Carnosine phenyl derivatives as specific and efficient sequestering agents of cytotoxic Reactive Carbonyl Species (RCS)

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Background

- *Reactive carbonyl species (RCS) (Fig. 1) are cytotoxic mediators generated by lipidoxidation of PUFAs, leading to alteration of the cellular function and inducing irreversible structural modifications to biomolecules (1,2).*
- *RCS and the corresponding adducts with proteins (that is, carbonylated proteins) are widely used as biomarkers of lipidperoxidation and, in general, of oxidative stress.*
- *Moreover, there are several convincing evidences supporting a pathogenic role for RCS, such as in the case of diabetic-related diseases, age-dependent tissue dysfunction, and metabolic distress syndrome.*
- *Consequently, RCS, in addition to being a predictive biomarker, also represents a biological target for drug discovery (Fig.1).*

❖ *The most promising strategy to neutralize/reduce RCS is based on nucleophilic compounds capable to form covalent and unreactive adducts with RCS (RCS sequestering agents) such as pyridoxamine (PYR), hydralazine (HY), dihydralazine (di-HY), aminoguanidine (AG), and metformin (MF).*

❖ *However these compounds are characterized by a severe aspecificty since they react also with physiological aldehydes such as pyridoxal (3).*

- *We recently found that the endogenous dipeptide carnosine (β-alanyl-L-histidine) is a specific quencher of α,β-unsaturated aldehydes due to its peculiar mechanism involving the Schiff base formation between the β-alanine amino group and the RCS aldehyde followed by the Michael adduction between the C3 of the aldehyde and the Nτ of the histidine group (4)(Fig. 2).*
- *However, the therapeutic use of carnosine is limited since it is unstable in human plasma due to the serum carnosinase activity (5). Moreover the reactivity of carnosine towards RCS is significant lower in respect to that of AG, HY and PYR.*

Aim of the work

Aim of the work was to derive carnosine analogues characterized by ***(I) carnosinase stability and (II) a grater reactivity towards RCS in respect to carnosine, even maintaining the same specificity.***

Results

Stability*: The stability was reached by the isomerization of L- to D-histidine aminoacid, leading to β-alanyl-D-histidine (D-carnosine) which is not recognized by carnosinase but conserves the same quenching activity of L-carnosine .*

Reactivity*: Although the simplest approach to increase the reactivity would be to enhance the nucleophilicity of the amino group, this is not largely exploitable since it would mine the specificity and favor the protonated amino form. Hence we focused our attention to the specific Michael adduction, by modulating the conformational profile of the Schiff base intermediate in order to favor a close conformation in which the imidazole ring approaches enough the C3 of the Schiff base to form the corresponding Michael adduct.*

A series of D-carnosine derivatives was then designed by in silico approaches to find out those characterized by a favorable folded conformational profile. In detail, the conformational profile of the corresponding imine of these D-carnosine analogues was explored through 5 ns MD simulations in water and their ability to assume a favorable conformation was assessed by monitoring the distance between the barycentre of imidazole ring and C3 atom as obtained by MD runs (Fig. 4). The most promising analogues were then synthesized and the quenching ability, stability in human plasma, basicity and the abundance of folded conformations evaluated. (Table 1). Noticeably, a marked correlation between quenching ability and the percentage of folded conformations was determined.

The key role of conformational profile:
the case of **Schiff base carnosine-HNE**

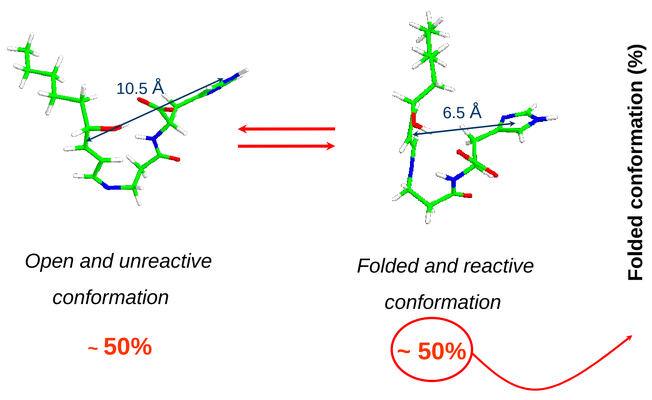
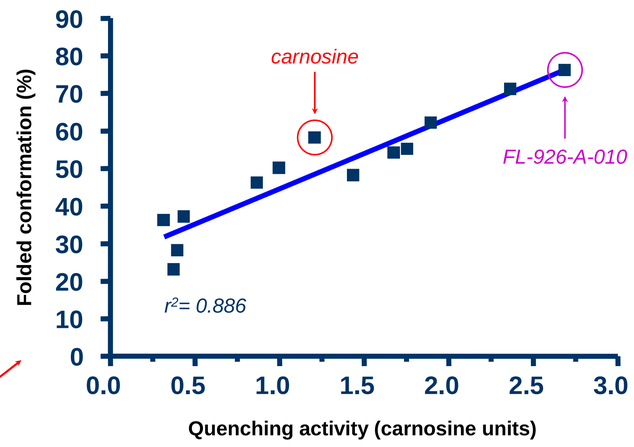


Fig. 4 - QSAR study



Noticeably, *Fig. 4* shows that suitable modifications in considered analogues enhances the folded percentage up to 75% with a corresponding increase of quenching activity.

Experimentals (Legend to *Table 1*)

1 – ***The quenching activity*** was determined by monitoring (HPLC analysis) the HNE consumption after incubation (60 min at 37° C) with the tested compound. The results are reported as carnosine units (C.U.), taking the value of 1 the quenching ability of carnosine.

2 – ***Specificity*** was evaluated by mass spectrometry (direct infusion method) and using pyridoxal as a model of physiological aldehyde. The method consists to incubate the target compound with pyridoxal and to evaluate the residual amount of the physiological aldehyde after 60 min at 37° C. The results are reported as percentage of the fre e aldehyde consumed in respect to a blank incubated in the absence of the tested compound.

3 – ***Plasma stability*** was evaluated in human serum (from healthy donors), by incubating the tested compound for 60 min at 37° C and determining the residual content by usi ng a LC-MS/MS method and Tyr-His as internal standard. The results are reported as percentage loss in respect to a blank incubated in the absence of serum.

4 – ***The basicity*** (pK_b) of the β-alanyl amino group was determined in silico (ACD/pKa version 8.19).

5 – ***Folded conformation:*** the conformational profile was assessed by 5 ns MD runs simulating for each designed analogue the corresponding Schiff base with HNE as inserted in a 15 Å radius sphere of water molecules. The percentage of folded conformations was evaluated by monitoring the distance between the barycentre of imidazole ring and C3 atom. In this analysis a distance lesser than 7.5 Å was considered conducive to Michael adduction.

6 – ***Synthesis:*** D-carnosine derivatives were synthesized either by solid phase synthesis or by suitable coupling methods starting from protected unnatural aromatic aminoacids and D-Histidine or its derivatives. The unnatural aminoacidic building block which are not commercially available, were prepared by suitable enantioselective synthesis.

Fig. 1 - RCS as biological target for drug discovery

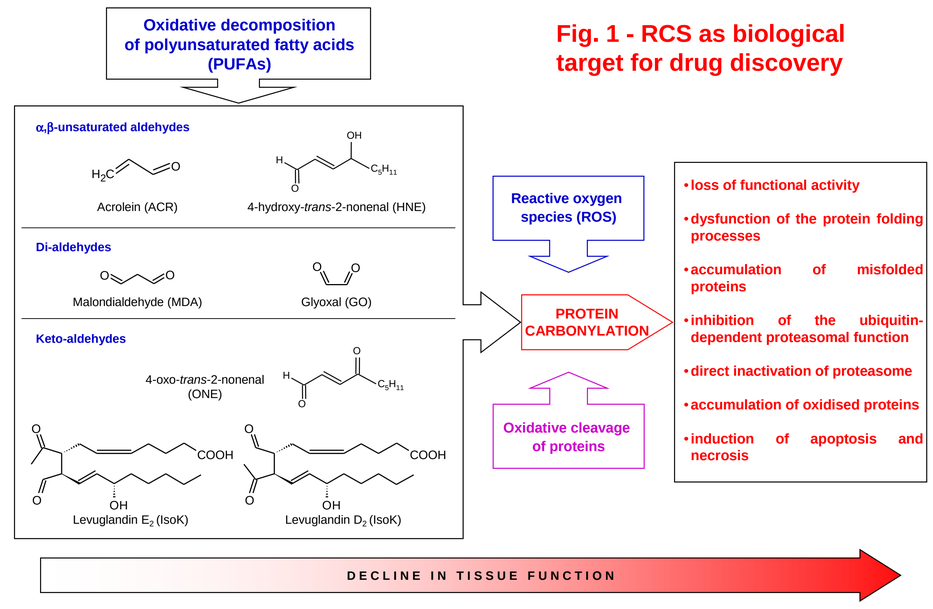


Fig. 2 - Reaction mechanism of carnosine with α,β-unsaturated aldehydes

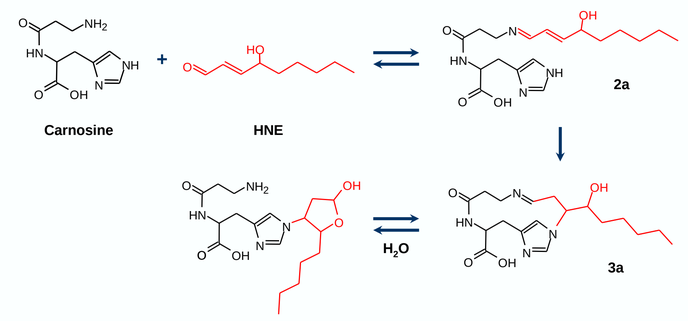
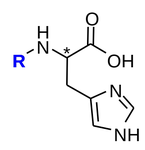


Table 1- β-alanyl-D-histidine analogues



Compound	Quenching activity (C.U.)[1]	Specificity[2]	Plasma stability[3]	pK_b[4]	Folded conformation (%)[5]
FL-926-A-010	2.69 ±0.06	98.3 ±2.4	96.3 ±4.4	8.88	76.8
FL-926-A-007	2.37 ±0.06	99.7±0.8	105.6 ± 7.8	9.21	71.5
FL-926-A-001	1.90 ±0.08	97.5±2.7	97.4 ±4.4	9.25	62.5
FL-926-A-004	1.76 ±0.07	102.9±3.2	102.5±3.7	8.27	55.2
FL-926-A-006	1.68 ±0.04	100.4±3.2	101.4±2.7	8.22	53.7
CS70	1.21±0.03	99.0±1.4	99.4±4.1	8.17	58.3
D-carnosine	1	0	0	9	49.8

Conclusion

By this way a set of phenyl derivatives was identified (Table 1), characterized by high stability in human plasma and by a quenching activity towards HNE increased uo tp almost 3 folds in respect to D-carnosine. Finally, the reaction products of the β-alanyl-D-histidine analogues with HNE were fully characterized by MS and assigned to the Nτ Michael adducts.

References

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