COMBINED IN SILICO APPROACHES FOR DRUG DESIGN AND PHARMACOKINETIC OPTIMIZATION OF A SET OF CARNOSINE ANALOGUES AS POTENT AND SELECTIVE CARBONYL QUENCHERS

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Background

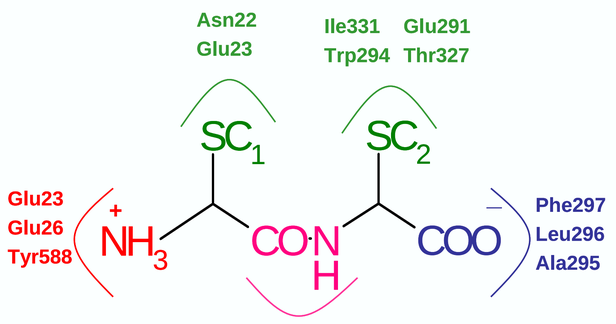
- *Reactive carbonyl species (RCS) are cytotoxic mediators generated by lipidoxidation of PUFAs, leading to alteration of cellular functions and inducing irreversible structural modifications to biomolecules.*
- *RCS and the corresponding adducts with proteins (that is, carbonylated proteins) are widely 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.*

- *We recently found that the endogenous dipeptide carnosine (β-alanyl-L-histidine) is a specific quencher of α,β-unsaturated aldehydes [1]. Although carnosine is actively absorbed by intestinal transporter hPepT1, its therapeutic use is limited since it is unstable in human plasma due to the serum carnosinase activity (5). Moreover, the reactivity of carnosine towards RCS is markedly lower compared to other known quenchers.*
- *Hence, the rational design of new carnosine analogues should (1) increase the quenching activity of carnosine, maintaining its selectivity (2) confer plasma stability against human serum carnosinase, and (3) conserve an optimal recognition by hPepT1.*
- *Accordingly, in silico approaches can support the design of carnosine derivatives by (1) parameterizing the factors which govern the quenching activity, (2) predicting the effects of serum carnosinase and (3) modeling the recognition by hPepT1.*
- *When the modifications were so significant to prevent the active transport, the marked hydrophilicity of carnosine analogues was modulated by designing prodrugs whose hydrolysis was predicted in silico by docking simulations with the major human carboxylesterases.*

Predicting the active absorption: the homology model for hPepT1

The hPepT1 structure was modeled by fragments based on the resolved structure of lactose permease, *LacY*. Docking analyses involved a set of 50 known substrates and allowed the identification of a common pattern of interactions [3].

The residues which interact with the side chains are heterogeneous, justifying the ability of hPepT1 to interact with structurally diverse substrates. It is possible to recognize a set of residues involved in the interaction with the N-terminal side chain (SC1) such as **Asn22**, **Glu23**, and **Phe293**, while the C-terminal side chain (SC2) contacts **Trp294**, **Ile331**, **Glu291** and **Thr327**.



The ammonium head probably plays the most critical role since it realizes a reinforced H-bond with **Tyr588** as well as ion-pairs with **Glu23** and/or **Glu26**. Notably, the contact between Tyr588 and ammonium head characterizes the most affinitive ligands.

The carboxy terminus appears less involved in ligand recognition, since it stabilizes only H-bonds with the backbone of **Ala295**, **Leu296**, and **Phe297** without forming strong ionic interactions

The central peptide bond can stabilize H-bonds with backbone atoms of **Phe293** and **Trp294**.

Docking results suggest that the most affinitive compounds have a distance between charged termini about equal to 6 Å. **Δdistance** defines the difference between the distance value of a given ligand and the optimal distance (6.02 Å) as evidenced by the most affinitive ligand.

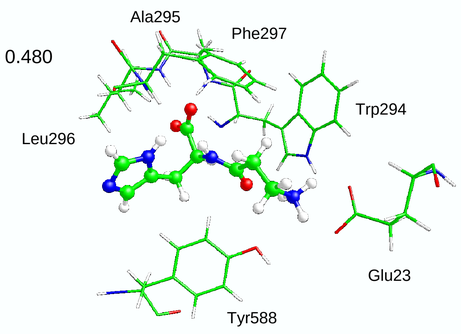
Zapbind accounts for the ionic interactions. Despite the known predilection of hPepT1 for hydrophobic ligands, the role of Zapbind score emphasizes the relevance of the polar interactions mostly realized by the ligand's charged groups.

Given the beneficial role of the interaction between Tyr588 and ammonium head, we introduced a binary descriptor (**Int_Tyr588**), which is equal to 1 for substrates which realize such a reinforced H-bond and 0 otherwise.

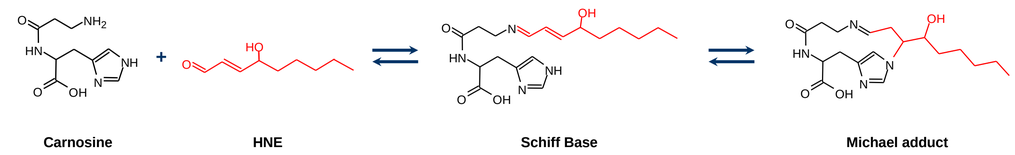
$$pKi = 0.235\,\Delta distance - 7.912\,10^{-3}\,Zapbind + 1.534\,Int\_Tyr588 - 0.480$$

$n = 50;\ r^2 = 0.85;\ s = 0.45;\ F = 89.92$

Docking results confirmed the key role of chiral centers in hPepT1 recognition, thus explaining why D-car derivatives are not actively transported. Docking results also allowed to derive a reliable correlative equation which was used to predict the affinity of carnosine analogues. The figure shows the putative complex carnosine-hPepT1. The predicted affinity is in line with the experimental value: 1.41 mM (pred) *vs*. 2.48 mM (exp).

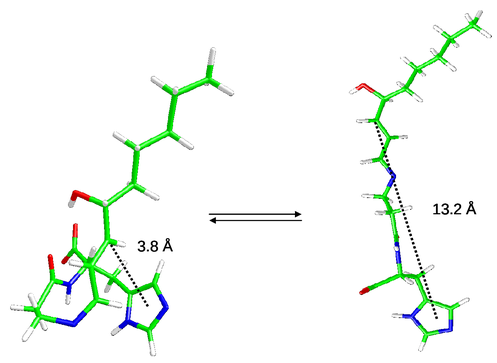


Enhancing the quenching activity



The modifications aimed to enhance the reactivity of the amino group are not largely exploitable since they would mine the specificity quenching also physiological aldehydes. Conversely, the specific Michael adduction can be optimized, by (1) modifying the imidazole ring to increase its nucleophilicity and (2) modulating the conformational profile of the Schiff base intermediate in order to favor a close conformation in which the imidazole ring approaches the reactive C3 to form the corresponding Michael adduct.

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

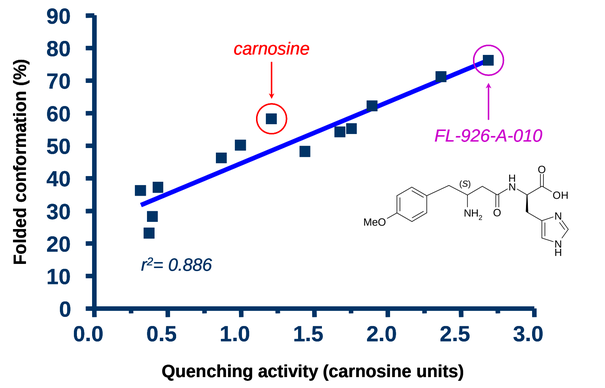


Folded and reactive conformation

~ 50%

Open and unreactive conformation

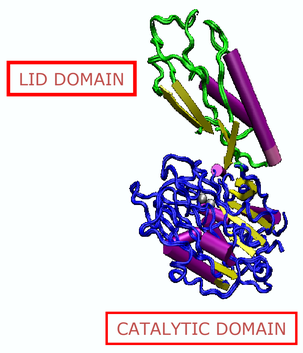
~ 50%

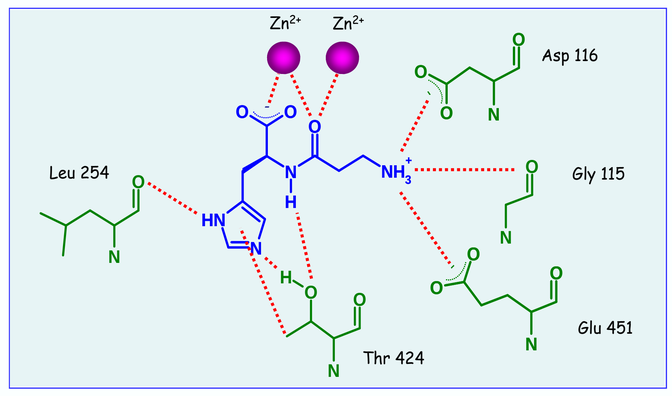


Noticeably, suitable modifications in aryl-analogues enhances the folded percentage up to 75% with a corresponding increase of quenching activity as seen in 4-metoxybenzyl derivative [2].

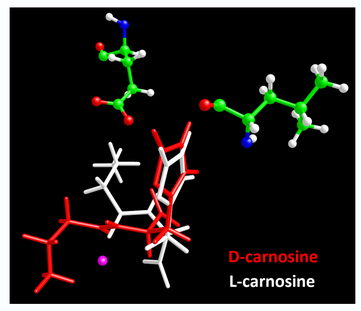
The Michael adduction can be parameterized calculating the nucleophilicity of new analogues using suitable indices mainly based on *ab initio* simulations. Preliminary analyses confirmed the significant reactivity of imidazole ring and unveiled the promising activity of furan ring.

Predicting the plasma stability: the serum carnosinase

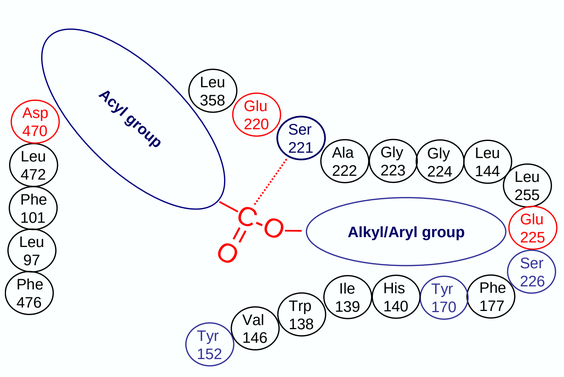


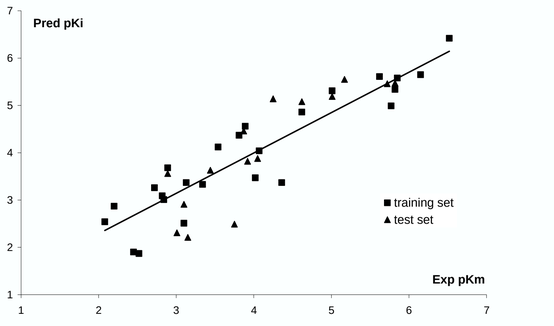


The human serum carnosinase was modeled using the structure of β-alanine synthase as template. Docking analyses allowed to identify the ligand moieties which are critically involved in enzyme recognition, emphasizing the key role of the contacts with metal ions [4].
Furthermore, docking studies can be used to predict the plasma stability of novel analogues. For instance, they can well explain why D-carnosine is not recognized by serum carnosinase and is stable in human plasma.



Predicting the prodrug hydrolysis: the carboxylresterase 1





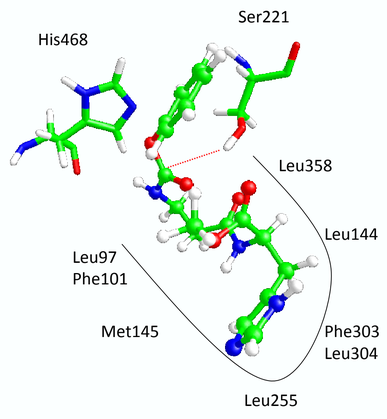
$$pKm = -1.66MLP_{InS} - 0.381Dist_{Ser221} + 4.18$$

$n = 40;\ r^2 = 0.85;\ q^2 = 0.73;\ SE = 0.49;\ F = 64.06;\ p < 0.0001$

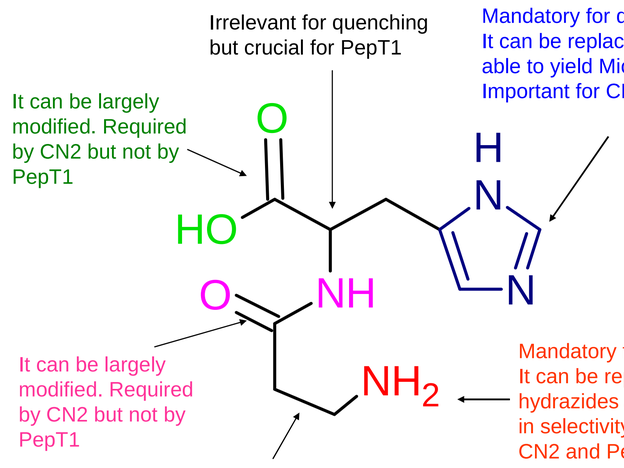
The design of suitable prodrugs for D-carnosine was supported by docking analyses involving the human carboxylesterase-1. Such studies firstly involved a set ok known substrates and allowed the identification of key residues involved in complex stabilization as well as the development of a robust predictive equations [5].

The obtained results were then exploited to rationalize the stability of a set of D-carnosine prodrugs also predicting the more labile function for the bi-protected derivatives.

Globally, the performed simulations lead to the design of octyl-D-carnosine as most promising prodrug for clinical studies.



Conclusions: summarizing the obtained SARs



Based on obtained SARs and performed simulations, the rational design of new carnosine analogues followed such a pathway:

L-carnosine

Active, absorbed but unstable

⬇

D-carnosine

Active, stable but not absorbed

⬇

Octyl-D-carnosine

Active, stable, absorbed but toxic

⬇

FL-927-A

Active, stable and absorbed

References

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