"*Sensorial Chemistry*": functional characterization and structure activity relationship understanding of OR5K1 and OR2AG1 allow to design and synthetize new selective compounds

Figure 1. - Absence of paralogy between OR2AG1 and OR5K1 illustrated by their relative distance on the human ORs cladogram.

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Introduction:

ChemCom has deorphanized more than 120 human ORs relying on (i) an efficient proprietary screening system and (ii) libraries of thousands of odorant compounds. Accordingly, ChemCom is currently identifying and characterizing new modulating molecules (enhancers or blockers) and novel odorant compounds for the whole range of hORs.

We previously made structure-activity relationship (SAR) studies on two unrelated and well known hORs[1, 2, 3,4], OR5K1 and OR2AG1, highly expressed in the whole olfactory mucosa[5] and we wanted to go one step further by linking *in vitro* profile of the best identified ligands of these 2 hORs to their *in vivo* organoleptic properties. If we were able, among a large set of commercially available compounds (>120), to identify ligands displaying good selectivity for OR5K1 compared to OR2AG1, or dual acting OR5K1 – OR5AG1, we could not identify compounds displaying a good selectivity for OR2AG1 versus OR5K1. Thus, by making an in depth analysis on SARs established on both hORs, subtle differences on aza heterocycles were identified enabling us to design ligands likely to display good to remarkable selectivity for OR2AG1 versus OR5K1. After chemical synthesis and *in vitro* characterization, those compounds effectively display the expected selectivity:

- allowing the sensorial assessment of OR2AG1, OR5K1 and dual OR1AG1/OR5K1 ligands.
- Aiming to demonstrate that "Sensorial Chemistry" might allow to modulate the olfactory properties of volatile organic compounds

Methods:

In vitro functional assay: performed in HEK293T-hRTP1S/hRTP2 cells using the CRE-Luciferase reporter assay system[6]. Briefly, cells plated (96 well plates, 25.000 cells per well) one day before, were transfected with OR and pGL4.29 plasmids using TransIT®-LT1 (Mirus) according to the manufacturer's protocol. Twenty hours after transfection and four hours after incubation with tested compounds (range of concentrations from 30nM to 1mM), cells were lysed and processed for luminescence measurement using a Spectra Max M5 reader (Molecular Devices). Results of concentration-response analyses are expressed as the percentage of the response induced by 10 µM of Forskolin (FSK) and were fitted to the Hill's equation.

Sensory assessment: Tedlar bags (previously conditioned 24 hours in a ventilated oven at 90°C in order to eliminate intrinsic plastic smell) is first loaded with a known volume of pure air (from oil free compressor with additional charcoal filters). Then the compound of interest (EtOH solution) is injected with a high accuracy micro-syringe through a septum placed on the tap-valve. In case of deposit, visible haloes can be observed on the bag walls. Gentle heating is then applied until no more haloes are visible. We assume complete vaporisation of the solute. The so prepared tedlar bags are presented to expert panel, trained to ISO5496 and the "**field of odour**"® but also used to ISO12219-7. ®

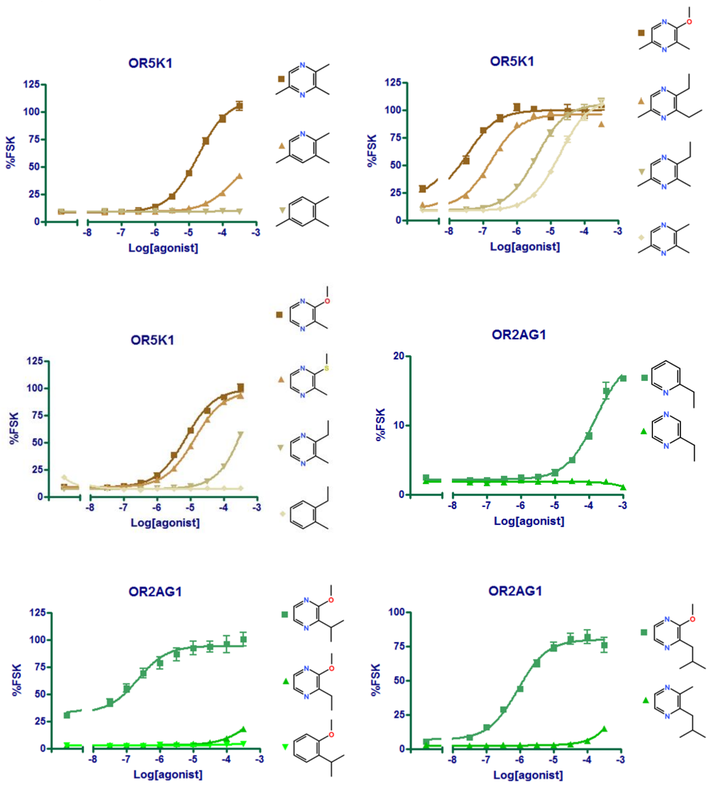


Figure 1: *In vitro* activity of key ligands of OR5K1 and OR2AG1.

These ligands, among others, were quite instrumental for the understanding of the differential structure activity relationship of commercially available small heterocycles on both OR5K1 and OR2AG1. Thus, they enable us to identify key structural requirements to achieve good functional activity on the targets.

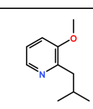
	Sample name	Structure	OR5K1 - logEC$_{50}$	OR5K1 E_{max} [% FSK]	OR2AG1 - logEC$_{50}$	OR2AG1 E_{max} [% FSK]
A	2-Ethyl-3,5(or 6)-dimethylpyrazine		6.3	104	-	-
B	2-Methoxy-3-(1-methylpropyl)pyrazine		5.3	27	7.2	72
C	2-(sec-butyl)-3-methoxypyridine		-	-	5.9	69
D	2-isobutyl-3-methoxypyridine		4.8	45	5.7	77

Table 1.- *In vitro* activity and sensory relationships of OR5K1 and OR2AG1.
Description of the organoleptic properties are given by The Good Scents Company.

Potency of agonists is measured by the logEC$_{50}$ calculated from concentration-response analyses of an experiment representative of at least two independent experiments. Efficacy of agonists is measured by the E_{max}, or to the highest measured value when an E_{max} plateau is not obtained; values are % of the response induced by 10 µM Forskolin [% FSK] ; '-' = not active.

Using the so acquired SAR's on both olfactory receptors, OR2AG1 and OR5K1, we proceed to a differential analysis trying to pick-up key pharmacophoric differences enabling the design of OR2AG1 selective compounds. Indeed, according to this differential analysis, a pyridine core scaffold should have a tendency to give better activity on OR2AG1 versus OR5K1, particularly when substituted by small alkoxy substituents in R1 and bulky alkyl in R2. Obviously, R3 and R4 are kept unsubstituted to maintain a good level of OR2AG1 activity. Thus, we arrived at the hypothesis that those 2 compounds: 2-isobutyl-3-methoxypyridine and 2-(sec-butyl)-3-methoxypyridine, and preferentially the later one, 2-(sec-butyl)-3-methoxypyridine will have a better selectivity for OR2AG1 versus OR5K1 (figure 5).

These compounds are not commercially available, so we synthesized these compounds using our available external chemistry platform. Obviously, these compounds, as well as all the chemical intermediates, were tested on both targets.

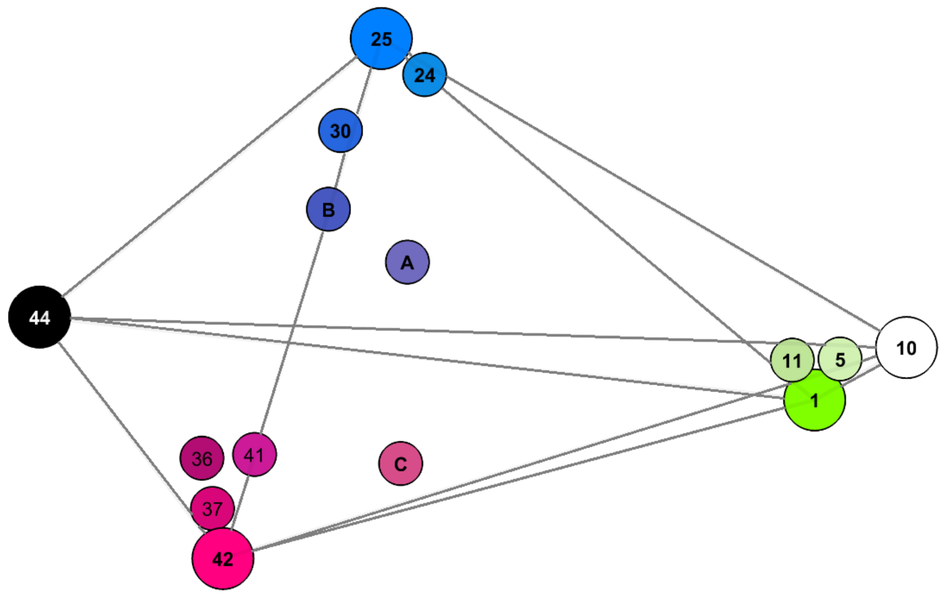


Figure 3: localization of the few ligands in the field of odour®.
The field of odour is a purely objective way to characterize odorant compound regarding their hedonic proximity towards a selected set of reference compounds. Here, selective OR5K1 and dual OR5K1/OR2AG1 are close together while selective OR2AG1 appears quite different.

OR5K1 and OR2AG1 main structural requirements:

This comparative SAR between both olfactory receptors allowed us to draw some clear differences around key substitution patterns and central core scaffolds allowing to guide selectivity for one or the other OR:

- For OR5K1, the preferred core scaffold seems to be pyrazines where both nitrogen atoms allow better potency compared to pyridines. Regarding preferred substitution patterns, substitution in R1 is mandatory and small linear alkoxy group in R1 are preferred. In R2, steric hindrance leads to a decrease in EC_{50} while linear alkyl derivatives are preferred. Small methyl group in R3 and R4 tends to enhanced functional activity but only few compounds are commercially available to make this analysis.
- For OR2AG1, substitution on R1 and R2 are mandatory for functional activity. Preferred substitution pattern seems to be small linear alkoxy in R1, bulky alkyl substituent en R2, while substitution in R3 and R4 seems detrimental to its functional activity. The preferred core scaffold seems to be pyridine since the loss of the upper nitrogen of pyrazine seems to increase EC_{50}.

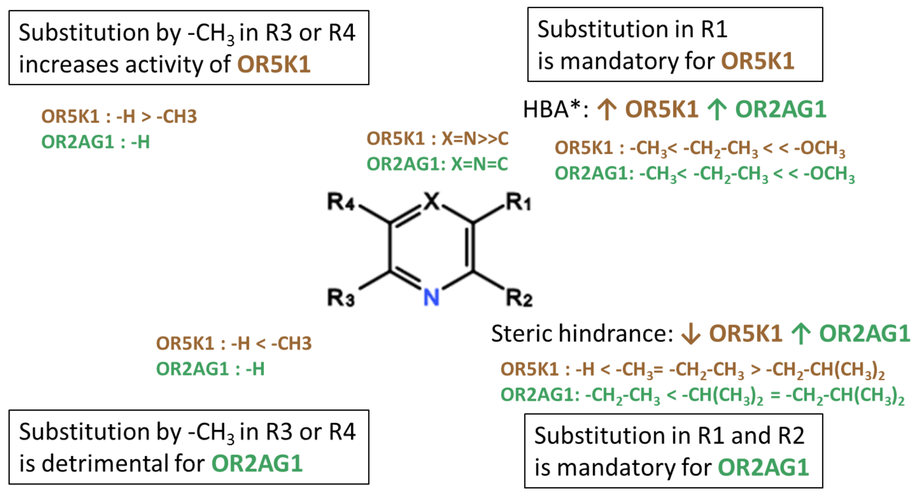


Figure 2: Olfactophore of pyrazine agonists of OR5K1 and OR2AG1 based on the potency (logEC$_{50}$) and the efficacy (E_{max}) obtained in concentration-response experiments.
*HBA = H bond acceptor.

Discussion:

In this study, we demonstrated that "Sensorial Chemistry" allowed us to modulate the olfactory *in vitro* profile of volatile organic compounds and control the selectivity of those ligands on two unrelated hORs, OR5K1 and OR2AG1, both highly expressed in the whole olfactory mucosa and activated by closely related ligands. Some of those ligands were characterized in human sensory assessment using the field of odor® from Jaubert[7] showing that their different *in vitro* profile effectively translate into quite different hedonic properties. Indeed, OR5K1 contribution appears to be more important than OR2AG1 since the dual acting ligand is very close to the OR5K1 selective one (both in the β-caryophyllene proximity) while the selective OR2AG1 is quite far from them.

This study tends to demonstrate that "Sensorial Chemistry" might be a valuable tool since it should enable to optimise a "hit" coming from screening on different parameters (potency, selectivity, physicochemical properties, cost…) and will be a strong driver towards market accessibility, exactly as medicinal chemistry is in the pharmaceutical area.

The identification of different antagonists acting selectively on OR5K1 or OR2AG1 and their evaluation as odor blockers will help to clarify the implication of each hORs in the perception of pyrazines. Moreover, as some of the key components of french fries and roasted beef odors correspond to the best agonists identified so far for both ORs, we may predict that the identification of such antagonists will lead to the formulation of new air cleaners, more effective in reducing the corresponding malodors.

References

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