Abstract: A series of novel hybrid 8-hydroxyquinoline-indole derivatives (7a–7e, 12a–12b and 18a–18h) were synthesized and screened for inhibitory activity against self-induced and metal-ion induced \( \alpha \)-amyloid\(_{1-42} \) aggregation as potential treatments for Alzheimer’s disease (AD). In vitro studies identified the most inhibitory compounds against self-induced \( \alpha \)-amyloid\(_{1-42} \) aggregation as 18c, 18d and 18f (EC\(_{50} \) = 1.72, 1.48 and 1.08 \( \mu \)M, respectively) compared to the known anti-amyloid drug, clioquinol (1, EC\(_{50} \) = 9.95 \( \mu \)M). The fluorescence of thioflavin T-stained amyloid formed by \( \alpha \)-amyloid\(_{1-42} \) aggregation in the presence of Cu\(^{2+}\) or Zn\(^{2+}\) ions was also dramatically decreased by treatment with 18c, 18d and 18f. The most potent hybrid compound 18f afforded 82.3% and 88.3% inhibition, respectively, against Cu\(^{2+}\)-induced and Zn\(^{2+}\)-induced \( \alpha \)-amyloid\(_{1-42} \) aggregation. Compounds 18c, 18d and 18f were shown to be effective in reducing protein aggregation in HEK-tau and SY5Y-APP\(_{Sw} \) cells. Molecular docking studies with the most active compounds performed against \( \alpha \)-amyloid\(_{1-42} \) peptide indicated that the potent inhibitory activity of 18d and 18f were predicted to be due to hydrogen bonding interactions, \( \pi \)-\( \pi \) stacking interactions and \( \pi \)-cation interactions with \( \alpha \)-amyloid\(_{1-42} \), which may inhibit both self-aggregation as well as metal ion binding to \( \alpha \)-amyloid\(_{1-42} \) to favor the inhibition of \( \alpha \)-amyloid\(_{1-42} \) aggregation.

Keywords: Alzheimer’s disease; clioquinol analogues; hybrid 8-hydroxyquinoline-indole analogs; \( \alpha \)-amyloid aggregation; metal chelating agents

1. Introduction

Alzheimer’s disease (AD) is the most debilitating age-associated neurodegenerative disorder leading to dementia, affecting millions of elderly people, and the number of patients is expected to reach 130 million worldwide by 2050 [1,2]. AD remains incurable due to the low efficacy and the very limited number of available drugs to treat this neurodegenerative disease. Salient features of AD are the accumulation of amyloid-\( \beta \) (A\( \beta \)) plaques and tangles containing hyperphosphorylated tau protein. These proteins misfold and aggregate in the brains of affected individuals as amyloid...
senile plaques outside the neurons and neurofibrillary tangles (NFTs) within neurons, which are
diagnostic for AD but accompanied by dyshomeostasis of biometals [3,4]. Current treatments for AD
include acetylcholinesterase (AChE) inhibitors (i.e., tacrine, donepezil, rivastigmine, and galantamine),
and N-methyl-D-aspartate (NMDA) antagonists, the only drugs approved for AD which provide a
symptomatic relief strategy for mild forms of AD [5]. Unfortunately, there is currently no means to cure
or even slow the progression of AD [6], spurring increased efforts to develop more effective drugs to
prevent or treat AD. Due to the complexity of AD and the multitude of factors potentially involved in
its progression, a strategy that uses multi-target directed ligands (MTDLs) has drawn much attention
as a mainstream therapeutic approach for treatment of this disease [7–11].

The deposition of Aβ plays a crucial role in the pathogenesis of AD [12]. Many studies have shown
that Aβ forms consist of several different types of aggregates, such as oligomers and fibrils [13,14].
Among these aggregates, soluble Aβ oligomers produce the most potent neurotoxicity and are generally
regarded as the main neurotoxins in AD. Soluble Aβ oligomers not only lead to cognitive impairment in
rodents but also induce neuronal death in primary neurons and neuronal cell cultures [15]. Soluble Aβ
oligomers rapidly interact with neuronal cell membranes, produce free radicals, and increase the levels
of intracellular reactive oxygen species (ROS) [16]. Moreover, Aβ oligomers induce neuronal apoptosis
by regulating signaling pathways, such as glycogen synthase kinase 3β (GSK3β) and mitogen-activated
protein kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling [16]. In addition, metal
ions such as Cu^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+} are required for neuronal activity within synapses. Due to
the necessity of these metal ions, cells have a sophisticated system to maintain metal-ion homeostasis.
Breakdown of these mechanisms alters the ionic balance and can result in aggregation of Aβ peptide
into plaques, and production of reactive oxygen species induced by Aβ. Elevated levels of these metal
ions can readily bind to Aβ via histidine residues H6, H13, and H14, facilitating Aβ aggregation [17–21]
and generating reactive oxygen species (ROS) via Fenton-like reactions, which lead to oxidative stress
and eventual neuronal death in AD patients [22]. Thus, clearance of Aβ amyloid in the brain by
targeting metal ions effectively detoxifies the Aβ plaques, and has become a promising approach
for inhibition of Aβ aggregation [17,20,22]. These metal ions are also implicated in the formation of
hyperphosphorylated tau and tau tangles [23].

Clioquinol (CQ, Figure 1), and PBT2 (2, Figure 1) exhibit moderate affinity for Cu^{2+} and
Zn^{2+} and can inhibit metal-induced Aβ aggregation and ROS generation in vitro [24–27]. Recently,
8-hydroxyquinoline-based multi-target-directed ligands (MTDLs), such as M30, HLA20, WBQ5187,
and tacrine-8-hydroxyquinoline hybrids, have been developed for treatment of AD. These compounds
significantly inhibit Aβ aggregation in vitro and improve cognition in vivo in mouse models [28–31].
Interestingly, the melatonin-N-benzylamine hybrids (3, Figure 1), which have an indole framework, can
promote the effective development of neural stem cells into the neuronal phenotype [32,33]. The current
study focuses on a series of molecules that incorporate the indole moiety and the 8-hydroxyquinoline
moiety into a novel 8-hydroxyquinoline-indole hybrid structure (Figure 2; left panel). The fifth and
seventh position of the 8-hydroxyquinoline moiety has also been varied by replacing the hydrogen atoms
with chloro or bromo groups, followed by conjugation of these 8-hydroxyquinoline moieties to indole
or 5 fluoro-, 5-chloro- or 5-methoxyindole scaffolds to improve binding affinity and metal ion selectivity.
In addition, recent studies have reported that incorporating a piperazine moiety into these MTDL
molecules affords significant inhibition of Aβ aggregation [34], which prompted us to design a second
set of hybrid molecules by inserting a piperazine bridging moiety between the 8-hydroxyquinoline
and indole scaffolds (Figure 2; right panel) to afford a series of novel 8-hydroxyquinoline indole ester
and amide analogs as multitarget-directed drugs in AD.
For the synthesis of hybrid 8-hydroxyquinoline indoles (7a–7e), we initially prepared tert-butyl (2-(hydroxymethyl)quinolin-8-yl) carbonates 4a and 4b utilizing literature procedures [35], and carried out EDC coupling of each compound with a variety of indole-2-carboxylic acids (5a–5c) to afford Boc-protected ester intermediates 6a–6e. Boc-deprotection of intermediates 6a–6e was carried out with trifluoroacetic acid in dichloromethane at room temperature to afford 7a–7e (Scheme 2).

For the synthesis of hybrid N-((5,7-dichloro-8-hydroxyquinolin-2-yl)methyl)-1H-indole-2-carboxamides (12a–12b), the key intermediate 2-(aminomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate (10) was prepared by reacting intermediate 4b [35] with iodine in the presence of PPh3 and imidazole in dichloromethane, for 1 h at room temperature, to afford iodo-intermediate 8. The product
was then reacted with sodium azide, at reflux temperature in acetone for 6 h, to afford the azido intermediate 9. Compound 9 was then reduced to the amino compound 10 by treatment with PPh3 in water at 60 °C for 12 h (Scheme 1).

Scheme 1. Synthesis of 2-(aminomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate intermediate 10.

Finally, EDC coupling of 10 with indole-2-carboxylic acids 5a or 5b afforded the Boc-protected amide intermediates 11a and 11b. Boc-deprotection of 11a and 11b was carried out with trifluoroacetic acid in dichloromethane at room temperature to afford 12a and 12b, respectively (Scheme 3).

Scheme 2. Synthesis of (8-hydroxyquinolin-2-yl)methyl 1H-indole-2-carboxylate analogs 7a–7e.

Scheme 3. Synthesis of N-((5,7-dichloro-8-hydroxyquinolin-2-yl)methyl)-1H-indole-2-carboxamide analogs (12a and 12b).

For the synthesis of hybrid (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylates 18a–18h, initially we synthesized 8-(tert-butoxycarbonyloxyquinolin-2-yl)methyl...
1H-1,2,4-triazole-1-carboxylate intermediates 14a–14c by reacting tert-butyl (2-(hydroxymethyl)quinolin-8-yl) carbonates 4a–4c with di-(1H-1,2,4-triazol-1-yl)methanone (13, CDT) in dichloromethane at room temperature. Compounds 14a–14c were then each reacted with piperazine (15) in dichloromethane at room temperature to afford the corresponding piperazine-1-carboxylates 16a–16c, which were subjected to EDC coupling with indole-2-carboxylic acids 5a–5c to afford the Boc-protected intermediates 17a–17h. Subsequent deprotection of 17a–17h with trifluoroacetic acid in dichloromethane at room temperature over 4 h afforded the desired series of (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylates 18a–18h in 75–90% yields (Scheme 4). Structural confirmation of all the above intermediates and final synthetic products was obtained from 1H-NMR, 13C-NMR, and HR-MS analysis (See Supplementary Materials).

Scheme 4. Synthesis of (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate analogs 18a–18h.

2. Results and Discussion

The novel hybrid 8-hydroxyquinoline-indole derivatives, 7a–7e, 12a, 12b, and the (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate analogs 18a–18h were evaluated for inhibitory activity against self-induced Aβ1-42 aggregation at five different concentrations (0.3, 1.0, 3, 9 and 27 µM) with 25 µM Aβ1-42, utilizing a thioflavin T (ThT) fluorescence assay with clioquinol, 1 as a positive control (Figure 3). In the absence of inhibitor, the self-induced Aβ1-42 aggregation ThT fluorescence was recorded as 115 arbitrary units (a.u.). The self-induced Aβ1-42 aggregation results for all the above synthetic analogs from the ThT fluorescence assay are shown in Table 1. The results indicate that analogs lacking a piperazino bridging moiety in their structure (i.e., 7a–7e and 12a–12b) were found to be either weak inhibitors or inactive against self-induced Aβ1-42 aggregation. Of the piperazino-containing compounds 18a–18h, five analogs exhibited EC50 values >3 µM. 8-Hydroxyquinolin-2-yl compounds containing the piperazino bridge linked to indole (18e), or 5-methoxy indole (18d and 18f) (EC50 = 1.72, 1.48 and 1.08 µM, respectively) were the most potent inhibitors of self-induced Aβ1-42 and were superior to both clioquinol (1, EC50 = 9.95 µM) and 1H-indole-2-carboxylic acid (5a, EC50 > 20 µM). The structure activity study (SAR) revealed that incorporation of the piperazino bridging moiety between a 5-methoxy indole group and a 5,7-dichloro-8-hydroxyquinoline group affords the most potent compound, 18f, which exhibited a 10-fold improvement in inhibitory potency over 1.
The fluorescence intensity of Aβ1–42 after treatment with Zn2+ was designated as 100%. The fluorescence intensity of Aβ1–42 incubated with or without drugs for 24 h at 37 °C was found to induce amyloid plaques [8,14]. Due to their inhibitory activity against Aβ1–42 aggregation in thioflavin T (ThT)-fluorescence assays.

Table 1. EC50 (µM) values of compound 1, and hybrid 8-hydroxyquinoline-indole derivatives 7a–7e, 12a–12b and 18a–18h against self-induced Aβ1–42 aggregation in thioflavin T (ThT)-fluorescence assays.

| Compd. | 1 | 7a | 7b | 7c | 7d | 7e | 12a | 12b |
|--------|---|----|----|----|----|----|-----|-----|
| Aβ1–42| 9.95 | 4.26 | 9.28 | 3.22 | 6.34 | 9.52 | >10  | >10  |
| Compd. | 18a | 18b | 18c | 18d | 18e | 18f | 18g | 18h |
| Aβ1–42| >10 | >10 | 1.72 | 1.48 | 2.49 | 1.08 | >10  | 2.58 |

Analogs 18c, 18d, and 18f, showed potent self-induced Aβ1–42 aggregation (<2 µM) and were selected for further investigation for inhibition of Cu2+ - and Zn2+-induced Aβ1–42 aggregation. Metal ions such as Cu2+ and Zn2+ can react with Aβ to form Aβ oligomers, and then eventually to form Aβ amyloid plaques [8,14]. Due to their inhibitory activity against Aβ1–42, 18c, 18d, and 18f were expected to decrease metal ion-induced Aβ1–42 aggregation.

Initially, CuSO4 or ZnCl2 was added to Aβ1–42 peptides at room temperature and the mixture incubated with or without drugs for 24 h at 37 °C. The fluorescence intensity of Aβ1–42 peptide alone was designated as 100%. The fluorescence intensity of Aβ1–42 peptide after treatment with Cu2+ was 135.1% and after treatment with Zn2+ was 129.3% compared to Aβ1–42 alone (Figure 4A,B), indicating that both Cu2+ and Zn2+ ions significantly enhanced Aβ1–42 aggregation.

![Figure 3](image-url)  
Figure 3. Dose-response curves of (A) compounds 7a–7e, 12a–12b and 1; (B) compounds 18a–18h and 1 against self-induced Aβ1–42 aggregation.

![Figure 4](image-url)  
Figure 4. Effect of (A) Zn2+- induced and (B) Cu2+-induced Aβ1–42 aggregation in the presence of clioquinol (1), 18c, 18d, and 18f at 50 µM. Data shown are presented as the mean ±SD of three independent experiments.
In contrast, the fluorescence produced by Aβ1-42 treated with Cu²⁺ or Zn²⁺ ions in the presence of 18c, 18d, and 18f dramatically decreased (Figure 4), indicating inhibition of metal ion-induced Aβ1-42 aggregation (i.e., 18c, 76.6% decrease (Cu²⁺) and 70.3% decrease (Zn²⁺); 18d, 66.1% decrease (Cu²⁺) and 59.3% decrease (Zn²⁺); 18f, 88.3% decrease (Cu²⁺) and 82.3% decrease (Zn²⁺); and for comparison compound 1, 62.5% decrease (Cu²⁺) and 57.2% decrease (Zn²⁺)). These results show that 18f exhibited significantly greater inhibitory activity in Cu²⁺- and Zn²⁺-induced Aβ1-42 aggregation than compound 1.

Based on the above results from self-induced Aβ1-42 aggregation and metal-induced Aβ1-42 aggregation studies, the most potent compounds 18f was selected for further investigation into its ability to chelate metal ions such as Cu²⁺, Zn²⁺, and Fe²⁺ using UV-vis spectroscopy at wavelengths ranging from 200 to 500 nm. Compound 18f (50 µM) was treated with 50 µM concentrations of CuSO₄, ZnCl₂, or FeSO₄ for 30 min in HEPES buffer at room temperature, and displayed maximum absorption wavelength shifts from 252 to 274, 269 and 255 nm in the presence of Cu²⁺, Zn²⁺, and Fe²⁺, respectively (Figure 5). These wavelength shifts are indicative of the formation of 18f-Cu²⁺, 18f-Zn²⁺, and 18f-Fe²⁺ and show that Zn²⁺ and Cu²⁺ undergo significant chelation with compound 18f. The spectrum of 18f-Fe²⁺ showed only a moderate increase in wavelength. Furthermore, the stoichiometry of formation of metal complexes 18f-Cu²⁺ and 18f-Zn²⁺ was also determined by using Job's method (Figure 6) [36]. The isosbestic points shown in Figure 6A,B at 0.6 and 0.5 imply a stoichiometry of 1.5:1 for 18f-Cu²⁺ and 1:1 for 18f-Zn²⁺.

![Figure 5](image_url)

*Figure 5.* UV-absorbance spectra of compound 18f (50 µM) in the presence of Cu²⁺, Zn²⁺, or Fe²⁺ (each at 50 µM) in buffer (20 mM HEPES, 150 mM NaCl; pH = 7.4). 18f displayed maximum absorption wavelength shifts from 252 to 274, 269 and 255 nm in the presence of Cu²⁺, Zn²⁺, and Fe²⁺, respectively.

Copper and zinc dysregulation in the brain is considered to arise as a consequence of age-associated neurodegenerative disease such as Alzheimer’s disease. Zinc has also been shown to play an important role in pre- and post-synaptic responses [37]. Our drug design approach is to target dysregulation of metal homeostasis and protein aggregation. Dysregulation of metal ions is implicated in the elevated formation of reactive oxygen species and in susceptibility to oxidative stress. These metal ions were shown to interact with protein aggregation seed proteins such as tau and Aβ1-42, increasing misfolding...
Among these analogs, compounds 7b–7d, 18c–18f associated neurotoxicity. Analogs 8-hydroxyquinoline-indole analogs reduces tau- and amyloid-mediated protein aggregation, and their influence trans-synaptic and promoting protein aggregation. The 8-hydroxyquinoline compound, PBT2, has been shown to reduce protein aggregation in SY5Y-APPSw cells. SY5Y-APPSw cells were exposed to the above protein inhibitors effective inhibition of both self-induced and metal ion-induced Aβ1–42 aggregation in cell culture is assessed after 48 h. Another important difference is that in vitro aggregation is assessed after 24 h, whereas thioflavin in cell-culture stains not just amyloid aggregates but also other amyloid-like fibrillar deposits by staining with 0.1% w/v thioflavin T, counterstaining nuclei with DAPI, and mean ThT fluorescence signal per nucleus was calculated over multiple fields [39]. All the analogs reduced fluorescence intensity (indicative of protein aggregation) by 26–62% (P < 0.001 by 2-tailed t-test). Among these analogs, compounds 7b–7d, 18c, and 18f were the most effective as protein aggregation inhibitors in HEK-tau cells, and were more potent than clioquinol (1) (Figure 7). In our study, we have shown that restoring metal homeostasis by treatment with hybrid 8-hydroxyquinoline-indole analogs reduces tau- and amyloid-mediated protein aggregation, and their associated neurotoxicity. Analogs 7a–7d, 18c–18f, and 18h were evaluated for their ability to reduce protein aggregation in HEK-tau cells. HEK-tau cells were exposed to the above Aβ1–42 aggregation inhibitors and parent drug clioquinol (1) at 1 µM for 48 h at 37 °C. Cells were assessed for amyloid deposits by staining with 0.1% w/v thioflavin T, counterstaining nuclei with DAPI, and mean ThT fluorescence signal per nucleus was calculated over multiple fields [39]. All the analogs reduced fluorescence intensity (indicative of protein aggregation) by 26–62% (P < 0.001 by 2-tailed t-test). Among these analogs, compounds 7b–7d, 18c, and 18f were the most effective as protein aggregation inhibitors in HEK-tau cells, and were more potent than clioquinol (1) (Figure 7A,B).
In another study compounds 7a–7d, 18c–18f, and 18h were also evaluated for their ability to reduce protein aggregation in SY5Y-APP<sub>Sw</sub> cells. SY5Y-APP<sub>Sw</sub> cells were exposed to the above protein aggregation inhibitors and parent compound 1 at 1 µM for 48 h at 37 °C. Cells were assessed for amyloid deposits by staining with 0.1% w/v ThT, counterstaining nuclei with DAPI, and mean ThT fluorescence signal per nucleus calculated over multiple fields. All the analogs reduced fluorescence intensity (indicative of protein aggregation) by 22–67% (P < 0.001 by 2-tailed t-test). Analogs 7a and 18d were the most effective in SY5Y-APP<sub>Sw</sub> cells and were more potent than the parent compound, clioquinol (1) (Figure 7C,D). Thioflavin-T fluorescence assays for in vitro amyloid aggregation and cell culture models of amyloid aggregation overlap but are not identical. This is due to the fact that thioflavin in cell-culture stains not just amyloid aggregates but also other amyloid-like fibrillar structures. Another important difference is that in vitro aggregation is assessed after 24 h, whereas aggregation in cell culture is assessed after 48 h.

The binding interactions of the active compounds 18d and 18f were also investigated utilizing a crystal structure of the target Aβ<sub>1–42</sub> peptide (PDB code: 1IYT) [40] employing Schrodinger Maestro 11.4 software (Figure 8) [41]. For analogue 18d, the quinoline ester carbonyl group of this hybrid molecule is involved in a hydrogen bonding interaction with Lys16 at a distance of 3.12 Å units and the 8-hydroxyquinoline moiety is involved in a π–π stacking interaction with residue Phe20; this is similar to that observed with the deoxyvasicinone-donepezil hybrid molecule binding region on Aβ<sub>1–42</sub> reported in the literature [41]. In addition, the quinoline and indole ring systems of compound 18d are involved in π–cation interactions with Lys16. With the potent analog 18f, the hydroxyl group of the 8-hydroxyquinoline moiety is involved in a hydrogen bonding interaction with the His13 residue at 2.17 Å units, and the 8-hydroxyquinoline moiety of 18f is also involved in a π–π stacking interaction with Phe20, similar to the binding mode of compound 18d. In addition, the quinoline ring system of 18f is involved in a π–cation interaction with Lys16. These docking results indicate that the effective inhibition of both self-induced and metal ion-induced Aβ<sub>1–42</sub> aggregation by 18d and 18f may be due to hydrogen bonding interactions, π–π stacking interactions, and π–cation interactions of these molecules. Of particular interest is the interaction the most potent compound 18f with the His13 residue on the Aβ<sub>1–42</sub> peptide. His13 has been implicated in the binding of metal ions with Aβ<sub>1–42</sub> (17–21); thus, 18f may compete with metal ions for this binding site on Aβ<sub>1–42</sub>, thereby inhibiting metal ion induction of Aβ<sub>1–42</sub> aggregation.

![Figure 8. Proposed binding modes of (A) compound 18d and (B) 18f with Aβ<sub>1–42</sub> peptide.](image-url)

In summary, a series of hybrid 8-hydroxyquinoline-indole derivatives 7a–7e, 12a–12b, and 18a–18h have been synthesized and evaluated as inhibitors of Aβ<sub>1–42</sub> self-aggregation and metal chelation-induced aggregation, and have also been evaluated against HEK-tau and SY5Y-APP<sub>Sw</sub> cells to determine their effect on cellular protein aggregation. In vitro studies showed that hybrid 8-hydroxyquinoline-indole analogs lacking the piperazine bridging unit (i.e., compounds 7a–7e and 12a–12b) exhibited poor-to-moderate inhibitory activities against Aβ<sub>1–42</sub> peptide self-aggregation. However, the majority of the hybrid 8-hydroxyquinoline-indole compounds incorporating the piperazine bridge unit (i.e., 18c–18f, and 18h) were potent inhibitors against Aβ<sub>1–42</sub> self-aggregation.
Analogue 18f was the most potent inhibitor of Aβ1–42 aggregation in both self-induced and metal chelation-induced assays, and in protein aggregation assays in HEK-tau cells. Compound 18f afforded 82.3% and 88.3% inhibition against Cu2+ -induced and Zn2+ -induced Aβ1–42 aggregation assays, and was also shown to be a chelator of Cu2+, Zn2+, and Fe2+ ions in physiological buffer solutions. Molecular docking studies with 18f showed that this molecule has the potential to interact with the His13 residue on Aβ1–42 peptide, which suggests that its potent anti-aggregation properties may also be due in part to its ability to competitively inhibit the binding of metal ions that interact with this site to induce Aβ1–42 aggregation. Thus, the present study has identified 18f, an analog that incorporates a piperazine bridging group between a 5-methoxyindole moiety and a 5,7-dichloro-8-hydroxyquinoline moiety, as the most potent Aβ1-42 aggregation inhibitor; this analog exhibited a 10-fold improvement in inhibitory potency over clioquinol (1) as an inhibitor of Aβ1-42 self-aggregation. We consider 18f to be a lead compound worthy of further structural optimization and preclinical development as a treatment for AD.

3. Experimental Section

3.1. Chemistry

All reagents, solvents, and chemicals utilized in the synthesis of the hybrid 8-hydroxyquinoline-indole analogs were purchased from Oakwood Chemicals Fisher Scientific and Adooq Bioscience. 1H and 13C-NMR spectra were recorded on a Varian 400 MHz spectrometer equipped with a Linux workstation running on vNMRj software. Spectral analyses were carried out in CDCl3 or DMSO-d6 for both 1H and 13C spectra. Chemical shifts were measured in δ parts per million (ppm) and coupling constants (J) were measured in hertz (Hz). High-resolution mass spectra (HR-MS) were recorded on an Agilent 6545 ESI/APCI TOF MS. Thin-layer chromatography (TLC) was carried out on pre-coated silica gel glass plates (F254 Merck).

3.2. Experimental Procedure for the Synthesis of Hybrid 8-Hydroxyquinoline-Indole Analogs

Synthesis of (8-hydroxyquinolin-2-yl)methyl-1H-indole-2-carboxylate hybrid analogs 7a–7e

To a stirred solution of an appropriate indole-2-carboxylic acid (5a–5c) (0.15 mmol) in dry dichloromethane (5 mL) under an argon atmosphere at 0 °C was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.181 mmol), and the mixture allowed to stir for 15 min. A catalytic amount of 4-dimethylaminopyridine (DMAP , 0.045 mmol) was then added, followed by addition of an appropriate tert-butyl (2-(hydroxymethyl)quinolin-8-yl) carbonate (4a–4b) (0.151 mmol) in dry dichloromethane (3 mL) at 0 °C. The resulting mixture was stirred for 8 h at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was washed with water (10 mL), and the dichloromethane layer separated, dried over anhydrous sodium sulfate, filtered, concentrated, and purified by silica gel column chromatography using 2–5% methanolic dichloromethane as eluent to afford the corresponding Boc-protected 8-hydroxyquinoline-indole derivatives (6a–6e).

The above Boc-protected 8-hydroxyquinoline-indole derivatives (6a–6e) were each dissolved in a mixture of DCM (10 mL) and TFA (1.0 mL) and stirred for 6 h until the Boc-deprotection was completed. A saturated NaHCO3 solution (20 mL) was then added and the resulting mixture was extracted with dichloromethane. The organic layer was separated and washed with water, followed by brine solution, dried over anhydrous Na2SO4, and concentrated to afford the appropriate hybrid 8-hydroxyquinoline indole derivatives (7a–7e).

(8-Hydroxyquinolin-2-yl)methyl-1H-indole-2-carboxylate (7a): 1H-NMR (400 MHz, DMSO-d6): δ 12.00 (s, 1H), 9.67 (s, 1H), 8.38 (d, J = 8.4 Hz, 1H), 7.76–63 (m, 2H), 7.52–7.37 (m, 3H), 7.35–7.22 (m, 2H), 7.13–7.07 (m, 2H), 5.64 (s, 2H) ppm. 13C-NMR (100 MHz, DMSO-d6): δ 161.44, 154.70, 153.40, 138.08, 138.01, 137.56, 128.57, 127.99, 127.16, 127.14, 125.34, 122.59, 120.73, 120.35, 118.15, 113.06, 112.19, 108.99, 67.50 ppm. HR-MS (ESI) m/z calcd for C19H14N2O3 (M + H)+ 319.1075, found 319.1076.
(5,7-Dichloro-8-hydroxyquinolin-2-yl)methyl-1H-indole-2-carboxylate (7b): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 12.02 (s, 1H), 10.67 (s, 1H), 8.58 (d, \(J = 8.8\) Hz, 1H), 7.91 (d, \(J = 8.8\) Hz, 1H), 7.84 (s, 1H), 7.7 (d, \(J = 8.0\) Hz, 1H), 7.49 (d, \(J = 8.8\) Hz, 1H), 7.33 (s, 1H), 7.29 (t, \(J = 7.6\) Hz, 1H), 7.10 (t, \(J = 7.6\) Hz, 1H), 5.70 (s, 2H) ppm. \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 161.35, 156.98, 149.23, 138.68, 138.05, 134.50, 128.27, 127.15, 126.97, 125.42, 124.61, 122.62, 121.61, 120.77, 119.58, 116.56, 113.06, 109.14, 67.02 ppm. HR-MS (ESI) \(m/z\) calcd for \(\text{C}_{19}\text{H}_{22}\text{Cl}_2\text{N}_2\text{O}_3\) (M + H\(^+\)) 387.0291, found 387.0296.

(8-Hydroxyquinolin-2-yl)methyl-5-methoxy-1H-indole-2-carboxylate (7c): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 11.87 (s, 1H), 9.67 (s, 1H), 8.38 (d, \(J = 8.4\) Hz, 1H), 7.70 (d, \(J = 8.4\) Hz, 1H), 7.46–7.35 (m, 3H), 7.2 (s, 1H), 7.12 (d, \(J = 2.8\) Hz, 2H), 6.95 (d, \(J = 8.8\) Hz, 1H), 5.63 (s, 2H) ppm. \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 161.36, 154.76, 154.44, 153.40, 138.08, 137.55, 133.40, 128.57, 127.98, 127.49, 127.30, 120.33, 118.15, 117.03, 113.98, 112.18, 108.55, 102.39, 67.40, 55.65 ppm. HR-MS (ESI) \(m/z\) calcd for \(\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_4\) (M + H\(^+\)) 349.1174, found 349.1178.

(5,7-Dichloro-8-hydroxyquinolin-2-yl)methyl-5-fluoro-1H-indole-2-carboxylate (7d): \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 12.13 (s, 1H), 9.67 (s, 1H), 8.38 (d, \(J = 8.4\) Hz, 1H), 7.71 (d, \(J = 8.4\) Hz, 1H), 7.50–7.40 (m, 4H), 7.28 (s, 1H), 7.19–7.11 (m, 2H), 5.64 (s, 2H) ppm. \(^{13}\)C-NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 161.17, 158.86, 156.54, 154.58, 153.41, 138.09, 137.57, 137.45, 125.80, 125.88, 125.01, 127.24, 127.13, 120.37, 118.15, 114.49, 114.39, 114.20, 112.20, 108.88, 108.83, 106.66, 106.43, 67.62 ppm. HR-MS (ESI) \(m/z\) calcd for \(\text{C}_{19}\text{H}_{13}\text{F}_2\text{N}_2\text{O}_3\) (M + H\(^+\)) 337.0969, found 337.0978.

Synthesis of tert-butyl (5,7-dichloro-2-(iodomethyl)quinolin-8-yl) carbonate (8):
To a stirred solution of tert-butyl (5,7-dichloro-2-(hydroxymethyl)quinolin-8-yl) carbonate \(4\) \(b\) (1 mmol) was added triphenyl phosphine (1.2 mmol), imidazole (1.3 mmol) in dichloromethane, and iodine (1.2 mmol) at room temperature. The resulting reaction mixture was stirred for 1 h. After completion of the reaction, aqueous sodium dithionite solution was added and the resulting mixture extracted with dichloromethane, the separated organic layer was dried over \(\text{Na}_2\text{SO}_4\) and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford compound 8.

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.42 (d, \(J = 8.4\) Hz, 1H), 7.62 (s, 1H), 7.61 (d, \(J = 8.8\) Hz, 1H), 4.61 (s, 2H), 1.60 (s, 9H) ppm. \(^{13}\)C-NMR (101 MHz, CDCl\(_3\)): \(\delta\) 160.22, 150.41, 142.71, 141.54, 134.27, 128.76, 127.40, 127.34, 124.70, 122.56, 84.43, 27.67, 5.41 ppm.

Synthesis of 2-(azidomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate (9):
To a stirred solution of tert-butyl (5,7-dichloro-2-(iodomethyl)quinolin-8-yl) carbonate 8 (1 mmol) in acetone was added sodium azide (3 mmol). The resulting mixture was stirred at reflux temperature for 6 h. After completion of the reaction, the reaction mixture was cooled to room temperature then solvent was evaporated. Water was added to the crude mass and mixture extracted with dichloromethane, the separated organic layer dried over \(\text{Na}_2\text{SO}_4\) and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, 1% methanol in dichloromethane) to afford compound 9.

\(^1\)H-NMR (CDCl\(_3\), 400 MHz): \(\delta\) 8.49 (d, \(J = 8.8\) Hz, 1H), 8.00 (s, 1H), 7.60 (d, \(J = 8.4\) Hz, 1H), 4.64 (s, 2H), 1.59 (s, 9H) ppm. \(^{13}\)C-NMR (CDCl\(_3\), 101 MHz): \(\delta\) 13C-NMR (CDCl\(_3\), 101 MHz): \(\delta\) 158.07, 150.27, 144.91, 141.76, 137.04, 133.13, 126.94, 121.31, 118.93, 117.11, 84.41, 77.30, 76.98, 76.67, 55.60, 27.61 ppm.

Synthesis of 2-(aminomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate (10):
To a stirred solution of 2-(azidomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate 9 (1 mmol) in THF/H₂O (3 mL, 9:1) was added PPh₃ (1.2 mmol) at room temperature. The resulting reaction mixture was stirred at 60 °C for 12 h. After completion of reaction, the reaction mixture was concentrated under vacuum and 1 M aqueous HCl added to the residue. The mixture was stirred for 30 min, then the mixture was filtered to get aqueous phase. The aqueous phase was basified to pH 10 with NaHCO₃ and extracted with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄, and the solution was evaporated under vacuum to afford target compound 10.

Synthesis of N-((8-hydroxyquinolin-2-yl)methyl)-1H-indole-2-carboxamide hybrid analogs 12a and 12b:

To a stirred solution of indole-2-carboxylic acid (1 mmol) in dry dichloromethane (5 mL) under an argon atmosphere at 0 °C was added EDCI (0.181 mmol) and the mixture allowed to stir for 15 min. A catalytic amount of DMAP (0.045 mmol) was then added followed by addition of 2-aminomethyl)-5,7-dichloroquinolin-8-yl tert-butyl carbonate (10) (0.151 mmol) in dry dichloromethane (3 mL) at 0 °C, and the resulting mixture was stirred for 8 h at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was washed with water (10 mL), and the dichloromethane layer separated, dried over anhydrous sodium sulfate, filtered, concentrated, and purified by silica gel column chromatography, using 2–5% methanolic dichloromethane as eluent, to afford the corresponding Boc-protected 8-hydroxyquinoline-indole derivatives 11a or 11b as a solid. Products were obtained in 56–74% yield.

The above Boc-protected 8-hydroxyquinoline-indole derivatives 11a and 11b were each dissolved in a mixture of DCM (10 mL) and trifluoroacetic acid (TFA, 1.0 mL) and the resulting mixture stirred for 6 h until the Boc-deprotection was completed. A saturated NaHCO₃ solution (20 mL) was then added and the resulting mixture extracted with dichloromethane. The organic layer was separated and washed with water, followed by brine solution, dried over anhydrous Na₂SO₄, and concentrated to afford the appropriate hybrid 8-hydroxyquinoline indole derivatives 12a and 12b.

N-((5,7-Dichloro-8-hydroxyquinolin-2-yl)methyl)-1H-indole-2-carboxamide (12a): ¹H-NMR (400 MHz, DMSO-δ₆): δ 12.01 (s, 1H), 10.71 (s, 1H), 10.07 (s, 1H), 8.52 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.33 (s, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 5.71 (s, 2H) ppm. ¹³C-NMR (100 MHz, DMSO-δ₆): δ 161.34, 156.89, 151.03, 138.59, 138.05, 137.00, 133.70, 127.14, 126.97, 125.25, 125.41, 122.61, 122.08, 120.77, 113.06, 109.39, 109.14, 106.03, 66.91 ppm. HR-MS (ESI) m/z calcd for C₁₉H₁₃Cl₂N₂O₃ (M + H)⁺ 386.0379, found 386.0382.

N-((5,7-Dichloro-8-hydroxyquinolin-2-yl)methyl)-5-methoxy-1H-indole-2-carboxamide (12b): ¹H-NMR (400 MHz, DMSO-δ₆): δ 11.87 (s, 1H), 10.64 (s, 1H), 10.02 (s, 1H), 8.56 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.8 (s, 1H), 7.35 (d, J = 9.2 Hz, 1H), 7.2–7.19 (m, 1H), 7.11 (d, J = 2.4 Hz, 1H), 6.94 (dd, J = 8.8, 2.4 Hz, 1H), 5.67 (s, 2H), 3.74 (s, 3H) ppm. ¹³C-NMR (100 MHz, DMSO-δ₆): δ 161.26, 157.04, 154.46, 149.21, 138.68, 134.49, 133.43, 128.26, 127.47, 127.10, 124.60, 121.59, 119.58, 117.13, 116.55, 113.98, 108.68, 102.37, 66.93, 55.65 ppm. HR-MS (ESI) m/z calcd for C₂₀H₁₅Cl₂N₂O₃ (M + H)⁺ 416.0485, found 416.0492.

Synthesis of (8-((tert-butoxycarbonyloxy)quinolin-2-yl) methyl-1H-1,2,4-triazole-1-carboxylates 14a–14c:

To a stirred solution of an appropriate tert-butyl (2-(hydroxymethyl)quinolin-8-yl) carbonate (4a–4o) (1 mmol) in dichloromethane was added carbonyldimidazole (2 mmol) at room temperature. The reaction mixture was stirred for 30 min. After completion of the reaction, water was added and the resulting mixture was extracted with dichloromethane, the separated organic layer dried over Na₂SO₄ and concentrated under reduced pressure to afford triazole intermediates 14a–14c.
(8-((tert-Butoxycarbonyl)oxy)quinolin-2-yl)methyl-1H-1,2,4-triazole-1-carboxylate (14a):

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.88 (s, 1H), 8.27 (d, $J = 8.5$ Hz, 1H), 8.11 (s, 1H), 7.74 (dd, $J = 6.6, 3.0$ Hz, 1H), 7.63 (d, $J = 8.5$ Hz, 1H), 7.58–7.50 (m, 2H), 5.77 (s, 2H), 1.59 (s, 9H) ppm. $^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 153.83, 153.68, 151.83, 147.28, 145.79, 140.60, 137.46, 128.89, 126.90, 125.61, 121.67, 119.87, 83.70, 71.17, 27.62 ppm.

(8-((tert-Butoxycarbonyl)oxy)-5,7-dichloroquinolin-2-yl)methyl-1H-1,2,4-triazole-1-carboxylate (14b):

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.89 (s, 1H), 8.59 (d, $J = 8.8$ Hz, 1H), 8.11 (s, 1H), 7.71 (d, $J = 8.4$ Hz, 2H), 5.78 (s, 2H), 1.58 (s, 9H) ppm. $^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 155.53, 153.88, 150.16, 147.26, 145.86, 144.92, 141.67, 137.30, 133.51, 127.12, 120.74, 118.92, 117.35, 84.52, 70.23, 27.55 ppm.

(8-((tert-Butoxycarbonyl)oxy)-5,7-dibromoquinolin-2-yl)methyl-1H-1,2,4-triazole-1-carboxylate (14c):

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.89 (s, 1H), 8.54 (d, $J = 8.7$ Hz, 1H), 8.08 (d, $J = 30.9$ Hz, 2H), 7.71 (d, $J = 8.8$ Hz, 1H), 5.78 (s, 2H), 1.58 (s, 9H) ppm. $^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 155.59, 153.75, 153.35, 152.22, 137.99, 137.42, 128.43, 127.80, 119.91, 118.08, 112.07, 67.84, 45.81, 27.52 ppm.

Synthesis of hybrid (8-((tert-butoxycarbonyloxy)quinolin-2-yl)methylpiperazine-1-carboxylates 16a–16c:

An appropriate triazole intermediate (14a–14c) (1 mmol) was dissolved in dichloromethane (2 mL) and treated with piperazine (15) (1.0 mmol) at room temperature for 1 h. Completion of the reaction was monitored by TLC. Water (2 mL) was then added and the resulting mixture was extracted with dichloromethane (2 × 3 mL), the organic layer separated, dried over anhydrous Na$_2$SO$_4$ and concentrated to afford the appropriate Boc-protected product (16a–16c). The crude product was purified by column chromatography (silica gel, 2% methanol in dichloromethane) to afford the desired product (16a–16c).

(8-((tert-Butoxycarbonyl)oxy)quinolin-2-yl)methyl-piperazine-1-carboxylate (16a): $^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.33 (d, $J = 8.4$ Hz, 1H), 7.52 (d, $J = 8.4$Hz, 1H), 7.45–7.35 (m, 2H), 7.10 (dd, $J = 7.2, 1.2$ Hz, 1H), 5.33 (s, 2H), 3.37 (m, 4H), 2.72–2.61 (m, 4H), 1.58 (s, 9H) ppm; $^{13}$C-NMR (101 MHz, DMSO-d$_6$): $\delta$ 155.59, 154.75, 153.35, 153.22, 137.99, 137.42, 128.43, 127.80, 119.91, 118.08, 112.07, 67.84, 45.81, 27.52 ppm.

(8-((tert-Butoxycarbonyl)oxy)-5,7-dichloro-8-hydroxyquinolin-2-yl)methyl-piperazine-1-carboxylate (16b):

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.39 (d, $J = 8.4$ Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 1H), 7.48 (s, 1H), 5.37 (s, 2H), 3.59 (m, 4H), 2.88 (br-s, 4H), 1.57 (s, 9H) ppm; $^{13}$C-NMR (101 MHz, CDCl$_3$): $\delta$ 156.66, 153.64, 154.82, 147.78, 137.93, 134.32, 128.07, 124.18, 120.40, 120.31, 115.78, 67.22, 53.42, 45.49, 27.52 ppm.

(8-((tert-Butoxycarbonyl)oxy)-5,7-dibromo-8-hydroxyquinolin-2-yl)methyl-piperazine-1-carboxylate (16c):

$^1$H-NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.40 (d, $J = 8.8$ Hz, 1H), 7.97 (s, 1H), 7.68 (d, $J = 8.8$ Hz, 1H), 5.38 (s, 2H), 3.46 (m, 4H), 2.80 (br-s, 4H), 1.58 (s, 9H) ppm; $^{13}$C-NMR (101 MHz, DMSO-d$_6$): $\delta$ 156.92, 154.60, 153.55 153.13, 139.44, 136.71, 133.63, 126.37, 121.45, 106.98, 106.32, 67.49, 45.01, 44.33, 27.51 ppm.

Synthesis of (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate hybrid derivatives (18a–18h):

To the stirred solution of an appropriate indole-2-carboxylic acid (5a–5c) (0.15 mmol) in dry dichloromethane (5 mL) under an argon atmosphere at 0 °C was added EDCI (0.181 mmol), and the mixture allowed to stir for 15 min. A catalytic amount of DMAP (0.045 mmol) was then added, followed by addition of an appropriate (8-((tert-butoxycarbonyloxy)quinolin-2-yl)methyl piperazine-1-carboxylate (16a–16c) (0.151 mmol) in dry dichloromethane (3 mL) at 0 °C. The resulting mixture was stirred for 8 h at room temperature. After completion of the reaction (monitoring by TLC), the reaction mixture was washed with water (10 mL) and
the dichloromethane layer separated, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel column chromatography, using 2–5% methanolic dichloromethane as eluent, to afford the corresponding (8-((tert-butoxycarbonyl)oxy)quinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate hybrid derivatives (17a–17h), which was dissolved in a mixture of DCM (10 mL) and TFA (1.0 mL) and stirred for 6 h until Boc-deprotection was completed. Saturated NaHCO₃ solution (20 mL) was then added to the resulting mixture which was extracted with dichloromethane. The organic layer was separated and washed with water, followed by brine solution, dried over anhydrous Na₂SO₄ and concentrated to afford the corresponding (8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate hybrid derivative (18a–18h).

(8-Hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate (18a): ¹H-NMR (400 MHz, CDCl₃): δ 9.67 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.52–7.42 (m, 3H), 7.35–7.2 (m, 2H), 7.20–7.12 (m, 2H), 6.78 (s, 1H), 5.45 (s, 2H), 3.96 (s, 4H), 3.70 (s, 4H) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ 162.82, 154.94, 154.26, 151.88, 137.07, 127.85, 124.64, 121.87, 120.66, 120.06, 117.73, 111.71, 110.44, 105.49, 68.12, 43.74 ppm. HR-MS (ESI) m/z calcd for C₂₅H₂₂N₄O₄ (M + H)⁺ 431.1708, found 431.1712.

(5,7-Dibromo-8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate (18b): ¹H-NMR (400 MHz, CDCl₃): δ 9.50 (s, 1H), 8.44 (d, J = 8.8 Hz, 1H), 7.86 (s, 1H), 7.61 (dd, J = 15.2, 8.4 Hz, 2H), 7.41 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 6.76 (s, 1H), 5.46 (s, 2H), 3.96 (s, 4H), 3.68 (s, 4H) ppm; ¹⁵N-CMR (100 MHz, CDCl₃): δ 162.75, 156.35, 154.69, 149.35, 137.77, 137.09, 135.75, 133.78, 128.62, 127.32, 125.92, 124.69, 121.91, 121.05, 120.73, 111.78, 110.07, 105.50, 104.33, 67.38, 44.03 ppm. HR-MS (ESI) m/z calcd for C₂₅H₂₂Br₂N₂O₄ (M + H)⁺ 588.9879, found 588.9885.

(5,7-Dichloro-8-hydroxyquinolin-2-yl)methyl-4-(1H-indole-2-carbonyl)piperazine-1-carboxylate (18c): ¹H-NMR (400 MHz, CDCl₃): δ 10.08 (s, 1H), 8.41 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.5 (s, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 7.13 (t, J = 7.2 Hz, 1H), 6.76 (s, 1H), 5.45 (s, 4H), 3.99 (s, 4H), 3.7 (s, 4H) ppm. ¹³C-NMR (100 MHz, CDCl₃): δ 162.98, 156.40, 154.75, 147.29, 137.69, 135.95, 134.42, 128.61, 128.14, 127.22, 124.54, 124.13, 121.84, 120.68, 120.61, 120.45, 115.68, 111.89, 105.52, 67.48, 43.82 ppm. HR-MS (ESI) m/z calcd for C₂₄H₂₀Cl₂N₂O₄ (M + H)⁺ 499.0925, found 499.0929.

(8-Hydroxyquinolin-2-yl)methyl-4-(5-methoxy-1H-indole-2-carbonyl)piperazine-1-carboxylate (18d): ¹H-NMR (400 MHz, DMSO-d₆): δ 11.46 (s, 1H), 9.64 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.45–7.38 (m, 2H), 7.35 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 6.8 Hz, 1H), 7.07 (s, 1H), 6.86 (dd, J = 8.8, 2.4 Hz, 1H), 6.75 (s, 1H), 5.58 (s, 2H), 3.81 (br-s, 4H), 3.75 (s, 3H), 3.63 (br-s, 2H) ppm; ¹³C-NMR (100 MHz, DMSO-d₆): δ 162.65, 155.37, 154.78, 154.18, 153.37, 153.08, 137.43, 131.66, 130.37, 128.50, 127.83, 127.52, 120.01, 118.13, 114.89, 113.36, 112.13, 104.51, 102.31, 68.09, 55.66, 44.04 ppm. HR-MS (ESI) m/z calcd for C₂₅H₂₄N₄O₅ (M + H)⁺ 461.1811, found 461.1813.

(5,7-Dibromo-8-hydroxyquinolin-2-yl)methyl-4-(5-methoxy-1H-indole-2-carbonyl)piperazine-1-carboxylate (18e): ¹H-NMR (400 MHz, DMSO-d₆): δ 11.46 (s, 1H), 8.48 (d, J = 8.8 Hz, 1H), 8.05 (s, 1H), 7.8 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 9.2 Hz, 1H), 7.07 (d, J = 2.0 Hz, 1H), 6.85 (dd, J = 8.8, 2.4 Hz, 1H), 6.75 (s, 1H), 5.45 (s, 2H), 3.821 (s, 4H), 3.75 (s, 3H), 3.65 (br-s, 4H) ppm. ¹³C-NMR (100 MHz, DMSO-d₆): δ 162.66, 157.61, 154.61, 154.17, 149.09, 138.58, 134.32, 131.65, 131.50, 130.32, 130.18,
128.09, 127.49, 127.45, 124.49, 121.23, 119.58, 116.43, 114.88, 113.34, 113.29, 104.49, 104.45, 102.25, 67.63, 44.09, 40.54, 40.33, 40.12, 39.91, 39.70, 39.49, 39.28 ppm. HR-MS (ESI) m/z calcd for C_{25}H_{22}Cl_{2}N_{3}O_{5} (M + H)^{+} 529.1026, found 529.1031.

(5,7-Dibromo-8-hydroxyquinolin-2-yl)methyl-4-(5-fluoro-1H-indole-2-carbonyl)piperazine-1-carboxylate (18g):
{\textsuperscript{1}H-NMR (400 MHz, DMSO-d_{6}):} \delta 11.73 (s, 1H), 8.52 (d, J = 8.8 Hz, 1H), 7.80 (s, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.43 (dd, J = 8.8, 4.8 Hz, 1H), 7.37 (d, J = 10.0 Hz, 1H), 7.05 (t, J = 9.2 Hz, 1H), 6.83 (s, 1H), 5.44 (s, 2H), 3.82 (s, 4H), 3.66 (br-s, 2H), 3.55 (br-s, 2H) ppm. \textsuperscript{13}C-NMR (100 MHz, DMSO-d_{6}): \delta 162.35, 158.72, 157.60, 156.40, 154.62, 149.28, 138.66, 134.30, 133.13, 131.83, 128.11, 127.33, 127.22, 124.50, 121.22, 119.58, 116.47, 113.77, 113.67, 112.57, 112.31, 106.04, 105.81, 104.72, 104.67, 67.66, 43.92 ppm. HR-MS (ESI) m/z calcd for C_{24}H_{19}FN_{3}O_{4} (M + H)^{+} 517.0829, found 517.0833.

(5,7-Dibromo-8-hydroxyquinolin-2-yl)methyl-4-(5-fluoro-1H-indole-2-carbonyl)piperazine-1-carboxylate (18h):
{\textsuperscript{1}H-NMR (400 MHz, DMSO-d_{6}):} \delta 11.72 (s, 1H), 9.65 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.45–7.35 (m, 4H), 7.12–7.03 (m, 2H), 6.83 (d, J = 1.6 Hz, 1H), 5.38 (s, 2H), 3.81 (br-s, 4H), 3.63 (br-s, 2H), 3.54 (br-s, 2H) ppm. \textsuperscript{13}C-NMR (101 MHz, DMSO-d_{6}): \delta 162.33, 158.71, 156.39, 155.35, 154.78, 153.35, 138.01, 137.44, 133.12, 131.83, 128.50, 127.84, 127.33, 127.22, 120.01, 118.14, 113.77, 113.67, 112.57, 112.31, 112.14, 106.05, 105.82, 104.74, 104.69, 68.09, 44.0 ppm. HR-MS (ESI) m/z calcd for C_{24}H_{19}FN_{3}O_{4} (M + H)^{+} 494.1621, found 494.1616.

3.3. Methodology for the In Vitro Self-Induced Aβ_{1–42} Aggregation Assay

The thioflavin T (ThT)-fluorescence assay was used to measure the inhibition of Aβ aggregation [42]. Aβ_{1–42} (Adooq Bioscience, CA, USA) was pretreated with 1 mL of hexafluoroisopropanol (HFIP) to afford a stock solution, which was aliquoted into small samples. The solvent was evaporated at room temperature, and samples were stored at −80 °C. For Aβ_{1–42} aggregation inhibition experiments, phosphate buffer (pH 7.4) was added to the Aβ stock solution to afford a 50 μM concentration before use. A mixture of the Aβ_{1–42} peptide (10 μL, 25 μM final concentration) with or without the test compound (10 μL; 0.3, 1.0, 3, 9, and 27 μM) was incubated at 37 °C for 48 h. Blanks using phosphate buffer (pH 7.4) instead of Aβ, with or without test compound, were also assessed. Then 50 mM glycine-NaOH buffer (pH 8.0) containing ThT (5 μM) was added to 20 μL of the sample to afford a final volume of 200 μL. The fluorescence intensities were recorded (excitation, 450 nm; emission, 485 nm).

The fluorescence intensities were background corrected to the no enzyme control and 100% of intensity reflected no inhibition of aggregation. To obtain the normalized amyloid aggregation inhibition relative to the control group, the amyloid aggregation/cell values were further divided by the total amyloid fluorescence of the control group. Using GraphPad Prism, the amyloid aggregation values (relative to control group) were entered along the corresponding concentrations for the triplicates and then a log transformation was obtained. Using the dose–response simulation module in GraphPad Prism, nonlinear regression fit (curve) was then generated for each small molecule with various concentrations used. Curves were then compared to determine if they were statistically different, by performing the best-fit values of selected unshared parameters between data sets and extra sum-of-squares F test, allowing for selection of the simpler model unless the P value was less than 0.05. The logEC_{50} output parameter was then selected to obtain the EC_{50} for each of the dose–response curves for the small molecules.

3.4. Methodology for the In Vitro Metal-Induced Aβ_{1–42} Aggregation Assay

For the inhibition of copper- and zinc-mediated Aβ_{1–42} aggregation, 20 μM HEPES (pH 6.6) in 150 μM NaCl was added to the Aβ_{1–42} stock solution to afford a 25 μM solution. Mixtures of the peptide (10 μL, 25 μM final concentration) with or without Cu^{2+} or Zn^{2+} (10 μL, 25 μM final concentration) and the test compound (10 μL, 50 μM final concentration) were incubated at 37 °C for 24 h. Then 50 mM glycine-NaOH buffer (pH 8.0) containing ThT (5 μM) was added to 20 μL of the sample and diluted to afford a final volume of 200 μL. Fluorescence intensities of the solutions were recorded.
(excitation, 450 nm; emission, 485 nm). The percentage of aggregation was calculated by the expression 
\[(1 - \text{IFi}/\text{IFc}) \times 100\], in which IFi and IFc are the fluorescence intensities obtained for Aβ in the presence and absence of inhibitors, respectively.

3.5. Methodology for the Metal-Chelation Study

The chelating studies were performed with a UV–vis spectrophotometer. The absorption spectra of test compound (50 µM, final concentration) alone or in the presence of CuSO₄, FeSO₄, or ZnCl₂ (50 µM, final concentration) in buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) were incubated for 30 min and then recorded at room temperature, respectively. For the stoichiometry of the test compound–Cu²⁺ complex or the test compound–Zn²⁺ complex, a fixed amount of test compound (50 µM) was mixed with increasing amounts of copper ion or Zn ion (0–100 µM), and the UV–vis difference spectra were analyzed to determine the ratio of ligand/metal ion in the complex.

3.6. Methodology for In Vitro HEK-Tau and SY5Y-APPsw Cell Aggregation Assays

Human Embryonic Kidney cells that overexpress tau protein (HEK-tau) cells or SY5Y neuroblastoma cells that express a familial-AD mutant of amyloid precursor protein (SY5Y-APPsw cells) were each seeded in 96-well plates at 8000 cells per well. After 24 h, cells were supplemented with test compound at 1 µM after diluting with medium. Vehicle only control was also included for each experiment. The cells were cultured for an additional 48 h at 37 °C. Both the cell lines were able to reach approximately 80% confluency in 48 h. Cells were assessed for amyloid deposits by staining with 0.1% w/v ThT, counterstaining nuclei with DAPI, and calculating mean ThT fluorescence signal per nucleus over multiple fields.

3.7. Methodology for Molecular Docking

Molecular docking was performed using Schrodinger software Maestro 11.4 suite. The ligands were prepared using the LigPrep module. The protein crystal structure PDB ID: 1IYT was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). The downloaded protein crystal structure was prepared in Protein Preparation Wizard wherein the missing hydrogens were added and bond orders assigned. A grid for docking the ligand was generated with the centroid of the co-crystallized ligand from PDB ID: 1IYT as the center of the grid with no constraints. The prepared ligands 18d and 18f were docked into this grid using Glide module. Molecular docking was performed at XP precision and results were analyzed in the Maestro visualizer.

Supplementary Materials: The following are available online.

Author Contributions: S.K.B.: Design, Synthesis and In vitro self-induced and metal-Induced Aβ₁–₄₂ aggregation assay; N.M. and S.K. (Seshu Krishnamachari): In vitro HEK-Tau and SY5Y-APPsw cell culture experiments; S.A.: Design of screening the drugs against Aβ₁–₄₂ aggregation and cell culture experiments; N.R.P.: Molecular docking studies; S.K. (Samuel Kakraba): Calculations of in vitro self-induced and metal-Induced Aβ₁–₄₂ aggregation assay results; R.J.S.R.: Supervision and design of overall biological experiments; P.A.C.: Supervision and design of overall project; S.K.B. and N.R.P.: Manuscript preparation with additional inputs from P.A.C. All authors have read and agreed to the published version of the manuscript.

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**Sample Availability:** Samples of the compounds are available from the authors.