New multifunctional diamine AGE/ALE inhibitors to prevent oxidative and carbonyl stress exacerbation in Alzheimer's disease

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INTRODUCTION

Reactive carbonyl species (RCS) are now considered to play an important role in Alzheimer's disease (AD) pathogenesis. Indeed, methylglyoxal (MGO) or malondialdehyde (MDA) are endogenously formed during the sugar glycoxidation and lipid peroxidation of polyunsaturated fatty acids induced by oxidative stress exacerbation. Their condensation with amino groups of tissue proteins leads to **AGE (Advanced Glycation Endproducts) and ALE (Advanced Lipid peroxidation Endproducts) accumulation** in amyloid *β* (A*β*) plaques and tau-associated neurofibrillary tangles (NFT). In AD, A*β*-oligomers induce oxidative stress whereas transition metals (Zn^{2+}, Cu^{2+} and Fe^{3+}) stimulate A*β* aggregation and APP (amyloid precursor protein) processing. **Carbonyl stress takes part in this vicious downward redox amyloid spiral leading to neurodegeneration as oxidative stress promoter and toxic mediator.**[1,2] Glycated A*β* cross-linking promotion accelerates its deposition and its protease resistance. Moreover, AGE promote oxidative stress and inflammation as well as cell apoptosis via their receptors RAGE.[3] Previously, we have successfully demonstrated the efficacy of 2,3-diaminopropionic acid (Dap) derivatives to trap RCS.

With this in mind and taking into account the multifactorial pathogenesis of AD, we developed **new promising multifunctional drugs that are simultaneously able to trap RCS (primary vicinal diamine function) as well as ROS and biometals (phenolic acid or hydroxypyridinone moiety)** (Figure 1) .[4]

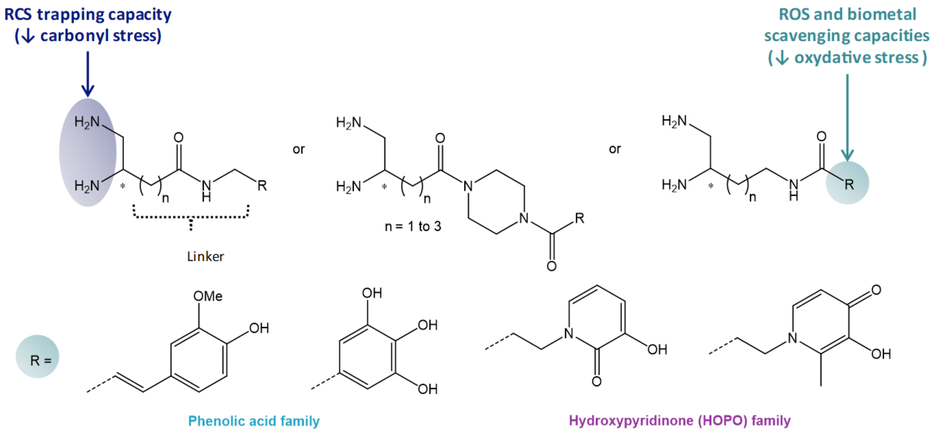


Figure 1. New multifunctional diamine AGE/ALE inhibitors.

RESULTS AND DISCUSSION

Synthesis of new hybrid diamine AGE/ALE inhibitors

As shown in figure 2, our synthetic strategy involves: i) the transformation of *α*-carbonyl group of **easily available amino acids** (D or L) such as aspartic acid, glutamic acid, ornithine and lysine into amine group in order to create **vicinal diamines**, ii) the coupling of the carboxylic or amino group of the side chain of these amino acids with the **second functional moieties possessing antioxidant and siderophoric properties like phenolic acids** (ferulic acid or gallic acid) **or hydroxypyridinones** (3,2-HOPO or 2-methyl-3,4-HOPO). The two functions are linked by different amide or piperazine spacers.

Physicochemical and biological evaluations

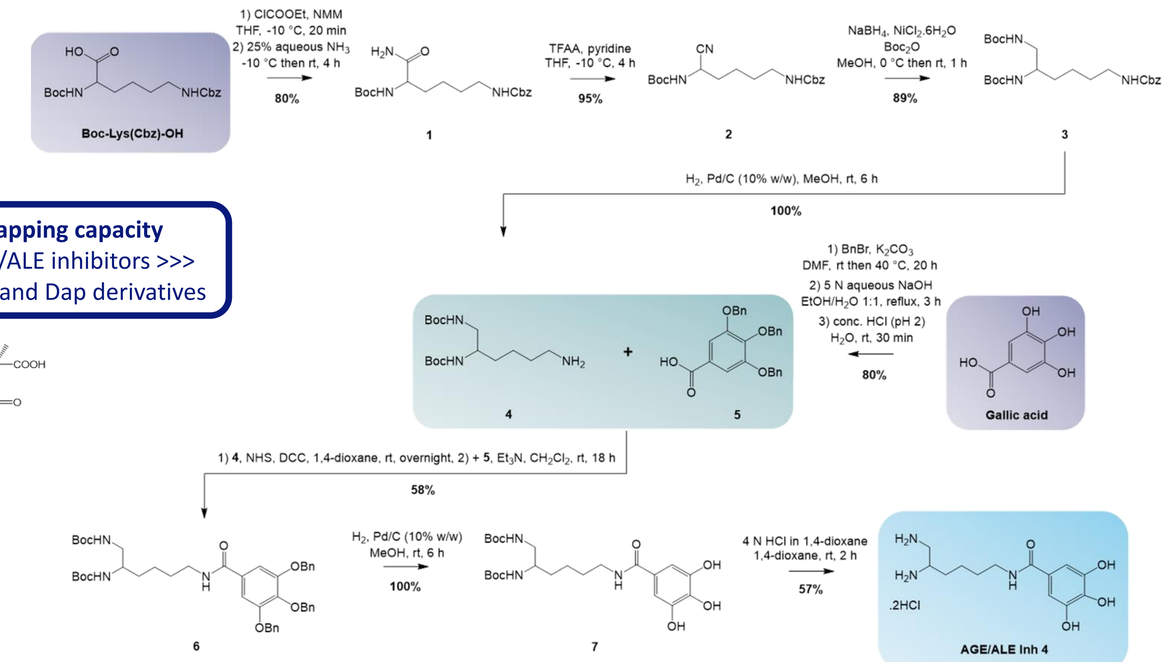


Figure 2.

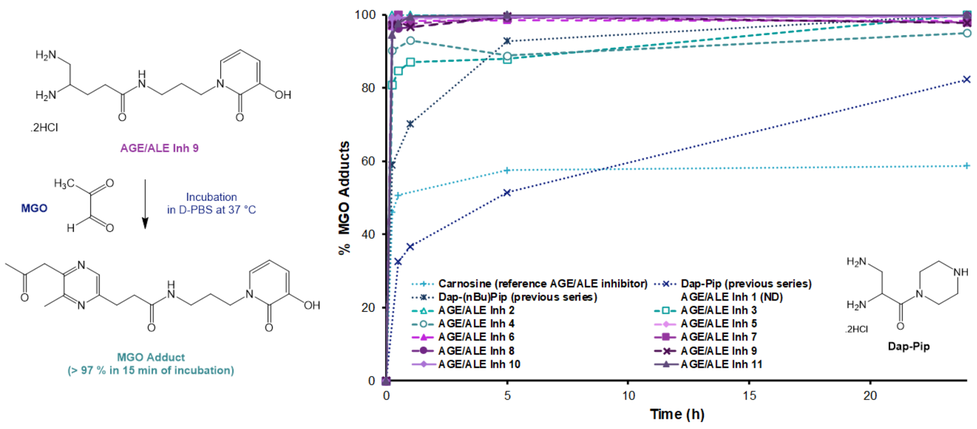
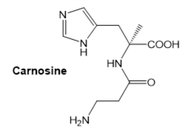


Figure 3. Kinetic curves representing the formation of MGO adducts in the presence of different scavengers. Tested compounds (10 mM) dissolved in D-PBS were incubated with MGO (20 mM) at 37 °C for 24 h. Samples collected at regular time intervals were subsequently analyzed by LCMS. Data are expressed as % MGO adducts compared with remaining free scavenger for a representative single sample. ND: Not determined.

RCS trapping capacity
New AGE/ALE inhibitors >>> Carnosine and Dap derivatives



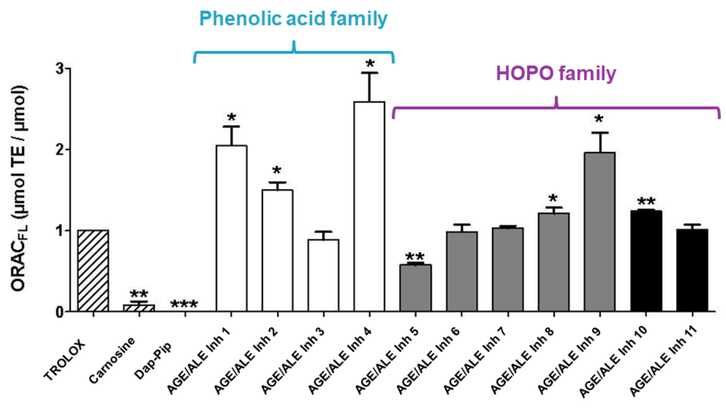


Figure 4. $ORAC_{FL}$ (Oxygen Radical Absorbance Capacity assay using fluorescein) values of new hybrid diamine derivatives and references. Peroxyl radicals generated from AAPH at 37 °C reacted with this fluorescent probe to form a non fluorescent product. The protective effect of the tested compounds was determined following FL fluorescence decay in time and measuring AUC of the sample in comparison with the control corresponding to an absence of antioxidant. Trolox was used as standard for the calculation of $ORAC_{FL}$ values at 10 µM expressed as µmol trolox equivalent (TE)/µmol of tested compound with respect to the linear equation of its calibration curve. Bars represent the means ± SEM calculated at 10 µM of at least three independent experiments performed in triplicate.

Antioxidant properties
New AGE/ALE inhibitors >>> Trolox (vitamin E analog)

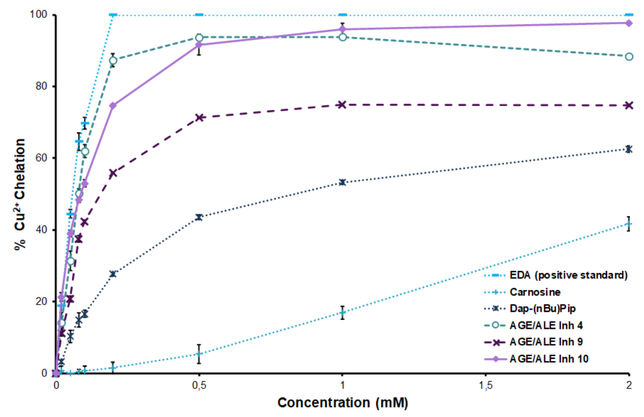


Figure 5. Evaluation of Cu^{2+}-chelating capacity of new hybrid diamine derivatives and references. Tested compounds (0 - 2 mM) were incubated with $CuSO_4.5H_2O$ (120 µM) for 10 min at rt. Murexide (50 µM), used as a complexometric indicator, was added. The absorbance ratio A_{485}/A_{520} (λmax of Cu^{2+}/murexide complex: 485 nm and λ max of free murexide: 520 nm) provided remaining free Cu^{2+} concentration with respect to calibration curves and % Cu^{2+} chelation by tested compounds was calculated by difference. Data are presented as means ± SEM of triplicates.

Cu^{2+}-chelating capacity
New AGE/ALE inhibitors >>> Carnosine and Dap derivatives

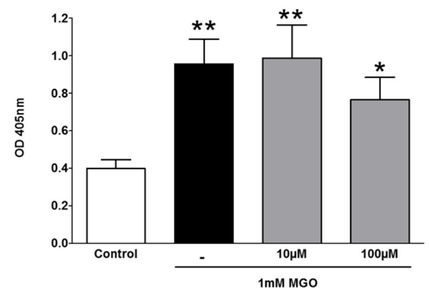


Figure 6. *In vitro* evaluation of MGO-induced apoptosis inhibition. PC12 cells were incubated for 24 h in media (control), and with MGO (1 mM) in the absence or the presence of either 10 µM or 100 µM of **AGE/ALE Inh 9**. At the end of the incubation, the cells were lysed and analyzed for DNA fragmentation. Bars represent the means ± SEM of at least three independent experiments performed in triplicate.

Reduction of MGO-induced apoptosis in the presence of AGE/ALE Inh 9 at 100 µM on a model AD cell-line

CONCLUSION

New synthesized AGE/ALE inhibitors demonstrated particularly **interesting RCS, ROS and Cu^{2+}-scavenging capacities** and no cytotoxicity in the model AD cell-line PC12. Furthermore, **AGE/ALE Inh 9** offered promising protective biological activity reducing *in vitro* MGO-induced apoptosis. Finally, at least two lead compounds (**AGE/ALE Inh 4 and 9**) seem to be able to **prevent oxidative and carbonyl stress extension implicated in the pathogenesis of AD** and further investigations to improve their druglikeness are currently in progress.

REFERENCE

1. D.A. Butterfield, J. Drake, C. Pocernich and A. Castegna, *Trends Mol. Med.*, **2001**, *7*, 548-554; 2. A. Tiiman, P. Palumaa and V. Tougu, *Neurochem. Int.*, **2013**, *62*, 367-378; 3. M. Krautwald and G. Münch, *Exp. Gerontol.*, **2010**, *45*, 744-751; 4. E. Lohou, N.A. Sasaki, A. Boullier and P. Sonnet, *Eur. J. Med. Chem.*, **2016**, *122*, 702-722.