GLUCOSYL POLYPHENOLS WITH THE POTENTIAL TO CHANGE THE PARADIGM IN ALZHEIMER'S DISEASE THERAPEUTICS: SYNTHESIS, INHIBITION OF Aβ-INDUCED FYN ACTIVATION AND TAU PHOSPHORYLATION

AUTHORS: Ana M. Matos, M. Teresa Blásquez-Sánchez, Cleide S. Sousa, Beining Chen, Amélia P. Rauter

R&D UNIT: CQE | 11HC

CONTACT: aprauter@fc.ul.pt | amamatos@fc.ul.pt

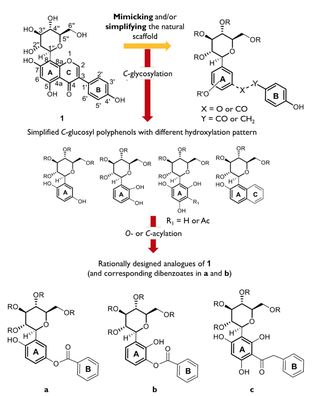
Introduction

- Alzheimer's disease (AD) is characterized by the presence of extracellular deposits of amyloid beta (Aβ) in the senile plaques and by intracellular neurofibrillary tangles induced by deposits of hyperphosphorylated Tau protein. These deposits are known be one of the leading causes of synaptic dysfunction that ultimately result in neuronal death in AD patients.[1]
- The cellular prion protein (PrP^C), located in the neuronal cell surface, works as a high-affinity binding partner of Aβ oligomers (Aβo), leading to the activation of Fyn kinase, which triggers a cell signaling pathway culminating in Tau hyperphosphorylation.[2]
- Fyn activity was found to be increased in AD brain by exposure of neurons to Aβo via PrP^C.[3,4] Moreover, genetic deletion of *FYN* prevents Aβo-induced cell death in hippocampus and Fyn inhibition restores synapse density and memory function in transgenic mice.[5,6]
- **Taken together, these data suggest that the inhibition of Aβ-induced Fyn activation and subsequent tau phosphorylation may have important disease-modifying effects in AD.**

Objectives

Our primary goal was to explore the bioactivity of a small library of *C*-glucosyl polyphenols based on the structure of 8-β-D-glucosyl genistein (**1**), a natural glucosyl isoflavone with potential against Aβ-induced neurotoxicity[7]. For this, we aimed at:

1. Synthesizing **simplified analogues of 1 with different hydroxylation patterns in ring A**, maintaining the sugar β-C linkage found in the original compound.[8]
2. **Inserting benzoate moieties** in C-glucosyl **(a)** hydroquinone and **(b)** catechol derivatives, or **(c)** ketone moieties in C-glucosyl phloroglucinol derivatives, to keep rings A and B linked by a 3-bond spacer moiety for the mimicking of **1**.[8]
3. Generating **more lipophilic analogues of 1** with higher chances of crossing the blood-brain barrier (BBB), namely by ***O*-methyl protection of sugar hydroxy groups,** due to the extremely polar nature of the lead compound.[8]



All synthesized compounds were evaluated against a variety of therapeutic targets in the context of AD and diabetes-induced dementia.[8] In this poster, Aβ-induced FYN activation assays are presented, as well as the assessment of Aβ-promoted hyperphosphorylated Tau levels.

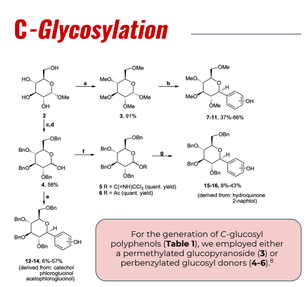
Acknowledgements The European Union is gratefully acknowledged for the support of the project entitled "Diagnostic and Drug Discovery Initiative for Alzheimer's Disease" (D3I4AD), FP7-PEOPLE-2013-IAPP, GA 612347. The authors also thank the Portuguese Foundation for Science and Technology (FCT) for the PhD grant attributed to AMM (SFRH/BD/93170/2013), and for the support of Centro de Química Estrutural (project UIDB/0100/2020).

References | **1.** Matos A. M.; Macedo M. P.; Rauter A. P. Bridging type 2 diabetes and Alzheimer's disease: assembling the puzzle pieces in the quest for the molecules with therapeutic and preventive potential. Med. Res. Rev. 2018, 38(1), 261-324. **2.** Nygaard H. B. Targeting Fyn kinase in Alzheimer's Disease. Biol. Psychiatry. 2018, 83(4), 369-376. **3.** Um J. W.; Strittmatter S. M. Amyloid-β induced signaling by cellular prion protein and Fyn kinase in Alzheimer disease. Prion. 2013, 7(1), 37-41. **4.** Smith L. M.; Zhu R.; Strittmatter S. M. Disease-modifying benefit of Fyn blockade persists after washout in mouse Alzheimer's model. Neuropharmacology. 2018, 130, 54-61. **5.** Kaufman A. C.; Salazar S. V.; Haas L. T.; Yang J.; Kostylev M. A., Jeng A. T.; Robinson S. A.; Gunther E. C.; van Dyck C. H.; Nygaard H. B.; Strittmatter S. M. Fyn inhibition rescues established memory and synapse loss in Alzheimer mice. Ann. Neurol. 2015, 77(6), 953-971. **6.** Lambert M. P.; Barlow A. K.; Chromy B. A.; Edwards C.; Freed R.; Liosatos M.; Morgan T. E.; Rozovsky I.; Trommer B.; Viola K. L.; Wals P.; Zhang C.; Finch C. E.; Krafft G. A.; Klein W. L. Diffusible, nonfibrillar ligands derived from Abeta(1–42) are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U. S. A. 1998, 95(11), 6448-6453. **7.** Jesus A. R.; Dias C.; Matos A. M.; de Almeida R. F.; Viana A. S.; Marcelo F.; Ribeiro R. T.; Macedo M. P.; Airoldi C.; Nicotra F.; Martins A.; Cabrita E. J.; Jiménez-Barbero J.; Rauter A. P. Exploiting the therapeutic potential of 8-β-D-glucopyranosylgenistein: synthesis, antidiabetic activity, and molecular interaction with islet amyloid polypeptide and amyloid β-peptide (1–42). J. Med. Chem. 2014, 57(22), 9443-9472. **8. Matos, A.M.; Blázquez-Sánchez M.T.; Bento-Oliveira A.; de Almeida R.F.M.; Nunes R.; Lopes, P.E.M.; Machuqueiro M.; Cristóvão J.S.; Gomes C.M.; Souza C.S.; El Idrissi I.G.; Colabufo N.A.; Diniz A.; Marcelo F.; Oliveira M.C.; López O.; Fernandez-Bolaños J.G.; Dätwyler P.; Ernst B.; Ning K.; Garewood C.; Chen B.; Rauter A.P. 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. J. Med. Chem. 2020, 63 (20) 11663–11690.**

Synthesis

- The methyl protected glucosyl donor **3** (**Scheme 1**) gave, by reaction with all the acceptors tested, *C*-glucosyl derivatives as the major products (**7-11**, **Table 1**).[8]
- In the case of catechol and hydroquinone, when using benzyl protected sugar donors **4-6**, glycosylation yields were drastically lower when compared to reactions either with phloroglucinol or trihydroxyacetophenone as sugar acceptors (**Table 1**).[8]
- In the synthesis of *C*-glucosyl catechol derivatives **7** and **12** (**Table 1**), the *para*-isomers are preferably formed, thus indicating that the Lewis acid-promoted Friedel–Crafts-type C-glycosylation is the favoured reaction mechanism, prevalent over the Fries-type rearrangement described for unprotected phenols.[8]
- 2-Naphthol was also used to generate *C*-glucosyl analogue **11** and **16** with two fused planar rings, in order to mimic rings A and C in the original structure (**Table 1**).[8]
- A benzoyl group was regioselectively introduced in *C*-glucosyl hydroquinone derivatives **10** and **15** to afford compounds **17** and **19** together with the dibenzoate analogues **18** and **20** (**Scheme 2**). After catalytic hydrogenation, **21** and **22** were also generated.[8]
- The hydroquinone and catechol per-*O*-benzyl *O*-glycosides **27α,β** and **31** (**Scheme 3**), obtained as major products under the *C*-glucosylation reaction conditions (**Table 1**), were also benzoylated and deprotected to afford the corresponding α-glycosides **29** and **33** as major products in excellent overall yield.[8]
- The phloroglucinol *C*-glucosyl derivative **13** (**Scheme 4**) was originally chosen as the precursor of the planned analogue of compound **1** with the *meta* hydroxylation pattern.[8]
- The use of TfOH 2% in MeCN rendered a mixture of the *O*/*C*-acylated products **34** and **35** (**Scheme 4**). Then, using an excess of TfOH, compound **35** was rearranged into the *C*-acylated analogue **34**, which was subsequently *C*-glycosylated to afford **36**. The final compound, analogue **37**, was achieved after catalytic hydrogenation.[8]

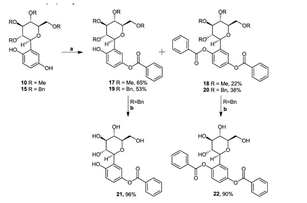
C-Glycosylation



For the generation of *C*-glucosyl polyphenols (**Table 1**), we employed either a permethylated glucopyranoside (**3**) or perbenzylated glucosyl donors (**4-6**).[8]

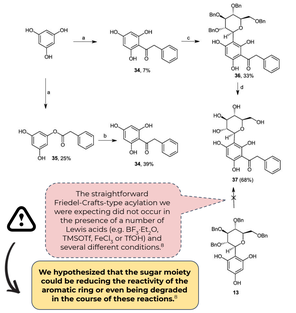
Scheme 1. Preparation of glucosyl donors and protected *C*-glucosyl phenols. Reagents and conditions: **a)** DMF, NaH, MeI, 0 °C, 3 h; **b)** dry MeCN, polyphenol, dioxane, -78 °C → r.t., TMSOTf, 18 h – 48 h; **c)** DMF, NaH, BnBr, 0 °C → r.t., 20 h; **d)** AcOH, H_2SO_4, reflux, 36 h; **e)** dichloromethane/MeCN, dioxane, -78 °C → r.t. or 40 °C, TMSOTf, 8 h – 64 h; **f)** for compound **5**: dichloromethane, molecular sieves 3Å, 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, dioxane, -78 °C → r.t., $BF_3{\cdot}Et_2O$, 40 h; for compound **16**: dichloromethane, molecular sieves 3Å, 0 °C → r.t., TMSOTf, 20 h.

O-Acylation

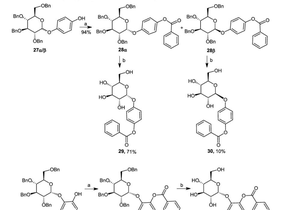


Scheme 2. Preparation of *C*-glucosyl hydroquinone benzoates. Reagents and conditions: **a)** Dichloromethane, imidazole, DMAP, BzCl, 0 °C → r.t., 60 h – 120 h; **b)** EtOAc, Pd/C, H_2, r.t., 16 h – 22 h (R=Bn).

C-Acylation



Scheme 4. Preparation of compound **40**. Reagents and conditions: **a)** phenylacetyl chloride, 2% TfOH/MeCN, 0°C → r.t., overnight, 32%; **b)** TfOH, 100 °C, 2 h, 39%; **c)** dichloromethane/MeCN, compound 4, dirietrie, -40 °C → r.t., 16 h, 33%; **d)** MeOH/EtOAc, Pd/C, H_2, r.t., 3 h, 68%.



Scheme 3. Preparation of hydroquinone and catechol *O*-glycoside benzoates. Reagents and conditions: **a)** Dichloromethane, imidazole, DMAP, BzCl, 0 °C → r.t., 60 h – 120 h; **b)** EtOAc, Pd/C, H_2, r.t., 16 h – 22 h.

Other compounds

For comparison purposes:

- Compounds **14** and **16** (**Table 1**) were debenzylated through catalytic hydrogenation to afford compounds **23** and **24**, respectively.[8]
- The dibenzoate catechol analogues of compounds **18** and **22** (**Scheme 2**) were also synthesized (compounds **25** and **26**, respectively, after benzoylation and removal of the sugar protecting groups[8]).

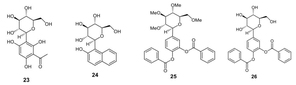


Table 1. *C*-Glucosylation of polyphenols carried out with TMSOTf as promoter.

Phenol	Glycosyl donor	Isolated Yield (%)	Glycosyl donor	Isolated Yield (%)
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)
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 $BF_3{\cdot}Et_2O$ as promoter.

Biological Activity

Inhibition of Aβ-Induced Fyn Activation

Fyn activation levels in hiPSC (human induced pluripotent stem cells) derived neural progenitor cells from healthy donors **increased upon treatment with Aβ**, i.e. pFyn (phosphorylated Fyn) production was increased.[8]

However, **Aβ-induced Fyn activation was reduced back to normal control values in the presence of the commercial Fyn kinase inhibitor PP1.**[8]

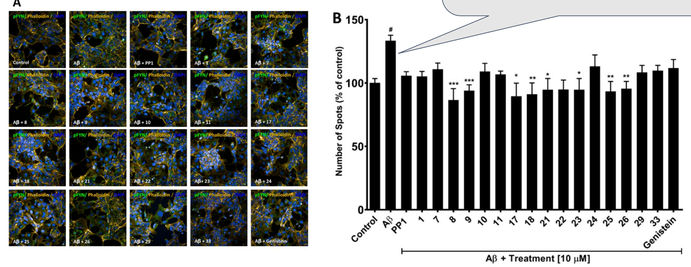


Figure 1. Effect of glucosylphenols in Aβ-induced Fyn activation. The indirect activation of Fyn kinase was measured by immunofluorescence using Opera High Content Screening System **(A)**. Cells were exposed to 10 μM of compounds in association with Aβ. The results were normalized against the control group, which was considered as 100% **(B)**. Percentage of number of pFyn + spots in each treatment group. Results are expressed as the mean ± standard error mean (SEM); n = 3. Significant differences between control are indicated with #(p≤0.05) and *(p(<0.05) when compared to Aβ treatment (*p < 0.05) or **(p=0.01) or ***(p=0.001).

Inhibition of Aβ-Induced Tau hyperphosphorylation

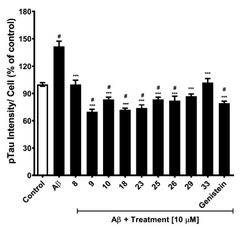


Figure 2. Effect of compounds against hyperphosphorylation of Tau induced by Aβ. Neurons treated with Aβ oligomers were evaluated against pTau (AT270). Tau hyperphosphorylation was measured by immunofluorescence using the Opera High Content Screening System. Cells were exposed to 10 μM of each compound 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≤0.05) ***(p=0.001) when compared with with Aβ treatment.

- Aβ-induced Fyn activation was generally reduced by all tested glucosyl polyphenols, and significantly inhibited in the presence of compounds **8, 9, 17, 18, 21, 23** and **26** (**Figure 1**). Compounds **8** and **9** presented the result with the highest statistical significance (p<0.001).[8]
- Neither the lead compound **1**, nor its aglycone, **genistein**, which was also tested, were found to Aβ-induced Fyn activation to a significant extent (**Figure 1**).[8]
- Neural progenitor cells were treated with the compounds without addition of Ab to determine whether the effects observed are independent of Ab treatment, and it was confirmed that tested compounds and PP1 alone do not reduce the basal levels of pFyn (data not shown).[8]
- All compounds that were tested against Aβ-induced Tau hyperphosphorylation (**8, 9, 10, 18, 23, 25, 26, 29** and **genistein**) were able to significantly (p<0.001) reduce the levels of pTau when compared to Aβ treatment alone (**Figure 2**).[8]
- Compounds **9, 19, 18, 25, 26** and **genistein** were furthermore able to reduce pTau levels when compared to untreated controls (p≤0.05) (**Figure 2**).[8]

Other Assays

- Neural progenitor cells (NPC) were treated with each compound for 24 hours, and **none presented any signs of cytotoxicity at 10 μM**.[8]
- Furthermore, compounds **23**, **26**, and **29** were not cytotoxic in concentrations up to 100 μM, while **9** is safe to administer up to a 50 μM concentration (data not shown).[8]
- Compounds **9** and **26** presented an effective permeability (log P_e = - 4.74 ± 0.02 and - 5.06 ± 0.08, respectively determined in a PAMPA assay) and log *D* values (2.3 ± 0.3 and n.d., respectively) that are compatible with the desired pharmacokinetic profile.[8]

[Optimal effective permeability: log P_e > -5; log *D* 1-4 for a good compromise between solubility and membrane permeability.]

Conclusions

- Although this work was primarily inspired by a natural *C*-glucosyl isoflavone (**1**), we herein disclose that **much simpler *C*-glucosyl polyphenols embody the right scaffold to tackle the chain of Aβ-induced processes culminating in Tau hyperphosphorylation.**[8]
- The most promising compounds are **9**, synthesized in only 2 steps, and **26**. Both were found to inhibit Aβ-induced Fyn kinase activation, and to consequently reduce the levels of hyperphosphorylated Tau. Moreover, these compounds are not toxic at relevant concentrations and have the right balance between effective permeability and lipophilicity to be orally available and brain penetrant.[8]
- Our results suggest that C-glucosyl polyphenols, in particular compounds **9** and **26**, should be regarded as new promising scaffolds for further development against Aβ-induced Tau pathology in AD.[8]

These and other results were published in the most recent issue of the *Journal of Medicinal Chemistry*.[8]