Abstract: The present invention has found that inhibitors reducing the glycolytic flux are not only useful for the treatment of cancer but can also be applied for the treatment of pathological angiogenesis such as for example macular degeneration. In particular the invention provides siRNAs directed against PFKFB3 for the treatment of pathological angiogenesis. The invention also provides the use of a therapeutically effective amount of aza chalcones or a pharmaceutically acceptable salt thereof for the treatment of pathological angiogenesis such as for example macular degeneration.
MEANS AND METHODS FOR THE TREATMENT OF PATHOLOGICAL ANGIogenesis

Field of the invention
The present invention relates to the field of angiogenesis, more particularly to the field of pathological angiogenesis. In particular the invention has found that inhibitors reducing the glycolytic flux can be used for treatment of diseases in which pathological angiogenesis is involved. In particular the invention provides siRNAs directed against PFKFB3 for the treatment of pathological angiogenesis. The invention also provides the use of a therapeutically effective amount of aza chalcones or a pharmaceutically acceptable salt thereof for the treatment of pathological angiogenesis.

Introduction to the invention
Angiogenesis and vasculogenesis are processes involved in the growth of blood vessels. Angiogenesis is the process by which new blood vessels are formed from extant capillaries, while vasculogenesis involves the growth of vessels deriving from endothelial progenitor cells. Angiogenesis is a complex, combinatorial process that is regulated by a balance between pro- and anti-angiogenic molecules. Angiogenic stimuli (e.g. hypoxia or inflammatory cytokines) result in the induced expression and release of angiogenic growth factors such as vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF). These growth factors stimulate endothelial cells (EC) in the existing vasculature to proliferate and migrate through the tissue to form new endothelialized channels. Angiogenesis and vasculogenesis, and the factors that regulate these processes, are important in embryonic development, inflammation, and wound healing, and also contribute to pathologic conditions such as tumor growth, diabetic retinopathy, rheumatoid arthritis and a variety of chronic inflammatory diseases. Both angiogenesis and vasculogenesis involve the proliferation of endothelial cells. Endothelial cells line the walls of blood vessels; capillaries are comprised almost entirely of endothelial cells. The angiogenic process involves not only increased endothelial cell proliferation, but also comprises a cascade of additional events, including protease secretion by endothelial cells, degradation of the basement membrane, migration through the surrounding matrix, proliferation, alignment, differentiation into tube-like structures, and synthesis of a new basement membrane. Vasculogenesis involves recruitment and differentiation of mesenchymal cells into angioblasts, which then differentiate into endothelial cells which then form de novo vessels. Inappropriate, or pathological, angiogenesis is involved for example in the growth of atherosclerotic plaque, diabetic retinopathy, degenerative maculopathy, retrolental fibroplasia, idiopathic pulmonary fibrosis, acute adult respiratory distress syndrome, and asthma. There is a need in the art for methods of reducing pathological angiogenesis. The present invention addresses this need.
Summary of the invention
Several glycolytic inhibitors have been described which are currently being used in pre-clinical uses for cancer treatment (Pelicanò H et al (2006) Oncogene 25, 4633-4646. Some examples of glycolytic inhibitors include 2-deoxyglucose, lonidamine, 3-bromopyruvate, imatinib, oxythiamine and aza chalcones, the latter being described in WO2008/156783. Aza chalcones specifically inhibit the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3). One example of such an aza chalcone is 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) which has been shown to actively suppresses the glycolytic flux in tumors (Clem B et al (2008) Mol. Cancer Ther. 7(1):10-120). We have surprisingly shown that aza chalcones (as described in WO2008/156783) also have an effect on endothelial cells, i.e. they can actively suppress the outgrowth and proliferation of endothelial cells. The present invention therefor provides methods of reducing angiogenesis in an individual. The methods generally involve administering to the individual an effective amount of a siRNA with a specificity for PFKFB3 and/or administering a therapeutically effective amount of aza chalcones (the latter being described in WO2008/156783) or a pharmaceutically acceptable salt thereof. The methods are useful to treat conditions associated with or resulting from excess angiogenesis, particularly pathological angiogenesis. The invention further provides methods of treating a condition associated with or resulting from angiogenesis. The present invention also features methods of treating a disorder associated with pathological angiogenesis. In some embodiments, the invention features a method of inhibiting inflammatory arthritis. In some embodiments, the invention features a method of inhibiting a proliferative retinopathy in a mammal. In some of these embodiments, the proliferative retinopathy occurs as a result of diabetes in the mammal. The methods generally involve administering to a mammal PFKFB3 antagonist in an amount effective to reduce pathological angiogenesis. In some other embodiments the invention provides methods of inhibiting age-related macular degeneration. In some embodiments, the methods further comprise administering a second angiogenesis inhibitor.

Figure legends
Figure 1: CNV was induced in C57BL/6 mice by laser burn. Treated mice were injected intraperitoneally with 3PO while untreated mice were injected with the solvent (i.e. DMSO). The graph shows the ratio between the neovascular area versus the lesion area. Filled (black) circles depict the treated mice and the white circles represent the control (untreated) mice.

Figure 2: The left panel shows the neovascular area of the mice subjected by the CNV model (control mice (DMSO) versus the 3PO-treated mice). The right panel depicts the %neovascular area in control mice (DMSO) versus the 3PO-treated mice.
Figure 3: Panels A and B show the FITC-perfused flat mounts, A is derived from a control (i.e. non treated mice subjected to a CNV model) and B represents the 3PO-treated mice subjected to a CNV model.

Detailed description to the invention

Increased aerobic glycolysis is commonly seen in a wide spectrum of human cancers, and hypoxia is present in most tumor microenvironments, the development and the use of glycolytic inhibitors is currently been explored in a variety of pre-clinical tumor models. In the present invention we show that compounds able to inhibit the glycolytic flux (or compounds inhibiting the glycolysis or compounds inhibiting the aerobic glycolysis which are equivalent wordings) are also able to inhibit endothelial cell outgrowth and hence are able to inhibit angiogenesis, more particularly pathological angiogenesis.

Accordingly in a first embodiment the invention provides a compound inhibiting the glycolytic flux for treatment of angiogenesis, more particularly to inhibit pathological angiogenesis. In particular the compounds inhibit the glycolytic flux and enhance the pentose-phosphate pathway.

In a particular embodiment a compound is a siRNA with a specificity for PFKFB3 for the treatment of pathological angiogenesis.

In a specific embodiment the siRNA with a specificity for PFKFB3 is expressed by an expression construct incorporated into an adenoviral associated (AAV) vector.

The term "siRNA" refers to a small interfering RNA(s), which also has been referred to in the art as short interfering RNA and silencing RNA, among others. siRNAs generally are described as relatively short, often 20-25 nucleotide-long, double-stranded RNA molecules that are involved in RNA interference (RNAi) pathway(s). Generally, siRNAs are, in part, complementary to specific mRNAs (such as PFKFB3) and mediate their down regulation (hence, "interfering"). siRNAs thus can be used for down regulating the expression of specific genes and gene function in cells and organisms. siRNAs also play a role in related pathways. The general structure of most naturally occurring siRNAs is well established. Generally, siRNAs are short double-stranded RNAs, usually 21 nucleotides long, with two nucleotides single stranded "overhangs" on the 3' of each strand. Each strand has a 5' phosphate group and a 3' hydroxyl (--OH) group. In vivo, the structure results from processing by the enzyme "dicer," which enzymatically converts relatively long dsRNAs and relatively small hairpin RNAs into siRNAs. The term siRNA refers to a nucleic acid that acts like a siRNA, as described herein, but may be other than an RNA, such as a DNA, a hybrid RNA:DNA or the like. siRNAs function like siRNAs to down regulate expression of gene products. The term "RNA interference" which
also has been called "RNA mediated interference" refers to the cellular processes by which RNA (such as siRNAs) down regulate expression of genes; i.e., down regulate or extinguish the expression of gene functions, such as the synthesis of a protein encoded by a gene. Typically, double-stranded ribonucleic acid inhibits the expression of genes with complementary nucleotide sequences. RNA interference pathways are conserved in most eukaryotic organisms. It is initiated by the enzyme dicer, which cleaves RNA, particularly double-stranded RNA, into short double-stranded fragments 20-25 base pairs long. One strand of the double-stranded RNA (called the "guide strand") is part of a complex of proteins called the RNA-induced silencing complex (RISC). The thus incorporated guide strand serves as a recognition sequence for binding of the RISC to nucleic acids with complementary sequences. Binding by RISC to complementary nucleic acids results in their being "silenced." The best studied silencing is the binding of RISCs to RNAs resulting in post-transcriptional gene silencing. Regardless of mechanism, interfering nucleic acids and RNA interference result in down regulation of the target gene or genes that are complementary (in pertinent part) to the guide strand. A polynucleotide can be delivered to a cell to express an exogenous nucleotide sequence, to inhibit, eliminate, augment, or alter expression of an endogenous nucleotide sequence, or to affect a specific physiological characteristic not naturally associated with the cell. The polynucleotide can be a sequence whose presence or expression in a cell alters the expression or function of cellular genes or RNA.

In addition, the present invention contemplates polynucleotide-based expression inhibitors of PFKFB3 which may be selected from the group comprising: siRNA, microRNA, interfering RNA or RNAi, dsRNA, ribozymes, antisense polynucleotides, and DNA expression cassettes encoding siRNA, microRNA, dsRNA, ribozymes or antisense nucleic acids. SiRNA comprises a double stranded structure typically containing 15 to 50 base pairs and preferably 19 to 25 base pairs and having a nucleotide sequence identical or nearly identical to an expressed target gene or RNA within the cell. An siRNA may be composed of two annealed polynucleotides or a single polynucleotide that forms a hairpin structure. MicroRNAs (miRNAs) are small noncoding polynucleotides, about 22 nucleotides long, that direct destruction or translational repression of their mRNA targets. Antisense polynucleotides comprise a sequence that is complimentary to a gene or mRNA. Antisense polynucleotides include, but are not limited to: morpholinos, 2'-O-methyl polynucleotides, DNA, RNA and the like. The polynucleotide-based expression inhibitor may be polymerized in vitro, recombinant, contain chimeric sequences, or derivatives of these groups. The polynucleotide-based expression inhibitor may contain ribonucleotides, deoxyribonucleotides, synthetic nucleotides, or any suitable combination such that the target RNA and/or gene is inhibited. Polynucleotides may contain an expression cassette coded to express a whole or partial protein, or RNA. An expression cassette refers to a natural or recombinantly produced polynucleotide that is
capable of expressing a sequence. The cassette contains the coding region of the gene of interest along with any other sequences that affect expression of the sequence of interest. An expression cassette typically includes a promoter (allowing transcription initiation), and a transcribed sequence. Optionally, the expression cassette may include, but is not limited to, transcriptional enhancers, non-coding sequences, splicing signals, transcription termination signals, and polyadenylation signals. An RNA expression cassette typically includes a translation initiation codon (allowing translation initiation), and a sequence encoding one or more proteins. Optionally, the expression cassette may include, but is not limited to, translation termination signals, a polyadenosine sequence, internal ribosome entry sites (IRES), and non-coding sequences. The polynucleotide may contain sequences that do not serve a specific function in the target cell but are used in the generation of the polynucleotide. Such sequences include, but are not limited to, sequences required for replication or selection of the polynucleotide in a host organism.

Based on the RNA sequence of PFKFB3, siRNA molecules with the ability to knock-down PFKFB3 activity can be obtained by chemical synthesis or by hairpin siRNA expression vectors. There are numerous companies that provide the supply of costumer-designed siRNAs on a given RNA sequence, e.g. Ambion, Imgenex, Dharmacon. The PFKFB3 siRNAs of the invention may be chemically modified, e.g. as described or example in US20030143732, by phosphorothioate internucleotide linkages, 2'-0-methyl ribonucleotides, 2'-deoxy-2'fluoro ribonucleotides, "universal base" nucleotides, 5-C-methyl nucleotides, and inverted deoxyabasic residue incorporation. The sense strand PFKFB3 siRNAs may also be conjugated to small molecules or peptides, such as membrane-permeant peptides or polyethylene glycol (PEG). Other siRNA conjugates which form part of the present invention include cholesterol and alternative lipid-like molecules, such as fatty acids or bile-salt derivatives.

In a further embodiment, the present invention relates to an expression vector comprising any of the above described polynucleotide sequences encoding at least one PFKFB3 siRNA molecule in a manner that allows expression of the nucleic acid molecule, and cells containing such vector. The polynucleic acid sequence is operably linked to regulatory signals (promoters, enhancers, suppressors etc.) enabling expression of the polynucleic acid sequence and is introduced into a cell utilizing, preferably, recombinant vector constructs. A variety of viral-based systems are available, including adenoviral, retroviral, adeno-associated viral, lentiviral, herpes simplex viral vector systems. Selection of the appropriate viral vector system, regulatory regions and host cell is common knowledge within the level of ordinary skill in the art.
As gene delivery and gene silencing techniques improve, the selective deletion of PFKFB3, for example in the eye, may prove useful in order to limit the impact of protein deletion to a particular system under study. The PFKFB3 siRNA molecules of the invention may be delivered by known gene delivery methods, e.g. as described in US20030143732, including the use of naked siRNA, synthetic nanoparticles composed of cationic lipid formulations, liposome formulations including pH sensitive liposomes and immunoliposomes, or bioconjugates including siRNAs conjugated to fusogenic peptides. Delivery of siRNA expressing vectors can also be systemic, such as by intravenous, intraperitoneal, intracocular, intravitreal or intramuscular administration or even by intrathecal or by intracerebral injection that allows for introduction into the desired target cell (see US 20030143732).

In yet another embodiment the compound is a small molecule compound able to inhibit the enzyme PFKFB3 for the treatment of a pathological angiogenesis, excluding cancer.

In a specific embodiment a compound is a molecule with the formula (I) for the treatment of an age-related macular degeneration, diabetic retinopathy, proliferative retinopathies, choroidal and other intraocular disorders with an excessive angiogenesis component

\[
\text{(I)}
\]

wherein:

\(X, X_2,\) and \(X_3\) are each C or CH;

\(X_1\) is selected from the group consisting of O, S, NR\(_1\), C(R\(_2\))\(_2\), OR\(_3\), SR\(_4\), NR\(_5\)R\(_6\), and C(R\(_7\))\(_3\), wherein \(R_1, R_3, R_4, R_5\) and \(R_6\) are each independently selected from the group consisting of H, alkyl, aryl, aralkyl, and acyl, and each \(R_2\) and \(R_7\) is independently selected from the group consisting of H, halo, hydroxyl, alkoxy, alkyl, aralkyl, and aryl;

\(R_{10}\) is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;

\(R_{11}\) is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;

\(A_1, A_2, A_3, A_4,\) and \(A_5,\) are each independently N or CR\(_{12}\), wherein each \(R_{12}\) is independently selected from the group consisting of H, alkyl, halo, nitro, cyano,
hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:

wherein:

\( X_4 \) is \( NR_{14} \), wherein \( R_{14} \) is selected from the group consisting of H, alkyl, hydroxyl, aralkyl, and aryl;

\( X_5 \) is selected from the group consisting of O, S, C\( (R_{15})^2 \), and \( NR_{14} \), wherein each \( R_{15} \) is independently selected from the group consisting of H, hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and

\( X_6 \) is selected from H, alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein \( R_{10} \) and one \( R_{12} \) are together alkylene;

\( A_{r_2} \) is selected from the group consisting of

wherein:

each \( Y_1, Y_2, Y_3, Y_4, Y_6, Y_7, Y_8, Y_9, Y_{10}, Y_{11}, Y_{12}, Y_{13}, Y_{14}, Y_{15}, Y_{16}, Y_{17}, Y_{18}, Y_{19} \) is independently selected from the group consisting of N and CR\( _{13} \), wherein each \( R_{13} \) is independently selected from the group consisting of H, alkyl, halo, nitro, cyano, hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:
wherein:

$X_4$ is $NR_{14}$, wherein $R_{14}$ is selected from the group consisting of $H$, alkyl, hydroxyl, aralkyl, and aryl;

$X_5$ is selected from the group consisting of $O$, $S$, $C(R_i 2)$, and $NR_{14}$, wherein each $R_{16}$ is independently selected from the group consisting of $H$, hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and

$X_6$ is selected from $H$, alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein $R_{16}$ and one $R_{13}$ are together alkylene; and

wherein at least one of $A_1$, $A_2$, $A_3$, $A_4$, $A_5$, $Y_1$, $Y_2$, $Y_3$, $Y_4$, $Y_5$, $Y_6$, $Y_7$, $Y_8$, $Y_9$, $Y_{10}$, $Y_{11}$, $Y_{12}$, $Y_{13}$, $Y_{14}$, $Y_{15}$, $Y_{16}$, $Y_{17}$, $Y_{18}$, and $Y_{19}$ is $N$; or a pharmaceutically acceptable salt thereof.

In yet another embodiment a compound is according to the previous embodiment wherein $X_1$ is $O$ and $X$ is $C$.

In yet another embodiment in the compound of formula (I) $Ar_2$ is:

$X$, $X_2$, and $X_3$ are each $C$;

$X_1$ is selected from the group consisting of $O$, $S$, $NR_1$, and $C(R_2 2)$, wherein $R_1$, is selected from the group consisting of $H$ and alkyl, and each $R_2$ is independently selected from the group consisting of $H$, halo, hydroxyl, alkoxy, and alkyl; and the compound of Formula (I) has a structure of Formula (II):
In yet another embodiment the compound of formula (II) is selected from the group consisting of:

- \( \text{O} \equiv \text{C} \text{N} \)
- \( \text{O} \equiv \text{C} \text{Cl} \)
- \( \text{O} \equiv \text{C} \text{OCH}_3 \)
- \( \text{O} \equiv \text{C} \text{Cl} \)

and

In yet another embodiment the compound according to formula (I) is wherein \( \text{Ar}_2 \) is:

- \( X, X_1, X_2, X_3 \) are each \( \text{C} \);
$X_1$ is selected from the group consisting of O, S, NR$_1$, and C(R$_2$)$_2$, wherein R$_1$, is selected from the group consisting of H and alkyl, and each R$_2$ is independently selected from the group consisting of H, halo, hydroxyl, alkoxy, and alkyl; and the compound of Formula (I) has a structure of Formula (III):

In a specific embodiment the compound of Formula (III) is

In yet another embodiment the compound according to formula (I) is wherein Ar$_2$ is

$X$, $X_2$, and $X_3$ are each C;

$X_1$ is selected from the group consisting of O, S, NR$_1$, and C(R$_2$)$_2$, wherein R$_1$, is selected from the group consisting of H and alkyl, and each R$_2$ is independently selected from the group consisting of H, halo, hydroxyl, alkoxy, and alkyl; and the compound of Formula (I) has a structure of Formula (IV):
In a specific embodiment a compound according to Formula (IV) is:

\[
\text{(IV)}
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In yet another embodiment the invention provides a compound which is a molecule with the formula (I) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, particularly a pharmaceutically acceptable salt thereof, or a mixture of the same for the treatment of a condition involving pathological angiogenesis excluding cancer:

\[
\text{(I)}
\]

wherein:

- \(X, X_2,\) and \(X_3\) are each \(C\) or \(\text{CH}\);
- \(X_1\) is \(O\);
- \(R_{10}\) is selected from the group consisting of \(H,\) alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;
- \(R_{11}\) is selected from the group consisting of \(H,\) alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;
- \(A_1, A_2, A_3, A_4,\) and \(A_5\), are each independently \(N\) or \(\text{CR}12\), wherein each \(R_{12}\) is independently selected from the group consisting of \(H,\) alkyl, halo, nitro, cyano,
hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyi, dialkylcarbamoyi, sulfate, and a group having the structure:

\[ X_4 \]

wherein:

5

- \( X_4 \) is \( \mathbf{N}R_{14} \), wherein \( R_{14} \) is selected from the group consisting of \( H \), alkyl, hydroxyl, aralkyl, and aryl;

- \( X_5 \) is selected from the group consisting of \( \mathbf{O}, \mathbf{S}, \mathbf{C}(\mathbf{R}_{15})^2 \), and \( \mathbf{N}R_{14} \), wherein each \( R_{15} \) is independently selected from the group consisting of \( H \), hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and

10

- \( X_6 \) is selected from \( H \), alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein \( R_{10} \) and one \( R_{12} \) are together alkylene;

- \( A_{R_2} \) is selected from the group consisting of

15

\[ Y_1, Y_2, Y_3, Y_4, Y_5 \] are each \( \mathbf{C} \);

each \( Y_6, Y_7, Y_8, Y_9, Y_{10}, Y_{11}, Y_{12}, Y_{13}, Y_{14}, Y_{15}, Y_{16}, Y_{17}, Y_{18}, Y_{19} \) is independently selected from the group consisting of \( \mathbf{N} \) and \( \mathbf{CR}_{13} \), wherein each \( R_{13} \) is independently selected from the group consisting of \( H \), alkyl, halo, nitro, cyano, hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyi, dialkylcarbamoyi, sulfate, and a group having the structure:
wherein:

\( X_4 \) is \( NR_4 \), wherein \( R_4 \) is selected from the group consisting of \( H \), alkyl, hydroxyl, aralkyl, and aryl;

\( X_5 \) is selected from the group consisting of \( O, S, C(R_5)_2 \), and \( NR_4 \), wherein each \( R_5 \) is independently selected from the group consisting of \( H \), hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and

\( X_6 \) is selected from \( H \), alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein \( R_{10} \) and one \( R_{13} \) are together alkylene; and

wherein at least one of \( A_1, A_2, A_3, A_4, A_5, Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, Y_{10}, Y_{11}, Y_{12}, Y_{13}, Y_{14}, Y_{15}, Y_{16}, Y_{17}, Y_{18}, \) and \( Y_{19} \) is \( N \); or a pharmaceutically acceptable salt thereof.

In yet another embodiment \( Ar_2 \) is:

\[ \text{Diagram} \]

\( X, X_2, \) and \( X_3 \) are each \( C \);

and the compound of Formula (I) has a structure of Formula (III):

\[ \text{Diagram} \]

(III)
In specific embodiments the previous compounds for the treatment of pathological angiogenesis excluding cancer are used for the treatment of age-related macular degeneration, diabetic retinopathy, choroidal, proliferative retinopathies and other intraocular disorders with an excessive angiogenesis component, rheumatoid arthritis, psoriasis and atherosclerosis. The term “excessive angiogenesis component with respect to intraocular disorders” refers to the fact that in certain pathological eye diseases an excess angiogenesis occurs. A medical doctor such as an eye doctor or eye surgeon is well positioned to determine if excessive pathological angiogenesis occurs in the eye.

In yet another embodiment the invention provides a siRNA with a specificity for PFKFB3 for the treatment of conditions and disorders resulting from pathological angiogenesis including diseases from the list macular degeneration, atherosclerosis, proliferative retinopathies, arthritis and psoriasis.

In a specific embodiment said siRNA with a specificity for PFKFB3 is expressed by an expression construct incorporated into a viral vector.

In yet another specific embodiment said siRNA with a specificity for PFKFB3 is expressed by an expression construct incorporated into an adenoviral-2 associated (AAV-2) vector.

The instant invention provides a method of reducing angiogenesis in a mammal. The method generally involves administering to a mammal a siRNA with a specificity for PFKFB3 and/or a compound as herein described before which inhibits the enzyme PFKFB3 in an amount effective to reduce angiogenesis. An effective amount of a siRNA with a specificity for PFKFB3, in combination with, or applied separately with a compound as herein described before which inhibits the enzyme PFKFB3, reduces angiogenesis by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or more, when compared to an untreated (e.g. a placebo-treated) control. Whether angiogenesis is reduced can be determined using any known method. Methods of determining an effect of an agent on angiogenesis are known in the art and include, but are not limited to, inhibition of neovascularization into implants impregnated with an angiogenic factor; inhibition of blood vessel growth in the cornea or anterior eye chamber; inhibition of endothelial cell proliferation, migration or tube formation in vitro; the chick chorioallantoic membrane assay; the hamster cheek pouch assay; the polyvinyl alcohol sponge disk assay; the formation of blood vessels in zebrafish larvae. Such assays are well known in the art and have been described in numerous publications.

The term "pathological angiogenesis" as used herein refers to the excessive formation and growth of blood vessels during the maintenance and the progression of several disease states.
Examples where pathological angiogenesis can occur are blood vessels (atherosclerosis, bone and joints (rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation), skin (warts, pyogenic granulomas, hair growth, scar keloids, allergic oedema), liver, kidney, lung, ear and other epithelia (inflammatory and infectious processes (including hepatitis, glomerulonephritis, pneumonia), asthma, nasal polyps, otitis, transplantation, liver regeneration), uterus, ovary and placenta (dysfunctional uterine bleeding (e.g. due to intrauterine contraceptive devices), follicular cyst formation, ovarian hyperstimulation syndrome, endometriosis), brain, nerves and eye (retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders (e.g. macular degeneration), leukomalacia), heart and skeletal muscle due to work overload, adipose tissue (obesity), endocrine organs (thyroiditis, thyroid enlargement, pancreas transplantation). While it is generally known in the art that pathological angiogenesis is also associated with neoplasms and metastasis, the latter conditions are herein specifically excluded (or disclaimed) from the claim scope of the invention.

The term "alkyl" refers to C₁₋₂₀ inclusive, linear (i.e., "straight-chain"), branched, or cyclic, saturated or at least partially and in some cases fully unsaturated (i.e., alkenyl and alkynyl) hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, octyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, octenyl, butadienyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, and allenyln groups. " Branched" refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain. "Lower alkyl" refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C₁₋₈ alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about 20 carbon atoms, e.g., 10, 11, 12... or 20 carbon atoms. In certain embodiments, "alkyl" refers, in particular, to C₁₋₈ straight-chain alkyls. In other embodiments, "alkyl" refers, in particular, to C₁₋₈ branched-chain alkyls.

An "alkyl group substituent" includes but is not limited to alkyl, substituted alkyl, haloarylamino, acyl, hydroxyl, arylhydroxyl, alkoxy, alkoxythio, aralkyloxyl, aralkylthio, aralkylcarboxyl, alkoxycarbonyl, oxo, and cycloalkyl. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as "alkylaminoalkyl"), or aryl.

"Aryl" is used herein to refer to an aromatic substituent that can be a single aromatic ring, or multiple aromatic rings that are fused together, linked covalently, or linked to a common group, such as, but not limited to, a methylene or ethylene moiety. The term "aryl" specifically encompasses heterocyclic aromatic compounds. The aromatic ring(s) can comprise phenyl, naphthyl, biphenyl, diphenylether, diphenylamine and benzophenone, among others. The aryl group can be optionally substituted (a "substituted aryl") with one or more aryl group
substituents, which can be the same or different, wherein "aryl group substituent" includes alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, hydroxyl, alkoxyl, aryloxyl, aralkyloxyl, carboxyl, acyl, halo, nitro, alkoxy carbonyl, aryloxy carbonyl, aralkoxy carbonyl, acyloxyl, acylamino, arolylamino, carbamoyl, alkyl carbamoyl, dialkyl carbamoyl, arythio, alkylthio, alkylene.

Thus, as used herein, the term "substituted aryl" includes aryl groups, as defined herein, in which one or more atoms or functional groups of the aryl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxy, hydroxyl, nitro, amino, alkyaminio, dialkylamino, sulfate, and mercapto.

The term "aza" refers to a heterocyclic ring structure containing at least one nitrogen atom. Specific examples of aza groups include, but are not limited to, pyrrolidine, piperidine, quinuclidine, pyridine, pyrrole, indole, pyridazine, pyrimidine, and pyrazine.

The term "aza-aryl" refers to a heterocyclic aryl group wherein one or more of the atoms of the aryl group ring or rings is nitrogen.

"Alkylene" refers to a straight or branched bivalent aliphatic hydrocarbon group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more "alkyl group substituents," including hydroxyl, halo, nitro, alkyl, aryl, aralkyl, carboxyl and the like. There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as "alkylaminoalkyl"), wherein the nitrogen substituent is alkyl as previously described. The term "acyl" refers to an organic carboxylic acid group wherein the -OH of the carboxyl group has been replaced with another substituent (i.e., as represented by RCO — , wherein R is an alkyl, aralkyl or aryl group as defined herein, including substituted alkyl, aralkyl, and aryl groups). As such, the term "acyl" specifically includes aryl acyl groups, such as an acetylfuran and a phenacyl group. Specific examples of acyl groups include acetyl and benzoyl.

"Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, alkyl, substituted alkyl, aryl, or substituted aryl, thus providing a heterocyclic group. "Alkoxy" refers to an alkyl-O- group wherein alkyl is as previously described. The term "alcohoyl" as used herein can refer to, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, butoxyl, f-butoxyl, and pentoxy. The term "oxyalkyl" can be used interchangably with "alcohoyl".
"Aryloxyl" refers to an aryl-O- group wherein the aryl group is as previously described, including a substituted aryl. The term "aryloxyl" as used herein can refer to phenyloxyl or hexyloxyl, and alkyl, substituted alkyl, halo, or alkoxy substituted phenyloxyl or hexyloxyl.

"Aralkyl" refers to an aryl—alkyl— group wherein aryl and alkyl are as previously described, and included substituted aryl and substituted alkyl. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

"Alkoxycarbonyl" refers to an alkyl-O-CO- group. Exemplary alkoxycarbonyl groups include methoxycarbonyl, ethoxycarbonyl, butyloxycarbonyl, and f-butyloxycarbonyl.

"Aryloxycarbonyl" refers to an aryl-O-CO- group. Exemplary aryloxycarbonyl groups include phenoxy- and naphthoxy-carbonyl.

"Aralkoxycarbonyl" refers to an aralkyl-O-CO- group. An exemplary aralkoxycarbonyl group is benzyloxy carbonyl. "Carbamoyl" refers to an H₂N-CO- group.

"Alkylcarbamoyl" refers to a R'RN-CO- group wherein one of R and R' is hydrogen and the other of R and R' is alkyl and/or substituted alkyl.

"Acylxyl" refers to an acyl-O- group wherein acyl is as previously described.

"Acylamino" refers to an acyl-NR- group wherein acyl is as previously described and R is H or alkyl. Thus, the "acylamino" group can have the structure -NR-C(=0)-R', wherein R' is alkyl, aryl, aralkyl, and like.

The term "amino" refers to the -NH₂ group.

The term "carbonyl" refers to the -(C=0)- group.

The term "carboxyl" refers to the -COOH group.

The terms "halo", "halide", or "halogen" as used herein refer to fluoro, chloro, bromo, and iodo groups.

The term "hydroxyl" refers to the -OH group.

The term "hydroxyalkyl" refers to an alkyl group substituted with an -OH group.

The term "mercapto" refers to the -SH group. The term "oxo" refers to a compound described previously herein wherein a carbon atom is replaced by an oxygen atom.

The term "aza" refers to a compound wherein a carbon atom is replaced by a nitrogen atom.

The term "nitro" refers to the -NO₂ group. The term "thio" refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term "sulfate" refers to the -SO₄ group.

When the term "independently selected" is used, the substituents being referred to (e.g., R groups, such as groups R₁ and R₂, or groups X and Y), can be identical or different. For example, both R₁ and R₂ can be substituted alkyls, or R₁ can be hydrogen and R₂ can be a substituted alkyl, and the like.
Medicinal uses:
This invention also relates to pharmaceutical compositions containing one or more compounds of the present invention. These compositions can be utilized to achieve the desired pharmacological effect by administration to a patient in need thereof. A patient, for the purpose of this invention, is a mammal, including a human, in need of treatment for the particular condition or disease, i.e. a disease wherein pathological angiogenesis is involved excluding cancer (excluding tumors or excluding neoplasia which are equivalent terms). Therefore, the present invention includes pharmaceutical compositions that are comprised of a pharmaceutically acceptable carrier and a pharmaceutically effective amount of a compound, or salt thereof, of the present invention. A pharmaceutically acceptable carrier is preferably a carrier that is relatively non-toxic and innocuous to a patient at concentrations consistent with effective activity of the active ingredient so that any side effects ascribable to the carrier do not vitiate the beneficial effects of the active ingredient. A pharmaceutically effective amount of compound is preferably that amount which produces a result or exerts an influence on the particular condition being treated. The compounds of the present invention can be administered with pharmaceutically-acceptable carriers well known in the art using any effective conventional dosage unit forms, including immediate, slow and timed release preparations, orally, intraperitoneally, parenterally, topically, nasally, ophthalmically, optically, sublingually, rectally, vaginally, intrathecally, intracerebroventricularly and the like.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, troches, lozenges, melts, powders, solutions, suspensions, or emulsions, and may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions. The solid unit dosage forms can be a capsule that can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, calcium phosphate, and corn starch.

In another embodiment, the compounds of this invention may be tableted with conventional tablet bases such as lactose, sucrose and cornstarch in combination with binders such as acacia, corn starch or gelatin, disintegrating agents intended to assist the break-up and dissolution of the tablet following administration such as potato starch, alginic acid, corn starch, and guar gum, gum tragacanth, acacia, lubricants intended to improve the flow of tablet granulation and to prevent the adherence of tablet material to the surfaces of the tablet dies and punches, for example talc, stearic acid, or magnesium, calcium or zinc stearate, dyes, coloring agents, and flavoring agents such as peppermint, oil of wintergreen, or cherry flavoring, intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient. Suitable excipients for use in oral liquid dosage forms include dicalcium phosphate and diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable
surfactant, suspending agent or emulsifying agent. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance tablets, pills or capsules may be coated with shellac, sugar or both.

Dispersible powders and granules are suitable for the preparation of an aqueous suspension. They provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example those sweetening, flavoring and coloring agents described above, may also be present.

The pharmaceutical compositions of this invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as liquid paraffin or a mixture of vegetable oils. Suitable emulsifying agents may be (1) naturally occurring gums such as gum acacia and gum tragacanth, (2) naturally occurring phosphatides such as soy bean and lecithin, (3) esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, (4) condensation products of said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil such as, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as, for example, beeswax, hard paraffin, or cetyl alcohol. The suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate; one or more flavoring agents; one or more sweetening agents such as sucrose or saccharin. Syrups and elixirs may be formulated with sweetening agents such as, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, and preservative, such as methyl and propyl parabens and flavoring and coloring agents.

The parenteral compositions of this invention will typically contain from about 0.5% to about 25% by weight of the active ingredient in solution. Preservatives and buffers may also be used advantageously. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophilic-lipophilic balance (HLB) preferably of from about 12 to about 17. The quantity of surfactant in such formulation preferably ranges from about 5% to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high
molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The pharmaceutical compositions may be in the form of sterile injectable aqueous suspensions. Such suspensions may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents such as, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents which may be a naturally occurring phosphatide such as lecithin, a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate, a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadeca-ethyleneoxyxetanol, a condensation product of ethylene oxide with a partial ester derived form a fatty acid and a hexitol such as polyoxyethylene sorbitol monoooleate, or a condensation product of an ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride, for example polyoxyethylene sorbitan monooleate.

The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Diluents and solvents that may be employed are, for example, water, Ringer's solution, isotonic sodium chloride solutions and isotonic glucose solutions. In addition, sterile fixed oils are conventionally employed as solvents or suspending media. For this purpose, any bland, fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables.

A composition of the invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritation excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are, for example, cocoa butter and polyethylene glycol.

Another formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art (see for example US 5,023,252). Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents. Controlled release formulations for parenteral administration include liposomal, polymeric microsphere and polymeric gel formulations that are known in the art. It may be desirable or necessary to introduce the pharmaceutical composition to the patient via a mechanical delivery device. The construction and use of mechanical delivery devices for the delivery of pharmaceutical agents is well known in the art. Direct techniques for, for example, administering a drug directly to the
brain usually involve placement of a drug delivery catheter into the patient's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of agents to specific anatomical regions of the body, is described in US 5,011,472.


In a specific embodiment ocular delivery (or delivery to the eye) is preferred. For local delivery to the eye, the pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum. Preferred methods of local ocular administration include e.g. choroidal injection, transscleral injection or placing a scleral patch, selective arterial catheterization, intraocular administration including transretinal, subconjunctival bulbar, intravitreous injection, suprachoroidal injection, subtenon injection, scleral pocket and scleral cutdown injection, by osmotic pump, etc. In choroidal injection and scleral patching, the clinician uses a local approach to the eye after initiation of appropriate anesthesia, including painkillers and ophthalmoplegics. A needle containing the therapeutic composition of the invention is directed into the subject's choroid or sclera and inserted under sterile conditions. When the needle is properly positioned the compound is injected into either or both of the choroid or sclera. When using either of these methods, the clinician can choose a sustained release or longer acting formulation. Thus, the procedure can be repeated only every several months, depending on the subject's tolerance of the treatment and response. Intraocular administration of drugs intended for treatment of macular degeneration and other intraocular conditions is well known in the art. See, e.g. U.S. Pat. Nos. 5,632,984 and 5,770,589. U.S. Pat. No. 6,378,526 provides methods for intrascleral injection of a therapeutic at a location overlying the retina, which provide a minimally invasive technique for delivering the agent to the posterior segment of the eye. In certain embodiments of the invention a composition is delivered to the vicinity of the eye, e.g. in close proximity to the posterior segment of the eye.
The "vicinity of the eye" refers to locations within the orbit, which is the cavity within the skull in which the eye and its appendages are situated. Typically the compositions would be delivered close to their intended target within the eye, e.g. close to (within several millimeters of) the portion of the sclera that overlies the posterior segment of the eye, or immediately adjacent to the exterior surface of the sclera. A number of polymeric delivery vehicles for providing controlled release have been used in an ocular context and can be used to administer the compositions of the invention. Various polymers, e.g., biocompatible polymers, which may be biodegradable, can be used. For example, U.S. Pat. No. 6,692,759 describes methods for making an implantable device for providing controlled release of therapeutic agents in the eye.

Other useful polymers and delivery systems for ocular administration of a therapeutic agent have been described. The active agent may be released as the polymer degrades. Polymers that have been used for drug delivery include, but are not limited to, poly(lactic-co-glycolic acid), polyanhydrides, ethylene vinyl acetate, polyglycolic acid, chitosan, polyorthoesters, polyethers, polylactic acid, and poly (beta amino esters). Peptides, proteins such as collagen and albumin, and dendrimers (e.g., PAMAM dendrimers) have also been used. Any of these can be used in various embodiments of the invention. Poly(ortho-esters) have been introduced into the eye and demonstrated favorable properties for sustained release ocular drug delivery (Einmahl S. (2002), Invest. Ophthal. Vis. Sci., 43(5). Polylactide particles have been used to target an agent to the retina and RPE following intravitreous injection of a suspension of such particles (Bourges, J.L. et al (2003) Invest. Ophthal. Vis. Sci., 44(8). A macroscopic implantable device suitable for introduction into the posterior or anterior segment of the eye is referred to herein as an ocular implant (Jaffe, G. (2000) Invest. Ophthal. Vis. Sci., 41(11). Such devices may be comprised of a plurality of nanoparticles less than or microparticles impregnated with the agent. Methods for making microparticles and nanoparticles are known in the art. Generally, a microparticle will have a diameter of 500 microns or less, e.g., between 50 and 500 microns, between 20 and 50 microns, between 1 and 20 microns, between 1 and 10 microns, and a nanoparticle will have a diameter of less than 1 micron. Preferably the device is implanted into the space occupied by the vitreous humor. The ocular implant may comprise a polymeric matrix. The invention also provides periorcular implants, which are macroscopic implantable device suitable for introduction in the vicinity of the eye, e.g., in close proximity to the eye. In certain embodiments the periorcular implant is made of similar materials to those described above.

Pharmaceutical compositions according to the present invention can be illustrated as follows:

- Sterile IV Solution: A 5 mg/mL solution of the desired compound of this invention can be made using sterile, injectable water, and the pH is adjusted if necessary. The solution is
diluted for administration to 1 - 2 mg/mL with sterile 5% dextrose and is administered as an IV infusion over about 60 minutes.

- Lyophilised powder for IV administration: A sterile preparation can be prepared with (i) 100 - 1000 mg of the desired compound of this invention as a lyophilised powder, (ii) 32 - 327 mg/mL sodium citrate, and (iii) 300 - 3000 mg Dextran 40. The formulation is reconstituted with sterile, injectable saline or dextrose 5% to a concentration of 10 to 20 mg/mL, which is further diluted with saline or dextrose 5% to 0.2 - 0.4 mg/mL, and is administered either IV bolus or by IV infusion over 15 - 60 minutes.

Intramuscular suspension: The following solution or suspension can be prepared, for intramuscular injection:

- 50 mg/mL of the desired, water-insoluble compound of this invention
- 5 mg/mL sodium carboxymethylcellulose
- 4 mg/mL TWEEN 80
- 9 mg/mL sodium chloride
- 9 mg/mL benzy alcohol

Combination therapies
The compounds of this invention can be administered as the sole pharmaceutical agent or in combination with one or more other pharmaceutical agents where the combination causes no unacceptable adverse effects. The present invention relates also to such combinations. For example, the compounds of this invention can be combined with other anti-angiogenic agents. Anti-angiogenic agents include, but are not limited to, angiostatic steroids such as heparin derivatives and glucocorticosteroids; thrombospondin; cytokines such as IL-12; fumagillin and synthetic derivatives thereof, such as AGM 12470; interferon-alpha; endostatin; soluble growth factor receptors; neutralizing monoclonal antibodies directed against growth factors such as vascular endothelial growth factor and the like.

Dose and administration:
Based upon standard laboratory techniques known to evaluate compounds useful for the treatment of diseases where excessive (or pathological) angiogenesis occurs, by standard toxicity tests and by standard pharmacological assays for the determination of treatment of the conditions identified above in mammals, and by comparison of these results with the results of known medicaments that are used to treat these above described conditions, the effective dosage of the compounds of this invention can readily be determined for treatment of each desired indication. The amount of the active ingredient to be administered in the treatment of one of these conditions can vary widely according to such considerations as the particular
compound and dosage unit employed, the mode of administration, the period of treatment, the age and sex of the patient treated, and the nature and extent of the condition treated.

The total amount of the active ingredient to be administered will generally range from about 0.001 mg/kg to about 200 mg/kg body weight per day, and preferably from about 0.01 mg/kg to about 20 mg/kg body weight per day. Clinically useful dosing schedules will range from one to three times a day dosing to once every four weeks dosing. In addition, "drug holidays" in which a patient is not dosed with a drug for a certain period of time, may be beneficial to the overall balance between pharmacological effect and tolerability. A unit dosage may contain from about 0.5 mg to about 150 mg of active ingredient, and can be administered one or more times per day or less than once a day. The average daily dosage for administration by injection, including intravenous, intramuscular, intraocular, intravitreal, subcutaneous, intrathecal, intracereventricularly and parenteral injections, and use of infusion techniques will preferably be from 0.01 to 200 mg/kg of total body weight. The average daily rectal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The average daily vaginal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The average daily topical dosage regimen will preferably be from 0.1 to 200 mg administered between one to four times daily. The transdermal concentration will preferably be that required to maintain a daily dose of from 0.01 to 200 mg/kg. The average daily inhalation dosage regimen will preferably be from 0.01 to 100 mg/kg of total body weight.

It is evident for the skilled artesan that the specific initial and continuing dosage regimen for each patient will vary according to the nature and severity of the condition as determined by the attending diagnostician, the activity of the specific compound employed, the age and general condition of the patient, time of administration, route of administration, rate of excretion of the drug, drug combinations, and the like. The desired mode of treatment and number of doses of a compound of the present invention or a pharmaceutically acceptable salt or ester or composition thereof can be ascertained by those skilled in the art using conventional treatment tests.

Examples

1. Pharmacological inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) reduces ocular angiogenesis in a murine model for age-related macular degeneration.

Several small molecules able to specifically inhibit the PFKFB3 enzyme have been described in the art and these molecules have been shown to suppress the glycolytic flux (see Clem B et al (2008) Mol. Cancer Ther, 7(1): 110 and also patent application WO2008/156783). One of these compounds, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) has been shown to suppress the glycolytic flux and the compound was shown to be cytostatic to neoplastic cells
(Clem B. et al (2008) Mol. Cancer Ther. 7(1): 110). We have surprisingly demonstrated that 3PO not only is capable of inhibiting the glycolysis in tumor cells but that this compound also significantly inhibits the angiogenesis in murine models of pathological angiogenesis. Our experiments show that the enzyme PFKFB3 is a target for the inhibition of pathological angiogenesis and that aza chalcones, of which a representative member, 3PO, can be used for the treatment of pathological angiogenesis. The protein extravasation and hemorrhage associated with choroidal neovascularization (CNV) are primary causes of severe vision loss in retinal diseases such as age-related macular degeneration (ARMD). In ARMD the normal barrier function of Bruch’s membrane is compromised, and CNV can develop either under the retinal pigment epithelium (RPE) and photoreceptor outer segments. Choroidal neovascularization (CNV) was induced in C57BL/6 mice by laser burn. Mice were injected intraperitoneally (i.p.) with 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) (0.047 mg/g) (n=6) or control DMSO (n=5) daily until day 14. Laser burn (400 mW) was performed with Alcon Purepoint equipment. CNV was measured by investigators masked to treatment. Mice were pretreated 2 days before injury with 3PO or vehicle. Treatment was interrupted on day 6 and day 7. Eyes were enucleated after retrobulbar perfusion with FITC-dextran (HMW) and flat mounted. The CNV area, total lesion area, and their ratio were analyzed using Zeiss Axio Imager Z1 microscope with macros (KS300 image analysis software) on FITC-perfused (200 μL; 25 mg/mL; 10 min) flat mounts. Figure 1 shows the ratio between the neovascular area and the lesion area (filled circles represent 3PO-treated mice and unfilled circles depict untreated (control) mice. Figure 2, left panel, shows that pathological angiogenesis in an eye model for age-related macular degeneration is reduced by at least 60%. Figure 3 shows the FITC perfused flat mounts of the control mice (panel A) versus the 3PO-treated mice (panel B). In a next experimental setup the intraocular administration of 3PO is carried out in a murine model for age-related macular degeneration.

2. Selective inhibition of PFKFB3 can be used to treat ocular angiogenesis in an animal model for age-related macular degeneration

Intraocular delivery of small interfering RNAs specific for PFKFB3 to the eye of a mouse is subjected to a model for age-related macular degeneration as in example 1 is accomplished by delivery of a specific small interfering RNA for PFKFB3 into the eye via intraocular delivery. The three representative examples of siRNA sequences directed against murine PFKFB3 used are the sequence 5’-GGATAGGTTTCCAAGGAA-3’ (SEQ ID NO: 1), 5’-CCACATCGAGCCGATT-3’ (SEQ ID NO: 2) and 5’-GCTGCCTACTAGCCTTCTT-3’ (SEQ ID NO: 3).

Alternatively SEQ ID NO: 1, 2 and 3 are modified with phosphorothioate modifications throughout and 2'-0-(2-methoxy)ethyl substitutions on the sugars of the first and last 5
nucleotides to increase biological half-lives and binding affinity. Clinical analysis of the mice is carried out essentially as described in example 1.
Claims

1. A compound which is a molecule with the formula (I) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, particularly a pharmaceutically acceptable salt thereof, or a mixture of the same for the treatment of age-related macular degeneration, diabetic retinopathy, proliferative retinopathies, choroidal and other intraocular disorders with an excessive angiogenesis component

![Chemical Structure](image)

wherein:

- $X_1$, $X_2$, and $X_3$ are each CO or CH;
- $X_i$ is selected from the group consisting of O, S, NR$_1$, C(R$_2$)$_2$, OR$_3$, SR$_4$, NR$_5$R$_6$, and C(R$_7$)$_3$ , wherein $R_1$, $R_3$, $R_4$, $R_5$, and $R_6$ are each independently selected from the group consisting of H, alkyl, aryl, aralkyl, and acyl, and each $R_2$ and $R_7$ is independently selected from the group consisting of H, halo, hydroxyl, alkoxy, alkyl, aralkyl, and aryl;
- $R_{i1}$ is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, ary1, and aralkyl;
- $R_{i1}$ is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, ary1, and aralkyl;
- $A_i$, $A_2$, $A_3$, $A_4$, and $A_5$, are each independently N or CR$_{12}$, wherein each $R_{12}$ is independently selected from the group consisting of H, alkyl, halo, nitro, cyano, hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkyl carbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:

![Chemical Structure](image)

wherein:

- $X_4$, $X_5$, $X_6$, $X_7$, and $X_8$ are each CO or CH;
$X_4$ is $NR_{14}$, wherein $R_{14}$ is selected from the group consisting of H, alkyi, hydroxyl, aralkyi, and aryl;

$X_5$ is selected from the group consisting of O, S, C(R$\text{I}_3$)$_2$, and $NR_{14}$, wherein each $R_{15}$ is independently selected from the group consisting of H, hydroxyl, alkoxy, alkyi, aralkyi, and aryl; and

$X_6$ is selected from H, alkyi, aralkyi, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein $R_{10}$ and one $R_{12}$ are together alkylene;

$A_{r_2}$ is selected from the group consisting of

![Diagram](image)

wherein:

each $Y_1$, $Y_2$, $Y_3$, $Y_4$, $Y_5$, $Y_6$, $Y_7$, $Y_8$, $Y_9$, $Y_{10}$, $Y_{11}$, $Y_{12}$, $Y_{13}$, $Y_{14}$, $Y_{15}$, $Y_{16}$, $Y_{17}$, $Y_{18}$, $Y_{19}$, $Y_{20}$, $Y_{21}$, $Y_{22}$, $Y_{23}$, $Y_{24}$, $Y_{25}$, $Y_{26}$, $Y_{27}$, $Y_{28}$, $Y_{29}$, and $Y_{30}$ is independently selected from the group consisting of N and CR$\text{I}_3$, wherein each $R_{13}$ is independently selected from the group consisting of H, alkyi, halo, nitro, cyano, hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:

![Diagram](image)

wherein:

$X_4$ is $NR_{14}$, wherein $R_{14}$ is selected from the group consisting of H, alkyi, hydroxyl, aralkyi, and aryl;

$X_5$ is...
X₅ is selected from the group consisting of O, S, C₁₁₁₉₂, and NR₁₄, wherein each R₁₅ is independently selected from the group consisting of H, hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and X₆ is selected from H, alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy; or wherein R₁₉ and one R₃ are together alkyene; and wherein at least one of A₁, A₂, A₃, A₄, A₅, Y₁, Y₂, Y₃, Y₄, Y₅, Ye, Y₇, Ye, Y₉, Y₁₀, Y₁₁, Y₁₂, Y₁₃, Y₁₄, Vis, Y₁₆, Y₁₇, Y₁₈, and Y₁₉ is N; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein X₁ is O and X is C.

3. A compound according to claim 1 wherein A₂ is:

\[
\begin{array}{c}
\text{Y}_1 \\
\text{Y}_2 \\
\text{Y}_3 \\
\text{Y}_4 \\
\text{Y}_5 
\end{array}
\]

X₁, X₂, and X₃ are each C;
X₄ is selected from the group consisting of O, S, NR₁₄, and C₁₁₂₁₂, wherein R₁₄ is selected from the group consisting of H and alkyl, and each X₄ is independently selected from the group consisting of H, halo, hydroxyl, alkoxy, and alkyl; and the compound of Formula (I) has a structure of Formula (II):

4. A compound according to claim 3 wherein the compound of formula (II) is selected from the group consisting of:
5. A compound according to claim 1 wherein $A_{r2}$ is:

\[ X, X_2, \text{and } X_3 \text{ are each } C; \]

$X_1$ is selected from the group consisting of $O, S, NR_1$, and $C(R_2)_2$, wherein $R_1$ is selected from the group consisting of $H$ and alkyl, and each $R_2$ is independently selected from the group consisting of $H, \text{halo, hydroxyl, alkoxy, and alkyl};$ and the compound of Formula (I) has a structure of Formula (III):
6. A compound according to claim 5 wherein the compound of Formula (III) is

![Chemical Structure](image)

7. A compound according to claim 1 wherein $A_{r_2}$ is

![Chemical Structure](image)

$X, X_2, \text{and } X_3$ are each $C$;

$x_1$ is selected from the group consisting of $O, S, N^{\text{R_1}}, \text{and } C(R_2)_{2}$, wherein $R_1$, is selected from the group consisting of $H$ and alkyl, and each $R_2$ is independently selected from the group consisting of $H, \text{halo, hydroxyl, alkoxy, and alkyl}$; and the compound of Formula (I) has a structure of Formula (IV):

![Chemical Structure](image)
8. A compound according to claim 7 wherein the compound of Formula (IV) is:

![Compound Diagram]

(IV)

9. A compound which is a molecule with the formula (I) or a stereoisomer, a tautomer, a hydrate, a solvate, or a salt thereof, particularly a pharmaceutically acceptable salt thereof, or a mixture of the same for the treatment of a condition involving pathological angiogenesis excluding cancer:

![Compound Diagram]

(I)

wherein:

- $X_1, X_2,$ and $X_3$ are each C or CH;
- $X_1$ is O;
- $R_{10}$ is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;
- $R_{11}$ is selected from the group consisting of H, alkyl, halo, cyano, hydroxyl, aryl, and aralkyl;
- $A_1, A_2, A_3, A_4,$ and $A_5,$ are each independently $N$ or $CR_{12},$ wherein each $R_{12}$ is independently selected from the group consisting of H, alkyl, halo, nitro, cyano, hydroxyl, mercapto, amino, alkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:
wherein:

- $X_4$ is $N\, R_4$, wherein $R_4$ is selected from the group consisting of $H$, alkyl, hydroxyl, aralkyl, and aryl;
- $X_5$ is selected from the group consisting of $O$, $S$, $C(\,R_i\,)$, and $N\, R_4$, wherein each $R_i$ is independently selected from the group consisting of $H$, hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and
- $X_6$ is selected from $H$, alkyl, aralkyl, aryl, heteroaryl, dialkylamino, and alkoxy;

or wherein $R_{10}$ and one $R_{12}$ are together alkylene;

$A_{r_2}$ is selected from the group consisting of

wherein:

- $Y_i$, $Y_2$, $Y_3$, $Y_4$, $Y_5$ are each $C$;
- each $Y_6$, $Y_7$, $Y_8$, $Y_9$, $Y_{10}$, $Y_{11}$, $Y_{12}$, $Y_{13}$, $Y_{14}$, $Y_{15}$, $Y_{16}$, $Y_{17}$, $Y_{18}$, and $Y_{19}$ is independently selected from the group consisting of $N$ and $CR_{13}$, wherein each $R_{13}$ is independently selected from the group consisting of $H$, alkyl, halo, nitro, cyano, hydroxyl, mercapto, amino, dialkylamino, dialkylamino, carboxyl, acyl, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, sulfate, and a group having the structure:
wherein:

X₄ is N R₄, wherein R₄ is selected from the group consisting of H, alkyl, hydroxyl, aralkyl, and aryl;

X₅ is selected from the group consisting of O, S, C(Rᵢ₅)₂, and N R₁₄, wherein each Rᵢ₅ is independently selected from the group consisting of H, hydroxyl, alkoxy, alkyl, aralkyl, and aryl; and

X₆ is selected from H, alkyl, aralkyl, aryl, heteroaryl, alkylamino, dialkylamino, and alkoxy;

or wherein R₁₀ and one R₁₃ are together alkylene; and

wherein at least one of A₁, A₂, A₃, A₄, A₅, Y₁, Y₂, Y₃, Y₄, Y₅, Y₆, Y₇, Y₈, Y₉, Y₁₀, Y₁₁, Y₁₂, Y₁₃, Y₁₄, Y₁₅, Y₁₆, Y₁₇, Y₁₈, and Y₁₉ is N; or a pharmaceutically acceptable salt thereof.

10. A compound according to claim 9 wherein Ar₂ is:

\[
\begin{align*}
\text{X, X₂, and X₃ are each C;} \\
\text{and the compound of Formula (I) has a structure of Formula (III):}
\end{align*}
\]
11. A compound according to claim 10 wherein the compound of Formula (III) is

\[
\text{O} \quad \text{N}
\]

12. A compound according to claim 9 wherein \( A_{r2} \) is

\[
Y_{13} \quad Y_{14} \quad Y_{15} \\
Y_{16} \quad Y_{17} \quad Y_{18}
\]

and the compound of Formula (I) has a structure of Formula (IV):

\[
\text{O} \quad \text{N}
\]

13. A compound according to claim 12 wherein the compound of Formula (IV) is:
14. A compound according to any one of claims 9 to 13 for the treatment of age-related macular degeneration, diabetic retinopathy, proliferative retinopathies, choroidal and other intraocular disorders with an excessive angiogenesis component, rheumatoid arthritis, psoriasis and atherosclerosis.

15. A compound which is an siRNA with a specificity for PFKFB3 for the treatment of age-related macular degeneration, diabetic retinopathy, proliferative retinopathies, choroidal and other intraocular disorders with an excessive angiogenesis component, rheumatoid arthritis, psoriasis and atherosclerosis.
## INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K31/44 A61K31/4409 A61P9/10 A61P27/02

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K

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

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>WO 2008/156783 A2 (UNIV LOUISVILLE RES FOUND [US]; CHESNEY JASON [US]; TRENT JOHN 0 [US];) 24 December 2008 (2008-12-24) cited in the application on claims 2, 6, 9, 26, 8 page 13, line 9</td>
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**Further documents are listed in the continuation of Box C.**

**See patent family annex.**

### Special categories of cited documents:

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Date of the actual completion of the international search: 3 September 2012

Date of mailing of the international search report: 07/09/2012

Name and mailing address of the ISA:

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

Authorized officer:

Gutke, Hans-JLirgen

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<td>E. M. DIOUM ET AL: &quot;Haploinsufficient Mice Have Blunted Retinal Neovascularization Due to Impaired Expression of a Proangiogenic Gene Battery&quot;. INVESTIGATIVE OPHTHALMOLOGY &amp; VISUAL SCIENCE, vol. 49, no. 6, 1 January 2008 (2008-01-01), pages 2714-2720, XP55035613, ISSN: 0146-0404, DOI: 10.1167/iovs.07-1469 page 2718</td>
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