Combination comprising DHODH inhibitors and methotrexate

A combination which comprises (a) methotrexate and (b) a non-hepatotoxic DHODH inhibitor.
The present invention relates to new combinations of methotrexate with DHODH inhibitors. These combinations are useful in the treatment, prevention or suppression of diseases and disorders known to be susceptible to improvement with methotrexate and/or by inhibition of dihydroorotate dehydrogenase, such as autoimmune diseases, immune and inflammatory diseases, destructive bone disorders, malignant neoplastic diseases, angiogenic-related disorders, viral diseases, and infectious diseases.

BACKGROUND OF THE INVENTION

Methotrexate (MTX) is an antimetabolite and immunomodulator that affects many intracellular pathways of purine metabolism. It is effective in reducing the signs and symptoms of rheumatoid arthritis (RA), as well as in slowing or halting radiographic damage. Due to its efficacy, ease of administration and relatively low cost, MTX has become the first-line oral therapy in most patients with RA. In those patients who have an incomplete response to MTX, another DMARD (disease modifying anti-rheumatic drug) is added on top of it. Thus, combination therapy with MTX is more and more frequent in the clinical practice.

Leflunomide is an example of such a DMARD. It was approved in September 1998 for use in RA. It has been shown to reduce the signs and symptoms of the disease, to inhibit structural damage (evidenced by X-ray erosions and joint space narrowing) and to improve physical function. Teriflunomide is the active metabolite of Leflunomide.

Methotrexate is thought to act primarily on purine pathways of cellular metabolism, whereas Leflunomide affects pyrimidine pathways. Given the diverse intracellular pathways affected by both drugs, the combination of Leflunomide and methotrexate has the potential for biochemical synergy. In fact, it has been reported that the combination of both agents led to considerable clinical improvement (see for example, Kremer JM et al. "Concomitant Leflunomide therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate". Ann. Intern. Med., 2002; 137, 726-733).

Unfortunately, both methotrexate and leflunomide have serious adverse effects, in particular hepatotoxicity. Methotrexate may cause fatal liver damage such as fibrosis and cirrhosis after prolonged use. Liver enzyme increases are frequently seen during treatment with methotrexate. Hence, regular and careful monitoring of patients taking MTX is essential, particularly when MTX is combined with other DMARDS.

Leflunomide include diarrhea, dyspepsia, rash, hair loss, hypertension and elevated hepatic enzymes. The hepatotoxicity potential is of special relevance and regular laboratory tests, including blood tests of liver function, must be performed for all patients taking this medication. Leflunomide is not recommended for use in patients with evidence of hepatitis B or C infection or significant hepatic impairment.

Clinical trials have reported that the number of patients experiencing an increase in liver markers (measured as transaminase levels) is notably higher in the group of Leflunomide plus MTX than in the group of MTX alone. The product information for Leflunomide warns against combination with methotrexate on the basis that such combination therapy can lead to additive or even synergistic hepatotoxicity.

The adverse hepatic effects of leflunomide, and in particular of its active metabolite, teriflunomide, have been attributed to its activity as inhibitor of dihydroorotate dehydrogenase (DHODH). Liver toxicity has thus been identified as an adverse effect directly derived from the mechanism of action of DHODH-inhibitors, which has hampered the development of this class of compounds.

DESCRIPTION OF THE INVENTION

We have now found that, contrary to general belief, the inhibition of DHODH is not responsible for the liver damage produced by leflunomide and that DHODH inhibitors are particularly suitable for combination with methotrexate.

It is known that inhibition of DHODH produces immunosuppressant and antiproliferative effects. DHODH inhibitors could therefore be used as immunosuppressants and as antiproliferatives in the treatment of autoimmune, inflammatory and proliferative diseases, like RA.

Our invention is based on the surprising finding that the inhibition of DHODH is not linked to hepatotoxicity and, consequently, DHODH inhibitors devoid of hepatotoxic potential represent an important contribution to the treatment of these diseases, thanks to their advantageous combinability with MTX, the most commonly used first-line drug in RA treatment.

We have developed an in vivo model of hepatotoxicity assessment in mice, in which test compounds are administered by intraperitoneal route to maximise liver exposure. In this model, Teriflunomide, the active metabolite of Leflunomide, has shown a drastic increase in the levels of transaminases and bilirubin in plasma, whereas DHODH inhibitors do not show an increase in any of the plasma liver markers in the same model, while maintaining their efficacy in arthritis.

Thus, the present invention is directed to a combination product which comprises (a) Methotrexate and (b) and...
DHODH inhibitor, in particular a non-hepatotoxic DHODH inhibitor.

In a preferred embodiment the DHODH inhibitor is other than Leflunomide or any active metabolite thereof.

Most preferably, the DHODH inhibitor (b) is a compound of formula (I):

wherein

- one of the groups \( G^1 \) represents a nitrogen atom or a group \( \text{CR}^c \) and the other represents a group \( \text{CR}^c \)
- \( G^2 \) represents a nitrogen atom or a group \( \text{CR}^d \)
- \( R^1 \) represents a group selected from hydrogen atoms, halogen atoms, \( C_{1-4} \) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and \( C_{3-8} \) cycloalkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
- \( R^2 \) represents a group selected from hydrogen atoms, halogen atoms, hydroxyl groups, \( C_{1-4} \) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, \( C_{1-4} \) alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and \( C_{3-8} \) cycloalkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
- \( R^a, R^b \) and \( R^c \) independently represent groups selected from hydrogen atoms, halogen atoms, \( C_{1-4} \) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and \( C_{1-4} \) alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
- \( R^d \) represents a group selected from hydrogen atoms, halogen atoms, hydroxyl groups, \( C_{1-4} \) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, \( C_{1-4} \) alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and \( C_{3-8} \) cycloalkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
- one of the groups \( G^3 \) and \( G^4 \) is a nitrogen atom and the other is a \( \text{CH} \) group,
- \( M \) is a hydrogen atom or an pharmaceutically acceptable cation

with the proviso that, when at least one of the groups \( R^a \) and \( R^b \) represent a hydrogen atom and \( G^2 \) is a group \( \text{CR}^c \), then \( R^d \) represents a groups selected from \( C_{1-4} \) alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, \( C_{3-8} \) cycloalkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups; and the pharmaceutically acceptable salts and N-oxides thereof.

As used herein the term alkyl embraces optionally substituted, linear or branched hydrocarbon radicals having 1 to 4 carbon atoms. Preferred substiuents on the alkyl groups are halogen atoms and hydroxy groups, and are more preferably halogen atoms.

Examples include methyl, ethyl, \( n \)-propyl, \( i \)-propyl, \( n \)-butyl, \( sec \)-butyl and \( tert \)-butyl radicals.

As used herein the term alkoxy embraces optionally substituted, linear or branched oxy-containing radicals each having 1 to 4 carbon atoms. Preferred substituents on the alkoxy groups are halogen atoms and hydroxy groups, and are more preferably halogen atoms.

Examples include methoxy, ethoxy, \( n \)-propoxy, \( i \)-propoxy, \( n \)-butoxy, \( sec \)-butoxy and \( tert \)-butoxy radicals.

As used herein, the term cycloalkyl embraces saturated carbocyclic radicals and, unless otherwise specified, a cycloalkyl radical typically has from 3 to 8 carbon atoms.

Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. When a cycloalkyl radical
carries 2 or more substituents, the substituents may be the same or different. Preferred substituents on the cycloalkyl
groups are halogen atoms and hydroxy groups, and are more preferably halogen atoms.

[0022] As used herein, the term cycloalkoxy embraces saturated oxy-containing carboxyclic radicals and, unless
otherwise specified, a cycloalkoxy radical typically has from 3 to 8 carbon atoms.

[0023] Examples include cyclopropoxy, cyclobutyloxy, cyclopentxyloxy, cyclohexyloxy and cycloheptyloxy. When a
cycloalkoxy radical carries 2 or more substituents, the substituents may be the same or different. Preferred substituents
on the cycloalkoxy groups are halogen atoms and hydroxy groups, and are more preferably halogen atoms.

[0024] As used herein, some of the atoms, radicals, moieties, chains or cycles present in the general structures of the
invention are "optionally substituted". This means that these atoms, radicals, moieties, chains or cycles can be either
unsubstituted or substituted in any position by one or more, for example 1, 2, 3 or 4, substituents, whereby the hydrogen
atoms bound to the unsubstituted atoms, radicals, moieties, chains or cycles are replaced by chemically acceptable
atoms, radicals, moieties, chains or cycles. When two or more substituents are present, each substituent may be the
same or different.

[0025] As used herein, the term halogen atom embraces chlorine, fluorine, bromine or iodine atoms typically a fluorine,
chlorine or bromine atom, most preferably fluorine or bromine. The term halo when used as a prefix has the same meaning.

[0026] M may be a hydrogen atom or a pharmaceutically acceptable cation. When M is a pharmaceutically acceptable
cation, the compound represented by formula (I) may alternatively be represented by formula (I*) below.

\[ (I^*) \]

[0027] As used herein, the term pharmaceutically acceptable cation embraces both inorganic cations, for example
alkali metal cations (Li\(^+\), Na\(^+\), K\(^+\)), alkaline earth cations (Ca\(^{2+}\), Mg\(^{2+}\)) and other pharmaceutically acceptable inorganic
cations known in the art (Zn\(^{2+}\), Al\(^{3+}\)), and organic cations, for example ammonium ion (i.e., NH\(_4^+\)) and substituted
ammonium ions, such as NH\(_3\)R\(_1^+\), NH\(_2\)(R\(_1^\))\(_2^+\), NH(R\(_1^\))\(_3^+\) and N(R\(_1^\))\(_4^+\), where each R\(_1^\) is independently selected from a
phenyl group, a benzyl group, C\(_1-4\) alkyl and C\(_3-8\) cycloalkyl.

[0028] Examples of some suitable substituted ammonium ions are EtNH\(_3^+\), Et\(_2\)NH\(_2^+\), Et\(_3\)NH\(_+\), (C\(_6\)H\(_5\))\(_2\)NH\(_2^+\),
CH\(_3\)CH\(_2\)CH\(_2\)CH\(_2\)NH\(_3^+\), PhCH\(_2\)NH\(_3^+\) and (Ph)(PhCH\(_2\))NH\(_2^+\). An example of a common quaternary ammonium ion is N
(CH\(_3\))\(_4^+\).

[0029] Typically, M is a hydrogen atom or a pharmaceutically acceptable cation selected from Li\(^+\), Na\(^+\), K\(^+\), Ca\(^{2+}\) and
Mg\(^{2+}\). It is preferred that M is a hydrogen atom or a pharmaceutically acceptable cation selected from Li\(^+\), Na\(^+\) and K\(^+\).
More preferably M is a hydrogen atom or Li\(^+\), and most preferred is when M is a hydrogen atom.

[0030] If M of formula (I) is a pharmaceutically acceptable cation having a charge greater than +1, then additional
anions are present to maintain the electroneutrality of the compound.

[0031] The counteranion may be an anion X\(^-\) as defined below or an anion as represented in formula (I*) above.

[0032] As used herein, the term pharmaceutically acceptable salt embraces salts with a pharmaceutically acceptable
acid or base. Pharmaceutically acceptable acids include both inorganic acids, for example hydrochloric, sulphuric,
phosphoric, diphosphoric, hydrobromic, hydroiodic and nitric acid and organic acids, for example citric, fumaric, maleic,
malic, mandelic, ascobic, oxalic, succinic, tartaric, benzoic, acetic, methanesulphonic, ethanesulphonic, benzenesulphonic,
cyclohexylsulfamic (cyclamic) or p-toluenesulphonic acid. Pharmaceutically acceptable bases include alkali metal (e.g. sodium or potassium) and alkali earth metal (e.g. calcium or magnesium) hydroxides and organic bases, for
example alkyl amines, arylalkyl amines and heterocyclic amines.

[0033] Other preferred salts according to the invention are quaternary ammonium compounds wherein an equivalent
of an anion (X\(^-\)) is associated with the positive charge of the N atom. X\(^-\) may be an anion of various mineral acids such
as, for example, chloride, bromide, iodide, sulphate, nitrate, phosphate, or an anion of an organic acid such as, for
example, acetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, trifluoroacetate, methanesulphonate and p-toluenesulphonate. X is preferably an anion selected from chloride, bromide, iodide, sulphate, nitrate, acetate, maleate, oxalate, succinate or trifluoroacetate. More preferably X is chloride, bromide, trifluoroacetate or methanesulphonate.

[0034] As used herein, an N-oxide is formed from the tertiary basic amines or imines present in the molecule, using a convenient oxidising agent.

[0035] Typically, R is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl, cyclopropyl and cyclobutyl.

[0036] Typically, G represents a nitrogen atom and G represents a group CH.

[0037] Typically, G represents a group CH and G represents a nitrogen atom.

[0038] Typically, G represents a group CR.

[0039] Typically, each R is independently selected from the groups consisting of hydrogen atoms, fluorine atoms, chlorine atoms and C alkyl groups.

[0040] Typically, G represents a group CR.

[0041] Typically, R is selected from the groups consisting of hydroxy, C alkoxy groups, 2,2,2-trifluoroethoxy and C cycloalkoxy groups. Preferably, C alkoxy groups, 2,2,2-trifluoroethoxy and C cycloalkoxy groups.

[0042] Typically, R is selected from the groups consisting of fluorine atoms, methyl groups and trifluoromethoxy groups.

[0043] Typically, R is selected from the group consisting of hydrogen atoms, fluorine atoms and chlorine atoms.

[0044] Typically, R is selected from the group consisting of hydrogen atoms and halogen atoms, preferably hydrogen atoms and fluorine atoms.

[0045] Typically, both groups G represent C(R ) groups, G represents a C(R ) group, preferably G is a group selected from C(OH), C(OMe) and C(OEt); R is a fluorine atom, R is selected from the group consisting of hydrogen atoms and R is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl and cyclopropyl groups. Preferably, both G represent CH groups, G is a group selected from C(OMe) and C(OEt); R is a fluorine atom, R is selected from the group consisting of hydrogen atoms and fluorine atoms and R is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl and cyclopropyl groups.

[0046] Preferably, R is a hydrogen atom, R is a hydroxy or a C alkoxy groups and R is a hydrogen atom, preferably R is a hydrogen atom, R is a C alkoxy and R is a hydrogen atom.

[0047] Preferably, G represents a nitrogen atom, G represents a group CH and R is a fluorine atom and the compounds wherein G represents a group CH, G represents a nitrogen atom.

[0048] More preferably, both groups G represent C(R ) groups, G represents a C(R ) group, R is a fluorine atom, R is selected from the group consisting of hydrogen atoms and fluorine atoms and R is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl and cyclopropyl groups. Preferably R is a hydrogen atom, R is selected from the group consisting of C alkoxy and C cycloalkoxy groups and R is a hydrogen atom. Particularly preferred are the compounds wherein G represents a nitrogen atom, G represents a group CH and R is a fluorine atom and the compounds wherein G represents a group CH, G represents a nitrogen atom.

[0049] Preferably, the DHODH inhibitor is one of:

1. 2-(3-Fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2. 2-(3'-Ethoxy-3-fluorobiphenyl-4-ylamino)nicotinic acid
3. 2-(3-Fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
4. 2-(3'-Ethoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
5. 2-(3'-Methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
6. 2-(2,5-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
7. 2-(3'-Ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
8. 2-(2,3-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
9. 2-(2-Methyl-3'-trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
10. 2-(3-Chloro-3'-trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
11. 2-(3-Chloro-3'-ethoxybiphenyl-4-ylamino)nicotinic acid
12. 2-(3-Methyl-3'-trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
13. 2-(3-Chloro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
14. 2-(3'-Difluoromethoxy)-3-fluorobiphenyl-4-ylamino)nicotinic acid
15. 2-(3'-Cyclobutoxy-3-fluorobiphenyl-4-ylamino)nicotinic acid
16. 2-(3'-Fluoro-3'-(2,2,2-trifluorooctoxy)biphenyl-4-ylamino)nicotinic acid
17. 2-(3'-Cyclobutoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
18. 2-(3,5-Difluoro-3'-trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
19. 2-(3'-Ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
20. 2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
21. Lithium 3-(3'-ethoxy-3-fluorobiphenyl-4-ylamino)isonicotinate
22. Lithium 3-(3-fluoro-3'-methoxybiphenyl-4-ylamino)isonicotinate
23. Lithium 3-(3'-methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)isonicotinate
24. Lithium 3-(3-fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)isonicotinate
25. 2-(3'-Ethoxybiphenyl-4-ylamino)nicotinic acid
26. 2-(5-Fluoro-2-methyl-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
27. 2-(2'-Difluoro-5'-(isopropoxy)biphenyl-4-ylamino)nicotinic acid
28. 2-(3'-Fluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
29. 2-(3,5-Difluoro-3'-hydroxybiphenyl-4-ylamino)nicotinic acid
30. 5-Bromo-2-(3-fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
31. 5-Bromo-2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
32. 5-Bromo-2-(3-fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
33. 2-(3-Fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)-5-methylnicotinic acid
34. 5-Cyclopropyl-2-(3-fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
35. 2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
36. 2-(3'-Ethoxy-5-fluoro-2-methylbiphenyl-4-ylamino)nicotinic acid
37. 2-(5-Fluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)nicotinic acid
38. 2-(3'-ethoxy-3,5-difluorobiphenyl-4-ylamino)-5-methylnicotinic acid
39. 5-cyclopropyl-2-(3'-ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
40. 2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)-5-ethylnicotinic acid
41. 5-bromo-2-(3'-ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
42. 5-cyclopropyl-2-(3'-ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
43. 2-(5-fluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)-5-methylnicotinic acid
44. 5-cyclopropyl-2-(5-fluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)nicotinic acid
45. 2-(2',3,5-trifluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
46. 2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
47. 2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
48. 2-(3,5-difluoro-2-methylbiphenyl-4-ylamino)nicotinic acid
49. 5-cyclopropyl-2-(2,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
50. 2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)-5-cyclopropynicotinic acid
51. 5-chloro-2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
52. 5-cyclopropyl-2-(3,5-difluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
53. 2-(2,3,5-trifluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
54. 2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)-5-cyclopropynicotinic acid
55. 2-(2,5-difluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)nicotinic acid
56. 2-(2,5-difluoro-2-methyl-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
57. 2-(2'-chloro-3,5-difluoro-2-methylbiphenyl-4-ylamino)nicotinic acid
58. 5-chloro-2-(3,5-difluorobiphenyl-4-ylamino)nicotinic acid
59. 5-chloro-2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
60. 2-(2,3,5,6-tetrafluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
61. 2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid
62. 3-(3'-cyclopropoxy-3-fluorobiphenyl-4-ylamino)isonicotinic acid

or a pharmaceutically acceptable salt or N-oxide thereof.

[0050] More preferably, the DHODH inhibitor is one of:

2-(3'-Ethoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3'-Methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3'-Ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
Lithium 3-(3'-ethoxy-3-fluorobiphenyl-4-ylamino)isonicotinate
Lithium 3-(3'-methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)isonicotinate
2-(3-Fluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
5-Bromo-2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
5-Cyclopropyl-2-(3-fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
2-(2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)-5-ethylnicotinic acid
2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid
5-cyclopropyl-2-(2,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
5-cyclopropyl-2-(3,5-difluoro-3'-trifluoromethoxybiphenyl-4-ylamino)nicotinic acid
2-(2',3,5,6-tetrafluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid

or a pharmaceutically acceptable salt or N-oxide thereof.

[0051] Most preferably, the DHODH inhibitor is 2-(3'-Ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid, 2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid, 2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid, 2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid or 5-cyclopropyl-2-(3,5-difluoro-3'-trifluoromethoxybiphenyl-4-ylamino)nicotinic acid, or a pharmaceutically acceptable salt or N-oxide thereof.

[0052] Preferably the active ingredients (a) and (b) form part of a single pharmaceutical composition.

[0053] Further provided is a combination as described above which further comprises (c) another compound selected from:

(i) Anti-TNF-alpha monoclonal antibodies such as Infliximab, Certolizumab pegol, Golimumab, Adalimumab and AME-527 from Applied Molecular Evolution
(ii) TNF-alpha Antagonists such as Etanercept, Lenercept, Oncept and Pegsnercept
(iii) Calcineurin (PP-2B) Inhibitors / INS Expression Inhibitors such as cyclosporine A, Tacrolimus and ISA-247 from Isotechnika
(iv) IL-1 Receptor Antagonists such as Anakinra and AMG-719 from Amgen
(v) Anti-CD20 monoclonal antibodies such as Rituximab, Ofatumumab, Ocrelizumab and TRU-015 from Trubion Pharmaceuticals
(vi) p38 Inhibitors such as AMG-548 (from Amgen), ARRY-797 (from Array Biopharma), Chlormethazole edisylate, Doramapimod, PS-540446 (from BMS), SB-203580, SB-242235, SB-235699, SB-281832, SB-681323, SB-856553 (all from GlaxoSmithKline), KC-706 (from Kemia), LEO-1606, LEO-15520 (all from Leo), SC-80036, SD-06 (all from Pfizer), RWJ-67657 (from R.W. Johnson), RO-321195, RO-4402257 (all from Roche), AVE-9940 (from Aventis), SCIO-323, SCIO-469 (all from Scios), TA-5493 (from Tanabe Seiyaku), and VX-745 and VX-702 (all from Vertex)
(vii) NF-kappaB (NFKB) Activation Inhibitors such as Sulfasalazine and Igruratimod
(viii) Another dihydrofolate reductase (DHFR) inhibitor such as Aminopterin and CH-1504 from Chelsea
(ix) Janus kinase (JAK) inhibitors, such as CP-690, 550 from Pfizer and INCB-18424, from Incyte.
(x) MEK inhibitor, such as ARRY-162 from Array
(xi) Sphingosine-1 phosphate receptor agonists, such as fingolimod (Novartis)

[0054] The present invention further provides use of (a) methotrexate and (b) a DHODH inhibitor of the invention for the preparation of a medicament for simultaneous, separate or sequential use in the treatment of a pathological condition or disease susceptible to amelioration by inhibition of dehydroorotate dehydrogenase.

[0055] Diseases or disorders in which DHODH inhibition plays a role include without limitation autoimmune diseases, immune and inflammatory diseases, destructive bone disorders, malignant neoplastic diseases, angiogenic-related disorders, viral diseases, and infectious diseases.

[0056] Autoimmune diseases which may be prevented or treated include but are not limited to rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, multiple sclerosis, psoriasis, ankylosing spondylitis, Wegener's granulomatosis, polyarticular juvenile idiopathic arthritis, inflammatory bowel disease such as ulcerative colitis and Crohn's disease, Reiter's syndrome, Reiter's syndrome and type-1 diabetes.

[0057] Immune and inflammatory diseases which may be prevented or treated include but are not limited to asthma, COPD, respiratory distress syndrome, acute or chronic pancreatitis, graft versus-host disease, chronic sarcoidosis, transplant rejection, contact dermatitis, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, Behcet syndrome, inflammatory eye conditions such as conjunctivitis and uveitis.

[0058] Destructive bone disorders which may be prevented or treated include but are not limited to osteoporosis, osteoarthritis and multiple myeloma-related bone disorder.

[0059] Malignant neoplastic diseases that may be prevented or treated include but are not limited to prostate, ovarian and brain cancer.

[0060] Angiogenesis-related disorders that may be prevented or treated include but are not limited to hemangiomas, ocular neovascularization, macular degeneration or diabetic retinopathy.

[0061] Viral diseases which may be prevented or treated include but are not limited to HIV infection, hepatitis and cytomegalovirus infection.
Infectious diseases which may be prevented or treated include but are not limited to sepsis, septic shock, endotoxic shock, Gram negative sepsis, toxic shock syndrome, Shigellosis and other protozoal infestations such as malaria.

Preferably, the pathological condition or disease is selected from rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, multiple sclerosis, Wegener’s granulomatosis, systemic lupus erythematosus, psoriasis and sarcoidosis. More preferably the pathological condition or disease is rheumatoid arthritis, psoriatic arthritis or psoriasis. Most preferably it is rheumatoid arthritis.

Preferably in said method, (a) methotrexate and (b) the DHODH inhibitor are the sole active components.

Typically the medicament is for use in treating a human or animal patient suffering or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity. More typically, the said human or animal patient is suffering from liver fibrosis, hepatitis (typically hepatitis A to G), cirrhosis (typically caused by alcoholism) or liver cancer.

The present invention therefore also provides the use of a DHODH inhibitor of the invention, as defined above, in the manufacture of a medicament for use in treating or preventing a pathological condition or disease, as defined above, in a human or animal patient which is suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity, as defined above.

Preferably in said method, (a) methotrexate and (b) the DHODH inhibitor are the sole active components.

Also provided is a method for use in treating a human or animal patient suffering from or susceptible to a pathological condition or disease as defined above, wherein the human or animal patient is suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity as defined above, which method comprises administering to said human or animal patient a therapeutically effective amount of a DHODH inhibitor as defined above.

The active compounds in the combinations of the invention may be administered by any suitable route, depending on the nature of the disorder to be treated, e.g. orally (as syrups, tablets, capsules, lozenges, controlled-release preparations, fast-dissolving preparations, etc); topically (as creams, ointments, lotions, nasal sprays or aerosols, etc); by injection (subcutaneous, intradermic, intramuscular, intravenous, etc.) or by inhalation (as a dry powder, a solution, a dispersion, etc).

The active compounds in the combination may be administered together in the same pharmaceutical composition or in different compositions intended for separate, simultaneous, concomitant or sequential administration by the same or a different route.

The combinations of the invention may conveniently be presented in unit dosage form and may be prepared...
by any of the methods well known in the art of pharmacy.

[0080] Combinations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil- in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

[0081] A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerine or water with flavouring or colouring agent.

[0082] Where the combination is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, t alc, gelatine, acacia, stearic acid, starch, lactose and sucrose.

[0083] A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

[0084] Where the combination is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatine capsule. Where the composition is in the form of a soft gelatine capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatine capsule.

[0085] The combination may be in the form of a dry powder composition for topical delivery to the lung by inhalation. Dry powder compositions may, for example, be presented in capsules and cartridges of for example gelatine or blisters of for example laminated aluminium foil, for use in an inhaler or insufflator. Formulations generally contain a powder mix for inhalation of the compound of the invention and a suitable powder base (carrier substance) such as lactose or starch. Use of lactose is preferred. Each capsule or cartridge may generally contain between 2µg and 150 µg of each therapeutically active ingredient. Alternatively, the active ingredient (s) may be presented without excipients.

[0086] Packaging of the formulation for inhalation may be carried out by using suitable inhaler devices such as the Novolizer SD2FL which is described in the following patent applications: WO 97/000703, WO 03/000325 and WO 03/061742.

[0087] The combination may be in the form of a composition for nasal delivery. Typical compositions for nasal delivery include those mentioned above for inhalation and further include non-pressurized compositions in the form of a solution or suspension in an inert vehicle such as water optionally in combination with conventional excipients such as buffers, anti-microbials, tonicity modifying agents and viscosity modifying agents which may be administered by nasal pump.

[0088] Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

[0089] Preferably the combination is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

[0090] The amount of each active which is required to achieve a therapeutic effect will, of course, vary with the particular active, the route of administration, the subject under treatment, and the particular disorder or disease being treated.

[0091] Typically all active agents in the combination are administered at the same time, or very close in time. Alternatively, one or two actives could be taken in the morning and the other (s) later in the day. Or in another scenario, one or two actives could be taken twice daily and the other (s) once daily, either at the same time as one of the twice-a-day dosing occurred, or separately. Preferably at least two, and more preferably all, of the actives would be taken together at the same time. Preferably, at least two, and more preferably all actives would be administered as an admixture.

[0092] Preferably the drug combination of the invention is for administration as a dosage regime which involves administration of (i) 0.015 to 3 mg/kg/week of methotrexate, more preferably 0.07 to 0.7 mg/kg/week of methotrexate and most preferably 0.15 to 0.35 mg/kg/week of methotrexate, and (ii) 0.03 to 30 mg/kg/day of DHODH inhibitor, more preferably 0.07 to 14 mg/day of DHODH inhibitor and most preferably 0.15 to 0.3 mg/kg/day of DHODH inhibitor.

EXAMPLES

Example 1 - inhibition of human DHODH activity assay

[0093] DHODH activity and its inhibition were studied using a chromogen reduction assay with DCIP (2,6-dichlorophenol-indophenol). The substrate oxidation (Dihydroorotate, L-DHO), as well as cosubstrate reduction (coenzyme Q, CoQ) is coupled to the chromogen reduction, hence enzymatic activity results in a loss of chromogen absorbance at 600 nm.

[0094] Enzyme extracts (8 µl, ~1.5 µg of human protein) were incubated in 96-well plates. The assay mixture (200
μl) contained 200 μM CoQD, 100 μM L-DHO, 120 μM DCIP in the assay buffer (100 mM HEPES pH 8.0, 150 mM NaCl, 10% Glicerol, 0.05% Triton X-100) and 2 μl of test compound. The compounds were dissolved in DMSO at a stock concentration of 1 mM, and tested at different concentrations varying from 10 μM to 1 pM to calculate an IC₅₀ (concentration of inhibitor required for 50% of inhibition).

[0095] The reaction was initiated by adding the enzyme and then incubated for 10 min at room temperature before measuring DCIP reduction by counting a decrease in absorbance at 600 nm using standard instrumentation (Spectramax).

[0096] All reactions were carried out in duplicate and graphs, determining IC₅₀ values for each compound, were plotted using the ABase software.

[0097] Table 1 shows the activities in human DHODH inhibition assay of some compounds of the present invention showing that these compounds are potent DHODH inhibitors.

### TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>hDHODH IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>88</td>
</tr>
<tr>
<td>13</td>
<td>150</td>
</tr>
<tr>
<td>17</td>
<td>90</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>24</td>
<td>200</td>
</tr>
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<td>110</td>
</tr>
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<td>34</td>
<td>33</td>
</tr>
<tr>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>37</td>
<td>99</td>
</tr>
<tr>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>45</td>
<td>53</td>
</tr>
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</tr>
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</tr>
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<td>6</td>
</tr>
<tr>
<td>57</td>
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</tr>
<tr>
<td>58</td>
<td>8</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>61</td>
<td>11</td>
</tr>
</tbody>
</table>

Example 2 - reduced hepatotoxicity

[0098] Acute hepatotoxicity assays were performed in Swiss mice. Animals received a single administration of either vehicle, or 100 mg/kg of teriflunomide or a compound of the present invention by intraperitoneal route. Twenty-four hours
later, animals were sacrificed and the levels of liver markers AST (aspartate aminotransferase), ALT (alanine aminotransferase) and BIL (total bilirubin) in plasma were determined.

Table 2: Plasma levels of liver markers of mice after administration of 100 mg/kg of the compound, 100 mg/kg Teriflunomide or vehicle (IU: International Units).

<table>
<thead>
<tr>
<th>Example</th>
<th>ALT (IU/l)</th>
<th>AST (IU/l)</th>
<th>BIL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99</td>
<td>84</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>83</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>108</td>
<td>0.05</td>
</tr>
<tr>
<td>19</td>
<td>60</td>
<td>92</td>
<td>0.05</td>
</tr>
<tr>
<td>20</td>
<td>73</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>35</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>47</td>
<td>66</td>
<td>91</td>
<td>0.14</td>
</tr>
<tr>
<td>48</td>
<td>44</td>
<td>95</td>
<td>0.08</td>
</tr>
<tr>
<td>57</td>
<td>60</td>
<td>109</td>
<td>0.06</td>
</tr>
<tr>
<td>Vehicle</td>
<td>55</td>
<td>78</td>
<td>0.05</td>
</tr>
<tr>
<td>Teriflunomide</td>
<td>423</td>
<td>542</td>
<td>0.5</td>
</tr>
</tbody>
</table>

[0099] As it can clearly be seen from Table 2, Teriflunomide-treated mice showed a dramatic increase in the three liver markers compared to vehicle-treated mice, clearly indicating a high hepatotoxicity, whereas the DHODH inhibitors disclosed in the present invention did not cause a significant increase in any of the parameters measured.

EXAMPLE 3: Efficacy assay in adjuvant-induced arthritis of the combination product of the present invention.

[0100] The effect of DHODH inhibitor compounds were tested in combination with methotrexate (0.05 mg/Kg/ day) in the rat adjuvant-induced arthritis model (AIA) in animals with established disease (curative protocol). Briefly, Complete Freund Adjuvant (CFA) was injected into the left hind footpad of Wistar rats, and 10 days later the swelling of the two rear paws was measured with a plethysmometer. Rats exhibiting a similar degree of inflammation in both paws were randomized into treatment groups (n=7 per group). Compounds were administered orally once a day for 10 days and paw volumes were determined every two days up to day 21.

Table 3. Effects of compound A (3mg/Kg/day), Methotrexate (0.05 mg/Kg/day) and their combination on the inhibition of paw inflammation in arthritic rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% inhibition of the inflammation (AUC) Right paw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound A (3 mg/Kg)</td>
<td>48±7</td>
</tr>
<tr>
<td>MTX (0.05 mg/Kg)</td>
<td>40±9</td>
</tr>
<tr>
<td>Compound A (3 mg/Kg) + MTX (0.05 mg/Kg)</td>
<td>66±5</td>
</tr>
</tbody>
</table>

[0101] Results are expressed as the mean±SEM inhibition of the inflammation measured as the area under the curve (AUC) of the right paw volumes in the period comprised between days 10 and 21 post-induction. The percentage of inhibition for every group was calculated versus values from vehicle-treated rats.

[0102] Results from Table 3 show that Compound A inhibits the inflammation caused by experimental arthritis in rats. Furthermore, the co-administration of MTX and compound A resulted in an increased efficacy (38 %) versus compound A alone, thus indicating the feasibility of administering the compound in patients treated with MTX.
Table 4: Effects of Teriflunomide (3mg/Kg/day), Methotrexate (0.05 mg/Kg/day) and their combination on the inhibition of paw inflammation in arthritic rats.

The results are expressed as the mean ± SEM inhibition of inflammation measured as the area under the curve (AUC) of the right paw volumes in the period comprised between days 10 and 21 post-induction. The percentage of inhibition for every group was calculated versus values from vehicle-treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% AUC inhibition right paw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teriflunomide (3 mg/Kg)</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>MTX (0.05 mg/Kg)</td>
<td>21 ± 9</td>
</tr>
<tr>
<td>Teriflunomide (3 mg/Kg) + MTX</td>
<td>58 ± 5</td>
</tr>
</tbody>
</table>

The DHODH inhibitor compounds disclosed in the present invention show antiarthritic effect alone as well as in combination with MTX, like teriflunomide but a clearly diminished hepatotoxic potential.

Claims

1. A combination which comprises (a) methotrexate and (b) a non-hepatotoxic DHODH inhibitor.

2. A combination which comprises (a) methotrexate and (b) a DHODH inhibitor wherein the DHODH inhibitor is other than Leflunomide or any active metabolite thereof.

3. A combination according to claim 1 or 2, wherein the DHODH inhibitor (b) is a compound of formula (I):

![Chemical structure](image)

wherein

- one of the groups G\(^1\) represents a nitrogen atom or a group CR\(^c\) and the other represents a group CR\(^d\)
- G\(^2\) represents a nitrogen atom or a group CR\(^d\)
- R\(^1\) represents a group selected from hydrogen atoms, halogen atoms, C\(_{1-4}\) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and C\(_{3-8}\) cycloalkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
- R\(^2\) represents a group selected from hydrogen atoms, halogen atoms, hydroxyl groups, C\(_{1-4}\) alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, C\(_{1-4}\) alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and C\(_{3-8}\) cycloalkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups
• $R^a$, $R^b$ and $R^c$ independently represent groups selected from hydrogen atoms, halogen atoms, C$_{1-4}$ alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and C$_{1-4}$ alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups.

• $R^d$ represents a group selected from hydrogen atoms, halogen atoms, C$_{1-4}$ alkyl groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and C$_{1-4}$ alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and C$_{3-8}$ cycloalkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups.

• one of the groups $G^3$ and $G^4$ is a nitrogen atom and the other is a CH group.

• $M$ is a hydrogen atom or a pharmaceutically acceptable cation

with the proviso that, when at least one of the groups $R^a$ and $R^b$ represent a hydrogen atom and $G^2$ is a group CR$^d$, then $R^d$ represents a groups selected from C$_{1-4}$ alkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, C$_{3-8}$ cycloalkoxy groups which may be optionally substituted by 1, 2 or 3 substituents selected from halogen atoms and hydroxy groups, and the pharmaceutically acceptable salts and N-oxides thereof.

4. A combination according to claim 3 wherein each of the C$_{1-4}$ alkyl groups, the C$_{3-8}$ cycloalkyl groups, the C$_{1-4}$ alkoxy groups and the C$_{3-8}$ cycloalkoxy groups is optionally substituted by 1, 2 or 3 halogen atoms.

5. A combination according to claim 3 or 4 wherein $R^1$ is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl, cyclopropyl and cyclobutyl.

6. A combination according to any one of claims 3 to 5 wherein $G^3$ represents a nitrogen atom and $G^4$ represents a group CH.

7. A combination according to any one of claims 3 to 5 wherein $G^3$ represents a group CH and $G^4$ represents a nitrogen atom.

8. A combination according to any one of claims 3 to 7 wherein both groups $G^1$ represent a group CR$^c$.

9. A combination according to any one of claims 3 to 8 wherein each $R^c$ is independently selected from the groups consisting of hydrogen atoms, fluorine atoms, chlorine atoms and C$_{1-3}$ alkyl groups.

10. A combination according to any one of claims 3 to 9 wherein the group $G^2$ represents a CR$^d$ group.

11. A combination according to claim 10 wherein $R^d$ is selected from the groups consisting of hydroxy groups, C$_{1-3}$ alkoxy groups, 2,2,2-trifluoroethoxy and C$_{3-4}$ cycloalkoxy groups.

12. A combination according to claim 11 wherein $R^d$ is selected from the groups consisting of C$_{1-3}$ alkoxy groups, 2,2,2-trifluoroethoxy and C$_{3-4}$ cycloalkoxy groups.

13. A combination according to any one of claims 3 to 12 wherein $R^3$ is selected from the groups consisting of fluorine atoms, methyl groups and trifluoromethoxy groups.

14. A combination according to claim 13 wherein $R^b$ is selected from the group consisting of hydrogen atoms, fluorine atoms and chlorine atoms.

15. A combination according to any one of claims 3 to 14 wherein $R^2$ is selected from the group consisting of hydrogen atoms and halogen atoms.

16. A combination according to claim 15 wherein $R^2$ is selected from the group consisting of hydrogen atoms and fluorine atoms.

17. A combination according to claim 3 or 4 wherein both groups $G^1$ represent C(R$^c$) groups, $G^2$ represents a C (R$^d$) group, $R^4$ is a fluorine atom, $R^b$ is selected from the group consisting of hydrogen atoms and fluorine atoms and $R^1$ is selected from the group consisting of hydrogen, bromine and fluorine atoms, methyl, ethyl and cyclopropyl...
18. A combination according to claim 17 wherein G^2 is a group selected from C(OH), C(OMe) and C(OEt).

19. A combination according to claim 18 wherein both G^1 represent CH groups and G^2 is a group selected from C(OMe) and C(OEt).

20. A combination according to claim 17, wherein R^2 is a hydrogen atom, R^d is selected from the group consisting of C_{1-3} alkoxy and C_{3-4} cycloalkoxy, and R^2 is a hydrogen atom.

21. A combination according to claim 20 wherein R^d is a hydroxy group or a C_{1-3} alkoxy group.

22. A combination according to claim 21 wherein R^d is a C_{1-3} alkoxy group.

23. A combination according to claim 20 or 21 wherein G^3 represents a nitrogen atom, G^4 represents a group CH and R^2 is a fluorine atom.

24. A combination according to claim 20 or 21 wherein G^3 represents a group CH, G^4 represents a nitrogen atom.

24. A combination according to claim 3 or 4 wherein the DHODH inhibitor is one of:

2-(3'-Fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3'-Ethoxy-3-fluorobiphenyl-4-ylamino)nicotinic acid
2-(3'-Fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3'-Ethoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3'-Methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(2,5-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3'-Ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(2',3-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(2'-Methyl-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3-Chloro-3'-ethoxybiphenyl-4-ylamino)nicotinic acid
2-(3-Chloro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3'-Difluoromethoxy)-3-fluorobiphenyl-4-ylamino)nicotinic acid
2-(3'-Cyclobutoxy-3-fluorobiphenyl-4-ylamino)nicotinic acid
2-(3'-Fluoro-3'-(2,2,2-trifluoroethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3'-Cyclobutoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3',5'-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3'-Ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
Lithium 3-(3'-ethoxy-3-fluorobiphenyl-4-ylamino)isonicotinate
Lithium 3-(3-fluoro-3'-methoxybiphenyl-4-ylamino)isonicotinate
Lithium 3-(3'-methoxy-3-(trifluoromethoxy)biphenyl-4-ylamino)isonicotinate
Lithium 3-(3-fluoro-3'-methoxybiphenyl-4-ylamino)isonicotinate
2-(3'-Ethoxybiphenyl-4-ylamino)nicotinic acid
2-(5-Fluoro-2-methyl-3'-trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3',5'-Difluoro-5'-isopropoxybiphenyl-4-ylamino)nicotinic acid
2-(3-Fluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
2-(3,5-Difluoro-3'-hydroxybiphenyl-4-ylamino)nicotinic acid
5-Bromo-2-(3-fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
5-Bromo-2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
5-Bromo-2-(3-fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)nicotinic acid
2-(3-Fluoro-3'-(trifluoromethoxy)biphenyl-4-ylamino)-5-methylnicotinic acid
5-Cyclopropyl-2-(3-fluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3,5-Difluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid
2-(3'-Ethoxy-5-fluoro-2-methylbiphenyl-4-ylamino)nicotinic acid
2-(5-Fluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)nicotinic acid
2-(3'-ethoxy-3,5-difluorobiphenyl-4-ylamino)-5-methylnicotinic acid
5-cyclopropyl-2-(3'-ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)-5-ethylnicotinic acid
5-bromo-2-(3'-ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
5-cyclopropyl-2-(3'-ethoxy-2,5-difluorobiphenyl-4-ylamino)nicotinic acid
5-cyclopropyl-2-(5'-fluoro-3'-methoxy-2-methylbiphenyl-4-ylamino)nicotinic acid
2-(2',3,5-trifluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(2-chloro-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid
5-cyclopropyl-2-(2,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)-5-cyclopropylnicotinic acid
5-chloro-2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(2,3,5-trifluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)-5-cyclopropynicotinic acid
2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid
5-chloro-2-(2'-chloro-3,5-difluorobiphenyl-4-ylamino)nicotinic acid
2-(2,3,5,6-tetrafluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid
2-(3,5-difluoro-2'-methylbiphenyl-4-ylamino)nicotinic acid
3-(3'-cyclopropoxy-3-fluorobiphenyl-4-ylamino)isonicotinic acid

or a pharmaceutically acceptable salt or N-oxide thereof.

26. A combination according to claim 3 or 4 wherein the DHODH inhibitor is 2-(3'-ethoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid or a pharmaceutically acceptable salt or N-oxide thereof.

27. A combination according to claim 3 or 4 wherein the DHODH inhibitor is 2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)nicotinic acid or a pharmaceutically acceptable salt or N-oxide thereof.

28. A combination according to claim 3 or 4 wherein the DHODH inhibitor is 2-(3'-cyclopropoxy-3,5-difluorobiphenyl-4-ylamino)nicotinic acid or a pharmaceutically acceptable salt or N-oxide thereof.

29. A combination according to claim 3 or 4 wherein the DHODH inhibitor is 2-(3,5-difluoro-3'-methoxybiphenyl-4-ylamino)-5-methylnicotinic acid or a pharmaceutically acceptable salt or N-oxide thereof.

30. A combination according to any one of the preceding claims characterised in that the active ingredients (a) and (b) form part of a single pharmaceutical composition.

31. A combination according to any one of the preceding claims, which further comprises (c) another compound selected from:

(i) Anti-TNF-alpha monoclonal antibodies such as Infliximab, Certolizumab pegol, Golimumab, Adalimumab and AME-527 from Applied Molecular Evolution
(ii) TNF-alpha Antagonists such as Etanercept, Lenercept, Onercept and Pegsnercept
(iii) Calcineurin (PP-2B) Inhibitors / INS Expression Inhibitors such as cyclosporine A, Tacrolimus and ISA-247 from Isotechnika
(iv) IL-1 Receptor Antagonists such as Anakinra and AMG-719 from Amgen
(v) Anti-CD20 monoclonal antibodies such as Rituximab, Ofatumumab, Ocrelizumab and TRU-015 from Trubion Pharmaceuticals
(vi) p38 Inhibitors such as AMG-548 (from Amgen), ARRY-797 (from Array Biopharma), Chlormethiazole edisylate, Doramapimod, PS-540446 (from BMS), SB-203580, SB-242235, SB-235699, SB-281832, SB-681323, SB-856553 (all from GlaxoSmithKline), KC-706 (from Kemia), LEO-1606, LEO-15520 (all from Leo), SC-80036, SD-06 (all from Pfizer), RWJ-67657 (from R.W. Johnson), RO-3201195, RO-4402257 (all from Roche), AVE-
9940 (from Aventis), SCIO-323, SCIO-469 (all from Scios), TA-5493 (from Tanabe Seiyaku), and VX-745 and VX-702 (all from Vertex).

(v) NF-kappaB (NFkB) Activation Inhibitors such as Sulfasalazine and Igraytimod

(viii) Another dihydrofolate reductase (DHFR) inhibitor such as Aminopterin and CH-1504 from Chelsea.

(ix) Janus kinase (JAK) inhibitors, such as CP-690, 550 from Pfizer and INCB-18424, from Incyte.

(x) MEK inhibitor, such as ARRY-162 from Array, and

(xi) Sphingosine-1 phosphate receptor agonists, such as fingolimod (Novartis).

32. Use of (a) methotrexate and (b) a DHODH inhibitor as defined in any one of claims 1 to 29 for the preparation of a medicament for simultaneous, separate or sequential use in the treatment of a pathological condition or disease susceptible to amelioration by inhibition of dehydroorotate dehydrogenase.

33. Use according to claim 32 wherein the pathological condition or disease is selected from rheumatoid arthritis, psoriatic arthritis, ankylosing spondilitis, multiple sclerosis, Wegener’s granulomatosis, systemic lupus erythematosus, psoriasis and sarcoidosis.

34. A product comprising (a) methotrexate and (b) a DHODH inhibitor as defined in any one of claims 1 to 29, as a combined preparation for simultaneous, separate or sequential use in the treatment of a human or animal patient suffering from or susceptible to a pathological condition or disease as defined in claim 32 or 33.

35. A product according to claim 34, which further comprises an active compound (c), as defined in claim 31.

36. A kit of parts comprising (b) a DHODH inhibitor as defined in any one of claims 1 to 29 together with instructions for simultaneous, separate or sequential use in combination with (a) methotrexate for the treatment of a human or animal patient suffering from or susceptible to a pathological condition or disease as defined in claim 32 or 33.

37. A kit according to claim 36, which further comprises an active compound (c), as defined in claim 31.

38. A package comprising (b) a DHODH inhibitor as defined in any one of claims 1 to 29 and (a) methotrexate, for simultaneous, separate or sequential use in the treatment of a pathological condition or disease as defined in claim 32 or 33.

39. A package according to claim 38, which further comprises an active compound (c), as defined in claim 31.

40. Use of (b) a DHODH inhibitor as defined in any one of claims 1 to 29 for the preparation of a medicament, for use in combination with (a) methotrexate, for the treatment of a pathological condition or disease as defined in claim 32 or 33.

41. Use of (a) methotrexate, for use in combination with (b) a DHODH inhibitor as defined in any one of claims 1 to 29 for the treatment of a pathological condition or disease as defined in claim 32 or 33.

42. Use according to claim 40 or 41, wherein the methotrexate (a) is for administration at a dosage regime which involves administration of 0.015 to 3 mg/kg/week of methotrexate and the DHODH inhibitor (b) is for administration at a dosage regime which involves administration of 0.03 to 30 mg/kg/day of DHODH inhibitor.

43. Use according to any one of claims 32, 33 and 40 to 42, wherein the medicament is for use in treating a human or animal patient suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity.

44. Use according to claim 43, wherein said condition that would be aggravated by hepatotoxicity is liver fibrosis, hepatitis, cirrhosis or liver cancer.

45. Use of a DHODH inhibitor as defined in any one of claims 1 to 29 in the manufacture of a medicament for use in treating a human or animal patient suffering from or susceptible to a pathological condition or disease as defined in claim 32 or 33, wherein the human or animal patient is suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity, as defined in claim 43 or 44.

46. A method of treating a human or animal patient suffering from or susceptible to a pathological condition or
disease as defined in claim 32 or 33, which method comprises simultaneously, separately or sequentially administering to said human or animal patient a therapeutically effective amount of (a) methotrexate and (b) a DHODH inhibitor as defined in any one of claims 1 to 29.

47. A method of treating a human or animal patient suffering from or susceptible to a pathological condition or disease as defined in claim 32 or 33, wherein the human or animal patient is suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity as defined in claim 43 or 44, which method comprises administering to said human or animal patient a therapeutically effective amount of a DHODH inhibitor as defined in any one of claims 1 to 29.

48. A combination according to any of claims 1 to 31 for use in treating a pathological condition or disease as defined in claim 32 or 33.

49. A DHODH inhibitor as defined in any one of claims 1 to 29 for use in treating a human or animal patient suffering from or susceptible to a pathological condition or disease as defined in claim 32 or 33, wherein the human or animal patient is suffering from or susceptible to hepatic impairment or a condition that would be aggravated by hepatotoxicity as defined in claim 43 or 44.
## DOCUMENTS CONSIDERED TO BE RELEVANT

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The present search report has been drawn up for all claims.

Place of search: Berlin
Date of completion of the search: 13 November 2008
Examiner: Siatou, Evangelia

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For more details about this annex: see Official Journal of the European Patent Office, No. 12/82
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