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New polyphenols active on β-amyloid aggregation

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Abstract—New polyphenol classes have been tested against amyloid-β peptide aggregation. We have identified four novel polyphenols which could be efficient fibril inhibitors in Alzheimer's disease: malvidin and its glucoside and curculigosides B and D. We suggest that molecules with the particular C_6-linkers-C_6 structure could be potent inhibitors. From the results reported for the flavan-3-ol family, their anti-amyloidogenic effects against whole peptides (1–40 and 1–42) could involve several binding sites.

For demographic reasons, neurodegenerative diseases affect an increasing percentage of population. Alzheimer's disease (AD) is the most common type of neurodegenerative disorders. It accounts for 65% of all dementias, with an estimated prevalence between 1% and 5% among people aged 65, which doubles every 4 years to reach about 30% at 80 years.[1] Histopathology reveals that one of the major hallmarks of AD is the abundant protein deposits in neurons that trigger neuronal degeneration. These deposits result from the extracellular and intracellular accumulation of amyloid-β peptides (Aβ) and phosphorylated tau protein (Pτ), respectively.[2] Aβ and Pτ accumulation leads to the formation and deposition of senile plaques and neurofibrillary tangles, respectively, which promote pro-inflammatory responses and activate neurotoxic pathways, leading to dysfunction and death of brain cells.[3]

Finding molecules to prevent the aggregation of Aβ could be a therapeutic goal in AD.[4] In a preview study, we described an original routine in vitro assay to search for inhibitors of Aβ fibril formation by using UV–visible measurements and electron microscopy. We used the Aβ 25–35 amino-acid peptide ($A\beta_{25-35}$) that preserves the properties of neurotoxicity and aggregation.[5,6] Our results suggested that the stilbenes resveratrol and piceid could be important molecules of therapeutic interest.[7]

The goal of this study was to investigate the effects of other polyphenols on $A\beta_{25-35}$ fibril formation using the same routine assays (Fig. 1) and eventually to draw hypotheses on the structure–activity relationship of polyphenols against Aβ aggregation. Phenolic acids were purchased from Sigma. Flavan-3-ols and anthocyanins were purchased from Extrasynthèse. Curculigosides were extracted from *Curculigo orchioides*.[8] Polyphenol purity was controlled by HPLC measurements. Initial screening for inhibition was performed at a concentration of 10 μM and their inhibiting effect was compared to that of curcumin used as reference.[9] The compounds exhibiting inhibitory activity at least equal to that of curcumin were further analysed to determine their effective inhibitory concentration (EC_{50}) values. Data concerning inhibition of amyloid fibril formation are shown in Table 1.

Concerning non-flavonoïd polyphenols, we identified two novel potential inhibitors, curculigosides B and D, which have an EC_{50} value of 8.5 and 9.5 μM, respectively. These molecules have a benzylbenzoate structure with two phenolic rings at both extremities linked by three atoms. The results confirm the importance of this particular C_6-linkers-C_6 structure on fibril inhibition.

Keywords: Alzheimer's disease; Polyphenols; UV–visible spectroscopy.
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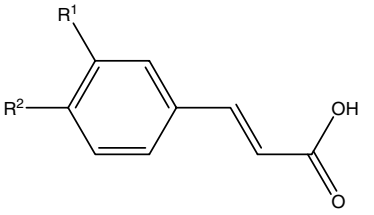
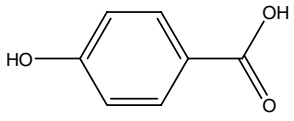
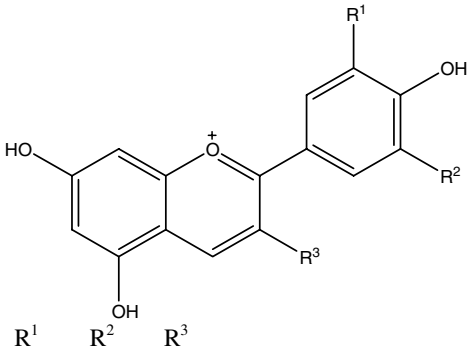


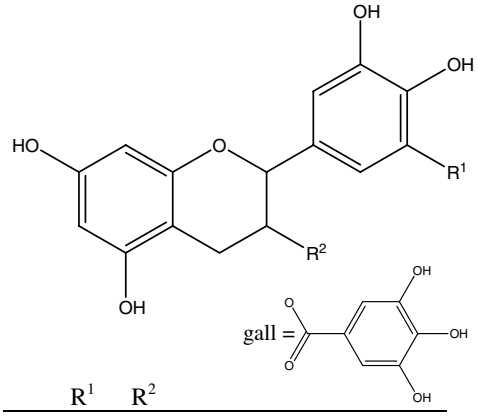
	R^1	R^2	
1	H	OH	**p-coumaric acid**
2	OH	OH	**caffeic acid**



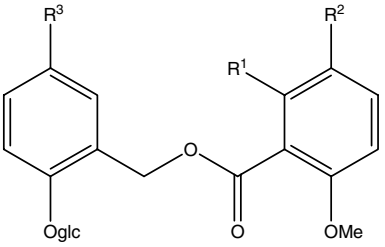
3 **p-hydroxybenzoic acid**



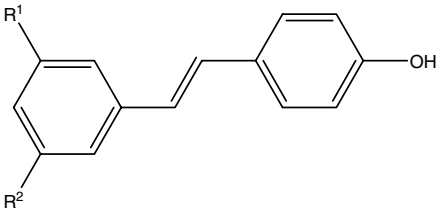
	R^1	R^2	R^3	
4	OH	H	OH	**cyanidin**
5	OH	H	Oglc	**cyanidin glucoside**
6	OMe	OMe	OH	**malvidin**
7	OMe	OMe	Oglc	**malvidin glucoside**
8	OH	OH	OH	**delphinidin**



	R^1	R^2	
9	OH	OH	**epigallocatechin**
10	OH	gall	**epigallocatechin gallate**
11	H	gall	**epicatechin gallate**



	R^1	R^2	R^3	
12	OMe	H	OH	**curculigoside A**
13	OH	H	OH	**curculigoside B**
14	OMe	OH	H	**curculigoside D**



	R^1	R^2	
15	OH	OH	**resveratrol**
16	OMe	OMe	**pterostilben**

Figure 1. Polyphenolic molecules tested on the $A\beta_{25-35}$ fibril formation.

Indeed, many polyphenol inhibitors are composed of two phenolic rings at both extremities with several atom linkers: resveratrol,[7] curcumin, rosmarinic acid and nordihydroguaiaretic acid.[9] To reinforce this hypothesis, three phenolic acids were tested: coumaric acid, *p*-hydroxy benzoic acid and caffeic acid. Compared to resveratrol, these molecules have a structure with only one phenol ring. They have a weak inhibitory activity on $A\beta_{25-35}$ fibril formation compared to resveratrol. Thus, the presence of the two rings at the extremities of the molecule seems to be necessary in order to have potent inhibition. The activity of curculigoside A is two orders less than that of curculigosides B and D. Curculigoside A differs from B only in the substitution of a hydroxyl group (curculigoside B) by an *O*-methyl group (curculigoside A) on the benzoate ring. Curculigoside D, which has two *O*-methyl and hydroxyl groups on the benzoate ring, has activity similar to that of curculigoside B. Taken together, these results indicate that the presence of a hydroxyl group on the benzoate ring could be important for inhibitory activity via a possible hydrogen bond binding with the peptide, which could reinforce the hydrophobic ring interactions.

Concerning flavonoïd polyphenols, anthocyanins and flavan-3-ols, the results are contrasted. Flavan-3-ols are not efficient inhibitors of $A\beta_{25-35}$ fibril formation. They are known to interact with proteins and studies have shown that epicatechin and epicatechin gallate have a potent inhibitory effect on the aggregation of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides.[10,11] From our results, we

Table 1. Inhibition of $A\beta_{25-35}$ fibril formation

Compound	Inhibition %	EC_{50} (μM)
No-flavonoids		
Curcumin	**45 ± 9**	**10**
Stilbenes		
Resveratrol	**63 ± 8**	**6.0**
Pterostilben	35 ± 7	—
Phenolic acids		
p-Coumaric acid	17 ± 3	—
Caffeic acid	15 ± 4	—
p-Hydroxybenzoic acid	29 ± 6	—
Curculigosides		
Curculigoside A	28 ± 6	—
Curculigoside B	**53 ± 8**	**8.5**
Curculigoside D	**49 ± 7**	**9.5**
Flavonoids		
Anthocyanins		
Cyanidin	35 ± 3	—
Cyanidin glucoside	31 ± 5	—
Malvidin	**46 ± 3**	**10**
Malvidin glucoside	**44 ± 8**	**10**
Delphinidin	26 ± 11	—
Favan-3-ols		
Epigallocatechin	18 ± 6	—
Epigallocatechin gallate	13 ± 7	—
Epicatechin gallate	13 ± 5	—

Bold values indicate the molecules exhibiting inhibitory activity at least equal to that of reference curcumin.

therefore conclude that there are other sites of polyphenol interaction on the whole peptides leading to inhibition of aggregation.

Most of the anthocyanins tested showed weak inhibition of about 30%. However, malvidin and its glucoside demonstrated activity comparable to that of curcumin with an EC_{50} of 10 μM. The fact that the inhibitory activity of anthocyanins was higher than that of flavan-3-ols could be due to the properties of anthocyanins. In aqueous solutions at pH ~ 7, the anthocyanin structure is in equilibrium with the chalcone structure (Fig. 2).[12] Thus, the activity of anthocyanins could be due to the chalcone, which has a C_6-linkers-C_6 structure.

Nevertheless, the activity of anthocyanins also seems to be highly governed by the nature of the constituents on the rings, as shown when pterostilbene activity is compared to that of resveratrol or when the activities of curculigosides are compared (Table 1). Furthermore, the hydrophobic character of polyphenolic molecules is likely not their only key property, as suggested by the equal inhibitory activity of resveratrol and piceid,[7] of malvidin and malvidin glucoside (Table 1) or by the inhibitory activity of rosmarinic acid, a hydrophilic polyphenol.[9] For these polyphenols and particularly the glucoside derivatives, hydrogen bonds might play an important role in fibril inhibition by reinforcing the hydrophobic interaction between the aromatic rings.

In conclusion, we have identified four novel polyphenols which could be efficient fibril inhibitors in AD: malvidin and its glucoside and curculigosides B and D. We suggest that molecules with the particular C_6-linkers-C_6 structure could be potent inhibitors. Finally, flavan-3-ols did not inhibit $A\beta_{25-35}$ aggregation, suggesting that their anti-amyloidogenic effects against whole peptides (1–40 and 1–42) could involve other binding sites within the peptide sequence different from the fragment 25–35.

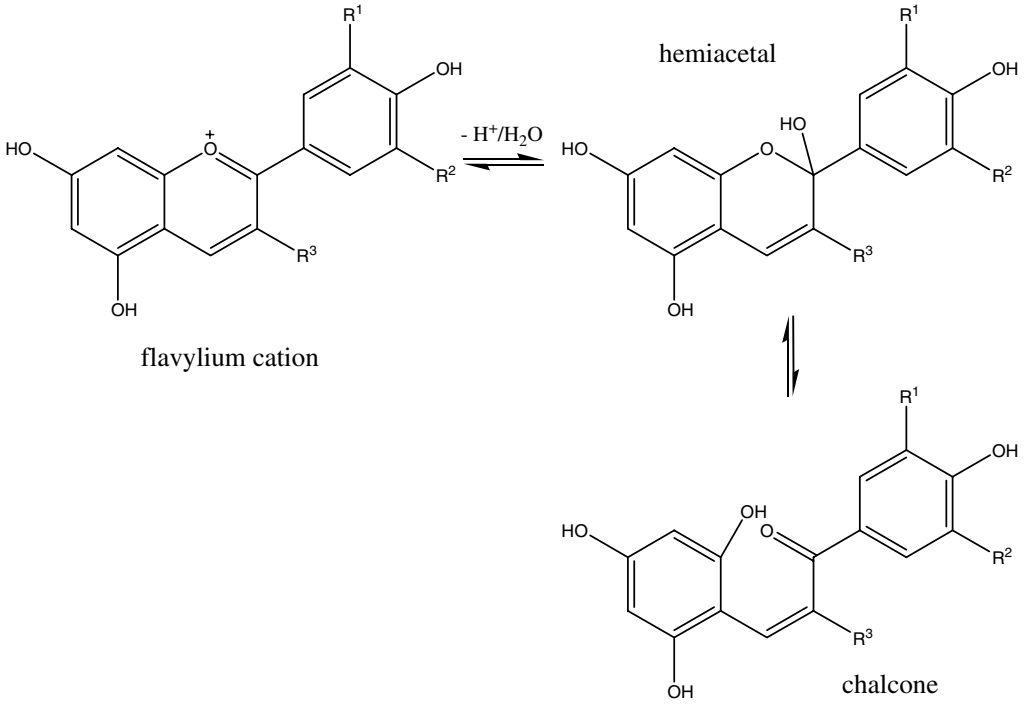


Figure 2. Possible structures of anthocyanins in solution.

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