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Search for Constituents with Neurotrophic Factor Activity from Medicinal Plants and their Application to Drug Development

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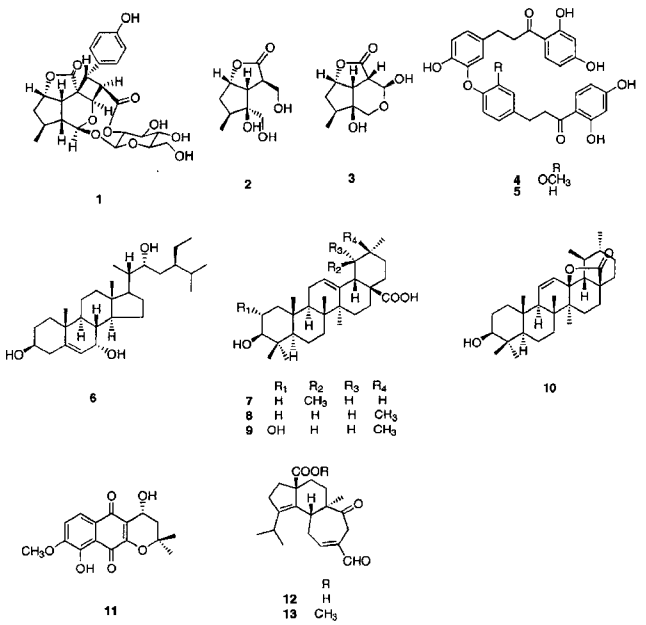
The use of neurotrophic factors (NGF, BDNF, NT-3 and GDNF) as therapeutic agents for neurodegenerative disorders is a just approach to maintain neuronal function (Fukunaga and Miyamoto, 1998; Woodruff and Franklin, 1997; Partrick *et al.*, 1996). However, many of them including NGF are polypeptides of large molecule, can not cross the blood-brain barrier and are easily metabolized by peptidases when administered peripherally. A useful strategy of addressing the drug delivery problem is to administer drugs which are the low molecular substances possessing the characteristic that either enhance the action of or increase the expression of neuritogenic factors such as NGF in the central nervous system (Levi-Montalcini, 1987).

1. The natural products that enhance the activity of neurotrophic factors

20 Paraguayan medicinal plants were examined on NGF-potentiating activities in PC12D cells and the trail results demonstrated that *Verbena littoralis* and *Scoparia dulcis* markedly enhanced the neurite outgrowth from PC12D cells. Furthermore, utilizing the bioactivity-guided separation we successfully isolated 31 and 3 constituents from *V. littoralis* and *S. dulcis*, respectively, including nine iridoid and iridoid glucosides, two dihydrochalcone dimers, two flavonoids and three flavonoid glycosides, two sterols, ten triterpenoids, one naphthoquinone, one benzenepropanamide, four phenylethanoid glycosides. Among which, 11 compounds were new natural products. The results of pharmacological trails demonstrated that littoralisone (**1**) (Li *et al.*, 2001a), gelsemiol (**2**), 7a-hydroxysemperoside aglucone (**3**) (Li *et al.*, 20012), verbenachalcone (**4**) (Li *et al.*, 2001d), littorachalcone (**5**) (Li *et al.*, 2003a), stigmast-5-ene 3β,7α,22α-triol (**6**), ursolic acid (**7**), oleanolic acid (**8**) (Li *et al.*, 2003b), 2a,3b-dihydroxyolean-12-en-28-oic acid (**9**), 3β-hydroxyurs-11-en-28, 13β-olide (**10**), and (4*R*)-4,9-dihydroxy-8-methoxy-α-lapachone (**11**) (Li *et al.*, 2003d) markedly enhanced NGF's action. These substances may contribute to the basic study and the medical treatment of dementia.

In particular, littoralisone (**1**) is a unique heptacyclic iridolactone bearing four-, five-, six-, and nine-membered rings. It was first description that the biosynthesis of the four-membered ring may be formed by the intramolecular [2+2] cycloaddition of



trans-cinnamate moiety on the convex side of iridolactone. Verbenachalcone (**4**) and littorachalcone (**5**) are the first examples of the dimeric dihydrochalcone with a biphenyl ether linkage. These dihydrochalcone dimers showed strong NGF-potentiating activity. Verbenachalcone (**4**) has been regarded as lead compound and successfully been synthesized by American

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Cunys group (Xing *et al.*, 2002). Gelsemiol (**2**) markedly enhanced an increase in the proportion of neurite-bearing cells and an extension of the neurite length in the presence of NGF. It is interesting that in the presence of NGF, 7α-hydroxysemperoside aglucone (**3**) markedly enhanced the elongation of the neurite length, whereas the increase in the population bearing neurites was not affected by it. The data may be interpreted in that gelsemiol (**2**) enhanced NGF-signaling pathways resulting in an increase in the population bearing neurites and neurite elongation, but that 7α-hydroxysemperoside aglucone (**3**) preferentially potentiated the NGF-signal transduction of neurite elongation. Gelsemiol (**2**) and 7α-hydroxysemperoside aglucone (**3**) may provide useful pharmacological tools for studying the mechanism of neurotrophic action of NGF.

For the triterpenoid compounds, ursolic acid (**7**) and oleanolic acid (**8**) showed the most powerful activities. Study of the structure-activity relationships showed that during the ursane type triterpene compounds, ursolic acid and 3b-hydroxyurs-11-en-28,13β-olide exhibited significant activity and 2α,3β-dihydroxyurs-12-en-28-oic acid was secondary, but the other compounds, which possess one hydroxyl group at C-19, C-23, or C-24 position more than ones, were not found activity for this examination. Among the oleanane type triterpene compounds, oleanolic acid and 2α,3β-dihydroxyolean-12-en-28-oic acid showed powerful activities. However, the other compounds were inactivity. These results suggest that with the increase of hydroxyl group number the activity will be weakened or lost in this bioassay system, accordingly.

2. The natural products that enhance secretion of neurotrophic factors

We have shown that the scabronine G (**12**) isolated from *Sarcodon scabrosus* enhanced the secretion of neurotrophic factors from 1321N1 human astrocytoma cells (Obara *et al.*, 2001). We examined the mechanism of newly synthesized scabronine G-methylester (ME, **13**)-induced secretion of neurotrophic factors from 1321N1 cells. Scabronine G-ME increased the secretion of nerve growth factor (NGF) and interleukin-6 (IL-6) from 1321N1 cells with the enhancement of their mRNA expressions. GF109203X inhibited the scabronine G-ME-induced mRNA expressions of both NGF and IL-6 and the differentiation of PC-12 cells, showing that scabronine G-ME activated PKC. Recombinant PKC-ζ activity was also increased by scabronine G-ME, suggesting the involvement of PKC-ζ in the effect of scabronine G-ME. Scabronine G-ME translocated nuclear factor-kB to nucleus, and enhanced its transcriptional activity. These results suggest that scabronine G-ME potentially enhances the secretion of neurotrophic factors from 1321N1 cells mediated via the activation of PKC-ζ.

3. The natural products that induce neuronal differentiation

Recently F1, a flavonoid isolated from citrus fruits, was observed to induce neurite outgrowth from a rat pheochromocytoma cell line, PC12D cell, in a concentration-dependent manner. F1-induced neurite outgrowth was prevented by specific MEK inhibitors, PD98059 and Uo126. It was also found that this natural product promoted and sustained MAPK and CREB phosphorylations which were blocked by an adenylate cyclase inhibitor, SQ22536 and a PKA inhibitor, H89, but not by a TrkA inhibitor, K252a, indicating a cAMP-dependency of the actions of this compound. Consistently, F1 enhanced a CRE-mediated transcriptional activation in PC12D cells. F1 also stimulated synaptic transmission among rat primary hippocampal neurons in culture. Moreover, mice exhibiting the impairment of learning and memory-related behavior caused by olfactory bulbectomy (OBX) were used to examine the effect of F1 on the learning and memory in vivo. F1 markedly improved the impairment of learning and memory-related behavior in a dose-dependent manner in the OBX mice. These findings suggest that F1 is a new lead compound for drug development for the CNS neurodegenerative diseases, including Alzheimer's disease, and is useful to clarify the mechanism which controls the CNS neuronal differentiation and functions.

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