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Efficient and flexible synthesis of new photoactivatable propofol analogs

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ABSTRACT

Propofol is a widely used general anesthetic, which acts by binding to and modulating several neuronal ion channels. We describe the synthesis of photoactivatable propofol analogs functionalized with an alkyne handle for bioorthogonal chemistry. Such tools are useful for detecting and isolating photolabeled proteins. We designed expedient and flexible synthetic routes to three new diazirine-based crosslinkable propofol derivatives, two of which have alkyne handles. As a proof of principle, we show that these compounds activate heterologously expressed Transient Receptor Potential Ankyrin 1 (TRPA1), a key ion channel of the pain pathway, with a similar potency as propofol in fluorescence-based functional assays. This work demonstrates that installation of the crosslinkable and clickable group on a short nonpolar spacer at the *para* position of propofol does not affect TRPA1 activation, supporting the utility of these chemical tools in identifying and characterizing potentially druggable binding sites in propofol-interacting proteins.

Propofol (2,6-diisopropylphenol) is an intravenous general anesthetic used in millions of surgical procedures worldwide.[1] In addition to the sedative effects it exerts on the central nervous system, propofol also elicits a "burning" sensation upon injection in many patients.[2] The γ-aminobutyric acid A ($GABA_A$) receptor is the principal target leading to the anesthetic properties of propofol,[3] whereas Transient Receptor Potential Ankyrin 1 (TRPA1), a calcium-permeable ion channel expressed in peripheral sensory neurons, has been suggested to be the key mediator of propofol-induced pain.[4] We recently used a photocrosslinkable propofol analog to demonstrate that this analog does indeed bind in the TRPA1 site identified by mutagenesis.[5] Similar strategies have identified propofol-binding sites in the $GABA_A$ receptor.[6,7] Also, molecular modeling and mutagenesis of the binding pocket residues corroborate photolabeled sites.[8,9] These studies illustrate the power of photoaffinity labeling (PAL) to identify the binding-site location in complex biological systems, particularly where multiple binding sites may exist.[5,9] In addition, PAL probes may elucidate new drug targets and off-target proteins, facilitating drug discovery efforts.[10,11]

PAL finds widespread utility in drug discovery as a method to identify new macromolecular targets and as a complement to structure determination techniques like cryo-EM and X-ray crystallography to probe the location and structure of ligand binding sites. Although several photoaffinity analogs of propofol exist,[6,7,12,13] there is a dearth of propofol probes that also contain a chemical handle to enable subsequent isolation of photolabeled protein, limiting the discovery of novel propofol-interacting proteins. Thus, it is important to expand the library of crosslinkable analogs to provide more tools to understand how propofol and similar molecules, like thymol, carvacrol and zingerone, which are plant phenols with multiple biological activities, interact with their physiological targets.

One of the ideal traits of a photoaffinity probe is structural similarity to the parent molecule. In addition, the choice of crosslinker is important; diazirines have favorable specific activities due to their activation at relatively long wavelengths, the small size of the photoreactive group, and the short half-life of the reactive carbene intermediate.[14] The rapid quenching of carbenes by reaction with water molecules minimizes unspecific labeling, but also causes low yields of photolabeling. To facilitate the detection and isolation of photolabeled protein, clickable handles such as alkynes provide a convenient post-crosslinking method to incorporate an azide-containing biotin or fluorescent tag into the photoprobe.[15] To the best of our knowledge, the only report of a bifunctional propofol analogue has only been demonstrated to retain biological activity for the $GABA_A$ receptor,[6] and has not been tested for other targets such as TRPA1 activation.

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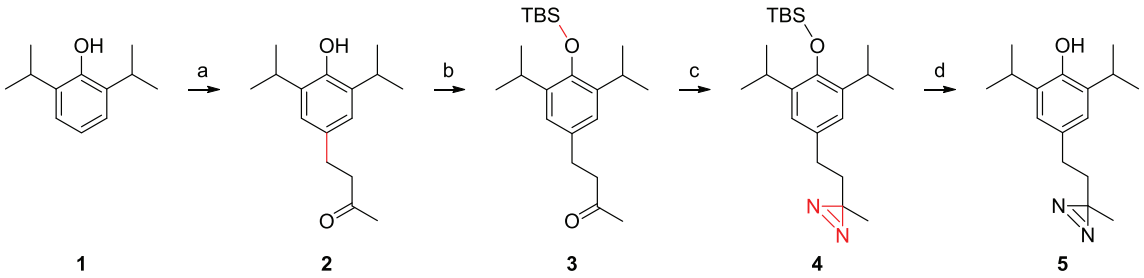
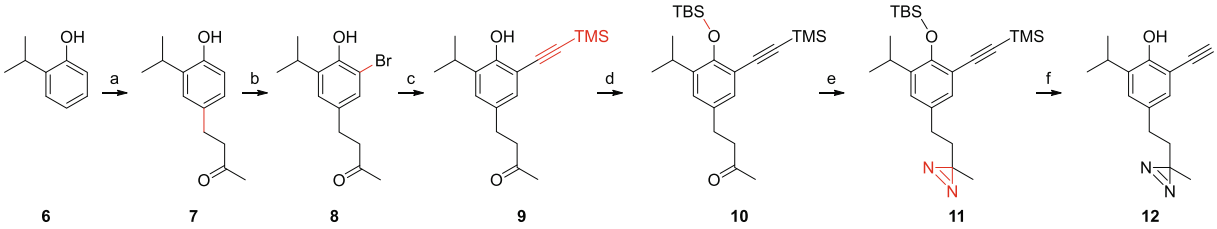
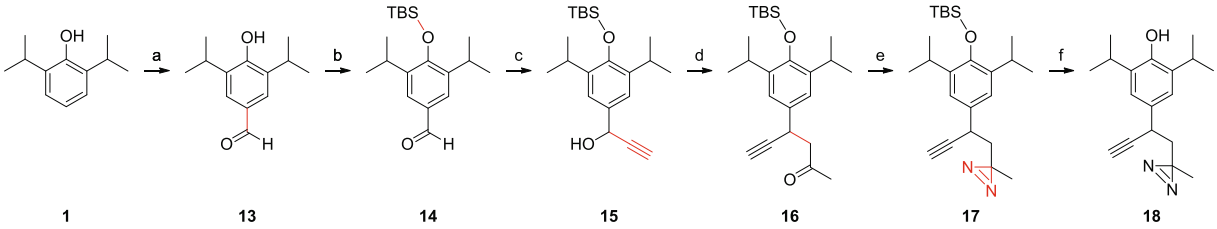


Scheme 1. Synthesis of XPRO. Reagents and conditions: (a) Methyl vinyl ketone, Amberlyst-15, toluene, 50 °C, N_2, 6 h, 80%; (b) TBSCl, imidazole, DMF, rt, N_2, 16 h, 79%; (c) NH_3, MeOH, NH_2OSO_3H, −40 °C, N_2, 7 h; TEA, iodine, MeOH, 45%; (d) TBAF-acetic acid, THF, rt, N_2, 16 h, 76%.



Scheme 2. Synthesis of *o*-XPRO-Click. Reagents and conditions: (a) Methyl vinyl ketone, Amberlyst-15, toluene, 40 °C, N_2, 8 h, 44%; (b) NBS, DCM, rt, N_2, 16 h, 54%; (c) PPh_3, $PdCl_2(PPh_3)_2$, CuI, ethynyltrimethylsilane, TEA, rt, N_2, 16 h, 59%; (d) TBSCl, imidazole, DMF, rt, N_2, 16 h, 82%; (e) NH_3, MeOH, NH_2OSO_3H, − 40 °C, N_2, 7 h; TEA, iodine, MeOH, 32%; (f) TBAF-acetic acid, THF, rt, N_2, 16 h, 53%.



Scheme 3. Synthesis of *p*-XPRO-Click. Reagents and conditions: (a) Hexamethylenetetramine, TFA, HCl, reflux, 9 h, 90%; (b) TBSCl, imidazole, DCM, rt, N_2, 16 h, 63%; (c) Ethynylmagnesium bromide, THF, −78 °C, N_2, 16 h, 83%; (d) cat. $Cp^*RuCl(\mu_2\text{-}SMe)_2RuCP^*Cl$, NH_4BF_4, acetone, reflux, N_2, 5 h, 83%; (e) NH_3, MeOH, NH_2OSO_3H, − 40 °C, N_2, 7 h; TEA, iodine, MeOH, 52%; (f) TBAF-acetic acid, THF, rt, N_2, 16 h, 55%.

Although photoactive propofol derivatives do exist, several are unstable,[13] or have cumbersome synthetic routes,[12] and only one has a click handle.[6] We hypothesized that linking the propofol core with a short nonpolar aliphatic spacer to separate the diazirine moiety from the aryl ring would not only preserve the minimal pharmacophore but also facilitate synthetic expediency.[7,13] We designed three probes that fulfill these criteria, and we synthesized them using relatively short and efficient synthetic sequences (Schemes 1–3), which could readily be applied to other similar scaffolds. Following synthesis, we used functional assays using calcium influx and binding to a fluorescent indicator as a measure of probe activation of TRPA1 to determine their promise in future crosslinking experiments.

We designed our initial crosslinkable probe, XPRO (**5**), bearing the linker and diazirine moiety in the *para* position to minimize potential intramolecular interactions of the reactive carbene intermediate while maintaining overall hydrophobicity of the propofol starting point. Indeed, a propofol analog with a trifluoromethyl diazirine directly at the *para* position was reported to be a poor photolabel.[7,13] Synthesis of XPRO was achieved in an expedient 5-step sequence (Scheme 1). Initial functionalization of the para-position was achieved through an Amberlyst-15-mediated alkylation[16] followed by protection of the phenol hydroxyl as a TBS silyl ether. The pendant methyl ketone was subsequently converted to the requisite diazirine via a two-step sequence.[17,18] Lastly, deprotection of the TBS ether with TBAF afforded XPRO. Overall yield for this sequence was 22%.

We next sought to generate an analog with a clickable group to enable potential labeling studies. We envisioned placing an alkyne at two possible locations within XPRO—in one analog one of the two isopropyl groups could be substituted with an alkyne (*o*-XPRO-Click) and in a second analog the alkyne could be appended proximal to the diazirine itself (*p*-XPRO-Click). At the outset, we chose to synthesize and profile both designs, since it was unclear which would have the best potency/reactivity profile.

To synthesize *o*-XPRO-Click (**12**; Scheme 2) starting from 2-isopropylphenol (**6**), Amberlyst-15 was again used to generate the alkyl ketone **7**. Subsequent bromination with NBS, Sonogashira coupling with TMS-acetylene, and phenol protection yielded the bis-silyl-protected intermediate **10**. Functionalization of the ketone using previously established conditions, followed by deprotection, generated *o*-XPRO-Click. In the case of *p*-XPRO-Click (**18**; Scheme 3), Duff formylation generated a benzaldehyde intermediate which, after phenol protection, was subjected to a Grignard reaction with ethynylmagnesium bromide. Subsequent propargylic alkylation[19] followed by diazirination and deprotection yielded *p*-XPRO-Click. Moreover, *o*-XPRO-Click and *p*-XPRO-conjugation experiments with 3-Azido-7-hydroxycoumarin, whose fluorescence increases upon a successful click reaction with an alkyne,[20] demonstrate their ability to react with azide partners (Supplementary fig. 1).

As a first assessment of the biological activity of our three new photocrosslinkable propofol analogs, we tested their ability to activate

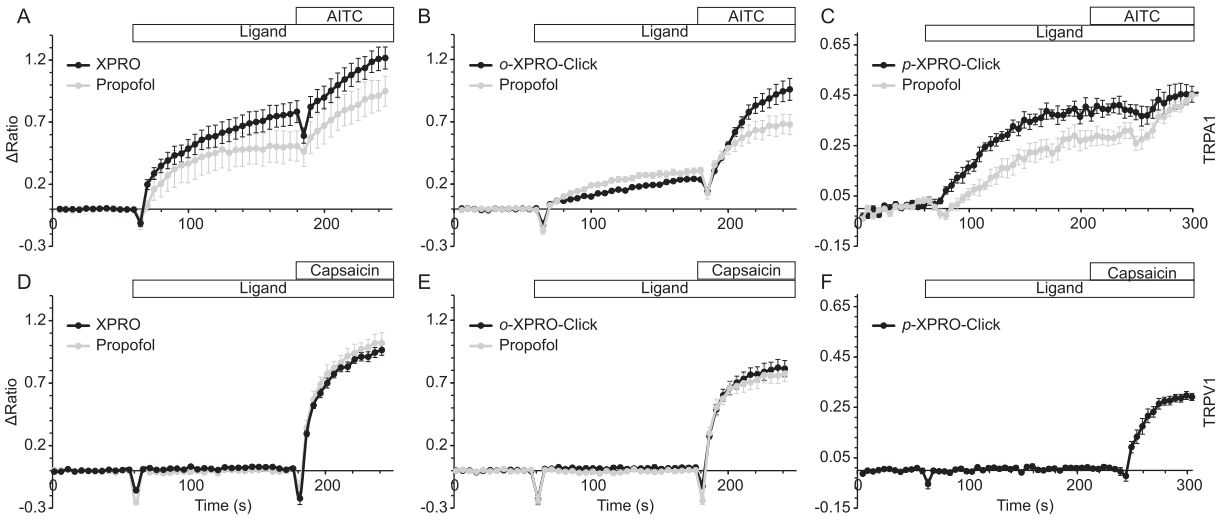


Fig. 1. XPRO, *o*-XRO-Click, and *p*-XPRO-Click and propofol elicit calcium influx in Sf21 insect cells heterologously expressing TRPA1 but not TRPV1. (A, B, C) Time traces showing the net change in Fura-2 340/380 fluorescence ratio (ΔRatio) in TRPA1-expressing insect cells. Adding XPRO (A), *o*-XPRO-Click (B) or *p*-XPRO-Click (C) at 60 s caused a rise in $[Ca^{2+}]_i$. Traces showing activation of the equivalent batches of cells with 60 μM propofol (grey traces) are included as positive controls in each panel. Addition of 1 mM allyl isothiocyanate (AITC) at 180 s provided a positive control for mouse TRPA1 expression. Data represent mean ± SEM (n = 4–14). (D, E, F) Addition of 60 μM XPRO (D), *o*-XPRO-Click (E) or *p*-XPRO-Click (F) or propofol (grey traces) at 60 s did not cause a rise in $[Ca^{2+}]_i$ in rat TRPV1-expressing insect cells. Addition of 75 nM capsaicin, a TRPV1-specific agonist, at 180 s did elicit a rise in $[Ca^{2+}]_i$, confirming TRPV1 expression in all samples. Data represent mean ± SEM for n = 3–12.

TRPA1, leveraging our previously developed cell-based calcium-influx assay.[5] For TRPA1 production, we chose baculovirus-infected insect cells, a common heterologous expression system for eukaryotic integral membrane proteins.[21] Cells were loaded with Fura-2, a calcium-sensitive ratiometric fluorescent dye. Ligand binding and activation causes ion channels like TRPA1 to open, allowing calcium to flow into cells and bind Fura-2, resulting in an absorption shift.[22]

After measuring background fluorescence levels, addition of 60 μM XPRO or propofol at 60 s elicited a rise in $[Ca^{2+}]_i$ in TRPA1-expressing cells, resulting in an increase in the 340/380 ratio of the Fura-2 dye (Fig. 1A). Expression of TRPA1 in all samples was confirmed by activation at 180 s with 1 mM AITC, an electrophilic TRPA1 agonist.[23,24] Interestingly, *o*-XPRO-Click was a weaker TRPA1 agonist than propofol (Fig. 1B). We hypothesize that the potency difference was due to replacement of a key structural feature of propofol, one of the 2-isopropyl group, with a smaller and non-branched alkyne. Consistent with this hypothesis, *p*-XPRO-Click, which preserves both 2-isopropyl substituents, was at least as efficient as propofol (Fig. 1C).

To rule out nonspecific calcium influx due to increased hydrophobicity of XPRO relative to propofol, we also tested activation of TRPV1, a related channel found on TRPA1-expressing sensory nerves and crucial for pain sensation.[25] As expected,[26] neither propofol nor XPRO activated TRPV1 while capsaicin, which serves as a positive control for functional TRPV1 expression, was a potent TRPV1 agonist (Fig. 1D). In addition, neither *o*-XPRO-Click nor *p*-XPRO-Click elicited calcium influx in TRPV1-expressing cells (Fig. 1E and 1F). Taken together, calcium-influx assays demonstrate that XPRO, *o*-XPRO-Click, and *p*-XPRO-Click share similar TRPA1 potency with propofol.

In summary, the structure-function studies highlight promising photoactivatable propofol analogs to move forward in crosslinking experiments. We envision our analogs and synthesis schemes will benefit future photolabeling strategies in several ways. For example, XPRO and *p*-XPRO-Click retain both isopropyl arms, unlike most previously published photoactivatable propofol analogs except *p*-4-AziC5-Pro,[13] and both of our new analogs have a shorter linker to the diazirine than *p*-4-AziC5-Pro and a simpler synthetic route. Of note, given their structural similarities, we anticipate that XPRO and *p*-XPRO-Click will activate the $GABA_A$ receptor both *in vitro* and *in vivo* similarly to *p*-4-AziC5-Pro, which was tested in zebrafish tadpoles,[13] although pharmacokinetic assessments should be performed before using these new compounds *in vivo*. Furthermore, our bifunctional probes, *o*-XPRO-Click and *p*-XPRO-Click, will facilitate profiling of the propofol proteome under cellular conditions, for example enabling tagging to a fluorophore to determine the localization of propofol-interacting proteins. The synthetic strategies described here are also applicable to alkyl phenols like propofol, such as carvacrol and thymol, and molecules that contain an analogous linker in the *para* position, such as zingerone, all of which include TRPA1 modulation among their biological activities.[27] Thus, the combination of the diazirine and alkyne functionality enables not only detection and enrichment of photolabeled biological targets but also interrogation of the propofol-interacting proteome and generation of novel ion channel modulators through click chemistry reactions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127927.

References

1 Jalota L, Kalira V, George E, et al. Prevention of pain on injection of propofol: systematic review and meta-analysis. *BMJ*. 2011;342(mar15 1). https://doi.org/10.1136/bmj.d1110. d1110.

2 Picard P, Tramer MR. Prevention of pain on injection with propofol: a quantitative systematic review. *Anesth Analg*. 2000;90(4):963–969. https://doi.org/10.1097/00000539-200004000-00035.

3 Jurd R, Arras M, Lambert S, et al. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J*. 2003;17(2):250–252. https://doi.org/10.1096/fj.02-0611fje.

4 Matta JA, Cornett PM, Miyares RL, Abe K, Sahibzada N, Ahern GP. General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. *Proc Natl Acad Sci U S A*. 2008;105(25):8784–8789. https://doi.org/10.1073/pnas.0711038105.

5 Woll KA, Skinner KA, Gianti E, et al. Sites contributing to TRPA1 activation by the anesthetic propofol identified by photoaffinity labeling. *Biophys J*. 2017;113(10):2168–2172. https://doi.org/10.1016/j.bpj.2017.08.040.

6 Woll KA, Murlidaran S, Pinch BJ, et al. A novel bifunctional alkylphenol anesthetic allows characterization of gamma-aminobutyric acid, type A (GABAA), receptor subunit binding selectivity in synaptosomes. *J Biol Chem*. 2016;291(39):20473–20486. https://doi.org/10.1074/jbc.M116.736975.

7 Yip GMS, Chen Z-W, Edge CJ, et al. A propofol binding site on mammalian GABAA receptors identified by photolabeling. *Nat Chem Biol*. 2013;9(11):715–720. https://doi.org/10.1038/nchembio.1340.

8 Ton HT, Phan TX, Abramyan AM, Shi L, Ahern GP. Identification of a putative binding site critical for general anesthetic activation of TRPA1. *Proc Natl Acad Sci U S A*. 2017;114(14):3762–3767. https://doi.org/10.1073/pnas.1618144114.

9 Ton HT, Phan TX, Ahern GP. Inhibition of ligand-gated TRPA1 by general anesthetics. *Mol Pharmacol*. 2020;98(3):185–191. https://doi.org/10.1124/mol.119.118851.

10 Hill JR, Robertson AAB. Fishing for drug targets: a focus on diazirine photoaffinity probe synthesis. *J Med Chem*. 2018;61(16):6945–6963. https://doi.org/10.1021/acs.jmedchem.7b01561.

11 Prevet H, Collins I. Labelled chemical probes for demonstrating direct target engagement in living systems. *Future Med Chem*. 2019;11(10):1195–1224. https://doi.org/10.4155/fmc-2018-0370.

12 Hall MA, Xi J, Lor C, et al. m-Azipropofol (AziPm) a photoactive analogue of the intravenous general anesthetic propofol. *J Med Chem*. 2010;53(15):5667–5675. https://doi.org/10.1021/jm1004072.

13 Stewart DS, Savechenkov PY, Dostalova Z, et al. p-(4-Azipentyl)propofol: a potent photoreactive general anesthetic derivative of propofol. *J Med Chem*. 2011;54(23):8124–8135. https://doi.org/10.1021/jm200943f.

14 Dubinsky L, Krom BP, Meijler MM. Diazirine based photoaffinity labeling. *Bioorg Med Chem*. 2012;20(2):554–570. https://doi.org/10.1016/j.bmc.2011.06.066.

15 Li Z, Hao P, Li L, et al. Design and synthesis of minimalist terminal alkyne-containing diazirine photo-crosslinkers and their incorporation into kinase inhibitors for cell- and tissue-based proteome profiling. *Angew Chem Int Ed Engl*. 2013;52(33):8551–8556. https://doi.org/10.1002/anie.201300683.

16 Bunce RA, Reeves HD. Amberlyst-15 catalyzed addition of phenols to α, β-unsaturated ketones. *Synth Commun*. 1989;19(5-6):1109–1117.

17 Church RFR, Kende AS, Weiss MJ, Diazirines I. Some observations on the scope of the ammonia-hydroxylamine-o-sulfonic acid diaziridine synthesis. The preparation of certain steroid diaziridines and diazirines. *J Am Chem Soc*. 1965;87(12):2665–2671. https://doi.org/10.1021/ja01090a025.

18 Church RFR, Diazirines II WMJ. Synthesis and properties of small functionalized diazirine molecules. Observations on the reaction of a diaziridine with the iodine-iodide ion system. *J Organic Chem*. 1970;35(8):2465–2471. https://doi.org/10.1021/jo00833a001.

19 Nishibayashi Y, Wakiji I, Ishii Y, Uemura S, Hidai M. Ruthenium-catalyzed propargylic alkylation of propargylic alcohols with ketones: straightforward synthesis of gamma-keto acetylenes. *J Am Chem Soc*. 2001;123(14):3393–3394. https://doi.org/10.1021/ja015670z.

20 Sivakumar K, Xie F, Cash BM, Long S, Barnhill HN, Wang Q. A fluorogenic 1,3-dipolar cycloaddition reaction of 3-azidocoumarins and acetylenes. *Org Lett*. 2004;6(24):4603–4606. https://doi.org/10.1021/ol047955x10.1021/ol047955x.s001.

21 He Y, Wang K, Yan N. The recombinant expression systems for structure determination of eukaryotic membrane proteins. *Protein Cell*. 2014;5(9):658–672. https://doi.org/10.1007/s13238-014-0086-4.

22 Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J Biol Chem*. 1985;260(6):3440–3450.

23 Hinman A, Chuang HH, Bautista DM, Julius D. TRP channel activation by reversible covalent modification. *Proc Natl Acad Sci U S A*. 2006;103(51):19564–19568. https://doi.org/10.1073/pnas.0609598103.

24 Macpherson LJ, Dubin AE, Evans MJ, et al. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature*. 2007;445(7127):541–545. https://doi.org/10.1038/nature05544.

25 Fernandes ES, Fernandes MA, Keeble JE. The functions of TRPA1 and TRPV1: moving away from sensory nerves. *Br J Pharmacol*. 2012;166(2):510–521. https://doi.org/10.1111/j.1476-5381.2012.01851.x.

26 Nishimoto R, Kashio M, Tominaga M. Propofol-induced pain sensation involves multiple mechanisms in sensory neurons. *Pflugers Arch*. 2015;467(9):2011–2020. https://doi.org/10.1007/s00424-014-1620-1.

27 Lee SP, Buber MT, Yang Q, et al. Thymol and related alkyl phenols activate the hTRPA1 channel. Br J Pharmacol. 2008;153(8): 1739–49. 10.1038/bjp.2008.85.