Metal Complexes for Therapy and Diagnosis of Drug Resistance

Vijay Sharma and David Piwnica-Worms*

Laboratory of Molecular Radiopharmacology, Mallinckrodt Institute of Radiology, and Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, Missouri

Received March 1, 1999 (Revised Manuscript Received June 14, 1999)

Contents

I. Metal Complexes and Multidrug Resistance in Cancer

A. Introduction

Resistance to chemotherapy represents a major obstacle in the treatment of cancer patients. Many tumors are intrinsically resistant to chemotherapy, whereas others initially respond to treatment but acquire resistance to selected cytotoxic drugs during chemotherapy. Multidrug resistance (MDR) is the phenomenon by which cultured cells in vitro and tumor cells in vivo show resistance simultaneously to a variety of structurally and functionally dissimilar cytotoxic and xenobiotic compounds.[1−5] While several different genes have been shown to be associated with a multidrug resistance phenotype,[6] MDR mediated by overexpression of the *MDR1* gene product, P-glycoprotein (Pgp), represents one of the best characterized barriers to chemotherapeutic treatment in cancer. Pgp, a 170 kDa plasma membrane protein, is predicted by sequence analysis to comprise two symmetrical halves that share both homology with a family of ATP-binding cassette (ABC) membrane transport proteins and a common ancestral origin with bacterial transport systems.[5,7] Characterized by 12 transmembrane domains and two nucleotide-binding folds,[3,5] the protein is thought to hydrolyze ATP to affect outward transport of substrates across the cell surface membrane.[5,8] Although the specific protein domains and amino acids involved in substrate recognition have not been formally identified, genetic and biochemical evidence has conventionally been interpreted to show putative membrane-associated domains interacting directly with selected cytotoxic agents to affect transport.[9−11] The net effect is that Pgp decreases the intracellular concentration of substrate compounds in Pgp-expressing multidrug resistant cells compared with non-Pgp-expressing drug sensitive cells.

In addition to the standard paradigm of *MDR1* Pgp as an ATP-dependent efflux transporter of chemotherapeutic drugs, recent studies provide evidence supporting alternative mechanisms for the diminished Pgp-mediated drug content in MDR cells. For example, a flippase model has been proposed for Pgp.[12] This model suggests that Pgp flips hydrophobic cytotoxic compounds from the inner to the outer leaflet of the lipid bilayer wherein the agents can diffuse away, thereby accounting for the observed decrease in intracellular concentration of drug and providing an explanation for the broad specificity of Pgp. Roepe and colleagues[13,14] have shown a more alkaline cytosolic pH and decreased membrane potential in cells transfected with *MDR1* Pgp compared with normal cells. In addition, cells expressing *MDR1* maintain intracellular vesicular compartments that are more acidic than non-Pgp-expressing cells.[15,16] These variations in internal pH and membrane potential may decrease the intracellular concentration of cationic cytotoxic agents and/or lead to sequestration of cytotoxic agents away from their target sites. Furthermore, *MDR1* Pgp may interfere with or alter pathways of apoptosis (programmed cell death),[17,18] therefore offering protection to malignant cells from cytotoxic compounds. Thus, although de-

* Author to whom correspondence should be addressed at the Mallinckrodt Institute of Radiology, Washington University Medical School, Box 8225, 510 S. Kingshighway Blvd., St. Louis, MO 63110. Tel: 314-362-9356. Fax: 314-362-0152. E-mail: piwnica-worms@mirlink.wustl.edu.

10.1021/cr980429x CCC: $35.00
Published on Web 08/07/1999

Born in Chandigarh, a joint capital of the states of Punjab and Haryana, located about 190 miles north of New Delhi, India, Vijay Sharma received his B.Sc., M.Sc., and Ph.D. from Panjab University, Chandigarh, India. Following his Ph.D. dissertation in chemistry, he joined the group of Prof. James D. Wuest, Department of Chemistry, University of Montreal, Quebec, Canada. During his postdoctoral tenure, Dr. Sharma worked on the synthesis and design of multidentate Lewis acids capable of holding metals in well-defined nearby positions. These compounds can be used to bind and orient guests to increase their chemical reactivity. Presently, he is Research Assistant Professor at the Mallinckrodt Institute of Radiology, Washington University Medical School. He is broadly interested in medicinal applications of metal complexes, with specific emphasis on therapy of infectious diseases such as chloroquine-resistant *Plasmodium falciparum* in malaria and exploring coordination compounds as probes of mechanism(s) of drug resistance.

Born and raised in Minneapolis, MN, David Piwnica-Worms received his B.Sc. in Mechanical Engineering from Stanford University. He pursued combined M.D.–Ph.D. degrees with dissertation studies under Prof. Melvyn Lieberman in membrane biophysics and physiology at Duke University Medical School. After a residency in diagnostic radiology at the Brigham and Women's Hospital and a postdoctoral fellowship in magnetic resonance, he joined the faculty of Harvard Medical School. He was promoted to Associate Professor before moving to Washington University Medical School in 1994. Dr. Piwnica-Worms is a Professor in the Departments of Radiology and Molecular Biology & Pharmacology, Washington University Medical School. His research interests include the biochemistry and molecular pharmacology of multidrug resistance mediated by P-glycoprotein and homologous transporters, the utility of novel coordination complexes as radiopharmaceuticals and medicinals, as well as translational research using ^{99m}Tc-labeled radiopharmaceuticals for functional molecular imaging in vivo. As Director of the Laboratory of Molecular Radiopharmacology, he leads a multidisciplinary group of scientists focused on research investigations arising at the interface of cancer cell biology, inorganic chemistry and the radiopharmaceutical sciences.

tails of the molecular mechanism have not been elucidated, the observed combined net effect is a decreased intracellular concentration of cytotoxic drugs that correlates with overexpression of *MDR1* Pgp, thereby making chemotherapeutic treatment ineffective in cancer.

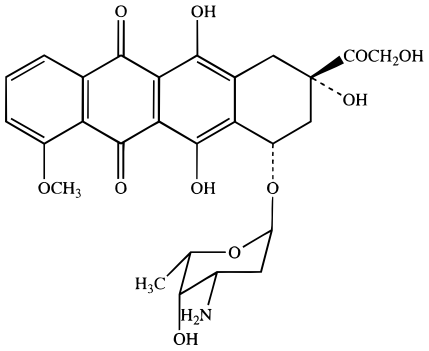
In addition to its overexpression in tumors, *MDR1* Pgp is normally located in several tissues involved in excretory functions, including the brush border of proximal tubule cells in the kidney, the biliary surface of hepatocytes, and the apical surface of mucosal cells in the small intestine and colon.[19,20] *MDR1* Pgp also is located on the luminal surface of endothelial cells lining capillaries in the brain and in the testis[21,22] as well as on the apical surface of choroid plexus epithelial cells.[23] However, despite its widely disseminated expression, the function of *MDR1* Pgp in normal physiology has not been clearly defined, although Pgp may have a role in protection from xenobiotics[24] and intracellular cholesterol trafficking.[25]

B. Multidrug Resistance in the Chemotherapy of Cancer Patients

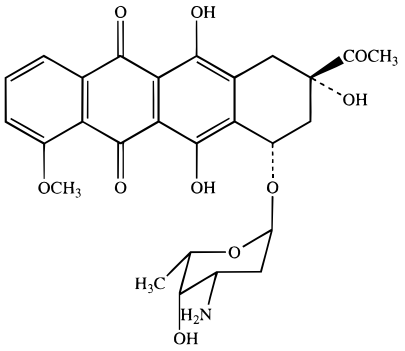
Compounds recognized by Pgp are typically characterized as modestly hydrophobic (octanol/water partitioning coefficient, log P > 1), often contain titratable protons with a net cationic charge under physiological conditions, and are predominately "natural products" with a single aromatic moiety.[26] Among an extensive list of compounds, anthracyclines (doxorubicin **1**, daunorubicin **2**), taxanes (paclitaxel **3**, docetaxel **4**), *Vinca* alkaloids (vincristine **5**, vinblastine **6**, vindesine **7**), and etoposides (VP-16 **8**) (Chart 1) are examples of clinically important chemotherapeutic compounds recognized by *MDR1* Pgp.[6,26,27] The diversity of these agents emphasizes the key characteristic feature of multidrug resistance, i.e., the apparent capacity of Pgp to recognize a large group of cytotoxic compounds sharing little or no structural or functional similarities.

Because clinical studies have documented the poor outcomes associated with *MDR1* Pgp expression in tumors,[27,28] reversal of multidrug resistance by nontoxic agents that block the transport activity of MDR1 Pgp has been an important target for pharmaceutical development. When coadministered with a cytotoxic agent, these compounds, known as MDR modulators or reversal agents, enhance net accumulation of relevant cytotoxic drugs within the tumor cells.[26,29] Many compounds known to have other pharmacological sites of action initially were used to reverse MDR in cancer cells grown in culture and several underwent pilot clinical trials.[26] These compounds included verapamil (**9**), cyclosporin A (**10**), quinidine (**11**), trifluperazine (**12**), and their derivatives (Chart 2).[26] However, these agents had limited clinical utility because of unacceptable side effects at the serum concentrations needed to modulate *MDR1* Pgp.[27] New second-generation modulators (dexverapamil, an optically pure verapamil (**9**),[30] and PSC 833 (**13**), a cyclic undecapeptide analogue of cyclosporin A[31]) and third-generation modulators (GF120918 (**14**), a substituted isoquinolinyl acridonecarboxamide;[32] LY335979 (**15**), a difluorocyclopropyl dibenzosuberane;[33] and VX-710 (**16**), an amido keto pipecolinate[34]) have been developed recently, and phase I/II clinical trials are currently in progress with these new, more specific and potent compounds

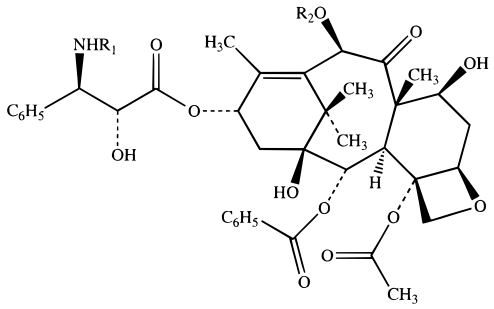
Chart 1



1

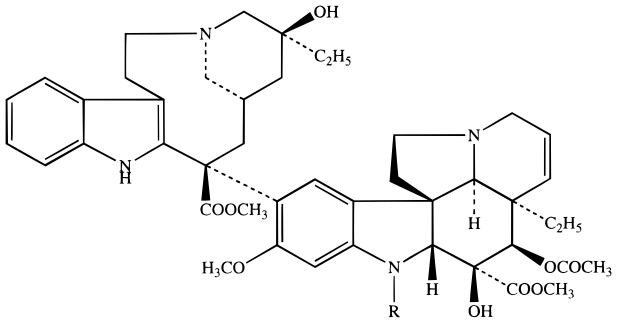


2



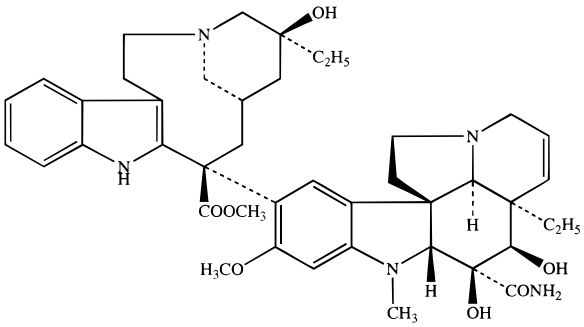
R_1 = COC_6H_5, R_2 = $COCH_3$ (Paclitaxel) **3**

R_1 = $COOC(CH_3)_3$, R_2 = H (Docetaxel) **4**

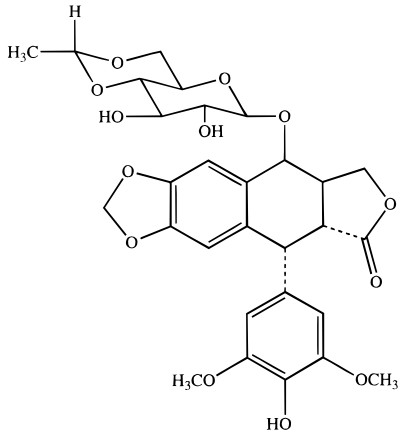


R = CHO (Vincristine) **5**

R = Me (Vinblastine) **6**



7



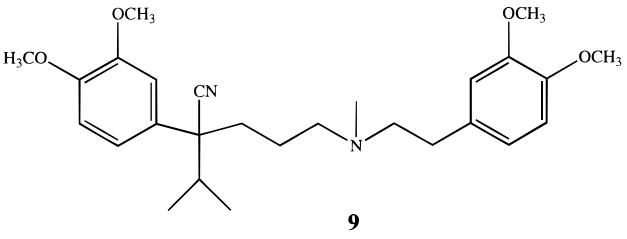
8

(Chart 3). Thus, the MDR phenotype may be modulated more effectively with these more selective reversal agents to improve the efficacy of chemotherapy.

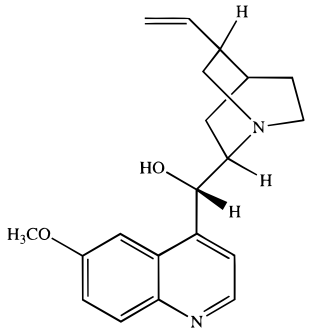
C. Metal Complexes for Imaging the Transport Activity of P-Glycoprotein

Increasingly, the choice of systemic therapy for cancer is based on a priori analysis of tumor markers to assess the presence or absence of a molecular pathway or target (such as a key receptor or enzyme activity) for a given therapeutic agent. Identification of tumor markers with diagnostic agents assists in the proper selection of patients most likely to benefit from targeted therapy. Measurement of MDR is one potentially important marker in planning systemic therapy. However, expression of *MDR1* Pgp, as detected at the level of messenger RNA or protein, does not always correlate with the functional assessment of Pgp-mediated transport activity. Because Pgp transport activity is affected by specific mutations as well as the phosphorylation state of the protein,[5,35,36] altered or less active forms of Pgp may be detected by polymerase chain reaction (PCR) or immunohistochemistry that do not accurately reflect the status of tumor cell resistance. Thus, methods to functionally interrogate Pgp transport activity have been sought.[37] Imaging with a radiopharmaceutical that is transported by *MDR1* Pgp may identify noninvasively those tumors in which the transporter is not only expressed but is functional. Thus, significant effort has been directed toward the noninvasive detection of transporter-mediated resistance utilizing γ-emitting metal complexes characterized as transport substrates for *MDR1* Pgp. These now will be reviewed in detail.

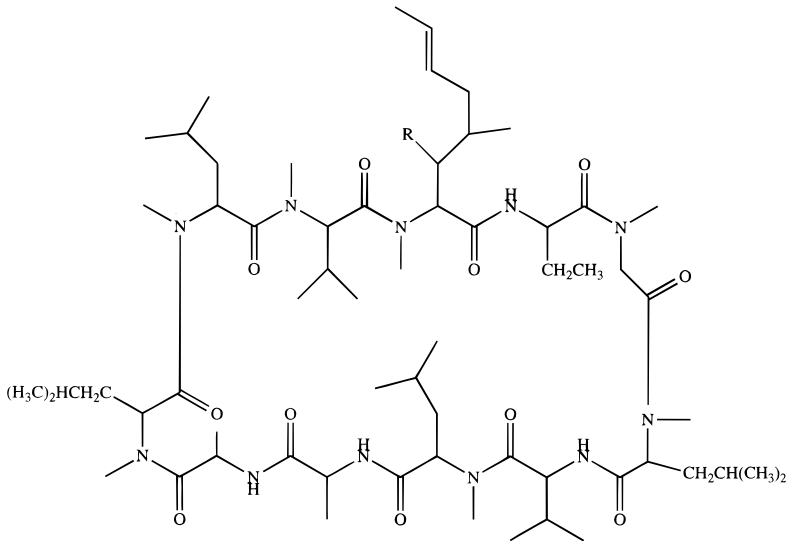
Chart 2



9

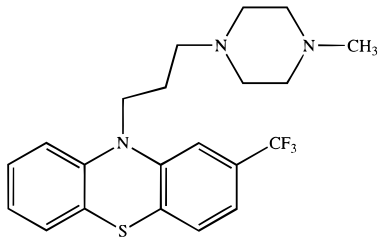


11



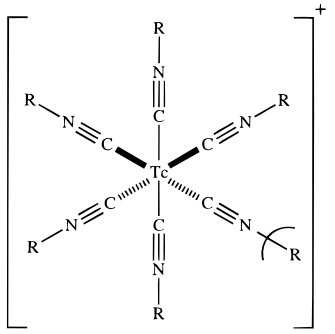
R = OH (Cyclosporin A) **10**

R = ketone (PSC 833) **13**

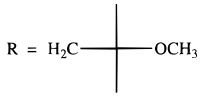


12

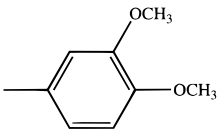
Hexakis(2-methoxyisobutylisonitrile)—^{99m}Tc(I) (commonly known as [^{99m}Tc]sestamibi) (**17a**), although



17



17a

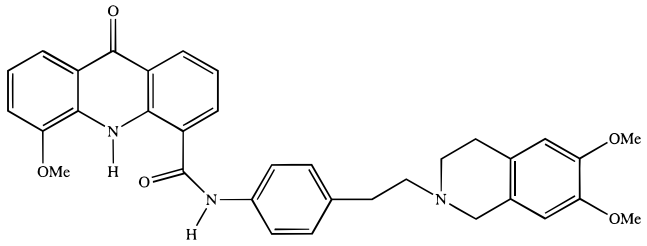


17b

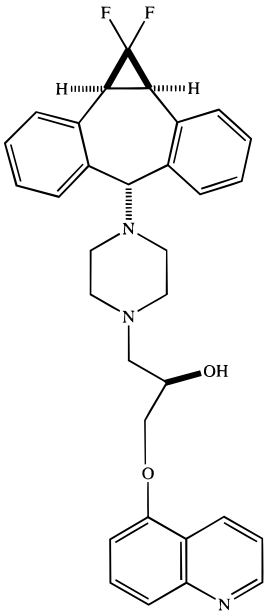
originally developed as a radiopharmaceutical for myocardial perfusion imaging,[38,39] subsequently was the first metal complex shown to be a Pgp transport substrate.[40] Characterized by octahedral geometry around the central technetium(I) core,[38,41,42] this nonmetabolized radiopharmaceutical possesses a cationic charge and modest hydrophobicity similar to many chemotherapeutic agents in the MDR phenotype. In the absence of Pgp expression, this [^{99m}Tc]-isonitrile complex accumulates within the interior of cells in response to the physiologically negative mitochondrial and plasma membrane potentials maintained within cells.[43] However, in Pgp-expressing multidrug resistant tumor cells, net cellular accumulation levels of [^{99m}Tc]sestamibi are inversely proportional to the level of *MDR1* Pgp expression.[40,44−47] Furthermore, complete reversal of the Pgp-mediated exclusion of [^{99m}Tc]sestamibi has been affected by treatment with conventional *MDR1* Pgp inhibitors such as verapamil (**9**), cyclosporin A (**10**), and quinidine (**11**) or newer more potent reversal agents such as PSC 833 (**13**), GF120918 (**14**), or LY335979 (**15**).[25,40,44,45,48−50] Of interest, [^{99m}Tc]sestamibi is also recognized as a transport substrate for the multidrug resistance-associated protein (MRP),[51−53] a close homologue of Pgp,[54] thereby providing a two-edged sword. On one hand, cross-reactivity with MRP may reduce the specificity of the tracer for functional imaging of *MDR1* Pgp in tumors, but alternatively, this property may favorably enable [^{99m}Tc]sestamibi to be a more general probe of transporter-mediated multidrug resistance in cancer.

Recently, clinical studies with cancer patients have validated use of [^{99m}Tc]sestamibi to functionally detect transporter-mediated resistance in tumors in vivo using planar scintigraphy or single-photon emission computed tomography (SPECT).[55−62] For example, rates of efflux of [^{99m}Tc]sestamibi were 2.7-fold greater in breast tumors expressing increased *MDR1* Pgp compared with tumors that expressed Pgp at a level comparable to benign breast lesions.[55] In addition, initial experience with [^{99m}Tc]sestamibi suggests that this radiopharmaceutical also can be used to detect modulator-induced inhibition of Pgp function in patients.[57−59] The prospective value of high tumor clearance rates of [^{99m}Tc]sestamibi to predict poor therapeutic outcomes also has been validated in locally advanced breast cancer,[62] and

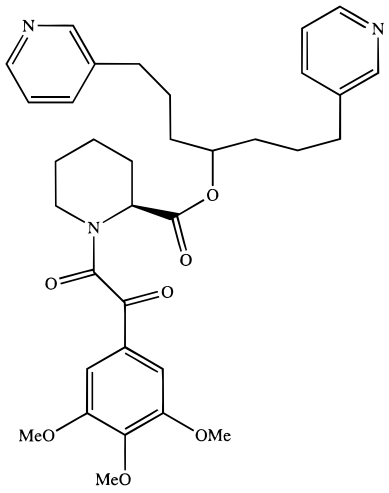
Chart 3



14



15

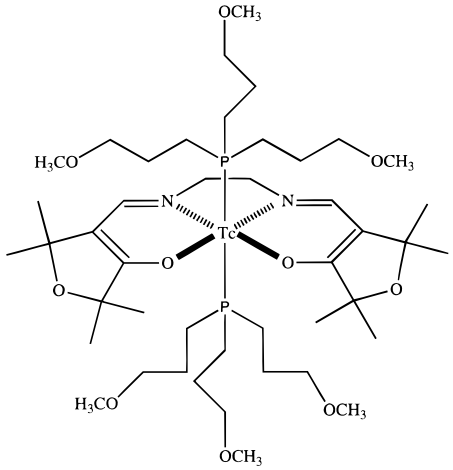


16

larger clinical trials to rigorously test the application of these in vivo functional assays of *MDR1* Pgp in tumors are underway.

To potentially optimize the transport and Pgp-targeting characteristics of [^{99m}Tc]isonitrile complexes, several studies investigating structure–activity relationship have been performed. In one study, the alkyl chains in [^{99m}Tc]sestamibi were replaced with longer chain ether functionalities. The hexakis(2-ethoxyisobutylisonitrile)–^{99m}Tc complex ([^{99m}Tc]EIBI) was shown to be a transport substrate recognized by Pgp, but with slightly greater nonspecific cell binding than [^{99m}Tc]sestamibi.[63] Substituted aromatic functionalities were also explored.[64] A series of substituted arylisonitrile analogues was synthesized from their corresponding amines through a reaction with dichlorocarbene under phase transfer catalyzed conditions, and noncarrier-added hexakis(arylisonitrile)–^{99m}Tc complexes were produced by reaction with pertechnetate in the presence of sodium dithionite. The lead compound of the series, **17b**, demonstrated an overall encouraging transport profile in Pgp-expressing cells, but significant nonspecific adsorption to hydrophobic compartments was identified. The results also suggested that methoxy substituents, compared with other substituents, preferentially contributed to enhanced Pgp recognition for this class of compounds. However, none of these radiolabeled complexes exceeded [^{99m}Tc]sestamibi in their Pgp-mediated transport properties.

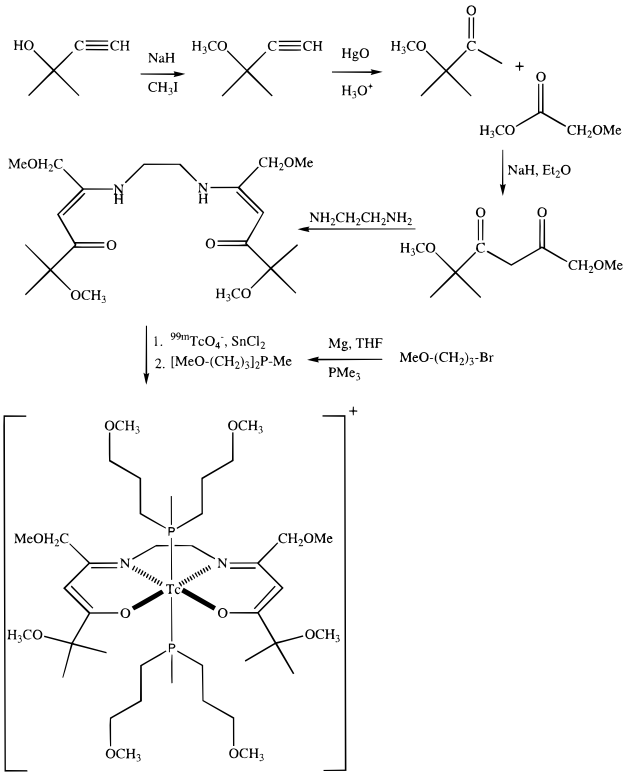
Recently, several entirely different classes of technetium complexes have been identified as Pgp transport substrates. Using a planar Schiff-base moiety and hydrophobic phosphines, nonreducible Tc(III) monocationic compounds known as "Q-complexes" were developed a decade ago for applications in myocardial perfusion imaging.[65,66] The lead complex for clinical development was trans[(1,2-bis(dihydro-2,2,5,5-tetramethyl-3(2*H*)furanone-4-methyleneimino)ethane) bis(tris(3-methoxy-1-propyl)phosphine)]-technetium(III), known as [^{99m}Tc]furifosmin (**18**)[67] Because the hydrophobicity and Pgp-targeting prop-



18

erties of these complexes could be readily adjusted by varying functionalities on the Schiff base or phosphine moieties independently, a variety of novel [^{99m}Tc]Q complexes with subtle structural differences were amenable to synthesis.[48,68] This approach allowed the coordination environment of the Tc(III) metal core to be maintained while the overall electronic environment of the periphery of the molecule was altered, thereby enabling refined exploration and evaluation of features conferring Pgp-mediated transport properties. Ether functionalities can be incorporated into the equatorial Schiff base ligand by condensation of ethylenediamines with ether-containing β-dicarbonyl compounds (Scheme 1).[69] The

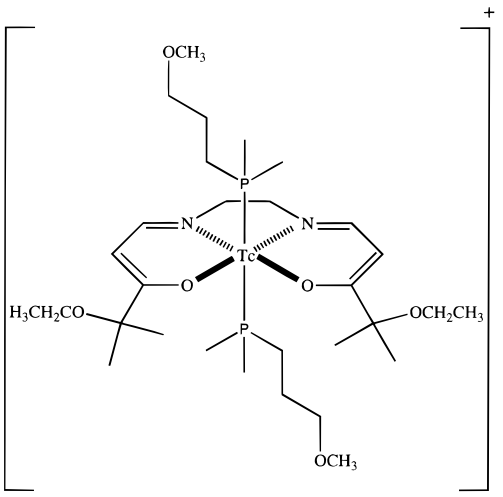
Scheme 1



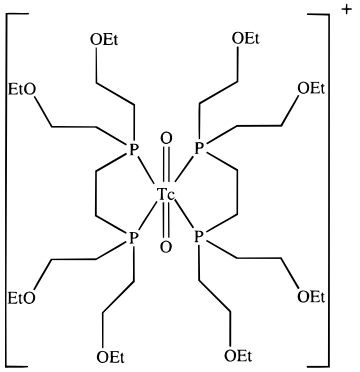
19

presence of *gem*-dimethyl groups sterically hinder the attack of diamine at the adjacent carbonyl, and the strategy results in regioselective condensation at the less hindered carbonyl. Preparation of the tertiary phosphines was accomplished in a two-step, one-pot reaction involving treatment of 1-chloro-3-methoxypropane with magnesium in tetrahydrofuran and subsequent reaction of the reagent with dimethylchlorophosphines or dichloromethylphosphines to provide the necessary substituted phosphines with overall yield of 50−70%.[68] The desired [^{99m}Tc]Q complexes were then obtained by a two-step synthetic approach using the phosphines as both reductants and ligands.[70] From MDR transport assays in vitro, the *trans*-[2,2′-(1,2-ethanediyldiimino)bis(1,5-methoxy-5-methyl-4-oxohexenyl)]bis[methylbis(3-methoxy-1-propyl)phosphine]technetium(III) complex (**19**) and the *trans*-[5,5′-(1,2-ethanediyldiimino)bis(2-ethoxy-2-methyl-3-oxo-4-pentenyl)]bis[dimethyl(3-methoxy-1-propyl)phosphine]technetium(III) complex (**20**) (Chart 4) were discovered. These complexes are nearly

Chart 4



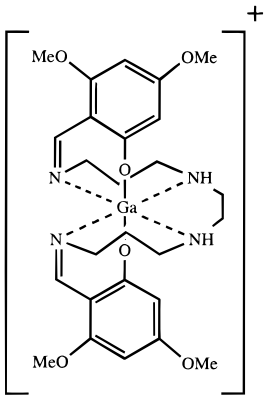
20



21

identical to [^{99m}Tc]sestamibi in their Pgp recognition properties in vitro.[48,68] In addition, a Tc(V) complex known as [^{99m}Tc]tetrofosmin,[71] [1,2-bis{bis(2-ethoxyethyl)phosphino}ethane]$_2$−O_2Tc(V) (**21**), has been identified as another ^{99m}Tc complex with highly favorable *MDR1* Pgp-mediated transport properties.[68,72] While these metal complexes do not share any obvious structural homology, they do share the common features of a cationic charge and modest hydrophobicity. Overall, which of these selected ^{99m}Tc complexes may be most clinically useful in evaluation of the Pgp status of tumors by SPECT imaging remains under intense investigation.

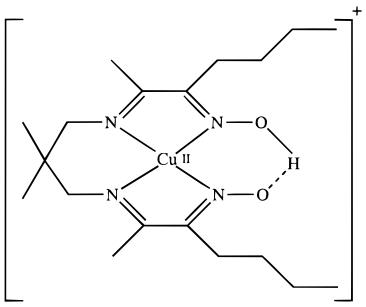
Ligands coordinating other metals have also been explored. Multidentate ligands with an N_4O_2 donor core have the ability to form stable monomeric, monocationic, hydrophobic complexes with a variety of main group[73,74] and transition metals.[75−77] Schiff-base Ga(III) complexes were previously reported as potential positron emitting (PET) radiopharmaceuticals with utility as myocardial perfusion imaging agents.[78,79] These complexes **22** possess several characteristics indicating potential utility as PET probes of *MDR1* Pgp activity in tumors.[80,81] The triaryl precursors containing a central imidazolidine ring were synthesized by condensation of an appropriate linear tetramine with substituted salicylaldehydes. The desired Schiff-base ligands comprising substituted ethylenediamine-*N*,*N*′-bis[propyl(2-hydroxy-



22

benzylimino)] (ENBPT) were obtained by cleavage of the imidazolidine ring, and the corresponding monocationic metal complexes were produced by reaction with appropriate acetylacetonates of Al(III), Fe(III), Ga(III), and In(III). In human epidermal carcinoma KB-3-1 (non-Pgp) cells, cytotoxic potencies of racemic mixtures of these complexes were in the low micromolar range and depended strongly on the identity of the coordinating central metal in the potency rank order Fe(III) > Al(III) > Ga(III) ≥ In(III).[80] The active metal complexes containing 4,6-dimethoxy-substituted aromatic rings were more potent than their corresponding 3-methoxy analogues. Furthermore, when assayed for cytotoxic potency in tumor cells, modest expression of *MDR1* Pgp in, for example, drug resistant KB-8-5 tumor cells, reduced the cytotoxic activity of these complexes relative to drug-sensitive KB-3-1 tumor cells, consistent with recognition of these metal complexes by *MDR1* Pgp[80] (Figure 1). These results further suggested that radiolabeled analogues of these Ga(III) complexes could provide templates for ^{68}Ga PET radiopharmaceuticals to probe Pgp transport activity in tumors.[81]

On the basis of previous work,[82,83] a stable, monocationic radiolabeled complex of copper(II) **23** was



23

obtained as a potential ^{64}Cu PET radiopharmaceutical targeting Pgp.[84] The desired diiminedioxime ligand was synthesized from 2,3-dimethylpropane-1,2-diamine and heptane-2,3-dione 3-oxime. Cellular accumulation studies demonstrated significantly more accumulation of the radiolabeled compound in MES-SA (non-Pgp) cells compared with MES-SA/Dx5 (Pgp expressing) cells in vitro. Addition of the MDR reversal agent cyclosporin A completely reversed the accumulation profile in MES-SA/Dx5 cells, rendering uptake comparable to control (non-Pgp) cells. Biden-

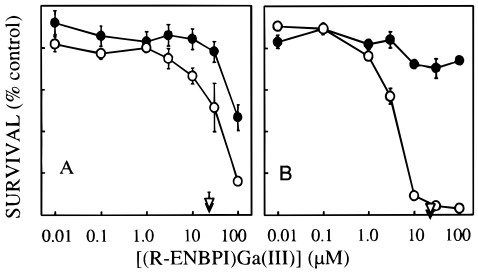
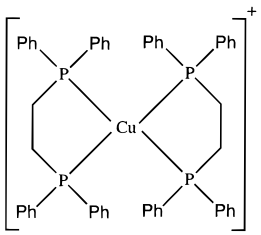


Figure 1. Cell survival studies and LC_{50} determination. Survival of parental KB-3-1 (○, ▽) and multidrug resistant KB-8-5 (●, ▼) cells in increasing concentrations of R-ENBPI−gallium(III) complexes (○, ●) {R = 3-OMe (A); 4,6-diOMe (B)} or 25 μM colchicine (▽, ▼). Each point represents the mean of triplicate determinations; bars represent ±SEM when larger than the symbol; solid lines are a spline presentation of the data.

tate tertiary phosphine ligands have the ability to generate stable copper(I) complexes through a one-step synthesis in quantative yields[85] and represent another class of potential ^{64}Cu PET radiopharmaceuticals targeting Pgp. These complexes previously demonstrated potent antitumor properties compared with their free ligands alone.[86] As with [^{99m}Tc]Q complexes, herein phosphines were exploited as both ligands and reducing agents to generate cationic, hydrophobic, tetrahedral copper(I) complexes **24** with



24

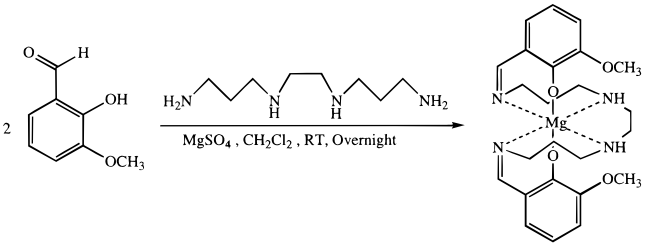
1,2-bis(diphenylphosphino)ethane.[87,88] These potential PET radiopharmaceuticals show Pgp-targeting properties.[89] Thus, several leads exist for a ^{64}Cu-based radiopharmaceutical for interrogation of Pgp by PET.

Parenthetically, it should be mentioned that in addition to these metal complexes several organic compounds based on structures of known MDR cytotoxic drugs or classic modulators have been evaluated as potential PET agents for targeting *MDR1*.[90−93] While promising preliminary data have been generated, these agents generally suffer from low radiochemical yields and complex pharmacokinetics in vivo mediated, at least in part, by rapid metabolism of the radiolabeled compounds.

In summary, *MDR1* Pgp recognizes a wide variety of radioactive compounds comprising metals in various oxidation states and a broad diversity of chelation scaffolds which may enable diagnostic imaging technologies to be exploited for functional interrogation of the MDR phenotype in cancer patients. In particular, several clinically approved ^{99m}Tc complexes have already shown promise for use in the functional evaluation of *MDR1* Pgp-mediated transport activity in human tumors in vivo.

D. Neutral Metal Complexes

Most, but not all, compounds that interact with Pgp are hydrophobic and cationic at physiological pH,[26] and indeed, several cationic Ga(III) complexes have been shown to be recognized as Pgp transport substrates for possible use in PET evaluation of the MDR phenotype.[81] To further explore the impact of the charge of a candidate agent in promoting interactions with Pgp, a neutral analogue of these Ga(III) complexes was synthesized. A neutral Mg complex of *N*,*N*′-bis{3-[(2-hydroxy-3-methoxybenzyl)imino]propyl}ethylenediamine (**25**), incorporating a Schiff-base



25

phenolic ligand structurally similar to the analogous Ga(III) complex, was synthesized by a one-step condensation reaction.[94] ^{1}H NMR data suggested that 85% of the parent compound remained after a 72 h incubation in solution containing an equimolar mixture of Mg^{2+} and $HPO_4^{2-}/H_2PO_4^-$ ions at pH 7.4. Lowering the pH or deleting Mg^{2+} ions increased the rate of hydrolysis.[94] Cytotoxicity studies (Figure 2) in non-Pgp- and Pgp-expressing tumor cell lines demonstrated that the cytotoxicity profiles were not modified by expression of *MDR1* Pgp. These data suggested that compound **25** was not interacting with Pgp. These results confirmed that charge is relevant for recognition of these compounds by Pgp and, furthermore, suggested indirectly that membrane potential differences between Pgp and non-Pgp cells

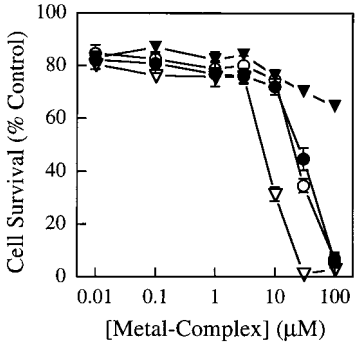


Figure 2. Cell survival studies and LC_{50} determination. Survival of drug sensitive KB-3-1 (○, ▽) and Pgp-expressing KB-8-5 (●, ▼) cells grown in the presence of increasing concentrations of **25** (○, ●) or the cationic Fe(III) analogue (▽, ▼). Cells grown in the presence of vehicle (0.85% ethanol/0.15% DMSO) alone served as control preparations; data for cell survival in the presence of complexes are plotted as a percentage of vehicle control. LC_{50} (μM), mean ± SEM: **25**, KB-3-1, 26 ± 1.6; KB-8-5, 34 ± 2.5; Fe(III)-complex, KB-3-1, 9.0 ± 0.5; KB-8-5, >200. Each point represents the mean of triplicate determinations; bars represent ±SEM when larger than symbol.

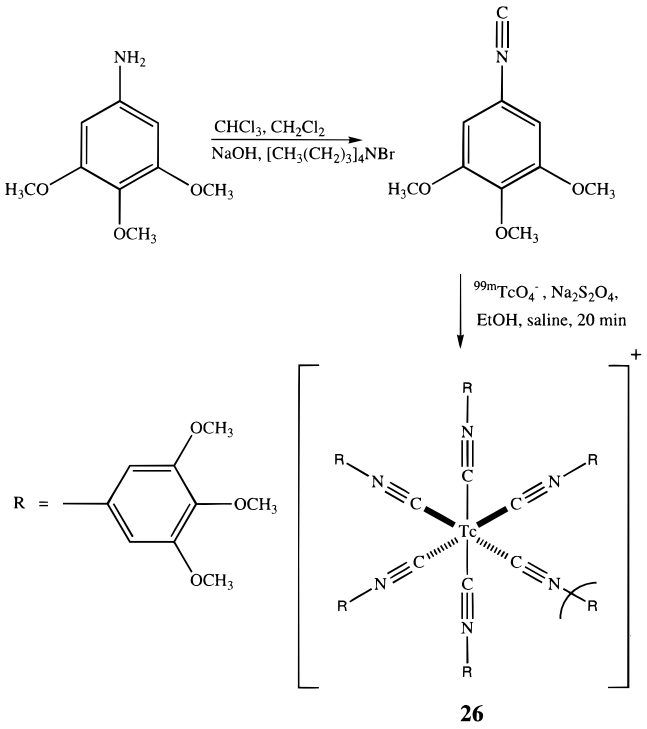
could potentially play a role in the intracellular trafficking of a variety of these metal complexes.

E. Metal Complexes for Reversal of Multidrug Resistance

As reviewed above, many organic compounds have been identified which can reverse MDR.[26] Most reversal agents are hydrophobic compounds and contain a basic nitrogen that can be protonated at physiological pH. These agents putatively inhibit the transporter either by direct interactions with hydrophobic domains of the protein or partitioning into the lipid bilayer to impact membrane permeability or bilayer-induced changes in protein function.

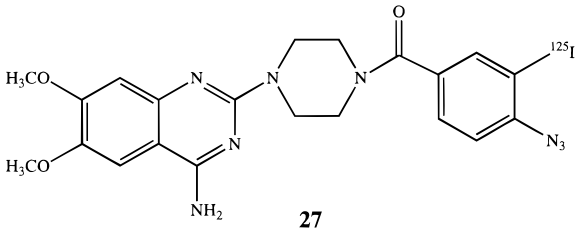
In contrast to the wide variety of organic compounds identified that possess MDR reversal activity, metal complexes with efficacy as reversal agents have only begun to be explored. For example, from a series of novel substituted areneisonitrile analogues of [^{99m}Tc]sestamibi emerged the monocationic hexakis-(3,4,5-trimethoxyphenylisonitrile)−Tc(I) complex (Tc−TMPI) (**26**, Scheme 2) as a potential modulator of

Scheme 2



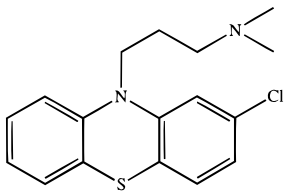
26

Pgp.[95] In tumor cells in culture, tracer [^{99m}Tc]TMPI showed net cellular accumulation in inverse proportion to expression of Pgp and enhancement upon addition of other classic MDR modulators. At pharmacological concentrations, the carrier-added [^{99}Tc]-TMPI complex showed potent inhibition of Pgp-mediated [^{99m}Tc]sestamibi transport (EC_{50}, 1.1± 0.2 μM) and displacement of the Pgp-specific photolabel [^{125}I]IAP (iodoarylazidoprazosin, **27**) in a concentration-dependent manner. It was concluded that [^{99}Tc]-TMPI directly inhibited Pgp transport activity and provided a convenient template for development of nonradioactive Re(I) complexes as novel inhibitors of MDR.



27

Calcium-dependent protein kinases, such as protein kinase C (PKC), may contribute to positive regulation of the transport functions of Pgp. For example, PKC-mediated phosphorylation of Pgp decreases accumulation of cytotoxic compounds in MDR cells.[96] Furthermore, evidence indicates that inhibition of PKC can reverse the MDR phenotype.[97] Because phenothiazines have been used as modulators of MDR[98] and, in particular, chlorpromazine (**28**)(CPZ) and trifluperazine (**12**) (TFP) are PKC



28

inhibitors, it was of interest to explore complexes of CPZ and TFP with various metals such as vanadium(IV), copper(II), and nickel(II).[99,100] The data indicate that TFP complexes of vanadium(IV) and copper(II) are more potent MDR reversal agents than their corresponding free ligands alone. However, in peripherally related work with another metal complex incorporating a pharmaceutical ligand, the Fe chelator desferrioxamine has been evaluated as a therapeutic agent in a xenograft model of human neuroblastoma, a tumor commonly associated with the MDR phenotype, unfortunately with little efficacy.[101] In addition, a ferrocene derivative of hydroxytamoxifen ("ferrocifene") has been reported.[102]

Thus, while less explored than diagnostic radiopharmaceuticals, several nonradioactive metal complexes with broad structural diversity have shown potential efficacy as antagonists of MDR in tumor cells.

II. Metal Complexes and Drug Resistance in Tropical Diseases: Malaria

A. Introduction

Nearly half of the global population lives under the continuous threat of malaria, and the disease is responsible for approximately 2 million deaths each year.[103] Caused by a protozoan parasite of the genus *Plasmodium*, there are four species that infect humans, the most deadly of which is *Plasmodium falciparum*. The malarial life cycle is complex, requiring a female mosquito (anopheles) as vector, parasitic sporozoite and merozoite stages which mature in the host liver, and intraerythrocytic parasitic phases known as ring, trophozoite, and schizont stages.[104,105] Most serious symptoms and deaths occur due to complications of infections with *P. falciparum* strains, not uncommonly occurring in the context of inappropriate host immune reactions and/or when infected erythrocytes containing mature stage parasites adhere to the vascular endothelium of host capillary venules in the brain, leading to vascular occlusion.[106]

Given the worldwide importance of malaria and long-standing efforts to eradicate the disease, the inventory of antimalarial compounds is immense.[105] However, the single most important driving force for continued interest in innovative antimalarials is the emergence of drug-resistance throughout the world.[107] Many conventional antimalarial agents have become ineffective. These can be roughly divided into two distinct groups based on their putative mechanisms of action. The first major group of agents, represented by chloroguanide, pyrimethamine, sulfonamides, and derivatives, have mechanisms of action that are slow in onset and appear to target the synthesis of folinic acid (folate) from *p*-aminobenzoic acid (PABA). Acquired or natural drug resistance to these agents, largely due to mutations in the target enzymes, can be demonstrated readily in the laboratory and has been reported from every major endemic malarial region.

The second group, characterized by older well-known agents such as chloroquine (CQ), primaquine, quinine, and their derivatives, demonstrates rapid cytological action on the intraerythrocyte parasite. Of this group, chloroquine has been the most common, well-tolerated, and cost-effective drug for prophylaxis and therapy of malaria.[108] Chloroquine-like agents are thought to selectively accumulate within the parasite and interfere with the function of the digestive vacuole. In fact, chloroquine-sensitive strains of *Plasmodium* parasites concentrate chloroquine approximately 800-fold in their digestive vacuoles.[109,110] The digestive vacuole is a crucial organelle for the parasite, responsible for degradation of the host's hemoglobin, the main source of nutrients for the parasite. The digestive vacuole has lysosomal characteristics such as an acid pH[111] and a high content of acid hydrolases.[112] Hemoglobin degradation inside the digestive vacuole generates large amounts of toxic heme monomers which are subsequently detoxified by polymerization into hemozoin, the black pigment of malaria. Furthermore, the existence of a putative heme polymerase in the digestive vacuole to ennucleate the polymerization process has been supported[113] but remains somewhat controversial.[114] Nonetheless, chloroquine inhibits this heme polymerization reaction in vitro at concentrations which are found in the digestive vacuole of chloroquine-sensitive parasites.[115−120] Chloroquine may also function by raising the pH of the vacuolar compartment, thereby disrupting pH-dependent enzymatic activities.[105,121] However, like the first group, except in the Middle East and much of Central America, chloroquine-resistant organisms are now reported from every other major region where malaria is endemic.[122] Despite the reliance of patients

with malaria on chemotherapy, the effectiveness of available antimalarials is dwindling.

B. Candidate Mechanism(S) of Chloroquine Resistance in Malaria

Because chloroquine derivatives have been the therapeutic choice in treatment of malaria, we will briefly examine several mechanisms hypothesized to render these 4-aminoquinolines ineffective in *P. falciparum*.

Seminal observations by Krogstad and colleagues demonstrated that the MDR reversal agent verapamil (**9**) and the Pgp transport substrates daunorubicin (**2**) and vinblastine (**6**) enhanced the accumulation of chloroquine in drug resistant *P. falciparum* clones.[123] These observations suggested the existence of common resistance mechanisms in both malaria and cancer. The efflux rate of preaccumulated chloroquine from resistant parasites was 40 times faster than the efflux rate from sensitive parasites ($t_{1/2} = 2$ min vs 75 min).[123] However, others demonstrated that the rate of drug efflux is a function of vacuolar concentration rather than the forces for either accumulation or efflux.[124] Earlier studies suggested gene amplification of *pfmdr1*, an *MDR1* homologue, in chloroquine resistant isolates and none in chloroquine susceptible isolates.[125] Immunofluorescence and immunoelectron microscopy demonstrated expression of Pgh1, the product of *pfmdr*, throughout the erythrocytic life cycle, and furthermore, in trophozoites, the protein was located predominantly on the vacuolar membrane.[126] The location was thought to be consistent with its putative role as a chloroquine transporter. However, quantification of Pgh1 by immunoblot analysis did not show any correlation between overexpression and resistance, and equivalent amounts were present in several chloroquine sensitive and resistant lines.[126] Subsequent efforts did not correlate *pfmdr1* gene copy number with chloroquine resistance.[127]

To further examine chloroquine resistance mechanisms, Wellems and co-workers performed a genetic cross between the chloroquine susceptible HB3 clone and chloroquine resistant Dd2 clone of *P. falciparum*. The cross generated independent offspring which exhibited drug response characteristics of either the resistant or susceptible parent, suggesting a single genetic locus may be responsible for the drug susceptibility phenotype.[128] Analysis of restriction fragment polymorphism enabled the parental origin of *pfmdr1* in the offspring to be identified. Further analysis of inheritance patterns demonstrated that the parental *pfmdr1* did not segregate with chloroquine resistance. Thus, it was concluded that chloroquine resistance is independent of MDR genes.[128] Further studies of genetic crosses using restriction fragment length polymorphism markers were able to distinguish an inheritance from chloroquine resistant and chloroquine susceptible parents.[129] An exhaustive search of the 14 chromosomes disclosed a perfect linkage of the chloroquine resistant phenotype to a genetic locus of around 36 kilobase on chromosome 7. This segment harbors eight potential genes, including the recently sequenced *cg2* which encodes CG2, a unique ~330 kDa protein with complex polymorphisms.[130]

Recently, these *P. falciparum* clones have been examined for chloroquine transport. It has been shown that chloroquine accumulation is temperature dependent, saturable, and inhibitable.[131] Kinetic analysis was consistent with these *P. falciparum* clones differentially encoding a protein which facilitates chloroquine import. The kinetics of chloroquine accumulation differs in isolates from both chloroquine susceptible and resistant parasites derived from the genetic cross and again center on the locus containing the 36 kilobase segment. Further analysis of these crosses showed competitive inhibition of chloroquine uptake by amiloride derivatives, suggesting that chloroquine influx is mediated by a *Plasmodial* Na^+/H^+ exchanger.[131] While some sequence similarities in CG2 and Na^+/H^+ exchangers were recently identified,[132] this has been refuted.[133] Thus, CG2 remains under scrutiny as the key mediator of chloroquine resistance in malaria.

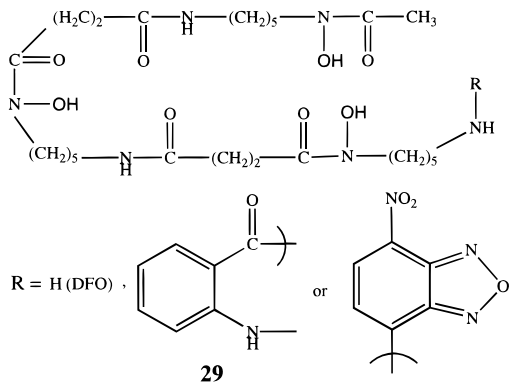
C. Metal Chelators for Treatment of Malaria

In the following two sections, we will review the classes of bioinorganic compounds that have shown promising efficacy as antimalarials, including both metal chelators used as the free ligands to bind metabolically active metals such as iron(III) as well as recently reported intact metal complexes.

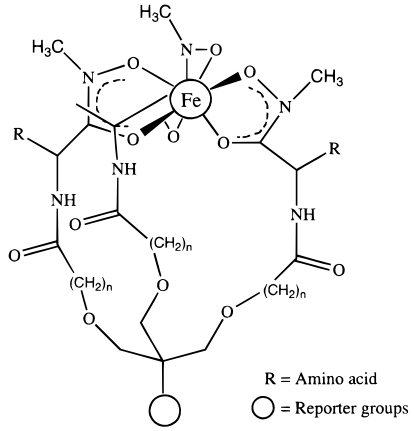
Given the importance of iron metabolites in malarial physiology and toxicity, various metal chelators have been used as antimalarials.[134] The strategy is based upon the principles that, (a) all microorganisms need iron for their growth and replication, (b) microorganisms secrete siderophores, small molecular weight, water soluble molecules that bind extracellular iron(III) with high affinity with the resulting metal complexes binding to selective surface receptors for internalization through a multistep process, and (c) iron deprivation impacts preferentially parasite growth relative to mammalian cells.

Thus, iron chelators such as deferoxamine **29** and its derivatives[135−138] and other iron-chelating drugs such as polyanioinic amines,[139] 3-hydroxypyridin-4-ones,[140] and reversed siderophores **30**[141−143] have been evaluated as antimalarials (Chart 5). The tripodal design of reversed siderophores enables incorporation of fluorescent or radioactive markers as reporter groups to facilitate biochemical and pharmacological studies. The antimalarial effect of these chelators is attributed to their interaction with a labile iron pool within parasitized erythrocytes[137] and possible chelation of ferric iron in association with hemozoin,[144] thereby depriving the parasite of a necessary trace metal. Previously synthesized for imaging applications[145,146] and known to bind iron with high affinity, aminethiol multidentate chelators, such as ethane-1,2-bis(*N*-1-amino-3-ethylbutyl-3-thiol) (BAT, **31**) and *N′*,*N′*,*N′*-tris(2-methyl-2-mercaptopropyl)-1,4,7-triazacyclononane (TAT, **32**), were evaluated for their

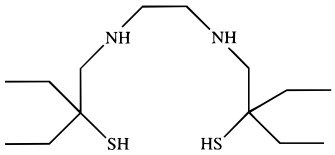
Chart 5



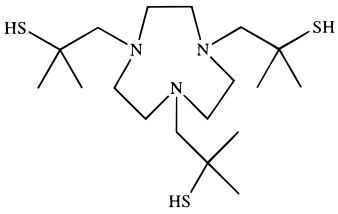
29



30



31



32

efficacy as antimalarials.[147] These compounds inhibited parasitic growth as evaluated through hypoxanthine incorporation with IC_{50} values of 7.6 and 3.3 μM, respectively, and were 5−10 times more potent than desferrioxamine.[147] On the basis of earlier data demonstrating the low stability constants for zinc(II) complexes of desferrioxamine (DFO) versus iron(III) complexes of DFO,[148] a zinc(II)−DFO complex was synthesized recently as a potential antimalarial compound.[149] Thought to possess improved membrane permeability properties, the results suggested that zinc(II) complexes were engaged in transmetalation reactions with iron(III) within intracellular compartments; the strategy provided improved potency in inhibiting the growth of parasites compared with DFO alone.

However, it could be argued that metal chelators may lack selective parasitic targeting and, therefore, undesired biological side effects may appear in the host when these compounds are administered as the free ligand. Furthermore, administration of free ligands may result in the formation of metal complexes with other cations in the extracellular and intracellular compartments of the host, thus leading to unanticipated biological effects.

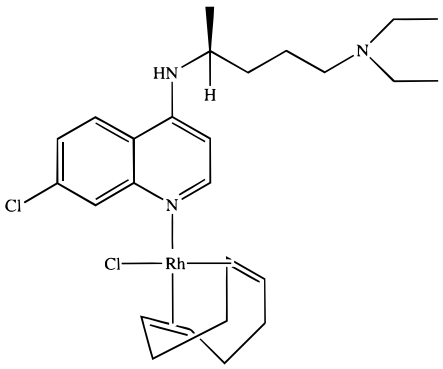
D. Metal Complexes as Antimalarials

Encouraged by the success of *cis*-platin and several other platinum compounds as antitumor agents as well as gadolinium complexes as contrast agents for magnetic resonance imaging,[150] medicinal chemists have begun to evaluate the utility of intact metal complexes as potential antimalarials. Herein, we report recent efforts to design metal complexes for antimalarial therapies.

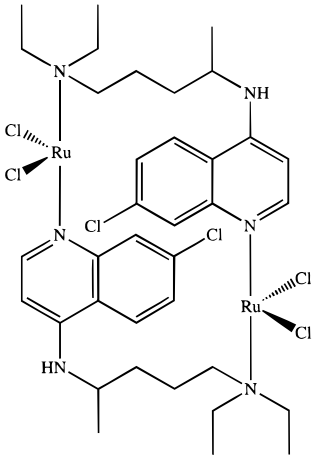
Exploiting the potent antimalarial activity of CQ against many malarial strains, direct incorporation of CQ into metal complexes or incorporation of CQ into the organic scaffolds of metal complexes have been explored. For example, treatment of chlorobis(cyclooctadiene)rhodium(I)[151] with 3 equiv of CQ under mild conditions provided air-stable yellow microcrystals of a metal complex **33** (Chart 6). Alternatively, interaction of ruthenium(III) chloride hydrate with 5 equiv of CQ in the presence of reducing agent provided another metal complex, **34**.[152] Growth inhibition studies indicated that rhodium complex **33** was equipotent to chloroquine diphosphate (CQDP), whereas ruthenium complex **34** was 4 times more potent than CQDP against strains of *Plasmodium berghei*. However, the ruthenium complex was about 4 times more potent than CQDP in FCB1 strains and 2 times more potent than CQDP in drug-resistant FCB2 strains of *P. falciparum*.[152] In further efforts to obtain metal complexes of greater efficacy, the investigators isolated a gold complex of CQ **35** through a reaction of $AuClPPh_3$[153] in acetonitrile in the presence of potassium hexafluorophosphate with 2 equiv of CQ under reflux conditions.[154] The gold complex **35** was a more potent antimalarial compared with rhodium **33** and ruthenium **34** complexes against strains of both *P. berghei.* and *P. falciparum*.[154]

Organometallic compounds such as ferrocenes (dicyclopentadienyliron) are stable and nontoxic, i.e., compatible with biomedical applications. These eighteen electron systems (10 from rings and eight from iron in the zero oxidation state) are amenable to a variety of aromatic substitutions.[155−157] Recently, carbon chains of chloroquine were substituted with a hydrophobic ferrocenyl group, while the position of two exocyclic nitrogens was maintained. Using this synthetic strategy,[158] a ferrocene−chloroquine analogue, 7-chloro-4-{{{2-{(*N*,*N*-dimethylamino)methyl}-ferrocenyl}methyl}amino}quinoline (**36**), was obtained. Compared with chloroquine, **36** was 22 times more potent against drug-resistant strains of *P. falciparum*. Analysis of structure−activity relation-

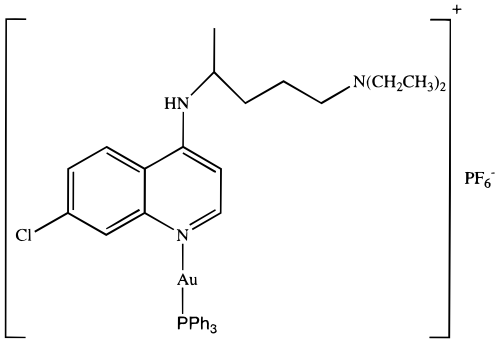
Chart 6



33

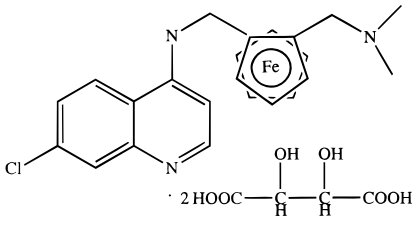


34



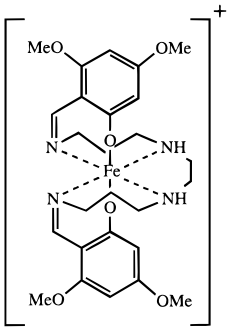
35

ships demonstrated that ferrocene was required to be covalently bound to chloroquine to antagonize the drug resistance of the parasites. Thus, while ferrocene alone did not show any efficacy, ferrocene enhanced the potency of chloroquine when enclosed within the molecule.[159]



36

Schiff-base phenolate metal complexes, a class of coordination complexes with favorable cell membrane permeability properties which have been exploited in cancer MDR, have also been tested as antimalarials.[160] These scaffolds are amenable to accommodating a variety of metals,[73,74] including Al(III), Ga(III), and In(III), in addition to biocompatible metals relevant to malaria-host interactions such as Fe(III). These metal(III) complexes, such as **37** inhibited the



37

intraerythrocytic malarial parasitic growth in a stage specific manner. Inhibiting both chloroquine sensitive and resistant strains (Figure 3), the efficacy of these

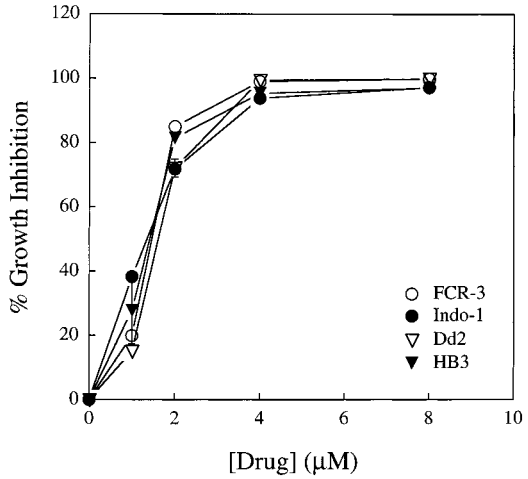


Figure 3. Effect of 4,6-dimethoxy-ENBPI Fe(III) complex **37** on intraerythrocytic *P. falciparum* in culture. Concentration−effect curve of antimalarial activity: chloroquine sensitive (HB3) and resistant (FCR-3, Indo-1, Dd2) lines were grown in the absence or presence of various concentrations of inhibitor. Growth inhibition relative to control was measured by the [^{3}H]hypoxanthine incorporation assay.[160] Data are shown as mean values of triplicate determinations; error bars (when larger than symbol) represent ±SEM.

metal complexes correlated with their ability to inhibit heme polymerization.[160] Of note, it was recently reported that high valent iron−oxo species may mediate the molecular mechanism of action of artemisinin, another antimalarial under active investigation.[161]

E. Probing Chloroquine Resistance Mechanisms

While characterizing the antimalarial properties of Schiff-base and amine phenolic complexes of Ga(III) and Fe(III),[160,162] an unusual selectivity profile was found with complexes of one particular ligand. This compound, equipotent as the Ga(III) or Fe(III) com-

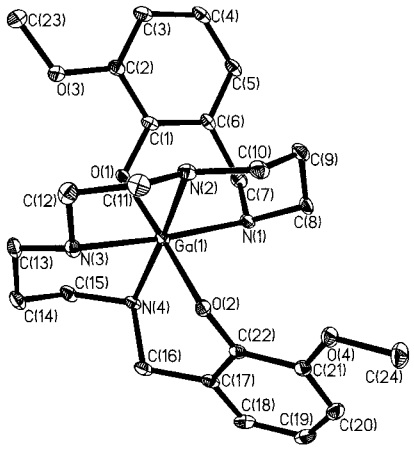


Figure 4. ORTEP drawing of the 3-methoxy-ENBPA Ga(III) cation that targets chloroquine resistant clones of *P. falciparum*. Atoms are represented by thermal ellipsoids corresponding to 20% probability.

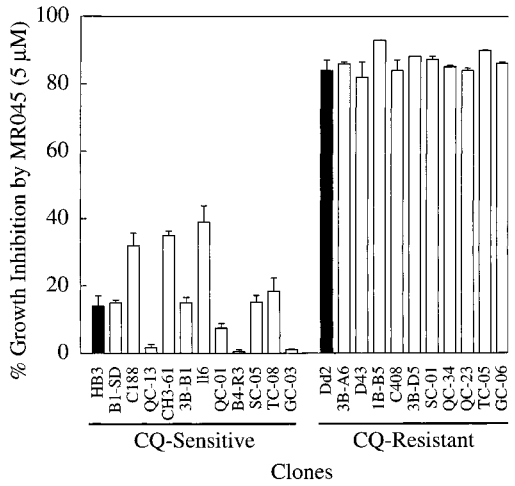


Figure 5. Effect of 3-methoxy-ENBPA Ga(III) complex (MR045) on the growth of a *P. falciparum* genetic cross in intraerythrocytic culture. Parental chloroquine-sensitive (HB3) and resistant (Dd2) clones (solid bars) and 21 independent recombinant progeny with crossovers in the chloroquine-resistance segment of chromosome 7 (open bars) were grown in the absence or presence of a saturating concentration of inhibitor (5 μM). Growth inhibition was measured by the [^{3}H]hypoxanthine incorporation assay. Data represent percent growth inhibition presented as mean values of 3−6 determinations; error bars represent + SEM. Data are replotted from ref 160.

plex, was shown to possess a pseudo-octahedral environment for the N_4O_2 donor core (Figure 4) and inhibited the same vital target as chloroquine, heme polymerization, with an IC_{50} of 0.5 μM. Curiously, in a genetic cross of 21 independent recombinant progeny, the susceptibility of this complex mapped in perfect linkage with the chloroquine resistance phenotype (Figure 5), suggesting that a locus for susceptibility to this compound was located on the same 36 kilobase segment of chromosome 7 previously identified as the chloroquine-resistance determinant.[160] This scaffold offers an interesting template for the development of antimalarial metal complexes that selectively target chloroquine resistance and, in addition, may be useful as a novel probe of chloroquine resistance mechanisms in *P. falciparum*.

III. Metal Complexes and Drug Resistance in Tropical Diseases: Leishmaniasis

A. Introduction

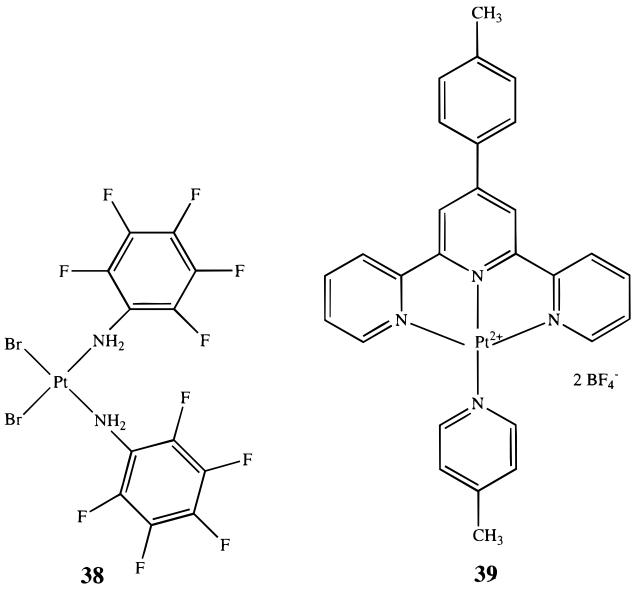
Leishmaniasis is a significant cause of morbidity and mortality worldwide. Approximately 10−15 million people are estimated to be infected with *Leishmania* species.[163−165] The vertebrate host becomes infected with flagellated extracellular promastigote via the bite of the sandfly. Promastigotes rapidly transform into nonflagellated amastigotes which divide within the mononuclear phagocytes of the vertebrate host. Among the three forms of leishmaniasis, cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis, the latter is the most dangerous and is often fatal if untreated.

In the chemotherapy of leishmanisis, pentamidine, amphotericin B, aminosidine, and antimonials such as sodium stibogluconate and meglumine antimoniate are among the primary drugs of choice in treatment regimens. Antimonials also have been encapsulated in liposomes and these are reported to be 700 times more active when compared with the free compounds.[166] Due to the emergence of resistance to pentavalent antimonials in mucocutaneous leishmaniasis and visceral leishmaniasis,[167] efforts are continuing in the pursuit of alternative therapeutic choices. In this section, we present a few examples of metal complexes that have been evaluated for their efficacy in various species of *Leishmania*.

B. Metal Complexes for Treatment of Leishmaniasis

Several scaffolds of metal complexes known to possess antitumor properties also have been tested in tropical diseases. Among these, several rhodium(I), rhodium(III), and rhodium(IV), as well as antimony(III), metal complexes have been synthesized and evaluated for growth inhibition of *Leishmania donovani*.[168] Rhodium(III) cationic complexes containing 2-hydroxybenzothiazole ligands and antimony(III) complexes containing *N*,*N*′-piperazinedithiocarbamate ligands inhibited 100% growth of *L. donovani*. Similarly, an osmium(III) complex containing a 2,4-dinitroimidazole dithiocarbamate ligand[169] was shown to inhibit growth of *L. donovani* after 48 h. Recently, another class of organometallic complexes of platinum has been derived[170] and tested in vitro against promastigote forms of *L. donovani*. Of these, platinum(2,3,4,5,6-pentafluoroaniline)$_2$Br$_2$ **38** (Chart 7) and platinum−pentamidine−I_2 were found to be extremely potent. Terpyridine−platinum complexes synthesized earlier had been shown to bind double-stranded DNA through intercalation.[171] Recently, several 2,2′:6′2″-terpyridine−platinum(II) complexes **39** were obtained, and the lead compounds were shown to cause almost 100% growth inhibition of intracellular amastigote forms of *L. donovani*.[172] The complexes were proposed to be capable of platinating DNA, based upon the activity profile of related compounds.

Chart 7



IV. Summary

Conventionally, medicinal chemistry within the context of pharmaceutical research has been primarily focused on organic compounds with therapeutic efficacy. However, the emergence of drug resistance as a worldwide problem in several diseases, specifically in cancer and tropical diseases, makes it mandatory for scientists to broaden the domain of available therapies. Herein, bioinorganic complexes have been explored which provide diversity and unique scaffolds for potential exploitation of therapeutic effect. Several radiopharmaceuticals incorporating γ-emitting metals, exemplified by [^{99m}Tc]sestamibi, have been validated as diagnostic tumor markers for the identification of cancer patients exhibiting the multidrug resistance phenotype and may provide information to assist optimal chemotherapeutic treatments. Several organometallic compounds, led by metallocene derivatives, offer interesting alternatives to chloroquine and its analogues in the chemotherapy of malaria. These metal complexes are extremely potent against both chloroquine resistant and sensitive strains and have demonstrated encouraging in vivo activity. Other Schiff-base phenolic complexes of gallium(III) and iron(III) offer unique templates for examining the molecular mechanism(s) of chloroquine resistance in *P. falciparum* strains. In addition, selected metal complexes have shown encouraging results in the growth inhibition of drug resistant *L. donovani*. Thus, application of metal complexes to the therapy and diagnosis of drug resistance promises to be an area of intense investigation into the future.

V. Acknowledgments

The authors thank all our colleagues and collaborators (both past and present) for their contributions that led to successful execution of this review. Financial assistance for work from the Laboratory of Molecular Radiopharmacology was provided by the U.S. Department of Energy (DE-FG02-94ER61885) and the National Institutes of Health (RO1 CA65735 and R21 AI45640).

VI. References

(1) Ling, V.; Thompson, L. H. *J. Cell Physiol.* **1974**, *83*, 103−111.
(2) Gerlach, J. H.; Endicott, J. A.; Juranka, P. F.; Henderson, G.; Sarangi, F.; Deuchars, K. L.; Ling, V. *Nature* **1986**, *324*, 485−489.
(3) Gros, P.; Ben Neriah, Y.; Croop, J. M.; Housman, D. E. *Nature* **1986**, *323*, 728−731.
(4) Endicott, J. A.; Ling, V. *Annu. Rev. Biochem.***1989**, *58*, 137−171.
(5) Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.* **1993**, *62*, 385−427.
(6) Bosch, I.; Croop, J. *Biochim. Biophys. Acta* **1996**, *1288*, F37−F54.
(7) Ames, G. *Annu. Rev. Biochem.* **1986**, *55*, 397−425.
(8) Gros, P.; Dhir., R.; Croop, J.; Talbot, F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7289−7293.
(9) Bruggemann, E. P.; Germann, U. A.; Gottesman, M. M.; Pastan, I. *J. Biol. Chem.* **1989**, *264*, 15483−15488.
(10) Yoshimura, A.; Kuwazuru, Y.; Sumizawa, T.; Ichikawa, M.; Ikeda, S.; Uda, T.; Akiyama, S. *J. Biol. Chem.* **1990**, *264*, 16282.
(11) Raviv, Y.; Pollard, H.; Bruggemann, E.; Pastan, I.; Gottesman, M. *J. Biol. Chem.* **1990**, *265*, 3975.
(12) Higgins, C. F.; Gottesman, M. M. *Trends Biochem. Sci.* **1992**, *17*, 18−21.
(13) Hoffman, M.; Wei, L.; Roepe, P. *J. Gen. Physiol.* **1996**, *108*, 295−313.
(14) Hoffman, M.; Roepe, P. *Biochemistry* **1997**, *36*, 11153−11168.
(15) Altan, N.; Chen, Y.; Schindler, M.; Simon, S. *J. Exp Med.* **1998**, *187*, 1583−1598.
(16) Hurwitz, S.; Terashima, M.; Mizunuma, N.; Slapak, C. *Blood* **1997**, *89*, 3745−3754.
(17) Robinson, L.; Roberts, W.; Ling, T.; Lamming, D.; Sternberg, S.; Roepe, P. *Biochemistry* **1997**, *36*, 11169−11178.
(18) Smyth, M. J.; Krasovskis, E.; Sutton, V. R.; Johnstone, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7024−7029.
(19) Thiebaut, F.; Tsuruo, T.; Hamada, H.; Gottesman, M. M.; Pastan, I.; Willingham, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7735−7738.
(20) Hitchins, R. N.; Harman, D. H.; Davey, R. A.; Bell, D. R. *Eur. Proc. Natl. Acad. Sci. Cancer Clin. Oncol.* **1988**, *24*, 449−54.
(21) Cordon-Cardo, C.; OBrien, J. P.; Casals, D.; Rittman, G. L.; Biedler, J. L.; Melamed, M. R.; Bertino, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 695−698.
(22) Croop, J. M.; Raymond, M.; Haber, D.; Devault, A.; Arceci, R. J.; Gros, P.; Housman, D. E. *Mol. Cell Biol.* **1989**, *9*, 1346−1350.
(23) Rao, V.; Dahlheimer, J.; Bardgett, M.; Snyder, A.; Finch, R.; Sartorelli, A.; Piwnica-Worms, D. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3900−3905.
(24) Schinkel, A.; Mayer, U.; Wagenaar, E.; Mol, C.; van Deemer, L.; Smit, J.; van der Valk, M.; Voordouw, A.; Spits, H.; van Tellingen, O.; Zijlmans, J.; Fibbe, W.; Borst, P. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4028−4033.
(25) Luker, G.; Nilsson, K.; Covey, D.; Piwnica-Worms, D. *J. Biol. Chem.* **1999**, *274*, 6979−6991.
(26) Ford, J. M.; Hait, W. N. *Pharmacol. Rev.* **1990**, *42*, 155−199.
(27) Sandor, V.; Fojo, T.; Bates, S. *Drug Resistance Updates* **1998**, *1*, 190−200.
(28) Trock, B.; Leonessa, F.; Clarke, R. *J. Natl. Cancer Inst.* **1997**, *89*, 917−931.
(29) Beck, W. T. *Eur. J. Cancer* **1990**, *26*, 513−515.
(30) Wilson, W.; Jamis-Dow, C.; Bryant, G.; Balis, F.; Klecker, R.; Bates, S.; Chabner, B.; Steinberg, S.; Kohler, D.; Wittes, R. *J. Clin. Oncol.* **1995**, *13*, 1985−1994.
(31) Gaveriaux, C.; Boesch, D.; Jachez, B. *J. Cell. Pharmacol.* **1991**, *2*, 225−234.
(32) Hyafil, F.; Vergely, C.; Du Vignaud, P.; Grand-Perret, T. *Cancer Res.* **1993**, *53*, 4595−4602.
(33) Dantzig, A.; Shepard, R.; Cao, J.; Law, K.; Ehlhardt, W.; Baughman, T.; Bumol, T.; Starling, J. *Cancer Res.* **1996**, *56*, 4171−4179.
(34) Germann, U.; Ford, P.; Schlakhter, D.; Mason, V.; Harding, M. *Anticancer Drugs* **1997**, *8*, 141−155.
(35) Germann, U. A.; Willingham, M. C.; Pastan, I.; Gottesman, M. M. *Biochemistry* **1990**, *29*, 2295−2303.
(36) Ahmad, S.; Safa, A. R.; Glazer, R. I. *Biochemistry* **1994**, *33*, 10313−10318.
(37) Homolya, L.; Hollo, Z.; Germann, U. A.; Pastan, I.; Gottesman, M. M.; Sarkadi, B. *J. Biol. Chem.* **1993**, *268*, 21493−21496.
(38) Abrams, M. A.; Davison, A.; Jones, A. G.; Costello, C. E.; Pang, H. *Inorg. Chem.* **1983**, *22*, 2798−2800.
(39) Wackers, F. J.; Berman, D.; Maddahi, J.; Beller, G.; Straus, H.; Boucher, C.; Picard, M.; Holman, B.; Fridrich, R. *J. Nucl. Med.* **1989**, *30*, 301−309.

(40) Piwnica-Worms, D.; Chiu, M. L.; Budding, M.; Kronauge, J. F.; Kramer, R. A.; Croop, J. M. *Cancer Res.* **1993**, *53*, 977−984.
(41) Tulip, T.; Calabrese, J.; Kronauge, J.; Davison, A.; Jones, A. In, Technetium in Chemistry and Nuclear Medicine 2; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, **1986**; 119.
(42) Kronauge, J. F.; Davison, A.; Roseberry, A. M.; Costello, C. E.; Maleknia, S.; Jones, A. G. *Inorg. Chem.* **1991**, *30*, 4265−4271.
(43) Backus, M.; Piwnica-Worms, D.; Hockett, D.; Kronauge, J.; Lieberman, M.; Ingram, P.; LeFurgey, A. *Am. J. Physiol. (Cell)* **1993**, *265*, C178−C187.
(44) Piwnica-Worms, D.; Rao, V.; Kronauge, J.; Croop, J. *Biochemistry* **1995**, *34*, 12210−12220.
(45) Ballinger, J.; Hua, H.; Berry, B.; Firby, P.; Boxen, I. *Nucl. Med. Commun.* **1995**, *16*, 253−257.
(46) Ballinger, J. R.; Sheldon, K. M.; Boxen, I.; Erlichman, C.; Ling, V. *Q J. Nucl. Med.* **1995**, *39*, 122−128.
(47) Cordobes, M.; Starzec, A.; Delmon-Moingeon, L.; Blanchot, C.; Kouyoumdjian, J.-C.; Prevost, G.; Caglar, M.; Moretti, J.-L. *J. Nucl. Med.* **1996**, *37*, 286−289.
(48) Luker, G.; Rao, V.; Crankshaw, C.; Dahlheimer, J.; Piwnica-Worms, D. *Biochemistry* **1997**, *36*, 14218−14227.
(49) Bosch, I.; Crankshaw, C.; Piwnica-Worms, D.; Croop, J. *Leukemia* **1997**, *11*, 1131−1137.
(50) Barbarics, E.; Kronauge, J.; Cohen, D.; Davison, A.; Jones, A.; Croop, J. *Cancer Res.* **1998**, *58*, 276−282.
(51) Crankshaw, C.; Piwnica-Worms, D. *J. Nucl. Med.* **1996**, *37*, 247P.
(52) Hendrikse, N.; Franssen, E.; van der Graaf, W.; Meijer, C.; Piers, D.; Vaalburg, W.; de Vries, E. *Br. J. Cancer* **1998**, *77*, 353−358.
(53) Moretti, J.-L.; Cordobes, M.; Starzec, A.; de Beco, V.; Vergote, J.; Benazzouz, F.; Boissier, B.; Cohen, H.; Safi, N.; Piperno-Neumann, S.; Kouyoumdjian, J.-C. *J. Nucl. Med.* **1998**, *39*, 1214−1218.
(54) Cole, S. P. C.; Bhardwaj, G.; Gerlach, J. H.; Mackie, J. E.; Grant, C. E.; Almquist, K. C.; Stewart, A. J.; Kurz, E. U.; Duncan, A. M. V.; Deeley, R. G. *Science* **1992**, *258*, 1650−1654.
(55) Del Vecchio, S.; Ciarmiello, A.; Potena, M. I.; Carriero, M. V.; Mainolfi, C.; Botti, G.; Thomas, R.; Cerra, M.; D′Aiuto, G.; Tsuruo, T.; Salvatore, M. *Eur. J. Nucl. Med.* **1997**, *24*, 150−159.
(56) Del Vecchio, S.; Ciarmiello, A.; Pace, L.; Potena, M.; Carriero, M.; Mainolfi, C.; R, T.; D'Aiuto, G.; Tsuruo, T.; Salvatore, M. *J. Nucl. Med.* **1997**, *38*, 1348−1351.
(57) Chen, C.; Meadows, B.; Regis, J.; Kalafsky, G.; Fojo, T.; Carrasquillo, J.; Bates, S. *Clin. Cancer Res.* **1997**, *3*, 545−552.
(58) Luker, G. D.; Fracasso, P. M.; Dobkin, J.; Piwnica-Worms, D. *J. Nucl. Med.* **1997**, *38*, 369−372.
(59) Bom, H.; Kim, Y.; Lim, S.; Park, K. *J. Nucl. Med.* **1997**, *38*, 240P.
(60) Kostakoglu, L.; Elahi, N.; Kirarli, P.; Ruacan, S.; Sayek, S.; Baltali, E.; Sungur, A.; Hayran, M.; Bekdik, C. *J. Nucl. Med.* **1994**, *38*, 1003−1008.
(61) Kostakoglu, L.; Kirath, P.; Ruacan, S.; Hayran, M.; Emri, S.; Ergun, E.; Bekdik, C. *J. Nucl. Med.* **1998**, *39*, 228−234.
(62) Ciarmiello, A.; Del Vecchio, S.; Silvestro, P.; Potena, M. I.; Carriero, M. V.; Thomas, R.; Botti, G.; D'Aiuto, G.; Salvatore, M. *J. Clin. Oncol.* **1998**, *16*, 1677−1683.
(63) Piwnica-Worms, D.; Kronauge, J. *J. Nucl. Med.* **1994**, *35*, 230P.
(64) Herman, L. W.; Sharma, V.; Kronauge, J. F.; Barbarics, E.; Herman, L. A.; Piwnica-Worms, D. *J. Med. Chem.* **1995**, *38*, 2955−2963.
(65) Jurisson, S.; Berning, D.; Jia, W.; Ma, D.-S. *Chem. Rev.* **1993**, *93*, 1137−1156.
(66) Deutsch, E.; Vanderheyden, J.-L.; Gerundini, P.; Libson, K.; Hirth, W.; Colombo, F.; Savi, A.; Fazio, F. *J. Nucl. Med.* **1987**, *28*, 1870−1880.
(67) Rossetti, C.; Vanoli, G.; Paganelli, G.; Kwiatkowski, M.; Zito, F.; Colombo, F.; Bonino, C.; Carpinelli, A.; Casati, R.; Deutsch, K.; Marmion, M.; Woulfe, S. R.; Lunghi, F.; Deutsch, E.; Fazio, F. *J. Nucl. Med.* **1994**, *35*, 1571−1580.
(68) Crankshaw, C.; Marmion, M.; Luker, G.; Rao, V.; Dahlheimer, J.; Burleigh, B.; Webb, E.; Deutsch, K.; Piwnica-Worms, D. *J. Nucl. Med.* **1998**, *39*, 77−86.
(69) Woulfe, S.; Dunn, T.; Marmion, M.; MacDonaold, J.; Rogic, M.; Deutsch, E. *J. Nucl. Med.* **1991**, *32*, 1101.
(70) Jurisson, S. S.; Dancey, K.; McPartlin, M.; Tasker, P. A.; Deutsch, E. *Inorg. Chem.* **1984**, *23*, 4743−4749.
(71) Kelly, J.; Forster, A.; Higley, B.; Archer, C.; Booker, F.; Canning, L.; Chiu, K.; Edwards, B.; Gill, H.; McPartlin, M.; Nagle, K.; Latham, I.; Pickett, R.; Storey, A.; Webbon, P. *J. Nucl. Med.* **1993**, *34*, 222−227.
(72) Ballinger, J. R.; Bannerman, J.; Boxen, I.; Firby, P.; Hartman, N. G.; Moore, M. J. *J. Nucl. Med.* **1996**, *37*, 1578−1582.
(73) Wong, E.; Liu, S.; Lugger, T.; Hahn, F. E.; Orvig, C. *Inorg. Chem.* **1995**, *34*, 93−101.
(74) Yang, L. W.; Liu, S.; Wong, E.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1995**, *34*, 2164−2178.
(75) Sarma, B. D.; Bailar, J. C. *J. Am. Chem. Soc.* **1955**, *77*, 5476−5480.
(76) Sarma, B. D.; Ray, K. R.; Sievers, R. E.; Bailar, J. C. *J. Am. Chem. Soc.* **1964**, *86*, 14−16.
(77) Tweedle, M. F.; Wilson, L. J. *J. Am. Chem. Soc.* **1976**, *98*, 4824−4834.
(78) Tsang, B. W.; Mathias, C. J.; Green, M. A. *J. Nucl. Med.* **1993**, *34*, 1127−1131.
(79) Tsang, B. W.; Mathias, C. J.; Fanwick, P. E.; Green, M. A. *J. Med. Chem.* **1994**, *37*, 4400−4406.
(80) Sharma, V.; Crankshaw, C.; Piwnica-Worms, D. *J. Med. Chem.* **1996**, *39*, 3483−3490.
(81) Sharma, V.; Wey, S. P.; Bass, L.; Crankshaw, C. L.; Green, M. A.; Welch, M. J.; Piwnica-Worms, D. *J. Nucl. Med.* **1996**, *37*, 51P.
(82) Anderson, O.; Packard, A. *Inorg. Chem.* **1980**, *19*, 2941−2945.
(83) Anderson, O.; Packard, A. *Inorg. Chem.* **1979**, *18*, 1940−1947.
(84) Packard, A.; Barbarics, E.; Kronauge, J.; Treves, S.; Jones, A. 215th American Chemical Society Meeting, Dallas, TX 1998.
(85) Lewis, J.; Zweit, J.; Dearling, J.; Rooney, B.; Blower, P. *Chem. Commun.* **1996**, 1093−1094.
(86) Berners-Price, S.; Johnson, R.; Mirabelli, C.; Faucette, L.; McCabe, F.; Sadler, P. *Inorg. Chem.* **1987**, *26*, 3383−3387.
(87) Camus, A.; Marsich, N.; Nardin, G. *Transition Met. Chem.* **1976**, *1*, 205.
(88) Blower, P. *Transition Met. Chem.* **1998**, *23*, 109−112.
(89) Zweit, J.; Lewis, J.; Dearling, J.; Rooney, B.; Coley, H.; Kelland, L. *J. Nucl. Med.* **1997**, *38*, 133P.
(90) Mehta, B. M.; Rosa, E.; Fissekis, J. D.; Bading, J. R.; Biedler, J. L.; Larson, S. M. *J. Nucl. Med.* **1992**, *33*, 1373−1377.
(91) Mehta, B.; Rosa, E.; Biedler, J.; Larson, S. *J. Nucl. Med.* **1994**, *35*, 1179−1184.
(92) Elsinga, P. H.; Franssen, J. F.; Hendrikse, N. H.; Fluks, L.; Weemaes, A.-M. A.; van der Graaf, W. T. A.; de Vries, E. G. E.; Visser, G. M.; Vaalburg, W. *J. Nucl. Med.* **1996**, *37*, 1571−1575.
(93) Hendrikse, N.; Franssen, E.; van der Graaf, W.; Vaalburg, W.; de Vries, E. *Eur J. Nucl. Med.* **1999**, *26*, 283−293.
(94) Polyakov, V. R.; Sharma, V.; Crankshaw, C. L.; Piwnica-worms, D. *Inorg. Chem.* **1998**, *37*, 4740−4742.
(95) Rao, V.; LW, H.; Kronauge, J.; Piwnica-Worms, D. *Nucl. Med. Biol.* **1998**, *25*, 225−232.
(96) Chambers, T. C.; McAvoy, E. M.; J. W., J.; Eilon, G. *J. Biol. Chem.* **1990**, *265*, 7679−7686.
(97) Yu, G.; Ahmad, S.; Aquino, A.; Fairchild, C. R.; Trepel, J. B.; Ohno, S.; Suzuki, K.; Tsuruo, T.; Cowan, K. H.; Glazer, R. I. *Cancer Commun.* **1991**, *3*, 181−189.
(98) Tsuruo, T.; Lida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1982**, *42*, 4730−4733.
(99) Nasca, J.; NAgy, L.; Sharples, D.; Hever, A.; Szabo, D.; Ocsovski, I.; Varga, A.; Konig, S.; Molnar, J. *Anticancer Res.* **1998**, *18*, 3093−3098.
(100) Nagy, L.; Korecz, L.; Kiricsi, I.; Zsikla, L.; Burger, K. *Struct. Chem.* **1991**, *2*, 231−238.
(101) Selig, R.; White, L.; Gramacho, C.; Sterling-Levis, K.; Fraser, I.; Naidoo, D. *Cancer Res.* **1998**, *58*, 473−478.
(102) Top, S.; Tang, J.; Vessieres, A.; Carrez, D.; Provot, C.; Jaouen, G. *Chem. Commun.* **1996**, 955−956.
(103) Sturchler, D. *Parasitol. Today* **1989**, *5*, 39−40.
(104) Foley, M.; Tilley, L. *Parasitol. Today* **1995**, *11*, 436−439.
(105) Wernsdorfer, W.; McGregor, I. (Eds.) *Malaria: Principles and Practice of Malariology*; Churchill Livingstone, New York, 1988; p 1818.
(106) Grau, G.; De Kossodo, S. *Parasitol. Today* **1994**, *10*, 408−409.
(107) Olliaro, P.; Cattani, J.; Wirth, D. *J. Am Med. Assoc.* **1996**, *275*, 230−233.
(108) Muller, R.; Baker, J. R. *Medical Parasitology*; Gower Medical Publishing: London, **1990**.
(109) Veignie, E.; Moreau, S. *Ann. Trop. Med. Parasitol.* **1991**, *85*, 229−237.
(110) Krogstad, D. J.; Gluzman, I. Y.; Herwaldt, B. L.; Schlesinger, P. H.; Wellems, T. E. *Biochem. Pharmacol.* **1992**, *43*, 57−62.
(111) Krogstad, D. J.; Schlesinger, P. H. *Am. J. Trop. Med. Hyg.* **1987**, *36*, 213−220.
(112) Goldberg, D. E.; Slater, A. F. G. *Parasitol. Today* **1992**, *8*, 280−283.
(113) Sullivan, D.; Gluzman, I.; Goldberg, D. *Science* **1996**, *271*, 219−222.
(114) Dorn, A.; Stoffel, R.; Matile, H.; Bubendorf, A.; Ridley, R. *Nature* **1995**, *374*, 269−271.
(115) Slater, A.; Swiggard, W.; Orton, B.; Flitter, W.; Goldberg, D.; Cerami, A.; Henderson, G. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 325−329.(1) Ling, V.; Thompson, L. H. *Proc. Natl. Acad. Sci.Cell Physiol* 1974, *83*, 103−111.
(116) Slater, A. F.; Cerami, A. *Nature (London)* **1992**, *355*, 167−169.
(117) Bendrat, K.; Berger, B.; Cerami, A. *Nature* **1995**, *378*, 138.
(118) Ridley, R.; Dorn, A.; Matile, H.; Kansy, M. *Nature* **1995**, *378*, 138−139.
(119) Ridley, R.; Dorn, A.; Vippagunta, S.; Vennerstrom, J. *Ann. Trop. Med. Parasit.* **1997**, *91*, 559−566.
(120) Dorn, A.; Vippagunta, S.; Matile, H.; Bubendorf, A.; Vennerstrom, J.; Ridley, R. *Biochem. Pharmacol.* **1998**, *55*, 737−747.

(121) Fitch, C.; Chevli, R.; Banyal, H.; Phillips, G.; Pfallar, M.; Krogstad, D. *Antimicrob. Agents Chemother.* **1982**, *21*, 819−822.
(122) van Es, H. H.; Skamene, E.; Schurr, E. *Clin. Invest. Med.* **1993**, *16*, 285−293.
(123) Krogstad, D. J.; Gluzman, I. Y.; Kyle, D. E.; Oduola, A. M.; Martin, S. K.; Milhous, W. K.; Schlesinger, P. H. *Science* **1987**, *238*, 1283−1285.
(124) Ginsburg, H.; Stein, W. D. *Biochem. Pharmacol.* **1991**, *41*, 1463−1470.
(125) Foote, S. J.; Thompson, J. K.; Cowman, A. F.; Kemp, D. J. *Cell* **1989**, *57*, 921−930.
(126) Cowman, A. F.; Karcz, S.; Galatis, D.; Culvenor, J. G. *J. Cell Biol.* **1991**, *113*, 1033−1042.
(127) Ekong, R. M.; Robson, K. J. H.; Baker, D. A.; Warhurst, D. E. *Parasitology* **1993**, *106*, 107−115.
(128) Wellems, T. E.; Panton, L. J.; Gluzman, I. Y.; do Rosario, V. E.; Gwadz, R. W.; Walker, J. A.; Krogstad, D. J. *Nature* **1990**, *345*, 253−255.
(129) Wellems, T. E.; Walker-Jonah, A.; Panton, L. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3382−3386.
(130) Su, X.; Kirkman, L. A.; Fujioka, H.; Wellems, T. E. *Cell* **1997**, *91*, 593−603.
(131) Sanchez, C.; Wunsch, S.; Lanzer, M. *J. Biol. Chem.* **1997**, *272*, 2652−2658.
(132) Sanchez, C. P.; Paul, H.; Lanzer, M. *Cell* **1998**, *92*, 601−602.
(133) Wellems, T. E.; Wootton, J. C.; Fujioka, H.; Su, X.; Cooper, R.; Baruch, D.; Fidock, D. A. *Cell* **1998**, *94*, 285−286.
(134) Mabeza, G.; Loyevsky, M.; Gordeuk, V.; Weiss, G. *Pharmacol. Ther.* **1999**, *81*, 53−75.
(135) Pollack, S.; Rossan, R.; Davidson, D.; Escajadillo, A. *Proc. Soc. Exp. Biol. Med.* **1987**, *184*, 162−164.
(136) Stahel, E.; Mazier, D.; Guillouzo, A.; Miltgen, F.; Landau, I.; Mellouk, S.; Beaudoin, R.; Langlois, P.; Gentilini, M. *Am. J. Trop. Med. Hygiene* **1988**, *39*, 236−40.
(137) Hershko, C.; Peto, T. *J. Exp. Med.* **1988**, *168*, 375−87.
(138) Gordeuk, V.; Thuma, P.; Brittenham, G.; Biemba, G.; Zulu, S.; Simwanza, G.; Kalense, P.; M′Hango, A.; Parry, D.; Poltera, A.; Aikawa, M. *Am. J. Trop. Med. Hygiene* **1993**, *48*, 193−197.
(139) Yinnon, A.; Theanacho, E.; Grady, R.; Spira, D.; Hershko, C. *Blood* **1989**, *74*, 2166−2171.
(140) Hershko, C.; Gordeuk, V.; Thuma, P.; Theanacho, E.; Spira, D.; Hider, R.; Peto, T.; Brittenham, G. *J. Inorg. Biochem.* **1992**, *47*, 267−277.
(141) Lytton, S.; Cabantchik, Z.; Libman, J.; Shanzer, A. *Mol. Pharma.* **1991**, *40*, 584−590.
(142) Shanzer, A.; Libman, J.; Lytton, S. D.; Glickstein, H.; Cabantchik, Z. I. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6585−6589.
(143) Lytton, S.; Loyevsky, M.; Mester, B.; Libman, J.; Landau, I.; Shanzer, A.; Cabantchik, Z. *Am. J. Hematol.* **1993**, *43*, 217−220.
(144) van Zyl, R.; Havlik, I.; Hempelmann, E.; MacPhail, A.; McNamara, L. *Biochem. Pharmacol.* **1993**, *45*, 1431−1436.
(145) Kung, H.; Molnar, M.; Billings, J.; Wicks, R.; Blau, M. *J. Nucl. Med.* **1984**, *25*, 326−332.
(146) John, C.; Costello, C.; Schlemper, E. *Polyhedron* **1992**, *11*, 2651−2655.
(147) Loyevsky, M.; John, C.; Zaloujnyi, I.; Gordeuk, V. *Biochem. Pharmacol.* **1997**, *54*, 451−458.
(148) Keberle, H. *Ann. N.Y. Acad. Sci.* **1964**, *119*, 758−768.
(149) Chevion, M.; Chuang, L.; Golenser, J. *Antimicrob. Agents Chemother.* **1995**, *39*, 1902−1905.
(150) Abrams, M.; Murrer, B. *Science* **1993**, *261*, 725−730.
(151) Herde, J.; Lambert, J.; Senoff, C. *Inorg. Synth.* **1974**, *15*, 19.
(152) Sanchez-Delgado, R.; Navarro, M.; Perez, H.; Urbina, J. *J. Med. Chem.* **1996**, *39*, 1095−1099.
(153) McAuliffe, C.; Parish, R.; Randall, P. *J. Chem. Soc., Dalton Trans* **1979**, 1730−1735.
(154) Navarro, M.; Perez, H.; Sanchez-Delgado, R. *J. Med. Chem.* **1997**, *40*, 1937−1939.
(155) Wang, K.; Munoz, S.; Zhang, L.; Castro, R.; Kaifer, A.; Gokel, G. *J. Am. Chem. Soc.* **1996**, *118*, 6707−6715.
(156) Plesske, K. *Angew. Chem.* **1962**, *1*, 312−327.
(157) Plesske, K. *Angew. Chem.* **1962**, *1*, 394−399.
(158) Biot, C.; Glorian, G.; Maciejewski, L.; Brocard, J. *J. Med. Chem.* **1997**, *40*, 3715−3718.
(159) Domarle, O.; Blampain, G.; Agnaneit, H.; Nzadiyabi, T.; Lebibi, J.; Brocard, J.; Maciejewski, L.; Biot, C.; Georges, A.; Millet, P. *Antimicrob. Agents Chemother.* **1998**, *42*, 540−544.
(160) Goldberg, D. E.; Sharma, V.; Oksman, A.; Gluzman, I. Y.; Wellems, T. E.; Piwnica-Worms, D. *J. Biol. Chem.* **1997**, *272*, 6567−6572.
(161) Posner, G.; Cumming, J.; Ploypradith, P.; Oh, C. *J. Am. Chem. Soc.* **1995**, *117*, 5885−5886.
(162) Sharma, V.; Beatty, A.; Goldberg, D. E.; Piwnica-Worms, D. *J. Chem. Soc. Chem. Commun.* **1997**, 2223−2224.
(163) Croft, S. *Trends Pharmacol. Sci.* **1988**, *9*, 376−381.
(164) Berman, J. *J. Infect. Dis.* **1988**, *10*, 560−586.
(165) Ashford, R.; Desjeux, P.; deRaadt, P. *Parasitol. Today* **1992**, *8*, 104−105.
(166) Alving, C.; Steck, E.; Chapman, W.; Waits, V.; Hendricks, L.; Swartz, G.; Hanson, W. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2959−2963.
(167) Ouellette, M.; Papadopoulou, B. *Parasitol. Today* **1993**, *9*, 150−153.
(168) Mesa-Valle, C.; Moraleda-Lindez, V.; Craciunescu, D.; Alonso, M.; Osuna, A. *Arzneim.-Forsch./Drug Res.* **1993**, *43*, 1010−1013.
(169) Castilla, J.; Mesa-Valle, C.; Sanchez-Moreno, M.; Arnedo, T.; Rosales, M.; Mascaro, C.; Craciunescu, D.; Osuna, A. *Arzneim.-Forsch./Drug Res.* **1996**, *46*, 990−996.
(170) Mesa-Valle, C.; Moraleda, V.; Lazuen, J.; Craciunescu, D.; Osuna, A. *J. Antimicrob. Chemother.* **1997**, *40*, 47−57.
(171) Jennette, K.; Lippard, S.; Vassiliades, G.; Bauer, W. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3839−3843.
(172) Lowe, G.; Droz, A.; Vilaivan, T.; Weaver, G.; Tweedale, L.; Pratt, J.; Rock, P.; Yardley, V.; Croft, S. *J. Med. Chem.* **1999**, *42*, 999−1006.

CR980429X