Supplementary Materials

Structural modeling of TRPA1 ion channel – determination of the binding site for antagonists

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Table S1: SiteMap TRPA1 binding site prediction results

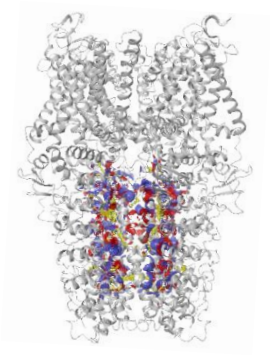
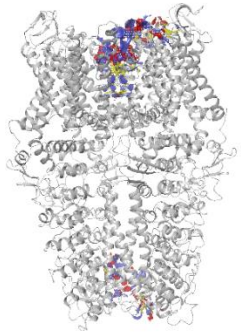
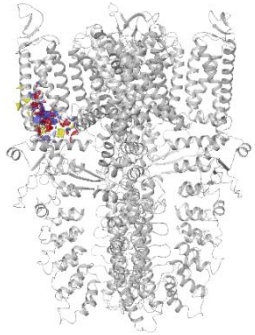
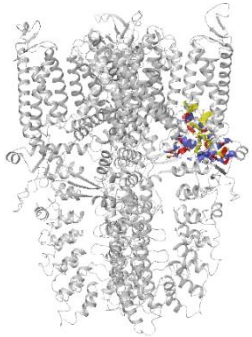
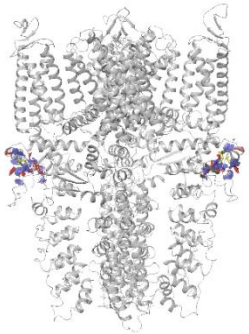
		Ankyrin repeats 	channel gap 	Transmembrane region 	pre-S1 / S4 / S5 / TRP-like domain 	below TRP-like domain area 
3J9P-based model	number of sites	6	3	1	5	5
	DScore - site	0.955 - s1 1.013 - s4 1.026 - s7 1.184 - s15 1.000 - s17 0.994 - s18	1.017 - s3 0.985 - s10 1.189 - s19	1.213 - s20	1.030 - s2 1.010 - s5 1.024 - s6 0.980 - s13 1.054 - s14	1.052 - s8 1.064 - s9 1.065 - s11 1.068 - s12 0.977 - s16
	average score	1.029	1.064	1.213	1.019	1.045
6PQQ-based model	number of sites	4	4	1	4	6
	DScore - site	1.023 - s1 1.016 - s2 1.015 - s3 0.991 - s5	1.075 - s4 1.029 - s13 0.961 - s15 0.976 - s17	1.041 - s19	1.089 - s6 1.118 - s7 1.172 - s8 1.172 - s9	0.989 - s12 0.958 - s14 0.932 - s16 0.929 - s18 0.939 - s20 1.076 - s12
	average score	1.011	1.010	1.041	1.137	0.971

Table S2: Deepsite TRPA1 binding site prediction results

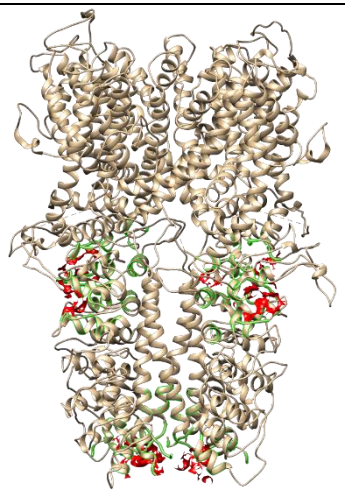
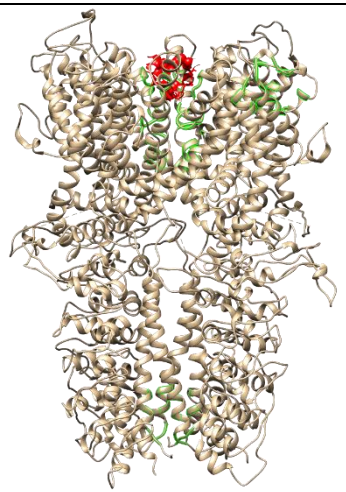
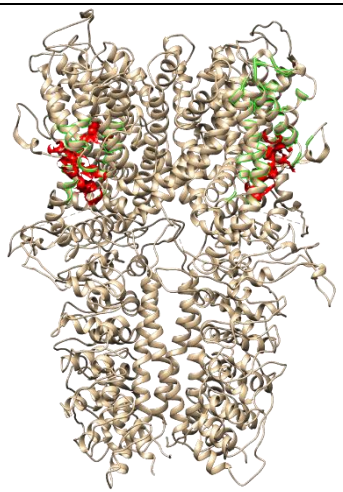
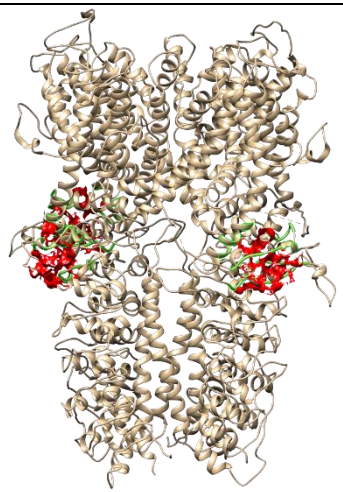
		Binding site 1		Binding site 2		Binding site 3	
3J9P-based model	score	0.9988941550254822		0.996812641620636		0.9990683197975159	
	site	linker domain		transmembrane domain (S1 / S2 / S3)		ankyrin repeats	
		Tyr-655	Asn-619	Asn-724	Tyr-812	Tyr-629	Leu-1051
		Ile-656	Cys-608	Tyr-726	Ser-780	Lys-593	Arg-1050
		Glu-657	Leu-609	Tyr-849	Ser-781	Ile-627	Tyr-1049
		Tyr-658	Lys-610	Gly-729	Ile-782	Gln-594	Lys-1048
		Asn-659	Ile-611	Tyr-842	Phe-783	Arg-592	Glu-1047
		Phe-660	Lys-620	Asn-845	Gly-784	Lys-591	Lys-1044
		Leu-686	Cys-621	Glu-808	Tyr-785	Asn-999	Leu-1045
		Tyr-680	Gly-618	Tyr-809		Asp-1053	Met-1042
		Ala-685	Glu-607			Lys-1052	
6PQQ-based model	score	0.9997281432151794		0.9987033605575562			
	site	pre-S1 / S4 / S5 / TRP-like domain		ankyrin repeats			
		Arg-852	Gln-1028	Lys-591	Lys-1052		
		Met-720	Asn-855	Asn-590	Leu-1051		
		His-719	Gln-854	Arg-592	Tyr-1049		
		Phe-716	Cys-856	Arg-557	Lys-1048		
		Trp-711	Phe-853	Gln-558	Lys-1046		
		Phe-1020	Gln-979	Leu-1054	Leu-1045		
		Leu-1023	Phe-1024	Asp-1053			

Table S3: Metapocket 2.0 TRPA1 binding site prediction results

		Binding site 1			Binding site 2			Binding site 3		
3J9P-based model	score	4.39			3.76			3.70		
	Site	pre-S1 / S4 / S5 / TRP-like domain			Ankyrin repeats			pre-S1 / S4 / S5 / TRP-like domain		
		Ile-858B	Ser-873C	Glu-705B	Ile-611C	Asp-606C	Leu-683C	Glu-705C	Gln-1031C	Asn-855C
		Met-862B	Asn-855B	Arg-975B	Gly-618C	Leu-584C	Leu-588C	Glu-1032C	Pro-1034C	Arg-872D
		Val-967B	Cys-856B	Glu-854B	Lys-620C	Ile-633C	Leu-552C	Ile-1033C	Ile-695C	Ile-858C
		Gln-968B	Val-875C	Ser-972B	Tyr-658C	Thr-634C	Ala-556C	Asn-699C	Lys-969C	Gln-768C
		Ala-971B	Ile-860B	Asp-1037B	Leu-609C	Leu-640C	Val-564C	Pro-701C	His-970C	Cys-856C
		Thr-869C	Lys-868C	Ile-695B	Asn-619C	Pro-631C	Phe-583C	His-700C	Ala-971C	Val-865D
		Leu-959C	Pro-1034B	Asn-699B	Cys-608C	Met-634C	Val-595C	Val-1005C	Asp-1037C	Asp-966D
		Ile-964B	Gln-1031B	His-970B	Glu-607C	Ile-600C	Leu-568C	Arg -652C	Ser-972C	Val-967D
		Phe-859B	Glu-1032B	Leu-973B	Tyr-654C	Ile-599C	Thr-598C	Tyr-706C	Leu-973C	Lys-974D
		Leu-955C	Gly-958C	Lys-969B	Ile-656C	Lys-635C	Cys-621C	Val-702C	Arg-975C	Asp-963D
		Val-865C	Leu-871C	Met-709B	Glu-657C	Ala-685C	Ala-688C	Met-709C	Glu-854C	
		Arg-872C	Ile-1033B	Tyr-706B	Asn-659C	Lys-704C	Leu-597C			
		Asp-963C			Lys-603C	Met-689C	Cys-633C			
					Arg-604C	Leu-686C	Arg-601C			
					Trp-605C	Leu-707C	Leu-637C			
						Leu-708C	Val-596C			

		Binding site 1			Binding site 2			Binding site 3		
6PQQ-based model	score	8.34			5.21			4.52		
	Site	Channel gap			pre-S1 / S4 / S5 / TRP-like domain			pre-S1 / S4 / S5 / TRP-like domain		
		Val-942A	Pro-949D	Gly-914D	Val-875D	Arg-852B	Thr-874D	Arg-1011C	Phe-1024A	His-719A
		Leu-881C	Asn-954D	Ile-916B	Phe-853B	Gln-979B	Cys-1025B	Cys-1021A	Leu-708A	Phe-716A
		Ile-905C	Ile-957D	Asn-917B	Leu-1023B	Leu-707B	Phe-1020B	Pro-1010A	Leu-712A	Met-720A
		Phe-909C	Leu-913B	Tyr-918B	Thr-1026B	His-983B	Cys-1021B	Phe-1017A	Glu-854A	Leu-723A
		Ile-906C	Pro-949B	Asn-917C	Leu-850B	Leu-708B	Phe-1017B	His-1018A	Arg-852A	Gly-715A
		Ile-878C	Met-953B	Tyr-918C	Phe-879D	Arg-975B	Arg-1011B	Val-1005A	Phe-853A	Leu-982A
		Leu-941A	Asn-954B	Asn-917A	Phe-846B	Glu-1028B	Leu-712B	Tyr-1006A	Gln-979A	Arg-975A
		Ile-916A	Ile-957B	Ser-910D	Leu-723B	Ile-1029B	Leu-698B	Pro-1007A	Asn-855A	Met-978A
		Ser-910C	Leu-902C	Ser-910A	Cys-856B	Gly-1027B	Cys-703B	Phe-1022A	Glu-1028A	Ile-858A
		Leu-913C	Val-935A	Arg-919C	Phe-716B	Lys-704B	Asn-699B	Cys-1025A	Cys-856A	Lys-974A
		Gly-914A	Tyr-926A	Glu-920C	Met-720B	Glu-1032B	Ile-1019B	Phe-800B	Leu-707A	Gln-851A
		Asp-915C	Pro-934A	Asn-917D	Asn-855B	Ile-976B	His-1018B	Met-801B	His-983A	Glu-981A
		Gly-914C	Leu-903C	Glu-920D	Phe-1024B	Glu-705B	Phe-1022B	Thr-1026A	Lys-704A	Ser-985A
		Phe-938A	Ile-946A	Ile-950C	Glu-854B	Leu-871D	Pro-1010B	Trp-711A	Glu-705A	Leu-848A
		Tyr-918A	Leu-867C	Ser-910B	Trp-711B	Arg-872D		Phe-1020A	Ile-976A	Tyr-849A
		Ala-939A	Leu-870C	Ile-916D						
		Leu-913A	Leu-871C	Tyr-918D						
		Asp-915A	Thr-874C	Phe-909B						
		Pro-949A	Ile-950A	Ile-950D						
		Met-953C	Phe-877C	Glu-924C						
		Pro-949C	Val-951A	Phe-909D						
		Met-953A	Met-912C	Thr-945B						
		Asn-954C	Leu-956C	Phe-909A						
		Ile-957C	Asp-915D	Leu-941B						
		Asn-954A	Ile-916C	Ile-906A						
		Ile-957A	Gly-914B	Val-942B						
		Leu-913D	Asp-915B	Ser-900C						
		Met-953D								

Table S4: Castp3.0 TRPA1 binding site prediction results

		Ankyrin repeats		Channel gap		Transmembrane region		TRP-like domain area	
3J9P-based model	number of sites	8		3		3		4	
									
	Sites' details	Area(SA)	Volume(SA)	Area(SA)	Volume(SA)	Area(SA)	Volume(SA)	Area(SA)	Volume(SA)
		376.322 379.370 362.644 345.352 234.976 234.714 234.632 143.230	119.040 118.614 112.002 108.928 96.300 96.083 96.028 63.240	661.154 225.302 177.676	521.175 79.644 56.523	234.741 225.302 165.347	96.208 79.644 77.629	262.030 252.168 241.639 177.457	192.827 169.087 131.245 123.297

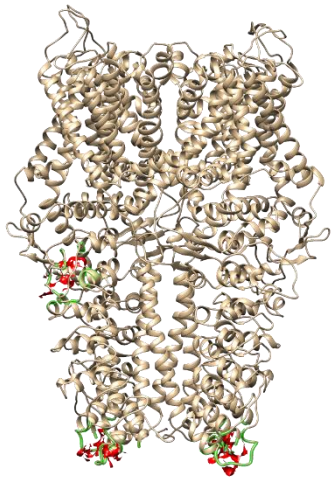
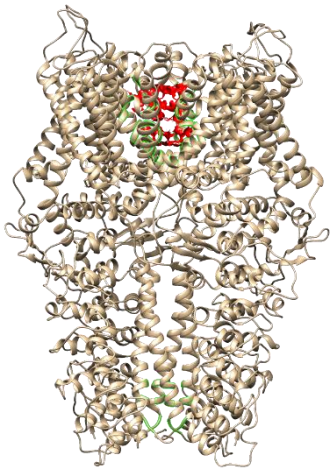
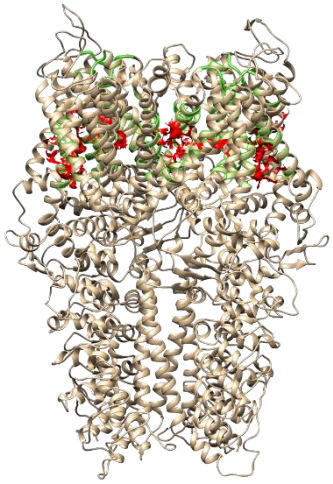
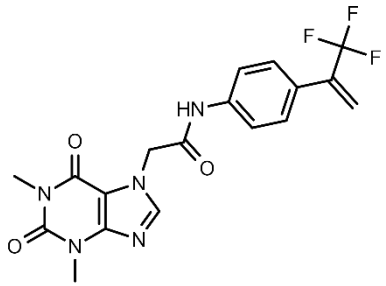
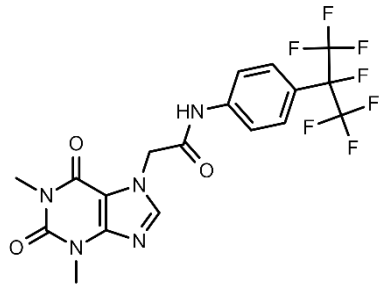
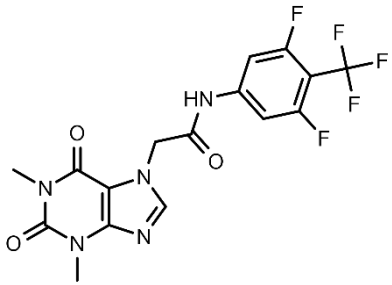
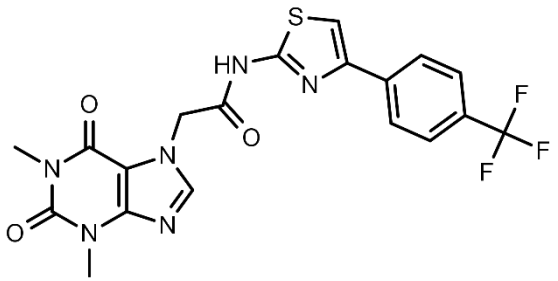
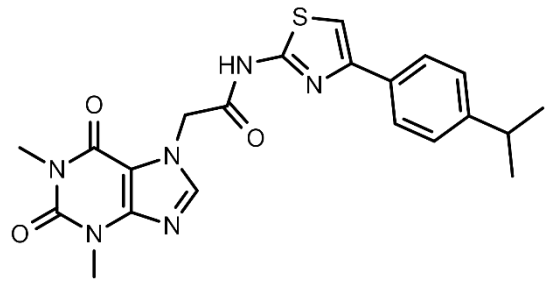
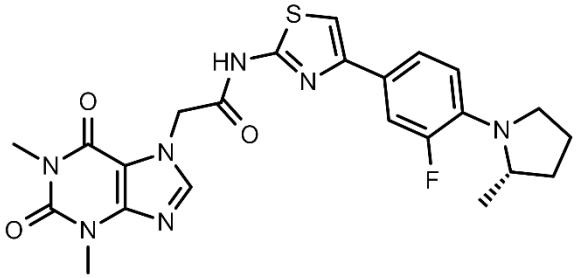
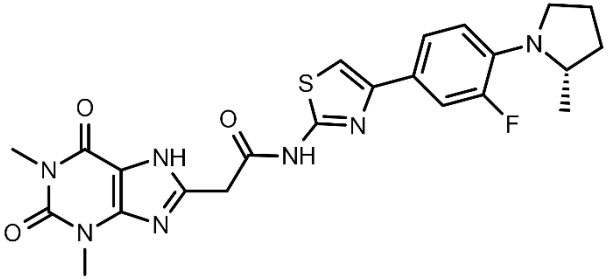
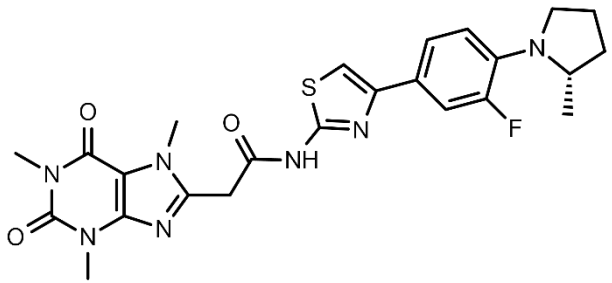
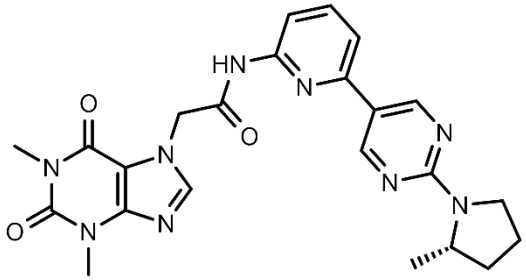
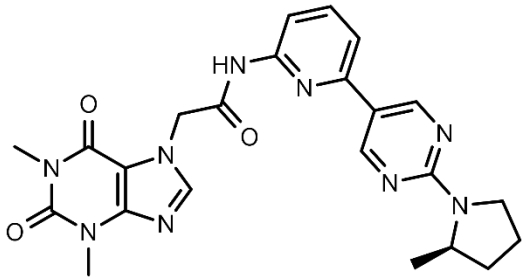
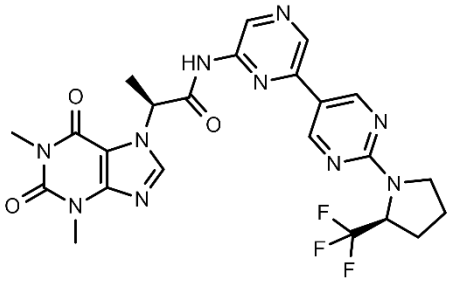
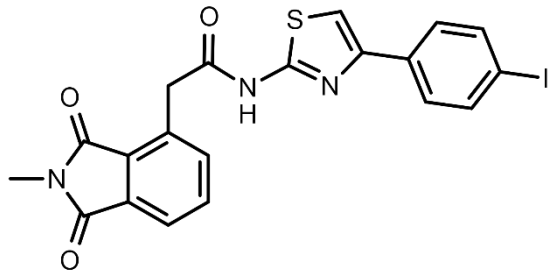
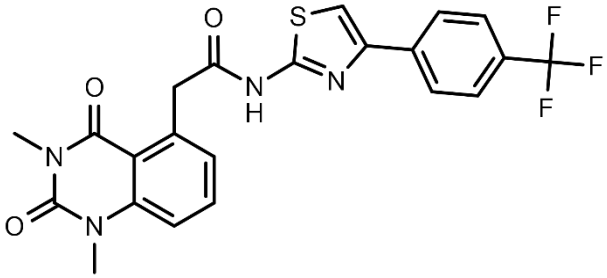
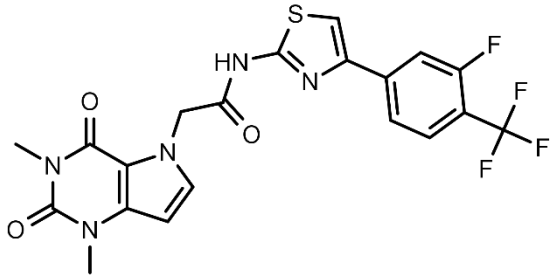
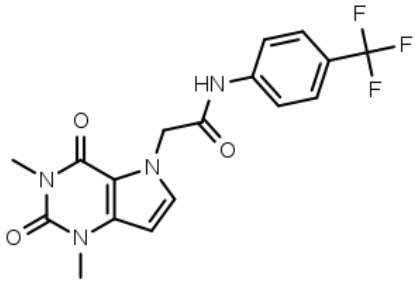
		Ankyrin repeats		Channel gap		Transmembrane region		TRP-like domain area
6PQQ-based model	number of sites	5		2		11		
								
	Sites' details	Area(SA)	Volume(SA)	Area(SA)	Volume(SA)	Area(SA)	Volume(SA)	
		171.229 123.463 79.200 74.575 118.121	87.070 61.535 64.834 52.827 50.780	602.144 155.912	556.536 81.334	157.806 155.348 150.966 139.597 78.232 102.544 110.502 97.413 53.778 90.840	89.004 79.688 74.437 64.020 54.091 36.835 36.183 33.812 31.333 31.313	

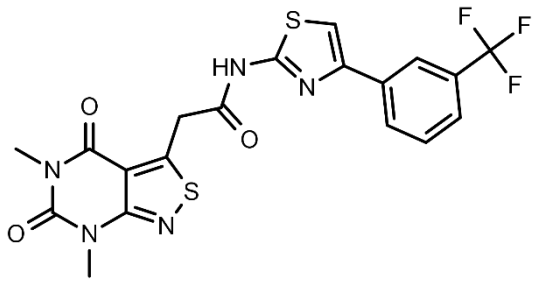
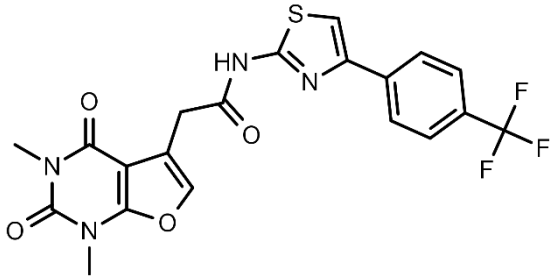
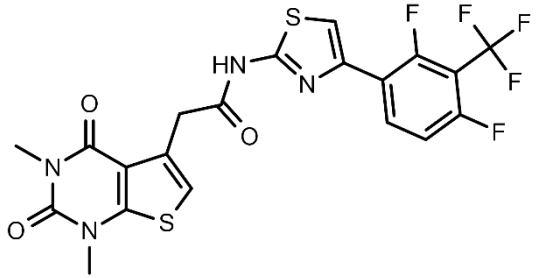
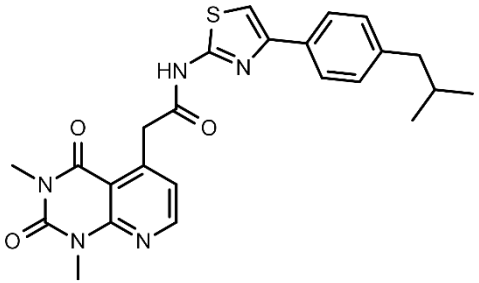
Table S5: List of active ligands used in retrospective virtual screening.

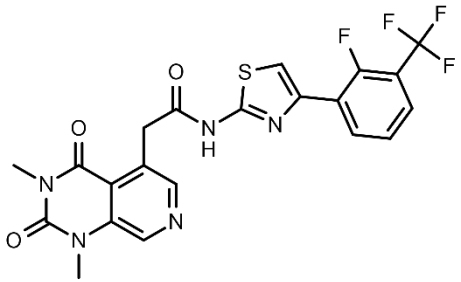
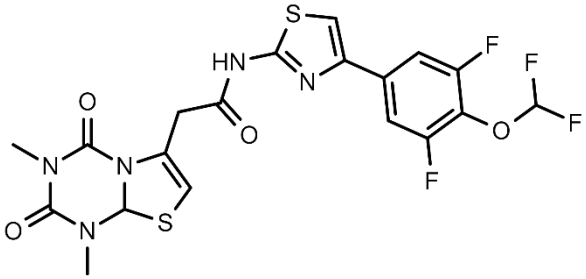
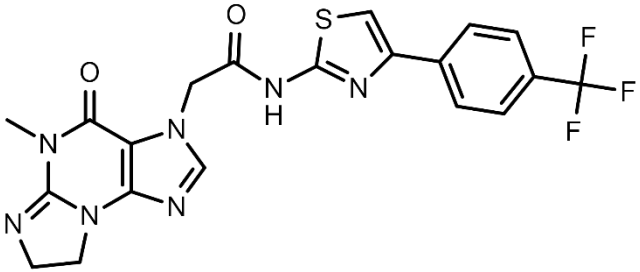
Ligand	Structure	SMILES	Patent	TRPA1 IC50
1		CN1C2=C(N(CC(=O)NC3=CC=C(C=C3)C(=C)C(F)(F)F)C=N2)C(=O)N(C)C1=O	WO2010138879	< 250 nM
2		CN1C2=C(N(CC(=O)NC3=CC=C(C=C3)C(F)(C(F)(F)F)C(F)(F)F)C=N2)C(=O)N(C)C1=O	WO2010138879	<250 nM
3		CN1C2=C(N(CC(=O)NC3=CC(F)=C(C(F)=C3)C(F)(F)F)C=N2)C(=O)N(C)C1=O	WO2010138879	250-499 nM

4		CN1C2=C(N(CC(=O)NC3=NC(=CS3)C3=CC=C(C=C3)C(F)(F)F)C=N2)C(=O)N(C)C1=O	WO2009140519	<1 μM
5		CC(C)C1=CC=C(C=C1)C1=CSC(NC(=O)CN2C=NC3=C2C(=O)N(C)C(=O)N3C)=N1	WO2009002933	<1 μM
6		C[C@@H]1CCCN1C1=CC=C(C=C1F)C1=CSC(NC(=O)CN2C=NC3=C2C(=O)N(C)C(=O)N3C)=N1	WO2010075353	4 nM
7		C[C@@H]1CCCN1C1=CC=C(C=C1F)C1=CSC(NC(=O)CC2=NC3=C(N2)C(=O)N(C)C(=O)N3C)=N1	WO2010132838	310 nM

8		C[C@@H]1CCCN1C1=CC=C(C=C1F)C1=CSC(NC(=O)CC2=NC3=C(N2C)C(=O)N(C)C(=O)N3C)=N1	WO2010132838	81 nM
9		C[C@@H]1CCCN1C1=NC=C(C=N1)C1=CC=CC(NC(=O)CN2C=NC3=C2C(=O)N(C)C(=O)N3C)=N1	WO2013023102	93 nM
10		C[C@H]1CCCN1C1=NC=C(C=N1)C1=CC=CC(NC(=O)CN2C=NC3=C2C(=O)N(C)C(=O)N3C)=N1	WO2013023102	77 nM
11		C[C@H](N1C=NC2=C1C(=O)N(C)C(=O)N2C)C(=O)NC1=NC(=CN=C1)C1=CN=C(N=C1)N1CCC[C@H]1C(F)(F)F	WO2014113671	< 25 nM

12		CN1C(=O)C2=CC=CC(CC(=O)NC3=NC(=CS3)C3=CC=C(I)C=C3)=C2C1=O	WO2009118596	< 250 nM
13		CN1C(=O)N(C)C2=CC=CC(CC(=O)NC3=NC(=CS3)C3=CC=C(C=C3)C(F)(F)F)=C2C1=O	US20090325987	< 50 nM
14		CN1C2=C(N(CC(=O)NC3=NC(=CS3)C3=CC=C(C(F)=C3)C(F)(F)F)C=C2)C(=O)N(C)C1=O	WO2010109287	< 50 nM
15		CN1C2=C(N(CC(=O)NC3=CC=C(C=C3)C(F)(F)F)C=C2)C(=O)N(C)C1=O	-	400 nM

16		CN1C2=NSC(CC(=O)NC3=NC(=CS3)C3=CC=CC(=C3)C(F)(F)F)=C2C(=O)N(C)C1=O	WO2010109328	< 50 nM
17		CN1C2=C(C(CC(=O)NC3=NC(=CS3)C3=CC=C(C=C3)C(F)(F)F)=CO2)C(=O)N(C)C1=O	WO2010109287	< 50 nM
18		CN1C2=C(C(CC(=O)NC3=NC(=CS3)C3=CC=C(F)C(=C3F)C(F)(F)F)=CS2)C(=O)N(C)C1=O	WO2010109334	< 50 nM
19		CC(C)CC1=CC=C(C=C1)C1=CSC(NC(=O)CC2=C3C(=O)N(C)C(=O)N(C)C3=NC=C2)=N1	WO2010125469	< 50 nM

20		CN1CN(C)C2=CN=CC(CC(=O)NC3=NC(=CS3)C3=CC=CC(=C3F)C(F)(F)F)=C2C1=O	WO2011132017	< 50 nM
21		CN1C2SC=C(CC(=O)NC3=NC(=CS3)C3=CC(F)=C(OC(F)F)C(F)=C3)N2C(=O)N(C)C1=O	WO2011114184	< 50 nM
22		CN1C2=NCCN2C2=C(N(CC(=O)NC3=NC(=CS3)C3=CC=C(C=C3)C(F)(F)F)C=N2)C1=O	WO2009144548	< 500 nM