Bioorganic & Medicinal Chemistry Letters 28 (2018) 689–693

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Triptolide derivatives as potential multifunctional anti-Alzheimer agents: Synthesis and structure–activity relationship studies

Chengqing Ning [a], Liumei Mo [b,c], Xuwei Chen [d], Wentong Tu [e], Jun Wu [b,*], Shengtao Hou [d,*], Jing Xu [a,*]

[a] SUSTech Academy for Advanced Interdisciplinary Studies and Department of Chemistry, Southern University of Science and Technology, Shenzhen 518055, China
[b] Department of Neurology, Peking University Shenzhen Hospital, Shenzhen 518036, China
[c] Shantou University Medical College, Shantou 515041, China
[d] Brain Research Centre and Department of Biology, Southern University of Science and Technology, Shenzhen 518055, China
[e] Department of Chemistry, Southern University of Science and Technology, Shenzhen 518055, China

ARTICLE INFO

Article history:
Received 11 September 2017
Revised 5 January 2018
Accepted 12 January 2018
Available online 16 January 2018

Keywords:
Triptolide
Memantine
Neuroprotection
Neuroinflammation
Aβ

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 sub-nanomolar 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.

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.

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

* Corresponding authors.
E-mail address: xuj@sustc.edu.cn (J. Xu).

https://doi.org/10.1016/j.bmcl.2018.01.019
0960-894X/

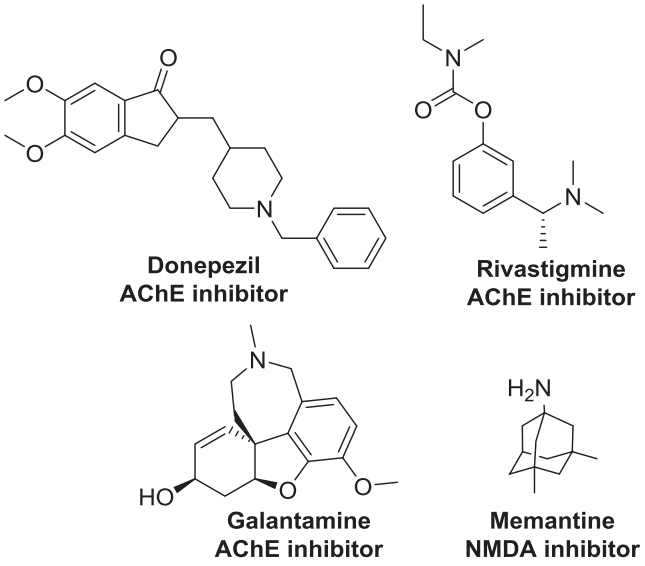


Fig. 1. Chemical structure of FDA-approved anti-AD drugs.

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

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),

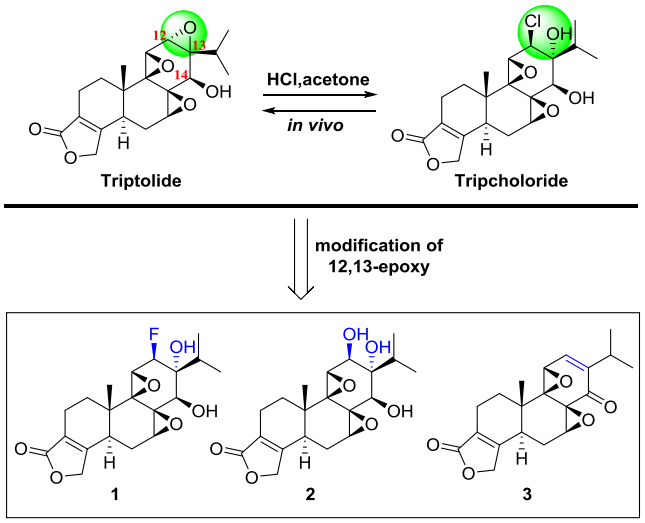


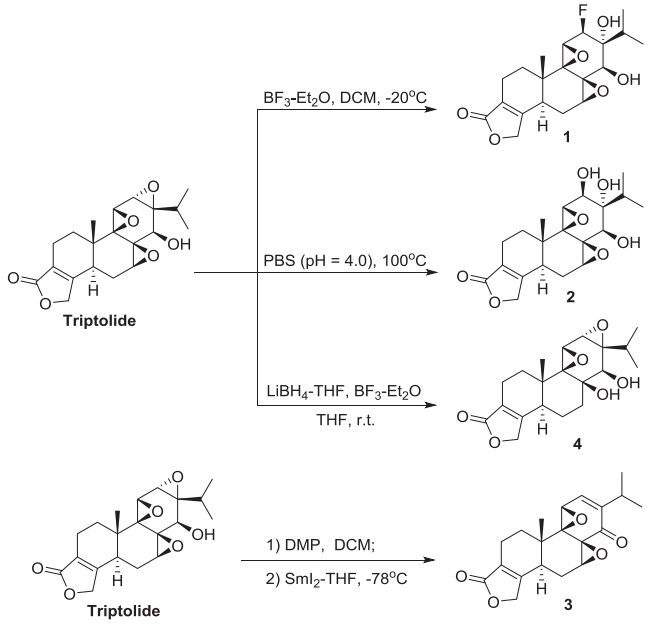
Fig. 2. Modification of the 12,13-epoxy moiety.

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 °C.[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.

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**



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