RESEARCH ARTICLE

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2018, 9, 1891

New amyloid beta-disaggregating agents: synthesis, pharmacological evaluation, crystal structure and molecular docking of *N*-(4-((7-chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)amines†

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In the journey towards the development of potent multi-targeted ligands for the treatment of Alzheimer's disease, a series of A β aggregation inhibitors having quinoline scaffold were designed utilizing computational biology tools, synthesized and characterized by various spectral techniques including single-crystal X-ray crystallography. Organic syntheses relying upon convergent synthetic routes were employed. Investigations via ThT fluorescence assay, electron microscopy and transmission electron microscopy revealed the synthesized derivatives to exhibit A β self-aggregation inhibition. Molecules **5g** and **5a** showed the highest inhibitory potential, 53.73% and 53.63% at 50 μ M respectively; higher than the standard A β disaggregating agent, curcumin. Molecules **5g** and **5a** disaggregated AChE-induced (58.26%, 47.36%) A β aggregation more than two fold more than the standard drug-donepezil (23.66%) and inhibited Cu²⁺-induced A β aggregation. A docking study significantly showed their interaction with key residues of A β and the results were in accordance with the study. Besides, these compounds also exhibited potential antioxidant activity (**5a**, 2.7240 Trolox equivalent by ORAC assay) and metal chelating property. Furthermore, the stoichiometric ratio of Cu (II)–**5a** and Cu(II)–**5g** complexes were found by Job's method (0.5:1 for **5a** and 0.8:1 for **5g**). *In silico* ADMET profiling showed these derivatives to have drug like properties with very low toxicity effects in the pharmacokinetic study. Overall, these results displayed a multi-activity profile with promising A β aggregation inhibition and antioxidation and metal chelation activity that could be helpful for developing new multifunctional agents against Alzheimer's disease.

Received 23rd June 2018,
Accepted 6th August 2018

DOI: 10.1039/c8md00312b

rsc.li/medchemcomm

1. Introduction

Disorders caused by dementia are a societal burden and the demand for a therapeutic breakthrough for brain degenerating disorders is accelerating. The most common dementia spread over all nations is Alzheimer's disease (AD).¹ AD results in the destruction of vital brain cells, and severely affects behavior, thinking and memory and leads to the loss of independence. The disease causes a serious economic burden for

patients. Extensive research to gain insight into the cellular and molecular pathomechanism in AD has been done, however, four acetylcholinesterase inhibitors (rivastigmine, galantamine, donepezil and tacrine) and a *N*-methyl D-aspartate (NMDA) receptor antagonist (memantine) are the only medicines available for its treatment.² Insufficient effective treatments makes this neurological impairment a serious public health concern. New drugs beyond these symptomatic drugs beyond these symptomatic drugs and effective disease modifying strategies are desired. The etiology of Alzheimer's remains elusive, but multiple factors, like decreased acetylcholine, amyloid β formation, τ -protein, biometal dyshomeostasis and oxidative stress, supposedly play a significant part in Alzheimer's development. In view of the complexity of Alzheimer's disease any molecule that hits one of these targets could be a great importance towards treating the pathological activities involved in the neurodegenerative cascade.^{3–7}

A miscellany of biochemical, pathological and genetic studies portrays the crucial functions of amyloid β in AD pathogenesis. Amyloid β plays a very important role in

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† Electronic supplementary information (ESI) available. CCDC 1828487 and 1828488. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8md00312b

various factors that are conducive towards AD progression. Its creation and accumulation are a central event in the pathogenesis of AD.⁸ Accumulation of amyloid β peptide has been postulated as a significant factor responsible for cognitive decline and memory deficits in Alzheimer's. It may be due to a physical perturbation in synapse communication. The "amyloid hypothesis," depicts the modified synthesis and aggregation of amyloid β that leads to the formation of amyloid plaques.⁹ Impaired clearance and/or overproduction of amyloid precursor protein (APP) are possible explanations for the aggregation of amyloid β . The proteolytic cleavage of APP *via* β - and γ -secretase produces amyloid β . The soluble oligomers of amyloid β initiate the activity that give rise to neuronal dysfunction. Extracellular deposits of amyloid β plaques as well as soluble oligomeric aggregates of amyloid β contribute towards neurotoxicity.¹⁰ Therefore, a salient curative strategy for AD involves the development of therapeutic agents targeted to inhibit amyloid β formation, destabilize & disaggregate them and clear the preformed fibrils. Aggregation of this natural product in the brain and in cerebral blood vessel walls causes extracellular lesions that induce development of neuritic plaques, thus triggering neurotoxicity. Amyloid β can effectively produce reactive oxygen species (ROS) in the presence of a few transition metals. It forms dityrosine cross-linked dimers that are stable and are generated *via* attack of free radical in oxidative conditions.¹¹

Oxidative damage exists inside the brain of AD patients and affects almost all forms of biological macromolecules.¹² Consequently antioxidant protection is beneficial throughout aging and particularly in AD patients since the endogenous antioxidant protection system fails quickly. Neuroinflammation and oxidative stress, with successive activation of glial cells have also been considered to participate, likely downstream, in AD-related neurodegeneration.¹³ Complementarily, growing evidence indicates that the generation of free-radicals plays a more significant role in the cognitive dysfunctioning seen in AD patients.¹⁴

Elevated levels of metals such as mercury cause learning and memory problems, as well as serious and fatal neurological diseases and movement disorders.¹⁵ Furthermore, disproportionate levels and distribution of these metals particularly zinc, copper and iron, in the brain, may play a role in dementias like AD. Metal ion (Fe^{2+} , Cu^{2+} , Zn^{2+}) levels are 3–7 fold higher in AD patients with respect to healthy individuals.¹⁶

The metal ion and β amyloid aggregate accumulation found in brains with AD has been suggested to be involved in AD pathogenesis. In order to investigate metal-A β -associated pathways development of chemical tools that target metal-A β species is necessary. The current literature reports a number of metal-A β -targeted systems.^{17–20} In light of the above facts, inhibition of amyloid β aggregation as well as toxic free radicals in the brain are presently portrayed as potential targets for anti-AD treatment.²¹ Therapeutic options presently available for the treatment of AD such as a group of acetylcholinesterase inhibitors (AChEIs) remain very few and afford only minimal symptomatic relief. Consequently, the development

of novel amyloid beta disaggregators with improved biological profiles like antioxidation and metal chelation continues to be of great interest to medicinal chemists. In order to fulfill this requirement medicinal chemists are switching to find suitable biologically active molecules that either consist of a potent scaffold or structurally fit to the site of action. The proposed molecules 5a–g contain the biologically important moiety "quinoline".

Quinoline is amongst the useful biologically active molecules that have been studied extensively to determine their bioactivity against various diseases, including AD,²² malaria,²³ tumour,²⁴ viral²⁵ and tuberculosis.²⁶ A wide variety of quinoline based anti-neurodegenerative compounds has been reported like chloroquine and mefloquine (Fig. 1).²⁷ Compounds such as 8-hydroxyquinoline derivative PBT2 and clioquinol (CQ) have proceeded towards clinical trials and show improved cognition. There are other analogues with anti-inflammation activity,²⁸ anti-aggregation²⁹ and antioxidant activity³⁰ also. Furthermore, quinolines show effective metal chelation and free radical scavenging ability. These promising candidates exemplifying quinoline hybrids, could enhance cognitive properties along with the deceleration of neurodegeneration by de-escalating the amyloid β level.

The versatile nature of quinolines has encouraged us to optimize the pharmacological profile of these structural types as significant scaffolds for AD treatment. Continuing with the novel approach to disrupt the noble targets of amyloid β aggregation, metal chelation, antioxidation and inflammation, in this paper, we describe the study of novel quinoline analogues that potentially inhibit self-induced, AChE-induced, and metal-induced amyloid β aggregation, and exhibit antioxidant and metal chelation properties.

2. Results and discussion

2.1. Chemistry

Our general strategy for the synthesis of potential A β aggregation inhibitors, possessing antioxidant and metal chelating

Fig. 1 Anti-Alzheimer's agents (a) donepezil,³¹ (b) tacrine,³¹ (c) galantamine,³¹ (d) huperzine-A,³¹ (e) rivastigmine,³¹ and some anti-neurodegenerative quinoline analogs (f) CQ,³² (g) mefloquine,²⁷ (h) PBT2,^{31,32} (i) chloroquine²⁷.

Scheme 1 Molecular strategy towards quinoline hybrid (4) obtained from hybridization of reference molecules (1), (2) and (3).

properties is illustrated in Scheme 1. Donepezil, an acetylcholinesterase inhibitor, is an approved drug for the treatment of AD and is the current first choice drug for AD as it is a very potent, has low toxicity and is also well tolerated. Donepezil was found also to significantly improve A β induced memory impairment and contributes to the amelioration of neurodegeneration and memory impairment.³³ We designed a synthesis of these disaggregators by reductive amination of a quinoline based aldehyde and the appropriate pyridine, substituted-pyridine or substituted-benzene derivative 4a–g.³⁴

Furthermore Scheme 2 outlines the synthetic path used to synthesize the title compounds (5a–5g). The 4-((7-chloroquinolin-4-yl)oxy)-3-ethoxybenzaldehyde was prepared

by a substitution reaction of ethyl vanillin at the 4-position of 4,7-dichloroquinoline. Derivatives of quinoline, 5a–g were prepared from the quinoline–ethyl vanillin hybrid (3) simply by reductive amination with the appropriate aryl amine using sodium triacetoxyborohydride in anhydrous DCE in low yields (Scheme 2). The synthesized molecules were confirmed by ¹H, ¹³C NMR, mass spectrometry and 5b and 5f were further characterized by X-ray crystallography (Fig. S1–S28, ESI†). TLC and spectral analysis supported the purity of the compounds. With the help of crystal structure data and the enzyme model in the PDB, we also report a computational study, rationalizing the binding modes of molecules with the enzyme.

The ESI mass spectra of the synthesized molecules 3, 5a–g in MeOH showed primarily the [M + H]⁺ peak in accordance with the calculated *m/z* values. The isotopic distribution pattern indicated the presence of compounds having a unipositive charge on them (Fig. S1–S8, ESI†). In the ¹H NMR spectra a prominent singlet peak for the NH group and characteristic multiplets for CH₃ and OCH₂ groups were observed at distinct δ values for all the synthesized compounds. The ¹³C resonance data showed the presence of the various carbon atoms of the quinoline ring, aryl/heteroaryl system in the structure of the synthesized compounds. The characteristic peak of CHO present in the intermediate compound 3 was found absent in the ¹³C spectra of the final compounds 5a–g indicating utilization of the CHO group in the reaction.

Compound	-X	-R
4a, 5a	-CH	-H
4b, 5b	-CH	4-CH ₃
4c, 5c	-N	3-CH ₃
4d, 5d	-N	-H
4e, 5e	-CH	3-CF ₃
4f, 5f	-CH	2-F,4-F
4g, 5g	-CH	4-OCH ₃

Scheme 2 Synthesis of the quinoline derivatives 5a–g. Reagents and conditions: (i) NaH, dry DMF, reflux, 72 h (ii) NaBH(OAc)₃, anhyd. DCE, N₂, RT.

2.2. Crystallography study

Compounds 5b and 5f were structurally characterized by single-crystal X-ray crystallography (Fig. 2, Table 1, and Fig. S25–S28, ESI†). Molecule 5b crystallized in the *P* $\bar{1}$ triclinic space group crystal system (Fig. 2a) and 5f crystallized in the *Pn*2₁*c* monoclinic space group crystal system (Fig. 2b). There

Fig. 2 An ORTEP view of compounds **5b** (a) and **5f** (b) showing thermal ellipsoids at a 50% probability level. Color codes: O blue, F grey, Cl purple, N green, and C black. Hydrogen atoms are omitted for clarity.

Table 1 Crystal data and structure refinement for *N*-(4-((7-chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-4-methylaniline (**5b**) and *N*-(4-((7-chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-2,4-difluoroaniline (**5f**)

	5b	5f
Empirical formula	C ₂₅ H ₂₃ ClN ₂ O ₂	C ₂₄ H ₁₉ ClF ₂ N ₂ O ₂
Formula weight	418.92	440.87
Crystal system	Triclinic	Monoclinic
Space group	<i>P</i> $\bar{1}$	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	5.350(4)	10.474(3)
<i>b</i> /Å	14.080(11)	11.271(3)
<i>c</i> /Å	14.717(11)	17.518(5)
α /deg	101.00(3)	90
β /deg	90.44(3)	96.136(9)
γ /deg	97.24(3)	90
<i>V</i> /Å ³	1078.9(15)	2056.2(11)
<i>Z</i>	2	4
<i>T</i> , K	296(2)	296(2)
ρ_{calcd} /g cm ⁻³	1.289	1.411
λ /Å (Mo-K α)	0.71073 Å	0.71073 Å
Data/restraints/param	6626/0/275	6283/0/284
<i>F</i> (000)	220	904
GOF	1.172	1.029
<i>R</i> (<i>F</i> _o), ^a <i>I</i> > 2 σ (<i>I</i>) [<i>wR</i> (<i>F</i> _o) ^b]	0.0582 [0.1973]	0.0520 [0.1054]
<i>R</i> (all data) [<i>wR</i> (all data)]	0.3659 [0.4426]	0.0941 [0.1246]
Largest diff peak, hole (e Å ⁻³)	0.457, -0.404	0.287, -0.254
<i>w</i> = 1/[$(\sigma F_o)^2 + (AP)^2 + (BP)$]	<i>A</i> = 0.2000	<i>A</i> = 0.0410 <i>B</i> = 0.8737

$$^a R = \sum |F_o| - |F_c| / \sum |F_o|, \quad ^b wR = \{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)] \}^{1/2},$$

where $P = (F_o^2 + 2F_c^2)/3$.

is no hydrogen bonding or pi-pi stacking interactions observed for either **5b** or **5f**, although a short contact bonding with their adjacent atoms was observed.

2.3. Biological evaluation and docking study

2.3.1. Evaluation of self-mediated A β ₁₋₄₂ aggregation inhibition. Amyloid precursor protein gives rise to amyloid beta peptides which play a critical role in the pathology of AD. The major forms of amyloid proteins involved in senile plaques are A β ₁₋₄₂ and A β ₁₋₄₀. A β ₁₋₄₂ is found to have higher pathogenicity, as it has a higher propensity towards fibrillar aggregates which aids in neurodegeneration.³⁵

Therefore, inhibiting A β ₁₋₄₂ could be an important therapeutic strategy for the treatment of AD. With this aim, all newly synthesized compounds **5a-g** were evaluated for their inhibitory action against self-mediated A β ₁₋₄₂ aggregation using the thioflavin-T fluorescence method using curcumin (a known anti-amyloidogenic agent) as reference compound. Fluorescence of the A β peptide treated with **5a** or **5g** decreased in comparison to A β alone (Fig. 3a and b respectively) demonstrating that the compounds decelerate A β aggregation. The data for their effects on A β ₁₋₄₂ peptide aggregation at a concentration of 50 μ M is summarized in Table 2, with the percentage inhibition for the reference as well as for all the compounds. The results indicated that most of the target compounds showed potencies ranging from 30% to 53.72% (Fig. 4) relative to that of the curcumin (52.7%) indicating the inhibition of the A β ₁₋₄₂ self-aggregation process. With maximum inhibition percents of more than that of the reference compound, compounds **5g**, followed by **5a**; were found to be the most effective compounds with inhibitory potencies 53.72% and 53.62% respectively. The result implies the importance of the presence of electron donating groups like methoxy on the phenyl ring. Compounds with a phenyl substituent having a more compact electron cloud density (like CF₃) showed lower disaggregation and substituents with a more diffused electron charge density resulted in better disaggregation which is assumed to be due to more disruption caused by a more scattered electron cloud among the A β fibril layers causing aggregates to resolve. In accordance to this, compounds with electron donating groups like methoxy (**5g**), methyl (**5b**) showed higher potency while those with electron withdrawing groups like fluorine (**5f**) and trifluoromethyl (**5e**) showed the lowest potency for inhibition of A β ₁₋₄₂ self-aggregation (Table 3). Another factor that drives the potency of compounds is structural fitting as the absence of the phenyl substituent (**5a**) facilitates the molecule to intercalate better. A drop in activity was observed for compounds with pyridine in place of the phenyl ring, which is expected as the electronegative nitrogen in the pyridine ring makes it relatively electron deficient and hence having a constricted electron cloud.

2.3.2. Evaluation of AChE-induced A β ₁₋₄₂ peptide aggregation inhibition. Acetylcholinesterase (AChE) is an enzyme that hydrolyzes the neurotransmitter acetylcholine. It colocalizes with the amyloid beta peptide which is characteristic of AD and may be involved in the generation of fibril assembly.³⁶ Based on the inhibition of self-induced A β ₁₋₄₂ aggregation the best two disaggregators **5a** and **5g** were further

Fig. 3 ThT emission fluorescence spectra (range 450–550 nm) of (a) Aβ₁₋₄₂ alone in red, and in the presence of compound **5a** (50 μM) in blue, incubated at 37 °C for 48 h. (b) Aβ₁₋₄₂ alone in red, and in the presence of compound **5g** (50 μM) in blue, incubated at 37 °C for 48 h (c) **5a** and **5g** respectively in the absence of Aβ₁₋₄₂.

Table 2 Self-induced Aβ aggregation inhibition and antioxidant ORAC assay

Entry	Inhibitor	Self-induced Aβ ₁₋₄₂ aggregation inhibition ^a (%)	Trolox equivalent ^b
1	5a	53.62 ± 0.121	2.72 ± 0.21
2	5b	46.79 ± 0.121	2.28 ± 0.34
3	5c	41.59 ± 0.1715	0.87 ± 0.09
4	5d	41.45 ± 0.238	0.55 ± 0.03
5	5e	30.03 ± 0.1044	1.56 ± 0.05
6	5f	27.76 ± 0.271	2.37 ± 0.12
7	5g	53.72 ± 0.0451	1.95 ± 0.08
8	Curcumin	52.77 ± 1.67	n.t.

^a Inhibition of self-induced Aβ₁₋₄₂ aggregation (100 μM) by tested inhibitors at 50 μM by thioflavin-T based fluorescence method (means ± SD of three experiments). ^b Data are expressed as μmol of Trolox equivalent per μmol of tested compound.

evaluated for AChE-induced Aβ₁₋₄₂ peptide aggregation inhibition using the same ThT-based fluorometric assay. The standard drug donepezil was taken as the reference compound against AChE-induced Aβ₁₋₄₂ peptide aggregation inhibition. In comparison to the reference compound, test compounds were screened at 50 μM. After 48 h of incubation, a remarkable increase in the intensity of the ThT fluorescence signal from co-incubated samples of AChE-Aβ was observed indicating the acceleration of Aβ fibrillogenesis by AChE (Fig. 5a).

Fig. 4 Percent inhibition of self-induced Aβ₁₋₄₂ aggregation (100 μM) by tested inhibitors (50 μM) and curcumin (50 μM) incubation at 37 °C, 48 h using the thioflavin-T based fluorescence method.

As shown in Fig. 5b, compounds **5a** and **5g** inhibited AChE induced Aβ aggregation by ~47.36% and ~58.26%, respectively, which was higher than that of standard drug donepezil (23.66%). The results of the ThT assay suggest clearly that targeted compounds **5a** and **5g** may have a potential disease-modifying role in AD therapy. In accordance with the results of Aβ₁₋₄₂ self-aggregation inhibition, here also the compound containing a methoxy group was found to show about 11% more inhibition than the compound without a methoxy group. Thus, confirming the important role of substitution at the aryl tail for potential Aβ₁₋₄₂ disaggregation.

2.3.3. Evaluation of copper-induced Aβ₁₋₄₂ peptide aggregation inhibition. To explore the ability of the quinoline derivatives to inhibit metal-induced Aβ aggregation, we

Table 3 Table for IC₅₀ values of **5a** and **5g**

Compound	Self-induced Aβ ₁₋₄₂ aggregation inhibition ^a (%)			
	25 μM	50 μM	75 μM	IC ₅₀ (μM)
5a	37.11	53.62	3.37	44.52
5g	26.72	53.72	6.89	46.42

^a Inhibition of self-induced Aβ₁₋₄₂ aggregation (100 μM) by tested inhibitors at 50 μM by thioflavin-T based fluorescence method (means ± SD of three experiments).

Fig. 5 ThT binding assay for hAChE-induced Aβ aggregation, and test compound induced Aβ disaggregation; (a) fluorescence of the Aβ peptide alone (100 μM), treated with AChE (1 μM), Aβ with AChE and 5a (50 μM), Aβ with AChE and 5g (50 μM), incubated 48 h, 37 °C; (b) percent inhibition of AChE-induced Aβ aggregation by donepezil, values are reported as the mean ± SD of three independent experiments.

evaluated the most potent compounds 5a and 5g on metal-induced Aβ_{1–42} aggregation using the ThT fluorescence assay, with curcumin as reference against copper-induced Aβ_{1–42} peptide aggregation inhibition. Fluorescence of the Aβ peptide treated with Cu²⁺ increased in comparison to Aβ alone (Fig. 6a) demonstrating that Cu²⁺ accelerates Aβ aggregation. While the fluorescence of Aβ treated with Cu²⁺ and the tested compounds decreased (5a, 22.39% inhibition of Cu²⁺-induced Aβ aggregation; 5g, 32.67% inhibition and curcumin, 52.93% inhibition) (Fig. 6b). The results show that test compounds could inhibit Cu²⁺-induced Aβ_{1–42} aggregation through chelation with Cu²⁺ ions. Furthermore, better chelation could be achieved with a more electron donating substituent at aryl tail of the quinoline derivative.

2.3.4. Metal binding studies. The ability of 5a and 5g to chelate biometals such as Cu²⁺, Zn²⁺, Fe²⁺ and Al³⁺ was studied *via* UV-vis spectroscopy.³⁷ The maximum absorption wavelength at 301 nm exhibited a bathochromic shift when CuSO₄, FeSO₄, ZnCl₂, or AlCl₃ was added, suggesting that 5a binds the biometals: Cu²⁺, Fe²⁺, Zn²⁺ and Al³⁺. The spectrum of the 5a-Cu²⁺ and 5a-Fe²⁺ complex was considerably different from that of 5a alone. Upon the addition of CuSO₄ and FeSO₄, a red shift in the maximum absorption wavelength from 301 nm to 305 nm and increase in absorbance indicated the formation of a 5a-Cu²⁺ complex (Fig. 7 for 5a). The

Fig. 6 ThT binding assay for Cu²⁺-induced Aβ aggregation, and test compound induced Aβ disaggregation; (a) fluorescence of the Aβ peptide alone (100 μM), treated with Cu²⁺ (100 μM), Aβ with Cu²⁺ and 5a (50 μM), Aβ with Cu²⁺ and 5g (50 μM), incubated at 37 °C, 24 h; (b) percent inhibition of Cu²⁺-induced Aβ aggregation by curcumin, 5a and 5g, values are reported as the mean ± SD of three independent experiments.

Fig. 7 UV spectra (in range 230 to 350 nm) of compound 5a (30 μM) alone and in the presence of 60 μM AlCl₃, ZnCl₂, FeSO₄, and CuSO₄.

maximum absorption at 301 nm exhibited a considerable shift to 304 nm and significant drop in absorbance when AlCl₃ and ZnCl₂ was added, suggesting that 5a binds to Al³⁺ and Zn²⁺.^{38,39} Interestingly, when the same experiment was carried out with compound 5g, the maximum absorption wavelength at 300 nm was shifted to 304 nm, 305 nm, 304 nm, 304 nm respectively, suggesting complex formation for 5g-Cu, 5g-Fe, 5g-Al, and 5g-Zn respectively (Fig. 8 for 5g). These observations show that the test compounds possess the ability to chelate biometals and hence could act as potential metal chelators for AD therapy.

The stoichiometry of the Cu(II)-5a and Cu(II)-5g complex was determined using Job's method. UV spectroscopy was used to determine the absorbance of the complexes of 5a-CuSO₄ and 5g-CuSO₄ at different concentrations. As indicated in Fig. 9, when the absorbance changes at 305 nm were plotted, it was observed that the maximum intensity of the difference spectra was reached at 0.5 : 1 for 5a and 0.8 : 1 ratio for 5g, demonstrating that the stoichiometric ratio of metal ion/ligand in the complex was 0.5 : 1 for 5a and 0.8 : 1 for 5g.

2.3.5. Antioxidant activity *in vitro* using oxygen radical absorbance capacity (ORAC-FL) assay. The surge of events involving AD pathogenesis is initiated by oxidative stress. Thus, reduction of oxidative stress is another crucial phase for designing multifunctional agents in AD therapy.^{40,41} The

Fig. 8 UV spectra (in range 230 to 350 nm) of compound 5g (30 μM) alone and in the presence of 60 μM AlCl₃, ZnCl₂, FeSO₄, and CuSO₄.

Fig. 9 Determination of the stoichiometry of (a) complex Cu(II)-5a and (b) complex Cu(II)-5g respectively by using Job's method.

antioxidant activities of all test compounds were evaluated by measuring the oxygen radical absorbance capacity of fluorescein (ORAC-FL),⁴² and the results are presented in Table 2. Their potential to scavenge radicals is expressed as Trolox (a vitamin E analog) equivalent (μmol of Trolox equiv. per μmol of tested compound), in a relative scale, where ORAC (Trolox) = 1. As given in Table 2, most of the test compounds showed potent oxygen radical absorbance capacities, ranging from 0.55- to 2.72-fold of the value for Trolox. Amongst the tested quinoline derivatives, compound 5a gave the best results with 2.72 Trolox equivalents. Interestingly, compounds possessing a methoxy or methyl group at the *para*-position or fluorine at the *ortho*, *para*-position of the aryl tail showed antioxidant activity around 2 Trolox equivalents or more. Furthermore, compounds with a pyridine tail (5c and 5d) showed the lowest antioxidant activity. The result demonstrates the presence of methyl, fluoro, and methoxy substituents plays a significant role in radical absorbance capacity.

2.3.6. Transmission electron microscopy (TEM) assay. TEM was used to further confirm the ability of 5a and 5g compounds to inhibit $\text{A}\beta_{1-42}$ aggregation. As shown in (Fig. 10b), $\text{A}\beta_{1-42}$ alone after incubation at 37 °C for 48 h, formed mature, denser and bulky aggregates as compared to $\text{A}\beta_{1-42}$ alone (Fig. 10a) at 0 h kept at 0 °C. Whereas, disaggregation effects were significantly visible upon addition of compounds 5g and 5a (50 μM each), as sparse $\text{A}\beta$ fibrils were observed (Fig. 10c and d), respectively, as compared to $\text{A}\beta_{1-42}$ alone (Fig. 10b). The results obtained from TEM analysis are in accordance with the ThT binding assay results, suggesting compounds 5g and 5a to be good inhibitors of $\text{A}\beta$ aggregation.

2.3.7. Docking study of possible conformations of 5g and 5a with $\text{A}\beta$. The amyloid peptide neurotoxicity is associated with the formation of amyloid fibrils. The formation of a β -sheet structure may promote the aggregation of $\text{A}\beta_{1-42}$. Thus, molecules that can inhibit the formation of a β -sheet structure were a benefit for the inhibition of aggregation of $\text{A}\beta$.⁴³ In the process of fibrillogenesis, β -sheet conformational changes are mainly stabilized by hydrophobic interactions and a salt bridge between Asp23/Glu22 and Lys28 residue in $\text{A}\beta_{1-42}$.⁴⁴ To demonstrate the possible binding modes of the compounds with $\text{A}\beta_{1-42}$, 5g and 5a were selected for molecular docking studies using the AutoDock software and figures were generated through PyMOL. The Protein data Bank 1IYT was chosen for the docking study of $\text{A}\beta$ with designed compounds.

All docked conformations are ranked based on docking scores. As shown in Fig. 11 the compounds are mainly

Fig. 10 TEM image analysis of $\text{A}\beta_{1-42}$ aggregation in the presence of compounds 5a and 5g; (a) $\text{A}\beta_{1-42}$ (100 μM), 0 °C, 0 h; (b) $\text{A}\beta_{1-42}$ alone (100 μM) was incubated at 37 °C for 48 h; (c) $\text{A}\beta_{1-42}$ (100 μM) and 5g (50 μM) were co-incubated at 37 °C for 48 h; (d) $\text{A}\beta_{1-42}$ (100 μM) and 5a (50 μM) were co-incubated at 37 °C for 48 h.

Fig. 11 A pose of the docked structure of 5a (colored green) and 5g (colored red) with Aβ₁₋₄₂ (PDB code 1IYT); (a) molecule 5a (colored green) interacting with Aβ₁₋₄₂ in one possible binding conformation. The hydrogen-bonding interaction between the ligand and residue Lys16 is indicated by the red line. (b) Interaction of 5g (colored red) and the Aβ₁₋₄₂ peptide obtained from docking calculations. The hydrogen-bonding interaction between the ligand and residue Leu17 is indicated by the red line.

stabilized through hydrophobic binding with residues Leu17, Val18, Phe18, Phe20, Ala21, Val24, Ile31 and Leu34, Met35. In the case of compound 5a, an intermolecular hydrogen bond interaction was formed between the O atom attached to the quinoline group and the backbone NH₂ group of Lys16, at a close distance of 2.1 Å (Fig. 11(a)). Additionally, an intermolecular hydrogen bonding interaction was observed between the NH group of 5g and the carbonyl group of Leu17 with an average distance of 2.0 Å (Fig. 11(b)). On the basis of the outcomes of molecular docking results, we propose that compounds 5a and 5g share a similar binding mode with Aβ₁₋₄₂ near the stabilizing residues of the α-helix Aβ and can thus interfere and inhibit Aβ₁₋₄₂ aggregation *via* interfering with the formation of β-sheets. Further analysis of the protein–ligand interaction through Ligplots⁴⁵ for molecules 5b, 5c, 5d, 5e and 5g showed a strong hydrogen bonding between the NH group of the test compound and the carbonyl group of Leu17. A strong hydrogen bonding was also observed between the oxygen atom linked to the quinoline group in 5a and the backbone NH group of Lys16 (Fig. 12). Furthermore, hydrogen bonding was apparent between the backbone NH group of Lys16 and the fluorine atom of 5e and the oxygen of the ethoxy group in 5f respectively.

2.3.8. Pharmacokinetic properties and drug likeness of compounds 5a–5g. In course of drug discovery and development, many drugs are rejected owing to poor pharmacokinetic profiles. Thus, the determination of ADMET properties of the desired molecules is vital for lead generation. In our study, the ‘drug likeness’ of the newly designed inhibitors was analyzed *via* ADMET descriptors of Accelrys Discovery studio 4.0.

We studied various pharmacological properties with special emphasis on the requirements of the CNS (central nervous system). The ability to penetrate the blood–brain barrier

(BBB) is the fundamental physiochemical feature of CNS drugs. ADME properties such as BBB level, absorption,

Fig. 12 Plot (A–G) shows Ligplot representations of the inhibitors (5a, 5b, 5c, 5f, 5e, 5g, 5d, respectively). The intermolecular hydrogen bonds are shown with green dashed lines. Residues involved in hydrophobic interactions with the ligand are shown with red rays.

Table 4 Predicted ADME properties of the target compounds **5a–5g**

Compound	BBB level	Abs.	Solubility	Sol.	EXT hepatotoxic	EXT PPB	AlogP98	Unknown AlogP98	PSA 2D	EXT_CYP2D6
5a	0	1	−6.929	1	−2.91828	9.21525	5.965	0	41.931	5.29135
5b	0	1	−7.351	1	−2.31792	9.81105	6.451	0	41.931	3.81523
5c	0	0	−6.733	1	0.857639	6.92752	5.636	0	53.192	3.23374
5d	0	0	−6.478	1	−0.109283	5.9083	5.354	0	53.192	4.58168
5e	4	2	−7.972	1	−6.66075	12.5443	6.907	0	41.931	8.38337
5f	0	1	−7.456	1	4.43074	7.76103	6.376	0	41.931	5.01493
5g	0	1	−6.838	1	−0.928399	8.88486	5.949	0	50.861	3.10442

BBB level: blood brain barrier level 0–4, having high penetration to no penetration, Abs.: absorption level, Sol. (solubility level): ideal value of solubility level is 3, EXT hepatotoxic: hepatotoxicity level, PPB: plasma protein binding, AlogP value should not be greater than 3.0, PSA: polar surface area, polar surface area ≤ 100 is ideal, CYP2D6: cytochrome P450-14DM enzyme inhibition using 2D chemical structure as input, CYP2D6 < 0.5 is good and denoted with level 0.

aqueous solubility, AlogP98, PSA and CYP2D6 were studied for tested quinoline derivatives. The standard ADME model was generated for predicting the human intestinal absorption (HIA) after oral administration of the tested inhibitors. The absorption model includes 95% and 99% confidence ellipses in the PSA_2D and AlogP98 plane. The ellipses (Fig. 13) define the regions where well-absorbed inhibitors are expected to be located. For intestinal absorption, 95% and 99% of well-absorbed inhibitors are expected to fall within the ellipses colored in red and green, respectively. Similarly, for crossing the blood–brain–barrier, 95% and 99% of well absorbed inhibitors are expected to fall within the ellipses colored with magenta and aqua, respectively.

Furthermore, there are prediction levels for compound absorption rated as good (0), moderate (1), poor (2) and very poor (3). Most of our compounds were inside the BBB-99 and Absorption-99 ellipses, satisfying the required conditions for absorption by the intestines and the brain (Table 4). Additionally, the prediction level for the BBB was 0 except for compound 5e, which lies outside the ellipse area. Absorption levels were found between 0 and 1 for most of the compounds suggesting good and moderate absorption. The aqueous solubility logarithmic level of all compounds was found to be 1 which indicates better aqueous solubility. To penetrate the BBB, inhibitors should satisfy criteria such as PSA $< 60\text{--}70 \text{ \AA}^2$ and MW < 450 .⁴⁶ All compounds fulfilled these criteria. Among other calculated parameters are AlogP98,

CYP2D6 and PPB that mainly account for the ability of the compound's distribution inside the body. As per the *in silico* toxicity studies, listed in Table 5, the inhibitors showed no Ames mutagenicity, weight of evidence (WOE) prediction showed that they are non-carcinogenic. In the rodent carcinogenicity test, all tested inhibitors were found to be non-carcinogenic for the female mouse model however, they exhibited single carcinogenic and multi-carcinogenic potential in the male rat and male mouse model. The LD₅₀ values ranged from 0.281 g kg^{-1} to 1.347 g kg^{-1} . Higher LD₅₀ values suggest higher safety of these derivatives. The predicted ADMET properties suggest that most of the compounds fulfill drug-likeness criteria and would be good candidates for drug development.

3. Conclusions

In this work new quinoline based compounds have been designed, synthesized and biologically tested as potential inhibitors of self-induced, AChE-induced, and metal-induced amyloid β aggregation, and exhibit antioxidant and metal chelation properties. Furthermore, acetylcholinesterase inhibition studies were also carried out, but no inhibition was observed. Compounds among these quinoline derivatives effectively inhibited amyloid β aggregation *in vitro* and 5a, 5g have proven to be better than the standard anti-Alzheimer's agent curcumin and the standard drug donepezil according to some results. Two of the synthesized molecules were characterized by single-crystal X-ray crystallography. Overall, the variation in results for different derivatives 5a–g gives a trend of the activity–substitution relationship. According to the results substituents with more diffused electron charge density resulted in better aggregation inhibition and those with compact electron cloud showed lower activity owing to lesser interaction with fibrils. Further modifications in substitution pattern may furnish even better results. Theoretic ADMET analysis predicts these molecules to exhibit drug-likeness. Overall, these results support our assertion that the heterocyclic quinoline derivatives synthesized using reductive amination could act as templates in the field of multifunctional anti-Alzheimer's agents.

Fig. 13 Plot of polar surface area (PSA) vs. log *P* for a standard and test set showing the 95% and 99% confidence limit ellipses corresponding to the blood brain barrier and intestinal absorption models.

Table 5 Predicted toxicity properties of the target compounds 5a–5g

Compound	Aerobic bio-degradability prediction	AMES score	AMES Mut.	Rat rodent carcinogenicity_FDA								Skin sensitization	Skin irritancy	WOE_prediction	WOE_probability	DTP_prediction
				Rat oral LD ₅₀ (gm kg ⁻¹)	Female		Male		Male							
					mouse	mouse	mouse	rat	mouse	rat						
5a	ND	-6.81832	NM	0.814	NC	NC	SC	NC	NC	NC	Moderate	None	None	NC	0.334721	NT
5b	ND	-6.01923	NM	1.347	NC	NC	NC	NC	NC	NC	Moderate	None	None	NC	0.294863	NT
5c	ND	-7.85765	NM	0.281	NC	NC	SC	SC	C	NC	Severe	None	None	NC	0.354711	NT
5d	ND	-9.24268	NM	0.386	NC	NC	SC	SC	C	NC	Severe	None	None	NC	0.326389	NT
5e	ND	-15.0457	NM	0.493	NC	NC	SC	SC	NC	NC	Moderate	Strong	None	NC	0.321882	NT
5f	ND	-5.70038	NM	0.460	NC	NC	NC	NC	NC	NC	Moderate	None	None	NC	0.338684	T
5g	ND	-5.9757	NM	0.964	NC	NC	SC	SC	NC	NC	Moderate	None	None	NC	0.312561	T

Ames Mut: ames mutagen prediction; Prob.: ames probability; enrichment: ames enrichment; WOE: weight of evidence; M: (mutagen); NM: (non-mutagen); C: (carcinogen); NC: (non-carcinogen); SC: (single-carcinogen); T: (toxic); NT: (non-toxic); ND: (non-degradable).

4. Experimental

4.1. General procedures

All the required reagents used in this work were available commercially and solvents were dried using standard methods. The purity of intermediates was verified by using TLC and NMR techniques. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were obtained from a Bruker Avance 400 NMR spectrometer. Mass spectrometric analyses were done on an Agilent 6538 Ultra High Definition Accurate Mass-Q-TOF (LC-HRMS) instrument. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60–120 and F254 pre-coated aluminum-backed TLC plates (0.25 mm) thickness. TLC plates were visualized under short (254 nm) and long (365 nm) wavelength UV light. Column chromatography was performed with 100–120 mesh silica gel. Unless otherwise stated the commercial chemicals and solvents were of reagent grade (RG) and were used without further purification.

4.2. Experimental procedure for the synthesis of 4-((7-chloroquinolin-4-yl)oxy)-3-ethoxybenzaldehyde (3)

A solution of ethyl vanillin (7 g, 42 mmol) in anhydrous DMF was stirred in an ice bath with the addition of sodium hydride (46 mmol) in small lots under nitrogen atmosphere. 4,7-Dichloroquinoline (10 g, 50.5 mmol) was added and the reaction mixture was refluxed for 72 h under nitrogen atmosphere. Completion of the reaction was checked by TLC. On completion of the reaction the mixture was cooled to room temperature and extracted with ethyl acetate and water. The organic extract was dried using anhydrous sodium sulphate and was concentrated on a rota-evaporator to give crude product, which was purified *via* column chromatography with 20% EtOAc/hexane. White powder (30%); ¹H NMR (300 MHz, CDCl₃): δ (ppm) 9.998 (ss, 1H, CHO), 8.678 (d, 1H, *J* = 5.1 Hz, –C=N), 8.341 (d, 1H, *J* = 9 Hz, Ar-H), 8.106 (d, 1H, *J* = 1.8 Hz, Ar-H), 7.576–7.527 (m, 3H, *J* = 14.7, Ar-H), 7.369 (d, 1H, *J* = 7.8 Hz, Ar-H), 6.472 (d, 1H, *J* = 5.1 Hz, Ar-H), 4.120–4.051 (m, 2H, *J* = 20.7 Hz, CH₂O), 1.155 (t, 3H, *J* = 13.9 Hz, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 190.75, 160.99, 15.06, 151.51, 147.94, 136.18, 135.07, 128.08, 127.19, 124.98, 123.44, 122.91, 119.51, 112.66, 104.20, 64.71, 14.30; HRMS in MeOH for C₁₈H₁₄ClNO₃ (mol. wt = 327.76 g mol⁻¹) (ESI, *m/z*): observed, 328.0742; calculated, 328.0740 [M + H]⁺.

4.3. General procedure for the synthesis of compounds 5a–g

A dry round-bottomed flask, equipped for magnetic stirring was loaded with the respective amine 4a–g (3.8 mmol) and aldehyde 3 (3.8 mmol) in anhydrous 1,2-dichloroethane (10 ml). Sodium triacetoxyborohydride (7.6 mmol) was then added in small portions under N₂ atmosphere. The reaction mixture was stirred at RT for 2–3 h. Completion of the reaction was checked by TLC. On completion of the reaction it was quenched by adding an aqueous saturated solution of sodium bicarbonate and then extracted with dichloromethane.

The organic extract was dried over anhydrous NaSO₄, filtered off and concentrated to give the crude product which was purified by silica gel column chromatography.

4.3.1. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-aniline (5a). White powder (48%): ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.67 (d, 1H, *J* = 4 Hz, Ar-H), 8.42 (d, 1H, *J* = 8 Hz, Ar-H), 8.35 (d, 1H, *J* = 4 Hz, Ar-H), 7.63–7.61 (dd, 1H, *J* = 8 Hz, Ar-H), 7.40 (s, 1H, Ar-H), 7.22–7.16 (m, 3H, Ar-H), 7.11 (d, 1H, *J* = 4 Hz, Ar-H), 7.07 (d, 1H, *J* = 4 Hz, Ar-H), 6.76 (t, 1H, Ar-H), 16.68 (d, 1H, *J* = 8 Hz, Ar-H), 6.56 (d, 1H, *J* = 8 Hz, Ar-H), 4.90 (s, 1H, NH), 4.39 (s, 2H, CH₂N), 4.00–3.94 (q, 2H, *J* = 24 Hz, OCH₂), 1.09 (t, 3H, *J* = 12 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 150.72, 149.61, 147.89, 140.89, 139.68, 129.72, 129.48, 128.32, 125.45, 124.07, 122.77, 120.23, 119.38, 118.23, 113.43, 113.20, 103.70, 77.48, 77.16, 76.84, 64.63, 48.31, 14.61; HRMS in MeOH for C₂₄H₂₁ClN₂O₂ (mol. wt = 404.8887 g mol⁻¹) (ESI, *m/z*): observed, 405.1387; calculated, 405.1370 [M + H]⁺.

4.3.2. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-4-methylaniline (5b). White powder (48%): ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.68 (d, 1H, *J* = 8 Hz, Ar-H), 8.41 (m, 2H, *J* = 20 Hz, Ar-H), 7.65–7.62 (d, 1H, *J* = 12 Hz, Ar-H), 7.18–7.00 (m, 6H, Ar-H), 6.61–6.55 (m, 2H, Ar-H), 4.87 (s, 1H, NH), 4.36 (s, 2H, CH₂N), 4.01–3.94 (q, 2H, OCH₂), 2.25 (ss, 3H, CH₃-Ar), 1.11 (t, 3H, *J* = 4 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 163.61, 151.23, 148.58, 145.98, 141.51, 139.81, 130.28, 127.99, 127.78, 126.98, 124.27, 123.18, 120.54, 119.86, 113.83, 113.68, 104.03, 64.94, 51.28, 49.03, 20.88, 14.96; HRMS in MeOH for C₂₅H₂₃ClN₂O₂ (mol. wt = 418.9153 g mol⁻¹) (ESI, *m/z*): observed, 419.1523; calculated, 419.1526 [M + H]⁺.

4.3.3. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-6-methylpyridin-2-amine (5c). White powder (48%): ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.67 (d, 1H, *J* = 4 Hz, Ar-H), 8.36 (d, 1H, *J* = 8 Hz, Ar-H), 8.07 (s, 1H, Ar-H), 7.68–7.66 (dd, 1H, *J* = 8 Hz, Ar-H), 7.28 (d, 1H, *J* = 4 Hz, Ar-H), 7.25 (d, 1H, *J* = 8 Hz, Ar-H), 7.02 (s, 1H, NH), 6.95 (t, 1H, Ar-H), 6.46 (d, 1H, *J* = 4 Hz), 6.38 (d, 1H, *J* = 8 Hz, Ar-H), 6.33 (d, 1H, *J* = 12 Hz, Ar-H), 4.51 (s, 2H, CH₂N), 3.98–3.99 (q, 2H, *J* = 4 Hz, OCH₂), 2.28 (s, 3H, CH₃), 1.00 (t, 3H, *J* = 16 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 141.13, 127.59, 124.75, 123.27, 120.94, 114.83, 111.73, 105.63, 104.63, 64.81, 44.92, 41.06, 40.85, 40.64, 40.44, 40.23, 40.02, 39.81, 25.11, 15.25; HRMS in MeOH for C₂₄H₂₂ClN₃O₂ (mol. wt = 419.9034 g mol⁻¹) (ESI, *m/z*): observed, 420.1480; calculated, 420.1479 [M + H]⁺.

4.3.4. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-pyridin-2-amine (5d). White powder (48%): ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.64 (d, 1H, *J* = 4 Hz, Ar-H), 8.36 (d, 1H, *J* = 8 Hz, Ar-H), 8.15 (s, 1H, Ar-H), 7.89 (d, 1H, *J* = 4 Hz, Ar-H), 7.80 (m, 1H, Ar-H), 7.56–7.54 (dd, 1H, *J* = 8 Hz, Ar-H), 7.19 (d, 1H, *J* = 8 Hz, Ar-H), 7.11 (d, 1H, *J* = 8 Hz, Ar-H), 7.02 (t, 2H, *J* = 8 Hz), 6.78 (t, 1H, *J* = 20 Hz, Ar-H), 6.45 (m, 1H, *J* = 8 Hz, Ar-H), 5.21 (s, 1H, NH), 4.59 (s, 2H, CH₂N), 4.02 (q, 2H, *J* = 4 Hz, OCH₂), 1.11 (t, 3H, *J* = 12 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 155.15, 151.91, 144.07, 142.55, 138.67, 137.80, 127.80, 127.61, 124.09, 123.69, 123.25, 120.13, 119.88, 113.37,

112.85, 110.12, 104.06, 77.78, 77.47, 77.15, 65.28, 64.98, 46.62, 14.92; HRMS in MeOH for C₂₃H₂₀ClN₃O₂ (mol. wt = 405.8768 g mol⁻¹) (ESI, *m/z*): observed, 406.1273; calculated, 406.1322 [M + H]⁺.

4.3.5. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-3-(trifluoromethyl)aniline (5e). White powder (48%); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.69 (s, 1H, Ar-H), 8.47 (m, 2H, *J* = 8 Hz, Ar-H), 7.73 (t, 1H, *J* = 8 Hz, Ar-H), 7.58 (t, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.21 (m, 1H, *J* = 16 Hz, Ar-H), 7.07 (m, 1H, Ar-H), 7.00–6.95 (m, 2H, *J* = 20 Hz, Ar-H), 6.80 (d, 1H, *J* = 8 Hz, Ar-H), 6.65 (d, 1H, Ar-H), 4.92 (s, 1H, NH), 4.43 (s, 1H, Ar-H), 4.00–3.93 (m, 2H, CH₂O), 1.11 (t, 3H, *J* = 16 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 169.13, 165.04, 157.59, 154.74, 152.78, 150.50, 150.22, 140.00, 133.45, 116.88, 108.83, 108.69, 95.59, 61.63, 61.22, 53.61, 52.83, 48.49, 41.05, 40.84, 40.63, 40.42, 40.21, 40.00, 39.79, 25.48; HRMS in MeOH for C₂₅H₂₀ClF₃N₂O₂ (mol. wt = 472.8867 g mol⁻¹) (ESI, *m/z*): observed, 473.1248; calculated, 473.1244 [M + H]⁺.

4.3.6. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-2,4-difluoroaniline (5f). White powder (48%); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.67 (d, 1H, *J* = 8 Hz, Ar-H), 8.41 (d, 1H, *J* = 8 Hz, Ar-H), 8.29 (d, 1H, *J* = 4 Hz, Ar-H), 7.61 (d, 1H, *J* = 12 Hz, Ar-H), 7.20 (d, 1H, *J* = 12 Hz, Ar-H), 7.08 (s, 1H, Ar-H), 7.05 (d, 1H, *J* = 8 Hz, Ar-H), 6.82 (m, 1H, *J* = 20 Hz, Ar-H), 6.74 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H), 6.53 (d, 1H, *J* = 8, Ar-H), 4.38 (s, 2H, CH₂N), 4.22 (s, 1H, NH), 3.98 (t, 2H, *J* = 20 Hz, OCH₂), 1.10 (t, 3H, *J* = 12 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 151.13, 149.65, 141.35, 139.42, 128.80, 124.39, 123.21, 120.47, 119.67, 113.59, 112.82, 111.26, 111.08, 104.32, 104.01, 65.01, 48.60, 14.91; 441.1181; HRMS in MeOH for C₂₄H₁₉ClF₂N₂O₂ (mol. wt = 440.8697 g mol⁻¹) (ESI, *m/z*): observed, 441.0981; calculated, 441.1181 [M + H]⁺.

4.3.7. *N*-(4-((7-Chloroquinolin-4-yl)oxy)-3-ethoxybenzyl)-4-methoxyaniline (5g). Wine red viscous liquid (40%); ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.526 (d, 1H, *J* = 5.1 Hz, C=N), 8.268 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.979 (d, 1H, *J* = 1.8 Hz, Ar-H), 7.413 (d, 1H, *J* = 9 Hz, Ar-H), 7.022 (t, 2H, *J* = 20.4 Hz, Ar-H), 6.926 (d, 1H, *J* = 7.8 Hz, Ar-H), 6.706 (d, 2H, *J* = 9 Hz, Ar-H), 6.539 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.336 (d, 1H, *J* = 5.4 Hz, Ar-H), 4.207 (ss, 2H, CH₂N), 4.046–3.975 (m, 1H, *J* = 21.3 Hz, NH), 3.888–3.819 (m, 2H, *J* = 20.7 Hz, CH₂O), 3.634 (ss, 3H, OCH₃), 0.982 (t, 3H, *J* = 13.8 Hz, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 171.09, 161.99, 152.41, 152.14, 150.95, 150.01, 142.29, 141.47, 139.14, 127.80, 126.82, 123.67, 122.83, 120.09, 119.60, 114.98, 114.25, 113.54, 103.61, 64.48, 60.36, 55.79, 49.07, 21.01, 14.51, 14.20, 1.04; HRMS in MeOH for C₂₅H₂₃ClN₂O₃ (mol. wt = 434.9147 g mol⁻¹) (ESI, *m/z*): observed, 435.1469; calculated, 435.1475 [M + H]⁺.

4.4. X-ray crystal structure determination

Three-dimensional X-ray data were collected on an automated Bruker SMART APEX CCD diffractometer equipped with a fine focus 1.75 kW sealed tube Mo Kα X-ray source (λ = 0.71073 Å) with increasing ω (width of 0.3° per frame) at 5 s per frame scan speed for **5b** and **5f**.

Crystal structures of **5b** and **5f** were obtained *via* the single-crystal X-ray diffraction method. Colorless rectangular block crystals of compounds **5b** and **5f**, which were obtained from a mixture of ethyl acetate and hexane on slow evaporation, were mounted on loops with mineral oil. The ω - 2θ scan mode was used for collecting intensity data and then corrected for Lorentz-polarization and absorption effects.⁴⁷ The WinGx suite of programs (version 1.63.04a) was used for solving and refining structures by the SHELXL-2013 method.⁴⁸ The coordinates of non-hydrogen atoms were allowed to be on their respective carbons and non-hydrogen atoms were refined with anisotropic displacement coefficients. Atomic positions of all atoms, anisotropic and isotropic thermal parameters for all the non-hydrogen and hydrogen atoms respectively, were included in the final refinement. The structural views of crystallized molecules were drawn using ORTEP.⁴⁹ Crystallographic parameters, angles, and bond distances data are shown in Table 1. The CCDC deposition numbers are 1828488 and 1828487.

4.5. Self-mediated A β _{1–42} aggregation assay

Commercially available peptides were first treated with hexafluoroisopropanol (HFIP) at 5 mg ml^{–1} to avoid self-aggregation. The clear solution containing the dissolved peptide was then aliquoted in a microcentrifuge tube. The HFIP was allowed to evaporate under a stream of nitrogen until a clear film remained in the test tube. The pretreated A β _{1–42} samples were then dissolved in DMSO in order to have a stable stock solution (A β 5 mM). For the inhibition of self-mediated A β _{1–42} aggregation experiment, the A β stock solution was diluted with 50 mM phosphate buffer (pH 7.4). A mixture of the peptide (10 μ L, 100 μ M, final concentration) with or without the tested compound (50 μ M) was incubated at 37 °C for 48 h. To quantify amyloid fibril formation, the thioflavin-T fluorescence method was used.^{50,51} Blanks containing 50 mM phosphate buffer (pH 7.4) in place of A β , with or without inhibitors were also carried out. After incubation, samples were diluted to a final volume of 200 μ L with 50 mM glycine-NaOH buffer (pH 8.0).

4.6. Inhibition of AChE-induced A β _{1–42} peptide aggregation assay

For conducting co-incubation experiments^{52,53} AChE from electric eels (E.C.3.1.1.7) and aliquots of A β _{1–42} peptide, in the presence or absence of the test inhibitors were incubated for 48 h at 37 °C. The final concentrations of AChE (dissolved in 0.1 M sodium phosphate buffer, pH 8.0) and A β (dissolved in DMSO and diluted 0.215 M sodium phosphate buffer, pH 8.0) were 0.02 U and 100 μ M, respectively. Following co-incubation at room temperature for 48 h, 180 μ L of 5 μ M thioflavin-T, in 50 mM glycine-NaOH buffer (pH 8.0) was added. To analyse co-aggregation inhibition, the ThT fluorescence method was used and the fluorescence was measured at excitation λ 450 nm and emission λ 485 nm. The percent inhibi-

tion of the AChE-induced aggregation was calculated by the following expression:

$$[100 - (IF_i/IF_o \times 100)]\%$$

where, IF_i and IF_o being the fluorescence intensities with and without the test compound, respectively, minus the fluorescence intensities due to the respective blanks. Assays were conducted in triplicate, and each reaction was repeated at least three times independently.

4.7. Inhibition of copper-induced A β _{1–42} peptide aggregation assay

For the inhibition of copper-mediated A β _{1–42} aggregation experiment, the A β stock solution was diluted in 20 μ M HEPES (pH 6.6) with 150 μ M NaCl. The mixture of the peptide (10 μ L, 100 μ M, final concentration) with or without copper (100 μ M, final concentration) and the tested compound (50 μ M, final concentration) was incubated at 37 °C for 24 h. Then 20 μ L of the sample was diluted to a final volume of 200 μ L with 50 mM glycine-NaOH buffer (pH 8.0) containing thioflavin T (5 μ M). The detection method was the same as that of the AChE-mediated A β _{1–42} aggregation experiment.

4.8. Transmission electron microscopy (TEM) assay

A β _{1–42} peptide was dissolved in 50 mM phosphate buffer (pH 7.4), which was incubated in the presence and absence of test compounds at 37 °C. The final concentrations of A β _{1–42} and test inhibitors were 100 μ M and 50 μ M, respectively. After 48 h of incubation, aliquots of 10 μ L samples were placed on carbon-coated copper grid. The grid was treated with A β aggregated samples and dried for 15 min at room temperature. Images from each sample were taken by FEI Tecnai G2 HRTEM transmission electron microscope.⁵⁴

4.9. Oxygen radical absorbance capacity (ORAC-FL) assay

The antioxidant activity was determined based on the oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay.^{55,56} The reaction was carried out at pH 7.4, with phosphate buffer (75 mM), and the final reaction mixture volume was 200 μ L. Antioxidant (20 μ L) and FL (120 μ L; 70 nM, final concentration) solutions were kept in a black 96-well microplate (96F untreat, Nunc). The mixture was preincubated for 15 min (37 °C), followed by rapid addition of AAPH solution (60 μ L, 12 mM, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and fluorescence was recorded for every minute for the duration of 80 minutes (excitation, 485 nm; emission, 520 nm). Samples were measured at eight concentrations (1–8 μ M). Additionally a blank (FL + AAPH) using the phosphate buffer instead of the tested compound and eight calibration solutions using Trolox (1–8 μ M, final concentration) as antioxidant were carried out in each assay. All of the reaction mixtures were prepared in triplicate, and were run at least three independent times. The antioxidant curves (fluorescence *vs.* time) were

normalized to the curve of the blank. The area under the fluorescence decay curve (AUC) was calculated using the following equation:

$$\text{AUC} = 1 + \sum_{i=1}^{i=80} (f_i/f_0)$$

where, f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i . The net AUC was calculated by the following equation: $\text{AUC}_{\text{sample}} - \text{AUC}_{\text{blank}}$. The regression equations between the net AUC and the Trolox concentrations were calculated. The ORAC-FL value for each sample was calculated using the standard curve, and the ORAC-FL value of each tested compound is thus expressed as Trolox equivalents.

4.10. Metal binding studies

The metal binding studies were carried out in a Perkin-Elmer Multimode Reader (Thermo Scientific). The UV absorption of the tested compounds **5g** and **5a** (30 μM , final concentration) alone or in the presence of CuSO_4 , FeSO_4 , AlCl_3 or ZnCl_2 (60 μM , final concentration) for 30 min in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) was recorded with wavelength ranging from 220 to 400 nm. The final volume of reaction mixture was 200 μL .

Further the metal binding studies were done through Job's method.^{57,58} This method studies the ratio of metal ion/ligand in the complex. A fixed amount of compounds **5a** & **5g** (50 μM) was mixed with growing amounts of copper ion. The stoichiometry of the Cu(II) -**5a** and Cu(II) -**5g** complex was determined.

4.11. Docking calculations

Atomic coordinates of amyloid β_{1-42} were taken from the Protein Data Bank (www.rcsb.org)⁵⁹ (from PDB ID: 1IYT). The amyloid β_{1-42} structure file was saved in pdbqt format to perform the docking analysis. AutoDock 4.2 was used with the standard protocol to perform docking of the synthesized compounds with amyloid β_{1-42} . The Lamarckian genetic algorithm (LGA) was applied to assess the protein-ligand interactions.⁶⁰ The most promising binding free energy as well as docking orientations lying within a 2.0 Å range in root-mean square deviation (RMSD) tolerance were enrolled to cluster the molecule and rank-up accordingly. The PyMOL visualization tool was used to visualize the molecular interactions.

4.12. ADME property prediction and TOPKAT analysis

A variety of key ADME (absorption, distribution, metabolism and excretion) properties of compounds were predicted using the module of Accelrys Discovery studio 4.0. This software generates an estimate of the physicochemical properties and the bioavailability of the compounds. Parameters such as polar surface area (PSA), P log BB (Predicted brain/blood parti-

tion coefficient), human oral absorption (Predicted human oral absorption on 0–100% scale) were calculated.

Virtual toxicity risk assessment was performed using the TOPKAT module of Accelrys Discovery studio 4.0. TOPKAT accurately and rapidly predicts the toxicity of chemicals from their 2D molecular structure. It employs a range of robust, cross-validated quantitative structure toxicity relationship (QSTR) models for assessing various measures of toxicity. Compounds were monitored for dose dependent toxicity parameters including FDA and NTP rodent carcinogen models, skin irritancy, ocular irritancy, mutagenicity, aerobic biodegradability and developmental toxicity potential.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

T. U. wishes to thank the University Grants Commission (UGC), Government of India for the Basic Scientific Research (BSR) fellowship. The Center for Nanoscience and Nanotechnology, JMI, is acknowledged for the TEM facility. T. U. also thanks Halima Khatoon. The author S. S. would like to thank Ministry of AYUSH for the financial assistance (JRF). Author M. T. is thankful to the University of Delhi for sanctioning research funds to carry out this study.

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