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(54) Title: USE OF A POLYPHENOLIC TYPE COMPOUND FOR PREVENTING OR TREATING A POLYGLUTAMINE EXPANSION NEURODEGENERATIVE DISEASE

(57) Abstract: The present invention relates to the use of polyphenolic type compounds as disclosed in the specification for preventing or treating a polyglutamine expansion neurodegenerative disease.



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TITLE OF THE INVENTION

Use of a polyphenolic type compound for preventing or treating a polyglutamine expansion neurodegenerative disease

FIELD OF THE INVENTION

The present invention relates to the field of human therapeutically useful compounds that are active against neurodegenerative diseases.

More particularly, the present invention relates to the use of a polyphenolic type compound for the manufacture of a medicament intended for preventing or treating polyglutamine expansion neurodegenerative diseases.

BACKGROUND OF THE INVENTION

Polyglutamine expansion neurodegenerative diseases, including Huntington's disease, spinocerebellar ataxia 1, 2, 3, 6, 7, and 17, spinal and bulbar muscular atrophy (SBMA) and dentatorubral pallidoluysian atrophy (DRPLA), are hereditary neurodegenerative disorders caused by CAG repeat expansions encoding polyglutamine stretches in the corresponding proteins.

These CAG repeat expansions result in the synthesis and accumulation of ubiquitously expressed polyglutamine-containing proteins, some of still unknown function (e.g. Huntingtin in Huntington's disease and ataxins in spinocerebellar ataxias).

The accumulation of polyglutamine-containing proteins in various brain regions is responsible for neurologic problems. Proteins with expanded polyglutamine domains aggregate and indeed aggregation is a pathologic hallmark of the polyglutamine repeat diseases.

All diseases in the CAG repeat family show genetic anticipation, meaning that the disease usually appears at an earlier age and increases in severity with each generation. Genetic anticipation is linked to increasing numbers of CAG repeats, which result from expansion of the unstable CAG sequence when reproductive cells divide to form eggs and sperm. In general, neurodegenerative disorders are progressive (i.e., their symptoms are not apparent until months or more commonly years after the disease has begun), and caused by an initial reduction of neuronal function, followed by a complete dysfunction upon neuronal death.

Huntington's Disease (HD) is a devastating, degenerative brain disorder for which there is, at present, no effective treatment or cure. Early symptoms of Huntington's Disease may affect cognitive ability or mobility and include depression, mood swings, forgetfulness, clumsiness, involuntary twitching and lack of coordination. As the disease progresses, concentration and short-term memory diminish and involuntary movements of the head, trunk and limbs increase. Walking, speaking and swallowing abilities deteriorate. Death follows from complications such as choking, infection or heart failure. HD typically begins in midlife, between the ages of 30 and 45, though onset may occur as early as the age of 2 in its juvenile, more

severe form. Children who develop the juvenile form of the disease rarely live to adulthood. HD affects males and females equally and crosses all ethnic and racial boundaries. Each child of a person with HD has a 50/50 chance of inheriting the fatal gene. HD is an autosomal dominant condition and thus everyone who carries the mutant allele of the gene will develop the disease.

5 The Huntington's Disease (HD) gene was cloned in 1993 (The Huntington's Disease Collaborative Research Group. Cell. 1993) and found to contain a CAG repeat within its 5'-end coding sequence. This CAG repeat is expanded in individuals with HD (who may or may not be symptomatic, depending upon their age). However, the presence of a CAG repeat expansion is found in virtually all symptomatic HD individuals. Individuals affected with HD typically have at
10 least one HD allele with 36 or more CAG repeat. The polyglutamine expansion results in the formation of insoluble, high molecular weight protein aggregates similar to those seen in Alzheimer's disease.

 Spinobulbar muscular atrophy (SBMA, also named Kennedy disease) is caused by a specific mutation (an expansion of the normally polymorphic CAG trinucleotide repeat) in the
15 first exon of the androgen receptor gene (which encodes polyglutamine tracts) which is located on the X-chromosome. Similar to Huntington's disease, in this disease CAG is also abnormally repeated. The CAG repeat range in the general population is approximately 12 to 32 repeats. In patients with Kennedy disease, the repeats may vary from 40 to 55 repeats. Symptoms appear when the repeats exceed about 40. A larger number of repeats has been suggested to cause
20 symptoms to begin earlier in life and progress more rapidly.

 Spinocerebellar ataxias are a group of autosomal dominantly inherited ataxias with heterogeneous presentation. Characteristic CAG repeat expansions in the coding sequences at several loci have been detected for certain of these disorders. For example, Spinocerebellar ataxia 7 (SCA7) is a progressive autosomal dominant neurodegenerative disorder characterized
25 by cerebellar ataxia and visual impairment (David et al., 1997) due to moderate to severe neuronal loss in the cerebellum and associated structures and degeneration of cone and rod photoreceptors. The function of the protein encoded by the SCA7 gene was elucidated, in 2004. The SCA7 gene product, ataxin-7 (ATXN7), is a component of the TBP-free TAF-containing complex (TFTC) and the SPT3/TAF9/GCN5 acetyltransferase complex (STAGA), which are
30 implicated in several steps of transcriptional regulation, such as histone acetylation and recruitment of the preinitiation complex to promoters.

 Current treatment of the polyglutamine expansion neurodegenerative diseases includes number of medications to help control some symptoms like emotional and movement problems associated with polyglutamine disorders.

35 However, there is currently no efficient treatment to prevent, stop and/or reverse the course of the polyglutamine expansion neurodegenerative diseases.

Thus, new methods for the treatment of neurodegenerative polyglutamine diseases, including but not limited to Huntington's disease, spinocerebellar ataxias, and spinobulbar muscular atrophy (Kennedy's Disease) that are effective and convenient are really needed.

A new therapeutic strategy to fight against these polyglutamine expansion neurodegenerative diseases, consists in the identification of partners to polyglutamine mutant proteins, as well as developmental and longevity pathways, to influence the variability of neurodegenerative diseases through the modulation of cell maintenance mechanisms.

Regarding for example Huntington's disease (HD), the notion that partners of polyglutamine mutant proteins could influence the variability of HD was illustrated for the transcriptional regulator and huntingtin (htt) partner protein CA150 (See Holbert et al., Proc. Natl. Acad. Sci. USA. 2001 ; 98 : 1811-1816, Arango et al. Neurotoxicity. Journal of Neuroscience 2006 ; 26 : 4649-4659)

It was also shown that key longevity modulators like the NAD-dependent deacetylase sir-2.1 and its target, the transcription factor DAF-16/FoxO, protect nematode neurons from the burden of expanded polyQ cytotoxicity (Parker et al., 2005, Nat Genet, Vol. 37 : 349-350).

It was moreover demonstrated that the antioxidant resveratrol rescued the neuronal dysfunction induced by expanded polyQ cytotoxicity through an action on sir-2.1 and on the transcription factor daf-16/FoxO (Parker et al., 2005, *supra*); these result was confirmed in neuronal cell lines derived from the striatum of HdhQ111 knock-in mice where resveratrol decreased cell death induced in cells homozygous with respect to an allele encoding mutant htt (109Q) (Parker et al., 2005 *supra*).

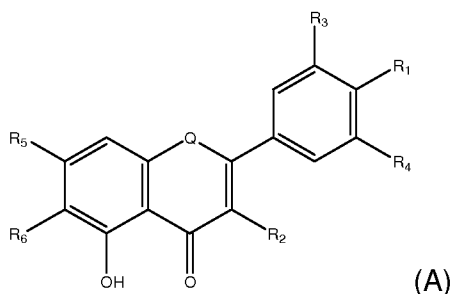
While resveratrol protects the HD neuron through the activity of key modulators of neuron cell survival like sirtuins (sir-2.1) or FoxO proteins, its bioavailability and blood-brain barrier (BBB) properties may be limited.

There is us a need in the art for other new molecules showing a similar mode of action on cell maintenance/longevity mechanisms and having optimal drug properties, and which could be used for the treatment of polyglutamine expansion neurodegenerative diseases.

SUMMARY OF THE INVENTION

The present invention relates to the use a polyphenolic type compound which is selected from the group of compounds having the following formulae A, B or C:

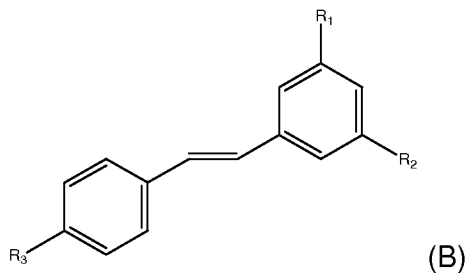
(A)



wherein:

- R1 and R2, one independently from the other, are selected from the group consisting of a saccharide group, an alkoxy and a hydroxy,
- R3, R4, R5 and R6, one independently from the other, are selected from the group consisting of a hydrogen atom, an alkoxy and a hydroxy,
- Q is selected from the group consisting of oxygen, sulfur or nitrogen,

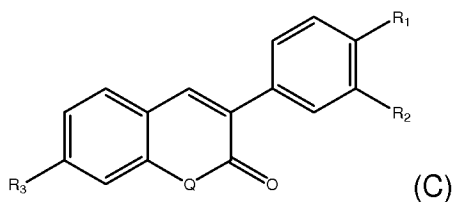
(B)



wherein

- R1 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a hydroxy and an alkoxy,
 - R2 is selected from the group consisting of a saccharide group, a hydroxy and an alkoxy,
 - with the proviso that R1, R2 and R3 do not mean simultaneously a hydroxyl group,
- and

(C)



wherein :

- R1, R2 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a halogen atom, a hydroxy and an alkoxy,
 - Q is selected from the group consisting of oxygen, sulfur or nitrogen,
- for manufacturing a medicament for preventing or treating a polyglutamine expansion neurodegenerative disease.

The present invention also relates to an active polyphenolic type compound selected from the group as defined above, advantageously for the use as a medicament.

This invention concerns also a pharmaceutical composition comprising a compound as above mentioned, in combination with one or more pharmaceutically acceptable excipients, advantageously to prevent or to treat polyglutamine expansion neurodegenerative disease.

5 **BRIEF DESCRIPTION OF THE FIGURES**

Figures 1 to 11 show dose-response curves for means $\pm$ s.e.m percent rescue of neuronal dysfunction by polyphenolic type compounds in worms expression control 19Q or 128Q, in different genotypes.

The resveratrol dose-response curve is repeated for comparison.

10 $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus DMSO-treated 128Q animals.

Abscissa : final concentration of the tested compound, expressed in μ M. Ordinate : percent rescue of neuronal dysfunction.

In figures 1 to 11 :

- 15 - the black curve with the square symbol “-■-” illustrate the results obtained with the tested compound on 128Q worms,
- the grey curve with the triangle symbol “-▲-” illustrate the results obtained with the tested compound on 19Q worms,
- the black curve with the inversed triangle symbol “-▼-” illustrate the results obtained with the tested compound on *sir-2.1(ok434)*, 128Q worms, and
- 20 - the black curve with the losange symbol “-◆-” illustrate the results obtained with resveratrol on 128Q worms.

Figure 12A shows dose-response curves for means $\pm$ s.e.m percent rescue of neuronal dysfunction by the hyperoside compound in worms expression control 19Q or 128Q, in different genotypes.

25 The resveratrol dose-response curve is repeated for comparison.

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus DMSO-treated 128Q animals.

Abscissa : final concentration of the tested compound, expressed in μ M. Ordinate : percent rescue of neuronal dysfunction.

In figure 12A :

- 30 - the black curve with the circle symbol “-●-” illustrate the results obtained with resveratrol on 128Q worms,
- the black curve with the triangle symbol “-▲-” illustrate the results obtained with hyperoside on 128Q worms,
- the grey curve with the circle symbol “-●-” illustrate the results obtained with hyperoside on
- 35 19Q worms,
- the black curve with the inversed triangle symbol “-▼-” illustrate the results obtained with hyperoside on *sir-2.1(ok434)*, 128Q worms,

- the grey curve with the losange symbol “-◆-” illustrate the results obtained with resveratrol on *ucp-4(ok195)*, 128Q worms, and
- the black curve with the square symbol “-■-” illustrate the results obtained with hyperoside on *daf-16(mgDf50)*, 128Q worms.

Figure 12B shows that hyperoside reduces the mortality of 109Q/109Q cells (*p<0.001 versus DMSO controls) with no effect in 7Q/7Q cells. Ordinate : fold change in cell mortality versus untreated.

Figure 13 illustrate the effect of the polyphenolic type compounds on the reduction of cell death in a cellular model of Huntington's disease pathogenesis.

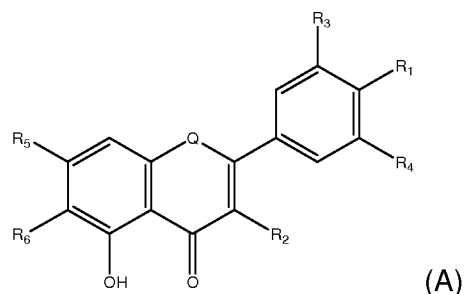
Abscissa : Control cultures or the identity of the tested compound; Grey bars on the left side of the figure illustrate the results obtained with wild-type cells. Black bars on the right side of the figure illustrate the results obtained with cells expressing the mutated (109Q) huntingtin. Ordinate : fold change in mortality versus untreated.

DETAILED DESCRIPTION OF THE INVENTION

It has been found according to the invention a new group of polyphenolic compound that are active against polyglutamine expansion neurodegenerative diseases, in particular of the Huntington's disease.

A first object of the invention consists of the use of a substance selected from the group of compounds having the formulae A, B or C, for manufacturing a medicament for preventing or treating a polyglutamine expansion neurodegenerative disease, in which said formulae A, B or C are the following ones :

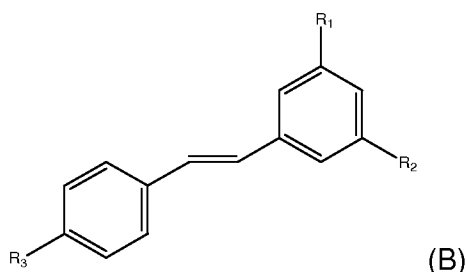
(A)



wherein :

- R1 and R2, one independently from the other, are selected from the group consisting of a saccharide group, an alkoxy and a hydroxy,
- R3, R4, R5 and R6, one independently from the other, are selected from the group consisting of a hydrogen atom, an alkoxy and a hydroxy,
- Q is selected from the group consisting of oxygen, sulfur or nitrogen,

(B)



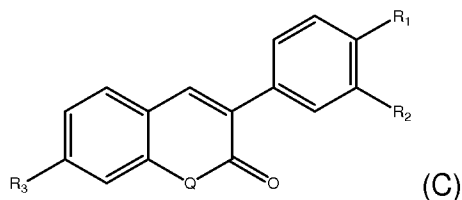
wherein

- R1 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a hydroxy and an alkoxy,

- R2 is selected from the group consisting of a saccharide group, a hydroxy and an alkoxy,

- with the proviso that R1, R2 and R3 do not mean simultaneously a hydroxyl group, and

(C)



wherein :

- R1, R2 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a halogen atom, a hydroxy and an alkoxy,

- Q is selected from the group consisting of oxygen, sulfur or nitrogen.

The inventors demonstrated, in *C. elegans* and in mouse strial cells, that this type of compounds protects against expanded polyQ toxicity, with no effect on normal polyQs.

Specific embodiments of the compound of formula (A) of the invention.

It will be described thereafter various specific embodiments of the compound of formula (A) (which are quercetin analogs), that have been found by the inventors to possess activity against polyglutamine expansion neurodegenerative diseases.

In a preliminary specific aspect, in the compound of formula (A), R1 and R2 do not mean simultaneously a saccharide group.

In a first embodiment, in the compound of formula (A):

- R1 means a saccharide group,

- R3, R4 and R6 each means a hydrogen atom, and

- R2 and R5 each mean a hydroxyl.

In a second embodiment, in the compound of formula (A):

- R2 means a saccharide group,
- R3 is selected from the group consisting of a hydrogen atom, an alkoxy and a hydroxy,
- R1 means a hydroxy,
- R4 is selected from the group consisting of a hydrogen atom and, notably when R3 is
- 5 an alkoxy group, a methoxy,
- R5 means a hydroxy, and
- R6 means a hydrogen atom.

The inventors demonstrated that a specific compound of this second embodiment shows surprisingly a significantly better activity against polyglutamine expansion neurodegenerative

10 disease, i.e. the compound known also as quercetin-3-O-galactoside in which:

- R1, R3 and R5 mean each a hydroxy group,
- R4 and R6 mean each a hydrogen atom, and
- R2 means a monosaccharide group, particularly in a galactoside form.

And in a third embodiment, in the compound of formula (A):

- 15
- R1, R2, R5 and R6 each mean an alkoxy,
 - R3 is selected from the group consisting of a hydrogen atom and an alkoxy, and
 - R4 is selected from the group consisting of a hydrogen atom and a hydroxyl.

Notably, specific embodiments of the compound of formula (A) are the following ones:

2-(3,4-dihydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one or

20 quercetin 3-O-hexapyranoside (compound hyperoside of figure 12);

2-(phenyl 3-O-hexapyranoside)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (compound of figure 7);

2-(3-methoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one (compound of figure 8);

25 2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxyl-5-hydroxy-4H-1-benzopyran-4-one (compound of figure 9);

2-(3,5-dimethoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one (compound of figure 10);

2-(3,4-dimethoxyphenyl)-3,6,7-trimethoxy-5-hydroxy-4H-1-benzopyran-4-one (compound

30 of figure 11).

Specific embodiments of the compound of formula (B) of the invention.

It is described thereafter various specific embodiments of compounds of formula (B) (which are resveratrol analogs), that have also been found by the inventors to possess activity

35 against polyglutamine expansion neurodegenerative diseases.

In a first embodiment, in the compound of formula (B):

- R1 means an alkoxy group,
- R2 is selected from the group consisting of a hydroxy and an alkoxy, and

- R3 is selected from the group consisting of a hydrogen atom and a hydroxyl.

In a second embodiment, in the compound of formula (B):

- R2 means a saccharide group, and

- R1 and R3 each mean a hydroxyl.

5 Notably, specific embodiments of the compound of formula (B) are the following ones:

(E) 3-methoxy-5-hydroxystilbene (compound of figure 1);

(E) 4',5 - dihydroxystilbene-3-hexopyranoside or polydatin (compound of figure 2);

(E) 3',5'-dimethoxy-4-hydroxystilbene or pterostilbene (compound of figure 3).

10 If any discrepancy exists between the structures of the compounds shown in figures 1 to 12 and their corresponding chemical names above, then the identity of the compounds to be retained is that which is illustrated in figures 1 to 12.

Specific embodiments of the compound of formula (C) of the invention.

15 The inventors found additionally various specific embodiments of compound of formula (C), which are active against polyglutamine expansion neurodegenerative diseases.

In a first embodiment, in the compound of formula (C):

- R3 means a hydroxy, and

- R1 means an alkoxy or R2 means a bromine atom, the other between R1 and R2 consisting of a hydrogen atom.

20 In a second embodiment, in the compound of formula (C):

- R3 means a hydrogen atom,

- R1 means a hydroxy, and

- R2 means a hydrogen atom.

Notably, specific embodiments of the compound of formula (C) are the following ones:

25 7-hydroxy-3-(4-methoxyphenyl)-chromen-2-one (compound of figure 4) ;

3-(4-hydroxyphenyl)-chromen-2-one (compound of figure 5); or

3-(3-bromophenyl)-chromen-2-one (compound of figure 6).

Additional features of the compounds of formulae (A), (B) or (C) of the invention.

30 In certain embodiments of any one of the polyphenolic type compounds of formulae (A), (B) and (C), one or more of the alkoxy groups, when present, consist advantageously of a methoxy group.

35 In certain other embodiments of any one of the polyphenolic type compounds of formulae (A), (B) and (C), the saccharide group, when present, consists advantageously of a monosaccharide group. Preferably, the said monosaccharide group, when present, consists of an hexose group, in particular in pyranose form, and especially of galactoside type

Such glycosylated compounds of formulae (A) (B) and (C) may be particularly interesting: the interaction of the glycosyl group with GLUT-1, a glucose transporter highly

expressed in the BBB, may indeed help generate active compound concentrations in the brain (Fernandez et al., 2003).

The general structural features of the compounds of formulae (A), (B) and (C) which are described herein, because they belong to the widely known chemical class of the polyphenolic compounds, are very well known from the one skilled in the art. Generally, the compounds of formulae (A), (B) and (C) as described in the present specification may thus be easily available or alternatively may be synthesized by the one skilled in the art by known methods.

Upon request, the compounds of formulae (A), (B) and (C) as described in the present specification may be furnished by numerous providers of chemical compounds, including companies like, for example, Cerep (Paris, France), Chemdiv Inc. (San Diego, United States), Chembridge (San Diego, United States), Maybridge subsidiary of Thermo Fischer Scientific International Inc. (Trevillet, UK) and Evotec Inc. (North Potomac, United States).

Compositions comprising a polyphenolic type compound of the invention

A further object of the invention consists of compositions comprising at least a polyphenolic type compound as defined in the present description in combination with one or more auxiliary agents.

The polyphenolic type compounds of the invention, consisting of formula (A), (B) or (C), may be used *per se* for preventing or treating of polyglutamine expansion neurodegenerative diseases, and in particular Huntington's disease.

Generally, for preventing or treating polyglutamine expansion neurodegenerative diseases, the polyphenolic type compounds of the invention are used under the form of pharmaceutical compositions for human use, said pharmaceutical compositions comprising at least one polyphenolic type compounds of the invention in combination with one or more pharmaceutically acceptable excipients.

In some embodiments, a pharmaceutical composition of the invention comprises more than one polyphenolic type compounds of the invention.

The pharmaceutical compositions in accordance with this invention alternatively can be formulated for parenteral administration, including subcutaneous administration, intraperitoneal administration, intramuscular administration, intrathecal administration and intravenous administration. Such parenteral dosage forms are typically in the form of aqueous solutions or dispersions utilized in a pharmaceutically acceptable carrier such as isotonic saline, 5% glucose or other well known pharmaceutically accepted liquid carrier compositions. The compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders or lyophilizates for the extemporaneous preparation of sterile injectable solutions or dispersions.

Parenteral dosage forms of the present pharmaceutical compositions can also be formulated as injectable prolonged release formulations in which the polyphenolic type compound(s) is combined with one or more natural or synthetic biodegradable or biodispersible

polymers such as carbohydrates, including starches, gums and etherified or esterified cellulosic derivatives, polyethers, polyesters, polyvinyl alcohols, gelatins or alginates. Such dosage formulations can be prepared, for example, in the form of microsphere suspensions, gels (of hydrophilic or hydrophobic constitution), or shaped-polymer matrix implants that are well known in the art for their function as "depot-type" drug delivery systems that provide prolonged release of the biologically reactive components. Such compositions are prepared using art recognized formulation techniques, and can be designed for any of a wide variety of drug release profiles.

The administration of polyphenolic type compounds in accordance with this invention, can be intermittent or at a gradual, continuous, constant or controlled rate to a patient in need of treatment. In addition, the daily dosage amount can be divided and administered in two or more daily doses depending on patient condition and environment. The optimal dosage amounts and dosage form for implementing the present method in accordance with this invention is dependent not only on the absorption and pharmacokinetic properties of the compound, but also on patient and patient condition and adjustable within reasonable ranges in the judgment of the attending physician.

The formulation is typically administered over a suitable period of time.

To treat a patient suffering from polyglutamine neurodegenerescence disease, an effective amount of a polyphenolic type compound(s) or a pharmaceutically-acceptable salt thereof, is administered to said patient.

Effective dosage forms, modes of administration and dosage amounts of the polyphenolic type compounds, may be determined empirically, and making such determinations is within the skill in the art. It is understood by those skilled in the art that the dosage amount will vary with the activity of the particular compound employed, the route of administration, the rate of excretion of the compound, the duration of the treatment, the identity of any other drugs being administered to the animal, the age, size and species of the animal, and like factors well known in the medical arts. In general, a suitable daily dose will be that amount which is the lowest dose effective to produce a therapeutic effect. The total daily dosage will be determined by an attending physician within the scope of sound medical judgment. If desired, the effective daily dose of a composition of the present invention, may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

The present composition comprising a polyphenolic type compound(s) may be administered to a patient by any suitable route of administration, including orally, nasally, rectally and parenterally, for example under the form of powders, or drops, including buccally and sublingually. The preferred routes of administration are oral and parenteral administration.

While it is possible for the active ingredient(s) to be administered alone, it is preferable to administer the active ingredient(s) as a pharmaceutical formulation (composition). The pharmaceutical compositions of the invention comprise the active ingredient(s) in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other

compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

In the present case, the carrier is advantageously suitable to optimise the bioavailability and blood-brain barrier properties of the associated active ingredient(s). For example, suitable carriers are any chemical groups that may preferentially or significantly interact with the brain blood barrier (BBB), including, without being limited to, glycosyl groups

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, typically sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of the active ingredient(s). The active ingredient(s) may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient(s) is/are mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient(s) moistened with an inert liquid diluent.

EXAMPLES

A. Materials and Methods

A.1. *Nematodes*

The wild-type strain of *C. elegans* used was Bristol N2. Standard methods of culturing and handling worms were used. All strains were scored at 20°C.

Touch tests, scoring of PLM cell processes, drug response assays and quantitative Real-Time PCR were performed as previously described (Parker et al., 2005, Nat Genet, Vol. 37 : 349-350).

All strains were obtained from the *C. elegans* Genetics Center (University of Minnesota, Minneapolis).

For strain construction with polyQ transgenes, mutants were verified by visible phenotypes :

- A posterior Mec phenotype is defined as touch-insensitive at the tail.
- An anterior Mec phenotype is defined as touch-insensitive at the head.

Transgenic animals expressing normal polyQs were essentially touch sensitive at the tail (Parker et al, 2001, Proc. Natl. Acad. Sci. USA. 2001 ; 98 : 13318 – 13323;]; Parker et al. 2005, Nat Genet, Vol. 37 : 349-350).

In contrast, animals expressing mutant polyQs showed significant posterior touch insensitivity. Posterior touch insensitivity was highly penetrant in these animals (Parker et al, 2001, *Supra*; Parker et al. 2005, *Supra*). Additionally, transgenic animals show accumulation of the transgenic proteins in neurons (Parker et al, 2001, *Supra*; Parker et al. 2005, *Supra*). Finally, animals expressing mutant polyQs show a strong level of axonal dystrophy not observed for animals expressing normal polyQs, which may reflect altered neurotransmission (Parker et al, 2001, *Supra*; Parker et al. 2005, *Supra*).

A.2. *Striatal cells*

Culture of htt knock-in mouse striatal cells expressing full-length htt with either wild-type (7Q/7Q) or mutant glutamines (109Q/109Q) was performed as described previously (Arango et al., 2006, J Neurosci, Vol. 26 : 4649-4659).

A.3. *Chemicals*

Chemicals were either purchased from Sigma (Resveratrol) and Calbiochem (BIO, LiCl) or obtained from the High Q Foundation (all other compounds described herein).

A.4. *C. elegans* transgenics

Transgenic strain (integrated array) derived from *C. elegans* lin-15 (n765) hermaphrodites (*C. elegans* Genetic Center, St. Paul, MN) injected with a wild type lin-15 marker, a mec-7 promoter_YFP plasmid, and a mec-3 promoter_Huntingtin 1-57(128 Glus)::CFP plasmid — are synchronized at the L1 larvae stage. L1s are manually seeded in 96-well assay plates (Costar white plates, #3596) 50 microliters/well for an animal density of

around 30 L1s/well. Compounds are added to reach a final concentration of 100-0.0001 μ M (diluted with assay medium that contains no bacteria and no DMSO, by 100-800x if from a 10 mM stock solution in DMSO). Dilutions are made by hand always using the same pipettes. Initial compound plates and dilution plates are stored at 80°C immediatly after thawing and use.

5 However, in some instances, dilution plates may be stored at room temperature, here 20°C. Refreezing plates is performed on dry ice prior to storing them in -80°C freezers. Animals are incubated with the test compounds at 20 degrees C for ~72 hours (3 wells/compound). After incubation, animals (now young adults) are transfered to Petri dishes without bacteria (3 dishes/compound) and allowed to rest for about 15-30'. Animals are then tested for response to

10 gentle touch at the tail using a fine eyebrow hair mounted on a tooth pick, and the percentage of animals backing away from touch at the tail is determined. Additionnaly, the animals can be tested for a reduction of aggregates after incubation. Dose-response tests are usually performed in a duplicate or triplicate, 6- to 8-point dose-response assay. Results are expressed as a percentage of Rescue. Rescue is calculated as $(\% \text{ compound treated response} - \% \text{ baseline response}) / (1 - \% \text{ baseline response})$. While the calculation of R relies on the total

15 number of animals tested, a quality control is performed on triplicates in order to use the two most robust values if the variation on triplicates happens to be too high ($SE > 7$). This situation is not frequent. Indeed, around 85% of the compounds tested usually show consistent triplicate values with a standard ecart less or equal to 7, a value above which the two most robust values

20 are possibly retained for calculating R. The assay has now been used for greater than several years and no contamination has been encountered. The baseline in the assay ('negative control') is determined from incubation -on a regular basis- of trangenic animals expressing 128 Glns with no compound in the growth medium, and expressed as the percentage of animals backing away from touch at the tail (percentage of responders). The baseline has been stable

25 around 20% since the assay is in use for drug screening. Three categories of compounds are usually identified: compounds found to be toxic at the concentration tested, compounds that worsened the mechanosensory defective phenotype induced by 128 Glns (negative R value), and compounds that corrected the mechanosensory defective phenotype induced by 128 Glns (positive R value). Several cut-off methods can be applied depending on the type of drug screen

30 performed. In large-scale single-pass screening, a cut-off method based on a Poisson distribution is applied, and R values above $SEM \times 2.47$ are considered significant. In multiple dose-response assays for the same compound, R values above the SEM are considered significant.

35

Example 1 : Dose-response curves relative to the percent rescue of neuronal dysfunction by polyphenolic compounds

The results obtained are shown in figures 1 to 12, which demonstrate that 13 compounds effectively protect against expanded polyQ toxicity with no effect on normal polyQs.

Hits were quercetin analogs and true resveratrol analogs, and they were all dependent on *sir-2.1* for activity. Among the most active hits were 4 quercetin analogues carrying a glucoside group. Interestingly, glycosyl derivatives have been shown in the art as having brain prodrug delivery potential.

Particularly high neuronal rescue activity has been obtained with hyperoside (quercetin-3-O-galactoside – See figure 12A and 12B). These effects were not due to a reduction in transgenic (polyQ) protein expression.

Hyperoside indeed shows a better $R_{\max}$ and a better EC_{50} compared to resveratrol, and the $R_{\max}$ of hyperoside is very close to the maximum achievable rescue in the assay. These data indicate that quercetin-glucosides are promising compounds to elicit FoxO-dependent neuroprotection in the diseased brain.

Example 2: additional assays with hyperoside:

For example in the case of Huntington's disease models, this particular compound hyperoside indeed presents a better $R_{\max}$ and a better EC_{50} compared to resveratrol, and a $R_{\max}$ very close to the maximum achievable rescue in the assay.

Additionally, this compound specifically enhanced the survival of 109Q/109Q striatal cells with no effect on *htt* expression.

Protocol:

The culture of *htt* knock-in mouse striatal cells expressing full-length *htt* with either wild-type (7Q/7Q) or mutant (109Q/109Q) glutamines, the treatment of these cells to induce cell death, and the incubation of these cells with compounds were performed as described previously (Arango et al., 2006).

The results are shown in figure 12B

Example 3 : Effect of the polyphenolic type compounds according to the invention on reducing cell death in a cellular model of Huntington's disease pathogenesis.

htt knock-in mouse striatal cells expressing full-length *htt* with either wild-type (7Q/7Q) or mutant glutamines (109Q/109Q) were incubated *in vitro* with various polyphenolic type compounds according to the invention, respectively :

- Compound 1 which is the 3',5'-dimethoxy-4-hydroxystilbene or pterostilbene illustrated in figure 3,
- Compound 2 which is the 4',5' - dihydroxystilbene-3-hexopyranoside or polydatin illustrated in figure 2,

- Compound 3 which is the 2-(3-methoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one illustrated in figure 8,
- Compound 4 which is 2-(3,4-dimethoxyphenyl)-3,6,7-trimethoxy-5-hydroxy-4H-1-benzopyran-4-one illustrated in figure 11.
- 5 - Compound 5 which is 2-(3,5-dimethoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one illustrated in figure 10,
- Compound 6 which is 3-(4-hydroxyphenyl)-chromen-2-one illustrated in figure 5,
- Compound 7 which is 2-(phenyl 3-O-hexapyranoside)-3,5,7-trihydroxy-4H-1-benzopyran-4-one illustrated in figure 7
- 10 - Compound 8 which is 2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxyl-5-hydroxy-4H-1-benzopyran-4-one illustrated in figure 9,
- Compound 9 which is 3-(3-bromophenyl)-chromen-2-one illustrated in figure 6,
- control cultures incubated only with the culture medium.

The results are shown for Compound Hyperoside in figure 12B and for the other
15 compounds in figure 13.

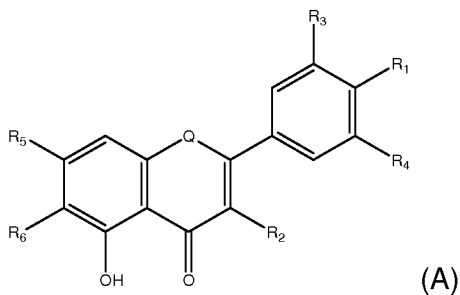
The results presented in the left portion of figure 13 (grey bars), show that none of the polyphenolic type compounds tested exert any effect on the mortality of the (7Q/7Q) mouse striatal cells, as compared with control.

However, the results presented in the right portion of figure 13 (black bars) clearly show
20 that almost all the polyphenolic type compounds tested greatly reduce the mortality of the (109Q/109Q) mouse striatal cells, as compared with control.

CLAIMS

1. The use of a polyphenolic type compound which is selected from the group of compounds having the following formulae A, B or C:

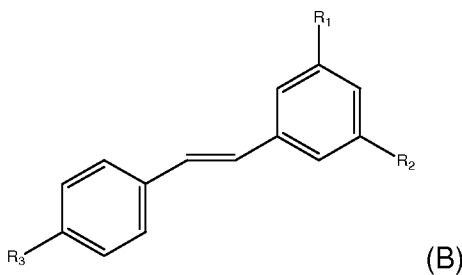
(A)



wherein :

- R1 and R2, one independently from the other, are selected from the group consisting of a saccharide group, an alkoxy and a hydroxy,
- R3, R4, R5 and R6, one independently from the other, are selected from the group consisting of a hydrogen atom, an alkoxy and a hydroxy,
- Q is selected from the group consisting of oxygen, sulfur or nitrogen,

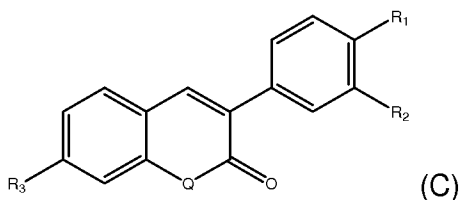
(B)



wherein

- R1 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a hydroxy and an alkoxy,
- R2 is selected from the group consisting of a saccharide group, a hydroxy and an alkoxy,
- R1, R2 and R3 do not mean simultaneously hydroxy group,

(C)



wherein :

- R1, R2 and R3, one independently from the other, are selected from the group consisting of a hydrogen atom, a halogen atom, a hydroxy and an alkoxy,

- Q is selected from the group consisting of oxygen, sulfur or nitrogen,
for manufacturing a medicament for preventing or treating a polyglutamine expansion neurodegenerative disease.

2. The use according to claim 1, wherein, in a compound of formula (A), R1 and R2 do not mean simultaneously a saccharide group.

3. The use according to claims 1 or 2, wherein the said polyphenolic type compound consists of a compound of formula (A1) wherein :

(A1)

- R1 means a saccharide group,
- R3, R4 and R6 each mean a hydrogen atom, and
- R2 and R5 each mean a hydroxyl.

4. The use according to claims 1 or 2, wherein the said polyphenolic type compound consists of a compound of formula (A2) wherein:

(A2)

- R2 means a saccharide group,
- R3 is selected from the group consisting of a hydrogen atom, an alkoxy and a hydroxy,
- R1 means a hydroxy,
- R4 is selected from the group consisting of a hydrogen atom and a methoxy,
- R5 means a hydroxy, and
- R6 means a hydrogen atom.

5. The use according to claims 1 or 2, wherein the said polyphenolic type compound consists of a compound of formula (A3) wherein :

(A3)

- R1, R2, R5 and R6 each means an alkoxy,
- R3 is selected from the group consisting of a hydrogen atom and an alkoxy, and
- R4 is selected from the group consisting of a hydrogen atom and a hydroxyl.

6. The use according to claim 1, wherein the said polyphenolic type compound consists of a compound of formula (B1) wherein :

(B1)

- R1 means an alkoxy group,
- R2 is selected from the group consisting of a hydroxy and an alkoxy, and
- R3 is selected from the group consisting of a hydrogen atom and a hydroxyl.

5 7. The use according to claim 1, wherein the said polyphenolic type compound consists of a compound of formula (B2) wherein :

(B2)

- R2 means a saccharide group, and
- R1 and R3 each mean a hydroxyl.

10 8. The use according to claim 1, wherein the said polyphenolic type compound consists of a compound of formula (C1) wherein :

(C1)

- R3 means a hydroxy, and
- 15 - R1 means an alkoxy or R2 means a bromine atom, the other between R1 and R2 consisting of a hydrogen atom.

9. The use according to claim 1, wherein the said polyphenolic type compound consists of a compound of formula (C2) wherein :

20 (C2)

- R3 means a hydrogen atom,
- R1 means a hydroxy, and
- R2 means a hydrogen atom

25 10. The use according to any one of claims 1 to 9, wherein one or more of the alkoxy groups of formulae A, B and C, when present, consist of a methoxy group.

11. The use according to any one of claims 1 to 10, wherein the saccharide group of formulae A, B and C, when present, consists of a monosaccharide.

30 12.- The use according to claim 11, wherein the monosaccharide of formulae A, B and C, when present, consists of an hexose group, in particular in pyranose form, and in particular of galactoside type .

35 13.- The use according to claim 1, wherein the polyphenolic type compound is selected from the group consisting of:

- 2-(3,4-dihydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one or quercetin 3-O-hexapyranoside;
- 2-(phenyl3-O-hexapyranoside)-3,5,7-trihydroxy-4H-1-benzopyran-4-one;

- 2-(3-methoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one;
- 2-(3-hydroxy-4-methoxyphenyl)-3,6,7-trimethoxy-5-hydroxy-4H-1-benzopyran-4-one;
- 5 - 2-(3,5-dimethoxy-4-hydroxyphenyl)-3-hexapyranosyl-5,7-dihydroxy-4H-1-benzopyran-4-one;
- 2-(3,4-dimethoxyphenyl)-3,6,7-trimethoxy-5-hydroxy-4H-1-benzopyran-4-one;
- (E) 3-methoxy-5-hydroxystilbene;
- (E) 4',5 - dihydroxystilbene-3-hexopyranoside or polydatin (compose B);
- 10 - (E) 3',5'-dimethoxy-4-hydroxystilbene or pterostilbene;
- 7-hydroxy-3-(4-methoxyphenyl)-chromen-2-one ;
- 3-(4-hydroxyphenyl)-chromen-2-one; or
- 3-(3-bromophenyl)-chromen-2-one.

15 14. The use according to any one of claims 1 to 13, wherein the said polyglutamine expansion neurodegenerative disease is selected from the group consisting of spinocerebellar ataxia 1, 2, 3, 6, 7 and 17, Huntington's disease, spinar and bulbar muscular atrophy and dentatorubral pallidolusyan atrophy.

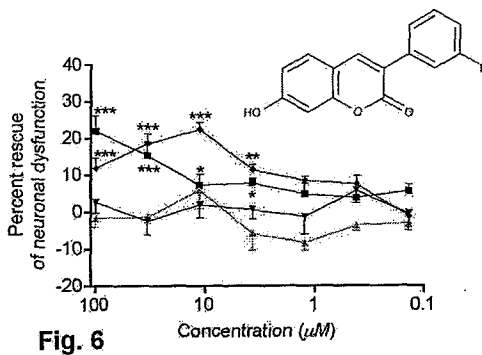
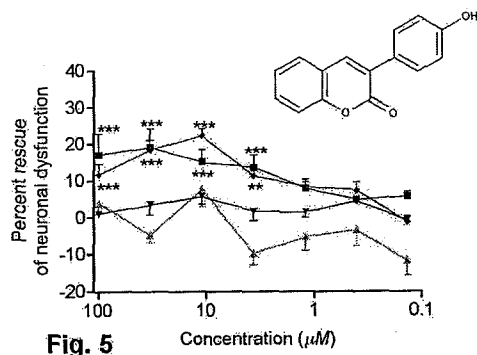
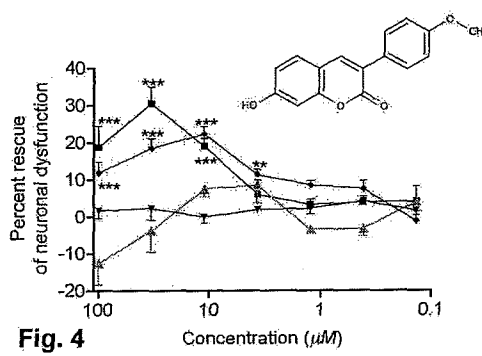
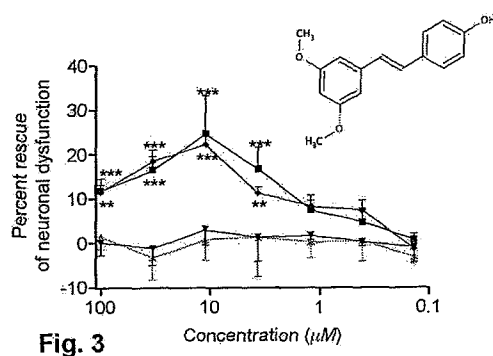
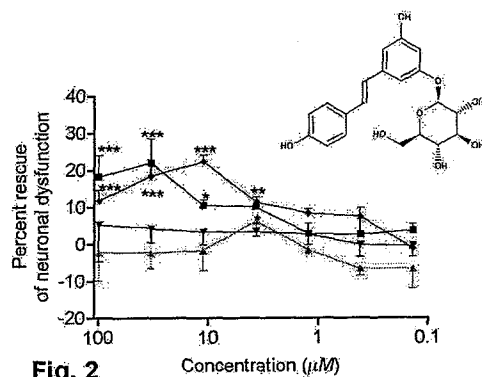
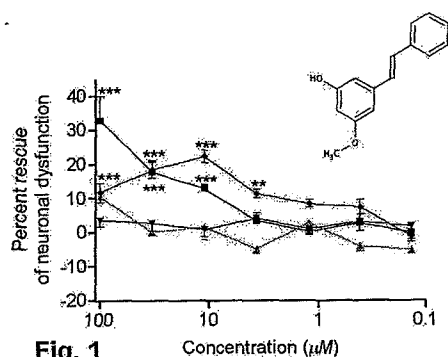
20 15. A polyphenolic type compound selected from the group of compounds which are defined in any one of claims 1 to 14.

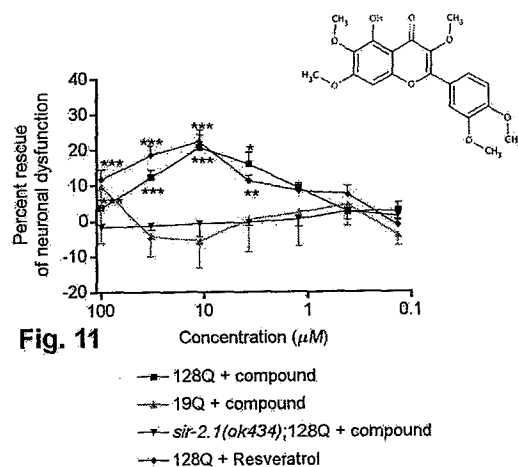
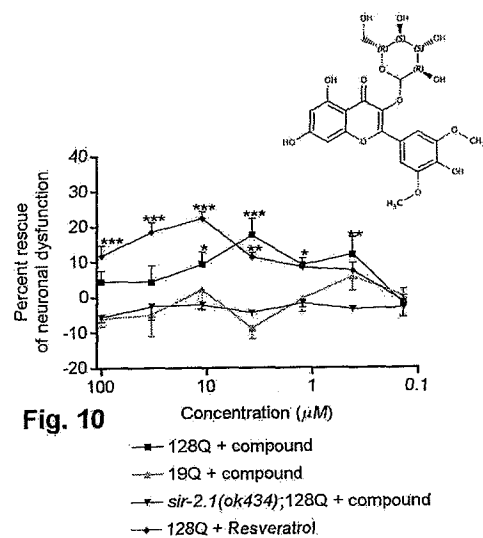
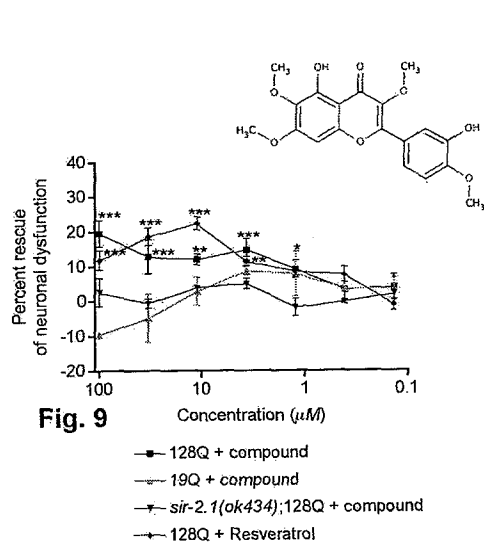
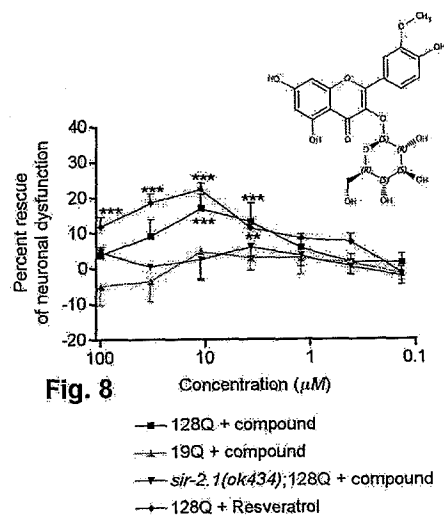
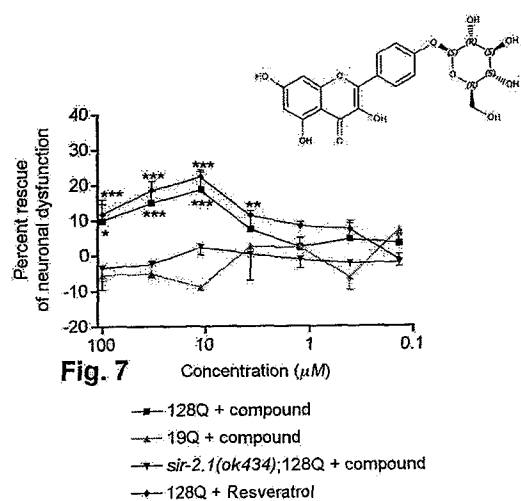
16. A polyphenolic type compound according to claim 15, for use as a medicament.

25 17. A pharmaceutical composition comprising a polyphenolic type compound according to claim 15 in combination with one or more pharmaceutically acceptable excipients.

18. A pharmaceutical composition according to claim 17, for preventing or treating polyglutamine expansion neurodegenerative disease.

30 19. A pharmaceutical composition according to claim 18, for preventing or treating Huntington's disease.





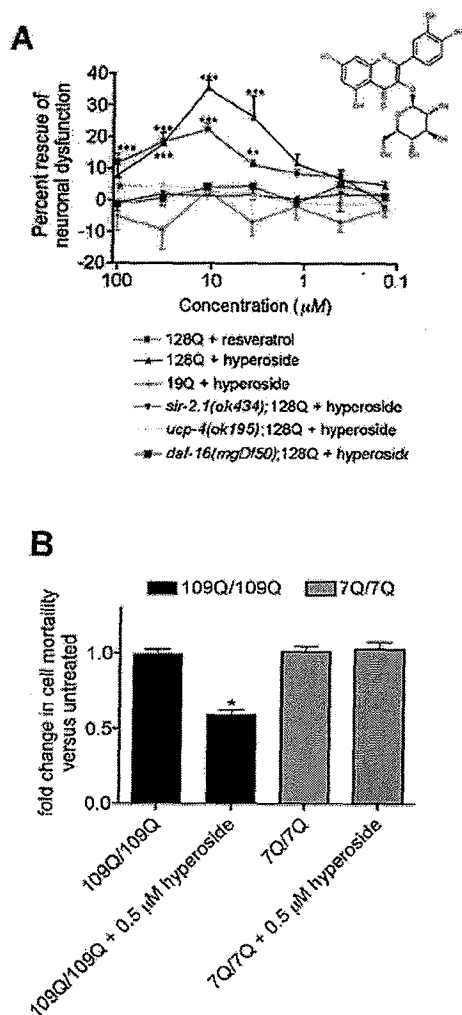


Figure 12

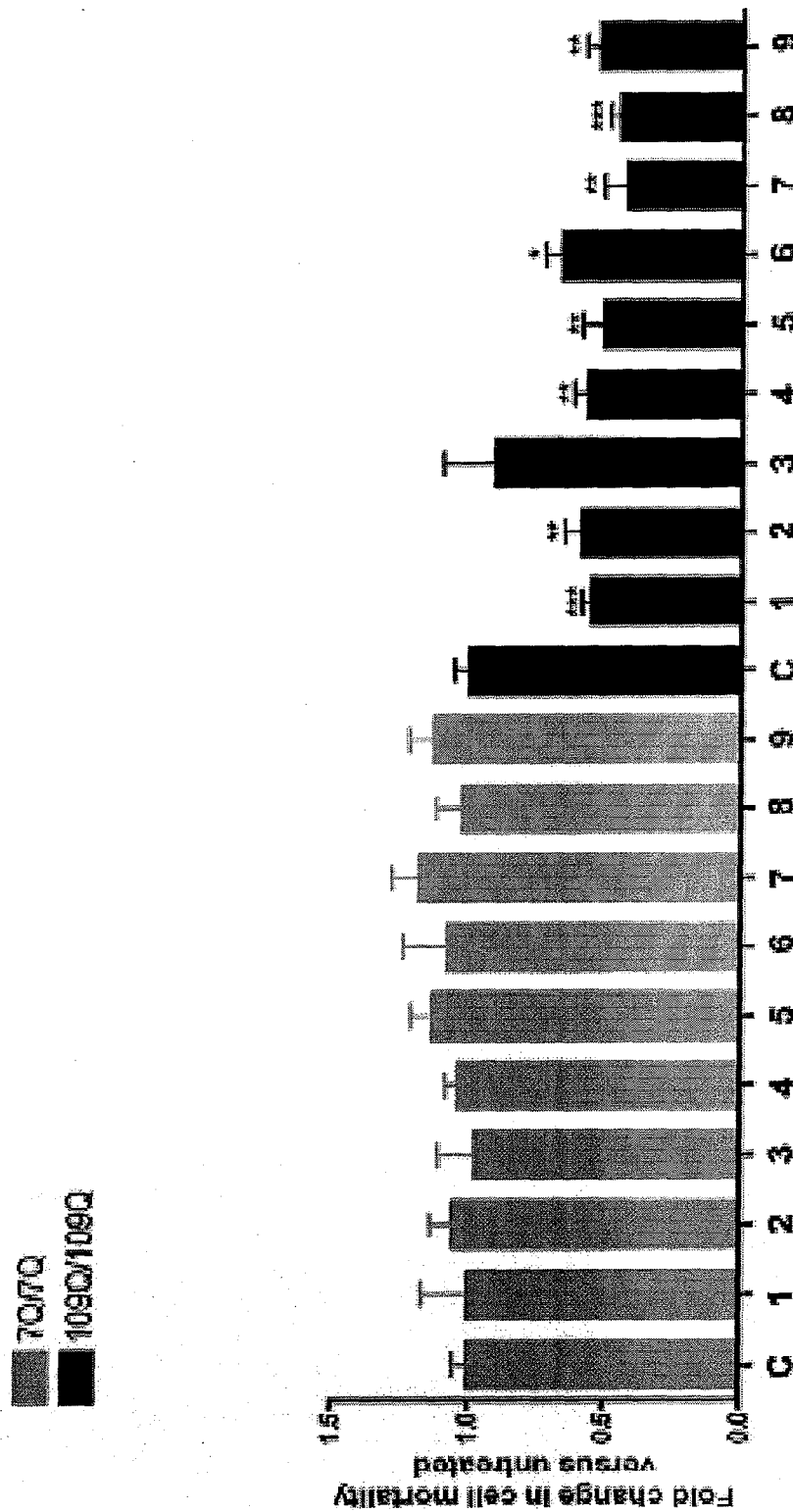


Figure 13