Evaluation of comprehensive read-across assessment of compounds with limited toxicological data using *in silico* tools

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INTRODUCTION

Efficient strategies are needed to assess the general biological and toxicological profiles of novel compounds with no or limited experimental data. *In silico* approaches have emerged as indispensable for addressing this challenge. This study presents an *in silico* methodology that uses predictive pharmacology, toxicology profiling, target-based cluster analysis, chemical fingerprinting, and metabolic similarity assessment to support read-across with weight of the evidence by selecting more suitable target analogues with available toxicological information. We present a case-study using this approach via carvone and structurally related substances used as food ingredients.

METHODS

- 11 Carvone and structurally related substances (hereafter "carvones") were selected (Figure 1)
- Biological binding targets (from 4799 biological targets including enzymes, protein kinase, Cyp450, G protein coupled receptor, transporters, ion channels, nuclear receptors and others) with 11 Carvones were predicted using Chemotargets CLARITY (v4.x.18-0-ge1130d78) which is a commercially available statistical software tool.
- Uniprot ID (https://www.uniprot.org/) was used to identify species in which biological targets are expressed and only targets expressed in mammalian were selected.
- Biological process of each biological target was mapped using GO term mapper (https://go.princeton.edu/cgi-bin/GOTermMapper).
- Target-based cluster analysis was performed using R Studio (version 4.4.0).
- t-SNE and PCA analysis were also performed based on the structure-based information using RDKit which was integrated in KNIME. For t-SNE visualization, the perplexity was 3 and iteration was 1,000.
- Mapping using the predictions and structural alerts given from OECD QSAR Toolbox 4.5.

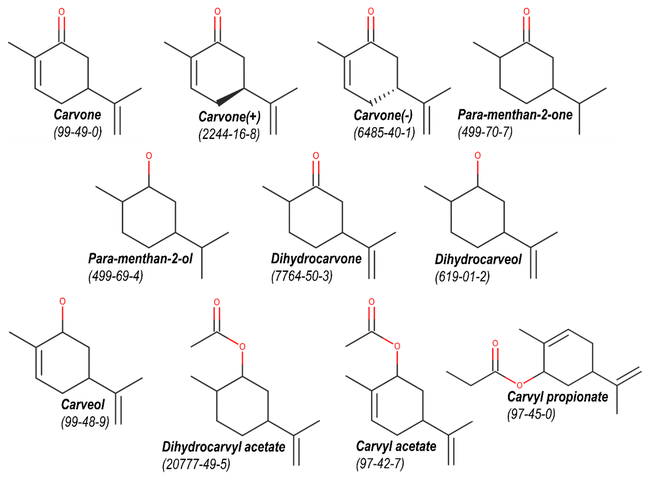


Figure 1: A total of 11 compounds (unique CAS and SMILES) were identified, three of which were the racemic mixture and two enantiomers of carvone. Nine of the compounds were classified as "Carvone derivatives".

BIOLOGICAL TARGETS

A

Biological target	No. Carvones	Families
Cocaine esterase	11	EC
Cytochrome P450 2A13	11	CP,EC
Glutamate receptor ionotropic, NMDA 2A	11	IC
N-acylethanolamine-hydrolyzing acid amidase	11	EC,OF
Transporter	11	OF
Synaptic vesicle glycoprotein 2A	11	TC
Neuronal acetylcholine receptor subunit alpha-4	10	IC
High affinity choline transporter 1	10	TC
Liver carboxylesterase 1	9	EC
Neuronal acetylcholine receptor subunit alpha-7	9	IC
Glutamate receptor ionotropic, NMDA 2D	9	IC
4-hydroxyphenylpyruvate dioxygenase	9	EC,OF
Lysophosphatidic acid receptor 3	9	GR
Nitric oxide synthase, endothelial	9	EC
Carbonic anhydrase 5B, mitochondrial	8	EC
Carbonic anhydrase 6	8	EC
Tyrosinase	8	EC
Carboxypeptidase B2	7	EC,OF
Glutamate receptor ionotropic, kainate 2	7	IC
Ryanodine receptor 2	7	IC
Alcohol dehydrogenase 1A	6	EC
Carbonic anhydrase 15	6	EC
Lysophosphatidic acid receptor 4	6	GR
Nischarin	6	UC
Nitric oxide synthase, inducible	6	EC
Polyphenol oxidase 2	6	EC
Alpha-2A adrenergic receptor	5	GR
Cytochrome P450 2A6	5	CP,EC
Dipeptidyl peptidase 9	5	EC,OF
Epoxide hydrolase 1	5	EC,OF
Glutamate receptor ionotropic, NMDA 2B	5	IC
Beta-hexosaminidase subunit alpha	5	EC,OF
Lysine-specific demethylase 2A	5	EC,OF

- Only those biological targets were selected which are expressed in mammalian and have predicted active binding.
- 7 key targets for 11 carvones were identified.
- 37 unique targets were identified.
- Major protein classes were enzymes (43%), ion channels (13%) and G protein coupled receptor (8%).

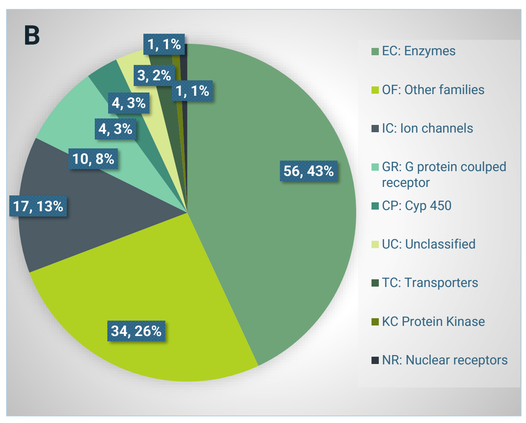


Figure 2: (A)The list of biological targets binding to at least.(B) Protein families of predicted binding targets.

BIOLOGICAL PROCESS

- 50 biological processes were identified based on the predicted biological binding targets.
- 55.3% of predicted targets are included in signaling, and major biological processes were anatomical structure development, nervous system process, lipid metabolic process, transmembrane transport and programmed cell death, respectively.

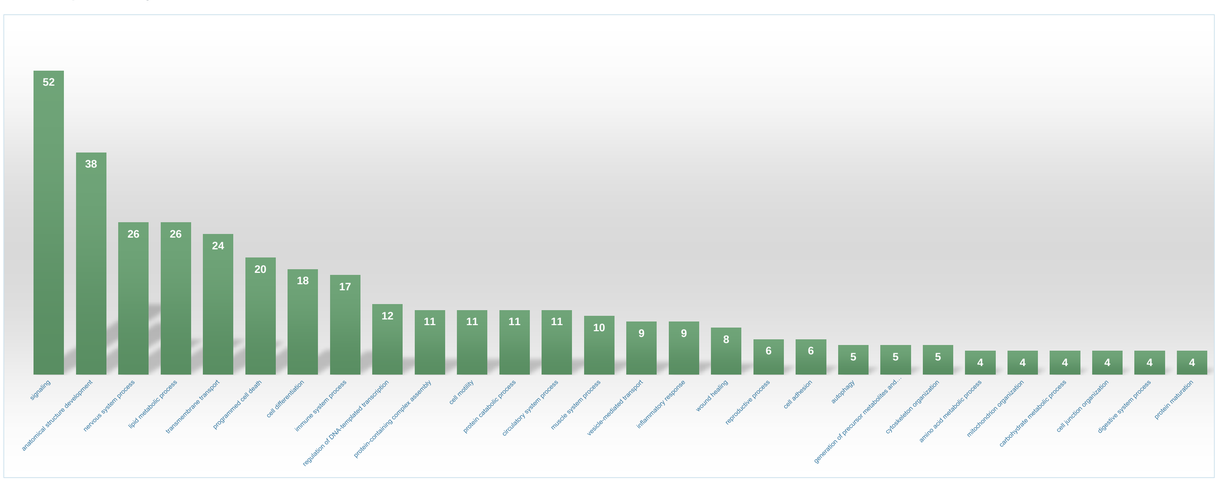
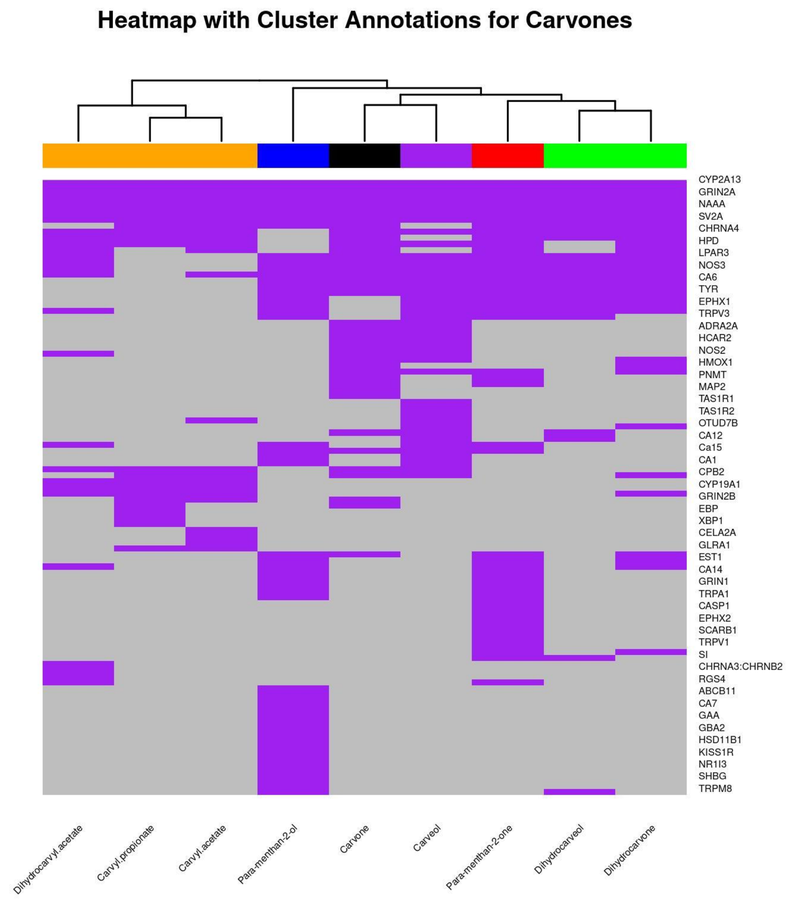


Figure 3 : Biological process obtained from all the predicted targets binding with carvones.

CLUSTERING ANALYSIS



- Similarity among carvones was determined using all the predicted targets binding carvones, active binding in purple and negative binding in grey.
- Dendrogram showing 2 large clusters of carvones which were categorized as 3 substances containing esters and structurally similar carvones.
- The racemic mixture and two enantiomers of carvone were predicted as a single molecule in Chemotarget CLARITY.
- Dihydrocarveol is predicted as a metabolite of dihydrocarɐvone, which is probably why they are clustered together.

Figure 4: Heat map with cluster annotation for carvones

t-SNE AND PCA

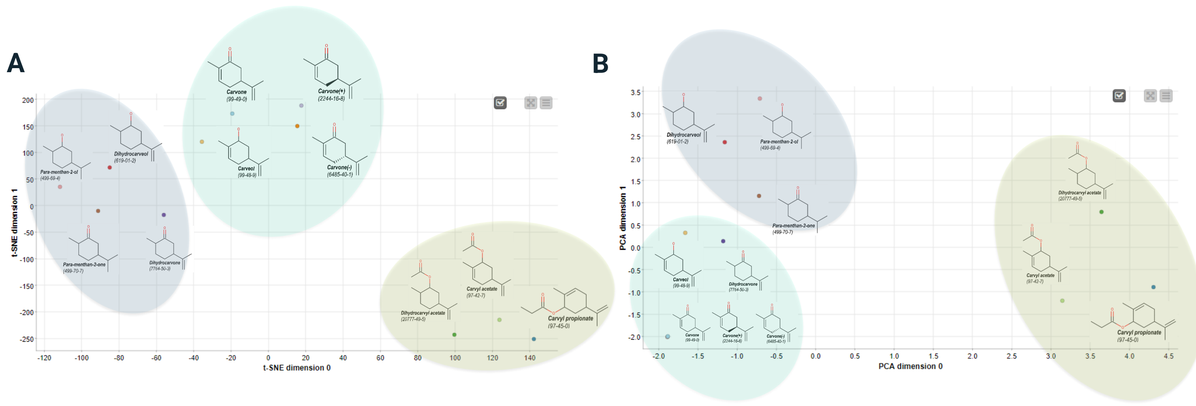


Figure 5: Chemical structural similarity analysis using (A) t-SNE and (B) PCA

OECD QSAR TOOLBOX

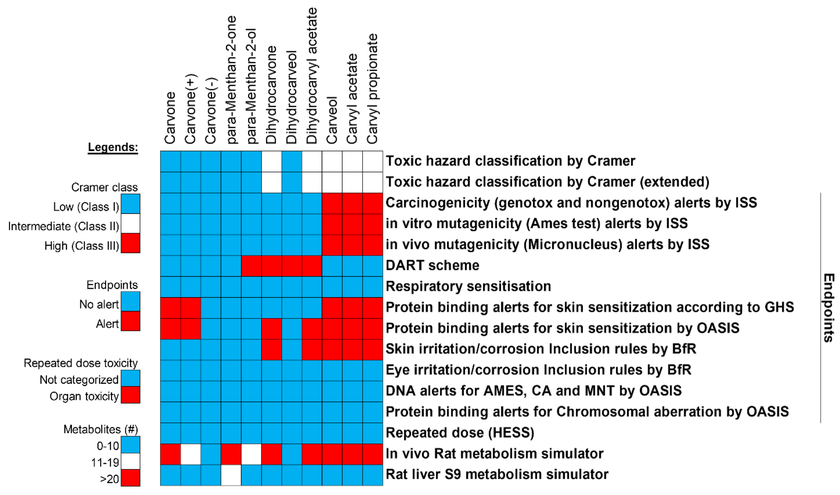


Figure 6: *In silico* human health alerts and metabolism predictions (OECD QSAR Toolbox).

- The *in silico* predictions and structural alerts from OECD Quantitative Structure-Activity Relationship (QSAR) Toolbox are summarized.
- The *in silico* predictions for the number of metabolites based on simulated *in vivo* rat metabolism were examined.
- These predictions included >20 metabolites for seven carvones, 11 to 19 metabolites for two carvones, and 0 to 10 metabolites for two carvones.
- Regarding rat liver S9 metabolism simulations, 11 to 19 metabolites were predicted for one carvone, whereas the remaining carvones were predicted to have 0 to 10 metabolites.

CONCLUSION

We utilized Chemotargets CLARITY to perform in silico target predictions for 11 Carvone and structurally related substances. The results demonstrated similarity among carvones based on predicted biological targets, which aligned with the findings from chemical structural similarity analyses. Overall, these predictions can support the selection of more suitable targets, particularly for the assessment of substances with limited available toxicological data.

REFERENCES

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