) tricarbonyl complexes anchored by tridentate chelators.

of particular importance to obtain Ga complexes which are resistant to hydrolysis. Usually, this requires the saturation of its coordination sphere. Such saturation is achieved through the formation of six-coordinated and octahedral complexes, as the less saturated ones (five- or four-coordinated) easily undergo ligand-exchange or hydrolysis reactions. For this reason, hexadentate ligands with hard donor groups (*e.g.* nitrogen or oxygen) are among the most used chelators to prepare Ga(III) complexes as potentially relevant radiopharmaceuticals (Fig. 6).

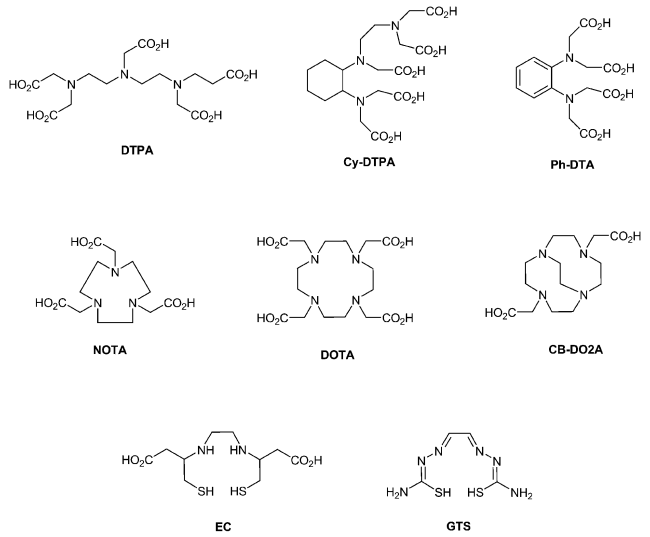


Fig. 6 Selected acyclic and macrocyclic chelators for stabilization of Ga(III) and/or Cu (II).

Copper presents several non-traditional positron-emitting radionuclides suitable for PET imaging, ^{64}Cu being the most explored for Nuclear Medicine applications (see Table 1). From the three accessible oxidation states (I–III)) of copper under aqueous solution, Cu(II) has been the most studied to synthesize complexes potentially useful as radiopharmaceuticals.[45,47,48] This is due to the increased kinetic inertness of Cu(II) complexes compared with Cu(I), reflecting the presence of some crystal-field stabilization in the case of Cu(II). On the other hand, Cu(III) is relatively rare and difficult to stabilize in aqueous solution. Like Ga, the

stabilization of Cu(II) under physiological conditions requires the use of polydentate ligands, in order to obtain kinetically inert and thermodynamically stable complexes. Usually, Cu(II) forms complexes with coordination numbers ranging from 4 to 6, with approximately square planar, square pyramidal, trigonal bypiramidal and octahedral coordination geometries. Square-planar coordination geometries are favored by acyclic tetradentate N_2O_2, N_2S_2 or N_4 chelators, while acyclic and cyclic hexadentate chelators, such as DTPA-based ligands, and macrocycles (N_3O_3, N_4O_2 donor atom set), favor the formation of octahedral complexes (Fig. 6).

Technetium organometalic complexes

Homoleptic Tc(I) isonitrile complexes

Hexakis(2-methoxyisobutylisonitrile)technetium-99m, $[^{99m}Tc(MIBI)_6]^+$ (**1**) (Fig. 7) is a radiopharmaceutical used clinically to study myocardial perfusion (Cardiolite®),[49,50] and was the first metal complex shown to be a Pgp transport substrate.[18] In this stable cationic and lipophilic complex, the metal center is six-coordinated by six identical isonitrile ligands, displaying an octahedral coordination geometry, as confirmed by the chemical characterization of the complex prepared at a macroscopic level with ^{99}Tc.[49] Compound **1** can be routinely synthesized by its users at any hospital, in a 'kit-like' preparation by reduction of $^{99m}TcO_4^-$ in saline with $SnCl_2$ in the presence of $[Cu(MIBI)_4]^+$, which acts as the source of the isonitrile (MIBI) ligand.

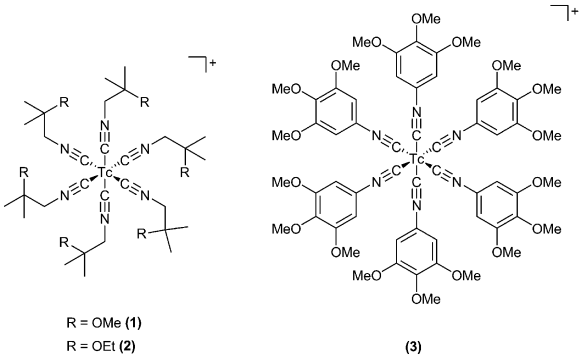


Fig. 7 Chemical structure of homoleptic Tc(I) complexes $[^{99m}Tc(MIBI)_6]^+$ (**1**), $[^{99m}Tc(EIBI)_6]^+$ (**2**) and $[^{99m}Tc(TMPI)_6]^+$ (**3**).

$[^{99m}Tc(MIBI)_6]^+$ enters the cell *via* a passive pathway due to its lipophilicity and accumulates in the mitochondria in response to the physiologically negative mitochondrial and plasma membrane potentials.[51,52] Due to their elevated number of mitochondria, the heart, muscles, liver and kidneys present a high uptake of this radiopharmaceutical. Cancer cells and tumours also maintain a more negative potential owing to increased metabolic requirements, and as a result, there is an increased accumulation of $[^{99m}Tc(MIBI)_6]^+$ in malignant tumours.[53] This feature permits the use of this radiotracer in clinical practice for the detection of various tumours.[54–58]

Following the synthesis of $[^{99m}Tc(MIBI)_6]^+$, other homoleptic Tc(I) complexes anchored by aliphatic and aromatic isonitriles were synthesized and biologically evaluated. From these complexes, hexakis(2-ethoxy-2-methyl-1-isocyanopropane)-technetium $[^{99m}Tc(EIBI)_6]^+$ (**2**) and hexakis(3,4,5-trimethoxyphenylisonitrile)technetium $[^{99m}Tc(TMPI)_6]^+$ (**3**) were shown to be the most promising, but none exceeded $[^{99m}Tc(MIBI)_6]^+$ in their Pgp-targeting properties.[21,59,60]

Piwnica-Worms was the pioneer of the evaluation of $[^{99m}Tc(MIBI)_6]^+$ as a Pgp substrate, encouraged by its cationic charge and modest hydrophobicity, features common to many chemotherapeutic agents used for the MDR phenotype.[18] Using lung fibroblasts and derivative cell lines expressing modestly low, intermediate, and very high levels of Pgp, an enhanced extrusion of the imaging agent by Pgp-enriched cells was observed.[18]

Further studies in a large variety of cellular models confirmed that in Pgp-expressing multidrug-resistant tumour cells, net cellular accumulation levels of $[^{99m}Tc(MIBI)_6]^+$ are inversely proportional to the level of Pgp expression.[61–64] Furthermore, reversal of the Pgp-mediated exclusion of $[^{99m}Tc(MIBI)_6]^+$ has been observed after treatment with a broad range of classic, second and third generation Pgp modulators.[18,62,65–73]

Different studies have also been performed to evaluate whether $[^{99m}Tc(MIBI)_6]^+$ is an MRP1 substrate. Comparing MDR negative cell lines with MRP1-/Pgp+ or MRP1+/Pgp- it was reported that $[^{99m}Tc(MIBI)_6]^+$ uptake was significantly lower in cells expressing MRP1 as well as Pgp, compared to MDR negative cells.[74,75] Depletion of GSH resulted in an increase of $[^{99m}Tc(MIBI)_6]^+$ uptake in multidrug resistant cells overexpressing MRP1 but not expressing Pgp.[76] Further studies performed in the presence of various inhibitors of Pgp and/or MRP1 confirmed that the radiotracer was a substrate of both transporters,[77] a result which reduces its specificity for Pgp; however, it shows that $[^{99m}Tc(MIBI)_6]^+$ can be a general probe for functional imaging of the two multidrug resistance pumps. Recently, Gomes *et al.* showed that $[^{99m}Tc(MIBI)_6]^+$ can detect Pgp and MRP1-mediated drug resistance, but MRP1 seems to be more effective than Pgp on outward transport of the radiotracer. The authors postulate that this finding can be useful to distinguish between the two resistance mechanisms.[78]

In vivo studies, using nude mouse xenograft models, have also demonstrated the usefulness of $[^{99m}Tc(MIBI)_6]^+$ for the functional imaging of Pgp, by differentiating drug-sensitive and drug-resistant tumours,[18,62] as well as the effect of Pgp modulators.[68,79,80] In knockout MDR mice the uptake of $[^{9m}Tc(MIBI)_6]^+$ increased in liver, lung and spleen, in comparison to wild-type mice.[81] More recently, $[^{99m}Tc(MIBI)_6]^+$ was shown to be a sensitive probe to monitor Pgp inhibition by WKX34 (a third generation modulator) and *MDR1* antisense nucleotides.[73,82] However, it has been described that in certain tumour xenografts, which lack a high number of mitochondria, $[^{99m}Tc(MIBI)_6]^+$ cannot detect either resistant nor sensitive tumours *in vivo*.[83]

Following these promising results, clinical studies were undertaken to establish correlations between *in vivo* uptake/efflux rates of $[^{99m}Tc(MIBI)_6]^+$ and Pgp expression levels and, more importantly, on prediction of chemotherapy outcome.[67,72,84] Many of the clinical studies have been performed on breast cancer patients, a type of cancer with frequent chemotherapeutic failure associated with Pgp.[85] Del Vecchio and others demonstrated a higher efflux rate from breast carcinomas with high Pgp expression compared with tumours with low Pgp expression,[86] and several other studies showed that $[^{99m}Tc(MIBI)_6]^+$ uptake is reduced in tumours where there is Pgp expression.[87–89] A correlation between the tumour-to-background ratio and Pgp expression was established, and such

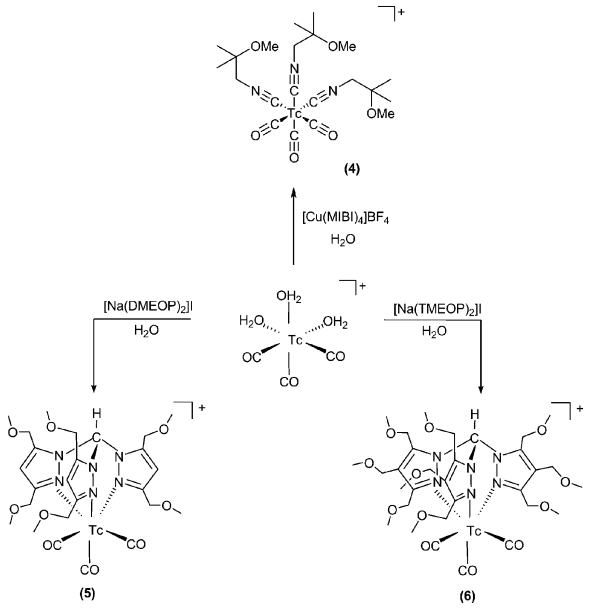
a ratio could be used to predict response to therapy.[90] Positive and negative predictive values of 81.0%, 96.0%, and a diagnostic accuracy of 89.1% were found. Other studies with breast cancer patients confirmed $[^{99m}Tc(MIBI)_6]^+$ prognostic value.[91,92]

A similar trend was found for patients with small cell lung carcinoma (SCLC) or squamous cell lung carcinoma.[58,93,94] Zhou *et al.* also found that the uptake was significantly higher and the washout rate lower in the Pgp negative patients compared with the Pgp+ group, and that such correlations were not observed for the expression of MRP or lung resistant protein (LRP), suggesting that $[^{99m}Tc(MIBI)_6]^+$ may be useful for the noninvasive detection of Pgp, but not MRP and LRP, in lung cancer patients.[95] In groups of patients with SCLC and non-small cell lung cancer (NSCLC) statistically significant differences in tumour-to-normal lung ratios were seen between responders and non-responders to chemotherapy.[96,97] Recently, a meta-analysis showed that $[^{99m}Tc(MIBI)_6]^+$ could play a significant role in the management of lung cancer as it can predict which patients will respond to chemotherapy with 94% of sensitivity, 90% of specificity and an accuracy of 92%. Such pre-selection for chemotherapy has significant cost savings in the health care system without a significant loss of life expectancy for patients.[98]

Analogous studies have been performed for other types of cancer.[58] For brain cancer, however, the results are not conclusive: some studies suggest that $[^{99m}Tc(MIBI)_6]^+$ imaging results might correlate with the presence of functional Pgp in neural crest tumours without MYCN amplification,[99] while others suggest that there is no clear relationship between Pgp expression and imaging results.[100] It has also been reported that $[^{99m}Tc(MIBI)_6]^+$ cannot be used for predicting response to chemotherapy in gliomas.[101] In malignant lymphomas it was demonstrated that their is correlation between good chemotherapeutic response with positive $[^{99m}Tc(MIBI)_6]^+$ scintigraphy and negative Pgp or MRP1 expression.[102–104] The usefulness of $[^{99m}Tc(MIBI)_6]^+$ in predicting the presence of Pgp in patients with hepatocellular carcinoma has also been reported.[105] In osteosarcoma patients, both the expression of MRP1 and Pgp, and response to chemotherapy, were correlated with the washout rate of $[^{99m}Tc(MIBI)_6]^+$.[106] Although *in vitro* and clinical studies have suggested that $[^{99m}Tc(MIBI)_6]^+$ is transported by MRP1 (see above), several other clinical studies demonstrated that $[^{99m}Tc(MIBI)_6]^+$ scintigraphy may not be used to evaluate the MDR phenotype associated with MRP1 expression in lung carcinoma,[95,94] breast carcinoma,[107] or gastric cancers.[108]

Tc(I) tricarbonyl complexes

Using the tricarbonyl core several research groups have prepared cationic and lipophilic complexes to be evaluated as myocardial imaging probes.[109–114] Most probably based on their favourable biological profile and heart uptake, the usefulness of some of these complexes for functional assessment of MDR was also evaluated. Piwnica-Worms *et al.* prepared the tri-substituted complex $[^{99m}Tc(CO)_3(MIBI)_3]^+$ (**4**) (Scheme 1) by reacting *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ with $[Cu(MIBI)_4]BF_4$.[109,115] Compound **4** was obtained in *ca.* 90% yield, characterized by comparing its high performance liquid chromatography (HPLC) chromatogram with the one of the corresponding Re complex, and its potential usefulness as a reporter of Pgp transport activity was studied.[115] A sixty-fold higher accumulation in human epidermal carcinoma KB 3–1 drug



Scheme 1 Synthesis of Tc(I) tricarbonyl complexes $[^{99m}Tc(CO)_3(MIBI)_3]^+$ (**4**), $[^{99m}Tc(CO)_3(DMEOP)]^+$ (**5**) and $[^{99m}Tc(CO)_3(TMEOP)]^+$ (**6**).

sensitive cells compared to KB 8–5 drug resistant cells was found for **4**, but its transport by MRP1 was modest. *In vivo* studies with *MDR1a/1b* knockout mice revealed a delayed liver clearance and enhanced brain uptake compared to wild-type mice. The authors suggest that, as the uptake profile is inversely proportional to Pgp expression, **4** is recognized as a transport substrate.[115]

More recently, Santos *et al.* explored pyrazole-based tripods in the design of cationic tricarbonyl complexes for heart imaging. Starting with the tris(pyrazolyl)methane tricarbonyl complex *fac*-$[^{99m}Tc(CO)_3(k^3\text{-}HC(pz)_3]^+$ as a lead structure, a family of complexes was prepared by functionalization of the tris(pyrazolyl)methane chelator with different ether groups.[113,114] Two of the resulting complexes $[^{99m}Tc(CO)_3(DMEOP)]^+$ (DMEOP: di-methoxy-tris-pyrazolylmethane) (**5**) and $[^{99m}Tc(CO)_3(TMEOP)]^+$ (TMEOP: tri-methoxy-tris-pyrazolylmethane) (**6**) (Scheme 1) showed excellent pre-clinical results as myocardial imaging agents, exhibiting a high initial and persistent heart uptake associated with rapid blood and liver clearance. Remarkably, $[^{99m}Tc(CO)_3(TMEOP)]^+$ reached a heart/liver ratio of 1 in about half the time of the compounds in clinical use, allowing a much better heart image.[116] Complexes **5** and **6** were obtained under aqueous conditions, in an almost quantitative yield, by reaction of the sodium salts of the respective substituted tris(pyrazolyl)methanes, $[Na(DMEOP)_2]I$ and $[Na(TMEOP)_2]I$, with *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ (Scheme 1).

Taking into consideration the characteristics of these new complexes, and in particular of $[^{99m}Tc(CO)_3(TMEOP)]^+$, their usefulness for the functional assessment of MDR has been studied (Fig. 8). The uptake kinetics of **6** are comparable with $[^{99m}Tc(MIBI)_6)]^+$, being significantly reduced in the cells overexpressing Pgp (*e.g.* in MCF7 breast cancer cells with no expression of Pgp *versus* MCF7 Pgp cells, which overexpress Pgp). In a different type of cancer line, small cell lung carcinoma cell line (H69) and the derivative drug-resistant lines H69 Lx4 (Pgp overexpression), the uptake of $[^{99m}Tc(CO)_3(TMEOP)]^+$ is significantly lower in the drug resistant line, similar to $[^{99m}Tc(MIBI)_6)]^+$. Interestingly, in the derivative line overexpressing MRP1, H69AR, the uptake

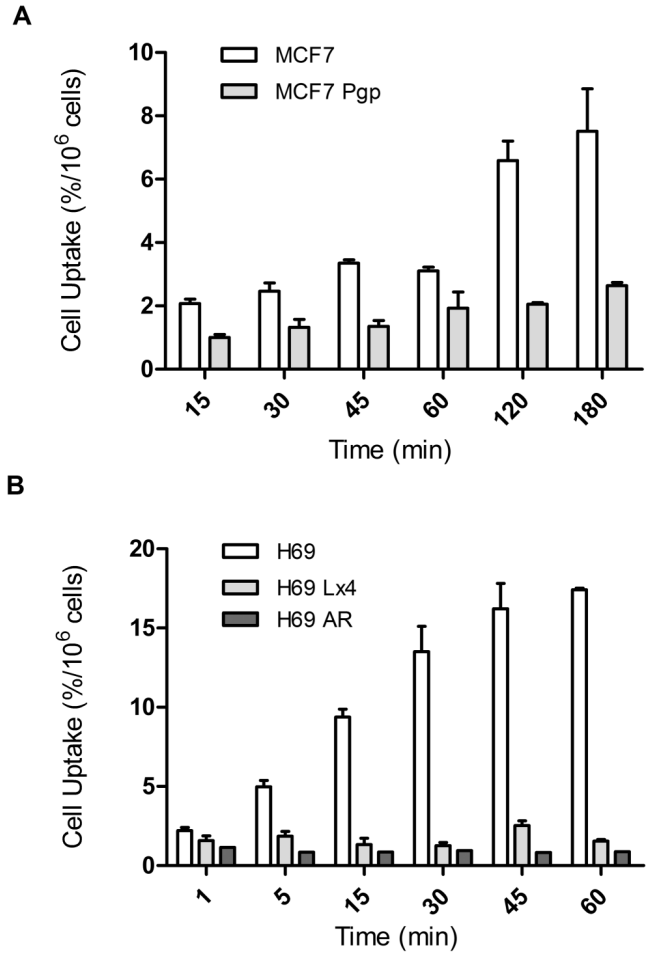


Fig. 8 *In vitro* uptake studies of $[^{99m}Tc(CO)_3(TMEOP)]^+$ (**6**). A: Uptake kinetics of $[^{99m}Tc(CO)_3(TMEOP)]^+$ in MCF7 breast cancer cells (with no expression of Pgp) and MCF7 Pgp cells (which overexpress Pgp); B: Uptake kinetics of $[^{99m}Tc(CO)_3(TMEOP)]^+$ by human small cell lung cancer H69 (with no expression of Pgp) and derivative drug-resistant lines H69 LX4 (known to express Pgp) and H69 AR (which overexpresses MRP1).

of $[^{99m}Tc(CO)_3(TMEOP)]^+$ (**6**) is also reduced compared with the parental H69. This seems to suggest that this complex functions as a substrate of both Pgp and MRP1. Moreover, an enhanced intracellular concentration of the complex is observed following inhibition of Pgp by verapamil, confirming that low uptake in the resistant cell lines was due to the overexpression of ABC transporters.[117] *In vivo*, $[^{99m}Tc(CO)_3(TMEOP)]^+$ (**6**) presents rapid liver clearance.[114] To elucidate the relationship of this behaviour with MDR function, the effect of cyclosporin A on the biodistribution profile of $[^{99m}Tc(CO)_3(TMEOP)]^+$ in rats was assessed. The obtained biodistribution data indicate that cyclosporin A treatment induces a significant decrease in the washout rate of $[^{99m}Tc(CO)_3(TMEOP)]^+$ from the liver, kidneys and lungs, organs with a high expression of Pgp. These results suggest that the excretion of $[^{99m}Tc(CO)_3(TMEOP)]^+$ from these organs is mediated by Pgp and may indicate that this complex is efficiently recognized *in vivo* by the MDR efflux pumps.[118]

Preliminary results in nude mice bearing MDR-negative and MDR-positive tumour xenografts showed that the biodistribution of $[^{99m}Tc(CO)_3(TMEOP)]^+$ is similar in noncancerous tissues. However, the tumour uptake is almost 2 times higher in the MCF7 xenografts compared with the MCF7 Pgp tumours. The *in vivo* MDR phenotype of the tumours was confirmed by detection of protein expression levels by Western blot.[118] Altogether, the results in human cancer cell lines and animal models indicate that $[^{99m}Tc(CO)_3(TMEOP)]^+$ is a promising candidate for tumour imaging and functional assessment of MDR mediated drug resistance.

Tc(III) complexes

Several monocationic Tc(III) complexes stabilized by different Schiff-bases and hydrophobic phosphines have been synthesized, characterized and evaluated as myocardial perfusion imaging agents. Both the ^{99m}Tc and ^{99}Tc complexes are obtained by a two-step synthesis which involves the formation of a Tc(V) intermediate that is further reduced to Tc(III) by the incoming phosphine ligand in a substitution/reduction reaction.[119] X-Ray structural analysis of the *trans*-$[Tc\{(acac)_2en\}(PPh_3)_2]^+$ confirmed the octahedral coordination sphere of this type of complex, containing an equatorial tetradentate Schiff base ligand and two monodentate phosphine ligands *trans* to each other.[120] The lead complex of this family was Q12 – trans((1,2-bis(dihydro-2,2,5,5-tetramethyl-3(2*H*)furanone-4-methyleneimino)ethane)bis(tris(3-methoxy-1-propyl) phosphine))Tc(III), known as ^{99m}Tc-Furifosmin (**7**) (Fig. 9).[119,121] From ^{99m}Tc-Q12, a series of 38 structurally variable complexes were prepared, using Schiff bases and phosphines with different substituent groups.

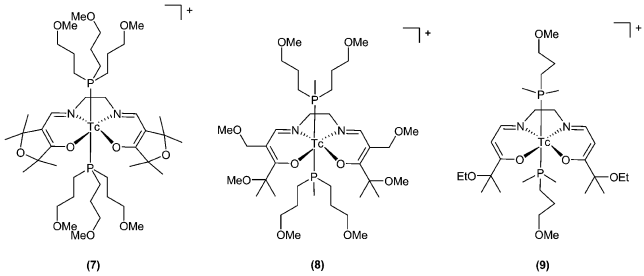


Fig. 9 Chemical structure of ^{99m}Tc-Q12 (**7**), ^{99m}Tc-Q58 (**8**), ^{99m}Tc-Q63 (**9**).

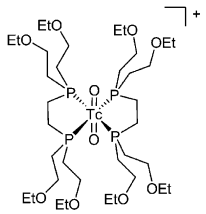
In vitro, the accumulation of ^{99m}Tc-Q12 (^{99m}Tc-Furifosmin) in a rat breast cancer cell line, MatB/WT, and its doxorubicin-selected resistant variant, MatB/AdrR, were compared to those of $[^{99m}Tc(MIBI)_6]^+$ (**1**) and $[^{99m}Tc(tetrofosmin)_2O_2]^+$ (**10**). Drug-sensitive cells accumulate much more ^{99m}Tc-Q12 (**7**) than drug-resistant cells, and the addition of PSC833, a Pgp modulator, increased the accumulation only in the resistant line. *In vivo*, and over the course of 30 min, ^{99m}Tc-Furifosmin washed out of the MatB/AdrR tumours more rapidly than it did from MatB/WT tumours. However, washout of $[^{99m}Tc(MIBI)_6)]^+$ from MatB/WT tumours was slower than that of ^{99m}Tc-Furifosmin. The authors concluded that ^{99m}Tc-Furifosmin was suitable for functional imaging of MDR.

From the series prepared, and through functional *in vitro* screening, two compounds, ^{99m}Tc-Q58 trans-(2,2′-(1,2-ethanediyldiimino)bis(1,5-methoxy-5-methyl-4-oxo-hexenyl))bis[methylbis(3-methoxy-l-propyl)phosphine]Tc(III) (**8**) and ^{99m}Tc-Q63 trans-[5,5′-(1,2- ethanediyldiimino)bis(2-ethoxy-2-methyl-3-oxo-4-pentenyl)]bis[dimethyl(3-methoxy-1-propyl)phosphine]]Tc(III) (**9**) were selected for further studies (Fig. 9).[122,123] In human drug-sensitive KB 3–1 cells and multidrug-resistant KB 8–5 and

8–5-11 derivative cell lines, expressing none, low, and high levels of P-glycoprotein, respectively, accumulation of ^{99m}Tc-Q58 and ^{99m}Tc-Q63 was inverse to expression of the transporter. The uptake of ^{99m}Tc-Q58 and ^{99m}Tc-Q63 was enhanced up to 60-fold in MDR cells by known modulators of *MDR1* P-glycoprotein. *In vitro* these Q complexes have properties that mimic $[^{99m}Tc(MIBI)_6]^+$ and are superior to ^{99m}Tc-Furifosmin in terms of difference of accumulation in sensitive and resistant cells, and in response to Pgp modulation. Despite these promising results, no new studies with the Q complexes have been published hitherto.

Tc(v) oxocomplexes

The cationic and lipophilic trans-dioxo-bis(diphosphine)-technetium(v) complex $[^{99m}Tc(tetrofosmin)_2O_2]^+$ (**10**) (Fig. 10) is currently used as a myocardial perfusion imaging agent (Myoview®).[124,125] Complex **10** offers advantages over $[^{99m}Tc(MIBI)_6]^+$, such as a room temperature synthesis from a lyophilized kit containing the diphosphine ligand, $SnCl_2$ to reduce $^{99m}TcO_4^-$ and sodium gluconate as a labile co-ligand. The crystal structure of the complex synthesized with the long-lived ^{99}Tc shows a linear trans-oxo core, with the four phosphorus atoms of the two bidentate diphosphine ligands equatorial, forming an exactly planar array. The main deviation from an idealized octahedral geometry arises from the steric requirements of the five-membered ring formed by the diphosphine ligand.[124]



(10)

Fig. 10 Chemical structure of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ (**10**).

Because the *in vivo* behavior of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ demonstrates similarities with $[^{99m}Tc(MIBI)_6]^+$ it was initially suggested that the mechanism determining cellular distribution was also similar. The first studies indicated that the uptake is through a metabolism-dependent process, most likely by potential-driven transport of the lipophilic cation.[126,127] However, subsequently it was shown that inhibition of the Na^+/K^+ ATPase, partly inhibited the uptake of Myoview®, indicating that lipophilicity is not the only factor involved in the cellular uptake. Moreover, $[^{99m}Tc(tetrofosmin)_2O_2]^+$ (**10**) appears to be more associated with the cytosol than with mitochondria, contrary to $[^{99m}Tc(MIBI)_6]^+$ (**1**) which accumulates in mitochondria.[128,129] Nevertheless, it is consensual that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ uptake depends on both cell membrane and mitochondrial potentials. In addition to accumulating in myocardial cells, complex **10** has been shown to accumulate in a variety of tumours and has found particular utility for imaging cancers of the breast, lung, brain and parathyroid adenomas.[130,131] $[^{99m}Tc(tetrofosmin)_2O_2]^+$ was also evaluated as a substrate for Pgp, both *in vitro* and *in vivo*. Ballinger *et al.* studied $[^{99m}Tc(tetrofosmin)_2O_2]^+$ uptake in wild-type (sensitive) and doxorubicin-resistant variants of rat and human breast tumour cell lines, and found that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ accumulated extensively in the sensitive cell lines. In contrast, multidrug resistant cell lines accumulated very little of either tracer, but the accumulation was increased by the addition of Pgp modulators in a dose dependent manner.[19] Similar results were obtained in studies with other resistant cells lines,[132,133] suggesting that the sensitivity of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ is similar to that of $[^{99m}Tc(MIBI)_6]^+$ for the detection of functional Pgp.

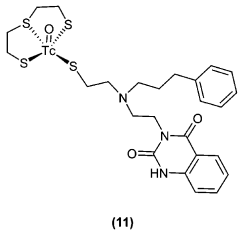
Further studies focused on the characterization of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ as a substrate of other ABC transporters. Chen *et al.* found that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ is a substrate of MRP1, although the differences in the net uptake produced by MRP1 expression were significantly less than the ones due to Pgp. $[^{99m}Tc(MIBI)_6]^+$ and $[^{99m}Tc(tetrofosmin)_2O_2]^+$ do not seem to act as substrates for the BCRP/MXR/ABCP half-transporter.[134] In several MRP1+/Pgp– carcinoma cell lines, $[^{99m}Tc(tetrofosmin)_2O_2]^+$ accumulation was increased and efflux decreased after addition of MRP1 inhibitors.[135,136]

In pre-clinical studies with knockout MDR mice $[^{99m}Tc(tetrofosmin)_2O_2]^+$ showed greater brain uptake and retention compared with wild-type mice, with no net change in blood pharmacokinetics, consistent with transport *in vivo* by Pgp expressed at the capillary blood–brain barrier.[134] In severe combined immunodeficient mouse models with human breast-cancer xenografts, the washout rates of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ in drug-resistant tumours were significantly greater than those in drug-sensitive tumours, being superior to $[^{99m}Tc(MIBI)_6]^+$.[79] After treatment with a modulator, there was a greater increase in the accumulation of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ *versus* $[^{99m}Tc(MIBI)_6]^+$. The authors concluded that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ may be at least comparable with $[^{99m}Tc(MIBI)_6)]^+$ in recognizing Pgp expression and modulation *in vivo*.[137]

The ability of $[^{99m}Tc(tetrofosmin)_2O_2]^+$ to functionally assess MDR was also evaluated in several clinical studies. Kao and colleagues extensively studied $[^{99m}Tc(tetrofosmin)_2O_2]^+$ scintigraphy correlation with Pgp or MRP1 expression in patients with small cell lung cancer,[138,139] non-small cell lung cancer,[140] lymphoma,[141,142] parathyroid adenomas,[143,144] and breast cancer.[145] Altogether the results showed that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ retention was higher in tumours that did not express Pgp or MRP1, and that higher retention was correlated with a favourable response to chemotherapy. Therefore it can be concluded that $[^{99m}Tc(tetrofosmin)_2O_2]^+$ is useful for predicting the response to chemotherapy, and a reduced retention can be considered a bad prognosis factor. Other groups have also demonstrated a correlation between $[^{99m}Tc(tetrofosmin)_2O_2]^+$ retention and therapeutic resistance in patients with lung cancer.[146] In musculoskeletal tumours, Soderlund found a wide variability in tumour/background ratios that were attributed in part to differing expression of Pgp,[147] while Yapar *et al.* reported that Pgp overexpression was not related with $[^{99m}Tc(tetrofosmin)_2O_2]^+$ uptake but with washout rate.[148] $[^{99m}Tc(tetrofosmin)_2O_2]^+$ has similar but not identical properties to those of $[^{99m}Tc(MIBI)_6]^+$, therefore clinical studies involving functional imaging of MDR and *in vivo* modulation of MDR could be performed with $[^{99m}Tc(tetrofosmin)_2O_2]^+$ or $[^{99m}Tc(MIBI)_6]^+$, but the two should probably not be used interchangeably.[133]

Other Tc(v) complexes that have been explored and evaluated for MDR imaging are mixed ligand oxocomplexes of the type "3+1". From all the complexes studied by Bergman *et al.*, the most

promising was [^{99m}Tc(O)(SSS)(SR)] (**11**) (Fig. 11), where SSS = (3-thiapentane-1,5-dithiolato) and SR = [[*N*-(3-phenylpropyl)-*N*-2(3-quinazoline-2,4-dionyl)ethyl]aminoethylthiolato]. The corresponding ^{99}Tc complex presents effective inhibition of efflux of [^{99m}Tc(MIBI)$_6$]$^+$ (**1**) and [^{99m}Tc(tetrofosmin)$_2$O$_2$]$^+$ (**10**) from rat brain RBE4 endothelial cells expressing Pgp.[149] The effects of ^{99}Tc-**11** on the *in vivo* distribution of **1** and ^{18}F-FDG in rats were comparable with the effects of verapamil. The authors concluded that **11** is a transport substrate and a potential inhibitor of Pgp and can serve as a template for the development of nonradioactive Re analogues as Pgp inhibitors. However, further studies are necessary to fully evaluate the potential of **11** as a radiopharmaceutical for monitoring the function of Pgp *in vivo*.

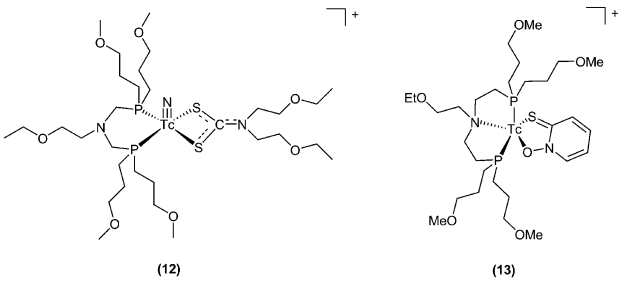


(11)

Fig. 11 Chemical structure of Tc(V) complex (**11**).

Tc(V)-nitrido complexes

Duatti *et al.* have introduced the metal fragment [M(N)(P–N–P)]$^{2+}$ (M = Re, Tc) that exhibits a selective reactivity toward nucleophilic bidentate ligands (X–Y) having π-donors as coordinating atoms.[150] Based on this metal fragment, and using monoanionic co-ligands of the dithiocarbamate or 2-mercaptopyridine oxide types, the monocationic Tc(V) nitrido complexes [^{99m}Tc(N)(DBODC)(PNP5)]$^+$ (DBODC: bis-(*N*-ethoxyethyl)dithiocarbamato; PNP5: bis-(dimethoxypropylphosphinoethyl) ethoxyethylamine) (**12**) and [^{99m}Tc-N(mpo)(PNP5)]$^+$ (Hmpo: 2-mercaptopyridine *N*-oxide) (**13**) were obtained and evaluated as metalloprobes for SPECT imaging of MDR (Fig. 12).[151–155] Complexes **12** and **13** were synthesized by reacting ^{99m}Tc-nitrido precursors with a mixture of the tridentate phosphine ligand and respective bidentate co-ligand. The precursors were obtained by reduction of ^{99m}TcO$_4^-$ with tin chloride in the presence of succinic dihydrazide (SDH) and an appropriate polyaminocarboxylic acid.



(12) (13)

Fig. 12 Chemical structure of [^{99m}Tc (N)(DBODC)(PNP5)]$^+$ (**12**) and [^{99m}Tc-N(mpo)(PNP5)]$^+$ (**13**).

Complex **12** is the lead compound of a series of monocationic ^{99m}Tc(N)-based potential myocardial imaging agents that exhibit a high and persistent myocardial accumulation, with an uptake mechanism and a kinetic behaviour identical to those of the commercially available myocardial imaging agents.[151,153] Interestingly, [^{99m}Tc (N)(DBODC)(PNP5)]$^+$ (**12**) presents a rapid efflux from lungs and liver and an enhanced intracellular concentration following inhibition of the Pgp function by cyclosporin A.[151] Preliminary results show that the uptake of **12** was reduced in MDR tumour cell lines *versus* drug-sensitive lines, and that an enhancement of this uptake could be observed after blocking of Pgp.[152] Altogether, these results led the authors to propose that this class of ^{99m}Tc(N)-tracers (**12** and analogues) might be recognized more specifically by Pgp than the ^{99m}Tc-complexes in clinical use.

The other nitride complex [^{99m}Tc-N(mpo)(PNP5)]$^+$ (**13**) is also promising for myocardial perfusion imaging,[154] as it presents a faster liver clearance with significantly higher heart/liver ratios at early times (<30 min p.i.) than [^{99m}Tc(MIBI)$_6$]$^+$ (**1**). To elucidate the relationship between the MDR transport function of hepatocytes with the fast liver clearance of [^{99m}Tc-N(mpo)(PNP5)]$^+$ (**13**), the authors used a pre-treatment with cyclosporin A that resulted in a significant increase in the kidney and liver uptakes. The authors suggest that the complex [^{99m}Tc-N(mpo)(PNP5)]$^+$ (**13**) might be more efficiently recognized by the MDR Pgp and MRPs.[155] Further studies on the potential of complex [^{99m}Tc-N(mpo)(PNP5)]$^+$ as a radiotracer to monitor the MDR transport function in different tumour-bearing animal models are warranted.

Gallium complexes

Several lipophilic and monocationic Ga(III) complexes anchored by N_4O_2-hexadentate Schiff-bases, derived from the linear tetraamine *N*,*N*′-bis(3-aminopropyl)-*N*,*N*′-ethylenediamine, have been synthesized and characterized (Fig. 13). The backbone of this tetraamine is very versatile, allowing the synthesis of different final chelators, since it can be condensed with aromatic aldehydes bearing different substituents at different positions of the phenyl rings. Moreover, different alkyl groups can be introduced at the propylenic chains of the chelator and/or at the central nitrogen atoms. Such versatility has been explored to modulate the physicochemical properties of the corresponding Ga(III) complexes, namely their size, topology and lipophilicity. Initially, these N_4O_2 Schiff-base Ga(III) complexes were evaluated as SPECT (^{67}Ga) or PET (^{68}Ga) radiopharmaceuticals for myocardial imaging.[156–158] However, studies at the macroscopic level indicated that this class of compounds has a pharmacological profile consistent with their recognition as Pgp substrates,[159] which prompted their evaluation as radioactive probes for imaging of MDR.

Fig. 13 shows the structures of the gallium complexes (**14–20**) that have been pre-clinically evaluated as metalloprobes for functional imaging of MDR. In these complexes the N_4O_2-hexadentate Schiff-bases were obtained by condensation of a tetraamine backbone with 2-hydroxysalicylaldehyde or 2-hydroxynaphthaldeyde derivatives.

At a macroscopic or at a carrier-free level (^{67}Ga/^{68}Ga), the complexes were obtained by ligand-exchange reactions with Ga(III) acetylacetonate in ethanol, or by reacting ^{67}GaCl$_3$ in aqueous solution containing 5% ethanol, with the corresponding ligand.[160–163]

X-Ray crystallography of some complexes (**14** and **19**) has confirmed that the metal is symmetrically coordinated by the N_4O_2

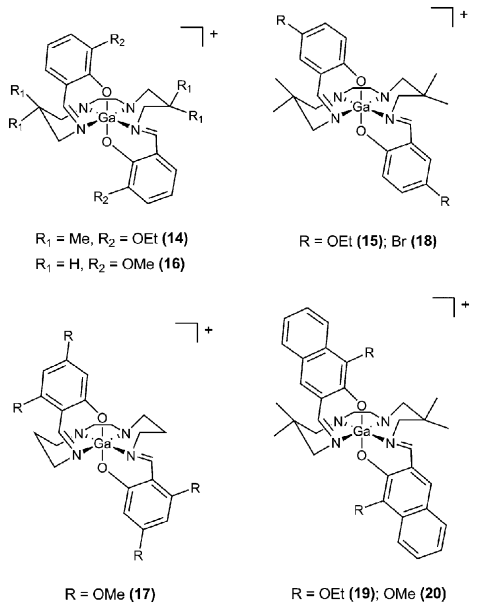


Fig. 13 Chemical structure of Ga(III) complexes (**14–20**).

donor-atom set of the ligands, displaying a pseudo-octahedral coordination environment with a trans-arrangement of the phenoxy oxygens.[157,160,163] Such a coordination environment is retained in solution, as shown by the ^{1}H NMR data. At the no carrier added level (^{67}Ga/^{68}Ga), the majority of the complexes were characterized uniquely by thin layer chromatography (TLC).[160–162] However, the fully characterized Ga complexes **19** and **20** have been applied as surrogates in the chemical identification of the ^{67}Ga congeners by HPLC.[163]

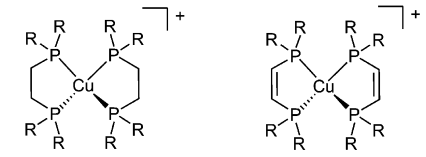
From all the complexes shown in Fig. 13, complex **14** has been the most explored for SPECT (^{67}Ga)/PET (^{68}Ga) functional imaging of MDR.[160,161] This metabolically stable compound, was evaluated *in vitro* using sensitive and resistant cell lines and *in vivo* using a nude mouse xenograft tumour model and *MDR1a/1b*(−/−) mice. The ^{67}Ga-complex showed high accumulation in drug-sensitive KB3–1 cells, and a low accumulation in MDR KB8–5 cells, that could be enhanced in the presence of different Pgp inhibitors. Moreover, using a variety of cells expressing Pgp, MRP1–MRP6 and BCRP, it has been demonstrated that the complex is readily transported by Pgp and, to a much lesser extent by MRP1, but not by MRP2–MRP6 or BCRP. *In vivo* this ^{67}Ga complex produced a readily detected 3-fold difference between Pgp-expressing and drug-sensitive tumours in a nude mouse xenograft tumour model. In *MDR1a/1b*(−/−) gene–deleted mice, both the ^{67}Ga- and ^{68}Ga-complexes showed an enhanced brain uptake and retention compared with wild-type mice, which is consistent with their transport *in vivo* by Pgp at the capillary blood–brain barrier. Altogether, these findings point out that the $^{67/68}$Ga-**14** complexes are sensitive and rather selective probes for monitoring PgP activity *in vivo* by SPECT or PET, respectively.[160,161] The cellular uptake of the congener ^{67}Ga-**15** (Fig. 13), containing the ethoxy group at the 5-position of the aromatic ring, has been measured using the same drug-sensitive and resistant cell lines. Complex ^{67}Ga-**15** showed a less pronounced increase of uptake in the drug-sensitive cell line compared with ^{67}Ga-**14**, indicating a less efficient Pgp-mediated extrusion. The same trend was observed for complexes ^{67}Ga-**16** and ^{67}Ga-**17**, anchored by N_4O_2-hexadentate Schiff-base ligands without methyl substituents at the propylenic chains of the *N*,*N*′-bis(3-aminopropyl)-*N*,*N*′-ethylenediamine backbone, and having one or two methoxy groups at the aromatic rings. According to the authors, these results highlighted that the unique Pgp transport properties of **14** most likely reflect the spatial orientation of the peripheral constituents of the complex instead of the inner coordination sphere. Consistently, it has been shown more recently that ^{68}Ga-**18**, having bromide substituents instead of alkoxide substituents at the phenyl rings of the anchor ligand, is also a substrate of Pgp. This complex was used to measure functional differences in the activity of Pgp in tumour-bearing mice, due to variations in the pH of the tumour.[162] This study demonstrated that an acidic extracellular environment activates Pgp, with enhancement of the drug efflux, which is possibly one of the factors responsible for the reduced chemosensitivity of hypoxic tumours. Finally, the pre-clinical evaluation of the complexes ^{67}Ga-**19** and ^{67}Ga-**20**, containing hexadentate Schiff-base ligands derived from 2-hydroxynaphthaldeyde, has been recently reported.[163] Similarly to ^{67}Ga-**14**, complexes ^{67}Ga-**19** and ^{67}Ga-**20** have a cellular uptake inversely proportional to Pgp-expression in different human tumour cell lines. In *MDR1a/1b*(−/−)mice, biodistribution studies of ^{67}Ga-**20** showed an 8-fold increase in the brain uptake and retention compared to the wild-type control.

Copper complexes

A small number of Cu(I) and Cu(II) complexes have been evaluated as ^{64}Cu-based radiopharmaceuticals for functional imaging of MDR.

As shown in Fig. 14 a family of monocationic diphosphine copper(I) complexes (**21–25**) were readily obtained in a high radiochemical yield by a one step synthesis that involved the reduction of $^{64}CuCl_2$ with the phosphorus donor ligands.[164,165] The same synthetic approach was successfully applied in the synthesis of the non-radioactive congeners, which were fully characterized as tetrahedral Cu(I) complexes and used as surrogates in the identification of the ^{64}Cu congeners by instant thin layer chromatography (ITLC).[164–167] The cell uptake of the ^{64}Cu complexes was studied in CH1 human ovarian carcinoma cells, which do not express detectable levels of Pgp, as well as in hooded rat sarcoma (HSN) cells which express Pgp. Besides complex **24**, which is anchored by 1,2-dimethylphosphinoethane (DMPE), all the other complexes are lipophilic and were avidly taken up by CH1 cells. Treatment of CH1 cells with doxorubicin induced an increase in Pgp expression and reduced significantly the uptake of the complexes. It has also been shown that the uptake of complexes **22**, **23** and **25** in HSN cells is increased by the presence of the Pgp modulator cyclosporin A. Taken together, these findings suggested that these lipophilic diphosphine Cu(I) complexes are substrates of Pgp.[165]

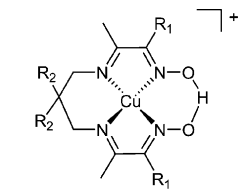
^{64}Cu(II) complexes, anchored by tetradentate diiminedioxime ligands, were also investigated as PET probes for myocardial imaging and for functional imaging of MDR.[168,169] As shown in Fig. 15, three different ^{64}Cu complexes (**26–28**) with diiminedioxime chelators bearing methyl and/or *n*-propyl substituents were synthesized and biologically evaluated. These compounds present a pseudomacrocyclic structure that results from the formation of a strong hydrogen bond between the remaining oxime hydrogen atom and the two oxime oxygen atoms, as confirmed by X-ray diffraction analysis in the case of **26** (synthesized with



R = Ph **(21)**
R = CH_2CH_3 **(23)**
R = CH_3 **(24)**
R = CH_2CH_2OEt **(25)**

R = Ph **(22)**

Fig. 14 Chemical structures of Cu(I) complexes (**21**–**25**).

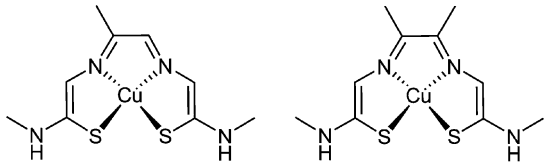


$R_1 = CH_3$, R_2 = H **(26)**
$R_1 = R_2 = CH_3$ **(27)**
$R_1 = (CH_2)_3CH_3$, $R_2 = CH_3$ **(28)**

Fig. 15 Chemical structures of copper(II) complexes (**26**–**28**).

cold copper).[170] Complexes **26** and **27** were initially investigated as potential radiopharmaceuticals for myocardial perfusion imaging. However, biodistribution studies in mice have shown a low heart uptake, as a consequence of their high hydrophilicity (log *P* values: –1.96 (**26**); –1.60 (**27**)).[164]

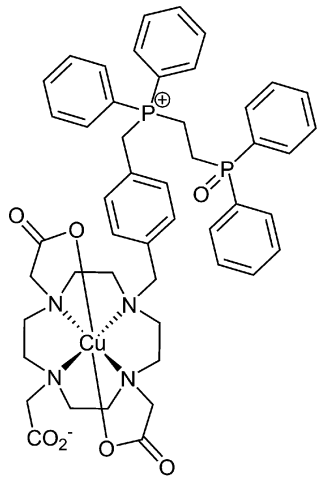
The use of a diiminedioxime chelator containing two *n*-propyl substituents led to a more lipophilic Cu(II) complex (**28**: log P = 0.9), which was evaluated for functional imaging of MDR. *In vitro* cell uptake studies, using resistant and non-resistant MES-SA human uterine sarcoma cells, in the presence or absence of cyclosporin A were performed. The complex ^{64}Cu-**28** showed a much reduced cell accumulation in the resistant cell line, strongly enhanced by the presence of the Pgp modulator. According to the authors, these findings suggest that lipophilic Cu(II) complexes anchored by diiminedioxime chelators are suitable platforms to design ^{64}Cu metalloprobes for PET imaging of MDR.[169] Using the same cell line, it has been shown that the overexpression of Pgp also diminishes the retention of ^{64}Cu-PTSM (**29**) (PTSM: pyruvaldehyde-bis(*N*-4-methylthiosemicarbazone) and ^{64}Cu-ATSM (**30**) (ATSM: diacetyl-bis(*N*-4-methylthiosemicarbazone) (Fig. 16), which are neutral and lipophilic bis(thiosemicarbazone) Cu(II) complexes with potential as PET perfusion tracers or as hypoxia-specific PET tracers, respectively.[171]



(29) **(30)**

Fig. 16 Chemical structure of Cu(II) complexes (**29**) and (**30**).

S. Liu *et al.* have explored a quite different approach for finding potential radiotracers for PET imaging of MDR. From a relatively large family of macrocyclic Cu(II) complexes, containing different linkers and different lipophilic cations of the triphenylphosphonium or triphenylarsonium type,[172,173] compound **31** has been identified as the most promising for tumour detection. The ^{64}Cu-**31** complex is anchored by a bifunctional DOTA-like chelator functionalized with a 2-(diphenylphosphoryl)ethyldiphenylphosphonium (TPEP) group (Fig. 17). The TPEP group acts as a "mitochondrion-targeting" moiety that carries the complex into tumour cells with a much higher mitochondrial transmembrane potential than normal cells.



(31)

Fig. 17 Chemical structure of Cu(II) complex (**31**).

Complex ^{64}Cu-**31** has been further evaluated in several xenografted tumour models, expressing different multidrug resistance proteins (Pgp, MRP2 and MRP4). The results of this study prompted the authors to propose that this compound might be a more efficient probe for functional imaging of MDR compared to $[^{99m}Tc(MIBI)_6]^+$, since **31** has shown a greater tumour uptake difference between tumours overexpressing MDR transporters and those not overexpressing the same transporters.[174]

Conclusion and perspectives

In the last decade, due to the high worldwide incidence of cancer, radiopharmaceutical research has been significantly focused on target-specific radiopharmaceuticals for early detection and targeted radionuclide therapy of cancer. Some advances and successes have been achieved in this multidisciplinary area fuelled by the convergence of biology, chemistry, physics and engineering. However, despite a satisfactory progress in diagnosis and therapy, the overall success rate of targeted approaches still remains low, due to the different aggressiveness and responsiveness displayed by each particular malignancy to therapy. Thus, chemotherapy is still one of the major therapeutic options in many malignant tumours, but the intrinsic or acquired resistance to chemotherapeutic agents (MDR) constitutes a major drawback to its success. A key point would be the *in vivo* functional monitoring of tumour MDR, to assist on the selection of patients and treatment planning, saving lives or increasing life expectancy. The cationic and lipophilic complexes $[^{99m}Tc(MIBI)_6]^+$ and $[^{99m}Tc(tetrofosmin)_2O_2]^+$,

initially developed for nuclear cardiology, are in clinical use for non-invasive assessment of tumour MDR, despite their limited diagnostic and prognostic value. Searching for better radioprobes, several attempts have been made to find other ^{99m}Tc, $^{67/68}$Ga or ^{64}Cu complexes with improved biological properties. These complexes, in general cationic and lipophilic, despite being biologically promising, did not prove to be more sensitive and specific than the ones in clinical use. Cytotoxic drugs and MDR modulators and/or substrates have also been labelled with ^{11}C and ^{18}F. Although they showed some promising biological behaviour, these organic molecules were obtained in low radiochemical yield, metabolize easily and their preparation needed a cyclotron nearby.

An interesting alternative for designing better SPECT or PET MDR probes could be to explore the bifunctional approach. This approach, intensively explored in radiopharmaceutical sciences, consists of the synthesis of metal fragments bearing small organic molecules recognized by target proteins. Examples of possible organic molecules that can be labelled with PET or SPECT radioprobes are the conventional cytotoxic compounds and MDR modulators and/or substrates. This bifunctional approach would probably allow the introduction of more specific and easily available radioprobes for functional monitoring of MDR.

In accordance with current knowledge, from the ABC transporters clearly related to MDR (Pgp, MRP's, BCRP), Pgp contributes to MDR in about half of human cancers. However, controversy remains as to how and why this protein recognizes such a broad variety of drugs sharing little or no structural or functional similarities. A better understanding of these problems would certainly help in producing a rational probe design. After several attempts, the X-ray crystal structure of mouse Pgp, which presents 87% sequence identity with human Pgp, was recently reported. Such an achievement may be a significant breakthrough and will likely help develop a better understanding of Pgp–drug interactions, opening new avenues for probe design. There is no doubt that for a rational drug design chemists and radiochemists need a significant input from biology on the mechanisms of MDR, role of related ABC transporters, and types of interactions of these proteins with drugs. We believe that the scarcity of such information is one of the likely reasons for the reduced number of strategies explored for the design of probes for MDR functional assessment. Nevertheless, as a final comment, we would like to stress that, although extensive research efforts have focused on the characterization of the mechanisms of multidrug resistance in cancer, the translation of this knowledge to the clinic still represents a major challenge, as evidenced by the failure of trials to modulate Pgp expression.

Acknowledgements

The financial support of Covidien, Petten, The Netherlands, is acknowledged. The authors wish to acknowledge Prof Piwnica-Worms for the kind gift of the MCF7-MDR cell line and Dr Sofia Gama for her invaluable cooperation in the preparation of the figures.

References

1 World Health Organization, Fact Sheet No. 297 – Cancer, 2009, http://www.who.int/mediacentre/factsheets/fs297/en/index.html.
2 American Cancer Society, Cancer Facts & Figures, 2010, http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-026238.pdf.
3 D. Majumdar, X. H. Peng and D. M. Shin, *Curr. Top. Med. Chem.*, 2010, **10**, 1211–1226.
4 L. E. Jennings and N. J. Long, *Chem. Commun.*, 2009, 3511–3524.
5 S. L. Pimlott and A. Sutherland, *Chem. Soc. Rev.*, 2011, **40**, 149–162.
6 P. Blower, *Dalton Trans.*, 2006, 1705–1711.
7 M. M. Gottesman and I. Pastan, *Annu. Rev. Biochem.*, 1993, **62**, 385–427.
8 J. P. Gillet and M. M. Gottesman, *Methods Mol. Biol.*, 2010, **596**, 47–76.
9 M. M. Gottesman, *Annu. Rev. Med.*, 2002, **53**, 615–627.
10 V. Sharma, *Bioconjugate Chem.*, 2004, **15**, 1464–1474.
11 H. K. Mohan and K. A. Miles, *J. Nucl. Med.*, 2009, **50**, 376–381.
12 P. Kannan, C. John, S. S. Zoghbi, C. Halldin, M. M. Gottesman, R. B. Innis and M. D. Hall, *Clin. Pharmacol. Ther.*, 2009, **86**, 368–377.
13 K. A. Kurdziel, J. D. Kalen, J. I. Hirsch, J. D. Wilson, R. Agarwal, D. Barrett, H. D. Bear and J. F. McCumiskey, *Nucl. Med. Biol.*, 2007, **34**, 823–831.
14 A. Levchenko, B. M. Mehta, J. B. Lee, J. L. Humm, F. Augensen, O. Squire, P. J. Kothari, R. D. Finn, E. F. Leonard and S. M. Larson, *J. Nucl. Med.*, 2000, **41**, 493–501.
15 E. Eriks-Fluks, P. H. Elsinga, N. H. Hendrikse, E. J. Franssen and W. Vaalburg, *Appl. Radiat. Isot.*, 1998, **49**, 811–813.
16 P. H. Elsinga, E. J. Franssen, N. H. Hendrikse, L. Fluks, A. M. Weemaes, W. T. van der Graaf, E. G. de Vries, G. M. Visser and W. Vaalburg, *J Nucl. Med.*, 1996, **37**, 1571–1575.
17 N. Lazarova, S. S. Zoghbi, J. Hong, N. Seneca, E. Tuan, R. L. Gladding, J. S. Liow, A. Taku, R. B. Innis and V. W. Pike, *J. Med. Chem.*, 2008, **51**, 6034–6043.
18 D. Piwnica-Worms, M. L. Chiu, M. Budding, J. F. Kronauge, R. A. Kramer and J. M. Croop, *Cancer Res.*, 1993, **53**, 977–984.
19 J. R. Ballinger, J. Bannerman, I. Boxen, P. Firby, N. G. Hartman and M. J. Moore, *J. Nucl. Med.*, 1996, **37**, 1578–1582.
20 J. R. Ballinger, T. Muzzammil and M. J. Moore, *J. Nucl. Med.*, 1997, **38**, 1915–1919.
21 V. V. Rao, L. W. Herman, J. F. Kronauge and D. Piwnica-Worms, *Nucl. Med. Biol.*, 1998, **25**, 225–232.
22 V. Sharma and D. Piwnica-Worms in *Contrast Agents I I I Radiopharmaceuticals –From Diagnostics to Therapeutics*, Springer, 2005, pp. 155–178.
23 J. Sivapackiam, S. T. Gammon, S. E. Harpstrite and V. Sharma, *Methods Mol. Biol.*, 2010, **596**, 141–181.
24 H Akizawa, T. Uehara and Y. Arano, in *Technetium-99m Radiopharmaceuticals: Status and Trends*, IAEA, 2010, ch. 12, pp. 255–267.
25 R. G. Deeley, C. Westlake and S. P. Cole, *Physiol. Rev.*, 2006, **86**, 849–899.
26 C. F. Higgins and M. M. Gottesman, *Trends Biochem. Sci.*, 1992, **17**, 18–21.
27 M. M. Gottesman and S. V. Ambudkar, *J. Bioenerg. Biomembr.*, 2001, **33**, 453–458.
28 P. Borst and R. O. Elferink, *Annu. Rev. Biochem.*, 2002, **71**, 537–592.
29 R. L. Juliano and V. Ling, *Biochim. Biophys. Acta, Biomembr.*, 1976, **455**, 152–162.
30 K. Ueda, C. Cardarelli, M. M. Gottesman and I. Pastan, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 3004–3008.
31 S. G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P. M. Harrell, Y. T. Trinh, Q. Zhang, I. L. Urbatsch and G. Chang, *Science*, 2009, **323**, 1718–1722.
32 M. D. Hall, M. D. Handley and M. M. Gottesman, *Trends Pharmacol. Sci.*, 2009, **30**, 546–556.
33 S. P. Cole, G. Bhardwaj, J. H. Gerlach, J. E. Mackie, C. E. Grant, K. C. Almquist, A. J. Stewart, E. U. Kurz, A. M. Duncan and R. G. Deeley, *Science*, 1992, **258**, 1650–1654.
34 C. C. Paulusma, P. J. Bosma, G. J. Zaman, C. T. Bakker, M. Otter, G. L. Scheffer, R. J. Scheper, P. Borst and R. P. Oude Elferink, *Science*, 1996, **271**, 1126–1128.
35 R. Allikmets, B. Gerrard, A. Hutchinson and M. Dean, *Hum. Mol. Genet.*, 1996, **5**, 1649–1655.
36 L. A. Doyle, Y. Gao, W. Yang and D. D. Ross, *Int. J. Cancer*, 1995, **62**, 593–598.
37 A. Haimeur, G. Conseil, R. G. Deeley and S. P. Cole, *Curr. Drug Metab.*, 2004, **5**, 21–53.
38 M. Maliepaard, G. L. Scheffer, I. F. Faneyte, M. A. van Gastelen,

A. C. Pijnenborg, A. H. Schinkel, D. van, V R. J. Scheper and J. H. Schellens, *Cancer Res.*, 2001, **61**, 3458–3464.
39 U. Abram and R. Alberto, *J. Braz. Chem. Soc.*, 2006, **17**, 1486–1500.
40 R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, U. Abram and T. A. Kaden, *J. Am. Chem. Soc.*, 1998, **120**, 7987–7988.
41 R. Alberto, *Topics Current Chemistry*, 2005, **252**, 1–44.
42 R. Alberto, *Eur. J. Inorg. Chem*, 200921–31.
43 J. D. Correia, A. Paulo and I. Santos, *Current Radiopharmaceuticals*, 2009, **2**, 277–294.
44 R. Garcia, A. Paulo and I. Santos, *Inorg. Chim. Acta*, 2009, **362**, 4315–4327.
45 T. J. Wadas, E. H. Wong, G. R. Weisman and C. J. Anderson, *Chem. Rev.*, 2010, **110**, 2858–2902.
46 M. D. BartholomaÍ, A. S. Louie, J. F. Valliant and J. Zubieta, *Chem. Rev.*, 2010, **110**, 2903–2920.
47 P. Barnard, S. Bayly, H. Betts, P. Bonnitcha, M. Christlieb, J. Dilworth, J. Holland and S. Pascu, *Q. J. Nucl. Med Mol Imaging.*, 2008, **52**, 174–184.
48 M. Shokeen and C. J. Anderson, *Acc. Chem. Res.*, 2009, **42**, 832–841.
49 M. A. Abrams, A. Davison, A. G. Jones, C. E. Costello and H. Pang, *Inorg. Chem.*, 1983, **22**, 2798–2800.
50 F. J. Wackers, D. S. Berman, J. Maddahi, D. D. Watson, G. A. Beller, H. W. Strauss, C. A. Boucher, M. Picard, B. L. Holman and R. Fridrich, *J. Nucl. Med.*, 1989, **30**, 301–311.
51 D. Piwnica-Worms, J. F. Kronauge and M. L. Chiu, *Circulation*, 1990, **82**, 1826–1838.
52 M. Backus, D. Piwnica-Worms, D. Hockett, J. Kronauge, M. Lieberman, P. Ingram and A. LeFurgey, *Am. J. Physiol.*, 1993, **265**, C178–C187.
53 L. Maffioli, J. Steens, E. Pauwels and E. Bombardieri, *Tumori.*, 1996, **82**, 12–21.
54 I. M. Hassan, A. Sahweil, C. Constantinides, A. Mahmoud, M. Nair, Y. T. Omar and H. M. bdel-Dayem, *Clin. Nucl. Med.*, 1989, **14**, 333–340.
55 L. Kostakoglu, S. Ruacan, E. L. Ergun, I. Sayek, N. Elahi and C. F. Bekdik, *J. Nucl. Med.*, 1998, **39**, 1021–1026.
56 B. Caner, M. Kitapci, T. Aras, G. Erbengi, O. Ugur and C. Bekdik, *J. Nucl. Med.*, 1991, **32**, 1977–1978.
57 K. Schomacker and H. Schicha, *Eur. J. Nucl. Med. Mol. Imaging*, 2000, **27**, 1845–1863.
58 O. Schillaci, A. Spanu and G. Madeddu, *Q. J. Nucl. Med. Mol. Imaging.*, 2005, **49**, 133–144.
59 L. W. Herman, V. Sharma, J. F. Kronauge, E. Barbarics, L. A. Herman and D. Piwnica-Worms, *J. Med. Chem.*, 1995, **38**, 2955–2963.
60 D. Piwnica-Worms and J. Kronauge, *J Nuclear Medicine*, 1994, **35**, 230P.
61 D. Piwnica-Worms, V. V. Rao, J. F. Kronauge and J. M. Croop, *Biochemistry*, 1995, **34**, 12210–12220.
62 J. R. Ballinger, H. A. Hua, B. W. Berry, P. Firby and I. Boxen, *Nucl. Med. Commun.*, 1995, **16**, 253–257.
63 J. R. Ballinger, K. M. Sheldon, I. Boxen, C. Erlichman and V. Ling, *Q. J. Nucl. Med.*, 1995, **39**, 122–128.
64 M. D. Cordobes, A. Starzec, L. mon-Moingeon, C. Blanchot, J. C. Kouyoumdjian, G. Prevost, M. Caglar and J. L. Moretti, *J. Nucl. Med.*, 1996, **37**, 286–289.
65 I. Bosch, C. L. Crankshaw, D. Piwnica-Worms and J. M. Croop, *Leukemia*, 1997, **11**, 1131–1137.
66 G. D. Luker, P. M. Fracasso, J. Dobkin and D. Piwnica-Worms, *J. Nucl. Med.*, 1997, **38**, 369–372.
67 C. C. Chen, B. Meadows, J. Regis, G. Kalafsky, T. Fojo, J. A. Carrasquillo and S. E. Bates, *Clin. Cancer Res.*, 1997, **3**, 545–552.
68 E. Barbarics, J. F. Kronauge, D. Cohen, A. Davison, A. G. Jones and J. M. Croop, *Cancer Res.*, 1998, **58**, 276–282.
69 G. D. Luker, K. R. Nilsson, D. F. Covey and D. Piwnica-Worms, *J. Biol. Chem.*, 1999, **274**, 6979–6991.
70 R. A. Peck, J. Hewett, M. W. Harding, Y. M. Wang, P. R. Chaturvedi, A. Bhatnagar, H. Ziessman, F. Atkins and M. J. Hawkins, *J. Clin. Oncol.*, 2001, **19**, 3130–3141.
71 A. Pichler, J. L. Prior and D. Piwnica-Worms, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1702–1707.
72 M. Agrawal, J. Abraham, F. M. Balis, M. Edgerly, W. D. Stein, S. Bates, T. Fojo and C. C. Chen, *Clin. Cancer Res.*, 2003, **9**, 650–656.
73 V. Jekerle, W. Klinkhammer, D. A. Scollard, K. Breitbach, R. M. Reilly, M. Piquette-Miller and M. Wiese, *Int. J. Cancer*, 2006, **119**, 414–422.
74 N. H. Hendrikse, E. J. Franssen, W. T. van der Graaf, C. Meijer, D. A. Piers, W. Vaalburg and E. G. de Vries, *Br. J. Cancer*, 1998, **77**, 353–358.
75 J. Vergote, J. L. Moretti, E. G. de Vries and A. Garnier-Suillerot, *Eur. J. Biochem.*, 1998, **252**, 140–146.
76 J. L. Moretti, C. M. Duran, A. Starzec, B. de, V J. Vergote, F. Benazzouz, B. Boissier, H. Cohen, N. Safi, S. Piperno-Neumann and J. C. Kouyoumdjian, *J. Nucl. Med.*, 1998, **39**, 1214–1218.
77 K. Utsunomiya, J. R. Ballinger, M. Piquette-Miller, A. M. Rauth, W. Tang, Z. F. Su and M. Ichise, *Eur. J. Nucl. Med. Mol. Imaging*, 2000, **27**, 1786–1792.
78 C. M. Gomes, A. J. Abrunhosa, E. K. Pauwels and M. F. Botelho, *Cancer Biother. Radiopharm.*, 2009, **24**, 215–227.
79 Z. Liu, G. D. Stevenson, H. H. Barrett, G. A. Kastis, M. Bettan, L. R. Furenlid, D. W. Wilson and J. M. Woolfenden, *Nucl. Med. Biol.*, 2004, **31**, 53–65.
80 C. M. Gomes, M. Welling, I. Que, N. V. Henriquez, P. G. van der, S. Romeo, A. J. Abrunhosa, M. F. Botelho, P. C. Hogendoorn, E. K. Pauwels and A. M. Cleton-Jansen, *Eur. J. Nucl. Med. Mol. Imaging*, 2007, **34**, 1793–1803.
81 C. A. Slapak, J. Dahlheimer and D. Piwnica-Worms, *J. Clin. Pharmacol.*, 2001, 29S–38S.
82 V. Jekerle, J. H. Wang, D. A. Scollard, R. M. Reilly, M. Wiese and M. Piquette-Miller, *Mol. Imaging Biol.*, 2006, **8**, 333–339.
83 D. E. Lorke, M. Kruger, R. Buchert, K. H. Bohuslavizki, M. Clausen and U. Schumacher, *J. Nucl. Med.*, 2001, **42**, 646–654.
84 H. S. Bom, S. C. Lim, Y. C. Kim, H. C. Song, J. J. Min, H. J. Jeong, J. Y. Kim, J. Lee and K. O. Park, *Clin. Nucl. Med.*, 1999, **24**, 97–101.
85 B. J. Trock, F. Leonessa and R. Clarke, *J. Natl. Cancer Inst.*, 1997, **89**, 917–931.
86 S. D. Vecchio, A. Ciarmiello, M. I. Potena, M. V. Carriero, C. Mainolfi, G. Botti, R. Thomas, M. Cerra, G. D'Aiuto, T. Tsuruo and M. Salvatore, *Eur. J. Nucl. Med. Mol. Imaging*, 1997, **24**, 150–159.
87 J. L. Moretti, H. Azaloux, D. Boisseron, J. C. Kouyoumdjian and J. Vilcoq, *Eur. J. Nucl. Med. Mol. Imaging*, 1996, **23**, 980–986.
88 C. H. Kao, S. C. Tsai, T. J. Liu, Y. J. Ho, J. J. Wang, S. T. Ho and S. P. ChangLai, *Cancer Res.*, 2001, **61**, 1412–1414.
89 H. Palmedo, *J. Nucl. Med.*, 2002, **43**, 526–530.
90 Y. Takamura, Y. Miyoshi, T. Taguchi and S. Noguchi, *Cancer*, 2001, **92**, 232–239.
91 A. Ciarmiello, V. S. Del, P. Silvestro, M. I. Potena, M. V. Carriero, R. Thomas, G. Botti, G. D'Aiuto and M. Salvatore, *J. Clin. Oncol.*, 1998, **16**, 1677–1683.
92 R. Sciuto, R. Pasqualoni, S. Bergomi, G. Petrilli, P. Vici, F. Belli, C. Botti, M. Mottolese and C. L. Maini, *J. Nucl. Med.*, 2002, **43**, 745–751.
93 L. Kostakoglu, P. Kiratli, S. Ruacan, M. Hayran, S. Emri, E. L. Ergun and C. F. Bekdik, *J. Nucl. Med.*, 1998, **39**, 228–234.
94 X. Y. Duan, J. S. Wang, M. Liu and Y. M. Guo, *Ann. Nucl. Med.*, 2008, **22**, 49–55.
95 J. Zhou, K. Higashi, Y. Ueda, Y. Kodama, D. Guo, F. Jisaki, A. Sakurai, T. Takegami, S. Katsuda and I. Yamamoto, *J. Nucl. Med.*, 2001, **42**, 1476–1483.
96 H. S. Bom, Y. C. Kim, H. C. Song, J. J. Min, J. Y. Kim and K. O. Park, *J. Nucl. Med.*, 1998, **39**, 91–94.
97 C. H. Kao, J. F. Hsieh, S. C. Tsai, Y. J. Ho and J. K. Lee, *Clin. Cancer Res.*, 2000, **6**, 820–824.
98 H. K. Mohan and K. A. Miles, *J. Nucl. Med.*, 2009, **50**, 376–381.
99 M. B. De, W. C. Van de, C. Dhooge, J. Philippe, F. Speleman, Y. Benoit, G. Laureys and R. A. Dierckx, *Eur. J. Nucl. Med. Mol. Imaging*, 1999, **26**, 396–403.
100 Y. Shibata, A. Matsumura and T. Nose, *Neurol. Medico-Chir.*, 2002, **42**, 325–330.
101 S. Nagamachi, S. Jinnouchi, K. Nabeshima, R. Nishii, L. Flores, T. Kodama, K. Kawai, S. Tamura, K. Yokogami, T. Samejima and S. Wakisaka, *Neuroradiology*, 2001, **43**, 1023–1030.
102 A. mitrakopoulou-Strauss, L. G. Strauss, H. Goldschmidt, W. J. Lorenz, W. Maier-Borst and K. G. van, *Eur. J. Nucl. Med. Mol. Imaging*, 1995, **22**, 434–442.
103 C. H. Kao, S. C. Tsai, J. J. Wang, Y. J. Ho, S. T. Ho and S. P. ChangLai, *Br. J. Haematol.*, 2001, **113**, 369–374.
104 J. A. Liang, Y. C. Shiau, S. N. Yang, F. J. Lin, A. Kao and C. C. Lee, *Jpn. J. Clin. Oncol.*, 2002, **32**, 140–145.
105 Y. S. Kim, S. W. Cho, K. J. Lee, K. B. Hahm, H. J. Wang, H. Yim, Y. M. Jin and C. H. Park, *Clin. Nucl. Med.*, 1999, **24**, 874–879.
106 Z. Burak, J. L. Moretti, O. Ersoy, U. Sanli, M. Kantar, F. Tamgac and G. Basdemir, *J. Nucl. Med.*, 2003, **44**, 1394–1401.

107 X. M. Zhang, Z. W. Zhang and H. Wu, *Zhonghua Zhong. Liu Za Zhi.*, 2004, **26**, 353–355.
108 K. Kawata, M. Kanai, T. Sasada, S. Iwata, N. Yamamoto and A. Takabayashi, *Clin. Cancer Res.*, 2004, **10**, 3788–3793.
109 M. Marmion and J. MacDonald, *J Nuc. Med.*, 2000, **41**, 40.
110 G. Y. Hao, J. Y. Zang, L. Zhu, Y. Z. Guo and B. L. Liu, *J. Labelled Compd. Radiopharm.*, 2004, **47**, 513–521.
111 K. P. Maresca, J. F. Kronauge, J. Zubieta and J. W. Babich, *Inorg. Chem. Commun.*, 2007, **10**, 1409–1412.
112 Z. He, W. Y. Hsieh, Y. S. Kim and S. Liu, *Nucl. Med. Biol.*, 2006, **33**, 1045–1053.
113 L. Maria, S. Cunha, M. Videira, L. Gano, A. Paulo, I. C. Santos and I. Santos, *Dalton Trans.*, 2007, 3010–3019.
114 L. Maria, C. Fernandes, R. Garcia, L. Gano, A. Paulo, I. C. Santos and I. Santos, *Dalton Trans.*, 2009, 603–606.
115 M. Dyszlewski, H. M. Blake, J. L. Dahlheimer, C. M. Pica and D. Piwnica-Worms, *Mol. Imaging*, 2002, **1**, 24–35.
116 L R Goethals, I. Santos, V. Caveliers, A. Paulo, F De Geeter, L. Gano, C. Fernandes and T. Lahoutte, *Contrast. Media Mol. Imaging.*, 2010, DOI: 10.1002/cmmi.413.
117 L. Gano, F. Mendes, S. Cunha, C. Fernandes, A. Paulo and I. Santos, *Q. J. Nucl. Med. Mol. Imaging.*, 2010, **54**, 10–11.
118 F. Mendes, L. Gano, C. Fernandes, A. Paulo and I. Santos, *Eur J Nucl. Med Mol Imaging.*, 2010, **37**, S358.
119 C. Rossetti, G. Vanoli, G. Paganelli, M. Kwiatkowski, F. Zito, F. Colombo, C. Bonino, A. Carpinelli, R. Casati and K. Deutsch, *J Nucl. Med.*, 1994, **35**, 1571–1580.
120 S. S. Jurisson, K. Dancey, M. McPartlin, P. A. Tasker and E. Deutsch, *Inorg. Chem.*, 1984, **23**, 4743–4749.
121 C. Rossetti, G. Paganelli, G. Vanoli, L. C. Di, M. Kwiatkowski, F. Zito, F. Colombo, C. Bonino, A. Carpinelli and E. Deutsch, *J Nucl. Biol. Med.*, 1992, **36**, 29–31.
122 C. L. Crankshaw, M. Marmion, G. D. Luker, V. Rao, J. Dahlheimer, B. D. Burleigh, E. Webb, K. F. Deutsch and D. Piwnica-Worms, *J. Nucl. Med.*, 1998, **39**, 77–86.
123 G. D. Luker, V. V. Rao, C. L. Crankshaw, J. Dahlheimer and D. Piwnica-Worms, *Biochemistry*, 1997, **36**, 14218–14227.
124 J. D. Kelly, A. M. Forster, B. Higley, C. M. Archer, F. S. Booker, L. R. Canning, K. W. Chiu, B. Edwards, H. K. Gill and M. McPartlin, *J Nucl. Med.*, 1993, **34**, 222–227.
125 B. S. Sridhara, S. Braat, P. Rigo, R. Itti, P. Cload and A. Lahiri, *Am. J. Cardiol.*, 1993, **72**, 1015–1019.
126 E. A. Platts, T. L. North, R. D. Pickett and J. D. Kelly, *J Nucl. Cardiol.*, 1995, **2**, 317–326.
127 A. J. Sinusas, J. D. Bergin, N. C. Edwards, D. D. Watson, M. Ruiz, R. W. Makuch, W. H. Smith and G. A. Beller, *Circulation.*, 1994, **89**, 2332–2341.
128 A. Younes, J. A. Songadele, J. Maublant, E. Platts, R. Pickett and A. Veyre, *J Nucl. Cardiol.*, 1995, **2**, 327–333.
129 A. S. Arbab, K. Koizumi, K. Toyama and T. Araki, *J Nucl. Med.*, 1996, **37**, 1551–1556.
130 L. Mansi, P. F. Rambaldi, E. Procaccini, F. D. Gregorio, A. Laprovitera, B. Pecori and W. D. Vecchio, *Eur. J. Nucl. Med. Mol. Imaging*, 1996, **23**, 932–939.
131 O. Schillaci, R. Danieli, R. Tavolaro and F. Scopinaro, *Clin. Nucl. Med.*, 1997, **22**, 46–47.
132 T. Muzzammil, M. J. Moore and J. R. Ballinger, *Cancer Biother. Radiopharm.*, 2000, **15**, 339–346.
133 J. R. Ballinger, *J. Clin. Pharmacol.*, 2001, 39S–47S.
134 W. S. Chen, K. E. Luker, J. L. Dahlheimer, C. M. Pica, G. D. Luker and D. Piwnica-Worms, *Biochem. Pharmacol.*, 2000, **60**, 413–426.
135 K. Utsunomiya, J. R. Ballinger, M. Piquette-Miller, A. M. Rauth, W. Tang, Z. F. Su and M. Ichise, *Eur. J. Nucl. Med. Mol. Imaging*, 2000, **27**, 1786–1792.
136 X. F. Li, S. Kinuya, K. Yokoyama, S. Konishi, Y. Y. Ma, N. Watanabe, N. Shuke, H. Bunko, T. Michigishi and N. Tonami, *Cancer Biother. Radiopharm.*, 2001, **16**, 17–23.
137 Z. Liu, G. D. Stevenson, H. H. Barrett, L. R. Furenlid, D. W. Wilson, G. A. Kastis, M. Bettan and J. M. Woolfenden, *Nucl. Med. Biol.*, 2005, **32**, 573–583.
138 Y. C. Shiau, S. C. Tsai, J. J. Wang, Y. J. Ho, S. T. Ho and C. H. Kao, *Cancer Lett.*, 2001, **169**, 181–188.
139 T. H. Kuo, F. Y. Liu, C. Y. Chuang, H. S. Wu, J. J. Wang and A. Kao, *Nucl. Med. Biol.*, 2003, **30**, 627–632.
140 Y. C. Shiau, S. C. Tsai, J. J. Wang, Y. J. Ho, S. T. Ho and C. H. Kao, *Lung*, 2001, **179**, 197–207.
141 Y. C. Shiau, S. C. Tsai, J. J. Wang, Y. J. Ho, S. T. Ho and C. H. Kao, *Cancer Lett.*, 2001, **20**, 139–146.
142 J. A. Liang, Y. C. Shiau, S. N. Yang, F. J. Lin, C. C. Lin, A. Kao and C. C. Lee, *Oncol. Rep.*, 2002, **9**, 307–312.
143 Y. C. Shiau, S. C. Tsai, J. J. Wang, S. T. Ho and A. Kao, *Nucl. Med. Biol.*, 2002, **29**, 339–344.
144 H. S. Wu, Y. C. Liu, A. Kao, J. J. Wang and S. T. Ho, *Surgery*, 2002, **132**, 456–460.
145 S. S. Sun, J. F. Hsieh, S. C. Tsai, Y. J. Ho and C. H. Kao, *Anticancer Res.*, 2000, **20**, 1467–1470.
146 M. Fukumoto, D. Yoshida, N. Hayase, A. Kurohara, N. Akagi and S. Yoshida, *Cancer*, 1999, **86**, 1470–1479.
147 V. Soderlund, C. Jonsson, H. C. Bauer, O. Brosjo and H. Jacobsson, *J. Nucl. Med.*, 1997, **38**, 682–686.
148 Z. Yapar, M. Kibar, A. F. Yapar, A. Uguz, S. Ozbarlas and G. Gonlusen, *Ann. Nucl. Med.*, 2003, **17**, 443–449.
149 R. Bergmann, P. Brust, M. Scheunemann, H. J. Pietzsch, S. Seifert, F. Roux and B. Johannsen, *Nucl. Med. Biol.*, 2000, **27**, 135–141.
150 C. Bolzati, A. Boschi, L. Uccelli, F. Tisato, F. Refosco, A. Cagnolini, A. Duatti, S. Prakash, G. Bandoli and A. Vittadini, *J. Am. Chem. Soc.*, 2002, **124**, 11468–11479.
151 C. Bolzati, M. Cavazza-Ceccato, S. Agostini, S. Tokunaga, D. Casara and G. Bandoli, *J. Nucl. Med.*, 2008, **49**, 1336–1344.
152 C. Bolzati, M. Cavazza-Ceccato, V. Gandin, C. Marzano, F. Refosco, A. Dolmella, N. A. Colabufo, F. Berardi, R. Perrone and G. Bandoli, *Eur J Med Mol Imaging*, 2009, **36**, S309.
153 C. Bolzati, M. Cavazza-Ceccato, S. Agostini, F. Refosco, Y. Yamamichi, S. Tokunaga, D. Carta, N. Salvarese, D. Bernardini and G. Bandoli, *Bioconjugate Chem.*, 2010, **19**, 928–939.
154 Y. S. Kim, J. Wang, A. Broisat, D. K. Glover and S. Liu, *J. Nucl. Cardiol.*, 2008, **15**, 535–546.
155 Y. S. Kim, J. Shi, S. Zhai, G. Hou and S. Liu, *J. Nucl. Cardiol.*, 2009, **16**, 571–579.
156 B. W. Tsang, C. J. Mathias and M. A. Green, *J Nucl. Med.*, 1993, **34**, 1127–1131.
157 B. W. Tsang, C. J. Mathias, P. E. Fanwick and M. A. Green, *J. Med. Chem.*, 1994, **37**, 4400–4406.
158 Y. M. Hsiao, C. J. Mathias, S. P. Wey, P. E. Fanwick and M. A. Green, *Nucl. Med. Biol.*, 2009, **36**, 39–45.
159 V. Sharma, C. L. Crankshaw and D. Piwnica-Worms, *J. Med. Chem.*, 1996, **39**, 3483–3490.
160 V. Sharma, A. Beatty, S. P. Wey, J. Dahlheimer, C. M. Pica, C. L. Crankshaw, L. Bass, M. A. Green, M. J. Welch and D. Piwnica-Worms, *Chem. Biol.*, 2000, **7**, 335–343.
161 V. Sharma, J. L. Prior, M. G. Belinsky, G. D. Kruh and D. Piwnica-Worms, *J. Nucl. Med.*, 2005, **46**, 354–364.
162 O. Thews, W. Dillenburg, M. Fellner, H. G. Buchholz, N. Bausbacher, M. Schreckenberger and F. Rosch, *Eur J Nucl. Med Mol Imaging.*, 2010, **37**, 1935–1942.
163 J. Sivapackiam, S. E. Harpstrite, J. L. Prior, H. Gu, N. P. Rath and V. Sharma, *Dalton Trans.*, 2010, **39**, 5842–5850.
164 J. S. Lewis, J. Zweit, J. L. J. Dearling, B. C. Rooney and P. J. Blower, *Chem. Commun.*, 1996, 1093–1094.
165 J. S. Lewis, J. L. Dearling, J. K. Sosabowski, J. Zweit, P. Carnochan, L. R. Kelland, H. M. Coley and P. J. Blower, *Eur. J. Nucl. Med. Mol. Imaging*, 2000, **27**, 638–646.
166 S. J. Berners-Price, R. K. Johnson, C. K. Mirabelli, L. F. Faucette, F. L. McCabe and P. J. Sadler, *Inorg. Chem.*, 1987, **26**, 3383–3387.
167 J. S. Lewis, J. Zweit and P. J. Blower, *Polyhedron*, 1998, **17**, 513–517.
168 A. B. Packard, J. F. Kronauge, P. J. Day and S. T. Treves, *Nucl. Med. Biol.*, 1998, **25**, 531–537.
169 A. B. Packard, J. F. Kronauge, E. Barbarics, S. Kiani and S. T. Treves, *Nucl. Med. Biol.*, 2002, **29**, 289–294.
170 O. P. Anderson and A. B. Packard, *Inorg. Chem.*, 1979, **18**, 1940–1947.
171 J. Liu, A. Hajibeigi, G. Ren, M. Lin, W. Siyambalapitiyage, Z. Liu, E. Simpson, R. W. Parkey, X. Sun and O. K. Oz, *J. Nucl. Med.*, 2009, **50**, 1332–1339.
172 J. Wang, C. T. Yang, Y. S. Kim, S. G. Sreerama, Q. Cao, Z. B. Li, Z. He, X. Chen and S. Liu, *J. Med. Chem.*, 2007, **50**, 5057–5069.
173 C. T. Yang, Y. S. Kim, J. Wang, L. Wang, J. Shi, Z. B. Li, X. Chen, M. Fan, J. J. Li and S. Liu, *Bioconjugate Chem.*, 2008, **19**, 2008–2022.
174 S. Liu, Y. S. Kim, S. Zhai, J. Shi and G. Hou, *Bioconjugate Chem.*, 2009, **20**, 790–798.