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# The Ongoing Search for Small Molecules to Study Metal-Associated Amyloid-$\beta$ Species in Alzheimer's Disease

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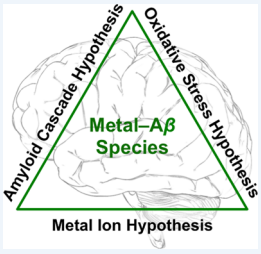
**CONSPECTUS:** The development of a cure for Alzheimer's disease (AD) has been impeded by an inability to pinpoint the root cause of this disorder. Although numerous potential pathological factors have been indicated, acting either individually or mutually, the molecular mechanisms leading to disease onset and progression have not been clear. Amyloid-$\beta$ (A$\beta$), generated from proteolytic processing of the amyloid precursor protein (APP), and its aggregated forms, particularly oligomers, are suggested as key pathological features in AD-affected brains. Historically, highly concentrated metals are found colocalized within A$\beta$ plaques. Metal binding to A$\beta$ (metal−A$\beta$) generates/stabilizes potentially toxic A$\beta$ oligomers, and produces reactive oxygen species (ROS) in vitro (redox active metal ions; plausible contribution to oxidative stress). Consequently, clarification of the relationship between A$\beta$, metal ions, and toxicity, including oxidative stress via metal−A$\beta$, can lead to a deeper understanding of AD development.




To probe the involvement of metal−A$\beta$ in AD pathogenesis, rationally designed and naturally occurring molecules have been examined as chemical tools to target metal−A$\beta$ species, modulate the interaction between the metal and A$\beta$, and subsequently redirect their aggregation into nontoxic, off-pathway unstructured aggregates. These ligands are also capable of attenuating the generation of redox active metal−A$\beta$-induced ROS to mitigate oxidative stress. One rational design concept, the incorporation approach, installs a metal binding site into a framework known to interact with A$\beta$. This approach affords compounds with the simultaneous ability to chelate metal ions and interact with A$\beta$. Natural products capable of A$\beta$ interaction have been investigated for their influence on metal-induced A$\beta$ aggregation and have inspired the construction of synthetic analogues. Systematic studies of these synthetic or natural molecules could uncover relationships between chemical structures, metal/A$\beta$/metal−A$\beta$ interactions, and inhibition of A$\beta$/metal−A$\beta$ reactivity (i.e., aggregation modes of A$\beta$/metal−A$\beta$; associated ROS production), suggesting mechanisms to refine the design strategy.

Interdisciplinary investigations have demonstrated that the designed molecules and natural products control the aggregation pathways of metal−A$\beta$ species transforming their size/conformation distribution. The aptitude of these molecules to impact metal−A$\beta$ aggregation pathways, either via inhibition of A$\beta$ aggregate formation, most importantly of oligomers, or disaggregation of preformed fibrils, could originate from their formation of complexes with metal−A$\beta$. Potentially, these molecules could direct metal−A$\beta$ size/conformational states into alternative nontoxic unstructured oligomers, and control the geometry at the A$\beta$-ligated metal center for limited ROS formation to lessen the overall toxicity induced by metal−A$\beta$. Complexation between small molecules and A$\beta$/metal−A$\beta$ has been observed by nuclear magnetic resonance spectroscopy (NMR) and ion mobility-mass spectrometry (IM-MS) pointing to molecular level interactions, validating the design strategy. In addition, these molecules exhibit other attractive properties, such as antioxidant capacity, prevention of ROS production, potential blood-brain barrier (BBB) permeability, and reduction of A$\beta$-/metal−A$\beta$-induced cytotoxicity, making them desirable tools for unraveling AD complexity. In this Account, we summarize the recent development of small molecules, via both rational design and the selection and modification of natural products, as tools for investigating metal−A$\beta$ complexes, to advance our understanding of their relation to AD pathology.

## INTRODUCTION

Dementia is a growing problem with the aging worldwide demographic, and Alzheimer's disease (AD) is the most prevalent type, accounting for 50−80% of cases.[1] There is no cure for this neurodegenerative illness, primarily due to the difficulty of determining disease etiology.[2−4] This is a consequence of the multiple factors, operating either individually or mutually, that are proposed to contribute to disease development.[5] One recent approach examines the relation among several hypotheses to further understanding of AD etiology: the amyloid cascade hypothesis,[2,3] the metal ion hypothesis,[6] and the oxidative stress hypothesis[6,7] (Figure 1).

The amyloid cascade hypothesis suggests that the amyloid-$\beta$ (A$\beta$) peptide is the principal contributor to neurodegeneration. A$\beta$ is a proteolytic product of the amyloid precursor protein (APP) in the brain (Figure 1).[2,3] APP cleavage by $\alpha$-/$\gamma$-secretases yields shorter, nonamyloidogenic A$\beta$ peptides (ca. 25 residues) that lack the self-recognition sequence (Figure 1),

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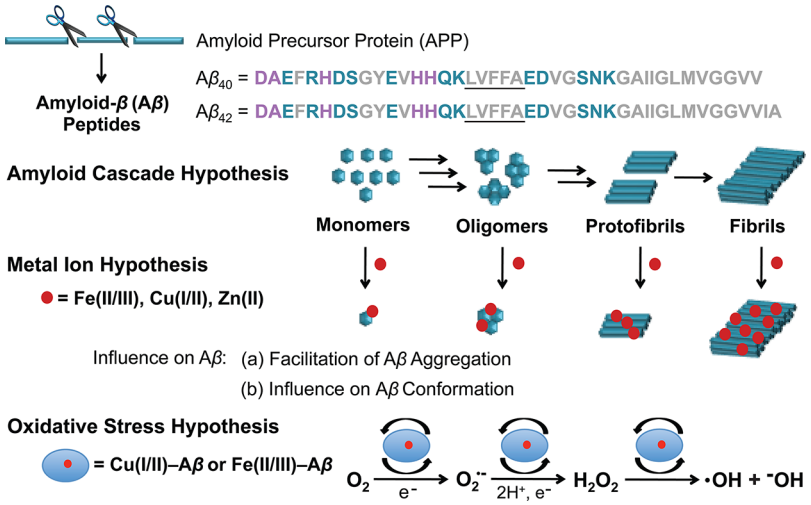


**Figure 1.** Schematic representation of the amyloid cascade hypothesis, the metal ion hypothesis, and the metal–A$\beta$-mediated oxidative stress hypothesis. The amino acid sequences of A$\beta_{40}$/A$\beta_{42}$ are shown: metal binding residues (purple); hydrophilic and hydrophobic residues (blue and gray, respectively); self-recognition sequence (underlined).

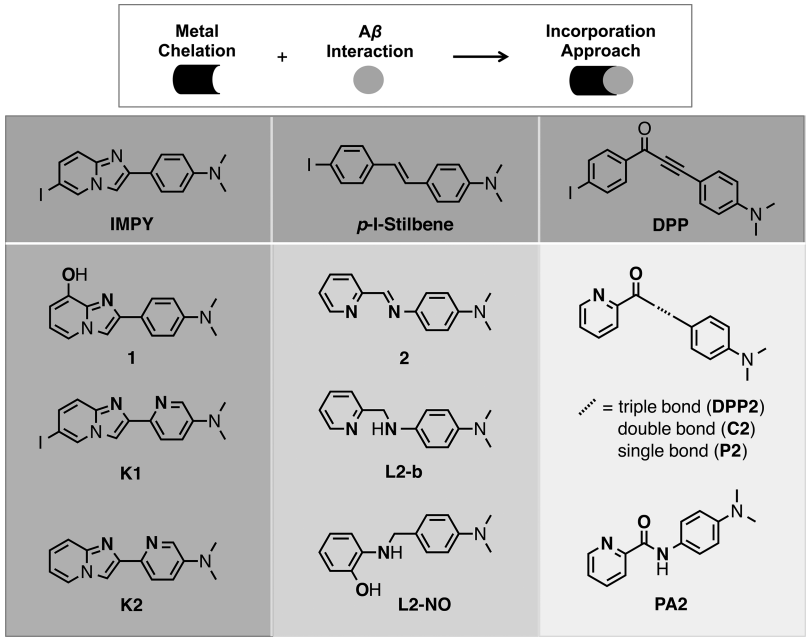


**Figure 2.** Chemical structures of small molecules designed by the incorporation approach (top). Bottom: IMPY, 4-(6-iodoimidazo[1,2-*a*]pyridin-2-yl)-*N*,*N*-dimethylaniline; **1**, 2-(4-(dimethylamino)phenyl)imidazo[1,2-*a*]pyridin-8-ol; **K1**, 6-(6-iodoimidazo[1,2-*a*]pyridin-2-yl)-*N*,*N*-dimethylpyridin-3-amine; **K2**, 6-(imidazo-[1,2-*a*]pyridin-2-yl)-*N*,*N*-dimethylpyridin-3-amine; *p*-I-stilbene, *N*,*N*-dimethyl-4-[(1*E*)-2-(4-iodophenyl)ethenyl]-benzenamine; **2**, (*E*)-*N*,*N*-dimethyl-4-((pyridin-2-ylmethylene)amino)aniline; **L2-b**, $N^1$,$N^1$-dimethyl-$N^4$-(pyridin-2-ylmethyl)benzene-1,4-diamine; **L2-NO**, 2-((4-(dimethylamino)benzyl)amino)phenol; DPP, 3-(4-(dimethylamino)phenyl)-1-(4-iodophenyl)prop-2-yn-1-one; **DPP2**, 3-(4-(dimethylamino)phenyl)-1-(pyridin-2-yl)prop-2-yn-1-one; **C2**, (*E*)-3-(4-(dimethylamino)phenyl)-1-(pyridine-2-yl)prop-2-en-1-one; **P2**, 3-(4-(dimethylamino)phenyl)-1-(pyridin-2-yl)propan-1-one; **PA2**, *N*-(4-(dimethylamino)phenyl)picolinamide). **L2-a**, **DPP1**, **C1**, **P1**, and **PA1** are the analogues of **L2-b**, **DPP2**, **C2**, **P2**, and **PA2**, respectively, that do not contain the dimethylamino group.

while $\beta$-/$\gamma$-secretases' activity produces longer, amyloidogenic peptides, A$\beta_{40}$ and A$\beta_{42}$ (ca. 90% and 9% soluble monomers, respectively). A$\beta_{40}$/A$\beta_{42}$ monomers have a propensity to aggregate into oligomers, protofibrils, and fibrils (Figure 1), which, with exogenous species, such as proteins and metal ions, form dense senile plaques (SP), a histopathological feature of AD brains.[2,3] A$\beta$ drives neurotoxicity via multiple possible routes, operating mainly through soluble A$\beta$ oligomers, leading to neuronal atrophy and death.[3]

High levels of metals (ca. 0.4 mM Cu; 1.0 mM Zn; 0.9 mM Fe) are found within SP from AD brain tissue compared to healthy tissue.[5,6,8,9] Metal binding to A$\beta$ has been widely confirmed in vitro.[5,6,8,9] Metals could influence A$\beta$ toxicity through multiple mechanisms (see the metal ion hypothesis in Figure 1): (i) A$\beta$ aggregation acceleration; (ii) various neurotoxic oligomer generation/stabilization;[6,8,10] and (iii) Fenton-like reactions at A$\beta$-bound redox active metal ions (i.e., Cu(I/II); Fe(II/III)).[5−7] Reactive oxygen species (ROS) overproduction by Cu(I/II)− and/or Fe(II/III)−A$\beta$ could damage biomolecules or overwhelm antioxidant mechanisms leading to elevated oxidative stress and inflammation (see the oxidative stress hypothesis in Figure 1).


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Metal chelators have been considered as potential therapeutic candidates, partly due to the metal ion hypothesis, and the possible contribution of metal−Aβ to AD. Two metal chelators, clioquinol (**CQ**) and its derivative (**PBT2**), reached pilot phase II and phase II clinical trials, respectively.[11,12] Administration of **CQ** and **PBT2** demonstrated a statistically significant deceleration in cognitive decline in AD patients compared to placebo control groups.[11,12] **CQ**-/**PBT2**-treated AD transgenic mice had reduced Aβ plaque burden, and altered metal ion distribution in their brains suggesting that they contain ionophore activity as a possible mode of action (MoA)].[13,14] Despite the fact that **CQ** and **PBT2** have not made it past phase II clinical trials, they have spurred an expansive exploration into metal chelation therapy. In addition, the role of metal−Aβ species in AD pathogenesis is still unknown; thus, suitable chemical tools would be valuable for offering a promising route to resolve this uncertainty. Small molecules have been developed for this purpose, either through rational structure-based design or the selection of natural products and their derivatives. These small molecules target metal−Aβ species and modulate the interaction between the metal and Aβ, thus redirecting the aggregation pathway into off-pathway, nontoxic unstructured aggregates that are different from neurotoxic metal−Aβ oligomers.[10] Additionally, these ligands could alter the geometry at the metal center of metal−Aβ complexes to attenuate ROS formation.[15,16] Investigations employing chemical tools can uncover the molecular level interactions between metal ions and Aβ, involved in AD pathology, and illustrate how exogenous ligands could influence the reactivity of metal−Aβ (i.e., metal−Aβ aggregation; metal−Aβ-induced ROS generation), possibly reducing toxicity and validating metal−Aβ as a viable therapeutic target.

## MULTIFUNCTIONAL SMALL MOLECULE DESIGN

To explore the relation of metal−Aβ to AD pathology, multifunctional molecules have been prepared via a rational structure-based design strategy, named the incorporation approach (Figure 2).[4,15,16] In this tactic, metal binding nitrogen (N) and oxygen (O) donor atoms are integrated, without significant structural alterations, into Aβ imaging frameworks (ca. nM binding affinity against Aβ fibrils).[17−27] These hybrid compounds are tested in vitro for metal binding by UV−visible spectroscopy; optical variable-pH titration experiments in aqueous solution to determine their metal binding affinities ($K_d$).[17,18,20,24,25,27] Interactions of the molecules with Aβ or metal−Aβ indicate close contact with the residues of Aβ (Figure 1), responsible for metal binding, and/or generate Aβ−ligand or Aβ−metal−ligand complexes as demonstrated by NMR and/or MS.[17−19,21,22,24,25,27] The dually equipped compounds (for metal chelation and Aβ interaction) target metal−Aβ species and form ternary Aβ−metal−ligand complexes, believed to redirect the aggregation pathway into off-pathway, nontoxic or less toxic conformations, which could contribute to the MoA of these molecules.[17,18] Therefore, implementation of this strategy might address the first aspect of metal−Aβ-induced toxicity, by guiding metal−Aβ complexes into off-pathway unstructured forms and rendering them nontoxic.

The design of ligands that exhibit reactivity with metal−Aβ species can be further refined. For example, ligand donor atoms can be varied to modulate the binding affinity for Cu(II) over Zn(II) in accordance with the Irving-Williams series, which can result in metal selectivity (i.e., for Cu(II)−Aβ over Zn(II)−Aβ).[19] Optimization of the geometry and coordination number at the metal center can further enhance metal selectivity of ligands. A relation between metal binding affinity and reactivity toward metal−Aβ has been noted; a relatively high metal binding affinity of compounds (ca. $K_d \sim 10^{-12}-10^{-9}$ for Cu(II), $K_d \sim 10^{-9}-10^{-6}$ for Zn(II))[17,18] results in reactivity against both Cu(II)− and Zn(II)−Aβ ($K_d$ of Aβ for Cu(II) and Zn(II), ca. $10^{-11}-10^{-7}$ and ca. $10^{-9}-10^{-6}$, respectively).[5,6] A relatively low $K_d$ of ligand for Cu(II) and Zn(II) (ca. $K_d > 10^{-5}$) leads to less or no noticeable reactivity against Cu(II)−/Zn(II)−Aβ.[20] Thus, matching a ligand's $K_d$ to Aβ's $K_d$ could direct marked reactivity and ternary complexation. For biological applications, the ligand's metal binding affinity needs to be within an appropriate range so that they disrupt the interaction between the metal and Aβ without sequestering essential metal ions from metalloproteins (most weakly bound $K_d$ range of $10^{-10}-10^{-8}$).[6] In addition, redox cycling can be prevented by considering the preferred geometry at the metal center, another MoA of the designed molecules.[18] This decreases metal-mediated ROS production, alleviating Cu(I/II)−Aβ-associated oxidative stress. Moreover, a number of other properties require deliberation as well: (i) Lipinski's rules set guidelines for drug-likeness of small molecules.[28] Thus, ligands are designed to conform to these constraints,[17−24] which may result in their BBB permeability to be balanced against relative water solubility;[17,18] (ii) ligand stability in aqueous media[17,21] and in vivo, with low cytotoxicity, would be necessary;[17,18,20,22−24] (iii) incorporation of antioxidant capacity. Consideration of these properties produces improved molecules,[18] and keeping these criteria in mind,[16,18] metal binding sites have been integrated into several Aβ imaging scaffolds (Figure 2).[17−24] These chemical classes have been employed to elaborate the relationships between chemical structures, metal/Aβ/metal−Aβ interactions, and inhibitory activity toward metal−Aβ reactivity (e.g., modulation of toxic oligomer formation; ROS formation).

### Class 1

The incorporation approach was initially applied to create compounds **1** and **2**,[21] with minimal structural modification to two Aβ imaging frameworks, IMPY and *p*-I-stilbene, by inserting oxygen (O) and nitrogen (N) donor atoms, respectively (Figure 2). Compared to conventional chelators (e.g., EDTA), these molecules impacted the resultant Cu(II)−Aβ conformations by regulating Cu(II)-induced Aβ aggregation via (i) inhibition of Aβ aggregate formation (e.g., oligomers; fibrils), and (ii) disaggregation of preformed Aβ fibrils. This demonstrated that combining moieties for metal chelation and Aβ interaction imparted reactivity against Cu(II)−Aβ.[21] Gel electrophoresis/Western blot (Gel/Western blot) of compound-treated Cu(II)−Aβ indicated that **1** and **2** altered the molecular weight (MW) distribution of Aβ species compared to compound-free samples. Transmission electron microscopy (TEM) studies presented that the molecules triggered the formation of smaller, amorphous Aβ aggregates, proposed to be nontoxic,[29] upon treatment of either monomeric Cu(II)−Aβ or preformed fibrils. Both compounds could moderate hydrogen peroxide ($H_2O_2$) generation by Cu(I/II)−Aβ in the presence of $O_2$/ascorbate as determined by an Amplex Red assay; **2** could reduce toxicity from Cu(II)−Aβ in living cells.[21] The direct interaction of **1** or **2** with Aβ was confirmed by 2D NMR.[21] $^1$H and $^{15}$N amide backbone signals near and/or at metal binding residues of Aβ shifted the most upon addition of **1** or **2**, suggesting that the molecules may have close contacts


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with the metal binding site of A$\beta$.[21] Follow-up studies employing an analogue of **2** without the dimethylamino moiety showed weakened reactivity against Cu(II)−A$\beta$, indicating the critical nature of this functional group for the reactivity of **2** with Cu(II)−A$\beta$.[22]

### Class 2

**1** and **2** demonstrated a proof of concept for the incorporation approach and the feasibility of designing small molecules to target metal−A$\beta$ species and modulate their reactivity; however, optimization of the compounds' properties was needed. One drawback of **2** was its limited aqueous stability due to imine hydrolysis. To address this, **L2-b** (Figure 2) was prepared by reduction of the imine (**2**) to amine.[17] The compound **L2-a**, an analogue of **L2-b** that does not contain a dimethylamino group, was also synthesized to reinforce the importance of this functionality for reactivity toward A$\beta$/metal−A$\beta$.[17] **L2-b** indicated that (i) reduction of imine to amine did not abrogate the interaction and reactivity toward metal−A$\beta$, but improved aqueous solubility and stability; (ii) comparison to **L2-a** underscored the importance of the dimethylamino functionality influencing of metal−A$\beta$ aggregation pathways. **L2-b** exhibited $K_d$ ∼$10^{-9}$ and ∼$10^{-6}$ for Cu(II) and Zn(II), respectively, in a range similar to A$\beta$, which enabled this compound to affect metal-induced A$\beta$ aggregation leading to the modified size and conformation distribution of the resultant A$\beta$ species' size and conformation distribution.[17] **L2-b**'s ex vivo efficacy toward aggregated A$\beta$ in AD brain tissue homogenates was evaluated, affording a greater amount of gel permeable, lower MW A$\beta$ species, compared to **L2-b**-untreated samples. Thus, **L2-b** could affect the distribution of A$\beta$, even in the complex, heterogeneous environment of brain tissue homogenates.[17]

**L2-NO** (Figure 2) was also designed as an **L2-b** derivative by replacing (N^N) for metal chelation with (N^O) within the scaffold of *p*-I-stilbene.[19] This change was intended to lower the ligand's affinity for Cu(II) and Zn(II) to a range in which its $K_d$ only made it competitive for Cu(II)−A$\beta$, for which it was selective.[19] The reactivity of **L2-NO** toward Cu(II)−A$\beta$ was affirmed by Gel/Western blot and TEM, and it was shown to interact with A$\beta$ and control ROS formation in a manner akin to **L2-b**.[19]

### Class 3

To expand the repertoire of scaffolds, IMPY derivatives similar to **1** (class 1) were explored in greater detail to yield **K1** and **K2** (Figure 2), but these exhibited a relatively weak ability to influence the size/conformation distribution of metal−A$\beta$.[23] A diphenylpropynone framework, also reported as an A$\beta$ imaging agent,[30] was used to generate **DPP1** and **DPP2** (Figure 2).[24] Metal chelation via N and O (carbonyl) donor atoms resulted in $K_d$ ∼ $10^{-7}$ of **DPP2** for Cu(II).[24] As in previous studies,[17,22] **DPP2** controlled metal-free and metal-triggered A$\beta$ aggregation pathways to a greater extent than **DPP1** that lacks a dimethylamino group. **DPP1/2** were limited in their biological applications due to their micromolar cytotoxicity,[24] possibly due to their triple bond in combination with a carbonyl group (ynone) that acts as a Michael acceptor to form covalent adducts with biomolecules.[31] This aspect was addressed in a class of DPP analogues where the triple bond was systematically reduced or modified.[20]

### Class 4

A family of DPP derivatives was prepared to mitigate cytotoxicity and establish a structure−reactivity−toxicity relationship. The triple bond of **DPP1/2** was reduced to a double or single bond to produce **C1/2** or **P1/2** (Figure 2).[20] **PA1/2** were constructed by connecting the 2-pyridyl ketone group from **DPP1/2** to **L2-b**'s aniline portion through an amide group, rather than the carbon linker of **DPP1/2**.[20] This compound library reasserted the importance of the dimethylamino functionality for pronounced reactivity against metal−A$\beta$ species, and offered insight on the effect of the structural portion on cytotoxicity and reactivity. **C1/2**, although somewhat less toxic than **DPP1/2** (ca. 60% cell viability for **C1/2** compared to 20% for **DPP1/2** at 50 $\mu$M), was still relatively toxic whereas **P1/2** and **PA1/2** were relatively nontoxic, indicating that the $\alpha,\beta$-unsaturated moiety to a carbonyl group[31] was responsible for cytotoxicity of **DPP1/2** and **C1/2**. In contrast to the cytotoxicity trend, the reactivity of **P1/2** and **PA1/2** against A$\beta$/metal−A$\beta$ was diminished, relative to **DPP1/2** and **C1/2**, which could be attributed to the overall loss of structural rigidity as well as their lowered metal binding affinity ($K_d$ ∼ $10^{-5}$ for Cu(II)) compared to **DPP1/2** and **C1/2** ($K_d$ ∼ $10^{-7}$ and ∼$10^{-6}$ for Cu(II), respectively).[20,24] This weak metal affinity renders it difficult for **P1/2** and **PA1/2** to interact with A$\beta$−bound metal ions, they are thus unable to form complexes with metal−A$\beta$ which is generally believed to be a suggested MoA for successful candidates.[20]

### Class 5

Multiple structural features can be combined within a single framework by careful consideration of chemical properties. This tactic was employed to yield a multifunctional ligand (**ML**)[18] by incorporating structural portions from the *p*-I-stilbene framework for A$\beta$ interaction, **L2-b** for metal−A$\beta$ interaction/reactivity, and (8-aminoquinolin-2-yl)methanol for metal chelation (increased denticity for Cu(II) and Zn(II) with 1:1 metal-to-ligand stoichiometry) (Figure 3).[17,18] **ML**'s metal binding property was designed to accommodate a slightly distorted square planar geometry for Cu(II) that could not easily adopt the preferred tetrahedral geometry of Cu(I); thus, this compound could hinder ROS generation by controlling

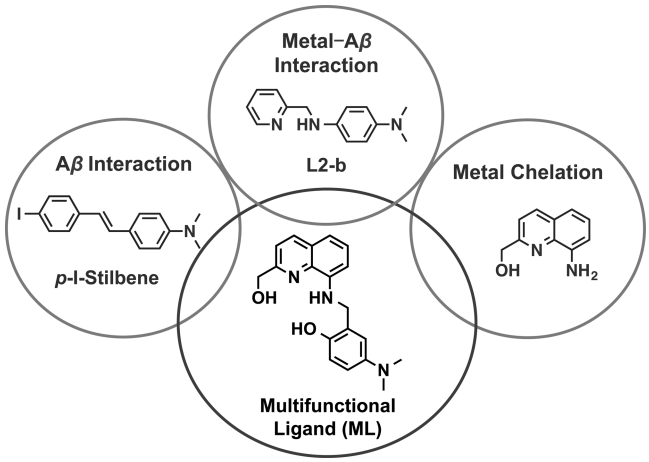


**Figure 3.** Illustration of integrating the structures for A$\beta$ interaction, metal−A$\beta$ interaction, and metal chelation into a single molecule (**ML**, 4-dimethylamino-2-(((2-(hydroxymethyl)-quinolin-8-yl)amino)-methyl)phenol).


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redox cycling at the copper center.[18] Quinoline and phenolic groups were included into the structure of **ML** for antioxidant capability, and hydroxyl and amino groups for enhanced aqueous solubility.[18] The overall scaffold of **ML** still adhered to the restricted terms of Lipinski's rules for drug-likeness; its BBB permeability was confirmed in vitro and in vivo.[18]

The ability of **ML** to interact with A$\beta$/metal−A$\beta$ was demonstrated with MS/IM-MS and NMR, which revealed a possible MoA for the compound.[18] NMR studies revealed interactions of **ML** with both soluble monomeric and insoluble, fibrillar forms of A$\beta_{40}$. The MS/IM-MS data indicated that **ML** could interact with metal-free A$\beta_{40}$/A$\beta_{42}$, producing A$\beta$−**ML** complexes, and with Cu(II)− or Zn(II)−A$\beta_{40}$/A$\beta_{42}$ forming ternary complexes of A$\beta$−metal−**ML**.[18] Additionally, **ML** could modify the aggregation pathways of A$\beta$/metal−A$\beta$ into nontoxic unstructured aggregates (e.g., off-pathway oligomers).[18] If A$\beta$ and metal−A$\beta$ species contribute to AD pathology, then the ability of **ML** and similar molecules to generate complexes with A$\beta$/metal−A$\beta$ and subsequently transfigure the size/conformation of the resultant species with less or no toxicity could provide therapeutic solutions. Supporting this notion, **ML** could diminish A$\beta$-/metal−A$\beta$-induced toxicity in an AD model neuroblastoma cell line, and reduce ROS production presumably by constraining the geometry at the Cu(II) center from redox cycling.[18] Although much remains to be revealed, the studies on **ML** provided the first demonstration that a designed, single structural entity can target and regulate multiple facets found in AD with comprehensive molecular level details on the molecule's MoA.[18]

## OTHER BIFUNCTIONAL MOLECULES

The incorporation approach has been utilized by other groups employing various backbones. Rodríguez-Rodríguez et al. reported, for the first time, the small molecules [2-(2-hydroxyphenyl)benzoxazole (**HBX**), 2-(2-hydroxyphenyl)-benzothiazole (**HBT**), and 2-(2-aminophenyl)-1*H*-benzimidazole (**BM**)] that were based on the incorporation approach employing the thioflavin T (ThT) backbone, a well-known A$\beta$ fibril imaging agent.[25] These compounds bound Cu(II) and Zn(II), and modulated the aggregation pathway of Cu(II)−A$\beta$, as observed by a turbidity assay. Scott et al. presented new small molecules designed by conjugating the ThT backbone with the metal binding moiety, 3-hydroxy-4-pyridinone, to inhibit metal-triggered A$\beta$ aggregation.[26] Jones et al. employed a modular click reaction to prepare triazole ligands with a metal chelating motif.[27] These compounds bound Cu(II) and Zn(II), and impacted metal-mediated aggregation as determined by TEM and turbidity investigations.

## NATURAL PRODUCTS

Epidemiological studies have indicated a possible relationship between AD prevalence and diet, which may arise from the molecular composition of food.[32,33] Therefore, investigating natural molecules found in food could be a useful strategy to identify frameworks that bind A$\beta$/metal−A$\beta$ or may have therapeutic value for AD treatment. In this sense, naturally occurring flavonoids have been screened against AD targets due to their rich structural variation. Several molecules have been identified as useful candidates due to their ability to redirect A$\beta$ aggregation pathways into off-pathway, unstructured oligomers.[29,34]

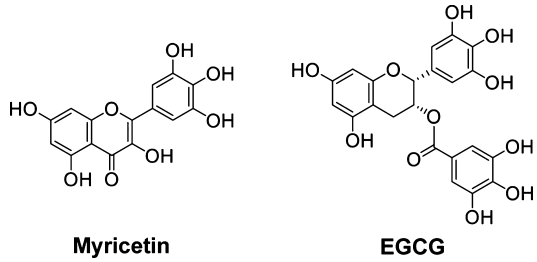


**Figure 4.** Structures of **myricetin** (3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one) and **EGCG** [(−)-epigallocatechin-3-gallate; (2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate].

The green tea extract epigallocatechin (**EGCG**, Figure 4) is one such natural flavonoid to have been investigated for its antiamyloidogenic properties.[35] **EGCG** could affect the extent of APP cleavage, reducing plaque load in an AD transgenic mouse model,[35] and interact directly with A$\beta$ monomers, redirecting A$\beta$ conformations into less toxic forms.[29] Similar work with **myricetin** (Figure 4), a natural product in berries, demonstrated its propensity to interact with A$\beta$ and alter fibril morphology.[34] The inherent capability of natural flavonoids for metal chelation,[36] in conjunction with their ability to interact with A$\beta$, suggests that they could be employed as tools to investigate the relationship between metal ions, A$\beta$, and metal−A$\beta$, in a manner similar to the designed molecules (infra supra). Ensuing rational structural modifications can be applied to simplify lead candidates and extract the structure−reactivity relationships to uncover the structural features that endow them with their ability to interact with metal−A$\beta$ and subsequently modulate metal−A$\beta$ reactivity.

The potential bifunctionality (i.e., metal chelation and A$\beta$ interaction) of naturally occurring flavonoids was initially investigated in vitro to survey **myricetin's** aptitude to modulate metal−A$\beta$ aggregation pathways and metal−A$\beta$-induced toxicity.[37] Myricetin could impact the size distribution of A$\beta$ species in the presence of Cu(II) or Zn(II), generating unstructured conformations, underlining the bifunctionality postulated for this natural product.[37] TEM studies of the resultant metal-induced A$\beta$ aggregates upon incubation with **myricetin** showed smaller sized, amorphous species, in contrast to **myricetin**-treated metal-free A$\beta$ analogues, which remained mainly fibrillar.[37] Therefore, **myricetin** preferentially interacted with metal−A$\beta$, controlling their aggregation pathways. Furthermore, **myricetin** could attenuate the toxicity of metal-free and metal-induced A$\beta$ in living cells.[37] Following these initial findings, additional insights on the A$\beta$−flavonoid interaction in the absence and presence of metal ions were uncovered using **EGCG** (Figure 4).[38] Previous studies suggested that a direct interaction of **EGCG** with metal-free A$\beta$ facilitated an alternative aggregation pathway that could generate nontoxic, amorphous oligomers.[29] This finding was recently corroborated through IM-MS/2D NMR investigations reported by Hyung et al. that identified the formation of a compact A$\beta$−**EGCG** complex with dissociation constants in the $\mu$M range.[38] Coordination of Cu(II) and Zn(II) through **EGCG**'s ortho phenol groups allowed the characterization of **EGCG**'s reactivity with metal−A$\beta$ species.[38] In vitro aggregation studies demonstrated that the size and morphology of **EGCG**-treated metal−A$\beta$ species were altered more noticeably than for metal-free A$\beta$ species.[38] To account for this reactivity, **EGCG**-treated Cu(II)−A$\beta$ and Zn(II)−A$\beta$


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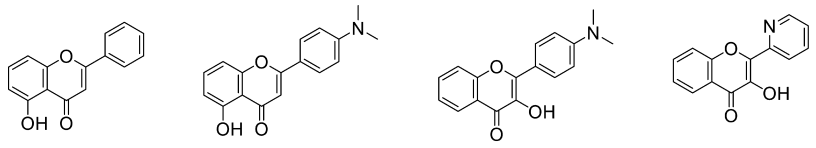


**Figure 5.** Chemcial structures of synthetic flavonoid derivatives (**FL1**−**FL4**). Left to right: **FL1**, 5-hydroxy-2-phenyl-4*H*-chromen-4-one; **FL2**, 2-(4-(dimethylamino)phenyl)-5-hydroxy-4*H*-chromen-4-one; **FL3**, 2-(4-(dimethylamino)phenyl)-3-hydroxy-4*H*-chromen-4-one; **FL4**, 3-hydroxy-2-(pyridin-2-yl)-4*H*-chromen-4-one.

samples were subjected to IM-MS/2D NMR, which detected ternary A$\beta$−metal−**EGCG** complexes.[38] Ternary complexation was also suggested to be responsible for the altered MW/conformation distribution of metal−A$\beta$ incubated with the compounds **L2-b** and **ML**.[17,18] Therefore, this type of ternary complex intermediate, a possible MoA, may instigate a conformational change that transforms the anticipated metal-induced A$\beta$ aggregation pathways into alternative, presumably nontoxic or less toxic species.[29,38] Recognizing the unique features of this system, new platforms, including surface plasmon resonance imaging (SPRi) and electrochemical methods, were developed to characterize the A$\beta$−metal−**EGCG** interaction.[39,40] It is evident from these investigations that **EGCG** is capable of modulating the metal−A$\beta$ interaction leading to modified aggregation products. Taken together, the research employing naturally occurring flavonoids demonstrates the ability of these scaffolds to interact with metal−A$\beta$ species and ultimately direct metal−A$\beta$ reactivity leading to decreased toxicity.

## SYNTHETIC NATURAL PRODUCT ANALOGUES

A small library of flavone and flavonol derivatives was rationally selected and prepared (Figure 5).[41] These compounds contain one or two potential metal chelation sites, compared to multiple sites in naturally occurring flavonoids (Figure 4). Among some of these synthetic structures, a dimethylamino group was incorporated to facilitate A$\beta$ interaction, which has been demonstrated by rationally designed molecules (vide supra).[17,20,22,24] The structural change was expected to maintain reactivity of the synthetic flavonoids toward metal−A$\beta$, as observed in their natural counterparts, but with greater ease of characterization. Unexpectedly, these compounds with only one metal chelation site and/or a dimethylamino moiety were unable to modify metal−A$\beta$ aggregation pathways.[41] Despite **FL4**'s binding affinity for Cu(II) ($K_d \sim 10^{-9}$), NMR/MS investigations suggested that it could interact only weakly with A$\beta$ monomers.[41] Therefore, these results indicate that both metal binding and A$\beta$ binding affinities should be appropriately balanced to ensure a significant interaction with metal−A$\beta$ to modulate metal−A$\beta$ aggregation pathways.

## CONCLUDING REMARKS

To gain a greater understanding of the role of metal−A$\beta$ species in AD, chemists have strived to develop small molecules as chemical tools to target metal−A$\beta$ species and modulate their interaction and reactivity. Several families of compounds with various frameworks have been used to uncover nuances of the metal−A$\beta$ interaction and its impact on toxicity-related events (i.e., metal−A$\beta$ aggregation pathways, such as neurotoxic oligomer formation; metal−A$\beta$-induced ROS generation), and this work will be valuable to elucidate the potential involvement of metal−A$\beta$ in AD pathogenesis. Furthermore, pharmacological properties (e.g., toxicity; metabolic stability) for therapeutic applications of these and other lead compounds will need to be evaluated and, if needed, optimized by structural modifications, including a prodrug formulation. Sufficient brain penetration would be required for these molecules to have a chance of being successful for in vivo applications. Their efficacy through assessment of neuroprotective ability on primary neuronal cultures and amelioration of cognitive deficit in AD mouse models could provide an indication if these candidates may have treatment potential. Collaborations across disciplines, such as medicinal chemistry, biology, and neuroscience, will enhance these efforts.

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### Notes

The authors declare no competing financial interest.

### Biographies

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**Dr. Alaina S. DeToma** obtained a B.S. in Chemistry from Elizabethtown College in Elizabethtown, PA. She received her Ph.D. in Chemistry from the University of Michigan under the direction of Professor Mi Hee Lim. Her research interests lie broadly in the bioinorganic chemistry of neurodegenerative diseases, particularly in the use of flavonoid derivatives to mediate metal−A$\beta$ interactions in vitro.

**Jeffrey S. Derrick** is currently an undergraduate student in the Department of Chemistry at the University of Michigan, pursuing research in the laboratory of Professor Mi Hee Lim. His research interests include the design of multifunctional molecules to target metal ions or metal−protein complexes involved in human diseases, and regulating their reactivity.

**Professor Mi Hee Lim** received her M.Sc. under Professor Wonwoo Nam at Ewha Womans University, Seoul, Korea. In 2002, she moved to MIT where she obtained her Ph.D. under the supervision of Professor Stephen J. Lippard. After her Ph.D., Dr. Lim pursued her postdoctoral research as a TRDRP postdoctoral fellow in the laboratory of Professor Jacqueline K. Barton at Caltech. In the summer of 2008, she began her independent position as an Assistant Professor of Chemistry and Research Assistant Professor in the Life Sciences Institute at the University of Michigan. Since September

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