

**Supplementary materials**

**A  $K_{Ca}3.1$  channel opener, ASP0819, modulates nociceptive signal processing from peripheral nerves in fibromyalgia-like pain in rats**

Nobuaki Takeshita<sup>1\*</sup>, Tomoya Oe<sup>1</sup>, Tetsuo Kiso<sup>1</sup>, and Shuichiro Kakimoto<sup>1</sup>

<sup>1</sup>Drug Discovery Research, Astellas Pharma Inc., Tsukuba, Japan

Correspondence:

Nobuaki Takeshita, PhD

Drug Discovery Research

Astellas Pharma Inc.

21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

Telephone number: +81-29-852-1111

Fax number: +81-29-850-5120

E-mail: [nobuaki.takeshita@astellas.com](mailto:nobuaki.takeshita@astellas.com)

## Supplemental Table 1

### Inhibitory effect of ASP0819 on radioligand binding to various receptors, ion channels, transporters

Assay name	Inhibition (%)	
	ASP0819	Positive substance
Adenosine A1 (Rat)	3.72	100.00 (DPCPX)
α1-Adrenergic (Non-selective) (Rat)	6.59	100.00 (Prazosin)
α2-Adrenergic (Non-selective) (Rat)	26.41	100.00 (Yohimbine)
β-Adrenergic (Non-selective) (Rat)	9.02	97.95 (Propranolol)
Angiotensin AT1 (Human)	0.00	100.00 (Angiotensin II)
Angiotensin AT2 (Mouse)	0.66	100.00 (Angiotensin II)
Bradykinin B2 (Human)	8.05	96.05 (HOE140)
Ca channel (Type L, Dihydropyridine) (Rat)	9.25	100.00 (Nitrendipine)
Ca channel (Type N) (Rat)	0.66	100.00 (ω-Conotoxin)
CCK A (Human)	0.00	99.32 (CCK-8 sulfated)
CCK B (Human)	2.28	100.00 (CCK-8 sulfated)
CRF1 (Human)	4.96	100.00 (Urocortin human)
Dopamine D1 (Rat)	1.77	99.95 (SCH23390)
Dopamine D2 short (Human)	0.00	98.85 ((+)-Butaclamol)
Dopamine transporter (Human)	30.09	100.00 (GBR12909)
Estrogen (Rat)	1.72	99.70 (β-Estradiol)
Endothelin ETA (Human)	0.00	100.00 (Endothelin-1)
Endothelin ETB (Human)	1.91	99.92 (Endothelin-1)
GABA A (Agonist site) (Rat)	1.01	100.00 (Muscimol)
GABA A (BZ central) (Rat)	0.27	100.00 (Diazepam)
GABA B (Rat)	16.61	100.00 (GABA)
Glutamate (AMPA) (Rat)	0.00	99.06 (AMPA)
Glutamate (Kainate) (Rat)	4.32	100.00 (Kainic acid)
Glutamate (NMDA agonist site) (Rat)	0.00	99.07 (Glutamic acid)
Glutamate (NMDA glycine site) (Rat)	0.00	100.00 (MDL105519)
Glycine (Strychnine sensitive) (Rat)	28.20	100.00 (Strychnine)
Histamine H1 (Central) (Guinea pig)	6.50	100.00 (Pyrilamine)
Histamine H2 (Rat)	15.21	99.79 (Cimetidine)
Histamine H3 (Rat)	3.94	96.14 (α-Methyl histamine)

Test substance concentration: 10 μM; positive substance concentration: 1 μM for HOE140, urocortin human and endothelin-1, or 10 μM for all others. Data are expressed as the mean of duplicate samples. The inhibition rate was calculated from “100 – binding ratio.”

$$\text{Binding ratio: } [(B - N)/(B_0 - N)] \times 100 (\%)$$

B: Total bound radioactivity in the presence of the test substance (individual value)

$B_0$ : Total bound radioactivity in the absence of the test substance (mean value)

N: Non-specific bound radioactivity (mean value)

**Supplemental Table 2****Inhibitory effect of ASP0819 on radioligand binding to various receptors, ion channels, transporters**

Assay name	Inhibition (%)	
	ASP0819	Positive substance
K channel KATP (Rat)	0.00	98.06 (Glibenclamide)
K channel SkCa (Rat)	1.18	98.99 (Apamin)
Leukotriene B4 (Guinea pig)	15.60	100.00 (Leukotriene B <sub>4</sub> )
Leukotriene D4 (Guinea pig)	0.00	100.00 (Leukotriene D <sub>4</sub> )
Melatonin MT1 (Human)	0.83	99.64 (Melatonin)
Muscarinic (Non-selective) (Rat)	2.14	99.92 (Atropine)
Muscarinic M1 (Human)	6.15	100.00 (Atropine)
Muscarinic M2 (Human)	2.86	100.00 (Atropine)
Na channel Site 2 (Rat)	83.71	100.00 (Dibucaine)
Neurokinin NK1 (Human)	25.32	100.00 (L-703,606)
Neurokinin NK2 (Human)	20.11	100.00 (Neurokinin A)
Neurokinin NK3 (Human)	6.46	97.47 (Senktide)
Norepinephrine transporter (Human)	15.35	98.90 (Desipramine)
Nicotinic Ni (Rat)	1.36	100.00 (Nicotine)
Opiate (Non-selective) (Rat)	5.52	100.00 (Naloxone)
Opiate $\mu$ (Human)	9.86	100.00 (DAMGO)
Oxytocin (Rat)	8.68	99.39 (Oxytocin)
PAF (Rabbit)	2.24	100.00 (PAF)
Serotonin 5HT1 (Non-selective) (Rat)	10.89	100.00 (Serotonin)
Serotonin 5HT2B (Human)	25.84	100.00 (Serotonin)
Serotonin transporter (Human)	13.38	100.00 (Imipramine)
Sigma (Non-selective) (Guinea pig)	57.57	92.60 (Haloperidol)
Testosterone (Human)	0.00	99.39 (Testosterone)
Vasopressin V1 (Rat)	2.48	95.68 ([Arg <sup>8</sup> ]-Vasopressin)
VIP 1 (Human)	0.00	100.00 (VIP)
Histamine H4 (Human)	9.39	100.00 (Histamine)
Serotonin 5HT4 (Guinea pig)	73.52	100.00 (Serotonin)
Serotonin 5HT3 (Human)	4.13	98.31 (MDL72222)

Test substance concentration: 10  $\mu$ M; positive substance concentration: 1  $\mu$ M for leukotriene B<sub>4</sub>, leukotriene D<sub>4</sub> and VIP, or 10  $\mu$ M for all others. Data are expressed as the mean of duplicate samples. The inhibition rate was calculated from “100 – binding ratio.”

$$\text{Binding ratio: } [(B - N)/(B_0 - N)] \times 100 (\%)$$

B: Total bound radioactivity in the presence of the test substance (individual value)

$B_0$ : Total bound radioactivity in the absence of the test substance (mean value)

N: Non-specific bound radioactivity (mean value)

### Supplemental Table 3

#### Inhibitory effect of AS0819 on various enzymes

Assay name	Inhibition (%)	
	ASP0819	Positive substance
Acetylcholinesterase (Eel)	0.68	97.59 (Eserine)
MAO-A (Rat)	0.00	98.35 (Clorgyline)
MAO-B (Rat)	0.00	93.70 (Ro 16-6491)

Test substance concentration: 10  $\mu$ M; positive substance concentration: 100  $\mu$ M for Ro 16-6491, or 10  $\mu$ M for all others. Data are expressed as the mean of duplicate samples. The inhibition rate was calculated from “100 – reaction ratio.”

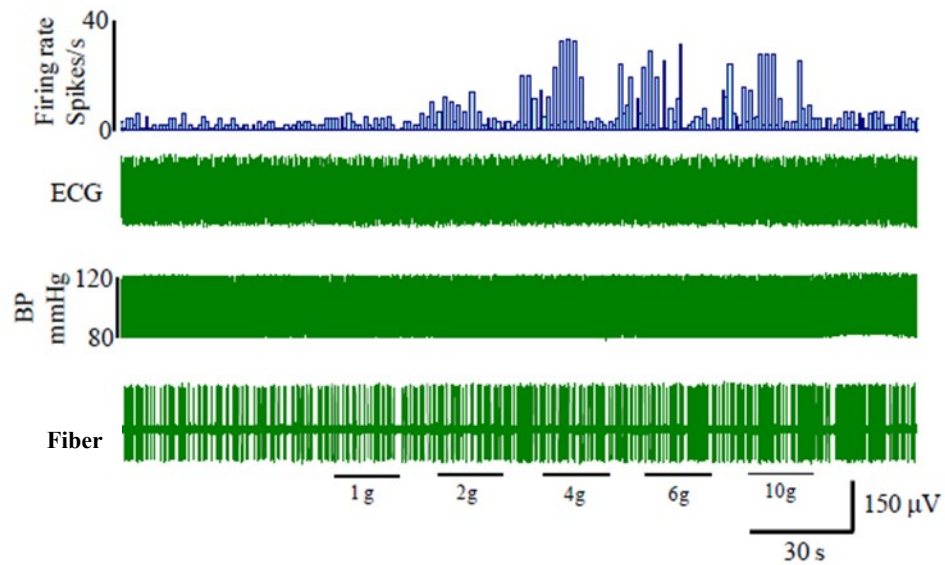
Reaction ratio:  $[(B - N)/(B_0 - N)] \times 100$  (%)

B: Radioactivity or fluorescence intensity in the tube for calculation of inhibition rate (individual value)

B<sub>0</sub>: Radioactivity or fluorescence intensity in the tube for calculation of total reaction (mean value)

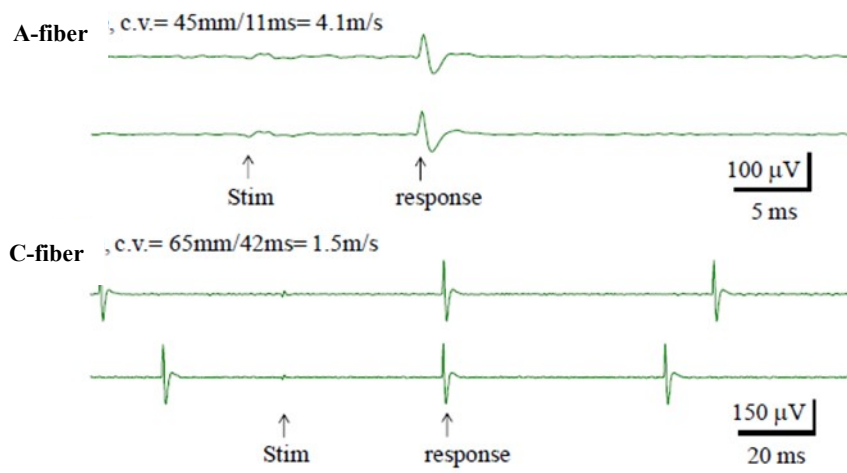
N: Radioactivity or fluorescence intensity in the tube for calculation of non-specific reaction (mean value)

## Supplemental Figure 1



A typical in vivo electrophysiology experiment set-up for dorsal root filament recording. 1g, 2g to 10g: von Frey hair 1 g, 2 g to 10 g, repeated 10 times using a one second-on-one-second-off protocol. ECG: electrocardiogram, BP: blood pressure.

## Supplemental Figure 2

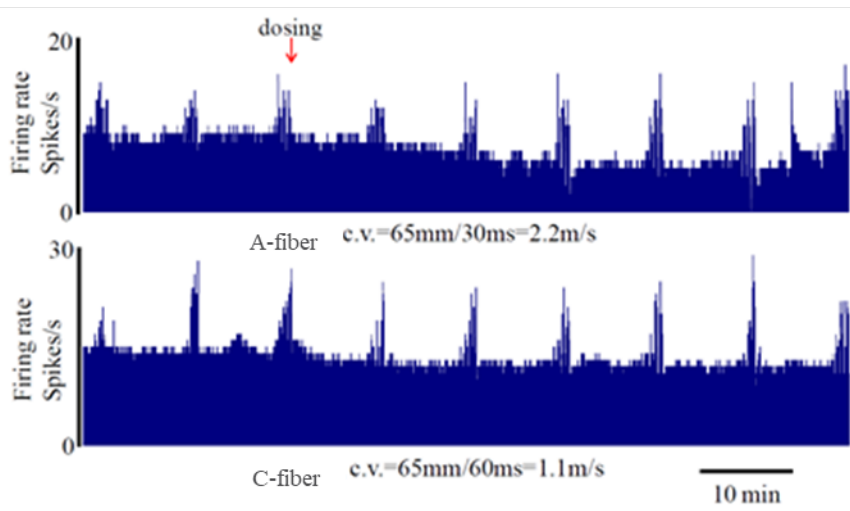


An example of the approach used for identification of fiber categories.



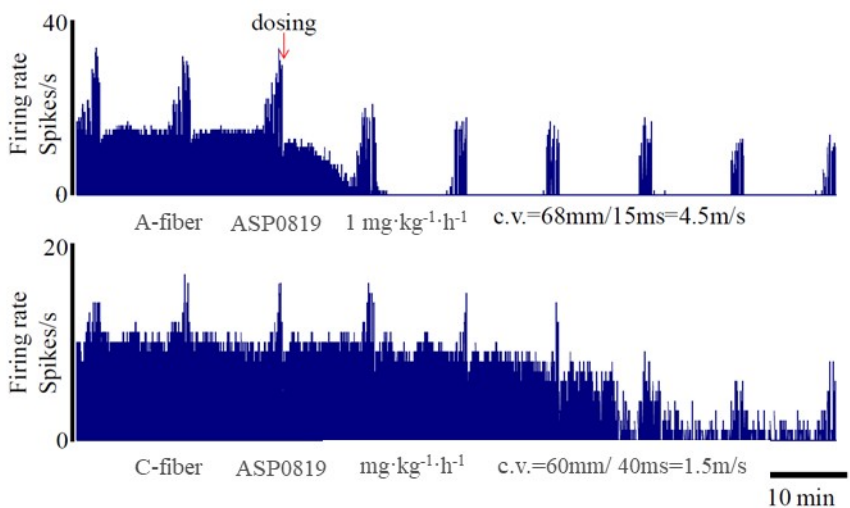
### Supplemental Figure 3

A



Example recordings on spontaneous activity and mechanically-evoked responses of an A- (upper trace) and C-fiber (lower trace) in RIM models following vehicle perfusion.

B



Example recordings on spontaneous activity and mechanically-evoked responses of an A- (upper trace) and C-fiber (lower trace) in RIM models inhibited by ASP0819 at 1 mg·kg<sup>-1</sup>·h<sup>-1</sup>.