Supporting Information

Bitter odorants and odorous bitters: toxicity and human TAS2R targets

Eitan Margulis[1], Tatjana Lang[2], Anne Tromelin[3], Evgenii Ziaikin[1], Maik Behrens[2], Masha Y. Niv[1*]

1. The Institute of Biochemistry, Food Science and Nutrition, The Robert H Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, 76100, Rehovot, Israel.

2. Leibniz Institute for Food Systems Biology at the Technical University of Munich, 85354, Freising,

Germany.

3. Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne Franche-Comté, F-21000 Dijon, France.

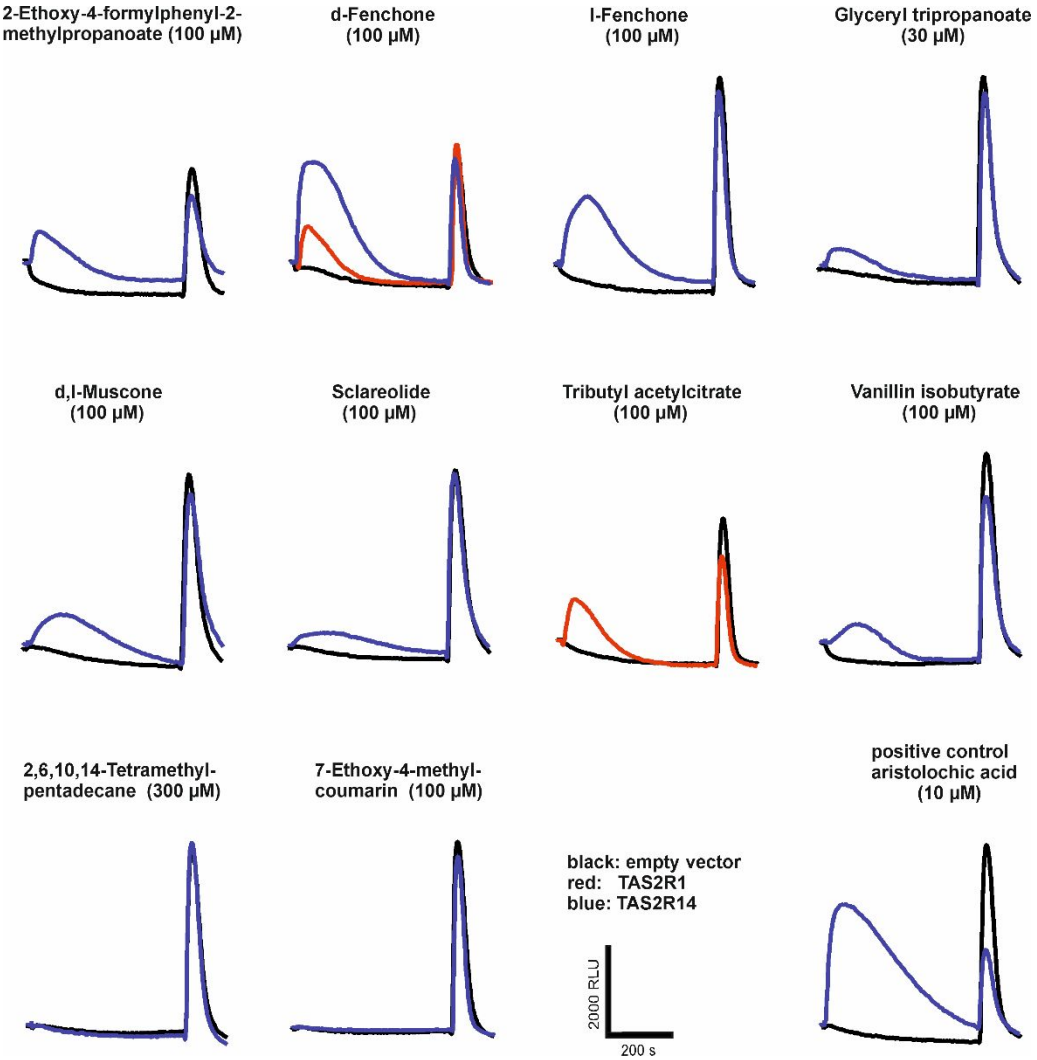

Figure S1. ***Screening of TAS2Rs with odorants.*** *Representative raw fluorescence traces of TAS2R14 (blue), TAS2R1 (red) or mock transfected cellular responses are shown. TAS2R14 transfected cells stimulated with 10 µM aristolochic acid served as positive control (bottom right trace). The second peak in each trace shows the cellular responses of endogenous somatostatin receptors stimulated with 100 nM somatostatin-14 serving as viability control. Scale bar: y-axis, relative light units (RLU); x-axis, time (seconds).*

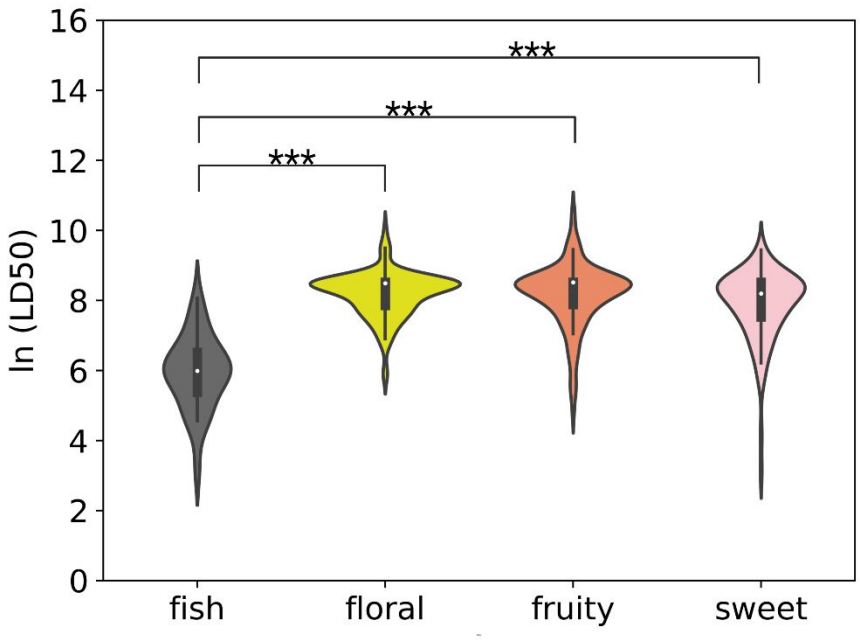

Figure S2. ***Comparison between ln(LD_{50}) values of fishy smelling odorants (grey) and floral (yellow), fruity (orange), and sweet (pink) odorants****. A significant difference was observed using Dunnett's test, P < 0.05. *** P < 0.0001.*

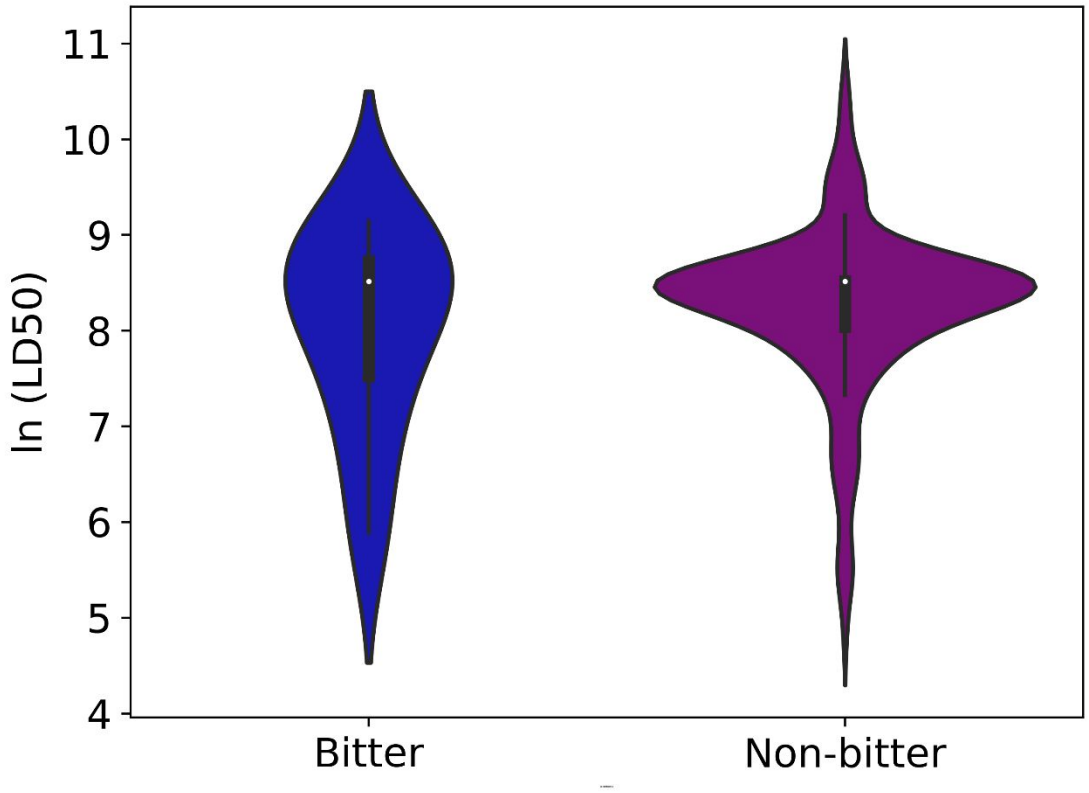

Figure S3. ***Comparison between ln(LD50) values of pleasant-smelling odorants with a bitter predicted taste (blue), non-bitter predicted taste (purple).*** *No significant difference was observed using a two-tailed t-test, $P < 0.05$.*

Table S1. Test compounds, their purity and suppliers.

No.	compound	CAS	purity & suppliers
1	2-Ethoxy-4-formyl phenyl 2-methylpropanoate	188417-26-7	98%, BLD Pharmatech Ltd.
2	7-Ethoxy-4-methylcoumarin	87-05-8	98%, AP OLLD Scientific
3	d-Fenchone	4695-62-9	≥98%, Sigma-Aldrich
4	l-Fenchone	7787-20-4	≥98%, Sigma-Aldrich
5	Glyceryl tripropanoate	139-45-7	97%, Thermo Scientific
6	d,l-Muscone	541-91-3	≥95%, MP Biomedicals
7	Sclareolide	564-20-5	97%, Sigma-Aldrich
8	2,6,10,14- Tetramethylpentadecane	1921-70-6	95%, Ehrenstorfer GmbH
9	Tributyl acetylcitrate	77-90-7	98%, Sigma-Aldrich
10	Vanillin isobutyrate	20665-85-4	≥98%, Sigma-Aldrich

Table S2. Bitter Predicted odorants (high confidence)

Acetin	CC(=O)OCC(COC(=O)C)OC(=O)C
2-Acetyl-3,5-dimethylpyrazine	CC(=O)c1ncc(C)nc1C
2-Acetyl-1,4,5,6-tetrahydropyridine	CC(=O)C1=CCCCN1
2-Acetyl-3,4,5,6-tetrahydropyridine	CC(=O)C1=NCCCC1
Acetylthiazolidine	CC(=O)C1NCCS1
Ammonia	N
Ammonium hydrogen sulphide	[NH4+].S
Ammonium hydroxide	[NH4+].[OH-]
Ammonium sulfide	[NH4+].[S-2]
Benzyl isothiocyanate	S=C=NCc1ccccc1
Bismethyltetrahydrofurylspirotrithiolane	CC1OCCC12SSC3(CCOC3C)S2
sec-Butylamine	CC[CH](C)N
Butylmethylpyrrolidinodithiazine	CCCCC1SC(C)N2CC=CC2S1
Calone	Cc1ccc2OCC(=O)COc2c1
Carvone acetate	CC(=O)OCC(=C)C1CC=C(C)C(=O)C1
Cyclohexyl anthranilate	Nc1ccccc1C(=O)OC2CCCCC2
Cyclohexyl formate	O=COC1CCCCC1
2,5-Dimethyl-4-(1-pyrrolidinyl)-3(2H)furanone	CC1OC(=C(N2CCCC2)C1=O)C

Dehydromenthofurolactone	C[CH]1CCC2=C(C)C(=O)OC2=C1
(S)-8,9-dehydrotheaspirone	CC1=CC[C]2(O1)C(=CC(=O)CC2(C)C)C
Diethylamine	CCNCC
Dihydroactinidiolide	CC1(C)CCC[C]2(C)OC(=O)C=C12
Dihydrotriethyldithiazine	CCC1NC(CC)SC(CC)S1
Dihydrotrismethylpropyldithiazine	CC(C)CC1NC(CC(C)C)SC(CC(C)C)S1
Dimethyl menthyl succinamide	CC(C)[C@@H]1CC[C@@H](C)C[C@H]1OC(=O)CCC(=O)N(C)C
Dimethylamine	CNC
Dimethylphenethylamine	C[CH](N(C)C)c1ccccc1
Dimethylpyrrolidinodithiazine	C[CH]1S[CH](C)N2CCC[CH]2S1
Ethoxymethylcoumarin	CCOc1ccc2C(=CC(=O)Oc2c1)C
Ethyl acetate	CCOC(=O)C
Ethyl formate	CCOC=O
Ethyl maltol isobutyrate	O=C(OC=1C(=O)C=COC=1CC)C(C)(C)[H]
2-Ethoxy-4-formyl phenyl 2-methylpropanoate	CCOc1cc(C=O)ccc1OC(=O)C(C)C
Ethylamine	CCN
d-Fenchone	CC12CC[C@H](C1)C(C)(C)C2=O
l-Fenchone	C[C@@]12CC[C@@H](C1)C(C)(C)C2=O
Geranyl anthranilate	CC(=CCC\C(=C\COC(=O)c1ccccc1N)\C)C
Glyceryl tripropanoate	CCC(=O)OCC(COC(=O)CC)OC(=O)CC
Heliotropin propylene glycol acetal	CC1COC(O1)c2ccc3OCOc3c2
Hexalactam	O=C1CCCCCN1
Hexylamine	CCCCCCN
Hydroxypyridine	Oc1ccccn1
Indole	c1ccc2[nH]ccc2c1
Isobutyl anthranilate	CC(C)COC(=O)c1ccccc1N
Isobutylamine	CC(C)CN
Isobutyldimethyldihydrodithiazine	CC(C)CC1S[CH](C)N[CH](C)S1
Isodecyl acetate	CC(C)CCCCCCCOC(=O)C
Isopentylamine	CC(C)CCN
Isopropanolamine	C[CH](O)CN
Isopropylamine	CC(C)N
Isoquinoline	c1ccc2cnccc2c1
Methyl acetate	COC(=O)C
Methyl formyloxydodecanoate	CCCCCCCC(CCCC(=O)OC)OC=O
Methylamine	CN
Methylbutylamine	CC[CH](C)CN
1-Methylpiperidine	CN1CCCCC1

2-Methylpiperidine	C[CH]1CCCCN1
Methylpyrrolidine	CN1CCCC1
Methylthiazolidine	C[CH]1NCCS1
Methylthiopropylamine	CSCCCN
d,l-Muscone	CC1CCCCCCCCCCCCC(=O)C1
Oxotetradecanoic acid glyceride	CCCCCCCCCCCC(=O)CC(=O)OC[CH](O)CO
Pentylamine	CCCCCN
1-Amino-2-phenylethane	NCCc1ccccc1
Piperazine	C1CNCCN1
Piperidine	C1CCNCC1
Propionylpyrroline	CCC(=O)C1=CCCN1
Propiosyringone	CCC(=O)c1cc(OC)c(O)c(OC)c1
Propyl acetate	CCCOC(=O)C
Propylamine	CCCN
Propylthiazolidine	CCC[CH]1NCCS1
Pyrazine	c1cnccn1
Pyridine	c1ccncc1
Pyrrole	c1cc[nH]c1
Pyrrolidine	C1CCNC1
Pyrroline	C1CC=NC1
Sclareolide	CC1(C)CCC[C@@]2(C)[C@H]1CC[C@@]3(C)OC(=O)C[C@H]23
Terpinyl anthranilate	CC1=CC[CH](CC1)C(C)(C)OC(=O)c2ccccc2N
Tetrahydrotrimethylthiadiazine	CC1NC(C)SC(C)N1
Tributyl acetylcitrate	CCCCOC(=O)CC(CC(=O)OCCCC)(OC(=O)C)C(=O)OCCCC
Trimethyldihydrodithiazine	C[CH]1N[CH](C)SC(C)S1
Tyramine	NCCc1ccc(O)cc1
Vanillin isobutyrate	COc1cc(C=O)ccc1OC(=O)C(C)C
Vanillin menthoxypropanediol acetal	COc1cc(ccc1O)[C@@H]2OC[C@@H](CO[C@H]3C[C@H](CC[C@@H]3C)C(C)C)O2
Vomifoliol	C[C@@H](O)\C=C\[C@@]1(O)C(=CC(=O)CC1(C)C)C
WS-5	CCOC(=O)CNC(=O)[C@@H]1C[C@H](C)CC[C@H]1C(C)C

Table S3. Very Bitter Predicted odorants

Name	SMILES
Carvone acetate	CC(=O)OCC(=C)C1CC=C(C)C(=O)C1
Citral glyceryl acetal(s)	CC(=CCCC(=CC1OCC(O)CO1)C)C
Ethyl ricinoleate	CCCCCCC(O)C\C=C/CCCCCCCC(=O)OCC
Glyceryl monooleate	CCCCCCCC\C=C/CCCCCCCC(=O)OC[C@H](O)CO
Lactic acid	C[CH](O)C(=O)O
Methyl jasmonate	CC\C=C/C[CH]1[CH](CC(=O)OC)CCC1=O

Oxohexadecanoic acid glyceride	CCCCCCCCCCCCCC(=O)CC(=O)OC[CH](O)CO
Oxotetradecanoic acid glyceride	CCCCCCCCCCCC(=O)CC(=O)OC[CH](O)CO
Sorbitan monostearate	CCCCCCCCCCCCCCCCCC(=O)OCC(O)[C@H]1OC[C@@H](O)[C@@H]1O
Vanillin menthoxypropanediol acetal	COc1cc(ccc1O)[C@@H]2OC[C@@H](CO[C@H]3C[C@H](CC[C@@H]3C)C(C)C)O2

Table S4. Odorous-predicted bitterants list (from BitterDB):

BitterDB ID	canonical_smiles	name
15	C1=CC=C(C=C1)C=O	benzaldehyde
37	C1=CC=C(C=C1)C(C(=O)C2=CC=CC=C2)O	Benzoin
49	C1=CC=C2C(=C1)C=CC(=O)O2	Coumarin
51	CCCCCCCC=CC(C#CC#CC(C=C)O)O	Falcarindiol
57	C1=CC=C(C=C1)C(=O)N	Benzamide
61	CN(C)CCOC(C1=CC=CC=C1)C2=CC=CC=C2	Diphenhydramine
64	C=CS(=O)C=C	Divinyl sulfoxide
77	CN1CCC23CCCCC2C1CC4=C3C=C(C=C4)OC	dextromethorphan
90	CC1C2CC2(CC1=O)C(C)C	(-)-alpha Thujone
108	C=CCN=C=S	allyl isothiocyanate
109	C1CCC(=O)NCC1	Caprolactam
110	CN(C)C=S	dimethylthioformamide
111	CCC1=NC=CN=C1	Ethylpyrazine
118	C1=CC=C(C=C1)CCN=C=S	phenethyl isothiocyanate
130	CC1=CC=CC=C1C(C2=CC=CC=C2)OCCN(C)C	orphenadrine
134	CC(C(C1=CC=CC=C1)O)N(C)CC=CC2=CC=CC=C2	Cinnamedrine
161	CC(CNC)C1CCCCC1.Cl	Cyclexedrine Hydrochloride
165	CC(CC1CCCC1)NC.Cl	Cyclopentamine Hydrochloride
188	CS(=O)C	Dimethyl Sulfoxide
192	C1=CN=CC=C1C2=CC=NC=C2	4,4'-Bipyridine
214	CC1=CC=C(C=C1)F	Fluorotoluene
217	C1=COC(=C1)CO	Furfuryl Alcohol
228	C(CC#N)CC#N	Glutaronitrile
232	CC1=NC=CC2=C1NC3=CC=CC=C23	Harman
248	OO	Hydrogen Peroxide
275	CC(C)O	Isopropyl Alcohol
290	CC1CC23CCCN4C2(C1)C(CCC4)C(=O)CC3	Lycopodine
299	CC1CCC(C(=O)C1)C(C)C	Menthone
313	CC(CC1=CC=CC=C1)NC.Cl	Methamphetamine Hydrochloride
369	C(CCO)CCO	1,5-Pentanediol

440	C1=CNN=C1	Pyrazole
443	CCC12CCCN(C1)CCC3=C(CC2)NC4=CC=CC=C34	Quebrachamine
449	C1=CC=C2C(=C1)C=NC=N2	Quinazoline
511	CC[N+](CC)(CC)CC.[OH-]	Tetraethylammonium Hydroxide
546	CCCC(CCC)C(=O)N	Valpromide
588	CC1=CC(=O)CC(C1)C(C)C	Homocamfin
650	CN1CCCC1C2=CN=CC=C2	nicotine
667	C1CCNCC1	piperidine
673	CCC=CCC=CCC=CCCCCCCCC(=O)O	alpha-Linolenic Acid
684	CCOC(=O)C1=CC=CC=C1	Ethyl Benzoate
685	CC1=CC=CC=N1	Picoline
686	CC(C)CNCC(C)C	Diisobutylamine
687	C1CCCC(=O)CCC1	Cyclooctanone
693	C1CCN2CCCC(C2C1)CO	Lupinine
694	CN(C)CCC=C1C2=CC=CC=C2COC3=CC=CC=C31	Doxepin
695	CC(=S)NC1=CC=CC=C1	Thioacetanilide
701	CC1CCCC(N1)C	2,6-Dimethylpiperidine
702	CN1C(=O)C=CC2=CC=CC=C21	1-methy-2-quinolinone
711	COC1=C(C=C(C=C1)CC=C)OC	Eugenyl methyl ether
713	CC(=O)C1=CC=CC=C1	Acetophenone
714	CC(=O)C1=CC=C(C=C1)OC	Acetanisole
715	CC(C)(CC1=CC=CC=C1)O	A,a-dimethylphenethyl alcohol
716	CCOC(=O)CC1=CC=CC=C1	Ethyl phenylacetate
717	CCC(=O)OCCC(C)C	Isoamyl propionate
718	CC(C)CCCC(C)CCO	3,7-dimethyl-1-octanol
719	CC(CCO)O	1,3-butylene glycol
720	CCOC=O	Ethyl formate
721	CC(C)COC(=O)C	Isobutyl acetate
723	CC(=O)OCC1=CC=CC=C1	Benzyl acetate
724	CCC(=O)OCCC(C)CCC=C(C)C	Citronellyl propionate
725	CC(CCC=C(C)C)CCO	D-citronellol
726	CCCCCCOC(=O)C	Hexyl acetate
727	COC1=CC=C(C=C1)OC	P-dimethoxybenzene
728	CCC(=O)OCC(C)C	Isobutyl propionate
729	CCCCCC(C)O	2-heptanol
730	CCCCC(CC)O	3-heptanol
731	CCC(=O)OC(C)C	Isopropyl propionate
732	CC1(C2CCC(C2)(C1=O)C)C	D-fenchone
733	CC1(C2CCC(C2)(C1O)C)C	Fenchyl alcohol
736	CC(C)CCOC(=O)C	Isoamyl acetate

738	CC1(C2CCC1(C(=O)C2)C)C	D-camphor
739	CC=CC1=CC(=C(C=C1)OC)OC	Isoeugenyl methyl ether
740	CC(=CCCC(=CC=O)C)C	Citral (neral)
741	CC(=CCCC(=CCOC(=O)C)C)C	Geranyl acetate
742	CC(=CCCC(=CCOC=O)C)C	Geranyl formate
743	C1=CC=C(C=C1)C=CCO	Cinnamyl alcohol
744	C1=CC=C(C=C1)C=CCOC=O	Cinnamyl formate
745	CCC(=O)OCC=C(C)CCC=C(C)C	Geranyl propionate
746	C1=CC=C(C=C1)CC=O	Phenylacetaldehyde
750	C1=CC=C(C=C1)CCO	Phenethyl alcohol
753	CC(=O)OC	Methyl acetate
754	CC1=CC2=C(C=C1)OC(=O)C=C2	6-methylcoumarin
755	CC1=CC(=C(C=C1)O)OC	2-methoxy-4-methylphenol
756	CC1=CCC(=CC1)C(C)C	P-mentha-1,4-diene
757	CC1=CC=C(CC1)C(C)C	1-isopropyl-4-methyl
758	CC(C)C(=O)OCCC1=CC=CC=C1	Phenethyl isobutyrate
759	CC(C)C(=O)OCCCC1=CC=CC=C1	3-phenylpropyl isobutyrate
760	C1=CC=C(C=C1)CCOC=O	Phenethyl formate
761	CCCOC(=O)CC	Propyl propionate
762	CCCOC(=O)C	Propyl acetate
763	CCCOC=O	Propyl formate
764	CCCCCCC(=O)C	2-octanone
767	COC1=CC=CC=C1C=O	O-methoxybenzaldehyde
768	CC(C)CC(=O)OCCC1=CC=CC=C1	Phenethyl isovalerate
769	CC(CCCC(=C)C)CCOC(=O)C	Rhodinyl acetate
770	CCCCCCCCCO	Nonyl alcohol
771	CCCCCCCCCOC(=O)C	Nonyl acetate
773	CC(=CCCC(=O)C)C	6-methyl-5-hepten-2-one
775	CCCOC(=O)CC(C)C	Propyl isovalerate
776	CCC(C=C)O	1-penten-3-ol
777	CC1=CCC(CC1)C(C)(C)OC=O	Terpinyl formate
778	CCC(C)C(=O)OC1CC(CCC1C(C)C)C	Menthyl isovalerate
779	CC(=O)OCCCC1=CC=CC=C1	3-phenylpropyl acetate
780	COC1=CC=C(C=C1)C=O	P-methoxybenzaldehyde
781	COC(CC1=CC=CC=C1)OC	Phenylacetaldehyde dimethyl acetal
782	CC(=CCCC(C)(C=C)OC=O)C	Linalyl formate
783	CC(CCCC(=C)C)CCOC=O	Rhodinyl formate
786	CCC(=O)OC(C)(C)C1CCC(=CC1)C	Terpinyl propionate
787	CC(C1=CC=CC=C1)OC(=O)C	A-methylbenzyl acetate
790	CC1=CCC(CC1)C(C)(C)OC(=O)CC(C)C	Terpinyl isovalerate
791	CC(=CCCC(=CCO)C)C	Nerol

793	CCCCCCCCCCC=CC=O	2-tridecenal
794	CC(C)CC(=O)OCC=C(C)CCC=C(C)C	Neryl isovalerate
796	CC(C)C(C=O)C1=CC=CC=C1	3-methyl-2-phenylbutyraldehyde
797	CC(C)CC(=O)OCCC(C)CCCC(=C)C	Rhodinyl isovalerate
805	C1CC2=CC=CC=C2C(=O)C1	Alpha-tetralone
839	CC(C)C1=CC=C(C=C1)C=NO	4-Isopropylbenzaldoxime
868	C1=CC=C(C=C1)C2=CC(=O)C3=CC=CC=C3O2	flavone
893	C1C(OC2=CC=CC=C2C1=O)C3=CC=CC=C3	flavanone
902	C1=CC=C(C=C1)C=CC(=O)C2=CC=CC=C2	chalcone
905	C1=CC=C(C=C1)C=CC(=O)C2=CC=C(C=C2)O	4-hydroxychalcone
919	C1=CC=C(C=C1)C2=COC3=CC=CC=C3C2=O	isoflavone
925	C1=CC=C2C(=C1)C(=O)C3=CC=CC=C3O2	xanthonee
926	COC1=CC=C(C=C1)O	4-hydroxyanisol
929	C1=CC=C2C(=C1)C=CC=C2C(=O)O	1-Naphthoic acid
946	CC1=CCCC(=CC2C(CC1)C(=C)C(=O)O2)C	costunolide
955	C1CCN2CC3CC(C2C1)CN4C3CCCC4	sparteine
961	CC12CCC(CC1CCC3C2CCC4(C3CCC4=O)C)O	Androsterone
964	CC(C)CCCCCCCC=CC(=O)O	cis-11-Methyl-2-dodecenoic acid
972	C1=CC=C(C(=C1)O)O	pyrocatechin
974	C1=CC=C(C=C1)CCN=C=O	phenylethyl isothiocyanate
1006	CCCCCCCC=CCC#CC#CC(C=C)O	falcarinol
1112	CC1CC2=C(C1=O)NCCC=C2	7-methyl-2,3,6,7-tetrahydrocyclopenta[b]azepin-8(1H)-one
1176	CC1CCC(C(C1)O)C(C)C	L-menthol
1223	C1CC2C=CC1C=C2C=NO	(E)-1-(Bicyclo[2.2.2]octa-2,5-dien-2-yl)-N-hydroxymethanimine
1228	C1=CC=C2C(=C1)C(=CN2)CCO	2-(1H-indol-3-yl)ethanol
1298	C1=CN=CN1	imidazole
1299	C1=CC=NN=C1	Pyridazine