Drugs of the Future 2002, 27(11): 1039-1048

CCC: 0377-8282/2002

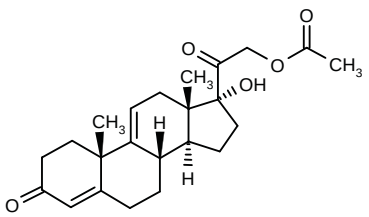
Anecortave Acetate

Prop INN; USAN

Treatment of Age-Related Macular Degeneration
Angiogenesis Inhibitor

AL-3789

21-Acetoxy-17α-hydroxypregna-4,9(11)-diene-3,20-dione



$C_{23}H_{30}O_5$
Mol wt: 386.493
CAS: 007753-60-8
EN: 198105

Abstract

Angiogenesis is a normal process that is strictly controlled. If this fine control is disrupted, chronic activation can occur resulting in inappropriate tissue responses that can lead to pathologic neovascularization. Many chronic ocular diseases are due to chronic stimulation of angiogenesis and they are the major cause of blindness worldwide. Treatment for these ocular neovascular disorders should involve delay, arrest or prevention of new capillary proliferation with the absence of or the presence of only minimal adverse events. To date, surgery, laser photocoagulation and glucocorticoid therapy are the usual treatment options. However, they may be ineffective, worsen the condition or, in the case of glucocorticoids, be associated with steroid-induced adverse events. Several classes of antiangiogenic agents have been described and they include antibiotics, polypeptides, polycations, polyanions, steroids, VEGF antagonists and integrin antagonists. Angiostatic steroids in particular have been shown to inhibit angiogenesis without the typical steroid activity that is associated with side effects. One such novel angiostatic steroid chosen for further development is anecortave acetate. It has shown excellent preclinical antiangiogenic efficacy and promising clinical activity as a treatment for ocular neovascular disorders.

Synthesis

Anecortave acetate has been obtained by several different ways:

1) Reaction of 9α-hydroxyandrost-4-ene-3,17-dione (I) with benzenesulfinyl chloride and pyridine gives the corresponding sulfinate (II), which by treatment with TsOH in refluxing chloroform yields androsta-4,9(11)-diene-3,17-dione (III). Reaction of the androstadienedione (III) with acetylene by means of potassium *tert*-butoxide in THF affords the 17α-ethynyl derivative (IV) (1), which is treated with phenylsulfenyl chloride and TEA at –70 °C to provide the sulfenate ester (V). Rearrangement of sulfenate (V) by warming at –40 °C gives the allene sulfoxide (VI), which is treated with sodium methoxide in methanol at 25 °C to yield the enol ether sulfoxide (VII). Then, by refluxing in methanol, an equilibrium between sulfoxide (VII) and sulfenate (VIII) occurs. Reaction of the non-isolated sulfenate (VIII) with the thiophile trimethyl phosphite affords the 17α-hydroxy enol ether (IX) (1, 2), which is finally converted to anecortave acetate by either treatment with peracetic acid and $NaHCO_3$ in dichloromethane (1) or bromination with Br_2 and pyridine in dichloromethane to give compound (X) followed by treatment with potassium acetate, KI and AcOH in refluxing acetone (1, 2). Scheme 1.

2) Reaction of 4,9(11)-androstadien-3,17-dione (III) with trimethyl orthoformate and TsOH in dioxane gives 3-methoxy-3,5,9(11)-androstatrien-17-one (XI), which is condensed with 1,2-dichloroethylene (XII) by means of MeLi in ethyl ether/THF to yield 17α-(chloroethynyl)-17β-hydroxy-4,9(11)-androstadien-3-one (XIII). Esterification of the OH group of (XIII) with fuming HNO_3 and acetic anhydride affords the 17β-(nitrooxy) derivative (XIV), which is treated with formic acid and $AgNO_3$ in *N*-methylpyrrolidone to provide 21-chloro-17α-(formyloxy)-4,9(11)-pregnadien-3,20-dione (XV). Hydrolysis of the formyloxy group of (XV) by means of $KHCO_3$ in methanol/water gives 21-chloro-17α-hydroxy-4,9(11)-

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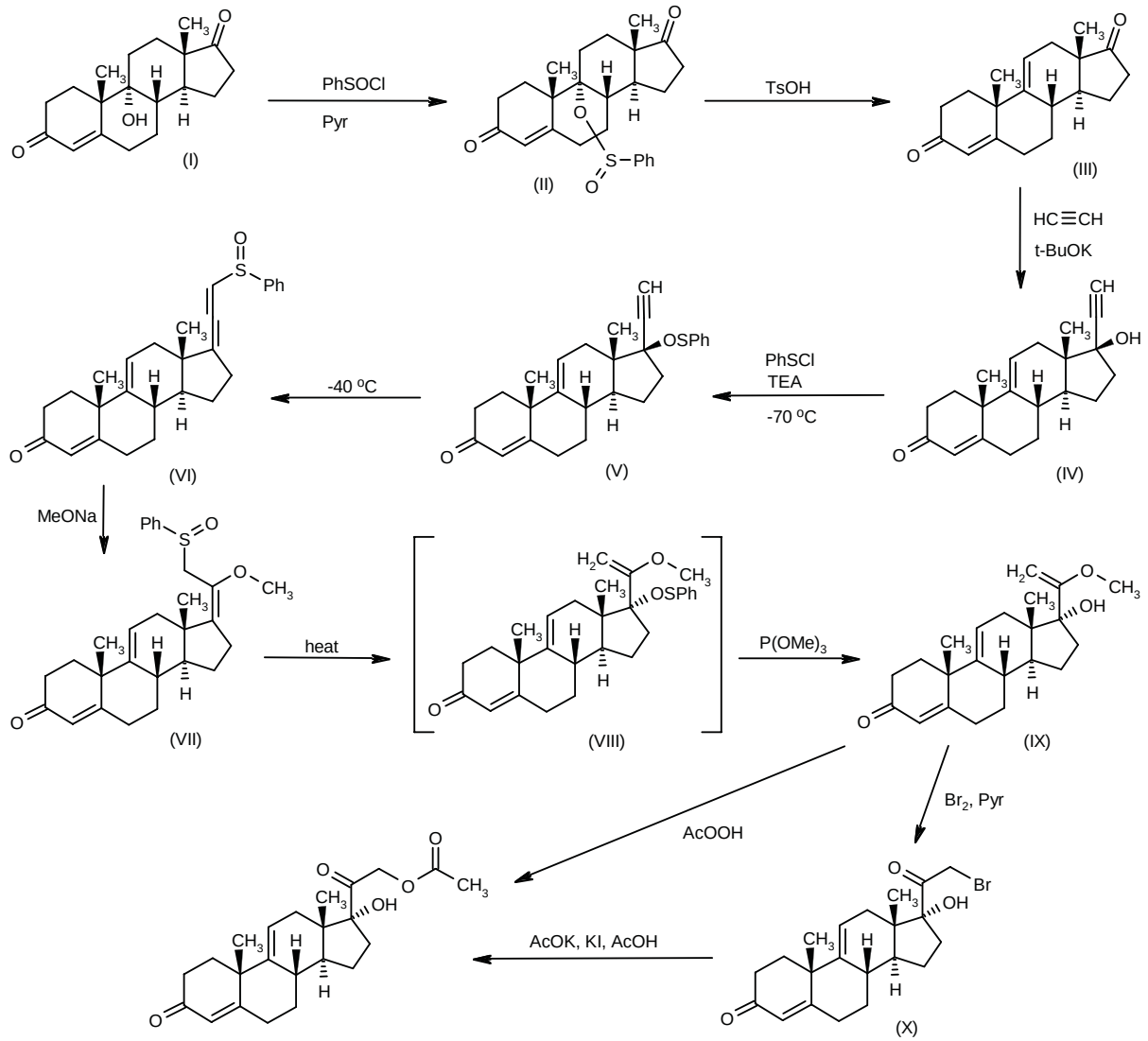
Scheme 1: Synthesis of Anecortave Acetate

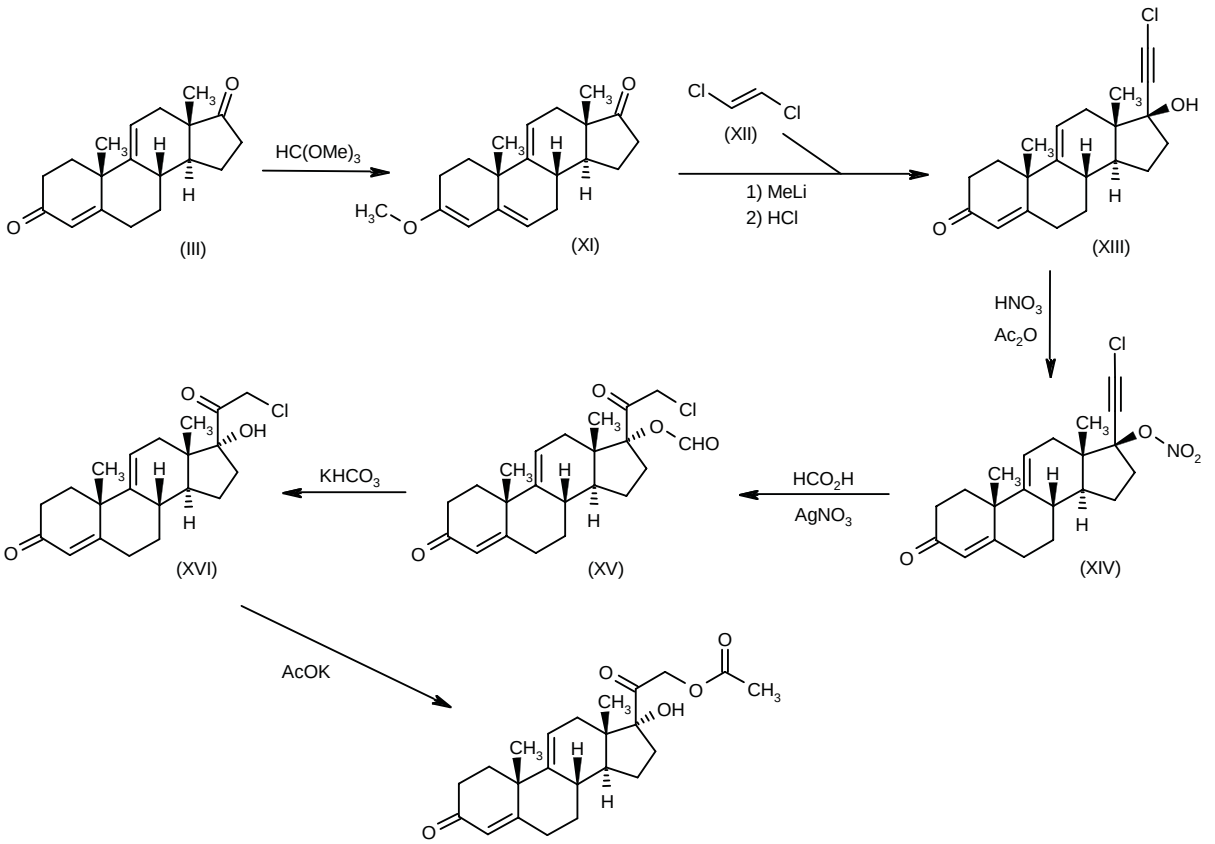


pregnadien-3,20-dione (XVI), which is finally acetylated by means of AcOK and KI in hot DMF (3). Scheme 2.

3) Reaction of 3-methoxyandrosta-3,5,9(11)-trien-17-one (XI) with KCN and AcOH in methanol gives a 1:1 mixture of the epimeric cyanohydrins (XVII) and (XVIII) and the desired β-cyano epimer (XVIII) is obtained in a 95% yield by selective crystallization under equilibrating conditions. Protection of the 17-OH group of (XVIII) with TMS-Cl and imidazole yields the silyl ether (XIX), which is reduced with DIBAL and AcOH to afford the carbaldehyde (XX). Reaction of compound (XX) with dibromomethane and LDA provides the dibromo derivative (XXI), which by reaction with more LDA gives the lithium enolate (XXII). Hydrolysis of enolate (XXII) in acid medium yields the α-bromoketone (XXIII), which is acylated with AcOH and TEA in hot acetone to afford the silylated steroid (XXIV). Finally, compound (XXIV) is deprotected by means of HF and TEA in dichloromethane (4). Scheme 3.

4) Condensation of 3-methoxyandrosta-3,5,9(11)-trien-17-one (XI) with 3-hydroxypropionitrile (XXV) by means of LDA in THF provides 17α-(1-cyano-2-hydroxyethyl)-17β-hydroxyandrosta-4,9(11)-dien-3-one (XXVI), which is selectively monoacetylated by means of Ac_2O in pyridine to yield monoacetate (XXVII). Dehydration of (XXVII) by means of $SOCl_2$ in pyridine gives 20-cyano-21-acetoxypregna-4,9(11),17(20)-trien-3-one (XXVIII), which is treated with ethylene glycol, trimethyl orthoformate and TsOH in dichloromethane to

Scheme 2: Synthesis of Anecortave Acetate



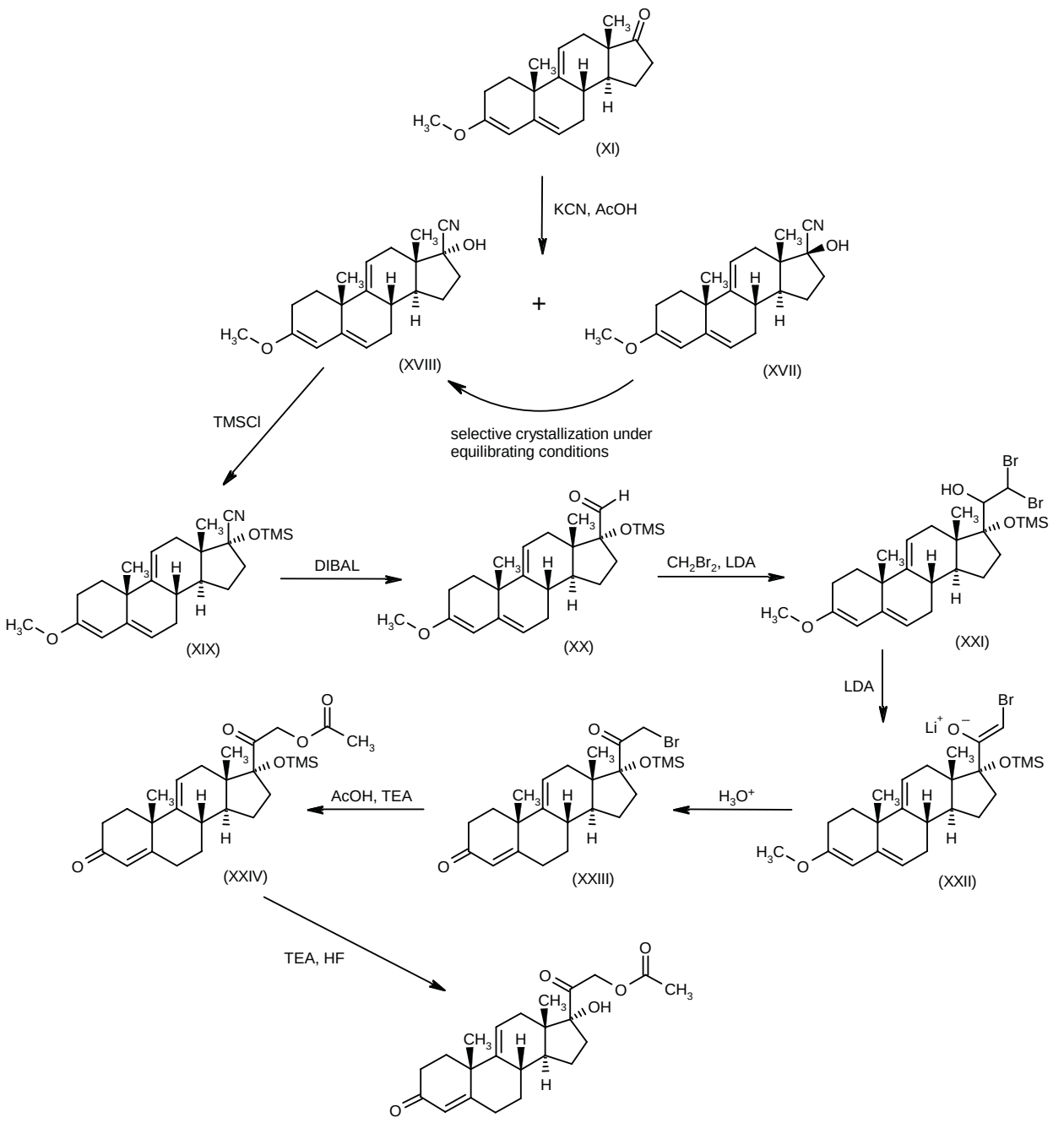
afford the ethylene ketal (XXIX). Finally, compound (XXIX) is oxidized with $KMnO_4$ in acetone/ethylene glycol and treated with $NaHSO_3$ and HCOOH (5). Scheme 4.

5) Condensation of 3-methoxyandrosta-3,5,9(11)-trien-17-one (XI) with (*E*)-1,2-dichloroethylene (XII) by means of BuLi in toluene gives 17α-[(*E*)-1,2-dichlorovinyl]-17β-hydroxyandrosta-4,9(11)-dien-3-one (XXX), which is treated with phenylsulfenyl chloride and TEA in dichloromethane to yield 20,21-dichloro-21-(phenylsulfinyl)pregna-4,9(11),17(20)-trien-3-one (XXXI). Reaction of compound (XXXI) with MeONa in acetone/MeOH at 0 °C affords 21-chloro-20-methoxy-21-(phenylsulfinyl)-pregna-4,9(11),17(20)-trien-3-one (XXXII), which by treatment with more MeONa at 35 °C provides 21-chloro-17α-hydroxy-20-methoxypregna-4,9(11),20-trien-3-one (XXXIII). Hydrolysis of the enol ether of (XXXIII) with HCl in THF/acetone/MeOH gives the 3,20-dione derivative (XVI), which is finally treated with AcOK and KI in hot acetone/dichloromethane (6). Scheme 5.

6) Reaction of 17α-ethynyl-9,11-didehydrotestosterone (IV) with phenylsulfinyl chloride and TEA in dichloromethane gives 21-(phenylsulfinyl)pregna-4,9(11),17(20),20-tetraen-3-one (VI), which is treated with phenol and NaOH in refluxing toluene to provide 20-(phenoxy)-21-(phenylsulfinyl)pregna-4,9(11),20-trien-3-one (XXXIV). The reaction of compound (XXXIV) with trimethyl phosphite and TEA in hot methanol affords 17α-hydroxy-20-(phenoxy)pregna-4,9(11),20-trien-3-one (XXXV) (7), which is finally treated with Oxone and KOH in hot dichloromethane, and then acetylated with Ac_2O, TEA and DMAP in THF/water (8). Scheme 6.

7) Condensation of 3-methoxyandrosta-3,5,9(11)-trien-17-one (XI) with 2-chlorovinyl ethyl ether (XXXVI) by means of BuLi in THF gives 20-chloro-3-oxopregna-4,9(11),17(20)-trien-21-al (XXXVII), which is treated with Ac_2O and AcOK in hot DMF to yield 21-(acetoxy)pregna-4,9(11),16-triene-3,20-dione (XXXVIII) (9), Reaction of compound (XXXVIII) with $RhCl(PPh_3)_3$ and triethylsilane in hot dichloromethane affords 21-(acetoxy)-20-(triethylsilyloxy)pregna-4,9(11),17(20)-trien-3-one (XXXIX), which is finally oxidized with peracetic acid in toluene, quenched with SO_2 and treated with TEA (10). Scheme 7.

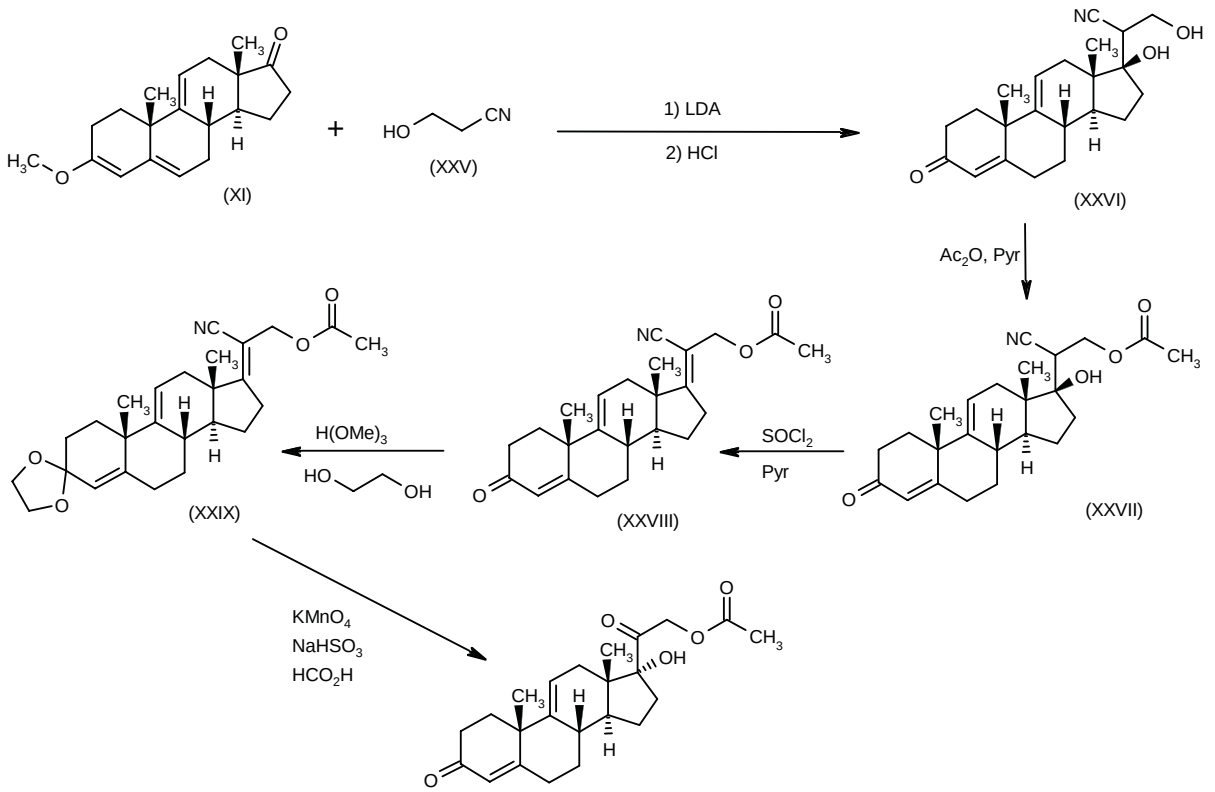
Scheme 3: Synthesis of Anecortave Acetate



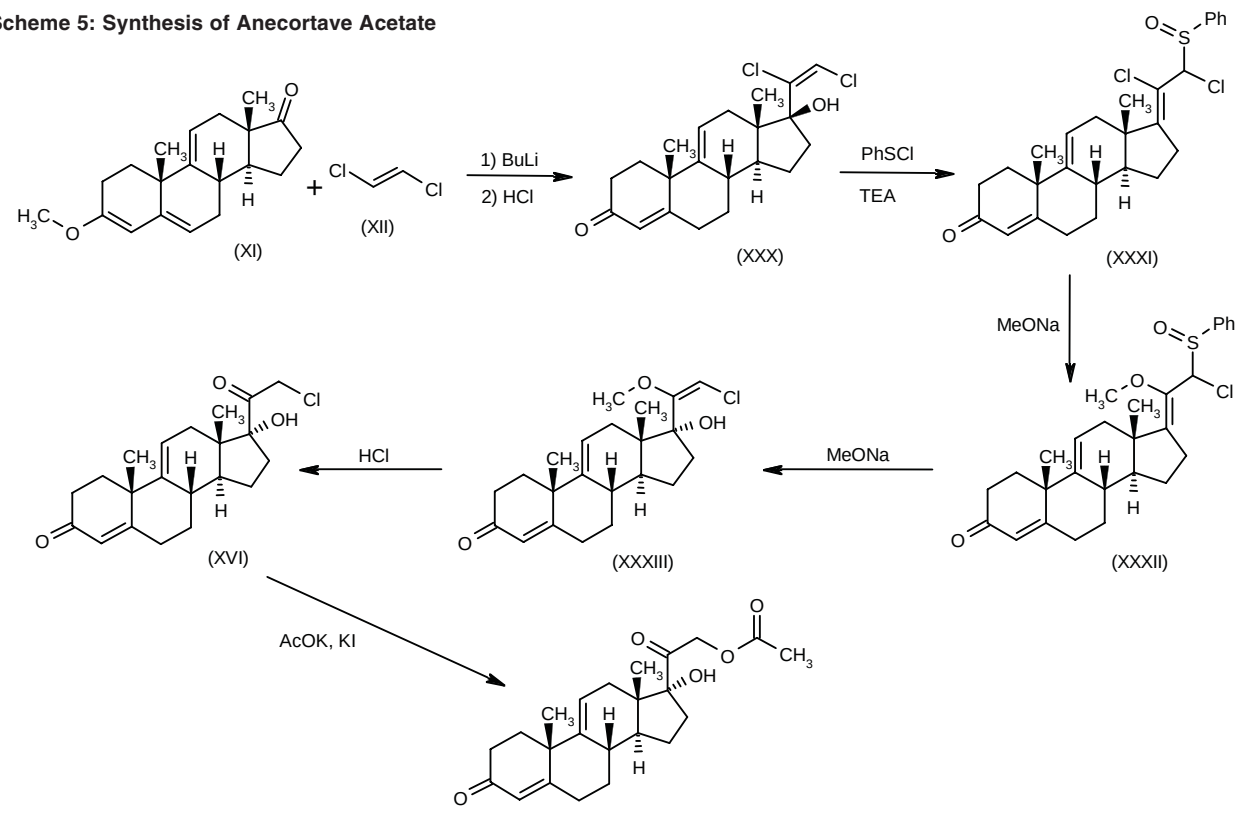
Introduction

Angiogenesis or neovascularization is a normal process involving the formation and growth of new capillaries from existing vessels. It is essential for embryonic development, female reproductive cycle, normal growth and tissue repair and is strictly modulated by angiogenic and antiangiogenic factors. Disruption of this fine control such as a constant release of stimulating factors resulting in chronic activation, results in inappropriate tissue responses leading to pathologic neovascularization. Solid tumors are dependent on angiogenesis for growth and metastasis and many chronic ocular diseases are due to chronic stimulation of angiogenesis; arthritis can also involve neovascularization (11, 12).

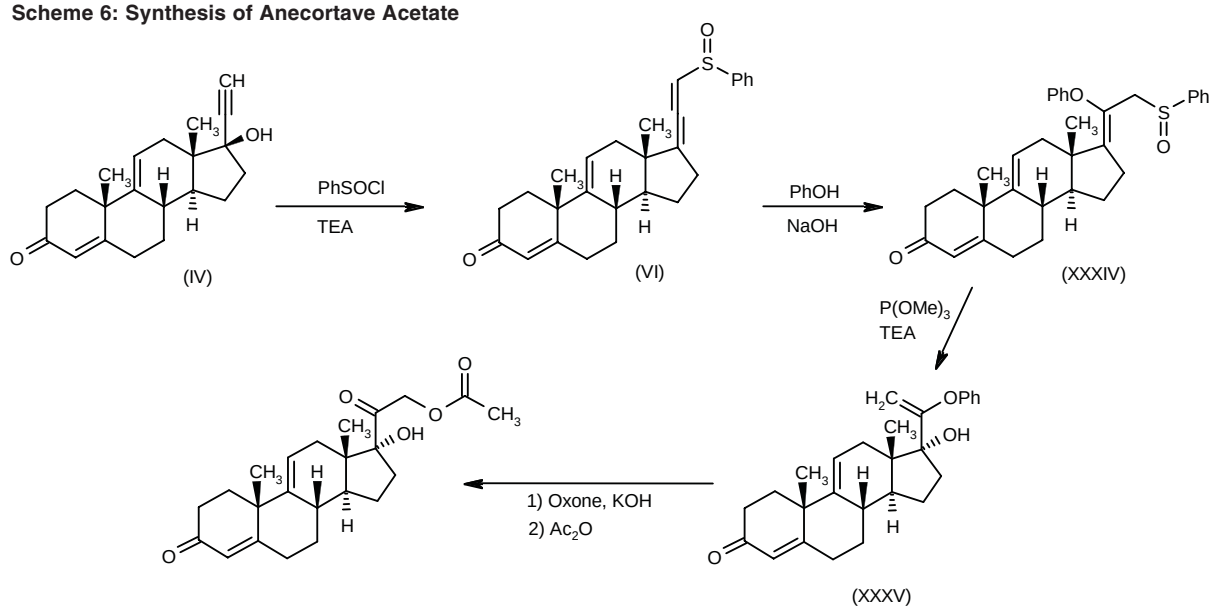
Scheme 4: Synthesis of Anecortave Acetate



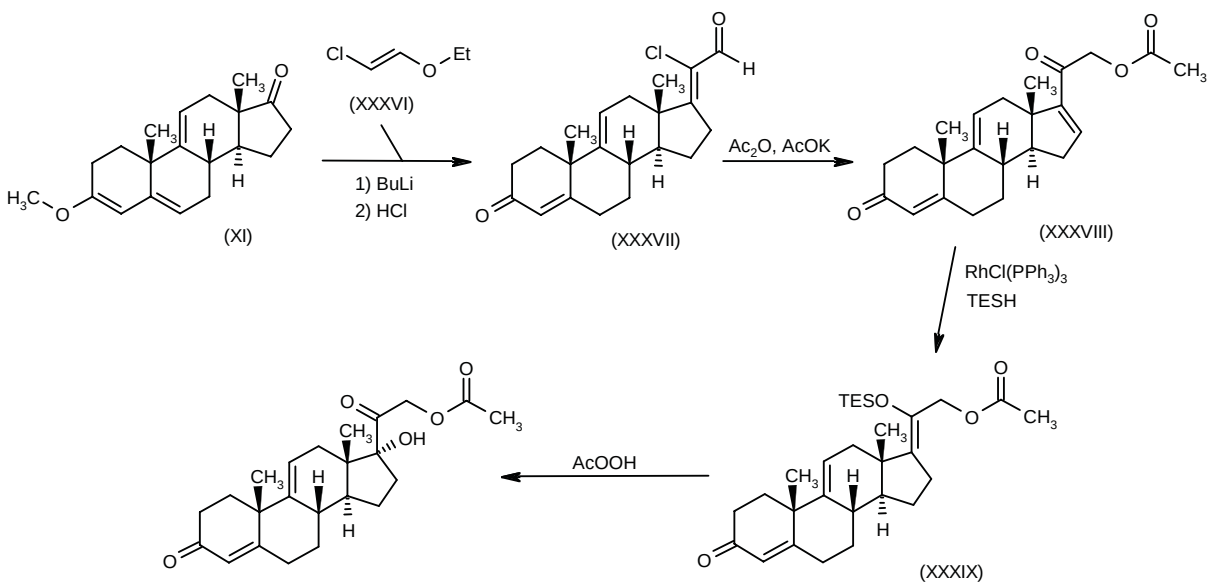
Scheme 5: Synthesis of Anecortave Acetate



Scheme 6: Synthesis of Anecortave Acetate



Scheme 7: Synthesis of Anecortave Acetate



The major causes of blindness in the world are neovascular ocular diseases such as age-related macular degeneration, proliferative diabetic retinopathy, retinopathy of prematurity (ROP), ischemia-induced retinopathy, central retinal vein occlusion, neovascular glaucoma, iritis rubeosis, cystitis, pterygium, corneal neovascularization, corneal graft rrejection and chronic inflammation (13). Effective treatment for these disorders would have to involve delay, arrest or prevention of new capillary proliferation with the absence of or presence of only minimal adverse events. Treatment options for ocular neovascular diseases to date include surgery which is not always effective, laser photocoagulation which can worsen the disorder and glucocorticoid therapy which is associated with steroid-induced ocular hypertension, posterior cataracts and delayed healing of surgical wounds. In

terms of drug therapy, several classes of antiangiogenic agents have been described. The different classes include antibiotics (*e.g.*, fumagillin analogues, minocycline, herbimycin A), polypeptides (*e.g.*, platelet factor 4, interferon-γ, angiostatin), polycations and polyanions (*e.g.*, suramin, protamine), VEGF antagonists (*e.g.*, VEGF antibodies, VEGF antisense) steroids (*e.g.*, medroxyprogesterone acetate, angiostatic steroids) and integrin antagonists (*e.g.*, antibodies to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, the cyclic peptide RDGfV) (14-21).

Of particular interest are the angiostatic steroids which were first described in 1985 by Crum and colleagues (20). These steroids have antiangiogenic activity without typical steroid activity. Thus, angiostatic steroids would lack the side effects associated with glucocorticoids and therefore are an attractive option for the treatment of ocular neovascular disease. Tetrahydrocortexolone (3α,17α,21-trihydroxy-5β-pregnan-20-one; THS), a corticosteroid metabolite with no known endogenous biological activity, and tetrahydrocortisol (3α,11α,17α,21-tetrahydroxy-5β-pregnan-20-one; THF), a metabolite of cortisol, are 2 angiostatic steroids that have shown efficacy in animal models of corneal neovascularization (21-24). However, the search for more potent angiostatic steroids continues.

One novel angiostatic steroid discovered is anecortave acetate (AL-3789). It has potent antiangiogenic activity ranging from 30-99% inhibition of new capillary growth in a number of preclinical neovascularization models. Moreover, although retaining several structural features of glucocorticoids such as a partially oxidized A-ring, 3- and 20-keto groups and 17α- and 21-hydroxyl groups, it lacks the 11β-hydroxyl group essential for glucocorticoid agonist activity and therefore is not associated with glucocorticoid-induced adverse effects. Anecortave acetate was selected for further development as a treatment for ocular neovascular diseases (25, 26).

Pharmacological Actions

The antiangiogenic efficacy of anecortave acetate and its lack of glucocorticoid activity have been demonstrated in several preclinical models of neovascularization.

An *in vitro* study using TPA-differentiated human U937 lymphoma cells examined the ability of anecortave acetate to inhibit LPS-stimulated IL-1β production, an indicator of glucocorticoid activity. In contrast to standard glucocorticoids (dexamethasone, hydrocortisone and prednisolone acetate) which resulted in inhibition of IL-1β production, anecortave acetate (up to 1 μM) showed no inhibitory activity in this antiinflammatory assay. The agent was also shown not to have antiinflammatory activity in a rat and rabbit model of endotoxin uveitis and in the carrageenan-induced rat paw edema model of inflammation. Together, these results indicate that anecortave lacks glucocorticoid activity (26).

The angiostatic activity of anecortave acetate was shown in studies using the chick embryo chorioallantoic membrane (CAM) assay and the rabbit corneal pocket model of LPS-induced neovascularization. After evaluating the activity of more than 100 steroidal compounds (10 μg; including corticosteroids, androgens, estrogens, progestins) in the CAM assay, and determining relative angiostatic factors (RAFs) for each by comparing activity to THF (RAF = 1.0), anecortave was one of the more potent agents (RAF = 1.41). In addition, anecortave acetate was the most active compound tested in inhibiting in LPS-stimulated capillary growth in the rabbit cornea (26).

The mechanism of action of anecortave acetate responsible for its angiostatic activity was investigated in in vitro experiments using cultured human vascular endothelial cells (HUVECs) and human microvascular endothelial cells (MVECs). Preliminary results indicate that the efficacy of the agent may be via inhibition of proteolysis since urokinase-type plasminogen activator (uPA) and stromelysin (MMP-3) were inhibited in HUVECs and plasminogen activator inhibitor 1 (PAI-1) activity or expression was stimulated. In addition, preliminary data suggest that anecortave acetate alters vascular endothelial cell gene expression (25, 26).

A study using a rat model of ROP has also reported that the angiostatic activity of anecortave acetate may occur via increases in PAI-1 expression. In this study, ROP was induced by placing rats at birth in varying oxygen (between 50 and 10% every 24 h)-containing atmospheres. Rats were treated with the agent in the left eye (5 μl of a 10% solution intravitreal) on day 14 (after being placed in room air) and in the right eye after 2 days in room air. The severity of abnormal retinal vascularization was significantly reduced in ROP animals treated with the agent although there was no significant difference between normal total retinal vascular area; treatment had no effect on eyes of control animals reared in room air. Moreover, anecortave acetate-treated eyes displayed a 6- to 9-fold increase in PAI-1 mRNA at 1-3 days postinjection (27).

A topical formulation (0.1 and 1% 2 or 4 drops/day for 2 weeks) of anecortave acetate was shown to be effective in inhibiting corneal neovascularization (induced by corneal implantation of a LPS-containing pellet) in rabbits by 76-100% at 1 week after the last treatment; a 0.01% formulation was ineffective (28). Anecortave acetate (0.1 and 1% at 2 weeks of glucocorticoid treatment) also successfully inhibited glucocorticoid (*i.e.*, dexamethasone acetate injections for 4 weeks)-induced ocular hypertension in rabbits. Treatment with the agent dose-dependently and significantly reduced intraocular pressure (IOP) by 50-70% (29).

Anecortave acetate was found to be effective against a highly vascularized angiogenic intraocular tumor. Topical treatment with anecortave acetate (1% t.i.d. for 28 days starting on the day of transplantation) was effective in inhibiting growth of murine uveal melanoma tumors (99E1) transplanted in athymic nude BALB/c mice so that net tumor weight on days 21 and 28 were 30-40% of the controls; tumor growth was not only significantly slower

Table I: Clinical studies of anecortave acetate (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Pterygium	Double-blind	Anecortave, 1.0% opthalm susp 1-2 drops top tid x 12 mo Placebo		Topical anecortave as an opthalmic suspension was safe, well tolerated and effective in reducing reneovascularization rates and the incidence of recurrence in patients with recurrent malignant pterygium who underwent bare-scleral surgical excision	31
Macular degeneration	Multicenter, pooled/ meta-analysis	Anecortave acetate, sub-Tenon's retrobulbar injection 1x/6 mo Visudyne photodynamic therapy → Anecortave acetate, sub-Tenon's retrobulbar injection 1x/6 mo	264	The sub-Tenon's retrobulbar injection of anecortave acetate was safe and no clinically significant safety issues were identified	34, 35
Macular degeneration	Randomized, double-blind, multicenter	Anecortave, sub-Tenon's retrobulbar injection Placebo	128	The sub-Tenon's retrobulbar injection of anecortave was safe and no significant safety issues were identified	37

but tumors did not perforate the eye. The effects of anecortave were concluded to be angiostatic since the agent (0.1 or 1 μM) had no effect on tumor cell proliferation *in vitro* (30).

Clinical Studies

The efficacy of topical anecortave acetate (1% t.i.d. for up to 20 weeks) in inhibiting glucocorticoid-induced ocular hypertension was demonstrated in a compassionate use protocol involving 15 patients (average IOP = 25 mmHg) receiving concomitant glucocorticoid therapy. Treatment with the agent decreased IOP by 12-34% during the treatment period, with reductions maintained for at least 11 weeks in most patients (29).

The efficacy and safety of topical anecortave acetate (1% ophthalmic suspension 1-2 drops t.i.d. for up to 1 year) as a treatment for recurrent pterygium was examined in a placebo-controlled, double-masked study involving patients who had undergone surgical excision of recurrent pterygium. The double-masked phase was followed by a compassionate use protocol allowing anecortave treatment for those patients who failed placebo. Treatment was concluded to be safe and well tolerated. The neovascularization rates (0.79 *vs.* 1.53 mm^2/week in placebo) and the frequency of recurrence (42.4 *vs.* 70.6% in placebo) were significantly reduced in the anecortave acetate-treated group as compared to placebo. The rate of neovascularization with anecortave acetate in the companionate use protocol was also significantly reduced in placebo-failed patients (31). The results of this study and some that follow are summarized in Table I.

The safety and efficacy of anecortave acetate administered as a sub-Tenon's retrobulbar bolus injection with or without Visudyne™ photodynamic therapy (PDT) to patients (n = 264) with predominantly classic or minimally classic subfoveal age-related macular degeneration are currently being evaluated in 2 multicenter, randomized, masked, placebo-controlled trials. The monotherapy study (C-98-03) which has enrolled and treated 128 patients, is examining 3 doses of anecortave acetate (with optional reinjections at 6-month intervals) as compared to placebo; so far, 56, 22 and 3 patients have received 2, 3 and 4 additional injections, respectively. Changes in both lesion size according to digital fluorescein and indocyanine green angiographies (evaluated by the Digital Angiography Reading Center [DARC]) and visual acuity are being compared between treatment groups. The second study (C-00-07) is a 6-month randomized study comparing the efficacy of 2 doses of anecortave acetate following up to 2 Visudyne™ PDT sessions. Of the 136 enrolled and treated patients, 115 have completed treatment. An independent safety committee comprised of 3 clinical retina specialists, an internist and medical monitor from Alcon, has been reviewing the safety data (from physical examinations and dilated ophthalmic examinations including indocyanine green and/or fluorescein angiograms) from these trials since 1998. The Committee has reviewed 400 and 180 safety events reported from a total of 149 patients from the C-98-03 and C-00-07 studies, respectively. The most common changes observed in the C-98-03 study were cataract, ptosis, decreased visual acuity, eye pain, retinal/subretinal hemorrhage, subconjunctival hemorrhage, ocular pruritus, tearing and abnormal vision. Foreign body sensation and ocular hyperemia were the most frequent changes reported in the C-00-07 study. It was concluded that none of the events were clinically significant and current study designs need not be changed (32-37).

Results at 12-months have been reported and demonstrate the efficacy of anecortave acetate (15 mg) in reducing vision loss (79 *vs.* 53% in placebo, patients who lost less than 3 lines of in vision acuity tests as compared to baseline), preventing severe vision loss and inhibiting lesion growth in the retina as compared to placebo in patients with the wet form of age-related macular degeneration. Even greater clinical efficacy was observed (84 *vs.* 50% in placebo, patients who lost less than 3 lines in

vision acuity tests as compared to baseline) in a subgroup of patients with classic lesions and treated with the agent (38).

A multicenter phase III trial (C-01-99) had been initiated involving approximately 500 patients with predominantly classic wet age-related macular degeneration. The randomized, masked, controlled trial will compare the efficacy of anecortave acetate to Visudyne™ PDT (39).

Source

Alcon Laboratories, Inc. (US).

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