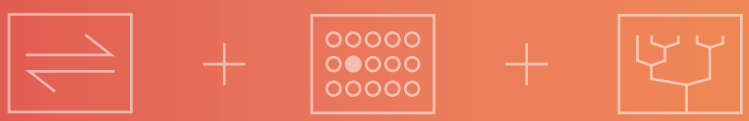
SENSE THE DIFFERENCE

Development of *Novel Transient Receptor Potential* (TRP)-based Bioactives



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Teaser for the **TRiP²Application Data Packet Development** of *Novel Transient Receptor Potential* (TRP)-based Bioactives

SYNOPSIS

The scope of TRiP²Application (Novel Ingredients for Food & Cosmetics) is to identify a portfolio of novel Transient Receptor Potential (TRP)-based bioactive natural products, extracts and/or, if applicable, (semi-) synthetic compounds for novel products in a joint innovation environment program. The core partners BRAIN AG and AnalytiCon Discovery (together the BRAIN Group) with their expertise in Natural Compound Access, Chemistry, Biotechnology & Cell Biology strive for an improved knowledge to develop the next generation TRP modulators for industrial applications.

TARGET PORTFOLIO AND CONTENT

Framework of the TRiP²Application program is an *in vitro* cell-based assay (CBA) screening of more than 15.000 natural products incl. enriched fractions of natural extracts and pre-filtered/pre-evaluated (semi-) synthetic compounds for modulating activity on three recombinant ScreenLines® expressing individual TRP ion channels (in particular TRPM8, -V1, -A1). Active samples are characterized by potency, efficacy and cross-selectivity measurements (see section 'Data Packet'). Validation and/or cross-check on a human primary cell line and/or other molecular targets is optional.
An extended partner has the chance to validate novel candidates with TRP modifying activities by *in vivo* efficacy testing (e.g. a sensory assessments) of selected hit candidates. The intention of the progam in a staggered approach is to integrate further working packages at later stages sequentially and based on the increasing knowledge gained in the course of TRiP²Application.

PROGRAM SCHEDULING

A face to face meeting could be scheduled in the near future to discuss the opportunities and options of the above outlined approach for TRiP²Application. High on the agenda should be an agreement on time lines and contributions of the partners as well as perspectives on the program. An extended partner has the opportunity to develop individual candidates for selected fields of application (on an exclusive or non-exclusive basis).

DATA PACKET

The packet is based on *in vitro* activity data of more than 15.000 screening samples: natural products incl. enriched fractions of natural extracts and pre-filtered/pre-evaluated (semi-)synthetic compounds. Data will be

collected, processed and provided in Excel format (ongoing process). Included in the data packest are samples with effect on the activity of at least one TRP ion channel.

Data packet provides information concerning the following topics:

- Compound ID*
- Common Name
- CAS number
- Structure* (e.g. SMILES)
- Physico-chem parameter*
- Biosource(s) e.g. taxonomy
- TRPV1 (agonistic/antagonistic)
- TRPA1 (agonistic/antagonistic)
- TRPM8 (agonistic/enhancement)
- Initial in vivo efficacy (e.g. off-taste)
- In silico Tox categories*
- Initial toxicological evaluation (e.g. Treshold of Toxicological Concern (TTC) levels)
- Value-Added Comments & additional data

* Mandatory data; remaining datasets are optional (if applicable)

Compound Identifier

The compound structure will be offered as a SMILES (Simplified Molecular Input Line Entry Specification) code. A CAS (Chemical Abstracts Service) number will be provided if available. Basic physico-chemical data and the source (including taxonomy; if available) are included as well.
Structures can be submitted in a SDF format as well.

In vitro Activity

Effects on the activity of at least one TRP ion channels (TRPM8, -V1, -A1) are given as the half maximal effective concentration (EC50) for agonists as well as the half maximal inhibitory concentration (IC50) for antagonists as concentrations in micromoles per litre [µM].
Furthermore, efficacy (the relative ability of a modulator to produce a maximal response compared to a reference ligand) will be determined for agonists [%, normalized to reference]. Antagonists will be characterized by mode of action (MoA) analysis.

In vivo Efficacy

If possible and applicable, an initial assessment of the *in vivo* efficacy is conducted. Feasibility is based on the initial toxicological evaluation and the application field (depending on readout and product-specific matrices). For food application (for example) an Sip & Spit analysis could be performed (based on the TTC concept) to examine and classify intrinsic taste and/or off-taste as well as general taste modifying activities.

In silico Toxicology

The BRAIN Group is applying computational (*in silico*) techniques and tools to predict toxicity. *In silico* toxicology is a time- and cost-effective method to assess the potential risks of novel bioactive compounds, thereby supporting their development. The methodology has gained increasing attention in the last years, not least because of rapid technical development and strong governmental support. Furthermore, the approach is in line with the 3Rs (Replacement, Refinement and Reduction) philosophy for the replacement of animals in testing and meets the legal requirements connected to the animal testing ban for cosmetic purposes announced by the European Commission.

To predict potential health risks the BRAIN Group is applying well-established software, including knowledge-based expert systems and data-driven statistical tools. Free computer programs like Toxtree (IDEAconsult Ltd.), TEST (US EPA) and Vega (Mario Negri Institute) as well as commercial tools like DEREK and SARAH (Lhasa Ltd.) are used for the analysis. Where possible, concerns according to structure, substructure(s) and/or fragment(s) are correlated with toxicological studies and/or published data. Note: *in silico* tox does not accurately predict and cannot replace real tox.

Value-Added Comments

Building on the long-standing expertise and experience in the field the BRAIN Group provides guidance for various samples regarding potential applications. Comments are based on *in vitro* activity (efficacy and potency) and cross-selectivity as well as physico-chemical data and *in silico* toxicological evaluation (as alerts might be connected to application field by the route of administration).

Preview on the Data Packet Excel Format:

Preview adjusted to handout paper format. Original data set will we provided as one searchable table that can be filtered according to partner-specific criteria.

Sample			TRPV1 (*in vitro*)		TRPA1 (*in vitro*)		TRPM8 (*in vitro*)		[...]
TRiP-ID	Name	CAS	Agonistic act.	Antagonistic act.	Agonistic act.	Antagonistic act.	Agonistic act.	PAMs (Menthol)	[...]
TRP-00001	XXX acid	-	tbd	tbd	-	+ [~10µM]	ND	ND	
TRP-00002	-	-	-	-	-	+ [25µM]	ND	ND	
TRP-00003	Dehydroabietic acid	1740-19-8	-	+ [30µM], ND	-	+ [25µM], competitive	-	-	
TRP-00004	Fersinin	-	-	+ [35µM], ND	-	+ [2.5µM], non-competitive	-	-	
...									
			EC50 [µM], Efficacy	IC50 [µM], MoA	EC50 [µM], Efficacy	IC50 [µM] (Reference AITC or Menthol), MoA	EC50 [µM], Efficacy	Effective conc. [µM]	

(continuation)

Sensory	Physico-chem. Data			Supporting information			Summary	
In vivo efficacy	MW	Formula	SMILES	Source(s)	*In silico* tox profile	External evaluation	Comment	Additional data
ND	234.34	C15H22O2	C/C(=C\CC	Genus spec.	1	TTC (90µg/p/day)		
ND	510.58	C26H27FN4O4S	CC(=O)NC	Synthetic	2-3	ND		
yes	300.44	C20H28O2	CC(C)C1=C	botanical, constit. of *Pinus sp.*	1	TDI (3.5mg/p/day)		
yes (topical)	346.42	C20H26O5	COC1=C(C	botanical, constit. of *Ferula soongorica*	1	TTC (1.8mg/p/day)	Skin care, soothing	tested neg. for skin sensitization
If applicable			Structure	Botanical (genus, spec), MO (fungi, bacteria; taxonomy if available)	Score (considering genotox, cramer cl., (Q)SAR predicitons, etc.)	External evaluation if available	Classification acc. to activity profile	

Regarding the *in silico* tox profile:
1 = no alerts
2 = minor concerns
3 = needs further evaluation

HOW TO WORK WITH THE BRAIN GROUP

A partner has to pay a disclosure fee to get access to the described data packet. The following joint program is based on deliverables and milestones. Market shares for successful candidates have to be negotiated.

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