CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

## Accepted Article

**Title:** A (+)-larixol congener with high affinity and subtype selectivity towards TRPC6

**Authors:** Stephanie Häfner, Finn Burg, Martina Kannler, Nicole Urban, Peter Mayer, Alexander Dietrich, Dirk Trauner, Johannes Broichhagen, and Michael Schaefer



**To be cited as:** *ChemMedChem* 10.1002/cmdc.201800021

**Link to VoR:** http://dx.doi.org/10.1002/cmdc.201800021

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

# A (+)-larixol congener with high affinity and subtype selectivity towards TRPC6

Stephanie Häfner[a], Finn Burg[b], Martina Kannler[c], Nicole Urban[a], Peter Mayer[b], Alexander Dietrich[c], Dirk Trauner[b],[d] *, Johannes Broichhagen[b],[e] * and Michael Schaefer[a] *

[a] Stephanie Häfner (0000-0003-0501-2193), Nicole Urban, and Prof. Dr. Michael Schaefer (0000-0003-3662-4387)
Rudolf-Boehm-Institute for Pharmacology and Toxicology, Leipzig University
Härtelstr. 16-18, 04107 Leipzig, Germany.
E-mail: michael.schaefer@medizin.uni-leipzig.de

[b] Finn Burg (0000-0002-8038-5638), Peter Mayer (0000-0002-1847-8032), Prof. Dr. Dirk Trauner (0000-0002-6782-6056), Dr. Johannes Broichhagen (0000-0003-3084-6595),
Department of Chemistry, Ludwig-Maximilians-University Munich and Center for Integrated Protein Science Munich
Butenandtstr. 5–13, 81377 Munich, Germany.

[c] Martina Kannler (0000-0003-2152-3858), Prof. Dr. Alexander Dietrich (0000-0002-1168-8707)
Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University Munich
Nußbaumstr. 26, 80336 Munich, Germany.

[d] Prof. Dr. Dirk Trauner (0000-0002-6782-6056)
Present address: Department of Chemistry, New York University
Silver Center for Arts and Science, 100 Washington Square East, New York, NY 10003, USA.
E-mail: dirktrauner@nyu.edu

[e] Dr. Johannes Broichhagen (0000-0003-3084-6595)
Present address: Department of Chemical Biology, Max Planck Institute for Medical Research
Jahnstr. 29, 69120 Heidelberg, Germany.
E-mail: johannes.broichhagen@mpimf-heidelberg.mpg.de

Supporting information for this article is given via a link at the end of the document.

**Abstract:** Natural products have many health benefits and their application can improve the quality of life. Recently, the diterpene (+)-larixol and its acetylated congeners demonstrated a selective inhibition of the second messenger-gated cation channel transient receptor potential canonical 6 (TRPC6) over its close isoforms TRPC3 and TRPC7. Building on this knowledge, we expand these findings by chemical diversification of (+)-larixol mostly at position *C6*. Implementing High-Throughput $Ca^{2+}$ FLIPR screening assays and electrophysiological patch-clamp recordings, we showcase larixyl *N*-methylcarbamate, dubbed **SH045**, as a compound with nanomolar affinity and a 13-fold subtype selectivity over TRPC3 in stably expressing HEK293 cells. Expanding on this finding, TRPC6 inhibition was also achieved in rat pulmonary smooth muscle cells. Furthermore, treatment of isolated perfused lung preparations with **SH045** led to a reduction in lung ischaemia reperfusion oedema (LIRE), a life-threatening condition associated with TRPC6 that may occur after organ transplantation. Taken together, and given the cheap, straightforward and scalable preparation of **SH045**, we report a TRPC6 blocker that holds promise for translational treatment of LIRE.

## Introduction

The superfamily of transient receptor potential (TRP) channels consists of more than 28 members, grouped into seven subfamilies. The canonical transient receptor potential receptor 6 (TRPC6) is an isoform of seven encoded TRPC channels (that were the first TRP channels to be described; TRPC2 being a pseudogene in humans) forming a closely related subgroup together with TRPC3 and TRPC7 (Figure 1a). TRPC6 is a non-selective ion channel that, upon activation, mediates $Ca^{2+}$ entry and membrane depolarization (Figure 1b). Activated by diacylglycerol (DAG), a second messenger produced downstream of the GPCR signaling cascade by depletion of $PIP_2$[1] or by receptor mediated PLCγ-signaling[2], TRPC6 contributes to a variety of physiological and pathophysiological responses. As such, high expression levels of TRPC6 are observed in the lung, especially in vascular endothelial and smooth muscle cells, where it is associated with hypoxic vasoconstriction, idiopathic hypertension, lung ischaemia reperfusion oedema (LIRE)[3,4] and idiopathic pulmonary arterial hypertension (IPAH).[5] In addition, TRPC6 has also emerged as a potential target for the treatment of focal segmental glomerulosclerosis that seems to be a phenotype of both gain- and loss-of-function mutations in the TRPC6 gene.[6,7] Our latest reports were based on the natural product (+)-larixol, a labdane terpene occurring in the resin of the European larch, which showed that channel inhibition can be achieved by functionalizing the *C6* alcohol to an acetyl or a carbamate moiety.[8,9] Spurred by recent computational approaches alongside with Biology-Oriented Synthesis (BIOS)[10], which have ignited a renaissance in the use of natural products in drug discovery and chemical biology, and in particular for TRP-channel treatment[11,12], we intended to further explore the larixol scaffold. Our aim was to utilize the vast chemical space in natural products to obtain a channel blocker with unprecedented affinity and selectivity (Figure 1c).

(+)–Larixol (**1**) can be isolated from the oleoresin of *Larix decidua* as a crystalline solid between 10–12 wt% together with its congener *epi*-manool (**2**) that lacks the hydroxyl group at position *C6* (Figure 2; carbon numbering is depicted in centered structure).[13] While **1** is a subtype-prevalent blocker of TRPC6 over TRPC3 and TRPC7, *epi*-manool shows lower affinity and no selectivity, accounting for the importance of the *C6* alcohol function. The bioactivity of this alcohol can be further enhanced by converting it to the corresponding acetate **3** and carbamate **4**.[8,9] In accordance with previous studies, we aimed to expand the knowledge about the structure-activity relationship of **1** by i) further diversification of the alcohol at *C6*, ii) formation of a third ring structure and oxidation chemistry and iii) chemical engineering the side chain at *C9*. Detailed synthetic protocols and full characterization are outlined in the SI, and final structures are shown in Figure 2 for clarity.

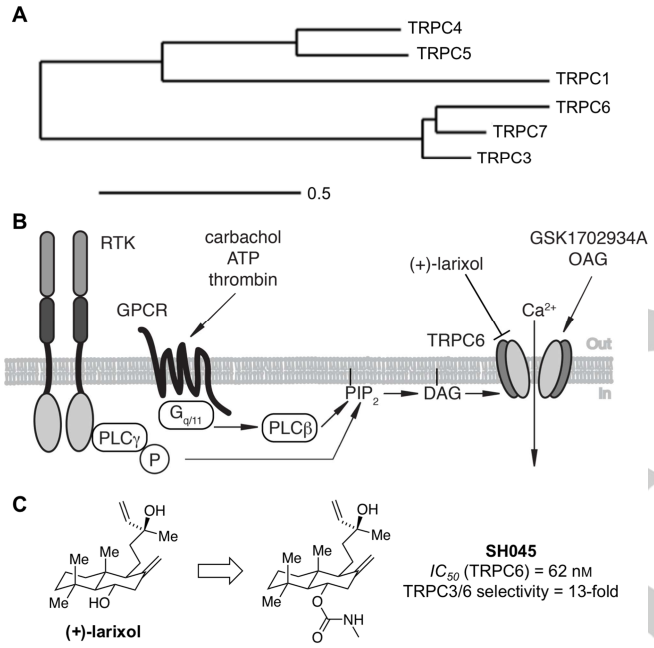



**Figure 1.** Larixol and TRPC6. a) Phylogenetic analysis of the human TRPC family shows close relation between TRPC3, 6 and 7. b) Schematic representation of TRPC6 activation by DAG and inhibition by (+)-larixol. c) (+)-Larixol derivatization for optimized blocking and selectivity towards TRPC6.

## Results and Discussion

### Design of a labdane compound library

At first, we wondered how important the atomic arrangement of the side chain at *C9* is, which bears a tertiary allylic alcohol linked by two methylene spacers. We therefore set out to bishydroxylate and glycol cleave the corresponding trisalcohol to obtain methyl ketone **5**[14], which could either be reduced to secondary alcohol **6** or treated with MeMgBr reagent to have access to dimethyl tertiary alcohol **7** (Scheme 1a and Figure 2 for final compounds overview). The new stereocenter created in **6** leads to a d.r. = 5.4/4.6, as acquired by $^1$H NMR (see SI).

In line with the modification solely at the *C9* chain, we were further interested to oxidize the larixol scaffold and lock the side chain by formation of a third ring structure. Oxidation was performed on the *exo*-methylene group at *C8* by ozonolysis starting from methyl ketone **5** to obtain bisketone **8**. Instead, by monooxidation of **1** with *m*CPBA, we were able to convert the *exo*-methylene group to its corresponding epoxide[14] that proved to be unstable and was subsequently reacted with LAH to give *C8* alcohol **9** (Scheme 1b). Treatment of **5** with $Tf_2O$ initiated a Prins reaction, resulting in ring formation between *C13* and *C17* with a double bond found between *C8* and *C9* to give **10**. Its congener **11**, in which the double bond is between *C8* and *C14* and the stereochemistry of the tertiary alcohol differs, can be obtained by Grubbs' metathesis of **1**, *i.e.* the fusion of the allylic and the exomethylene groups. Interestingly, ozonolysis of **1** provided spiroacetal **12**[15] but with unknown outcome of the stereochemistry at *C14*, which we solve herein by X-ray crystallography to be (*R*)-configured. Last but not least, the *C6* alcohol was oxidized to ketone **13** (Scheme 1c), which could be isomerized from its β,γ-unsaturated system to Michael system-containing compound **14**.[14]

Starting from ketone **13**, DIBAL reduction gave access to *epi*-larixol **15** with the *C6* alcohol inverted, shown by X-ray crystallography of glycol-cleaved compound **15b** (see SI). Activation of the alcohol with CDI gave larixyl imidazolo formate **16** (Scheme 1d) that could be isolated and further diversified to a series of compounds, such as hydrazide **17**, methyl carbonate **18**, methyl carbamate **19** (termed **SH045**), *n*-butyl carbamate **20** and cyclohexyl carbamate **21**. A completely different functional group was installed using $CS_2$ and MeI on **1** to yield xanthate **22** (Scheme 1e), which was unstable within days at –20 °C. Finally, carbamate **4** was further derivatized on the tertiary alcohol residing at *C13*, to get the mixed alcohol-modified structures **23** (biscarbamate) and **24** (carbamate and acetate) (Scheme 1f).

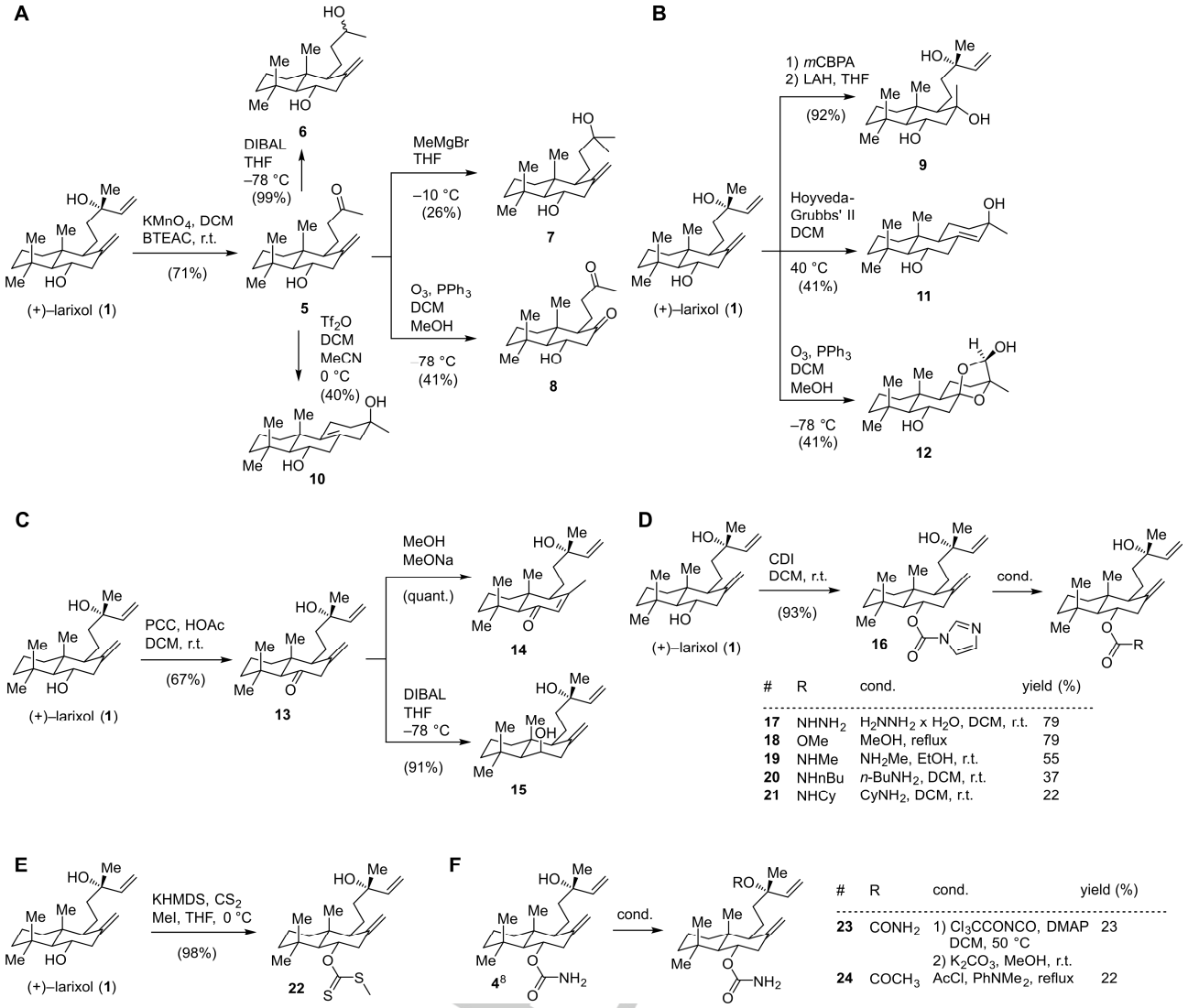
### SAR for the inhibition of TRPC3, 6 and 7

After having generated a small labdane-based library, blocking characteristics of all compounds were assessed by high throughput fluorescence imaging plate reader (FLIPR) assay. To determine subtype selectivity, the compounds were tested on HEK293 cells stably overexpressing hTRPC3, hTRPC6 or hTRPC7 (abbreviated as $HEK_{hTRPC3}$, $HEK_{hTRPC6}$, $HEK_{hTRPC7}$, respectively). Serial dilutions were applied to fluo-4/AM-loaded suspensions of $HEK_{hTRPC6\text{-}YFP}$, $HEK_{hTRPC3\text{-}YFP}$ and $HEK_{hTRPC7\text{-}YFP}$ cells that were subsequently stimulated with 50 µM 1-oleyl-2-acetyl-*sn*-glycerol (OAG) to induce $Ca^{2+}$ entry through TRPC channels for the evaluation of channel inhibition (representative traces and concentration-response curves of **SH045** are depicted in Figure 3a-c and 3d-e, respectively). A characteristic of TRPC6 is its lower basal activity compared to TRPC3 and TRPC7,[16] and treatment of $HEK_{hTRPC3}$ and $HEK_{hTRPC7}$ with inhibitors results in a diminution of basal $Ca^{2+}$ influx (of higher significance for TRPC7). With this in mind and in order to obtain comparable $IC_{50}$ values, the ordinate of the calculated concentration-response plots represents $\Delta F/F_{inh}$, where $\Delta F$ is calculated as follows

$$\Delta F = F_{stim} - F_{inh} \qquad \text{(Eq. 1)}$$

with $F_{stim}$ = fluorescence intensity after agonist stimulation and $F_{inh}$ = fluorescence intensity after addition of inhibitor.

For all compounds tested, the calculated logP values range from 4 to 7 and for some derivatives pluronic F127 was added to increase solubility, which had a positive effect on bioactivity (see SI section 1.2). Results are referred to $IC_{50}$ values in the text and




**Scheme 1.** Synthetic route for (+)-larixol congeners. a) Modification of the *C9* side chain. b) Modification of the exomethylene group. c) Oxidation and inversion of *C6* alcohol. d) Modification of the *C6* alcohol *via* imidazoyl formate **16**. e) Synthesis of xanthate **22**. f) Derivatives at *C13* alcohol with *C6* carbamate.

represented as p$IC_{50}$ values in Figure 4 for clarity (dose-response curves for all compounds can be found in the SI).

In previous studies, we reported that potent and subtype-selective TRPC6 inhibitors could be generated by acetylation and carbamylation of (+)-larixol,[8,9] suggesting the high importance of *C6* substituents. Nonetheless, a more extensive study of the effects of compound derivatization, *e.g.* the influence of an altered *C9* sidechain on TRPC6 inhibition and selectivity, was still of interest. Hence, we first tested *C9* side chain modified compounds, such as ketone **5**, secondary alcohol **6** and tertiary alcohol **7**. These derivatives still acted as TRPC6 inhibitors, but showed significantly higher $IC_{50}$ values (22, 13 and 7 μM for **5**, **6** and **7**, respectively) than **1** (see SI, Figure 4). The tertiary hydroxyl group in conjunction with the exact stereochemical arrangement of the methyl and vinyl substituents at *C13* seems to be a necessary moiety for selective channel binding. Given these results, we did not aim for higher diastereometric excess of **6**, for instance by CBS reduction. It should be noted that oxidation of the exocyclic double bond of **5** to yield biscarbonyl **8** leads to complete loss of its bioactivity (see SI), comparable with **9**, where the exocyclic double bond is oxidized. In a similar manner, strongly decreased activity can be observed when a third ring system is installed by joining the two exogenous double bonds via Prins reaction or Grubbs' metathesis (**10** and **11**, respectively), which also applies for spiroacetal **12** ($IC_{50}$ = 26, >50 and >50 μM for TRPC6, respectively). This is supported by previous studies, where larch turpentine components with a three-ring system, such as abietic acid or pimaric acid, displayed $IC_{50}$ values higher than larixol.[8] When testing **13** and **14**, both bearing a $sp^2$-hybridized carbonyl at position *C6*, the spatial position and the different hydrogen donor-acceptor capabilities of the oxygen atom seems to play a minor role. This observation becomes more evident by comparing the potency and the selectivity profile of alcohol inverted *epi*-larixol **15** to its diastereomer **1**. Despite this intervention in the molecular scaffold, compounds **13**, **14** and **15** still display lower $IC_{50}$ values than unmodified **1** (0.49, 0.98 and 0.89 μM on TRPC6, respectively).

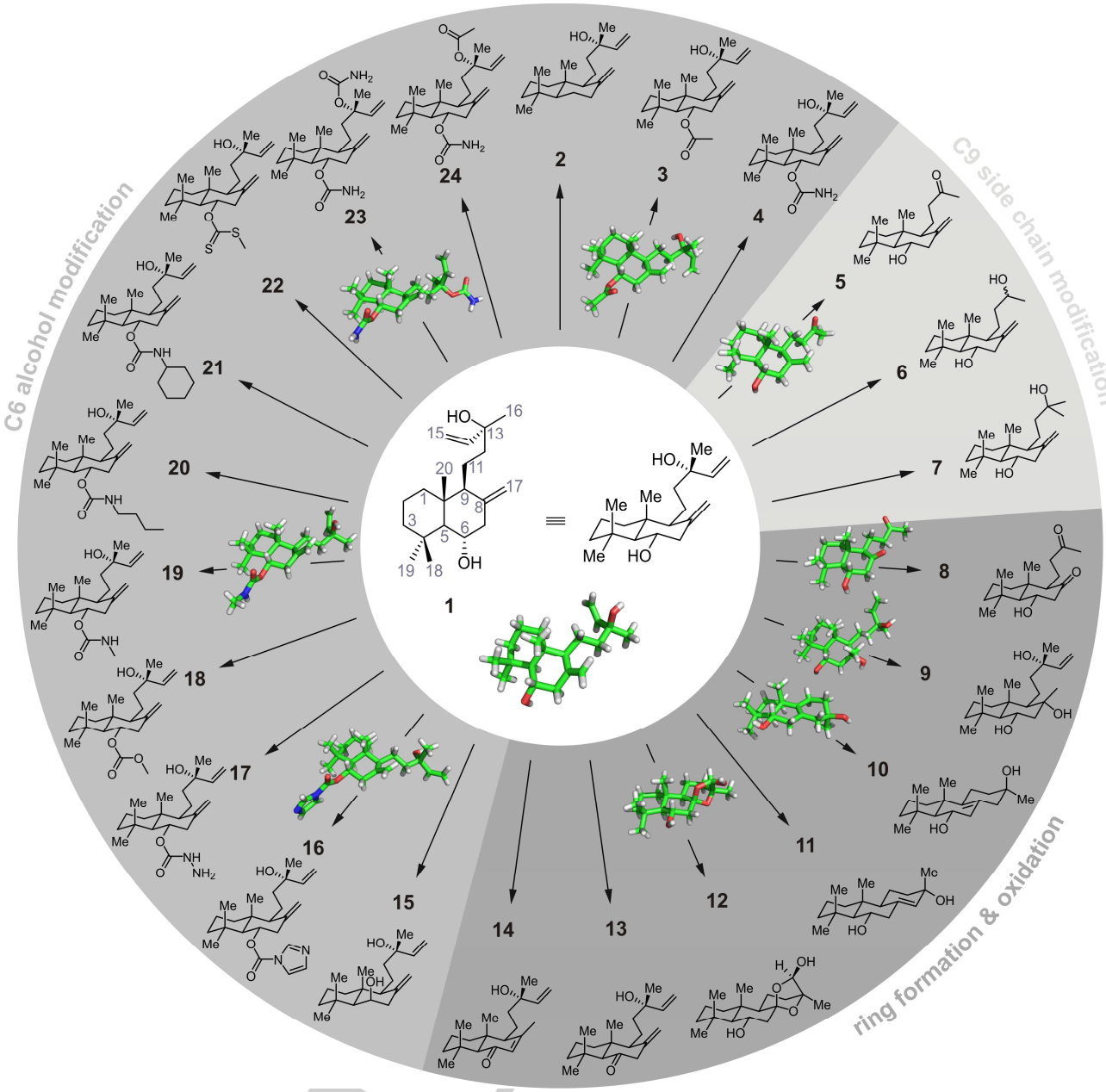



**Figure 2.** Overview of compounds derived from (+)-larixol (**1**). Carbon numbering is given in the centred structure. Obtained compounds were tested towards TRPC6 inhibition, and X-ray structures have been solved for compounds **1**, **3**, **5**, **8**, **9**, **10**, **12**, **16**, **19** (**SH045**) and **23**.

Further modifications of the *C6* alcohol were achieved using the imidazole-activated formate precursor **16,** which allowed the easy generation of the isosters **17**, **18** and **SH045**. While **16**, **17** and **18** displayed $IC_{50}$ values of 0.44, 0.45, and 0.17 µM, respectively, the introduction of a *N*-methylated carbamate function in compound **SH045** yielded a 15-fold improvement in potency over larixol (**1**) and a 3-fold improvement over the previously described larixyl carbamate **4**[9] with an $IC_{50}$ = 63 nM. Furthermore, FLIPR measurements on TRPC3 and TRPC7 provided a promising selectivity data for **SH045** ($IC_{50}$ = 0.84/0.22 µM, Figure 3d, e; for TRPC3 $p<0.05$, Figure 4) and therefore outperformed every known congener regarding affinity and selectivity to date. On the other hand, introducing sterically demanding or rigid elements at position *C6* reduced bioactivity in all cases (Figure 4). This confirms the hypothesis that sterically undemanding substituents at this site are crucial for binding and blocking and hints towards the existence of a narrow binding pocket that is suited best for the electronic environment of a carbamate. The observation that an unbranched alkyl chain substituent at this position (*n*-butyl, as in **20**) was better tolerated than a branched moiety (cyclohexyl as in **21**), which is shown by the $IC_{50}$ values of 0.55 and 1.54 µM on TRPC6, respectively, clearly supports this hypothesis. Interestingly, with an $IC_{50}$ = 13.6 µM, xanthate **22**, which is isolobal to **SH045,** did not block $Ca^{2+}$ influx as potently. This shows that small alterations and fine tuning of the electronic properties leads to pronounced effects

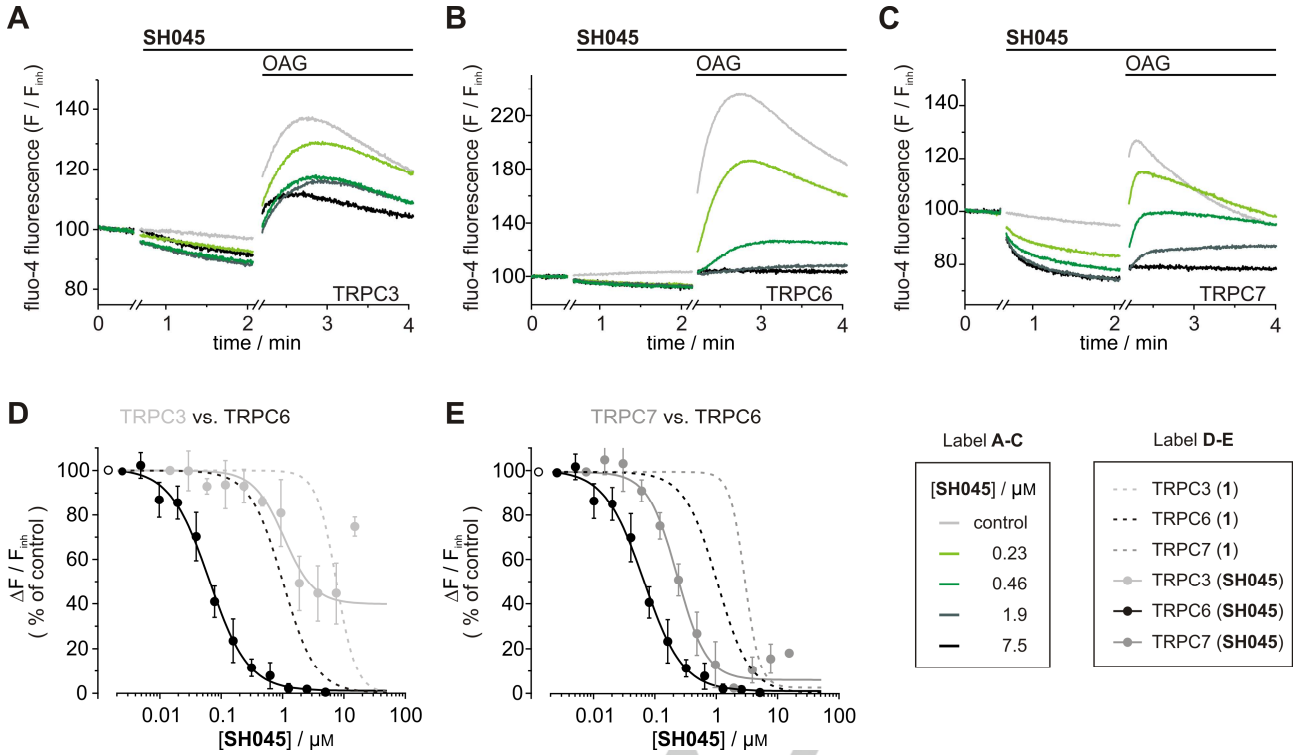



**Figure 3.** Compound testing by Fluorescent Imaging Plate Reader (FLIPR). a-c) Representative FLIPR $Ca^{2+}$ traces of OAG-stimulated TRPC currents blocked by various concentrations of **SH045**. d-e) Corresponding concentration-response curves of **SH045** were obtained from several experiments (n = 3-7) performed as in (a-c).

on bioactivity. Previous studies revealed that a bisacetate is less bioactive than the monoacetylated larixol at *C6*,[9] but the higher subtype selectivity towards TRPC3 seemed to indicate the benefit of modifying the secondary alcohol. While biscarbamylation of larixol led to the barely active compound **23** ($IC_{50}$ = 20 µM), the mixed carbamate/acetate **24** is highly potent ($IC_{50}$ = 0.25 µM) and shows higher subtype selectivity over TRPC7 than carbamate **4** (11-fold vs. 4-fold, Figure 4), albeit with lower potency than **SH045**. In addition, **SH045** was also preliminary screened towards TRPC4 and TRPC5 among other TRP isoforms from the ankyrin, vanilloid and the melastatin family. Compared to TRPC6, **SH045** showed high selectivity over TRPA1 (630-fold), TRPV1 (400-fold), TRPM2 (1400-fold) and did not have any notable effects on the other tested channels including TRPC4, 5 and TRPV2, TRPV3, TRPM3, TRPM8 (Figure S2). The high bioactivity of **SH045** could also be verified by stimulation of $HEK_{hTRPC3}$, $HEK_{hTRPC6}$ and $HEK_{hTRPC7}$ with a redundant mix of GPCR agonists (1 mM carbachol, 300 µM adenosine-5'-triphosphate and 0.5 U/µL thrombin). This mixture leads to the formation of DAGs via $G_{q/11}$, which represents the endogenous way of TRPC3/6/7 activation (Figure 1b). Interestingly, **SH045** is an even more potent blocker when TRPC6 is engaged via GPCR signalling ($IC_{50}$ = 8.9 nM, $p<0.05$) or via TRPC6 agonist GSK1702934A[17] ($IC_{50}$ = 14.2 nM, non-significant). One could therefore speculate that **SH045** induced block is more potent when TRPC6 is activated from the cytosolic site. Beneficially, the inhibitory performance of **SH045** on TRPC3 and TRPC7 was not considerably altered by using other agonists than OAG (Figure S3, S4 and Table S2, S5). Having these results in mind, we tested the most potent compounds with a MTT assay in HEK293 cells for their influence on cell viability (Figure S5). After 24-hour incubation with 5 or 15 µM of the compounds, no significant reduction in metabolic activity was detected when compared to vehicle alone. Taken together, and given the high potency, high selectivity on direct and upstream activation of TRPC6 and absent toxicity of **SH045**, this structure emerged as the most promising candidate for further evaluation in more physiologically relevant systems.

**Patch-clamp recordings in TRPC-stable HEK293 cells**

In a next step, we aimed to characterize **SH045** more conclusively by electrophysiological whole-cell recordings to assess the respective $IC_{50}$ values on TRPC3, TRPC6 and TRPC7. Indeed, the high potency on TRPC6 could be confirmed by patch-clamp measurements, which includes all cations that are transmitted via the plasma membrane due to the unselective pore properties of TRPC3, 6 and 7. More precisely, **SH045** blocked the inward and outward currents through TRPC6 with $IC_{50}$ values of 5.8 nM and 5.2 nM respectively (Figure 5a). With $IC_{50}$ values of 22 nM (inward) and 18 nM (outward), we could determine a 3-fold selectivity over TRPC7 (Figure 5b), similar to observations from fluorometric $[Ca^{2+}]_i$ assays. The corresponding $IC_{50}$ values of TRPC3 currents were 440 nM (inward) and 634 nM (outward), and thereby ~2 orders of magnitude higher compared to TRPC6 (Figure 5c). Original traces from whole cell patch clamp recordings are shown in Figure S6.

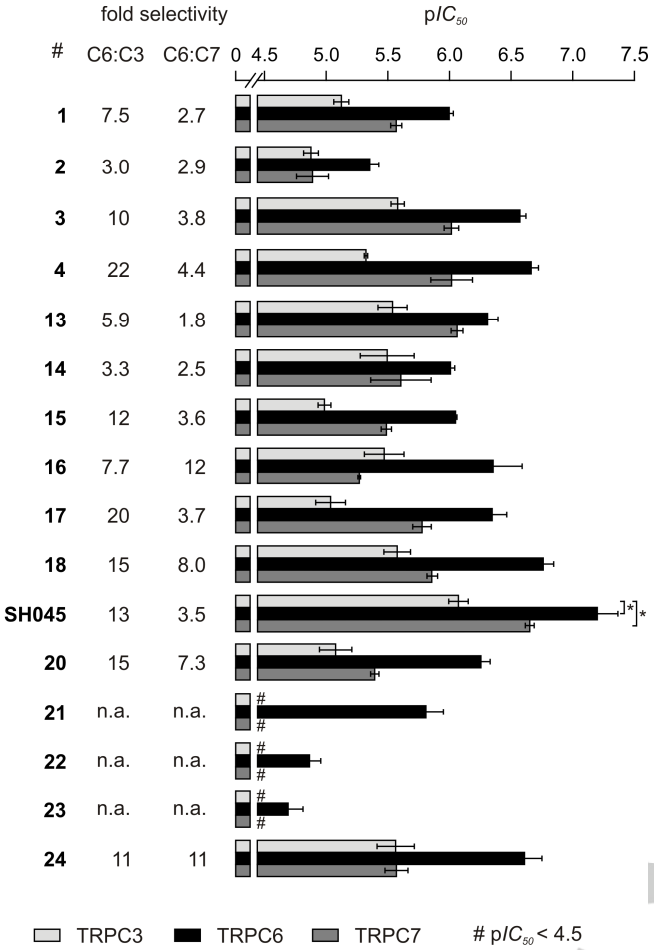



**Figure 4:** Selectivity profiling for larixol derived compounds. TRPC6:TRPC3 and TRPC6:TRPC7 fold-selectivity of all blockers including their p$IC_{50}$ (data represents means ± S.E.M.; for **SH045**: one-way ANOVA with Tukey's post hoc test, * p<0.05). Label legend is given in the Figure.

**SH045 in rPASMCs and explanted lungs**

In addition to the characterization of heterologously expressed TRPC channels, we intended to test the TRPC6 inhibitor **SH045** in its native, and therefore physiological environment. The expression of TRPC6 is well-described in pulmonary smooth muscle cells[18], thus we chose this system to validate the inhibition of OAG-mediated $Ca^{2+}$ influx by fura-2 $Ca^{2+}$ measurements. By testing five different concentrations of **SH045** we determined an $IC_{50}$ of 339 nM in rPASMCs, reflecting direct TRPC6 blocking independent of detected signals from PKC activation, which was ensured by preincubation with bisindolylmaleimide-1 (BIM) (Figure 5d, e).

TRPC6 also contributes to severe pathophysiological effects in the lung, such as oedema formation induced by excessive $Ca^{2+}$ influx in endothelial cells and subsequent disruption of the endothelial barrier.[19] This increase in endothelial permeability induces the formation of lung ischaemia-reperfusion oedema (LIRE), a primary graft failure, which causes alveolar damage and hypoxemia in patients after lung transplantations. Weissmann et al. reported a reduction of oedema formation in TRPC6$^{-/-}$ mice after ischaemic conditions compared to wildtype controls.[4] Hence, we tested if **SH045** is able to reduce oedema formation, which is correlated to increased lung weight in the isolated perfused lung. Accordingly, oedema were provoked by an interruption of lung perfusion, inducing ischaemia in explanted perfused mouse lungs for 90 minutes, before perfusion was re-initiated. Five µM of **SH045** were applied for 15 minutes before stopping the perfusion and led to significantly reduced oedema formation 60 and 90 minutes after reperfusion compared to ischaemic lungs perfused and reperfused with buffer (Figure 5f).

## Conclusions

In the present study, we showcase the synthesis and structure-activity relationship of several derivatives of the diterpene (+)-larixol (**1**). The newly generated analogues are divided into three categories: (i) alteration of the functionality at the *C9* side chain, (ii) third ring formation and (iii) functionalization of the hydroxyl group at *C6* with the introduction of different side chains. Analogues generated by modification according to types i) and ii) showed a diminished bioactivity in terms of TRPC6 inhibition. However, the derivatization of **1** according to iii) led to potent TRPC6 inhibitors if the modification was at the *C6* position. This validates the importance of this moiety for selectivity against TRPC6's closest relatives, TRPC3 and TRPC7, among more distant relatives, such as TRPC4 and TRPC5 and others from the ankyrin, vanilloid and melastatin family. Further testing towards other transmembrane proteins, for instance GPCRs and receptor-linked enzymes, can be considered for follow-up studies. The introduction of small modifications could improve the bioactivity and finally led to the highly potent and selective TRPC6 inhibitor **SH045**, assessed both in $Ca^{2+}$ FLIPR and patch-clamp recordings. We note that in the concentration-response curves generated by FLIPR-measurements steeper Hill slopes were observed for some compounds (*cf.* larixol, Figure 3e), especially those that display high logP and high $IC_{50}$ values, which may point to colloidal aggregation. Nevertheless, **SH045** performs over a broad dynamic range for all tested TRPC isoforms and we therefore conclude that it does not aggregate and is a suitable candidate for bioavailability. Although the *N*-methyl carbamate function is just a small alteration in the molecular framework, it led to a significant improvement in potency compared to the non-methylated carbamate. Interestingly, none of the compounds exhibited higher affinity for TRPC3/7 over TRPC6 suggesting that the TRPC binding site is conserved. Although our library covers <30 compounds, we derive a structure-activity relationship from our results. It can be clearly stated that alterations anywhere but on the *C6* alcohol lead to decreased blocking capacity, thereby suggesting that the *C9* chain is well-fitted onto the protein surface. By comparing the *C6* derivatives, we observe that a carbamate leads to the best results. This might be due to the installed nitrogen being a hydrogen donor, which is absent for an oxygen or sulfur atom. Furthermore, it is important for the *N*-linked alkyl chain to be small in size; even by expansion to a *n*-butyl group, a loss in affinity can be observed. This loss is even more pronounced when a cyclohexyl ring is installed. Given the fact that *C6* and *C9* is part of the same ring structure, we therefore propose that indeed this part is the main pharmacologic entity that is responsible for TRPC blocking.

After the description of several low potent TRPC6 channel blockers like SKF96365[20], 8009-5364[21] or norgestimate[22], more promising candidates for TRPC6 inhibition were discussed in recent years. In 2013, anilino-thiazole based small molecules were described to block $Ca^{2+}$ influx through TRPC3 and TRPC6 in the low nanomolar range, however, most of them were not

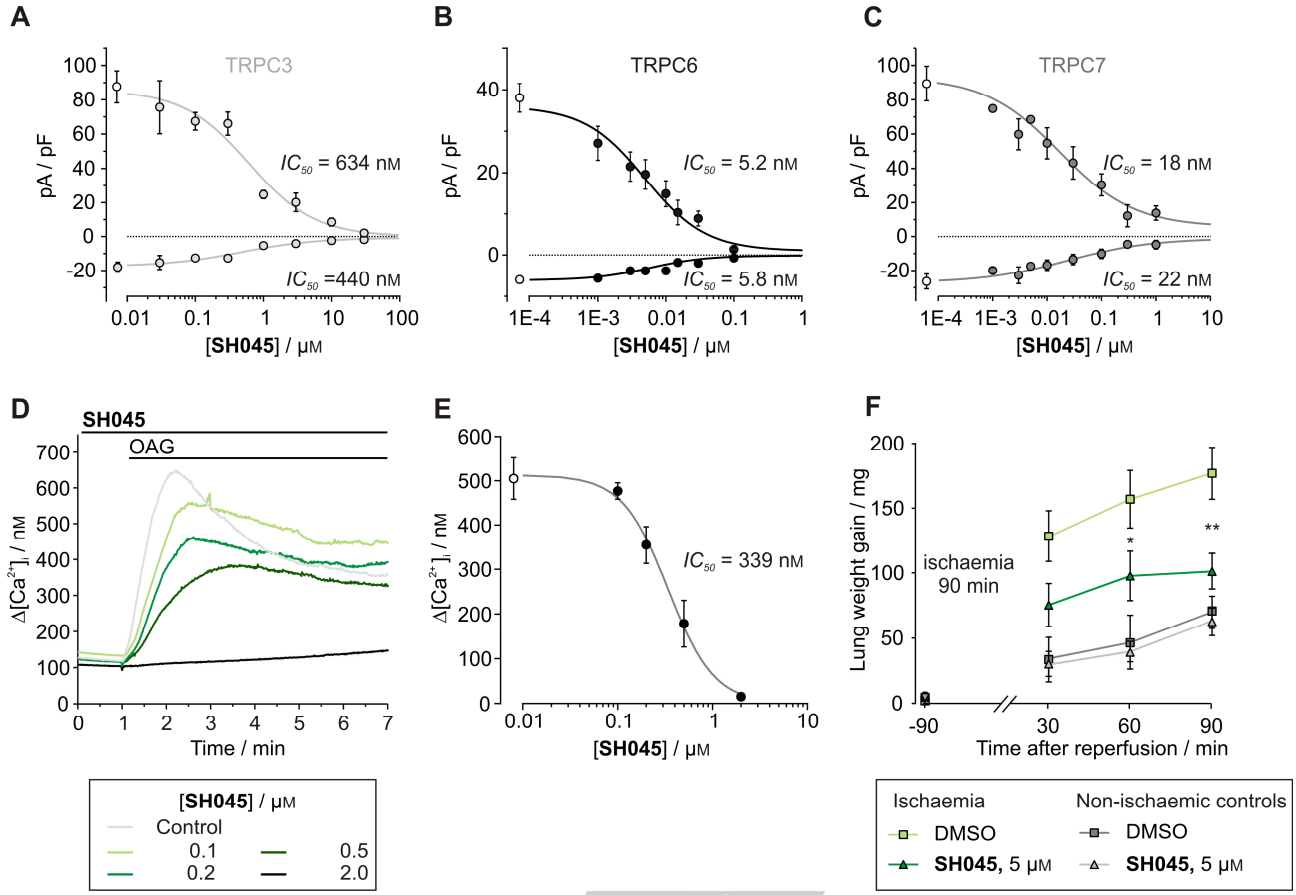



**Figure 5.** Compound **SH045** was evaluated *in cellulo* (TRPC stably-expressing HEK and rPASMC cells) and *ex vivo* (murine lungs). a-c) $IC_{50}$ determination of **SH045** in HEK293 cells stably expressing TRPC3, 6, or 7. Data represents means ± S.E.M. (n = 6-14). d) Concentration-response curves of **SH045** in rPASMCs. Data represents means ± S.E.M. (n = 6). e) Representative traces from fura-2 $[Ca^{2+}]_i$ measurements of **SH045** in rPASMC. f) LIRE in explanted mouse lungs could significantly be reduced with 5 µM of **SH045** (n = 6, two-way ANOVA).

suitable for *in vivo* exposure due to poor bioavailability.[23] Another compound, termed SAR7334, has become a benchmark blocker since its synthesis in 2015 as it displays an $IC_{50}$ value of 7.9 nM on TRPC6 with high selectivity over TRPC3/7 (36-/29-fold with $IC_{50}$ = 282/226 nM, respectively).[24] Therefore, our herein disseminated larixol congener **SH045** expands the repertoire of TRPC6 inhibitors with an $IC_{50}$ value of 5.8 nM that compares favorably to SAR7334. Beneficially, **SH045** can be generated in a semisynthetic two-step protocol from the abundant natural product (+)-larixol, whose isolation in bulk quantities is straightforward and inexpensive, and indeed **SH045** has been obtained in gram quantities. Finally, with its ability to reduce induced oedema formation in explanted murine lungs, pharmacological profiling will be performed to set the stage for studies *in vivo* in the near future.

## Experimental Section

The Supporting Information contains synthetic protocols including chemical and physical characterization of the described compounds and biological methods. All compounds were determined to be >95% pure by UHPLC (Shimadzu MS2020 connected to a Nexerra UHPLC system equipped with a Waters ACQUITY UPLC BEH C8 1.7 µm 2.1x50 mm column. Buffer A: 0.05% HCOOH in $dH_2O$ Buffer B: 0.05% HCOOH in MeCN. Analytical gradient was from 10% to 90% B within 5.0 min with 1.0 ml/min flow, absorbance was monitored at λ = 200 nm), unless stated otherwise in the synthetic procedure in the Supporting Information. CCDCs 1556986 (**1**), 1556990 (**3**), 1556984 (**5**), 1556987 (**8**), 1556991 (**9**), 1556982 (**10**), 1556985 (**12**), 1556988 (**15b**), 1556983 (**16**), 1572562 (**SH045**), 1556989 (**24**) contain the supplementary crystallographic data. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre under https://www.ccdc.cam.ac.uk/structures/.

## Acknowledgements

D.T. and J.B. thank the Munich Centre for Integrated Protein Science (CIPSM). This work was supported by the SFB TRR 152 (S.H., D.T., J.B. and M.S.). D.T. acknowledges support of the European Research Council for an Advanced Grant (268795). We thank Dominik Dosch for synthetic assistance and Narges Pourmandi for proofreading.

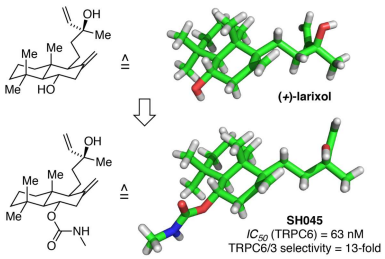
**Keywords:** larixol • diterpene • labdane • TRPC6 • LIRE

## References

[1] T. Hofmann, A. G. Obukhov, M. Schaefer, C. Harteneck, T. Gudermann, G. Schultz, *Nature* **1999**, *397*, 259–263.
[2] S. G. Rhee, *Ann. Rev. Biochem.* **2001**, *70*, 281–312.
[3] N. Weissmann et al., *Proc. Natl. Acad. Sci. U. S. A* **2006**, *103*, 19093–19098.

[4] N. Weissmann et al., *Nat. Commun.* **2012**, *3*, 649.
[5] Y. Yu, I. Fantozzi, C. V. Remillard, J. W. Landsberg, N. Kunichika, O. Platoshyn, D. D. Tigno, P. A. Thistlethwaite, L. J. Rubin, J. X.-J. Yuan, *Proc. Natl. Acad. Sci. U. S. A* **2004**, *101*, 13861–13866.
[6] M. P. Winn, *Science* **2005**, *308*, 1801–1804.
[7] M. Riehle et al., *J. Am. Soc. Nephrol.* **2016**, *27*, 2771–2783.
[8] N. Urban, L. Wang, S. Kwiek, J. Rademann, W. M. Kuebler, M. Schaefer, *Mol. Pharm.* **2016**, *89*, 197–213.
[9] N. Urban, S. Neuser, A. Hentschel, S. Köhling, J. Rademann, M. Schaefer, *Brit. J. Pharmacol.* **2017**, *174*, 4099–4122.
[10] S. Wetzel, R. S. Bon, K. Kumar, H. Waldmann, *Angew. Chem., Int. Ed. Engl.* **2011**, *50*, 10800–10826.
[11] I. Vetter, R. J. Lewis, *Adv. Exp. Med. Biol.* **2011**, *704*, 41–85.
[12] T. Rodrigues, F. Sieglitz, G. J. L. Bernardes, *Chem. Soc. Rev.* **2016**, *45*, 6130–6137.
[13] H. Wienhaus, W. Pilz, H. Seibt, H.-G. Dässler, *Chem. Ber.* **1960**, *93*, 2625–2630.
[14] M. G. Bolster, B. J. Jansen, A. de Groot, *Tetrahedron* **2001**, *57*, 5663–5679.
[15] P. F. Vlad, A. M. Kyl'chik, E. N. Schmidt, M. N. Koltsa, V. N. Odinokov, V. A. Pentegova, *Chem. Nat. Compd.* **1986**, *22*, 541–547.
[16] a) A. Dietrich, M. Mederos y Schnitzler, J. Emmel, H. Kalwa, T. Hofmann, T. Gudermann, *J. Biol. Chem.* **2003**, *278*, 47842–47852; b) T. Okada et al., *J. Biol. Chem.* **1999**, *274*, 27359–27370;
[17] X. Xu, I. Lozinskaya, M. Costell, Z. Lin, J. A. Ball, R. Bernard, D. J. Behm, J. P. Marino, C. G. Schnackenberg, *Biophys. J.* **2013**, *104*, 454a.
[18] A. Dietrich, V. Chubanov, H. Kalwa, B. R. Rost, T. Gudermann, *Pharmacol. Ther.* **2006**, *112*, 744–760.
[19] C. Tiruppathi, G. U. Ahmmed, S. M. Vogel, A. B. Malik, *Microcirculation* **2006**, *13*, 693–708.
[20] C. Harteneck, C. Klose, D. Krautwurst, *Adv. Exp. Med. Biol.* **2011**, *704*, 87–106.
[21] N. Urban, K. Hill, L. Wang, W. M. Kuebler, M. Schaefer, *Cell Calcium* **2012**, *51*, 194–206.
[22] S. Miehe, P. Crause, T. Schmidt, M. Lohn, H.-W. Kleemann, T. Licher, W. Dittrich, H. Rutten, C. Strübing, *PloS one* **2012**, *7*, e35393.
[23] D. G. Washburn et al., *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4979–4984.
[24] T. Maier et al., *Brit. J. Pharmacol.* **2015**, *172*, 3650–3660.

## Entry for the Table of Contents




The natural product (+)-larixol was derivatized to obtain **SH045**, a potent and selective TRPC6 channel blocker, comparable to synthetic benchmark blockers such as SAR7334. We assessed **SH045**'s pharmacological properties by means of electrophysiology and FLIPR assays in heterologously TRPC6 expressing HEK293 cells and in endogeneous pulmonary smooth muscle cells, and ultimately showcase a reduction of oedema in explanted murine lungs.