SUPPORTING INFORMATION

Pd-catalyzed direct modification of anti-Alzheimer's disease drug: Synthesis and biological evaluation of \( \alpha \)-arylDonepezil analogues

Lin-Xi Wan\textsuperscript{a,b}, Shi-Xing Miao\textsuperscript{a}, Zhen-Xiang He\textsuperscript{a}, Xiaohuan Li\textsuperscript{b}, Xian-Li Zhou\textsuperscript{a,*}, and Feng Gao\textsuperscript{a,*}

\textsuperscript{a} School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, PR China
\textsuperscript{b} Key Laboratory of Advanced Technology of Materials, Ministry of Education, Southwest Jiaotong University, Chengdu 610031, PR China

* Corresponding Author
Xian-Li Zhou, Email: zhouxl@swjtu.edu.cn
Feng Gao, Email: gaof@swjtu.edu.cn
1. General Methods

All reactions were conducted under an inert atmosphere of dry argon. Anhydrous 2-MeTHF was purchased from Aladdin and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (GF 254) using UV light to visualize the course of the reactions. Silica gel H (Qingdao Sea Chemical Factory, Qingdao, PR China) was used for column chromatography. NMR spectra were obtained using a Brüker 400 MHz Fourier-transform NMR spectrometer at Chengdu Institute of Biology, Chinese Academy of Sciences NMR facility. High resolution mass spectrometry (HRMS) data were obtained on a Waters LC-TOF mass spectrometer (model LCT-XE Premier) using chemical ionization (CI) or electrospray ionization (ESI) in positive or negative mode, depending on the analyze. Chemical shifts (δ) are reported in ppm with TMS as internal standard. Abbreviations for signal couplings are: s, singlet; d, doublet; t, triplet; m, multiplet. Bromides were purchased from Aladdin and used as received.

2. General procedure for Pd-catalyzed arylation of donepezil

The suspension of Pd(OAc)$_2$ (5 mol%), Butyldi-1-adamantylphosphine (10 mol %) in anhydrous 2-MeTHF (3.0 mL) was stirred at 30°C under an argon atmosphere for 2 hour to be a light pink solution. Then the light pink solution was added to a 10 mL sealed dry reaction vial contained Donepezil (1.0 mmol), naphthalenyl, pyridinyl or quinolinyl bromides (1.5 mmol) and NaHMDS (2.0 mmol) via syringe. The reaction mixture was stirred for 24 hours at 80°C before quenching with two drops of H$_2$O. The mixture was then diluted with ethyl acetate (3 mL), and filtered over a pad of MgSO$_4$. The pad was rinsed with additional ethyl acetate, and the solution was concentrated in vacuo. The crude residue was loaded onto a silica gel column and purified by flash chromatography, using Petroleum ether-EtOAc (2:1 to 1:2) as eluent.

3. Biological Materials and methods

3.1 Cholinesterases inhibition assay

AChE from Electrophorus electricus (electric eel), 5,5'-dithiobis-2-nitrobenzoic
acid (Ellman’s reagent, DTNB), and acetylthiocholine chloride (ATC) were purchased from Macklin. BuChE from Equine serum, butylthiocholine chloride (BTC) were purchased from Sigma Aldrich. The inhibitory activity of test compounds 1-15 against AChE/BuChE was assessed by Ellman’s method. Donepezil and the synthesized compounds were dissolved in DMSO to 100 μM or 50 μM. All the assays were under 0.1 M NaH₂PO₄/Na₂HPO₄ buffer (pH 6.7), using a spectraMax absorbance reader Instrument. Enzyme solutions were prepared by 2.5 mg (0.5U/mL) AChE in pH 8.0 buffer (1 mL). The assay medium contained phosphate buffer (pH 6.7, 140 μL), Donepezil derivatives (I=100 μM or 50 μM, 10μL), 0.5U/mL of enzyme (10 μL). After 20 min of incubation time, DTNB (0.75 mM, 10μL) and acetylthiocholine chloride (1.5 mM, 10μL) were added for incubation for another 20 min. The inhibitory activity was determined by measuring the increase in absorbance at 405 nm. Each concentration was assayed in triplicate. Enzyme Inhibitory activity (%) = [1– (A sample-blank/ A control-blank)] × 100%.

3.2 In vitro cytotoxicity assay against SH-SY5Y cell line

MTT (M2128) was purchased from Sigma-Aldrich (St. Louis, Mo, USA). The SH-SY5Y cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in DMEM with 10% fetal bovine serum and incubated with 5% CO₂. SH-SY5Y cells were seeded at a density of 5×10³/well in a 96-well plate for 12 hours, and then treated with different tested compounds(100μL, 50 μM/L) dissolved in 100% DMSO for 24 hours, with the final DMSO concentrations lower than 0.1%. Control cells were treated with Donepezil containing 0.1% DMSO. DMSO served as a negative control. Then, 120 μL MTT (5 mg/mL) were added into each well and incubated for another 4 hours. Finelly, 150μL DMSO was added and the absorbance was detected at 492 nm by a microplate reader (Thermo MK3, USA).

3.3 H₂O₂-induced cell death in SH-SY5Y cells

The neuroprotective activity was assessed using the MTT assay. The human SH-SY5Y neuroblastoma cells were cultured at 37 °C in a humidified atmosphere of
5% CO₂, in Gibco™ Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum. Briefly, SH-SY5Y cells were seeded at a density of 1×10⁴/well in 96-well plates for 12 hours, then pre-treated with 50 μM/L tested compounds. After 24 h of incubation, hydrogen peroxide solution (100μL, 500 μM/L) was added into cells, and incubated for 4 h. Then MTT (120μL, 5 mg/mL) was added into each well. After a 4 h treatment, the supernatant was removed, and DMSO (150 μL/well) was added to dissolve the insoluble formazan crystals. Then, the plate was vibrated, and the absorbance was measured at 492 nm using a microplate reader.
Figure S1 $^1$H NMR (400 MHz) spectrum of compound 1 in Acetone-$d_6$

Figure S2 $^{13}$C NMR (100 MHz) spectrum of compound 1 in CDCl$_3$
Figure S3 $^1$H NMR (400 MHz) spectrum of compound 2 in Acetone-$d_6$.

Figure S4 $^{13}$C NMR (100 MHz) spectrum of compound 2 in CDCl$_3$. 
Figure S5 $^1$H NMR (400 MHz) spectrum of compound 3 in Acetone-$d_6$.

Figure S6 $^{13}$C NMR (150 MHz) spectrum of compound 3 in CDCl$_3$. 
**Figure S7** $^1$H NMR (600 MHz) spectrum of compound 4 in CDCl$_3$.

**Figure S8** $^{13}$C NMR (150 MHz) spectrum of compound 4 in CDCl$_3$. 
Figure S9 $^1$H NMR (400 MHz) spectrum of compound 5 in Acetone-$d_6$.

Figure S10 $^{13}$C NMR (100 MHz) spectrum of compound 5 in CDCl$_3$. 
Figure S11 $^{19}$F NMR (565 MHz) spectrum of compound 5 in CDCl$_3$

Figure S12 $^1$H NMR (400 MHz) spectrum of compound 6 in Acetone-$d_6$
Figure S13 $^{13}$C NMR (100 MHz) spectrum of compound 6 in CDCl$_3$

Figure S14 $^1$H NMR (400 MHz) spectrum of compound 7 in Acetone-$d_6$
Figure S15 $^{13}$C NMR (100 MHz) spectrum of compound 7 in CDCl$_3$

Figure S16 $^{19}$F NMR (565 MHz) spectrum of compound 7 in CDCl$_3$
Figure S17 $^1$H NMR (400 MHz) spectrum of compound 8 in Acetone-$d_6$

Figure S18 $^{13}$C NMR (100 MHz) spectrum of compound 8 in CDCl$_3$
Figure S19 $^{19}$F NMR (565 MHz) spectrum of compound 8 in CDCl$_3$.

Figure S20 $^1$H NMR (600 MHz) spectrum of compound 9 in CDCl$_3$. 
Figure S21 $^{13}$C NMR (100 MHz) spectrum of compound 9 in CDCl$_3$.

Figure S22 $^1$H NMR (400 MHz) spectrum of compound 10 in Acetone-$d_6$. 
Figure S23 $^{13}$C NMR (150 MHz) spectrum of compound 10 in CDCl$_3$

Figure S24 $^1$H NMR (600 MHz) spectrum of compound 11 in CDCl$_3$
Figure S25 $^{13}$C NMR (150 MHz) spectrum of compound 11 in CDCl$_3$

Figure S26 HMBC spectrum of compound 11 in CDCl$_3$
Figure S27 HMQC spectrum of compound 11 in CDCl₃

Figure S28 ¹H NMR (400 MHz) spectrum of compound 12 in Acetone-d₆
Figure S29 $^{13}$C NMR (100 MHz) spectrum of compound 12 in CDCl$_3$

Figure S30 $^1$H NMR (600 MHz) spectrum of compound 13 in CDCl$_3$
Figure S31 $^{13}$C NMR (150 MHz) spectrum of compound 13 in CDCl$_3$

Figure S32 $^1$H NMR (600 MHz) spectrum of compound 14 in CDCl$_3$
Figure S33 $^{13}$C NMR (150 MHz) spectrum of compound 14 in CDCl$_3$.

Figure S34 $^1$H NMR (600 MHz) spectrum of compound 15 in CDCl$_3$.
Figure S35 $^{13}$C NMR (150 MHz) spectrum of compound 15 in CDCl$_3$
Table S1. $^1$H NMR data and $^{13}$C of compounds 11 (600 MHz, CDCl$_3$, $\delta$ in ppm, $J$ in Hz)

| No. | $^1$H NMR (ppm) | $^{13}$C NMR (ppm) |
|-----|----------------|-------------------|
| 1   |                | 149.6             |
| 2   |                | 156.0             |
| 3   | 6.95-6.89      | 107.5             |
| 4   |                | 149.5             |
| 5   |                | 128.5             |
| 6   | 7.13           | 105.0             |
| 7   |                |                   |
| 8   |                |                   |
| 9   | 3.13, 4.40     | 37.7              |
| 10  |                | 60.3              |
| 11  |                | 206.7             |
| 12  |                |                   |
| 13  | 2.45, 2.25-2.15| 45.6              |
| 14  | 1.93           | 33.8              |
| 15  | 1.84-1.68      | 33.4              |
| 16  | 2.73           | 53.9              |
| 17  |                |                   |
| 18  | 2.45           | 53.9              |
| 19  | 1.84-1.68      | 33.4              |
| 20  | 3.45-3.37      | 63.6              |
| 21  |                | 128.3             |
| 22  | 7.26           | 129.5             |
| 23  | 7.26           | 128.3             |
| 24  | 7.26           | 127.1             |
| 25  | 7.26           | 128.3             |
| 26  | 7.26           | 129.5             |
| 27  | 3.88           | 56.4              |
| 28  | 3.98           | 56.3              |
| 29  |                | 160               |
| 30  |                |                   |
| 31  |                | 157.3             |
| 32  | 6.95-6.89      | 121.2             |
| 33  | 7.47           | 136.6             |
| 34  | 7.47           | 119.0             |
| 35  | 2.45           | 24.8              |