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Sugar-linked polyphenols as inhibitors of Aβ-induced Fyn kinase activation and Tau phosphorylation in neural cells

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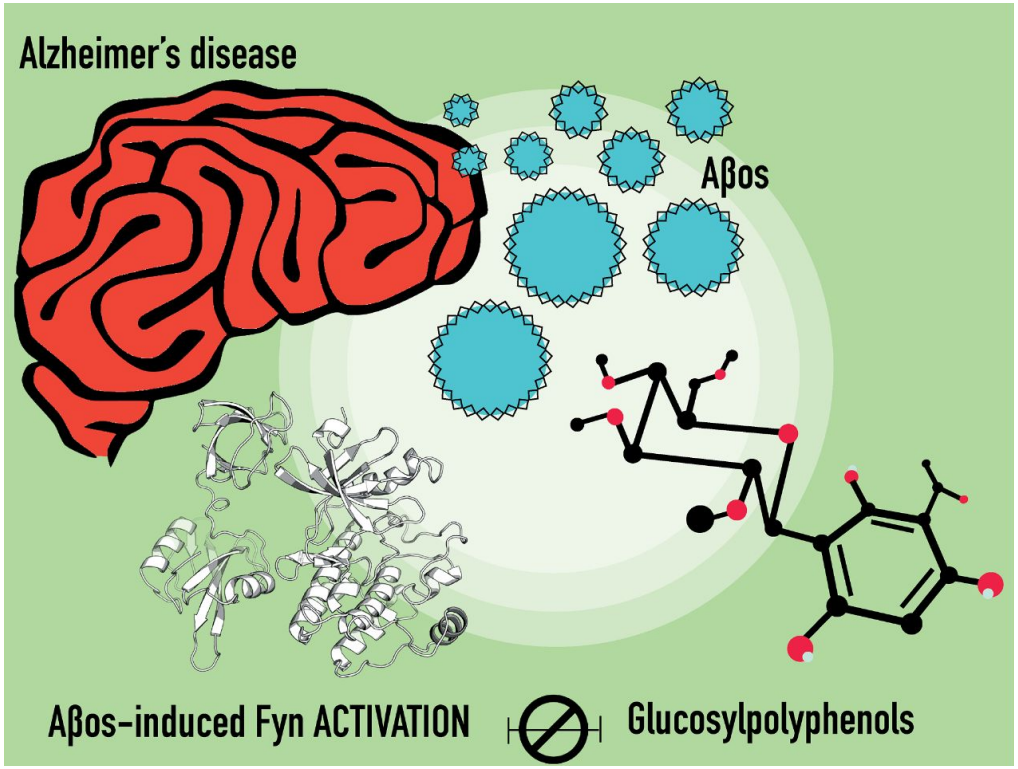
Sugar-linked polyphenols as inhibitors of Aβ-induced Fyn kinase activation and Tau phosphorylation in neural cells



Abstract: Alzheimer's disease (AD) is characterized by the presence of extracellular deposits of amyloid-beta (Aβ) in the brain, together with intracellular neurofibrillary tangles containing hyperphosphorylated Tau, which eventually lead to synaptic dysfunction and neuronal death. Importantly, the cellular prion protein (PrP^C), located at the neuronal cell surface, works as a high-affinity binding partner of Aβ oligomers, and the interaction between these two players results in Fyn kinase activation with subsequent Tau hyperphosphorylation. Therefore, the inhibition of Aβ-induced Fyn activation mediated by PrP^C is nowadays regarded as a promising strategy for the treatment of AD.
Inspired by 8-β-D-glucosylgenistein - a natural compound that has been shown to interact with the $Aβ_{1-42}$ peptide - this communication will focus on the synthesis and biological evaluation of a small library of sugar-linked polyphenols with neuroprotective potential. These *C*-glucosides were able to significantly inhibit PrP^C-dependent Aβ-induced Fyn activation and subsequent Tau phosphorylation at 10 μM in hiPSC-derived neural cells - a result that was not achieved by the natural lead molecule. The most promising *C*-glucosides were not neurotoxic in concentrations up to 100 μM and displayed favorable physicochemical characteristics that anticipate their ability to act in the central nervous system. Ultimately, in this work we show, for the first time, that *C*-glucosyl polyphenols are able to tackle Aβ-induced Fyn kinase activation with enough efficacy to reduce Tau phosphorylation, thus having the potential to be considered for further development against AD.

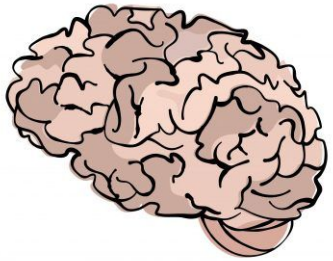
Keywords: Carbohydrate Chemistry; Polyphenols; Amyloid-beta; Fyn kinase; Alzheimer's disease.

Introduction

AD is the most common type of dementia

50 M

Current number of AD cases globally



10 M

Number of new cases every year

WHO: https://www.who.int/news-room/fact-sheets/detail/dementia.

Introduction

Life domains impacted by AD

Physical

Psychological

Social

Economic

Not only patients are impacted by AD, but also caregivers and society as a whole

WHO: https://www.who.int/news-room/fact-sheets/detail/dementia.

Introduction

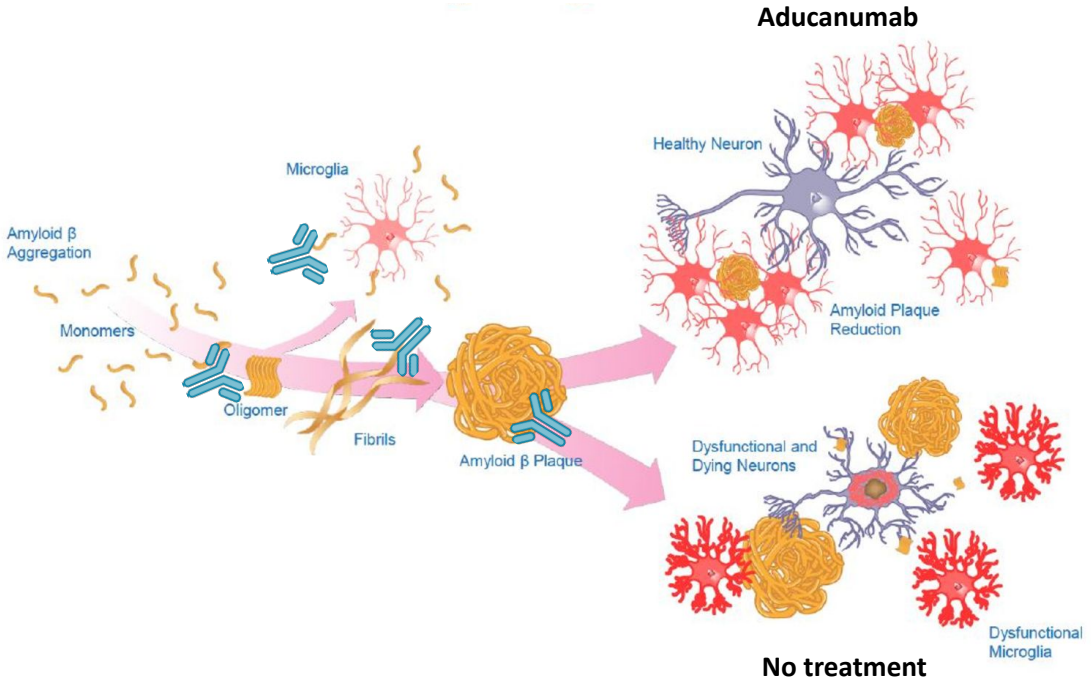
Only one disease-modifying therapy has been approved so far

Aducanumab

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

APPROVED

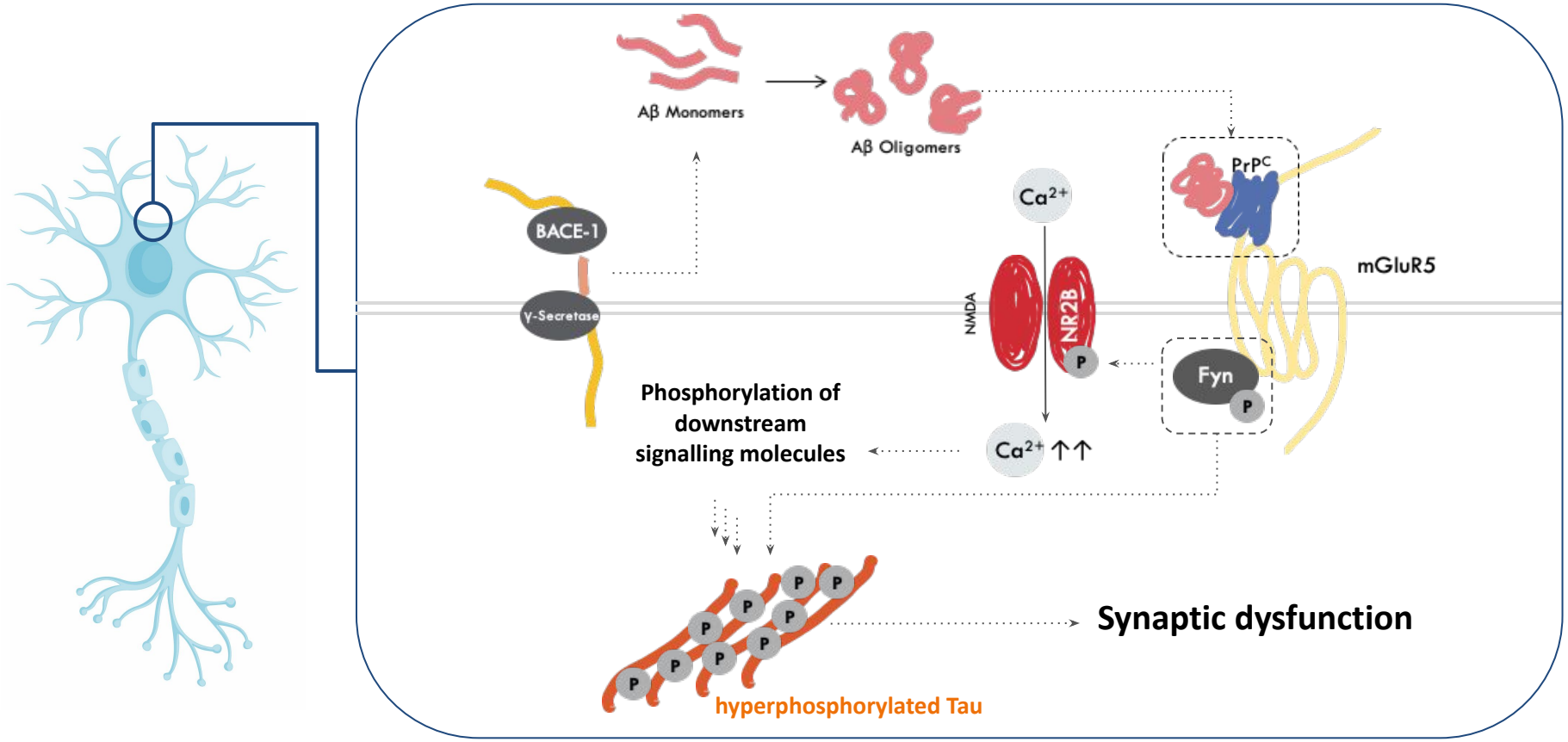
As of 7th June 2021



Adapted from: Esang M, Gupta M (August 31, 2021) Aducanumab as a Novel Treatment for Alzheimer's Disease: A Decade of Hope, Controversies, and the Future. Cureus 13(8): e17591. doi:10.7759/cureus.17591

Introduction

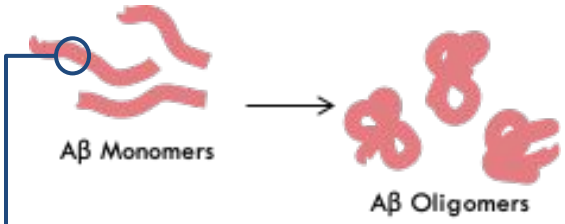
Therapeutic targets with potential to be explored



Nygaard HB, et al. *Alzheimers Res. Ther.* 2014 Feb 5;6(1):8.

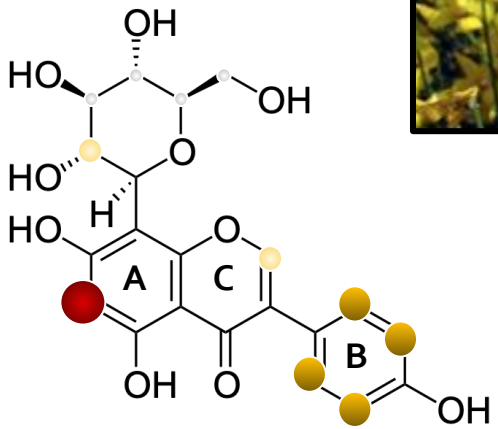
Introduction

Our previous findings



STD-NMR

Genista tenera



8-β-D-glucosylgenistein (8G)

100-80%

79-69%

59-40%

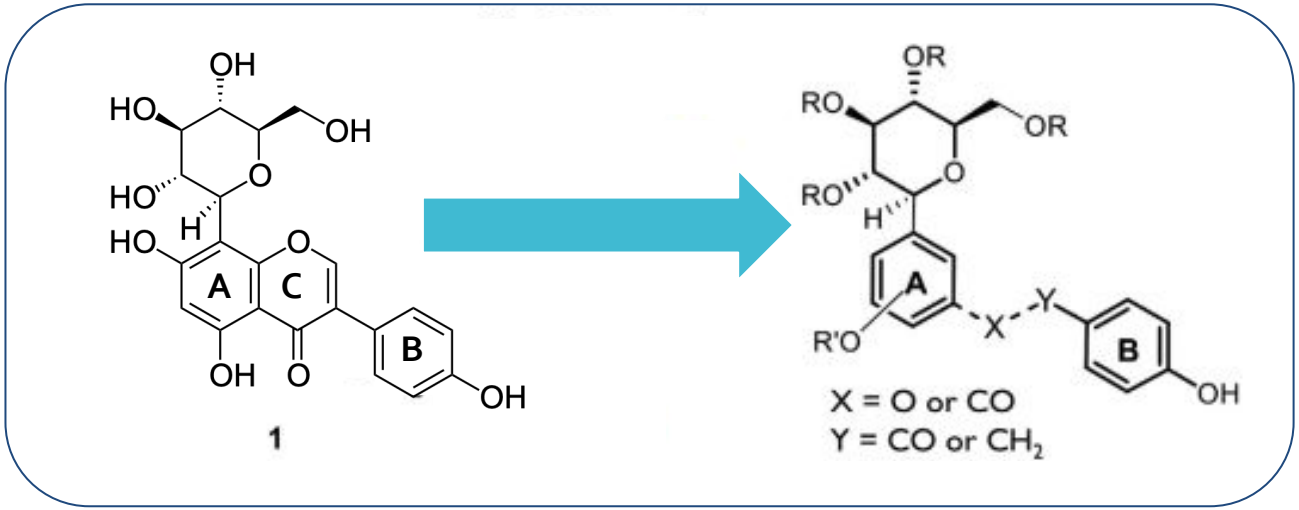
20-39%

Jesus AR, et al. *J. Med. Chem.* 2014 Nov 26;57(22):9463-72.

Introduction

Objectives of the present work

1. To mimic the natural compound | 2. To pursue structural simplification

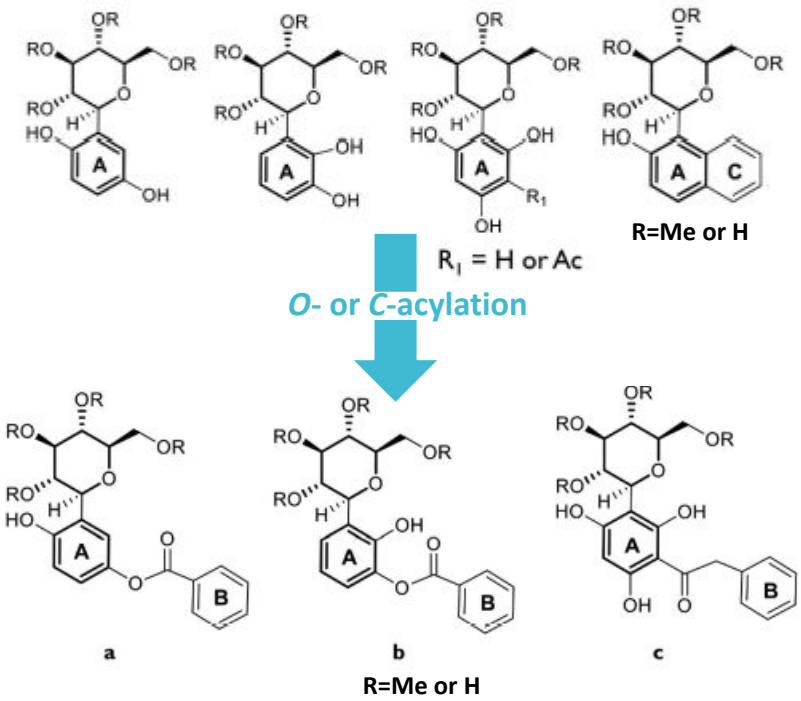


Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

Introduction

Strategy

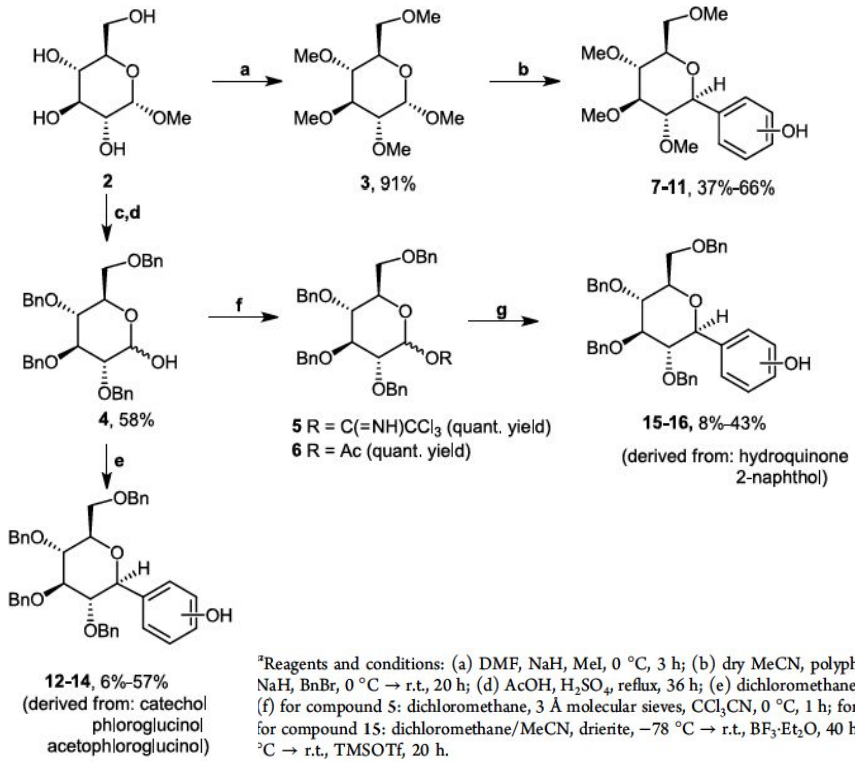
Simplified *C*-glucosyl polyphenols with different hydroxylation patterns



Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

Results and discussion

Synthesis of glycosyl donors



[a]Reagents and conditions: (a) DMF, NaH, MeI, 0 °C, 3 h; (b) dry MeCN, polyphenol, drierite, −78 °C → r.t., TMSOTf, 18–48 h; (c) DMF, NaH, BnBr, 0 °C → r.t., 20 h; (d) AcOH, H_2SO_4, reflux, 36 h; (e) dichloromethane/MeCN, drierite, −78 °C → r.t. or 40 °C, TMSOTf, 8–64 h; (f) for compound **5**: dichloromethane, 3 Å molecular sieves, CCl_3CN, 0 °C, 1 h; for compound **6**: pyridine, DMAP, 0 °C → r.t., Ac_2O, 2.5 h; (g) for compound **15**: dichloromethane/MeCN, drierite, −78 °C → r.t., $BF_3{\cdot}Et_2O$, 40 h; for compound **16**: dichloromethane, 3 Å molecular sieves, 0 °C → r.t., TMSOTf, 20 h.

Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

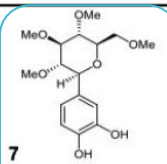
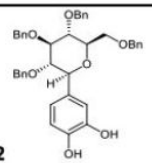
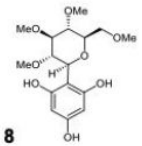
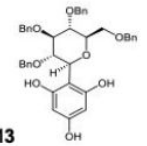
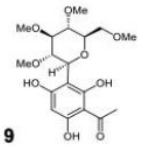
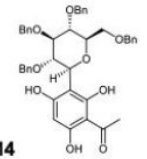
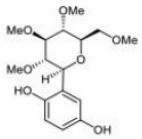
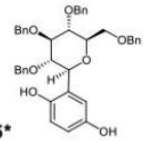
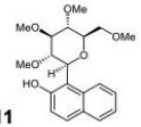
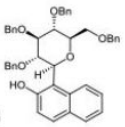
Results and discussion

C-Glucosylation reactions

The first per-*O*-methyl-β-glucosylated polyphenols accessed in good yields by using:

- **TMSOTf** as the promoter
- **Fully *O*-methylated methyl glucoside** as the glucosyl donor
- **Unprotected polyphenols** as glucosyl acceptors

No additional reaction steps required for the formation of "good leaving groups" typically used for the anomeric position.

Phenol	Glycosyl donor	Isolated Yield (%)	Glycosyl donor	Isolated Yield (%)
		Major products		
Catechol ***Ortho-** hydroxylation pattern*	 **7**	63	 **12**	6 (R = H)
Phloroglucinol ***Meta-** hydroxylation pattern*	 **8**	53	 **13**	42 (R = H)
Trihydroxyacetophenone ***Meta-** hydroxylation pattern*	 **9**	45	 **14**	57 (R = H)
	10			
Hydroquinone ***Para-** hydroxylation pattern*		37	 **15***	8 (R = Ac)
2-Naphthol *Monophenol*	 **11**	66	 **16**	43 (R = $CNHCCl_3$)

*Compound **15** was obtained using $BF_3.Et_2O$ as promoter.

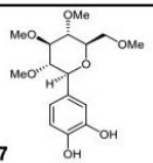
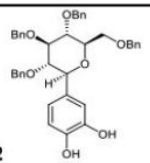
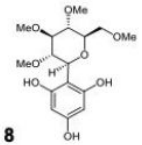
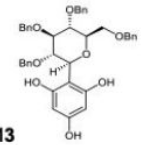
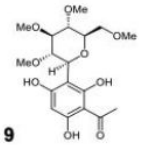
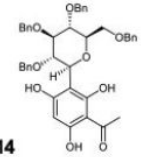
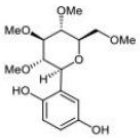
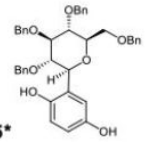
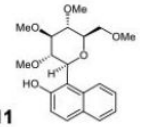
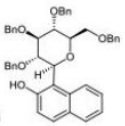
Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

Results and discussion

C-Glucosylation reactions

Similar yields obtained when using phloroglucinol or trihydroxyacetophenone, **regardless of the sugar protecting group**.

Electron-donating effects of the aglycone seem to prevail.

Phenol	Glycosyl donor	Isolated Yield (%)	Glycosyl donor	Isolated Yield (%)
		Major products		
Catechol ***Ortho-** hydroxylation pattern*	 7	63	 12	6 (R = H)
Phloroglucinol ***Meta-** hydroxylation pattern*	 8	53	 13	42 (R = H)
Trihydroxyacetophenone ***Meta-** hydroxylation pattern*	 9	45	 14	57 (R = H)
	10			
Hydroquinone ***Para-** hydroxylation pattern*		37	 15*	8 (R = Ac)
2-Naphthol *Monophenol*	 11	66	 16	43 (R = $CNHCCl_3$)

*Compound **15** was obtained using $BF_3.Et_2O$ as promoter.

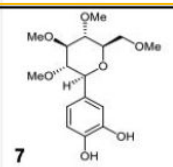
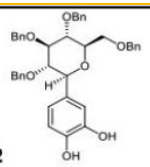
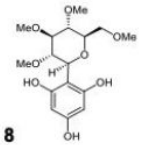
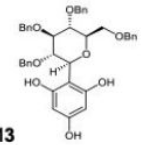
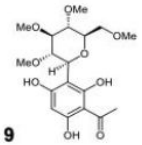
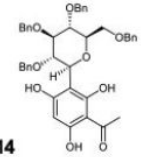
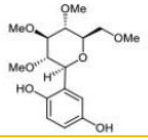
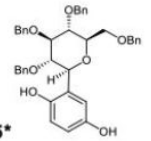
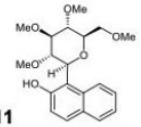
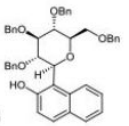
Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

Results and discussion

C-Glucosylation reactions

Glycosylation **yields were drastically lower** when using **benzyl** sugar protecting groups and either **catechol or hydroxyquinone**

No improvements when changing solvent proportion, promoter and/or polyphenol proportion, or temperature.

Phenol	Glycosyl donor	Isolated Yield (%)	Glycosyl donor	Isolated Yield (%)
Catechol *Ortho- hydroxylation pattern*	 **7**	63	 **12**	6 (R = H) Minor product
Phloroglucinol *Meta- hydroxylation pattern*	 **8**	53	 **13**	42 (R = H)
Trihydroxyacetophenone *Meta- hydroxylation pattern*	 **9**	45	 **14**	57 (R = H)
10 ***Hydroquinone*** *Para- hydroxylation pattern*		37	 **15***	8 (R = Ac) Minor product
2-Naphthol *Monophenol*	 **11**	66	 **16**	43 (R = $CNHCCl_3$)

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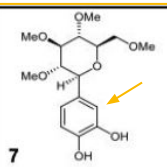
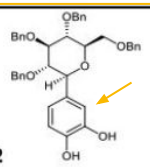
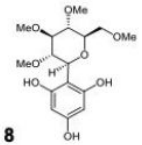
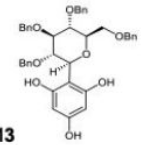
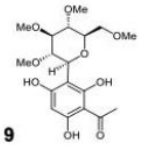
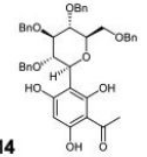
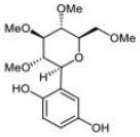
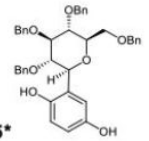
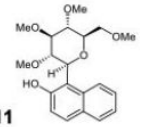
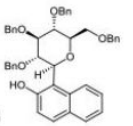
Matos AM, et al. *J. Med. Chem.* 2020, 63, 20, 11663–11690.

Results and discussion

C-Glucosylation reactions

Para-isomers are formed in the synthesis of catechol *C*-glucosides

Friedel–Crafts-type *C*-glucosylation is the favored reaction mechanism, **prevalent over the Fries-type rearrangement** described for unprotected phenols.

Phenol	Glycosyl donor	Isolated Yield (%)	Glycosyl donor	Isolated Yield (%)
Catechol *Ortho- hydroxylation pattern*	 7	63	 12	6 (R = H) Minor product
Phloroglucinol *Meta- hydroxylation pattern*	 8	53	 13	42 (R = H)
Trihydroxyacetophenone *Meta- hydroxylation pattern*	 9	45	 14	57 (R = H)
Hydroquinone *Para- hydroxylation pattern*	10 	37	 15*	8 (R = Ac)
2-Naphthol *Monophenol*	 11	66	 16	43 (R = $CNHCCl_3$)

*Compound **15** was obtained using $BF_3.Et_2O$ as promoter.

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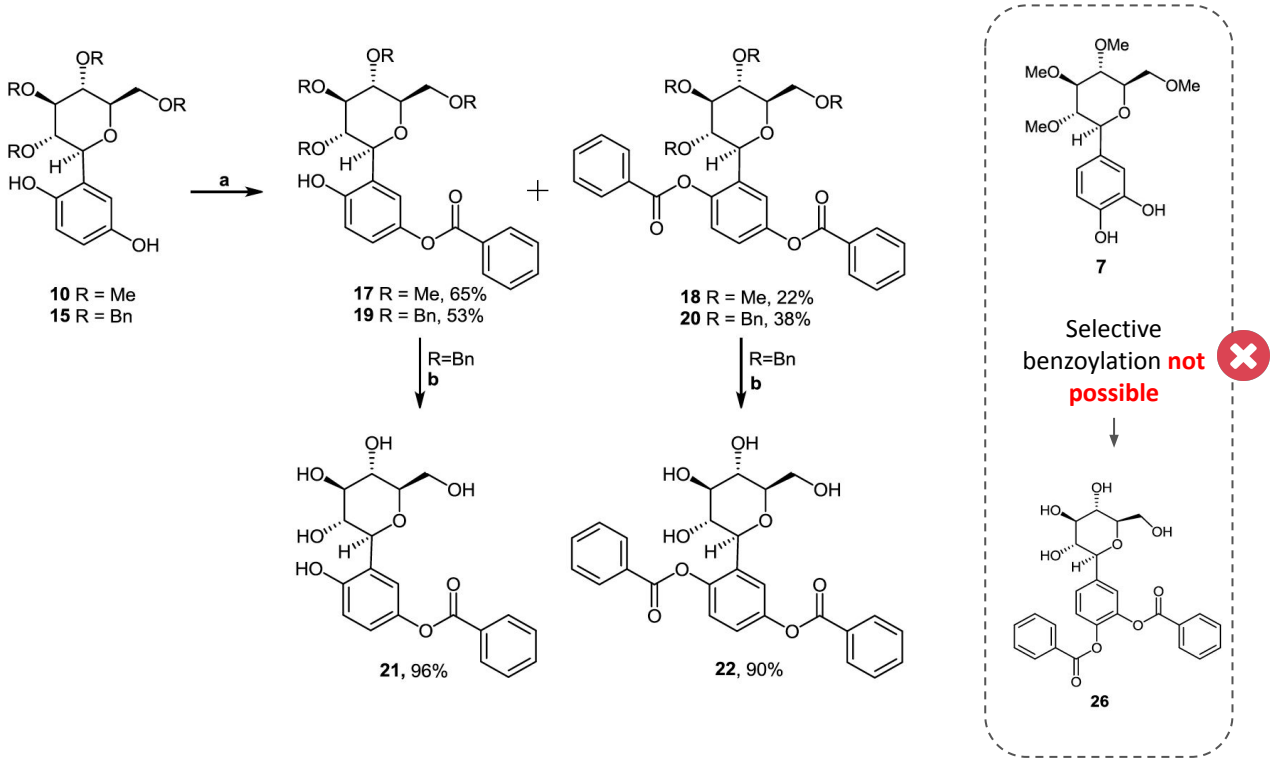
Results and discussion

O-Acylation reactions



[a]Reagents and conditions: (a) dichloromethane, imidazole, DMAP, BzCl, 0 °C → r.t., 60–120 h; (b) EtOAc, Pd/C, H_2, r.t., 16–22 h (R = Bn).

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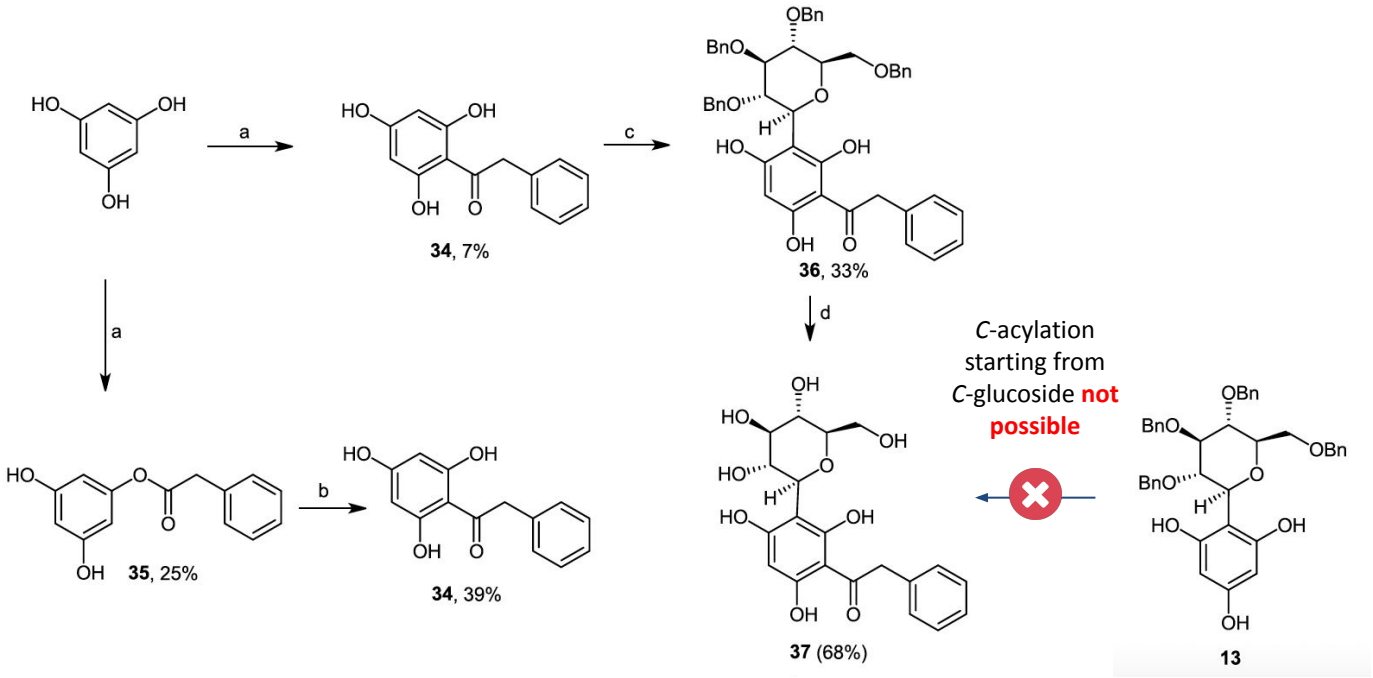
Results and discussion

C-Acylation reactions



[a]Reagents and conditions: (a) phenylacetyl chloride, 2% TfOH/MeCN, 0 °C → r.t., overnight; **34**, 7%; **35**, 25%; (b) TfOH, 100 °C, 2 h, 39%; (c) TMSOTf, dichloromethane/MeCN, compound **4**, drierite, −40 °C → r.t., overnight, 33%; (d) MeOH/EtOAc, Pd/C, H_2, r.t., 3 h, 68%.

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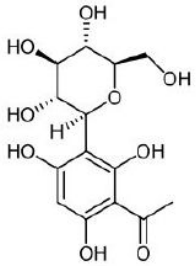
Results and discussion

Other compounds synthesized for comparison purposes



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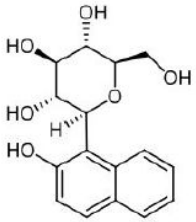

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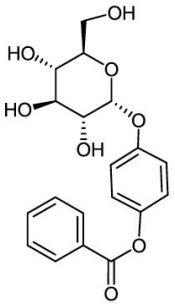

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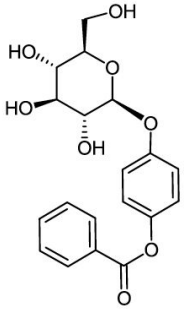

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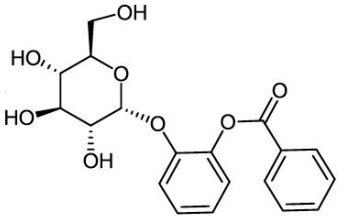

33

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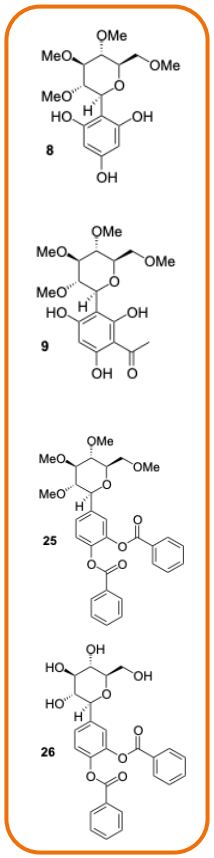
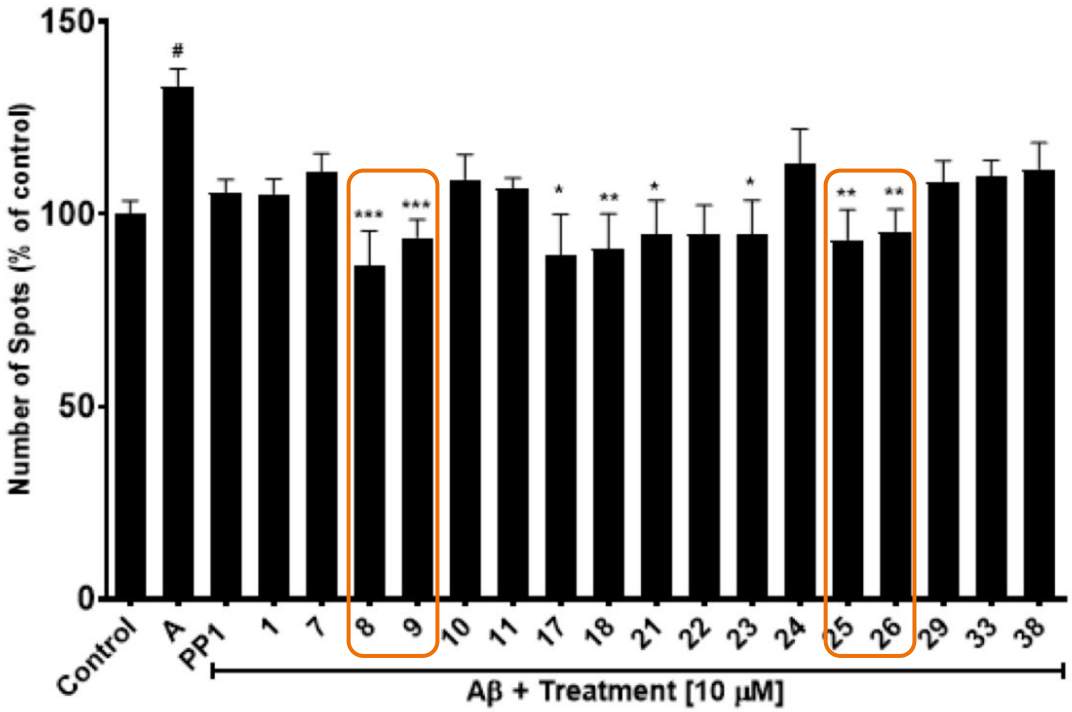
Results and discussion

Biological activity: Ability to inhibit Aβo-induced Fyn activation (pFyn)





Effect of glucosylphenols in Aβ-induced Fyn activation. The indirect activation of Fyn kinase was measured by immunofluorescence using Opera High Content Screening System. Cells were exposed to 10 μM of compounds in association with Aβ. Results were normalized against the control group, which was considered as 100%. Percentage of number of pFyn + spots in each treatment group. The morphological features assessed for both treated and control cells were the number of cells and intensity of Alexa 568 per cell. Results are expressed as the mean ± standard error mean (SEM); n = 3. Significant differences between control are indicated with #($p \leq 0.05$) and *($p < 0.05$) when compared to Aβ treatment *($p < 0.05$) or **($p < 0.01$) or ***($p < 0.001$).

Effects on the basal levels of pFyn were not found for any compound

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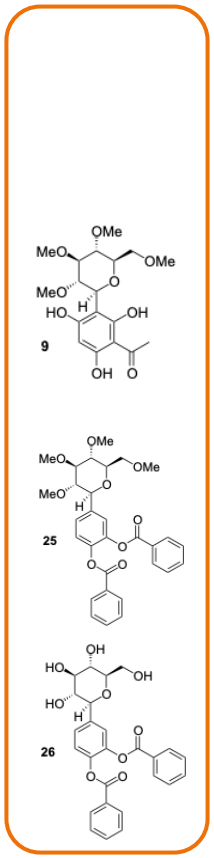
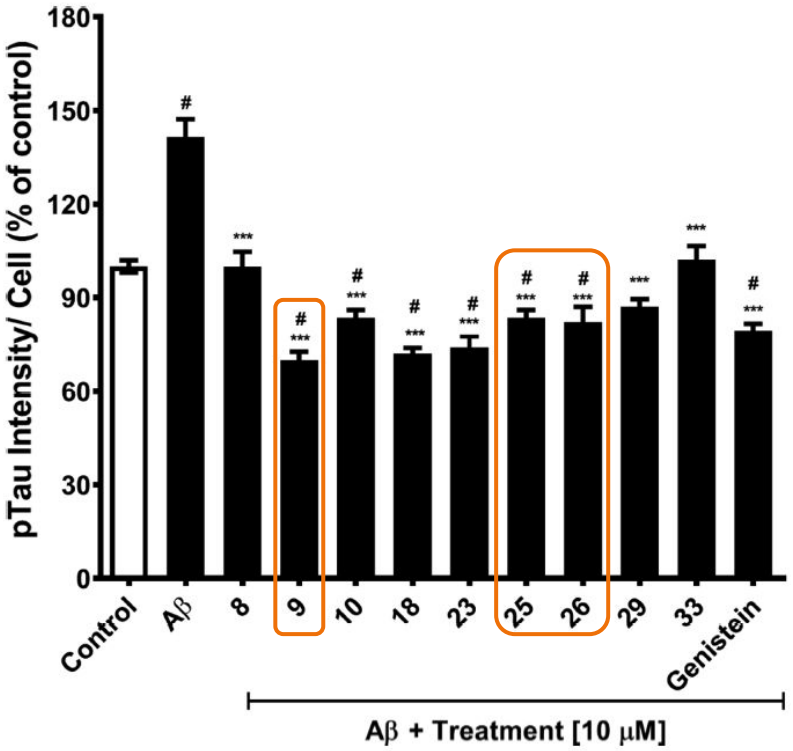
Results and discussion

Biological activity: Ability to inhibit Aβo-induced Tau phosphorylation





Effect of compounds against hyperphosphorylation of Tau induced by Aβ. Neurons treated with Aβ oligomers were evaluated against pTau (AT270). Tau hyperphosphorylation was measured by immunofluorescence using the Opera High Content Screening System. Cells were exposed to 10 μMof each compound in association with Aβ for 4 days. Results were normalized against the control group considered as 100%. The values are expressed as the mean ± SEM; n= 3. Significant differences between control are indicated with #($p \leq 0.05$) and ***($p < 0.001$) when compared with Aβ treatment.

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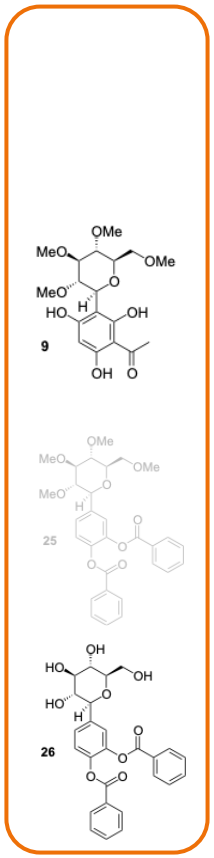
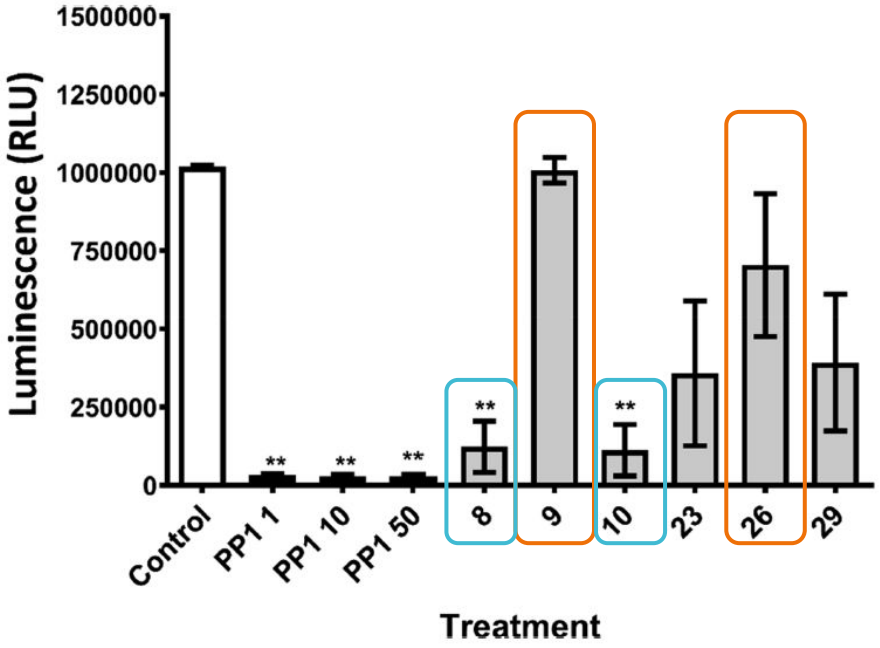
Results and discussion

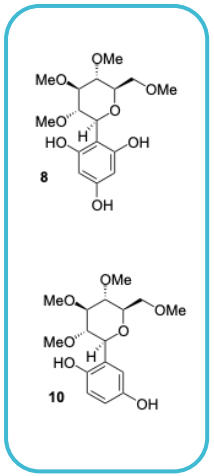
Investigation of the mechanism of action



No significant inhibition of Fyn activity



Effect of glucosylpolyphenols and the polyphenol glucoside 29 in the inhibition of Fyn kinase activity measured by the ADP-Glo kinase assay. Results are expressed as the mean ± SEM; n = 3. Significant differences between control are indicated with **($p < 0.01$) when compared with Aβ treatment.



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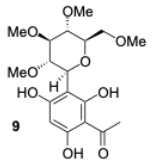
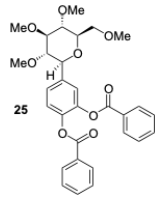
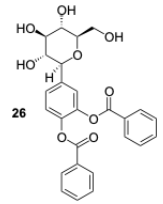
Results and discussion

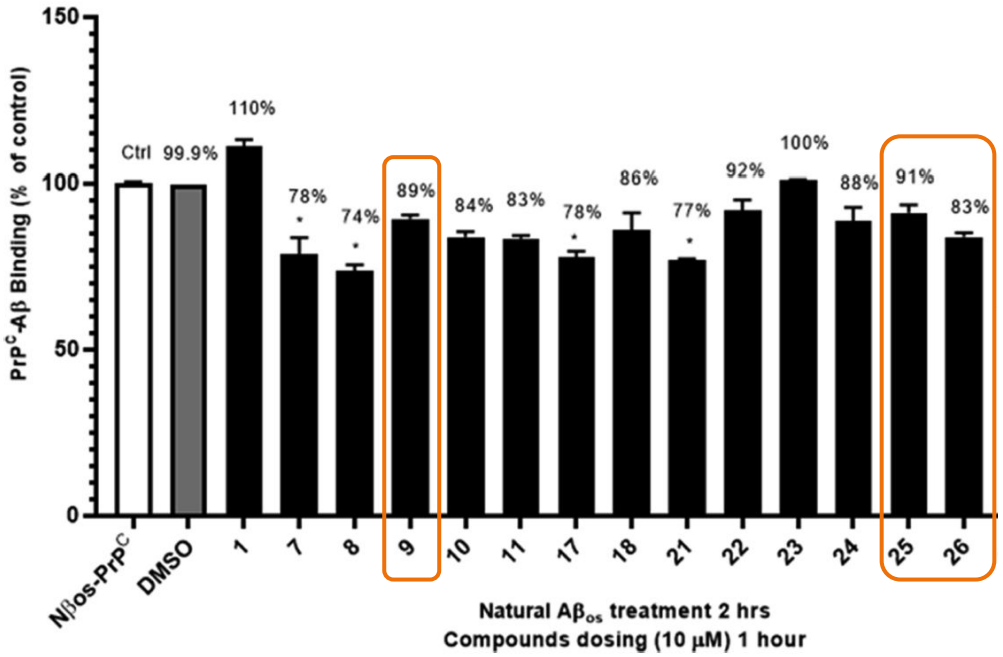
Investigation of the mechanism of action







No significant ability to inhibit Aβo-PrP^C binding in HEK 293 cells



Screening for compounds that are able to induce a PrP^C–NAβos binding inhibition. All compounds were tested at 10 μM as the final concentration. Results are expressed as the mean ± standard error mean (SEM); n = 3. Significant differences between control are indicated with *(p <0.05), **(p < 0.01) and ****(p ≤ 0.0001). The PrP^C–NAβ(1–42) binding (%) after treatment with the compounds is also indicated.

None of the compounds were significant inhibitors of Aβ amyloid aggregation

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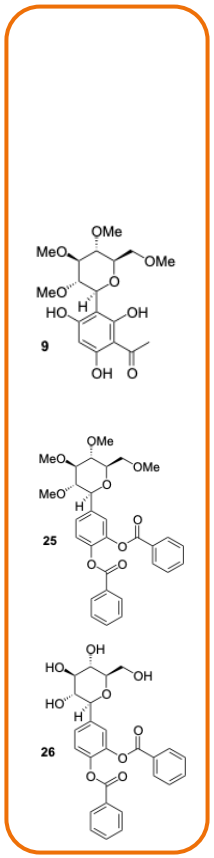
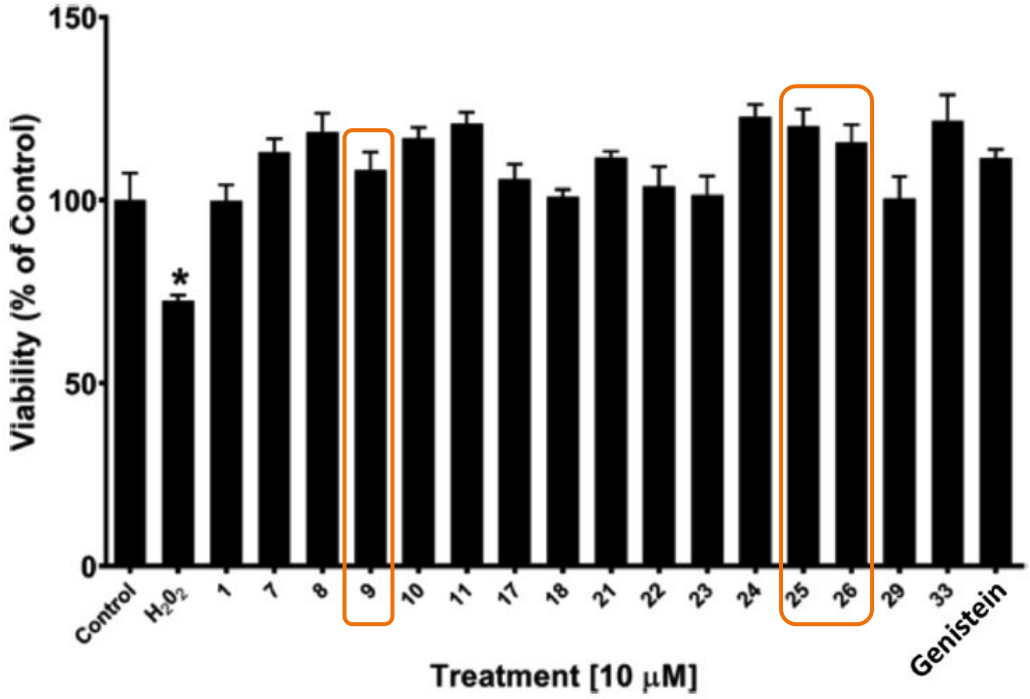
Results and discussion

Biological activity: Cytotoxicity towards hiPSC-derived neural cells





Cytotoxicity of C-glucosyl phenols and glucosides 29 and 33 in neunal cells derived from hiPSCs. Cell viability was measured in an MTT assay. Cells were exposed to 10 μM of each compound for 24 h. Results were normalized relative to a control group considered as 100%. The values are expressed as the mean ± SEM; n = 3. Significant differences between control are indicated with * ($p < 0.05$).

Compounds 9 and 26 not cytotoxic up to 50 and 100 μM, respectively

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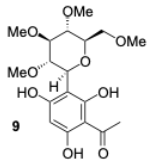
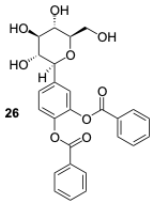
Results and discussion

Physicochemical properties: PAMPA and log*D* determination assays





permethylated sugar

non-protected sugar

compound no.	*c* log $P^{a,b}$	log P_e	log $D_{7.4}$
1	−0.17	−4.63 ± 0.15	−0.1 ± 0.1
7	0.74	−5.33 ± 0.08	1.1 ± 0.1
8	0.58	−5.24 ± 0.17	1.6 ± 0.2
9	1.06	−4.74 ± 0.02	2.3 ± 0.3
10	0.75	−5.52 ± 0.07	n.d.
11	1.96	−4.39 ± 0.04	2.7 ± 0.2
17	2.70	membrane retention over 80%	3.2 ± 0.1
18	3.95	equilibrated	>2.5
21	0.60	−6.35 ± 0.12	<0.5
22	1.93	−5.18 ± 0.61	2.0 ± 0.2
23	−1.23	below detection limit	n.d.
24	−0.44	−6.41 ± 0.24	n.d.
25	3.81	partial membrane retention	>2.5
26	1.95	−5.06 ± 0.08	n.d.
29	0.59	below detection limit	1.0 ± 0.1
33	0.58	−5.85 ± 0.54	0.1 ± 0.3
37	0.13	n.d.	n.d.
genistein	2.45	−4.49 ± 0.04	3.3 ± 0.2
testosterone	2.99	−4.42 ± 0.09	

Should be > -5.7

Should be between 1 and 4

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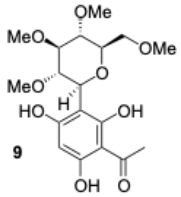
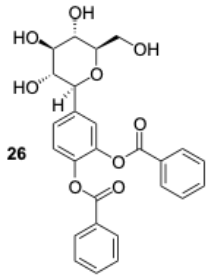
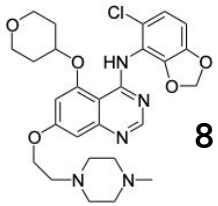
Conclusions



2 reaction steps



4 reaction steps



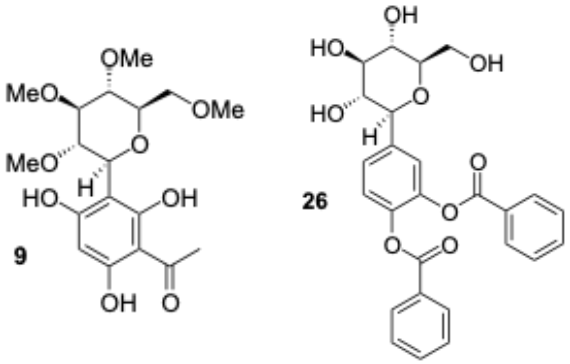
Saracatinib

8 reaction steps

- **First carbohydrate-based compounds displaying the ability to:***
  - **Inhibit Aβo-induced Fyn activation**
  - **Inhibit subsequent tau phosphorylation**
- **Not cytotoxic** up to 50 µM and 100 µM
- Favourable physicochemical properties (**potential to cross the BBB**)
- Much **more straightforward and efficient synthesis** compared to other molecules relevant in the same therapeutic context

*10 µM in neuronal cells.

Conclusions



Together, our results show that:

Compounds 9 and 26 are promising new leads and should be considered for further development against AD

For more details on this work

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Glucosylpolyphenols as Inhibitors of Aβ-Induced Fyn Kinase Activation and Tau Phosphorylation: Synthesis, Membrane Permeability, and Exploratory Target Assessment within the Scope of Type 2 Diabetes and Alzheimer's Disease

Ana M. de Matos,[¶] M. Teresa Blázquez-Sánchez,[¶] Andreia Bento-Oliveira, Rodrigo F. M. de Almeida, Rafael Nunes, Pedro E. M. Lopes, Miguel Machuqueiro, Joana S. Cristóvão, Cláudio M. Gomes, Cleide S. Souza, Imane G. El Idrissi, Nicola A. Colabufo, Ana Diniz, Filipa Marcelo, M. Conceição Oliveira, Óscar López, José G. Fernandez-Bolaños, Philipp Dätwyler, Beat Ernst, Ke Ning, Claire Garwood, Beining Chen,* and Amélia P. Rauter*

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Fundação para a Ciência e Tecnologia, Portugal, for supporting Centro de Química Estrutural (project UIDB/00100/2020) and for the PhD grant attributed to Ana Marta de Matos (**SFRH/BD/93170/2013**).

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