Towards musky odors: discovery of the broadly tuned human musk receptor

Sandra Huysseune, Mélodie Locrel, Alex Veithen, Magali Philippeau and Yannick Quesnel

ChemCom S.A., Route de Lennik 802, 1070 Anderlecht, Belgium.

Contacts: yaq@chemcom.be; shu@chemcom.be

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Introduction:

Musk odors have been used for medicine and fragrance from time immemorial because of their fascinating scent and potential effects on human health. The first natural musk, Muscone, originates from the musk deer where it has a pheromonal function. Nowadays, most of the musks used in the industry follows from chemical synthesis and are widely used in cosmetic and perfume industry due to their warmth, elegance, animal scent as well as for their fixative properties.

To date, four structurally diverse groups of chemical compounds sharing a musky-like note have been identified: nitro musks, polycyclic musks, macrocyclic musks and alicyclic (or linear) musks. Despite their big commercial success, the use of nitro and polycyclic musks has been reduced in recent years because of their potential health and environment damaging properties. Their replacement by safer and/or ecologically benign musks or compounds that enhance the musk perception remains an important goal.

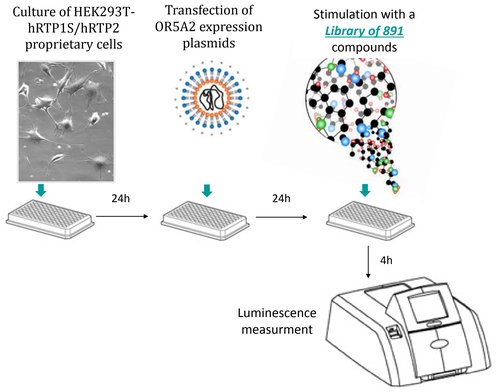
Materials and Methods:

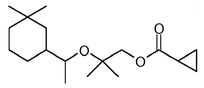
In vitro functional assay :

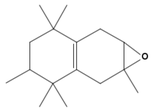
Deorphanization screening and dilution-response analysis were performed in the HEK293T-hRTP1S/hRTP2 proprietary cell line using the CRE-luciferase reporter assay system. Briefly, cells plated one day before were transfected with ORs of interest or empty vector plasmids using TransIT®-LT1 (Mirus) according to the manufacturer's protocol. Twenty hours after transfection and four hours after incubation with tested compounds, cells were lysed and processed for luminescence measurement using a Spectra Max M5 reader (Molecular Devices). Results of agonist concentration-response analyses are expressed as relative light unit (RLU) and were fitted to the Hill's equation

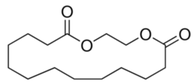
Results:

A. Deorphanization of OR5A2



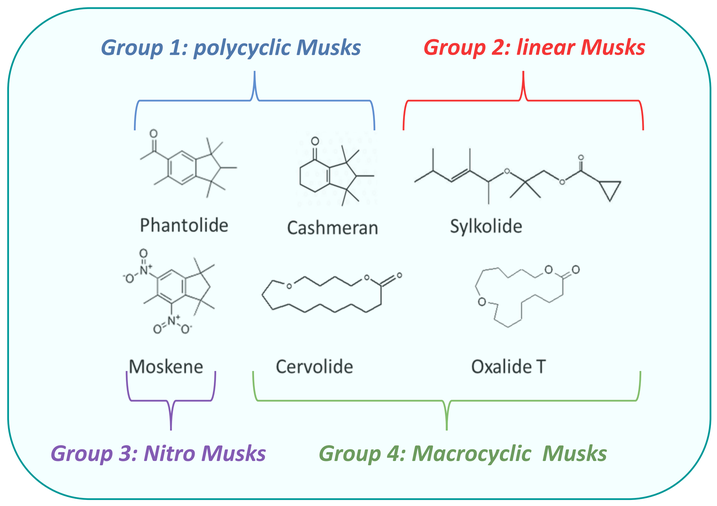

Serenolide (Givaudan)

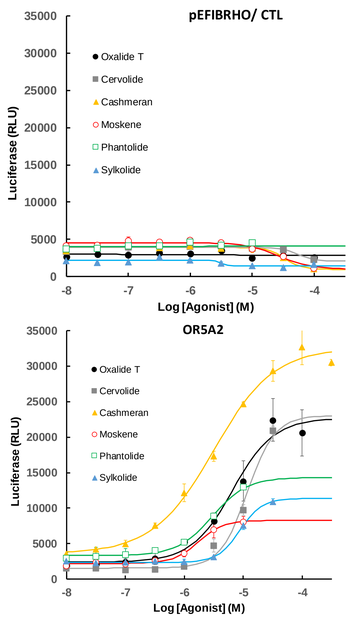

Moxalone (Givaudan)


Ethylene Brassylate

✓ Positive hits on OR5A2: Musk compounds

B. Concentration-response (CR) analysis of OR5A2 activated by archetypal compounds of the four different groups of musk





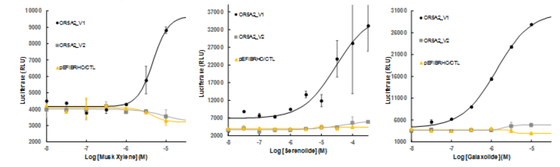
✓ OR5A2 is activated by the 4 different groups of Musk

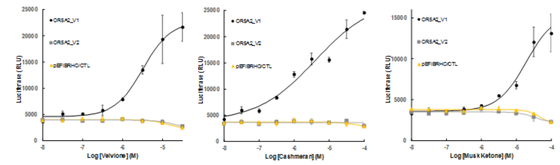
C. OR5A2 variants (haplotypes): effect of a mutation on OR5A2 functionality

	M302R	P172L	R123Q	F103L	% Freq	CORP
1	M	P	R	F	79.72	0.156
2	M	L	R	F	16.59	0.156
3	M	P	R	L	3.30	0.156
4	R	P	R	F	0.31	0.156
5	M	P	Q	F	0.08	0.767

>95%

https://genome.weizmann.ac.il/horde/card/index/symbol:OR5A2





- Tested in vitro with a luciferase reporter assay
- Transfection of the 2 haplotypes and an empty vector
  - V1: 172 Proline
  - V2: 172 Lysine
- Ligands representative of the 4 groups of musk

✓ The haplotype with the substitution in position 172 (P172L) loses its ability to be activated by musk compounds

D. Comparison of EC50 obtained in CR assays of OR5A2, its closest paralog OR5A1 and the two previously described musk receptors: OR11A1 and OR5AN1

OR activated; mean value n=3
OR non activated; n=3

Name	Organoleptic properties	Structure	Class	OR5A2 (log EC50)	OR5A1 (log EC50)	OR5AN1 (log EC50)	OR11A1 (log EC50)
Moskene	sweet **musk** ambrette ketone powdery dry		Nitro Musk	-5.73			
Musk ketone	fatty **musk** soapy dry powdery			-3.97		-6.54	
Musk xylol	fatty dry sweet soapy **musk**			-5.73		-6.18	-5.73
Musk ambrette	**musty** sweet ambrette seed			-5.06		-3.16	

Name	Organoleptic properties	Structure	Class	OR5A2 (log EC50)	OR5A1 (log EC50)	OR5AN1 (log EC50)	OR11A1 (log EC50)
Cyclopentenyl propionate musk	sweet **musk**		Linear	-4.55			
serenolide	**musk**			-4.66			
Sylkolide	Sylkolide™ is a **musk** by Givaudan			-4.75			
Helvetolide	**musky**, ambrette, pear			-3.86			

Name	Organoleptic properties	Structure	Class	OR5A2 (log EC50)	OR5A1 (log EC50)	OR5AN1 (log EC50)	OR11A1 (log EC50)
Crysolide	animal **musk** cedar ambergris woody		Polycyclic	-4.65		-3.92	
Tonalid	strong sweet amber fruity **musk** powdery			-6.12			-4.74
Phantolide	strong sweet **musk** amber powdery dry fruity			-5.34			-5.12
Cashmeran	rich spicy **musk** woody clean			-4.75			-4.67
Galaxolide	strong diffusive sweet floral **musk**			-5.98			
Traseolide	dry sweet amber **musk** herbal creamy			-5.71			-5.15
Moxalone	Moxalone® is a **musk** fragrance ingredient by Givaudan			-4.53		-3.51	
Vernolide	sweet intense **musk** ambrette macrocyclic			-5.17			-4.81
Fixal	powerful, very natural-warm, **musk**-like odor notes			-4.99			-5.05

Name	Organoleptic properties	Structure	Class	OR5A2 (log EC50)	OR5A1 (log EC50)	OR5AN1 (log EC50)	OR11A1 (log EC50)
Ethylene brassylate	powdery sweet floral ambrette **musk** woody		Macrocyclic	-4.31		-4.18	
Thibetolide = Exaltolide	**musk** animal powdery natural fruity			-4.36		-4.06	
1,16-Hexadecalactone	sweet **musk** balsam amber animal			-4.39		-3.49	
Exaltenone	**musk** animal natural			-4.81		-5.73	
Globanone (Animusk)	**musk** floral			-5.4		-5.65	
Musk R1	sweet oily incense **musk** amber animal			-4.72		-4.02	
Velvione	dry powdery **musk** amber civet			-4.54		-4.31	
Cyclopentadecanone	powdery **musk** animal natural greasy			6		-4.95	
Muscone	sweet **musk** animal powdery fatty natural			-5.25		-5.37	
Civetone	clean **musk** dry animal sweet			-5.29		-5.325	
Musk MC4	sweet clean waxy **musk** animal			-4.42		-4.12	
Cervolide	**musk** woody sweet brassylate fruity			-4.52		-4.13	
AAI des formules désactivé	sweet soapy **musk** amber fruity berry			-5.17		-4.68	
nirvanolide	intense **musky,** fruity, powdery odor with lactonic nuances			-4.71		-3.38	
Isoambrettolide	sweet **musk** ambrette fruity waxy			-4.72			
Habanolide	**musk**			-4.79		-4.18	
Musk 77	**musk** like note			-5.29			-5.04
Oxalide T	sweet **musk** oily fruity animal floral			-5.08		-4.49	

- ✓ OR5A1, the closest paralog of OR5A2, was not activated by any of the musk tested
- ✓ Musk-specific ORs (OR5AN1 and OR11A1) respond mainly to nitro musks and macrocyclic musks or polycyclic musks and nitro musks respectively
- ✓ None of the musk compounds tested was able to activate exclusively OR5AN1
- ✓ OR5A2 is activated by musk compounds belonging to the 4 groups of musky chemicals
- ✓ OR5A2 is the ***only*** OR activated by the linear musk family, known to be more environment friendly

- WO 2015/020158 A1
- Shirasu et al. 2014 Neuron 81, 165-78
- Sato-Akuhara N. et al. 2016 J Neurosci 36(16), 4482-91
- WO 2016/201152 A1
- WO 2019/110630 A1 (CHEMCOM PATENT; OR5A2)

Conclusion:

In this study, we screened the human set of functional olfactory receptors (OR) for additional "musk" receptors and discovered a new unexpected OR belonging to class 2 activated by archetypal representatives of the four different groups of musk. So far, none of the formerly identified musk-receptors (OR5AN1 and OR11A1) showed such levels of responsiveness for all musk groups. In this context, we have compared the selectivity, sensitivity and efficacy of these three receptors as well as the impact of amino acids mutations on the functionality of the receptor. Given the importance of musk in our everyday life, the understanding of the molecular mechanisms that support the perception of the musky odor is crucial and will allow the development of new generations of musk or musk enhancers useful for perfumers and F&F companies.