Received: 12 April 2019 | Revised: 6 July 2019 | Accepted: 24 July 2019

DOI: 10.1002/ddr.21585

RESEARCH ARTICLE

DDR WILEY

Design, synthesis, and biological activity evaluation of BACE1 inhibitors with antioxidant activity

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Funding information
Key Laboratory of Therapeutic Material of Chinese Medicine, Jiangsu Province; Natural Science Foundation of Jiangsu Province, Grant/Award Number: BK20151563; Program for Excellent Talents in School of Pharmacy of Nanjing University of Chinese Medicine, Grant/Award Number: 15ZYXET-1; Project Funded by the Flagship Major Development of Jiangsu Higher Education Institutions, Grant/Award Number: PPZY2015A070; Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions; State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine

Abstract

The proteolytic enzyme β-secretase (BACE1) plays a central role in the synthesis of the pathogenic β-amyloid peptides (Aβ) in Alzheimer's disease (AD), antioxidants could attenuate the AD syndrome and prevent the disease progression. In this study, BACE1 inhibitors (**D1**–**D18**) with free radical-scavenging activities were synthesized by molecular hybridization of 2-aminopyridine with natural antioxidants. The biological activity evaluation showed that **D1** had obvious inhibitory activity against BACE1, and strong antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^{+\bullet}$) assay, which could be used as a lead compound for further study.

KEYWORDS
Alzheimer's disease, antioxidant activity, BACE1, molecular docking, molecular hybridization

1 | INTRODUCTION

For older people, Alzheimer's disease (AD) is the main reason to cause dementia, it consists a progressive and chronic neurodegenerative disorder (Selkoe, 2001). The pathological features of AD include two kinds of lesions in the patient brain, the first kind is the extracellular accumulation of amyloid plaques which is composed of the β-amyloid (Aβ) peptide, and the second kind is the intracellular neurofibrillary tangles which is produced from the aggregation of hyperphosphorylated microtubule-associated tau protein (V. M. Lee, Balin, Otvos Jr, & Trojanowski, 1991).

The β-secretase, also called as beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), is an enzyme that initiates Aβ formation by hydrolysis of the APP extracellular domain, thus generates the membrane bound *C*-terminal fragment C99 and the *N*-terminal Aβ fragment (Vassar, Kovacs, Yan, & Wong, 2009). Subsequently, intramembrane processing of the former fragment C99 by γ-secretase affords Aβ (Sisodia & St George-Hyslop, 2002). Most Aβ peptides end at residue 40 but about 5–10% terminates at residue 42, which is a major actor in the pathogenesis of AD. The studies with knockout mice in the absence of BACE1 showed that both wild type and mutant forms of APP failed to produce Aβ, they were in good health and did not show any side effects of BACE1 deficiency, thus evinced the key role of BACE1 in APP processing (Luo et al., 2001) and further confirmed that BACE1 was very important as a target to therapeutically intervent AD.

The research on BACE1 inhibitors was initiated from the substrate-based design of transition-state compounds, the cleavable amide bond of APP was replaced by a stable mimetic of the putative transition-state structure (Evin, Lessene, & Wilkins, 2011). Thus some peptidomimetic

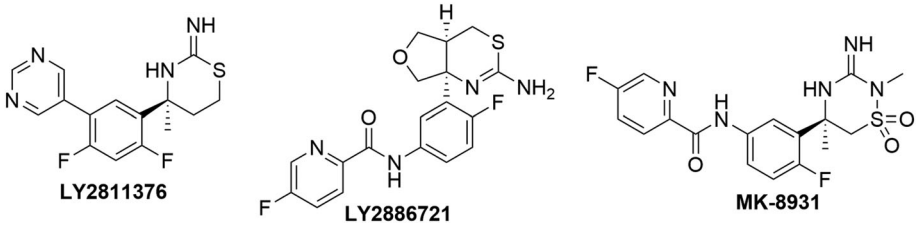


FIGURE 1 The chemical structures of **LY2811376**, **LY2886721**, and **MK-8931**

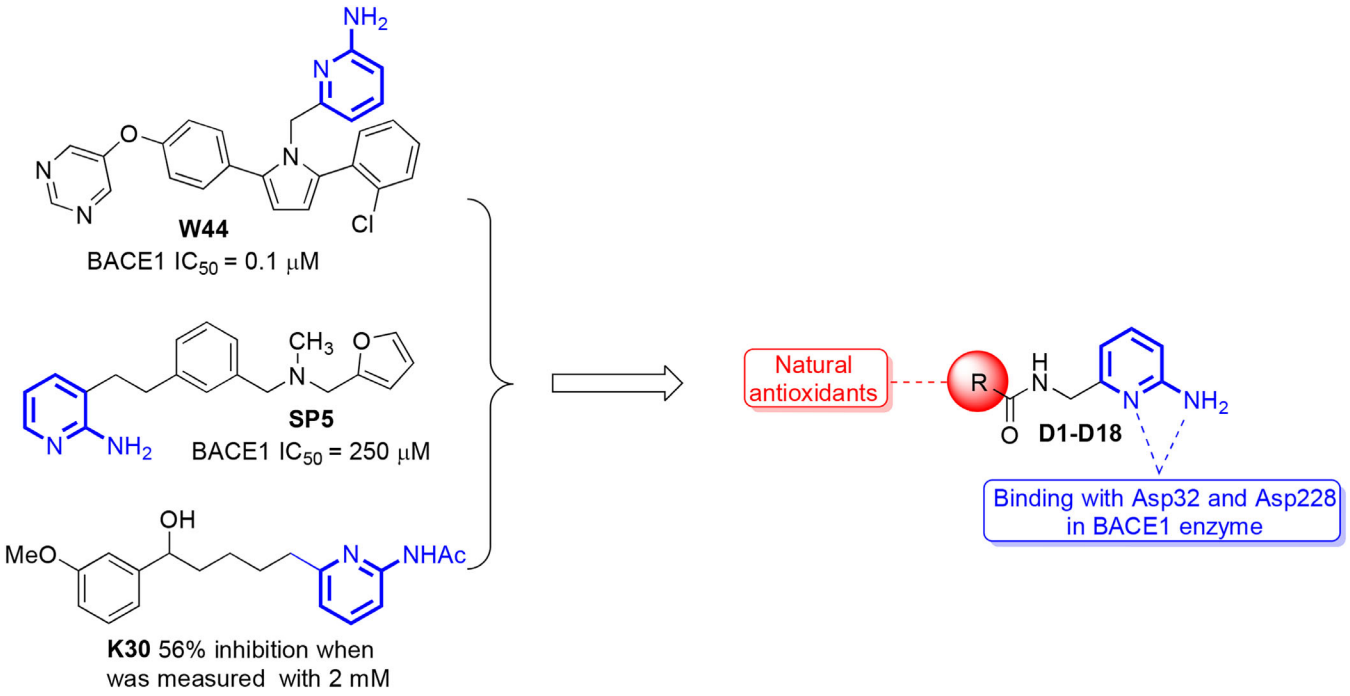


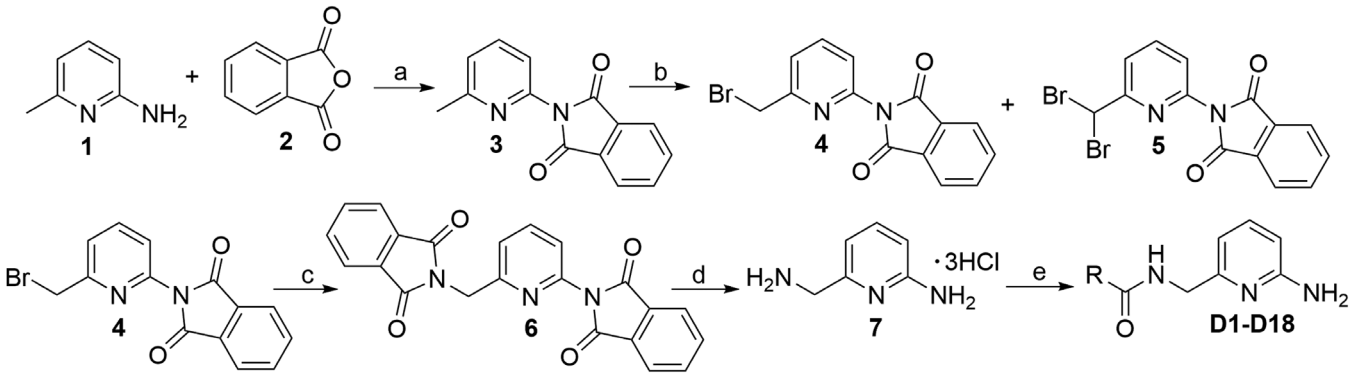
FIGURE 2 The chemical structures of some compounds with 2-aminopyridine scaffold, and the design of BACE1 inhibitors (**D1**–**D18**) bearing 2-aminopyridine and natural antioxidants

inhibitors have been designed, such as hydroxyethylen and statine-based agents, these compounds exhibited potent in vitro subnanomolar activities. However, some peptidic inhibitors suffered from poor pharmacological properties, because they had unfavorable physicochemical characteristics and poor brain penetration (Ghosh, Brindisi, & Tang, 2012; Hom et al., 2003; Hong et al., 2000). So the study to develop potent non-peptidic small-molecule BACE1 inhibitors has become the focus of many academic and pharmaceutical researchers (Albert, 2009), and some candidates have been put into clinical studies.

The first generation of orally active BACE1 inhibitor **BI1181181** (its chemical structure has not been publicly disclosed), failed in Phase I trials because of low oral bioavailability and low blood–brain barrier penetration. Then the second-generation BACE1 inhibitors **LY2811376** (Phase I) (Figure 1), **LY2886721** (Phase II) (Figure 1), and **RG7129** (Phase I; its chemical structure has not been publicly disclosed) also failed in clinical trials because they had liver toxicity. Although the third-generation BACE1 inhibitor **MK-8931** (Figure 1) reduces CNS Aβ in animal models and in AD patients, however, Merck has stopped the clinical trial in mild-to-moderate AD patients because it showed minor efficacy (Hung & Fu, 2017).

In recent years, more attention has been paid to oxidative stress because of its role in AD (H. A. Lee et al., 2018). Several studies indicated that the antioxidant defense system in some elderly people lost its ability to neutralize the oxidative substance. In the early AD stage, the oxidative stress could initiate the Aβ aggregation and tau protein hyperphosphorylation (Gomes et al., 2018). Many evidences have proved that the antioxidants could attenuate the AD syndrome, and prevent the disease progression (Gomes et al., 2018). Thus, drugs with specifically antioxidant activity might be useful for either the prevention or the treatment of AD.

So in this manuscript, we designed and synthesized a series of BACE1 inhibitors with antioxidant activities. Recently, BACE1 inhibitors bearing 2-aminopyridine scaffold have received great attention because they had a superior brain penetration. Wyeth (Malamas et al., 2010) identified compound **W44** (Figure 2) as a potent BACE1 inhibitor (IC_{50} = 0.1 μM) demonstrating high brain permeability with a brain to plasma ratio of 1.1. Schering-Plough (Y. S. Wang et al., 2010) optimized the 2-aminopyridine and led series to more potent small-molecule inhibitors of BACE-1, **SP5** (Figure 2) inhibited BACE-1 with an IC_{50} value of 250 μM in the BACE-1 homogeneous time-resolved fluorescence (HTRF) assay. Konno, Sato, Saito, Sakamoto, and Akaji (2015) prepared a variety of aminopyridine derivatives, and found that 6-alkyl-2-aminopyridine **K30** (Figure 2) exhibited the greatest inhibitory activity when it was screened for rBACE1 inhibition at a 2.0 mM concentration (56% inhibition). The X-ray structure crystal studies showed that the 2-aminopyridine interacted with the catalytic acids



SCHEME 1 Reagents and conditions: (a) *o*-phthalic anhydride (**2**) (1.0 equiv.), CH_3COOH, reflux, 5 hr, 95.2%; (b) NBS (1.1 equiv.), AIBN (0.1 equiv.), benzene, 85°C, 5 hr, **4** (46.7%), **5** (21.3%); (c) phthalimide potassium salt (1 equiv.), DMF, 160°C for 4 hr, room temperature overnight, 90.3%; (d) HCl, reflux, 20 hr, 93.7%; (e) acid, EDCI (1.2 equiv.), HOBT (1.2 equiv.), DIPEA (4.0 equiv.), room temperature overnight, 78.8–89.6%. EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole; DIPEA, *N*,*N*-diisopropylethylamine

Asp32 and Asp228 in BACE1 enzyme via a hydrogen-bounding network (Cole et al., 2006). Furthermore, some natural antioxidants including hydroxycinnamic acid derivatives, ferulic acid (Gay et al., 2018), sinapic acid (Tańska, Mikołajczak, & Konopka, 2018), and caffeic acid (J. Liu et al., 2018) have been shown to scavenge radicals effectively and to repair the oxidizing OH adducts of DNA via electron transfer reactions. So in this study, BACE1 inhibitors (**D1**–**D18**) (Figure 2) bearing 2-aminopyridine with natural antioxidants were designed and synthesized by molecular hybridization.

2 | MATERIALS AND METHODS

2.1 | Synthesis

The compounds were prepared according to Scheme 1. Treatment of aminopyridine **1** with *o*-phthalic anhydride (**2**) produced imide **3**, which was brominated by N-bromosuccinimide (NBS) to afford two benzyl bromides **4** and **5**. In order to improve the yield of important intermediate **4**, we subsequently optimized this reaction condition by changing the ratio of NBS and azodiisobutyronitrile (AIBN), and we found that the best result for the synthesis of **4** as the main product was that the ratio of NBS and AIBN should be 1.1 equiv. and 0.1 equiv., which afforded **4** and **5** in 46.7 and 21.3%, respectively. Then the reaction of **4** with phthalimide potassium salt afforded **6** which could produce amine hydrochloride **7** after hydrolysis with hydrochloric acid. At last, the target compounds **D1**–**D18** were obtained by reaction of **7** and substituted acids using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBT), and *N*,*N*-diisopropylethylamine (DIPEA) as condensation agents.

3 | EXPERIMENTAL

Reagents and solvents were used without further purification unless otherwise specified. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Organic solutions were concentrated below 45°C at approximately 20 mmHg by rotary evaporation. All nonaqueous reactions were carried out under anhydrous conditions using flame-dried glassware within a nitrogen atmosphere in dry, freshly distilled solvents, unless otherwise stated. Yields referred to chromatographical seperation, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.15–0.20 mm Yantai silica gel plates (RSGF 254; Yantai Chemical Industry Research Institute, Yantai, Shandong, China) using ultraviolet light as the visualizing agent. Chromatography was performed on Qingdao silica gel (160–200 mesh) (Qingdao Banke separation materials Company Limited, Qingdao, Shandong, China) using ethyl acetate in petroleum ether and methanol in dichloromethane as the eluting solvent. Melting points (mp) were measured on a WRS-1B apparatus and were uncorrected. ^{1}H Nuclear Magnetic Resonance (NMR) spectra were obtained using a Bruker AV-400 (400 MHz; Bruker Corporation, Germany). Chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. *J*-values were given in Hertz (Hz). Abbreviations used were singlet (s), doublet (d), triplet (t), quartet (q), broad (b), and multiplet (m). Electrospray ionization tandem mass spectrometry (ESI-MS) was recorded on a Waters Synapt high definition mass spectrometry spectrometer.

3.1 | 2-(6-Methylpyridin-2-yl)isoindoline-1,3-dione (3)

6-Methylpyridin-2-amine (**1**) (5.00 g, 46.30 mmol) and phthalic anhydride (**2**) (6.85 g, 46.30 mmol, 1.0 equiv.) were added into glacial acetic acid (20 ml), then the solution was refluxed for 5 hr under the nitrogen. After the reaction was complete by TLC analysis, it was poured into cold water (200 ml), the solid appeared was filtered, washed with water, and then dried under vacuum to afford **3** as white solid (10.49 g, 95.2% yield). mp 193–194°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H), 7.26 (dd, J_1 = 7.8 Hz, J_2 = 10.8 Hz, 2H), 7.77–7.83 (m, 3H), 7.99 (dd, J_1 = 3.0 Hz, J_2 = 5.5 Hz, 2H). ESI-MS: *m/z* 239 $[M + H]^+$, 261 $[M + Na]^+$.

3.2 | 2-(6-(Bromomethyl)pyridin-2-yl)isoindoline-1,3-dione (4)

3 (5.00 g, 21.01 mmol) was added into a solution of AIBN (344.56 mg, 2.101 mmol, 0.1 equiv.) in N,N-dimethylformamide (DMF) (90 ml),

NBS (4.114 g, 23.11 mmol, 1.1 equiv.) was then added into this solution in three parts every 30 min, after this mixture was refluxed for 5 hr, it was concentrated and then purified by column chromatography using 10% ethyl acetate in petroleum ether as eluent to afford **4** (3.10 g, 46.7%) as white solid. mp 177–179°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62 (s, 2H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.84 (dd, J_1 = 3.1 Hz, J_2 = 5.5 Hz, 2H), 7.93 (t, 1H), 8.00 (dd, J_1 = 3.1 Hz, J_2 = 5.5 Hz, 2H). ESI-MS: *m/z* 317 $[M + H]^+$, 239 $[M + Na]^+$.

3.3 | 2-(2-(1,3-dioxoisoindolin-2-yl)methyl) isoindoline-1,3-dione (6)

Phthalimide potassium salt (2.93 g, 15.82 mmol, 1.0 equiv.) was added into a solution of **4** (5.00 g, 15.82 mmol) in DMF (50 ml), after this mixture was refluxed for 4 hr, it was poured into cold water (500 ml), the solid appeared was filtered, dried, and then purified by column chromatography using 2% methanol in dichloromethane as eluent to afford **6** (5.47 g, 90.3%) as yellow solid. mp 114–116°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (s, 2H), 6.38 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 8.0 Hz, 1H), 7.35–7.39 (m, 1H), 7.71–7.82 (m, 4H), 7.88–7.97 (m, 4H). ESI-MS: *m/z* 384 $[M + H]^+$, 406 $[M + Na]^+$.

3.4 | 6-(Aminomethyl)pyridin-2-amine (7)

6 (5.00 g, 13.05 mmol) was added into 40 ml concentrated hydrochloric acid, after the mixture was refluxed for 20 hr, it was filtered and washed with water, then the crude product was recrystallized from ethanol to afford **7** (1.50 g, 93.7%) as yellow solid. mp 201–203°C. ^{1}H NMR (400 MHz, D_2O) δ 4.25 (s, 2H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 9.0 Hz, 1H), 7.85 (t, 1H). ESI-MS: *m/z* 124 $[M + H]^+$, 146 $[M + Na]^+$.

3.5 | Synthesis of D1–D18

The natural acid (0.52 mmol) was added into a solution of HOBT (84 mg, 0.62 mmol, 1.2 equiv.), EDCI (119 mg, 0.62 mmol, 1.2 equiv.), DIPEA (343 μL, 2.08 mmol, 4.0 equiv.) in 10 ml DMF at 0°C, 15 min later, **7** (76 mg, 0.62 mmol, 1.2 equiv.) was added into this solution. After the mixture was reacted at 25°C for 12 hr, it was poured into 100 ml cold water, and then extracted with ethyl acetate (30 ml × 4). The ethyl acetate layer was washed with saturated salt water, dried over anhydrous sodium sulfate, concentrated and purified by column chromatography using 10% ethyl acetate in petroleum ether as eluent to afford **D1**–**D18** (yields 78.8–89.6%).

3.5.1 | (E)-*N*-((6-Aminopyridin-2-yl)methyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (D1)

Yield 81.2%, white solid. mp 97–99°C. ^{1}H NMR (400 MHz, dimethyl sulfoxide (DMSO)-d_6) δ 3.81 (s, 3H), 4.23 (d, *J* = 8.0 Hz, 2H), 5.89 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.40 (d, *J* = 8.0 Hz, 1H), 6.57 (d, *J* = 16.6 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 7.01–7.03 (m, 1H), 7.14 (s, 1H), 7.32–7.38 (m, 2H), 8.36 (t, 1H). ESI-MS: *m/z* 300 $[M + H]^+$, 322 $[M + Na]^+$.

3.5.2 | *N*-((6-aminopyridin-2-yl)methyl) cinnamamide (D2)

Yield 78.8%, white solid. mp 140–142°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.25 (d, *J* = 8.0 Hz, 2H), 5.91 (s, 2H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 16.0 Hz, 1H), 7.31–7.35 (m, 1H), 7.38–7.49 (m, 4H), 7.57–7.59 (m, 2H), 8.54 (t, 1H). ESI-MS: *m/z* 254 $[M + H]^+$, 276 $[M + Na]^+$.

3.5.3 | (E)-*N*-((6-aminopyridin-2-yl)methyl)-3-(3,4-dihydroxyphenyl)acrylamide (D3)

Yield 83.5%, white solid. mp 195–197°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.23 (s, 2H), 5.89 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.39–6.44 (m, 2H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.95 (s, 1H), 7.23–7.35 (m, 2H), 8.41 (s, 1H). ESI-MS: *m/z* 286 $[M + H]^+$, 308 $[M + Na]^+$.

3.5.4 | (E)-*N*-((6-aminopyridin-2-yl)methyl)-3-(3-hydroxy-4-methoxyphenyl)acrylamide (D4)

Yield 85.8%, white solid. mp 214–216°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.80 (s, 3H), 4.23 (s, 2H), 5.91 (s, 2H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 6.51 (d, *J* = 16.0 Hz, 1H), 6.93–7.00 (m, 3H), 7.30–7.36 (m, 2H), 8.43 (t, 1H), 9.19 (s, 1H). ESI-MS: *m/z* 300 $[M + H]^+$, 322 $[M + Na]^+$.

3.5.5 | (E)-*N*-((6-aminopyridin-2-yl)methyl)-3-(3,5-dihydroxy-4-methoxyphenyl)acrylamide (D5)

Yield 82.1%, white solid. mp 97–99°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.80 (s, 6H), 4.26 (s, 2H), 6.06 (s, 2H), 6.35 (d, *J* = 8.0 Hz, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 6.63 (d, *J* = 16.0 Hz, 1H), 6.88 (s, 2H), 7.35–7.39 (m, 2H), 8.41 (t, 1H), 8.84 (s, 1H). ESI-MS: *m/z* 316 $[M + H]^+$, 338 $[M + Na]^+$.

3.5.6 | (E)-*N*-((6-aminopyridin-2-yl)methyl)-3-(4-hydroxyphenyl)acrylamide (D6)

Yield 89.6%, white solid. mp 70–72°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.24 (s, 2H), 5.90 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.40 (d, *J* = 8.0 Hz, 1H), 6.53 (d, *J* = 16.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 12.2 Hz, 2H), 8.42 (t, 1H), 9.97 (s, 1H). ESI-MS: *m/z* 270 $[M + H]^+$, 292 $[M + Na]^+$.

3.5.7 | (E)-*N*-((6-aminopyridin-2-yl)methyl)-3-(2-hydroxyphenyl)acrylamide (D7)

Yield 80.3%, white solid. mp 85–87°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.24 (d, *J* = 8.0 Hz, 2H), 5.91 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.40 (d,

J = 8.0 Hz, 1H), 6.74–6.91 (m, 3H), 7.19 (t, 1H), 7.33 (t, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 16.0 Hz, 1H), 8.50 (t, 1H), 10.07 (s, 1H). ESI-MS: *m/z* 270 $[M + H]^+$, 292 $[M + Na]^+$.

3.5.8 | *N*-((6-aminopyridin-2-yl)methyl)-4-hydroxybenzamide (D8)

Yield 88.1%, white solid. mp 200–202°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.30 (d, *J* = 8.0 Hz, 2H), 5.89 (s, 2H), 6.29 (d, *J* = 8.0 Hz, 1H), 6.38 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 2H), 7.29–7.33 (m, 1H), 7.78 (d, *J* = 8.0 Hz, 2H), 8.67 (t, 1H), 9.99 (s, 1H). ESI-MS: *m/z* 244 $[M + H]^+$, 266 $[M + Na]^+$.

3.5.9 | *N*-((6-aminopyridin-2-yl)methyl) benzamide (D9)

Yield 82.7%, white solid. mp 142–144°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.34 (d, *J* = 8.0 Hz, 2H), 5.90 (s, 2H), 6.30 (d, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 7.30–7.34 (m, 1H), 7.47–7.55 (m, 3H), 7.92 (d, *J* = 8.0 Hz, 2H), 8.95 (t, 1H). ESI-MS: *m/z* 228 $[M + H]^+$, 260 $[M + Na]^+$.

3.5.10 | *N*-((6-aminopyridin-2-yl)methyl)-4-methoxybenzamide (D10)

Yield 80.9%, white solid. mp 178–180°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H), 4.32 (d, *J* = 8.0 Hz, 2H), 5.87 (s, 2H), 6.30 (d, *J* = 8.0 Hz, 1H), 6.39 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 7.31 (t, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 8.79 (t, 1H). ESI-MS: *m/z* 258 $[M + H]^+$, 280 $[M + Na]^+$.

3.5.11 | *N*-((6-aminopyridin-2-yl)methyl)-2-hydroxybenzamide (D11)

Yield 79.5%, white solid. mp 169–171°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.37 (d, *J* = 8.0 Hz, 2H), 5.91 (s, 2H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.43 (d, *J* = 8.0 Hz, 1H), 6.89–6.93 (m, 2H), 7.33 (t, 1H), 7.42 (t, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 9.27 (t, 1H). ESI-MS: *m/z* 244 $[M + H]^+$, 266 $[M + Na]^+$.

3.5.12 | *N*-((6-aminopyridin-2-yl)methyl)-3,4-dihydroxybenzamide (D12)

Yield 83.9%, white solid. mp 116–118°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.27 (d, *J* = 8.0 Hz, 2H), 5.88 (s, 2H), 6.29 (d, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 7.25–7.33 (m, 3H), 8.60 (t, 1H). ESI-MS: *m/z* 260 $[M + H]^+$, 282 $[M + Na]^+$.

3.5.13 | *N*-((6-aminopyridin-2-yl)methyl)-4-hydroxy-3-methoxybenzamide (D13)

Yield 85.3%, white solid. mp 88–90°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.79 (s, 3H), 4.30 (d, *J* = 8.0 Hz, 2H), 5.87 (s, 2H), 6.29 (d, *J* = 8.0 Hz, 1H), 6.38 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 7.30 (t, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 8.62 (t, 1H) ESI-MS: *m/z* 274 $[M + H]^+$, 296 $[M + Na]^+$.

3.5.14 | *N*-((6-aminopyridin-2-yl)methyl)-4-fluorobenzamide (D14)

Yield 82.8%, white solid. mp 158–160°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (d, *J* = 8.0 Hz, 2H), 5.93 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.40 (d, *J* = 8.0 Hz, 1H), 7.32 (t, 3H), 7.99 (t, 2H), 9.00 (t, 1H). ESI-MS: *m/z* 246 $[M + H]^+$, 268 $[M + Na]^+$.

3.5.15 | *N*-((6-aminopyridin-2-yl)methyl)-3-bromobenzamide (D15)

Yield 84.4%, white solid. mp 162–164°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.31 (d, *J* = 8.0 Hz, 2H), 5.91 (s, 2H), 6.31 (d, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 7.30–7.34 (m, 1H), 7.45–7.48 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 9.10 (t, 1H). ESI-MS: *m/z* 306 $[M + H]^+$, 328 $[M + Na]^+$.

3.5.16 | *N*-((6-aminopyridin-2-yl)methyl)-2-chlorobenzamide (D16)

Yield 87.3%, white solid. mp 161–163°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.30 (d, *J* = 8.0 Hz, 2H), 5.92 (s, 2H), 6.32 (d, *J* = 8.0 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 7.35–7.50 (m, 4H), 7.90 (t, 1H), 8.89 (t, 1H). ESI-MS: *m/z* 262 $[M + H]^+$, 284 $[M + Na]^+$.

3.5.17 | *N*-((6-aminopyridin-2-yl)methyl)-2-hydroxy-2-phenylacetamide (D17)

Yield 84.2%, white solid. mp 78–80°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.14 (t, 2H), 4.99 (s, 1H), 5.88 (s, 2H), 6.29 (d, *J* = 8.0 Hz, 2H), 7.25–7.44 (m, 4H), 7.45 (d, *J* = 8.0 Hz, 2H), 8.37 (t, 1H). ESI-MS: *m/z* 258 $[M + H]^+$, 280 $[M + Na]^+$.

3.5.18 | *N*-((6-aminopyridin-2-yl)methyl)-2-(2-hydroxyphenyl)acetamide (D18)

Yield 80.6%, white solid. mp 168–170°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.48 (s, 2H), 4.12 (d, *J* = 8.0 Hz, 2H), 5.88 (s, 2H), 6.29 (d, *J* = 8.0 Hz, 1H), 6.39 (d, *J* = 8.0 Hz, 1H), 6.75 (t, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 7.11–7.28 (m, 2H), 7.31 (t, 1H), 8.37 (t, 1H), 9.72 (s, 1H). ESI-MS: *m/z* 258 $[M + H]^+$, 280 $[M + Na]^+$.

4 | BIOLOGICAL SCREENING

4.1 | BACE1 inhibitory activity assay

The enzyme inhibition assay was evaluated according to the manufacturer instructions (Al-Tel et al., 2011) using BACE1 fluorescence resonance energy transfer assay kit, Red (P2985, Thermo fisher).

Epigallocatechin gallate (EGCG) was used as a positive control. The BACE1 enzyme (purified baculovirus-expressed enzyme) was diluted with the assay buffer (50 mM sodium acetate, pH 4.5) to make a 3× working solution (1 Unit/ml). The peptide substrate (Rh-EVNLDAEFK-Quencher) was also diluted with the same assay buffer to provide the 3× stock solution (750 nM). The inhibitor stock solutions in DMSO were diluted with assay buffer to provide 3× solution of test compounds at different concentrations. The 3× solution of BACE1 enzyme (10 μl) and each inhibitor sample (10 μl) were placed in the 96-well plate and gently mixed. The substrate 3× solution (10 μl) was then added to this mixture in each well to start the reaction, and the final reaction volume was 30 μl (the concentration of DMSO in each sample and control wells was less than 4%). The reaction mixtures were incubated at 25°C for 90 min in the dark and then the reaction was stopped with 10 μL of 2.5 mM sodium acetate. Fluorescence was monitored at 545 nm (excitation wavelength) and 585 nm (emission wavelength). The percentage of enzyme inhibition for each concentration of test compound was calculated compared to maximum enzyme activity wells (containing substrate plus enzyme) and baseline wells (containing substrate). IC_{50} values (concentration of the compound that induces 50% inhibition of enzymatic activity) were calculated with the CurveExpert software version 1.34 for Windows.

4.2 | 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity assay was measured according to the method (H. Wang, Gao, Zhou, Cai, & Yao, 2008) with a few modifications. The sample (4–250 μmol/L, 100 μl) which was dissolved in ethanol was added to ethanol solution (100 μl) containing DPPH radicals (80 μmol/L) in 96-well plates. The mixture was shaken and left at room temperature for 30 min in the dark, and the absorption was measured at 517 nm, the Trolox was used as the positive control. A lower absorbance represents a higher DPPH scavenging activity. The percentage scavenging effect was calculated as scavenging rate (%) = $[1 - (A_1 - A_2)/A_0] \times 100\%$, where A_0 was the absorbance of the control (without sample), A_1 was the absorbance in the presence of the sample, and A_2 was the absorbance without DPPH.

4.3 | 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) radical-scavenging activity assay

The 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^{+\bullet}$) radical-scavenging activity assay was measured according to a literature procedure (Z. Q. Liu, 2010). $K_2S_2O_8$ (2 mmol/L) and ABTS (7 mmol/L) were dissolved and mixed in deionized water, reacting at room temperature for 12–16 hr. The mixture ($ABTS^{+\bullet}$ stock solution) was then diluted by phosphate buffer solution to give an absorbance at 734 nm near 0.7, defined as the reference absorbance (A_0). A_0 decreased to a stable value (A_1) when $ABTS^{+\bullet}$ (100 μl) was mixed with the sample (1–62.5 μmol/L, 100 μl) for 6 min. The percentage scavenging effect was calculated as scavenging rate (%) = $[1 - (A_1 - A_2)/A_0] \times 100\%$, where A_0 was the absorbance of the control (without sample), A_1 was the absorbance in the presence of the sample, and A_2 was the absorbance without $ABTS^{+\bullet}$.

4.4 | Docking studies

Compound **D1** was docked into the crystal structures of BACE 1 (PDB ID: 3MSL) by using Discovery Studio 2.5. Standard precession with standard deviation (SD) was used to dock **D1**. Optimization was performed on the following conditions: CHARMm for force field setting, an active site radius was 10.0 Å. Physicochemical properties and their scoring functions (a combination of interaction energy, hydrogen bonding, and electrostatic interactions) were used to select the final pose.

5 | RESULTS AND DISCUSSION

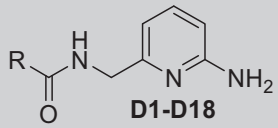
5.1 | BACE1 inhibiting activity

Epigallocatechin gallate has been proved to be a BACE1 inhibitor with its IC_{50} value was 1.6 μmol L^{-1} (Jeon, Bae, Seong, & Song, 2003). So in this research, EGEG was used as positive control to evaluate the BACE1 inhibiting activity, and the results were shown in Table 1. From the results, we could see that **D1**–**D7** had potent inhibitory activity against BACE1, compared to EGCG with its IC_{50} was 1.18 μM. For compounds **D2**, **D3**, **D6**, and **D7** with benzene ring or hydroxyl substituted benzene ring that were in the *R* substituent, the inhibition rates were less than 50% in the concentration of 2 μmol L^{-1}. Interestingly, for compounds **D1**, **D4**, and **D5** in which the methoxy group was introduced, the inhibitory activities against BACE1 were improved with their inhibition rates were all above 50%, and the IC_{50}s for these three compounds were 1.80, 2.07, and 1.96 μM, respectively, this information indicated that introduction of methoxy group was very important for the inhibitory activities against BACE1. When the vinyl group was removed, the obtained compounds **D8**–**D13** and **D18** lost their inhibitory activities dramatically with their inhibition rates were all below 10%, this information confirmed that cinnamyl group in the side chain was very important to inhibit BACE1. Unfortunately, compounds **D14**–**D17** with no phenolic hydroxyl group was substituted in the benzene ring were inactive in this research, these results indicated that the phenolic hydroxyl group in the side chain contributed to the BACE1 inhibitory activities.

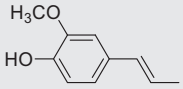
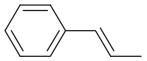
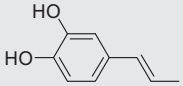
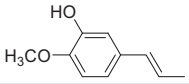
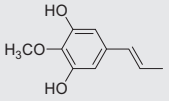
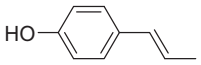
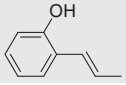
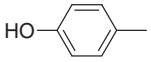
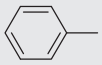
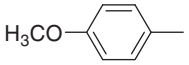
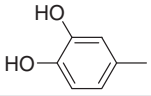
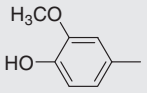
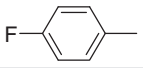
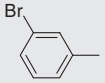
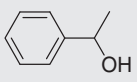
5.2 | Antioxidant activities

The in vitro antioxidant activities of these compounds were assessed by DPPH and $ABTS^{+\bullet}$ assays. DPPH and $ABTS^{+\bullet}$ (both stable radicals) were widely used to evaluate the antioxidant capacity of complex mixtures and individual compounds (Z. Q. Liu, 2010). The in vitro antioxidant activity of the target compounds in comparison with Trolox was evaluated by DPPH and $ABTS^{+\bullet}$ assays. The IC_{50} value was defined as the concentration of sample that causes 50% loss of the radical. The data obtained were summarized in Table 1. The IC_{50}s for

TABLE 1 The inhibitory activity against BACE1 and antioxidant activities of the target compounds D1–D18

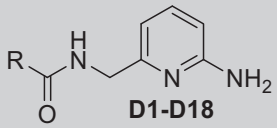


D1-D18

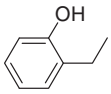
Compd.	*R*	Inhibition rate (%) (at 2 μm)	Activity (IC_{50}, μM)	Compd.	DPPH assay IC_{50} (μM)	ABTS assay IC_{50} (μM)
EGCG		56.41 ± 0.47	1.18 ± 0.41	**Trolox**	21.53	26.83
D1		53.22 ± 1.19	1.80 ± 0.22	**D1**	67.85	27.14
D2		43.52 ± 3.59	ND	**D2**	ND	ND
D3		47.53 ± 3.34	ND	**D3**	18.67	24.83
D4		51.61 ± 2.50	2.07 ± 0.51	**D4**	ND	27.79
D5		52.61 ± 1.71	1.96 ± 0.60	**D5**	53.64	23.76
D6		47.29 ± 0.50	ND	**D6**	ND	45.82
D7		40.18 ± 3.22	ND	**D7**	ND	40.11
D8		9.03 ± 2.24	ND	**D8**	ND	83.45.
D9		6.54 ± 1.76	ND	**D9**	ND	ND
D10		4.07 ± 2.34	ND	**D10**	ND	ND
D11		3.12 ± 3.04	ND	**D11**	ND	72.91
D12		4.79 ± 0.51	ND	**D12**	35.21	35.58
D13		4.98 ± 3.06	ND	**D13**	ND	40.63
D14		0	ND	**D14**	ND	ND
D15		0	ND	**D15**	ND	ND
D16		0	ND	**D16**	ND	ND
D17		0	ND	**D17**	ND	ND

(Continues)

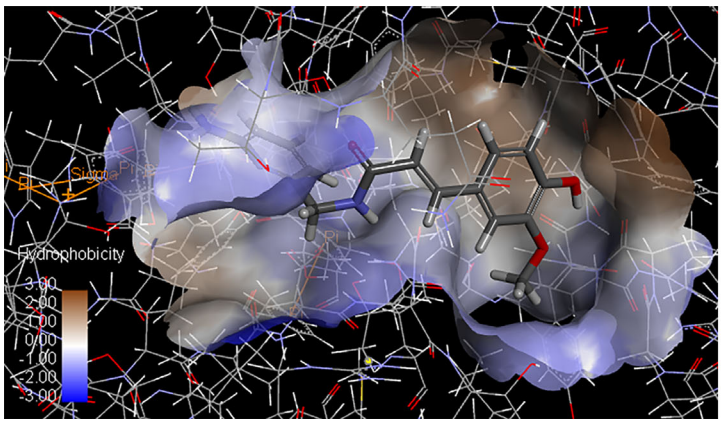
TABLE 1 (Continued)



D1-D18

Compd.	R	Inhibition rate (%) (at 2 μm)	Activity (IC_{50}, μM)	Compd.	DPPH assay IC_{50} (μM)	ABTS assay IC_{50} (μM)
D18		1.80 ± 2.29	ND	**D18**	ND	27.56

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate); DPPH, 1,1-diphenyl-2-picrylhydrazyl; EGCG, epigallocatechin gallate; ND, not determined.



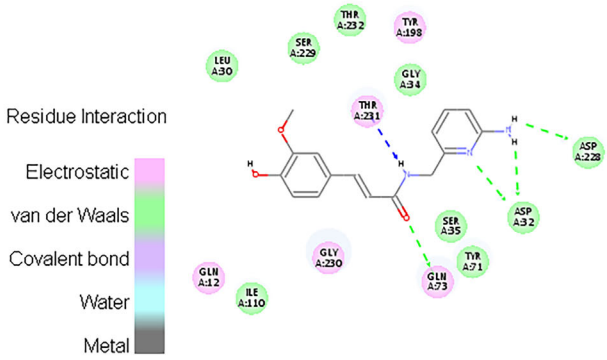


FIGURE 3 Molecular docking studies of compound **D1** with BACE 1 protein

Trolox in DPPH and $ABTS^{+\bullet}$ assays were 21.53 and 26.83 μM. Only one phenolic hydroxyl group substituted compounds showed good antioxidant activity in $ABTS^{+\bullet}$ assay, for example, the IC_{50}s for **D1**, **D4**, **D6**, **D7**, **D8**, **D11**, **D13**, and **D18** were 27.14, 27.79, 45.82, 40.11, 83.45, 72.91, 40.63, and 27.56 μM, respectively. When the two phenolic hydroxyl groups were introduced into the benzene ring, the antioxidant activities were enhanced. For example, the IC_{50}s for **D3** in DPPH and $ABTS^{+\bullet}$ assays were 18.67 and 24.83 μM, the IC_{50}s for **D5** in DPPH and $ABTS^{+\bullet}$ assays were 53.64 and 23.76 μM, and the IC_{50}s for **D12** in DPPH and $ABTS^{+\bullet}$ assays were 35.21 and 35.58 μM. This information confirmed that the phenolic hydroxyl group was very important for antioxidant activities.

5.3 | Molecular docking studies

Considering that compound **D1** exhibited the most potent BACE1 inhibitory activity with its IC_{50} was 1.80 μM, and good antioxidant activities with its IC_{50}s in DPPH and $ABTS^{+\bullet}$ assays were 67.85 and 27.14 μM, so **D1** was selected for molecular docking studies. As shown in Figure 3, compound **D1** could extend deeply into the cavity of BACE1, and combine excellently with hydrophilic surface and hydrophobic surface (Figure 3), thus contributing to the ligand affinity. As expected, the amino group in the aminopyridine moiety made two hydrogen bonds with Asp32 and Asp228, and the nitrogen atom in the aminopyridine moiety made one hydrogen bond with Asp32. Furthermore, the amide bond in the side chain also formed two hydrogen bonds with the active sites, the carbonyl group formed one hydrogen bond with Gln73, and the amino group formed one hydrogen with Thr231. These actions might verify that **D1** had potent inhibitory activity against BACE1.

6 | CONCLUSIONS

In summary, we have synthesized new class of BACE1 inhibitors with antioxidant activities by molecular hybridization of the 2-aminopyridine with some antioxidant natural products. The aminopyridine could form hydrogen bonds with Asp32 and Asp228, and the amide bond in the side chain also formed two hydrogen bonds with Gln73 and Thr231. Furthermore, the phenolic hydroxyl group in the side chain contributed to the antioxidant activities. These information were very important to design and synthesize BACE1 inhibitors with good antioxidant activities for the treatment of AD.

ACKNOWLEDGMENTS

This work was financially supported by Natural Science Foundation of Jiangsu Province (BK20151563), Program for Excellent Talents in

School of Pharmacy of Nanjing University of Chinese Medicine (15ZYXET-1), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, Project Funded by the Flagship Major Development of Jiangsu Higher Education Institutions (PPZY2015A070), Key Laboratory of Therapeutic Material of Chinese Medicine, Jiangsu Province, State Key Laboratory Cultivation Base for TCM Quality and Efficacy, Nanjing University of Chinese Medicine.

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How to cite this article: Li H-M, Yu S-P, Fan T-Y, et al. Design, synthesis, and biological activity evaluation of BACE1 inhibitors with antioxidant activity. *Drug Dev Res*. 2019;1–9. https://doi.org/10.1002/ddr.21585