Multi-target-directed triazole derivatives as promising agents for the treatment of Alzheimer’s disease

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ABSTRACT

A novel series of triazole-based compounds have been designed, synthesised and evaluated as multi-target-directed ligands (MTDLs) against Alzheimer disease (AD). The triazole-based compounds have been designed to target four major AD hallmarks that include Aβ aggregation, metal-induced Aβ aggregation, metal dys-homeostasis and oxidative stress. Among the synthesised compounds, 6n having o-CF₃ group on the phenyl ring displayed most potent inhibitory activity (96.89% inhibition, IC₅₀ = 8.065 ± 0.129 μM) against Aβ₄₂ aggregation, compared to the reference compound curcumin (95.14% inhibition, IC₅₀ = 6.385 ± 0.009 μM). Compound 6n disassembled preformed Aβ₄₂ aggregates as effectively as curcumin. Furthermore, 6n displayed metal chelating ability and significantly inhibited Cu²⁺-induced Aβ₄₂ aggregation and disassembled preformed Cu²⁺-induced Aβ₄₂ aggregates. 6n successfully controlled the generation of the reactive oxygen species (ROS) by preventing the copper redox cycle. In addition, 6n did not display cytotoxicity and was able to inhibit toxicity induced by Aβ₄₂ aggregates in SH-SYSY cells. The preferred binding regions and key interactions of 6n with Aβ₄₂ monomer and Aβ₄₂ protofibril structure was evaluated with molecular docking. Compound 6n binds preferably to the C-terminal region of Aβ₄₂ that play a critical role in Aβ₄₂ aggregation. The results of the present study highlight a novel triazole-based compound, 6n, as a promising MTDL against AD.

1. Introduction

The highly ordered peptide or protein aggregates in brain are thought to be responsible for a number of protein misfolding diseases in humans that include Alzheimer disease (AD), Parkinson disease, Creutzfeldt-Jakob disease, type II diabetes, etc. AD is the most common form of dementia, with no treatment available to completely cure or halt its progression. The characteristic symptoms of AD are progressive memory loss, cognitive impairment, and behaviour changes like depression, aggression, etc. [1]. Around 50 million people are affected with dementia worldwide and the number will reach to 152 million by 2050 [2]. The precise molecular mechanism behind the pathogenesis of AD remains elusive. A number of hypotheses have been proposed for the pathomechanism of AD that include β-amyloid deposits, abnormally phosphorylated and β-folded tau protein, metal ion dyshomeostasis and oxidative stress that highlight the multifactorial nature of AD [3]. The FDA approved drugs (rivastigmine [4], donepezil [5], galantamine [6] and memantine [7]) for AD target neurotransmitter abnormalities associated with the disease and do not affect the underlying etiology. These drugs at best provide only temporary and incomplete symptomatic relief [8]. Moreover, the recent failure of various moieties such as PPI-1019 [9], NAP (NAPVSIPQ) [10], tarenflurbil [11], semagacestat...
A large number of studies highlighted that amyloid plaques as soluble oligomers formed by the deposition of amyloid-β (Aβ) peptide (39–43 amino acid residues) play a critical role in the pathophysiology of AD [19]. Aβ results from the proteolytic cleavage of the amyloid precursor protein (APP) by enzymes α-secretase, β-secretase and γ-secretase [20]. The Aβ oligomers interact with neurons and glial cells that result in the activation of pro-inflammatory cascades, and finally cause neuronal apoptosis and cell death. Thus, designing anti-aggregation agents that inhibit the self-assembly of Aβ peptide and destabilize the protofibril structure will provide a potent therapeutics against AD [21].

The recent studies highlighted that dysregulation of brain metal ion (copper and zinc) homeostasis, play a critical role in the pathogenesis of AD [22]. The amyloid plaques are found to be enriched with Cu²⁺ and Zn²⁺. These metal ions are found to coordinate with histidine residues in senile plaque core, which results in the accumulation of the metal ions in the extracellular plaques. As a result, the intracellular copper stores become deficient in AD patient. This promotes a vicious cycle of metal ion accumulation and plaque formation [23]. Thus, modulating the concentration of these biometals has been highlighted as a possible therapeutic strategy for AD, and various metal chelators have been proposed in literature [24]. Moreover, studies highlighted that inappropriate accumulation of redox-active metal ions (Cu and Fe) cause oxidative stress that ultimately lead to neuronal cell death by the reaction between di-alkyne and Cu²⁺. These metal ions are able to modulate Aβ42 aggregation in the presence of metal ions [34] and are able to modulate Aβ42 aggregation in the presence of copper. The metal chelating property of the designed triazole based compounds helps to reduce the oxidative stress by disrupting the copper-ascorbate redox system. The designed di-triazole based compounds are assembled by highly regioslectivity copper catalysed Huisgen cycloaddition reaction [35]. Further, the synthetic strategy and structural variance assimilated by Huisgen cycloaddition reaction, highlights the influence of R-group variation on the ligand-Aβ₄₂ interactions.

The target compounds 6(a–p) were assembled according to the synthetic sequence depicted in Scheme 1. The designed di-triazole based compounds 6(a–p) were synthesized via Huisgen cycloaddition reaction between di-alkyne 4 and azides 5(a–p). To realize the designed strategy, we first assembled the di-alkyne building block 4 in three steps starting from ethyl isocyanoacetate 1 [36]. In this regard, compound 1 was propargylated in the presence of tetrabutylammonium hydrogen sulfate (TBAHS) to furnish the isonitrile derivative 2. Compound 2 was hydrolysed under acidic conditions to yield the amino ester 3. The acetylation of the NH₂ group in 3 delivered the desired di-alkyne building block 4. On the other hand, various azides 5(a–p) were synthesised from the corresponding amines according to the literature procedure [37]. Finally, the di-alkyne derivative 4 was reacted with differently substituted phenyl azides to yield the designed di-triazole based compounds 6(a–p) listed in Table 1. The formation of compounds 6(a–p) were confirmed by complementary spectral data (1H NMR, 13C NMR and HRMS/LRMS).

2.2. Inhibition of self-mediated Aβ₄₂ aggregation by synthesised MTDLs

The effect of di-triazole based compounds 6(a–p) on Aβ₄₂ aggregation was examined by performing a thioflavin-T (ThT) fluorescence binding assay. ThT fluorescence can be employed to monitor the formation of Aβ fibrils as ThT, a cationic benzothiazole dye, undergoes a large augmentation in its fluorescence emission upon binding to amyloid fibrils [38]. The low intensity of ThT fluorescence value indicates the less formation of β-sheet structure. In order to perform the ThT assay, we first optimized the experimental conditions, i.e., concentration of Aβ₄₂ and ThT to be used, and time of incubation and consequently the optimized conditions were used in the studies. Curcumin (a known Aβ aggregation inhibitor) was used as reference compound in the present assay [39]. The results shown in Fig. 2A indicate that most of the synthesised compounds display moderate to good potencies compare to curcumin. Among them, compounds 6g-6h, 6l and 6n, exhibited more than 50% inhibition of Aβ₄₂ aggregation. Compound 6n featuring o-CF₃ group on the phenyl ring exhibited most potent inhibitory activity against Aβ₄₂ aggregation, i.e., 96.89%, better than the reference compound curcumin (95.14%). The complete dose-response curve of compound 6n was evaluated. The curve shows that compound 6n inhibited Aβ₄₂ aggregation with an IC₅₀ value of 8.065 ± 0.129 μM as compared with the control, curcumin (IC₅₀ = 6.385 ± 0.009 μM). The ability of 6n to inhibit self-mediated Aβ₄₂ aggregation was further confirmed by transmission electron microscopy (TEM) assay. The incubation of Aβ₄₂ at 37 °C for 24 h produced long rope-like amyloid fibrils (Fig. 2Bb) as compared to Aβ₄₂ at 0 h (Fig. 2Ba). In contrast, the formation of amyloid fibrils was significantly reduced in the presence of compound 6n (Fig. 2Bc) under the similar experimental conditions, which highlight that 6n inhibited Aβ₄₂ aggregation. The excellent inhibitory activity of 6n can be attributed to the presence of strong electron withdrawing –CF₃ group, which increases the occurrence of non-covalent interaction and hydrogen bond formation. The –CF₃ group stabilize the interaction between protein and ligand through charge distribution and stabilize Aβ peptide in the a-helix conformation [40].
2.3. Effect of compound 6n on disaggregation of self-mediated Aβ42 aggregation fibrils

The disaggregation of preformed Aβ42 fibrils by 6n was investigated using ThT fluorescence assay. The Aβ42 fibrils were generated by incubating fresh Aβ42 monomer (20 µM) for 24 h at 37 °C. Compound 6n and curcumin (reference compound) were then added separately to the samples before incubating for another 24 h at 37 °C. The results of the ThT assay were shown in Fig. 3A, which indicated that 6n was as effective as curcumin in disaggregating preformed Aβ42 fibrils (6n: 81.89% at 40 µM; curcumin: 85.75% at 40 µM). The results of the ThT assay were further complimented by TEM assay. The results in Fig. 3B, indicated that Aβ42 alone had aggregated into amyloid fibrils (Fig. 3Bb) and after the addition of Cu2+ to the solution of Aβ42 fibrils, the percentage of formation of Aβ42 fibrils is 42% (3Ba), which in turn highlights a 2:1 stoichiometry for the Cu2+-Aβ42 complex. When Zn2+ was added to the solution, there was a decrease in the absorbance at 222 nm and a slight increase in absorbance at 262 nm was observed. Similarly, the absorbance at 262 nm increases on addition of Fe2+ to a solution of 6n, suggesting that 6n binds to Zn2+ and Fe2+. The metal chelating ability of 6n could be due to the donation of lone pair of electron on one of the π-bonded nitrogen atoms present in the triazole ring.

In order to determine the binding stoichiometry of 6n with Cu2+, a series of solutions were prepared according to the Job’s method [41]. The total concentration of compound 6n and CuSO4 remains constant, however, their proportion were varied. The absorbance of complex of CuSO4 and 6n at different concentrations were recorded on a UV–Vis spectrometer (Fig. S1, Supplementary material). As shown in Fig. 4b, the absorbance at 262 nm increases initially and thereafter decreases, which yield two straight lines that intersect at a mole fraction of 0.3, which in turn highlights a 2:1 stoichiometry for 6n–Cu2+ complex.

2.5. Ability of compound 6n to suppress copper-based redox activity

Copper play a critical role in the formation of ROS, which lead to an increase in the oxidative stress. The excessive generation of ROS may activate neuronal cell death in AD patients. Thus, compound 6n was examined for its ability to halt the production of ROS by utilizing the Cu-ascorbate redox system described in Scheme 2, as a model system [42]. The generation of the hydroxyl radicals (OH•) during the copper redox cycling were measured by using coumarin-3-carboxylic acid (CCA), which forms fluorescent 7-hydroxy-CCA species (emission at 450 nm). The results in Fig. 5, indicated that OH• produced by copper and ascorbate increases steadily with time and achieved plateau at nearly 12 min. However, this process was completely inhibited in presence of 6n, highlighting that 6n has the ability to prevent copper redox cycling involved in the oxidative stress by chelating the metal ions.

2.6. Effect of compound 6n on Cu2+-mediated Aβ42 aggregation fibrils

The inhibitory activity of 6n against Aβ42 aggregation in the presence of copper was investigated using ThT fluorescence assay. Fig. 6, clearly indicated that copper accelerates the Aβ42 aggregation. According to ThT assay, the percentage of formation of Aβ42 fibrils is 42% higher in the presence of Cu2+ than Aβ42 alone. However, the fluorescence of Aβ42 co-incubated with Cu2+ and the test compounds decreased notably. The fluorescence after treatment with 6n, and clioquinol [43] (CQ, a known metal chelator used as reference compound) was 49.01% and 42.25%, respectively. The above results strongly indicate that 6n was capable of reducing the Cu2+-mediated Aβ42 aggregation.

2.7. Ability of compound 6n to disaggregate Cu2+-mediated Aβ42 aggregation fibrils

The ability of compound 6n to disaggregate Cu2+-mediated Aβ42 fibrils was studied by ThT fluorescence assay and the results are shown in Fig. 7. The Aβ42 fibrils were generated by incubating fresh Aβ42 monomer with 1 equiv. of Cu2+ for 24 h at 37 °C. Then, 6n and CQ
(40 µM) were added separately to Aβ42 fibrils and incubated for another 24 h at 37 °C. The results shown in Fig. 7 indicated that both 6n and CQ can disaggregate Aβ42 fibrils resulting from Cu^{2+}-induced aggregation at 40 µM (6n: 61.42%; CQ: 65.34% disaggregation).

2.8. Effect of compound 6n on neuronal cell viability and on Aβ42 mediated neurotoxicity

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay [44] using SH-SY5Y cells were performed to check the cytotoxicity of compound 6n. The results shown in Fig. 8a, indicated that the incubation of the SH-SY5Y cells with up to 50 µM concentration of the compound 6n for 48 h did not influence the cell viability of the strain significantly (cell viability in the presence of 6n: 97.79% at 5 µM; 95.31% at 25 µM and 90.01% at 50 µM). These results indicated that the compound 6n is biocompatible.

The Aβ42 aggregates were toxic to the SH-SY5Y cells (Fig. 8) and reduced the cell viability to 59% as compared to the control (100%). However, Aβ42 co-incubated with 6n at different molar ratio resulted in higher cell viability. The cell viability increases with increasing the molar ratio of 6n to Aβ42. The cell viability increases to 74.79% at Aβ42/6n = 1:10. These results suggest that 6n was able to inhibit the toxicity induced by Aβ42 aggregates in SH-SY5Y cells.

2.9. Molecular docking of 6n with Aβ42 monomer and Aβ42 protofibril

To elucidate the binding regions and key interactions of 6n with Aβ42 monomer, 6n was docked to Aβ42 monomer (PDB ID: 1Z0Q). The negative binding energy (−5.52 kcal/mol, Table 2) highlights the favorable binding between 6n and Aβ42 monomer. A hydrogen bond was observed between the nitrogen atom of triazole ring of 6n and NH of the backbone of Ala42 of Aβ42 (Fig. 9a). 6n display hydrophobic contacts with Ala30, Ile31, Leu34, Met35, Gly38, Val39, Val40, Ile41, and Ala42 residues of Aβ42 monomer (Fig. 10a).

To determine the binding regions of 6n with Aβ42 protofibril, 6n was docked to Aβ42 protofibril (PDB ID: 2BEG). The visualization of

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**Table 1 (continued)**

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<th>Cpd</th>
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<th>Di-triazole derivatives</th>
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<sup>a</sup> Yields were of isolated and purified products.
docked pose highlights that 6n bind with chain E of Aβ42 protofibril and the observed binding energy is −6.75 kcal/mol (Table 2). 6n form two hydrogen bonds with Aβ 42 protofibril. The first hydrogen bond was observed between the nitrogen atom of triazole ring of 6n and NH of the backbone of Ile41(E) of Aβ42 protofibril and the second hydrogen bond was formed between the NH of the amide group of 6n and carbonyl of the backbone of Val39 (E) of Aβ42 protofibril (Fig. 9b). The 2D interaction map display hydrophobic contacts of 6n with Leu17(D), Phe19(D), Leu17(E), Phe19(E), Gly37(E), Gly38(E), Val39(E), Val40(E), Ile41(E) residues of Aβ42 protofibril (Fig. 10b). The molecular docking studies highlighted that 6n bind preferably to the C-terminus region (which is known to play a key role in Aβ42 aggregation [45], by making hydrogen bonds and hydrophobic contacts with Aβ42 residues.

3. Conclusions

By combining the metal chelating triazole moiety and anti-amyloid aggregation pharmacophore, a novel series of compounds 6(a-p) have been designed, synthesised, and evaluated as multi-target-directed ligands against AD. Among the synthesised library, compound 6n displayed most potent inhibitory activity against Aβ42, which is known to play a key role in Aβ42 aggregation by hydrogen bonds and hydrophobic contacts. In summary, a novel triazole-based scaffold has been identified that significantly controls Aβ42 aggregation, metal-induced Aβ42 aggregation, metal dys-homeostasis, oxidative stress and display neuroprotective action, and might be a promising pharmacotherapeutic lead for further development in AD.

4. Experimental section

4.1. General methods

Human Aβ42 was purchased from Anaspec. All the reagents were purchased from Sigma Aldrich and were used without further purification. The progress of the chemical reactions was monitored by thin layer chromatography (TLC) using an appropriate solvent system for development. The reported yields of the synthesised compounds are the isolated yields. In the 1H NMR the coupling constants (J) are given in hertz (Hz) and chemical shifts are stated in parts per million (ppm). The abbreviations s, d, t, m and ABq stand for singlet, doublet, triplet, multiplet and AB quartet, respectively. 1H NMR (400 MHz or 500 MHz, CDCl3) and 13C NMR (100 MHz or 125 MHz, CDCl3) spectra were recorded on a Bruker NMR spectrometer. HRMS or LRMS data were recorded using Waters Micromass Q-Tof Micro and AB Sciex QTRAP 5500 instrument. Melting points were recorded with a Perfit apparatus. HPLC analysis was recorded on Agilent Technologies 1260 Infinity series system, reverse phase C18 column eluted with 100% acetonitrile at a flow rate 1 mL/min.

Fig. 2. Inhibition of self-mediated Aβ42 aggregation by designed di-triazole based compounds: (A) The results of ThT fluorescence binding assay. The statistical significance was analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test: (**) p < 0.01, versus Aβ42 alone. [Aβ42] = 20 µM, [6(a-p)] = [Curcumin] = 100 µM. (B) TEM images of Aβ42 aggregation in the presence of compound 6n: [Aβ42] = 20 µM, [6n] = 40 µM, 37 °C, 24 h, constant agitation (a) Aβ42, 0 h; (b) Aβ42, 24 h; (c) Aβ42 + 6n, 24 h. Scale bar: 200 nm.
4.2. General procedure for synthesis of di-triazole based amino acids 6(a-p)

The di-alkyne building block 4 (1 mmol) was dissolved in t-BuOH/H₂O (3:3 mL) and the azide 5(a-p) (2.2 mmol), Cu(OAc)₂ (0.2 mmol) and sodium ascorbate (0.4 mmol) were then added. The resulting reaction mixture was stirred at rt till the completion of the reaction (monitored by TLC). The reaction mixture was diluted with ethyl acetate and washed with aq NH₄OH (0.2%) and brine. The aqueous phases were extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated invacuo. The

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**Fig. 3.** Disaggregation of self-mediated Aβ₄₂ aggregation fibrils by compound 6n: (A) The results of ThT binding assay. The statistical significance was analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test: (***) p < 0.01, versus Aβ₄₂ alone. [Aβ₄₂] = 20 µM, [6n] = [Curcumin] = 40 µM. (B) TEM images of disaggregation of self-mediated Aβ₄₂ aggregation fibrils in the presence of compound 6n: [Aβ₄₂] = 20 µM, [6n] = 40 µM, 37 °C, 24 h, constant agitation (a) Aβ₄₂, 0 h; (b) Aβ₄₂, 24 h; (c) Aβ₄₂ fibrils + 6n, 24 h. Scale bar: 200 nm.

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**Fig. 4.** (a) The UV–Vis spectrum of compound 6n (20 µM) alone and in the presence of 20 µM CuSO₄, ZnCl₂ and FeSO₄ in buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). (b) Determination of the stoichiometry of 6n-Cu²⁺ complex by the Job’s method.

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4.3. Synthesis of compounds 6(a-p)

4.3.1. Compound (6a)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), phenyl azide 5a (117.9 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 24 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6a (134.4 mg, 65%) as a white solid. Rf: 0.31 (70% ethyl acetate/petroleum ether). Mp: 83–85 °C. 1H NMR (400 MHz, CDCl3): δ = 1.33 (t, J = 7.2 Hz, 3H), 2.01 (s, 3H), 3.49, 3.87 (ABq, JAB = 14.6 Hz, 4H), 4.31 (q, J = 7.2 Hz, 2H), 6.81 (s, 1H), 7.41–7.45 (m, 2H), 7.49–7.54 (m, 4H), 7.69–7.73 (m, 4H), 7.91 (s, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.13, 14.18, 22.70, 23.98, 29.37, 29.70, 31.08, 31.93, 62.31, 63.16, 120.34, 121.38, 129.77, 136.97, 143.32, 170.28, 171.99 ppm. HRMS m/z: calcd. for C24H25N7O3Na [M + Na]+ 482.1136, found: 482.1917.

4.3.2. Compound (6b)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), o-fluorophenyl azide 5b (135.6 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 18 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6b (182.8 mg, 82%) as a white solid. Rf: 0.30 (70% ethyl acetate/petroleum ether). Mp: 96–98 °C. 1H NMR (400 MHz, CDCl3): δ = 1.36 (t, J = 7.3 Hz, 3H), 1.99 (s, 3H), 3.55, 3.97 (ABq, JAB = 14.5 Hz, 4H), 4.32 (q, J = 7.3 Hz, 2H), 6.62 (s, 1H), 7.27–7.34 (m, 4H), 7.40–7.45 (m, 2H), 7.94–8.01 (m, 4H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.12, 23.91, 31.14, 62.47, 63.68, 116.91, 117.11, 124.20, 124.29, 124.67, 125.24, 125.28, 130.01, 130.08, 142.97, 151.96, 154.45, 170.25, 171.90 ppm. LRMS m/z: calcd. for C24H24F2N7O3 [M+H]+ 496.5, found: 496.2.

4.3.3. Compound (6c)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), m-fluorophenyl azide 5c (135.6 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 12 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6c (118.2 mg, 53%) as a white solid. Rf: 0.30 (70% ethyl acetate/petroleum ether). Mp: 96–98 °C. 1H NMR (400 MHz, CDCl3): δ = 1.37 (t, J = 7.0 Hz, 3H), 2.03 (s, 3H), 3.49, 3.90 (ABq, JAB = 15.6 Hz, 4H), 4.34 (q, J = 7.0 Hz, 2H), 6.78 (s, 1H), 7.16–7.18 (m, 2H), 7.52–7.55 (m, 6H), 7.93 (s, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.13, 23.91, 31.14, 62.47, 63.68, 115.66, 117.11, 121.25, 131.29, 143.75, 164.34, 170.31, 171.90 ppm. LRMS m/z: calcd. for C24H24F2N7O3 [M+H]+ 496.5, found: 496.2.
4.3.4. Compound (6d)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), p-fluorophenyl azide 5d (135.6 mg, 0.99 mmol), Cu(OAc)$_2$ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H$_2$O (3:3 mL) was stirred at rt for 24 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6d (196.2 mg, 88%) as a white solid. $R_f$: 0.33 (70% ethyl acetate/petroleum ether). Mp: 133–135 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.36 (t, $J$ = 7.2 Hz, 3H), 2.02 (s, 3H), 3.49, 3.87 (ABq, $J_{AB}$ = 14.5 Hz, 4H), 4.33 (q, $J$ = 7.2 Hz, 2H), 6.82 (s, 1H), 7.18–7.27 (m, 4H), 7.66–7.75 (m, 4H), 7.89 (s, 2H) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 14.20, 24.00, 31.04, 62.35, 63.06, 116.66, 116.86, 121.58, 122.27, 122.34, 143.47, 170.19, 171.97 ppm. LRMS m/z: calcd. for C$_{24}$H$_{24}$F$_2$N$_7$O$_3$ [M+H]$^+$ 496.5, found: 496.1.

4.3.5. Compound (6e)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), o-chlorophenyl azide 5e (152.0 mg, 0.99 mmol), Cu(OAc)$_2$ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H$_2$O (3:3 mL) was stirred at rt for 12 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6e (128.3 mg, 54%) as a white solid. $R_f$: 0.33 (70% ethyl acetate/petroleum ether). Mp: 133–135 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.37 (t, $J$ = 7.1 Hz, 3H), 2.02 (s, 3H), 3.58, 3.97 (ABq, $J_{AB}$ = 14.4 Hz, 4H), 4.33 (q, $J$ = 7.1 Hz, 2H), 6.72 (s, 1H), 7.46–7.48 (m, 4H), 7.58–7.61 (m, 2H), 7.64–7.66 (m, 2H), 7.91 (s, 2H) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 14.20, 24.02, 31.19, 62.44, 63.57, 125.20, 127.20, 127.97, 128.42, 130.68, 130.80, 134.95, 142.40, 170.17, 171.97 ppm. LRMS m/z: calcd. for C$_{24}$H$_{23}$Cl$_2$N$_7$O$_3$ [M]+ 528.4, found: 528.1.

4.3.6. Compound (6f)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), m-chlorophenyl azide 5f (152.0 mg, 0.99 mmol), Cu(OAc)$_2$ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H$_2$O (3:3 mL) was stirred at rt for 24 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6f (168.8 mg, 71%) as a white solid. $R_f$: 0.29 (70% ethyl acetate/petroleum ether). Mp: 97–99 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.37 (t, $J$ = 7.3 Hz, 3H), 2.03 (s, 3H), 3.49, 3.89 (ABq, $J_{AB}$ = 14.2 Hz, 4H), 4.34 (q, $J$ = 7.3 Hz, 2H), 6.79 (s, 1H), 7.42–7.44 (m, 2H), 7.46–7.50 (m, 2H), 7.62–7.64 (m, 2H), 7.79 (s, 2H) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 14.26, 24.06, 31.07, 62.44, 63.10, 118.26, 120.63, 121.31, 128.78, 130.85, 135.66, 137.77, 143.61, 170.21, 171.93 ppm. LRMS m/z: calcd. for C$_{24}$H$_{23}$Cl$_2$N$_7$O$_3$ [M]+ 528.4, found: 528.1.

Fig. 7. Disaggregation of Cu$^{2+}$-mediated Aβ$_{42}$ aggregation in the presence of compound 6n: The results of ThT binding assay. The statistical significance was analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test: (**) $p < 0.01$, [Aβ$_{42}$] = 20 µM, [Cu$^{2+}$] = 20 µM, [6n] = [CQ] = 40 µM.

Fig. 8. MTT cell viability assay: (a) Cell viability in the presence of 6n alone at variable concentrations (5 µM, 25 µM and 50 µM) after 48 h of incubation. (b) Cell viability in the presence of Aβ$_{42}$ alone at a concentration of 5 µM and mixture of Aβ$_{42}$-6n at molar ratios of 1:1, 1:5, 1:10. The data is expressed as mean values ± SEM from three independent experiments. The statistical significance was analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test: (**) $p < 0.01$. In both panel (a) and (b) error bars represent the average of three replicate experiments.
4.3.7. Compound (6g)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), p-chlorophenyl azide 5 g (152.0 mg, 0.99 mmol), Cu(OAc)₂ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-
BuOH/H₂O (3:3 mL) was stirred at rt for 12 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6 g (128.4 mg, 54%) as a white solid. Rf: 0.30 (70% ethyl acetate/petroleum ether). Mp: 98–100 °C. ¹H NMR (500 MHz, CDCl₃): δ = 1.36 (t, J = 7.2 Hz, 3H), 2.02 (s, 3H), 3.49, 3.89 (ABq, Jₐ₉ = 14.8 Hz, 4H), 4.34 (q, J = 7.2 Hz, 2H), 6.79 (s, 1H), 7.51–7.55 (m, 4H), 7.68–7.70 (m, 4H), 7.91 (s, 2H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ = 14.11, 21.25, 32.66, 61.26, 62.15, 121.32, 121.50, 123.41, 129.98, 130.79, 132.82, 140.83, 170.44, 171.20 ppm. LRMS m/z: calcd. for C₁₅H₁₉Cl₂N₇O₃ [M]+ 528.4, found: 528.1.

4.3.8. Compound (6h)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), o-bromophenyl azide 5 h (196.0 mg, 0.99 mmol), Cu
(OAc)₂ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-
BuOH/H₂O (3:3 mL) was stirred at rt for 8 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6 h (144.5 mg, 52%) as a white solid. Rf: 0.33 (70% ethyl acetate/petroleum ether). Mp: 73–75 °C. ¹H NMR (500 MHz, CDCl₃): δ = 1.37 (t, J = 7.1 Hz, 3H), 2.03 (s, 3H), 3.58, 3.97 (ABq, Jₐ₉ = 14.2 Hz, 4H), 4.32 (q, J = 7.1 Hz, 2H), 6.75 (s, 1H), 7.40–7.43 (m, 2H), 7.49–7.52 (m, 2H), 7.56–7.58 (m, 2H), 7.76–7.78 (m, 2H), 7.86 (s, 2H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ = 14.22, 24.14, 31.15, 62.44, 63.55, 118.56, 125.31, 128.20, 128.55, 131.13, 133.92, 136.58, 142.30, 170.09, 171.89 ppm. LRMS m/z: calcd. for C₁₅H₁₉Br₂N₇O₃ [M+H]+ 618.3, found: 618.0.

4.3.9. Compound (6i)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), m-bromophenyl azide 5 i (196.0 mg, 0.99 mmol), Cu(OAc)₂ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-
BuOH/H₂O (3:3 mL) was stirred at rt for 20 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6 i (161.1 mg, 58%) as a white solid. Rf: 0.31 (70% ethyl acetate/petroleum ether). Mp: 96–98 °C. ¹H NMR (500 MHz, CDCl₃): δ = 1.37 (t, J = 7.2 Hz, 3H), 2.03 (s, 3H), 3.49, 3.89 (ABq, Jₐ₉ = 14.6 Hz, 4H), 4.34 (q, J = 7.2 Hz, 2H), 6.79 (s, 1H), 7.40–7.43 (m, 2H), 7.58–7.59 (m, 2H), 7.67–7.68 (m, 2H), 7.91 (s, 2H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ = 14.24, 24.15, 31.14, 62.53, 63.17, 118.84, 121.38, 123.54, 131.17, 131.80, 137.90, 143.75, 172.90 ppm. LRMS m/z: calcd. for C₁₅H₁₉Br₂N₇O₃ [M+H]+ 618.3, found: 618.0.

4.3.10. Compound (6j)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), p-bromophenyl azide 5 j (196.0 mg, 0.99 mmol), Cu(OAc)₂ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-
BuOH/H₂O (3:3 mL) was stirred at rt for 6 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6 j (147.2 mg, 53%) as a white solid. Rf: 0.30 (70% ethyl acetate/petroleum ether). Mp: 108–110 °C. ¹H NMR (400 MHz, CDCl₃): δ = 1.30 (t, J = 7.1 Hz, 3H), 2.06 (s, 3H), 3.58, 3.81 (ABq, Jₐ₉ = 14.8 Hz, 4H), 4.28 (q, J = 7.1 Hz, 2H), 7.48 (s, 1H), 7.57–7.57 (m, 8H), 8.19 (s, 2H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.13, 23.63, 30.84, 62.39, 121.80, 122.05, 122.58, 123.20, 133.09, 135.45, 142.59, 170.76, 171.37 ppm. HRMS m/z: m/z: calcd. for C₁₅H₁₉Br₂N₇O₃NaM + Na+: 640.2823, found: 639.9523.

4.3.11. Compound (6k)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), o-iodophenyl azide 5 k (242.6 mg, 0.99 mmol), Cu(OAc)₂ (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-
BuOH/H₂O (3:3 mL) was stirred at rt for 30 h. The crude mixture was...
purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6k (188.8 mg, 59%) as a white solid. Rf: 0.31 (70% ethyl acetate/petroleum ether). Mp: 77–79 °C. 1H NMR (500 MHz, CDCl3): δ = 1.37 (t, J = 7.1 Hz, 3H), 2.05 (s, 3H), 3.58, 3.96 (ABq, JAB = 14.4 Hz, 4H), 4.33 (q, J = 7.1 Hz, 2H), 6.79 (s, 1H), 7.24–7.27 (m, 2H), 7.45–7.46 (m, 2H), 7.78 (s, 1H), 8.05–8.02 (m, 2H) ppm. 13C NMR (125 MHz, CDCl3): δ = 14.36, 24.40, 31.27, 62.53, 63.55, 94.05, 125.32, 127.99, 129.39, 131.55, 140.32, 142.48, 170.19, 171.97 ppm. LRMS m/z: calcd. for C24H24I2N7O3 [M+H]+ 712.3, found 712.0.

4.3.12. Compound (6l)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), m-iodophenyl azide 5l (242.6 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 20 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6l (188.8 mg, 59%) as a pale yellow solid. Rf: 0.31 (70% ethyl acetate/petroleum ether). Mp: 94–96 °C. 1H NMR (400 MHz, CDCl3): δ = 1.34 (t, J = 7.2 Hz, 3H), 2.01 (s, 3H), 3.48, 3.86 (ABq, JAB = 14.5 Hz, 4H), 4.31 (q, J = 7.2 Hz, 2H), 6.79 (s, 1H), 7.24–7.27 (m, 2H), 7.45–7.46 (m, 2H), 7.78 (s, 1H), 8.05–8.02 (m, 2H) ppm. 13C NMR (125 MHz, CDCl3): δ = 14.36, 24.40, 31.27, 62.53, 63.55, 94.05, 125.32, 127.99, 129.39, 131.55, 140.32, 142.48, 170.19, 171.97 ppm. LRMS m/z: calcd. for C24H24I2N7O3 [M+H]+ 712.3, found 712.0.

Fig. 9. The docked complex of 6n with Aβ42 monomer (PDB ID: 1Z0Q) and Aβ42 protofibril (PDB ID: 2BEG) are shown in panel (a), and (b), respectively. The N- and C-terminal is labelled in Aβ42 monomer structure shown in panel (a). The Aβ42 protofibril structure with five peptide chains, A–E, is shown in the cartoon representation in cyan and 6n is shown in the stick representation. The hydrogen bonds between Aβ42 monomer-6n, and Aβ42 protofibril-6n are shown as yellow dashed lines with distance in nm in panel (a), and (b), respectively.

Fig. 10. The 2D interaction maps displaying the hydrophobic contacts of 6n with Aβ42 monomer (PDB ID: 1Z0Q) and Aβ42 protofibril (PDB ID: 2BEG) are shown in panel (a), and (b), respectively. The maps are generated using LigPlot + software. The hydrogen bonds between Aβ42 monomer-6n and Aβ42 protofibril-6n are shown as green lines.
2.72–7.24 (m, 2H), 7.67–7.70 (m, 2H), 7.75–7.77 (m, 2H), 7.88 (s, 2H), 8.08–8.10 (m, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.12, 23.92, 31.14, 62.47, 63.68, 124.20, 124.29, 124.67, 125.24, 125.27, 120.01, 142.97, 151.96, 170.25, 171.90 ppm. LRMS m/z: calcd. for C26H24F6N7O3[M+H]+ 596.5, found: 596.1.

4.3.13. Compound (6m)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), p-iodosophenyl azide 5m (242.6 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.45 mmol), in t-BuOH/H2O (3:3 mL) was stirred at rt for 24 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6m (208.1 mg, 65%) as a white solid. Rf = 0.31 (70% ethyl acetate/petroleum ether). Mp: 113–115 °C. 1H NMR (400 MHz, CDCl3): δ = 1.33 (t, J = 7.0 Hz, 3H), 1.98 (s, 3H), 3.46, 3.89 (ABq, JAB = 14.5 Hz, 4H), 4.30 (q, J = 7.0 Hz, 2H), 6.77 (s, 1H), 7.46–7.49 (m, 4H), 7.83–7.85 (m, 4H), 7.88 (s, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.17, 23.95, 29.30, 31.04, 62.38, 63.10, 93.51, 121.12, 121.82, 136.51, 138.82, 143.59, 170.30 ppm. LRMS m/z: calcd. for C26H24F6N7O3[M+H]+ 596.5, found: 596.19.

4.3.14. Compound (6n)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), o-trifluoromethylphenyl azide 5n (185.3 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 24 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6n (176.9 mg, 66%) as a white solid. Rf = 0.29 (70% ethyl acetate/petroleum ether). Mp: 78–80 °C. 1H NMR (400 MHz, CDCl3): δ = 1.34 (t, J = 7.1 Hz, 3H), 1.98 (s, 3H), 3.54, 3.96 (ABq, JAB = 14.5 Hz, 4H), 4.30 (q, J = 7.1 Hz, 2H), 6.65 (s, 1H), 7.55–7.57 (m, 2H), 7.65–7.76 (m, 6H), 7.84–7.86 (m, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.24, 23.77, 31.27, 62.66, 63.80, 128.08, 128.61, 129.01, 130.66, 133.46, 135.23, 142.61, 170.44, 171.84 ppm. HRMS m/z: calcd. for C24H24F6N7O3[M+H]+ 485.3, found: 485.38. HPLC purity: 99.98%.

4.3.15. Compound (6o)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), m-trifluoromethylphenyl azide 5o (185.3 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 10 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6o (150.1 mg, 56%) as a white solid. Rf = 0.30 (70% ethyl acetate/petroleum ether). Mp: 96–98 °C. 1H NMR (400 MHz, CDCl3): δ = 1.35 (t, J = 7.2 Hz, 3H), 1.99 (s, 3H), 3.49, 3.89 (ABq, JAB = 14.5 Hz, 4H), 4.32 (q, J = 7.2 Hz, 2H), 6.78 (s, 1H), 7.64–7.71 (m, 4H), 7.93–7.90 (m, 2H), 7.97 (s, 2H), 8.02 (s, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.18, 24.19, 29.00, 62.55, 63.11, 117.46, 121.35, 124.38, 125.38, 130.67, 137.20, 143.87, 170.28, 171.95 ppm. HRMS m/z: calcd. for C26H24F6N7O3[M+H]+ 596.5042, found: 596.5042.

4.3.16. Compound (6p)

According to the general procedure, di-alkyne 4 (100 mg, 0.45 mmol), p-trifluoromethylphenyl azide 5p (185.3 mg, 0.99 mmol), Cu(OAc)2 (17.9 mg, 0.09 mmol) and sodium ascorbate (35.6 mg, 0.18 mmol) in t-BuOH/H2O (3:3 mL) was stirred at rt for 12 h. The crude mixture was purified by column chromatography (50% ethyl acetate/petroleum ether) to give compound 6p (142.0 mg, 53%) as a white solid. Rf = 0.30 (70% ethyl acetate/petroleum ether). Mp: 116–118 °C. 1H NMR (500 MHz, CDCl3): δ = 1.36 (t, J = 7.1 Hz, 3H), 2.02 (s, 3H), 3.48, 3.87 (ABq, JAB = 14.8 Hz, 4H), 4.33 (q, J = 7.1 Hz, 2H), 6.81 (s, 1H), 7.72–7.73 (m, 4H), 7.70–7.72 (m, 4H), 7.89 (s, 2H) ppm. 13C NMR (100 MHz, CDCl3): δ = 14.18, 24.47, 31.28, 61.30, 63.25, 116.90, 120.19, 121.63, 123.02, 125.99, 143.73, 170.56, 171.12 ppm. LRMS m/z: calcd. for C20H24F6N6O3[M+H]+ 596.4, found: 596.1.

4.4. Thiourea T (ThT) fluorescence assay

The chelating ability of compound 6n towards biomacromers such as Cu2+, Zn2+ and Fe3+ was examined by UV–Vis spectroscopy (Ultrospec 3000). The stock solution of compound 6n (4 mM), metal ions (8 mM)
were prepared in methanol. The solution of 6n (20 µM, final concentration) alone or in the presence of CuSO₄, ZnCl₂ or FeSO₄ (20 µM, final concentration) in (20 mM HEPES, 150 mM NaCl, pH 7.4) were incubated for 30 min at 25 °C. The spectra of each sample were recorded with wavelength ranging from 200 nm to 600 nm using blank containing 20 mM HEPES, 150 mM NaCl, pH 7.4.

The stoichiometry of the 6n-Cu²⁺ complex was determined by Job’s method, by preparing the separate solutions of 6n and CuSO₄ in which the total concentration remain constant (40 µM) but the proportion of each component was varied from 0% to 100%. The absorbance at 262 nm was plotted against mole fraction of Cu²⁺. The break point displays the stoichiometry of the complex.

4.8. Ascorbate assay

The ascorbate study was performed by using SpectraMax M5 spectrophotometer. The fluorescence intensities were recorded with excitation and emission wavelengths at 395 nm and 450 nm for the period of 16 min. The stock solution of 6n (in methanol), CuSO₄ (in Milli-Q water), CCA and ascorbate were dissolved and diluted in 50 mM PBS buffer (pH 7.4). The final volume of the sample is 200 µL. The production of hydroxyl radical was measured as the change of CCA into 7-hydroxy-CCA. The order of addition follows: CCA (50 µM), ligand (15 µM), or copper (5 µM) and ascorbate (150 µM). All test solutions contained 1 µM desferryl.

4.9. MTT cell viability assay

The potential role of 6n in the Aβ42-induced cell toxicity was studied using the MTT assay with SH-SY5Y cell line according to the literature procedure [44]. In high glucose Dulbecco’s modified eagle’s medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin and 100 U/mL streptomycin, the SH-SY5Y cells were maintained at 37 °C under 5% CO₂ in a CO₂ cell culture box. In a polystyrene 96-well plate, a total of 5 × 10³ cells (90 µL) were seeded for 24 h and then, the cells were treated with Aβ42 and 6n + Aβ42 (2.5 µL, 6n of different concentration was co-incubated with Aβ42 (2.25 µL) monomers at 37 °C for 24 h). The final concentration of DMSO was kept constant at 2.5% (v/v). For the incubation of cells was continued for an additional 24 h, and then 10 µL of MTT solution at the concentration of 5.5 mg/mL in PBS was added into each well and followed by another 4 h of incubation. Then the medium was discarded, and 100 µL of DMSO was used to dissolve the cells till the complete dissolution of purple crystals. By plate reader, the absorbance at 570 nm was measured. From each reading, the background was subtracted i.e. the wells containing only medium. The data obtained was normalized as a percentage of the control group without Aβ42 and inhibitor.

4.10. Molecular docking studies

The molecular docking was performed using AutoDock 4.2 [46]. To identify the binding region and key interactions of 6n with Aβ42 using molecular docking, the NMR structure of Aβ42 monomer (PDB ID: 1Z0Q) and Aβ42 protofibril (PDB ID: 2BEG) were employed. The 3D structure of 6n was optimized by Gaussian using Hatree–Fock (HF) theory with basis set 6-31G(d). The grid spacing was kept default (0.375 Å) and dimension of the box for 1Z0Q was set to 127 Å × 86 Å with grid center defined at x = −2.020, y = 0.250, and z = −8.949. The population of 150 individuals was employed to generate 100 conformations for 27,000 generations with 2,500,000 energy evaluations. The mutation rate of 0.02, a crossover rate of 0.80, and reference root-mean-square deviation (RMSD) were kept as default. The Lamarckian Genetic Algorithm that utilizes global search (Genetic Algorithm) and local search (Solis and Wets algorithm) was chosen for the present study [47]. The docked poses of 6n were clustered using a tolerance of 0.2 nm for RMSD and ranked on the basis of binding energy.

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Appendix A. Supplementary material

Supplementary data to this article can be found at https://doi.org/10.1016/j.bioorg.2019.03.058.

References


(h) X.T. Luo, C.M. Wang, Y. Liu, Z.G. Huang, New multifunctional metallophen–


(f) C. Kumar, V. Sim, D-amino–acid–based peptide inhibitors as early or pre-


