Structural Diversity of Neuritogenic Substances and their Application Perspective

J. Qi*, Y. Luo and L. Gao

College of Pharmaceutical Sciences, Zhejiang University, Yu Hang Tang Road 388, Hangzhou 310058, China

Abstract: Small molecular weight substances are functional, as neurotrophic factors can be developed as therapeutic drugs to treat neurodegenerative disorder. Recently, a large number of natural and synthetic neuritogenic compounds have been discovered. These compounds have various structural features, including terpenoids, lipids, alkaloids, steroid glycosides, small molecular peptides, and so on. Some of them possess not only neurotrophic properties but also neuroprotective activities. The structure-activity relationships (SARs) and mechanism of action of some important compounds have been studied intensively. Increasing experimental evidence suggests that several of these compounds can be promising candidates for drug development.

Keywords: Neuritogenic substances, Alzheimer's disease, natural products, neurotrophic factors, nerve growth factor, neuronal cells.

INTRODUCTION

Populations are aging worldwide. Aging is a natural process that involves the progressive deterioration of various biological functions of a mature organism. Neurodegenerative disorder is one of the major problems of an aging society. Alzheimer's disease (AD) is the most prevalent form of dementia among neurodegenerative diseases in the aged population, and the number of cases is expected to increase exponentially worldwide. However, the pathogenesis of AD is not yet fully understood. Various hypotheses with respect to disease aetiology have been proposed. First, the progressive production and subsequent accumulation of β-amyloid (Aβ) play a central role in AD pathogenesis [1, 2]. Dysfunctional Aβ metabolism is the underlying cause of the neurodegeneration and dementia observed in AD. A leading strategy for the development of AD pharmacotherapies is the modulation of Aβ production, aggregation, and/or clearance. A large number of molecules, which show the capacity to prevent Aβ aggregation or even disaggregate Aβ oligomers, fibrils, and plaques, have been discovered. Several such molecules are under Phase III clinical trials [3, 4]. Second, oxidative stress is an important factor that acts in the early stages of AD [5]. Antioxidant approaches are used in the treatment of AD because oxidative damage has been suggested as a key pathological mechanism in AD. However, therapeutic approaches targeting oxidative damage have not been completely successful. Third, the inflammatory process has a fundamental role in the pathogenesis of AD [6]. Recent studies indicate that inflammation is not merely a bystander in neurodegeneration but a powerful pathogenetic force in the disease process. Furthermore, a few other therapeutic programs, such as reducing tau phosphorylation and/or aggregation, preserving mitochondrial structure and function, and so on, have been targeted [7, 8]. Current approaches for the treatment of AD provide only temporary symptomatic relief and do not inhibit and/or reverse the underlying disease mechanisms. To date, the only drugs available for the treatment of AD patients are acetylcholinesterase inhibitors (AChEIs) tacrine, donepezil, galantamine, and rivastigmine, which are considered symptomatic therapies [3]. AChEIs exert its effect by preventing the enzymatic degradation of the neurotransmitter acetylcholine (ACh), resulting in increased ACh concentrations in the synaptic cleft and enhanced cholinergic transmission [9]. Although intensive studies are being conducted to develop effective drugs to cure AD, effective treatment to cure AD is not yet available even when more than one century has passed. Therefore, the discovery and development of a new strategy is necessary for effective drug development. Aβ, oxidation, inflammation, and other AD-related pathological changes induce various types of toxic mechanisms that contribute to neuronal death [10]. Thus, the prevention and protection of neuron cell survival, as well as the maintenance of its function, is considered a promising strategy to treat AD. Neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF), play critical roles in neuronal development, survival, and functional maintenance of neurons [11-13]. However, because neurotrophic factors are high molecular weight polypeptides, they are unstable and are unable to cross the blood-brain barrier (BBB) [14]. Therefore, the application of neurotrophic factors as medicines for the treatment of neurodegenerative disorders is assumed to be difficult. To address this issue, considerable efforts have been made to find and/or synthesize small molecules that have neurotrophic properties and are able to pass through BBB.

In recent years, various achievements have been made in searching for small molecules with neurotrophic properties.

*Address correspondence to this author at the College of Pharmaceutical Sciences, Zhejiang University, Yu Hang Tang Road 388, Hangzhou 310058, China; Tel/Fax: +86-571-88208627; E-mail: qijianhua@zju.edu.cn

1389-5575/11 $58.00+.00

Hundreds of neuritogenic substances from animals, plants, and microorganisms were isolated, and their structures were characterized. Some of them were synthesized completely, and their structure-activity relationships (SARs) and mechanism of action were studied further. This review covers most of the literature reporting the isolation, structures, biological activity, SARs, and mechanism of action of small molecular weight neuritogenic substances from 1995 to the present. We review the natural and synthetic substances that can significantly induce neurite outgrowth on PC12, Neuro 2a, SH-SY5Y cells, and other related cells, including terpenoids, lipids, alkaloids, steroid glycosides, small molecular peptides, and other types of chemical compounds. The natural sources of neuritogenic substances, synthetic method of the natural compounds, and their derivatives are involved. Two neuritogenic agents, which are in clinical studies for drug development, are discussed. We also provide the perspective for their development as drugs to treat neurodegenerative disorders such as AD.

TERPENOIDS

Terpenoids are the second largest group of secondary metabolites. They have an incredibly diverse structure and activity even if they are all originally derived from a simple molecule called isoprene. Terpenoids have been reported to possess various biological activities, such as antimicrobial [15], anticancer [16], anti-inflammatory [17], cardioprotective [18], hepatoprotective [19], neuritogenic, and neuroprotective effects [20]. Most of the discovered terpenoids with neurogenic activity belong to iridoids, sesquiterpenes, and diterpenoids. Examples of important iridoids or sesquiterpenes are geniposide, genipin, and merrilactone A. Due to their structural and biological features, these natural products attract great attention from pharmaceutical chemists.

Geniposide and Genipin

Geniposide (**1**), an iridoid glucoside, was first isolated by Inouye and Saito in 1969 from the fruits of *Gardenia jasminoides* Ellis, a popular Chinese herb medicine used to treat febrile diseases (Fig. **1**) [21]. Geniposide (**1**) was rediscovered by Yamazaki and coworkers in 1996 as an inducer of neurite outgrowth on PC12h cells [22]. Geniposide-type iridoids (**2**-**6**) isolated from various medicinal herbs induce neurite outgrowth, which are similar to or more potent than geniposide (**1**) (Fig. **1**). Compounds (**3**) and (**5**) show a higher neuritogenic activity than its optical isomers **2** and **4** at position C-6, respectively. These results suggest that the configuration of geniposide-related compounds plays an important role in the interaction between iridoid compounds and the target molecule for neuritogenic function [23].

The mechanism of action of geniposide (**1**) in inducing neuronal differentiation of PC12 cells is to activate insulinotrophic hormone glucagon-like peptide-1 (GLP-1) receptor through the mitogen-activated protein kinase (MAPK) signaling cascade [24]. Geniposide (**1**) prevents PC12 cells from oxidative damage induced by H_2O_2 through the phosphatidylinositol 3-kinase (PI3K) signaling pathway [25] and peroxynitrite damage *via* the MAPK signaling pathway [26].

Genipin (**7**), the aglycone of geniposide, is an iridoid compound isolated from an extract of *Gardenia fructus*.

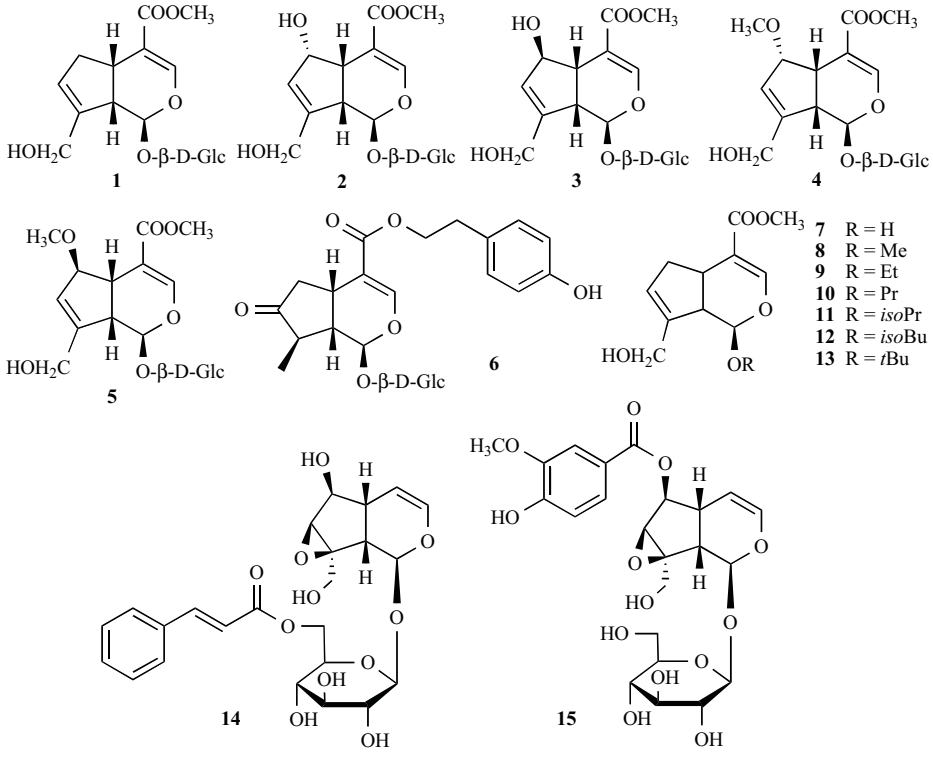


Fig. (1). Structures of neuritogenic iridoids.

Genipin (**7**) stimulates prominent neuritogenic activity in PC12h cells [22], and Neuro 2a cells [27]. Genipin (**7**) induces neurite outgrowth in Neuro 2a cells and PC12h cells through the nitric oxide (NO)-cyclic GMP (cGMP)-dependent protein kinase (PKG) signaling pathway, followed by phosphorylation of extracellular signal-regulated kinase (ERK). In Neuro 2a cells, the ERK phosphorylation induced by genipin is mediated by PKG activation rather than tyrosine receptor kinase A (TrkA) activation [28]. Apart from the neuritogenic activity, genipin is also known to have neuroprotective effects on cytotoxicity induced by Aß in hippocampal neurons [29], serum deprivation [27], and oxidative stress in Neuro 2a cells [30].

Ohkubo and coworkers used a geniposide overlay method to detect genipin/geniposide-binding proteins. In the cytosolic fraction of the rat brain cortex and PC12h cells, a major geniposide binding protein with a molecular weight of about 170 kDa was found. The major protein band was comparable with the protein band (about 170 kDa) using anti-neuronal nitric oxide synthase (nNOS) antiserum detected by simultaneous Western blot analysis. Therefore, nNOS is one of the target molecules of genipin/geniposide, suggesting that nNOS plays a crucial role in the neurotrophic activities of genipin/geniposide [31].

The results of metabolism and pharmacokinetics of genipin and geniposide in rats indicate that the detected genipin sulfate is a major metabolite in the blood stream instead of the parent forms of geniposide and genipin after oral administration of genipin or the Gardenia fruit decoction. More importantly, the oral administration of 200 mg/kg of genipin leading the high mortality in rats implies that genipin is toxic to rats [32]. Therefore, a more stable molecule during metabolic process that shows similar neuritogenic activity as genipin is required. Recently, a series of 1-alkyloxygenipins (**8**-**13**) has been designed and synthesized to improve the stability of genipins based on the structural and electronic properties of genipins. Its neuritogenic activities in PC12h cells were also examined. (1*R*)-isopropyloxygenipin (**11**) showed activity comparable with that of genipin (**7**), and unlike the parent compound genipin, it was found to be physiologically stable in rat liver homogenate [33].

Picrosides

Picrosides I and II (**14**, **15**) are natural iridoids isolated from *Picrorhiza scrophulariiflora* (Fig. **1**). Compounds (**14**) and (**15**) failed to induce neurite outgrowth from PC12D cells even at a high concentration of 60 μM, but caused a concentration-dependent enhancement of NGF-induced (2 ng/mL) neurite outgrowth from PC12D cells [34]. Picrosides I (**14**) and II (**15**) enhance the staurosporine-, basic fibroblast growth factor (bFGF)-, or dibutyryl cyclic AMP (dbcAMP)-stimulated neurite outgrowth from PC12D cells. These results are probably caused by amplifying a downstream step of MAPK in the intracellular MAPK-dependent signaling pathway. Therefore, picrosides I (**14**) and II (**15**) may become selective pharmacological tools to study the MAPK-dependent signaling pathway in the neurite outgrowth induced by neuritogenic substances, including bFGF [35].

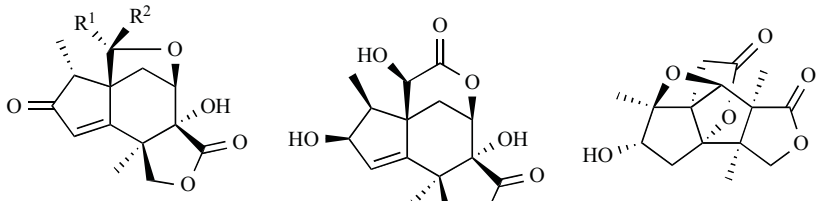
Jiadifenin

New seco-prezizaane-type sesquiterpenes, jiadifenin, isolated from the methanol extract of the pericarps of *Illicium jiadifengpi* and indigenous to the southern part of China, is an equilibrated mixture of epimers **16** and **17** on the acetal carbon C-10. The absolute structures of jiadifenins (**16**, **17**) were confirmed by the chemical conversion of the known sesquiterpene (2*S*)-hydroxy-3,4-dehydroneomajucin (**18**) [36, 37]. The structures of jiadifenins are shown in Fig. (**2**).

Sesquiterpenes **16**, **17**, and **18** significantly promote neurite outgrowth in primary cultures of fetal rat cortical neurons at levels as low as 0.1 μM [37]. In chemical terms, the densely oxygenated and highly compact but functionality-laden structure of jiadifenin poses significant issues in the propositions directed to its synthesis. From both biological and chemical perspectives, Danishefsky reported the first total synthesis of racemic jiadifenin and the establishment of a modality for its biological evaluation [38, 39].

Merrilactone A

Merrilactone A (**19**) (Fig. **2**), a kind of sesquiterpene, was obtained from the methanol extract of *Illicium merrillianum* in 2000 by Fukuyama and co-workers [40]. This natural product exhibits unusual neurotrophic activity, promoting the neurite outgrowth of fetal rat neurons at concentrations of 0.1-10 μM. The small molecule has attracted the attention of many synthetic groups because of its interesting structure and remarkable neuritogenic activity. To date, the total synthesis of merrilactone A was accomplished by three groups [41-44]. The flexible racemic approach or asymmetric route described by these groups can provide practical access to analogous structures and enantiomers for future detailed biological and SAR studies of merrilactone A.



16 $R^1 = OH$, $R^2 = CO_2Me$
17 $R^1 = CO_2Me$, $R^2 = OH$

18

19

Fig. (2). Structures of neuritogenic sesquiterpenes.

Neovibsanin

The rearranged vibsane-type diterpenoids, neovibsanins A (**20**) and B (**21**), were isolated from the leaves of *Viburnum awabuki* by Fukuyama in 1996 (Fig. **3**) [45]. Neovibsanins A (**20**) and B (**21**) are rare natural products containing more complex and tricyclic structures than other vibsane-type diterpenoids. Their unusual structure, based on the cyclohexene core fused with two tetrahydrofurans possessing five chiral carbons, includes two quaternary centers and two side chains. Both **20** and **21** show notable neurotrophic activity to promote neurite outgrowth in NGF-induced PC12 cells [46]. The architectural complexity, outstanding neurotrophic activity, and extreme scarcity of these vibsane-type diterpenoids have attracted increasing attention in recent years. Recently, two more new vibsane-type diterpenoids possessing neurogenic activity called neovibsanin L (**22**) and (8*Z*)-neovibsanin M (**23**) have been isolated from the leaves of *Viburnum sieboldii*. Compounds **22** and **23** significantly enhance the neurite outgrowth of NGF-induced PC12 cells at concentrations ranging from 20-40 μM [47].

Chen *et al.* synthesized a series of neovibsanin derivatives to study their neurotrophic ability on PC12 cells. Among the biological activity of several neovibsane derivatives, 4,5-bis-epi-neovibsanin A (**24**) shows a prominent ability to induce neurite outgrowth compared with control cultures [48].

Clerodane-Type Diterpenoids

Novel clerodane-type diterpenoids **25**, **26**, **27**, and **28** were isolated from the MeOH extract of *Ptychopetalum olacoides*, which is used for the treatment of chronic degenerative conditions of the nervous system in Brazilian folk medicine (Fig. **3**). Compounds **25** and **26** that contain a furan ring significantly enhance NGF-mediated neurite outgrowth in PC12 cells at concentrations ranging from 0.1-50.0 μM for **25** and 0.1-30.0 μM for **26**, whereas **27** and **28** have no activity on NGF-mediated PC12 cells in the same concentration range tested [49].

LIPIDS AND ITS DERIVATIVES

The analogues and derivatives of lipids are widespread in nature. The main biological functions of lipids include energy storage, structural components of cell membranes, and important signaling molecules. Lipids can be broadly defined as hydrophobic or amphiphilic small molecules that include ceramides, glycosphingolipids, gangliosides, N-acylated lipids, bacterial lipoproteins, and other natural complex compounds. A large number of lipids have been found to have neuritogenic activity, such as docosahexaenoic acid (DHA), ganglioside, polyacetylene, cerebroside, and the newly isolated gentiside.

Polyunsaturated Fatty Acids

DHA (22:6, n-3) (**29**) (Fig. **4**) is essential for normal brain development and function in humans and animals. It is

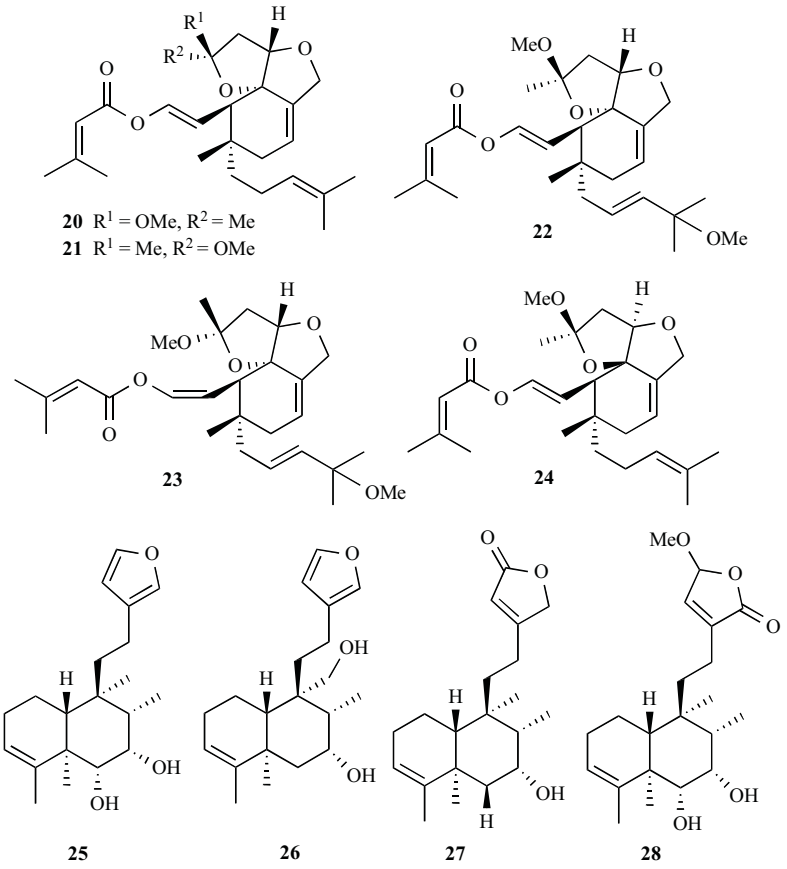


Fig. (3). Structures of vibsane- and clerodane-type diterpenoids.

also considered important for the prenatal development of the central nervous system. Clinical and laboratory studies indicate that DHA (**29**) deficiency causes a number of neurodegenerative diseases involve cell membrane abnormalities, and that supplementation with DHA (**29**) may protect neuronal damage and cell death [50, 51].

DHA (**29**) plays a crucial role in diverse cellular functions such as promoting neurite outgrowth of PC12 cells [52, 53], hippocampal cells [54], and cultured fetal rat cortical neurons *in vitro* [55]. The neuroprotective effects of DHA are also widely investigated [56].

However, there are only a few reports on the mechanism of the proteins responsible for DHA internalization and its subsequent metabolism. Recently, the study of Marszalek *et al.* has suggested that DHA may function to induce neuronal differentiation by being preferentially metabolized into phospholipids (PLs) and stimulating PL synthesis. They found that acyl-CoA synthetase long-chain (Acsl) 6 preferentially promotes DHA (**29**) metabolism reveal that perhaps other proteins may function to internalize DHA (**29**) efficiently [57]. Dagai and coworkers showed that DHA (**29**) significantly induces immediate-early neurogenesis events, as evident in both morphological and molecular markers [58].

Polyacetylene

Lembehyne A (LB-A) (**30**), B (**31**), and C (**32**) are novel neuritogenic polyacetylene isolated from an Indonesian marine sponge *Haliclona* sp. [59, 60]. Lembehyne A (**30**) induces neurite outgrowth in Neuro 2a and PC12 cells at 0.1 and 2 μg/mL, respectively. Lembehynes B (**31**) and C (**32**), which have different types of long carbon chain parts compared with those of lembehyne A (**30**), exhibit neuritogenic activity against Neuro 2a cells. To confirm the absolute stereostructure of lembehyne A and to explore simplified medicinal lead compounds, lembehyne A was first totally synthesized by utilizing alkyne formation with dimethyl-1-diazo-2-oxopropylphosphonate and asymmetric reduction with Alpine-borane as the key reactions [61]. Analogues **33**-**37** of lembehyne A with different types of long carbon chain parts were prepared to study the SAR. The result of the neuritogenic activity for lembehynes A-C and their analogues revealed that carbon chain length is important for the activity, whereas the unsaturated bonds in the long carbon chain parts are not. Analogue **35** with the 3*R* configuration showed a much stronger activity than analogue **36** with 3*S* configuration of the same carbon chain part. The result indicated that the stereochemistry of the hydroxyl group in lembehynes at position C-3 has an important effect on the activity [60]. Moreover, the importance of the *R*-configuration in the C-3 chiral center for the activity implies the presence of a target protein for LB-A. A radioactive photoaffinity probe [^{125}I]18-(2'-azido-5'-iodo-ben-zoyloxy)-LB-18 ([^{125}I]azido-LB-18) was synthesized to identify the target protein for LB-A. Results suggest that the protein of *M*r of 30 kDa is the target protein of LB-A and may play an important role in the neurite outgrowth in Neuro 2a cells

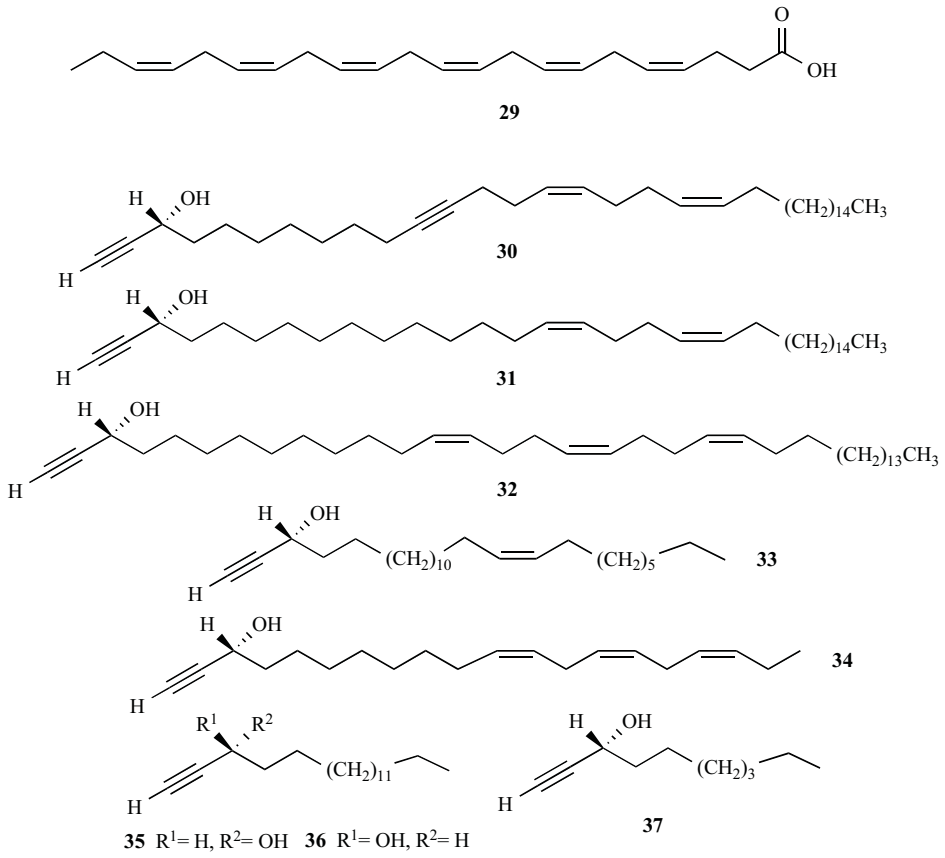
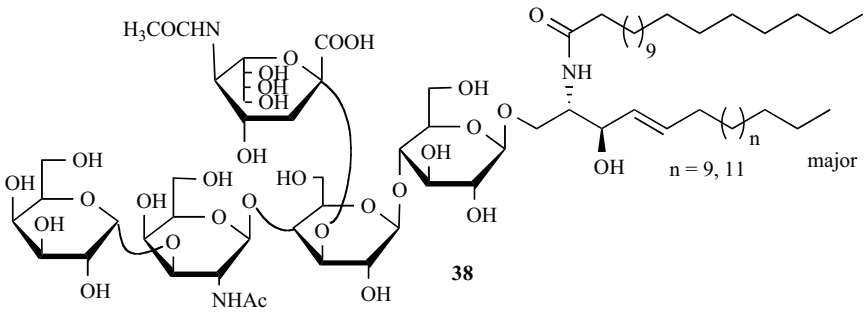
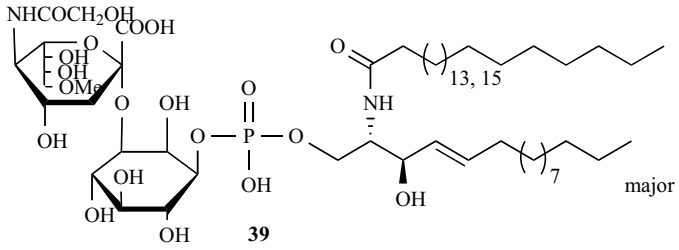


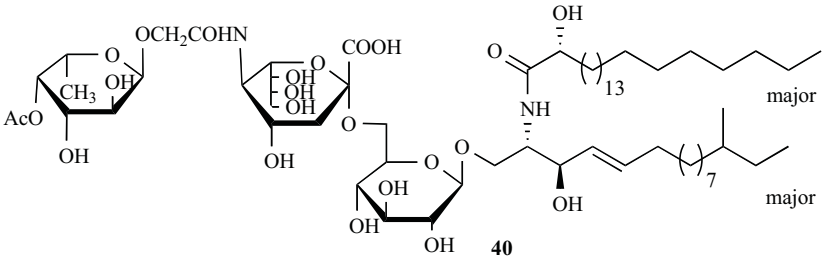
Fig. (4). Structures of DHA, lembehynes, and their analogues.



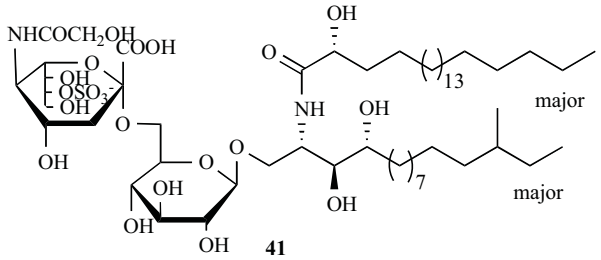
38



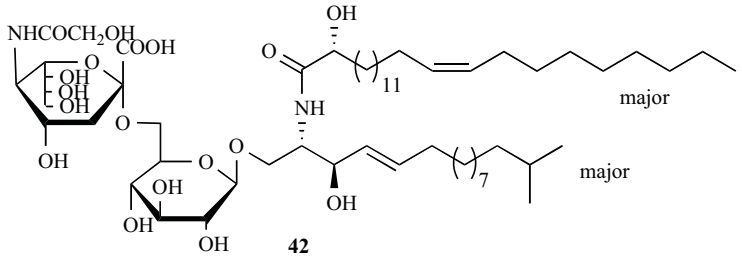
39



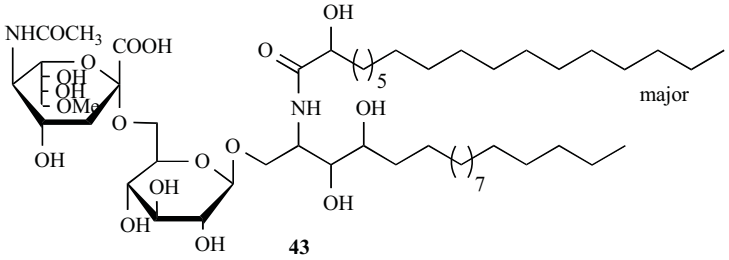
40



41



42



43

(Fig. 5). Contd…..

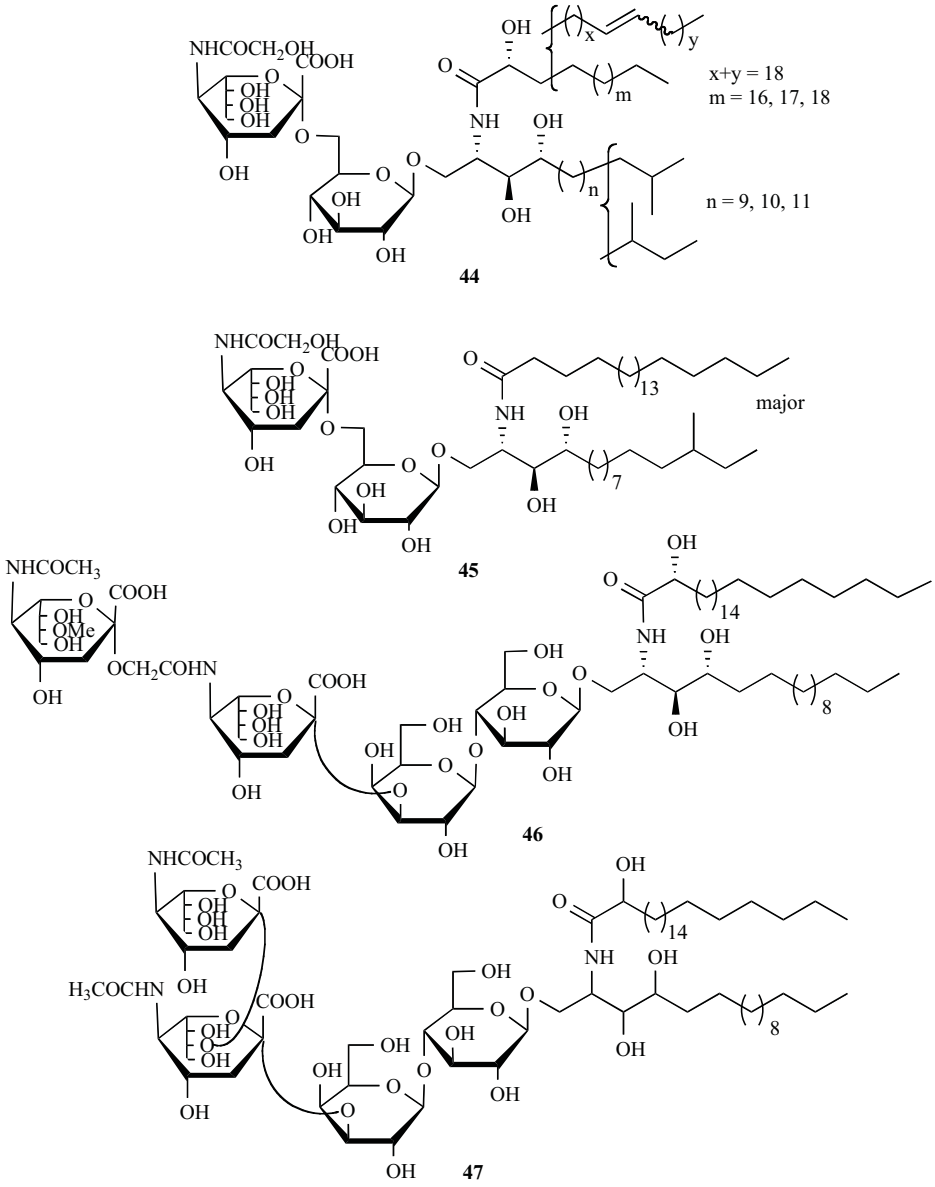


Fig. (5). Structures of selected neuritogenic gangliosides.

[62]. The structures of lembehynes A-C and their analogues are schematically illustrated in Fig. (**4**).

Gangliosides

Gangliosides, N-acetyl-neuraminic acids (sialic acid) containing glycosphingolipids, are enriched particularly in the nervous tissues and brain of echinoderms. The structural feature of the echinoderm gangliosides is its unique carbohydrate moieties. According to the number of sialic acid, gangliosides are classified into three types: monosialo-gangliosides, disialo-gangliosides, and trisialo-gangliosides. However, the ceramide parts of the gangliosides are composed of sphingosine- or phytosphingosine-type long-chain base (LCB) and fatty acids with or without α-hydroxy.

Recently, because of their biological functions especially their effects on neurological diseases, gangliosides have received much attention. Monosialo-ganglioside (GM1, **38**) (Fig. **5**) is a naturally occurring sialic acid containing glycosphingolipid and is a component of the plasma membrane in eukaryotic cells [63]. GM1 is known to show positive effects on neurological diseases and is one of the substances intensively evaluated for use as therapy for neurodegenerative disease. There are several publications describing the beneficial effect of GM1 administration on patients with the early onset of AD or on animal models of this disease [64, 65].

Higuchi and coworkers performed many studies on the isolation, structural elucidation, and biological test of gangliosides from echinoderms. More than 40 gangliosides with different structures were obtained, and over 75% of them displayed potential neuritogenic activity. Gangliosides have no ability to induce neurite outgrowth but they

significantly enhance neurite outgrowth in the presence of trace NGF (3 or 5 ng/mL) [66, 67]. However, further investigations of the SAR of gangliosides have been hampered because of the extensive complexity of their structures. Thus, we only gathered the major neurotrophic gangliosides (structures of selected neuritogenic gangliosides are shown in Fig. **5**). The possible SARs of these gangliosides are summarized as follows:

(1) The polar glycosphingolipids CJP-2 (**39**), CJP-3, and CJP-4 are inositolphosphoceramide derivatives isolated from the feather star *Comanthus japonica*. These unique glycosphingolipids with different numbers of sialic acids on the saccharide moiety show much higher activity than mammalian ganglioside GM1 (**38**). Therefore, the sugar and ceramide part linked by a phosphate may be more effective [68, 69].

(2) Among the monosialo-gangliosides, the neuritogenic activity of CEG-3 (**40**), CG-1 (**41**), SCG-1 (**42**), SCG-2, SCG-3, and DSG-A (**43**) is more effective than that of GM1 (**38**) [66, 70-72].

(3) The difference in the activity between HLG-1 (**44**) and SJG-1 (**45**), despite the similarity in the sugar moiety, may be due to the difference in the structure of ceramide [67].

(4) A comparison of the gangliosides with tandem disialoyl moiety [LLG-3 (**46**), LLG-5, HLG-2, LMG-4 (**47**), and HLG-3] and gangliosides with 8-*O*-Me silalic acid (LLG-3 and LLG-5) shows better activity than other gangliosides [67].

(5) Trisialo-gangliosides (SJG-2, LLG-5, CEG-8, CEG-9, and CJP-4) show a higher activity than GM1 [67, 69, 73].

Cerebrosides

Cerebrosides are a group of glycosphingolipids composed of a hexose, an amide-linked long-chain fatty acid, and a long-chain aminoalcohol (sphingoid). They are important components of a wide variety of tissues and organs in biological systems. They show antitumor, immunosuppressive, anti-HIV-1, antihepatotoxic, antifungal, anti-ulcerogenic, and neurite outgrowth activities, *etc.* [74].

Termitomycesphins A-F (**48**-**53**) (Fig. **6**) are isolated from the Chinese mushroom *Termitomyces albuminosus* (Berk.) Heim. ("Jizong" in Chinese) [75, 76]. These cerebrosides possess a hydroxy group around the middle of the LCB and show neuritogenic activity on PC12 cells. The initial SAR studies suggest that not only the fatty acyl-chain length but also the polarity of the molecules (i.e., the presence of the extra hydroxyl group around the middle of the LCB) play important roles in the activity of termitomycesphins. This is further reinforced by the known cerebroside (**54**) also isolated from the *T. albuminosus* (Fig. **6**). The compound (**54**) does not have a hydroxy group around the middle of the LCB, is inactive against PC12 cells. The inactive cerebroside is converted to the di- and tetrahydroxylated derivatives, which become neuritogenic [76].

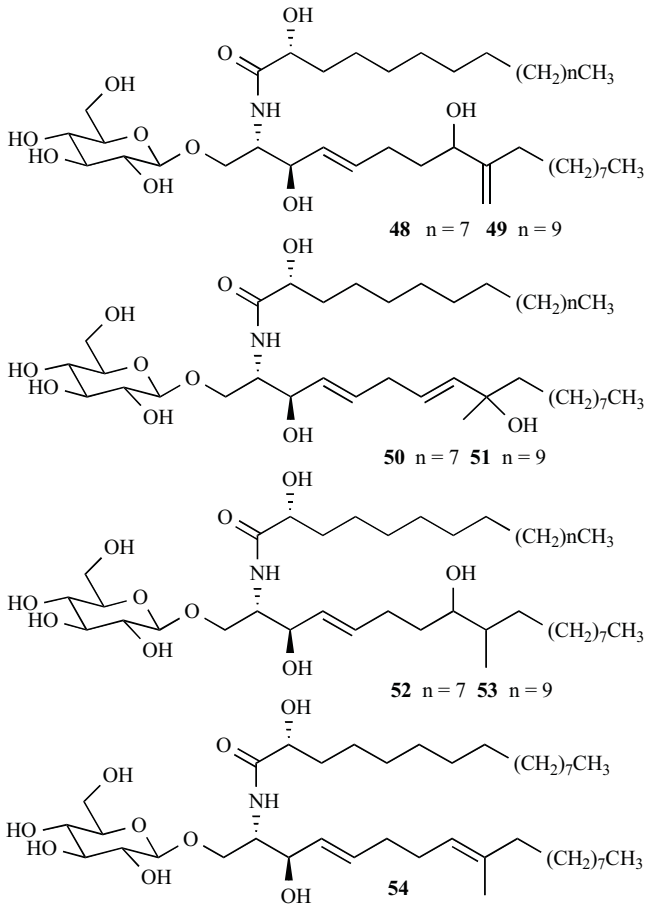


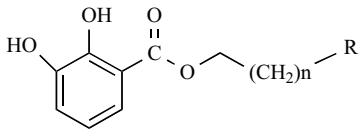
Fig. (6). Structures of neuritogenic (**48**-**53**) and inactive (**54**) cerebrosides.

Alkyl Benzoates

Gentisides A-K (**55**-**65**) (Fig. **7**), which are new alkyl 2,3-dihydroxybenzoates, are a novel class of neuritogenic compounds isolated from the traditional Chinese medicine *Gentiana rigescens* Franch [77, 78]. They are structurally different from one another because they possess varying alkyl chain lengths with or without an isopropyl or isobutyl group at the end of the alkyl chain. These compounds (**55**-**65**) are potent inducers of neurite outgrowth on PC12 cells. Gentiside C (**57**), which has the shortest alkyl chain length, exhibits the highest neuritogenic activity among all gentisides isolated. Gentiside C (**57**) shows a significant neuritogenic activity against PC12 cells at 1 μM comparable with that observed in the best NGF concentration (40 ng/mL). At a concentration as low as 0.03 μM, evident neuritogenic activity is also observed when treated with **57** in the PC12 cells. The SARs within the gentisides A-K (**55**-**65**) revealed that alkyl chain length is important for the activity but not the structure diversity at the end of the long alkyl chain [77, 78]. Further studies on the SARs and mechanism of action of gentisides are ongoing in our laboratory.

ALKALOIDS

Alkaloids are a group of chemical compounds that mostly contain basic nitrogen atoms. They are an important



NO.	n	R	NO.	n	R
55	17		**60**	13	
56	17		**61**	14	
57	11		**62**	15	
58	13		**63**	18	
59	15		**64**	19	
			65	21	

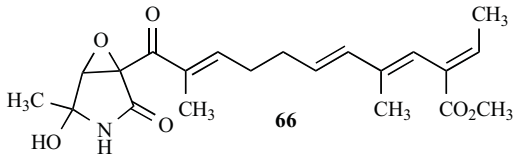
Fig. (7). Structures of neuritogenic gentisides.

group of diversely distributed and chemically, biologically, and commercially significant substances. To date, a great quantity of characterized natural alkaloids has been reported from a variety of sources including fungi, plants, marine microorganisms, mammals, and others. These natural alkaloids provide a wealth of pharmacologically active substances. Some of them are totally synthesized or semi-synthesized, and many alkaloids have been approved for medicinal use, usually in the form of salts. Recently, a lot of nitrogen heterocycle alkaloids, including epolactaenes, militarinones, farinosones, and manzamines, have been found to show dramatic neuritogenic activity.

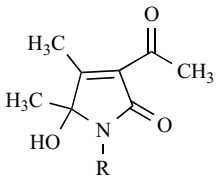
Epolactaenes

The novel microbial product epolactaene (**66**) (Fig. **8**) was isolated by Kakeya and coworkers from the fungal strain *Penicillium* sp. BM1689-P in 1995. Epolactaene was the first microbial metabolite that induced neurite outgrowth in the human neuroblastoma cell line SH-SY5Y [79]. However, this natural product contains a labile triene group on the side chain and an epoxide fused to a γ-lactam ring, which lead to instability under light. Thus, a number of epolactaene derivatives possessing a double bond in the γ-lactam ring have been designed and synthesized to find more effective neuritogenic compounds [80].

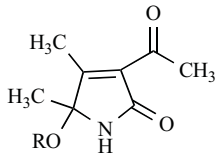
The results of the SAR of these epolactaene derivatives revealed that 1) an epoxide ring fused to the γ-lactam ring is not always necessary for biological activity in epolactaene, and 2) at least a carbonyl group at the 3-position and one straight long alkyl chain group are required for biological activity. Among these prepared epolactaene derivatives, compounds MT-21 (**67**), MT-20 (**68**), MT-19 (**69**), MT-17 (**70**), MT-6 (**71**), and MT-5 (**72**) with straight long-chain alkyls induce moderate neurite outgrowth in SH-SY5Y cells (Fig. **8**). Compounds **67**, **68**, **69** and **72** also show neuoritogenic activity in PC12 cells. However, the neuritogenic activity order in these two different cell lines is not the same. In fact, PC12 cells express much higher levels of Trk-A than SH-SY5Y cells. Therefore, the mode of action of these MT series compounds could be due to the different signal transductions in SH-SY5Y cells and PC12 cells. To study the mechanism of action, further studies on the SAR of epolactaene and the absolute stereochemistry of the epoxy moiety had been required. Thus, epolactaene was totally synthesized by Marumoto *et al.* [81] and Hayashi *et al*. in 1998 [82].



66



67 R = *n*-C_8H_{17}
68 R = *n*-$C_{12}H_{25}$
69 R = *n*-$C_{16}H_{33}$
70 R = *n*-$C_{18}H_{37}$



71 R = *n*-C_6H_{13}
72 R = *n*-$C_{18}H_{37}$

Fig. (8). Structures of neuritogenic epolactaene and its derivatives.

Importantly, except for the neuritogenic activity, epolactaene inhibits the activities of mammalian DNA polymerases and human DNA topoisomerase II *in vitro* [83]. Due to the interesting biological properties, highly unusual structure, and scarcity of this natural material, the total synthesis of epolactaene has become a hot research topic for organic chemists [84-86].

Militarinones

Novel pyridone alkaloids militarinone A, D (**73**, **76**) and 3-acyl tetramic acids militarinone B, C (**74**, **75**) are obtained from a mycelial extract of the entomofenous fungus *Paecylomyces militaris* RCEF 0095 (Fig. **9**) [87, 88]. The (+)-*N*-deoxymilitarinone A (**77**) is isolated from *Paecilomyces farinosus* RCEF 0097 [89]. Militarinone A (**73**) and (+)-*N*-deoxymilitarinone A (**77**) (Fig. **9**) induce neurite outgrowth in PC12 cells at concentrations of 10 and 33 μM, respectively, whereas militarinones B (**74**), C (**75**), and D (**76**) show a negligible neuritogenic activity. Militarinone D (**76**) exhibits significant cytotoxicity at a concentration of 100 μM [88]. Riese *et al.* demonstrated that the cellular differentiation induced by militarinone A (**73**) involves a delayed phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and the activation of protein kinase B (Akt) as well as the transcription factor

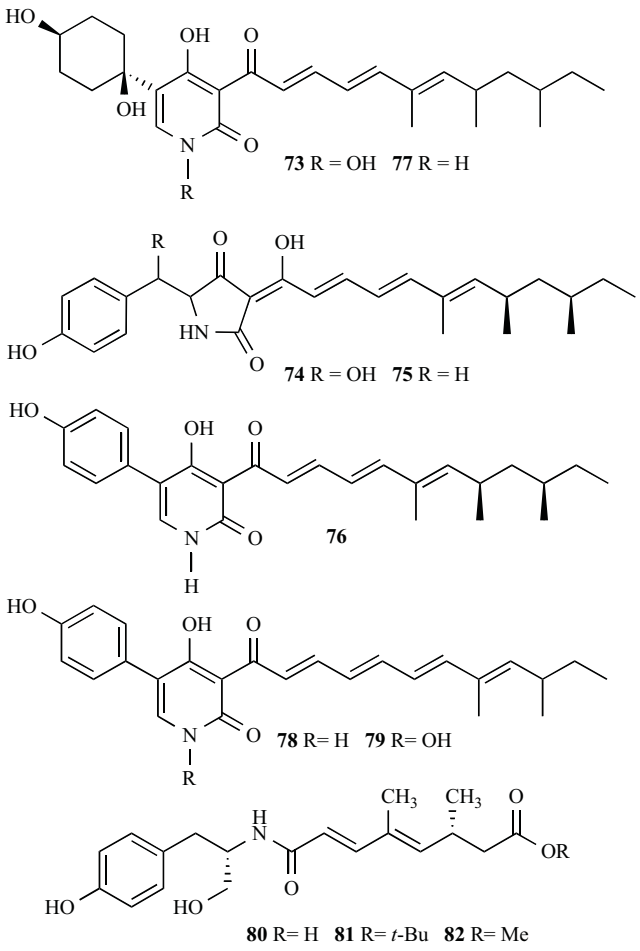


Fig. (9). Structures of neuritogenic militarinones, farinosones, and their derivatives.

cAMP responsive element binding protein (CREB) [90]. Militarinone A (**73**) stimulates neurite outgrowth in PC12 cells through the persistent activation of the PI3-K/PKB and MEK/ERK pathways, which was also involved in NGF-induced differentiation. However, treatment of **73** on murine neuroblastoma cell line N2a cells results in the rapid onset of apoptosis by the nuclear translocation of the apoptosis inducing factor (AIF) and activation of caspases and c-Jun/AP-1. The basal expression of p53 is main difference between the two cell types: high in N2a and low in PC12. The compound (**73**) induces the further activation, stabilization of p53 and the p53-dependent release as well as nuclear translocation of AIF in PC12 cells, which eventually results in apoptosis. It induces the self-same pathways in both of the two cell lines, initially leading to diverse and finally to identical results [91].

Farinosones

Farinosones A-C (**78**-**80**) are isolated from the entomologic fungus *Paecylomyces farinosus* RCEF 0101 (Fig. **9**). Farinosone C (**80**), derived from an early step of pyridone alkaloid biosynthesis, is a new metabolite. At a concentration of 50 μM, compounds **78** and **80** exhibit distinct neurite outgrowth in the PC12 cell line, whereas compound **79** is inactive [92]. As the configuration of these alkaloids is unknown, four possible stereoisomers of **80** were totally synthesized, and their neuritogenic activity in the PC12 cells was tested. The result of the preliminary SAR for these compounds revealed that the structural pattern of L-tyrosinol-amides is the most important for neurite outgrowth. However, the carboxylic acid moiety has no influence on these effects (both *tert*-butyl ester **81** and methyl ester **82** exert similar activity) (Fig. **9**) [93].

Labuanine A

Labuanine A (**83**) and three known pyridoacridine alkaloids (**84**-**86**) are isolated from the Indonesian marine sponge *Biemna fortis* (Fig. **10**). These pyridoacridine alkaloids (**83**-**86**) significantly induce multipolar neurite outgrowth in more than 50% of cells at concentration ranged from 0.03 to 3 μM. The compound **85** exhibits the strongest neuritogenic activity among them (Fig. **10**). The different neuritogenic activity between compounds **84** and **85** suggests the importance of the amino group at position C-9 in **85** for the activity [94].

Manzamine A

Manzamine A (**87**), a new alkaloid, was initially isolated as an antitumor agent from a marine sponge *Haliclona* sp. in the waters of the Okinawa [95]. Later on, manzamine A (**87**) (Fig. **10**) was found to exhibit broad biological activities, including cytotoxic, antibacterial, antimalarial, insecticidal, anti-inflammatory, anti-HIV activities and *etc.* [96]. However, the neuritogenic activity of manzamine A (**87**) reported by Zhang *et al*. was found more than 20 years later in 2008. Neuritogeneous manzamine A (**87**), 8-hydroxymanzamine (**88**), and two known alkaloids (**89**, **90**) were isolated from a Japanese marine sponge *Acanthostrongylophora* aff. *ingens* (Fig. **10**) [96]. These compounds were tested for their ability to induce neurite outgrowth against mouse neuroblastoma cell line. According

to the results, all the compounds showed an activity about two times stronger than that of the control.

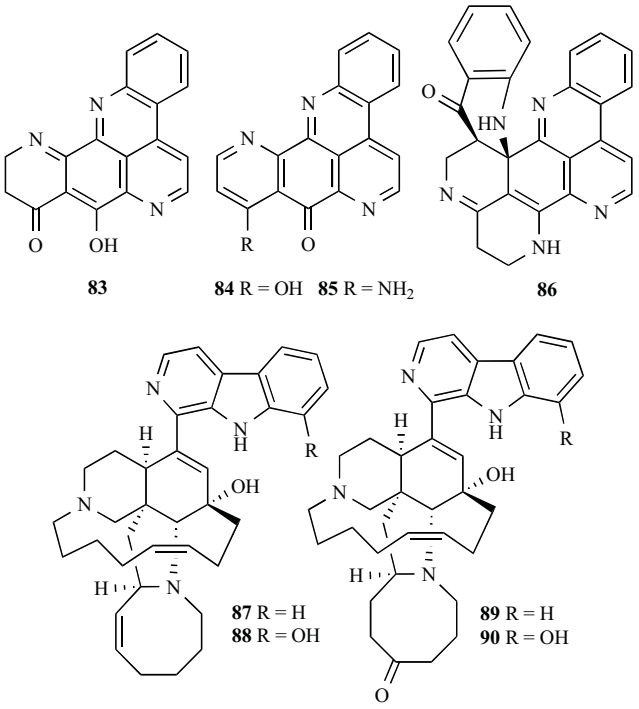


Fig. (10). Structures of neuritogenic labuanine A, manzamines, and their analogues.

STEROID

A steroid is a type of organic compound containing a specific three-dimensional arrangement of four rings (i.e., three cyclohexane rings and one cyclopentane ring) joined to each other. At present, hundreds of steroids have been found in plants and animals, and thousands more are synthesized or made by modifying natural steroids. Steroids have been given increasing attention due to their important role in the fields of organic chemistry and medicinal chemistry. Examples of steroid medicines include hormones (sex hormones), bile acids, sterols (cholesterol), oral contraceptives (contraception), and the anti-inflammatory drug dexamethasone. However, the number of steroids that induce neurite outgrowth is considerably limited. To date, only linckosides A-K and M-Q, granulatoside A, spicatoside A, NGA0187, and their analogues are reported.

Linckosides

Linckosides A-K (**91-101**) and M-Q (**102-106**), a series of new steroid glycosides, and known compounds **107**-**109** were isolated from the Okinawan starfish *Linckia laevigata* (Fig. **11**) [97-100]. All of the linckosides possess a polyhydroxylated steroid glycosylated with 2'-*O*-methyl-β-D-xylose or β-D-xylose at the C-3 position. These novel compounds can be classified into three series mainly according to the kind and number of saccharide in the side chain: linckosides A (**91**), E (**95**) and K (**101**) containing a carbon branch glycosylated with another arabinose; linckosides B-D (**92-94**), I (**99**) and M-P (**102-105**) glycosylated with another xylose at C-24 branch or terminal C-26; and linckosides F-H (**96-98**), J (**100**), and Q (**106**), without a saccharide.

Aside from this structural feature, the linckosides can induce neurite outgrowth in PC12 cells similar to NGF. All the metabolites significantly enhanced the neuritogenic activity of a trace amount of NGF (1.5 ng/mL). This NGF-mimic neuritogenic activity was highly dependent on the small differences among the structures of the linckosides. The SAR suggests the following:

1) 2'-*O*-methylxylopyranose at C-3 of the aglycon is important in neuritogenic activity. Compounds **96** and **107** possessing a 2'-*O*-methyl ether group are approximately three times as active as their corresponding non-methylated derivatives **98** and **100**, respectively (Fig. **11**) [99].

2) The kind and position of the pentose at the side-chain branch are important factors in neuritogenic activity. Xylosides **92-94** are twice as active as arabinosides **91** and **95** [98]. Steroid glycosides with xylose at the C-24 carbon branch (**92**, **104** and **105**) are much more active than those with xylose at the terminal C-26 (**102**, **103**) [100].

3) The carbon branch modified by a sugar on the side chain is necessary for neuritogenic activity. Monoglycosides **106** and the two known metabolites **108** and **109** are much less active than the bisglycosides (Fig. **11**) [100].

4) The C-24 carbon branch itself is not important. The activity of steroid glycoside **92** is much greater than that of **109** [100].

Granulatoside A

Granulatoside A (**110**) was originally isolated from the starfish *Choriaster granulatus* [101] and was rediscovered as a neuritogenic substance from starfish *Linckia laevigata* (Fig. **12**) [102]. Granulatoside A (**110**) does not induce any neurite outgrowth up to a concentration of 40 μM in PC12 cells despite its structural similarity to some active linckosides. However, it potently enhances the neurite outgrowth at a concentration of 40 μM in the presence of a trace amount of NGF as low as 1.5 ng/mL, scarcely inducing neurite outgrowth. The enhancement of NGF-induced neurite outgrowth by granulatoside A in PC12 cells is attributable to both the enhancement and maintenance of the phosphorylation of MAPK ERK1/2, even though the upstream pathways are unclear. The biological activity of this marine natural product has never been reported, and its potential for improving NGF deficiency can be explored further.

Spicatoside A

A steroidal saponin, spicatoside A (**111**), isolated from *Liriope platyphylla*, induces neuritogenic activity in PC12 cells (Fig. **12**). The mechanism of action on its neuritogenic activity was studied, and results showed that **111** activates ERK1/2 and PI 3-kinase/Akt *via* TrkA, which is responsible for the induction of the neurite outgrowth. The effects of NGF and spicatoside A on neurite outgrowth diminish in

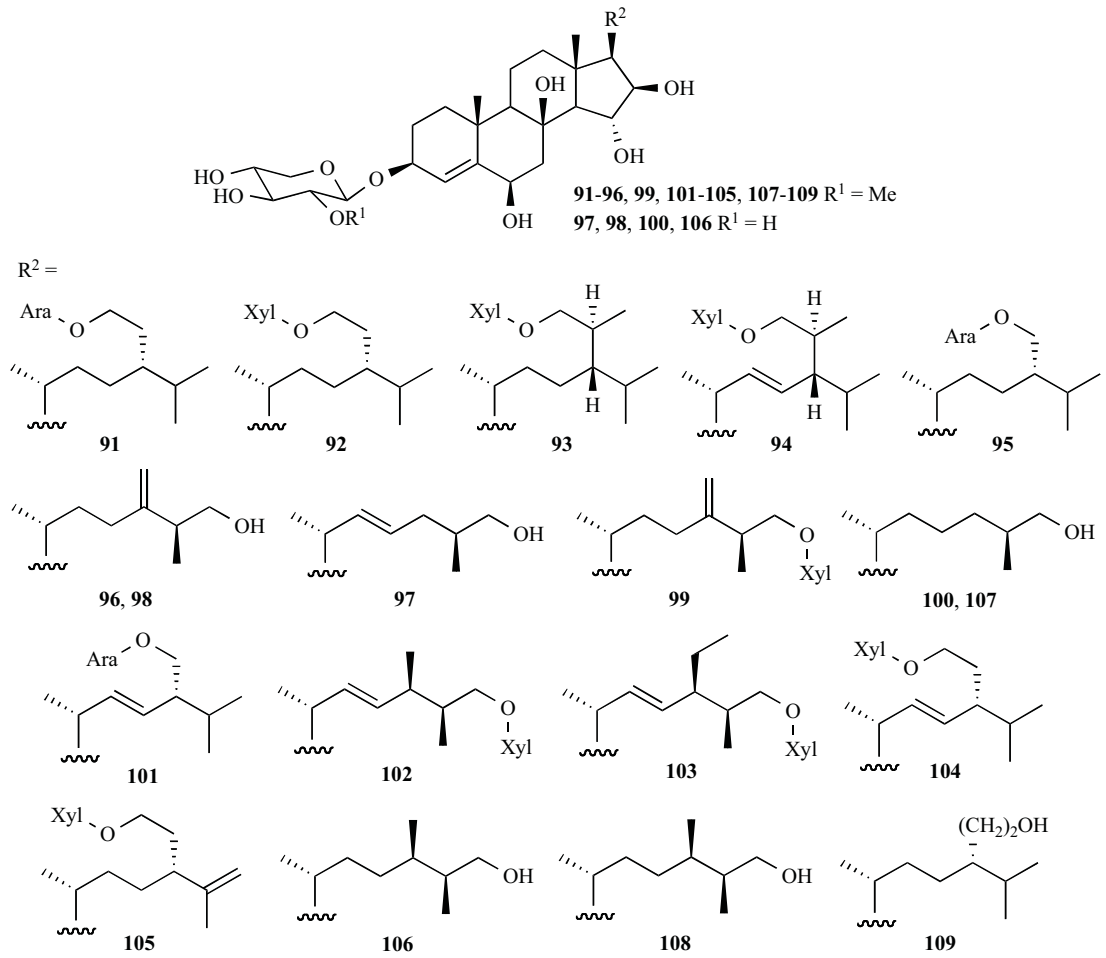


Fig. (11). Structures of neuritogenic linckosides and related compounds.

Fig. (12). Structures of granulatoside A and spicatoside A.

TrkA knockdown PC12 cells by siRNA indicated that the neuritogenic effects resulting from spicatoside A may be involved in TrkA activation [103].

NGA0187

Steroid NGA0187 (**112**) was isolated from a fermentation broth of *Acremonium* sp. TF-0356 by Nozawa and coworkers in 2002 (Fig. **13**). NGA0187 not only significantly induces neurite outgrowth at a dose of 30 μg/mL in PC12 cells but also shows survival effect in protecting rat cerebral cortical neuronal cells against damage [104]. The full account of the total synthesis of NGA0187 and the synthesis of its analogues **113**, **114**, and **115** were accomplished by Hua *et al*. (Fig. **13**). However, none of these analogues promote neurite outgrowth to any appreciable extent including compound **114** type, which that differs from NGA0187 (**112**) only in the C17 side chain and does not show any neuritogenic activity. Accordingly, the C17 side chain may play an important role in target recognition in PC12 cells for NGA0187 (**112**) [105].

SMALL MOLECULAR PEPTIDES

Peptide is made up of two or more amino acids connected by covalent bonds formed by the elimination of a molecule of H_2O from the amino group of one amino acid and the carboxyl group of the next amino acid. The term peptides describes molecules consists of fewer than 50 amino acids, peptides larger than about 50 amino acid

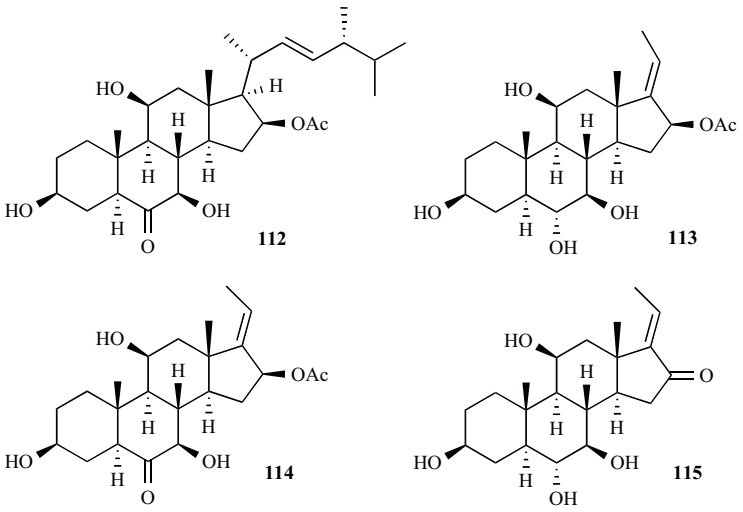


Fig. (13). Structures of NGA0187 (**112**) and its analogues (**113-115**).

residues are usually classified as proteins. Based on the screening from the combinatorial library of synthetic peptides, many small molecular peptides with homo- and heterophili-interactions of the active proteins have the same obvious activity as proteins. As the role of synthetic small peptide has become more important, small molecular peptides with neuritogenic activity have received great attention.

Pituitary Adenylyl Cyclase-Activating Peptide (PACAP)

PACAP (**116**) was originally isolated from ovine hypothalamic extracts based on its ability to stimulate cAMP formation in anterior pituitary cells [106]. PACAP, a 38-amino acid peptide, is a member of the vasoactive intestinal peptide (VIP) (**118**)/secretin/glucagon family of peptides (Fig. **14**) [107]. It exists in two amidated forms, PACAP38 (**116**) and PACAP27 (**117**). The **116** and **117** share an identical 27-amino-acid N-terminus and are alternatively processed from the preproPACAP precursor (Fig. **14**). PACAP38 (**116**) potently stimulates neuritogenesis of human neuroblastoma cells [108], neonatal rat chromaffin cells [109], and PC12 cells [110]. Barrie *et al.* demonstrated that PACAP38 (**116**) activates ERK1/2 for prolonged times and that this activation is dependent on MEK1/2 but independent of Ras in PC12 cells [111]. Sakai *et al*. indicated that PACAP induces the activation of Rac1 associated with neurite outgrowth for the first time. They suggested that the synergistic effect of PACAP and NGF on neurite outgrowth is due to the enhanced activation of ERK1/2 [112].

Neural Cell Adhesion Molecule (NCAM)-Derived Peptide

NCAM belongs to the cell adhesion molecules of the immunoglobulin (Ig) superfamily (IgSF) characterized by the Ig domain, which contains approximately 100 amino acids forming two β-sheets. NCAM plays a key role in neural regeneration, development, and synaptic plasticity [113]. The cell adhesion mediated by NCAM through homophilic and heterophilic interaction. Both homo- and heterophilic NCAM-interactions can be mimicked by synthetic peptides, which can stimulate NCAM-like signaling. Results obtained from *in vitro* and *in vivo* studies suggest that such NCAM mimetics may be used for the treatment of neurodegenerative disorders [114, 115].

NCAM binding peptide 10 (NBP10) (**119**) (Fig. **14**), a synthetic nonapeptide, modulates neurite outgrowth induced specifically by homophilic NCAM binding. The mechanism of action of NBP10 (**119**) stimulating neurite outgrowth is activated by a signal transduction pathway similar to that activated by NCAM itself [116].

The FGL-peptide (**120**) (Fig. **14**), a synthetic 15 amino acid peptide derived from the second fibronectin type III module of NCAM, binds to and induces phosphorylation of the fibroblast growth factor receptor (FGFR). This peptide can mimic NCAM heterophilic binding to the FGFR proved by inducing neurite outgrowth and promoting cell survival in three independent neuronal cell types of primary neurons (hippocampal, dopaminergic and cerebellar granule neurons). The effects of the FGL peptide were dependent of activation of the FGFR, the MAPK and PI3K-Akt signalling pathways [117]. Moreover, FGL-peptide (**120**) can enhance presynaptic function in primary hippocampal neurons *in vitro* and improve memory and learning in healthy adult rats [118].

A newly designed and synthesized peptide ligand of NCAM known as plannexin (**121**) (Fig. **14**) induces neurite extension in cultures of dopaminergic neurons and cerebellar granule neurons (CGNs). It also mimicks the neuritogenic effect of homophilic NCAM binding [119]. The synthetic peptide known as C3 (**122**) (Fig. **14**), which has the ability of binding the first immunoglobulin-like module of NCAM, promotes neurite outgrowth. The synthetic NCAM ligand C3-peptide can induce an increase in intracellular calcium in the PC12 E2 and primary hippocampal neurons cells. This presumably means that NCAM-dependent neurite outgrowth requires the mobilization of calcium from both intracellular and extracellular stores [120].

Other Peptides

Betrofin 3 (**123**) and Betrofin 4 (**124**), derived from the BDNF sequence, induce neurite outgrowth and promote

survival significantly in neuron (Fig. **14**). The mechanism of these activity peptides possibly suggests that they are bound to the cognate BDNF receptors, tyrosine receptor kinase B (TrkB) and p75 neurotrophin receptor ($p75^{NTR}$), stimulate the signaling pathways through the Akt and MAPK, and then induce neurite outgrowth and enhance neuronal survival [121]. Three selected peptides (**125**-**127**) from three groups of soluble combinatorial peptide libraries are found to induce neurite outgrowth from primary hippocampal neurons (Fig. **14**) [122].

116 HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK-NH_2
117 HSDGIFTDSYSRYRKQMAVKKYLAAVLG-NH_2
118 HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2

119 AKKMWKKTW
120 EVYVVAENQQGKSKA
121 KTKVDRDGRI
122 ASKKPKRNIKA

123 RGIDKRHWNSQ
124 SYVRALTMDSKKRIGWR

125 QSGKKF
126 QSGPLA
127 QSGKQG

Fig. (14). Sequences of neuritogenic small molecular peptides (**116**-**127**).

OTHERS

T-817MA

T-817MA [1-{3-[2-(1-benzothiophen-5-yl) ethoxy] propyl}-3-azetidinol maleate] (**128**) is a novel synthesized compound developed as a therapeutic agent for neurodegenerative disorders, such as AD (Fig. **15**). It has been reported that T-817MA (**128**) protects neurons against Aβ and oxidative stress-induced neurotoxicity, also promotes neurite outgrowth both in the reaggregation culture of rat cortical neurons and hippocampal slice cultures [123]. Fukushima *et al*. had reported that T-817MA (**128**) improves sodium nitoroprusside (SNP)-induced mitochondrial dysfunction in a newly synthesized protein-mediated mechanism in cortical neurons [124]. Furthermore, T-817MA (**128**) ameliorats learning deficits induced by Aβ infusion, which might be attributed to neuroprotection in the hippocampus [125] and reduces noise-induced cochlear damage and hearing loss, suggesting functional and morphological protection [126]. These neurotrophic and neuroprotective properties suggest that T-817MA (**128**) may be useful in the treatment of AD. A large Phase II study, initiated in April 2008 in patients with mild to moderate AD (N = 316), is assessing the safety and tolerability of T-817MA (**128**) and is powered to detect an efficacy signal using the Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog) as a primary outcome measure [4].

Verbenachalcone

The novel dimeric dihydrochalcone, verbenachalcone (**129**), was isolated from the aerial parts of *Verbena littoralis* by Li *et al*. in 2001 (Fig. **15**). This compound caused a significant enhancement of NGF-mediated neurite outgrowth from PC12D cells [127]. Verbenachalcone (**129**) and its derivative verbenachalcone pentaacetate (**130**) were prepared successfully to study preliminary SARs of verbenachalcone (Fig. **15**). Initial SAR data showed compound **130** was more active than the natural product **129**, the increased activity maybe due to an increase in cell permeability or an increase in intrinsic activity at the molecular target. Nevertheless, the ketones functional groups is crucial for activity, as the derivative of verbenachalcone which lacked the ketone functional groups was inactive [128].

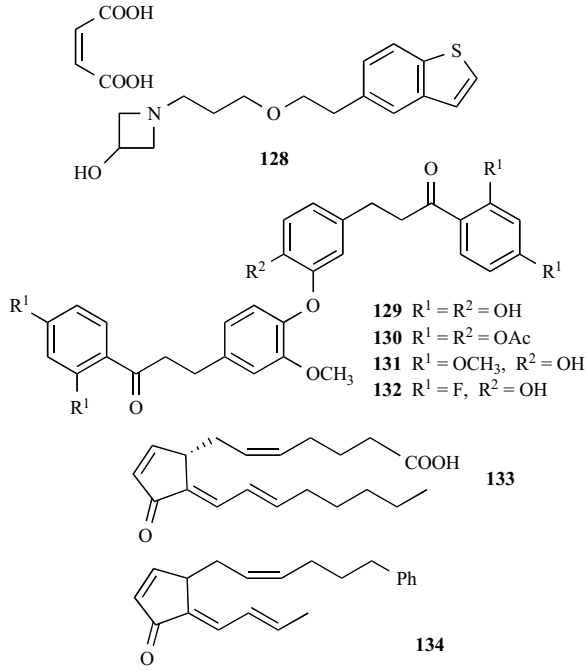


Fig. (15). Structures of T-817MA, verbenachalcone, 15d-PGJ_2 and their derivatives.

Yeh *et al*. reported the synthesis of another verbenachalcone derivative (**131**) which has increased hydrophobicity and its effects on neurite outgrowth in PC12 cells (Fig. **15**). They also decribed compound **131** protect N2a cells from caspase induction caused by serum starvation [129].

DSRB20-022 (**132**), a newly synthetic verbenachalcone derivative, showed significant enhancement of NGF-dependent neurite outgrowth in NeuroScreen-1 (NS-1) cells, a derivative of PC12 cells (Fig. **15**). The cell viability assays showed that compounds **132** and **129** are neuroprotective and enhanced survival of PC12, NS-1 and N2a cell lines under serum deprivation conditions. The enhancement of NGF-induced neurite outgrowth by compound **132** in NS-1 was dependent on MAP kinase. Furthermore, the neuroprotective function of compounds **132** and **129** was accompanied by suppression of caspase-3/7 activation, whereas these two compounds exerted their antagonistic effects on caspase-3/7 activation through potentially different mechanisms of action [130].

15-Deoxy-$\Delta^{12,14}$-PGJ_2 Derivatives

15-Deoxy-$\Delta^{12,14}$-prostaglandin (PG) J_2 (15d-PGJ_2) (**133**), a naturally occurring downstream metabolite of PGD_2, is a

high-affinity ligand of the peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear hormone receptor superfamily (Fig. **15**) [131]. It has been reported that 15d-PGJ_2 promote NGF-induced neurite outgrowth in PC12 cells [132]. Tanaka *et al*. had reported the efficient combinatorial synthesis of the 15d-PGJ_2 derivatives with variable α and ω chains based on a polymer-assisted solution-phase synthetic strategy. They also described that the carboxylic acid on the α chain was important for the enhancement of neurogenic activity, but that it was not absolutely necessary for biological activity. Furthermore, 15d-PGJ_2 derivative **134** which contains a phenyl group on the α chain showed comparable neuritogenic activity with 15d-PGJ_2 (Fig. **15**) [133].

But the mechanism by which 15d-PGJ_2 and its derivatives activates NGF-induced neurite outgrowth is not very clear. Jung and coworkers demonstrated that activation of p38 MAP kinase in conjunction with AP-1 signal pathway may be important on the neurite outgrowth of PC12 cells in the promoting activity of 15d-PGJ_2 [134]. Very recently, Hatanaka *et al*. had reported that 15d-PGJ_2 enhanced NGF-induced neurite outgrowth was blocked by the chemoattractant receptor-homologous molecule expressed on T-Helper type 2 cells (CRTH2) antagonist CAY10471 in PC12 cells [135].

In addition to the neuritogentic substances mentioned above, there were still many novel natural compounds were related to neurotrophic properties, such as luteolin [136], artepillin C [137], pheophytin a [138], (-)-3,5-dicaffeoyl-*muco*-quinic acid (DQ) [139], (-)-(7*R*, 8*S*)- dihydrodehydrodiconiferyl alcohol (DHDA) [140], honokiol [141], NG-061 [142], *etc*.. However, further study including SAR or mechanism of action of these neurogenic compounds were received little attention.

PERSPECTIVE

Small molecule substances with neurotrophic and neuroprotective properties may be useful in the treatment of brain disorders characterized by neurodegeneration, neuronal cell loss, and deficiencies in synaptic connectivity. Several agents with neurotrophic and neuroprotective properties are undergoing Phase II clinical trials. SK-PC-B70M, the oleanolic-glycoside saponin-enriched fraction of *Pulsatilla koreana* (Korean pasque flower), has been found to have cognition-enhancing and neuroprotective effects in animal models [4]. A Phase II study comparing SK-PC-B70M monotherapy with placebo with mild to moderate AD in 188 patients was completed. T-817MA, a synthetic small neurotrophic molecule, promotes neurite outgrowth in rat hippocampal slice cultures as well as exhibits potent neuroprotective effects against Aβ- and oxidative stress-induced neurotoxicity [4]. These neurotrophic and neuroprotective properties suggest that T-817MA may be useful in the treatment of AD. A large Phase II study in patients with mild to moderate AD is currently assessing the safety and tolerability of T-817MA that started in 2008.

In recent years, a large number of natural and synthetic small molecules with neuritogenic activity in various structural features have been discovered. Their structural and biological features have attracted the attention of researchers in different research fields. Most of them have shown the capacity to induce neurite outgrowth and prevent neuron cell death in several neuronal cells, such as, PC12, Neuro 2a, SH-SY5Y cells, and others. Comparing the biological activity of these neuritogenic substances is difficult due to the following reasons. First, the literature cited in this review are reported from different laboratories, and the cell culture conditions and bioassay methods have not been unified. Second, in the case of using PC12 cell line as a bioassay system, many authors used NGF as the positive control. However, NGF is unstable, and PC12 cell condition is not similar to that in different generations. Therefore, the bioassay results of NGF on PC12 cell are different.

At present, several natural and/or derivatives, such as DHA, ganglioside, linckoside, lembehyne, genipin/genipo-side, and epolactaene, have been studied intensively. Various data obtained reveal a promising application on the precaution and treatment of neurodegenerative diseases. Moreover, gentisides, which are a newly discovered class of neuritogenic substance possessing a very simple chemical structure and exhibiting a significant activity on PC12 cells, are a promising candidate for anti-AD drug development.

The structural features of these neuritogenic substances favoring their interaction with the target molecule are becoming clearer. Thus, collecting more scientific results that will allow the development of small molecules as therapeutics drugs for the treatment of neurodegenerative diseases such as AD is necessary.

To date, great achievements have been made in searching for substances with neurotrophic and neuroprotective properties. The biological activity evaluation systems *in vitro* and the action mechanism studies on the cell level of such substances are well established. However, suitable models for evaluating neurotrophic and neuroprotective properties of such compounds *in vivo* are lacking. Therefore, great attention to developing useful and suitable animal models that can connect *in vitro* tests and clinical trials is required. Indeed, there is still a long way to go in curing AD.

ACKNOWLEDGEMENTS

Thank every author of all the references cited herein for their contributions in this field. This work was financially supported by NSFC (30873152, and 81072536) and *Qianjiang Talent* Program of Zhejiang Province (2010R10052).

ABBREVIATION

ACh	=	acetylcholine
AchEIs	=	acetylcholinesterase inhibitors
Acsl	=	acyl-CoA synthetase long-chain
AD	=	Alzheimer's disease
ADAS-cog	=	Alzheimer's Disease Assessment Scale-cognitive subscale
AIF	=	apoptosis inducing factor

Aβ = β-amyloid

BBB = blood-brain barrier

BDNF = brain-derived neurotrophic factor

bFGF = basic fibroblast growth factor

CGNs = cerebellar granule neurons

C-jun/AP-1 = oncoprotein C-jun/active protein-1

CNS = central nervous system

CREB = cAMP responsive element binding

CRTH2 = chemoattractant receptor-homologous molecule expressed on T-Helper type 2 cells

15d-PGJ$_2$ = 15-deoxy-$\Delta^{12,14}$-prostaglandin (PG) J$_2$

dbcAMP = dibutyryl cyclic AMP

DHA = docosahexaenoic acid

DHDA = (-)-(7*R*, 8*S*)-dihydrodehydrodiconiferyl alcohol

DQ = (-)-3,5-dicaffeoyl-*muco*-quinic acid

ERK = extracellular signal-regulated kinase

FA = fatty acid

FGFR = fibroblast growth factor receptor

GDNF = glial cell line-derived neurotrophic factor

GLP-1 = glucagon-like peptide-1

GM1 = monosialo-ganglioside 1

Ins-1-P-Cer = inositolphosphoceramide

Ig = immunoglobulin

IgSF = immunoglobulin superfamily

LB-A = lembehynes A

LCB = long-chain base

MAPK = mitogen-activated protein kinase

NBP10 = NCAM binding peptide 10

NCAM = neural cell adhesion molecule

Neuro 2a = neuroblastoma 2a

NGF = nerve growth factor

NO-cGMP-PKG = nitric oxide-cyclic GMP-dependent protein kinase

nNOS = neuronal NO synthase

NS-1 = NeuroScreen-1

p75^{NTR} = p75 neurotrophin receptor

PACAP = pituitary adenylate cyclase-activating polypeptide

PC12 = pheochromocytoma 12

PI3K = phosphatidylinositol 3-kinase

PLs = phospholipids

PPAR-γ = peroxisome proliferator-activated receptor-γ

PUFAs = polyunsaturated fatty acids

SAR = structure-activity relationship

SNP = sodium nitroprusside

TrkA = tyrosine receptor kinase A

TrkB = tyrosine receptor kinase B

VIP = vasoactive intestinal peptide

REFERENCES

[1] Bharadwaj, P.R.; Dubey, A.K.; Masters, C.L.; Martins, R.N.; Macreadie, I.G. Aβ aggregation and possible implications in Alzheimer's disease pathogenesis. *J. Cell. Mol. Med*., **2009**, *13*, 412-21.

[2] Re, F.; Airoldi, C.; Zona, C.; Masserini, M.; La Ferla, B.; Quattrocchi, N.; Nicotra, F. Beta amyloid aggregation inhibitors: small molecules as candidate drugs for therapy of Alzheimer's disease. *Curr. Med. Chem*., **2010**, *17*, 2990-3006.

[3] Roberson, E.D.; Mucke, L. 100 Years and counting: prospects for defeating Alzheime's disease. *Science*, **2006**, *314*, 781-84.

[4] Sabbagh, M.N. Drug development for Alzheimer's disease: where are we now and where are we headed? *Am. J. Geriatr. Pharmacother.*, **2009**, *7*, 167-85.

[5] Lee, H.P.; Zhu, X.W.; Casadesus, G.; Castellani, R.J.; Nunomura, A.; Smith, M.A.; Lee, H.G.; Perry, G. Antioxidant approaches for the treatment of Alzheimer's disease. *Expert. Rev. Neurother*., **2010**, *10*, 1201-08.

[6] Salminen, A.; Ojala, J.; Kauppinen, A.; Kaarniranta, K.; Suuronen, T. Inflammation in Alzheimer's disease: amyloid-β oligomers trigger innate immunity defence *via* pattern recognition receptors, *Prog. Neurobiol*., **2009**, *87*, 181-94.

[7] Jeganathan, S.; von Bergen, M.; Mandelkow, E.M.; Mandelkow, E. The natively unfolded character of tau and its aggregation to Alzheimer-like paired helical filaments. *Biochemistry*, **2008**, *47*, 10526-39.

[8] Moreira, P.I.; Zhu, X.W.; Wang, X.L.; Lee, H.G.; Nunomura, A.; Petersen, R.B.; Perry, G.; Smith, M.A. Mitochondria: a therapeutic target in neurodegeneration. *Biochim. Biophys. Acta-Mol. Basis Dis*., **2010**, *1802*, 212-20.

[9] Lleó, A.; Greenberg, S.M.; Growdon, J.H. Current pharmacotherapy for Alzheimer's disease. *Annu. Rev. Med*., **2006**, *57*, 513-33.

[10] Donev, R.; Kolev, M.; Millet, B.; Thome, J. Neuronal death in Alzheimer's disease and therapeutic opportunities. *J. Cell. Mol. Med*., **2009**, *13*, 4329-48.

[11] Thoenen, H. Neurotrophins and neuronal plasticity. *Science*, **1995**, *270*, 593-98.

[12] Berninger, B.; Poo, M.M. Exciting neurotrophins. *Nature*, **1999**, *401*, 862-63.

[13] Kromer, L.F. Nerve growth factor treatment after brain injury prevents neuronal death. *Science*, **1987**, *235*, 214-16.

[14] Brinton, R.D.; Yamazaki, R.S. Advances and challenges in the prevention and treatment of Alzheimer's disease. *Pharm. Res*., **1998**, *15*, 386-98.

[15] Li, Y.Y.; Hu, Z.Y.; Lu, C.H.; Shen, Y.M. Four new terpenoids from *Xylaria* sp. 101. *Helv. Chim. Acta,* **2010**, *93*, 796-802.

[16] Robinson, A.; Kumar, T.V.; Sreedhar, E.; Naidu, V.G.M.; Krishna, S.R.; Babu, K.S.; Srinivas, P.V.; Rao, J.M. A new sesquiterpene lactone from the roots of *Saussurea lappa*: structure-anticancer activity study. *Bioorg. Med. Chem. Lett*., **2008**, *18*, 4015-17.

[17] Chen, B.W.; Chao, C.H.; Su, J.H.; Wen, Z.H.; Sung, P.J.; Sheu, J.H. Anti-inflammatory eunicellin-based diterpenoids from the cultured soft coral *Klyxum simplex*. *Org. Biomol. Chem*., **2010**, *8*, 2363-66.

[18] Liebgott, T.; Miollan, M.; Berchadsky, Y.; Drieu, K.; Culcasi, M.; Pietri, S. Complementary cardioprotective effects of flavonoid metabolites and terpenoid constituents of Ginkgo biloba extract (EGb 761) during ischemia and reperfusion. *Basic Res. Cardiol*., **2000**, *95*, 368-77.

[19] Li, C.J.; Zhang, D.M.; Luo, Y.M.; Yu, S.S.; Li, Y.; Lu, Y. Bis-sesquiterpenes and diterpenes from *Chloranthus henryi*. *Phytochemistry*, **2008**, *69*, 2867-74.

[20] Tundis, R.; Loizzo, M.R.; Menichini, F.; Statti, G.A.; Menichini, F. Biological and pharmacological activities of iridoids: recent developments. *Mini-Rev. Med. Chem*., **2008**, *8*, 399-420.

[21] Inouye, H.; Saito, S. New iridiglucosides gardenoside and genipinside from *Gardenia jasminoides*. *Tetrahedron Lett*., **1969**, *28*, 2347-50.

[22] Yamazaki, M.; Chiba, K.; Mohri, T. Neuritogenic effect of natural iridoid compounds on PC12h cells and its possible relation to signaling protein kinases. *Biol. Pharm. Bull*., **1996**, *19*, 791-95.

[23] Chiba, K.; Yamazaki, M.; Kikuchi, M.; Machida, K.; Kikuchi, M. Neuritogenesis of herbal geniposide-related compounds in PC12h cells. *J. Health Sci*., **2006**, *52*, 743-47.

[24] Liu, J.H.; Zheng, X.X.; Yin, F.; Hu, Y.H.; Guo, L.X.; Deng, X.H.; Chen, G.; Jing, J.J.; Zhang, H. Neurotrophic property of geniposide for inducing the neuronal differentiation of PC12 cells. *Int. J. Devl Neuroscience*, **2006**, *24*, 419-24.

[25] Liu, J.H.; Yin, F.; Guo, L.X.; Deng, X.H.; Hu, Y.H. Neuroprotection of geniposide against hydrogen peroxide induced PC12 cells injury: involvement of PI3 kinase signal pathway. *Acta Pharmacol. Sin*., **2009**, *30*, 159-65.

[26] Yin, F.; Liu, J.H.; Xiao, H.; Kong, S.Z. Geniposide prevents PC12 cells from peroxynitrite *via* the mitogen-activated protein kinase signaling pathway. *J. Health Sci*., **2010**, *56*, 195-99.

[27] Yamazaki, M.; Chiba, K. Neurotrophic effects of genipin on Neuro 2a cells. *J. Health Sci*., **2005**, *51*, 687-92.

[28] Yamazaki, M.; Chiba, K. Genipin exhibits neurotrophic effects through a common signaling pathway in nitric oxide synthase-expressing cells. *Eur. J. Pharmacol*., **2008**, *581*, 255-61.

[29] Yamazaki, M.; Sakura, N.; Chiba, K.; Mohri, T. Prevention of the neurotoxicity of the amyloid ß protein by genipin. *Biol. Pharm. Bull*., **2001**, *24*, 1454-55.

[30] Yamazaki, M.; Chiba, K.; Satoh, K. Neuro2a cell death induced by 6-hydroxydopamine is attenuated by genipin. *J. Health Sci*., **2008**, *54*, 638-44.

[31] Ohkubo, T.; Yamazaki, M.; Yoshida, A.; Chiba, K.; Mohri, T. Detection of genipin/geniposide-target molecules by a geniposide overlay method using anti-geniposide antibody. *J. Health Sci*., **2004**, *50*, 193-96.

[32] Hou, Y.C.; Tsai, S.Y.; Lai, P.Y.; Chen, Y.S.; Chao, P.D.L. Metabolism and pharmacokinetics of genipin and geniposide in rats. *Food Chem. Toxicol*., **2008**, *46*, 2764-69.

[33] Suzuki, H.; Yamazaki, M.; Chiba, K.; Uemori, Y.; Sawanishi, H. Neuritogenic activities of 1-alkyloxygenipins. *Chem. Pharm. Bull*., **2010**, *58*, 168-71.

[34] Li, P.; Matsunaga, K.; Yamakuni, T.; Ohizumi, Y. Potentiation of nerve growth factor-action by picrosides I and II, natural iridoids, in PC12D cells. *Eur. J. Pharmacol*., **2000**, *406*, 203-08.

[35] Li, P.; Matsunaga, K.; Yamakuni, T.; Ohizumi, Y. Picrosides I and II, selective enhancers of the mitogen-activated protein kinase-dependent signaling pathway in the action of neuritogenic substances on PC12D cells. *Life Sci.*, **2002**, *71*, 1821-35.

[36] Kouno, I.; Baba, N.; Hashimoto, M.; Kawano, N.; Takahashi, M.; Kaneto, H.; Yang, C.S. Sesquiterpene lactones from the pericarps of *Illicium majus*; 2-oxy derivatives of neomajucin and 3,4-dehydroxyneomajucin. *Chem. Pharm. Bull*., **1990**, *38*, 422-25.

[37] Yokoyama, R.; Huang, J.M.; Yang, C.S.; Fukuyama, Y. New *seco*-prezizaane-type sesquiterpenes, jiadifenin with neurotrophic activity and 1,2-dehydroneomajucin from *Illicium jiadifengpi*. *J. Nat. Prod*., **2002**, *65*, 527-31.

[38] Cho, Y.S.; Carcache, D.A.; Tian, Y.; Li, Y.M.; Danishefsky, S.J. Total synthesis of (±)-jiadifenin, a non-peptidyl neurotrophic modulator. *J. Am. Chem. Soc*., **2004**, *126*, 14358-59.

[39] Carcache, D.A.; Cho, Y.S.; Hua, Z.H.; Tian, Y.; Li, Y.M.; Danishefsky, S.J. Total synthesis of (±)-jiadifenin and studies directed to understanding its SAR: probing mechanistic and stereochemical issues in palladium-mediated allylation of enolate-like structures. *J. Am. Chem. Soc*., **2006**, *128*, 1016-22.

[40] Huang, J.M.; Yokoyama, R.; Yang, C.S.; Fukuyama, Y. Merrilactone A, a novel neurotrophic sesquiterpene dilactone from *Illicium merrillianum*. *Tetrahedron Lett*., **2000**, *41*, 6111-14.

[41] Huang, J.M.; Yang, C.S.; Tanaka, M.; Fukuyama, Y. Structures of merrilactones B and C, novel anislactone-type sesquiterpenes from *Illicium merrillianum*, and chemical conversion of anislactone B to merrilactone A. *Tetrahedron*, **2001**, *57*, 4691-98.

[42] Birman, V.B.; Danishefsky, S.J. The total synthesis of (±)-merrilactone A. *J. Am. Chem. Soc*., **2002**, *124*, 2080-81.

[43] Inoue, M.; Sato, T.; Hirama, M. Total synthesis of merrilactone A. *J. Am. Chem. Soc*., **2003**, *125*, 10772-73.

[44] Iriondo-Alberdi, J.; Perea-Buceta, J.E.; Greaney, M.F. A Paternò-Büchi approach to the synthesis of merrilactone A. *Org. Lett*., **2005**, *7*, 3969-71.

[45] Fukuyama, Y.; Minami, H.; Takeuchi, K.; Kodama, M.; Kawazu, K. Neovibsanines A and B, unprecedented diterpenes from *Viburnum awabuki*. *Tetrahedron Lett*., **1996**, *37*, 6767-70.

[46] Esumi, T.; Zhao, M.; Kawakami, T.; Fukumoto, M.; Toyota, M.; Fukuyama, Y. Synthetic studies toward neovibsanins A and B: construction of the neovibsanin core utilizing palladium(0)-catalyzed carbonylative cyclization with carbon monoxide. *Tetrahedron Lett*., **2008**, *49*, 2692-96.

[47] Kubo, M.; Kishimoto, Y.; Harada, K.; Hioki, H.; Fukuyama, Y. NGF-potentiating vibsane-type diterpenoids from *Viburnum sieboldii*. *Bioorg. Med. Chem. Lett*., **2010**, *20*, 2566-71.

[48] Chen, A.P.J.; Müller, C.C.; Cooper, H.M.; Williams, C.M. Synthetic neovibsanes and their ability to induce neuronal differentiation in PC12 cells. *Tetrahedron*, **2010**, *66*, 6842-50.

[49] Tang, W.X.; Kubo, M.; Harada, K.; Hioki, H.; Fukuyama, Y. Novel NGF-potentiating diterpenoids from a Brazilian medicinal plant, *Ptychopetalum olacoides*. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 882-86.

[50] Calon, F.; Lim, G.P.; Yang, F.S.; Morihara, T.; Teter, B.; Ubeda, O.; Rostaing, P.; Triller, A.; Salem, N.; Ashe, K.H.; Frautschy, S.A.; Cole, G.M. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron*, **2004**, *43*, 633-45.

[51] Lukiw, W.J.; Cui, J.G.; Marcheselli, V.L.; Bodker, M.; Botkjaer, A.; Gotlinger, K.; Serhan, C.N.; Bazan, N.G. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease, *J. Clin. Invest*., **2005**, *115*, 2774-83.

[52] Kaur, P.; Schulz, K.; Aschner, M.; Syversen, T. Role of docosahexaenoic acid in modulating methylmercury-induced neurotoxicity. *Toxicol. Sci*., **2007**, *100*, 423-32.

[53] Ikemoto, A.; Kobayashi, T.; Watanabe, S.; Okuyama, H. Membrane fatty acid modifications of PC12 cells by arachidonate or docosahexaenoate affect neurite outgrowth but not norepinephrine release. *Neurochem. Res*., **1997**, *22*, 671-78.

[54] Calderon, F.; Kim, H.Y. Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *J. Neurochem*., **2004**, *90*, 979-88.

[55] Cao, D.H.; Xue, R.H.; Xu, J.; Liu, Z.L. Effects of docosahexaenoic acid on the survival and neurite outgrowth of rat cortical neurons in primary cultures. *J. Nutr. Biochem*., **2005**, *16*, 538-46.

[56] Shimazawa, M.; Nakajima, Y.; Mashima, Y.; Hara, H. Docosahexaenoic acid (DHA) has neuroprotective effects against oxidative stress in retinal ganglion cells. *Brain Res*., **2009**, *1251*, 269-75.

[57] Marszalek, J.R.; Kitidis, C.; Dirusso, C.C.; Lodish, H.F. Long-chain acyl-CoA synthetase 6 preferentially promotes DHA metabolism. *J. Biol. Chem.*, **2005**, *280*, 10817-26.

[58] Dagai, L.; Peri-Naor, R.; Birk, R.Z. Docosahexaenoic acid significantly stimulates immediate early response genes and neurite outgrowth. *Neurochem. Res*., **2009**, *34*, 867-75.

[59] Aoki, S.; Matsui, K.; Tanaka, K.; Satari, R.; Kobayashi, M. Lembehyne A, a novel neuritogenic polyacetylene, from a marine sponge of *Haliclona* sp.. *Tetrahedron*, **2000**, *56*, 9945-48.

[60] Aoki, S.; Matsui, K.; Wei, H.; Murakami, N.; Kobayashi, M. Structure-activity relationship of neuritogenic spongean acetylene alcohols, lembehynes. *Tetrahedron*, **2002**, *58*, 5417-22.

[61] Murakami, N.; Nakajima, T.; Kobayashi, M. Total synthesis of lembehyne A, a neuritogenic spongean polyacetylene. *Tetrahedron Lett*., **2001**, *42*, 1941-43.

[62] Aoki, S.; Matsui, K.; Takata, T.; Kobayashi, M. In situ photoaffinity labeling of the target protein for lembehyne A, a neuronal differentiation inducer. *FEBS Lett*., **2003**, *544*, 223-27.

[63] Ledeen, R.W. Biology of gangliosides: neuritogenic and neuronotrophic properties. *J. Neurosci. Res*., **1984**, *12*, 147-59.

[64] Matsuoka, Y.; Saito, M.; LaFrancois, J.; Saito, M.; Gaynor, K.; Olm, V.; Wang, L.L.; Casey, E.; Lu, Y.F.; Shiratori, C.; Lemere, C.; Duff, K. Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to beta amyloid. *J. Neurosci*., **2003**, *23*, 29-33.

[65] Svennerholm, L.; Bråne, G.; Karlsson, I.; Lekman, A.; Ramström, I.; Wikkelsö, C. Alzheimer disease-effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. *Dement Geriatr Cogn Disord*, **2002**, *14*, 128-36.

[66] Higuchi, R.; Inagaki, M.; Yamada, K.; Miyamoto, T. Biologically active gangliosides from echinoderms. *J. Nat. Med*., **2007**, *61*, 367-70.

[67] Kaneko M.; Yamada, K.; Miyamoto, T.; Inagaki, M.; Higuchi, R. Neuritogenic activity of gangliosides from echinoderms and their structure-activity relationship. *Chem. Pharm. Bull*., **2007**, *55*, 462-63.

[68] Arao, K.; Inagaki, M.; Higuchi, R. Constituents of crinoidea. 2, isolation and structure of the novel type gangliosides from the feather star *Comanthus japonica*. *Chem. Pharm. Bull*., **2001**, *49*, 695-98.

[69] Arao, K.; Inagaki, M.; Miyamoto, T.; Higuchi, R. Constituents of crinoidea. 3. isolation and structure of a glycosyl inositolphosphoceramide-type ganglioside with neuritogenic activity from the feather star *Comanthus japonica*. *Chem. Pharm. Bull*., **2004**, *52*, 1140-42.

[70] Kisa, F.; Yamada, K.; Miyamoto, T.; Inagaki, M.; Higuchi, R. Constituents of holothuroidea, 17. isolation and structure of biologically active monosialo-gangliosides from the sea cucumber *Cucumaria echinata*. *Chem. Pharm. Bull*., **2006**, *54*, 982-87.

[71] Yamada, K.; Hamada, A.; Kisa, F.; Miyamoto, T.; Higuchi, R. Constituents of holothuroidea, 13. structure of neuritogenic active ganglioside molecular species from the sea cucumber *Stichopus chloronotus*. *Chem. Pharm. Bull*., **2003**, *51*, 46-52.

[72] Yamada, K.; Tanabe, K.; Miyamoto, T.; Kusumoto, T.; Inagaki, M.; Higuchi, R. Isolation and structure of a monomethylated ganglioside possessing neuritogenic activity from the ovary of the sea urchin *Diadema setosum*. *Chem. Pharm. Bull*., **2008**, *56*, 734-37.

[73] Kisa, F.; Yamada, K.; Miyamoto, T.; Inagaki, M.; Higuchi, R. Constituents of holothuroidea, 18. isolation and structure of biologically active disialo- and trisialo-gangliosides from the sea cucumber *Cucumaria echinata*. *Chem. Pharm. Bull*., **2006**, *54*, 1293-98.

[74] Tan, R.X.; Che, J.H. The cerebrosides. *Nat. Prod. Rep*., **2003**, *20*, 509-34.

[75] Qi, J.H.; Ojika, M.; Sakagami, Y.J. Termitomycesphins A-D, novel neuritogenic cerebrosides from the edible Chinese mushroom *Termitomyces albuminosus*. *Tetrahedron*, **2000**, *56*, 5835-41.

[76] Qi, J.H.; Ojika, M.; Sakagami, Y.J. Neuritogenic cerebrosides from an edible Chinese mushroom. part 2: structures of two additional termitomycesphins and activity enhancement of an inactive cerebroside by hydroxylation. *Bioorg. Med. Chem*., **2001**, *9*, 2171-77.

[77] Gao, L.J.; Li, J.Y.; Qi, J.H. Gentisides A and B, two new neuritogenic compounds from the traditional Chinese medicine *Gentiana rigescens* Franch. *Bioorg. Med. Chem*., **2010**, *18*, 2131-34.

[78] Gao, L.J.; Xiang, L.; Luo, Y.; Wang, G.F.; Li, J.Y.; Qi, J.H. Gentisides C-K: nine new neuritogenic compounds from the traditional Chinese medicine *Gentiana rigescens* Franch. *Bioorg. Med. Chem*., **2010**, *18*, 6995-7000.

[79] Kakeya, H.; Takahashi, I.; Okada, G.; Isono, K.; Osada, H. Epolactaene, a novel neuritogenic compound in human neuroblastoma cells, produced by a marine fungus. *J. Antibiot*., **1995**, *48*, 733-35.

[80] Kakeya, H.; Onozawa, C.; Sato, M.; Arai, K.; Osada, H. Neuritogenic effect of epolactaene derivatives on human neuroblastoma cells which lack high-affinity nerve growth factor receptors. *J. Med. Chem.*, **1997**, *40*, 391-94.

[81] Marumoto, S.; Kogen, H.; Naruto, S. Absolute configuration and total synthesis of (+)-epolactaene, a neuritogenic agent from *Penicillium* sp. BM 1689-P active in human neuroblastoma cells. *J. Org. Chem.*, **1998**, *63*, 2068-69.

[82] Hayashi, Y.; Narasaka, K. Asymmetric total synthesis of epolactaene. *Chem. Lett.*, **1998**, *4*, 313-14.

[83] Mizushima, Y.; Kobayashi, S.; Kuramochi, K.; Nagata, S.; Sugawara, F.; Sakaguchi, K. Epolactaene, a novel neuritogenic compound in human neuroblastoma cells, selectively inhibits the activities of mammalian DNA polymerases and human DNA topoisomerase II. *Biochem. Biophys. Res. Commun*., **2000**, *273*, 784-88.

[84] Hayashi, Y.; Kanayama, J.; Yamaguchi, J.; Shoji, M. Diastereoselective total synthesis of both enantiomers of epolactaene. *J. Org. Chem*., **2002**, *67*, 9443-48.

[85] Kuramochi, K.; Nagata, S.; Itaya, H.; Matsubara, Y.; Sunoki, T.; Uchiro, H.; Takao, K.; Kobayashi, S. A convergent total synthesis of epolactaene: an application of the bridgehead oxiranyl anion strategy. *Tetrahedron*, **2003**, *59*, 9743-58.

[86] Tan, Z.; Negishi, E. Selective synthesis of epolactaene featuring efficient construction of methyl (*Z*)-2-iodo-2-butenoate and (2*R*,3*S*,4*S*)-2-trimethylsilyl-2,3-epoxy-4-methyl-γ-butyrolactone. *Org. Lett*., **2006**, *8*, 2783-85.

[87] Schmidt, K.; Günther, W.; Stoyanova, S.; Schubert, B.; Li, Z.Z.; Hamburger, M. Militarinone A, a neurotrophic pyridone alkaloid from *Paecilomyces militaris*. *Org. Lett*., **2002**, *4*, 197-99.

[88] Schmidt, K.; Riese, U.; Li, Z.Z.; Hamburger, M. Novel tetramic acids and pyridone alkaloids, militarinones B, C, and D, from the insect pathogenic fungus *Paecilomyces militaris*. *J. Nat. Prod*., **2003**, *66*, 378-83.

[89] Cheng, Y.X.; Schneider, B.; Riese, U.; Schubert, B.; Li, Z.Z.; Hamburger, M. (+)-*N*-Deoxymilitarinone A, a neuritogenic pyridone alkaloid from the insect pathogenic fungus *Paecilomyces farinosus*. *J. Nat. Prod*., **2006**, *69*, 436-38.

[90] Riese, U.; Ziegler, E.; Hamburger, M. Militarinone A induces differentiation in PC12 cells *via* MAP and Akt kinase signal transduction pathways. *FEBS Lett*., **2004**, *577*, 455-59.

[91] Küenzi, P.; Kiefer, S.; Koryakina, A.; Hamburger, M. Promotion of cell death or neurite outgrowth in PC-12 and N2a cells by the fungal alkaloid militarinone A depends on basal expression of p53. *Apoptosis*, **2008**, *13*, 364-76.

[92] Cheng, Y.X.; Schneider, B.; Riese, U.; Schubert, B.; Li, Z.Z.; Hamburger, M. Farinosones A-C, neurotrophic alkaloidal metabolites from the entomogenous deuteromycete *Paecilomyces farinosus*. *J. Nat. Prod*., **2004**, *67*, 1854-58.

[93] Jessen, H.J.; Barbaras, D.; Hamburger, M.; Gademann, K. Total synthesis and neuritotrophic activity of farinosone C and derivatives. *Org. Lett.*, **2009**, *11*, 3446-49.

[94] Aoki, S.; Wei, H.; Matsui, K.; Rachmat, R.; Kobayashi, M. Pyridoacridine alkaloids inducing neuronal differentiation in a neuroblastoma cell line, from marine sponge *Biemna fortis*. *Bioorg. Med. Chem*., **2003**, *11*, 1969-73.

[95] Sakai, R.; Higa, T. Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc*., **1986**, *108*, 6404-05.

[96] Zhang, B.; Higuchi, R.; Miyamoto, T.; Van Soest, R.W.M. Neuritogenic activity-guided isolation of a free base form manzamine A from a marine sponge, *Acanthostrongylophora* aff. *ingens* (Thiele, 1899). *Chem. Pharm. Bull*., **2008**, *56*, 866-69.

[97] Qi, J.H.; Ojika, M.; Sakagami, Y. Linckosides A and B, two new neuritogenic steroid glycosides from the Okinawan starfish *Linckia laevigata*. *Bioorg. Med. Chem*., **2002**, *10*, 1961-66.

[98] Qi, J.H.; Ojika, M.; Sakagami, Y. Linckosides C-E, three new neuritogenic steroid glycosides from the Okinawan starfish *Linckia laevigata*. *Bioorg. Med. Chem*., **2004**, *12*, 4259-65.

[99] Han, C.G.; Qi, J.H.; Ojika, M. Structure-activity relationships of novel neuritogenic steroid glycosides from the Okinawan starfish *Linckia laevigata*. *Bioorg. Med. Chem*., **2006**, *14*, 4458-65.

[100] Han, C.G.; Qi, J.H.; Ojika, M. Linckosides M-Q: neuritogenic steroid glycosides from the Okinawan starfish *Linckia laevigata*. *J. Nat. Med*., **2007**, *61*, 138-45.

[101] Pizza, C.; Minale, L.; Laurent, D.; Menou, J.L. Starfish saponins. 27. steroidal glycosides from the starfish *Choriaster-granulatus*. *Gazz. Chim. Ital*., **1985**, *115*, 585-89.

[102] Qi, J.H.; Han, C.G.; Sasayama, Y.; Nakahara, H.; Shibata, T.; Uchida, K.; Ojika, M. Granulatoside A, a starfish steroid glycoside,

enhances PC12 cell neuritogenesis induced by nerve growth factor through an activation of MAP kinase. *ChemMedChem*, **2006**, *1*, 1351-54.

[103] Hur, J.Y.; Lee, P.; Moon, E.J.; Kang, I.; Kim, S.H.; Oh, M.S.; Kim, S.Y. Neurite outgrowth induced by spicatoside A, a steroidal saponin, *via* the tyrosine kinase A receptor pathway. *Eur. J. Pharmacol*., **2009**, *620*, 9-15.

[104] Nozawa, Y.; Sakai, N.; Matsumoto, K.; Mizoue, K. A novel neuritogenic compound, NGA0187. *J. Antibiotics*., **2002**, *55*, 629-34.

[105] Hua, Z.H.; Carcache, D.A.; Tian, Y.; Li, Y.M.; Danishefsky, S.J. The synthesis and preliminary biological evaluation of a novel steroid with neurotrophic activity: NGA0187. *J. Org. Chem*., **2005**, *70*, 9849-56.

[106] Miyata, A.; Arimura, A.; Dahl, R.R.; Minamino, N.; Uehara, A.; Jiang, L.; Culler, M.D.; Coy, D.H. Isolation of a novel-38 residue-hypothalamic polypeptide which stimulates adenylate-cyclase in pituitary-cells. *Biochem. Biophys. Res. Commun*., **1989**, *164*, 567-74.

[107] Vaudry, D.; Gonzalez, B.J.; Basille, M.; Yon, L.; Fournier, A.; Vaudry, H. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev*., **2000**, *52*, 269-324.

[108] Deutsch, P.J.; Schadlow, V.C.; Barzilai, N. 38-Amino acid form of pituitary adenylate cyclase activating peptide induces process outgrowth in human neuroblastoma cells. *J. Neurosci. Res*., **1993**, *35*, 312-20.

[109] Wolf, N.; Krieglstein, K. Phenotypic development of neonatal rat chromaffin cells in response to adrenal growth factors and glucocorticoids: focus on pituitary adenylate cyclase activating polypeptide. *Neurosci. Lett*., **1995**, *200*, 207-10.

[110] Hernandez, A.; Kimball, B.; Romanchuk, G.; Mulholland, M.W. Pituitary adenylate cyclase-activating peptide stimulates neurite growth in PC12 cells. *Peptides*, **1995**, *16*, 927-32.

[111] Barrie, A.P.; Clohessy, A.M.; Buensuceso, C.S.; Rogers, M.V.; Allen, J.M. Pituitary adenylyl cyclase-activating peptide stimulates extracellular signal-regulated kinase 1 or 2 (ERK1/2) activity in a ras-independent, mitogen-activated protein kinase/ERK kinase 1 or 2-dependent manner in PC12 cells. *J. Biol. Chem*., **1997**, *272*, 19666-71.

[112] Sakai, Y.; Hashimoto, H.; Shintani, N.; Katoh, H.; Negishi, M.; Kawaguchi, C.; Kasai, A.; Baba, A. PACAP activates Rac1 and synergizes with NGF to activate ERK1/2, thereby inducing neurite outgrowth in PC12 cells. *Mol. Brain. Res*., **2004**, *123*, 18-26.

[113] Rønn, L.C.B.; Hartz, B.P.; Bock, E. The neural cell adhesion molecule (NCAM) in development and plasticity of the nervous system. *Exp. Gerontol*., **1998**, *33*, 853-64.

[114] Walmod, P.S.; Kolkova, K.; Berezin, V.; Bock, E. Zippers make signals: NCAM-mediated molecular interactions and signal transduction. *Neurochem. Res*., **2004**, *29*, 2015-35.

[115] Hansen, S.M.; Berezin, V.; Bock, E. Signaling mechanisms of neurite outgrowth induced by the cell adhesion molecules NCAM and N-Cadherin. *Cell. Mol. Life Sci*., **2008**, *65*, 3809-21.

[116] Rønn, L.C.B.; Olsen, M.; Soroka, V.; Østergaard, S.; Dissing, S.; Poulsen, F.M.; Holm, A.; Berezin, V.; Bock, E. Characterization of a novel NCAM ligand with a stimulatory effect on neurite outgrowth identified by screening a combinatorial peptide library. *Eur. J. Neurosci*., **2002**, *16*, 1720-30.

[117] Neiiendam, J.L.; Køhler, L.B.; Christensen, C.; Li, S.Z.; Pedersen, M.V.; Ditlevsen, D.K.; Kornum, M.K.; Kiselyov, V.V.; Berezin, V.; Bock, E. An NCAM-derived FGF-receptor agonist, the FGL-peptide, induces neurite outgrowth and neuronal survival in primary rat neurons. *J. Neurochem*., **2004**, *91*, 920-35.

[118] Cambon, K.; Hansen, S.M.; Venero, C.; Herrero, A.I.; Skibo, G.; Berezin, V.; Bock, E.; Sandi, C. A synthetic neural cell adhesion molecule mimetic peptide promotes synaptogenesis, enhances presynaptic function, and facilitates memory consolidation. *J. Neurosci*., **2004**, *24*, 4197-204.

[119] Køhler, L.B.; Soroka, V.; Korshunova, I.; Berezin, V.; Bock, E. A peptide derived from a *trans*-homophilic binding site in neural cell adhesion molecule induces neurite outgrowth and neuronal survival. *J. Neurosci. Res*., **2010**, *88*, 2165-76.

[120] Rønn, L.C.B.; Dissing, S.; Holm, A.; Berezin, V.; Bock, E. Increased intracellular calcium is required for neurite outgrowth induced by a synthetic peptide ligand of NCAM. *FEBS Lett*., **2002**, *518*, 60-66.

[121] Fobian, K.; Owczarek, S.; Budtz, C.; Bock, E.; Berezin, V.; Pedersen, M.V. Peptides derived from the solvent-exposed loops 3 and 4 of BDNF bind TrkB and $p75^{NTR}$ receptors and stimulate neurite outgrowth and survival. *J. Neurosci. Res*., **2010**, *88*, 1170-81.

[122] Ralets, I.; Østergaard, S.; Holm, A.; Køhler, L.; Bock, E.; Berezin, V. Identification of neurite extension inducing peptides by means of soluble combinatorial peptide libraries. *J. Neurosci. Methods*., **2004**, *137*, 61-69.

[123] Hirata, K.; Yamaguchi, H.; Takamura, Y.; Takagi, A.; Fukushima, T.; Iwakami, N.; Saitoh, A.; Nakagawa, M.; Yamada, T. A novel neurotrophic agent, T-817MA [1-{3-[2-(1-Benzothiophen-5-yl) ethoxy] propyl}-3-azetidinol maleate], attenuates amyloid -β-induced neurotoxicity and promotes neurite outgrowth in rat cultured central nervous system neurons. *J. Pharmacol. Exp. Ther*., **2005**, *314*, 252-59.

[124] Fukushima, T.; Koide, M.; Ago, Y.; Baba, A.; Matsuda, T. T-817MA, a novel neurotrophic agent, improves sodium nitroprusside-induced mitochondrial dysfunction in cortical neurons. *Neurochem. Int*., **2006**, *48*, 124-30.

[125] Nguyen, P.T.H.; Kimura, T.; Ho, S.A.; Tran, A.H.; Ono, T.; Nishijo, H. Ameliorative effects of a neuroprotective agent, T-817MA, on place learning deficits induced by continuous infusion of amyloid-β peptide (1-40) in rats. *Hippocampus*, **2007**, *17*, 443-55.

[126] Yamashita, D.; Shiotani, A.; Kanzaki, S.; Nakagawa, M.; Ogawa, K. Neuroprotective effects of T-817MA against noise-induced hearing loss. *Neurosci. Res*., **2008**, *61*, 38-42.

[127] Li, Y.S.; Matsunaga, K.; Kato, R.; Ohizumi, Y. Verbenachalcone, a novel dimeric dihydrochalcone with potentiating activity on nerve growth factor-action from *Verbena littoralis*. *J. Nat. Prod*., **2001**, *64*, 806-08.

[128] Xing, X.C.; Padmanaban, D.; Yeh, L.A.; Cuny, G.D. Utilization of a copper-catalyzed diaryl ether synthesis for the preparation of verbenachalcone. *Tetrahedron*, **2002**, *58*, 7903-10.

[129] Yeh, L.A.; Padmanaban, D.; Ho, P.; Xing, X.C.; Rowley, P.; Morse, L.J.; Jensen, R.V.; Cuny, G.D. Effects of a verbenachalcone derivative on neurite outgrowth, inhibition of caspase induction and gene expression. *Bioorg. Med. Chem. Lett*., **2005**, *15*, 1193-96.

[130] Clement, C.M.; Dandepally, S.R.; Williams, A.L.; Ibeanu, G.C. A synthetic analog of verbenachalcone potentiates NGF-induced neurite outgrowth and enhances cell survival in neuronal cell models. *Neurosci. Lett*., **2009**, *459*, 157-61.

[131] Forman, B.M.; Tontonoz, P.; Chen, J.; Brun, R.P.; Spiegelman, B.M.; Evans, R.M. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. *Cell*, **1995**, *83*, 803-12.

[132] Satoh, T.; Furuta, K.; Suzuki, M.; Watanabe, Y. Prostaglandin J_2 and its metabolites promote neurite outgrowth induced by nerve growth factor in PC12 cells. *Biochem. Biophys. Res. Commun*., **1999**, *258*, 50-53.

[133] Tanaka, H.; Hasegawa, T.; Kita, N.; Nakahara, H.; Shibata, T.; Oe, S.; Ojika, M.; Uchida, K.; Takahashi, T. Polymer-assisted solution-phase synthesis and neurite-outgrowth-promoting activity of 15-deoxy-$\Delta^{12,14}$-PGJ_2 derivatives. *Chem. Asian J*., **2006**, *1*, 669-77.

[134] Jung, K.M.; Park, K.S.; Oh, J.H.; Jung, S.Y.; Yang, K.H.; Song, Y.S.; Son, D.J.; Park, Y.H.; Yun, Y.P.; Lee, M.K.; Oh, K.W.; Hong, J.T. Activation of p38 mitogen-activated protein kinase and activator protein-1 during the promotion of neurite extension of PC-12 cells by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. *Mol. Pharmacol*., **2003**, *63*, 607-16.

[135] Hatanaka, M.; Shibata, N.; Shintani, N.; Haba, R.; Hayata, A.; Hashimoto, H.; Baba, A. 15d-Prostaglandin J_2 enhancement of nerve growth factor-induced neurite outgrowth is blocked by the chemoattractant receptor-homologous molecule expressed on T-Helper type 2 cells (CRTH2) antagonist CAY10471 in PC12 cells. *J. Pharmacol. Sci*., **2010**, *113*, 89-93.

[136] Lin, C.W.; Wu, M.J.; Liu, I.Y.C.; Su, J.D.; Yen, J.H. Neurotrophic and cytoprotective action of luteolin in PC12 cells through ERK-dependent induction of Nrf2-driven HO-1 expression. *J. Agric. Food Chem*., **2010**, *58*, 4477-86.

[137] Kano, Y.; Horie, N.; Doi, S.; Aramaki, F.; Maeda, H.; Hiragami, F.; Kawamura, K.; Motoda, H.; Koike, Y.; Akiyama, J.; Eguchi, S.; Hashimoto, K. Artepillin C derived from propolis induces neurite

outgrowth in PC12m3 cells *via* ERK and p38 MAPK pathways. *Neurochem. Res*., **2008**, *33*, 1795-803.

[138] Ina, A.; Hayashi, K.I.; Nozaki, H.; Kamei, Y. Pheophytin a, a low molecular weight compound found in the marine brown alga *Sargassum fulvellum*, promotes the differentiation of PC12 cells. *Int. J. Devl Neurosci.*, **2007**, *25*, 63-68.

[139] Hur, J.Y.; Lee, P.; Kim, H.; Kang, I.; Lee, K.R.; Kim, S.Y. (-)-3,5-Dicaffeoyl-*muco*-quinic acid isolated from *Aster scaber* contributes to the differentiation of PC12 cells: through tyrosine kinase cascade signaling. *Biochem. Biophys. Res. Commun*., **2004**, *313*, 948-53.

[140] Shin, J.S.; Kim, Y.M.; Hong, S.S.; Kang, H.S.; Yang, Y.J.; Lee, D.K.; Hwang, B.Y.; Ro, J.S.; Lee, M.K. Induction of neurite outgrowth by (-)-(7*R*, 8*S*)-dihydrodehydrodiconiferyl alcohol from PC12 cells. *Arch. Pharm. Res.*, **2005**, *28*, 1337-40.

[141] Fukuyama, Y.; Nakade, K.; Minoshima, Y.; Yokoyama, R.; Zhai, H.F.; Mitsumoto, Y. Neurotrophic activity of honokiol on the cultures of fetal rat cortical neurons. *Bioorg. Med. Chem. Lett*., **2002**, *12*, 1163-66.

[142] Ito, M.; Sakai, N.; Ito, K.; Mizobe, F.; Hanada, K.; Mizoue, K. A novel fungal metabolite NG-061 enhances and mimics neurotrophic effect of nerve growth factor (NGF) on neurite outgrowth in PC12 cells. *J. Antibiotics*., **1999**, *52*, 224-30.

Received: October 28, 2010 Revised: April 10, 2011 Accepted: May 15, 2011