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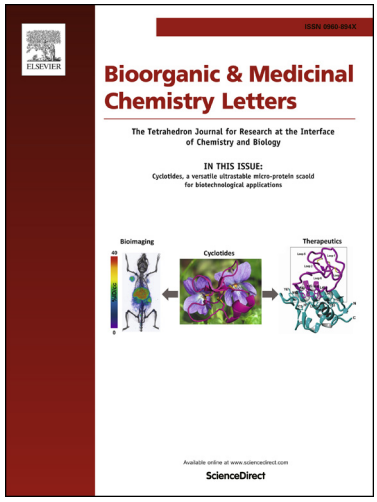

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Triptolide Derivatives as Potential Multifunctional Anti-Alzheimer Agents: Synthesis and Structure–Activity Relationship Studies

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ABSTRACT

Owning to the promising neuroprotective profile and the ability to cross the blood–brain barrier, triptolide has attracted extensive attention. Although its limited solubility and toxicity have greatly hindered clinical translation, triptolide has nonetheless emerged as a promising candidate for structure–activity relationship studies for Alzheimer's disease. In the present study, a series of triptolide analogs were designed and synthesized, and their neuroprotective and anti-neuroinflammatory effects were then tested using a cell culture model. Among the triptolide derivatives tested, a memantine conjugate, compound **8**, showed a remarkable neuroprotective effect against $A\beta_{1-42}$ toxicity in primary cortical neuron cultures as well as an inhibitory effect against LPS-induced TNF-α production in BV2 cells at a subnanomolar concentration. Our findings provide insight into the different pharmacophores that are responsible for the multifunctional effects of triptolide in the central nervous system. Our study should help in the development of triptolide-based multifunctional anti-Alzheimer drugs.

KEYWORDS: triptolide; memantine; neuroprotection; neuroinflammation; Aβ

Alzheimer's disease (AD), the most common form of dementia, is a major public health problem, with an ever-increasing number of affected individuals as the world's population ages. It is estimated that 47 million people worldwide were living with dementia in 2015, and this number will increase to more than 131 million by 2050. [1] At present, there is no cure for AD, and there is no way to halt the neurological damage. The current US FDA-approved drugs (**Fig. 1**) only help lessen or stabilize cognitive symptoms. [2] Thus, there is an urgent need for new effective therapies for AD.

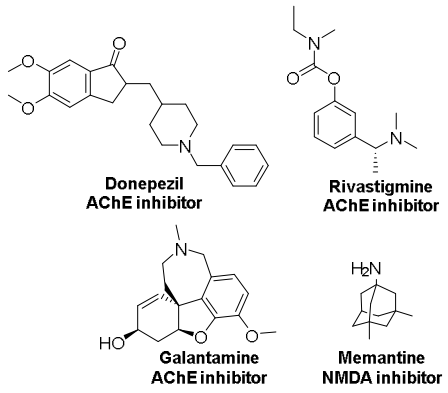


Fig. 1. Chemical structure of FDA-approved anti-AD drugs.

Although the neuropathology of AD is still not fully understood, deposition of β-amyloid peptides and neurofibrillary tangles consisting of hyperphosphorylated tau are the two primary pathological hallmarks of the disease. [3-5] Accumulating evidence indicates that the inflammatory response to these amyloid plaques and neurofibrillary tangles plays an important role in the pathogenesis of AD. [6-9] The inflammatory response, caused by the activation of microglial cells and astrocytes, leads to the overexpression of proinflammatory factors such as TNF-α and IL-1β, contributing to neuronal dysfunction and death.[10-12] In light of the highly complex pathology of AD and recent failures of several clinical candidates (such as solanezumab and verubecestat) targeting the amyloid or tau cascades, [13] multifunctional compounds with multiple modes of action targeting several specific pathogenetic AD processes might have promise as disease-modifying agents [14-15] and have better clinical efficacy.

Triptolide, a diterpene triepoxide, is one of the major active components of the Chinese herb *Tripterygium wilfordii* Hook F (Thunder God Vine), which has been used in Traditional Chinese Medicine to treat autoimmune and inflammatory diseases, such as rheumatoid arthritis, for decades. Since it was isolated, triptolide has been reported to possess a broad spectrum of biological actions, including anticancer, anti-inflammatory, immunosuppressive and anti-fertility activities. [16-19] More recently, triptolide was shown to reduce AD-like pathology in a transgenic mouse model of AD. [20-22] Although the mechanisms underlying the therapeutic effects of triptolide in the AD model remain to be fully elucidated, it was found that the activation of neurotrophic pathways, inflammatory cascades, modulation of oxidative stress and inhibition of BACE1 expression were involved, suggesting that triptolide might be a potent multifunctional natural lead compound for the treatment of AD.

Despite the promising neuroprotective activities of triptolide, poor aqueous solubility and toxicity have impeded preclinical development and clinical translation of the compound. Previous structure–activity relationship (SAR) studies[16] of triptolide identified the key pharmacophores that may account for its antitumor effect. In the present study, we explore whether these key pharmacophores are important for its neuroprotective activities. To this end, a series of triptolide derivatives were designed and synthesized, and their neuroprotective effects were evaluated with SAR studies, leading to the identification of conjugate **8**, a potential multifunctional anti-AD agent.

RESULTS AND DISCUSSION

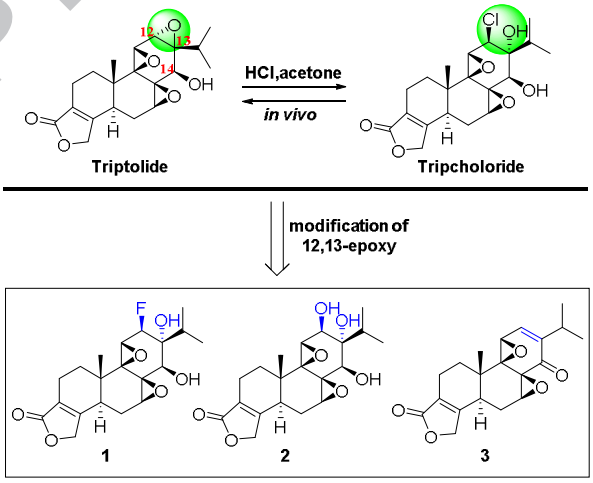


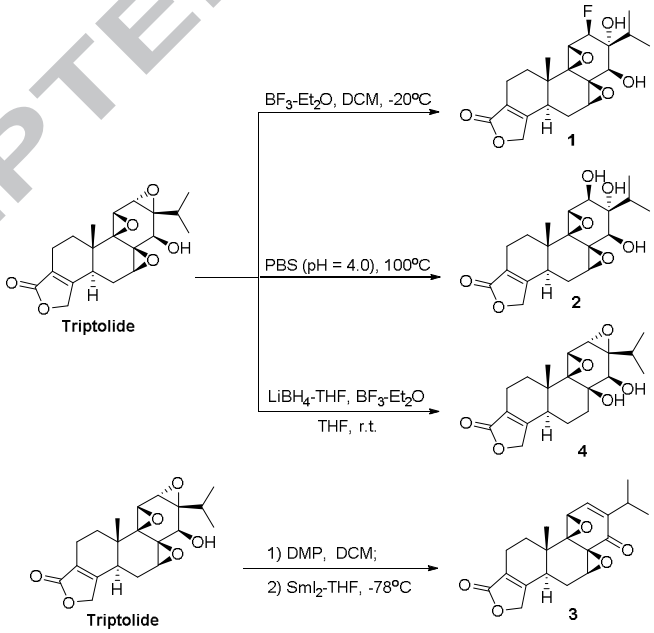
Fig. 2. Modification of the 12,13-epoxy moiety.

Epoxy groups and the C-14-hydroxyl group have been reported to be key functional groups, accounting for the antitumor effect of triptolide. [16] We therefore examined the role of these groups

in its neuroprotective action. We first focused on the epoxy groups. Among the three epoxy groups, C-12,13-epoxy is the most sensitive to nucleophilic attack and is the only group involved in the covalent binding with the cysteine of xeroderma pigmentosum B (XPB), [23] a receptor reported to be involved in its antitumor effect. [24] It has been demonstrated [25] that derivatives with nucleophilic ring-opening at the C-12 position, such as tripchlorolide (**Fig. 2**), completely lack immunosuppressive activity while retaining the anti-inflammatory activity. However, tripchlorolide can be converted into triptolide *in vivo*. We therefore synthesized C-12,13-epoxy opening analogs that cannot be efficiently converted to triptolide *in vivo* (**Fig. 2**). In addition, the C-7,8-β-epoxide-modified analog, compound **4** (**Scheme 1**), was synthesized to assess the roles of the different epoxy groups on neuroprotective activity.

The synthetic route is shown in **Scheme 1**. Compound **1** was synthesized via boron trifluoride etherate-mediated hydrofluorination, [26] while Compound **2** was prepared by hydrolysis of C-12,13-epoxy in PBS buffer (pH = 4.0) at 100 ºC. [27] SmI_2-induced reduction of α,β-epoxy ketone provided compound **3**. [28] When reacted with $LiBH_4$•THF and BF_3•Et_2O, [23] C-7,8-β-epoxy is selectively opened at room temperature, giving rise to product **4**.

We next evaluated the neuroprotective activities of these synthesized compounds. Because soluble Aβ oligomers play a key role in the pathogenesis of AD, [29-30] we used oligomeric $Aβ_{1–42}$ as a neurotoxin to establish a cell-based screen for neuroprotection. Primary cortical neurons[31] were incubated with Aβ oligomers in the absence or presence of varied concentrations of the different compounds for 24 h, and the neuroprotective effect was evaluated by measuring cell viability using the CCK8 assay. Doses showing no neurotoxicity were selected for the assay (data not shown). Aβ oligomers were prepared according to the reported method.[32] Triptolide was selected as positive control. As shown in **Fig. 3**, exposure to $Aβ_{1–42}$ at a concentration of 4 μM for 24 h resulted in a 40% reduction in primary cortical neuronal viability, compared with the control group. Pretreatment with triptolide completely rescued cells from $Aβ_{1–42}$ toxicity. An increase in cell viability, although slight, was also observed when primary cortical neurons were pretreated with compounds **1–4**, indicating that both C12,13-epoxy and C-7,8-β-epoxy are involved in neuroprotection. Hydrofluorination, hydrolysis or reduction resulted in the loss of the neuroprotective activity against Aβ toxicity.



Scheme 1. Synthesis of compounds **1–4.**

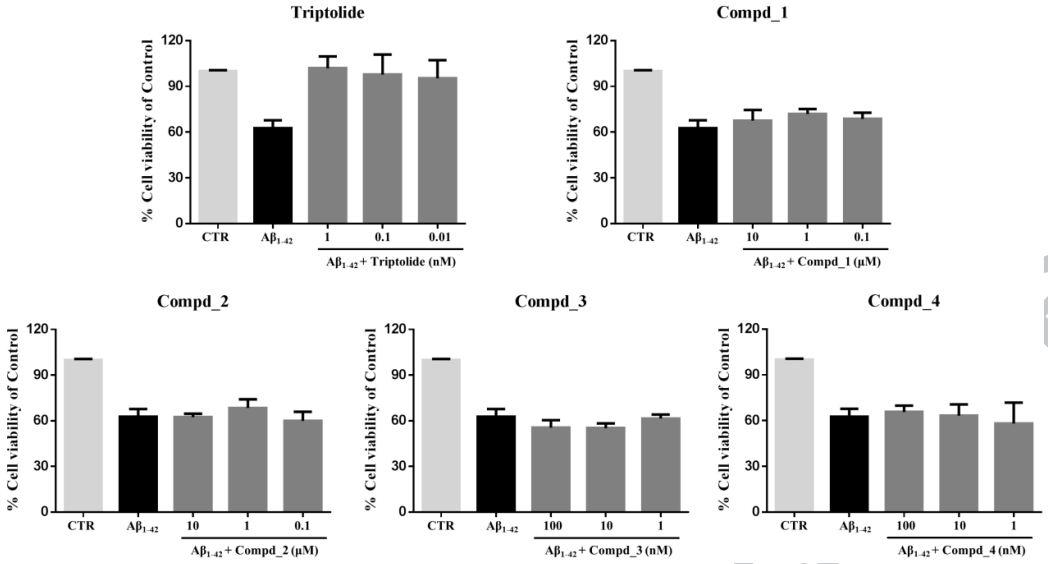


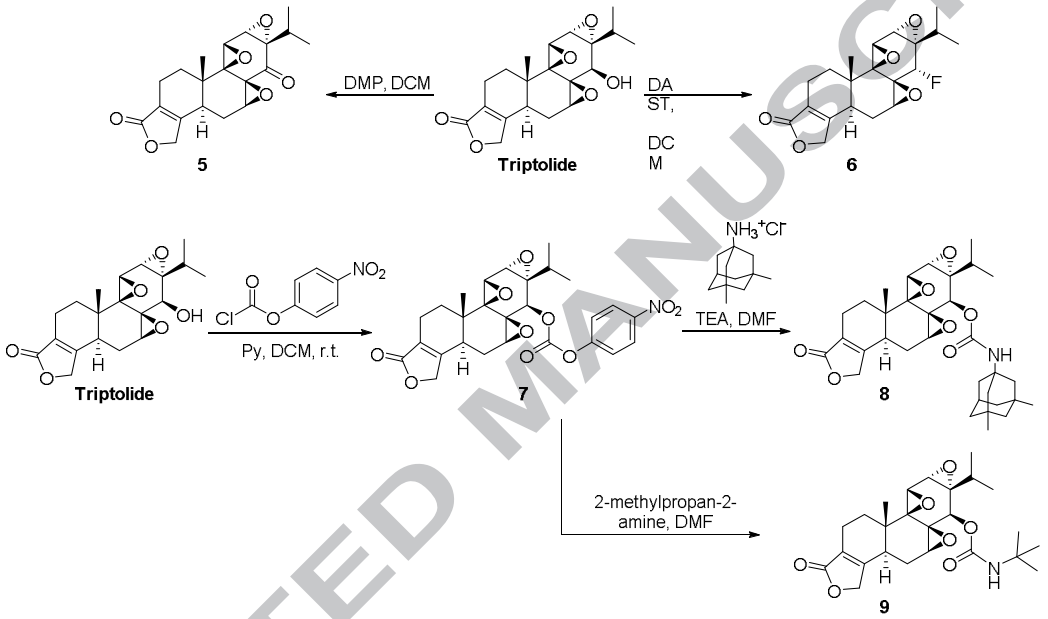
Fig. 3. Neuroprotective profiles of compounds **1–4** against $A\beta_{1-42}$ neurotoxicity. Primary cortical neurons were treated with $A\beta_{1-42}$ (4 μM) alone or in the presence of the indicated dose of triptolide derivatives for 24 h. Cell viability was determined by CCK8 assay. All results are presented as mean ± SD of three independent experiments.

We then turned our attention to the modification of the 14-β-OH group. As shown in **Scheme 2**, oxidation of the 14-OH group with Dess–Martin periodinane (DMP) at room temperature produced the ketone analog, **5**. Triptolide treated with DAST under ice bath conditions produced the 14-α-fluorinated derivative, **6**. The neuroprotective assay showed that compounds **5** and **6** completely lost their protective activities against Aβ cytotoxicity towards primary cortical neuronal cells (**Fig. 4**), suggesting that the 14-β-OH group is also a key pharmacophore for its neuroprotective action.

Given the importance of the 14-β-OH group for the bioactivity of triptolide, we next examined the effect of modifying this group by the addition of various moieties. We conjugated triptolide to memantine via a carbamate linker to generate a triptolide-memantine conjugate, compound **8**. The rational for selecting memantine as the moiety included the following: 1) the introduction of memantine might increase its neuroprotective activity, and the safety is known, as it is an NMDA receptor antagonist approved by the FDA for the treatment of AD; 2) memantine is a privileged scaffold used in CNS drug discovery, and a triptolide-memantine conjugate has not been reported in the literature. To explore the influence of moieties on neuroprotective effect, we also conjugated triptolide to a bulky moiety lacking neuroprotective activity via a carbamate linker to generate compound **9** as control. Conjugate **8** and **9** were prepared from triptolide via 2 steps as outlined in **Scheme 2**. CCK8 assay revealed that compound **8** dose-dependently reduced cell viability (**Fig. 4C**). No toxicity was observed at concentrations ≤ 10 nM. At 25, 50 and 100 nM, it induced a 7.7 ± 1.8%, 32.8 ± 0.7% and a 73.5 ± 2.6% reduction in viable cells, respectively. In comparison, cells exposed to triptolide at these concentrations showed a 27.2 ± 3.4%, 62.8 ± 5.9 and a 76.5 ± 2.9% reduction in cell viability, respectively. This demonstrates that the toxicity of conjugate **8** is slightly lower than that of triptolide in cortical neuronal cultures. Conjugate **8** completely protected against Aβ toxicity at a concentration of 0.01 nM, with a neuroprotective profile similar to triptolide, indicating that the conjugated large rigid moiety memantine does not reduce neuroprotective activity. Interestingly, when the triptolide conjugated to tert-butylamine (compound **9**), no neuroprotective effect against Aβ toxicity or even toxic effect on cell viability was observed at a concentration of 1000 nM (**Fig. 4E**). These results also confirm that 14-β-OH is an important pharmacophore for neuroprotective activity. Chemical modification of 14-β-OH may result in loss of activity. The different neuroprotective profile between compound **8** and **9** suggests that the conjugated moiety has a significant influence on the neuroprotective activity. We assumed

that a bulky moiety linked to 14-β-OH group would reduce the activity of triptolide, while a moiety having neuroprotective effect, such as memantine, might recover the activity. However, it's worth noting that only at concentrations ≥ 10 μM can memantine demonstrate neuroprotective effect against Aβ toxicity in cortical neurons.[33, 34] Thus, a possible explanation for the remarkable neuroprotective activity of conjugate **8** could be due to the synergistic effects of triptolide and memantine in cortical neuronal cells.

To investigate the stability of carbamate group under non-enzymatic hydrolysis conditions, conjugate **8** was incubated with neurobasal cell culture medium (supplemented with B-27 and N2) at 37 ^{o}C for 24h. The sample was then analyzed by LC-HRMS to determine the concentration of conjugate **8**. The results showed that 13% of conjugate **8** as well as positive control triptolide underwent decomposition or hydrolysis, which suggested that the conjugate **8** is stable under conditions used for neuroprotective assay.



Scheme 2. Synthesis of triptolide derivatives **5-6** and **8-9.**

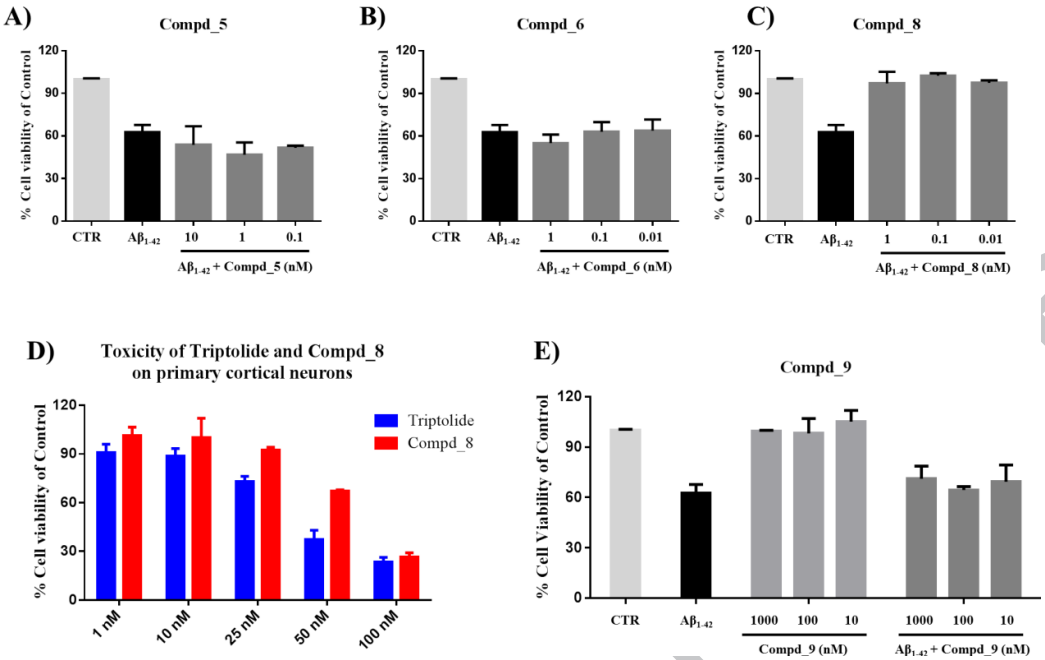


Fig. 4. Neuroprotective effects of triptolide derivatives against $A\beta_{1-42}$ cytotoxicity in primary cortical neuronal cultures. Primary cortical neurons were treated with $A\beta_{1-42}$ (4 μM) alone or in the presence of the indicated dose of triptolide analogs for 24 h. All results are presented as the mean ± SD of three independent experiments.

To further investigate whether the neuroprotective effects of these derivatives are related to their anti-inflammatory activities, we assessed the ability of these compounds to repress TNF-α production in LPS-activated BV-2 microglial cells, given that high TNF-α levels in the brain are reported to disrupt microglial clearance of Aβ and cause synaptic dysfunction.[10, 35] We measured the cytotoxicity of these compounds using the MTS assay for cell viability. BV-2 cells were incubated with various doses of the compounds for 24 h, and then cell viability was determined to evaluate the dose–effect relationship (data not shown). Doses that did not affect cell viability were selected for the anti-inflammation assay. BV-2 cells were pretreated with various doses of compounds for 1 h and stimulated with LPS (1 μg/mL) for another 23 h. ELISA kits from eBioscience (BMS607-3) were used to measure TNF-α levels at 24 h. As shown in **Fig. 5**, treatment with LPS produced a robust increase in TNF-α levels, compared with the vehicle control group. This significant increase in TNF-α expression was inhibited by the various compounds in a dose-dependent manner to different degrees, except for compounds **3** and **4**, which displayed only a weak anti-inflammatory activity in BV-2 cells. Compound **8** had the strongest anti-inflammatory activity, similar to that of triptolide, which completely suppressed the expression of TNF-α induced by LPS at a concentration of 10 nM. The anti-inflammatory effects of compounds **5** and **6** were slightly weaker than that of conjugate **8**. They completely inhibited TNF-α production at 20 nM.

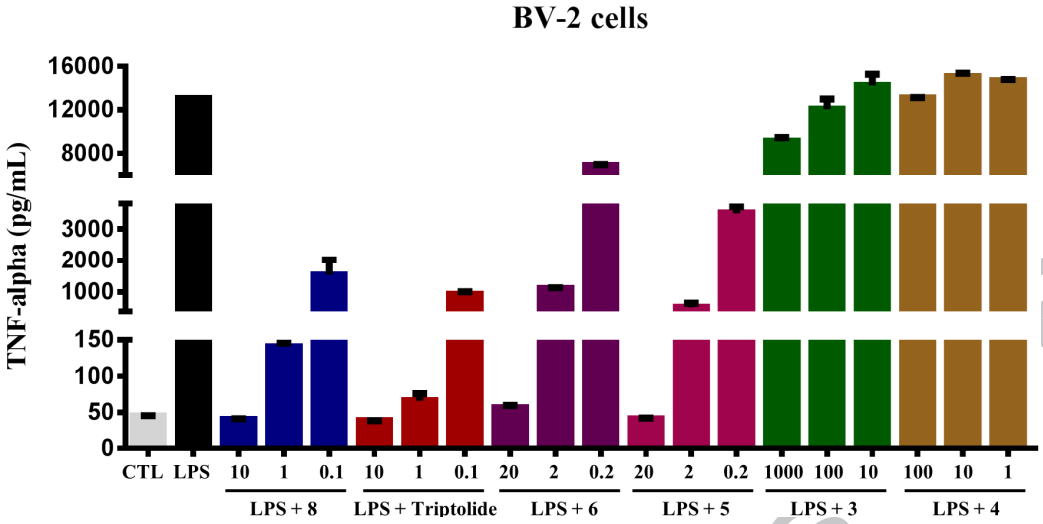


Fig. 5. Effects of triptolide derivatives on LPS-induced TNF-α expression in BV-2 cells. BV-2 cells were treated with LPS (1 μg/mL) alone or in the presence of the indicated concentrations (nM) of the various triptolide derivatives for 24 h. TNF-α levels in the cell supernatants were determined by ELISA assay. All results are presented as mean ± SD of three independent experiments.

Although the various compounds demonstrated moderate to potent anti-inflammatory activities, the activities of some of the derivatives are inconsistent with their neuroprotective activities towards Aβ cytotoxicity, such as compounds **5** and **6**. As described above, no neuroprotective activities were observed for compounds **5** and **6** in Aβ-treated cortical neurons, although they completely suppressed TNF-α expression. This suggests that different pharmacophores are responsible for the neuroprotective and anti-neuroinflammatory effects. It seems that both epoxy and 14-β-OH groups are required for the neuroprotective activities, whereas epoxy groups, but not the 14-β-OH group, are involved in the anti-neuroinflammatory activities. Indeed, triptolide has been reported to interact with several cellular proteins, including XPB, dCTP pyrophosphatase 1 (DCTPP1), polycystin-2 (PC-2), disintegrin and metalloprotease 10 (ADAM10).[36] Therefore, the various moieties in triptolide might participate in binding to these proteins.

In summary, we designed, synthesized and assessed the neuroprotective activities of triptolide derivatives in a cell culture model of AD. Preliminary SAR investigation revealed that both the epoxy and 14-β-OH groups were required for the neuroprotective activity against $A\beta_{1-42}$ toxicity towards primary cortical neurons. Because it was reported that the neuroprotective effect of triptolide was related to its anti-inflammatory activity, we examined the anti-neuroinflammatory activities of the various compounds. We found that the neuroprotective activities did not correlate well with the anti-inflammatory activities, indicating that different pharmacophores are responsible for the multifunctional effects of triptolide in the CNS. Together, our results demonstrate that the epoxy groups, but not the 14-β-OH group, are necessary for the anti-neuroinflammatory action of triptolide. SAR investigation showed that conjugate **8** exhibits promising neuroprotective and anti-neuroinflammatory profiles at subnanomolar concentration. In addition, a slightly lower cytotoxicity against cortical neurons was observed compared with triptolide. Given the encouraging cell-based neuroprotective and anti-neuroinflammatory activities, conjugate **8** merits further investigation, as it might open up new avenues for generating leads and drug candidates endowed with multifunctional neuroprotective profiles against AD. Thus, our study provides a foundation for the discovery of triptolide derivatives as potential anti-AD agents. Furthermore, our study provides a good lead (triptolide-memantine conjugate **8**) for further anti-AD drug discovery and development.

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SUPPLEMENTARY DATA

Detail about experimental information and spectra of target compounds.

REFERENCES AND NOTES

1. Prince M, Comas-Herrera A, Knapp M, Guerchet M, Karagiannidou M. World Alzheimer Report 2016. Alzheimer's Disease International (ADI), London.
2. Citron M. *Nat Rev Drug Discov*. 2010; 9:387.
3. Selkoe DJ. *Physiol Rev.* 2010; 81:741.
4. Blennow K, de Leon MJ, Zetterberg H. *Lancet*. 2006; 368:387.
5. Alberto SP, Matthew PF, Eliezer M, Bradley TH. *Perspect Med*. 2011; 1:a006189.
6. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. *Lancet Neurol.* 2015; 14:388.
7. Shadfar S, Hwang CJ, Lim MS, Choi DY, Hong JT. *Arch Pharm Res*. 2015; 38:2106.
8. McGeer PL, McGeer EG. *Acta Neuropathol*. 2013; 126:479.
9. Wyss-Coray T. *Nat Med.* 2006; 12:1005.
10. Koenigsknecht-Talboo J, Landreth GE. *J Neurosci.* 2005; 25, 8240.
11. Griffin WS, Sheng JG, Roberts GW, Mrak RE. *J Neuropathol Exp Neurol.* 1995; 54:276.
12. Griffin WS, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, Roberts G W, Mrak RE. *Brain Pathol*. 1998; 8:65.
13. Hardy J, De Strooper B. *Brain*. 2017; 140:853.
14. Zheng H, Fridkin M, Youdim M. *Pharmaceuticals (Basel)*. 2014; 7:113.
15. Rosini M, Simoni E, Caporaso R, Minarini A. *Future Med Chem*. 2016; 8:697.
16. Zhou ZL, Yang YX, Ding J, Li YC, Miao ZH. *Nat Prod Rep.* 2012; 29:457.
17. Zheng Y, Zhang WJ, Wang XM. *CNS Neurosci Ther*. 2013; 19:76.
18. Liu Q. *Int Immunopharmacol*. 2011; 11:377.
19. Qiu D, Kao PN. *Drugs R D.* 2003; 4:1.
20. Cui YQ, Wang Q, Zhang DM, Wang JY, Xiao B, Zheng Y, Wang XM. *Curr Alzheimer Res*. 2016; 13:288.
21. Wang Q, Xiao B, Cui S, Song H, Qian Y, Dong L, An H, Cui Y, Zhang W, He Y, Zhang J, Yang J, Zhang F, Hu G, Gong X, Yan Z, Zheng Y, Wang X. *Dis Model Mech*. 2014; 7: 1385.
22. Cheng S, LeBlanc KJ, Li L. *PLoS One*. 2014; 9:e108845.
23. He QL, Titov DV, Li J, Tan M, Ye Z, Zhao Y, Romo D, Liu JO. *Angew Chem Int Ed Engl*. 2015; 54:1859.
24. Titov DV, Gilman B, He QL, Bhat S, Low WK, Dang Y, Smeaton M, Demain AL, Miller PS,

Kugel JF, Goodrich JA, Liu JO. *Nat Chem Biol*. 2011; 7:182.

25. Yu DQ, Zhang DM, Wang HB, Liang XT. *YaoXueXueBao*. 1992; 27:830.
26. Zderic JA, Limon DC, Ringold HJ, Djerassi C. *J Am Chem Soc*. 1959; 81:3120.
27. Dai D, Yuan H, Musser JH. WIPO Pat Appl. 2002070472.
28. Molander GA, Hahn, G. *J Org Chem.* 1986; 51:2596.
29. Viola KL, Klein WL. *Acta Neuropathol*. 2015; 129:183.
30. Tu S, Okamoto S, Lipton SA, Xu H. *Mol Neurodegener*. 2014; 9:48.
31. Primary cortical neurons were prepared according to previously described: Hou ST, Jiang SX, Zaharia LI, Han X, Benson CL, Slinn J, Abrams SR. *J Biol Chem*. 2016; 291:27007.
32. Dahlgren KN, Manelli AM, Stine WB, Baker LK, Krafft GA, LaDu MJ. *J Biol Chem.* 2002; 277:32046.
33. Song MS, Rauw G, Baker GB, Kar S. *Eur J Neurosci.* 2008; 28:1989.
34. Tremblay R, Chakravarthy B, Hewitt K, Tauskela J, Morley P, Atkinson T, Durkin JP. *J Neurosci.* 2000; 20:7183.
35. Ralay Ranaivo H, Craft JM, Hu W, Guo L, Wing LK, Van Eldik LJ, Watterson DM. *J Neurosci.* 2006; 26:662.
36. Manzo SG. Zhou ZL, Wang YQ, Marinello J, He JX, Li YC, Ding J, Capranico G, Miao ZH. *Cancer Res*. 2012; 72:5363.

Graphical Abstract