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Ultra-Short Cyclo-Peptides as Bio-Inspired Therapeutics: Proline-Based 2,5-Diketopiperazines (DKP) †

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Abstract: 2,5-diketopiperazines (DKP) are the simplest cyclo-peptides in nature, which could play a key role in the origin of life. They are ubiquitous in microorganisms, higher species, and in food and beverages. These dipeptides have been known since the beginning of the 20th century, but they have only recently been gaining interest due to diverse, noteworthy bioactivities, such as, but not limited to, anticancer, antiviral, antioxidant, and neuroprotective properties. DKPs have relevance in quorum sensing, cell–cell signaling, or as drug delivery systems. They have less toxicity, increased cell permeability, and binding affinity. Proline-containing DKPs have an extra-rigid conformation and are more resistant to degradation by enzymes. They represent an attractive subclass of cyclo-peptides with a high potential in future therapies.

Keywords: short peptides; diketopiperazine; proline-derived cyclic dipeptides

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1. Introduction

The main aim of this mini-review is to provide an overview of the scientific findings on diversity and the enormous biological potential of the simplest natural cyclo-peptides in light of the newest literature, structural databases, and patents. Further, we have emphasized the attractiveness of their pharmacokinetic profile in relation to the future innovative effective therapeutical and bio-control agents.

2. Cyclo-Peptides: General Considerations

Recently, short peptides have been enjoyed greater and greater significant interest as a unique class of bio-molecules filling a therapeutic niche between small chemical drugs and macro-molecular agents with diverse well-known limitations. Oligopeptides are a primary source of bio-molecules, which are components of proteins participating in bio-processes. Therefore, they have significant advantages, such as diverse bioactivities, high selectivity due to specific interactions of peptides with targets, low toxicity, because they do not accumulate in the organs, and amino acids are degradation products.

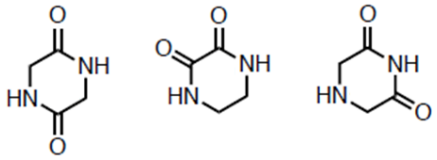
On the other hand, shortcomings of the peptides, such as poor oral absorption, low stability in vivo, high conformational freedom, or low cell permeability, can not be neglected [1].

Nevertheless, cyclic peptides have specific structural features resulting in a superior pharmacological profile [1,2]. Amino and carboxyl terminals are linked together with a peptide bond, forming a circular chain, which results in rigid conformation. This "head to tail" cyclization leads to increased stability against proteolysis and better bioavailability. Besides, cyclo-peptides have less cytotoxicity, higher bioactivity, specificity and efficacy, increased cell permeability, or binding affinity [3].

3. Diketopiperazines

Cyclic dipeptides containing a 2,5-diketopiperazine backbone, known also as cyclodipeptides, 2,5-diketopiperazines (DKPs), piperazine-2,5-diones, 2,5-dioxopiperazines, or dipeptide anhydrides, represent a unique class of compounds with extra advantages in drug development [4]. The DKP scaffold consists of a six-membered ring with, or without, various substituents orientated in a defined way. It provides three-dimensionality, increased rigidity, chiral nature, enables the control of the substituent's stereochemistry, stimulation of the pharmacophoric peptide groups, promotes the intermolecular H-bonding interactions with bio-targets via corresponding sites of donors and acceptors [5]. The rigid DKP core enables the preferential conformation of peptide to be mimicked, and allows the dual behavior of amino acids to be activated in an either constrained or flexible manner [6].

It should be mentioned that even though 2,5-DKPs are the most popular in nature and therapies [4,7], 2,3-DKPs and 2,6-DKPs are possible as well—see Scheme 1 [8].



Scheme 1. Structure of 2,5-DKP (**on the left**), 2,3-DKP (**in the middle**) and 2,6-DKP (**on the right**).

3.1. Historical Background and Occurence: Origin of Life

In nature, imperative fundamental functions of amino acids are indisputable. The cyclic dipeptides probably play a key role in the origin of life in the context of chemical evolution. They could have relevance in the process of catalyse chiral selection and act as precursors in the formation of the peptide, which is considered an essential condition of the beginning of life [9].

2,5-diketopiperazines (DKP) are the simplest naturally occurring cyclo-peptides. They are biosynthesized from amino acids, which are catalyzed through two enzyme families, both nonribosomal peptide synthetase and cyclodipeptide synthase enzymes, resulting in the formation of the two peptide bonds [10–14]. The DKP skeleton is ubiquitous in various environments, either in microorganisms, bacteria such as *Bacillus subtilis*, *Streptomyces*, *Pseudomonas aeruginosa*, or *Lactobacillus plantarum* [15–17], marine and terrestrial fungi [18] as *Aspergillus flavus* or *Alternaria alternata* and *Penicillium*, respectively [19,20], or higher species, such as marine sponges such as *Dysidea herbacea* and *fragilis* [21], but also proteobacteria *Alcaligenes faecalis*, algae, lichens, gorgonians, tunicates, plants, or animals venoms. Notably, they have been found in the central nervous system, gastrointestinal tract, and blood of humans [22]. Additionally, they are present in food and beverages [4,23,24]. DKPs can be chemical byproducts, e.g., in Pu-erh tea, cocoa, dried bonito, roasted coffee, sake, beer, cheese, casein, chicken extract, or stewed beef, giving a special metallic bitter taste [25–28], but also in drugs because of intramolecular cyclization of the dipeptidyl moiety in active peptide-based substances. The latter is a common phenomenon in different therapeutics, in aminopenicillin, amoxicillin, ACE inhibitors [29–31], etc. Notably, proteins and peptides can be cyclized to DKPs by heating [32].

Surprisingly, these natural dipeptides have been known since the beginning of the 20th century [33,34] but they have been neglected for a long time. Only recently, they have been enjoyed greater and greater significant attention, and their biological profile is investigated in detail.

3.2. Properties and Possibilities

The simple biomolecules containing the bis-lactam core of DKP have a wide spectrum of biological activities, *inter alia* anticancer, T-cell mediated immunity, antiviral, nootropic and neuroprotective in neurodegenerative diseases (Alzheimer's or Parkinson's diseases and amyotrophic lateral sclerosis), cytotoxic, immunosuppressive, antibacterial, antifungal, antimutagenic, anti-inflammatory, antihyperglycemic, antiarrhythmic, antimalarial, antiparasitic, anthelmintic, insecticidal, antifouling, anti-prion, vasorelaxant or metabolic regulatory [5,24,34–37]. They have relevance in quorum sensing, improving the classical theory of quorum sensing, unique communication manner between bacteria and gene regulation systems [38]. DKP ring plays a key role in fighting oxidative stress [39]. Moreover, they play role in ion-transport, cell–cell signaling, and a high affinity to many receptors and enzymes [4]. They are useful in delivery systems of drugs, which have low permeability to crossing the blood–brain barrier [34]. Additionally, cell-penetrating peptides containing DKP have better properties in relation to anti-cancer drugs. They provide high cell membrane penetration or transport cargo into the cell [3]. The DKPs can be easily synthesized through conventional procedures providing an attractive scaffold in new drug design due to its main simplicity and marvelous structural diversity [40,41]. They are an excellent model for theoretical studies in the context of the constrained structural scaffold with a relevant pharmacophore [4,5,42]. Furthermore, they are used in the synthesis of many natural products, alkaloids [23]. DKP framework is present in culture broths fermented with lactic acid bacteria. Therefore, they provide an eco-friendly approach for food and feed preservation [43].

3.3. DKP-Based Drugs

Interestingly, many relatively new drugs, such as tadalafil, phosphodiesterase-5 inhibitor for the treatment of erectile dysfunction [44], retosiban, as an oxytocin antagonist for preterm labor [45], epelsiban, as an oxytocin antagonist in premature ejaculation in men [4], the vascular disrupting and tubulin-depolymerizing pinabulin, on the base of the marine fungal halimide, potential therapeutical agent in lung cancer [46,47], as well as other anticancer natural agents as ambewelamides, phenylahistin, dehydrophenylahistin [48], verticillin A [49], but also Aplaviroc for HIV [50], antiviral and immunosuppressive activities (gliotoxin and sirodesmin PL), antimicrobial pulcherimin, antibacterial albonoursin, brevianamide S or bicyclomycin, avrainvillamide [51], anti-inflammatory agents, e.g., FR106969 and FR900452 [6], and many others, contain DKP core.

3.4. Cyclo-Dipeptides Containing Proline: Towards Effective Therapies

Currently, the growing attention in terms of cyclic dipeptides containing proline moiety is noticeable. Among the amino acids, proline is unique due to its specific structure and a lot of biological properties [52]. Both *L* and *D*-proline-based DKPs, widespread in nature, are an interesting sub-family of cyclo-dipeptides [53]. It could be mentioned that they are more predominant in heated and fermented foods than another type of DKPs, even at the level of ~90%. Pro-based DKPs are more easily generated in comparison to other DKPs and can be considered as significant components of flavor and bioactivity [28]. The structural complexity and bioactivity of them are highly impressive [54], *inter alia* in the stimulation of hematopoiesis, bacterial, viral, and fungal infections, food intake inhibition, control the activity of many receptors, as markers in the protein pyrolysates [55] etc. [28,36,56]. They have been potential as cytotoxic and anticancer agents, in treating renal inflammation [57] or cardio-metabolic disorders [58]. In the proline-containing cyclo-dipeptides, the 2,5-DKP a six-membered piperazine nucleus is fused to pyrrolidine ring, which leads to prominent bio-properties, such as extra rigid heterocyclic structure, and consequently, inter alia greater resistance to degradation by enzymes. Proline- and hydroxyproline-based DKPs can form novel essential quorum

sensing inhibitors, which are involved in intracellular communication [38]. They are also promising candidates in neurodegeneration prevention, e.g., in the treatment of Alzheimer disease [59], in flavor response [28], etc.

General mechanism of DKP formation was decribed by Gomes et al. [60], while mechanism of proline-based DKPs formation is proposed by Otsuka et al. [9,28].

The diverse mechanisms of DKP bio-actions peptides as well as their targets have not been precisely known and understood yet. Nevertheless, the growing number of scientific reports in this topic are observable. As an example, neuroprotective action of diketopiperazine-(proline) based agents via different mechanisms are discussed by Cornachia et al. [34], while antibacterial, antifungal activities are considered by Zhao et al. [18]. They proved that presence of specific substituents and other modifications have relevance for endowing bio-action. Propositions of mechanisms via inter alia different inhibitions were summarized.

3.5. Databases Survey

The survey of the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [61] revealed 42 bio-complexes related to DKP moiety, including proline-based one, signed by reference codes: 6SSG, 6SSF, 6SSE, 6SSD [62], 6F0B, 6F0C [63], 41CT, 41Q7, 41PS, 41PW, 41Q9 [64], 5YL4 [65], 6EZ3, 5MLQ, 5OCD, 5MLP [66], 4Q24 [67], 4E0T, 4E0U [68], 3S7T, 3OQJ, 3OQH, 3OQI [69], 3OQV [70], 3NC6, 3NC7, 3NC3, 3NC5 [71], 3N1A [72], 2X9Q [73], 3G5H, 3G5F [74], 1W1T, 1W1P, 1W1V, 1W1Y [75], 6VXV, 6VZB, 6WOS, 6VZA [76], 1O6I [77]. The latter represents proline-based cyclic-dipeptide as chitinase inhibitor with chemotherapeutic potential against fungi, insects and protozoan/nematodal parasites—see Figure 1. In the previous cases, insight into the mechanism of action of biomacromolecules, especially a new class of small proteins, cyclodipeptide sythases, the molecular bases of the interactions with DKP ring towards the design of more effective diverse therapetical agents are discussed. Interestingly, only one complex, 1QZR [78], contains 2,6-DKP core.

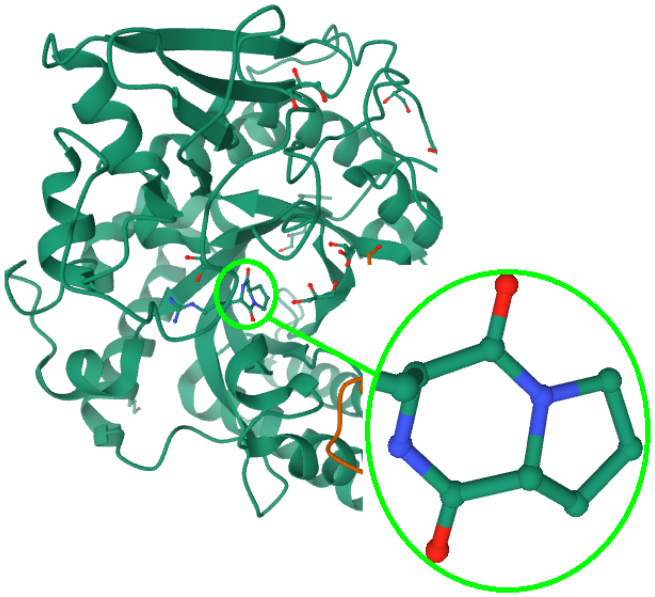


Figure 1. Bio-complex containing proline-based 2,5-DKP moiety, 1O6I.pdb [76].

On the other hand, the thorough analysis of the Cambridge Structure Database (CSD) [79,80] leads to 256 entries of 2,5-DKPs, 52 2,3-DKPs and 5 hits of 2,6-DKPs (see Scheme, and Supplementary Materials).

It is noteworthy that CSD collects a huge structural knowledge on potential peptide-based ligands that can be applicable at the macromolecular level.

Small-molecular crystal structures, especially peptides and their derivatives, have a natural synergy with proteins. The rational design of modern effective ligands should be based not only on the 3D-structure of macromolecular target but also on potential ligands. Groom and Cole [81] said that the designers should try to "*understand and exploit what small-molecule crystal structures tell them; it is just a matter of listening*".

4. Conclusions and Future Prospects

Taking all the above into account, we can conclude that DKPs in general, and proline-based DKPs peptides especially, offer a highly functionalized natural arsenal and huge potential as biological tools for either better understanding bio-mechanisms or future more effective therapies. These the simplest either natural or unnatural cyclo-peptides possessing economically beneficial biological properties are valuable molecular scaffolds in synthetic biology and protein engineering.

Supplementary Materials: The following are available online at www.mdpi.com/2504-3900/83/1/10/s1, Table S1: Crystal structures containing DKP moiety, retrieved from the CSD.

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