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(54) **Titre : TRAITEMENT DE LA GLOMERULONEPHRITE**
(54) **Title: GLOMERULONEPHRITIS TREATMENT**

(57) **Abrégé/Abstract:**
A compound of formula (I) R-L-CO-X (I) (wherein R is a C_{10-24} unsaturated hydrocarbon group optionally interrupted by one or more heteroatoms or groups of heteroatoms selected from S, O, N, SO, SO_2, said hydrocarbon group comprising at least 4 non-conjugated double bonds; L is a linking group forming a bridge of 1 to 5 atoms between the R group and the carbonyl CO; and X is an electron withdrawing group) or a salt thereof for use in the treatment of glomerulonephritis.

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(57) Abstract: A compound of formula (I) R-L-CO-X (I) (wherein R is a C_{10-24} unsaturated hydrocarbon group optionally interrupted by one or more heteroatoms or groups of heteroatoms selected from S, O, N, SO, SO_2, said hydrocarbon group comprising at least 4 non-conjugated double bonds; L is a linking group forming a bridge of 1 to 5 atoms between the R group and the carbonyl CO; and X is an electron withdrawing group) or a salt thereof for use in the treatment of glomerulonephritis.

Glomerulonephritis Treatment

This invention relates to the use of certain polyunsaturated long-chain ketones for the treatment of glomerulonephritis and in particular to ketones carrying electron withdrawing substituents alpha to the carbonyl functionality in such treatment.

Glomerulonephritis, also known as glomerular nephritis, abbreviated GN, is a renal disease characterized by inflammation of the glomeruli, or small blood vessels in the kidneys. It may present with isolated hematuria and/or proteinuria or as a nephrotic syndrome, acute renal failure, or chronic renal failure. Glomerulonephritis is categorised into several different pathological patterns, which are broadly grouped into non-proliferative or proliferative types. Diagnosing the pattern of GN is important because the outcome and treatment differs in different types.

Primary causes of glomerulonephritis are those which are intrinsic to the kidney, whilst secondary causes are associated with certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders (SLE, vasculitis) or cancers.

The glomerulus is a unique vascular network with three specialised types of cell: the endothelial cell, the mesangial cell and the visceral epithelial cell Mesangial cells (MC) serve a number of functions in the renal glomerular capillary including structural support of the capillary tuft, modulation of the glomerular hemodynamics and a phagocytic function allowing removal of macromolecules and immune complexes. The proliferation of MC is a prominent feature of glomerular disease including IgA nephropathy, membranoproliferative glomerulonephritis, lupus nephritis, and diabetic nephropathy.

Reduction of MC proliferation in glomerular disease models by treatment with, for example, a low protein diet has been shown to produce extracellular matrix expansion and glomerulosclerotic changes. MC proliferation inhibitors may therefore offer therapeutic opportunities for the treatment of proliferative glomerular disease.

Mesangial proliferative glomerulonephritis is a form of glomerulonephritis which involves inflammation at the kidney glomeruli. The mesangial cells which

are a part of the glomerular capillaries increase in size giving the glomeruli a lumpy appearance. The disorder usually causes nephritic syndrome which represents protein loss in the urine. It may be present as acute, chronic or rapidly progressive glomerulonephritis and may progress to chronic renal failure.

It is known that MC proliferation is inhibited by a variety of pharmacological drugs, for example inhibitors against angiotensin converting enzyme (ACE) , cyclin-dependent kinases (CDK), platelet derived growth factor and others.

The glomerulus also has potential to express several isoforms of nitric oxide synthase (NOS). Induction of inducible NOS occurs as part of a rapid initial response to immune injury in a glomerulonephritis. Whilst the role of NO generated by NOS in the glomerulus is still unclear, some studies have demonstrated that NO inhibition can alter the level of proteinuria and leukocyte infiltration and other manifestations of injury such as thrombosis, proliferation and matrix production.

It seems clear that reduction of NO will contribute to amerlioration of mesangial proliferation and hence offer alleviation of the symptoms of glomerulonephritis.

The present inventors seek new treatments for GN and related conditions. As noted above, current proposed therapies may be based on angiotensin converting enzyme (ACE) inhibitors such as lisinopril and similar compounds. These inhibitors reduce blood pressure, a feature common to all anti-hypertensive drugs but they also possess inhibitory activity of intra renal MC proliferation and also seem to lower proteinurea. Other treatments include the use of CDK2 antagonists or calcium antagonists.

The present inventors have realised that the compounds claimed herein, some of which are new, others known, have potential in the treatment of proliferative conditions in general and glomerulonephthritis in particular. The inventors have found that a certain class of compounds based upon long chain unsaturated fatty acid molecules are useful in the treatment of glomerulonephritis.

Some of the compounds proposed for use in this invention have been disclosed before, for example, in EP-A-1469859 but only there in the context of the treatment of psoriasis. The present inventors have realised that these compounds

and others have utility also in the treatment of glomerulonephritis or other proliferative diseases.

Thus, viewed from one aspect the invention a compound of formula (I)

R-L-CO-X (I)

(wherein R is a C_{10-24} unsaturated hydrocarbon group optionally interrupted by one or more heteroatoms or groups of heteroatoms selected from S, O, N, SO, SO_2, said hydrocarbon group comprising at least 4 non-conjugated double bonds;

L is a linking group forming a bridge of 1 to 5 atoms between the R group and the carbonyl CO; and

X is an electron withdrawing group) or a salt thereof

for use in the treatment of glomerulonephritis.

Viewed from another aspect the invention provides a method of treating glomerulonephritis comprising administering to an animal, preferably a mammal, e.g. human, an effective amount of a compound of formula (I) or a salt thereof as hereinbefore described.

Viewed from another aspect the invention provides use of a compound of formula (I) or a salt thereof as hereinbefore described for use in the manufacture of a medicament for treating glomerulonephritis.

Detailed Description

This invention involves the use of compounds of formula (I) or a salt thereof in the treatment of glomerulonephritis and related conditions. Glomerulonephritis is a renal disease characterized by inflammation of the glomeruli.

The group R preferably comprises 5 to 9 double bonds, preferably 5 or 8 double bonds, e.g. 5 to 7 double bonds such as 5 or 6 double bonds. These bonds should be non-conjugated. It is also preferred if the double bonds do not conjugate with the carbonyl functionality.

The double bonds present in the group R may be in the cis or trans configuration however, it is preferred if the majority of the double bonds present

(i.e. at least 50%) are in the cis configuration. In further advantageous embodiments all the double bonds in the group R are in the cis configuration or all double bonds are in the cis configuration except the double bond nearest the carbonyl group which may be in the trans configuration.

The group R may have between 10 and 24 carbon atoms, preferably 12 to 20 carbon atoms, especially 17 to 19 carbon atoms.

Whilst the R group can be interrupted by at least one heteroatom or group of heteroatoms, this is not preferred and the R group backbone preferably contains only carbon atoms.

The R group may carry up to three substituents, e.g. selected from halo, C_{1-6} alkyl e.g. methyl, C_{1-6} alkoxy. If present, the substituents are preferably non-polar, and small, e.g. a methyl group. It is preferred however, if the R group remains unsubstituted.

The R group is preferably linear. It preferably derives from a natural source such as a long chain fatty acid or ester. In particular, the R group may derive from AA, EHA or DHA.

The linking group L provides a bridging group of 1 to 5 backbone atoms, preferably 2 to 4 backbone atoms between the R group and the carbonyl. The atoms in the backbone of the linker may be carbon and/or be heteroatoms such as N, O, S, SO, SO_2. The atoms can form part of a ring and the backbone atoms of the linking group can be substituted with side chains, e.g. with groups such as C_{1-6} alkyl, oxo, alkoxy, or halo.

Preferred components of the linking group are $-CH_2-$, $-CH(C_{1-6}alkyl)-$, $-N(C_{1-6}alkyl)-$, -NH-, -S-, -O-, -CH=CH-, -CO- , -SO-, $-SO_2-$ which can be combined with each other in any (chemically meaningful) order to form the linking group. Thus, by using two methylene groups and an -S- group the linker $-SCH_2CH_2-$ is formed.

It is highly preferred if the linking group L contains at least one heteroatom in the backbone. It is also preferred if the first backbone atom of the linking group attached to the R group is a heteroatom or group of heteroatoms.

It is highly preferred if the linking group L contains at least one $-CH_2-$ link in the backbone. Ideally the atoms of the linking group adjacent the carbonyl are

$-CH_2-$.

It is preferred that the group R or the group L (depending on the size of the L group) provides a heteroatom or group of heteroatoms positioned α, β, γ, or δ to the carbonyl, preferably β or γ to the carbonyl. Preferably the heteroatom is O, N or S or a sulphur derivative such as SO.

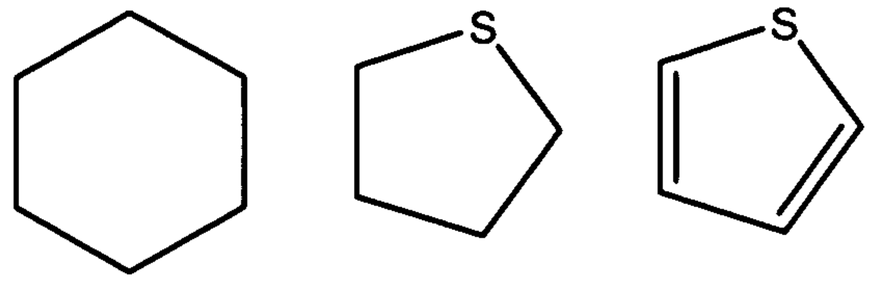
Highly preferred linking groups therefore are $-NH_2CH_2$, $-NH(Me)CH_2-$, $-SCH_2-$, $-SOCH_2-$, $-COCH_2-$

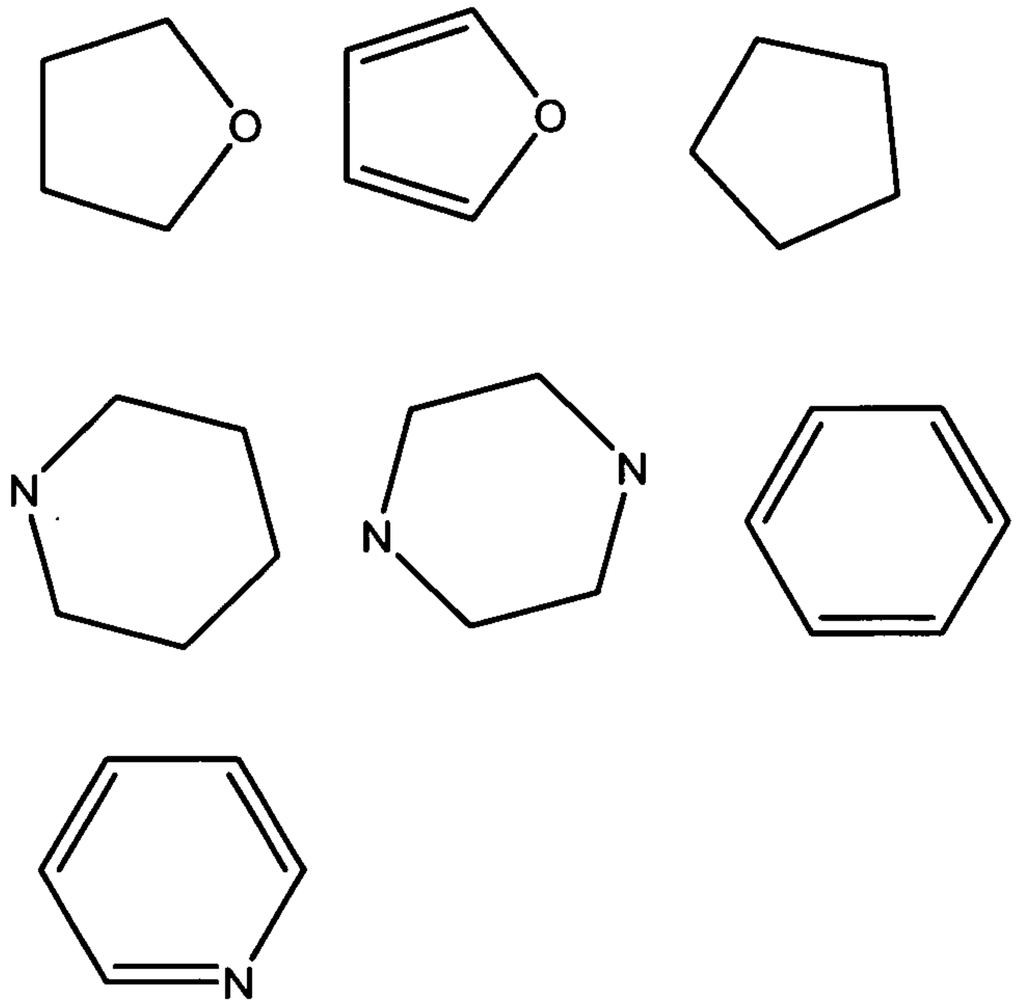
It is also within the invention for the linking group to be a ring or to comprise a ring. Thus for example, the linker might be thiophene, e.g. 2,4-thiophene which provides a two atom bridge to the carbonyl (via the shortest route). It would also be possible for the linker to be a ring such as furan, tetrahydrofuran, piperidine, cyclohexane, benzene or pyridine. Where the linker comprises a ring it is preferred if this is a 5 or 6 membered ring. It is preferred if the ring comprises at least one heteroatom or group of heteroatoms. It is preferred if the ring is unsaturated or aromatic. When the R and COX groups bind directly to such a ring, it is preferred if the R group and COX group bind on different atoms and preferred if they bind on carbon atoms of the ring.

The substitution pattern is preferably such that the R and carbonyl substituents are alpha, gamma to each other (i.e. 1,3 or 2, 4 or 3, 5-split).

For the avoidance of doubt, it is stressed that the 1 to 5 atom bridge should be counted as the shortest route from the start of the linker to the carbonyl.

Suitable ring linkers are shown below where the R group and carbonyl can bind to any two carbon atoms on these rings:





It is also within the scope of the invention for the linker to comprise a ring and non ring portion, e.g. CH_2-thiophene or NH_2-thiophene and so on. In such a linker it is preferred if the R group binds directly to the ring and that the carbonyl group binds to the non ring portion, e.g. a -CH_2- linkage. The skilled man will be able to devise all kinds of different linkers suitable for use in the invention.

Highly preferred linking groups are -CH_2-, -CH_2-CH_2-, -CH(Me), -CH(Me)CH_2-, -CH(Me)-CH(Me)-, SCH_2, $NHCH_2$, N(Me)CH_2, 2,4-thiophene and 2,5-thiophene.

The group X is an electron withdrawing group. Suitable groups in this regard include O-C_{1-6} alkyl, CN, OCO_2-C_{1-6} alkyl, phenyl, $CHal_3$,$CHal_2H$,$CHalH_2$ wherein Hal represents a halogen, e. g. fluorine, chlorine, bromine or iodine, preferably fluorine.

In a preferred embodiment the electron withdrawing group is $CHal_3$, especially CF_3.

Thus, preferred compounds of formula (I) are those of formula (I')

R-Y1-Y2-CO-X

wherein R and X are as hereinbefore defined;

Y1 is selected from O, S, NH, N(C_{1-6}-alkyl), SO or SO_2 and

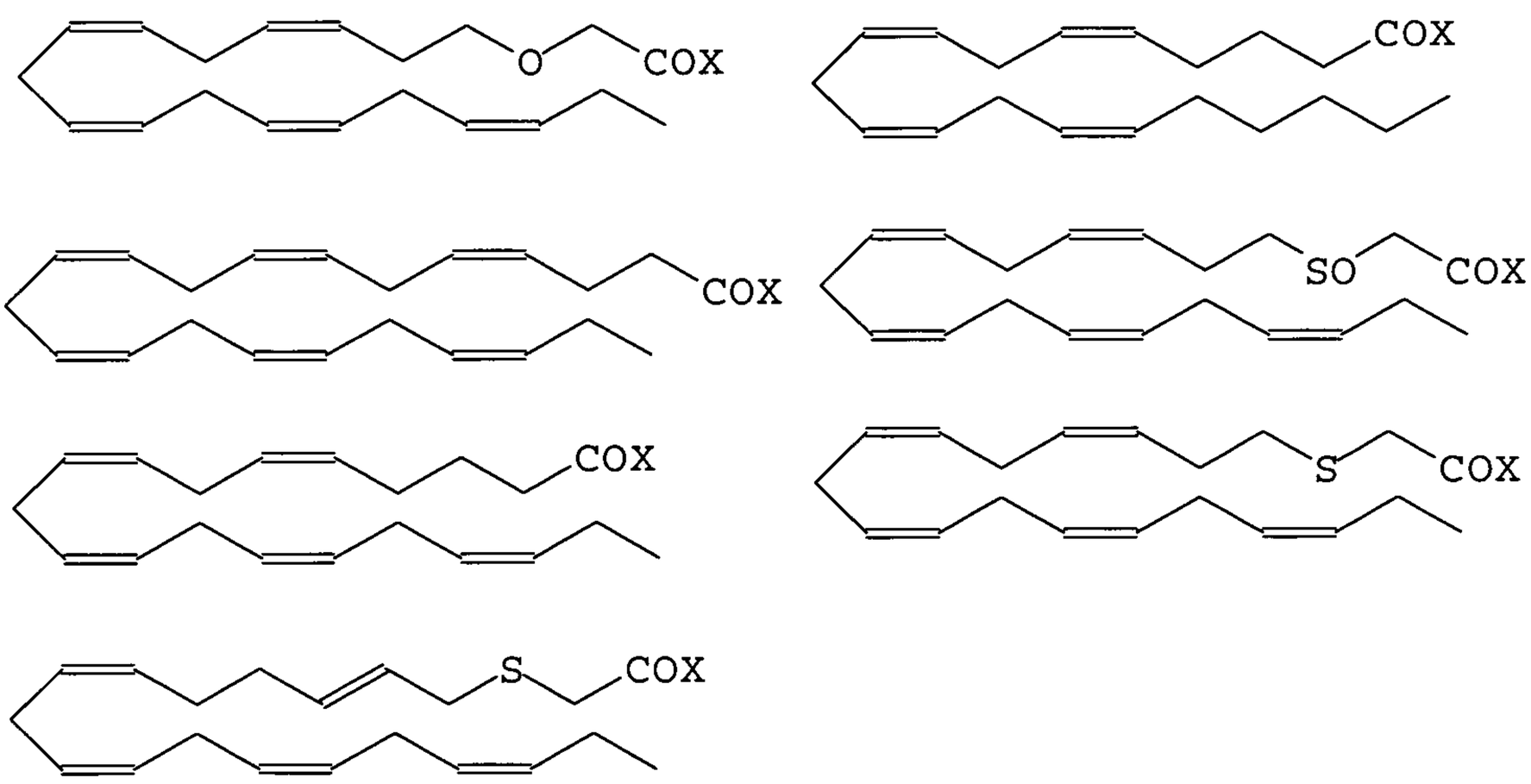
Y2 is $(CH_2)_n$ or CH(C_{1-6} alkyl); or

Y1 and Y2 taken together form a 5 or 6 membered homo or heterocyclic, optionally unsaturated or aromatic ring; or

Y1 forms a 5 or 6 membered homo or heterocyclic, optionally unsaturated or aromatic ring and Y2 is $(CH_2)_n$;

where n is 1 to 3, preferably 1.

Highly preferred (known) compounds for use in the invention are depicted below.



Certain compounds are new and form a further aspect of the invention.

Thus viewed from another aspect the invention provides a compound of formula (I")

R-Y1-Y2-CO-X (I")

wherein R and X are as hereinbefore defined;

Y1 and Y2 taken together form a 5 or 6 membered homo or heterocyclic, optionally unsaturated or aromatic ring; or

Y1 forms a 5 or 6 membered homo or heterocyclic, optionally unsaturated or aromatic ring and Y2 is $(CH_2)_n$;

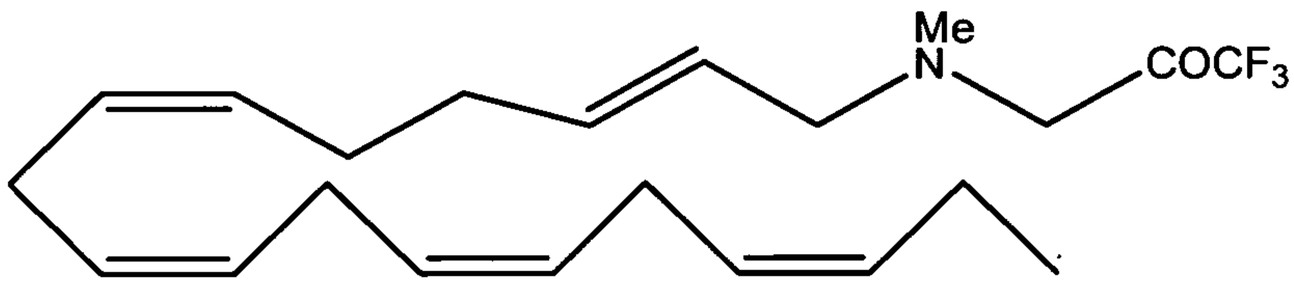
where n is 1 to 3, preferably 1.

Further compounds which are new include the compounds

$RN(C_{1-6}alkyl)COX$. Thus viewed from another aspect the invention provides a compound of formula (II)

$RN(C_{1-6}alkyl)(CH_2)_nCOX$ (II)

where R, n and X are as hereinbefore defined, especially the compound:

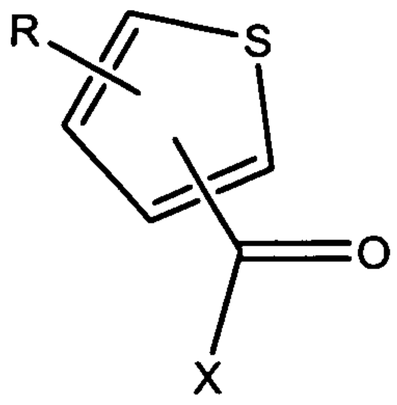


Further preferred compounds which are new are those in which the L group is a ring or comprises a ring. Viewed from another aspect therefore the invention provides a compound of formula (III)

R-L'-CO-X (III)

where R and X are as hereinbefore defined an L' represents a linking group forming a bridge of 1 to 5 atoms between the R group and the carbonyl CO wherein said L' linking group comprises a ring structure.

Preferred compounds of formula (III) are depicted below.



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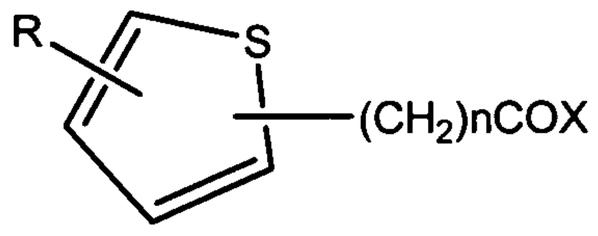


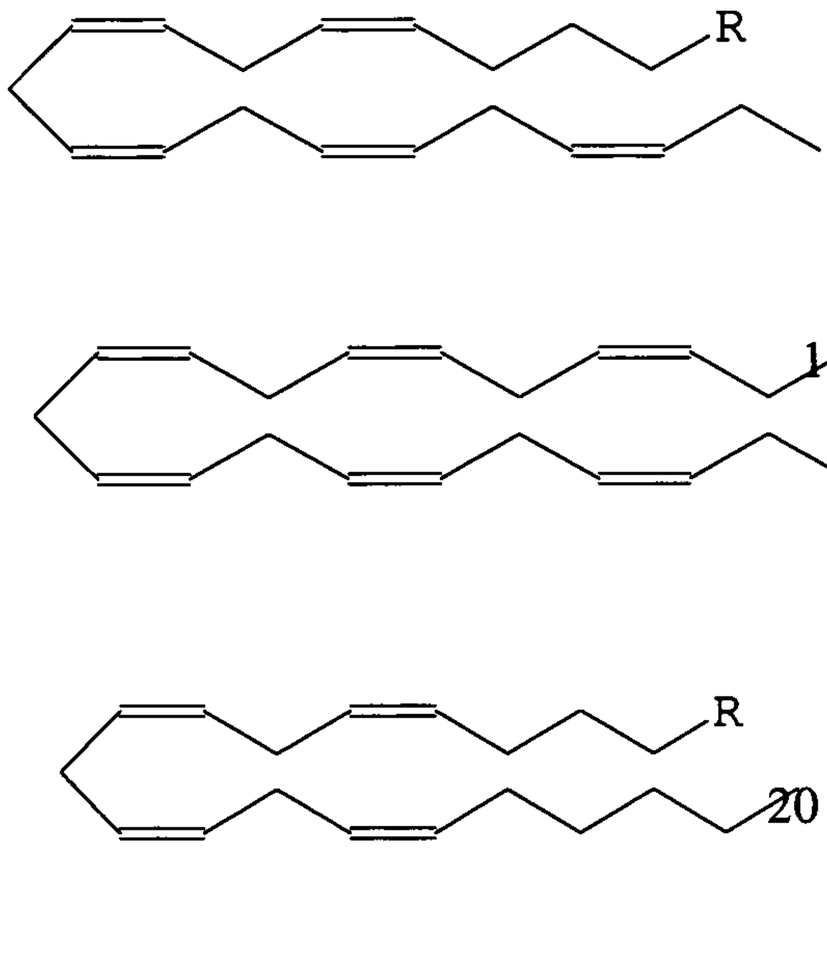
(V)

where n is 1 to 3, e.g. 1 to 2.

Especially preferably the groups bind to the 2 and 4 positions of the ring (where atom 1 is the S atom).

Viewed from another aspect the invention provides a pharmaceutical composition comprising any new compound as hereinbefore defined in combination with at least one pharmaceutically acceptable excipient.

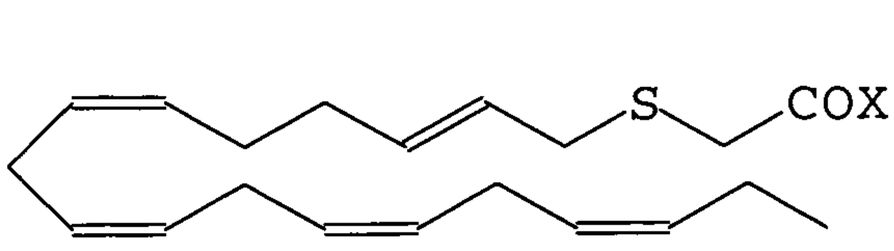
The following compounds are highly preferred for use in the invention:



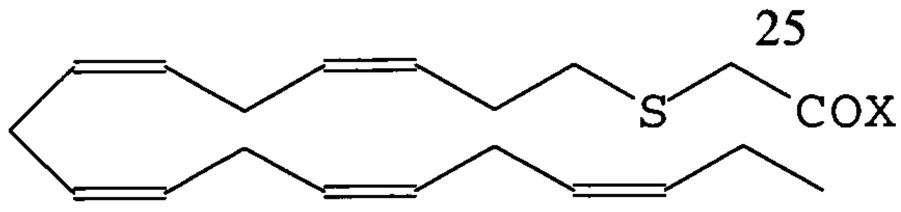
$R= COCF_3 = EPACOCF_3$

$R= COCF_3 = DHACOCF_3$

$R= COCF_3 = AACOCF3$



$X= CF_3 = AKH217$



$X= CF_3 = AVX002$

Where possible, the compounds of the invention can be administered in salt, solvate, prodrug or ester form, especially salt form. Preferably however, no such form is used.

Typically, a pharmaceutical acceptable salt may be readily prepared by using a desired acid. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. For example, an aqueous solution of an acid such as hydrochloric acid may be added to an aqueous suspension of a compound of formula (I) and the resulting mixture evaporated to dryness (lyophilised) to obtain the acid addition salt as a solid. Alternatively, a compound of formula (I) may be dissolved in a suitable solvent, for example an alcohol such as isopropanol, and the acid may be added in the same solvent or another suitable solvent. The resulting acid addition salt may then be precipitated directly, or by addition of a less polar solvent such as diisopropyl ether or hexane, and isolated by filtration.

Suitable addition salts are formed from inorganic or organic acids which form non-toxic salts and examples are hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, nitrate, phosphate, hydrogen phosphate, acetate, trifluoroacetate, maleate, malate, fumarate, lactate, tartrate, citrate, formate, gluconate, succinate, pyruvate, oxalate, oxaloacetate, trifluoroacetate, saccharate, benzoate, alkyl or aryl sulphonates (eg methanesulphonate, ethanesulphonate, benzenesulphonate or p-toluenesulphonate) and isethionate. Representative examples include trifluoroacetate and formate salts, for example the bis or tris trifluoroacetate salts and the mono or diformate salts, in particular the tris or bis trifluoroacetate salt and the monoformate salt.

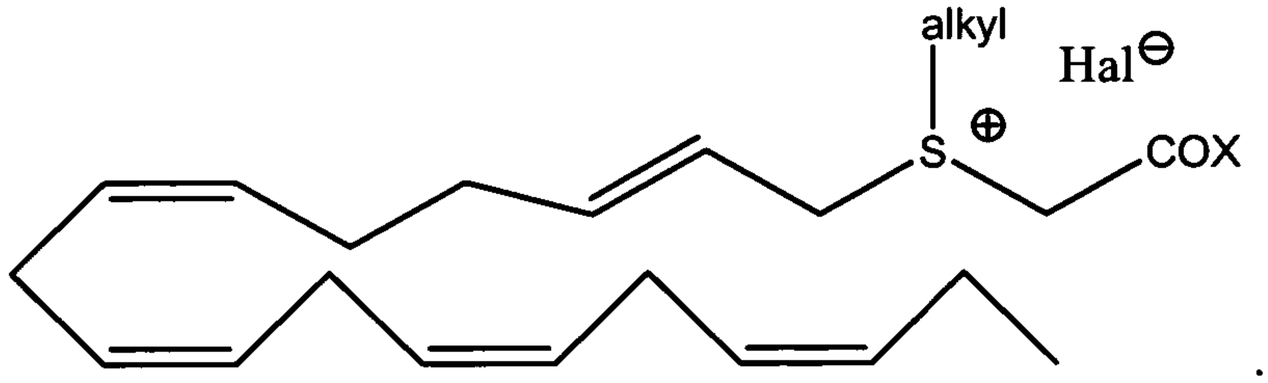
In a further highly preferred embodiment, the compound of the invention is a sulphonium salt. In such a compound, a sulphur atom in the backbone of the molecule, e.g. in the linker group, is functionalised to carry a C1-6-alkyl group. This can be achieved through reaction with an alkyl halide, e.g. methyl iodide. The halide ion forms the counterion of the salt.

In a further preferred embodiment therefore the invention provides a sulphonium salt of a compound of formula (I). Preferably the compound is of formula (VI)

$RS(C_{1-6}alkyl)CH_2\text{-}COX^+Z^-$ (VI)

where R and X are as hereinbefore defined and Z is a counterion, e.g. halide;

e.g. the compound

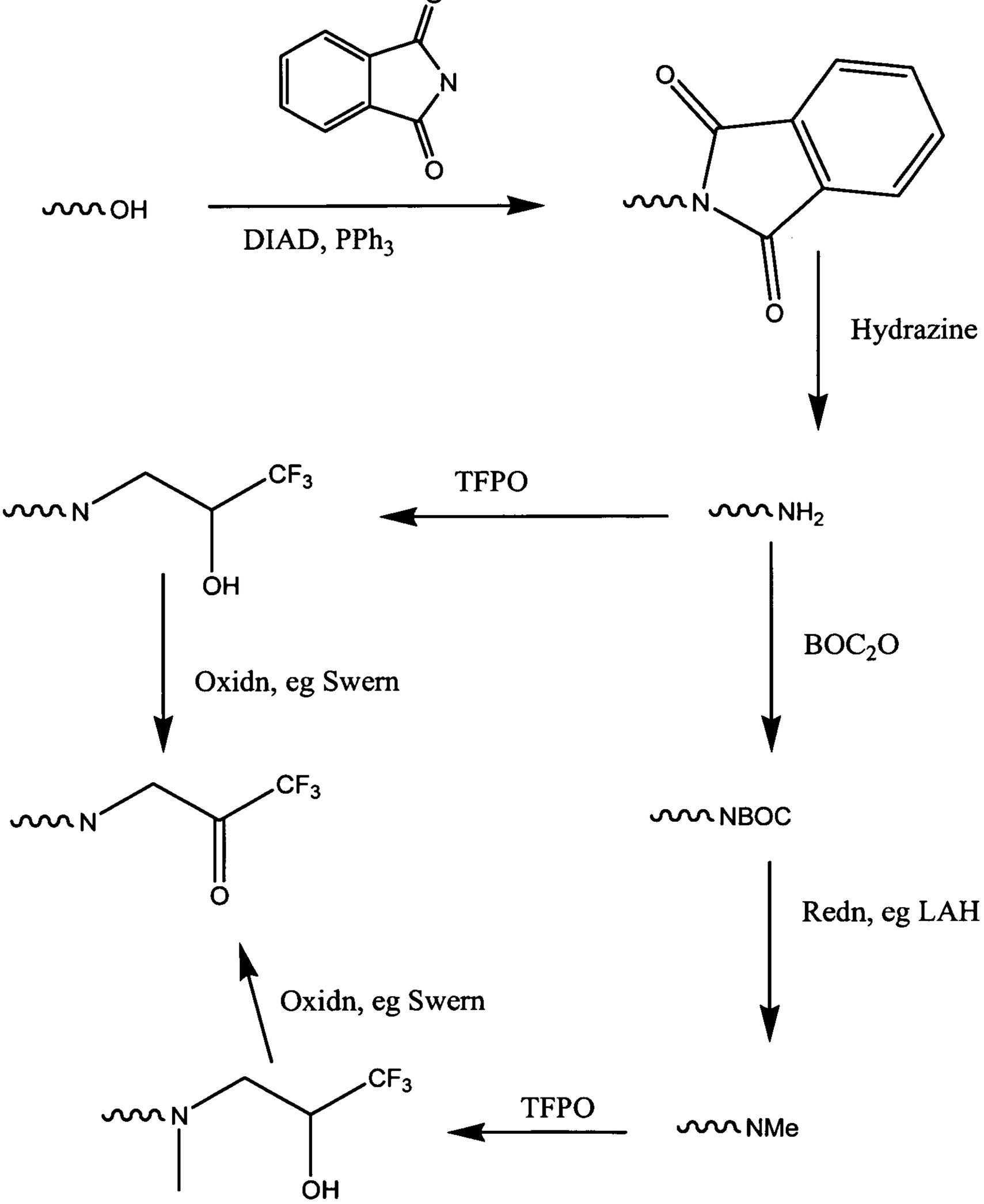


Compounds of formula (I) may be manufactured using known chemical synthetic routes. It is convenient to begin synthesis from the commercially available compounds arachidonic acid (AA), EPA (all-Z-eicosa-5,8,11,14,17-pentaenoic acid) or DHA (all-Z-docosa-4,7,10,13,16,19-hexaenoic acid). Conversion of the acid functionality of these compounds into, for example a $-COCF_3$ group can be achieved readily, e.g. by converting the carboxylic acid into its corresponding acid chloride and reacting the same with trifluoroacetic anhydride in the presence of pyridine.

Introduction of a heteroatom into the carbon chain is also achieved readily. Conveniently, for example, the starting acid is reduced to an alcohol and, if required, converted to the corresponding thiol. The nucleophilic thiol may then be reacted with a group such as $BrCH_2COCF_3$ thereby introducing the carbonyl and electron withdrawing species. Complete synthetic protocols may be found in J. Chem. Soc., Perkin Trans 1, 2000, 2271-2276 or J. Immunol., 1998, 161, 3421.

Where the backbone of the molecule contains a nitrogen atom, an alternative synthesis is required. Formation of a polyunsaturated alcohol can be achieved using protocols give in the above Perkin Trans paper. Thereafter, conversion of an alcohol -OH to $-NH_2$ with, for example, phthalimide and subsequent hydrazine reduction allows formation of a $-NH_2CH_2COCF_3$ group by reaction with trifluoropropyleneoxide (TFPO) and oxidation of the hydroxyl to a ketone. This reaction is shown below.

Methylation of the nitrogen can be effected before this reaction by the formation of an N-BOC group and reduction, e.g. with lithium aluminium hydride. Reaction with TFPO and oxidation yields the linker NMe-CH_2.



This forms a further aspect of the invention which therefore provides a process for the preparation of a compound of formula (I) comprising:

(I) converting the compound R-OH to R-NH_2;

(II) optionally methylating the N atom;

(III) reacting with TFPO; and

(IV) oxidising the formed hydroxyl to a ketone.

The compounds of the invention are proposed primarily for use in the treatment of, *inter alia*, glomerulonephritis. Whilst the compounds of the invention are generally of use in the treatment of glomerulonephritis, the compounds are of particular utility in the treatment of the proliferative type of the disease.

By treating or treatment is meant at least one of:

(i). preventing or delaying the appearance of clinical symptoms of the disease developing in a mammal;

(ii). inhibiting the disease i.e. arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or subclinical symptom thereof, or

(iii). relieving or attenuating one or more of the clinical or subclinical symptoms of the disease.

The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician. In general a skilled man can appreciate when "treatment" occurs.

The word "treatment" is also used herein to cover prophylactic treatment, i.e. treating subjects who are at risk of developing a disease in question.

The compounds of the invention can be used on any animal subject, in particular a mammal and more particularly to a human or an animal serving as a model for a disease (e.g., mouse, monkey, etc.).

In order to treat a disease an effective amount of the active agent needs to be administered to a patient. A "therapeutically effective amount" means the amount of a compound that, when administered to an animal for treating a state, disorder or condition, is sufficient to effect such treatment. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the

age, weight, physical condition and responsiveness of the subject to be treated and will be ultimately at the discretion of the attendant doctor.

While it is possible that, for use in the methods of the invention, a compound of formula I may be administered as the bulk substance, it is preferable to present the active ingredient in a pharmaceutical formulation, for example, wherein the agent is in admixture with a pharmaceutically acceptable carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The term "carrier" refers to a diluent, excipient, and/or vehicle with which an active compound is administered. The pharmaceutical compositions of the invention may contain combinations of more than one carrier. Such pharmaceutical carriers are well known in the art.. The pharmaceutical compositions may also comprise any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilizing agent(s) and so on. The compositions can also contain other active components, e.g. other drugs for the treatment of glomerulonephritis.

It will be appreciated that pharmaceutical compositions for use in accordance with the present invention may be in the form of oral, parenteral, transdermal, inhalation, sublingual, topical, implant, nasal, or enterally administered (or other mucosally administered) suspensions, capsules or tablets, which may be formulated in conventional manner using one or more pharmaceutically acceptable carriers or excipients. The compositions of the invention could also be formulated as nanoparticle formulations.

The compounds of the invention can be administered for immediate-, delayed-, modified-, sustained-, pulsed-or controlled-release applications.

The pharmaceutical compositions of the invention may contain from 0.01 to 99% weight - per volume of the active material.

A therapeutically effective amount of the compound of the present invention can be determined by methods known in the art. The therapeutically effective quantities will depend on the age and on the general physiological condition of the patient, the route of administration and the pharmaceutical formulation used. The therapeutic doses will generally be between about 10 and 2000 mg/day and preferably between about 30 and 1500 mg/day. Other ranges may be used, including, for example, 50-500 mg/day, 50-300 mg/day, 100-200 mg/day.

Administration may be once a day, twice a day, or more often, and may be decreased during a maintenance phase of the disease or disorder, e.g. once every second or third day instead of every day or twice a day. The dose and the administration frequency will depend on the clinical signs, which confirm maintenance of the remission phase, with the reduction or absence of at least one or more preferably more than one clinical signs of the acute phase known to the person skilled in the art.

It is advantageous if the medicament of the invention is taken orally.

The compounds of the invention may be used in the treatment of glomerulonephritis and related diseases. In particular, the compounds of the invention may be used to treat mesangioproliferative glomerulonephritis, nephrotic syndrome, chronic or acute renal failure, proteinuria, hematuria, IgA nephropathy, , membranoproliferative glomerulonephritis, lupus nephritis, diabetic nephropathy, and glomeruloscerlosis,

The compounds of the invention may be used to treat glomerulonephritis in combination with other known pharmaceuticals for said purpose and this forms a further aspect of the invention. Other useful pharmaceuticals include corticosteriods, immunosuppressive drugs, antihypertensive agents and diuretic medications.

The invention is described further below with reference-to the following non-limiting examples and figures.

Description of Figures:

Figure 1 shows the effect of the inhibitors of the examples on cytokine-stimulated PGE_2 formation in mesangial cells. Quiescent cells were stimulated with either DMEM (-), IL-1β (1nM), in the absence (-) or presence of the indicated concentrations of AKH-217 and AVX002. Supernatants were collected and taken for PGE2 quantification using an ELISA. Data are expressed as % of maximal IL-1β-stimulated PGE2 and are means ± S.D. (n=3).

Figure 2 shows the effect of the inhibitors of the examples on cytokine-stimulated $sPLA_2$ protein (A) and mRNA (B) expression and promoter activity in mesangial cells.

Quiescent cells were stimulated with either DMEM (-), or IL-1β (1nM) in the absence (-) or presence of AKH-217 (etOH), vehicle control.

(A) Supernatants were taken for protein precipitation and precipitated proteins were separated by SDS-PAGE and subjected to a Western blot analysis using a monoclonal antibody against rat $sPLA_2$. Data show duplicates of a representative experiment.

(B). Cells were taken for RNA extraction and subjected to quantitative PCR analysis of rat IIA-$sPLA_2$ and 18 S RNA. ΔΔCt values were calculated and results are expressed as % of maximal IL-1β-stimulated response and are means + S.D. (n=3).

(C) Cells were transfected with the sPLA2 promoter construct plus a plasmid coding for the Renilla luciferase. After transfection, cells were stimulated for 24h with vehicle (-), IL-1β (1nM), or IL-1β plus 10μM of AKH-217. sPLA2 promoter activity was calculated and results are expressed as relative luciferase units (RLU) and are means ±S.D. (n=3).

Figure 3 shows the effect of the inhibitors of the examples on cytokine-stimulated NO formation in mesangial cells. Quiescent cells were stimulated with either DMEM (-), IL-1β (1nM; +), in the absence or presence of the indicated concentrations of AKH-217, AVX002, or AACOCF3. Supernatants were collected and taken for nitric oxide (NO) quantification by using a Griess Reaction assay. Data are expressed as μM of NO in the supernatant and are means ± S.D. (n=3).

Figure 4 shows the effect of the inhibitors of the examples on cytokine-stimulated iNOS protein (A) and mRNA (B) expression and promoter activity in mesangiai cells. Quiescent cells were stimulated with either vehicle (DMEM), IL-1β (1nM), in the absence (-) or presence of the indicated concentrations (in μM) of AVX001 and AVX002. EtOH, vehicle control.(A) Cells were taken for protein extraction and equal amount of. protein were separated by SDS-PAGE and subjected to a Western blot analysis using a polyclonal antibody against iNOS at a dilution of 1:2000. Data are representative of at least 3 independent experiments giving similar results.

(B). Cells were taken for RNA extraction and subjected to quantitative PCR analysis of rat iNOS and 18 S RNA. ΔΔCt values were calculated and results are expressed as % of maximal IL-1β response and are means ± S.D (n=3).

(C) Cells were transfected with the rat iNOS promoter construct plus a plasmid coding for the Renilla luciferase. After transfection, cells were stimulated with vehicle (-), IL-1β (1nM), or IL-1β plus 10μM of AVX001. iNOS promoter activity was calculated and results are expressed as relative luciferase units (RLU) and are means ± S.D.(n=3).

Figure 5 shows the effect of the inhibitors of the examples on [^{3}H]thymidine incorporation in mesangial cells. Quiescent cells were stimulated for 24h with vehicle (DMEM), or 100μM of ATP in the absence (-) or presence of the indicated concentrations of AVX001 or AVX002 , in the presence of [^{3}H]thymidine.

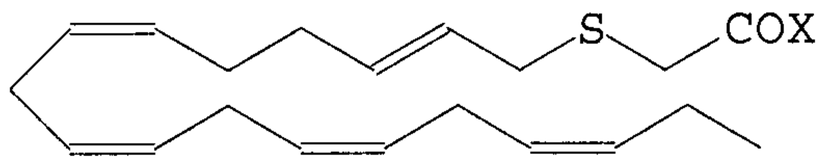
(B) Cells were stimulated for 24h with insuline (100ng/ml) or IGF (50ng/ml) in the absence (-) or presence of 10μM of AVX001 or AVX002 , in the presence of [^{3}H]thymidine. Thymidine incorporated into DNA was determined and results are expressed as % of control and are means ± S.D. (n=3).

Figure 6 shows the effect of the inhibitors of the examples on IL-1 β-stimulated NFκB activation in mesangial cells. Quiescent cells were stimulated for 30 min with either vehicle (DMEM), or IL-1β (2nM) in the absence (-) or presence (+) of AVX001 or AVX002 (10μM, pretreated 2h). Thereafter, cell lysates were separated by SDS-PAGE and subjected to a Western blot analysis using a polyclonal antibody against phospho-p65 (NFκB) (upper panel), HuR as a loading control (middle panel), and IκB (lower panel). Data in Fig 6A show duplicates of one representative experiments. Figures 6B and C show the densitometric evaluation of NFkB and IkB bands.

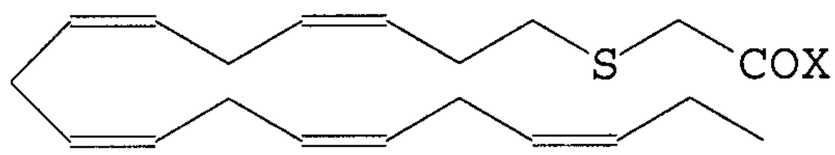
Examples

The following compounds were used in the Experiments:

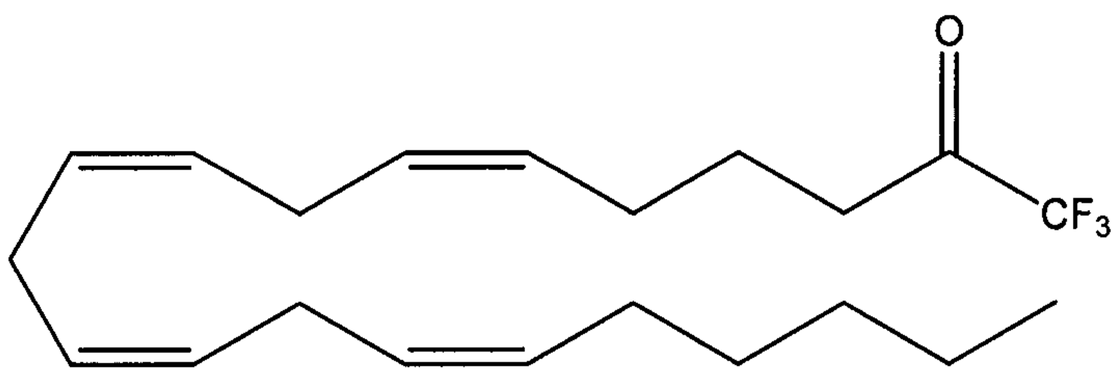
AKH-217/AVX001



AVX002



$X = CF_3$



AACOCF3

These compounds were synthesised based on . Chem. Soc., Perkin Trans 1, 2000, 2271-2276.

Example 1

Effect of inhibitors on PGE2 formation in rat renal mesangial cells

We investigated the effect of the inhibitors on PGE2 formation in mesangial cells. PGE2 formation is highly induced by stimulation of cells with the pro-inflammatory cytokine IL-1β. This induction of PGE2 is dose-dependently reduced in the presence of the inhibitors. Maximal effects were seen with 3-10 μM of AKH-217 (AVX001) and Compound B (AVX002) (Fig. 1).

It has been previously shown that the cytokine-induced PGE2 formation in mesangial cells involves both sPLA2 and cPLA2 activation, we then investigated the effect of the inhibitors above on sPLA2 protein and

mRNA expressions. As seen in Fig. 2, AKH-217 was able to reduce the IIA-sPLA2 protein expression and secretion (Fig. 2A), but also IIA-sPLA2 mRNA expression (Fig. 2B). This effect on sPLA2 mRNA expression was due to a reducing effect on gene transcription. This was shown by a luciferase reporter gene assay that reflected sPLA2 promoter activity.

To this end, a 2.26kb fragment of the rat IIA-sPLA2 promoter was cloned. This fragment was ligated into a luciferase-containing vector (pGL3) and used to transfect mesangial cells. As seen in Fig. 2C, the IL-1β-stimulated promoter activity was completely reduced by AKH-217.

These data suggest that the inhibitors of the invention could affect some transcription factors, which are activated by IL-1β and are essential for sPLA2 gene transcription. Potential candidates include NFkB and PPAR.

Example 2

Effect of inhibitors on nitric oxide (NO) formation in rat renal mesangial cells

Nitric oxide (NO) is also considered a pro-inflammatory mediator which is generated by the inducible NO synthase (iNOS) upon cytokine treatment of mesangial cells. Various previous studies have indicated that iNOS expression is regulated by the same transcription factors as sPLA2. We investigated whether cytokine-triggered iNOS expression is also affected by the inhibitors.

NO formation in mesangial cells was highly induced by IL-1 β treatment. This stimulated NO formation was reduced in a dose-dependent manner in the presence of AKH-217 and AVX002 (Fig. 3). Furthermore, the protein expression of iNOS, which is induced by IL-1 β (Fig. 4A), is down-regulated in the presence of AKH-217 and AVX002 (Fig. 4A). A similar reducing effect was also seen on iNOS mRNA expression when quantitative RealTime PCR analyses were performed (Fig. 4B). To see whether this effect is due to altered gene transcription of iNOS, luciferase reporter gene assays were performed to measure iNOS promoter activity. A 4.5kb fragment of the rat iNOS promoter was kindly provided by Dr. K.F. Beck (pharmazentrum frankfurt). As seen in Fig. 4C, IL-1 β stimulation of mesangial cells

stimulated iNOS promoter by 10-fold. In the presence of AKH217, the promoter activity was completely lost.

These data suggest, that also in the case of iNOS, AVX inhibitors have a reducing effect on gene transcription, most probably affecting the same transcription factors as in the case of sPLA2 transcription.

Example 3

Effect of inhibitors on mesangial cell proliferation

Glomerulonephritis is characterized in a first early phase by increased mesangial apoptosis which in a second phase is replaced by an opposite event, i.e. hyperproliferation of mesangial cells. Many previous studies have shown that quiescent mesangial cells in culture can re-enter the cell cycle when exposed to various growth factors, including PDGF, insulin, insulin-like growth factor (IGF), or extracellular nucleotides such as ATP and UTP. These data are confirmed here, as insulin, IGF and ATP trigger increased [^{3}H]thymidine incorporation into DNA (Fig. 5A and 5B). In the presence of either AKH217 or AVX002, agonist-stimulated [^{3}H]thymidine incorporation is reduced (Fig. 5A and 5B). Similar data were also obtained when cells were stimulated with PDGF. These data suggest an anti-proliferative potential of the inhibitors.

Example 4

Effect of inhibitors on NFkB activity in mesangial cells.

As we have seen in Examples 1 and 2, that iNOS and sPLA2 expression is regulated by the inhibitors in a similar manner, we further studied whether these inhibitors had an effect on NFkB activation. NFkB activation was measured by Western blot analyses by detecting the amount of phospho-p65 which represents the active transcription factor subunit. Short-term stimulation of cells with IL-1 β (3 revealed a small but clear increase of phospho-p65 (Fig. 6, upper panel) consistent with many previous reports that cytokines activate NFkB. This effect was reduced by AKH217 (Fig. 6). In addition, the inhibitor of kB (IkB), which is constitutively expressed in

unstimulated cells, is downregulated by IL-1 β stimulation, and this downregulation is reverted by the inhibitors (Fig. 6, lower panel). For equal loading, the nuclear HuR stabilization factor was stained (Fig. 6, middle panel)

Claims

1. A compound of formula (I)

R-L-CO-X (I)

wherein R is a C_{10-24} unsaturated hydrocarbon group said hydrocarbon group comprising at least 4 non-conjugated double bonds;

L is a linking group forming a bridge of 1 to 5 atoms between the R group and the carbonyl CO wherein L comprises at least one heteroatom; and

X is $CHal_3$ or a salt thereof

for use in the treatment of glomerulonephritis, lupus nephritis or diabetic nephropathy.

2. A compound as claimed in claim 1 wherein said hydrocarbon group has 5 to 7 double bonds.

3. A compound as claimed in any one of claims 1 to 2 wherein no double bond is conjugated with the carbonyl group.

4. A compound as claimed in any one of claims 1 to 3 wherein all double bonds are in the cis configuration.

5. A compound as claimed in any one of claims 1 to 3 wherein all double bonds are in the cis configuration except the double bond nearest the carbonyl.

6. A compound as claimed in any one of claims 1 to 5 wherein the R group comprises 17 to 19 carbon atoms.

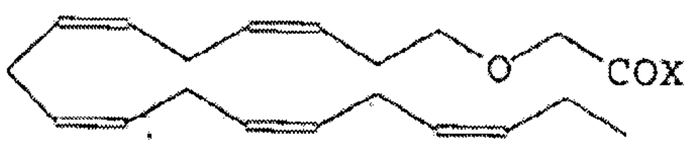
7. A compound as claimed in any one of claims 1 to 5 wherein L comprises O, S, N or SO.

8. A compound as claimed in any one of claims 1 to 7 wherein linking group L comprises $-CH_2-$, $-CH(C_{1-6}alkyl)-$, $-N(C_{1-6}alkyl)-$, -NH-, -S-, -O-, -CH=CH-, -CO-, -SO-, or $-SO_2-$ which can be combined with each other in any chemically meaningful order to form the linking group, wherein L comprises at least one heteroatom.

9. A compound as claimed in any one of claims 1 to 6 wherein L is $-NH_2CH_2$, $-NH(Me)CH_2-$, $-SCH_2-$, or $-SOCH_2-$.

10. A compound as claimed in any one of claims 1 to 9 wherein X is CF_3.

11. A compound as claimed in claim 1 having the formula:



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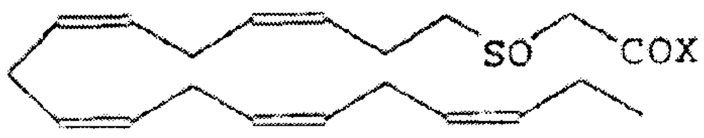

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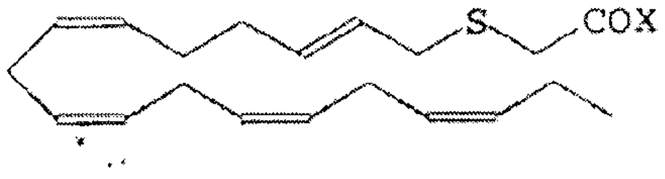

, or


.

12. A compound as claimed in claim 1 having the formula (I')

R-Y1-Y2-CO-X (I')

wherein R and X are as hereinbefore defined;

Y1 is selected from O, S, NH, $N(C_{1-6}$-alkyl), SO or SO_2 and

Y2 is $(CH_2)_n$ or $CH(C_{1-6}$ alkyl); and

where n is 1 to 3.

13. Use of a compound as claimed in any one of claims 1 to 12 or a salt thereof for treatment of glomerulonephritis, lupus nephritis or diabetic nephropathy in an animal.

14. Use of a compound as claimed in any one of claims 1 to 12 or a salt thereof for use in the manufacture of a medicament for treatment of glomerulonephritis, lupus nephritis or diabetic nephropathy.

Figures:

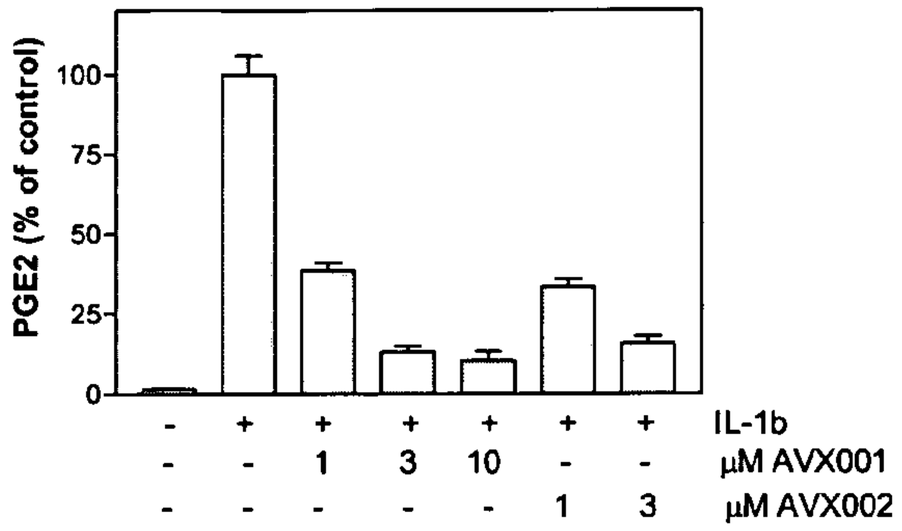

Fig. 1

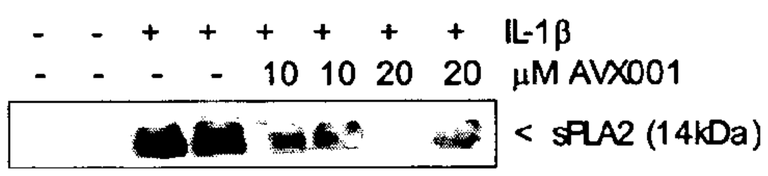

Fig. 2A

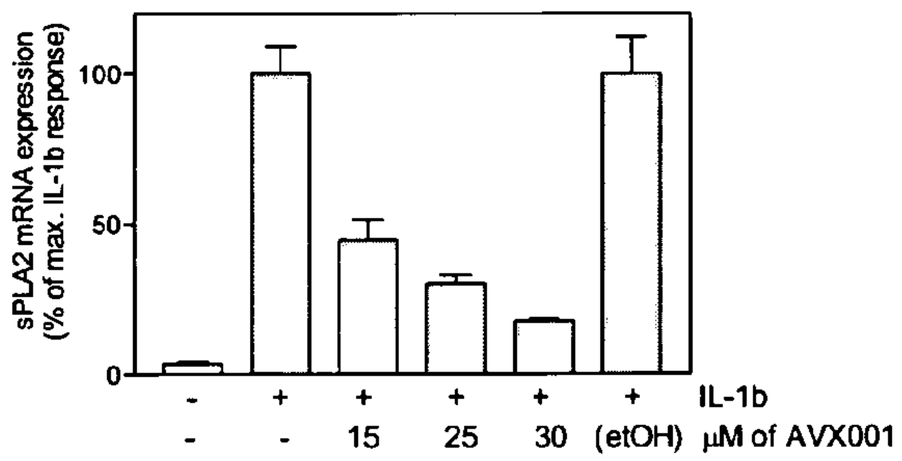

Fig. 2B

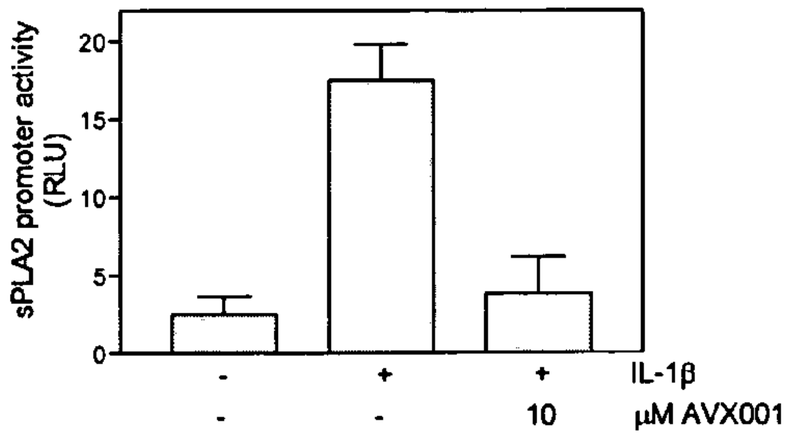

Fig. 2C

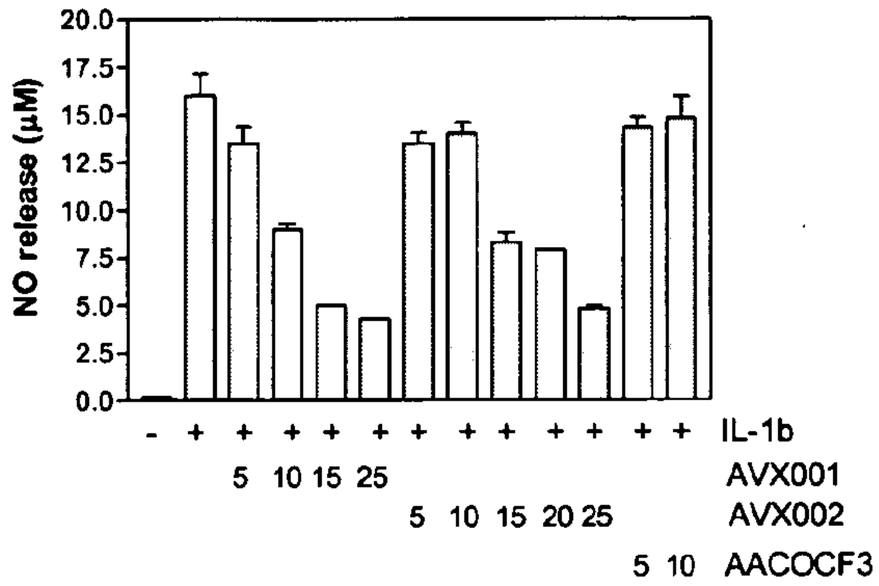

Fig. 3

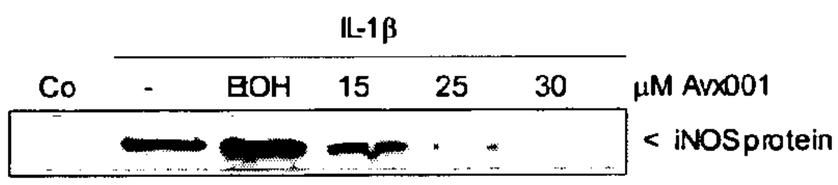

Fig. 4A

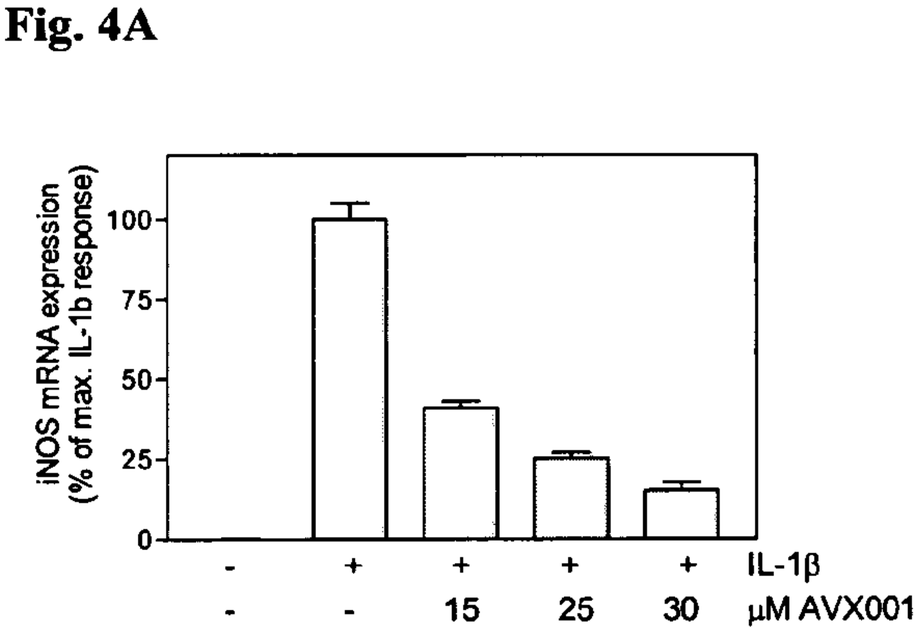

Fig. 4B

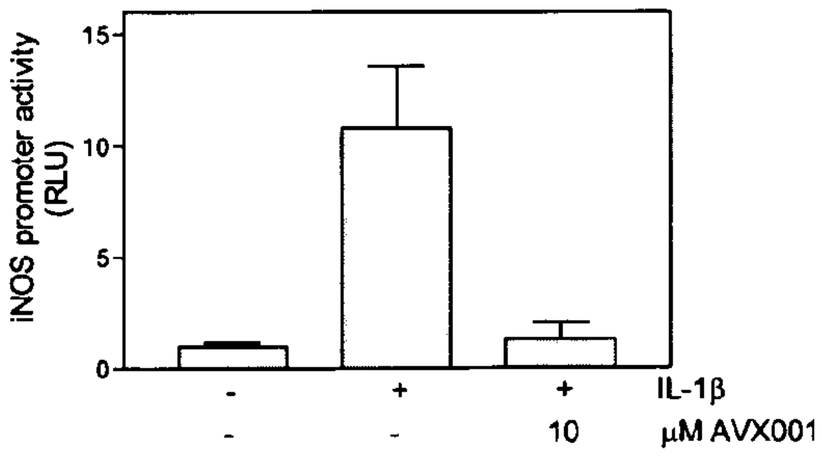

Fig. 4C

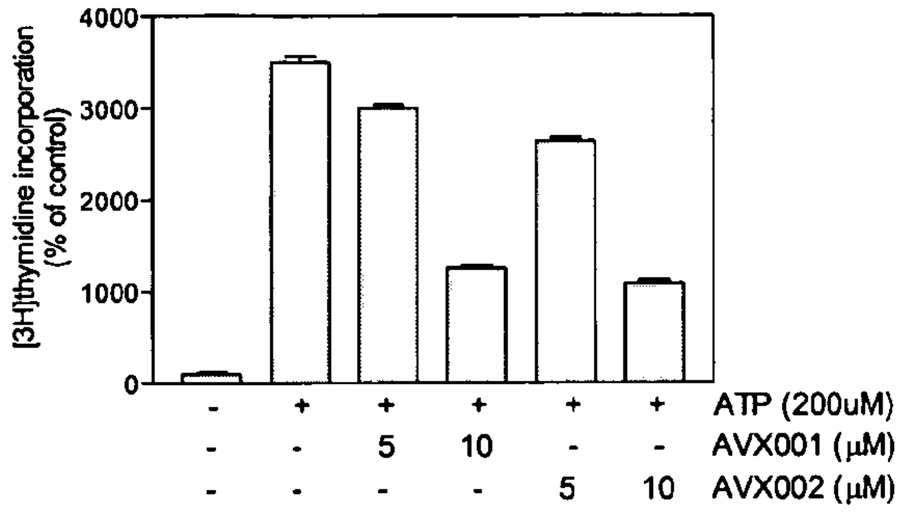

Fig. 5 A

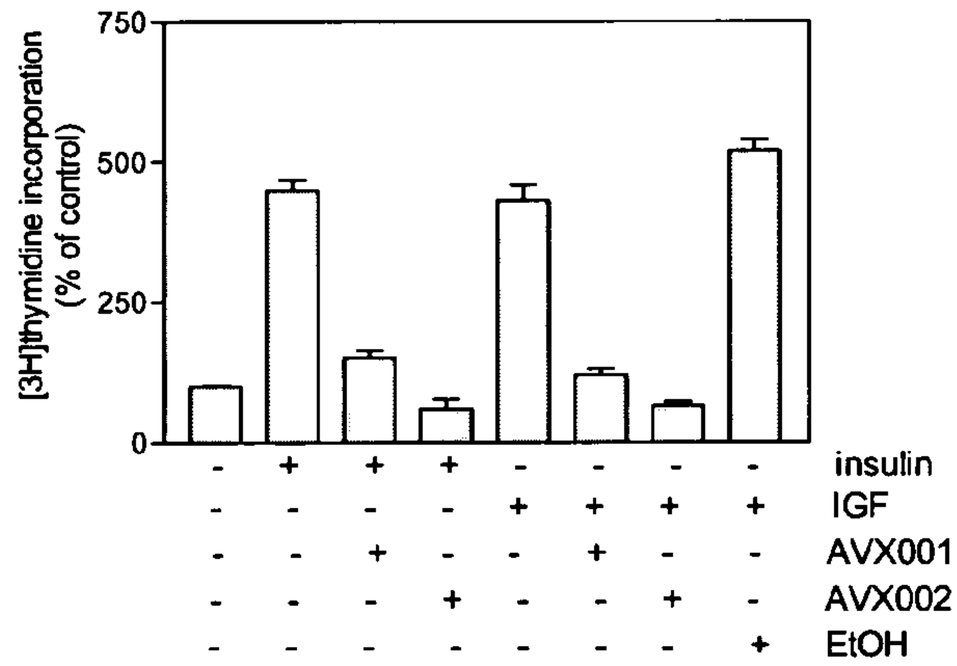

Fig. 5B

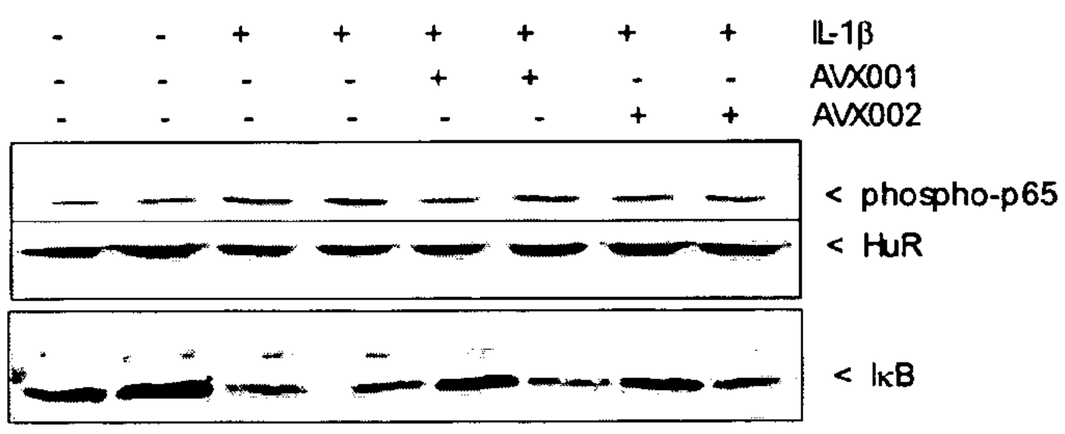

Fig. 6 A

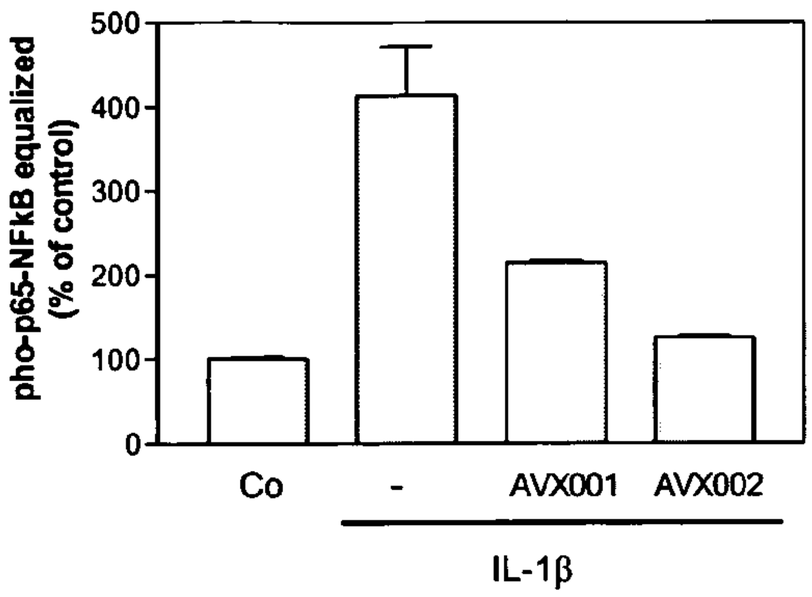
pho-p65-NFkB equalized (% of control)
500
400
300
200
100
0
Co
-
AVX001
AVX002
IL-1β

Fig. 6 B

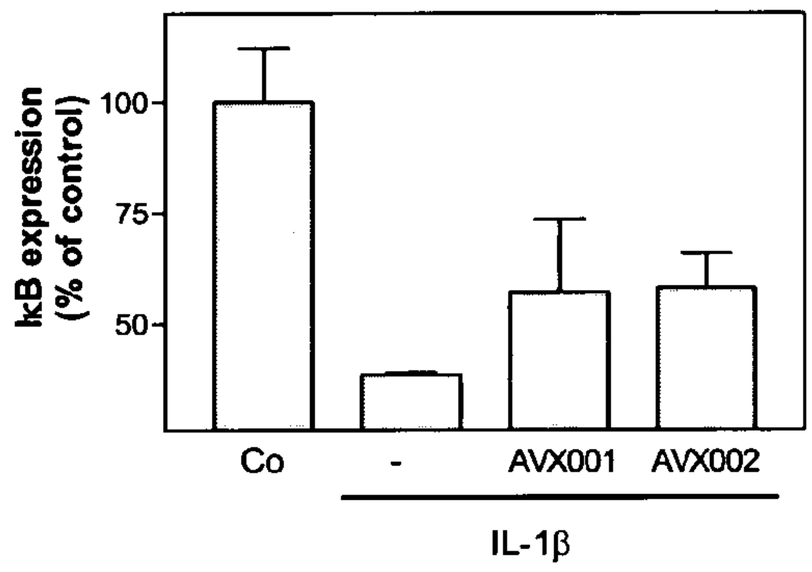
kB expression (% of control)
100
75
50
Co
-
AVX001
AVX002
IL-1β

Fig. 6 C