Electronic Supplementary Material (ESI) for Natural Product Reports.

SUPPORTING INFORMATION

Site-directed mutagenesis and substrate compatibility to reveal the structure-function relationships of plant oxidosqualene cyclases

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Table S1 Biochemically characterized plant OSCs.

No.	OSC	GenBank ID	Species	Product	Reference
1	LsOSC5	MG708186	*Lagerstroemia speciosa*	cycloartenol	1
2	McCAS	AB781677	*Momordica charantia*	cycloartenol	2
3	PPX	LC389070	*Polystichum polyblepharum*	cycloartenol	3
4	MiCAS	KX147271	*Maytenus ilicifolia*	cycloartenol	4, 5
5	PtCAS1	EU275203	*Polygala tenuifolia*	cycloartenol	6
6	PtCAS2	EU275205	*Polygala tenuifolia*	cycloartenol	6
7	NtCAS1	KM452913	*Nicotiana tabacum*	cycloartenol	7
8	WsOSC/CS	HM037907	*Withania somnifera*	cycloartenol	8
9	WsCAS	KX574828	*Withania somnifera*	cycloartenol	9
10	AmCAS1	AF216755	*Abies magnifica*	cycloartenol	10
11	AcACX	AB368375	*Adiantum capillus-veneris*	cycloartenol	10
12	AsCS1	AJ311790	*Avena strigosa*	cycloartenol	10
13	BpBPX1	AB055509	*Betula platyphylla*	cycloartenol	10
14	BpBPX2	AB055510	*Betula platyphylla*	cycloartenol	10
15	CsOSC1	AB058507	*Costus speciosus*	cycloartenol	10
16	CpCPX	AB116237	*Cucurbita pepo*	cycloartenol	10
17	DzCAS1	AM697885	*Dioscorea zingiberensis*	cycloartenol	10
18	GgCAS1	AB025968	*Glycyrrhiza glabra*	cycloartenol	10
19	KdCAS	HM623872	*Kalanchoe daigremontiana*	cycloartenol	10
20	KcCAS	AB292609	*Kandelia candel*	cycloartenol	10
21	LjOSC5	AB181246	*Lotus japonicus*	cycloartenol	10
22	LcCAS1	AB033334	*Luffa cylindrica*	cycloartenol	10
23	OsOSC2	AK121211	*Oryza sativa*	cycloartenol	10
24	PgPNX	AB009029	*Panax ginseng*	cycloartenol	10
25	PsPSX	D89619	*Pisum sativum*	cycloartenol	10
26	PnCAS	AB530328	*Polypodiodes niponica*	cycloartenol	10
27	RsCAS	AB292608	*Rhizophora stylosa*	cycloartenol	10
28	RcCAS	DQ268870	*Ricinus communis*	cycloartenol	10
29	ElCAS1	MH215230	*Euphorbia lathyris*	cycloartenol	11
30	TaOSC3	MK547513	*Terminalia arjuna*	cycloartenol	12
31	PpCAS	MN368727	*Paris polyphylla*	cycloartenol	13
32	TwOSC4	MH310939	*Tripterygium wilfordii*	cycloartenol	14
33	TwOSC6	MH310938	*Tripterygium wilfordii*	cycloartenol	14
34	TwOSC1(2)	MN621246	*Tripterygium wilfordii*	β-amyrin	15
35	TcOSC3	MK351898	*Taraxacum coreanum*	β-amyrin	16
36	LsOSC3	MG708184	*Lagerstroemia speciosa*	β-amyrin	1
37	LsOSC4	MG708185	*Lagerstroemia speciosa*	β-amyrin	1
38	TwOSC2	KY885468	*Tripterygium wilfordii*	β-amyrin	17
39	CsOSC2(2)	XM_025102137	*Citrus sinensis*	β-amyrin	18
40	McBAS	AB781676	*Momordica charantia*	β-amyrin	2
41	KsBAS	KT150523	*Kalopanax septemlobus*	β-amyrin	19
42	PlgOSC1	KY412556	*Platycodon grandiflorum*	β-amyrin	20
43	CbβAS	KX907781	*Conyza blinii*	β-amyrin	21
44	EsBAS	KX378998	*Eleutherococcus senticosus*	β-amyrin	22
45	GsAS2	KJ467352	*Gentiana straminea*	β-amyrin	23
46	GsAS1	FJ790411	*Gentiana straminea*	β-amyrin	23
47	GmBAS3	KF597523	*Glycine max*	β-amyrin	24
48	CqbAS1	KX343074	*Chenopodium quinoa*	β-amyrin	25
49	MlbAS	KF425519	*Maesa lanceolata*	β-amyrin	26
50	ObAS1	KF636411	*Sweet Basil*	β-amyrin	27
51	WsOSC/BS	JQ728553	*Withania somnifera*	β-amyrin	8
52	PtBS	EF107623	*Polygala tenuifolia*	β-amyrin	28
53	AtLUP4	NM_106544	*Arabidopsis thaliana*	β-amyrin	10, 29
54	AabAS	EU330197	*Artemisia annua*	β-amyrin	10

55	AsOXA1	AY836006	*Aster sedifolius*	β-amyrin	10
56	BpBPY	AB055512	*Betula platyphylla*	β-amyrin	10
57	BgbAS	AB289585	*Bruguiera gymnorrhiza*	β-amyrin	10
58	EtAS	AB206469	*Euphorbia tirucalli*	β-amyrin	10
59	GgbAS1	AB037203	*Glycyrrhiza glabra*	β-amyrin	10
60	LjOSC1	AB181244	*Lotus japonicus*	β-amyrin	10
61	MtBAS1	AJ430607	*Medicago truncatula*	β-amyrin	10
62	NsbAS1	FJ013228	*Nigella sativa*	β-amyrin	10
63	PgPNY1	AB009030	*Panax ginseng*	β-amyrin	10
64	PgPNY2	AB014057	*Panax ginseng*	β-amyrin	10
65	PsPSY	AB034802	*Pisum sativum*	β-amyrin	10
66	SlTTS1	HQ266579	*Solanum lycopersicum*	β-amyrin	10
67	VhBS	DQ915167	*Vaccaria hispanica*	β-amyrin	10
68	SAD1	AJ311789	*Avena species*	β-amyrin	30
69	TaOSC1	MK547511	*Terminalia arjuna*	β-amyrin	12
70	AeAS	HM219225	*Aralia elata*	β-amyrin	31
71	BcBAS	MN186093	*Bupleurum chinense*	β-amyrin	32
72	TwOSC8	MK541924	*Tripterygium wilfordii*	β-amyrin	14
73	LjOSC9	LC485316	*Lotus japonicus*	α-amyrin	33
74	OeOEA	AB291240	*Olea europaea*	α-amyrin	10, 34
75	BfOSC3	LC464980	*Bauhinia forficata*	α-amyrin	35
76	LsOSC1	MG708182	*Lagerstroemia speciosa*	lupeol	1
77	CsOSC3	XM_015526998	*Citrus sinensis*	lupeol	18
78	WsOSC/LS	JQ728552	*Withania somnifera*	lupeol	8
79	BpBPW	AB055511	*Betula platyphylla*	lupeol	10
80	BgLUS	AB289586	*Bruguiera gymnorrhiza*	lupeol	10
81	GgLUS1	AB116228	*Glycyrrhiza glabra*	lupeol	10
82	KdLUS	HM623871	*Kalanchoe daigremontiana*	lupeol	10
83	LjOSC3	AB181245	*Lotus japonicus*	lupeol	10
84	OeOEW	AB025343	*Olea europaea*	lupeol	10
85	RcLUS	DQ268869	*Ricinus communis*	lupeol	10
86	ToTRW	AB025345	*Taraxacum officinale*	lupeol	10
87	TaOSC4	MK547514	*Terminalia arjuna*	lupeol	12
88	QsOSC1	MN428315	*Quercus suber*	lupeol	36
89	ElLAS1	MH215229	*Euphorbia lathyris*	lanosterol	11
90	AtLSS1	NM_001339197	*Arabidopsis thaliana*	lanosterol	10, 37
91	LjLAS	AB244671	*Lotus japonicus*	lanosterol	10
92	PgPNZ1	AB009031	*Panax ginseng*	lanosterol	10
93	TwOSC4(2)	MN621249	*Tripterygium wilfordii*	friedelin	15
94	MiFRS2	MG677552	*Maytenus ilicifolia*	friedelin	4
95	MiFRS3	MG677553	*Maytenus ilicifolia*	friedelin	4
96	MiFRS4	MG677554	*Maytenus ilicifolia*	friedelin	4
97	MiFRS	KX147270	*Maytenus ilicifolia*	friedelin	4
98	KdFRS	HM623870	*Kalanchoe daigremontiana*	friedelin	10
99	McCBS	AB781675	*Momordica charantia*	cucurbitadienol	2
100	SgCbQ	HQ128567	*Siraitia grosvenorii*	cucurbitadienol	38
101	CcCDS2	KM821405	*Citrullus colocynthis*	cucurbitadienol	39
102	CpCPQ	AB116238	*Cucurbita pepo*	cucurbitadienol	10
103	CsBi	KM655855	*Cucumis sativus*	cucurbitadienol	40
104	AiOSC1	MK803262	*Azadirachta indica*	tirucalla-7,24-dien-3β-ol	18
105	MaOSC1	MK803261	*Melia azedarach*	tirucalla-7,24-dien-3β-ol	18
106	CsOSC1(2)	XM_006468053	*Citrus sinensis*	tirucalla-7,24-dien-3β-ol	18
107	TcOSC4	MK351899	*Taraxacum coreanum*	taraxerol	16
108	KdTAS	HM623868	*Kalanchoe daigremontiana*	taraxerol	10
109	McIMS	AB781678	*Momordica charantia*	isomultiflorenol	2
110	LcIMS1	AB058643	*Luffa cylindrica*	isomultiflorenol	10
111	OsPS	MG932724	*Oryza sativa*	parkeol	41

112	PgDS	GU183405	*Panax ginseng*	dammarenediol-II	42
113	PgPNA	AB265170	*Panax ginseng*	dammarenediol-II	10
114	ElBUT1	MH215231	*Euphorbia lathyris*	butyrospermol	11
115	OsOS	MG932696	*Oryza sativa*	orysatinol	41
116	AtTHAS1	NM_001085264	*Arabidopsis thaliana*	thalianol	10, 43
117	AtSHS1	AB609123	*Aster tataricus*	shionone	10
118	KdGLS	HM623869	*Kalanchoe daigremontiana*	glutinol	10
119	OsIAS	AK067451	*Oryza sativa*	isoarborinol	10
120	SrBOS	AB455264	*Stevia rebaudiana*	baccharis oxide	10
121	AaCAS	KM670093	*Artemisia annua*	cycloartenol; an unknown compound	44
122	BfOSC4	LC464981	*Bauhinia forficata*	cycloartenol; unidentified minor peaks	35
123	AtCAS1	NM_126681	*Arabidopsis thaliana*	cycloartenol; parkeol	10, 45, 46
124	TwOSC3(2)	MN621248	*Tripterygium wilfordii*	α-amyrin; β-amyrin	15
125	LsOSC2	MG708183	*Lagerstroemia speciosa*	α-amyrin; β-amyrin	1
126	EjAS	JX173279	*Eriobotrya japonica*	α-amyrin; β-amyrin	47
127	TrOSC	MH161182	*Tripterygium regelii*	α-amyrin; β-amyrin	48
128	IpAS1	MF062494	*Ilex pubescens*	α-amyrin; β-amyrin	49
129	MdOSC1	FJ032006	*Malus domestica*	α-amyrin; β-amyrin	50
130	MdOSC3	FJ032008	*Malus domestica*	α-amyrin; β-amyrin	50
131	IaAS1	KM111167	*Ilex Asprella*	α-amyrin; β-amyrin	51
132	ObAS2	JQ809437	*Sweet Basil*	α-amyrin; β-amyrin	27
133	CrAS	JN991165	*Catharanthus roseus*	α-amyrin; β-amyrin	52
134	TaOSC2	MK547512	*Terminalia arjuna*	α-amyrin; β-amyrin	12
135	CaDDS	AY520818	*Centella asiatica*	α-amyrin; β-amyrin; dammarenediol-II	53
136	AaOSC2	KF309252	*Artemisia annua*	α-amyrin; β-amyrin; δ-amyrin	44
137	SlTTS2	HQ266580	*Solanum lycopersicum*	α-amyrin; β-amyrin; δ-amyrin	10
138	PsPSM	AB034803	*Pisum sativum*	α-amyrin; β-amyrin; δ-amyrin; φ-taraxasterol; butyrospermol; lupeol; germanicol; taraxasterol	10
139	TkOSC6	MG646381	*Taraxacum koksaghyz*	β-amyrin; α-amyrin	54
140	IaAS2	KM111168	*Ilex Asprella*	β-amyrin; α-amyrin	51
141	QsOSC2	MN428316	*Quercus suber*	β-amyrin; α-amyrin	36
142	TcOSC1	MK351896	*Taraxacum coreanum*	β-amyrin; α-amyrin; φ-taraxasterol; taraxasterol; δ-amyrin; dammarenediol-II	16
143	TwOSC2(2)	MN621247	*Tripterygium wilfordii*	β-amyrin; lupeol	15
144	BfOSC1	LC464978	*Bauhinia forficata*	β-amyrin; α-amyrin; lupeol	35
145	AtLUP2	NM_001334861	*Arabidopsis thaliana*	β-amyrin; taraxasterol; tirucalla-7,24-dien-3β-ol; lupeol; bauerenol; butyrospermol; multiflorenol; φ-taraxasterol; α-amyrin	10, 45
146	TkLUP	MG646375	*Taraxacum koksaghyz*	lupeol; β-amyrin	55
147	MdOSC5	KT383436	*Malus domestica*	lupeol; β-amyrin	50
148	AaLUS	KM670094	*Artemisia annua*	lupeol; other unknown compounds	44
149	CsOSC2	AB058508	*Costus speciosus*	lupeol; germanicol; β-amyrin; other unknown compounds	10
150	KcMS	AB257507	*Kandelia candel*	lupeol; α-amyrin; β-amyrin	10
151	LjAMY2	AF478455	*Lotus japonicus*	lupeol; β-amyrin; other unknown compounds	10
152	QsOSC3	MN428317	*Quercus suber*	friedelin; β-amyrin	36
153	TwOSC1	KY885467	*Tripterygium wilfordii*	friedelin; β-amyrin; α-amyrin	17
154	TwOSC3	KY885469	*Tripterygium wilfordii*	friedelin; β-amyrin; α-amyrin	17
155	TkOSC1	MG646376	*Taraxacum koksaghyz*	taraxasterol; α-amyrin; β-amyrin; lup-19(21)-en-3β-ol)	54
156	RsM2	AB263204	*Rhizophora stylosa*	taraxerol; β-amyrin; lupeol	10
157	TcOSC2	MK351897	*Taraxacum coreanum*	bauerenol and an unknown triterpene	16
158	AtPEN6	NM_001334828	*Arabidopsis thaliana*	bauerenol; lupeol; α-amyrin; taraxasterol; φ-taraxasterol; multiflorenol; isoursenol; seco-α-amyrin; seco-β-amyrin	10; 45; 56
159	MdOSC4	KT383435	*Malus domestica*	germanicol; β-amyrin; lupeol	50
160	RsM1	AB263203	*Rhizophora stylosa*	germanicol; β-amyrin; lupeol	10
161	BfOSC2	LC464979	*Bauhinia forficata*	germanicol; β-amyrin; lupeol	35
162	AtLUP1	NM_001334865	*Arabidopsis thaliana*	lupane-3β,20-diol; β-amyrin; germanicol; taraxasterol; φ-taraxasterol; lupeol	10, 45

163	AtLUP5	NM_001334260	*Arabidopsis thaliana*	tirucalla-7,24-dien-3β-ol; isotirucallol; euferol; butyrospermol; tirucallol; 13βH-malabarica-14(27),17,21-trien-3β-ol;	57
164	AtPEN3	NM_001344127	*Arabidopsis thaliana*	tirucalla-7,24-dien-3β-ol; butyrospermol; tirucallol; isotirucallol; 13βH-malabarica-14(27),17,21-trien-3β-ol; dammara-20,24-dien-3β-ol	10
165	AtPEN1	NM_117622	*Arabidopsis thaliana*	arabidiol; 14-epithalianol	10, 45
166	AtMRN1	NM_123624	*Arabidopsis thaliana*	marneral; achilleol A; camelliol C; △8-polypodatetraenol	10, 45
167	OsOSC8	AK070534	*Oryza sativa*	achilleol B; other unknown compounds	10, 58
168	AtCAMS1	NM_148667	*Arabidopsis thaliana*	camelliol C; achilleol A; β-amyrin	10
169	Bra032185	XM_009142199	*Brassica rapa*	astertarone A and other 20 compounds (butyrospermol; euphol; dammarenediol-II$^{\#}$; boeticol; dammarenediol-I$^{\#}$; isoeuphol; dammara-20(21),24-dien-3β-ol$^{\#}$; camelliol C; isohelianol; 9αH-polypoda-7,13E,17E,21-tetraenol; isotirucallol; 20R-dammara-12,24-dien-3β-ol$^{\#}$; 9αH-polypoda-8(26),13E,17E,21-tetraenol; 14-epiarabidiol; 20S-dammara-12,24-dien-3β-ol$^{\#}$; dammara-20(22)Z,24-dien-3β-ol$^{\#}$; dammara-20(22)E,24-dien-3β-ol$^{\#}$; tirucalla-7,24-dien-3β-ol; β-amyrin; lupeol)	59
170	AtBARS1 (PEN2)	NM_117625	*Arabidopsis thaliana*	baruol and other 22 minor ones (columbiol; sasanqual; dammara-20,24-dien-3β-ol; podioda-7,17,21-trienol; δ-amyrin; 13βH-malabarica-14(27),17,21-trien-3β-ol; malabarica-14,17,21-trienol; isomultiflorenol; achilleol A; lemmaphylladienol; unknown; taraxerol; isotirucallol; camelliol C; taraxasterol; φ-taraxasterol; lupeol; β-amyrin; multiflorenol; α-amyrin; butyrospermol; tirucalla-7,24-dien-3β-ol	10, 45

$^{\#}$ Names of these compounds were different from the original reference (#59) because a difference carbon numbering system was used in #59.

Table S2 Summary of mutagenesis studies for plant OSCs.

Species	OSC	GenBank ID	Product	Mutant	Function of mutant	Reference
Bauhinia forficata	BfOSC1	LC464978	β-amyrin (major); α-amyrin and lupeol (trace)	M259T	lupeol (trace)	35
				W260L	lupeol (trace)	35
				M259T/W260L	lupeol (trace)	35
	BfOSC2	LC464979	germanicol (major); β-amyrin and lupeol (trace)	I257T	unchanged	35
				W258L	lupeol (major); germanicol (minor)	35
				A261C	germanicol (trace)	35
				I257T/A261C	unchanged	35
				I257T/W258L	lupeol (major); germanicol (minor)	35
				W258L/A261C	lupeol (major); germanicol (minor)	35
				I257T/W258L/A261C	germanicol and lupeol (at equal)	35
	BfOSC3	LC464980	α-amyrin	T257M	unchanged	35
				L258W	inactive	35
				T257M/L258W	inactive	35
Tripterygium wilfordii	TwOSC1	KY885467	friedelin (7.35 mg/L); β-amyrin (2.22 mg/L); α-amyrin (2.11 mg/L)	L486I	friedelin (6.2 mg/L); β-amyrin (7.57 mg/L); α-amyrin (1.86 mg/L)	17
				L486V	friedelin (1.44 mg/L); β-amyrin (6.36 mg/L)	17
				L486F/R/H/P	inactive	17
				T502I	friedelin (8.67 mg/L); β-amyrin (2.17 mg/L); α-amyrin (2.21 mg/L)	17
				T502K	friedelin (7.88 mg/L); β-amyrin (1.93 mg/L); α-amyrin (2.22 mg/L)	17
				T502E	friedelin (10.63 mg/L); β-amyrin (2.63 mg/L); α-amyrin (2.69 mg/L)	17
				T502P	friedelin (6.9 mg/L); β-amyrin (2.01 mg/L); α-amyrin (1.9 mg/L)	17
	TwOSC2	KY885468	β-amyrin (16.96 mg/L)	V485L	β-amyrin (1.71 mg/L)	17
	TwOSC3	KY885469	friedelin (2.2 mg/L); β-amyrin (1.22 mg/L); α-amyrin (0.58 mg/L)	L482I	friedelin (2.95 mg/L); β-amyrin (1.69 mg/L); α-amyrin (0.6 mg/L)	17
				L482V	β-amyrin (1.37 mg/L); α-amyrin (0.4 mg/L)	17
				L482S	β-amyrin (1.64 mg/L); α-amyrin (0.56 mg/L)	17

				L482F/P/R/A	inactive	17
Ilex Asprella	IaAS1	KM111167	α-amyrin (80%); β-amyrin	K372G	inactive	60
				W612F	inactive	60
	IaAS2	KM111168	β-amyrin (95%); α-amyrin	K372G	the ratios of these two products changed	60
				W611F	the ratios of these two products changed	60
Oryza sativa	OsPS (OsOSC2)	MG932724	parkeol	Y257A	nine tetracyclic skeletons including parkeol together with a tricyclic triterpene, 20R- and 20S-configured tetracycles as a 59:40 ratio	61
				Y257F/L/S/T/W/H	except H, other mutants all have 20R- and 20S-configured tetracycles	61
Oryza sativa	OsOS	MG932696	orysatinol (112.98 μg/g) and others	A732I	orysatinol (1.59 μg/g); parkeol (3.08 μg/g) and others	41
				L365F	orysatinol (2.72 μg/g) and others	41
				L124F	orysatinol (13.4 μg/g); parkeol (4.15 μg/g) and others	41
				L124F/L365F	orysatinol (1.05 μg/g); parkeol (6.33 μg/g) and others	41
				L124F/A732I	parkeol (5.07 μg/g) and others	41
				L365F/A732I	parkeol (3.94 μg/g) and others	41
				L124F/L365F/A732I	parkeol (17.59 μg/g) and others	41
				Y257L/F/A	inactive	41
				L124F/L365F/A732I/Y257L	parkeol (trace)	41
				L124F/L365F/A732I/Y257F	parkeol and others	41
				L124F/L365F/A732I/Y257A	parkeol (trace)	41
	OsPS	MG932724	parkeol (20.98 μg/g) and others	F124L	parkeol (4.98 μg/g)	41
				F365L	parkeol (2.62 μg/g)	41
				F124L/F365L	inactive	41
				I732A	orysatinol (5.76 μg/g); parkeol (55.93 μg/g) and others	41
				F124L/I732A	orysatinol (8.83 μg/g); parkeol (2.75 μg/g) and others	41
				F365L/I732A	orysatinol (7.98 μg/g); parkeol (4.85 μg/g) and others	41
				F124L/F365L/I732A	orysatinol (46.32 μg/g) and others	41
				Y257L/A	parkeol (trace)	41
				Y257F	inactive	41

				F124L/F365L/I732A/Y257(L/F/A)	inactive	41
Siraitia grosvenorii	SgCbQ	HQ128567	cucurbitadienol	C565A/M	products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol (tirucallenol)	62
				C487A/M	products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol	62
				D486N/A	no cucurbitadienol; but products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol	62
				Y535W/A	products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol	62
				C487R	a new unidentified compound appears	62
				C565R	a new unidentified compound appears	62
				D486E	a new unidentified compound appears	62
				Y535L	a new unidentified compound appears	62
				Y535L/C565R	another new unidentified compound appears	62
				H260A/C/G/I/L/M/N/P/Q/V	products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol	62
				H260D/E/F/K/R/S/T/W/Y	no cucurbitadienol; but products with a C-C-C configuration appear, such as euphol, dihydroeuphol, and tirucallol	62
Panax ginseng	PgDS	GU183405	dammarenediol-II (114.74 mg/L)	C568A	dammarenediol-II (98.92 mg/L)	42
				C264A	dammarenediol-II (55.33 mg/L)	42
				D488A, C489A, W421A*, F477A, W616A, Y263A, Y732A, Y268A*, I559A*	dammarenediol-II (trace)	42
				W538A	inactive	42
Gentiana straminea	GsAS1	FJ790411	β-amyrin	H560Y	conversion rate increased	23
	GsAS2	KJ467352	β-amyrin	Y560H/F	conversion rate reduced	23
Maytenus ilicifolia	MiFRS	KX147270	friedelin	L482V	friedelin (major); β-amyrin (minor)	5
				L482T	β-amyrin	5
				L482I	friedelin	5
Pisum	PsPSX	D89619	cycloartenol (major);	Y118L	cucurbitadienol (major); cycloartenol (minor)	63

sativum			cucurbitadienol (minor)			
				G617A*	cycloartenol (major); cucurbitadienol (minor)	63
				I365L, P480L, T531S*	cycloartenol	63
Cucurbita pepo	CpCPQ	AB116238	cucurbitadienol	L125Y	parkeol (major); cucurbitadienol (minor)	63
				L373I	cucurbitadienol (minor)	63
				L488P, A625G*	cucurbitadienol (major)	63
				S123G*/L125Y/L129M*	parkeol (major)	63
				S123G*/L125Y/L129M*/L228M*/L373I/L488P/L527I*/S539T*/T574A*/M575I*/E576Q*/C617S*/A625G*	parkeol (minor)	63
				S539T*	inactive	63
Cucurbita pepo	CpCPQ	AB116238	cucurbitadienol	L488P, I489V, L488P/I489V	cucurbitadienol	64
Avena sativa	SAD1	AJ311789	β-amyrin (major); epoxydammarane (minor)	S728F	epoxydammarane (major); β-amyrin (minor)	30
Arabidopsis thaliana	AtLUP1	NM_001334865	lupanediol (major); lupeol; epoxydammara-3,25-diol; 17,24-expoybaccharane diol	T729F	epoxydammara-3,25-diol (major); 17,24-expoybaccharane diol (major); lupanediol; lupeol;	30
Cucumis sativus	CsBi	KM655855	cucurbitadienol	L146Y	inactive	40
				Y445T (Y439T)□	conversion rate reduced	40
				L146Y/Y445T (L146Y/Y439T)□	lanosterol	40
				C397Y* (C393Y)□	inactive	40
				N402E*, P400C*, N406C* (N398E, P396C, N402C)□	conversion rate reduced	40
Arabidopsis thaliana	AtCAS1	NM_126681	cycloartenol (99%), parkeol (1%),	H477N/I481V	lanosterol (99%), parkeol (1%)	65
				H477Q/I481V	lanosterol (94%), parkeol (6%)	65
				I481L	cycloartenol (83%), lanosterol (1%), parkeol (16%)	66
				I481A	cycloartenol (12%), lanosterol (54%), parkeol (15%), achilleol A (13%), camelliol C (6%)	66

				I481G	cycloartenol (17%), lanosterol (23%), parkeol (4%), achilleol A (44%), camelliol C (12%)	66
				I481V	cycloartenol (55%), lanosterol (24%), parkeol (21%)	66
				Y410T/I481V	lanosterol (75%), parkeol (<1%), 9β-Δ7-lanosterol (25%)	66
				Y410C	lanosterol (75%), 9β-Δ7-lanosterol (24%), achilleol A (1%)	66
				Y410T	lanosterol (65%), parkeol (2%), 9β-Δ7-lanosterol (33%)	66
				Y532H	lanosterol (45%), parkeol (31%), achilleol A (24%)	66
				H477N	lanosterol (88%), parkeol (12%)	66
				H477Q	lanosterol (22%), parkeol (73%), 9β-Δ7-lanosterol (5%)	66
				Y410T/H477N/I481V	lanosterol (78%), parkeol (<1%), 9β-Δ7-lanosterol (22%)	66
				Y410T/H477Q/I481V	lanosterol (78%), parkeol (<1%), 9β-Δ7-lanosterol (22%)	66
				F472S	less lanosterol	67
Lotus japonicus	LjLAS	AB244671	lanosterol (99%), parkeol (1%)	N478H/V482I	parkeol (83%), cycloartenol (13%), lanosterol (4%)	68
Panax ginseng	PgPNY1	AB009030	β-amyrin	W259L	β-amyrin (30.3%), lupeol (54.6%), butyrospermol (3.6%), germanicol (3.4%)	69
				M258I/W259L	β-amyrin (40.5%), lupeol (53.4%), butyrospermol (3.6%), germanicol (2.5%)	69
				C262S	β-amyrin (100%)	69
				Y261H	lupeol (2.4%), germanicol (13.6%), dammaradienols (84%, including dammara-20(22)E,24-dien-3β-ol[#], dammara-20(22)Z,24-dien-3β-ol[#], and dammara-20(21),24-dien-3β-ol[#])	69
Olea europaea	OeOEW	AB025343	lupeol	L256W	β-amyrin (74.8%), lupeol (6.9%), butyrospermol (9.9%), germanicol (8.4%)	69
				L256F	β-amyrin (9.8%), lupeol (69.7%), butyrospermol (17.9%)	69
				L256Y	β-amyrin (1.6%), lupeol (54.8%), butyrospermol (22.7%), dammaradienols (18.7%)	69
				L256H	β-amyrin (3.8%), lupeol (69.5%), butyrospermol (10%), dammaradienols (13.5%)	69
				L256A	β-amyrin (<1%), lupeol (68.2%), butyrospermol (20.2%), dammaradienols (7.5%)	69
				Y258H	lupeol (43.6%), butyrospermol (6.2%), dammaradienols (42.4%)	69
Arabidopsis thaliana	AtLUP1	NM_001334865	lupeol	L255W	β-amyrin (14%), lupeol (55%), butyrospermol (16%), 3β,20-dihydroxylupane (15%, also called lupane-3β,20-diol)	69
Euphorbia	EtAS	AB206469	β-amyrin (major),	W612F/Y	β-amyrin (major), germanicol (minor), tirucalla-7,24-dien-3β-ol (trace),	70

tirucalli			butyrospermol (trace), tirucalla-7,24-dien-3β-ol (trace)		lupeol (trace)	
				W612H	conversion rate reduced	70
				W612A/V/M/I	inactive	70
				L734G/A	taraxerol was a little more than β-amyrin	70
				L734V/I/M	56–78% of wild-type activity	70
				L734F	conversion rate reduced	70
				L734Y/W	inactive	70
				Y736F	67% of wild-type activity	70
				Y736A/L	β-amyrin (trace)	70
				Y736S/W/M	inactive	70
Euphorbia tirucalli	EtAS	AB206469	oleanane skeleton (97%), dammarane skeleton (3%),	F413A	oleanane skeleton (24%), dammarane skeleton (73.8%), bicycle (2.1%)	71
				F413V	oleanane skeleton (89.5%), dammarane skeleton (10.5%)	71
				F413M	oleanane skeleton (92.5%), dammarane skeleton (7.5%)	71
				F413S	oleanane skeleton (17.5%), dammarane skeleton (79.9%), bicycle (2.6%)	71
				F413T	oleanane skeleton (28%), dammarane skeleton (65.2%), bicycle (6.7%)	71
				F413Y	oleanane skeleton (40.4%), dammarane skeleton (57.8%), bicycle (1.7%)	71
				F413H	oleanane skeleton (37.3%), dammarane skeleton (60.8%), bicycle (1.9%)	71
				F413W	oleanane skeleton (84.8%), dammarane skeleton (15.2%)	71
				Y259A	oleanane skeleton (20.8%), tetracyclic skeleton (79.2%)	71
				Y259V	oleanane skeleton (4.8%), tetracyclic skeleton (95.2%)	71
				Y259I	oleanane skeleton (0.7%), tetracyclic skeleton (99.3%)	71
				Y259L	oleanane skeleton (6.7%), tetracyclic skeleton (93.3%)	71
				Y259H	oleanane skeleton (20.3%), tetracyclic skeleton (76%), lupeol (3.7%)	71
				Y259F	oleanane skeleton (94.7%), tetracyclic skeleton (5.3%)	71
				Y259W	oleanane skeleton (40.9%), tetracyclic skeleton (49.6%), lupeol (9.5%)	71
				W257A	oleanane skeleton (30%), tetracyclic skeleton (7.2%), lupeol (62.8%)	71
				W257V	oleanane skeleton (44.3%), tetracyclic skeleton (11.5%), lupeol (44.2%)	71
				W257L	oleanane skeleton (52.6%), tetracyclic skeleton (3.4%), lupeol (42.9%)	71
				W257F	oleanane skeleton (44.3%), tetracyclic skeleton (5%), lupeol (44.2%)	71
				W257Y	oleanane skeleton (21.9%), tetracyclic skeleton (13.6%), lupeol (64.5%)	71
Euphorbia	EtAS	AB206469	β-amyrin (94.7%),	V483G	β-amyrin (8.3%), camelliol C (84.9%), achilleol A (2.8%), butyrospermol	72

tirucalli			butyrospermol (3.1%), tirucalla-7,24-dien-3β-ol (2.2%)		(3.5%), tirucalla-7,24-dien-3β-ol (0.5%)	
				V483A	β-amyrin (38.1%), camelliol C (56.9%), achilleol A (1.1%), butyrospermol (3.4%), tirucalla-7,24-dien-3β-ol (0.5%)	72
				V483I	β-amyrin (94%),butyrospermol (3.6%), tirucalla-7,24-dien-3β-ol (2.4%)	72
				V483F	inactive	72
				M729G	β-amyrin (53.5%), germanicol (30.6%), φ-taraxasterol (2.7%), lupeol (4.1%), butyrospermol (3.1%), tirucalla-7,24-dien-3β-ol (6.0%)	72
				M729A	β-amyrin (62.8%), germanicol (27.0%), φ-taraxasterol (1.1%), lupeol (2.6%), butyrospermol (1.8%), tirucalla-7,24-dien-3β-ol (4.7%)	72
				M729V	β-amyrin (88.6%), germanicol (4.8%), butyrospermol (3.3%), tirucalla-7,24-dien-3β-ol (3.3%)	72
				M729L	β-amyrin (98.9%), tirucalla-7,24-dien-3β-ol (1.1%)	72
				M729F	β-amyrin (87.8%), butyrospermol (3.2%), tirucalla-7,24-dien-3β-ol (9.0%)	72
				M729W	inactive	72
				M729N	β-amyrin (34.5%), germanicol (54.4%), lupeol (1.8%), butyrospermol (6.5%), tirucalla-7,24-dien-3β-ol (2.8%)	72
				W534A/V/I/M/H/F/Y	conversion rate reduced	72
Euphorbia tirucalli	EtAS	AB206469	tetracycle (3.3%), pentacycle (96.7%)	F474G	C-B type bicycle (1.6%), C-C type bicycle (92.5%), pentacycle (5.9%)	73
				F474A	C-B type bicycle (9.4%), C-C type bicycle (66.3%), tetracycle (3%), pentacycle (21.2%), polypoda-8(9),13,17,21-tetraen-3β-ol (0.3%)	73
				F474V	C-C type bicycle (14.2%), tetracycle (8.6%), pentacycle (77.2%)	73
				F474L	C-C type bicycle (12.0%), tetracycle (6.1%), pentacycle (81.9%)	73
				F474M	C-C type bicycle (2.0%), tetracycle (8.4%), pentacycle (89.6%)	73
				F474T	C-B type bicycle (1.8%), C-C type bicycle (36.6%), tetracycle (6.3%), pentacycle (55.3%)	73
				F474H	C-C type bicycle (3.0%), tetracycle (5.9%), pentacycle (91.1%)	73
				F474Y	tetracycle (5.0%), pentacycle (95.0%)	73
				F474W	C-C type bicycle (30.1%), pentacycle (69.9%)	73
Euphorbia tirucalli	EtAS	AB206469	tetracycle (3.4%), pentacycle from oleanyl cation (96.6%)	F728A	tetracycle (39.3%), pentacycle from lupanyl cation (5.6%), pentacycle from oleanyl cation (52.0%), unidentified products (3.1%)	74
				F728I	inactive	74

				F728M	tetracycle (16.0%), pentacycle from oleanyl cation (80.3%), unidentified products (3.7%)	74
				F728H	tricycle (5.6%), tetracycle (17.3%), pentacycle from lupanyl cation (15.1%), pentacycle from oleanyl cation (54.1%), pentacycle from taraxasteryl and ursanyl cation (7.9%)	74
				F728Y	tetracycle (4.4%), pentacycle from oleanyl cation (95.6%),	74
				F728W	tetracycle (27.3%), pentacycle from lupanyl cation (15.8%), pentacycle from oleanyl cation (20.5%), pentacycle from taraxasteryl and ursanyl cation (36.1%), unidentified products (0.3%)	74
Euphorbia tirucalli	EtAS	AB206469	β-amyrin	D485N/E	inactive	75
				C486A	50% activity of the wild type	75
□	□	□	□	C564A	1.6% activity of the wild type	75

* These mutagenesis were not shown in Fig. 2.

Names of these compounds were different from the original reference (#69) because a difference carbon numbering system was used in #69.

□ Numbers of these residues were different from those in the original reference (#40).

Table S3 Substrate compatibility of plant OSCs.

OSC	GenBank ID	Species	Substrate	Product	Refere-nce
OSCs utilize 2,3:22,23-dioxidosqualene (DOS) as the substrate					
AtLUP1	NM_001334865	*Arabidopsis thaliana*	DOS	20R,24S-epoxydammarane; 20S,24S-epoxydammarane; 17,24-epoxybaccharane diol	30, 76
SAD1	AJ311789	*Avena species*	DOS	20S,24S-epoxydammarane	30
AtPEN1	NM_117622	*Arabidopsis thaliana*	DOS	arabidiol 20,21-epoxide	77
AtTHAS1	NM_001085264	*Arabidopsis thaliana*	DOS	(3S,13S,14R,21S)-21(22)-epoxy-malabarica-8,17-dien-3-ol	43
SgCDS	HQ128567	*Siraitia grosvenorii*	DOS	epoxycucurbitadienol	78
LCC	LC053635	*Lycopodium clavatum*	DOS	pre-α-onocerin	79
LCD	LC053636	*Lycopodium clavatum*	pre-α-onocerin	α-onocerin	79
LCE	LC200804	*Lycopodium clavatum*	pre-α-onocerin	tohogenol; serratenediol	80
OsONS1	KY625496	*Ononis spinosa*	DOS	α-onocerin	81
OsONS2	KY625497	*Ononis spinosa*	DOS	α-onocerin	81
OSCs utilize substituted 2,3-oxidosqualenes as substrates					
AtLUP1	NM_001334865	*Arabidopsis thaliana*	(18E)-22,23-dihydro-20-oxaoxidosqualene	3β-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one (61%)	82
AtLUP1	NM_001334865	*Arabidopsis thaliana*	3-(ω-oxidogeranylgeranyl) indole	petromindole (0.46%), camelliol-like (2.35%), achilleol-like (0.56%)	83
PsPSY	AB034802	*Pisum sativum*	24,30-bisnoroxidosqualene (**7**)	29,30-bisnor-β-amyrin (**7a**, 19%), 29,30-bisnorgermanicol (**7b**, 7%), 29,30-bisnor-δ-amyrin (**7c**, 1%)	84
PsPSY	AB034802	*Pisum sativum*	22,23-dihydro-2,3-oxidosqualene	euph-7-en-3β-ol (4%), bacchar-12-en-3β-ol (1%)	85
EtAS	AB206469	*Euphorbia tirucalli*	26-noroxidosqualene (**2**)	25-nor-β-amyrin (**2a,** 0.8%)	86
			27-noroxidosqualene (**3**)	26-nor-β-amyrin (**3a**); 26-nor-germanicol (**3b**); 26-nor-δ-amyrin (**3c**). 3a:3b:3c=4:2:1 (34%)	86
			28-noroxidosqualene (**4**)	27-nor-β-amyrin (**4a**); (**4b**); 27-nor-germanicol (**4c**) 4a:4b:4c=8:2:1 (22%)	86
			6-ethyl-oxidosqualene (**8**), 10-ethyl-oxidosqualene (**9**), 15-ethyl-oxidosqualene (**10**)	inactive	86
EtAS	AB206469	*Euphorbia tirucalli*	24,30-bisnoroxidosqualene (**7**)	29,30-bisnor-β-amyrin (**7a**, 57.2%); 29,30-bisnor-germanicol (**7b**, 24.0%); 29,30-bisnor-δ-amyrin (**7c**, 8.9%); and another two pentacyclic scaffolds (9.9%). (total: 76%)	87
			24-noroxidosqualene (**1**)	30-nor-β-amyrin (**1a**, 95.5%); (19S)-30-norlup-12-en-3β-ol (**1b**, 1.5%); 29-nor-β-amyrin (**1c**, 1.5%) and 29-nor-germanicol (**1d**, 1.5%). (total: 66%)	87
			30-noroxidosqualene (**6**)	27-nordammara-20(21),24-dien-3β-ol (**6a**, 3.9%); 27-norbutyrospermol (27-noreuph-7(8)-24-diene-3β-ol) (**6b**, 7.7%); (19R)-30-norlup-12-en-3β-ol (**6c**, 7.1%); 27-nortirucalla-7(8)-24-diene-3β-ol (**6d**, 9.1%); 30-norbacchara-12,21-diene-3β-ol (**6e**, 10.3%); **1c** (18.6%); **1d** (12.9%); 29-nortaraxasterol (29-norurs-20(30)-en-3β-ol) (**6f**, 3.9%); 27-nordammara-3β,20-diol (**6g**, 19.5%); others (7%). (total: 29%)	87
EtAS	AB206469	*Euphorbia tirucalli*	19-ethyl-oxidosqualene (**11**)	(17β-H, 20S)-20-ethyldammara-12,24-diene (**11a**); β-homoamyrin (**11b**). (total: 73%). 11a:11b=3:1	86, 88
			29-noroxidosqualene (**5**)	a new 6,6,6,6-fused tetracycle (**5a**, 3.4%)	86, 88

EtAS	AB206469	*Euphorbia tirucalli*	(23*E*)-ethyl-oxidosqualene (**12**)	**12a** (49.2%)	89
			(23*Z*)-ethyl-oxidosqualene (**13**)	**13a** (40.3%)	89
			(23*E*)-ethyl-30-noroxidosqualene (**14**)	**14a** and other products. (negligible amount)	89
			(23*Z*)-ethyl-24-noroxidosqualene (**15**)	**15a** (37.9%)	89
			(23*Z*)-propyl-24-noroxidosqualene (**16**)	**16a** (3.8%)	89
			(23*E*)-hydroxymethyl-oxidosqualene (**17**)	**17a** (88%)	89
			(23*Z*)-hydroxymethyl-oxidosqualene (**18**)	**18a**; **18b**; **18c** (total: 19.2%) 18a:18b:18c=2.9:2.1:1	89
OSCs related to oxidopolyprene					
PsPSY	AB034802	*Pisum sativum*	C25 oxidopolyprene	pregn-7-en-3-ol, 4,4,14,20-tetramethyl-, (3β, 5α,13α,14β,17α)-(9CI)	90
			C35 oxidopolyprene	inactive	90

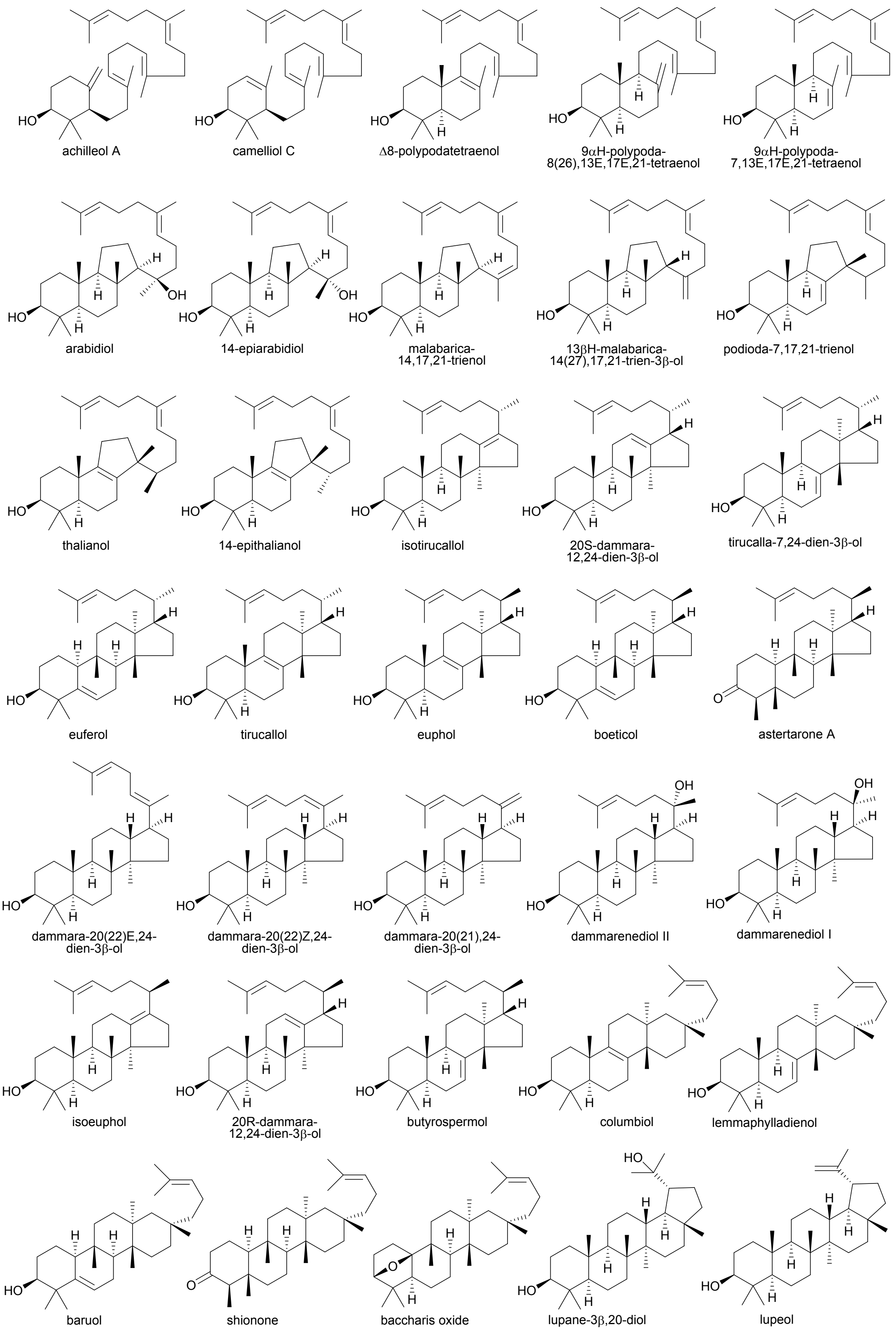


Fig. S1 Product structures for plant OSCs.

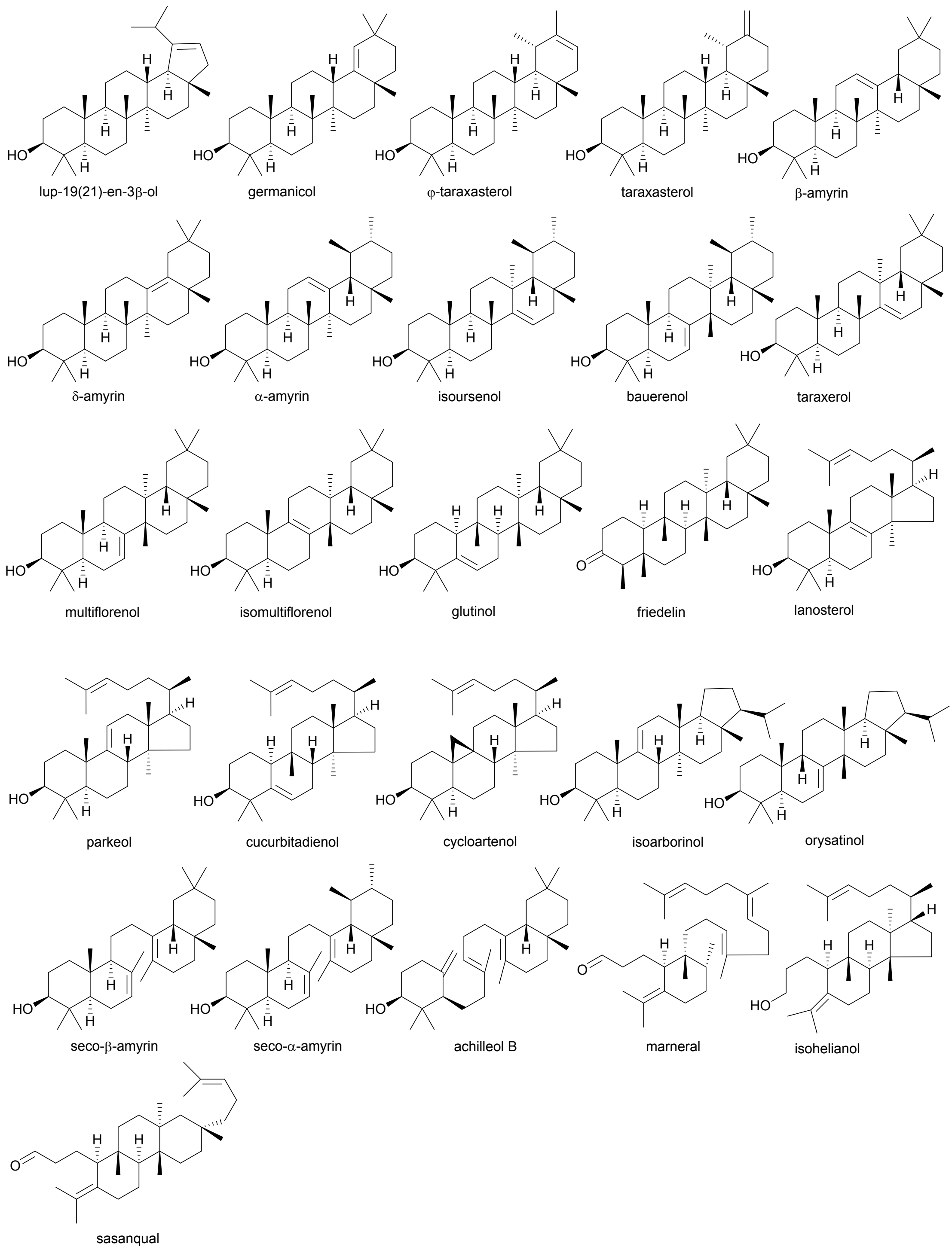
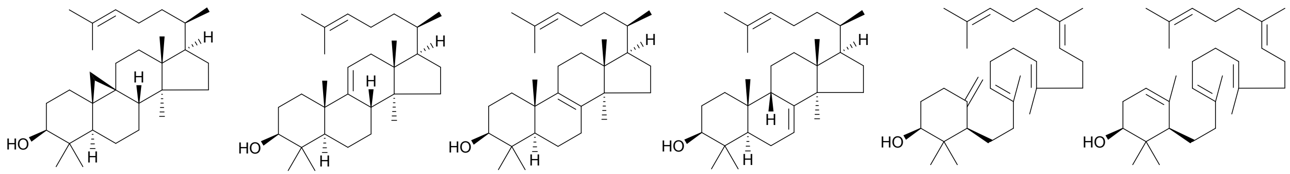
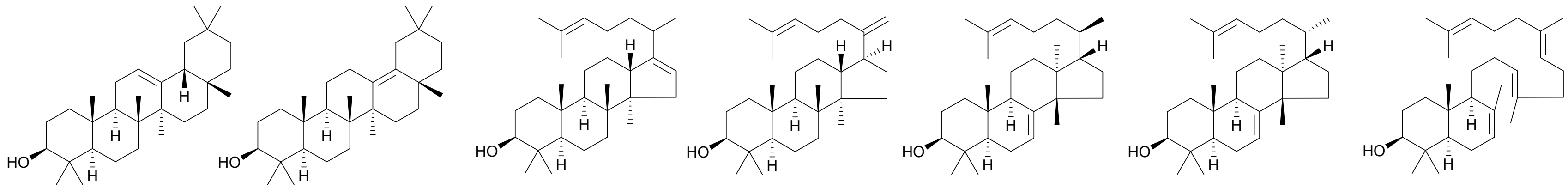

lup-19(21)-en-3β-ol
germanicol
φ-taraxasterol
taraxasterol
β-amyrin
δ-amyrin
α-amyrin
isoursenol
bauerenol
taraxerol
multiflorenol
isomultiflorenol
glutinol
friedelin
lanosterol
parkeol
cucurbitadienol
cycloartenol
isoarborinol
orysatinol
seco-β-amyrin
seco-α-amyrin
achilleol B
marneral
isohelianol
sasanqual

Fig. S1 (*Contd.*)



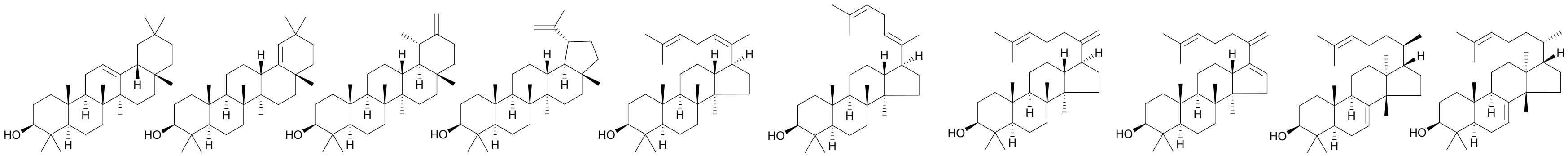
	cycloartenol	parkeol	lanosterol	9β-Δ7-lanosterol	achilleol A	camelliol C
AtCAS1-WT	99%	1%				
Y410C			75%	24%	1%	
Y410T		2%	65%	33%		
H477N		12%	88%			
H477Q		73%	22%	5%		
I481L	83%	16%	1%			
I481A	12%	15%	54%		13%	6%
I481G	17%	4%	23%		44%	12%
I481V	55%	21%	24%			
Y410T/I481V		<1%	75%	25%		
H477N/I481V		1%	99%			
H477Q/I481V		6%	94%			
Y410T/H477N/I481V		<1%	78%	22%		
Y410T/H477Q/I481V		<1%	78%	22%		
Y532H		31%	45%		24%	
F472S			less			

Fig. S2 Product structures and yields for AtCAS1 and its mutants.[65,66,67] WT, wild type.



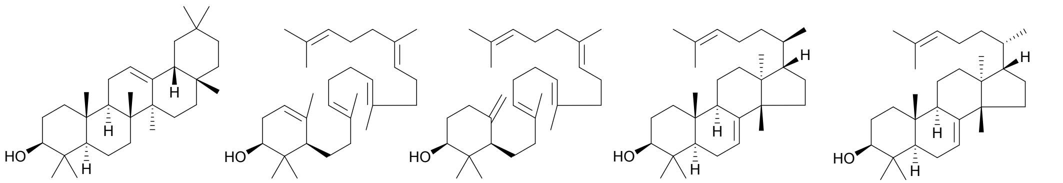
	Oleanane skeleton		Dammarane skeleton				Bicycle
	β-amyrin	δ-amyrin	dammara-16,24-dien-3β-ol	dammara-20(21),24-dien-3β-ol	butyrospermol	tirucalla-7,24-dien-3β-ol	γ-polypodatetraen-3β-ol
EtAS-WT	**97.0%**				**0.7%**	**2.3%**	
F413A	**17.9%**	**6.1%**	**2.0%**	**62.90%**	**6.8%**	**2.2%**	**2.1%**
F413V	**88.4%**	**1.1%**		**8.10%**	**0.2%**	**2.2%**	
F413M	**81.0%**	**11.5%**		**5.80%**	**0.6%**	**1.1%**	
F413S	**10.8%**	**6.7%**	**4.8%**	**69.30%**	**5.0%**	**0.8%**	**2.6%**
F413T	**22.0%**	**6.0%**	**3.9%**	**54%**	**5.6%**	**1.7%**	**6.7%**
F413Y	**35.0%**	**5.4%**	**1.8%**	**36.60%**	**15.7%**	**3.8%**	**1.7%**
F413H	**31.3%**	**6.0%**	**1.0%**	**44.40%**	**12.6%**	**2.8%**	**1.9%**
F413W	**84.8%**				**4.8%**	**10.4%**	

Fig. S3 Product structures and yields for EtAS and its F413 mutants.[71]



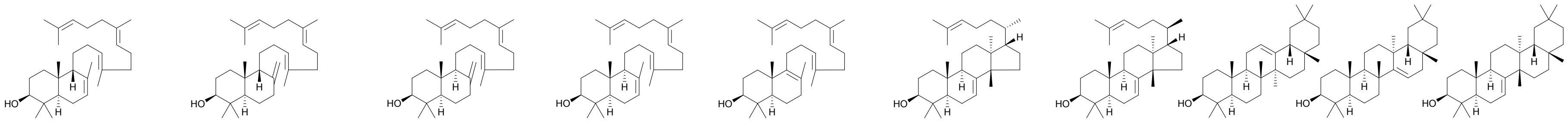
	Oleanane skeleton			Lupanyl skeleton	Tetracyclic skeleton					
	β-amyrin	germanicol	taraxasterol (urs-20(30)-en-3β-ol)	lupeol	dammara-(Z)-20(22)-24(25)-dien-3β-ol	dammara-(E)-20(22)-24(25)-dien-3β-ol	dammara-20(21),24-dien-3β-ol	dammara-16(17),20(21), 24-trien-3β-ol	butyrospermol	tirucalla-7,24-dien-3β-ol
EtAS-WT	97.0%								0.7%	2.3%
Y259A		19.0%	1.8%		1.9%		51.6%	18.1%	7.6%	
Y259V			4.8%				95.2%			
Y259I			0.7%				99.3%			
Y259L		4.6%	2.1%		8.7%		77.4%	7.2%		
Y259H		16.6%	3.7%	3.7%	69.6%	2.4%	2.0%			
Y259F	94.7%								1.5%	3.8%
Y259W	40.9%			9.5%					49.6%	
W257A	23.5%	6.5%		62.8%					5.9%	1.3%
W257V	38.2%	6.1%		44.2%					7.0%	4.5%
W257L	50.1%	2.5%		42.9%					2.6%	0.5%
W257F	38.2%	6.1%		44.2%					3.8%	1.2%
W257Y	21.2%	0.7%		64.5%					10.9%	2.7%

Fig. S4 Product structures and yields for EtAS and its W257 and Y259 mutants.[71]



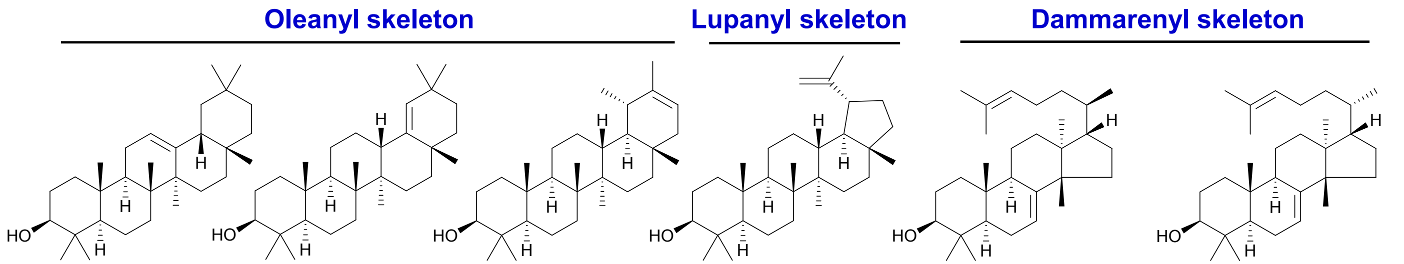
	β-amyrin	camelliol C	achilleol A	butyrospermol	tirucalla-7,24-dien-3β-ol
EtAS-WT	**94.7%**			**3.1%**	**2.2%**
V483G	**8.3%**	**84.9%**	**2.8%**	**3.5%**	**0.5%**
V483A	**38.1%**	**56.9%**	**1.1%**	**3.4%**	**0.5%**
V483I	**94.0%**			**3.6%**	**2.4%**
V483F					

Fig. S5 Product structures and yields for EtAS and its V483 mutants.[72]



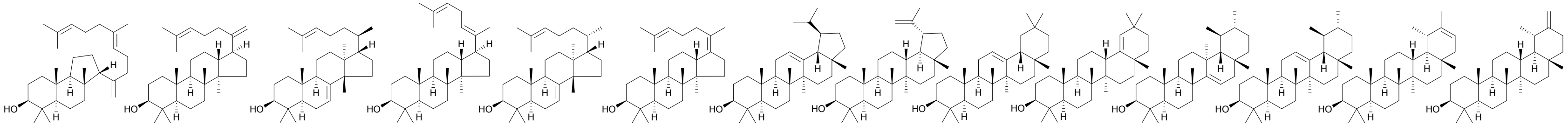
	Bicycle (C-B type)		Bicycle (C-C type)			Tetracycle		Pentacycle		
	9βH-polypoda-7,13E,17E,21-tetraenol	9βH-polypoda-8(26),13E,17E,21-tetraenol	9αH-polypoda-8(26),13E,17E,21-tetraenol	9αH-polypoda-7,13E,17E,21-tetraenol	Δ8-polypodatetraenol	tirucalla-7,24-dien-3β-ol	butyrospermol	β-amyrin	taraxerol	multiflorenol
EtAS-WT						1.5%	1.8%	96.7%		
F474G	1.6%		43.2%	49.3%				5.9%		
F474A	7.5%	1.9%	12.8%	53.5%	0.3%	0.5%	2.5%	12.5%	8.7%	
F474V				14.2%		4.2%	4.4%	71.3%	5.9%	
F474L				12.0%		2.0%	4.1%	81.9%		
F474M			1.1%	0.9%		3.8%	4.6%	80.5%	1.1%	8.0%
F474T	1.8%		8.8%	27.8%		2.0%	4.3%	48.0%	7.3%	
F474H				3.0%		2.2%	3.7%	89.6%	1.5%	
F474Y						1.9%	3.1%	90.1%	4.9%	
F474W			30.1%					69.9%		

Fig. S6 Product structures and yields for EtAS and its F474 mutants.[73]



	β-amyrin	germanicol	φ-taraxasterol	lupeol	butyrospermol	tirucalla-7,24-dien-3β-ol
EtAS-WT	**95.9%**				**2.0%**	**2.1%**
M729G	**53.5%**	**30.6%**	**2.7%**	**4.1%**	**3.1%**	**6.0%**
M729A	**62.8%**	**27.0%**	**1.1%**	**2.6%**	**1.8%**	**4.7%**
M729V	**88.6%**	**4.8%**			**3.3%**	**3.3%**
M729L	**98.9%**					**1.1%**
M729F	**87.8%**				**3.2%**	**9.0%**
M729W						
M729N	**34.5%**	**54.4%**		**1.8%**	**6.5%**	**2.8%**

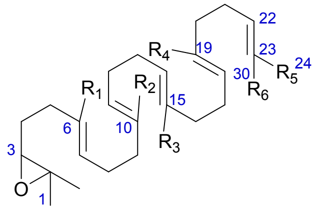
Fig. S7 Product structures and yields for EtAS and its M729 mutants.[72]



	Tricycle skeleton	Tetracycle skeleton					Pentacycle (lupanyl skeleton)		Pentacycle (oleanyl skeleton)		Pentacycle (taraxasteryl and ursanyl skeleton)				
	malabarica-14(27),17,21-trienol	dammara-20(21),24-dien-3β-ol	butyrospermol	dammara-(E)-20(22)-24(25)-dien-3β-ol	tirucalla-7,24-dien-3β-ol	(17E)-dammara-17(20),24-trien-3β-ol	lup-12-en-3β-ol	lupeol	β-amyrin	germanicol	isoursenol	α-amyrin	φ-taraxasterol	taraxasterol	unidentified product
EtAS-WT			1.8%		1.6%				96.6%						0.0%
F728A			5.3%		34.0%			5.6%	52.0%						3.1%
F728I															
F728M			4.7%		11.3%				72.9%	7.4%					3.7%
F728H	5.6%		1.8%	3.4%	6.1%	6.0%		15.1%	3.2%	50.9%			7.9%		0.0%
F728Y			1.1%		3.3%				95.6%						0.0%
F728W		9.8%	11.9%	1.9%	4.0%		6.7%	9.1%	17.2%	3.3%	13.2%	18.0%	3.8%	1.1%	0.3%

Fig. S8 Product structures and yields for EtAS and its F728 mutants.[74]

(A)



	R1	R2	R3	R4	R5	R6
1	Me	Me	Me	Me	H	Me
2	H	Me	Me	Me	Me	Me
3	Me	H	Me	Me	Me	Me
4	Me	Me	H	Me	Me	Me
5	Me	Me	Me	H	Me	Me
6	Me	Me	Me	Me	Me	H
7	Me	Me	Me	Me	H	H
8	Et	Me	Me	Me	Me	Me
9	Me	Et	Me	Me	Me	Me
10	Me	Me	Et	Me	Me	Me
11	Me	Me	Me	Et	Me	Me
12	Me	Me	Me	Me	Et	Me
13	Me	Me	Me	Me	Me	Et
14	Me	Me	Me	Me	Et	H
15	Me	Me	Me	Me	H	Et
16	Me	Me	Me	Me	H	Pr
17	Me	Me	Me	Me	CH_2OH	Me
18	Me	Me	Me	Me	Me	CH_2OH

(B)

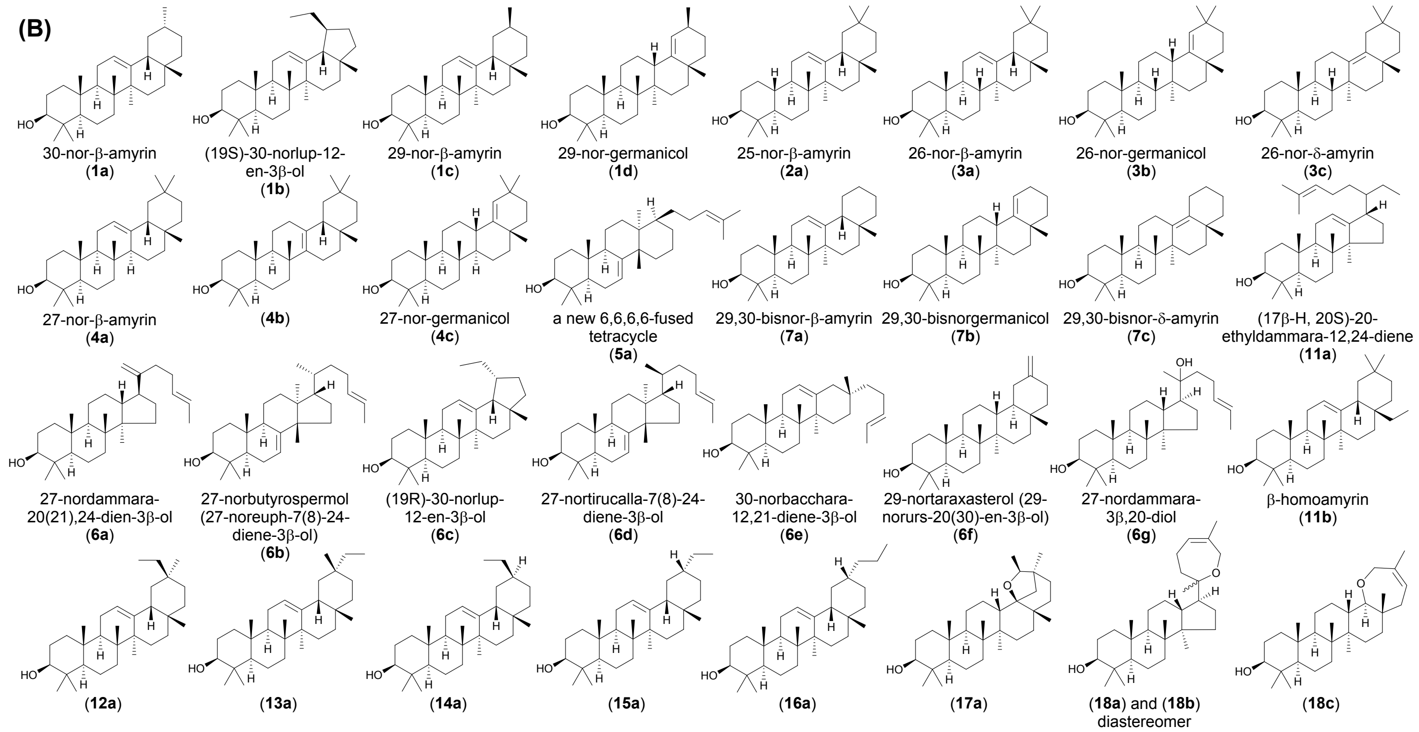


Fig. S9 Substituted 2,3-oxidosqualene and their corresponding products catalyzed by OSCs. Compounds **1-18** were studied using EtAS, and compound **7** was also studied using PsPSY.[84,86,87,88,89] (A) Structures of substituted 2,3-oxidosqualenes. (B) The corresponding products for substituted 2,3-oxidosqualenes. **Xa**, **Xb** etc. indicate the products of compound **X**. **1c** and **1d** are products of both compounds **1** and **6**.

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