Supporting Information

Structural Optimization and Biological Evaluation of Substituted Bisphenol A Derivatives as β-Amyloid Peptide Aggregation Inhibitors

Yu Zhou,[a,†] Chunyi Jiang,[b,†] Yaping Zhang,[c,†] Zhongjie Liang,[a] Wenfeng Liu,[a,d] Liefeng Wang,[e] Cheng Luo,[a] Tingting Zhong,[c] Yi Sun,[c] Linxiang Zhao,[d] Xin Xie,[f] Hualiang Jiang,[a] Naiming Zhou,[c,*] Dongxiang Liu[b,*] and Hong Liu[a,*]

[a]*The Center for Drug Discovery and Design;* [b]*Department of Molecular Pharmacology;* [f]*State key Laboratory of Drug Research, The National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China*

[c]*Institute of Biochemistry, College of Life Sciences, Zijingang Campus, Zhejiang University, Hangzhou, Zhejiang, 310058, China*

[d]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China*

[e]*Laboratory of Receptor-based Bio-medicine, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China*

[†]*These authors contributed equally to this work.*

CONTENT

Table S1. The HPLC analyses of all the target compounds (**1, 22-55**). The purity of identified compounds that was essential to the conclusions drawn in the text and determined by the same instrumentation with one system given in the following table. The peak purity was checked with UV spectra.

	Method		
Equipment	Agilent 1100 with binary pump, photodiode array detector (DAD), ESI and APCI mass spectrometer		
Column	Agilent Zorbax Eclipse XDB-C_{18} (4.6×150mm, 5 μm particle size)		
System condition	CH_3OH/H_2O, 85% (v/v) of CH_3OH gradient, flow rate: 1.0 ml/min, calculated the relative purity of each compound at 214 nm		
Results	**Compound No.**	**Retention Time (min)**	**Relative Purity (%)**
	1	5.004	98.09
	22	1.290	97.32
	23	3.992	95.49
	24	1.310	96.65
	25	3.585	98.97
	26	2.593	95.34
	27	1.316	100.00
	28	1.301	100.00
	29	6.585	97.07
	30	1.303	100.00
	31	1.274	99.64
	32	1.321	100.00
	33	1.314	100.00
	34	1.295	100.00

	35	1.316	100.00
	36	5.662	98.43
	37	1.298	100.00
	38	1.286	98.33
	39	1.311	99.63
	40	1.291	100.00
	41	1.301	100.00
	42	1.285	97.87
	43	1.306	100.00
	44	1.304	98.52
	45	1.989	96.16
	46	1.941	99.81
	47	1.280	100.00
	48	2.325	98.18
	49	1.295	100.00
	50	1.323	100.00
	51	1.311	100.00
	52	1.302	98.40
	53	3.003	100.00
	54	1.297	98.41
	55	1.297	100.00

γ-Secretase Fluorogenic Substrate Assay

Materials and methods

Reagents

The fluorogenic substrate, γ-secretase inhibitors and CHAPSO were purchased from Merck KGaA (Darmstadt, Germany). Cell culture medium and fetal bovine serum were bought from Invitrogen (Carlsbad, CA, USA). Tris base was purchased from Amresco (Solon, OH, USA). Complete™ protease inhibitor cocktail was purchased from Roche Applied Science (Basel, Switzerland). Other reagents and solvents used in the experiments were of analytical grade.

Cell culture and membrane protein preparation

HEK293T cells obtained from ATCC (Manassas, VA, USA) were maintained in high-glucose Dulbecco's modified Eagle's medium (HG-DMEM) supplemented with 10% fetal bovine serum, 100 mg/L penicillin and 100 mg/L streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Cells were trypsinized and seeded onto 10 cm plates at proper density for 48 h before harvesting. HEK 293T cells were washed with ice-cold PBS by centrifugation at 500 g for 5 min and the pellets were homogenized with a Dounce homogenizer (pestle B, 15 strokes) in lysis buffer contained 5 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM EGTA and Complete™ protease inhibitor cocktail. The homogenates were centrifuged at 800 g for 10 min to remove nuclei and large debris. The supernatants were centrifuged at 25,000 g for 1 h at 4°C and the membrane pellets were resuspended in reaction buffer containing 50 mM Tris-HCl (pH 6.8), 2 mM EDTA, 150 mM KCl and Complete™ protease inhibitor cocktail. After measuring the protein concentration, the membranes protein were diluted to 1 mg/ml in 50 mM Tris-HCl, pH 6.8, 2 mM EDTA, 150 mM KCl, 0.25% CHAPSO, flash-frozen in liquid N_2, and stored at –70 °C before use.

Fluorogenic substrate assay

10 μg membrane protein was added to each well of a 96-well plate with 6 μM

fluorogenic substrate. Compounds were added at the indicated concentrations and the final reaction system was adjusted to 100 μL/well with the reaction buffer. The plate was incubated at 37°C in a humidified atmosphere for 5h. Fluorescence was measured using a BIOTEK FLX800 microplate reader with the excitation wavelength at 355 nm and the emission wavelength at 440 nm. The γ-secretase inhibitor L685,458 was used as positive control. Data were analyzed with GraphPad Prism software (GraphPad, San Diego, CA, USA). Non-linear regression analyses were performed to generate dose-response curves and calculate IC_{50} values.

Results and discussion

We selected the most effective compounds (**43**, **45** and **52**), founded in our previous researches, to measured their inhibitory activities against γ-secretase with a γ-secretase fluorogenic substrate assay.[1,2] As shown in Table S1 and Figure S1, the reported γ-secretase inhibitor L685,458 displayed a strong inhibition of γ-secretase activity with an IC_{50} value at 0.22 nM, and our compound **43** and **52** also showed good inhibitory effect on γ-secretase activity, the IC_{50} values were 5.57 and 1.82 μM, respectively. In view of these positive results, these compounds also provide a promising potential to us for the development of Aβ aggregation and γ-secretase dual inhibitors.

Table S1. Inhibitory effects of three compounds against γ-secretase.

Compound	γ-Secretase Inhibitory Potency IC_{50} (μM)
43	5.57 μM
45	>10 μM
52	1.82 μM
L685,458 (control)	0.22 nM

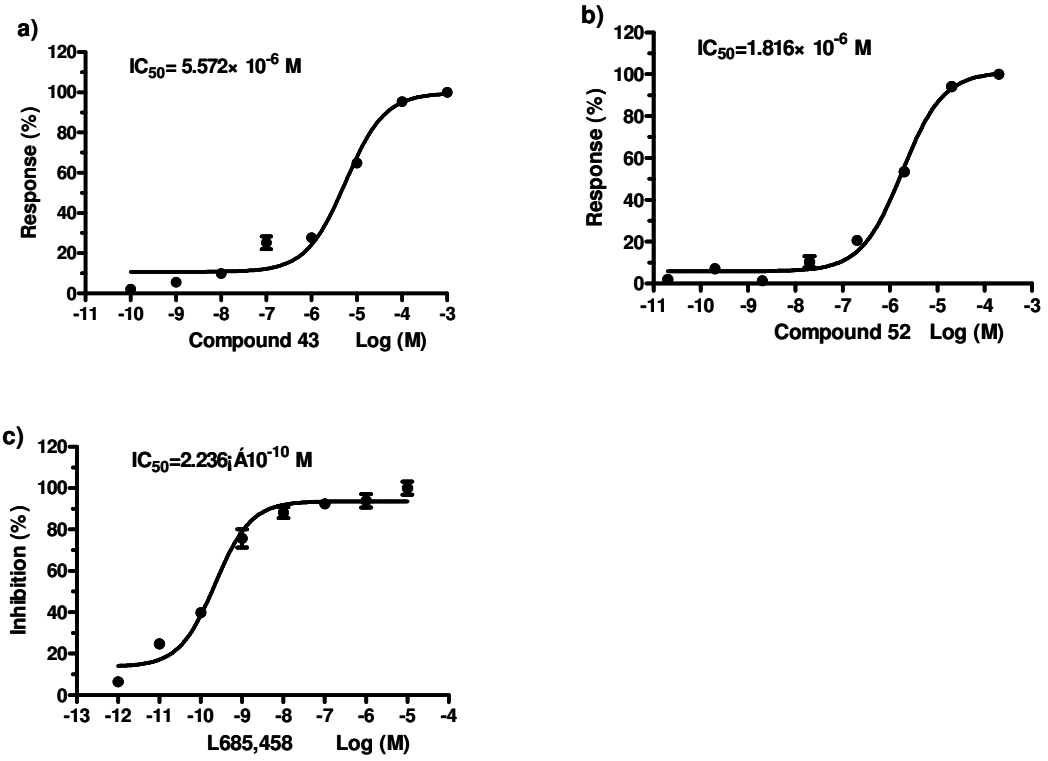

Figure S1. Inhibitory effects of three compounds against γ-secretase. (a) Compound **43**; (b) Compound **52**; (c) L685,458.

References

(1) Farmery, M. R.; Tjernberg, L. O.; Pursglove, S. E.; Bergman, A.; Winblad, B.; Naslund, J. Partial purification and characterization of gamma-secretase from post-mortem human brain. *J. Biol. Chem.* **2003**, *278*, 24277-24284.

(2) Wang, L. F.; Zhang, R.; Xie, X. Development of a high-throughput assay for screening of gamma-secretase inhibitor with endogenous human, mouse or Drosophila gamma-secretase. *Molecules*. **2009**, *14*, 3589-3599.