Supplementary Table S1. The influence of Sigma1R antagonists BD-1047 and NE-100 on mice behavior in the elevated plus-maze test.

Experimental groups	Number of entries into open arms (N open)	Number of entries into open arms, % (%N open)	Time spent in open arms, s (T open)	Time spent in open arms, % (%T open)	Number of entries into closed arms (N closed)	Number of total entries (N total)
Intact *n*=15	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	5.27 ± 2.34	10.80 ± 4,69
Veh1+ Veh2 *n*=15	0.0 (0.0-1.0)	0.0 (0.0-14.0)	0.0 (0.0-3.0)	0.0 (0.0-1.2) * *p*=0.048	5.07 ± 2.25	10.80 ± 5,52
BD-1047 1.0+ Veh2 *n*=15	0.0 (0.0-1.0)	0.0 (0.0-10.0)	0.0 (0.0-2.0)	0.0 (0.0-0.8)	6.67 ± 2.74	14.93 ± 5.97
NE-100 1.0+ Veh2 *n*=15	0.0 (0.0-1.0)	0.0 (0.0-10.0)	0.0 (0.0-6.0)	0.0 (0.0-2.6)	5.13 ± 2.13	11.47 ± 4.84
NE-100 3.0+ Veh2 *n*=15	0.0 (0.0-1.0)	0.0 (0.0-17.0)	0.0 (0.0-4.0)	0.0 (0.0-1.8)	6.67 ± 2.50	14.80 ± 4.90

Data are presented as median (q25 - q75) for experimental groups N open, %N open, T open, %T open and mean ± S.D for experimental groups N closed, N total. n – the number of animals in the experimental group. Experimental groups: intact BALB/c mice (Intact), vehicle 1 + vehicle 2 (Veh1+ Veh2), BD-1047 1.0 mg/kg + vehicle 2 (BD-1047 1.0+ Veh2), NE-100 1.0 mg/kg + vehicle 2 (NE-100 1.0+ Veh2), NE-100 3.0 mg/kg + vehicle 2 (NE-100 3.0+ Veh2). Kruskal–Wallis test, Dunn's multiple comparison test: * - statistical significance vs. Intact.

Supplementary Table S2. The influence of Sigma1R antagonists BD-1047 and NE-100 on the anxiolytic effect of diazepam in the elevated plus-maze test.

Experimental groups	Number of entries into open arms (N open)	Number of entries into open arms, % (%N open)	Time spent in open arms, s (T open)	Time spent in open arms, % (%T open)	Number of entries into closed arms (N closed)	Number of total entries (N total)
Veh1+ Veh2 *n*=15	0.0 (0.0-1.0)	0.0 (0.0-13.0)	0.0 (0.0-3.0)	0.0 (0.0-0.8)	5.73 ± 2.63	12.60 ± 5.71
Veh1+D 1.0 *n*=15	6.0 (5.0-9.0) * p<0.001	46.0 (33.0-72.0) * p<0.001	93.0 (77.0-201.0) * p<0.001	39.0 (32.0-85.0) * p<0.001	6.73 ± 4.22	28.0 ± 9.61 * p<0.001
BD-1047 1.0+D 1.0 *n*=15	4.0 (2.0-5.0)	25.0 (19.0-36.0) # p=0.025	26.0 (19.0-37.0) # p=0.0016	10.0 (7.2-15.0) # p=0.002	10.60 ± 3.68 # p=0.0054	29.40 ± 9.87
NE-100 1.0+D 1.0 *n*=14	6.0 (3.75-8.0)	50.0 (32.25-53.75)	79.5 (30.25-190.3)	34.95 (11.18-69.78)	7.36 ± 2.24	26.43 ± 6.27
NE-100 3.0+D 1.0 *n*=15	4.0 (3.0-6.0)	33.0 (20.0-39.0)	33.0 (14.0-59.0) # p=0.011	13.0 (5.2-24.0) # p=0.011	8.13 ± 2.59	25.27 ± 10.07

Data are presented as median (q25 - q75) for experimental groups N open, %N open, T open, %T open and mean ± S.D for experimental groups N closed, N total. n – the number of animals in the experimental group. Experimental groups: vehicle 1 + vehicle 2 (Veh1+Veh2), vehicle 1 + diazepam 1.0 mg/kg (Veh1+D 1.0), BD-1047 1.0 mg/kg + diazepam 1.0 mg/kg (BD-1047 1.0+D 1.0), NE-100 1.0 mg/kg + diazepam 1.0 mg/kg (NE-100 1.0+D 1.0), NE-100 3.0 mg/kg + diazepam 1.0 mg/kg (NE-100 3.0+D 1.0). Kruskal–Wallis test, Dunn's multiple comparison test for experimental groups N open, %N open, T open, %T open, one-way ANOVA and the post hoc Sidak multiple comparisons test for experimental groups N closed, N total: * - statistical significance vs. Veh1+Veh2, # - statistical significance vs. Veh1+D 1.0.

Supplementary Table S3. The influence of Sigma1R antagonists BD-1047 and NE-100 on the anxiolytic effect of phenazepam in the elevated plus-maze test.

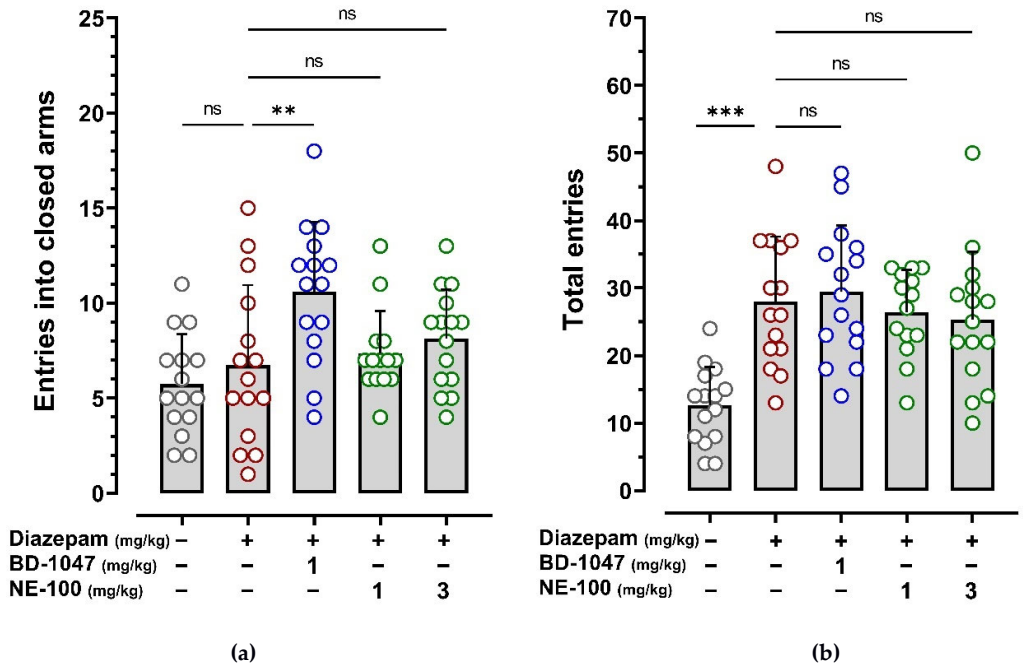
Experimental groups	Number of entries into open arms (N open)	Number of entries into open arms, % (%N open)	Time spent in open arms, s (T open)	Time spent in open arms, % (%T open)	Number of entries into closed arms (N closed)	Number of total entries (N total)
Veh1+Veh2 *n*=15	1.0 (0.0-1.0)	8.03 (0.0-17.0)	0.0 (0.0-3.0)	0.0 (0.0-1.1)	5.73 ± 2.60	12.93 ± 5.18
Veh1+Ph 0.1 *n*=15	5.0 (3.0-7.0) * p<0.001	47.0 (39.0-57.0) * p<0.001	119.0 (77.0-189.0) * p<0.001	56.3 (33.6-76.7) * p<0.001	5.73 ± 3.31	21.80 ± 9.03 * p=0.001
BD-1047 1.0+Ph 0.1 *n*=14	2.0 (1.0-3.0) # p=0.011	22.0 (20.0-36.5) # p=0.016	25.0 (12.75-40.25) # p=0.019	10.1 (4.47-14.93) # p=0.016	5.71 ± 2.27	15.43 ± 5.39 # p=0.031
NE-100 3.0+ Ph 0.1 *n*=14	1.0 (0.75-2.0) # p<0.001	19.0 (9.75-26.0) # p<0.001	13.5 (0.75-21.75) # p<0.001	5.0 (0.3-8.2) # p<0.001	5.57 ± 1.99	13.93 ± 5.28 # p=0.006

Data are presented as median (q25 - q75) for experimental groups N open, %N open, T open, %T open and mean ± S.D for experimental groups N closed, N total. n – the number of animals in the experimental group. Experimental groups: vehicle 1 + vehicle 2 (Veh1+ Veh2), vehicle 1 + phenazepam 0.1 mg/kg (Veh1+Ph 0.1), BD-1047 1.0 mg/kg + phenazepam 0.1 mg/kg (BD-1047 1.0+Ph 0.1), NE-100 3.0 mg/kg + phenazepam 0.1 mg/kg (NE-100 3.0+Ph 0.1). Kruskal–Wallis test, Dunn's multiple comparison test for experimental groups N open, %N open, T open, %T open, one-way ANOVA and the post hoc Sidak multiple comparisons test for experimental groups N closed, N total: * - statistical significance vs. Veh1+Veh2, # - statistical significance vs. Veh1+Ph 0.1.

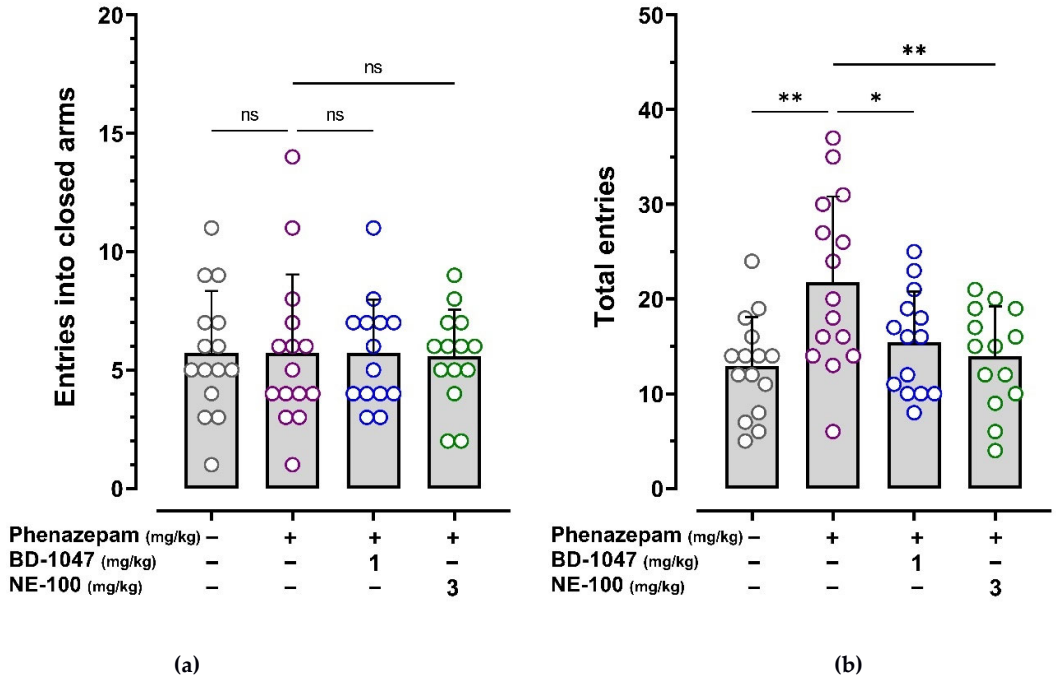
Supplementary Table S4. The influence of Sigma1R agonist PRE-084 on the anxiolytic effect of diazepam in the elevated plus-maze test.

Experimental groups	**Number of entries into open arms (N open)**	**Number of entries into open arms, % (%N open)**	**Time spent in open arms, s (T open)**	**Time spent in open arms, % (%T open)**	**Number of entries into closed arms (N closed)**	**Number of total entries (N total)**
Veh1+Veh2 *n*=8	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	4.63 ± 2.93	9.63 ± 5.98
Veh1+D 1.0 *n*=8	7.0 (6.25-9.75) **p*=0.001	56.5 (47.0-59.75) **p*<0.001	124.0 (118.5-144.5) **p*=0.004	49.05 (42.23-55.25) **p*=0.026	6.5 ± 1.77	28.88 ± 5.54 **p*<0.001
PRE-084 1.0+D 1.0 *n*=8	7.0 (5.25-10.5) **p*=0.002	47.5 (36.5-52.75) **p*=0.007	131.0 (120.3-186.0) **p*<0.001	62.5 (55.53-67.85) **p*<0.001	8.38 ± 3.46 **p*=0.042	32.25 ± 11.78 **p*<0.001

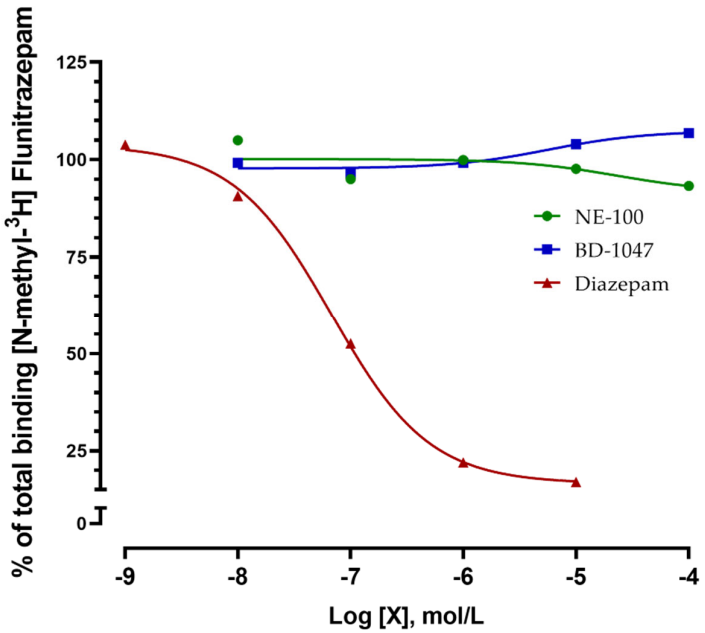
Data are presented as median (q25-q75) for experimental groups N open, %N open, T open, %T open and mean ± S.D for experimental groups N closed, N total. n – the number of animals in the experimental group. Experimental groups: vehicle 1 + vehicle 2 (Veh1+ Veh2), vehicle 1 + diazepam 1.0 mg/kg (Veh1+D 1.0), PRE-084 1.0 mg/kg + diazepam 1.0 mg/kg (PRE-084 1.0+D 1.0). Kruskal–Wallis test, Dunn's multiple comparison test for experimental groups N open, %N open, T open, %T open, one-way ANOVA and the post hoc Sidak multiple comparisons test for experimental groups N closed, N total: * - statistical significance vs. Veh1+Veh2.



Supplementary Figure S1. Influence of Sigma1R antagonists BD-1047 and NE-100 on the effect of diazepam evaluated by the parameters "entries into closed arm" and "total entries" in the elevated plus-maze test. (**a**) The number of entries into the closed arms (N closed); (**b**) The number of total entries into the open and closed arms (N total). Vehicle 2 and diazepam (1.0 mg/kg) were injected i.p. 30 min prior to the EPM exposition. Vehicle 1, selective Sigma1R antagonists BD-1047 (1.0 mg/kg) and NE-100 (1.0 and 3.0 mg/kg) were injected i.p. 30 min prior diazepam. Data are presented as mean with SD. Statistically significant differences according to the one-way ANOVA and the post hoc Sidak multiple comparisons test: ns - not significant; ** $p < 0.01$; *** $p < 0.001$.



Supplementary Figure S2. Influence of Sigma1R antagonists BD-1047 and NE-100 on the effect of phenazepam evaluated by the parameters "entries into closed arm" and "total entries" in the elevated plus-maze test. (**a**) The number of entries into the closed arms (N closed); (**b**) The number of total entries into the open and closed arms (N total). Vehicle 2 and phenazepam (0.1 mg/kg) were injected i.p. 30 min prior to the EPM exposition. Vehicle 1, selective Sigma1R antagonists BD-1047 (1.0 mg/kg) and NE-100 (3.0 mg/kg) were injected i.p. 30 min prior phenazepam. Data are presented as mean with SD. Statistically significant differences according to the one-way ANOVA and the post hoc Sidak multiple comparisons test: ns - not significant; * $p < 0.05$; ** $p < 0.01$.



Supplementary Figure S3. Competitive interaction of diazepam and Sigma1R antagonists BD-1047 and NE-100 with [N-methyl-^{3}H] Flunitrazepam. Data were obtained in the brain homogenates of BALB/c mice (n=2). IC_{50} diazepam = 68 nM.

Supplementary Table S5. The influence of Sigma1R antagonist BD-1047 on anticonvulsant activity of diazepam in the intravenous pentylenetetrazol infusion test on mice.

Experimental groups	**Dose of PTZ to induce clonic jerks,** mg/kg **Mean ± SEM (min;max)**	**Dose of PTZ to induce generalized clonic seizure,** mg/kg **Mean ± SEM (min;max)**	**Dose of PTZ to induce generalized tonic seizure,** mg/kg **Mean ± SEM (min;max)**
1% PTZ *n*=10	34.86 ± 1.65 (24;41)	43.52 ± 2.47 (34;60)	100.39 ± 5.12 (73;123)
BD-1047 1 mg/kg, 1% PTZ *n*=11	39.49 ± 1.30 (30;45)	46.76 ± 2.11 (34;56)	103.47 ± 5.82 (70;136)
BD-1047 10 mg/kg, 1% PTZ *n*=9	35.50 ± 2.26 (29;52)	47.06 ± 4.01 (35;77)	102.46 ± 9.59 (53;139)
BD-1047 20 mg/kg, 1% PTZ *n*=9	33.14 ± 2.60 (24;47)	42.86 ± 2.56 (32;53)	86.28 ± 10.02 (48;128)
Diaz 1 mg/kg, 1% PTZ *n*=8	102.46 ± 9.59 (55;83) #### $p<0.0001$	81.32 ± 3.31 (68;95) #### $p<0.0001$	181.09 ± 7.10 (146;299) #### $p<0.0001$
BD-1047 1 mg/kg, Diaz 1 mg/kg, 1% PTZ *n*=5	70.65 ± 3.55 (60;81)	91.14 ± 5.15 (73;103)	157.66 ± 18.51 (110;208)
BD-1047 10 mg/kg, Diaz 1 mg/kg, 1% PTZ *n*=8	68.31 ± 3.13 (56;81)	80.26 ± 2.69 (68;90)	149.79 ± 7.64 (115;174)
BD-1047 20 mg/kg, Diaz 1 mg/kg, 1% PTZ *n*=7	50.37 ± 1.58 (42;55) **** $p<0.0001$	64.39 ± 3.76 (55;81) ** $p=0.0057$	133.54 ± 10.58 (99;166) ** $p=0.0068$

n – the number of animals in the experimental group. Diaz – Diazepam. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: * - vs. Diaz 1 mg/kg, 1% PTZ; # - vs. 1% PTZ.

Supplementary Table S6. The influence of Sigma1R agonist PRE-084 on anticonvulsant activity of diazepam in the intravenous pentylenetetrazol infusion test on mice.

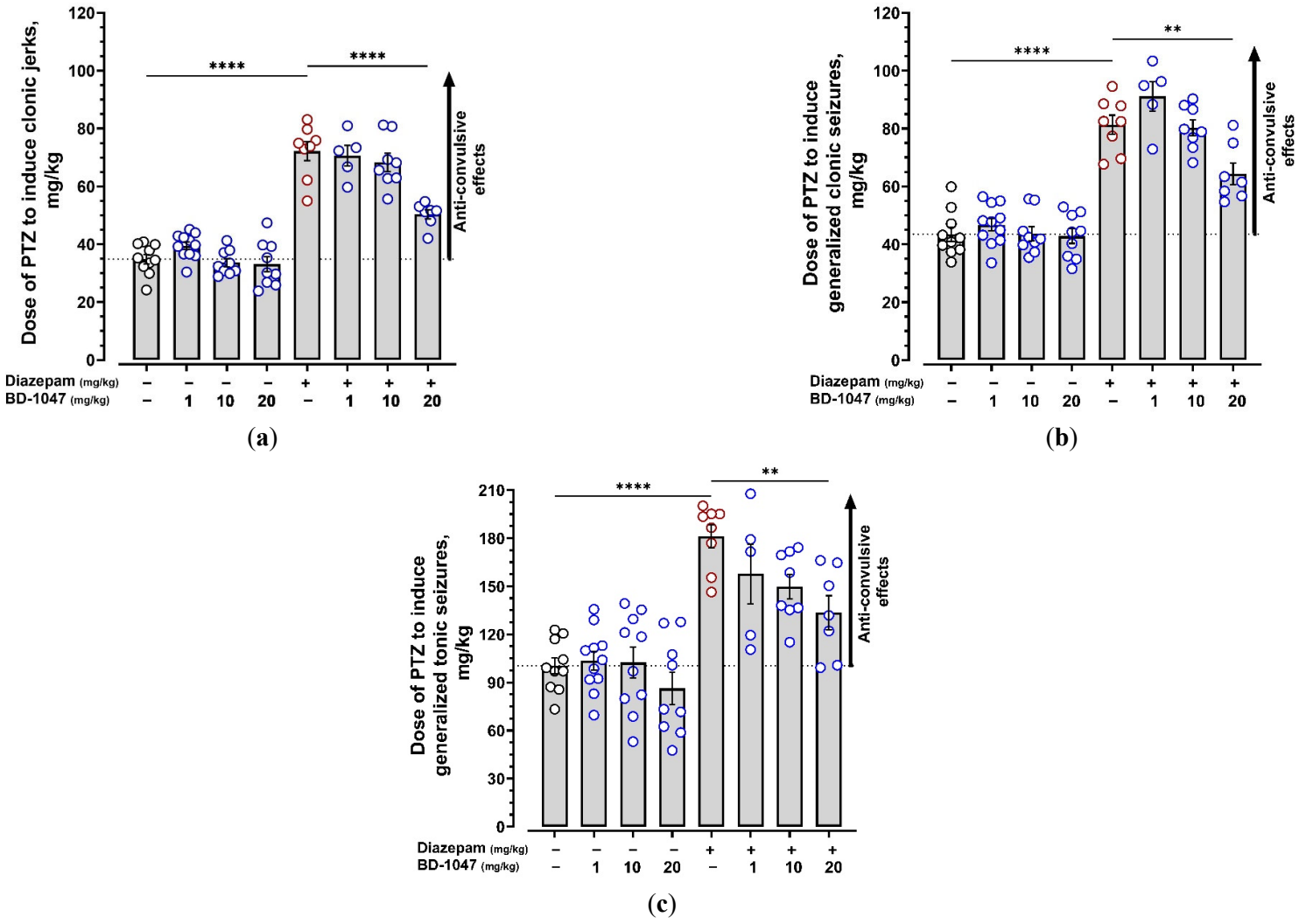
Experimental groups	**Dose of PTZ to induce clonic jerks,** mg/kg **Mean ± SEM (min;max)**	**Dose of PTZ to induce generalized clonic seizure,** mg/kg **Mean ± SEM (min;max)**	**Dose of PTZ to induce generalized tonic seizure,** mg/kg **Mean ± SEM (min;max)**
1% PTZ *n*=12	43.86 ± 1.47 (37; 53)	51.9 ± 1.53 (43; 63)	67.67 ± 3.42 (50; 83)
PRE–084 5 mg/kg, 1% PTZ *n*=12	49.36 ± 2.23 (40; 61)	57.81 ± 3.11 (44; 73)	74.7 ± 6.15 (50; 103)
PRE–084 20 mg/kg, 1% PTZ *n*=11	43.2 ± 1.33 (38; 50)	50.35 ± 2.23 (38; 66)	95.53 ± 5.66 (73; 127)
Diaz 1 mg/kg, 1% PTZ *n*=10	69.25 ± 2.31 (55; 78) #### $p<0.0001$	84.46 ± 2.22 (77; 94) #### $p<0.0001$	101 ± 5.21 (82; 127) ## $p=0.0055$
PRE–084 5 mg/kg, Diazepam 1 mg/kg, 1% PTZ *n*=11	85.2 ± 2.93 (67; 102) **** $p<0.0001$	101.5 ± 4.36 (82; 133) ** $p=0.0038$	130.6 ± 7.89 (108; 174) * p=0.0272
PRE–084 20 mg/kg, Diazepam 1 mg/kg, 1% PTZ *n*=12	81.4 ± 2.78 (68; 97) ** $p=0.002$	91.42 ± 3.91 (72; 115)	155.8 ± 11.46 (88; 218) **** $p<0.0001$

n – the number of animals in the experimental group. Diaz – Diazepam. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: * - vs. Diaz 1 mg/kg, 1% PTZ; # - vs. 1% PTZ.

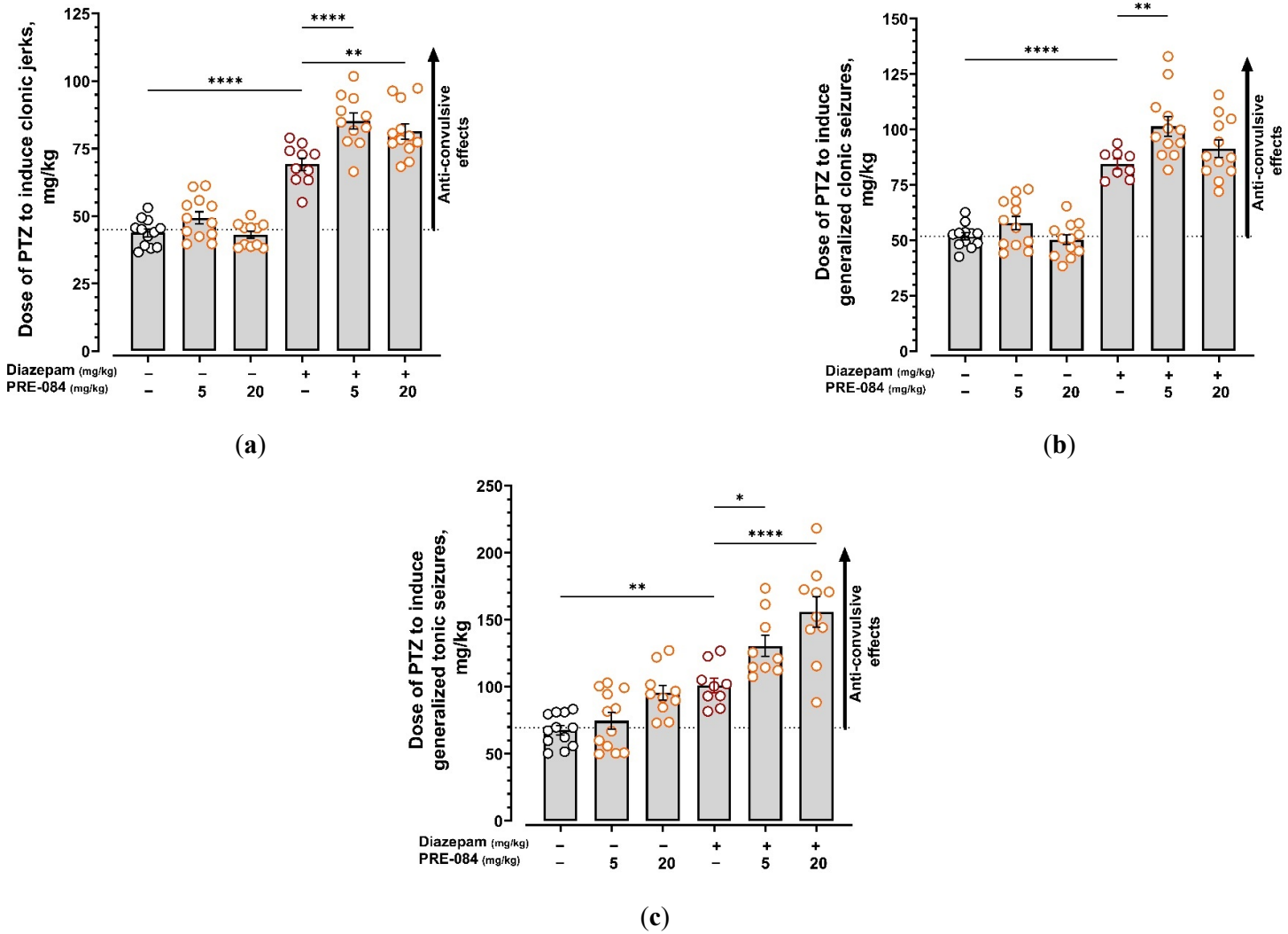
Supplementary Table S7. The influence of Sigma1R antagonist BD-1047 and agonist PRE-084 on mice falling asleep time and sleeping time in the pentobarbital-induced sleep test on mice.

Experimental groups	Falling asleep time, s Mean ± SEM (min;max)	Sleeping time, s Mean ± SEM (min;max)
Pentobarbital sodium 50 mg/kg *n*=10	229 ± 5.63 (206; 262)	5842.7 ± 277.05 (4763; 6870)
BD-1047 1 mg/kg **Pentobarbital sodium 50 mg/kg** *n*=10	260 ± 7.33 (233; 308) ** *p*=0.0023	4900.5 ± 84.14 (4370; 5213) ** *p*=0.0037
BD-1047 10 mg/kg **Pentobarbital sodium 50 mg/kg** *n*=10	240.2 ± 4.91 (216; 263)	4944.9 ± 171.45 (3935; 5625) ** *p*=0.0056
PRE-084 1 mg/kg **Pentobarbital sodium 50 mg/kg** *n*=10	222.8 ± 17.59 (150; 290)	11078.56 ± 1632.8 (4680; 19160) *** *p*=0.0008
PRE-084 5 mg/kg **Pentobarbital sodium 50 mg/kg** *n*=10	190.1 ± 8.98 (150; 230) * *p*=0.0473	12246 ± 665.69 (8980; 15579) **** *p*<0.0001
Diazepam 1 mg/kg **Pentobarbital sodium 50 mg/kg** *n*=10	244 ± 8.46 (210; 285)	11336.5 ± 641.45 (8730; 14580) *** *p*=0.0003

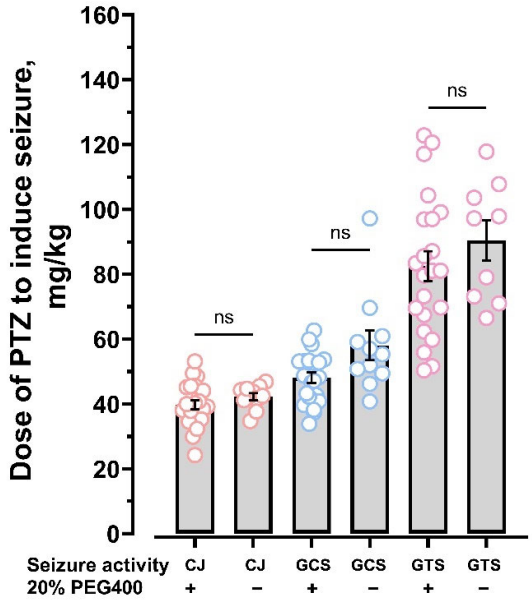
n – the number of animals in the experimental group. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: * - statistical significance vs. Pentobarbital sodium 50 mg/kg. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0,0001$.



Supplementary Figure S4. The influence of Sigma1R antagonist BD-1047 on anticonvulsant activity of diazepam in the intravenous pentylenetetrazol infusion test on mice. (**a**) Clonic jerks; (**b**) Generalized clonic seizure; (**c**) Generalized tonic seizure. Data are presented as mean ± S.E.M. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: ** $p < 0.01$; ****$p < 0.0001$.



Supplementary Figure S5. The influence of Sigma1R agonist PRE-084 on anticonvulsant activity of diazepam in the intravenous pentylenetetrazol infusion test on mice. (**a**) Clonic jerks; (**b**) Generalized clonic seizure; (**c**) Generalized tonic seizure. Data are presented as mean ± S.E.M. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: * $p < 0,05$; ** $p < 0.01$; **** $p < 0.0001$.



Supplementary Figure S6. The influence of 20% PEG 400 on convulsant activity of PTZ in the intravenous pentylenetetrazol infusion test on mice. CJ: clonic jerks, GCS: generalized clonic seizure, GTS: generalized tonic seizure. Data are presented as mean ± S.E.M. Statistically significant differences according to the one-way ANOVA and the post hoc Dunnet multiple comparisons test: ns - not significant