Title: Small molecule positive allosteric modulation of TRPV1 activation by vanilloids and acidic pH
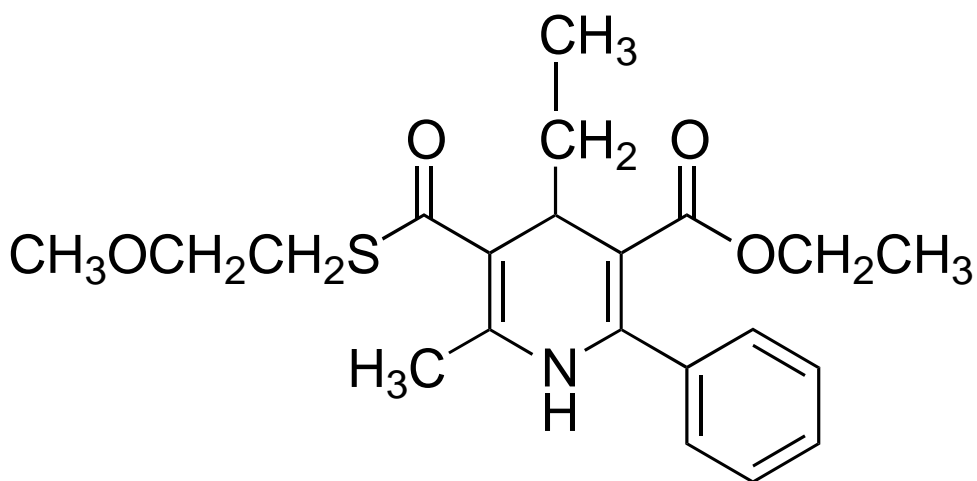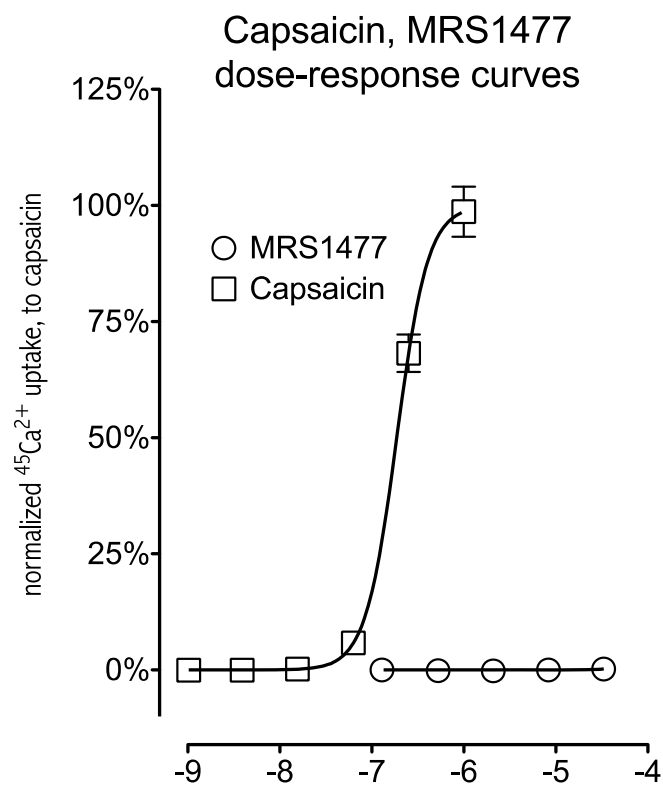
Authors: Krisztian Kaszas, Jason M Keller, Claudio Coddou, Santosh Mishra, Mark Hoon, Stanko Stojilkovic, Kenneth A. Jacobson and Michael J. Iadarola

Supplemental Figure 1. Full name and structure of MRS1477 4,5-diethyl-3-(2-methoxyethylthio)-2-methyl-6-phenyl-1,4-($\pm$)-dihydropyridine-3,5-dicarboxylate

Supplemental Figure 2: Dose-response curve of MRS1477 in the absence of any TRPV1

agonist; Capsaicin dose-response curve from the same experiment included for reference.
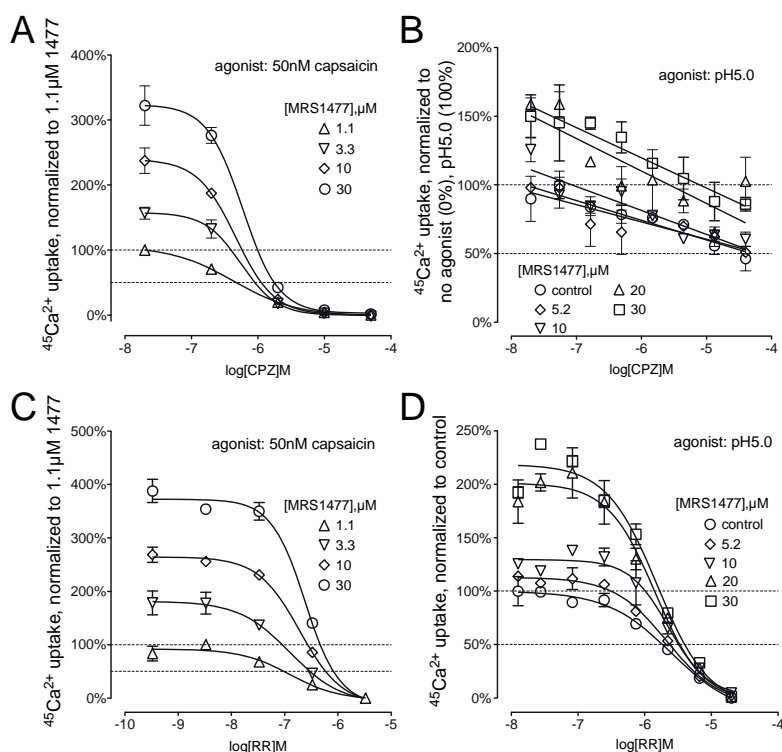
Supplemental Figure 3: MRS1477 does not affect TRPV1 inhibition by capsazepine or ruthenium red (A) Increasing concentrations of MRS1477 potentiate activation of TRPV1 by 50nM capsaicin up to three-fold. (B) CPZ is a partial inhibitor of TRPV1 activation by protons, yielding incomplete D-I curves that show a linear increase in inhibition with increasing doses of CPZ. (C) RR inhibition of capsaicin-activated TRPV1 in the presence of increasing amounts of MRS1477 shows a progressive potentiation of TRPV1 response, with the extent of MRS1477-induced potentiation comparable to what we have previously observed with CPZ on *panel A*. (D) RR is also an inhibitor of proton activation of TRPV1 but it does not interfere with potentiation by MRS1477. Curve-fit details are in Supplemental Table 3.

	EC50,nM	+/-SEM	Hill-Slope	+/-SEM
pH7.4	133.4	7.38	1.71	0.19
pH7.4 + PMA	82.6	5.66	1.85	0.2
pH7.4 + MRS1477	87.7	3.62	1.98	0.14
pH7.4 + PMA + MRS1477	29.5	3.98	1.29	0.24
pH6.0	31.4	4.23	1.44	0.3
pH6.0 + PMA	20.9	2.76	1.34	0.22
pH6.0 + MRS1477	18.4	2.78	1.8	0.4
pH6.0 + PMA + MRS1477	6.72	2.86	0.96	0.3

Supplemental Table 1: Detailed curve-fit results for Figure 1G-H. PMA and MRS1477 concentrations were 10μM and 27μM, respectively.

	[MRS1477] µM	Log IC50	+/-StdErr	Hill Slope	+/-StdErr	R^2
A - CPZ with 50nM Caps	30	-6.217	0.068	-1.609	0.216	0.968
	10	-6.321	0.057	-1.516	0.189	0.975
	3.33	-6.235	0.070	-1.590	0.221	0.966
	1.11	-6.307	0.057	-1.038	0.107	0.972
C - RR with 50nM Caps	30	-6.689	0.055	-1.218	0.176	0.969
	10	-6.781	0.047	-1.132	0.114	0.980
	3.33	-6.942	0.117	-1.020	0.220	0.900
	1.11	-7.072	0.140	-0.873	0.208	0.850
D - RR with pH5.0	control	-5.770	0.053	-1.104	0.134	0.967
	5.2	-5.759	0.036	-1.220	0.108	0.984
	10	-5.716	0.063	-1.337	0.232	0.943
	20	-5.916	0.066	-1.148	0.177	0.950
	30	-5.960	0.062	-0.989	0.125	0.957
	[MRS1477] µM	Slope	Slope 95% CI	Y-intercept when X=0.0	F	P_F
B - CPZ with pH5.0	control	0.00 ± 2.376	-5.097 to 5.097	100.0 ± 14.64	0.0000	1.0000
	5.2	2.73 ± 3.158	-4.044 to 9.504	115.8 ± 19.45	0.7475	0.4018
	10	-1.04 ± 4.145	-9.928 to 7.855	102.8 ± 25.53	0.0625	0.8062
	20	-0.39 ± 7.565	-16.62 to 15.83	140.2 ± 46.60	0.0027	0.9590
	30	0.43 ± 4.584	-9.406 to 10.26	156.0 ± 28.24	0.0087	0.9271

Supplemental Table 2: Detailed curve-fit results for Figure 5. Results in panel A, C and D were fit using least squares fit "log(inhibitor) vs. normalized response -- Variable slope". Panel B shows linear regression analysis of the data where F shows how close the slope of the line is to horizontal (horizontal = 0) while P_F is the probability associated with the regression line not being horizontal ($P<0.05$ means line is not horizontal)

	[MRS1477] μM	Log IC50	+/-StdErr	Hill Slope	+/-StdErr	R^2
A - CPZ with 50nM capsaicin	**30**	-6.227	0.099	-1.610	0.297	0.968
	10	-6.333	0.082	-1.509	0.264	0.975
	3.33	-6.250	0.102	-1.608	0.311	0.966
	1.11	-6.361	0.097	-0.919	0.168	0.974
C - RR with 50nM capsaicin	**30**	-6.613	0.063	-1.354	0.346	0.974
	10	-6.715	0.066	-1.101	0.166	0.982
	3.33	-6.899	0.202	-0.909	0.315	0.901
	1.11	-6.904	0.233	-0.954	0.388	0.863
D - RR with pH5.0 a	**control**	-5.604	0.162	-0.902	0.234	0.972
	5.2	-5.662	0.077	-1.086	0.173	0.987
	10	-5.637	0.099	-1.483	0.433	0.957
	20	-5.842	0.115	-1.216	0.352	0.955
	30	-5.810	0.098	-1.134	0.260	0.972

Supplemental Table 3: Detailed curve-fit results for Supplemental Figure 3. In the case of Panel B, CPZ with pH5.0 as agonists is not included since it was not possible to fit curves to the data. Straight lines were fit to illustrate trend.