2,5-Diketopiperazines as Neuroprotective Agents

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Abstract: 2,5-diketopiperazines are the simplest cyclic peptides found in nature, commonly biosynthesized from amino acids by different organisms, and represent a promising class of biologically active natural products. Their peculiar heterocyclic structure confers high stability against the proteolysis and constitutes a structural requirement for the active intestinal absorption. Furthermore, the diketopiperazine-based motif is considered as a novel brain shuttle for the delivery of drugs with limited ability to cross the blood-brain barrier (BBB) and can be proposed as an ideal candidate for the rational development of new therapeutic agents. Although these cyclic peptides have been known since the beginning of the 20th century, only recently have they attracted substantial interest with respect to the wide spectrum of their biological properties, including antitumor, antiviral, antifungal, antibacterial and antihyperglycemic activities. In addition to these, the most challenging function of the diketopiperazine derivatives is related with their remarkable neuroprotective and nootropic activity. The aim of the present paper is to provide an overview of the two major classes of diketopiperazines, the TRH-related and the unsaturated derivatives both characterized by a significant ability to protect against neurotoxicity in several experimental models.

The neuroprotective profile of these compounds suggests that they may have a future utility in the therapy of neuronal degeneration *in vivo*, potentially through several different mechanisms.

Keywords: Alzheimer's disease, Amyotrophic lateral sclerosis, cyclic dipeptides, 2,5-diketopiperazines, neuroprotective peptides, Parkinson's disease.

INTRODUCTION

2,5-diketopiperazines (DKPs) (Fig. **1**), also known as piperazine-2,5-diones, are a group of small cyclic peptides characterized by a peculiar heterocyclic system, commonly found in several natural products [1]. Although cyclic dipeptides are extensively obtained by extraction from natural sources, they may be easily prepared by conventional synthetic procedures, due to the relative structural simplicity of their essential nucleus [2]. The conformationally constrained DKP scaffold is constituted of a six-membered ring that orientates its substituents in a spatially defined manner and represents a significant pharmacophore in medicinal chemistry because of its stable structural characteristics [3, 4]. In addition, owing to their restricted conformational freedom and structural simplicity, DKPs have been extensively used as valuable models for conformational studies either in solution or in solid state, particularly with regard to the consequences (i.e. cis, trans-isomerism) deriving from the relative configuration of amino acid residues [5, 6].

In contrast with classical linear peptides, DKPs show some important chemical advantages, such as stability to proteolysis, ability to simulate peptidic pharmacophoric groups, control of the substituent stereochemistry, conformational rigidity and promotion of interactions with biological targets due to donor and acceptor groups for hydrogen bonding [7]. In addition, His-containing DKPs deserve a significant interest in view of their non-enzymatic catalytic properties [8, 9]. Furthermore, Teixidò *et al.* [10] described for the first time DKPs as a novel family of brain delivery systems for poorly BBB-permeable compounds. Therefore, the DKPs scaffold is considered a useful tool for the discovery of new lead compounds and the suitable properties of DKPs make them attractive and promising agents for the rational development of new therapeutic agents [2, 11-13].

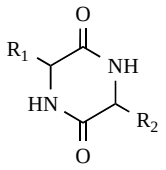

Fig. (1). General structure of 2,5-diketopiperazines.

Several important biological properties have been recently assigned to members of this family. In fact, DKPs have shown activity as antitumor, antiviral, antifungal, antibacterial, as well as antiarrhythmic and antihypertensive agents [7, 13-23]. Lately, a strong attention has been focused on the multifunctional neuroprotective and nootropic activity of DKPs. This new intriguing role was confirmed in several experimental models and suggests the possibility of using these cyclic dipeptides for the treatment of neurodegenerative disorders. As well-known, neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are age-dependent multifactorial disorders characterized by

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neuronal death and degeneration leading to a progressive functional decline. The current available treatments are symptomatic and do not affect the underlying course of the disease. Therefore, the challenge involved in the discovery and development of novel potential neuroprotective drugs for the treatment of these disorders is one of the major tasks for both the academic and pharmaceutical communities. The goal of this article is to provide the reader with a critic overview on various classes of DKPs able to protect against neuronal degeneration, on their different mechanisms of action, and finally on the structure-activity relationships that influence the neuroprotective activity of the DKPs backbone.

TRH-RELATED DKPs

Naturally occurring hormones, such as corticosteroids, progesterone and thyrotropin-releasing hormone (TRH), have been among the first compounds to be studied for their multipotential neuroprotective effects. The tripeptide TRH, L-pyroglutamyl-L-histidyl-L-prolineamide, is the first hypothalamic releasing factor to be characterized, and plays a neuromodulatory role within the central nervous system (CNS) [24]. Evidence for the significant neuroprotective role of TRH has considerably increased over recent years. In fact, substantial literature [25-27] demonstrates that TRH or TRH analogs can significantly improve neurological recovery after traumatic brain or spinal cord injuries. However, TRH is subject to rapid enzymatic degradation and shows autonomic, analeptic and endocrine actions that limit its clinical use [27]. Therefore, a series of TRH derivatives devoid of side physiological actions retaining the neuroprotective action of the parent drug, as well as a family of cyclized dipeptides related to TRH, have been investigated [24, 28-34].

Cyclo(His-Pro) (CHP) (**1**) (Fig. **2**) is a biologically active peptide produced by the enzymatic cleavage of the hypothalamic TRH (Fig. **3**) [35], and it has been the subject of much research over the last decades for its several biological roles in the CNS [36, 37].

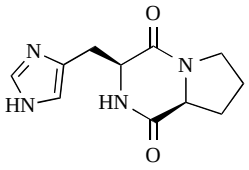

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Fig. (2). Chemical structure of Cyclo (His-Pro).

Among these, its neuroprotective activity seems to be one of the most innovative and promising feature for future applications. According to this, it has been reported that CHP and some of its recently synthesized derivatives are able to prevent neuronal death induced by free radicals, calcium mobilization, and traumatic injury, suggesting their potential use for the treatment of neurodegenerative diseases [38].

Lately, Minelli and co-workers [39] investigated the ability of CHP in *in vitro* PC12 cells cultures to affect cellular proliferation in presence of experimental conditions that cause cellular stress and to prevent apoptosis [40]. It has been hypothized that CHP acts *via* a receptor dually coupled to stimulatory and inhibitory G proteins, but the binding characteristics of this receptorial system are still not completely clear [39]. *In vitro* results suggested that this cyclic dipeptide exhibits a protective effect against oxidative stress in H_2O_2-injured cells through a mechanism that leads to a decrease in reactive oxygen species (ROS) generation and an increase in glutathione levels (Fig. **4**) [41]. The cytoprotection provided by CHP seems to occur through a mechanism involving the nuclear accumulation and activation of the Nf-E2-related factor-2, a transcription factor that up-regulates antioxidant-/electrophile-responsive element-related genes, in PC12 cells [42].

In order to detect the structural moiety responsible for the antioxidant effect and to enhance the neuroprotective action, a variety of CHP-related DKPs have been synthesized, modifying both the proline and histidine residues.

Keeping intact the proline residue, the histidine has been replaced by 3,5-di-*tert*-butyltyrosine (DBT), a phenolic amino acid known as scavenger of ROS. In this contest, Prakash *et al*. [38] reported the synthesis and the biological activity of two diastereomeric 2,5-diketopiperazines (**2**, **3**) (Fig. **5**) based on the CHP backbone.

The neuroprotective profile of these compounds was evaluated in several models of cell death, using the two components of the new drugs, 3,5-di-*tert*-butyltyrosine and cyclo(Gly-Pro) (CGP), as controls. Both diastereoisomers showed neuroprotective activity in a neuronal-glial model of trauma, in which several secondary injury factors are involved, and dose-dependently prevented calcium-induced necrosis, as well as cell death induced by $FeSO_4$, a free radical generator. Importantly, the new derivatives also protected against glutamate or β-amyloid neurotoxicity [29]. It is not surprising that both DBT and CGP components contribute to the overall neuroprotective effect of **2** and **3**,

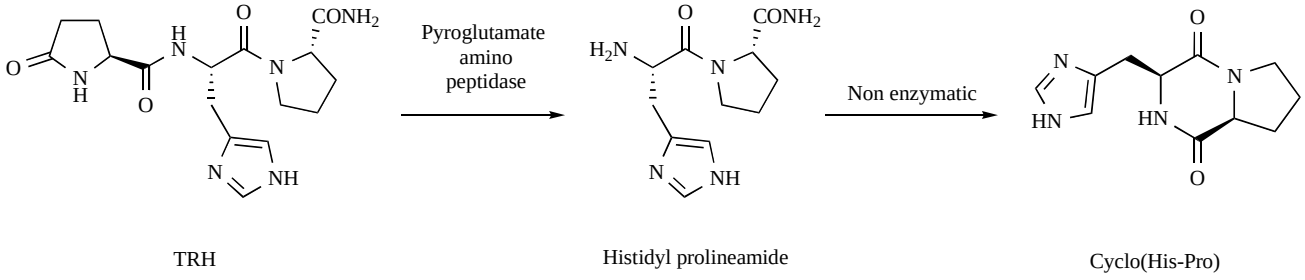


Fig. (3). Cyclo(His-Pro) formation.

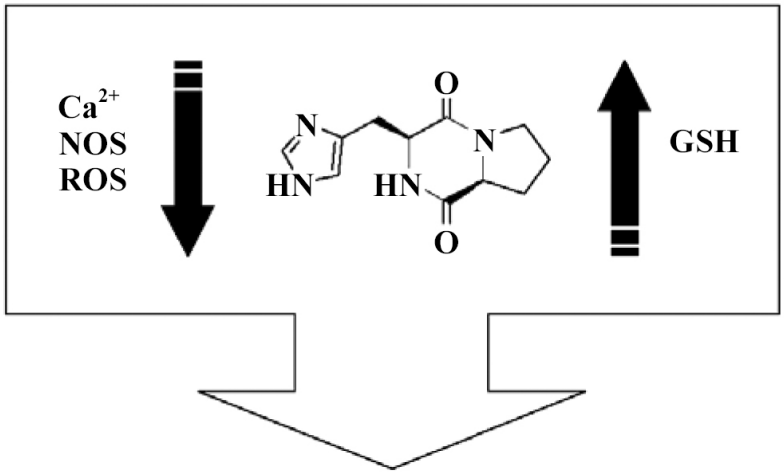
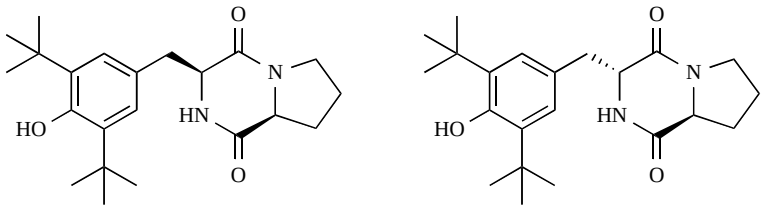


Fig. (4). Protection mechanism proposed for Cyclo(His-Pro) in H_2O_2-injured cells.

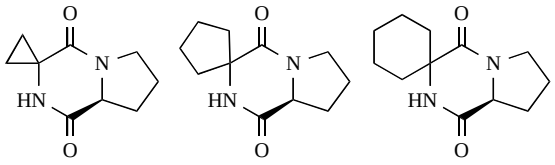


2 **3**

Fig. (5). Chemical structure of two diastereomeric DKPs based on the structure of CHP.

being DBT, as mentioned above, a phenolic antioxidant able to improve the neuroprotection and CGP a cyclic derivative of glycine, known in the literature as a protective agent against toxicity by reducing oxidative stress [43-45].

Subsequently, a series of three, five and six-membered ring 1-amino-1-cycloalkylcarboxylic acids have been inserted in the DKP scaffold in place of histidine, obtaining compounds **4**-**6** (Fig. **6**), which retain the physiological activity of TRH [29, 32, 33, 46, 47].



4 **5** **6**

Fig. (6). Chemical structure of DKPs containing three (**4**), five (**5**) and six (**6**)-membered ring 1-amino-1-cycloalkylcarboxylic acids in place of histidine.

All cyclic dipeptides exhibited remarkable neuroprotective effects in multiple *in vitro* models of neuronal injury and after controlled cortical impact (CCI) in mice, although the best neuroprotective profile *in vitro* is recorded for compound **6**, as detailed in Table **1** [29].

This cyclohexyl diketopiperazine, which is currently being developed by RemeGenix for clinical trials, resulted able to reduce both apoptotic and necrotic cell death in different *in vitro* models. Effects appeared to be pleiotropic, with treatment reducing multiple potential secondary injury factors (cyclins, calpains, cathepsins), while upregulating various endogenous neuroprotective and neurotrophic factors (BDNF, HSP-70, HIF-1) [30-33]. Further, DKP **6** protected against glutamate or β-amyloid neurotoxicity, as well as free-radical induced toxicity. In i*n vivo* experiments, intravenous administration of **6** as single bolus injection significantly reduced volumes of brain lesions and improved neurological outcome after either fluid percussion-induced traumatic brain injury (FPI) in rats or CCI injury in mice [29, 46]. In addition, Faden *et al*. [46] demonstrated that compound **6** also offers pharmacokinetic advantages compared with the parent drug TRH and TRH analogs. Derivative **6** is in fact reported to be several orders of magnitude more lipophilic (log P = 1.17) than TRH (log P = -2.46) and this high lipophilicity would be expected to increase the cellular permeability and thus to satisfy the requirements for CNS penetration. In addition, its cyclic structure confers high stability toward proteolytic degradation, making it likely a good candidate for oral administration [46]. The neuroprotective and nootropic activity of this novel diketopiperazine makes it a promising

Table 1. ***In Vitro*** **Neuroprotective Activity of DKPs 4-6 [29]**

	In vitro **models**						
DKPs	**Glutamate**	**Maitotoxin**	**$FeSO_4$**	**β Amyloid**	***In vitro*** **trauma**	**Trophic Factor Removal**	**Oxigen/Glucose Deprivation**
4	-	NT	NT	-	+	+	NT
5	-	NT	NT	-	+	+	NT
6	+	+	-	+	+	+	±

+, positive effect; -, no effect; ±, partial effect; NT, not tested.

agent for the treatment of both acute and chronic neurodegeneration.

More recently, in order to detect structurally novel neuroprotective lead compounds based on DKP **6**, pharmacophore modeling and three-dimensional structure-based database search approaches have been employed by Faden *et al.* [48]. Structure-activity relationship analysis revealed that all DKPs with neuroprotective activity contain common structural features, as shown in Fig. (**7**).

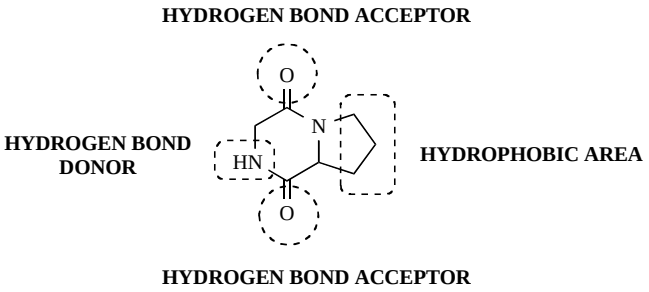


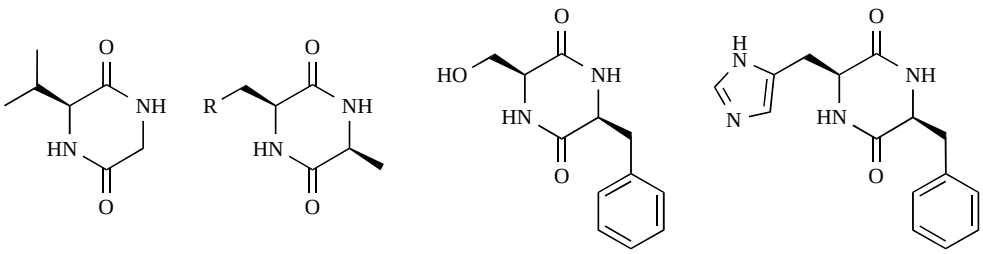
Fig. (7). Common structural features of active DKPs [48].

This pharmacophore model describes the necessary but not the sufficient structural features required for neuroprotection, thus providing a useful tool to search for structurally novel lead compounds. Through a preliminary screening, 115 compounds have been identified as potential candidates and among these 15 were selected for *in vitro* tests. All compounds were studied in a *in vitro* model of apoptotic neuronal cell death and five of these DKPs, **7**-**11** (Fig. **8**), afforded substantial neuroprotective activity at concentrations of 100 μM. The hydrophobic portion of these DKPs is guaranteed by the presence of isopropyl, isobutyl or aromatic side chain, and in the meantime the hydrogen bond donor-acceptor systems provided by the amide linkages are preserved. The best neuroprotective profile was recorded after administration of DKP **9** even at lower concentrations, remarkably reducing cell death at 10 μM concentration [48].

Compared to the lead compound **6**, in **9** the proline residue has been replaced with an alanine, and an isobutyl substituent has been inserted in place of the cyclohexyl. Although **9** and these other four DKPs did not show a significant efficacy compared to the potent lead **6**, they are nevertheless considered as a starting point for further optimization through chemical modifications and molecular modeling.

More recently, a series of potent, safe and neuroprotective DKPs, including DKPs **12**-**25** (Fig. **9**), and their pharmaceutical compositions, have been patented [49]. The histidine residue of the parent CHP has been replaced with different modified-amino acids bearing side chain moieties which act as radical scavengers, such as sulphur-containing residues (**19**-**25**), or as nitric oxide synthase (NOS) inhibitors, such as modified-arginine residues (**17**, **18**). High concentrations of ROS, accompanied by an inadequate antioxidant defense system, and an excessive production of NO, as a consequence of nitric oxide synthase induction in activated glia, have been in fact attributed to participate in neurodegeneration processes.

As DKPs with a sulfanyl group can form disulfide-bridged dimers with strong CNS activity, Kozikowski *et al.* [49] described in their invention a heterodimer (**24**) in which one monomer is a DKP scaffold, and the other is a 2-azetidinone TRH analogue, and alternatively a homodimer (**25**) in which both monomers are DKPs. There is no



7 **8** R = H **9** R = *i*-Pr **10** **11**

Fig. (8). Chemical structure of DKPs selected through pharmacophore modeling and three-dimensional structure-based database search approaches.

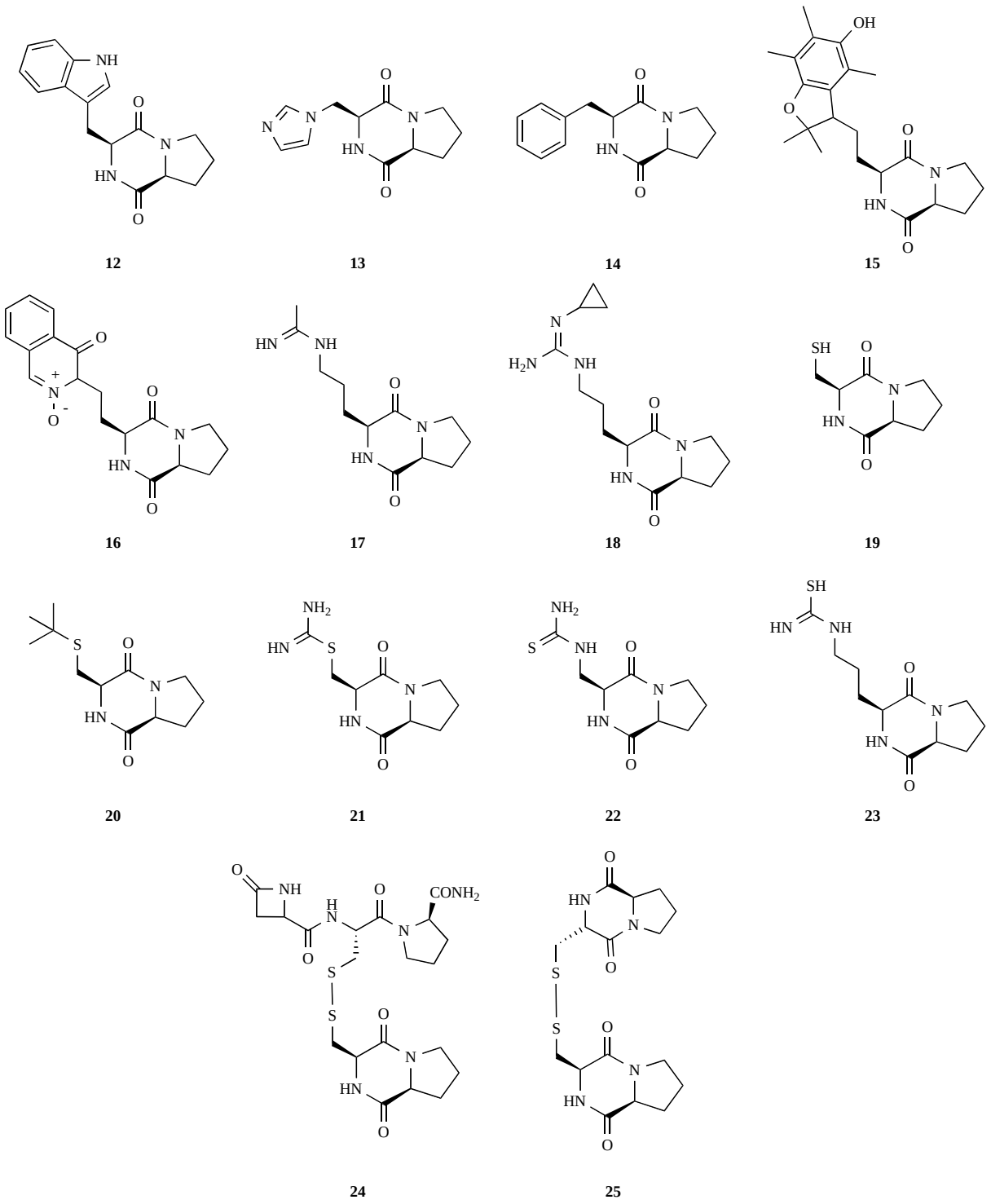


Fig. (9). Chemical structure of neuroprotective DKPs patented by Kozikowski *et al*. [49].

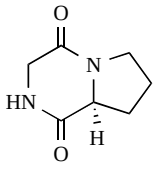
evidence that the mechanism of action of these disulfide-bridged dimers involves a direct interaction with TRH receptors but it has been proposed that they are converted *in vivo* to the sulfanyl-containing monomeric forms and that their neuroprotective action may be associated with their free-radical scavenging properties, similar to the GSH action. The common structural motif of these cyclic dipeptides **12-25** is the proline moiety. The proline is expected to contribute to the nootropic properties, since structurally related amides or esters of proline possess cognitive enhancing ability and proline dipeptides have been found much more potent than piracetam, which is the prototype of nootropic drugs [50-52]. The patented compounds showed in fact neuroprotective effects in standardized models of CNS injuries or neurodegenerative disorders, such as Alzheimer's mouse model, and a strong

CNS activity which allows them to be used to enhance memory function and to treat several neurological disorders. Therefore, these results strengthen the hypothesis that the incorporation of the proline residue in the DKP scaffold represents an important structural feature for the overall neuroprotective action exerted by this class of cyclic dipeptides.

UNSATURATED DKPs

Another prominent and recent class of neuroprotective cyclic dipeptides consists of a diketopiperazine scaffold containing double bonds in side chains. The unsaturated function is often present in α, β or γ, δ position, thus generating two main classes of unsaturated DKPs. It was of interest to explore the contribution of unsaturated systems in performing the neuroprotective effect. Importantly, it has been shown that the insertion of these unsaturated bonds is a major determinant of the potential protective and reparative effects on neural degeneration or cell death.

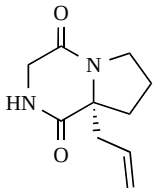
CGP (**26**) (Fig. **10**), as previously mentioned, is an endogenous cyclic derivative of glycine, which has been isolated from brain tissue, showing an impressive influence on memory consolidation. It has been in fact reported to improve cognitive function and to exhibit antiamnesic activity [43, 53].



26

Fig. (10). Chemical structure of CGP.

In order to improve the enzymatic resistance and thus the bioavailability of this endogenous compound, a CGP analogue (**27**) (Fig. **11**), namely NNZ 2591, was synthesized by inserting a γ, δ-allyl substituent at C-8a on the diketopiperazine skeleton, with the intention of increasing lipophilicity and thereby facilitating the ability to cross the cerebrospinal fluid (CSF)-brain barrier after the compound is delivered to the CSF [54, 55].

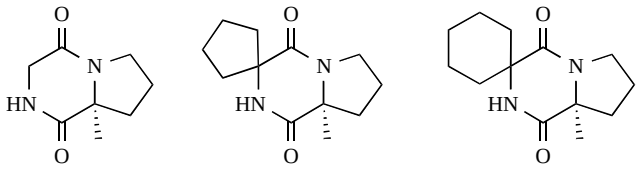


27

Fig. (11). Chemical structure of the allylated analogue of CGP (**27**).

Possible mechanisms underlying the neuroprotective activity of **27** have been suggested by studies examining the treatment effects of **27** on adult rats with hypoxic-ischemic brain injury [55]. Compared to the parent **26**, central administration of **27** resulted in a much stronger neuroprotective action, as the effective doses of the allyl DKP were in a lower range than those of the non allylated. The allylation process therefore caused an increase in lipophilicity which allowed an easier crossing of the BBB after the delivery to the CSF, where the drug remained detectable for several hours after peripheral administration. Guan *et al*. [55] also demonstrated that its peripheral administration improved somatosensory-motor function and log-term histological outcome. Although the neuroprotective mechanism of **27** has not completely been identified and needs further investigation, enhancement of astrocyte activity and inhibition of both caspase-3 and microglial reactivity seem to be the mechanisms responsible for the protective effects [55, 56]. A rat model of PD has also been used to confirm both the neuroprotective and neurotrophic actions of this allylated DKP [57]. The studies showed that an earlier treatment with **27** during the active phase of neuronal loss resulted in a significant long-lasting improvement of the motor function after the onset of 6-hydroxydopamine-induced motor deficit in rats. Finally, the effects of **27** on spatial learning and memory after scopolamine-induced amnesia in rats have been investigated [58]. The allylated diketopiperazine has been found to prevent scopolamine-induced acute memory impairment, playing a scopolamine-independent antagonistic role on muscarinic M2 acetylcholine receptors. This modulation of acetylcholine neurotransmission seems to be the mechanism of action responsible for its nootropic action [58]. In addition, **27** has recently been discovered to be effective in prophylaxis and treatment of PD and different methods for its therapeutic use to treat disorders characterized by degeneration and death of dopaminergic neurons have been patented [59].

The structure of **27** has been chemically modified with the aim of both investigating the role of the allyl group and improving the neuroprotective action. For this purpose, the allyl group has been replaced with a methyl group and cyclopentyl and cyclohexyl rings have been inserted into the alpha-position of glycine, as in compounds **5**-**6**, obtaining respectively DKPs **28**-**30** (Fig. **12**) [54, 59].

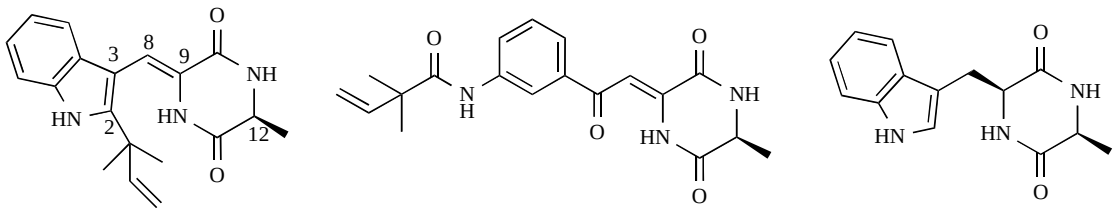


28 **29** **30**

Fig. (12). Chemical structure of DKPs containing a methyl group (**28**) and cyclopentyl (**29**) and cyclohexyl (**30**) rings.

Among these DKPs, the cyclopentyl derivative **29** significantly decreased or prevented glutamate-induced neurotoxicity, but in any case none of them exceeded the long-lasting therapeutic potency of the allylic parent **27** [59]. This finding confirms that the presence of the allylic side chain plays a critical role in the protective properties of the compound.

Another interesting structural modification of neuroprotective DKPs is present in bioactive metabolites isolated



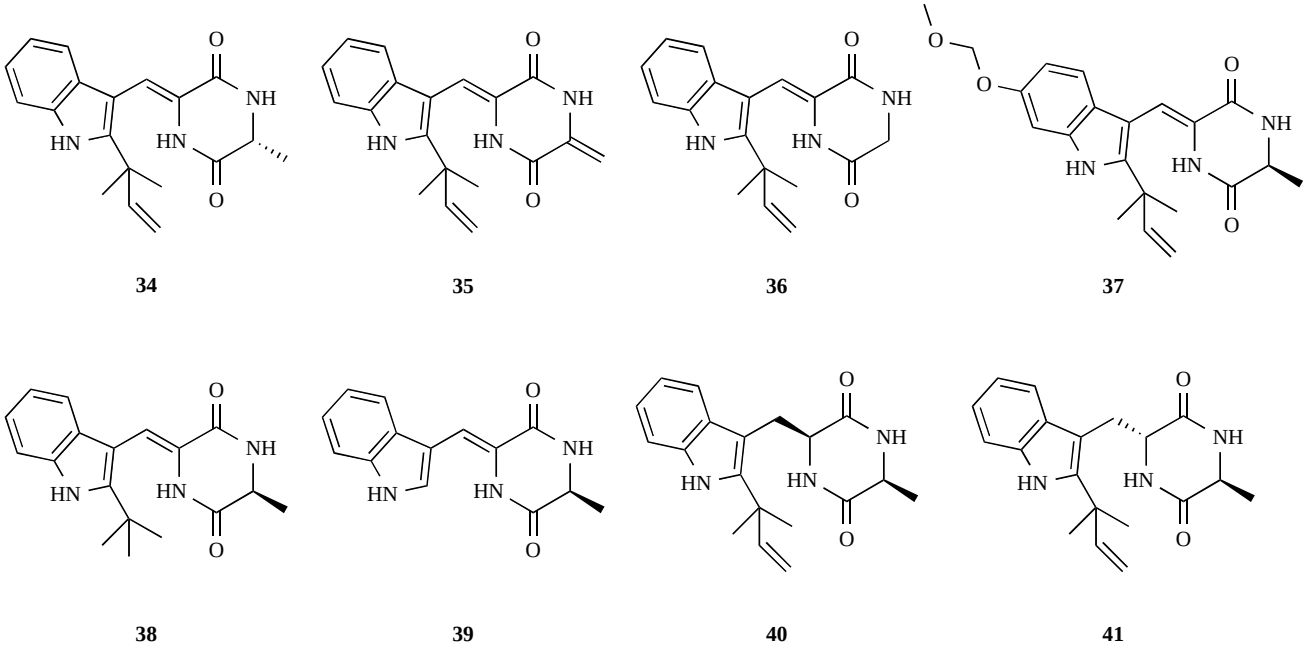
31 **32** **33**

Fig. (13). Chemical structure of by bioactive metabolites isolated from marine-derived fungus *Aspergillus* sp.

from the culture broth of the marine-derived fungus *Aspergillus* sp, including two α, β- unsaturated compounds, Neoechinulin A (**31**) and golmaenone (**32**), in addition to the saturated cyclo(Trp-Ala) (**33**) (Fig. **13**) [60, 61]. Neoechinulin A (**31**) is considered the lead of these metabolites and possesses scavenging, neurotrophic factor-like and anti-apoptotic activities [62]. Investigating the antioxidant activity of these metabolites, Li *et al.* [61] demonstrated that golmaenone **32** exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values comparable to those of Neoechinulin A (**31**). On the contrary, cyclo(Trp-Ala) (**33**), lacking the C-8/C-9 double bond, showed no activity.

Neoechinulin A (**31**) has been shown to protect primary neuronal cells and nerve growth factor (NGF)-differentiated PC12 cells against extracellular peroxynitrite ($ONOO^-$)-induced oxidative damage and against 1-methyl-4-phenylpyridine (MPP^+)-induced cytotoxicity [62, 63]. A substantial literature has provided strong evidence that peroxynitrite, a powerful oxidant produced from the reaction of superoxide with nitric oxide, is involved in oxidative damage of PD and AD [64-66]. Thus, it became apparent that compounds able to scavenge $ONOO^-$, such as DKP **31**, can represent potentially useful therapeutic agents for the treatment of these diseases. Neoechinulin A, probably biosynthesized from an L-tryptophan and an L-alanine, consists of three structural moieties, an indole, an isoprenyl substituent and a diketopiperazine nucleus. The stereochemistry of the C-8/C-9 double bond and of the stereocenter C-12 was assigned as Z and S, respectively. In order to investigate the potential mechanism of action and the structure-activity relationships of the parent **31** respect to anti-nitration, antioxidant and cytoprotective properties, a series of Neoechinulin A derivatives **34**-**41** (Fig. **14**) has been designed and prepared [67].

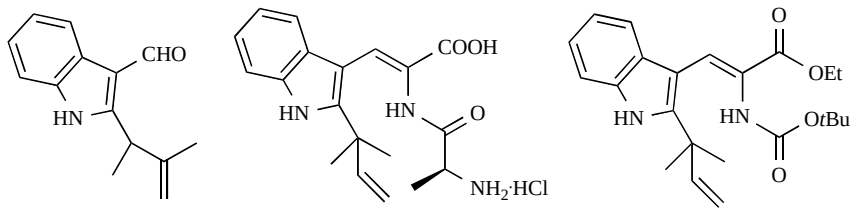
The anti-nitration activity of compounds **34**-**41** was evaluated by their ability to inhibit the formation of nitrotyrosine induced by 3-(4-morpholinyl)sydnonimine hydrochloride (SIN-1), a peroxynitrite generator. Compounds **34**-**39** showed a strong inhibitory action, while for **40** and **41**, lacking the C-8/C-9 double bond, a detectable inhibition even at high concentration was not recorded. The antioxidant activity was measured evaluating both the inhibition of peroxynitrite-induced luminol oxidation and the effects on lipid peroxidation in rat brain homogenates. As observed in the previous assay, DKPs **40** and **41** were devoid



34 **35** **36** **37**

38 **39** **40** **41**

Fig. (14). Chemical structure of Neoechinulin A derivatives.



42 **43** **44**

Fig. (15). Chemical structure of Neoechinulin A derivatives lacking the DKP ring.

of antioxidant activity. Once again, DKPs **34**-**39** showed cytoprotection against peroxynitrite derived from SIN-1 in NGF-differentiated PC12 cells and this cytoprotective activity is probably correlated with their ability to intensify the NAD(P)H-producing capacity of the cells. Diketopiperazine ring-opened analogs **42**-**44** (Fig. **15**) have been also synthesized in order to investigate the role of the DKP skeleton [67, 68].

The main aim of these studies was to determine the structural requirements necessary for the overall neuroprotective action. It became clear that the intact DKP ring is necessary for the anti-nitration activity but at the same time is not required for cytoprotection. In fact, opened analogs **42**-**44** showed no or slightly inhibitory effect on nitrotyrosine formation, but nevertheless treatment with the compound **44** resulted in a greater cytoprotective effect than the parent Neoechinulin A, thus demonstrating that the anti-nitration activity is not responsible for the cytoprotection in SIN-1-induced cell death. The lack of anti-nitrative effect observed with aldehyde **42** suggests that the presence of a suitable substitution at the C-3 of the indole ring is required for this activity.

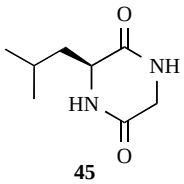
In addition, after treatment with the natural isomer of Neoechinulin A (**34**), the same degree of protection achieved with the parent (**31**) has been observed. Therefore, the orientation of the methyl group at C-12 does not provide a significant contribution to the cytoprotection against SIN-1 [69]. Finally, the total absence of any kind of neuroprotective action recorded with compound **40** and **41** underlines that the isoprenylated indole group at C-9 doesn't contribute to cytoprotection but above all that the presence of the C-8/C-9 double bond is a structural requirement necessary for all the anti-nitration, antioxidant as well as cytoprotective actions.

It is interesting to note that the presence of the sp^2-hybridized Cα and Cβ atoms in compounds **31**, **32** and **34**-**39** generates an achiral amino acid residue capable to exert conformational constraint on the DKP backbone and restricts at the same time the β-substituent to the Z or E orientation. Although the consequences of this structural alteration appears quite relevant with respect to the γ, δ-unsaturated models above reported (see compound **27**), an equivalent neuroprotective effect is observed for the two kinds of unsaturated DKPs.

Findings reported in this section confirm that the insertion of these different kinds of unsaturated systems in the diketopiperazine scaffold plays in any case a pivotal role in the protective and reparative properties of these DKPs, suggesting a potential therapeutic intervention in neurodegenerative diseases.

OTHER DKPs

In addition to the two main classes of DKPs above mentioned, it should be further noted that others cyclic dipeptides, have also been shown to exhibit neuroprotective and nootropic properties. Among these, cyclo(Leu-Gly) (**45**) (Fig. **16**), the cyclic analog of H-Pro-Leu-Gly-NH_2, which in turn is the C-terminal neurohypophyseal tripeptide of oxytocin, results in fact very effective in memory retention [70].



45

Fig. (16). Chemical structure of cyclo(Leu-Gly).

As reported by Walter and co-workers [70], this structurally simple DKP is able to reduce the blockade of memory induced by puromycin, a protein synthesis inhibiting drug which can impair memory processes. Recent advances in the study of neurodegenerative diseases have provided molecular and cellular models for studying the underlying disease mechanisms. In a recent study 1040 FDA-approved drugs have been screened by a consortium of investigators in multiple neurodegenerative disease *in vitro* assays, including a model of ALS [71]. As well-established, ALS is a nervous system disorder with uncertain pathogenesis characterized by progressive degeneration of motor neurons in the brainstem and spinal cord. Many causal and pathogenetic hypotheses for ALS have been proposed over the years, ranging from heavy-metal toxic effects to environmental and occupational exposures [72, 73]. Despite extensive research, the disorder remains poorly understood in terms of a unifying causal hypothesis and, therefore, the main objective of the consortium was to identify novel pharmacological agents for the treatment of ASL. Among the several compounds screened, cyclo(Leu-Gly) (**45**) has been found to reduce motor neuron death induced by 100 μM glutamate, at the 1 μM dose, even if its protective mechanism of action still remains unknown. Therefore, this

cyclic dipeptide, probably due to the incorporation of the neuroprotective glycine in the DKP skeleton [44], could be potentially able to slow the progression of the disease, providing a useful supplement to standard treatment.

CONCLUSIONS AND FUTURE PERSPECTIVES

In the recent decades two main classes of diketopiperazines, the TRH-related and the unsaturated compounds, have been proposed as new potential candidates with a remarkable neuroprotective profile. 2,5-diketopiperazines structurally related to TRH have been reported to prevent or reduce both necrotic and apoptotic cell death in different *in vitro* models and resulted able to significantly improve cognitive and motor outcome and to reduce lesion volumes following traumatic brain injury. Based on the biological activity results it is noteworthy that the proline residue could be identified as a structural element that contributes significantly to the overall neuroprotective action, as well as to the nootropic and cognitive-enhancing properties of this class of DKPs.

On the other hand, DKPs containing unsaturated systems possess the ability to enhance somatosensory-motor function and long-term histological outcome and exhibit a significant radical scavenging activity. In this context, biological activity results confirmed that the presence of unsaturated groups in the DKP skeleton of this class plays a critical role in the protective properties of these compounds.

In this review we have emphasized the attractiveness of exploiting the good pharmacokinetic profile and the long-lasting neuroprotective action of diketopiperazine-based agents, originated from synthetic chemistry as well as from natural sources, in order to provide a helpful assistance to conventional therapies for chronic neurodegenerative conditions. Despite the encouraging results reported in this field, further structure activity relationship studies will be required also to identify the key structural elements that confer neuroprotective activity to the DKP scaffold.

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Received: May 08, 2011 Revised: July 11, 2011 Accepted: August 26, 2011