Recent Advances on Molecular Modeling Studies of Transient Receptor Potential Ankyrin 1

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Abstract Transient receptor potential ankyrin 1 (TRPA1) is an ion channel involved in nociception. In addition, TRPA1 activation triggers an inflammatory response, which intensifies the sensation of pain. The role of TRPA1 in pain makes it an attractive target for painkiller drug design. The research on TRPA1 antagonists and agonists has increased in recent years with the focus on discovering novel and effective TRPA1 antagonists. This minireview describes some computational drug design methods that have been applied for examining TRPA1 and its ligands. So far, three ligand binding sites have been proposed for TRPA1. The binding of various ligands into TRPA1 has been explored with molecular docking and molecular dynamics. This is the first review that concentrates on TRPA1 computational studies.

Keywords TRPA1, agonist, antagonist, molecular modeling, drug design, painkiller

Introduction

Nowadays, around every fifth European suffers from chronic pain, which dramatically reduces his/her lifespan as well as increasing healthcare visits and need for medical help, posing a significant burden on health care budgets.[7] Some common analgesics such as NSAIDs (non-steroidal anti-inflammatory drugs) or opioids administered to treat chronic pain have side-effects, which limit their usage.[2] Normally, the sensation of nociception, i.e., the perception of pain, is induced by injured tissues, toxic molecules, or inflammatory mediators. These stimuli are detected by specialized peripheral sensory neurons (nociceptors).

TRP (Transient Receptor Potential) is a superfamily of cation channels that play critical roles in sensory physiology related to vision, taste, hearing, and touch.[3] Many TRP channels are activated by a variety of different stimuli and function as signal integrators. TRPA1 (Transient Receptor Potential Ankyrin 1), also known as the wasabi receptor, is one member of the TRP family. It is an ion channel that has been shown to play an important role in nociception.[4]

TRPA1 functions as a pivotal sensor for both neuropathic pain and inflammatory pain.[6] The receptor is a Ca²⁺-permeable non-selective cation channel and it is highly expressed in sensory neurons. Similar to other TRP-channels, TRPA1 also responds to a wide range of diverse stimuli, e.g., mechanical stress, cold temperature, and noxious chemical agents, both endogenous, and environmental compounds such as mustard oil, wasabi and cinnamaldehyde.[7-11] TRPA1 is also known to be a sensor of oxidative, nitrative and carbonyl stress because of its sensitivity to reactive oxygen species, reactive nitrogen, and carbonyl species.[12-14] It has also been reported to be involved in a molecular oxygen-sensing mechanism, where it is activated by both hyperoxia and hypoxia.[15] TRPA1 antagonists seem to target a single nociceptive signaling pathway and thus would be more potent than NSAIDs in pain therapy.[16]

Various agonists and antagonists of TRPA1 have been described in the literatures (Figure 1), for example, agonist JT010 (1) and antagonist HC-030031 (2), which have been tested in humans.[16,17] Although several antagonists of human TRPA1 have been developed in pharma companies, none of these compounds has actually entered the market. HC-030031 (2) is a xanthine derivative that has been shown to effectively antagonize TRPA1 in vivo. A-967079 (3) is also a well-known TRPA1 antagonist, and it is an oxime derivative. Five antagonists were evaluated in clinical trials during 2015—2019, but none of them is not yet accepted as a drug.[18] One reason for this might be the variation of TRPA1 structures between different species that complicates in vivo studies and can result in failures in clinical trials.[19]

The cryo-electron microscope (cryoEM) has been utilized to examine the six mammalian TRP-channel families with several experimental structures being identified, for example, human TRPV1[20] mouse TRPC5[21] human TRPM4,[22] and TRPML1[23]. However, only a few TRPA1 structures have been determined in the presence of agonists or antagonists, or without the application of cryoEM.[7,24-25] Although the earlier TRPA1 cryoEM structures had low resolution, they provided important information concerning the binding pockets for ligands. The recently published cryoEM structures with good resolution open new possibilities to identify novel TRPA1 antagonists with computer-aided drug design.

Structure of TRPA1

TRPA1 is a membrane protein consisting of four homologous subunits.[7] Each subunit has three parts (Figure 2). At the top is a transmembrane domain (TMD), which consists of six transmembrane α-helices S1-S6 situated between a voltage-sensor-like domain (VSD) (S1-S4) and a pore domain (SS-S6) with a pore-loop between SS and S6. The pore domain also contains two pore helices. A coupling domain (CD) is located in the center of the subunit, which has eight short helices (H1-H7 and pre-S1), a three-stranded beta sheet (βbcd: β1.1, β1.2, β1.3) and TRP-like helix (TRPL). At the bottom,
TRPA1 ligand binding sites

A few binding sites in TRPA1 have been determined for agonists and antagonists (Table 1). There is a binding site (A) located in the region of S5, S6 and pore helix 1 (PH1) of the pore domain. For instance, a known TRPA1 antagonist, A-967079 (3) was shown to bind in this pocket, undergoing interactions with residues F909 (Figure 2), S873, T874 and M912. Piperidine carboxamides (PIPCs) 1 (4) and 2 are fitted in a pocket by residues F909, M912, and L913 of PH1 and L881, F877, M953 and I957 of S5 and S6. PIPC1 and 2 are stabilized by residues of S6 (F938, V942 and I946) and residues of S5 (L870, S873, T874, I878 and L881). Decanol (5), phenethyl butanoate (6) and 2-ethyl-1-hexanol (7) interacted with hydrophobic residues L881 and F909 and hydrophilic residue T874. The conformational re-arrangement of S5, S6 and PH1 was associated with channel opening, especially the conformational movement of S6 was linked with the opening of the lower and upper gates. In addition, a novel TRPA1 antagonist, AZ868 (8), showed an interaction in the TRPA1 pore vestibule region, where the compound interacted with side chains of S6 residues and residues M911 and M912 near the selectivity filter.

The other binding site (B) is located in the CD, where the helices H1, H2 and H1-2 loop form the bottom and the region between turn β1.2 (strand of βCD) and H5 form the top half of the pocket. This binding site was found based on experiments conducted with two agonists, JT010 (1) and BITC (9) (benzy-
isothiocyanate). The pocket’s key residues are C621 (Figure 2) at the bottom of the pocket that forms a covalent bond with JT010 (1) and BITC (9) and other four aromatic residues F612, H614, F669 and Y680 from the both halves completing the pocket. In addition, C665 has been shown to be another critical cysteine in this pocket. The four aromatic residues and their opposite residues Y662 and W605 are suggested to function as the entry site for electrophiles and to allow the pocket to undergo conformational changes. The changes in the top of the pocket have been shown to be similar even though different agonists were applied, suggesting that a diverse set of agonists could activate the protein. Furthermore, the irreversible TRPA1 agonist iodoacetamide (IA (10)) showed an attachment with C621 in a similar manner in the study conducted by Zhao et al.[29]

The third identified pocket (C) in TRPA1 is situated in the linker region of S4 and S5. This pocket was identified based on the binding of antagonist HC-030031 (2). HC-030031 forms a hydrogen bond with N855 (Figure 2), which is located above the TRPL in the C-terminus.[19] This hydrophobic binding pocket is also demonstrated as the binding site for saikosaponins.[31]

### Modeling TRPA1 ligands

Molecular modeling has been widely applied in the development of novel drugs. The drug design strategies can be divided into ligand-based and structure-based methods. The ligand-based method was applied in the first study concerning TRPA1 agonists before there were any 3D protein structures available.[32] CoMFA and CoMSia models were generated by using various 114 dibenz[b,e]azepine (11) and dibenz[b,j,f][1,4]-oxazine derivatives. Although only 21 compounds were examined in the study, both CoMFA and CoMSia models displayed good statistical values. They were also evaluated with an external test set of five compounds, and the predictive correlation coefficients for the test set were 0.967 and 0.981 for the CoMFA and CoMSia models, respectively.

The first cryoEM structure of TRPA1 was published by Paulsen et al. in 2015, which made it possible to investigate the binding mode of various compounds.[15] Although the resolution of the first 3D structure of TRPA1 was low (4.2 Å), it has been extensively applied in the structure-based drug design.

Saikosaponins are triterpenoid saponin glycosides that have been shown to inhibit agonist-induced activation of TRPA1 transfected cells.[31] The binding mode of the saikosaponins was examined by molecular docking into the various binding sites of TRPA1. These studies revealed that saikosaponins can bind to the TRPA1’s hydrophobic pocket in the linker region of S4 and S5, i.e., the site that had been proposed for HC-030031 (2), while they failed to dock in the other two binding sites. The most potent saikosaponin D (12) formed hydrogen bonds with TRPA1’s residues N855, E854, and R975. The triterpenoid moiety of saikosaponin D (12) was surrounded by hydrophobic residues I878, V875, and L871.

A structure-based virtual screening campaign was performed to identify novel agonists for TRPA1.[29] In this study, 20 ns MD simulations were applied for the structure of TRPA1 by Paulsen et al. (2015) to examine and multiple pocket structures to be investigated with molecular docking. MD simulations produced 463 candidate pockets. In these pockets, 51 known agonists and 44 inactive compounds were docked to evaluate the binding mode of agonists. Finally, four pockets from MD were selected after analyzing candidates based on the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) and area under the curve (AUC). The selected four pockets were able to discriminate between active and inactive agonists, and they were selected as representative pocket structures of TRPA1. A library of natural products (1 555 compounds) was docked to these four pockets. As a result, 49 compounds were chosen for experimental testing in a TRPA1 agonist assay. Three compounds (decanol (5), phenethyl butanoate (6), and 2-ethyl-1-hexanol (7)) showed over 60% TRPA1 agonist activity. These compounds were able to form hydrogen bonds to the hydrophobic residues (in the S5 helix and in the PH1) and with a hydrophilic residue (in the S5 helix), which has been suggested to be important for the binding of agonists.[29]

PIPCs (10) have been identified as potent, noncovalent agonists of human TRPA1.[28] The most potent agonist (PIPC1 (4)) was estimated to have an EC50 = 6.5 nM towards human TRPA1. The binding site studies exploited the homology models of the open and closed states for TRPA1. These two states showed differences in the transmembrane part of the upper (D915) and lower (I957 and V961) gates. PIPCs were docked to both states with an induced fit protocol. The docking results revealed that binding of PIPCs to TRPA1 occurred primarily in the open state. PIPC1 (4) underwent interactions with residues F909, F877 and T874 in the open state of TRPA1.

Carvacrol (13), which is natural phenol found in oregano, is an agonist of the TRPA1 receptor.[33] Its binding has been explored via molecular docking, where carvacrol (13) was demonstrated to form interactions with several residues, i.e., Y726, Y842, S780, Y812, I811 and S781.[33] In addition, the binding mode of its derivative, carvacryl acetate was examined.[34] This derivative underwent interactions with several residues I803, L807, L848, L863, E864, L867, K868 and F947 in TRPA1. The acylate ring of carvacryl acetate also formed a hydrogen bond with residue K868.

In addition, other natural compounds have been studied with TRPA1 using molecular modeling tools. From a database of 156 natural chemicals, virtual screening revealed that cardamom (14) exerted effects on TRPA1.[35] Cardamom (14) was docked into the binding site 1 (Table 1). The results indicated that it underwent interactions with R928, P925, E930, Q895, and D896.

### Table 1: TRPA1 binding sites

| Binding site | Location | Key residues | Compounds | Ref. |
|--------------|----------|--------------|-----------|-----|
| A            | Pore domain: S5, S6 and pore-helix 1 | F909, S876, T874, F912 | A-967079, Phenethyl butanoate, Decanol, 2-ethyl-1-hexanol, AZ268, PIPCs | [27–30] |
| B            | Coupling domain: H1, H2, H1-2 loop, H5 and β1.2 | C621, C665 | JT010, BITC, Iodoacetamide | [7, 24, 25] |
| C            | Linker region of S4 and S5 | N855 | HC-030031, Saikosaponins | [19, 31] |

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(2). The most potent derivative of these compounds formed interactions with S934, F877 and T874.

Conclusions and Perspectives

TRPA1 can be viewed as a hot target of painkiller research at the moment. The identification of the TRPA1 antagonist binding site has been important in the development of novel antagonists as prospective painkillers. In this search, different drug design technologies have been utilized in recent years. Despite extensive research, only a few binding sites for antagonists and agonists have been identified, and only a partial explanation for antagonist action has been postulated. 3D structures with co-crystallized ligands and advanced modeling techniques are urgently needed for efficient TRPA1 antagonist-based drug design.

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Author Contributions

Following are the details of the contributions made by each of the authors for the manuscript: Carita Sallomy, writing of the manuscript; Maija Lahtela-Kakkonen, writing and editing of the manuscript; Juri Timonen, writing and editing of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] European Road map monitor 2019, www.sfp-platform.eu/press-area/article/impact-of-pain-on-society-costs-the-eu-up-to-441-billion-euros-annually visited 29.09.2020
[2] Martel, M. O.; Finan, P. H.; Dolman, A. J.; Subramanian, S.; Edwards, R. R.; Wasan, A. D.; Jamison, R. N. Self-reports of medication side effects and pain-related activity interference in patients with chronic pain: a longitudinal cohort study. Pain 2015, 156, 1092–1100.
[3] Venkataraman, K.; Montell, C. TRP Channels. Annu. Rev. Biochem. 2007, 76, 387–417.
[4] Talavera, K.; Startek, J. B.; Julio Alvarez-Collarizo; Boonen, B.; Alpizar, Y. A.; Sanchez, A.; Naert, R.; Nilius, B. Mammalian Transient Receptor Potential TRPA1 Channels: From Structure to Disease. Physiol. Rev. 2020, 100, 725–803.
[5] Moilanen, L. J.; Laavola, M.; Kukkonen, M.; Korhonen, R.; Leppänen, T.; Högestätt, E. D.; Zygmun, P. M.; Niemen, R. M.; Moilanen, E. TRPA1 contributes to the acute inflammatory response and mediates carrageenan-induced paw edema in the mouse. Sci. Rep. 2012, 2, 380.
[6] Nassini, R.; Materazzi, S.; Benemer, S.; Gepetti, P. The TRPA1 channel in inflammatory and neuropathic pain and migraine Rev. Physiol. Biochem. Pharmacol. 2014, 167, 1–43.
[7] Paulsen, C. E.; Armache, J. P.; Gao, Y.; Cheng, Y.; Julius, D. Structure of the TRPA1 ion channel suggests regulatory mechanisms. Nature 2015, 520, 511–517.
[8] Bandell, M.; Story, G. M.; Hwang, S. W.; Viswanath, V.; Eid, S. R.; Petrus, M. J.; Earley, T. J.; Patapoutian, A. Noxious Cold Ion Channel TRPA1 Is Activated by Pungent Compounds and Bradykinin. Neuron 2004, 41, 849–857.
[9] Story, G. M.; Peier, A. M.; Reeve, A. J.; Eid, S. R.; Mosbacher, J.; Hrick, T. R.; Earley, T. J.; Herberg, A. C.; Andersson, D. A.; Hwang, S. W.; McIntyre, P.; Jegla, T.; Bevan, S.; Patapoutian, A. ANKT1M1, a TRP-like Channel Expressed in Nocticeptive Neurons, Is Activated by Cold Temperatures. Cell 2003, 112, 819–829.
[10] Jordt, S.; Bautista, D. M.; Chuang, H.; McKemy, D. D.; Zygmun, P. M.; Högestätt, E. D.; Meng, I. D.; Julius, D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKT1M1. Nature 2004, 427, 260–265.
[11] Moore, C.; Gupta, R.; Jordt, S.; Chen, Y.; Liedtke, W. B. Regulation of Pain and Itch by TRP Channels. Neuronsci. Bull. 2018, 34, 120–142.
[12] Trevisani, M.; Siemens, J.; Materazzi, S.; Bautista, D. M.; Nassini, R.; Campi, B.; Imamachi, N.; André, E.; Patacchini, R.; Cottrell, G. S.; Gatti, R.; Basbaum, A. I.; Bunnett, N. W.; Julius, D.; Gepetti, P. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc. Natl. Acad. Sci. U. S. A. 2007, 104, 13519.
[13] De Logu, F.; Li Puma, S.; Landini, L.; Portelli, F.; Innocenti, A.; de Araújo, D.; Souza Monteiro; Janal, M. N.; Patacchini, R.; Bunnett, N. W.; Gepetti, P.; Nassini, R. Schwann cells expressing nociceptive channel TRPA1 orchestrate ethanol-evoked neuropathic pain in mice. J. Clin. Invest. 2019.
[14] Taylor-Clark, T.; Ghatta, S.; Bettner, W.; Undem, B. J. Nitroacidic, an endogenous product of nitrate stress, activates nociceptive sensory nerves via the direct activation of TRPA1. Mol. Pharmacol. 2009, 75, 820–829.
[15] Takahashi, N.; Kuwaki, T.; Kiyonaka, S.; Numata, T.; Kozai, D.; Mizuno, Y.; Yamamoto, S.; Naito, S.; Knevels, E.; Carmellet, P.; Oga, T.; Kaneko, S.; Sugia, S.; Nakomi, T.; Yoshida, J.; Mori, Y. TRPA1 underlies a sensing mechanism for O3. Nat. Chem. Biol. 2011, 7, 701.
[16] Kiovisto, A.; Jalava, N.; Bratty, R.; Pertovaara, A. TRPA1 Antagonists for Pain Relief. Pharmaceuticals 2018, 11, 117.
[17] Heber, S.; Gold-Binder, M.; Clout, C. I.; Witek, M.; Nidize, N.; Kress, H.; Fischer, M. J. M. A Human TRPA1-Specific Pain Model. J. Neurosci. 2019, 39, 3845.
[18] Chen, H.; Terrett, J. A. Transient receptor potential ankyrin 1 (TRPA1) antagonists: a patent review (2015–2019). Expert Opin. Ther. Patents 2020, 643–657.
[19] Gupta, R.; Saito, M.; Mori, Y.; Itoh, S. G.; Okumura, H.; Tominaga, M. Structural basis of TRPA1 inhibition by HC-030031 utilizing species-specific differences. Sci. Rep. 2016, 6, 37460.
[20] Wilkes, M.; Madej, M. G.; Kreuter, L.; Rhinow, D.; Heinz, V.; De Sanctis, S.; Ruppel, S.; Richter, R. M.; Joos, F.; Grieben, M.; Pike, A. C. W.; Huisken, J. T.; Carpenter, E. P.; Kühlbrandt, W.; Witzgall, R.; Ziegler, C. Molecular insights into lipid-assisted Ca2+ regulation of the TRP channel Polycystin-2. Nat. Struct. Mol. Biol. 2017, 24, 123–130.
[21] Duan, J.; Li, L.; Chen, G.; Ge, Y.; Liu, J.; Xie, K.; Peng, X.; Zhou, W.; Zhong, J.; Zhang, Y.; Xu, J.; Xue, C.; Liang, B.; Zhu, L.; Liu, W.; Zhang, C.; Tian, X.; Wang, J.; Clapham, D. E.; Zeng, B.; Li, Z.; Zhang, J. Cryo-EM structure of TRPC5 at 2.8Å resolution reveals unique and conserved structural elements essential for channel function. Sci. Adv. 2019, 5, eaaw7935.
[22] Autzen, H. E.; Myasnikov, A. G.; Campbell, M. G.; Asamow, D.; Julius, D.; Cheng, Y. Structure of the human TRPM4 ion channel in a lipid nanodisc. *Science* 2018, 362, 228.

[23] Schmiege, P.; Fine, M.; Biobel, G.; Li, X. Human TRPML1 channel structures in open and closed conformations. *Nature* 2017, 550, 366–370.

[24] Sudo, Y.; Wang, Z.; Zuberovic, L.; Hsu, A. L.; He, Q.; Borgia, M. J.; Ji, R.; Lee, S. Structural Insights into Electrophilic Irritant Sensing by the Human TRPA1 Channel. *Neuron* 2020, 105, 882–894.

[25] Zhao, J.; Lin King, J. V.; Paulsen, C. E.; Cheng, Y.; Julius, D. Irritant-evoked activation and calcium modulation of the TRPA1 receptor. *Nature* 2020, 585, 141–145.

[26] Dong, X.; Kashiio, M.; Peng, G.; Wang, X.; Tominaga, M.; Kadouâki, T. Isoform-specific modulation of the chemical sensitivity of conserved TRPA1 channel in the major honeybee ectoparasitic mite, *Tropilaelaps mercedesae*. *Open Biol.* 2016, 6, 160042.

[27] Ton, H. T.; Phan, T. X.; Abramyan, A. M.; Shi, L.; Ahem, G. P. Identification of a putative binding site critical for general anesthetic activation of TRPA1. *Proc. Natl. Acad. Sci. U. S. A.* 2017, 114, 3762.

[28] Chemov-Rogan, T.; Giangi, E.; Liu, C.; Villemure, E.; Cridland, A. P.; Hu, X.; Ballini, E.; Lange, W.; Desemann, H.; Li, T.; Ward, S. I.; Hackos, D. H.; Magnuson, S.; Safina, B.; Klein, M. L.; Volgraf, M.; Carnevale, V.; Chen, J. TRPA1 modulation by piperidine carboxamides suggests an evolutionarily conserved binding site and gating mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 2019, 116, 26008.

[29] Araki, M.; Kanda, N.; Iwata, H.; Sagae, Y.; Masuda, K.; Okuno, Y. Identification of a new class of non-electrophilic TRPA1 agonists by a structure-based virtual screening approach. *Bioorg. Med. Chem. Lett.* 2020, 30, 127142.

[30] Klement, G.; Eisese, L.; Malinowsky, D.; Nolting, A.; Svensson, M.; Terp, G.; Weigelt, D.; Dabrowski, M. Characterization of a Ligand Binding Site in the Human Transient Receptor Potential Ankyrin 1. *Biophys. J.* 2013, 104, 798–806.

[31] Lee, G.; Choi, J.; Nam, Y.; Song, M.; Kim, J. K.; Kim, W. J.; Kim, P.; Lee, J. S.; Kim, S.; No, K. T.; Lee, J. H.; Lee, J. K.; Choi, Y. Identification and characterization of salixosaponins as antagonists of transient receptor potential A1 channel. *Phytother. Res.* 2020, 34, 788–795.

[32] Ai, Y.; Song, F.; Wang, S.; Sun, Q.; Sun, P. Molecular Modeling Studies on 1H-Dibenzo[b,e]azepine and Dibenzo[a,f][1,4]oxazepine Derivatives as Potent Agonists of the Human TRPA1 Receptor. *Molecules* 2010, 15, 9364–79.

[33] Alvarenga, E. M.; Souza, L. K.; Araújo, T. S.; Nogueira, K. M.; Sousa, F. B.; Araújo, A. R.; Martins, C. S.; Pacifico, D. M.; de Brito, G. A.; Souza, E. P.; Sousa, D. P.; Medeiros, J. V. *Carvacrol reduces irinotecan-induced intestinal mucositis through inhibition of inflammation and oxidative damage via TRPA1 receptor activation. Chem. Biol. Interact.* 2016, 260, 129–140.

[34] Alvarenga, E. M.; Sousa, N. A.; de Araújo, S.; Júnior, J. L. P.; Araújo, A. R.; Illes, B.; Pacifico, D. M.; Brito, G. A. C.; Souza, E. P.; Sousa, D. P.; Medeiros, J. V. R. *Carvacryl acetate, a novel semisynthetic monoterpenic ester, binds to the TRPA1 receptor and is effective in attenuating irinotecan-induced intestinal mucositis in mice. J. Pharm. Pharmacol.* 2017, 69, 1733–1785.

[35] Wang, S.; Zhai, C.; Zhang, Y.; Yu, Y.; Zhang, Y.; Ma, L.; Li, S.; Qiao, Y. *Cardamonin, a Novel Antagonist of hTRPA1 Cation Channel, Reveals Therapeutic Mechanism of Pathological Pain. Molecules* 2016, 21, 1145.

[36] Chłor-Rzepa, G.; Ślusarczyk, M.; Jankowska, A.; Gawalska, A.; Bucki, A.; Kołaczkowski, M.; Świerczek, A.; Pociecha, K.; Wyska, E.; Zygmunt, M.; Kazek, G.; Salat, K.; Pawlowski, M. Novel amide derivatives of 1,3-dimethyl-2,6-dioxopurin-7-yl-alkylcarboxylic acids as multifunctional TRPA1 antagonists and PDE4/7 inhibitors: A new approach for the treatment of pain. *Eur. J. Med. Chem.* 2018, 158, 517–533.