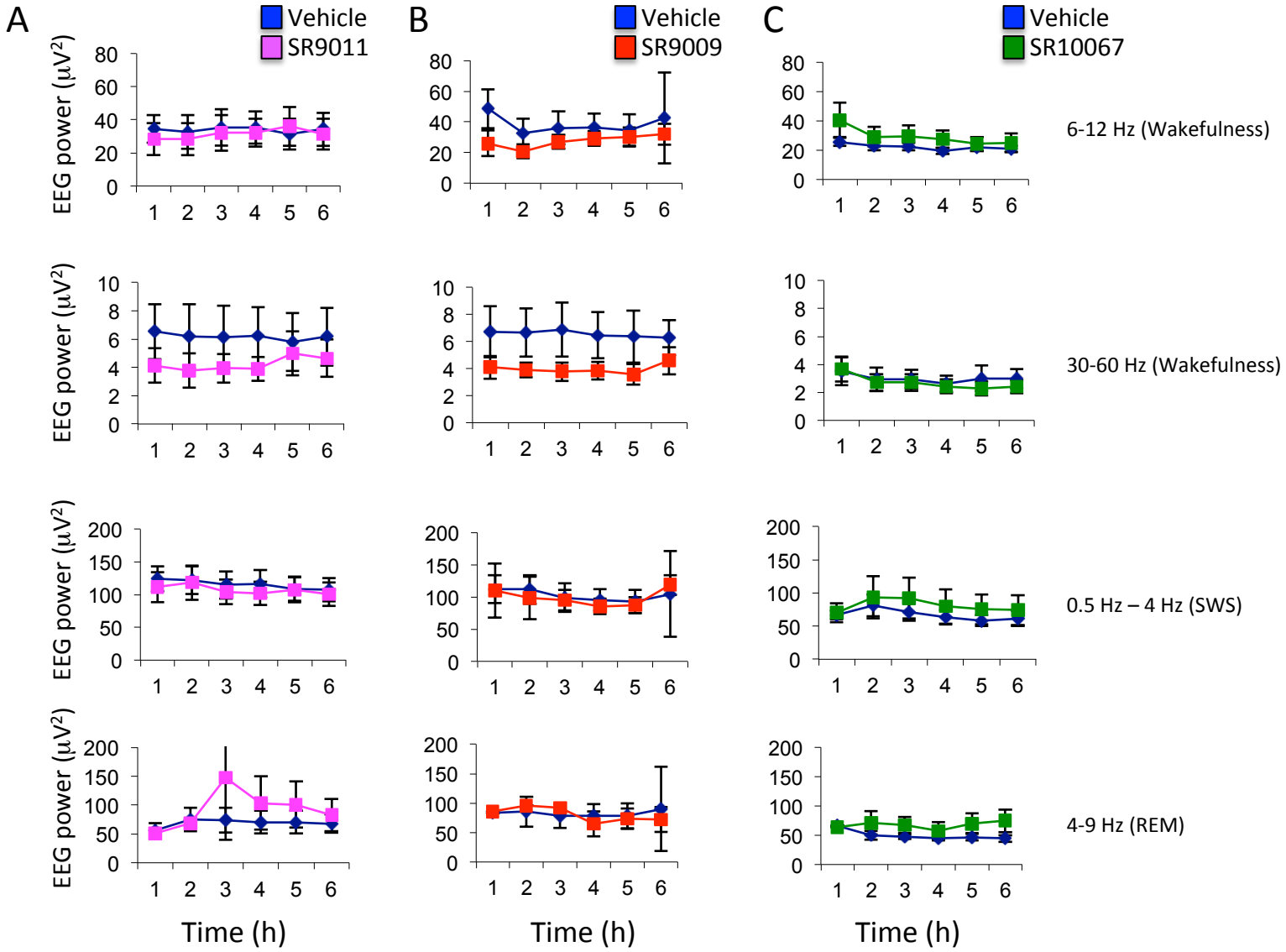
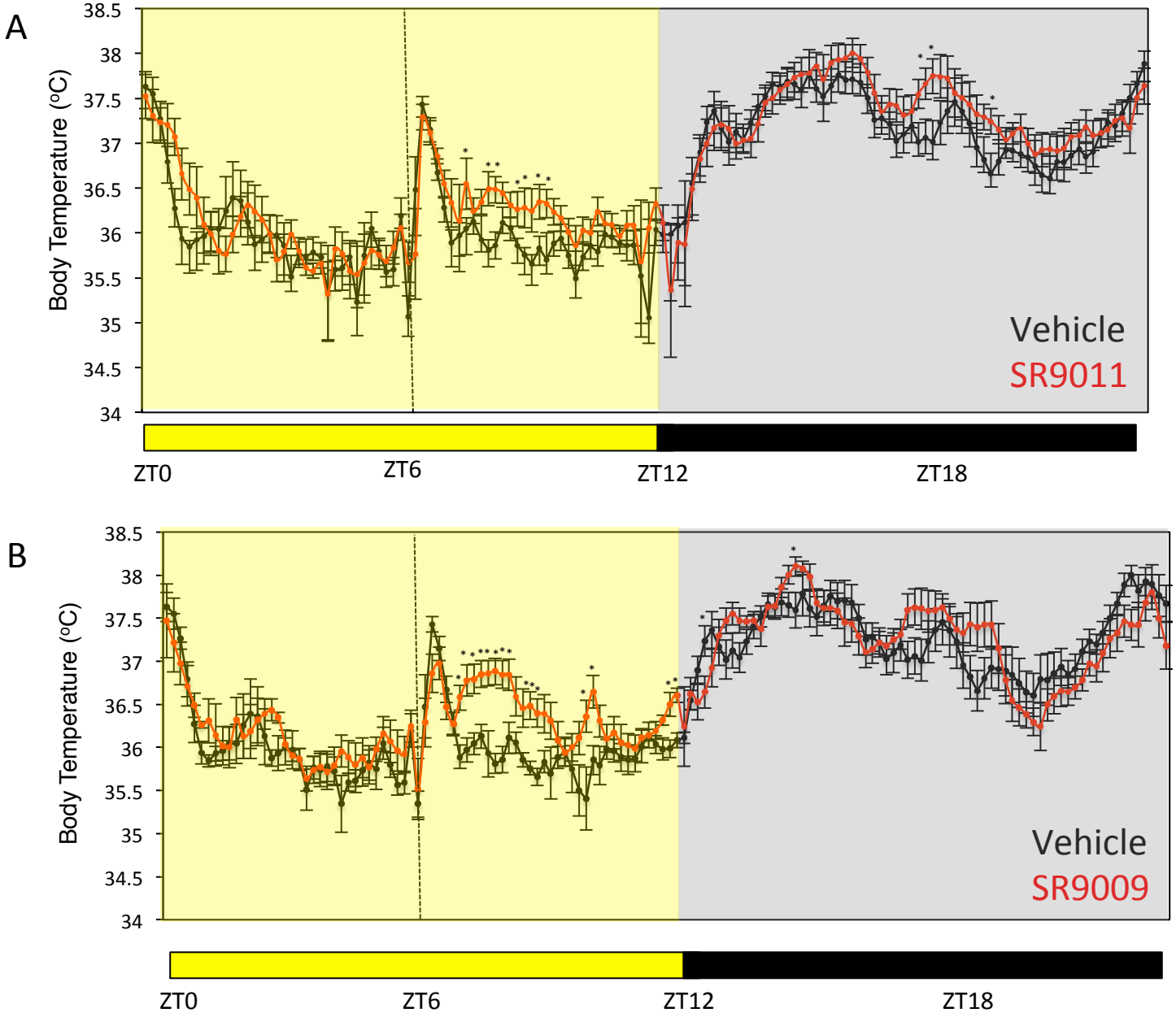
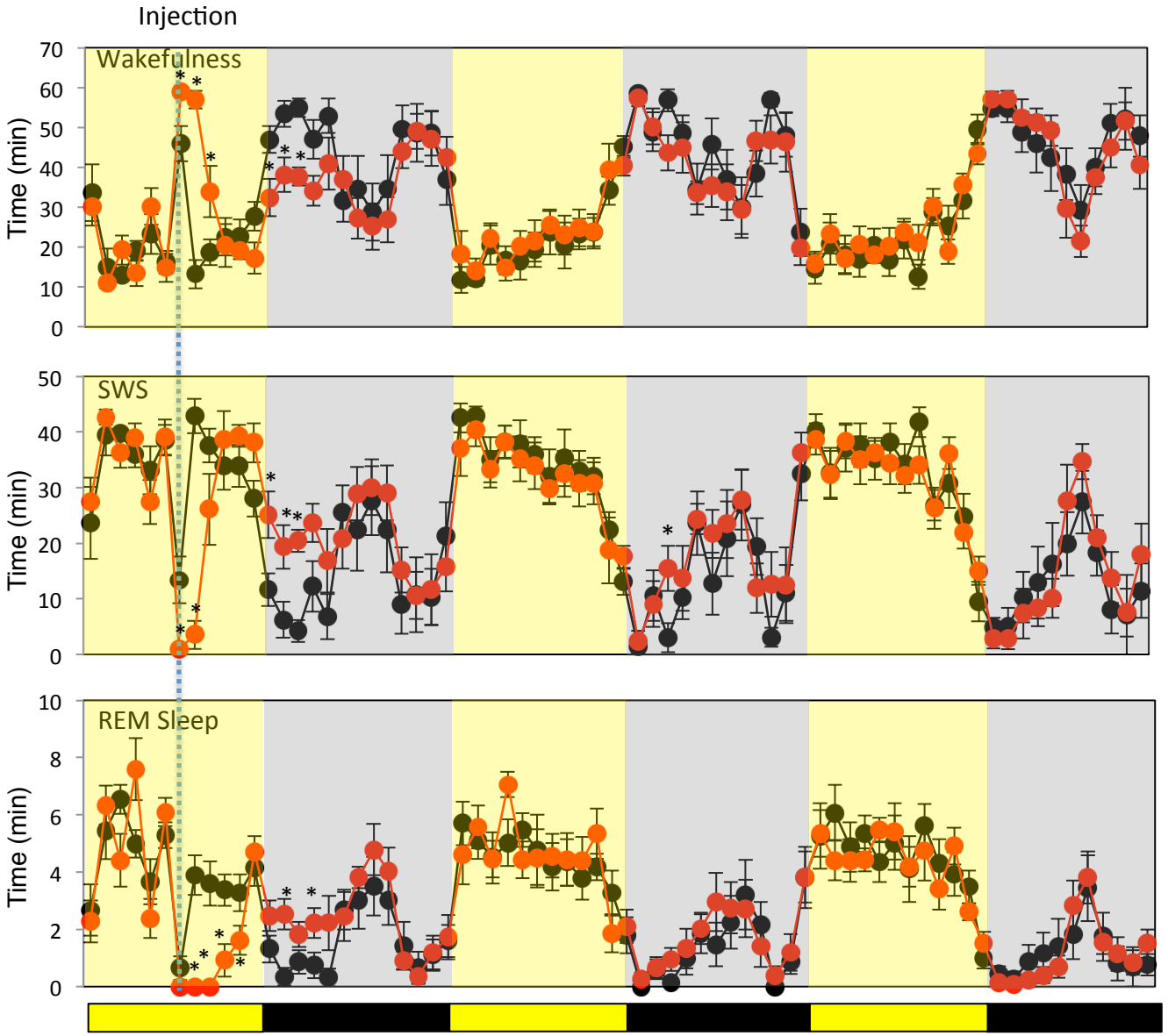


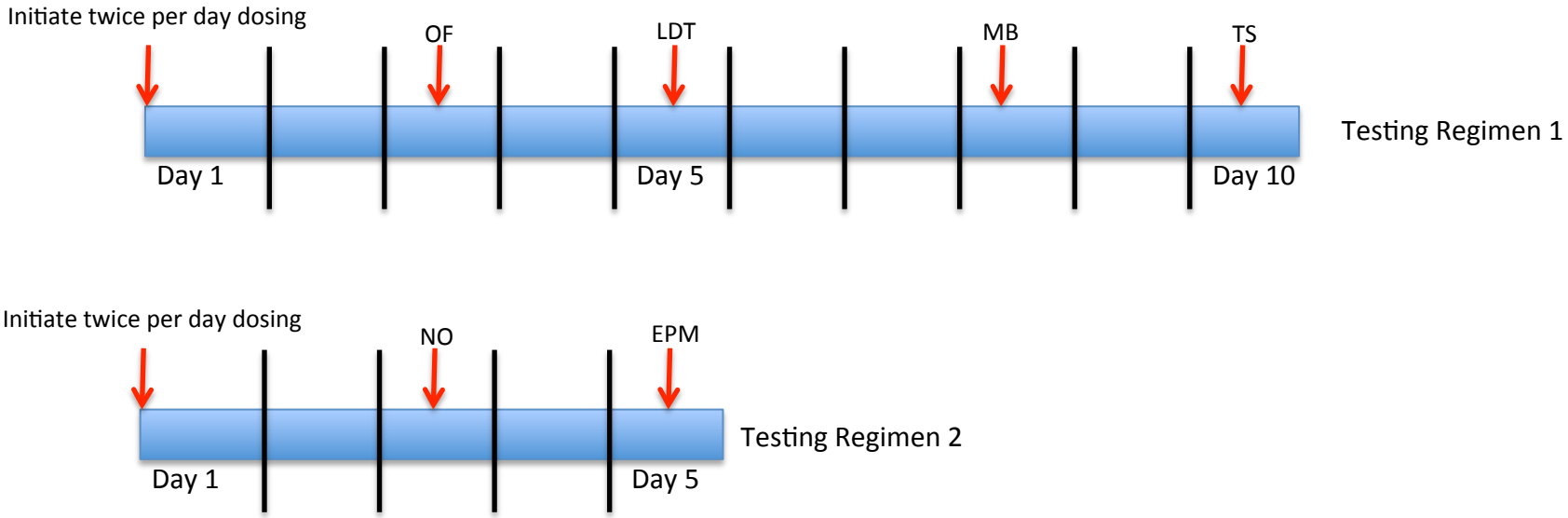
Supplementary Figure 1. Analysis of EEG power following administration of REV-ERB agonists. The effect of SR9011 (**a**), SR9009 (**b**), or SR10067 (**c**) on EEG power spectra for 6 h post administration is illustrated. EEG power was assessed for 4 distinct spectra: 6-12 Hz (wakefulness), 30-60 Hz (wakefulness), 0.5-4 Hz (SWS) and 4-9 Hz (REM Sleep). Potential differences between treatments were assessed by repeated measure two-way ANOVA followed by Bonferroni post hoc test. N=8 mice per group. No significant differences between SR compound and vehicle treatment was noted at any time point.



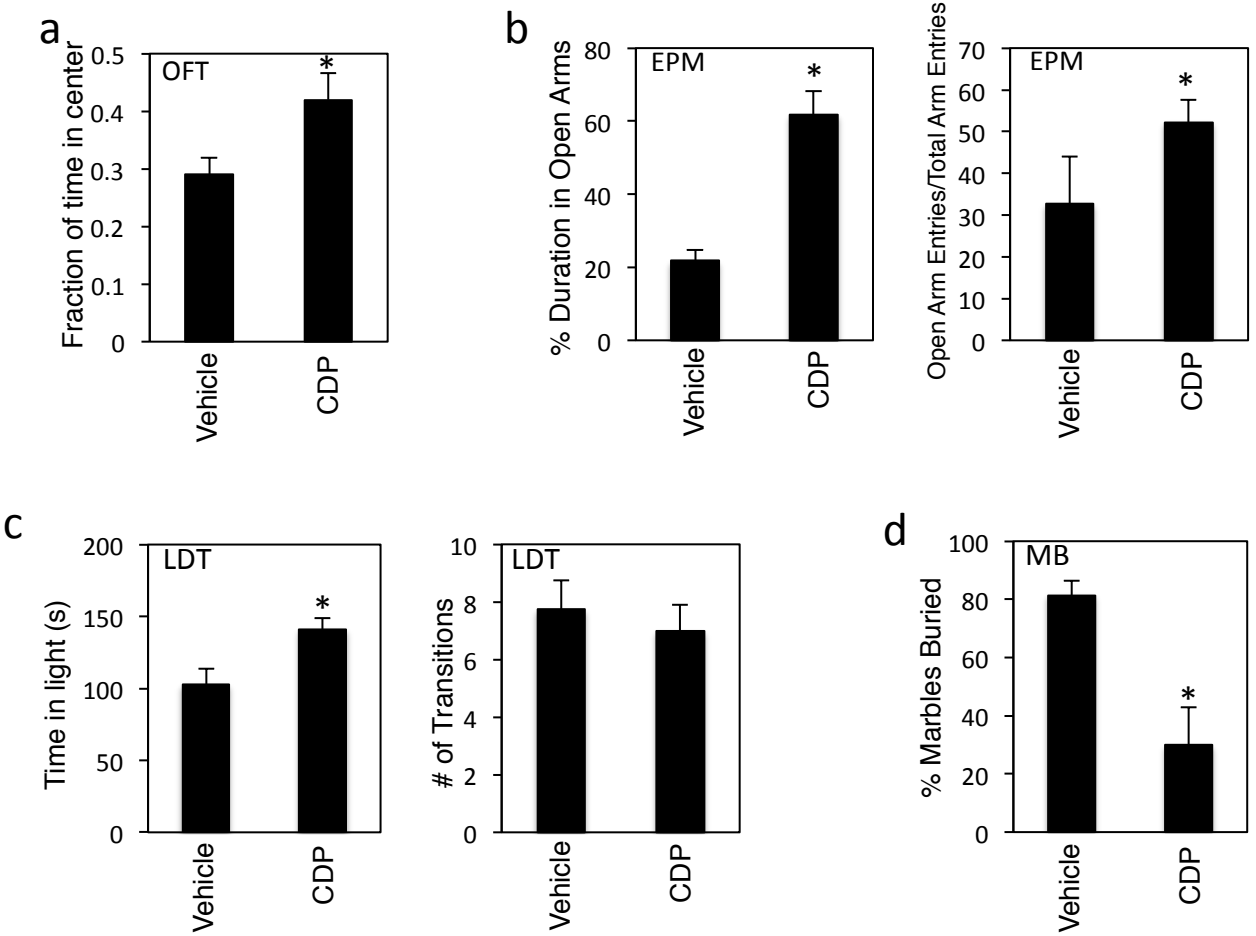
Supplementary Figure 2. REV-ERB agonists transiently increase core body temperature as assessed by telemetry. Data are expressed mean±SEM and core body temperature was assessed multiple times per hour. SR9009 and SR9011 were administered i.p. 100 mg kg^{-1} at ZT6. N=8. Potential differences between treatments were assessed by repeated measure two-way ANOVA followed by Bonferroni post hoc test. N=6 mice per group. $P<0.05$ is indicated by *.



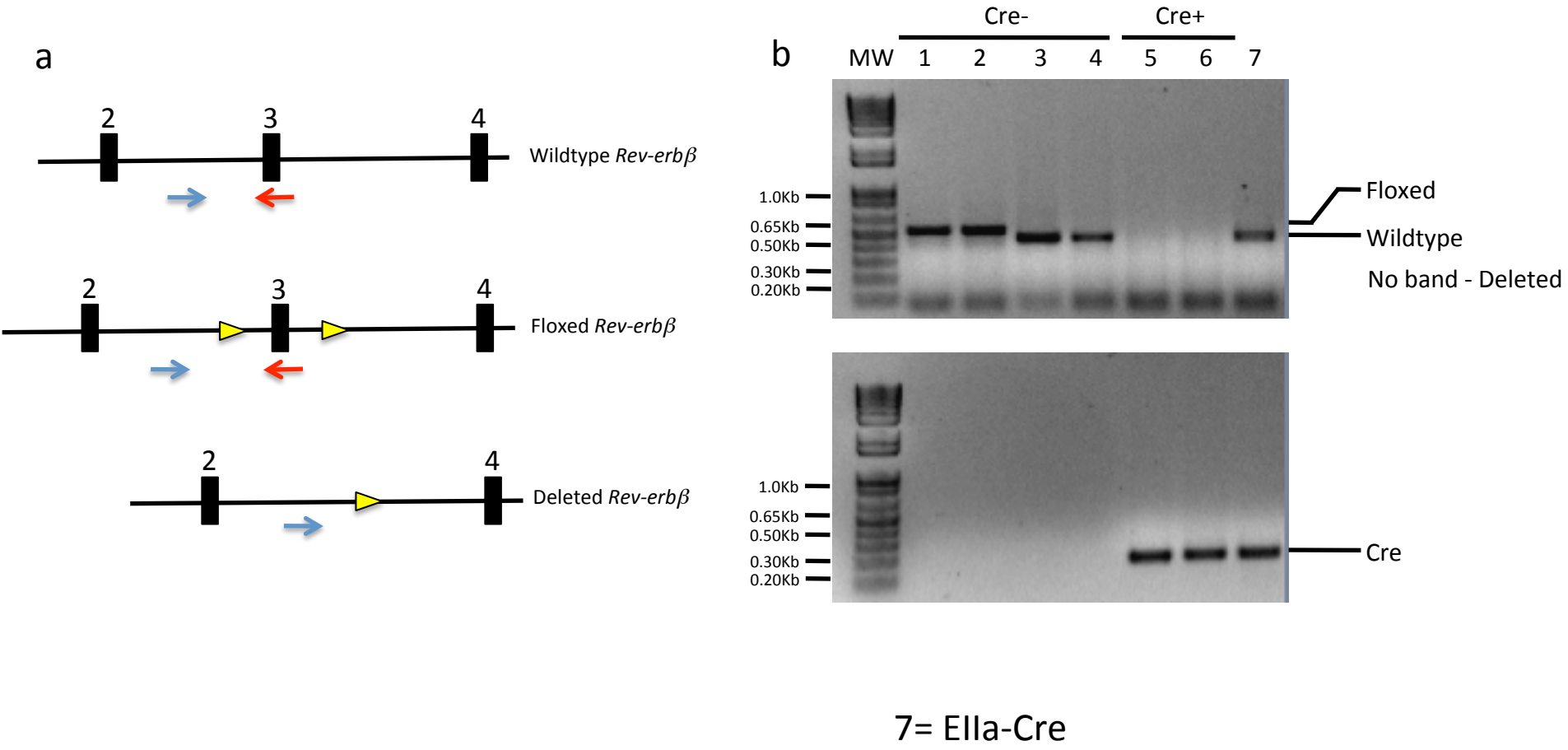
Supplementary Figure 3. The REV-ERB agonist, SR9009, induces wakefulness and suppresses sleep. Effect of SR9009 on sleep-wakefulness (wakefulness, slow wave sleep (SWS) and rapid eye movement (REM) sleep). This graph is an extension of the graph shown in Fig 3a demonstrating that normal sleep architecture returns in mice within 1 day of administration of the REV-ERB agonists. Data are expressed mean±SEM. All EEG graphs are plotted per 1h for a 24h period. N=8. Potential differences between treatments were assessed by repeated measure two-way ANOVA followed by Bonferroni post hoc test. N=8 mice per group. $P<0.05$ is indicated by *.



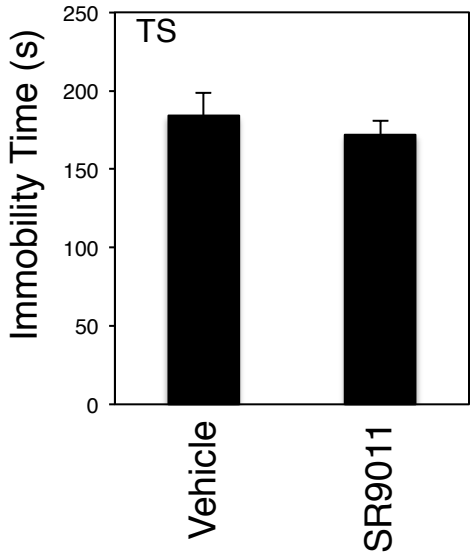
Supplementary Figure 4. Scheme for assessment of anxiety in mice treated with SR compounds or vehicle. Multiple behavioral tests were run on cohorts of mice as shown above in two distinct schemes. Both received compound twice per day at ZT0 and ZT12 based on observations made in metabolic studies where indications of anxiolytic action were noted. OF, open field assay; LDT, light-dark transition assay; MB, marble burying assay; TS, tail suspension assay; NO, novel object assay; EPM, elevated plus maze. Dosing and the testing regimens are described in greater detail in the methods.



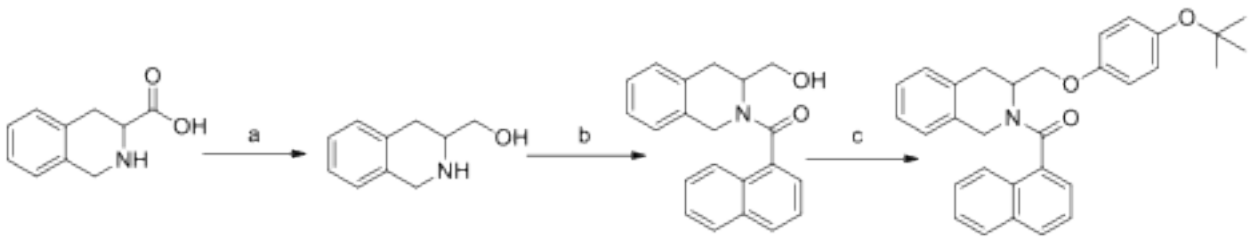
Supplementary Figure 5. The benzodiazepine, chlordiazepoxide (CDP; Librium®), exhibits activity in a range of anxiety assays. CDP displays anxiolytic activity in (**a**) the open field test (OFT), the (**b**) elevated plus maze assay (EPM), (**c**) the light dark transition assay (LDT) and (**d**) the marble burying assay (MB). Data are expressed mean ± SEM. N=12 to 15 mice per group with the exception of the MB assay that utilized n=8. Differences between treatment groups (vehicle vs. SR) were assessed by a two-tail t test (Student's) with significance *P<0.05.



Supplementary Figure 6. Generation of *Rev-erbβ* null mice. a) Schematic illustrating the strategy for generation of the *Rev-erbβ* deletion. Wildtype, floxed and deleted loci are shown. Primer binding regions and predicted PCR amplicons for each allele of *Rev-erbβ* are indicated. Insertion of the loxP sequence in the PCR amplicon in the floxed allele leads to an approximate 100 bp greater PCR fragment. The 3' primer recognizes sequences in exon 3 and thus there is no PCR product in the deletion. b) PCR genotyping of mice using tail DNA. Wild type *Rev-erbβ* are shown in lanes 3 and 4 while floxed mice are shown in lanes 1 and 2. Lanes 5 and 6 represent *Rev-erbβ* null mice (floxed mice crossed with Ella-Cre mice). Lane 7 displays results from Ella-Cre mice illustrating wildtype *Rev-erbβ* and the Cre transgene. The Cre transgene is also shown for the null mice (lanes 5 and 6). Fifth generation or later Ella-Cre X *nr1d2* floxed mice were used in all experiments.



Supplementary Figure 7. SR9011 Does Not Display Activity in the Tail Suspension Assay. Values are mean ± SEM, n=12 mice per group. Potential differences between treatment groups (vehicle vs. SR) were assessed by a two-tailed t test (Student's) with significance $P < 0.05$.



Supplementary Figure 8. Synthesis of SR10067. Experimental Protocol: a. $LiAlH_4$, THF; b. 1-Naphthoyl chloride, TEA; c. 4-(*tert*-Butoxy)phenol, DIAD, Ph_3P, THF.

a

Scripp's Discovery VP HPLC Report

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Solvent B = ACN
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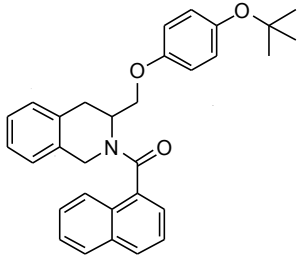
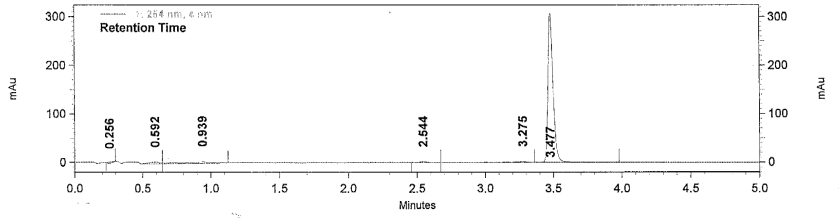
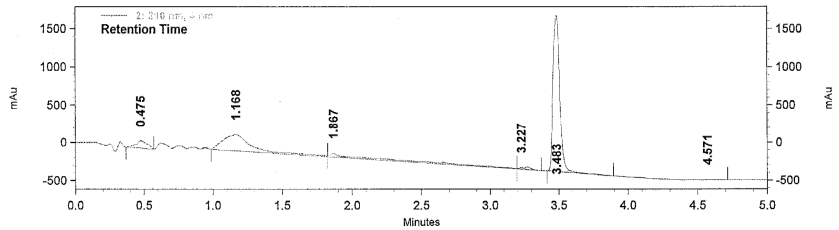
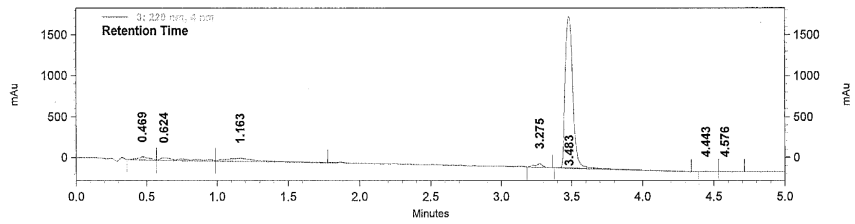
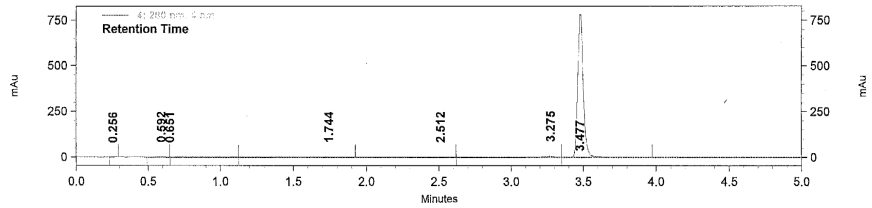
SR-10067



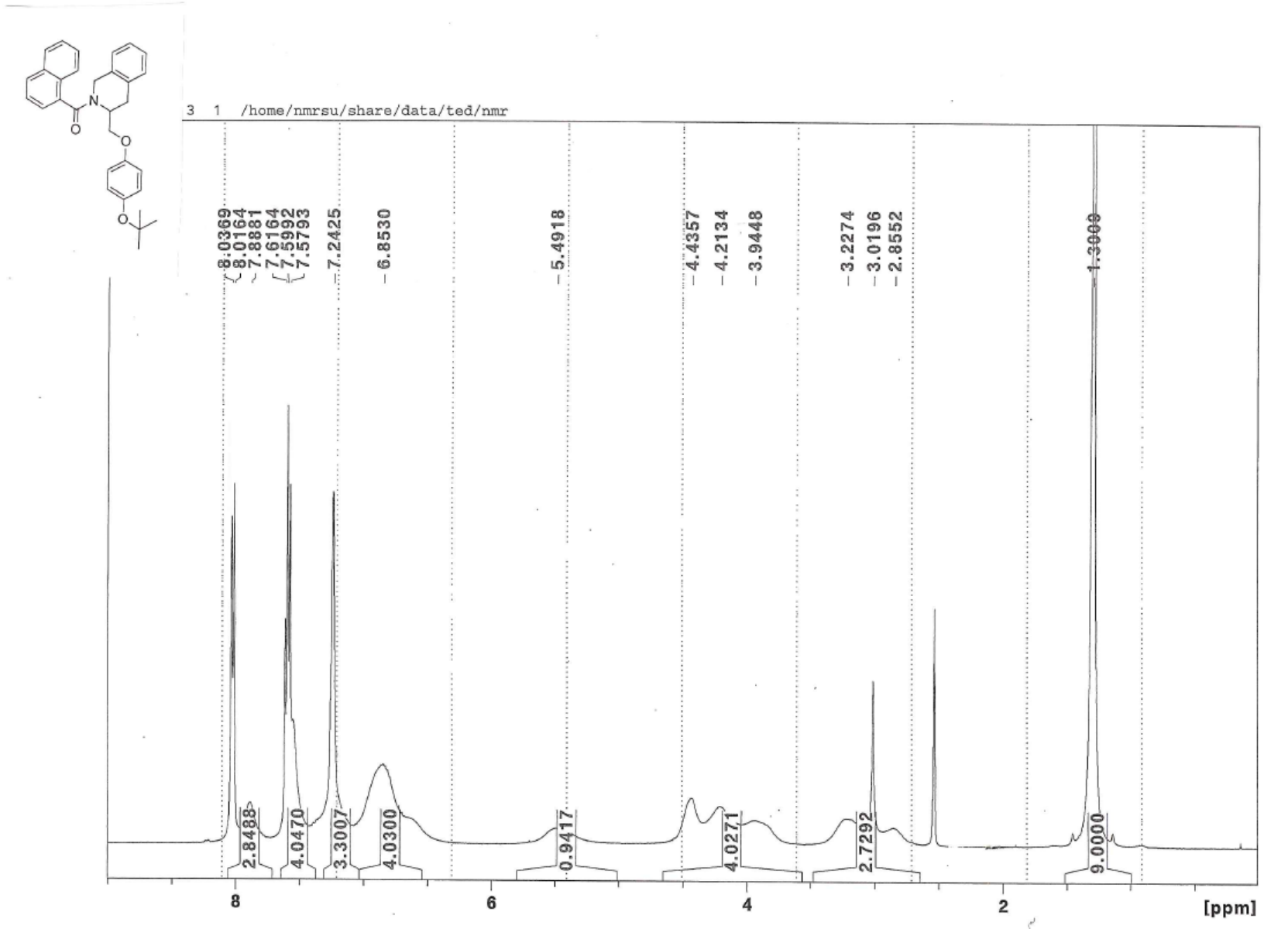








b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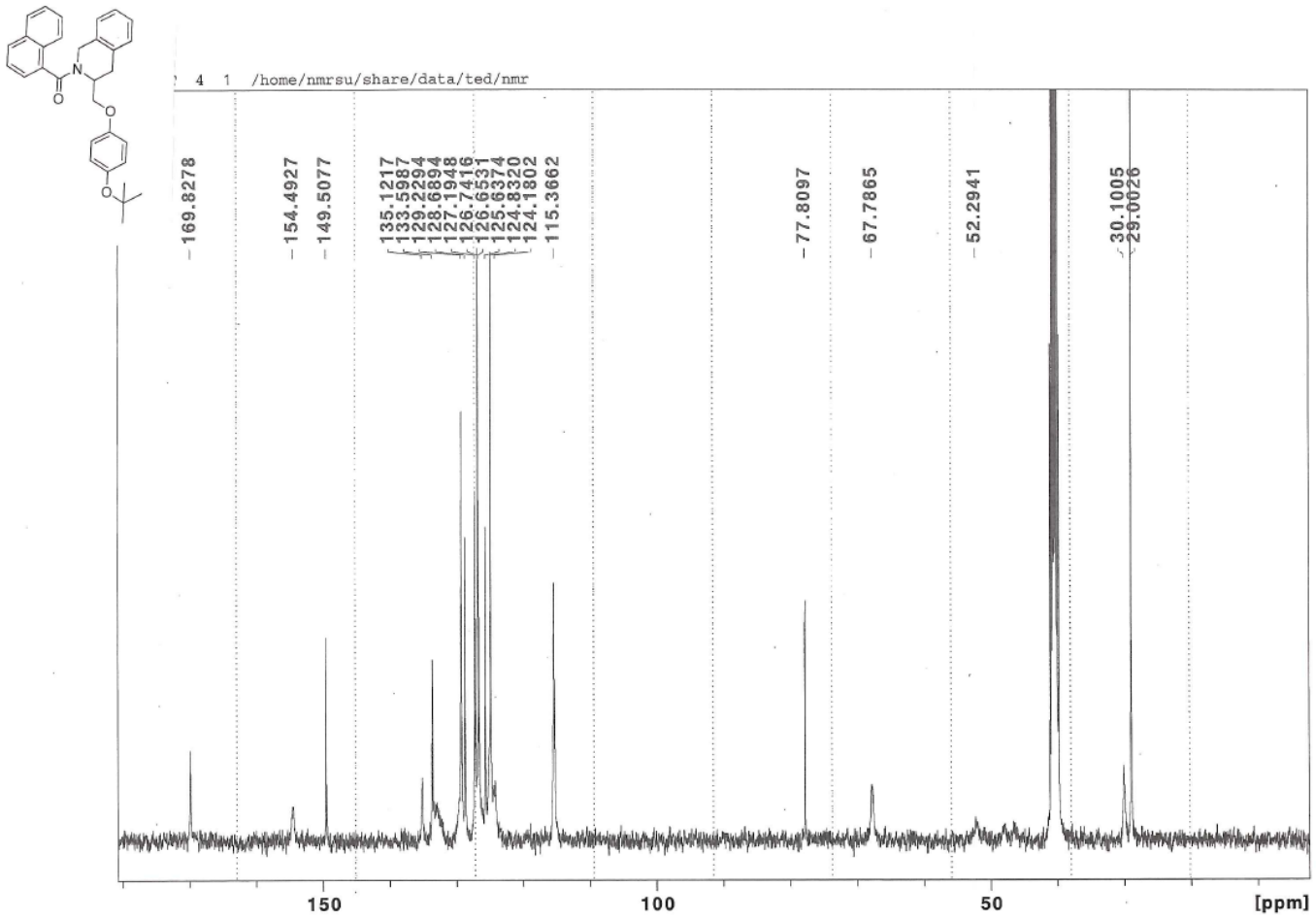
C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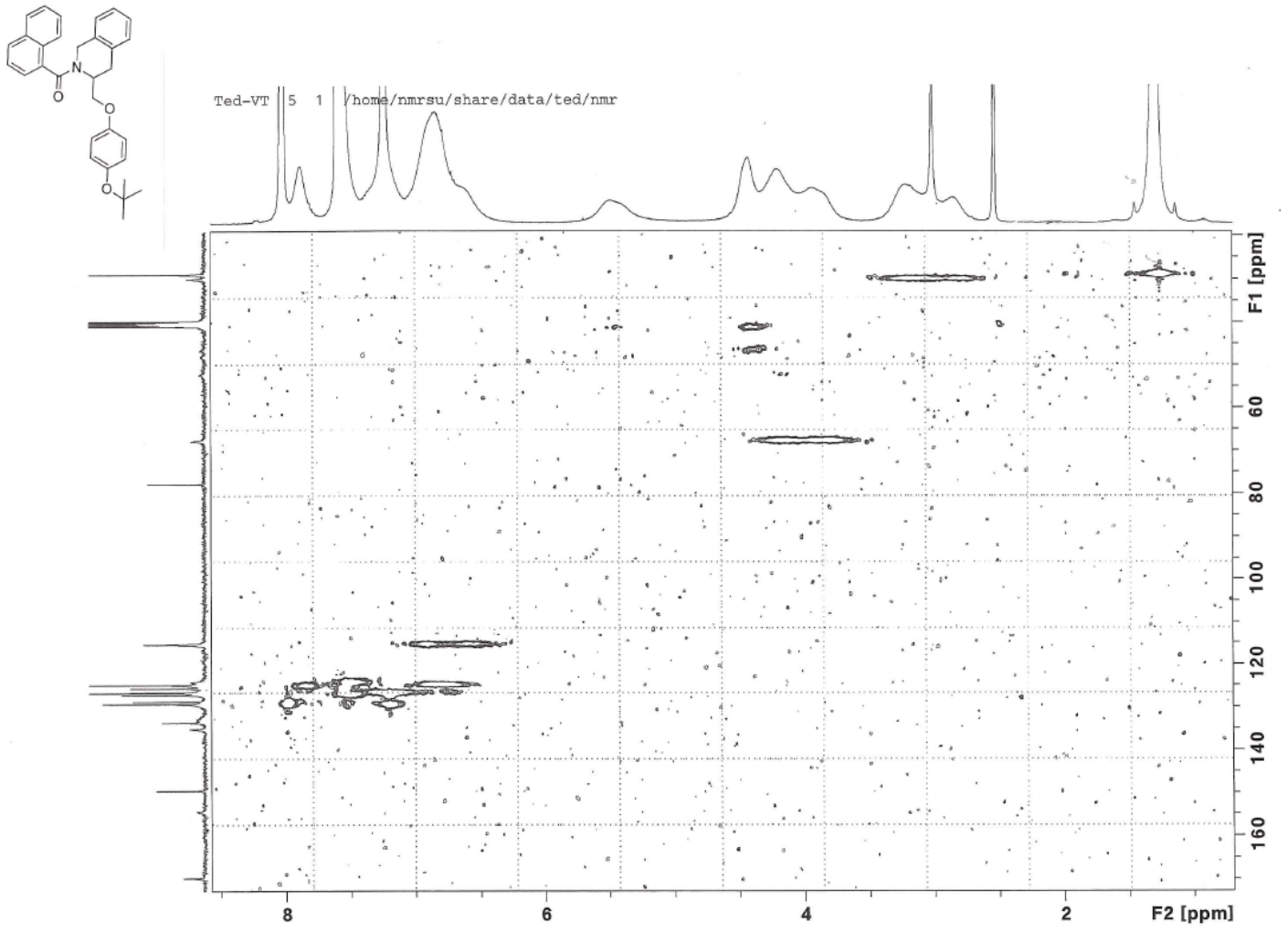

Supplementary Figure 9. Characterization of SR10067. **a**, Sample purity of SR10067 evidenced by reverse-phase analytical HPLC. Four traces indicate data collected at distinct wavelengths (210, 220, 254, and 280 nM). **b**, Identity of SR10067 evidenced by high temperature ^{1}H-NMR (110°C in d_6-DMSO) **c**, ^{13}C-NMR, and **d,** 2D ^{1}H-^{13}C correlation Spectrum

Serotonin Receptors

$5HT_{1A}$	K_i>10 μM
$5HT_{1B}$	K_i>10 μM
$5HT_{1D}$	K_i>10 μM
$5HT_{1E}$	K_i>10 μM
$5HT_{2A}$	K_i>10 μM
$5HT_{2B}$	K_i>10 μM
$5HT_{2C}$	K_i>10 μM
$5HT_{3}$	K_i>10 μM
$5HT_{5A}$	K_i>10 μM
$5HT_{6}$	K_i>10 μM
$5HT_{7}$	K_i>10 μM

Adrenergic Receptors

α_{1A}	K_i>10 μM
α_{1B}	K_i>10 μM
α_{1D}	K_i>10 μM
α_{2B}	K_i>10 μM
α_{2C}	K_i>10 μM
β_1	K_i>10 μM
β_2	K_i>10 μM
β_3	K_i>10 μM

Monoamine Transporters

DAT	K_i>10 μM
NET	K_i>10 μM
SERT	K_i>10 μM

Dopamine Receptors

D1	K_i>10 μM
D2	K_i>10 μM
D3	K_i>10 μM
D4	K_i>10 μM
D5	K_i>10 μM

Others

GABAA	K_i>10 μM
Sigma1	K_i>10 μM
Sigma2	K_i>10 μM
PBR	K_i>10 μM
hERG	NEGATIVE

Histamine Receptors

H1	K_i= 4 μM
H2	K_i>10 μM
H3	K_i>10 μM

Acetyl Choline Receptors

M1	K_i>10 μM
M2	K_i>10 μM
M3	K_i>10 μM
M4	K_i>10 μM

Opioid Receptors

DOR	K_i>10 μM
KOR	K_i>10 μM
MOR	K_i>10 μM

Supplementary Table 1. NIMH Psychoactive Drug Screen Program (PDSP) Results. SR10067 specificity against a range of drug targets was tested in the PDSP. Details of the assays are located on the PDSP website (http://pdsp.med.unc.edu/indexR.html).