placental formation and development, and hypoxia. There are currently no therapies which solve the underlying vascular causes of these conditions.

In addition to its function in endothelial NO generation, Tetrahydrobiopterin (BH4) is a redox cofactor for other enzymes, specifically a group of amino acid hydroxylases which convert the amino acids tryptophan, phenylalanine and tyrosine. These enzymes are used in the degradation of the amino acid phenylalanine and in the biosynthesis of the neurotransmitters serotonin (5-hydroxytryptamine, 5 HT), melatonin, dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline).

Numerous disorders are connected with dysfunction of these hydroxylase enzymes which can also be caused by a deficiency, or reduction in the bioavailability of, the required cofactor BH4. Such disorders include, for example, BH4 deficiency itself and phenylketonuria (PKU). In addition, due to its role in neurotransmitter production, BH4 is also implicated in neurological disorders such as autism, ADHD, depression, and Parkinson's. Low availability of BH4 in specific cells and tissues may not be related only to systemic BH4 levels, but may also be influenced by local BH4 oxidation or recycling, and the distribution of BH4, for example by cellular uptake or transport.

However, as with the treatment of cardiovascular and pregnancy-related disorders, many of these diseases rely on other therapeutics which are not based on the involvement of BH4.

Only one commercially available therapeutic comprising BH4 is available in the form of *sapropterin dihydrochloride* (BH4*2HCL) and is approved for use in the treatment of PKU. However, most people with PKU have little or no benefit from the drug (Camp KM, Parisi MA, Acosta PB, Berry GT, Bilder DA, Blau N, et al. (June 2014) Molecular Genetics and Metabolism. 112 (2): 87–122). Furthermore, administration of BH4 in an oral form had no benefit in patients with existing vascular disease and endothelial dysfunction (Cunnington C et al. Circulation 2012). Another significant problem with using BH4 is that it is susceptible to oxidation.

The present invention is intended to solve one or more of the above-mentioned problems by one or more of the following aspects.

~~**Statements of Invention**~~

## Summary

In a first aspect of the present disclosure, there is provided a use of a reduced folate in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability, wherein the reduced folate is not for administration in combination with any of the following:

- aspirin;
- a herbal extract.

In another aspect of the present disclosure, there is provided a use of a combination of a reduced folate and BH4, a precursor, or functional equivalent thereof in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability.

In yet another aspect of the present disclosure, there is provided a use of a composition comprising one or more active pharmaceutical ingredients in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability, wherein the active pharmaceutical ingredient(s) consist of a reduced folate and optionally BH4, a precursor, or functional equivalent thereof.

In a further aspect of the present disclosure, there is provided a method of selecting a subject that may benefit from treatment with a reduced folate and optionally BH4, a precursor or functional equivalent thereof, the method comprising determining the level of one or more markers of a disorder associated with low BH4 bioavailability in a subject, comparing the level(s) of the or each marker to a reference level(s) in a healthy subject, and selecting the subject for treatment if the subject exhibits an abnormal level of the one or more markers of a disorder associated with low BH4 bioavailability compared to the reference level.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising a reduced folate and BH4, a precursor, or functional equivalent thereof.

In yet another aspect of the present disclosure, there is provided a kit comprising:

- a reduced folate; and
- BH4, a precursor, or functional equivalent thereof.

In a further aspect of the present disclosure, there is provided a use of a reduced folate to prevent oxidation of BH4, a precursor, or a functional equivalent thereof, *in vitro*.

## Statements of Invention

According to a first aspect of the present invention, there is provided a reduced folate for use in the prevention or treatment of a disorder associated with low BH4 bioavailability , wherein the reduced folate is not for administration in combination with any of the following:

- aspirin;
- a herbal extract.

In one embodiment, the reduced folate is for administration in combination with BH4, a precursor, or functional equivalent thereof.

In one embodiment, the reduced folate, and optionally BH4, a precursor, or functional equivalent thereof, are the sole active pharmaceutical agents. In one embodiment, the reduced folate is the sole active pharmaceutical agent.

According to a second aspect of the present invention, there is provided a combination of a reduced folate and BH4, a precursor, or functional equivalent thereof for use in the prevention or treatment of a disorder associated with low BH4 bioavailability.

According to a third aspect of the present invention, there is provided a composition comprising one or more active pharmaceutical ingredients for use in the prevention or treatment of a disorder associated with low BH4 bioavailability, wherein the active pharmaceutical ingredients consist of a reduced folate and optionally BH4, a precursor, or functional equivalent thereof.

In one embodiment, the active pharmaceutical ingredient consists of a reduced folate.

According to a fourth aspect of the present invention, there is provided a method of treating a subject having a disorder associated with low BH4 bioavailability, the method comprising administering to said subject a reduced folate, wherein the reduced folate is not administered in combination with any of the following:

- aspirin;
- a herbal extract.

In one embodiment, the reduced folate is administered in combination with BH4, a precursor, or functional equivalent thereof.

**Claims**

1. Use of a~~A~~ reduced folate ~~for use~~ in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability, wherein the reduced folate is not for administration in combination with any of the following:
    - aspirin;
    - a herbal extract.
2. ~~A reduced folate for~~The use according to claim 1, wherein the reduced folate is in combination with BH4, a precursor, or functional equivalent thereof.
3. ~~A reduced folate for~~The use according to claim 1 or 2, wherein the reduced folate, and optionally BH4, a precursor, or functional equivalent thereof, are the sole active pharmaceutical agents.
4. ~~A reduced folate for~~The use according to claim 1, wherein the reduced folate is the sole active pharmaceutical agent.
5. Use of a~~A~~ combination of a reduced folate and BH4, a precursor, or functional equivalent thereof ~~for use~~ in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability.
6. Use of a~~A~~ composition comprising one or more active pharmaceutical ingredients ~~for use~~ in the manufacture of a medicament for the prevention or treatment of a disorder associated with low BH4 bioavailability, wherein the active pharmaceutical ingredient(s) consist of a reduced folate and optionally BH4, a precursor, or functional equivalent thereof.
7. ~~A composition for~~The use according to claim 6 wherein the active pharmaceutical ingredient consists of a reduced folate.
8. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-7, wherein the reduced folate is in its natural stereoisomeric form.
9. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-8, wherein the reduced folate increases or restores BH4 levels.
10. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-9, wherein the reduced folate prevents oxidation of BH4.
11. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-10, wherein the reduced folate is selected from: DHF; 5-FTHF; THF; 5,10-CH-THF; 5,10-$CH_2$-THF, 5-MTHF, and 10-FTHF, or a pharmaceutically acceptable salt thereof, preferably wherein the reduced folate is 5-MTHF or a pharmaceutically acceptable salt thereof.
12. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-11, wherein the BH4, a precursor thereof, or a functional equivalent thereof is a

precursor selected from: GTP; NH2TP; PTP; oxo-PH4; 7,8-BH2; HO-BH4; q-BH2; L-arginine and L-citrulline.

13. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-11, wherein the BH4, a precursor thereof, or a functional equivalent thereof is a functional equivalent selected from: neopterin; sepiapterin; biopterin; and primapterin.
14. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-13, wherein the disorder associated with low BH4 bioavailability is caused by impaired activity of one or more enzymes within the pterin biosynthetic pathway.
15. ~~A reduced folate, a combination, or a composition for~~The use according to claim 14, wherein the one or more enzymes within the pterin biosynthetic pathway are selected from: GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SP), dihydrofolate reductase (DHFR), dihydropteridine reductase (DHPR), pterin-4a-carbinolamine dehydratase (PCD), and endothelial NOS (eNOS).
16. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-15, wherein the disorder associated with low BH4 bioavailability is selected from: BH4 deficiency (Tetrahydrobiopterin deficiency), phenylketonuria (PKU), GTP cyclohydrolase deficiency, dopa-responsive dystonia, 6-pyruvoyl-tetrahydropterin synthase deficiency, sepiapterin reductase deficiency, dihydrofolate reductase deficiency, dihydropteridine reductase deficiency, and pterin-4a-carbinolamine dehydratase deficiency.
17. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-16, wherein the disorder associated with low BH4 bioavailability is a cardiac disease, a liver disease, a neurological disease or a vascular disease.
18. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-17, wherein the disorder associated with low BH4 bioavailability is a pregnancy-related disorder.
19. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-18, wherein the disorder associated with low BH4 bioavailability is a pregnancy-related vascular disorder.
20. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-19, wherein the disorder associated with low BH4 bioavailability is hypertension in pregnancy.
21. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-19, wherein the disorder associated with low BH4 bioavailability is pre-eclampsia.
22. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-21, wherein the reduced folate is for administration to a subject after having been diagnosed with a pregnancy-related vascular disorder.

23. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-22, wherein the reduced folate, combination or composition is effective in the prevention of a disorder associated with insufficient BH4 levels in subsequent generations.
24. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-23, wherein the prevention or treatment is in a subject who has previously suffered with a disorder associated with insufficient BH4 levels.
25. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-24, wherein the prevention or treatment is in a subject who has previously suffered with hypertension in pregnancy.
26. ~~A reduced folate, a combination, or a composition for~~The use according to any of claims 1-24, wherein the prevention or treatment is in a subject who has previously suffered with pre-eclampsia.
27. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-26, wherein the reduced folate is for administration at a dosage of between 1mg and 50mg per day, preferably between 2mg and 30mg per day, preferably between 3mg and 25mg per day, preferably between 4mg and 20mg per day, preferably between 5mg and 15mg per day.
28. ~~A reduced folate, a combination, or a composition for~~ The use according to any of claims 1-27, wherein the reduced folate is for administration once or twice daily.
29. A method of selecting a subject that may benefit from treatment with a reduced folate and optionally BH4, a precursor or functional equivalent thereof, the method comprising determining the level of one or more markers of a disorder associated with low BH4 bioavailability in a subject, comparing the level(s) of the or each marker to a reference level(s) in a healthy subject, and selecting the subject for treatment if the subject exhibits an abnormal level of the one or more markers of a disorder associated with low BH4 bioavailability compared to the reference level.
30. The method according to claim 29, wherein the method ~~further comprises~~is for providing a treatment to the selected subject.
31. The method according to claim 29 or 30, wherein the treatment comprises a reduced folate and optionally BH4, a precursor, or functional equivalent thereof.
32. The method according to any of claims 29-31, wherein the reduced folate is in its natural stereoisomeric form.
33. The method according to any of claims 31-32, wherein the reduced folate is to be provided to the selected subject before gestation.
34. The method according to any of claims 31-33, wherein the reduced folate is to be provided to the selected ~~-~~subject in the first trimester of pregnancy.

35. The method according to any of claims 31-34, wherein the reduced folate is to be provided to the selected -subject in the second or third trimester of pregnancy.
36. The method according to any of claims 31-35, wherein the reduced folate is to be provided to the selected subject after having measured an abnormal level of one or more markers of a disorder associated with low BH4 bioavailability in the subject.
37. A pharmaceutical composition comprising a reduced folate and BH4, a precursor, or functional equivalent thereof.
38. A kit comprising:
    - a reduced folate; and
    - BH4, a precursor, or functional equivalent thereof.
39. Use of a reduced folate to prevent oxidation of BH4, a precursor, or a functional equivalent thereof, *in vitro*.
40. The pharmaceutical composition of claim 37, the kit of claim 38, or the use of claim 39, wherein the reduced folate is in its natural stereoisomeric form.