REVIEW

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Comparative Neuroprotective Properties of Stilbene and Catechin Analogs: Action Via a Plasma Membrane Receptor Site?

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Various studies have reported on the neuroprotective effects of polyphenols, widely present in food, beverages, and natural products. For example, we have shown that resveratrol, a polyphenol enriched in red wine and other foods such as peanuts, protects hippocampal cells against β-amyloid (Aβ)-induced toxicity, a key protein involved in the neuropathology of Alzheimer disease. This effect involves, at least in part, the capacity of resveratrol to activate the phosphorylation of delta isoform of protein kinase C (PKC-δ). The neuroprotective action of resveratrol is shared by piceatannol, a stilbene derivative, as well as by tea-derived catechin gallate esters. The thioflavin T assay indicated that all these polyphenols inhibited the formation of Aβ fibrils, suggesting that this action likely also contributes to their neuroprotective effects. Binding and autoradiographic studies revealed that the effects of polyphenols might involve specific binding sites that are particularly enriched in the choroid plexus in the rat brain. Interestingly, the choroid plexus secretes transthyretin, a protein that has been shown to modulate Aβ aggregation and that may be critical to the maintenance of normal learning capacities in aging. Taken together, these data suggest that polyphenols target multiple enzymes/proteins, leading to their neuroprotective actions, possibly through action via specific plasma membrane binding sites.

Introduction

There is much evidence showing that polyphenols present in high amounts in fruits, vegetables, and natural products play a preventive role in the incidence of age-related neurological disorders. Indeed, epidemiological studies reported that older people have a lower risk of developing dementia if they regularly consume fruits and vegetables (more than three servings per day; relative risk of 0.72), drink fruit and vegetable juices at least three times per week (relative risk of 0.84), or drink red wine up to three glasses per day (relative risk ranging from 0.55 to 0.58). These epidemiological findings have been supported by *in vitro* models of toxicity and animal models of neurological disorders, reporting that polyphenol-rich fruit and plant extracts display neuroprotective abilities or reverse cognitive deficits [1–6]. For example, the *Ginkgo biloba* extract EGb 761, a plant extract prescribed in Europe for the treatment of age-related cognitive deficits [7], protected cultured hippocampal neuronal cells against toxicity induced by beta-amyloid (Aβ), a peptide that accumulates in Alzheimer disease (AD) brain [1]. The effects of EGb 761 were attributable to the polyphenolic constituents of the whole extract [1]. Other groups have reported that diets supplemented with berry fruits [8], pomegranate juice [9], a polyphenol-rich juice with purported greatest antioxidant activity [10], or moderate consumption of Cabernet Sauvignon [11] attenuated behavioral deficits in a rodent model of accelerated aging or in transgenic mice overexpressing Aβ peptides. Red wine and tea-derived polyphenols—called catechins or flavanols—have recently received particular attention because of the possible preventive role of these beverages in the incidence of age-related neurological disorders [12–17]. Using cultured rat hippocampal neuronal cells, the neuroprotective abilities of various ingredients enriched in green tea and red wine were investigated against toxicity induced by Aβ peptides, considering their purported deleterious role in AD [18,19]. Our results, along with those obtained by other groups, suggest

that the antioxidant activities of these ingredients do not solely contribute to their neuroprotective action but also involve intracellular signaling pathways or interaction with proteins (such as Aβ and transthyretin [TTR]) associated with cell death/survival. The purpose of this manuscript is to summarize data on the neuroprotective effects of stilbenes and catechin derivatives and discuss the possible molecular mechanisms involved in these effects. We will then review the characterization of specific binding sites for polyphenols in rat brain and their possible relevance in the neuroprotective action of these molecules.

Results

Neuroprotective Effect of Stilbene Analogs Against Aβ-Induced Neurotoxicity

The measurements of cell survival and cell death were performed, as described in detail elsewhere, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT; an indicator of mitochondrial activity) and Sytox® green assays (Molecular Probes, Eugene, OR, USA), respectively [21]. Briefly, 6-day old primary mixed (glial/neuronal) hippocampal cells were exposed to fresh solutions of either Aβ_{25-35} or the physiological fragment Aβ_{1-42} for 24 h in the presence or absence of different polyphenols. The MTT colorimetric assay revealed that treatment with either Aβ_{25-35} (20 μM) or Aβ_{1-42} (20 μM) resulted in about 40% cell death. These effects were concentration dependently reduced in the presence of resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, a naturally occurring stilbene found in red wine), with a maximal effect at 25 μM ($EC_{50} = 13 \pm 3$ μM vs Aβ_{25-35}-induced toxicity). A pretreatment with (2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF 109203X; a protein kinase C [PKC] inhibitor, 1 μM) significantly reduced the neuroprotective effects of resveratrol against Aβ_{25-35}-induced neurotoxicity. In contrast, inhibitors of mitogen-activated protein (MAP) kinase (2′-amino-3′-methoxyflavone [PD 98059], 25 μM), and phosphoinositol-3 (PI3) kinase (2-(4-morpholino)-8-phenyl-4*H*-1-benzopyran-4-one [LY 294002], 5 μM) failed to block the neuroprotective action of resveratrol [2]. Moreover, Western blot data suggested that resveratrol (20–30 μM) induced the phosphorylation of PKC and abolished the inhibitory effect of Aβ_{25-35} on phosphorylation of PKC-δ isoform. Taken together, these results support the role for this PKC isoform in the neuroprotective action of resveratrol [2]. The effect of resveratrol was shared by the resveratrol analog piceatannol (3,3′,4′,5-tetrahydroxy-*trans*-stilbene, 1–25 μM) against Aβ_{25-35}-induced toxicity ($EC_{50} = 11 \pm 2$ μM [MTT assay]), and this was confirmed when piceatannol (10 μM) was co-treated with Aβ_{1-42} (109 ± 5 μM vs 137 ± 7 μM; $P < 0.01$ [Sytox® green values]). Similarly, two other stilbenes analogs known as 3,5-dihydroxy-*trans*-stilbene ($EC_{50} = 17 \pm 3$ μM vs Aβ_{25-35}-induced toxicity) and 3,4,4′-trihydroxy-*trans*-stilbene ($EC_{50} = 6 \pm 1$ μM vs Aβ_{25-35}-induced toxicity) exerted neuroprotective action, whereas *trans*-stilbene, *trans*-4-stilbenemethanol, and diethylstilbestrol failed to protect hippocampal neuronal cells ($EC_{50} >> 50$ μM vs Aβ_{25-35}-induced toxicity) [20].

Effects of Tea Catechins and Gallate Derivatives Against Toxicity Induced by Aβ Peptides

We examined next the effects of tea extracts and their catechin constituents in the same model of toxicity [21]. The neurotoxic effect of Aβ_{25-35} was reduced, in a concentration-dependent manner, by treatment with either green or black tea extract, with a maximal protective effect at 25 μg/mL and 5 μg/mL, respectively. Similar neuroprotective effects were observed with the most abundant green tea polyphenol known as epigallocatechin gallate [EGCG; 1–20 μM]. EGCG appears to be the most potent ingredient in green tea since the effect is significant only at 5μM [21]. The other catechin gallate ester known as epicatechin gallate (ECG), which represents approximately 5% of the total extract [22], was less potent, with a significant effect only at the highest concentration tested here (20 μM), whereas non-gallate forms of catechins such as epicatechin (EC) and epigallocatechin (EGC) were ineffective [21].

The Sytox® green cytotoxic assay indicated that the green (25 μg/mL) and black (5 μg/mL) tea extracts were able to completely block cell death produced by the endogenous fragment Aβ_{1-42} (15 μM). Similar but albeit less potent neuroprotective action was observed with the catechin gallate ester EGCG (10 μM) [21].

Finally, gallic acid (3,4,5-trihydroxybenzoic acid) shared with derivatives such as tannic acid (a polymer of gallic acid molecules), n-propyl gallate (N-propyl 3,4,5-trihydroxybenzoate, an alkyl ester of gallic acid), and pyrogallol (1,2,3-trihydroxybenzene, a metabolite of gallic acid) the ability to protect hippocampal cells against Aβ_{25-35}-induced toxicity, whereas gallic acid methyl ester was ineffective (Table 1).

Effects of Polyphenols on Aggregated, Insoluble Forms of Aβ

On the basis of the purported fibril-destabilizing effects of various polyphenols [23–25], we investigated the *in vitro* effect of various stilbenes and catechins analogs on

Table 1 Effects of gallic acid and derivatives against toxicity induced by a 24-h exposure to 25 μM of Aβ_{25-35} (Sytox® green assay) in comparison to their inhibitory action on Aβ fibril formation produced by a 24-h incubation with 15 μM Aβ_{1-42} (ThT binding assay)

Treatment	Sytox® green values (% of control)	Fibrillar forms of Aβ_{1-42} (% of control)
Control	100 ± 2	100 ± 4
Aβ	190 ± 6	686 ± 109
+ Gallic acid (5 μM)	158 ± 4**	436 ± 46*
+ Gallic acid (10 μM)	163 ± 8**	389 ± 17**
+ Gallic acid (20 μM)	134 ± 5**	246 ± 16**
Control	100 ± 3	100 ± 4
Aβ	190 ± 11	637 ± 34
+ Tannic acid (1 μM)	167 ± 10	374 ± 4**
+ Tannic acid (2 μM)	146 ± 8**	340 ± 8**
+ Tannic acid (5 μM)	115 ± 7**	288 ± 34**
Control	100 ± 2	ND
Aβ	131 ± 4	ND
+ n-propyl gallic acid (1 μM)	126 ± 4	ND
+ n-propyl gallic acid (5 μM)	117 ± 3**	ND
+ n-propyl gallic acid (10 μM)	108 ± 4**	ND
+ n-propyl gallic acid (20 μM)	93 ± 3**	ND
Control	100 ± 2	ND
Aβ	124 ± 3	ND
+ Pyrogallol (1 μM)	120 ± 3	ND
+ Pyrogallol (5 μM)	111 ± 3*	ND
+ Pyrogallol (10 μM)	103 ± 3**	ND
+ Pyrogallol (20 μM)	103 ± 3**	ND
Control	100 ± 1	100 ± 3
Aβ	146 ± 5	425 ± 92
+ Gallic acid methyl ester (1 μM)	140 ± 7	379 ± 98
+ Gallic acid methyl ester (5 μM)	137 ± 9	436 ± 112
+ Gallic acid methyl ester (10 μM)	129 ± 4	400 ± 99
+ Gallic acid methyl ester (20 μM)	136 ± 3	284 ± 34

ND, not determined.

Values represent mean ± SEM of three (MTT) or two (ThT) separate experiments.

*$P < 0.05$, **$P < 0.01$ compared with groups treated with Aβ_{25-35} alone.

aggregated, insoluble forms of Aβ using the thioflavin T (ThT) fluorescence assay, as described previously [23,24 Bastianetto et al., 2003]; Briefly, a fresh solution of Aβ_{1-42} was incubated at 37°C for 24 h in phosphate-buffered saline (pH 7.4). After incubation, a 100-μL aliquot of the solution was added to a final volume of 300 μL of phosphate buffer (50 mM, pH 6.0) containing 5 μM ThT in the presence of different drugs. Fluorescence was then monitored (excitation wavelength = 450 nm, emission wavelength = 485 nm) using a fluorescent plate reader (Bio-Tek Instruments®, Ville St-Laurent, Québec, Canada).

A 24-h incubation with a fresh solution of Aβ_{1-42} (15 μM) resulted in an increase (+337–759% relative to control) in ThT fluorescence that was reduced in the presence of neuroprotective stilbenes (i.e., resveratrol and piceatannol), the green tea extract, EGCG and, to a lesser extent, ECG (although it did not reach significance). In contrast, nonprotective polyphenols (i.e., *trans*-4-stilbenemethanol, transtilbene, diethylstilbestrol, EC, and EGC) were ineffective (Table 2). Among phenolic acids tested here, gallic and tannic acids, but not gallic acid methyl ester, inhibited the formation of Aβ fibrils (Table 1).

Identification of [^{3}H]Resveratrol Binding Sites

Binding Assays and Receptor Autoradiography

Membranes were prepared from whole rat brains, as described previously [26], with minor modifications. In brief, binding assays were initiated by adding the membrane preparations in a solution of Krebs containing [^{3}H]resveratrol and competitors, as described earlier [20]. Saturation experiments were performed at room temperature in the presence of increasing concentrations of [^{3}H]resveratrol, whereas competition binding experiments were performed in the presence of 20 nM [^{3}H]resveratrol and various competitors (10^{-10} to 10^{-4} M). Nonspecific binding was determined in the presence of 100 μM resveratrol [20]. The concentration of competitor required to compete for 50% of specific [^{3}H]resveratrol binding (IC_{50}) was calculated from competition binding curves. Quantitative receptor autoradiography was performed as described previously [26].

Next, we characterized the existence of specific [^{3}H]resveratrol binding sites in rat brain subcellular fractions [20]. Significant specific [^{3}H]resveratrol binding was detected in the plasma membrane (PII fraction), and to a lesser extent, in nuclear and large cellular components. Binding to the PII fraction was significantly reduced by pretreatment with trypsin or boiling, suggesting that specific [^{3}H]resveratrol binding sites are of proteinaceous nature and are particularly abundant in the plasma membrane. Scatchard transformation of isotherm saturation binding experiments suggested that [^{3}H]resveratrol specifically binds to a single class of sites, with an apparent affinity of 220 ± 80 nM in the PII fraction [20].

Quantitative autoradiographic studies revealed significant amounts of specific [^{3}H]resveratrol binding sites in the choroid plexus and subfornical organ, and to a lesser extent, in other regions such as the hippocampal formation and the cortex [20]. We then evaluated a series of resveratrol and catechin derivatives for their ability to compete for specific

Table 2 Effects of catechins and stilbene derivatives on Aβ fibril formation produced by a 24-h incubation with Aβ_{1-42} (15 μM), as monitored by the ThT binding assay

Treatment	Fibrillar forms of Aβ_{1-42}
Neuroprotective polyphenols[a]	
Control	100 ± 2
Aβ_{1-42}	541 ± 79
+ Green tea extract (1 μg/mL)	165 ± 12**
+ Green tea extract (5 μg/mL)	134 ± 7**
+ Green tea extract (10 μg/mL)	119 ± 11**
+ Green tea extract (25 μg/mL)	110 ± 9**
Control	100 ± 3
Aβ_{1-42}	759 ± 57
+ Epigallocatechin gallate (1μM)	411 ± 53**
+ Epigallocatechin gallate (5 μM)	325 ± 22**
+ Epigallocatechin gallate (10 μM)	283 ± 16**
+ Epigallocatechin gallate (20 μM)	204 ± 7**
Control	100 ± 4
Aβ_{1-42}	609 ± 119
+ Epicatechin gallate (1 μM)	596 ± 100
+ Epicatechin gallate (5 μM)	510 ± 85
+ Epicatechin gallate (10 μM)	434 ± 84
+ Epicatechin gallate (20 μM)	362 ± 66
Control	100 ± 2
Aβ_{1-42}	561 ± 74
+ Resveratrol (1 μM)	373 ± 78*
+ Resveratrol (5 μM)	292 ± 59*
+ Resveratrol (10 μM)	270 ± 47**
+ Resveratrol (20 μM)	259 ± 53*
Control	100 ± 2
Aβ_{1-42}	372 ± 49
+ Piceatannol (1 μM)	156 ± 23**
+ Piceatannol (5 μM)	132 ± 24**
+ Piceatannol (10 μM)	106 ± 6**
+ Piceatannol (20 μM)	102 ± 8**
Nonneuroprotective polyphenols[a]	
Control	100 ± 2
Aβ_{1-42}	539 ± 85
+ Epigallocatechin (1 μM)	458 ± 87
+ Epigallocatechin (5 μM)	459 ± 87
+ Epigallocatechin (10 μM)	442 ± 84
+ Epigallocatechin (20 μM)	384 ± 60
Control	100 ± 3
Aβ_{1-42}	337 ± 37
+ Epicatechin (1 μM)	235 ± 44
+ Epicatechin (5 μM)	335 ± 53
+ Epicatechin (10 μM)	343 ± 56
+ Epicatechin (20 μM)	326 ± 51
Control	100 ± 3
Aβ_{1-42}	463 ± 93
+ *Trans*-4-stilbenemethanol[a] (10 μM)	578 ± 99
+ Transtilbene[a] (10 μM)	485 ± 88
+ Diethylstilbestrol[a] (10 μM)	363 ± 44

[a]Based on toxicity induced by either Aβ_{25-35} and/or Aβ_{1-42} [3,16,17]. Values represent mean ± SEM of at least three separate experiments. *$P < 0.05$, **$P < 0.01$ compared with group treated with Aβ_{1-42} alone.

[^{3}H]resveratrol binding in PII fraction. Interestingly, polyphenols that display neuroprotective action are the most potent to compete for specific [^{3}H]resveratrol binding, with IC_{50} values ranging from 45 nM (for EGCG) to 112 nM (for resveratrol), whereas other molecules including *trans*-4-stilbenemethanol and EC were inactive (IC_{50} > 10,000 nM). The affinity of polyphenols and various resveratrol analogs to compete for specific [^{3}H]resveratrol binding correlated very well ($r = 0.74$) with their neuroprotective activity against Aβ_{25-35}-induced toxicity in primary hippocampal cell cultures, suggesting the existence of polyphenol-specific plasma membrane binding sites underlying the neuroprotective action of these compounds [20].

Discussion

Our studies indicated that stilbenes and catechins analogs that are particularly enriched in red wine and teas are able to protect cultured hippocampal cells against Aβ-induced toxicity. These data extend findings showing that grape seed and tea extracts, and their main constituents, display neuroprotective actions in various *in vitro* and *in vivo* models of toxicity [3,11,27–33]. It also suggests that regular consumption of polyphenols may attenuate the deleterious effects of accumulation of Aβ peptides involved in neuronal death occurring in AD [14,34].

Resveratrol and Stilbene Analogs as Modulators of Intracellular Effectors

Resveratrol and its stilbene analog piceatannol concentration dependently protected cultured hippocampal neurons against Aβ-induced toxicity, in agreement with previous studies [32,33]. The mechanism(s) responsible for the neuroprotective effects of resveratrol likely includes PKC since GF 109203X, a potent PKC inhibitor, abolished both the neuroprotective action of resveratrol and the stimulation of the phosphorylation of this enzyme by resveratrol. In contrast, resveratrol failed to modulate the phosphorylation of extracellular signal-regulated kinase (ERK)1 and ERK2 [2, but see 35]. Other studies have shown that resveratrol reversed the phosphorylation of stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) [36] and the activation of caspase 7 [37] and heme oxygenase 1 [38]. Moreover, resveratrol was able to protect cells by increasing the activity of SIRT1, a member of the sirtuin family of protein deacetylases, resulting in the inhibition of intracellular effectors (e.g., p53) implicated in neuronal death/apoptosis [36]. Interestingly, it has been suggested that the decrease in brain levels of Aβ in a mouse model of AD under caloric

restriction (CR) can be reproduced *in vitro* by promoting NAD^+-dependent sirtuin and SIRT1-mediated deacetylase activity, and it may be a mechanism by which CR influences AD-type neuropathology [39]. Moreover, resveratrol was shown to promote intracellular Aβ degradation through a proteasome-dependent, secretases-independent mechanism [40], whereas piceatannol inhibited Aβ-induced DNA fragmentation, possibly by inhibiting the cleavage of poly(ADP-ribose) polymerase (PARP) and the activation of the proapoptotic enzyme, caspase-3 [33]. Taken together, these data and those obtained by other groups suggest that intracellular effectors including the PKC and SIRT1 pathways may play an important role in the neuroprotective action of stilbenes against Aβ-induced toxicity.

Polyphenols Directly Interact with Aβ Peptides

Catechins gallate esters (i.e., EGCG, and to a lesser extent, ECG), found as monomers and dimers in green and black teas, also displayed strong neuroprotective activities, in accordance with previous studies [3,27,29]. These findings suggest that neuroprotective activities of catechin gallate esters depend on the esterification of the pyran hydroxyl group of catechins by gallic acid, a phenolic acid present in tea and red wine with neuroprotective action against Aβ peptides [21].

The ThT assay revealed that phenolic compounds with neuroprotective actions inhibited the formation of Aβ fibrils [21 and present data], in accordance with previous studies, revealing that phenolic compounds display anti-amyloidogenic activities [23–25]. Among them, EGCG and piceatannol appeared to be the most potent polyphenols [present paper]. Moreover, Western blot analysis showed that EGCG was able to inhibit Aβ-derived diffusible ligands [21], small oligomers that have emerged as the potential critical player in the development of AD [19]. Taken together, these data suggest that the neuroprotective action of polyphenols against Aβ-induced neurotoxicity is due, at least in part, to their inhibitory action on Aβ fibril/oligomer formation.

Structure–Activity Relationship of Polyphenols

It is noteworthy that the activity of polyphenols tested here depends on their chemical structures. Resveratrol and its analogs piceatannol, *trans*-stilbene, 3,5-dihydroxy-*trans*-stilbene, and 3,4,4′-trihydroxy-*trans*-stilbene strongly inhibited either neurotoxicity or Aβ fibril formation produced by $A\beta_{1-42}$, suggesting that the hydroxyl groups at the 3,5-positions and 4′-position were important for their activities. Esterification of catechins with gallic acid was essential for the effectiveness in neuroprotection and binding assays. In support of this hypothesis, we have reported a strong neuroprotective/antiaggregation activity of tannic acid, a polymer of gallic acid molecules and glucose.

Polyphenols May Interact with TTR

The binding studies revealed the existence, at the level of the cell plasma membrane, of specific [^{3}H]resveratrol binding sites in the rat brain [20]. These specific binding sites may mediate some of the neuroprotective actions of polyphenols since the most potent compounds against Aβ-induced toxicity are those that bind to these sites with the highest affinities [20]. Autoradiographic studies revealed that the choroid plexus, a brain tissue composed of epithelial cells that makes the cerebrospinal fluid, is particularly enriched in specific [^{3}H]resveratrol binding sites. Interestingly, the choroid plexus synthesizes and secretes TTR, a homotetrameric protein known for the transport of thyroxine and retinol [41]. The misfolding of TTR, characterized by tetramer dissociation and partial monomer denaturation, is involved in amyloid diseases such as the senile systemic amyloidosis, familial amyloid polyneuropathy, and familial amyloid cardiomyopathy [42]. More recently, overexpression of a wild-type human TTR transgene has been shown to decrease Aβ burden in a transgenic murine model of human AD, whereas knocking down the endogenous TTR gene accelerated the development of the neuropathologic phenotype, suggesting that TTR is neuroprotective because of its ability to prevent Aβ aggregates [43–45] but see. It has been hypothesized that inhibition of TTR misfolding by small molecules should be effective against amyloid diseases and can be used as an effective treatment or prophylaxis for such diseases [44]. Interestingly, the same group mentioned above reported that resveratrol fluorescences when bound to at least one of the two thyroid hormone binding sites in the TTR tetramer [46], suggesting that resveratrol and other stilbenes may act as inhibitors that stabilize the native protein structure, thereby acting as Aβ inhibitors [47–49]. Finally, genistein, a phenolic compound present in soy, has been reported to inhibit TTR tetramer dissociation and amyloidogenesis, and exhibits highly selective binding to TTR in the plasma as compared with all other plasma proteins [50]. Recently, we reported a possible role of TTR in the maintenance of learning associated with age [45].

In summary, our results and those obtained by other groups demonstrate that the neuroprotective action of tea catechins gallate esters and resveratrol involves their interaction with genes (i.e., *SIRT1*) and enzymes/proteins located in the plasma membranes, nucleus, and cytoplasm (i.e., secretases, kinases, proteasomes, and PARP)

as well as involves their inhibitory action on fibril formation. It is also possible that polyphenols regulate Aβ fibrilization by binding to TTR, a protein with purported Aβ antifibrillization activities. To support this hypothesis, we have found that polyphenol-specific binding sites were particularly enriched in the choroid plexus, a brain tissue that produces TTR in large amounts. Further studies will be necessary to confirm this hypothesis. These findings support the purported prophylactic effects of regular intake of polyphenols, particularly catechins and stilbenes derived from red wine and teas, against age-related neurological disorders and suggest that polyphenols may be potent neuroprotective agents with pleiotropic activities.

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Abbreviations

Aβ, beta-amyloid;
AD, Alzheimer disease;
EC, epicatechin;
ECG, epicatechin gallate;
EGC, epigallocatechin;
EGCG, epigallocatechin gallate;
GF 109203X, 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl)maleimide;
LY 294002, 2-(4-morpholino)-8-phenyl-4*H*-1-benzopyran-4-one;
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide;
PD 98059, 2′-amino-3′-methoxyflavone;
PKC, protein kinase C;
Th-T, thioflavin T;
TTR, transthyretin.

Conflict of Interest

The authors declare no conflict of interest.

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