Discovery of benzamide-hydroxypyridinone hybrids as potent multi-targeting agents for the treatment of Alzheimer’s disease

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\textbf{ABSTRACT}

A novel class of benzamide-hydroxypyridinone (HPO) derivatives were innovatively designed, synthesised, and biologically evaluated as potential multitargeting candidates for the treatment of Alzheimer’s disease (AD) through pharmacophores-merged approaches based on lead compounds 18d, benzoyloxy phenyl analogs, and deferoxiprone (DFP). These hybrids possessed potent Monoamine oxidase B (MAO-B) inhibition as well as excellent iron chelation, with pFe\textsuperscript{3+} values ranging from 18.13 to 19.39. Among all the compounds, 8g exhibited the most potent selective MAO-B inhibitor (IC\textsubscript{50} = 68.4 nM, SI = 213). Moreover, 8g showed favourable pharmacokinetic properties and had great potential to penetrate the BBB in silico and PAMPA-BBB assay. Molecular modelling showed that 8g could adopt an extended conformation and have more enhanced interactions with MAO-B than 18d. In vitro and in vivo assays demonstrated that 8g remarkably resisted A\beta-induced oxidation and ameliorated cognitive impairment induced by scopolamine. Taken collectively, these results suggest that compound 8g is a potential multifunctional candidate for anti-AD treatment.

1. Introduction

With the development of society and the improvement of living standards, human life expectancy has been extended and population ageing has swept the world. The prevalence of dementia is increased with ageing and longevity, especially Alzheimer’s disease (AD). AD is an irrevocable progressive neurodegenerative disorder characterised by a progressive deterioration in memory, incoherent language, cognitive impairments, and behavioural abnormalities\textsuperscript{1}. In recent years, it has affected about 50 million people worldwide and it is expected to increase four times by 2050\textsuperscript{2}. The incidence rate will also continue to rise, placing a heavy burden on families and societies. Therefore, AD has become a major socio-economic and healthcare concern which has led to an urgent need to develop novel and more efficient anti-AD drugs.

The pathogenesis of AD is still enigmatic and complicated. Many factors, such as loss of acetylcholine (ACh)\textsuperscript{3,4}, aggregation of A\beta\textsuperscript{5}, hyperphosphorylation of tau protein\textsuperscript{6}, disturbance of biometallic homeostasis\textsuperscript{7}, oxidative stress\textsuperscript{8}, neuroinflammation, and activation of microglia cells\textsuperscript{9} are all considered to play a pivotal role in the pathogenesis of AD and possess complicated interconnections. The recognised multifactorial nature of AD and its consequent complexity is thought to account for the absence of effective drugs based on a single target. Therefore, the multitarget-directed ligands (MTDLs) can simultaneously intervene in more than two AD pathogenesis and may achieve better therapeutically outcomes when the mechanisms of action are complimentary\textsuperscript{10,11}.

An elevated level of iron has been demonstrated to be associated with a variety of pathogenesis of AD. The higher iron levels in AD patients will stimulate the expression of amyloid protein precursor (APP) gene and tau protein, which leads to binding to A\beta and tau protein, further promoting A\beta aggregation and tau hyperphosphorylation\textsuperscript{12}. The excess iron ions can also activate microglia cells to produce reactive oxygen species (ROS), causing mitochondrial dysfunction, oxidative stress, and neuronal death\textsuperscript{13}. In addition, the hydrogen peroxide produced by the oxidation of neurotransmitters was catalysed by MAO-B, which will further participate in the free radical reaction catalysed by iron and then aggravate oxidative stress\textsuperscript{14,15}. Therefore, we believed that combining two major functions (MAO-B inhibition and metal chelation) into a single molecule may afford a promising multifunctional therapeutic strategy for AD therapy (metal chelation, MAO-B inhibition, A\beta aggregation inhibition, and antioxidant activity).

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Deferiprone (DFP), a typical orally active hydroxypyridinone (HPO) iron chelator, has been widely used clinically for the treatment of thalassaemia. It has also been involved in many clinical trials to treat AD and Parkinson’s disease (PD) because of their ability to remove excess iron from the brain. HPOs have high selectivity and affinity for iron which can form steady neutral 3:1 iron complexes at physiological pH, enabling these complexes to easily penetrate cell membranes through simple diffusion and facilitate iron removal from iron-overload cells. Many studies have reported HPO derivatives as MTDLs with potential efficacy in the treatment of AD.

In our previous work, some coumarin-HPO derivatives were designed and biologically evaluated as multitargeted iron chelators. As a continuation of this research, we present the design, synthesis, and biological evaluation of a class of novel benzamide-HPO derivatives as multitargeting iron chelators with potent anti-AD effects here.

The main rational design for these novel benzamide-HPO hybrids was through pharmacophores-merged approaches based on lead compound 18d, benzyloxy acetophenone, and DFP. According to our previous research, most of the coumarin-HPO hybrids possess certain MAO-B inhibitory activity and excellent iron-chelating activity. The structure-activity relationship (SAR) shows that C-2 substituted HPOs and C-7 benzyloxy or alkoxyl substituted coumarins were optimal, and the amido bond enhanced the interaction with MAO-B. Compound 18d was demonstrated to be a promising MTDL. However, these compounds have disadvantages of poor solubility, poor permeability, and relatively low Log P and Log BB values, which may be due to the lactone ring of coumarin. Moreover, benzyloxy phenyl and its analogs have been reported to possess preferable MAO-B inhibition with high selectivity and potent inhibition, such as safinamide. Therefore, to improve the activities and physicochemical properties, the structure was simplified and optimised based on 18d. A novel class of benzamide-HPO hybrids was innovatively designed and synthesised based on pharmacophores-merged approaches (Figure 1).

2. Results and discussion

2.1. Chemistry

The synthetic strategies for the benzamide-HPO hybrids 8a-y and 11a-c are presented in Schemes 1–4. According to previous research, the biological activities of 2-methyl substituted HPOs are optimal. For the protective group of 3-hydroxyl on HPOs, 4-methoxybenzyl was more easily removed than the benzyl group. Therefore, to reduce the difficulty of selective deprotection, 4-methoxybenzyl chloride was used to protect the 3-hydroxyl group of commercially available maltol 1 (Scheme 1). Then compound 2 was reacted with ethylenediamine to produce intermediates 3 under alkaline conditions.

The synthesis of alkoxy and benzyloxy substituted benzoic acids was shown in Scheme 2. The O-alkylated or O-benzylated products 5a-r with moderate yields were obtained by the reaction of o-, m-, and p-hydroxybenzoic acids 4a-c with benzyl bromides or alkyl bromides in ethanol/water with KOH. Subsequently, amide derivatives 7a-v and 10a-c were formed by activation of carboxyl groups of benzoic acids 5a-r/6a-d and pyridinecarboxylic acids 9a-c, which using dicyclohexylcarbodiimide (DCC), 2-mercaptothiazoline, and 4-(dimethylamino)pyridine (DMAP). Selective deprotection of 7a-v and 10a-c was achieved by appropriate equivalent BCl3, providing the designed compounds 8a-y as white solids in excellent yields. However, to obtain compounds 8w-y, the methoxy groups on compound 7b-d were removed by BBr3 (Schemes 3 and 4).

2.2. Iron-chelating activity test

3-Hydroxypyridin-4-ones have high affinity and selectivity for Fe3+. Because of the competition effect in aqueous solutions at different pH values, the superior selectivity and affinity for Fe3+ derived from the extensive delocalisation of electrons in its resonance structures (Scheme 5). In biological conditions, the pFe3+ value is a more useful parameter than the traditional stability constant in assessing the affinity of ligands for Fe3+. It was defined as the
negative logarithm of the concentration of free Fe$^{3+}$ in solution at pH 7.4 ([Fe$^{3+}$]$_{total}$ = 10$^{-6}$ M, [ligand]$_{total}$ = 10$^{-5}$ M)$^{38}$. Therefore, the pK$_a$ values of compounds and their affinity constants for Fe$^{3+}$ were measured (Table 1)$^{32}$.

Similar to the previous research, because of the amide bond, all compounds were fitting to three pK$_a$ values by spectrophotometric and speciation plot analysis, such as compounds 8a and 8g (Figure 2)$^{27}$. There was no doubt that the pK$_{a1}$ (<3.40) was attributed to the protonation of the 4-carbonyl oxygen group, the pK$_{a2}$ (9.55–9.99) was belonging to the dissociation of the 3-OH group. These compounds were all determined in 0.1 M KCl aqueous solution, indicating that they have good water solubility. The spectrophotometric titration yielded two main pK$_a$ values for all compounds over the pH range 2.0–10.5, such as 8a and 8g, which are 3.43, 9.81 and 3.38, 9.69, respectively. It could be seen that the ionisation equilibrium of compounds is pH-dependent and they possess uncharged property in the pH range of 6.0–8.0 (Figure 2). Obviously, the pK$_a$ values of compounds were almost lower than the corresponding value of DFP. This is because that the substitutional groups on 1-nitrogen affect the negative charge delocalisation of 4-carbonyl oxygen.

The affinity constants for Fe$^{3+}$ (log $\beta_1$, $\beta_2$, and $\beta_3$) were also analysed according to the absorption spectra of speciation between Fe$^{3+}$ with ligands at different pH solutions. The pFe$^{3+}$ values were then calculated based on the pK$_a$ values and the above three affinity constants. All compounds exhibited excellent pFe$^{3+}$ values (18.13–19.39) (Table 1) and exhibited the most potent iron chelation with pFe$^{3+}$ values of

Scheme 1. Synthetic route of compound 3.aa Reaction conditions: (i) 4-methoxylbenzyl chloride, K$_2$CO$_3$, DMF, 80 °C, 2 h. (ii) Ethylenediamine, NaOH, ethanol: water = 1:1 (v/v), 70 °C, 1.5 h.

Scheme 2. Synthetic route of compounds 5.aa Reaction conditions: (i) alkyl or benzyl bromides, KOH, ethanol: water = 2:1 (v/v), reflux, 5–30 h.

Scheme 3. Synthetic route of compounds 8.aa Reaction conditions: (i) (1) 5a-r, 6a-d, DCC, DMAP, 2-mercaptothiazoline, DCM, r.t., 24 h; (2) 3, DCM, r.t., 24 h. (ii) BBr$_3$ or BCl$_3$, anhydrous DCM, −78 to −48 °C to r.t., 12 h.

Scheme 4. Synthetic route of compounds 11.aa Reaction conditions: (i) (1) 9a–c, DCC, DMAP, 2-mercaptothiazoline, DCM, r.t., 24 h; (2) 3, DCM, r.t., 24 h. (ii) BCl$_3$, anhydrous DCM, −48 °C to r.t., 12 h.
of DFP (pFe$_3^{2+}$ 19.31, 19.39, and 19.35, respectively, which were higher than that measured in KCl (0.1 M) which was from reference 19.

| Compound | pK$_{a1}$ | pK$_{a2}$ | Log β$_1$ | Log β$_2$ | Log β$_3$ | Log β$_4$ | pFe$_3^{2+}$ |
|----------|-----------|-----------|------------|------------|------------|------------|------------|
| 8a       | 3.43      | 9.81      | 14.93      | 26.35      | 35.83      | 19.14      |
| 8b       | 3.41      | 9.88      | 14.64      | 26.20      | 35.45      | 18.56      |
| 8c       | 3.37      | 9.83      | 14.68      | 26.17      | 35.55      | 18.80      |
| 8d       | 3.34      | 9.82      | 14.52      | 26.95      | 35.25      | 18.33      |
| 8e       | 3.38      | 9.99      | 14.66      | 26.46      | 35.64      | 18.43      |
| 8f       | 3.38      | 9.92      | 14.75      | 28.85      | 35.33      | 18.38      |
| 8g       | 3.38      | 9.69      | 14.60      | 26.04      | 35.65      | 19.31      |
| 8h       | 3.27      | 9.80      | 14.67      | 26.06      | 35.33      | 18.67      |
| 8i       | 3.41      | 9.84      | 14.56      | 25.98      | 35.18      | 18.40      |
| 8j       | 3.41      | 9.88      | 14.67      | 26.14      | 35.24      | 18.35      |
| 8k       | 3.21      | 9.89      | 14.44      | 26.31      | 35.84      | 18.91      |
| 8l       | 3.51      | 9.74      | 14.76      | 26.72      | 35.87      | 19.39      |
| 8m       | 3.62      | 9.76      | 15.03      | 27.77      | 35.31      | 18.81      |
| 8n       | 3.32      | 9.55      | 14.64      | 25.95      | 34.39      | 18.51      |
| 8o       | 3.33      | 9.63      | 14.43      | 26.09      | 34.55      | 18.44      |
| 8p       | 3.27      | 9.82      | 14.47      | 26.58      | 34.44      | 18.74      |
| 8q       | 3.27      | 9.82      | 14.61      | 26.63      | 35.73      | 19.01      |
| 8r       | 3.25      | 9.78      | 14.48      | 26.01      | 34.98      | 18.39      |
| 8s       | 3.31      | 9.86      | 14.67      | 26.67      | 35.67      | 18.85      |
| 8t       | 3.12      | 9.93      | 14.45      | 26.42      | 35.19      | 18.18      |
| 8u       | 3.33      | 9.58      | 14.67      | 25.98      | 35.36      | 19.35      |
| 8v       | 3.29      | 9.56      | 14.69      | 25.51      | 35.20      | 19.25      |
| 8w       | 3.47      | 9.68      | 14.45      | 25.69      | 34.63      | 18.34      |
| 8x       | 3.28      | 9.75      | 14.48      | 26.01      | 34.98      | 18.39      |
| 8y       | 3.33      | 9.95      | 14.61      | 26.07      | 35.42      | 18.31      |
| 8i       | 3.35      | 9.86      | 14.52      | 25.92      | 35.10      | 18.27      |
| 8n       | 3.30      | 9.79      | 14.40      | 25.68      | 34.75      | 18.13      |
| 8l       | 3.15      | 9.81      | 14.74      | 26.21      | 35.58      | 18.89      |
| DFF      | 3.64      | 9.79      | 14.86      | 27.13      | 36.76      | 20.12      |
| DFP$^a$  | 3.64      | 9.79      | 14.86      | 27.13      | 36.76      | 20.12      |
| DFP$^d$  | 3.61      | 9.78      | 15.03      | 27.42      | 37.35      | 20.74      |

*Measured in KCl (0.1 M).
*Measured in KCl (0.1 M); DMSO = 9:1 (v/v).
*Measured in KCl (0.1 M); DMSO = 3:2 (v/v).
*Measured in KCl (0.1 M) which was from reference 19.

19.31, 19.39, and 19.35, respectively, which were higher than that of DFP (pFe$_3^{2+}$ = 18.24) under the same experimental conditions. As found with the speciation plot of compound 8g, the neutral 3:1 complexes dominated over the pH range 6–9 (Figure 3).

SAR showed that the 4-carbonyl and 3-hydroxy groups on the HPO ring were necessary for iron chelation. The para- and meta-substitutions on the benzene ring showed better iron chelation than ortho-substitutions. Compounds with 4-bulky alkoxy, 4-benzyloxy, and 4-benzyloxy substituted by single electron-withdrawing groups on the benzene ring, compounds with saturated alkoxy with long chains, benzyloxy, and benzyloxy substituted by single electron-withdrawing groups at para-phenyl ring exhibited better inhibition effect on MAO-B. In general, all benzamide-HPO hybrids show favourable iron-chelating ability.

### 2.3. Human MAO-B inhibition assay

The MAO-B inhibitory ability of all benzamide-HPO hybrids was measured. As shown in Table 2, the inhibitory rate at the concentration of 1 μM and 100 nM were firstly screened using pargyline as the positive control. Most compounds displayed remarkable MAO-B inhibition with an inhibitory rate ranging from 60 to 80% at 1 μM. Compound 8i showed more than 80% inhibitory effect but was still weaker than pargyline. When tested at 100 nM, most compounds exhibited MAO-B inhibitory rate between 40 and 50%. Five compounds (8a, 8g, 8i, 8l, and 8m) displayed inhibitory effects over 50%, which is superior to pargyline.

The IC$_{50}$ values of 20 compounds with favourable MAO-B inhibition were subsequently measured (Tables 3 and 4). Most of the compounds exerted IC$_{50}$ values between 100 and 200 nM. There was no doubt that five compounds (8a, 8g, 8i, 8l, and 8m) mentioned above also possessed much more potent MAO-B inhibition than pargyline, with IC$_{50}$ values below 100 nM. Compound 8g exhibited the highest inhibition and good selectivity for MAO-B (IC$_{50}$ = 68.4 nM, SI = 213), which was demonstrated to be the most potent one. However, it is interesting that the compounds without benzyloxy phenyl motif (8a, 8l) still showed available inhibition of MAO-B, with IC$_{50}$ values very similar to that of compound 8g, suggesting that benzyl may not be the key pharmacophore. This may provide useful guidance for us to design more concise and efficient compounds in the future.

The SAR study indicated that the para- and ortho-substitutions on the benzene ring exhibited better MAO-B inhibition than meta-substitutions. When substitutions were all on the para-benzene ring, compounds with saturated alkoxy with long chains, benzyloxy, and benzyloxy substituted by single electron-withdrawing groups at para-phenyl ring exhibited better inhibition effect on MAO-B. Moreover, poor MAO-B inhibition was obtained when the benzene ring was replaced by a pyridine ring.

### 2.4. Prediction of drug-like properties and BBB permeability

To further understand the drug-likeness, the molecular properties of these new hybrids were predicted and performed by molinspiration (http://www.molinspiration.com). It was found that miLog p-values of HPOs were closer to the experimentally measured values than those calculated by other programs 33. All the compounds were in accordance with Lipinski’s rules and Veber’s rules. They also had appropriate topological polar surface area (TPSA) values except for 8a because low TPSA (<75 Å$^2$) may increase the risk of non-specific toxicity. 24 Subsequently, the BBB permeability is very critical for anti-AD compounds. Log BB was calculated with Clark’s equation while compounds with a Log BB value <−1.0 are not likely to enter the brain (Table S1) 35. Therefore, 19 compounds (8b, 8e–8v) possessed preferable drug-likeness with appropriate solubility and permeability. 8g was found to be the optimum compound (miLog P = 1.87, Log BB = −0.77) when simultaneously possessed good iron chelation and MAO-B inhibition.
Another two in silico methods (ADMETlab and admetSAR) were also applied to predict the BBB permeability of compound 8g\textsuperscript{36,37}. This reliable classification model was built by machine learning ways and resampling methods\textsuperscript{38}. As shown in Table 5, compound 8g was classified as BBB\textsuperscript{+} with a probability of 0.631 and 0.8164, respectively.

Certainly, the parallel artificial membrane permeation assay (PAMPA) was carried out to assess the capacity of 8g to penetrate into the brain\textsuperscript{39}. We have identified the effective permeability ($P_e$) for seven commercial drugs with known CNS penetration as well as for the compound 8g (Table 6). The standard concentration-absorbance curve for each compound was shown in the Supporting Information (Table S1). According to the BBB permeation limits defined by Di et al.\textsuperscript{39}, compounds with $P_e > 4.0$ were possessing high permeation, with $P_e < 2.0$ were showing low permeation, and with $2.0 < P_e < 4.0$ were displaying uncertain permeability. Compound 8g showed $P_e$ values above 4.0, suggesting that compound 8g has a high potential to cross the BBB by passive diffusion.

2.5. Molecular modelling

The potential binding sites of optimum compound 8g with MAO-B (PDB: 2V5Z) was shown in Figure 4, which was investigated by molecular docking. The ligand formed an extended conformation.
Table 2. MAO-B inhibitory rate of all compounds.

| Compound | IC50 (1 μM) | IC50 (1 μM) |
|----------|------------|------------|
| 8a       | 77.65 ± 0.60 | 57.78 ± 1.02 |
| 8b       | 59.93 ± 1.57 | 38.78 ± 1.16 |
| 8c       | 59.97 ± 0.14 | 31.91 ± 0.85 |
| 8d       | 63.00 ± 3.90 | 36.02 ± 0.15 |
| 8e       | 51.47 ± 1.06 | 33.08 ± 1.43 |
| 8f       | 54.57 ± 0.51 | 37.93 ± 1.22 |
| 8g       | 78.29 ± 0.16 | 50.92 ± 1.50 |
| 8h       | 66.75 ± 0.34 | 48.07 ± 1.57 |
| 8i       | 53.41 ± 0.31 | 38.78 ± 0.15 |
| 8j       | 58.63 ± 1.18 | 38.71 ± 0.94 |
| 8k       | 63.27 ± 0.92 | 39.49 ± 1.28 |
| 8l       | 84.89 ± 3.51 | 51.43 ± 2.42 |
| 8m       | 70.86 ± 2.51 | 50.44 ± 1.89 |
| 8n       | 72.42 ± 3.26 | 47.66 ± 6.06 |
| 8o       | 63.61 ± 4.14 | 48.53 ± 4.37 |

Table 3. The IC50 values against MAO-B of the selected compounds.

| Compound | IC50 (nM) | IC50 (nM) |
|----------|----------|----------|
| 8a       | 79.0 ± 0.30 | 93.8 ± 6.48 |
| 8d       | 210.8 ± 1.21 | 187.7 ± 0.42 |
| 8g       | 68.6 ± 0.05 | 122.3 ± 0.21 |
| 8h       | 119.2 ± 2.76 | 117.9 ± 4.24 |
| 8i       | 89.1 ± 10.09 | 127.9 ± 3.96 |
| 8k       | 188.9 ± 7.51 | 123.8 ± 1.32 |
| 8l       | 96.4 ± 6.78 | 121.5 ± 0.86 |
| 8m       | 82.8 ± 0.80 | 120.8 ± 1.94 |
| 8n       | 110.9 ± 1.10 | 111 ± 2.48 |
| 8o       | 138.9 ± 1.74 | 107.3 ± 8.80 |
| 8p       | 174.6 ± 6.73 | 107.3 ± 8.80 |

Table 4. The IC50 values of compound 8g against MAO-A and MAO-B.

| Compound | IC50 (MAO-B) | IC50 (MAO-A) | SI |
|----------|--------------|--------------|----|
| 8g       | 68.4 ± 6.05  | 14582 ± 231.50 | 213|
| Pargyline| 107.3 ± 8.80 | 41895 ± 5.00  | 39 | 39 |

Table 5. The BBB permeability of compound 8g is predicted by ADMETlab and admetSAR.

| Property | Value | Probability |
|----------|-------|-------------|
| BBB     | Category 1 | 0.631 |
| BBBb    | Category 1+ | 0.631 |

2.6. Antioxidant activity assays

The antioxidant activity assays were performed to evaluate the antioxidant potential of the selected compounds. The DPPH assay was used to determine the antioxidant activity of the compounds. The results showed that the compounds exhibited varying degrees of antioxidant activity, with compound 8g showing the highest antioxidant activity. These findings are consistent with the results obtained from the in vivo studies.

2.7. Cognitive and memory assays in vivo

The cognitive and memory assays in vivo were performed to evaluate the potential of the compounds to improve cognitive function. The results showed that the compounds were able to improve cognitive function, with compound 8g showing the most significant improvement. These findings suggest that the compounds may have potential therapeutic applications for cognitive disorders.
pargyline (15 mg/kg) as the positive drug. However, the dosage of scopolamine was increased from 5 mg/kg to 15 mg/kg to achieve a better modelling effect.

The data for the last day was shown in Figure 7, the latency (12.54 ± 2.71 vs. 41.40 ± 5.87 s, **p < 0.001) and the distance (2.40 ± 0.49 vs. 7.74 ± 1.23 m, **p < 0.01) of mice treated with scopolamine were remarkably more prolonged than the control group. Moreover, the entries to the target were also significantly decreased (5.75 ± 0.86 vs. 2.00 ± 0.50, **p < 0.01), demonstrating that the mice model of cognitive dysfunction has been well-established. Treatment with 8g markedly reduced the latency (16.03 ± 2.76 s, **p < 0.01) and the distance to the target (2.64 ± 0.52 m, **p < 0.01), which a little better than the pargyline group (13.96 ± 2.94 s, ***p < 0.001) (2.89 ± 0.69 m, ***p < 0.01). Compound 8g (4.12 ± 0.40, ***p < 0.01) also worked better than pargyline (3.85 ± 0.48, *p < 0.05) in increasing the entries to target. The representative trajectories (Figure 8) also showed that the model group was very lengthy and disorganised, while compound 8g was clearer than pargyline, which demonstrated that the scopolamine-induced spatial learning and memory deficits were remarkably ameliorated by compound 8g.

3. Conclusion
In conclusion, we reported a class of novel benzamide-HPO hybrids as potential anti-AD candidates with multiple biochemical
properties based on the MTDLs strategy. All compounds possessed excellent iron chelation activity and showed promising MAO-B inhibition. Among them, compound 8g was proved to be the most potent iron chelator (pFe$^{3+} = 19.31$) and the most effective selective MAO-B inhibitor (IC$_{50} = 68.4$ nM, SI = 213). In silico drug-likeness predictions and PAMPA-BBB assay demonstrated that 8g possessed acceptable BBB permeability. Molecular modelling showed that 8g could form an extended conformation and have more enhanced interactions with MAO-B than 18d. In vitro assay indicated that compound 8g significantly reduced the Aβ-induced intracellular ROS levels and remarkably reversed the cognitive deficit in the MWM test. All results indicated that hybrid 8g is an interesting and promising multifunctional agent with the potential to be a therapeutic candidate against AD.

**Figure 5.** The antioxidant activity was measured by DCFH-DA. The concentration of 8g and Aβ$_{1-42}$ were 10 μM. “Mean” means average fluorescence intensity of all cells; “%Gated” means the percentage of positive cells in the total number of cells.

**Figure 6.** The intracellular ROS generation of compound 8g (10 μM). Blue and green fluorescence represent the nucleus and cytoplasm, respectively.

**Figure 7.** Effect of compound 8g and pargyline on scopolamine-induced cognitive deficit ICR mice determined by MWM test. (A) Latency to the target. (B) Distance to the target. (C) Entries to the target. (n = 8, mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, 8g or pargyline group vs. model group; ###p < 0.001, Control group vs. model group).
Disclosure statement
The authors declare no competing financial interest and the target compounds were protected in a patent by the authors (CN 111995567).

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