Antidepressants Are Poor Inhibitors of Heat-Evoked Ion Currents Mediated by TRPM2

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\textbf{Keywords}

TRPM2 · Duloxetine · Patch clamp · Antidepressant

\textbf{Abstract}

\textbf{Introduction:} The heat and redox-sensitive ion channel TRPM2 was reported to be a causative mechanism for depression in a mouse model and to be upregulated in the hippocampus in patients suffering from depressive disorders. TRPM2 may thus be a novel target for antidepressants, but so far, selective TRPM2-inhibitors have not yet been developed. In this in vitro study, we examined the inhibitory effects of several established antidepressants on heat-evoked inward currents of TRPM2.\textbf{Methods:} Human (h) TRPM2 expressed in HEK293 cells was examined by means of whole-cell patch clamp recordings. Effects of duloxetine, amitriptyline, sertraline, fluoxetine, paroxetine, citalopram, escitalopram, ketamine, pregabalin, lidocaine, and QX-314 were explored on heat-evoked currents in cells pretreated with ADP-ribose (ADPR). \textbf{Results:} While inward currents induced by 1 mM ADPR in the pipette solution displayed a strong rundown hampering pharmacological experiments, heat-evoked currents in cells loaded with 200 \textmu M APDR remained stable upon repetitive activation. Among all substances examined, only inhibition induced by duloxetine displayed a clear concentration-dependency. Thirty micromolar duloxetine was required for 50\% inhibition, the same degree of inhibition was also induced by 30 \textmu M amitriptyline, fluoxetine, and paroxetine. While citalopram, escitalopram, ketamine, and pregabalin failed to robustly modify TRPM2, sertraline and low concentrations of lidocaine even potentiated heat-evoked currents. \textbf{Conclusion:} Our data indicate that some, but not all established antidepressants inhibit hTRPM2 when it is activated by heat and ADPR in vitro, e.g., presumably relevant endogenous agonists. However, none of the examined substances exhibited a potent inhibition which is likely to translate into a clinically relevant effect at effective plasma concentrations. Whether or not TRPM2 may be a relevant target for antidepressants cannot be conclusively assessed by a single in vitro study, thus further studies are required along these lines. Nevertheless, future studies may get simplified by the novel approach we developed for in vitro pharmacological analysis of TRPM2.
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Introduction

Within the family of transient receptor potential (TRP) channels, the nonselective cation channel TRPM2 is of particular importance for sensing oxidative stress and subsequently inducing cell death [1]. Activation of TRPM2 triggers sodium influx of cations along the electrochemical gradient with the resulting increase of intracellular free calcium causing cellular damage and apoptosis [2]. Besides its function in terms of oxidative stress, TRPM2 plays a role in a number of other physiological processes including insulin secretion [3], cytokine production [4, 5], immune cell activation [6], and temperature regulation [7, 8]. Channel activation is evoked by intracellular adenosine 5′-diphosphoribose (ADPR) through interaction with the specific Nudix hydrolase 9 homology domain (NUDT9-H) [9, 10], nicotinamide adenine dinucleotide, or reactive oxygen species such as hydrogen peroxide [11–13]. TRPM2 is almost ubiquitously expressed in various tissues, including the heart, lung, pancreas, and inflammatory cells [14]. The highest expression of TRPM2, however, is found in the central nervous system [15]. This finding, considered in conjunction with the abovementioned significance for oxidative stress-induced cellular damage leads to the assumption that TRPM2 might be involved in the pathophysiology of neurodegenerative and psychiatric diseases. Thus, identifying pharmacological agents for inhibition or modulation of TRPM2 is of utmost interest. An increasing number of reports describe promising effects of TRPM2-selective inhibitors identified in preclinical drug development [16–18]. Clinically well-established substances like the nonsteroidal anti-inflammatory drug flufenamic acid and the antifungal agents clotrimazole and econazole are known to quite potently inhibit TRPM2 [19, 20]. However, these substances are not selective for TRPM2.

There is accumulating evidence that TRPM2 contributes to the genesis of depression and bipolar disorder [21, 22]. Ko et al. [23] demonstrated that TRPM2-deficient mice showed antidepressant-like behavior and reduced stress-induced reactive oxygen species accumulation. Accordingly, recent studies described an inhibitory effect of the serotonin-noradrenaline reuptake inhibitor duloxetine and other antidepressants on TRPM2 [24–26]. As these studies primarily utilized ratiometric calcium imaging in order to examine TRPM2; however, it is not clear if and how effective antidepressants directly inhibit ion currents mediated by TRPM2. Therefore, we aimed to establish an experimental protocol.

Chemicals

Amitriptyline, duloxetine, ketamine, lidocaine, pregabalin, ADP-ribose were obtained from Sigma-Aldrich (Taufkirchen, Germany). QX-314 was obtained from Hello Bio (Bristol, UK). Citalopram, escitalopram, fluoxetine, paroxetine, and sertraline were purchased from Biozol (Munich, Germany). Dilution series of stock solutions of these substances were prepared in nominal calcium-free saline containing (in mM) 140 NaCl, 5 KCl, 2 MgCl₂, 10 glucose,10 HEPES, and 5 EGTA, adjusted to pH 7.4 using NaOH. All test solutions were prepared directly before experiments.

Cell Culture

Human embryonic kidney (HEK) 293 cells stably overexpressing human (h) TRPM2 were kept under standard culture conditions (5% CO₂, 37°C) and cultivated in Dulbecco’s Modified Eagle Medium (Gibco/Invitrogen, Darmstadt, Germany) supplemented with 10% fetal calf serum (Biochrom, Berlin, Germany), 1-glutamine, penicillin/streptomycin, and blasticidin (Gibco/Invitrogen). Prior to experiments, cells were incubated for 24 h in an induction medium containing 100 mg/ml zeocin and 500 μg/ml tetracycline (both Sigma-Aldrich) and tested in a time interval of 24 h to maximum 36 h. It was previously demonstrated that TRPM2 is not functionally expressed in non-treated HEK 293 cells [10].

Patch Clamp Electrophysiology

Patch clamp recordings were performed in the whole-cell configuration using an EPC10 amplifier (HEKA Elektronik, Langen, Germany) and the PATCHMASTER software v2x73 (HEKA Elektronik) for data acquisition. Application of heated solutions was achieved by using the WAS02 system as described previously [27]. Briefly, the temperature of the applied solution was regulated by a thermocouple fixed at the orifice of the capillary tip placed close to the cells under investigation. Heated solutions were repeatedly applied to the cells in order to evoke reproducible inward currents. Except for heat stimuli, all experiments were performed at room temperature. Glass pipettes (2–3 MΩ) were prepared from borosilicate glass (Science Products, Hofheim, Germany) using a PC-10 puller (Narishige International, Tokyo, Japan). Currents were filtered at 2 kHz and sampled at 10 kHz. The extracellular solution contained (in mM) 140 NaCl, 5 KCl, 2 MgCl₂, 10 glucose, 10 HEPES, and 5 EGTA and was adjusted to pH 7.4 using NaOH. The pipette solution contained (in mM) 140 KCl, 2 MgCl₂, 10 glucose, 10 HEPES, and 5 EGTA and was adjusted to pH 7.4 using KOH. Two hundred micromolar ADPR was added to the pipette solution directly before experiments. A gravity-driven application system continuously applied the prepared test solutions in close proximity to the cell. One cell per dish was used.

Analyses

Data analysis was performed using the Fitmaster software v2x67 (HEKA Electronics) and Origin 8.5 (Origin Lab, Northampton, MA, USA). Statistical analyses were calculated with Origin 8.5 (Origin Lab). Tests for multiple comparisons were calculated with one-way ANOVA followed by the post hoc Tukey test. In both cases ANOVA was calculated, Mauchly’s test of sphericity indicated that sphericity can be assumed (Prob > F > 0.05). p < 0.05 was regarded as significant. In all bar graphs, data are shown as mean ± SEM.

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Materials and Methods

Pharmacology 2022;107:472–479
DOI: 10.1159/000524934
Results

Heat-Induced Inward Currents Allow Pharmacological Experiments on TRPM2

Several previous studies using whole-cell patch clamp to examine in vitro pharmacology of TRPM2 included ADPR to the pipette solution for robust channel activation [20, 24, 26]. We adapted this approach and indeed observed large inward currents when 1 mM ADPR was included in the pipette solution. Throughout such experiments, however, we repeatedly observed a rather rapid current rundown in presence of control solution (Fig. 1a). This rundown strongly hampers the interpretation of effects observed following the application of possible channel blockers. In case of the antidepressants duloxetine (Fig. 1b), amitriptyline (Fig. 1c), and fluoxetine (Fig. 1d) applied at 30 μM, we unfortunately observed slow and irreversible current reductions which we could not differentiate from this rundown (n = 4–6 cells for each substance). In contrast to these rather vague effects induced by antidepressants, the previously described potent and rapid inhibition induced by clotrimazole (30 μM) could be readily identified with this protocol (Fig. 1e; [20]). Attempting to establish an experimental protocol allowing us to more accurately identify effects on TRPM2 induced by antidepressants, we took use of the heat sensitivity of TRPM2 [3]. We found that reproducible inward currents could be evoked by brief applications of heated solution when a low concentration of APDR (200 μM) was included in the pipette solution. As a control experiment, we observed that 30 μM clotrimazole induced an almost complete inhibition of these heat-evoked currents in HEK 293 cells expressing TRPM2 (Fig. 1f, 90 ± 4% block, n = 6).

Duloxetine Inhibits TRPM2-Mediated Heat-Evoked Currents in a Concentration-Dependent Manner

We next applied the approach with heat-induced ion currents to examine the effects of antidepressants on TRPM2. As duloxetine was previously suggested to inhibit TRPM2 [24], we first conducted experiments with this substance. As is shown in Figure 2a–e, we indeed observed a concentration-dependent current inhibition from 29 ± 14% with 3 μM, 43 ± 7% with 10 μM, 52 ± 4% with 30 μM, and 95 ± 1% with 100 μM duloxetine (n = 4–6 cells for each concentration). While the degree of inhibition did not significantly differ between 3 and 30 μM duloxetine, the inhibition induced by 100 μM duloxetine was significantly stronger than the lower concentrations (one-way ANOVA with post hoc Tukey test). This inhibition was partly reversible and could be repeated with a second application of duloxetine on the same cell (not shown).

Several Antidepressants Inhibit Heat-Evoked Currents of TRPM2

We proceeded with further commonly used antidepressant substances, including the tricyclic antidepressants, the monoamine oxidase inhibitors (MAOIs) such as amitriptyline and the selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine. We chose these substances because of their widespread clinical use and the high frequency of side effects, especially the electrocardiogram (ECG) alterations. Our results showed that these substances also inhibited heat-evoked currents from 31 ± 12% with 3 μM, 44 ± 6% with 10 μM, 53 ± 3% with 30 μM, and 96 ± 1% with 100 μM fluoxetine and 30 ± 4% with 3 μM, 44 ± 7% with 10 μM, 52 ± 1% with 30 μM, and 95 ± 1% with 100 μM amitriptyline (n = 4–6 cells for each concentration). While the degree of inhibition did not significantly differ between 3 and 30 μM fluoxetine, the inhibition induced by 100 μM fluoxetine was significantly stronger than the lower concentrations (one-way ANOVA with post hoc Tukey test). This inhibition was partly reversible and could be repeated with a second application of fluoxetine on the same cell (not shown).
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Sant amitriptyline and the selective serotonin reuptake inhibitors fluoxetine, paroxetine, and citalopram. Similar to duloxetine, inhibition induced by amitriptyline was concentration-dependent with 30 ± 12% with 3 μM up to 44 ± 6% with 30 μM (Fig. 3a, n = 7–9 for each concentration). While 30 μM of both fluoxetine (Fig. 3b, 49 ± 5%, n = 6) and paroxetine (Fig. 3c, 49 ± 7%, n = 5) induced an inhibition of TRPM2, lower concentrations of both substances failed to robustly reduce current amplitudes of heat-evoked currents. Showing that not all antidepressants induce more or less identical effects on TRPM2, 30 μM of citalopram completely failed to reduce current amplitudes (Fig. 3d, n = 7). Similarly, escitalopram also failed to inhibit TRPM2 (data not shown). Of note, the degrees of inhibition induced by 30 μM duloxetine, amitriptyline, fluoxetine, and paroxetine were not significantly different (one-way ANOVA with post hoc Tukey test).

Of note, application of sertraline resulted in distinct channel activation instead of inhibition. Thus, the initial amplitudes increased by 283 ± 95% by 30 μM sertraline (Fig. 4a, n = 5). Subsequent application of control solution led to a washout effect (not shown). Activation of TRPM2 by sertraline was also induced at room temperature without heat stimulus (Fig. 4b).

Being used for treatment of depressions as well, we also investigated the effects of the dissociative anesthetic ketamine and the anticonvulsant pregabalin on TRPM2. At
the concentrations up to 30 μM, both ketamine and pregabalin failed to inhibit TRPM2 (Fig. 4c, d, respectively, n = 6 and 4). If anything, both substances slightly potentiated heat-evoked currents (pregabalin 143 ± 35%; ketamine 107 ± 21%).

Discussion
As recent studies identified the ion channel TRPM2 as a key molecule for depressive disorders, the current study aimed to perform a thorough pharmacological characterization on the effects of clinically used antidepressants on TRPM2. To be able to display a direct inhibition induced by applied substances, a steady channel activation had to be established. Previous studies addressing TRPM2 with a focus on pharmacological questions only rarely utilized the patch clamp technique, and in most published reports, channel activation was induced by ADPR included to the pipette solution [20, 24, 26]. Due to a rather arbitrarily rundown of the ADPR-induced current, however, we had to discard this approach. Instead, we established a novel experimental protocol taking use of the heat sensitivity of TRPM2. Thus, the inhibitory effects of antidepressants on TRPM2 were determined on heat-evoked inward currents in cells treated with a low concentration of ADPR. In our hands, this approach allowed for a more solid pharmacological characterization and identified most antidepressants as rather weak inhibitors of TRPM2.
TRPM2 is almost ubiquitously expressed throughout the body. Accordingly, the number of reports identifying important physiological as well pathophysiological roles of TRPM2 is steadily increasing. Focusing on the central nervous system where TRPM2 is widely expressed, this ion channel seems to be involved in several neurological diseases including depression, ischemic neuronal injury, neuropathic pain, and Alzheimer’s, as well as Parkinson’s disease [29]. For all these diseases, there is an unmet need for more effective pharmacological interventions, and thus, TRPM2 may be a good molecular target. In terms of depressive disorders, an excellent study from Ko et al. [30] showed that genetic deletion of TRPM2 results in antidepressant-like behavior in mice. Adding a translational perspective, they also demonstrated that TRPM2 is upregulated in the hippocampus in patients who suffered from depressive disorders. The study did not only suggest that TRPM2 may be a suitable target for the development of new antidepressant drugs, but it also raised the question as to whether clinically established drugs employed for treatment of depressive disorders may modulate TRPM2. Adding significance to this note, many antidepressant drugs are also employed for treatment of neuropathic pain. Accordingly, TRPM2 was shown to play an important role in neuropathic pain-like behavior in various mouse models [30, 31].

Our data demonstrate a concentration-dependent inhibitory effect of the antidepressant duloxetine on TRPM2-mediated heat-induced currents. In a previous study addressing the role of TRPM2 for cerebral ischemia-reperfusion injury, Toda et al. [24] already described an inhibitory effect of duloxetine primarily on H2O2-induced calcium-influx mediated by TRPM2. Importantly, this exciting property of duloxetine may not only be due to a direct inhibition but also to a possible antioxidant effect. Toda et al. [24] also demonstrated that 10 μM duloxetine induced a ~50% inhibition of ADPR-induced inward currents. This effect required 200–300 s and was described to be hampered by the occurrence of nonspecific leak currents. Considering the impressive rundown of ADPR-induced currents observed in our experimental setting, we were critical of this finding and aimed to reassess it. Nonetheless, the inhibitory efficacy of duloxetine on heat-induced currents through TRPM2 was almost identical to the effect demonstrated by Toda et al. [24]. Altogether, it seems like duloxetine indeed directly inhibits TRPM2 at micromolar concentrations. As effective plasma levels of duloxetine hardly exceed 1 μM, however [32], the relevance of this property of duloxetine remains unclear. This note raises the question, if possibly other depressants may exhibit a more potent and thus a likely relevant inhibition of TRPM2. Looking at our data, however, none of the investigated substances induced a potent block of TRPM2. While amitriptyline, fluoxetine, and paroxetine induced a comparable degree of inhibition as was determined for duloxetine, all other tested substances failed to induce a robust inhibition of TRPM2. In case of sertraline, we even observed a clear potentiation or even activation of TRPM2. The same effect was observed for low concentrations of lidocaine, while higher concentrations induced an almost complete block. One can only speculate about a possible relevance of these TRPM2-activating properties of lidocaine and sertraline. However, both substances may be interesting tools for further functional studies on TRMP2. Even ketamine, known to functionally interact with a variety of ion channels did not inhibit but rather slightly potentiated heat-evoked currents at concentrations up to 30 μM. This observation is confirmative to a recent study reporting that an acute application of 300 μM ketamine did not influence TRPM2-mediated calcium influx in hippocampal and dorsal root ganglion neurons [33]. Ending up with the finding that also pregabalin fails to inhibit or modify TRPM2, our and previous reports indicate that TRPM2 does not seem to be a primary target for clinically applied antidepressants. This notion is supported by the fact that the plasma concentrations of antidepressants such as duloxetine are usually in the nanomolar range and thus considerably lower than such concentrations which were found to inhibit TRPM2 in this study [32].

Although we believe that our approach to examining in vitro pharmacology of TRPM2 delivered quite solid data, it has the clear disadvantage with the fact that it is technically demanding and rather time-consuming. While it allows for focused and small-scaled studies like the present study, other approaches must be developed in order to enable compound libraries to be investigated on TRPM2. Fluorescence-based imaging techniques are a commonly applied approach, but they stay short when it comes to more detailed and mechanistic pharmacological analyses. The main obstacle that needs to be overcome is the pronounced instability of TRPM2 once being activated, e.g., TRPM2-mediated currents exhibit a strong rundown that cannot be controlled [34]. Saying this, however, some recent reports on the development of selective TRPM2-inhibitors are promising and demonstrate in vitro pharmacology of TRPM2 where this rundown may not be a limiting problem [16, 18]. Thus, identified substances from these reports may now be used in order to further explore the possible role of TRPM2 as a target for
novel antidepressants. Another important limitation of our study is the use of only recombinant TRPM2 channels which are overexpressed in HEK 293 cells. While this approach is state-of-art to study pharmacological properties of TRPM2 [16–20, 35, 36], the physiological and pathophysiological relevance of such data needs so be further validated in more intact in vitro and in vivo assays [37–39]. Furthermore, we cannot rule out the possibility that certain properties of TRPM2 may differ from a highly overexpressing system like in our study as compared to native cell likely to express lower levels of TRPM2.

**Acknowledgments**

The authors thank Mrs. Heike Bürger and Mrs. Kerstin Gutt (both Hannover Medical School) for excellent technical assistance.

**Statement of Ethics**

As a pure in vitro study, ethical approval was not required for this study in accordance with local/national guidelines.

**References**

1 Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, et al. LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol Cell.* 2002 Jan 1;9(1):163–73.
2 McHugh D, Flemming R, Xu SZ, Perraud AL, Beech DJ. Critical intracellular Ca2+-dependence of transient receptor potential melastatin 2 (TRPM2) cation channel activation. *J Biol Chem.* 2003 Mar 28;278(13):11002–6.
3 Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, et al. TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. *EMBO J.* 2006 May 3;25(9):1804–15.
4 Wehrhahn J, Kraft R, Harteneck C, Hausbaldi S. Transient receptor potential melastatin 2 is required for lipopolysaccharide-induced cytokine production in human monocytes. *J Immunol.* 2010 Mar 1;184(5):2386–93.
5 Yamamoto S, Shimizu S, Kiyonaka S, Taka-hashi N, Wajima T, Hara Y, et al. TRPM2-mediated Ca2+ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat Med.* 2008 Jul 1;14(7):738–47.
6 Knowles H, Li Y, Perraud AL. The TRPM2 ion channel, an oxidative stress and metabolisic sensor regulating innate immunity and inflammation. *Immunol Res.* 2013 Mar 1;55(1-3):241–8.
7 Song K, Wang H, Kamm GB, Pohle J, Reis FC, Heppenstall P, et al. The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia. *Science.* 2016 Sep 23;353(6306):1393–8.
8 Tan CH, McNaughton PA. The TRPM2 ion channel is required for sensitivity to warmth. *Nature.* 2016 Aug 25;536(7617):460–3.
9 Fonfria E, Marshall IC, Benham CD, Boyfield I, Brown JD, Hill K, et al. TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase. *Br J Pharmacol.* 2004 Sep 1;143(1):186–92.
10 Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature.* 2001 May 31;411(6837):595–9.
11 Wehage E, Eislfeld J, Heiner I, Jungling E, Zitt C, Lackhoff A. Activation of the cation channel long transient receptor potential channel 2 (LTRPC2) by hydrogen peroxide. A splice variant reveals a mode of activation independent of ADP-ribose. *J Biol Chem.* 2002 Jun 28;277(26):23150–6.
12 Sano Y, Inamura K, Miyake A, Mochizuki S, Yoko H, Matsushima H, et al. Immunocyte Ca2+ influx system mediated by LTRPC2. *Science.* 2001 Aug 17;293(5533):1327–30.
13 Kraft R, Grimm C, Grosse K, Hoffmann A, Sauerbruch S, Kettenmann H, et al. Hydrogen peroxide and ADP-ribose induce TRPM2-mediated calcium influx and cation currents in microglia. *Am J Physiol Cell Physiol.* 2004 Jan 1;286(1):C129–37.
14 Fonfria E, Murdock PR, Cusdin FS, Benham CD, Kelsell RE, McNulty S. Tissue distribution profiles of the human TRPM cation channel family. *J Recept Signal Transduct Res.* 2006;26(3):159–78.
15 Nagamine K, Kudoh J, Minoshima S, Kawasaki K, Asakawa S, Ito F, et al. Molecular cloning of a novel putative Ca2+-channel protein (TRPC7) highly expressed in brain. *Genomics.* 1998 Nov 15;54(1):124–31.
16 Cruz-Torres I, Backos DS, Herson PS. Characterization and optimization of the novel transient receptor potential melastatin 2 antagonist tatM2NX. *Mol Pharmacol.* 2020;97(2):102–11.
17 Luo X, Li M, Zhan K, Yang W, Zhang L, Wang K, et al. Selective inhibition of TRPM2 channel by two novel synthesized ADPR analogues. *Chem Biol Drug Des.* 2018;91(2):552–66.
18 Fourgeaud L, Dvorak C, Faouzzi M, Starkus J, Sahdeo S, Wang Q, et al. Pharmacology of JNJ-28583113: a novel TRPM2 antagonist. *Eur J Pharmacol.* 2019;853:299–307.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

**Funding Sources**

The study was funded by internal funds of the Hannover Medical School.

**Author Contributions**

Franziska Bracke performed all experiments, performed data analysis, and wrote the manuscript. Helge Frieling supervised the experiments and wrote the final version of the manuscript. Andreas Leffler supervised the experiments and wrote the final version of the manuscript.

**Data Availability Statement**

All electrophysiological patch clamp generated and analyzed for this study are including in this report. Further inquiries can be directed to the corresponding author.
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19 Hill K, Benham CD, McNulty S, Randall AD. Flufenamic acid is a pH-dependent antagonist of TRPM2 channels. *Neuropharmacology*. 2004 Sep; 47(3):450–60.

20 Hill K, McNulty S, Randall AD. Inhibition of TRPM2 channels by the antifungal agents clotrimazole and econazole. *Naunyn Schmiedebergs Arch Pharmacol*. 2004; 363(3):355–66.

21 Belrose J, Jackson MF. TRPM2: a candidate therapeutic target for treating neurological diseases. *Acta Pharmacol Sin*. 2018; 39(5):722–32.

22 Ko S, Wang SE, Lee HK, Jo S, Han J, Lee SH, et al. Transient receptor potential melastatin 2 governs stress-induced depressive-like behaviors. *Proc Natl Acad Sci U S A*. 2019; 116(5):1770–5.

23 Toda T, Yamamoto S, Umehara N, Mori Y, Wakamori M, Shimizu S. Protective effects of duloxetine against cerebral ischemia-reperfusion injury via transient receptor potential melastatin 2 inhibition. *J Pharmacol Exp Ther*. 2019; 368(2):246–54.

24 Akpinar A, Uğuz AC, Nazıroğlu M. Agomelatine and duloxetine synergistically modulates apoptotic pathway by inhibiting oxidative stress triggered intracellular calcium entry in neuronal PC12 cells: role of TRPM2 and voltage-gated calcium channels. *J Membr Biol*. 2014; 247(5):451–9.

25 Demirdaş A, Nazıroğlu M, Övey İS. Duloxetine reduces oxidative stress, apoptosis, and Cal(2+) entry through modulation of TRPM2 and TRPV1 channels in the hippocampus and dorsal root ganglion of rats. *Mol Neurobiol*. 2017; 54(6):4683–95.

26 Tóth B, Csanády L. Pore collapse underlies irreversible inactivation of TRPM2 cation channel currents. *Proc Natl Acad Sci U S A*. 2012; 109(33):13440–5.

27 Eberhardt MJ, Schillers F, Eberhardt EM, Riser L, de la Roche J, Herzog C, et al. Reactive metabolites of acetaminophen activate and sensitize the capsacin receptor TRPV1. *Sci Rep*. 2017 Oct 6; 7(1):12775–3.

28 McKamey SG, Jira LR, Tweed CM, Blake SD, Powell DP, Daghistani AT, et al. Antagonism of the transient receptor potential melastatin-2 channel leads to targeted antitumor effects in primary human malignant melanoma cells. *Int J Oncol*. 2022; 60(4):43.

29 Zhao S, Zhang H, Jin H, Cai X, Zhang R, Jin Z, et al. Design, synthesis and biological activities of benzo[d]imidazo[1,2-a]imidazole derivatives as TRPM2-specific inhibitors. *Eur J Med Chem*. 2021; 225:113750.