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Lipoic-Based TRPA1/TRPV1 Antagonist to Treat Orofacial Pain

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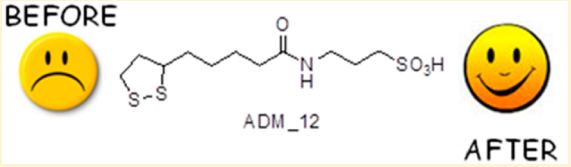
Supporting Information

ABSTRACT: Inflammation of the trigeminal nerve is considered one of the most painful conditions known to humankind. The diagnosis is often difficult; moreover, safe and effective pharmacological treatments are lacking. A new molecule, ADM_12, formed by a lipoic and omotaurine residues covalently linked, is here reported. In vitro and in vivo tests showed that ADM_12 is a very attractive original compound presenting (i) a remarkable safety profile; (ii) a high binding constant versus TRPA1; (iii) an intriguing behavior versus TRPV1; and (iv) the ability to significantly and persistently reduce mechanical facial allodynia in rats. Noteworthy, by testing ADM_12, we shed light on the unprecedented involvement of TRPA1 and TRPV1 channels in orofacial pain.



KEYWORDS: *Lipoic acid, trigeminal pain, antiallodynic agents, TRP channels*

Pain is the subjective, evolved response to noxious stimuli[1] deputed to alert the individual from potential sources of tissue destruction. Typically, local injury or infections trigger the release of peripheral chemical mediators, which activate the local inflammatory response and sensitize *nociceptors* (neurons responsible for the transmission of pain), enhancing their response to pain. Orofacial pain is an unpleasant emotional experience, which affects a large proportion of the general population. It can originate from either hard or soft tissues of the head, neck, face, or mouth;[2] hence, an accurate diagnosis is often not trivial. In addition, since the pharmacological treatment of orofacial pain is far from satisfying, it represents a significant healthcare issue. This is the reason why the International Association for the Study of Pain (IASP) devoted the year 2014 as the Global Year Against Orofacial Pain. Temporomandibular joint (TMJ) disorders are among the most common causes of orofacial pain which, in this case, mostly originates from TMJ inflammation. As a consequence, the sensitization of trigeminal ganglion (TG) nociceptors occurs, which, in turn, gives rise to cutaneous allodynia at head and facial districts: the trigeminal neuralgia (TGN).[1,3]

Transient receptor potential (TRP) channels are a large family of nonselective cation channels.[4] Several TRP channel family members, including TRP cation channel subfamily V member 1 (TRPV1), subfamily A member 1 (TRPA1), and TRP channel melastatin 8 (TRPM8), are expressed in TG somatosensory neurons, which also project within the oral and nasal cavities and are deputed to detect a great deal of external stimuli, including pressure, temperature, and chemicals.[5] In particular, the TRPA1 channel seems to play a pivotal role in the generation and maintenance of inflammatory pain.[6] As a matter of fact, the TRPA1 antagonists HC-030031[7] and AP-18[8] significantly reduce mechanical hypersensitivity due to Complete Freund's Adjuvant (CFA) injection in the paw[7,8] and, conversely, a number of inflammatory agents, such as nerve growth factor (NGF) or proteinase-activated receptor-2 (PAR2),[9,10] can sensitize TRPA1 channels. Moreover, TRPA1-knockout mice displayed a significant reduction of formalin-evoked pain behavior.[11] The TRPV1 channel is the heat transducer of our body,[12] and it is activated by inflammatory mediators and tissue damaging stimuli.[13] TRPM8 is the molecular target of menthol[14] and is also involved in injury- or inflammation-evoked hypersensitivity to cold as well as in cold-mediated analgesia.[15]

Despite all the efforts devoted to the discovery of new analgesics and in particular of compounds active on both inflammatory and neuropatic pain, a real improvement still lags behind. Thus, capitalizing on recent results[16] about the ability of the synthetic TRPA1 antagonist ADM_09 (Scheme 1) to revert oxaliplatin-induced neuropatic pain, and given the evident role of TRP channels in TG-related pain, we report herein on the synthesis of a new lipoic-containing antagonist, namely, ADM_12. This water-soluble small molecule, structurally simpler and more stable than ADM_09, showed (i) a remarkable safety profile; (ii) a low inhibition constant (IC_{50}) versus TRPA1; (iii) a double behavior versus TRPV1; and (iv) a significant reduction of mechanical facial allodynia in rats.

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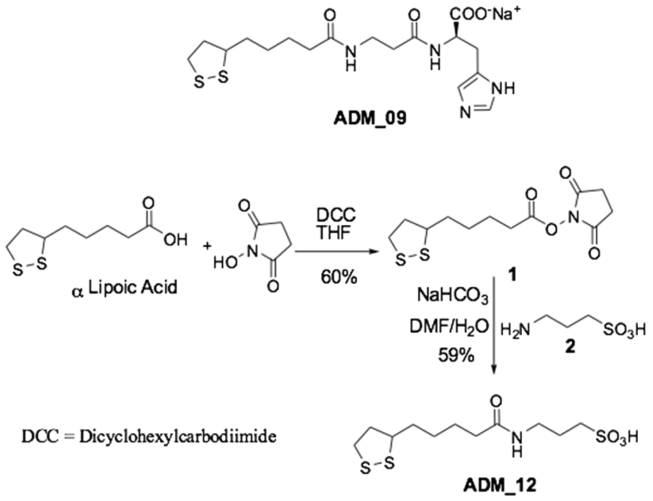
Scheme 1. Structure of ADM_09 and Synthesis of ADM_12



RESULTS AND DISCUSSION

Under very mild conditions, ADM_12 was synthesized in two steps with good yield. Commercially available (±) α-lipoic acid was treated with *N*-hydroxysuccinimide in tetrahydrofuran (THF) and in the presence of dicyclohexylcarbodiimide (DCC) to give the reactive lipoic derivative **1**[16] which, after crystallization, was reacted at room temperature with omotaurine **2** in H_2O/DMF as solvent to afford, after purification, ADM_12 as a white powder (59% yield) (Scheme 1).

The potencies of ADM_09 and ADM_12 as TRPA1 antagonists were compared by running patch-clamp experiments using covalent (allyl isothiocyanate from mustard oil, AITC) and noncovalent (menthol at low concentration) TRPA1 activators. Graded drug concentrations (0.1−100 μM) were applied to the cell culture (see the Supporting Information), recording the resulting whole-cell current measured during voltage ramps protocol from −100 to +100 mV repeated every 2 s (Figure 1C−E). The IC50 values

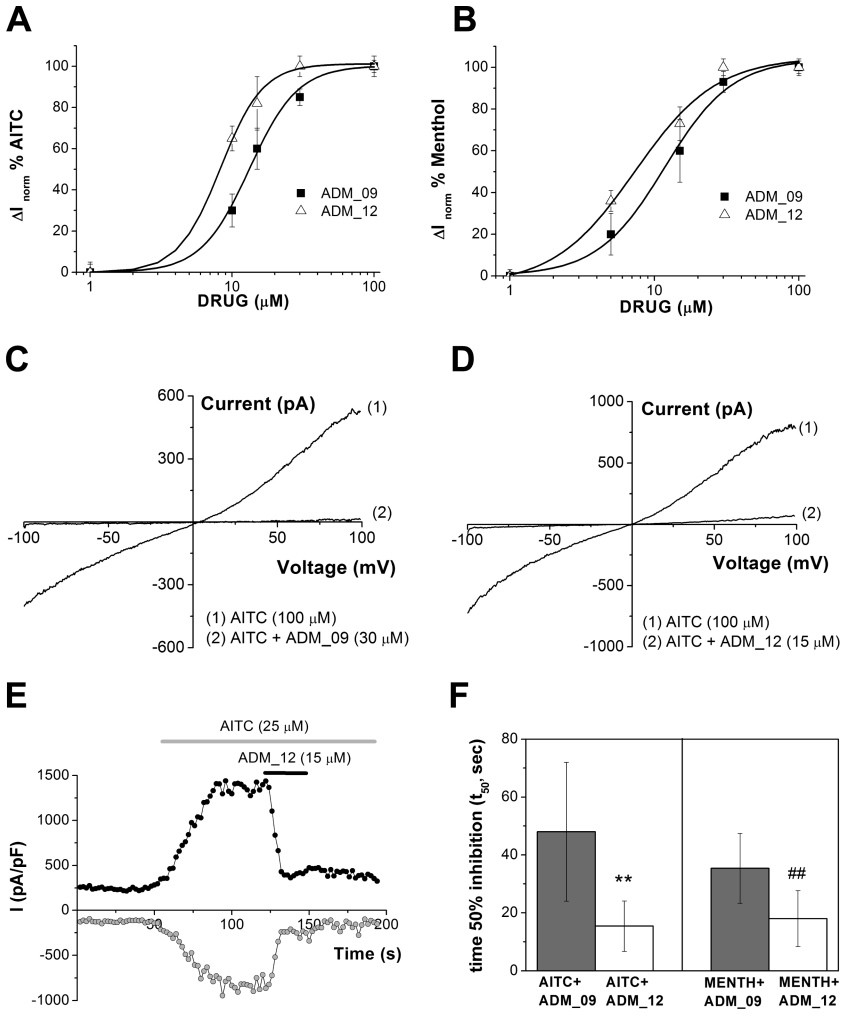


Figure 1. (A) Dose−response curve for the inhibition of 100 μM AITC-induced currents by ADM_09 and ADM_12. (B) Dose−response curve for the inhibition of 30 μM menthol-induced currents by ADM_09 and ADM_12. (C, D) Representative *I*−*V* traces of whole-cell currents through TRPA1-CHO transfected cells obtained with voltage ramps from −100 to +100 mV, activated by allyl isothiocyanate (AITC; 100 μM), followed by ADM_12 (15 μM) (C) or ADM_09 (30 μM) (D). (E) Representative time courses of whole-cell currents through TRPA1-CHO transfected cells measured at membrane potential of +50 mV (black curve) and −50 mV (gray curve), during administration of AITC and ADM_12 at the indicated concentrations and time intervals (see Supporting Information, ref S6). (F) t_{50} for ADM_09 and ADM_12, after TRPA1 activation by AITC (25 μM) or menthol (30 μM). $^{**}p < 0.05$, $^{\#\#}p < 0.005$.

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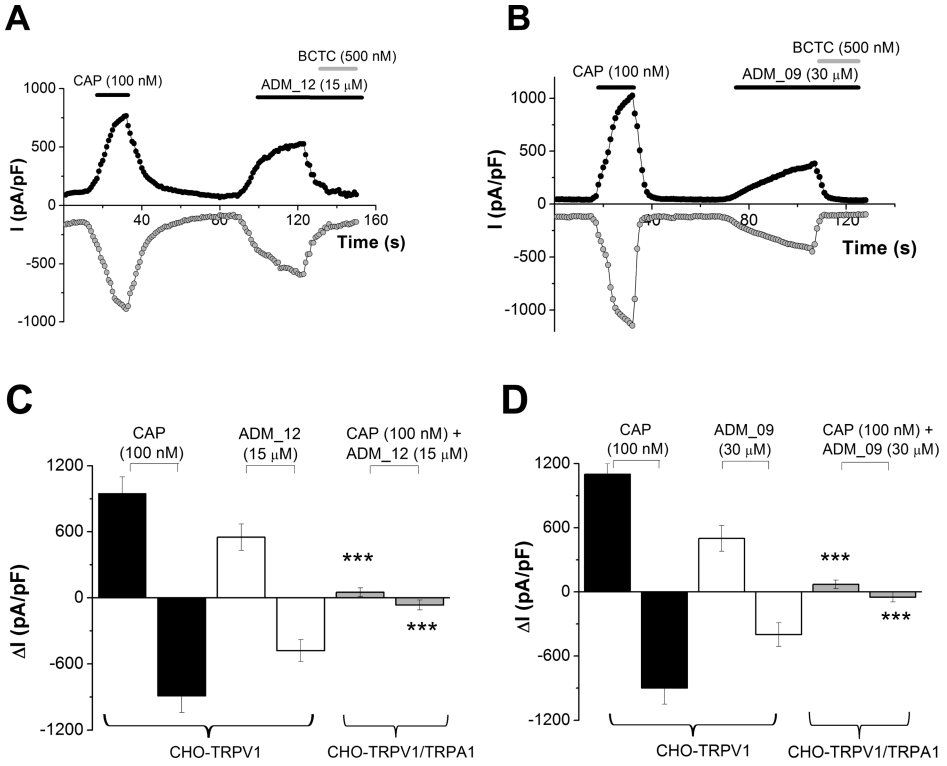

Figure 2. (A, B) Representative time courses of whole-cell currents through TRPV1-CHO transfected cells measured at membrane potential of +50 mV (black curve) and −50 mV (gray curve), activated by CAP (100 nM) and ADM_12 (15 μM), and CAP (100 nM) and ADM_09 (30 μM), respectively. (C, D) Pooled data of whole-cell currents through CHO cells expressing TRPV1 (CHO-TRPV1) or CHO cells coexpressing TRPA1/TRPV1 channels (CHO-TRPA1/TRPV1) evoked by CAP, ADM_12, and CAP + ADM_12 (C) or CAP, ADM_09, and CAP + ADM_09 (D). Each column represents mean ± SEM of $n > 5$ cells. ***$p < 0.001$ vervus CAP (unpaired Student's t test).

obtained for ADM_09 and ADM_12, after activation of TRPA1 by AITC, were 13.2 ± 0.5 μM and 8.2 ± 0.8 μM, respectively (Figure 1, A). The IC50 value for the inhibition of 30 μM menthol-induced currents were 10.3 ± 1.7 μM for ADM_09 and 7.3 ± 2.1 μM for ADM_12 (Figure 1, B). This means that ADM_12 is a more effective TRPA1 antagonist than ADM_09.

Of note, once activated, the time required to block 50% of the activity of TRPA1 channel (t_{50}) depended on the agonist used (AITC or menthol) as well as on the antagonist tested (ADM_09 or ADM_12) (see Figure 1F). A certain variability from cell to cell was observed for the kinetics of TRPA1 blockage which was likely due to possible differences in the patch size or geometries; nonetheless, we can reasonably conclude that ADM_12 blocks the TRPA1 channels faster than ADM_09.

Since TRPA1 channels coexist in TG somatosensory neurons with other TRP channels (see above), we explored the effect of ADM_09 and ADM_12 on TRPV1, TRPM8, and TRPV4 channels as well. Whole-cell patch clamp data showed that ADM_9 and ADM_12 have no effect on CHO cells expressing-TRPM8 channel, activated by menthol (100 μM; see Supporting Information Figure S1A, B), and a very small modulatory effect on CHO cells expressing-TRPV4 channel, activated by the specific agonist GSK1016790A (100 nM; see Supporting Information Figure S1C, D). Only data related to ADM_12 are shown.

On the contrary, both ADM_09 and ADM_12 elicited outward and inward currents in TRPV1 channels, which were blocked by the TRPV1 antagonist BCTC[17] (500 nM) (Figure 2A, B). Furthermore, as there is evidence for a TRPV1-TRPA1 interlink in the generation of pain and neurogenic inflammation,[18] we tested the effect of ADM_09 and ADM_12 on TRPA1/TRPV1-coexpressing cells. Interestingly, we observed that both ADM_09 and ADM_12 behave as dual TRPA1 and TRPV1 antagonists; indeed, they do block the current elicited by the TRPA1 agonist AITC, and by capsaicin (CAP), a specific TRPV1 agonist (Figure 2C, D).

Finally, we evaluated the effect of ADM_09 and ADM_12 on rat TG neurons, fully confirming the results obtained with transfected cells. As expected, capsaicin-sensitive TG neurons expressing TRPV1, but not TRPA1 channels,[19] are activated by both ADM_09 and ADM_12 (see Supporting Information Figure S2). Noteworthy, the two compounds blocked currents induced by AITC or capsaicin in neurons coexpressing TRPA1 and TRPV1, acting as antagonists of both channels (Figure 3).

Recent investigations on TRPA1/TRPV1 expressed on heterologous systems[20] or trigeminal neurons[21] suggested that the two channels could form heteromultimeric complexes. Keeping this in mind, on the basis of our data, we might hypothize that ADM_09 and ADM_12 likely act as modulators of a TRPA1/TRPV1 heterodimer. In fact, only in the presence of both the ion channels, ADM_09 and ADM_12 are capable to block AITC and capsaicin-elicited currents. Conversely, if only TRPA1 channel is expressed, ADM_09 and ADM_12 are able to block MO- and menthol-activated TRPA1 current, thus acting as a TRPA1 antagonist with different affinities; if only TRPV1 channel is expressed, the two lipoic derivatives act as TRPV1 agonists.

The toxicity of ADM_12 was assessed in a 300−3000 mg/kg dose range. Measures of neurological and motor functions were evaluated over time after per os administration (see Supporting

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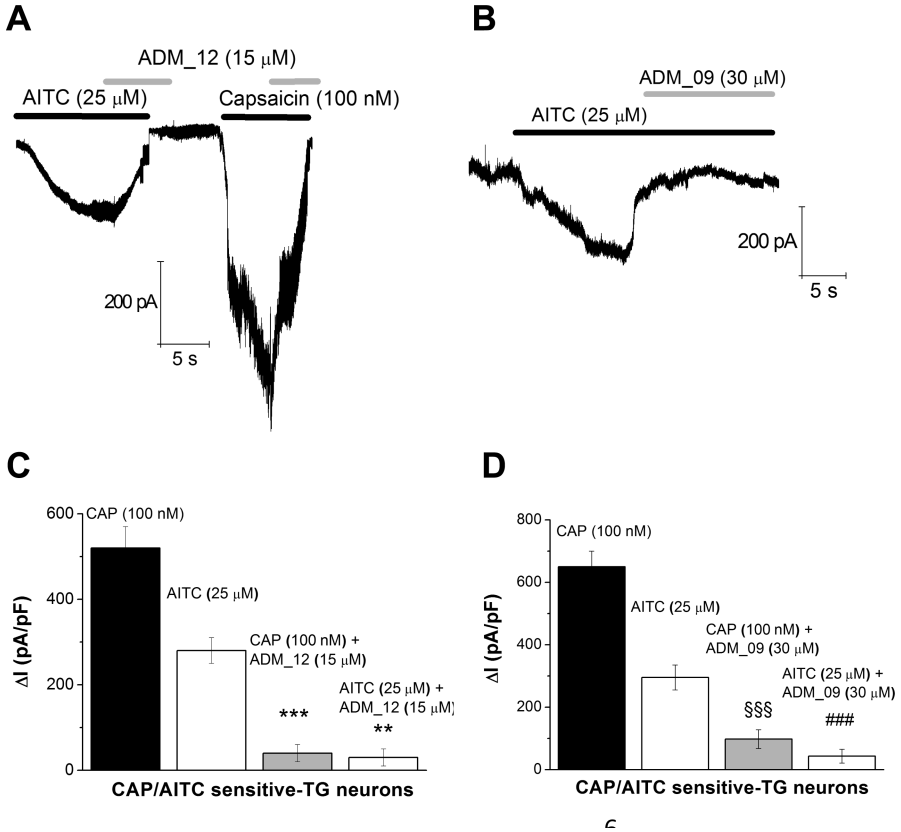

Figure 3. (A, B) Representative whole-cell current traces recorded at −60 mV, through CAP and AITC-sensitive rat TG neurons, activated by application of AITC 25 μM and capsaicin 100 nM, followed by the blocking effect of 15 μM ADM_12 (A) and 30 μM ADM_09 (B). (C) Pooled data of whole-cell currents through CAP/AITC-sensitive TG neurons evoked by CAP (100 nM), AITC (25 μM), CAP + ADM_12 (15 μM), AITC + ADM_12 (15 μM). (D) Pooled data of whole-cell currents through CAP/AITC-sensitive TG neurons evoked by CAP (100 nM), AITC (25 μM), CAP + ADM_09 (30 μM), AITC + ADM_09 (30 μM). Each column represents mean ± SEM of $n > 5$ cells. ***$p < 0.001$ versus CAP, **$p < 0.01$ versus AITC, $^{§§§}p < 0.001$ versus CAP, $^{###}p < 0.001$ versus AITC (unpaired Student's t test).

Information text and Table S1 for details). Data obtained suggested 1000 mg/kg as the maximum nontoxic dose. This is 30-fold higher than the minimum active pain-relieving dose (see below). When ADM_12 was administered at the antiallodynic dosage (30 mg/kg p.o.) daily for 14 days, neurological and motor functions were not altered.

The recent United States Food and Drug Administration recommendation has pointed out the possible unrecognized potential of analgesics (opioid or nonopioid) to prolong the QT interval and to the importance to include the hERG assay in the early stage of drug-discovery process.[20] For this reason, cardiotoxicity of ADM_12 (and of ADM_09 as a comparison) was assessed by patch-clamp experiments, recording whole-cell currents through HEK293 cells expressing hERG channels (see Supporting Information text and Figure S3). Remarkably, both ADM_09 and ADM_12 showed no inhibitory effects on the hERG channel activity at concentration up to 1 mM, demonstrating no overt cardiotoxicity.

The antiallodynic effect of ADM_09 and ADM_12 was next investigated in vivo. Animals subjected to TG inflammatory sensitization (9 per group; see the Supporting Information) were treated with a single dose of ADM_09 or ADM_12 (30 mg/kg) by gavage, and the evolution of mechanical allodynia evaluated thereafter. As expected, 24 h after CFA injection, a significant reduction in the head withdrawal threshold was observed in all animals ipsilaterally to the injection side, and this was maintained up to 72 h after CFA injection in vehicle-treated animals (CMC; Figure 4A). Administration of ADM_09 or ADM_12 attenuated facial allodynia with a significant increase in the head withdrawal threshold at 24, 48, and 72 h post CFA injection. A lower effect was observed after administration of ADM_09, further confirming our in vitro results. The development of facial allodynia was accompanied by satellite glial cell (SGCs) activation in the ipsilateral TG as evident by a significant up-regulation of glial fibrillar acidic proteic (GFAP) (Figure 4B). Either ADM_09 or ADM_12 administration did not alter SGC activation despite the observed reduction in mechanical allodynia (Figure 4C). These data indicate that the biological target(s) of ADM_09 and ADM_12 are not expressed by SGCs. Instead, based on the in vitro data, we demonstrated for the very first time that both TRPA1 and TRPV1 channels are involved in the development of mechanical allodynia and facial pain associated with inflammatory TG sensitization, either alone or combined in heteromeric form.[21,22] Recently, by employing the same experimental model described in Figure 4, a critical role for TRPV4 channels expressed by TG sensory neurons was incidentally demonstrated in pain,[23] whereas a possible role played by other members of the TRP family of receptors was not ascertained.

Concluding, we synthesized a nontoxic, structurally simple lipoic derivative, named ADM_12, able to revert inflammatory TG allodynia, a widespread painful condition lacking effective and safe pharmacological treatments. Unprecedentedly, our

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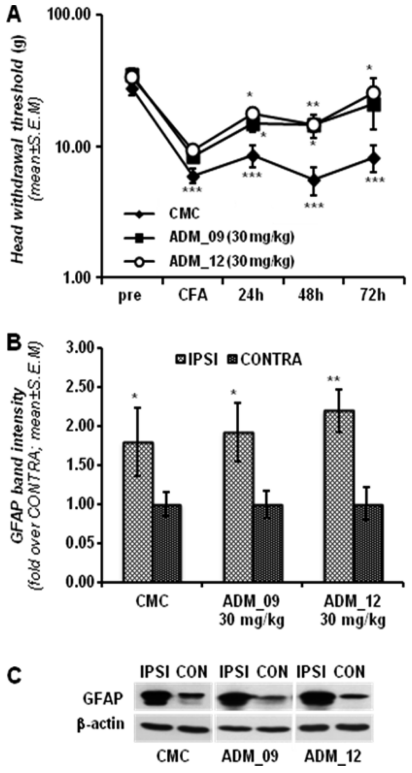

Figure 4. (A) von Frey test performed before CFA injection (PRE) 24 h later (CFA) and 30 min, 24 h, and 48 h (corresponding to 24 h, 48 h, and 72 h post CFA injection, respectively) after the pharmacological treatment. The mean head withdrawal threshold force, in grams (g), at the ipsilateral vibrissae pad side is shown. *Y*-axis = $\log_{10}$ scale. Values are the mean ± SEM of 6 rats (CMC), 10 rats (ADM_09, 30 mg/kg), 9 rats (ADM_12, 30 mg/kg), from 3 independent experiments. (B) Densitometric analysis of GFAP protein bands at 72 h post-CFA injection, normalized for β-actin, and expressed as fold over corresponding contra (CON) value set to 1. Values are the mean ± SEM of 6 (CMC), 9 (ADM_09 30 mg/kg), and 8 rats (ADM_12, 30 mg/kg) from 3 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ with respect to CON; unpaired Student's *t* test. (C) Representative Western blotting experiment. β-Actin is used as an internal loading control.

data highlighting the involvement of TRPA1 and TRPV1 channels in orofacial pain open the way for the discovery or development of effective drugs to treat inflammatory TG allodynia. Altogether our data also suggest an intriguing physiological role of TRPV1 channels as well as an interesting mode of action of ADM_12 as its modulator. These data extend our previous results, demonstrating the efficacy of lipoic-containing molecules against chemotherapy-induced neuropatic pain.[16] Mechanistic models for a deeper elucidation of the interaction of ADM_09 and ADM_12 with TRP channels are under evaluation.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of ADM_12, details concerning cell cultures, animals, "in vivo" and "in vitro" tests, patch-clamp experiments on TRPM8 cells, TG neurons, and hERG-HEK293 cells, ^{1}H and ^{13}C spectra of ADM_12. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

#R.G. and S.C. equally contributed to the work. C.N., C.G., and M.R.M. designed research; R.G., S.C., L.D.C.M., G.M., D.M., O.F., B.R., and G.l.M. performed research; C.N., C.G., R.G., and S.C. analyzed data; C.N. wrote the paper.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sherrington, C.-S. (1905) Observation on the scratch-reflex in the spinal dog. *J. Physiol. 34*, 1−50.

(2) (a) Nascimento, G.-C., Rizzi, E., Gerlach, R.-F., and Leite-Panissi, C.-R. (2013) Expression of MMP-2 and MMP-9 in the rat trigeminal ganglion during the development of temporomandibular joint inflammation. *Braz. J. Med. Biol. Res. 46*, 956−967. (b) Hargreaves, K.-M. (2011) Orofacial pain. *Pain 152*, S25−S32.

(3) (a) Kumar, S., Rastogi, S., Kumar, S., Mahendra, P., Bansal, M., and Chandra, L. (2013) Pain in trigeminal neuralgia: Neurophysiology and measurement: A comprehensive review. *J. Med. Life 6*, 383−388. (b) Materazzi, S., Benemei, S., Fusi, C., Gualdani, R., De Siena, G., Vastani, N., Andersson, D.-A., Trevisan, G., Moncelli, M. R., Wei, X., Dussor, G., Pollaro, F., Patacchini, R., Appendino, G., Geppetti, P., and Nassini, R. (2013) Parthenolide inhibits nociception and neurogenic vasodilatation in the trigeminovascular system by targeting the TRPA1 channel. *Pain 154*, 2750−2758.

(4) Clapham, D.-E. (2003) TRP channels as cellular sensors. *Nature 426*, 517−524.

(5) Gerhold, K.-A., and Bautista, D.-M. (2009) Molecular and cellular mechanisms of trigeminal chemosensation. *Ann. N. Y. Acad. Sci. 1170*, 184−189.

(6) Bautista, D.-M., Pellegrino, M., and Tsunozaki, M. (2013) TRPA1: A gatekeeper for inflammation. *Annu. Rev. Physiol. 75*, 181−200.

(7) Eid, S.-R., Crown, E.-D., Moore, E.-L., Liang, H.-A., Choong, K.-C., Dima, S., Henze, D.-A., Kane, S.-A., and Urban, M.-O. (2008) HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Mol. Pain 4*, 48.

(8) Petrus, M., Peier, A. M., Bandell, M., Hwang, S. W., Huynh, T., Olney, N., Jegla, T., and Patapoutian, A. (2007) A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Mol. Pain 3*, 40.

DOI: 10.1021/cn500248u
ACS Chem. Neurosci. 2015, 6, 380−385

(9) Diogenes, A., Akopian, A.-N., and Hargreaves, K.-M. (2007) NGF up-regulates TRPA1: Implications for orofacial pain. *J. Dent. Res. 86*, 550−555.
(10) Dai, Y., Wang, S., Tominaga, M., Yamamoto, S., Fukuoka, T., Higashi, T., Kobayashi, K., Obata, K., Yamanaka, H., and Noguchi, K. (2007) Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J. Clin. Invest. 117*, 1979−1987.
(11) McNamara, C.-R., Mandel-Brehm, J., Bautista, D. M., Siemens, J., Deranian, K. L., Zhao, M., Hayward, N.-J., Chong, J.-A., Julius, D., Moran, M.-M., and Fanger, C.-M. (2007) TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. U. S. A. 104*, 13525−13530.
(12) Cao, E., Cordero-Morales, J.-F., Liu, B., Qin, F., and Julius, D. (2013) TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron 77*, 667−679.
(13) Julius, D., and Basbaum, A.-I. (2001) Molecular mechanisms of nociception. *Nature 413*, 203−210.
(14) Bautista, D.-M., Siemens, J., Glazer, J.-M., Tsuruda, P.-R., Basbaum, A. I., Stucky, C.-L., Jordt, S.-E., and Julius, D. (2007) The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature 448*, 204−208.
(15) Colburn, R.-W., Lubin, M.-L., Stone, D.-J., Jr., Wang, Y., Lawrence, D., D'Andrea, M.-R., Brandt, M.-R., Liu, Y., Flores, C.-M., and Qin, N. (2007) Attenuated cold sensitivity in TRPM8 null mice. *Neuron 54*, 379−386.
(16) Nativi, C., Gualdani, R., Dragoni, E., Di Cesare Mannelli, L., Sostegni, S., Norcini, M., Gabrielli, G., la Marca, G., Richichi, B., Francesconi, O., Moncelli, M.-R., Ghelardini, C., and Roelens, S. (2013) A TRPA1 antagonist reverts oxaliplatin-induced neuropathic pain. *Sci. Rep. 3*, 2005 DOI: 10.1038/srep02005.
(17) Pomonis, J.-D., Harrison, J.-E., Bristol, D.-R., Valenzano, K.-J., and Walker, K. (2003) N-(4-Tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide (BCTC), a novel, orally effective vanilloid receptor 1 antagonist with analgesic properties: II. In vivo characterization in rat models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther 306* (1), 387−393.
(18) Fernandes, E.-S., Fernandes, M.-A., and Keeble, J.-E. (2012) The functions of TRPA1 and TRPV1: Moving away from sensory nerves. *Br. J. Pharmacol. 166*, 510−521.
(19) As known, 97% of TRPA1-positive sensory neurons also express TRPV1 while only 30% of TRPV1-positive neurons coexpress TRPA1. See Story, G.-M., Peier, A.-M., Reeve, A.-J., Eid, S.-R., Mosbacher, J., Hricik, T.-R., Earley, T.-J., Hergarden, A.-C., Andersson, D.-A., Hwang, S.-W., McIntyre, P., Jegla, T., Bevan, S., and Patapoutian, A. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell 112*, 819−829.
(20) Staruschenko, A., Jeske, N.-A., and Akopian, A.-N. (2010) Contribution of TRPV1-TRPA1 interaction to the single channel properties of the TRPA1 channel. *J. Biol. Chem. 285*, 15167−15177.
(21) Fischer, M.J.-M., Balasuriya, D., Jeggle, P., Goetze, T.-A., McNaughton, P.-A., Reeh, P.-W., and Edwardson, J.-M. (2014) Direct evidence for functional TRPV1/TRPA1 heteromers. *Pfluegers Arch. 466*, 2229−2241.
(22) Raffa, R.-B., Burmeister, J.-J., Yuvasheva, E., and Pergolizzi, J.-V., Jr. (2012) QTc interval prolongation by *d*-propoxyphene: What about other analgesics? *Expert Opin. Pharmacother. 13*, 1397−13409.
(23) Chen, Y., Williams, S.-H., McNulty, A.-L., Hong, J.-H., Lee, S.-H., Rothfusz, N.-E., Parekh, P.-K., Moore, C., Gereau, R.-W., 4th, Taylor, A.-B., Wang, F., Guilak, F., and Liedtke, W. (2013) Temporomandibular joint pain: A critical role for Trpv4 in the trigeminal ganglion. *Pain 154*, 1295−1304.

DOI: 10.1021/cn500248u
ACS Chem. Neurosci. 2015, 6, 380−385