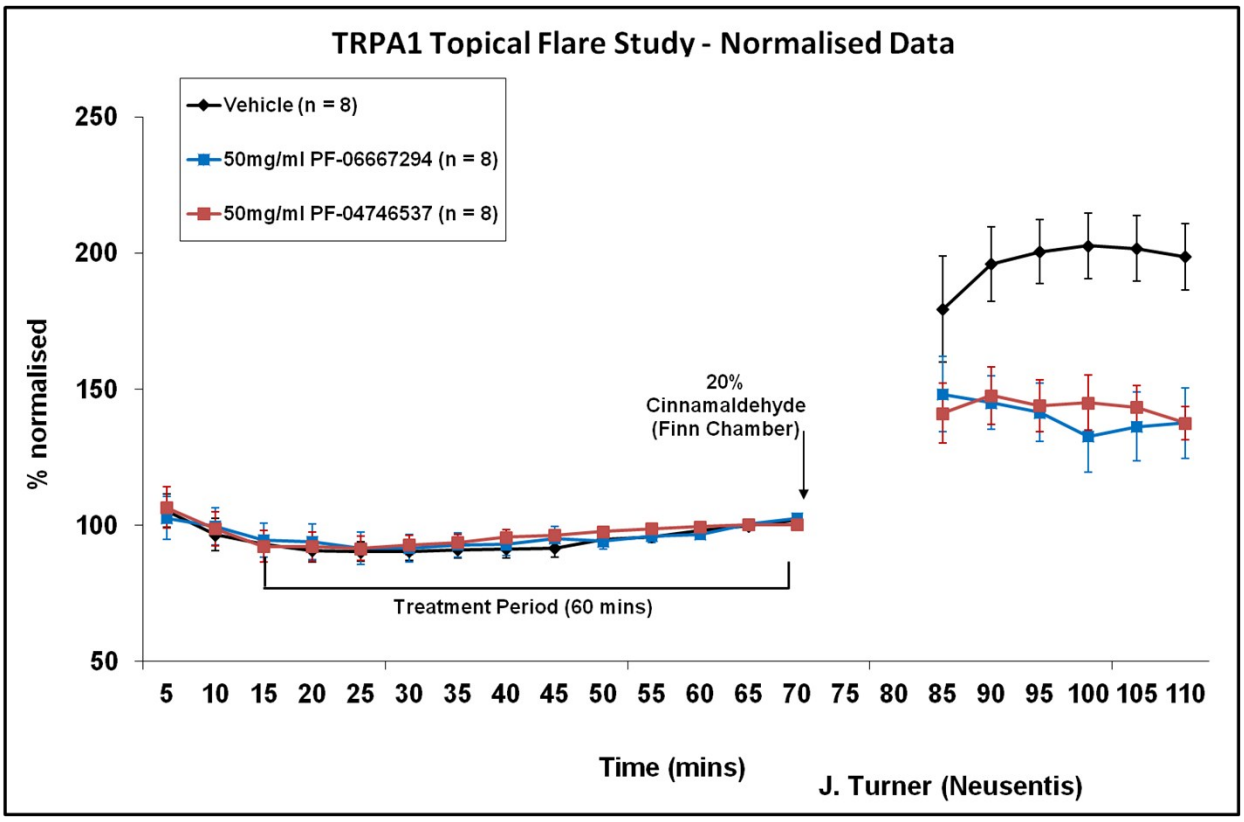
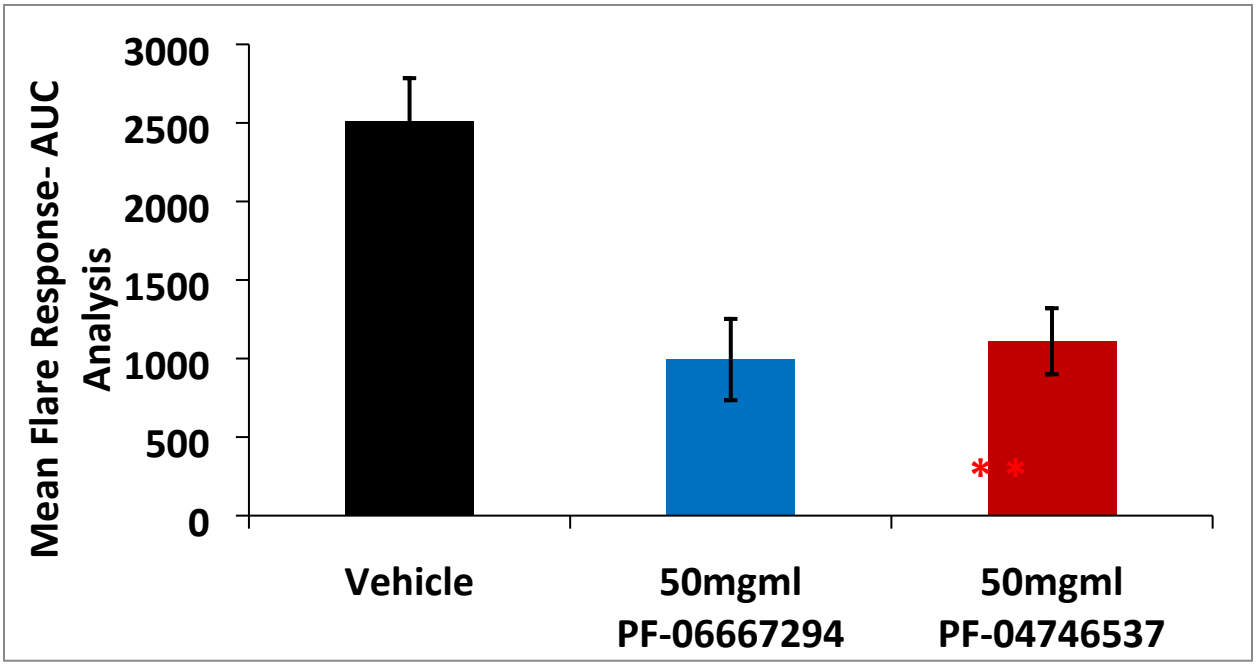
Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Figures

1. In vivo efficacy graphs and charts



Normalised flare response after a topical cinnamaldehyde challenge with equivalent doses of compounds 4 and 8. Please note PF-6667294 is Compound 4 and PF-4746537 is Compound 8.



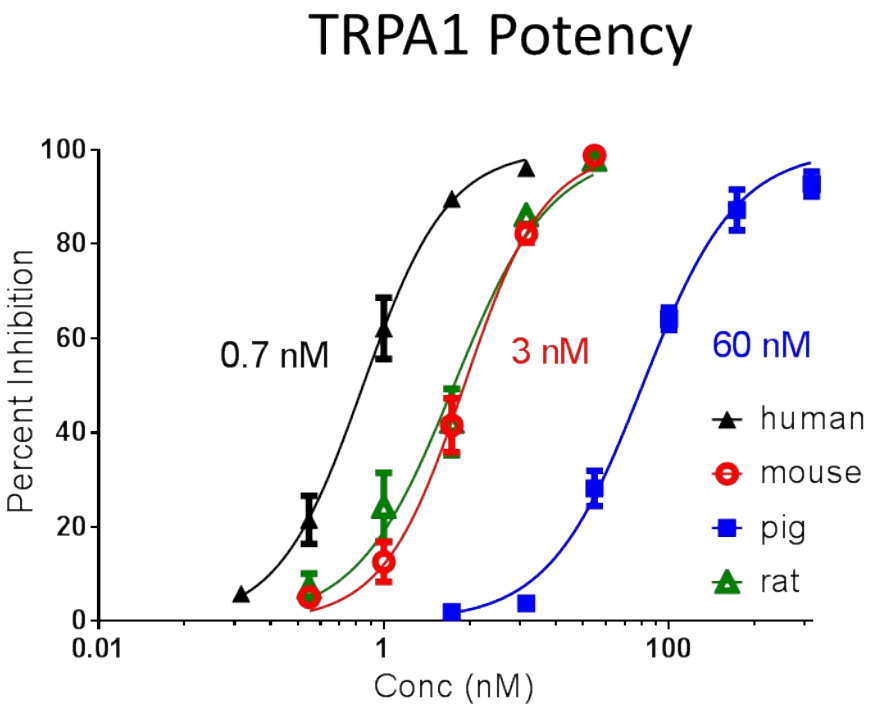
Mean flare response after a topical cinnamaldehyde challenge with equivalent doses of compounds 4 and 8. Please note PF-6667294 is Compound 4 and PF-4746537 is Compound 8.

2. In vitro pharmacology

Compound 39 broad TRP channel selectivity and Nav channel activity

	% block ± SEM @ 1 microM	Selectivity vs. hTRPA1
hTRPM8	6.44 ± 3.27	> 1000
hTRPV1	-17.44 ± 1.80	> 1000
TRPV1 mouse DRG	35.07 ± 4.25	> 1000
TTX-S Na mouse DRG	22.40 ± 0.84	> 1000
TTX-R Na mouse	62.03 ± 7.56	> 500

DRG		



Broad ion channel selectivity

Compound 8	
Channel	**Activity (IC_{50}, nM)**
TRPA1 human	17
TRPV1 human	>3000
TRPM8 human	>3000
hERG	>20000
LQT1	1830
SKCa channel	>3000
L-type Ca	>3000
Sodium channel site 2	660
GABAA chloride channel	850
nAChR	>3000
AMPA	>3000

Data were the mean of at least 2 experiments. Experiments were carried out either in-house or at CEREP. Assay formats were either patch clamp, radioligand binding displacement or ion flux.

Compound 39	
Channel	**Activity (IC_{50}, nM)**
TRPA1 human	8
TRPV1 human	>3000
TRPM8 human	>3000
TRPA1 rat	3
TRPA1 mouse	4
TRPA1 pig	65
hERG	>20000
Kv1.5	>3000
Nav1.5	>3000
LQT1	>3000
SKCa channel	>3000
L-type Ca	>3000
Sodium channel site 2	730
GABAA chloride channel	2300
nAChR	>3000
AMPA	>3000

Data were the mean of at least 2 experiments. Experiments were carried out either in-house or at CEREP. Assay formats were either patch clamp, radioligand binding displacement or ion flux.

3. Metabolite ID study on Compound 8

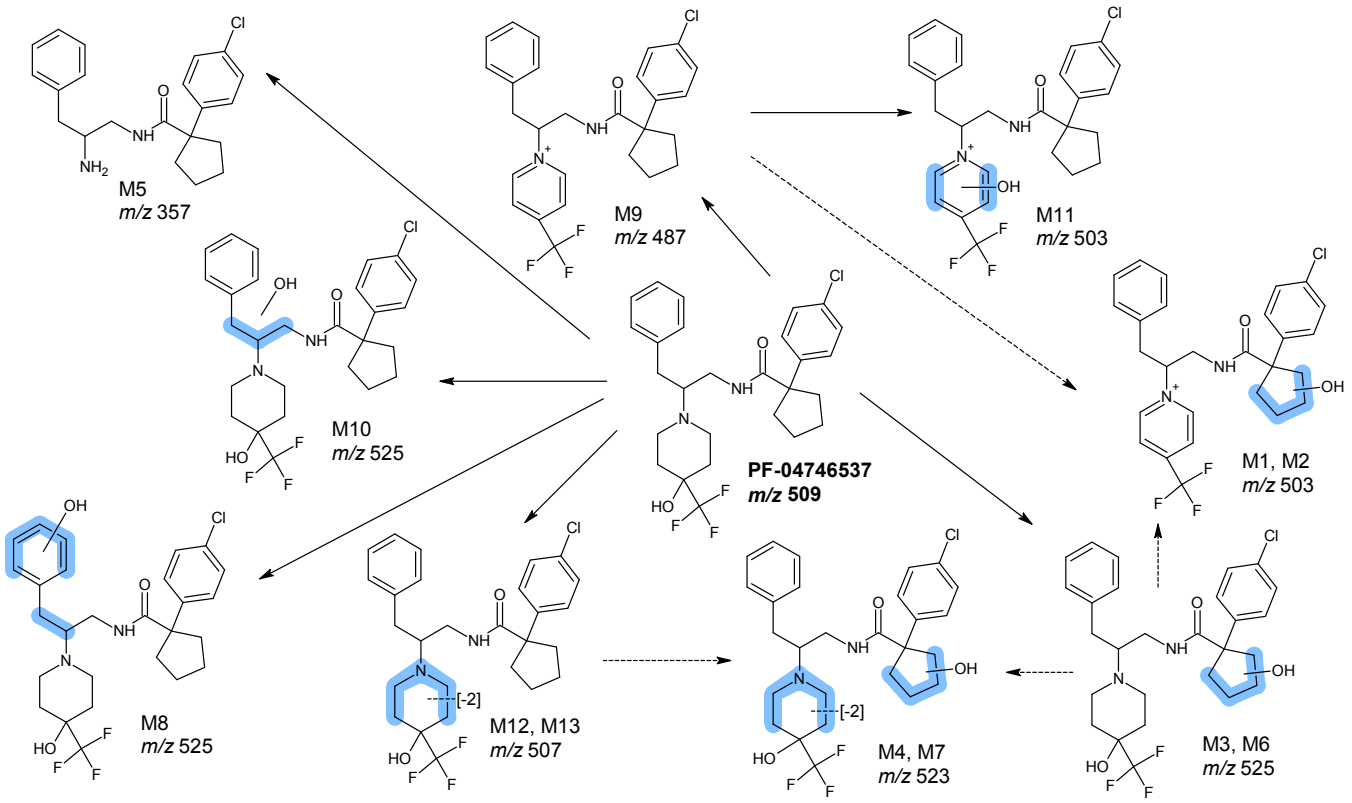
Compound 8 - Metabolism in Human Liver Microsomes

Summary:

PF-04746537 (m/z 509) was incubated with human liver microsomes (15min; 37°C; 10µM) and the resulting samples analysed by LC-MS/UV to enable detection of drug-related material. The positive control incubations (verapamil) formed the expected metabolites at appropriate levels.

In human liver microsomes unchanged PF-04745637 was the major drug-related component detected. The major metabolite observed resulted from dehydration and aromatisation at the 4-hydroxypiperidine moiety (**M9**; *m/z* 487; P-22); several mono-oxidised derivatives of **M9** were also observed (**M1**, **M2** (involving oxidation of the cyclopentyl ring) and **M11** (oxidation of the pyridinium group); *m/z* 503; P-6). Metabolite **M5** was identified as a product of N-dealkylation resulting in loss of the piperidine ring (m/z 357; P-152), whilst products of mono-oxidation in the cyclopentane (**M3** and **M6**; *m/z* 525; P+16) and phenylpropyl moieties (**M8** and **M10**; *m/z* 525; P+16) were also detected. Additional metabolites were postulated to arise from oxidation and dehydration in the piperidine ring (**M12** and **M13**; *m/z* 507; P-2), and mono-oxidation in the cyclopentane ring with oxidation and dehydration in the piperidine ring (**M4** and **M7**; *m/z* 523; P+14). There was also evidence of further minor products involving di-oxidation and dehydration (not shown).

Metabolite Scheme:



UV and MS Chromatograms Following Incubation of Compound 8 in HLM: