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Discovery of betamethasone 17α-carbamates as dissociated glucocorticoid receptor modulators in the rat

Amjad Ali [a,*], James M. Balkovec [a], Mark Greenlee [a], Milton L. Hammond [a], Greg Rouen [a], Gayle Taylor [a], Monica Einstein [b], Lan Ge [b], Georgianna Harris [b], Terri M. Kelly [b], Paul Mazur [b], Shilpa Pandit [b], Joseph Santoro [b], Ayesha Sitlani [b], Chuanlin Wang [b], Joann Williamson [c], Michael J. Forrest [c], Ester Carballo-Jane [c], Silvi Luell [d], Karen Lowitz [d], Denise Visco [d]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, United States
[b] Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, United States
[c] Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, United States
[d] Department of Laboratory Animal Resources, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, United States

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ABSTRACT

A series of betamethasone 17α-carbamates were designed, synthesized, and evaluated for their ability to dissociate the two main functions of the glucocorticoid receptor, that is, transactivation and transrepression, in rat cell lines. A number of alkyl substituted betamethasone 17α-carbamates were identified with excellent affinity for the glucocorticoid receptor (e.g., **7**, GR IC_{50} 5.1 nM) and indicated dissociated profiles in functional assays of transactivation (rat tyrosine aminotransferase, TAT, and rat glutamine synthetase, GS) and transrepression (human A549 cells, MMP-1 assay). Gratifyingly, the in-vivo profile of these compounds, for example, **7**, also indicated potent anti-inflammatory activity with impaired effects on glucose, insulin, triglycerides, and body weight. Taken together, these results indicate that dissociated glucocorticoid receptor modulators can be identified in rodents.

1. Introduction

Glucocorticoids (GCs), for example, betamethasone **1** and prednisolone **2**, are among the most effective therapies available for the treatment of allergic and chronic inflammatory diseases.[1–3] However, the long-term use of GCs is limited by their severe, and sometimes irreversible, side effects such as diabetes, osteoporosis, glaucoma, skin atrophy, and muscle wasting. An unmet medical need, therefore, is the development of agents with improved therapeutic ratios compared to currently available steroids, particularly upon systemic administration. The last decade has provided important advances in our understanding of the molecular basis of GC-mediated actions or gene expression, and this work may lead to the development of improved GCs and more specific therapies in the future.[4,5] Indeed, efforts to identify a new generation of compounds with improved therapeutic profiles are currently underway, and recent investigations have focused on the molecular mechanisms of glucocorticoid receptor (GR) action.[6–9] The effects of GCs in target tissues are mediated by GR, the expression of which is ubiquitous. GR is a member of a superfamily of nuclear receptors, and it modulates gene expression by two different regulatory mechanisms, namely transactivation (TA) and transrepression (TR).[10,11] Transactivation (TA) depends on the binding of GR to *cis*-acting glucocorticoid response elements (GREs) in the promoter region of the glucocorticoid target genes leading to an increase in the transcription rate of the respective genes. In contrast, TR is mediated via protein–protein interactions with other transcription factors such as AP-1[12] and NF-κB,[13] thereby suppressing the transcriptional activities of these factors. The dissection of these two mechanisms has been achieved by a single amino acid substitution in the GR, such as A458T, Y735F, and I747T, which results in reduced transactivational activity.[14–16] In addition, studies of genetically engineered GR^{dim} mice that carry the substitution A458T in the dimerization domain, which impairs the ability of the GR to bind to DNA, revealed that thymocyte apoptosis and erythroblast proliferation require the transactivating function of GR, whereas the anti-inflammatory activity does not.[17] These findings therefore suggest that GCs lacking transactivation activity, so-called dissociated GCs, would quite likely sepa-

* Corresponding author. Tel.: +1 732 594 8549; fax: +1 732 594 2210.
E-mail address: amjad_ali@merck.com (A. Ali).

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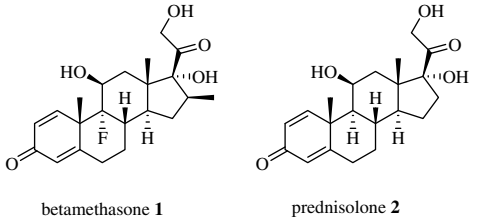
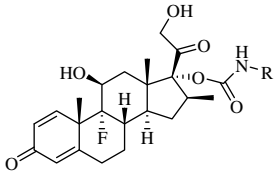
rate the therapeutic effects from the major side effects. In a program aimed at discovering such novel GC ligands while retaining a major portion of the steroid nucleus for glucocorticoid-like activity, we have synthesized a series of betamethasone derived 17α-carbamates represented by the general structure **3**. The advantage of these 17α-carbamate structures is the possible dissociation of anti-inflammatory activity and the associated side effects when compared to other compounds containing the intact steroidal skeleton. Identification of such an anti-inflammatory steroid would represent a significant structure–activity relationship breakthrough. Our initial interest was peaked by recent reports in the literature indicating that certain betamethasone derived steroidal GCs displayed intriguing in-vivo characteristics. For example, it has been reported that betamethasone valerate exerts much less thymolytic activity in rats than in mice, although it exhibits strong therapeutic activity in both species.[18] In addition, species specific differences in TA function were observed for a series of betamethasone esters.[19] Also, maternal administration of betamethasone esters induced adrenal hypertrophy in rat fetuses,[20] but adrenal atrophy in mice fetuses,[21] whereas betamethasone, dexamethasone, and esterified dexamethasone induced adrenal atrophy in both species.[22] These observations raise the possibility that betamethasone derivatives can act as dissociated GCs. In the present study, we have characterized the TA and TR function of a series of 17α-carbamates derived from betamethasone, both in-vitro and in-vivo. We demonstrate that these dissociated GCs have potent anti-inflammatory activity in the rat with impaired effects on glucose, insulin, triglyceride, and body weight.



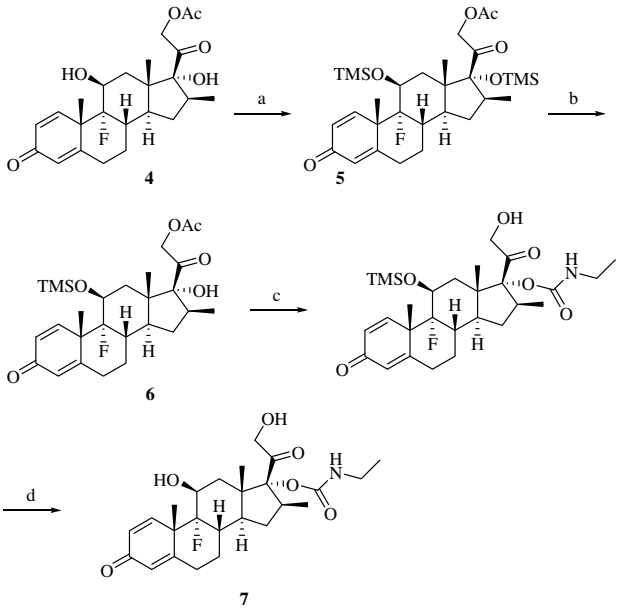
betamethasone **1** prednisolone **2**



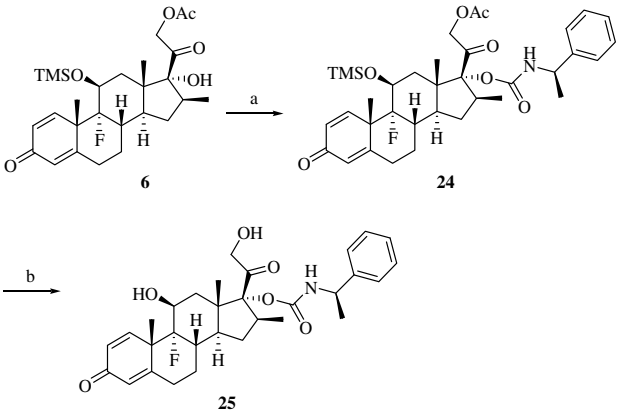
3 R= alkyl, alkenyl, benzyl

2. Chemistry

The target compounds were synthesized by the routes as shown in Schemes 1 and 2. The key step involved reaction of the appropriately substituted isocyanates with the monosilyl-protected betamethasone derivative **6**. The preparation of **6** began by bis-silyl protection of betamethasone acetate **4** to afford **5**, which was selectively deprotected using 1.0 M TBAF in acetic acid. Treatment of **6** with a variety of commercially available isocyanates yielded the desired carbamates, and clean deprotection of both the silyl and acetyl groups was achieved under acidic conditions (Scheme 1). Hindered carbamates, for example, **25** were synthesized by treatment of **6** with excess isocyanate in the presence of a catalytic amount of copper (I) chloride (Scheme 2). Non-commercially available isocyanates of interest were prepared by treating a mixture of



Scheme 1. Reagents and conditions: (a) TMS–Cl, imidazole, DMF, rt, 16 h; (b) AcOH, 1.0 M TBAF in THF, 0 °C, 4 h; (c) ethyl isocyanate, toluene, rt, 48 h; (d) MeOH/$CHCl_3$/6 N HCl (10:2:1), rt, 21 h.



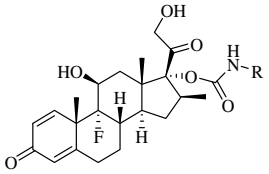
Scheme 2. Reagents and conditions: (a) CuCl DMF, (*R*)-1-phenethyl isocyanate, rt, 4.5 h; (b) MeOH/$CHCl_3$/6 N HCl (10:2:1), rt, 24 h.

the corresponding amine and 1,8-bis(dimethylamino)naphthalene with trichloromethyl chloroformate. [23] The target 17α-carbamates **7**–**25** were prepared in good overall yields, and their structures were confirmed on the basis of ^{1}H NMR, LCMS, high-resolution mass spectra and C, H, N analyses.

3. Biological evaluation

The results of the biological evaluation of the compounds are presented in Tables 1–3. Each compound was first evaluated for its ability to specifically bind to the α-isoform of GR in a competition binding assay, which constitutes the primary event in the action of a GR modulator. Counter screen binding assays were routinely performed on a subset of compounds against PR to monitor steroid receptor selectivity and many of the newly synthesized analogs displayed good selectivity toward GR over PR (Table 3). The ability of the compounds to repress transcription was evaluated by glucocorticoid mediated inhibition of MMP-1 expression as determined by ELISA measurement of secreted MMP-1 protein levels from PMA stimulated A549 cells. The transactivation potential of each compound was evaluated by its ability to induce tyro-

Table 1
Evaluation of betamethasone 17α carbamate glucocorticoid modulators



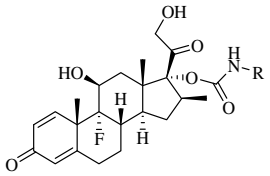
Compound	R	GR[a]	TAT[b,c]		GS[d,c]		MMP-1[e,c]	
		IC_{50} (nM)	EC_{50}[b] (nM)	% dex	EC50 (nM)	% dex	EC50 (nM)	% dex
7	Ethyl	5.1	n.r.[f]	10.2	n.r.[f]	23.4	15.9	104
8	Propyl	3.6	n.r.[f]	10.3	n.r.[f]	34.7	18.7	103
9	Butyl	2.8	n.r.[f]	6.1	n.r.[f]	31.3	22	98
10	Pentyl	2.1	117	37.3	0.4	60.3	18	97.5
11	*iso*-propyl	21.5	n.r.[f]	8.6	n.r.[f]	26	1.9	105
12	Allyl	17.4	n.r.[f]	10.9	n.r.[f]	16	39	106
13	*tert*-Butyl	2.4	5.3	70.1	0.37	64	1.6	94
14		28.6	211	99.9	47	103	83	107
15		0.5	n.r.[f]	15.8	0.3	54	0.8	97.5
16		55.1	705	40.7	n.r.[f]	35	53.3	65.9
17		352	n.r.[f]	30.6	—	—	0.1	44.7
18		12.3	646	54.6	576	61.8	21	56.3
19		5.7	156	63.2	223	50	23.2	80.3

[a] Data reported were derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration response curves and standard errors were ⩽20%.
[b] Rat tyrosine amino transferase (TAT) assay in rat H4 cells.
[c] Experiments were run in duplicate. EC_{50} values were determined from eight-point, one-third log concentration response curves and standard errors were ⩽20%.
[d] Rat glutamine synthetase (GS) assay in skeletal muscle cells.
[e] Human matrix metalloproteinase I (MMP-1) assay in A549 cells.
[f] n.r.: not reported; EC_{50} values not reported in cases where % dex ⩽35%.

sine aminotransferase (TAT) and glutamine synthetase (GS) activity in rat H4 and skeletal muscle cells, respectively. TAT is a liver specific, GR responsive gene, the product of which initiates tyrosine catabolism.[24] GS catalyzes the conversion of glutamate to glutamine, an important regulator of skeletal muscle.[25–27] These TAT and GS functional assays were used as markers for hepatic- and muscle-specific GR transactivation. In-vivo activity readouts included effects on glucose, insulin, triglyceride, depression of body weight gain without a change in food consumption, and anti-inflammatory activity, which constitute significant aspects of the in-vivo profile of a glucocorticoid in the rat. The hGR, transactivation (rTAT and rGS), and transrepression (MMP-1) activities for a selection of compounds are provided in Tables 1 and 2.

Most of the compounds presented in Table 1 exhibited significant affinity and selectivity toward the glucocorticoid receptor and many bound with approximately the same or better affinity than prednisolone (IC_{50} = 13.8 nM). Small unbranched alkyl derivatives, for example, R = Et **7**, bound with exceptional affinity and selectivity for GR versus PR (IC_{50} 5.1 = nM vs >1000 nM) (Table 3). The *N*-Et derivative **7** was evaluated further in functional assays of TA and TR and, quite gratifyingly, afforded a completely dissociated profile in rat. Interestingly, however, **7** was found to be a potent inducer of GS activity in mouse and human skeletal muscle cells.[28] This apparent species specific difference in TA activity is not well understood, but is in line with literature reports for a series of structurally related betamethasone esters.[19] In rat, however, as shown in Table 1, **7** exhibited a weak agonist profile in functional assays of TA, yet displayed characteristics of a full agonist in functional assays of TR. Larger alkyl substituents (e.g., **16**, **17**) generally led to reduced affinity and selectivity for GR. The introduction of larger substituents also led to partial agonist profiles and produced analogs that no longer appeared to be dissociated. Indeed, an *n*-butyl substituent (i.e., **9**) appeared to be the longest linear group that could be tolerated while maintaining a fully dissociated profile. Both branched alkyl substituents (e.g., **13**, **16**) and the introduction of polarity (e.g., **14**) led to decreases in affinity and to less interesting partial agonist profiles in our cellular based assays of TA and TR. In fact, our SAR studies have shown that among those variations examined any departure from small straight-chain alkyl substituents such as *N*-Et of **7,** resulted in either loss of affinity for GR or to a partial agonist profile. As shown in Table 2, sterically bulkier substituents, for example, *N*-cyclohexyl **21**, were

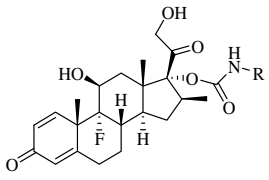
Table 2
Sterically hindered 17α carbamates



Compound	R	GR[a]	TAT[b,c]		GS[d,c]		MMP-1[e,c]	
		IC_{50} (nM)	EC_{50}[b] (nM)	% dex	EC_{50} (nM)	% dex	EC_{50} (nM)	% dex
20	Cyclopentyl	3.8	n.r.[f]	6.0	n.r.[f]	23	2.8	76.1
21	Cyclohexyl	3.5	2.0	42.9	n.r.[f]	24.7	1.3	82
22	Adamantyl	5.8	9.8	80.8	—	—	3.4	84.1
25		5.2	70.3	66.1	1.6	92	21.5	87.5
26		1.3	37.3	71.7	0.6	49	4.9	93

[a] Data reported were derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration response curves and standard errors were ⩽20%.
[b] Rat tyrosine amino transferase (TAT) assay in rat H4 cells.
[c] Experiments were run in duplicate. EC_{50} values were determined from eight-point, one-third log concentration response curves and standard errors were ⩽20%.
[d] Rat glutamine synthetase (GS) assay in skeletal muscle cells.
[e] Human matrix metalloproteinase I (MMP-1) assay in A549 cells.
[f] n.r.: not reported; EC_{50} values not reported in cases where % dex ⩽35%.

Table 3
Binding affinities of 17α carbamates against human GR versus other steroidal receptors



Compound	R	Binding IC_{50} (nM)[a]	
		hGR	hPR
7	Ethyl	5.1	>1000
8	Propyl	3.6	394
12	Allyl	17.4	>1000
19		5.7	>1000
21	Cyclohexyl	3.5	120
26		5.2	176

[a] Data reported were derived from duplicate wells and three independent experiments. Mean IC_{50} values were determined from 10-point, one-third log concentration response curves and standard errors were ⩽20%.

tolerated but these derivatives generally exhibited reduced selectivity for GR versus the other steroidal receptors (Table 3) and many had partially dissociated profiles in functional assays of TA and TR.

3.1. In-vivo evaluation

A number of the *N*-alkyl 17-α carbamates displayed good levels of oral bioavailability in the rat including the fully dissociated derivatives *N*-Et **7** (Cl_p 26 mL/min/kg, $t_{1/2}$ 2.2 h, V_{dss} 3.22 L/kg, %F 43) and *N*-Pr **8**. On the basis of their dissociated profiles in our in-vitro assays and promising pharmacokinetic data above, **7** and **8** were selected for further in-vivo evaluation. We initially examined **7** under acute inflammatory TR conditions using the murine LPS challenge model of inflammation.[29] Thus, upon oral administration of **7**, a dose-dependent inhibition of TNF-α was observed, similar for that of prednisolone (ED_{50} = 0.1 mg/kg for **7** vs 0.5 mg/kg for prednisolone). Figure 1 depicts the dose–response curve for prednisolone versus **7**. The anti-inflammatory efficacy of **7** and **8** was also determined in a chronic 7 day rat cotton pellet granuloma assay[30] at total daily doses of 5, 10, and 19 mg/kg given p.o. b.i.d from day 1. As shown in Table 4, compounds **7** and **8** produced dose-dependent inhibition of the cotton pellet granuloma in female Lewis rats on days 4 and 7. At 19 mg/kg, compounds **7** and **8** inhibited the cotton pellet granuloma by 45% and 51%, respectively, and this compared to prednisolone at 2.5 mg/kg which showed 49% inhibition at this dose. We next turned our attention toward examining the in-vivo side effect profile of **7** and **8**.

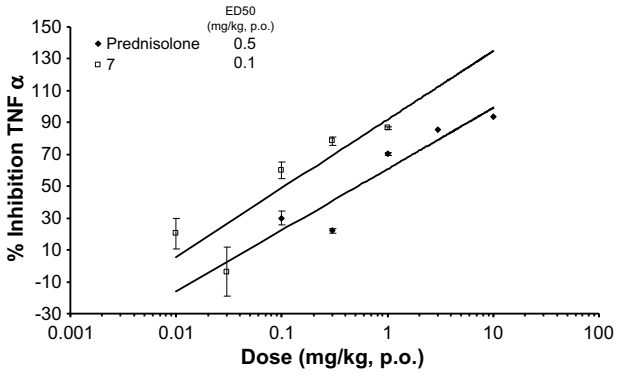


Figure 1. Prednisolone and **7** dose dependently inhibit mouse LPS-induced TNFα production.

Table 4
The effects of prednisolone, compounds **7** and **8** on change in body weight, spleen, thymus, and adrenal weights and inhibition of granuloma formation in female Lewis rats with implanted cotton pellets

Treatment (mg/kg)	Change in body weight on Day 4 (g)	Change in body weight on Day 7 (g)	Spleen weight (mg)	Thymus weight (mg)	Adrenal weight (mg)	% Inhibition granuloma
Vehicle	7.0 ± 3.0	14.4 ± 4.3	438.3 ± 878.7	401.3 ± 76.8	41.3 ± 7.6	
Prednisolone						
1.0	1.5 ± 2.1**	3.0 ± 2.3**	334.7 ± 16.2**	219.5 ± 39.6**	38.7 ± 5.3	37.6 ± 15.9
2.5	−1.0 ± 2.1**	−0.4 ± 2.7**	266.8 ± 11.2**	117.8 ± 38.0**	26.9 ± 4.7**	49.3 ± 6.0
6.0	−3.6 ± 2.5**	−4.1 ± 3.4**	260.0 ± 22.1**	104.0 ± 26.8**	27.9 ± 6.3**	56.2 ± 6.8
*Compound **7***						
5.0	5.9 ± 4.4	11.0 ± 4.2	330.9 ± 17.5**	170.7 ± 29.1**	39.0 ± 3.4	35.6 ± 18.3
10	7.3 ± 3.7	10.0 ± 3.9	311.2 ± 18.4**	144.9 ± 23.2**	36.s8 ± 7.4	33.5 ± 14.5
19	5.6 ± 3.7	7.5 ± 4.6**	305.3 ± 15.4**	119.9 ± 36.7**	30.8 ± 6.6**	44.6 ± 8.6
*Compound **8***						
5.0	1.1 ± 4.5**	5.0 ± 6.6**	296.1 ± 11.7**	105.5 ± 15.7**	34.7 ± 5.7	32.7 ± 12.8
10	3.9 ± 2.1	7.1 ± 3.5**	274.3 ± 15.2**	91.4 ± 21.6**	34.6 ± 10.3	40.0 ± 15.7
19	3.9 ± 3.6	6.6 ± 3.4**	258.0 ± 13.1**	82.9 ± 19.3**	25.6 ± 2.8**	51.0 ± 8.6

Significant difference from vehicle at $p < 0.05$ (*) or $p < 0.01$(**) with Dunnett's *t*-test.

Increases in glucose, insulin, and triglyceride levels and decreases in body weight gain constitute significant undesired activity of currently available GCs. Samples collected from the rat cotton pellet granuloma study allowed measurement of all of the above parameters and indicated an improved side effect profile for **7** and **8** consistent with a dissociated GR modulator. Thus, whereas administration of prednisolone at 2.5 mg/kg led to significant decreases in body weight on day 7 (−0.4 g ± 2.7 g) versus vehicle (+14.4 g ± 4.3 g), weight loss with compound **7** at 19 mg/kg on day 7 was less pronounced (+7.5 g ± 4.6 g). The 7 day organ weights for the prednisolone treated groups (spleen, thymus, and adrenal) indicated that these lymphoid organs were subject to the catabolic actions of the steroid. Interestingly, compounds **7** and **8** had similar effects on organ weights compared to the prednisolone treated groups. Decreases in plasma insulin and triglyceride levels were also evident (Fig. 2) and in the case of plasma triglyceride levels these differences were not statistically significant compared to vehicle. On day 7, the rats were administered an oral glucose test (OGTT) with dextrose dosed at 3 mg/kg (Fig. 3). Rats dosed with **7** exhibited a glucose excursion similar to vehicle 2 h post dose, while the prednisolone dosed animals possessed a strong spike consistent with conventional steroid therapy. Rats dosed with **8** exhibited a glucose excursion at the higher doses of 10 and 19 mg/kg, similar to that observed with prednisolone at 2.5 and 6.0 mg/kg (Fig. 3). To further profile **7**, effects on plasma glucose, insulin, triglyceride, and % body weight change were assessed in the Zucker Rat chronic dosing model.[31] In this five day study, **7** was dosed at 30 mg/kg alongside dexamethasone at 0.3 mg/kg and the GR antagonist RU486 at 30 mpk. As shown in Figure 4, dexamethasone exhibited a profound effect on all four parameters at low doses. In comparison, the *N*-Et derivative **7** showed little effect on body weight, glucose, insulin, and triglyceride levels and the differences were not statistically significant compared to vehicle or the GR antagonist RU 486. Interestingly, co-administration of compound **7** (30 mg/kg) with dexamethasone (0.3 mg/kg) led to normalization of glucose, insulin, and body weight change parameters indicating that **7** was able to antagonize the TA activity of dexamethasone. A similar effect was observed by co-administration of the GR antagonist RU 486.

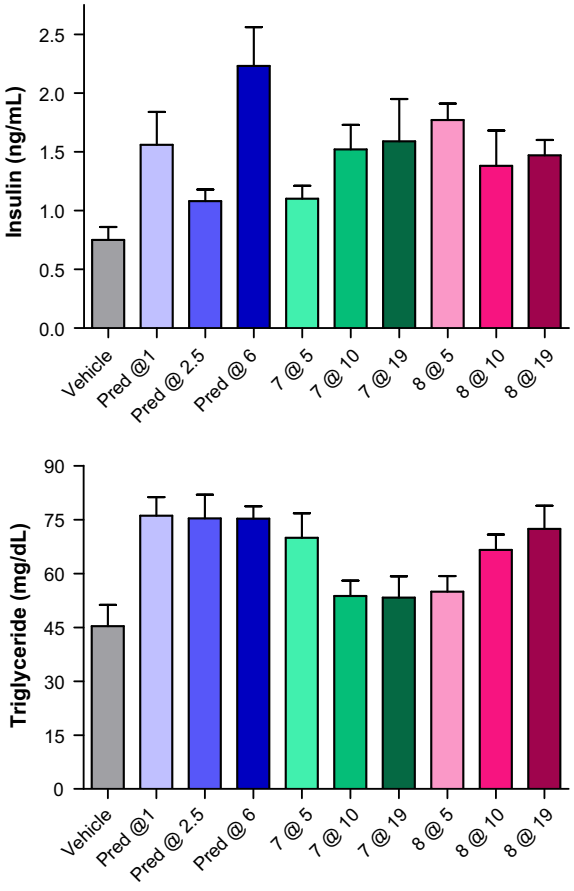


Figure 2. The effects of prednisolone, vehicle, and compounds **7** and **8** on plasma insulin and triglyceride levels in female Lewis rats with implanted cotton pellets.

4. Conclusion

In conclusion, we have demonstrated that a series of 17-α carbamates derived from betamethasone retain significant activity and selectivity for the glucocorticoid receptor. In addition, many of these compounds exhibit a fully dissociated profile in functional assays of TA and TR in the rat. In-vivo administration of **7** caused a significant anti-inflammatory response yet showed little effects on glucose, insulin, and triglyceride levels. A reduced catabolic effect was also observed. Taken together, these results indicate that dissociated GR modulators can be identified in the rat starting from traditional steroidal platforms and that these compounds may offer therapeutic advantages in the treatment of diseases in which GR plays a critical role, including inflammatory and immunological disorders.

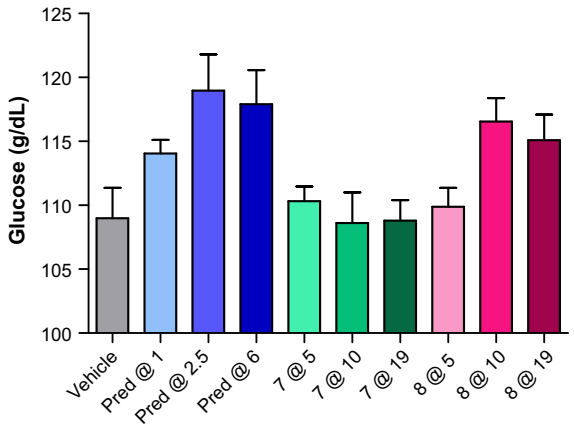


Figure 3. The effects of prednisolone, vehicle, and compounds **7** and **8** on plasma glucose levels following an oral glucose tolerance test (OGTT) in female Lewis rats with implanted cotton pellets.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were recorded on a Varian InNova 500 MHz instrument in $CDCl_3$ or DMSO-d^6 solutions, unless otherwise stated. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. All compounds were detected as single spots on TLC plates and visualized using UV light and phosphomolybdic acid stain. Low-resolution mass spectra (MS) were determined on a Micromass Platform LC by electrospray positive ionization. High-resolution mass spectra (HRMS) were determined on a Thermo Electron LTQ FT Ultra using ESI ionization. LCMS analyses were performed on a Waters Micromass ZQ single quadrupole mass spectrometer. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ, and are within ±0.4% of the calculated values unless otherwise stated. Reagents were purchased commercially and used without further purification unless otherwise stated.

5.1.1. (11β,16β)-9-Fluoro-16-methyl-3,20-dioxo-11,17-bis[(trimethylsilyl)oxy]pregna-1,4-dien-21-yl acetate (5)

To a solution of (11β,16β)-9-fluoro-11,17-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-21-yl acetate (18.79 g, 43.25 mmol) and imidazole (58.89 g, 864.9 mmol) in 200 mL of dry *N*,*N*-dimethylformamide was added neat trimethylsilyl chloride (55.0 mL, 433 mmol) dropwise during 15 min. The resulting pale yellow solution was stirred at room temperature for 16 h. The solution was diluted with ethyl acetate and washed successively with water, satd NH_4Cl, water, satd $NaHCO_3$, water, and brine. The organic phase was dried over Na_2SO_4, filtered, and evaporated in vacuo to afford 24.8 g (99%) of **5** as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.25 (s, 9H), 0.26 (s, 9H), 0.94 (s, 3H), 1.15–1.25 (m, 1H), 1.21 (d, J = 7.3 Hz, 3H), 1.49 (s, 3H), 1.50–1.65 (m, 1H), 1.61 (d, J = 13.7 Hz, 1H), 1.85–2.0 (m, 3H), 2.18 (s, 3H), 2.15–2.25 (m, 1H), 2.25–2.50 (m, 3H), 2.55–2.65 (m, 1H), 4.35–4.40 (m, 1H), 4.75 (d, J = 17.5 Hz, 1H), 4.84 (d, J = 17.5 Hz, 1H), 6.13 (s, 1H), 6.36 (dd, J = 10.1, 1.8 Hz, 1H), 7.06 (d, J = 10.1 Hz, 1H). MS (ESI): m/z = 579.3 (MH^+).

5.1.2. (11β,16β)-9-Fluoro-17-hydroxy-16-methyl-3,20-dioxo-11-[(trimethylsilyl)oxy]-pregna-1,4-dien-21-yl acetate (6)

A solution of (11β,16β)-9-fluoro-16-methyl-3,20-dioxo-11,17-bis[(trimethylsilyl)oxy]pregna-1,4-dien-21-yl acetate (26 g, ~43 mmol) in 430 mL of tetrahydrofuran (THF) was cooled to 0 °C and neat acetic acid (4.95 mL, 86.5 mmol) was added followed

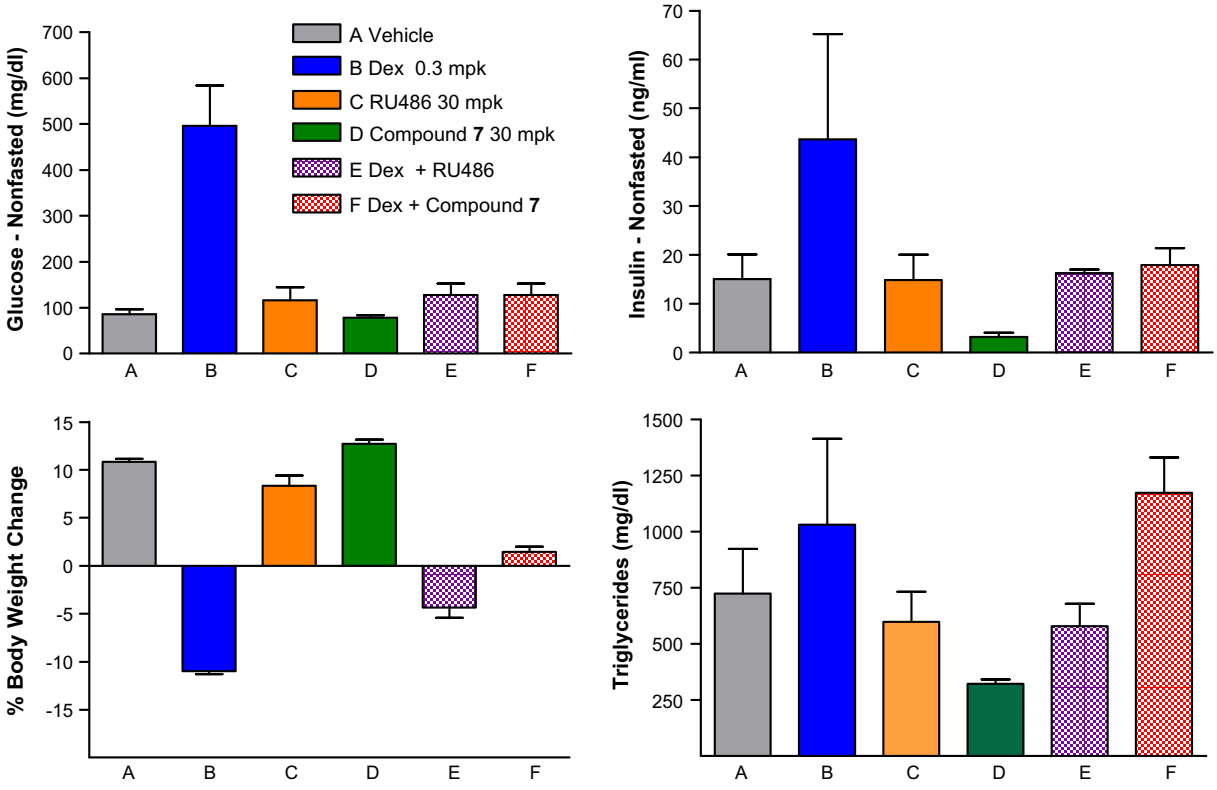


Figure 4. The effects of dexamethasone, vehicle, compound **7**, and RU486 on plasma glucose, insulin, triglyceride, and % body weight change in Zucker fatty rat chronic dosing model.

by dropwise addition of a 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (43.3 mL, 43.3 mmol). After stirring at 0 °C for 4 h, the ice bath was removed and the reaction was allowed to warm to room temperature and stirred for an additional 16 h. Most of the THF was removed by rotary evaporation in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was washed successively with water, satd NH_4Cl, water, satd $NaHCO_3$, water, and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum to give an off-white solid. Purification by flash chromatography through a 1.6 kg column of silica gel eluting with 4% MeOH in dichloromethane provided 16.1 g (74%) of **6** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.25 (s, 9H), 1.06 (s, 3H), 1.15–1.25 (m, 1H), 1.17 (d, J = 7.1 Hz, 3H), 1.49 (s, 3H), 1.45–1.60 (m, 1H), 1.64 (d, J = 14 Hz, 1H), 1.85–2.2 (m, 5H), 2.18 (s, 3H), 2.35–2.55 (m, 3H), 2.55–2.65 (m, 1H), 4.35–4.40 (m, 1H), 4.94 (AB, 2H), 6.11 (s, 1H), 6.35 (dd, J = 10.3, 1.8 Hz, 1H), 7.05 (d, J = 10 Hz, 1H). MS (ESI): m/z = 507.2 (MH^+).

5.1.3. (11β,16β)-21-(Acetyloxy)-9-fluoro-16-methyl-3,20-dioxo-11-[(trimethylsilyl)-oxy]pregna-1,4-dien-17-yl ethylcarbamate

To a mixture of (11β,16β)-9-fluoro-17-hydroxy-16-methyl-3,20-dioxo-11-[(trimethyl-silyl)oxy]-pregna-1,4-dien-21-yl acetate (3.11 g, 6.15 mmol) in 41 mL of toluene was added neat ethyl isocyanate (20.5 mL, 259 mmol), and the mixture was refluxed for 48 h. The resulting solution was cooled to room temperature and evaporated under vacuum to leave a slightly gummy solid. Flash chromatography through a 1 kg column of silica gel eluting with *tert*-butyl methyl ether/hexane/chloroform (1:1:1) gave 2.22 g (67%) of the title compound as a white solid. ^{1}H NMR (500 MHz, CDCl3): δ 0.26 (s, 9H), 0.94 (s, 3H), 1.1–1.3 (m, 4H), 1.34 (d, J = 6.7 Hz, 3H), 1.48 (s, 3H), 1.45–1.60 (m, 1H), 1.78 (d, J = 13.5 Hz, 1H), 1.8–2.1 (m, 3H), 2.16 (s, 3H), 2.15–2.55 (m, 4H), 2.55–2.65 (m, 1H), 3.1–3.3 (m, 2H), 4.35–4.45 (m, 1H), 4.54 (d, J = 16 Hz, 1H), 4.82 (d, J = 16 Hz, 1H), 5.0–5.1 (m, 1H), 6.11 (s, 1H), 6.34 (d, J = 10 Hz, 1H), 7.04 (d, J = 10 Hz, 1H). MS (ESI): m/z = 578.3 (MH^+).

5.1.4. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl ethylcarbamate (7)

Solid (11β,16β)-21-(acetyloxy)-9-fluoro-16-methyl-3,20-dioxo-11-[(trimethylsilyl)oxy]-pregna-1,4-dien-17-yl ethylcarbamate (2.22 g, 3.84 mmol) was dissolved in 128 mL of a pre-mixed solution of methanol/chloroform/6 N HCl (10:2:1) and stirred at room temperature. After 16 h, an additional 2 mL of 6 N HCl was added. After an additional 5 h, the solution was partitioned between ethyl acetate and water. The organic phase was washed successively with satd $NaHCO_3$, water, and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to give a pale yellow oil. Purification by flash chromatography through a column of 500 g of silica gel, eluting with hexane/ethyl acetate/dichloromethane/methanol (3:3:3:1) yielded 0.890 g (50%) of **7** as a white powder. 1H NMR (500 MHz, CDCl3): δ 0.96 (s, 3H), 1.15 (t, J = 7 Hz, 3H), 1.15–1.30 (m, 1H), 1.41 (d, J = 7.3 Hz, 3H), 1.47 (d, J = 13.9 Hz, 1H), 1.56 (s, 3H), 1.50–1.65 (m, 1H), 1.9–2.1 (m, 3H), 2.2–2.7 (m, 7H), 3.1–3.3 (m, 2H), 4.03 (d, J = 18 Hz, 1H), 4.26 (d, J = 18 Hz, 1H), 4.30–4.45 (m, 1H), 5.2–5.3 (m, 1H), 6.14 (s, 1H), 6.34 (dd, J = 10, 1.6 Hz, 1H), 7.21 (d, J = 10 Hz, 1H). MS (ESI): m/z = 464.2 (MH^+). C, H, N ($C_{25}H_{34}FNO_6$) calcd %C 64.78, %H 7.39, %N 3.02; found %C 64.78, %H 7.66, %N 3.02.

5.1.5. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl propylcarbamate (8)

From **6** and propyl isocyanate. MS (ESI): m/z = 478.3 (MH^+). C, H, N ($C_{26}H_{36}FNO_6$) calcd %C 65.39, %H 7.60, %N 2.93; found %C 65.40, %H 7.85, %N 2.85.

5.1.6. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl butylcarbamate (9)

From **6** and *n*-butyl isocyanate. MS (ESI): m/z = 492.3 (MH^+). C, H, N ($C_{27}H_{38}FNO_6$) calcd %C 65.97, %H 7.79, %N 2.85; found %C 65.86, %H 7.93, %N 2.69.

5.1.7. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl isopropylcarbamate (11)

From **6** and isopropyl isocyanate. MS (ESI): m/z = 478.3 (MH^+). C, H, N ($C_{26}H_{36}FNO_6$) calcd %C 65.39, %H 7.60, %N 2.93; found %C 65.23, %H 7.64, %N 2.97.

5.1.8. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl allylcarbamate (12)

From **6** and allyl isocyanate. MS (ESI): m/z = 476.3 (MH^+). C, H, N ($C_{26}H_{34}FNO_6$) calcd %C 65.67, %H 7.21, %N 2.95; found %C 65.08 ,%H 7.30, %N 3.15.

5.1.9. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl *tert*-butylcarbamate (13)

From **6** and *tert*-butyl isocyanate. MS (ESI): m/z = 492.3 (MH^+). C, H, N ($C_{27}H_{38}FNO_6$) calcd %C 65.97, %H 7.79, %N 2.85; found %C 65.83, %H 8.07, %N 2.76.

5.1.10. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl cyclopentylcarbamate (20)

From **6** and cyclopentyl isocyanate. MS (ESI): m/z = 504.2 (MH^+). C, H, N ($C_{28}H_{38}FNO_6$) calcd %C 66.78, %H 7.61, %N 2.78; found %C 66.62, %H 7.75, %N 2.65.

5.1.11. (11β,16β)-21-(Acetyloxy)-9-fluoro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1R)-1-phenylethylcarbamate (24)

To a mixture of (11β,16β)-9-fluoro-17-hydroxy-16-methyl-3,20-dioxo-11-[(trimethyl-silyl)oxy]-pregna-1,4-dien-21-yl acetate (100 mg, 0.197 mmol) and copper (I) chloride (0.5 mg, 0.005 mmol) in 1 mL of *N*,*N*-dimethylformamide was added neat (*R*)-1-phenylethyl isocyanate (0.085 mL, 0.60 mmol). The pale green reaction mixture was stirred at room temperature in the dark for 4.5 h before being partitioned between ethyl acetate and water. The organic layer was washed successively with water, sat. NH_4Cl, water, and brine and then dried over Na_2SO_4. Filtration and evaporation in vacuo gave a foam which was purified by flash chromatography through a 40 g column of silica gel, eluting with hexane/*tert*-butyl methyl ether/chloroform (1:1:1), to provide 67 mg of the title compound as a colorless oil. MS (ESI): m/z = 654.4 (MH^+).

5.1.12. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1*R*)-1-phenylethylcarbamate (25)

(11β,16β)-21-(Acetyloxy)-9-fluoro-11-hydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1*R*)-1-phenylethylcarbamate **24** (42 mg, 0.064 mmol) was dissolved in 1.25 mL of a pre-mixed solution of methanol/chloroform/6 N HCl (10:2:1) and stirred at room temperature for 24 h. The solution was then partitioned between ethyl acetate and water, and the organic phase was washed with water (twice) and brine and dried over Na_2SO_4. Filtration and evaporation under vacuum gave a white film which was purified by preparative thin-layer chromatography on silica gel, eluting with ethyl acetate/hexane/dichloromethane/methanol (95:95:95:15), to yield 20 mg of (11β,16β)-9-fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1*R*)-1-phenylethylcarbamate as a white solid. MS (ESI): m/z = 540.3 (MH^+). HRMS for $C_{31}H_{39}FNO_6$ $(M+1)^+$: calcd 540.2757; found 540.2756.

5.1.13. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1*S*)-1-phenylethylcarbamate (26)

From **6** and (1*S*)-1-phenyl isocyanate. MS (ESI): $m/z = 540.3$ (MH^+). HRMS for $C_{31}H_{39}FNO_6$ $(M+1)^+$: calcd 540.2757; found 540.2756.

5.1.14. (11β,16β)-9-Fluoro-11,21-dihydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl (1*S*)-1-(methoxycarbonyl)-ethylcarbamate (14)

From **6** and (*S*)-2-isocyanatopropionic acid methyl ester. MS (ESI): $m/z = 522.2$ (MH^+). HRMS for $C_{27}H_{37}FNO_8$ $(M+1)^+$: calcd 522.2497; found 522.2498.

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Supplementary data

Cell-based assay procedures for hGRα, hPR, glutamine synthetase activity, tyrosine amino transferase activity and MMP-1 assay can be found in the online version. Procedures are also available for the in-vivo evaluation in the mouse LPS challenge assay, rat cotton pellet granuloma assay and Zucker fatty rat model. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.07.037.

References and notes

1. Barnes, P. J. *Clin. Sci.* **1998**, *94*, 557.
2. Boumpas, D. T.; Chrousos, G. P.; Wilder, R. L.; Cupps, T. R.; Balow, J. E. *Ann. Intern. Med.* **1993**, *12*, 1198.
3. Moreland, L. W.; O'Dell, J. R. *Arthritis Rheum.* **2002**, *46*, 2553.
4. Schaecke, H.; Rehwinkel, H. *Curr. Opin. Invest. Drugs* **2004**, *5*, 524.
5. Song, I.-H.; Gold, R.; Straub, R. H.; Murmester, G.-R.; Buttgereit, F. *J. Rheum.* **2005**, *7*, 1199.
6. (a) Kym, P. R.; Kort, M. E.; Coghlan, M. J.; Moore, J. L.; Tang, R.; Ratajczyk, J. D.; Larson, D. P.; Elmore, S. W.; Pratt, J. K.; Stashko, M. A.; Falls, H. D.; Lin, C. W.; Nakane, M.; Miller, L.; Tyree, C. M.; Miner, J. N.; Jacobson, P. B.; Wilcox, D. M.; Nguyen, P.; Lane, B. C. *J. Med. Chem.* **2003**, *46*, 1016. and reference therein; (b) Ali, A.; Thompson, C. F.; Balkovec, J. M.; Graham, D. W.; Hammond, M. L.; Quraishi, N.; Tata, J. R.; Einstein, M.; Ge, L.; Harris, G.; Kelly, T. M.; Mazur, P.; Pandit, S.; Santoro, J.; Sitlani, A.; Wang, C.; Williamson, J.; Miller, D. K.; Thompson, C. M.; Zaller, D. M.; Forrest, M. J.; Carballo-Jane, E.; Luell, S. *J. Med. Chem.* **2004**, *47*, 2441; (c) Thompson, C. F.; Quraishi, N.; Ali, A.; Tata, J. R.; Hammond, M. L.; Balkovec, J. M.; Einstein, M.; Ge, L.; Harris, G.; Kelly, T. M.; Mazur, P.; Pandit, S.; Santoro, J.; Sitlani, A.; Wang, C.; Williamson, J.; Miller, D. K.; Yamin, T. D.; Thompson, C. M.; O'Neill, E. A.; Zaller, D.; Forrest, M. J.; Carballo-Jane, E.; Luell, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2163; (d) Smith, C. J.; Ali, A.; Balkovec, J. M.; Graham, D. W.; Hammond, M. L.; Patel, G. F.; Rouen, G. P.; Smith, S. K.; Tata, J. R.; Einstein, M.; Ge, L.; Harris, G. S.; Kelly, T. M.; Mazur, P.; Thompson, C. M.; Wang, C. F.; Williamson, J. M.; Miller, D. M.; Pandit, S.; Santoro, J. C.; Sitlani, A.; Yamin, T. D.; O'Neill, E. A.; Zaller, D. M.; Carballo-Jane, E.; Forrest, M. J.; Luell, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2926.
7. (a) Betageri, R.; Zhang, Y.; Zindell, R. M.; Kuzmich, D.; Kirrane, T. M.; Bentzien, J.; Cardozo, M.; Capolino, A. J.; Fadra, T. N.; Nelson, R. M.; Paw, Z.; Shih, D. T.; Shih, C. K.; Zuvela-Jelaska, L.; Nabozny, G.; Thomson, D. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4761; (b) Shah, N.; Scanlan, T. S. *Bioorg. Med. Chem.* **2004**, *14*, 5199.
8. Fenske, D.; Husmann, M.; Apotheke, d.-K.; Johannes, J. *Pharm. Zeitung* **2004**, *15*, 16, 22.
9. Eberhardt, W.; Kilz, T.; Akool, E.-S.; Mueller, R.; Pfeilschifter, J. *Biochem. Pharmacol.* **2005**, *3*, 433.
10. Truss, M.; Beato, M. *Endocr. Rev.* **1993**, *14*, 459.
11. (a) Beato, M.; Herrlich, P.; Schultz, G. *Cell* **1995**, *83*, 851; (b) Resche-Rigon, M.; Gronemeyer, H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 501.
12. Jonat, C.; Rahmsdorf, H. J.; Park, K. K.; Cato, A. C.; Gebel, S.; Ponta, H.; Herrlich, P. *Cell* **1990**, *62*, 1189.
13. Vander Burg, B.; Liden, J.; Okret, S.; Delaunay, F.; Wissink, S.; Vander Saag, P. T.; Gustafsson, J. A. *Trends Endocrinol. Metab.* **1997**, *8*, 152.
14. Heck, S.; Kullmann, M.; Gast, A.; Ponta, H.; Rahmsdorf, H. J.; Herrlich, P. A. *EMBO J.* **1994**, *17*, 4087.
15. Ray, D. W.; Suen, C. S.; Brass, A.; Soden, J. *Mol. Endocrinol.* **1999**, *11*, 1855.
16. Roux, S.; Terouanne, B.; Couette, B.; Rafestin-Oblin, M. E.; Nicolas, J. C. *J. Biol. Chem.* **1999**, *15*, 10059.
17. (a) Reichardt, H. M.; Kaestner, K. H.; Tuckermann, J.; Kretz, O.; Wessely, O.; Bock, R. *Cell* **1998**, *4*, 531; (b) Reichardt, H. M.; Tuckermann, J. P.; Bauer, A.; Schutz, G. *Z. Rheumatol.* **2000**, *59*, II/1.
18. Child, K. J.; English, A. F.; Gilbert, H. G.; Hewitt, A.; Woollett, E. A. *Arch. Dermatol.* **1968**, *4*, 407.
19. Kiyoshi, T.; Hideki, N.; Koichi, O.; Katsunao, T.; Hidekazu, M.; Mamoru, K.; Koichi, I. *Int. Immunopharmacol.* **2002**, *2*, 941.
20. Hasegawa, Y.; Okamoto, A.; Sakaguchi, I. *Acta Endocrinol.* **1978**, *4*, 828.
21. Nakano, M.; Nishiuchi, M.; Takeuchi, M.; Yamada, H. *Steroids* **1981**, *5*, 511.
22. Nakano, M.; Takeuchi, M.; Sugeno, K. *J. Pharmacobio. Dyn.* **1981**, *3*, 211.
23. (a) Majer, P.; Randad, R. S. *J. Org. Chem.* **1965**, *59*, 1937; (b) Dragovich, P. S.; Barker, J. E.; French, J.; Imbacuan, M.; Cáliz, V. J.; Kissinger, C. R.; Knighton, D. R.; Lewis, C. T.; Moomaw, E. W.; Parge, H. E.; Pelletier, L. A. K.; Prins, T. J.; Showalter, R. E.; Tatlock, J. H.; Tucker, K. D.; Villafranca, J. E. *J. Med. Chem.* **1996**, *39*, 1872.
24. Jantzen, H. M.; Strahle, U.; Gloss, B.; Stewart, F.; Schmidt, W.; Boshart, M.; Miksicek, R.; Schutz, G. *Cell* **1987**, *49*, 29.
25. Rowe, W. B.; Ronzio, R. A.; Wellner, V. P. *Methods Enzymol.* **1970**, *17*, 900.
26. Minet, R.; Villie, F.; Marcollet, M.; Meynial-Denis, O.; Cynober, L. *Clin. Chim. Acta* **1997**, *268*, 121.
27. Santoro, J. C.; Harris, G.; Sitlani, A. *Anal. Biochem.* **2001**, *289*, 18.
28. Merck Research Laboratories, unpublished data.
29. Gonzales, J. C.; Johnson, D. C.; Morrison, D. C.; Freudenberg, M. A.; Galanos, C.; Silverstein, R. *Infect. Immun.* **1993**, *61*, 970.
30. Rassaert, C. L.; Dipasquale, G.; O'Donoghue, S. *Inflamm. Res.* **1975**, *2*, 128.
31. Ogawa, A.; Johnson, J. H.; Ohneda, M.; McAllister, C. T.; Inman, J.; Alam, T.; Ynger, R. H. *J. Clin. Invest.* **1992**, *90*, 497.