Donated Chemical Probe

TRPA1 Inhibitor Probe BAY-390

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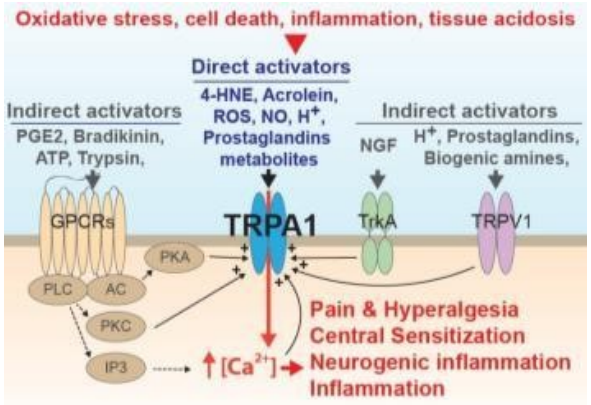
September, 2020

TRPA1 Mediates Pain, Neurogenic Inflammation and Inflammation

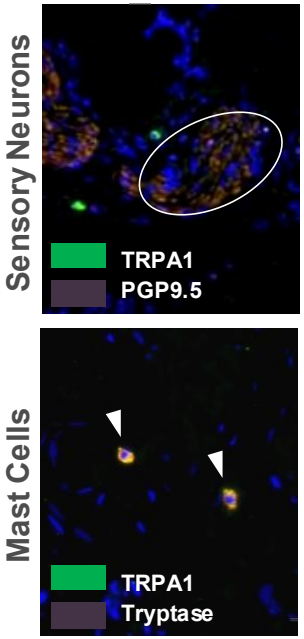
Background & Rationale

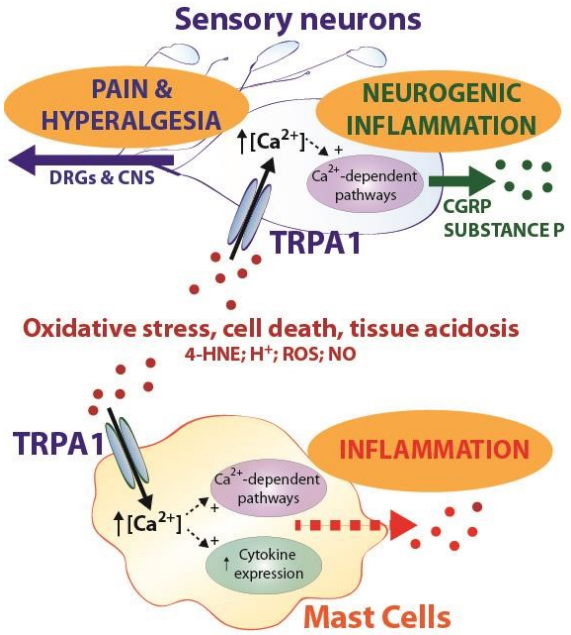
TRPA1, a damage sensor activated by a wide range of endogenous mediators



TRPA1 activation induces pain, central sensitization, neurogenic inflammation and mast cell-driven inflammation

TRPA1 in human endometriotic lesions

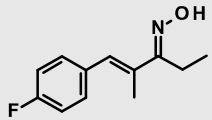




SECRET

Commercially available TRPA1 antagonists

A-079 donated as chemical probe

Origin	Code	Structure	hTRPA1	r | m TRPA1	References
Abbvie	A-079		67 nM (FLIPR Ca2+ assay)	289 | 298 nM (FLIPR Ca2+ assay)	PMID: 22319196 PMID: 21402443

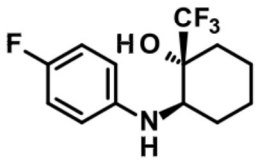
Reference of TRPA1 probe:

https://www.sgc-ffm.uni-frankfurt.de/chemProbes#!specificprobeoverview/A-079

https://www.sgc-ffm.uni-frankfurt.de/APP/connector/0/146/url/A-079+Antagonist+for+TRPA1.pdf

BAY-390 is a potent and selective TRPA1 inhibitor

Technical Profile



Absolute Stereochemistry: ***R,R*** by VCD measurement

▪ Molecular Properties

MW_{corr} [g/mol]	277
TPSA [A^2]	32
logD 7.5 (calc/exp)	3.3 / 3.3

▪ PhysChem

Sol_{Flask} pH 6.5, 4, 2 [mg/L]	n.d. (oil)
Plasmastab (4h) hum/rat [%]	n.d.
Stability (24h) pH1/7/10 [%]	95/100/100

▪ Pharmacology

hTRPA1 FLIPR IC_{50} [nM]	16
hTRPA1 Ephys IC_{50} [nM]	82
rTRPA1 FLIPR IC_{50} [nM]	63
rDRG EPhys IC_{50} [nM]	35
m | gp | dog | monkey TRPA1 IC_{50} [nM]	73 | 68 | 81 | 19

▪ Selectivity/ Safety

TRP selectivity IC_{50} [µM] hTRPV1 | V4 | TRPC3 | C5 | C6	> 25 | > 25 | >25 | 5.6 | > 25
hCa$_v$3.2 | TASK-3 IC_{50} [µM]	> 25 | > 30
hERG IC_{50} [µM]	> 10
Panlabs Lead Profiling [µM] | Cardiac Profiler [%] inhibition| transactivation Erα @10 uM	Ki hPR-B: 4.0; Ki hDAT : 0.9| no findings | EC_{50} ERα: 2.1
Ames MPF	negative

Cardiac profiler: hNav1.5; hCav1.2; hKir2.1; hKvLQT1; hKv4.3

▪ Pharmacokinetics

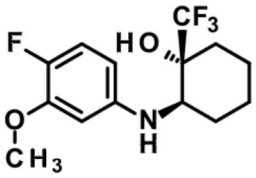
	CL_{bl} [L/h/kg]		Fmax [%]	
in vitro PK (hHep/rHep)	0.38	2.4	71	42
	Vss [L/kg]	CL_{bl} [L/h/kg]	t1/2 [h] iv	F [%]
in vivo PK	2.1	1.4	2.0	38
	Hum [%]	Rat [%]	Mouse [%]	Williams E [%]
Protein binding fu	11.5	6.0	8.1	57

	A-B [nm/s]		B-A [nm/s]		Efflux	
CaCo	293				0.63	
	1A2	2C8	2C9	2D6	3A4	TDI
CYP Inhibition [µM]	>20	>20	>20	>20	>20	no
	PXR		NOEL 1A2 [µg/L]		NOEL 3A4 [µg/L]	
CYP Induction	YELLOW		10000		30000	

m: mouse; gp: guinea pig; DRG: dorsal root ganglion

Negative Probe BAY-9897

Technical Profile



Molecular Properties

MW_{corr} [g/mol]	307
TPSA [A²]	41
logD 7.5 (calc/exp)	3.2 / 2.9

PhysChem

Sol_{Flask} pH 6.5, 4, 2 [mg/L]	n.d. (oil)
Plasmastab (4h) hum/rat [%]	n.d.
Stability (24h) pH1/7/10 [%]	n.d.

Pharmacology

hTRPA1 FLIPR (WUP) IC_{50} [nM]	> 25000
hTRPA1 Ephys (Evo) IC_{50} [nM]	n.d.
rTRPA1 FLIPR (WUP) IC_{50} [nM]	> 25000
rDRG EPhys (Evo) IC_{50} [nM]	n,.d.

Selectivity/ Safety

TRP selectivity IC_{50} [µM] hTRPV1 | V4 | TRPC5 | C6	>25 | >25 | 10 | >30
$hCa_v3.2$ | TASK-3 IC_{50} [µM]	n.d.
hERG IC_{50} [µM]	> 10
Panlabs Lead Profiling [µM] | Cardiac Profiler [%] inhibition	n.d. | all > 10
Ames MPF	n.d.

Cardiac profiler: hNav1.5; hCav1.2; hKir2.1; hKvLQT1; hKv4.3

Pharmacokinetics

11.	CL_{bl} [L/h/kg]		Fmax [%]	
in vitro PK (hHep/rHep)	0.85	3.5	36	17
	Vss [L/kg]	CL_{bl} [L/h/kg]	t1/2 [h] iv	F [%]
in vivo PK	n.d.	n.d.	n.d.	n.d.
	Hum [%]	Rat [%]	Mouse [%]	Williams E [%]
Protein binding fu	n.d.	n.d.	n.d.	n.d.

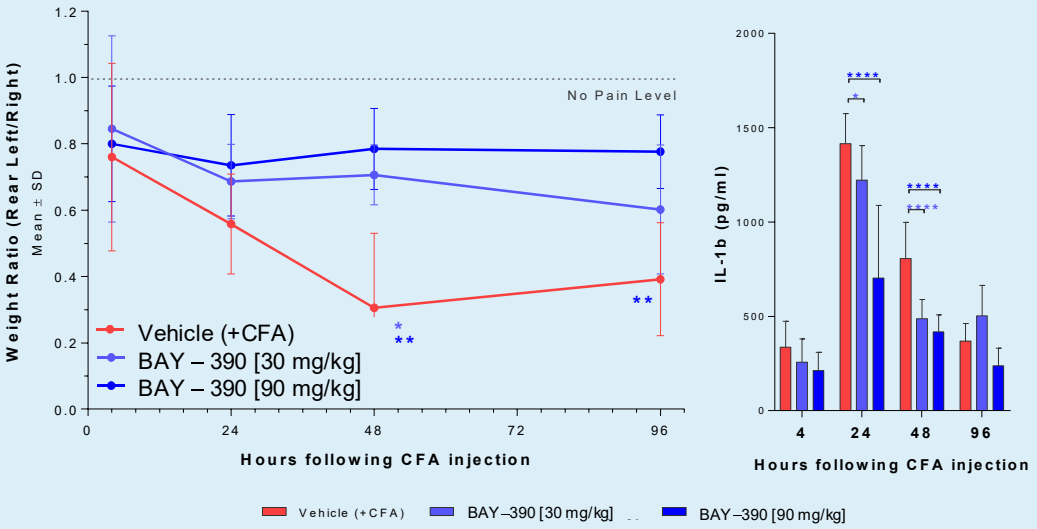
	A-B [nm/s]	B-A [nm/s]	Efflux
CaCo	375		0.55

	1A2	2C8	2C9	2D6	3A4	TDI
CYP Inhibition [µM]	> 20	> 20	> 20	> 20	> 20	

	PXR	NOEL 1A2 [µg/L]	NOEL 3A4 [µg/L]
CYP Induction	RED	30000	2610

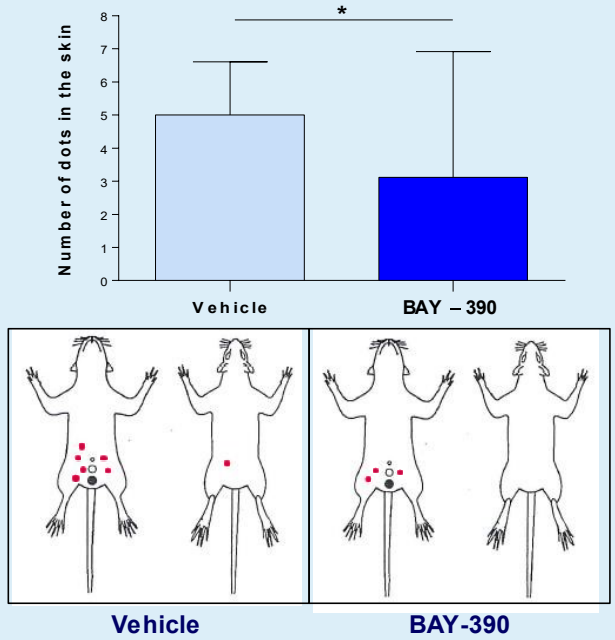
BAY-390 shows efficacy in inflammatory pain and neurogenic inflammation models

Reduction of mechanical hyperalgesia and inflammation in mouse inflammatory pain models



Inflammatory Pain and Peripheral Inflammation

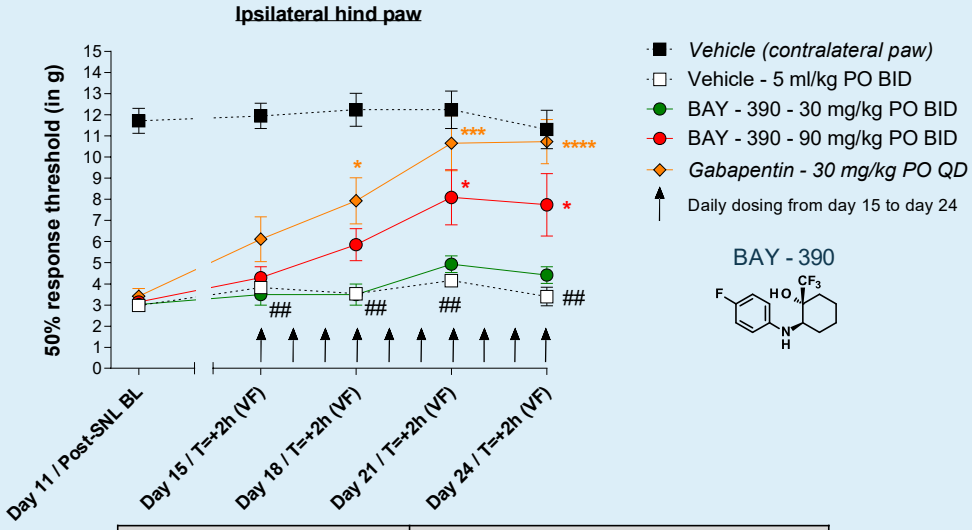
Reduction of neurogenic inflammation (plasma extravasation) in a rat neurogenic inflammation model



Neurogenic Inflammation

BAY-390 shows efficacy in neuropathic pain and interstitial cystitis models

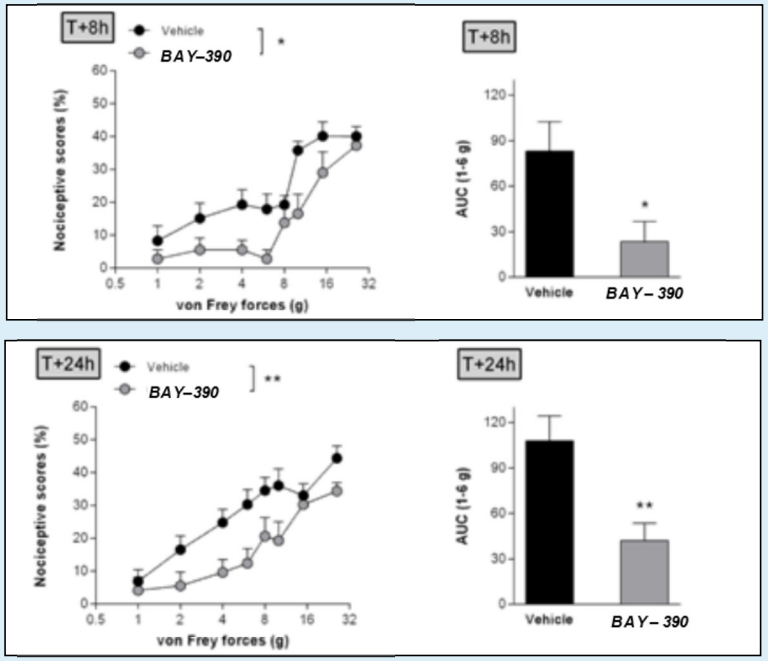
Reduction of mechanical hyperalgesia in rodent neuropathic pain model



Exposures	Chronic therapeutic treatment (BID for 10 days, 13x dosing)	
Compound ID	BAY-390	
(mg/kg), p.o.	30	90
Plasma C_{ub} [nM] @ 2.5h	115	201
Estimated Brain Cub [nM] @ 2.5h	33	61
rIC50 / kpu,u	63 nM / 0.3	

Neuropathic Pain

Reduction of visceral pain in a rat cyclophosphamide induced cystitis model



IC/BPS

BAY-390

Is a potent, across species active and brain penetrating TRPA1 antagonist

Probe criteria	
Inhibitor/agonist potency: goal is < 100 nM (IC_{50}, Kd)	Potency as inhibitor of TRPA1 ion channel demonstrated in FLIPR Ca^{2+} assay (CHO or HEK): human TRPA1 IC_{50} = 16 nM, dog IC_{50} = 19 nM, rat IC_{50} = 63 nM; Equipotent on rat, mouse, guinea pig and monkey TRPA1
Selectivity within target family: goal is >30-fold	Surpasses criteria; Selectivity against family members was tested, all >30fold
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	Surpasses criteria; Selectivity in Eurofins Lead Profiling Screen, GPCR Profiling Screen and Bayer Kinase Panel was performed (only relevant Ki: hDAT 0.9 µM; hPR-B: 4 µM || Erα EC_{50}: 2.1 µM)
On target cell activity for cell-based targets: goal is < 1 micromolar IC_{50}/EC_{50}	Surpasses criteria; Ephys human TRPA1 IC_{50} at 82 nM (Patchliner, CHO) Efficacy in several in vitro and in vivo mechanistic models demonstrated; efficacy in PD pain inflammation model
On target cell activity for secreted targets: appropriate alternative such as mouse model or other mechanistic biological assay, e.g., explant culture	Surpasses criteria; Suitable pharmacokinetic profile for in vivo studies in rodents
Neg ctrl: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	Surpasses criteria; Structure related compound BAY-9897 with high micromolar TRPA1 activity (human TRPA1 IC_{50} >25 µM (CHO, Ca^{2+})

We ask for acceptance of TRPA1 inhibitor BAY-390 as chemical probe, accompanied by BAY-9897 as negative control

Acknowledgement

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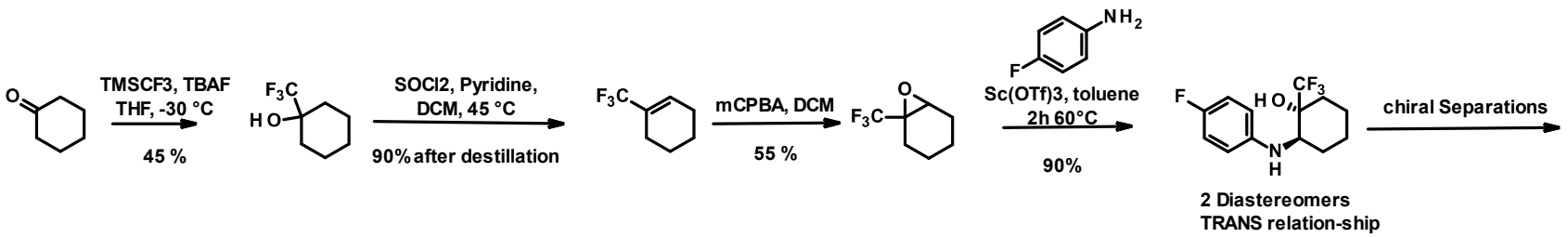
Anne-Marie Coelho
Stuart Flanagan
Stephen Hess
Schanila Nawaz
Daryl Walter

* Now at Nuvisan

Thank you

Questions?

Synthesis


TMSCF3, TBAF
THF, -30 °C
45 %
SOCl2, Pyridine,
DCM, 45 °C
90% after destillation
mCPBA, DCM
55 %
Sc(OTf)3, toluene
2h 60°C
90%
chiral Separations
2 Diastereomers
TRANS relation-ship

CEREP results

BAY-390

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH039-2017, PT #: 1207556						
107000	Aldose Reductase	401131	rat	2	10 µM	3
107710	ATPase, Na^+/K^+, Heart, Pig	401160	pig	2	10 µM	3
112020	Carbonic Anhydrase II	401132	hum	2	10 µM	7
104010	Cholinesterase, Acetyl, ACES	401128	hum	2	10 µM	-2
116020	Cyclooxygenase COX-1	401157	hum	2	10 µM	8
118010	Cyclooxygenase COX-2	401158	hum	2	10 µM	23
124010	HMG-CoA Reductase	401134	hum	2	10 µM	8
132000	Leukotriene LTC_4 Synthase	401133	gp	2	10 µM	-15
199017	Lipoxygenase 15-LO	401198	hum	2	10 µM	23
140010	Monoamine Oxidase MAO-A	401130	hum	2	10 µM	3
140120	Monoamine Oxidase MAO-B	401159	hum	2	10 µM	7
142000	Nitric Oxide Synthase, Neuronal (nNOS)	401135	rat	2	10 µM	-3
199010	Nitric Oxide Synthetase, Inducible (iNOS)	401197	mouse	2	10 µM	5
107300	Peptidase, Angiotensin Converting Enzyme	401129	rabbit	2	10 µM	3
152000	Phosphodiesterase PDE3	401193	hum	2	10 µM	3
154000	Phosphodiesterase PDE4	401194	hum	2	10 µM	-11
156000	Phosphodiesterase PDE5	401393	hum	2	10 µM	7
194020	Thromboxane Synthase	401136	hum	2	10 µM	6
200510	Adenosine A_1	401253	hum	2	10 µM	18
200610	Adenosine A_{2A}	401253	hum	2	10 µM	3
200720	Adenosine A_3	401176	hum	2	10 µM	4
203100	Adrenergic α_{1A}	401240	rat	2	10 µM	18
203630	Adrenergic α_{2A}	401239	hum	2	10 µM	7
203710	Adrenergic α_{2B}	401142	hum	2	10 µM	2
203810	Adrenergic α_{2C}	401211	hum	2	10 µM	-1
204010	Adrenergic β_1	401230	hum	2	10 µM	3
204110	Adrenergic β_2	401239	hum	2	10 µM	11
204200	Adrenergic β_3	401359	hum	2	10 µM	1
206000	Androgen (Testosterone)	401322	hum	2	10 µM	47
210030	Angiotensin AT_1	401212	hum	2	10 µM	4
210120	Angiotensin AT_2	401213	hum	2	10 µM	1
212510	Bradykinin B_1	401177	hum	2	10 µM	9
212620	Bradykinin B_2	401170	hum	2	10 µM	4
217030	Cannabinoid CB_1	401143	hum	2	10 µM	-15

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
gp=Guinea pig; hum=Human

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
217100	Cannabinoid CB_2	401141	hum	2	10 µM	21
219500	Dopamine D_1	401243	hum	2	10 µM	-14
219600	Dopamine D_{2L}	401370	hum	2	10 µM	12
219700	Dopamine D_{2S}	401241	hum	2	10 µM	13
219800	Dopamine D_3	401243	hum	2	10 µM	9
224010	Endothelin ET_A	401229	hum	2	10 µM	7
224110	Endothelin ET_B	401366	hum	2	10 µM	-4
226010	Estrogen ERα	401171	hum	2	10 µM	75
226810	$GABA_A$, Chloride Channel, TBOB	401172	rat	2	10 µM	-8
226600	$GABA_A$, Flunitrazepam, Central	401246	rat	2	10 µM	-17
228510	$GABA_B$, Non-Selective	401185	rat	2	10 µM	3
232030	Glucocorticoid	401208	hum	2	10 µM	4
232600	Glutamate, AMPA	401173	rat	2	10 µM	8
232700	Glutamate, Kainate	401296	rat	2	10 µM	-3
232810	Glutamate, NMDA, Agonism	401224	rat	2	10 µM	5
232910	Glutamate, NMDA, Glycine	401224	rat	2	10 µM	9
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	401154	hum	2	10 µM	3
239610	Histamine H_1	401249	hum	2	10 µM	6
239710	Histamine H_2	401256	hum	2	10 µM	-8
239820	Histamine H_3	401166	hum	2	10 µM	-4
243000	Insulin	401420	rat	2	10 µM	8
252200	Motilin	401156	hum	2	10 µM	13
252610	Muscarinic M_1	401216	hum	2	10 µM	-1
252710	Muscarinic M_2	401231	hum	2	10 µM	-2
252810	Muscarinic M_3	401231	hum	2	10 µM	21
252910	Muscarinic M_4	401217	hum	2	10 µM	14
258590	Nicotinic Acetylcholine	401227	hum	2	10 µM	-7
260130	Opiate δ_1 (OP1, DOP)	401150	hum	2	10 µM	-6
260210	Opiate κ(OP2, KOP)	401150	hum	2	10 µM	7
260410	Opiate μ(OP3, MOP)	401232	hum	2	10 µM	5
299005	Progesterone PR-B	401321	hum	2	10 µM	65
268700	Purinergic P2X	401151	rabbit	2	10 µM	3
268810	Purinergic P2Y	401152	rat	2	10 µM	-6
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	401182	hum	2	10 µM	10
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	401181	hum	2	10 µM	-2

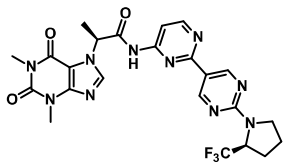
Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
* Batch: Represents compounds tested concurrently in the same assay(s).
gp=Guinea pig; hum=Human

Experimental Results

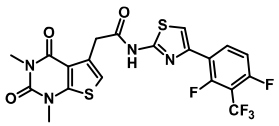
Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	401168	hum	2	10 µM	-4
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	401180	hum	2	10 µM	3
202000	Transporter, Adenosine	401137	gp	2	10 µM	-2
220320	Transporter, Dopamine (DAT)	401164	hum	2	10 µM	84
226400	Transporter, GABA	401186	rat	2	10 µM	-5
204410	Transporter, Norepinephrine (NET)	401164	hum	2	10 µM	16
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	401182	hum	2	10 µM	3
287530	Vasopressin V_{1A}	401219	hum	2	10 µM	-7

// IC_{50}s were determined,
→ Please refer to page 5

Literature known TRPA1 Inhibitors



Cpd 1a
Hydra
WO 2015164643



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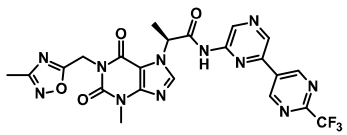
Glenmark ●
WO 2013183035



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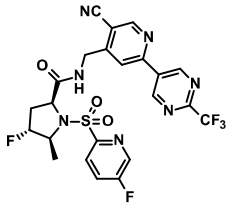
Eli Lilly
WO 2019152465



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Roche
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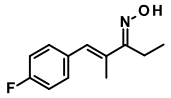
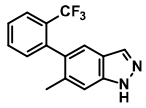
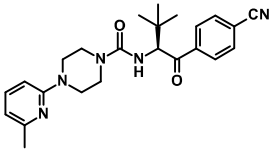


Cpd 5a
Abbvie
***Probe A-079**



Cpd 6a
Novartis ●
J. Med. Chem., **2014**, 57, 5129-5140



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AstraZeneca
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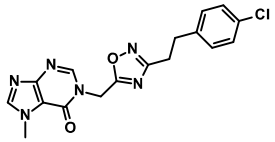

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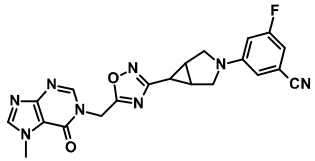
Amgen
J. Med. Chem., **2016**, 59, 2794-2809



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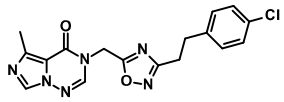
Genentech Inc.
WO 2018162607



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Almirall
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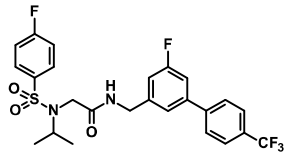

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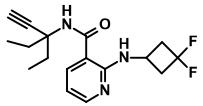
Janssen
WO 2014049047



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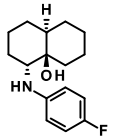
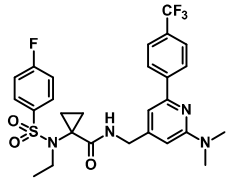
WO 2014053694



Cpd 13a
Merck Sharp & Dome ●
WO 2011043954



Cpd 14a
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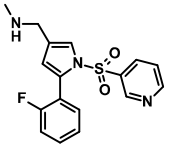
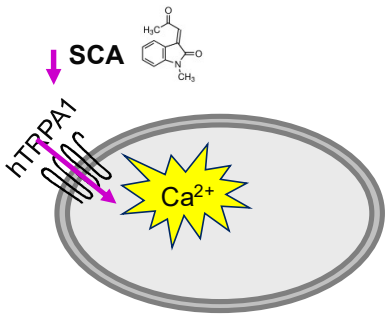
WO2014135617



Cpd 15a
Shanghai Jing'an Pharma. ●
CN 108558831

* Probe details see page 3
● Commercially available

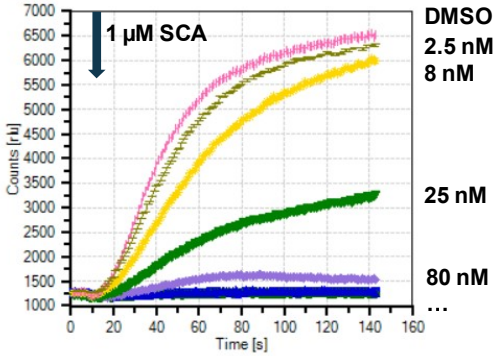
FLIPR assay for TRPA1 inhibitors



CHO-hTRPA1 GCaMP

- TRPA1 overexpressing cell line (e.g. CHO hTRPA1 GCaMP6)
- Add test compounds for 10 min
- Activation with agonist supercinnemaldehyde (SCA)
- Readout: calcium sensor GCaMP6 (or Fluo8)

Ca^{2+}-transients of BAY-390



BAY-390 hTRPA1 IC_{50} fit