Excited-State Intramolecular Hydrogen Transfer of Compact Molecules Controls Amyloid Aggregation Profiles

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ABSTRACT: Developing chemical methodologies to directly modify harmful biomolecules affords the mitigation of their toxicity by persistent changes in their properties and structures. Here we report compact photosensitizers composed of the anthraquinone (AQ) backbone that undergo excited-state intramolecular hydrogen transfer, effectively oxidize amyloidogenic peptides, and, subsequently, alter their aggregation pathways. Density functional theory calculations showed that the appropriate position of the hydroxyl groups in the AQ backbone and the consequent intramolecular hydrogen transfer can facilitate the energy transfer to triplet oxygen. Biochemical and biophysical investigations confirmed that these photoactive chemical reagents can oxidatively vary both metal-free amyloid-β (Aβ) and metal-bound Aβ, thereby redirecting their on-pathway aggregation into off-pathway as well as disassembling their preformed aggregates. Moreover, the in vivo histochemical analysis of Aβ species produced upon photoactivation of the most promising candidate demonstrated that they do not aggregate into oligomeric or fibrillar aggregates in the brain. Overall, our combined computational and experimental studies validate a light-based approach for designing small molecules, with minimum structural complexity, as chemical reagents targeting and controlling amyloidogenic peptides associated with neurodegenerative disorders.

KEYWORDS: photosensitizers, small molecules, ESIHT, amyloid-β, peptide aggregation

INTRODUCTION

Modifications of peptides or proteins in nature lead to diverse structural frameworks that allow unprecedented activities, folding, location, and interactions.1−5 Chemical methodologies to tactically manipulate certain amino acid residues installed in disease-related peptides or proteins have been developed for controlling the reactivities of these toxic biomolecules.6 Structural variations on amyloid-β (Aβ) peptides have recently been recognized to be effective for altering their aggregation pathways that are linked to the pathology of Alzheimer’s disease (AD).7 These approaches include oxidation, covalent bond formation, and hydrolytic cleavage that offer permanent changes in peptides with minimum probability to aggregate into toxic forms.8−11 In particular, the oxidative modifications of Aβ peptides with photosensitizers12 or redox-active molecules13−18 are the simplest strategy to change their aggregation profiles and improve protective defects in AD transgenic mice. A variety of photosensitizers ranging from fullerene or porphyrin-based macromolecules19,20 organometallic complexes,21−24 nanoparticles,27,28 and organic molecules29−34 were designed to modulate the aggregation of Aβ. Despite their noticeable potential, the complexity of synthesis, the low blood–brain barrier (BBB) permeability,35,36 and the potential risk of toxicity37−39 remain significant hurdles to overcome. In addition, the dysregulation of metal ions [e.g., Fe(II/III), Cu(I/II), and Zn(II)] induces biologically detrimental outcomes.40,41 Especially in the brains of AD patients, high concentrations of these metal ions are found in senile plaques, which implies that they could be a critical factor in the pathology of AD.42 According to extensive studies, metal ions could bind to Aβ to form metal-bound Aβ (metal–Aβ) and, subsequently, influence the aggregation pathways of Aβ.7,43,44 Indeed, the aggregation kinetics and morphology of Aβ species are significantly dependent on the concentration and type of metal ions. For example, the increased ratio of Cu(II) for Aβ could induce a shift from fibrillar structures to amorphous and spherical aggregates.7,44 In the case of Zn(II), nonfibrillar assemblies could be produced and stabilized.7,44

We questioned if small organic photosensitizers carrying simple functionalities such as hydroxyl and carboxyl groups can...
Figure 1. Rational selection of compact molecules that can modify the aggregation of Aβ with photoactivation. (a) Chemical structures and properties of 9,10-AQ (Parent); HQ, Ali, Qui, Pur (Group A); Dan, Rhe, Alo (Group B). 9,10-AQ, anthracene-9,10-dione; HQ, 1-hydroxyanthracene-9,10-dione; Ali (alizarin), 1,2-dihydroxyanthracene-9,10-dione; Qui (quinizarin), 1,4-dihydroxyanthracene-9,10-dione; Pur (purpurin), 1,2,4-trihydroxyanthracene-9,10-dione; Dan (dantron), 1,8-dihydroxyanthracene-9,10-dione; Rhe (rhein), 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid; Alo (alo-emodin), 1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione. (b) Sequence of Aβ Hydrophobic residues are underlined. (c) Schematic description of the on-pathway aggregation of Aβ with the modulatory strategy employing the photoactivated AQ series.

oxidatively modify both metal-free Aβ and metal–Aβ and alter their aggregation profiles. Small molecule-based biosensors or probes have been reported to have notable sensitivity against peptides or other biomolecules. As shown in Figure 1a, we rationally selected a series of anthraquinone (AQ)-based compounds and tested their capacity to oxidize metal-free Aβ and metal–Aβ upon photoirradiation and, consequently, vary their aggregation. In addition, the detailed mechanism for such reactivity was determined. Moreover, the aggregation behaviors of Aβ species produced upon light activation of the most promising molecule were probed in vivo. Collectively, our studies demonstrate that compact molecules with proper structural and photophysical features can be developed for manipulating Aβ aggregation with light.

RESULTS AND DISCUSSION

AQ-based dyes have been used as photosensitizers for various applications such as photovoltaics, photocatalysts, and photoactive peptides, and cell imaging; thus, we chose the AQ backbone to identify effective photosensitizers that can oxidatively modify Aβ peptides. As displayed in Figure 1a, a series of AQ-based molecules with simple structural variations composed of only three basic carbon, hydrogen, and oxygen atoms were selected for this work. Based on the parent structure 9,10-AQ, the hydroxyanthraquinone derivatives were classified into two groups: Group A (HQ, Ali, Qui, and Pur) possesses a hydroxyl group on the R1 position forming one quasi-ring with the adjacent ketone and additional hydroxyl groups on R2, R6, or both; Group B (Dan, Rhe, and Alo) contains hydroxyl groups on both R1 and R2 generating two quasi-rings and structural variance on R3. The anthraquinone skeleton and the aforementioned structural variation can offer several advantages in targeting Aβ. The AQ series has amphipathic structures that can interact with Aβ consisting of both hydrophilic and hydrophobic amino acid residues, as visualized in Figure 1b. The addition of hydroxyl and carbonyl groups with the ketone functionality can provide additional binding sites with Aβ through hydrogen bonds. The π-plane backbone is not only essential for the photosensitizing ability but also enables hydrophobic interactions with the β-sheet in oligomeric and fibrillar Aβ. As illustrated above, amphiphilicity has been known to be one of the essential assets for molecules to interact with proteins or peptides. Moreover, hydroxyl groups and the neighboring ketone functionality are envisioned to serve as potential metal-binding sites that can interact with other metal ions bound to Aβ. Upon photoactivation, anthraquinones are reported to generate singlet oxygen (¹O₂), which are highly reactive toward diverse biological substrates. As such, we anticipated that AQ derivatives could readily oxidize amino acid residues in Aβ, as depicted in Figure 1c. We evaluated the ¹O₂ production of compact molecules in the AQ series with mechanistic investigations and their reactivities toward both metal-free Aβ and metal–Aβ.

¹O₂ Production

The photochemical properties of the AQ series used in this study were examined by ultraviolet–visible (UV–Vis) and fluorescence spectroscopies. As summarized in Figure S1, the absorption spectra of the AQ series in dimethyl sulfoxide (DMSO) or the buffered solution showed maxima in the range of 326–486 and 335–516 nm, respectively, denoting photon absorbance in the blue-light region. In particular, the absorption of Ali and Pur in the buffered solution displayed bathochromic shifts (λ = 82 and 21 nm, respectively) relative...
to the maximum in DMSO because the hydroxyl group at the R₂ position is deprotonated at neutral pH. Moreover, the luminescence spectra of the AQ series in DMSO and the buffered solution were measured, as presented in Figure S2. While 9,10-AQ had a maximum emission wavelength at 425 nm in DMSO and diminished absorbance in the buffered solution, the rest of the AQ series emitted light within the range of 554−612 nm upon illumination. Luminescence of Ali and Pur was not observed in the buffered solution.

The capability of the AQ series to produce ¹O₂ in the wavelength of the blue light spectrum (ca. 467 nm) was evaluated employing the ABDA assay [ABDA = 9,10-anthracenediyl-bis(methylene)dimalonic acid]. As illustrated in Figures 2a and S3, the absorbance attenuation (ΔA) of ABDA in the presence of the AQ series under aerobic conditions revealed the energy transfer from the compounds in Group B toward O₂, exhibiting a range of Φ from 0.062 up to 0.188 comparable to [Ru(bpy)₃]²⁺ (Φ = 0.18 in H₂O) as a reference molecule. [Ru(bpy)₃]²⁺ was reported to oxidize Aβ species, resulting in inhibiting the self-aggregation of Aβ and dismantling Aβ aggregates under illumination. The molecules in Group A displayed moderate or low ¹O₂ formation with a Φ range from 0.013 to 0.047. Note that the Φ value of 9,10-AQ could not be obtained due to the minimum changes of the absorption peaks of ABDA. Therefore, the photochemical measurements confirm that the AQ series generates ¹O₂ and the molecules in Group B are most effective.

Mechanism for ¹O₂ Generation

Density functional theory (DFT) calculations were performed to estimate the ability of our selected molecules to produce ¹O₂ upon photoirradiation. As illustrated in Figure 2b, the energy transfer occurs from the triplet excited photosensitizer (³AQ) to the acceptor triplet oxygen (³O₂) resulting in a singlet ground state photosensitizer (¹AQ) and a singlet excited state acceptor (¹O₂).

Hydroxyanthraquinones exhibit excited-state intramolecular hydrogen transfer (ESIHT) from hydroxyl groups toward the adjacent ketone functionality during light activation. Moreover, hydroxyanthraquinones with deuterium-substituted hydroxyl groups are reported to have longer emission lifetimes, compared to nonsubstituted analogues. Through notable isotope effects on the emission decay time, it has been proposed that the O−H vibration of hydroxyl groups is strongly coupled with the relaxation process. Therefore, we can hypothesize that the donor relaxation could be also linked to the reverse intramolecular hydrogen transfer (RIHT) process. The relationship between the energy transfer and RIHT was investigated through our simulations. In support of this notion, the DFT-calculated barrier (ΔG⧧) of the energy transfer for 9,10-AQ to produce ¹O₂ is unrealistically high at 138.9 kcal/mol if intramolecular
hydrogen transfer is not considered. The incorporation of the hydroxyl moiety enables intramolecular hydrogen transfer and reduces the triplet energy transfer barrier considerably, suggesting that hydroxyanthraquinones are suitable scaffolds for $^1$O$_2$ production. Specifically, calculations on Group A (HQ, Ali, Qui, and Pur; Figure 1a) that share a common backbone of 1-hydroxyl group next to the ketone functionality reveal low energy transfer barriers ranging from 1.3 up to 11.2 kcal/mol. The molecules in Group B (Dan, Rhe, and Alo) with 1- and 8-hydroxyl moieties are predicted to be most efficient and barrierless.

We questioned why the position of hydroxyl groups has such a dramatic impact on the energy transfer barriers toward $^1$O$_2$ excitation. The triplet energy transfer barrier can be divided into two principal components: thermodynamic driving force ($\Delta G$) and reorganization energy ($\lambda$). $\Delta G$ is the energy difference between the singlet–triplet energy gap of the donor and the acceptor. Comparing the frontier molecular orbitals of the donor scaffolds, all of our selected AQ derivatives bear delocalized singly occupied molecular orbital (SOMO) in their triplet state, as presented in Figure S4. The molecules in Group A present a smaller singlet–triplet energy gap of the

Figure 3. Analysis of Aβ$_{40}$ species produced by treatment of the AQ series with light exposure by ESI–MS and ESI–MS$^2$. (a) ESI–MS spectra of the samples containing Aβ$_{40}$ and compounds with and without light treatment. The number of red dots represents the number of oxygen atoms incorporated into the Aβ$_{40}$ monomer [Aβ$_{40}$ + nO]$^{2+}$ (n = 1, 2, or 3). (b) Sequence of Aβ$_{40}$ and structures of oxidized His and Met residues. (c, d) ESI–MS$^2$ analyses of the singly oxidized peak (m/z = 1448) obtained by treatment of Pur as well as the singly and doubly oxidized peaks (m/z = 1448 and 1453, respectively) generated upon incubation with Alo. In the ESI–MS$^2$ studies, monooxidized and dioxidized b and y ions are illustrated in red and green, respectively. Conditions: [Aβ$_{40}$] = 25 μM; [compound] = 50 μM; 20 mM ammonium acetate (1% v/v DMSO), pH 7.4; 37 °C; 3 h; constant agitation (250 rpm); Kessil lamp (467 nm) for 1 h. The samples were diluted by 5-fold with H$_2$O before injection into the mass spectrometer.
donor where $\Delta G$ varies from $-13.3$ to $3.6$ kcal/mol. This small energy gap is due to the biased position of electron-donating groups and the resulting localized highest occupied molecular orbital (HOMO), which leads to a relatively unstable singlet ground state. The consequence is dramatic for the deprotonated Ali and Pur ($\Delta G$ of $3.6$ kcal/mol and $-0.1$ kcal/mol, respectively) possessing phenolate moieties with a strong electron-donating character at neutral pH.\(^{38}\) In contrast to Group A, the compounds in Group B retain symmetrically positioned hydroxyl groups with delocalized HOMO in the singlet ground state maintaining the singlet–triplet energy gap to a range of $-16.2$ to $-14.7$ kcal/mol. Another factor in determining the barrier is $\lambda$ associated with structural changes required for the energy transfer. Ranging from $24.6$ to $38.5$ kcal/mol, the molecules in Group A generally require higher $\lambda$ values than those in Group B that show $\lambda$ values in the range of $16.8$ to $17.2$ kcal/mol. To better understand this trend, HQ ($\lambda = 24.6$ kcal/mol) and Dan ($\lambda = 16.8$ kcal/mol) were chosen to represent molecules in Group A and Group B, respectively, as depicted in Figure 2c. The hydrogen bond in $^3$HQ shortens from $1.64$ to $0.99$ Å during donor relaxation, while the hydrogen bond involved in the hydrogen transfer in $^3$Dan displays a smaller change from $1.58$ to $0.99$ Å, indicating a reduced reorganization penalty compared to that of $^3$HQ. Two explanations can be offered to unveil the role of the additional hydroxyl group at the $R_1$ position of Dan. In the context of intramolecular resonance-assisted hydrogen bonding, the extra hydroxyl functionality can serve as a $\pi$-electron-donating group to strengthen the hydrogen bond motif and, subsequently, shorten it.\(^{38}\) As another aspect, the additional hydrogen bond donor can withdraw the electron density from the interacting hydroxyl group and lower its pK$_a$ facilitating the hydrogen transfer process back to the singlet ground state structure. We reoptimized the triplet structure of $^3$Dan with the supplementary hydroxyl group to face the opposite direction and observed a slight elongation of the hydrogen bond length to $1.61$ Å, which validates the dual effect of the hydroxyl group at $R_1$ in Group B, as described in Figure S5.

An alternative mechanism can be proposed for the excitation and relaxation of the photosensitizers during the triplet energy transfer: the stepwise procedure where the vertical relaxation of the triplet state comes first and RIHT comes later, as shown in Figure S6. Note that the mechanism of the concerted or stepwise pathway is still under debate because of the undetectable phosphorescence for hydroxynaphthaquinones. Two types of vertical relaxation were examined through DFT and time-dependent density functional theory (TD-DFT) calculations: the AQ series that (i) undergoes ESIHT in a stepwise manner or (ii) does not transfer their hydrogen upon excitation. In both of these cases, the reorganization energy is negligible and the singlet–triplet energy gap is the only factor that determines the triplet energy transfer. According to our calculations, the ESIHT-assisted models exhibit an adequate energy gap to facilitate the triplet energy process. Ali, Qui, and Pur have an energy gap ranging from $10.9$ to $19.9$ kcal/mol that is insufficient for converting $^{3}$O$_2$ to $^{1}$O$_2$, while the energy gap of HQ, Dan, Rhe, and Alo ranges from $21.6$ to $23.2$ kcal/mol comparable to the energy of $22.5$ kcal/mol needed to activate inert $^{3}$O$_2$. On the other hand, our hypothetical model that does not go through ESIHT and directly to intersystem crossing yields a larger singlet–triplet energy gap. HQ has an energy gap of $42.6$ kcal/mol, but Ali and Pur present an energy gap between $12.5$ and $18.9$ kcal/mol that is too small. Dan, Rhe, and Alo have energy gaps in the range of $40.6$ to $43.4$ kcal/mol, rendering them incompetent to achieve effective triplet energy transfer to the oxygen acceptor. Therefore, ESIHT plays a significant role for the AQ series not only in the concerted but also in the stepwise mechanism to become appropriate photosensitizers by controlling the singlet–triplet energy gap. Taken together, our computational studies highlight the importance of additional hydroxyl groups and reveal that the position of hydrogen bond donors is key to facilitate the triplet energy transfer.

### Aβ Oxidation

To determine whether the AQ series can oxidatively modify metal-free Aβ and metal–Aβ with photoinirradiation under aerobic conditions, the resultant Aβ species were analyzed by electrospray ionization–mass spectrometry (ESI–MS) and tandem MS (ESI–MS$^2$). As displayed in Figures 3a and S7, no significant change in metal-free Aβ$_{40}$ was observed in the absence of light. Upon photoactivation of the AQ series, the peaks corresponding to oxidized Aβ$_{40}$ species were monitored. The parent molecule 9,10-AQ did not affect Aβ$_{40}$ despite light exposure. When HQ, Ali, Qui, and Pur (Group A) were exposed to Aβ$_{40}$, a new peak was detected at $1,448$ m/z, indicative of the incorporation of a single oxygen atom into monomeric Aβ$_{40}$. In the case of Dan, Rhe, and Alo (Group B), three new peaks were detected at $1,448, 1,453,$ and $1,459$ m/z corresponding to the singly, doubly, and triply oxidized Aβ$_{40}$ monomers, respectively. The singly, doubly, or triply oxidized Aβ$_{40}$ monomer induced by the light-activated AQ series was also noticed in both Zn(II)−Aβ$_{40}$ and Cu(II)−Aβ$_{40}$ as shown in Figure S8.

The peaks assigned to oxidized Aβ$_{40}$ species generated by photoactivation were further probed by ESI–MS$^2$ to identify the oxidized amino acid residues (Figure 3b). Aβ$_{40}$$_{40}$ peptides photooxyxygenated with Pur (Group A) and Alo (Group B) to different extents were selected for tandem MS measurements. Figure 3c exemplifies the collision-induced dissociation (CID) experiments on the singly oxidized peak of Aβ$_{40}$ (1,448 m/z) obtained by photoexcited Pur. We observed $b$ fragments from $b_{13}$ in their nonoxidized and oxidized forms, implying that Pur oxidizes either His13, His14, or Met35. It should be noted that the oxidation is unable to simultaneously modify more than one residue. On the contrary, as disclosed in Figure 3d, ESI–MS$^2$ studies on the singly and doubly oxidized peaks verified that Alo can concurrently oxidize all three aforementioned amino acid residues. The fragmentation analysis on the singly oxidized Aβ$_{40}$ by Alo was identical to the results obtained by Pur. For the doubly oxidized Aβ$_{40}$ we could not monitor oxidized fragments smaller than $b_{13}$, and doubly oxidized forms were detected from $b$ ions larger than $b_{13}$. Note that the Met residue was oxidized to sulfone in the doubly oxidized Aβ$_{40}$. Overall, our MS studies substantiate that the AQ series can possibly oxidize Aβ at the His13, His14, and Met35 residues to varying degrees.

### Influence on Aβ Aggregation

To test if the different degree of Aβ oxidation by light activation of the molecules in Group A and Group B alters the aggregation of both metal-free Aβ and metal–Aβ in a detectable manner, the molecular weight (MW) distribution of the resultant Aβ species was first analyzed by gel electrophoresis with Western blotting (gel/Western blot) using an anti-Aβ antibody (6E10). The morphological change of metal-free Aβ or metal–Aβ aggregates produced by

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 treatment of the AQ series was visualized with transmission electron microscopy (TEM). Aβ aggregates larger than ca. 270 kDa are not detectable in gel/Western blot but can be probed by TEM. In this work, we conducted two experiments: (i) inhibition experiments for determining the influence of the inhibition experiments with Zn(II) and Cu(II); (ii) disaggregation experiments for assessing the ability of the AQ series to disassemble preformed Aβ aggregates.

In the inhibition experiments, metal-free Aβ or metal–Aβ was freshly prepared and treated for 24 h with the AQ series, as shown in Figure 4a. For light-exposed samples, Aβ species added with the AQ series were photoradiated for 1 h prior to 24 h incubation. As portrayed in Figure 4b, under dark conditions (gray gels), no significant change in the MW distribution of both metal-free Aβ40 and metal–Aβ40 was observed even with the addition of the AQ series. Notably, in the presence of light, the resultant Aβ40 species with the AQ series (blue gels) showed a divergent MW distribution, relative to Aβ40 only. Treatment of metal-free Aβ40 with light-activated 9,10-AQ, HQ, Ali, Qui, and Pur gave rise to increased signal intensities between ca. 15–35 kDa. Note that the AQ series can produce superoxide anion radicals; however, we could not monitor them because the excitation and emission wavelengths used for the assays overlapped with those of the compounds. On the contrary, the illuminated Aβ40 samples with Dan, Rhe, and Alo greatly affected the MW distribution exhibiting new gel bands throughout ca. 15–240 kDa. Smearing bands over ca. 240 kDa were spotted for the samples added with both Group A and Group B indicating that the light-driven oxidation of metal-free Aβ40 leads to smaller peptide ensembles that can penetrate the gel matrix. In the inhibition experiments with Zn(II)–Aβ40, 9,10-AQ, HQ, Ali, Qui, and Pur upon photoactivation mildly affected the MW distribution while Dan, Rhe, and Alo significantly enhanced the intensities of the bands between ca. 15–240 kDa. Various aggregates were detected upon incubation of Cu(II)–Aβ40 with photoreactive Dan, Rhe, and Alo, but moderate effects were identified with the illumination of the rest of the AQ series.

As described in Figure 4c, metal-free Aβ40 and metal–Aβ40 aggregates generated with and without the treatment of 9,10-AQ, Pur, or Alo were further examined by TEM. 9,10-AQ, Pur, and Alo were chosen as the representative molecules of Parent, Group A, and Group B, respectively. Without light,
thick fibrils were formed by incubation of metal-free Aβ40 in
the presence of all three compounds, compared to fibrillar
aggregates produced in the sample of metal-free Aβ40 only.
Morphologies of the resultant metal−Aβ40 aggregates were not
significantly changed even with the compounds, however. This
suggests that the AQ backbone itself may interact with metal-
free Aβ40 and affect its aggregation. When 9,10-AQ was
incubated with metal-free Aβ40 with photoirradiation, long
and thick fibrils as well as similar fibrils to those formed from Aβ40
only were detected. The conformational transformation of
metal-free Aβ40 by photoexcited Pur and Alo was more
noticeable than that by 9,10-AQ, resulting in smaller and
thinner aggregates. In the case of metal−Aβ40 species, small
amorphous assemblies and thin fibrils were visualized with the
illumination of Pur and Alo, but 9,10-AQ did not noticeably
alter their morphologies. These amorphous aggregates are
reported to be less toxic than structured assemblies.9,13,15,17
Modest or no changes found in the structures of metal-free and
metal-bound Aβ40 species with photoactivated 9,10-AQ
denote the importance of efficient 1O2 production in modifying
their aggregation pathways.

Inhibition experiments were also conducted employing Aβ42
(Figure S9a). As described in Figure S9b, the application of
light with 9,10-AQ toward metal-free Aβ42 enhanced the band
intensity in the high MWs (ca. over 70 kDa). HQ and Ali did
not significantly change MW distribution with photoirradiation.
The addition of light-exposed Qui, Pur, and Dan reduced the
intensities of the bands in the lower MW region (ca. below
15 kDa) but increased them in the higher MW region (ca. over
70 kDa). This phenomenon was distinct from the samples of
Rhe and Alo with light. In the presence of Zn(II), smearing
bands emerged in the case of Pur, Dan, Rhe, and Alo
throughout ca. 4–270 kDa. In Ali- and Qui-added samples, a
new band at ca. 7 kDa and amplified the intensities of bands
over ca. 35 kDa were monitored. Such change of the band
above ca. 70 kDa was also observed upon treatment of 9,10-
AQ and HQ. The Cu(II)-added samples with the photo-
illuminated AQ series presented the changes in the MW
distribution to different extents. Figure S9c illustrates the TEM
results of the inhibition experiments using Aβ42. Similar to
Aβ40, TEM studies showed that 9,10-AQ, Pur, and Ali did not
noticeably alter the morphologies of metal-free Aβ42 and
metal−Aβ42 aggregates without light. The size of metal-free
Aβ42 and metal−Aβ42 aggregates was greatly diminished by
photoexcited Alo, and thinner fibrils and smaller aggregates
were spotted by the addition of Pur, compared to compound-
free and metal-treated Aβ42 aggregates.

Moving forward, as depicted in Figures S10 and S11, metal-
free Aβ or metal−Aβ was preincubated for 24 h to form
peptide aggregates and the AQ series was treated with and
without 1 h photoexcitation followed by incubation for an
additional 24 h. The gel/Western blots in the disaggregation
experiments afforded shifts in the MW distribution of Aβ species
to various degrees upon photosensitization of the AQ
series. Specifically, the MW distribution of preformed metal-
free Aβ40 aggregates was influenced by 9,10-AQ, HQ, Ali, Qui,
Pur, Dan, and Rhe exhibiting new bands between ca. 15–35
kDa. Light-exposed Ali, Qui, Pur, Dan, Rhe, and Alo showed
dimmer or no bands in the MW region below ca. 7 kDa.
Substantial MW changes of Zn(II)−Aβ40 aggregates incubated
with Dan, Rhe, and Alo were observed with new bands
throughout ca. 15–270 kDa. In the case of Qui and Pur, the
intensities at ca. 7 kDa and above 240 kDa were increased, but
photoactivated 9,10-AQ, HQ, and Ali manifested a less
pronounced impact on the MW distribution. The gel/Western
blot of preformed Cu(II)−Aβ40 aggregates also indicated an
amplified variation in the MW distribution with Dan, Rhe, and
Alo, while the treatment of 9,10-AQ and the molecules in
Group A resulted in minor or no changes. For metal-free Aβ42
aggregates, we were not able to detect a significant MW change
with 9,10-AQ, but slight alterations were found with HQ and
Alo. The addition of Qui and Pur displayed lower band
intensities below ca. 15 kDa. On the other hand, the intensities
of the bands throughout the detectable region observed in the
sample of Aβ42 only were all diminished in the presence of
Dan, Rhe, and Alo. Likewise, the MW distribution of metal−
Aβ42 aggregates with photoactivated 9,10-AQ was negligibly
varied. Against Zn(II)−Aβ42 aggregates, the illumination of
Group A and Group B increased the intensity in the higher-
order region above ca. 35 kDa. Furthermore, HQ and Qui
produced a new band at ca. 7 kDa with smearing near ca. 15
kDa. Pur, Dan, Rhe, and Alo decreased the intensity below ca.
15 kDa. A change in the MW distribution of Cu(II)−Aβ42
aggregates was also monitored by treatment of Group A and
Group B in different ranges. Photoactivated HQ lowered the
intensity between ca. 7–35 kDa, where the bands in the
(corresponding region became more obscure for Ali, Qui, and
Pur. Dan resulted in the smearing throughout ca. 7–240 kDa
while the bands almost disappeared in the region below ca. 70
kDa for Cu(II)−Aβ42 aggregates added with Rhe and Alo. As
presented in Figures S10c and S11c, the fibrillar aggregates of
metal-free and metal-bound Aβ40 and Aβ42 were monitored by
treatment of compounds without light, similar to those of
compound-free samples. In contrast, thin and short fibrils were
detected when Pur was incubated and photoexcited with
preformed metal-free and metal-bound Aβ aggregates,
presenting their morphological changes, different from 9,10-
AQ that showed fibrillar aggregates. When Alo was added
with preformed metal-free Aβ and Cu(II)−Aβ aggregates upon
light activation, thick and well-ordered fibrils without and with
amorphous aggregates, respectively, were visualized, and small-
sized Aβ aggregates were indicated in the presence of Zn(II).

Taken together, the gel/Western blot and TEM studies
implicitly demonstrate the ability of photoexcited AQ series for
modulating the formation of metal-free Aβ or metal−Aβ
taggregates as well as the disassembly of preformed metal-free
or metal-added Aβ aggregates. The impact of photoreactive
AQ series against diverse Aβ species reflects the significance
of 1O2 production and the number of oxygen atoms incorporated
into Aβ; thus, the compounds in Group B are able to
remarkably modify the aggregation pathways of both metal-free
Aβ and metal−Aβ. Furthermore, taking account to minimum
changes noticed in the parent structure 9,10-AQ, these in vitro
aggregation investigations reveal the relationship between the
structural features (e.g., the position and number of hydroxyl
groups placed in the framework) of the AQ series and the
modulatory reactivity toward the aggregation of metal-free Aβ
and metal−Aβ. Note that such structural variations can change
photophysical properties of compounds, with their direct
contacts on Aβ species (vide infra).

Biological Efficacies

The cell viability and BBB permeability of the AQ series were
examined prior to in vivo studies. Photodegradation of
photosensitizers during illumination was reported to yield
unidentified fragments that can cause toxicity.22 Thus, the
toxicity of the compounds prepared with and without photoirradiation was determined in human neuroblastoma SH-SY5Y cells by the MTT assay [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. As described in Figure S12, the cells in the presence of both Group A and Group B without photoactivation exhibited greater survival of over ca. 80% at up to 25 μM. The parent moiety 9,10-AQ displayed over 70% cell viability. Intriguingly, the toxicity of all molecules preilluminated for 1 h before 24 h incubation was lowered by up to ca. 14% at 25 μM. This suggests that the AQ series does not yield more toxic products upon photoactivation.

Crossing the BBB is an essential factor for chemical reagents to be utilized in the brain. Thus, the brain uptake of the AQ series was predicted by the parallel artificial membrane permeability assay adapted for the BBB. As summarized in Table S1, most of the molecules selected in this study were expected to have sufficient BBB penetration with a−logPc value below 5.4 except for 9,10-AQ [5.54 (±0.11)] and (Rhe [5.97 (±0.03)]. The permeability of 9,10-AQ could not be determined due to limited solubility in water. Moving forward, we chose to examine Alo in histochemical investigations in vivo because it showed a relatively high 1O2 quantum yield in the AQ series, significant modulatory impact on Aβ aggregation, relatively low cytotoxicity, and potential BBB permeability.

To assess the aggregation propensity of both Aβ40 and Aβ42 species treated with Alo and light in the brain, we conducted histochemical studies on the brain samples injected with Alo-treated Aβ species. As illustrated in Figures S13a and 5a, Aβ40 and Aβ42 were freshly prepared in 20 mM HEPES, pH 7.4, 150 mM NaCl (1% v/v DMSO; vehicle) and exposed to light for 1 h with and without Alo, incubated for an additional 2 h, and directly injected into the hippocampus of murine brains. The hippocampal region is vital for memory formation and retrieval in both murine and human brains and is highly affected by Aβ aggregates formed during the progression of AD. As expected, the brain samples administered with compound-free Aβ species exhibited Aβ aggregates such as oligomers or fibrils visualized with an antibody and fluorescent dyes (e.g., detection of Aβ oligomers with an anti-Aβ antibody [primary antibody, yellow] and A11 [antioligomer antibody; red] with fluorescent-conjugated secondary antibodies or fluorescence dyes [ThS for Aβ fibrils (green) and DAPI for fibrillary Aβ and nucleus (blue)]. Images were taken by a confocal microscope (left; ×10) or a scanning microscope (right; ×10). Detected amyloid species are marked with white arrows. Scale bars = 250 and 500 μm for confocal or scanning microscopies, respectively. Animal number: n = 4 (for Aβ42 and Alo-added Aβ42) and n = 3 (for vehicle).
CONCLUSIONS

Regulating the aggregation of Aβ peptides with synthetic small chemical tools has been the long-lasting interest and challenge to alleviate the progression of AD. Incorporation of oxygen atoms in early stage amyloid species as well as mature fibrils resulted in dramatic changes of their morphologies and toxicity while preventing the restoration of their original form. There has been controversy of the specific role of Aβ in AD, but recent reports continue to emphasize the necessity of our study. The demand of finding appropriate reagents oxidatively modifying pathological factors in AD requires both effective O₂ production and nontoxic products. Our structure-based computational modeling of small AQ-based molecules highlights the importance of the adequate level of the singlet–triplet energy gap associated with the reorganization energy throughout the ESIHT process. These conditions were met by incorporating additional hydroxyl groups to form two quasi-ring moieties that facilitate O₂ production. Spectroscopic and biochemical studies demonstrate the enhanced photoreactivity of AQ-based reagents with two quasi-rings mediated by hydrogen bonding against metal-free Aβ and metal–Aβ, compared to the molecules that possess one or no intramolecular quasi-ring. The AQ series also displayed relatively lower cellular toxicity upon photoactivation. In the brains of mice, Aβ species generated by Alo with light exposure exhibited minimum aggregation into amyloid assemblies such as oligomers or fibrils known to be linked to the pathology of AD.

Furthermore, selectivity is another crucial aspect for Aβ-targeting small molecules. As illustrated in Figure S14, Pur and Alo could bind with Aβ₄₀ species, as observed by docking studies, where both hydroxyl groups and the three-membered ring can interact with hydrophilic and hydrophobic residues in Aβ, respectively. This interplay may indicate the importance of both hydrophilic and hydrophobic structural moieties of the AQ series for targeting the self-recognition site (i.e., LVFFA; Figure 1b) of Aβ and affecting its aggregation pathways. Like Aβ oxidation, Alo has the ability to oxidize other disease-related amyloidogenic proteins or peptides such as α-synuclein and human islet amyloid polypeptide (hIAPP) and modify their aggregation pathways, as depicted in Figure S15. The oxidation of each peptide in a mixture of Aβ₄₀ and hIAPP was further monitored. Thus, our AQ series should be optimized to achieve the selectivity against Aβ species for biological applications. Overall, our combined experimental and computational studies illuminate how compact photoactivatable molecules that can readily modify amyloidogenic peptides and, consequently, control their aggregation process can be rationally developed.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.2c00281.

Experimental Section, Table S1, and Figures S1–S15 (PDF)

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Notes

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