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Soft glucocorticoid design: structural elements and physicochemical parameters determining receptor-binding affinity

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Following rational, retrometabolism-based drug design strategies, already two generations of cortienic acid-based soft corticosteroids have been designed. During their development, a large number of receptor-binding affinity (RBA) data for the glucocorticoid receptor (GR) were determined. RBA is a major determinant of therapeutic potential for corticosteroids, because GRs from different tissues and even from different species seem to be essentially the same. A quantitative analysis of these RBA data obtained from more than sixty structures was performed. Within both generations of soft steroids, good receptor-binding affinity could be achieved with adequate substitution at the sensitive 17α or 17β pharmacophores. For soft steroids that satisfy the main binding criteria at the glucocorticoid receptor, an indicator variable for a structural element (6α- or 9α-halogenation) and a physicochemical parameter (lipophilicity as measured by log $P_{o/w}$) account for a large portion of the variability in RBA. Following a classical, regression-type analysis, a QSAR model that accounts for close to 80% of the variability in the log RBA data could be built using only these two descriptors. According to these data, receptor binding affinity at the GR is dramatically increased by 6α- or 9α-halogenation and it also tends to increase with increasing lipophilicity.

1. Introduction

1.1. Glucocorticoids

Corticosteroids exert profound biological effects in almost every organ, and they are one of the most widely used drug classes (Schimmer and Parker 1996). Today, they are commonly used in a variety of clinical diseases mainly for their antiinflammatory and immunosuppressive effects, and the range of diseases that are considered as responsive to steroid therapy is indeed bewildering (Table 1) (Le Fanu 1999; Martindale 1996). Naturally occurring corticosteroids are synthesized by the adrenal cortex together with androgens. Historically, their actions were described as glucocorticoid and mineralocorticoid being related to the regulation of carbohydrate metabolism and electrolyte balance, respectively.

Based on activity data accumulated for a variety of natural and synthetic corticosteroids (see Fig. 1 for the numbering and notation system of these structures), a number of structural requirements for glucocorticoid and mineralocor-

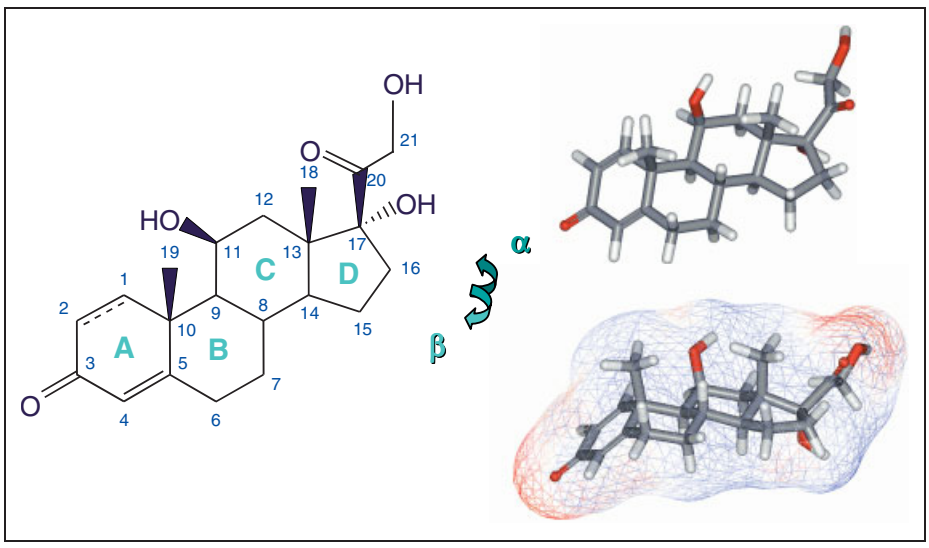


Fig. 1:
Common numbering and notation system of the steroid structure. To illustrate the corresponding three-dimensional structure, two views from different angles are also included on the right side; the bottom one also includes a soft wire-mesh surface

Table 1: Alphabetical listing of diseases currently considered as responsive to steroid therapy (Le Fanu 1999; Martindale 1996)

- Addison's disease
- Anaphylactic shock
- Aspiration syndromes
- Behcet's syndrome
- Bites and stings
- Blood disorders
  - Cold hemagglutinin disease
  - Hemangioma
  - Hemolytic anemia
  - Hypereosinophilia
  - Hypoplastic anemia
  - Macroglobulanemia
  - Thrombocytopenic purpura
- Cancer
  - Hodgkin's disease
  - Leukemia
- Cerebral edema
- Cogan's syndrome
- Congenital andrenal hyperplasia
- Connective tissue disorders
  - Dermatomyositis
  - Polymyalgia rheumatica
  - Polymyositis
  - Systemic lupus erythematosus
- Epilepsy
- Eye disorders
  - Allergic conjunctivitis
  - Corneal graft rejection
  - Iritis
  - Keratitis
  - Optic neuritis
  - Post cataract surgery
  - Retinal vasculitis
  - Scleritis
  - Sympathetic ophthalmia
  - Uveitis
- Gastrointestinal disorders
  - Crohn's disease
  - Hemorrhoids (piles)
  - Hypercalcemia
  - Ulcerative colitis
- Infections
  - Glandular fever
  - Leishmaniasis
  - Leprosy
  - Meningitis
  - Pneumocystis carinii pneumonia
  - Septic shock
  - Tuberculosis
- Kidney disorders
  - Lupus nephritis
  - Membranous nephropathy
  - "Minimal change" nephritis
  - Renal transplant
- Liver disorders
  - Alcoholic liver disease
  - Biliary cirrhosis
  - Chronic active hepatitis
  - Sclerosing cholangitis, liver transplant
- Male infertility
- Neurological disorders
  - Bell's palsy
  - Coma
  - Multiple sclerosis
  - Myasthenia gravis
  - Polyneuropathies
- Organ and tissue transplantation
- Respiratory disorders
  - Acute eosinophilic pneumonia
  - Asthma
  - Chronic obstructive pulmonary disease
  - Croup
  - Fat embolism syndrome
  - Fibrosing alveolitis
  - Pulmonary eosinophilia
  - Sarcoidosis
- Rheumatoid disease and osteoarthritis
- Rhinitis
- Skin disorders
  - Alopecia
  - Atopic dermatitis
  - Contact dermatitis
  - Dermatitis herpetiformis
  - Eczema
  - Infantile eczema
  - Lichen sclerosis
  - Neurodermatitis
  - Pemphigoid
  - Pemphigus
  - Psoriasis
  - Pyoderma gangrenosum
  - Seborrhoeic dermatitits
  - Urticaria
- Spinal cord injury
- Thyroid disorders
- Vascular disorders

ticoid activities are now commonly accepted (Schimmer and Parker 1996); the more important ones are summarized in Fig. 2. In ring A, the 3-keto group and the 4,5 double bond ($\Delta^{4,5}$) seem essential for both gluco- and mineralocorticoid activities, whereas an additional 1,2 double bond ($\Delta^{1,2}$) seems to selectively increase glucocorticoid activity. In ring B, halogenation (most commonly fluorination) at the 6α and 9α position enhances both glucocorticoid and mineralocorticoid activity. In ring C, an 11β hydroxyl substitution seems required for glucocorticoid, but not for mineralocorticoid activity. In ring D, there seems to be some freedom in choosing substitutions at C^{16} and C^{17}. Substitutions at C^{16} tend to eliminate mineralocorticoid activity. By all indications, a hydroxyl group at C^{21} seems required for mineralocorticoid activity, but it is not an absolute requirement for glucocorticoid activity. Many of these structural requirements have been recently reinforced by solving the structure of a glucocorticoid receptor with a bound ligand as it will be briefly discussed in the next subchapter.

1.2. The glucocorticoid receptor

Glucocorticoids have been in use as potent antiinflammatory agents for more than 50 years, and the addition of cortisone to the therapeutic arsenal is usually considered

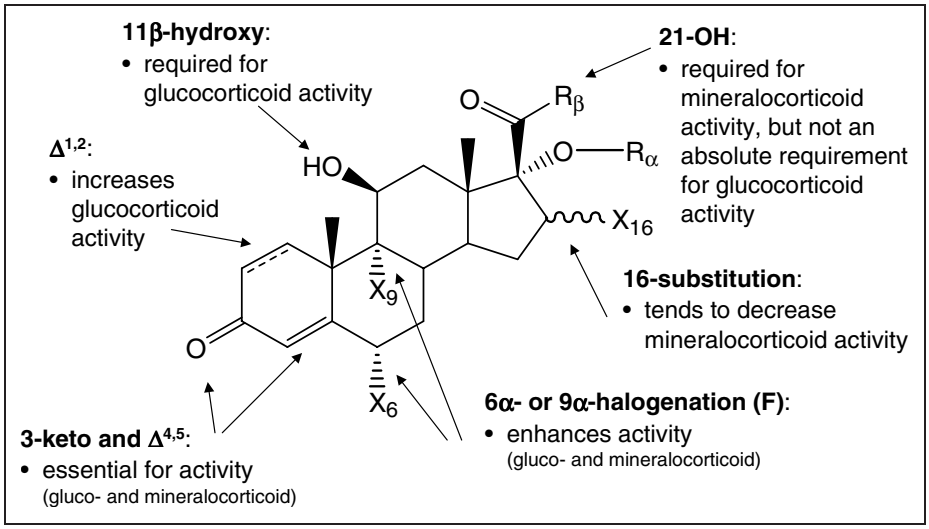


Fig. 2:
Commonly accepted structure-activity relationship for corticosteroid structures according to current knowledge

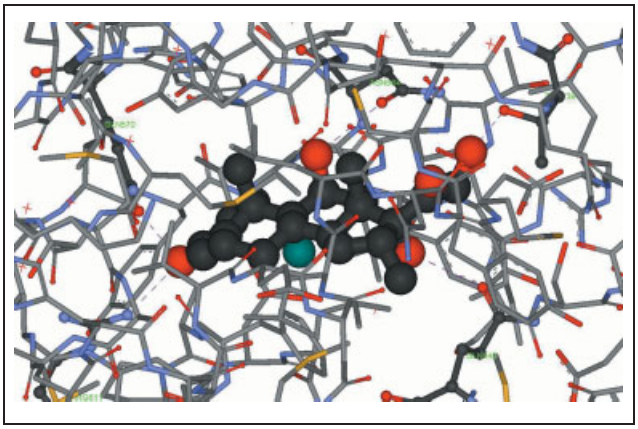


Fig. 3: Dexamethasone with the surrounding amino acid residues in the crystal structure of the human glucocorticoid ligand-binding domain. Structure 1M2Z (Bledsoe et al. 2002) was obtained from the Protein Data Bank and is displayed using DS ViewerPro 5.0 (Accelrys, Inc., San Diego, CA). Dexamethasone is shown as a darker, scaled ball-and-stick structure; residues indicated by the HBond monitor tool of the software as hydrogen bonded to the dexamethasone ligand (Asn^{564}, Gln^{570}, Arg^{611}, Gln^{642}, and Thr^{739}) are also shown as somewhat smaller scaled ball-and-stick structures; and the corresponding hydrogen bonds are shown as dashed lines

as one of the ten definitive moments of modern medicine (Le Fanu 1999). Nevertheless, the molecular mechanism of glucocorticoid action started to emerge only recently (Baraniuk 1996; Barnes 2001). These steroids exert their action by binding to glucocorticoid receptors (GRs). GRs are predominantly localized to the cytoplasm of target cells and move into the nuclear compartment only on binding of the glucocorticoid. The binding affinity of cortisol (hydrocortisone) to the GR seems to be around 30 nM, which is within the normal plasma concentration range of free cortisol (Barnes et al. 1998).

Glucocorticoid receptors (GRs) are soluble, intracellular proteins that act as ligand-regulated transcription factors controlling specific gene expression in most mammalian cells (Muller and Renkawitz 1991). GR structural studies have been hampered by problems related to the expression and purification of an active protein. Mostly due to solubility problems, GR was more difficult to express than other members of the nuclear receptor super-family. Besides GR, this steroid-thyroid-retinoid receptor super-family includes receptors for steroids, such as receptors for mineralocorticoids (MR), estrogens (ER), progestins (PR), and androgens (AR), as well as receptors for thyroid hormone, vitamins A- an D-derived hormones, and certain fatty acids (Kumar and Thompson 1999). Nevertheless, the crystal structure of the human GR ligand binding domain (LBD) bound to dexamethasone has been recently determined by using a receptor with a single point mutation (F602S) (Bledsoe et al. 2002). Interestingly, and in good agreement with the results of the present study, GR seems to have an additional branch compared to the steroid-shaped pocket of AR, ER, or PR, and it can accommodate the larger 17α substituents of glucocorticoids that are not present in estrogen, progesterone, or testosterone. In the crystal structure (Fig. 3), dexamethasone seems to occupy only about 65% of the volume of the GR steroid pocket, whose total volume was estimated to be around 580 $Å^3$. Nevertheless, nearly every atom of the steroid core of dexamethasone is in contact with one or more hydrophobic residue of GR, and there also are specific protein-ligand hydrogen bonds at all hydrophilic moieties (e.g., at the C^3 ketone, at the 11-, 17α-, and 21-hydroxyl) (Fig. 3). These interactions are likely to provide the binding specificity at GR and probably also are responsible for many of the SARs indicated in Fig. 2. For example, steroids active at the mineralocorticoid receptor (MR), such as aldosterone or corticosterone, lack the 17α-hydroxyl group, which is hydrogen-bonded to Gln^{642} in the GR (Fig. 3). At this position, MR has a hydrophobic residue (Leu^{848}) that cannot form hydrogen bonds (Bledsoe et al. 2002).

Receptor-binding affinity (RBA) is, in general, a major determinant of therapeutic potential. For corticosteroids, it is even more so, because glucocorticoid receptors from different tissues and even from different species seem to be essentially the same. Hence, relative RBAs (RRBA; usually expressed using dexamethasone as reference, $RRBA_{Dex} = 100$) and various *in vitro* and *in vivo* pharmacological properties tend to correlate closely (Kelly 1998). For example, RBA has been shown to be related to the clinical efficacy of inhaled glucocorticoids (Rohdewald 1998), to side effects such as cortisol suppression (Derendorf et al. 1998; Rohatagi et al. 2003), or to immunosuppressive potency (Mager et al. 2003). For the same reason, a simple noninvasive model (skin blanching from vasoconstriction) can be a good predictor of the *in vivo* potency for glucocorticoids.

Corticosteroids exert their main action via binding to these hormone receptors that regulate the expression of corticosteroid-responsive genes, but there is also increasing evidence that corticosteroids can exert non-genomic effects as well. Unfortunately, because of their intrinsic multiple activity and because of the ubiquitous distribution of the corticosteroid receptors, unwanted side effects (Table 2) tend to closely parallel therapeutic effectiveness. This still represents a serious impediment despite chemical manipulations that yielded highly potent compounds with greater separation of glucocorticoid and mineralocorticoid activity. Furthermore, corticosteroids are also subject to different oxidative and/or reductive metabolic conversions. Forma-

Table 2: Alphabetical listing of the common side effects of glucocorticoid therapy

Receptor mediated effects
Adrenal suppression causing dependency on glucocorticoid therapy (withdrawal symptoms)
Cushingoid features (moon face, truncal obesity, wasted limbs – generally associated with diabetes and hypertension)
Easy bruising and skin thinning (for topical application)
Growth inhibition in children
Mineralocorticoid side effects
Osteoporosis
Suppression of the hypothalamic-pituitary-adrenal (HPA) axis
Other effects (resulting from reactivity with macromolecules)
Cataract formation
Immunogenecity
IOP (intraocular pressure) – elevation

tion of various steroidal metabolites can lead to undesirably complex situations. A considerable number of attempts were aimed to improve this situation, and soft drug approaches are particularly well suited for this purpose.

1.3. Soft steroids

Soft drugs are new, active therapeutic agents, often isosteric-isoelectronic analogues of a lead compound, with a chemical structure specifically designed to allow predictable metabolism into inactive metabolites after exerting the desired therapeutic effect. Inclusion of a metabolically sensitive moiety into the drug molecule makes possible the design and prediction of the major metabolic pathway and avoids the formation of undesired toxic, active, or high-energy intermediates (Bodor 1984; Bodor and Buchwald 2000; 2003).

Within the various retrometabolism-based drug design and targeting approaches, soft corticosteroids represent one the most successful areas (Bodor 1991; 1993; 1999; Bodor and Buchwald 2002; 2003). Following rational, soft drug design strategies, already two generations of soft corticosteroids have been designed. Despite both approaches starting from the same known inactive metabolite (Δ^1-cortienic acid), there are considerable structural differences between the corresponding two generations of steroid structures. Loteprednol etabonate (LE) and etiprednol dicloacetate (ED) are representatives of the first- and second-generation of soft steroids, respectively that were selected for development. LE has already reached the market for ocular administration (Alrex, Lotemax) and is garnering increasing market share (Bodor and Buchwald 2002; Noble and Goa 1998). Both LE and ED are in various stages of clinical development for a full spectrum of other possible applications, such as nasal spray for rhinitis, inhalation products for asthma, oral tablet for IBD, or topical cream or lotion for dermatological applications.

1.3.1. First generation soft steroids (loteprednol etabonate and analogs)

During the design and development of the first generation of soft steroids, more than 120 compounds that resulted from modifications of the 17β carboxyl function and the 17α hydroxy function together with other changes intended to enhance corticosteroid activity (introduction of Δ^1, fluorination at 6α and/or 9α, methylation at 16α or 16β) have been synthesized (Bodor 1991; 1996; Druzgala et al. 1991; Hochhaus et al. 1991). This process was the result of a classic inactive metabolite-based soft drug approach that started from cortienic acid. Hydrocortisone (no Δ^1; R_α, X_6, X_9, $X_{16} = H$; $R_\beta = CH_2OH$) undergoes a variety of oxidative and reductive metabolic conversions (Monder and Bradlow 1980). Oxidation of its dihydroxyacetone side chain leads to formation of cortienic acid (no Δ^1; R_α, X_6, X_9, $X_{16} = H$; $R_\beta = OH$) through a 21-aldehyde (21-dehydrocortisol) and a 21-acid (cortisolic acid). Cortienic acid is an ideal lead for the inactive metabolite approach because it lacks corticosteroid activity and is a major metabolite excreted in human urine. Starting from cortienic acid, active compounds can be obtained if the important pharmacophores found in the 17α and 17β side chains can be restored. Suitable isosteric/isoelectronic substitution of the α-hydroxy and β-carboxy substituents with esters or other types of functions should restore the original corticosteroid activity and also incorporate hydrolytic features to help avoid accumulation of toxic levels. As mentioned, numerous such soft steroids that resulted from modifications of the 17β carboxyl function and the 17α hydroxy function together with other changes intended to enhance corticosteroid activity have been synthesized at Otsuka Pharmaceutical Co., Japan.

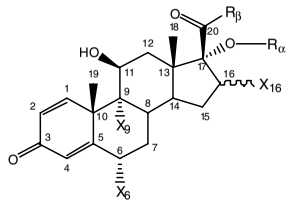
A haloester in the 17β position and a novel carbonate (Bodor 1991; Druzgala et al. 1991) or ether (Druzgala and Bodor 1991) substitution in the 17α-position were found as critical functions for activity. Incorporation of 17α carbonates or ethers was preferred over 17α esters to enhance stability and to prevent formation of mixed anhydrides that might be produced by reaction of a 17α ester with a 17β acid functionality. Such mixed anhydrides were assumed toxic and probably cataractogenic. A variety of 17β esters was synthesized. Because this position is an important pharmacophore that is sensitive to small modifications, the freedom of choice was relatively limited. For example, although chloromethyl or fluoromethyl esters showed very good activity, the chloroethyl or α-chloroethylidene derivatives demonstrated very weak activity (see data of Table 3). Simple alkyl esters also proved virtually inactive. Consequently, the 17β chloromethyl ester was held constant ($R_\beta = OCH_2Cl$) and 17α-carbonates with different substituents on the steroid skeleton were varied for further investigation. LE (Δ^1; X_6, X_9, $X_{16} = H$; $R_\alpha = COOCH_2CH_3$, $R_\beta = OCH_2Cl$), and some of the other soft steroids, provided a significant improvement of the therapeutic index determined as the ratio between the antiinflammatory activity and the thymus involution activity (Bodor 1988; 1989; Bodor and Buchwald 2002). Furthermore, as indicated by the RBA data, some of these compounds approach and even exceed the binding affinity of the most potent corticosteroids known (see data of Table 3). LE was selected for development based on various considerations including the therapeutic index, availability, synthesis, and "softness" (the rate and easiness of metabolic deactivation).

1.3.2. Second generation soft steroids (etiprednol dicloacetate and analogs)

More recently, a second generation of soft steroids with 17α-dichloroester substituent has been developed ($R_\alpha = COCHCl_2$) (Bodor 1999). This is a unique design: no known corticosteroid contains halogen substituents at the 17α position. Nevertheless, the pharmacophore portions of these steroids, including the halogen atoms at 17α, can be positioned so as to provide excellent overlap with those of the traditional corticosteroids, including LE (Fig. 4). Dichlorinated substituents seem required for activity and sufficiently soft nature. At present, two justifications seem likely. First, with dichlorinated substituents, one of the Cl atoms will necessarily point in the direction needed for pharmacophore overlap, but with monochlorinated substituents, steric hindrance will force the lone Cl atom to point away from this desired direction. Second, whereas dichloro substituents increase the second order rate constant k_{cat}/K_M of enzymatic hydrolysis in acetate esters by a factor of about 20 compared to the unsubstituted ester, monochloro substituents do not cause any change (Barton et al. 1994).

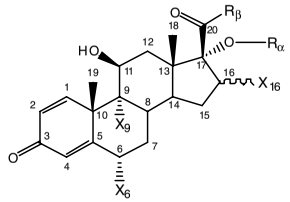
Contrary to the first generation, in this second generation of soft steroids, hydrolysis primarily cleaves not the 17β-positioned, but the 17α-positioned ester. Nevertheless, the corresponding metabolites are also inactive. Members of this class, including ED, have shown better receptor binding than LE. ED has also been proven as, or even more

Table 3: Structures and data for the present RBA study



Compound	$\Delta^{1,2}$	X_6	X_9	X_{16}	R_α	R_β	log P	RRBA	R.*	MW	Ve	ACD log P	CLOGP	QLogP	Avg.	log RRBA no $X_{6,9}$	log RRBA w $X_{6,9}$
Beclomethasone	+	H	Cl	βCH_3	H	CH_2OH		100		408.9	306.4	2.42	2.13	2.52	2.36		2.00
Betamethasone	+	H	F	βCH_3	H	CH_2OH	1.94	60		392.5	298.2	2.06	1.79	2.26	2.04		1.78
Corticosterone	–	H	H	H	no OH	CH_2OH	1.94	10		346.5	279.5	1.76	2.32	2.21	2.10	1.00	
Dexamethasone	+	H	F	αCH_3	H	CH_2OH	1.83	100		392.5	298.0	2.06	1.79	2.26	2.03		2.00
ED (BNP-166)	+	H	H	H	$COCHCl_2$	OCH_2CH_3		200		485.4	347.3	4.71	4.46	4.15	4.44	2.30	
LE5601	–	H	H	H	$COOCH_2CH_3$	OCH_2Cl		150		469.0	348.7	3.43	3.61	2.97	3.34	2.18	
LE5602	+	H	H	H	$COO(CH_2)_3CH_3$	OCH_2Cl		110		495.0	370.5	4.75	4.40	3.61	4.25	2.04	
LE5603	–	H	H	H	$COOCH(CH_3)_2$	OCH_2Cl		70		483.0	362.7	3.77	3.92	3.38	3.69	1.85	
LE5606	+	H	H	H	$COOCH_2CH_3$	OCH_2SCH_3		3	r1	478.6	362.0	3.85	3.56	3.57	3.66		
LE5608	+	H	H	H	$COOCH_2CH_3$	OCH_2CH_3		<1	r1	446.5	345.3	3.59	3.75	2.87	3.41		
LE5610	+	H	H	H	$COOCH(CH_3)_2$	OCH_2Cl		200		481.0	356.5	4.04	3.65	3.20	3.63	2.30	
LE5613	+	H	H	H	$COOCH_2Cl$	OCH_3		<1	r1	452.9	328.5	3.04	3.31	3.23	3.19		
LE5614	+	H	F	H	$COOCH_2CH_3$	OCH_2CH_3		<1	r1	464.5	348.6	3.47	3.60	2.97	3.35		
LE5618	+	H	H	H	$COOCH_2OCH_3$	OCH_2Cl		16		483.0	350.0	3.00	2.61	2.46	2.69	1.20	
LE5621	+	H	H	H	$COOCOOCH_3$	OCH_2Cl		<1	r2	496.9	350.2	3.43	2.37	2.47	2.76		
LE5623	+	H	H	H	$COOCH_2OCH_3$	CH_2Cl		10		467.0	343.0	2.99	2.35	2.24	2.53	1.00	
LE5628	+	H	F	αCH_3	$COOCH_2CH_3$	OCH_2Cl		740		499.0	359.7	4.06	3.70	3.30	3.69		2.87
LE5629	+	H	F	αCH_3	$COOCH(CH_3)_2$	OCH_2Cl		560		513.0	373.9	4.41	4.01	3.71	4.04		2.75
LE5638	+	H	F	βCH_3	$COOCH(CH_3)_2$	OCH_2Cl		440		513.0	374.1	4.41	4.01	3.72	4.04		2.64
LE5639	+	H	F	βCH_3	$COOCH_2CH_3$	OCH_2Cl		820		499.0	360.0	4.06	3.70	3.30	3.69		2.91
LE5643	+	H	H	H	$COOC_6H_5$	OCH_2Cl		80		515.0	373.2	4.50	3.94	3.69	4.04	1.90	
LE5644	–	H	H	H	$COO(CH_2)_2CH_3$	OCH_2Cl		210		483.0	362.6	3.96	4.14	3.38	3.83	2.32	
LE5648	+	H	F	αCH_3	$COO(CH_2)_2CH_3$	OCH_2Cl		870		513.0	373.9	4.59	4.23	3.71	4.18		2.94
LE5649	+	H	H	H	$COOCH(CH_3)_2$	OCH_3		3	r1	446.5	345.0	3.40	3.53	3.75	3.56		
LE5651	+	H	H	H	$COOCH(CH_3)_2$	$OCH_2OCH_2CH_3$		<1	r1	490.6	380.3	3.94	3.70	3.43	3.69		
LE5654	+	H	H	H	$COOCH(CH_3)_2$	$OCH(CH_3)Cl$		10	r3	495.0	370.4	4.38	4.18	3.61	4.05		
LE5657	+	F	H	βCH_3	$COOCH(CH_3)_2$	$OCH(CH_3)Cl$		11	r3	527.0	387.8	4.42	4.74	4.12	4.42		
LE5658	+	H	F	αCH_3	$COO(CH_2)_4CH_3$	OCH_2Cl		840	r4	541.1	401.9	5.66	5.29	4.53	5.16		
LE5660	+	H	F	αCH_3	$COOCH_2CH_3$	OCH_2Cl, 11-keto		16		497.0	354.9	4.34	3.89	3.09	3.77		
LE5671	+	H	F	αCH_3	$COOCH(CH_3)_2$	OCH_2F		820		496.5	365.9	3.95	3.57	3.47	3.66		2.91
LE5673	+	F	F	αCH_3	$COOCH_2CH_3$	OCH_2Cl		2100		517.0	363.1	3.77	3.74	3.39	3.64		3.32
LE5679	+	H	F	αCH_3	$COOCH_2CH_2Cl$	OCH_3		<1	r1	499.0	359.7	3.70	3.65	3.30	3.55		
LE5683	+	H	F	αCH_3	$COOCH_2CH_3$	OCH_2CH_2Cl		19	r5	513.0	373.7	4.23	4.18	3.70	4.04		
LE5685	+	H	H	H	$COOCH(CH_3)_2$	OCH_2CH_2Cl		1	r5	495.0	370.4	4.20	4.13	3.61	3.98		
LE5687	+	H	F	αCH_3	H	OCH_2Cl		7	r6	426.9	309.5	2.56	2.65	3.07	2.76		
LE5689	+	F	F	αCH_3	$COOCH(CH_3)_2$	OCH_2Cl		1100		531.0	377.1	4.12	4.05	3.80	3.99		3.04
LE5690	+	F	F	αCH_3	$COO(CH_2)_2CH_3$	OCH_2Cl		1000		531.0	377.3	4.30	4.27	3.81	4.13		3.00
LE5693	+	H	F	αCH_3	$COO(CH_2)_2CH_3$	OCH_2F		990		496.5	366.0	4.14	3.79	3.48	3.80		3.00

Table 3: (continued)



Compound	$\Delta^{1,2}$	X_6	X_9	X_{16}	R_α	R_β	log P	RRBA	R.*	MW	Ve	ACD log P	CLOGP	QLogP	Avg.	log RRBA no $X_{6,9}$	log RRBA w $X_{6,9}$
LE5698	+	F	H	αCH$_3$	COO(CH$_2$)$_2$CH$_3$	OCH$_2$Cl		1000		513.0	373.8	4.26	4.43	3.71	4.13		3.00
LE5699	+	F	H	αCH$_3$	COOCH(CH$_3$)$_2$	OCH$_2$Cl		820		513.0	374.0	4.07	4.21	3.71	4.00		2.91
LE5702	+	H	H	H	COOCH$_3$	OCH$_2$Cl		180		452.9	328.5	3.16	2.81	3.23	3.07	2.26	
LE5704	+	F	H	αCH$_3$	COOCH$_3$	OCH$_2$Cl		1200		484.9	345.8	3.19	3.37	2.89	3.15		3.08
LE5707	+	H	F	βCH$_3$	COOCH$_3$	OCH$_2$Cl		990		484.9	346.0	3.19	3.17	2.89	3.09		3.00
LE5709	+	H	F	βCH$_3$	COO(CH$_2$)$_2$CH$_3$	OCH$_2$Cl		1460		513.0	374.0	4.26	4.23	3.71	4.07		3.16
LE5711	+	H	H	H	COOCH$_2$CH$_3$	OCH$_2$F		200		450.5	334.5	3.23	2.90	3.42	3.18	2.30	
LE5712	+	H	H	H	COOCH(CH$_3$)$_2$	OCH$_2$F		70		464.5	348.5	3.58	3.21	2.97	3.25	1.85	
LE5715	+	H	H	βCH$_3$	H	OCH$_2$Cl		3	r6	408.9	306.0	2.68	2.81	2.97	2.82		
LE5718	+	H	H	H	CH$_3$	OCH$_3$		<1	r1	374.5	295.3	2.41	2.64	2.67	2.57		
LE5720	+	H	H	H	(CH$_2$)$_2$CH$_3$	O(CH$_2$)$_2$CH$_3$		10	r1	430.6	351.4	4.53	4.62	4.27	4.47		
LE5721	+	H	F	αCH$_3$	CH$_3$	OCH$_3$		25	r1	406.5	312.5	2.78	3.00	3.16	2.98		
LE5725	+	H	H	H	CH$_3$	OCH$_2$Cl		10		408.9	306.7	3.04	2.75	2.99	2.93	1.00	
LE5726	+	H	H	H	(CH$_2$)$_2$CH$_3$	OCH$_2$Cl		315		437.0	334.7	4.11	3.67	3.79	3.86	2.50	
LEGH01	–	H	H	H	CH$_3$	OCH$_2$Cl		3	r6	410.9	312.8	2.78	3.03	3.17	2.99		
LEGH02	–	H	H	H	CH$_2$CH$_3$	OCH$_2$Cl		29		425.0	326.7	3.31	3.42	3.56	3.43	1.46	
LEGH03	–	H	H	H	(CH$_2$)$_2$CH$_3$	OCH$_2$Cl		132		439.0	340.7	3.84	3.94	3.96	3.92	2.12	
LEGH04	–	H	H	H	(CH$_2$)$_3$CH$_3$	OCH$_2$Cl		124		453.0	354.7	4.37	4.47	4.37	4.40	2.09	
LEGH05	–	H	H	H	CH$_2$SCH$_3$	OCH$_2$Cl		54		457.0	343.1	3.68	3.34	3.43	3.48	1.73	
LEGH06	–	H	H	H	CH$_2$OCH$_3$	OCH$_2$Cl		6	r6	441.0	334.1	2.89	2.78	3.17	2.95		
LEGH07	–	H	H	H	CH$_2$OCH$_2$CH$_3$	OCH$_2$Cl		10	r6	455.0	348.1	3.43	3.17	3.58	3.39		
LEGH08	–	H	H	H	CH(CH$_3$)OCH$_3$	OCH$_2$Cl		9	r6	455.0	348.0	3.24	3.09	3.58	3.30		
LEGH09	–	H	H	H	CH(CH$_3$)OCH$_2$CH$_3$	OCH$_2$Cl		2	r6	469.0	362.2	3.77	3.48	3.99	3.74		
LE	+	H	H	H	COOCH$_2$CH$_3$	OCH$_2$Cl	3.03	150		467.0	345.9	3.69	3.34	2.89	3.31	2.18	

* Remarks: r1 – no halogen or hydroxyl substituent in R_β, r2 – unsuitable R_α, r3 – inadequately positioned halogen substituent in R_β (branched R_β), r4 - R_α too large, r5 – inadequately positioned halogen substituent in R_β, r6 – inadequate R_α

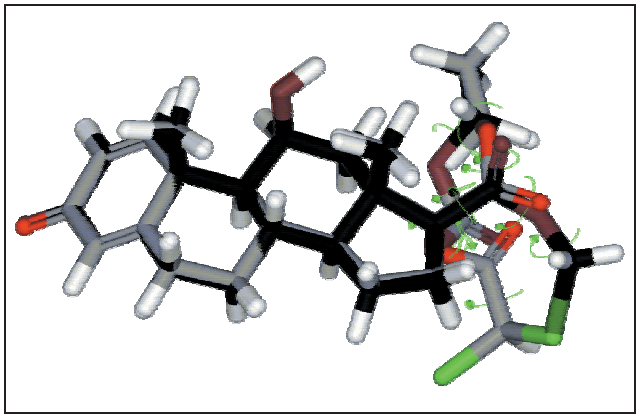


Fig. 4: Overlap of the soft corticosteroids loteprednol etabonate (LE, in darker colors) and etiprednol dicloacetate (ED, in lighter colors) generated by Discovery Studio's (Accelrys, Inc., San Diego, CA) Molecular Overlay algorithm within ViewerPro 5.0. The overlap was generated starting from individually AM1-optimized structures (CAChe 5.0, Fujitsu, Ltd., Chiba, Japan), and then allowing for conformational change during a consensus overlap along the rotatable bonds shown in the figure. Atoms in the steroid ring structure, a pair of chlorine atoms, and the two pairs of carbon atoms in the alkyl side-chains were uses as tethers in generating the overlap. Note the good overlap even between oxygen atoms that were not required to overlap. The view is from the β side, from above the steroid ring system

effective, than budesonide in various asthma models, and, in agreement with its soft nature, was found as having low toxicity in animal models and in human clinical trials (Bodor 1999; Kurucz et al. 2003; Miklós et al. 2002).

2. Investigations, results, and discussion

In the present study, available soft steroid relative receptor binding affinity (RRBA) data were collected and subjected to a QSAR-type analysis in an attempt to establish the structural and physicochemical requirements of adequate receptor-binding. The overwhelming majority of structures are from the first generation of soft steroids with beclomethasone, betamethasone, corticosterone, and dexamethasone, as well as ED and LE included for reference; data are presented in Table 3. Not surprisingly, a number of structures were inactive as they did not satisfy the known structural requirements of receptor binding; these were omitted from the regression study, and a corresponding footnote in Table 3 (r1–r6) indicates the reason for inactivity (e.g., no halogen or hydroxyl substituent in R_β, inadequately positioned halogen substituent in R_β, or unsuitable R_α).

For the remaining 38 structures, a classical, regression-type QSAR study was performed with descriptors known to be relevant for receptor binding: molecular size descriptors (molecular weight, volume, and surface area), hydrogen bond descriptors (number of hydrogen-bond acceptors, donors, and the *N* descriptor of QLogP), lipophilicity descriptors (calculated log octanol-water partition coefficients log $P_{o/w}$), and indicator variables for halogenation at X_6 and/or X_9. As required by free energy considerations, log RRBA was used in all correlations. This corresponds to ΔΔG values compared to dexamethasone, the standard reference used in calculating relative receptor-binding affinities. It became immediately apparent that halogenation of the steroid structure at the 6α or 9α position and molecular size or lipophilicity are two major determinants of receptor affinity as long as all the other structural requirements of binding to the GR are satisfied.

Structures that are halogenated (fluorinated) at the 6α- and/or 9α-position have, on average, an almost tenfold increased RBA compared to their unsubstituted parent compound. Use of a simple indicator variable (I_F, 0 or 1) for these substitutions already accounts for 51% of the variability in log RRBA data on these 38 structures with an average increase of 0.92 in log RRBA for the 18 halogenated compounds. There was no major difference between substitutions at the 6α- or 9α-position. Furthermore, a second fluorination seems to cause some improvement, but far less than the approximately tenfold increased caused by the first fluorination, and only three such examples were available in the present data; see LE5689 (RRBA of 1100) vs. LE5699 (820), LE5673 (2100) vs. LE5628 (740), and LE5690 (1000) vs. LE5648 (870) in Table 3. Therefore, a single indicator variable was used in the final correlation for all halogenated compounds. This was somewhat surprising as 6α- or 9α-fluorination (halogenation) is usually considered as activity enhancing for any structure, and a previous SAR study on antiinflammatory activity indicated not just additive, but even synergistic effects for this pair of substituents (Bodor et al. 1983). It also has to be noted that, because of synthetic considerations, all halogenated compounds also contained 16α or 16β methyl substitutions, but we assumed halogenation to be the more specific, activity-enhancing substitution. It still remains to be clarified why does 6α- or 9α-halogenation increases glucocorticoid-receptor binding so significantly. The crystal structure of the dexamethasone-receptor complex (Fig. 3) does not seem to indicate the presence of any special interaction. Interestingly, 6α-halogenation (F, Cl) has no effect on binding of progesterone to its receptor (Seeley et al. 1982).

Addition of molecular size (e.g., molecular volume, V) or lipophilicity (log $P_{o/w}$) results in models of similar and very good quality, with lipophilicity giving somewhat better description than size: r^2 of 0.79 vs. 0.73. To increase the reliability of the lipophilicity estimate, three different log $P_{o/w}$ values were calculated (ACD/LogP, CLOGP, and QLogP) for every structure, and their average was used in the final correlation. Nevertheless, use of any one of these calculated log $P_{o/w}$ values by itself in the final correlation has no significant effect on the final conclusions. The final model uses only two parameters and accounts for close to 80% in the variability of log RRBA:

$$\log \text{RRBA} = 0.101\ (\pm 0.280) + 0.512\ (\pm 0.078) \log P_{o/w} + 0.880\ (\pm 0.102) I_F$$

$$n = 38, \qquad r^2 = 0.780, \qquad \sigma = 0.313, \qquad F = 62.0$$

Introduction of further descriptors could improve the correlation, but it only worsened the F-statistics; therefore, we settled on this model, which is far from being complete, but already gives good quantitative description. In fact, the standard error of this regression (0.31) is already somewhere within the range of the experimental error, as threefold differences among measured receptor binding affinities are considered quite common. Furthermore, available RRBA data for a variety of other steroids seem to fit this very same model very well (Buchwald and Bodor, to be submitted).

It is also somewhat surprising that a nonspecific parameter such as lipophilicity (or size) has such a significant influence on RRBA (Fig. 5), because one would expect receptor binding more strongly dependent on specific elements, such as the presence or absence of "pharmacophore" moieties. However, the compounds used in the regression

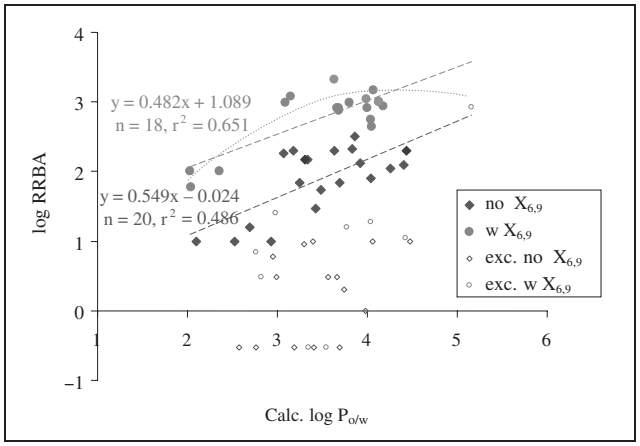


Fig. 5: Glucocorticoid relative receptor binding affinity (log RRBA) for the structures of the present study as a function of calculated log octanol-water partition coefficient (log $P_{o/w}$). If 6α- or 9α-halogenated compounds are considered separately, lipophilicity alone accounts for a large portion of the variance in the log RRBA data. Two separate trendlines for halogenated and non-halogenated steroids are shown together with the corresponding regressions. Data for compounds that do not satisfy known requirements for GR-binding were not used in the regression and are shown separately (open symbols). To allow graphical representation on a logarithmic scale, all RRBA < 1 values were arbitrarily set to 0.3 for the inactive compounds not used in the correlations

already satisfy the main structural requirements of binding to the GR, and they already contain the essentials pharmacophores required for adequate binding. Because GR seems to have a relatively large side pocket compared to other steroid receptors, as revealed by crystallographic studies (Bledsoe et al. 2002) and discussed earlier, unspecific (van der Waals-type) interactions in this side pocket may be mostly responsible for the RRBA variations seen, and such interactions may indeed be mainly size- and/or lipophilicity-dependent. Hence, as long as the substituents are not prohibitively large, increasing size (and consequently increasing lipophilicity) will result in increasing receptor binding (Fig. 5). In fact, some of the largest structures are probably already approaching the size limitations of the steroid-binding pocket of GR, and binding affinity seems to start declining for compounds with a calculated effective molecular volume V_e approaching 400 $Å^3$. This is especially evident in the halo-substituted group (see the curved trendline for this group in Fig. 5). By the same account, ED is approaching the maximum limit for a non-fluorinated steroid.

In conclusion, good receptor-binding affinity could be achieved within both generations of soft steroids with adequate substitution at the sensitive 17α or 17β pharmacophores. For soft steroids that satisfy the main binding criteria at the GR, 6α- or 9α-halogenation and lipophilicity (as characterized by log $P_{o/w}$) account for a large portion of the variability in log RRBA. A QSAR model of good quality could be built using only these two descriptors for a total of 38 structures, and it indicates that GR binding affinity is dramatically increased by 6α- or 9α-halogenation and it also tends to increase with increasing lipophilicity.

3. Experimental

Relative receptor-binding affinity data (RRBA, $RRBA_{Dex} = 100$) were determined using standard methodology and have been mostly published before (Bodor 1996; Bodor and Buchwald 2002; Druzgala et al. 1991; Hochhaus et al. 1991). Calculated log octanol-water partition coefficients (log $P_{o/w}$) were obtained using our own QLogP program (Bodor and Buchwald 1997; Buchwald 2000; Buchwald and Bodor 1998) from 3D molecular structures built using Alchemy (Tripos Assoc., St. Louis, MO, USA). The same software was used to calculate molecular volumes and surface areas. In addition, calculated log $P_{o/w}$ values were also used from CLOGP (ChemDraw Ultra 7.0, CambridgeSoft, Cambridge, Massachusetts, USA) and ACD/LogP (Advanced Chemistry Development, Toronto, Ontario, Canada). Statistical analyses were performed using a standard spreadsheet program (Microsoft Excel 2002).

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