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Research paper

Design, synthesis and biological evaluation of tricyclic diterpene derivatives as novel neuroprotective agents against ischemic brain injury

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ABSTRACT

Lead compound **7** has neuroprotective effects, and it was discovered by screening a small synthetic natural product-like (NPL) library. Based on the lead, a series of tricyclic diterpene derivatives was designed and synthesized, and their neuroprotective effects were further evaluated against glutamate-, oxygen and glucose deprivation (OGD)- and nutrient deprivation-induced neuronal injury using cell-based assays. To our delight, most of these synthetic compounds exhibited increased neuroprotective effects and blood–brain barrier (BBB) permeability without cellular toxicity. The most potent compound, compound **30**, showed significantly improved neuroprotection against neuronal injury in primary neurons. Furthermore, compound **30** exhibited remarkable neuroprotection in transient middle cerebral artery occlusion (tMCAO) rats by reducing their infarct sizes and neurological deficit scores. A mechanistic exploration using in vitro and in vivo experiments showed that the neuroprotection of these compounds was at least partly mediated by improving the levels of glutathione (GSH), superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) protein. Therefore, these tricyclic diterpene derivatives could be used as promising leads for the development of a new type of neuroprotective agents against ischemic brain injury.

1. Introduction

Ischemic stroke, which results from a transient or permanent reduction in cerebral blood flow, accounts for more than 80% of all strokes and is a leading cause of human morbidity and mortality throughout the world [1]. In general, brain ischemia is characterized by excess glutamate release, insufficient nutrient supply, and oxygen and glucose deprivation (OGD), which result in neuronal death. Glutamate is a major excitatory neurotransmitter that plays an important role in the mammalian brain [2]. However, excessive release of this amino acid can induce excitotoxicity, which is a major factor in neuronal injury that is associated with many acute and chronic brain disorders, such as neurodegenerative diseases (for example, amyotrophic lateral sclerosis, Parkinson's, Alzheimer's and Huntington's diseases) [3,4], traumatic brain injury [5], and especially brain ischemic stroke [6]. An excessive level of the excitatory neurotransmitter glutamate is not only a key factor in ischemia-induced neuronal injury, but it also produces reactive oxygen species (ROS) and further leads to the inhibition of antioxidants such as superoxide dismutase (SOD) and glutathione (GSH) synthesis [7]. Oxidative stress is also considered to be one of the major mechanisms that triggers the pathogenic actions of ischemic stroke [8], which is produced when ROS surpass the endogenous antioxidant system, leading to the injury of essential components in neural cells [9]. Therefore, the reduction of ROS has been considered a promising remedy to attenuate neuronal damage from ischemia [10]. This reduction includes elements such as the antioxidative enzyme heme oxygenase-1 (HO-1), which

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protects neurons from the deleterious effects of ROS [11]. To date, the only drug that is currently approved by the FDA to treat ischemic strokes is tissue plasminogen activator (t-PA), which has a very short therapeutic window (3 h) and is used in less than 5% of stroke patients, thus significantly limiting the percentage of treatable subjects [12,13]. This deficiency indicates an urgent need to search for novel agents with neuroprotective effects to provide treatments for brain ischemic strokes.

Diterpenoids are a large family of natural products that exhibit a wide range of biological activities, such as anti-inflammatory, anti-HIV, anti-tumor, anti-diabetic, antibacterial and especially neuroprotective activity [14–16]. For instance (Fig. 1), carnosic acid, a component of rosemary, strongly promotes neurite outgrowth by activating antioxidant responsive element (ARE)-mediated transcription through the activation of Nrf2 [17]; serofendic acid, a sulfur-containing diterpenoid derived from fetal calf serum, exhibits potent neuroprotective actions in neurons against the cytotoxicity of glutamate and nitric oxide [18,19]; triptolide, the major active component of Tripterygium extracts, exerts both neuroprotective and neurotrophic activities in Parkinson's disease models [20,21]. Natural products have provided a rich resource for drug discovery in recent years [22]. However, the scarcity and poor activities of these products frequently limit their development. Recently, the production of modified, natural-based compounds and synthetic natural analogs has been shown to be an effective strategy in drug discovery.

In our search for novel and potent anti-ischemic agents, we report the discovery of neuroprotective leads that were uncovered by screening our small synthetic natural product-like (NPL) library against glutamate-induced neuronal injury. This novel small NPL library contains approximately 200 tricyclic diterpene analogs, and it was constructed based on the cyclization reaction [23,24]. Compound **7** (Scheme 1) of the NPL library was chosen as a novel neuroprotective lead, and its neuroprotective percentage against glutamate-induced neuronal injury was 53.7% (Table 1), respectively. A series of tricyclic diterpene analogs was synthesized based on the lead compound. These newly synthesized analogs were evaluated for their neuroprotective effects against glutamate-, OGD- and nutrient deprivation-induced injury in cell-based assays. The most potent analog was further evaluated for its neuroprotective effects in a rat cerebral ischemia model.

2. Chemistry

The lead compound and a series of tricyclic diterpene derivatives (methylol, esters, acyls and amides) was synthesized according to the pathways described in Schemes 1–3.

The synthesis of lead compound and diterpenoid methylol and esters is outlined in Scheme 1. Compound **6** was synthesized according to our previously reported procedure [25]. The details were as follows. The coupling reaction of 6,7-epoxygeranyl acetate with 4-methoxybenzylmagnesium chloride in the presence of Li_2CuCl_4 yielded compound **1**. Key intermediate **2** was obtained by the cyclization of **1** under the Lewis acid Et_2AlCl. The bromination of compound **2** with Br_2 in CH_2Cl_2 furnished intermediate **3**. Compound **4** was produced by the protection of the 3-hydroxyl group of **3** with TBSCl. The treatment of **4** with n-butyllithium, and then with dry CO_2 afforded compound **5**. The deprotection of the TBS group under boron trifluoride etherate afforded compound **6**. According to the Stork-Eschenmoser hypothesis [26,27], compound **2** is a racemate, thus all the synthesized diterpenoids herein belong to racemates.

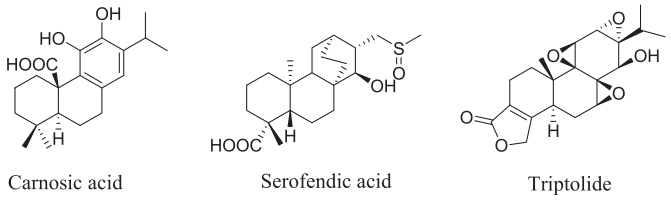

Fig. 1. Chemical structures of neuroprotective diterpenoids.

The esterification of **6** with corresponding alcohols in the presence of a catalytic amount of H_2SO_4 gave lead compound **7** and compounds **8** and **9**. The methylol **11** was obtained by the reduction of **5** with $LiAlH_4$ and then the deprotection of the TBS group under boron trifluoride etherate.

The synthesis of diterpenoid acyls is outlined in Scheme 2. Compounds **13**–**17** were furnished by the esterification of compound **2** with acetic anhydride under DMAP, followed by Friedel–Crafts acylation with various acyl chlorides respectively. The hydrolysis of **13**–**17** with NaOH in MeOH produced compounds **18**–**22**. Treatment of **18** with BBr_3 produced compound **23**. The oxidation of **18** with 2-iodoxybenzoic acid (IBX) formed compound **24**. Compound **25** was prepared by the esterification of **18** with trifluoroacetic anhydride.

The synthesis of diterpenoid amides is outlined in Scheme 3. Amide derivatives **26**–**32** were synthesized by the condensation of **6** with amines or their hydrochlorides under 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) in CH_2Cl_2. The hydrolysis of **29** with LiOH in THF afforded **33**.

3. Result and discussion

3.1. Neuroprotective effects against neuronal damage induced by glutamate

Glutamate is the primary excitatory amino acid in the mammalian brain. The glutamate-induced over-excitation of neurons plays a pivotal role in the pathogenesis of neurodegeneration [28]. For instance, the ischemic stroke is related to a dramatic increase in excitatory glutamate in the extracellular space. The neuroprotective effects of the lead compound **7** and their synthetic derivatives **6** and **8**–**33** were evaluated against glutamate-induced injury in primary rat cerebellar granule neuronal cells. The results are shown in Table 1.

Compared with lead compound **7** (53.7%, neuroprotection), most of these derivatives showed increased or equivalent cell viability against glutamate-induced neuronal injury. Compounds **13**, **18**, **23**, **25**–**27** and **30**–**32** exhibited more neuroprotective abilities than the leads. Compounds **18** and **30** in particular possessed much more neuroprotective potency than **7**.

For acyl-substituted compounds, the small acetyl group (**18**) showed much more potent activity than the relatively large propionyl (**19**), 3-methoxypropionyl (**20**), isobutyryl (**21**) and octanoyl (**22**) groups. Notably, **21** and **22** showed almost no activity against glutamate-induced neuronal injury. If the methoxy group on the benzene ring was hydrolyzed to a hydroxyl group (Scheme 2), the activity was clearly decreased, and a similar situation occurred as the 3-hydroxyl group was oxidized to carbonyl (**18** vs **23** and **24**).

For amide substituted compounds, the butyramide group (**30**) was better than other amide substituents (**26**–**28** and **31**–**33**). Compound **30** (10 μM) showed the most potent neuroprotective activity against neuronal damage induced by glutamate, and was more effective than positive control edaravone (50 μM).

For ester-substituted compounds, regardless of the ethyl and butyl esters (**8** and **9**), they had no obvious improving effects in terms of neuroprotection compared with **7**, and a similar situation