CHEMICAL REVIEWS

Review

Synthesis and Pharmacology of Anti-Inflammatory Steroidal Antedrugs

M. Omar F. Khan, and Henry J. Lee

Chem. Rev., **2008**, 108 (12), 5131-5145 • DOI: 10.1021/cr068203e • Publication Date (Web): 26 November 2008

Synthesis and Pharmacology of Anti-Inflammatory Steroidal Antedrugs

M. Omar F. Khan*,† and Henry J. Lee‡

College of Pharmacy, Southwestern Oklahoma State University, 100 Campus Drive, Weatherford, Oklahoma 73096, and College of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee, Florida 32307

Received June 7, 2007

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1. Introduction

The purpose of this review is to give a comprehensive understanding about anti-inflammatory steroidal antedrugs. The concept of antedrug first came into light in 1982, and since then, a few academic institutions and some pharmaceutical industries worldwide are performing active research in this avenue in search of safer therapeutic agents. Although the concept primarily focused on the discovery of safer topical anti-inflammatory steroids, the scope has broadened involving other therapeutic drug classes as well. A recent review has outlined briefly all different currently available approaches of antedrug discovery. Because of the increasing interests on antedrug approach and vastness of steroid chemistry, the focuses of the present review have been concentrated on anti-inflammatory steroidal antedrugs. The research efforts since the 1980s in the chemical synthesis and pharmacological actions of the steroidal antedrugs have dictated the breadth of this article. For the sake of completeness of information and consistency, historical materials and references to earlier relevant works have also been cited. After a brief introduction about the glucocorticoid therapy, especially their clinical applications and deleterious shortcomings and earlier medicinal chemical efforts to overcome those shortcomings, the antedrug approach is introduced outlining the rationale, scopes, and successes. Borderline between the other related concepts such as prodrug has also been explained. The synthetic approaches of all chemical classes of anti-inflammatory steroidal antedrugs and their pharmacological activities have been the main emphasis, and a section has also been devoted to pro-antedrugs. Finally, to reflect the scopes and future of the concept of antedrug, drugs in clinical use or in pipeline that are developed based on this concept have been placed before the concluding remarks.

1.1. Introduction to Glucocorticoid Therapy

The beneficial effects of glucocorticoids in the treatment of chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, and autoimmune disorders have been appreciated for over 50 years but not without serious complications, which have imposed limitations on the clinical use of this class of drugs.[1,2] Suppression on the hypothalamic-pituitary-adrenal (HPA) axis, the immune system, aggravation of diabetes, hypertension, osteoporosis, and retardation of growth in children are a few of the most deleterious side effects.[3−7] A considerable research effort has been devoted to the structural modifications of glucocorticoids, with a hope of increasing their potencies while minimizing their propensity to elicit systemic adverse effects, and some success has been evident in producing potent glucocorticoids with minimum salt-retaining activity.[8−11] The introduction of fluorine at the 9α-position of the natural hydrocortisone (**1**) increased the binding affinity to glucocorticoid (GC) receptors and retarded the oxidation of the proximal 11-OH group, for example,

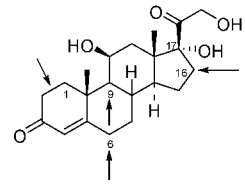
* To whom correspondence should be addressed. Tel: (1)-580-774-3064. Fax: (1)-680-774-7020. E-mail: faruk.khan@swosu.edu.
† Southwestern Oklahoma State University.
‡ Florida A & M University.

10.1021/cr068203e CCC: $71.00
Published on Web 11/26/2008

M. O. Faruk Khan joined the College of Pharmacy in January 2007 as an Assistant Professor of Medicinal Chemistry. He received his Bachelor of Pharmacy (Honors) (1989) and Master of Pharmacy (1993) Degrees from the University of Dhaka, Bangladesh and then taught there for about two years (1993−1995) as a faculty member before attending the University of Manchester, UK for completing his PhD in Medicinal Chemistry under the Commonwealth Scholarship program. On completion of his PhD (1999), he resumed his position in the University of Dhaka and was promoted to Assistant Professor. Later, he was a postdoctoral fellow at Hiroshima University, Japan (2001−2002), University of Mississippi, Oxford, MS (2002−2004), and Florida A&M University, Tallahassee, FL (2004−2006). He published about 40 peer-reviewed articles in various areas including antiparasitic drug discovery, anti-inflammatory steroidal antedrug design, and drug discovery from natural products including several review articles and book chapters. Currently he is pursuing active funded research, both independently and in collaboration, in these areas and also serves as a reviewer and editorial board member of several peer-reviewed journals.

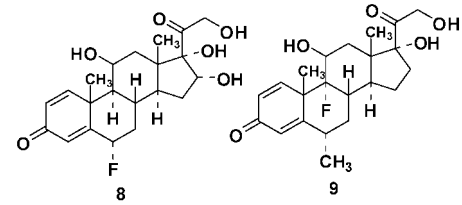
Henry Joung Lee came to Florida A&M University in 1973 as an assistant professor in the College of Pharmacy and Pharmaceutical Sciences. Since 1982, he has been a professor in the graduate program and head of the Center for Anti-inflammatory Research. In the summer of 1975, Lee was a visiting scientist at Mt. Sinai School of Medicine in New York, NY, and in the fall 1986, he was visiting professor at the University de Geneva, Geneva, Switzerland. In 1973, he completed postdoctoral training in steroidal chemistry at the Mt. Sinai School of Medicine in New York. Lee received the PhD degree (1971) in biochemistry from Oklahoma State University, Stillwater, OK, the M.S. degree (1966) also in biochemistry from Seoul National University, Seoul, Korea, and the B.S. degree (1964) in agricultural chemistry also from Seoul National University. Lee's research involves the discovery of safer and yet potent drugs based on his new concept called "antedrug" (*Science*, **1982**, *215*, 989−991). He has been working on the synthesis and evaluation of mainly three therapeutic classes of agents, i.e., anti-inflammatory steroids, anti-AIDS, and anticancer agents.

fludrocortisone (**2**). The introduction of a Δ^1-double bond, as in prednisolone (**3**), led to increased potency with reduced salt retaining activity. The incorporation of C6α-methyl, to prevent hydroxylation at this position as in 6α-methylprednisolone (**4**), increased both potency and duration of action. The incorporation of 16-methyl, in addition to the 9α-flouro and Δ^1-double bond, resulted in an increase in anti-inflammatory potency by about 25 folds, for example, dexamethasone (**6**) and betamethasone (**7**).

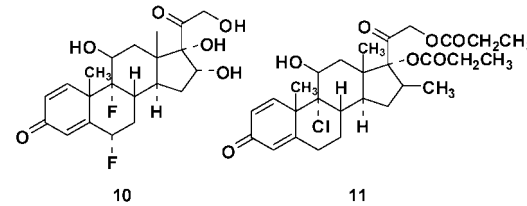


Drugs	Functional groups			
	Δ^1	C6	C9	C16
Hydrocortisone (**1**)	-	H	H	H
Fludrocortisone (**2**)	-	H	F	H
Prednisolone (**3**)	+	H	H	H
6 -Methylprednisolone (**4**)	+	CH_3	H	H
Triamcinolone (**5**)	+	H	F	OH
Dexamethasone (**6**)	+	H	F	CH_3
Betamethasone (**7**)	+	H	F	CH_3

The main challenge or shortcoming with GC therapy, however, has been in the separation of anti-inflammatory effects on target tissues or organs from systemic glucocorticoid effects, which are largely inherent in the nature of steroids themselves. Not only do they possess multiple biological activities, but structural requirements for various activities seem to be overlapping and inseparable.[1−5] Furthermore, GC receptors are present in virtually all tissues, and these receptors appear to be similar or identical on the basis of the relative affinities of GC ligands for these receptors. The introduction of C16,C17-acetonides[12] and esterification at C17- and/or C21-OH groups that increased lipophilicity of glucocorticoids proved to be useful for topical application.[13,14] Other changes more significant from the chemistry viewpoint rather than pharmacotherapeutic viewpoint included the replacement of C21- and C11-OH with chlorine and incorporation of fused phenylpyrazole ring at C2 and C3 or fused oxazole ring at C16 and C17.[15,16] The relatively newer potent anti-inflammatory agents like flurandrenolone (**8**), fluorometholone (**9**), and flucinolone (**10**) are used only topically for the treatment of psoriasis due to their systemic toxicities.



8 **9**



10 **11**

1.2. Concept of Antedrug

The introduction of beclomethasone dipropionate (**11**) in topical treatment of asthma by Glaxo Wellcome was a revolutionary discovery by Barnes et al.[17] Local delivery of the drug to the lungs allowed the dose to be greatly reduced, thereby dramatic reduction of undesirable systemic side effects. Later in early 1980s, several observations served as guidelines in developing the new concept of antedrug: (i) corticoid pharmacotherapy appears to offer an abundance of agents, but no truly safe drug, (ii) systemic manifestations

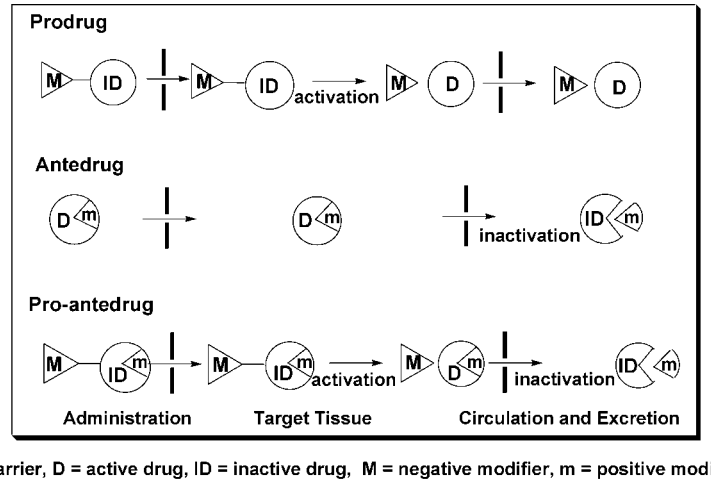


Figure 1. (I) Prodrug, needing bioactivation to remove the negative modifier (M). (II) Antedrug, the active drug, needing metabolic inactivation by removing the positive modifier to eliminate or minimize unwanted systemic effects. (III) Pro-antedrug, contains both negative and positive modifiers where the negative modifier is removed first at the site of action (as in prodrug) and the positive modifier is removed later when reach systemic circulation after its activity (as in antedrug). Adapted with permission from Ref 31. Copyright 2005 Bentham Science Publishers Ltd.

of steroids are unnecessary complications which accompany treatment of many inflammatory conditions, (iii) an intact ketol chain is not an absolute requirement for the anti-inflammatory activity of glucocorticoids,[18−21] and (iv) steroid acid esters with intact structures of potent glucocorticoids retain anti-inflammatory activity of the parent compound, but upon entry into the systemic circulation are hydrolyzed to inactive readily excretable steroid acids.[13,22] These factors led to the development of the concept of antedrug (Figure 1).

The antedrug concept was introduced by Lee and Soliman in 1982 in designing potent, yet safer locally active anti-inflammatory steroids.[18] Antedrug is defined as an active synthetic derivative that is designed to undergo biotransformation to the readily excretable inactive form upon entry in the systemic circulation, thus minimizing systemic side effects they are increasing the therapeutic indices. A hybrid of prodrug and antedrug concepts came out as the pro-antedrug.[23] The other concept diametric to antedrug is the prodrug coined by Albert in 1958,[24] which is defined as an inactive compound that undergoes metabolic transformation *in vivo* to furnish the active drug. The objectives of prodrugs are to increase therapeutic indices by the (a) alteration of the physicochemical properties of drug e.g., solubility, stability, *in vivo* bioavailability and other pharmacokinetic properties and (b) alteration of pharmacodynamic profiles so as to increase duration of pharmacological effects (for review, see ref 25). These three concepts are illustrated in Figure 1. Incorporation of a metabolically labile functional group into the active molecule gives rise to the antedrug, which is active at the application site, and on entering into the systemic circulation is quickly metabolized to inactive molecule and eliminated; in this way, it eliminates or reduces the systemic side effects. Since its first introduction, the antedrug concept is well accepted by the Pharmaceutical industries like Glaxo Wellcome Inc., who is actively pursuing development of steroidal anti-inflammatory antedrug for treatment of bronchial asthma,[26−29] and Johnson & Johnson, who is developing cytokine-inhibiting antedrugs for treatment of asthma.[30] Later, other steroidal and nonsteroidal antedrugs have also been developed (reviewed in ref 31).

2. Synthesis of Anti-Inflammatory Steroidal Antedrugs

2.1. Carboxylic Esters and Amides

2.1.1. 21-Carboxylate Esters and Amides

Prolonged oxidation (1 week) of prednisolone (3) with methanolic cupric acetate applying the procedure of Lewbart and Mattox[32] and Monder et al.[33] furnished two epimers **12α** and **12*β*** which were separated using semipreparative HPLC. The treatment of compounds **12α** and **12*β*** with acetone in the presence of catalytic amount of perchloric acid gave the corresponding acetonides **13α** and **13*β*** (Scheme 1).[22]

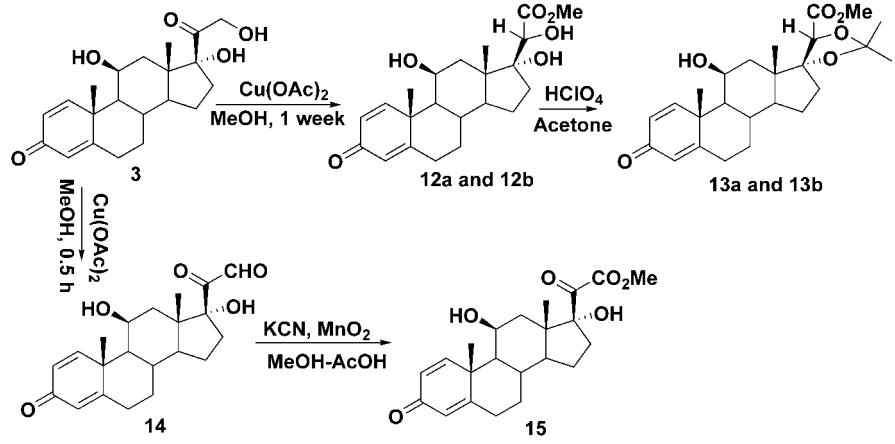
Mild oxidation of prednisolone with methanolic cupric acetate for a short period (0.5 h) gave corresponding aldehydes (**14**), which on further oxidation with KCN and MnO_2 in a mixture of methanol and acetic acid gave corresponding methyl esters (**15**).[22] This reaction is assumed to proceed through a cyanohydrin that is oxidized to an α-ketonitrile, which is then converted into methyl ester (**15**). Treatment of prednisolone with triethylorthobenzoate in presence of *p*-TsOH, resulted a cyclic orthobenzoate, which on hydrolysis gave prednisolone-17α-benzoate (**16**). Mild oxidation of its 21-alcoholic function furnished the aldehyde (**17**) as above. Interestingly, the treatment of **17** with KCN and MnO_2 in a mixture of methanol and acetic acid furnished the 17-deoxy-21-carboxylate methyl ester. It is suggested that this reaction possibly proceeds through the formation of cyanohydrin, which is enolized to yield *bis*-enol followed by *β*-elimination of benzoate to give 17-deoxy-α-ketonitrile. This keto nitrile is converted to the corresponding 17-deoxy-21-carboxylate methyl ester (**18**) in a similar fashion as described above (Scheme 2).[34]

The hydrolysis and then DCC coupling of the methyl ester **12α** and **12*β*** with amines furnished the 21-amides **20α−23α** and **20*β*−23*β*** as shown in Scheme 3.[35]

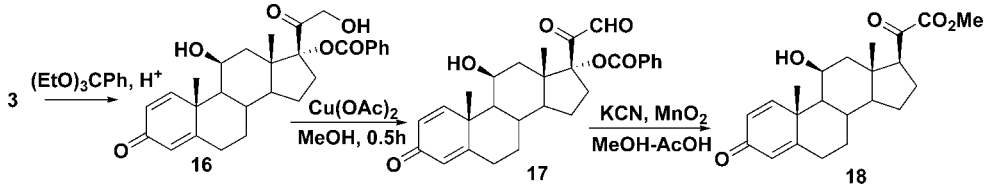
2.1.2. 16-Carboxylate Esters

The synthesis of 16-carboxylate ester was possible with the discovery of the key intermediates, 16,17-unsaturated corticosteroids. Generally, the 16,17-double bond is intro-

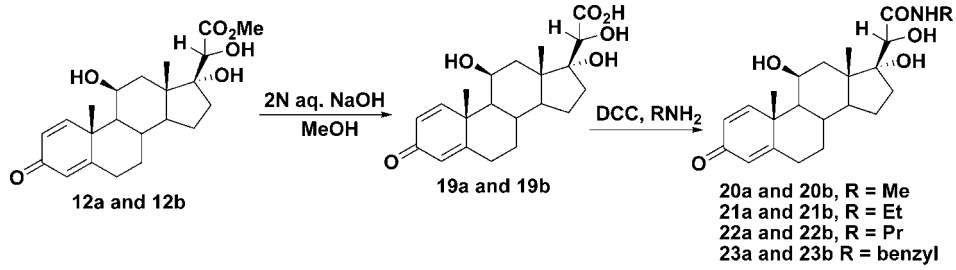
Scheme 1. Synthesis of 20-Carboxylate Esters



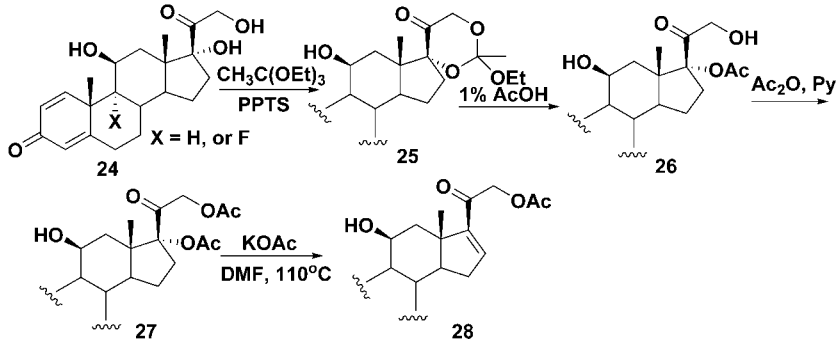
Scheme 2. Synthesis of 17-Dehydro-21-Carboxylate Ester



Scheme 3. Synthesis of 21-Carboxylic Amides



Scheme 4. Synthesis of 16,17-Unsaturated Corticosteroid by Route 1



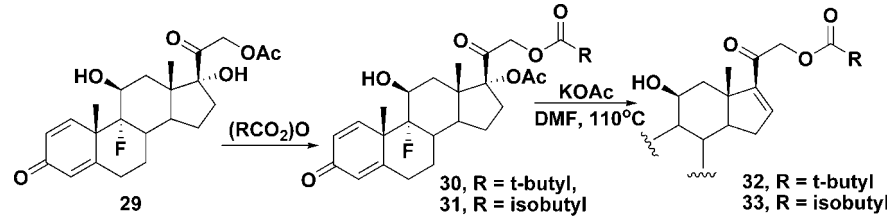
duced by the elimination of 17-acyloxy group.[36,37] Acylation of 17α-OH involves the synthesis of 17α,21-orthoester (**25**), which on hydrolysis yields the desired 17α-acetate (**26**), in addition to some 21-acetate side product. It is subsequently separated and esterified at 21-OH to furnish **27**. Heating the DMF solution of **27** with KOAc effects the deacylation furnishing the desired olefins (**28**) (Scheme 4).[37−40]

Relatively simpler and direct procedure for the synthesis of 16,17-unsaturated glucocorticoid was reported by Ramesh et al.,[41] which is shown in Scheme 5. This approach took the advantage of the steric hindrance of 11β-OH and used bulkier reagent to esterify the 17α-OH. Thus reaction of 9-fluoroprednisolone-21-acetate (**29**) with pivalic acid and isobutyric anhydrides at 150 °C gave diesters **30** and **31**, respectively as sole products that undergo deacylation smoothly under standard conditions to give corresponding olefins (**32** and **33**).

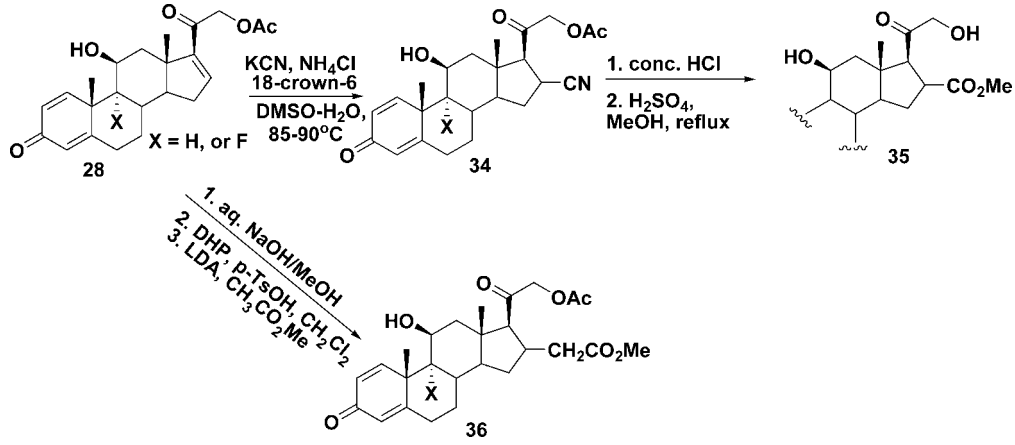
The addition of KCN to the 16,17-unsaturated glucocorticoids (**28**) afforded 16-nitrile (**34**), which on hydrolysis followed by esterification of the acid product (**35**) gave 16-ester **36** (Scheme 6).[42] Hydrolysis of the 21-acetate group of **28**, protection of 21-OH as THP ether, and subsequent 1,4-addition of lithiomethyl acetate enolate afforded steroid 16-ester **36**.[43]

To synthesize the 17-hydroxy-16-carboxyesters, the corresponding 16-nitrile is the crucial intermediate, synthesis of which is one of the most challenging steps. Formerly it had been prepared with triethylamine from isoxazoline

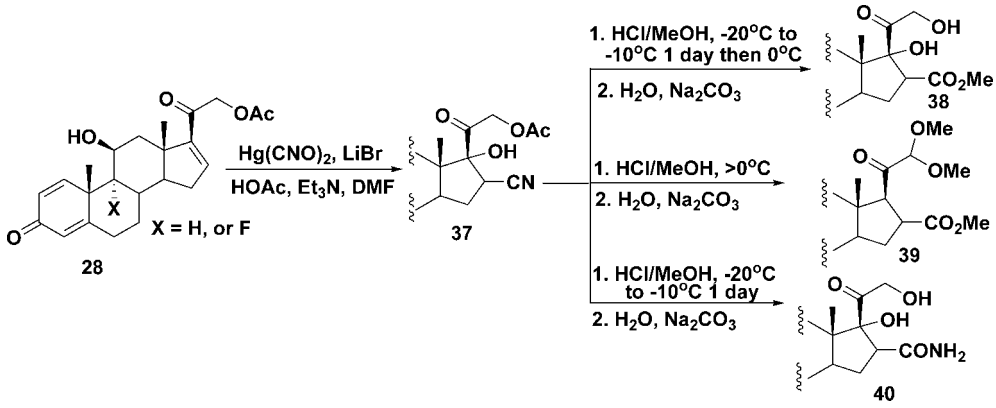
Scheme 5. Synthesis of 16,17-Unsaturated Corticosteroid by Route 2



Scheme 6. Synthesis of 16-Carboxylate Esters



Scheme 7. Synthesis of 17-Hydroxy-16-Carboxyesters



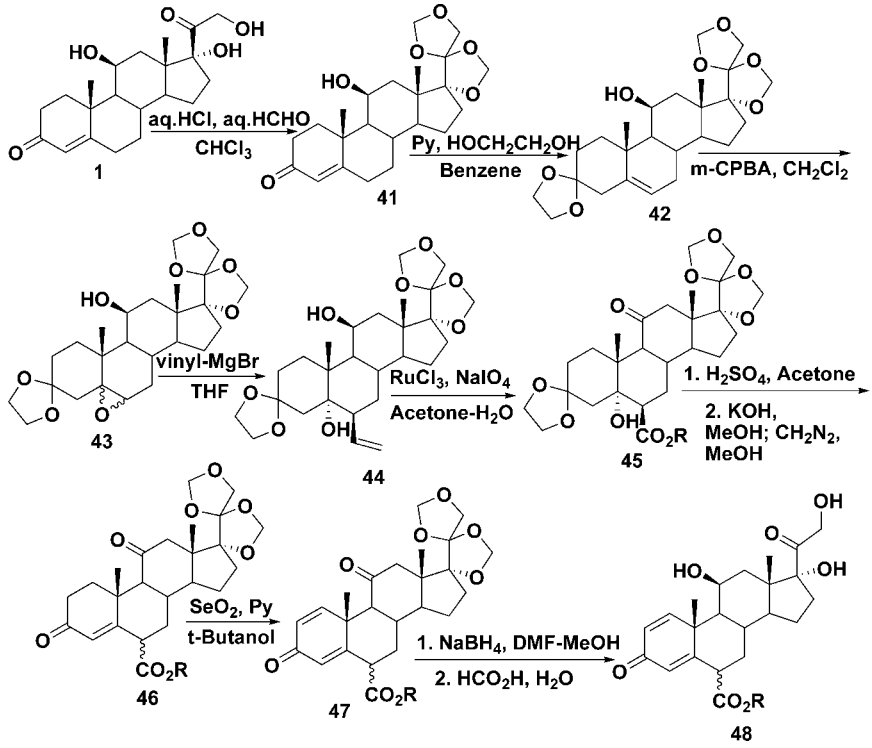
intermediate which in turn obtained by 1,3-dipolar cycloaddition of fulminic acid (HCNO) to the olefin **28**, where an oxime always remained as significant side product.[44] The reaction was later modified to effect a one step conversion of highly dipolariphilic olefin (**28**) with metal fulminate to the corresponding α-hydroxy-*β*-cyano adduct (**37**) (Scheme 7).[45]

In this procedure, mercuric fulminate, lithium bromide, acetic acid, and triethylamine at optimum ratios were used, with DMF as the superior solvent, and the reaction condition was strictly maintained at 50 °C to get better yield (72%) after purification. The methanolysis of the 16-cyanoprednisolone derivative (**37**) to convert it into 16-methylcarboxylate (**38**) needs careful thermodynamic control of the reaction to suppress the Mattox rearrangement[46,47] leading to **39**, and/or the side reaction leading to **40**.[48] The methanolic solution of **37** is saturated with HCl gas at −20 °C and maintained the temperature at −10 °C overnight. The temperature of the reaction mixture is then elevated slowly to 0 °C over 5 h to force the reaction to completion, before pouring it into water and neutralizing with sodium bicarbonate powder. Pouring the reaction mixture in water for hydrolysis to occur at the final stage of reaction at low temperature (−20 to −10 °C) leads to the formation of amide **40** as major product, whereas allowing the temperature to be >0 °C before pouring it into water causes Mattox rearrangement, furnishing compound **39** as major product. A careful balance of temperature is important to yield the desired methyl ester (**38**) at higher yield.[48]

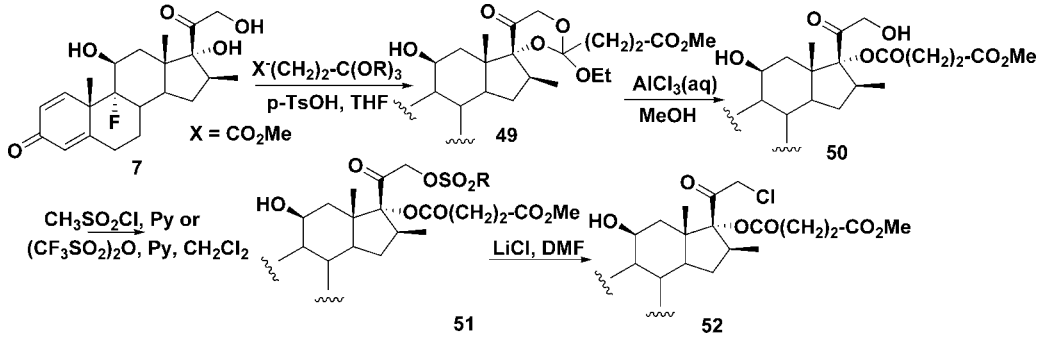
2.1.3. 6-Carboxylate Esters

Scheme 8[49] shows the 10-step synthesis of prednisolone-6-carboxylate ester (**48**) from hydrocortisone (**1**). The 21-OH and 17-OH groups of hydrocortisone were protected as bismethylene dioxyether (**41**) by treating with formaldehyde and conc. HCl in $CHCl_3$ and the 3-keto function was converted to corresponding cyclic ketal, with the spontaneous shift of the 4,5-double bond to the 5,6-position giving **42**. Oxidation of the olefinic bond by *m*-chloroperbenzoic acid gave epoxide **43** as a 1:1 mixture of α- and *β*-isomers. Nucleophilic ring opening of α-epoxide with vinyl magnesium bromide provided alcohol **44**, which by $RhCl_3-NaIO_4$ (2:1 in acetone−water)-catalyzed oxidation of terminal alkene and concomitant oxidation of 11-OH to ketone, provided carboxylic acid **45**. Esterification of the product

Scheme 8. Synthesis of Prednisolone 6-Carboxylate Ester



Scheme 9. Synthesis of 17α-Ester Containing a Functional Group

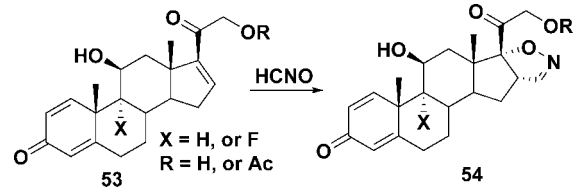


(**45**) with diazomethane in methanol yielded the methyl ester. Deprotection of cyclic ketal with 2N H_2SO_4 in acetone, then dehydration at 5α-OH in methanolic KOH followed by reesterification of the hydrolyzed 6-carboxylate gave 2:1 mixture of **46α** and **46*β***. SeO_2-catalyzed oxidation at 1,2-position afforded **47**, which on reduction at 11-ketone with $NaBH_4$ gave 11*β*-OH and subsequent deprotection with aq. formic acid gave 3:1 inseparable mixture of prednisolone 6-carboxylate derivatives **48α** and **48*β***.[49]

2.1.4. 17α-Esters Containing a Terminal Methoxycarbonyl Functional Group

On the basis of the antedrug principle, a series of 21-desoxy-21-chloro-glucocorticoids containing a functionalized ester group at 17α for topical anti-inflammatory therapy were synthesized by Ueno et al.[50] Introduction of the functionalized ester group at 17α of commercially available betamethasone (**7**) was carried out by an acid catalyzed formation of cyclic *ortho* ester (**49**) with 17α,21-OH groups of betamethasone and subsequent Lewis-acid-catalyzed hydrolysis to afford **50**. The methoxycarbonyl functional group was introduced at the terminal carbon atom of the 17α-alkanoate group. The 21-OH group was transformed into a mesylate (or triflate) (**51**), an excellent leaving group, which was treated with LiCl in DMF to give 21-desoxy-21-chloro- derivative (**52**) (Scheme 9).[50]

Scheme 10. Synthesis of Isoxazoline Derivatives



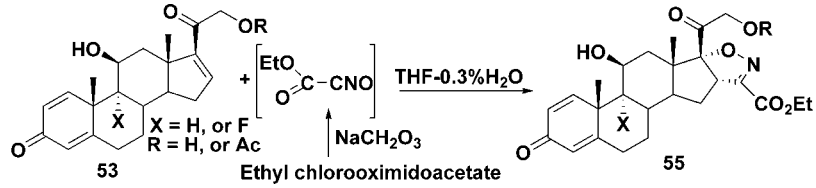
2.2. Ring-Fused Heterocyclo-Steroidal Antedrugs

2.2.1. Ring-Fused Isoxazolines and Oxime Derivatives

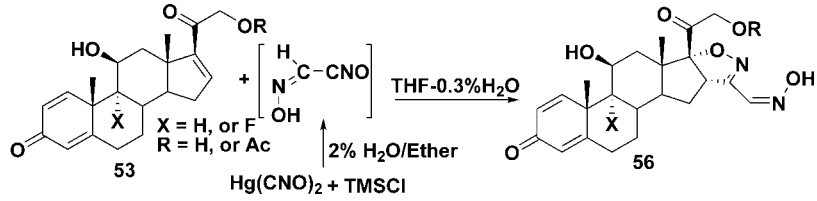
The novel 16α,17α-d-isoxazoline derivatives of anti-inflammatory steroids (**54**) were synthesized via 1,3-dipolar cycloaddition of fulminic acid to the corresponding olefins (**53**). The fulminic acid was prepared from mercuric fulminate and bromotrimethyl selane in anhydrous ether under nitrogen. The reaction is highly regio- and stereoselective, and no isomer was detected (Scheme 10).[44,51]

The steroid, 3′-ethoxycarbonyl-[16α,17α-d]isoxazolino-prednisolone derivatives (**55**) were prepared by 1,3-dipolar

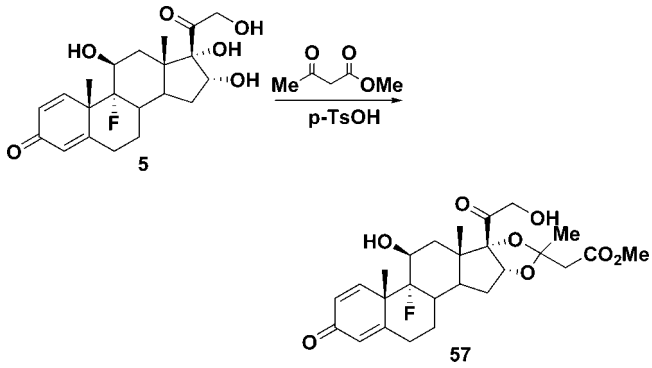
Scheme 11. Synthesis of Carbethoxyisoxazolines



Scheme 12. Synthesis of Oxime Derivatives



Scheme 13. Synthesis of Ketal Derivatives



cyclization of carbethoxyformonitrile oxide (CEFNO) to α,β-unsaturated enone (**53**). CEFNO was *in situ* generated by the treatment of ethyl chlorooximidoacetate with sodium bicarbonate. Using the 21-acylated steroids furnished the acylated products (Scheme 11).[52]

The efforts were continued leading to the development of the series of hydroxyiminoformyl isoxazoline and their 21-acetate derivatives (**56**). A similar method as in Scheme 11 was employed. Slow *in situ* generation of fulminic acid in THF-H_2O in absence of a dipolarophile yielded a trimer and a tetramer via the unstable intermediates. Quenching the reactive intermediates by a dipolarophile competing with the cyclization process would lead to a corresponding cycloadduct. Therefore the optimization of temperature and reaction time to trap the fulminic acid dimer is vital to obtain a high yield of the target compounds (**52**). 1,3-Dipolar cycloaddition of fulminic acid dimer at 4 °C to the olefins (**53**) afforded the corresponding oxime derivatives (**56**) along with several minor side products, which are not shown (Scheme 12).[53]

2.2.2. Ring-Fused Ketal Derivatives

The ketalization of triamcinolone (**5**) with methyl acetoacetate a catalytic amount of perchloric acid afforded (22*R*)-9α-fluoro-11β,21-dihydroxy-3,20-dioxo-16α,17α-(methyl, methoxycarbonylmethyl)-methylenedioxy-1,4-pregnadiene (**57**) (Scheme 13).[54]

2.3. Spiro Enone Derivatives

Condensation of prednisolone and its derivatives (**58**) containing free 21- and 17-OH groups with diethyl oxalate using NaH in benzene yielded novel spiro enones (**59**) as the major product (37%) along with the aldehyde (**60**) and other undetected minor side products (Scheme 14).[55] Molecular modeling studies with this group of compounds suggested that the spiro enones adopt rigid planner geometry with the ester group in the plane.

2.4. 20-Thioester Derivatives

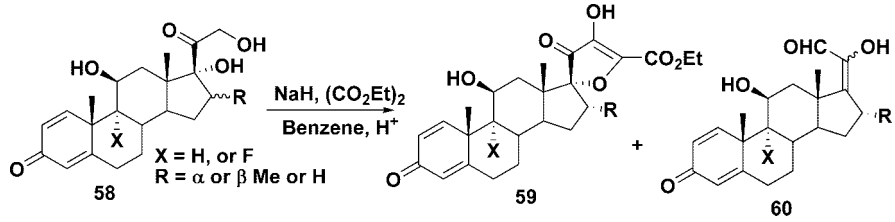
The search for safer steroids based on antedrug concept focused its primary attention toward the plasma labile carboxylic esters. Because the esterase is ubiquitous, which is present even in lungs, the attention was also extended toward the synthesis and study of thioester derivatives. Milioni et al. first reported such an attempt by synthesizing series of local anti-inflammatory thiosteroid antedrugs.[56] The most striking example of the thiosteroid is the clinically used drug fluticasone propionate (**65**).[57] The synthetic route of fluticasone propionate is shown in Scheme 15.[58] The oxidative cleavage of 21-hydroxypregnane-20-one derivative (**61**) with periodic acid in aqueous dioxane or THF furnished 17α-hydroxyandrostane-17β-carboxylic acid intermediate (**62**). The reaction of carboxylic acid and 1,1′-carbonyldiimidazole (CDI) to give thioesters reported by Gais[59] and applied by Keresz and Marx[60] in the synthesis of alkylandrostane-17β-carbothioates was employed by Phillipps et al.[58] to synthesize the carbothioic acid derivative (**63**), which was readily and selectively 17α-acylated without concomitant 11β-acylation to yield **64**. Fuloromethyl carbothioate (**65**) was prepared from carbothioate salt by alkylation with bromofluoromethane or fluoroiodomethane in dimethylacetamide.

2.5. γ-Butyrolactone Derivatives

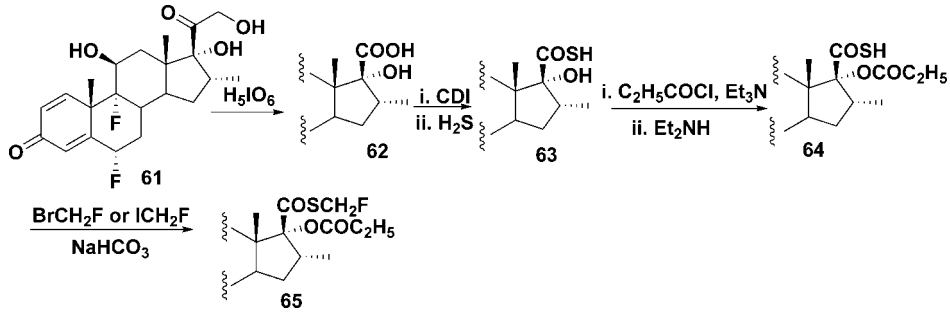
The incorporation of γ-butyrolactones onto the glucocorticoid nucleus has been the most recent approach in the search for better steroidal antedrugs for treatment of asthma at Glaxo Wellcome.[26−29,61] The practical approach to the synthesis of glucocorticoid antedrug **GR250495X** (**70**) has been outlined in Scheme 16.[28]

The most convenient starting material is the commercially available fluocinolone acetonide (**66**). The sequential transformations included: (i) selective reduction of Δ^1 olefin in presence of 5 mol % of Wilkinson′s catalyst in EtOH at 50 °C to yield **67**, (ii) oxidation at C20 with aq. H_2O_2 and K_2CO_3 in MeOH at room temperature cleanly afforded the 1,2-dihydro acid **68**, (iii) selective conversion of the 16,17-acetonide to the 16,17 (*R*-butylidenedioxy) group by acetalization of **68** with butyraldehyde in the presence of

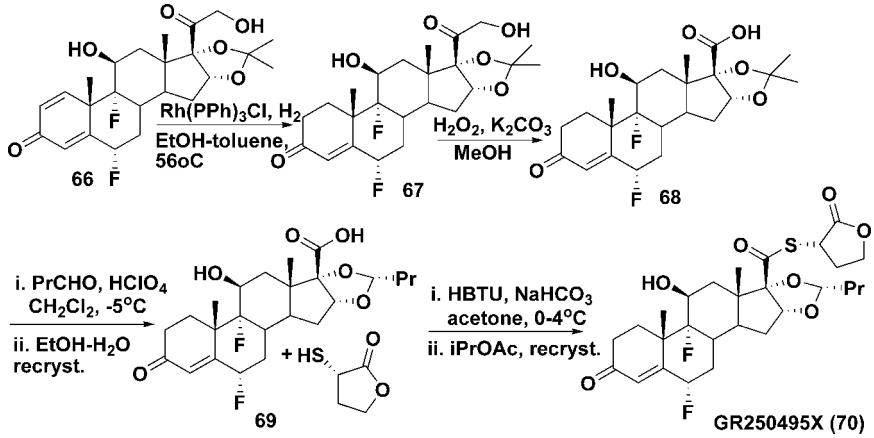
Scheme 14. Synthesis of the Spiro Enone Derivatives



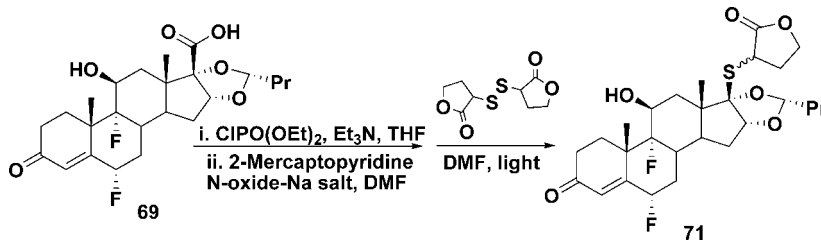
Scheme 15. Synthesis of Fluticasone Propionate



Scheme 16. Synthesis of GR250495X



Scheme 17. Synthesis of 17β-(γ-Butyrolactone) Derivative

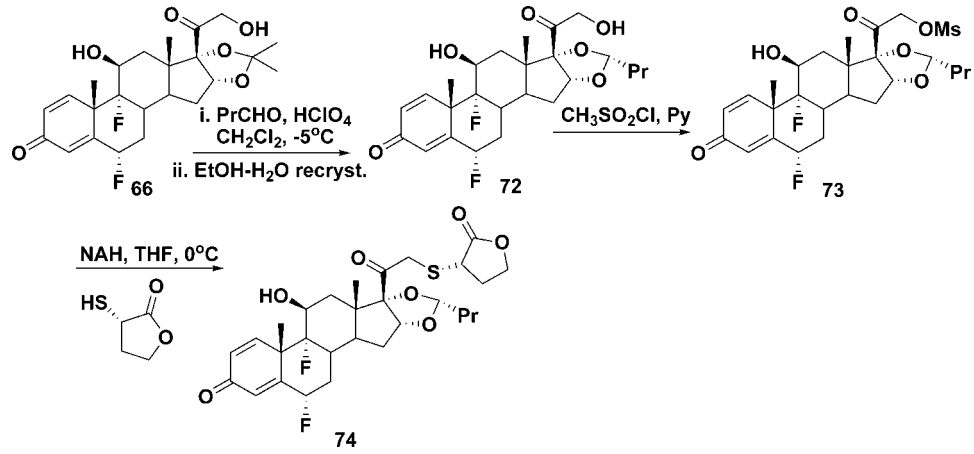


catalytic perchloric acid in CH_2Cl_2 at −5 °C gave the desired (21*R*)-acetal **69** as the major diastereomer (10:1 R/S ratio), recrystallization of which from EtOH-H_2O removed the undesired diastereomer, affording the desired (21*R*)-acetal (**69**) in 65% overall yield, (iv) stereoselective incorporation of (*S*)-2-mercapto-γ-butyrolacton was accomplished by first activating the COOH group of **69** with HBTU in presence of excess solid $NaHCO_3$ in acetone, which was then coupled cleanly with (^{2}S)-mercapto-γ-butyrolactone at 0−4 °C with minimal detectable epimerization during the reaction. Crystallization of the crude product from reaction mixture, then recrystallization from iPrOAc-heptane provided the drug candidate **GR250495X** (**70**) in 72% recovery.[28]

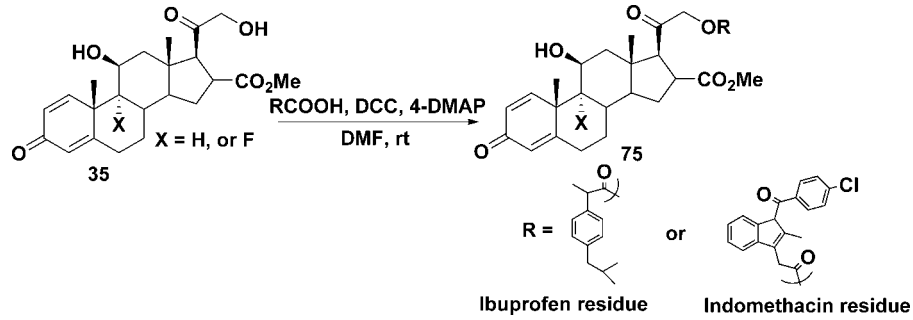
Conversion of the acid **69** to the corresponding mixed anhydride followed by Barton ester formation and then decarboxylation in presence of butyrolactone disulfide gave 1:1 diastereomeric mixture of γ-lactone sulfide **71** which were separated by HPLC (Scheme 17).[29]

Novel glucocorticoid antedrugs possessing 21-(γ-butyrolactone) ring (e.g., **74**) was prepared in 1:1 diastereomeric mixture by displacement of the 21-methanesulfonate with α-mercapto-γ-butyrolactone, which were separated by reverse phase preparative HPLC (Scheme 18). The butylidene (**72**) was prepared as described in Scheme 16. The reaction with methansulfonyl chloride in pyridine gave the methane sulfonate **73** which was reacted with the thiol in presence of NaH in THF at 0 °C yielded the desired product (**74**) after HPLC separation of the isomeric mixture.[61] A series of γ-butyrolactones fused at the 16β,17β position of 9α-fluoro11β-hydroxy-3-oxandrosta-1,4-diene nucleus and possessing oxygen substituents at 16α,17α positions had also been synthesized by Biggadike et al.[27]

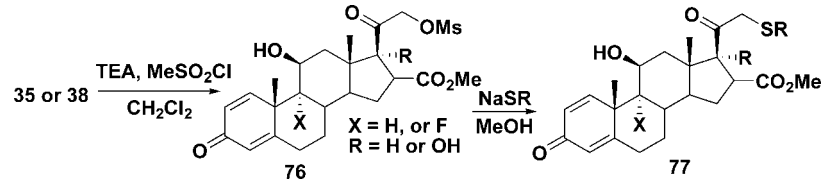
Scheme 18. Synthesis of 21-(γ-Lactone) Derivatives



Scheme 19. Synthesis of Steroid-NSAID Conjugates



Scheme 20. Synthesis of 21-Thioalkylether Derivatives



2.6. Synthesis of Derivatives of Steroidal Antedrugs

A persistent pitfall of the steroidal antedrugs synthesized so far is that, although their systemic toxicities are eliminated or reduced, their intrinsic anti-inflammatory activity is similar to or less than prednisolone whose potency is less than that of the most potent anti-inflammatory steroids, such as dexamethasone and fluocinolone. Thus, derivatives of the antedrugs have been synthesized in search of more potent agents.

2.6.1. Steroid-NSAID Conjugates

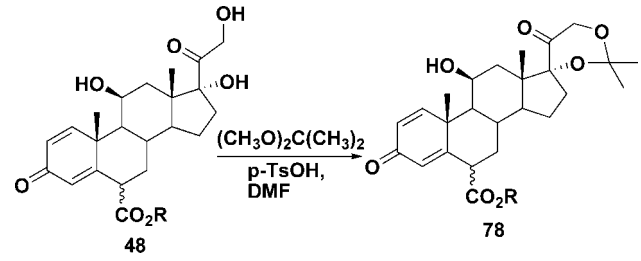
The conjugation of steroidal antedrug 21-OH with non-steroidal anti-inflammatory drugs was accomplished as shown in Scheme 19. Ibuprofen/indomethacin-steroid conjugates were synthesized by esterification of the 21-OH of steroidal antedrugs (**35**) with the COOH of ibuprofen or indomethacin using DCC and 4-DMAP in DMF.[62]

2.6.2. 21-Thioalkylether Derivatives

The synthesis of 21-thioalkylether derivatives of methyl 16-prednisolone carboxylates was performed as shown in Scheme 20.

The 21-OH of steroidal antedrugs (**35** or **38**) were mesylated using methyl sulfonylchloride and triethylamine at 0 °C to yield steroids with general structure **76**, which were reacted with sodium thioalkoxides to yield corresponding thioalkylethers (**77**).[48]

Scheme 21. Synthesis of 17,21-Acetonide Derivatives of 6-Carboxylates

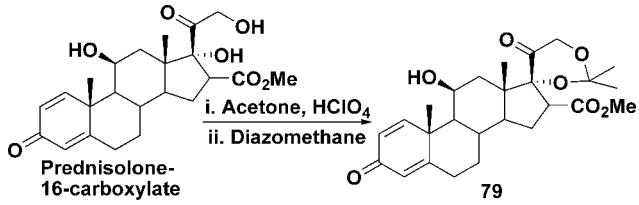


2.6.3. 17,21-Acetonide Derivatives

Treatment of steroidal antedrug **48** with 2,2-dimethoxy propane and a catalytic amount of *p*-TsOH in DMF gave a 6:1 mixture of corresponding acetonides **78α** and **77*β*** (Scheme 21).[49]

Treatment of methyl prednisolone-16-carboxylate with dry acetone in presence of catalytic amount of perchloric acid and reesterification of the 16-carboxylic acid with diazomethane afforded corresponding α-acetonide (**78**) (Scheme 22).[63]

Scheme 22. Synthesis of 17α,21-Acetonide Derivatives of 16-Carboxylates



3. Pharmacology of Anti-Inflammatory Steroidal Antedrugs

Whole cell glucocorticoid receptor binding assays are carried out using rat liver cytosolic preparations as previously described by Lee[64] in an approach to study the activity of the steroidal antedrugs and in comparison to their respective parent drug in molecular level.[65−67] It is believed that the anti-inflammatory potency of the steroids and their antedrugs are related to their GC receptor binding affinity. The plasma GC level is determined by radioimmunoassay according to the procedure recommended in RSL Rat [^{3}H]corticosterone kit (Radioassay System Laboratories, Carson, USA). The carrageenan (CGN)-soaked sponge model of acute inflammation experiments were conducted using a modified method,[68] where 0.4 mL of sterile 1% CGN in saline was injected into the sponges which were excised at 24 h.[63] Elastase activity was measured by the modified method of Saklatvala.[68] Protein was measured by method of Lowry et al.[69] and enzyme activity expressed as nmols *p*-nitroanilide generated/mg protein/h.[63] Concomitant biological activity associated with the occupancy of the GC receptor is assessed as inhibition of NO generation by pro-inflammatory macrophages stimulated with lipopolysaccharide (LPS). Thus, in addition to the receptor binding affinity, the IC_{50} values for inhibition of NO generation guide as a measure of the anti-inflammatory potency of the steroids and their antedrugs.[52,67,70−73] Anti-inflammatory activity of steroids has also been evaluated in cotton pellet granuloma bioassay, using the modification of the Meier bioassay.[74] The effects of topically applied steroids on edema formation were measured using the croton oil-induced ear edema bioassay[68] a well-established pharmacological method for evaluation of anti-inflammatory activity of the steroids in animal model and is employed in comparing the efficacy of the antedrugs with their parent steroids. The parameters of undesirable side effects are evaluated by measuring the alterations of body weight, thymus weight, and plasma corticosteroid levels after 5−7 consecutive days of drug treatment.[52,55,67,70−73,75,76] Clinically used topical anti-inflammatory adrenal steroids inhibit skin collagen synthesis. This impaired collagen synthesis may result in dermal atrophy. An approach to histopathology of skin was made to demonstrate the effects of the steroids and their antedrugs on the skin.[77]

Within the antedrug paradigm, efforts have been made to incorporate a metabolically labile group into the potent GC nucleus and hydrolytic inactivation has been considered to be the most important metabolic process for the antedrugs. Thus, the *in vitro* hydrolysis rates of steroidal anti-inflammatory antedrugs in rat or human blood plasma have been serving as very important criteria in antedrug study.[28,67,71,72,75] The hydrolysis rates in most studies were expressed as half-life ($t_{1/2}$), which is the time it takes for 50% of the compounds to be hydrolyzed to inactive metabolites as determined by analytical HPLC.

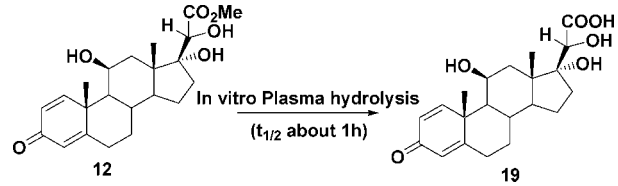


Figure 2. *In vitro* hydrolysis of steroidal antedrug **12**.

3.1. Carboxylic Esters and Amides

Series of pharmacological studies with the steroid 21-carboxylic esters were performed that included cotton pellet granuloma bioassay, relative thymus weight, plasma corticosterone level, ear-edema bioassay, and rat liver cytosol GC receptor binding study and compared with those of prednisolone.[22,68] In steroids **12**, 20-OH group has two different configurations; the β form is few fold more potent than the α-epimers as shown by their ID_{50} values in granuloma bioassay (anti-inflammatory). On the other hand, the **13α** is several times more potent than the **13*β***** in the same assay. (Reviewed in refs 9 and 30.) While their potencies were little less than prednisolone the systemic effects were significantly reduced. The order of potency in ear-edema bioassay was prednisolone > **13α** > **13*β*** > **12*β*** > **12α**, which correlated well with the values of their 1-octanol/water partition coefficients. All the steroids also showed competitive [^{3}H]dexamethasone displacement for binding to rat liver cytosolic GC receptors, which are comparable to that of prednisolone. The 20-keto analogs **15** and **18** showed anti-inflammatory activities in granuloma bioassays comparable to that of prednisolone. In accordance with their mechanism of action that is GCs indirectly inhibit phospholipase A_2 and thereby prevents biosynthesis of prostaglandins, thromboxanes, and leucocytes,[76−78] the antedrugs were also found to decrease to a varying degrees, the liberation of PGF_{1a} and PGE_2 into inflammatory exudates.[68]

In vitro metabolism study with the antedrugs showed that steroids **12α** and **12*β*** undergo rapid hydrolysis giving corresponding carboxylic acid (**19**) (Figure 2).[79] From *in vitro* plasma hydrolysis study, it was found that **12*β*** was completely hydrolyzed and **12α** was 30% hydrolyzed within one hour at 35 °C.[80]

The carboxylic acid (**19**) showed no affinity for the rat liver or thymus cytosolic GC receptors or any significant activity in other pharmacological tests[64] suggesting the inactivation is related to their rapid hydrolysis in accordance with the concept of antedrug. Most importantly, all of these steroidal antedrugs were devoid of any significant suppression on the thymus and adrenal weights and plasma corticosterone levels.[22,34] Clinically used topical anti-inflammatory steroids inhibit skin collagen synthesis leading to dermal atrophy. As shown in Figure 3 by the cross sections of rat skins, none of the tested antedrugs (**12** and **15**) did cause any dermal atrophy in contrast to triamcinolone and prednisolone, which resulted in marked skin atrophy after topical treatment with equivalent doses.[76]

Epimers at C20 of methyl 11β,17α,20-trihydroxy-3-oxo-1,4-pregnadiene-21-oates, their 9α-fluoro analogs and their carbonate and acetonide derivatives (**80**−**82**) were subjected to similar *in vitro* hydrolysis rate study in rat plasma and liver homogenate.[72] In rat plasma, the carboxy ester bonds of 20β-triols and their acetonides were hydrolyzed remarkably faster ($t_{1/2}$ 5.7 − 7.7 min) than their α-epimers ($t_{1/2}$ >2.5

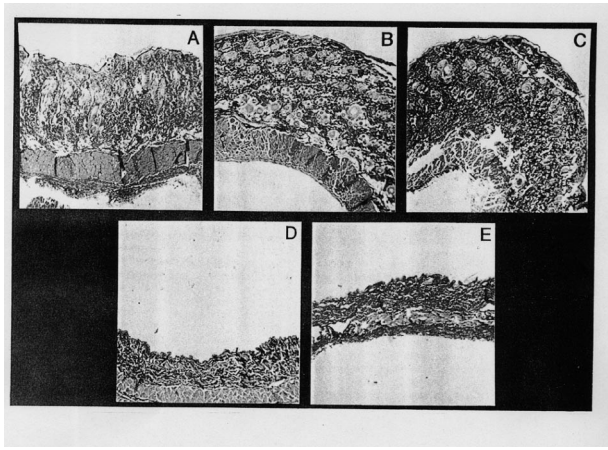
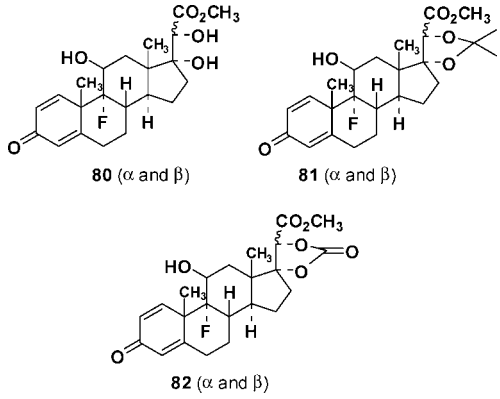


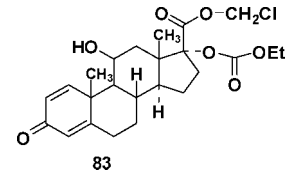
Figure 3. Cross sections of rat skins topically treated with 0.5% adrenal steroid creams for 30 days, once daily. (A) Control; (B) antedrug **12**; (C) antedrug **15**; (D) prednisolone; and (E) triamcinolone. Two rats were in each treatment group. These are representative data (Masson trichrome, X8).[77] Copyright 1984, American Medical Association. All rights reserved.

h) and those for the carbonate derivatives were even more profound with the $t_{1/2}$ of <1 min and ~3 min, respectively. In rat liver homogenate, the β- and α-isomers of the triols and the acetonides were much more stable than in plasma ($t_{1/2}$ 54−108 min and 7 h, respectively); however, the carbonates showed essentially the same hydrolytic patterns as in the rat plasma.[72]



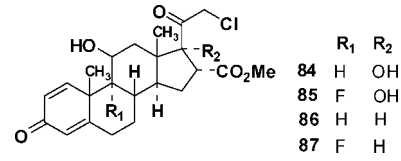
Loteprednol etabonate (LE) (Lotemax or Alrex, **83**) is an example of oral antedrug (or soft drug),[81−109] originally designed for a safe ophthalmic anti-inflammatory drug and received FDA approval in 1998. It is used for topical treatment of all types of inflammatory diseases including gastrointestinal inflammation via oral and rectal administration in rats. It was originally designed based on the soft drug principle with a minor modification which is overlapping with the antedrug principle (reviewed in 110). After oral administration about 90% of the LE remained in the alimentary tract up to 8 h and after rectal administration it remained intact into the rectal loop for more that 5 h with a slow rate of distribution into rectal membranes to some extent. Very low concentrations of the LE or its inactive metabolites (<0.1 mg/ml) were observed in plasma after both oral and rectal administrations during the experiments thus showed very high local/systemic anti-inflammatory activity ratio rendering it to be an important candidate for the treatment of inflammatory bowl syndromes.

The topical anti-inflammatory activities of all the 21-carboxamides (**20**−**23**) were less than prednisolone as shown



by ear edema bioassays, the *N*-benzyl derivative **23α** being the most potent. They also possessed comparable systemic effects including the undesirable side effects, which might be due to their slow rate of hydrolysis than the esters.[35]

Steroids with intact 17β-ketal side chain with the metabolically labile carboxylic ester at C16 position (**35** or **36** where **X = H**)[43] exhibited half of the local activity of prednisolone without any significant systemic anti-inflammatory or other undesirable side effects. Later Lee et al.[65,66] showed that the modifications of 9α-fluoro-prednisolone by 16α-methoxycarbonyl group alone (**36** where **X = F**) or in conjunction with modifications at C21 retained topical activity with much reduced systemic side effects. Compounds with intact 17-OH and replacement of 21-OH of four different prednisolone derivatives with chlorine (**84**−**87**) did not change the topical anti-inflammatory activity but noticeably improved the local vs systemic activity ratios and also reduced systemic side effects.[70] The potencies of steroidal antedrugs showed little dependencies on the 17α-OH functional group. The improved potency of the fluorinated GCs is in consistence with previous finding that fluorination particularly at 9α-position enhances all the biological activities of glucocorticoids.[111−113] Esterification of 21-OH[67] of 9α-fluoro prednisolone derivatives (**38** where **X = F** and 21-OH is replaced with different alkyl esters) demonstrated that 9α-fluoro, 16α-methoxy carbonyl, and 21-pivalyl group increased the topical anti-inflammatory potencies by 2-folds with the exception of other esters. The systemic side effects were insignificant as shown by unaltered body weight, thymus weight or plasma corticosteroid levels while cytosolic GC receptor affinity were comparable or slightly weaker than that of prednisolone.



The *in vitro* hydrolysis rate study in rat plasma for the 21-ester derivatives of antedrug (**38**) showed that the 21-*O*-acyl groups were hydrolyzed much faster ($t_{1/2}$ 6−23 min and for pivalyl, 60 min) than that of 16-methoxycarbonyl group providing active compounds, which are then hydrolyzed ($t_{1/2}$ 90−100 min) to inactive 16-carboxylic acids (Figure 4).[71] The effect of increasing the size of the group at C16 was also examined by synthesizing 16α-malonyl and carboxymethyl compounds (e.g., **36**), which showed reduced potency suggestive of a critical steric size requirement at this position.[43]

Incorporation of the carboxylate ester function at C6 of the steroid nucleus slightly reduced the overall potency; however, the potency was comparable to that of prednisolone when its 21-OH was esterified (**48** with 21-O-Acyl) or 17,21-OH functions were ketalized (e.g., **78**).[49] The ω-methoxycarbonyl group at 17α-ester function (**52**) where the 21-OH is replaced with a Cl-group furnished potent topical anti-inflammatory agent (1.3-fold over parent steroid) with

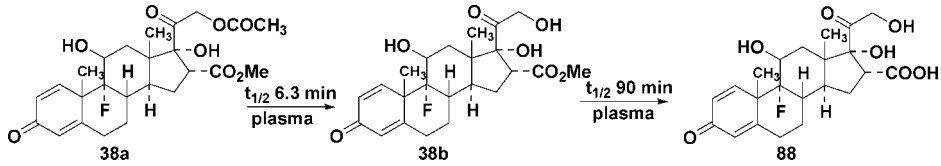


Figure 4. Hydrolysis of steroid 16-carboxylate-21-acetate.

reduced thymolytic activity and thus excellent separation of systemic thymus involution from topical anti-inflammatory activity (the ration of topical/systemic >130). The metabolic study in human skin homogenates with this ester (**52**) demonstrated the main metabolite to be the free carboxylic acid which showed dramatically reduced binding affinity to the GC receptor and showed neither topical anti-inflammatory activity nor the thymolytic activity, suggestive of another excellent strategy for providing an antedrug.[50]

3.2. Ring-Fused Isoxazolines and Oxime Derivatives

A series of carbethoxyisoxazolines with structures similar to **56** where R = H or Ac and X = H or F were synthesized and tested for their biological activity, which were found to retain the anti-inflammatory activity with reduced systemic side effects as desired with the antedrugs. This finding prompted the synthesis of newer oxime derivatives (**55**) and isoxazolines (**54**) where R = H or Ac and X = H or F. Surprisingly, their biological evaluation demonstrated that they possess even greater therapeutic indices than those with the carboxyethyl moiety, although devoid of any metabolically labile ester group. Results of the 5-day rat croton oil ear edema assay indicated that most of these antedrugs increased anti-inflammatory activities without significantly altering body weight, thymus weights or plasma corticosterone level rendering them as a promising class of antedrugs.[44,51,52,73] Most recently, the receptor binding study demonstrated that most of these compounds have high binding affinity with GC receptor. 21-Acetyloxy-9α-fluoro-11β-hydroxy-3,20-dioxo-1,4-pregnadieno[16α,17α-d] isoxazoline (**54**, R = H) and 11β−21-dihydroxy-9α-fluoro-3,20-dioxo-1,4-pregnadieno[16α,17α-d] isoxazoline (54, R = Ac) were found 5.0- and 5.3-fold more potent than prednisolone, respectively. Overall, they were less potent in inhibiting the nitric oxide (NO) production in LPS-stimulated RAW 264.7 murine macrophage cells.[114] The metabolic mechanisms by which they exert their antedrug property are unknown, and the identification of the metabolites of these compounds is in progress.

3.3. Spiro Enone Derivatives

This is based upon the same principle as described in Figure 4; however, the ester moiety is added through a rigid spiroenone ring exemplified by the structure **59** where the spiroenone ring is added to α- and β-dexamethasone and prednisolone.[55] Receptor binding study showed that all of these spiroenones had weaker affinities for the GC receptor and found to be less potent anti-inflammatory agents than prednisolone. Their anti-inflammatory potency in croton oil-induced edema bioassay was 1/2-fold less than that of prednisolone suggesting that the rigid spiroenone ring dramatically reduced the anti-inflammatory potency of the compounds and that the flexible 17α-side chain played an important role in anti-inflammatory action.[55]

3.4. 20-Thioester Derivatives

The incorporation of thioester at C20 was based on the fact that hepatic inactivation will afford the corresponding carboxylic acid as shown in Figure 5.

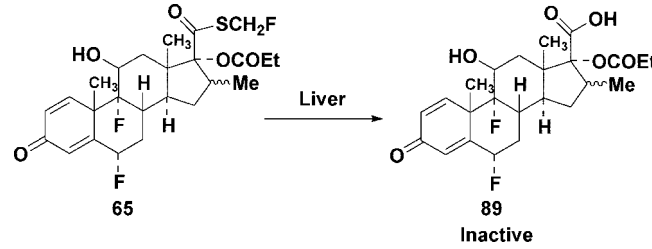


Figure 5. Plasma hydrolytic study with fluticasone propionate.

Thus much reduced systemic exposure was achieved with the introduction of fluticasone propionate (**65**) in which the hepatic inactivation affords the inactive carboxylic acid **89**.[56] However, one study reported that it may be metabolized by an oxidative thiol cleavage mechanism instead of hydrolysis[115] to the inactive carboxylic acid and is thus stable for longer time in the blood rendering it not to be an ideal antedrug.

3.5. γ-Butyrolactone Derivatives

Aggressive research activity in Glaxo Wellcome in the antedrug paradigm enabled the discovery that lactone hydrolysis to steroidal acid is an ideal antedrug candidate for inhaled steroids. The lactones were found to be remarkably stable in human lung S9 preparations while the ethyl esters were rapidly cleaved under the same conditions (Table 1).[26]

Table 1. Stability in Human Plasma and Human Lung S9 and GC Receptor Affinities of GR250495X (70) and its Metabolites (90)

compound	human plasma $t_{1/2}$ (min)	human lung S9 $t_{1/2}$ (min)	GC receptor affinity IC_{50} (nM)
70			
Isomer A	<1	>480	3.4
Isomer B	<1	>480	6.6
90			
Isomer A	–	–	194
Isomer B	–	–	827

Although as early as 1966 it had been known that lactonase activity exists in human plasma, recent work at Glaxo Wellcome suggests that this enzyme is human paraoxonase, which is known to be absent from the lung, thus explaining the unique selectivity observed with this class of steroids.[26] Importantly, the hydrolysis of the lactone moiety (Figure 6) resulted in marked inactivation. While the lactones showed high affinity for GC receptor the corresponding hydroxyl acids (**90**) showed much lower affinity. Thus, the lactone-

moiety containing glucocorticoids display the ideal lung selective antedrug profile of rapid inactivation in plasma with stability in the target tissue.[26]

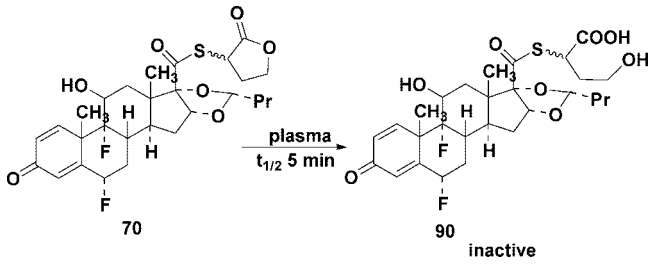


Figure 6. Inactivation of **GR250495X** in blood.

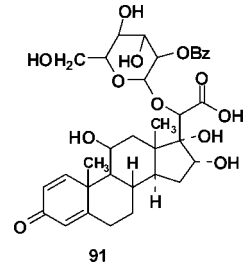
3.6. Steroid-NSAID Conjugates

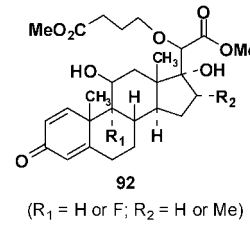
Another effort was made to test the hypothesis that the topical potencies of moderately potent steroidal antedrugs could be enhanced by conjugation to nonsteroidal anti-inflammatory drug, ibuprofen or indomethacin (**75**).[63,116] However, the topical potencies were not enhanced which might be attributed to the decreased affinity to GC receptor of the conjugates, but showed remarkable reduction in systemic side effects with significantly improved local/systemic activity ratio.

4. The Prodrugs and Pro-Antedrugs

The design of beclomethasone dipropionate and ciclesonide use the prodrug approach for lung selectivity. Both of these steroids are modified by incorporating lipophilic moiety to increase their lipid solubility and thus to localize the action in lung to reduce the episodes of systemic side effects. The US Food and Drug Administration (FDA) announced October 2006 the approval of ciclesonide nasal spray for the treatment of nasal symptoms associated with seasonal and perennial allergic rhinitis in adults and children 12 years of age and older. Although its short-term safety is documented, the long-term data are lacking. The prolongation of intraluminal retention time by significantly increasing the lipophilicity of the steroids has also been well investigated.[117]

Based upon the antedrug and the prodrug concepts, Kimura et al.[24] introduced a new concept of pro-antedrug by incorporating 21-oate ester of the prodrug (**91**), to reduce the systemic side effects of dexamethasone-α-D-glycoside, an effective colon-specific prodrug used in oral treatment of ulcerative colitis. This hydrophilic glycoside was stable in small intestine, but in the large intestine the glycoside bond was cleaved by the action of bacteria to release the antedrug. Suzuki et al.[117,118] synthesized novel steroid-17-methyl glycolates of dexamethasone and dihydroprednisolone by incorporating a succinyl group at C20. Among them the (20*S*)-succinyl dexamethasone derivatives (**92**) displayed more or less potent anti-inflammatory activity than the parent dexamethasone and did not show corticosteroidal systemic side effects. Hydrolysis of the pro-antedrug afforded the antedrug within 2 min, which was inactivated by further hydrolysis to carboxylic acid ($t_{1/2}$ = 2 h).[118,119]





5. Antedrugs in Clinical Use or in Pipeline

Fluticasone propionate (**65**, 6,9-difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxopropoxy)-androsta-1,4-diene-17-carbothioic acid (6α,11β,16α,17α)-S-(fluoromethyl) ester, Flonase) is an anti-inflammatory steroid approved by FDA in 1994 for use in asthma and allergic rhinitis, which is believed to work in the same principle as antedrug. It is a highly popular nasal spray for allergic lung conditions including asthma and rhinitis. It was not developed originally as an antedrug. It may not be a true antedrug as it has also been shown that it may be metabolized by oxidative cleavage of the thiol ester bond rather than by hydrolysis[115] and thus is relatively slowly eliminated with a terminal half-life of 7.7−8.3 h and produces a dose-related cortisol suppression.[120,121]

Loteprednol etabonate (**83**, chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene, 17β-carboxylate) is the first corticosteroid synthesized based on soft drug approach, received the final FDA approval in 1998. Since this drug is also deactivated into systemic circulation and intended to work topically in the eye it is considered that the concept is overlapping with that of antedrug approach. It lacks serious side effects and is the active principle of two ophthalmic preparations, Lotemax and Alrex[81−109] for use in all inflammatory and allergy-related ophthalmic disorders including inflammation following post-contact surgery, uveitis, allergy conjunctivitis, and giant papillary conjunctivitis. It is also being developed for treatment of asthma, rhinitis, colitis, and dermatological problems.

GR250495X(**70**) is a true antedrug candidate developed by Glaxo Welcome for asthma treatment which is in clinical trial. The most important profile for this class of compounds is that they are stable in human lung S9 but is rapidly hydrolyzed to the corresponding inactive hydroxy acid **90** in blood plasma ($t_{1/2}$ 5 min) (Figure 6).[26]

6. Conclusion

The very stringent safety issues of therapeutic drugs demand a new approach for developing existing drugs with further safety parameters which may save much more time and money needed to develop a new drug from the concept. From medicinal chemical point of views, prodrug approach has shown much success in correcting many issues of drugs' safety by modification of their physicochemical properties. Other technologies like targeted drug delivery to the different organs and tissues has also shown remarkable success from the pharmaceutical or molecular biological view points. However, since the antedrug approach is strictly to localize the drug action at the site of application and deactivate into the system, it is much more logical medicinal chemical approach to deal specifically with the safety issues of potent therapeutic agents. Since its first introduction, the approach showed some remarkable success in developing safer anti-

asthmatic drugs for both steroids and nonsteroids. This can also work in concert with other technologies to strengthen and diversify the focus. This review will be an important guideline not only for further development of anti-inflammatory steroids but also for other classes of therapeutic drugs.

7. Acknowledgments

This research was supported in part by NIH grants AM 21627, GM 08111, and RR 02660.

8. References

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