Drofenine: a 2-APB analog with improved selectivity for human TRPV3

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Abstract
Transient receptor potential vanilloid-3 (TRPV3) is a member of the TRPV subfamily of TRP ion channels. The physiological functions of TRPV3 are not fully understood, in part, due to a lack of selective agonists and antagonists that could both facilitate the elucidation of roles for TRPV3 in mammalian physiology as well as potentially serve as therapeutic agents to modulate conditions for which altered TRPV3 function has been implicated. In this study, the Microsource Spectrum Collection was screened for TRPV3 agonists and antagonists using alterations in calcium flux in TRPV3 overexpressing human embryonic kidney-293 (HEK-293) cells. The antispasmodic agent drofenine was identified as a new TRPV3 agonist. Drofenine exhibited similar potency to the known TRPV3 agonists 2-aminoethoxydiphenylboronate (2-APB) and carvacrol in HEK-293 cells, but greater selectivity for TRPV3 based on a lack of activation of TRPA1, V1, V2, V4, or M8. Multiple inhibitors were also identified, but all of the compounds were either inactive or not specific. Drofenine activated TRPV3 via interactions with the residue, H426, which is required for TRPV3 activation by 2-APB. Drofenine was a more potent agonist of TRPV3 and more cytotoxic than either carvacrol or 2-APB in human keratinocytes and its effect on TRPV3 in HaCaT cells was further demonstrated using the antagonist icilin. Due to the lack of specificity of existing TRPV3 modulators and the expression of multiple TRP channels in cells/tissue, drofenine may be a valuable probe for elucidating TRPV3 functions in complex biological systems. Identification of TRPV3 as a target for drofenine may also suggest a mechanism by which drofenine acts as a therapeutic agent.

Abbreviations
2-APB, 2-aminoethoxydiphenylboronate; HEK-293, human embryonic kidney-293 cells; TRPA1, transient receptor potential ankyrin-1; TRPV1, transient receptor potential vanilloid-1; TRPV3, transient receptor potential vanilloid-3.

Introduction
The transient receptor potential vanilloid (TRPV) subfamily of TRP channels consists of six structurally similar, but functionally unique proteins (TRPV1-V6). Mammalian TRPV and other TRP family proteins (i.e., TRPA, TRPC, and TRPM) are differentially expressed in neurons and non-neuronal cells of animals and humans. Unlike many other TRP channels, TRPV3 expression in sensory neurons appears low, and it may exist in some cells as a heteromultimer with TRPV1 resulting in a functionally unique ion channel (Cheng et al. 2012). TRPV3 is abundantly expressed by keratinocytes, particularly intrafollicular keratinocytes (Peier et al. 2002; Chung et al. 2003, 2004b) as well as cells of the tongue, palate, testes, cornea, nasal epithelium, distal colon, and inner ear (Xu et al. 2006).
TRPV3 is currently being investigated for its role in cutaneous physiology, specifically its role in thermal perception, inflammation, irritation and pain, wound healing, maintenance of skin barrier integrity, and hair growth (Nilius and Biró 2013; Nilius et al. 2013; Kaneko and Szallasi 2014). Known TRPV3 agonists include natural products such as thymol, carvacrol, camphor, 6-tert-butyl-m-cresol, menthol, eugenol, farnesyl pyrophosphate, incensole acetate, as well as synthetic 2-aminooethoxydiphenylboronate (2-APB), and z-hydroxy acids which are widely used in the cosmetic industry as skin peeling agents (Chung et al. 2004a; Xu et al. 2006; Vogt-Eisele et al. 2007; Moussaieff et al. 2008; Bang et al. 2010; Earley et al. 2010; Cao et al. 2012). These agents exhibit EC50 values between ~130 nmol/L (farnesyl pyrophosphate), ~34 to ~500 μmol/L (carvacrol), and up to >6 mmol/L (cresol), depending on the model used. The fact that essentially all known agonists of TRPV3 are skin sensitizing agents and irritants suggests that aberrant activation of TRPV3 may play key roles in regulating skin homeostasis and dermatopathologies, a notion supported by the identification of a constitutively active TRPV3 variant as a cause of Olmsted Syndrome (Lin et al. 2012). However, conflicting evidence has been obtained regarding the role of TRPV3 in skin using both transgenic mice and known TRPV3 agonists, essentially all of which also modulate the activity of other TRP channels including TRPA1 and TRPM8.

Known TRPV3 antagonists include 17(R and S)-resolvin D1, naturally occurring anti-inflammatory/proresolving omega-3 lipid derivatives (Bang et al. 2012), the TRPM8 agonist icilin (Sherkheli et al. 2012), 2,2-diphenyltetrahydrofuran (a 2-APB analog) (Chung et al. 2005), and isopentenyl pyrophosphate (Bang et al. 2011). However, much like the known TRPV3 agonists, these antagonists are not selective for TRPV3. Numerous TRPV3 modulators are currently under development as possible therapeutic agents (e.g., GRC15300 by Glenmark Pharmaceuticals and others by Hydra Biosciences), with potential utility in conditions such as itch, psoriasis, hirsutism, dermatitis, pain, etc. Thus, there is not only a need to identify selective agonists and antagonists of TRPV3 that can be used to understand its role in mammalian physiology but there is also an ongoing effort to target TRPV3 for treating human disorders and diseases.

The purpose of this study was to identify new commercially available agonists and antagonists with increased selectivity for TRPV3. Here we report that drofenine (CAS#: 0001679-76-1), a 2-APB analog, is an agonist of human TRPV3, with greater selectivity for TRPV3 versus other TRP channels than both 2-APB and carvacrol. As such, drofenine may be a valuable chemical probe to more specifically ascertain the complex physiological and pathophysiological roles of TRPV3. Furthermore, one might speculate that TRPV3 may have some role in the purported antispasmodic activity of drofenine (Spasmorel; Novartis, Muscat, Oman).

## Materials and Methods

### Chemicals

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise stated. 2-(5-trifluoromethyl-pyridine-2ylsulfanyl)-1-(8-methyl-3,4-dihydro-2H-quinolin-1-yl)-ethanone (TRPV3 antagonist) was synthesized as previously described (Chong et al. 2010) and the purified product verified by 1H-NMR.

### Screening library

The Microsource Spectrum Collection was obtained from the Drug Screening Resource at The University of Utah. This library consisted of 2320 compounds including drug components (60%), natural products (25%), and other bioactive compounds (15%). The compound library was prepared for screening as 3× (300 μmol/L) stock solutions in calcium assay buffer (1× HBSS [Hank’s Balanced Salt Solution], 20 mmol/L HEPES [4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid], pH 7.3) in 384-well plates.

### Cell culture

Human embryonic kidney-293 (HEK-293) cells (ATCC, Rockville, MD) were engineered to stably overexpress human TRPV3 (in pcDNA3.1 D V5/His-TOPO) and other human TRP channels (TRPA1, TRPM8, TRPV1, TRPV2, and TRPV4) as previously described (Deering-Rice et al. 2011). HEK-293 cells were grown in DMEM:F12 (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (FBS) and 1× penicillin/streptomycin (Invitrogen). HEK-293 cells stably overexpressing the various TRP channels were maintained in DMEM:F12 supplemented with 5% FBS, 1× penicillin/streptomycin, and 350 μg/mL Geneticin (Invitrogen).

Cells were subcultured using trypsin and plated into 1% gelatin-coated 96- or 384-well plates for calcium imaging experiments. Human immortalized keratinocytes, HaCaT cells, were provided by Dr. Douglas Grossman, M.D., Ph.D., of the Department of Dermatology, University of Utah. HaCaT cells were cultured in DMEM containing 5% FBS and 1× penicillin/streptomycin/glutamine. Cells were subcultured using trypsin and plated into 96-well plates for experiments.

### Site-directed mutagenesis and transient transfection of TRPV3

The following TRPV3 mutants were constructed using the QuickChange XL site-directed mutagenesis kit (Stratagene,
La Jolla, CA): H426N and R696K, residues previously identified as being critical for activation of TRPV3 by 2-APB (Hu et al. 2009). The primers were: TRPV3–H426N (+) 5′-CTGGAGCGGTGCTAACGCTGTCG-3′ and (−) 5′-GCAGACGGGTTCAGCGCTCCAG-3′; and TRPV3–R696K (+) 5′-CATCTGGCCCTGCAGAAAGGCGAGC-3′ and (−) 5′-GTCCTGGTTTCGTACCAGGGCAGATG-3′. For assays, HEK-293 cells were transfected with mutant or wild-type constructs using 175 ng purified plasmid DNA complexed with Lipofectamine 2000 (Invitrogen), at a 2:1 ratio of lipid to DNA. Transiently transfected cells were assayed 48 h post transfection.

Calcium imaging

Calcium imaging experiments were performed as described previously (Deering-Rice et al. 2011, 2012; Shapiro et al. 2013). Briefly, cells were loaded with Fluo-4 AM using the Fluo-4 Direct Kit (Invitrogen) diluted in LHC-9 (HEK-293 cells) or calcium assay buffer (HaCaT cells) for 60 min at 37°C (HEK-293) or room temperature (HaCaT). The loading solution was removed and the cells were subsequently incubated 30 min at 37°C (HEK-293) or room temperature (~23°C) (HaCaT) in the dark with LHC-9 (HEK-293) or calcium buffer (HaCaT) containing 0.5 mM probenecid and 0.75 mM trypan red (ATT Bioquest, Sunnyvale, CA). Treatment solutions were prepared in LHC-9 (HEK-293) or calcium assay buffer (HaCaT) at 3× concentration and 10 or 25 μL was added to 20 or 50 μL of media on the cells in 384- or 96-well plates. Calcium flux responses were measured using either a NOVOstar plate reader (BMG LABTECH, Offen-berg, Germany) or microscopically as described previously in the references above. Where specified, data were corrected for nonspecific responses observed with HEK-293 cells, and normalization to the maximum attainable change in fluorescence elicited by ionomycin (10 μmol/L).

Electrophysiology

Whole-cell recordings were made using borosilicate pipettes (3–5 MΩ). Coverslips were coated with concanavalin A as described previously (Ryskamp et al. 2011) and rinsed with phosphate buffer solution. Suspended TRPV3-overexpressing HEK-293 cells were plated on these coverslips in supplemented DMEM:F12 and used within 24 h. Throughout experiments, cells were perfused with external saline containing 145 mM NaCl, 5 mM KCl, 3 mM MgCl₂, 10 mM HEPES, 10 mM CaCl₂, and 2 mM MgCl₂ (pH 7.4, 320 mosM). Drofenine was bath applied via perfusion (250 and 500 μmol/L) or injection (1 μmol/L). The internal pipette solution consisted of 133 mM CsCl, 10 mM HEPES, 5 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 1 mM CaCl₂, and 1 mM MgCl₂ (pH of 7.3 set with CsOH) (Bae et al. 2011). Pipette and membrane capacitances were automatically compensated and current recordings were Bessel-filtered at 10 kHz using an EPC-10 amplifier (Patchmaster; HEKA Instruments Inc., Bellmore, NY). Cells were voltage clamped at −60 or −70 mV to measure inward currents. The holding potential was adjusted to approximately normalize the baseline current from cell to cell. For each drofenine concentration, we only used cells that were recorded at the same holding potential. Fresh cells were used for each recording to prevent variability due to sensitization or desensitization. Data were collected at 1.0 kHz and analyzed with IGOR Pro 6 software (WaveMetrics Inc., Lake Oswego, OR). Every 100th data point was plotted in example traces (Fig. 3A).

Cell viability assay

HaCaT cells were subcultured in 96-well plates, grown until the cells reached 80–90% confluence, and incubated with increasing concentrations of 2-APB, drofenine, or carvacrol for 24 h. Cytotoxicity was measured using the Cell Counting Kit-8 assay (Cell Counting Kit CCK-8, Dojindo Laborato ries, Gaithersberg, MD) as per manufacturer’s instructions.

Results

The Microsource Spectrum Collection of compounds was screened to identify modulators of TRPV3 activity, as represented graphically in Figure 1. Calcium influx (i.e., increased fluorescence upon compound addition; t = ~8–17 sec) was used as an indicator of TRPV3 activation by an agonist. Attenuated calcium influx (i.e., reduced fluorescence change) upon addition of the known TRPV3 agonist carvacrol (300 μmol/L; t = ~17–30 sec) was indicative of an antagonist. Initial screening identified 22 of 2320 as possible TRPV3 agonists, and 102 of 2320 as possible antagonists; 1873 had insignificant effects on TRPV3 function. Upon further analysis of the potential agonists and antagonists, selected based on the magnitude of the effect and compound availability, all were either known agonists (e.g., 2-APB), false-positives (e.g., autofluorescent such as fluorescein), not reproducible, or nonspecific for TRPV3; activating or inhibiting other TRP channels (Tables 1 and 2), and/or causing an equivalent degree of calcium flux response in native HEK-293 cells (e.g., ace tycholine). A brief summary of the confirmation studies and selectivity data generated using other TRP channel overexpressing HEK-293 cell lines and specific agonists for each receptor, are summarized in Tables 1 and 2.

Drofenine was selective for TRPV3 in that activation of TRPA1, M8, V1, V2, and V4, which are known to be
activated by the TRPV3 agonists 2-APB and/or carvacrol, was not observed at concentrations up to 1 mmol/L (Fig. 2A). A detectable change in TRPV3 activity was observed at concentrations as low as \( \sim 30 \mu \text{mol/L} \) and increased in a concentration-dependent manner. At concentrations greater than 500 \( \mu \text{mol/L} \), calcium flux exhibited some evidence of TRPV3 independence based on small and comparable responses in both native HEK-293 cells and other TRP channel overexpressing cell lines. The maximum change in cytosolic calcium (\( \Delta F \)) for drofenine (EC\(_{50} = 207 \mu \text{mol/L} \)) was comparable to the known TRPV3 agonists 2-APB (EC\(_{50} = 78 \mu \text{mol/L} \)) and carvacrol (EC\(_{50} = 438 \mu \text{mol/L} \)) (Fig. 2B). A TRPV3 antagonist was synthesized and used to block calcium flux in TRPV3 overexpressing HEK-293 cells. Equal inhibition of calcium flux was observed for all three agonists with an IC\(_{50} \) of 1–3 \( \mu \text{mol/L} \).

Consistent with calcium influx across the plasma membrane, drofenine dose-dependently induced inward currents in TRPV3-overexpressing HEK-293 cells held at...
negative membrane potentials (Fig. 3A–D). Drofenine (250 μmol/L) tended to increase the frequency and amplitude of stochastic and transient inward currents, whereas 500 μmol/L and 1 mmol/L drofenine induced higher amplitude, more sustained responses with faster onset. Drofenine-induced inward currents were similar to 2-APB-evoked TRPV3 currents (Chung et al. 2004a). These data demonstrate that drofenine gates plasmalemmal calcium influx.

The chemical structure of drofenine is similar to the known nonspecific TRPV3 agonist 2-APB (Fig. 4A). The contributions of the residues previously shown to determine 2-APB sensitivity were evaluated as determinants of drofenine sensitivity by comparing calcium flux elicited by carvacrol, 2-APB, and drofenine in HEK-293 cells transiently transfected with wild-type human TRPV3 (TRPV3-WT), TRPV3-H426N, or TRPV3-R696K. The TRPV3-H426N mutant exhibited reduced activation relative to TRPV3-WT using both drofenine and 2-APB at a concentration of 100 μmol/L (Fig. 4B). Mutation of H426 had no effect on calcium flux induced by carvacrol (100 μmol/L), as has been previously reported for the structurally similar monoterpenoid agonist camphor (Hu et al. 2009). At 200 μmol/L drofenine, the H426N mutation did not reduce TRPV3 activation, indicating only a shift in the binding of drofenine to TRPV3 or that drofenine may potentially interact with TRPV3 similar to, but not identically to 2-APB. The TRPV3-R696K mutant displayed diminished-to-no-function using all three agonists. Of significance, based on structural similarity and screening results, the 2-APB and drofenine analogs dicyclomine (Bentyl; anticholinergic, antispasmodic), was neither a TRPV3 agonist nor antagonist, while diphenhydramine was a nonselective TRPV3 inhibitor.

Drofenine also induced calcium flux in HaCaT cells, which are an immortalized human keratinocyte cell line that expresses high levels of TRPV3 and other TRP channels. Drofenine was a more potent agonist of TRPV3 in HaCaT cells than both 2-APB and carvacrol (Fig. 5A). The EC50 for drofenine was 605 μmol/L, versus >1000 μmol/L for carvacrol and 2-APB (Fig. 5A), and the maximum change in fluorescence achieved by treatment with drofenine was ~80% that of ionomycin. Fluorescence images of HaCaT cells 1 min after treatment with 100 μmol/L carvacrol, 100 μmol/L 2-APB, or drofenine (50 and 100 μmol/L) show the differences in potency for the three TRPV3 agonists in HaCaT cells (Fig. 5B). Icilin, a potent TRPM8 agonist, also inhibits TRPV3 at low micromolar concentrations (Sherkheli et al. 2012). Icilinameliorated drofenine-induced calcium flux with an IC50 of 3.3 μmol/L (Fig. 5C). The synthesized TRPV3 antagonist could not be used in the HaCaT cells due to apparent off target effects characterized by increases in calcium flux at low (<300 nmol/L) and high (>1 μmol/L) concentra-

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Table 2. List of potential TRPV3 antagonists identified by initial screening and re-evaluated as TRPV3 antagonists.

| Name                   | CAS #       | Description/Use | Comment(s)                        |
|------------------------|-------------|-----------------|-----------------------------------|
| Erythromycin           | 114-07-8    | Macrolide antibiotic | Not inhibitory on rescreen        |
| Tetracaine HCl         | 136-47-0, 94-24-6 | Anesthetic; antipruritic; ryanodine receptor antagonist; Ca++-induced Ca++ release/SOCE inhibitor | Structural similarity with dyclonine, pramoxine, tetracaine, and mifepristone; inhibited TRPA1>V3>V1; apparent TRPV1 agonist <25 μmol/L |
| Etoposide              | 33419-42-0 | Antineoplastic | Cytotoxic, false positive |
| Diphenhydramine HCl    | 147-24-0    | Antihistaminic | Inhibitory at high concentrations. TRPV1>TRPV3>TRPA1. |
| Clomipramine HCl       | 17321-77-6, 303-49-1 | Tricyclic antidepressant; nonselective neurotransmitter reuptake inhibitor | Inhibitory in rescreen; nonselective SOCE inhibitor. |
| Spectinomycin HCl      | 22189-32-8, 21736-83-4 | Aminocyclitol antibiotic | Not inhibitory on rescreen |
| Sulfinpyrazone          | 57-96-5     | Uricosuric diuretic | Not inhibitory on rescreen |
| Chloramphenicol         | 56-75-7     | Bacteriostatic antibiotic | Not inhibitory on rescreen |
| Dyclonine HCl          | 536-43-6, 586-60-7 | Topical anesthetic | Not inhibitory on rescreen |
| Tamoxifen citrate       | 54965-24-1, 10540-29-1 | Estrogen receptor antagonist | Structural similarity with dyclonine, pramoxine, tetracaine, and mifepristone; inhibited TRPV3>V1>V4>M8>V3 |
| Warfarin               | 81-81-2, 2610-86-8 | Anticoagulant | Nonselective; also inhibited TRPV1 |
| Fluufenamic acid        | 530-78-9    | NSAID; voltage-gated Na+ channel inhibitor | Nonselective; also inhibited TRPV1 |
| Pramoxine HCl          | 637-58-1, 140-65-8 | Topical anesthetic; antipruritic; Na+ channel blocker | Structural similarity with dyclonine, tetracaine, tamoxifen, and mifepristone; inhibited TRPA1, V3, and V1 |
| Mifepristone (RU 486)  | 84371-65-3 | Progesterone receptor antagonist; abortifacient | Structural similarity with dyclonine, pramoxine, and tamoxifen; inhibited TRPA1>V3>V1 |
Characteristics and specificity of TRPV3 activation by drofenine. (A) Drofenine selectively increased calcium flux in HEK-293 cells overexpressing TRPV3, but not HEK-293 cells overexpressing TRPA1, M8, V1, V2, or V4 at concentrations up to 1000 μmol/L. (B) HEK-293 cells overexpressing TRPV3 were exposed to increasing concentrations of drofenine, carvacrol, and 2-APB. Changes in fluorescence were monitored. (C) TRPV3 overexpressing HEK-293 cells were preincubated with increasing concentrations of the TRPV3 antagonist for 30 min followed by treatment of drofenine (200 μmol/L), carvacrol (300 μmol/L), or 2-APB (300 μmol/L), inhibition of calcium flux was measured and an IC_{50} value was calculated. Data were collected using a NOVOSTAR plate reader, blank subtracted, and are expressed as the change in the maximal fluorescence (ΔF_{max}) or the initial rate of change in cellular fluorescence (ΔF/sec). Antagonist data were normalized to the baseline current and the peak response detected using a one-tailed, paired t-test.

**Discussion**

The roles TRPV3 plays in mammalian physiology are not completely understood. This is due, in part, to the lack of selective agonists and antagonists. In this study, we sought to identify new compounds that may be used as pharmacological tools for the study of TRPV3, as well as molecules that may have potential for treating pathologies in which TRPV3 may be involved. The Microsource Spectrum Collection, containing 2320 compounds including drugs, natural products, and other bioactive compounds, was screened.
identified substance \( P \), resulting from TRPA1 activation in peripheral C-fibers, as a mediator of pruritus (Liu et al. 2013). Therefore, inhibition of TRPV3 in conjunction with other TRP channels such as TRPV1 and TRPA1 by diphenhydramine, tetracaine, or other molecules found to inhibit multiple TRP channels, in addition to inhibition histamine \( H_1 \) receptors or other receptor targets, may have complimentary effects in treating itch. This concept is supported by finding that the antitussive properties of dextromethorphan and chlorpheniramine are attributable to both inhibition of histamine \( H_1 \) receptors and TRPV1 (Sadofsky et al. 2008).

Multiple potential TRPV3 agonists were also identified and evaluated (Table 1). These included 2-APB (previously known) and drofenine. Here, drofenine was shown to activate TRPV3 (Figs. 2 and 3), but not other TRP channels that are known to have overlap in agonist preference with TRPV3 (Fig. 2A). For example, 2-APB was also identified in our screen, but 2-APB is known to be nonselective for TRPV3, based on numerous reports documenting the activation of other TRP channels including TRPA1, M6, V1, and V2, and inhibiting TRPC4, C5, C6, M2, and M8 (Clapham 2007).

Amino acid residues comprising a binding site for drofenine on TRPV3 were similar to 2-APB, H426 and R696 (Hu et al. 2009). However, these sites do not appear to solely explain the activation mechanism for TRPV3 since inhibition of TRPV3 was not achieved with the H426N mutant when higher concentrations of drofenine were used, similar to carvacrol (Fig. 4B). The differences in the relative potency of the three agonists between TRPV3 overexpressing HEK-293 and HaCaT cells may be due to differences in cell permeability and activation of different subcellular pools of TRPV3. Thus, additional studies are required to more fully elucidate the molecular basis for TRPV3 activation by drofenine.

In the human keratinocyte (HaCaT) cell line, drofenine also induced calcium flux (Fig. 5A–C) and caused cytotoxicity (Fig. 5D), exhibiting greater potency in both measures than the known TRPV3 agonists 2-APB and carvacrol. These results suggested a role for TRPV3 activation in cell death as has been suggested by others (Yamada et al. 2010). Pretreatment of HaCaT cells with icilin completely inhibited TRPV3-induced calcium flux (Fig. 5C) (Sherkheli et al. 2012), but icilin was also potently cytotoxic to HaCaT cells (data not shown) preventing the use of icilin to definitively link drofenine-induced calcium flux with cell death. The TRPV3 antagonist was also evaluated, but was not informative in this cell line due to apparent off target effects. This emphasizes the continued need for new TRPV3 antagonists for elucidating precisely what roles TRPV3 plays in biological systems such as skin cells and others.
Drofenine, product name Spasmo-Cibalgin (Novartis, Oman), is an antispasmodic/anticholinergic agent used for relaxing smooth muscle, treating dysmenorrhea, and relieving pain in the gastrointestinal tract, biliary passages, and urogenital tract. Information regarding the clinical utility of drofenine is limited, but obscure sources suggest use for treatment of visceral spasm and urinary retention. Our results may suggest that TRPV3 could be involved in regulating these phenomena, if the concentration is high enough at the site of action, thus potentially providing new insights into mechanisms by which these events transpire. Additionally, drofenine has also been identified as an inhibitor of neomycin-induced hair cell loss in a zebrafish model of ototoxicity (Coffin et al. 2010). Similarly, several of the nonselective TRPV3 inhibitors listed in Table 2 were also identified as ototoxins (Coffin et al. 2010). Although not investigated, the results presented herein could indicate a critical role for TRPV3 in hair cell physiology and ototoxicity, as has been suggested for TRPA1, V1, and V4 (Mukherjea et al. 2008; Stepanyan et al. 2011; Lee et al. 2013).

In summary, drofenine has been characterized as a TRPV3 agonist with improved selectivity for TRPV3 relative to other TRP channels. The ability to target individual TRP channels is important not only for identifying key mediators of physiological and pathological processes but also in identifying the correct TRP channels for potential pharmacological interventions. Our results demonstrate that drofenine may be a new and valuable TRPV3 agonist for further unraveling some of the mysterious and occasionally paradoxical properties that have been attributed to TRPV3 thus far.

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Disclosures

None declared.
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