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MINIREVIEW ARTICLE

Carnosine derivatives: new multifunctional drug-like molecules

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Abstract Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide widely and abundantly distributed in the muscle and nervous tissues of several animal species. Many functions have been proposed for this compound because of its antioxidant and metal ion-chelator properties. Many potential therapeutic properties have been recognized especially related to the antioxidant activity, but the therapeutic uses are strongly limited by the mechanism governing its homeostasis. This fact has been the main reason for developing the synthesis of carnosine derivatives with interesting potentiality, but until now there have been very few applications. These derivatives could represent the future drugs for many pathologies related to oxidative stress and metal ion dyshomeostasis.

Keywords Carnosine · Dipeptide · Derivatisation · Carnosinase

Introduction

L-Carnosine (β-alanyl-L-histidine, L-Car) is an endogenous dipeptide widely and abundantly distributed in the muscle and nervous tissues of several animal species. Its homeostasis is regulated by a specific synthase (Drozak et al. 2010) and by the carnosinases which are the serum-circulating form ('serum carnosinase', CN1, EC 3.4.13.20) (Jackson et al. 1991; Teufel et al. 2003) and the cytosolic isoform ('tissue carnosinase', CN2, EC 3.4.13.18) (Lenney et al. 1985; Otani et al. 2005).

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Many biological functions have been proposed for L-Car, including its use as a physiological buffer, a wound healing promoter, a metal-chelating agent, an antioxidant and a free-radical scavenger (Decker et al. 2000; Kang et al. 2002) against both nitrosative and oxidative stress (Calabrese et al. 2007; Nicoletti et al. 2007). L-Car has been shown to trap reactive carbonyl species (RCS) and this quenching ability is considered one of the main protection mechanisms in vivo (Aldini et al. 2005). Its properties, such as antiaging and antioxidant (Kohen et al. 1988; Hipkiss 1998; Calabrese et al. 2010), make L-Car extensively used for nutraceutical applications (Ferrari 2004; Shytle et al. 2007; Hipkiss 2009).

Many claims have been made about the therapeutic actions of L-Car. These also include antihypertensive effects, as well as immunomodulating actions, wound healing and acting as an anti-inflammatory agent, as observed on lung injuries caused by bleomycin administration (Cuzzocrea et al. 2007), and on ischemia/reperfusion liver injuries in rats (Fouad et al. 2007). It is also used in the treatment of acute spinal cord injury (Di Paola et al. 2011). Favorable effects of carnosine on survival and learning ability of animals under ischemic injury were also showed (Gallant et al. 2000; Dobrota et al. 2005). In the context of neurodegenerative disorders, L-Car has been suggested as an inhibitor of Aβ toxicity in vitro (Preston et al. 1998). Moreover, it has been reported that carnosine has a strong effect in restoring mitochondrial functioning and in counteracting amyloid pathology in triple-transgenic Alzheimer's disease model mice (Corona et al. 2011). Very recently, a proteomic approach revealed that L-Car affects tumor cell growth by causing an interference with protein folding/processing and HIF-1α signaling in gliobastomas (Asperger et al. 2011).

The metal binding ability of L-Car especially for copper(II) and zinc(II) ions has extensively been studied

(Baran 2000). The copper- and zinc-mediated neurotoxicity involved in several pathologies, such as amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases (Barnham and Bush 2008), might be reduced or prevented by endogenous metal-chelating agents, such as L-Car (Fu et al. 2008; Trombley et al. 2000). Recently, it has been also proven that polaprezinc, the zinc(II)–carnosine complex, is effective for the recovery of ulcers and other lesions in the alimentary tract (Katayama et al. 2000; Matsukura and Tanaka 2000).

The peptide nature of carnosine imposes limitations in its therapeutical uses, mainly associated with the breakdown caused by the carnosinases. Several attempts have been made to overcome this limitation, essentially through the derivatization of carnosine or the synthesis of its structural analogs (Guiotto et al. 2005), but only *N*-acetyl carnosine has been used for some pharmaceutical applications (Babizhayev and Yegorov 2010). In many cases, the role of β-alanine as a precursor of carnosine has been investigated to increase the concentration of muscle carnosine (Sale et al. 2010). This approach has been pursued in the sports medicine field with a number of results (Derave et al. 2010; Artioli et al. 2010).

The conjugation of carnosine with several types of organic molecules has the main purpose of reducing the carnosinase action on the peptide moiety, improving the multifunctional activity, because of the potential beneficial role of the conjugating moiety and the synergism with the peptide properties as well. Finally, the functionalized group could aim at the delivery to a specific target.

The chemical derivatization of carnosine is a very promising approach to realize therapeutic carnosinase-resistant molecules based on carnosine. In addition, the enantiomer D-carnosine (β-alanyl-D-histidine, D-Car) has been reported as a very promising dipeptide because it maintains the same activity of L-enantiomer. D-Car is not hydrolyzed by carnosinase and so its concentration could be maintained in the serum (Vistoli et al. 2009). The reasonable beneficial effect of D-Car due to the carnosinase resistance in the treatment of acute spinal cord injury has been recently reported (Di Paola et al. 2011). However D-Car is less bioavailable than L-Car, because it is not recognized by hPepT1, a transporter responsible for the uptake of a broad array of small peptides in the colon. Very recently, increasing attention has been paid on the functionalization of D-Car and several compounds have been produced with the aim of increasing the bioavailability of D-Car (Orioli et al. 2011). Some of these derivatives have been patented by Flamma (Negrisoli et al. 2009).

In this review, we will collect the data reported on L- and D-Car derivatives and their tested/potential applications. We will focus our attention on carnosine derivatives, in which the peptide unit has been modified at the amino or the carboxyl group. We will not include functional analogs of carnosine, widely described elsewhere (Guiotto et al. 2005), in this review.

These data could be a starting point for the researchers who would like to chemically modify carnosine, thus widening the spectrum of carnosine derivatives which could be potentially useful for several applications.

Derivatives of L-Carnosine

Derivatives on the amino group

A large number of carnosine derivatives have been synthesized modifying the amino group of the dipeptide. The lack of the primary amino group makes this class of derivatives not able to react with RCS species. However, they could be recognized from hPepT1 because the amino group has been tested not to be essential for the recognition of the transporter (Bai et al. 1991).

These derivatives are reported in Fig. 1. A largely used synthetic procedure consists in the alkylation of carnosine using tosylate, iodide or bromide in a nucleophilic substitution reaction. In some cases, the condensation reaction was used, in the presence of activating agents.

The amino group of carnosine has been variously modified with trolox, a well-known antioxidant compound, L-Dopa, cyclodextrins, lactose, trehalose and glucose. Most of these kinds of derivatives have been synthesized with the aim of obtaining carnosinase-resistant compounds with antioxidant activity. Several derivatives have been tested by very different assays to evaluate the antioxidant activity; for this reason, a structure–activity relationship cannot be derived. The antioxidant activity is generally due to carnosine moiety and its activity has widely reported in various reviews (Babizhayev et al. 2011; Bellia et al. 2011; Boldyrev 1993; Kliment and Oury 2010; Guney et al. 2006).

The carnosine derivatives with *R* and *S*-trolox, the water-soluble analog of alpha-tocopherol acylated derivatives [(*S*,*S*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)], have been designed to exploit the cooperative effect of the beneficial activities showed by the constituents (Stvolinsky et al. 2010b). The antioxidant activity of trolox conjugates has been tested by means of several assays (red blood cell hemolysis, DPPH, and lipoprotein oxidation). As a matter of fact, the conjugates generally show an average activity between those of trolox and carnosine. A higher quenching activity against the DPPH radical has been reported for the conjugates with respect to that of the constituents. In this assay, the enantioselective activity has been shown and the conjugate with *R*-trolox (Fig. 1, **1R**) is more active than the epimer with *S*-trolox (Fig. 1, **1S**). A similar trend has been shown in the

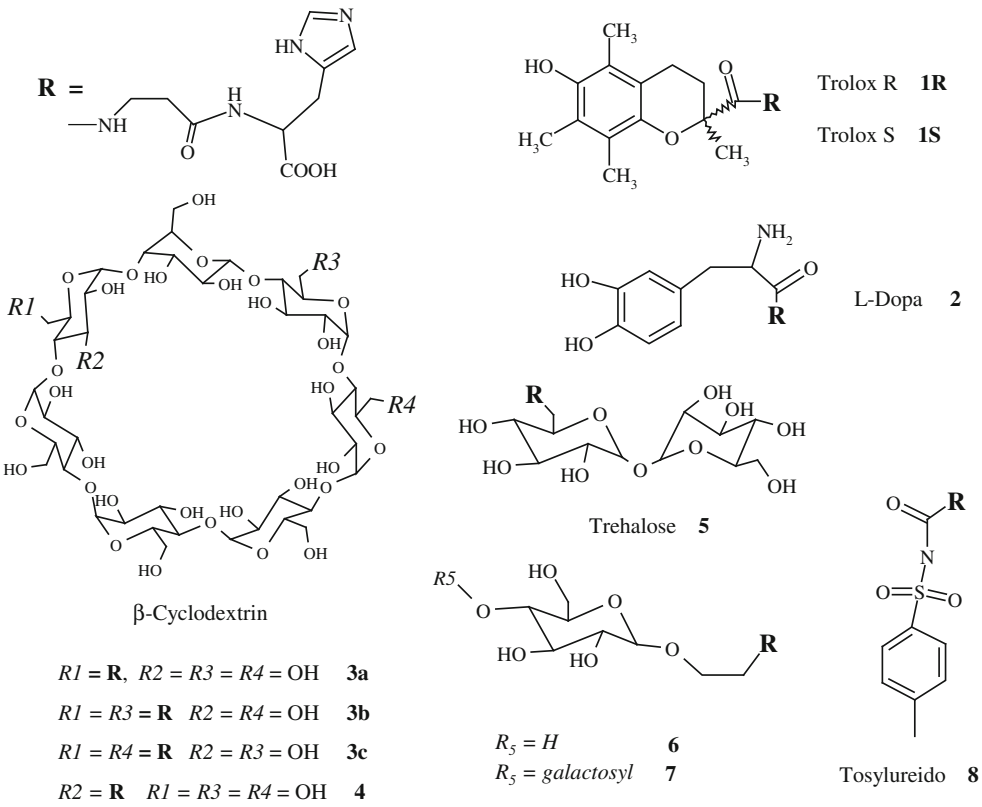


Fig. 1 L-Car derivatives at the amino group

protection of rat neurons from oxidative stress: **1R** > **1S** > L-Car. No hydrolysis by carnosinase in the human serum has been observed for these conjugates. The antioxidant effects of trolox conjugates have also been tested on the lifespan of the fruit fly, *Drosophila melanogaster*. The findings obtained in that study show that **1S** is more active than carnosine. On the contrary, **1R** is less active than the dipeptide. These data suggest the presence of additional cellular targets in comparison with simple neuronal cells to be acted upon by exposing *D. melanogaster* to these compounds (Stvolinsky et al. 2010a).

L-Car has also been modified with L-Dopa (L-3,4-dihydroxyphenylalanine) (Fig. 1, **2**) with the aim of reducing the reactive oxygen species generated by the L-Dopa treatment in Parkinson's disease (Sozio et al. 2008). Very interestingly, the sustained release of L-Dopa in the rat striatum after the administration of the new compounds has been shown. Anyway, the conjugate 2, designed as a potential prodrug for Parkinson's disease, has not shown significant antioxidant activity in vivo.

Another class of promising carnosine derivatives are the glycoconjugates. Carnosine has been functionalized with oligosaccharides, such as *β*-cyclodextrin, in different positions of the sugar (Fig. 1, **3a**, **3b**, **3c**, **4**). Cyclodextrins are particularly used in pharmaceutical science for their ability to include and stabilize drugs. Therefore, several inclusion complexes are commercially available. *β*-cyclodextrin functionalized at the upper rim (primary OHs) with one (**3a**) or two carnosine moieties (**3b, 3c**) (La Mendola et al. 2002a, b; Bellia et al. 2008; Mineo et al. 2004) and mono-functionalized at the lower rim (secondary OHs) (**4**) have been reported. The conjugation stabilizes the dipeptide to the carnosinase hydrolysis and confers a higher antioxidant activity than that of the natural dipeptide, as it has been found by the pulse radiolysis method.

It is worth noting that the prevention of low-density-lipoprotein (LDL) oxidation induced by copper ions has also been observed at concentrations 10–20 times lower than that reported for L-Car.

Other glycoconjugates with monosaccharides and disaccharides have been synthesized (Fig. 1, **5**, **6**, **7**) (Lanza et al. 2011). The trehalose derivative of carnosine (**5**) has also been synthesized to combine the properties of trehalose and carnosine (Rizzarelli et al. 2007). Trehalose is a sugar distributed in many living systems and used in cosmetics with protective and moisturising functions. Its ability to protect proteins against the denaturation process and conformational changes has been focused on and related to potential application in the treatment of Huntington's disease. The carnosine trehalose conjugate has been tested in the LDL assay. As in the case of the cyclodextrin moiety, the trehalose increased the antioxidant properties and protected carnosine from the degradation by carnosinase.

With the aim of selectively addressing carnosine and its antioxidant function, glucose and lactose functionalizations

have been carried out (**6**, **7**). An important physiological role of the conjugating moiety in the carnosine derivatization is enhancing the bioavailability of the dipeptide by facilitating the site-specific transport to different tissues. In recent years, it has been shown that the animal lectins and galectins are important mediators in inflammatory diseases (Almkvist and Karlsson 2004; Gabius 1997; Elgavish and Shaanan 1997). The key role that lectins play in recognition processes has prompted efforts to synthesize the glycoconjugates of small molecules (D'Agata et al. 2006; Hashida et al. 1997; Hashida et al. 1999) or proteins, such as albumin or SOD (Nishikawa et al. 1995), to be specifically bound to a selected lectin. In keeping with the results reported in the literature, these kinds of derivatives are stable to carnosinases (Lanza et al. 2011).

4-Toluensulfonylureido has been conjugated to carnosine and tested as a target moiety for the delivery to tumor cells (Nielsen et al. 2002). Compounds containing this aromatic moiety have been shown to act as anticancer agents for their ability to inhibit the carbonic anhydrase in tumor cells (Supuran et al. 2001).

4-Toluensulfonylureido carnosine (Fig. 1, **8**) has been shown to be stable to serum carnosinase and to have good affinity for the hPetT1 transporter. However, its transepithelial transport was very low thus excluding such an application for carnosine tosylate.

Nanoparticles (NPs) are a new class of carriers in nanomedicine, with special applications in the case of cancer. Recently, the interest for NPs has been increasing and also NPs based on carnosine have been obtained. Carnosine has been functionalized with L-lipoic acid to synthesize gold NPs (Saada et al. 2011). These NPs have been synthesized as activators of carbonic anhydrase. The role of carnosine could be related to the presence of histidine, being that the activity of carnosine nanoparticles is very similar to that of histidine nanoparticles.

Derivatives at the carboxylic group

A number of these derivatives are amides of carnosine (Lanza et al. 2011; Bertinaria et al. 2011) (Fig. 2). The simplest example of this family of derivatives is the amide **9**. The most used strategy for the synthesis of a carnosine amide involves the conjugation of Boc-protected beta-alanine with a histidine amide, as reported in Fig. 3.

The amide functionalization seems to be a very promising strategy because it deeply modifies the resistance of the carnosine versus the human serum carnosinase, while maintaining some important biological functions of the dipeptide. It has been reported that the carboxylic group is important in the recognition done by the carnosinase enzymes (Unno et al. 2008). So, the conversion of carboxyl

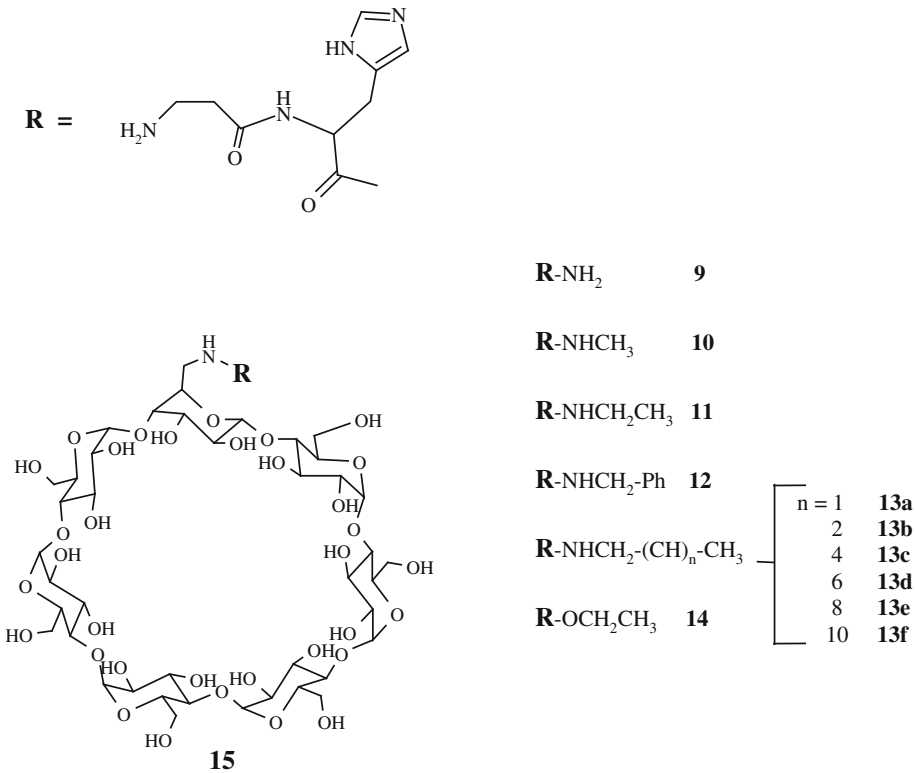


Fig. 2 L-Car derivatives at carbonyl group

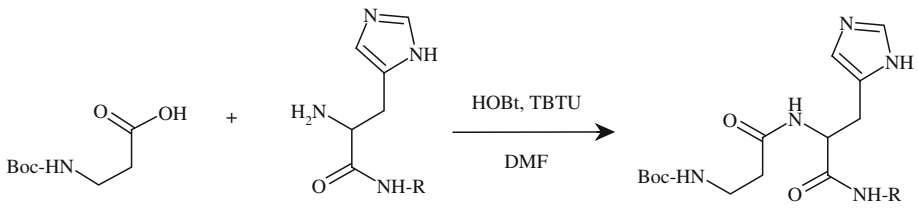


Fig. 3 Synthetic scheme for carbonamides of L-Car (HOBt *N*-Hydroxybenzotriazole; HBTU *O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate)

group into amide makes the derivatives very stable to the carnosinase action.

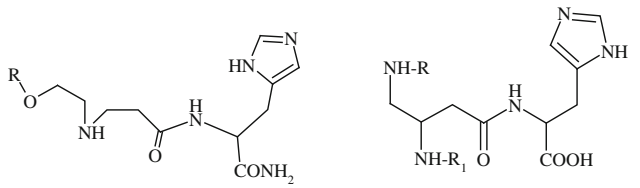
In addition to simple amido-carnosine (Fig. 2, **9**), other amides have been synthesized (**10**–**14**). Amino-β-cyclodextrin has also been used to prepare the amide of carnosine (**15**) and the stability in the human serum has been reported (Bellia et al. 2008).

A recent study reports on the comparative investigation in dependence of the amine lipophilicity (Bertinaria et al. 2011). The antioxidant capability of these derivatives has been tested in comparison to that of carnosine and it is disfavored from the liphophilicity. It is interesting that the modification of the carboxyl group maintains the HNE (4-hydroxy-trans-2-nonenal) quenching activity of the carnosine moiety, though the derivatives show a lower activity than that of the dipeptide. The activity has also been studied in cell cultures. The amido derivative **13a** (Fig. 2), which is moderately more hydrophobic with respect to **9**, has been able to protect primary hippocampus neurons against HNE-induced death, showing a very significant increase in comparison to L-Car. In fact, the dipeptide is demolished by the carnosinases and it is not able to exploit any protective activity. Derivative **13a** is also able to cross the blood brain barrier (BBB) and to concentrate in the rat brain after intravenous administration. This finding renders **13a** very promising as a neuroprotective agent.

Other derivatives

Other derivatives with double functionalization or modified at the ethylenic chain have also been reported. For instance, the amide of carnosine has also been glycoconjugated at amino group in order to study the metal complexing ability (Lanza et al. 2011) (Fig. 4, **16**, **17**). As expected, these derivatives were resistant to the carnosinases.

Carnosine derivatives whose side chain has been introduced on the ethylenic chain of beta alanine have also been reported (Fig. 4, **18**, **19**) (Cacciatore et al. 2005). Carnosinase resistance was higher in the case of **19**. Compound **18** showed only partial carnosinase resistance. Interestingly enough, derivative **19** is also able to inhibit carnosinase. These compounds have shown antioxidant ability quenching $^{\cdot}$OH and peroxynitrite in vitro (Cacciatore et al. 2005).



R = glucosyl **16**

R = lactosyl **17**

R = CH_3CO, R_1 = H **18**

R = R_1 = CH_3CO **19**

Fig. 4 Double functionalized L-Car derivatives

Derivatives of D-carnosine

The design and synthesis of D-Car prodrugs have been inspired by the RCS scavenger ability of L-Car. In addition to being a reliable biomarker of oxidative damage, the protein carbonylation has been considered a novel target for drug discovery (Aldini et al. 2007).

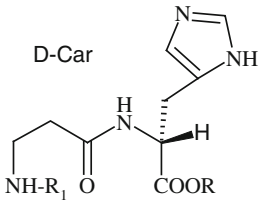
The prodrugs of D-Car have been designed mainly based on computed lipophilicity (Orioli et al. 2011). Derivatives with both amine and carboxyl groups were synthesized (Fig. 5) in order to study the hydrolysis mechanism in rat plasma. The most stable derivatives have been excluded from an in vivo investigation. The octyl ester of D-Car **(20)** has been selected to undergo an extensive evaluation in the Zucker rat. Several protective actions have been observed: the reduction of markers of carbonyl stress, such as advanced glycoxidation product (AGE), of hyperlipidemie and the prevention of renal and vascular injuries.

The D-Car derivatives have also been synthesized to compare the stereochemical differences of the L- and D-Car conjugates. Cyclodextrin (**21**) and trehalose (**22**) conjugates of D-Car have been structurally investigated and their ability to complex metal ions has been correlated to the chirality of His (Grasso et al. 2011a, b). These systems are very interesting examples in the field of stereochemistry, but their potentialities as therapeutic agents have not been investigated yet.

Metal complexes of carnosine derivatives

The metal binding properties of L-Car have also been widely investigated due to the interest in the development of clinical approaches for the regulation of metal ion

Fig. 5 D-Car derivatives



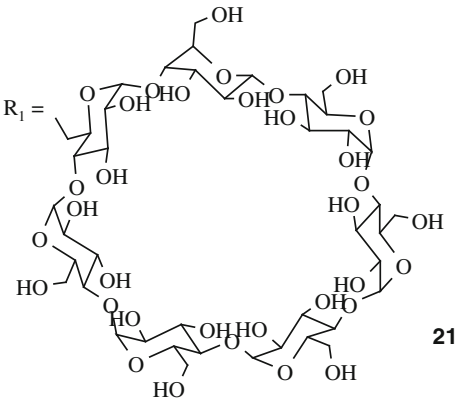
R_1 = CH_3CO, R = H
R = CH_3
R = CH_2CH_3
R = C_8H_{17}

R_1 = EOC R = CH_3
R = $CH(CH_3)_2$
R = $(CH_2)_3CH_3$

R_1 = cbz R = H
R = CH_3
R = CH_2CH_3

R_1 = H R = CH_2CH_3
R = $CH(CH_3)_2$
R = C_8H_{17}
R = $C_{16}H_{33}$

20



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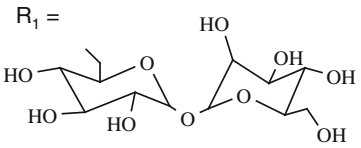

22

cbz is $C_6H_5CH_2O(C=O)$-

EOC is $CH_3CH_2O(C=O)$-

homeostasis in the medicinal inorganic chemistry field. Metal homeostasis is highly correlated to a number of diseases: Alzheimer's, Parkinson's, cancer and aging. Redox metal ions, such as iron and copper ions, are especially involved in these pathologies, though the role of zinc has also been reported (Jomova and Valko 2011).

Although L-Car is able to complex various transition metal ions, the copper(II) complexes have been the most widely studied due to their thermodynamic stability (Sigel and Martin 1982). The copper(II)-L-Car system has largely been investigated through different techniques (Brown and Antholine 1979; Daniele et al. 1993; Mineo et al. 2002). A detailed thermodynamic characterization of the complex species has been also reported (Daniele et al. 1982; Brookes and Pettit 1975; Agarwal and Perrin 1975). Stability constants of Cu-L-Car system are reported in Table 1.

The equilibria for the formation of the copper(II) complexes are given in Eq. (1), where L is the anionic form of the ligand for amino derivatives (**3c, 32, 4, 5, 22**) or L is uncharged ligand for the amide derivatives (**9, 12, 13a, 13d, 13f, 14**) (the charges of the copper(II) complexes have been omitted for the sake of clarity).

$$m\mathrm{Cu}^{2+} + l\mathrm{L} + h\mathrm{H} + \mathrm{Cu}_m\mathrm{L}_l\mathrm{H}_h \quad (1)$$

The overall stability constant β_{mlh} is defined by the following equation:

$$\beta_{m/h} = \frac{[\mathrm{Cu}_m\mathrm{L}_l\mathrm{H}_h]}{[\mathrm{Cu}]^m[\mathrm{L}]^l[\mathrm{H}]^h}$$

The main species formed at physiological pH and *m*M concentration of both the ligand and the Cu^{2+} are the monomeric species [Cu(L-Car)H_{-1}], together with a secondary dimeric species [Cu_2(L-Car)$_2H_{-2}$] (Fig. 6).

In the monomeric species the amino, amido deprotonated and imidazole groups complex the metal ion as shown in Fig. 6. A similar environment is in the dimeric species, but in that case every metal ion is coordinated by imidazole nitrogen of a different ligand unit.

Table 1 Formation constants (logβ) for the copper(II) complexes of the carnosine derivatives (L)

Equilibrium	Carnosine derivative (L)												
	L-Car	3c	21	4	5	22	9	12	13a	13d	13f	14
$Cu^{2+} + L + H^{+} \rightleftarrows [CuLH]$	13.54	11.58	11.42	11.68	12.1	11.78	12.62	12.22	12.47	12.71	12.69	13.58
$Cu^{2+} + L \rightleftarrows [CuL]$	8.46	6.92	6.36	8.33	7.27	7.25	6.82	6.72	6.59	7.05	7.01	7.30
$Cu^{2+} + L \rightleftarrows [CuLH_{-1}] + H^{+}$	2.98	1.3	–	–	–	–	1.18	1.28	1.55	1.53	1.50	0.16
$2Cu^{2+} + 2L \rightleftarrows [Cu_2L_2H_{-2}] + 2H^{+}$	8.06	6.33	3.47	7.77	8.83	5.23	5.09	5.40	5.52	6.35	6.20	–
$2Cu^{2+} + L \rightleftarrows [Cu_2LH_{-1}] + H^{+}$	5.35	–	3.51	2.83	–	–	–	–	–	–	–	–
$Cu^{2+} + L \rightleftarrows [CuLH_{-2}] + 2H^{+}$	–	–	−9.30	−3.95	−7.52	−7.43	–	–	–	–	–	–
$2Cu^{2+} + 2L \rightleftarrows [Cu_2L_2H_{-3}] + 3H^{+}$	–	–	–	–	–	–	−2.82	−3.09	−2.46	−2.41	−2.84	–
$2Cu^{2+} + 2L \rightleftarrows [Cu_2L_2H_{-4}] + 4H^{+}$	–	–	–	–	–	–	−12.23	−12.67	−11.68	−12.32	−12.74	–
$Cu^{2+} + 2L \rightleftarrows [CuL_2]$	–	–	–	12.73	–	–	13.86	13.47	13.81	13.27	13.30	–

The charge of L and the complex species are omitted for simplicity. L is negatively charged for L-Car, **3c, 32, 4, 5, 22.** L is uncharged for **9, 12, 13a, 13d, 13f, 14**

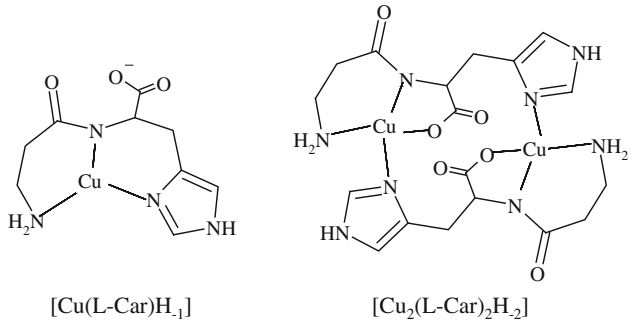

Fig. 6 Copper(II)-L-Car complexes

Some of the derivatives described above have also been investigated for their ability to form metal complexes. The stability constants of copper(II) complexes reported for carnosine derivatives are summarized in Table 1. In general, the functionalization does not reduce the complexing ability of the carnosine moiety.

The copper(II) complexes with L-Car-cyclodextrin conjugates **3** (Fig. 1) are the first example ever reported of carnosine derivative complexes (Bonomo et al. 2003). In spite of the steric hindrance due to the β-cyclodextrin bound to the amino group, these L-Car derivatives show a similar metal binding behavior of the natural dipeptide in terms of the stoichiometry of the copper complex species and their thermodynamic stabilities (Table 1). A full characterization of the first member of glycoconjugate family (**3a**) and its copper(II) complexes by means of potentiometric and calorimetric measurements, ESI–MS, EPR and CD spectroscopy has been reported. The cyclodextrin L-Car conjugate (**4**) form the monomeric species involving a secondary OH of cyclodextrin (Fig. 7a) in the metal ion coordination.

β-cyclodextrins di-functionalized with L-Car have also been characterized for their ability to form metal complexes (Fig. 7). In the case of the L-Car conjugates in the 6A,6C positions (Fig. 1, **3b**), the formation of oligomeric complexes is induced by the coordination of Cu^{2+} with the L-Car units (Fig. 7b). Interestingly, from the point of view of supramolecular chemistry, it has been found that the regioisomer 6A,6D (Fig. 1, **3c**) forms only dimeric species involving two ligands and four metal ions (Fig. 7c).

Copper(II) complexes of D-Car conjugates of β-cyclodextrin (Fig. 5, **21**) (Grasso et al. 2011a) and trehalose (**22**) (Grasso et al. 2011b) have also been reported. In these cases, the dimeric species showed a significantly lower stability constant than those reported for the analogs of derivatives with L-Car (Table 1). The difference between the stability constants of the dimeric species with L- or D-systems is 2.6 and 3.6 logarithm units, respectively. This stereoselectivity is driven by non-covalent interactions, namely hydrogen bonds, CH–π interactions, hydrophobic

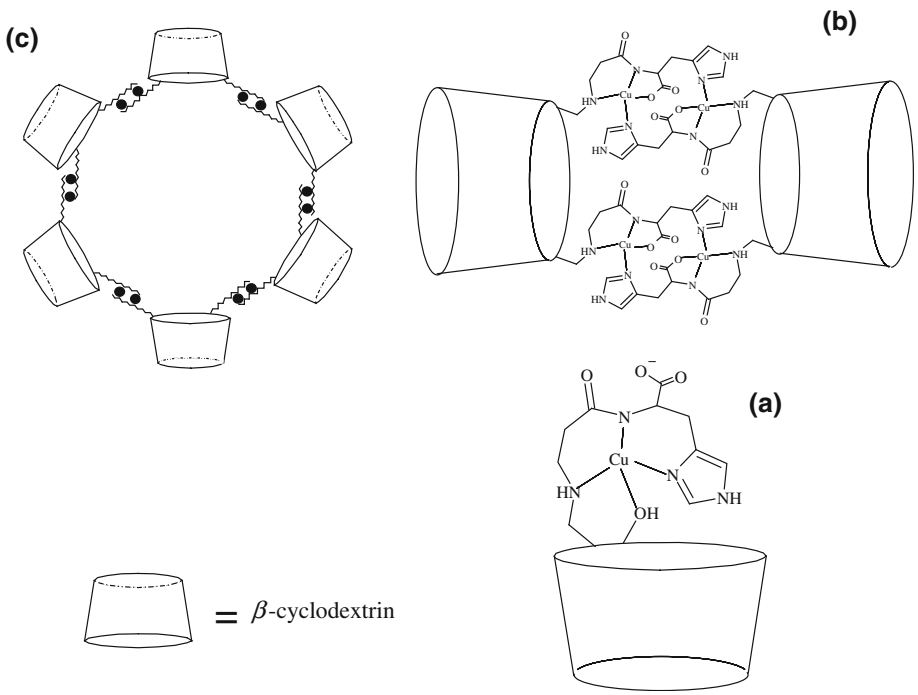

Fig. 7 Copper(II) complex species with **3** at physiological pH (**a**), proposed structures of the dimeric species relative to the Cu^{2+}–**3c** system (**b**) and the sketch of the proposed structure of the hexamer for the Cu^{2+}–**3b** system (**c**)

and steric effects involving the chiral moieties, such as cyclodextrin and trehalose.

The copper(II) complex with L-Car show SOD-like activity. The ability to catalyze the dismutation of the superoxide anion is also found for the copper complexes with the L-Car-cyclodextrin derivatives. Moreover, it is interesting to note that the carnosine derivatization with β-cyclodextrin induces an increase of the SOD-like activity with respect to that of the L-Car complexes, as observed by the Fridovich assay. The antioxidant activity of the complexes depends on the functionalization position (Bonomo et al. 2003).

The ester (**14**) and amide derivatives of carnosine **13a–f** and **14** have also shown the ability to bind copper(II) ion, forming complex species very similar to those of the copper(II)-L-Car system (Table 1). Only amide derivatives are able to form dimeric species, though with lower thermodynamic stability (Lanza et al. 2011; Bertinaria et al. 2011). The amide group is able to coordinate the metal ion in the deprotonated form (Table 1). L-Carnosine ethyl ester is not able to form dimeric species due to the lack of carboxylate, differently from L-Car.

Conclusion

A large number of carnosine derivatives have been synthesized and structurally characterized, though few biological assays have highlighted the potentiality of these derivatives.

The main goals reached by the carnosine functionalization have been (a) avoiding or, at least, reducing the carnosinase hydrolysis, (b) conferring a lipophilic character with the aim to aid the BBB-crossing, (c) enhancing or, at least, maintaining the beneficial effects of the dipeptide, (d) counterbalancing the side effects of the grafted compound and (e) aiming at the targeted delivery.

The carnosinase resistance has been reported for most of all the carnosine conjugates, whether the dipeptide is derivatized through the amine or the carboxyl group. This is because both the functional groups are essential for the carnosinase recognition. The effect against the oxidative stress has generally been the most studied activity of the carnosine derivative and, in several cases, it has been improved with respect to that of the natural dipeptide. Finally, the carnosine ability to bind several metal ions, mainly copper(II) and zinc(II), has been exploited to design several conjugates which are able to form metal complex species with different stoichiometry and stability.

On the basis of the wide interest in carnosine, these derivatives represent very promising systems; among them, a system having at least the main important therapeutic actions of carnosine improved by the resistance to carnosinase will probably be selected in the future.

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