New Steroidal Anti-Inflammatory Antedrugs: 21-Thioalkylether Derivatives of Methyl 16-Prednisolone Carboxylates

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Abstract: Antedrug approach of the corticosteroids has been described as a fundamentally sound approach for the development of safer anti-inflammatory steroids devoid of systemic side effects. In our continued efforts under the antedrug paradigm, we have recently extended this effort to synthesize the 21-thioalkylether derivatives of methyl 16-prednisolonecarboxylates. The 21-mesylate of the methyl-16-perdnisolonecarboxylates and 9-fluoro-17-dehydro methyl 16-prednisolonecarboxylate were reacted with Na-thioalkoxides to furnish the desired thioalkylethers in 60-75% yields. These newly synthesized thioalkylether steroid series were tested for their *in vitro* metabolism and corticosteroid receptor binding affinity. They were metabolized in predictable manner to inactive 16-carboxylic acids. All the newly synthesized antedrugs showed lowered glucocorticoid receptor binding affinity than prednisolone indicating that the replacement of the 21-OH function with thioalkylether of the 16-prednisolone carboxylate esters decreases their receptor binding affinity.

Key Words: Antedrug, thioalkylether, anti-inflammatory steroids, dissociated steroids, receptor binding.

INTRODUCTION

Glucocorticoids are widely used for the suppression of chronic inflammatory and allergic conditions such as asthma, Chronic Obstructive Pulmonary Diseases (COPD) rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases. Although the beneficial effects of corticosteroids in the treatment of inflammatory and allergic conditions have been appreciated for over 60 years, complications arising from the steroid therapy have imposed limitations on the clinical use of this class of drugs [1,2]. Among the deleterious systemic effects that they cause, suppression on the hypothalamic-pituitary-adrenal (HPA) axis, the immune system, aggravation of diabetes, hypertension, osteoporosis, and retardation of growth in children have proven to be quite debilitating effects that limit their therapeutic uses [3-6]. 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 [2-4]. Several considerations of steroid therapy that led to the development of a new concept, "antedrug" by Lee [7] were: (i) corticosteroid pharmacotherapy appears to offer an abundance of agents, but no truly safe drug; (ii) systemic effects of steroids are unnecessary complications which accompany treatment of many inflammatory conditions; (iii) the intact 17-ketol side chain is not an absolute requirement for the anti-inflammatory activity of corticosteroids [8,9]; and (iv) steroid acid esters derived from potent glucocorticoids would retain anti-inflammatory activity but upon entry into the circulatory system from the site of administration would be hydrolyzed to steroid acids that are inactive and readily excretable [7,10].

The concept of antedrug coined by Lee [7] has generated renewed interests not only in the area of anti-inflammatory steroids but also expanded to other therapeutic classes. Various chemical classes of steroidal antedrugs e.g., (i) the carboxylic esters and amides, (ii) ring-fused isoxazolines and oximes (iii) spiroenones, (iv) 20-thioesters and (v) γ-butyrolactone derivatives have been developed (reviewed in [11]). Metabolic biotransformation is the common characteristic of an antedrug that leads to inactivation of a molecule as illustrated in Fig. (**1**). Thus, a true antedrug acts locally and can be used for long term therapy.

One possible way of separation of beneficial effects from the deleterious effects of glucocorticoid-based drugs has recently been reported [12,13]. Screening a library of compounds using a trans-activation and activator protein -1 (AP-1) trans-repression models in transiently transfected cells the dissociated glucocorticoids were identified (Fig. (**2**), RU 24782 & RU 24858), which exerts strong AP-1 inhibition but little or no trans-activation. A series of molecular biological, biochemical and animal model studies revealed that these dissociated glucocorticoids retained the anti-inflammatory and immunosuppressive potential of classic glucocorticoid prednisolone whereas are weak agonists and are much less potent in exerting the metabolic activity responsible for the deleterious systemic side effects of the glucocorticoids. This provides another possible way for the discovery of safer anti-inflammatory steroid [13].

The merging of the concepts of "antedrug" and the "dissociated glucocorticoids" might provide a new leverage in developing safer anti-inflammatory steroids. Thus, in the present study a series of nine thioalkylether analogs of 16-prednisolone carboxylates as shown in Table **1** (**1**-**9**) are synthesized and studied for their *in vitro* metabolism and corticosteroid receptor binding affinity.

RESULTS AND DISCUSSION

The 21-mesylate of the corresponding 16-prednisolone carboxylate derivatives were reacted with the Na-thialkoxides to yield the desired products (**1**-**9**) in 60-75% yields [14].

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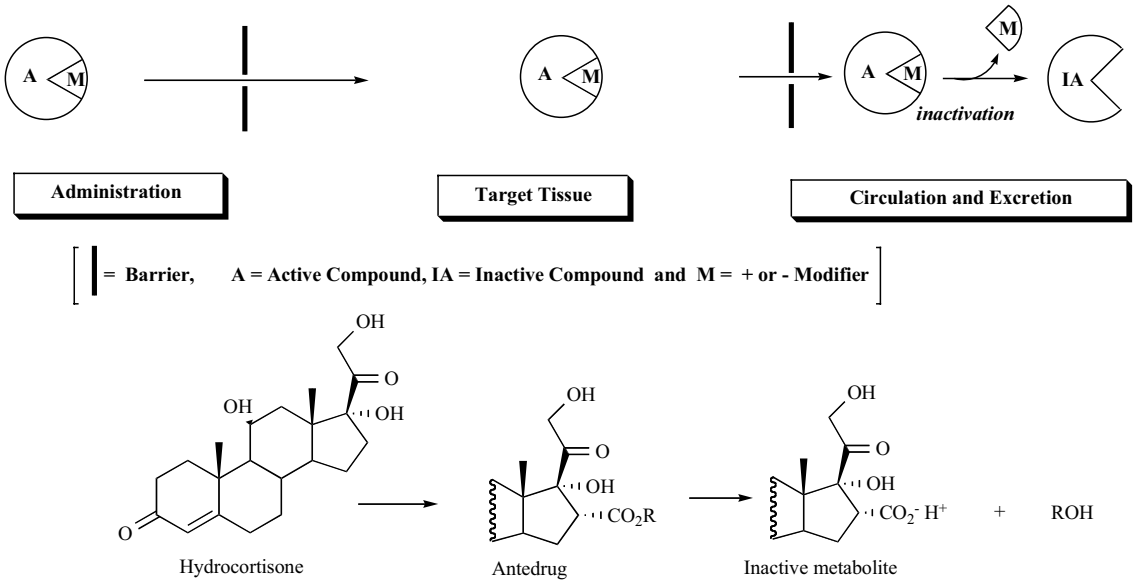


Fig. (1). Concept of antedrug showing chemical example.

This set of study provided a new convenient method of synthesizing 21-thioalkyl derivatives of the anti-inflammatory steroids which will guide to develop newer derivatives.

All the compounds were subjected to metabolic degradation in blood plasma *in vitro* according to the previously reported method [15,16]. To determine the hydrolysis rates, each compound was incubated in rat plasma for 24 h. All the compounds were hydrolyzed to inactive steroid-16-carboxylate, with half-lives of 90-100 minutes and were nondetectable after 6h incubation. This is in consistent with our previous reports of series of antedrugs which were hydrolyzed in blood plasma to their corresponding inactive 16-carboxyxlic acids [16]. Stability tests showed that the compounds tested were stable and more than 90% of them were recovered after 1 h incubation in the phosphate buffer as well as in the plasma, which was treated in boiling water. This result indicates that hydrolysis of these compounds is an enzyme-catalyzed reaction. The hydrolysis of these steroidal antedrugs is catalyzed by nonspecific esterases which are widely distributed in biological fluids and tissues, including liver and plasma [17]. In previous study with 16-carboxylate esters, it was shown that the metabolites, 16-carboxylic acids, were inactive and have higher polarities [16,18].

Competitive receptor binding assay was carried out with cytosol glucocorticoid receptor preparations from Sprague Dawley rat liver. All compounds tested in this assay showed lower binding affinity than prednisolone. When IC_{50} values (Concentration of the compounds required to displace 50% of bound [^{3}H]dexamethasone from the glucocorticoid receptor) were compared, compounds **2**, **5**, and **8** exhibited less than 2 μM (prednisolone = 92.4 nM).

These results indicate that the replacement of the 21-OH group with a thioalkyl group of 16-prednisolone carboxylate is responsible for reduction in the receptor binding affinity. Among the newly synthesized compounds those with ethyl substituents showed relatively better receptor binding affinities. The methylated ones were also good in binding affinity but lower than those with the ethylated ones. However, the too bulky butyl group dramatically reduced the binding affinities of the compounds as evident in the Fig. (**3**). The binding affinities of both the methylated and ethylated ones are in lower micromolar range. The receptor binding affinity might not be the sole determinant for their *in vivo* anti-inflammatory activity, as other factors like lipophilicity and solubility in biological fluid, cell uptake and distribution, transactivation, and transrepresion are also important to consider. Previously it had been shown that topical application of 16-prednisolone methyl carboxylates demonstrated 14-times more potent anti-inflammatory activity than prednisolone in the croton oil ear edema model in rats and that it had

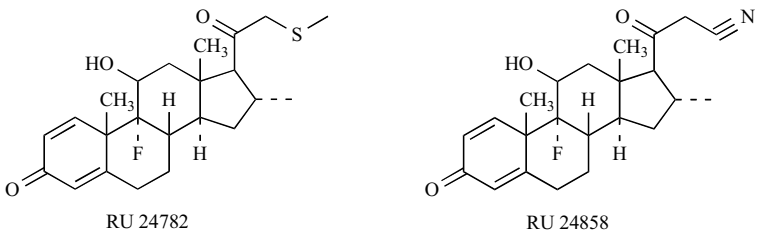
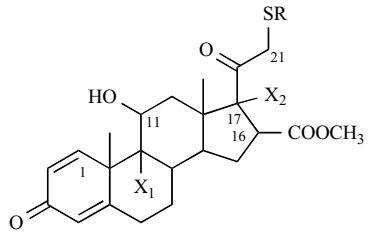


Fig. (2). Structures of dissociated glucocorticoids (taken from [13]).

Table 1. Structures of the 21-Thioalkylether Derivatives of Methyl 16-Prednisolone Carboxylates



(In prednisolone, X_1 = H, X_2 = OH, 16-H and 21-OH)

Compound	X_1	X_2	R
1	H	OH	CH_3
2	H	OH	C_2H_5
3	H	OH	$C(CH_3)_3$
4	F	H	CH_3
5	F	H	C_2H_5
6	F	H	$C(CH_3)_3$
7	F	OH	CH_3
8	F	OH	C_2H_5
9	F	OH	$C(CH_3)_3$

a greatly reduced tendency to cause systemic side-effects. Following multiple topical applications, no thymolysis was noted. In the carrageenan model of acute inflammation, they were potent inhibitors of leukocyte migration, generation of prostaglandin (PGE2), and release of elastase. Overall the C-16 methoxycarbonyl derivatives found to increase the topical activity without concomitantly increasing the risk of side-effects [19]. Detail anti-inflammatory studies with these newly developed 21-thioalkyl derivatives of the 16-prednisolone methyl carboxylates are in progress.

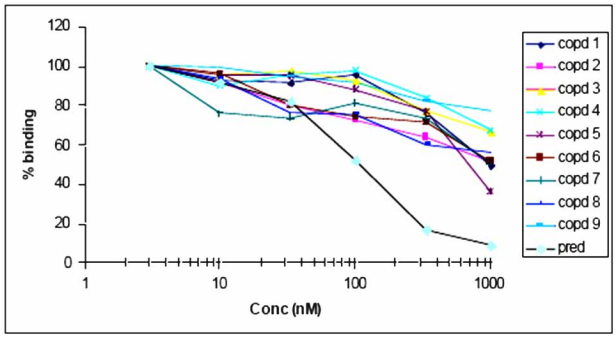


Fig. (3). Competitive receptor binding assay in liver cytosol.

EXPERIMENTAL

Chemistry

Details synthesis, purification and characterization by ^{1}HNMR, ^{13}CNMR, C, H analyses and melting point data of the compounds published elsewhere [14] and are available on request from the authors.

In Vitro Hydrolysis in Blood Plasma

Animals were anesthetized with Halothane. Blood was collected by cardiac puncture into a test tube containing heparin and pooled in order to reduce individual variation. The blood was centrifuged at 3000×*g* for 10 min, 4 °C. The plasma was stored in a −20 °C freezer immediately and used within a week. All steroid stock solutions (1 mg/ml) were dissolved in ethanol and stored in −20 °C freezer prior to use. No degradation of these steroids was noticed for about 1 month at room temperature. Equal volumes of plasma (20 ml) were allowed to equilibrate for 30 min at 37 °C, 95% humidity, and 5% CO_2, and then were spiked with 5 µg/ml of steroids. Samples were collected at selected intervals. A 1.0 ml aliquot of plasma was collected in a 15 ml tube and acidified with three drops of 2N HCl (pH=2.0). Then, 10 ml of ethyl acetate and 5 µg of dexamethasone as an internal standard were added. The tubes were shaken for 10 min and centrifuged at 10,000×*g* for 10 min. The organic phase was evaporated under reduced pressure. The residue was immediately reconstituted with 100 µl of methanol. A 40 µl aliquot of the sample solution was injected for HPLC analysis. To test the stability of compounds, rat plasma (1 ml) was put in boiling water for 10 min to stop enzyme activity and centrifuged. The supernatant was considered to be medium that was devoid of enzymatic activity. Each compound (5 µg/ml) was incubated in both the plasma medium and phosphate buffer for 1 h.

The HPLC system consisted of a Varian auto-sampler (9100), pump (9012), and UV detector (9050) (Varian, Walnut Creek, CA). The column was a Discovery-C18 (5 µm, 4.0 mm×15 cm i.d.), coupled with a Pelliguard-C18 guard column (4.6 mm×5 cm i.d.) from Supelco (Belleponte, PA). The detector wavelength was set at 254 nm. The mobile phase was a mixture of methanol and water. Flow rate was maintained at 1.5 ml/min.

Liver Cytosol Preparation

The liver from male Sprague Dawley rats, weighing 150-200 g, were minced and homogenized in a polytron homogenizer at 4°C in 5 volume of TEDM buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 25 mM sodium molybdate, and 10% glycerol, pH 7.4). The homogenate was centrifuged at 10,000 g for 10 minutes at 4°C, and recentrifuged at 100,000 g for 60 minutes at 4°C. The supernatant was used

as liver cytosol. To remove endogenous steroids, the cytosol was treated with dextran coated charcoal (DCC). Briefly, 1 g of DCC was added to 40 mL of liver cytosol allowed to mix with gentle shaking for 2 hours at 4°C. The cytosol was centrifuged at 5,000 g for 10 minutes at 4°C. The supernatant was recentrifuged two more times to remove remaining charcoal particles, and stored in a –70°C freezer until used.

Competitive Binding Assay

Competitive binding assays were performed in triplicate in a total volume of 160 µL, containing 5 nM [^{3}H]dexamethasone (Amersharm Pharmacia Biotech; specific activity 89.0 Ci/mmol) and various concentrations (0-1000 nM) of compounds. After 24 h incubation at 4°C, unbound [^{3}H]dexamethasone was removed by the treatment with DCC in TEDM buffer on ice. A 150 µL sample was pipetted into scintillation vial and 5 ml scintillation cocktail were added. Nonspecific binding was determined in the presence of 1000-fold excess of unlabelled dexamethasone. The radioactivity was measured with scintillation counter (Beckman Instruments, Fullerton, CA). IC_{50} values (concentrations at which 50% of specific binding is displaced by the compounds) were determined from the best fit lines derived by least square regression lines of competitive displacement graph.

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