

# Medicinal Inorganic Chemistry Approaches to Passivation and Removal of Aberrant Metal Ions in Disease

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## 1. Introduction

The field of medicinal inorganic chemistry has evolved with three conceptual aims: the introduction of metal ions to the biological system, manipulation and redistribution of metal ions within the system, and removal of metal ions from the system. This review focuses on the latter two goals: binding of metal ions for redistribution or removal. Metal ions play a pivotal role in the development and pathology of a range of conditions and, in some cases, are implicated in redox chemistry leading to oxidative stress. This review places particular focus on Alzheimer's disease (AD) with additional coverage of Parkinson's disease (PD), Friedreich's ataxia (FRDA), transfusion-related iron overload, and Wilson's disease (WD). All of these conditions involve elevated levels of metal ions in particular tissues or cell compartments of the body and present challenges in the field of medicinal inorganic chemistry to present, among other possible interventions, new chelators for therapeutic application.

This paper will review the development and testing over the past 20 years of therapeutic chelators for the aforementioned conditions. Only those molecules that have been tested for applicability to the target condition on *in vitro* disease models or those that at the highest stage of development are in clinical use have been included in this review. Finally, the future of the design and utility of metal chelating drugs for disease therapy will be addressed; this will include the development of therapeutics for amyotrophic lateral sclerosis (ALS), transmissible spongiform encephalopathies (TSEs), cancer, and malaria, as well as a glimpse into the importance of multifunctionality in said design.

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Originally from Victoria, BC, Canada, Lauren E. Scott earned a B.Sc. (Hons.) at McMaster University in Hamilton, Ontario, Canada, in Biological Chemistry (2004). Following up on an interest in medicinal applications of synthetic inorganic chemistry, she joined the Orvig research group in 2004 and is currently a Ph.D. Candidate with Prof. Orvig at the University of British Columbia. Her research interests center on the design, synthesis, and *in vitro* testing of novel small-molecule metal ion binders as potential Alzheimer's therapeutics.

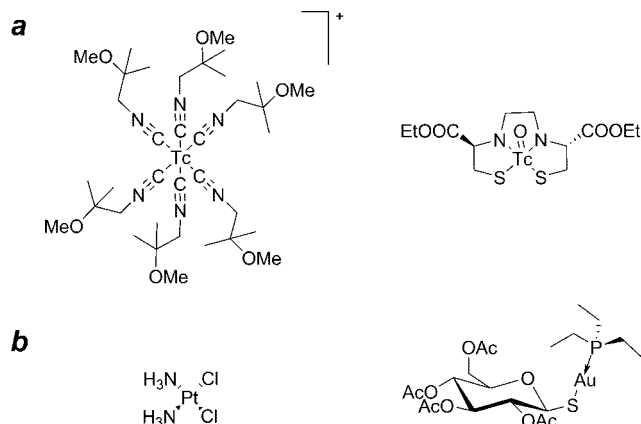


Chris Orvig was born and raised in Montreal. He received his Hons. B.Sc. in chemistry from McGill University in 1976 and subsequently completed his doctorate (as a Natural Sciences and Engineering Research Council, NSERC, of Canada scholar) in technetium chemistry at MIT with Prof. Alan Davison, FRS. After an NSERC postdoctoral fellowship with Prof. Kenneth N. Raymond at the University of California, Berkeley (1981–1983), and one year with the late Prof. Colin J. L. Lock at McMaster University, he joined the Department of Chemistry at the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences, and Director of the Medicinal Inorganic Chemistry Group, as well as graduate advisor. His scientific interests are firmly based in the areas of medicinal inorganic chemistry and coordination chemistry; he has been involved over the years with radiopharmaceutical chemistry, metal ion decorporation, and metal ion neurotoxicology, as well as chemotherapeutic metal complexes and ligands. Orvig chairs the editorial board of *Dalton Transactions*, has received various research and teaching awards, has published more than 170 research papers, and is a co-inventor on many issued patents; he is also a certified ski instructor.

## 2. Background: Metal-Based Therapies in Medicine

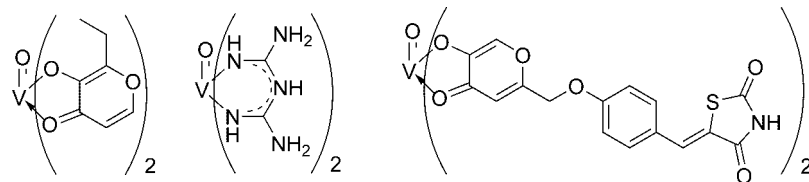
### 2.1. Introduction of Metal Ions into the Biological System: Imaging Agents, Therapeutics, and Biomolecule Mimetics

Metal complexes are introduced into the biological system as imaging agents for the diagnosis of disease. These complexes generally incorporate  $\gamma$ -emitting radionuclides for use in single-photon emission computed tomography (SPECT)

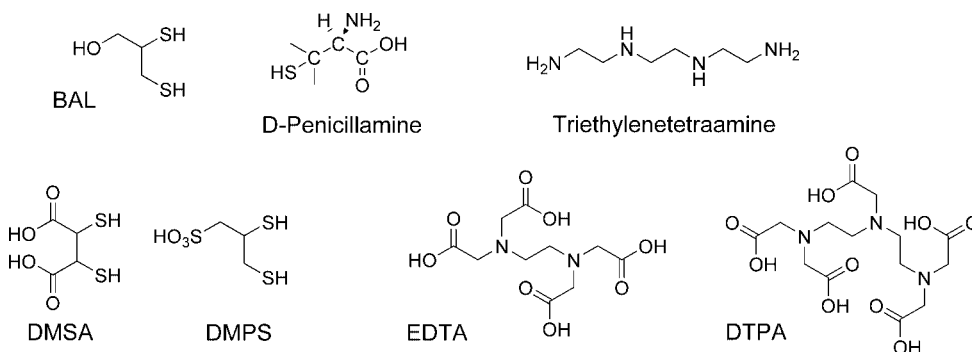


**Figure 1.** (a) First-generation imaging agents  $^{99m}\text{Tc}$ -sestamibi (Cardiolite, left) and  $^{99m}\text{Tc}$ -bicisate (Neurolite, right) are targeted to the tissue of interest by the chemical and physical properties of the overall compound.<sup>1</sup> (b) Cisplatin and auranofin for cancer and rheumatoid arthritis treatment, respectively.

or positron-emitting isotopes for positron emission tomography (PET).<sup>1</sup> So-called “first-generation” complexes are targeted to the tissue or organ of interest solely by the chemical and physical properties of the complex. For example, the technetium-based imaging agent  $^{99m}\text{Tc}$ -sestamibi (Cardiolite, Figure 1a) is lipophilic and monocationic; it is taken up by the sodium/potassium pump in hard-working heart tissue for cardiac imaging, whereas  $^{99m}\text{Tc}$ -bicisate (Neurolite, Figure 1a) is an uncharged complex and thus is capable of permeating the blood–brain barrier (BBB) for measurement of cerebral blood flow. On the other hand, “second-generation” imaging agents use an incorporated biomolecule to interact with a specific receptor within the body, resulting in preferential uptake of the complex in a certain type of organ, tissue, or cell. This bioconjugate approach requires a biomolecule, a linker, and a metal ion-binding moiety within the pro-ligand. Examples of second-generation complexes include carbohydrate-linked  $^{99m}\text{Tc}$  agents for SPECT-based cancer imaging<sup>2</sup> and antibody- or peptide-linked  $^{99m}\text{Tc}$  complexes such as  $^{99m}\text{Tc}$ -apcitide (AcuTect) or  $^{99m}\text{Tc}$ -arcitumomab (CEA-Scan) for selective imaging of deep-vein thromboses and colorectal cancer, respectively. In addition to their use as diagnostic agents, metal complexes may be introduced to the body for therapeutic use. Therapeutic radiopharmaceuticals generally incorporate  $\beta$ -particle-emitting radiometals such as  $^{90}\text{Y}$  or other lanthanides, and like their diagnostic counterparts, these complexes may be targeted to their preferred site of action by complexation with bifunctional chelators (with metal ion-binding and biological activities). Examples include the non-Hodgkin's lymphoma radiotherapeutic  $^{90}\text{Y}$ -ibritumomab tiuxetan (Zevalin), targeted to cancer cells by an incorporated monoclonal antibody.<sup>3</sup> In addition to radiotherapeutic applications, stable isotopes of transition metals are administered in complexes designed for treatment of various conditions including cancer (e.g., platinum in cisplatin) and rheumatoid arthritis (e.g., gold complexes such as auranofin, both Figure 1b). The intended localization and activity of the metal ion is achieved via the ligands imparting various physical and chemical characteristics to the complexes; cisplatin (*cis*-diamminedichloroplatinum(II), Figure 1b) hydrolyzes within cells, yielding a positively charged complex that is trapped within the cell, binds to DNA, and, through cross-linking adducts, effects antiproliferative activity (recently reviewed in the context of new platinum compound



**Figure 2.** Vanadium complexes as insulin-enhancing agents: bis(ethylmaltolato)oxovanadium(IV) (BEOV), bis(metformin),<sup>10</sup> and bis(thiazolidinedione)<sup>11</sup> complexes.



**Figure 3.** Selected pro-ligands developed for metal ion binding and removal to treat overload and intoxication conditions, 1940–1980.

development).<sup>4</sup> Auranofin ((2,3,4,6-tetra-*O*-acetyl-1-thio- $\beta$ -D-glycopyranosato-*S*)-(triethylphosphine)gold, Figure 1b) is formulated for oral availability, unlike its injectible predecessors such as gold sodium thiomalate. Though the mechanisms for therapeutic (and toxicological) effects are still unclear, auranofin is thought to effect immunosuppression by a number of different actions.<sup>5</sup> In these and other metal complexes administered for therapy, the metal ion is integral to drug activity, which is targeted and modulated by ligands.

Metal compounds may also be introduced to the biological system as biomolecule mimetics. For example, the Meggers group has developed protein kinase inhibitors using organometallic moieties as structural scaffolds for the design of biologically active compounds.<sup>6</sup> In contrast to the metal-dependent activities of radionuclide complexes or drugs such as cisplatin and auranofin, in this construct the metal ion performs no direct action in the biological system. Instead, it is incorporated into a kinetically inert coordination complex such that the metal plays the role of “innocent bystander” yet organizes the organic ligands to mimic enzyme substrates and become very high-affinity inhibitors for enzymes (for example, glycogen synthase kinase 3).<sup>7</sup> A similar use of biologically active inorganic complexes uses vanadium compounds for enhancement (often misnamed mimicry) of the effect of a larger biomolecule (insulin) for therapy of diabetes mellitus. Phosphate ( $[\text{PO}_4]^{3-}$ ) and vanadate ( $[\text{VO}_4]^{3-}$ ) are chemically similar. Thus, vanadate can enter into cell signaling cycles and replicate the overall effect of insulin, increasing glucose transport and oxidation, stimulating glycogen synthesis in the liver, and inhibiting glucose synthesis.<sup>8</sup> Development of these vanadium insulin therapeutics has involved complexation of vanadyl with maltol or ethylmaltol to improve bioavailability<sup>9</sup> or with small molecules that enhance the activity of insulin such as biguanides<sup>10</sup> (metformin) or thiazolidinediones<sup>11</sup> to try to get synergistic effects (Figure 2).

## 2.2. Removal of Metal Ions from the System: The Origins of Metal Binding Therapy

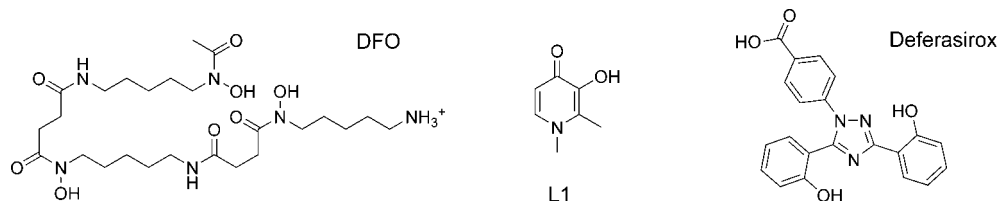
The use of chelating agents to adjust metal ion/metalloid toxicity began in the early 1900s with researchers such as

Alfred Werner, Paul Ehrlich, and Carl Voegtlin aiming to reduce toxicity of arsenic- and antimony-containing drugs for such parasitic diseases as syphilis, trypanosomiasis, and schistosomiasis; small-molecule chelators were applied to relieve the effects of heavy metal and metalloid overload. The use of small-molecule chelators to relieve accidental overexposure to metal ions began in 1941 with the (questionably appropriate) use of citrate for lead intoxication.<sup>12</sup> First, an historical overview of the major compounds for metal ion binding and excretion in overload conditions will be discussed, to be followed with further discussion in the heavy metal overload section at the end of this review. Considerations for development of therapeutic metal binding agents will be discussed, followed by a survey of disease conditions treated with metal ion passivation.

## 2.3. Development of Metal Ion Binding Agents in the 20th Century

The most obvious medicinal application of metal chelators is in the treatment of metal overload conditions. Since the industrial revolution, the advent of metalloid-containing drugs such as arsenic-based treatments, and chemical warfare, this application has focused on binding and removing metals such as lead, mercury, antimony, and arsenic from the human body. Because these target metals are in the “borderline” or “soft” ion classification of the hard–soft acid–base system,<sup>13</sup> most of the early ligands applied to the treatment of heavy metal overload have included sulfur donors. 2,3-Dimercaptopropanol (British anti-Lewisite, BAL, Figure 3) was first developed in the 1940s against Lewisite (dichlorovinyl arsine)<sup>14</sup> but was never used against the chemical weapon. Its first use was against the toxicity associated with arsenic-containing syphilis therapies and against cases of arsenical industrial accidents.<sup>15</sup> The development of less toxic, more hydrophilic BAL analogs *meso*-2,3-dimercaptosuccinic acid (DMSA) and *D,L*-2,3-dimercapto-1-propanesulfonic acid (DMPS, both Figure 3) followed in later years, and these are now registered for use in some countries for treatment of mercury intoxication. The necessity of painful intramuscular injections for lipophilic BAL delivery however led to the development of (*2S*)-2-amino-3-methyl-3-sulfanyl-bu-





**Figure 4.** Multidentate pro-ligands used for treatment of iron-overload and other conditions: DFO; **L1**; deferasirox.

tanoic acid (D-penicillamine, D-pen, Figure 3) as an alternative orally active copper chelator; D-pen has been utilized since 1956 for treatment of copper-overloaded WD patients (see section 3.5.2). Ethylenediaminetetraacetic acid (EDTA, Figure 3) was the next chelator in clinical use, being introduced in the 1950s for treatment of lead toxicity and also used in cases of accidental radionuclide dosing. Eventually, EDTA was replaced by diethylenetriaminepentaacetic acid (DTPA, Figure 3); particularly for radionuclide removal. Triethylenetetraamine (TETA, Figure 3) was introduced in 1980s for use in WD patients intolerant of D-pen; it is considered to be less potent, but to be an effective alternative to D-pen and by some to be the first choice for metal passivation and removal in WD. Desferrioxamine (DFO, Figure 4) has been the standard chelator used to treat iron overload since the 1970s; the pyridinone derivative 3-hydroxy-1,2-dimethyl-4(1H)-pyridinone (deferiprone, **L1**) and the tridentate chelator deferasirox (also known as **ICL670**) have been used orally for the same purpose since the late 1980s and the 1990s, respectively (Figure 4, see also section 3.4.2). A selection of other metal binding agents will be discussed in their respective sections, grouped by their target clinical condition (*vide infra*).

## 2.4. Considerations in Therapeutic Metal Binding Agent Development

In introducing metal ion complexes or pro-ligands to the biological system, it is imperative to consider the reactivity and distribution characteristics of all parts of the coordination complex: metal ion, pro-ligand, and complex. Because the passive permeation of biological membranes (lipid bilayers) depends primarily on hydrophobicity and ionic charge, charged metal ions require some sort of transport mechanism to cross biological membranes such as transmembrane ion channels or transfer proteins (also called ionophores). Whereas some neutral chelators will be able to permeate cell membranes, other charged ions will achieve significant permeation only upon metal ion complexation and formation of an uncharged complex. In many cases, the corresponding metal complexes are more lipophilic and mobile throughout the biological system than are pro-ligands.<sup>16</sup> The importance of this consideration is demonstrated by the design of cadmium ( $\text{Cd}^{2+}$ )-targeting chelators, because some chelators will mobilize metal ions to magnify their toxicity by increasing deposition in the brain.<sup>17</sup>

In designing a therapeutic metal ion chelator, one must consider the hard–soft acid–base (HSAB) theory for the incorporation of appropriate donor atoms for the target metal. This interaction will alter not only the stability of the final complex but also the selectivity of the pro-ligand for the intended metal ion in the same environment as many other ions in free solution, such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$ . The hard–soft acid–base theory describes the propensity of metal ions (electron acceptors) and coordinating molecules (electron donors) to bind preferentially with those of similar

“hardness” or “softness”, where “hardness” and “softness” refer to, for metal ions, how readily the empty orbitals of the metal ions accept electron density and, for donor groups, how deformable are the outer electron orbitals.<sup>13</sup> “Hard” metal ions such as  $\text{Fe}^{3+}$  or  $\text{Al}^{3+}$  bind preferentially with “hard” donors such as oxygen in carboxylate groups; thus, EDTA is a good ligand for these and other metal ions. On the other end of the spectrum, “soft” metal ions such as  $\text{Hg}^{2+}$  are well-coordinated by ligands with “soft” donor atoms such as sulfur; BAL or D-pen form strong complexes with these metal ions. Of course, the other consideration in ligand design is the *chelate effect* describing the favorable entropy change upon exchange of many monodentate ligands for fewer multidentate ligands binding a given metal ion. The higher denticity the ligand, the more thermodynamically stable will be its complex. Thus, higher-denticity ligands such as EDTA and DTPA form extremely stable complexes with a wide range of metal ions classified as both “hard” and “intermediate” and, in practice, are less selective metal binders. A more in-depth review of the factors governing coordination complex stability in aqueous environments can be found in that of Martell and Hancock,<sup>18</sup> and considerations for the design of clinically useful chelators, particularly for  $\text{Fe}^{3+}$ , is discussed by Liu and Hider.<sup>19</sup>

The introduction of chelating agents to bind specific metal ions requires that affinities must be compared: that of the proposed chelator vs that of the endogenous ligand within the body. Metal ions (especially metal ions, which are redox-active under physiological conditions, such as  $\text{Cu}^{1+/2+}$  or  $\text{Fe}^{2+/3+}$ ) are generally not in free form but are bound, with varying mixes of aquo or other ligands, to biomolecules such as proteins, nucleic acids or others. Thus, the introduced metal-binding therapeutic must participate in a series of ligand exchange reactions to form the new, desired complex before metal ion repartitioning or removal (via excretion) can be achieved. Principles governing metal speciation in the body can be found elsewhere,<sup>20</sup> in addition to in-depth discussion of the mathematical approaches to modeling *in vivo* competitive metal ion chelation.<sup>21</sup> A final consideration must be made of the reactivity of the metal ion in complexed form; for example,  $\text{Fe}^{3+}$  complexation by DFO involves complete encapsulation of the metal ion, preventing any possible redox reaction,<sup>22</sup> but  $\text{Fe}^{3+}$  complexation by EDTA exposes the metal ion and actually increases its reactivity to promote production of reactive oxygen species (ROS).<sup>23</sup>

Overall, manipulating the distribution of metal ions in biological systems in a specific way is a very complicated process. It is exceedingly difficult to effectively model the expected pharmacokinetics of both the free pro-ligand and the complex and to take into account all of the biological contributions to every chemical reaction and all biochemical implications of such reactions. Thus the efficacy and activity of metal ion chelators are generally not well quantified or theoretically modeled but are probed by and discussed in context of empirical evidence from biological experiments.

Model systems are used to characterize the action of new therapeutics; these include metal-loaded cultured cells, *ex vivo* tissue and cells, and animal models (including genetic mimics of disease states). In the end, assessment of drug effect and efficacy in humans can only be done with clinical observations and formal clinical trials.

The challenge of achieving metal binding selectivity has already been mentioned. For example, when one aims to counteract iron overload, one risks binding and affecting concentrations of other essential metals such as copper and zinc to produce deficiencies. The design of the metal ion chelator should localize its activity to the target tissue or even to the target cell compartment. A number of groups have tried different strategies to achieve this localization of activity utilizing such methods as masked prochelator synthesis. The Franz group uses boronic esters to mask the metal-binding moiety of salicylaldehyde isonicotinoyl hydrazone or salicylaldehyde benzoyl hydrazone molecules; these inactive prodrugs have negligible interaction with  $\text{Fe}^{3+}$ , but upon  $\text{H}_2\text{O}_2$  oxidation of the aryl boronic ester to a phenol group, the molecule becomes a high-affinity ligand for trivalent metal ions.<sup>24,25</sup> Another way to target the metal binding activity of a proposed ligand is to append targeting molecules to the pro-ligand, and this can be particularly important when designing a therapeutic molecule to the brain.

The blood–brain barrier, or BBB, is formed by the endothelium of the brain blood vessels, the basal membrane, and the neuroglial cells and separates the brain interstitial fluid from the circulating blood to insulate the brain from fluctuations in blood levels of metal ions and small molecule metabolites. Its extreme selectivity means that from a drug design perspective physicochemical properties such as lipophilicity and molecular weight must be considered if the drug is to permeate to the central nervous system (CNS). For passive BBB penetration, drugs or prodrugs must be uncharged at physiological pH, relatively lipophilic (octanol/water partition coefficient,  $\log P > 1.5$ ), compact (small polar surface area, PSA), and of low molecular weight (less than about 500 g/mol). Functional group modification can be used to increase passive BBB uptake; generally this would focus on increasing drug lipophilicity, for instance, by esterification of carboxyl groups. Other transport mechanisms exist in the BBB, however, such as hexose, amino acid, and neuropeptide transporters, and these can be utilized to impart brain uptake to other therapeutics not meeting the above criteria for passive BBB permeation. The glucose transporter (GLUT) family of membrane transport proteins can be utilized by conjugation of the drug to a glucose molecule; this has been used to increase brain uptake of HIV,<sup>26</sup> AD,<sup>27</sup> and PD<sup>28</sup> therapeutics, among others. The amino acid transporter can be exploited by creation of a “pseudonutrient”, modifying the structure of the drug to mimic nutrient structure (e.g., using L-DOPA as substrate for amino acid transporter for brain delivery of dopamine<sup>29</sup>). The “Trojan horse” approach uses peptides such as insulin or transferrin bound to the drug to exploit receptor-mediated transcytosis mechanisms in the BBB.<sup>30</sup> Similarly, nanoparticles are the newest vector to be suggested for BBB permeation of CNS drugs.<sup>31</sup> A more exhaustive summary of approaches for increased BBB penetration has recently been presented elsewhere.<sup>32</sup>

### 3. Manipulation of Metal Ions for Disease Therapy

#### 3.1. Alzheimer’s Disease

##### 3.1.1. Introduction to Alzheimer’s Disease and the Amyloid Hypothesis

A few of the erroneous assumptions existing today about Alzheimer’s disease are that *the disease is not fatal, it is a natural part of the aging process, and it only affects the elderly*. Although the etiology of AD is still poorly understood, its pathology is known and has been characterized for over 100 years since Alois Alzheimer first described “a peculiar disease of the cerebral cortex”.<sup>33</sup> Now, AD affects more than 24 million people worldwide, with this number expected to reach over 81 million by 2040.<sup>34</sup> AD patients experience multiple cognitive deficits including memory loss and disorientation linked with the breakdown of neuronal function and neuron death. This section will outline the prevailing understanding of the biochemical causes of AD, with a special focus on the role of metal ions and their interactions with Alzheimer’s-associated proteins (including redox chemistry). Current therapeutic approaches will be mentioned, followed by an introduction to metal binding molecules as applied to AD treatment, with considerations for their design. Next, an overview will be given of compounds tested and developed for AD intervention, and finally, recent developments will be discussed in the theory of metal ion passivation for AD therapy.

##### 3.1.2. The Etiology of Alzheimer’s Disease and Current Focus of Treatment

There are two types of AD currently recognized: early-onset, in which symptoms appear prior to age 65, and late-onset, which manifests after age 65, with the latter comprising 95% of all diagnoses. While genetic factors have been identified in the development of early-onset AD, increased age is the major risk factor for late-onset AD.<sup>35</sup> In both early- and late-onset forms of the condition, the pathology leading to a positive diagnosis is the same: the presence of extracellular plaques formed from a peptide called  $\beta$ -amyloid ( $A\beta$ ) and intracellular deposits of a peptide called tau. These tissue markers of AD are generally accompanied by high levels of oxidative stress, inflammation in the brain, and neurodegeneration. Linking the noted pathologies to a causative agent, the *amyloid cascade hypothesis* has defined the fibrillization of  $A\beta$  into amyloid deposits as a toxic “gain-of-function.”<sup>36</sup> Indeed, the genetic, biochemical, and neuropathological evidence strongly suggest that  $A\beta$  amyloidogenesis is central to AD pathogenesis, with age-related increases in metal ion concentration associated with  $A\beta$  plaque deposition, redox reactions, and oxidative damage in brain tissue.<sup>37</sup>  $A\beta$  peptide is cleaved by secretase enzymes from membrane-bound amyloid precursor protein, APP. Although the function of APP is unknown, recent evidence suggests that it functions in the maintenance of copper homeostasis.<sup>38</sup> While  $A\beta$  is a natural product and is present in the brain (and the cerebrospinal fluid, CSF) normally throughout life, a particular self-association occurs in AD to form  $A\beta$  plaques. These plaques are extracellular fibrillized deposits of amyloid  $\beta$  peptide (40–43 amino acid residues long)<sup>39,40</sup> and first deposit in the glutamatergic synapse in the cortex and hippocampus, which is important for formation of the

physical substrate of memory. Notably, this is the only place in the body where exchangeable copper and zinc are found together;  $Zn^{2+}$  is thought to be released into the extracellular space in either a free or exchangeable form,<sup>41</sup> and Cu has been shown to be released in ionic form by postsynaptic neurons.<sup>42,43</sup> These  $A\beta$  plaques are known to be toxic, and aggregated  $A\beta$  is likely more neurotoxic than the native peptide.<sup>44</sup> More recent evidence points to oligomers being particularly toxic;<sup>45–47</sup> however, it is generally conceded that many forms of  $A\beta$  peptide, from small oligomers to large fibrils, are harmful.<sup>48</sup> Recent progress in delineating the mechanism of  $A\beta$  toxicity has been reviewed by Cappai and Barnham.<sup>49</sup>

Another myth surrounding Alzheimer's disease is that *there are treatments available to halt the progression of the disease*. In fact, current therapies are not able to stop disease progression but offer only symptomatic relief and can, in the best case, slow cognitive decline. Generally these therapies attempt to address neurotransmitter defects, bolstering neuronal activity by enhancing the amount of acetylcholine neurotransmitter in the synaptic cleft (through acetylcholinesterase inhibition), by protecting neurons from further damage (via glutamate blockers/NMDA receptor inhibitors), or by restoring nerve activity (through supplementation of nerve growth factor). In addition, statins are thought to show promise in slowing neurodegeneration. Other therapies aim to alleviate the associated inflammation and oxidative stress in brain tissue (i.e., administration of nonsteroidal anti-inflammatory drugs or antioxidants such as vitamin E, respectively); current AD therapies have recently been reviewed in detail.<sup>50</sup> These treatments target only the symptoms, and as it stands, new therapies are needed to target the underlying pathology of AD.

### 3.1.3. The Involvement of Metals in the Pathology of Alzheimer's Disease

Increased age is the major risk factor for neurodegenerative disease, and it is known that brain metal concentration increases as a result of normal aging.<sup>51–53</sup> It is also clear that metal ions mediate the oxidative stress mechanism of  $A\beta$  toxicity.<sup>54,55</sup> While accurate analysis can be problematic (the processes of plaque isolation and tissue fixation can introduce metal ions to the sample or alter their distribution), new methods obviating these processes are being developed.<sup>56</sup>

Copper in the AD brain appears to be miscompartmentalized rather than universally elevated; it is concentrated within  $A\beta$  plaques<sup>57</sup> with observed levels of up to  $400\ \mu\text{M}$ ,<sup>58</sup> approximately 2 orders of magnitude higher than the normal brain extracellular level<sup>59–61</sup> and higher even than the levels reached in the synaptic cleft ( $250\ \mu\text{M}$  on average).<sup>59</sup> This elevation, however, is not matched by results from bulk brain studies, which show no change in overall copper levels<sup>62,63</sup> or even a decrease in copper concentration vs age-matched controls.<sup>64</sup> Serum copper levels are elevated in AD patients, which suggests that this may serve as a peripheral diagnostic tool for AD.<sup>65</sup>

Zinc has been shown to colocalize with dense (but not diffuse)  $A\beta$  plaques,<sup>66</sup> showing levels of up to approximately  $1\ \text{mM}$  in plaques,<sup>58</sup> but zinc levels in bulk brain are more difficult to quantify. There is evidence for elevated zinc levels in AD tissue vs control (non-AD) tissue in various brain regions;<sup>58,64</sup> other reports however, show a *decrease* in zinc levels in the AD brain.<sup>63,67</sup> More focused studies on the  $A\beta$

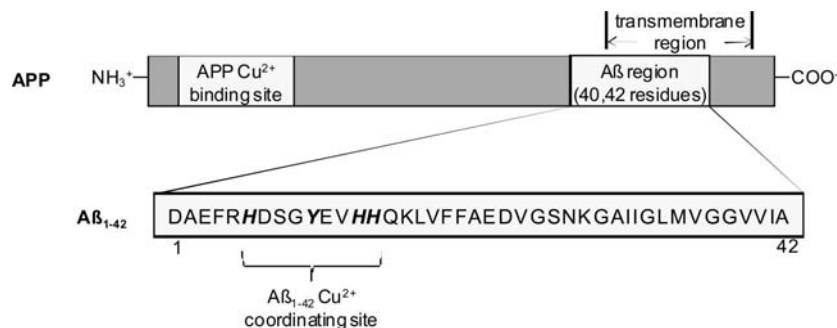
plaques of the AD brain show colocalization of zinc within the plaques,<sup>57,68</sup> and zinc is also elevated in the CSF of AD patients.<sup>69</sup>

The status of iron in the Alzheimer's brain is somewhat complicated as well. Iron imbalance in the AD brain was first reported in 1953 with Prussian blue staining of iron deposits in the cytoplasm of neurons containing neurofibrillary tangles (NFT) and in senile plaques.<sup>70</sup> Similar experiments have been performed more recently showing iron localization in senile plaques and NFT, which both is redox-active and may be removed with tissue pretreatment with DFO.<sup>71</sup> Separate analyses of bulk AD brain matter indicate iron elevation in a number of AD brain regions vs controls,<sup>72,73</sup> elevation in NFT-bearing neurons at the cellular level,<sup>74</sup> and elevation in senile plaques.<sup>58</sup> Indeed, it has been proposed that this iron elevation may be exploited as a biomarker for AD imaging by MRI.<sup>75</sup> Unlike copper and zinc, iron does not copurify with plaque-extracted  $A\beta$ ; instead, it may be that iron is elevated in the neurons surrounding and extending within the plaques, but not closely associated with the plaque peptide itself.<sup>76</sup> Clearly there is evidence of dishomeostasis and overall miscompartmentalization of metals such as copper, zinc, and iron in the AD brain, with accumulation of copper and zinc in amyloid deposits and iron in plaque-associated neurons.

### 3.1.4. Amyloid- $\beta$ ( $A\beta$ ) Peptide as Metalloprotein

The amyloid precursor protein, APP, is a ubiquitous transmembrane protein of unknown biological function. It has specific and saturable binding sites for zinc and copper ions with dissociation constants  $K_d$  of  $764^{77}$  and  $10\ \text{nM}$ ,<sup>78</sup> respectively (reviewed by Kong et al.<sup>79</sup>). Because these binding sites seem to be conserved across the APP superfamily of proteins, it seems that zinc and copper binding may play an important role in APP function and metabolism.<sup>80</sup> Putative functions of APP include regulation of cell growth and adhesion and metal ion homeostasis, among others.<sup>81</sup> The amyloid peptide fragment  $A\beta_{1-40}$  specifically and saturably binds  $Zn^{2+}$ ; early solution studies showed one high-affinity binding site ( $K_d = 107\ \text{nM}$ ) with 1:1 stoichiometry and one low-affinity binding site ( $K_d = 5.2\ \mu\text{M}$ ) exhibiting 2:1 zinc/ $A\beta$  stoichiometry.<sup>82</sup> Variable pH trials have pointed to this binding being histidine-mediated, because it was inhibited by low pH and by chemical alteration of histidine residues.<sup>83</sup> *In vitro*, low micromolar concentrations of  $Zn^{2+}$  rapidly precipitate soluble  $A\beta$  into amyloid aggregates<sup>82,84</sup> and intermolecular  $\text{His}(N_\tau)\text{-Zn}^{2+}\text{-His}(N_\tau)$  bridges are thought to mediate this reaction.<sup>85</sup> At physiological pH (7.4),  $Zn^{2+}$  is the only relevant biometal able to precipitate  $A\beta$ ,<sup>82</sup> while at slightly acidic conditions  $\text{Cu}^{2+}$  and even  $\text{Fe}^{3+}$  are known to induce  $A\beta$  aggregation.<sup>83</sup> As for  $Zn^{2+}$ ,  $A\beta_{1-40}$  displays both high- and low-affinity binding sites for  $\text{Cu}^{2+}$  with  $K_d = 0.05$  (estimated) and  $13\ \text{nM}$ , respectively.<sup>86</sup> The other major peptide fragment,  $A\beta_{1-42}$ , displays higher  $\text{Cu}^{2+}$  affinity with  $K_d = 7\ \text{nM}$  and  $5\ \text{nM}$  for the high- and low-affinity  $\text{Cu}^{2+}$  binding sites, respectively.<sup>86</sup> Nuclear magnetic resonance (NMR) studies have characterized the structure of the  $A\beta$  metal binding site and implicate three histidine residues at positions 6, 13, and 14 on the peptide.<sup>87</sup> In the same study, electron paramagnetic resonance (EPR) experiments indicated a square-planar  $N_3O$  coordination site, with N donors from the imidazole rings of the three histidine residues and the oxygen proposed to be donated from the position-10





**Figure 5.** Location of  $A\beta_{1-42}$  region within the larger transmembrane protein APP.  $Cu^{2+}$  binding sites both on APP and within the  $A\beta_{1-42}$  fragment are indicated, and putative  $Cu^{2+}$ -binding residues within  $A\beta_{1-42}$  are highlighted. Adapted with permission from Wiley-Blackwell Publishing, ref 94, copyright 2005.

tyrosine side chain hydroxyl group.<sup>87,88</sup> Conversely, other studies have postulated that the oxygen donor is the carboxylate group of Glu5 or the N-terminal aspartate residue,<sup>88</sup> the peptide amino terminus itself,<sup>88,89</sup> or some other exogenous ligand such as water.<sup>85</sup> Most recently, X-ray absorption spectroscopy (XAS) combined with density functional theoretical (DFT) analysis points to a  $N_3O_3$  distorted six-coordinate binding mode for the high-affinity  $Cu^{2+}$ -binding site of  $A\beta$  consisting of three histidine N-donors, glutamic or aspartic acid, and axial water.<sup>90</sup> The coordination chemistries of copper and zinc ions by  $A\beta$  have recently been reviewed.<sup>91</sup> Interestingly, recent evidence has implicated  $A\beta$ -Zn interactions in promoting  $A\beta$  accumulation, possibly by preventing normal  $A\beta$ -degrading protease activity.<sup>92</sup> While the main body of study into  $A\beta$ -metal binding has certainly focused on copper and zinc, there is some evidence for an  $A\beta_{1-42}$ -Fe complex with calculated  $K_d$  of 36  $\mu M$ ; furthermore, it seems that fibrillized  $A\beta_{1-42}$  binds  $Fe^{2+}$  much more tightly ( $K_d = 0.2 \mu M$ ) than does the monomeric form.<sup>93</sup> Relative locations of  $Cu^{2+}$  binding sites on APP and the  $A\beta_{1-42}$  region of APP are depicted in Figure 5.<sup>94</sup>

The conformation and kinetics of  $A\beta_{1-40}$  aggregation upon pH and metal ion challenge has been examined with a variety of methods. Atomic force microscopy (AFM) has identified two different types of  $A\beta_{1-40}$  aggregates formed depending on pH and the presence of metal ions ( $Fe^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ); furthermore, these two aggregate types were found to differ in cytotoxicity by *in vitro* assay.<sup>95</sup> Surface plasmon resonance bioimaging has been employed to monitor the kinetics of  $A\beta_{1-40}$  aggregation upon exposure to a number of metal ions including  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ , and  $Ca^{2+}$ .<sup>96</sup> A range of biophysical techniques such as AFM, Fourier transform infrared spectroscopy (FT-IR), thioflavin T fluorescence assay, and secondary ion mass spectrometry (SIMS) have been used to examine  $A\beta_{1-40}$  and  $A\beta_{1-42}$  deposition induced by metal ions; aggregate structure varied with metal ion used.<sup>97</sup> Electron microscopy, gel electrophoresis, thioflavin T, and light scattering methods were used to demonstrate the dependence of  $A\beta_{1-42}$  aggregation state on  $Cu^{2+}/A\beta$  peptide ratio; subequimolar ratios led to thioflavin T-reactive amyloid, and superequimolar ratios led to larger oligomers and amorphous aggregates.<sup>98</sup>

Beyond simple conformational/fibrillization state change, the reaction of  $A\beta$  with various metal ions *in vitro* has been probed. As mentioned above, the metal ions of interest in amyloid-mediated Alzheimer's pathology are zinc, copper, and iron. Of these three, only copper and iron are redox-active under physiological conditions. It was noted in 1999 that  $A\beta$  peptide can directly produce  $H_2O_2$  through  $Cu^{2+}$  and  $Fe^{3+}$  reduction<sup>99</sup> and that this catalytic production of  $H_2O_2$

### Chart 1. Redox Chemistry Involving Metal Ion Cycling and the $A\beta$ Peptide<sup>a</sup>

- $A\beta + M^{(n+1)+} \rightarrow A\beta^{n+} + M^{n+}$
- $M^{n+} + O_2 \rightarrow M^{(n+1)+} + O_2^-$
- $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
- $M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + HO^- + HO^*$
- $O_2^- + H_2O_2 \rightarrow HO^- + O_2 + HO^*$

<sup>a</sup> (a) Metal ion reduction by  $A\beta$ ; (b) redox cycling of metal ion (i.e.  $Fe^{3+}$ ,  $Cu^{2+}$ ); (c) production of  $H_2O_2$ ; (d) Fenton and (e) Haber-Weiss chemistry to produce the hydroxyl radical.

in the presence of biological reductants,  $A\beta$ , and  $Cu^{2+}$  was inhibited by the addition of copper chelators.<sup>100</sup> In fact,  $A\beta$  can rapidly reduce  $Cu^{2+}$  and  $Fe^{3+}$  and promote redox cycling at near-physiological buffered conditions.<sup>102</sup> This is important because the generated  $H_2O_2$  can then react with the reduced metal ions via Fenton chemistry (Chart 1) to produce the hydroxyl radical ( $HO^*$ ). The hydroxyl radical is particularly reactive, abstracting hydrogen atoms from organic molecules extremely quickly and unselectively causing immediate oxidative damage in the vicinity of its production.<sup>103</sup> Oxidative conditions can influence the mono- or oligomeric state of  $A\beta$  because free radical attack on the tyrosine residue at position 10 can lead to stable dityrosine cross-linked dimers.<sup>104</sup> The biological implications of these  $A\beta$ -associated redox reactions are observed in cell system experiments wherein redox-active  $Cu^{2+}$  and  $Fe^{3+}$  increase  $A\beta$  toxicity, but  $Zn^{2+}$  attenuates  $A\beta$  toxicity *in vitro*.<sup>105</sup> There are conflicting data implicating the methionine-35 (Met35) residue of  $A\beta$  in the redox-mediated toxicity of  $A\beta$  peptide. Free radicals could oxidize the sulfur-containing side chain of Met35 to form a radical cation, which then can abstract H from surrounding lipids to initiate lipid peroxidation or from proteins to initiate protein oxidation. On the other hand, Met35 could donate an electron for metal ion reduction, which could participate in Fenton chemistry. Methionine is at least important to the redox chemistry of the  $A\beta$  peptide, and an in-depth discussion of this residue's role in  $A\beta$  redox chemistry can be found elsewhere.<sup>54</sup>

### 3.1.5. The Role of Aluminum in Alzheimer's Disease

Some researchers have linked aluminum to the etiology of AD due to evidence of its elevation in NFT-bearing neurons. Specifically, X-ray spectrometric evidence showing aluminum accumulation in NFT-bearing neurons was given in 1980,<sup>106</sup> laser microprobe techniques were used to demonstrate a small increase in aluminum level within AD neurons,<sup>107</sup> and some epidemiological studies have made an

association between Al in drinking water and AD occurrence.<sup>108</sup> Indeed, Al was the only metal monitored at all in participants in the desferrioxamine (DFO) AD clinical trial.<sup>109</sup> While convincing evidence is still pending (after decades) to link this metal with the pathology of Alzheimer's disease, its role is still a controversial issue in many circles, and research continues today to prove this connection. Some researchers posit that aluminum is linked to amyloid deposition both *in vitro* and *in vivo*.<sup>110</sup> A new staining method was recently used to demonstrate elevated Al<sup>3+</sup> levels in *ex vivo* AD neurons,<sup>111</sup> and a recent study on the AD transgenic mouse demonstrated impaired cognition and increased amounts of A $\beta$  fragments in the brain with oral Al<sup>3+</sup> supplementation.<sup>112</sup> Despite these findings, it is generally accepted that although Al<sup>3+</sup> is neurotoxic and can cause Alzheimer's-like lesions in the brain, there is no convincing causal link between aluminum and AD initiation or progression.<sup>113</sup>

### 3.1.6. The Use of Metal Chelators To Attenuate A $\beta$ -Mediated Toxicity

Because of the detrimental interactions of metal ions with Alzheimer's A $\beta$  peptide, considerable focus has been placed on developing novel therapeutic approaches to modulate the metal-protein interactions.<sup>80,114</sup> The term metal-protein attenuating compound (MPAC) was coined to describe the approach of chelator introduction to disrupt specific, abnormal metal-protein interactions,<sup>38</sup> and it is distinct from the process of chelation and excretion of bulk metal ions, as is the case in copper removal in Wilson's disease. The two approaches differ conceptually in the localization (targeted vs systemic) of chelator activity and the affinity with which the chelator binds metal ions. While use of MPACs is meant to repartition and normalize metal ion distribution, traditional chelation sequesters and clears metal ions from the body using agents for the most part originally developed for treatment of heavy metal poisoning. With the MPAC concept in mind, some groups have attempted to rationally design metal ion binding agents to target the metal ions associated with Alzheimer's disease A $\beta$ . Targeting of chelator activity to the amyloid plaques may be attempted via structural manipulation, as in the case of the chelating AD therapeutic **XH1** (Figure 8)<sup>115</sup> and others.<sup>116</sup> Because in AD therapy the desired site of action is in the brain, the BBB permeation of the chelator must be considered. Diffusion through the BBB requires a high level of hydrophobicity that is generally not seen in hard base-containing metal chelators, but other methods can be employed to imbue the chelator with the ability to permeate the BBB. For example, pendant glucose molecules have been attached to metal binding drugs to increase their CNS uptake, attempting to take advantage of the many GLUT1 hexose transporter proteins localized within the BBB.<sup>117</sup> The glycosylation strategy has been applied to dopamine,<sup>28</sup> 3-hydroxy-4-pyridinone,<sup>27</sup> and tetrahydroxalen pro-ligands.<sup>118,119</sup>

Another strategy involves the use of nanoparticles (NP) to carry putative brain drugs across the BBB via the low-density lipoprotein receptor-mediated transport system.<sup>120</sup> One advantage of this approach is that the lipophilicity of the chelator itself no longer needs to be considered, neither in the structural design of chelator nor regarding expected drug toxicity. The use of NPs to transport drugs also obviates molecular weight concerns generally accompanying brain drug design.<sup>121</sup> Nanoparticle binding will likely also signifi-

cantly change how the complexes exit the brain after chelation. It has been postulated that if the nanoparticles are not biodegradable they may be able to leave the brain via the apolipoprotein carrier-mediated transport system.<sup>122</sup> A number of metal ion binding agents have been prepared based on the 3-hydroxy-4-pyridinone scaffold and proposed for conjugation to NPs for therapy of CNS diseases such as AD or PD; in addition to imparting greater BBB permeation, it is thought that NP linkage of many bidentate chelators will increase the effective ligand denticity and impart greater complex stability to complexes.<sup>120</sup> In a similar bid to improve CNS uptake of metal chelators, the Mumper group has covalently linked D-penicillamine to NPs for brain delivery.<sup>123</sup>

### 3.1.7. Testing Biological Activity and Applicability of Putative Alzheimer's Disease Therapeutics

**Turbidity Assay for Inhibition of Metal Ion-Mediated A $\beta$  Aggregation.** In aqueous medium, Zn<sup>2+</sup> and Cu<sup>2+</sup> promote the aggregation of synthetic human A $\beta$  peptide, which may be reversed by the addition of metal chelating agents;<sup>27</sup> the process is most readily observed by a light scattering-based "turbidity" assay and allows the comparison of different metal chelators based on efficacy of their interaction with and attenuation of metal ion-promoted amyloid aggregation. However, the turbidity assay posits no structural characterization of the fibrillization state of A $\beta$ . The metal-induced aggregation of human A $\beta$ <sub>1-40</sub> was first observed *in vitro* by Bush et al. in 1994 wherein Zn<sup>2+</sup> exposure reduced the recovery of A $\beta$ <sub>1-40</sub> from solutions passed through a 0.2- $\mu$ M filter.<sup>82</sup> The aggregation of A $\beta$ <sub>1-40</sub> at low pH was then visualized by simple light scattering ("turbidity") and Congo Red binding in 1996.<sup>124</sup> Since then, turbidity assays have marked Cu<sup>2+</sup>-induced A $\beta$ <sub>1-40</sub> aggregation at decreased pH; a process that is reversible with treatment with metal chelators such as EDTA.<sup>83</sup> Various metal chelators have been challenged in the turbidity assay and attenuated A $\beta$ <sub>1-40</sub> aggregation induced by both Zn<sup>2+</sup> and Cu<sup>2+</sup> at pH 7.4 and 6.6, respectively. These are outlined in the following sections and include **XH1**,<sup>115</sup> a series of multifunctional 3-hydroxy-4-pyridinones,<sup>27,125</sup> and a number of tetrahydroxalen multifunctional ligands,<sup>118,119</sup> in addition to representative multidentate chelators such as EDTA and DTPA.

**Enzyme-Linked Immunosorbent Assay (ELISA) for A $\beta$ <sub>1-42</sub> Fibril Binding.** The Yang group has developed an enzyme-linked immunosorbent assay (ELISA) technique to screen for the association of small molecules with insoluble deposits of aggregated A $\beta$  peptides.<sup>126</sup> Synthetic A $\beta$ <sub>1-42</sub> peptide is fibrillized by incubation in distilled water, adsorbed onto multiwell spectrophotometry plates, and then exposed to putative binding agents. After overnight incubation, visualization is performed using antibodies specific to the A $\beta$ <sub>1-42</sub> peptide; any small molecule binding the fibrils and obscuring the site of antibody binding will give a positive result by this assay.

**Solution Fibrillization Assays Monitored by Fluorescence.** Conformation-specific ligands to probe peptide aggregation states are in constant development.<sup>127</sup> One *in vitro* method available relies on the binding of thioflavin T to fibrillized A $\beta$ <sub>1-42</sub> to screen small molecules for development into Alzheimer's therapeutics.<sup>128</sup> The test compound is added to a solution of A $\beta$ <sub>1-42</sub> peptide; after incubation, the extent of A $\beta$ <sub>1-42</sub> fibrillization (or inhibition thereof) is monitored via thioflavin T binding and characteristic changes in fluores-



**Table 1. Metal Binding Compounds Designed or Tested for AD Therapy**

compound name	figure	state of development for AD	selected other recorded uses
<i>N</i> -acetylcysteine amide (NACA, AD4)	6a	<i>in vitro</i> study: rat <sup>145</sup> and human cells <sup>146</sup> exposed to AD-related biochemical challenge	derivative of <i>N</i> -acetylcysteine, a mucolytic drug and antidote for acetaminophen overdose
clioquinol	10b	clinical trial <sup>169</sup>	antifungal, antibacterial
deferiprone (L1)	4	<i>in vitro</i> mouse neuron study <sup>137</sup>	treatment of iron overload
desferrioxamine (DFO)	4	clinical trial <sup>109</sup>	treatment of iron overload
DP-109	8	transgenic mouse study <sup>158</sup>	
Feralex	8	removal of Al <sup>3+</sup> from preloaded human brain cell nuclei <sup>148</sup> <i>ex vivo</i> AD tissue study <sup>147</sup>	
glucose-bearing pyridinone derivatives	7	A $\beta$ turbidity study, mouse brain uptake study <sup>27,125</sup>	
glucose-bearing tetrahydroalans	9	A $\beta$ turbidity studies <sup>118,119</sup>	
hydroxychloroquine	10a	clinical trial <sup>165</sup>	antimalarial, anti-inflammatory
bis(8-hydroxyquinoline)	10c	<i>in vitro</i> A $\beta$ resolubilization study <sup>174</sup> and inhibition of A $\beta$ -Cu <sup>2+</sup> redox chemistry <sup>175</sup>	
JKL 169	8	rat study <sup>161</sup>	anti-HIV and others
PBT2		clinical trial <sup>173</sup>	
D-penicillamine (D-pen)	3	<i>ex vivo</i> AD tissue study <sup>133</sup> nanoparticle conjugation <sup>123</sup>	Cu <sup>2+</sup> chelation for WD therapy
triethylenetetraamine (TETA)	3	transgenic mouse study <sup>140</sup>	Cu <sup>2+</sup> chelation for WD therapy
XH1	8	transgenic mouse study <sup>115</sup>	

cence. This approach has been used recently to test various natural products,<sup>129</sup> *N*-methylated peptides,<sup>130</sup> and bis(styrylpyridine) or bis(styrylbenzene) derivatives<sup>131</sup> for inhibition of A $\beta$ <sub>1–42</sub> fibrillization. A similar assay has been put forward using bis(ANS) (4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonate) to probe the aggregation state of A $\beta$ <sub>1–40</sub> in solution upon exposure to various small molecules.<sup>132</sup>

**In Vitro Studies on Brain-Deposited Amyloid Plaque: Ex Vivo Tissue Assays.** In this type of experiment, Alzheimer's-affected postmortem brain tissue sections are exposed to test chelators, which can effect solubilization of A $\beta$  plaques<sup>133</sup> and inhibit A $\beta$ -mediated redox activity.<sup>134</sup> Both human AD-affected and transgenic AD-model mouse tissue have been analyzed in this way with a number of different chelators.<sup>135</sup>

**In Vitro Neuron Studies.** Cell study is the most basic way to probe the efficacy of a putative Alzheimer's therapeutic chelator in a biological environment and is the first line of testing beyond *in vitro* peptide/metal binding studies. A few chelators have been shown to impart neuroprotection to cell samples exposed to AD-relevant reagents or conditions such as synthetic A $\beta$ <sub>1–40</sub> or A $\beta$ <sub>1–42</sub> fibrils.<sup>136,137</sup> Specifically, DFO pretreatment of synthetic A $\beta$ <sub>1–42</sub> fibrils attenuates their neurotoxicity vs nonpretreated fibrils.<sup>136</sup> Similarly, deferiprone has been shown to protect cultured primary neurons from damage following exposure to a variety of AD-related chemical insults including Fe<sup>3+</sup>, H<sub>2</sub>O<sub>2</sub>, and A $\beta$ <sub>1–40</sub>.<sup>137</sup> Beyond showing efficacy of metal ion chelators in attenuating AD-related damage, experiments such as these support the concept that redox-active metal ions are the mediators of amyloid toxicity.

**Model Animal Studies.** Transgenic mouse models have been developed based on the knowledge of genes involved in familial AD and via manipulation of those genes to alter expression of protein deposited in plaques (and NFTs); these models have been recently reviewed.<sup>138</sup> Typically the mouse models exhibit the behavioral, biochemical, and pathological abnormalities reminiscent of AD. For example, the Tg2576 mouse model displays increased levels of A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> and A $\beta$  plaques,<sup>139</sup> which upon zinc supplementation show increased and preferential localization of zinc within the plaques.<sup>66</sup>

### 3.1.8. Compounds Tested or Designed as Alzheimer's Disease Therapeutics

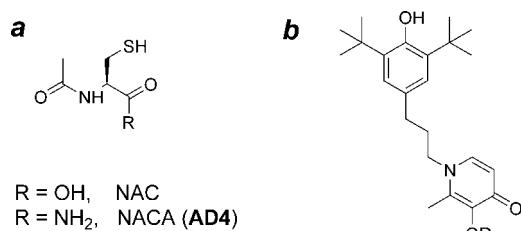
A wide range of metal-binding compounds have been designed and tested for use as AD therapeutics based on the metal ion-linked amyloid hypothesis. Some of these were originally developed for treatment of other metal-associated disease conditions and are discussed in the "crossover compound" section, while others were rationally designed as brain-permeating metal binders to target AD pathology and are discussed in the subsequent section; all compounds are summarized in Table 1.

**Crossover Compounds.** A number of "crossover compounds" have been put forward as possible Alzheimer's therapeutics, based on their proven utility for therapy of other metal-overload conditions such as copper overload in WD (see section 3.5.2) and thalassemia-related iron overload (see section 3.4.2).

D-Penicillamine ((*2S*)-2-amino-3-methyl-3-sulfanylbutanoic acid, D-pen), one of the original WD therapeutic chelators, has been used in *ex vivo* AD plaque resolubilization studies and can markedly enhance the solubilization of A $\beta$ .<sup>133</sup> In addition, it has been conjugated to nanoparticles in a bid to increase its delivery to the brain;<sup>123</sup> however, the applicability of this conjugate has not yet been tested.

Triethylenetetraamine (*N,N'*-bis(2-aminoethyl)ethane-1,2-diamine, TETA) has been used since 1982 for the treatment of WD but has been tested for AD application in the transgenic mouse model. Treatment with TETA over 12 weeks had no significant effect on A $\beta$  deposition and was likely too hydrophilic to penetrate the brain and interact with brain metals or plaques directly; at the same time, higher doses of TETA were found to cause significant toxicity in wild-type mice.<sup>140</sup>

*N*-Acetylcysteine ((*R*)-2-acetamido-3-sulfanylpropanoic acid, NAC; Figure 6a) is FDA-approved for medicinal use mainly as a mucolytic agent and for treatment for acetaminophen overdose. Being a small thiol-containing compound, it is also an effective copper chelator and has antioxidant activity. Its radical scavenging abilities and pharmacokinetics have recently been reviewed.<sup>141</sup> *N*-Acetylcysteine has relatively low bioavailability due to its carboxylate group (negatively charged at physiological conditions) and does



**Figure 6.** (a) "Crossover compounds" for AD therapy NAC and NACA (**AD4**). (b) "Hybrid" metal binding and antioxidant ligands in prodrug (nonchelating) form incorporating structural features of deferiprone (metal binder) and BHT (antioxidant); R = any of a series of acyl groups.<sup>152</sup>

not readily permeate membranes by diffusion. However, the analog *N*-acetylcysteine amide (NACA, or **AD4**) has the carboxyl group replaced by an amide group such that the molecule remains uncharged and is thus more able to permeate biological membranes, even penetrating the BBB after oral administration in animals.<sup>142</sup> *In vitro* examination of the antioxidant activities of NAC, NACA, and many known antioxidants has been carried out by six different tests with NACA demonstrating superior antioxidant activity vs NAC.<sup>143</sup> *Ex vivo* studies on human  $\beta$ -thalassemic blood cells have demonstrated the ability of NAC and NACA to attenuate oxidative stress in a biological setting, with NACA being slightly more effective.<sup>144</sup> *N*-Acetylcysteine amide and a few other thiol-containing amide derivatives of oligopeptides prevented protein oxidation and protected rat neurons from *in vitro*  $A\beta_{1-42}$  toxicity,<sup>145</sup> and NACA successfully relieved the indicators of oxidative stress present in AD fibroblasts.<sup>146</sup>

Desferrioxamine, (*N'*-[5-(acetyl-hydroxy-amino)pentyl]-*N*-[5-[3-(5-aminopentyl-hydroxy-carbamoyl) propanoylamino]pentyl]-*N*-hydroxy-butane diamide also known as deferoxamine or DFO, Figure 4) is a hexadentate hydroxamate trivalent metal ion chelator and is the most widely used chelator for treatment of iron overload. To investigate its applicability to AD therapy, DFO has been used in  $A\beta$  solution studies, neuron culture studies, and *ex vivo* brain tissue experiments. Early *ex vivo* tissue studies with DFO demonstrated the colocalization of redox-active iron with plaques.<sup>71</sup> Desferrioxamine can provide protection for neurons from metal ion-mediated toxicity of synthetic  $A\beta$ ,<sup>136</sup> inhibit  $A\beta$ -mediated redox activity,<sup>134</sup> remove  $\text{Fe}^{3+}$  from hyperphosphorylated tau,<sup>147</sup> and, used in concert with Feralex (*vide infra*), efficiently remove  $\text{Al}^{3+}$  from neurons.<sup>148</sup> Desferrioxamine was the focus of the first clinical attempt to target metal ions for amelioration of neurodegenerative disease; a 1991 study used sustained low doses of DFO to see whether the clinical progression of AD-related dementia could be slowed.<sup>109</sup> The drug was able to slow the progression of Alzheimer's-related dementia, and at the time, it was hypothesized that DFO targeted iron or aluminum in the patients; however, neither blood nor CSF metal concentrations were monitored over the course of the trial, and only urinary Al levels were monitored but not reported. No conclusion was made at the time on DFO's metal binding action in AD patients, and because DFO has significant affinity for metal ions beside  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  (DFO stability constants,  $\log K$ , for  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$  are 30.6, 22.0, 14.1, and 11.1, respectively)<sup>149</sup> it is possible that the drug acted by targeting  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , or  $\text{Zn}^{2+}$ . Furthermore, it has been suggested (Cuajungco et al.)<sup>80</sup> that the study authors

verbally reported a decrease in postmortem brain zinc and iron levels after study completion.

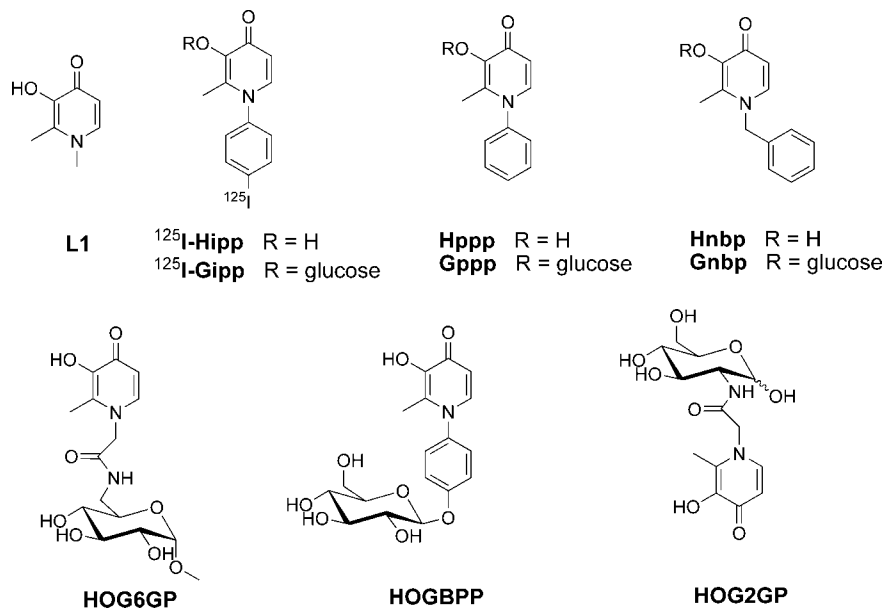
Deferiprone (3-hydroxy-1,2-dimethyl-4(1*H*)-pyridinone, **L1**) is approved for use as therapeutic iron chelator for iron overload conditions in Europe and in India.<sup>150</sup> Its relative lipophilicity gives it high oral activity and BBB penetration, it is as effective as DFO for iron removal, and while deferiprone appears to mobilize iron primarily from the serum pool, it is an effective binder of other metal ions in the order:<sup>150</sup>  $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Al}^{3+} > \text{Zn}^{2+}$  at pH 7.4. Deferiprone has recently been shown to protect mouse cortical neurons exposed to AD-related insults  $\text{Fe}^{3+}$ ,  $\text{H}_2\text{O}_2$ , and  $A\beta_{1-40}$  and to prevent neuronal death.<sup>137</sup>

**Rationally-Designed Multifunctional Molecules.** Because of deferiprone's precedent for clinical application, its formation from cheap precursors (i.e., 3-hydroxy-2-methyl-4-pyrone (maltol), an FDA-approved food additive), and its ease of derivatization, many such derivatives have been made for therapeutic metal ion binding, and some of these have been applied to the treatment of AD. Bebbington et al. imbued the basic structure of deferiprone with antioxidant activity by incorporating butylated hydroxytoluene (BHT, a known antioxidant) into the structure to make "hybrid" metal binding and antioxidant compounds.<sup>151</sup> To improve solubility, prodrugs were formed by esterifying the 3-hydroxyl group of the pyridinone moiety with various groups including amino acids (Figure 6b).<sup>152</sup> Select functionalizations significantly increased the water solubility of hybrid molecules and *in vitro* ester hydrolysis testing showed good stability in buffer, with limited to complete hydrolysis in rat plasma, demonstrating promise for the prodrug strategy.<sup>152</sup> The suitability of these deferiprone analogs for metal binding in an Alzheimer's disease model has not been assayed.

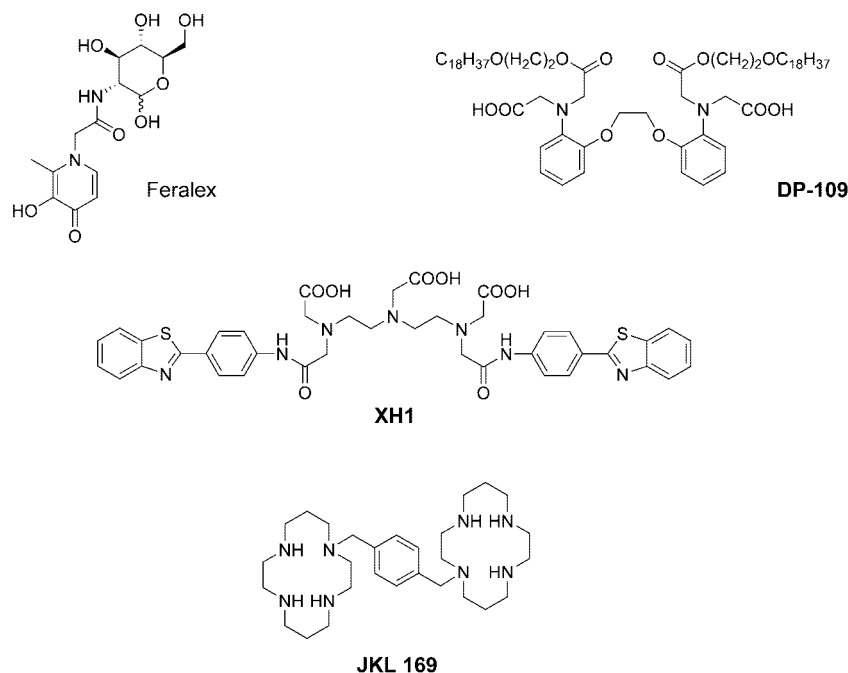
Other variations on the deferiprone theme have been constructed by incorporating secondary ring structures and functionalizing the pyridinone ring with glycosylation of the 3-hydroxyl group (Figure 7 (top)). This glucose group is readily hydrolyzed enzymatically by  $\beta$ -glucosidases, as shown via *Agrobacterium faecalis* assay.<sup>153</sup> Some analogs have been radiolabeled and assayed for murine brain uptake with promising results.<sup>27</sup> In addition, the compounds show antioxidant activity by *in vitro* assay and ability to inhibit metal ion-mediated  $A\beta_{1-40}$  aggregation *in vitro*.<sup>27,125</sup> A number of pyridinone compounds bearing pendant carbohydrate groups were synthesized<sup>154</sup> and, though originally designed for  $\text{Ga}^{3+}$  and  $\text{Al}^{3+}$  complexation, retain the same metal binding moiety and have in fact shown effective inhibition of  $\text{Zn}^{2+}$ - and  $\text{Cu}^{2+}$ -mediated  $A\beta_{1-40}$  aggregation.<sup>155</sup>

Feralex, also known as Feralex-G (2-deoxy-2-(*N*-carbamoylmethyl-[*N'*-2'-methyl-3'-hydroxypyrid-4'-one])-D-glucopyranose, Figure 7) is a glucose-bearing deferiprone derivative.<sup>156</sup> In AD brain tissue experiments, Feralex was comparable to DFO in  $\text{Fe}^{3+}$  removal from AD-related neurofibrillary tangles.<sup>147</sup> In human brain cell cultures exposed to  $\text{Al}^{3+}$ , Feralex exerted a cooperative effect with DFO for  $\text{Al}^{3+}$  removal, possibly by participating in *molecular shuttle chelation* in which Feralex released  $\text{Al}^{3+}$  from within the cells to DFO acting as an extracellular high-affinity metal ion sink.<sup>148</sup>

**DP-109** (1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid, Figure 8) is the more lipophilic diester derivative of BAPTA (1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid), a known calcium chelator. **DP-109** is the prodrug form of a hexadentate chelator and is



**Figure 7.** Selected 3-hydroxy-4-pyridinone compounds showing activity in various relevant assays, with **L1** shown as a comparison. 3-( $\beta$ -D-Glucopyranosyloxy)-2-methyl-1-(4-[ $^{125}\text{I}$ ]iodophenyl)-4(*H*)-pyridinone ( $^{125}\text{I}$ -Gipp) shows brain uptake in the mouse;<sup>27</sup> **L1**, **Hppp**, **Hnbp**, and all pendant carbohydrate-bearing compounds (bottom) are able to inhibit metal-induced  $\text{A}\beta_{1-40}$  aggregation *in vitro*.



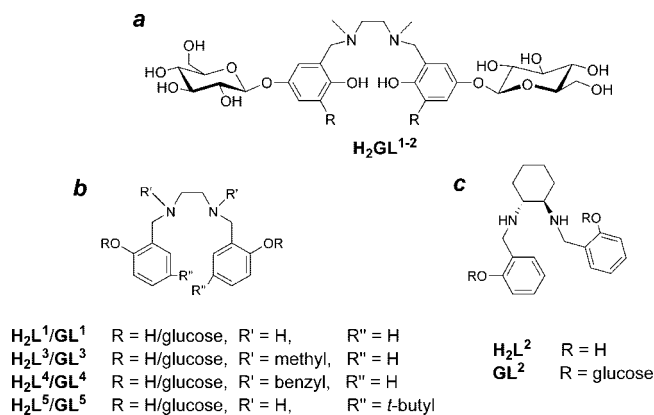
**Figure 8.** Compounds developed for therapeutic metal ion manipulation: Feralex, a glucose-bearing deferiprone derivative; prodrug compound **DP-109**;<sup>157</sup> putatively  $\text{A}\beta$ -associating chelator **XH1**;<sup>115</sup> bicyclam **JKL 169**.<sup>161</sup>

designed for oral administration, greater brain penetration, increased residence time in the brain, and selective chelation of  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Fe}^{3+}$  within membrane compartments; it demonstrates better chelating efficacy for  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  than for other divalent metal ions.<sup>157</sup> A trial on the Alzheimer's transgenic mouse model has demonstrated **DP-109**'s ability to reduce the level of aggregated insoluble  $\text{A}\beta$  while increasing the level of soluble  $\text{A}\beta$  forms; tissue staining showed a reduction in plaque number, density, and percent area in cortical sections as well as reduced zinc content in AD tissue samples.<sup>158</sup> Finally, the authors report that the effect of a lower daily dosage of **DP-109** was comparable to that of clioquinol (CQ, *vide infra*).<sup>158</sup> No cognitive testing was reported, and further study must demonstrate a positive link between these physiological effects and positive neu-

rological effects for further development of **DP-109** as a viable candidate for AD therapy.

**XH1** [(4-benzothiazol-2-yl-phenylcarbamoyl)-methyl]-{2-[(2-[(4-benzothiazol-2-yl-phenylcarbamoyl)methyl]-carboxymethyl-amino)-ethyl]-carboxymethyl-amino}-ethyl)-amino)-acetic acid was developed to target metal binding activity to  $\text{A}\beta$  by covalent linkage of an amyloid-binding functionality (benzothiazole) with a DTPA-like metal ion binding core.<sup>159</sup> The result is a relatively lipophilic molecule, which, in computations, shows putative binding to the  $\text{A}\beta_{1-40}$  peptide and is able to effectively inhibit  $\text{Zn}^{2+}$ -induced  $\text{A}\beta_{1-40}$  aggregation in solution.<sup>115</sup> In addition **XH1** reduces APP protein expression in human neurons and attenuates amyloid pathology in the brains of APP transgenic mice.<sup>115</sup>





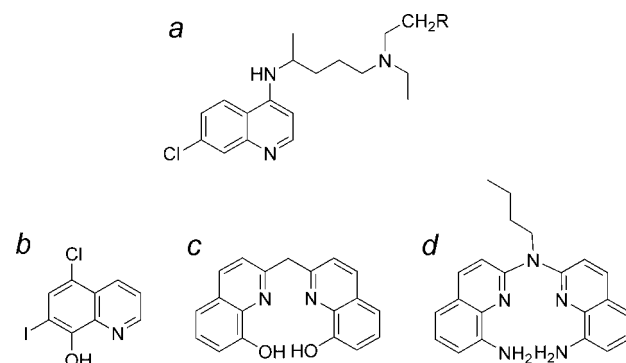
**Figure 9.** Tetrahydro-salen pro-ligands (a) with pendant glucose molecules<sup>118</sup> or (b, c) with glucose linkages installed as part of the prodrug concept, masking metal ion binding until activation by enzymatic deglycosylation.<sup>119</sup>

After the fortuitous discovery of a high-potency HIV inhibitor **JM1657**,<sup>160</sup> bicyclams have been developed for potential HIV therapy and other applications such as stem cell mobilization. The bicyclam **JKL 169** (1,1'-xylyl bis-1,4,8,11-tetraazacyclotetradecane, Figure 8) has been directly compared with CQ (below) in rat studies, with both compounds decreasing CSF copper concentration and slightly decreasing serum copper concentration and **JKL 169** significantly increasing copper levels in the brain cortex.<sup>161</sup> Thus, **JKL 169** is capable of affecting body distribution of copper and may be a candidate for further development into a viable AD therapeutic.

A number of tetrahydro-salen compounds have been designed, prepared, and evaluated for potential use in AD therapy.<sup>118,119</sup> A series has been produced bearing pendant glucose molecules, which demonstrate significant antioxidant activity, Zn<sup>2+</sup> and Cu<sup>2+</sup> coordinating ability, and inhibition of metal ion-induced A $\beta$ <sub>1-40</sub> aggregation in solution (Figure 9a).<sup>118</sup> A second series of tetrahydro-salen compounds was synthesized utilizing the prodrug approach with glucose masking of the metal binding site to potentially provide for more brain-specific metal binding (Figure 9b).<sup>119</sup> Antioxidant activity, facile enzymatic deglycosylation, and inhibition of metal ion-mediated A $\beta$ <sub>1-40</sub> aggregation were demonstrated,<sup>119</sup> paving the way for further biological investigation of these compounds for application to AD therapy.

Though not a small-molecule metal ion binder, the metalloprotein metallothionein-3 (Zn<sub>7</sub>MT-3) has been used to protect cultured neurons from A $\beta$  toxicity.<sup>162</sup> Divalent copper is reduced by protein thiolates to form an air-stable Cu(I)<sub>4</sub>-thiolate cluster, which inhibits Cu<sup>2+</sup>-mediated ROS generation and neurotoxicity and is an interesting modification of the concept of metal ion binding for AD therapy by inhibition of redox reaction and prevention of oxidative stress.

**Hydroxyquinoline Derivatives.** Both chloroquine (*N'*-(7-chloroquinolin-4-yl)-*N,N*-diethyl-pentane-1,4-diamine) and its analog hydroxychloroquine (2-[(4-[(7-chloroquinolin-4-yl)amino]pentyl)-(ethyl)amino]ethanol, Figure 10a), have been used as antimalarial agents and anti-inflammatory agents. Metal-binding agent chloroquine has been shown to inhibit iron uptake into cultured cells<sup>163</sup> and into rat tissue,<sup>164</sup> however, a double-blind trial of hydroxychloroquine on patients with minimal or mild AD indicated no significant advantage with the drug vs the control on the rate of cognitive decline or quality of life.<sup>165</sup>



**Figure 10.** (a) Chloroquine (R = H) and hydroxychloroquine (R = OH), metal-chelating antimalarial agents tested for AD application, (b) clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), and tetradentate quinoline-based chelators (c) bis(3-hydroxyquinoline)<sup>174</sup> and (d) bis(8-aminoquinoline).<sup>175</sup>

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ, also known as **PBT1**, Figure 10b) was initially approved decades ago for use as an antibiotic. It is an 8-hydroxyquinoline that binds Zn<sup>2+</sup> and Cu<sup>2+</sup> (with greater affinity than it binds Ca<sup>2+</sup> and Mg<sup>2+</sup>), is hydrophobic, and freely crosses the blood–brain barrier.<sup>166</sup> Clioquinol has been shown to reduce or prevent the formation of amyloid plaques in the transgenic AD mouse model, and the effect is correlated with improved cognitive activity with the proposed mechanism of action involving removal of metals from brain amyloid plaques.<sup>140</sup> In another strain of APP transgenic mouse, CQ supplementation significantly reduced plasma levels of copper, zinc, and iron, whereas supplementation with Cu<sup>2+</sup> and CQ increased cerebral copper; it is thought that CQ's role as an intracellular Cu<sup>2+</sup> transporter is responsible for its effects.<sup>167</sup> Although promising, the same authors report reduced survival of another strain of APP transgenic mouse with CQ supplementation in food.<sup>167</sup> Radiolabeling experiments with CQ have shown that *in vitro*, it saturably binds synthetic A $\beta$  precipitated by Zn<sup>2+</sup> ( $K_d = 0.45$  and 1.40 nM for A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub>, respectively) and localizes to the A $\beta$ - and Zn<sup>2+</sup>-enriched fraction of human *ex vivo* AD brain homogenates.<sup>168</sup> The distribution of CQ differs in the APP transgenic mouse and AD human brain vs their respective controls; [<sup>125</sup>I]CQ retention is higher in the AD mouse model, and its uptake is significantly more rapid into AD patient brains.<sup>168</sup> A 2003 clinical trial showed that CQ can significantly affect A $\beta$  metabolism in AD patients; in cases of severe AD, CQ slows cognitive decline with a concurrent reduction of plasma A $\beta$ <sub>1-42</sub> and increase in plasma Zn<sup>2+</sup> concentration and no effect on plasma Cu<sup>2+</sup> concentration.<sup>169</sup> The drug was overall well-tolerated enough to leave the possibility of human use in the future. Based on these data, CQ's mechanism of action is thought to be (a) inhibition of A $\beta$ –metal interaction and prevention of A $\beta$  aggregation and associated generation of ROS,<sup>140</sup> (b) scavenging of redox-active metal ions like Cu<sup>2+</sup>, and (c) lowering of cellular A $\beta$  production by increasing cellular levels of metal ions, and elevating levels of matrix metalloproteinase activity to degrade A $\beta$ .<sup>170</sup>

Because of difficulties encountered in scale-up of CQ synthesis for clinical trials (cited as the presence of a di-iodo-8-hydroxyquinoline impurity), further studies pursuing CQ clinical use have been postponed. Instead, researchers close to the CQ project are now focusing on other analogs, the most promising among them being a compound of undisclosed structure, **PBT2**.<sup>171</sup> This 8-hydroxyquinoline derivative contains no iodine and thus is not capable of

forming the di-iodo impurity. The results of a number of *in vitro* and *in vivo* tests on both CQ and **PBT2** were recently published and explain the current focus on **PBT2** for further clinical testing and development by Prana Biotechnology.<sup>172</sup> While both compounds performed well in *in vitro* assays such as Zn<sup>2+</sup>-mediated turbidity assays and inhibition of A $\beta$ /Cu redox chemistry, **PBT2** exhibited greater membrane permeability than did CQ for increased cellular and brain permeation and effected no change in tissue levels of other metals such as copper, zinc, iron, or manganese.<sup>172</sup> Finally, **PBT2** showed better performance than CQ in reduction of soluble brain A $\beta$  and improvement of cognitive functioning in the AD mouse model.<sup>172</sup> Furthermore, a phase IIa clinical trial was performed for **PBT2** similar to that done on CQ in 2001; **PBT2** caused no serious adverse toxicity events and had no effect on serum copper or zinc levels but significantly reduced the level of the AD biomarker A $\beta$ <sub>1–42</sub> in cerebrospinal fluid.<sup>173</sup>

Compound clioquinol analogs have been synthesized with varying linker lengths between and attachment points on the two quinoline moieties.<sup>174</sup> This forms a tetradentate proligand that chelates Cu<sup>2+</sup> and Zn<sup>2+</sup> in a 1:1 manner with much higher apparent affinity than clioquinol<sup>175</sup> and is significantly more effective than clioquinol in restoring A $\beta$ <sub>1–42</sub> solubility after metal ion-induced precipitation, particularly at low metal ion concentrations.<sup>174</sup> The bis(8-hydroxyquinoline) compound (Figure 10c) is also able to inhibit A $\beta$ <sub>1–42</sub>/Cu-mediated H<sub>2</sub>O<sub>2</sub> production *in vitro*.<sup>174</sup> Further derivations have generated a bis(8-aminoquinoline) version (Figure 10d), which displays extra selectivity for Cu<sup>2+</sup> and may thus be particularly useful in mitigating the oxidative stress observed in the AD brain.<sup>176</sup>

The results from the latest **PBT2** trials and others have given rise to the newest theory on metal ion distribution in AD and its treatment: that extracellular A $\beta$  peptide interacts with metal ions (Cu<sup>2+</sup>, Zn<sup>2+</sup>) to form oligomers and aggregates. In addition to the related ROS production and oxidative damage to brain tissue, this leads to depletion of intracellular metal ion reserves. It is hypothesized that metal-binding compounds such as CQ or **PBT2** bind metal ions from extracellular A $\beta$  aggregates to dissolve the aggregates, then redistribute metals via ionophore action (carriage of charged ions across the cell membrane) to restore depleted intracellular concentrations. This can up-regulate matrix metalloprotein expression, which then degrades and clears aggregated extracellular A $\beta$ .<sup>170</sup> Based on this hypothesis, the focus of new AD therapeutic development is on small, relatively lipophilic chelators that can enter the brain and capture metal ions from oligomerized and precipitated interstitial A $\beta$ , ideally forming redox-neutral complexes (for reduced neuronal damage) and dissolving oligomeric and aggregated A $\beta$  to facilitate brain clearance.<sup>177</sup> In addition, the complexes should permeate cell membranes to increase cellular copper and zinc concentrations.

## 3.2. Parkinson's Disease

### 3.2.1. Introduction to Parkinson's Disease

Parkinson's disease (PD) was initially described as "shaking palsy" by James Parkinson, an English surgeon, in 1817;<sup>178</sup> now affecting 1 in 100 persons over the age of 65, PD is the second-most common neurodegenerative disorder after Alzheimer's disease,<sup>179</sup> and like AD, it occurs most frequently as a sporadic condition with fewer than 10% of

cases classified as familial.<sup>180</sup> The condition is histologically characterized by the progressive loss of dopaminergic neurons in one region of the brain, leading to depleted neurotransmitter levels and neurological decline. While the pathogenesis of the disease is not completely understood, some insight has been gleaned from recent studies of familial forms of the disease,<sup>181</sup> and it is thought that a combination of genetic factors and environmental triggers are responsible for disease development. Current treatments are symptomatic only and focus on restoring brain function through enhancement of neurotransmitter activity. This can be achieved by increasing the levels of dopamine itself: administration of its precursor (3-(3,4-dihydroxyphenyl)-L-alanine, L-DOPA) to supplement its formation or monoamine oxidase (MAO) inhibitors to prevent its degradation.<sup>182</sup> Alternatively, dopaminergic receptor agonists can be used to mimic the effect of the neurotransmitter itself; a comprehensive assessment of current PD therapies has recently been compiled.<sup>183</sup>

### 3.2.2. The Biochemical Pathology of Parkinson's Disease

Although the etiology of PD is poorly understood, the molecular factors leading to dopaminergic neuron degeneration may include impairment of mitochondrial function, oxidative stress, accumulation of metal ions and of misfolded/aberrant proteins, and abnormal protein phosphorylation.<sup>184</sup> The metal of focus in PD is iron; Fe<sup>3+</sup> is elevated in the substantia nigra of the PD brain,<sup>185–187</sup> the site of neurological damage in PD.<sup>188</sup> Introduction of excess iron in the form of FeCl<sub>3</sub> salts directly into the brain induces PD-like symptoms,<sup>189</sup> while iron sequestration (with chelators or overexpressed ferritin, an iron storage protein) provides neuroprotection in animal models.<sup>190</sup> Although the mechanisms leading to iron accumulation are unclear, very recent evidence has revealed a PD-related increase in the expression of a divalent metal transporter, which may play a role.<sup>191</sup> Elevation of redox-active iron in the PD brain may contribute to oxidative stress and the other typical histological feature, deposition of  $\alpha$ -synuclein protein.

Like the  $\beta$ -amyloid peptide cited in the Alzheimer's amyloid cascade hypothesis, a small amyloidogenic peptide is implicated in the progression of PD. Here,  $\alpha$ -synuclein (140 amino acid residues long), a cytoplasmic, ubiquitous, and normally soluble protein, forms oligomers and larger aggregates, eventually fibrillizing and forming intracellular deposits of protein (in  $\beta$ -pleated sheets) known as Lewy bodies.<sup>192</sup> Like that of the A $\beta$  peptide in AD, this fibrillization process is thought to be accompanied by interactions with metal ions (of metals such as Fe, Cu, Zn, and Al) and increased levels of oxidative stress in the surrounding tissue.<sup>193</sup>  $\alpha$ -Synuclein is able to directly interact with metal ions, leading to redox reactions and protein aggregation. For example, when small amounts of Fe<sup>2+</sup> are added to  $\alpha$ -synuclein in solution, immediate generation of HO $\cdot$  is observed by EPR analysis.<sup>194</sup> Although a few different forms of  $\alpha$ -synuclein are identifiable, including monomers, oligomers, and filaments, the identification of the toxic species is not trivial, and examination of  $\alpha$ -synuclein aggregation is ongoing.<sup>195</sup> Despite the uncertainty surrounding the toxic mechanism of PD, the aberrant peptide and its associated elevation in metal ion concentration remain a therapeutic target for treatment. To this end, groups have designed a number of chelators targeting Fe<sup>3+</sup> and have tested their applicability in model PD systems.

### 3.2.3. Methods of Testing Putative Parkinson's Disease Therapeutics

A number of different models have been developed for PD experimentation, but the most widely used is the toxin-induced PD animal model, using compounds such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce biochemical and cellular changes that are remarkably similar to those seen in PD<sup>196</sup> including selective degeneration of dopaminergic neurons, formation of intracellular  $\alpha$ -synuclein aggregates, and significant increase in iron levels in the substantia nigra of the brain.<sup>197,198</sup> The putative neuroprotective agent is administered before or after the toxin challenge and evaluated for its effect. For example, a common test involves the administration of test therapeutic, followed by introduction of 6-OHDA directly to the test animal's brain (it does not readily permeate the BBB); 6-OHDA is readily taken up by catecholamine transporters into neurons where it is oxidized and produces hydrogen peroxide and paraquinone, both of which are highly toxic, particularly in the presence of redox active metal ions.<sup>199</sup> The degree of dopaminergic neuron loss is then measured, most commonly with drug-induced behavioral challenges and assessment of the animal's rotational movement. The full range of animal models for PD has been recently reviewed.<sup>196</sup>

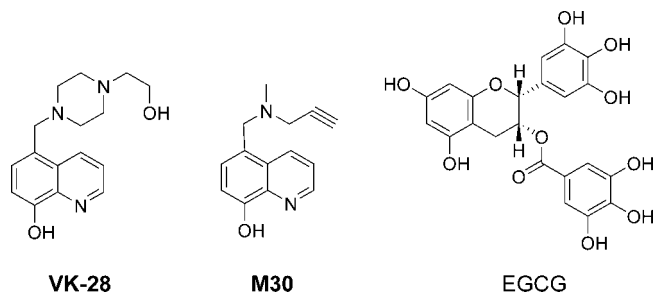
### 3.2.4. Compounds Used in Parkinson's Disease Therapy

**Crossover Compounds for Metal Binding in PD.** A few metal ion binding agents developed for other therapeutic uses have been tested in animal models of PD. Specifically, DFO pretreatment can protect against 6-OHDA-induced<sup>200,201</sup> and also MPTP-induced<sup>202</sup> neurodegeneration in animal models. The more lipophilic chelator **DP-109** (see section 3.1.8) was neuroprotective in the 6-OHDA PD model when administered even 2 weeks after lesion induction,<sup>203</sup> and clioquinol effected neuroprotection in the same animal model.<sup>190</sup> Conversely, the administration of a known copper chelator, D-penicillamine, did not seem to have similar neuroprotective capacity,<sup>204</sup> indicating that it is the targeting of the excess iron, not any other redox-active metal ion, that is a viable target for PD therapy by metal passivation.

**Drugs Designed or Tested for PD Therapy.** Particular focus has been placed on the development of multifunctional brain therapeutics that combine antioxidant activity, MAO inhibition, and iron binding into one compound. Iron binding is thought to be useful to prevent Fenton chemistry (production of ROS and therefore oxidative damage) and reduce the elevated levels of iron in the brain. Although a large range of compounds have been synthesized and tested,<sup>205</sup> a selection of the more fully developed compounds is discussed here.

The multifunctional chelator 5-(4-(2-hydroxyethyl)piperazin-1-ylmethyl)-8-hydroxyquinoline (**VK-28**, Figure 11) combines metal binding with MAO inhibition to increase overall neuronal function.<sup>206</sup> This compound was shown to be able to penetrate into the brain and to provide preventive neuroprotection against 6-OHDA damage in the rat system.<sup>207,208</sup>

Another combination drug, 5-(*N*-methyl-*N*-propargylaminoethyl)-8-hydroxyquinoline, dubbed **M30** (Figure 11), has been put forth by the same group. Based on the same hydroxyquinoline iron-chelating pharmacophore of **VK-28**, **M30** is also a combination iron chelator/antioxidant/MAO



**Figure 11.** Bifunctional  $\text{Fe}^{3+}$  chelators/MAO inhibitors **VK-28** and **M30** and  $\text{Fe}^{3+}$  chelator/antioxidant (–)-epigallocatechin-3-gallate, **EGCG**. All are being investigated by the Youdim group for PD therapy.<sup>208,210,212</sup>

inhibitor<sup>205</sup> and provides neuroprotection in MPTP-mediated PD animal trials.<sup>209</sup>

The green tea polyphenol (–)-epigallocatechin-3-gallate (**EGCG**, Figure 11) has also undergone investigation for application to PD; in addition to its antioxidant activity, the compound chelates iron,<sup>210</sup> crosses the BBB in animal studies,<sup>211</sup> and provides neuroprotection in the murine system vs MPTP challenge.<sup>212</sup>

## 3.3. Friedreich's Ataxia

### 3.3.1. Introduction to Friedreich's Ataxia

The condition is named after Nikolaus Friedreich, a German pathologist and neurologist who described a condition consisting of “degenerative atrophy of the posterior columns of the spinal cord” in 1863.<sup>213</sup> Now, Friedreich's ataxia (FRDA) is the most common form of inherited *ataxia*, or unsteady gait; however there is currently no effective treatment for the condition, which leads to degeneration of the nerve and muscle tissue and, in many cases, premature death associated with heart disease.<sup>213</sup> The most widely used therapy focuses on antioxidant supplementation (vitamin E, *N*-acetylcysteine, or coenzyme Q analogs such as idebenone), and because only inconclusive results are available, trials are ongoing to probe efficacy and safety of these treatments.<sup>214</sup> The autosomal recessive condition is caused by a repeat in the gene coding for the frataxin protein leading to decreased expression and reduced concentrations of the protein.<sup>215</sup> Although the full extent of frataxin's function is still a subject of debate, it is a mitochondrial iron chaperone participating in the synthesis and maintenance of heme as well as iron–sulfur clusters; deficiency leads to impaired iron–sulfur cluster assembly, increased cell susceptibility to oxidative stress, and mitochondrial dysfunction.<sup>213</sup> As with a number of other neurological disorders, there is evidence indicating elevated iron levels in the FRDA brain,<sup>216,217</sup> and there is also evidence for increased iron deposition in the heart, liver, and spleen.<sup>218</sup> It is thought that cellular iron distribution is altered; while it has been challenged by some,<sup>219</sup> a few studies have shown moderate but significant increases in mitochondrial iron,<sup>220–222</sup> and X-ray absorption spectroscopic methods have suggested excess iron storage in mitochondrial ferritin.<sup>223</sup> This evidence showing altered iron distribution as well as the hypersensitivity of FRDA cells to iron and oxidative stress challenges<sup>222</sup> has focused future therapeutic development not only on antioxidants but also on iron complexing agents.

In the more common transfusion-related or hemochromatosis-based iron overload conditions, plasma iron levels are elevated due to repeated blood transfusion or enhanced



alimentary uptake: as iron-transport proteins such as transferrin are saturated, iron is taken up into cells and accumulates in tissues such as the liver and heart.<sup>217</sup> In contrast, genetic disorders such as FRDA do not necessarily entail systemic metal ion overload; metal can accumulate in select tissues while plasma levels are normal or even deficient.<sup>217</sup> Based on this lack of systemic overload and on trials demonstrating iron regulation of frataxin expression, some propose that an ideal FRDA therapy should not simply chelate and remove iron from the body, leading to overall iron deprivation, but redistribute iron from the mitochondria to increase cytosolic levels, possibly alleviating frataxin suppression by cytosolic iron deficiency.<sup>224</sup>

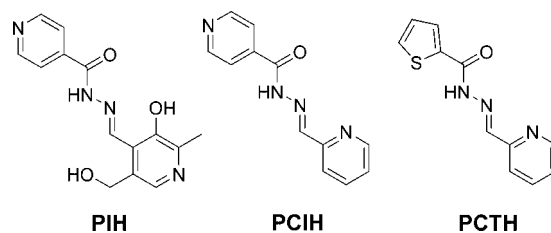
### 3.3.2. Metal Binding Therapeutics for Friedreich's Ataxia

The concept requires a metal ion-binding agent that can permeate membranes, compete with mitochondrial ferritin to chelate iron, and subsequently donate that iron to physiological acceptors in deficient regions (ideally, to the cytosol) or, perhaps more simply, remove the iron from the system entirely (for excretion).

Desferrioxamine primarily binds and permits excretion of iron from plasma pools; its considerable hydrophilicity makes its permeation of biological membranes particularly slow,<sup>225</sup> and mobilization of excess mitochondrial iron does not occur in cell models.<sup>226,227</sup> Given DFO's high affinity for Fe<sup>3+</sup> and stability of the complex, it is more likely to cause systemic Fe<sup>3+</sup> deficiency before inducing therapeutic redistribution of Fe<sup>3+</sup> in FRDA-affected cells.

Deferiprone (**L1**) was the first pro-ligand used to test the feasibility of this iron-redistribution approach, because it is membrane-permeable and able to shuttle iron between cellular compartments,<sup>228</sup> from intracellular organelles to extracellular apotransferrin,<sup>228</sup> or to extracellular DFO.<sup>229</sup> The chelation of organellar or cytosolic Fe<sup>3+</sup> by both deferiprone and deferasirox has been imaged with fluorescence microscopy,<sup>230</sup> and deferiprone has been used on FRDA cells *in vitro* to induce a loss in mitochondrial function by iron chelation.<sup>231</sup> It should be noted that although deferiprone toxicity was noted in the latter investigation, more detailed study of the metabolic processes affected by deferiprone has been performed examining deferiprone effect on the mitochondrial labile iron pool (reduced), cellular oxidative damage (reduced), mitochondrial activity (increased), and aconitase activity (restored) *in vitro*.<sup>232</sup> It is clear that while such *in vitro* cell exposure experiments are useful and necessary, sustained direct application of chelator to human cell culture may yield conflicting results depending on the measured observables and may or may not be predictive of subsequent *in vivo* results. In an efficacy–toxicity phase 1–2 open trial of deferiprone, the drug reduced iron accumulation in the brains of treated FRDA patients, reduced the neuropathy and ataxic gait in some, and induced no apparent hematologic or neurologic side effects.<sup>217</sup>

Pyridoxal isonicotinoyl hydrazone (PIH, Figure 12) is a tridentate NO<sub>2</sub> chelator, forming 2:1 L/M complexes with Fe<sup>3+</sup> with high affinity and selectivity over other biological metal ions.<sup>233</sup> Synthesis is via Schiff base condensation of pyridoxal (3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinecarboxaldehyde, a form of vitamin B<sub>6</sub>) and isonicotinic acid hydrazide (pyridine-4-carbohydrazide).<sup>226</sup> The resultant pro-ligand PIH is relatively lipophilic, and the basic structure is predominantly neutral at physiological pH, making the molecules membrane-permeable for intracellular iron target-



**Figure 12.** A selection of pyridoxal isonicotinoyl hydrazone (PIH) derivatives demonstrating good iron removal and low toxicity in iron-loaded FRDA model systems.

ing as well as for oral bioavailability (although somewhat susceptible to acid hydrolysis).<sup>234</sup> The bis-Fe<sup>3+</sup> complexes are also relatively lipophilic,<sup>16</sup> facilitating their passage through membranes after intracellular Fe<sup>3+</sup> binding. It was first demonstrated 30 years ago that PIH induces iron clearance from preloaded cells,<sup>226</sup> since then, various *in vitro* and *in vivo* trials have been performed (discussed by Richardson),<sup>235</sup> and these trials have led to a phase I clinical trial showing low toxicity and significant iron excretion in normal and iron-overloaded patients.<sup>236</sup>

Since then, a range of similar compounds have been synthesized by varying the aldehyde or the hydrazone starting material; compared with PIH, these have a better chance of seeing further pharmaceutical development because they have been patented for use as iron-overload therapeutics.<sup>237</sup>

Synthesized using 2-pyridylcarboxaldehyde instead of pyridoxal,<sup>238</sup> 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) and its derivatives are predominantly N<sub>2</sub>O chelators, which demonstrate good Fe<sup>3+</sup> binding activity and efficient iron excretion from iron-overloaded cells, with PCIH (Figure 12) showing the greatest efficacy (in some trials better than the parent compound PIH) and PCTH (2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone, Figure 12) also showing efficient iron removal from cells; both exhibited much higher efficacy than DFO for iron removal.<sup>227</sup> A selection of PCIH derivatives including PCIH and PCTH were found to exhibit low toxicity,<sup>238</sup> and one derivative (PCTH) demonstrated fast cell permeation and effective protection from H<sub>2</sub>O<sub>2</sub>-mediated oxidative insult of FRDA cells *in vitro*, providing better protection to cells than conventional radical scavengers or idebenone, one of the most common compounds used in FRDA.<sup>239</sup> Other biological assays have been performed on bis(PCIH)–Fe<sup>3+</sup> complexes, with sufficiently little biological redox activity observed for continued consideration of PCIH derivatives as therapeutic chelators.<sup>240</sup>

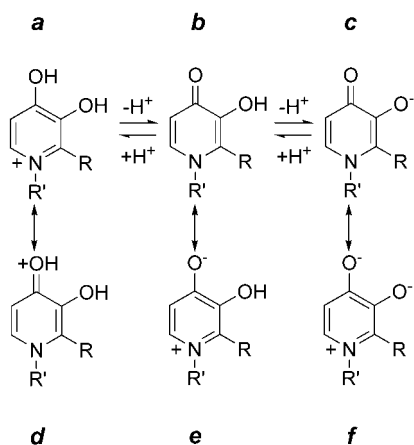
## 3.4. Transfusion-Related Iron Overload

### 3.4.1. Introduction to Transfusion-Related Iron Overload

Patients with hereditary or difficult-to-treat anemias such as  $\beta$ -thalassemia or sickle-cell disease require frequent and ongoing blood transfusions; because regular iron excretion is low, these transfusions lead to iron overload. Without treatment, iron accumulates in the heart, liver, and joints, among other tissues, and can lead to organ failure and death.

### 3.4.2. Compounds Used for Iron Overload Therapy

Since the mid-1960s, the only chelator in widespread clinical use has been desferrioxamine, also known as deferoxamine, desferrioxamine-B, or Desferal, *N'*-[5-(acetylhydroxy-amino)pentyl]-*N*-[5-[3-(5-aminopentyl)-hydroxy-car-

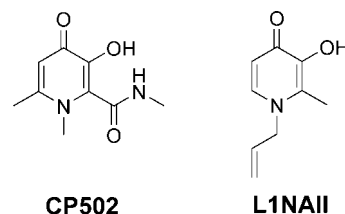
**Chart 2. Resonance Forms of 3-Hydroxy-4-pyridinone pro-ligands in Various Protonation States**

bamoyl) propanoylamino]pentyl]-*N*-hydroxy-butane diamide, DFO-B, or DFO (Figure 4). A fungal siderophore, DFO is an hydroxamate-based hexadentate trivalent metal ion chelator. Because of its high hydrophilicity and peptidic nature, DFO displays very poor oral bioavailability and must be administered via long subcutaneous infusions ( $\geq 8$  h,  $\geq 5$  days per week), leading to high cost of treatment and low patient compliance. In addition, DFO can induce allergic reactions and is linked with a host of other side effects including auditory, ophthalmic, and neurotoxicities, as well as skin rashes.<sup>241</sup>

Due to DFO's shortfalls in cost effectiveness and patient compliance, hundreds of chelators have been tested both *in vitro* and in iron-overload animal models as replacement therapeutics. Because of the "hard" nature of  $\text{Fe}^{3+}$ , many of these chelators contain oxygen donors as part of groups such as polyaminocarboxylic acids (EDTA, DTPA, Figure 3), catechols, or hydroxamates; a few are discussed below.

EDTA is a potentially hexadentate aminocarboxylate chelator, while DTPA is a potentially octadentate aminocarboxylate chelator, which has been used in patients who are intolerant of DFO.<sup>242</sup> Like DFO, neither EDTA nor DTPA are orally active; unlike DFO, they are not very selective for  $\text{Fe}^{3+}$  and thus can lead to depletion of other metals, such as zinc, in patients. To mitigate this effect, DTPA may be administered with zinc salts for treatment of iron overload.

3-Hydroxy-4-pyridinone compounds (pioneered by Hider's lab) have been designed with the action of pyranones such as maltol (3-hydroxy-2-methyl-4-pyranone, a common food additive) and natural products such as L-mimosine ((*S*)- $\alpha$ -amino- $\beta$ -[1-(3-hydroxy-4-oxopyridine)]propionic acid) in mind, having shown previous oral activity of effective *in vivo* iron binding.<sup>243</sup> Pyridinone synthesis can be achieved in one step from the appropriate primary amine and pyranone such as maltol;<sup>244</sup> however, better yields are frequently obtained via aminolysis with the benzyl- or methyl ether-protected pyranone.<sup>245,246</sup> Although their complexation constants may be lower in some cases, 3-hydroxy-4-pyridinones have considerably higher affinity for  $\text{Fe}^{3+}$  than do similar oxygen-donating bidentate ligands like the equivalent pyranones or catechols at physiological pH,<sup>247</sup> due to resonance structures. The aromatic resonance forms (Chart 2a,e,f) place additional electron density on the 4-position oxygen donor, making the deprotonated pyridinone pro-ligand (f) a double oxo donor for metal ions. This oxo rather than keto character is observed by X-ray structural analysis wherein the C—O

**Figure 13.** 3-Hydroxy-4-pyridinone-based iron chelators CP502 and L1NAII.

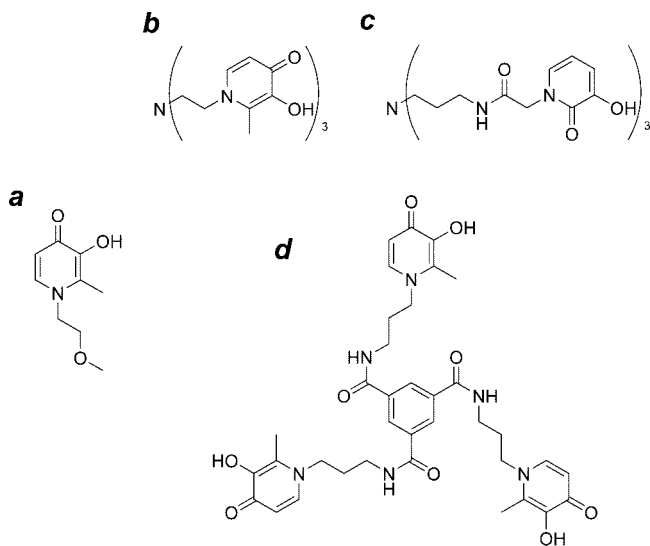
bond distances are longer than would normally be expected for a carbonyl functional group.<sup>247–250</sup> A great many 3-hydroxy-4-pyridinone variations have been designed and synthesized with variation in the *N*-substituent to modulate physical characteristics such as lipophilicity, to obtain targeting effects, or to integrate multifunctional activity without greatly affecting iron-chelating efficacy.<sup>247</sup> However only a few have progressed to the clinical trial stage including 3-hydroxy-1,2-dimethyl-4(1*H*)-pyridinone (**L1**, deferiprone, Figure 4), 1-ethyl-3-hydroxy-2-methyl-4(1*H*)-pyridinone (**L1NEt**), 1,2-diethyl-3-hydroxy-4(1*H*)-pyridinone, 3-hydroxy-1,6-dimethyl-2-(*N*-methyl-amido)-4(1*H*)-pyridinone (**CP502**, Figure 13), and 1-allyl-3-hydroxy-2-methyl-4(1*H*)-pyridinone (**L1NAII**, Figure 13).<sup>251</sup>

Also known as **L1**, **CP20**, or Ferriprox, deferiprone has been used as an oral alternative to DFO since the late 1980s. Commercially available in Europe and some other parts of the world, it is not effective in some patients,<sup>252</sup> and while its use is highly controversial due to concerns about side effects,<sup>253</sup> it is possible that lower and more frequent dosing of the drug could attenuate these effects.<sup>241</sup> Because of these side effect concerns, deferiprone is approved for use only as a second-line treatment after DFO in the EU and is not FDA-approved. Nevertheless, deferiprone offers a significant advantage over DFO in its ability to remove cardiac iron<sup>254</sup> and is now in consideration for combination therapy with DFO in iron-overloaded thalassemia patients.<sup>255</sup> The drug appears to mobilize iron primarily from the serum pool and is as effective as DFO for iron removal; historical details on the development and pharmacokinetics of deferiprone have been recently reviewed.<sup>256</sup>

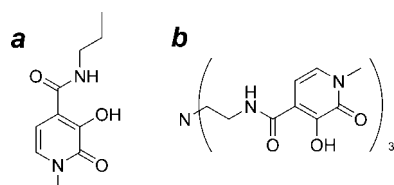
Developed for the treatment of iron overload, **L1NAII** is more lipophilic than deferiprone and more effective than DFO or deferiprone for iron excretion in animal trials; this higher apparent efficacy could be due to different pharmacokinetics such as clearance time from the blood.<sup>257</sup> It has shown oral activity and good tolerability in phase I clinical trials on normal volunteers.<sup>258</sup>

Dobbin et al. synthesized a large range of 3-hydroxy-4-pyridinones with varying alkyl substitution at the 1- and 2-positions in an early attempt to develop effective, orally active iron chelators for overload therapy.<sup>247</sup> A number of the compounds were tested for and demonstrated oral activity and iron removal from iron-overloaded mice, with one compound (Figure 14a) showing higher *in vivo* activity than did DFO.<sup>247</sup>

A number of groups have conjugated bidentate pyridinones via various linkers to produce new hexadentate chelators. Sheppard et al. have synthesized a tripodal tris-**L1**-type hexadentate chelator around a simple tertiary amine core (Figure 14b); its iron-binding properties at varying pH were compared with those of DFO, with the oligomeric pyridinone chelator showing a higher iron binding capacity than did DFO at low pH.<sup>259</sup> Similarly, Streater et al. have produced



**Figure 14.** (a) 3-Hydroxy-1-(2'-methoxyethyl)-2-methyl-4(1H)-pyridinone;<sup>247</sup> (b, c, d) hexadentate tripodal pyridinone chelators.

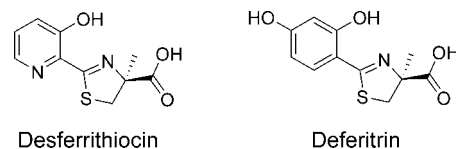


**Figure 15.** (a) Bidentate pyridinone metal chelator Pr(Me-3,2-HOPO) and (b) hexadentate analog TREN-Me-3,2-HOPO, both synthesized by the Raymond group for iron chelation.

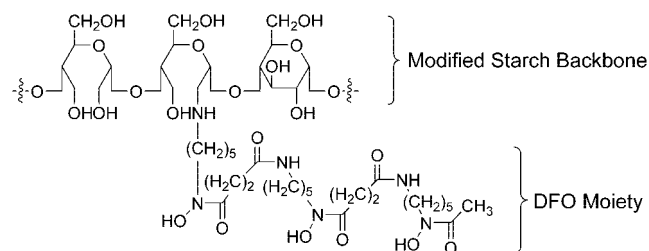
hexadentate tripodal compound 3-hydroxy-2-pyridinones with longer linkers; stable 1:1 complexes are formed with  $\text{Fe}^{3+}$ , and significant iron mobilization (from human hepatocytes, iron-overloaded mice) was achieved by one such compound (Figure 14c).<sup>260</sup> While chelation was effective (comparable to that of DFO), the overall formation constant was lower than that for the analogous tris-bidentate complex, indicating that the stereochemistry of the linked pyridinone chelating moieties can be improved.<sup>260</sup> Liu et al. have also produced hexadentate pyridinone chelators by covalent linkage of 3-hydroxy-4-pyridinone moieties around a trifunctionalized benzene core (Figure 14d).<sup>261</sup> In general, although they show improved  $\text{Fe}^{3+}$  binding over the bidentate forms, the low solubility of these compound pyridinones hinders their *in vivo* testing.

Over the past three decades, the Raymond group has developed and tested a range of derivatized chelators comprising hydroxypyridinone, hydroxamate, and catecholate metal-binding moieties. In recent work, one such hexadentate chelator TREN-Me-3,2-HOPO (*N,N',N''*-tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyrid-4-yl)carboxamidoethyl]-amine, Figure 15b) has been studied in an iron-overloaded rat model, found to be orally active, and, as expected, is a more efficient iron chelator than its "parent" bidentate compound Pr(Me-3,2-HOPO) (3-hydroxy-1-methyl-4-(1-propylcarbamoyl)-2-(1H)-pyridinone, Figure 15a).<sup>262</sup> Further to this idea, the group has formed mixed tripodal chelators by substituting in other functional groups such as 2-hydroxyisophthalamide or 2,3-dihydroxyterephthalamide for at least one of the hydroxypyridinone moieties in a bid to allow further functionalization and modification of chelator properties such as solubility.<sup>263</sup>

Deferasirox (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid, or **ICL670**, Figure 4) was first reported in



**Figure 16.** Desferrithiocin and deferitrin, orally active  $\text{Fe}^{3+}$  chelators.



**Figure 17.** Starch deferoxamine polymer **40SD02**. Adapted with permission from Wiley-Blackwell Publishing, ref 273, copyright 2007.

1999;<sup>264</sup> it is a tridentate chelator with high selectivity for  $\text{Fe}^{3+}$ , and its  $\text{NO}_2$  donation arises from one triazole nitrogen and two phenolate oxygen donors. It selectively binds  $\text{Fe}^{3+}$  over  $\text{Fe}^{2+}$  and shows little affinity for other divalent ions such as  $\text{Zn}^{2+}$  or  $\text{Cu}^{2+}$ .<sup>265</sup> *In vivo*, this selectivity is demonstrated by conserved plasma Zn and Cu levels in patients taking deferasirox, and while its efficacy is rather low for inducing negative iron balance, it is effective and well-tolerated.<sup>266</sup> In 2005, deferasirox became the first FDA-approved oral alternative for treatment of iron overload (with orphan drug designation) and was subsequently approved in the EU in 2006.<sup>267</sup> Its relatively long half-life before excretion allows once-daily dosage and good overall patient compliance, as well as cost-effectiveness, and deferasirox is considered to be superior to DFO. Likely because of its significant aromatic structure, the drug is able to permeate biological membranes; *in vitro* and *in vivo* assays demonstrate its ability to enter into and chelate iron from human cells.<sup>230</sup> The pharmacokinetics of deferasirox have been recently reviewed.<sup>267</sup>

Desferrithiocin (2-(3-hydroxypyrid-2yl)-4-methyl- $\Delta^2$ -thiazoline-4(S)-carboxylic acid or DFT, Figure 16) is a tridentate bacterial siderophore, forming stable 2:1 complexes with  $\text{Fe}^{3+}$  with  $\text{NO}_2$  donation from the thiazole nitrogen, phenolate, and carboxylate groups. While DFT itself is severely nephrotoxic, it was found to be orally effective and thus developed further in a bid to formulate a safer analog. Deferitrin (4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-methylthiazole-4(S)-carboxylic acid, or **GT-56-252**; also known as 4'-(HO)-DADFT or 4'-hydroxydesazadesferrithiocin, Figure 16) is one of the most effective and nontoxic compounds in this class of desferrithiocin-derived iron chelators. Deferitrin has been developed as an orally available treatment for thalassemia-related iron overload, and it is now in phase II clinical trials.<sup>268</sup> New analogs are being developed, and structure-activity studies are being performed in an effort to identify a lower toxicity effective iron chelator for clinical use.<sup>269</sup>

Starch deferoxamine polymers (known as S-DFO) such as hydroxyl-ethyl starch-DFO (HES-DFO) and **40SD02** (Figure 17) may be synthesized by covalent attachment of DFO to modified starch polymers in such a way that the affinity and specificity for iron are unaffected, while the plasma half-life is extended and the toxicity is lowered compared with the parent drug.<sup>270,271</sup> The HES-DFO polymer



has shown good tolerability in trials on healthy volunteers<sup>271</sup> and has been tested on a small group of iron-overloaded patients.<sup>272</sup> The **40SD02** polymer differs slightly in structure in that it contains a higher ratio of DFO to starch unit; in a phase I clinical trial in thalassemia patients, treatment with **40SD02** was well tolerated and effected a substantial increase in iron excretion.<sup>273</sup> Like DFO, starch polymers of the drug must be administered intravenously; however, unlike DFO they persist in patient plasma for a longer period of time such that the polymeric forms may be given on a much less frequent schedule (**40SD02** is effective when administered on a weekly basis). This dosage schedule is expected to greatly increase patient compliance vs DFO.

Over the past 30 or 40 years, hundreds of iron chelators have been designed and investigated for clinical use in the treatment of iron overload; inefficacy or toxicity issues have halted the development of the majority of them. These molecules comprise all manner of compounds including synthetic hydroxamic acids, catechols, and pyridoxalisonicotinoylhydrazone (PIH) analogs (see section 3.3.2) of varying denticity and lipophilicity.<sup>251</sup>

### 3.4.3. The Future of Iron Chelation for Treatment of Overload Conditions

It is very likely that the future of therapeutic iron binding and removal involves the use of more than one chelating drug at one time, that is, a smaller bidentate pro-ligand alongside a hexadentate one, to effect greater iron removal with lower total drug dose. *In vitro*, this concept has been demonstrated since the mid-1970s with various small molecule chelators used in conjunction with DFO,<sup>274</sup> and recently the concept has been confirmed using clinically relevant concentrations of deferiprone and DFO together to remove iron more quickly from transferrin than either chelator used alone.<sup>275</sup> Deferiprone and DFO have been used together in iron-overloaded patients; not only was combination therapy well-tolerated, but it caused significant improvement in at least one additional observed end point (such as cardiac function) vs treatment with DFO alone,<sup>254,276</sup> even over a longer treatment span.<sup>255</sup> The smaller bidentate chelator functions as a “shuttle” to first bind the biological metal ion then transfer it to the multidentate chelator, which acts as a “sink” for the ion. Deferiprone and DFO are particularly good representatives for this model, because deferiprone is the smaller, more lipophilic pro-ligand, able to permeate the cell and bind intracellular metal ions, while DFO, with higher lipophilicity but with high affinity for metal ions, acts as the extracellular ion sink. Combination therapies are thought to provide higher efficacies and lower toxicities than current monotherapies and, in addition to new chelators in development, will provide new options for clinical treatment of iron overload.

## 3.5. Wilson’s Disease

### 3.5.1. Introduction to Wilson’s Disease

Wilson’s disease (WD) is an autosomal recessive genetic disorder of copper metabolism; specifically,  $\text{Cu}^{2+}$  uptake into hepatocytes is impaired, providing for reduced copper excretion and resulting in the accumulation of copper in many organs and tissues of the body.<sup>277</sup> The condition affects between one in 30 000 and one in 100 000 people, and while it was first described by Kinnier Wilson in 1912,<sup>278</sup> the

accumulation of copper was not demonstrated until 1948.<sup>279</sup> The disease is caused by mutations in the gene *ATP7B*; its product (ATP7B), also known as the Wilson’s disease protein (WND), is a transmembrane protein ATPase that transports copper into the secretory pathway for incorporation into ceruloplasmin for biliary excretion (the only mechanism for copper excretion from the body).<sup>280</sup> Little is known about the role of this protein in the CNS, although it is known to be expressed in the brain. Neurological symptoms are present in 40–50% of patients with WD<sup>281</sup> and include difficulty with speech, coordination, and movement; changes in brain pathology are visible by MRI, and “Kayser–Fleischer” rings around the corneas are also typical. The tissue effects of copper overload are seen in mitochondrial damage and lipid oxidation in the liver, likely attributable to ROS generation by high concentrations of the redox-active copper ions.<sup>282</sup> Once the liver has stored copper to capacity, copper is released to the circulation and taken up by other tissues, depositing selectively in the brain.<sup>283</sup>

### 3.5.2. Wilson’s Disease Treatment

There are usually two phases of treatment for symptomatic WD patients: first, a chelating agent is administered to effect systemic depletion of metals by urinary excretion (i.e., D-penicillamine or TETA), then for maintenance of treated symptomatic patients or for treatment of asymptomatic patients, either a chelating agent or zinc salts are administered to prevent accumulation or reaccumulation of copper to toxic levels.<sup>284</sup> Superficially, the treatment of WD is similar to that of AD by metal ion binding; however, BBB penetration has not been an important driving force in WD therapeutic development. In WD therapy, systemic metal ion depletion is achieved vs specific “metal–protein attenuation” or redistribution of metal ions within the system as has been suggested for AD and FRDA therapy.

**Metal Binding Compounds Used Historically for WD Therapy.** The first use of a metal binding agent for therapy of WD copper overload was by neurologist Derek Denny-Brown in 1951 when he administered intramuscular BAL to a patient.<sup>285</sup> British anti-Lewisite (2,3-dimercaptopropanol, dimercaprol, Figure 3) contains two thiol groups that compete with body protein for copper binding; once copper is chelated, the complex is excreted in the urine.

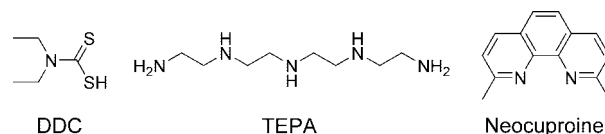
D-Penicillamine ((2*S*)-2-amino-3-methyl-3-sulfanylbutanoic acid, D-pen, Figure 3) was developed to improve upon BAL, because it is orally active, and was first used in WD therapy in 1956.<sup>286</sup> The selection of D-pen was based on an observation that patients receiving parental administration of penicillin excreted penicillamine (3,3-dimethyl-D-cysteine) in their urine, suggesting that the compound may be bioavailable in its reduced state and therefore useful for metal ion passivation. D-pen is a reductive chelator for redox-active  $\text{Cu}^{2+}$  and is most effective for WD treatment in combination with  $\text{Zn}^{2+}$  salts. It is considered to be an aggressive treatment in that it binds and removes a significant amount of copper from the body very quickly and it induces a negative copper balance over time. Another possible disadvantage to D-pen is its considerable hydrophilicity, which would prevent the drug from being able to passively permeate the BBB. More lipophilic hexyl, benzyl, and methyl ester derivatives of D-pen have been generated, which could theoretically increase cell uptake of the chelator.<sup>287</sup> This approach does not fall under the prodrug approach however, because the active thiol donor is not masked.

Triethylenetetraamine (*N,N'*-bis(2-aminoethyl)-1,2-ethanediamine, trientine, or TETA, Figure 3) was introduced in 1982 to be used in patients intolerant of D-penicillamine.<sup>288</sup> Interestingly, because of the narrow scope of its FDA-approval for WD therapy (only for use in D-pen intolerant patients), no formal human toxicity study has been performed. Like D-pen, TETA is hydrophilic, is orally available, complexes copper in the body, and increases its urinary excretion; however, it is considered to be less potent than D-pen.<sup>289</sup> In neurologically presenting WD patients, the use of copper chelators (D-pen, TETA) has been observed to actually worsen neurological symptoms at the beginning of treatment;<sup>290,291</sup> it is likely that freeing protein-bound  $\text{Cu}^{2+}$  (especially by mobilizing hepatic copper) temporarily elevates blood and brain copper levels. During this period of neurological deterioration, an increased ratio of albumin in the CSF/serum is noted, indicating BBB perturbation.<sup>60</sup> It has been suggested that monitoring this CSF/serum albumin ratio may be useful to guide the calculation of chelator dosage and modulation to less aggressive treatment as required.<sup>60</sup>

Both DMSA (*meso*-2,3-dimercaptosuccinic acid) and DMPS (D,L-2,3-dimercapto-1-propanesulfonic acid) were developed as less toxic, more hydrophilic BAL analogs (Figure 3); DMSA has been used to treat hundreds of patients with WD in China for over 40 years.<sup>292</sup> Both compounds were reviewed by Aposhian in 1995.<sup>293</sup>

**Non-Chelation-Based WD Therapies.** Zinc salts are now administered as zinc sulfate or acetate for treatment of WD after it was noticed in 1978 that zinc therapy in sickle cell anemia patients elicited copper deficiency.<sup>294</sup> The application of zinc salts to the therapy was developed at about the same time by both Brewer and Hoogenraad.<sup>295</sup> Zinc ions are thought to interfere with the intestinal absorption of  $\text{Cu}^{2+}$ , possibly by induction of intestinal cell zinc metallothionein production and excretion; alimentary  $\text{Cu}^{2+}$  then displaces  $\text{Zn}^{2+}$  in metallothionein and thus is not absorbed. Another possible mechanism for the efficacy of  $\text{Zn}^{2+}$  for WD therapy is by induction of hepatic metallothionein to bind hepatic  $\text{Cu}^{2+}$  and reduce further damage in the liver. Today zinc salts are administered with chelators to WD patients, and in fact, it has been suggested by some that chelators such as D-pen be discontinued after initial treatment, leaving only zinc supplementation for maintenance therapy.<sup>296</sup>

**Other Metal Binding Agents Tested for WD Therapy.** 3-Hydroxy-1,2-dimethyl-4(1*H*)-pyridinone (deferiprone, Figure 4, sections 3.1.8 and 3.4.2) was tested in a transgenic mouse model of WD with no significant decrease of the elevated copper levels (or iron levels); in fact, deferiprone increased the brain level of copper in the animals.<sup>297</sup> In the same study, however, it was noted that tetrathiomolybdate (TTM,  $[\text{MoS}_4]^{2-}$ ) was highly effective in removing elevated copper from the mouse model brain. The compound is a specific copper chelator developed for treatment of Wilson's disease<sup>284</sup> and is typically administered as the diammonium salt. Its application to reduction of body copper burden was discovered in the analysis of a particular ruminant disease in New Zealand and Australia caused by high levels of molybdenum in the soil. The molybdenum was converted in the animals' rumen to thiomolybdates, which were effecting copper deficiency.<sup>298</sup> Its mode of action differs from those of other WD therapeutics already discussed in that TTM forms stable ternary complexes with copper ions and protein, which are unavailable for cellular uptake.<sup>299</sup> Two



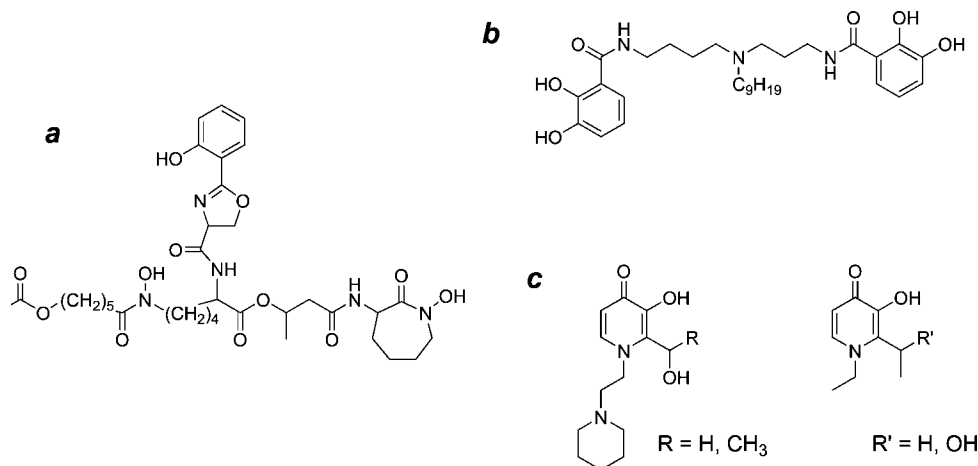
**Figure 18.** Small-molecule chelators inhibiting ALS-related oxidative reactions in cell-free, *in vitro* cell culture, and animal models of the disease: diethyldithiocarbamate (DCC), tetraethylenepentamine (TEPA), and 2,9-dimethyl-1,10-phenanthroline (neocuproine).

modes of action are biologically important: first, TTM binds alimentary  $\text{Cu}^{2+}$  in the gastrointestinal tract and prevents its absorption; if administered without food, TTM is absorbed into the blood and complexes  $\text{Cu}^{2+}$  along with plasma albumin. The complex is then metabolized by liver such that copper excretion is achieved via the bile. Tetrathiomolybdate is now administered to patients for initial therapy followed by zinc acetate for maintenance therapy with good results<sup>284</sup> and is thought to be a better choice than traditional chelation (such as by TETA) alone for treatment of neurologically affected WD patients.<sup>300</sup> Overall, a combination of therapies is recommended comprising chelators such as D-pen, TETA, or TTM and zinc supplementation to avoid worsening of neurological symptoms and excessive depletion of copper.

## 3.6. Other Therapeutic Applications of Small-Molecule Chelators for Metal Ion Passivation and Removal

### 3.6.1. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is among the most common neuromuscular diseases today with about 5–7 cases per 100 000 population.<sup>301</sup> Commonly affecting those in middle age, ALS involves a loss of motor neurons in the spinal cord and in the brain leading to muscle wasting, respiratory failure, and death in approximately 5 years or less from time of onset. Current drug therapies generally focus on bolstering neuron function.<sup>301</sup> Like the other neurodegenerative diseases AD and PD, ALS comprises both a sporadic (SALS) and a more rare familial form of the disease (FALS), with over 100 identified mutations in the superoxide dismutase (SOD) gene alone.<sup>302</sup> The condition emulates AD in that it is linked with the deposition of protein aggregates in the neural tissue, as aggregates of mutant copper/zinc superoxide dismutase enzyme, SOD1, are observed in ALS transgenic mice.<sup>303</sup> A recent review<sup>304</sup> gives an overview of the pathological processes involved in ALS, including a putative role for zinc in the development of the condition. There is also evidence implicating metal ion-mediated redox reactions in the pathology of the disease; mutant enzymes catalyze  $\text{H}_2\text{O}_2$  oxidation of a given substrate at a higher rate than does the wild-type enzyme and can cause death of cultured neuronal cells. This mutant-catalyzed reaction is particularly sensitive to inhibition by diethyldithiocarbamate (DDC, Figure 18) and penicillamine chelators,<sup>305</sup> and cell death can be prevented by addition of tetraethylenepentamine (TEPA, Figure 18).<sup>306</sup> Separate experiments using the transgenic mouse model of FALS demonstrated delayed onset of the disease<sup>307</sup> and extended survival with oral administration of D-pen<sup>307</sup> or BAPTA diesters such as **DP-109** (Figure 8);<sup>308</sup> copper chelators such as DDC and 2,9-dimethyl-1,10-phenanthroline (neocuproine, Figure 18) inhibited death of cells cultured from the same



**Figure 19.** (a) **D-Exo 772SM**, a desferri-exochelin siderophore tested for antiproliferative activity,<sup>316</sup> (b) dicaticholate compound **FR160** showing *in vitro* inhibition of *P. falciparum*,<sup>322</sup> and (c) four 3-hydroxy-4-pyridinone derivatives showing effective iron removal from overloaded rats and inhibition of *in vitro* *P. falciparum* growth.<sup>325</sup>

mouse FALS model.<sup>309</sup> These results suggest that redox-active metal ion (i.e.,  $\text{Cu}^{2+}$ ) binding may be useful in ALS therapy.

### 3.6.2. Transmissible Spongiform Encephalopathy

The transmissible spongiform encephalopathies (TSEs, including mad cow and Creutzfeldt–Jakob diseases) are predominantly sporadic (80–85%) but may be inherited (10–15%) and are characterized by rapidly progressing dementia and early death, frequently accompanied by other neurological features such as ataxia and involuntary muscle spasms.<sup>310</sup> The TSEs are caused by a change in protein conformation; the protein of interest (cellular prion protein, PrP<sup>c</sup>) is membrane-associated and is transformed by some unclear mechanism into the pathogenic form called scrapie (PrP<sup>Sc</sup>).<sup>311</sup> Like the APP peptide implicated in AD, while the normal function of PrP<sup>c</sup> is not yet established, the protein is thought to bind  $\text{Cu}^{2+}$ , and in fact, much recent research has focused on the metal ion binding properties of PrP<sup>c</sup>. The functions of PrP<sup>c</sup> and PrP<sup>Sc</sup>, their  $\text{Cu}^{2+}$  binding modes, the involvement of metal ions in prion disease, and its parallels to AD have been reviewed.<sup>312,313</sup> While 30 years of study on human prion disease and possible treatments have made little progress in identifying effective treatments,<sup>310</sup> metal ion-focused interventions may show some promise, because copper chelation with D-pen has been shown to significantly delay the onset of prion disease in mice.<sup>314</sup>

### 3.6.3. Other Emerging Applications for Metal Ion Passivation and Removal

Other conditions for which metal ion binding and removal have been proposed or tested include cancer and malaria. Only brief mention will be made of these applications of metal chelation, and the reader will be referred to other more comprehensive reviews.

While cancer does not involve true metal ion overload, iron is a requirement for cell cycle progression and for DNA synthesis; thus, cancer cells have a higher demand for the ion and are particularly susceptible to its depletion. Iron chelators including DFO and other hexadentate naturally occurring siderophores such as desferri-exochelin (i.e., **D-Exo 772SM**, Figure 19a),<sup>315</sup> DFT, PIH, and their respective analogs, hydroxypyridinone derivatives including defer-

iprone, and TTM have been investigated as antiproliferatives for cancer therapy.<sup>316</sup>

Half the world's population is at risk of malaria, and in 2006 alone an estimated 247 million cases led to nearly a million deaths, mostly in young children.<sup>317</sup> Malaria is a blood-borne disease caused by one of a few parasites, particularly *Plasmodium falciparum*, which require iron for many processes involved in growth and multiplication; thus, the use of iron chelators to limit the amount of  $\text{Fe}^{3+}$  available for parasite use has been suggested as an alternative to current therapies against which the parasites increasingly develop resistance.<sup>247</sup> Desferrioxamine has demonstrated antimalarial activity in humans;<sup>318</sup> however, is not used clinically for this purpose. A number of iron chelators can inhibit *Plasmodium* sp. growth both *in vitro* and *in vivo* including deferasirox<sup>319</sup> (section 3.4.2), PIH and derivatives<sup>320</sup> (section 3.3.2), dicaticholate<sup>321</sup> (Figure 19b), and a number of hydroxypyridinones,<sup>322,323</sup> among others.<sup>324</sup> In the case of hydroxypyridinones, host toxicity has been a problem. Thus, to better target the compound to the parasite, a range of hydroxypyridinones with basic moieties has been designed and tested; it is thought that the protonated conjugate acid will accumulate in acidic vesicles within the infected red blood cells.<sup>325</sup> A number of these hydroxypyridinones (Figure 19c) showed good *in vivo* iron-scavenging ability and effective *in vitro* antimalarial activity, whether or not basic moieties were incorporated.<sup>325</sup>

### 3.6.4. Heavy Metal Intoxication

Elements such as aluminum, antimony, arsenic, bismuth, copper, lead, mercury, nickel, tin, and zinc can be toxic upon adventitious exposure, which usually occurs by overingestion, pesticide exposure, or other environmental or occupational exposures. (Extensive reviews have been provided by Andersen.<sup>21,326</sup>) Because it is relatively rare, there is little pressure to develop new chelators specifically for treatment of intoxication; however, those chelating drugs approved for other indications such as neurodegenerative diseases or genetic disorders involving metal dishomeostasis may prove to benefit this medicinal application as well.

## 4. Summary and Future Research Directions

Therapeutic metal ion manipulation, including redistribution or removal, is one of the main applications of inorganic



chemistry to the field of medicine. The most widely recognized clinical use of metal ion chelators is in treatment of adventitious overload of heavy metals. Iron overload and WD are two other conditions for which the current treatment of choice is chelation, in these cases for excretion of excess metal ions accumulated either as a side effect of another intervention or due to inherited deficiencies in metal ion homeostatic processes. Based on relatively recent hypotheses assigning a causative role to metal ions in diseases such as AD, PD, and FRDA, metal ion targeting therapeutics may be the future of treatment for these conditions as well, serving not only to attenuate the symptoms of the conditions but also to intervene in their respective progressions. In contrast with conventional chelation, metal-targeted strategies for these conditions are based on inhibiting abnormal metal ion–protein interactions or remediating localized elevations or deficiencies in metal ion concentrations as opposed to increasing systemic excretion of metals. Although the state of development of chelating therapeutics for each of these neurodegenerative diseases vary, the use of *in vitro* assays mimicking disease conditions and animal disease models have identified many potentially useful compounds for their treatment. As more chelators reach the point of clinical use, it is very likely that both off-label and combination uses of these chelators will be prove useful, and in fact, combination therapy is already a valuable approach for passivation and removal of excess iron.

Within such a complicated system as the body, it is exceedingly difficult to target the specific metal ion of interest in a particular region. Thus, the future of designed chelators for disease therapy rests on the multifunctional approach, which incorporates not only metal ion binding but tissue targeting with appended biomolecules, ligands for particular tissue receptors, or manipulation of physical properties. There are multiple biochemical aberrations in any given disease state that also need to be addressed, and although these could be addressed with combinations or “cocktails” of different therapeutics, it may be more desirable to build these functionalities into one molecule for delivery. For example, building a true multifunctional therapeutic for metal-associated neurodegenerative disease will require the construction of a binding agent specific for the metal ion of interest. It should be able to access the brain, via incorporation of appended biomolecules, biomolecule mimicry, or the appropriate physical properties to passively diffuse across the BBB, and it must be targeted to the location of interest within the brain. Finally, it should address the peripheral pathologies involved, for example, mitigate oxidative stress through incorporation of antioxidant functionality.

## 5. Abbreviations

6-OHDA	6-hydroxydopamine, 2,4,5-trihydroxyphenylethylamine
<b>40SD02</b>	one type of starch desferrioxamine polymer (S-DFO)
A $\beta$	$\beta$ -amyloid peptide
AD	Alzheimer's disease
AFM	atomic force microscopy
APP	amyloid precursor protein
BAL	British anti-Lewisite, 2,3-dimercaptopropanol
BAPTA	1,2-bis(2-aminophenoxy)ethane- <i>N,N,N',N'</i> -tetraacetic acid
BBB	blood–brain barrier
BHT	butylated hydroxytoluene
CQ	5-chloro-7-iodo-8-hydroxyquinoline, clioquinol, <b>PBT1</b>
CNS	central nervous system
<b>CP502</b>	1,2-diethyl-3-hydroxy-4(1 <i>H</i> )-pyridinone, 3-hydroxy-1,6-dimethyl-2-( <i>N</i> -methyl-amido)-4(1 <i>H</i> )-pyridinone
CSF	cerebrospinal fluid
DDC	diethyldithiocarbamate
<b>D-Exo 772SM</b>	a member of the desferri-exochelin family of bacterial siderophores
D-pen	D-penicillamine, (2 <i>S</i> )-2-amino-3-methyl-3-sulfanyl-butanoic acid
deferasirox	4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid, <b>ICL670</b>
deferitritin	4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-methylthiazole-4( <i>S</i> )-carboxylic acid, <b>GT-56-252</b> , (S)-4'-(HO)-DADFT, 4'-hydroxydesazadesferriothiocin
desferrithiocin	2-(3-hydroxypyrid-2yl)-4-methyl- $\Delta^2$ -thiazoline-4( <i>S</i> )-carboxylic acid, DFT
DFO	<i>N'</i> -[5-(acetyl-hydroxy-amino)pentyl]- <i>N</i> -[5-[3-(5-aminopentyl-hydroxy-carbamoyl)propanoylamino]pentyl]- <i>N</i> -hydroxy-butane diamide, desferrioxamine, desferrioxamine-B, deferoxamine, Desferal, DFO-B
DFT	density functional theory
DMPS	D,L-2,3-dimercapto-1-propanesulfonic acid
DMSA	<i>meso</i> -2,3-dimercaptosuccinic acid
<b>DP-109</b>	1,2-bis(2-aminophenoxy)ethane- <i>N,N,N',N'</i> -tetraacetic acid
DTPA	diethylenetriaminepentaacetic acid
EDTA	ethylenediaminetetraacetic acid
EGCG	(-)-epigallocatechin-3-gallate
ELISA	enzyme-linked immunosorbent assay
EPR	electron paramagnetic resonance
EU	European union
FALS	familial amyotrophic lateral sclerosis
FDA	the U.S. Food and Drug Administration
FRDA	Friedreich's ataxia
FT-IR	Fourier transform infrared spectroscopy
GLUT	a protein belonging to the glucose transport facilitator family
HES-DFO	hydroxyethyl starch-desferrioxamine polymer
HIV	human immunodeficiency virus
<b>Hnbp</b>	1-benzyl-3-hydroxy-2-methyl-4(1 <i>H</i> )-pyridinone
<b>Hppp</b>	3-hydroxy-2-methyl-1-phenyl-4(1 <i>H</i> )-pyridinone
<b><sup>125</sup>I-Gipp</b>	3-( $\beta$ -D-glucopyranosyloxy)-2-methyl-1-(4-[ <sup>125</sup> I]iodophenyl)-4(1 <i>H</i> )-pyridinone
<b>JKL 169</b>	1,1'-xylyl bis-1,4,8,11-tetraazacyclotetradecane
L-DOPA	3-(3,4-dihydroxyphenyl)-L-alanine
<b>L1</b>	3-hydroxy-1,2-dimethyl-4(1 <i>H</i> )-pyridinone, deferiprone, <b>CP20</b> , Ferriprox
<b>L1NAII</b>	1-allyl-3-hydroxy-2-methyl-4(1 <i>H</i> )-pyridinone
<b>L1Net</b>	1-ethyl-3-hydroxy-2-methyl-4(1 <i>H</i> )-pyridinone
<b>M30</b>	5-( <i>N</i> -methyl- <i>N</i> -propargylaminomethyl)-8-hydroxyquinoline
MAO	monoamine oxidase
Met35	methionine-35 (residue at position 35 of the peptide)
MPAC	metal–protein attenuating compound
MPTP	<i>N</i> -methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
NAC	( <i>R</i> )-2-acetamido-3-sulfanyl-propanoic acid, <i>N</i> -acetyl cysteine
NACA	<i>N</i> -acetyl cysteine amide, <b>AD4</b>
NFT	neurofibrillary tangle
NMDA	<i>N</i> -methyl-D-aspartate
NMR	nuclear magnetic resonance
NP	nanoparticle
<b>PBT2</b>	dichloro analog of clioquinol (structure undisclosed)

PCIH	2-pyridylcarboxyaldehyde isonicotinoyl hydrazone
PCTH	2-pyridylcarboxyaldehyde 2-thiophenecarboxyl hydrazone
PD	Parkinson's disease
PET	positron emission tomography
PIH	pyridoxal isonicotinoyl hydrazone
Pr(Me-3,2-HOPO)	3-hydroxy-1-methyl-4-(1-propylcarbamoyl)-2-(1H)-pyridinone
PSA	polar surface area
ROS	reactive oxygen species
S-DFO	starch desferrioxamine polymers of varying structure
SALS	sporadic amyotrophic lateral sclerosis
SIMS	secondary ion mass spectrometry
SPECT	single photon emission computed tomography
TEPA	tetraethylenepentaamine
TETA	triethylenetetraamine
TREN-Me-3,2-HOPO	<i>N,N',N''</i> -tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydro-4-pyridyl)carboxamidoethyl]-amine
TTM	tetrathiomolybdate
VK-28	(5-[4-(2-hydroxyethyl) piperazine-1-ylmethyl]-quinoline-8-ol)
WD	Wilson's disease
WND	Wilson's disease protein, also known as ATP7B
XAS	X-ray absorption spectroscopy
XH1	[(4-benzothiazol-2-yl-phenylcarbamoyl)-methyl]-{2-[(2-[(4-benzothiazol-2-yl-phenylcarbamoyl)-methyl]-carboxymethyl-amino)-ethyl]-carboxymethyl-amino}-ethyl]-amino)-acetic acid

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