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(71) Applicant: **MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN**; Robert-Rössle-Straße 10, 13125 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **WANKER, Erich** [AT/DE]; Wolfshagener Str. 85, 13187 Berlin (DE). **WIGLEND, Thomas** [DE/DE]; Hendrichplatz 19, 10367 Berlin (DE). **BABILA, Julius, Tachu** [DE/DE]; Perleberger Str. 8, 10559 Berlin (DE). **BÖDDRICH, Annett** [DE/DE]; Friedrich-Ludwig-Jahn-Str. 68, 14612 Falkensee (DE). **SCHMIDT, Michael** [DE/DE]; Ingwäonenweg 131, 13125 Berlin (DE). **NEUENDORF, Sandra** [DE/DE]; Bahnstrasse 1, 16540 Hohen Neuendorf (DE). **SCHIELE, Franziska** [DE/DE]; Kiebingenstr. 38, 72108 Rottenburg-Kiebingen (DE).

(74) Agent: **WEIß, Wolfgang**; Weickmann & Weickmann, Postfach 860 820, 81635 München (DE).

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(54) Title: ENHANCERS OF PROTEIN DEGRADATION

(57) Abstract: The present invention relates to compounds suitable for modulating huntingtin protein processing and useful for treating or preventing huntingtin-related disorders. The invention provides pharmaceutical compositions comprising said compounds and methods of syntheses thereof.



WO 2011/020883 A1

- 1 -

Enhancers of Protein Degradation

5 The present invention relates to hydrazone compounds effective in inhibiting protein misfolding, accumulation of misfolded proteins and inducing degradation of misfolded proteins and aggregates thereof. Further, the compounds of the present invention are suitable for treating or preventing conditions associated with the occurrence of protein misfolding and aggregate formation. The invention provides compositions comprising a
10 hydrazone compound in a stabilized form and methods of producing thereof.

Protein metabolism comprises a complex interaction of a plurality of strongly regulated intracellular processes. After synthesis of gene encoded proteins on ribonucleoprotein complexes, the newly synthesized polypeptide chain is
15 processed, i.e. subjected to various posttranslational modifications including folding of the polypeptide chain into the destined three dimensional conformation resulting in a functional protein molecule. It is a prerequisite for the viability of any cell that besides the anabolism also the protein catabolism is strongly regulated. Dysfunctions in the posttranslational protein processing
20 may result in changing the steady state level of one or more protein species effecting the physiology of the cell. Proteins which are not properly folded or misfolded, respectively, are subjected to refolding and if refolding does not lead to a correct conformation to degradation. However, some misfolded and/or abundantly expressed proteins tend to form aggregates by
25 accumulating with each other and/or with other (macro)molecules within compartments of the cell or extracellular, but within multicellular organisms. Reasons therefor are amongst others mutations in protein encoding genes and/or an imbalance of protein synthesis and degradation.

30 A number of mutations is known in the art leading to mutated proteins which have a considerably tendency to be misfolded and/or form protein aggregates. Following translation of the mutated protein encoding gene locus the synthesized polypeptide chain will be misfolded into a conformation

- 2 -

allowing on the one hand to circumvent or escape the protein degradation machinery of the cell or organism, respectively, and on the other hand to accumulate with each other and/or other protein species. Aggregate formation may result in damaging organelles and finally in cell death and tissue degeneration. If the affected tissue is one which is hardly regenerative (as for example nerve tissue), occurrence of protein misfolding and aggregate formation will result in the death of the organism.

Fatal diseases in mammals, associated with and/or caused by protein misfolding and formation of intracellular protein aggregates are known in the art and accompanied mostly with successive loss of brain functions due to degeneration of affected nerve cells and tissue. Such prominent diseases are for example Alzheimer's disease, Parkinson's disease and Prion diseases as bovine spongiform encephalopathy (BSE), Scrapie and Creutzfeldt-Jakob disease.

Likewise, other diseases such as Chorea Huntington or Spinocerebellar ataxia are caused by expression of a protein species which undergoes pathological misfolding and accumulation, wherein misfolding is due to the presence of a polyglutamine stretch or an expanded polyglutamine stretch, respectively, within the primary structure of said protein species.

Chorea Huntington, also called Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by motor disturbance, cognitive loss and psychiatric manifestations. First symptoms arise in the fourth to fifth decade of life and gradually becomes more severe over the course of approximately 15 years until death. HD has been shown to be an autosomal dominant hereditary disorder caused by expression of a pathogenic variant of the protein huntingtin (htt). Compared to wild-type (wt) htt, toxic htt exhibit an abnormally extended polyglutamine stretch at its N-terminal end resulting from expression of a mutated htt (mhtt) encoding gene, the open reading frame of which has an expansion of more than 35 repeats of the triplet CAG. Inter alia, deleterious effects of HD are generated due to aggregation of mhtt

- 3 -

with itself and/or with other proteins within the nucleus and cytoplasm of neuronal cells resulting in degradation of nerve tissue.

Although htt is ubiquitously expressed throughout the whole body, HD pathology predominantly concerns the central nervous system (CNS). Therefore, the current therapeutics for HD have been targeted at preventing neuronal damage or treating symptoms of neuronal damage. Most of the agents used for HD therapy are well-known psychopharmaceuticals and neuroepileptics designed to alleviate diverse psychological symptoms (e.g. depression, epilepsy) and motor dysfunctions (e.g. hyperkinesia, dance-like movements).

Another approach for HD therapy is focussed on the htt protein itself. Appropriate therapeutically effective agents therefore act on the stage of preventing mhtt aggregation by enhancing protein-folding activity and proteolysis performance of mhtt expressing cells. Such agents modulate htt conformations or influence enzymes involved in post-translational protein modification and processing. One example therefor is geldanamycin which elevates the expression of heat shock proteins (Hsps). Hsps are members of the chaperone protein family involved in refolding of several aberrantly folded proteins. Therefore, geldanamycin may limit the accumulation of mhtt aggregates. However, compounds from which have been shown to possess some degree of efficacy in treating HD symptoms via alteration of the protein folding or degradation machinery of the cell are chemically unrelated and partially harmful for other physiological processes. Moreover, known agents acting on the stage of the primary mediator of HD itself are not suitable for an efficient HD therapy when used as the only active ingredient. Further, gene therapy based on RNA interference (RNAi) directed against the mhtt encoding gene or application of antibodies specific for mhtt are discussed. However, due to the ubiquitary occurrence of mhtt throughout the whole body and the relatively late occurrence of HD specific symptoms, it seems to be difficult to establish an effective and durable gene targeting therapy without undesired germline manipulation. An effective antibody therapy in

- 4 -

turn requires the selective supply of anti-mhtt antibodies to affected nerve tissue and overcoming the blood/brain barrier.

In summary it can be held that despite the progress made in Huntington research, an efficient or primal therapy could not be found, and thus a healing of this disease has not been possible. By means of medicaments which are available today, the symptoms of this disease can merely be alleviated. Therefore, there is a need for a medicament which can efficiently stop the progress of HD.

Surprisingly, it was found out by the present inventors that hydrazone compounds of formula I or a physiologically acceptable salt, hydrate, solvate, tautomer, stereoisomer, metabolite or prodrug thereof, as defined below, are useful for modulating, e.g. reducing the level of misfolded proteins, proteins which tend to be misfolded and proteins forming toxic aggregates. Especially they are useful for reducing the intracellular amount of misfolded proteins which are abundantly present in a cell, e.g. due to accumulation and/or overexpression. Further, the compounds as described therein are useful for inhibiting or preventing pathological accumulation of proteins, protein misfolding and/or degrading aggregates mainly comprising misfolded proteins. In particular the hydrazone compounds of formula I as described herein are useful for htt protein processing in prokaryotic or eukaryotic cells expressing htt or toxic variants thereof and especially in mhtt expressing mammalian cells.

For a better understanding and unless otherwise specified various terms used in the description and in the claims are defined as follows:

"htt" or "wt htt" refers to huntingtin or wild-type huntingtin protein having an amino acid sequence of SEQ ID NO:1 and homologs thereof comprising a sequence having at least 70%, preferably 70-80%, more preferably 80-90% and 90-95%, and most preferably $\geq 95\%$ identity with SEQ ID NO:1 over the whole length of SEQ ID NO:1, as long as they do not possess a poly-Q-

- 5 -

stretch of more than 35, i.e. an amino acid sequence of more than 35 contiguous glutamine residues. Further, "htt" or "wthtt" may refer to a portion of a htt as defined above, wherein said portion is encoded by one or more exons of a htt encoding gene, preferably by exon 1 of a htt encoding gene, e.g. the human htt encoding gene, provided that said portion comprises a polyglutamine stretch of up to 35, i.e. an amino acid sequence of up to 35 contiguous glutamine residues.

"mhtt" refers to a mutated variant of htt, wherein said mutated variant has compared to wild-type htt a sequence portion on the primary structure of 27 or more, e.g. more than 35 contiguous glutamine residues and is characterized by its tendency to misfolding and the ability to form toxic aggregates with each other and/or with wild-type htt or diverse other proteins under physiological conditions. Mhtt can exhibit up to 180 or more contiguous glutamine residues and can be further modified, for example, by post-translational modifications.

Htt or mhtt as well as homologues thereof as described herein, further can be a part of a chimeric protein. Such chimeric constructs are also comprised by the terms "htt" or "mhtt" as long as they show physiological characteristics comparable to huntingtin or toxic variants thereof.

"Alkyl" refers to straight or branched chain unsubstituted hydrocarbon groups of 1 to 16 carbon atoms, preferably of 1 to 8 carbon atoms, more preferably 1-4 carbon atoms.

"Alkenyl" refers to a straight or branched hydrocarbon group containing at least one double bond, having from 2 to 16 carbon atoms, preferably 2 to 8 carbon atoms and more preferably 2 to 4 carbon atoms.

"Alkynyl" refers to a straight or branched hydrocarbon group containing at least one triple bond, optionally further containing at least one double bond, having from 2 to 14 carbon atoms, preferably 2 to 8 carbon atoms.

- 6 -

"Aryl" refers to monocyclic, bicyclic or tricyclic, preferably monocyclic, aromatic hydrocarbon groups having 6 to 14 carbon atoms in the ring portion, such as phenyl, naphthyl, biphenyl and diphenyl groups. Preferably, aryl is phenyl.

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"Heteroalkyl", "heteroalkenyl" and "heteroalkynyl" refer to an alkyl, alkenyl or alkynyl group, respectively, as described herein, wherein at least one carbon atom of the alkyl, alkenyl or alkynyl portion is replaced a heteroatom, e.g. selected from N, O and S. A preferred heteroalkyl is propanenitrile.

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"Heteroaryl" refers to a 5-14 membered fully or partially aromatic ring group containing one to five heteroatoms selected from the group consisting of nitrogen (N), oxygen (O) and sulfur (S). For purposes of this invention, the heteroaryl may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl may be optionally oxidized; the nitrogen atom may be optionally quaternized. Preferably, heteroaryl is selected from indol and benzothiazol.

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"Cycloalkyl" refers to saturated cyclic hydrocarbon ring systems, preferably containing 3 to 14 carbons.

"Cycloalkenyl" refers to a cyclic hydrocarbon chain group, containing at least one double bond, having from 3 to 14 carbon atoms.

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"Cycloalkynyl" refers to a cyclic hydrocarbon chain group, containing at least one triple bond, having from 3 to 14 carbon atoms.

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"Cycloheteroalkyl", "cycloheteroalkenyl" and "cycloheteroalkynyl" refer to cycloalkyl, cycloalkenyl or cycloalkynyl groups, respectively, as described herein, wherein at least one carbon atom of the cycloalkyl, cycloalkenyl or cycloalkynyl portion, respectively, is replaced by a heteroatom, e.g. selected from N, O and S.

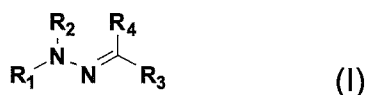
- 7 -

“Prodrug” refers to a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound of the invention. Thus, the term “prodrug” refers to a metabolic precursor of a compound of the invention that is pharmaceutically acceptable. The term “prodrug” is also meant to include any covalently bonded carriers, which release the active compound of the invention in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of the invention may be prepared by modifying functional groups present in the compound of the invention in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compound of the invention.

“Halogen” refers to fluorine, chlorine, bromine and iodine. Preferred are fluorine and chlorine.

“Proteids” refer to conjugated proteins composed of proteinogenic and/or non-proteinogenic amino acids and a non-protein portion like carbohydrates, lipids or nucleic acids. Preferred proteids used within the context of the invention are glyco-, lipo- and nucleo-proteins.

The present inventions provides in a first aspect a compound of formula I



or a physiologically acceptable salt, hydrate, solvate, tautomer, stereoisomer, metabolite or prodrug thereof, and a composition comprising at least one compound of formula I or a physiologically acceptable salt, hydrate, solvate, tautomer, stereoisomer, metabolite or prodrug thereof,

wherein

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at least one of R₁ and R₂ is selected from, preferably both of R₁ and R₂ are independently selected from

- 8 -

(i) H,

(ii) CN, or

5 (iii) an acyclic radical selected from C_{1-16} alkyl, C_{2-16} alkenyl, C_{2-16} alkynyl, wherein each acyclic radical is optionally mono- or poly-substituted with halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-
10 8, OR₅, CN, or

(iv) a C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each cyclic radical is optionally mono-
15 or poly-substituted with R₅, halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN, or

20 (v) N, R₁ and R₂ form together a heterocyclic radical selected from mono- or bicyclic, saturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each heterocyclic radical is optionally mono- or poly-substituted with R₅, halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂,
25 -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN,

(vi) -S(O)_nR₅, -S(O)_nN(R₅)₂, with n=0-3, -OR₅, -OCOR₅, -COR₅, -CON(R₅)₂, -COOR₅, -COC_nH_{2n}COR₅ with n=0-4, -OCOC_nH_{2n}COOR₅ with n=0-4, -
30 (CH₂O)_nR₅ with n=0-7, -(CO)_nR₅ with n=1-3, -CO-NHN=R₅, -CO-NHNHR₅, -CO-NHNHR₅, -CONHCOR₅, -CH=NNHR₅, -CH=NN=R₅, -C_nH_{2n}R₅ with n=0-8.

In one embodiment of the invention R₁ and/or R₂ is not CONH₂. In another

- 9 -

aspect of the invention R_1 and/or R_2 is not pyrazolopyrimidine or substituted pyrazolopyrimidine. In another aspect of the invention R_1 and/or R_2 is not $-\text{COR}_5$.

- 5 In another aspect of the invention R_1 and/or R_2 is not $-\text{CONH}_2$ and/or pyrazolopyrimidine or substituted pyrazolopyrimidine and/or $-\text{COR}_5$.

At least one of R_3 and R_4 is a C_{3-14} carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein
10 each cyclic radical is mono- or poly-substituted and preferably poly-substituted, e.g. 2- or 3 times substituted with $-\text{OH}$, $-\text{OR}_6$, and/or $-\text{OCOR}_6$, and one of R_3 and R_4 may be H or CH_3 , wherein H is preferred.

- 15 In one embodiment at least one of R_3 and R_4 is a C_{3-14} carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals, which may contain up to five heteroatoms, e.g. N, S, or O, wherein each cyclic radical is 2- or 3-times substituted with $-\text{OH}$, $-\text{OR}_6$, and/or $-\text{OCOR}_6$.

20 Preferably, the other one of R_3 and R_4 may be H or CH_3 , wherein H is preferred. The term "2- or 3-times substituted" means that the cyclic radical contains only 2 or only 3 above defined substituents (and no further substitutions).

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R_5 is selected from

(i) H,

- 30 (ii) CN,

(iii) an acyclic radical selected from C_{1-16} alkyl, C_{2-16} alkenyl, C_{2-16} alkynyl, wherein each acyclic radical is optionally mono- or poly-substituted with halo,

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-CN, -NO₂, =NR₇, -N(R₇)₂, -N(R₇)₃⁺, -NR₅COR₇, -S(O)_nR₇, S(O)_nN(R₇)₂, with n=0-3, -OR₇, OCOR₇, -CON(R₇)₂, -COOR₇, -COR₇, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₇, with n=0-8, OR₇, CN, or

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(iv) a C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each cyclic radical is optionally mono- or poly-substituted with R₇, halo, -CN, -NO₂, =NR₇, -N(R₇)₂, -N(R₇)₃⁺,
 10 -NR₇COR₇, -S(O)_nR₇, S(O)_nN(R₇)₂, with n=0-3, -OR₇, OCOR₇, -CON(R₇)₂, -COOR₇, -COR₇, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₇, with n=0-8, OR₇, CN,

R₆ is selected from C₁₋₄ alkyl which may be optionally halogenated.

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In one embodiment R₆ is selected from unsaturated C₁₋₄-alkyl.

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R₇ is selected from an acyclic radical selected from C₁₋₁₆ alkyl, C₂₋₁₆ alkenyl, C₂₋₁₆ alkynyl, C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e. g. N, S, or O, wherein each acyclic and or cyclic radical is optionally mono- or poly-substituted with H, halo, -NO₂, -NH₂, -CN, -OH, -COOH, -COH, -OCH₃, -OC₂H₅, -OC₃H₇, -COCH₃, -COC₂H₅, -OCOCH₃, -OCOC₂H₅, C₁₋₃ F₁₋₃ (haloalkyl), C₁₋₆ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, -C₆H₅SO₃⁻,
 25 -SO₃⁻, -C₅H₅NCH₃⁻, -C₅H₅N⁺ O⁻, -N(CH₃)₂⁺ and/or -N(C₂H₅)₂⁺.

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Preferably, one of R₁ and R₂ is a carbocyclic radical, i.e. an aryl, cycloalkyl, cycloalkenyl or cycloalkynyl radical, wherein at least one carbon atom may be replaced by a heteroatom, e.g. N, O or S.

In another embodiment, at least one of R₁ and R₂ is selected from aryl, preferably phenyl, or heterocyclyl, preferably benzothiazolyl, piperidinyl, pyrazolyl, pyridinyl, morpholinyl, piperazinyl, pyridazinyl, phthalazinyl,

- 11 -

dihydroimidazolyl, quinolinyl, more preferably benzothiazolyl, quinolinyl, pyridinyl, phthalazinyl, dihydroimidazolyl, unsubstituted or mono- or polysubstituted with halo, e.g. Br, I, F and/or Cl, CN, C₁₋₃(halo)alkyl, particularly CF₃, COOR₅ and/or NO₂.

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In another embodiment of the invention, N, R₁ and R₂ together form a heterocyclic radical selected from a monocyclic, saturated and/or aromatic radicals, which may contain up to five heteroatoms, particularly O, N, or S, more particularly O and/or N, wherein each heterocyclic radical is unsubstituted or mono- or fully substituted with R₅, -S(O)_nR₅ with n = 0-3, a C₃₋₁₄ carbocyclic or heterocyclic moiety, preferably pyridinyl, which may be substituted with halo or C_nH_{2n}R₅ with n = 0-8.

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In one embodiment, preferably N, R₁ and R₂ together form a piperidinyl, pyrazolyl, morpholinyl or piperazinyl moiety.

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In another embodiment, at least one of R₁ and R₂ is an acyclic radical selected from C₁₋₁₆-alkyl, wherein each acyclic radical is optionally mono- or polysubstituted with halo, CN, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety, preferably phenyl, which may be substituted with halo, C_nH_{2n}R₅, with n = 0-8, OR₅, or CN.

20

In another embodiment, at least one of R₁ and R₂ is -COR₅.

More preferably, R₁ and R₂ is selected from aryl, e.g. phenyl, or heteroaryl, e.g. benzothiazolyl, unsubstituted or mono- or poly-substituted with halo, e.g. Br, I, F and/or Cl, CN, C₁₋₃ (halo)alkyl, e.g. CF₃ and/or NO₂.

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In another embodiment, one of R₁ and R₂ is selected from phenyl or benzothiazolyl unsubstituted or substituted with

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(i) CF₃

(ii) CF₃ and at least one Cl,

(iii) at least one of F, Br, Cl and/or -I

- 12 -

(iv) -CN and at least one of F or Cl,

(v) NO₂ and optionally CF₃, or

(vi) COOH.

Most preferably, one of R₁ and R₂ is selected from phenyl or benzothiazolyl
 5 unsubstituted or substituted with (i) -CF₃, (ii) -CF₃ and at least one -Cl, (iii) at
 least one of -F, -Br, -Cl, and/or -I, (iv) -CN and at least one -F or -Cl. In this
 context, it is further preferred that the other one of R₁ and R₂ is H, C₁₋₃ alkyl,
 e.g. methyl or aryl, e.g. phenyl.

10 In one embodiment, the other one of R₁ and R₂ is H, or C₁₋₃-alkyl,
 unsubstituted, mono- or polysubstituted with halo, -CN, -NO₂, =NR₅, -N(R₅)₂,
 -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅,
 -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety
 which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN; or aryl,
 15 particularly phenyl, optionally mono- or polysubstituted with R₅, halo, -CN,
 -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3,
 -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or
 heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8,
 OR₅ or CN.

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In another embodiment, the other one of R₁ and R₂ is H, or C₁₋₃-alkyl or
 phenyl.

In another embodiment, the other one of R₁ and R₂ is H, or C₁₋₃-alkyl.

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In another embodiment, the other one of R₁ and R₂ is H or CH₃.

In another embodiment, the other one of R₁ and R₂ is H or phenyl.

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It is preferred that at least one of R₃ and R₄ is selected from aryl, particularly
 phenyl, mono- or polysubstituted, particularly 2- or 3-times substituted with
 -OH and/or -OR₆.

- 13 -

It is preferred that at least one of R_3 and R_4 is selected from aryl, e.g. phenyl mono- or poly-substituted and preferably poly-substituted, e.g. 2- or 3 times substituted with OH. Especially preferred at least one of R_3 and R_4 is phenyl disubstituted or trisubstituted with OH.

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In one embodiment, the other one of R_3 and R_4 is H or CH_3 , wherein H is preferred.

In another embodiment, the other one of R^3 and R^4 is CH_3 .

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Further preferred are compounds of formula I, wherein one of R_1 and R_2 is phenyl substituted with CF_3 and/or with 1-5 halogen residues selected from Cl and/or F and the other one of R_1 and R_2 is H, CH_3 or phenyl, and wherein one of R_3 and R_4 is phenyl substituted with 1, 2 or 3 OH groups and the other

15

one of R_3 and R_4 is H.
In another embodiment compounds of formula (I) are preferred, wherein one of R_1 and R_2 is phenyl substituted with CF_3 and/or with 1-5 halogen residues selected from Cl and/or F and the other one of R_1 and R_2 is H, CH_3 or phenyl, and wherein one of R_3 and R_4 is phenyl substituted with two or three OH

20

and/or OR_6 groups, and the other one of R_3 and R_4 is H.

In another embodiment compounds of formula (I) are preferred, wherein one of R_1 and R_2 is phenyl substituted with CF_3 and/or with 1-5 halogen residues selected from Cl and/or F and the other one of R_1 and R_2 is H, CH_3 or phenyl,

25

and wherein one of R_3 and R_4 is phenyl substituted with 2 or 3 OH groups and the other one of R_3 and R_4 is H.

In one embodiment, preferred are compounds selected from
1-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-(2,4-dimethoxybenzylidene)
hydrazine,

30

5-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,

4-((2-(4-Fluoro-phenyl)hydrazono)methyl)benzene-1,3-diol,

4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,3-diol,

- 14 -

- 5-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,
 4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2-diol,
 4-((2-Phenylhydrazono)methyl)benzene-1,3-diol,
 2-((2-Phenylhydrazono)methyl)phenol,
 5 4-((2-(2-Chloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
 diol,
 4-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,3-diol,
 5-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,2,4-triol,
 4-((2,2-Diphenylhydrazono)methyl)benzene-1,3-diol,
 10 4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,3-diol,
 5-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2,4-triol,
 5-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2,4-triol,
 4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2-diol,
 5-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2,4-triol,
 15 4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,3-diol,
 4-((Piperidin-1-ylimino)methyl)benzene-1,3-diol,
 3-(2-(2,4-Dihydroxybenzylidene)hydrazinyl)propanenitrile
 2-((2-Phenylhydrazono)methyl)phenol
 4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)phenol
 20 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)phenol
 4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)phenol
 1-(2,4-Dimethoxyphenyl)-2-(4-(trifluoromethyl)benzylidene)hydrazine
 2-(2-(Pyridin-2-ylmethylene)hydrazinyl)quinoline
N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-2-hydroxybenzohydrazide
 25 2-Chloro-*N'*-(1-(2,4-dihydroxyphenyl)ethylidene)-4-nitrobenzohydrazide
N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-3-iodobenzohydrazide

Most preferred are compounds

- 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
 30 1-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-(2,4-dimethoxybenzylidene)hydrazine,
 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-

diol,

- 4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-diol,
5-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,
5 4-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,3-diol,
3-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2-diol,
4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,3-diol,
5-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,
4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2-diol,
10 4-((2-Phenylhydrazono)methyl)benzene-1,3-diol,
2-((2-Phenylhydrazono)methyl)phenol,
4-((2-(2,5-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-5-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-phenyl)hydrazono)methyl)benzene-1,3-diol,
15 4-((2-(2,4-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,3-diol,
5-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,2,4-triol,
5-((2,2-Diphenylhydrazono)methyl)benzene-1,2,4-triol,
20 4-((2,2-Diphenylhydrazono)methyl)benzene-1,3-diol,
4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,2-diol,
4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2-diol,
4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,3-diol,
25 5-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2,4-triol,
3-Chloro-4-(2-(2,4,5-trihydroxybenzylidene)hydrazinyl)benzonitrile,
3-Chloro-4-(2-(2,4-dihydroxybenzylidene)hydrazinyl)benzonitrile,
3-Chloro-4-(2-(3,4-dihydroxybenzylidene)hydrazinyl)benzonitrile,
4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2-diol,
30 5-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2,4-triol,
4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((Piperidin-1-ylimino)methyl)benzene-1,3-diol,
4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol,

- 16 -

- 3-(2-(2,4-Dihydroxybenzylidene)hydrazinyl)propanenitrile,
2-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3,5-triol
2-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,3,5-triol
5 2-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3,5-triol
4-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,3-diol
4-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,2,3-triol
10 2-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,3,5-triol
4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,3-diol
5-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,3-triol
15 5-((3-Mercapto-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-ylimino)methyl)benzene-
1,2,4-triol
5-((2-(5-Chloro-3-iodopyridin-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((Morpholinoimino)methyl)benzene-1,2,3-triol
4-(2-(3,4-Dihydroxybenzylidene)hydrazinyl)benzoic acid
20 5-((4-Methylpiperazin-1-ylimino)methyl)benzene-1,2,4-triol
5-((2-(6-Chloropyridazin-3-yl)hydrazono)methyl)benzene-1,2,4-triol
5-((2-(Phthalazin-1-yl)hydrazono)methyl)benzene-1,2,4-triol
5-((2-(4,5-Dihydro-1*H*-imidazol-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((2-Benzyl-2-methylhydrazono)methyl)benzene-1,2-diol
25 4-((2-(5-(4-Methoxyphenylamino)pyridin-2-yl)hydrazono)methyl)benzene-
1,3-diol
4-((2-(4-(4-Methoxyphenylamino)phenyl)hydrazono)methyl)benzene-1,3-diol
4-((2-(4-(4-Methoxybenzyl)thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol
N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-2-hydroxybenzohydrazide
30 2-Chloro-N'-(1-(2,4-dihydroxyphenyl)ethylidene)-4-nitrobenzohydrazide
N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-3-iodobenzohydrazide.
1-(4-Cyanophenyl)-N'-(2,4-dihydroxybenzylidene)piperidine-4-
carbohydrazide

- 17 -

N'-(2,4-Dihydroxybenzylidene)-2-phenylacetohydrazide.

In a preferred embodiment, within a compound of formula I, the hydrogen of at least one -NH and/or =CH group, e.g. at least one -CH group of an aromatic radical as defined above, may be replaced by deuterium atoms. Preferably, the average degree of deuterization (number of deuterium atoms/(number of deuterium + hydrogen atoms) x 100) in the molecule is at least 50-100%, preferably 60-100%, more preferably 70-100%, more preferably 80-100%, more preferably 95-100%, and most preferably 96-99%.

Further, it is preferred that a compound of formula I as defined above is present in isomeric pure form, wherein "isomeric pure" means that a preparation of the compound comprises more than 90%, preferably more than 95%, more preferably more than 98% and most preferably more than 99% of one defined isomer of said compound compared to the total amount of said compound.

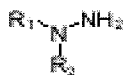
In one embodiment a compound of formula I as described herein is present as a salt. Preferred is a salt which is soluble in an aqueous solution. Therefore, the salt may be in the form of a metal salt. The metal may be selected from a transition metal, preferably from the group consisting of Fe, V, Mo, Ni, Cu, Co, Zn. The oxidation state of the metal may be -2, -1, 0, +1, +2, +3, +4, +5 or +6 depending on the metal used. Mostly preferred is

- Fe in the oxidation state +2 and/or +3,
- Ni, Cu, Co, Zn in the oxidation state +2,
- Mo in the oxidation state +4 and/or +6,
- V in the oxidation state +5.

The present invention further provides a composition comprising at least one compound of formula I as described above, wherein in a preferred embodiment at least one of said compounds is present in an isomeric pure form.

- 18 -

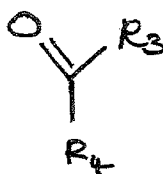
In general, hydrazones of present formula I are synthesized by reacting an appropriate hydrazine of formula II



(II)

5

with an aldehyde of formula III



(III)

10 wherein R_1 - R_4 are as defined above, at a temperature of between 10-100°C, preferably 20-80°C. The molar ratio of aldehyde and hydrazine is from 1:10 to 10:1, preferred is a substantially 1:1 (equimolar) ratio. The reaction is carried out in an organic solvent, preferably alkanol. Especially preferred is absolute ethanol.

15

A few hydrazones are known to be instable in aqueous solutions. Thus, hydrazones in aqueous solution are in equilibrium with hydrazine and aldehyd, the equilibrium being shifted towards hydrazine and aldehyde. It was therefore another object of the present invention to stabilize hydrazones in aqueous solutions and to provide aqueous compositions comprising at
20 at least one hydrazone compound of formula I as described above, wherein the hydrazone is stabilized, i.e. protected from decomposition.

25

Surprisingly, it was found by the present inventors that an addition of a stabilizing agent selected from amino acids, peptides, polypeptides, proteins, proteids and/or transition metal ions to an aqueous solution of a compound of formula I, does dramatically increase the stability of the hydrazone

- 19 -

compound. Therefore, the present invention provides a method of stabilizing a compound of formula I within an aqueous solution comprising adding to the solution a stabilizer selected from amino acids, peptides, polypeptides, proteins, proteids or transition metal ions or combinations thereof. Suitable amino acids, peptides and polypeptides are for example naturally occurring proteinogenic and/or non-proteinogenic amino acids or (poly-)peptides, respectively, which optionally can be modified. Preferred are the water-soluble proteinogenic amino acids. Suitable (poly-)peptides are naturally occurring water-soluble peptides having a sequence length of 2-100 amino acids, preferably 2-10, more preferably 2-4 amino acids and polypeptides having more than 100 amino acids in length, preferably up to 200 amino acids, each of which can be further modified. Preferred stabilizing agents are naturally occurring proteins, preferably globular proteins, each of which can be further modified. Especially preferred is a serum albumin, e.g. bovine serum albumin (BSA). Preferred transition metal ions are Fe, Cu, Zn, Ni, Mo, Co. In one embodiment, stabilizers are used in sub-equimolar ratios compared to hydrazone, i.e. in a molar ratio between stabilizer and hydrazone of $< 1:1$. Suitable molar ratios of stabilizing agent to hydrazone are 0.001:1, preferably 0.01:1. Mostly preferred, the molar ratio of stabilizing agent to hydrazone is 0.1:1.

The invention further provides a composition comprising at least one hydrazone compound of formula I as described above, wherein the hydrazone is stabilized. In this context, "stabilized" preferably means that the content of hydrazone within a composition is decreased over a period of 24 hours after formulating said composition, e.g. by dissolving hydrazone, by less than 20%, preferably less than 10%, and more preferably less than 5%. In other words, a stabilized composition according to the invention is one, in which after a time period of 24 hours after its formulation, e.g. by dissolving hydrazone, at least 80%, preferably at least 90%, and more preferably at least 95% of hydrazone is present, given that the content of hydrazone dissolved at time point 0 hours is set to 100%.

- 20 -

As mentioned above, the compounds and compositions according to the invention can be used according to another aspect of the invention for

5 (i) modulating the processing, e.g. mediating, activating or enhancing degradation of proteins which are misfolded, tend to be misfolded and/or form toxic aggregates,

(ii) preventing misfolding of proteins,

10 (iii) inhibiting accumulation of misfolded proteins, and/or

(iv) decomposing cytotoxic aggregates, e.g. formed by or mainly comprising misfolded proteins.

15 One group of targets for the compounds of formula I as described herein comprises protein species which are characterized by the presence of a poly-Q stretch, i.e. an amino acid sequence of more than 15, preferably more than 20, 27, more preferably more than 35 and most preferably more than 50 contiguous glutamine residues within their primary structures, and by
20 misfolding of said poly-Q-stretch. Exemplary proteins having a poly-Q-stretch which may be subjected to misfolding are Androgen receptor, Ataxin-1, Ataxin-2, Ataxin-3, CACNA1A, Ataxin-7, TATA-binding protein, Atrophin-1 and htt. A preferred target protein is a mutated variant of a wildtype form of a protein listed above, wherein compared to the wildtype form the mutated
25 variant has an expanded poly-Q-stretch and wherein said mutated variant is subjected to misfolding due to the expansion of the poly-Q-stretch. An example of a preferred target protein is htt or a toxic variant of htt like mhtt.

Therefore, according to a further aspect of the invention, the compounds of
30 formula I as described herein can be used for mediating, activating or enhancing degradation of a protein having a poly-Q-stretch as defined above, e.g. aberrant htt or mhtt, and/or decomposition of aggregates formed by or mainly comprising a protein species having a poly-Q-stretch, e.g. mhtt,

- 21 -

when applied to cells expressing said protein species.

Without being limited to a specific mode of action it was found out by the present inventors that hydrazone compounds of formula I are able to interact
5 or directly interact, respectively, with pathologically misfolded protein molecules or protein molecules having a tendency to be misfolded leading to conformational changes of said protein molecules allowing them to undergo degradation or preventing them from misfolding. Moreover, it was surprisingly found that the hydrazone compounds of formula I as described
10 herein do not influence formation of proteinaceous structures as for example tubulin polymerization necessary for cell viability (i.e. they are non-toxic) when applied in concentrations effecting degradation of misfolded proteins and aggregates thereof or prevention of protein misfolding. In other words, the hydrazone compounds according to the present invention are selective
15 for misfolded proteins, proteins having a tendency to be subjected to misfolding and aggregates thereof.

According to the invention, the compounds of formula I as described herein or a composition comprising at least one compound of formula I as the active
20 ingredient can be used as a medicament for treating, alleviating and/or preventing conditions associated with and/or caused by protein misfolding and/or accumulation of (misfolded) proteins. Non-limiting examples of conditions or diseases within the context of the invention are Huntington's disease (HD), spinobulbar muscular atrophy (Kennedy disease),
25 dentatorubral-pallidoluysian atrophy (Haw River syndrome), spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3 (Machado-Joseph disease), spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Alzheimer's disease, Parkinson's disease, primary systemic amyloidosis, secondary systemic
30 amyloidosis, senile systemic amyloidosis, familial amyloid polyneuropathy 1, hereditary cerebral amyloid angiopathy, hemodialysis-related amyloidosis, familial amyloid polyneuropathy III, Finnish hereditary systemic amyloidosis, type 2 diabetes, medullary carcinoma of thyroid, atrial amyloidosis,

- 22 -

- hereditary non-neuropathic systemic amyloidosis, injection-localized amyloidosis, hereditary renal amyloidosis, amyotrophic lateral sclerosis, schizophrenia, sickle cell anaemia, unstable haemoglobin inclusion body haemolysis, α_1 -antitrypsin deficiency, antithrombin deficiency, thromboembolic disease, Transmissible spongiform encephalopathies (prion diseases): bovine spongiform encephalopathy, kuru, Gerstmann-Straeussler-Scheinker syndrome, fatal familial insomnia, scrapie, Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease.
- 10 In one preferred embodiment the compounds of formula I as described herein or a composition comprising at least one compound of formula I can be used as a medicament for treating, alleviating and/or preventing diseases, the causative agent of which is a pathological intracellular amount of a misfolded protein species having a poly-Q-stretch as defined above.
- 15 More preferably, the condition is caused by cytotoxic accumulation of htt or expression of mhtt. Most preferably, a compound of formula I or a composition as described herein, is for HD therapy and/or preventing symptoms accompanying said disease.
- 20 For use as a medicament, the compound according to the invention may be formulated as a pharmaceutical composition with pharmaceutically acceptable diluents and/or carriers known in the art. Optionally, the composition may contain other active ingredients suitable for treating symptoms coinciding with pathological protein misfolding and accumulation.
- 25 In case of treating HD symptoms, such additional active ingredients may be selected from psychoactive agents as for example antipsychotics, antidepressants and mood stabilizers. The content of the at least one hydrazone compound of formula I as described herein, within the pharmaceutical composition can vary over a broad range. Preferably, the
- 30 hydrazone concentration per dosage unit is about 0.1 mg – 1000 mg or higher. The dosage can be administered all in one administration per day or partially several times per day.

- 23 -

The medicament comprising a compound of formula I as described above can be formulated to be suitable for any known dosage form. Thus, the pharmaceutical formulation according to the invention can be in the form of a solution, syrup, suspension, tablet, powder, capsule, aerosol, suppository, crème, lotion, ointment, gel or patch for topical, transdermal, enteral (e.g. orally, rectally or by feeding tube) or parenteral (e.g. by intravenous, intraarterial, intramuscular, subcutaneous, intrathecal, interperitoneal injection or infusion) application. Preferably, the medicament is formulated as an aqueous solution for parenteral application. More preferably, the medicament comprises at least one stabilizing agent e.g. selected from amino acids, peptides, polypeptides, proteins, proteids and/or transition metal ions for stabilizing a compound of formula I, and optionally further agents suitable for treating HD symptoms as defined above. Further, the pharmaceutical composition is preferably in the form of an aqueous solution comprising at least one compound of formula I as the active ingredient and a serum albumin, e.g. BSA as the stabilizing agent. In one preferred embodiment, the compound of formula I is present in an isomeric pure form.

In a further aspect, the present invention provides a method for modulating protein processing, e.g. degrading misfolded proteins or aggregates thereof and inhibiting protein misfolding or pathological accumulation of proteins comprising administration of a compound of formula I, or a composition comprising at least one of said compounds as described above to a eukaryotic or prokaryotic cell, cell culture, cell tissue or subject containing said proteins or aggregates. The cell or subject may be homozygous or heterozygous for at least one of said proteins, preferably heterozygous. Preferably, the protein is mhtt and the cell, cell culture, cell tissue or subject expresses mhtt. In one embodiment, the cell or subject expresses mhtt as well as htt, i.e. said cell or subject is heterozygous for mhtt.

30

Especially preferred is a method for modulating mhtt processing, wherein the mhtt protein itself and/or protein aggregates comprising mhtt are degraded or decomposed and wherein said degradation or decomposition is mediated

- 24 -

by a compound of formula I as described above.

The method according to the invention can be carried out in vivo as well as in vitro.

5

In a further aspect, the present invention relates to a method for treating and/or preventing conditions caused by expression of proteins showing a tendency to misfolding and/or aggregate formation, e.g. htt or mhtt, comprising administration of a compound of formula I or a composition
10 comprising said compound as described above to a subject in need thereof. In one embodiment, the conditions to be treated and/or prevented are characteristic for a disease, the causative agent of which is mhtt. Especially, the conditions are characteristic for HD.

15 Another aspect of the present invention refers to a kit for modulating processing of proteins showing a tendency to misfolding and/or aggregate formation, e.g. htt and/or mhtt. The kit comprises at least one compound of formula I as defined above and optionally means for detecting protein processing, e.g. htt and/or mhtt protein processing.

20

The invention will be described in further detail by the following Figures and Examples.

Description of Figures

25

Figure 1 illustrates the general synthesis of arylated and alkylated hydrazones comprised by formula I (product examples 1-67) from various hydrazines (68-99) and aldehydes (100-108) and the combination of the used educts.

30

Figure 2 illustrates the structures of the hydrazine (68-99) and aldehyde (100-108) used to synthesize hydrazones according to the invention (product examples 1-67).

- 25 -

Figure 3 illustrates the stability of hydrazone compounds according to the invention (product examples 1, 2, 12, 23 and 25) in a concentration of 20 μ M in an aqueous ammonium acetate solution (10 mM).

5

Figure 4 illustrates the stability of a hydrazone compound according to the invention (product example 1) in the presence of BSA in a submolar ratio.

10

Figures 5 and 6 illustrate the amount of HTT-Q103-protein in PC12 cells after incubation with product examples 1-36 and 40-42 in concentration dependence as percent of control DMSO.

15

Figure 7 illustrates the effect of product example 1 on the HTT-Q25 and -Q103 protein levels (A) and of product examples 4, 23, 25, 32, 35 and 36 on the HTT-Q103-protein level (B).

20

Figures 8 and 9 illustrate the influence of product examples 1, 4, 5, 8, 16 and 20, 21, 23, 25, 27, 33, 37-43, 46, 61, 62-66 on the cell viability of HEP-G2 cells in concentration dependence.

25

Figure 10 illustrates the time-dependent change in conformation of the HTT-Q53 protein caused by product examples 1 and 23, detected by the conformational CAG antibody.

Figure 11 illustrates that product example 1 shows no effect on tubulin polymerization at various concentrations.

Example 1: Synthesis of hydrazones

30

The synthesis of alkyl/aryl and bisaryl hydrazones was carried out in ethanol at ambient temperature under dry conditions as described in Figure 1. An equimolar ratio of aldehyde and hydrazine was used for the hydrazones in hydrazines preparation. 32 hydrazones and 9 aldehydes were condensed in

- 26 -

selected combinations to 59 hydrazones (Figure 2). The reaction times differ between 16 to 24 hours. In some cases the precipitated products were directly separated from the reaction mixture by filtration. The rest of the compounds were separated per filtration after varied treatments with different organic solvents. Based on the hydrazine consumption, yields from 10% to 97% were achieved (see experimental). For the vast majority of compounds, purities (RP-HPLC) from >96% were confirmed. In five cases (example 12, 14, 32, 56, 57) a purity from 64-80% was ascertained. In three cases (example 12, 14, 32) a purity from 66-80% was ascertained. All compounds were characterized by ¹H- and ¹³C-MNR and GC-MS measurements (see experimental).

Example 2: Stability of hydrazones

The stability of hydrazones was estimated in a 10 mM ammonium acetate solution at room temperature (Figure 3). Freshly prepared 10 mM methanol solutions of product examples 1, 2, 12, 23 and 25 were diluted in a 10 mM ammonium acetate solution to a final concentration of 20 μM. These solutions were analyzed via RP-HPLC over a sample period of 24 h. The injection volume was 20 μL, the sample was stored at room temperature. The hydrazone peak was exploited for the determination of the hydrazone concentration. Depending on the substitution pattern, strong distinctions were observed. For all analyzed compounds an equilibration was detectable. Through the allowance of organic solvents and proteins, or transition metal ions in subequimolar ratios, the stability of the labile compounds dramatically increased. The level of the establishment of equilibrium for such instable compounds was raised to a level, where only small traces from the hydrazines and aldehydes were detectable.

Figure 4 illustrates the difference in the hydrazone hydrolysis with and without the addition of BSA of product example 1. A freshly prepared 10 mM methanol solution of product example 1 was diluted with an ammonium acetate (10 mM) BSA (5 μM) solution to a final concentration of 20 μM. The

- 27 -

free soluble hydrazone was analyzed via RP-HPLC over a sample period of 24 h. The injection volume was 20 μ L, the sample was stored at room temperature. The hydrazone peak was exploited for the determination of the hydrazone concentration. After the addition of BSA approximately 17% of the available hydrazone was directly associated to the protein. Afterwards, the free hydrazone concentration was almost constant, between 96% and 91%, over the sample period.

Example 3: Hydrazones

3.1 General Synthetic Methods

All chemicals were purchased from commercial suppliers and used without further purification. The reaction was monitored by thin-layer chromatography on silica gel 60 F₂₅₄ coated aluminium sheets (Merck KGaA). All filtration was performed using a Büchner funnel with filter circles MN 615 and MN 640 (Macherey-Nagel). NMR spectra were recorded on Bruker Avance 300 MHz spectrometer. Chemical shifts are reported in parts per million downfield from the internal standard Tetramethylsilane (0.0 ppm) for ¹H-NMR and ¹³C-NMR spectra. All samples were measured in d₆-DMSO. The high performance liquid chromatography (HPLC) measurements were carried out on an Agilent System from the 1200 Series including binary pump, auto sampler and diode array detector. A Chromolith RP-18 SpeedRod column (50 mm x 4.6 mm) was used for the analytical reverse phase HPLC analysis (Merck KGaA, model 1.51450.0001). For the evaluation of the syn-anti isomerism an Ascentis RP-Amide column (100 mm x 3.0 mm, 3 μ m particle size, Supelco, model 565312-U) and a Astec Cyclobond I 2000 DNP column (250 mm x 4.6 mm, 5 μ m particle size, Supelco, model 25042AST) was used. Solvent A was 10 mM ammonium acetate solution and solvent B was acetonitrile. The following running conditions were used: a) B: 70%, 0-10 min, flow 0.7 mL/min; b) B: 40% (0 min) $\rightarrow$ 60% (8 min) $\rightarrow$ 100% (10 min), flow 1.0 mL/min; c) B: 50% (0 min) $\rightarrow$ 70% (8 min) $\rightarrow$ 100% (10 min), flow 1.0 mL/min; d) B: 40% (0 min) $\rightarrow$ 70% (8

- 28 -

min) → 100% (10 min), flow 0.8 mL/min; e) B: 40%, 0-10 min, flow 1.0 mL/min; f) B: 40%, 0-10 min, flow 0.65 mL/min; g) B: 50% (0-8 min) → 100% (10 min), flow 0.5 mL/min; h) B: 50%, 0-10 min, flow 1.0 mL/min; i) B: 20% (0 min) → 60% (8 min) → 100% (10 min), flow 0.5 mL/min; j) B: 30% (0 min) → 50% (8 min) → 100% (10 min), flow 1.0 mL/min; k) B: 30% (0 min) → 70% (8 min) → 100% (10 min), flow 1.0 mL/min; l) B: 40% (0 min) → 70% (8 min) → 100% (10 min), flow 1.0 mL/min; m) B: 50% (0 min) → 70% (8 min) → 100% (10 min), flow 1.2 mL/min; n) B: 5% (0 min) → 40% (8 min) → 100% (10 min), flow 0.9 mL/min; o) B: 25% (0 min) → 50% (8 min) → 100% (10 min), flow 1.0 mL/min; p) B: 0% (0 min) → 10% (8 min) → 100% (10 min), flow 1.0 mL/min. All runs were performed at 43°C. All mass spectrometry data were collected at IntraServ GmbH & Co. Knapsack KG and ChiroBlock GmbH. The Uv-vis spectra were recorded with a SPECORD 250 spectrometer from Analytik Jena AG. All spectra were collected in water containing 2% Acetonitril.

3.2 General Synthesis of Hydrazones

An equimolar ratio of aldehyde and hydrazine was stirred for 24-48h in absolute ethanol under dry conditions at ambient temperature. The product was dried at 75°C for 15-24h.

3.3 Hydrazine Preparation

Hydrazines, which are only available as hydrochloride, were stirred in a saturated aqueous NaHCO₃ solution for 1h. The free hydrazine was extracted twice with ethyl acetate, the organic layers were combined, dried with Na₂SO₄ and the solvent evaporated. The resulting oil was dried under reduced pressure at 50°C for 2h.

3.4 Purification

Method 1

- 5 The solid product was separated with a Büchner funnel and washed with cool ethanol.

Method 2

- 10 The reaction was quenched with the 5-7-fold volume of cyclohexane. The organic solvents were removed under reduced pressure. The product starts to precipitate at the sixth of the starting volume. The distillation was stopped and the batch stored at 15°C for 1h. The product was separated with a Büchner funnel and washed with cyclohexane.

15

Method 3

- Ethanol was removed under reduced pressure. The crude product was resolved in diethyl ether (1 part) and diluted with 4-6 parts of cyclohexane.
- 20 The vast majority of diethyl ether and parts of cyclohexane were removed under reduced pressure, until the product starts to precipitate. The distillation was stopped and the batch stored at 15°C for 1h. The product was separated with a Büchner funnel and washed with cyclohexane.

25

Method 4

- Ethanol was removed under reduced pressure. The crude product was resolved in ethyl acetate. The organic phase was washed twice with water. The organic layer was dried with Na₂SO₄ and the solvent removed.

30

Method 5

Solid impurities were separated with a Büchner funnel. The filtrate was

- 30 -

diluted with n-hexane. The product starts to crystallized after a few minutes. The batch was stored at room temperature for 1h; the product was separated with a Büchner funnel and washed with n-hexane.

5 **Method 6**

Ethanol was removed under reduced pressure. The crude product was resolved in diethyl ether (1 part) and diluted with 4-6 parts of cyclohexane. The vast majority of diethyl ether and parts of cyclohexane was removed
10 under reduced pressure, until the product starts to refine as oil. The distillation was stopped and the batch stored at room temperature for 1h. The oil phase was separated, washed twice with cyclohexane and dried under reduced pressure at 50°C for 2h. The oil starts to crystallize after several hours at room temperature.

15

Method 7

The reaction solution was poured on crushed ice. The precipitated product was separated with a Büchner funnel and washed with a mixture of ethanol
20 and water (1:4).

Method 8

Ethanol was removed under reduced pressure. The crude product was
25 resolved in diethyl ether (1 part) and diluted with 10 parts of n-hexane. The solution was treated in an ultrasonic bath. The product precipitated rapidly. It was separated with a Büchner funnel and washed with n-hexane.

Method 9

30

The reaction solution was diluted with a mixture of methanol and water (1:2). The product was extracted once with ethyl acetate, the organic layer was washed twice with water and dried with Na₂SO₄. The solvent was removed

- 31 -

under reduced pressure.

Method 10

5 Ethanol was removed under reduced pressure. The crude product was resolved in ethyl acetate (1 part) and diluted with 10 parts of cyclohexane. The organic solvents were removed under reduced pressure. The product starts to precipitate at the fourth to sixth of the starting volume. The distillation was stopped and the batch stored at 20°C for 1h. The product
10 was separated with a Büchner funnel and washed with cyclohexane.

Method 11

Ethanol was removed under reduced pressure. The product starts to
15 precipitate at the sixth of the starting volume. The distillation was stopped and the batch stored at 5°C for 1h. The product was separated with a Büchner funnel and washed with a mixture of cyclohexane and diethyl ether (6:4).

20 3.5 Product Examples 1-67

Example 1: 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol

(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazine (200 mg, 0.8 mmol), 2,4-
25 Dihydroxybenzaldehyde (113 mg, 0.8 mmol);
Purification: Method 3; Yield: 265 mg, 89%; C₁₄H₉Cl₂F₃N₂O₂, M = 365,13 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.29 (d, 1H, J = 1.5 Hz), 6.33 (dd, 1H, J = 1.9 Hz, J = 8.5 Hz), 7.25 (d, 1H, J = 8.4 Hz), 7.81 (s, 2H), 8.41 (s, 1H),
30 9.75 (s, 2H), 10.42 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 102.98, 108.14, 111.58, 122.49 (q, J_{C-F} = 33.7 Hz), 123.53 (d, J_{C-F} = 271.9 Hz), 123.57, 127.18 (q, J_{C-F} = 3.8 Hz), 130.66, 141.551, 145.27, 158.72, 160.36; GC-MS 364 m/z [M-H]⁺; Purity: 98.9% (324 nm, t_r = 3.89 min, Method: c)

- 32 -

Example 2: 1-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-(2,4-dimethoxybenzylidene)hydrazine

(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazine (200 mg, 0.8 mmol), 2,4-Dimethoxybenzaldehyde (136 mg, 0.8 mmol);

5 Purification: Method 1; Yield: 197 mg, 61%; $C_{16}H_{13}Cl_2F_3N_2O_2$, $M = 393,19$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.80 (s, 3H), 3.85 (s, 3H), 6.59 (dd, 1H, $J = 2.6$ Hz, $J = 11.2$ Hz), 6.1 (d, 1H, $J = 3.0$ Hz), 7.72 (d, 1H, $J = 8.5$ Hz), 7.77 (s, 2H), 8.50 (s, 1H), 9.88 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm:
10 55.28, 55.62, 98.16, 106.27, 115.89, 121.39 (q, $J_{C-F} = 33.7$ Hz), 122.96, 123.05 (d, $J_{C-F} = 271.7$ Hz), 125.89, 126.51 (q, $J_{C-F} = 3.7$ Hz), 137.98, 141.49, 158.18, 161.51; GC-MS 392 m/z $[M-H]^+$; Purity: 92.0% (372 nm, $t_r = 4.68$ min, Method: a)

15 **Example 3: 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-diol**

(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazine (300 mg, 1.2 mmol), 3,4-Dihydroxybenzaldehyde (169 mg, 1.2 mmol);

Purification: Method 3; Yield: 322 mg, 72%; $C_{14}H_9Cl_2F_3N_2O_2$, $M = 365,13$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.76 (d, 1H, $J = 8.1$ Hz), 6.87 (dd, 1H, $J = 1.7$ Hz, $J = 8.1$ Hz), 7.15 (d, 1H, $J = 1.7$ Hz), 7.79 (s, 2H), 8.10 (s, 1H), 9.13 (s, 1H), 9.22 (s, 1H), 9.69 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm:
20 112.19, 115.48, 119.18, 121.32 (q, $J_{C-F} = 33.4$ Hz), 122.82, 123.06 (d, $J_{C-F} = 271.8$ Hz), 126.40, 126.53 (q, $J_{C-F} = 3.7$ Hz), 141.43, 143.09, 145.53, 146.98; GC-MS 364 m/z $[M-H]^+$; Purity: 98.5% (326 nm, $t_r = 3.08$ min, Method: c)

Example 4: 4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol

30 (4-(Trifluoromethyl)phenyl)hydrazine (200 mg, 1.1 mmol), 2,4-Dihydroxybenzaldehyde (156 mg, 1.1 mmol);

Purification: Method 2; Yield: 269 g, 80%; $C_{14}H_{11}F_3N_2O_2$, $M = 296,24$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.33-6.36 (m, 2H), 7.05 (d, 2H, $J = 8.5$

- 33 -

Hz), 7.41 (d, 1H, $J = 9.1$ Hz), 7.54 (d, 2H, $J = 8.6$ Hz), 8.15 (s, 1H), 9.72 (s, 1H), 10.38 (s, 1H), 10.57 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.50, 107.69, 111.05, 111.95, 117.96 (q, $J_{\text{C-F}} = 31.9$ Hz), 125.05 (d, $J_{\text{C-F}} = 270.3$ Hz), 125.54 (q, $J_{\text{C-F}} = 3.5$ Hz), 128.78, 140.23, 148.03, 157.57, 159.51; GC-MS 296 m/z $[\text{M}]^+$; Purity: 100.0% (342 nm, $t_r = 2.89$ min, Method: d)

Example 5: 4-((2-(4-**(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-diol**

(4-(Trifluoromethyl)phenyl)hydrazine (1.2 g, 6.8 mmol), 3,4-Dihydroxybenzaldehyde (940 mg, 6.8 mmol); Purification: Method 6 or 8; Yield: 1.789 g, 89%; $\text{C}_{14}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_2$, $M = 296.24$ g/mol ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.76 (d, 1H, $J = 8.1$ Hz), 6.90 (dd, 1H, $J = 1.8$ Hz, $J = 8.2$ Hz), 7.12 (d, 2H, $J = 8.5$ Hz), 7.19 (d, 1H, $J = 1.8$ Hz), 7.52 (d, 2H, $J = 8.6$ Hz), 7.79 (s, 1H), 9.10 (s, 1H), 9.20 (s, 1H), 10.49 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 111.16, 112.10, 115.51, 117.60 (q, $J_{\text{C-F}} = 31.86$ Hz) 118.94, 125.05 (d, $J_{\text{C-F}} = 270.2$ Hz) 126.35 (q, $J_{\text{C-F}} = 3.5$ Hz), 126.67, 139.86, 145.54, 146.63, 148.49; GC-MS 296 m/z $[\text{M}]^+$; Purity: 99.8% (344 nm, $t_r = 3.67$ min, Method: h)

Example 6: 5-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2,4-triol

(4-Fluorophenyl)hydrazine (236 mg, 1.9 mmol), 2,4,5-Trihydroxybenzaldehyde (288 mg, 1.9 mmol); Purification: Method 2; Yield: 402 mg, 82%; $\text{C}_{13}\text{H}_{11}\text{FN}_2\text{O}_3$, $M = 262.24$ g/mol ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.30 (s, 1H), 6.88 (t, 3H, $J = 6.8$ Hz), 7.05 (t, 2H, $J = 8.8$ Hz), 7.97 (s, 1H), 8.38 (s, 1H), 9.20 (s, 1H), 9.81 (s, 1H), 9.98 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 103.35, 111.03, 112.29 (d, $J_{\text{C-F}} = 7.5$ Hz), 113.12, 115.57 (d, $J = 22.3$ Hz), 138.30, 141.96 (d, $J_{\text{C-F}} = 1.7$ Hz), 147.12, 149.59, 153.48 (d, $J_{\text{C-F}} = 233.5$ Hz); GC-MS 262 m/z $[\text{M}]^+$; Purity: 100.0% (350 nm, $t_r = 2.69$ min, Method: f)

30

Example 7: 4-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,3-diol

(4-Fluorophenyl)hydrazine (202 mg, 1.6 mmol), 2,4-Dihydroxybenzaldehyde (221 mg, 1.6 mmol); Purification: Method 1; Yield: 35 mg, 89%; $\text{C}_{13}\text{H}_{11}\text{FN}_2\text{O}_2$,

- 34 -

M = 246,24 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.28-6.32 (m, 2H), 6.88 (t, 2H, J = 6.6 Hz), 7.06 (t, 2H, J = 8.8), 7.28 (d, 1H, J = 10.9 Hz), 8.03 (s, 1H), 9.64 (s, 1H), 10.05 (s, 1H), 10.60 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 102.42, 107.42, 111.95, 112.40 (d, J_{C-F} = 8.2 Hz), 115.65 (d, J_{C-F} = 22.4 Hz), 128.92, 139.09, 141.78 (J_{C-F} = 1.7 Hz), 155.62 (J_{C-F} = 233.7 Hz), 157.36, 158.92; GC-MS 246 *m/z* [M]⁺; Purity: 97.7% (338 nm, t_r = 1.49 min, Method: c)

Example 8: 3-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2-diol

(4-Fluorophenyl)hydrazine (267 mg, 2.1 mmol), 2,3-Dihydroxybenzaldehyde (292 mg, 2.1 mmol); Purification: Method 2; Yield: 218 mg, 42%; C₁₃H₁₁FN₂O₂, M = 246,24 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.70 (t, 1H, J = 7.7 Hz), 6.76 (dd, 1H, J = 7.8 Hz, J = 1.7 Hz), 7.03-6.91 (m, 3H), 7.11 (t, 2H, J = 8.9 Hz), 8.12 (s, 1H), 9.18 (s, 1H), 10.06 (s, 1H), 10.35 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 112.64 (d, J_{C-F} = 7.6 Hz), 115.38, 115.73 (d, J_{C-F} = 22.2 Hz), 117.85, 119.10, 120.66, 138.05, 141.42 (d, J_{C-F} = 1.8 Hz), 144.09, 145.29, 155.86 (d, J_{C-F} = 234.32 Hz); GC-MS 246 *m/z* [M]⁺; Purity: 100.0% (310 nm), 100.0% (342 nm, t_r = 2.64 min, Method: e)

Example 9: 4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,3-diol

(2,4-Difluorophenyl)hydrazine (300 mg, 2.1 mmol), 2,4-Dihydroxybenzaldehyde (287 mg, 2.1 mmol); Purification: Method 2; Yield: 526 mg, 96%; C₁₃H₁₀F₂N₂O₂, M = 264,23 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.31 (s, 1H), 6.33 (br d, 1H, J = 7.5 Hz), 7.00 (br t, 1H, J = 8.6 Hz), 7.16-7.26 (m, 2H), 7.32 (d, 1H, J = 8.4 Hz), 8.27 (s, 1H), 9.67 (s, 1H), 9.92 (s, 1H), 10.94 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 102.39, 103.84 (dd, J = 22.3 Hz, J = 27.2 Hz), 107.47, 111.31 (dd, J_{C-F} = 3.3 Hz, J_{C-F} = 21.8 Hz), 111.89, 113.25 (dd, J_{C-F} = 4.7 Hz, J_{C-F} = 9.0 Hz), 128.90, 130.36 (dd, J_{C-F} = 2.9 Hz, J_{C-F} = 10.1 Hz), 141.35, 148.36 (dd, J_{C-F} = 12.0 Hz, J_{C-F} = 242.7 Hz), 154.38 (dd, J_{C-F} = 10.9 Hz, J_{C-F} = 236.7 Hz), 157.48, 159.22; GC-MS 264 *m/z* [M]⁺; Purity: 98.0% (336 nm, t_r = 1.70 min,

- 35 -

Method: c)

Example 10: 5-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2,4-triol

5 (2,4-Difluorophenyl)hydrazine (365 mg, 2.5 mmol), 2,4,5-Trihydroxybenzaldehyde (390 mg, 2.5 mmol); Purification: Method 8; Yield: 693 mg, 97%; $C_{13}H_{10}F_2N_2O_3$, $M = 280,23$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.38 (s, 1H), 6.93 (s, 1H), 7.01 (br t, 1H, $J = 8.7$ Hz), 7.15-7.26 (m, 3H), 8.24 (s, 1H) 8.44 (s, 1H), 9.27 (s, 1H),
10 9.76 (s, 1H), 9.82 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 103.40, 103.80 (dd, $J_{C-F} = 22.3$ Hz, $J_{C-F} = 26.9$ Hz), 110.96, 111.27 (dd, $J_{C-F} = 3.3$ Hz, $J_{C-F} = 21.7$ Hz), 113.10, 113.18 (dd, $J_{C-F} = 4.6$ Hz, $J_{C-F} = 8.8$ Hz) 130.57 (dd, $J_{C-F} = 2.8$ Hz, $J_{C-F} = 10.1$ Hz) 138.39, 140.84, 147.58, 148.31 (dd, $J_{C-F} = 11.5$ Hz, $J_{C-F} = 243.2$ Hz), 149.86, 154.30 (dd, $J_{C-F} = 10.9$ Hz, $J_{C-F} = 236.5$ Hz);
15 GC-MS 280 m/z $[M]^+$; Purity: 100.0% (348 nm, $t_r = 1.31$ min, Method: c)

Example 11: 4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2-diol

(2,4-Difluorophenyl)hydrazine (300 mg, 2.1 mmol), 3,4-
20 Dihydroxybenzaldehyde (287 mg, 2.1 mmol); Purification: Method 8; Yield: 326 mg, 59%; $C_{13}H_{10}F_2N_2O_2$, $M = 264,23$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.75 (d, 1H, $J = 8.1$ Hz), 6.86 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 6.99 (br t, 1H, $J = 8.7$ Hz), 7.13-7.21 (m, 1H), 7.15 (d, 1H, $J = 1.9$ Hz), 7.41 (td, 1H, $J = 1.9$ Hz, $J = 9.3$ Hz), 7.95 (s, 1H), 9.06 (s,
25 1H), 9.16 (s, 1H), 9.83 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 103.61 (dd, $J_{C-F} = 22.3$ Hz, $J_{C-F} = 26.9$ Hz), 111.18 (dd, $J_{C-F} = 3.6$ Hz, $J_{C-F} = 21.6$ Hz), 112.05, 113.59 (dd, $J_{C-F} = 4.7$ Hz, $J_{C-F} = 8.9$ Hz), 115.50, 118.62, 126.93, 130.79 (dd, $J_{C-F} = 2.8$ Hz, $J_{C-F} = 10.0$ Hz), 140.38, 145.49, 146.40, 148.11 (dd, $J_{C-F} = 12.0$ Hz, $J_{C-F} = 242.2$ Hz), 154.19 (dd, $J_{C-F} = 10.9$ Hz, $J_{C-F} = 236.2$ Hz);
30 GC-MS 264 m/z $[M]^+$; Purity: 98.2% (338 nm, $t_r = 1.44$ min, Method: c)

Example 12: 4-((2-Phenylhydrazono)methyl)benzene-1,3-diol

Phenylhydrazine (200 mg, 1.8 mmol), 2,4-Dihydroxybenzaldehyde (255 mg,

- 36 -

1.8 mmol);

Purification: Method 3; Yield: 221 mg, 52%; $C_{13}H_{12}N_2O_2$, $M = 228,25$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.30 (s, 1H), 6.32 (dd, 1H, $J = 2.1$, $J = 8.9$ Hz), 6.73 (t, 1H, $J = 7.3$ Hz), 6.89 (d, 2H, $J = 8.0$ Hz), 7.17-7.30 (m, 3H),
5 8.04 (s, 1H), 9.65 (s, 1H), 10.10 (s, 1H), 10.73 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.43, 107.39, 111.37, 111.88, 118.43, 129.09, 129.15, 139.22, 144.93, 157.46, 158.90; GC-MS 228 m/z $[M]^+$; Purity: 79.8% (340 nm, $t_r = 2.46$ min, Method: b)

10 **Example 13: 2-((2-Phenylhydrazono)methyl)phenol**

Phenylhydrazine (250 mg, 2.3 mmol), 2-Hydroxybenzaldehyde (282 mg, 2.3 mmol);

Purification: Method 1; Yield: 203 mg, 41%; $C_{13}H_{12}N_2O$, $M = 212,25$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.79 (t, 1H, $J = 7.3$ Hz), 6.89 (t, 2H, $J = 7.7$ Hz), 6.98 (d, 2H, $J = 8.0$ Hz), 7.18 (t, 1H, $J = 7.4$ Hz), 7.26 (t, 2H, $J = 7.8$ Hz), 7.55 (d, 1H, $J = 7.8$ Hz), 8.16 (s, 1H) 10.40 (s, 1H), 10.54 (s, 1H); ^{13}C -
15 NMR (75 MHz, d_6 -DMSO) ppm: 111.66, 115.83, 118.91, 119.30, 120.38, 127.29, 129.10, 129.20, 137.31, 144.67, 155.61; GC-MS 212 m/z $[M]^+$;
Purity: 97.1% (342 nm, $t_r = 2.81$ min, Method: c)

20

Example 14: 4-((2-(2,5-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol

(2,5-Dichlorophenyl)hydrazine (500 mg, 2.8 mmol), 2,4-Dihydroxybenzaldehyde (390 mg, 2.8 mmol);

25 Purification: Method 10; Yield: 533 mg, 63%; $C_{13}H_{10}Cl_2N_2O_2$, $M = 297,14$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.32 (s, 1H), 6.34 (d, 1H, $J = 7.2$ Hz), 6.78 (d, 1H, $J = 2.4$, $J = 8.4$), 7.27 (d, 1H, $J = 2.4$ Hz), 7.34 (d, 1H, $J = 8.4$ Hz), 7.44 (d, 1H, $J = 9.0$ Hz), 8.49 (s, 1H) 9.72 (s, 1H), 9.87 (s, 1H), 10.22 (s,
30 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.38, 107.70, 112.02, 112.11, 114.47, 118.15, 128.42, 130.66, 132.66, 142.29, 142.45, 157.57, 159.69; GC-MS 296 m/z $[M-H]^+$; Purity: 66.0% (338 nm, $t_r = 3.70$ min, Method: d)

- 37 -

Example 15: 4-((2-(2-Chloro-5-**(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol**

(2-Chloro-5-(trifluoromethyl)phenyl)hydrazine (250 mg, 1.2 mmol), 2,4-Dihydroxybenzaldehyde (163 mg, 1.2 mmol);

5 Purification: Method 10; Yield: 360 mg, 92%; $C_{14}H_{10}ClF_3N_2O_2$, $M = 330,69$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.34 (s, 1H), 6.35 (d, 1H, $J = 7.4$ Hz), 7.06 (d, 1H, $J = 8.4$), 7.45 (d, 1H, $J = 8.7$ Hz), 7.53 (s, 1H), 7.56 (d, 1H, $J = 9.0$ Hz), 8.54 (s, 1H) 9.74 (s, 1H), 10.04 (s, 1H), 10.21 (s, 1H); ^{13}C -NMR (75
10 MHz, d_6 -DMSO) ppm: 102.49, 107.83, 108.80 (q, $J_{C-F} = 4.0$ Hz), 112.07, 114.79 (q, $J_{C-F} = 3.61$ Hz), 119.64, 124.03 (d, $J_{C-F} = 272.4$ Hz), 128.42, 128.87 (q, $J_{C-F} = 31.8$ Hz), 130.41, 142.09, 142.65, 157.71, 159.87; GC-MS 330 m/z $[M]^+$; Purity: 97.5% (338 nm, $t_r = 3.31$ min, Method: c)

Example 16: 4-((2-(2-Chlorophenyl)hydrazono)methyl)benzene-1,3-diol

(2-Chlorophenyl)hydrazine hydrochloride (500 mg, 2.8 mmol), 2,4-Dihydroxybenzaldehyde (386 mg, 2.8 mmol);

Purification: Method 10; Yield: 638 mg, 69%; $C_{13}H_{11}ClN_2O_2$, $M = 262,69$ g/mol

20 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.30-6.38 (m, 2H), 6.73-6.82 (m, 1H), 7.22-7.29 (m, 2H), 7.29-7.36 (m, 2H), 8.44 (s, 1H), 9.68 (d, 1H), 10.51 (br s, 2H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.46, 107.60, 111.77, 116.10, 119.17, 128.04, 129.02, 129.34, 141.17, 142.43, 157, 71, 159.54; GC-MS 262 m/z $[M]^+$; Purity: 100.0% (338 nm, $t_r = 2.71$ min, Method: d)

25

Example 17: 4-((2-(2,4-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol

(2,4-Dichlorophenyl)hydrazine (200 mg, 1.1 mmol), 2,4-Dihydroxybenzaldehyde (129 mg, 0.9 mmol);

30 Purification: Method 3; Yield: 86 mg, 26%; $C_{13}H_{10}Cl_2N_2O_2$, $M = 297,14$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.32-6.34 (m, 2H), 7.30 (s, 2H), 7.38 (d, 2H, $J = 9.1$), 7.44 (s, 1H), 8.46 (s, 1H), 9.79 (s, 1H) 10.31 (br s, 2H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.41, 107.61, 111.91, 113.99, 116.43,

- 38 -

121.50, 127.99, 128.51, 128.58, 140.49, 142.22, 157.59, 159.60; GC-MS 296 m/z $[M-H]^+$; Purity: 98.2% (336 nm, t_r = 1.70 min, Method: c)

Example 18: 4-((2-(2-Chloro-4-

5 **(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol**

(2-Chloro-4-(trifluoromethyl)phenyl)hydrazine (250 mg, 1.2 mmol), 2,4-Dihydroxybenzaldehyde (163 mg, 1.2 mmol);

Purification: Method 3; Yield: 314 mg, 80%; $C_{14}H_{10}ClF_3N_2O_2$, $M = 330,69$ g/mol

10 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.34-6.37 (m, 2H), 7.47 (d, 2H, $J = 9.0$), 7.59 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.69 (d, 1H, $J = 1.8$ Hz), 8.58 (s, 1H) 9.77 (s, 1H), 10.14 (s, 1H), 10.23 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.44, 107.77, 111.95, 112.61, 115.53, 118.51 (q, $J_{C-F} = 32.8$ Hz), 124.15 (d, $J_{C-F} = 270.8$ Hz), 125.28 (q, $J_{C-F} = 3.6$ Hz), 126.42 (q, $J_{C-F} = 3.8$ Hz), 128.57, 143.21, 144.28, 157.78, 159.93; GC-MS 330 m/z $[M]^+$; Purity: 98.4% (342 nm, t_r = 3.57 min, Method: c)

Example 19: 4-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,3-diol

(4-Iodophenyl)hydrazine (200 mg, 0.9 mmol), 2,4-Dihydroxybenzaldehyde
20 (118 mg, 0.9 mmol);

Purification: Method 3; Yield: 264 mg, 87%; $C_{13}H_{11}IN_2O_2$, $M = 354,14$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.31 (s, 1H), 6.33 (d, 1H, $J = 7.4$ Hz), 6.77 (d, 2H, $J = 8.6$ Hz), 7.34 (d, 1H, $J = 8.7$ Hz), 7.51 (d, 2H, $J = 8.6$ Hz), 8.06 (s, 1H), 9.68 (s, 1H), 10.24 (s, 1H), 10.49 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -
25 DMSO) ppm: 79.29, 102.34, 107.44, 111.91, 113.86, 128.69, 137.41, 139.19, 144.74, 157.31, 159.06; GC-MS 354 m/z $[M]^+$; Purity: 96.7% (346 nm, t_r = 2.39 min, Method: c)

Example 20: 5-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,2,4-triol

30 (4-Iodophenyl)hydrazine (300 mg, 1.3 mmol), 2,4,5-Trihydroxybenzaldehyde (198 mg, 1.3 mmol);

Purification: Method 3; Yield: 430 mg, 91%; $C_{13}H_{11}IN_2O_3$, $M = 370,14$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.31 (s, 1H), 6.75 (d, 2H, $J = 8.7$ Hz),

- 39 -

6.92 (s, 1H), 7.49 (d, 2H, J = 8.7 Hz), 7.99 (s, 1H), 8.40 (s, 1H), 9.22 (s, 1H), 9.69 (s, 1H), 10.15 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 79.02, 103.34, 110.99, 112.86, 113.83, 137.36, 138.34, 138.59, 144.90, 147.38, 149.65; GC-MS 370 *m/z* [M]⁺; Purity: 96.1% (352 nm, t_r = 1.72 min, Method: c)

Example 21: 5-((2,2-Diphenylhydrazono)methyl)benzene-1,2,4-triol

1,1-Diphenylhydrazine (520 mg, 2.8 mmol), 2,4,5-Trihydroxybenzaldehyde (539 mg, 2.8 mmol);

Purification: Method 5; Yield: 491 mg, 53%; C₁₉H₁₆N₂O₃, M = 320,34 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.28 (s, 1H), 7.01 (s, 1H) 7.09 (d, 4H, J = 7.8 Hz), 7.19 (t, 2H, J = 7.3 Hz), 7.34 (s, 1H), 7.45 (t, 4H, J = 7.8 Hz), 8.40 (s, 1H), 9.18 (s, 1H), 9.46 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 103.31, 111.40, 112.42, 121.85, 124.09, 129.88, 135.91, 143.35, 147.46, 149.45; GC-MS 320 *m/z* [M]⁺; Purity: 96.2% (354 nm, t_r = 2.48 min, Method: i)

Example 22: 4-((2,2-Diphenylhydrazono)methyl)benzene-1,3-diol

1,1-Diphenylhydrazine (337 mg, 1.8 mmol), 2,4-Dihydroxybenzaldehyde (230 mg, 1.7 mmol);

Purification: Method 9; Yield: 501 mg, 90%; C₁₉H₁₆N₂O₂, M = 304,34 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.28-6.30 (m, 2H), 7.10 (d, 4H, J = 7.8 Hz), 7.20 (t, 2H, J = 7.3 Hz), 7.35 (d, 1H, J = 9.1 Hz), 7.39 (s, 1H) 7.45 (t, 4H, J = 7.8 Hz), 9.64 (s, 1H), 10.39 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 102.35, 107.52, 112.16, 121.92, 124.30, 128.77, 129.96, 137.16, 143.15, 157.27, 159.21; GC-MS 304 *m/z* [M]⁺; Purity: 97.6% (344 nm, t_r = 3.85 min, Method: c)

Example 23: 4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,3-diol

(Perfluorophenyl)hydrazine (200 mg, 1. mmol), 2,4-Dihydroxybenzaldehyde (139 mg, 1.0 mmol);

Purification: Method 11; Yield: 254 mg, 79%; C₁₃H₇F₅N₂O₂, M = 318,20 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.30 (d, 1H, J = 1.8 Hz), 6.34 (dd, 1H, J

- 40 -

= 2.0 Hz, $J = 8.4$ Hz), 7.23 (d, 1H, $J = 8.4$ Hz), 8.27 (s, 1H), 9.81 (s, 1H), 10.18 (s, 1H), 10.56 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.41, 107.45, 110.81, 121.12 (t, $J_{\text{C-F}} = 10.9$ Hz), 131.44 (d,), 134.41-139.83 (m, $J_{\text{C-F}} = 13.4$ Hz), 144.72, 158.09, 159.67; GC-MS 318 m/z $[\text{M}]^+$; Purity: 99.8%
5 (322 nm, $t_r = 2.22$ min, Method: c)

Example 24: 4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,2-diol

(Perfluorophenyl)hydrazine (300 mg, 1.5 mmol), 3,4-Dihydroxybenzaldehyde (209 mg, 1.5 mmol);

10 Purification: Method 3; Yield: 447 mg, 93%; $\text{C}_{13}\text{H}_7\text{F}_5\text{N}_2\text{O}_2$, $M = 318,20$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.74 (d, 1H, $J = 8.1$ Hz), 6.82 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.10 (d, 1H, $J = 1.7$ Hz), 7.96 (s, 1H), 9.11 (s, 1H), 9.20 (s, 1H), 9.95 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 112.03, 115.47, 119.15, 121.78 (t, $J_{\text{C-F}} = 11.7$ Hz), 126.23, 131.48-131.84 (m),
15 134.70-136.32 (m), 138.54-139.55 (m), 143.14, 145.55, 146.92; GC-MS 318 m/z $[\text{M}]^+$; Purity: 96.0% (318 nm, $t_r = 1.72$ min, Method: c)

Example 25: 4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2-diol

1-Methyl-1-phenylhydrazine (289 μL , 2.5 mmol), 3,4-Dihydroxybenzaldehyde (339 mg, 2.5 mmol);

20 Purification: Method 3; Yield: 226 mg, 38%; $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2$, $M = 242,27$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.35 (s, 3H), 6.74 (d, 1H, $J = 8.1$ Hz), 6.83 (t, 1H, $J = 7.0$ Hz), 6.92 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.21 (d, 1H, $J = 1.8$ Hz), 7.27 (t, 2H, $J = 7.9$ Hz), 7.35 (d, 2H, $J = 8.1$ Hz), 7.54 (s, 1H), 9.02
25 (br s, 2H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 32.49, 112.01, 114.12, 115.47, 118.59, 119.26, 128.20, 128.80, 133.31, 145.43, 145.76, 147.65; GC-MS 242 m/z $[\text{M}]^+$; Purity: 97.3% (340 nm, $t_r = 1.54$ min, Method: c)

Example 26: 4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,3-diol

30 1-Methyl-1-phenylhydrazine (289 μL , 2.5 mmol), 2,4-Dihydroxybenzaldehyde (339 mg, 2.5 mmol);

Purification: Method 3; Yield: 382 mg, 64%; $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2$, $M = 242,27$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.38 (s, 3H), 6.30 (d, 1H, $J = 2.2$ Hz),

- 41 -

6.34 (dd, 1H, J = 8.3.0 Hz, J = 2.3 Hz), 6.89 (t, 1H, J = 7.2 Hz), 7.21 (d, 2H, J = 8.0 Hz), 7.27-7.37 (m, 3H), 7.88 (s, 1H), 9.62 (s, 1H), 10.98 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 32.94, 102.44, 107.34, 112.47, 114.31, 120.03, 129.12, 129.58, 135.96, 147.20, 157.30, 158.73; GC-MS 242 *m/z* [M]⁺; Purity: 98.8% (336 nm, t_r = 1.85 min, Method: c)

Example 27: 5-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2,4-triol
1-Methyl-1-phenylhydrazine (289 μL, 2.5 mmol), 2,4,5-Trihydroxybenzaldehyde (378 mg, 2.5 mmol);
Purification: Method 3; Yield: 295 mg, 47%; C₁₄H₁₄N₂O₃, M = 258,27 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.36 (s, 3H), 6.31 (s, 1H), 6.87 (t, 1H, J = 7.1 Hz), 6.96 (s, 1H), 7.22 (d, 2H, J = 7.9 Hz), 7.30 (t, 2H, J = 7.9 Hz), 7.79 (s, 1H), 8.40 (s, 1H), 9.16 (s, 1H), 10.15 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 32.83, 103.33, 111.57, 113.83, 114.14, 119.72, 129.02, 134.93, 138.20, 146.92, 147.31, 149.51; GC-MS 258 *m/z* [M]⁺; Purity: 98.4% (348 nm, t_r = 1.34 min, Method: c)

Example 28: 3-Chloro-4-(2-(2,4,5-trihydroxybenzylidene)hydrazinyl)benzonitrile
3-Chloro-4-hydrazinylbenzonitrile (250 mg, 1.5 mmol), 2,4,5-Trihydroxybenzaldehyde (230 mg, 1.5 mmol);
Purification: Method 1; Yield: 301 mg, 66%; C₁₄H₁₀ClN₃O₃, M = 303,70 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.34 (s, 1H), 7.07 (s, 1H), 7.42 (d, 1H, J = 8.7 Hz), 7.66 (dd, 1H, J = 1.5 Hz, J = 8.7 Hz), 7.83 (d, 1H, J = 1.6 Hz), 8.49 (s, 1H), 8.56 (s, 1H), 9.36 (s, 1H), 9.45 (s, 1H), 10.25 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 99.13, 103.40, 110.88, 112.18, 112.61, 115.35, 118.85, 132.36, 132.93, 138.65, 143.25, 144.87, 148.61, 150.40; GC-MS 303 *m/z* [M]⁺; Purity: 100.0% (362 nm, t_r = 1.23 min, Method: c)

Example 29: 3-Chloro-4-(2-(2,4-dihydroxybenzylidene)hydrazinyl)benzonitrile
3-Chloro-4-hydrazinylbenzonitrile (250 mg, 1.5 mmol), 2,4-Dihydroxybenzaldehyde (206 mg, 1.5 mmol);

- 42 -

Purification: Method 1; Yield: 258 mg, 60%; $C_{14}H_{10}ClN_3O_2$, $M = 287,70$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.32-6.35 (m, 2H), 7.43 (d, 1H, $J = 8.7$ Hz), 7.50 (d, 1H, $J = 9.1$ Hz), 7.64 (dd, 1H, $J = 1.4$ Hz, $J = 8.7$ Hz), 7.84 (d, 1H, $J = 1.5$ Hz), 8.60 (s, 1H), 9.78 (s, 1H), 10.15 (s, 1H), 10.29 (s, 1H); ^{13}C -
NMR (75 MHz, d_6 -DMSO) ppm: 99.37, 102.42, 107.83, 111.89, 112.70,
115.46, 118.81, 128.45, 132.37, 132.94, 143.72, 144.75, 157.86, 160.11;
GC-MS 287 m/z $[M]^+$; Purity: 98.7% (354 nm, $t_r = 1.58$ min, Method: c)

Example 30: 3-Chloro-4-(2-(3,4-**dihydroxybenzylidene)hydrazinyl)benzonitrile**

3-Chloro-4-hydrazinylbenzonitrile (250 mg, 1.5 mmol), 3,4-Dihydroxybenzaldehyde (206 mg, 1.5 mmol);

Purification: Method 1; Yield: 200 mg, 47%; $C_{14}H_{10}ClN_3O_2$, $M = 287,70$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.78 (d, 1H $J = 8.1$ Hz), 6.93 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.20 (d, 1H, $J = 1.7$ Hz), 7.55 (d, 1H, $J = 8.7$ Hz), 7.67 (dd, 1H, $J = 1.7$ Hz, $J = 8.7$ Hz), 7.85 (d, 1H, $J = 1.7$ Hz), 8.26 (s, 1H), 9.15 (s, 1H), 9.33 (s, 1H), 10.24 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 99.12, 103.39, 110.87, 112.15, 112.60, 115.34, 118.84, 132.36, 132.93, 138.65, 143.23, 144.87, 148.61, 150.39; GC-MS 287 m/z $[M]^+$; Purity: 99.1%
(354 nm, $t_r = 1.37$ min, Method: c)

Example 31: 4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2-diol

(4-Nitrophenyl)hydrazine + H_2O (500 mg, 70%, 2.3 mmol), 4,5-Dihydroxybenzaldehyde (316 mg, 2.3 mmol);

Purification: Method 7; Yield: 381 mg, 61%; $C_{13}H_{11}N_3O_4$, $M = 273,24$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.77 (d, 1H, $J = 8.1$ Hz), 6.95 (dd, 1H, $J = 1.8$ Hz, $J = 8.1$ Hz), 7.09 (d, 2H, $J = 8.8$ Hz), 7.21 (d, 1H, $J = 1.8$ Hz), 7.89 (s, 1H), 8.12 (d, 2H, $J = 9.2$ Hz), 9.24 (br s, 2H), 11.07 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 110.69, 112.37, 115.52, 119.61, 126.02, 126.14, 137.65, 142.76, 145.58, 150.68; GC-MS 273 m/z $[M]^+$; Purity: 98.8% (426 nm, $t_r = 1.19$ min, Method: c)

Example 32: 5-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2,4-triol

- 43 -

(4-Nitrophenyl)hydrazine + H₂O (500 mg, 70%, 2.3 mmol), 2,4,5-Trihydroxybenzaldehyde (352 mg, 2.3 mmol);

Purification: Method 1; Yield: 497 mg, 75%; C₁₃H₁₁N₃O₅, M = 289,24 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.35 (s, 1H), 7.01 (d, 2H, J = 9.2 Hz), 7.09 (s, 1H), 8.12 (d, 2H, J = 9.2 Hz), 8.21 (s, 1H), 8.51 (s, 1H), 9.38 (s, 1H), 9.47 (s, 1H), 11.06 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 103.38, 110.41, 110.80, 111.96, 126.25, 137.44, 138.69, 141.18, 148.60, 150.25, 150.42; GC-MS 289 *m/z* [M]⁺; Purity: 76.5% (428 nm, t_r = 1.13 min, Method: c)

Example 33: 4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,3-diol

(4-Nitrophenyl)hydrazine + H₂O (500 mg, 70%, 2.3 mmol), 4,5-Dihydroxybenzaldehyde (316 mg, 2.3 mmol);

Purification: Method 1; Yield: 598 mg, 96%; C₁₃H₁₁N₃O₄, M = 273,24 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.33-6.36 (m, 2H), 7.03 (d, 2H, J = 9.1 Hz), 7.51 (d, 1H, J = 9.1 Hz), 8.12 (d, 2H, J = 9.2 Hz), 8.25 (s, 1H), 9.79 (s, 1H), 10.18 (s, 1H), 11.11 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 102.38, 107.84, 110.52, 111.80, 126.22, 128.23, 137.63, 141.58, 150.31, 157.68, 160.04; GC-MS 273 *m/z* [M]⁺; Purity: 98.1% (420 nm, t_r = 1.34 min, Method: c)

Example 34: 4-((Piperidin-1-ylimino)methyl)benzene-1,3-diol

Piperidin-1-amine (300 mg, 3.0 mmol), 2,4-Dihydroxybenzaldehyde (414 mg, 3.0 mmol);

Purification: Method 1; Yield: 462 mg, 70%; C₁₂H₁₆N₂O₂, M = 220,27 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 1.39-1.52 (m, 2H), 1.59-1.72 (m, 4H), 2.96-3.03 (m, 4H), 6.21 (d, 1H, J = 2.2 Hz), 6.28 (dd, 1H, J = 2.3 Hz, J = 8.3 Hz), 7.11 (d, 1H, J = 8.4 Hz), 7.83 (s, 1H), 9.62 (s, 1H), 11.80 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 23.35, 24.34, 51.91, 102.30, 106.78, 111.68, 130.52, 140.57, 158.48, 158.60; GC-MS 220 *m/z* [M]⁺; Purity: 97.% (290 nm), 99.2%, (308 nm, t_r = 1.56 min, Method: c)

Example 35: 4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3-

- 44 -

diol

2-Hydrazinylbenzo[d]thiazole (300 mg, 1.8 mmol), 2,4-Dihydroxybenzaldehyde (251 mg, 1.8 mmol);

Purification: Method 1; Yield: 290 mg, 56%; $C_{14}H_{11}N_3O_2S$, $M = 285,32$ g/mol

5 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.34 (s, 1H), 6.36 (dd, 1H, $J = 2.1$ Hz, $J = 9.8$ Hz), 7.07 (t, 1H, $J = 8.1$ Hz), 7.23-7.31 (m, 2H), 7.39 (d, 1H, $J = 8.2$ Hz), 7.71 (d, 1H, $J = 7.7$ Hz), 8.34 (s, 1H), 9.89 (s, 1H), 10.59 (s, 1H), 11.95 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.34, 107.74, 111.19, 121.18, 121.75, 126.10, 160.22; GC-MS 285 m/z $[M]^+$; Purity: 100.0% (332 nm, $t_r =$
10 1.30 min, Method: c)

Example 36: 3-(2-(2,4-Dihydroxybenzylidene)hydrazinyl)propanenitrile

3-Hydrazinylpropanenitrile (300 mg, 3.5 mmol), 2,4-Dihydroxybenzaldehyde (251 mg, 3.5 mmol);

15 Purification: Method 1; Yield: 75 mg, 10%; $C_{10}H_{11}N_3O_2$, $M = 205,21$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 2.69 (t, 2H, $J = 6.4$ Hz), 3.28-3.33 (m, 2H), 6.23 (d, 1H, $J = 2.1$ Hz), 6.27 (dd, 1H, $J = 2.2$ Hz, $J = 8.3$ Hz), 7.08 (d, 1H, $J = 8.3$ Hz), 7.86 (d, 1H), 10.11 (br s, 1H) 11.02 (br s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 17.04, 44.61, 102.29, 106.91, 111.50, 119.52, 129.59,
20 141.67, 157.93, 158.64; GC-MS 205 m/z $[M]^+$; Purity: 93,4% (290 nm), 91.5% (306 nm, $t_r = 3.47$ min; Methode: i)

Example 37: 2-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3,5-triol

25 (2,6-Dichloro-4-(Trifluoromethyl)phenyl)hydrazine (500 mg, 2.8 mmol), 2,4,6-Trihydroxybenzaldehyde (437 mg, 2.8 mmol);

Purification: Method 10; Yield: 640 mg, 72%; $C_{14}H_{11}F_3N_2O_3$, $M = 312,24$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 5.86 (s, 2H), 6.92 (d, 2H, $J = 8.5$ Hz),
30 7.55 (d, 2H, $J = 8.6$ Hz), 8.41 (s, 1H), 9.68 (s, 1H), 10.59 (s, 1H), 10.62 (s, 2H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 94.29, 99.29, 110.66, 117.95 (q, $J_{C-F} = 31.92$ Hz), 124.87 (d, $J_{C-F} = 264.9$ Hz), 126.65 (q, $J_{C-F} = 3.8$ Hz), 140.34, 147.39, 158.34, 160.28; GC-MS 312 m/z $[M-H]^+$; Purity: 100.0% (350 nm, $t_r =$

- 45 -

1.89 min, Method: c)

Example 38: 2-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3,5-triol

5 (2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazine (500 mg, 2.0 mmol), 2,4,6-Trihydroxybenzaldehyde (314 mg, 2.0 mmol);

Purification: Method 10; Yield: 700 mg, 90%; $C_{14}H_9Cl_2F_3N_2O_3$, $M = 381.13$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 5.82 (s, 2H), 7.81 (s, 2H), 8.72 (s, 1H),
10 9.68 (s, 1H), 9.73 (s, 1H), 10.48 (s, 2H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 94.23, 99.07, 121.87 (q, $J_{C-F} = 33.8$ Hz), 122.88, 122.96 (d, $J_{C-F} = 271.9$ Hz), 126.60 (q, $J_{C-F} = 3.7$ Hz), 140.96, 143.54, 158.81, 160.57; ppm: GC-MS 380 m/z $[M-H]^+$; Purity: 99.2% (342 nm, $t_r = 3.11$ min, Method: c)

15 **Example 39: 2-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3,5-triol**

2-Hydrazinylbenzo[d]thiazole (500 mg, 3.0 mmol), 2,4,6-Trihydroxybenzaldehyde (466 mg, 3.0 mmol);

Purification: Method 3; Yield: 435 mg, 48%; $C_{14}H_{11}N_3O_3S$, $M = 301.32$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 5.87 (s, 2H), 7.06 (t, 1H, $J = 7.9$ Hz),
20 7.27 (pt, 2H, $J = 6.9$ Hz), 7.68 (d, 1H, $J = 5.9$ Hz), 8.56 (s, 1H), 9.80 (s, 1H), 10.65 (bs, 2H), 11.87 (bs, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 94.32, 99.23, 121.20, 121.99, 126.32, 159.22, 161.18, 164.06; GC-MS 301 m/z $[M]^+$; Purity: 100.0% (354 nm, $t_r = 1.21$ min, Method: c)

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Example 40: 4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)phenol
(4-(Trifluoromethyl)phenyl)hydrazine (500 mg, 2.8 mmol), 4-Hydroxybenzaldehyde (347 mg, 2.8 mmol)

Purification: Method 10; Yield: 483 mg, 61%; $C_{14}H_{11}F_3N_2O$, $M = 280.25$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.81 (d, 2H, $J = 8.5$ Hz), 7.14 (d, 2H, $J = 8.4$ Hz), 7.51 (d, 2H, $J = 8.9$ Hz), 7.52 (d, 2H, $J = 8.6$ Hz), 7.87 (s, 1H), 9.71 (s, 1H), 10.53 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 111.22, 115.51, 117.66 (q, $J_{C-F} = 31.8$ Hz), 125.02 (d, $J_{C-F} = 270.2$ Hz), 126.18, 126.31 (q, J_{C-F}

30

- 46 -

= 3.7 Hz), 127.55, 139.49, 148.47, 158.13; GC-MS 280 m/z $[M]^+$; Purity: 100.0% (344 nm, t_r = 2.68 min, Method: c)

Example 41: 4-((2-(2,6-Dichloro-4-

5 **(trifluoromethyl)phenyl)hydrazono)methyl)phenol**

(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazine (500 mg, 2.0 mmol), 4-Hydroxybenzaldehyde (249 mg, 2.0 mmol)

Purification: Method 10; Yield: 520 mg, 73%; $C_{14}H_9Cl_2F_3N_2O$, $M = 349.14$ g/mol

10 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.81 (d, 2H, $J = 8.5$ Hz), 7.47 (d, 2H, $J = 8.5$ Hz), 7.77 (s, 2H), 8.17 (s, 1H), 9.72 (s, 1H), 9.75 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 115.22, 121.44 (q, $J_{C-F} = 33.6$ Hz), 122.97, 125.92, 126.52 (q, $J_{C-F} = 3.6$ Hz), 126.63 (d, $J_{C-F} = 271.6$ Hz), 127.69, 141.42, 142.67, 158.47; GC-MS 348 m/z $[M-H]^+$; Purity: 100.0% (334 nm, t_r = 4.66 min,
15 Method: c)

Example 42: 4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)phenol

2-Hydrazinylbenzo[d]thiazole (500 mg, 3.0 mmol), 4-Hydroxybenzaldehyde (369 mg, 3.0 mmol) Purification: Method 1; Yield: 620 mg, 76%; $C_{14}H_{11}N_3OS$,
20 $M = 269.32$ g/mol

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.83 (d, 2H, $J = 8.5$), 7.08 (t, 1H, $J = 7.6$ Hz), 7.28 (t, 1H, $J = 7.5$ Hz), 7.41 (bs, 1H), 7.53 (d, 2H, $J = 8.5$ Hz), 7.53 (d, 2H, $J = 8.5$ Hz), 7.53 (d, 2H, $J = 8.5$ Hz), 7.74 (d, 1H, $J = 7.6$ Hz), 8.03 (s, 1H), 9.86 (s, 1H), 12.02 (bs, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 115.62,
25 121.20, 121.32, 125.26, 125.75, 128.14, 158.92, 166.69; GC-MS 269 m/z $[M]^+$; Purity: 100.0% (338 nm, t_r = 1.44 min, Method: c)

Example 43: 4-((2-Methyl-2-(2-nitro-4-

(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol

30 1-Methyl-1-(2-nitro-4-(trifluoromethyl)phenyl)hydrazine (400 mg, 1.7 mmol), 2,4-Dihydroxybenzaldehyde (117 mg, 1.7 mmol)

Purification: Method 10; Yield: 479 mg, 79%; $C_{15}H_{12}F_3N_3O_4$, $M = 355.27$ g/mol

- 47 -

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.26 (dd, 1H, J = 2.2 Hz, J = 8.5 Hz), 7.28 (d, 1H, J = 8.53 Hz), 7.56 (d, 1H, J = 8.9 Hz), 7.85 (dd, 1H, J = 1.9 Hz, J = 8.9 Hz), 7.91 (s, 1H), 8.06 (d, 1H, J = 1.5 Hz), 9.66 (bs, 1H), 9.79 (bs, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 33.81, 102.24, 107.64, 112.55, 119.11, 119.56 (q, J_{C-F} = 33.6 Hz), 122.80 (q, J_{C-F} = 3.9 Hz); 123.49 (d, J_{C-F} = 271.23 Hz), 127.06, 128.78 (q, J_{C-F} = 3.5 Hz), 135.77, 139.63, 143.21, 157.19, 159.70; GC-MS 355 *m/z* [M]⁺; Purity: 100.0% (338 nm, t_r = 2.33 min, Method: c)

Example 44: 4-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2,3-triol

1-Methyl-1-(2-nitro-4-(trifluoromethyl)phenyl)hydrazine (500 mg, 2.1 mmol), 2,3,4-Trihydroxybenzaldehyde (327 mg, 2.1 mmol)

Purification: Method 1; Yield: 368 mg, 47%; C₁₅H₁₂F₃N₃O₅, M = 371.27 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.47 (s, 3H), 6.33 (d, 1H, J = 8.6 Hz), 7.58 (d, 1H, J = 8.6 Hz), 7.58 (d, 1H, J = 8.9 Hz), 7.87 (dd, 1H, J = 1.8 Hz, J = 8.9 Hz), 7.93 (s, 1H), 8.08 (d, 1H, J = 1.3 Hz), 8.41 (s, 1H), 9.06 (s, 1H), 9.40 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 34.04, 107.64, 113.29, 116.76, 119.38, 119.72 (d, J_{C-F} = 33.6 Hz), 122.85 (q, J_{C-F} = 3.8 Hz), 123.46 (d, J_{C-F} = 271.1 Hz), 128.86 (q, J_{C-F} = 3.4 Hz), 132.64, 137.10, 139.65, 143.28, 145.86, 147.47; GC-MS 371 *m/z* [M]⁺; Purity: 96.9% (334 nm), 92.2 (404 nm, t_r = 1.61 min, Method: m)

Example 45: 2-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3,5-triol

1-Methyl-1-(2-nitro-4-(trifluoromethyl)phenyl)hydrazine (500 mg, 2.1 mmol), 2,4,6-Trihydroxybenzaldehyde (327 mg, 2.1 mmol)

Purification: Method 6; Yield: 160 mg, 20%; C₁₅H₁₂F₃N₃O₅, M = 371.27 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.46 (s, 3H), 5.84 (s, 2H), 7.67 (d, 1H, J = 8.8 Hz), 7.95 (dd, 1H, J = 1.8 Hz, J = 8.8 Hz), 8.18 (s, 2H), 9.75 (s, 1H), 10.11 (s, 2H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 35.85, 94.29, 99.03, 121.06 (q, J_{C-F} = 33.5 Hz), 121.50, 123.19 (d, J_{C-F} = 273.2 Hz), 123.40 (q, J_{C-F} = 4.0 Hz), 129.62 (q, J_{C-F} = 3.1 Hz), 139.70, 140.43, 143.87, 158.46, 160.83;

- 48 -

GC-MS 371 m/z $[M]^+$; Purity: 100.0% (336 nm, t_r = 1.88 min, Method: c)

Example 46: 4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,3-diol

2-Hydrazinylquinoline (200 mg, 1.3 mmol), 2,4-Dihydroxybenzaldehyde (174 mg, 1.3 mmol) Purification: Method 1; Yield: 252 mg, 72%; $C_{16}H_{13}N_3O_2$, M = 279.29 g/mol ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.33-6.36 (m, 2H), 7.24-7.31 (m, 2H), 7.39 (d, 1H, J = 9.1 Hz), 7.54-7.61 (m, 2H), 7.75 (d, 1H, J = 7.9 Hz), 8.13 (d, 1H, J = 9.0 Hz), 8.24 (s, 1H), 9.73 (bs, 1H), 10.75 (bs, 1H), 11.12 (bs, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 102.43, 107.47, 109.40, 111.89, 122.30, 123.81, 125.55, 127.68, 128.94, 129.61, 137.77, 140.87, 147.07, 154.75, 157.80, 159.42; GC-MS 279 m/z $[M]^+$; Purity: 93.4% (250 nm); 98.6% (326 nm); 100.0% (362 nm, t_r = 1.40 min, Method: c)

Example 47: 5-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,4-triol

2-Hydrazinylquinoline (500 mg, 3.2 mmol), 2,4,5-Trihydroxybenzaldehyde (516 mg, 3.2 mmol) Purification: Method 1; Yield: 704 mg, 76%; $C_{16}H_{13}N_3O_3$, M = 295.29 g/mol ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.35 (s, 1H), 7.00 (s, 1H), 7.25 (t, 1H, J = 6.1 Hz), 7.33 (d, 1H, J = 8.7 Hz), 7.53-7.60 (m, 2H), 7.74 (d, 1H, J = 7.9 Hz), 8.13 (d, 1H, J = 8.8 Hz), 8.21 (s, 1H), 8.48 (bs, 1H), 9.4 (bs, 1H), 9.91 (bs, 1H), 10.97 (bs, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 103.38, 109.27, 110.93, 112.92, 122.12, 123.73, 125.49, 127.66, 129.59, 137.68, 138.38, 140.33, 147.11, 147.84, 150.16, 154.93; GC-MS 295 m/z $[M]^+$; Purity: 82.9% (374 nm, t_r = 5.22 min, Method: o)

Example 48: 4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,3-triol

2-Hydrazinylquinoline (500 mg, 3.2 mmol), 2,3,4-Trihydroxybenzaldehyde (516 mg, 3.2 mmol) Purification: Method 1; Yield: 750 mg, 81%; $C_{16}H_{13}N_3O_3$, M = 295.29 g/mol ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 6.39 (d, 1H, J = 8.4 Hz), 6.85 (d, 1H, J = 8.5 Hz), 7.21-7.30 (m, 2H), 7.55-7.60 (m, 2H), 7.75 (d, 1H, J = 7.9 Hz), 8.14 (d, 1H, J = 9.1 Hz), 8.22 (s, 1H), 9.31 (bs, 2H), 10.77 (bs, 2H); ^{13}C -NMR (75

- 49 -

MHz, d₆-DMSO) ppm: 107.44, 109.43, 112.16, 119.02, 122.30, 123.80, 125.55, 127.68, 129.66, 132.59, 137.79, 142.61, 146.25, 147.04, 147.50, 154.38; GC-MS 295 *m/z* [M]⁺; Purity: 85.5% (248 nm), 82.1% (320 nm, t_r = 4.43 min, Method: I)

5

Example 49: 5-((3-Mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)methyl)benzene-1,2,4-triol

4-Amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (350 mg, 1.8 mmol), 2,4,5-Trihydroxybenzaldehyde (279 mg, 1.8 mmol)

10 Purification: Method 1; Yield: 568 mg, 95%; C₁₄H₁₁N₅O₃S, M = 329.33 g/mol
¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.43 (s, 1H), 7.24 (s, 1H), 7.86 (d, 2H, J = 6.1 Hz), 8.76 (d, 2H, J = 6.0 Hz), 8.83 (s, 1H), 9.53 (s, 1H), 9.77 (s, 1H), 9.98 (s, 1H), 14.30 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 103.16, 108.34, 111.50, 121.60, 132.92, 139.21, 146.16, 150.10, 152.75, 153.84,
 15 162.93, 163.86; GC-MS 178 *m/z* [C₅NH₄-C₂N₃-SH + H]⁺, 151 *m/z* [2,3,4-tri-OH-C₆H₃CN]⁺; Purity: 87.1% (254 nm, t_r = 1:05 min, Method: n)

Example 50: 5-((2-(5-Chloro-3-iodopyridin-2-yl)hydrazono)methyl)benzene-1,2,4-triol

20 5-Chloro-2-hydrazinyl-3-iodopyridine (250 mg, 0.9 mmol), 2,4,5-Trihydroxybenzaldehyde (143, 0.9 mmol)

Purification: Method 1; Yield: 376 mg, 100%; C₁₂H₉ClIN₃O₃, M = 405.58 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.30 (s, 1H), 6.73 (s, 1H), 8.21 (s, 2H),
 25 8.41 (s, 1H), 8.46 (s, 1H), 9.38 (s, 1H), 10.00 (s, 1H), 11.08 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 77.47, 103.41, 109.66, 115.04, 120.46, 138.05, 145.52, 145.78, 146.09, 148.15, 151.37, 151.93; GC-MS 405 *m/z* [M]⁺; Purity: 100.0% (368 nm, t_r = 1.66 min, Method: c)

30 **Example 51: 4-((Morpholinoimino)methyl)benzene-1,2,3-triol**

Morpholin-4-amine (25 μL, 2.5 mmol), 2,3,4-Trihydroxybenzaldehyde (400 mg, 2.5 mmol) Purification: Method 1; Yield: 474 mg, 75%; C₁₁H₁₄N₂O₄, M = 238.24 g/mol

- 50 -

¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.02 (pt, 4H), 3.77 (pt, 4H), 6.33 (d, 1H, J = 8.4 Hz), 6.66 (d, 1H, J = 8.4 Hz), 7.88 (s, 1H), 8.22 (s, 1H), 9.15 (s, 1H), 11.49 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 51.86, 65.35, 106.99, 111.72, 120.21, 132.36, 142.46, 146.55, 147.09; GC-MS 238 *m/z* [M]⁺;
5 Purity: 99.0% (300 nm, t_r = 1.04 min, Method: d)

Example 52: 4-(2-(3,4-Dihydroxybenzylidene)hydrazinyl)benzoic acid

4-Hydrazinylbenzoic acid (500 mg, 3.4 mmol), 3,4-Dihydroxybenzaldehyde (454 mg, 3.4 mmol) Purification: Method 1; Yield: 370 mg, 41%; C₁₄H₁₂N₂O₄,
10 M = 272.26 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.75 (d, 1H, J = 8.1 Hz), 6.89 (dd, 1H, J = 1.6 Hz, J = 7.7 Hz), 7.03 (d, 2H, J = 8.7 Hz), 7.18 (d, 1H, J = 1.6 Hz), 7.78-7.81 (m, 3H), 9.14 (bs, 1H), 10.52 (s, 1H) 12.20 (bs, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 110.67, 112.05, 115.49, 118.94, 119.50, 126.68, 131.09,
15 139.85, 145.50, 146.60, 149.02, 167.23; GC-MS 272 *m/z* [M]⁺; Purity: 100.0% (356 nm, t_r = 1.95 min, Method: c)

Example 53: 5-((4-Methylpiperazin-1-ylimino)methyl)benzene-1,2,4-triol

4-Methylpiperazin-1-amine (200 mg, 1.7 mmol), 2,4,5-

20 Trihydroxybenzaldehyde (268 mg, 1.7 mmol) Purification: Method 1; Yield: 330 mg, 76%; C₁₂H₁₇N₃O₃, M = 251.28 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 2.23 (s, 3H), 3.01 (pt, 4H), 3.43 (bs, 4H), 6.24 (s, 1H), 6.07 (s, 1H), 7.75 (s, 1H), 8.37 (bs, 1H), 9.19 (bs, 1H), 10.87 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 45.25, 50.94, 53.59,
25 103.15, 110.28, 115.28, 137.79, 141.00, 147.08, 150.74; GC-MS 251 *m/z* [M]⁺; Purity: 82.0% (294 nm), 100.0% (332 nm, t_r = 1.05 min, Method: j)

Example 54: 5-((2-(6-Chloropyridazin-3-yl)hydrazono)methyl)benzene-1,2,4-triol

30 3-Chloro-6-hydrazinylpyridazine (200 mg, 1.4 mmol), 2,4,5-

Trihydroxybenzaldehyde (213 mg, 1.4 mmol)

Purification: Method 1; Yield: 361 mg, 93%; C₁₁H₉ClN₄O₃, M = 280.67 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.34 (s, 1H), 7.03 (s, 1H), 7.40 (d, 1H, J

- 51 -

= 9.4 Hz), 7.65 (d, 1H, J = 9.4 Hz), 8.26 (s, 1H), 8.43 (s, 1H), 9.34 (s, 1H), 9.49 (s, 1H), 11.33 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 103.34, 110.84, 112.17, 115.26, 129.68, 138.52, 141.36, 146.52, 148.29, 150.08, 158.26; GC-MS 280 *m/z* [M]⁺; Purity: 100.0% (354 nm, t_r = 1.41 min, Method: k)

Example 55: 5-((2-(Phthalazin-1-yl)hydrazono)methyl)benzene-1,2,4-triol

1-Hydrazinylphthalazine hydrochloride (300 mg, 1.5 mmol), 2,4,5-

Trihydroxybenzaldehyde (235 mg, 1.5 mmol)

Purification: Method 1; Yield: 449 mg, 99%, C₁₅H₁₂N₄O₃, M = 296.28 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 6.54 (s, 1H), 7.71 (s, 1H), 8.06-8.23 (m, 3H), 8.98 (s, 1H), 9.12 (d, 1H, J = 7.8 Hz), 9.12 (s, 1H), 9.73 (bs, 1H), 10.13 (bs, 1H), 13.91 (s, 1H), 14.29 (bs, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm:

102.93, 109.82, 113.32, 118.92, 125.00, 127.53, 128.04, 133.53, 135.52, 138.89, 144.09, 146.69, 150.30, 151.34, 152.68; GC-MS 296 *m/z* [M]⁺; Purity: 100.0% (392 nm, t_r = 1.26 min, Method: l)

Example 56: 5-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)methyl)benzene-1,2,4-triol

2-Hydrazinyl-4,5-dihydro-1H-imidazole hydrobromide (300 mg, 1.7 mmol), 2,4,5-Trihydroxybenzaldehyde (255 mg, 1.7 mmol)

Purification: Method 1; Yield: 379 mg, 97%, C₁₀H₁₂N₄O₃, M = 236.23 g/mol

¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.69 (s, 4H), 6.39 (s, 1H), 7.18 (s, 1H), 8.28 (s, 1H), 8.33 (s, 1H), 8.46 (bs, 1H), 9.31 (s, 1H), 9.71 (s, 1H), 11.93 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 42.58, 102.99, 109.65, 112.42,

138.67, 145.88, 149.90, 151.03, 157.30; ¹³C-NMR (75 MHz, D₄-MeOH) ppm: 44.34, 104.14, 110.90, 115.00, 140.09, 150.31, 151.66, 153.55, 159.24; GC-MS 236 *m/z* [M]⁺; Purity: 76.6% (346 nm, t_r = 0.96 min, Method: p)

Example 57: 4-((2-Benzyl-2-methylhydrazono)methyl)benzene-1,2-diol

1-Benzyl-1-methylhydrazine (100 mg, 0.7 mmol), 3,4-Dihydroxybenzaldehyde (113 mg, 0.7 mmol)

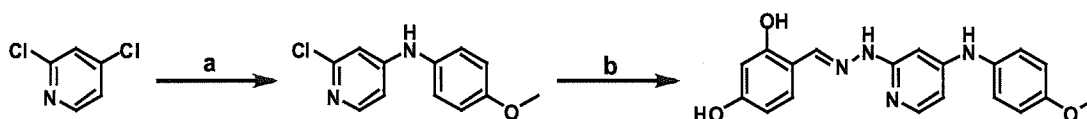
- 52 -

Purification: Method 6; Yield: 30 mg, 16%, $C_{15}H_{16}N_2O_2$, $M = 256,30$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 2.78 (s, 3H), 4.39 (s, 2H), 6.68 (d, 1H, J
 $= 7.98$ Hz), 6.76 (dd, 1H, $J = 1.7$ Hz, $J = 8.1$ Hz), 7.06 (d, 1H, $J = 1.7$ Hz),
 7.14 (s, 1H), 7.23-7.35 (m, 7H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 37.19,
 61.57, 111.67, 115.34, 117.79, 126.96, 128.12, 128.25, 128.54, 132.92,
 137.77, 145.19, 145.28; Purity: 64.6% (294 nm), 66.0% (298 nm) $t_r = 2.51$
 min, Method: I)

Example 58: 4-((2-(5-(4-Methoxyphenylamino)pyridin-2-yl)hydrazono)methyl)benzene-1,3-diol

$C_{19}H_{18}N_4O_3$, $M = 350,37$ g/mol

Synthesis



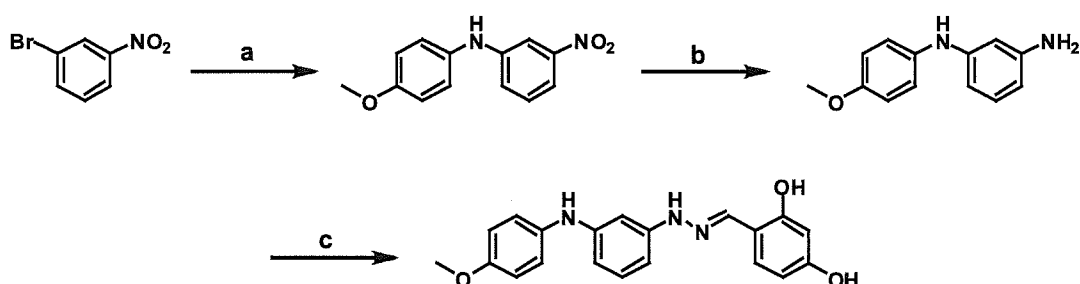
Example 58

a) 2,4-Dichloropyridine (2.0 g, 13.5 mmol) + 1.0 equiv. 4-Methoxyaniline +
 2.0 equiv. p-Toluenesulfonic acid, Dioxane, 64h reflux, aqueous workup,
 chromatography, yield: 53%, purity: 95%;

b) 1. 2-Chloro-N-(4-methoxyphenyl)pyridin-4-amine (0.5 g, 2.1 mmol) +
 193.0 equiv. Hydrazine hydrate (64%), 16h, 110°C, aqueous workup, 2. +
 2.0 equiv. Aldehyde, Ethanol (EtOH), 2h, 25°C, recrystallization, yield: 54%,
 purity: 99%

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.71 (s, 3H), 6.24 (dd, 1H, $J = 1.5$ Hz, J
 $= 5.7$ Hz), 6.30-6.35 (m, 3H), 6.84 (d, 2H, $J = 9.0$ Hz), 6.87 (d, 1H, $J = 9.1$
 Hz), 7.51 (d, 2H, $J = 9.0$ Hz), 7.80 (d, 1H, $J = 5.8$ Hz), 8.09 (s, 1H), 8.55 (s,
 1H), 9.77 (bs, 1H), 10.41 (s, 1H), 10.46 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -
 DMSO) ppm: 55.07, 89.37, 99.78, 102.37, 107.51, 111.69, 113.72, 120.07,
 128.90, 135.32, 140.49, 147.50, 151.03, 153.32, 157.23, 157.56, 159.41;

- 53 -

ppm: ; LC-MS 350 m/z $[M]^+$; Purity: 99.3% (344 nm, t_r = 1.44 min, Method: c)**Example 59: 4-((2-(4-(4-Methoxyphenylamino)phenyl)hydrazono)methyl)benzene-1,3-diol**5 $C_{20}H_{19}N_3O_3$, $M = 349,38$ g/mol,**Synthesis****Example 59**

10 **a)** Nitrobenzene (1.0 g, 4.9 mmol) + 1.2 equiv. 4-Methoxyaniline + 1.4 equiv. NaOtBu + 0.1 equiv. Tris(dibenzylideneacetone)dipalladium(0) + 0.1 equiv. [1,1'-Binaphthalene]-2,2'-diylbis(diphenylphosphine), Toluene, 16 h, 100°C, chromatography, yield: 73%, purity: 95%;

15 **b)** N-(4-Methoxyphenyl)-3-nitroaniline (2.0 g, 8.2 mmol) + EtOH, 2h 25°C, chromatography, yield: 80%, purity: 95%;

c) 1. Aniline (0.8 g, 3.7 mmol) + 1.0 equiv. NaNO₂, 6M HCl, 20 min, in 3.0 equiv. SnCl₂ x 2H₂O, -15°C, 30 min, aqueous workup, 2. + 1.0 equiv. Aldehyde in Dichloromethane (DCM):EtOH (2:1), 16h, 25°C, chromatography, recrystallization, yield: 19%, purity: 99%.

20

¹H-NMR (300 MHz, d₆-DMSO) ppm: 3.72 (s, 3H), 6.26-6.34 (m, 4H), 6.55 (t, 1H, J = 2.0 Hz), 6.87 (d, 2H, J = 8.9 Hz), 6.99 (t, 1H, J = 8.0 Hz), 7.05 (d, 2H, J = 8.9 Hz), 7.21 (d, 1H, J = 8.3 Hz), 7.78 (s, 1H), 7.98 (s, 1H), 9.64 (s, 1H), 9.98 (s, 1H), 10.81 (s, 1H); ¹³C-NMR (75 MHz, d₆-DMSO) ppm: 55.12, 97.80, 102.18, 102.37, 105.85, 107.26, 111.82, 114.34, 120.50, 129.14, 129.65, 136.12, 138.90, 145.70, 146.04, 153.61, 157.43, 158.72; ppm: ; LC-MS 350 m/z $[M+H]^+$; Purity: 97.4 (248 nm); 100.0% (294 nm); 99.2 (350 nm, t_r = 2.01

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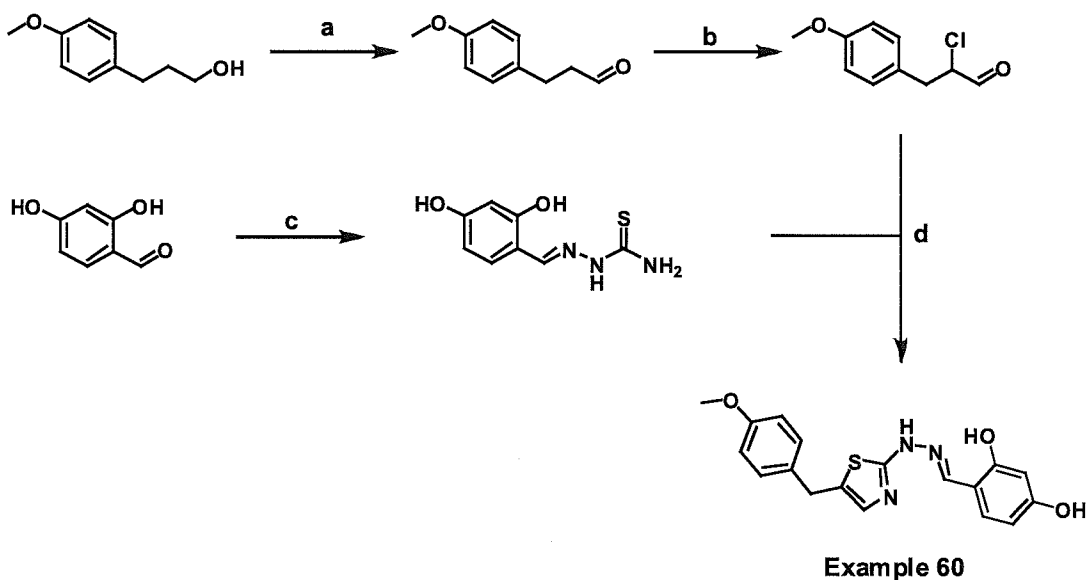
- 54 -

min, Method: c)

Example 60: 4-((2-(4-(4-Methoxybenzyl)thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol

5 $C_{18}H_{17}N_3O_3S$, M = 355,41 g/mol,

Synthesis



- 10 **a)** Propanol (18.5 g, 111.3 mmol) + 1.8 equiv. P_4O_{10} + 2.0 equiv. Dimethyl sulfoxide + 3.5 equiv. Triethylamine, DCM, 1h at 0°C to 25°C, aqueous workup yield: 90%, purity: 95%;
- b)** Aldehyde (2.4 g, 14.6 mmol) + 1.2 equiv. *N*-Chlorosuccinimide + cat. D-Proline at 0°C in DCM, stirring for 3h at 22°C, aqueous workup, chromatography, yield: 87%, purity: 85%;
- 15 **c)** Aldehyde (3.0 g, 21.7 mmol) + 1.0 equiv. Thiosemicarbazide, EtOH, 3h reflux, recrystallization, yield: 72%, purity: 95%;
- d)** Hydrazinecarbothioamide (1.5 g, 7.1 mmol) + 1.0 equiv. 2-chloropropanal, EtOH, 2h reflux, aqueous workup, chromatography, yield: 17%, purity: 100%.
- 20

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.73 (s, 3H), 3.86 (s, 2H), 6.28 (d, 1H, J = 2.2 Hz), 6.30 (dd, 1H, J = 2.3 Hz, J = 8.4 Hz), 6.88 (d, 2H, J = 8.6 Hz), 6.91

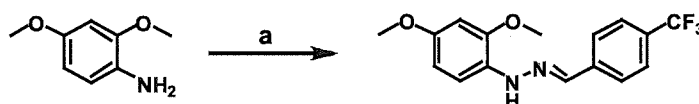
- 55 -

(s, 1H), 7.17 (d, 2H, J = 8.6 Hz), 7.26 (d, 1H, J = 8.4 Hz), 8.15 (s, 1H), 9.76 (s, 1H), 10.50 (bs, 1H), 11.36 (bs, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 31.74, 54.91, 102.31, 107.58, 111.32, 113.80, 129.20, 129.39, 131.81, 157.78, 158.05, 159.70, 167.34; ppm: ; LC-MS 355 m/z $[\text{M}]^+$; Purity: 87.8% (250 nm); 100.0% (376 nm, t_r = 6.22 min, Method: c)

Example 61: 1-(2,4-Dimethoxyphenyl)-2-(4-(trifluoromethyl)benzylidene)hydrazine

$\text{C}_{16}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_2$, M = 324,30 g/mol,

Synthesis



Example 61

a) 1. Aniline (10.0 g, 65.3 mmol) + 1.0 equiv. NaNO_2 , aq. HCl (6M), 20 min, in 3.0 equiv. $\text{SnCl}_2 \times 2\text{H}_2\text{O}$, -15°C , 30 min, aqueous workup, 2. + 0.6 equiv. Aldehyde in DCM:EtOH (2:1), 1h, 25°C , chromatography, recrystallization, yield: 17%, purity: 91%.

^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.72 (s, 3H), 3.84 (s, 3H), 6.51 (dd, 1H, J = 2.6 Hz, J = 8.22 Hz), 6.61 (d, 1H, J = 2.6 Hz), 7.31 (d, 1H, J = 8.7 Hz), 7.69 (d, 2H, J = 8.4 Hz), 7.77 (d, 2H, J = 8.2 Hz), 8.11 (s, 1H), 9.81 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 55.28, 55.60, 99.20, 104.96, 112.58, 124.32 (d, $J_{\text{C-F}}$ = 271.6 Hz), 125.40 (q, $J_{\text{C-F}}$ = 3.8 Hz), 125.56, 127.05 (q, $J_{\text{C-F}}$ = 31.6 Hz), 127.87, 134.64, 140.18, 146.31, 153.49; LC-MS 323 m/z $[\text{M-H}]^+$; Purity: 91.3% (354 nm, t_r = 6.22 min, Method: c)

Example 62: 2-(2-(Pyridin-2-ylmethylene)hydrazinyl)quinoline

Alfa Aesar, Karlsruhe, Germany

- 56 -

Example 63: *N'*-(1-(2,4-Dihydroxyphenyl)ethylidene)-2-hydroxybenzohydrazide

TimTec, Newark, USA

Example 64: 2-Chloro-*N'*-(1-(2,4-dihydroxyphenyl)ethylidene)-4-nitrobenzohydrazide

TimTec, Newark, USA

Example 65: *N'*-(1-(2,4-Dihydroxyphenyl)ethylidene)-3-iodobenzohydrazide

TimTec, Newark, USA

Example 66: 1-(4-Cyanophenyl)-*N'*-(2,4-dihydroxybenzylidene)piperidine-4-carbohydrazide

1-(4-Cyanophenyl)piperidine-4-carbohydrazide (250 mg, 1.0 mmol), 2,4-Dihydroxybenzaldehyde (141 mg, 1.0 mmol)
Purification: Method 1; Yield: 157 mg, 42%, $C_{20}H_{20}N_4O_3$, $M = 364,40$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 1.61-1.71 (m, 2H), 1.82 (pd, 2H), 2.80-3.04 (m, 3H), 3.98 (pd, 2H), 6.28-6.35 (m, 2H), 7.03 (d, 2H, $J = 9.0$ Hz), 7.26 (s, 1H, $J = 8.4$ Hz), 7.56 (d, 2H, $J = 8.7$ Hz), 8.25 (s, 1H), 9.91 (s, 0.8H), 11.02 (s, 0.2H), 11.33 (s, 1H), 11.47 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 18.45, 27.22, 46.14, 97.54, 102.51, 107.50, 110.33, 114.01, 120.00, 131.11, 133.28, 147.77, 152.73, 159.21, 160.45, 169.72; GC-MS 364 m/z $[M]^+$, 184; Purity: 98.6% (298 nm, $t_r = 1.17$ min, Method: c)

25

Example 67: *N'*-(2,4-Dihydroxybenzylidene)-2-phenylacetohydrazide

2-Phenylacetohydrazide (200 mg, 1.3 mmol), 2,4-Dihydroxybenzaldehyde (183 mg, 1.3 mmol)

Purification: Method 1; Yield: 179 mg, 49%, $C_{15}H_{14}N_2O_3$, $M = 270,28$ g/mol
 ^1H -NMR (300 MHz, d_6 -DMSO) ppm: 3.53(s, 2H), 6.29-6.36 (m, 3H), 7.23-7.32 (m, 5H), 8.28 (s, 1H), 9.92 (bs, 0.8H), 11.15 (0.2H) 11.26 (s, 1H), 11.63 (s, 1H); ^{13}C -NMR (75 MHz, d_6 -DMSO) ppm: 40.84, 102.50, 107.53, 110.31, 126.51, 128.22, 128.96, 131.08, 135.48, 147.88, 159.21, 160.52, 165.86;

30

- 57 -

GC-MS 270 m/z $[M]^+$; Purity: 94.6.0% (240 nm); 99.5% (294 nm), 100.0 (322 nm) t_r = 1.10 min, Method: c)

Example 4. Modulation of htt protein processing by compounds of formula I

4.1 Cultivation of PC12-Htt-Ex1-Q25 and PC12-Htt-Ex1-Q103

Inducible PC12 cells were kept in high glucose D-MEM supplemented with 5% horse serum, 2.5% FCS, 1% glutamine, 100 $\mu\text{g/ml}$ penicillin, and 100 $\mu\text{g/ml}$ streptomycin at 37°C and 5% CO_2 . 100 $\mu\text{g/ml}$ zeocine and 50 $\mu\text{g/ml}$ G418 (geneticine) were added to the culture medium, for selection. Cells were grown in 75 cm^2 culture flasks coated with collagen up to 90% confluence and split once a week either 1:4 for PC12-Q25 or 1:6 for PC12-Q103.

4.2 Test of chemical compounds in inducible PC12 cells

Method 1

To test the effect of chemical compounds on htt-processing, PC12-Q103 cells were plated in 96-well tissue culture plates coated with collagen at approximately 2300 cells/well in a total volume of 80 μl . For counting, a Neubauer haemocytometer using a 1:1 dilution of cell suspension and trypan blue was used.

After 24 hours, 10 μl of culture medium containing either 2.5 μM (0.25 μl of a 1 mM solution/well) ponasterone A (dissolved in 100% EtOH) for induction of htt-expression or 0.25 μl 100% ethanol as a negative control were added. One hour later, the substances diluted in DMSO were added in six different concentrations in a total volume of 10 μl culture medium. Control samples were analysed in triplicates, the wells containing the respective substance in four replicates.

- 58 -

Following 72 hours incubation at 37°C, cell morphology and confluency was evaluated and htt-expression and formation of htt-aggregates in PC12-Q103 were examined via GFP-fluorescence using an Olympus IX70 microscope. Afterwards, the medium was removed and 65 µl of lysis buffer (20 mM Hepes pH 7.3, 150 mM NaCl, 2 mM EDTA, 1% Tween-20 + 1x complete protease inhibitor cocktail [Roche], 10 mM NaF, 0.25 units/µl benzonase) was added to each well. Cell lysis was performed for 30 minutes on ice.

To estimate the effect of the compounds on the processing in PC12-Q103 cells, htt levels were measured. For the detection of htt-aggregates in the lysate, a cellulose acetate membrane and two thin Whatman filter papers were equilibrated in lysis buffer and mounted in a vacuum filtration unit. The membrane was washed once with 100 µl of lysis buffer per well of the filter unit. Then, 100 µl of lysis buffer was pipetted in each well and the total cell lysate was added. The mixture was filtered through the membrane. Finally, the membrane was washed once with 100 µl lysis buffer, taken out of the filter unit and the detection of the GFP signal was performed immediately under blue light with the LAS-3000 photo imager and quantified by using the AIDA software.

The results are shown in Figures 5 and 6. As to the compounds according to the invention not listed in these figures, promising results have been obtained in preliminary tests.

Method 2

The Htt-Q25 and -Q103 protein levels were evaluated in total lysate and in the supernatant and pellet of stably transfected PC12 cells. PC12-Htt-Ex1-Q25 and PC12-Htt-Ex1-Q103 cells were cultured on collagen coated 24-well plates. 24 hours after plating, expression was induced by adding ponasterone A (2.5 µM). Then drugs (5 µM) or DMSO as a control were added. After 48 h, Htt-expression levels were examined. For Htt-expression analysis, cells were washed once with D-PBS and lysed with lysis buffer (20

- 59 -

mM Hepes pH 7.3, 2 mM EDTA, 150 mM NaCl, 1% Tween-20 + 1x complete protease inhibitor cocktail [Roche], 10 mM NaF, 0.25 units/ μ l benzonase) for 30 minutes on ice. An aliquot of total cell lysate was ultra centrifuged (30 min, 55.000 rpm, [TLA-55 rotor] 4°C) to obtain soluble and pellet fractions of each sample. The pellet was then resuspended in lysis buffer. 5-fold SDS sample buffer containing DTT was added to total lysate, supernatant and pellet fractions and heated to 95°C for 5 minutes. SDS-PAGE was performed in 12.5% polyacrylamide resolving gels and 4% polyacrylamide stacking gels. Gels were transferred onto nitrocellulose membranes, which were then blocked with skim milk (3%) in TBS-Tween-20. Membranes were incubated over night at 4°C with anti-GAPDH^{rab} antibody (1:1000 in 3% skim milk in TBS-Tween-20), washed three times and subsequently incubated with anti-HD721^{rab}-antibody (1:2000 in 3% skim milk) for 1 h at room temperature. This was followed by incubation with a secondary antibody (anti-rabbit 1:5000 in 3% skim milk in TBS-Tween-20) conjugated to alkaline phosphatase (AP). Membranes were washed and treated for 3 minutes in a 2000-fold dilution of AttoPhosTM reagent in AttoPhosTM buffer. Signals were recorded under blue light in the LAS-3000 photo imager. Evaluation was carried out with AIDA software. The GAPDH concentration was used for normalizing protein levels.

The results are shown in Figure 7.

Method 3

GST-Htt-Q53-protein stock solution was ultra-centrifugated (40 min, 55.000 rpm [TLA-55 rotor], 4°C); the supernatant was diluted with H₂O to a final concentration of 2 μ M. DMSO as a control, or drugs at various concentrations were added to the supernatant of GST-Htt-Q53 protein which was preincubated with PreScission Protease (GE Healthcare, #27-0843-01) in Tris-buffer (A:B=1:1; A:50 mM Tris, 150 mM NaCl, pH = 6.8; B:1 mM EDTA, pH = 8.0 + 10 mM DTT) and incubated at 30°C (300 rpm). Samples were taken at 0, 4, 5 and 6 h. The samples were diluted with SDS-buffer (4%

- 60 -

SDS, 100 mM DTT) and heated at 98°C for 5 min. Samples were filtered through a cellulose-acetate membrane which was equilibrated with SDS solution (0.1%). The membrane was washed twice (SDS, 0.1%), blocked with skim milk (3% in PBS) for 30 min and developed with CAG antibody
5 over night at 4°C and with peroxidase labeled anti rabbit- antibody for 1 h at room temperature. The membrane was washed (2x TBS-0.05% Tween-20, 2x TBS) and luminescence was recorded with Chem Glow.

The results are shown in Figure 10.

10

Example 5. Viability assay

Method 1

15 Cell viability was assayed on HepG2 cells. HepG2 cells were cultured in 96-well black culture plates with transparent bottom in RPMI medium (1% L-Glu, 10% FCS). 3 hours after plating, different drug concentrations (0.1 µM – 100 µM) or DMSO as control were added. Chlorpromazine hydrochloride (Sigma, # C0982) and cycloheximide (Sigma, # C1988) were used as control
20 substances. Each concentration was run in triplicates. After 48 hours of incubation, Alamar Blue reagent (BIOSOURCE, # DAL1100) was added and plates were incubated for 4 hours at 37°C. Absorbance was measured in the Tecan-plate reader (Tecan, infinite M200) at 570 nm and 600 nm. Product example 1 was insoluble from a concentration of 50 µM, product example 33
25 from 30 µM and product example 23 at 100 µM.

The results are shown in Figures 8 and 9.

Method 2

30

The tubulin polymerization assay (Cytoskeleton, Inc., Denver, CO, USA, # BK011) was performed in accordance to the instruction manual.

- 61 -

The results are shown in Figure 11.

- 62 -

Claims

1. A compound of formula I



Formula I,

or a physiologically acceptable salt, hydrate, solvate, tautomer, stereoisomer
metabolite, or prodrug thereof,

wherein

at least one of R_1 and R_2 is selected from (i) H,

(ii) CN, or

(iii) an acyclic radical selected from C_{1-16} alkyl, C_{2-16} alkenyl, C_{2-16} alkynyl, wherein each acyclic radical is optionally mono- or poly-substituted with halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN,

(iv) a C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each cyclic radical is optionally mono- or poly-substituted with R₅, halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN, or

- 63 -

- (v) N, R₁ and R₂ form together a heterocyclic radical selected from mono- or bicyclic, saturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each heterocyclic radical is optionally mono- or poly-substituted with R₅, halo, -CN, -NO₂, =NR₅, -N(R₅)₂, -N(R₅)₃⁺, -NR₅COR₅, -S(O)_nR₅, S(O)_nN(R₅)₂, with n=0-3, -OR₅, OCOR₅, -CON(R₅)₂, -COOR₅, -COR₅, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₅, with n=0-8, OR₅, CN,
- (vi) -S(O)_nR₅, -S(O)_nN(R₅)₂, with n=0-3, -OR₅, -OCOR₅, -COR₅, -CON(R₅)₂, -COOR₅, -COC_nH_{2n}COR₅ with n=0-4, -OCOC_nH_{2n}COOR₅ with n=0-4, -(CH₂O)_nR₅ with n=0-7, -(CO)_nR₅ with n=1-3, -CO-NHN=R₅, -CO-NHNHR₅, -CO-NHNHR₅, -CONHCOR₅, -CH=NNHR₅, -CH=NN=R₅, -C_nH_{2n}R₅ with n=0-8,
- at least one of R₃ and R₄ is a C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each cyclic radical is 2- or 3 times substituted with -OH, -OR₆, and/or OCOR₆, and one of R₃ and R₄ may be H or CH₃,
- R₅ is selected from
- (i) H,
- (ii) CN,
- (iii) an acyclic radical selected from C₁₋₁₆ alkyl, C₂₋₁₆ alkenyl, C₂₋₁₆ alkynyl, wherein each acyclic radical is optionally mono- or poly-substituted with halo, -CN, -NO₂, =NR₇, -N(R₇)₂, -N(R₇)₃⁺, -NR₅COR₇, -S(O)_nR₇, S(O)_nN(R₇)₂, with n=0-3, -OR₇, OCOR₇, -CON(R₇)₂, -COOR₇, -COR₇, and/or a C₃₋₁₄ carbocyclic or heterocyclic moiety which may be substituted with halo, C_nH_{2n}R₇, with n=0-8, OR₇, CN, or
- (iv) a C₃₋₁₄ carbocyclic or heterocyclic radical selected from mono- or bicyclic

- 64 -

saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e.g. N, S, or O, wherein each cyclic radical is optionally mono- or poly-substituted with R_7 , halo, $-CN$, $-NO_2$, $=NR_7$, $-N(R_7)_2$, $-N(R_7)_3^+$, $-NR_7COR_7$, $-S(O)_nR_7$, $S(O)_nN(R_7)_2$, with $n=0-3$, $-OR_7$, $OCOR_7$, $-CON(R_7)_2$,
 5 $-COOR_7$, $-COR_7$, and/or a C_{3-14} carbocyclic or heterocyclic moiety which may be substituted with halo, $C_nH_{2n}R_7$, with $n=0-8$, OR_7 , CN ,

R_6 is selected from C_{1-4} alkyl (optionally halogenated),

10 R_7 is selected from an acyclic radical selected from C_{1-16} alkyl, C_{2-16} alkenyl, C_{2-16} alkynyl, C_{3-14} carbocyclic or heterocyclic radical selected from mono- or bicyclic saturated, unsaturated and/or aromatic radicals which may contain up to 5 heteroatoms, e. g. N, S, or O, wherein each acyclic and or cyclic radical is optionally mono- or poly-substituted with H, halo, $-NO_2$, $-NH_2$, $-CN$,
 15 $-OH$, $-COOH$, $-COH$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-COCH_3$, $-COC_2H_5$, $-OCOCH_3$, $-OCOC_2H_5$, $C_{1-3}F_{1-3}$ (haloalkyl), C_{1-6} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, $-C_6H_5SO_3^-$, $-SO_3^-$, $-C_5H_5NCH_3^-$, $-C_5H_5N^+O^-$, $-N(CH_3)_2^+$ and/or $-N(C_2H_5)_2^+$.

2. The compound according to claim 1, wherein one of R_1 and R_2 is a
 20 carbocyclic radical, particularly aryl, e.g. phenyl, or heteroaryl, e.g. benzothiazolyl, unsubstituted or mono- or poly-substituted with halo, e.g. Br, I, F and/or Cl, CN , C_{1-3} (halo)alkyl, e.g. CF_3 and/or NO_2 , and more particularly phenyl or benzothiazolyl unsubstituted or substituted with (i) $-CF_3$, (ii) $-CF_3$ and at least one $-Cl$, (iii) at least one of $-F$, $-Br$, $-Cl$, and/or $-I$, (iv) $-CN$ and at
 25 least one $-F$ or $-Cl$.

3. The compound according to claim 2, wherein the other one of R_1 and R_2 is H, C_{1-3} alkyl, e.g. methyl or aryl, e.g. phenyl.

30 4. The compound according to claim 1 or 2, wherein at least one of R_3 and R_4 is selected from aryl, e.g. phenyl, 2- or 3 times substituted with OH , $-OR_6$ and/or $-OCOR_6$.

- 65 -

5. The compound according to claim 4, wherein at least one of R₃ and R₄ is selected from aryl, e.g. phenyl, 2 or 3 time substituted with OH.

6. The compound according to claim 1, which is selected from

- 5 4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
1-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-(2,4-dimethoxybenzylidene)hydrazine,
4-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-diol,
10 4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,2-diol,
5-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,
4-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,3-diol,
15 3-((2-(4-Fluorophenyl)hydrazono)methyl)benzene-1,2-diol,
4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,3-diol,
5-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2,4-triol,
4-((2-(2,4-Difluorophenyl)hydrazono)methyl)benzene-1,2-diol,
4-((2-Phenylhydrazono)methyl)benzene-1,3-diol,
20 4-((2-(2,5-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-5-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-phenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2,4-Dichlorophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((2-(2-Chloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3-diol.
25 4-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,3-diol,
5-((2-(4-Iodophenyl)hydrazono)methyl)benzene-1,2,4-triol,
5-((2,2-Diphenylhydrazono)methyl)benzene-1,2,4-triol,
4-((2,2-Diphenylhydrazono)methyl)benzene-1,3-diol,
4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,3-diol,
30 4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,2-diol,
4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2-diol,
4-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,3-diol,
5-((2-Methyl-2-phenylhydrazono)methyl)benzene-1,2,4-triol,

- 3-Chloro-4-(2-(2,4,5-trihydroxybenzylidene)hydrazinyl)benzonitrile,
3-Chloro-4-(2-(2,4-dihydroxybenzylidene)hydrazinyl)benzonitrile,
3-Chloro-4-(2-(3,4-dihydroxybenzylidene)hydrazinyl)benzonitrile,
4-((2-(4-Nitrophenyl)hydra-zono)methyl)benzene-1,2-diol,
5 5-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,2,4-triol,
4-((2-(4-Nitrophenyl)hydrazono)methyl)benzene-1,3-diol,
4-((Piperidin-1-ylimino)methyl)benzene-1,3-diol,
4-((2-(Benzo[d]-thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol,
3-(2-(2,4-Dihydroxybenzyl-idene)hydrazinyl)propanenitrile.
10 2-((2-(4-(Trifluoromethyl)phenyl)hydrazono)methyl)benzene-1,3,5-triol
2-((2-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,3,5-triol
2-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3,5-triol
4-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
15 1,3-diol
4-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,2,3-triol
2-((2-Methyl-2-(2-nitro-4-(trifluoromethyl)phenyl)hydrazono)methyl)benzene-
1,3,5-triol
20 4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,3-diol
5-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((2-(Quinolin-2-yl)hydrazono)methyl)benzene-1,2,3-triol
5-((3-Mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)methyl)benzene-
1,2,4-triol
25 5-((2-(5-Chloro-3-iodopyridin-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((Morpholinoimino)methyl)benzene-1,2,3-triol
4-(2-(3,4-Dihydroxybenzylidene)hydrazinyl)benzoic acid
5-((4-Methylpiperazin-1-ylimino)methyl)benzene-1,2,4-triol
5-((2-(6-Chloropyridazin-3-yl)hydrazono)methyl)benzene-1,2,4-triol
30 5-((2-(Phthalazin-1-yl)hydrazono)methyl)benzene-1,2,4-triol
5-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)methyl)benzene-1,2,4-triol
4-((2-Benzyl-2-methylhydrazono)methyl)benzene-1,2-diol
4-((2-(5-(4-Methoxyphenylamino)pyridin-2-yl)hydrazono)methyl)benzene-

- 67 -

1,3-diol

4-((2-(4-(4-Methoxyphenylamino)phenyl)hydrazono)methyl)benzene-1,3-diol

4-((2-(4-(4-Methoxybenzyl)thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol

N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-2-hydroxybenzohydrazide5 2-Chloro-*N'*-(1-(2,4-dihydroxyphenyl)ethylidene)-4-nitrobenzohydrazide*N'*-(1-(2,4-Dihydroxyphenyl)ethylidene)-3-iodobenzohydrazide1-(4-Cyanophenyl)-*N'*-(2,4-dihydroxybenzylidene)piperidine-4-carbohydrazide*N'*-(2,4-Dihydroxybenzylidene)-2-phenylacetohydrazide.

10

7. The compound according to any one of claims 1-6, wherein at least one hydrogen of a hydrocarbon group of at least one aromatic radical is replaced by Deuterium and/or wherein at least one hydrogen of a nitrogen-hydrogen binding is replaced by Deuterium.

15

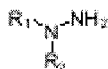
8. The compound according to any one of claims 1-7, wherein said compound is present as a metal salt, preferably as a Fe, Mo, Ni, Cu, Zn, V or Co salt.

20

9. The compound according to any one of claims 1-8 for use as a medicament, e.g. in human or veterinary medicine.

10. A method for producing a compound according to any one of claims 1-8 comprising reacting a compound of formula II

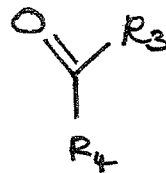
25



(II),

with a compound of formula III

- 68 -



(III),

to obtain a compound of formula I,

5 wherein the compound of formula I and R₁-R₄ are as defined in any one of claims 1-8.

11. The compound according to any one of claims 1-8 for use as an inhibitor of protein misfolding and/or the accumulation of misfolded protein,
10 and/or as an activator of degradation of a misfolded protein and aggregates thereof.

12. The compound according to claim 11, wherein the protein has a polyglutamine stretch within its primary structure, wherein the polyglutamine
15 stretch is more than 15, preferably more than 27 glutamine residues in length.

13. The compound or composition of claim 12, wherein the protein is mutated htt.
20

14. The compound according to any one of claims 1-8 for treating and/or preventing a disease or condition associated with and/or caused by protein misfolding or formation of protein aggregates.

25 15. The compound according to any one of claims 1-8 for treating and/or preventing Huntington's disease (HD), spinobulbar muscular atrophy (Kennedy disease), dentatorubral-pallidoluysian atrophy (Haw River syndrome), spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3 (Machado-Joseph disease), spinocerebellar

- 69 -

ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Alzheimer's disease, Parkinson's disease, primary systemic amyloidosis, secondary systemic amyloidosis, senile systemic amyloidosis, familial amyloid polyneuropathy 1, hereditary cerebral amyloid angiopathy, hemodialysis-related amyloidosis, familial amyloid polyneuropathy III, Finnish hereditary systemic amyloidosis, type 2 diabetes, medullary carcinoma of thyroid, atrial amyloidosis, hereditary non-neuropathic systemic amyloidosis, injection-localized amyloidosis, hereditary renal amyloidosis, amyotrophic lateral sclerosis, schizophrenia, sickle cell anaemia, unstable haemoglobin inclusion body haemolysis, α_1 -antitrypsin deficiency, antithrombin deficiency, thromboembolic disease, transmissible spongiform encephalopathies (prion diseases): bovine spongiform encephalopathy, kuru, Gerstmann-Straeussler-Scheinker syndrome, fatal familial insomnia, scrapie, Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease.

15

16. A kit for modulating protein misfolding comprising a compound of formula I as defined in any one of claims 1-8, and an agent for detecting protein misfolding.

Figure 1

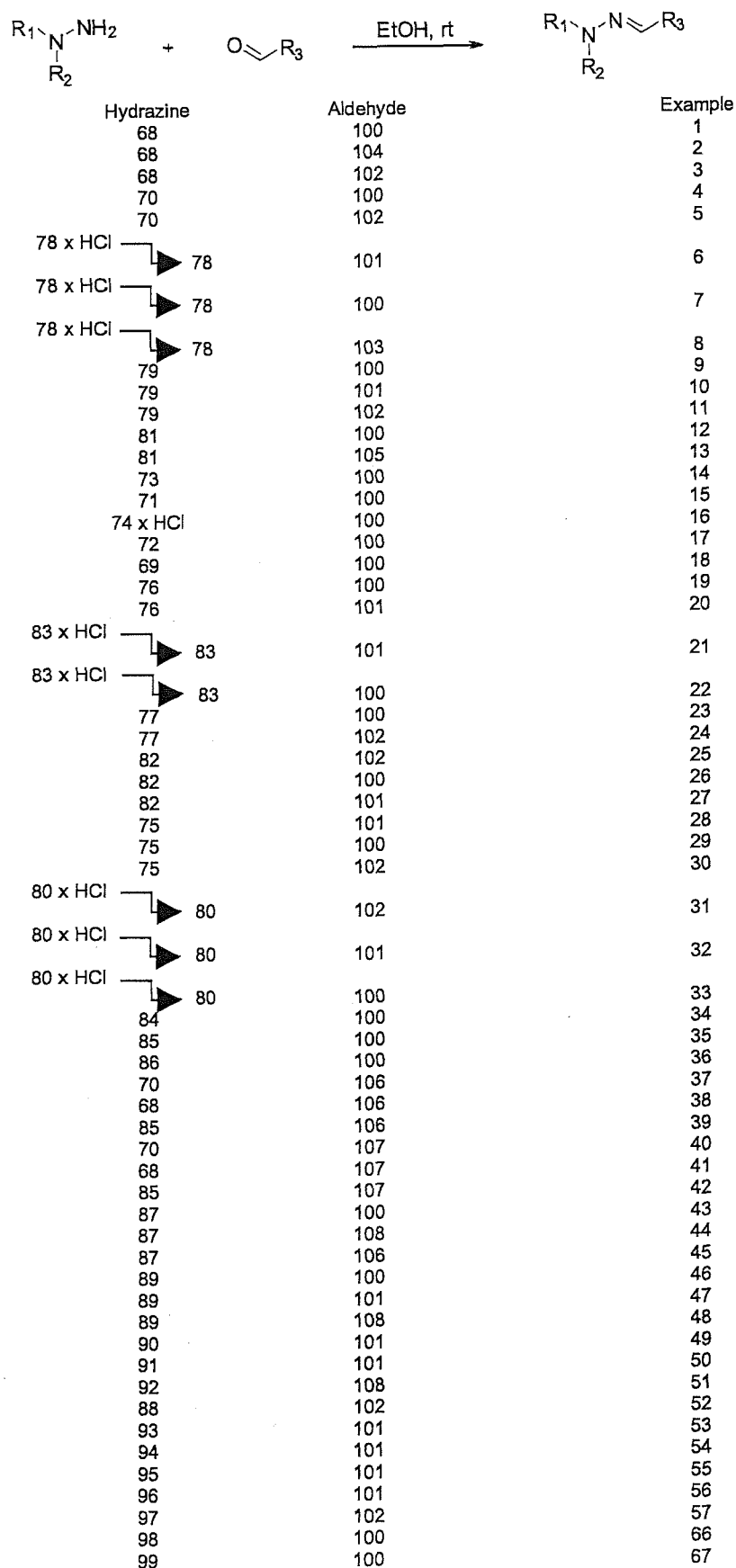


Figure 2

Hydrazine: $\begin{array}{c} R_1 \\ \\ R_2-NH_2 \end{array}$			Aldehyde: $O=CH-R_3$	
Entry	R_1	R_2	Entry	R_3
68			100	
69			101	
70			102	
71			103	
72			104	
73			105	
74			106	
75			107	
76			108	
77				
78				
79				
80				
81				
82				
83				
84		$=R_1$		
85				

Figure 2 (continued)

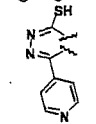
Hydrazine: $\begin{array}{c} R_1 \\ \diagdown \\ N \\ \diagup \\ R_2 \end{array} - NH_2$			Aldehyde: $O=CH-R_3$	
Entry	R_1	R_2	Entry	R_3
86				
87				
88				
89				
90				
91				
92		$= R_1$		
93		$= R_1$		
94				
95				
96				
97				
98				
99				

Figure 3

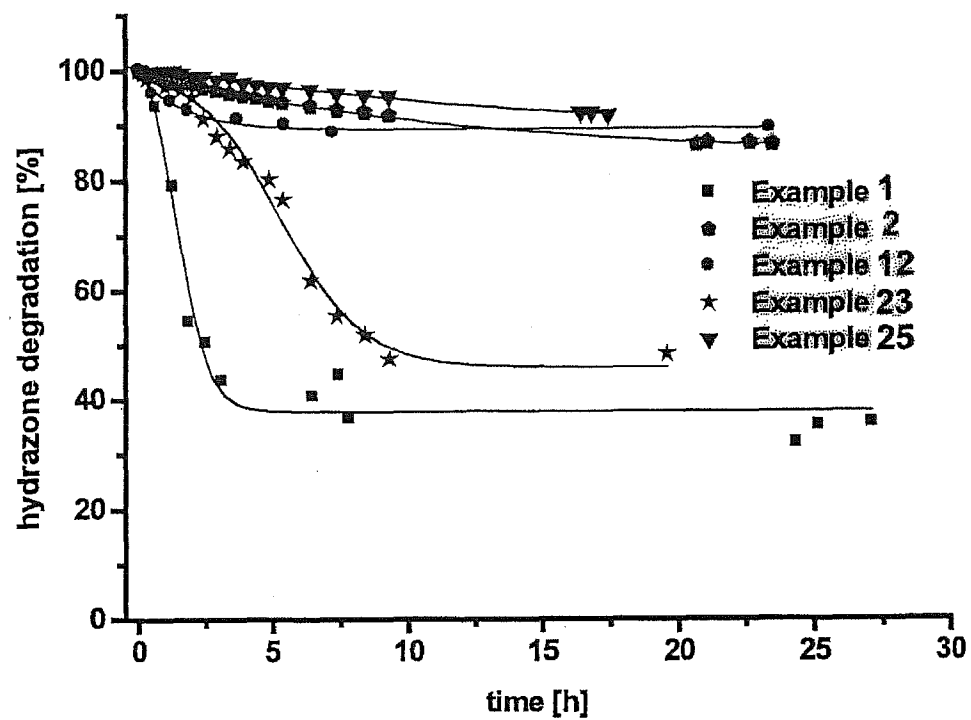


Figure 4

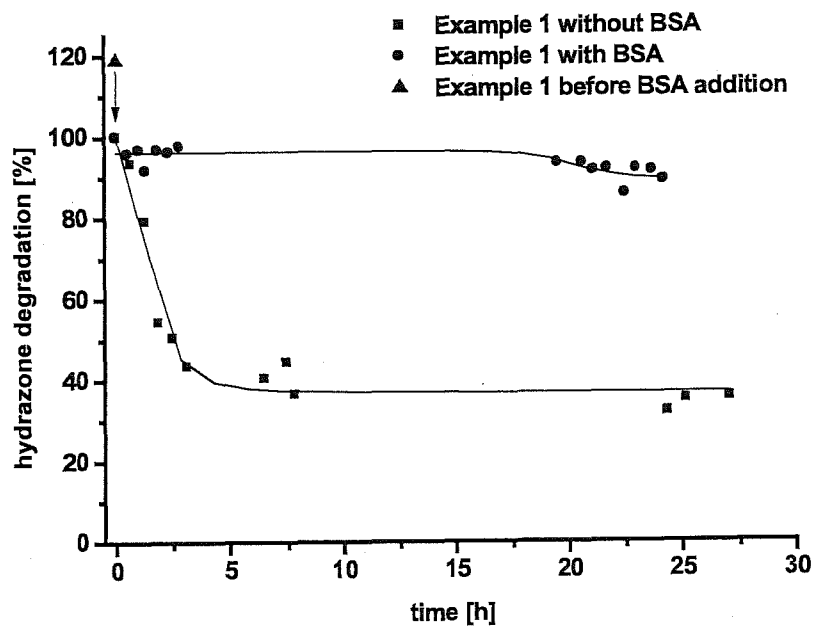


Figure 5

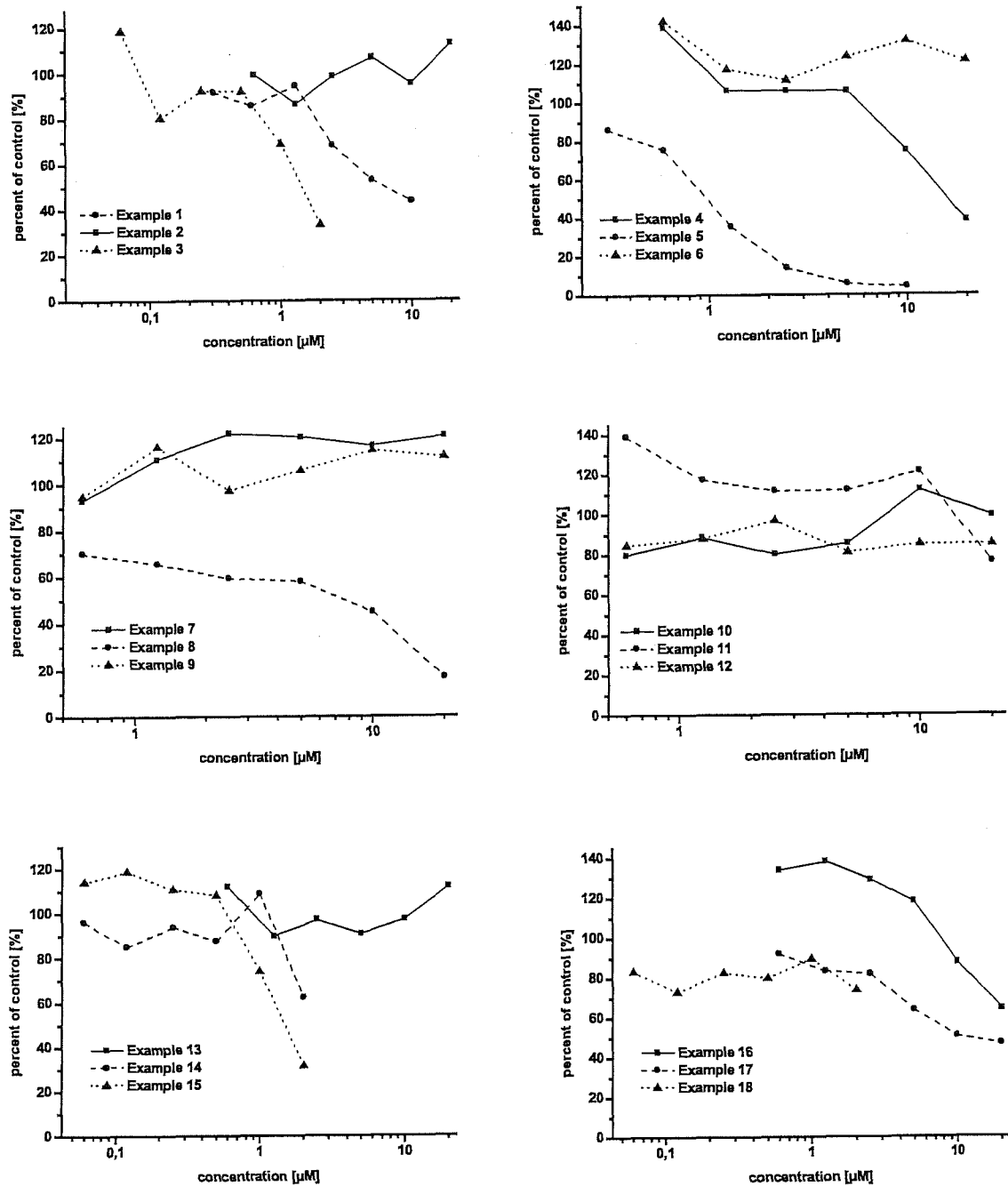


Figure 6

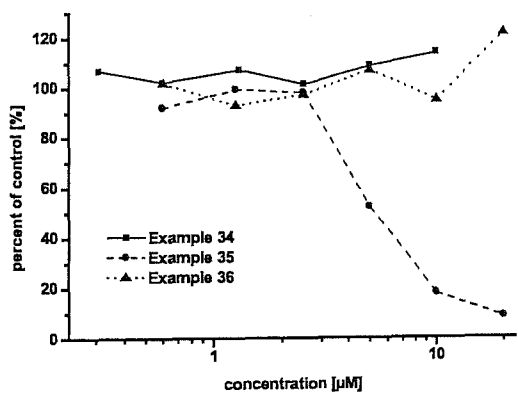
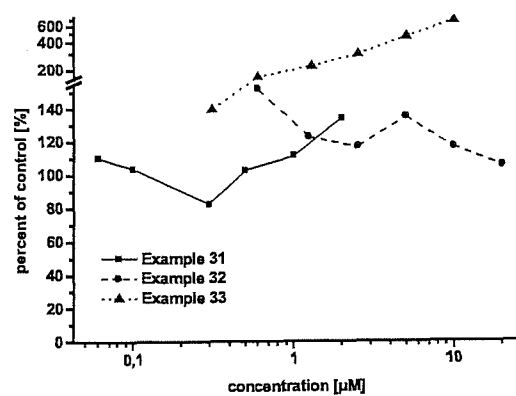
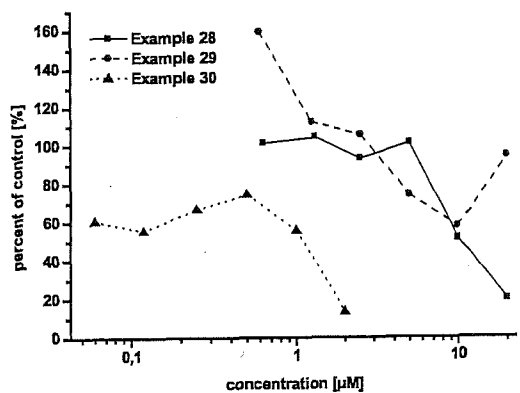
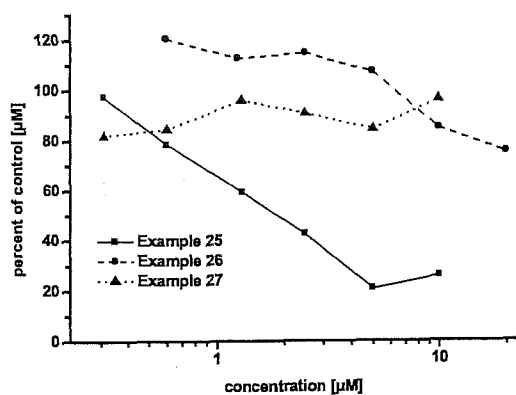
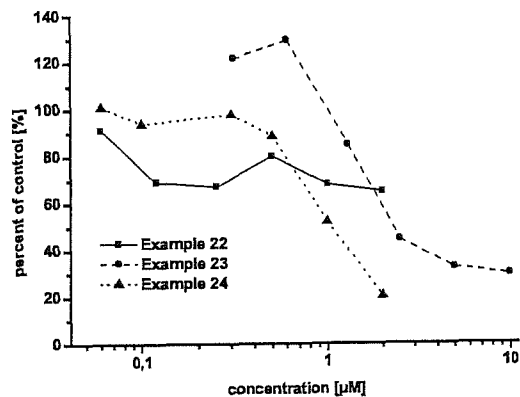
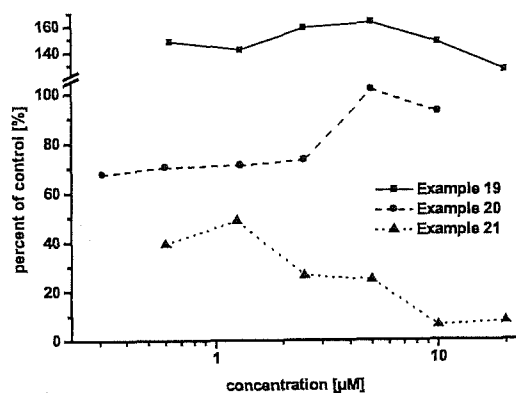


Figure 6 (continued)

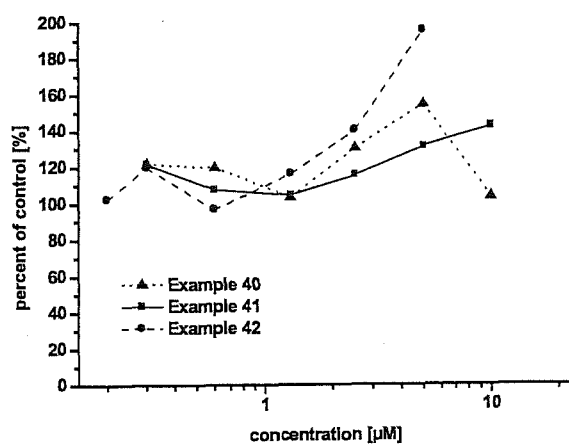
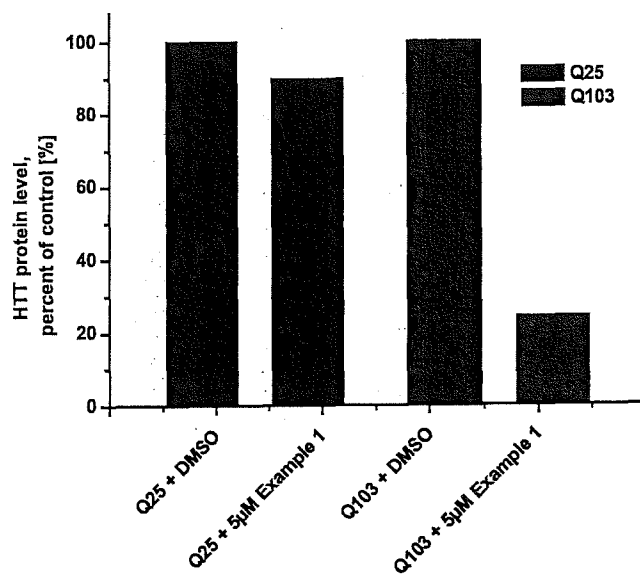


Figure 7

A)



B)

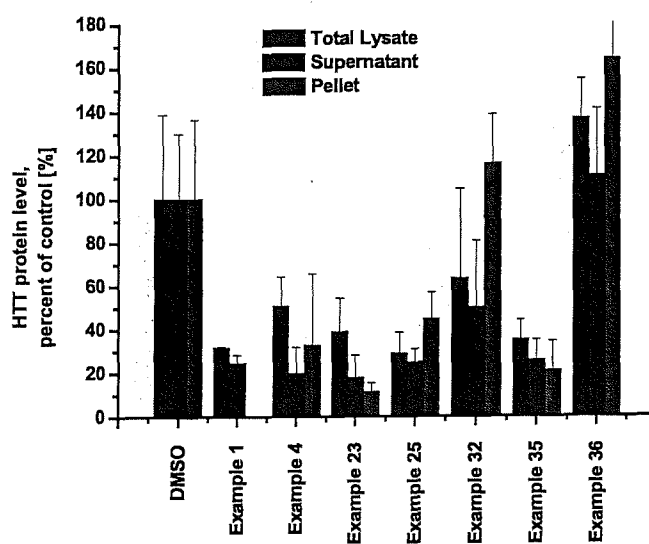


Figure 8

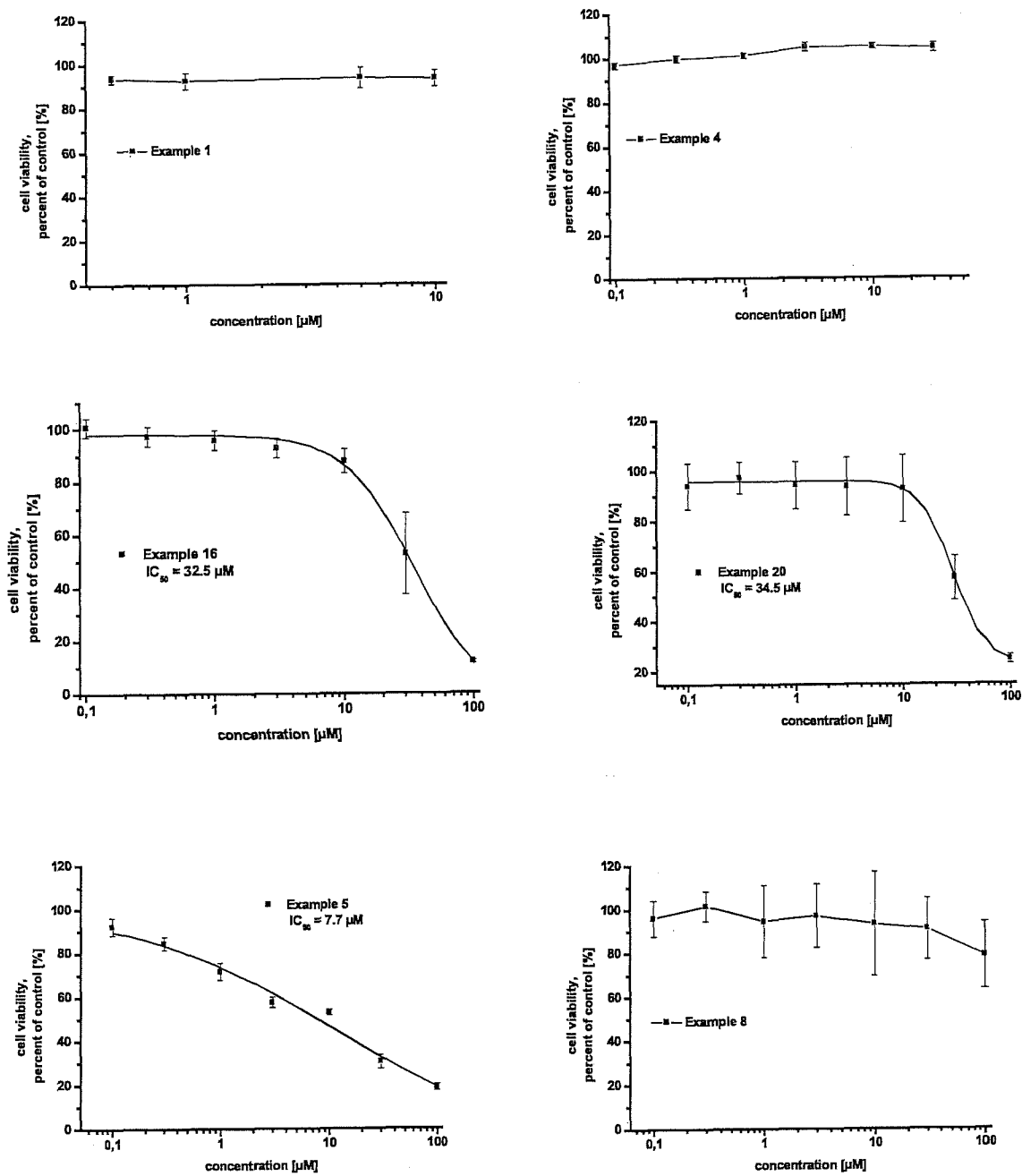


Figure 9

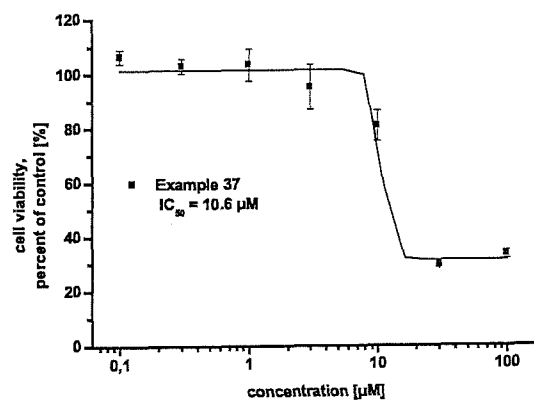
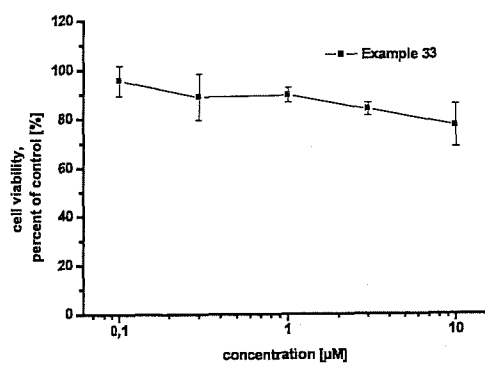
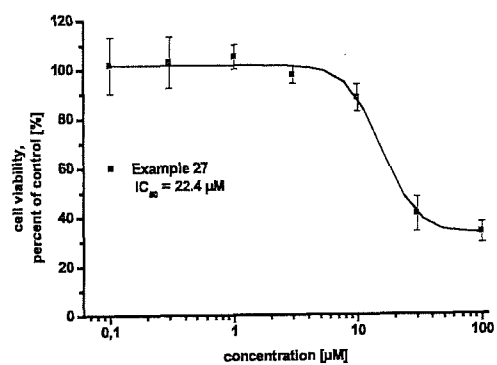
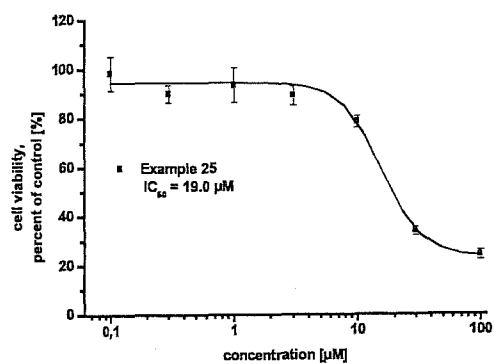
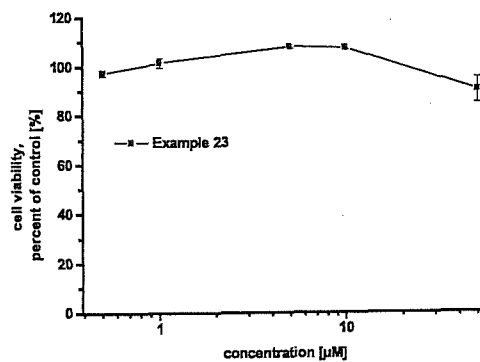
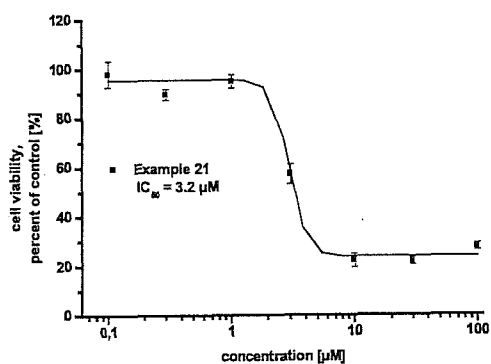


Figure 9 (continued)

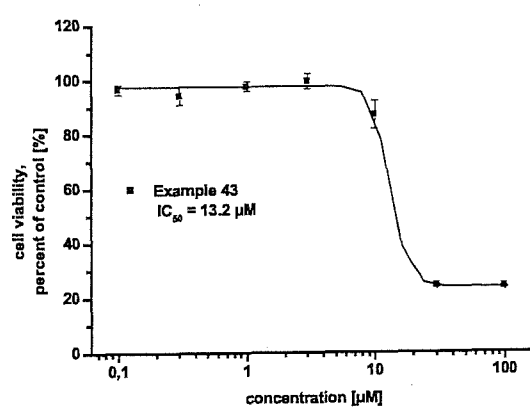
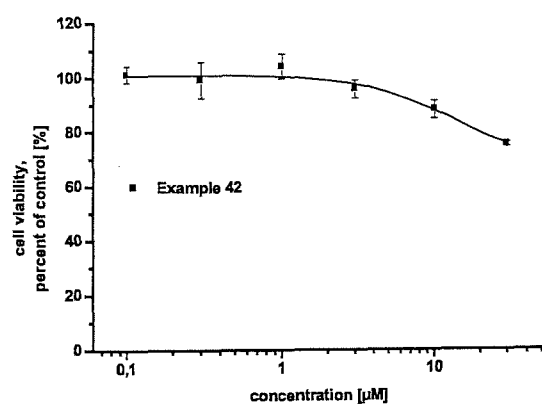
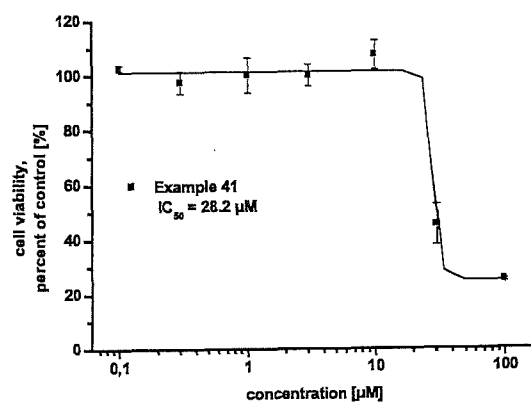
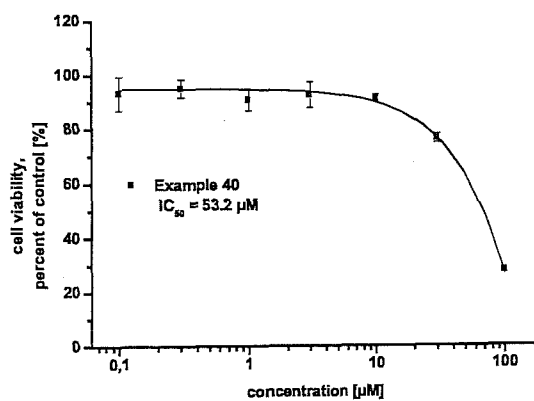
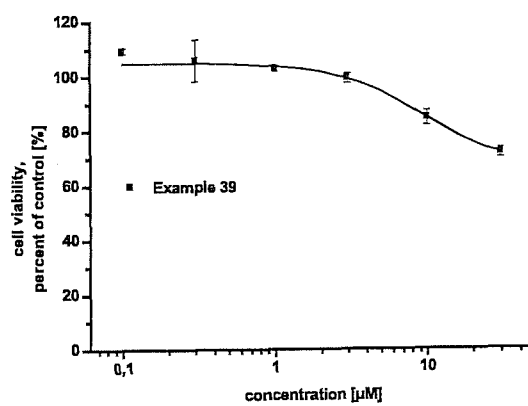
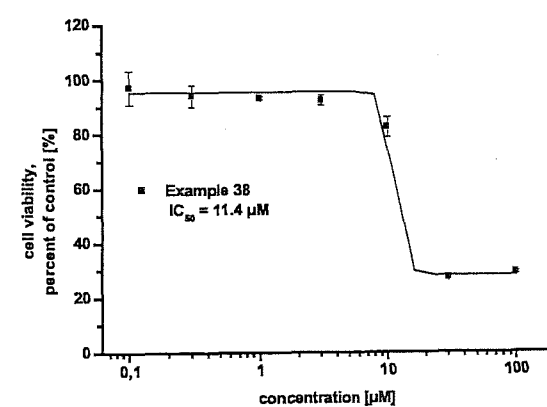


Figure 9 (continued)

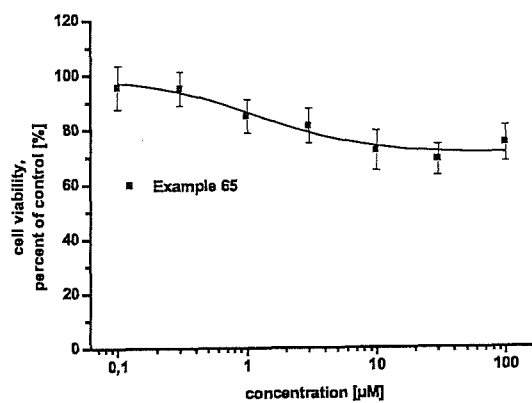
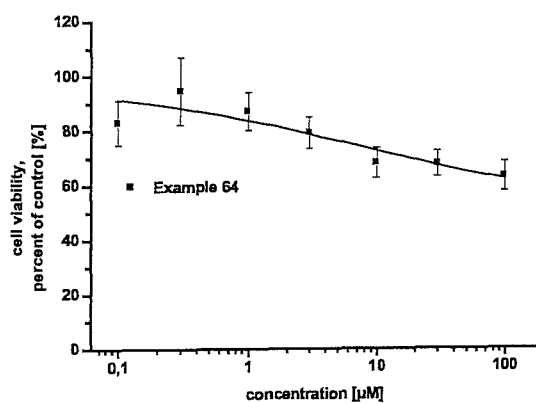
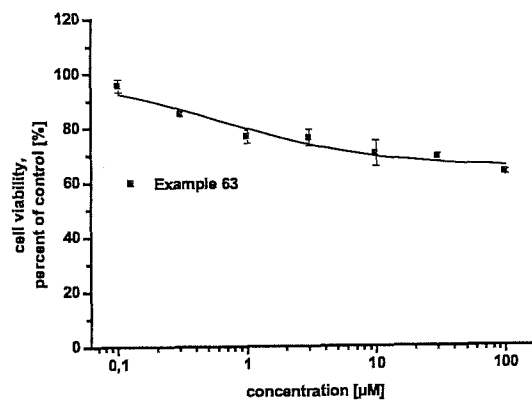
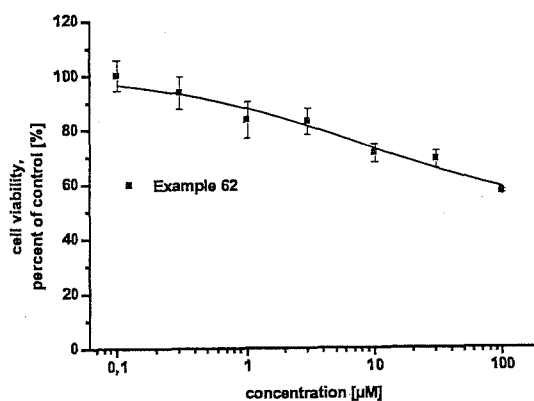
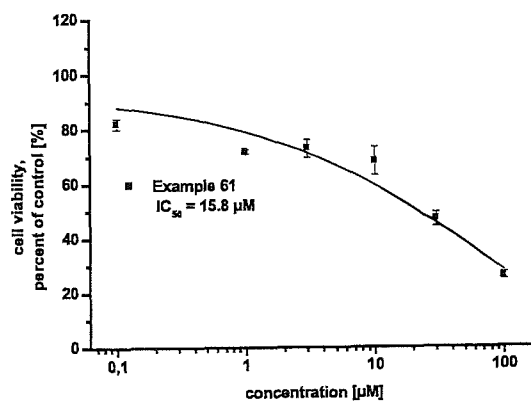
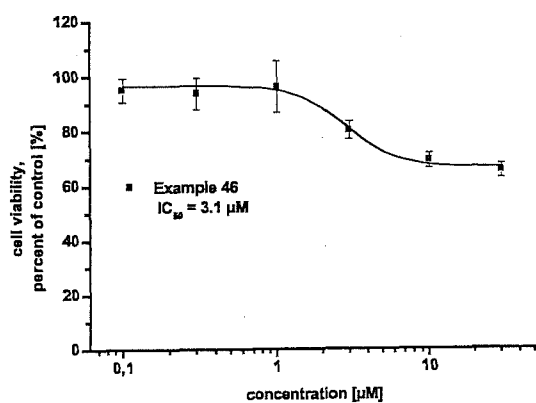


Figure 9 (continued)

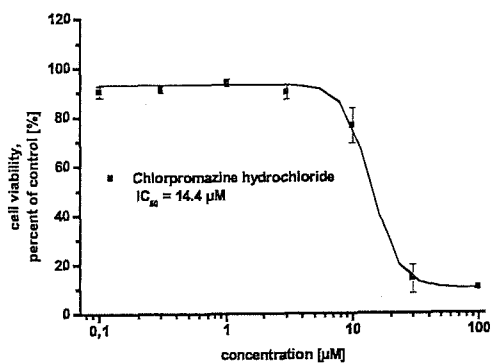
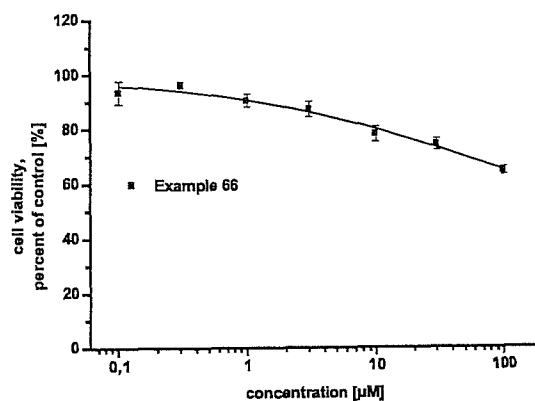


Figure 10

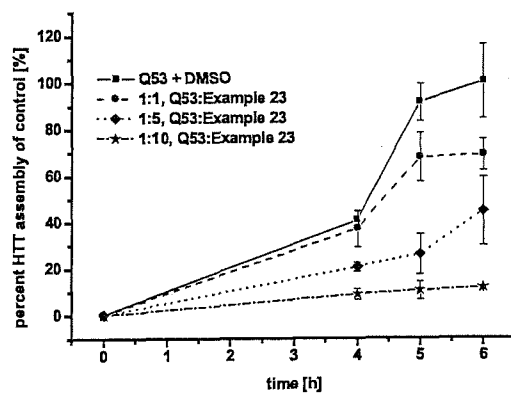
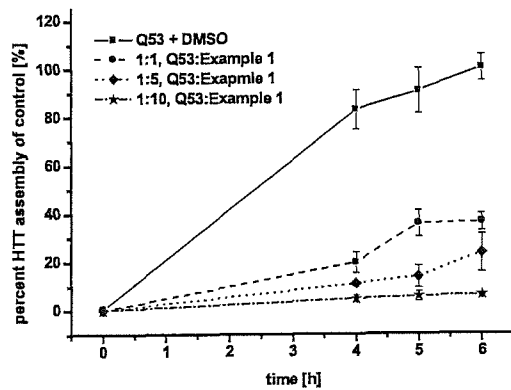
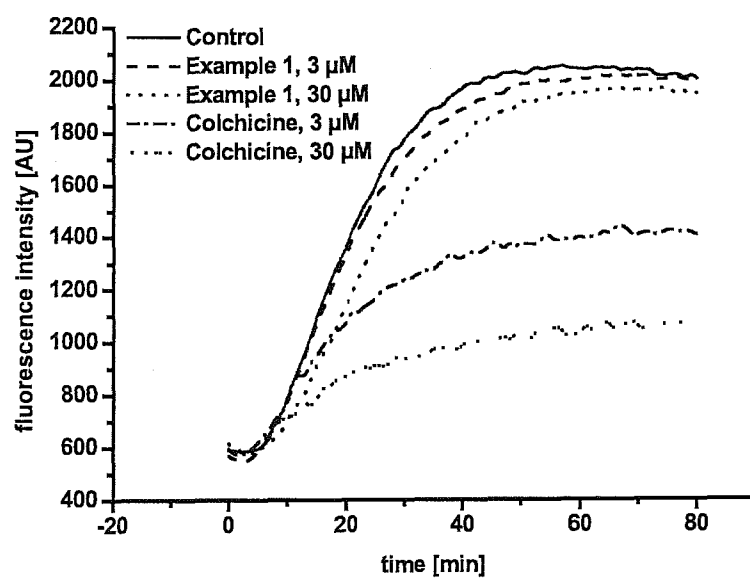


Figure 11



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/062111

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C251/86 C07C251/74 C07C251/84
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 458 843 B1 (WANG YAN [US] ET AL) 1 October 2002 (2002-10-01) example 23	1,2,7
X	US 2009/093480 A1 (GUENTHENSBERGER KATHERINE A [US] ET AL) 9 April 2009 (2009-04-09) table 1	1,2,7
X	WO 2004/009602 A (SMITHKLINE BEECHAM CORP [US]; BROWN MATTHEW LEE [US]; CHEUNG MUI [US];) 29 January 2004 (2004-01-29) example 3x	1,2,7
X,P	EP 2 119 698 A (ZEON CORP [JP]) 18 November 2009 (2009-11-18) example 1	1-4,7
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Further documents are listed in the continuation of Box C.



See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

10 December 2010

Date of mailing of the international search report

20/12/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Butkowskyj-Walkiw, T

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/062111

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/112015 A (THE FEINSTEIN INST MEDICAL RES [US]; AL-ABED YOUSEF [US]) 4 October 2007 (2007-10-04) example 1	1-4,7
X	US 6 613 942 B1 (LING ANTHONY [US] ET AL) 2 September 2003 (2003-09-02) example 39	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564356, Database accession no. BRN6534122 * abstract & BARBERA ET AL: MOLECULAR CRYSTALS AND LIQUID CRYSTALS, vol. 126, 1985, pages 259-268,	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564357, Database accession no. BRN7820445 * abstract & BUTORA ET AL: JACS, vol. 119, no. 33, 1997, pages 7694-7701,	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564358, Database accession no. BRN7821579 * abstract & BUTORA ET AL: JACS, vol. 119, no. 33, 1997, pages 7694-7701,	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564359, Database accession no. BRN3162328 * abstract & MARTIN ET AL: JACS, vol. 79, 1957, pages 2533-2540,	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564360, Database accession no. BRN8482217 * abstract -/--	1,2,7

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/062111

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	& ASIS ET AL: FARMACO, vol. 54, no. 8, 1999, pages 517-523, ----- DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564361, Database accession no. BRN943300 * abstract & KUGATOWA ET AL: CHEM. ABSTRACTS, 1960, page 5506, -----	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564362, Database accession no. BRN1830841 * abstract & PAQUETTE ET AL: JACS, vol. 94, 1972, page 8124, -----	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564363, Database accession no. BRN3329810 * abstract & HUDSON ET AL: JOURNAL OF THE CHEMICAL SOCIETY, 1946, page 76, -----	1,2,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564364, Database accession no. BRN3485601 * abstract & GAY ET AL: JOURNAL OF THE CHEMICAL SOCIETY, 1955, page 2530, -----	1-4,7
X	DATABASE BEILSTEIN BEILSTEIN INSTITUTE FOR ORGANIC CHEMISTRY, FRANKFURT-MAIN, DE; XP002564365, Database accession no. BRN8502154 * abstract & ASIS ET AL: FARMACO, vol. 54, no. 8, 1999, pages 517-523, ----- -/--	1-4,7

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/062111

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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