Supporting Information

Different Benzodiazepines Bind with Distinct Binding Modes to $GABA_A$ Receptors

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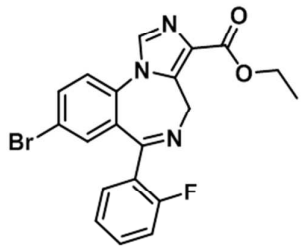
Supplementary Figure S1:

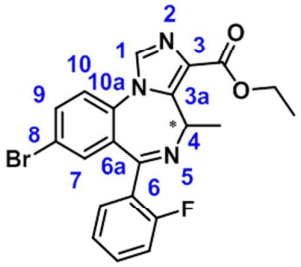
IUPAC nomenclature of the structures used in the study.

a



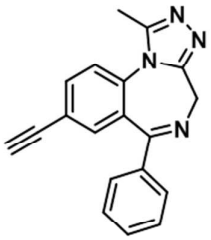
Ethyl 8-bromo-6-(2-fluorophenyl)-4*H*-benzo[*f*]-imidazo[1,5-*a*][1,4]diazepine-3-carboxylate
Compound 1

d



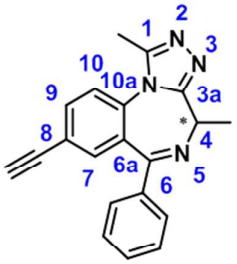
Ethyl 8-bromo-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate

b



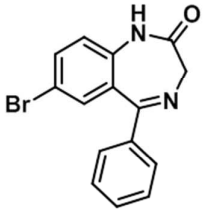
8-Ethynyl-1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]-triazolo[4,3-*a*][1,4]diazepine
Compound 2

e



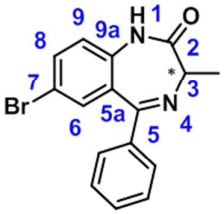
8-Ethynyl-1,4-dimethyl-6-phenyl-4*H*-benzo-[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine

c



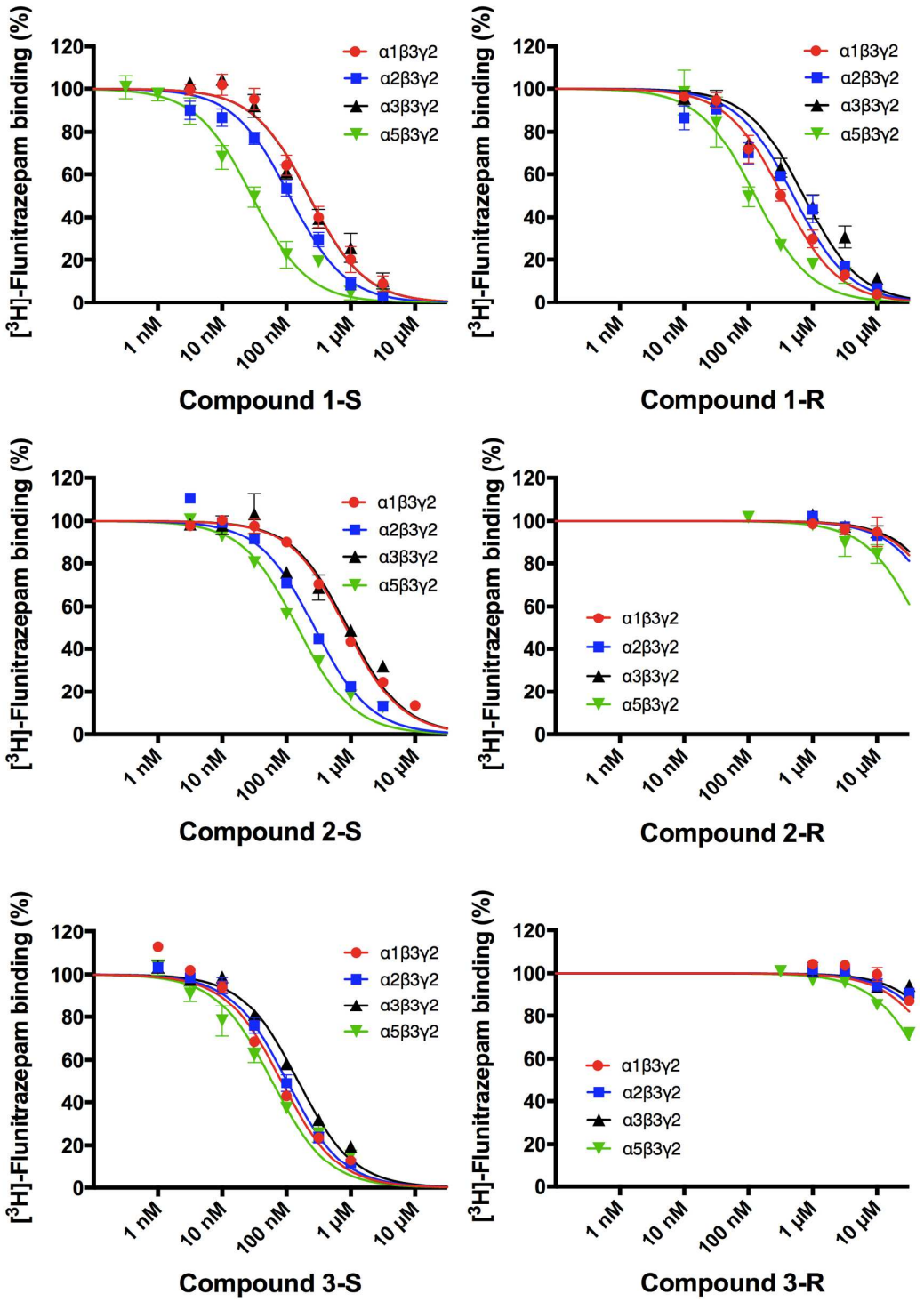
7-Bromo-5-phenyl-1,3-dihydro -2*H*-benzo[*e*][1,4]diazepin-2-one
Compound 3

f



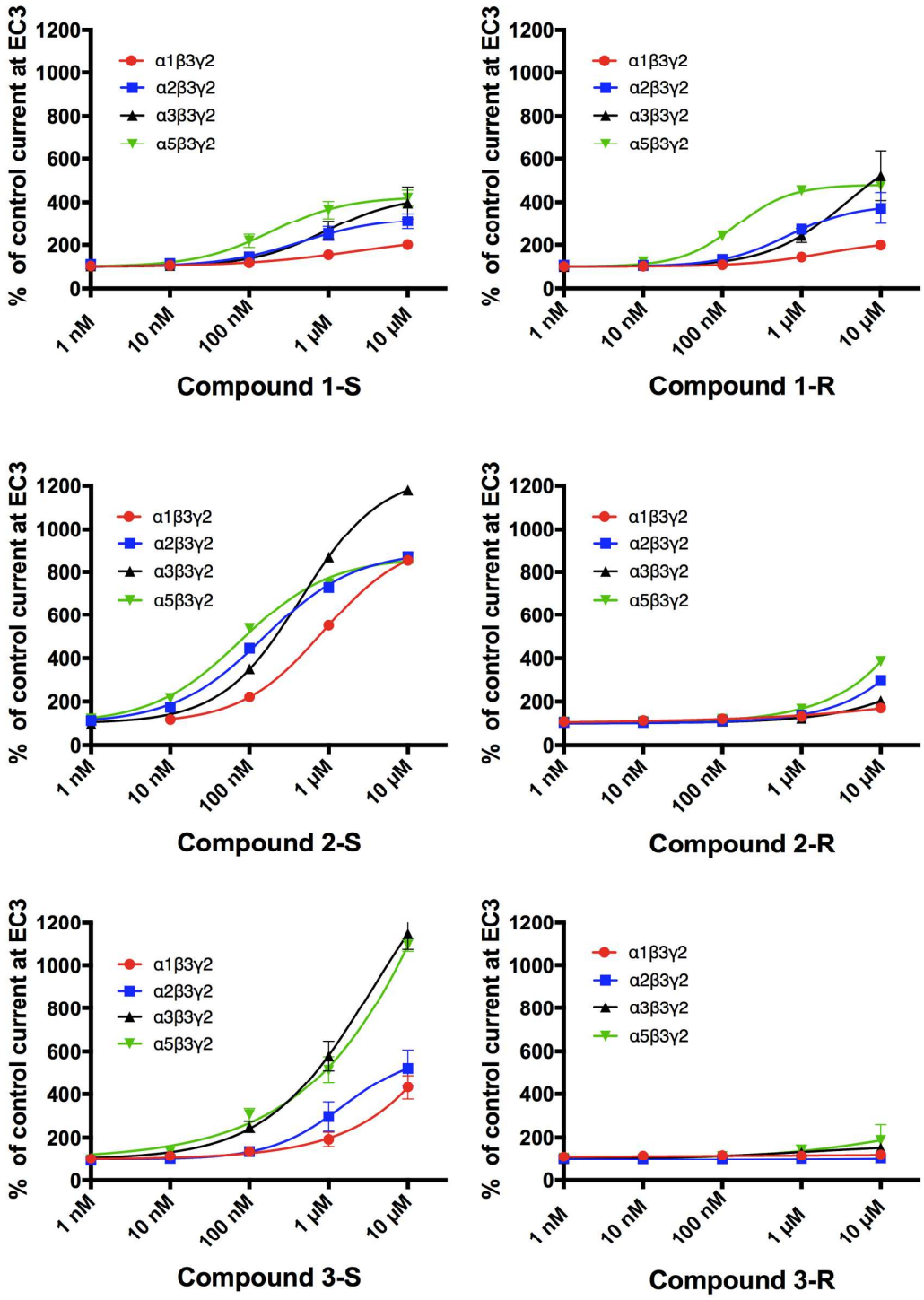
7-bromo-3-methyl-5-phenyl-1,3-dihydro -2*H*-benzo[*e*][1,4]diazepin-2-one

Supplementary Figure S2:



Inhibition of [^{3}H]flunitrazepam binding to recombinant α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2 $GABA_A$ receptors subtypes. Membranes from HEK 293 cells transfected with $GABA_A$ receptor subunit combination, (α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2) were incubated with 2 nM [^{3}H]flunitrazepam in the presence of various concentrations of $GABA_A$-R ligands investigated. Data shown represent mean ± SEM of three experiments performed in duplicates each.

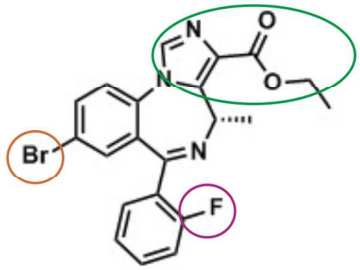
Supplementary Figure S3:



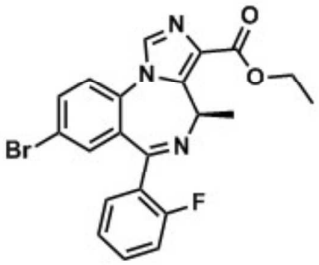
Concentration-response curves of drug-induced enhancement of GABA currents. *Xenopus laevis* oocytes were injected with mRNA for different $GABA_A$-Receptor subunit combination, ($\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma2$, $\alpha3\beta3\gamma2$, $\alpha5\beta3\gamma2$) and studied using two-electrode voltage clamp recordings. GABA induced currents (3% of maximum) were modulated by drug application. Data shown represent mean ± SEM of three experiments.

Supplementary Figure S4:

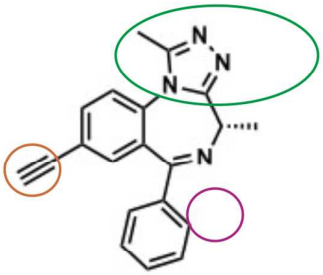
Comparison of the substitutions on the basic benzodiazepine core scaffold of the compound pairs investigated in the current study.



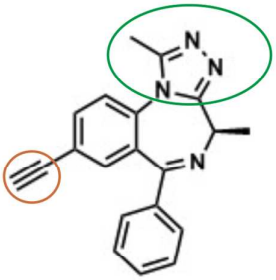
Compound 1-S
(SH-I-048B)



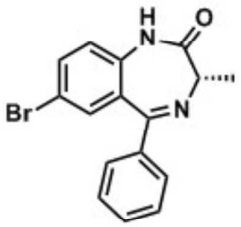
Compound 1-R
(SH-I-047)



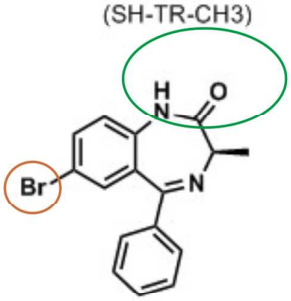
Compound 2-S
(SH-TS-CH3)



Compound 2-R
(SH-TR-CH3)



Compound 3-S
(SH-I-030)



Compound 3-R
(SH-I-053B)