Indole Glucocorticoid Receptor Antagonists Active in a Model of Dyslipidemia Act via a Unique Association with an Agonist Binding Site.

John Gately Luz[‡], Matthew W. Carson[†], Bradley Condon[‡], David Clawson[†], Anna Pustilnik[‡], Daniel T. Kohlman[†], Robert J. Barr[†], James S. Bean[†], M. Joelle Dill[†], Dana K Sindelar[†], Milan Maletic[‡], Michael J. Coghlan*[†]

†. Lilly Research Laboratories, A Division of Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN, 46285 USA.

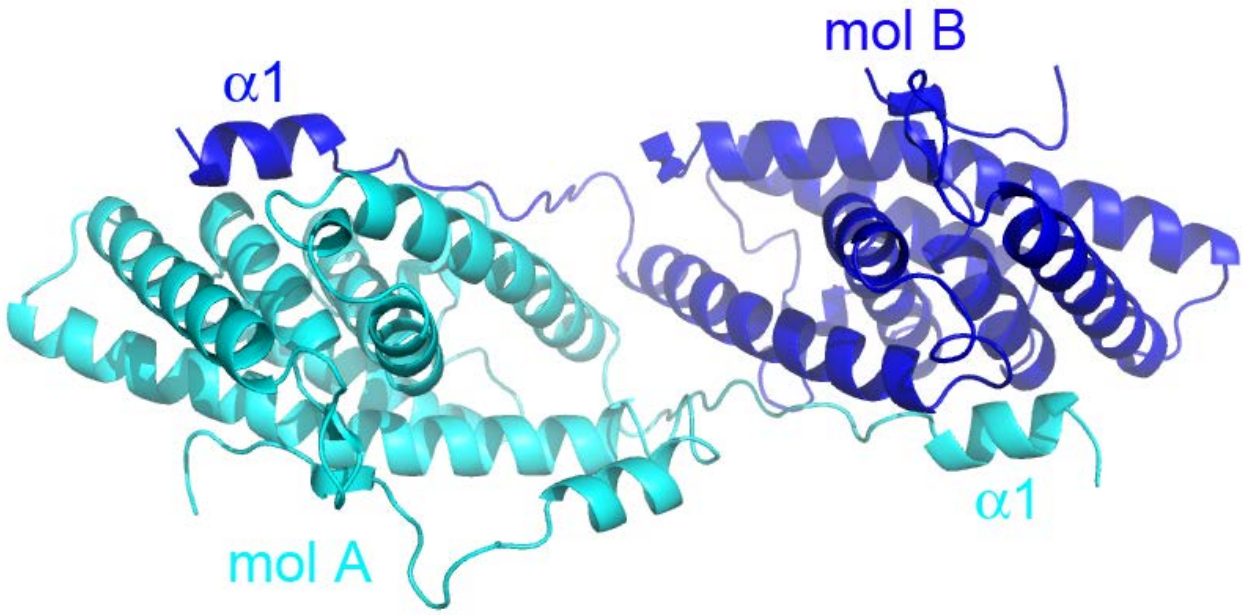
‡. Eli Lilly Biotechnology Center, 10300 Campus Point Drive, Suite 200, San Diego, CA, 92121 USA.

Supplemental Information

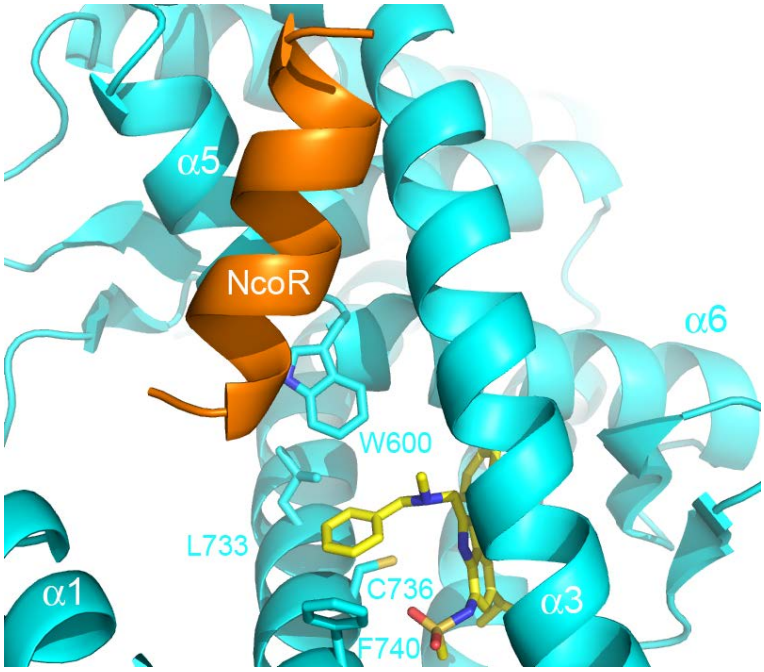
	(% efficacy @ 10μM)	
	GR Agonism	Transrepression
8	5.80 (2.22, n=5)	-
15	3.65(0.428)2	-
16	16.9	-
18	15.7	-
19	7.55 (1.14)2	14.3 (9.49)2
21	7.34	-7.00 (18.4)2
22	8.70 (1.66)2	87.0 (0.856)2
MIF	17.7 (1.71)7	19.4 (20.0)7

Table S1: Agonist and Transrepression data for selected GR ligands: Compounds with GR binding Ki < 10 nM were assayed in models of agonism (agonist activity in HEK293 cells transiently transfected with GREs controlling a luciferase (*luc*) reporter gene) and transrepression (Inhibition of IL-1β - induced IL-6 in CCD-39SK cells).[1] Both methods employed prednisolone at 10μM to define maximal efficacy (100%). In all but one case the compounds showed minimal functional efficacy as agonists or transrepressors at the highest concentration tested (10μM) and none had activity at lower concentrations.

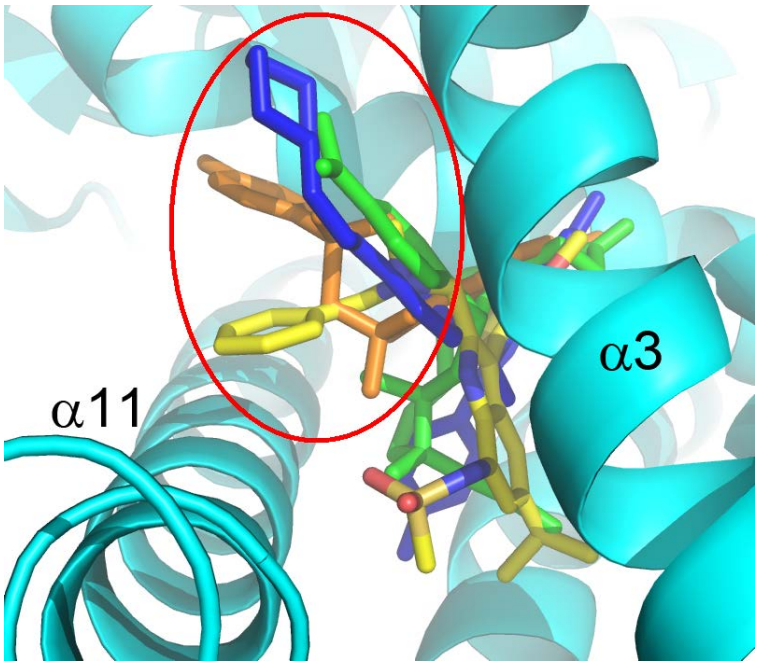
- No activity at 10μM



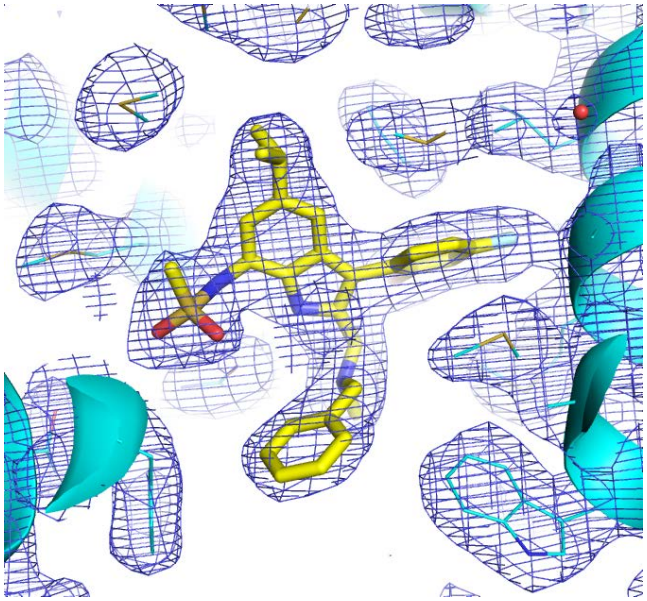
Supplemental Figure 1. Domain swapping through the α-1 helix in the asymmetric unit of the GR-LBD/Compound 8/NcoR crystal structure. GR-LBD is represented as a ribbon diagram with molecule A colored cyan and molecule B colored blue. The α-1 helix of molecule A extends to the right and interacts with molecule B. The α-1 helix of molecule B extends to the left and interacts with molecule A.



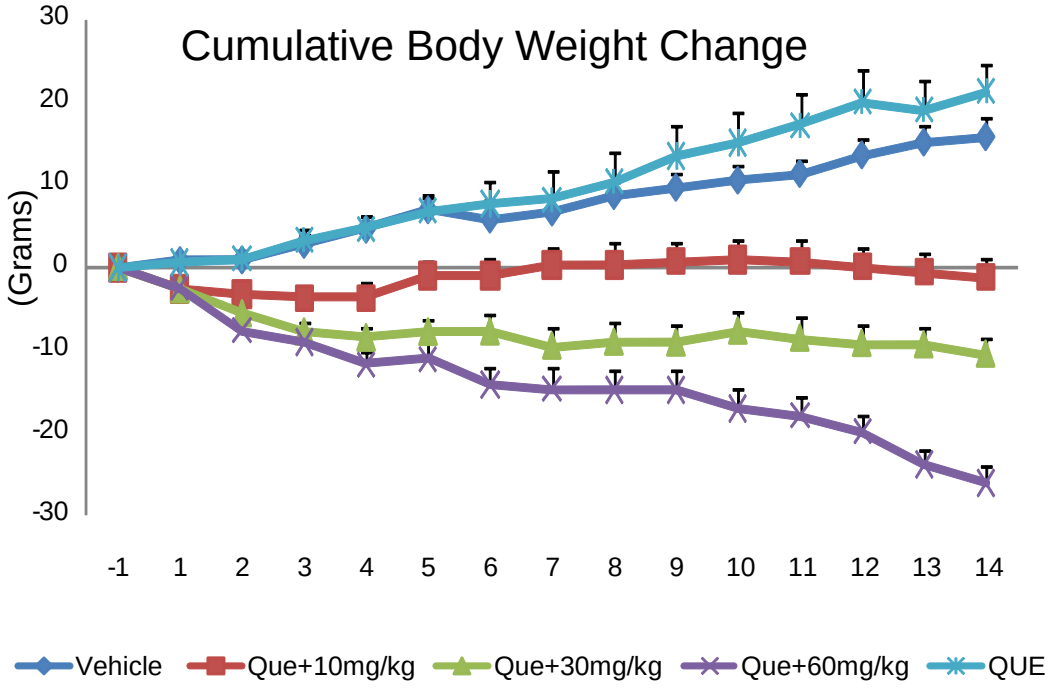
Supplemental Figure 2. Orientation of the NcoR corepressor peptide and the benzylamine moiety of compound 8. The GR-LBD (cyan) and NcoR (orange) are depicted as ribbons. compound **8** is represented as sticks and colored by atom (as previous) GR-LBD side chains that form Van der Waals interaction with the compound **8** phenyl are represented by sticks colored by atom (as previous). The NcoR peptide is bound in a groove formed from the α-3 and α-5 helices. The compound **8** phenyl is flanked by side chains extending from the α-11 helix and the tryptophan side chain extending from the first turn of the α-6 helix.



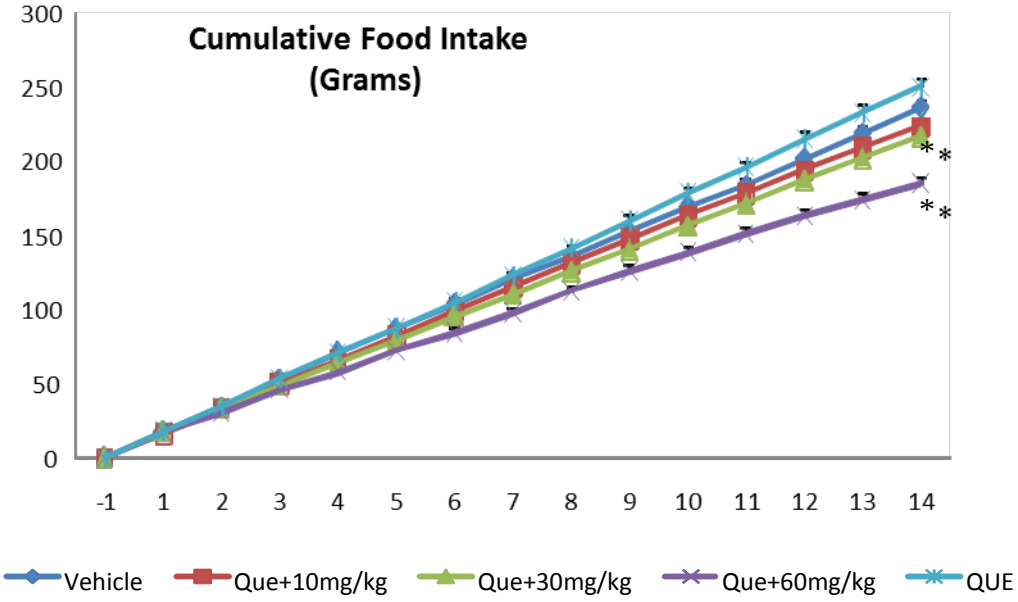
Supplemental Figure 3. Relative orientation of antagonists bound to steroid NHRs. The GR-LBD/ **8**, GR-LBD/MIF, ER/raloxifene, and AR/bicalutamide structures were superimposed. The NHR-LBD is represented by ribbons (cyan). Compound **8** (yellow), MIF (green), raloxifene (blue), and bicalutamide (orange) are represented by sticks. The red oval highlights differences of the benzyl group of compound **8** versus the 'blocking groups' of other NHR antagonists resulting in the antagonist conformation of the h12 helix and a closer association of **8** with α helix 11.



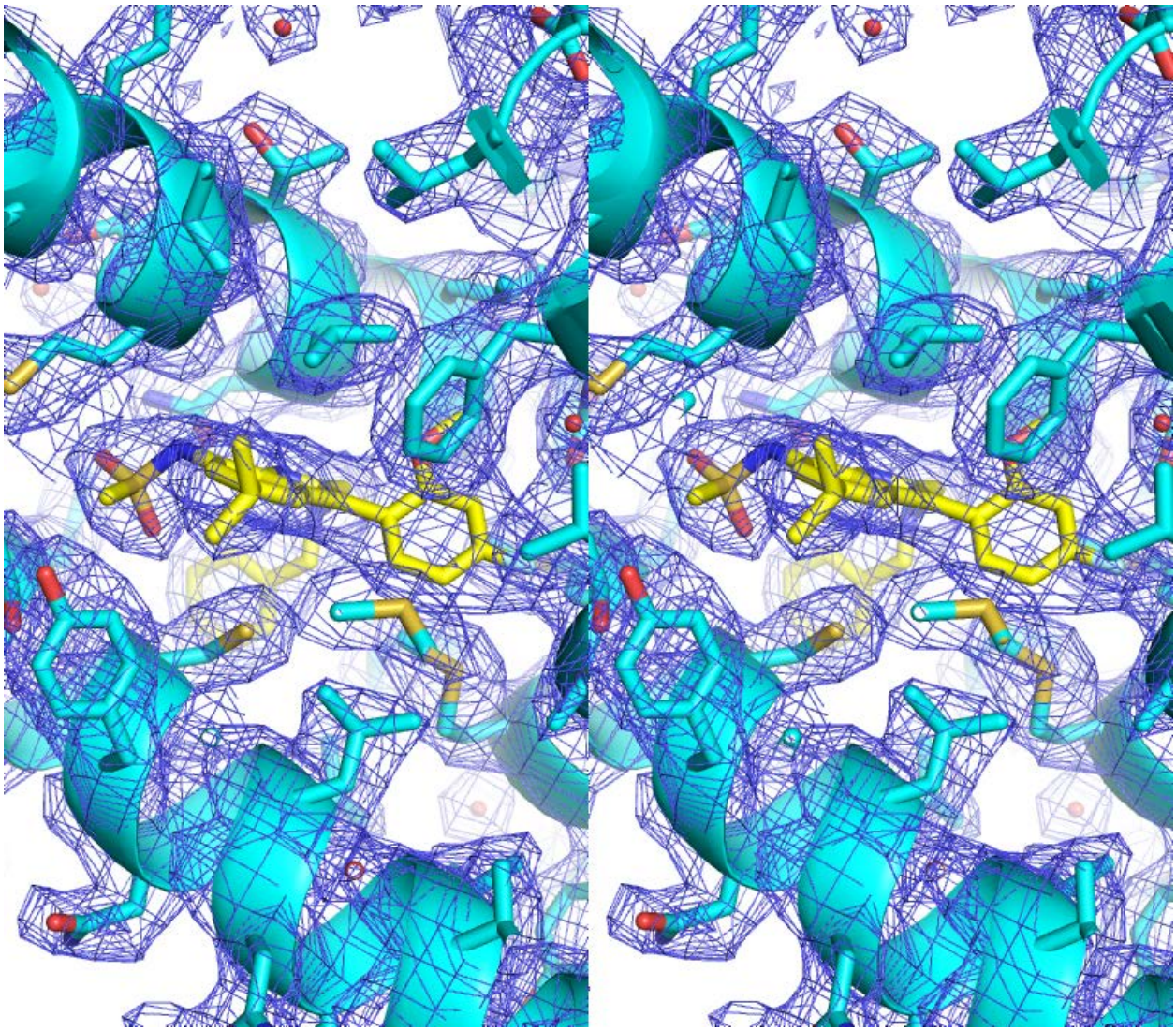
Supplemental Figure 4. Electron density of ligand binding site contoured at 1.0 σ The GR-LBD (cyan ribbons and side chains as lines colored by atom as previous) and compound **8** (sticks colored by atom as previous) are shown. The 2Fo-Fc electron density map, shown as mesh and contoured at 1.0 σ demonstrates that the entirety of the ligand is represented by unambiguous electron density.



Supplemental Figure 5a: Time course of weight gain in female Wistar rats dosed qd with quetiapine (10 mg) and varied doses of compound 8 po.



Supplemental Figure 5b: Food consumption during the 14d concurrent dosing of quetiapine and compound 8:



Stereo image of 2Fo-Fc electron density map contoured at 1.0 σ for quality check.

1. Carson, M. W.; Luz , J. G.; Suen, C.; Montrose, C.; Zink, R.; Ruan, X.; Cheng, C.; Cole, H.; Adrian, M. D.; Kohlman, D. T.; Mabry, T.; Snyder, N.; Condon, B.; Maletic, M.; Clawson, D.; Pustilnik, A.; Coghlan, M. J. Glucocorticoid Receptor Modulators Informed by Crystallography Lead to a New Rationale for Receptor Selectivity, Function and Implications for Structure-based Design. *J Med Chem* **2014,** *57*, 849-860.