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Alkylated resveratrol prodrugs and metabolites as potential therapeutics for neurodegenerative diseases

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KEYWORDS

Resveratrol, neuroprotection, inflammation, prodrug, zebra fish, neurodegenerative diseases

ABSTRACT

Resveratrol is a naturally occurring stilbene which has shown promising results as treatment for several neurodegenerative diseases. However, its application is limited due to its low efficacy and bioavailability. Here, we have designed and synthesized alkylated resveratrol prodrugs combining structural modification to improve antioxidant and anti-inflammatory properties and the preparation of prodrugs to extend drug bioavailability. For comparison we also studied resveratrol prodrugs and alkylated resveratrol derivatives. Methylated and butylated resveratrol derivatives showed the best *in vitro* neuroprotective and anti-inflammatory activity. The glucosyl- and glucosyl-acyl- prodrugs of these derivatives showed lower toxicity on zebra fish embryo. When neuroprotection was examined on pentylenetetrazole challenged zebra fish, they were capable of reverting neuronal damage but to a lower extent than resveratrol. Nevertheless, 3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside resveratrol (compound **8**) recovered AChE activity over 100% whereas resveratrol only up to 92%. In a 3-nitropropionic acid mice model of Huntington's disease, resveratrol derivative **8** delayed the onset and reduced the severity of HD-like symptoms, by improving locomotor activity and protecting against weight loss. Its effects involved an equal antioxidant but better anti-inflammatory profile than resveratrol as shown by SOD2 expression in brain tissue and circulating levels of IL-6 (11 vs 18 pg/mL), respectively. Finally, the octanoyl chain in compound **8** could be playing a role in inflammation and neuronal development indicating it could be acting as a double-drug, instead of as a prodrug.

HIGHLIGHTS

- A series of alkylated resveratrol (RES) prodrugs has been synthesized
- Cytotoxicity, neuroprotective and anti-inflammatory activity were measured
- 3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside resveratrol (**8**) was better neuroprotector than RES
- Compound **8** reduced the severity in a Huntington's disease mice model
- Compound **8** could be a double-drug due to the role of octanoic acid in its structure

1. Introduction

Neurodegenerative diseases are the result of progressive loss of neurons and neuronal connections in the central nervous system (CNS) which normally lead to cognition and motor dysfunction. Despite the differences in clinical manifestations of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS), among others, the pathological processes appear similar, suggesting common neurodegenerative pathways [1]. Mitochondrial malfunction and oxidative stress [2-4] together with the activation of innate immune responses leading to inflammation [5] are known to play a major role in the pathophysiology of neurodegenerative diseases.

A huge effort is being made to obtain drugs to prevent, treat or cure neurodegenerative diseases. The majority of drug discovery programs are based on a 'one-target-one-disease' approach that has not been successful up to now. Thus, alternative approaches such as 'multi-target' strategies have been proposed [6-9]. The main aim is to modulate simultaneously different targets involved in the disease with just a single drug. Natural products have been proposed as sources of multi-targeted lead compounds with proven biological efficacy and safety [8, 10]. In fact, they can be considered as pre-optimized leads for additional structural optimization by improving their bioactivity and/or their pharmacokinetic properties.

Resveratrol **1** (Figure 1) is a natural product with a stilbene scaffold that has shown antiplatelet [11], antitumor [12, 13], anti-inflammatory [14] and neuroprotective activities [15-17]. Its bioactivity comes from direct interactions of resveratrol with multiple molecular targets involved on inflammation, cell cycle arrest, cell signaling, metabolism and posttranslational modification [18, 19]. Resveratrol has shown promising results as treatment of neurodegenerative diseases and thus, has reached clinical trials for AD [20, 21], PD and recently for HD (clinicaltrials.gov). When patients with mild-moderate Alzheimer's disease were treated with resveratrol (1 g by mouth twice daily), declines in cerebrospinal fluid A β 40 levels and activities of daily living scores were attenuated. However, high amounts of resveratrol need to

be administered due to its low bioavailability. These results indicate that resveratrol could be considered a lead compound that needs optimization.

Fig. 1. Resveratrol and related natural products

Two simple natural resveratrol derivatives pinostilbene **2** and pterostilbene **3** (Figure 1) have shown improved neuroprotective capacity with respect to resveratrol. Pinostilbene **2** was reported to exert a potent neuroprotective effect in SH-SY5Y neuroblastoma cells with a wider effective concentration than resveratrol [22]. Low doses of pterostilbene **3** improved significantly radial arm water maze function in SAMP8 mice, a model of AD, together with positive modulation of inflammation and cellular stress whereas resveratrol showed no effect [23]. These results have inspired several groups to prepare *O*-alkylated and *C*-alkylated resveratrol derivatives and to examine their *in vitro* neuroprotective potential. Villalonga-Barber *et al.* [24] reported that 3',5'-*C*-alkylated-resveratrol derivatives were 100-fold more potent than resveratrol modulating oxidative stress on glutamate-challenged HT22 hippocampal neurons. Puksasook *et al.* [25] found that 4-prenylresveratrol exhibited better anti-A β aggregation activity than resveratrol and similar anti-BACE inhibitory activity. Other modifications on resveratrol such as imine resveratrol derivatives, [26] resveratrol tacrine hybrids [27] and resveratrol clioquinol hybrids [28] have also been explored as neuroprotective agents.

Due to the low bioavailability, rapid metabolism and low water solubility of resveratrol, several resveratrol prodrugs have been prepared [29, 30]. Our group previously reported a series of resveratrol

glucosylated and acylated prodrugs (Figure 2) and evaluated their anti-inflammatory activity. Compounds **7** and **8** were capable of decreasing colon inflammation in a mouse dextran sulfate sodium model to a much higher extent than resveratrol [31]. Surprisingly, piceid **4** (Figure 1) and resveratrol lipoconjugates [32] are the only resveratrol prodrugs that have been examined as neuroprotective drugs. Piceid, was able to protect the brain on an ischemic stroke animal model, possibly by up-regulating the expression of Gli1, Ptch1 and SOD1 and down-regulating the expression of NF- κ B [33].

Fig. 2. Resveratrol prodrugs prepared previously.

In this work, our aim was to combine two strategies in the same molecule to optimize resveratrol, i.e. structural optimization through the synthesis of alkylated resveratrol derivatives to increase efficacy and the improvement of bioavailability through the preparation of their prodrugs (Figure 3, Table 1). We have also added resveratrol sulfate metabolites to our approach since metabolites of several drugs have shown to be more active than the drugs themselves, as in the case of morphine-6-glucuronide [34]. Cytotoxicity, neuroprotective capacity and anti-inflammatory activity of alkylated resveratrol derivatives **11-25** were first evaluated *in vitro* in order to optimize the resveratrol scaffold. Then, prodrugs and metabolites of the best alkylated derivatives were synthesized and their toxicity and neuroprotection capacity was evaluated on zebrafish. Finally, the best resveratrol derivative, compound **8**, was investigated as potential treatment

for neurodegeneration using a preclinical model for Huntington's disease. We have chosen this disease because it is a hereditary and degenerative brain disorder (characterized by behavioural, cognitive and motor dysfunctions), for which there is no cure or treatments which can slow down its progression. New approaches in order to develop new therapeutics are strongly needed.

Fig. 3. Strategy for the design of novel resveratrol derivatives, metabolites and prodrugs.

Table 1. Resveratrol derivatives examined in this work.

Comp.	R ₁	R ₂	R ₃	R ₄	Comp.	R ₁	R ₂	R ₃	R ₄					
<i>RES prodrugs</i>					24	iPr	H	H	-					
5	Glc	Glc	H	H	25	H	H	iPr	-					
6	Glc	H	Glc	H	<i>Butyl RES prodrugs</i>									
7	Glc	H	H	COC ₃ H ₇	26	But	Glc	But	H					
8	Glc	H	H	COC ₇ H ₁₅	27	But	Glc	Glc	H					
<i>RES metabolites</i>					28	Glc	Glc	But	H					
9	SO ₃ ⁻	H	H	-	<i>Butyl RES metabolites</i>									
10	SO ₃ ⁻	H	SO ₃ ⁻	-	29	But	SO ₃ ⁻	But	-					
<i>Alkylated RES</i>					30	But	SO ₃ ⁻	SO ₃ ⁻	-					
11	Me	Me	Me	-	31	SO ₃ ⁻	SO ₃ ⁻	But	-					
12	Me	H	Me	-	<i>Methyl RES prodrugs</i>									
13	H	H	Me	-	32	Me	Glc	Me	H					
14	Et	Et	Et	-	33	Glc	Glc	Me	H					
15	Et	H	Et	-	34	Me	Me	Glc	H					
16	Et	H	H	-	35	Me	Glc	Me	COC ₇ H ₁₅					
17	H	H	Et	-	36	Glc	Glc	Me	COC ₇ H ₁₅					
18	But	But	But	-	37	Me	Me	Glc	COC ₇ H ₁₅					
19	But	H	But	-	38	Me	Glc	Me	COC ₃ H ₇					
20	But	H	H	-	39	Me	Me	Glc	COC ₃ H ₇					
21	H	H	But	-	40	Me	Me	Malt	H					
22	iPr	iPr	iPr	-	41	Me	Me	Malt	COC ₇ H ₁₅					
23	iPr	H	iPr	-										

2. Chemistry

Resveratrol glucosylated prodrugs **5** and **6** were synthesized by chemical glycosylation of TBDMS-protected resveratrol derivatives using a peracylated trichloroacetimidate donor, and posterior one-step deprotection with NaOH in MeOH/THF, as previously reported [31]. Piceid acylated prodrugs **7** and **8** were prepared by enzymatic acylation of piceid **4** using *Thermomyces lanuginosus* lipase immobilized on

granulated silica (Lipozyme TL IM). The reactions were carried out in tert-butyl alcohol with the corresponding fatty acid vinyl esters to obtain high yields of the acylated compounds (92-97%) [31]. Resveratrol sulfate metabolites **9** and **10** were prepared following the procedure described by Hoshino *et al.* [35] with an improved purification methodology reported by our group.[36] Briefly, TBDMS-protected resveratrol derivatives were treated with $\text{SO}_3 \cdot \text{NMe}_3$ and NEt_3 in acetonitrile under microwave irradiation. The crude was first purified by LH-20 chromatography, then desilylated with KF in MeOH and finally purified by reversed-phase chromatography.

Methyl, ethyl, isopropyl and butyl resveratrol derivatives were synthesized by random alkylation in DMF using potassium carbonate and the corresponding 1-iodoalkane (Scheme 1) [25, 37]. Subsequent chromatographic separation afforded mono-, di- and trialkyl resveratrol derivatives **11-25** although several derivatives such as pterostilbene (**3**) could not be isolated under the chromatographic conditions used. Compound characterization was easily carried out using NMR spectroscopy due to the differences in symmetry between the mono-substituted regioisomers and the same applies to the di-substituted regioisomers.

Scheme 1. Synthesis of alkylated resveratrol derivatives **11-25**. Reagents and conditions: (a) MeI or EtI or BuI or iPrI, K_2CO_3 , DMF.

Since butyl resveratrol derivatives **18-21** showed the best overall *in vitro* neuroprotective activity and lowest cytotoxicity among the alkylated derivatives examined (see below), we decided to prepare their glucosylated prodrugs **26-28** and sulfate metabolites **29-31** for further investigation. Reaction of a

peracylated glucosyl trichloroacetimidate donor with the corresponding butyl resveratrol derivatives and posterior basic deprotection with NaOMe in MeOH (Scheme 2) resulted on compounds **26-28**. Sulfation of butyl resveratrol derivatives was carried out using the same conditions described above for the silyl resveratrol derivatives and compounds **29-31** were obtained (Scheme 2).

Scheme 2. Synthesis of butylated resveratrol prodrugs and metabolites **26-31**. Reagents and conditions: (a) BF₃·Et₂O, CH₂Cl₂; (b) NaOMe/MeOH; (c) SO₃·NMe₃, TEA, CH₃CN, 100°C, 20 min, MW.

Since pterostilbene (3,5-dimethyl resveratrol) **3** has been reported to exert better neuroprotective and anti-inflammatory activity than resveratrol [23, 38], and the methyl resveratrol derivatives **11-13** were the second best family of alkylated derivatives on *in vitro* neuroprotection studies (see below), we decided to prepare several methyl resveratrol prodrugs **32-41**. Methyl resveratrol glucosylated derivatives **32-34** and **40** were prepared from the corresponding methyl resveratrol derivatives by reaction with peracylated glucosyl or maltotriosyl trichloroacetimidate donors and posterior basic deprotection with NaOMe in MeOH (Scheme 3). Since resveratrol sulfate metabolites showed high toxicity on the zebra fish embryo

acute toxicity assay (see below), we decided to prepare acylated derivatives of methyl glucosyl resveratrol derivatives instead. Moreover, the butanoyl or octanoyl fatty acids could be adding an intrinsic anti-inflammatory effect (see below). Thus, enzymatic acylation of compounds **32-34** and **40** was carried out with Lipozyme TL IM and butyric or octanoic acid vinyl esters to obtain compounds **35-39** and **41** (Scheme 3).

Scheme 3. Synthesis of methylated resveratrol prodrugs **32-41**. Reagents and conditions: (a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 ; (b) NaOMe/MeOH ; (c) Lipozyme TL IM, vinyl fatty acid ester, 55°C , 18h.

3. Results and discussion

3.1. Screening for neuroprotection against oxidative stress

To test if the alkylated modifications on resveratrol had any effect on its reported neuroprotective capacity [39], we evaluated viability of SH-SY5Y neuroblastoma cells after oxidative stress challenge with hydrogen peroxide in the presence or absence of the prepared alkylated resveratrol derivatives **11-25** [40]. We previously determined their cytotoxic effects at 10 μ M concentration on SH-SY5Y cells (Fig. S1) and found none of the compounds were toxic at the examined concentration. All alkylated resveratrol compounds **11-25** investigated counteracted to some extent oxidative damage on neuroblastoma cells produced by hydrogen peroxide at 1 and 10 μ M concentration. Methylated and butylated resveratrol derivatives, **11-13** and **18-21** respectively, showed a recovery up to 80-100% cell viability whereas resveratrol **1** only recovered up to 55% cell viability. These results are in accordance with previously reported results on the capacity to reduce oxidative stress of methylated resveratrol derivatives [22] and other alkylated derivatives [25] on similar cellular systems. At 100 μ M concentration several alkylated derivatives (**12**, **16**, **17**, **19-21** and **23-25**) showed no neuroprotection possibly due to their toxicity at higher concentrations as it could be deduced from toxicity data in zebra fish embryo (see below).

Fig. 4. Neuroprotective activity of alkylated resveratrol derivatives **11-25** on hydrogen peroxide-treated SH-SY5Y cells. Cell viability was evaluated in SH-SY5Y cells after 24h of hydrogen peroxide incubation (100 μ M) in combination with the compounds at 1, 10, and 100 μ M. Dashed line: reference value for viability found on the H₂O₂ control without compounds; black bar: control SH-SY5Y cell viability; white bar: SH-SY5Y cell viability after hydrogen peroxide incubation. Each bar represents mean $\pm$ standard deviation of at least two different experiments run in quadruplicate.

3.2. Screening for inhibitory activity against LPS-induced TNF- α and IL-6 release

Resveratrol has been reported to decrease inflammation by inhibiting the induced production of pro-inflammatory cytokines, such as TNF- α , IL-1 β , IL-6, IL-8 and matrix metalloproteinases MMP-2, MMP-3, MMP-9 and MMP-13, both on *in vitro* and on *in vivo* models [41-43]. We measured two inflammatory markers (TNF- α and IL-6) on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells in the presence or absence of the alkylated resveratrol derivatives prepared. Peralkylated resveratrol derivatives were discarded due to their high hydrophobicity and no possibility of further modification. We found that the selected alkylated resveratrol compounds showed a higher inhibition of TNF- α production than

resveratrol, except for 4'-ethylresveratrol **17** and 4'-isopropylresveratrol **25** (Figure 5A). The levels of IL-6 produced were similar for resveratrol and all the investigated alkylated derivatives (Figure 5B).

Fig. 5. Anti-inflammatory profile of alkylated resveratrol derivatives in RAW macrophages. TNF- α (A) and IL-6 (B) production was determined in RAW cells after 24h of LPS incubation (100 ng/ml) in combination with the compounds at 10 μ M concentration. Data are shown as percentage of cytokine inhibition and they were normalized using the values corresponding to LPS and basal- production in RAW cells as reference values for maximal and minimum cytokine production, respectively. Dashed line: reference values for anti-inflammatory activity exerted by unmodified resveratrol **1**. Each bar represents mean $\pm$ standard deviation of at least two different experiments run in quadruplicate.

3.3. Assessment of acute toxicity on zebra fish

To screen compound toxicity on zebra fish is a cost-effective model due to the high fecundity, rapid embryonic development and high homology to mammalian species of zebrafish [44]. Moreover, the embryo is preferred to adult fish because it is predicted that early life stages feel less pain and distress than adult fish. Fast acute toxicity was measured by incubation of zebrafish embryos with increasing concentration of each compound and cumulative mortality/toxicity was observed after 96 hpf (hours post

fertilization) [45]. It is important to note that cumulative mortality/toxicity is due to both developmental impact and organotoxicity.

In vivo toxicity on zebrafish embryo was measured for representative compounds of each family of resveratrol derivatives under study, alkylated resveratrol derivatives **11-25** and their corresponding synthesized prodrugs and metabolites **26-41**. We also measured toxicity for several resveratrol prodrugs **5-8**, metabolites **9-10** and resveratrol **1** itself. Table 2 and Figure S3 show NOEC, LOEC and LC₅₀ values for acute toxicity on zebrafish embryos. We observed that alkylated resveratrol derivatives **16**, **20** and **24** were quite toxic with LC₅₀ values from 11 to 13 µM whereas methylated resveratrol derivative **13** and pterostilbene **3** were slightly less toxic with LC₅₀ values of 32 and 36 µM, respectively. Addition of a sulfate group to the butyl resveratrol structure as in compound **30** did not improve toxicity (10 µM). In contrast, adding a glucose unit to the butyl resveratrol structure as in compounds **27** and **28** resulted in less toxic derivatives.

In the case of the methylated resveratrol derivatives, their modification with glucosyl or glucosyl-acyl groups (compounds **34-41**) resulted in similar toxicity to the parent compounds with LC₅₀ values from 29-45 µM. The exceptions were compounds **36** (8 µM) and **40** (379 µM). The least toxic compounds of the entire series were resveratrol **1** itself together with resveratrol prodrugs piceid **4**, diglucosyl resveratrol derivatives **5** and **6**, piceid butyrate **7**, piceid octanoate **8** and 4'-*O*-maltotriosyl pterostilbene **40**.

Table 2. NOEC, LOEC and LC₅₀ values for acute toxicity on zebra fish embryos for resveratrol prodrugs, metabolites and derivatives.

Compound	NOEC (μM)	LOEC (μM)	LC ₅₀ (μM)
1	1000	>1000	>1000
3	10	100	36
4	100	1000	114
<i>RES prodrugs</i>			
5	1000	>1000	>1000
6	1000	>1000	>1000
7	100	1000	315
8	1	10	138
<i>RES metabolites</i>			
9	10	100	29
10	0.1	1	17
<i>Alkylated RES</i>			
13	10	100	32
16	1	10	13
20	0.1	1	11
24	0.1	1	11
<i>Butylated RES prodrugs</i>			
27	10	100	35
28	10	100	21
<i>Butylated RES metabolites</i>			
30	0.1	1	10
<i>Methylated RES prodrugs</i>			
34	10	100	29
35	10	100	32
36	1	10	8
37	10	100	45
38	10	100	32
39	10	100	44
40	10	100	379
41	10	100	38

LC₅₀ (median lethal dose), calculated by fitting sigmoidal curve to mortality data ($y = \text{Bot} + (\text{Top} - \text{Bot}) / (1 + 10^{-(k \cdot (x_0 - \text{Log}(C)))})$). Bot, minimum mortality; Top, maximum mortality; k, curve slope; x₀, LC₅₀ estimated. NOEC (No observed effect concentration, with mortality score > 20% assumed as the effect). LOEC (Lowest observed effect concentration, with mortality score > 20% assumed as the effect). Negative control: 0.1% DMSO, in three replicates. Positive controls: 4-Diethylaminobenzaldehyde (DEAB) at 5 different concentrations (0.1 μM, 1 μM, 10 μM, 100 μM, 1 mM). DEAB is a competitive inhibitor of aldehyde dehydrogenases known to generate toxic and teratogenic effects.

3.4. Assessment of neuroprotective activity on zebra fish

Pentylentetrazole (PTZ) is a competitive GABA antagonist which acts blocking Cl^- anion conductance and the formation of inhibitory postsynaptic potentials [46]. In zebra fish, PTZ acts as a pro-convulsant agent that blocks the GABAergic inhibitory synaptic transmission [47]. PTZ has been used previously in rats to induce neurodegeneration associated to an increase of damaged neurons, oxidative stress and neuroinflammation [48-51]. We measured acetylcholinesterase (AChE) activity on PTZ challenged zebra fish embryo since acetylcholine is a neurotransmitter involved in movement control and an important modulator of cognitive functions such as learning and memory [52]. Therefore, appropriate levels of AChE reflect a healthy neuronal state. Five days post fertilization (dpf) zebra fish larvae were pre-incubated with each of the resveratrol derivatives for 1h. Then, media was changed and larvae were co-incubated with PTZ and each of the resveratrol derivatives for 6h. Finally, larvae were processed to measure AChE activity under each condition. A group treated with physostigmine (Phys), a commercial inhibitor of the enzyme AChE, was included as positive control.

In a first assay we examined two prodrugs, 3,5-diglucosyl-resveratrol **5** and piceid octanoate **8** and the three butylated resveratrol glucosylated prodrugs **26-28** together with control resveratrol **1**. Butylated resveratrol sulfate metabolites were not included due to their toxicity on zebrafish embryo. We found that AChE activity of resveratrol-3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside **8** group was fully recovered even over 100% (Figure 6A). In fact, its AChE activity was higher than the one found for resveratrol (92%) and for the other resveratrol prodrugs in this series. In a second assay we evaluated a series of methylated resveratrol prodrugs (**35**, **37**, **39** and **41**) to check the possible relevance of the methyl group position, the number of glucosyl groups and the optimum fatty acid group (Figure 6B). In this second assay all the compounds examined possessed a fatty acid chain because the results on the first assay pointed to their possible relevant role in neuroprotection. We found the methyl resveratrol prodrugs examined were capable of partially recovering from PTZ damage but none of them showed higher AChE activity than resveratrol.

Fig. 6. Acetylcholinesterase (AChE) activity of resveratrol derivatives. The percentage of AChE (mU) enzyme activity normalized to total protein (μg) vs control (considered as 100%) is shown. A) Series of resveratrol glucosylated prodrugs and the butyl resveratrol prodrugs examined: B) Series of methyl resveratrol prodrugs examined. Resveratrol was used as internal control. PTZ is pentylenetetrazole and Phys is physostigmine. Two independent experiments were carried out with ten replicates of each experimental condition. Each bar represents mean $\pm$ standard error of the mean (SEM). ANOVA statistics were carried out followed by Dunnett's test with multiple comparisons. It is considered significant when # $P < 0.05$, ## $P < 0.01$ respect to the control; ** $P < 0.01$ respect to the Control + PTZ.

We observed in our neuroprotective assay in zebra fish that 3,5-*O*-di- β -D-glucopyranosyl resveratrol **5**, a similar prodrug to resveratrol-3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside **8** failed to prevent AChE activity loss. This result indicates that the octanoyl chain could be playing a key active role. Short and medium chain fatty acids have been reported to possess anti-inflammatory activity[53] and octanoic acid was capable of partly restoring mitochondrial respiration on starved human endothelial cells and monocytes under inflammatory conditions [54]. Octanoic acid is the main component of medium chain triglycerides (MCT), and diets with a carbohydrate-reduction and an increase in MCT intake, constitute an alternative for the treatment of drug-resistant epilepsy in children [55, 56]. Moreover, octanoic acid induces neurite outgrowth in PC12 neuronal cells and shows a maximum effect in comparison with shorter or longer fatty acids [57]. Growth of neurite processes from the cell body is a critical step in neuronal development. Kamata *et al.* [57] suggested that the mechanism responsible is related with the activation of p38 mitogen-activated protein kinase (MAPK) and the extracellular signal-regulated kinase (ERK). This scenario, where the medium chain fatty acid and resveratrol would be playing a role side-by-side, indicates that compound **8** could indeed be a double-drug. Other double-drugs have been reported earlier as antimalarial [58], anti-HIV [59] or as antiproliferative agents [60].

Taking together, these results indicate that piceid octanoate **8** appeared to be one of the most effective resveratrol derivatives on neuroprotection. Therefore, we decided to evaluate its protective effect in a preclinical model of neurodegeneration.

3.5. Neuroprotective activity on a 3-nitropropionic acid mice model

3-Nitropropionic acid (3-NP) is an irreversible inactivator of the succinate dehydrogenase and an inducer of mitochondrial dysfunction, which leads to an energy deprivation and oxidative stress exacerbation that promotes neuronal damage. 3-NP-induced neurodegeneration results in a striking preferential damage of striatum [61-63], the mainly anatomical region affected in Huntington's disease (HD). HD is a mortal progressive disorder caused by a dominant inherited expansion of CAG repeats in the Huntington gene.

HD is characterized by neurological and systemic manifestations including cognitive, psychiatric and motor defects which are accompanied and even preceded in time by an increment of circulating pro-inflammatory cytokines (*i.e.* IL-6, TNF- α) [64, 65]. A plethora of evidences shows that 3-NP chronically administrated in rodents and primates can replicate key abnormalities underlying HD pathogenesis, including molecular alterations dependent of mitochondrial dysfunction (energetic deficit, oxidant species accumulation) as well as clinical (motor and cognitive) signs [61, 63, 66, 67] supporting the potential utility of the 3-NP intoxication model in HD studies [68, 69].

To analyze the neuroprotective and immune actions exerted by compound **8**, we used the 3-NP intoxication model (as described in the experimental section). Mice were given increasing amounts of the toxin accompanied by the administration of compound **8** or resveratrol **1** which was used as reference.

Fig. 7. Evaluation of the activity of compound **8** in a 3-NP-induced neurodegeneration animal model. Mice were treated with vehicle (Veh), 3-NP, 3-NP plus resveratrol (**1**) or 3-NP plus compound **8**, and semi-quantitative motor-deficits (score from 0 to 8) (**A**) and body weight changes (relative to initial weight) (**B**) were evaluated at different time points (n = 7 mice). [&]*P* < 0.05 3-NP vs. veh, [#]*P* < 0.05 3-NP vs. 1+3-NP, ^a*P* < 0.05 3-NP vs. 8+3-NP. At the end of experiment, (**C**) SOD2 and p53 mRNA levels in whole brain-derived samples were determined by qRT-PCR (n = 4-7 mice) and (**D**) serum IL-6 levels detected were assayed by ELISA (n = 6-7 mice). Results represent mean ± SEM. **P* < 0.05, ****P* < 0.005, n.s.: non-significant.

We found an increment of the motor deficit-score scale and the weight loss in response to increasing doses of 3-NP (Figure 7A-B). Furthermore, administration of compound **8** had a protective effect similar to resveratrol on ameliorating loss of both motor coordination (Figure 7A) and weight induced by toxin (Figure 7B). To evaluate the mechanisms of action of compound **8** on molecular hallmarks of HD such as oxidative stress, neuronal death, and pro-inflammatory cytokines overproduction, we measured the expression of SOD2 and p53, as well as the serum circulating level of IL-6 (Figure 7C-D). 3-NP increased the expression of the proapoptotic p53 but neither resveratrol nor compound **8** counteracted its effect. However, we found that although 3-NP did not appear to modify the basal level of SOD2, both resveratrol and compound **8** induced in a similar way the overexpression of SOD2, an indicative result of their antioxidant capacity (Figure 7C). In addition, 3-NP administration replicated the pro-inflammatory phenotype of HD leading an increase of circulating IL-6 production. Interestingly, IL-6 stimulation was efficiently counteracted by compound **8**, while resveratrol produced a slight but not significant amelioration (Figure 7D). All together these results show that compound **8** could exert neuroprotective actions accompanied by antioxidant and anti-inflammatory effects *in vivo* as good as, or even better than resveratrol itself, making of compound **8** a promising candidate for the therapeutic treatment of neurodegenerative diseases such as HD.

4. Conclusions

In this study, we have combined two approaches for lead optimization of resveratrol for neurodegenerative diseases, structural optimization through the synthesis of resveratrol derivatives to improve its efficacy and an improvement in bioavailability through the preparation of their glucosylated and acylated prodrugs. We have designed and prepared resveratrol prodrugs, alkylated resveratrol derivatives and alkylated resveratrol prodrugs, together with sulfate metabolites of some of these resveratrol derivatives. Methylated and butylated resveratrol derivatives showed better neuroprotective and anti-inflammatory activity than resveratrol in the initial *in vitro* drug screening. When we examined toxicity in animals using a zebra fish

embryo model, we observed that alkylated resveratrol derivatives were highly toxic whereas their glycosylated prodrugs showed lower toxicity. When we investigated neuroprotection activity on zebra fish on a pentylenetetrazole challenged zebra fish, resveratrol-3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside **8** displayed better neuroprotective activity than resveratrol. Methylated and butylated resveratrol prodrugs also could partially recover zebra fish embryo from neuronal damage although to a lower extent than resveratrol. We cannot rule out whether the intrinsic toxicity of the alkylated resveratrol derivatives are preventing a better recovery from the PTZ damage. We also observed that resveratrol-3-*O*-(6'-*O*-octanoyl)- β -D-glucopyranoside **8** was capable of ameliorating the onset and reducing the severity of HD-like symptoms induced by 3-nitropropionic acid in mice, used as a model of HD. Compound **8** improved mice locomotor activity and prevented weight loss in a similar way to resveratrol. Whereas their antioxidant effect seems to be comparable as revealed by SOD2 expression levels in brain tissue, the anti-inflammatory activity of compound **8** appears to be better than that of resveratrol as indicated by the circulating levels of IL-6. Finally, the octanoic acid chain within the structure of compound **8** could be playing an active role on inflammation and neuronal development what suggests **8** could be acting as a double-drug.

5. Experimental section

5.1. Chemistry

All solvents and chemicals were used as purchased without further purification. All reactions were monitored by TLC on precoated silica gel 60 plates F₂₅₄ (Merck) and detected by heating after staining with H₂SO₄:EtOH (1:9, v/v), anisaldehyde (450 ml ethanol, 25 ml anisaldehyde, 25 ml H₂SO₄ and 1 ml AcOH) or Mostain (500 ml of 10% H₂SO₄, 25g of (NH₄)₆Mo₇O₂₄•4H₂O, 1g Ce(SO₄)₂•4H₂O). Products were purified by flash chromatography with silica gel 60 (200-400 mesh). Eluents are indicated for each

particular case. NMR spectra were recorded on Bruker Advance 300, 400 or 500 MHz [300, 400 or 500 MHz (^1H), 75, 101 or 126 (^{13}C)] NMR spectrometers, at room temperature for solutions in CDCl_3 , or CD_3OD . Chemical shifts are referred to the solvent signal. 2D experiments (COSY, TOCSY, ROESY, and HMQC) were done when necessary to assign the new compounds. Chemical shifts are in ppm. Low resolution mass spectra were obtained on an ESI/ion trap mass spectrometer. High resolution mass spectra (HRMS) were obtained on an ESI/quadrupole mass spectrometer (WATERS, ACQUITY H CLASS). If necessary, the purity was determined by high performance liquid chromatography (HPLC). Purity of all final compounds was 95% or higher. The instrument used for chromatographic separation was a Waters Acquity UPLCTM H-class system (Waters, Manchester, UK). The column was an Acquity UPLC^R BEH C18 (2.1 x 100 mm, 1.7 μM). A QDA single quadrupole mass spectrometer (Waters) equipped with an orthogonal Z-sprayTM electrospray ionization (ESI) source was used for metabolites detection. Empower 3 software was used for instrument control, peak detection and integration.

5.1.1. General procedure for etherification. Resveratrol (1 eq.) and potassium carbonate (2 eq.) were added to DMF (2.85 ml/mmol of resveratrol) under agitation in a round-bottomed flask. 1-Iodoalkane (1-1.5 eq) was added dropwise and reaction was stirred for 6 h at room temperature. The reaction mixture was filtered, diluted with water and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried with MgSO_4 and the mixture filtered, concentrated. The crude was purified by flash column chromatography using different hexane/ethyl acetate mixtures.

5.1.1.1. Preparation of butylated resveratrol derivatives (18-21). Following the general procedure and starting from resveratrol (513 mg, 2.25 mmol) and 1-iodobutane (0.38 mL, 3.37 mmol) the reaction yielded **18-21** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (8:1 to 1:1).

5.1.1.1.1. (E)-1-(3,5-dibutoxyphenyl)-2-(4-butoxyphenyl)ethene (18). Yield=16%; R_f = 0.9 (Hexane/ethyl acetate, 2:1). Compound characterization in accordance to literature [70].

5.1.1.1.2. (*E*)-1-(3-butoxy-5-hydroxyphenyl)-2-(4-butoxyphenyl)ethene (**19**). Yield=31%; R_f = 0.6 (Hexane/ ethyl acetate, 2:1). ^1H NMR (400 MHz, CDCl_3) δ = 7.44 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 16.3 Hz, 1H), 6.97 – 6.82 (m, 3H), 6.74 – 6.63 (m, 1H), 6.63 – 6.54 (m, 1H), 6.35 (t, J = 2.2 Hz, 1H), 4.04 – 3.94 (m, 4H), 1.89 – 1.76 (m, 4H), 1.60 – 1.43 (m, 4H), 1.08 – 0.94 (m, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 160.64, 158.97, 156.84, 140.02, 129.76, 128.90, 127.83, 126.17, 115.70, 114.77, 105.61, 105.38, 105.00, 101.12, 67.86, 31.33, 19.28, 13.89; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{22}\text{H}_{28}\text{O}_3$ 341.2111, found 341.2104.

5.1.1.1.3. (*E*)-1-(3-butoxy-5-hydroxyphenyl)-2-(4-hydroxyphenyl)ethene (**20**). Yield=13%; R_f = 0.25 (Hexane/ ethyl acetate, 2:1). ^1H NMR (400 MHz, CD_3OD) δ = 7.38 (d, J = 8.3 Hz, 2H), 7.01 (d, J = 16.3 Hz, 1H), 6.86 (d, J = 16.3 Hz, 1H), 6.79 (d, J = 8.3 Hz, 2H), 6.57 (s, 2H), 6.27 (s, 1H), 3.95 (t, J = 6.4 Hz, 2H), 1.81 – 1.69 (m, 2H), 1.51 (dd, J = 14.9, 7.4 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 160.58, 158.22, 157.00, 139.91, 129.02, 128.27, 127.51, 125.62, 115.12, 105.16, 103.76, 100.57, 67.30, 31.20, 18.96, 12.87; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{18}\text{H}_{20}\text{O}_3$ 285.1485, found 285.1480.

5.1.1.1.4. (*E*)-1-(3,5-dihydroxyphenyl)-2-(4-butoxyphenyl)ethene (**21**). Yield=35%; R_f = 0.3 (Hexane/ethyl acetate, 2:1). ^1H NMR (400 MHz, CD_3OD) δ = 7.40 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 16.3 Hz, 1H), 6.90 – 6.81 (m, 3H), 6.51 (s, 2H), 6.23 (s, 1H), 3.92 (t, J = 6.4 Hz, 2H), 1.77 – 1.65 (m, 2H), 1.48 (dd, J = 15.0, 7.5 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ = 158.90, 158.28, 139.87, 129.91, 127.85, 127.36, 126.24, 114.33, 104.56, 101.43, 67.39, 31.14, 18.93, 12.87; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{18}\text{H}_{20}\text{O}_3$ 285.1485, found 285.1484.

5.1.1.2. Preparation of isopropylated resveratrol derivatives (22-25). Following the general procedure and starting from resveratrol (529 mg, 2.32 mmol) and 2-iodopropane (0.23 mL, 2.32 mmol) the reaction yielded **22-25** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (8:1 to 2:1).

5.1.1.2.1. (*E*)-1-(3,5-di-isopropoxyphenyl)-2-(4-isopropoxyphenyl)ethene (**22**). Yield=2%; R_f = 0.8 (Hexane/ ethyl acetate, 2:1). Compound characterization in accordance to literature [71].

5.1.1.2.2. (*E*)-1-(3-isopropoxy-5-hydroxyphenyl)-2-(4-isopropoxyphenyl)ethene (**23**). Yield=15%; R_f = 0.5 (Hexane/ethyl acetate, 2:1). ¹H NMR (400 MHz, CDCl₃) δ = 7.39 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 16.3 Hz, 1H), 6.91 – 6.77 (m, 3H), 6.58 (s, 1H), 6.56 (s, 1H), 6.28 (s, 1H), 4.62 – 4.41 (m, 2H), 1.28 (dd, *J* = 8.5, 6.4 Hz, 12H). ¹³C NMR (101 MHz, CDCl₃) δ = 159.31, 157.64, 156.89, 140.07, 129.82, 128.86, 127.89, 126.23, 116.16, 106.91, 105.66, 102.37, 70.21, 22.11, 22.07; ESI-HRMS [*M* - H] calcd for C₂₀H₂₄O₃ 311.1647, found 311.1633.

5.1.1.2.3. (*E*)-1-(3-isopropoxy-5-hydroxyphenyl)-2-(4-hydroxyphenyl)ethene (**24**). Yield=12%; R_f = 0.15 (Hexane/ethyl acetate, 2:1). ¹H NMR (400 MHz, CD₃OD) δ = 7.36 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 16.2 Hz, 1H), 6.89 – 6.75 (m, 3H), 6.56 (s, 2H), 6.56 (s, 1H), 6.29 (s, 1H), 4.46 (dt, *J* = 12.0, 6.0 Hz, 1H), 1.24 (d, *J* = 6.1 Hz, 6H). ¹³C NMR (101 MHz, CD₃OD) δ = 158.26, 157.55, 139.99, 129.89, 128.01, 127.50, 126.27, 115.79, 104.77, 101.56, 69.75, 21; ESI-HRMS [*M* + H] calcd for C₁₇H₁₈O₃ 271.1329, found 271.1323.

5.1.1.2.4. (*E*)-1-(3,5-dihydroxyphenyl)-2-(4-isopropoxyphenyl)ethene (**25**). Yield=33%; R_f = 0.4 (Hexane/ethyl acetate, 1:1). ¹H NMR (400 MHz, CD₃OD) δ = 7.38 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 16.3 Hz, 1H), 6.85 (d, *J* = 16.3 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 2H), 6.58 (s, 1H), 6.56 (s, 1H), 6.28 (s, 1H), 4.56 (dt, *J* = 12.0, 6.0 Hz, 1H), 1.31 (d, *J* = 6.0 Hz, 6H). ¹³C NMR (101 MHz, CD₃OD) δ = 159.22, 158.25, 156.98, 139.98, 129.05, 128.29, 127.54, 125.65, 115.16, 105.40, 105.23, 102.03, 69.64, 21.10; ESI-HRMS [*M* + H] calcd for C₁₇H₁₈O₃ 271.1329, found 271.1331.

5.1.2.1. General Procedure for glycosylation. 2,3,4,6-tetra-*O*-benzoyl-α/β-*D*-glucopyranosyl trichloroacetimidate **42**⁵³ (1 mmol) was dissolved in anhydrous CH₂Cl₂ (15 mL) in a 50 mL round-bottomed flask under argon atmosphere. Butylated resveratrol derivative (0.33 mmol) and boron trifluoride diethyl etherate (0.033 mmol) were added to the stirring solution and after 30 min, the reaction was stopped with triethylamine (5 mL) and concentrated. The crude product was purified by flash column chromatography using different hexane/ethyl acetate mixtures with 1% TEA.

5.1.2.1. (*E*)-1-(3-butoxy-5-(2,3,4,6-tetra-*O*-benzoyl- β -*D*-glucopyranosyloxy)phenyl)-2-(4-butoxyphenyl)ethene (**43**). Following the general procedure and starting from 3,4'-di-*O*-butyl-resveratrol **19**, the reaction yielded **43** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (6:1). Yield=95%; *R*_f = 0.6 (Hexane/ethyl acetate, 2:1). ¹H NMR (300 MHz, CDCl₃) δ = 7.93 (d, *J* = 7.4 Hz, 1H), 7.90 – 7.77 (m, 5H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 7.4 Hz, 1H), 7.32 – 7.24 (m, 3H), 7.17 (dd, *J* = 16.9, 8.6 Hz, 8H), 7.09 (d, *J* = 3.7 Hz, 2H), 6.83 (d, *J* = 16.2 Hz, 1H), 6.76 – 6.57 (m, 3H, CH), 6.51 (s, 1H), 6.42 (s, 1H), 6.35 (s, 1H), 6.05 – 5.85 (m, 1H), 5.79 – 5.59 (m, 2H), 5.50 – 5.37 (m, 1H), 4.66 – 4.47 (m, 1H), 4.47 – 4.33 (m, 1H), 4.33 – 4.20 (m, 1H), 3.79 (t, *J* = 6.5 Hz, 2H), 3.68 (t, *J* = 5.5 Hz, 2H), 1.66 – 1.54 (m, 2H), 1.49 (dd, *J* = 14.4, 6.6 Hz, 2H), 1.40 – 1.15 (m, 4H), 0.88 – 0.67 (m, 6H); ¹³C NMR (75 MHz, CDCl₃) δ = 166.49, 166.36, 166.12, 165.99, 165.91, 165.60, 165.51, 163.92, 160.73, 159.38, 158.55, 140.29, 133.87, 133.68, 133.56, 133.45, 130.28, 130.11, 130.00, 129.80, 129.76, 129.54, 129.37, 129.06, 129.03, 129.00, 128.78, 128.71, 128.67, 128.21, 126.16, 117.69, 115.00, 107.92, 107.46, 103.30, 99.90, 92.47, 89.13, 73.21, 72.90, 72.17, 70.74, 69.99, 68.64, 68.02, 63.70, 62.55, 31.60, 31.50, 31.28, 30.02, 19.55, 19.50, 14.19, 14.17; ESI-HRMS [*M* + *H*] calcd for C₅₆H₅₄O₁₂ 919.3694, found 919.3720.

5.1.2.2. (*E*)-1-(3-butoxy-5-(2,3,4,6-tetra-*O*-benzoyl- β -*D*-glucopyranosyloxy)phenyl)-2-(4-(2,3,4,6-tetra-*O*-benzoyl- β -*D*-glucopyranosyloxy)phenyl)ethene (**44**). Following the general procedure and starting from 3-*O*-butyl-resveratrol **20** (0.33 mmol), the reaction yielded **44** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (4:1). Yield = 88%; *R*_f = 0.3 (Hexane/ethyl acetate, 2:1). ¹H NMR (300 MHz, CDCl₃) δ = 7.92 (d, *J* = 7.7 Hz, 2H), 7.85 (t, *J* = 8.6 Hz, 10H), 7.76 (d, *J* = 7.6 Hz, 4H), 7.39 – 7.12 (m, 24H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.77 (d, *J* = 16.2 Hz, 1H), 6.67 – 6.54 (m, 3H, CH), 6.38 (s, 1H), 5.95 (t, *J* = 9.5 Hz, 2H), 5.75 (dd, *J* = 13.3, 5.5 Hz, 2H), 5.66 (dd, *J* = 12.4, 6.4 Hz, 2H), 5.43 (d, *J* = 7.6 Hz, 1H), 5.37 (d, *J* = 7.7 Hz, 1H), 5.12 (s, 1H), 4.62 (t, *J* = 9.1 Hz, 2H), 4.54 – 4.38 (m, 3H), 4.34 – 4.22 (m, 2H), 3.73 (t, *J* = 5.7 Hz, 2H), 1.61 – 1.49 (m, 2H), 1.30 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.81 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ = 171.40, 166.42, 166.28, 166.05, 165.55, 160.70, 158.50, 156.80, 139.84, 133.83, 133.65, 130.05, 129.93,

129.35, 128.96, 128.70, 128.63, 128.03, 117.62, 108.06, 107.61, 99.78, 73.08, 72.94, 72.02, 69.89, 68.09, 63.49, 60.65, 53.75, 31.46, 30.19, 29.91, 21.28, 19.45, 14.46, 14.11; ESI-HRMS [M + Na] calcd for C₈₆H₇₂O₂₁ 1463.4464, found 1463.4453.

5.1.2.3. (*E*)-1-(3,5-di-(2,3,4,6-tetra-*O*-benzoyl- β -D-glucopyranosyloxy)phenyl)-2-(4-butoxy)phenyl ethene (**45**). Following the general procedure and starting from 4'-*O*-butyl-resveratrol **21** (0.33 mmol), the reaction yielded **45** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (4:1). Yield = 87%; R_f = 0.3 (Hexane/ethyl acetate, 2:1). ¹H NMR (300 MHz, CDCl₃) δ = 7.84 (dd, *J* = 13.3, 7.5 Hz, 12H), 7.74 (d, *J* = 7.5 Hz, 4H), 7.40 – 7.08 (m, 27H), 6.74 (dd, *J* = 17.5, 11.8 Hz, 4H), 6.60 – 6.42 (m, 2H), 5.90 (t, *J* = 9.5 Hz, 2H), 5.65 (dd, *J* = 19.6, 9.6 Hz, 4H), 5.29 (s, 1H), 5.26 (s, 1H), 4.56 (d, *J* = 9.7 Hz, 2H), 4.41 (dd, *J* = 12.0, 5.7 Hz, 2H), 4.21 – 4.05 (m, 2H), 3.82 (t, *J* = 6.5 Hz, 2H), 1.60 (dd, *J* = 14.4, 6.5 Hz, 2H), 1.34 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.82 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ = 171.40, 166.45, 166.06, 165.54, 165.29, 159.47, 158.33, 140.81, 133.82, 133.64, 133.46, 130.13, 130.02, 129.66, 129.50, 129.38, 129.00, 128.76, 128.72, 128.64, 128.29, 125.34, 114.92, 110.33, 99.68, 73.02, 72.71, 72.05, 70.01, 68.01, 63.56, 60.66, 31.56, 29.98, 21.29, 19.51, 14.47, 14.15; ESI-HRMS [M + Na] calcd for C₈₆H₇₂O₂₁ 1463.4464, found 1463.4445.

5.1.3. General Procedure for benzoyl deprotection. Butyl perbenzoylated glucopyranosyl resveratrol derivative (0.35 mmol) was dissolved in CH₂Cl₂ (5 mL) in a 25 mL round-bottomed flask to give a yellow solution. A solution of 25% sodium methoxide in methanol (5 mL) was added and left under stirring for 5 h. CH₂Cl₂ (20 mL) was then added and extracted with water (10 mL x 3). The aqueous phase was neutralized with IR-120 H⁺ and concentrated. In the case of derivative **45**, the organic phase was concentrated and purified by flash column gel chromatography eluting with Hexane:EtOAc (1:3).

5.1.3.1. (*E*)-1-(3-butoxy-5-(β -D-glucopyranosyloxy)phenyl)-2-(4-butoxyphenyl)ethene (**26**). Yield = 63%, white solid; ¹H NMR (300 MHz, CDCl₃) δ = 7.44 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 16.4 Hz, 1H), 6.92 (d, *J* = 16.4 Hz, 1H), 6.91 – 6.85 (m, 3H), 6.72 (s, 1H), 6.58 (s, 1H), 4.91 (d, *J* = 7.2 Hz, 1H), 3.97 (q, *J* = 6.5 Hz, 4H), 3.93 (dd, *J* = 12.2, 1.8 Hz, 2H), 3.71 (dd, *J* = 12.0, 6.1 Hz), 3.54 – 3.44 (m, 2H), 3.39 (d, *J* = 9.0

Hz, 1H), 1.81 – 1.70 (m, 4H), 1.56 – 1.45 (m, 4H), 0.99 (td, $J = 7.4, 3.2$ Hz, 6H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 160.34, 159.05, 159.00, 139.83, 129.82, 128.59, 127.47, 127.31, 125.83, 116.52, 114.28, 106.56, 106.48, 102.18, 101.18, 76.90, 76.63, 73.58, 70.16, 67.45, 67.35, 61.23, 31.14, 31.12, 18.93, 18.90, 12.83, 12.81$; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{28}\text{H}_{38}\text{O}_8$ 503.2632, found 503.2645.

5.1.3.2 *(E)-1-(3-butoxy-5-(β -D-glucopyranosyloxy)phenyl)-2-(β -D-glucopyranosyloxy)phenyl ethene (27)*. Yield = 62%, white solid; ^1H NMR (300 MHz, CDCl_3) $\delta = 8.01$ (d, $J = 7.4$ Hz, 1H), 7.47 (d, $J = 7.4$ Hz, 1H), 7.45 (d, $J = 7.3$ Hz, 1H), 7.10 (d, $J = 16.3$ Hz, 1H), 6.95 (s, 1H), 6.88 (d, $J = 8.3$ Hz, 2H), 6.76 (s, 1H), 6.66 (s, 1H), 4.94 (d, $J = 6.3$ Hz, 2H), 4.01 – 3.94 (m, 2H), 3.94 – 3.70 (m, 4H), 3.70 – 3.60 (m, 2H), 3.59 – 3.51 (m, 2H), 3.50 – 3.44 (m, 2H), 3.37 (d, $J = 9.4$ Hz, 2H), 1.80 – 1.68 (m, 2H), 1.57 – 1.42 (m, 2H), 0.98 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 168.54, 159.17, 158.87, 140.01, 132.74, 129.40, 129.01, 128.16, 127.62, 125.6, 114.40, 108.50, 103.70, 100.94, 76.95, 76.71, 73.63, 70.35, 67.47, 61.44, 61.33, 31.22, 19.01, 12.90$; ESI-HRMS $[\text{M} + \text{Na}]$ calcd for $\text{C}_{30}\text{H}_{40}\text{O}_{13}$ 631.2367, found 631.2421.

5.1.3.3 *(E)-1-(3,5-di-(β -D-glucopyranosyloxy)phenyl)-2-(4-butoxy)phenyl ethene (28)*. Yield = 71%, white solid; ^1H NMR (300 MHz, CDCl_3) $\delta = 8.03 - 8.02$ (m, 1H), 8.01 (d, $J = 1.3$ Hz, 1H), 7.58 (ddd, $J = 7.0, 2.5, 1.3$ Hz, 1H), 7.47 – 7.43 (m, 2H), 7.11 – 7.07 (m, 1H), 6.98 – 6.91 (m, 1H, m, 1H), 6.74 (s, 1H), 6.59 (s, 1H), 4.94 (s, 2H), 4.01 – 3.88 (m, 4H), 3.94 – 3.88 (m, 2H), 3.75 – 3.70 (m, 2H), 3.56 – 3.39 (m, 6H), 1.73 (tt, $J = 13.5, 6.8$ Hz, 2H), 1.50 (td, $J = 14.9, 7.5$ Hz, 2H), 0.98 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) $\delta = 168.54, 160.34, 158.99, 157.32, 132.70, 130.43, 129.32, 128.08, 127.41, 116.53, 114.36, 101.09, 100.74, 76.83, 76.68, 76.50, 73.57, 73.50, 70.16, 69.98, 67.51, 61.21, 61.10, 31.11, 18.93, 12.87$; ESI-HRMS $[\text{M} + \text{Na}]$ calcd for $\text{C}_{30}\text{H}_{40}\text{O}_{13}$ 631.2367, found 631.2366.

5.1.4. General Procedure for sulfation. Butylated resveratrol derivative (1.4 mmol) and sulfur trioxide trimethylamine complex (14 mmol) were dissolved in anhydrous acetonitrile (10 mL) in a 25 mL round-bottomed flask. Triethylamine (28 mmol) was then added. The reaction was carried out under agitation in a microwave reactor (150 W, 60 °C, 1 h). After completion the mixture was filtered and concentrated. The

product was resuspended in 2-propanol, filtered and purified by flash column gel chromatography eluting with Hexane:EtOAc (1:1).

5.1.4.1 Potassium (E)-3-(4-butoxystyryl)-5-butoxyphenyl sulfate (29). Yield=66%, dark yellow oil, Eluents = Hexane:AcOEt (1:1), ^1H NMR (300 MHz, CD_3OD) δ = 7.27 (d, J = 8.7 Hz, 2H), 6.96 – 6.85 (m, 2H), 6.76 (d, J = 16.4 Hz, 1H), 6.69 (d, J = 8.4 Hz, 3H), 6.61 (d, J = 1.9 Hz, 1H), 3.83 – 3.72 (m, 4H), 1.61 – 1.47 (m, 4H), 1.30 (dt, J = 14.6, 7.3 Hz, 4H), 0.79 (td, J = 7.3, 5.3 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ = 187.82, 160.35, 159.40, 154.06, 139.91, 129.83, 129.23, 127.94, 125.70, 114.70, 111.13, 108.94, 106.78, 67.88, 67.68, 31.41, 19.25, 19.23, 13.25; ESI-HRMS $[\text{M} + \text{Na}]$ calcd for $\text{C}_{22}\text{H}_{27}\text{O}_6$ 419.1521, found 419.1528.

5.1.4.2. Dipotassium (E)-5-(4-butoxystyryl)phenyl-1,3-disulfate (30). Yield=24%, yellow oil; R_f = 0.6 (Hexane:AcOEt - 1:1), Column eluents = Hexane:AcOEt (1:1); ^1H NMR (300 MHz, CDCl_3) δ = 7.31 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 16.5 Hz, 1H), 6.80 (d, J = 16.5 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 6.56 (s, 1H), 6.52 (s, 1H), 6.25 (s, 1H), 3.86 (t, J = 6.4 Hz, 2H), 1.73 – 1.58 (m, 2H), 1.48 – 1.36 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ = 188.77, 172.99, 161.76, 159.33, 158.10, 141.11, 130.25, 129.52, 128.81, 126.89, 116.39, 106.43, 105.11, 101.88, 68.54, 32.41, 20.20, 14.21; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{18}\text{H}_{18}\text{O}_9\text{S}_2$ 443.0470, found 443.0461.

5.1.4.3. Dipotassium (E)-3-(4-sulphate-styryl)-5-butoxyphenyl sulfate (31). Yield=32%, yellow oil; R_f = 0.65 (Hexane:AcOEt - 1:1), Eluents = Hexane:AcOEt (1:1); ^1H NMR (300 MHz, CD_3OD) δ = 7.31 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 16.2 Hz, 1H), 6.80 (d, J = 16.2 Hz, 1H), 6.76 (d, J = 8.0 Hz, 2H), 6.56 (s, 1H), 6.52 (s, 1H), 6.25 (s, 1H), 3.85 (t, J = 5.7 Hz, 2H), 1.68-1.61 (m, 2H), 1.52 – 1.33 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ = 187.82, 160.85, 158.58, 157.39, 140.05, 129.15, 128.60, 127.90, 125.79, 115.50, 105.42, 100.91, 100.88, 67.60, 31.46, 19.25, 13.21; ESI-HRMS $[\text{M} + \text{H}]$ calcd for $\text{C}_{18}\text{H}_{18}\text{O}_9\text{S}_2$ 443.0470, found 443.0476.

5.1.5. Preparation of methylated resveratrol prodrugs (32-41). General Procedure for glycosylation. 2,3,4,6-tetra-*O*-acetyl- α/β -D-glucopyranosyl trichloroacetimidate **46** [72] or per-*O*-acetyl- α/β -D-

maltopyranosyl trichloroacetimidate **47** [73] (1 mmol) was dissolved in anhydrous CH₂Cl₂ (10 mL) in a 50 mL round-bottomed flask under argon atmosphere. Methylated resveratrol derivative (0.33 mmol) and boron trifluoride diethyl etherate (0.033 mmol) were added to the stirring solution. After 30 min the reaction was stopped with triethylamine (5 mL) and concentrated. The crude product was purified by flash column chromatography using different hexane/ethyl acetate mixtures with 1% TEA.

5.1.5.1. (E)-1-(3-methoxy-5-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl)-2-(4'-methoxyphenyl)ethene (48). Following the general procedure and starting from 3,4'-di-O-methyl-resveratrol **12**, the reaction yielded **48** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (4:1 to 1:2). Yield= 97%; ¹H NMR (400 MHz, CDCl₃) δ ppm : 7.36 (d, *J* = 8.7 Hz, 2H), 6.96 (d, *J* = 16.2 Hz, 1H), 6.81 (dd, *J* = 12.6, 6.7 Hz, 3H), 6.67 (d, *J* = 6.3 Hz, 2H), 6.39 (s, 1H), 5.23 (dt, *J* = 16.9, 9.4 Hz, 2H), 5.11 – 5.01 (m, 2H), 4.20 (dd, *J* = 12.2, 5.9 Hz, 1H), 4.07 – 3.98 (m, 1H), 3.81 (ddd, *J* = 9.1, 5.4, 2.0 Hz, 14H), 3.73 (d, *J* = 3.0 Hz, 6H), 2.00 – 1.95 (m, 12H); ¹³C NMR (101 MHz, CDCl₃): δ = 170.48, 170.07, 169.35, 169.24, 160.80, 159.48, 158.02, 139.89, 129.55, 129.17, 127.80, 125.92, 117.05, 114.13, 107.11, 106.36, 102.32, 98.77, 72.69, 71.93, 71.12, 68.39, 62.05, 55.31, 55.21, 55.18, 20.53, 20.50, 20.49, 20.46. HRMS (ES⁺) Calcd. for C₃₀H₃₄O₁₂Na: (M+Na) 609.1948, found; 609.1970.

5.1.5.2. (E)-1-(3,5-di-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl)-2-(4'-methoxy-phenyl)ethene (49). Following the general procedure and starting from 5-O-methyl-resveratrol **13**, the reaction yielded **49** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (2:1). Yield: 80%, R_f = 0.2 (4:1 – Hexane:EtOAc). ¹H NMR (300 MHz, CDCl₃) δ 7.36 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 16.2 Hz, 1H), 6.90 – 6.79 (m, 3H), 6.66 (d, *J* = 3.9 Hz, 2H), 6.39 (s, 1H), 5.29 – 5.16 (m, 2H), 5.16 – 5.03 (m, 4H), 4.29 – 4.12 (m, 3H), 4.12 – 3.99 (m, 4H), 3.89 – 3.79 (m, 1H), 3.75 (s, 3H), 2.02 – 1.92 (m, 24H). ¹³C NMR (75 MHz, CDCl₃) δ 171.46, 170.93, 170.49, 169.66, 161.10, 159.76, 158.28, 140.23, 129.86, 129.52, 128.09, 126.21, 114.43, 107.38, 106.67, 102.64, 99.09, 72.98, 72.27, 71.42, 68.67, 62.36, 60.65, 55.69, 55.55, 21.28, 20.92, 20.83, 20.79, 14.42. TOF MS ES⁺ Calculated Mass [M + Na]: 925.2742, Expected Mass [M + Na]: 925.2747

5.1.5.3. (*E*)-1-(3-methoxy-4'-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyloxy)phenyl)-2-(5-methoxyphenyl)ethene (**50**). Following the general procedure and starting from 3,5-di-*O*-methyl-resveratrol (pterostilbene, **3**), the reaction yielded **50** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (4:1 to 1:2). Yield= 96%; ^1H NMR (400 MHz, CD_3OD) δ ppm : 7.49 – 7.42 (m, 2H), 7.07 (d, J = 16.3 Hz, 1H), 7.00 – 6.92 (m, 3H), 6.67 (d, J = 2.2 Hz, 2H), 6.36 (s, 1H), 5.39 – 5.30 (m, 1H), 5.24 (dd, J = 7.8, 2.7 Hz, 1H), 5.14 (dd, J = 9.3, 8.1 Hz, 1H), 5.12 – 5.03 (m, 1H), 4.27 (dd, J = 12.3, 5.3 Hz, 1H), 4.11 – 4.03 (m, 1H), 4.03 – 3.93 (m, 1H), 3.77 (d, J = 1.6 Hz, 6H), 2.00 (dd, J = 13.0, 6.4 Hz, 12H); ^{13}C NMR (101 MHz, CD_3OD): δ = 170.81, 170.15, 169.81, 169.66, 161.08, 156.38, 139.45, 132.43, 127.83, 127.42, 116.57, 104.06, 99.33, 98.20, 72.72, 71.56, 71.29, 68.30, 61.67, 54.35, 19.21, 19.13, 19.11, 13.02; HRMS (ES^+) Calcd. for $\text{C}_{30}\text{H}_{35}\text{O}_{12}$: ($\text{M}+\text{H}$) 587.2129, found; 587.2112.

5.1.5.4. (*E*)-1-(3-methoxy-4'-(*per-O*-acetyl- β -D-maltopyranosyloxy)phenyl)-2-(5-methoxyphenyl)ethene (**51**). Following the general procedure and starting from 3,5-di-*O*-methyl-resveratrol (Pterostilbene, **3**), the reaction yielded **51** after purification with flash chromatography whilst eluting with a gradient concentration of hexane:ethyl acetate (3:2 to 1:3). Yield= 41%; ^1H NMR (400 MHz, CDCl_3): 7.39 (dt, J = 7.0, 1.6 Hz, 2H), 7.03 – 6.83 (m, 4H), 6.60 (t, J = 2.2 Hz, 2H), 6.37 – 6.30 (m, 1H), 5.42 – 5.21 (m, 6H), 5.14 – 4.96 (m, 3H), 4.80 (dtd, J = 8.3, 4.4, 2.0 Hz, 1H), 4.75 – 4.64 (m, 1H), 4.41 (ddd, J = 12.4, 5.5, 2.8 Hz, 2H), 4.10 – 3.93 (m, 4H), 3.89 (q, J = 9.1, 7.1 Hz, 4H), 3.77 (q, J = 1.8 Hz, 6H), 2.14 – 1.92 (m, 30H); ^{13}C NMR (101 MHz, CDCl_3): δ = 170.98, 170.48, 170.41, 170.32, 170.28, 170.00, 169.75, 169.61, 169.55, 169.34, 160.92, 156.25, 139.27, 132.42, 128.17, 127.80, 127.69, 117.10, 104.41, 99.84, 98.26, 95.81, 95.65, 75.13, 73.82, 72.57, 72.20, 71.94, 71.67, 71.63, 70.42, 70.01, 69.31, 68.96, 68.50, 68.43, 67.88, 62.95, 62.38, 61.37, 60.28, 55.27, 20.94, 20.82, 20.80, 20.76, 20.73, 20.70, 20.58, 20.55, 20.50, 20.47, 20.41, 14.12; HRMS (ES^+) Calcd. for $\text{C}_{54}\text{H}_{66}\text{O}_{28}\text{Na}$: ($\text{M}+\text{Na}$) 1185.3638, found; 1185.3632.

5.1.6. General Procedure for acetyl deprotection. Methyl peracetylated glucopyranosyl- or maltopyranosyl- resveratrol derivative (0.75 mmol) was dissolved in methanol (10 mL) in a 25 mL round-bottomed flask. A solution of 25% sodium methoxide in methanol (0.5 mL) was added slowly and left

under stirring at room temperature for 1 h. The solution was filtered first through IR-120 H⁺ to neutralize it, filtered through celite to remove salt excess and finally concentrated.

5.1.6.1. (E)-1-(3-methoxy-5-(β-D-glucopyranosyloxy)phenyl)-2-(4'-methoxyphenyl)ethene (32). Following the general procedure and starting from **48**, the reaction yielded **32** (98%), which characterization was in accordance to the literature [74].

5.1.6.2. (E)-1-(5-methoxy-3-(β-D-glucopyranosyloxy)phenyl)-2-(4'-(β-D-glucopyranosyloxy)phenyl)ethene (33). Following the general procedure and starting from **49** after purification with flash chromatography whilst eluting with a gradient concentration of ethyl acetate: methanol (20:1- 5:1) the reaction yielded **33**. Yield: 82%, R_f = 0.1 (20:1 - EtOAc:MeOH). Compound **33** characterization was in accordance to the literature [75].

5.1.6.3. (E)-1-(3-methoxy-4'-(β-D-glucopyranosyloxy)phenyl)-2-(5-methoxyphenyl)ethene (34). Following the general procedure and starting from **50**, compound **34** was afforded (100%). Characterization was in accordance to the literature [76].

5.1.6.4. (E)-1-(3-methoxy-4'-(per-O-acetyl-β-D-maltopyranosyloxy)phenyl)-2-(5-methoxyphenyl)ethene (40). Following the general deprotection procedure and starting from **51**, the reaction yielded **40** as a white solid. Yield= 96%; ¹H NMR (400 MHz, CD₃OD) δ ppm : 7.47 – 7.37 (m, 2H), 7.08 – 6.87 (m, 4H), 6.64 (t, *J* = 1.8 Hz, 2H), 6.38 – 6.31 (m, 1H), 5.25 – 5.12 (m, 2H), 4.94 (d, *J* = 7.7 Hz, 1H), 3.95 – 3.79 (m, 7H), 3.75 (d, *J* = 1.5 Hz, 6H), 3.72 – 3.58 (m, 5H), 3.58 – 3.42 (m, 6H); ¹³C NMR (101 MHz, CD₃OD): δ = 161.02, 157.18, 139.58, 131.67, 128.13, 127.39, 126.97, 116.55, 104.06, 101.44, 101.36, 101.23, 100.56, 99.29, 79.86, 79.46, 78.88, 76.21, 75.25, 73.68, 73.58, 73.33, 73.08, 72.78, 72.34, 71.95, 70.07, 61.33, 60.81, 60.65, 54.46, 54.45, 48.55, 48.53; HRMS (ES⁺) Calcd. for C₃₄H₄₆O₁₈Na: (M+Na) 765.2582, found; 765.2584.

5.1.7. General procedure for esterification. Starting material (1 eq.) and vinyl ester (3 eq.) were dissolved in *t*-BuOH (10ml) in a round-bottomed flask. Lipozyme[®] TL IM was then added to the solution (same mass of enzyme than that of starting material) and reaction was stirred overnight at 55°C in an orbital

shaker. The reaction mixture was filtered to remove the enzyme and the crude was purified by flash column chromatography using different hexane/ethyl acetate/ methanol mixtures.

5.1.7.1. (E)-1-(3-methoxy-5-(6-O-octanoyl- β -D-glucopyranosyloxy)phenyl)-2-(4'-methoxyphenyl)ethene (35). Following the general procedure and starting from **32**, the reaction yielded compound **35**. The crude was purified by flash column chromatography (hexane: ethyl acetate from 1:20 to 0:100 and then ethyl acetate: methanol 50:1) to afford **35** (21%); ^1H NMR (400 MHz, CD_3OD) δ ppm : 7.46 (d, J = 8.8 Hz, 2H), 7.13 – 6.83 (m, 5H), 6.77 – 6.63 (m, 1H), 6.54 (t, J = 2.2 Hz, 1H), 4.94 – 4.90 (m, 1H), 4.44 (td, J = 11.3, 10.8, 2.2 Hz, 1H), 4.23 (td, J = 11.9, 7.3 Hz, 1H), 3.83 – 3.78 (m, 6H), 3.71 (ddd, J = 10.0, 7.6, 2.2 Hz, 1H), 3.56 – 3.44 (m, 2H), 3.42 – 3.33 (m, 1H), 2.27 (t, J = 7.4 Hz, 2H), 1.60 (dq, J = 10.3, 7.1, 6.0 Hz, 2H), 1.31 (m, 8H), 0.95 – 0.86 (t, J = 13.8, 6.7 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD): 176.25, 173.95, 160.84, 159.55, 158.77, 139.73, 129.83, 128.58, 127.48, 126.01, 116.60, 113.76, 106.41, 105.69, 102.11, 100.66, 76.46, 73.95, 73.40, 70.59, 63.52, 54.45, 54.34, 33.57, 31.45, 28.80, 28.69, 24.69, 22.25, 13.03; HRMS (ES^+) Calcd. for $\text{C}_{30}\text{H}_{40}\text{O}_9\text{Na}$: ($\text{M}+\text{Na}$) 567.2570, found; 567.2547.

5.1.7.2. (E)-1-(3,5-di-(6-O-octanoyl- β -D-glucopyranosyloxy)phenyl)-2-(4'-methoxy-phenyl)ethene (36). Following the general procedure and starting from **33** after purification with flash chromatography whilst eluting with a gradient concentration of ethyl acetate: methanol (5:1- 2:1) the reaction yielded compound **36**. Yield: 18%, R_f = 0.3 (4:1 - EtOAc:MeOH); ^1H NMR (500 MHz, CD_3OD) δ 7.51 – 7.44 (m, 2H), 7.18 (d, J = 5.0 Hz, 1H), 7.12 (d, J = 15.5 Hz, 1H), 6.98 (d, J = 6.0 Hz, 1H), 6.95 – 6.89 (m, 2H), 6.72 (s, 1H), 6.67 (s, 1H), 4.95 (d, J = 7.1 Hz, 1H), 4.82 (dd, J = 12.6, 6.3 Hz, 2H), 4.47 (dd, J = 21.7, 9.4 Hz, 2H), 4.26 – 4.15 (m, 3H), 3.80 (s, 3H), 3.68 (dd, J = 12.7, 6.1 Hz, 2H), 3.49 (dd, J = 5.5, 2.6 Hz, 2H), 3.42 – 3.36 (m, 2H), 1.62 – 1.58 (m, 4H), 1.39 – 1.26 (m, 20H), 0.91 (t, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.32, 174.01, 173.89, 159.68, 158.76, 158.53, 128.85, 127.55, 113.83, 113.77, 107.80, 100.43, 96.79, 92.55, 76.52, 73.94, 73.52, 73.36, 72.37, 72.03, 70.60, 70.24, 69.26, 63.54, 61.39, 54.37, 33.62, 31.49, 28.83, 28.73, 24.72, 22.28, 13.06. HRMS (ES^+) Calcd. for $\text{C}_{43}\text{H}_{62}\text{O}_{15}$ ($\text{M}+\text{Na}$) 841.3986, found 841.3993.

5.1.7.3. *(E)*-1-(3-methoxy-4'-(6-*O*-octanoyl- β -D-glucopyranosyloxy)phenyl)-2-(5-methoxyphenyl)ethene (**37**). Following the general procedure and starting from **34**, compound **37** was afforded (61%). The crude was purified by flash column chromatography (hexane: ethyl acetate from 1:20 to 0:100 and then ethyl acetate: methanol 50:1); ^1H NMR (400 MHz, CD_3OD) δ (d, $J = 8.1$ Hz, 2H), 7.16 – 6.87 (m, 4H), 6.66 (s, 2H), 6.36 (s, 1H), 4.89 (d, $J = 5.8$ Hz, 1H), 4.40 (d, $J = 11.8$ Hz, 1H), 4.22 (dd, $J = 11.8, 7.1$ Hz, 1H), 3.78 (s, 6H), 3.62 (dt, $J = 15.3, 7.9$ Hz, 1H), 3.52 – 3.41 (m, 2H), 3.38 – 3.31 (m, 1H), 2.28 (dt, $J = 21.6, 7.5$ Hz, 2H), 1.56 (h, $J = 8.2$ Hz, 2H), 1.27 (m, 8H), 0.84 (dt, $J = 28.5, 6.5$ Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD): $\delta = 176.3, 173.9, 161.1, 157.1, 139.5, 131.7, 128.0, 127.2, 127.0, 116.6, 104.0, 100.6, 99.2, 76.4, 74.0, 73.4, 70.4, 63.2, 54.3, 33.7, 33.5, 31.5, 31.4, 28.8, 28.75, 28.7, 24.7, 22.3, 22.2, 13.0$. HRMS (ES^+) Calcd. for $\text{C}_{30}\text{H}_{40}\text{O}_9$: (M+H) 545.2751, found; 545.2728.

5.1.7.4. *(E)*-1-(3-methoxy-5-(6-*O*-butanoyl- β -D-glucopyranosyloxy)phenyl)-2-(4'-methoxyphenyl)ethene (**38**). Following the general procedure and starting from **32**, the reaction yielded compound **38**. The crude was purified by flash column chromatography (hexane: ethyl acetate from 1:20 to 0:100 and then ethyl acetate: methanol 50:1) to afford **38** (20%); ^1H NMR (600 MHz, CD_3OD) δ 7.48 – 7.42 (m, 2H), 7.08 (d, $J = 16.3$ Hz, 1H), 6.96 – 6.88 (m, 3H), 6.84 (t, $J = 1.7$ Hz, 1H), 6.77 – 6.74 (m, 1H), 6.54 (t, $J = 2.2$ Hz, 1H), 4.91 (d, $J = 7.1$ Hz, 1H), 4.44 (td, $J = 12.3, 2.2$ Hz, 1H), 4.22 (ddd, $J = 17.0, 11.9, 7.1$ Hz, 1H), 3.81 – 3.78 (m, 6H), 3.73 – 3.63 (m, 1H), 3.52 – 3.43 (m, 2H), 3.40 – 3.32 (m, 1H), 2.26 (t, $J = 7.5$ Hz, 2H), 1.53 (h, $J = 7.4$ Hz, 2H), 0.80 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD): δ 173.8, 161.08, 160.89, 159.58, 158.77, 139.81, 129.86, 128.61, 127.47, 125.97, 113.74, 106.57, 105.50, 103.99, 102.09, 100.70, 76.45, 74.01, 73.43, 70.52, 63.43, 54.44, 54.33, 35.47, 17.94, 12.44; HRMS (ES^+) Calcd. for $\text{C}_{26}\text{H}_{33}\text{O}_9$: (M+H) 489.2125, found; 489.2108.

5.7.1.5. *(E)*-1-(3-methoxy-4'-(6-*O*-butanoyl- β -D-glucopyranosyloxy-butyrate)phenyl)-2-(5-methoxyphenyl)ethene (**39**). Following the general esterification procedure and starting from **34** compound **39** was obtained. The crude was purified by flash column chromatography (hexane: ethyl acetate from 1:20 to 0:100 and then ethyl acetate: methanol 50:1) to afford compound **39** (159 mg, 56%); ^1H NMR (400 MHz, CD_3OD) δ ppm : 7.56 – 7.35 (m, 2H), 7.13 – 7.04 (m, 3H), 6.97 (d, $J = 16.3$ Hz, 1H), 6.69 (d, $J = 2.2$ Hz,

2H), 6.39 (t, $J = 2.2$ Hz, 1H), 4.98 – 4.88 (m, 1H), 4.47 (dd, $J = 11.9, 2.2$ Hz, 1H), 4.27 (dd, $J = 11.9, 6.8$ Hz, 1H), 4.12 (q, $J = 7.1$ Hz, 1H), 3.83 (s, 6H), 3.75 – 3.61 (m, 1H), 3.56 – 3.50 (m, 1H), 3.45 – 3.36 (m, 1H), 2.35 (t, $J = 7.4$ Hz, 2H), 1.65 (h, $J = 7.3$ Hz, 2H), 0.96 (t, $J = 7.4$ Hz, 3H).; ^{13}C NMR (101 MHz, CD_3OD): $\delta = 173.8, 171.7, 161.0, 157.1, 139.5, 131.8, 128.1, 127.3, 127.1, 116.7, 104.1, 100.7, 99.4, 78.0, 77.7, 77.4, 76.4, 74.0, 73.3, 70.3, 63.3, 60.2, 54.6, 35.7, 18.1, 12.8$. HRMS (ES^+) Calcd. for $\text{C}_{26}\text{H}_{33}\text{O}_9$: (M+H) 489.2125, found; 489.2108.

5.7.1.6. (*E*)-1-(3-methoxy-4'-(6,6',6''-O-trioctanoyl- β -D-maltopyranosyloxy)phenyl)-2-(5-methoxyphenyl) ethene (**41**). Following the general deprotection procedure and starting from **40**, the reaction yielded compound **41** as a white solid. Yield= 9%; ^1H NMR (500 MHz, CD_3OD) δ ppm : 7.53 – 7.47 (m, 2H), 7.15 – 6.94 (m, 4H), 6.70 (d, $J = 2.3$ Hz, 2H), 6.39 (t, $J = 2.0$ Hz, 1H), 5.35 – 5.09 (m, 2H), 5.08 – 4.90 (m, 1H), 4.02 (d, $J = 5.7$ Hz, 1H), 3.89 (m, 6H), 3.81 (s, 6H), 3.71 – 3.57 (m, 5H), 3.57 – 3.40 (m, 6H), 2.10 – 1.94 (m, 2H), 1.72 – 1.54 (m, 2H), 1.43 – 1.24 (m, 8H), 0.98 – 0.85 (m, 3H); ^{13}C NMR (126 MHz, CD_3OD): $\delta = 174.09, 161.10, 157.30, 139.62, 131.75, 128.07, 127.29, 126.97, 116.53, 103.97, 101.74, 101.27, 100.67, 99.25, 96.69, 80.72, 79.52, 76.26, 75.36, 73.61, 73.52, 73.11, 72.81, 72.30, 72.08, 70.93, 70.75, 70.23, 70.07, 68.54, 66.27, 63.51, 62.05, 54.72, 54.36, 33.51, 33.34, 31.47, 28.78, 28.69, 24.61, 13.01$; HRMS (ES^+) Calcd. for $\text{C}_{42}\text{H}_{60}\text{O}_{19}\text{Na}$: (M+Na) 891.3627, found; 891.3636.

5.2. Biological evaluation

5.2.1. *Cell cultures*. SH-S5Y5 neurons were cultured in collagen-pretreated petri-dishes with DMEM-F12 medium supplemented with Penicillin/Streptomycin and 10 % inactivated fetal bovine serum (iFBS). RAW 264.7 macrophages were cultured in DMEM high glucose medium supplemented with Penicillin/Streptomycin and 10 % iFBS.

5.2.2. *Cell viability assays*. Neuron assays were done in collagen-pretreated 96 well plates by seeding 2×10^4 neurons per well in a 100 μL volume and with 24 h of incubation time before compound addition.

Macrophage assays were done in 96 well plates by seeding 2.5×10^4 macrophages per well in a 100 μ L volume with 4 h of incubation time before compound addition. 10, 1 and 0.1 mM DMSO stocks of the tested compounds were prepared. 1:100 dilutions of each stock in cell culture media were carried out upon addition of the compounds to the well plate. Thus, the final compound concentrations in the plate were 100, 10 and 1 μ M respectively, whereas the DMSO percentage in each well was 1%. Cell viability was evaluated 24 hours after compound addition by mitochondrial MTT assay, according to manufacturer. Averages and standard deviations of at least two experiments in quadruplicate were calculated.

5.2.3. Neuroprotective assay. Neurons were cultured and plated as described in the cell viability assay. Tested compounds were added at different concentrations (1, 10 and 100 μ M dissolved in cell culture media with 1% DMSO) and after 10 min incubation 100 μ M of hydrogen peroxide was added. Final DMSO percentage in each well was adjusted to 1% DMSO. Cell viability was evaluated 24 hours after compound addition by mitochondrial MTT assay, according to manufacturer. Averages and standard deviations of at least two experiments in quadruplicate were calculated. Neuron recovery was calculated by normalizing the results from H_2O_2 -neuron viability to the H_2O_2 positive control.

5.2.4. Cytokine Production studies. To determine cytokine production, 5×10^5 RAW 264.7 macrophages were seeded in 24-well plates (in 0.5 ml). Compounds (10 μ M) were then added and macrophages were either stimulated or not by adding LPS (1 μ g/ml) to the medium. After 24 hr, levels of IL-6 and TNF- α in the supernatants were determined by ELISA using capture/biotinylated detection antibodies from BD PharMingen and PreproTech [77, 78]. A minimum of two independent sets of experiments and three replicates per experiment were carried out. All data are expressed as mean $\pm$ SEM.

5.2.5. Zebra fish toxicity assay. This assay was subcontracted to Zeclinics (Barcelona, Spain). Fertilized embryos of zebrafish (*Danio rerio* – strain AB) were harvested 3 hours post fertilization and grouped into

wells (20 embryos per well) in E3 medium with the desired compounds in gradient concentrations (5 different concentrations, from 0.1 μ M to 1 mM). They were further incubated at 28.5 °C for 93 more hours and then LC50 values (median lethal dose) were calculated by fitting sigmoidal curve to mortality data ($y = \text{Bot} + (\text{Top}-\text{Bot}) / (1 + 10^{-(k*(x_0 - \text{Log}(C))})$). Bot, minimum mortality; Top, maximum mortality; k, curve slope; x_0 , LC50 estimated. Negative controls were 0.1% of DMSO and as positive controls 4-diethylaminobenzaldehyde (DEAB) at 5 different concentrations (from 0.1 μ M to 1 mM).

5.2.6. PTZ neuroprotection assay. This assay was subcontracted to Neuron Bio (Granada, Spain). Fertilized embryos of zebrafish (*Danio rerio* – strain AB) were seeded into a Petri dish with 50 mL of dilution water until they reached larva state (5 days post fertilization). After verification of no abnormalities, five larvae per well were transferred into a 24 well plate and ten replicates of each experimental condition were carried out. Two independent experiments were performed. Pretreatment: larvae were incubated for 1h at 26 °C in a volume of 2 mL of dilution water containing 0.1% DMSO (control and control+PTZ groups), the positive control group (Phys) included physostigmine (20 μ M) and the groups with each compound to be examined included 10 μ M of the compound. After this pretreatment, media was changed and larvae were incubated for 6h at 26 °C with Phys (20 μ M) or each compound (10 μ M) in combination with a final concentration of 5 mM PTZ. Then, larvae were examined and their overall status was normal, without visible abnormalities and with normal behavior. Finally, in order to determine the levels of AChE activity, the larvae were mechanically homogenized and the samples centrifuged to obtain the supernatant, which was then directly tested. AChE activity was measured following methodology by Ellman et al [79]. Total protein was quantified using BCA methodology in order to normalize the obtained enzymatic values. Lastly, the negative control values were considered to be the 100% values. A statistical analysis was carried out using the Dunnett multiple comparison test (One-way ANOVA) with the GraphPad Prism program.

5.2.7. *Mice.* C57BL/6 mice 12 weeks old were obtained from Charles River and housed under standard conditions (12h light/dark cycle) with access to food and water *ad libitum*. Animals were handled and habituated to the experimenters one week before any behavioral assessment. All experiments with animals were performed in accordance with the European ethical guidelines and approved by the Animal Care Unit Committee from the Institute of Parasitology and Biomedicine Lopez-Neyra - Consejo Superior de Investigaciones Científicas. Procedures were designed to minimize the number of animals used and their suffering.

5.2.8. *3-Nitropropionic acid intoxication.* 3-nitropropionic acid (3-NP, Sigma) was prepared and administered as previously indicated with minor modifications [80-82]. 3-NP was dissolved in distilled water final pH 7.4, filtered (0.22 μ m, Millipore), protected from light and kept at 4° C until use. Resveratrol (compound **1**) and compound **8** were dissolved in an aqueous solution containing 13% (2-hydroxypropyl)- β -cyclodextrin (average MW $\approx$ 1380) and 2% Tween-80. 28 animals were grouped as follow for treatment administration: vehicle (veh, n=7), 3-NP (n=7), resveratrol (compound **1**) + 3-NP (1+3-NP, n=7), and compound **8** + 3-NP (8+3-NP, n=7). For 3-NP intoxication, animals received twice daily i.p. injections (8:00 am: 6:00 pm) 10 h apart with the next regimen 4x20 mg/kg, 4x40 mg/kg, 2x60 mg/kg (cumulated dose: 360 mg/kg in 5 days) (Figure S4). Resveratrol (5.5 mg/kg [38.75 μ M]) and compound **8** (12.46 mg/kg [38.75 μ M]) were injected only and immediately before the morning dose of 3-NP. All treatments were administered in a volume of $\approx$ 100 μ L and always after behavioral evaluations.

5.2.9. *Behavioral assessment.* Mice were weighed and then behavioral semi-quantitative assessments were carried out. Evaluations (weight and behavioral register) were performed just before injections, excepting the last one, performed three hours after the final 3-NP injection (60 mg/kg). Clinical semi-quantitative motor symptoms assessment was based on a previously reported motor scale with minor modification.[80, 82] Included items were: global locomotor activity, hindlimb claspings, hindlimb dystonia and truncal dystonia, each rated on a three levels scale of severity (0-absent, 1-slight to moderate and 2-severe) resulting in a total score ranging from 0 to 8.

5.2.10. Tissue collection. At the end of the experiment and at least three hours after any animal manipulation, mice were sacrificed in a CO₂ chamber and blood and brain tissue were immediately collected. Blood was centrifuged for serum recovering, and collected serum samples were stored at -20 °C from obtaining until the analysis of interleukin-6 (IL-6) content. Brains were frozen in liquid nitrogen and subsequently processed to qRT-PCR assay.

5.2.11. IL-6 detection. IL-6 content in serums from healthy or 3-NP treated mice was measured by a sandwich ELISA. In brief, a 96-well ELISA plate was coated overnight at 4°C with IL-6 capture antibody in 0.1 M phosphate buffer pH 9.0. The plate was washed and blocked with 0.1 M PBS containing 10% fetal bovine serum at room temperature for 3h. Then, serum samples or different concentrations of the recombinant IL-6 were added and incubated overnight at 4° C, followed by a 2 h period incubation with biotinylated secondary antibody at RT. Finally, avidin peroxidase (Sigma) was added followed by the ABTS substrate addition at RT, and plate was protected from light until color development. Three washing buffer (0.1 M PBS plus 0.05% Tween-20) washes were applied between steps. Absorbance was obtained in a reader plate at 405 nM, and amount of IL-6 was calculated from the standard curve.

5.2.12. qRT-PCR. RNA was isolated from brain tissues using Tripure (Roche) accordingly the manufacturer's indications. Total RNA (1 µg) was reverse transcribed in a 20 µL volume using the reagents supplied in the synthesis cDNA kit (*Thermo Scientific* RevertAid First Strand *K1622*, Vilnius, Lithuania). 20 ng cDNA (2 µL) were amplified by real-time PCR using SensyFast and sequences of specific primers including: iNOS, Nitric oxide synthetase 2; CAT, catalase; SOD2, dismutase duperioxide; p53, and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as housekeeping [see Table 1]. All PCR reaction was carried out in a Bio-Rad CFX equipment (Bio-Rad). Thermal cycling profile consisted of a preincubation step at 94°C for 5 min, followed by 40 cycles of denaturation (94°C, 30 sec), annealing (temperature adjusted for each gene (Table S1), 30 sec), and extension (72°C, 30 sec).

5.2.13. Statistical analysis. Data analysis was performed using the Sigma Stat 3.5 (Systat Software Inc.) and the GraphPad 5 (GraphPad Software, Inc.) software. In case of normal distribution and/or equal variance data, statistical differences were determined by two-tails Student's t test for a 2-groups comparison, or by One-way ANOVA followed by Bonferroni's post-Hoc test to compare more than three groups. Data with nonparametric distribution were evaluated for differences by Mann Whitney's or Kruskal-Wallis followed by Dunn's post-Hoc test when two or more than three groups were compared, respectively. $P < 0.05$ was considered statistically significant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at:

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Graphical abstract