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(71) Applicant: HEINRICH HEINE UNIVERSITÄT DÜSSELDORF [DE/DE]; Universitätsstrasse 1, 40225 Düsseldorf (DE).

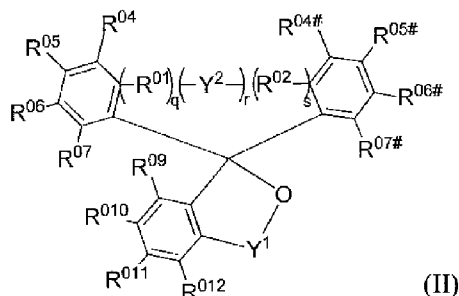
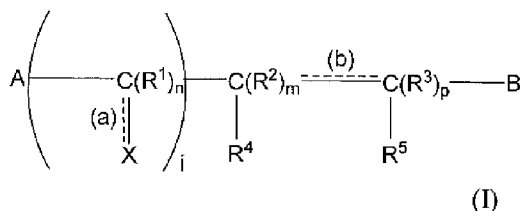
(72) Inventors: GROTH, Georg; Hasenpfad 8, 52428 Jülich (DE). PAULUS, Judith, Katharina; Dianastrasse 5, 40223 Düsseldorf (DE). SCHLIEPER, Daniel; Wissmannstrasse 21, 40219 Düsseldorf (DE). WESTHOFF, Peter; Heribertstr. 13, 41468 Neuss (DE).

(74) Agent: DICK, Alexander; Herzog Fiesser & Partner, Patentanwälte, Dudenstrasse 46, 68167 Mannheim (DE).

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(54) Title: SELECTIVE INHIBITION OF C4-PEP CARBOXYLASES





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Selective Inhibition of C4-PEP carboxylases

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In the past several different targets for selective and non-selective herbicides were identified. However, there still is a great need for new herbicides, especially selective herbicides. In particular specific herbicides against C4 plants would be of outmost importance for the agribusiness and a major turning point in the control of the worst weeds in the world. These weeds reduce the crop yield of the most important C3 cereals such as rice, wheat, barley and oat.

The C4 metabolism offers three potential targets for a selective herbicide. The first target is the enzyme phosphoenol pyruvate (PEP) carboxylase, which has a role in CO₂ fixation. The second target is the malic enzyme (ME) or the enzyme PEP carboxykinase (PEP CK) which catalyze the release of carbon dioxide in the bundle sheath cells. The third target is the enzyme pyruvate phosphate dikinase (PPDK), which regenerates PEP.

The substances unguinol and ilimaquinone were identified as selective inhibitors of PPDK from C4 plants maize, millet and proso millet. These substances were isolated from marine organisms (Motti *et al.*, 2007). The only universal target, that is common to all variants of C4 metabolism, is the enzyme PEP carboxylase. Due to the high sequence homology between the C4 specific PEP carboxylase and the C3 isoforms, which have further roles in the general C3 metabolism in plants and other organism, it is not obvious to use this enzyme as selective target.

The PEP carboxylase inhibitors described so far (Jenkins *et al.*, 1987; Jenkins, 1989; McFadden *et al.*, 1989; Mancera *et al.*, 1995) are based on PEP analogues (e. g., 3,3-Dichloro-2-(dihydroxyphosphinoylmethyl) propenoate (DCDP)). However, these compounds also inhibit C3 type PEP carboxylases at rates between 12-46% (Jenkins, 1989) and do not inhibit growth of C4 plants (Doyle *et al.*, 2005). Pairoba *et al.* (1996) showed that PEP carboxylase from C4 plant *Amaranthus viridis* is inhibited by several flavonoles and flavones. The inhibiting flavonoles are quercetin, quercitrin, rutin, kaempferol, fisetin, morin, and myricetin. The inhibiting flavone was baicalein/baicalin.

But in that work, neither the binding site of the inhibiting flavonoles (or flavones, resp.), nor the molecular mechanism of inhibition were identified. Thus, the mechanism of the inhibiting flavonoles and flavones is unknown. Furthermore, the substances were also described as inhibitors of the malic enzyme from *A. viridis*, a fact that implies a fairly
5 unspecific inhibition. Furthermore, the authors failed to show that the tested flavonoles and flavones inhibit C4 type PEP carboxylases only, but not C3 type isoforms.

The crystal structures of PEP carboxylase from *Escherichia coli* (C3 type) and from the C4 plant maize are known (Matsumura *et al.*, 2002). In the publications of these structures,
10 several amino acid side chains were identified as important for the binding of the feedback inhibitors malate and aspartate. However, the authors failed to note the structural difference of the malate binding site that we use in the development of C4 selective herbicides.

15 Thus, there is a need for effective C4 specific herbicides for all-purpose use, since no selective inhibitors of PEP carboxylase from C4 plants are known.

As set forth above, ilimaquinone and unguinol were identified as inhibitors of PPDK. These inhibitors, however, have several disadvantages, since they inhibit a subgroup of C4
20 plants. Tropical grasses, which use PEP carboxykinase for CO₂ release and PEP regeneration, are not inhibited. Further, the inhibitors need complex biosynthesis pathways and are expensive to produce (100 µg ilimanquinone cost 150 Euro). Also, both compounds have a low solubility in water (high clog values), which hampers the resorption by the plants.

25

Moreover, the inhibitory concentration of the compounds is high and they show poor solubility (unguinol ClogP = 5.7 and ilimaquinone ClogP = 6.0). The half maximal inhibitory concentration (IC₅₀) is 40 µM for unguinol and 292 µM for ilimanquinone for the isolated enzyme (Motti *et al.* 2007). Therefore, an inhibition of the plant would need
30 much higher concentrations up to millimolar range. Due to the high production costs, the use of these compounds is not economical.

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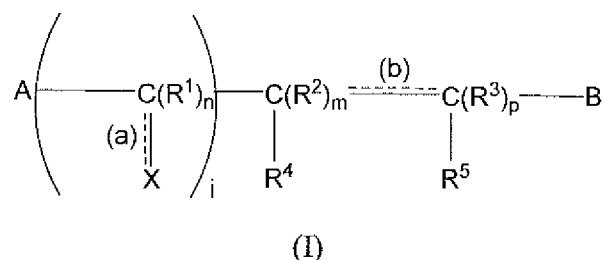
Finally, unguinol is used as growth hormone for animals, has anti-microbial and cytotoxic activity and inhibits the enzyme bile salt hydrolase. Therefore, it is unsuitable as herbicide.

For those flavonoles and flavones that were identified as inhibitors of PEP carboxylase from C4 plant *A. viridis*, the control experiments with C3 plants are missing to show the selective inhibition of the C4 isoform. The title of the publication suggests inhibitory effect of the complete group of flavonoids (Pairoba *et al.*, 1996), but the authors failed to show this claim. Flavonoids are with approx. 7000 different substances the largest class of plant secondary metabolites and have a high structural diversity. From this structurally heterogeneous class of substances, only a few flavonoles and flavones were tested. Flavanones, flavandioles, flavanes, flavanoles, isoflavones and anthocyanes, which also belong to the flavanoides, were not tested.

Furthermore, an inherent disadvantage of naturally occurring flavonoids is their low solubility in water, impeding the application as well the resorption of these substances.

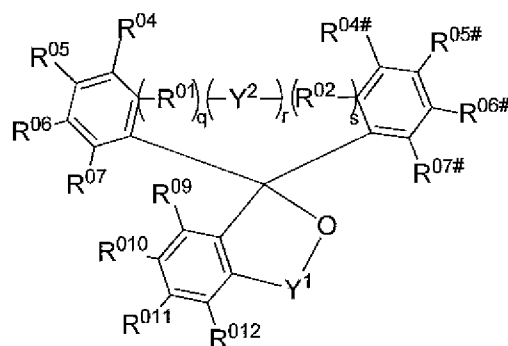
The inherent disadvantage of all tested PEP analogues is the fact that they also inhibit C3 type PEP carboxylases to a certain degree. They do not show any effect on the growth of C4 plants.

Thus, there is a need for effective C4 selective inhibitors. Thus, the present invention relates to use of at least one compound, salt or solvate thereof, preferably of a compound, salt or solvate thereof, as C4 plant selective herbicide, wherein said compound has a structure according to formula (I)



wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and wherein R¹, R², R³, R⁴ and R⁵ are, independently of each other H or an alkyl group, and the bond (a) is a single or double

bond, and wherein integer i is 0 or 1, and wherein in case (a) is a double bond, n is 0 and X is O or S, and wherein in case (a) is a single bond n is 1, and X is H or an alkyl group, and wherein the bond (b) is a single or double bond, and wherein in case (b) is a double bond, m and p are 0, and wherein in case (b) is a single bond m and p are both 1, and/or
 5 according to formula (II)



(II)

including tautomeric structures thereof, wherein R^{01} and R^{02} are independently of each other selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and
 10 halogen, wherein Y^1 is selected from the group consisting of $(S(=O)_2)$, $S(=O)$ and $(C(=O))$, and wherein Y^2 is O, and wherein r is 0 or 1 and wherein in case r is 0, q and s are 1, and wherein in case r is 1, q and s are 0, and wherein R^{01} , R^{02} , R^{04} , R^{05} , R^{06} , R^{07} , $R^{04\#}$, $R^{05\#}$, $R^{06\#}$, $R^{07\#}$, R^{09} , R^{10} , R^{11} and R^{12} are independently of each other selected from the group consisting of H, -OH, -SO₃H, carboxylic acid, ester, alkyl, alkoxy and halogen, said
 15 compound being capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C4 plant, thereby inhibiting said phosphoenolpyruvate carboxylase.

It is to be understood that also mixtures of two or more of the above mentioned compounds
 20 may be used as C4 inhibitors, thus mixtures of two or more compounds according to formula (I) or mixtures of two or more compounds according to formula (II) or mixtures of at least one compound according to formula (I) and at least one compound according to formula (II).

25 Within the meaning of the present invention, the term "alkyl" relates to non-branched alkyl residues and branched alkyl residues, as well as residues comprising one or more heteroatoms or functional groups, such as, by way of example, -O-, -S-, -NH-, -NH-

C(=O)-, -C(=O)-NH-, and the like. The term also encompasses alkyl groups which are further substituted by one or more suitable substituent.

5 Within the meaning of the present invention, the term "cyclic alkyl" or "cycloalkyl" relates to optionally suitably substituted 4 to 8-membered single-ring alkyl groups as well as optionally suitably substituted multicyclic alkyl residues.

10 Within the meaning of the present invention, the term "heterocycloalkyl" relates to optionally suitably substituted 4 to 8-membered single-ring alkyl groups as well as optionally suitably substituted multicyclic alkyl residues comprising one or more, preferably from 1 to 4 such as 1, 2, 3 or 4, heteroatoms, wherein in case the cycloalkyl residue comprises more than 1 heteroatom, the heteroatoms may be the same or different.

15 The term "alkyl", "cycloalkyl", "cyclic alkyl" and "heterocycloalkyl", also encompasses groups which are further substituted by one or more suitable substituent.

The term "substituted" as used in this context of the present invention preferably refers to alkyl, cycloalkyl, cyclic alkyl, aryl, heteroaryl and heterocycloalkyl groups being substituted in any position by one or more substituents, preferably by 1, 2, 3, 4, 5 or 6 substituents, more preferably by 1, 2, or 3 substituents. If two or more substituents are present, each substituent may be the same or may be different from the at least one other substituent. There are in general no limitations as to the substituent.

25 The substituents may be, for example, selected from the group consisting of aryl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino, acylamino, including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido, amidino, nitro, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, 30 trifluoromethyl, cyano, azido, cycloalkyl such as e.g. cyclopentyl or cyclohexyl, heterocycloalkyl such as e.g. morpholino, piperazinyl or piperidinyl, alkylaryl, arylalkyl and heteroaryl. Preferred substituents of such organic residues are, for example, halogens,

such as fluorine, chlorine, bromine or iodine, amino groups, hydroxyl groups, carbonyl groups, thiol groups and carboxyl groups.

Preferred examples for cycloalkyl groups are, e.g., cyclopentyl or cyclohexyl or steroids,
5 such as, e.g., steroids having a pregnane skeleton, such as 12, 20-dioxypregnane-3-yl.

Preferred examples for heterocycloalkyl groups are, e.g., morpholino, piperazinyl or piperidinyl.

10 Within the meaning of the present invention, the term "aryl" refers to, but is not limited to, optionally suitably substituted 5- and 6-membered single-ring aromatic groups as well as optionally suitably substituted multicyclic groups, for example bicyclic or tricyclic aryl groups. The term "aryl" thus includes, for example, optionally substituted phenyl groups or optionally suitably substituted naphthyl groups. Aryl groups can also be fused or bridged
15 with alicyclic or heterocycloalkyl rings are not aromatic so as to form a polycycle, e.g., benzodioxolyl or tetraline. The term "aryl" further includes aromatic groups which linked via a single bond to further aromatic groups so as to form, e.g., biphenyl groups.

The term "heteroaryl" as used within the meaning of the present invention includes
20 optionally suitably substituted 5- and 6-membered single-ring aromatic groups as well as substituted or unsubstituted multicyclic aryl groups, for example bicyclic or tricyclic aryl groups, comprising one or more, preferably from 1 to 4 such as 1, 2, 3 or 4, heteroatoms, wherein in case the aryl residue comprises more than 1 heteroatom, the heteroatoms may be the same or different. Such heteroaryl groups including from 1 to 4 heteroatoms are, for
25 example, benzodioxolyl, pyrrolyl, furanyl, thiophenyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, tetrazolyl, pyrazolyl, oxazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyridazinyl, benzoxazolyl, benzodioxazolyl, benzothiazolyl, benzoimidazolyl, benzothiophenyl, methylenedioxyphenyl, naphthyridinyl, quinolinyl, isoquinolinyl, indolyl, isoindolyl, benzofuranyl, purinyl, deazapurinyl, pteridinyl, or indolizinyl.

30

The term "substituted aryl" and the term "substituted heteroaryl" as used in the context of the present invention describes moieties having substituents replacing a hydrogen on one or more atoms, e.g. C or N, of an aryl or heteroaryl moiety. Again, there are in general no

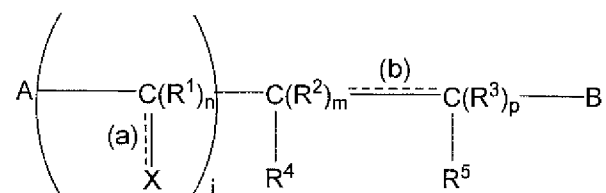
limitations as to the substituent. The substituents may be, for example, selected from the group consisting of alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy, carbonyloxy, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonate, phosphinato, amino, acylamino, including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido, amidino, nitro, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, trifluoromethyl, cyano, azido, cycloalkyl such as e.g. cyclopentyl or cyclohexyl, heterocycloalkyl such as e.g. morpholino, piperazinyl or piperidinyl, alkylaryl, arylalkyl and heteroaryl.

Preferred substituents of such organic residues are, for example, halogens, such as fluorine, chlorine, bromine or iodine, amino groups, hydroxyl groups, carbonyl groups, thiol groups, amino groups, and carboxyl groups.

Thus, the cyclic alkyl (cycloalkyl), heterocycloalkyl, aryl or heteroaryl group is, e.g., selected from the group consisting of, optionally suitably substituted, benzodioxolyl, pyrrolyl, furanyl, thiophenyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, tetrazolyl, pyrazolyl, oxazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyridazinyl, benzoxazolyl, benzodioxazolyl, benzothiazolyl, benzoimidazolyl, benzothiophenyl, methylenedioxyphenyl, naphthyridinyl, quinolinyl, isoquinolinyl, indolyl, isoindolyl, benzofuranyl, purinyl, deazapurinyl, pteridinyl, indolizinyl, phenyl, biphenyl and steroids.

The compounds according to the general formula (I)

According to a first preferred embodiment, the present invention relates to a use of a compound a salt or solvate thereof as C4 plant selective herbicide wherein said compound comprises a cyclic alkyl, aryl, heterocycloalkyl or heteroaryl group, wherein the compound has a structure according a structure according to formula (I)



wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group,

and wherein R^1 , R^2 , R^3 , R^4 and R^5 are, independently of each other H or an alkyl group,

5

wherein integer i is 0 or 1,

and the bond (a) is a single or double bond,

10 and wherein in case (a) is a double bond, n is 0 and X is O or S, and wherein in case (a) is a single bond n is 1, and X is H or an alkyl group,

and wherein the bond (b) is a single or double bond, and wherein in case (b) is a double bond, m and p are 0, and wherein in case (b) is a single bond m and p are both 1,

15

said compound being capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C4 plant, thereby inhibiting said phosphoenolpyruvate carboxylase.

20 It is contemplated that the binding to the malate binding site of a phosphoenolpyruvate carboxylase from a C3 plant is inhibited by steric or electrostatic constraints of a conserved arginine in the binding site of the C3 PEPC (Arg-884 in *Flaveria pringlei*).

Thus, the present invention also relates to the use of a compound of formula (I) as described above, wherein binding to the malate binding site of a phosphoenolpyruvate

25 carboxylase from a C3 plant is inhibited by steric or electrostatic constraints of a conserved arginine in the binding site of the C3 PEPC, preferably by Arg-884 in *Flaveria pringlei*.

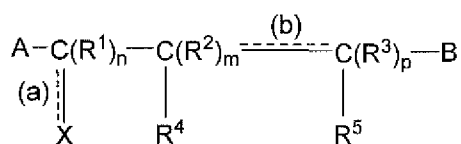
The amino acid sequence of the C3 PEPC of *Flaveria pringlei* is shown in SEQ ID NO: 2.

The sequence can be also found in GenBank, see GenBank Accession number GenBank: CAA88829.1 GI:763097).

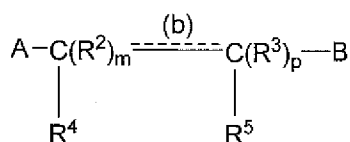
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Preferably i is 1 and the compound has the structure

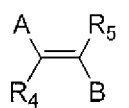
- 9 -



According an alternative embodiment i is 0, and the compound has the structure



5 , in particular wherein



Residue A

10

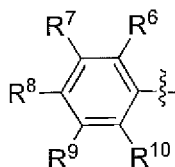
A is selected from the group consisting of cyclic alkyl, aryl, heterocycloalkyl and heteroaryl group. As described above, the terms cyclic alkyl, aryl, heterocycloalkyl and heteroaryl group may be substituted or unsubstituted. Thus, A is selected from the group consisting of unsubstituted cyclic alkyl, unsubstituted aryl, unsubstituted heterocycloalkyl, unsubstituted heteroaryl group, substituted cyclic alkyl, substituted aryl, substituted heterocycloalkyl and substituted heteroaryl group.

15

Preferably, A is a substituted or unsubstituted phenyl, benzodioxolyl or benzodioxinyl group.

20

In particular A is



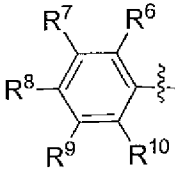
wherein R^6 , R^7 , R^8 , R^9 and R^{10} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen or wherein two

25

- residues in ortho position to each other form a cyclic or heterocyclic ring. The term “wherein two residues in ortho position to each other form a cyclic or heterocyclic ring” refers to such residues as R^6 and R^7 , or R^7 and R^8 , or R^8 and R^9 , or R^9 and R^{10} . In case two residues in ortho position to each other form a cyclic or heterocyclic ring, preferably R^7 and R^8 or R^8 and R^9 form a ring.
- 5 Preferably, R^6 , R^7 , R^8 , R^9 and R^{10} are, independently of each other, selected from the group consisting of H, OH, -COOH, -C(=O)-alkyl, -O-alkyl, alkyl, Cl, Br and I, wherein alkyl is in particular methyl or ethyl, in particular methyl.

- 10 The following preferred groups A are mentioned by way of example:

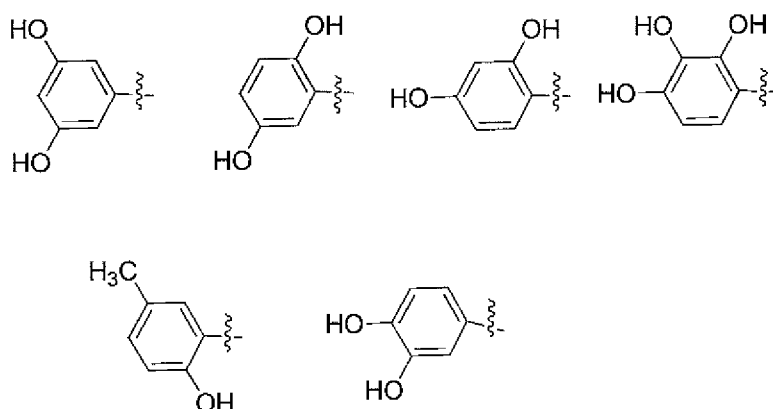
Table 1:

				
R^6	R^7	R^8	R^9	R^{10}
Selected from the group consisting of H, OH, -COOH, -C(=O)-Me, -OMe, methyl, Cl, Br and I	H, OH	H, OH	H, OH	H, OH
H, OH	Selected from the group consisting of H, OH, -COOH, -C(=O)-Me, -OMe, methyl, Cl, Br and I	H, OH	H, OH	H, OH
H, OH	H, OH	Selected from the group consisting of H,	H, OH	H, OH

- 11 -

		OH, -COOH, - C(=O)-Me, - OMe, methyl, Cl, Br and I		
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In particular A is selected from the group consisting of:

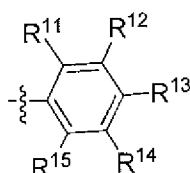


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Residue B

B is selected from the group consisting of cyclic alkyl, aryl, heterocycloalkyl and heteroaryl group. As described above, the terms cyclic alkyl, aryl, heterocycloalkyl and heteroaryl group may be substituted or unsubstituted. Thus, B is selected from the group consisting of unsubstituted cyclic alkyl, unsubstituted aryl, unsubstituted heterocycloalkyl, unsubstituted heteroaryl group, substituted cyclic alkyl, substituted aryl, substituted heterocycloalkyl and substituted heteroaryl group.

Preferably, B is a substituted or unsubstituted phenyl, benzodioxolyl or benzodioxinyl group. In particular B is



wherein R^{11} , R^{12} , R^{13} , R^{14} and R^{15} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, or wherein

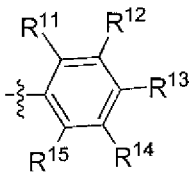
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two residues in ortho position to each other form a cyclic or heterocyclic ring. The term “wherein two residues in ortho position to each other form a cyclic or heterocyclic ring” refers to such residues as R¹¹ and R¹², or R¹² and R¹³, or R¹³ and R¹⁴, or R¹⁴ and R¹⁵. In case two residues in ortho position to each other form a cyclic or heterocyclic ring, preferably or R¹² and R¹³ or R¹³ and R¹⁴ form a ring.

Preferably, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are, independently of each other, selected from the group consisting of H, OH, -COOH, -C(=O)-alkyl, -O-alkyl, alkyl, Cl, Br and I, or wherein two residues in ortho position to each other form together the group O-CH₂-O-, -O-CH₂-CH₂-O-, wherein alkyl is in particular methyl or ethyl, in particular methyl.

The following preferred groups B are mentioned by way of example:

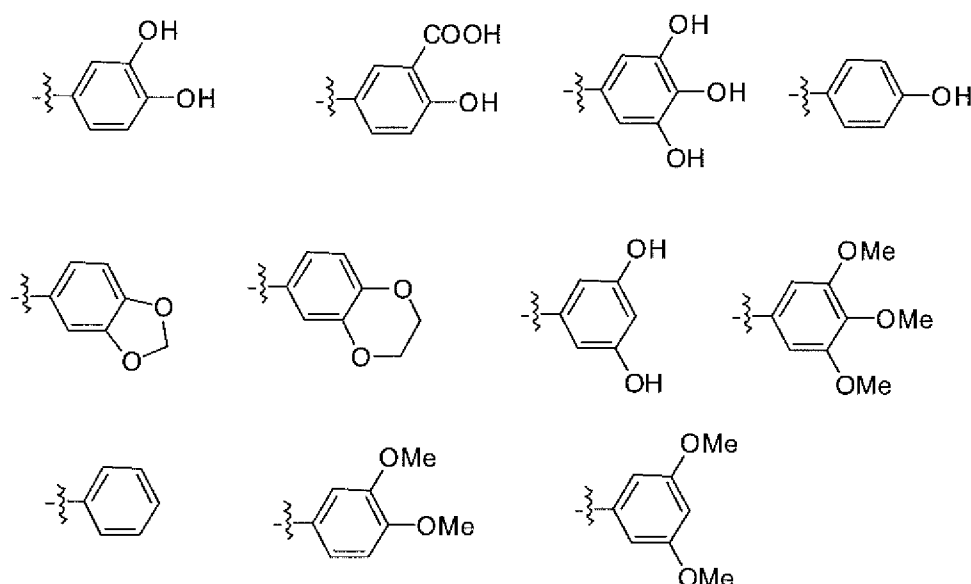
Table 2:

				
R ¹¹	R ¹²	R ¹³	R ¹⁴	R ¹⁵
Selected from the group consisting of H, OH, -COOH, -C(=O)-Me, -OMe, methyl, Cl, Br and I	H, OH	H, OH	H, OH	H, OH
H, OH	Selected from the group consisting of H, OH, -COOH, -C(=O)-Me, -OMe, methyl, Cl, Br and I	H, OH	H, OH	H, OH

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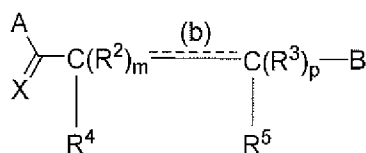
H, OH	H, OH	Selected from the group consisting of H, OH, -COOH, -C(=O)-Me, -OMe, methyl, Cl, Br and I	H, OH	H, OH
H, OH	H, OH	-O-CH ₂ -O-, -O-CH ₂ -CH ₂ -O-		H, OH

In particular B is selected from the group consisting of:



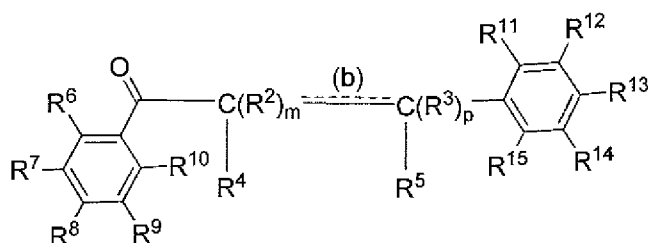
- 5 Preferably, integer n in formula (I) is 0 and (a) is a double bond. In this case, X is preferably O or S, in particular O.

Thus, the present invention also relates to the use of a compound, as described above, having the following structure:

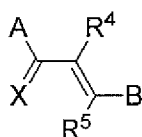


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wherein X is preferably O or S, in particular O. More preferably the compound described above has the structure



More preferably, (b) is a double bond and p is 0 and the compound has the structure

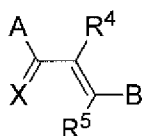


5

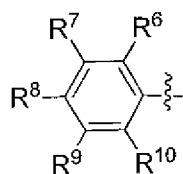
wherein X is preferably O or S, in particular O and with A and B, R⁴ and R⁵ being as described above.

Thus, the present invention also relates to the use of a compound as described above,

10 having the structure

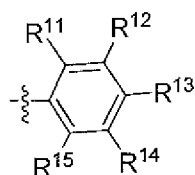


wherein X is preferably O or S, in particular O and with A and B, R⁴ and R⁵ being as described above, preferably with A being



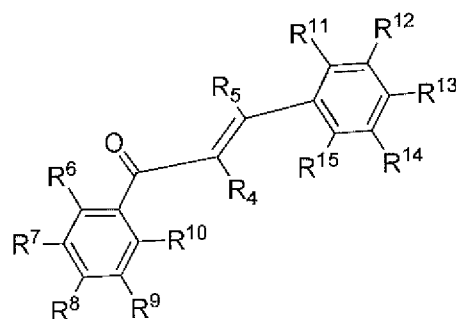
15

and/or B being

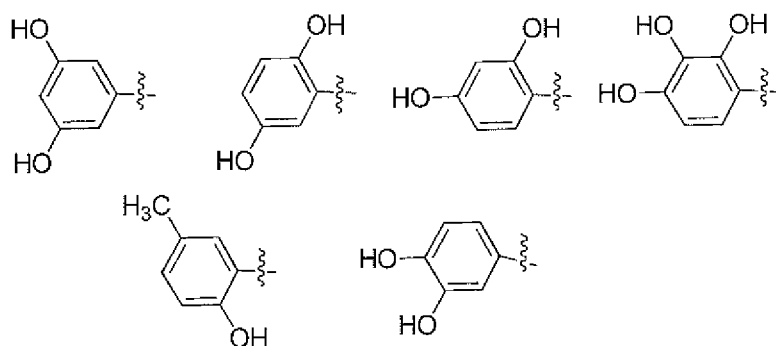


more preferably having the structure

- 15 -

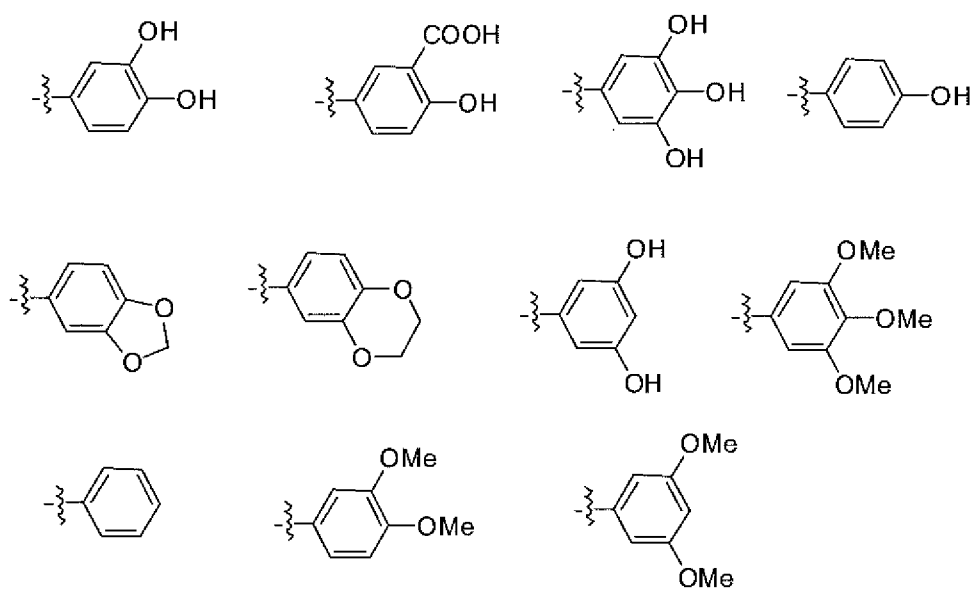


more preferably with A being selected from the group



5

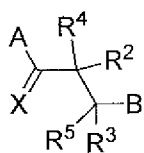
and/or B being selected from the group



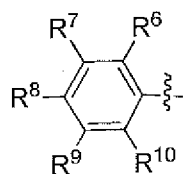
10

According to a further preferred embodiment, (b) is a single bond and the compound has the structure

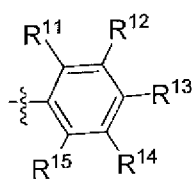
- 16 -



wherein X is preferably O or S, in particular O, and preferably with A being

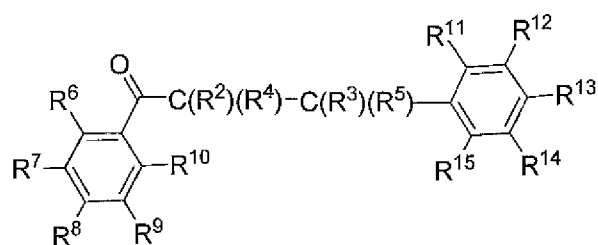


and/or B being

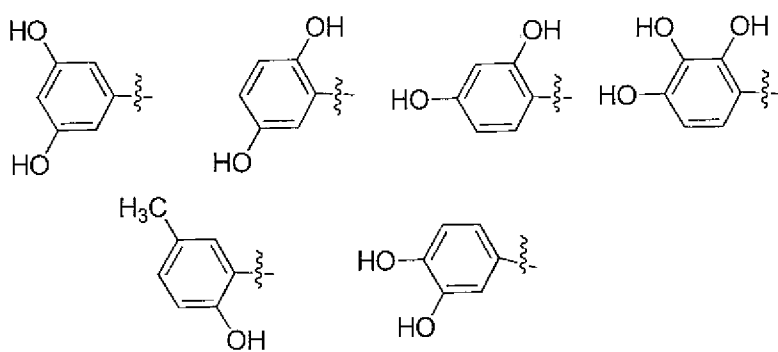


5

more preferably having the structure



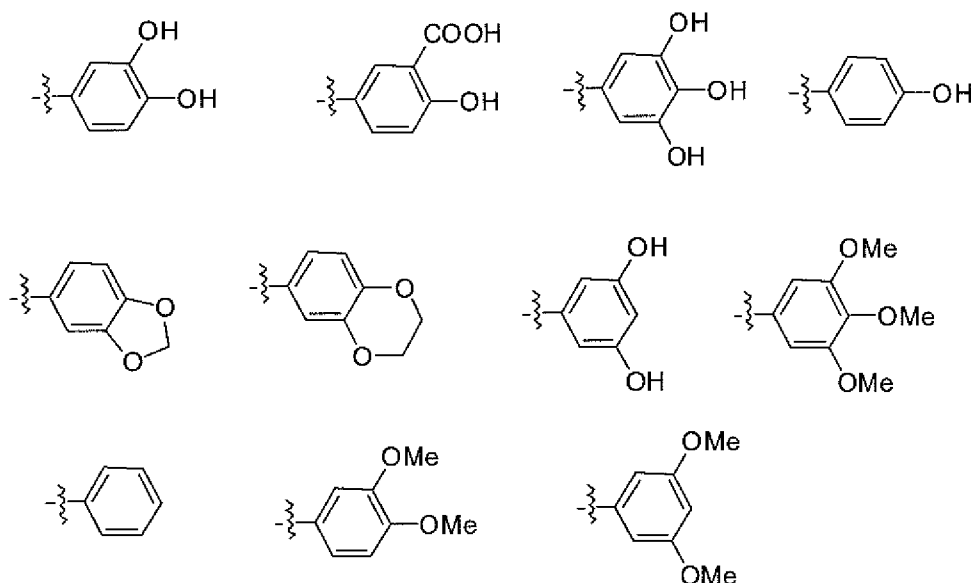
more preferably with A being selected from the group



10

and/or B being selected from the group

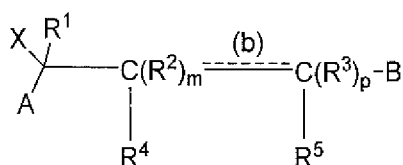
- 17 -



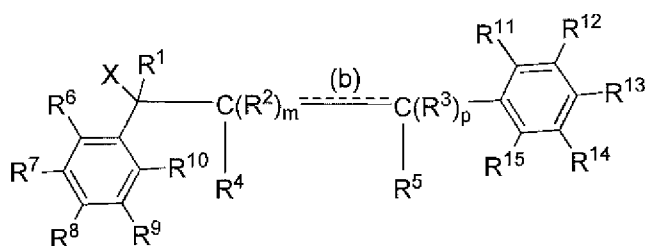
Most preferably in this case, R² and R³ are independently of each other, selected from the group, H, methyl, ethyl, n-butyl, sec-butyl and tert-butyl, in particular both H.

5

According to a further preferred embodiment, wherein n in formula (I) is 1, (a) is a single bond and X is H or alkyl, the compound thus having preferably the following structure

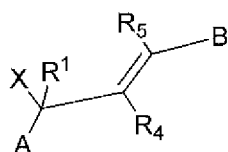


more preferably wherein the compound has the structure



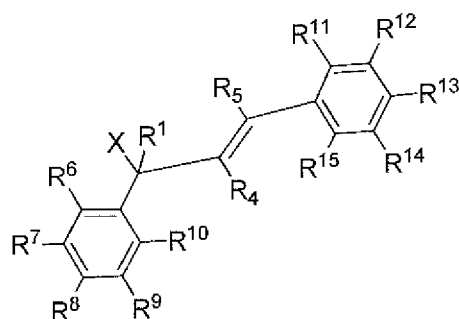
10

According to this embodiment, B is in particular a double bond and the compound has the structure



more preferably the structure

15

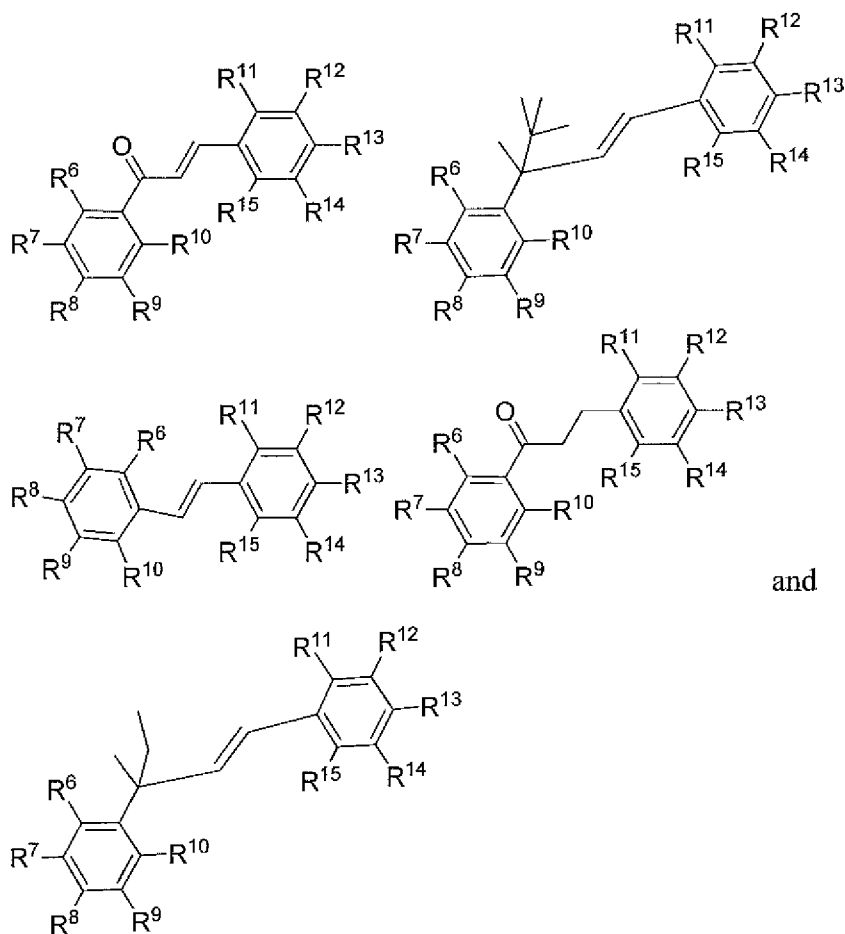


In case X is an alkyl group, X is preferably methyl, ethyl, n-butyl, sec-butyl or tert-butyl.

5 Residues R^4 and R^5

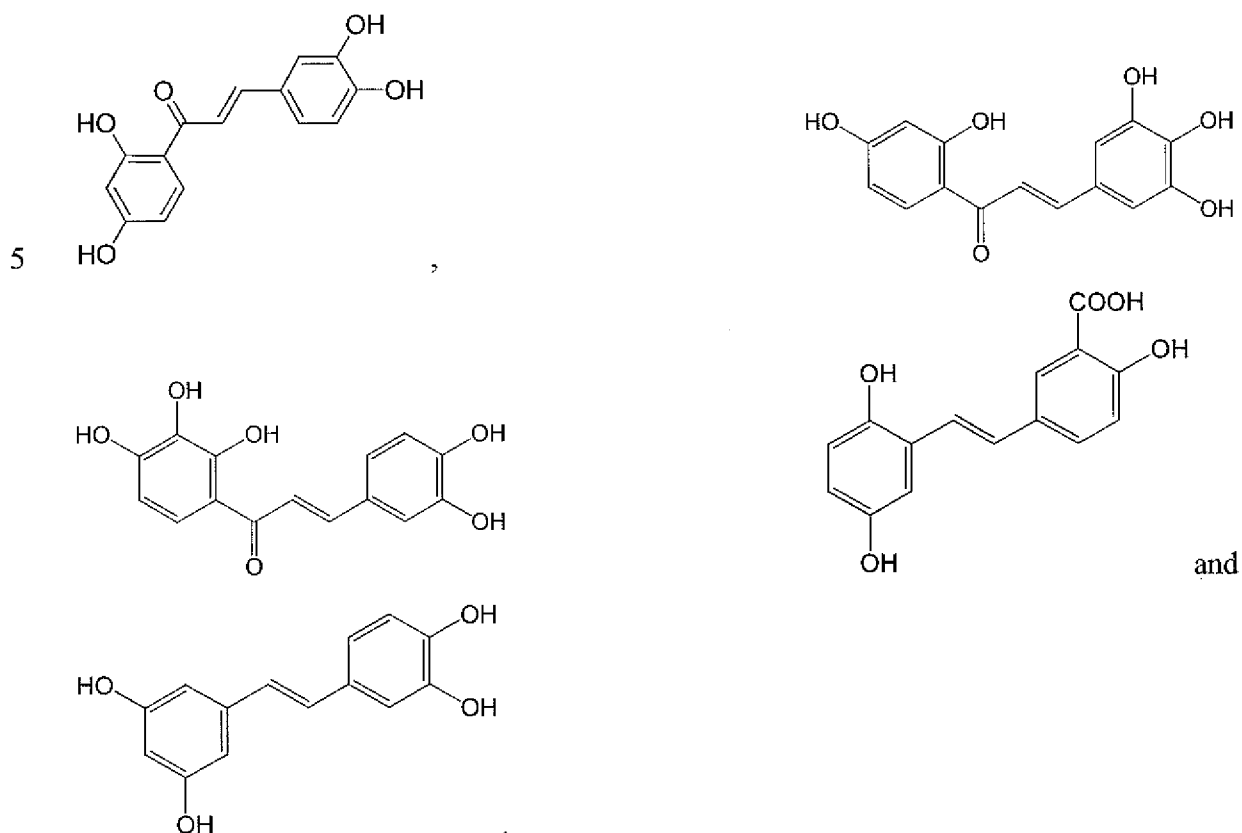
Residues R^4 and R^5 are preferably H or alkyl, more preferably, independently of each other, selected from the group, H, methyl, ethyl, n-butyl, sec-butyl and tert-butyl.

10 Most preferably, the compound, described above, is selected

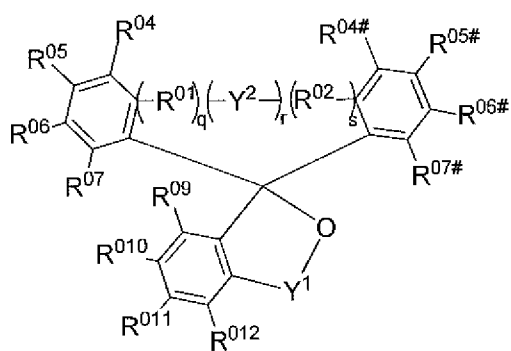


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According to a particularly preferred embodiment, the compound is selected from the group consisting of butein, Piceatannol, trans-1-(3'-Carboxy -4'-hydroxyphenyl)-2-(2,5-dihydroxy-phenyl)ethane, Robtein, and Okanin, thus selected from the group consisting of



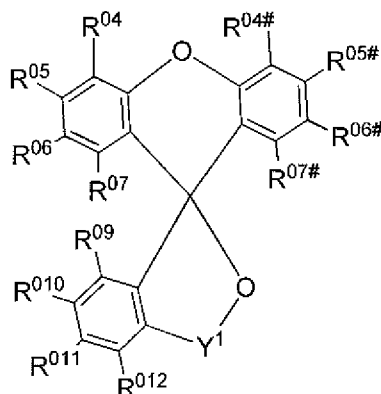
10 According to an alternative embodiment of the present invention, the compound has the structure (II)



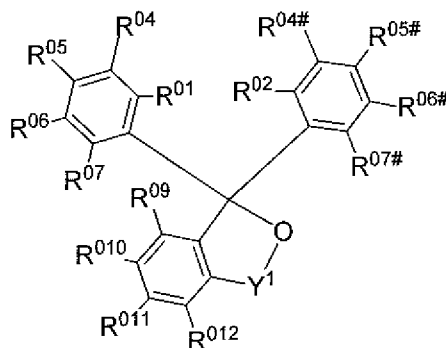
as described above or a tautomeric structure thereof. The term tautomeric structure is denoted to mean that at least one of residues R⁰¹, R⁰², R⁰⁴, R⁰⁵, R⁰⁶, R⁰⁷, R^{04#}, R^{05#}, R^{06#}

or $R^{07\#}$ comprises a proton, such as is a OH group, and in the tautomeric form this H is attached to the O adjacent to Y1 to give ring opened structure:

Preferably, in formula (II) Y^2 is O, and r is 1. In this case, q and s are 0, and the compound
5 has the structure



More preferably, r is 0 and wherein q and s are 1, the compound has the structure

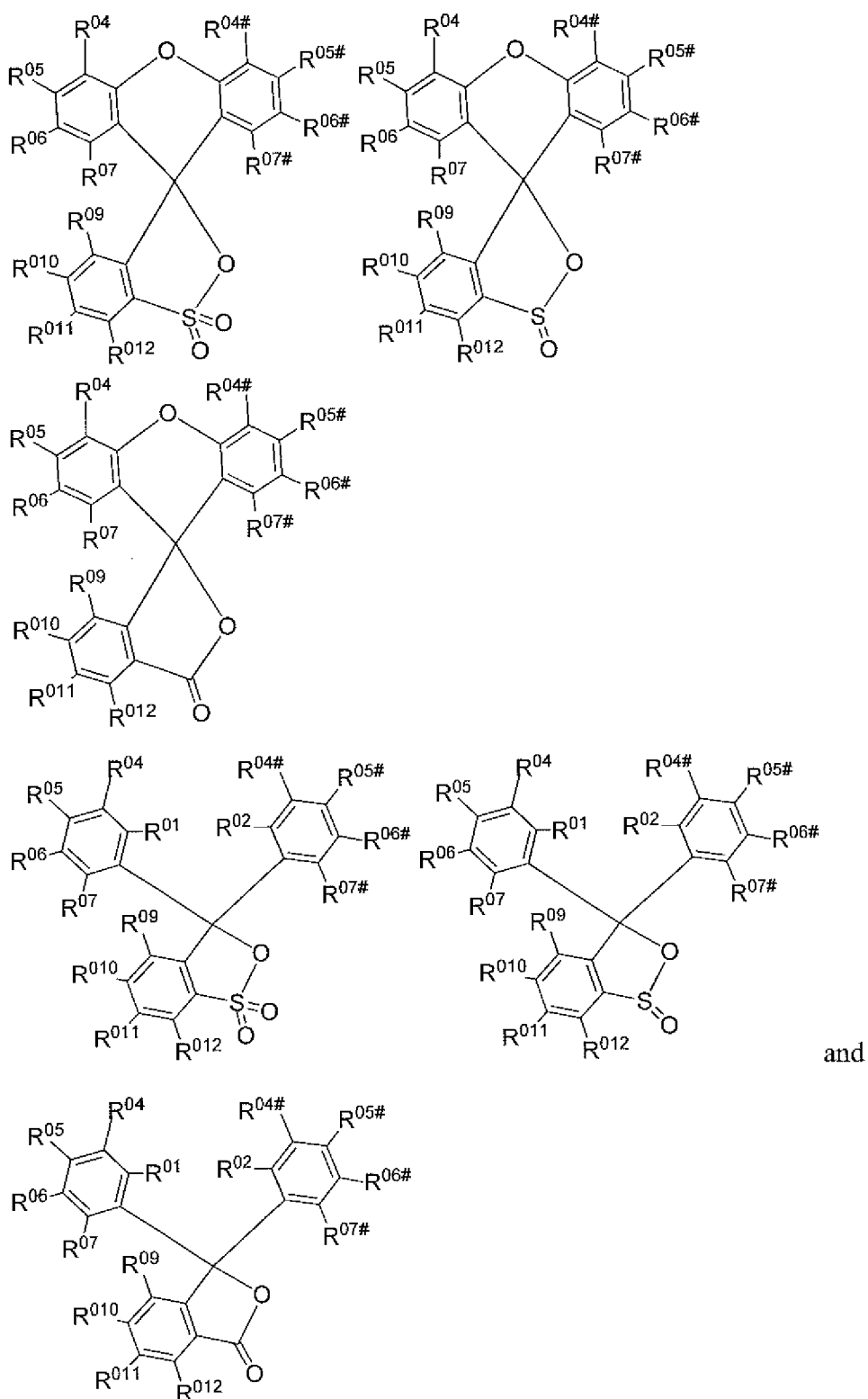


The group Y^1

As described above, Y^1 is preferably selected from the group consisting of $(S(=O)_2)$,
15 $S(=O))$ and $(C(=O))$.

Thus, the present invention also relates to a use of a compound, as described above, the compound having a structure selected from the group consisting of

- 21 -



and

5

As described above, R⁰¹, R⁰², R⁰⁴, R⁰⁵, R⁰⁶, R⁰⁷, R^{04#}, R^{05#}, R^{06#}, R^{07#}, R⁰⁹, R¹⁰, R¹¹ and R¹² are independently of each other selected from the group consisting of H, OH, -SO₃H, carboxylic acid, ester, alkyl, alkoxy and halogen. Preferably, R⁰¹, R⁰², R⁰⁴, R⁰⁵,

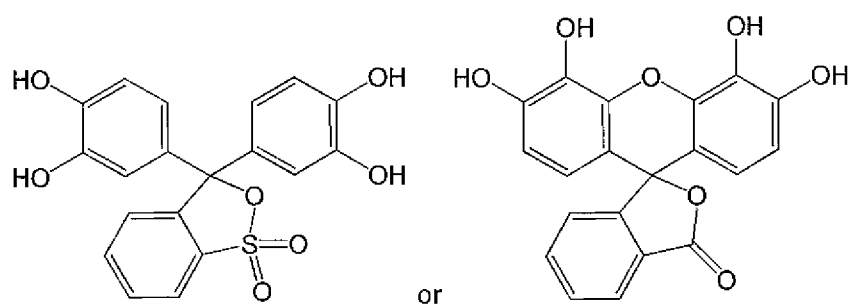
R^{06} , R^{07} , $R^{04\#}$, $R^{05\#}$, $R^{06\#}$, $R^{07\#}$, R^{09} , R^{010} , R^{011} and R^{012} are independently of each other selected from the group consisting of H, OH, -COOH, -SO₃H, -C(=O)-alkyl, -O-alkyl, alkyl, Cl, Br and I, wherein alkyl is preferably methyl or ethyl.

- 5 Preferably, the compound is selected from the group consisting of Pyrocatechol Violet, Pyrogallol Red, Gallein, Chrome Cyanine R, Cresol Red, Chlorphenol Red, Sulfonylfluorescein, BLAH (spiro[benzo[c][1,2]oxathiole-3,9'-xanthene] 1-oxide), 9,9-bis(4-methoxyphenyl)-8-oxa-7{4}-thiabicyclo[4.3.0]nona-2,4,10-triene, 2-hydroxy-5-[3-(4-hydroxy-3-isopropyl-5-sulfo-phenyl)-1,1-dioxo-benzo[c]oxathiol-3-yl]-3-isopropyl-b,
- 10 phenol red, chlorophenol red, bromphenol red, bromochlorophenol blue, bromophenol blue, iodophenol blue, Pyrogallolsulfonphthalein, brompyrogallored, bromochlorophenol blue, bromopheno blue, iodphenol blue, kresol red, bromokresol red, kresolpurpur (m-Kresolsulfonphthalein), bromokresolpurpur, kresolpurpur, bromokresol green, thymol blue, bromothymol blue, xlenol blue, bromoxlenol blue

15

Preferably, the compound is selected from the group consisting of Pyrocatechol Violet, Gallein, Chrome Cyanine R and Cresol Red.

- According to one particularly preferred embodiment, R^{05} , R^{06} , $R^{05\#}$, $R^{06\#}$ are OH, more
- 20 preferably the compound has the structure



Suitable salt

25

As described above, the compounds of the present invention can be formulated as suitable salt or solvate.

Typical acceptable salts include those salts prepared by reaction of the compounds of the present invention with an acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts. Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such suitable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, hydrochloride, dihydrochloride, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Preferred suitable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid. Salts of amine groups may also comprise quaternary ammonium salts in which the amino nitrogen carries a suitable organic group such as an alkyl, alkenyl, alkynyl, or aralkyl moiety. Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred. It should be recognized that the particular counter ion forming a part of any salt of this invention is usually not of a critical nature, so long as the salt as a whole is suitable and as long as the counter ion does not contribute undesired qualities to the salt as a whole.

The term acceptable solvate encompasses also suitable solvates of the compounds of the invention, wherein the compound combines with a solvent such as water, methanol,

ethanol, DMSO, acetonitrile or a mixture thereof to form a suitable solvate such as the corresponding hydrate, methanolate, ethanolate, DMSO solvate or acetonitrilate.

The compound of the present invention may be provided in form of an herbicide formulation. Accordingly, the compounds to be used in the context of the present invention can be formulated in a variety of ways. The following possibilities may be suitable for formulation: e.g. emulsifiable concentrates, emulsions, suspension concentrates, capsule suspension, water-soluble concentrates, water-soluble powders, water-soluble granules, water-dispersible powders, water-dispersible granules, in the form of microgranules, microcapsules and waxes. These formulations are well-known in the art and are e.g. described, for example, by van Valkenburg, "Pesticide Formulations", Marcel Dekker N.Y 1973 ISBN: 9780824716950 0824716957 which is herewith incorporated by reference with respect to its entire disclosure content.

The C4 plant selective herbicide according to the invention is preferably identified using a method, comprising screening a compound library for a compound, which is

- i) capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C4 plant (in particular, the allosteric feedback inhibitor binding site of PEPC from a C4 plant), thereby inhibiting the activity of said phosphoenolpyruvate carboxylase, and
- ii) not capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C3 plant (in particular, to the allosteric feedback inhibitor binding site of PEPC from a C3 plant).

The term „herbicide“ is well understood in the art. As used herein, the term refers to a compound that inhibits the growth of plants. The inhibition of the growth of plants shall include all deviations from natural development. In particular, the term refers to reducing the growth of the plant or to kill a plant. Moreover, the term may include inhibition of the development. Preferably, the term further includes dwarfing.

The herbicide according to the present invention shall be a C4 plant selective herbicide. A C4 plant selective herbicide, preferably, shall inhibit the growth of a C4 plant, but shall not inhibit the growth of a C3 plant. Also preferably, a C4 plant selective herbicide shall

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significantly inhibit the growth of a C4 plant, whereas growth of C3 plant shall remain unchanged, in particular essentially unchanged, in the presence of said herbicide (as compared to the absence of the herbicide). Whether growth of a plant is essentially unchanged or significantly inhibited can be determined by the skilled person without
5 further ado.

Preferably, the compound is capable of binding to the malate binding site comprised by the phosphoenolpyruvate carboxylase from a C4 plant, but is not capable of binding to the malate binding site comprised by the phosphoenolpyruvate carboxylase from a C3 plant or
10 binds only with significant lower affinity to the malate binding site comprised by the phosphoenolpyruvate carboxylase from a C3 plant.

The malate binding site of phosphoenolpyruvate carboxylase is well known in the art. In particular, is well known in the art that the regulation of the PEP carboxylase is, inter alia, effected by allosteric effectors, L-aspartate and L-malate. L-aspartate or L-malate which
15 bind at the enzyme to an allosteric binding domain, the malate binding site (also called "aspartate binding site" or "aspartate/malate binding site", or "allosteric feedback inhibitor binding site", or "allosteric malate feedback inhibitor site"), thereby inhibiting the activity of the PEP carboxylase (see elsewhere herein). Accordingly, the malate binding site is,
20 preferably, the allosteric feedback inhibitor binding site. The allosteric feedback inhibitor binding site from a C3 plant PEPC and from a C4 plant PEPC are, e.g., described and defined in Paulus et al., (2013a and 2013b) and by crystal structures 3ZGB and 3ZGE cited in these references. Both documents are herewith incorporated by reference with respect to their entire disclosure content.

25

Accordingly, the compound according to the use of the present invention, preferably, shall be capable of binding to the allosteric feedback inhibitor binding site of a PEPC from a C4 plant, but shall be not capable of binding to the allosteric feedback inhibitor binding site of a PEPC from a C3 plant or shall bind only with significant lower affinity to the allosteric
30 feedback inhibitor binding site comprised by the phosphoenolpyruvate carboxylase from a C3 plant.

Thus, is also envisaged that the compound, preferably, shall be capable of binding to the malate binding site of a PEPC from a C4 plant as depicted in Figure 7 A or Figure 8, but shall be not capable of binding to the malate binding site of a PEPC from a C3 plant as depicted in Figure 7 B (or shall bind only with significant lower affinity to the malate binding site of a PEPC from a C3 plant as depicted in Figure 7 B).

As set forth above, the term "inhibiting the growth" also includes a "reducing the growth". Accordingly, it is envisaged that the growth of a C4 plant shall be reduced in the presence of said herbicide (in particular in the presence of an effective amount) as compared to the growth in the absence of said herbicide. In contrast, the growth of a C3 plant shall not be affected by the presence of the herbicide, in particular, shall not be significantly affected in the presence of said herbicide. Accordingly, a C3 plant, preferably, shows the same growth (in particular, essentially the same growth), regardless whether the herbicide is present or not. Thus, it is envisaged, that a C4 plant selective herbicide shall reduce the growth of a C4 plant, but shall not reduce the growth of a C3 plants.

It is to be understood that the inhibition of the growth of the C4 plant may dependent on the concentration. Preferably, the growth of the C4 plant is inhibited if the plant is contacted with an effective amount of the herbicide, i.e.. with a herbicidal effective amount. Whether an amount of the herbicide is effective, or not, can be determined by methods known in the art.

The terms "C3 plant" and "C4 plant" are well understood by the skilled person.

A C3 plant, in particular, shall be a plant in which the CO₂ is first fixed into a compound containing three carbon atoms (phosphoglyceric acid) before entering the Calvin cycle of photosynthesis. Preferably, it uses the C3 carbon fixation pathway as the sole mechanism to covert CO₂ into 3-phosphoglycerate. C3 plants are well known in the art and include tobacco, tomato, soybeans, potato, cucumber, cotton, wheat, rice and barley.

30

Particularly preferred C3 plants are *Oryza sativa*, *Hordeum vulgare*, *Brassica napus*, *Triticum aestivum*, and, in particular, *Flaveria pringlei*. Further preferred are *Glycine max* (soybean), cucumber, grapes, potato, tomato, cassava, sugar beet und watermelon.

Moreover, it is also preferred that the C3 plant is selected from the group of C3 plants consisting of asparagus, green beans, cabbages and other brassicas, carrots and turnips, cauliflowers and broccoli, green chilies and peppers, gherkins, eggplants, lettuce and chicory, pumpkins, squash and gourds, spinach, sunflower seed, and yams.

5

A C4 is plant in which the CO₂ is first fixed into a compound containing four carbon atoms before entering the Calvin cycle of photosynthesis. In particular, a C4 plant shall utilize the C4 carbon fixation pathway in which the CO₂ is first bound to a phosphoenolpyruvate resulting in the formation of four-carbon compound (oxaloacetate). C4 plants are well known in the art and include monocotyledonous plants such as maize, sugarcane, and sorghum, as well as dicotyledonous plants such as Amaranthus.

10

Preferably, the C4 plants belong to the C4 plants of the family selected from Aizoaceae (Genus, in particular, Cypselea, Gisekia, Trianthema, Zalaeya), Amaranthaceae (Acanthochiton, Aerva, Alteranthera, Amaranthus, Brayulinea), Caryophyllaceae (Polycarpaea), Chenopodiaceae (Anabis, Aellenia, Arthrophytum, Atriplex, Bassia, Bienerta, Camphorosma, Chenolea, Climacoptera, Comulaca, Cytobasis, Echinopsilon, Gamanthus, Girgensohnia, Halanthium, Halimocnemis, Halocharis, Halogeton, Halostigmara, Haloxylon, Hammada, Horaninovia, Hypocyclix, Kochia, Londesia, Noaea, Pandertia, Petrosimonia, Salsola, Seidlitzia, Suaeda, Theleophyton, Traganum) Molluginaceae (Glinis, Mollugo) Nyctaginaceae (Ilionia, Boerhaavia, Okenia), Portulacaceae (Portulaca), Polygonaceae (Calligonum), Euphorbiaceae (Chamaesyce, Euphorbia) Capparaceae (Gynandropsis), Zygophyllaceae (Kallstroemia, Tribulus, Zygophyllum) Asteraceae (Glossocordia, Glossogyne, Isostigma, Pectis), Boraginaceae, Convolvulaceae, Acanthaceae, Scrophulariaceae, Poaceae (Alloteropsis, Andropogon, Arundinella, Bouteloua, Cynodon, Echinochloa, Leptochloa, Microstegium, Panicum, Paspalum, Setaria, Sorghum, Spartina, Sporobolus, Zea). Preferred genera are indicated in brackets.

20

25

Particularly preferred C4 plants are selected from the group consisting of Bothriochloa saccharoides, Bothriochloa ischaemum, Imperata cylindrica (Cogon grass), Panicum capillare (Witchgrass), Panicum coloratum, Panicum fluviicola, Panicum miliaceum, Panicum phragmitoides, Panicum turgidum, Saccharum officinarum (Wild sugarcane), Sorghum bicolor, Zea mays (Maize), in particular Flaveria trinervia, Setaria italica (Foxtail

30

millet), *Setaria palmifolia*, *Setaria plicata*, *Paspalum conjugatum* (Buffalo Gras), *Paspalum quadrifarium*, *Paspalum dilatatum*, *Amaranthus hypochondriacus*, *Amaranthus spinosus* (spiny amaranth), *Sorghum halepense* (Johnsongrass), *Rottboellia cochinchinensis* (itchgrass), *Commelina benghalensis* (tropical spiderwort), *Trianthema portulacastrum*
5 (desert horse purslane), *Ageratum conyzoides* (Chick weed, Goatweed, Whiteweed), *Bidens pilosa* (Spanish Needle), *Euphorbia hirta*, *Portulaca oleracea* (Pigweed). Further preferred C4 plants are *Alopecurus myosuroides* and *Gallium aparine*. Particularly preferred C4 plants are *Flaveria trinervia*, *Flaveria australasica*, *Zea mays*, and *Sorghum bicolor*.

10

The phosphoenolpyruvate carboxylases to be used in the context of the present invention shall be derived from a C3 and from a C4 plant, respectively. Thus, it is particular envisaged that the phosphoenolpyruvate carboxylase to be used is naturally present in a C3 and a C4 plant, respectively. It is also contemplated that the phosphoenolpyruvate
15 carboxylase may be a recombinant and/or mutated phosphoenolpyruvate carboxylase present in plant as transgene. Preferred C3 and C4 plants are disclosed herein above.

The term “phosphoenolpyruvate carboxylase” is well understood by the skilled person. Frequently, phosphoenolpyruvate carboxylases are also referred to as PEP carboxylase,
20 PEPCase, or PEPC. A phosphoenolpyruvate carboxylase as referred to herein shall be capable of catalyzing the formation of oxaloacetate from phosphoenolpyruvate (PEP) and bicarbonate (EC 4.1.1.31). In particular, a phosphoenolpyruvate carboxylase shall be capable of catalyzing the irreversible beta-carboxylation of phosphoenolpyruvate by bicarbonate to yield oxalacetate and phosphate. The aforementioned enzymatic activity can
25 be determined by assays well known in the art.

It is well known in the art, that the regulation of the PEP carboxylase is, inter alia, effected by allosteric effectors, L-aspartate and L-malate. L-aspartate or L-malate bind at the enzyme to an allosteric binding domain, the malate binding site (also called “aspartate
30 binding site” or “aspartate/malate binding site”, or “allosteric feedback inhibitor binding site”, or “allosteric malate feedback inhibitor site”), thereby inhibiting the activity of the PEP carboxylase. The malate binding site of a PEP carboxylase comprises four conserved residues which participate directly in the binding of malate and aspartate. The conserved

residues are well known in the art, and e.g. described by Jacobs et al. (Plant Cell and Environment (2008), 31, 793-803). In *Flaveria*, the four conserved residue which participate in the binding of aspartate/malate are R641, K829, R888 and N964 (see the sequences disclosed herein for the PEPC from *Flaveria pringlei* and *Flaveria trinervia*).

5

In a preferred embodiment of the present invention the PEP carboxylase from a C4 plant is derived from *Flaveria trinervia* (GenBank Accession Number CAA43601.1 GI:498699) , and the PEP carboxylase from a C3 plant is derived from *Flaveria pringlei* (GenBank Accession number CAA45505.1 GI:18458), i.e. two highly homologous PEP carboxylases.

10 The amino acid sequence of the PEP carboxylase from *Flaveria trinervia* is shown in SEQ ID NO: 1, the amino acid sequence of the PEP carboxylase from *Flaveria pringlei* is shown in SEQ ID NO: 2.

Thus, in a preferred embodiment, the phosphoenolpyruvate carboxylase derived from a C4
15 plant is, in particular, encoded by a polynucleotide comprising a nucleic acid selected from the group consisting of:

- a. a nucleic acid encoding a polypeptide having an amino acid sequence as shown in SEQ ID No: 1;
- b. a nucleic acid encoding a polypeptide having an amino acid sequence being at
20 least 90%, 95%, 97% or in particular 99% identical to the amino acid sequence shown in SEQ ID No: 1, wherein said polypeptide has phosphoenolpyruvate carboxylase activity.

The enzymatic activity of a PEP carboxylase is disclosed elsewhere herein. Thus, the
25 phosphoenolpyruvate carboxylase shall be capable of catalyzing the formation of oxaloacetate from phosphoenolpyruvate (PEP) and bicarbonate (EC 4.1.1.31, see above). Preferably, the phosphoenolpyruvate carboxylase derived from a C4, comprises, within its malate binding site, a glycine residue which corresponds to the conserved glycine residue found at position 884 of the PEP carboxylase from *Flaveria pringlei*. Moreover, the
30 inventors have found that some of the PEP carboxylases from C4 plants comprise at a position which corresponds to the glycine residue, a serine, glutamine or isoleucine residue, i.e. amino acid residues which will also not inhibit binding of the identified herbicides by their side-chain. Alternatively, the said phosphoenolpyruvate carboxylase

derived from a C4, comprises, within its malate binding site, a serine, glutamine or isoleucine residue which corresponds to said glycine residue. The malate binding site of the PEP Carboxylase from *Flaveria trinervia* is well known in the art, and, e.g. described by Jacobs et al. (Plant Cell and Environment (2008), 31, 793-803). Preferably, the variants
5 of the phosphoenolpyruvate carboxylase set forth in b. above are capable of binding malate.

Thus, in a preferred embodiment, the phosphoenolpyruvate carboxylase derived from a C3 plant is, in particular, encoded by a polynucleotide comprising a nucleic acid selected from
10 the group consisting of:

- a. a nucleic acid encoding a polypeptide having an amino acid sequence as shown in SEQ ID No: 2;
- b. a nucleic acid encoding a polypeptide having an amino acid sequence being at least 90%, 95%, 97% or in particular 99% identical to the amino acid sequence
15 shown in SEQ ID No: 2, wherein said polypeptide has phosphoenolpyruvate carboxylase activity.

The activity of a PEP carboxylase is disclosed elsewhere herein. Preferably, the phosphoenolpyruvate carboxylase derived from a C3, comprises, within its malate binding
20 site, a conserved arginine residue which corresponds to the conserved arginine residue found at position 884 of the PEP carboxylase from *Flaveria pringlei*. Preferably, the conserved arginine residue is within the region of amino acid 875 to 895. The malate binding site of the PEP Carboxylase from *Flaveria pringlei* is well known in the art, and, e.g. described by Jacobs et al. (Plant Cell and Environment (2008), 31, 793-803), which is
25 herewith incorporated by reference with respect to its entire disclosure content. Preferably, the variants of the phosphoenolpyruvate carboxylase set forth in b. above are capable of binding malate.

The term "polynucleotide" as used herein refers to a linear or circular nucleic acid
30 molecule. It encompasses DNA as well as RNA molecules. The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. isolated from its natural context) or in genetically modified form. The term encompasses single as well as double stranded polynucleotides. Moreover, comprised are also

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chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified one such as biotinylated polynucleotides. The polynucleotide of the present invention is characterized in that it shall encode a polypeptide as referred to above. The polynucleotide, preferably, has a specific nucleotide sequence as mentioned above. Moreover, due to the degeneracy of the genetic code, polynucleotides are encompassed which encode a specific amino acid sequence as recited above.

Moreover, the term "polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides. Said variants may represent orthologs, paralogs or other homologs of the polynucleotide of the present invention. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences by at least one nucleotide substitution, addition and/or deletion whereby the variant nucleic acid sequence shall still encode a polypeptide having the activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 x sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids are preferably for example 0.1 x SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are preferably, for example, 0.1 x SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined

for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer-based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequence of the polynucleotide or the amino acid sequence of the polypeptide of the present invention with sequences of other members of the enzyme families referred to in accordance with this invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are, in increasing order of preference, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the specific nucleic acid sequences. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding amino acid sequences which are, in increasing order of preference, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the specific amino acid sequences referred to herein. The percent identity values as set forth herein are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (Higgins 1989, CABIOS, 5 1989: 151-153) or the programs Gap and BestFit (Needleman 1970, J. Mol. Biol. 48; 443-453 and Smith 198, Adv. Appl. Math. 2; 482-489), which are part of the GCG software packet from Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711, version 1991, are to be used. The sequence identity values recited above in percent (%) are to be determined,

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preferably, using the program GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, shall always be used as standard settings for sequence alignments.

5

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has the activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

15

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Specifically, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part peptide sequences for monitoring expression or protein-protein interaction (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

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25

Further preferred PEP carboxylases from C3 plants are derived from the following plants (indicated is the organism, the GenBank Accession Nummer, and the position of the conserved arginine residue. For some enzymes, only the sequences of fragments are known, the full length sequences can be determined by the skilled person without further ado):

30

	Oryza sativa (Rice) EMBL AAG00180.1	R879
	Brassica napus (Rape) TrEMBL Q42634	R883
	Triticum aestivum (Wheat) EMBL CAA07610.1	R891
	Phaseolus vulgaris (Kidney bean) UniProtKB/Swiss-Prot Q9AU12	R887
5	Hordeum vulgare (Barley) EMBL ABB01326.1 (Fragment)	R146
	Vicia faba (Broad bean) (Faba vulgaris) EMBL CAA09588.1	R885
	Cucumis sativus (Cucumber) EMBL CAD10147.1 (Fragment)	R117
	Vitis vinifera (Grape) EMBL AAL83719.1 (Fragment)	R258
	Solanum tuberosum (Potato) UniProtKB/Swiss-Prot P29196	R885
10	Gossypium hirsutum (Upland cotton) EMBL AAB80714.1	R884
	Glycine max (Soybean) UniParc P51061	R886
	Nicotiana tabacum (Common tobacco) UniParc P27154	R884

Further preferred PEP carboxylases from C3 plants are derived from *Hordeum vulgare*
 15 (BAJ88050.1 GI:326516054) or from *Arabidopsis thaliana* (AAC24594.1 GI:3264805)

Further preferred PEP carboxylases from C4 plants are derived from the following plants
 (indicated is the organism, the GenBank Accession Nummer, and the position of the
 conserved glycine residue, alternatively of the serine, glutamine or isoleucine, see
 20 elsewhere herein. For some enzymes, only the sequences of fragments are known, the full
 length sequences can be determined by the skilled person without further ado):

	<i>Bothriochloa saccharoides</i> (Fragment) TrEMBL A7DX44	G429
	<i>Bothriochloa ischaemum</i> (Fragment) TrEMBL A7DX82	G429
	<i>Imperata cylindrica</i> (Cogon grass) (Fragment) TrEMBL A7DX63	G429
25	<i>Panicum capillare</i> (Witchgrass) (Fragment) EMBL CAM84110.1	G429
	<i>Panicum coloratum</i> (Fragment) TrEMBL A7DXB1	G429
	<i>Panicum fluviicola</i> (Fragment) TrEMBL D9UAH3	G721
	<i>Panicum miliaceum</i> (Fragment) TrEMBL E1XUD1	G721
	<i>Panicum phragmitoides</i> (Fragment) TrEMBL D9UAH8	G721
30	<i>Panicum turgidum</i> (Fragment) TrEMBL D9UAH5	G721
	<i>Saccharum spontaneum</i> (Wild sugarcane) EMBL CAC85930.1	G881
	<i>Saccharum officinarum</i> (Wild sugarcane) EMBL CAC08829.1	G881
	<i>Sorghum bicolor</i> Swiss-prot P15804	G881

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	Zea mays (Maize) EMBL CAA33317.1	G890
	Flaveria trinervia Swiss-Prot P30694	G884
	Flaveria australasica NCBI GenBank Z25853.1	G884
	Panicum laetum (Fragment) EMBL CAM84117.1	S429
5	Setaria italica (Foxtail millet) TrEMBL Q8S2Z8	Q884
	Setaria palmifolia (Fragment) TrEMBL A7DXC5	Q429
	Setaria plicata (Fragment) TrEMBL A7DXC6	Q429
	Paspalum conjugatum (Buffalo Gras) (Fragment) TrEMBL A7DXB6	Q429
	Paspalum quadrifarium (Fragment) EMBL CAM84120.1	Q429
10	Paspalum dilatatum (Fragment) TrEMBL A7DXB7	Q429
	Amaranthus hypochondriacus SwissProt Q43299	I884

How to determine the activity of a PEP carboxylase is well known in the art. E.g, the formation of oxaloacetate can be determined spectrophotometrically, e.g., in a malate
 15 dehydrogenase coupled system. The reaction velocity can be measured as a decrease in A_{340} resulting from the oxidation of NADH.

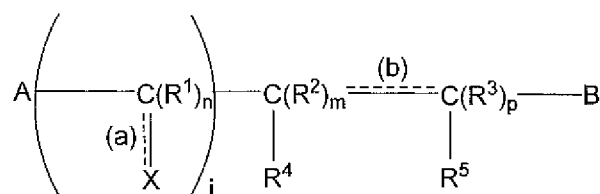
Preferably, a compound which inhibits the growth of a C4 plant, but which does not inhibit the growth of the C3 plant can be used as C4 selective herbicide.

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By way of example, the following particularly preferred embodiments of the invention are described:

1. Use of at least one compound, or salt or solvate thereof, as C4 plant selective
 25 herbicide wherein said compound has a structure according to

(a) formula (I)



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(I)

wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group,

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wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group,

and wherein R^1 , R^2 , R^3 , R^4 and R^5 are, independently of each other H or an alkyl group,

10

and wherein integer i is 0 or 1, preferably 1

and wherein the bond (a) is a single or double bond,

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and wherein in case (a) is a double bond, n is 0 and X is O or S, and wherein in case (a) is a single bond n is 1, and X is H or an alkyl group,

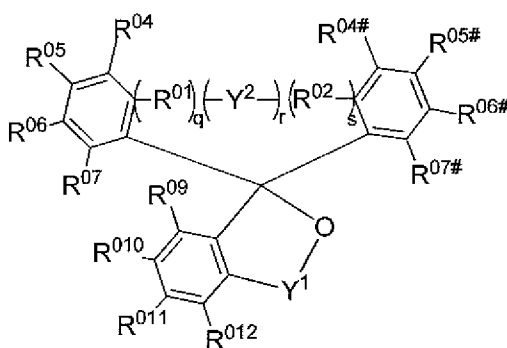
and wherein the bond (b) is a single or double bond, and wherein in case (b) is a double bond, m and p are 0, and wherein in case (b) is a single bond m and p are both 1,

20

and/or

(b) formula (II)

25



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(II)

wherein R^{01} and R^{02} are independently of each other selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen,

5

wherein Y^1 is selected from the group consisting of $(S(=O)_2)$, $S(=O)$ and $(C(=O))$

10

and wherein Y^2 is O, and wherein r is 0 or 1 and wherein in case r is 0, q and s are 1, and wherein in case r is 1, q and s are 0,

15

and wherein R^{01} , R^{02} , R^{04} , R^{05} , R^{06} , R^{07} , $R^{04\#}$, $R^{05\#}$, $R^{06\#}$, $R^{07\#}$, R^{09} , R^{010} , R^{011} and R^{012} , are independently of each other selected from the group consisting of H, OH, $-SO_3H$, carboxylic acid, ester, alkyl, alkoxy and halogen,

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said compound being capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C4 plant, thereby inhibiting said phosphoenolpyruvate carboxylase.

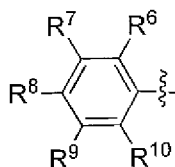
2. Use according to embodiment 1, wherein the binding to the malate binding site of a phosphoenolpyruvate carboxylase from a C3 plant is inhibited, preferably by steric or electrostatic constraints of a conserved arginine in the binding site of the C3 PEPC, preferably by Arg-884 in *Flaveria pringlei*.

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3. Use according to embodiment 1 or 2, wherein compound has a structure according to

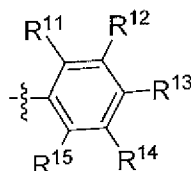
formula (I) and wherein A is

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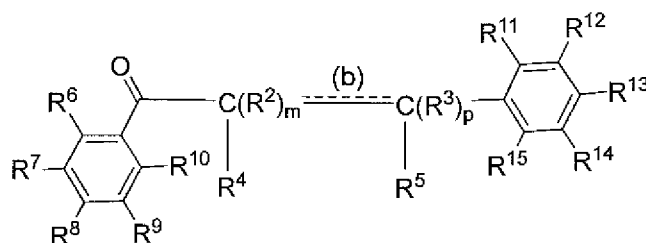
wherein R^6 , R^7 , R^8 , R^9 and R^{10} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, or wherein two residues in ortho position to each other form a cyclic or heterocyclic ring, preferably wherein at least one of R^6 , R^7 , R^8 , R^9 and R^{10} is OH.

4. Use according to any one of embodiments 1 to 3, wherein compound has a structure according to formula (I) and wherein B is



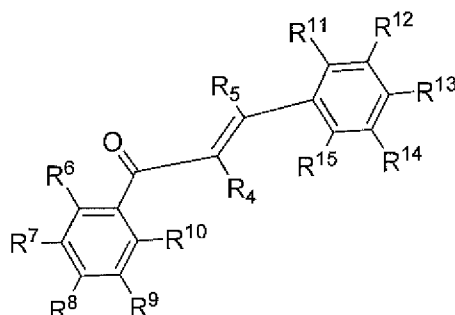
wherein R^{11} , R^{12} , R^{13} , R^{14} and R^{15} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, or wherein two residues in ortho position to each other form a cyclic or heterocyclic ring.

5. Use according to any one of embodiments 1 to 3, wherein compound has a structure according to formula (I) and wherein n is 0, (a) is a double bond and X is O or S, preferably O, more preferably wherein the compound has the structure



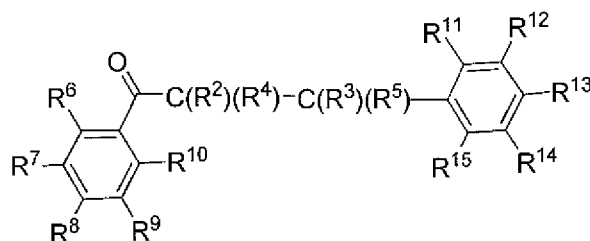
wherein R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, more preferably wherein at least one of R^6 , R^7 , R^8 , R^9 and R^{10} is OH.

6. Use according to embodiment 5, wherein b is a double bond and the compound has the structure

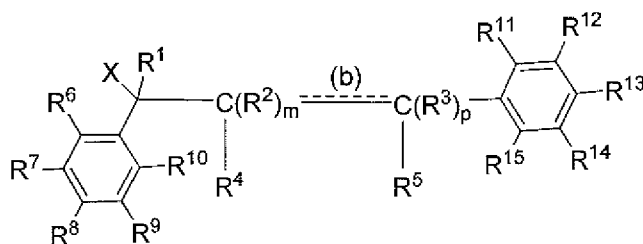


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7. Use according to embodiment 5, wherein b is a single bond and the compound has the structure

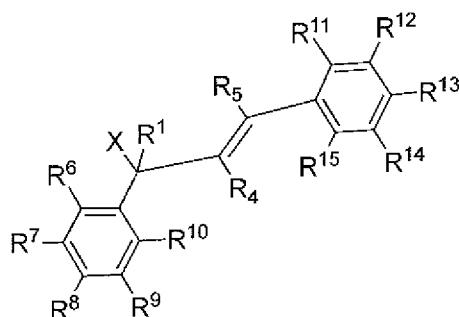


- 10 8. Use according to any one of embodiments 1 to 3, wherein n is 1, (a) is a single bond and X is H or alkyl, more preferably wherein the compound has the structure

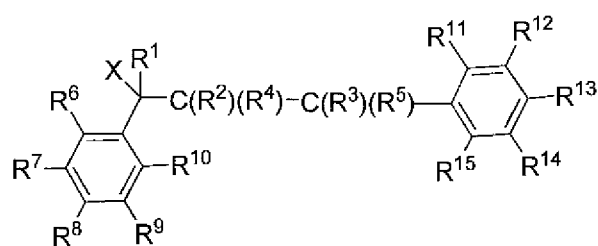


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9. Use according to embodiment 8, wherein b is a double bond and the compound has the structure

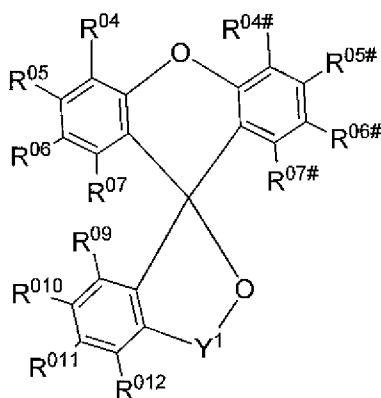


10. Use according to embodiment 5, wherein b is a single bond and the compound has the structure



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11. Use according to embodiment 1 or 2, wherein in formula (II) Y^2 is O, and wherein r is 1 and wherein q and s are 0, the compound having the structure

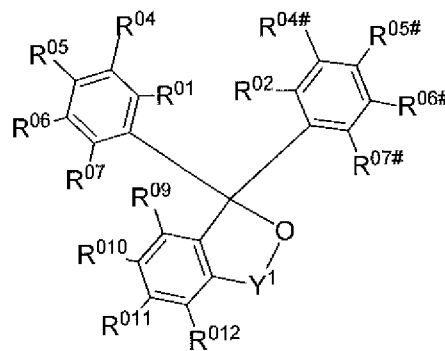


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12. Use according to embodiment 1 or 2, wherein in formula (II) wherein r is 0 and wherein q and s are 1, the compound having the structure

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13. Use according to embodiment 11 or 12, wherein R^{04} , R^{07} , $R^{04\#}$, $R^{07\#}$, R^{09} , and R^{012} are H.
14. Use according to any one of embodiments 11 to 13, wherein Y^1 is (S(=O)2).
15. Use according to any one of c embodiments 11 to 13, wherein Y^1 is (S(=O)).
16. Use according to any one of embodiments 11 to 13, wherein Y^1 is (C(=O)).
17. Use according to any one of embodiments 11 to 116, wherein R^{05} , R^{06} , $R^{05\#}$, $R^{06\#}$ are OH.

The figures show:

Fig. 1: Partial alignment of PEP carboxylases derived from various C3 and C4 plants. Indicated are the conserved arginine residues in C3 PEP carboxylases, and the conserved glycine residues in C4 PEP carboxylases. Numbering is according to *Flaveria pringlei* and *Flaveria trinervia*.

Fig. 2: Selective inhibition of a C4-PEPC by butein. Activity of C4 PEPC was measured over a range of butein concentrations. The half maximal (50 %) inhibitory concentration (IC₅₀) of butein for C4 PEPC was determined from these data that were measured in triplicate for each concentration. Corresponding activity of a C3 PEPC at the IC₅₀ concentration of butein for the C4 enzyme is also indicated in the Figure.

Fig. 3: Selective inhibition of a C4-PEPC by pyrocatechol violet. Activity of C4 PEPC was measured over a range of pyrocatechol violet concentrations. The half maximal (50 %) inhibitory concentration (IC₅₀) of pyrocatechol violet for C4 PEPC was determined from these data that were measured in triplicate for each concentration. Corresponding activity of a C3 PEPC at the IC₅₀ concentration of pyrocatechol violet for the C4 enzyme is also indicated in the Figure.

Fig. 4: Selective inhibition of a C4-PEPC by 1-(3'-carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl(ethene)). Activity of C4 PEPC was measured over a range of 1-(3'-carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl(ethene) concentrations. The half maximal (50 %) inhibitory concentration (IC₅₀) of 1-(3'-carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl(ethene) for C4 PEPC was determined from these data that were measured in triplicate for each concentration. Corresponding activity of a C3 PEPC at the IC₅₀ concentration of 1-(3'-carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl(ethene) for the C4 enzyme is also indicated in the Figure.

Fig. 5: Inhibition of a C4-PEPC by 2-(3,4-Dihydroxyphenyl)-6,7-dimethoxyquinoxaline (compound not according to the invention). Activity of C4 PEPC was measured over a range of 2-(3,4-Dihydroxyphenyl)-6,7-dimethoxyquinoxaline concentrations. The half maximal (50 %) inhibitory concentration (IC₅₀) of 2-(3,4-Dihydroxyphenyl)-6,7-dimethoxyquinoxaline for C4 PEPC was determined from these data that were measured in triplicate for each concentration. Corresponding activity of a C3 PEPC at the IC₅₀ concentration of 2-(3,4-Dihydroxyphenyl)-6,7-dimethoxyquinoxaline for the C4 enzyme is also indicated in the Figure.

Fig. 6: Inhibition of a C4-PEPC by catechin (compound not according to the invention). Activity of C4 PEPC was measured over a range of catechin concentrations. The half maximal (50 %) inhibitory concentration (IC₅₀) of catechin for C4 PEPC was determined from these data that were measured in triplicate for each concentration. Corresponding activity of a C3 PEPC at the IC₅₀ concentration of catechin for the C4 enzyme is also indicated in the Figure.

Fig. 7: Side by side comparison of the Allosteric Feedback Inhibitor Binding Site (Malate Binding Site) in PEPC. (A) Inhibitor-binding site of C4-PEPC (*F. trinervia*). (B) Inhibitor Binding Site of C3-PEPC (*F. pringlei*). Residues Arg641, Lys829, Arg888 and Asn964 have been identified as the malate-binding motif. In C3-PEPC, Arg884 provides an additional hydrogen bond for inhibitor binding. In C4-PEPC, this residue is replaced by glycine, which is 6.9 Å away from the inhibitor molecule. The bound feedback inhibitor is shown in the center of the binding site and labelled Asp1. Figures were made using LIGPLOT (Wallace *et al.*, 1995).

Fig. 8: Allosteric Feedback Inhibitor Binding Site (Malate Binding Site) of C4-type PEPCs. Residues Arg641, Lys829, Arg888 and Asn964 (*Flaveria* numbering) have been identified as the malate-binding motif. In C3-PEPCs (see Figure 7 B) Arg884 provides an additional hydrogen bond for inhibitor binding. In C4-PEPCs, this residue is replaced by glycine, glutamine, serine or glutamate (see Paulus *et al.*, 2013a and 2013b). The bound feedback inhibitor is shown in the center of the binding site and labelled Asp1. Figures were made using LIGPLOT (Wallace *et al.*, 1995).

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Example 1:

The histidine-tagged PEP carboxylase from *Flaveria pringlei* or from *Flaveria trinervia* was heterologously expressed in *E. coli* and purified via immobilized metal affinity chromatography (IMAC). Crystals of the C3 (*F. pringlei*) and the C4 (*F. trinervia*) PEP carboxylase were obtained from the purified proteins by microbatch vapor diffusion. Crystals were analyzed by synchrotron radiation and PEPC structures were determined from the diffraction data by molecular replacement using XDS, coot and the CCP4 suite (Kabsch, 2010; Emsley *et al.* 2004; CCP4, 1994).

Crystal structures of PEP carboxylase from *Flaveria pringlei* (PDB code: 3ZGB) or from *Flaveria trinervia* (PDB code: 3ZGE) were superimposed by the alignment algorithm of

PyMOL (DeLano, 2002) which performs a BLAST-like BLOSUM62-weighted dynamic programming sequence alignment followed by a series of refinement cycles intended to improve the fit by eliminating pairing with high relative variability. The malate binding site in the aligned crystal structures was visually inspected for structural differences in the region of 10-15 Å around the bound aspartate inhibitor. From this alignment the structural difference in position 884 was identified. The C3 type PEP carboxylase of *Flaveria pringlei* has a voluminous arginine side chain in this position, while the C4 type PEP carboxylase of *Flaveria trinervia* carries a small glycine residue in the corresponding position.

Potential selective inhibitors of C4 PEP carboxylase were selected from a Virtual Drug Screening (VDS) approach using the program PyRX (Wolf, 2009), standard compound libraries (ChemBank, NCI DataBase, KEGG Database), a library assembled from the Plant Metabolome Database and the high resolution crystal structures of PEP carboxylases from *F. pringlei* and *F. trinervia*.

In particular, the potential selective inhibitors of C4 PEP carboxylase shown in Table 3 and Table 4 as well as in Figures 5 and 6 were identified:

Example 2: Confirmation of selective inhibition of selected compounds

Inhibition of purified PEP carboxylases from *F. pringlei* and *F. trinervia* by compounds selected from the VDS was monitored by a spectrophotometric coupled assay with malate dehydrogenase which reduces the oxaloacetate formed by PEP carboxylase to malate. The simultaneous oxidation of NADH is followed at 340 nm with standard optical equipment. The concentration of the inhibitors selected from the VDS was varied in the coupled assay to determine the half maximal inhibitory concentration (IC₅₀).

With butein and pyrocathol violet, the inhibition shown in Fig. 2 and 3 of the PEP carboxylase activity of the C3 and the C4 enzyme was obtained. With 1-(3'-carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl(ethene), the inhibition shown in Fig. 4 of the PEP carboxylase activity of the C3 and the C4 enzyme was obtained. Further results are given

in Table 3 and Table 4. Comparative examples for compounds described earlier in PCT/EP2012/076648 are given in Figure 5 and 6.

Example 3: Determination of the binding constant of C4-selective inhibitors in the presence and in the absence of the natural feedback inhibitor malate.

The dissociation constants (K_d) of PEPC from *F. trinervia* for putative C4-selective inhibitors (for details see Table below) will be determined by microscale thermophoresis (MST, Duhr and Braun, 2006; Jerabek-Willemsen *et al.*, 2011) using the Monolith NT.115 (NanoTemper Technologies, Munich, Germany). Measurements will be done in the absence and in the presence of the natural feedback inhibitor malate (20 mM) in order to allow or to protect binding of the C4-selective inhibitor to the allosteric malate feedback inhibitor site.

Purified protein is buffered in 50 mM potassium phosphate (pH 7,6), 150 mM NaCl, 0,05 % Tween 20 and labelled with NT-547-Maleimide (NanoTemper Technologies) according to the protocol of the manufacturer. The compounds are dissolved in organic solvents like DMSO, acetone or ethanol or in aqueous buffer solutions at high concentrations.

For MST measurements the stock solutions are diluted with protein buffer (50 mM potassium phosphate (pH 7,6), 150 mM NaCl, 0,05 % Tween 20). The above solvent is used to prepare a 1:1 serial dilution in 16 dilution steps. 10 μ l of the compound dilution is mixed with 10 μ l protein solution and filled into capillaries. The compound concentration is highest in the first capillary and decreases in the next capillaries according to the 1:1 dilution. Data are fitted according to a cooperative binding model.

Tab. 1 Substances to be tested in binding studies by microscale thermophoresis

Butein
Robtein
Piceatannol
trans-1-(3'-Carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl)ethene
Pyrocatechol Violet
Gallein
Cresol Red
Chrome Cyanine R

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Collaborative Computational Project, Number 4 (1994)
The CCP4 Suite: Programs for Protein Crystallography. Acta Cryst. D50, 760-763.

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DeLano, WL, (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA, USA.

Doyle, JR, Burnell, JN, Haines, DS, Llewellyn, LE, Motti, CA and Tapiolas, DM (2005) A
Rapid Screening Method to Detect Specific Inhibitors of Pyruvate Orthophosphate
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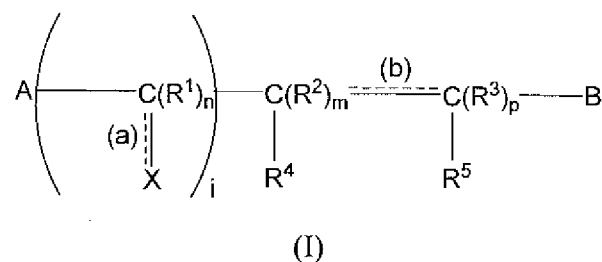
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Claims:

1. Use of at least one compound, a salt or solvate thereof as C4 plant selective herbicide wherein said compound has a structure according to

(a) formula (I)



wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group,

wherein A is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group, and B is a cyclic alkyl, aryl, heterocycloalkyl, or heteroaryl group,

and wherein R^1 , R^2 , R^3 , R^4 and R^5 are, independently of each other H or an alkyl group,

and wherein integer i is 0 or 1, preferably 1

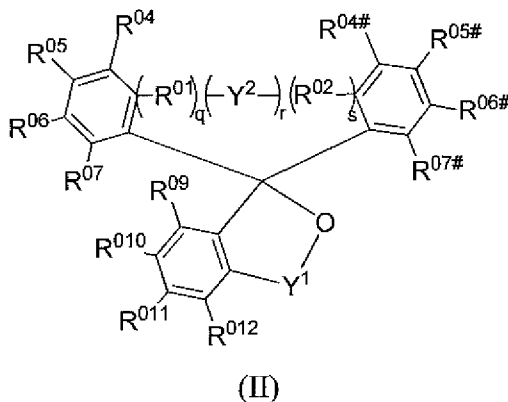
and the bond (a) is a single or double bond,

and wherein in case (a) is a double bond, n is 0 and X is O or S, and wherein in case (a) is a single bond n is 1, and X is H or an alkyl group,

and wherein the bond (b) is a single or double bond, and wherein in case (b) is a double bond, m and p are 0, and wherein in case (b) is a single bond m and p are both 1,

and/or

(a) formula (II)



including tautomeric structures thereof

wherein R^{01} and R^{02} are independently of each other selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen,

wherein Y^1 is selected from the group consisting of $(S(=O)_2)$, $S(=O)$ and $(C(=O))$

and wherein Y^2 is O, and wherein r is 0 or 1 and wherein in case r is 0, q and s are 1, and wherein in case r is 1, q and s are 0,

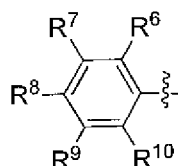
and wherein R^{01} , R^{02} , R^{04} , R^{05} , R^{06} , R^{07} , $R^{04\#}$, $R^{05\#}$, $R^{06\#}$, $R^{07\#}$, R^{09} , R^{10} , R^{11} and R^{12} are independently of each other selected from the group consisting of H, OH, $-SO_3H$, carboxylic acid, ester, alkyl, alkoxy and halogen,

said compound, preferably, being capable of binding to the malate binding site comprised by a phosphoenolpyruvate carboxylase from a C4 plant, thereby inhibiting said phosphoenolpyruvate carboxylase.

2. Use according to claim 1, wherein the binding to the malate binding site of a phosphoenolpyruvate carboxylase from a C3 plant is inhibited, preferably by steric or electrostatic constraints of a conserved arginine in the binding site of the C3 PEPC, preferably by Arg-884 in *Flaveria pringlei*.

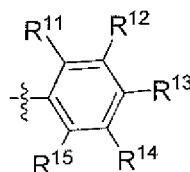
3. Use according to claim 1 or 2, wherein compound has a structure according to

formula (I) and wherein A is



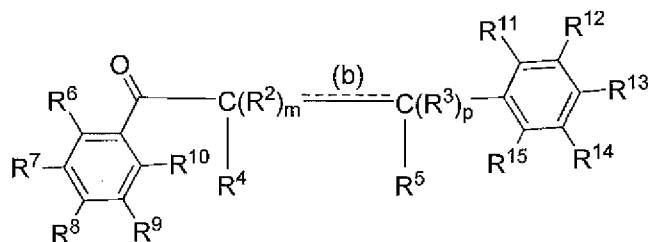
wherein R⁶, R⁷, R⁸, R⁹ and R¹⁰ are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, or wherein two residues in ortho position to each other form a cyclic or heterocyclic ring, preferably wherein at least one of R⁶, R⁷, R⁸, R⁹ and R¹⁰ is OH.

4. Use according to any one of claims 1 to 3, wherein compound has a structure according to formula (I) and wherein B is



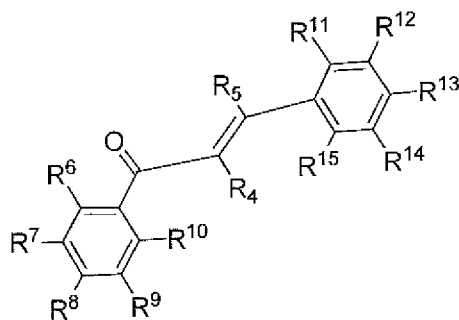
wherein R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen or wherein two residues in ortho position to each other form a cyclic or heterocyclic ring.

5. Use according to any one of claims 1 to 3, wherein compound has a structure according to formula (I) and wherein n is 0, (a) is a double bond and X is O or S, preferably O, more preferably wherein the compound has the structure

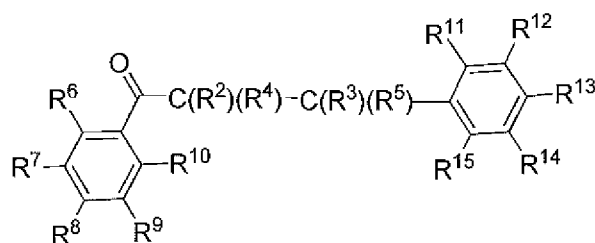


wherein $R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}$ and R^{15} are, independently of each other, selected from the group consisting of H, OH, carboxylic acid, ester, alkyl, alkoxy and halogen, more preferably wherein at least one of R^6, R^7, R^8, R^9 and R^{10} is OH.

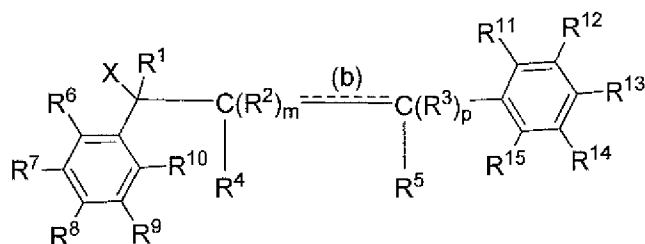
6. Use according to claim 5, wherein b is a double bond and the compound has the structure



7. Use according to claim 5, wherein b is a single bond and the compound has the structure

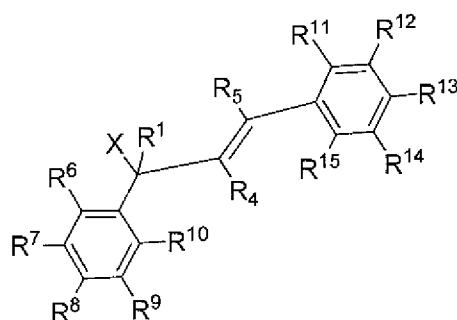


8. Use according to any one of claims 1 to 3, wherein n is 1, (a) is a single bond and X is H or alkyl, more preferably wherein the compound has the structure



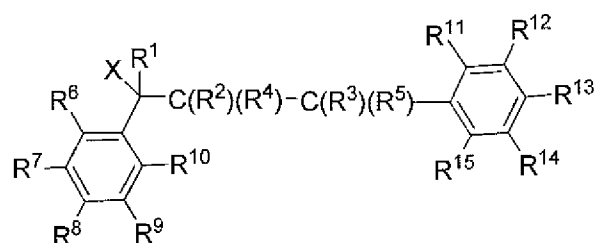
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9. Use according to claim 8, wherein b is a double bond and the compound has the structure



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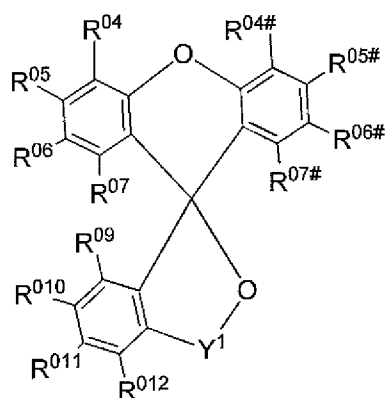
10. Use according to claim 5, wherein b is a single bond and the compound has the structure



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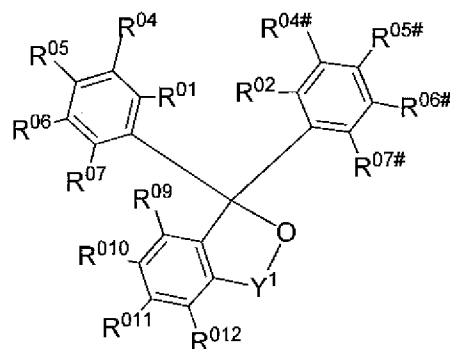
11. Use according to claim 1 or 2, wherein in formula (II) Y² is O, and wherein r is 1 and wherein q and s are 0, the compound having the structure

- 54 -



12. Use according to claim 1 or 2, wherein in formula (II) wherein r is 0 and wherein q and s are 1, the compound having the structure

5



13. Use according to claim 11 or 12, wherein R^{04} , R^{07} , $R^{04\#}$, $R^{07\#}$, R^{09} , and R^{012} are H.

- 10 14. Use according to any one of claims 11 to 13, wherein Y^1 is $(S(=O)_2)$.

15. Use according to any one of claims 11 to 13, wherein Y^1 is $(S(=O))$.

16. Use according to any one of claims 11 to 13, wherein Y^1 is $(C(=O))$.

15

17. Use according to any one of claims 11 to 17, wherein R^{05} , R^{06} , $R^{05\#}$, $R^{06\#}$ are OH.

Fig. 1

C3 -Type

884	
F_pringlei	PYLKQ R IRLRDSYITTLNVCQAYTLKRIRDPNY (SEQ ID NO: 7)
Hordeum vulgare	PYLKQ R LLRLRDPYITTLNVCQAYTLKRIRDPSE (SEQ ID NO: 3)
Oryza_sativa	LYLKQ R LLRLRNAYITTLNVCQAYTMKRIRDPDY (SEQ ID NO: 4)
ssp. indica	
Triticum_aestiv	PYLKQ R LLRLRDAYITTMNVCQAYTLKRIRDPDY (SEQ ID NO: 5)
A_thaliana	PYLKQ R LLRLRDSYITTLNVCQAYTLKRIRDANY (SEQ ID NO: 6)

C4 -Type

884	
F_trinervia	PYLKQ G IRLRDPYITTLNVCQAYTLKRIRDPNY (SEQ ID NO: 8)
F_australasica	PYLKQ G IRLRDPYITTLNVCQAYTLKRIRDPNY (SEQ ID NO: 9)
Zea mays	PFLKQ G LVLRNPYITTLNVFQAYTLKRIRDPNF (SEQ ID NO: 10)
Sorghum_bicolor	PYLKQ G LLRLRNPYITTLNVFQAYTLKRIRDPSE (SEQ ID NO: 11)
Saccharum_spont	PYLKQ G LLRLRNPYITTLNVLQAYTLKRIRDPSE (SEQ ID NO: 12)
Saccharum_offic	PYLKQ G LLRLRNPYITTLNVLQAYTLKRIRDPCE (SEQ ID NO: 13)

Fig. 2

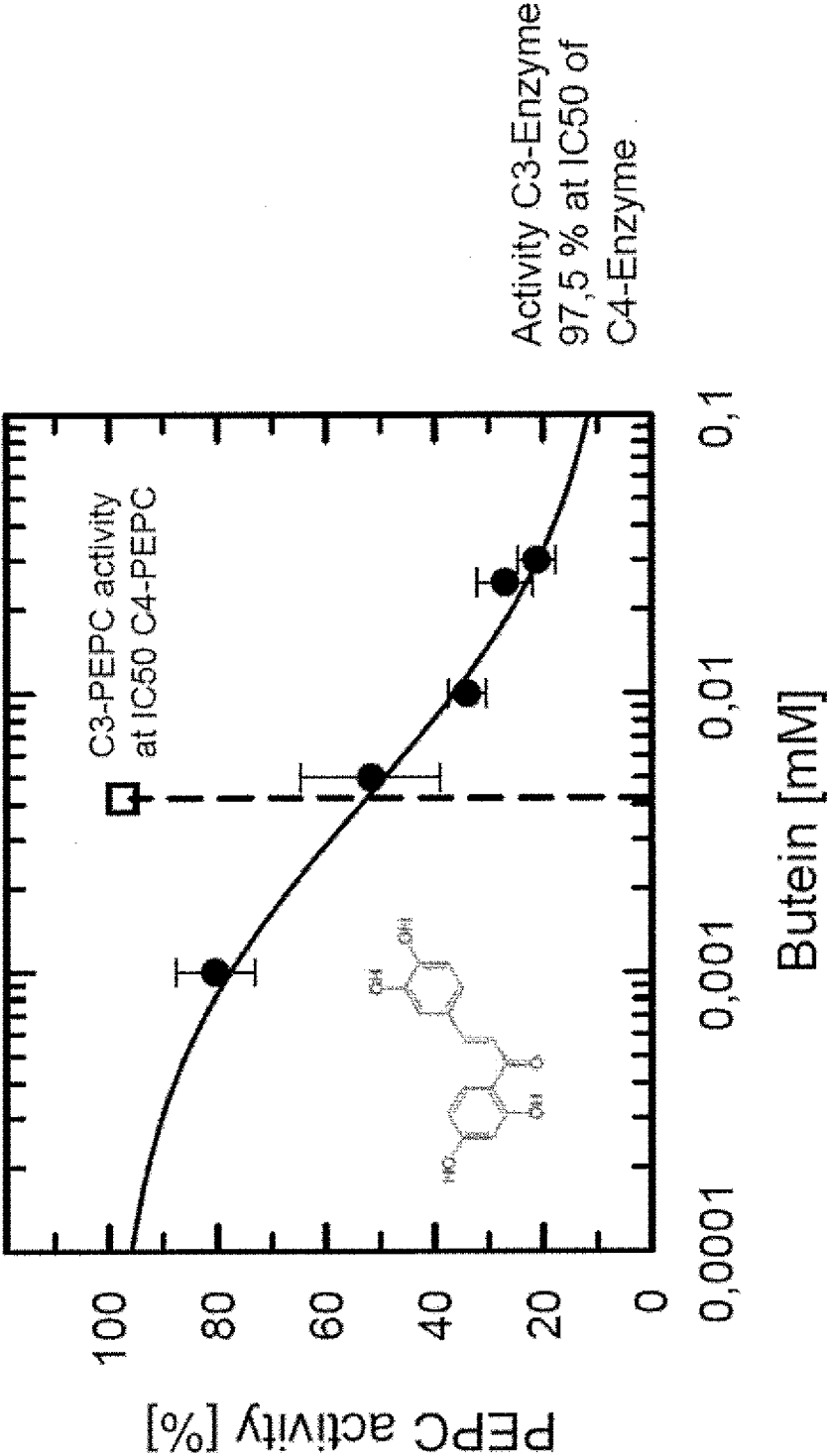


Fig. 3:

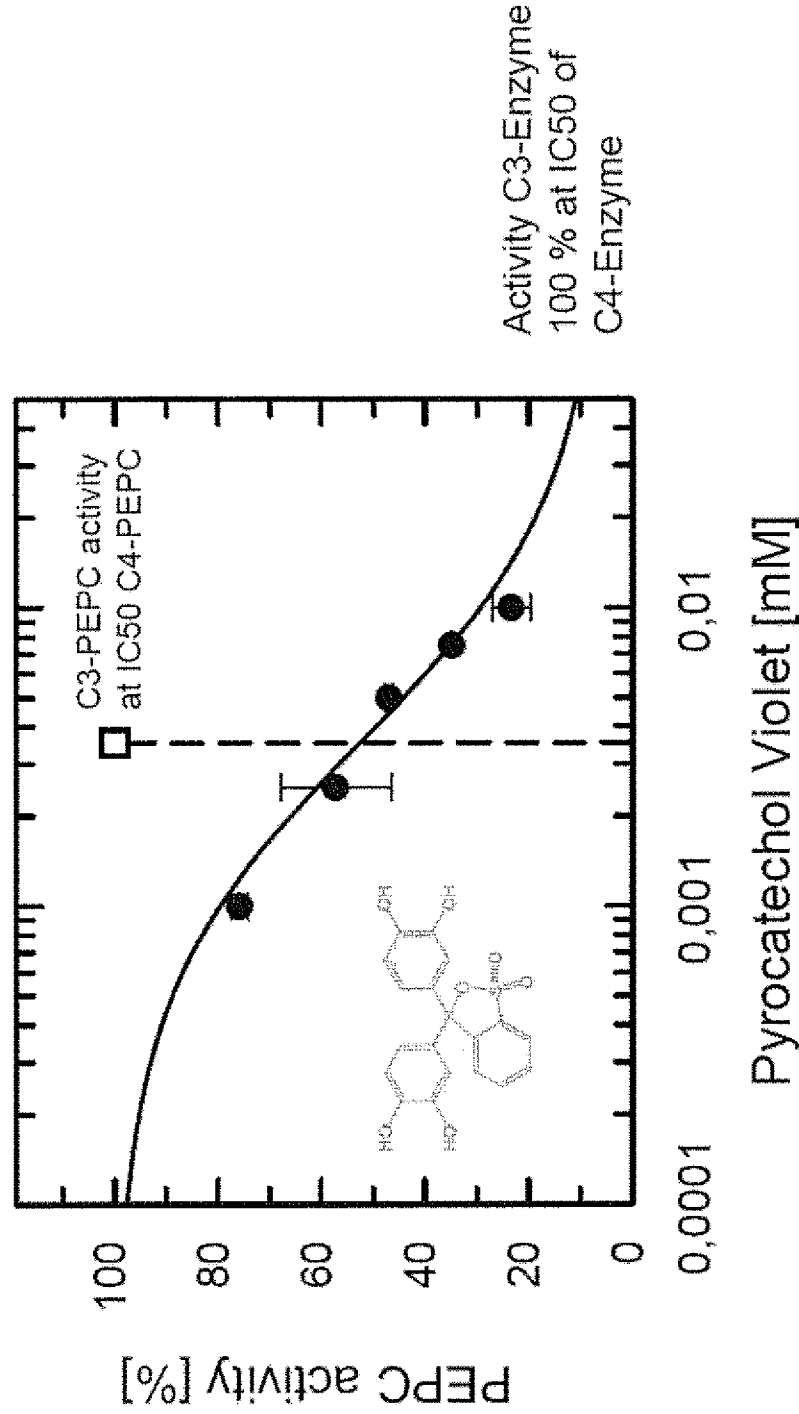


Fig. 4

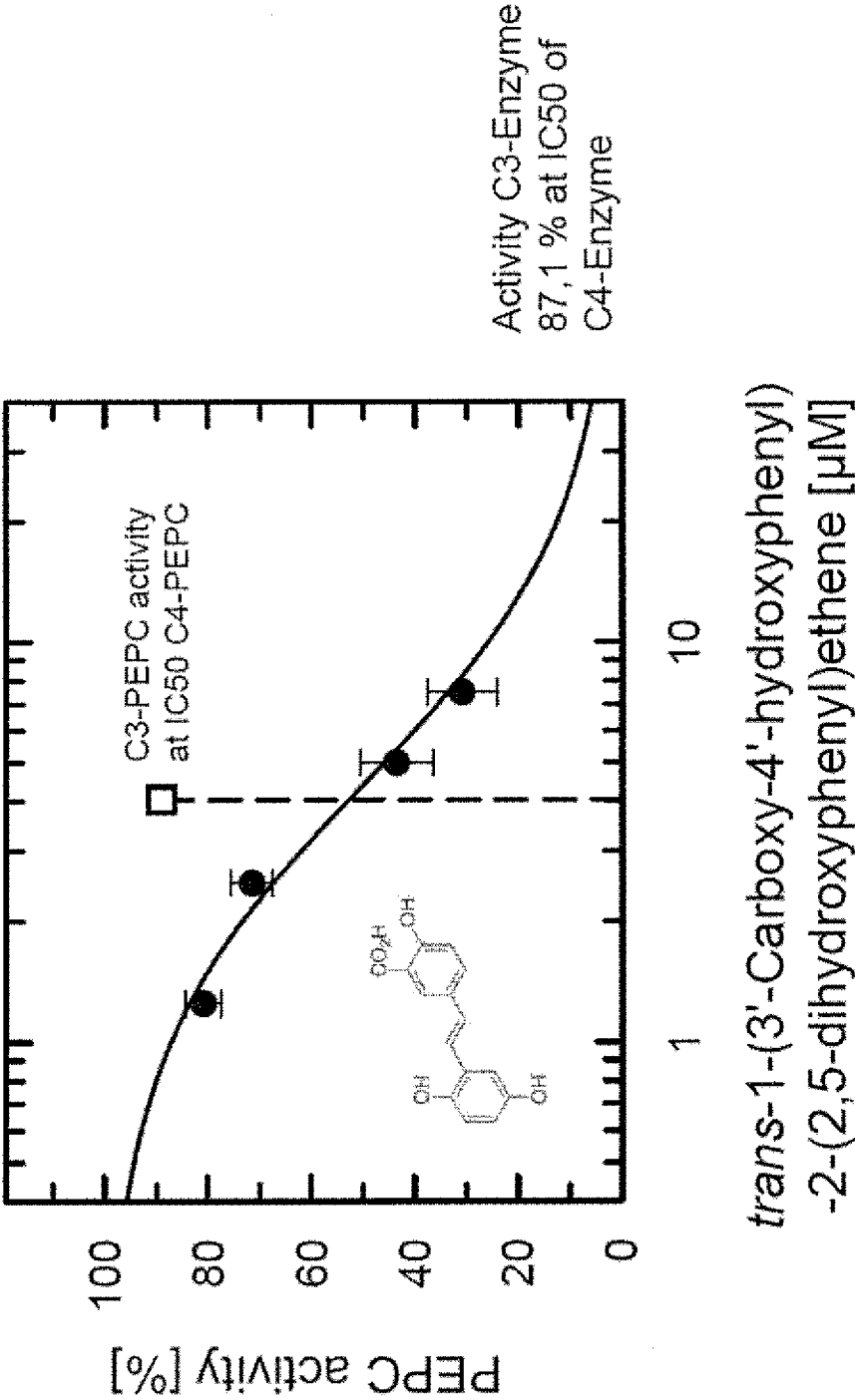
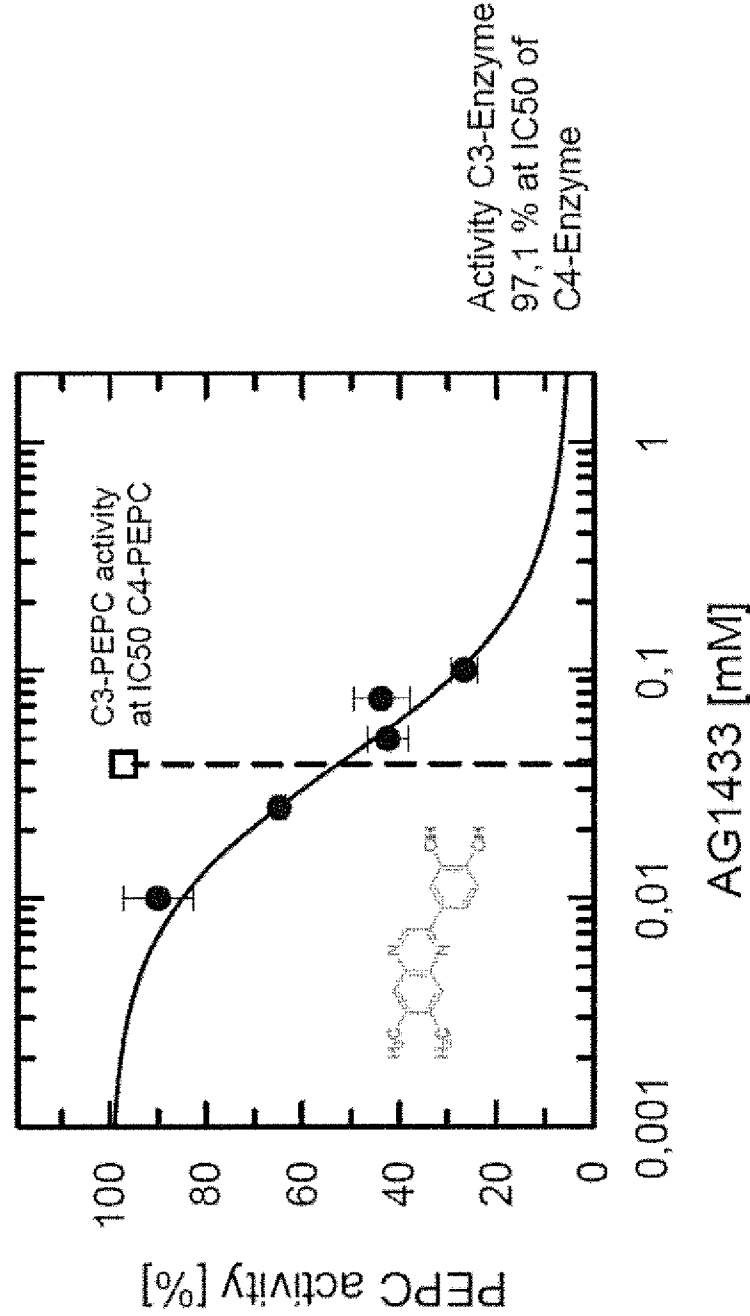


Fig. 5:



2-(3,4-Dihydroxyphenyl)-6,7-dimethylquinoxaline

Fig. 6:

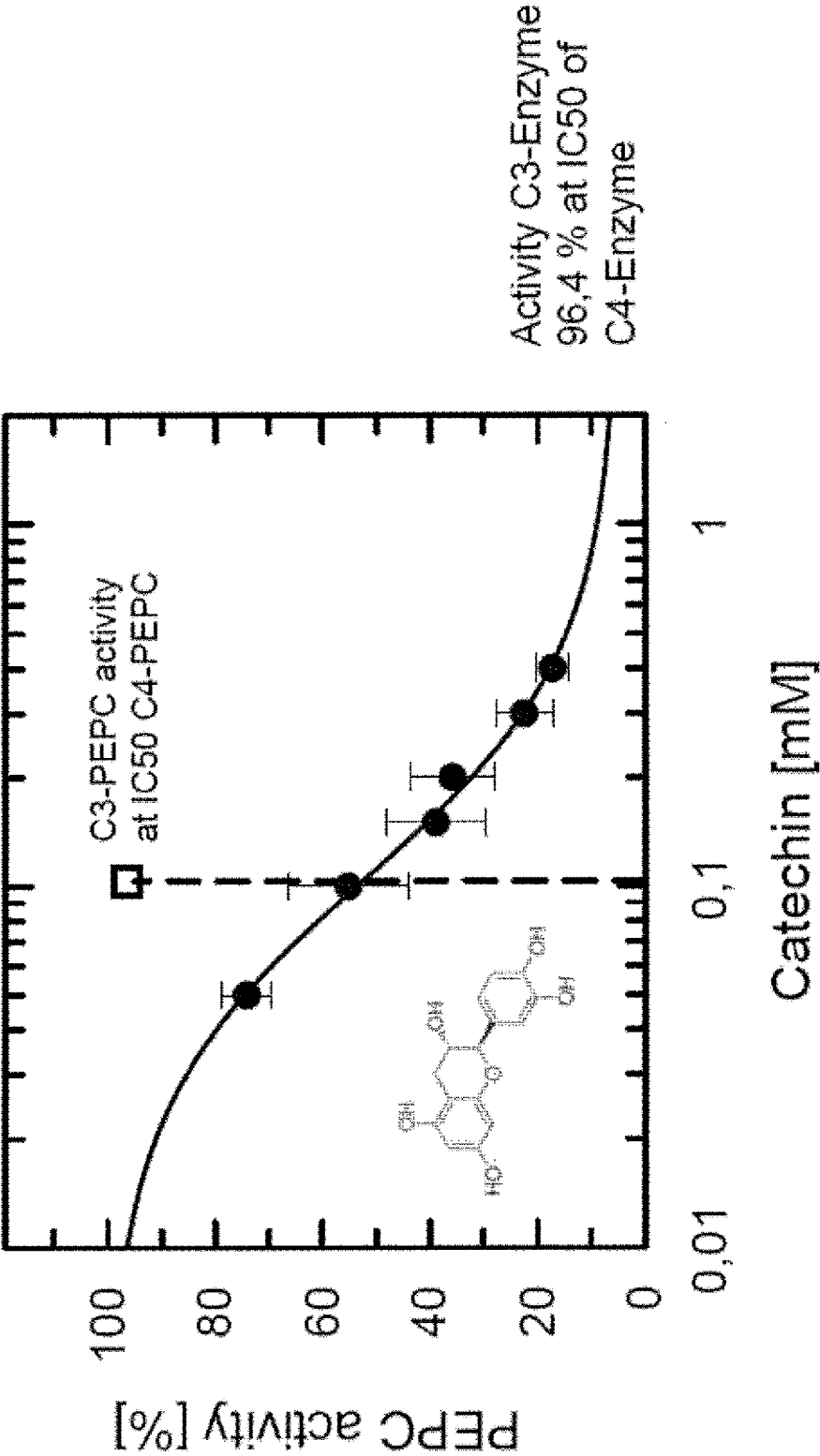
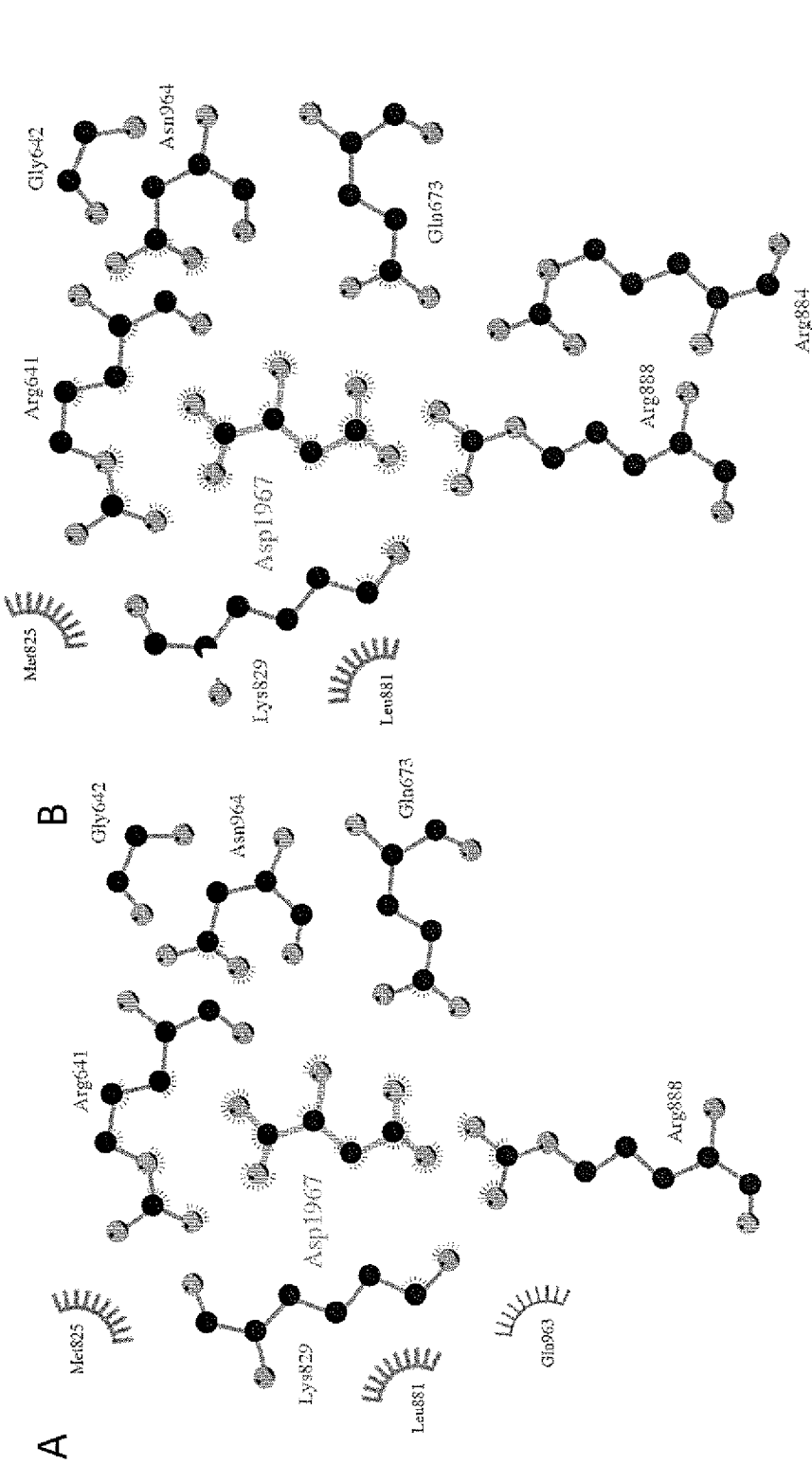


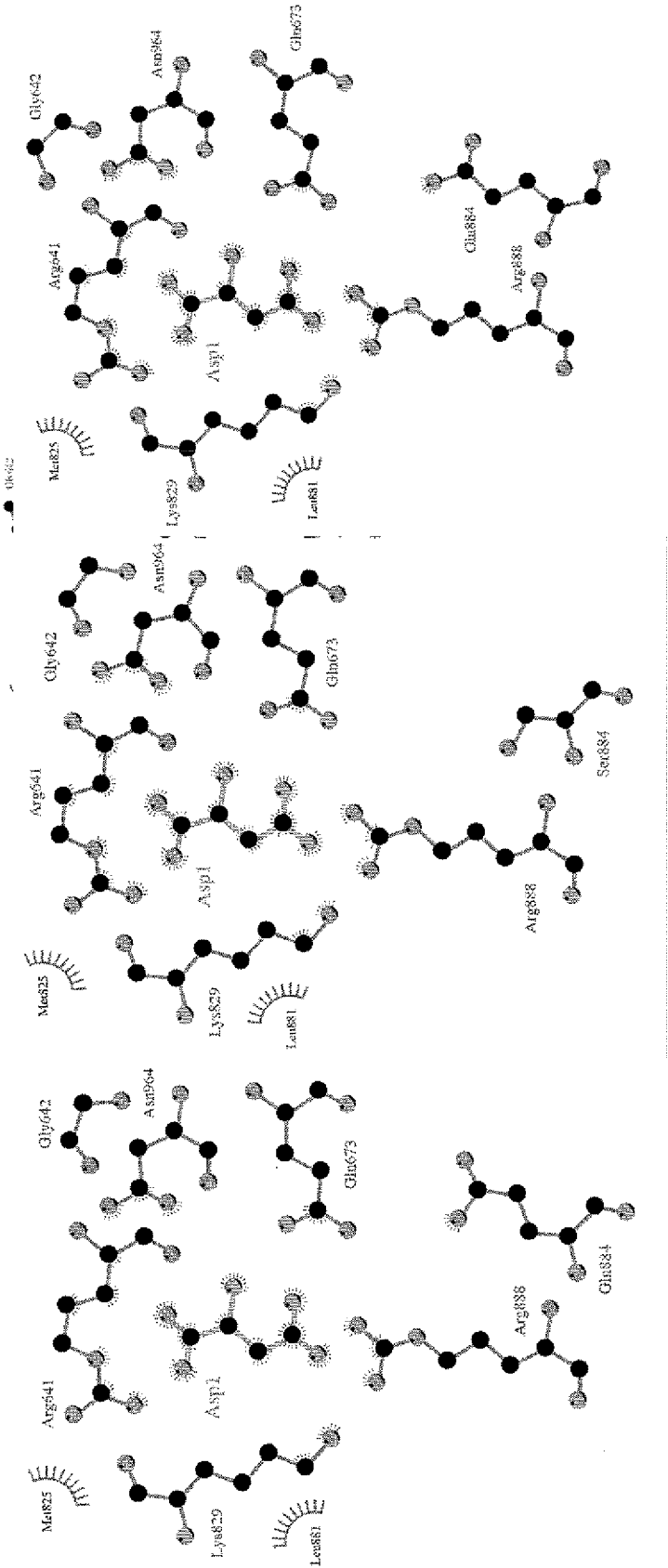
Figure 7



C4-PEPC from *F. trinervia*

C3-PEPC from *F. pringlei*

Figure 8

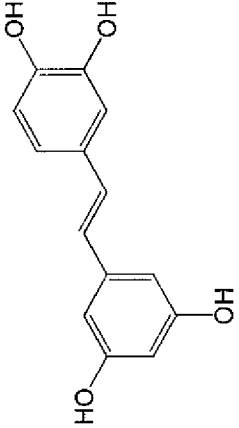
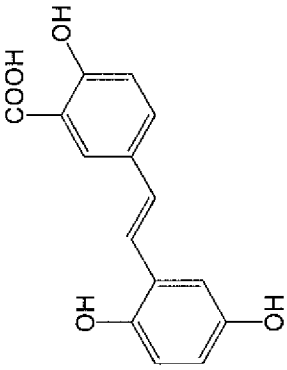
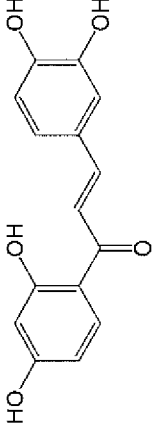


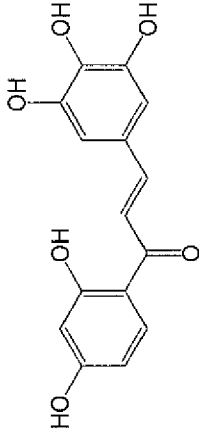
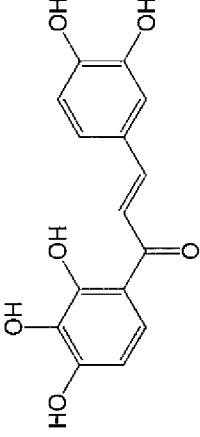
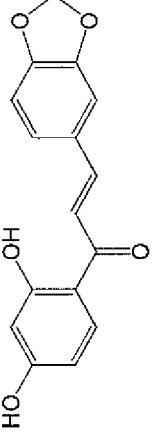
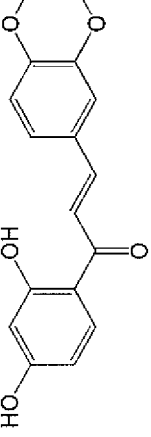
C4-type PEPC R884E

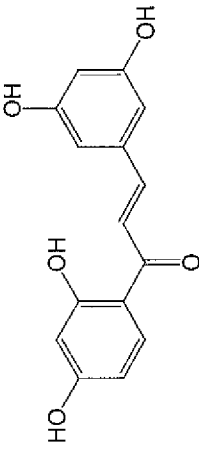
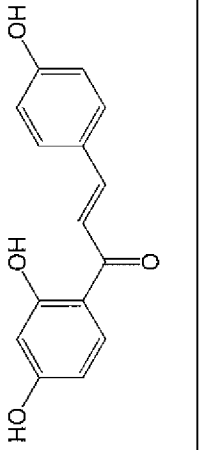
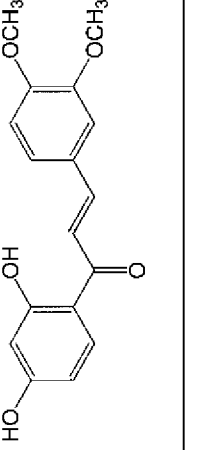
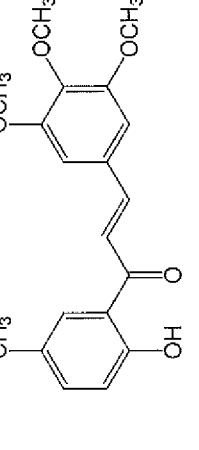
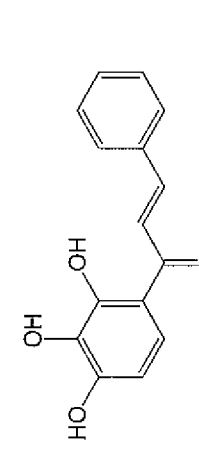
C4-type PEPC R884S

C4-type PEPC R884Q

Table 3:

Structure	Name	Predicted Binding Affinity [kcal/mol]	IC50 for C4-PEPC	Activity C3 PEPC at IC50 C4 PEPC
	Piceatannol	-8.4	14 μ M	81.8 %
	trans-1-(3'-Carboxy-4'-hydroxyphenyl)-2-(2,5-dihydroxyphenyl)ethene	-8.8	4 μ M	87.1 %
	Butein	-8.9	4.2 μ M	97.5 %

	Robtein	-9.2	13.8 μ M	92.5 %
	Okanin	-9.3		
	3-benzo[1,3] dioxol-5-yl-1-(2,4-dihydroxyphenyl)-prop-2-en-1-one	-9.0		
	1-(2,4-dihydroxyphenyl)-3-(2,5-dioxabicyclo[4.4.0]dec a-6,8,10-trien-8-yl)prop-2-en-1-one	-9.0		

	Pseudoindorin	-8.7		
	Isoliquiritigenin	-8.7		
	2',4'-Dihydroxy-3,4-Dimethoxy-chalcone	-8.7		
	(2E)-1-(2-hydroxy-5-methylphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one	-8.7		
	2',3',4'-Trihydroxychalcone	-8.6		

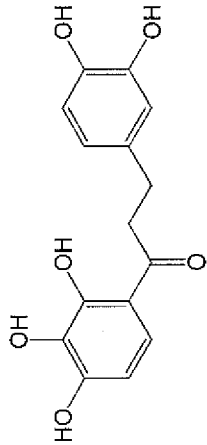
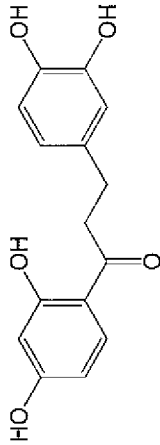
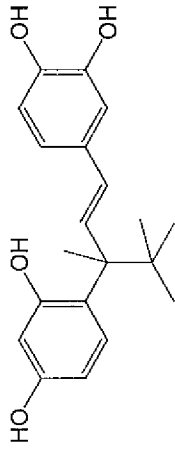
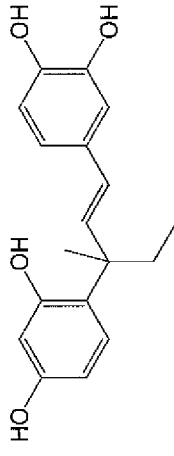
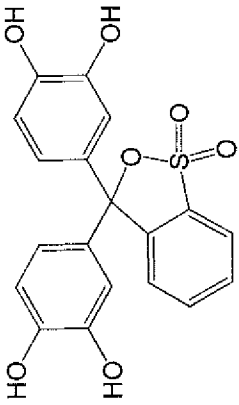
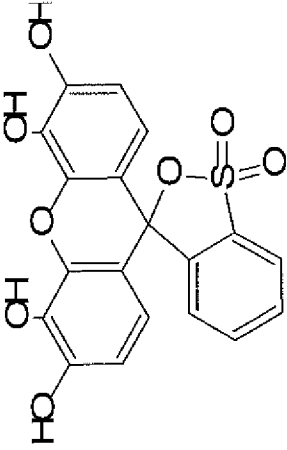
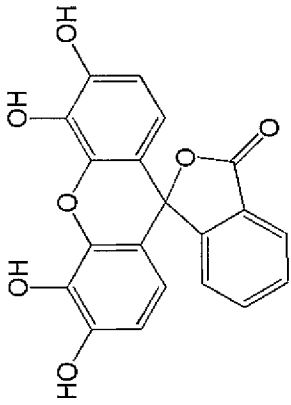
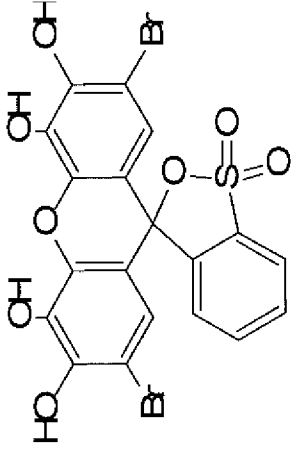
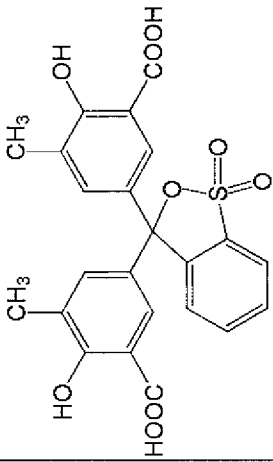
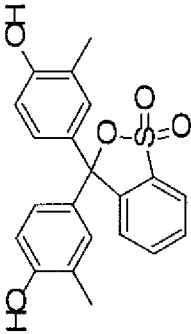
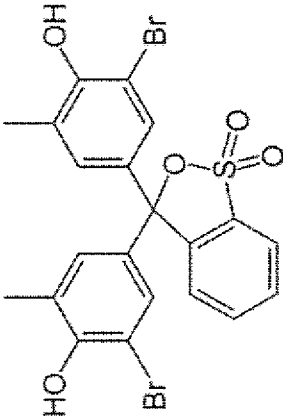
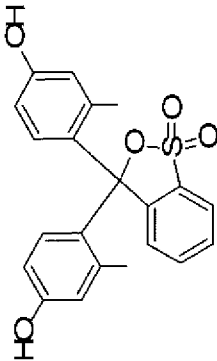
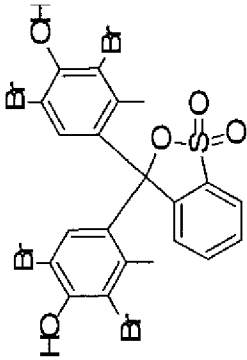
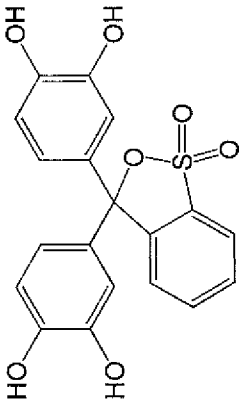
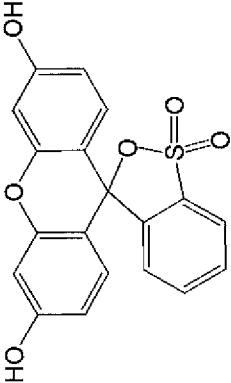
	3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)propan-1-one	-8.9		
	1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)propan-1-one	-8.6		
		-9.3		
		-9.2		

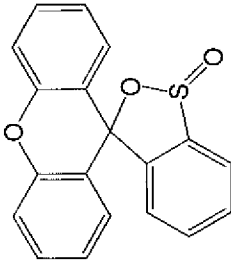
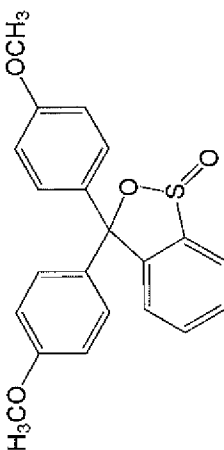
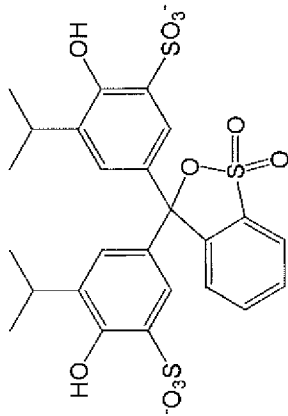
Table 4:

Structure	Name	Predicted Binding Affinity [kcal/mol]	IC50 for C4-PEPC	Activity C3 PEPC at IC50 C4 PEPC
	Pyrocatechol Violet	-9.9	3.5 μ M	100 %
	Pyrogallol Red	9.7		

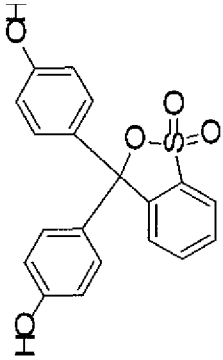
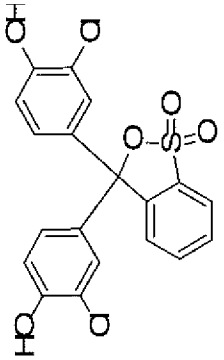
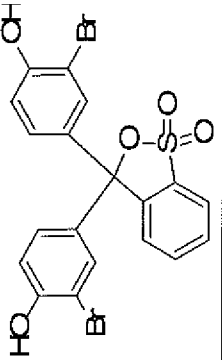
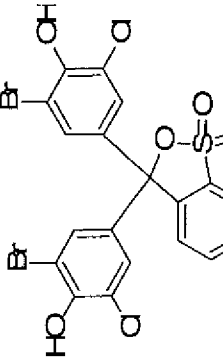
	Gallein	-10.9	7.9 μ M	95.9 %
	Bromopyrogallol Red			
	Chrome Cyanine R	-9.6	17.7 μ M	89.5 %

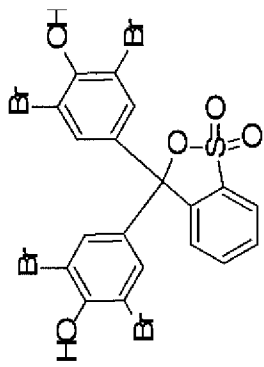
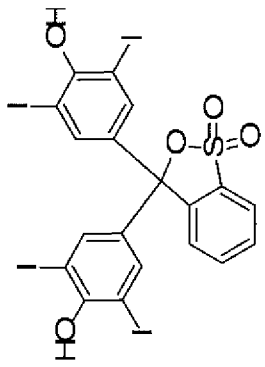
	Cresol Red	-9.3	106 μM	82.3 %
	Bromocresol purple			
	Cresol purple			

	Bromocresol green			
	Chlorophenol Red	-9.8		
	Sulfonfluorescein	-9.9		

	BLAH spiro[benzo[c][1,2]oxathiole-3,9'-xanthene] 1-oxide	-9.7		
	3,3-bis(4-methoxyphenyl)-3H-benzo[c][1,2]oxathiole 1-oxide	-9.9		
	5,5'-(1,1-dioxido-3H-benzo[c][1,2]oxathiole-3,3-diyl)bis(2-hydroxy-3-isopropylbenzenesulfonate)	-9.3		

Thymol blue	Bromothymol blue	Xylenol Blue	Bromoxylenol blue

Phenol red	Chlorophenol red	Bromophenol red	Bromochlorophenol blue
			

Bromophenol blue	Iodophenol blue
	

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/050766

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A01P13/02 A01N43/30	A01N31/16 A01N43/32
	A01N35/04 A01N43/90	A01N37/40 A01N43/26
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) A01N		
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 practicable, search terms used) EPO-Internal, BIOSIS, 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,P	WO 2013/093007 A1 (UNIV DUESSELDORF H HEINE [DE]) 27 June 2013 (2013-06-27) page 64, line 22; compound 2 page 81; compound 5 claims 1,11	1-4,8-10
X	----- DATABASE CAPLUS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; GRONOWSKA, JANINA ET AL: "Method of obtaining novel isomeric 3,3-diarylpthalides", XP002720583, retrieved from STN Database accession no. 1995:682843 abstract & PL 164 191 B1 (UNIWERSYTET MIKOLAJA KOPERNIKA, POL.) 30 June 1994 (1994-06-30) ----- -/-	1,2, 11-17
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "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 19 February 2014		Date of mailing of the international search report 06/03/2014
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 Marie, Gérald

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/050766

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R Harold Brown ET AL: "Assessing the Degree of C4 Photosynthesis in C3-C4 Species Using an Inhibitor of Phosphoenolpyruvate Carboxylase", Plant Physiol., 1 January 1991 (1991-01-01), pages 985-989, XP055025771, Retrieved from the Internet: URL:http://www.plantphysiol.org/content/97/3/985.full.pdf [retrieved on 2012-04-26] abstract	1-17
A	----- CLAUDE CRÉTIN ET AL: "Differential inhibition of phosphoenol-pyruvate carboxylases by 2,4-dichlorophenoxyacetic acid and two newly synthesized herbicides", PHYTOCHEMISTRY, vol. 22, no. 12, 1 January 1983 (1983-01-01), pages 2661-2664, XP055025854, ISSN: 0031-9422, DOI: 10.1016/S0031-9422(00)97668-4 abstract	1-17
A	----- MANCERA R L ET AL: "The molecular binding interactions of inhibitors and activators of phosphoenolpyruvate carboxylase", JOURNAL OF MOLECULAR STRUCTURE (THEOCHEM), ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL, vol. 755, no. 1-3, 30 November 2005 (2005-11-30), pages 151-159, XP027667700, ISSN: 0166-1280 [retrieved on 2005-11-30] abstract -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2014/050766

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013093007	A1	27-06-2013	NONE
