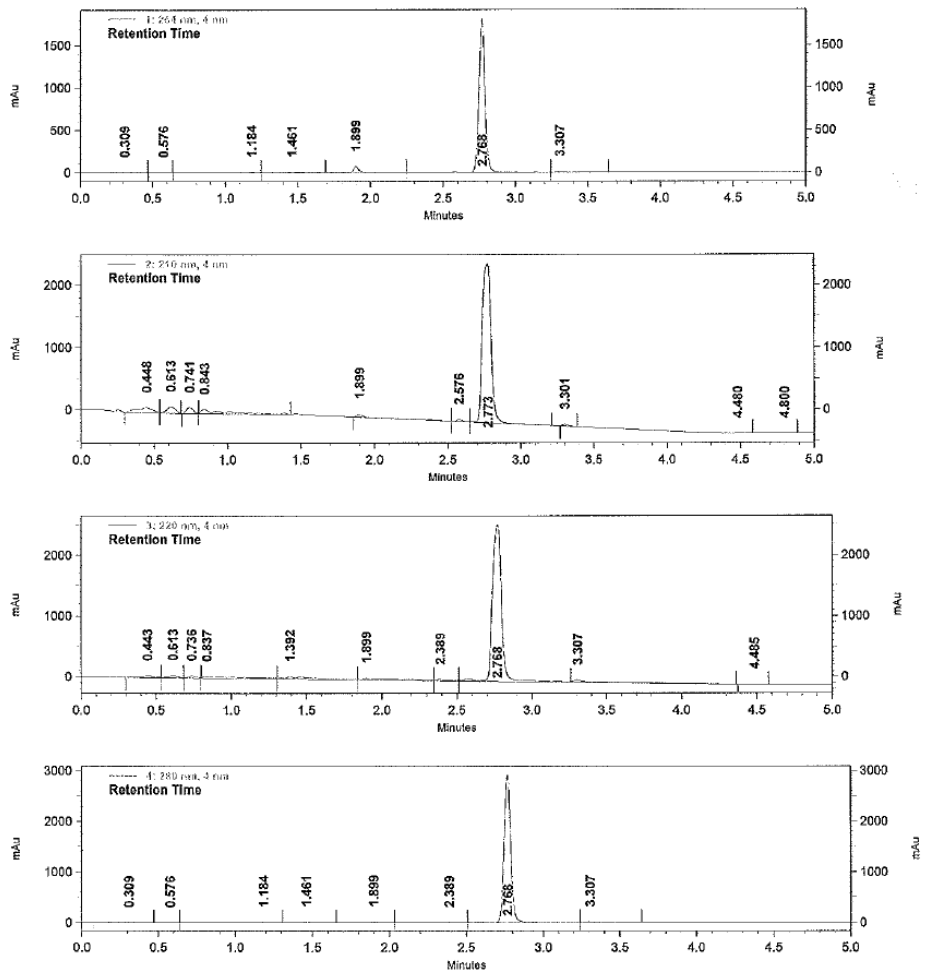
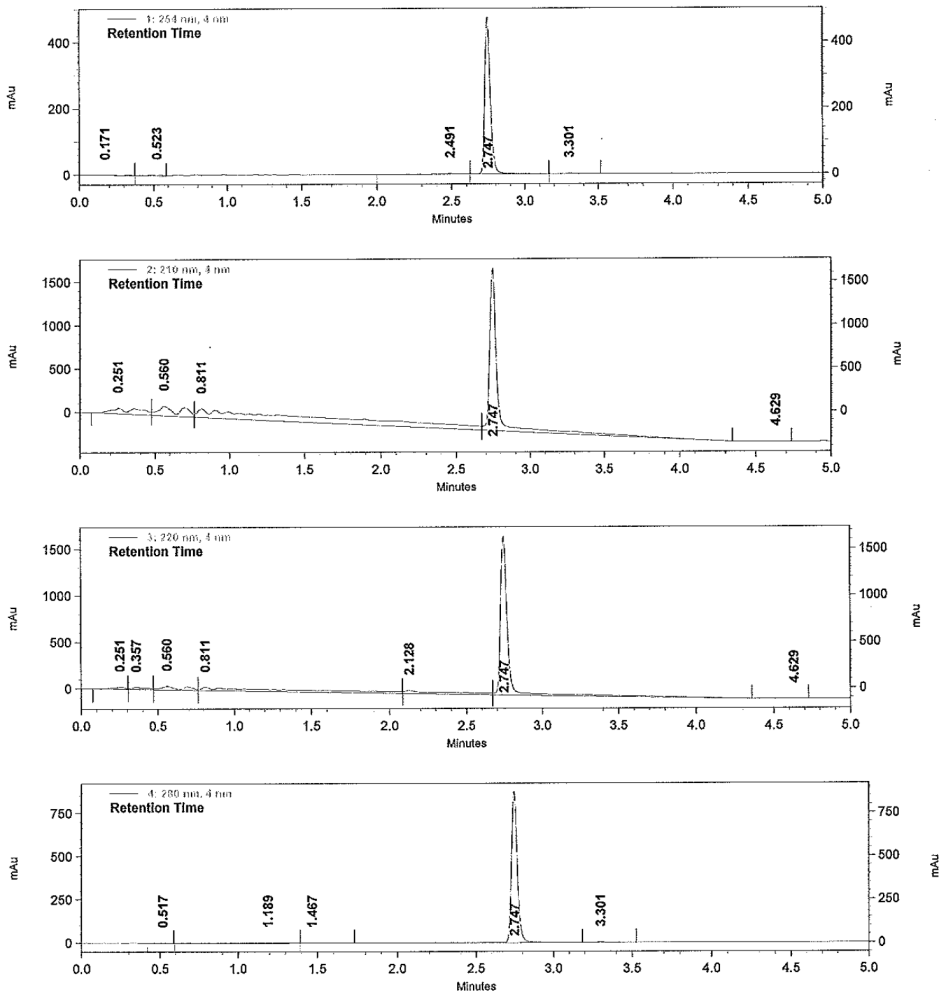
a



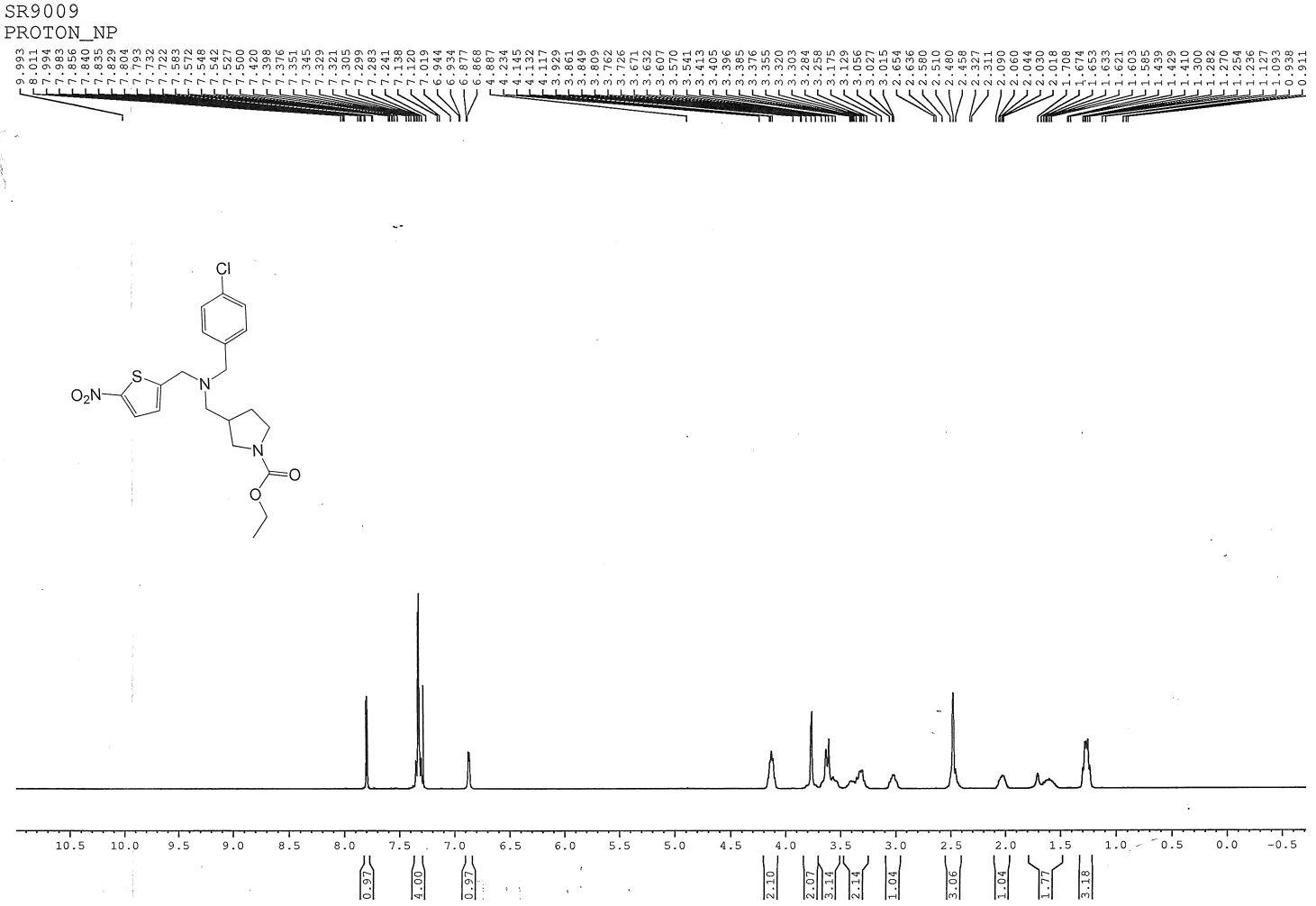
Start % B = 5
Final % B = 95
Gradient Time = 4 min
Flow Rate = 3 ml/min
Wavelength = 254
PDA WL1: 254 WL2: 210 WL3: 220 WL4: 280
Solvent A = H20+0.1%TFA
Solvent B = ACN
Column 1 : Shimadzu VP-ODS 4.6 x 50 mm (fast analyt)
SR-9009

b



Start % B = 5
Final % B = 95
Gradient Time = 4 min
Flow Rate = 3 ml/min
Wavelength = 254
PDA WL1: 254 WL2: 210 WL3: 220 WL4: 280
Solvent A = H20+0.1%TFA
Solvent B = ACN
Column 1 : Shimadzu VP-ODS 4.6 x 50 mm (fast analyt)
SR-9011

C


SR9009
PROTON_NP
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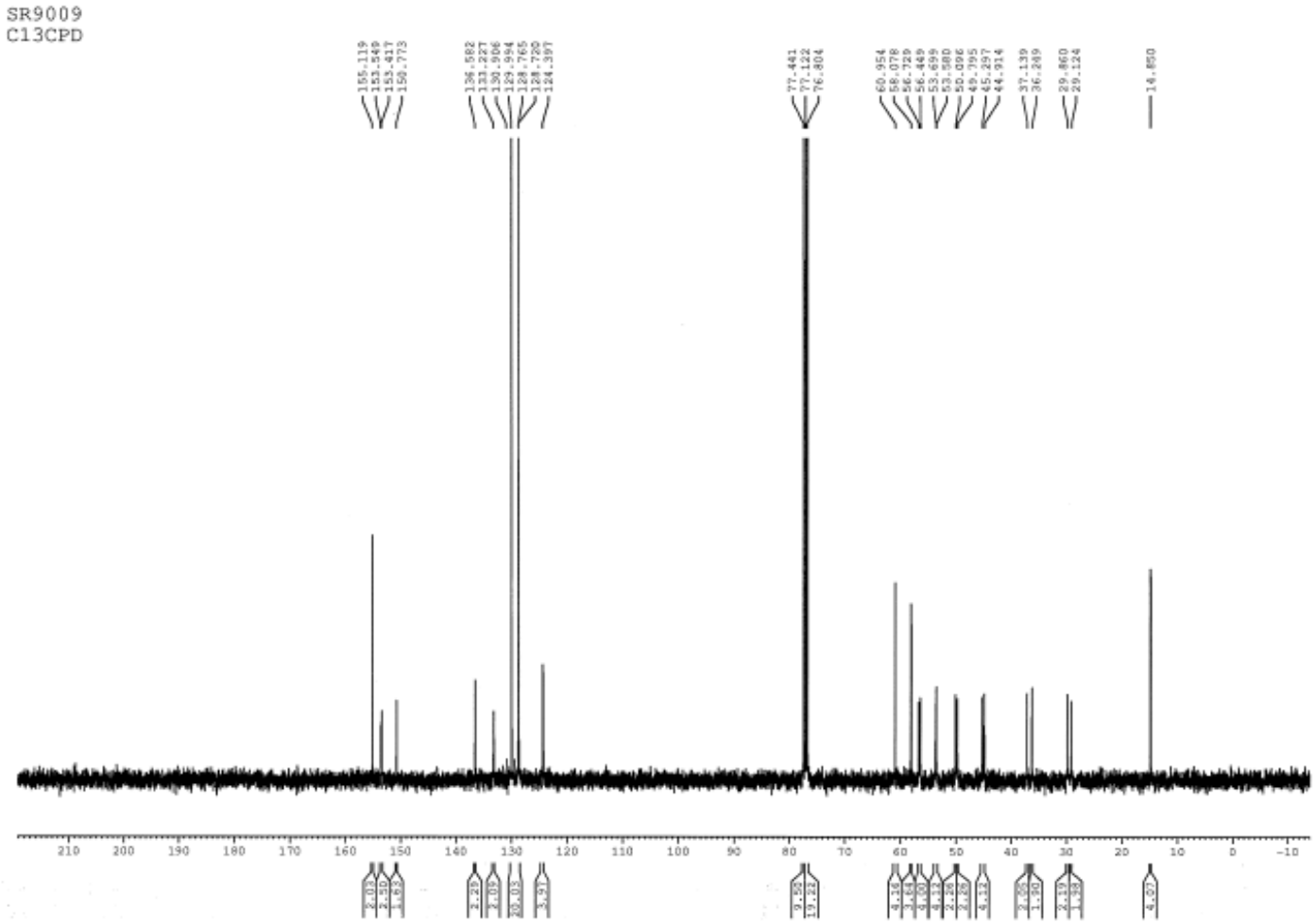
Cl
S
O2N
N
N
O
O
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
0.97 4.00 0.97 2.10 2.07 3.14 2.14 1.04 3.06 1.04 1.77 3.18

d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153.549
153.417
150.773
136.582
133.227
130.906
129.994
128.765
128.720
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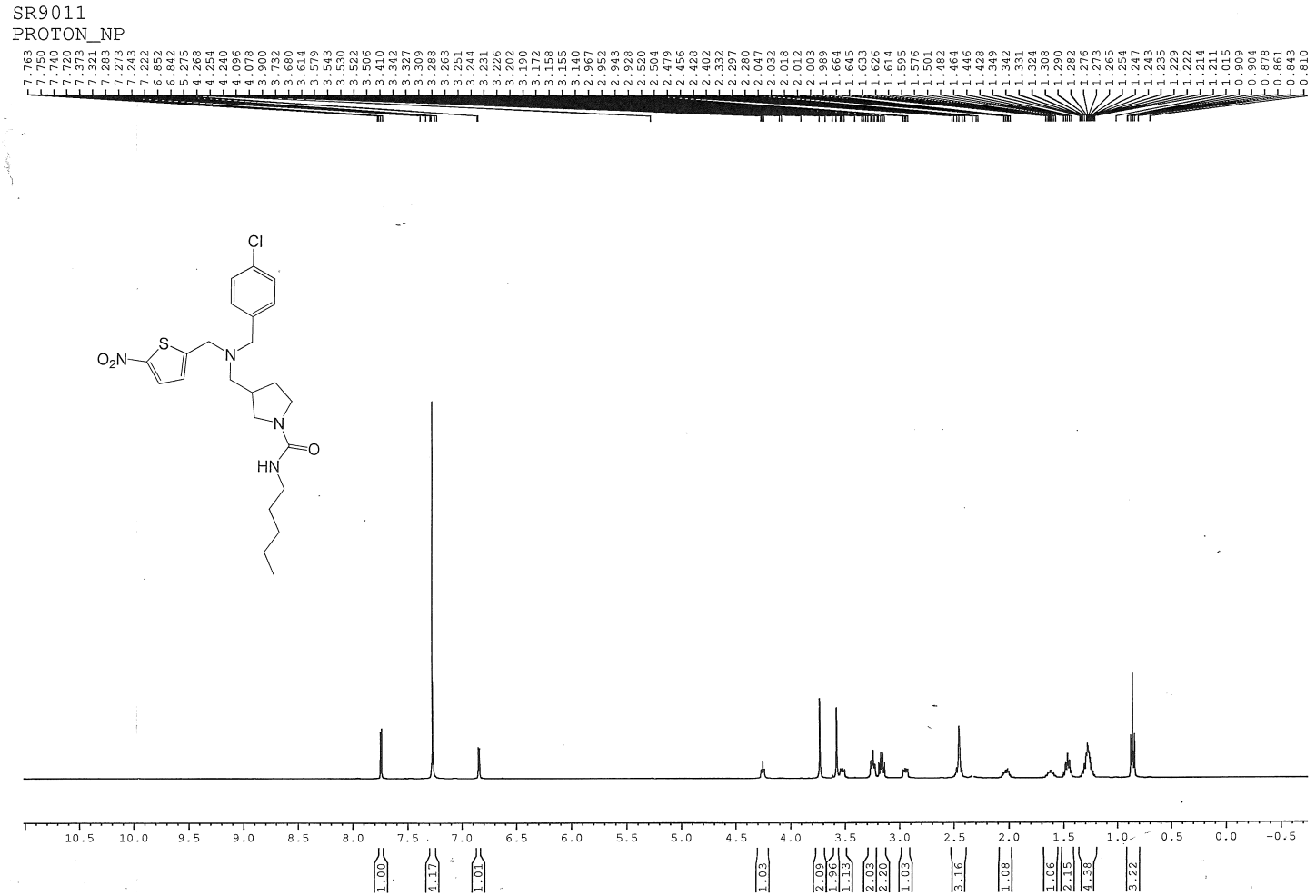
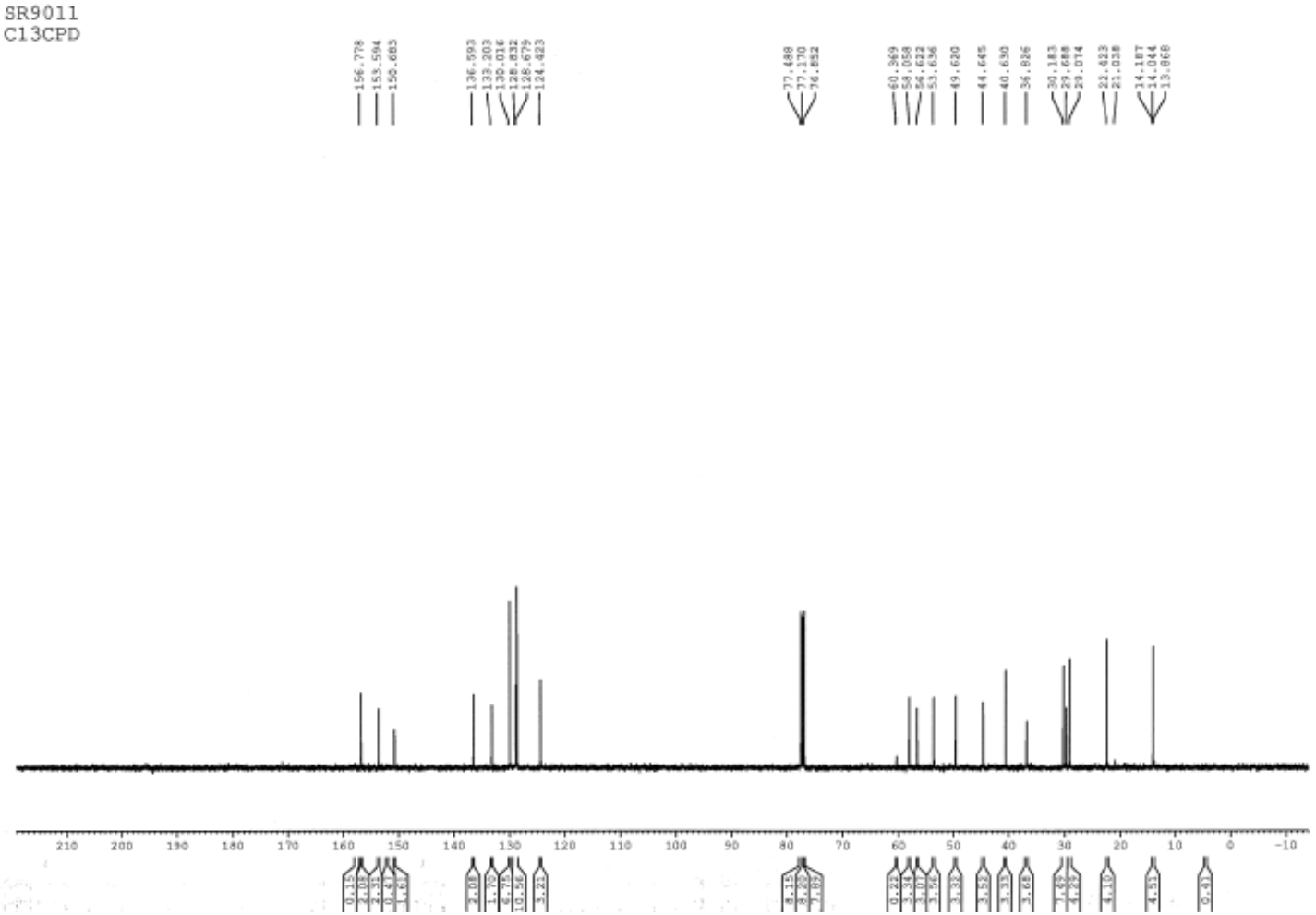
77.441
77.122
76.804
60.954
58.078
56.729
56.449
53.699
53.580
50.096
49.795
45.297
44.914
37.139
36.269
29.860
29.124
14.850

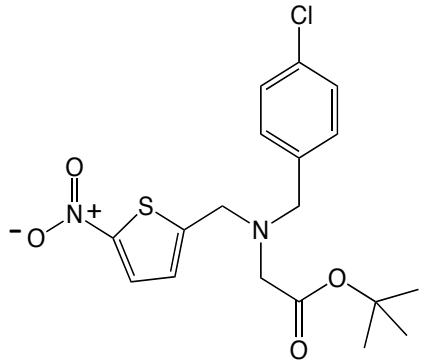
e


SR9011
PROTON_NP
7.763 7.750 7.740 7.720 7.373 7.321 7.283 7.273 7.243 7.222 6.852 6.842 5.275 4.268 4.254 4.240 4.096 4.078 3.900 3.732 3.680 3.614 3.579 3.543 3.530 3.522 3.506 3.410 3.342 3.327 3.309 3.288 3.263 3.251 3.244 3.231 3.226 3.202 3.190 3.172 3.158 3.155 3.140 2.967 2.952 2.943 2.928 2.520 2.504 2.479 2.456 2.428 2.402 2.332 2.297 2.280 2.047 2.032 2.018 2.012 2.003 1.989 1.664 1.645 1.633 1.626 1.614 1.595 1.576 1.501 1.482 1.464 1.446 1.428 1.349 1.342 1.331 1.324 1.308 1.290 1.282 1.276 1.273 1.265 1.254 1.247 1.243 1.235 1.229 1.222 1.214 1.211 1.015 0.909 0.904 0.878 0.861 0.843 0.810
Cl
S
O2N
N
N
O
HN
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
1.00 4.17 1.01 1.03 2.09 1.96 1.13 2.03 2.20 1.03 3.16 1.08 1.06 2.15 4.38 3.22

f

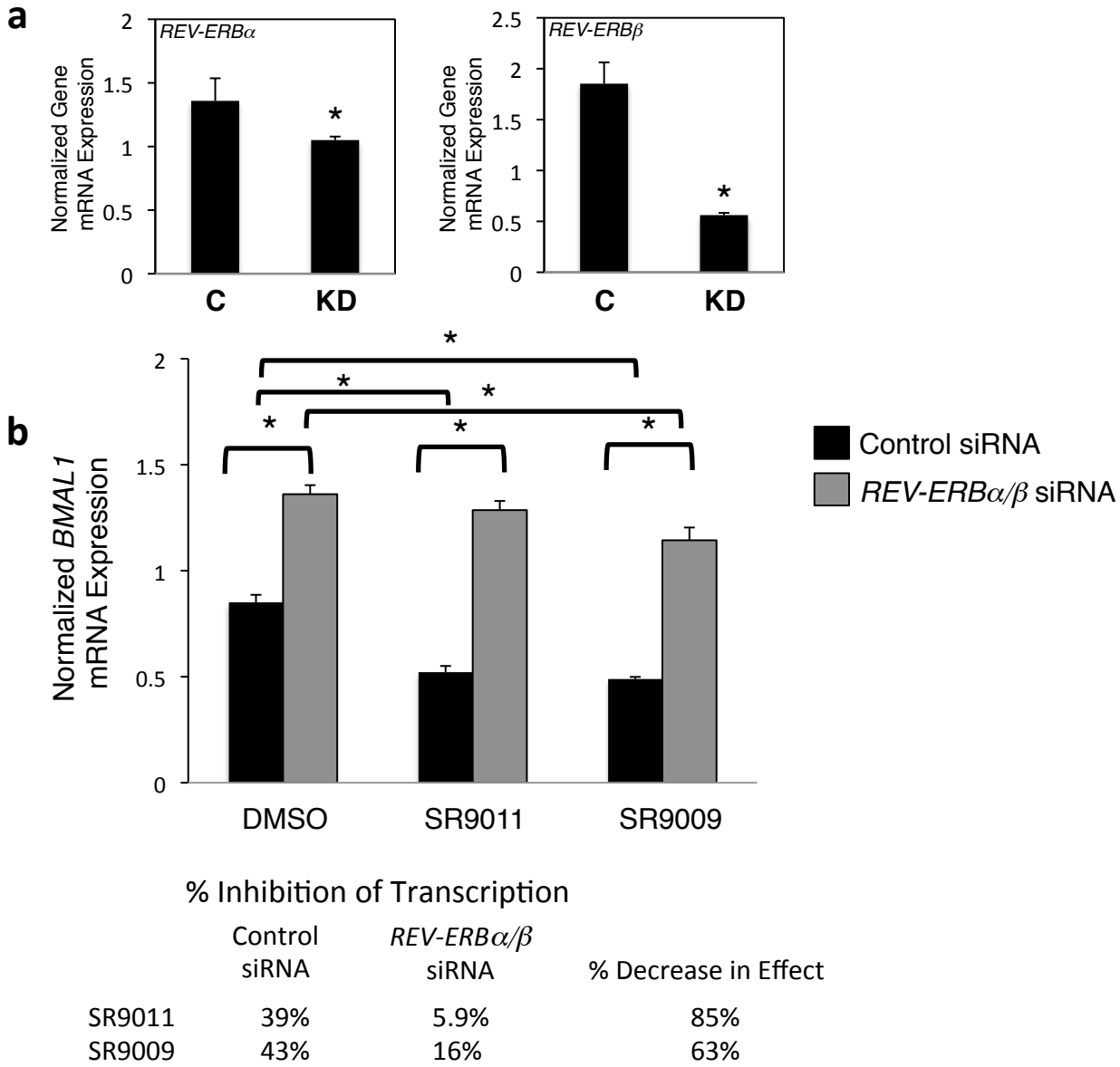


Supplementary Figure 1. Characterization of SR9011 and SR9009. **a**, Sample purity of SR9009 evidenced by HPLC. Four traces indicate data collected at distinct wavelengths (210, 220, 254 and 280 nM). **b**, Sample purity of SR9011 evidenced by HPLC. Chemical identity of SR9009 through **c**, ^{1}H-NMR and **d**, ^{13}C-NMR. Chemical identity of SR9011 through **e**, ^{1}H-NMR and **f**, ^{13}C-NMR.



GSK4112/SR6452

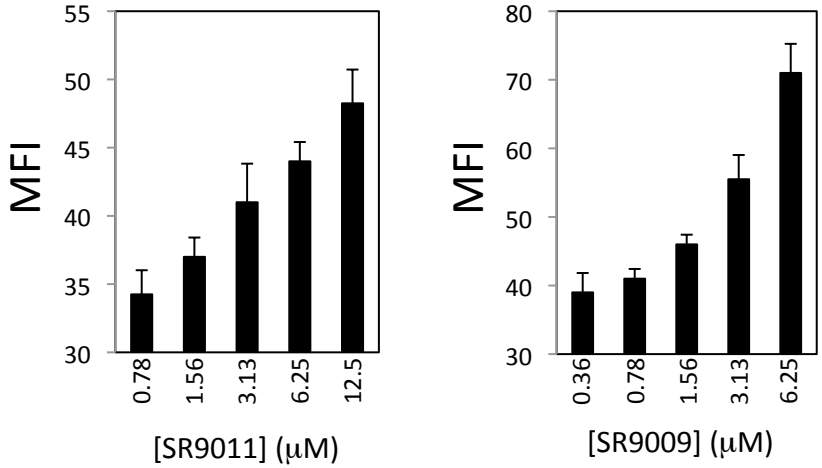
Supplementary Figure 2. Chemical structure of GSK4112, also known as SR6452.



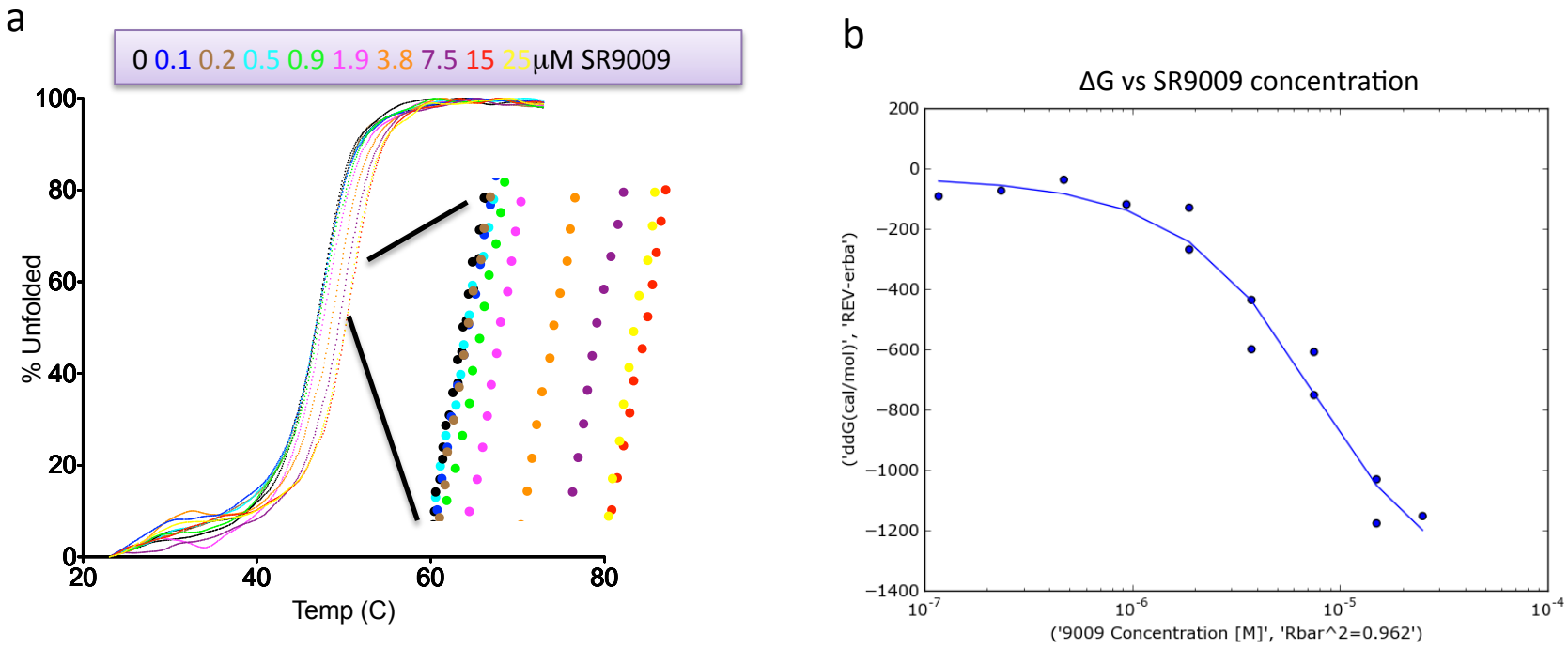
% Inhibition of Transcription

	Control siRNA	*REV-ERBα/β* siRNA	% Decrease in Effect
SR9011	39%	5.9%	85%
SR9009	43%	16%	63%

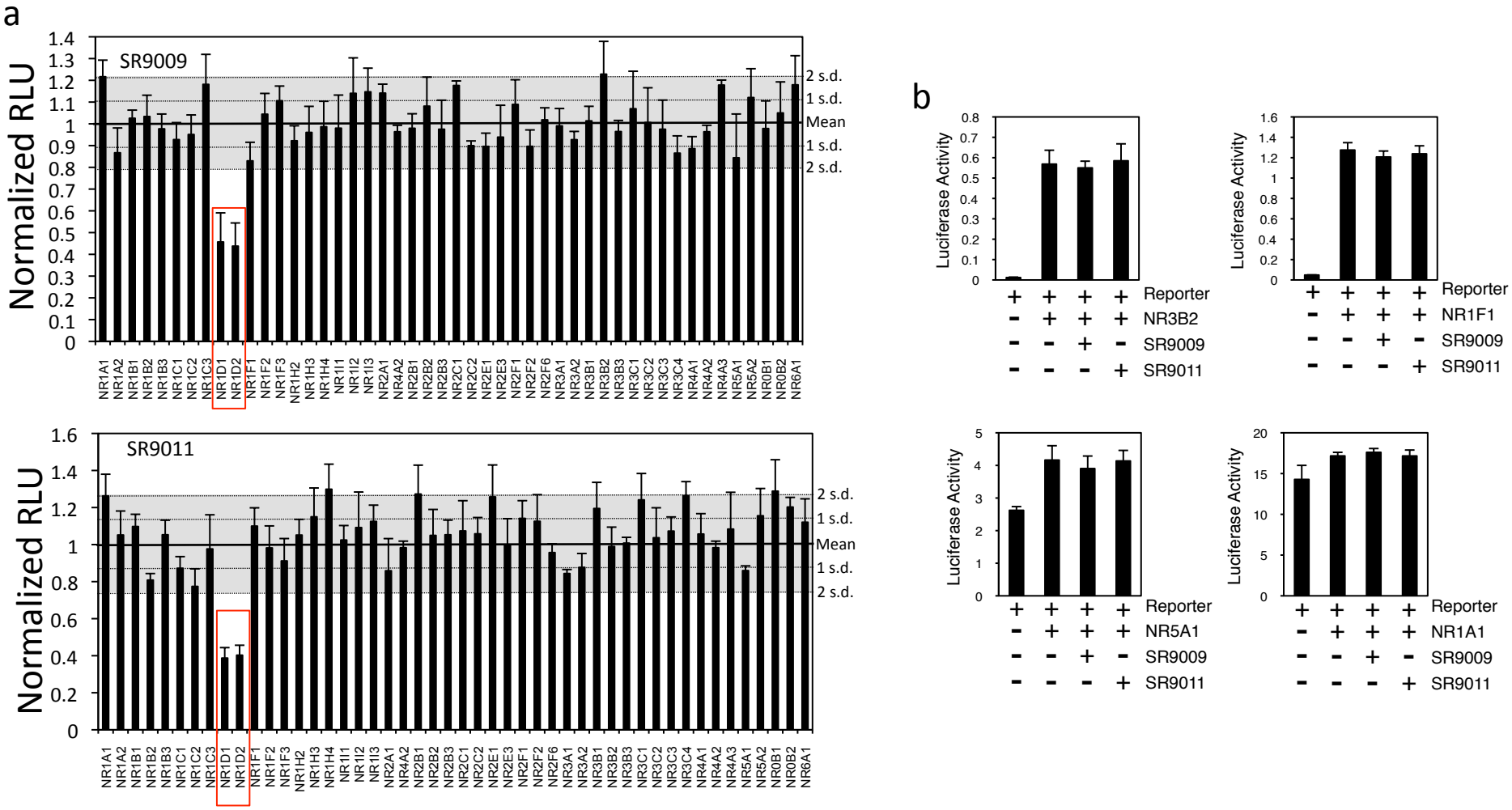
Supplementary Figure 3. SR9009 and SR9011 suppress BMAL1 mRNA expression in a REV-ERBα/β-dependent manner. a, Knock-down of expression of *REV-ERBα* and *REV-ERBβ* in HepG2 cells mediated by siRNA. C=Control siRNA and KD=*REV-ERBα/β* siRNA. There is an ~25% reduction in *REV-ERBα* expression and an ~72% reduction in *REV-ERBβ* expression. Before knock-down expression of *REV-ERBα* and *REV-ERBβ* was roughly equivalent. **b**, SR9011 and SR9009 suppress BMAL1 mRNA expression in HepG2 cells and the effect is suppressed when *REV-ERBα* and *REV-ERBβ* expression is knocked down. Gene expression was monitored by QPCR and normalized to *cyclophilin* expression.The increase in *BMAL1* expression with REV-ERB siRNA treatment is due to relief of constitutive repression of *BMAL1* expression by the REV-ERBs *, indicates $p<0.05$. Error bars indicate mean ± s.e.m. and n=3.



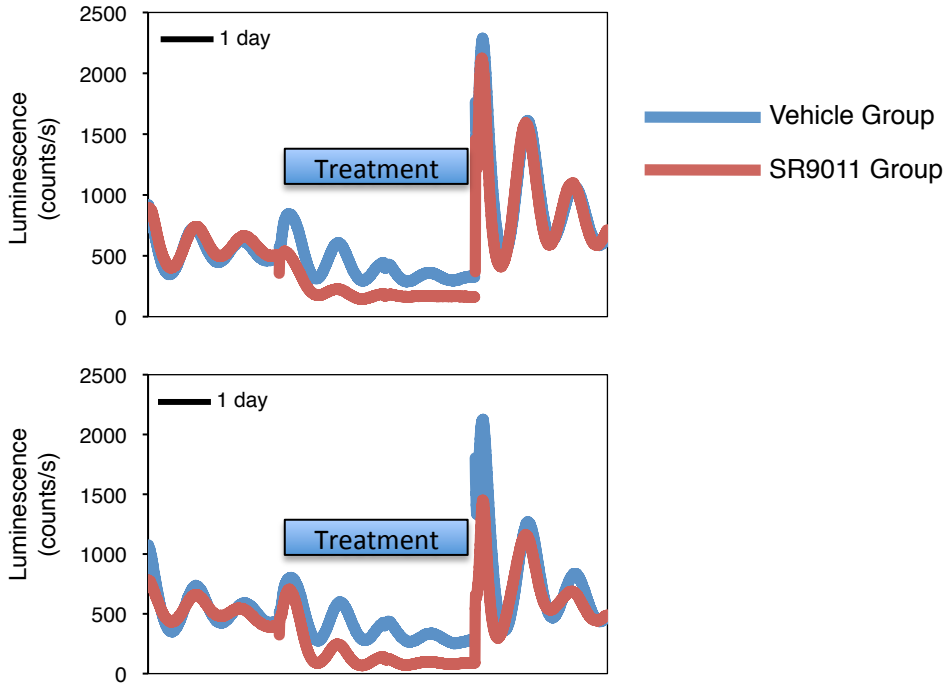
Supplementary Figure 4. Biochemical corepressor interaction assay illustrating the ability of REV-ERBα to recruit the NCoR CoRNR box peptide in a SR9011- or SR9009- dependent manner. Luminex technology was used as described in the Methods. Error bars indicate mean ± s.e.m. and n=3.



Supplementary Figure 5. Circular dichroism analysis demonstrates direct binding of SR9009 to REV-ERBβ. **a**, Increasing concentrations of SR9009 were added to 9μM REV-ERBα and melting was observed via circular dichroism while heating. **b**, Change in enthalpy upon unfolding and melting temperature (Tm) for the melt curves in panel a were fit and used to calculate the change in Gibbs energy of ligand binding at various concentrations. The change in Gibbs energy of ligand binding at various concentrations of SR9009 were used to calculate the K_d (800 nM). The T_m for apo-receptor was 47.1±0.1 °C and with saturating ligand it was 50.7±0.3 °C.



Supplementary Figure 6. Nuclear receptor specificity assay illustrating the activity of SR9009 and SR9011 on REV-ERBα (NR1D1) and REV-ERBβ (NR1D2). **a**, All 48 human nuclear receptors are represented in the specificity assay and the compounds were tested at a concentration of 20 μM. The format of the assay was a cotransfection assay with Gal4 DNA binding domain – nuclear receptor fusions in HEK293 cells as previously described (see Methods). **b**, Additional specificity assays utilizing full length receptor cotransfection assays for NR5A1, NR1A1, NR3B2 and NR1F1 in HEK293 cells. Luciferase reporters that were utilized include DR4-luc (NR1A1), 3xERE-luc (NR3B2), 3xSFRE (NR5A1), and 5xRORE (NR1F1). Compounds were tested at 20 μM. Error bars indicate mean ± s.e.m. and n=3.



Supplementary Figure 7. SR9011 Treatment Suppresses the Amplitude of Oscillations in MEFs Isolated from *Per2*^{luc} transgenic mice. Bioluminescence record from fibroblasts isolated from *Per2*^{luc} transgenic mice treated with 5 μM SR9011 as indicated. The results from two independent experiments are shown.

Time Post Injection	[Plasma] (μM)	[Brain] (μM)
1h	1.55±0.08	
2h	0.53±0.05	0.24±0.08
4h	0.11±0.01	
8h	0.01±0.01	not detected

SR9011, 10mg/kg i.p., 10mg/ml suspension in 10/10/80 DMSO/Tween80/water

Time Post Injection	[Plasma] (μM)
2h	15.3±0.7
8h	0.74±0.17

SR9011, 100mg/kg i.p., 10mg/ml solution in 15% Cremophor

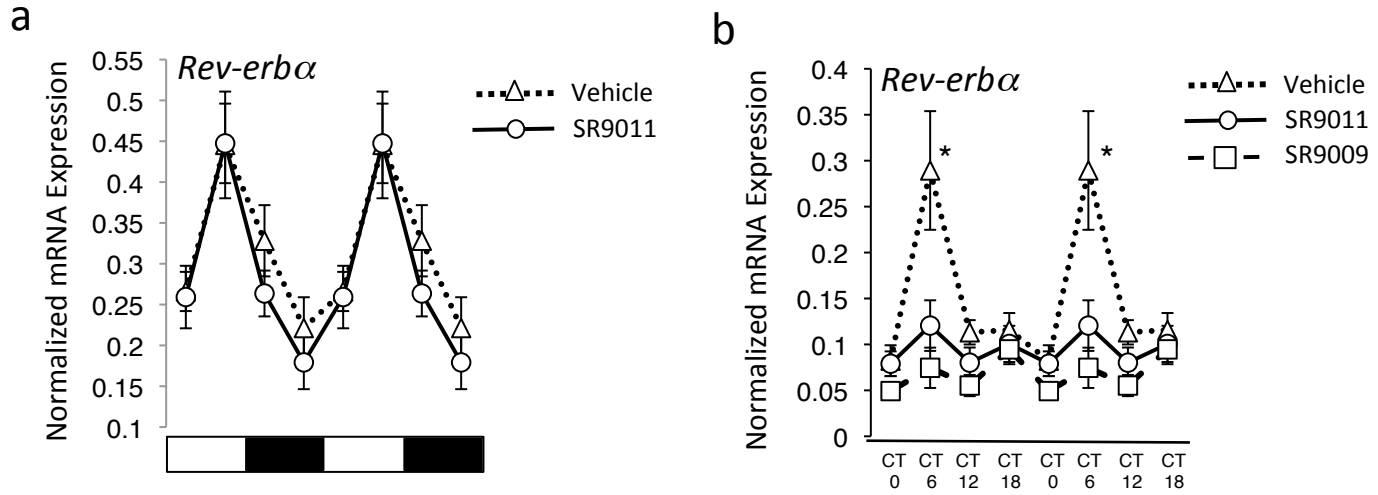
Time Post Injection	[Plasma] (μM)	[Brain] (μM)
1h	1.40±0.12	
2h	0.53±0.08	0.53±0.04
4h	0.22±0.04	
8h	0.13±0.03	0.11±0.01

SR9009, 10mg/kg i.p., 10mg/ml suspension in 10/10/80 DMSO/Tween80/water

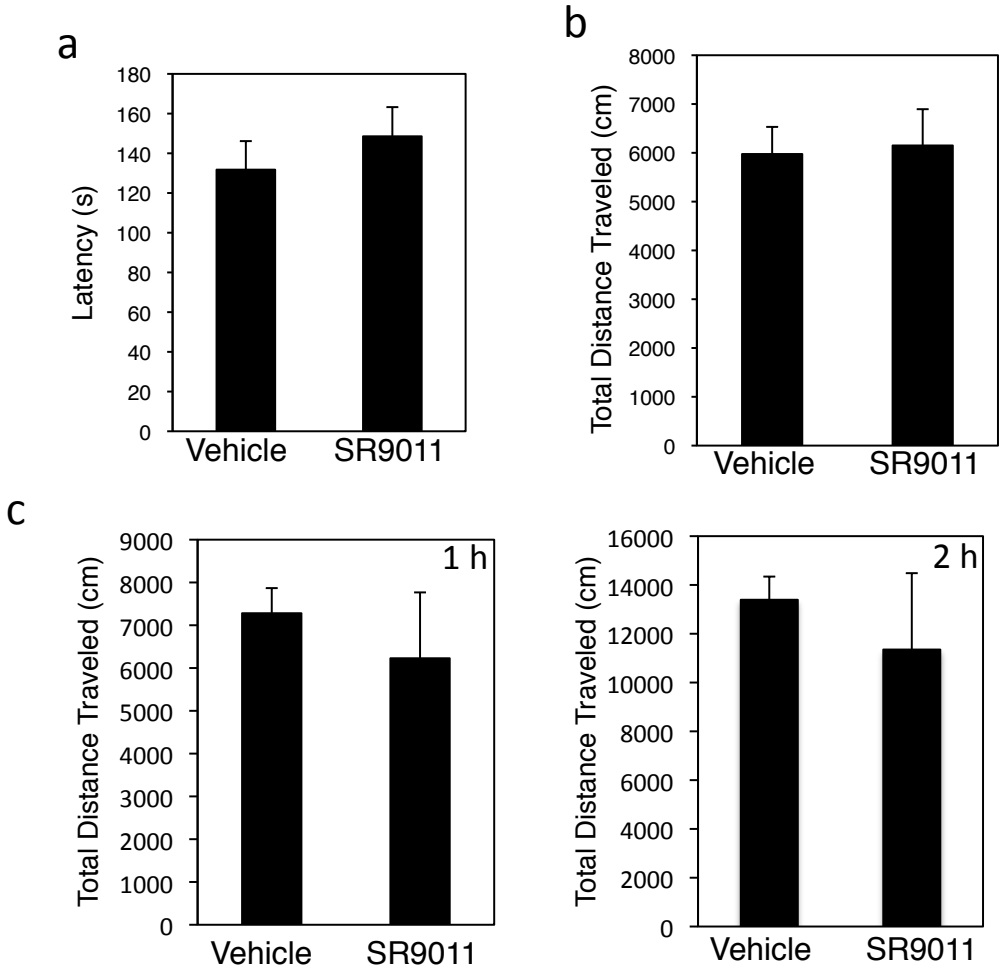
Time Post Injection	[Plasma] (μM)
2h	22.9±3.4
8h	1.5±0.1

SR9009, 100mg/kg i.p., 10mg/ml solution in 15% Cremophor

Supplementary Figure 8. Pharmacokinetic analysis of SR9011 and SR9009 in mice. Error bars indicate mean ± s.e.m. and n=3.



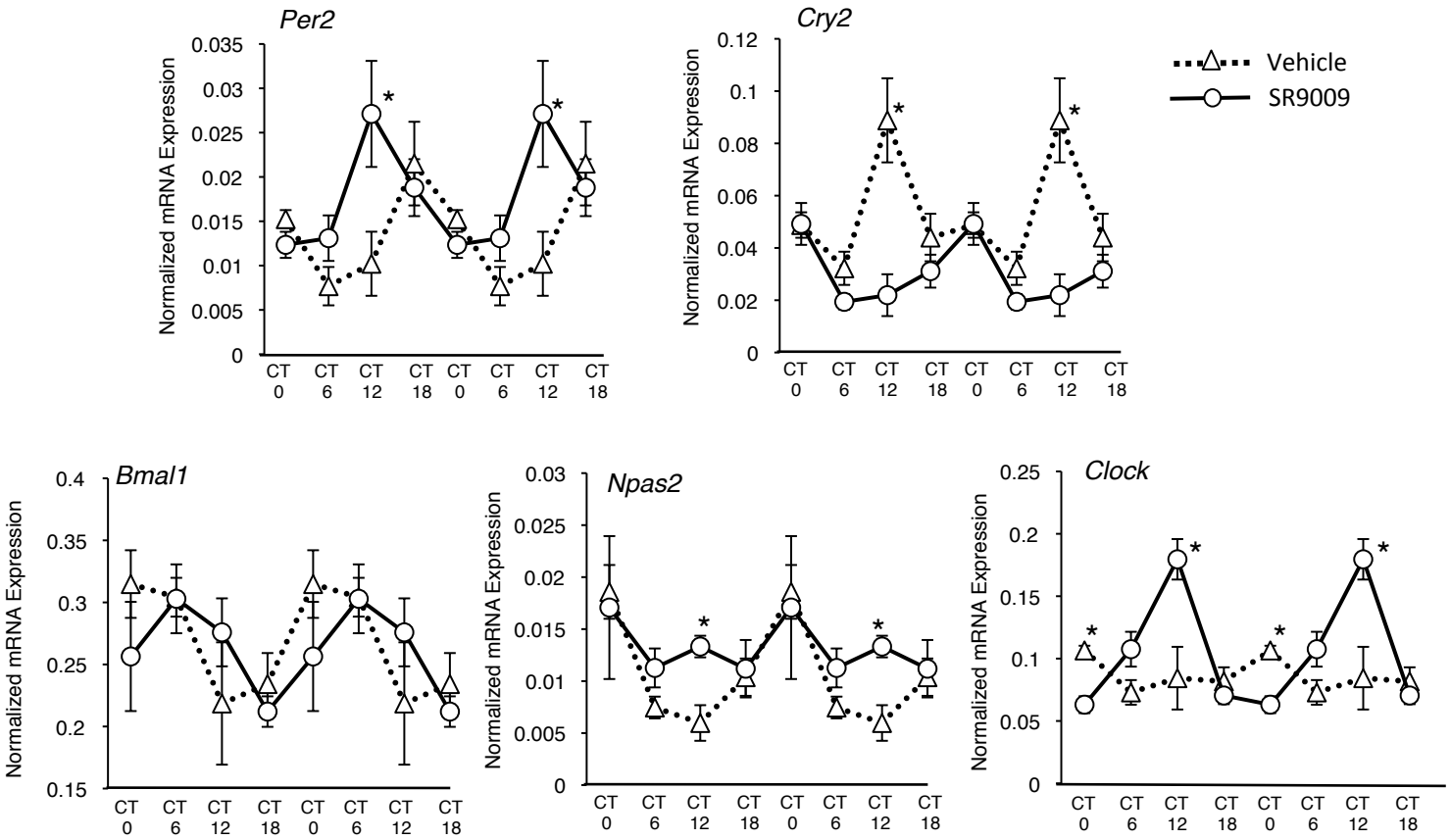
Supplementary Figure 9. Normalized expression levels of *Rev-erbα* in the hypothalamus following administration of SR9011, SR9009 or vehicle under L:D (12:12) conditions (**a**) or D:D (12:12) conditions (**b**). Expression was normalized to *Cyclophilin b* expression. Methods were identical to those described in Figure 2b and 2d. Error bars indicate mean ± s.e.m. and n=6.



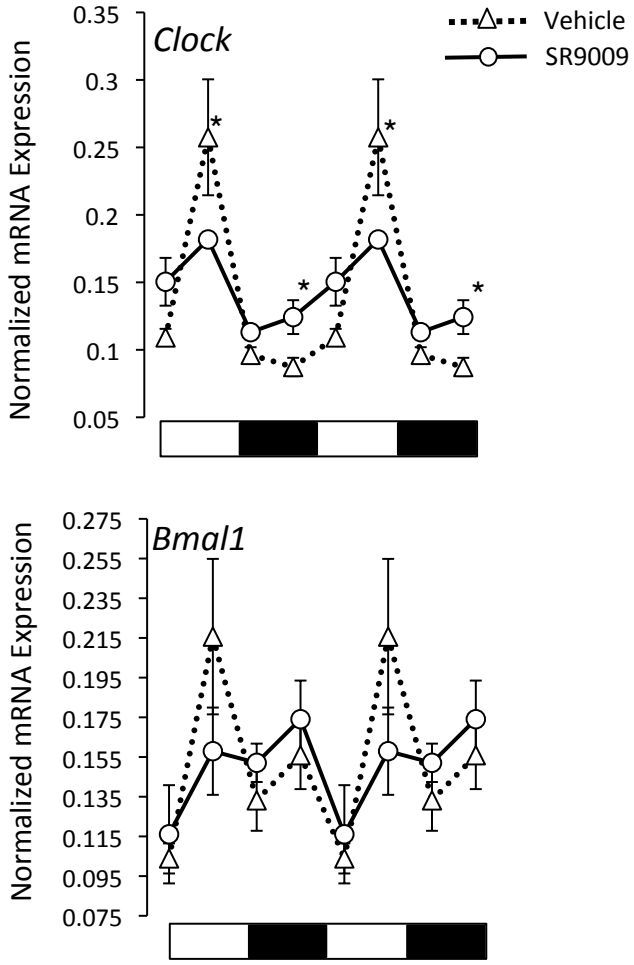
Supplementary Figure 10. SR9011 does not alter strength of mice or render the mice incapable of movement. **a**, Results from a rotorod assay demonstrating that mice treated with vehicle or SR9011 display similar strength. C57Bl6 mice were treated chronically with vehicle or SR9011 (100 mg/kg, b.i.d.) for 4-days prior to the assay. **b**, Mice treated with SR9011 do not display movement deficits. C57Bl6 mice were treated chronically with vehicle or SR9011 (100 mg/kg, b.i.d.) for 4-days prior to assessment of movement in an open field assay. This particular open field assay was conducted during the subject day period over 6h. **c**, Mice do not display movement deficits under constant darkness after injection with SR9011 (100 mg/kg CT6) during the subject dark period. C57Bl6 mice were subjected to total darkness for 4 days followed by injection of drug at CT6. Total distance traveled over 1h and 2h durations during subject dark period are shown (measurement initiated at CT14). Error bars indicate mean ± s.e.m. and n=6-10.

	Vehicle	SR9011	
WBC#	2.45±0.31	1.98±0.22	x1000/μl
NE#	0.61±0.13	0.47±0.08	x1000/μl
LY#	1.64±0.19	2.93±1.54	x1000/μl
MO#	0.14±0.02	0.18±0.09	x1000/μl
EO#	0.047±0.001	0.06±0.01	x1000/μl
BA#	0.013±0.004	0.016±0.004	x1000/μl
NE%	22.7±2.6	18.0±3.6	%
LY%	67.9±2.3	73.7±4.5	%
MO%	5.9±0.5	4.9±0.7	%
EO%	2.0±0.3	2.6±0.7	%
BA%	0.5±0.1	0.8±0.3	%
RBC	8.6±0.4	8.9±0.6	$x10^6/\mu l$
Hb	11.0±0.6	11.3±1.2	g/dl
HCT	35.5±1.5	36.8±2.7	%
MCV	41.0±0.2	41.5±0.4	fl
MCH	12.7±0.2	12.7±0.6	pg
MCHC	31.0±0.5	30.5±1.3	g/dl
RDW	16.7±0.1	17.0±0.5	%
PLT	422±61	503±67	x1000/μl
MPV	5.2±0.1	5.4±0.2	fl

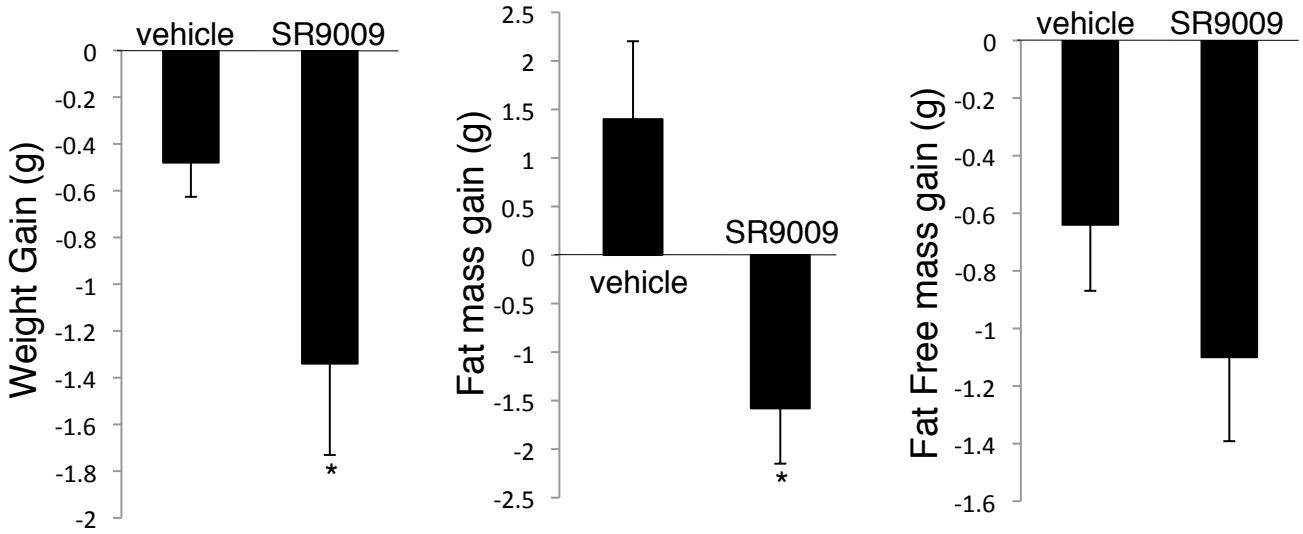
Supplementary Figure 11. Comparison of complete blood count parameters in vehicle vs. SR9011 treated C57Bl6 mice. Mice were treated chronically with SR9011 (100 mg/kg, b.i.d.) for 7 days. No significant differences were noted among any of the parameters examined consistent with no overt toxicity of SR9011. WBC, white blood cells; NE, neutrophils; LY, lympocytes; MO, monocytes; BA, basophils; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelet count; MPV, mean platelet volume. Error bars indicate mean ± s.e.m. and n=6.



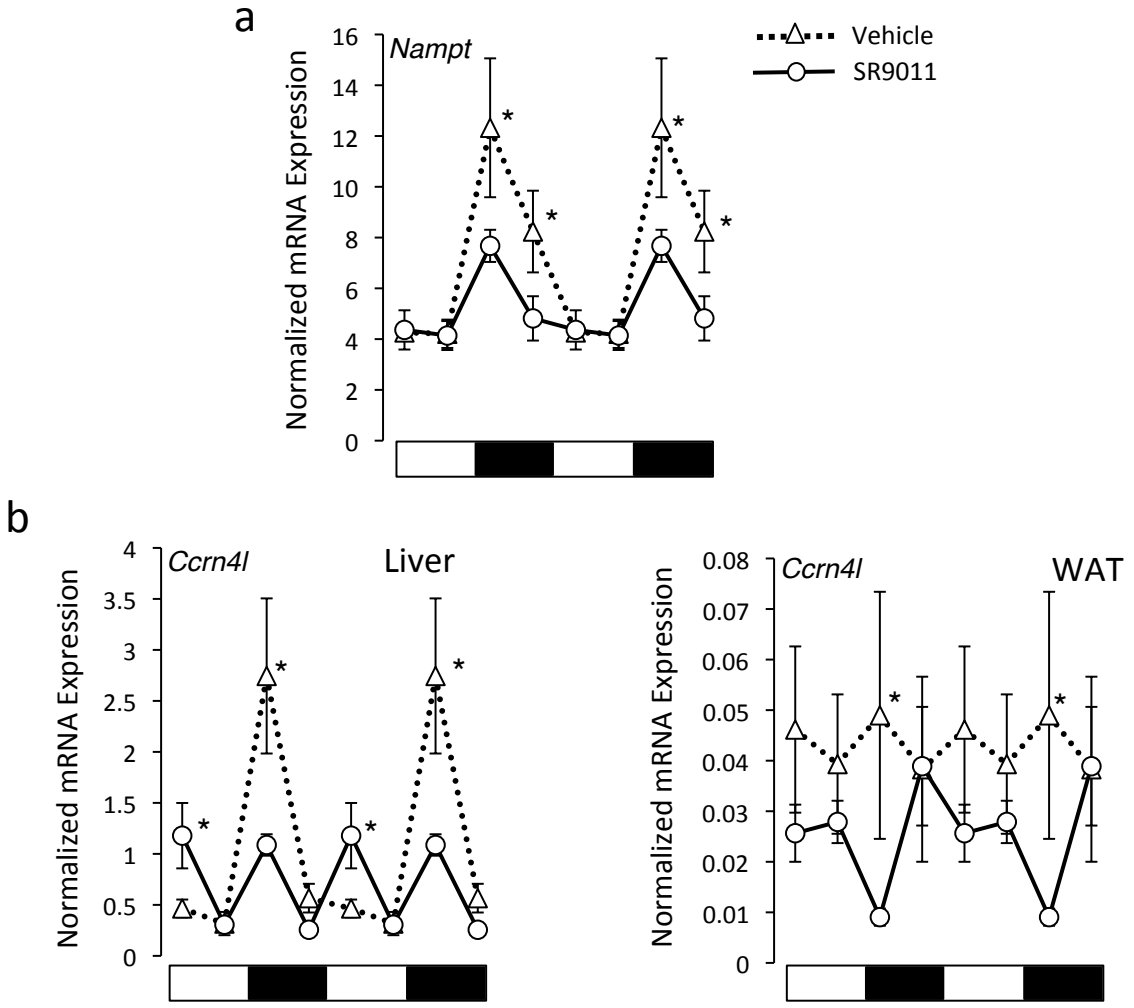
Supplementary Figure 12. SR9009 alters clock gene expression in the hypothalamus.
Normalized expression levels of several core clock genes following administration of SR9009 or vehicle under constant dark conditions. C57Bl6 mice were administered SR9009 (100 mg/kg, i.p.) at CT0 on a day of constant darkness. Hypothalami were collected at CT0, CT6, CT12, and CT18 and gene expression was determined and normalized to *Cyclophilin b.* Data were double plotted.* indicates $p<0.05$. Error bars indicate mean ± s.e.m. and n=6.



Supplementary Figure 13. Normalized expression levels of several core clock genes following administration of SR9009 or vehicle during L:D conditions. C57Bl6 mice were administered SR9009 (100 mg/kg, i.p.) at CT0. Hypothalami were collected at CT0,CT6, CT12, and CT18 and gene expression was determined and normalized to *Cyclophilin b*. Data were double plotted.* indicates $p<0.05$. Error bars indicate mean ± s.e.m. and n=6.



Supplementary Figure 14. Results from C57BL6 animals dosed with SR9009 for 7-days (100 mg/kg, i.p., b.i.d.). * indicates $p<0.05$. Error bars indicate mean ± s.e.m. and n=6.



Supplementary Figure 15. SR9011 suppresses both *Nampt* and *Ccrn4l* expression. C57Bl6 mice were treated identical to that described for Figure 4 and gene expression was assessed by RTQ-PCR. a, Hepatic expression of *Nampt* was suppressed by SR9011 treatment. b, *Ccrn4l* (*Nocturnin*) gene expression was suppressed by SR9011. The liver displayed a clear circadian rhythm of expression whereas the white adipose tissue (WAT) did not. Gene expression was determined and normalized to *Cyclophilin b*. Data were double plotted.* indicates $p<0.05$. Error bars indicate mean ± s.e.m. and n=6.