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(54) Use of steroids for the inhibition of angiogenesis
Verwendung von Steroiden zur Inhibierung von Angiogenesis
Utilisation des stéroides pour l'inhibition d'angiogenèse

(56) References cited:
US-A- 3 300 483

• THE JOURNAL OF ORGANIC CHEMISTRY, vol.
  44, no. 9, 27 April 1979, American Chemical
  Society, V. VANRHEENEN et al.: "New synthesis
  of cortico steroids from 17-keto steroids:
  Application and stereochemical study of the
  unsaturated sulfoxide-sulfenate
  rearrangement", pages 1582-1584, see page
  1582, compound 10

• THE JOURNAL OF MEDICINAL CHEMISTRY, vol.
  33, no. 4, April 1990, American Chemical Society,
  E.J. JACOBSEN et al.: "Novel 21-aminosteroids
  that inhibit iron-dependent lipid peroxidation
  and protect against central nervous system
  trauma", pages 1145-1151, see page 1147,
  compound 25; page 1150, method A

• STEROIDS, vol. 44, no. 4, October 1984, G.
  ZOMER et al.: "The synthesis of [1,2,3,4-13C]
  cortisol", pages 293-300, see page 295,
  compound 10

Remarks:
The file contains technical information submitted
after the application was filed and not included in
this specification

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Description

Field of the Invention

[0001] The present invention relates to the treatment of angiogenesis in mammals, using certain angiostatic Δ4,9(11)-steroids. These steroids are useful in treating diseases of neovascularization such as cancer, diabetes and arthritis.

Description of the Related Art

[0002] Angiogenesis is the development of blood vessels which typically would lead to a vascular bed capable of sustaining viable tissue. Angiogenesis is a necessary process in the establishment of embryonic tissue and development of a viable embryo. Similarly, angiogenesis is a necessary step in the establishment and development of tumor tissue as well as certain inflammatory conditions. The inhibition of angiogenesis would be useful in the control of embryogenesis, inflammatory conditions, and tumor growth, as well as numerous other conditions.


[0005] Heparin is presently used with inhibitors of angiogenesis, especially angiostatic steroids to treat diseases involving neovascularization, see Biochem. Pharmacol. 34, 905 (1985) and Annals of Surgery 206, 374 (1987). The heparin potentiates the angiogenesis inhibiting activity of other drugs, for example of collagen biosynthesis inhibitors such as L-azetidine carboxylic acid. The problem with using heparin is that the efficacy of each preparation/batch of heparin differs due to the chemical heterogeneity of the heparin molecules.

[0006] Suramin inhibits the binding of fibroblast growth factor to its receptor during in vitro experiments. Fibroblast growth factor is one of a number of known angiogenic growth factors. See, J. Cell Physiol. 132, 143 (1987).


[0008] US Patent 4,599,331 discloses 20-substituted Δ1,4,16-methyl steroids which do not have a Δ9(11) double bond, which are useful as angiogenetics when combined with heparin.

[0009] US Patent 4,771,042 discloses 21-hydroxysteroids which are useful in the inhibition of angiogenesis involving the co-administration of steroids with heparin or heparin fragments. The compounds of the present invention do not include -CH2- at C21.


[0011] The Journal of the National Cancer Institute 81, 1346 (1989) discloses that "Suramin also appears to have antiangiogenesis activity ...".

[0012] The combination of suramin-type compounds and angiostatic steroids has been reported to treat angiogenesis in a warm blooded mammal; see the Journal of the National Cancer Institute 81, 1346 (1989).

[0013] It is known that steroids alone can inhibit angiogenesis, see National Academy of Sciences USA 78, 1176 (1981) [medroxyprogesterone], Cancer Letters 43, 85 (1988) [medroxyprogesterone acetate], International Journal of Cancer 35, 549 (1985) [cortisone], JNCI 74, 869 (1985) [cortisone], Cancer Research 47, 5021 (1987) [cortisone acetate] and European Journal of Cancer and Clinical Oncology 23, 93 (1987) [cortisone acetate]. The data reported in these publications are consistent with the clinical practice of using steroids to inhibit tumors.


SUMMARY OF THE INVENTION

[0017] A first aspect of the present invention is the use of a steroid of formula (II)
A second aspect of the present invention is the use of any of those skilled in the art.

[0020] Compounds for use in the second aspect of this invention are also known. The first three are disclosed in, respectively, US-A-3980778 (column 2, compound 3) and US-A-4018918 (column 10, compound 17); J. Org. Chem. 33:1695 (1968); and US-A-4704358 and US-A-4771042. The fourth compound is also known; see also Example 1, below. The fifth and sixth compounds may be made by methods well known to those skilled in the art.

[0022] The steroids to which this invention relates are useful in treating angiogenesis, and especially diseases of neovascularisation. It is preferred that the indication for treatment is selected from solid tumours, diabetes, arthritis, atherosclerosis, neovascularisation of the eye, glaucoma, parasitic diseases, psoriasis, abnormal wound healing processes, hypertrophy following surgery, burns, injury, inhibition of hair growth, inhibition of ovulation and corpus luteum formation, inhibition of implantation and inhibition of embryo development in the uterus. It is more preferred that the neovascular disease is solid tumours, diabetes or arthritis.

[0023] The dose of the steroid is from 0.1 to 100 mg/kg/day, preferably from 0.1 to 50 mg/kg/day. The exact dosage and frequency of administration depends on the particular steroid being used, the particular condition being treated, the severity of the condition being treated, the age, weight, general condition of the particular patient, and other medication that the individual may be taking, as is well known to those skilled in the art. These factors can be more accurately determined by measuring the blood level or concentration of the steroid in the patient's blood and/or the patient's response to the particular condition being treated.

[0024] For the inhibition of angiogenesis, the steroids of the invention may be combined with each other or with other agents such as suramin (in the first aspect of the invention only), sulfated glycosaminoglycans and sulfated polysaccharides, or effective fragments of these molecules. The preferred glycosaminoglycans include heparin and heparan sulfate. Fragments of heparin or heparan sulfate may also be used if they contain a minimum of six saccharide residues; fragments of heparin and heparan sulfate may be prepared from heparin or heparan sulfate isolated from natural sources, or they may be prepared by chemical synthesis. The steroids of the invention may also be combined with polysaccharides including pentosan polysulphate, cyclodextrins, or other sulfated polysaccharides isolated from natural sources. The preferred polysaccharides are sulfated forms of glycosaminoglycans and include heparin and heparan sulfate isolated from natural sources. The preferred glycosaminoglycans include heparin and heparan sulfate.

[0025] The steroids of the invention may also be used in combination treatments containing compounds which...
interfere with collagen biosynthesis. Preferred compounds in this group include L-azetidine-2-carboxylic acid, thioproline, and related proline analogues. Also included are other inhibitors of basement membrane collagen synthesis such as 8,9-dihydroxy-7-methylbenzo(b)quinolizinium bromide.

[0026] The following Example illustrates the preparation of a steroid for use in this invention.

All temperatures are in degrees Centigrade.
TLC refers to thin-layer chromatography.
MS refers to mass spectrometry expressed as m/e or mass/charge unit. [M + H]+ refers to the positive ion of a parent plus hydrogen atom. EI refers to electron impact. CI refers to chemical ionisation.
FAB refers to fast atom bombardment.

EXAMPLE 1

[0027]

A. Methanol (20 ml) and sodium methoxide (25%, 0.2 ml) are added to 6α-fluoro-17α,21-dihydroxy-16α-methylpregna-4,9(11)-diene-3,20-dione 21-acetate (US Patent 3,291,815, 1.0 g) in methanol. The reaction mixture is neutralized with acetic acid and concentrated to dryness under reduced pressure. The concentrate is distributed between water and chloroform. The organic layer is separated and washed twice with water and dried over anhydrous sodium sulfate. The crude solid is chromatographed over silica gel eluting with ethyl acetate/hexane (35/65). The appropriate fractions are pooled and concentrated to give 6α-fluoro-17α,21-dihydroxy-16α-methylpregna-4,9(11)-diene-3,20-dione, mp 206-207°.

B. THF (26 ml) and periodic acid (0.677 g) in water (10 ml) are added to 611 mg (1.62 mmol) of 6α-fluoro-17,21-dihydroxy-16α-methylpregna-4,9(11)-diene-3,20-dione (Part A, 611 mg). The resulting solution is heated at reflux for 2 hours, then cooled to 25° and concentrated under reduced pressure to a volume of 5 ml. Water (15 ml) is added to the residue and the resulting mixture is extracted with ethyl acetate (2 x 25 ml). The ethyl acetate extracts are combined, dried over anhydrous sodium sulfate, filtered, and concentrated to give 6α-fluoro-17α,21-dihydroxy-16α-methylpregna-4,9(11)-diene-3,20-dione, mp 206-207°.

D. The crude 17-acetate (Part C) is dissolved in THF (8 ml) and then treated with freshly prepared diazomethane in ether until all of the starting material appeared to have reacted by TLC. The crude product is purified by chromatography over silica gel eluting with ethyl acetate/hexane (25/75). The appropriate fractions are pooled and concentrated to give 6α-fluoro-17α-hydroxy-16α-methylandrost-4,9(11)-dien-3-one 17β-carboxylic acid methyl ester 17-acetate, TLC Rf = 0.6 (ethyl acetate/hexane (35/65); MS calculated 419.2234, found 419.2212.

Claims

1. Use of a steroid of formula (II) wherein

\[ \text{CH}_3 \text{OR}_{21} \]

\[ \text{C} = \text{O} \]

\[ \text{OH} \]

\[ \text{R}_6 \]

\[ \text{R}_7 \]

\[ \text{R}_8 \]

\[ \text{R}_{21} \]

wherein

\[ \text{R}_8 \] is H, F or CH₃;

\[ \text{R}_7 \] is H or CH₃; and

\[ \text{R}_{21} \] is -CO(C₁₋₁₂ alkyl);

for the manufacture of a medicament for use in treating angiogenesis in a human or other warm-blooded mammal.

2. Use according to claim 1, where the steroid (II) is

6α-methyl-17α,21-dihydroxyprognav-4,9(11)-diene-3,20-dione 21-acetate.
3. Use of any of

6α-fluoro-17α,21-dihydroxy-16β-methylpregna-4,9(11)-dien-3,20-dione 17,21-diace-
tate,
21-bromo-3α,17α-dihydroxy-5β-pregnan-20-
one,
17α,21-dihydroxypregna-1,4,9(11)-triene-3,20-
dione,
6α-fluoro-17α-hydroxy-16α-methylandrosta-
4,9(11)-dien-3-one 17β-carboxylic acid methyl
ester 17-butyrate,
17β-carboxy-9β,11β-epoxy-6α-fluoro-17α-
hydroxy-16α-methylandrosta-1,4-dien-3-one
17α-butyrate 17β-methyl ester, and
17β-carboxy-6α-fluoro-17α-hydroxy-16β-methyl-
yandrosta-4,9(11)-dien-3-one 17α-butyrate,
in the absence of a suramin-type compound,
for the manufacture of a medicament for use in
treating angiogenesis in a human or other
warm-blooded mammal.

4. Use according to any of claims 1 to 3, where
the medicament is adapted for oral or parenteral
administration.

5. Use according to any of claims 1 to 4, for the treat-
mant of any of solid tumours, diabetes, arthritis,
atherosclerosis, neovascularisation of the eye,
glaucoma, parasitic diseases, psoriasis, abnormal
wound-healing processes, hypertrophy following
surgery, burns, injury, inhibition of hair growth, inhi-
bition of ovulation and corpus luteum formation,
inhibition of implantation and inhibition of embryo
development in the uterus.

Patentansprüche

1. Verwendung eines Steroids der Formel (II)

Revendications

1. Utilisation d'un stéroïde de formule (II),
dans laquelle $R_6$ représente $H$, $F$ ou $CH_3$; $R_7$ représente $H$ ou $CH_3$; et $R_{21}$ représente $-CO(alkyle en C_{1-12})$; pour la fabrication d'un médicament à utiliser dans le traitement de l'angiogenèse chez l'homme ou un autre mammifère à sang chaud.

2. Utilisation selon la revendication 1, dans laquelle le stéroïde (II) est le 21-acétate de $6\alpha$-méthyl-17$\alpha$,21-dihydroxypregna-4,9(11)-diène-3,20-dione,

3. Utilisation de l'un quelconque parmi le 17,21-diacétate de $6\alpha$-fluoro-17$\alpha$,21-dihydroxy-16$\beta$-méthyl[pregna-4,9(11)-diène-3,20-dione, le 21-bromo-3$\alpha$,17$\alpha$-dihydroxy-5$\beta$-pregnan-20-one, le $17\alpha$,21-dihydroxypregna-1,4,9(11)-triène-3,20-dione, la méthyl ester 17-butyrate d'acide $6\alpha$-fluoro-17$\alpha$-hydroxy-16$\alpha$-méthylandrosta-4,9(11)-dien-3-one, 17$\beta$-carboxylique, le 17$\beta$-méthyl ester 17$\alpha$-butyrate de 17$\beta$-carboxy-9$\beta$-11$\beta$-époxy-6$\alpha$-fluoro-17$\alpha$-hydroxy-16$\alpha$-méthylandrosta-1,4-dien-3-one, et le 17$\alpha$-butyrate de 17$\beta$-carboxy-6$\alpha$-fluoro-17$\alpha$-hydroxy-16$\beta$-méthylandrosta-4,9(11)-dien-3-one, en l'absence d'un composé de type suramine, pour la fabrication d'un médicament à utiliser dans le traitement de l'angiogenèse chez l'homme ou un autre mammifère à sang chaud.

4. Utilisation selon l'une quelconque des revendications 1 à 4, pour le traitement de l'une quelconque parmi les tumeurs massives, le diabète, l'arthrite, l'athérosclérose, la néovascularisation de l'œil, le glaucome, des maladies parasitaires, le psoriasis, des processus anormaux de guérison de blessures, l'hypertrophie suite à de la chirurgie, des brûlures, des blessures, l'inhibition de la croissance des cheveux, l'inhibition de l'ovulation et de la formation du corpus luticum, l'inhibition de l'implantation et l'inhibition du développement de l'embryon dans l'utérus.

5. Utilisation selon l'une quelconque des revendications 1 à 4, pour le traitement de l'une quelconque parmi les tumeurs massives, le diabète, l'arthrite, l'athérosclérose, la néovascularisation de l'œil, le glaucome, des maladies parasitaires, le psoriasis, des processus anormaux de guérison de blessures, l'hypertrophie suite à de la chirurgie, des brûlures, des blessures, l'inhibition de la croissance des cheveux, l'inhibition de l'ovulation et de la formation du corpus luticum, l'inhibition de l'implantation et l'inhibition du développement de l'embryon dans l'utérus.