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Short communication

Synthesis of new local anti-inflammatory thiosteroids based on antedrug concept

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Summary — The synthesis and the *in vitro* pharmacological evaluation of a number of topical corticosteroid derivatives designed on the basis of the antedrug concept are reported. Three series of compounds were synthesized in which sulfur-containing amino acids were incorporated to the steroidal structure in the 21 position. Compounds of series I are diesters of cystine with the 21-hydroxyl groups of the corticosteroids, while series II and III contain 21-amino and 21-thio corticosteroid derivatives, respectively. The new compounds were less potent than the parent corticosteroids *in vitro*. The 21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-S-acetylamino cysteine **13** was the most interesting compound of the series and is now under further evaluation.

Résumé — Synthèse de nouveaux thiostéroïdes anti-inflammatoires à usage topique. *La synthèse et l'évaluation pharmacologique d'un certain nombre de dérivés corticoïdes conçus en accord avec le concept d'antedrug sont décrits. Trois séries de composés sont synthétisés dans lesquels des amino-acides soufrés sont incorporés en position 21 de la structure stéroïdienne. Les composés de la série I sont des diesters de la cystine avec le groupement hydroxyl en position 21, tandis que les séries II et III sont des amino-21 et thio-21 stéroïdes. Les nouveaux composés sont moins actifs* in vitro *que les stéroïdes de départ. Le composé 21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-S-acétylamino cystéine* **13** *était le plus intéressant des trois séries et il est en cours d'études plus poussées.*

topical anti-inflammatory steroids / sulfur amino acids / corticosteroid activity

Introduction

The success of corticosteroids in the therapy of inflammatory disorders of the skin has led to the intensive research and development of new corticosteroids. More potent corticosteroids were obtained by chemical modifications of the natural corticosteroid hydrocortisone [1]. Furthermore, great efforts have been directed towards the improvement of the skin absorption efficiency of corticosteroids, either by chemical modifications (*eg* esterification of the 17- and/or 21-hydroxyl groups, formation of 17,16 acetonides) or by the development of new galenic formulations and the use of new methods of topical application [2]. However, despite these efforts, long-term use of corticosteroids is limited because of their many adverse systemic effects. Recently the concept of 'antedrugs' was introduced to describe a new synthetic approach towards safer corticosteroids [3]. The term 'antedrug' describes an active synthetic derivative which is inactivated by the first metabolic step upon entry into the systemic circulation. Thus, an antedrug acts only locally and is transformed to one (or more) inactive metabolite(s) offering a kind of site-specificity of action. The synthesis of a number of topical corticosteroid derivatives designed on the basis of the antedrug concept has been reported [4–6]. These compounds, although less potent than other available corticosteroids were devoid of systemic effects, following topical application.

We report here the synthesis of new anti-inflammatory thiosteroids for topical use, designed according to the antedrug concept. Three series of compounds were synthesized, in which sulfur-

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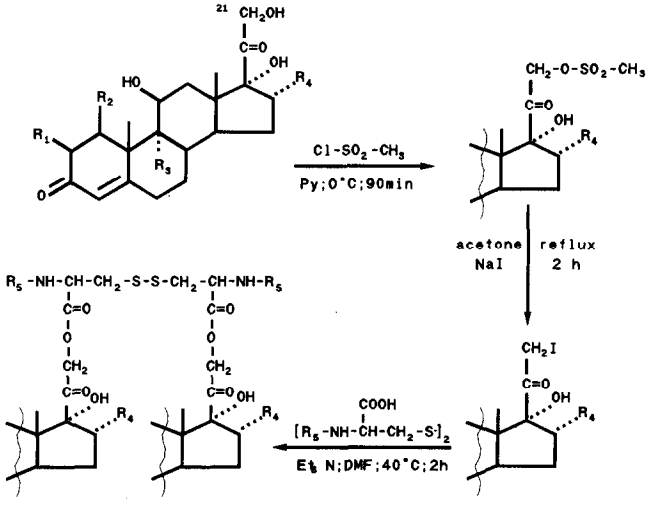
Abbreviations: ACTH, adrenocorticotropin hormone; α-MSH, α-melanin stimulating hormone; CRF, corticotropin releasing factor.

containing amino-acids were incorporated to the steroidal structure in the 21 position. An evaluation of the corticosteroid activity of the compounds *in vitro* is also reported.

Chemistry

The synthesis of the derivatives for each series of compounds is shown in schemes 1–3. Compounds of series I are diesters of cystine with the 21-hydroxyl group of the corticosteroids. They were obtained in three steps with an overall yield of about 30% (scheme 1). In the first step, the steroid was transformed to the 21-mesylate derivative, by treatment with mesyl chloride in pyridine. The 21-mesylate was converted to the corresponding 21-iodo derivative by the addition of NaI in acetone [7]. The final product was obtained in good yield by the reaction of the 21-iodo derivative with cystine, the amine function of which was previously protected by various groups as the *t*-butyloxycarbonyl (*t*-BOC), benzyloxycarbonyl, acetyl and the formyl group. In the case of *t*-BOC, this protecting group was subsequently eliminated by treating the diester with hydrochloric acid in methanol.

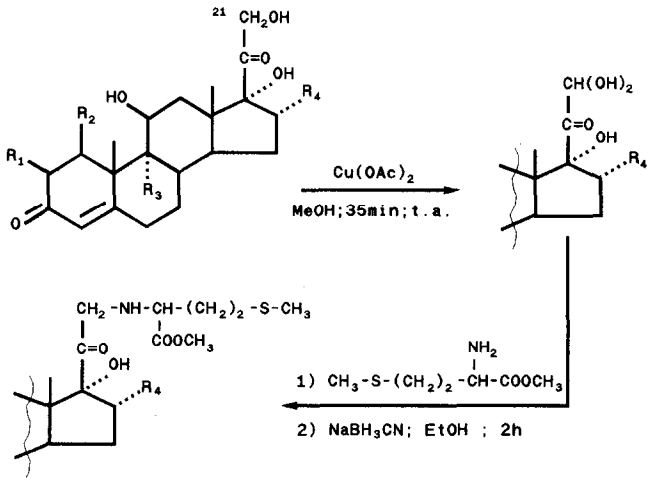


Compd	R_1	R_2	R_3	R_4	R_5	*mp (°C)*	*Yield %*
1	H_2	H_2	H	H	HCO	180	30
2	H_2	H_2	H	H	CH_3CO	160	30
3	H_2	H_2	H	H	$(CH_3)_3COCO$	190	25
4	H_2	H_2	H	H	$C_6H_5CH_2OCO$	200	25
5	H	H	F	CH_3	HCO	160	30
6	H	H	F	CH_3	CH_3CO	150	30
7	H	H	F	CH_3	$(CH_3)_3COCO$	170	25
8	H	H	F	CH_3	$C_6H_5CH_2OCO$	190	25

Scheme 1.

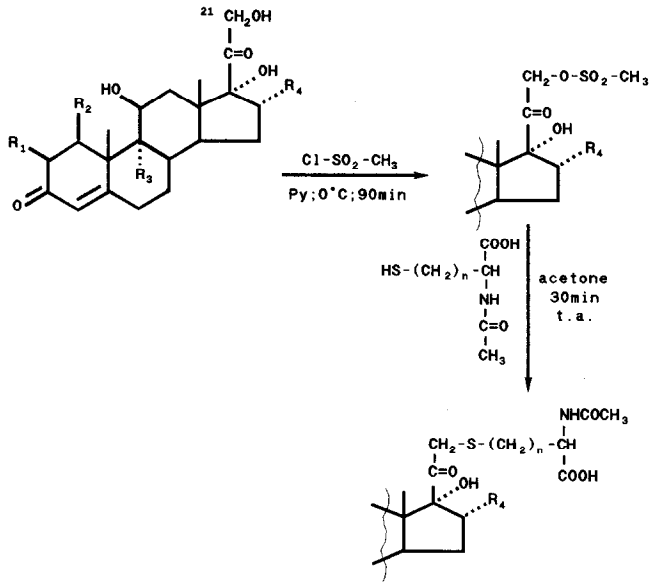
Series II contains 21-amino derivatives of corticosteroids. The preparation of these derivatives is shown in scheme 2. The α-ketol function of the steroid was transformed to glyoxal (20-oxo 21-aldehyde), by the action of cupric acetate in methanol [8]. This reagent gave the highest yields and the shortest reaction time. A ratio steroid/cupric acetate of 1/8 was used, the reaction time being 35 min. The reaction of the glyoxal derivative with the sulfur aminoacid, of which the carboxyl function was previously protected (methyl ester), afforded the corresponding Schiff's base. The reduction of the Schiff's base was carried out by the addition of $NaBH_3CN$ and afforded the final product. The overall yield was about 30%. Attempts to convert the 21-mesylate derivative of the steroid to corresponding 21-amino derivative were not satisfactory because of very low yields (< 10%).

The preparation of the 21-thiosteroid derivatives (series III) is presented in scheme 3. In the first step the steroid was transformed into its 21-mesylate derivative as described above (series I). This derivative was subsequently converted to the 21-thioether, by the addition of the N-acetyl cysteine in acetone. Yields were very high, in the order of 90%. The reactivity of the α-keto mesylate group of the corticosteroids towards the thiol group was reported previously [9]. This reactivity is enhanced by the presence of an hydroxyl group in the β-position of the α-ketol side chain. The choice of the N-acetyl cysteine was related to the fact that this compound is a metabolite of natural cysteine and to its properties as a scavenger of free



Compd	R_1	R_2	R_3	R_4	*mp (°C)*	*Yield %*
9	H_2	H_2	H	H	160	35
10	H	H	F	CH_3	176	32

Scheme 2.



Compd	R_1	R_2	R_3	R_4	*n*	*mp* (°C)	*Yield* %
11	H_2	H_2	H	H	1	210	89
12	H_2	H_2	H	H	2	189	90
13	H	H	F	CH_3	1	215	89
14	H	H	F	CH_3	2	210	90

Scheme 3.

radicals and as a precursor of glutathione [10]. Free radical formation is involved in inflammatory reactions [11].

Corticosteroid activity

The synthesis and secretion of glucocorticoids in man and other animal species is directly controlled by ACTH, the secretion of which is controlled by free cortisol (inhibitory effect) and by CRF (stimulating effect) [12]. Synthetic glucocorticoids, like dexamethasone, can inhibit the secretion of ACTH from hypophysis [13–17]. We used this property of glucocorticoids to evaluate the glucocorticoid activity of the new compounds *in vitro*. Murine AtT-20 corticotroph tumor cells can synthesise ACTH and this effect can be inhibited by synthetic (*eg* dexamethasone, triamcinolone) and natural (*eg* cortisol) glucocorticoids [18]. Furthermore, a correlation has been found between this inhibitory activity of glucocorticoids and their specific binding to glucocorticoid receptors [19]. The effect of the synthesized molecules of series I and II to the secretion of α-MSH and of the molecules of the third series to the secretion of ACTH, both induced by CRF, in cultured AtT-20 corticotroph cells is represented in figures 1 and 2 respectively.

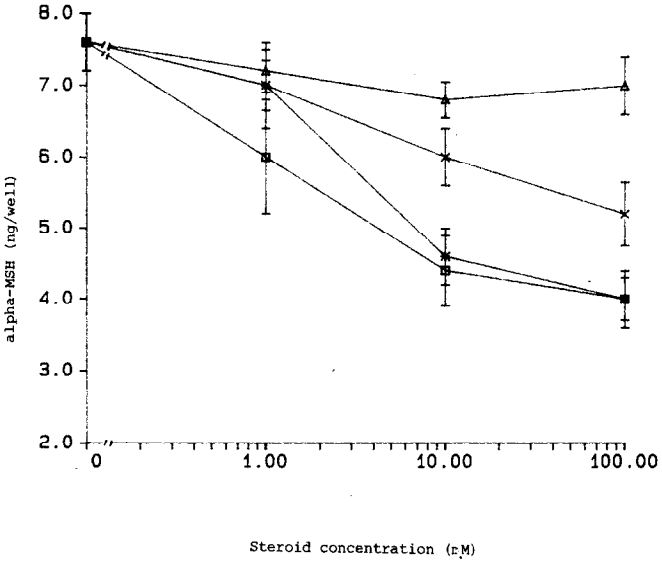


Fig 1. Effect of dexamethasone (□) and of the molecules of series I (✳ 5, x 6) and II (△ 10) on the secretion of α-MSH, induced by CRF (10 nM), in cultured AtT-20 corticotroph cells.

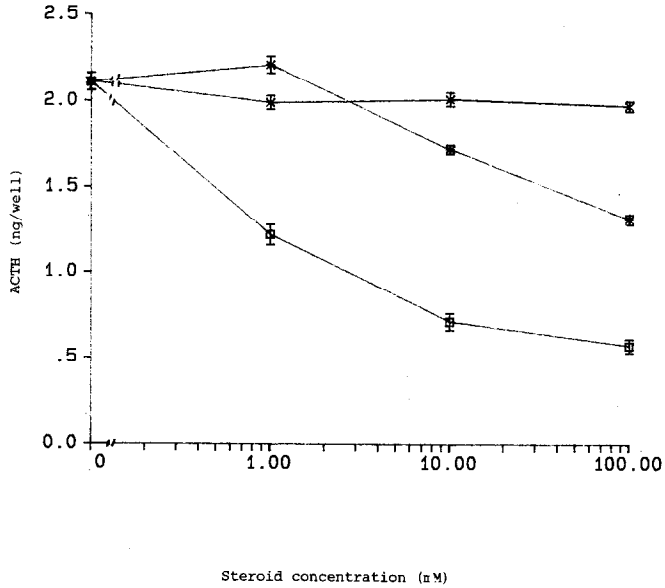


Fig 2. Effect of dexamethasone (□) and of molecules of series III (x 11, ✳ 13) on the secretion of ACTH, induced by CRF (10 nM), in cultured AtT-20 corticotroph cells.

The diacetylated diester derivative of dexamethasone **6** showed no significant glucocorticoid activity (fig 1). However, the activity of the diester of dexa-

methasone with di-formyl cystine **5** is equipotent, for the elevated concentrations, with dexamethasone. This difference of activity may be attributed to the more important steric hindrance provoked by the acetyl group. The substitution of the hydroxyl group in 21 position of the steroid by an amino group (series II, compound **10**) has led to a significant decrease of the glucocorticoid activity.

On the other hand, the effect of the thioether derivative of dexamethasone (series III, compound **13**) is about 8 times less potent than dexamethasone. The corresponding derivative of hydrocortisone **11** is much less active than dexamethasone, but this result can be explained by the original difference in the activity of the starting compounds (*ie* hydrocortisone is about 100 times less active than dexamethasone).

Discussion

Since the first topical use of hydrocortisone in 1952, several potent corticosteroid derivatives have been developed and used in dermatology [1]. The major drawbacks in their use are their limited cutaneous absorption and, mainly, the important side effects provoked especially in long-term therapies. Thus, the development of new more potent steroids with the same profile is not advisable. On the contrary, new compounds synthesized according to the recently developed antedrug concept offer the advantage of a site-specific (topical) activity [20, 21].

The presence of sulfur atoms in the structure of a compound contributes to its relative rapid biotransformation and to its fast elimination, the oxidized metabolites being inactive in most of the cases [22]. Furthermore, sulfur containing compounds have shown, generally, a good cutaneous distribution (*ie* affinity for the skin) [23, 24]. Therefore, the incorporation of sulfur atoms in the structure of corticoids seemed to us an attractive way of creating new compounds for topical use, which is in agreement with the antedrug concept.

The compounds of series I which showed a glucocorticoid activity (*eg* compound 5), can be considered as partial antedrugs, since they can be hydrolyzed affording the active parent steroid. The inactive compounds of series I (*eg* compound 6) can be considered as prodrugs, since they can be metabolized to the active parent steroid. However, in both cases, the intact molecules may be retained in the skin by interacting with endogenous keratin through the formation of sulfur bonds, resulting in prolonged activity. Such a mechanism has been suggested for sulfur containing anti-inflammatory steroids with prolonged activity [24].

The pharmacological evaluation *in vitro* of the compounds synthesized, confirmed the structure–activity relationships of corticosteroids reported in the literature regarding modifications in the 21-position [25–28]. Our results confirmed that relatively bulk substituants can be introduced in this position of steroids without total loss of the glucocorticoid activity. Furthermore, the results obtained for the compound of series III showed that a sulfur atom can replace the oxygen atom at the 21-position without a significant loss of glucocorticoid activity (bioisosterism of sulfur and oxygen atoms). This modification, when introduced in other steroidal derivatives described in the literature, has led to similar results [28]. For example, tixocortol (another 21-thiosteroid derivative) maintains its glucocorticoid activity *in vitro*, evaluated in thymic cells [29].

After the first evaluation *in vitro* we proceeded with the development of one of these compounds: the 21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-S-acetylamino cysteine **13**. This compound possesses the characteristics of a steroid suitable for topical use: it is a potential antedrug with physicochemical characteristics appropriate for topical use and has the additional advantage of a rapid synthesis with a very high yield. The anti-inflammatory activity of compound 13 has been evaluated *in vivo* using animal models [30]. A relative anti-inflammatory activity of about 1/10 compared to dexamethasone was observed *in vivo*, in agreement with the *in vitro* results reported in the present paper. However, compound 13 was practically devoid of systemic activity (about 970 times less active than dexamethasone) suggesting that it behaves according to the antedrug concept. On the other hand, the lipophilicity of compound 13 is similar to that of readily absorbed steroids as clobetasol 17-propionate, hydrocortisone 17-butyrate, betamethasone 21-propionate [31] and close to the upper limit of lipophilicity appropriate for a maximum flux through the skin [32].

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra of solid samples were run as KBr disks on a Beckman 4230 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker 200 MHz spectrometer (chemical shifts are given in ppm (δ) downfield from tetramethyl silane (TMS). Chemical ionization (CI) mass spectra using NH_3 as reactant gas were recorded on a Nermag mass spectrometer. All compounds had IR, NMR and mass spectra that were fully consistent with their structure. When microanalyses are indicated by symbols of the elements, the analytical results were within ± 0.4% of the theoretical value.

Dexamethasone-21-mesylate and dexamethasone-21-iodide
These were synthesized as described by Cousel *et al* [7].

Diformyl cystine di-21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-diester ***5***

One g (3.4 mmol) of N,N'-di-formyl cystine and 5.5 ml of Et_3N were added in 20 ml of anhydrous DMF. The solution was stirred for a few minutes, then 3.01 g (6.3 mmol) of the iodo derivative of dexamethasone were added. After 2 h of stirring at 40°C the solution was poured in 200 ml of ethyl acetate. The organic phase was successively washed with H_2O, with a solution at 5% of $NaHCO_3$, with an aqueous solution of 1% HCl and finally with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness. The diester was purified by column chromatography (eluent AcOEt:$CHCl_3$) (7:3). Recrystallization from $CHCl_3$ afforded 30% of the analytical pure product. mp = 160°C; IR (KBr): 3400, 1740, 1720, 1500–1550 and 890 cm^{-1}. NMR ($CDCl_3$): 7.24 (d); 6.25 (dd); 5.70 (dd); 4.36 (m); 4.04 (m); 1.45 (s); and 0.95 (s). Mass spectral peaks were observed in the CI mode with ammonia at m/e 508 [MH^+] and [MNH_4^+] 525.

9α-fluoro-11β,17α,21,21-tetrahydroxy-16α-methyl-1,4-pregnadiene-3,20-dione (dexamethasone aldehyde)

To a solution of 25 g (64 mmol) of dexamethasone in 50 ml of methanol was added 50 ml (1.5 g) of methanolic cupric acetate. Air was bubbled into the solution for 35 min and following the addition of 100 mg of EDTA in 30 ml of water, the methanol was evaporated. The residue was diluted with water and extracted with $CHCl_3$. The yellow extract, after being washed with dilute sodium bicarbonate and water, was concentrated to dryness. Recrystallization from acetone gave the final product at a very high yield (80%). mp = 127–130°C; IR (KBr): 3400–3500, 1720, 1660, 1620 and 890 cm^{-1}; NMR ($CDCl_3$): 7.24 (d); 6.25 (dd); 5.70 (dd); 4.36 (m); 4.04 (m); 1.45 (s); and 0.95 (s).

21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-N-methionine methyl ester ***10***

To a solution of 2 g (5 mmol) of dexamethasone aldehyde in 30 ml of ethanol, 4.8 g (24 mmol) of methyl ester of methionine were added. To this solution were added 10 ml of a buffer solution of KH_2PO_4 (pH 7.4) and 0.5 g of $NaBH_3CN$. The mixture was stirred at 25°C for 48 h. Afterwards the mixture was acidified at pH 2 with HCl concentrated and the ethanol was evaporated to dryness. The residue was diluted with 20 ml of water, and the solution was alcalinized with KOH 6 N and extracted with 3 x 15 ml of ethyl acetate. The extracts were dried over $MgSO_4$ and AcOEt was removed. The product was dissolved in methanol and gaseous HCl was added. Recrystallization from ethanol afforded the final product with a 32% yield. mp = 176°C. Anal $C_{27}H_{41}N$ (C, H, N). IR (KBr): 3440, 1740, 1720, 1660 and 890 cm^{-1}. NMR ($CDCl_3$): 7.25 (d); 6.30 (dd); 6.04 (dd); 4.41 (m); 3.68 (s); 1.45 (s); and 0.95 (s).

21-yl-[9α-fluoro-11β,17α-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione]-S-acetylamino cysteine ***13***

10 g (10 mmol) of *N*-acetyl cysteine were dissolved in 32 ml of anhydrous acetone. 15 ml of Et_3N were added and the solution was stirred for a few minutes. After the addition of 5 g (0.010 mmol) of dexamethasone-21-mesylate, the mixture was stirred for 1 h. The solution was poured into 100 ml of ice-water and was acidified with concentrated HCl. The precipitate was filtered, washed with water and recrystallized with absolute ethanol. mp = 215°C (89%); IR (KBr) 3490, 1720, 1660, 1620, 1550 and 890 cm^{-1}. NMR ($CDCl_3$): 7.24 (d); 6.25 (dd); 5.90 (dd); 4.36 (m); 1.45 (s); and 0.95 (s). MS (CI/NH_3) m/z: 538 [MH^+].

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