

Supplement

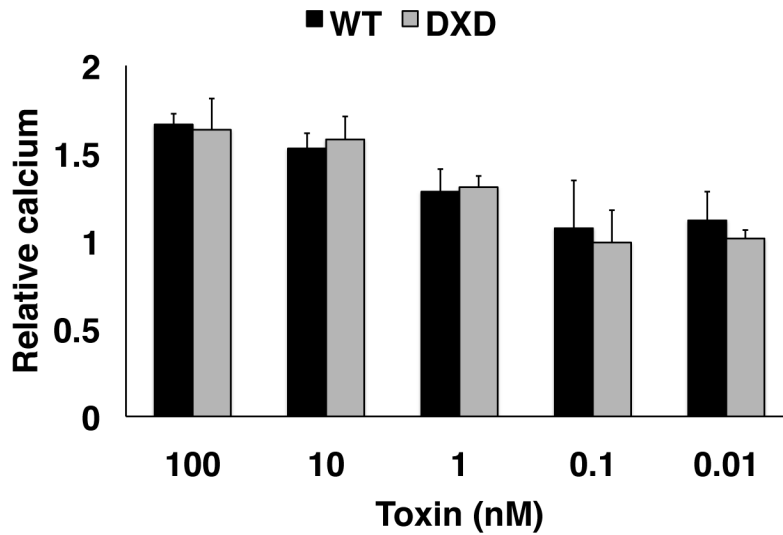
A small molecule inhibitor screen reveals calcium channel signaling as a mechanistic mediator of *Clostridium difficile* TcdB-induced necrosis

Melissa A. Farrow¹, Nicole M. Chumber¹, Sarah C. Bloch¹, McKenzie King¹, Kaycei Moton-Melancon¹, John Shupe¹, Mary K. Washington¹, Benjamin W. Spiller^{1,2}, D. Borden Lacy^{1,3*}

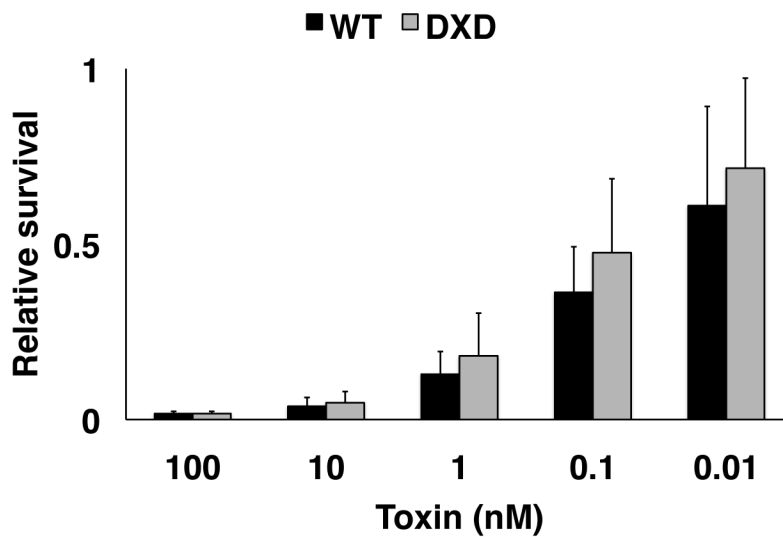
- Supplemental Figure 1
- Table 1

Supplemental Figure 1:

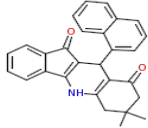
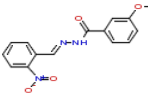
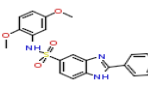
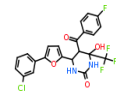
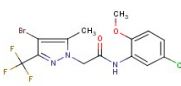
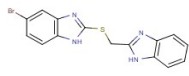
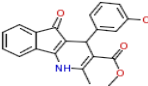
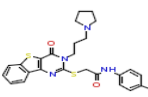
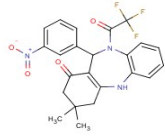
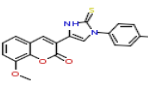
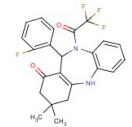
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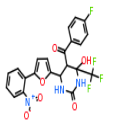
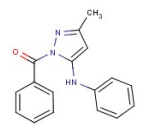
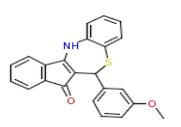
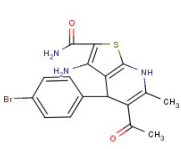
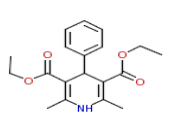
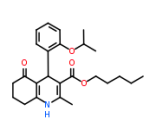
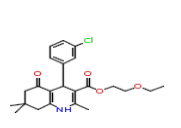
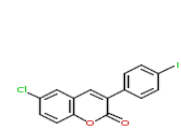
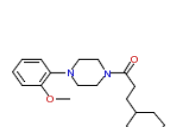
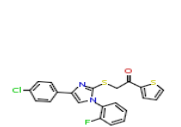
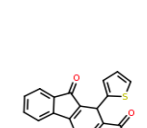


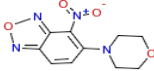
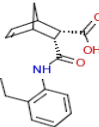
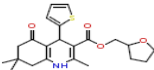
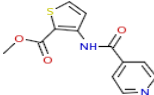
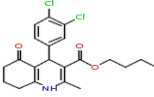
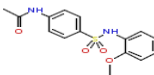
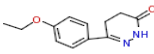
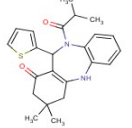
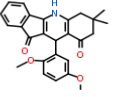
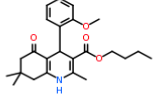
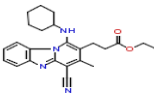
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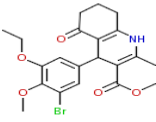
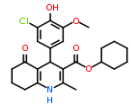
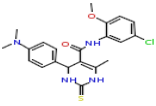
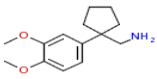
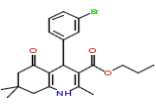
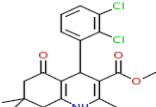
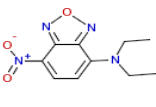
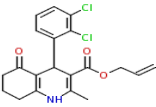
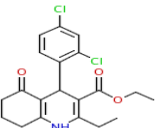
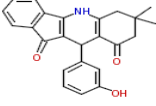
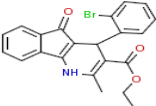


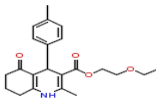
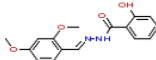
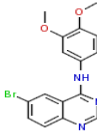
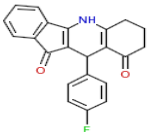
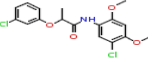
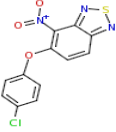
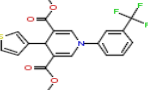
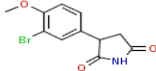
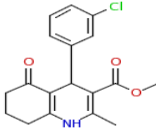
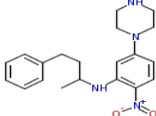
Supplemental Figure 1 (a). Calcium release in response to wild-type (WT) and the enzymatically inactive (DXD) toxin was measured in HeLa cells by pre-treating with FluoForte calcium indicator for one hour prior to intoxication with toxin for three hours. Relative calcium signal was determined by normalizing to unintoxicated cells. (b) HeLa cells in panel A were assessed for viability at 3 h post-intoxication. Relative survival was calculated by comparing to unintoxicated cells.

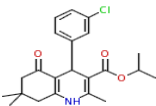
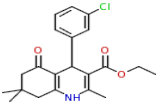
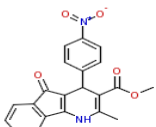
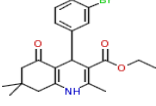
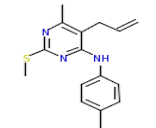
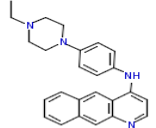
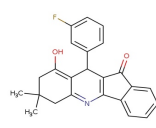
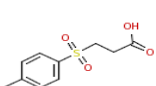
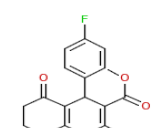
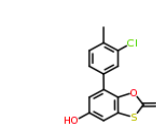
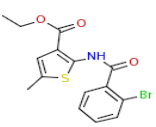
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0042524-1		7289888	8.7	DHP	6.60	14825	8
VU0014819-1		5337117	8.6		7.49	34094	
VU0076366-1		C261-0118	8.6		6.29	24822	
VU0064529-1		4618-0174	8.3		8.45	39457	
VU0028059-1		5672806	8.2		7.38	31298	
VU0027882-1		5669530	7.7		7.32	28350	
VU0042576-1		7315185	7.6	DHP-MPC	5.76	23059	
VU0079410-1		K263-0556	7.3		8.53	24718	
VU0028238-1		5676658	7.2		7.53	29889	2
VU0079201-1		K241-0366	7.2		5.71	18546	
VU0027852-1		5668847	7.2		8.19	29721	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0064519-1		4618-0171	7.1		7.81	31633	
VU0027966-1		5671120	7.0		6.02	27012	
VU0059223-1		3505-6154	6.9		6.66	17246	9
VU0028976-1		5691311	6.9		9.43	31490	11
VU0052186-1		0326-0433	6.9	DHP-MPC	6.91	34143	
VU0054188-1		1716-1851	6.9	DHP-MPC	7.81	32058	
VU0020926-1		5475796	6.7	DHP-MPC	6.47	29592	
VU0027308-1		5660100	6.7		6.58	39375	13
VU0042346-1		7245223	6.7		7.43	38804	6
VU0079212-1		K242-0148	6.6		7.41	27118	
VU0067632-1		5750-3240	6.5	DHP	7.78	26675	


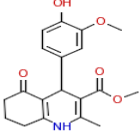
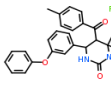
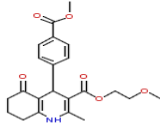
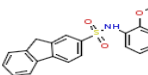
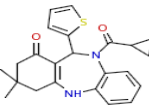
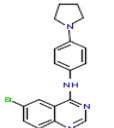
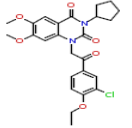
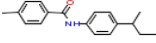
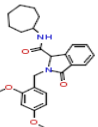
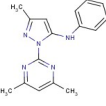
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
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VU0008625-1		5221993	6.5		6.54	38570	1
VU0059261-1		3542-5579	6.4	DHP	7.24	40358	
VU0042393-1		7254997	6.3		6.49	33382	3
VU0025442-1		5610970	6.2	DHP-MPC	6.25	21326	
VU0042114-1		7175407	6.2		6.41	37897	
VU0009014-1		5229227	6.1		6.29	37747	
VU0028090-1		5673431	6.0		8.22	40461	
VU0067642-1		5750-3315	6.0	DHP	6.68	26978	
VU0054158-1		1716-1823	5.9	DHP-MPC	7.99	29886	
VU0064640-1		4708-0206	5.9		8.25	29435	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0046401-1		7617800	5.8	DHP-MPC	6.70	30110	
VU0054168-1		1716-1834	5.8	DHP-MPC	6.27	30253	
VU0035230-1		6109032	5.8		4.96	25157	
VU0009481-1		5237522	5.7		6.98	36413	
VU0020357-1		5468377	5.7	DHP-MPC	6.89	21592	4
VU0020390-1		5468708	5.7	DHP-MPC	6.90	29966	
VU0009795-1		5241528	5.7		7.16	32715	12
VU0020433-1		5469189	5.7	DHP-MPC	6.60	31646	
VU0020827-1		5474652	5.6	DHP-MPC	6.58	34002	
VU0059259-1		3535-0411	5.6	DHP	5.23	22549	
VU0042752-1		7357077	5.6	DHP-MPC	5.54	23306	

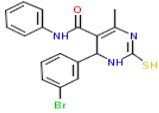
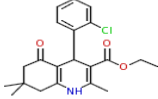
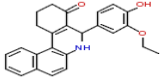
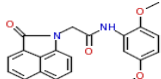
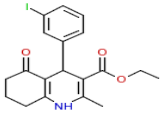
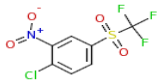
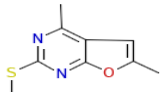
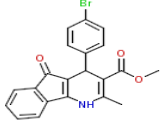
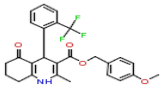
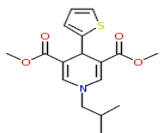
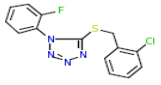
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0020901-1		5475562	5.5	DHP-MPC	6.22	31362	
VU0014813-1		5336992	5.5		5.83	41321	
VU0064813-1		4789-4114	5.5		5.70	26146	
VU0058592-1		3389-0934	5.4	DHP	6.52	25163	
VU0046705-1		7626982	5.4		6.88	30194	
VU0051917-1		0075-0087	5.4		6.33	22706	
VU0065007-1		4886-7641	5.4	DHP	7.31	25065	
VU0009006-1		5229152	5.4		6.33	38541	
VU0067612-1	Not available	5750-3219	5.4		6.84	13386	
VU0020994-1		5476838	5.4	DHP-MPC	6.50	33310	
VU0035419-1		6139451	5.3		6.86	26164	

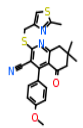
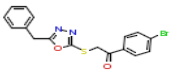
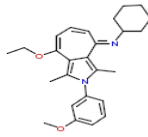
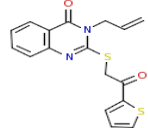
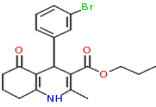
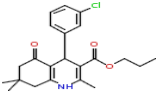
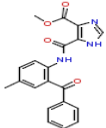
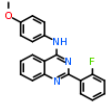
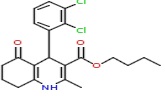
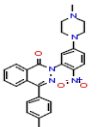
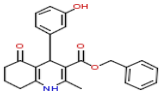
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0020386-1		5468671	5.3	DHP-MPC	6.46	26215	
VU0020490-1		5469930	5.3	DHP-MPC	6.11	23349	
VU0064817-1		4799-0441	5.3	DHP-MPC	5.29	20502	
VU0020495-1		5470016	5.3	DHP-MPC	6.48	26215	
VU0058491-1		3379-0901	5.3		7.47	31714	
VU0051907-1		0075-0061	5.2		7.74	36980	
VU0058610-1		3389-0449	5.2	DHP	7.63	20265	15
VU0007857-1		5210656	5.2		7.24	36444	
VU0042139-1		7182092	5.1	DHP-MPC	N/A		
VU0067788-1		5786-2840	5.1		6.56	22714	
VU0041833-1		7114797	5.0		N/A		

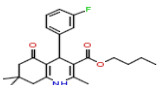
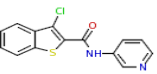
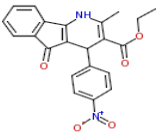
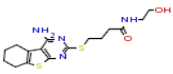
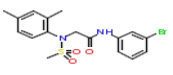
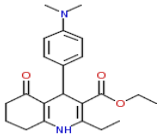
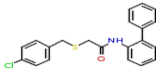
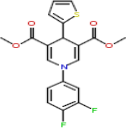
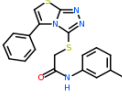
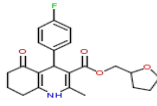
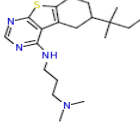
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0054138-1		1716-1812	5.0	DHP-MPC	7.91	33172	
VU0008620-1		5221584	5.0	scavenger	4.42	35384	
VU0020325-1		5468067	5.0	DHP-MPC	6.99	26294	
VU0014821-1		5337142	4.9	scavenger	3.61	38967	
VU0076317-1		C261-0140	4.9		6.29	20979	
VU0058572-1		3389-0931	4.9	DHP	6.99	24486	
VU0058582-1		3389-0933	4.9	DHP	7.71	19641	
VU0064532-1		4632-5664	4.9		7.38	27505	
VU0064827-1		4799-0463	4.9	DHP-MPC scavenger	5.05	16529	
VU0041890-1		7118586	4.8		N/A	34296	
VU0059269-1		3536-0017	4.8		5.61	38241	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0054031-1		1683-5278	4.8		7.64	30665	
VU0010015-1		5244967	4.8	DHP-MPC	7.64	38258	
VU0064499-1		4618-0109	4.8		8.00	39133	
VU0020884-1		5475397	4.8	DHP-MPC	6.51	32127	
VU0046748-1		7627643	4.7		6.41	25864	
VU0059270-1		3536-0196	4.7		7.40	31315	
VU0064833-1		4789-4126	4.7		6.27	29282	
VU0076343-1		C260-2670	4.6		7.77	26623	
VU0015122-1		5343696	4.6		6.57	33093	
VU0076126-1		C257-0153	4.6		7.67	35289	
VU0028417-1		5680134	4.6		7.30	33988	

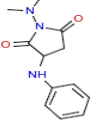
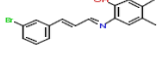
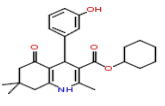
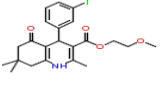
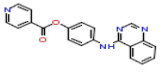
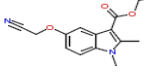
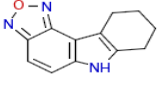
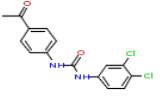
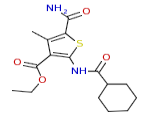
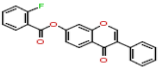
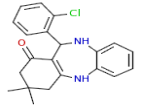
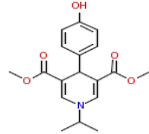
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0035265-1		6113618	4.5	scavenger	3.69	25363	
VU0021018-1		5477292	4.5	DHP-MPC	6.18	30983	
VU0059471-1		3591-0004	4.4		6.07	33996	
VU0064711-1		4767-0884	4.4	DHP-MPC scavenger	4.13	22977	
VU0014812-1		5336987	4.4	scavenger	4.49	31889	
VU0042818-1		7362684	4.4	DHP	8.66	32919	
VU0076365-1		C261-0025	4.3		7.35	23303	
VU0008617-1		5221571	4.3		5.89	37366	
VU0064690-1		4711-0021	4.3		10.87	32337	
VU0079236-1		K243-0277	4.3		6.98	29942	
VU0053928-1		1661-1366	4.3		7.45	28290	

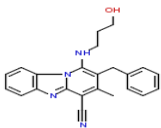
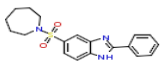
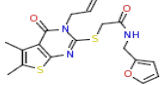
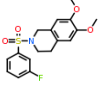
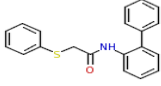
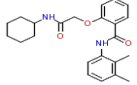
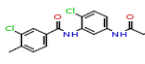
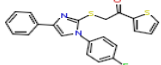
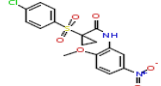
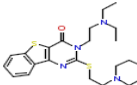
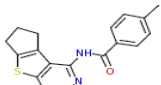
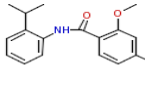
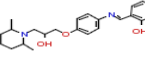
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0042577-1		7317241	4.2		4.92	29784	
VU0020992-1		5476779	4.2	DHP-MPC	5.86	29683	
VU0053908-1		1661-1316	4.1		10.34	31701	
VU0046068-1		7608357	4.1		6.30	33427	
VU0020878-1		5475328	4.1	DHP-MPC	6.70	25956	
VU0052065-1		0195-0039	4.0		7.73	32409	
VU0009298-1		5234658	4.0		6.97	35691	
VU0064701-1		4767-0881	4.0	DHP-MPC	6.29	21189	
VU0059447-1		3607-5700	4.0	DHP-MPC	7.82	27122	
VU0046905-1		7630823	4.0	DHP	7.65	34440	
VU0059484-1		3601-0375	3.9		7.75	34304	

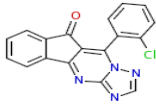
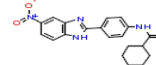
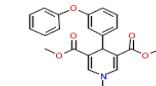
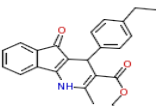
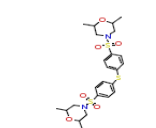
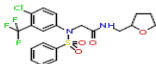
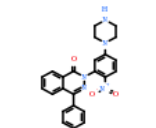
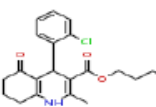
VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0067833-1		5867-1991	3.9		7.71	23644	
VU0042392-1		7254102	3.9		N/A	43316	
VU0064954-1		4861-0113	3.9		N/A	20359	
VU0042074-1		7163110	3.9		N/A		
VU0020456-1		5469512	3.8	DHP-MPC	5.65	28680	
VU0020306-1		5467855	3.8	DHP-MPC	5.86	23571	
VU0052179-1		0242-0741	3.8		8.22	39731	
VU0067802-1		5813-2310	3.8		7.85	26857	
VU0020183-1		5466310	3.8	DHP-MPC	6.02	24835	
VU0045675-1		7594306	3.8		7.15	35757	
VU0020973-1		5476344	3.8	DHP-MPC	6.59	31430	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0020230-1		5466975	3.8	DHP-MPC	6.42	22813	
VU0010062-1		5245463	3.8		6.93	37008	
VU0058658-1		3389-2494	3.7	DHP-MPC	8.73	17107	
VU0058304-1		3347-3506	3.7		7.54	41405	
VU0035923-1		6217152	3.7		6.73	31306	
VU0020682-1		5472460	3.7	DHP-MPC	N/A	35730	
VU0046331-1		7615345	3.7		6.64	31305	
VU0046854-1		7629977	3.7	DHP	7.50	26750	
VU0067896-1		5867-4068	3.7		7.30	38501	
VU0059281-1		3542-5611	3.7	DHP-MPC	7.33	35053	
VU0065010-1		4852-0396	3.6		8.20	32363	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0008763-1		5225941	3.6		5.92	39286	
VU0046063-1		7608209	3.6		6.14	40986	
VU0046642-1		7625879	3.6		6.65	31653	
VU0042278-1		7227672	3.6	DHP	6.85	28157	
VU0079351-1		K263-0587	3.6		8.89	32558	
VU0076367-1		C261-0191	3.6		5.75	26417	
VU0045672-1		7594153	3.6		6.46	34371	
VU0064914-1		4817-2931	3.6		7.79	33400	
VU0042604-1		7320193	3.5		7.21	31582	
VU0021017-1		5477268	3.5	DHP-MPC	6.02	31907	
VU0052191-1		0263-0386	3.5		8.18	33529	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0009275-1		5234485	3.5		7.09	34535	
VU0017877-1		5403371	3.5		5.28	30588	
VU0020260-1		5467399	3.5	DHP-MPC	6.03	32165	
VU0025481-1		5612811	3.4	DHP-MPC	7.43	31802	
VU0045625-1		7592297	3.4		6.00	36057	
VU0059385-1		3570-0574	3.4		7.22	38474	
VU0035268-1		6113948	3.4		6.96	34851	
VU0042547-1		7302436	3.3		6.90	37965	
VU0035311-1		6120096	3.3		6.28	27277	
VU0041945-1		7127045	3.3		6.29	35216	
VU0058633-1		3393-0111	3.3		8.08	34272	
VU0045666-1		7593765	3.2	DHP	6.33	39544	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0064659-1		4708-0097	3.2		9.21	31288	
VU0076326-1		C261-0077	3.2		7.11	32354	
VU0035220-1		6108147	3.2		6.80	34369	
VU0067882-1		5867-0961	3.2		8.21	33564	
VU0046601-1		7625042	3.2		7.07	38789	
VU0046744-1		7627582	3.2		6.88	37904	
VU0046961-1		7632067	3.2		6.78	35355	
VU0079241-1		K242-0048	3.2		6.07	27749	
VU0076114-1		C256-1538	3.2		7.89	26601	
VU0079389-1		K263-0286	3.2		7.83	30334	
VU0041603-1		7022242	3.2		6.67	34895	
VU0035193-1		6105404	3.2		6.51	32780	
VU0052192-1		0266-0168	3.1		5.94	40669	

VU Identifier	Structure	ChemDiv/ChemBridge Source ID	Initial Screen SDs > mean	Class	AmplexRed Signal	Calcium Signal	Table 2 Compound
VU0042384-1		7251883	3.1		6.46	33002	
VU0059399-1		3560-1942	3.1		7.60	26121	
VU0042775-1		7358535	3.1	DHP	7.42	31114	
VU0064919-1		4802-0254	3.1	DHP-MPC	7.01	29789	
VU0059359-1		3558-0117	3.1		7.63	33314	
VU0059304-1		3553-0392	3.0		7.32	35658	
VU0067803-1		5820-0741	3.0		9.43	22511	
VU0020972-1		5476338	3.0	DHP-MPC	7.23	27900	