



# The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)

01-30 NOVEMBER 2022 | ONLINE

## C-Glucosyl Flavone Derivatives as Non-PAIN Therapeutic Leads for Alzheimer's Disease

Chaired by **DR. ALFREDO BERZAL-HERRANZ**;  
Co-Chaired by **PROF. DR. MARIA EMÍLIA SOUSA**



*pharmaceuticals*



**Ana Marta de Matos<sup>1,\*</sup>**

<sup>1</sup> Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Campo Grande, 1749-016, Lisboa, Portugal

\* Corresponding author: [amamatos@fc.ul.pt](mailto:amamatos@fc.ul.pt)

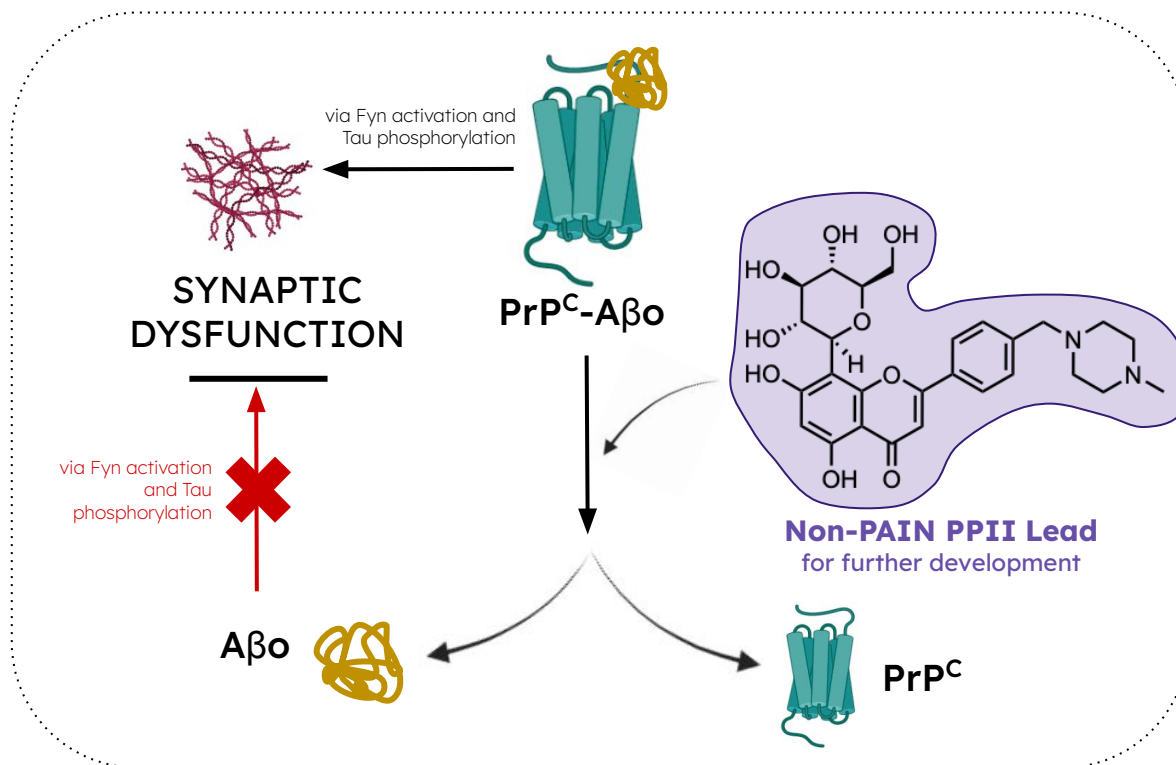


**Ciências  
ULisboa**

Faculdade  
de Ciências  
da Universidade  
de Lisboa



# C-Glucosyl Flavone Derivatives as Non-PAIN Therapeutic Leads for Alzheimer's Disease



## Abstract:

Alzheimer's Disease (AD) and other dementias are ranked by the WHO as the World's 7<sup>th</sup> leading cause of death. Aiming to respond to this international public health priority, the Carbohydrate Chemistry Group of CQE-IMS has been dedicated to uncovering the AD-modifying potential of carbohydrate-based molecules. In this communication, the rational design, synthesis, and biological evaluation of C-glucosyl flavone analogues with neuroprotective activity against H<sub>2</sub>O<sub>2</sub>- and A $\beta$ -induced cell death are explored. Furthermore, based on the well-established role of the binding between the PrP<sup>C</sup> and A $\beta$  oligomers (A $\beta$ <sub>o</sub>) for Tau hyperphosphorylation in the brain, the structural optimization process leading up to the discovery of *N*-methylpiperazinyl flavones and their C-glucosyl derivatives as protein-protein interaction inhibitors (PPII) against PrP<sup>C</sup>-A $\beta$ <sub>o</sub> is also presented. Importantly, because many planar lipophilic polyphenols such as the ones we have developed are Pan-Assay Interference CompoundS (PAINS), we were also interested in clarifying their ability to induce alterations of cell membrane properties in a non-specific manner. Our results show, for the first time, that well-known membrane disruptors such as resveratrol and genistein cease to alter the membrane dipole potential when linked to a glucosyl moiety through a C-C bond, suggesting that our C-glucosides should not raise concerns regarding membrane-related PAINS-type behavior. This communication ultimately highlights the promising neuroprotective and PPII activity of C-glucosyl flavones and corresponding aglycones in the context of AD, while exploring the role of the sugar moiety in favorably tuning aglycone bioactivity, cytotoxicity, and unspecific membrane modifying effects.

**Keywords:** Drug Discovery; Structural Optimization; Neurodegenerative Disorders; Carbohydrate Chemistry

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

## Introduction

### Why should we care about dementia?



**55**  
million people  
live with dementia

**7th**  
leading cause  
of death

#### Caused by

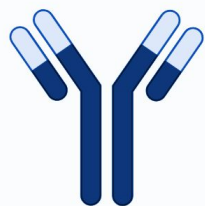
diseases and injuries that affect the brain, such as Alzheimer's disease and stroke

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry

01-30 NOVEMBER 2022 | ONLINE

# Introduction



Aducanumab

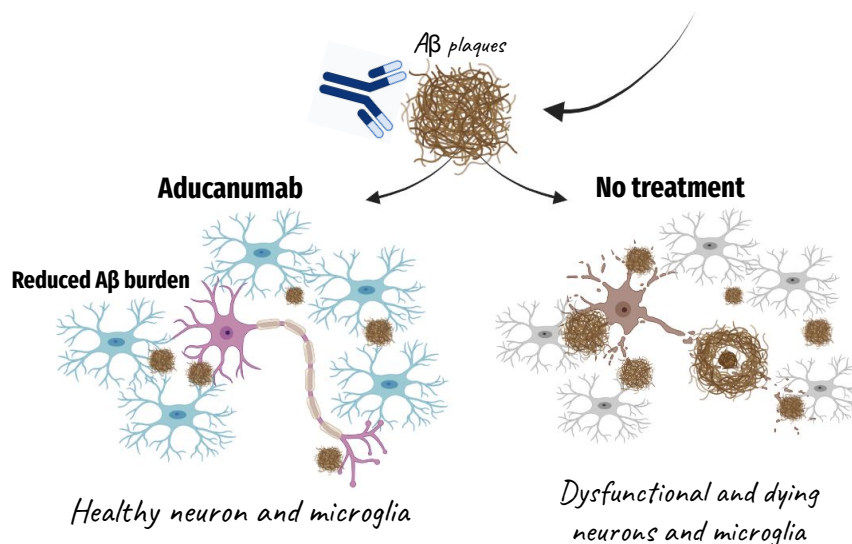
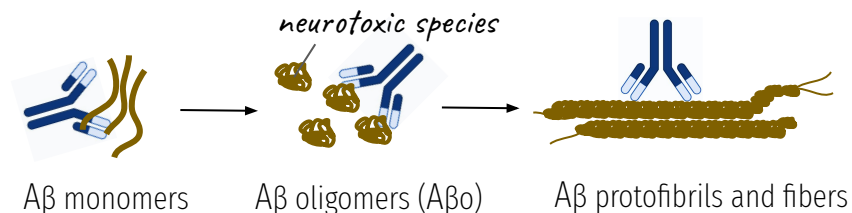
Indicated for its use in AD patients with mild cognitive impairment or mild dementia stage of the disease



Approved on  
7<sup>th</sup> June 2021<sup>1</sup>



Negative opinion  
from CHMP on  
December 2021<sup>2</sup>



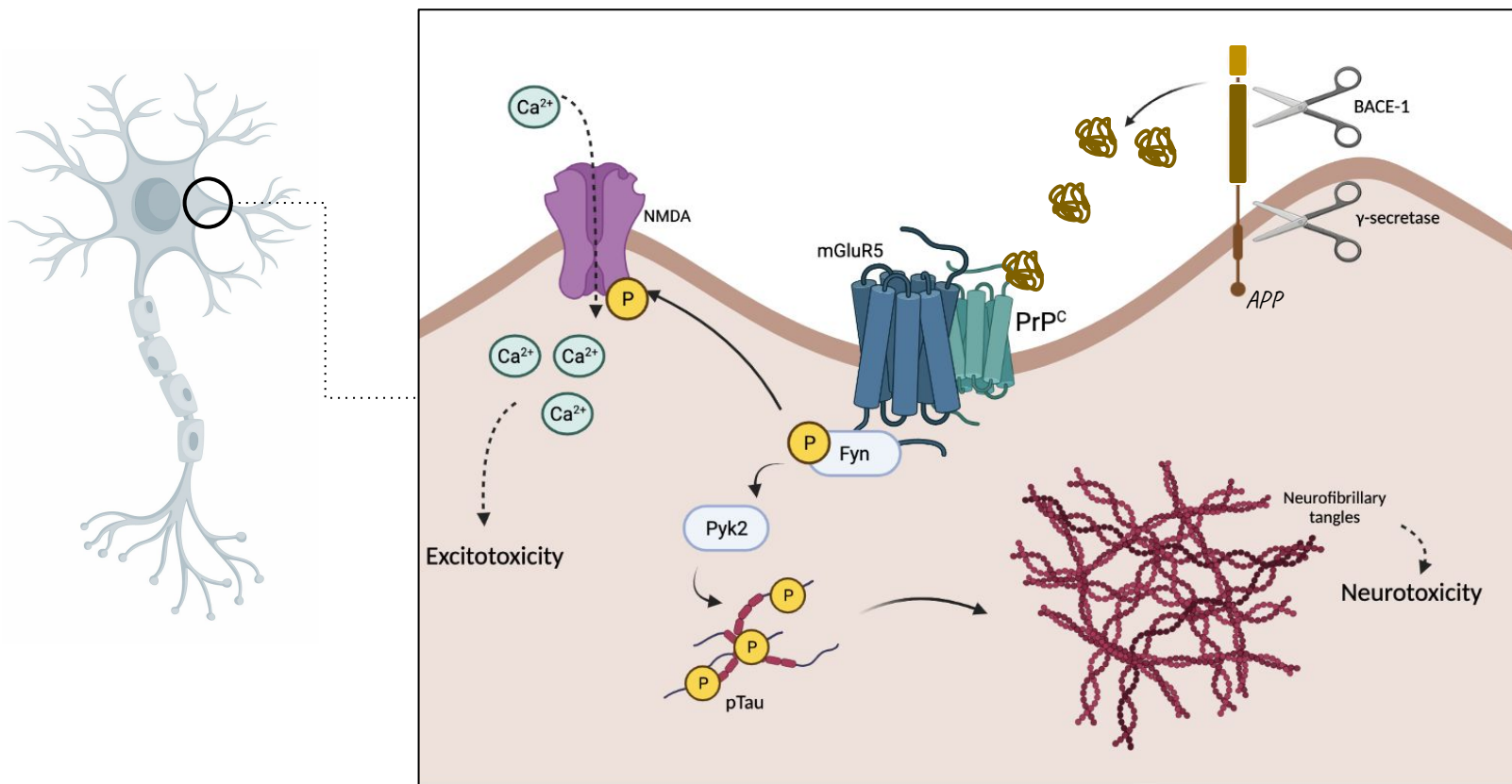
[1] <https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/aducanumab-marketed-aduhelm-information> [2]

<https://investors.biogen.com/news-releases/news-release-details/update-regulatory-submission-aducanumab-european-union-0>

Figure built with BioRender and adapted from: Esang M, et al. *Cureus*. 2021;13(8):e17591.

# Introduction

What other therapeutic targets can be explored?



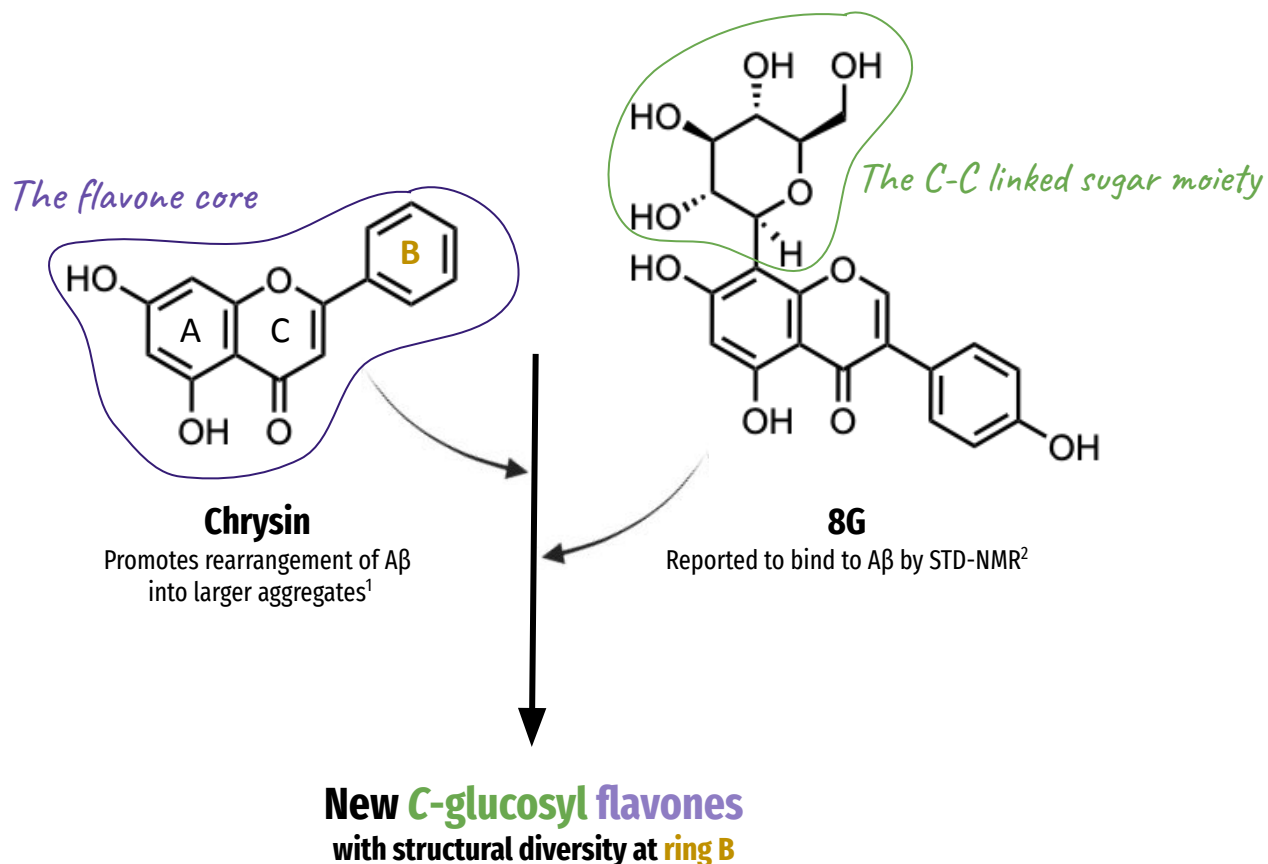
Build using BioRender and adapted from: Zhang Y, et al. *Front Cell Neurosci.* 2019;13:339 and van Nygaard CH, et al. *Alzheimers Res Ther.* 2014;6(1):8.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Introduction

## Our rationale



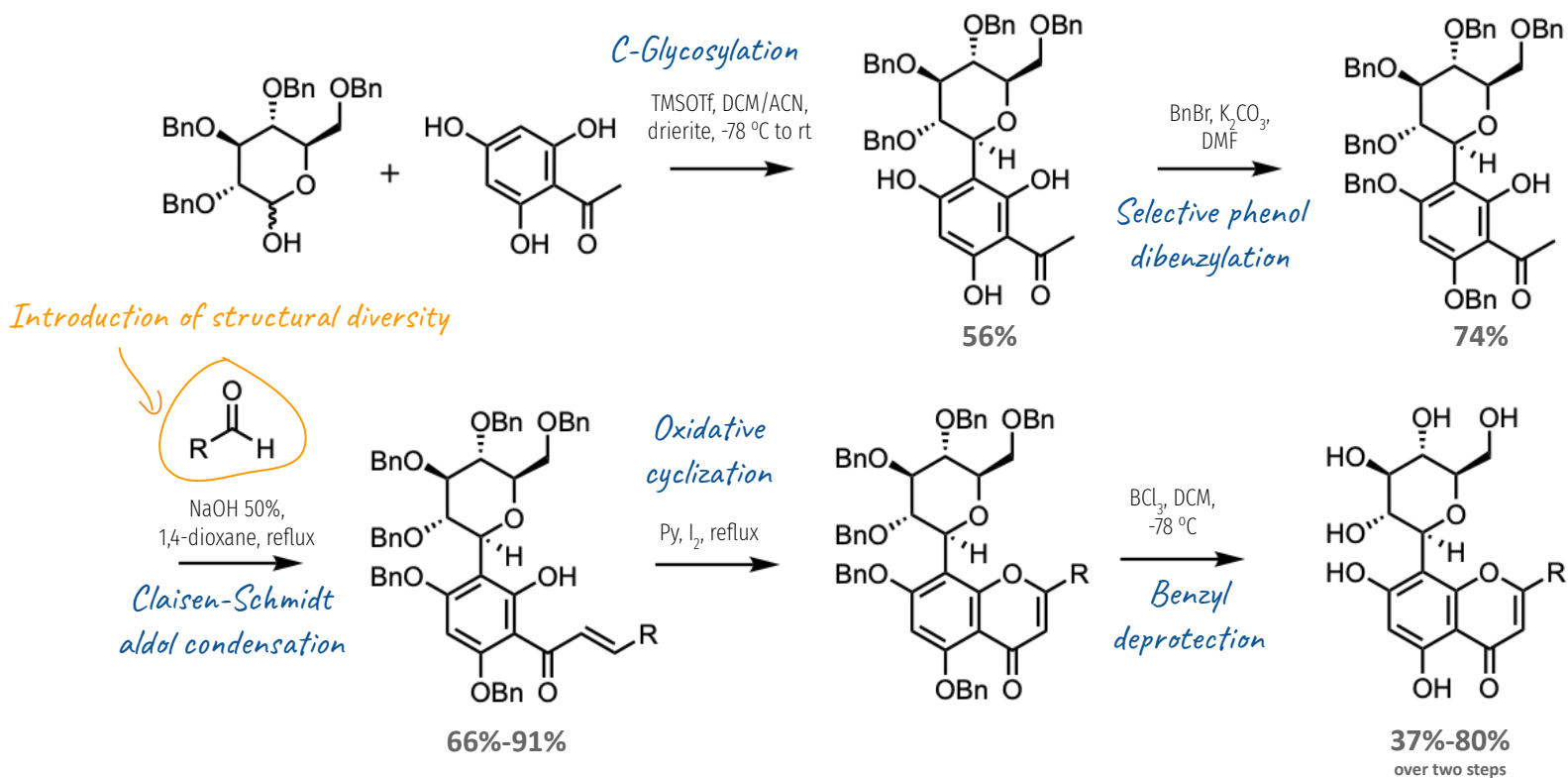
[1] Matos AM, et al. *Pure Appl Chem.* 2017;89(9):1305-1320. [2] Jesus AR, et al. *J Med Chem.* 201457(22):9463-9472.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## C-GLYCOSIDE Synthesis | General Procedure



Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

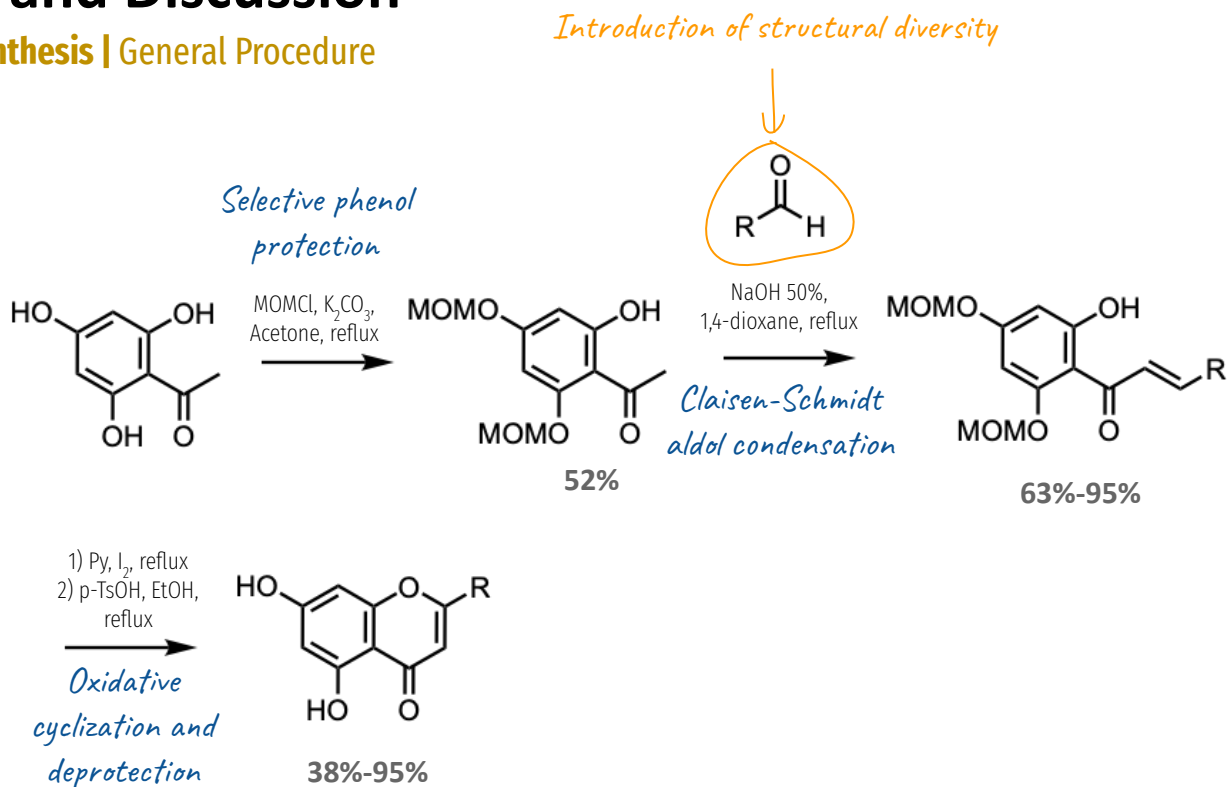
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE



# Results and Discussion

## AGLYCONE Synthesis | General Procedure



Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

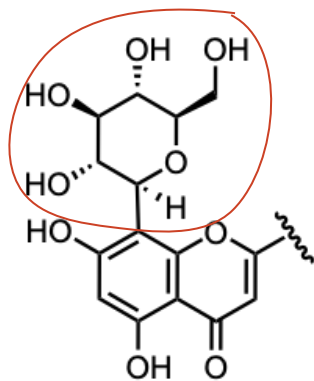
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

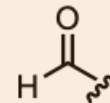
# Results and Discussion

## COMPOUND SELECTION LIBRARY

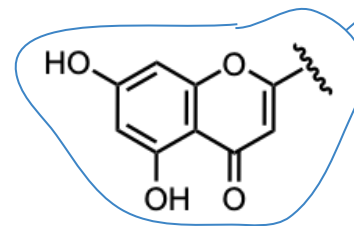
*CNS-MPO algorithm does not take into account the natural protein-binding profile of sugars*  
*e.g.: the sugar may work as a compound shuttle through GLUT transporters, despite extreme overall compound polarity*



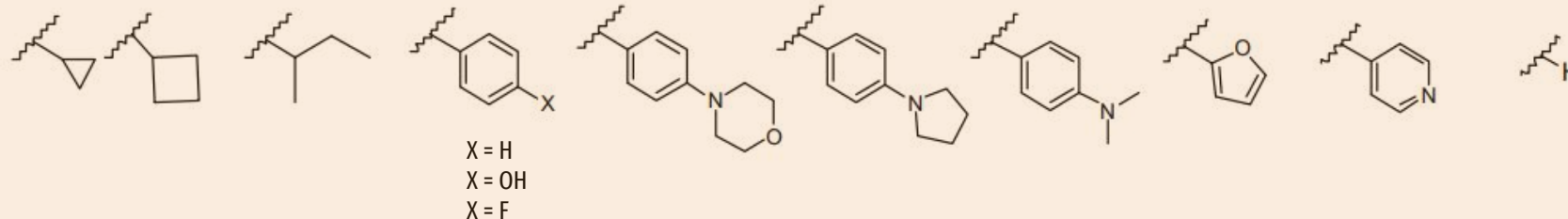
*Commercially available aldehydes*



selected based on the **CNS-MPO score\*** of aglycones



*cLogP*  
*cLogD*  
*PKA*  
*MW*  
*TPSA*  
*HBD*



\*From 1-6.

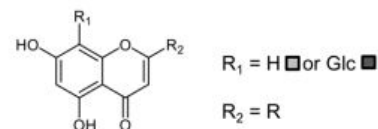
Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

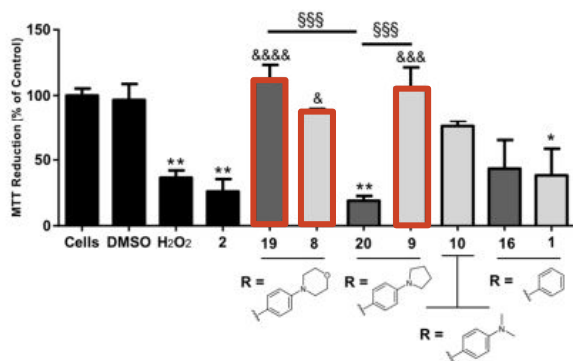
# Results and Discussion

## BIOLOGICAL TESTING

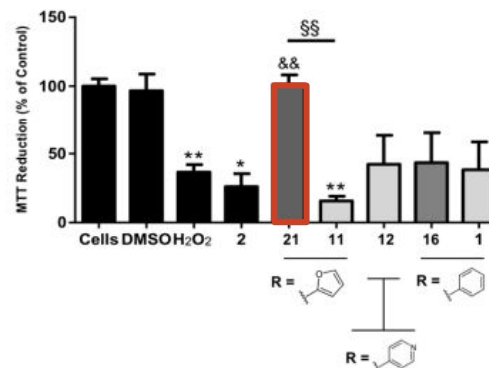


SH-SY5Y human neuroblastoma cells  
at 50  $\mu\text{M}$

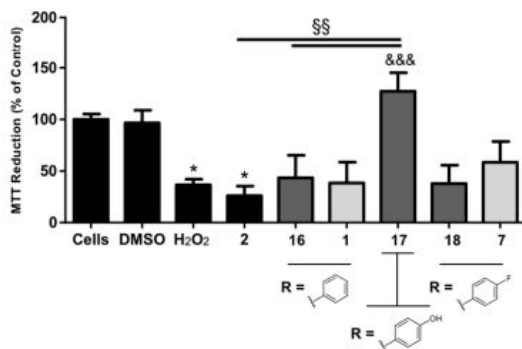
Addition of  
amine moieties in  
para-position of  
ring B



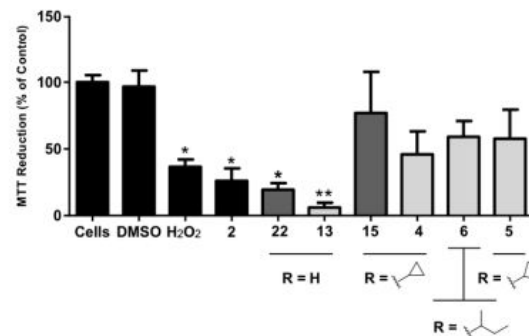
Replacement of  
ring B with  
heteroaromatic  
groups



Addition of  
EW groups in  
para-position of  
ring B



Replacement of  
ring B with  
aliphatic  
moieties



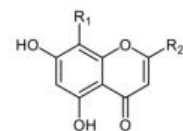
Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## BIOLOGICAL TESTING

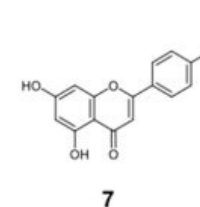
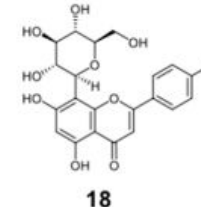
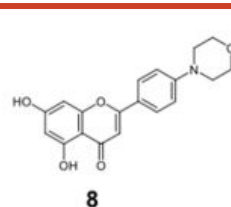
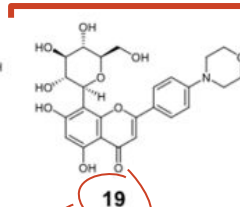
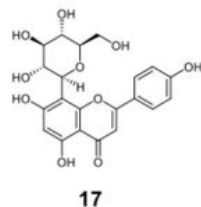
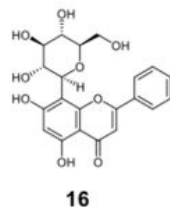
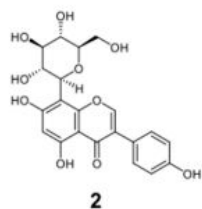
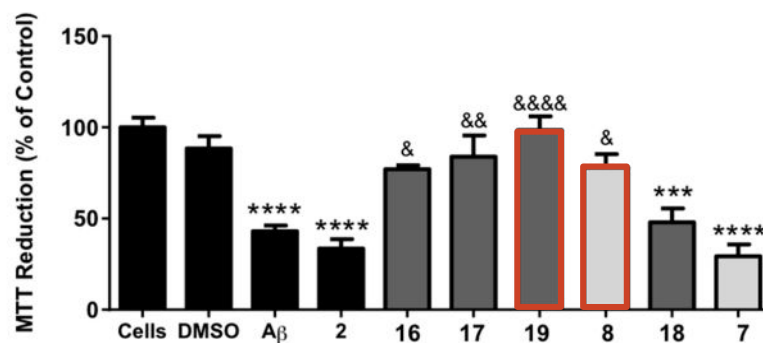


$R_1 = H$  or Glc

$R_2 = R$



SH-SY5Y human neuroblastoma cells  
at 50  $\mu M$



*Not toxic in HepG2 and Caco-2 cells at 100  $\mu M$*

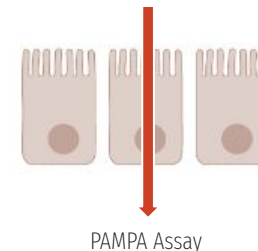
Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## BIOLOGICAL TESTING



Compound Nr.	Log P <sub>e</sub> <sup>a</sup>	Log D <sub>7.4</sub> <sup>b</sup>
1	-4.65 ± 0.09	3.6 ± 0.4
4	-4.66 ± 0.09	2.9 ± 0.1
5	-4.51 ± 0.06	>2.5
6	-4.48 ± 0.04	>2.5
7	-4.37 ± 0.12	>2.5
8	-4.56 ± 0.04	>2.5
9	-5.31 ± 0.12	n.d. <sup>c</sup>
10	-4.70 ± 0.14	3.4 ± 0.2
11	-4.93 ± 0.20	>2.5
12	-4.64 ± 0.02	n.d. <sup>c</sup>
13	-4.76 ± 0.02	2.4 ± 0.1
15	Below detection limit	-0.6 ± 0.2
16	-8.94 ± 1.83	0.8 ± 0.3
17	-8.70 ± 1.50	0.1 ± 0.1
18	Below detection limit	-0.2 ± 0.1
19	-7.08 ± 0.91	1.2 ± 0.1
20	-6.52 ± 0.41	1.8 ± 0.2
21	-6.94 ± 0.50	-0.2 ± 0.1
22	-6.76 ± 0.11	-2.0 ± 0.2
Testosterone	-4.42 ± 0.09	-

*Excellent membrane permeability, but extreme lipophilicity*

*Bioactive compound with a good balance between solubility, lipophilicity and membrane permeability*



**A new compound with neuroprotective effects, low cytotoxicity and potential for blood-brain barrier permeability**

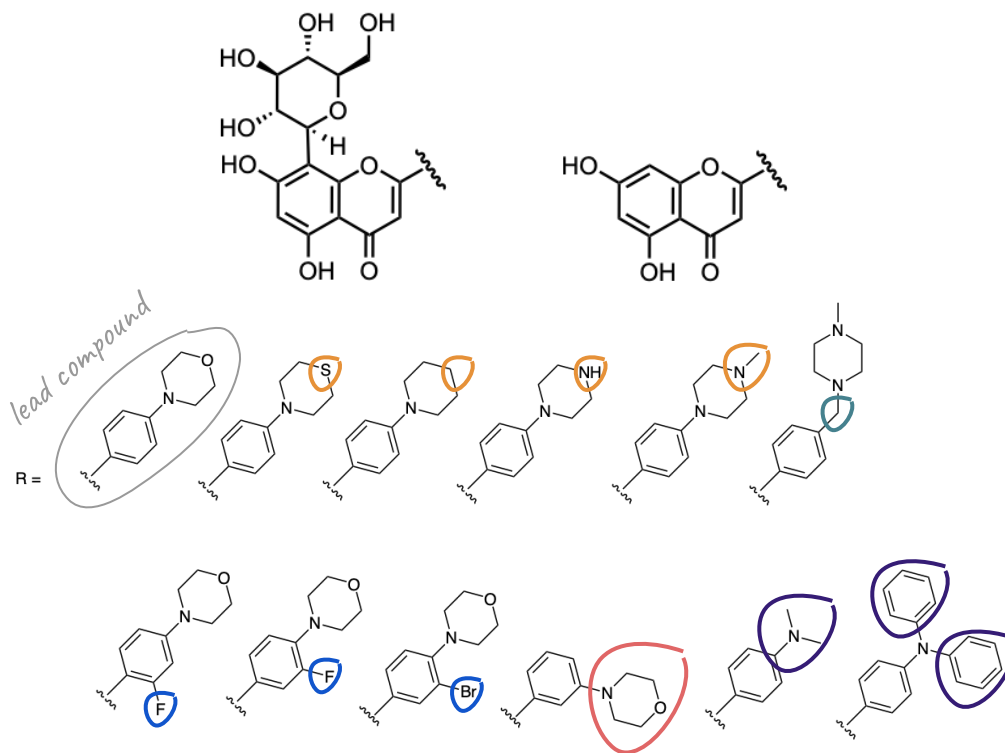
Matos AM, et al. *Pharmaceuticals*. 2019;12(2):98.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## IN-DEPTH STRUCTURAL DIVERSIFICATION



Bioisosteric replacement of the oxygen atom

Add  $\text{CH}_2$  bridge between ring B and D to improve water solubility

Add halogens to ring B

Move ring D to meta position

Replace ring D altogether

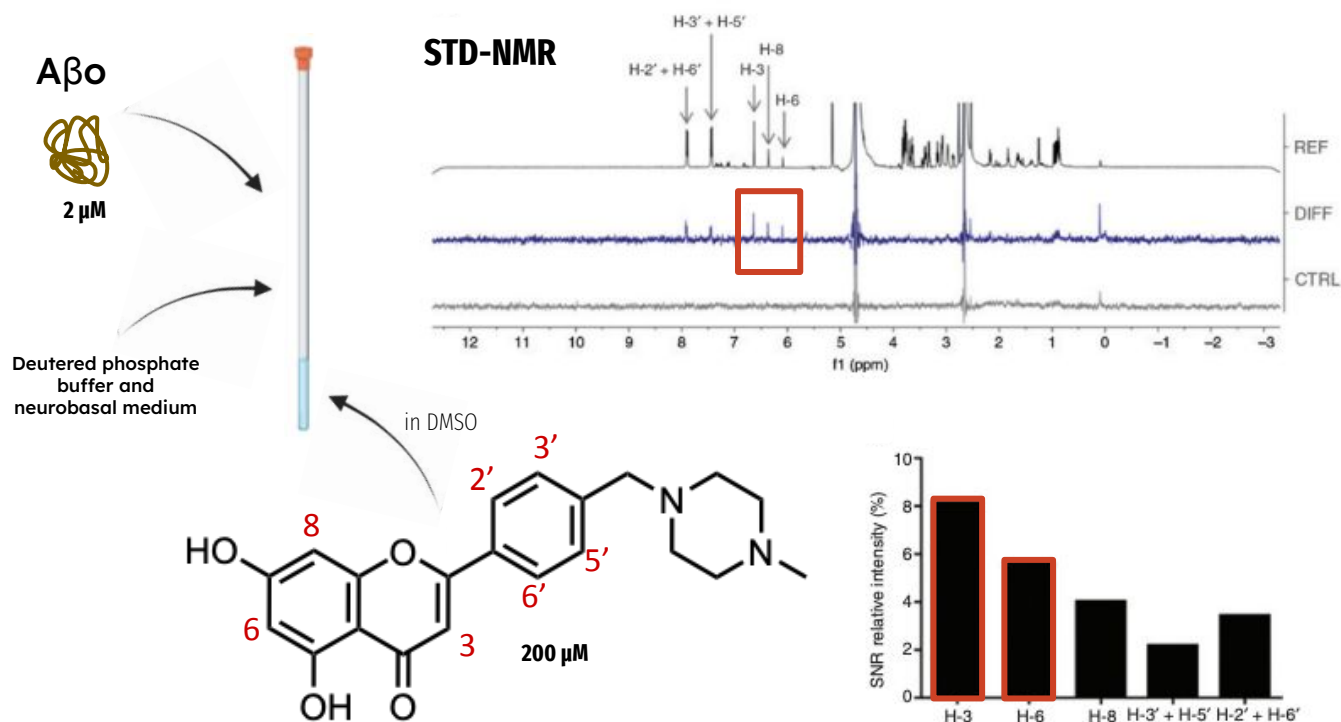
Matos AM, et al. *Pure Appl Chem.* 2019; 91(7): 1107-1136.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

**BIOLOGICAL TESTING** | The most significant screening result



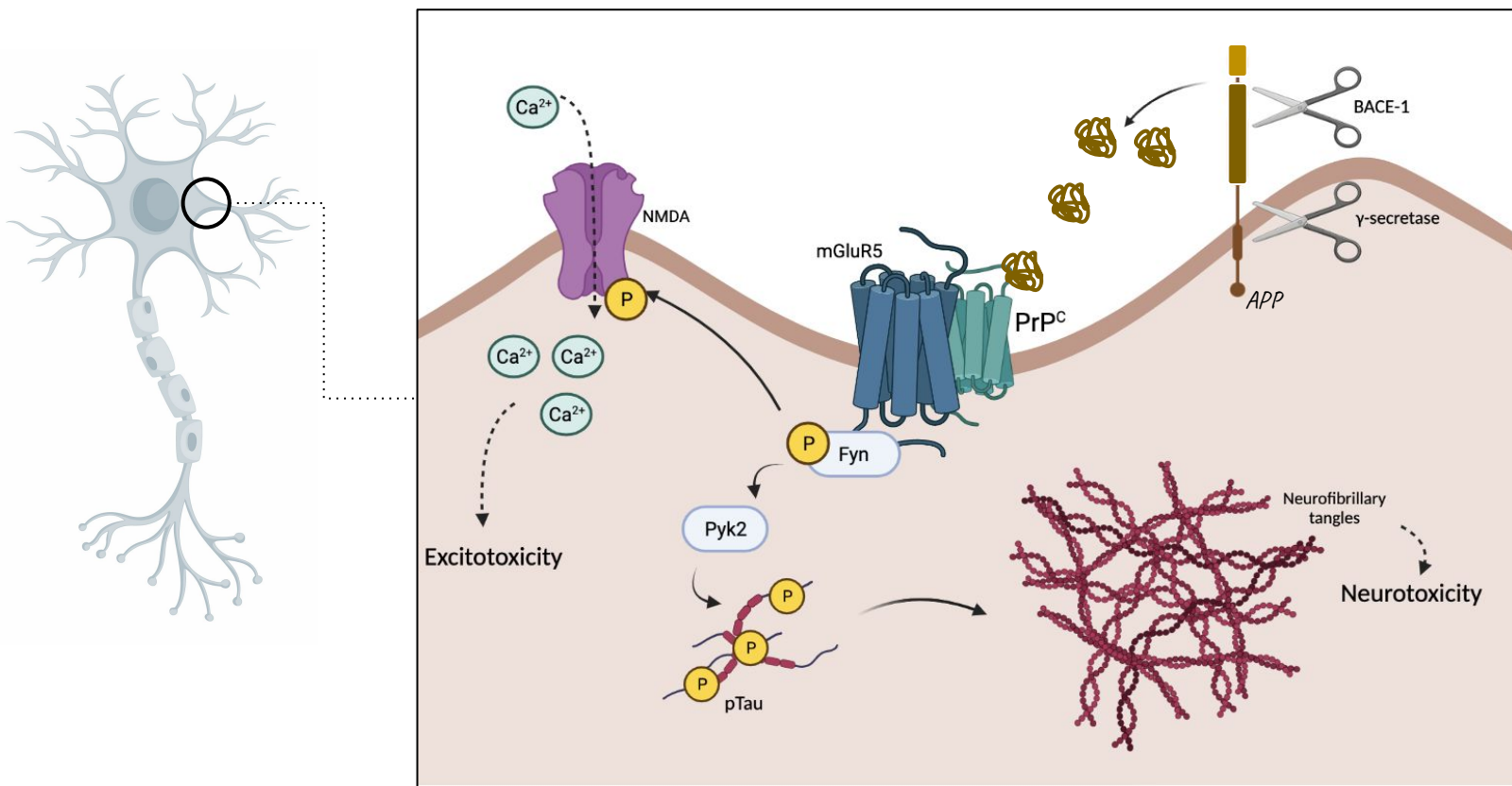
Matos AM, et al. *Pure Appl Chem.* 2019; 91(7): 1107-1136.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

Remember the pathophysiological cascade...



Build using BioRender and adapted from: Zhang Y, et al. *Front Cell Neurosci.* 2019;13:339 and van Nygaard CH, et al. *Alzheimers Res Ther.* 2014;6(1):8.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

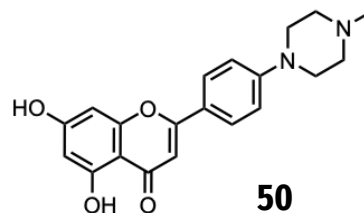
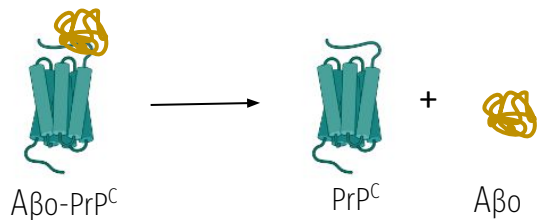




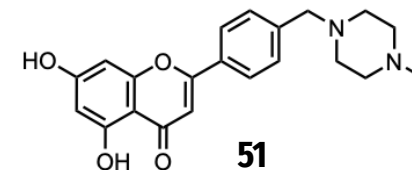
HEK cells  
at 10  $\mu\text{M}$

# Results and Discussion

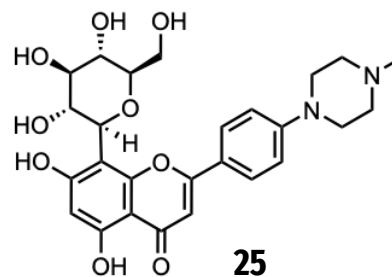
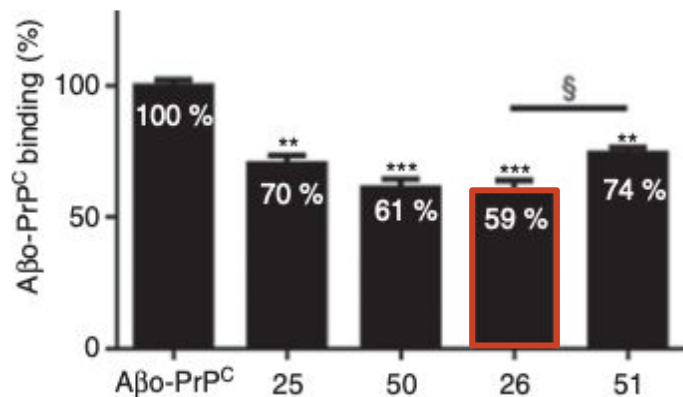
## BIOLOGICAL TESTING



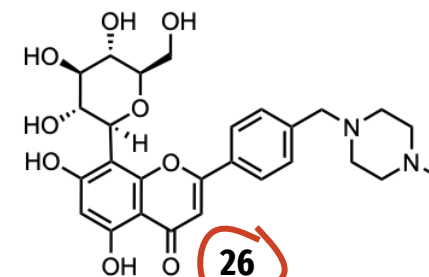
56%  $\pm$  3% cell viability at 50  $\mu\text{M}$



36%  $\pm$  6% cell viability at 50  $\mu\text{M}$



91%  $\pm$  4% cell viability at 50  $\mu\text{M}$



79%  $\pm$  1% cell viability at 50  $\mu\text{M}$

***N*-methyl piperazinyl flavones C-glucosides: The first sugar-based PPIIs against Aβo-PrP<sup>c</sup>**

Matos AM, et al. *Pure Appl Chem*. 2019; 91(7): 1107-1136.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

**Are these compounds safe bets for drug discovery?**

**DO C-GLUCOSYL POLYPHENOLS BEHAVE AS  
MEMBRANE-MODIFYING PAINS?**

**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**

**01-30 NOVEMBER 2022 | ONLINE**

# Results and Discussion

## DO C-GLUCOSYL POLYPHENOLS BEHAVE AS MEMBRANE-MODIFYING PAINS?



## Chemical con artists foil drug discovery

Naivety about promiscuous, assay-duping molecules is polluting the literature and wasting resources, warn **Jonathan Baell** and **Michael A. Walters**.

Baell J., Walters M. *Nature*, 2014, 513: 481-483.

## PAINS

### Pan-Assay Interference Compounds

Molecules capable of interfering with high-throughput screening assays, often times leading to "false hits".

They tend to react or interfere with numerous biological targets rather than specifically affecting one desired target.

ECMC  
2022

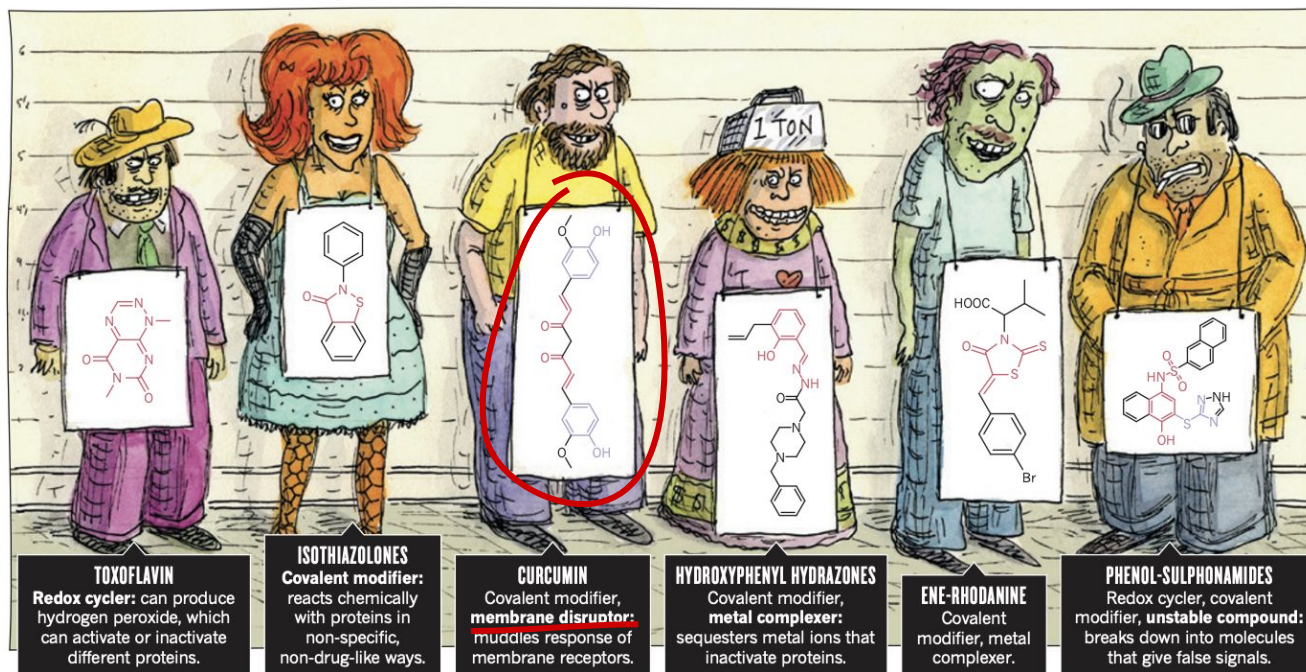
The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## DO C-GLUCOSYL POLYPHENOLS BEHAVE AS MEMBRANE-MODIFYING PAINS?

### WORST OFFENDERS

Membrane disruptors: Polyhydroxylated natural phytochemicals, e.g., curcumin, resveratrol and genistein



Baell J., Walters M. *Nature*, 2014, 513: 481-483.

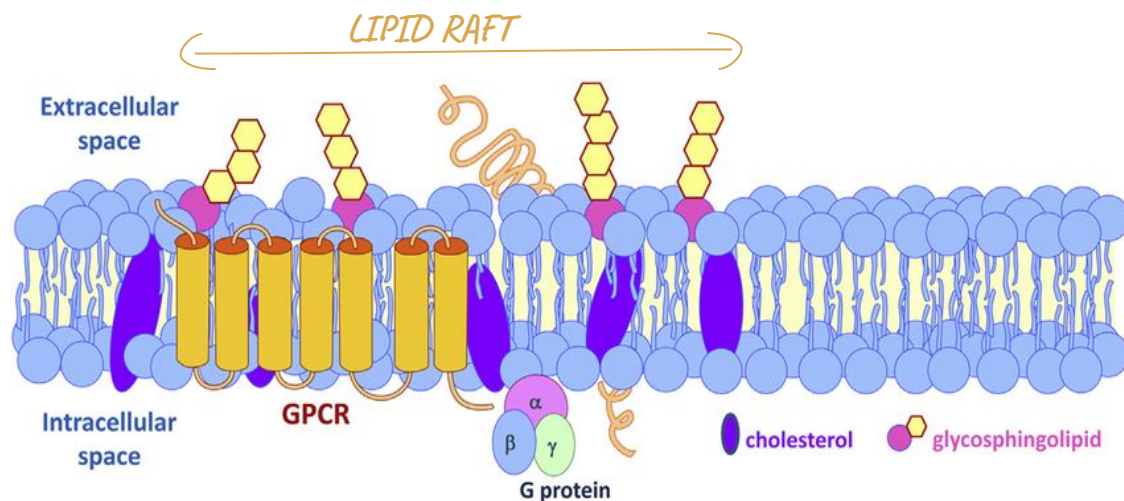
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## HOW TO GENISTEIN AND RESVERATROL INTERFERE WITH THE CELL MEMBRANE?

Plays a crucial role in the regulation of membrane protein activity, protein and lipid trafficking, and, ultimately, signal transduction



Affected by the membrane dipole potential

---

Alteration of cell membrane properties



Alteration of conformation and function of transmembrane proteins



Non-specific interference with intracellular signalling pathways



Drug-like profile

---

Villar CAM, et al. *Methods Cell Biol.* 2016; 132:3-23. Inglóffsson HI, et al. *ACS Chem. Biol.* 2014; 9(8):1788-1798.

ECMC  
2022

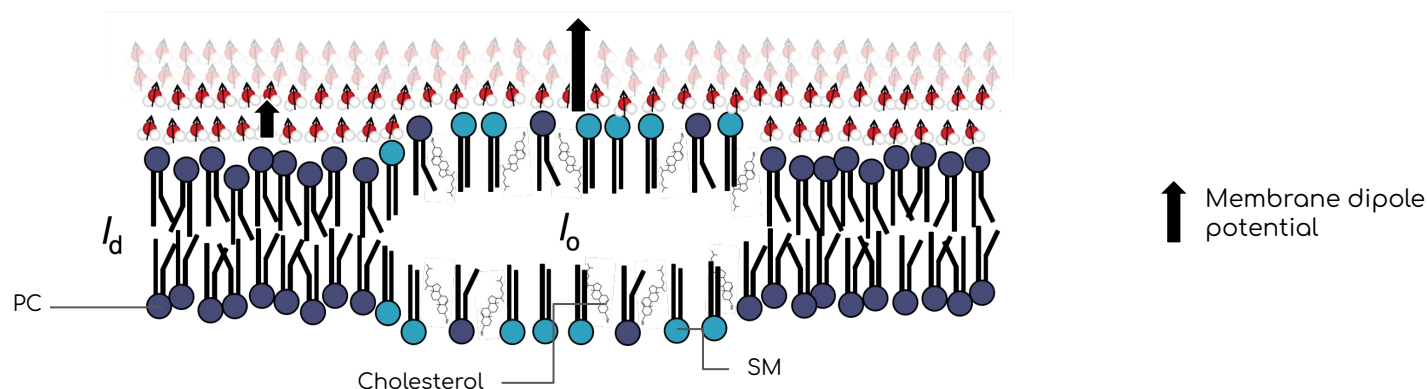
The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## WHAT IS THE MEMBRANE DIPOLE POTENTIAL?

- Part of the electric profile of cell membranes
- Key in ion transport, lipid-protein interactions, regulation of protein conformation and function, among other processes

### Results from the relative orientation between the electric dipoles of lipid headgroups and membrane-adsorbed water molecules.



Peterson U et al. *Chem. Phys. Lipids*. 2002;117(1-2):19-27.

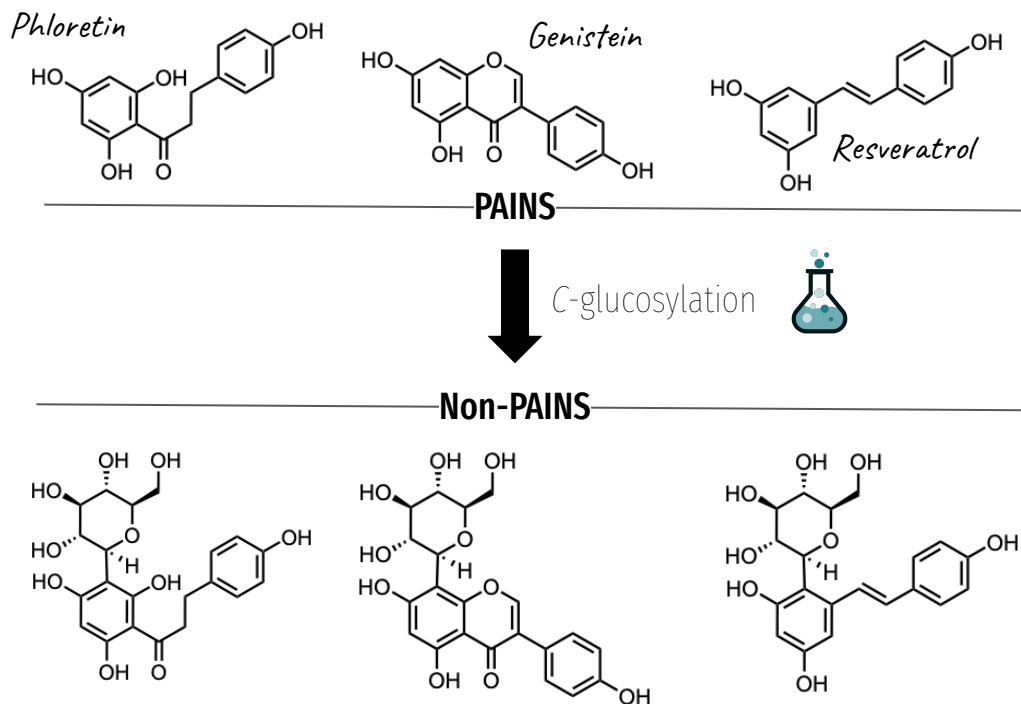
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## DO C-GLUCOSYL POLYPHENOLS BEHAVE AS MEMBRANE-MODIFYING PAINS?

Lipophilic polyphenols acting as membrane modifiers:



Matos AM, et al. *Sci Rep.* 2021; 11:4443.

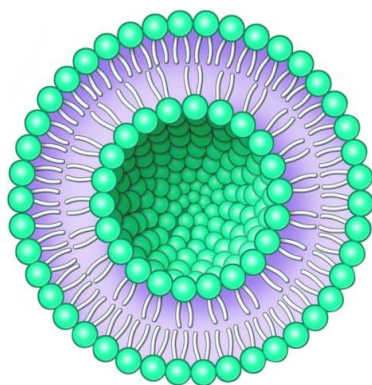
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

## DO C-GLUCOSYL POLYPHENOLS BEHAVE AS MEMBRANE-MODIFYING PAINS?

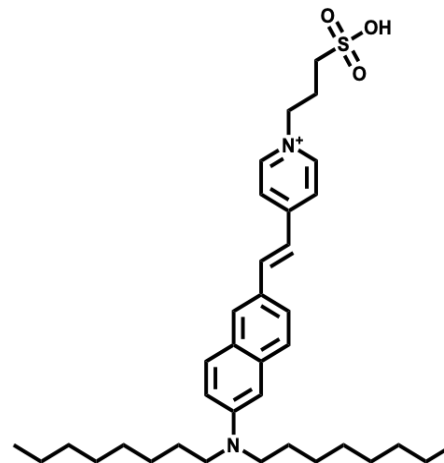
Our artificial membrane model



*LUVs*

Large unilamellar vesicles

Our potentiometric probe



*di-8-ANEPPS*

Established fluorescent probe for membrane dipole potential measurements

Matos AM, et al. *Sci Rep.* 2021; 11:4443.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE



# Results and Discussion

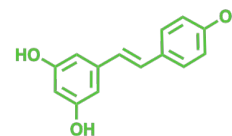
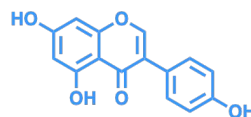
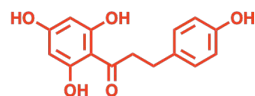
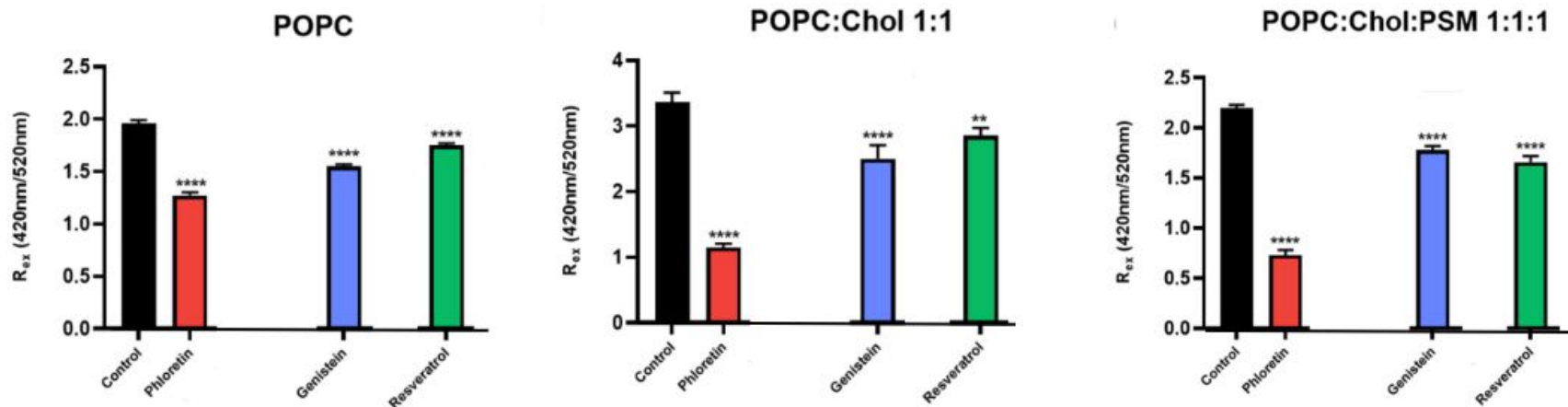
## EFFECTS OF PAIR LIPOPHILIC POLYPHENOLS ON MEMBRANE DIPOLE POTENTIAL

Determined by di-8-ANEPPS fluorescence ratiometric measurements,  $R_{ex}$ , between the intensity of the excitation spectra at 420 and 520 nm

*Liquid disordered phase (ld)*

*Liquid ordered phase (lo; lipid rafts)*

*ld + lo (ld + lipid rafts)*



Matos AM, et al. *Sci Rep.* 2021; 11:4443.

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Results and Discussion

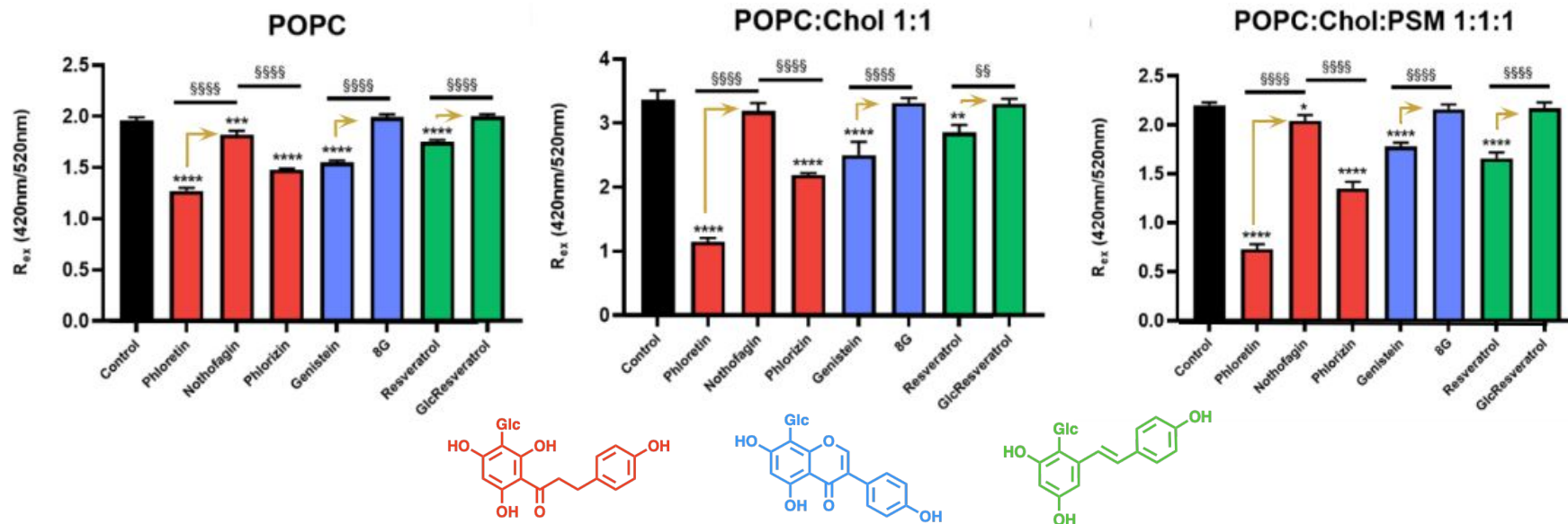
## EFFECTS OF PALE LIPOPHILIC POLYPHENOLS ON MEMBRANE DIPOLE POTENTIAL

Determined by di-8-ANEPPS fluorescence ratiometric measurements,  $R_{ex}$ , between the intensity of the excitation spectra at 420 and 520 nm

*Liquid disordered phase (ld)*

*Liquid ordered phase (lo; lipid rafts)*

*ld + lo (ld + lipid rafts)*



**C-glycosylation prevents reductions in the membrane dipole potential caused by lipophilic polyphenols**

Matos AM, et al. *Sci Rep.* 2021; 11:4443.

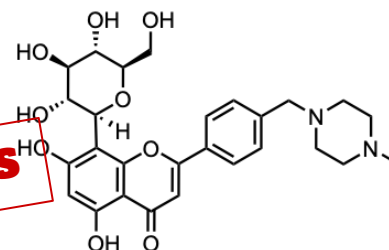
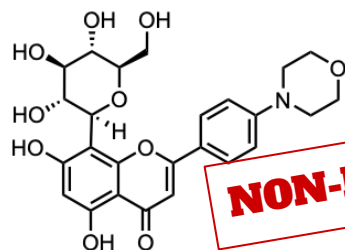
ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Conclusions

**C-glycosyl flavones** are promising lead molecules for the development of **disease-modifying treatments against AD:**

Neuroprotective activity against  $H_2O_2$ - and  $A\beta$ -induced cell death



Fist sugar-based PPII against  $PrP^C$ - $A\beta$

- Good characteristics for BBB passage into the CNS: improved balance between effective permeability, lipophilicity and water solubility vs. aglycone
- C-glycosyl moiety minimizes cytotoxic effects caused by the aglycone

**C-glycosylation** prevents reductions in membrane dipole potential caused by lipophilic polyphenols with PAINS-type membrane disrupting behaviour

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE

# Acknowledgments



Prof. Dr. Amélia Pilar Rauter  
Prof. Dr. Rodrigo Almeida  
Prof. Dr. Ana Viana  
Dr. Carla Sousa  
Dr. M. Teresa Blázquez-Sánchez  
Dr. Alice Martins  
Prof. Dr. Maria Eduarda Araújo  
Prof. Dr. Cláudio Gomes  
Dr. Joana Cristóvão  
Rafael S. Nunes  
João M. Barros



Prof. Dr. Maria Paula Macedo  
Dr. Inês Lima  
Dr. Fátima Martins  
Dr. Maria João Menezes  
Dr. Ana Farinho



Imane El Idrissi  
Nicola Colabufo



Dr. Magnus Walter  
Dr. Teresa Man  
Dr. David Evans  
Dr. Gary Sharman  
Dr. Peter Lindsay-Scott  
Dr. Andrew Williams



Prof. Dr. Beat Ernst  
Dr. Philipp Dätwyler



Prof. Dr. Óscar López



# FCT

Fundação para a Ciência e a Tecnologia  
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

SFRH/BD/93170/2013

CEECInst-LA 2021  
2022.07037.CEECIND  
UIDB/00100/2020  
UIDP/00100/2020  
LA/P/0056/2020



Dr. M. Conceição Oliveira



Dr. Beining Chen  
Dr. Cleide Souza  
James Grayson

ECMC  
2022

The 8th International Electronic  
Conference on Medicinal Chemistry  
01-30 NOVEMBER 2022 | ONLINE



<https://cqe.tecnico.ulisboa.pt/>

**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE

Thank you for your kind attention.

I am happy to answer your questions at:

**[amamatos@fc.ul.pt](mailto:amamatos@fc.ul.pt)**

**ECMC  
2022**

**The 8th International Electronic  
Conference on Medicinal Chemistry**  
01-30 NOVEMBER 2022 | ONLINE