New 4-N-phenylaminoquinoline derivatives as antioxidant, metal chelating and cholinesterase inhibitors for Alzheimer’s disease

Rong Cai, Li-Ning Wang, Jing-Jing Fan, Shang-Qi Geng, Yu-Ming Liu

**ABSTRACT**

A series of new 4-N-phenylaminoquinoline derivatives were designed, synthesized, and their anticholinesterase activities, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, metal-chelating ability were tested. Among them, compounds 11j, 11k and 11l had comparable inhibition activities to reference drug galantamine both in AChE and in BChE. Especially, compound 11j revealed the most potent inhibition to eAChE and eBChE with IC\textsubscript{50} values of 1.20 μM and 18.52 μM, respectively. Furthermore, both kinetic analysis of AChE inhibition and molecular docking study indicated that compound 11j was mixed-type inhibitor, binding simultaneously to the catalytic anionic site (CAS) and the peripheral anionic site (PAS) of AChE, and propidium iodide displacement assay showed significant displacement of propidium iodide with compound 11k (25.80%) from PAS of eAChE. More importantly, compound 11l displayed excellent DPPH radical scavenging activity (84% at 1 mg/mL), and its EC\textsubscript{50} value was 0.328 μM. In addition, compounds 11a, 11j, 11k and 11l exhibited obvious biometal chelating abilities toward Al\textsuperscript{3+}, Fe\textsuperscript{2+}, Cu\textsuperscript{2+} and Zn\textsuperscript{2+} ions. Taken together, 4-N-phenylaminoquinoline derivatives targeting multiple pathogenetic factors deserve further investigation for treatment of AD.

**1. Introduction**

Alzheimer’s disease (AD) is one of the most common types of dementia, and has seriously threatened the health of old people. The illness progress is well-known by the advent of aphasia, apraxia, executive disorders, mood disturbances, and psychiatric symptoms [1,2]. The exact origin of AD is ambiguous [3] and combinatorial reasons such as genetic, lifestyle, and environmental factors are involved in the onset and progression of the disease [4]. Now, such cellular processes as neurotransmitter systems [5], excitotoxicity [6], beta-amyloid aggregation, abnormal tau phosphorylation [7], oxidative stress, metal ion deregulation and inflammation [8] have been considered as the main causes of AD. Though several novel therapeutic approaches were explored in the last few decades like apolipoprotein E, CREB signaling pathways, insulin resistance, etc. to identify the potential leads, but were unable to elicit significant clinical outcomes [6].

One therapeutic approach to enhance cholinergic neurotransmission is to increase acetylcholine (ACh) availability by inhibiting acetylcholinesterase (AChE) [9–13]. Thus far, several anti-AD drugs targeting AChE have become available, including tacrine, donepezil, and the alkaloid galantamine. Compared with AChE, butyrylcholinesterase (BChE), the sister enzyme of AChE, plays a supportive role in the cholinergic neurotransmission. In the brain of healthy adults, AChE is very more active than BChE, and cause almost 80% ACh hydrolysis in the brain [14,15]. However, with the gradual severity of AD, AChE in the brain drops to 90% of the normal level, while the level of BChE is about doubled and tends to increase continuously [16]. And then the regulation of ACh is increasingly dependent on BChE in progressed AD [17–20]. Thus, the synergistic inhibition of both AChE and BChE enzymes may be one more valuable approach in the treatment of AD [21]. It is worth mentioning that AChE possesses two binding sites including the catalytic anionic site (CAS) and the peripheral anionic site (PAS). The new agents that serve as dual binding inhibitors for both CAS and PAS of AChE are more efficient in treating AD [22], as they can alleviate cognitive deficits by restoring cholinergic activity and prevent the deposition of beta-amyloid in the brain promoted by PAS of AChE [23]. As a result, novel AChE inhibitors targeting both CAS and PAS attract the attentions of medicinal chemists throughout the world.

Among the various causative factors involved in the pathogenesis of AD, oxidative stress has emerged as an important factor. Oxidative stress is caused by the imbalance of antioxidant defense system and intracellular reactive oxygen species (ROS) accumulation [24]. Unregulated reaction of molecular oxygen with the redox active metals can lead to the generation of ROS [25]. What’s more, the excessive...
accumulation of metals promotes beta-amyloid fibril aggregates, which induce inflammation and activate neurotoxic pathways, leading to the dysfunction and death of brain cells [26,27]. Recent studies have shown that antioxidants can inhibit lipid peroxidation by acting as metal ion-chelating and free-radical scavenging agents [28]. Clinical studies have shown the particular therapeutic efficacy of several antioxidants. Therefore, antioxidant can be a key factor in AD treatment.

Quinoline derivatives are important compounds that exhibited various biological activities, such as anti-tumor, anti-malaria, and cholinesterase (ChE) inhibitory activity [29,30], and tacrine was before used as the first AChE inhibitor in the treatment of AD [31]. Recently cholinesterase(ChE) inhibitory activity [29,30], and tacrine was before used as the first AChE inhibitor in the treatment of AD [31]. Recently cholinesterase(ChE) inhibitory activity [29,30], and tacrine was before used as the first AChE inhibitor in the treatment of AD [31].

According to scheme 1, compounds 11a–11m were synthesized starting from the commercially available material vanillic acid, which was esterified with methanol under acidic conditions to obtain compound 2. Compound 2 was alkylated with 1-bromo-3-chloropropane in acetone under basic condition to provide compound 3, which was converted to nitro compound 4 using fuming nitric acid as nitration reagent in dichloromethane for 5h with 98.98% yield. Compound 4 was reduced using iron powder and catalytic amounts of ammonium chloride in ethanol to obtain compound 5, which was further hydrolyzed with NaOH to provide compound 6. Next, intermediate 7 was formed by the reaction of compound 6 and 2-nitroacetaldoxime, which was prepared beforehand from nitromethane in the presence of NaOH. Compound 7 was cyclized with acetic anhydride to yield compound 8, which was subsequently treated with phosphorus oxychloride to afford compound 9. Compound 9 was further reacted with the corresponding aniline, affording compounds 10a–10m. Then, final compounds 11a–11m were achieved upon the substitution of compound 10a–10m with excessive 4-methyl-piperidine, respectively. All target compounds were purified by column chromatography and characterized by 1H NMR, 13C NMR, and HR-ESI-MS.

2.2. Biological evaluation

2.2.1. AChE and BChE inhibition assay

All synthesized quinoline derivatives (11a–11m) were evaluated for their ChE inhibitory activities on electric eel acetylcholinesterase (etAChE) and equine serum butyrylcholinesterase (eqBChE), by the Ellman’s method with galanthamine as the reference drug. The results showed that most 4-N-phenylaminoquinoline derivatives have inhibitory effects on AChE. Compared with compounds 11k and 11l, compounds 11e, 11f and 11g had relatively weaker inhibitory activities. It seems that AChE inhibitory activities can be enhanced when there is electron-donating group (such as hydroxyl) in 4-N-substituted aniline ring, and that relative activities are reduced when electron-withdrawing group is present (such as chlorine atom).

As shown in Table 1, what exhibited more potent on AChE than galanthamine were compound 11j with a para methoxy group (IC50 = 1.20 μM) and compound 11k with a meta hydroxyl group (IC50 = 1.23 μM). According to the different substituents, the order of the inhibitory potency of these derivatives bearing different o-substituted groups was hydrogen atom > chloro group > methoxy group > methyl group; the order with different m-substituted groups was as followed: hydroxyl group > hydrogen atom > chloro group > methyl group > methoxy group > trifluoromethyl group; and the order with different p-substituted groups was: methoxy group > hydrogen atom > hydroxyl group > methyl group > chloro group. On the other hand, based on the substituted position in the 4-N-substituted aniline ring, the order of inhibitory potency against AChE was: Meta > Ortho > Para, but it turned to be: Para > Meta > Ortho after methoxy substitution. Therefore, their anti-AChE activities directly depended on the electronic properties and position of different substituents on the aniline ring.

In terms of inhibitory activity against eqBChE, although the activities of compounds 11a–11g, 11m were weak, compounds 11j, 11k, 11l showed considerable activities. And compared with compound 11a, compounds with hydroxyl or methoxy group (i.e., compounds 11h–11l) were more favorable to the inhibitory activity. Interestingly, the position of methoxy substitution on phenyl ring has an essential effect on inhibiting BChE. When the methoxy group was shifted from 2-position or 3-position to 4-position of the phenyl ring, almost a 3-fold improvement in BChE inhibition was observed, and the obtained compound 11j...
The ability of the synthesized compounds in metal chelation would be an added advantage in the treatment of AD. Herein, compounds 11a,
11j, 11k and 11l were selected for their chelating abilities toward Cu\(^{2+}\), Fe\(^{2+}\), Al\(^{3+}\) and Zn\(^{2+}\), using UV spectrophotometer with wavelength ranging from 200 nm to 600 nm. As is seen in Fig. 5, the spectrum of compound 11l changed significantly by adding CuCl\(_2\). The maximum absorption at 308 nm shifted to 282 nm, and the blue shift was 26 nm, which indicated the formation of 11l-Cu\(^{2+}\) complex. In addition, the absorption decreased significantly when AlCl\(_3\), FeCl\(_2\), and ZnCl\(_2\) were added, indicating a possible interaction between the compound 11l and these biometals. However, no remarkable shifts were observed upon the addition of AlCl\(_3\), FeCl\(_2\) or ZnCl\(_2\). Similarly, the dramatic decreases in absorbance indicated a possible interaction between these biometals and compounds 11a, 11j and 11k, which suggested that all compounds 11a, 11j and 11k are also able to chelate biometals. The chelating ability of compound 11l towards Cu\(^{2+}\) could be due to the donation of lone pair of electron on the nitrogen atom present in 4-N-substituted anilinering, which might be easily stabilised by resonance through the phenol-quinone tautomerization, and strongly interfere in conjugated system between 4-N-substituted aniline ring and quinoline ring leading to a relative blue shift.

To further determine the stoichiometry of 11l-Cu\(^{2+}\) complex, molar ratio method was used by preparing the methanol solutions of compound 11l with increasing amounts of CuCl\(_2\). The UV spectra were used to obtain the absorbance of the 11l complex and different concentrations of CuCl\(_2\) at 282 nm (Fig. 6). Accordingly, the absorbance linearly decreased initially and then plateaued. The two straight lines intersected at a mole fraction of 0.50, revealing a 2:1 stoichiometry for 11l-Cu\(^{2+}\) complex.

### 2.3. Docking studies

In order to explore the possible interaction mode in the active sites of AChE, the most active compound 11j was selected for molecular modeling research using CDOCKER in Discovery Studio 3.0 software. The crystal complex of AChE with galanthamine (PDB: 1DX6) was selected for the docking research [38]. Fig. 7 showed that compound 11j interacted with the CAS site of AChE by the following moieties: the propylether fragment attached to quinolinenucleus showed one carbon hydrogen bond with His440; 4-N-phenylamine fragment created one \(\pi-\pi\) interaction with Phe331; 4-methyl-piperidine fragment of compound 11j formed one \(\pi\)-alkyl interaction and one carbon hydrogen bond concurrently with Trp84. It is worth mentioning that there are three amino acids of PAS site of AChE binding to compound 11j: Asp72 interacted with nitro group through one attractive charge, and with C-2 hydrogen through one carbon hydrogen bond; Tyr334 interacted with 4-N-phenylaminoquinoline skeleton through two \(\pi-\pi\) interactions; and Tyr121 interacted with the NH group via one conventional hydrogen bond. The binding mode suggests that compound 11j behaves as dual binding site AChE inhibitors, which is consistent with our kinetic analysis.

Noteworthy, nitrogen cation of the nitro group could form the attractive force with carboxyl anion of Asp72, an ion of opposite charge. And the strong electron-withdrawing ability of the nitro group could decrease the electron density of quinoline ring, and induces face-to-face \(\pi-\pi\) electron-donor-acceptor interaction with the aromatic amino acid residue (Try334) of AChE. Furthermore, other relative reports also suggested its important role in the AChE inhibition [45,46]. Unexpectedly, it was found from recent literatures that the nitro substituent of aromatic compounds could cause surprisingly monoamine oxidase (MAO) inhibitory and \(N\)-methyl-D-aspartate (NMDA) antagonistic effects [47,48]. So, the presence of nitro group might represent a valuable pharmacophoric feature for the treatment of AD.

The interaction of compound 11j with BChE (PDB code: 4BDS) was also carried out. As seen in Fig. 8, compound 11j was bound to the residues Gly117 and Gly116 from the oxyanion hole (OAH), Ser198 from esteratic site (ES), Trp82 from anionic substrate binding site (AS), three PAS amino acid residues such as Asp70, Pro285 and Tyr332, but...
also bound to Leu286, Ser287, Trp231 and Thr120 residues, via two π-ion interactions, two conventional hydrogen bond, four carbon hydrogen bonds, two amide-π interactions, one π-sigma interaction, one van der Waals, and two hydrophobic interactions. Briefly, it is noteworthy to say that compound 11j interacted with important amino acid residues in the OAH, ES, AS and PAS active sites of BChE, which makes it worthy of further study.

3. Conclusion

In summary, a series of novel 4-N-phenylaminoquinoline derivatives were designed, synthesized and evaluated as multifunctional agents for the treatment of AD. Among them, compounds 11j, 11k and 11l had comparable inhibition activities to galantamine in both AChE and BChE. Especially, compound 11j demonstrated the most potent inhibition to eeAChE and eqBChE with IC50 values of 1.20 μM and 18.52 μM, respectively. The kinetic analysis inferred that compound 11j was reversible inhibitor, and showed a mixed type of inhibition on AChE. What’s more, through molecular docking studies, it was found that compound 11j interacted with CAS and PAS of both AChE and BChE. Meanwhile, compound 11k exhibited significant displacement of propidium iodide from the PAS of AChE (25.80%). More importantly, the DPPH radical scavenging activity of compound 11l reached 84% (at 1 mg/mL), and its EC50 value of compound 11l was 0.328 μM, which

Fig. 3. Left: Lineweaver-Burk plots for the inhibition of eeAChE by compounds 11a, 11j, 11k and 11l at different concentrations of substrate (ATCh). Right: Secondary plots for calculation of steady-state inhibition constants (K_i) of compounds 11a, 11j, 11k and 11l.
was almost as high level as that of Vc (EC₅₀ = 0.095 μM). Additionally, compounds 11a, 11j, 11k and 11l showed satisfactory metal-chelating properties toward Al³⁺, Fe²⁺, Cu²⁺ and Zn²⁺ ions. From our in vitro and in silico results, 4-aza-N-phenylaminoquinoline might be a multifunctional potential scaffold for the treatment of AD, and compounds 11j, 11k and 11l could provide a starting point for further developments of new therapeutic agents.

4. Experimental section

4.1. Chemistry

The chemical reactions were monitored by TLC using commercially available alumina plates coated with silica gel 60 F254 (Merck). Chromatographic separation was performed on self-packed columns with silica gel from Qingdao Haiyang Chemical Group Co., Ltd. (PR China), and MPLC was carried out on a BUCHI apparatus equipped with a C-605 pump. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance III 400 spectrometer with TMS as an internal standard. Coupling constant (J) values were presented in Hz, and spin multiplicities were given as s (singlet), d (doublet), t (triplet), q (quartet), brs (broad) and m (multiplet). The purity of all compounds for biological evaluation was confirmed to be higher than 95% by analytical HPLC performed on Shimadzu SPD-M20A (Column: WondaSil C18, 5 μm particle size, 4.6 mm × 250 mm, S/N: 3k9701-01; mobile phase: methanol/H₂O (30/70–100/0); flow rate = 1 mL/min; λ = 308 nm). High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired on a Bruker microTOF-Q II spectrometer.

Compounds 2–9, 10a–10m were synthesized as our early reported procedures [16,35].

4.1.1. Synthesis of 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-phenylquinolin-4-amine derivatives (11a–11m)

A mixture of 6-Methoxy-7-(3-chloropropoxy)-3-nitro-N-phenylquinolin-4-amine (10a–10m) (0.59 mmol), NaI (0.098 g, 0.65 mmol), K₂CO₃ (0.013 g, 1.77 mmol) was refluxed in CH₃CN (45 mL) for 1 h, then added 4-methyl-piperidine (0.71 mmol) refluxed for 24 h. Excess CH₃CN was rotated off, and the residue was partitioned between CH₂Cl₂ and H₂O and filtered through Celite. The organic portion was worked up to give a solid which was chromatographed on silica gel. CH₂Cl₂/MeOH (9:2) eluted products 11a–11m.

4.1.2. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-phenylquinolin-4-amine (11a)

Yield: 46.78%, yellow solid, mp: 177.8–180.3°C. Purity 95.65% by HPLC. ¹H NMR (400 MHz, CDCl₃) δ: 0.93 (d, 3H, J = 6.3 Hz, CH₃), 1.26 (m, 2H, H-3″ax and H-5″ax), 1.35 (m, 1H, CH), 1.63 (d, 2H, J = 12.4 Hz, H-3″ eq and H-5″ eq), 1.95 (t, 2H, J = 11.4 Hz, H-2″ax and H-6″ax), 2.10 (m, 2H, CH₂CH₂CH₂), 2.52 (t, 2H, J = 7.1 Hz, NCH₂), 2.91 (d, 2H, J = 11.4 Hz, H-2″ eq and H-6″ eq), 3.34 (s, 3H, OCH₃), 4.23 (t, 2H, J = 6.6 Hz, OCH₂), 6.88 (s, 1H, H-5), 7.19 (d, 2H, J = 7.7 Hz, H-2′, H-6′), 7.25 (t, 1H, J = 7.7 Hz, H-4′), 7.35 (s, 1H, H-8), 7.41 (t, 2H, J = 7.7 Hz, H-3′, H-5′), 9.36 (s, 1H, NH), 13.43 (s, 1H, OCH₃). ¹³C NMR (100 MHz, CDCl₃) δ: 153.7, 148.3, 148.1, 145.2, 145.0, 141.2, 129.7 (2C), 128.5, 126.0, 124.0 (2C), 112.6, 109.9, 106.4, 67.8, 55.3, 55.2.

![Fig. 3. (continued)](image-url)

Table 2

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Propidium iodide displacement (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>9.73 ± 0.62</td>
</tr>
<tr>
<td>11j</td>
<td>14.85 ± 0.59</td>
</tr>
<tr>
<td>11k</td>
<td>25.80 ± 1.37</td>
</tr>
<tr>
<td>11l</td>
<td>13.78 ± 0.96</td>
</tr>
<tr>
<td>Donepezil</td>
<td>18.50 ± 1.13</td>
</tr>
</tbody>
</table>

Table 2

Propidium iodide displacement (eeAChE) assay.

a All values are expressed as the mean ± SD (n = 3).

Fig. 4. Free radical scavenging activities of compounds 11a, 11j, 11k, 11l and Vc.

![Fig. 4](image-url)
4.1.3. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(o-tolyl)quinolin-4-amine (11b)

Yield: 43.36%, yellow solid, mp: 166.6–168.9 °C. Purity 99.85% by HPLC. $^1$H NMR (400 MHz, CDCl$_3$) δ: 0.92 (d, 3H, J = 6.3 Hz, CH$_3$), 1.25 (m, 2H, H-3″ax and H-5″ax), 1.35 (m, 1H, CH), 1.62 (d, 2H, $J$ = 12.6 Hz, H-3″eq and H-5″eq), 1.93 (t, 2H, $J$ = 11.4 Hz, H-2″ax and H-6″ax), 2.08 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.35 (s, 3H, ArCH$_3$), 2.50 (t, 2H, $J$ = 7.0 Hz, NCH$_2$), 2.89 (d, 2H, $J$ = 11.4 Hz, H-2″eq and H-6″eq), 3.27 (s, 3H, OCH$_3$), 4.21 (t, 2H, $J$ = 6.6 Hz, OCH$_2$), 6.77 (s, 1H, H-5), 7.10 (m, 1H, ArH), 7.23 (m, 2H, ArH), 7.33 (s, 1H, H-8), 7.37 (m, 1H, ArH), 9.36 (s, 1H, H-2), 10.52 (s, 1H, NH). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 153.5, 148.3, 147.8, 146.1, 145.6, 139.6, 133.5, 134.1, 127.3, 127.2, 127.1, 125.7, 112.4, 110.0, 105.5, 67.8, 55.3, 55.1, 54.0 (2C), 34.3 (2C), 30.8, 26.5, 21.9, 18.3. HRMS (ESI) calculated for (C$_{25}$H$_{30}$N$_4$O$_4$+H$^+$) 451.2345, found 451.2351.

4.1.4. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(m-tolyl)quinolin-4-amine (11c)

Yield: 48.29%, yellow solid, mp: 120.0–122.3 °C. Purity 96.39% by HPLC. $^1$H NMR (400 MHz, CDCl$_3$) δ: 0.94 (d, 3H, J = 5.9 Hz, CH$_3$), 1.37 (m, 2H, H-3″ax and H-5″ax), 1.42 (m, 1H, CH), 1.67 (d, 2H, $J$ = 11.3 Hz, H-3″eq and H-5″eq), 2.07 (t, 2H, $J$ = 11.5 Hz, H-2″eq and H-6″eq), 2.16 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.34 (s, 3H, ArCH$_3$), 2.62 (t, 2H, $J$ = 7.6 Hz, NCH$_2$), 3.00 (d, 2H, $J$ = 11.5 Hz, H-2″eq and H-6″eq), 3.34 (s, 3H, OCH$_3$), 4.22 (t, 2H, $J$ = 6.5 Hz, 2H, OCH$_2$), 6.90 (s, 1H, H-5), 6.98 (d, 2H, $J$ = 8.0 Hz, 1H, H-4′), 7.01 (s, 1H, H-2′), 7.06 (d, 1H, $J$ = 7.5 Hz, H-6′), 7.28 (t, 1H, $J$ = 7.7 Hz, H-5′), 7.32 (s, 1H, H-8), 9.32 (s, 1H, H-2′), 10.42 (s, 1H, NH). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 153.3, 148.2, 147.9, 145.3, 141.0, 139.9, 129.5, 126.9, 124.7, 121.2, 112.9, 110.0, 106.6, 67.3, 55.2, 55.1, 53.6 (2C), 33.1 (2C), 31.8, 25.6, 21.4, 21.3. HRMS (ESI) calculated for (C$_{26}$H$_{32}$N$_4$O$_4$+H$^+$) 465.2502, found 465.2516.

4.1.5. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(p-tolyl)quinolin-4-amine (11d)

Yield: 46.47%, yellow solid, mp: 187.3–190.1 °C. Purity 98.91% by HPLC. $^1$H NMR (400 MHz, CDCl$_3$) δ: 0.95 (d, 3H, J = 5.5 Hz, CH$_3$), 1.40 (m, 2H, H-3″ax and H-5″ax), 1.41 (m, 1H, CH), 1.67 (d, 2H, $J$ = 10.3 Hz, H-3″eq and H-5″eq), 2.10 (t, 2H, $J$ = 11.1 Hz, H-2″eq and H-6″eq), 2.18 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.36 (s, 3H, ArCH$_3$), 2.65 (t, 2H, $J$ = 7.6 Hz, NCH$_2$), 3.03 (d, 2H, $J$ = 11.1 Hz, H-2″eq and H-6″eq), 3.32 (s, 3H, OCH$_3$), 4.20 (t, 2H, $J$ = 6.5 Hz, OCH$_2$), 6.73 (s, 1H, H-5), 7.00 (s, 1H, H-2′), 7.07 (d, 1H, $J$ = 7.5 Hz, H-6′), 7.30 (m, 1H, ArH), 7.32 (s, 1H, H-8), 9.33 (s, 1H, H-2′), 10.43 (s, 1H, NH). $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 153.3, 148.8, 147.9, 145.3, 141.0, 139.9, 129.5, 126.9, 124.7, 121.2, 112.9, 110.0, 106.6, 67.3, 55.2, 55.1, 53.6 (2C), 33.1 (2C), 31.8, 25.6, 21.4, 21.3. HRMS (ESI) calculated for (C$_{26}$H$_{32}$N$_4$O$_4$+H$^+$) 465.2502, found 465.2510.
4.1.6. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(2-chlorophenyl)quinolin-4-amine (11e)

Yield: 44.97%, yellow solid, mp: 135.4–137.7 °C. Purity 98.85% by HPLC. $^1$H NMR (400 MHz, CDCl$_3$) δ: 0.92 (d, 3H, J = 6.4 Hz, CH$_3$), 1.25 (m, 2H, H-3′″ax and H-5′″ax), 1.35 (m, 1H, CH), 1.62 (d, 2H, J = 12.1 Hz, H-3″$_{ax}$ and H-5″$_{ax}$), 1.93 (t, 2H, J = 11.4 Hz, H-2″$_{ax}$ and 6″$_{ax}$), 2.10 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.51 (t, 2H, J = 7.1 Hz, NCH$_2$), 2.90

Fig. 7. 3D binding mode of compound 11j with AChE (PDB code: 1DX6), highlighting the protein residues that participate in the main interactions with the inhibitor.

Fig. 8. 3D binding mode of compound 11j with BChE (PDB code: 4BDS), highlighting the protein residues that participate in the main interactions with the inhibitor.
11j, 9.23 (s, 1H, H-2), 10.27 (s, 1H, NH). 13C NMR (100 MHz, CDCl3) δ 153.5, 153.2, 148.2, 147.0, 145.1, 143.3, 143.3, 132.5, 127.8, 124.1, 121.3, 119.3, 114.8, 114.0, 112.8, 110.9, 108.6, 106.5, 65.7, 55.3, 55.2, 53.9 (2C), 33.7 (2C), 30.6, 26.0, 21.7. HRMS (ESI) calculated for (C25H23N4O4+H+) 481.2451, found 481.2475.

11k. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(3-hydroxylphenyl)quinolin-4-amine

Yield: 46.20%, yellow solid, mp: 84.6–87.2°C. Purity 97.90% by HPLC. 1H NMR (400 MHz, CDCl3) δ 0.93 (d, 3H, J = 6.3 Hz, CH3), 1.30 (m, 2H, H-3″′ ax and H-5″′ ax), 1.38 (m, 1H, CH), 1.63 (2H, J = 12.4 Hz, H-3″′ eq and H-5″′ eq), 2.03 (m, 2H, H-2″′ eq and 6″′ eq), 2.06 (m, 2H, CH2CH2CH2), 2.55 (t, 2H, J = 7.1 Hz, NCH3), 2.93 (d, 2H, J = 10.8 Hz, H-2″′ eq and H-6″′ eq), 3.39 (3H, OCH3), 4.06 (t, 2H, J = 6.0 Hz, OCH3), 6.38 (s, 1H, H-2′), 6.75 (d, 2H, J = 7.6 Hz, H-4′, H-6′), 6.91 (s, 1H, H-5), 7.10 (s, 1H, H-8), 7.27 (t, 1H, J = 7.6 Hz, H-5′), 9.23 (s, 1H, H-2), 10.27 (s, 1H, NH). 13C NMR (100 MHz, CDCl3) δ 158.5, 153.7, 148.2, 147.0, 145.2, 144.8, 143.1, 130.9, 128.3, 113.4, 114.0, 112.8, 110.9, 108.6, 106.5, 65.7, 55.3, 55.2, 53.9 (2C), 33.7 (2C), 30.6, 26.0, 21.7. HRMS (ESI) calculated for (C25H23N4O4+H+) 467.2294, found 467.2294.

11l. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(4-hydroxybenzyloxy)quinolin-4-amine

Yield: 47.12%, brown solid, mp: 124.8–126.3°C. Purity 96.70% by HPLC. 1H NMR (400 MHz, CDCl3) δ 0.94 (d, 3H, J = 5.2 Hz, CH3), 1.35 (m, 2H, H-3″′ ax and H-5″′ ax), 1.43 (m, 1H, CH), 1.68 (2H, J = 11.4 Hz, H-3″′ eq and H-5″′ eq), 2.11 (m, 2H, CH2CH2CH2), 2.09 (m, 2H, H-2″′ eq and 6″′ eq), 2.59 (t, 2H, J = 10.0 Hz, H-2″′ eq and H-6″′ eq), 3.29 (3H, OCH3), 4.11 (t-lke, 2H, OCH2), 6.87 (d, 2H, J = 7.4 Hz, H-2′, H-6′), 6.97 (s, 1H, H-5), 7.09 (d, 2H, J = 7.4 Hz, H-2′, H-5′), 7.25 (s, 1H, H-9), 8.31 (s, 1H, H-2′), 10.65 (s, 1H, NH); 13C NMR (100 MHz, DMSO-d6) δ 155.1, 152.5, 148.2, 146.5, 144.8, 143.3, 132.5, 127.8, 124.1 (2C), 115.8 (2C), 113.4, 106.9, 104.5, 66.6, 55.4, 54.1, 52.8 (2C), 32.7, 29.5, 25.1, 21.5. HRMS (ESI) calculated for (C26H23N4O4+H+) 467.2294, found 467.2299.

11m. 6-Methoxy-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitro-N-(3-trifluoromethyl)phenyl)quinolin-4-amine

Yield: 56.79%, brown solid, mp: 136.0–139.3°C. Purity 97.97% by HPLC. 1H NMR (400 MHz, CDCl3) δ 1.05 (d, 3H, J = 6.2 Hz, CH3), 1.73–2.07 (m, 5H, H-3″′, H-4″′ and H-5″′ of piperidyl moiety), 2.60 (brs, 2H, CH2CH2CH2), 2.90 (brs, 2H, H-3″′ ax and 6″′ ax), 3.53 (t-lke, 2H, NCH2), 3.38 (3H, OCH3), 3.68 (brs, 2H, H-3″′ eq and H-6″′ eq), 4.29 (t-lke, 2H, OCH2), 6.77 (s, 1H, H-8), 7.29 (s, 1H, H-8), 7.31 (d, 1H, J = 7.7 Hz, H-4′), 7.39 (s, 1H, H-2′), 7.46 (d, 1H, J = 7.7 Hz, H-6′), 7.52 (t, 1H, J = 7.7 Hz, H-5′), 9.30 (s, 1H, H-2), 10.20 (s, 1H, NH); 13C NMR (100 MHz, CDCl3) δ 153.1, 148.7, 145.0, 143.5, 141.8, 132.1 (q, J = 32.9 Hz), 130.3 (2C), 129.6, 126.1, 123.5 (q, J = 270.9 Hz), 121.9 (q, J = 4.2 Hz), 119.4 (q, J = 4.1 Hz), 113.4, 110.5, 105.8, 66.3, 55.3,
55.2, 53.4 (2C), 30.8 (2C), 29.0, 23.7, 21.1. HRMS (ESI) calculated for (C_{26}H_{29}F_{3}N_{4}O_{4} + H^+) 519.2219, found 519.2224.

4.2. Biological evaluation

4.2.1. AChE and BChE inhibition assay

AChE and BChE inhibitory activities of compounds were determined by using modified Ellman’s method [49]. AChE (EC 3.1.1.7, from electric eel), BChE (EC 3.1.1.8, from equine serum), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), and butyrylthiocholine iodide (BTCl) were purchased from Sigma-Aldrich. Compounds were dissolved in DMSO (10 mM). Reaction mixture contained 140 μL of 100 mM sodium phosphate buffer (pH 8.0), the enzyme solution (either 0.05 U/mL of AChE, 20 μL; or 0.05 U/mL of BChE, 20 μL), and test compound (20 μL). Assayed solutions of test compounds were pre-incubated with corresponding ChE at 25 °C for 15 min. The reaction was initiated by addition of 10 μL of 10 mM DTNB and 10 μL of 7.5 mM substrate (ATCI or BTCl). The activity was determined by measuring the increase in absorbance at 412 nm at 37 °C in 10 min intervals using microplate reader (BioTek Epoch). The percentage inhibition was calculated from the measured data as follows: (Ac − Ai)/Ac × 100%, where Ai and Ac represent the change in the absorbance in the presence of inhibitor and without inhibitor, respectively.

4.2.2. In vitro propidium iodide displacement assay

150 μL of a 20 μM solution of the test compound or standard donepezil (from Sigma) was incubated with 5 units of eAChE at 25 °C for 6 h in the 96-well plate. After incubation, 50 μL of 1 μM propidium iodide solution was added to make the final assay volume of 200 μL. After 10 min, the fluorescence intensity was observed at an excitation wavelength (λex) of 535 nm and an emission wavelength (λem) of 595 nm using a fluorescence plate reader. The percentage inhibition was calculated by following expression: 100 − (IFi/IF0 × 100), where IFi and IF0 are the fluorescence intensities with and without the test compounds, respectively. Each assay was repeated at three different times [50,51].

4.2.3. Enzyme kinetic analysis against AChE

In order to investigate the reversibility of the compounds (11a, 11j, 11k, 11l) against AChE, the absorbance was measured at 412 nm after two minutes of incubation at 37 °C for different concentrations of the compounds (0.0–2.0 μM) and five different concentrations of AChE (0.025–0.10 U/mL). The resulting values were measured in triplicate for the velocity and enzyme concentration curves. The velocity (V) was calculated from the measured data as follows: V = (Ac − Ai)/2, where Ai and Ac represent the change in the absorbance in the presence of AChE and without AChE, respectively. All data were analyzed by OriginPro 8.

For estimates of the inhibition model and inhibition constant Kᵢ, the rate of enzymatic reaction was obtained with different concentrations of compounds (0.0–2.0 μM) and at least five different concentrations of ATCl (3.75–15.00 mM). For each experiment, reaction was started by adding substrate and progress curves were recorded at 412 nm after two minutes of incubation at 37 °C. Next, double reciprocal plots (1/v vs 1/ [s]) were made using the slopes of progress curves to obtain the type of inhibition. Slopes of these reciprocal plots were then drawn against the concentration of a compound in a related analysis, and Kᵢ was determined as the intercept on the negative x axis [52]. All rate measurements were performed in triplicate and data analysis was performed with OriginPro 8.0.

4.2.4. DPPH radical scavenging activity

The antioxidant activity was determined by the DPPH radical scavenging assay. The mixture was incubated for 30 min in the dark. The absorbance of the resulting solution was measured at 517 nm. Different concentrations (0.03125–1 mg/mL) of the extract were tested. The control sample was a mixture of methanol and DPPH, and Vc was used as reference. Percentage of DPPH radical scavenging activity was obtained as (1 − As/Ac) × 100. As and Ac represent the absorbance of the sample and control, respectively [44], and then EC50 (effective concentration of a compound that decreases the initial concentration of DPPH by 50%) can be calculated.

4.2.5. Metal-chelating properties

All solutions used in metal-chelating study were prepared in methanol, and Fe²⁺, Cu²⁺, Al³⁺ and Zn²⁺ solutions were prepared from FeCl₂, CuCl₂, AlCl₃ and ZnCl₂ respectively. To study the metal binding ability, a mixture of test compound (1 mL) and metal solution (1 mL) with the same concentration (20 μM) in a 1 cm quartz cuvette was incubated at room temperature for 30 min. Then, the absorption spectra were recorded with wavelength ranging from 200 to 600 nm [43]. The stoichiometry of complex 11l-Cu²⁺ was also studied using the molar ratio method [4,53]. The concentration of tested compound 11l was 20 μM and the final concentration of Cu²⁺ ranged from 0 to 60 μM at 282 nm. The plot was obtained by the corresponding absorption versus mole fraction of Cu²⁺.

4.3. Docking study

4.3.1. Molecular docking studies on AChE

The binding modes were generated by using the Discovery Studio CDOCKER software (Accelrys, San Diego, USA). The crystal structure of AChE from Torpedo californica (TcAChE; Code ID: 1DX6) was obtained from the Protein Data Bank. The binding pattern of galanthamine in TcAChE is similar to that observed in human recombinant AChE (rhAChE) [54], hence TcAChE was selected for docking studies [42,55]. TcAChE was prepared for receptor protein by a sequence of operations including Clean Protein, Hydrogen Add and Apply Forcefield. All ligands were performed using the default settings, and docked in all possible stereoisomeric forms in an active site located sphere with 10 Å radius for TcAChE. Then receptor-ligand interactions were operated by the CDOCKER protocol with the default parameters. By using the DS CDOCKER program, the crystallographic binding mode of galanthamine was reproduced into the TcAChE binding site: the best ranked solution presented a root-mean-square deviations (RMSD) value of 0.50 Å with respect to the experimental pose, indicating that the docking methods and parameters used in this study were approximate for the AChE system.

4.3.2. Molecular docking studies on BChE

Flexible docking was conducted using Discovery Studio 2017 R2 (Accelrys, San Diego, USA). The crystal structure of BChE from Homo sapiens (Code ID: 4BDS) was extracted from the Protein Data Bank. And all water molecules and co-crystallized ligand were removed, which was followed by protein preparation protocol with CHARMM force field. The prepared ligands were subjected to minimization also with CHARMM force field before being used for docking analyses. The docking results were analyzed using Discovery Studio Visualizer 17.2.0.16349. The docking parameters were first validated by re-docking of co-crystallized ligand into the active site of the enzyme. The re-docked tacrine was found to bind in a similar manner as its respective crystallographic conformation, and the RMSD value for tacrine conformation is 0.41 Å, indicating that the selected docking parameters are acceptable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Acknowledgement

This research was supported by the Training Project of Innovation Team of Colleges and Universities in Tianjin (TD13-5020).

References


