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Multifunctional diamine AGE/ALE inhibitors with promising properties for treating Alzheimer's disease

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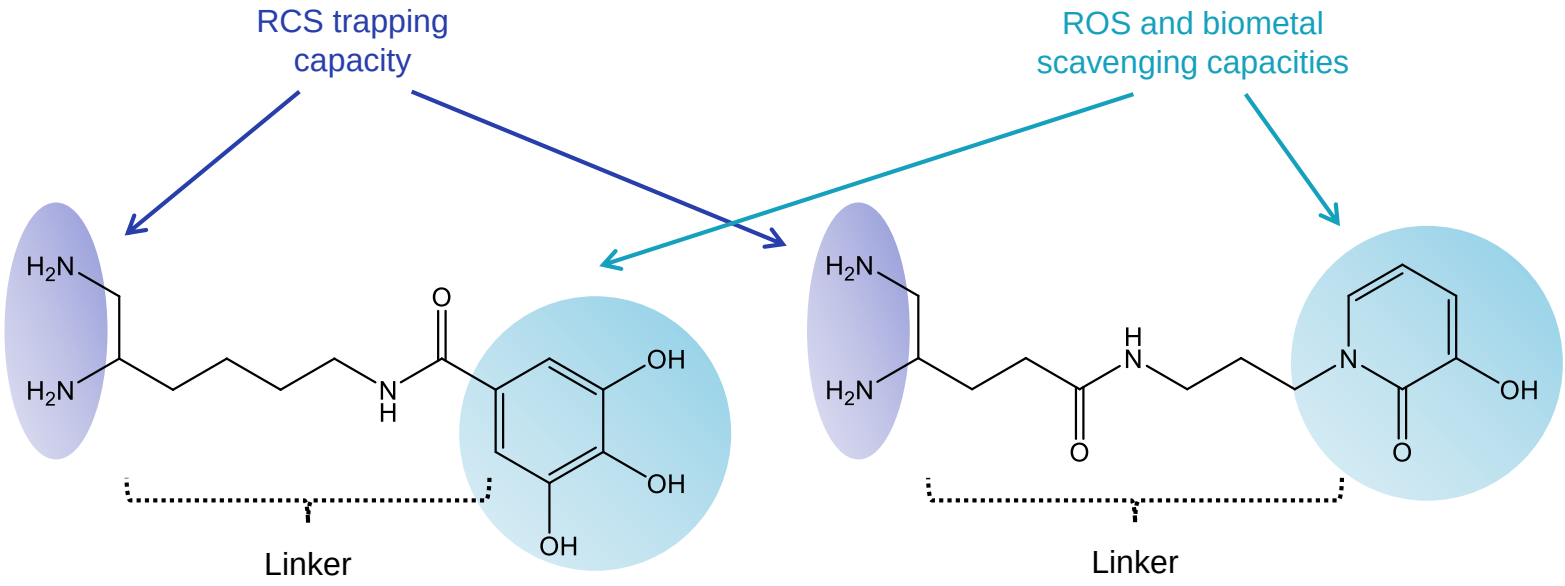
Multifunctional diamine AGE/ALE inhibitors with promising properties for treating Alzheimer's disease

Graphical Abstract

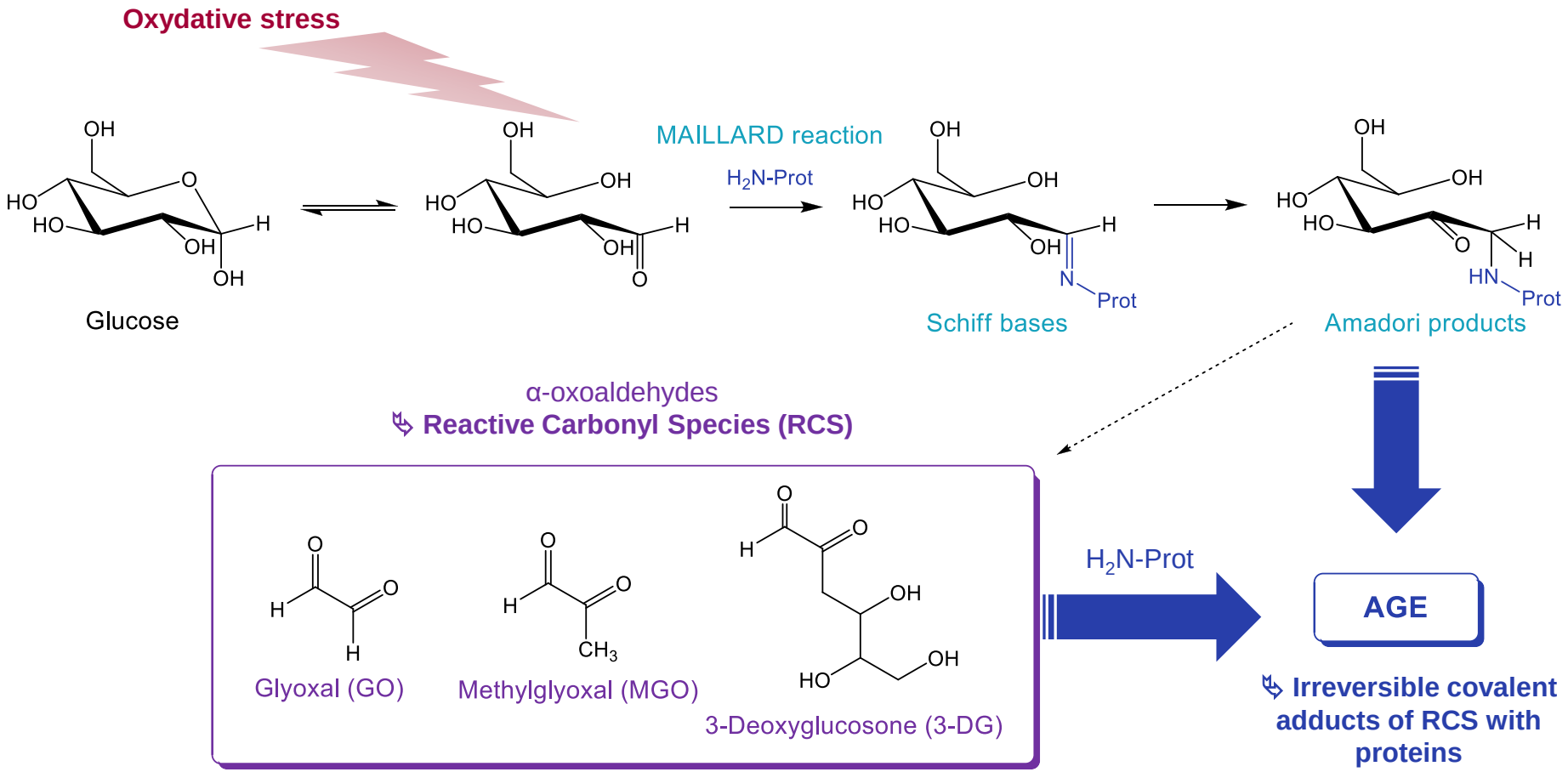


Abstract: Reactive carbonyl species (RCS) such as methylglyoxal or malondialdehyde are endogenously formed during the sugar glycoxidation and lipid peroxidation of polyunsaturated fatty acids induced by oxidative stress exacerbation. Their condensation with amino groups of tissue proteins gives AGE (Advanced Glycation Endproducts) and ALE (Advanced Lipid peroxidation Endproducts). In Alzheimer's disease (AD), extensive AGE/ALE accumulation has been reported in extracellular amyloid *β* (A*β*) plaques and intracellular tau-associated neurofibrillary tangles. Indeed, a critical imbalance between cerebral reactive oxygen species (ROS) production and endogenous antioxidant capacities associated with biometal dyshomeostasis has been suggested to be a driving force for AD onset and progression. Consequently, RCS accumulation takes part in the vicious downward redox amyloid spiral leading to neurodegeneration. Taking into account the multifactorial pathogenesis of AD, we designed new multifunctional drugs that are simultaneously able to trap RCS as well as ROS and biometals. In the presentation, synthesis of these new promising hybrid AGE/ALE inhibitors and evaluation of their physicochemical and biological properties are reported.

Keywords: Alzheimer's disease; AGE; ALE; Oxidative stress; Biometal dyshomeostasis

Introduction : AGE/ALE and carbonyl stress

✓ AGE = Advanced Glycation Endproducts



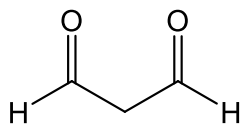
Introduction : AGE/ALE and carbonyl stress

✓ ALE = Advanced Lipid peroxidation Endproducts

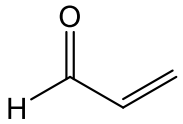
Lipid peroxydation

Stress oxydant

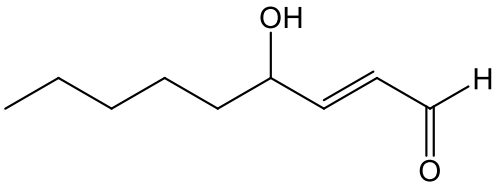
Polyunsaturated fatty acids



Malondialdehyde (MDA)



Acrolein



4-Hydroxy-2-nonenal (4-HNE)

α,β-unsaturated aldehydes

↳ **Reactive Carbonyl Species (RCS)**

MICHAEL addition

H_2N-Prot

ALE

↳ **Irreversible covalent adducts of RCS with proteins**

Introduction : AGE/ALE and carbonyl stress

✓ AGE/ALE physiopathological implications

❑ Age-related tissue and cell dysfunction

↳ **Reticulation of proteins** (like collagen, lens proteins…) and **loss of tissue elasticity** : skin ageing, cataract…

❑ Diabetic microvascular complications (nephropathy, retinopathy and neuropathy) and atherosclerosis

↳ **Reticulation of proteins** and **loss of vascular endothelium elasticity**

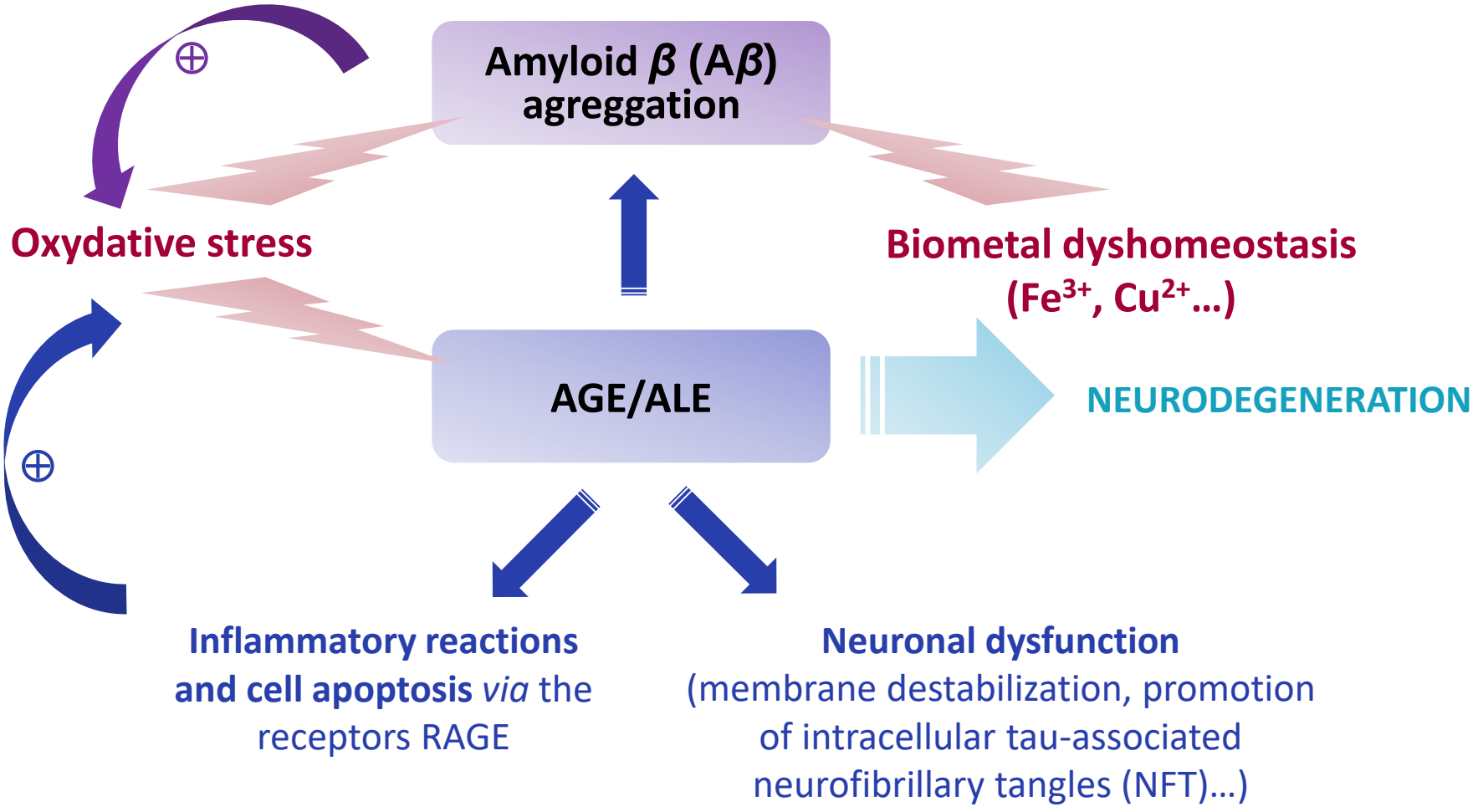
↳ **Formation of ApoB/MDA adducts** leading to modified oxidized LDL and atheroma

↳ **Oxydative stress exacerbation** associated with **inflammatory and thrombogenic reactions**

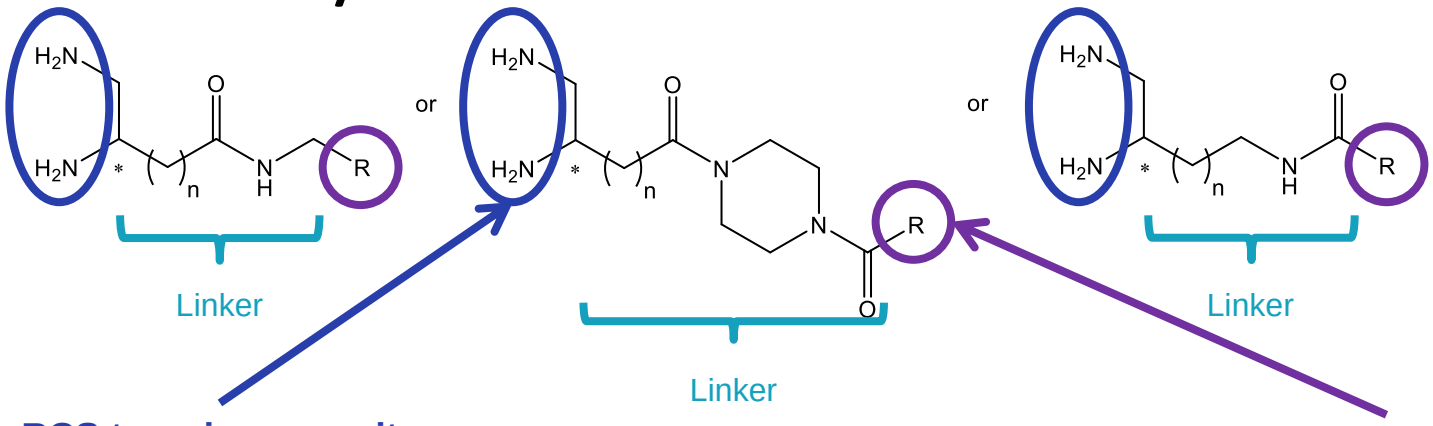
- ↳ Promotion *via* the **receptors RAGE**
- ↳ Damaging to antioxydant enzyme system

❑ Neurodegenerative diseases

Introduction : Downward redox amyloid spiral in Alzheimer's Disease (AD)



Results and discussion : Synthesis of new multifunctional diamine AGE/ALE inhibitors

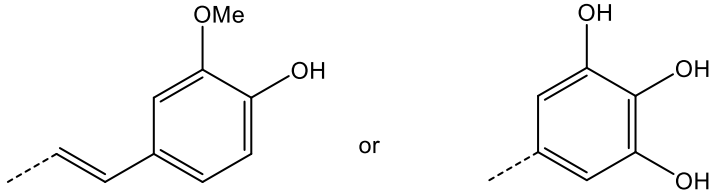


RCS trapping capacity (↓ carbonyl stress)

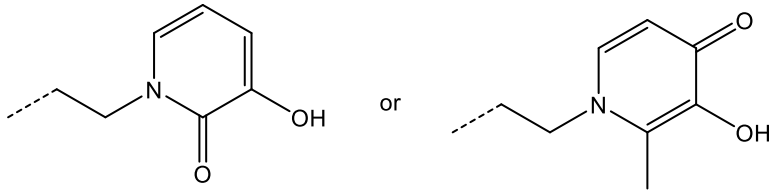
ROS (Reactive Oxygen Species) and biometal scavenging capacities (↓ oxydative stress)

n = 1 to 3

R =

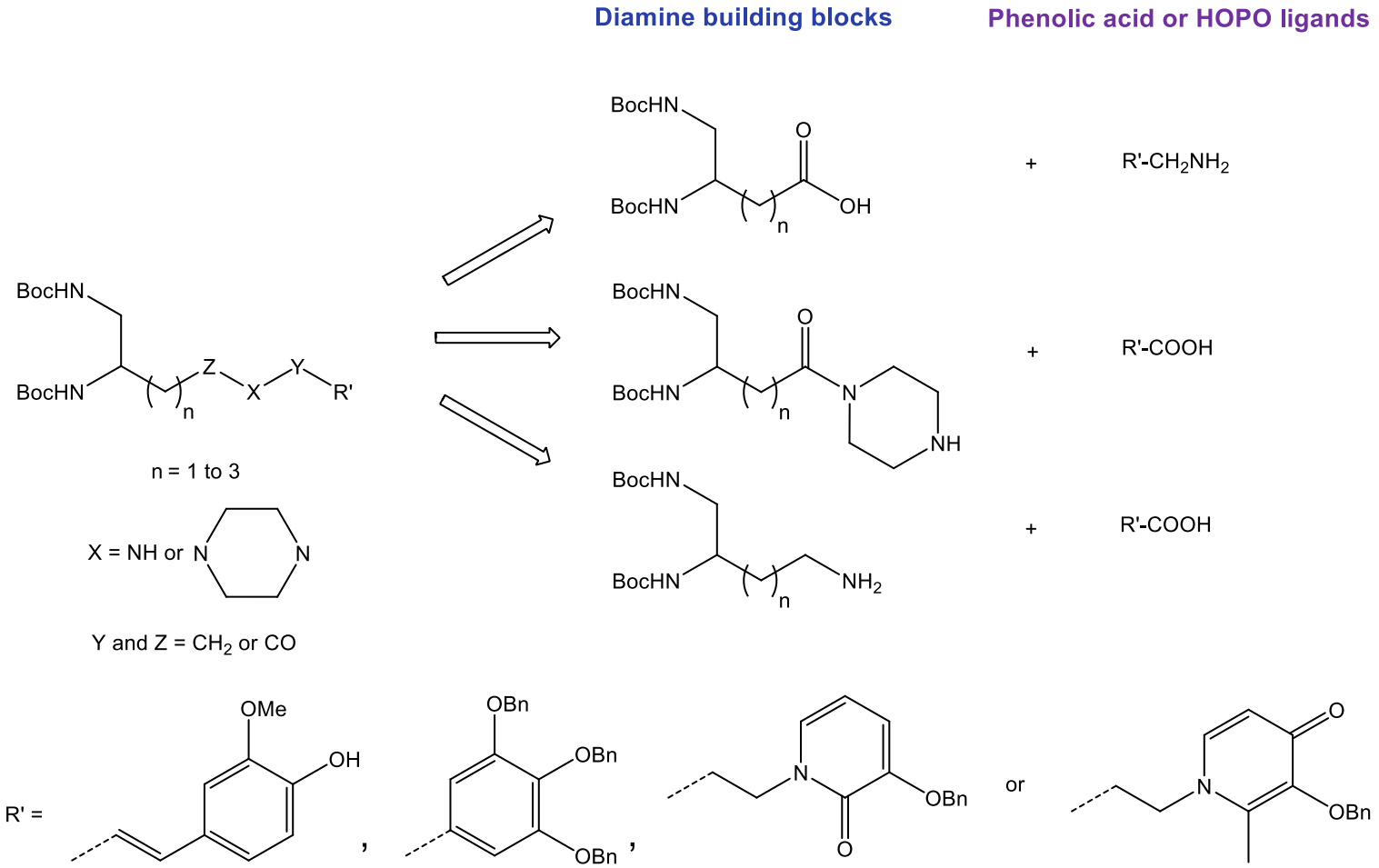


Phenolic acid family



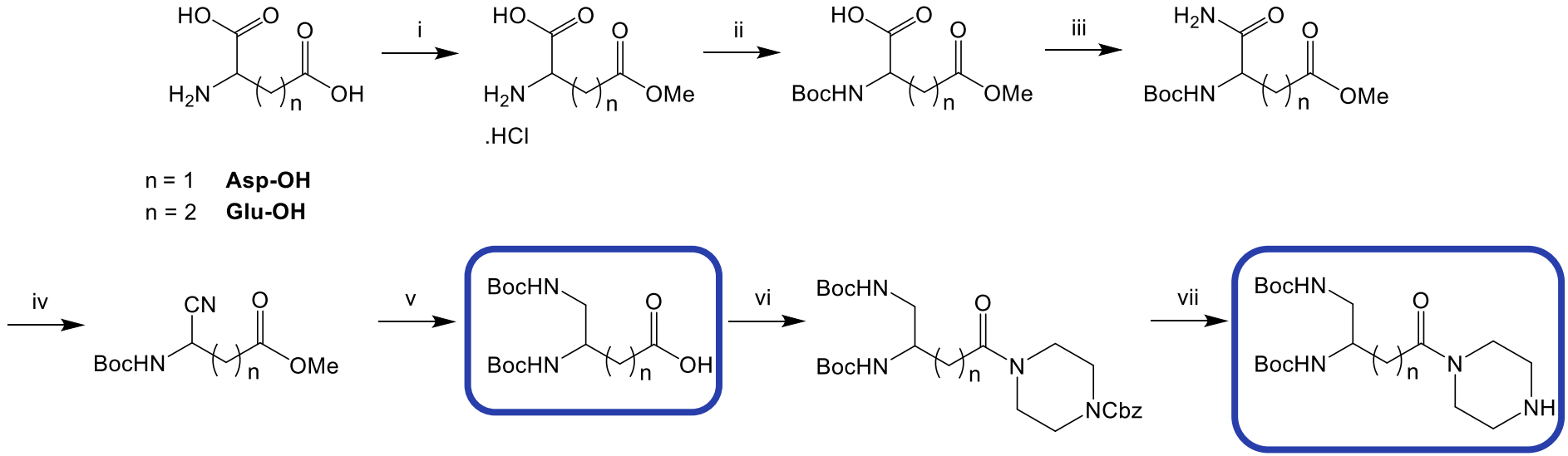
Hydroxypyridinone (HOPO) family

Results and discussion : Synthesis of new multifunctional diamine AGE/ALE inhibitors



Results and discussion : Synthesis of new multifunctional diamine AGE/ALE inhibitors

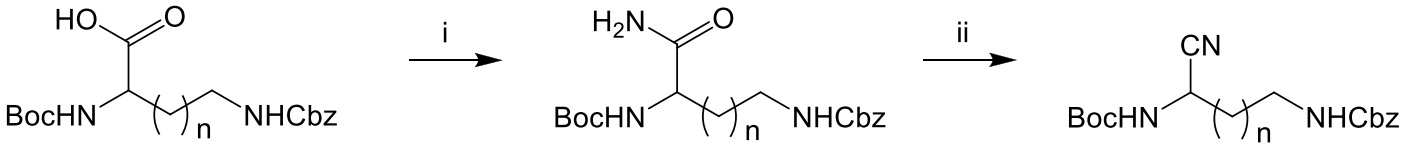
✓ Synthesis of diamine building blocks starting from aspartic acid or glutamic acid



(i) methanolic HCl, MeOH, 0°C, 3 h then rt, 1-1.5 h, **100%**; (ii) Boc_2O, $NaHCO_3$, 1,4-dioxane/H_2O 2:1, rt, 20-24 h, **75-77%**; (iii) 1) ClCOOEt, Et_3N, THF, -15°C, 30 min, 2) 25% aqueous NH_3, -15°C then rt, 18 h, **68-77%**; (iv) TFAA, Et_3N, THF, -10°C, 2-4 h, **60-74%**; (v) 1) $NaBH_4$, $NiCl_2.6H_2O$, Boc_2O, MeOH, 0°C then rt, 3 h, 2) 4 N aqueous LiOH, THF/H_2O 1:1, rt, 1-1,5 h, **50-67%**; (vi) 1) NHS, DCC, CH_2Cl_2, rt, overnight, 2) 1-Cbz-piperazine hydrochloride, Et_3N, CH_2Cl_2, rt, 18 h, **77-97%**; (vii) H_2, Pd/C (10% w/w), MeOH, rt, 6 h; **96-100%**.

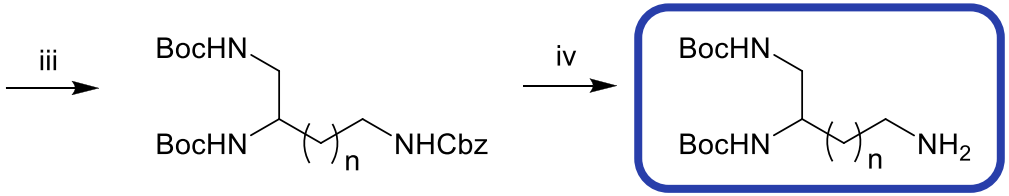
Results and discussion : Synthesis of new multifunctional diamine AGE/ALE inhibitors

✓ Synthesis of diamine building blocks starting from lysine or ornithine



n = 2 **Boc-Orn(Cbz)-OH**

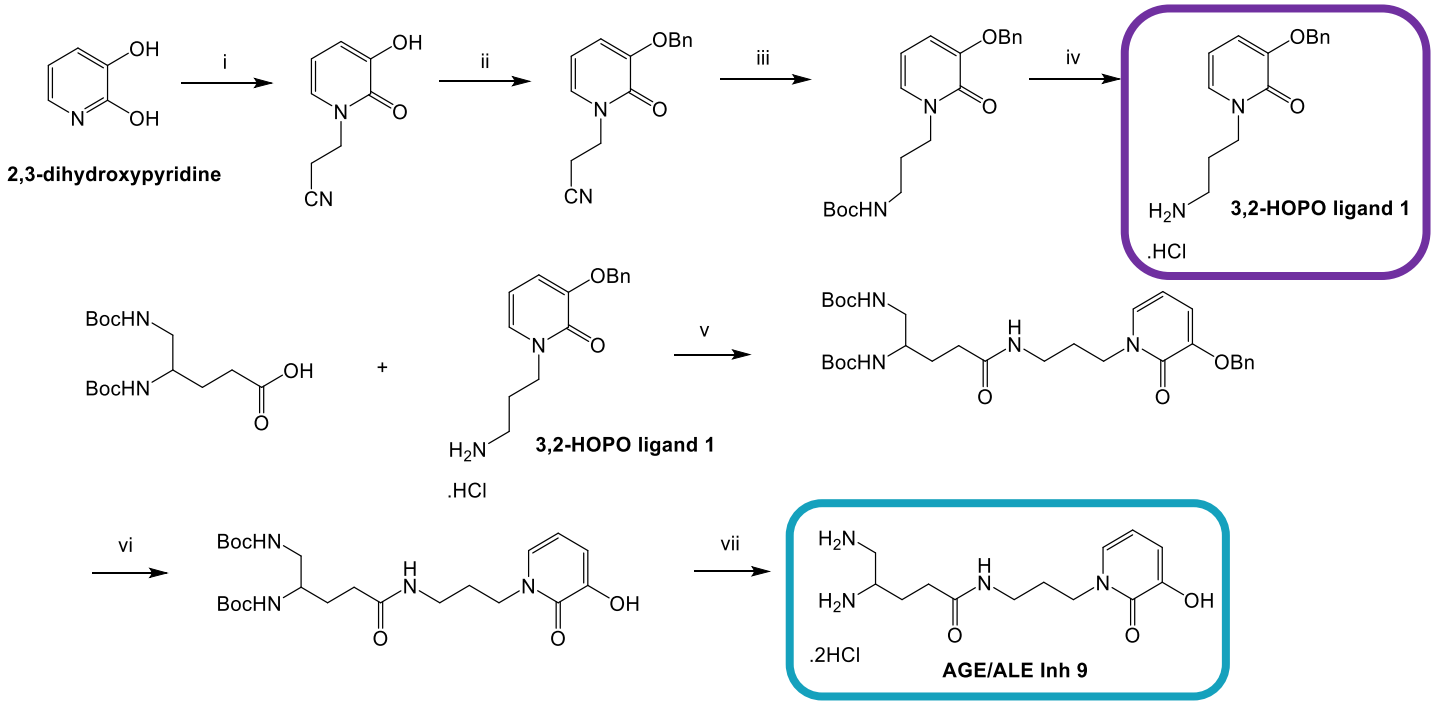
n = 3 **Boc-Lys(Cbz)-OH**



(i) 1) ClCOOEt, NMM, THF, -10°C, 20 min, 2) 25% aqueous NH_3, -10°C then rt, 4 h, **80-91%**; (ii) TFAA, pyridine, THF, -10°C, 2-4 h, **95-99%**; (iii) 1) $NaBH_4$, $NiCl_2.6H_2O$, Boc_2O, MeOH, 0°C then rt, 1 h, **89-92%**; (iv) H_2, Pd/C (10% w/w), MeOH, rt, 6 h; **92-100%**.

Results and discussion : Synthesis of new multifunctional diamine AGE/ALE inhibitors

✓ Coupling of 3,2-HOPO ligands



(i) Acrylonitrile, CsF, MeCN, reflux, 16 h, **93%**; (ii) BnBr, K_2CO_3, MeCN, reflux, 18 h, **90%**; (iii) $NaBH_4$, $NiCl_2.6H_2O$, Boc_2O, MeOH, 0°C then rt, 1 h, **84%**; (iv) 4 N HCl in 1,4-dioxane, 1,4-dioxane, rt, 2 h, **100%**; (v) 1) NHS, DCC, 1,4-dioxane or CH_2Cl_2, rt, overnight, 2) **3,2-HOPO ligand 1**, Et_3N, CH_2Cl_2, rt, 18 h, **68%**; (vi) H_2, Pd/C (10% w/w), MeOH, rt, 6 h; **100%**; (vii) 4 N HCl in 1,4-dioxane, 1,4-dioxane, rt, 2 h, **93%**.

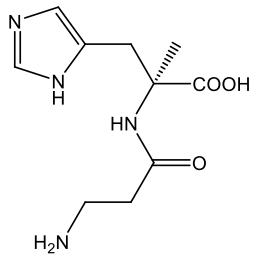
Results and discussion : Physicochemical and biological evaluations

✓ MGO and MDA trapping assay

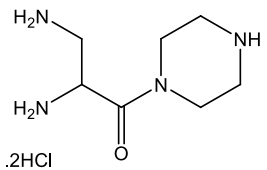
RCS trapping capacity of diamine function ?

- Incubation of tested compounds with MGO or MDA at 37°C for 24 h (pH 7.4)
- **Analysis by LCMS** of samples collected at regular time intervals **to perform a kinetic study of adduct formation**
  - Identification of major adducts with MGO and MDA on mass spectra
  - Comparison of area under the curve (AUC) of total peak of adducts with remaining free scavenger peak on UV chromatogram at 190 nm

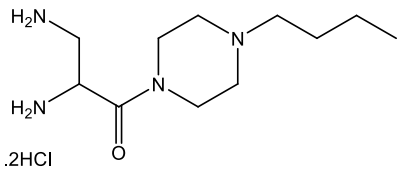
- Reference AGE/ALE inhibitors : **Carnosine** and **previously described Dap** (2,3-**Dia**mino**p**ropionic acid) **derivatives**



Carnosine



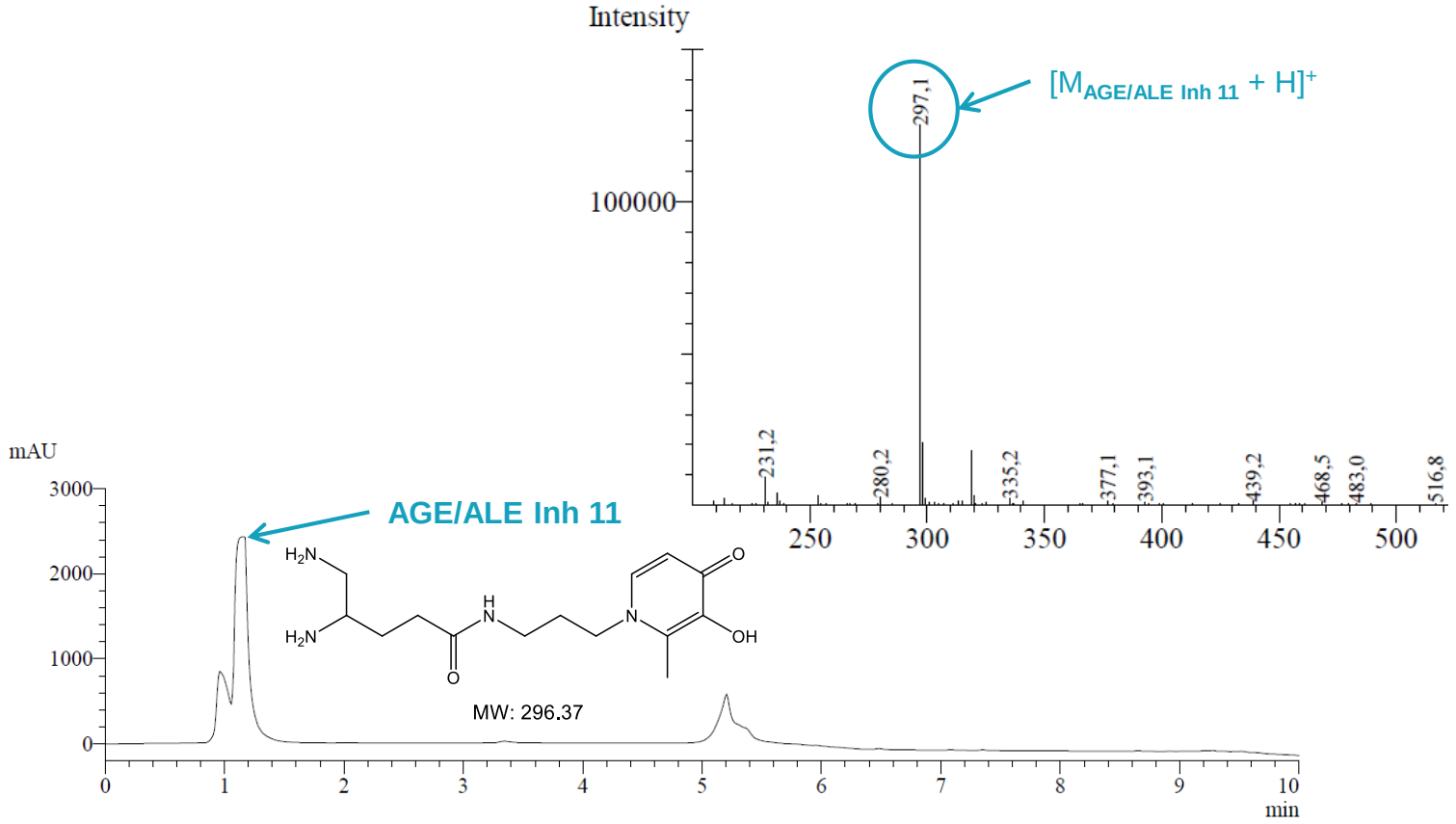
Dap-Pip



Dap-(nBu)Pip

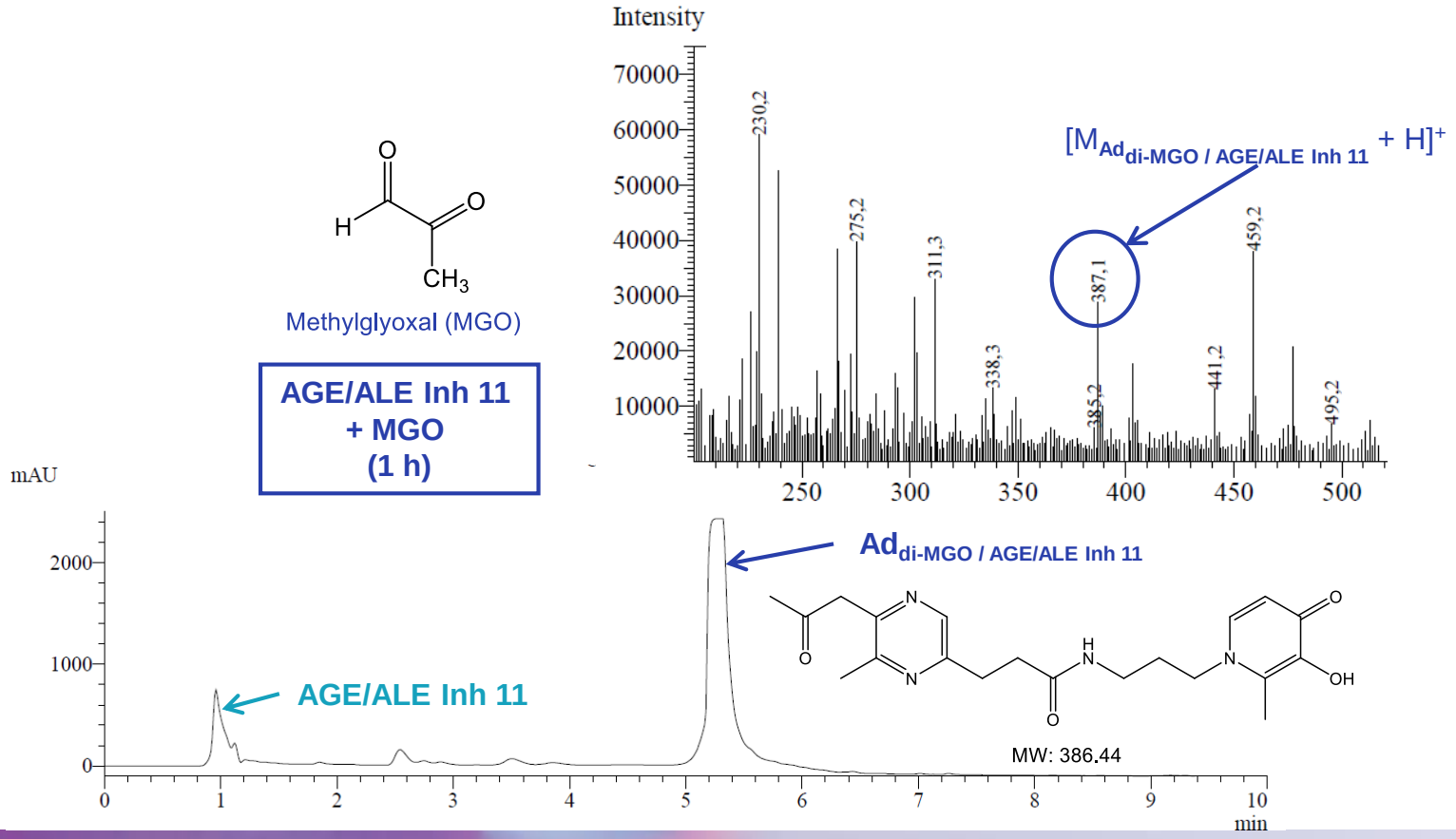
Results and discussion : Physicochemical and biological evaluations

✓ MGO and MDA trapping assay



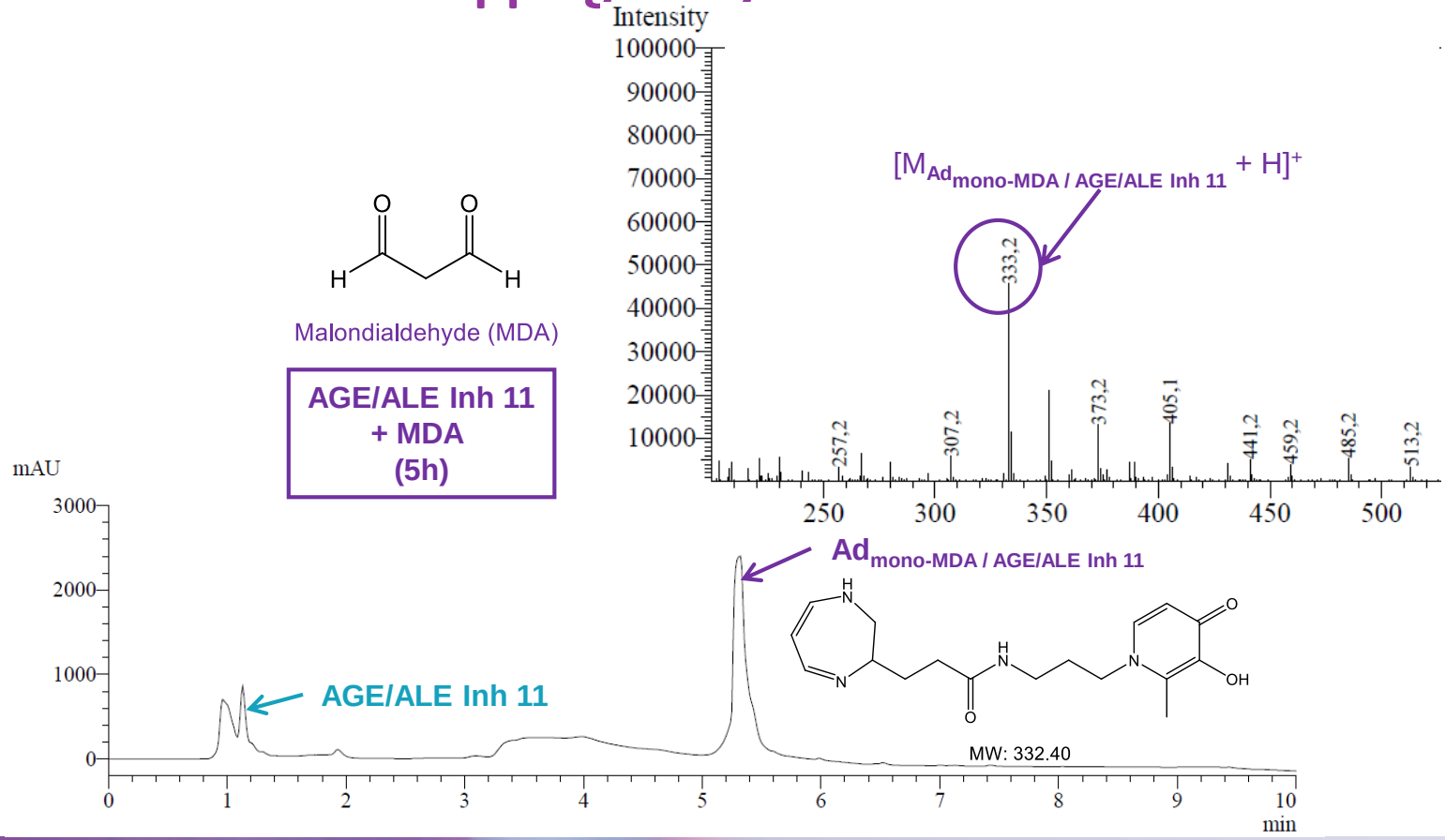
Results and discussion : Physicochemical and biological evaluations

✓ MGO and MDA trapping assay



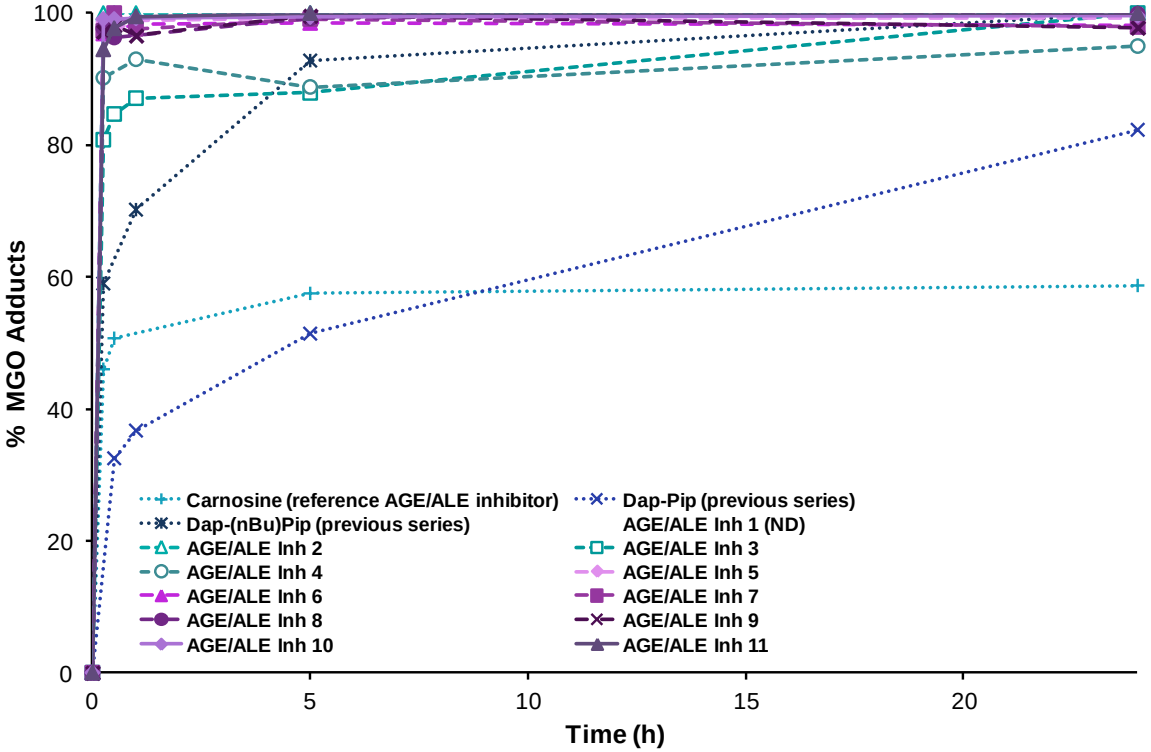
Results and discussion : Physicochemical and biological evaluations

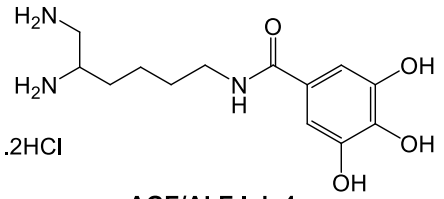
✓ MGO and MDA trapping assay



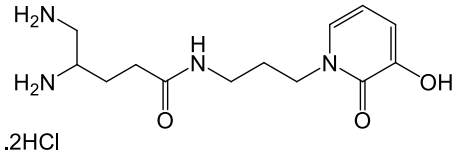
Results and discussion : Physicochemical and biological evaluations

✓ MGO and MDA trapping assay





AGE/ALE Inh 4



AGE/ALE Inh 9

Potent RCS trapping capacity of newly designed compounds

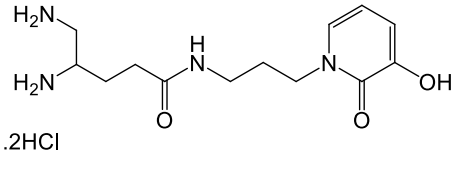
New AGE/ALE inhibitors >>> Carnosine and Dap derivatives

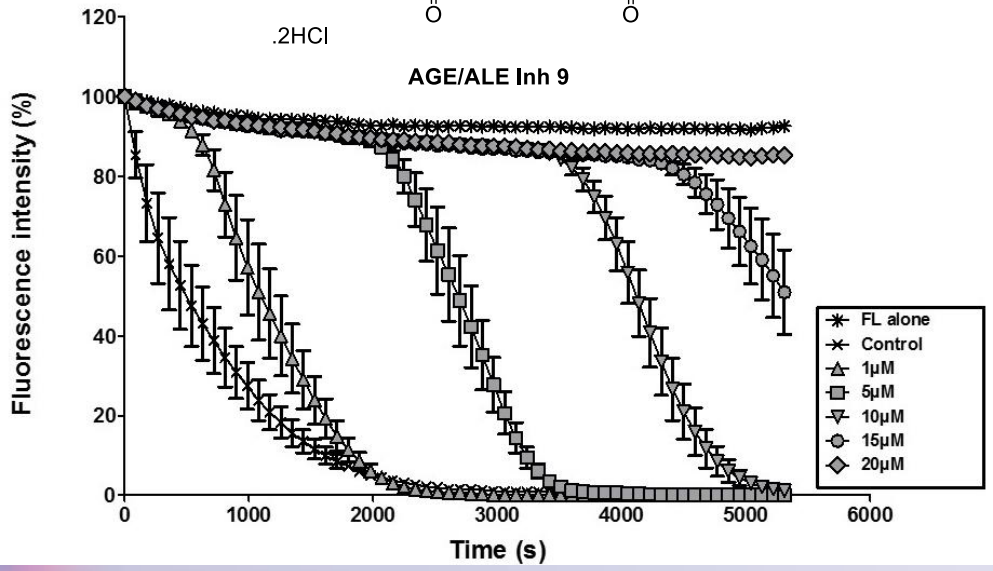
Results and discussion : Physicochemical and biological evaluations

✓ Oxygen radical absorbance capacity (ORAC) assay

Antioxidant properties ?

- Study of **fluorescein (FL) fluorescence decay, induced by AAPH used as a peroxyl radical generator**
  - Measurement of AUC of the samples in comparison with the control corresponding to an absence of antioxidant to highlight protective effect of tested compounds

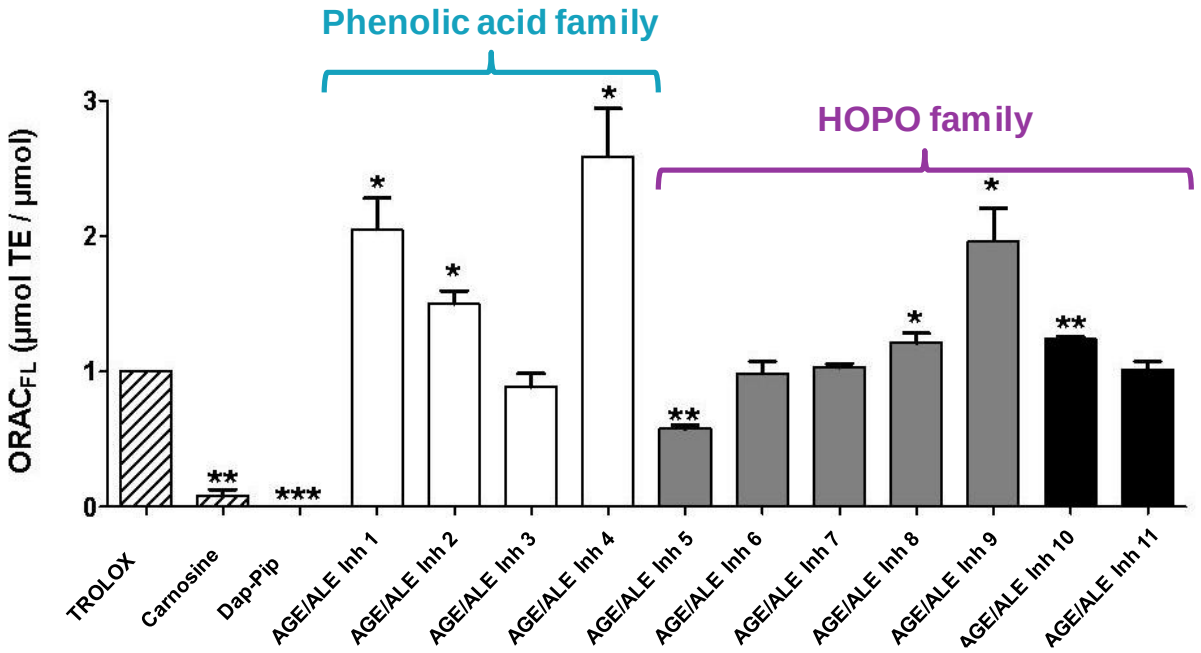




Results and discussion : Physicochemical and biological evaluations

✓ Oxygen radical absorbance capacity (ORAC) assay

↳ Use of trolox (a vitamin E analog) as standard for the calculation of $ORAC_{FL}$ values at 10 mM expressed as **µmol trolox equivalent (TE)/µmol of tested compound**



Interesting antioxidant properties of new hybrid compounds

AGE/ALE Inh 4 and 9 >>> Trolox

Results and discussion : Physicochemical and biological evaluations

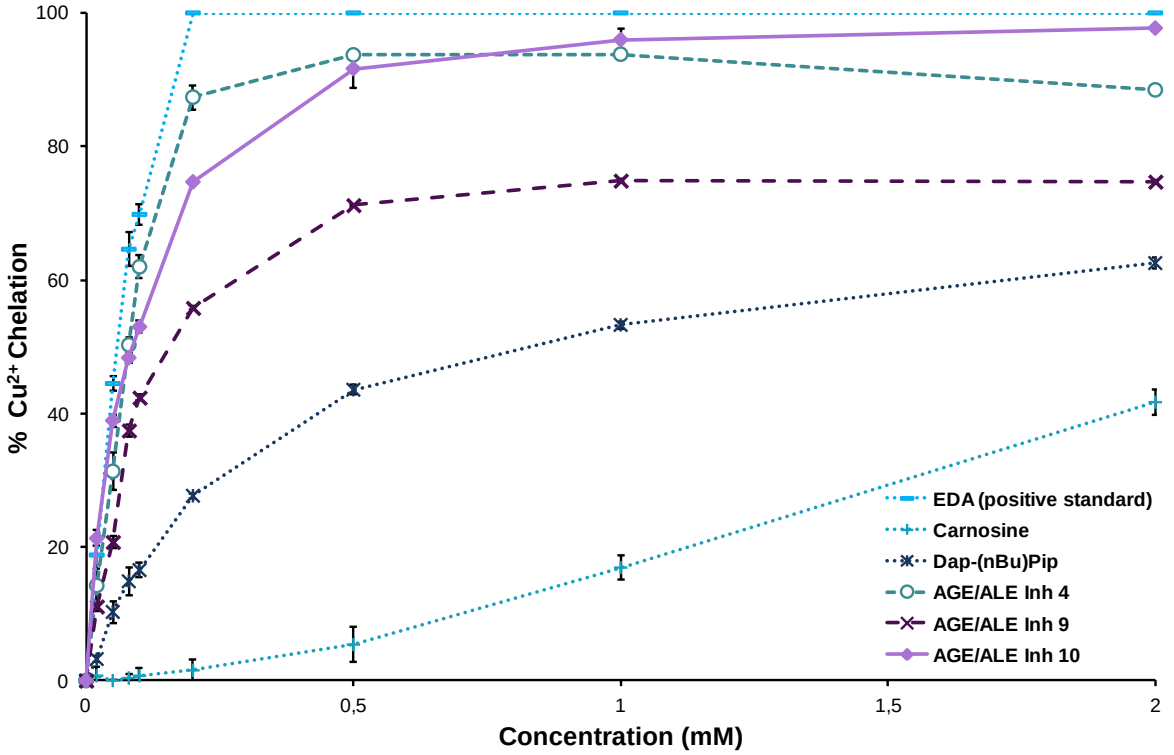
✓ Cu^{2+}-chelating assay

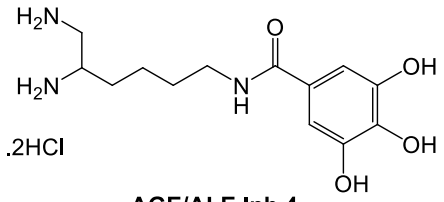
Biometal scavenging capacity ?

- Incubation of tested compounds with $CuSO_4.5H_2O$ at rt for 10 min (pH 5)
- **Analysis by UV/Vis spectrophotometry of remaining free Cu^{2+} concentration after complexation with murexide (complexometric indicator)**
  - Measurement of absorbance ratio A_{485}/A_{520} (λmax of Cu^{2+}/murexide complex: 485 nm and λmax of free murexide: 520 nm)
- Calculation by difference of % Cu^{2+} chelation by tested compounds

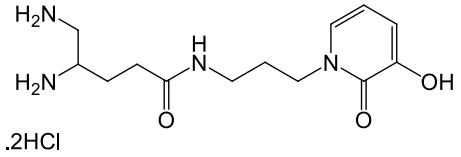
Results and discussion : Physicochemical and biological evaluations

✓ Cu^{2+}-chelating assay

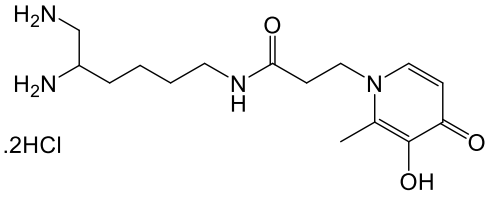




AGE/ALE Inh 4



AGE/ALE Inh 9



AGE/ALE Inh 10

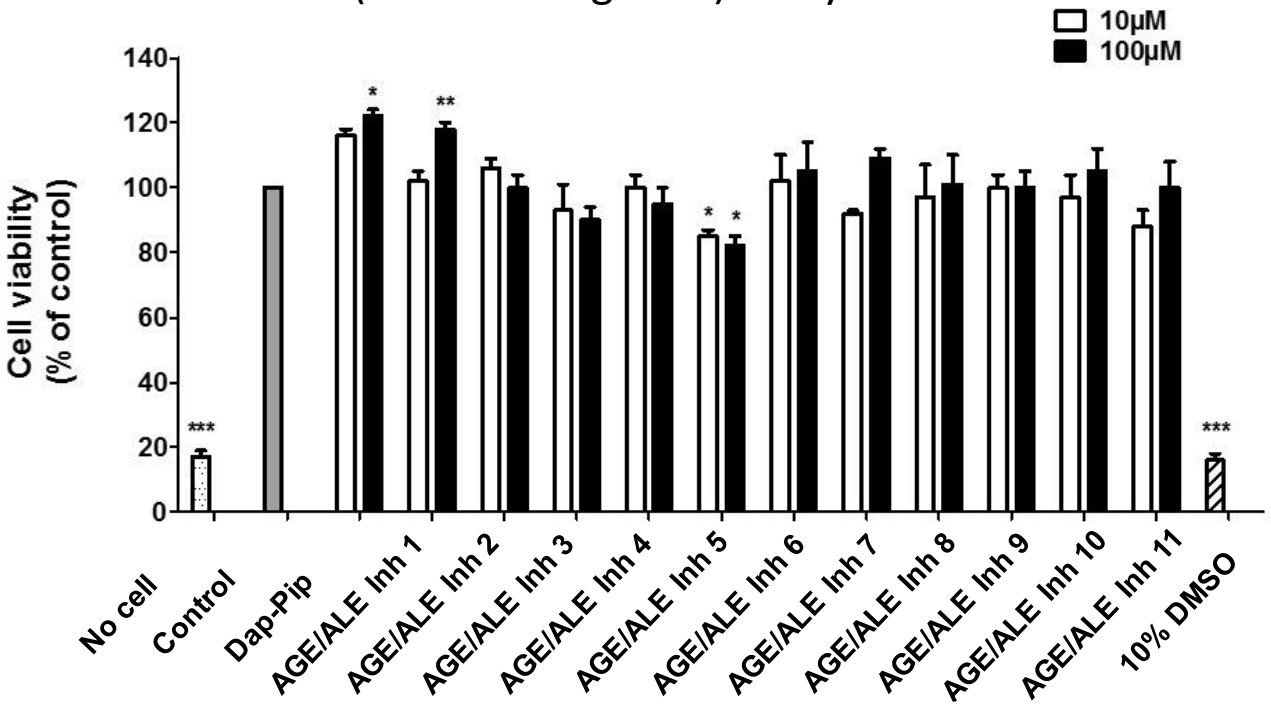
Important Cu^{2+}-chelating capacity of new multifunctional diamine compounds

New AGE/ALE inhibitors >>> Carnosine and Dap derivatives

Results and discussion : Physicochemical and biological evaluations

✓ Cell viability assay

Sensitive colorimetric CCK-8 (cell counting kit-8) assay



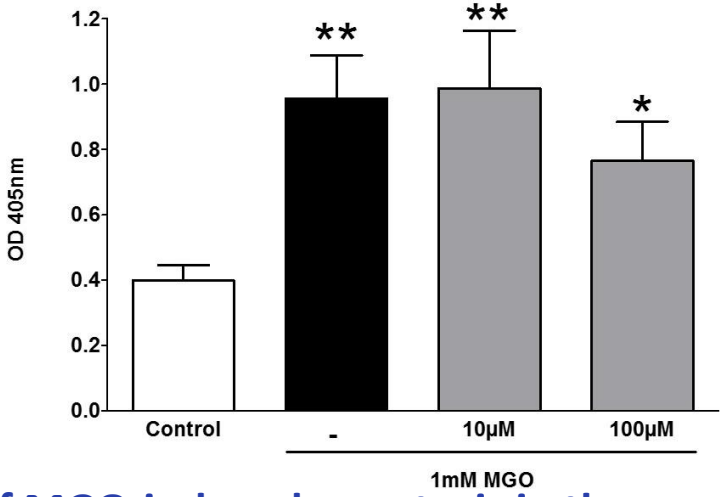
No cytotoxicity of new hybrid diamine derivatives on neuronal-like cell-line PC12 cells after 24 h of treatment at 10 mM as well as at 100 mM

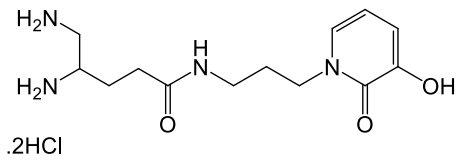
Results and discussion : Physicochemical and biological evaluations

✓ *In vitro* MGO-induced apoptosis inhibition assay

- Pretreatment of PC12 cells with the **lead compound AGE/ALE Inh 9** at 37°C before incubation in the presence of MGO
- Measurement of *in vitro* MGO-induced apoptosis using an ELISA detection of DNA fragmentation :

Optical density (OD) at 405 nm = Reflect of apoptosis level





AGE/ALE Inh 9

Attenuation of MGO-induced apoptosis in the presence of lead compound AGE/ALE Inh 9 at 100 mM on the model AD cell-line PC12 cells

Conclusions

✓ Synthesis of new hybrid diamine compounds

→ Phenolic acid family : overall yields = 10-32% (6 to 10 steps)
→ HOPO family : overall yields = 4-63% (7 to 10 steps)

✓ Demonstration of potent and synergetic multifunctional properties of the newly designed derivatives

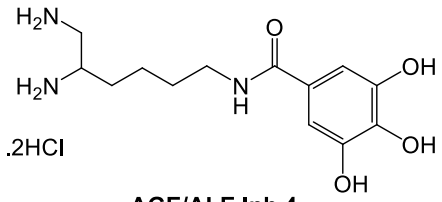
- ❑ AGE/ALE inhibitors

→ RCS trapping capacity of diamine function

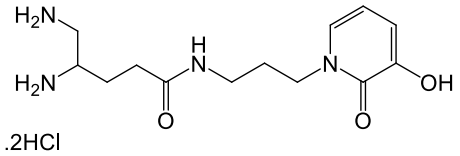
- ❑ ROS and biometal scavengers

→ Additional antioxidant and Cu^{2+}-chelating properties of phenolic acid or HOPO moiety

Two lead compounds



AGE/ALE Inh 4



AGE/ALE Inh 9

Promising biological evidence of the ability of new hybrid diamine compounds to limit the vicious downward carbonyl redox amyloid spiral that lead to neurodegeneration in Alzheimer's disease

Conclusions

✓ Valorisation of the research work

- Lohou, E.; Sasaki, N. A.; Boullier, A.; Sonnet, P. *Eur. J. Med. Chem.* **2016**, *122*, 702-722.
- Sasaki, N. A.; Lohou, E.; Boullier, A.; Sonnet, P. **PCT 2017**, WO 2017/006048 A1.

✓ Perspectives

→ Investigations to improve the **druglikeness** of new multifunctional AGE/ALE inhibitors and especially their capacity to cross the blood brain barrier are currently in progress.

→ Predictions of ADME properties performed using QikProp, a *Schrödinger* software :

$clogP_{o/w}$ = -2,110
and clogBB = -1,766 (QikProp-recommended values : -3<logBB<1,2)
for **AGE/ALE Inh 9**

Acknowledgments

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