JPET Fast Forward. Published on April 17, 2008 as DOI: 10.1124/jpet.107.132233
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Antihyperalgesic effects of AMG8562, a novel vanilloid receptor TRPV1 modulator that does not cause hyperthermia in rats

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Running title: Antihyperalgesic TRPV1 modulator devoid of hyperthermia

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Total number of pages including all figures & tables:	47
Figures:	7
Tables:	3
References:	30
Total number of words in abstract:	216
Total number of words in introduction:	562
Total number of words in discussion:	1422

Abbreviations: TRPV1: transient receptor potential vanilloid type 1; TRPA1: transient receptor potential ankyrin 1; TRPM8: transient receptor potential melastatin 8; TRPV2: transient receptor potential vanilloid type 2; TRPV3: transient receptor potential vanilloid type 3; TRPV4: transient receptor potential vanilloid type 4; 2-APB: 2-Aminoethoxydiphenyl borate; 4αPDD: 4-alpha-Phorbol 12,13-didecanoate; CHO: Chinese hamster ovary; CFA: complete Friend's adjuvant; MED: minimally effective dose; AMG 517: *N*-(4-[6-(4-trifluoromethyl-phenyl)-pyrimidin-4-yloxy]-benzothiazol-2-yl)-acetamide; AMG0347: [(E)-N-(7-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl)-3-(2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)acrylamide]; AMG8163: *tert*-butyl_2-(6-([2-(acetylamino)-1,3-benzothiazol-4-yl]oxy)pyrimidin-4-yl)-5-

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(trifluoromethyl)phenylcarbamate; compound M8-B: N-(2-aminoethyl)-N-((3-(methyloxy)-4-((phenylmethyl)oxy)phenyl)methyl)-2-thiophenecarboxamide; A-425619: (1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea); AMG8562: (*R*,*E*)-*N*-(2-hydroxy-2,3-dihydro-1*H*-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(trifluoromethyl)phenyl)acrylamide; AMG8563: (S,*E*)-*N*-(2-hydroxy-2,3-dihydro-1*H*-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(trifluoromethyl)phenyl)acrylamide; AMG7905: *N*-(6-(2-(cyclohexylmethylamino)-4-(trifluoromethyl)phenyl)pyrimidin-4-yl)benzo[d]thiazol-6-aminecompound V3-H: 1-(((5-chloro-1,3-benzothiazol-2-yl)thio)acetyl)-8-methyl-1,2,3,4-tetrahydroquinoline; SB-366791: 4'-Chloro-3-methoxycinnamanilide

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Abstract

Antagonists of the vanilloid receptor TRPV1 have been reported to produce anti-hyperalgesic effects in animal models of pain. These antagonists, however, also caused concomitant hyperthermia in rodents, dogs, monkeys and humans. Antagonist-induced hyperthermia was not observed in TRPV1 knockout mice, suggesting that the hyperthermic effect is exclusively mediated through TRPV1. Since antagonist-induced hyperthermia is considered a hurdle for developing TRPV1 antagonists as therapeutics, we investigated the possibility of eliminating hyperthermia while maintaining anti-hyperalgesia. Here, we report four potent and selective TRPV1 modulators with unique *in vitro* pharmacology profiles (Profiles A through D) and their respective effects on body temperature. We found that Profile C modulator, AMG8562, blocks capsaicin activation of TRPV1, does not affect heat activation of TRPV1, potentiates pH 5 activation of TRPV1 *in vitro* and does not cause hyperthermia *in vivo* in rats. We further profiled AMG8562 in an on-target (agonist) challenge model, rodent pain models and tested for its side effects. We show that AMG8562 significantly blocks capsaicin-induced flinching behavior, produces statistically significant efficacy in CFA- and skin incision-induced thermal hyperalgesia, and acetic acid-induced writhing models, with no profound effects on locomotor activity. Based on the data shown here, we conclude that it is feasible to modulate TRPV1 in a manner that does not cause hyperthermia while maintaining efficacy in rodent pain models.

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Introduction

The vanilloid receptor TRPV1 emerged as a therapeutic target for pain based on the fact that i) TRPV1 agonists cause pain (Szallasi and Blumberg, 1999; Jones et al., 2004), ii) TRPV1 expression is up-regulated during painful conditions (Ji et al., 2002; Matthews et al., 2004), iii) TRPV1 knockout mice display attenuated pain behaviors (Caterina et al., 2000; Davis et al., 2000; Keeble et al., 2005), and iv) TRPV1 antagonists reverse pain behavior in rodent models of pain (Walker et al., 2003; Gavva et al., 2005b; Ghilardi et al., 2005; Honore et al., 2005; Rami et al., 2006). We have recently reported the identification of AMG 517, a selective, and orally bioavailable TRPV1 antagonist as a clinical candidate. AMG 517 acted as a potent antagonist of TRPV1 *in vitro*, blocked capsaicin-induced flinch in rats (an "on-target" challenge model) and acted as an anti-hyperalgesic in a model of inflammatory pain (CFA-induced thermal hyperalgesia) (Doherty et al., 2007; Gavva et al., 2007a). Similar to other TRPV1 antagonists, AMG 517 caused hyperthermia in rodents, dogs and monkeys, but hyperthermia attenuated upon repeated administration of AMG 517 (Gavva et al., 2007a). During Phase I clinical trials in healthy volunteers, AMG 517 elicited a marked but reversible hyperthermia. Furthermore, AMG 517 administered after third molar extraction, a surgical cause of acute pain, elicited long-lasting hyperthermia with body temperature surpassing 40 °C, demonstrating that TRPV1 blockade elicits an undesirable magnitude of hyperthermia in some individuals (Gavva et al., 2008).

Due to the undesirable hyperthermia observed for AMG 517 in humans, we examined the effect of TRPV1 antagonists on body temperature regulation more closely and sought possible ways to eliminate this on-target effect. Initially, we examined the effects of

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several TRPV1 antagonists represented by various chemotypes, including: cinnamides, ureas, amides, benzimidazoles, and piperazine carboxamides on rat body temperature. We found that all chemotypes that we have tested cause 0.5-1.5 °C increase in body temperature suggesting that antagonist–induced hyperthermia is not chemotype specific but rather occurs because of TRPV1 blockade *in vivo* (Gavva et al., 2007b). Further, TRPV1 selective antagonists (AMG0347 and AMG 517) did not cause hyperthermia in TRPV1 knockout mice, demonstrating that entire hyperthermic effect was TRPV1 mediated (Steiner et al., 2007). The second approach that we examined was to study the effect of peripherally restricted TRPV1 antagonists. We postulated that we may be able to separate the analgesic effect of TRPV1 antagonists from their hyperthermic effect if the TRPV1 antagonists were excluded from the central nervous system. Unfortunately, this approach also did not prove to be successful because peripheral restriction of antagonists did not eliminate hyperthermia, suggesting that the site of action is predominantly outside of the blood-brain barrier (Gavva et al., 2007b; Tamayo et al., 2008). The site of action for antagonist-induced hyperthermia to be present outside of blood brain barrier was further confirmed by TRPV1 desensitization experiments (with RTX) that demonstrated visceral TRPV1 channels were responsible for antagonist-induced hyperthermia (Steiner et al., 2007). The final approach to eliminate TRPV1 antagonist-induced hyperthermia was to evaluate the compounds that display differential pharmacologies (i.e., compounds that differentially modulate distinct modes of *in vitro* TRPV1 activation such as capsaicin, pH 5, and heat) *in vivo*.

Here, we describe the differential pharmacology of four TRPV1 modulators and their effects on body temperature. We further characterized AMG8562, an orally bioavailable

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TRPV1 modulator and its effects on pain behavior in rodent models.

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Methods

Reagents

All the cell culture reagents were purchased from Invitrogen (Carlsbad, CA). All TRPV1 antagonists used in this study were synthesized at Amgen Inc, (Thousand Oaks, CA; Figure 1).

Agonist-induced $^{45}Ca^{2+}$ uptake assay

Two days prior to the assay, cells were seeded in Cytostar 96-well plates (Amersham) at a density of 20,000 cells/well. The activation of TRPV1 and TRPV2 was followed as a function of cellular uptake of radioactive calcium ($^{45}Ca^{2+}$, ICN). Capsaicin (0.5 μM), pH 5, and heat (45 ^{o}C) were used as agonists for TRPV1 and 2-APB (200 μM) was used as an agonist for TRPV2. To determine the ability of compounds to block agonist activation of TRPV1, compounds were incubated with CHO cells expressing the TRP channel for 2 minutes before the addition of agonist and $^{45}Ca^{2+}$ and cells were washed after further incubation of 2 min to determine the $^{45}Ca^{2+}$ uptake. All the antagonist $^{45}Ca^{2+}$ uptake assays were conducted as reported previously (Gavva et al., 2007a) and had a final $^{45}Ca^{2+}$ concentration of 10 μCi/mL. Radioactivity was measured using a MicroBeta Jet (Perkin-Elmer Inc.). Data were analyzed using GraphPad Prism 4.01 (GraphPad Software Inc, San Diego, CA).

Luminescence readout assay for measuring intracellular calcium

Stable CHO cell lines expressing human TRPA1, TRPM8, TRPV3, and TRPV4 were generated using tetracycline inducible T-REx^{TM} expression system from Invitrogen, Inc (Carlsbad, CA). In order to enable a luminescence readout based on intracellular increase

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in calcium (Le Poul et al., 2002), each cell line was also co-transfected with pcDNA3.1 plasmid containing jelly fish aequorin cDNA. Twenty four hours before the assay, cells were seeded in 96-well plates and TRP channel expression was induced with 0.5 μg/ml tetracycline. On the day of the assay, culture media was removed and cells were incubated with assay buffer (F12 containing 30 mM HEPES for TRPA1, TRPM8, and TRPV3; F12 containing 30 mM HEPES, 1 mM $CaCl_2$, and 0.3% BSA for TRPV4) containing 15 μM coelenterazine (P.J.K, Germany) for 2 hours. Antagonists were added for 2.5 min prior to addition of an agonist. The luminescence was measured by a CCD camera based FLASH-luminometer built by Amgen, Inc. The following agonists were used to activate TRP channels: 80 μM AITC for TRPA1, 1 μM icilin for TRPM8, 200 μM 2-APB for TRPV3, and 1 μM 4α-PDD for TRPV4. Compound activity was calculated using GraphPad Prism 4.01 (GraphPad Software Inc, San Diego, CA).

Animals

Adult male Sprague Dawley rats weighing 150-250g or CD-1 mice weighing 20-25g (Charles River Laboratories, Wilmington, MA) were allowed at least 1 week of acclimation in the Amgen's Association for Assessment and Accreditation of Laboratory Animal Committee approved animal care facility prior to being utilized. In one telemetry study, rats (n=6 per group, JVC from Taconic) were treated with various TRPV1 antagonists in 100% DMSO 1 ml/kg i.v. or vehicle control. In subsequent telemetry, efficacy and side effect studies in rats (n=6 or more per group), various doses of TRPV1 antagonists in a dosing volume of 5 ml/kg in an Ora-Plus/5% Tween-80 suspension p.o. or gabapentin (Sigma, St. Louis, MO) in saline i.p. 2 ml/kg or vehicle controls were

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administered at the treatment times specified for each model. Mice (8 or more per group) were treated with vehicle, TRPV1 antagonist or ibuprofen (Sigma, St. Louis, MO) in a dosing volume of 10 ml/kg in an Ora-Plus/5% Tween-80 suspension, p.o. at the doses and treatment times specified for each model. Blood samples were collected immediately following behavioral testing for pharmacokinetic analysis.

As a standard operation procedure, capsaicin-induced flinch and pain models were conducted in a blinded fashion by randomization of dosing groups, inclusion of a positive control and experimenters recording behavioral end points not participating in dosing of compounds.

Capsaicin-induced flinch model

AMG8562 at 0.1, 0.3, 1 and 3 mg/kg or AMG8163 at 3 mg/kg (used as the positive control) or vehicle was administered two hours prior to right hind paw intraplantar injection of 0.5 mg/25 ml capsaicin (Sigma-Aldrich Inc., St. Louis, MO; in a volume of 25 µl of 5% EtOH in phosphate-buffered saline without Ca^{2+} and Mg^{2+}). Immediately following the injection of capsaicin, the number of hind paw flinches was recorded over a 1-minute period by investigators fully blind to the randomized treatment conditions.

Radiotelemetry in rats

Rats were single-housed upon arrival to our animal facility. Prior to implanting the radiotelemetry probe (model ER-4000 PDT; Mini Mitter, Brend, OR), rats were lightly anesthetized using isoflourane (IsoFlo, Abbott Laboratories, Chicago, IL) at a concentration of 2% isoflourane at 2 L/min oxygen flow. While positioned in right lateral

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recumbence the left, lateral abdominal wall was clipped and cleaned with Betadine solution (Purdue Frederick Company, Stamford, CT) followed by 70% alcohol in water. A 3-4 mm incision was made through the skin and abdominal wall, into which a sterilized probe was inserted. The surgical site was closed with absorbable suture material (4-0 Vicryl, Ethicon Inc, Somerville, NJ) and silk suture (4-0, Ethicon, Inc. Somerville, NJ). Animals were returned to home caging for a three-day recovery period prior to the drug treatment experiments.

On the day of the experiment, single-housed animals were placed on the radiotelemetry receivers and acclimated to the test room for at least 1 hour before the baseline recording. The temperature in the room used for radiotelemetry experiments was maintained at 20 ± 2 °C. Baseline recording of core body temperature was recorded for 30 min prior to the drug administration. Rats (6 per group) were either treated with vehicle or TRPV1 antagonist and the body core temperature recordings were continued for 2-4 hours.

CFA-induced thermal hyperalgesia

Following three days of full habituation to the testing equipment, baseline thermal latencies were recorded in the rats using the modified Hargreaves' thermal stimulating apparatus (UCSD, CA) followed by injection of CFA into the right hind paw (50 µl of 0.1%). Twenty-one hours after the CFA injection animals were dosed (p.o.) with vehicle, AMG8562 at 30 or 100 mg/kg. AMG8163 at 3 mg/kg was used as the positive control. Two hours after drug dosing (23 hours after CFA injection), paw withdrawal latencies were measured by investigators fully blind to the randomized treatment conditions.

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Acetic acid induced writhing in mice

Mice were habituated to the testing room in their home cages overnight. Behavioral observations occurred in clear plastic testing cylinders 30 cm in height and 30 cm in diameter with opaque walls between cylinders such that mice were not visible to each other (Langford et al., 2006). TRPV1 antagonist, vehicle or ibuprofen 200 mg/kg was injected 1 hour before acetic acid injection. Immediately following i.p. injection of 0.6% acetic acid in saline 10 ml/kg, the number of writhes (characteristic lengthwise abdominal constrictions with torso elongations) was counted for 30 min.

Open field activity in mice and rats

Rats were habituated in a reversed light cycle room for at least one week and to the testing room for one hour prior to dosing. Mice were housed in a regular light cycle room and habituated overnight to the dark testing room in their home cages. TRPV1 antagonist or vehicle was injected one hour before placing mice or 1.5 hours before placing rats in the open-field apparatus. Open-field activity was measured using a system that counts interruptions of a set of photobeams (Hamilton-Kinder, Poway, CA) for the course of 60 min. To begin a session, animals are removed from the home cage and placed individually into an independent Plexiglas box (16”Lx16”Wx15”H) surrounded by a frame consisting of 32 photocells (16Y and 16X) that track the movement of the animal. Photobeam breaks are used as an indication of activity and are broken in to the following parameters per minute: basic movements (beam breaks), distance traveled (cm), time spent (s), number of repetitive beam breaks (i.e. stereotypic movement).

Statistical analysis

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Behavioral results were analyzed using one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons post hoc test for significance relative to vehicle. Multiple ANOVAs (one for each timepoint) were used for analysis of body core temperature data. The development of thermal hyperalgesia in baseline versus CFA or skin incision treated rats versus vehicle as well as the effect of gabapentin versus saline in SNL-induced mechanical allodynia was compared using Student's *t* test. Logarithmic transformations were made of von Frey threshold data in SNL-induced mechanical allodynia as described above. Statistical calculations and graphs were made using GraphPad Prism 5.01 (GraphPad Software Inc, San Diego, CA).

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Results

TRPV1 antagonists exhibit differential pharmacology

It has been reported that some TRPV1 antagonists exhibit differential pharmacology at distinct modes of activation (McIntyre et al., 2001; Gavva et al., 2005a; Gavva et al., 2007b). We have previously defined two profiles of TRPV1 antagonists, Group A, that block all modes of activation, and Group B, that block capsaicin and heat but not proton activation (Gavva et al., 2005a). Here, we evaluated several compounds for their ability to modulate three distinct modes of TRPV1 activation in agonist-induced $^{45}Ca^{2+}$ uptake assays using CHO cells stably expressing the rat TRPV1 channels (Fig. 2). AMG8163 potently inhibited capsaicin (0.5 μM), pH 5, and heat (45 ºC) activation of rat TRPV1, and represents 'Profile A' or Group A (Table 1; Fig. 2A-C, (Gavva et al., 2007b)). AMG8563 inhibited capsaicin and heat activation but only partially blocked pH 5 activation similar to capsazepine and AMG0610 that represent 'Profile B' or 'Group B' antagonists (Gavva et al., 2005a) (Fig. 2D-F). AMG8562 blocked capsaicin activation, did not affect heat activation and potentiated pH 5 activation, thus representing a new modulation profile, "Profile C" (Fig. 2G-I). AMG7905 inhibited capsaicin activation and potentiated both heat and pH 5 activation of rat TRPV1 representing yet another new modulation profile "Profile D" (Fig. 2J-L). Both AMG8562 and AMG7905 by themselves did not induce $^{45}Ca^{2+}$ uptake into CHO cells expressing the rat TRPV1 channels at physiological pH (pH 7.2), indicating that they are not partial agonists. In addition to blocking capsaicin activation, all four compounds inhibited putative endogenous ligand (AEA and NADA) activation of TRPV1 (Table 1).

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Potentiators of proton activation do not cause hyperthermia

We have recently demonstrated that TRPV1 antagonists that represent Profiles A and B cause transient hyperthermia (Gavva et al., 2007b). Since we are interested in dissecting the pharmacology of TRPV1 antagonists to evaluate if hyperthermia can be separated from their ability to act as anti-hyperalgesics, we tested all four molecules that represent Profiles A through D for their effect on body temperature. In the first experiment, AMG8163, AMG8563 and AMG8562 (representing Profiles A, B and C, respectively) were administered intravenously to rats implanted with radiotelemetry probes to monitor body temperature (Fig. 3). Overall, there were significant effects of these compounds on body temperature (Fig. 3A) from 10 min post dosing ($F_{3,20}$ = 14.0, $p<0.01$) through 120 min post dosing ($F_{3,20}$ = 42.3, $p<0.01$). Both AMG8563 and AMG8163 produced significant 1.0-1.5 ºC increase in body temperature as predicted for Profile A and B antagonists (Gavva et al., 2007b). AMG8163 caused a maximum increase in temperature relative to vehicle of 1.1 ºC at 110 min (vehicle = 37.4 ± 0.2 ºC; AMG8163 = 38.7 ± 0.1 ºC; $p<0.01$). AMG8563 caused a maximum increase in temperature of 1.2 ºC at 40 min (vehicle = 37.3 ± 0.2º C; AMG8563 = 38.5 ± 0.2 ºC; $p<0.01$). AMG8562, however, produced a significant decrease of 1.0 ºC in body temperature at 20 min post administration (vehicle = 37.2 ± 0.1 ºC; AMG8562 = 36.2 ± 0.3 ºC; $p<0.01$).

We further profiled AMG8562 at 1, 3, 10 and 30 mg/kg doses by oral administration to rats in order to assess dose dependency and maximal temperature effects using the same route used for *in vivo* efficacy studies (Fig. 3B). During the first three time-points post-dosing, there is no significant effect of AMG8562 on body temperature ($F_{4,25}$ = 0.65 - 2.5, $p>0.05$). At 40 min and 70 min post-dosing, there is a significant effect of AMG8562

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($F_{4,25}$ = 3.4 and 2.3, respectively; $p<0.05$) with significance relative to vehicle of only the 30 mg/kg dose ($p<0.01$). At 80 and 160 min post dosing, all four doses of AMG8562 significantly decrease body temperature ($F_{4,25}$ = 3.8 and 3.3, respectively; $p<0.05$). This decrease in body temperature was considered modest because the greatest change from vehicle was only -0.6 ºC (following 1 mg/kg at 160 min post dosing where vehicle = 37.5 ± 0.1 ºC and 1mg/kg = 36.8 ± 0.1 ºC). Oral administration of AMG8562 produced a smaller decrease in body temperature than intravenous administration. We assume that intravenous administration might have resulted in greater exposure compared to oral administration that resulted in a larger decrease in body temperature. Profile C compounds such as AMG8562 may cause decrease in body temperature as opposed to the hyperthermia elicited by Profile A and B compounds, AMG8163 and AMG8563, respectively.

We also profiled AMG7905 at 0.3, 1, 3, 10 and 30 mg/kg doses by oral administration in order to assess dose dependency and maximal temperature effects (Fig. 3C). Beginning at 30 min post-dosing and throughout the testing period, AMG7905 significantly and dose-dependently decreased body temperature 30 mg/kg ($F_{6,52}$ = 3.7, $p<0.01$). The maximum effect occurred at 160 min post-dosing ($F_{6,52}$ = 40.0, $p<0.01$) with vehicle group temperature of 37.1 ± 0.1 ºC and 30 mg/kg AMG7905 administered group temperature of 34.2 ± 0.4 ºC. Since AMG7905 potentiated both heat and pH 5 activation and caused a relatively greater magnitude of decrease in body temperature relative to vehicle, we concluded that potentiation of both heat and pH 5 appears to result not only in a lack of hyperthermia, but actually a marked hypothermia. TRPV1 modulator-induced hypothermia returned to baseline values within 12 hours.

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AMG8562 is a selective modulator of TRPV1 with acceptable pharmacokinetic properties

Since AMG8562 did not cause hyperthermia in rats, we evaluated its ability to modulate activation of closely related TRP channels in cell-based assays that measure agonist-induced increases in intracellular calcium in CHO cells recombinantly expressing the appropriate TRP channel. The IC_{50} value for AMG8562 was >20 μM against allyl isothiocyanate activated TRPA1, icilin activated TRPM8, 2-APB activated TRPV2, and TRPV3. AMG8562 IC_{50} value against 4-αPDD activated TRPV4 was approximately 3 μM (Fig. 4). In these assays, ruthenium red served as a positive control for TRPA1 (IC_{50} value: 367 nM), TRPV2 (IC_{50} value: 2028 nM), and TRPV4 (IC_{50} value: 24 nM). Compound M8-B and compound V3-H served as positive controls for TRPM8 (IC_{50} value: 5 nM) and TRPV3 (IC_{50} value: 72 nM), respectively. Similar to AMG8562, AMG7905 was also found to be selective for TRPV1 (IC_{50} against TRPA1, TRPM8, TRPV1-4 was > 4 μM). Similarly, AMG8562 IC_{50} value against human TRPA1, TRPM8, TRPV3, and TRPV4 was > 4 μM (data not shown). We acknowledge that most of the selectivity data generated here was with exogenous ligands and it may or may not necessarily correlate with yet to be discovered endogenous ligands for each of the TRP channels.

Interestingly, AMG8562 profile at human TRPV1 is different compared to its effect on rat TRPV1. AMG8562 behaved like a 'Profile B' antagonist of human TRPV1; i.e., blocked capsaicin and heat activation fully and partially blocked pH 5 activation of human TRPV1 (Figure 5).

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Further, in a selectivity screen conducted by Cerep, a 10 μM concentration of AMG8562 did not show significant binding (≥ 45% inhibition) to any of the targets that included receptors, enzymes, and ion channels (Table 2). AMG8562 demonstrated acceptable metabolic stability in rat liver microsomes (RLM $CL_{\text{in vitro}}$ = 61 μL/min/mg) and superior *in vivo* pharmacokinetic properties in rats ($t_{1/2}$ = 4.4 h, $CL_{\text{in vivo}}$ = 0.85 L/h/kg, and F_{oral} = 61% at 5 mg/kg), hence deemed suitable for oral administration during *in vivo* studies.

AMG8562 potently blocks capsaicin-induced flinch behavior in rats

First, we evaluated the ability of AMG8562 to block capsaicin-induced flinch because other TRPV1 antagonists that potently block capsaicin activation *in vitro* block capsaicin-induced flinching *in vivo* (Seabrook et al., 2002; Gavva et al., 2007a). AMG8562 significantly and dose-dependently decreased capsaicin-induced flinching in rats ($F_{7,80}$ = 15.7, $p<0.01$; Fig. 6A) with a 100% blockade of flinching following the 3 mg/kg. Baseline number of flinches in the vehicle administered group was 10.8 ± 1.7 flinches. The minimally effective dose (MED) to block capsaicin-induced flinch for AMG8562 was 1 mg/kg, which corresponds to a minimally effective plasma concentration (MEC) of 120.2 ± 7.9 ng/ml, $p<0.05$. The positive control, 3 mg/kg of AMG8163 showed 100% blockade of flinching in the same experiment.

AMG8562 significantly reduces CFA-induced thermal hyperalgesia

Several TRPV1 antagonists have been shown to block hyperalgesia induced by CFA (Pomonis et al., 2003; Walker et al., 2003; Gavva et al., 2005b; Honore et al., 2005). Since AMG8562 modulates TRPV1 differentially compared to the molecules used in the studies

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mentioned above, we evaluated AMG8562 effect on CFA-induced thermal hyperalgesia (Fig.6B). Prior to CFA injection, baseline latency of all rats combined was 10.4 ± 0.1 sec. Twenty-three hours post CFA injection, rats exhibited an increase in thermal sensitivity as shown by the decrease in latency in the vehicle treated rats (3.6 ± 0.3 sec) relative to their baseline latency (t_{22} = 21.4, $p<0.01$). AMG8562 significantly reduced CFA-induced thermal hyperalgesia ($F_{3,44}$ = 3.9, $p>0.05$) at 30 and 100 mg/kg ($p <0.05$). The corresponding plasma concentrations immediately after behavioral data collection were 4153 ± 172 and 7699 ± 281 ng/ml, respectively (Fig. 6B). Latency to respond to the thermal stimulus was increased to 5.8 ± 0.7 sec following 30 mg/kg and to 5.8 ± 0.5 sec following 100 mg/kg of AMG8562. The maximum effect, expressed as percent reduction of the "window of hyperalgesia" (window being defined as vehicle treated post CFA latency minus baseline latency), is 27 ± 9%. As previously shown (Gavva et al., 2007a), the positive control AMG8163 significantly reduced CFA-induced thermal hyperalgesia ($p<0.01$) with latency increased to 6.2 ± 0.5 sec, constituting a 35 ± 7% effect in this experiment with 31 ± 4 ng/ml plasma concentration.

AMG8562 significantly reduces plantar skin incision-induced thermal hyperalgesia

TRPV1 antagonists such A-425619 and AMG8163 have been reported to reverse thermal hyperalgesia induced by surgical incision (Honore et al., 2005; Magal, 2005). Hence, we evaluated and compared AMG8562 effects with AMG8163 in the same model (Fig. 6C). Prior to skin incision, baseline latency of all rats combined was 11.3 ± 0.1 sec. Two-hours post plantar skin-incision, rats exhibited an increase in thermal sensitivity as shown by the decrease in latency in the vehicle treated group to 3.6 ± 0.3 sec relative to their baseline latency (t_{22} = 23.6, $p<0.01$). AMG8562 significantly reduced thermal hyperalgesia in

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post-incision rats ($F_{3,44} = 5.6$, $p<0.01$). Latency to respond to the thermal stimulus was increased to 5.5 ± 0.6 sec following 30 mg/kg and to 5.9 ± 0.5 sec following 100 mg/kg ($p<0.05$) thus constituting a significant reversal of hyperalgesia following both doses ($p<0.05$) with maximum effect of 29 ± 7%. Corresponding plasma concentrations immediately following collection of behavioral data were 1786 ± 75 and 5918 ± 155 ng/ml, respectively (Fig. 6C). As previously shown (Magal, 2005), the positive control AMG8163 significantly reduced thermal hyperalgesia relative to vehicle-treated rats ($p<0.01$) with latency increased to 6.6 ± 0.7 sec, constituting a 39 ± 9% effect in this experiment and with corresponding plasma concentration of 20 ± 1 ng/ml.

AMG8562 reduces acetic-acid induced writhing

Since some TRPV1 antagonists have been reported to block acetic acid-induced writhing (Tang et al., 2007), we evaluated AMG8562 in this model (Fig. 6D). In mice, intraperitoneally injected acetic acid caused 36.4 ± 7.8 writhes. AMG8562 significantly reduced the number of writhes ($F_{4,39} = 2.7$, $p<0.05$; Fig. 6D). The number of writhes observed in mice administered with 10, 30, and 100 mg/kg of AMG8562 were 19.22 ± 5.3, 14.8 ± 5.7, and 15.0 ± 5.9, respectively. The corresponding plasma concentrations were 879 ± 111, 3024 ± 268, and 11029 ± 1094 ng/ml, respectively. Although there is a trend of decreased acetic acid-induced writhing relative to vehicle for all treatment groups, only 30 mg/kg of AMG8562 and the positive control, ibuprofen, showed statistically significant reduction ($p<0.05$). The percent reduction in writhing counts was 59% following AMG8562 30 mg/kg. Overall variability was high in this assay with SEM accounting for 21% of mean writhing counts in the vehicle group (compared to SEM accounting for 9.2% and 7.2% of mean latency in the CFA- and skin incision-induced

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thermal hyperalgesia assays, respectively) thus decreasing the power to detect significant drug effects.

AMG8562 does not affect most open field activity parameters in mice and rats

Since it is possible that compounds that reduce locomotor activity could appear as "false positive" anti-hyperalgesics or analgesics in pain models, we evaluated AMG8562 in open field locomotor activity tests. Total distance traveled following vehicle treatment in mice was 19,297 ± 2012 cm in mice and 15,212 ± 1980 cm in rats. There was no significant effect of 100 mg/kg (corresponding plasma concentration was 5416 ±445 ng/ml) of AMG8562 on total distance traveled in mice ($F_{4,33} = 1.2$, $p>0.05$) (Fig. 7A). There was no significant effect of 100 mg/kg (corresponding plasma concentration was 5659 ±734 ng/ml) of AMG8562 on total distance traveled in 60 min in rats ($F_{3,28} = 0.31$, $p>0.05$; Fig. 7B). There was a significant 17.5 ± 1.6% increase in rest time in rats following 100 mg/kg of AMG8562 ($F_{3,28} = 3.0$, $p<0.05$) but not in mice ($F_{3,26} = 1.5$, $p>0.05$) (data not shown). In both rats and mice, there was no significant effect on any other open field parameters analyzed including: rest time, rearing (counts or seconds), fine movements, basic movements, and immobility (data not shown). These results suggest that AMG8562 efficacy in rat or mouse pain models is not confounded by locomotor impairment or sedation.

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Discussion

TRPV1 antagonists are being considered as next generation pain therapeutics because of their anti-hyperalgesic effects in rodent models of inflammation and cancer (reviewed in (Immke and Gavva, 2006; Szallasi et al., 2007)). In the present study, we have characterized four compounds that exhibited differential modulation of TRPV1 activation by capsaicin, pH 5 and heat. Similar to previous findings (Gavva et al., 2007b), 'Profile A' (AMG8163) and 'Profile B' (AMG8563) modulators of TRPV1 caused hyperthermia in rats. Here, we have identified two additional groups of modulators, Profile C (AMG8562) that blocks capsaicin but not heat activation, and potentiates pH 5 activation of TRPV1, and 'Profile D' (AMG7905) that blocks capsaicin activation and potentiates both heat and pH 5 activation of TRPV1. Interestingly, AMG8562 did not cause hyperthermia, whereas AMG7905 caused marked hypothermia. In addition, other compounds that exhibit the same profile as AMG7905 also caused marked hypothermia (data not shown). Relative to the AMG8562 effects, the marked hypothermia of 3º C elicited by AMG7905 was considered undesirable, hence, it was decided that 'Profile D' is not suitable for evaluation of its antihyperalgesic effects in pain models. Since our goal was to evaluate the feasibility of separating the hyperthermic effect of TRPV1 modulation from the anti-hyperalgesic effects, we have further characterized AMG8562 in rodent models of pain and show that AMG8562 acts as an antihyperalgesic and does not cause hyperthermia in rats. It is important to note here that AMG8562 behaved as a 'Profile B' antagonist at human TRPV1, a profile that was reported to cause hyperthermia (Gavva et al., 2007b). Hence, it is expected that AMG8562 would cause hyperthermia in humans. The feasibility of Profile C modulation of both rat and human TRPV1 by a single small molecule is yet to be demonstrated.

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Previously, we demonstrated that competitive antagonists of capsaicin that block all modes of TRPV1 activation block pH 5 and heat activation allosterically (Gavva et al., 2005a). This suggested that the allosteric inhibition of pH 5 and heat activation depends on the compound's ability to lock the channel conformation in the closed or non-conducting state (Gavva et al., 2005a). In fact, compounds such as SB-366791 appeared to promote conformations that result in potentiation of pH 5 activation (Gavva et al., 2005a). All four compounds described here interact with the capsaicin binding pocket on TRPV1 and are potent antagonists of capsaicin activation. However, based on their ability to allosterically potentiate pH 5 and/or heat activation, we have defined two new profiles (Profiles C and D). For example, AMG8562 (Profile C compound that blocks capsaicin activation and potentiates pH 5 activation) may be considered as a competitive antagonist at the capsaicin binding pocket and as a positive allosteric modulator for proton activation. Similarly, AMG7905 ('Profile D' compound that blocks capsaicin activation and potentiates both pH 5 and heat activation) may be considered as a competitive antagonist at the capsaicin binding pocket and as a positive allosteric modulator for both proton and heat activation.

Since AMG8562 is a selective modulator of rodent TRPV1, did not cause hyperthermia, and is orally bioavailable, we evaluated it by oral administration in an on-target challenge model (capsaicin-induced flinch), CFA- and surgical incision-induced thermal hyperalgesia in rats, and acetic-acid induced writhing in mice. As expected for potent capsaicin antagonists (Gavva et al., 2007a; Gavva et al., 2007b), AMG8562 blocked capsaicin-induced flinching potently. Similar to previous reports of TRPV1 antagonists

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(Gavva et al., 2005b; Honore et al., 2005; Magal, 2005; Gavva et al., 2007a), AMG8562 partially reversed thermal hyperalgesia in inflammatory (CFA) and acute (surgical-incision) pain models as well as acetic acid-induced writhing. Overall, these studies with AMG8562 suggest that hyperthermic effects can be separated from anti-hyperalgesic effects of TRPV1 modulation. Mechanisms of the AMG8562 anti-hyperalgesic effect in CFA and surgical-incision models and the blockade of acetic acid-induced writhing by AMG8562 may differ. For example, AMG8562 blocks capsaicin, anandamide and NADA activation and by inference it also blocks the other putative endogenous ligands, such as 12-hydroperoxyeicosatetraenoic acid and oleolyldopamine. It is possible that the increased concentrations of endogenous ligands and phospholipase C mediated activation of TRPV1 may predominantly contribute to inflammation or injury related hyperalgesia. Therefore, the anti-hyperalgesic effect of AMG8562 in inflammatory and injury-induced pain models may represent blockade of the endogenous activation mechanisms of TRPV1 *in vivo*. The efficacy in the acetic acid-induced writhing in mice could be mediated by AMG8562-induced desensitization of the visceral TRPV1 channels, since i) AMG8562 potentiates pH 5 activation of both rat and mouse TRPV1, ii) the mechanisms of acetic acid-induced writhing may include activation of visceral TRPV1 channels, and iii) visceral TRPV1 channels are tonically activated *in vivo* (Steiner et al., 2007). Identification of more Profile C compounds, evaluating them in different pain models, and detailed mechanistic studies *in vivo* should reveal more accurate mechanism(s) of action for this new class of compounds.

TRPV1 selective antagonist reversal of inflammation-induced hyperalgesia ranged from 35-50% in our experimental conditions ((Magal, 2005; Gavva et al., 2007a; Wang et al.,

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2007) and this study), which were conducted in a blinded fashion that involved randomization of dose groups and investigators that measured hyperalgesia not participating in dosing of compounds. Since these conditions eliminate subjectivity to the greatest degree possible and give the most stringent results, we believe that TRPV1 partially contributes to inflammatory pain.

The minimally effective dose of AMG8163 to block capsaicin-induced flinch was 0.03 mg/kg, which corresponds to plasma concentration of 2.6 ng/ml (Gavva et al., 2007a), and the minimally effective dose of AMG8562 to block capsaicin-induced flinch was 1 mg/kg, which corresponds to plasma concentration of 120 ng/ml (this study, Fig.5). There is a 46-fold higher plasma concentration requirement of AMG8562 for comparable efficacy. However, AMG8163 is only approximately two-fold more potent at TRPV1 and protein binding of AMG8562 and AMG8163 are comparable. One possible reason for the efficacy difference may be that AMG8562 blocks only putative endogenous ligands and AMG8163 blocks all modes of TRPV1 activation, and therefore, a higher dose of AMG8562 may be required to show comparable efficacy. Alternately, differential distribution of AMG8163 and AMG8562 into organs and tissues, such as central nervous system may contribute to different dose requirements for each compound. The minimally effective dose of AMG8163 to cause hyperthermia was 0.1 mg/kg (Tamayo et al., 2008). It is impressive that AMG8562 did not cause hyperthermia up to 100 mg/kg dose that is 1000-fold higher compared to AMG8163. An important point to note here that AMG8562 blocked TRPV4 (IC_{50} value: ~3 μM) in addition to modulating TRPV1 and it is not known if TRPV4 antagonism contributed to lack of hyperthermia or to anti-hyperalgesia. Detailed characterization of additional Profile C compounds should provide better

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understanding of efficacy (anti-hyperalgesia) and hyperthermia relationship (or lack thereof) for Profile C compounds.

The unique profile of AMG8562 indicates that it is feasible to eliminate hyperthermia while preserving antihyperalgesia by differential modulation of distinct modes of TRPV1 activation. Although all four molecules studied here were identified based on their ability to block capsaicin activation of TRPV1, it is their differential pharmacology at pH 5 and heat activation that helped us to discover that Profile C modulation of TRPV1 does not cause hyperthermia (summarized in Table 3). Potentiation of pH 5 activation alone appear to negate the classic TRPV1 blockade (profile A or B) mediated hyperthermia. Further, potentiation of both pH 5 and heat activation of TRPV1 causing marked hypothermia *in vivo* suggests Profile D modulators act as functional agonists with respect to their effects on body temperature.

The TRPV1 blockade elicited hyperthermia is a major hurdle for the development of 1st generation TRPV1 modulators (TRPV1 selective Profile A antagonists) as therapeutics because: i) Pharmacological blockade of TRPV1 elicited marked hyperthermia in healthy humans (Gavva et al., 2008), ii) TRPV1 antagonist elicited hyperthermia did not show clear attenuation in humans (Gavva et al., 2008), and iii) Some individuals are more susceptible to marked and persistent hyperthermia after TRPV1 blockade (Gavva et al., 2008). Potential of developing the 2nd generation modulators such as Profile C compounds as therapeutics is yet to be evaluated. Before considering Profile C modulators for clinical development, further studies are required to understand: i) If the modest efficacy and high exposure requirement of AMG8562 are common to Profile C

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compounds, ii) if it is possible to discover more efficacious Profile C compounds, and if they cause hyperthermia, iii) if Profile C compounds cause toxicities by damaging the viscera or gastric mucosa, and finally, iv) if it is possible to invent a compound that exhibits Profile C modulation of both rodent and human TRPV1.

Acknowledgements: We thank Yunxin Bo, Lillian Liao, Markian Stec, Chenera Balan, Partha Chakrabarti, and Phi Tang for synthesis of these compounds: AMG8163, AMG8562, AMG8563 and AMG7905.

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Legends for Figures

Figure 1. Chemical structures of compounds used in these studies: AMG8163, AMG8562, AMG8563, AMG7905.

Figure 2. Characterization of *in vitro* profiles of different modulators against rat TRPV1. AMG8163, a Profile A (Group A) modulator effects on capsaicin (0.5 μM), pH 5 and heat (45 ºC) activation (**A-C**). AMG8563, a Profile B modulator effects on capsaicin (0.5 μM), pH 5 and heat (45 ºC) activation (**D-F**). AMG8562, a Profile C modulator effects on capsaicin (0.5 μM), pH 5 and heat (45 ºC) activation (**G-I**). AMG9705, a Profile D modulator effects on capsaicin (0.5 μM), pH 5 and heat (45 ºC) activation (**J-L**). Each point in the graph is an average ± SD of an experiment conducted in triplicate.

Figure 3. Effect on core body temperature of AMG8562, AMG8563, AMG8162 and AMG7905. Data are presented as mean ± SEM every 10 min. Statistical significance is relative to vehicle (ANOVA, Dunnett's post-hoc test). Baseline body temperature was measured for 20 min prior to compound administration at time zero min post injection. (A) AMG8563 dosed at 3 mg/kg i.v. significantly increased temperature relative to vehicle with a maximum of 1.2 ºC occurring at 40 min ($p<0.01$). AMG8163 significantly increased body temperature relative to vehicle with a maximum increase of 1.1 ºC occurring at 120 min ($p<0.01$). AMG8562 dosed at 3 mg/kg i.v. significantly decreased body temperature relative to vehicle with the maximum decrease of 1.0 ºC occurring at 20 min post dosing ($p<0.01$). (B) AMG8562 dosed at 1, 3, 10, 30 mg/kg p.o. significantly decreased

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body temperature with a maximum decrease of 0.6 ºC occurring at 160 min post dosing in the 1 mg/kg dosing group ($p<0.01$). (C) AMG7905 dosed at 0.3, 1, 3, 10, 30 mg/kg p.o. significantly decreased body temperature with a maximum decrease of 2.9 ºC occurring at 160 min post dosing. AMG7905 induced hypothermia returns to baseline temperature within 12 hours. As a positive control, AMG8163 dosed at 3 mg/kg p.o. significantly increased body temperature as expected ($p<0.01$).

Figure 4. Effect of AMG8562 on the activation of different TRP channels. (**A**) Effect of AMG8562 on allyl isothiocyanate-induced increase in intracellular calcium in CHO cells expressing human TRPA1 measured in an aequorin-readout assay. IC_{50} values of the positive control, ruthenium red was 367 nM. (**B**) Effect of AMG8562 on icilin-induced increase in intracellular calcium in CHO cells expressing human TRPM8 measured in an aequorin-readout assay. IC_{50} value of a positive control antagonist, compound M8-B, (N-(2-aminoethyl)-N-((3-(methyloxy)-4-((phenylmethyl)oxy)phenyl)methyl)-2-thiophenecarboxamide), was 5 nM in this assay. (**C**) Effect of AMG8562 on 2-APB-induced $^{45}Ca^{2+}$ uptake into CHO cells expressing rat TRPV2. IC_{50} value of the positive control, ruthenium red was 2028 nM in this assay. (**D**) Effect of AMG8562 on 2-APB-induced increase in intracellular calcium in CHO cells expressing human TRPV3 measured in an aequorin-readout assay. IC_{50} value of the positive control antagonist, compound V3-H, (1-(((5-chloro-1,3-benzothiazol-2-yl)thio)acetyl)-8-methyl-1,2,3,4-tetrahydroquinoline), was 72 nM in this assay. (**E**) Effect of AMG8562 on 4-αPDD-induced increase in intracellular calcium in CHO cells

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expressing human TRPV4 measured in an aequorin-readout assay. IC_{50} value of the positive control, ruthenium red was 24 nM in this assay. AMG8562 did not show partial agonism at TRPA1, TRPM8, TRPV2, TRPV3, or TRPV4 in these assays.

Figure 5. Effects of AMG8562 on capsaicin (0.5 μM), pH 5 and heat (45 ºC) activation of human TRPV1 (**A-C**). Each point in the graph is an average ± SD of an experiment conducted in triplicate. Note that AMG8562 blocks pH 5 activation partially and capsaicin and Heat activation fully making it a Profile B compound.

Figure 6. In vivo efficacy of AMG8562 in biochemical challenge as well as inflammatory and surgical pain models. (**A**) AMG8562 significantly decreased capsaisin-induced flinching behavior ($F_{7,80} = 15.7$, $p<0.01$). There was a near 100% blockade at 3 mg/kg corresponding to a plasma level of 409 ± 21 ng/ml. The positive control, AMG8163 (3 mg/kg) blocked capsaicin-induced flinch completely (100%) (**B**) AMG8562 significantly reversed CFA-induced thermal hyperalgesia ($F_{3,44} = 3.9$, $p<0.05$). Both 30 and 100 mg/kg doses ($p<0.05$) and AMG8163 (3 mg/kg) $p<0.01$ significantly reversed thermal hyperalgesia. (**C**) AMG8562 significantly reversed skin incision-induced thermal hyperalgesia. The overall ANOVA from post-incision latencies is $F_{3,44} = 5.6$, $p<0.01$; both 30 and 100 mg/kg ($p<0.05$) and AMG8163 ($p<0.01$) significantly reduced thermal hyperalgesia. (**D**) AMG8562 reduced acetic acid-induced writhing in mice. The overall ANOVA was $F_{4,39} = 2.7$, $p<0.05$; only 30 mg/kg (n=9; corresponding to a plasma level of 3024 ± 268 ng/ml) and ibuprofen significantly reduced the number

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of writhes ($p<0.05$). Note: AMG8562 showed a similar profile at both rat and mouse TRPV1.

Figure 7. (A) There was no significant effect of AMG8562 on total distance traveled in 60 min in mice ($F_{4,33} = 1.2$, $p>0.05$) with plasma concentration of 5416 ±445 ng/ml. (**B**) There was no significant effect of AMG8562 on total distance traveled in 60 min in rats ($F_{3,28} = 0.31$, $p>0.05$) with plasma concentration of 5659 ±734 ng/ml.

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Table 1. Effect of different compounds used in the study on distinct modes of rat TRPV1 activation. Since putative endogenous ligands are weak agonists, activation by Anandamide and NADA was evaluated at sensitizing pH conditions (pH 6). The IC_{50} values shown are in nanomolar. NA: not available

	AMG8163	AMG8563	AMG8562	AMG7905
Capsaicin	0.6 ± 0.3	2.9 ± 0.49	1.75 ± 0.48	39 ± 17
Anandamide	1.79 ± 0.68	3.97 ± 2.2	9.29 + 1.4	6.26 ± 2.6
NADA	2.36 ± 1.5	6.37 ± 0.9	3.87 ± 1.8	4101 ± 1412
pH 5	0.59 ± 0.3	>4000	potentiator	potentiator
Heat	0.15 ± 0.1	1.46 ± NA	>4000	potentiator

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Table 2: Effect of 10 μM AMG8562 on the specific radio-ligand binding to the receptors, ion channels, or enzymes studied. Ki values are shown in parenthesis for compounds showed greater than 50% inhibition at 10 μM.

Target	**Ligand**	**% inhibition of Specific binding (Ki)**	
A2A *(h)*	[3H]CGS 21680	-16	
α1 (non-selective)	[3H]prazosin	1	
α2 (non-selective)	[3H]RX 821002	-2	
β1 *(h)*	[3H](-)CGP 12177	20	
AT2 *(h)*	[125I]CGP 42112A	-3	
B1 *(h)*	[3H]desArg10-KD	9	
B2 *(h)*	[3H]bradykinin	-2	
CGRP *(h)*	[125I]hCGRPα	2	
CB1 *(h)*	[3H]CP 55940	53	(9.5 μM)
CB2 *(h)*	[3H]WIN 55212-2	26	
CCKA *(h)*(CCK1)	[125I]CCK-8	70	(16 μM)
D1 *(h)*	[3H]SCH 23390	22	
D2S *(h)*(agonist site)	[3H]7-OH-DPAT	15	
D3 *(h)*(agonist site)	[3H]7-OH-DPAT	10	
ETA *(h)*	[125I]endothelin-1	6	
GABA (non-selective)	[3H]GABA	-3	
GAL2 *(h)*	[125I]galanin	10	
CXCR2 *(h)*(IL-8B)	[125I]IL-8	17	

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CCR1 *(h)*	[125I]MIP-1α	-13	
Ghrelin *(h)*(GHS)	[125I][His]-ghrelin	-13	
H1 *(h)*	[3H]pyrilamine	-3	
H2 *(h)*	[125I]APT	9	
H3 *(h)*	[3H]Nα-Me-histamine	15	
H4 *(h)*	[3H]histamine	1	
LTB4 *(h)*(BLT1)	[3H]LTB4	14	
LTD4 *(h)*(CysLT1)	[3H]LTD4	8	
Edg-2 (LPA) *(h)*	[3H]LPA	15	
MC4 *(h)*	[125I]NDP-α-MSH	17	
MT1 *(h)*	[125I]iodomelatonin	81	(2.9 μM)
M1 *(h)*	[3H]pirenzepine	33	
M3 *(h)*	[3H]4-DAMP	9	
NK1 *(h)*	[125I]BH-SP	25	
N (neuronal)			
(α-BGTXinsensitive) (α4β2)	[3H]cytisine	-4	
K (KOP)	[3H]U 69593	53	(14 μM)
μ *(h)*(MOP)(agonist site)	[3H]DAMGO	15	
PAF *(h)*	[3H]C16-PAF	4	
PCP	[3H]TCP	18	
TXA2/PGH2 *(h)* (TP)	[3H]SQ 29548	40	
P2X	[3H]α,β-MeATP	8	
P2Y	[35S]dATPαS	0	
5-HT1A *(h)*	[3H]8-OH-DPAT	8	

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5-HT2B *(h)*(agonist site)	[125I](±)DOI	82	(8.9 µM)
5-HT3 *(h)*	[3H]BRL 43694	2	
Sst (non-selective)	[125I]Tyr11-somatostatin-14	12	
VIP1 *(h)*(VPAC1)	[125I]VIP	-1	
VIP2 *(h)*(VPAC2)	[125I]VIP	5	
V1a *(h)*	[3H]AVP	5	
Ca2+ channel (L, DHP site)	[3H](+)PN 200-110	69	(1.7 µM)
Ca2+ channel (N)	[125I]ω-conotoxin GVIA	-3	
Na+ channel (site 2)	[3H]batrachotoxinin	52	(3.2 µM)

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Table 3. Summary of TRPV1 modulator profiles and their effects on body temperature. All TRPV1 modulators block capsaicin activation but differentially affect pH 5 and heat activation of TRPV1 are grouped in to different profiles A through D.

	Capsaicin	**pH 5**	**Heat**	**Body temp**
Profile A	Block	Block	Block	↑↑
Profile B	Block	Partial block	Block	↑↑
Profile C	Block	Potentiate	Block	- / ↓
Profile D	Block	Potentiate	Potentiate	↓↓↓

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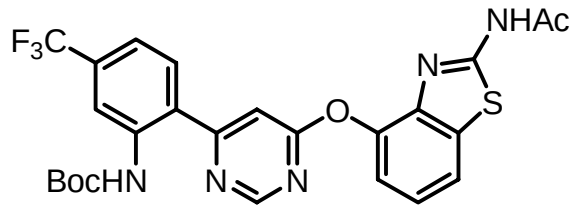
Figure 1



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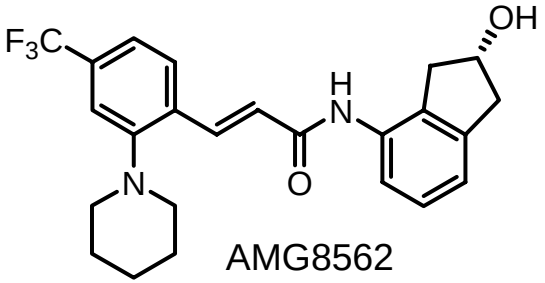

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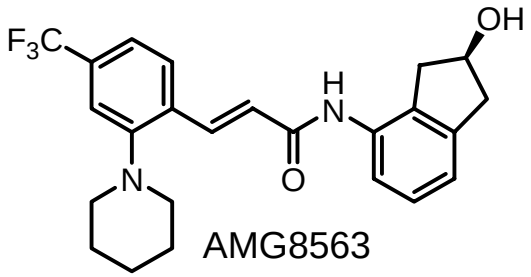

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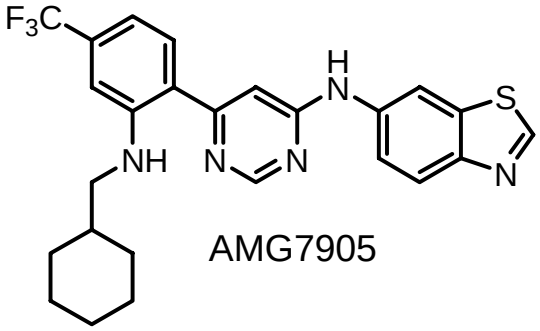

AMG7905

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Figure 2

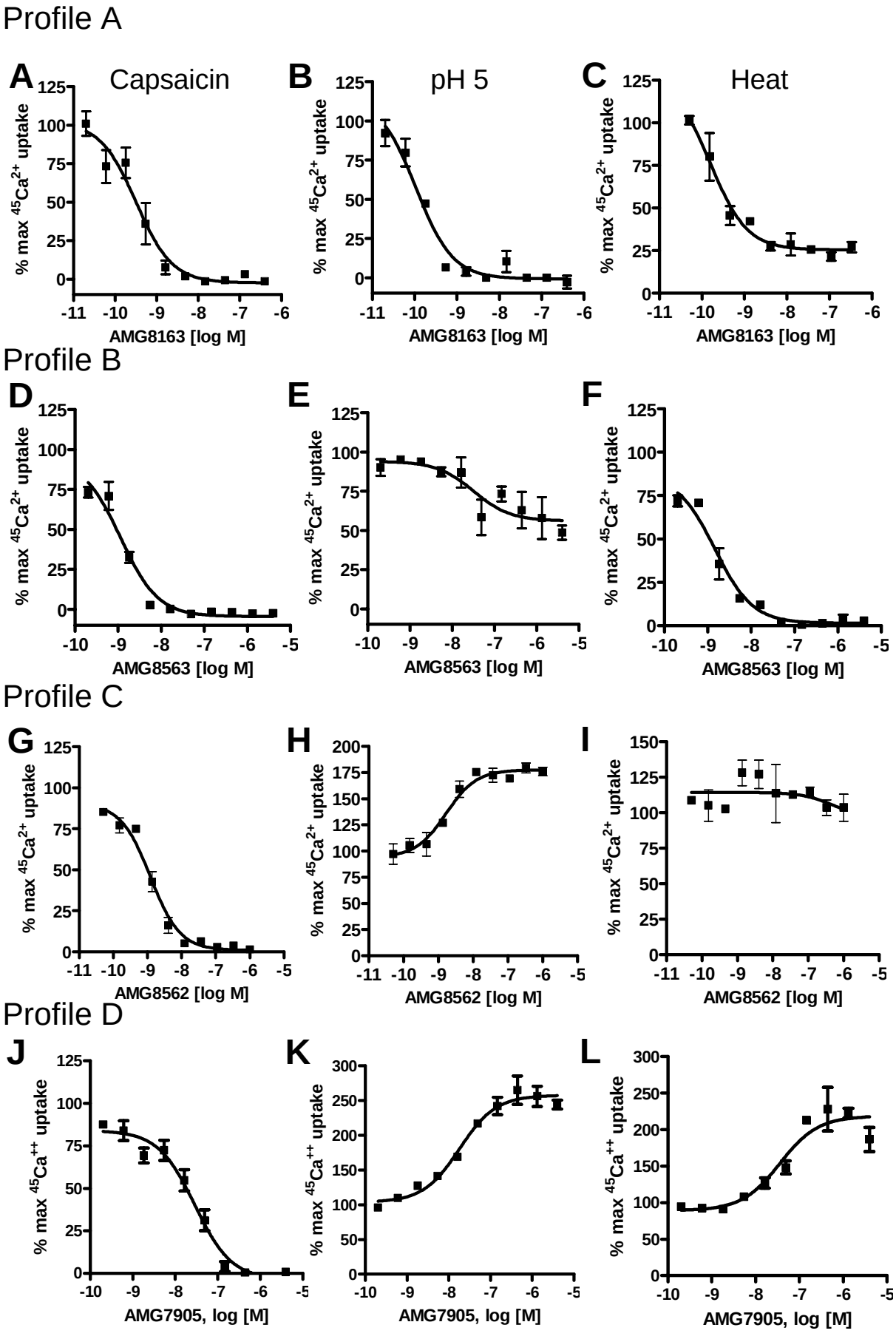

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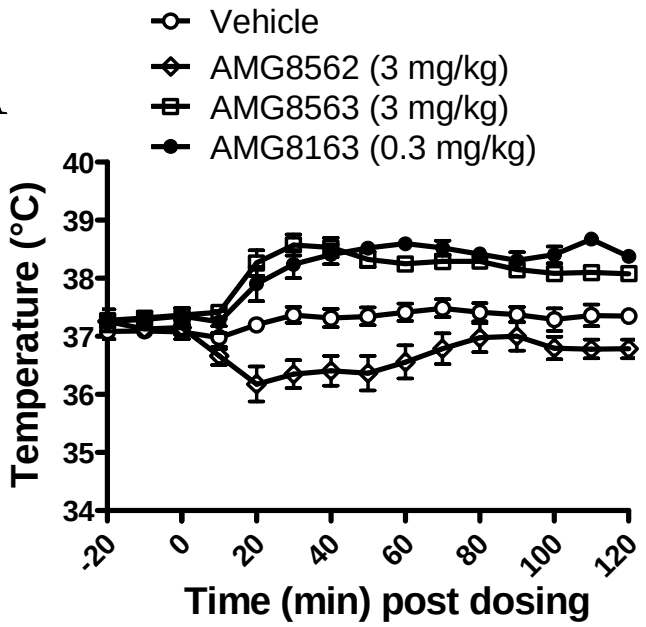
Figure 3

A



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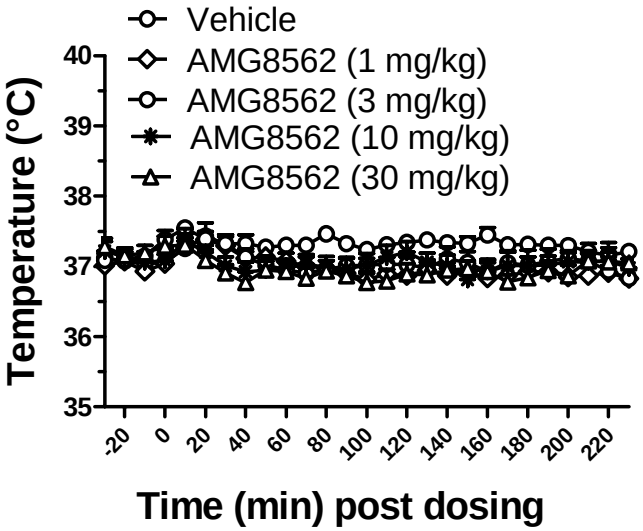

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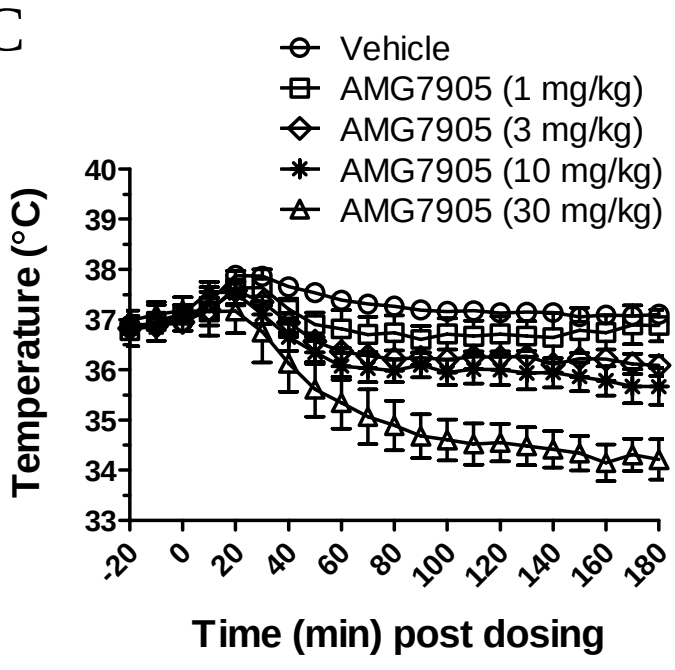

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Figure 4

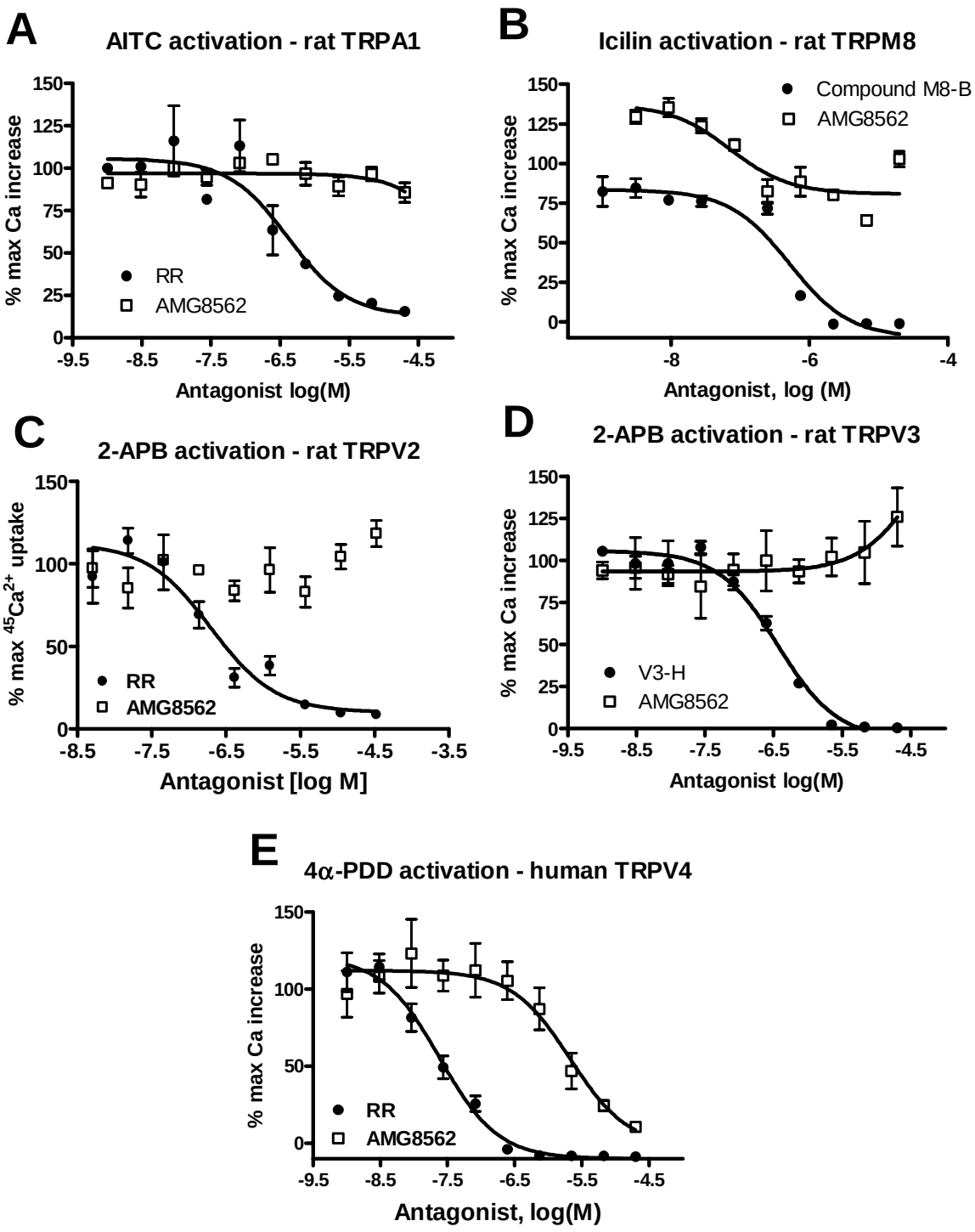

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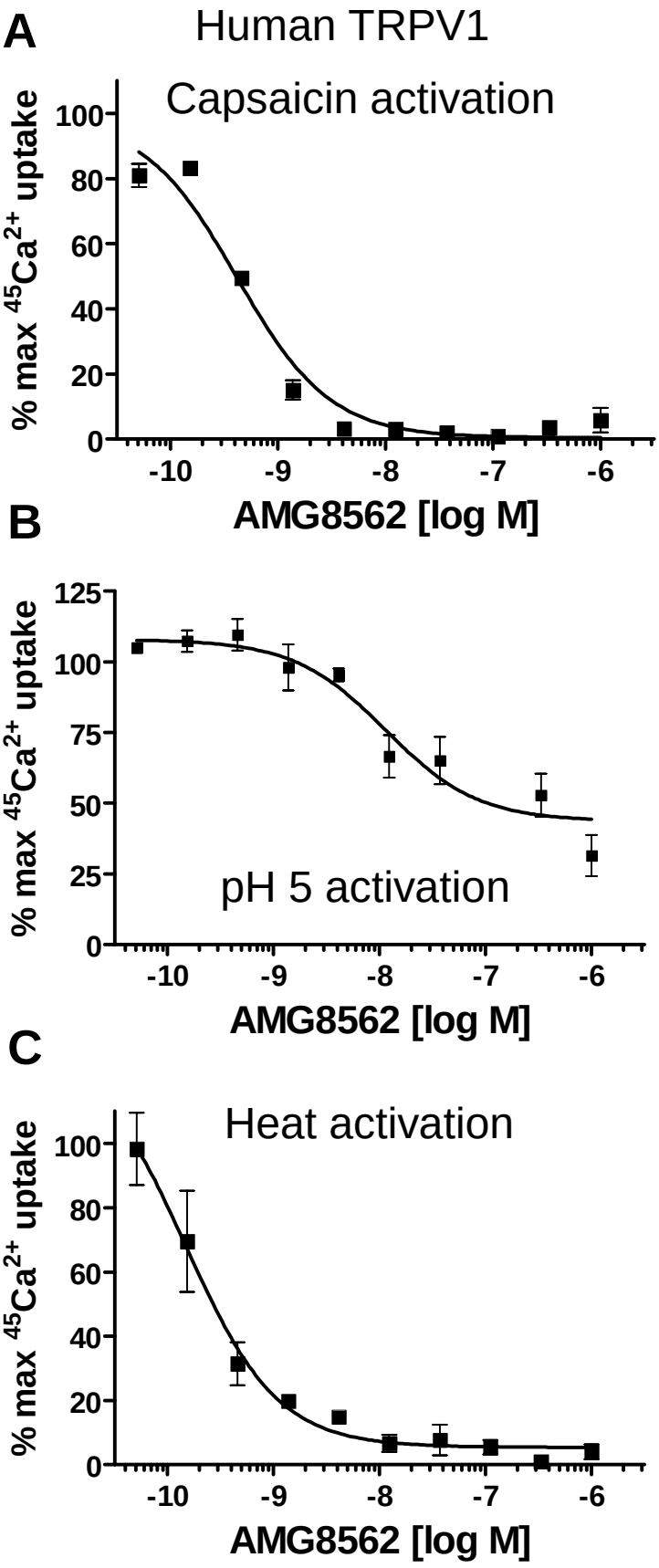
Figure 5



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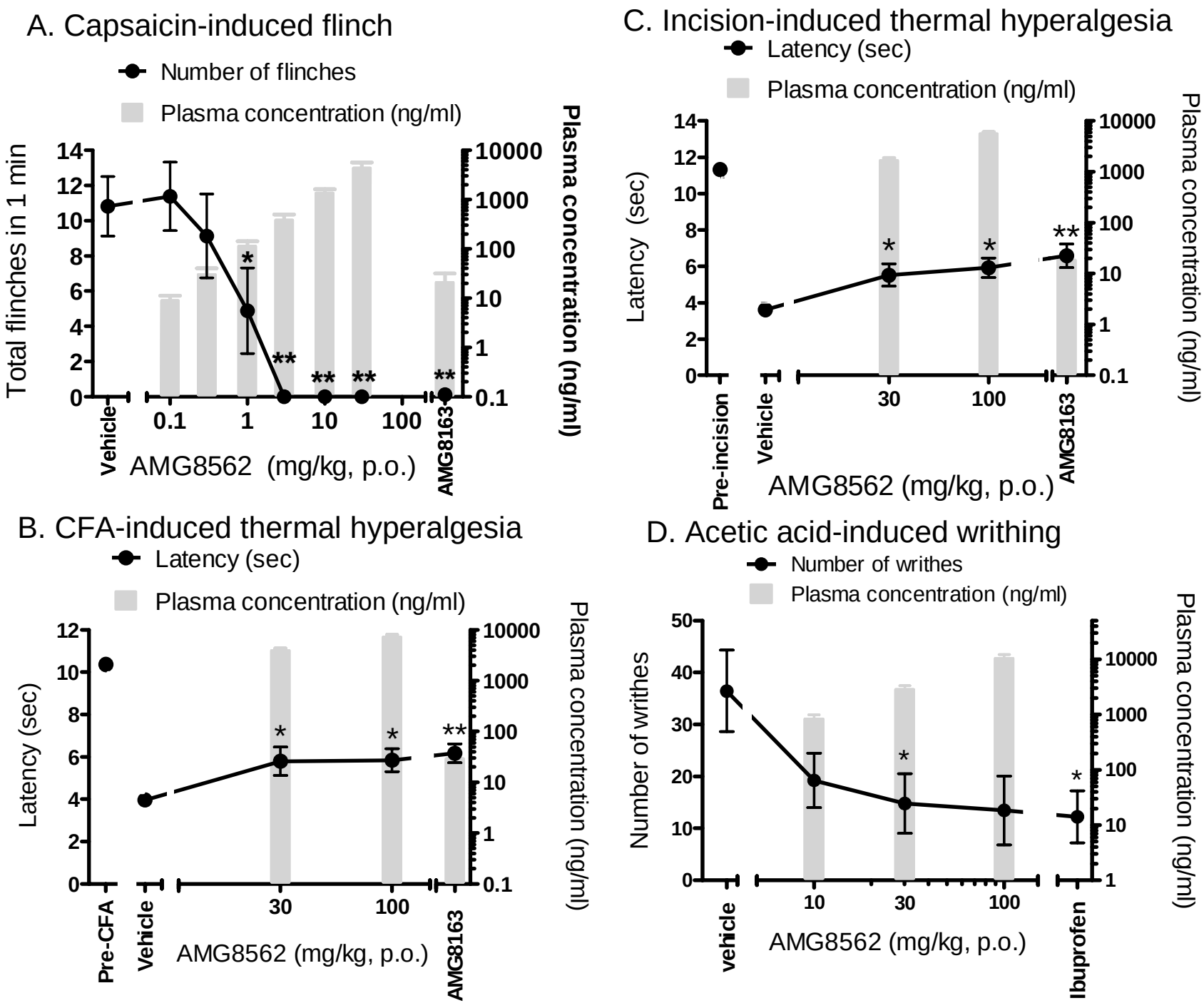

Figure 6

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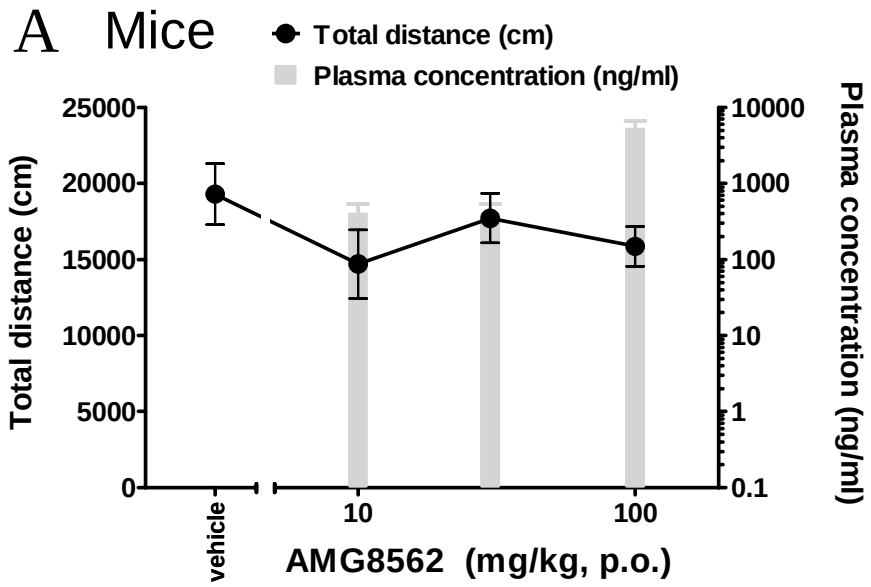
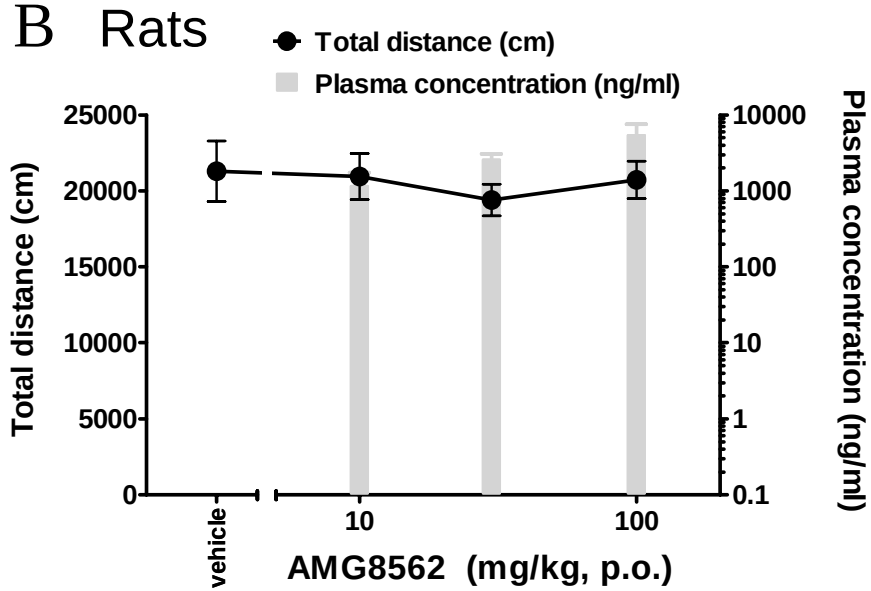
Figure 7





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