The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Sclareolide-based small molecules, TRPV4/$Ca_V1.2$ modulators, as new vasodilating agents

Gabriele Carullo[1,*], Sarah Mazzotta[2], Amer Ahmed[3], Alfonso Trezza[1], Ottavia Spiga[1], Simona Saponara[3], Fabio Fusi[1] and Francesca Aiello[4]

[1] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy;
[2] Department of Pharmaceutical Sciences, University of Milan, Via L. Mangiagalli, 20133 Milan, Italy;
[3] Department of Life Sciences, Via Aldo Moro 2, 53100 Siena, Italy;
[4] Department of Pharmacey, Health and Nutritional Sciences, University of Calabria, Via Savinio, 87036 Rende (CS), Italy.
* Corresponding author: gabriele.carullo@unisi.it

DBCF
DIPARTIMENTO DI BIOTECNOLOGIE, CHIMICA E FARMACIA

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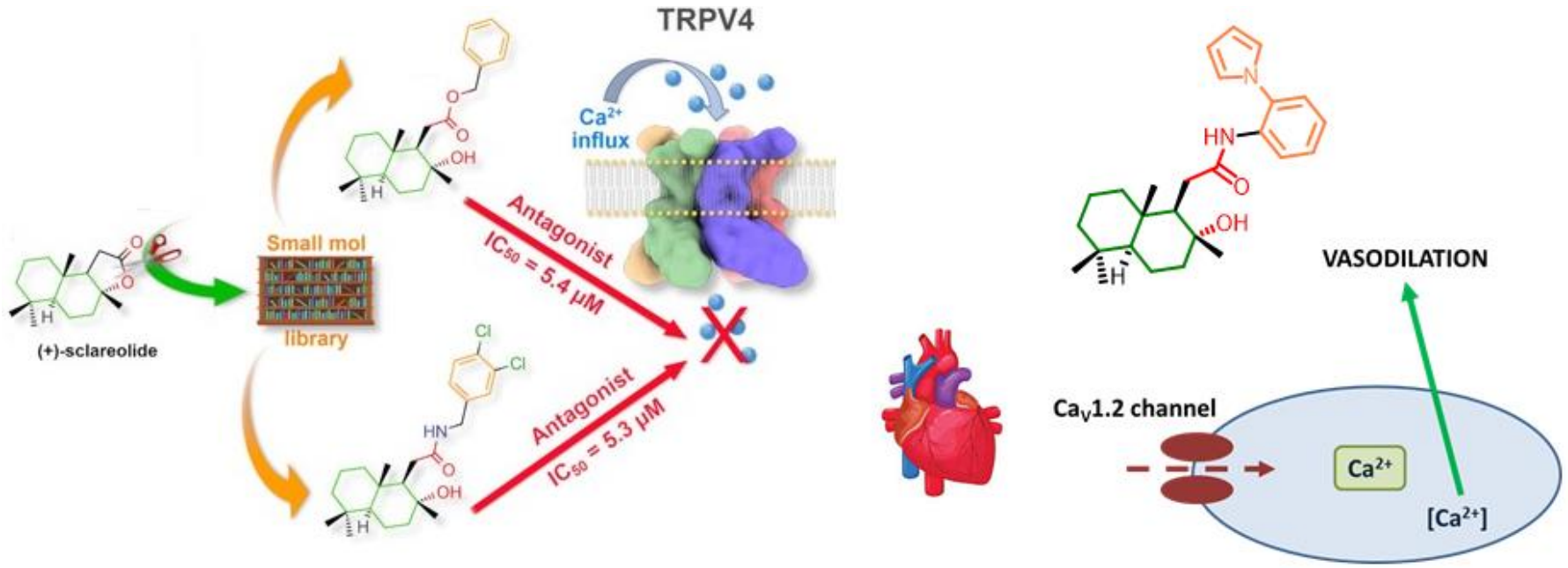
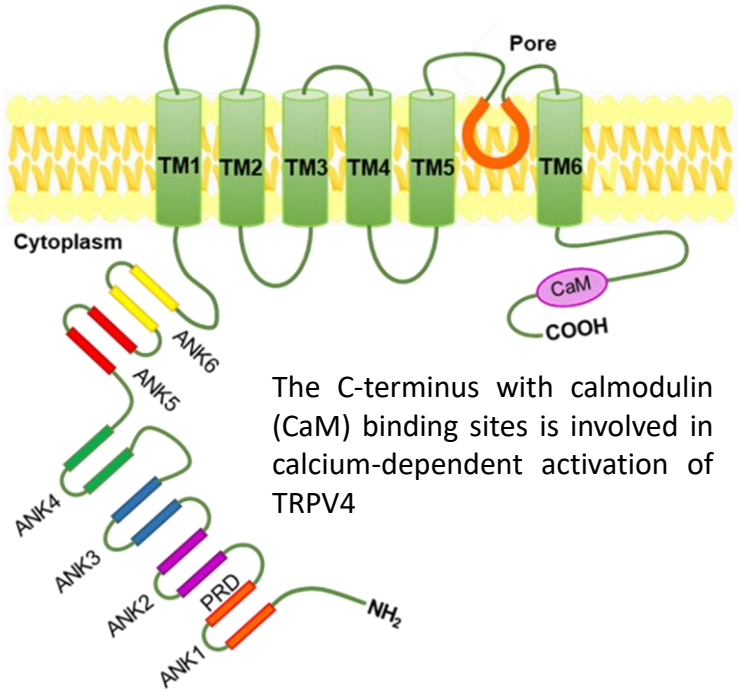
Sclareolide-based small molecules, TRPV4/$Ca_V1.2$ modulators, as new vasodilating agents



Abstract: Sclareolide is a fragrant sesquiterpene lactone found in *Salvia sclarea*, used as flavor additive in food. (+)-Sclareolide gained attention due to its versatility, since its lactone ring condensed with a trans-decalin-related homodrimane core can be easily opened and functionalized. Structural similarity between sclareolide and phorbol derivatives or onydecalin A, known TRPV4 modulators, prompted us to evaluate if sclareolide and its derivatives could modulate the activity of this channel. A small but diversified library of derivatives, characterized by the homodrimane backbone bearing flexible tails of different nature and chemical properties at position 1, was synthesized. In particular, the substituent groups, bound to the bicyclic nucleus by either an amide or ester or ether functionality, differ in size, flexibility, and electronic properties. The most interesting compounds were active in the submicromolar range and belong to the homodrimanyl acid amide series, in particular, benzyl and phenylethyl amides. Considering that inappropriate activation of TRPV4 produces acute circulatory collapse associated with endothelial activation/injury, and considering that the role of TRPV4 is confused, we investigated the best performing compounds as vasodilators in rat myocytes. The compounds were able to reduce the currents associated with $I_{Ba}1.2$; the mechanism of the interaction between the most intriguing compound and $Ca_V1.2$ channel was voltage-dependent and antagonized by a channel activator such as Bay K 8644. Furthermore, this compound stabilizes the inactivated state of the channel. Finally, it inhibits the contraction of the aorta rings induced by high potassium with an IC_{50} comparable to that obtained on the channels.

Keywords: sclareolide; TRPV4; $Ca_V1.2$; vasodilation.

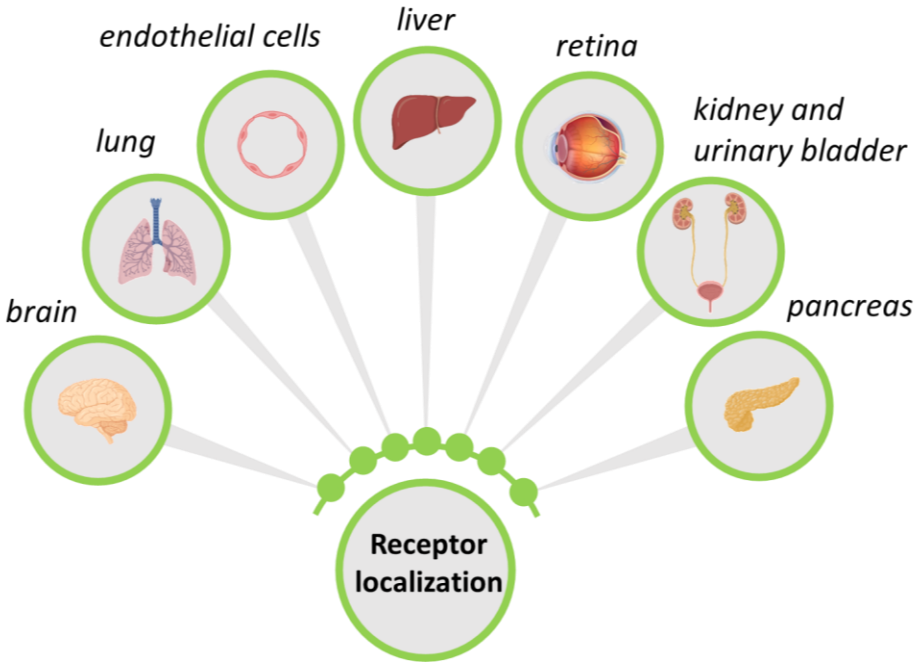
Introduction: TRPV4



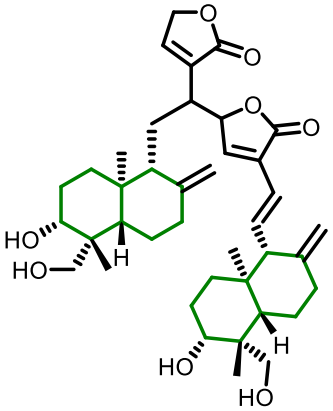
The C-terminus with calmodulin (CaM) binding sites is involved in calcium-dependent activation of TRPV4

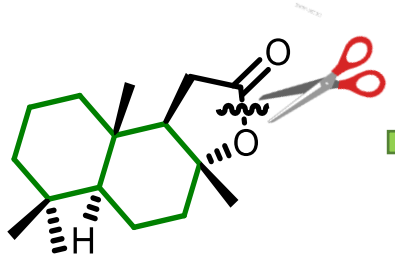
N-terminus presents 6 ankyrin (ANK) repeats implicated in protein interaction and in the assembly into a tetrameric structure

- Vanilloid cation channel
- High permeability to Ca^{2+}
- Homotetramer of 871 amino acids
- The pore channel associated to an S1-S4
- The cytosolic region includes N- and C- terminal domains

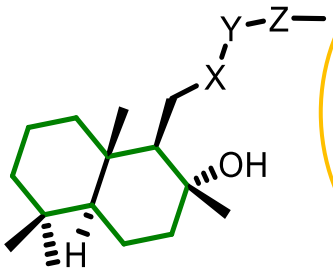


Introduction: Design of new TRPV4 antagonists



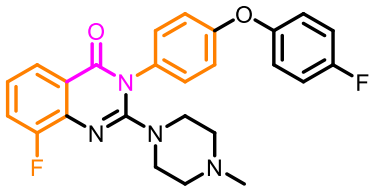


(+)-Sclareolide, 1

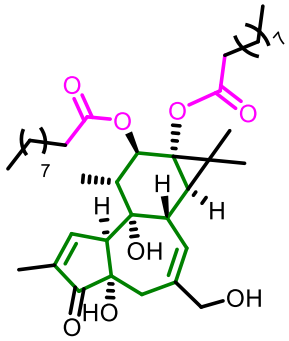


drimane-derived scaffold

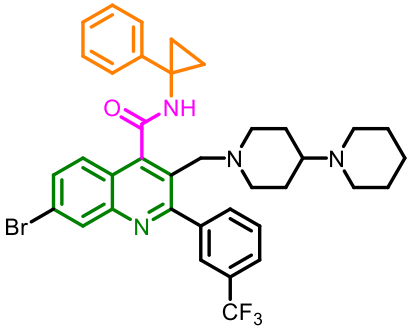
Aliphatic or aromatic moiety

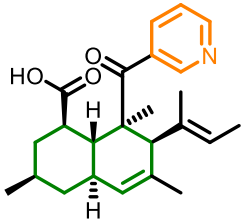


Agonists

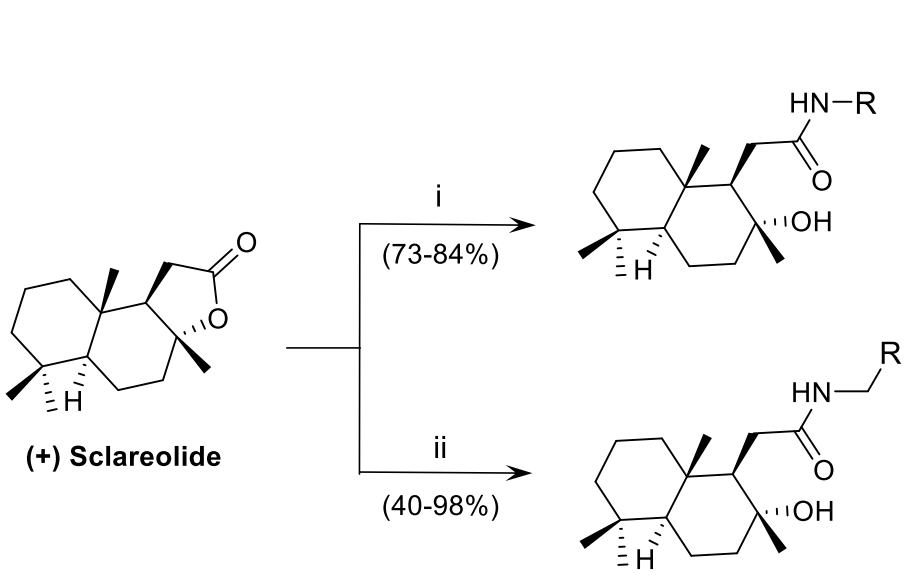


Antagonists



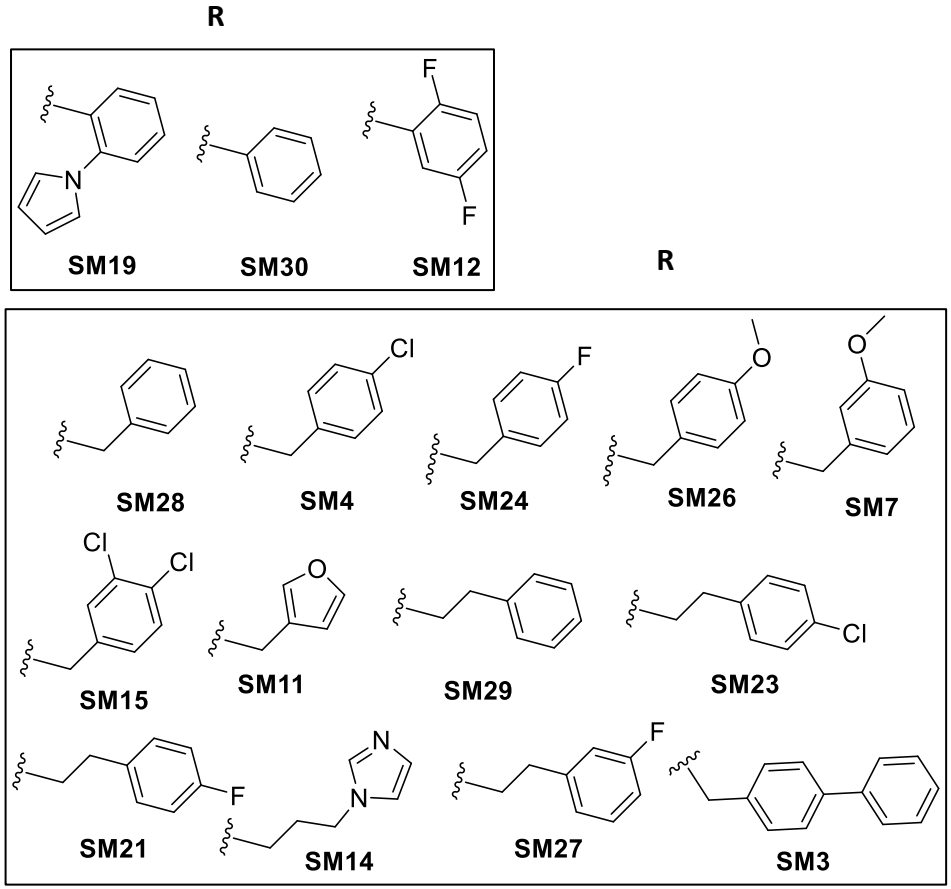


Results and discussion: Chemistry – part 1

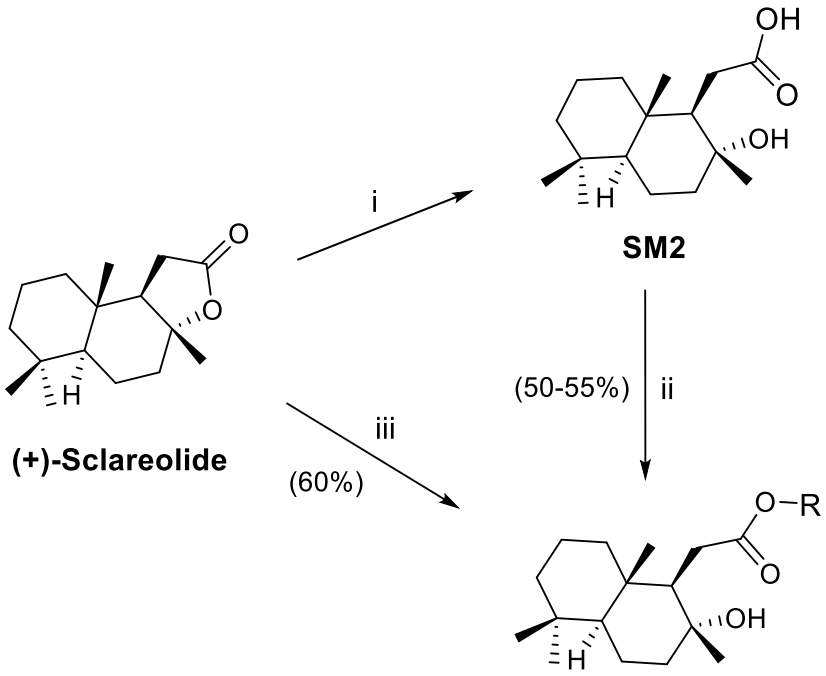


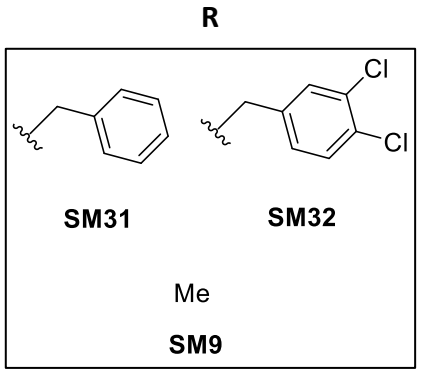
i: Aromatic amine 2.5 eq, DIBAL-H 3 eq, dry THF, rt, 3-5 h

ii: Aliphatic amine, dry THF, 45 ºC, 48 h



Results and discussion: Chemistry – part 2



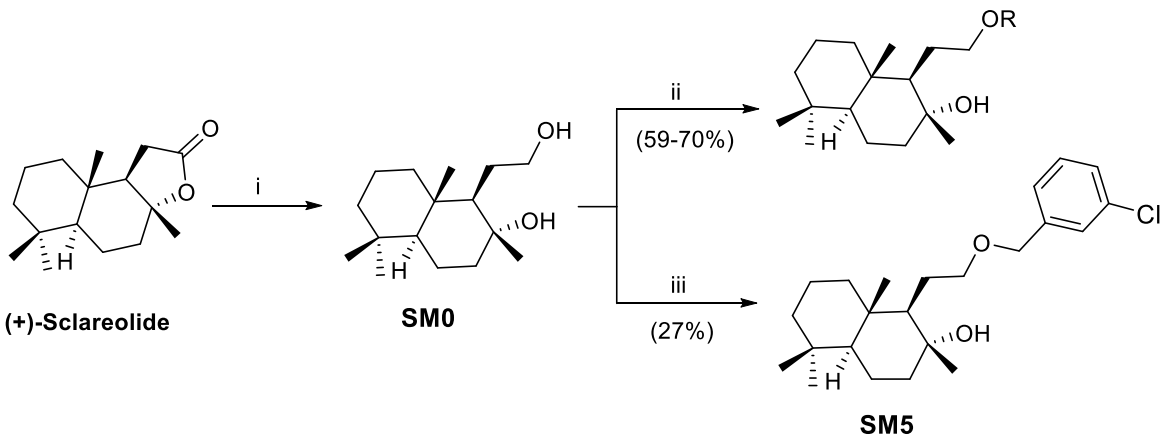


i: NaOH 4 eq, MeOH, rt, 2 h;

ii: Benzyl bromide 1 eq, K_2CO_3 1 eq, dry DMF, rt, 24 h;

iii: MeOH, 45 °C, 72 h

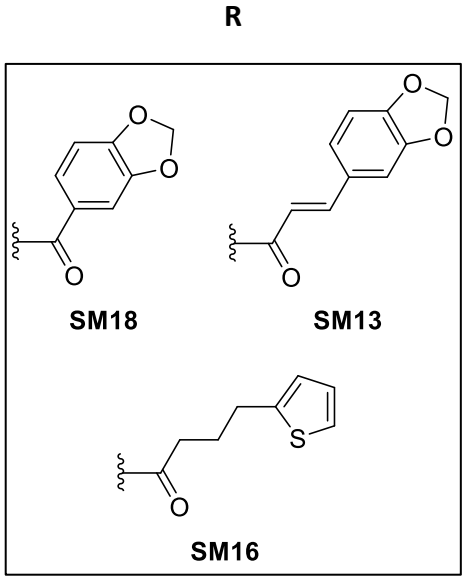
Results and discussion: Chemistry – part 3



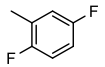
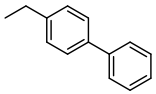
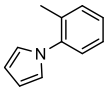
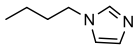
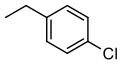
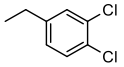
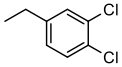
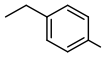
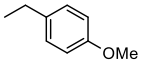
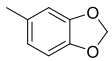
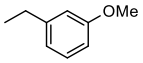
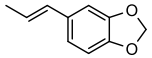
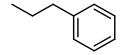
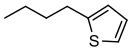
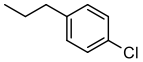
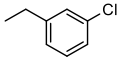
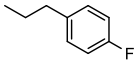
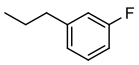
i: $LiAlH_4$ 10 eq, dry THF, rt, 6 h

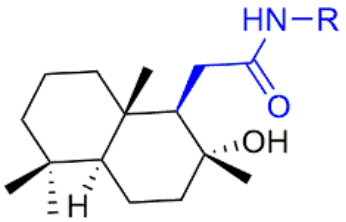
ii: Carboxylic acid 1 eq, EDCI 1.2 eq, DMAP 0.1 eq, DCM, rt, 48-72 h

iii: NaH 1.1 eq, 3-chlorobenzyl chloride 1.2 eq, dry THF, reflux, 48 h.

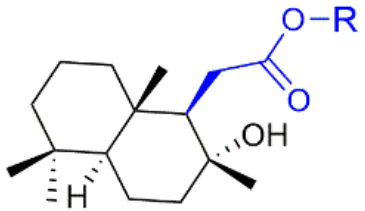


Results and discussion: TRPV4 assay

Cpd.	R	Efficacy[b] %	Potency EC_{50} (μM)	IC_{50} (μM)[c] inh TRPV4	Cpd.	R	Efficacy[b] %	Potency EC_{50} (μM)	IC_{50} (μM)[c] inh TRPV4
SM30 (a)		< 10	NA[d]	> 100	**SM11 (a)**		< 10	NA	53.5 ± 1.8
SM12 (a)		< 10	NA	> 100	**SM3 (a)**		< 10	NA	> 100
SM19 (a)		14.6± 1.5	1.1 ± 1.0	6.0 ± 0.1	**SM14 (a)**		< 10	NA	> 100
SM28 (a)		< 10	NA	32.0 ± 0.8	**SM31 (b)**		< 10	NA	5.41± 0.07
SM4 (a)		< 10	NA	7.7 ± 0.3	**SM32 (b)**		< 10	NA	> 100
SM15 (a)		< 10	NA	5.3 ± 0.3	**SM9 (b)**	Me	< 10	NA	> 100
SM24 (a)		15.8± 0.8	13.4± 2.6	16.9 ± 0.8	**SM0 (d)**	H	< 10	NA	> 100
SM26 (a)		< 10	NA	29.7 ± 0.7	**SM18 (c)**		<10	NA	NA
SM7 (a)		< 10	NA	18.1 ± 0.2	**SM13 (c)**		< 10	NA	> 100
SM29 (a)		< 10	NA	15.6 ± 0.3	**SM16 (c)**		< 10	NA	> 100
SM23 (a)		< 10	NA	7.0 ± 0.1	**SM5 (d)**		< 10	NA	> 100
SM21 (a)		< 10	NA	11.4 ± 0.1	**Scd[e]**	-	11.3± 0.7	> 10	> 100
SM27 (a)		< 10	NA	11.9 ± 0.4					



(a)



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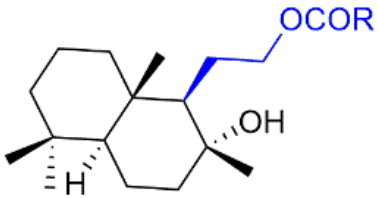
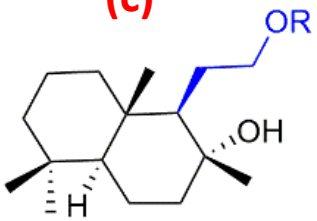


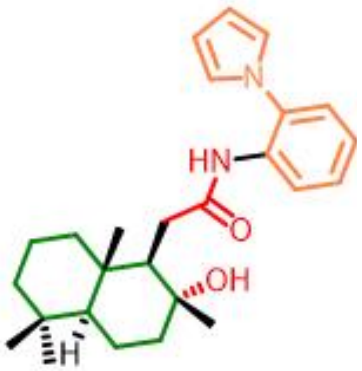
(c)

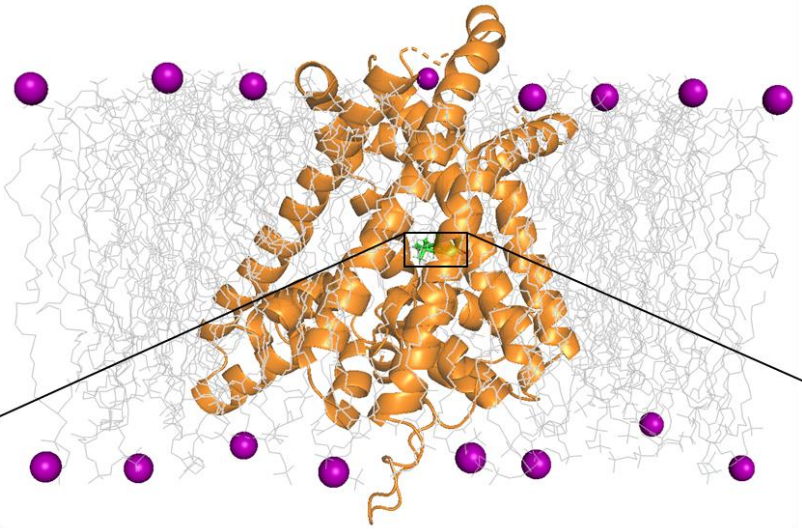


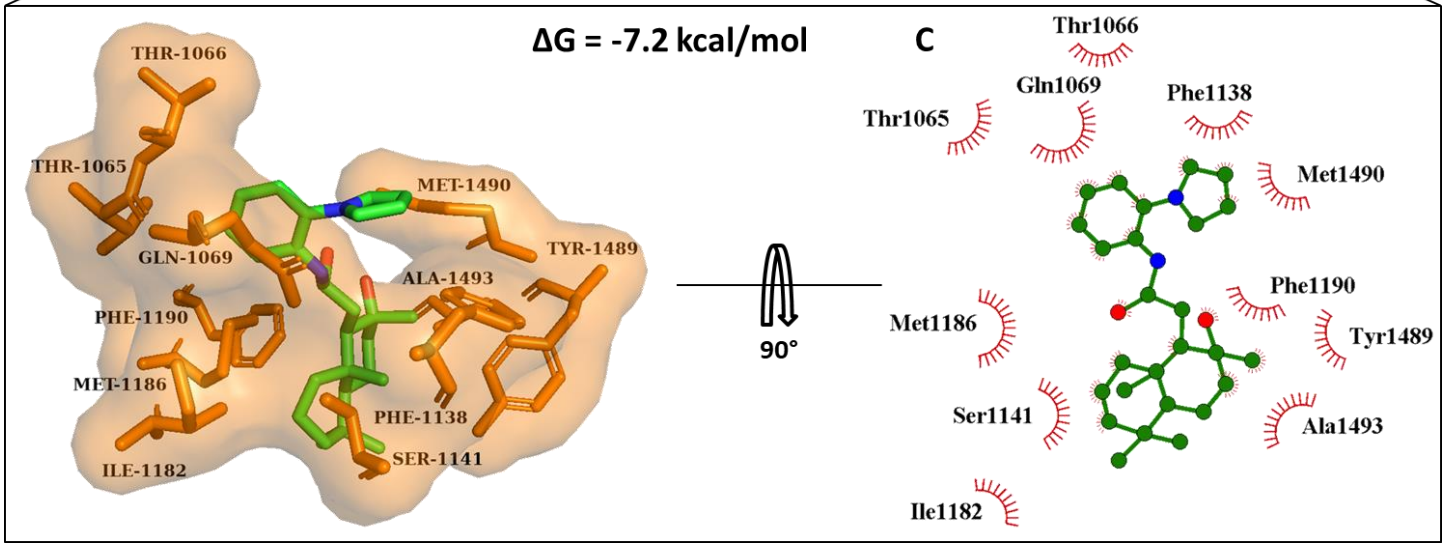
(d)

Results and discussion: $Ca_V1.2$ channels

The presence of chelating portions in sclareolide derivatives prompted us to evaluate if these compounds act as $Ca_V1.2$ channel blockers



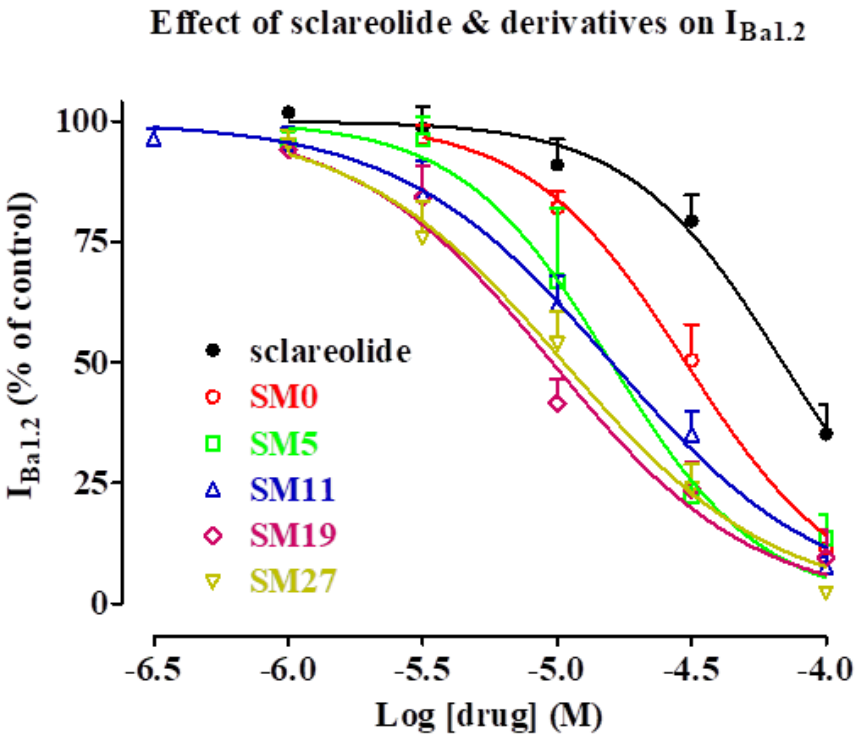




Results and discussion: Effect of sclareolide and its derivatives on Ba^{2+} current through $Ca_V1.2$ channels recorded in rat tail artery myocytes

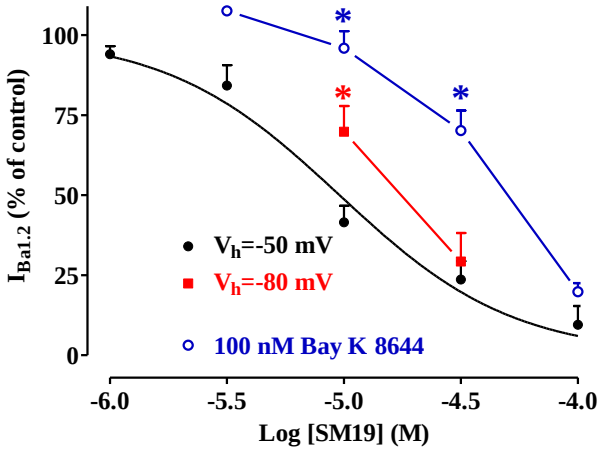
Compound	IC_{50} (μM)	E_{max} (at 100 μM)
SM19	13.2±5.4 (6)	90.5±5.8 (6)
SM27	15.3±5.3 (8)	97.8±0.8 (5)
SM11	16.7±3.0 (7)	92.4±2.2 (7)
SM5	20.8±6.1 (6)	86.4±4.9 (6)
SM0	33.2±6.8 (5)	88.7±4.0 (5)
Sclareolide	69.0§	64.8±6.1 (6)

Potency (IC_{50} values) and efficacy (E_{max}) are mean±s.e.m. n is the number of cells recorded, isolated from at least three animals.
§ Estimated

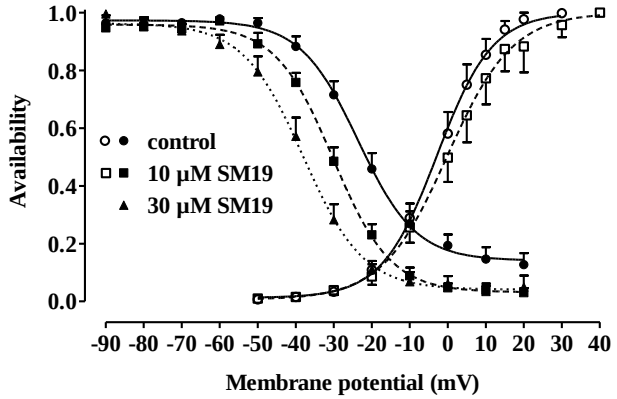


Results and Discussion: Effect of SM19 on rat tail artery myocytes

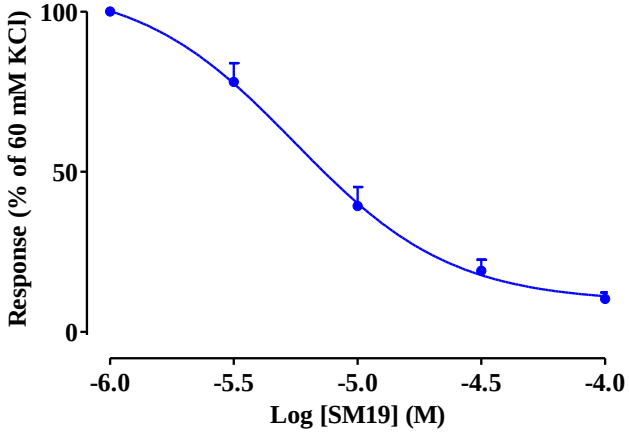
Effect of SM19 on $I_{Ba1.2}$
(RTA, V_h=-50/-80, 10 mV, 5 mM Ba^{2+}, -10 uM nifedipine)



Effect of SM19 on steady-state inactivation
(10 mV, 5 mM Ba^{2+}, V_h = -80 mV, -10 µM nifedipine)



Effect of SM19 on 60 mM KCl-induced contraction of endothelium-denuded abdominal rat aorta rings



Conclusions

- A new natural scaffold has been identified as tool for the development of TRPV4 modulators
- A library of compounds showed submicromolar activity against TRPV4 compared to TRPV1
- Selected compounds were able to inhbit $Ca_V1.2$ channel
- These showed to promote vasodilation also in rat aorta rings
- Further studies are ongoing to evaluate the role of TRPV4 in mediating vasodilation
- *In vivo* studies will be performed to ascertain the toxicity and efficacy profile

Acknowledgments

All my research group

The organizing Committee

And all of you for the kind attention