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Synthesis and evaluation of novel 1,2,3-triazole-based acetylcholinesterase inhibitors with neuroprotective activity

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ABSTRACT

A series of new 1,2,3-triazole derivatives were synthesized and evaluated for anticholinesterase and neuroprotective activities. Some synthetic derivatives, especially compound **32**, exhibited improved acetylcholinesterase (AChE) inhibitory activity by comparison with the hit **1**, high selectivity toward AChE over butyrylcholinesterase (BuChE), and suitable in vitro neuroprotective effect against amyloid-β_{25-35} ($A\beta_{25-35}$)-induced neurotoxicity in SH-SY5Y cells. Furthermore, these molecules have desired physicochemical properties in the range of CNS drugs and showed no cytotoxicity against two normal cells, including human keratinocytes HaCaT and murine fibroblasts NIH-3T3. The preliminary bioassay results and docking study indicated that compound **32** might be a promising lead compound with dual action for the treatment of Alzheimer's disease.

Alzheimer's disease (AD), the most common form of dementia, is a chronic neurodegenerative disorder, which is clinically characterized by impairment in memory, complex cognition, language, emotion, and behavior.[1] In 2015, approximately 46.8 million people worldwide were believed to suffer from AD, and this number was expected to triple by 2050 with the aging of the population.[2] Several factors, such as levels of acetylcholine (ACh)[3] and amyloid-β-peptide (Aβ) deposits,[4] play a significant role in the occurrence of AD.

Although the pathogenesis of AD is not fully understood, currently the most efficacious treatment approach for AD is considered to increase cholinergic neurotransmission in the brain by lowering Ach hydrolysis.[5] ACh can be degraded by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Compared with BuChE, AChE draws more attention from pharmaceutical chemists since it accounts for nearly 80% ACh hydrolysis in the brain.[5b] In this context, four AChE inhibitors including tacrine, donepezil, rivastigmine and galantamine had been clinically approved by the FDA.[5c] However, tacrine is rarely used due to its potential liver toxicity, and the others could only modestly improve memory and cognitive function in AD treatment but do not appear to stop or slow down the progress of AD. Because the pathogenesis of AD is very complicated and related to multiple systems' dysfunctions, there is still no ideal drug that can completely prevent and treat AD. Therefore, there is an urgent need for developing new effective anti-AD drugs.

Many studies indicate another hypothesis for AD pathogenesis, the so-called amyloid hypothesis.[6] Accumulated amyloid plaques made up of Aβ is a major hallmark of AD.[6a] Aβ is derived from amyloid precursor protein (APP) via sequential proteolytic cleavage by β- and γ-secretases.[6b] It is hypothesized that the accumulated Aβ is one of the primary causes of AD because its accumulation in the brain could trigger critical intracellular signaling pathways, resulting in neural cell stress and apoptosis.[6c] Thus, drugs which specifically protect neurons from injury and apoptosis induced by Aβ could be useful for both the prevention and treatment of AD.

Recently, a new synthetic 1,2,3-triazole derivative **1** with a morpholinoethanamine side chain (shown in Fig. 1) was screened out from our in-house compounds library. This compound showed AChE inhibitory activity (IC_{50} = 27.35 μM) and neuroprotective effect against $A\beta_{25-35}$-induced injuries in SH-SY5Y neuronal cells (85.2% of cell viability at 10 μM), indicating it was a potential dual-action anti-AD lead compound. As well as known, the 1,2,3-triazole scaffold is commonly found in synthetic products and

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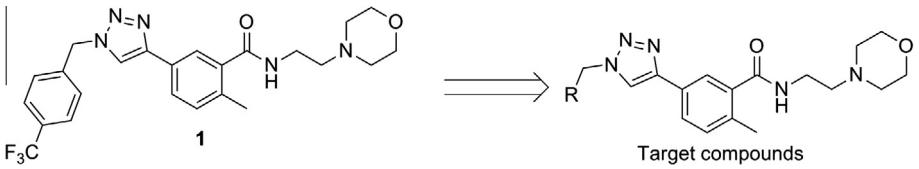


Figure 1. The structure of **1** and its structural derivatization.

represents an important pharmacophore in drug discovery.[7] Until now, lots of AChE inhibitors and neuroprotectants with 1,2,3-triazole unit have been reported.[8,9] Recently, Yadav et al. reported that a diaryltriazine possessing a morpholinoethanamine side chain showed potential multitarget-directed therapeutics, such as AChE inhibitory activity and neuroprotective effect against $A\beta_{1-42}$-induced apoptosis neurotoxicity, for the treatment of AD.[10] Basic side chain containing an amino group is an integral part of the structure of several reported AChE inhibitors such as donepezil, tacrine, and galantamine. The nitrogen atom was considered to play an important role in enzyme–inhibitor interaction.[11] In addition, the substituents attached to the 1,2,3-triazole unit usually affect the bioactivity.[12] Based on above observations and our experiences in the synthesis of neuroprotective agents,[13] a further improvement on the bioactivity profile of **1** through preliminary structural derivatization was carried out, by introducing different commercially available benzyl or naphthyl group into the 1,2,3-triazole unit.

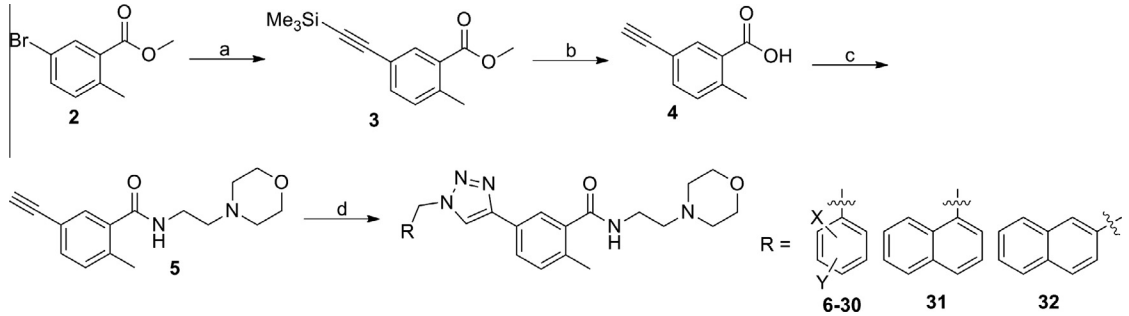
Herein, we present the synthesis and biological evaluation of a series of new 1,2,3-triazoles as selective AChE inhibitors with neuroprotective property, and molecular docking as well.

The synthetic strategy for target 1,2,3-triazoles **6**–**32** is depicted in Scheme 1. Briefly, it was started from the Sonogashira coupling reaction[14] of 5-bromo-2-methylbenzoic acid **2** and trimethylsilylacetylene to give **3**, which was subsequently deprotected and hydrolyzed in one step under basic condition[15] to yield **4**. Then compound **4** was converted into amide **5** by reacting with 2-morpholinoethanamine in the presence of EDCI and HOBT.[16] Finally, the addition **5** and CuI into the azide intermediates, which were freshly prepared from different bromides ($ArCH_2Br$) and NaN_3 in the mixture of H_2O/DMSO, yielded the corresponding target compounds **6**–**32** in the CuI-catalyzed Azide–Alkyne Cycloaddition.[17]

The inhibitory activities of **5**–**32** against AChE (from electric eel) and BuChE (from equine serum) were evaluated according to the Ellman's method[18] with galanthamine as the reference compound. The results were summarized in Table 1. All target compounds **6**–**32** showed moderately potent inhibition of AChE with IC_{50} values ranging from 64.17 to 7.23 μM, but stronger than that of their precursor compound **5**, clearly indicating that the introduction of substituted-1,2,3-triazole moiety could significantly increase their inhibitory activity.

Among the derivatives **1**, **6**, **8**–**13**, the compounds **8** with *para*-F and **11** with *para*-Br were the most potent inhibitor for AChE, revealing that the *para*-substituted group seems to be beneficial for the AChE inhibitory activity. However, the compounds **16**–**18** showed decreased activity by comparison with the above compounds bearing electron-withdrawing substituent, indicating that the electron-withdrawing substituent in benzene plays an important role in their bioactivities. This deduction was also supported by the observation that the activities of **11** and **13** were decreased when additional electron-donating groups (e.g., methoxyl or a methyl group) were introduced in their benzene ring to form **19** and **20**, respectively. Also, the selectivity of **6**–**20** toward AChE was not so satisfactory. Based on the observation that fluorinated functionalities are usually the key structural units, found in about 20% pharmaceuticals on the market,[19] the *para*-fluoro was conversed and a second small electron-withdrawing atom (such as F, Cl, and Br) was introduced to form **21**–**25**. To our light, the compounds **23** and **24** were found to show increased inhibitory activity against AChE, and higher selectivity toward AChE over BuChE by 6.2- and 6.6-fold compared with the positive control galanthamine, respectively. The *para*-fluor was further replaced by a more strong electron-withdrawing groups to yield derivatives **26**–**30**. Among them, compound **28** with *para*-CN also exhibited high inhibitory activity and selectivity toward AChE. Furthermore, the effect of aromatic property of the functional group on bioactivity was investigated by the introduction of naphthyl group, consequently resulting in the production of **31** and **32**. Surprisingly, compound **32** with 2-position substitution in naphthalene ring showed the strongest inhibition of AChE (IC_{50} = 7.23 μM) and highest selectivity toward AChE over BuChE by 12.6-fold among all the tested compounds. However, compound **31** showed significantly decreased activity, probably due to the steric hindrance resulting from 1-position substitution in naphthalene ring, compared with **32**.

The molecular docking was conducted to investigate the potential binding mode of compound **32** with the catalytic domain of AChE (PDB: 4EY7),[20] which was performed by using Autodock 4.2[21] with structure images created by Pymol 1.5. As shown in Figure 2, compound **32** was located in the active-site gorge of AChE, with 1,2,3-triazole pharmacophore oriented in the peripheral anionic site (PAS) and morpholine group oriented in catalytic active sites (CAS). This compound could form two kinds of primary



Scheme 1. Synthesis of **6**–**32**. Reagents and conditions: (a) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, DMF, Et_3N, 50 °C, 6 h, 73%; (b) KOH, MeOH, 0 °C to reflux 4 h, 98%; (c) 2-morpholinoethanamine, EDCI, HOBT, DIEPA, DCM, 12 h, 85%; (d) NaN_3,CuI, sodium ascorbate, substituted benzyl bromides for **6**–**30**, 1-(bromomethyl)naphthalene for **31** and 2-(bromomethyl)naphthalene for **32**, DMSO, H_2O, rt, overnight.