Bioorganic & Medicinal Chemistry Letters 20 (2010) 3441–3443

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New stilbene dimers against amyloid fibril formation

Céline Rivière [†], Yorgos Papastamoulis [†], Pierre-Yves Fortin, Nicolas Delchier, Soahary Andriamanarivo, Pierre Waffo-Teguo, Gilbert D. W. F. Kapche, H. Amira-Guebalia, Jean-Claude Delaunay, Jean-Michel Mérillon, Tristan Richard [*], Jean-Pierre Monti [*]

GESVAB (EA 3675), Université de Bordeaux, ISVV Bordeaux-Aquitaine, 71, Avenue Edouard Bourleaux, 33883 Villenave d'Ornon cedex, France

ARTICLE INFO

Article history:
Received 31 July 2009
Revised 16 September 2009
Accepted 18 September 2009
Available online 24 September 2009

Keywords:
Stilbene
Polyphenol
Alzheimer's disease
β-Amyloid peptide

ABSTRACT

Twenty stilbene derivatives and moracin M extracted from natural products were tested against amyloid-β peptide (Aβ) aggregation. Results of stilbene monomer derivatives indicated that interaction with resveratrol and piceid was specific. Concerning oligomers, scirpusin A and ε-viniferin glucoside demonstrated a strong inhibition of the aggregation process.

The accumulation of β-amyloid peptide (Aβ) is one of the major neurodegenerative processes occurring during the progression of pathogenesis in Alzheimer's disease (AD). Aβ is toxic to cells in culture via multiple pathways,[1,2] and its toxicity is correlated with the degree of peptide aggregation.[3]

Given the central importance of Aβ aggregation in the pathogenesis of AD, particular interest[4] is currently being shown in research aimed at therapy developments that target amyloid production, aggregation, clearance or toxicity. Reports that describe the effects of polyphenols on Alzheimer's Aβ aggregation in vitro are already available.[5–10] Using an original routine in vitro assay based on UV–visible measurements and electron microscopy, we recently reported, that two vine hydroxystilbenes, resveratrol and piceid, inhibit the aggregation of the peptide.[8] For this assay we used the 25–35 Aβ peptide fragment that preserves the properties of neurotoxicity and aggregation of the entire peptide.[3,11]

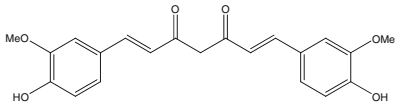
Using this routine assay, we examined the effects of other resveratrol derivative polyphenols (monomers, dimers and oligomers) on Aβ aggregation (Fig. 1). All stilbenes tested were extracted from plants: stem of *vitis vinifera* and stem bark of *Milicia excelsa* using the protocol described by Delaunay et al.[12] Briefly, stilbenoid extracts were fractionated by column chromatography followed by centrifugal partition chromatography. Pure compounds were obtained by semipreparative high-performance liquid chromatography. Their structures were elucidated by one- and two-dimensional NMR analysis. Polyphenol purity was controlled by HPLC–UV and HPLC–UV–ESI-MS^n spectroscopy. Screening for inhibition was carried out using an original routine in vitro assay to search for inhibitors of Aβ fibril formation by using UV–visible measurements and electron microscopy.[9] Initial screening for inhibition was performed at a concentration of 10 μM and their inhibiting effect was compared to that of curcumin as a Ref. 5 The compounds exhibiting inhibitory activity at least equal to that of curcumin were further analyzed to determine their IC_{50} values. The IC_{50} values of all compounds are summarized in Table 1. Among all the compounds tested, the dimer polyphenolic hydroxystilbenes, scirpusin A (**13**) and ε-viniferin glucoside (**15**), exhibited an extremely efficient inhibition of Aβ aggregation.

All the stilbene monomers tested were less active than resveratrol and its glucoside, both compounds being strong and specific potent inhibitors. Inspection of inhibitory data for stilbene monomers revealed several patterns in structure versus activity. First of all, substitutions on ring A reduced the protective activity against aggregation. Methoxy or glucosyl derivatives (**8**, 11%; **9**, 28%) and derivatives with an additional hydroxyl group (**5**, 25%; **7**, 32%) were weaker inhibitors than resveratrol (**3**, 63%) or piceid (**4**, 62%). These results indicate that the ring A structure is critical in the binding process. Furthermore, concerning ring B, piceid (**4**, 62%) showed a comparable activity to resveratrol, whereas the

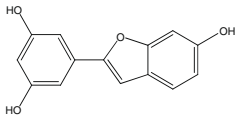
* Corresponding authors. Tel.: +33 557122710; fax: +33 557122717 (T.R.).
E-mail addresses: tristan.richard@u-bordeaux2.fr (T. Richard), jean-pierre.monti@u-bordeaux2.fr (J.-P. Monti).

† C.R. and Y.P. contributed equally to this work.

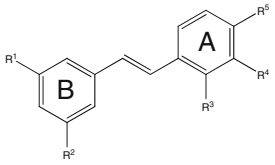
0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.09.074



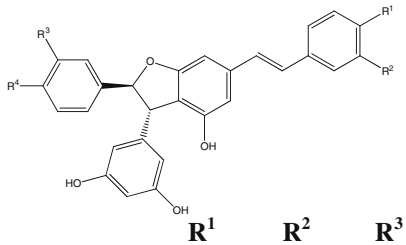
1 curcumin

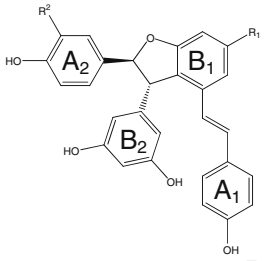


2 moracin M



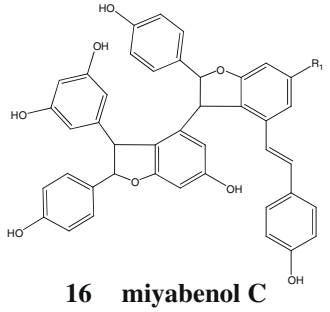
		R^1	R^2	R^3	R^4	R^5
3	**resveratrol**	OH	OH	H	H	OH
4	**piceid**	OGlc	OH	H	H	OH
5	**piceatannol**	OH	OH	H	OH	OH
6	**pterostilben**	OMe	OMe	H	H	OH
7	**oxyresveratrol**	OH	OH	OH	H	OH
8	**gob C**	OH	OH	H	H	OGlc
9	**gob D**	OH	OH	H	OMe	OGlc



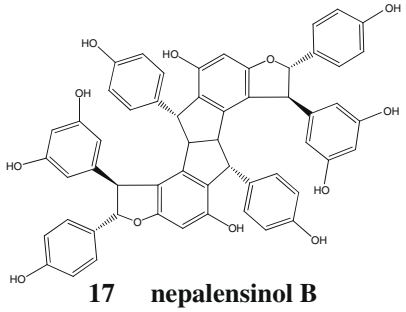


		R^1	R^2	R^3	R^4
10	**gnetin C**	OH	H	H	OH
11	**gneafricanin A**	OH	OMe	OMe	OH
12	**gnemenoside A**	OGlc	H	H	OGlc

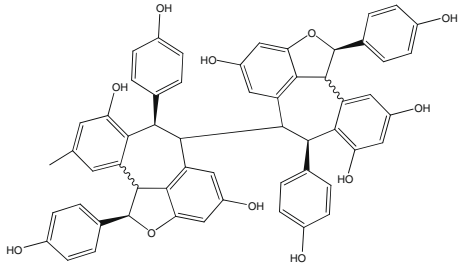
		R^1	R^2
13	**scirpusin A**	OH	OH
14	**ε-viniferin**	OH	H
15	**ε-viniferin glucoside**	OGlc	H



16 miyabenol C

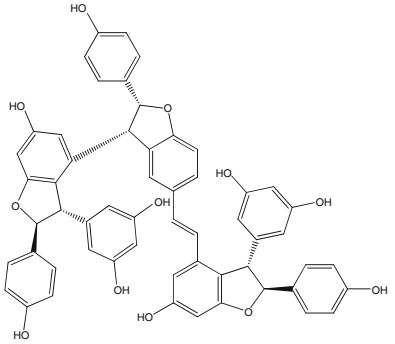


17 nepalensinol B

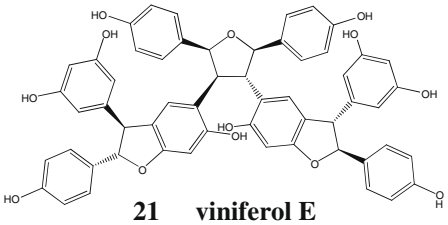


18 hopeaphenol

19 isohopeaphenol



20 vitisin C



21 viniferol E

Figure 1. Stilbene compounds tested on the Aβ fibril formation.

Table 1
Inhibition of Aβ fibril formation

	Compound	Inhibition (%)	EC_{50} (μM)
1	Curcumin	**45 ± 9**	**10 ± 2**
Monomers			
2	Moracin M	9 ± 7	—
3	Resveratrol	**63 ± 6**	**6 ± 2**
4	Piceid	**62 ± 6**	**6 ± 2**
5	Piceatannol	25 ± 9	—
6	Pterostilben	35 ± 7	—
7	Oxyresveratrol	32 ± 7	—
8	Gob C	11 ± 6	—
9	Gob D	28 ± 5	—
Dimers			
10	Gnetin C	39 ± 5	—
11	Gneafricanin A	40 ± 6	—
12	Gnemenoside A	**46 ± 7**	**10 ± 2**
13	Scirpusin A	**80 ± 9**	**0.7 ± 0.3**
14	ε-Viniferin	27 ± 5	—
15	ε-Viniferin glucoside	**93 ± 3**	**0.2 ± 0.3**
Oligomers			
16	Miyabenol C	15 ± 5	—
17	Nepalensinol B	17 ± 7	—
18	Hopeaphenol	13 ± 6	—
19	Isohopeaphenol	21 ± 9	—
20	Vitisin C	32 ± 9	—
21	Viniferol E	17 ± 10	—

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

activity of pterostilben (**6**, 35%) was weak. These results indicate that ring B is involved in the interaction. Piceid differs from resveratrol by the substitution of a hydroxyl group for a glucose unit, whereas pterostilbens have two methoxy groups on ring B. Thus steric hindering induced by the glucose moiety did not modify the inhibitory process, whereas both methoxy groups that involve a loss of hydrogen bond donors induced a decrease in inhibition. Indeed, many studies indicate that hydrogen bonds are crucial in the interactions between polyphenols and proteins.[13–16] Finally, the benzofuranyl group in moracin M (**2**, 9%) led to a decrease in activity. The greater rigidity induced by this group for the linkage between the two rings may play an important role.

Concerning stilbene dimers, apart from the ε-viniferin (**14**, 25%), all tested compounds had a considerable inhibitive activity compared to curcumin. Two dimers had a stronger inhibitory activity than curcumin (**13**, 80%; **15**, 93%). Strong inhibition of Aβ fibril formation by scirpusin A (**13**) and ε-viniferin glucoside (**15**) led to a very low IC_{50} ($\leqslant$1 μM). Thus, both stilbene dimers are potent inhibitor compounds. Scirpusin A and ε-viniferin glucoside differed only by their substituents on rings A_2 and B_1 (see Fig. 1). Scirpusin A had an additional hydroxyl group on ring A_2 and ε-viniferin glucoside had a glucose unit on ring B_1. Thus these compounds lead to a family of strong potent inhibitors. Nevertheless, ε-viniferin, with the same structure, is less active (**14**, 27%). At the present time it is very difficult to draw conclusions on structure–activity relationship because stilbene compounds are known for their capacity to form conformers which differ in their three-dimensional (3D) structure.[17] The activity difference between these samples could be due to the 3D conformation of the samples tested. To conclude, complementary studies are necessary for a better understanding of the structure–activity relationship of this new type of inhibitor. In particular, the three-dimensional conformation impact on Aβ aggregation of these particular stilbenes needs to be studied.

Concerning the stilbene oligomers trimer, miyabenol C (**16**, 15%), and tetramers, nepalensinol B (**17**, 17%), hopeaphenol (**18**, 13%), isohopeaphenol (**19**, 21%), vitisin C (**20**, 32%) and viniferol E (**21**, 17%), these were weaker inhibitors than curcumin. These results suggest that spatial constraints are critical in the binding process. Other oligomers need to be tested, however, to confirm that bulk compounds are not active. Indeed, our results showed that the inhibitory effect depends not only on the substituents of the ring but on the 3D structure too.

In conclusion, our results indicate that among the stilbene monomers, resveratrol and its glucoside are specific potent inhibitors of Aβ aggregation. We have identified two new resveratrol derivatives presenting strong, potent inhibiting activities: scirpusin A and ε-viniferin glucoside. They clearly showed a higher level of protective activity than resveratrol, indicating a new type of powerful inhibitor. Nevertheless, complementary studies are needed to understand the structure–activity relationship between these compounds. Finally, compounds with a high molecular weight appear to provide less active compounds, probably due to steric hindering in the binding process.

References and notes

1. Yankner, B. A.; Dawes, L. R.; Fisher, S. *Science* **1989**, *245*, 417.
2. Small, D. H.; Mok, S.; Bornstein, J. C. *Nat. Rev. Neurosci.* **2001**, *2*, 595.
3. Pike, C. J.; Walencewicz-Wasserman, A. J.; Kosmoski, J.; Cribbs, D. H.; Glabe, C. G.; Cotman, C. W. *J. Neurochem.* **1995**, *64*, 253.
4. Golde, T. E. *J. Neurochem.* **2006**, *99*, 689.
5. Ono, K.; Yoshiike, Y.; Takashima, A.; Hasegawa, K.; Naiki, H.; Yamada, M. *J. Neurochem.* **2003**, *87*, 172.
6. Ono, K.; Hasegawa, K.; Takashima, A.; Naiki, H.; Yamada, M. *J. Neurosci. Res.* **2004**, *75*, 742.
7. Taniguchi, S.; Suzuki, N.; Hisanaga, S. I.; Iwatsubo, T.; Goedert, M.; Hasegawa, M. *J. Biol. Chem.* **2005**, *280*, 7614.
8. Rivière, C.; Richard, T.; Quentin, L.; Krisa, S.; Mérillon, J. M.; Monti, J. P. *Bioorg. Med. Chem.* **2007**, *15*, 1160.
9. Rivière, C.; Richard, T.; Vitrac, X.; Mérillon, J. M.; Valls, J.; Monti, J. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 828.
10. Rivière, C.; Delaunay, J. C.; Immel, F.; Cullin, C.; Monti, J. P. *Neurochem. Res.* **2009**, *34*, 1120.
11. Klegeris, A.; Walker, D. G.; McGeer, P. L. *Biochem. Biophys. Res. Commun.* **1994**, *199*, 984.
12. Delaunay, J. C.; Castagnino, C.; Chèze, C.; Vercauteren, J. *J. Chromatogr., A* **2002**, *964*, 123.
13. Haslam, E. *J. Nat. Prod.* **1996**, *59*, 205.
14. Vergé, S.; Richard, T.; Moreau, S.; Nurich, A.; Mérillon, J. M.; Vercauteren, J.; Monti, J. P. *Biochem. Biophys. Acta* **2002**, *89*, 1571.
15. Richard, T.; Vitrac, X.; Mérillon, J. M.; Monti, J. P. *Biochim. Biophys. Acta* **2005**, *1726*, 238.
16. Richard, T.; Lefeuvre, D.; Descendit, A.; Quideau, S.; Monti, J. P. *Biochem. Biophys. Acta* **2006**, *951*, 1760.
17. Ito, T.; Abe, N.; Oyama, M.; Iinuma, M. *Helv. Chem. Acta* **2008**, *91*, 1989.