Bioorganic & Medicinal Chemistry Letters 19 (2009) 2139–2143

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel dihydro-9,10-ethano-anthracene carboxamides as glucocorticoid receptor modulators

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ARTICLE INFO

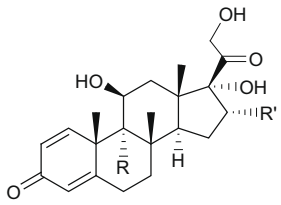
Article history:
Received 19 January 2009
Accepted 3 March 2009
Available online 9 March 2009

Keywords:
Glucocorticoid receptor modulators
Dihydro-9,10-ethano-anthracene-11-carboxamides
Dissociation
Transrepression
Transactivation
Anti-inflammatory activity

ABSTRACT

A series of dihydro-9,10-ethano-anthracene-11-carboxamides as novel glucocorticoid receptor modulators is reported. SAR exploration identified compounds from this series displaying a promising dissociation profile in discriminating between transrepression and transactivation activities. **17a** is a partial agonist of GR-mediated transactivation which elicits potent and efficacious transrepression in reporter gene assays. A hypothetical binding mode is provided which accounts for the induction of functional activity by a bridgehead methyl group.

Glucocorticoids, steroidal agonists of the glucocorticoid receptor (GR), such as prednisolone and dexamethasone, have been used for over 50 years to treat inflammatory diseases. However, their systemic use is limited by side effects including diabetes, osteoporosis and glaucoma. There is a strong medical need to develop agents that maintain the desirable anti-inflammatory efficacy of glucocorticoids while limiting side effects.[1] The anti-inflammatory activity of glucocorticoids is largely due to their ability to reduce the expression of pro-inflammatory genes via the inhibition of the transcription factors NFκB and AP-1 by ligand-activated GR. The primary mechanism of this inhibition, termed transrepression (TR),[2] is through a direct physical interaction that alters the transcription factor complex and reduces the ability of NFκB and AP-1 to stimulate transcription.[3] NFκB and AP-1 play key roles in the initiation and perpetuation of inflammatory and immunological disorders, as they are involved in regulating the expression of a number of important inflammatory and immunomodulatory genes including TNF-α, IL-1, IL-2, IL-5, adhesion molecules, chemokines, Cox-2, and others.[4] On the other hand, the interaction of a glucocorticoid with GR also results in the induction of transcription of certain genes. This process, termed transactivation (TA), requires dimerization of GR and binding to its cognate glucocorticoid response element (GRE) on DNA. Many of the side effects of glucocorticoid therapy are due to the ability of GR to induce transcription of various genes involved in metabolism. Previous studies using a transgenic GR dimerization-defective mouse have shown that the transactivation (DNA binding) activities of GR could be separated from the transrepressive effects (non-DNA binding) of GR.[5] Recent activities aimed at separating these different GR mechanisms have led to the discovery of several new classes of nonsteroidal GR modulators.[6–12] Characterization of such compounds indicates that it may be possible to develop dissociated GR agonists which separate TR and TA activities thereby maintaining an improved therapeutic index (TI) relative to glucocorticoids.



R = F, R' = Me, dexamethasone (dex)
R = H, R' = H, prednisolone (pred)

In this Letter, we wish to report our initial efforts on a novel class of dihydro-9,10-ethano-anthracene-based GR modulators.

Binding affinity of a compound to recombinant human GRα was measured by competition with FITC-dexamethasone in a fluores-

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doi:10.1016/j.bmcl.2009.03.006

cence polarization assay.[13] The transrepressive efficacy of a ligand was determined by its ability to inhibit AP-1 driven reporter activity, and its ability to repress E-selectin promoted transcription in a human A549 lung epithelial cell line.[14] The transactivation activity of a ligand (agonist activity) was evaluated by its ability to induce GAL4-GR-LBD binding to a GAL4 responsive promoter attached to a luciferase reporter gene in a human HeLa cell line (NP-1).[14]

Virtual screening of our compound repository using a GR homology model based on the progesterone receptor crystal structure[15] identified compound **1** as a potential GR ligand (Fig. 1). Compound **1** was found to have GR binding affinity of 158 nM but poor functional activity in the AP-1 repression assay (Table 1). Further structural exploration of **1** on amide functionalities identified N-(thiazol-2-yl)amide **2** as a novel, nonsteroidal GR modulator, which had high GR affinity (K_i = 2.9 nM) combined with >100-fold binding selectivity over PR and >1000-fold binding selectivity over AR and ERα. However, **2** had only weak AP-1 activity (EC_{50} = 268 nM with 43% dex efficacy).

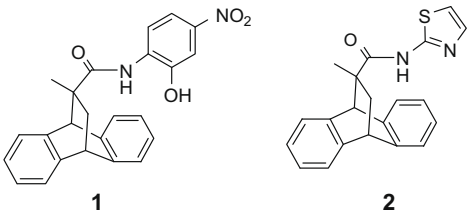
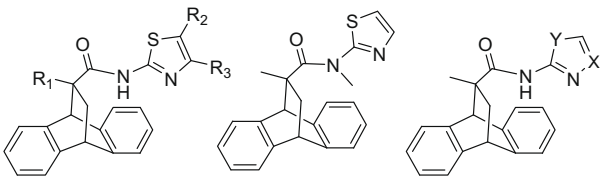


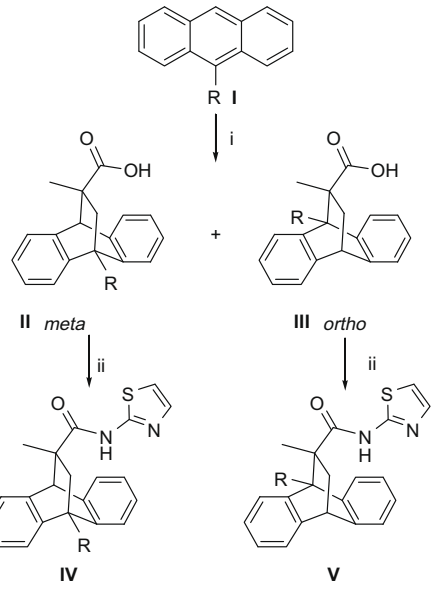
Figure 1.

Table 1
Modifications at the aminothiazole and α-position of the carboximides: GR binding, transrepression and transactivation data[a]



Compd	R_1	R_2	R_3	GR K_i (nM)	AP-1 repression (A549 cell) EC_{50}, nM (efficacy, %dex)[b]
Dex				1.1	2.5 (100)
Pred				1.5	15.8 (97)
1				157.7	>5000
2	Me	H	H	2.9	268.0 (43)
3	Me	Me	H	12.7	609.8 (30)
4	Me	H	Me	8.7	>5000
5	Me	Me	Me	22.6	922.6 (42)
6	Et	H	H	3.9	>2500
7	C_2H_4OH	H	H	303.0	>5000
8	—	—	—	82.5	1236.0 (67)
9	—	—	—	92.6	>5000
10	—	—	—	5.4	294.9 (40)
2a (R)	Me	H	H	1.3	114.9 (41)
2b (S)	Me	H	H	26.2	543.5 (45)
3a (A)[c]	Me	Me	H	4.1	345.0 (32)
3b (B)	Me	Me	H	187.0	972.5 (31)

Dex = dexamethasone; pred = prednisolone.
[a] Values are means of at least two experiments done in triplicate.
[b] Efficacy represented as a percentage of the maximal response of dexamethasone (100%).
[c] Where absolute stereochemistries are not defined, isomer A and isomer B designations are employed.[18]



Scheme 1. Reagents and conditions: (i) 2-methyl acrylic acid, toluene or nitrobenzene, 165 °C; or 2-methyl acrylic acid, neat 170 °C, 12–24 h, 40–60% (*meta* isomer); (ii) 2-amino thiazole, HOBT, EDC, CH_3CN, $^iPr_2NEt$, 85 °C, 12 h, 50–70% With other heteroaryl amines, the reaction times range from 12–24 h.

In order to improve the functional potency, we investigated modifications by employing the chemistry shown in Scheme 1.[16a] The 9,10-dihydro-9,10-ethano-anthracene carboxylic acids (II or III) were synthesized via the Diels–Alder reaction of 2-methyl acrylic acid with anthracene or 9-substituted anthracenes following modified literature procedures.[16] When using nitrobenzene as a solvent or in neat acrylic acid, at elevated temperatures of 165–170 °C, the Diels–Alder reaction gave products in higher *meta*/*ortho* ratio compared with those reported in the literature.[16b] For 9-chloro, 9-bromo and 9-methylanthracene, the *ortho* isomer products were minimal or undetectable under these reaction conditions. The coupling reaction of the acids and 2-aminothiazole or other heteroaryl amines yielded the corresponding carboxamides (IV or V).

We began our investigation by exploring the aminothiazole side chain. Initial SAR showed neither substitution at C-4 nor at C-5 of the thiazole was favorable for GR binding affinity (compounds **3–5**). Replacing the methyl group adjacent to the carboxamide with an ethyl group had minimal effect on GR binding (compound **6**). However, hydroxyethyl substitution (compound **7**) proved detrimental to binding potency. Also, introducing a methyl group to the amide nitrogen resulted in a considerable loss of binding affinity (compound **8**). To broaden the scope of our investigation, a survey of various heterocycles to replace the thiazole ring found the 1,3,4-thiadiazole to be a viable surrogate, with binding and functional activities comparable to those of the thiazole congener (compound **10**). In all cases, no significant improvement of the functional activity was observed in comparison to compound **2**.

Resolution of compounds **2** and **3** revealed that the binding activity resides mainly with one of the enantiomers **2a** or **3a**.[17]

We have applied computational modeling as an aid in exploring the SAR of the 9,10-ethano-anthracene series. Based on docking and minimization studies of **2a** with our GR homology model, we found a binding pose for the ligand which allows the deep hydrophobic pocket to be filled by the ethano-anthracenyl core, while the polar end consisting of the aminothiazole moiety engages in several H-bonds with Gln642 and Asn564 (Fig. 2b).

The overall binding pose is essentially similar to that published for the steroidal agonist, dexamethasone (Fig. 2a). Interestingly,

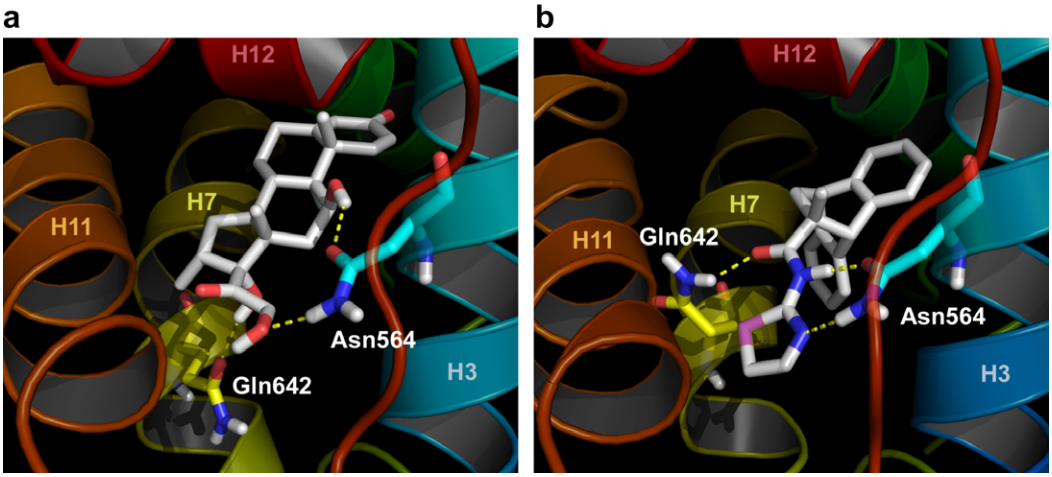


Figure 2. (a) Published X-ray structure of dexamethasone in the GR-LBD.[19] (b) Modeling of **2a** using a homology model and adjusting several residues in possible H-bonding contact with the ligand. Similar to their role in dexamethasone binding, Asn564 and Gln642 are thought to establish key interactions with the amidothiazole of **2a**. Dotted yellow lines represent potential H-bonds.

both dex and **2a** engage Asn564, wherein two H-bonds to the ligand can be formed. In addition, it was found that Gln642 could be repositioned to allow for a third H-bond to the amide carbonyl. The model of **2a** binding was consistent with the SAR findings described above. A region of particular interest for synthetic elaboration was the *meta*-bridgehead since the model suggested that the pocket was hydrophobic and capable of accommodating small functional groups.

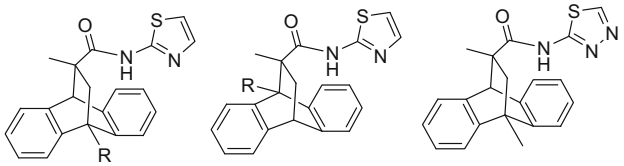
The effect of substitution at the bridgehead positions was then investigated (Table 2). The unsubstituted thiazole was first chosen as a fixed fragment for further optimization. To our delight, small, hydrophobic substituents at the *meta* position showed significant improvement of AP-1 inhibitory activity. A methyl group was found to be optimal at this position. Compound **17** had good potency in the AP-1 (EC50 = 15 nM) and E-selectin (EC50 = 8.3

nM) transrepression assays, with agonist efficacy reaching 79% of dex. Introducing polar or bulky groups at the *meta* position, such as NH_2, CO_2CH_3, CHO, CH_2NHR, $CONR_2$ led to compounds with reduced AP-1 activity ($EC_{50} > 1$ μM; data not shown). The *ortho* regioisomers (**12** and **14**) appeared to have weaker activity than their *meta* counterparts (**11** and **13**, respectively).

Selected *meta* isomers were resolved into their enantiomers either directly or indirectly using chiral column chromatography (Table 3).[18] Not surprisingly, the active enantiomer of **17**, compound **17a**, was the most potent GR agonist identified in the cellular assays, AP-1 (EC50 = 5.3 nM with 73% dex efficacy) and E-selectin (EC50 = 1.4 nM with 64% dex efficacy). To determine whether compounds demonstrated dissociated pharmacology, we compared their efficacies in the NP-1 GRE activation (TA) and AP-1 (TR) assays. Of particular interest was the TA activity of **17a**, which displayed agonism efficacy 31% of dex in the GRE activation assay, significantly weaker than the observed TR agonism efficacy (73% dex in the AP-1 assay). While exhibiting partial agonism (64–73% dex in TR assays), **17a** thereby demonstrated a good degree of separation of TR/TA efficacy. This is in sharp contrast to prednisolone which displayed equal maximal efficacy in the TR and TA assays (97% dex in both AP-1 and GRE activation assays). Lower TA agonism efficacy compared to TR agonism efficacy was also observed with compound **18a**, though its agonistic activity (potency and efficacy) in transrepression assays is slightly weaker than that of **17a**.

Docking of **17a** in the GR homology model illustrates a possible functional effect of the bridgehead *meta* methyl group (Fig. 3). This methyl group appears to form a tight van der Waals contact with Met646 (on H7), displacing the thiomethyl portion towards Leu732 (not shown) on H11. This subtle movement may have the indirect effect of forming a tighter hydrophobic interaction between H7 and H11, essentially leading to stabilization of H11 and a corresponding increase in agonist functional activity, as H11 stability may play a role in the equilibrium between open and closed H12 forms of the protein. Such stabilizing interactions are well precedented for steroid-binding nuclear hormone receptors.[20]

Table 2
Activities of bridgehead regioisomers[a]



meta
11 R = NO_2
13 R = CN
15 R = Br
16 R = Cl
17 R = Me

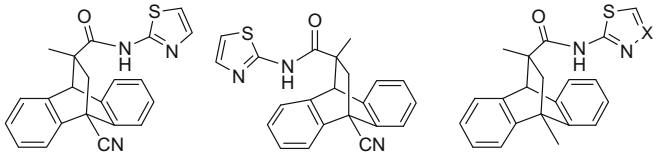
ortho
12 R = NO_2
14 R = CN

18

Compd	GR K_i (nM)	AP-1 repression EC_{50}, nM (Eff.%dex)[b]	E-selectin repression EC_{50}, nM (Eff.%dex)[b]	GRE activation EC_{50}, nM (Eff.% dex)[b]
Dex	1.1	2.5 (100)	1.1 (100)	4.2 (100)
Pred	1.5	15.8 (97)	13.6 (97)	82.7 (97)
2	2.9	268.0 (43)	30.7 (31)	>10,000
11	3.0	1067.3 (54)	>5000	>10,000
12	369.6	1159.6 (48)	>5000	>10,000
13	2.5	330.6 (52)	425.5 (32)	>10,000
14	343.0	874.2 (87)	678.1 (46)	>10,000
15	1.7	118.8 (82)	9.9 (60)	174.9 (38)
16	1.7	229.4 (71)	12.3 (38)	321.9 (9)
17	1.8	14.7 (79)	8.3 (56)	121.0 (30)
18	1.3	16.9 (59)	5.2 (51)	>10,000

Dex = dexamethasone; pred = prednisolone.
[a] Values are means of at least two experiments done in triplicate.
[b] Efficacy represented as a percentage of the maximal response of dexamethasone (100%).

Table 3
Activities of enantiomeric compounds[a,19]



13a (R) **13b (S)** **17a, 17b** X = CH **18a, 18b** X = N

Compd	Enantiomer[b]	GR K_i (nM)	AP-1 repression EC_{50}, nM (%dex)[c]	E-selectin repression EC_{50}, nM (%dex)[c]	GRE activation EC_{50}, nM (%dex)[c]
Dex		1.1	2.5 (100)	1.1 (100)	4.2 (100)
Pred		1.5	15.8 (97)	13.6 (97)	82.7 (97)
13a	R	0.9	243.1 (53)	19.4 (33)	>10,000
13b	S	37.8	836.8 (66)	>5000	>10,000
17a	A	1.0	5.3 (73)	1.4 (64)	117.0 (31)
17b	B	67.8	800.4 (61)	>5000	>10,000
18a	A	1.3	11.4 (69)	8.3 (55)	110.9 (20)
18b	B	49.7	3835.0 (51)	151.8 (44)	>2500

Dex = dexamethasone; pred = prednisolone.
[a] Values are means of at least two experiments done in triplicate.
[b] Where absolute stereochemistries are not defined, isomer A and isomer B designations are employed.
[c] Efficacy represented as a percentage of the maximal response of dexamethasone (100%).

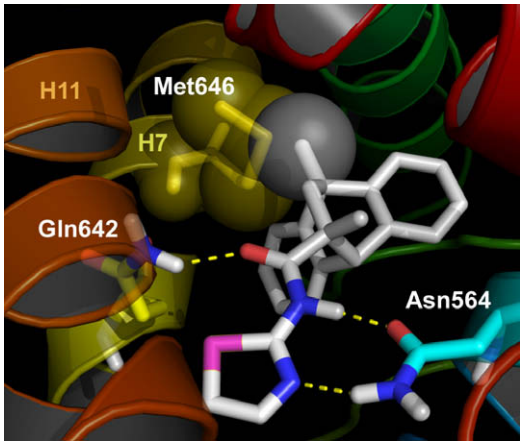


Figure 3. Modeling of **17a** in the ligand binding site of GR. Contact between the bridgehead methyl (gray sphere) and Met646 (yellow spheres) is illustrated. Dotted yellow lines represent potential H-bonds.

To monitor steroid receptor cross-reactivity, counter screen binding and activation assays were routinely performed on a subset of compounds against a panel of nuclear hormone receptors consisting of PR, AR, ER_α and MR (activation in A549 cell). Compounds **2a, 3a, 13a, 17a** and **18a** showed excellent selectivity between GR and AR or ER_α (Table 4). A modest 31–150-fold separation of binding affinity for GR over PR was observed with most of the compounds, except **3a** which had more than 2500-fold separation. The bridgehead substitution appeared to have a deleterious impact on selectivity over PR (**13a, 17a** and **18a**). In contrast, the substitution on the thiazole dramatically decreased affinity towards PR as manifested by **3a**. Finally, all compounds exhibited no agonistic activity at 5 μM in cellular MR activation assay. Conversely compounds bearing bridgehead substitution showed some antagonist activity at 0.1 μM with ~50% inhibition of the response of 1.25 nM aldosterone, an MR agonist.

In summary, we have described a novel class of dihydro-ethanoanthracene carboxamides as potent and selective non-steroidal ligands of the glucocorticoid receptor. Select compounds from this series showed a promising dissociation profile. **17a** elicits significantly reduced transactivation relative to steroidal agonists, while retaining good transrepression activities in reporter gene assays pertinent to the anti-inflammatory effect. A model of a compound from this class in the active site of the receptor is shown which can also account for the induction of the functional activity demonstrated by a bridgehead methyl group. Taken together, these findings indicate the attractiveness of this series for further studies which will be the subject of future reports.

Table 4
Cross-reactivity data for selected glucocorticoid receptor ligands[a]

Compd	GR	PR	AR	ERα	MR activation (A549 cell)[b]	
	K_i (nM)	K_i (μM)	K_i (μM)	K_i (μM)	Agonist mode % Ctl	Antagonist mode % inh.
2a	1.3	0.147	7.4	15.0	nd	3
3a	4.1	>10.0	>10.0	>5.0	nd	5
13a	0.9	0.034	>10.0	26.3	nd	47
17a	1.0	0.031	2.9	>5.0	nd	49
18a	1.3	0.035	9.3	>5.0	nd	53

Nd = not detectable; Ctl = control; inh. = inhibition.
[a] Values are means of at least two experiments done in triplicate.
[b] The mineralocorticoid receptor (MR) agonist aldosterone was used as a control in the MR transactivation assay. Agonist efficacies (at the concentration of 5 μM) represented as a percentage of the maximal response of 10 nM aldosterone (100%). Antagonist efficacies (at the concentration of 0.1 μM) represented as a percentage of inhibition of the induction of 1.25 nM aldosterone (100%).

Acknowledgments

The authors are grateful to the following colleagues for their support of the project, and their help in the preparation of this manuscript. Mary Ellen Cvijic, Ding Ren Shen, Melissa Yarde, Mary Malley, Dauh-Rurng Wu, Dawn Sun, and William J. Pitts.

References and notes

1. Buttgereit, F.; Burmester, G-R.; Lipworth, B. J. *Lancet* **2005**, *365*, 801.
2. For a review on transrepression, see: Laudet, V.; Gronemeyer, H. *The Nuclear Receptor Factsbook*; Academic Press, 2002. 42.
3. (a) Diamond, M. I.; Miner, J. N.; Yoshinaga, S. K.; Yamamoto, K. R. *Science* **1990**, *249*, 1266; (b) Caldenhoven, E.; Liden, J.; Wissink, S.; Van de Stolpe, A.; Raaijmakers, J.; Koenderman, L.; Okret, S.; Gustafsson, J.-A.; Van der Saag, P. T. *Mol. Endocrinol.* **1995**, *9*, 401.
4. (a) Baldwin, A. S. *J. Clin. Invest.* **2001**, *107*, 3; (b) Firestein, G. S.; Manning, A. M. *Arthritis and Rheumatism* **1999**, *42*, 609; (c) Peltz, G. *Curr. Opin. Biotechnol.* **1997**, *8*, 467.
5. Reichardt, H. M.; Kaestner, K. H.; Tuckermann, J.; Kretz, O.; Wessely, O.; Bock, R.; Gass, P.; Schmid, W.; Herrlich, P.; Angel, P.; Schütz, G. *EMBO J.* **2001**, *20*, 7168. and references cited herein.
6. For recent reviews on development in the discovery of dissociated glucocorticoid agonists, see: (a) Hudson, R.; Roach, S. L.; Higuchi, R. I. *Curr. Top. Med. Chem.* **2008**, *8*, 750; (b) Schcke, H.; Berger, M.; Hansson, T. G.; McKerrecher, D.; Rehwinkel, H. *Expert Opin. Ther. Patents* **2008**, *18*, 339; (c) Ragan, J.; Razavi, H.; Thomson, D. *Ann. Rep. Med. Chem.* **2008**, *43*, 141.
7. Abbott/Ligand: (a) Coghlan, M. J.; Kym, P. R.; Elmore, S. W.; Wang, A. X.; Luly, J. R.; Wilcox, D.; Stashko, M. l.; Lin, C.-W.; Miner, J.; Tyree, C.; Nakane, M.; Jacobson, P.; Lane, B. C. *J. Med. Chem.* **2001**, *44*, 2879; (b) Miner, J. N.; Ardecky, B.; Benbatoul, K.; Groffoths, K.; Larson, C. J.; Mais, D. E.; Marschke, K.; Rosen, J.; Vajda, E.; Zhi, L.; Negro-Vilar, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 19244.
8. Schering-Schacke, H.; Schottelius, A.; Docke, W.-D.; Strehlke, P.; Jaroch, S.; Schmees, N.; Rehwinkel, H.; Hennekes, H.; Asadullah, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 227.
9. Merck-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.
10. UCSF-Shah, N.; Scanlan, T. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5199.
11. Boehringer Ingelheim-Regan, J. L. T. W.; Zindell, R. M.; Bekkali, Y.; Bentzien, J.; Gilmore, T.; Hammach, A.; Kirrane, T. M.; Kukulka, A. J.; Kuzmich, D.; Nelson, R. M.; Proudfoot, J. R.; Ralph, M.; Pelletie, J.; Souza, D.; Zuvela-Jelaska, L.; Nabozny, G.; Thomson, D. S. *J. Med. Chem.* **2006**, *49*, 7887.
12. GlaxoSmithKline–Biggadike, K.; Boudjelal, M.; Clackers, M.; Coe, D. M.; Demaine, D. A.; Hardy, G. W.; Humphreys, D.; Inglis, G. G. A.; Johnston, M. J.; Jones, H. T.; House, D.; Loiseau, R.; Needham, D.; Skone, P. A.; Uings, I.; Veitch, G.; Weingarten, G. G.; McLay, I. M.; Macdonald, S. J. F. *J. Med. Chem* **2007**, *50*, 6519. and references cited herein.
13. GR, PR, ER and AR ligand binding assays were conducted in fluorescence polarization format which measures the competition between a test compound and a fluorescently labeled ligand for binding to the full length or ligand binding domain of the nuclear hormone receptor. IC_{50} values were determined by fitting the fluorescence polarization signal data using the four parameter logistic equation. The K_i values were determined by application of the Cheng–Prusoff equation to the IC_{50} values, where $K_i = IC_{50}/(1 + \text{ligand concentration}/K_d)$. Data shown represent the means of duplicate experiments.
14. Cellular assays: AP-1 activity is measured using an AP-1 response element (containing 5 copies) cloned into a luciferase reporter vector. This reporter is stably transfected into the human A549 lung epithelial cell line. AP-1 activity is induced by PMA (15 ng/ml), and inhibition of the induction by compounds is quantitated by measuring decreased luciferase activity. NFκB is measured using a truncated, NFκB dependant, E-selectin promoter (~300 bp) cloned into a luciferase reporter vector. This reporter is stably transfected into the human A549 lung epithelial cell line. NFκB activity is induced using IL-1β (0.5 ng/ml) and inhibition of the induction by compounds is quantitated by measuring decreased luciferase activity. NP-1 GRE activation activity is measured using a GR ligand binding domain (GR-LBD) chimera cloned into a GAL4 luciferase reporter system. This reporter system is stably transfected into a HeLa cell line (NP-1). Response to ligand induced binding is quantitated by measuring luciferase activity. Direct activation of the GR-LBD by compounds (agonist) can be measured as increased luciferase activity.
15. A GR homology model was built using the Modeler program with Insight II (Accelrys, Inc., San Diego, CA) based on the published progesterone receptor crystal structure (1A28.pdb); Williams, S. P.; Sigler, P. B. *Nature* **1998**, *393*, 329.
16. a Vaccaro, W.; Yang, B. V.; Kim, S.-H.; Huynh, T.; Tortolani, D. R.; Leavitt, K. J.; Doweyko, A.; Doweyko, L.; Chen, X.-T. Modulators of the glucocorticoid receptor and method, Patent US 2005-643462 (Jan 13, 2005).; (b) Alston, P. V.; Ottenbrite, R. M.; Newby, J. *J. Org. Chem.* **1979**, *44*, 4939.
17. A crystal structure of the enantiopure acid **II** (wherein R=H), the precursor of **2a**, was obtained which allowed the assignment of its absolute stereochemistry, therefore defined the configuration of compound **2a**. The absolute configuration of **12a** was assigned in a way analogous to that of **2a**.
18. Using flash chiral column technique, the final carboximides were separated into their enantiomers directly. If the absolute configurations of the two enantiomers have not been determined, enantiomer assignment is based on the order of elution from analytical chiral HPLC—isomer A is designated as the fast eluting isomer and isomer B as the slow eluting isomer. In some cases, the enantiopure carboximides were derived from the chiral acids when chiral separations were carried out with the precursor acids.
19. Bledsoe, R. B.; Montana, V. G.; Stanley, T. B.; Delves, C. J.; Apolito, C. J.; McKee, D. D.; Consler, T. G.; Parks, D. J.; Stewart, E. L.; Willson, T. M.; Lambert, M. H.; Moore, J. T.; Pearce, K. H.; Xu, H. E. *Cell* **2002**, *110*, 93.
20. Doweyko, A. M. *Drug Development Res.* **2007**, *68*, 95. and references cited herein.