Bioorg. Med. Chem. Lett. 43 (2021) 128092

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of novel diosgenin-triazole hybrids targeting inflammation as potential neuroprotective agents

Yi Huang [a], Weiwei Huang [a], Guixiang Yang, Rui Wang [*], Lei Ma [*]

Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

ARTICLE INFO

Keywords:
Diosgenin-triazole hybrids
Alzheimer's disease
Inflammation
Neuroprotective activities

ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disease, and its incidence is expected to increase as the global population ages. Recent studies provide increasing evidence that inflammation plays a key role in the pathogenesis and progression of AD. Diosgenin, an active ingredient in *Dioscorea nipponica* Makino, is a promising bioactive lead compound in the treatment of Alzheimer's disease, which exhibited anti-inflammatory activity. To search for more efficient anti-Alzheimer agents, a series of novel diosgenin-triazolyl hybrids were designed, synthesized, and their neuroprotective effects against oxygen-glucose deprivation-induced neurotoxicity and LPS-induced NO production were evaluated. Most of these new hybrids displayed better activities than DIO. In particular, the promising compound **L6** not only demonstrated an excellent neuroprotective effect but also showed the best anti-inflammatory activity. The structure-activity relationship study illustrated that the introduction of benzyl or phenyl triazole did improve the activity, and the introduction of benzyl triazole was better than that of phenyl triazole. The results we obtained showed that the diosgenin skeleton could be a promising structural template for the development of new anti-Alzheimer drug candidates, and compound **L6** has the potential to be an important lead compound for further research.

Introduction

Alzheimer's disease (AD) is one of the most serious and prevalent neurodegenerative diseases, affecting an estimated 47 million people worldwide and having a huge economic impact on families and societies.[1] An increasing number of experimental and clinical evidence suggests that AD is a complex disease manifesting as progressive memory loss and cognitive decline.[2,3] Although the etiology of AD is not fully understood, there are diverse factors that seem to play significant roles in the pathophysiology of the disease, including inflammation, β-amyloid (Aβ) deposition, neurofibrillary tangles, low levels of acetylcholine and oxidative stress.[1,4–8] Over the past few decades, much efforts have been devoted to understanding the role of Aβ in AD and determining whether disease progression can be alleviated by reducing Aβ.[9–10] The recent failure of drugs that target Aβ indicates that a better understanding of other etiological factors may shed light on new mechanistic pathways that can target to slow the progression of AD and improve clinical outcomes.[1]

Recent genomic, bioinformatics, and epidemiological studies provide increasing evidence that inflammation plays a crucial role in the pathogenesis and progression of AD.[1,11] Patients with asymptomatic AD exhibit neuroinflammation, which negatively affects nerve cell function during the onset and progression of AD.[12] Microglia and astrocytes are the two main types of nerve cells that mediate neuroinflammation.[13,14] Microglia are a type of immune cells inherent in the central nervous system with multiple synapses and highly functional plasticity.[15] Under normal conditions, microglia remain dormant and maintain nervous system homeostasis.[16,17] Aβ is a well-known inflammatory agent in AD pathogenesis.[18] Aβ can activate microglia and make them increase the secretion of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), macrophage inflammatory protein-1α (MIP-1α), and monocyte chemoattractant protein-1 (MCP-1).[19–21] Astrocytes are a class of large glial cells with multiple functions, including biochemical support to the endothelial cells of the blood–brain barrier (BBB), provision of nutrition to nerve tissue, maintenance of extracellular ion balance, and post-traumatic repair of the brain and spinal cord.[22] Similar to microglia, astrocytes secrete a variety of pro-inflammatory factors, such as interleukins (ILs), prostaglandins (PGs),

* Corresponding author.
E-mail addresses: ruiwang@ecust.edu.cn (R. Wang), malei@ecust.edu.cn (L. Ma).
[a] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmcl.2021.128092
Received 25 February 2021; Received in revised form 19 April 2021; Accepted 1 May 2021
Available online 6 May 2021

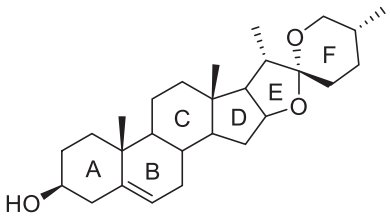


Fig. 1. The structure of Diosgenin.

leukotrienes (LTs), thromboxane (TXs), coagulation factors (CFs), complements, proteases, and protease inhibitors.[23,24] During AD progression, Aβ-mediated chronic inflammation leads to the dysfunction of microglia and astrocytes, which subsequently affects neuronal function and survival, and ultimately leads to cognitive deterioration.[1] Therefore, anti-inflammatory drugs may have therapeutic effects on AD, and the use of anti-inflammatory drugs may reduce the incidence of AD.[25]

Natural products play highly significant roles in drug discovery and development processes. Diosgenin (DIO), a steroidal sapogenin usually found in a variety of plants such as *Dioscorea nipponica* Makino (*D. nipponica* Makino) (Fig 1). DIO and its derivatives have proven to exhibit neuroprotective effects and have been efficiently used for AD. Wang et al. found that DIO suppressed the production of pro-inflammatory factors such as NO, IL-6, and TNF-α in rat microglia and BV-2 cells induced with lipopolysaccharides (LPS), thus achieving neuroprotective effects.[26] Besides, a previous study had shown that diosgenin derivatives with substituted 3-carbamate groups displayed significant neuroprotective activities.[27] Cai et al. designed and synthesized a series of amino acid derivatives of DIO, and found that compound DG-15 exhibited a good protective effect on the SH-SY5Y cell model induced by 2, 4, 5-trihydroxybutyrophenone (TBHP) (EC_{50} = 6.86 ± 0.69 μm).[28] These above findings suggest that DIO seems to be a promising compound for the discovery of new anti-AD candidates through structural modification.

Molecular hybridization is one of the most valuable structural modification tools for discovering ligands. In recent years, more and more hybrid drugs have been found by combining the pharmacological parts of different known lead compounds, bringing new hope for the treatment of multifactorial diseases.[29] Multiple studies have demonstrated that triazole-containing derivatives (Fig 2), which may show promising in vitro and in vivo Alzheimer's disease activities and might be able to prevent the drug-resistant to a certain extent, have attracted great interest in searching new drugs to treat Alzheimer's disease.[30–33]

Therefore, we designed and synthesized a series of diosgenin derivatives containing triazole. The BBB permeability of all the representative compounds was simulated by admetSAR (http://lmmd.ecust.edu.cn/admetsar2/, developed by Yang from ECUST). As the results listed in Table 1, we can see when the A ring becomes benzene, the BBB penetration was better. Besides, our previous research showed that the transformation of A ring into a benzene ring could improve the biological activity of esterified derivatives at 3-site. Therefore, we considered modifying the A ring of DIO to a benzene ring for further structural modification. In addition, diosgenin-triazole hybrid revealed much better BBB penetration. And the neuroprotective activity of the newly designed compounds was evaluated in OGD and LPS-induced NO production cell models.

The synthetic route for the target compounds is shown in Scheme 1. The intermediate **2** was synthesized by Oppenauer reaction with the raw material compound **1** (DIO). Then, intermediate **2** was oxidized by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) catalyzed by trimethylchlorosilane (TMSCl) to obtain intermediate **3** in a yield of 95%. Intermediate **3** was converted into intermediate **4** by arylation using biphenyl, diphenylmethane, and lithium under the protection of N_2 in a yield of 55%. Under alkaline conditions, the key intermediate **4** reacts with 3-bromoproparyne to form compound **5**. Substituted azides were

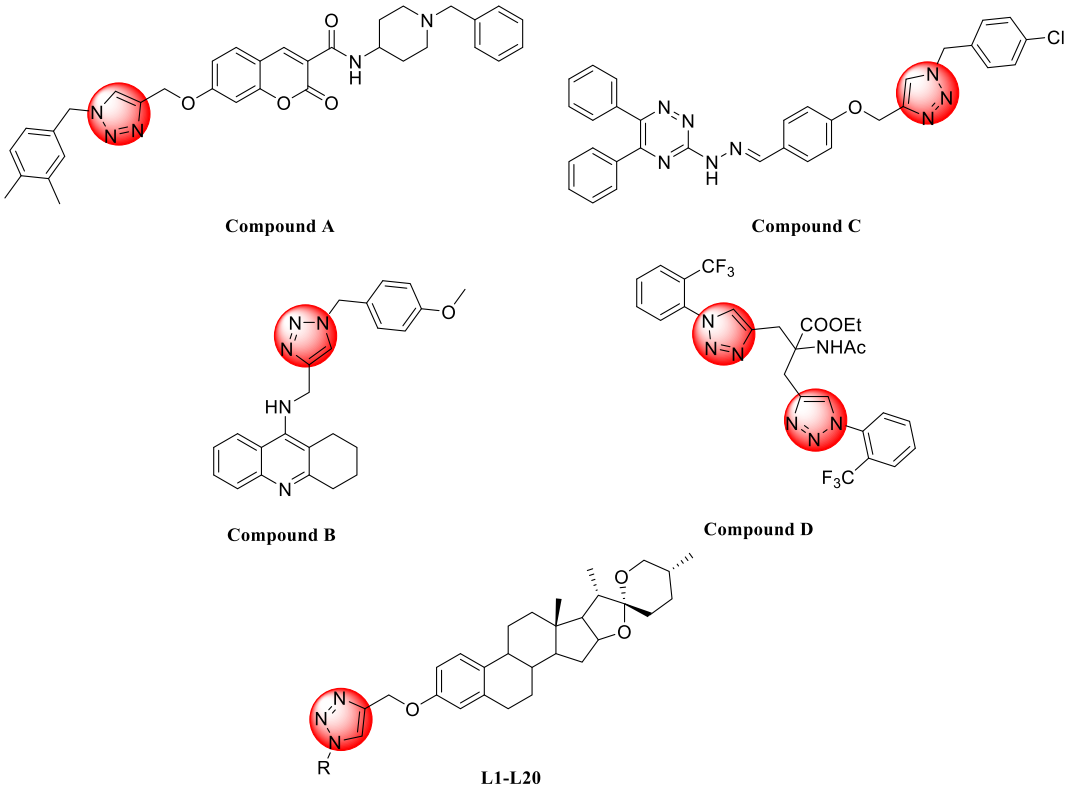
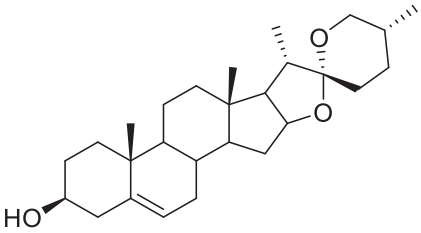
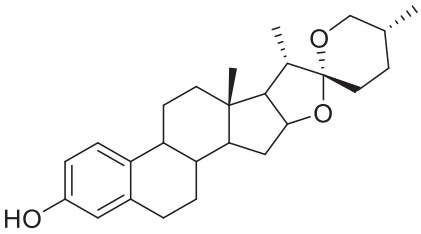
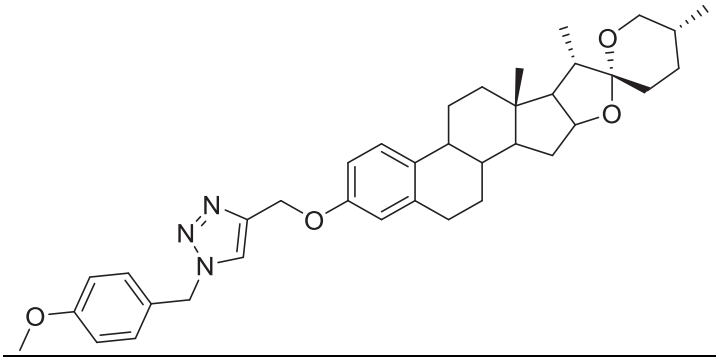


Fig. 2. Chemical structures of reported anti-Alzheimer's compounds containing triazole moiety and designed diosgenin-triazolyl compounds.

Table 1
The BBB permeant probability of the compounds.

Compd.	BBB permeant probability
	0.9063
	0.9194
	0.9817

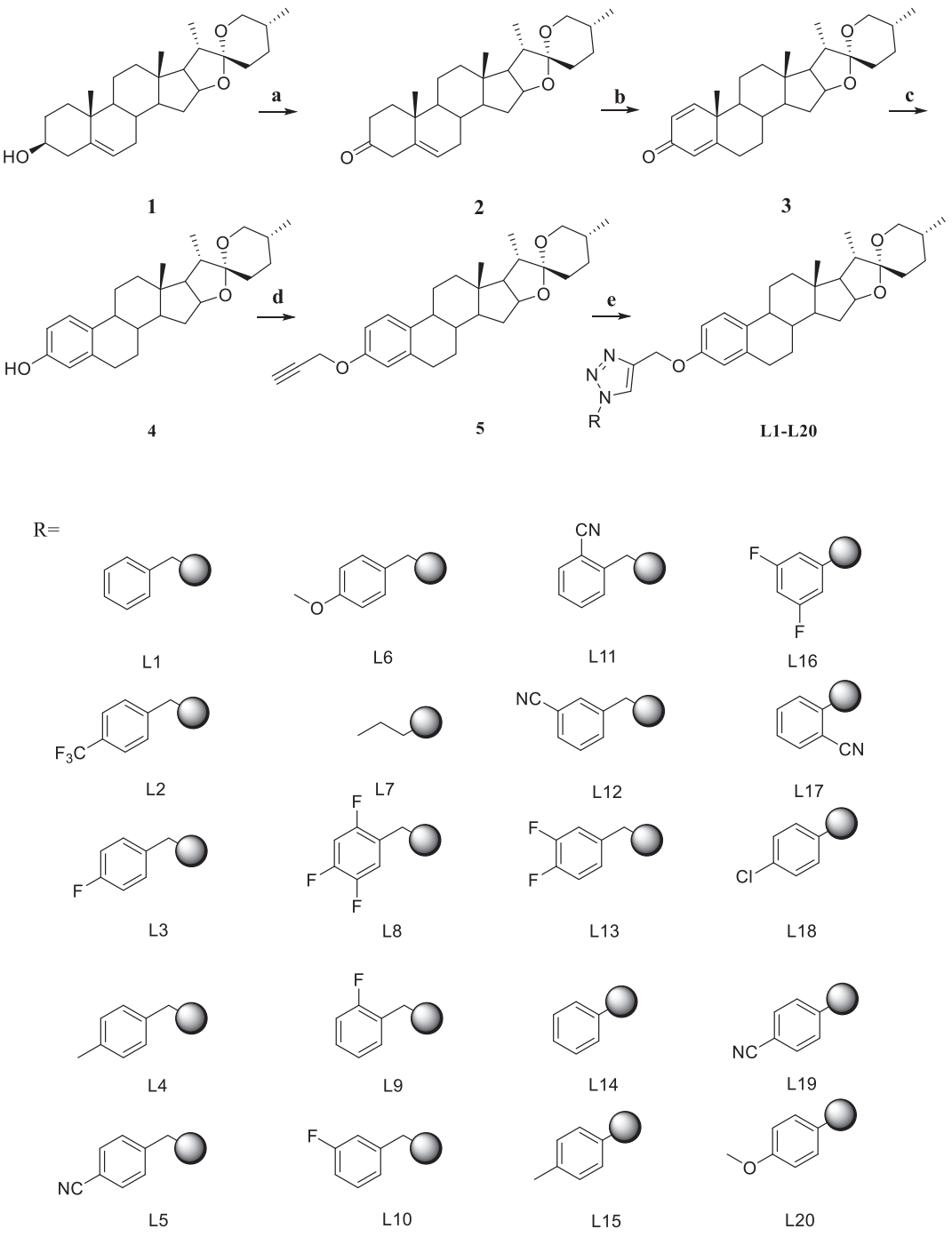
prepared from their corresponding bromoalkane with azidotrimethylsilane in hexamethylphosphoramide and used without further purification. Finally, a 1, 3-dipolar cycloaddition reaction of compound **5** with substituted azides in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate resulted in the formation of 1, 4-substituted triazolyl derivatives **L1-L20** in yields of 73%-89%.

The neuroprotective activity was investigated by assessing the ability of the diosgenin derivatives to protect human neuroblastoma SH-SY5Y cells against insult induced by an oxygen glucose deprivation (OGD) assay. The results were shown in Table 2.

Most of the compounds **L1-L20** showed good activity. The nice activity of **L1-L6** indicated that the introduction of benzyl or substituted benzyl groups at the end of 1, 2, 3-triazole can significantly increase the neuroprotection activity in vitro, and **L6** had the best activity (73.9 ± 3.6%). Besides, the introduction of 4-CF_3 (**L2**) would reduce the effect on the OGD model. Compared with the benzyl series, the introduction of saturated alkyl groups (such as compound **L7**) did not significantly increase the activity. The comparison of compounds **L3**, **L8,** and **L13** declared that it was not suitable to introduce multiple F atoms here. Furthermore, the site where F introduced was also important for activity, the priority order was 4-F (**L3**) > 3-F (**L10**) > 2-F (**L9**). Also, the introduction of 4-CN (**L5**) was far superior to 3-CN (**L12**) and 2-CN (***L11***). Compounds **L14-L20** were phenyl or substituted phenyl 1, 2, 3-triazole derivatives. The most active **L17** in this series was a 2-CN substituted derivative. Its cytoprotective rate on the OGD model was 81.2 ± 5.5% (10 μM), which was far better than other compounds. It probably because that the introduction of CN at this site increased the binding ability with related proteins. From the above, the results revealed that the introduction of triazole at 3-site of DIO can improve its neuroprotection activity against the OGD model. Then, the compounds with good activity, **L1-L6**, **L9**, **L10**, **L16**, **L17,** and **L20**, were selected for further exploration of anti-inflammation.

NO is a signaling molecule that plays a key role in immune and inflammatory responses, as well as neuronal transmission in the brain. Inhibitors of NO production have potential as anti-inflammatory drugs. As shown in Table 3, the selected compounds were tested on Abelson murine leukemia virus induced tumor (RAW 264.7), producing NO under LPS stimulation. The evaluation effect was quantified by Griess reaction. A typical non-steroidal anti-inflammatory drug indomethacin was used as positive control.

Most of this series compounds showed anti-inflammatory effects in the LPS-induced inflammatory model, and the strongest anti-inflammatory effect was **L6**, which was 40.4 ± 2.5%. In the comparison of compounds **L1-L6,** it was found that the introduction of electron-withdrawing groups such as 4-CF_3 (**L2**) and 4-CN (**L5**) was not conducive to the improvement of anti-inflammatory activity, while the introduction of electron-donating groups such as 4-CH_3 (**L4**) and 4-OCH_3 (**L6**) was beneficial to the improvement of anti-inflammatory activity. The priority sequence of substitution sites for different substituents was 3-F (***L10***) > 4-F (**L3**) > 2-F (**L9**). Compounds **L16, L17,** and **L20** were phenyl or substituted phenyl substituted derivative. The introduction of 3, 5-difluorine (**L16**) and 4-OCH_3 (**L20**), both decreased

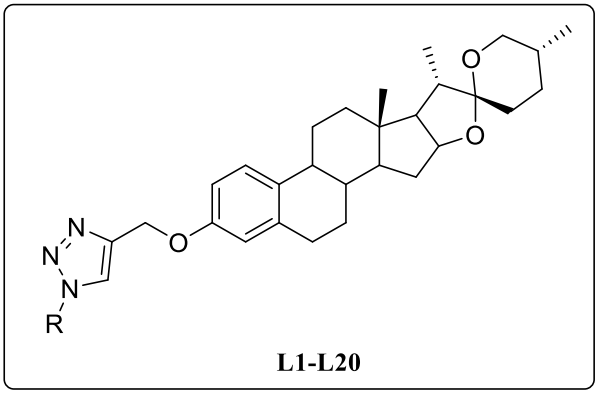


Scheme 1. General synthetic procedure for **L1-L20**. (a) aluminium isopropoxide, cyclohexanone, Toluene, 95 °C; (b) DDQ, TMSCl, THF, 68 °C, 18 h; (c) biphenyl, diphenylmethane lithium, Li, THF, 66 °C; (d) 3-bromopropine, K_2CO_3, MeCN, 65 °C, 3 h; (e) Sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, RN_3, DCM/H_2O, r.t, 8 h.

the anti-inflammatory activity in vitro. And the introduction of 2-CN (**L17**) increased the cytotoxic effect (Fig. 3), so no anti-inflammatory activity test was performed.

In order to identify new potential compounds for use as anti-AD agents, a total of 20 diosgenin-triazole derivatives were designed, synthesized and evaluated for their neuroprotective activities against oxygen-glucose deprivation-induced neurotoxicity and LPS-induced NO production. Preliminary studies revealed that triazole fragment was widely used in the design of drugs to treat Alzheimer's disease. Our results also found that the introduction of benzyl or phenyl triazole did improve the activity, and the substitution group had a great effect, but the introduction of benzyl triazole was better than that of phenyl triazole. 4-methoxybenzyl substituted triazole derivative **L6** was the most promising compound, which demonstrated the excellent neuroprotective and anti-inflammatory activity. Taken together, these findings may provide new insights into the development of neuroprotective drugs against Alzheimer's disease. Further work is underway to develop analogs with optimized structure and improved neuroprotective activity.

Table 2
Neuroprotective activity in OGD model in SH-SY5Y cell lines of compound **L1–L20**.



L1-L20

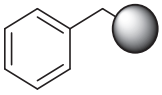
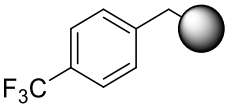
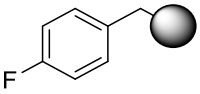
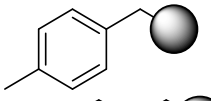
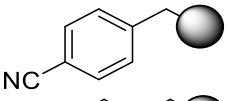
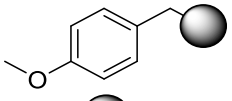
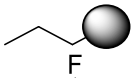
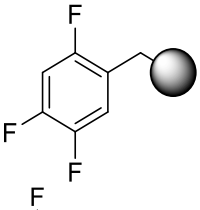
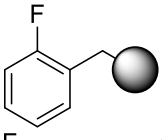
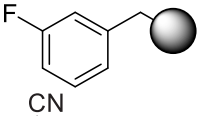
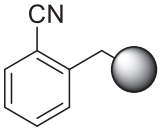
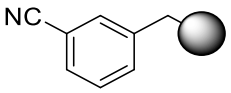
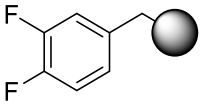
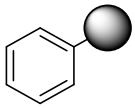
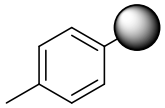
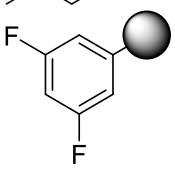
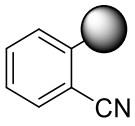
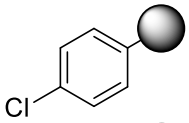
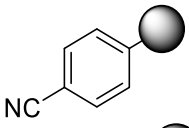
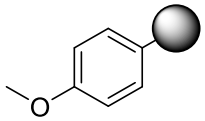
Compd.	R	Neuroprotection (%) (10 μM)
Diosgenin	–	3.5 ± 0.2
NAC[a]	–	84.3 ± 6.7
L1		50.7 ± 2.7
L2		35.5 ± 2.0
L3		57.6 ± 4.1
L4		66.4 ± 3.3
L5		40.1 ± 2.3
L6		73.9 ± 3.6
L7		16.5 ± 1.2
L8		12.1 ± 0.8
L9		34.1 ± 2.8
L10		51.6 ± 3.3
L11		NA

Table 2 (*continued*)

Compd.	R	Neuroprotection (%) (10 μM)
L12		NA
L13		NA
L14		12.1 ± 0.9
L15		3.3 ± 0.4
L16		71.4 ± 3.6
L17		81.2 ± 5.5
L18		NA
L19		NA
L20		40.1 ± 3.2

a:*N*-Acetyl-L-Cysteine (1 mM).

Table 3
NO inhibitory activities of the selected compounds.

Compound	NO inhibition %(10 μM)
Diosgenin	5.9 ± 0.6
Indo[a]	34.9 ± 2.7
L1	23.5 ± 1.4
L2	9.5 ± 0.7
L3	19.7 ± 1.3
L4	35.6 ± 1.8
L5	11.9 ± 0.8
L6	40.4 ± 2.5
L9	14.2 ± 0.9
L10	30.6 ± 1.7
L16	NA
L17	–
L20	9.9 ± 0.5

a:Indometacin.

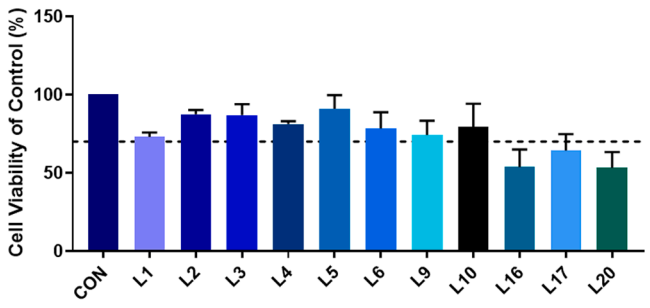


Fig. 3. The percent viability of RAW264.7 cell of selected compounds **L1, L2, L3, L4, L5, L6, L9, L10, L16, L17, L20** by MTT assay.

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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant 81973196), the National Science and Technology Major Project of China (Grant 2018ZX09735002), the 111 Project (Grant BP0719034) and the Natural Science Foundation of Shanghai, China (Grant 19ZR1413900).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128092.

References

1 Fu W-Y, Wang X, Ip NY. Targeting neuroinflammation as a therapeutic strategy for Alzheimer's disease: mechanisms, drug candidates, and new opportunities. *ACS Chem Neurosci*. 2019;10(2):872–879.
2 Hu J, Huang Y-D, Pan T, et al. Design, synthesis, and biological evaluation of dual-target inhibitors of acetylcholinesterase (AChE) and phosphodiesterase 9A (PDE9A) for the treatment of Alzheimer's disease. *ACS Chem Neurosci*. 2019;10(1):537–551.
3 Huang J-M, Huang F-I, Yang C-R. Moscatilin ameliorates tau phosphorylation and cognitive deficits in Alzheimer's disease models. *J Nat Prod*. 2019;82(7):1979–1988.
4 Walker KA, Ficek BN, Westbrook R. Understanding the role of systemic inflammation in Alzheimer's disease. *ACS Chem Neurosci*. 2019;10(8):3340–3342.
5 Wang W, Wang W, Yao G, et al. Novel sarsasapogenin-triazolyl hybrids as potential anti-Alzheimer's agents: design, synthesis and biological evaluation. *Eur J Med Chem*. 2018;151:351–362.
6 Cai L, Qu B, Hurtle BT, Dadiboyena S, Diaz-Arrastia R, Pike VW. Candidate PET radioligand development for neurofibrillary tangles: two distinct radioligand binding sites identified in postmortem Alzheimer's disease brain. *ACS Chem Neurosci*. 2016;7(7):897–911.
7 Popiolek M, Mandelblat-Cerf Y, Young D, et al. In vivo modulation of hippocampal excitability by M4 muscarinic acetylcholine receptor activator: implications for treatment of Alzheimer's disease and schizophrenic patients. *ACS Chem Neurosci*. 2019;10(3):1091–1098.
8 Kou X, Song L, Wang Y, et al. Design, synthesis and anti-Alzheimer's disease activity study of xanthone derivatives based on multi-target strategy. *Bioorg Med Chem Lett*. 2020;30(4):126927. https://doi.org/10.1016/j.bmcl.2019.126927.
9 Uddin MS, Kabir MT, Tewari D, et al. Revisiting the role of brain and peripheral Aβ in the pathogenesis of Alzheimer's disease. *J Neurol Sci*. 2020;416:116974. https://doi.org/10.1016/j.jns.2020.116974.
10 Fish PV, Steadman D, Bayle ED, Whiting P. New approaches for the treatment of Alzheimer's disease. *Bioorg Med Chem Lett*. 2019;29(2):125–133.
11 Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci*. 2015;16(6):358–372.
12 Pugazhenthi S. Chapter eight – metabolic syndrome and the cellular phase of Alzheimer's disease. *Prog Mol Biol Transl Sci*. 2017;146:243–258.
13 Wolf SA, Boddeke HWGM, Kettenmann H. Microglia in physiology and disease. *Annu Rev Physiol*. 2017;79(1):619–643.
14 Rossi D. Astrocyte physiopathology: at the crossroads of intercellular networking, inflammation and cell death. *Prog Neurobiol*. 2015;130:86–120.
15 Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. *J Neuroinflamm*. 2004;1(1):14.
16 Dorothy PS. Microglia function in central nervous system development and plasticity. Cold Spring Harbor Perspect Biol 2015, 10 (7), a020545.
17 Taipa R, Brochado P, Robinson A, et al. Patterns of microglial cell activation in Alzheimer disease and frontotemporal lobar degeneration. *Neurodegener Dis*. 2017;17(4-5):145–154.
18 VanItallie TB. Alzheimer's disease: innate immunity gone awry? *Metabolism*. 2017;69:S41–S49.
19 Manchikalapudi AL, Chilakala RR, Kalia K, Sunkaria A. Evaluating the role of microglial cells in clearance of Aβ from Alzheimer's brain. *ACS Chem Neurosci*. 2019;10(3):1149–1156.
20 Rogers J, Lue L-F. Microglial chemotaxis, activation, and phagocytosis of amyloid β-peptide as linked phenomena in Alzheimer's disease. *Neurochem Int*. 2001;39(5-6):333–340.
21 Ning C, Mo L, Chen X, et al. Triptolide derivatives as potential multifunctional anti-Alzheimer agents: synthesis and structure–activity relationship studies. *Bioorg Med Chem Lett*. 2018;28(4):689–693.
22 Morales JC, Ālvarez-Ferradas C, Roncagliolo M, et al. A new rapid kindling variant for induction of cortical epileptogenesis in freely moving rats. *Front Cell Neurosci*. 2014;8. https://doi.org/10.3389/fncel.2014.00200.
23 Cai Z, Wan C-Q, Liu Z. Astrocyte and Alzheimer's disease. *J Neurol*. 2017;264(10):2068–2074.
24 Acosta C, Anderson HD, Anderson CM. Astrocyte dysfunction in Alzheimer disease. *J Neurosci Res*. 2017;95(12):2430–2447.
25 Ozben T, Ozben S. Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. *Clin Biochem*. 2019;72:87–89.
26 Wang S, Wang F, Yang H, Li R, Guo H, Hu L. Diosgenin glucoside provides neuroprotection by regulating microglial M1 polarization. *Int Immunopharmacol*. 2017;50:22–29.
27 Yang G-X, Huang Y, Zheng L-L, et al. Design, synthesis and evaluation of diosgenin carbamate derivatives as multitarget anti-Alzheimer's disease agents. *Eur J Med Chem*. 2020;187:111913. https://doi.org/10.1016/j.ejmech.2019.111913.
28 Cai B, Seong K-J, Bae S-W, Chun C, Kim W-J, Jung J-Y. A synthetic diosgenin primary amine derivative attenuates LPS-stimulated inflammation via inhibition of NF-κB and JNK MAPK signaling in microglial BV2 cells. *Int Immunopharmacol*. 2018;61:204–214.
29 Xu M, Peng Y, Zhu L, Wang S, Ji J, Rakesh KP. Triazole derivatives as inhibitors of Alzheimer's disease: Current developments and structure-activity relationships. *Eur J Med Chem*. 2019;180:656–672.
30 Rastegari A, Nadri H, Mahdavi M, et al. Design, synthesis and anti-Alzheimer's activity of novel 1,2,3-triazole-chromenone carboxamide derivatives. *Bioorg Chem*. 2019;83:391–401.
31 Najafi Z, Mahdavi M, Saeedi M, et al. Novel tacrine-1,2,3-triazole hybrids: In vitro, in vivo biological evaluation and docking study of cholinesterase inhibitors. *Eur J Med Chem*. 2017;125:1200–1212.
32 Yazdani M, Edraki N, Badri R, Khoshneviszadeh M, Iraji A, Firuzi O. Multi-target inhibitors against Alzheimer disease derived from 3-hydrazinyl 1,2,4-triazine scaffold containing pendant phenoxy methyl-1,2,3-triazole: Design, synthesis and biological evaluation. *Bioorg Chem*. 2019;84:363–371.
33 Kaur A, Mann S, Kaur A, et al. Multi-target-directed triazole derivatives as promising agents for the treatment of Alzheimer's disease. *Bioorg Chem*. 2019;87:572–584.