Supplementary Information

"Allosteric modulation and direct activation of glycine receptors by a tricyclic sulfonamide"

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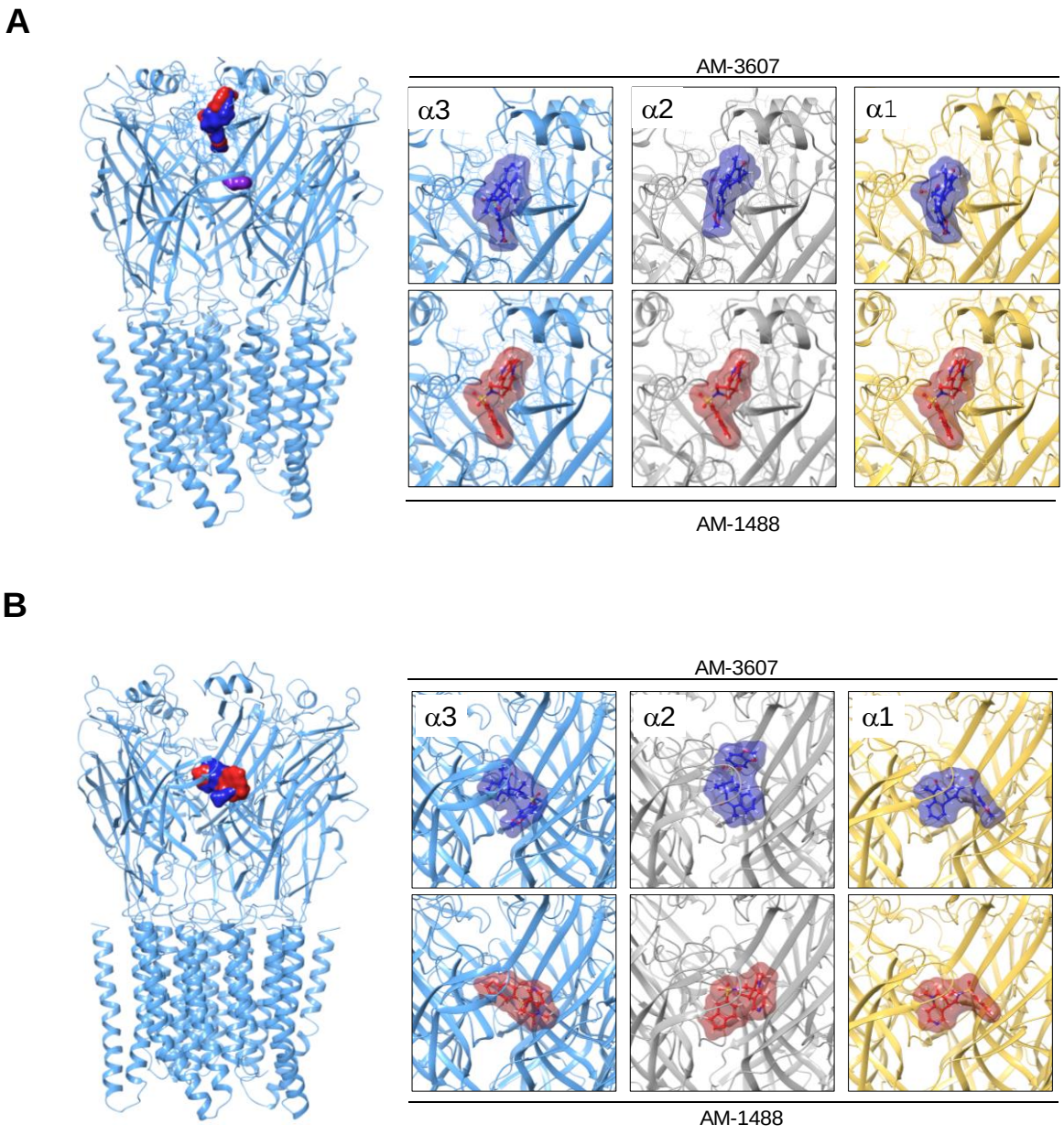

Figure S1. Interactions of AM-1488 and AM-3607 with GlyRs composed of α1, α2 and α3 subunits. A. The left panel show the AM-1488 (red) and AM-3607 (blue) binding to homomeric α3GlyRs in the presence of glycine (in purple). The right panels display enhanced views of the predicted binding of AM-1488 (in red) or AM-3607 (in blue) to the sulfonamide binding sites within α1, α2 and α3GlyRs. The image showing the AM-3607-α3GlyR complex, in the presence of glycine, was reproduced from the previously reported structural information (PDB:5TIN) [8]. **B.**

The left model displays the AM-1488 (red) and AM-3607 (blue) binding to the orthosteric site of homomeric α3GlyRs. The right panels are zoomed views of the predicted binding of the sulfonamides to the orthosteric sites of α1, α2 and α3GlyRs. The models were generated from the structural coordinates available (α1, PDB:7TU9; α2, PDB:7KUY and α3, PDB:5CFB).

Supplementary Table 1. Energetic parameters of tricyclic sulfonamide interactions with wild-type and mutated GlyRs

Allosteric site	**Docking score**			**$\Delta G_{binding}$ (kcal/mol)**		
	α3GlyR	**α2GlyR**	**α1GlyR**	**α3GlyR**	**α2GlyR**	**α1GlyR**
AM-1488	- 4.318	-4.083	-4.302	-68.21	-65.46	-65.51
AM-3607	-3.059	-3.202	-3.006	-58.92	-51.61	-65.32
	Docking score			**$\Delta G_{binding}$ (kcal/mol)**		
Orthosteric site	**α3GlyR**	**α2GlyR**	**α1GlyR**	**α3GlyR**	**α2GlyR**	**α1GlyR**
AM-1488	-4.468	-5.440	-6.138	-59.904	-60.69	-59.703
AM-3607	-4.899	-4.255	-6.173	-62.135	-58.55	-64.701

Docking score					
α3GlyR	**Wild-type**	**F13A**	**L14A**	**R27A**	**L83A**
AM-1488	- 4.318	-3.586	-3.139	-4.515	-3.986
AM-3607	-3.059	-3.123	-2.045	-2.570	-3.111
$\Delta G_{binding}$ (kcal/mol)					
α3GlyR	**Wild-type**	**F13A**	**L14A**	**R27A**	**L83A**
AM-1488	-68.21	-66.39	-61.91	-67.81	-62.05
AM-3607	-58.92	-65.70	-95.37	-62.35	-63.46

Supplementary Table 2. Allosteric potentiation and putative agonistic activity of wild-type and mutated GlyRs by AM-1488.

AM-1488 direct activation				
	α1GlyR	**α2GlyR**	**α3GlyR**	**α1βGlyR**
10 μM	20±9 % (n=6)	0.5±1 % (n=4)	2±1 % (n=6)	12±3 % (n=6)
50 μM	33±3 %*** (n=8)	6±3 % (n=8)	4±1 % (n=8)	50±10 % (n=7)

ANOVA followed by Bonferroni post-hoc test; ***: p<0.001, α1GlyR vs α2/α3 GlyRs.

α3GlyR allosteric potentiation (0.5 μM AM-1488)				
Wild-type	**F13A**	**L14A**	**R27A**	**L83A**
240±34 % (n=11)	59±5 %** (n=5)	56±14 %** (n=5)	193±40% (n=7)	5±4 %*** (n=6)

ANOVA followed Bonferroni post hoc test, wild-type vs L83A, ***, p<0.001; wild-type vs F13A or L14A, **, p<0.01

α3GlyR direct activation (100 μM AM-1488)				
Wild-type	**F13A**	**L14A**	**R27A**	**L83A**
14±3 % (n=3)	1±1 %* (n=3)	ND* (n=3)	0.5±0.3 %* (n=4)	ND* (n=3)

ND, not detected currents. ANOVA followed Bonferroni post hoc test, wild-type vs mutated receptors, p<0.05