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Synthesis of a second generation chroman/catechol hybrids and evaluation of their activity in protecting neuronal cells from oxidative stress-induced cell death

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ABSTRACT

A new generation of chroman/catechol hybrids bearing heterocyclic five-membered rings, such as 1,2,4-oxadiazole 1,3,4-oxadiazole, 1,2,3-triazole, tetrazole and isoxazole, were designed and synthesized. The activity of the new derivatives against oxidative stress induced neuronal damage, was evaluated using glutamate-challenged hippocampal HT22 cells.

Compound **3** in which a 3,4-dimethoxyphenyl moiety, is directly attached to the 1,2,4-oxadiazole ring was the most active among the 2-substituted chroman analogues, with EC_{50} = 254 ± 65 nM. Concerning the 5-subtituted chroman analogues, isoxazole derivative **29** exhibited the strongest activity (EC_{50} = 245 ± 38 nM). However, **29** was cytotoxic at concentrations higher than 1 μM, while the triazole analogue **24** (EC_{50} = 801 ± 229 nM), was non-toxic at all concentrations tested.

1. Introduction

The brain is particularly vulnerable to oxidative stress because of its high rate of oxidative metabolism (20% of total oxygen uptake in spite of its relatively small size) and reduced capacity for cellular regeneration compared to other organs. Neurodegenerative diseases of distinct pathophysiology are known to share common biochemical characteristics such as mitochondrial dysfunction and oxidative stress-induced cell damage.[1]

The oxidative stress-dependent neuronal death is due to multifactorial events. For instance, elevated levels of the excitatory amino acid glutamate are thought to cause oxidative stress by a non-receptor-mediated oxidative pathway which blocks cystine uptake and results in depletion of intracellular glutathione (GSH). There is also evidence that brain iron misregulation and oxidative stress, result in hydroxyl radical (·OH) generation from H_2O_2 and stimulation of inflammatory processes, triggering a cascade of events leading to apoptotic cell death in neurodegenerative disorders.[2,3]

Hence, developing of agents that are able to modulate multiple mechanisms of free radical production and scavenging, without dangerously hampering any essential physiological mechanism based on free radical cellular signalling, is a promising approach against neurodegeneration.[4,5]

HT22 hippocampal neurons do not express functional ionotropic glutamate receptors. When challenged with glutamate these cells undergo oxidative stress and oxytosis, a form of cell death involving glutamate blockage of cystine/glutamate antiporters, inhibition of cystine uptake and depletion of intracellular glutathione (GSH) as a consequence.[6] The exact steps in the HT22 cell death pathway have been the subject of extensive research in recent years but are yet poorly understood.[7,8] Nevertheless, the ability of HT22 cells to undergo glutamate-induced oxytosis independently of ionotropic glutamate receptor signalling renders them a valuable tool for high throughput screening of chemical libraries and natural products for potentially neuroprotective agents. It was recently reported that flavonoids screened positive against oxidative toxicity in HT22 cells were also tested positive in reducing stroke-induced behavioural defects in rabbits.[9]

In search of novel antioxidants, we have previously synthesized hybrids containing the chroman moiety of vitamin E and a catechol group (Fig. 1) and evaluated their activity against oxidative stress induced cellular damage. Specifically, the ability of the new molecules to protect cultured cells from H_2O_2-induced DNA damage was evaluated using single cell gel electrophoresis (comet assay), while their in vitro neuroprotective activity was assessed using glutamate-challenged HT22 cells. The inference from this previous study was that the activity of the hybrids against DNA damage could be attributed to their iron-chelating properties, while their

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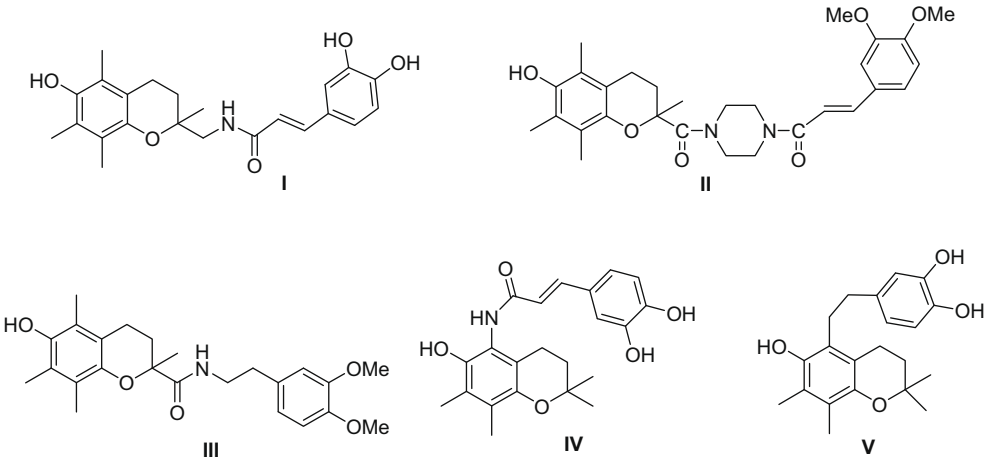


Figure 1. First generation of chroman/catechol hybrids.

HT22-protective activity could be due not only to their antioxidant properties but perhaps also to their capacity to interfere with other cell signaling cascades implicated in oxytosis.[10]

The present work involves the design and synthesis of new hybrids in which chroman and catechol moieties are connected trough heterocyclic five-membered rings, such as 1,2,4-oxadiazole 1,3,4-oxadiazole, 1,2,3-triazole, tetrazole and isoxazole. These heterocycles are potential pharmacophores and have been utilized as ester or amide bioisosteres in the search for compounds with superior pharmacokinetic profiles.[11]

The neuroprotective activity of the new hybrids was evaluated using glutamate-challenged HT22 cells.

2. Chemistry

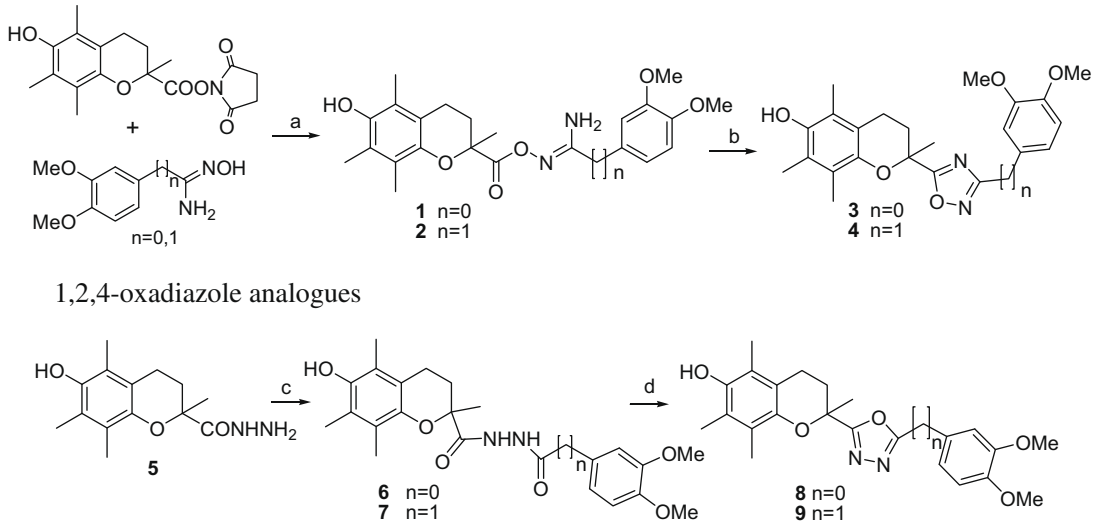
The synthesis of analogues bearing oxadiazoles at position 2 of the chroman moiety is depicted in Scheme 1. Specifically for the preparation of 1,2,4-oxadiazole derivatives, *N*-hydroxysuccinimidyl-trolox ester reacted with the appropriate *N*-hydroxy-amidines[12] to give the acyl amidoximes **1** and **2.** Subsequent intramolecular cyclization in the presence of tetrabutylammonium fluoride produced the 1,2,4-oxadiazole analogues **3** and **4**.

1,3,4-Oxadiazole analogues were synthesized from hydrazide **5** which was reacted with activated 3,4-dimethoxybenzoic acid or 3,4-dimethoxyphenylacetic acid to give intermediates **6** and **7**. Cyclodehydration in boiling $POCl_3$ produced the 2-substituted chroman analogues **8** and **9**.

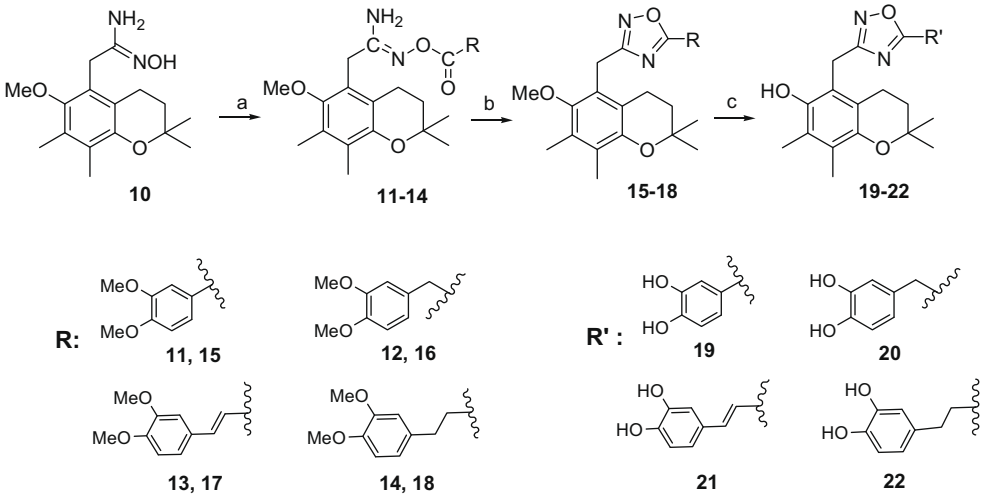
Analogues bearing 1,2,4-oxadiazoles at position 5 of the chroman moiety were prepared from *N*-hydroxy-amidine **10**[13] (Scheme 2), following similar procedure as for 2-substituted derivatives and were deprotected using $BF_3{\cdot}SMe_2$[14] to afford the final analogues **19–22**.

Scheme 3 depicts the synthesis of triazole **24** and its isomer **27**. Cu^I-catalyzed 'click' cycloaddition[15,16] between (3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-benzopyran-5-yl) methylazide, and 4-ethynyl-1,2-dimethoxybenzene, in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, afforded the 1,2,3-triazole analogue **23**, which was deprotected to produce the dihydroxy derivative **24**. Similarly, alkyne **25** (prepared by treatment of the corresponding chromanaldehyde with Bestmann-Ohira reagent[17]) reacted with 3,4-dimethoxy-benzylazide to afford compound **26**, which was treated with $BF_3{\cdot}SMe_2$ to produce **27**.

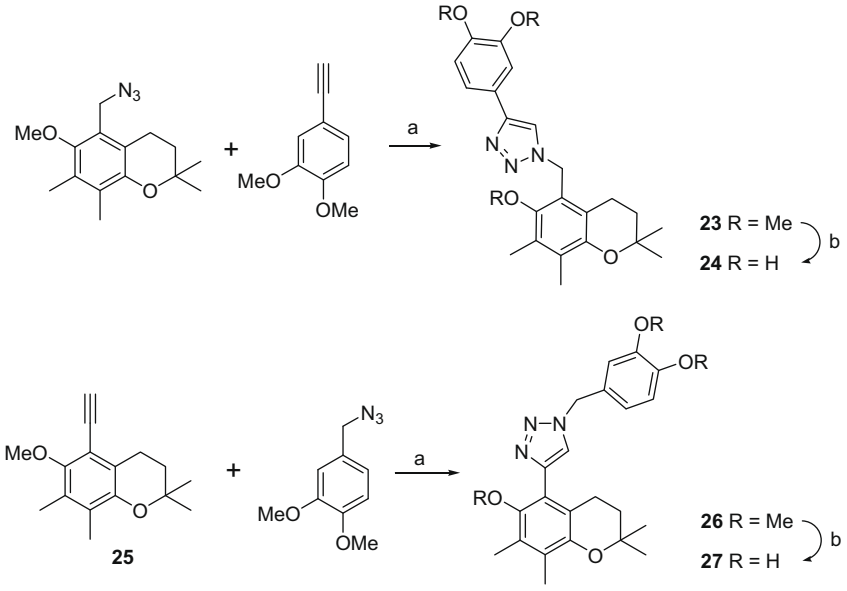
3,5-Disubstituted isoxazoles **29** and **32** were obtained as shown in Scheme 4. The appropriate aldoximes were transformed to the



Scheme 1. Synthesis of 2-substituted chromans. Reagents and conditions: (a) CH_2Cl_2, rt; (b) *n*-Bu_4NF, THF; (c) CDI, THF, 3,4-dimethoxybenzoic acid or 3,4-dimethoxyphenylacetic acid; (d) $POCl_3$.



Scheme 2. 5-Substituted chromans, 1,2,4-oxadiazole analogues. Reagents and conditions: (a) RCOOH, BOP, DMF, CH_2Cl_2; (b) *n*-Bu_4NF, THF; (c) $BF_3{\cdot}SMe_2$, CH_2Cl_2.



Scheme 3. 5-Substituted chromans, 1,2,3-triazole analogues. Reagents and conditions: (a) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH, H_2O; (b) $BF_3{\cdot}SMe_2$, CH_2Cl_2.

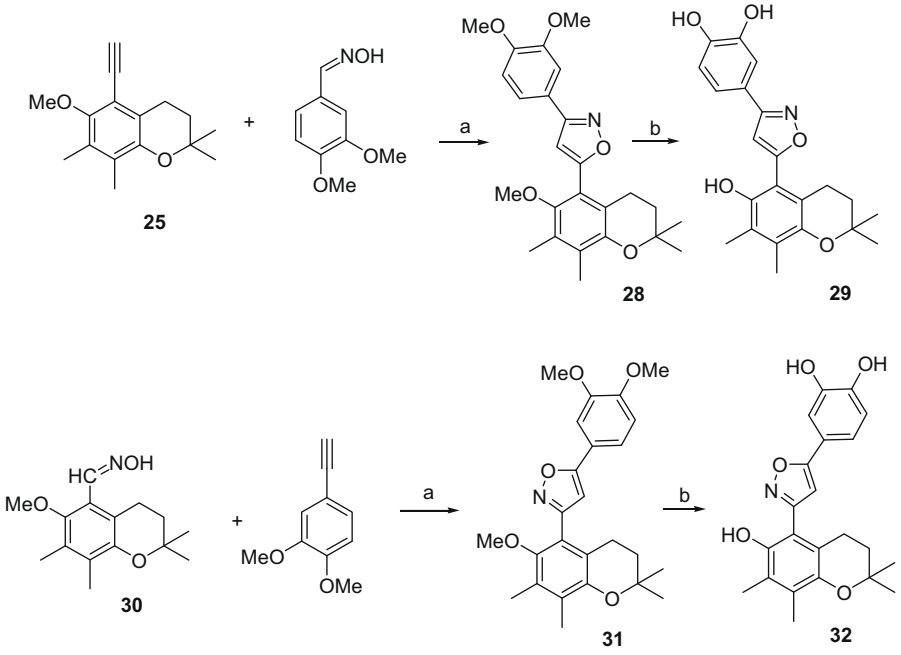
corresponding nitrile oxides using chloramine-T trihydrate, which acts as both a halogenating agent and a base.[18] In the presence of a catalytic amount of copper(I), obtained from comproportionation of Cu metal and $CuSO_4{\cdot}5H_2O$, the in situ generated nitrile oxides reacted with **25** or 4-ethynyl-1,2-dimethoxybenzene, respectively, furnishing the 3,5-disubstituted isoxazoles **28** and **31** which were deprotected to afford **29** and **32**.

The synthesis of tetrazole derivatives is depicted in Scheme 5. Acylation of the appropriate amines synthesized by our group[19,20] with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) activated 3-(3,4-dimethoxyphenyl)propanoic acid, as previously reported,[10] afforded amides **33** and **34**, which in turn were converted to thioamides **35** and **36** by treatment with Lawesson's reagent. Tetrazoles **37** and **38** were obtained by treatment of thioamides with trimethylsilyl azide ($TMSN_3$), in the presence of triphenylphosphine and diisopropylazodicarboxylate (DIAD).[21] Analogue **39** was obtained by deprotection of **37** with $BF_3{\cdot}SMe_2$.

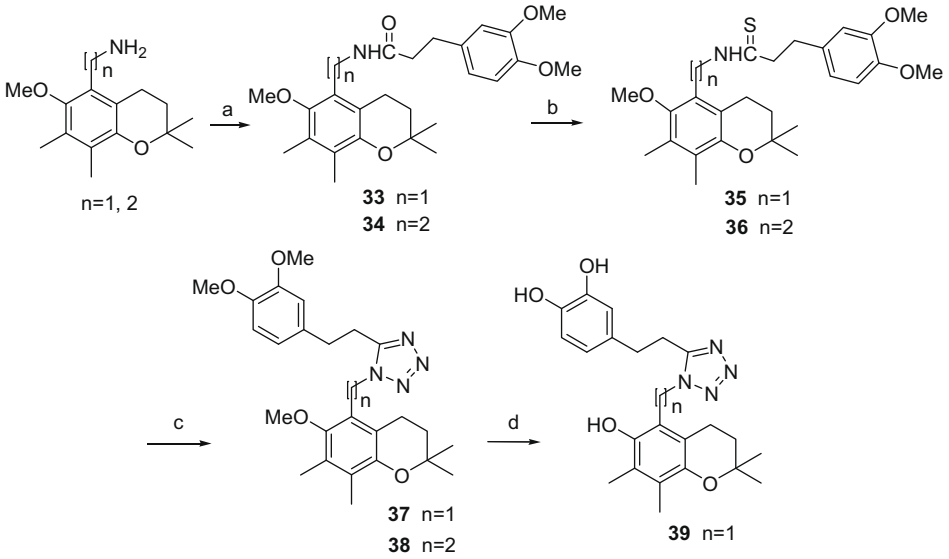
3. Results and discussion

The mouse hippocampal cell line HT22 has been used to elucidate sequential cellular events during programmed cell death from oxidative stress (oxytosis) caused by glutamate-induced depletion of intracellular glutathione.[6–8] Although HT22 cells lack ionotropic glutamate receptors that could mediate excitotoxicity, they undergo oxytosis within 24 h following exposure to 1–5 mM glutamate. Recent findings suggest that oxytosis faithfully mimics oxidative cytotoxicity in cerebral ischemia, Alzheimer's disease and other neurodegenerative disorders with an oxidative stress component.[6,8,22]

Among the 2-substituted chroman analogues (Table 1) compound **3** in which the 3,4-dimethoxyphenyl moiety is directly attached to the 1,2,4-oxadiazole ring, showed the highest activity (EC_{50} = 254 ± 65 nM) while its 3,4-dimethoxybenzyl derivative **4** was less active (EC_{50} = 741 ± 183 nM). Compound **4** was almost equipotent with the piperazine analogue **II** of the first generation



Scheme 4. 5-Substituted chromans, isoxazole analogues. Reagents and conditions: (a) $TsN(Cl)Na{\cdot}3H_2O$, $CuSO_4{\cdot}5H_2O$, Cu^0, *t*-BuOH/H_2O; (b) $BF_3{\cdot}SMe_2$, CH_2Cl_2.

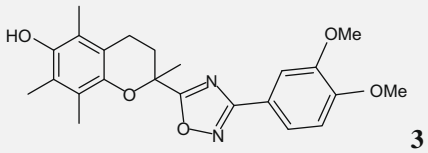
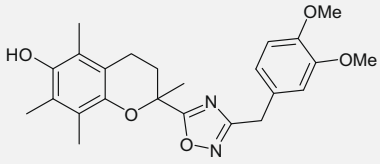
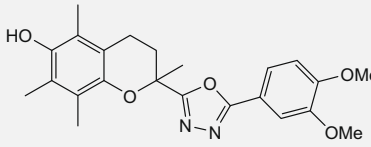
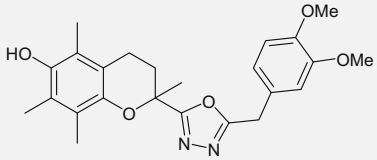
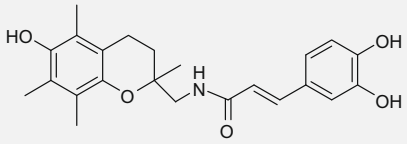
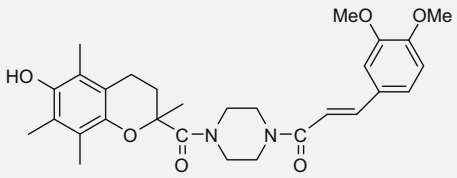
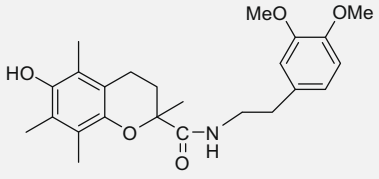


Scheme 5. 5-Substituted chromans, tetrazole analogues. Reagents and conditions: (a) 3-(3,4-dimethoxyphenyl)propanoic acid, DMF, Et_3N, BOP, CH_2Cl_2; (b) Lawesson's reagent, THF, reflux; (c) DIAD, $TMSN_3$, Ph_3P, THF; (d) $BF_3{\cdot}SMe_2$, CH_2Cl_2.

analogues and more active than the caffeic acid amide **I** and the 3,4-dimethoxyphenethyl derivative **III** (prepared from the *N*-hydroxysuccinimidyl-trolox ester and 3,4-dimethoxyphenethylamine). Replacement of the 1,2,4-oxadiazole ring by 1,3,4-oxadiazole, in the phenyl derivatives **3** and **8** strongly affected the neuroprotective activity (compound **8** was inactive), with the benzyl derivatives **4** and **9** being almost equipotent. It seems that the presence of conjugated system in **8** reduces its activity. Other factors, not directly related to oxidative stress pathways but differentially affected by 1,2,4-oxadiazole versus 1,3,4-oxadiazole derivatives,[23] might indirectly influence cell fate. Although the activity of our compounds at the cellular level depends on the nature of heterocycle and its substituents, whether and how these may differentially impact on the various pathways of ROS production and/or oxytosis modulation is presently unexplored.

Concerning the 5-substituted chroman analogues (Table 2), styrene analogue **21** was the most active of 1,2,4-oxadiazole derivatives with $EC_{50} = 637 \pm 321$ nM. 3,4-Dihydroxyphenyl derivative **19** was less active ($EC_{50} = 1340 \pm 214$ nM), while the more flexible 3,4-dihydroxyphenethyl derivative **22** exhibited the lowest activity ($EC_{50} = 2552 \pm 258$ nM) compared to the other 1,2,4-oxadiazole analogues as well as to the 3,4-dihydroxyphenethyl derivative **V** of the first generation hybrids. In addition, **22** was equipotent to its tetrazole analogue **39** ($EC_{50} = 2823 \pm 437$ nM).

Table 1
Efficacy and potency of 2-substituted chroman analogues to protect glutamate-challenged HT22 cells from oxytosis

Compound	EC_{50}[a] (nM)	Relative potency[b]	Efficacy[c]
 **3**	254 ± 65	2.6	100% (full)
 **4**	741 ± 183	0.9	75% (full)
 **8**	⩾10000	⩽0.1	ns
 **9**	843 ± 175	0.8	80% (full)
 **I**	1140 ± 350	0.6	94% (full)
 **II**	650 ± 13	1	100% (full)
 **III**	1707 ± 321	0.4	92% (full)

[a] EC_{50} values are test compound concentrations able to maintain the viability of glutamate-challenged HT22 cells to a level equal to 50% of that of non-challenged cells. Values are mean ± SEM of at least three independent experiments similar to those shown in Figures 2 and 3.

[b] Relative potencies were calculated by [$EC_{50\ \text{reference compound}}/EC_{50\ \text{compound}}$]. For the 2-substituted chroman analogues, the reference compound was hybrid II.

[c] Hybrids exhibiting statistically significant effects in protecting cell viability at 10 μM were classified as exhibiting full, partial or weak neuroprotective efficacy depending on whether their % neuroprotective effect was, respectively, 67–100%, 34–66% and ⩽33%. Values are the mean of at least three independent experiments similar to those shown in Figures 2 and 3. ns = non-significant.

Interestingly, triazole analogue **24** was ~10 times more active (EC_{50} = 801 ± 229) than its isomer **27** (EC_{50} = 7093 ± 929 nM) and ~2 times more potent than its 1,2,4-oxadiazole counterpart **19**. Isoxazole derivatives **29** and its regioisomer **32** exhibited the strongest activity with EC_{50} = 245 ± 38 nM and 584 ± 33 nM, respectively.

Comparing 1,2,4-oxadiazole derivatives, 2-substituted chromans bearing the protected catechols showed highest activity than the 5-substituted chromans with the free catechols. Although in lipoic acid conjugates bearing nitrogen heterocycles, the presence of a catechol moiety was requisite for activity,[12] replacement of the dithiolane by a chroman scaffold strongly enhanced the neuroprotective activity even when the catechol group was masked. Moreover, the presence of catechol moiety in compounds **21**, **22**, **29** and **32** is associated with cytotoxicity. Catechols can be converted in two one-electron oxidative steps to redox-active electrophilic *o*-quinones that in the presence of NAD(P)H are readily recycled in a non-enzymatic way back to catechols, thus amplifying ROS production and cell damage.[24] The prooxidant catechol-*o*-quinone redox cycling is apparently inhibited by catechol *O*-methyltransferases,[25] explaining in part why **3** is a better choice than any of the catechol derivatives of Table 2.

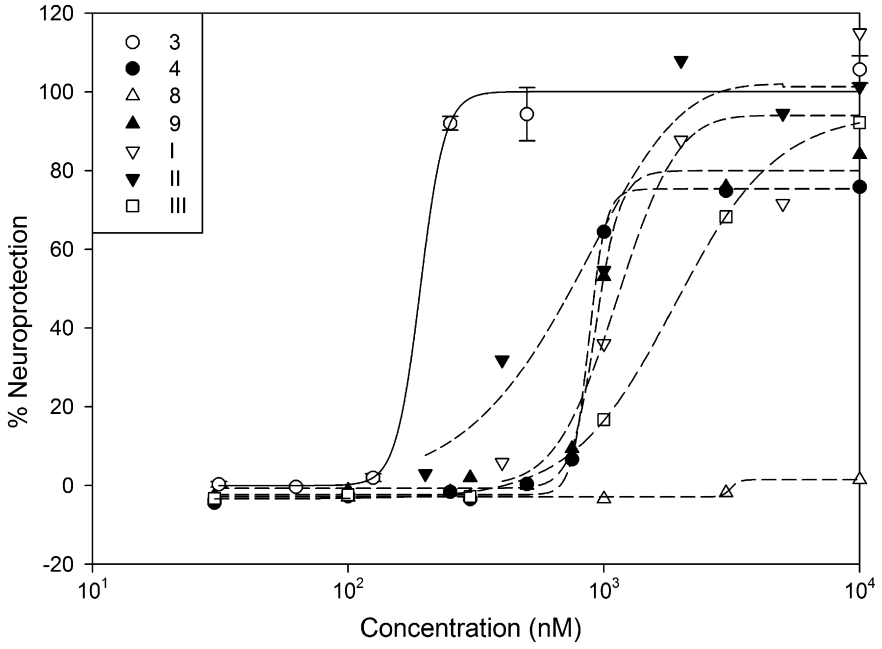


Figure 2. Protection of HT22 cells from oxytosis by 2-substituted chroman analogues. Cells were challenged with 5 mM glutamate in the absence or presence of increasing concentrations of the hybrids for 24 h and relative numbers of viable cells were assessed as described in Section 5. Values are mean ± SEM of three independent experiments with an intra-assay variation similar to that shown for **3**.

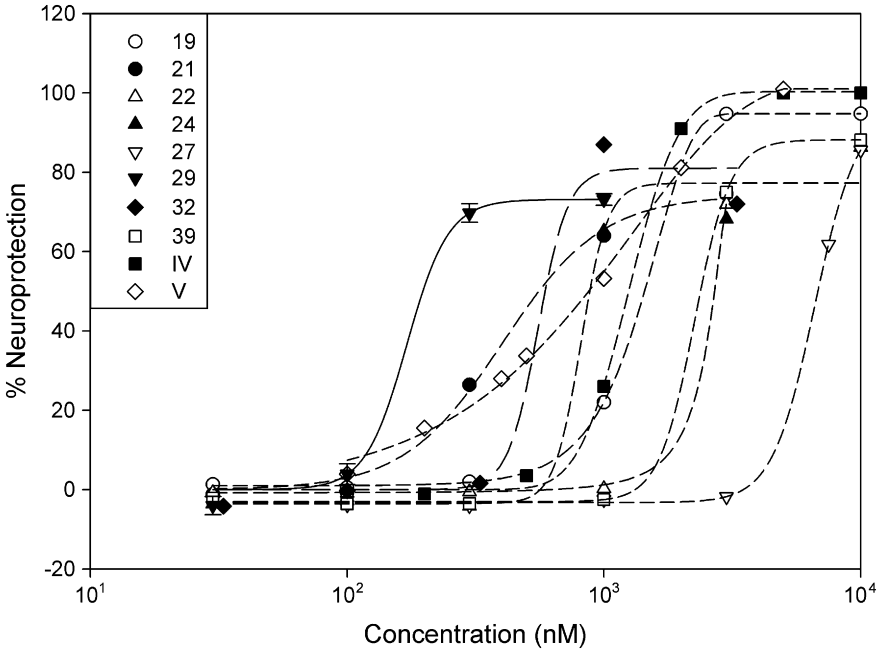


Figure 3. Protection of HT22 cells from oxytosis by 5-substituted chroman analogues. Cells were treated and data are presented as described in the legend to Figure 2.

4. Conclusion

Our results show that chroman/catechol hybrids bearing five membered heterocycles exhibited considerably higher in vitro neuroprotective activity compared to the majority of the first generation hybrids, with compounds **3**, **4**, **9**, **21**, **24**, **29** and **32** displaying EC_{50} values below 1 μM. Some 5-substituted chroman hybrids with isoxazole and oxadiazole moieties (**21**, **22**, **29** and **32**) displayed cytotoxicity at concentrations higher than 1–3 μM. However, chroman analogues bearing isoxazole substituents merit further investigation and the synthesis of new derivatives is currently in progress.

5. Experimental part

5.1. Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. NMR spectra were recorded on a Varian 300 spectrometer operating at 300 MHz for ^1H and 75.43 MHz for ^{13}C

Table 2
Efficacy and potency of 5-substituted chroman analogues to protect glutamate-challenged HT22 cells from oxytosis

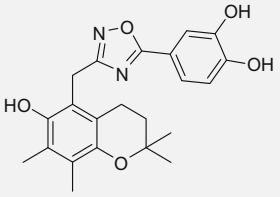
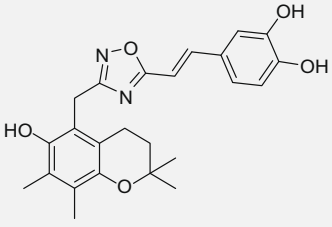
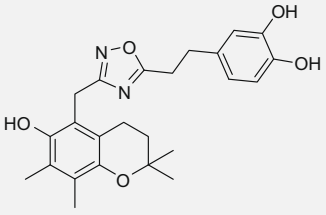
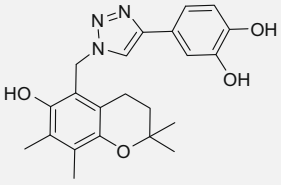
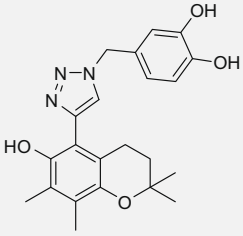
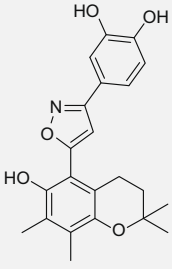
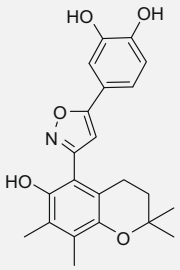
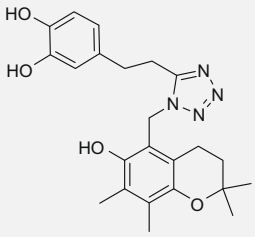
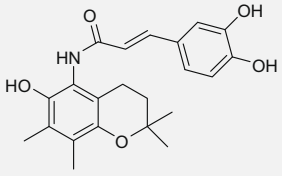
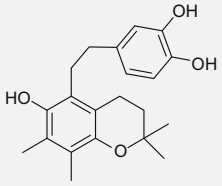
Compound	EC_{50}[a] (nM)	Relative potency[b]	Efficacy[c]
 **19**	1340 ± 214	0.7	95% (full)
 **21**	637 ± 321	1.5	73%* (full)
 **22**	2552 ± 258	0.4	72%* (full)
 **24**	801 ± 229	1.2	77% (full)
 **27**	7093 ± 929	0.1	86% (full)
 **29**	245 ± 38	3.8	73%◇ (full)

Table 2 (*continued*)

Compound	EC_{50}[a] (nM)	Relative potency[b]	Efficacy[c]
 **32**	582 ± 33	1.6	87%◇ (full)
 **39**	2823 ± 437	0.3	66% (full)
 **IV**	1230 ± 260	0.8	100% (full)
 **V**	930 ± 190	1	100% (full)

[a,b,c] as for legend to Table 1.
* Assessed at 3 μM **21** or **22.**
◇ Assessed at 1 μM **29**; ns = non-significant.

spectra with $CDCl_3$ as solvent. Silica gel plates Macherey-Nagel Sil G-25 UV_{254} were used for thin layer chromatography. Chromatographic purification was performed with silica gel (200–400 mesh). Mass spectra were recorded on TSQ 7000 Finigan instrument in the ESI mode. HRMS were recorded in FAB mode, at the University of Notre Dame, IN, USA.

5.1.1. *N*-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carbonyloxy)-*N*′-(3,4-dimethoxy)benzimidamide (1)

To a solution of *N*′-hydroxy-3,4-dimethoxybenzimidamide[12] (80 mg, 0.41 mmol) in 4 mL anhyd CH_2Cl_2 were added *N*-hydroxysuccinimidyl trolox ester (142 mg, 0.41 mmol) and the mixture was stirred at rt overnight. CH_2Cl_2 and water were then added, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, the solvent evaporated and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 97/3). Yield: 125 mg (72%), white solid, mp 218–220 °C. ^{1}H NMR δ: 7.12 (s, 1H, Ar*H*), 7.04 (d, *J* = 8.4 Hz, 1H, Ar*H*), 6.72 (d, *J* = 8.4 Hz, 1H, Ar*H*), 4.92 (br s, 1H, –O*H*), 4.51 (s, 2H, –NH_2), 3.82 (s, 3H, CH_3O–), 3.77 (s, 3H, CH_3O–), 2.61–2.48 (m, 3H), 2.19 (s, 3H, ArCH_3), 2.12 (s, 3H, ArCH_3), 2.01 (s, 3H, ArCH_3), 1.91–1.82 (m, 1H, C*H*–), 1.71 (s, 3H, CH_3). ^{13}C NMR δ: 172.0, 158.0, 151.5, 149.0, 146.3, 145.7, 123.3, 122.2, 122.1, 119.9, 119.6, 117.7, 110.7, 109.9, 78.1, 56.2, 56.1, 31.4, 26.2, 21.3, 12.5, 12.0, 11.6. MS *m/z*: 429.5 $[M+H]^+$.

5.1.2. *N*(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carbonyloxy)-*N*′-(3,4-dimethoxyphenyl) acetimidamide (2)

This compound was prepared according to the procedure described for **1**. Yield: 72%, yellowish gummy solid. ^{1}H NMR δ: 6.77–6.75 (m, 1H), 6.71 (br s, 2H), 4.06 (br s, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.46 (s, 2H), 2.63–2.54 (m, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 1.94–1.82 (m, 1H), 1.72 (s, 3H). ^{13}C NMR δ: 171.9, 158.8, 149.5, 148.7, 146.3, 145.7, 127.2, 121.6, 121.5, 112.1, 111.5, 78.1, 56.2, 56.1, 36.9, 31.3, 25.2, 21.3, 12.5, 12.0, 11.5. HRMS: calcd for $C_{24}H_{31}N_2O_6$ $[M+H]^+$ 443.2182, found 443.2169.

5.1.3. 3-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)-5-(3,4-dimethoxyphenyl)-1,2,4-oxadiazole (3)

Compound **1** (40 mg, 0.093 mmol) in 3.72 mL anhyd THF, was treated with 0.1 mL TBAF. After 2 h the solvent was evaporated

and the residue was diluted by AcOEt. The organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, the solvent evaporated and the residue was purified by flash chromatography (AcOEt/pet. ether, 95/5) to afford compound **3** as yellow foam. Yield: 38 mg (100%). ^{1}H NMR δ: 7.65 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 6.91 (d, J = 8.4 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 2.72–2.67 (m, 3H, CH_2 and CH*H*), 2.22 (s, 3H), 2.15 (s, 3H), 1.82 (s, 3H), 1.49–1.45 (m, 1H, C*H*), 1.25 (s, 3H). ^{13}C NMR δ: 181.1, 167.9, 151.4, 149.1, 145.7, 144.8, 123.0, 121.4, 120.9, 119.3, 118.4, 116.7, 110.9, 109.8, 73.7, 56.0, 55.9, 53.4, 31.4, 26.9, 20.5, 12.2, 11.9. MS *m/z*: 411 [M+H]$^+$. HRMS: calcd for $C_{23}H_{26}N_2O_5$ [M]$^+$ 410.1842, found 410.1846.

5.1.4. 5-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran)-3-(3,4-dimethoxybenzyl)-1,2,4-oxadiazole (4)

This compound was prepared according to the procedure described for **3**. Yield: 64%, yellow solid, mp 170–172 °C. ^{1}H NMR δ: 6.79 (br s, 3H), 3.98 (s, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 2.63–2.56 (m, 4H), 2.19 (s, 3H), 2.15 (s, 3H), 2.03 (s, 3H), 1.75 (s, 3H). ^{13}C NMR δ: 181.3, 169.4, 148.9, 148.0, 145.6, 144.8, 127.8, 122.9, 121.0, 118.3, 116.7, 112.1, 111.2, 73.6, 55.9, 55.8, 31.9, 31.4, 26.8, 20.5, 12.2, 11.9, 11.2. MS *m/z*: 425.3 [M+H]$^+$. HRMS: calcd for $C_{24}H_{28}N_2O_5$ [M]$^+$ 424.1998, found 424.1978.

5.1.5. *N*-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carbonyl)-*N'*-(3,4-dimethoxyphenyl-carbonyl) hydrazine (6)

CDI (26.8 mg, 0.165 mmol) was added to a solution of 3,4-dimethoxybenzoic acid (27 mg, 0.15 mmol) in 2.9 mL anhyd THF and the mixture was stirred at rt for 90 min. A solution of **5** (40 mg, 0.15 mmol) in 2.9 mL anhyd THF was then added and the new mixture was stirred at rt overnight. THF was evaporated and the residue was taken up by AcOEt and washed with satd aqueous NaCl. The organic layer was dried and evaporated to dryness and the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 97/3) affording **6** as yellow solid. Yield: 50 mg (77%), mp 198–200 °C. ^{1}H NMR δ: 9.32 (br s, 1H), 9.17 (br s, 1H), 7.75–7.56 (m, 1H), 7.38 (s, 1H), 6.98–6.81 (m, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 2.66–2.56 (m, 2H), 2.25 (s, 3H), 2.17 (s, 3H), 1.54–1.43 (m, 5H), 1.25 (s, 3H). MS *m/z*: 429.7 [M+H]$^+$.

5.1.6. 2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (8)

A mixture of compound **6** (39 mg, 0.09 mmol) and 0.18 mL $POCl_3$ was refluxed for 3 h. After completion of the reaction cold water was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with satd aqueous NaCl, dried and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 95/5) affording **8** as yellowish gummy solid. Yield: 23 mg (64%). ^{1}H NMR δ: 7.92 (d, J = 8.4 Hz, 1H), 7.70 (s, 1H), 6.97 (d, J = 8.4 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 2.71 (br s, 3H), 2.51–2.45 (m, 1H), 2.17 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.73 (s, 3H). ^{13}C NMR δ: 164.8, 154.2, 153.5, 148.8, 141.9, 127.8, 125.5, 124.3, 121.8, 116.9, 112.4, 110.4, 93.3, 82.7, 56.1, 56.0, 29.7, 13.1, 12.2, 11.9. HRMS: calcd for $C_{23}H_{26}N_2O_5$ [M]$^+$ 410.1842, found 410.1849.

5.1.7.2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran)-5-(3,4-dimethoxybenzyl)-1,3,4-oxadiazole (9)

Compound **7** (77 mg, 0.17 mmol) was treated as described for the synthesis of **8**. Yield: 10 mg (14 %), white gel. ^{1}H NMR δ: 6.79–6.67 (m, 3H), 4.08 (s, 2H), 3.88 (s, 3H), 3.78 (s, 3H), 2.82–2.63 (m, 4H), 2.13 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.73 (s, 3H). ^{13}C NMR δ: 168.8, 165.9, 149.1, 148.3, 145.7, 144.6, 126.2, 122.8, 120.8, 118.5, 117.2, 111.6, 111.3, 72.2, 55.9, 55.8, 31.4, 30.8, 26.6, 20.6, 12.1, 11.7, 11.2. MS *m/z*: 425.3 [M+H]$^+$. HRMS: calcd for $C_{24}H_{28}N_2O_5$ [M]$^+$ 424.1998, found 424.1981.

5.1.8. O-(3,4-Dimethoxybenzoyl)-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-acetamidoxime) (11)

To a solution of compound **10**[13] (40 mg, 0.14 mmol) in 4 ml anhyd CH_2Cl_2 were added 3,4-dimethoxybenzoic acid (25 mg, 0.14 mmol), DCC (34 mg, 0.16 mmol) and the mixture was stirred at rt overnight. CH_2Cl_2 was then added, the organic layer was washed with satd aqueous NaCl, dried and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95/5). Yield: 56 mg (90%), yellow solid, mp 198–200 °C. ^{1}H NMR δ: 7.63 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 6.87 (d, J = 8.4 Hz, 1H), 5.29 (br s, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.73 (s, 3H), 3.64 (s, 2H), 2.87 (t, J = 6.8 Hz, 2H), 2.21 (s, 3H), 2.10 (s, 3H), 1.79 (t, J = 6.8 Hz, 2H), 1.29 (s, 6H). ^{13}C NMR δ: 165.2, 158.7, 149.2, 141.5, 128.1, 123.3, 123.1, 112.1, 110.3, 73.3, 60.8, 56.1, 34.9, 29.7, 26.8, 25.5, 24.7, 20.3, 12.9, 12.1. MS *m/z*: 457.6 [M+H]$^+$.

5.1.9. O-(3,4-Dimethoxyphenylacetyl)-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-acetamidoxime) (12)

In 2 mL anhyd DMF were added, 3,4-dimethoxyphenylacetic acid (34 mg, 0.17 mmol) and 0.05 mL anhyd Et_3N. After 30 min the mixture was cooled and a solution of BOP (75 mg, 0.17 mmol) in 2 mL anhyd CH_2Cl_2 followed by a solution of **10** (50 mg, 0.17 mmol) in 3 mL anhyd CH_2Cl_2 were added. The mixture was stirred at 0 °C for 1 h and at rt overnight. The reaction mixture was then acidified by 10% HCl, diluted by water and extracted with AcOEt. The organic layer was washed with satd aqueous $NaHCO_3$, satd aqueous NaCl, dried and concentrated. Analogue **12** was obtained as a yellow gummy solid after purification by flash chromatography (CH_2Cl_2/MeOH, 97/3). Yield: 76 mg (93%). ^{1}H NMR δ: 6.86 (s, 1H), 6.83 (br s, 2H), 3.89 (s, 6H), 3.73 (s, 3H), 3.71 (s, 2H), 3.57 (s, 2H), 2.84 (t, J = 6.5 Hz, 2H), 2.24 (s, 3H), 2.14 (s, 3H), 1.81 (t, J = 6.5 Hz, 2H), 1.33 (s, 6H). ^{13}C NMR δ: 169.3, 158.7, 149.4, 149.2, 149.0, 148.4, 128.4, 126.8, 126.0, 123.4, 121.7, 118.7, 112.6, 111.5, 73.5, 61.1, 56.2, 40.5, 32.9, 28.4, 27.1, 20.6, 13.2, 12.4. MS *m/z*: 471.4 [M+H]$^+$ HRMS: calcd for $C_{26}H_{35}N_2O_6$ [M+H]$^+$ 471.2495, found 471.2481.

5.1.10. O-(3,4-Dimethoxyphenylpropenoyl)-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-acetamidoxime) (13)

According to the procedure described for **12** and using analogue **10** (40 mg, 0.14 mmol), 3,4-dimethoxyphenylpropenoic acid (29 mg, 0.14 mmol) and BOP (61 mg, 0.14 mmol), compound **13** was obtained as a yellow gummy solid after purification by flash chromatography (CH_2Cl_2/MeOH, 97/3). Yield: 18 mg (27%). ^{1}H NMR δ: 7.69 (d, J = 15.9 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 6.86 (d, J = 8.3 Hz, 1H), 6.36 (d, J = 15.9 Hz, 1H), 5.29 (br s, 2H), 3.91 (s, 6H), 3.71 (s, 3H), 2.84 (t, J = 6.8 Hz, 2H), 2.21 (s, 3H), 2.09 (s, 3H), 1.77 (t, J = 6.8 Hz, 2H), 1.28 (s, 6H). ^{13}C NMR δ: 165.3, 158.3, 151.4, 149.5, 149.4, 148.9, 145.2, 128.3, 127.7, 125.9, 123.4, 122.8, 118.8, 114.2, 111.3, 109.9, 73.5, 61.1, 56.2, 56.1, 32.8, 28.4, 27.1, 20.5, 13.1, 12.3. MS *m/z*: 483.2 [M+H]$^+$.

5.1.11. 5-(3,4-Dimethoxyphenyl)-3-[(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)methyl]-1,2,4-oxadiazole (15)

To a solution of acylated amidine **11** (46 mg, 0.11 mmol) in anhyd THF (C = 0.025 M), was added TBAF (0.04 mL, 0.11 mmol) and the mixture was stirred at rt for 2 h. After dilution with water the mixture was extracted with AcOEt, the organic layer was washed with satd aqueous $NaHCO_3$, satd aqueous NaCl, dried and

concentrated. Analogue **15** was obtained as a white gummy solid after purification by flash chromatography (pet. ether/AcOEt, 80/20). Yield: 27 mg (56%). ^{1}H NMR δ: 7.71 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 4.17 (s, 2H), 3.95 (s, 6H), 3.71 (s, 3H), 2.73 (t, J = 6.8 Hz, 2H), 2.21 (s, 3H), 2.11 (s, 3H), 1.77 (t, J = 6.8 Hz, 2H), 1.29 (s, 6H). ^{13}C NMR δ: 175.5, 170.6, 152.9, 150.1, 149.4, 148.5, 128.5, 125.6, 123.8, 122.2, 118.2, 117.2, 111.2, 110.7, 73.2, 61.5, 56.4, 56.3, 32.9, 27.1, 24.1, 20.8, 13.1, 12.3. MS m/z: 439.1 $[M+H]^+$.

5.1.12. 5-[2-(3,4-Dimethoxyphenyl)vinyl]-3-[(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)methyl]-1,2,4-oxadiazole (17)

According to the procedure described for **15** and using **13** (16 mg, 0.04 mmol) and TBAF (0.012 mL, 0.04 mmol), analogue **17** was obtained, as a yellow gummy solid. Yield: 13 mg (87%). ^{1}H NMR δ: 7.74 (d, J = 16.3 Hz, 1H), 7.33 (s, 1H), 7.18 (br s, 2H), 6.95 (d, J = 16.3 Hz, 1H), 4.29 (s, 2H), 3.99 (s, 6H), 3.77 (s, 3H), 2.75 (t, J = 6.5 Hz, 2H), 2.28 (s, 3H), 2.17 (s, 3H), 1.83 (t, J = 6.5 Hz, 2H), 1.35 (s, 6H). MS m/z: 465.4 $[M+H]^+$.

5.1.13. 5-[2-(3,4-Dimethoxyphenethyl)]-3-[(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)methyl]-1,2,4-oxadiazole (18)

According to the procedure described for **15** and using **14** (94 mg, 0.19 mmol) and TBAF (0.07 mL, 0.19 mmol), compound **18** was obtained as a yellow oil. Yield: 75 mg (86%). ^{1}H NMR δ: 6.73–6.67 (m, 3H), 4.08 (s, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.65 (s, 3H), 3.09–3.04 (m, 4H), 2.64 (t, J = 6.7 Hz, 2H), 2.20 (s, 3H), 2.09 (s, 3H), 1.75 (t, J = 6.7 Hz, 2H), 1.28 (s, 6H). ^{13}C NMR δ: 178.9, 169.9, 149.9, 149.1, 148.4, 147.9, 132.3, 128.5, 125.6, 123.6, 120.4, 117.9, 111.7, 111.5, 73.2, 61.4, 56.1, 55.9, 32.9, 32.4, 28.9, 27.1, 23.8, 20.7, 13.9, 13.1, 12.3. MS m/z: 467.6 $[M+H]^+$.

5.1.14. 5-[(3,4-Dihydroxyphenyl)]-3-[(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,4-oxadiazole (19)

A solution of **15** (25 mg, 0.06 mmol) in anhyd CH_2Cl_2 (C = 0.026 M), was cooled at 0 °C and $BF_3{\cdot}SMe_2$ (0.19 mL, 1.8 mmol) was added. The mixture was stirred at 0 °C for 1 h and an additional 1 h at rt. Excess of $BF_3{\cdot}SMe_2$ was evaporated under argon, water was added and the mixture was extracted with AcOEt. The organic layer was washed with satd aqueous NaCl, dried and concentrated. Analogue **19** was obtained as a white gel after purification by flash chromatography (CH_2Cl_2/MeOH, 97/3). Yield: 9 mg (40%). ^{1}H NMR δ: 7.59 (br s, 2H), 6.97 (br s, 1H), 4.09 (s, 2H), 2.84 (t, J = 6.7 Hz, 2H), 2.25 (s, 3H), 2.12 (s, 3H), 1.82 (t, J = 6.7 Hz, 2H), 1.31 (s, 6H). MS m/z: 397.3 $[M+H]^+$.

5.1.15. 5-[1-(3,4-Dihydroxyphenyl)methyl]-3-[(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,4-oxadiazole (20)

Oxadiazole analogue **16** (9.0 mg, 0.02 mmol) was treated as described for **19**. Compound **20** was obtained as a yellow gummy solid after purification by flash chromatography (CH_2Cl_2/MeOH, 97/3). Yield: 5 mg (64%). ^{1}H NMR δ: 6.81–6.79 (m, 2H), 6.71 (d, J = 7.5 Hz, 1H), 4.05 (s, 2H), 4.02 (s, 2H), 2.78 (t, J = 6.8 Hz, 2H), 2.23 (s, 3H), 2.11 (s, 3H), 1.78 (t, J = 6.8 Hz, 2H), 1.29 (s, 6H). MS m/z: 411.2 $[M+H]^+$.

5.1.16. 5-[2-(3,4-Dihydroxyphenyl)vinyl]-3-[(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,4-oxadiazole (21)

Analogue **17** (13 mg, 0.03 mmol) was treated as described for **19**. Compound **21** was obtained as a yellow gummy solid after purification by flash chromatography (CH_2Cl_2/MeOH, 95/5). Yield: 7 mg (64%). ^{1}H NMR δ: 7.61 (d, J = 16.3 Hz, 1H), 7.1–6.89 (m, 2H), 6.75 (s, 1H), 6.72 (d, J = 16.3 Hz, 1H), 4.07 (s, 2H), 3.51 (s, 3H), 2.83 (t, J = 6.7 Hz, 2H), 2.26 (s, 3H), 2.12 (s, 3H), 1.81 (t, J = 6.7 Hz, 2H), 1.31 (s, 6H). ^{13}C NMR δ: 175.2, 169.2, 146.4, 145.5, 143.8, 143.3, 127.6, 125.4, 125.3, 122.8, 116.5, 115.7, 114.1, 113.9, 72.7, 32.8, 29.7, 26.7, 23.4, 22.7, 20.8, 12.0. HRMS: calcd for $C_{24}H_{26}N_2O_5$ $[M]^+$ 422.1842, found 422.1842.

5.1.17. 5-[2-(3,4-Dihydroxyphenyl)ethyl]-3-[(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,4-oxadiazole (22)

Treatment of compound **18** (48 mg, 0.11 mmol) as described for **19**, gave **22** as yellow gummy solid. Yield: 25 mg (58%). ^{1}H NMR δ: 6.72 (d, J = 8.1 Hz, 1H), 6.63 (s, 1H), 6.54 (d, J = 8.1 Hz, 1H), 4.03 (s, 2H), 3.10–2.92 (m, 4H), 2.75 (t, J = 6.8 Hz, 2H), 2.09 (s, 3H), 2.06 (s, 3H), 1.77 (t, J = 6.8 Hz, 2H), 1.27 (s, 6H). ^{13}C NMR δ: 179.4, 168.6, 146.5, 145.1, 143.7, 142.6, 131.6, 125.4, 120.5, 118.8, 116.7, 115.4, 72.8, 32.8, 31.5, 29.7, 28.5, 26.7, 23.2, 20.7, 13.5, 12.5. MS m/z: 425.3 $[M+H]^+$. HRMS: calcd for $C_{24}H_{28}N_2O_5$ $[M]^+$ 424.1998, found 424.1997.

5.1.18. 4-(3,4-Dimethoxyphenyl)-1-[(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,3-triazole (23)

A solution of 4-ethynyl-1,2-dimethoxybenzene (40 mg, 0.25 mmol), of azidomethyl-chroman (137 mg, 0.50 mmol), $CuSO_4{\cdot}5H_2O$ (18.7 mg, 0.075 mmol) and sodium ascorbate (30 mg, 0.15 mmol) in 4.16 mL t-BuOH/H_2O (2:1) was stirred at rt for 24 h. After cooling at 0 °C, aqueous NH_4OH was added and the mixture was exracted with AcOEt. The organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered and the solvent evaporated. Compound **27** was obtained as a yellow oil. Yield: 110 mg (100%). ^{1}H NMR δ: 7.58 (s, 1H), 7.43 (s, 1H), 7.21 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 5.57 (s, 2H), 3.92 (s, 3H), 3.87 (s, 3H), 3.69 (s, 3H), 2.69 (t, J = 6.8 Hz, 2H), 2.23 (s, 3H), 2.12 (s, 3H), 1.73 (t, J = 6.8 Hz, 2H), 1.25 (s, 6H). ^{13}C NMR δ: 150.1, 149.2, 148.9, 148.6, 147.6, 127.9, 123.8, 122.2, 118.8, 118.2, 118.1, 111.2, 108.8, 73.3, 61.7, 55.9, 45.7, 32.3, 26.7, 19.9, 12.9, 12.2. MS m/z: 438.4 $[M+H]^+$.

5.1.19. 4-(3,4-Dihydroxyphenyl)-1-[(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-methyl]-1,2,3-triazole (24)

To a solution of protected triazole analogue **23** (38 mg, 0.08 mmol) in 3.5 mL anhyd CH_2Cl_2 were added at 0 °C, $BF_3{\cdot}SMe_2$ (0.28 mL, 2.61 mmol) and the mixture was stirred at 0 °C for 1 h and overnight at rt. Workup as described for **19** and purification by flash chromatography (CH_2Cl_2/MeOH, 95/5) afforded **24** as a yellowish solid, mp 265–268 °C. Yield: 26 mg (77%). ^{1}H NMR δ: 7.63 (s, 1H), 7.18 (s, 1H), 6.91 (d, J = 8.1 Hz, 1H), 6.75 (d, J = 8.1 Hz, 1H), 5.52 (s, 2H), 2.72 (t, J = 6.5 Hz, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 1.72 (t, J = 6.5 Hz, 2H), 1.22 (s, 6H). ^{13}C NMR δ: 147.6, 146.0, 145.8, 145.1, 144.6, 127.1, 123.6, 122.3, 119.4, 117.8, 117.6, 117.4, 115.3, 112.3, 72.9, 45.8, 32.5, 26.5, 20.1, 12.4, 12.2. MS m/z: 395.4 $[M]^+$. HRMS: calcd for $C_{22}H_{26}N_3O_4$ $[M+H]^+$ 396.1923, found 396.1964.

5.1.20. 1-(3,4-Dimethoxybenzyl)-4-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-1,2,3-triazole (26)

A solution of 5-ethynyl-3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran **25** (20 mg, 0.08 mmol), 4-azidomethyl-1,2-dimethoxy-benzene (31 mg, 0.16 mmol), $CuSO_4{\cdot}5H_2O$ (6.0 mg, 0.02 mmol), and sodium ascorbate (10 mg, 0.05 mmol) in t-BuOH/H_2O (2:1) (1.3 mL) was treated as described for **23**. Purification by flash chromatography (cyclohexane/AcOEt, 70/30) afforded compound **26**, as a yellow gummy solid. Yield: 34 mg (97%). ^{1}H

NMR δ: 7.60 (s, 1H), 6.68–6.81 (m, 3H), 5.52 (s, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 3.27 (s, 3H), 2.75–2.71 (m, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 1.69–1.65 (m, 3H), 1.30 (s, 3H), 1.24 (s, 3H). ^{13}C NMR δ: 149.4, 149.3, 149.1, 148.3, 128.1, 127.5, 126.5, 123.6, 120.6, 120.4, 118.3, 111.2, 110.9, 73.3, 60.6, 55.9, 53.9, 32.8, 29.7, 26.9, 21.9, 12.5, 12.2. MS *m/z*: 438.4 $[M+H]^+$.

5.1.21. 1-(3,4-Dihydroxybenzyl)-4-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)-1,2,3-triazole (27)

Triazole analogue **26** (18 mg, 0.04 mmol) was treated as described for **24** to produce deprotected derivative **27**, as a yellowish gummy solid. Yield: 13 mg (82%). ^{1}H NMR δ: 7.65 (s, 1H), 6.81–6.71 (m, 3H), 5.42 (s, 2H), 2.60 (t, J = 6.1 Hz, 2H), 2.16 (s, 3H), 2.13 (s, 3H), 1.72 (t, J = 6.1 Hz, 2H), 1.26 (s, 3H), 1.22 (s, 3H). HRMS: calcd for $C_{22}H_{25}N_3O_4$ $[M]^+$ 395.1845, found 395.1833.

5.1.22. 3-(3,4-Dimethoxyphenyl)-5-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)isoxazole (28)

3,4-Dimethoxybenzaldehyde oxime (11 mg, 0.06 mmol) was added in 1 mL of *t*-BuOH/H_2O (1/1), followed by addition of chloramine-T trihydrate (18 mg, 0.06 mmol) in small portions over 5 min, $CuSO_4{\cdot}5H_2O$ (2 mg, 0.02 mmol), copper turnings (catalytic amount) and alkyne **25** (15 mg, 0.06 mmol) and the mixture was stirred overnight. The reaction mixture was poured into ice/water and after addition of dilute NH_4OH, was extracted with AcOEt. The organic layer was washed with satd aqueous NaCl, dried and evaporated to dryness. Compound **28** was obtained as yellow gummy solid, after purification by flash chromatography (pet. ether/AcOEt, 80/20). Yield: 20 mg (81%). ^{1}H NMR δ: 7.52 (s, 1H), 7.39 (d, J = 8.3 Hz, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.72 (s, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.53 (s, 3H), 2.72 (t, J = 6.6 Hz, 2H), 2.24 (s, 3H), 2.18 (s, 3H), 1.74 (t, J = 6.6 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR δ: 168.1, 162.2, 150.5, 149.3, 148.3, 129.0, 128.9, 112.1, 119.9, 118.2, 111.1, 109.3, 102.5, 73.6, 61.6, 56.1, 56.0, 32.6, 26.9, 21.4, 12.6, 12.4. MS *m/z*: 424.5 $[M+H]^+$.

5.1.23. 3-(3,4-Dihydroxyphenyl)-5-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)isoxazole (29)

Isoxazole analogue **28** (10 mg, 0.03 mmol) was treated as described for **24** to produce deprotected derivative **29**, as an orange gummy solid, after purification by flash chromatography (CH_2Cl_2/MeOH, 95/5. Yield: 6 mg (67%). ^{1}H NMR δ: 7.43 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.60–6.57 (m, 2H), 5.57 (br s, 2H), 2.68–2.67 (m, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 1.71–1.68 (m, 2H), 1.31 (s, 6H). HRMS: calcd for $C_{22}H_{23}NO_5$ $[M]^+$ 381.1576, found 381.1584.

5.1.24. 5-(3,4-Dimethoxyphenyl)-3-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)isoxazole (31)

Oxime **30** (58 mg, 0.22 mmol) and 4-ethynyl-1,2dimethoxybenzene (37 mg, 0.23 mmol), were treated as described for **28**. Purification by flash chromatography (pet. ether/AcOEt, 85/15) afforded compound **31** as a yellow solid, mp 140–142 °C. Yield: 60 mg (65%). ^{1}H NMR δ: 7.40 (d, J = 8.4 Hz, 1H), 7.35 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.54 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.49 (s, 3H), 2.69 (t, J = 6.8 Hz, 2H), 2.21 (s, 3H), 2.15 (s, 3H), 1.69 (t, J = 6.8 Hz, 2H), 1.31 (s, 6H). ^{13}C NMR δ: 169.2, 160.8, 150.8, 149.8, 149.5, 148.6, 128.8, 127.9, 120.9, 119.9, 119.3, 118.1, 111.5, 108.9, 100.9, 73.7, 61.8, 56.3, 56.2, 32.9, 27.2, 21.8, 12.7, 12.5. MS *m/z*: 424.1 $[M+H]^+$. HRMS: calcd for $C_{25}H_{30}NO_5$ $[M+H]^+$ 424.2124, found 424.2122.

5.1.25. 5-(3,4-Dihydroxyphenyl)-3-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl)isoxazole (32)

Compound **31** (32 mg, 0.08 mmol), was treated as described for **24**. Purification by flash chromatography (CH_2Cl_2/MeOH, 95/5) gave isoxazole **32** as a white gummy solid. Yield: 9 mg (32%). ^{1}H NMR δ: 7.45 (s, 1H), 7.32 (d, J = 8.3 Hz, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.64 (s, 1H), 2.81 (t, J = 6.7 Hz, 2H), 2.23 (s, 3H), 2.18 (s, 3H), 1.75 (t, J = 6.7 Hz, 2H), 1.35 (s, 3H), 1.25 (s, 3H) HRMS: calcd for $C_{22}H_{23}NO_5$ $[M]^+$ 381.1576, found 381.1606.

5.1.26. *N*-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-methyl)-3-(3,4-dimethoxyphenyl-propanethioamide) (35)

To a solution of carboxamide **33** (76 mg, 0.17 mmol) in 6 mL THF was added Lawesson's reagent (69 mg, 0.17 mmol) and the mixture was refluxed for 6 h. The solvent was evaporated and the residue was purified by flash chromatography (pet. ether/AcOEt, 60/40), to afford thioamide **35**, as an off white gummy solid. Yield: 75 mg (96%). ^{1}H NMR δ: 6.71 (s, 1H), 6.68 (br s, 2H), 4.71 (d, J = 4.6 Hz, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.58 (s, 3H), 3.05 (t, J = 7.2 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 2.56 (t, J = 6.7 Hz, 2H), 2.16 (s, 3H), 2.09 (s, 3H), 1.72 (t, J = 6.7 Hz, 2H), 1.28 (s, 6H). ^{13}C NMR δ: 202.9, 150.2, 148.8, 148.4, 147.4, 132.8, 128.5, 126.7, 123.6, 120.2, 117.6, 111.6, 111.1, 73.3, 61.2, 49.0, 42.6, 35.0, 32.5, 26.8, 21.0, 20.3, 14.2, 12.6, 12.1. MS *m/z*: 458.5 $[M+H]^+$.

5.1.27. *N*-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-ethyl)-3-(3,4-dimethoxy-phenylpropanethioamide) (36)

Amide **34** (22 mg, 0.05 mmol) was treated as described for **35**. Yield: 19 mg (86%). ^{1}H NMR δ: 6.79–6.72 (m, 3H), 3.85 (s, 6H), 3.65–3.63 (m, 5H), 3.03–2.98 (m, 2H), 2.89–2.85 (m, 4H), 2.68 (t, J = 6.7 Hz, 2H), 2.18 (s, 3H), 2.10 (s, 3H), 1.80 (t, J = 6.7 Hz, 2H), 1.32 (s, 6H). ^{13}C NMR δ: 203.6, 148.9, 148.8, 148.7, 147.4, 133.2, 128.0, 126.3, 125.1, 120.1, 116.9, 111.7, 111.2, 73.1, 60.8, 55.9, 55.8, 48.9, 47.5, 34.8, 32.7, 26.8, 24.0, 20.3, 12.8, 11.9. MS *m/z*: 471.5 $[M]^+$.

5.1.28. 1-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl-methyl)-5-(3,4-dimethoxy-2-phenyl- ethyl) tetrazole (37)

To a solution of thioamide **35** (75 mg, 0.16 mmol) in 1.2 mL anhyd THF, were added DIAD (0.05 mL, 0.24 mmol), triphenylphosphine (65 mg, 0.24 mmol) and after 5 min $TMSN_3$ (0.03 mL, 0.24 mmol). The reaction mixture was stirred at rt for overnight. The solvent was evaporated in vacuo and the residue was purified by flash chromatography (pet. ether/AcOEt 50:50). Yield: 76 mg (100%), white gummy solid. ^{1}H NMR δ: 6.74 (d, J = 8.0 Hz, 1H), 6.61–6.57 (m, 2H), 5.22 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.53 (s, 3H), 3.14 (t, J = 7.6 Hz), 2.89 (t, J = 7.6 Hz), 2.53 (t, J = 6.7 Hz, 2H), 2.17 (s, 3H), 2.09 (s, 3H), 1.72 (t, J = 6.7 Hz, 2H), 1.26 (s, 3H), 1.24 (s, 3H). ^{13}C NMR δ: 154.7, 149.8, 148.9, 148.6, 147.7, 132.3, 128.4, 125.1, 120.8, 120.3, 118.3, 111.6, 111.2, 73.3, 61.5, 60.4, 55.9, 42.9, 33.3, 32.3, 26.7, 21.0, 20.4, 14.2, 12.8, 12.3. MS *m/z*: 467.6 $[M+H]^+$.

5.1.29. 1-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl-ethyl)-5-(3,4-dimethoxy-2-phenylethyl) tetrazole (38)

Thioamide **36** (19 mg, 0.04 mmol) was treated as described for **37**. Yield: 9 mg (47%), white gummy solid. ^{1}H NMR δ: 6.74 (d, J = 8.1 Hz, 1H), 6.58–6.53 (m, 2H), 4.29 (t, J = 6.9 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.64 (s, 3H), 3.04 (t, J = 6.9 Hz, 2H), 2.91–2.86 (m, 2H), 2.66 (t, J = 7.7 Hz, 2H), 2.28 (t, J = 6.7 Hz, 2H), 2.14 (s, 3H), 2.07 (s, 3H), 1.67 (t, J = 6.7 Hz, 2H), 1.24 (s, 6H). ^{13}C NMR δ: 149.8, 148.9, 148.4, 147.7, 132.2, 128.4, 125.5, 124.3, 120.1, 117.3, 111.6, 111.3, 73.0, 60.7, 55.9, 55.8, 46.7, 32.9, 32.6, 27.4, 26.7, 24.7, 20.2, 12.7, 12.0 MS *m/z*: 481.4 $[M+H]^+$. HRMS: calcd for $C_{27}H_{37}N_4O_4$ $[M+H]^+$ 481.2815, found 481.2809.

5.1.30. 1-(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-1-benzopyran-5-yl-methyl)-5-(3,4-dihydroxy-2-phenylethyl) tetrazole (39)

Tetrazole analogue **37** (34 mg, 0.07 mmol) was treated as described for **24**. Purification by flash chromatography (pet. ether/AcOEt, 50/50). Yield: 18 mg (60%), white gummy solid. ^{1}H NMR ($CDCl_3$) δ: 6.73 (d, J = 7.8 Hz, 1H), 6.55 (s, 1H), 6.45 (d, J = 7.8 Hz, 1H), 5.26 (s, 2H), 3.19–3.15 (m, 2H), 2.85–2.81 (m, 2H), 2.74–2.70 (m, 1H), 2.09 (s, 6H), 1.78–1.74 (m, 3H), 1.25 (s, 6H). HRMS: calcd for $C_{23}H_{28}N_4O_4$ $[M]^+$ 424.2111, found 424.2136.

5.2. Evaluation of the activity of chroman/catechol hybrids against oxidative stress-induced cell death of HT22 hippocampal neurons

The hybrids were tested as previously described,[10,13] with minor modifications. Briefly, HT22 cells were plated in a 96-well flat bottom plate at a density of 4000 cells per well in 100 μl of DMEM-Hepes-GlutaMAX medium containing 10% of fetal bovine serum. 24 h after plating, the cells were challenged with 5 mM glutamate in the absence or presence of increasing concentrations of the hybrids in fresh medium for 24 h prior to assessing the relative numbers of living cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. MTT conversion to coloured formazan was assessed from the difference in optical density (dOD) at 550 and 670 nm. Direct interference of the test compounds with MTT conversion to formazan was excluded using mock cultures deprived of HT22 cells. Interference of the hybrids with mitochondrial conversion of MTT to formazan was excluded using the trypan blue exclusion assay to directly determine the number living cells. No challenged cells served to test cytotoxicity at different hybrid concentrations, whereas challenged cells served to assess neuroprotective activity by comparison. Cells exposed only to vehicle (DMSO) or glutamate served as controls. Cell death (CD) in the absence of hybrids was calculated by $CD_{Vehicle} = [(dOD_{Vehicle} - dOD_{Glutamate}) * 100/dOD_{Vehicle}$, whereas cell death in their presence was calculated by $CD_{Compound} = [(dOD_{Compound} - dOD_{Compound+Glutamate}) * 100/dOD_{Compound}$. Neuroprotection (%) was calculated by $[(CD_{Vehicle} - CD_{Compound}) * 100/CD_{Vehicle}$.

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