Supplementary Materials

for

Pentacyclic triterpenoid acids in Styrax as potent and highly specific inhibitors against human carboxylesterase 1A

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This file contains one supplementary table and twenty-one supplementary figures

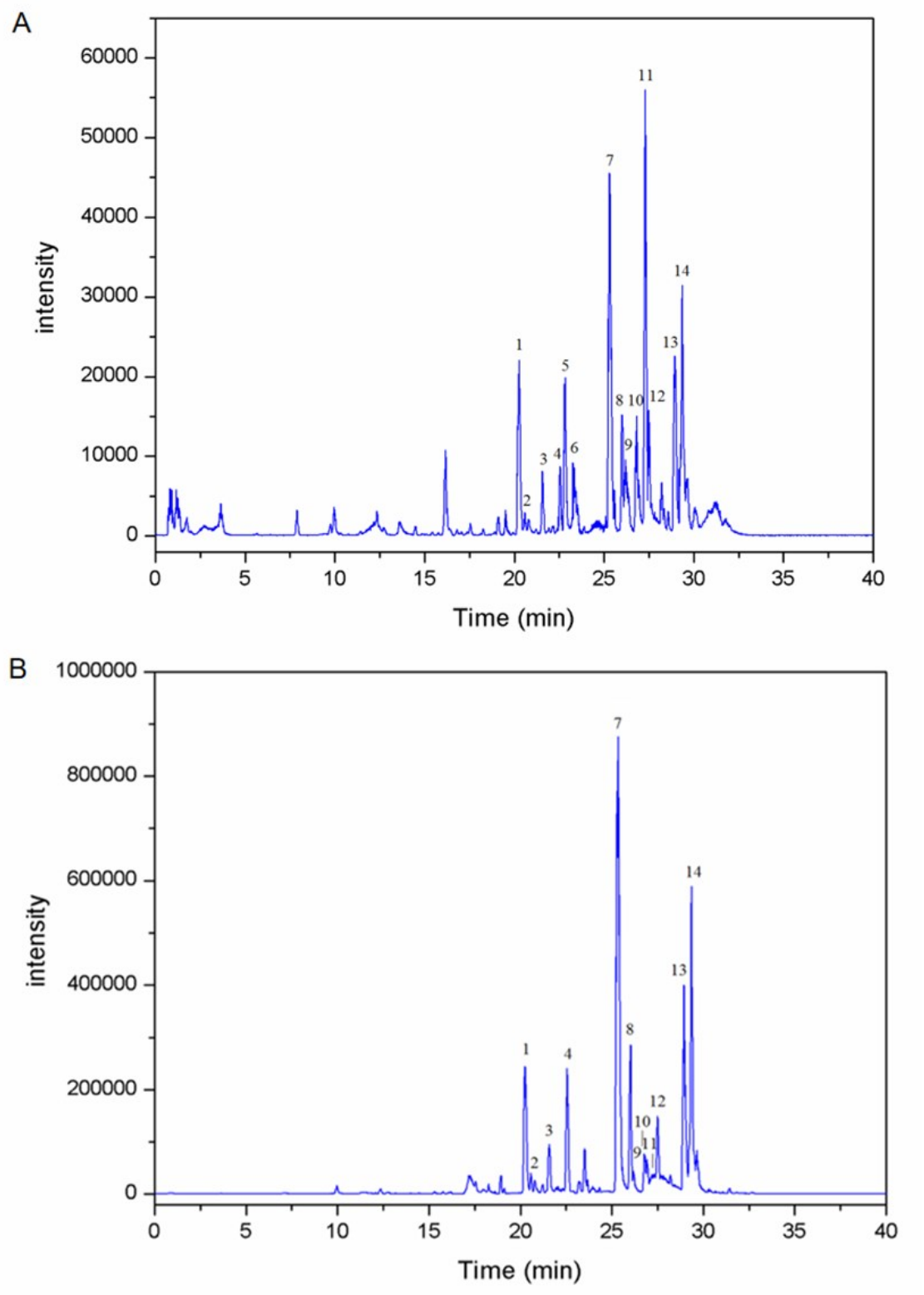

Fig. S1 Total ion chromatograph of styrax bioactive fraction (25-35 min) in positive ion mode (A) and negative ion mode (B).

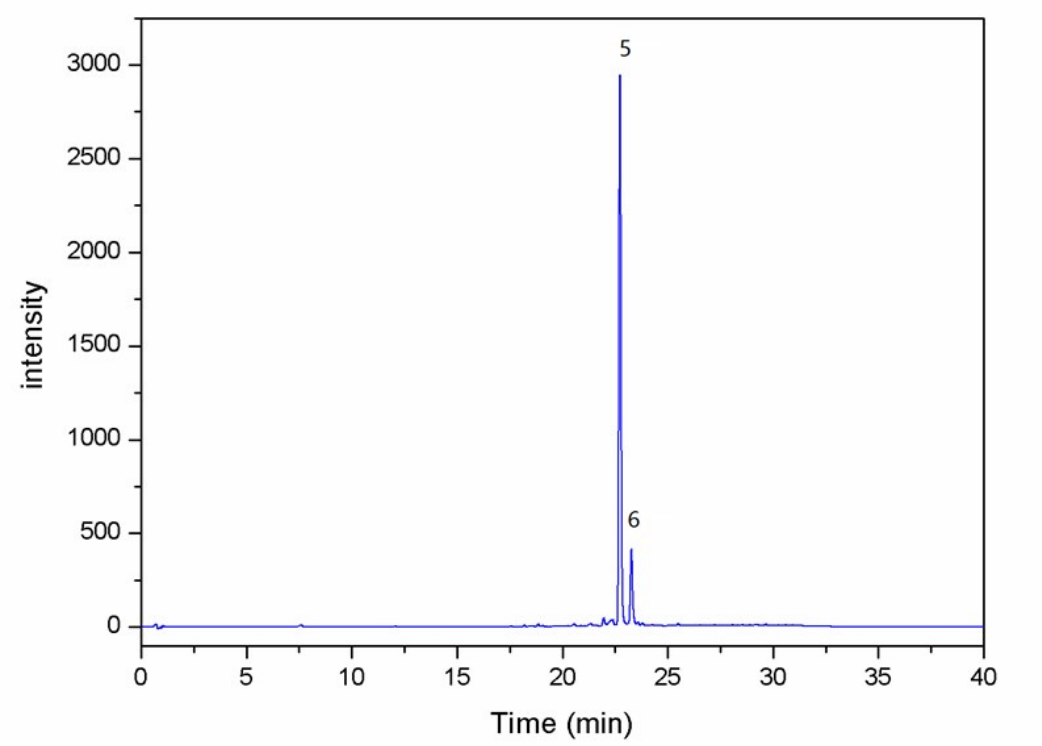

Fig. S2 LC-UV fingerprinting of styrax bioactive fraction (25-35 min) monitored at 254 nm.

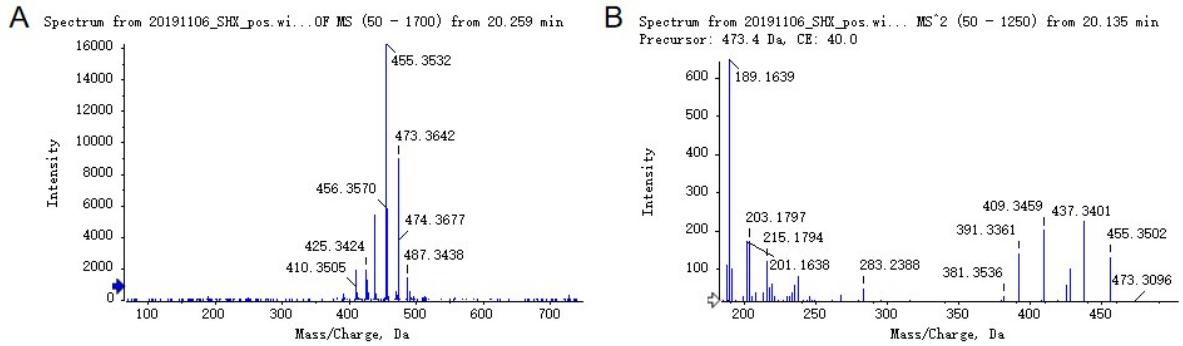

Fig. S3 MS^1 (A) and MS^2 (B) spectrum of compound 1.

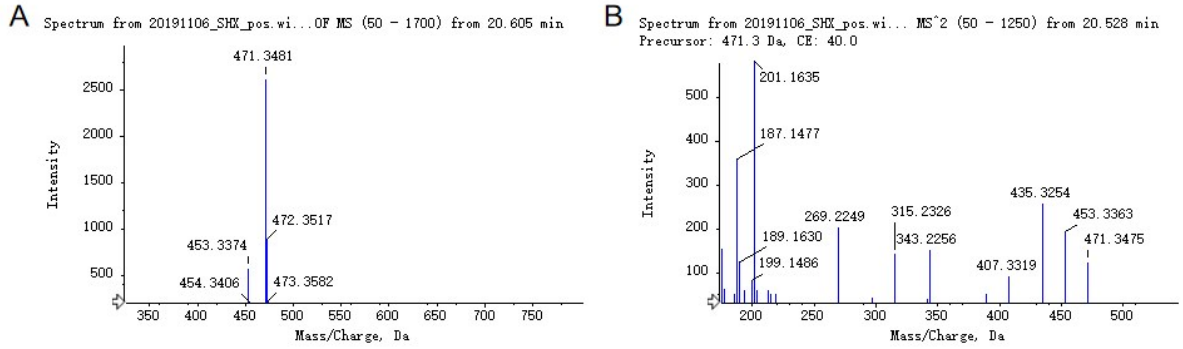

Fig. S4 MS^1 (A) and MS^2 (B) spectrum of compound 2.

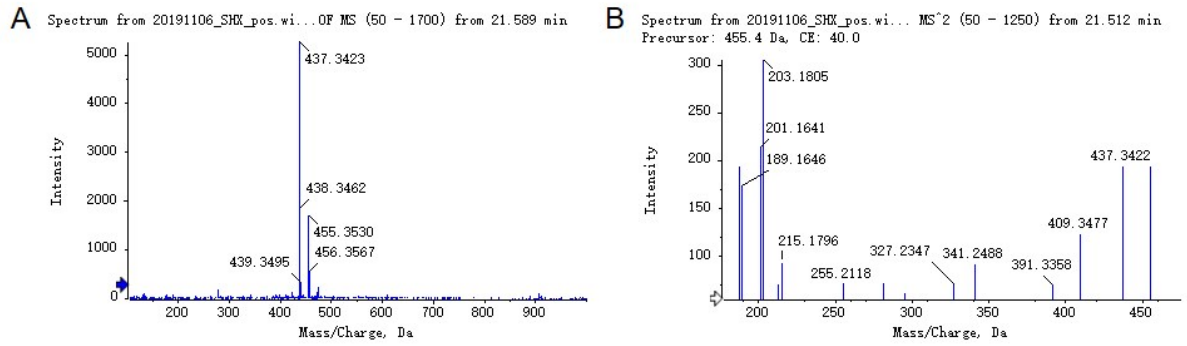

Fig. S5 MS^1 (A) and MS^2 (B) spectrum of compound 3.

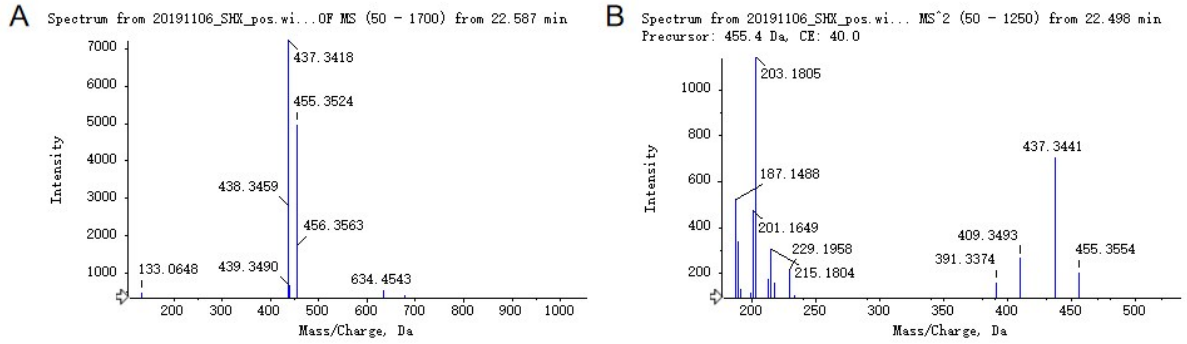

Fig. S6 MS^1 (A) and MS^2 (B) spectrum of compound 4.

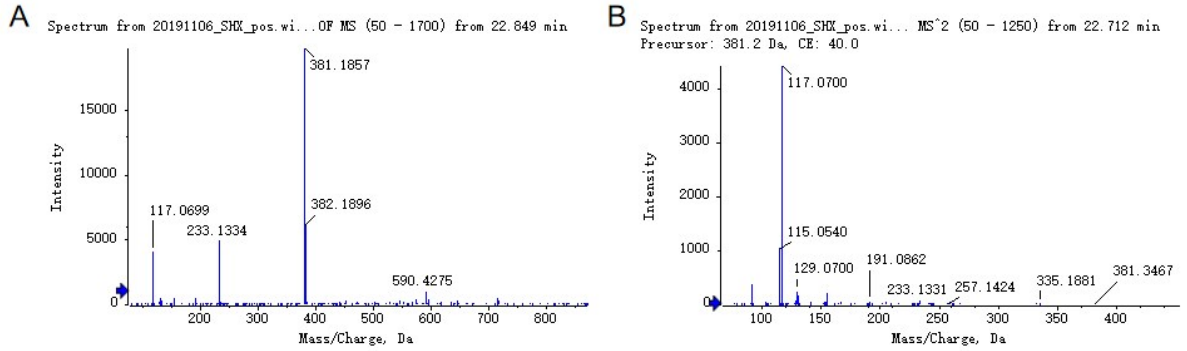

Fig. S7 MS^1 (A) and MS^2 (B) spectrum of compound 5.

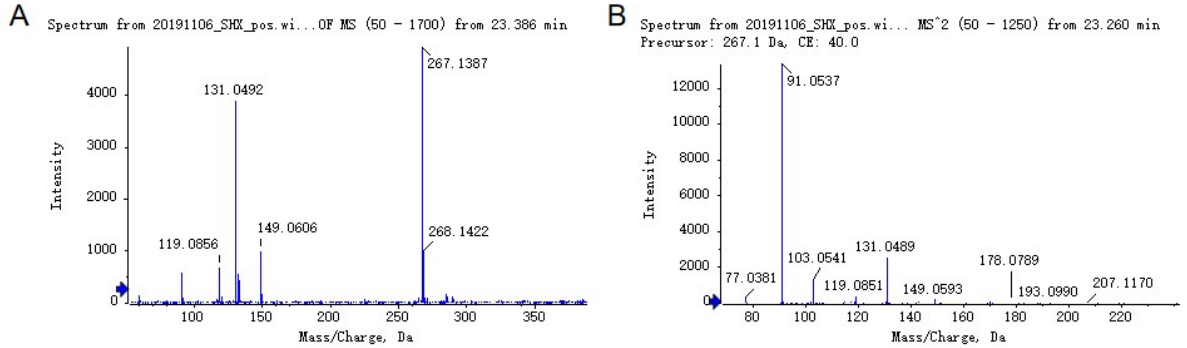

Fig. S8 MS^1 (A) and MS^2 (B) spectrum of compound 6.

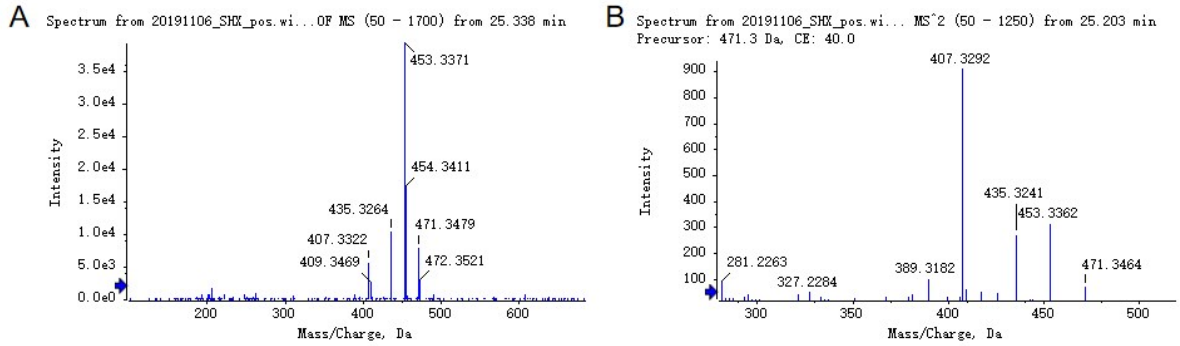

Fig. S9 MS^1 (A) and MS^2 (B) spectrum of compound 7.

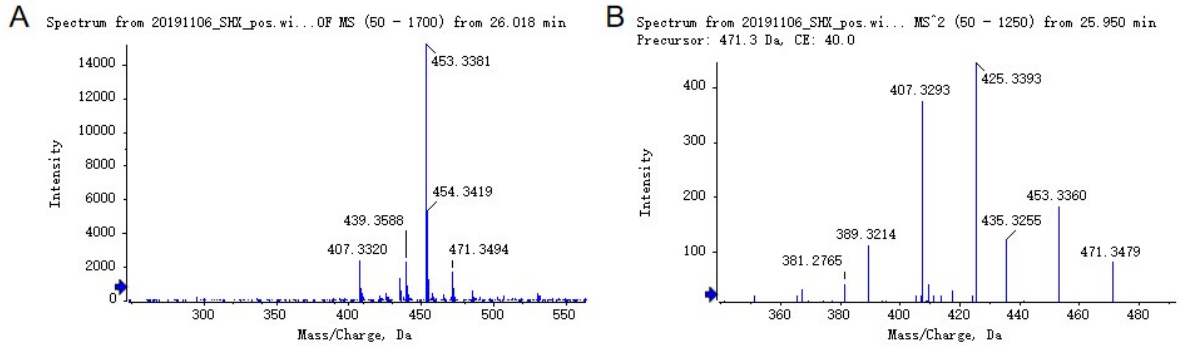

Fig. S10 MS^1 (A) and MS^2 (B) spectrum of compound 8.

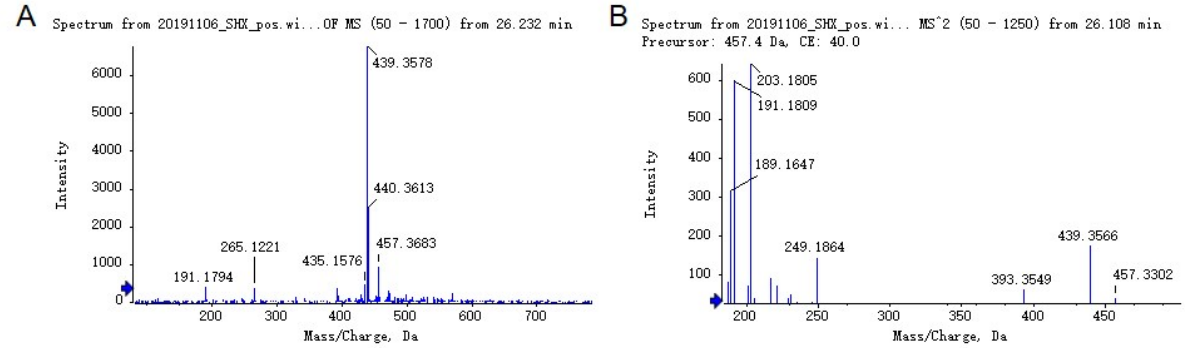

Fig. S11 MS^1 (A) and MS^2 (B) spectrum of compound 9.

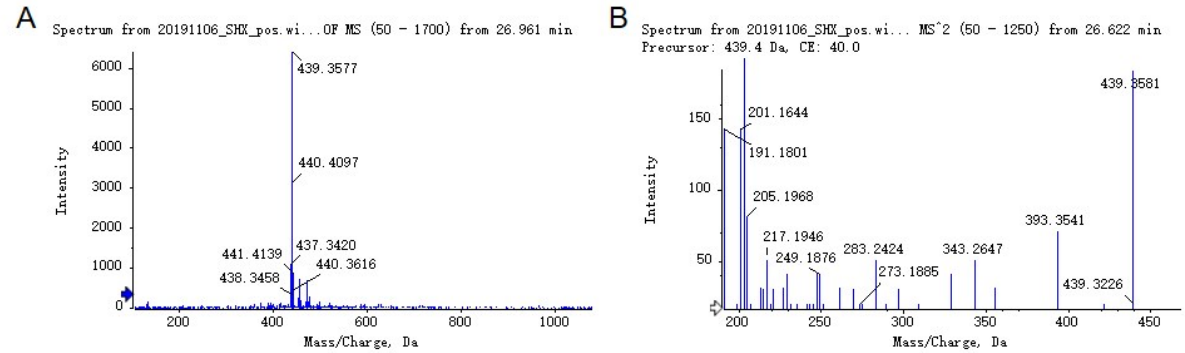

Fig. S12 MS^1 (A) and MS^2 (B) spectrum of compound 10.

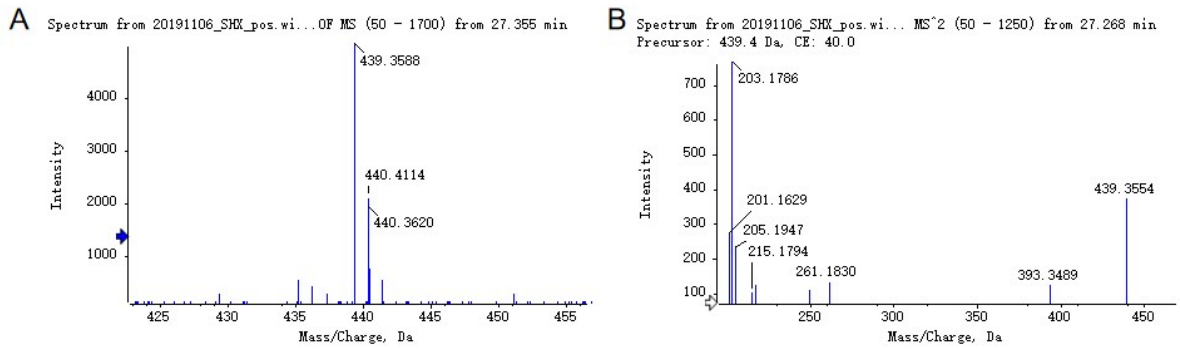

Fig. S13 MS[1] (A) and MS[2] (B) spectrum of compound 11.

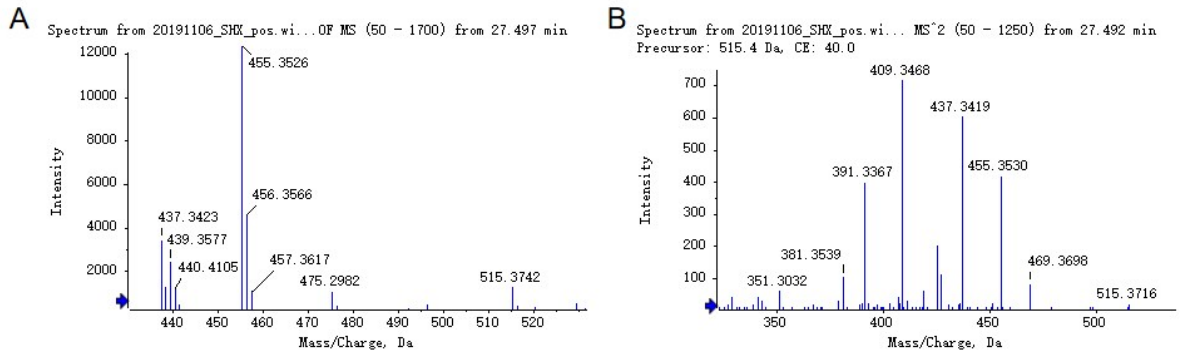

Fig. S14 MS[1] (A) and MS[2] (B) spectrum of compound 12.

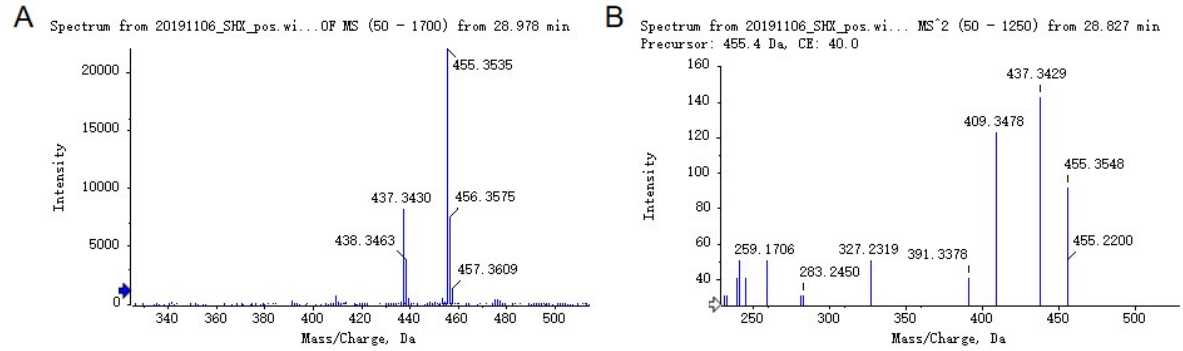

Fig. S15 MS[1] (A) and MS[2] (B) spectrum of compound 13.

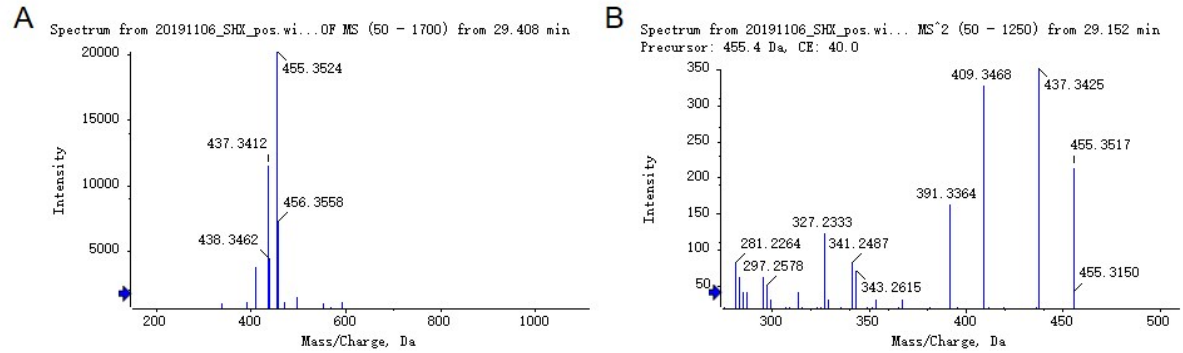

Fig. S16 MS[1] (A) and MS[2] (B) spectrum of compound 14.

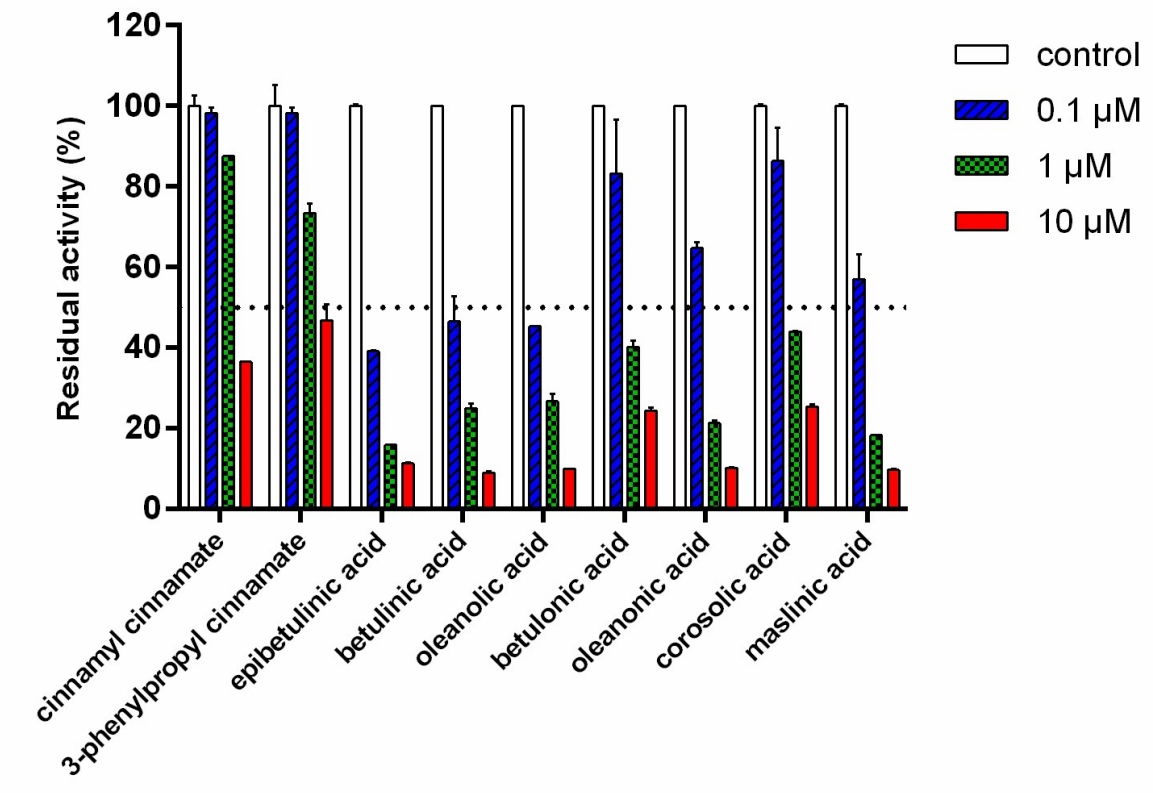

Fig. S17 Inhibitory effects of nine major constituents in the bioactive fraction from Styrax against hCES1A-mediated DME hydrolysis in HLM. Data are expressed as mean ± SD.

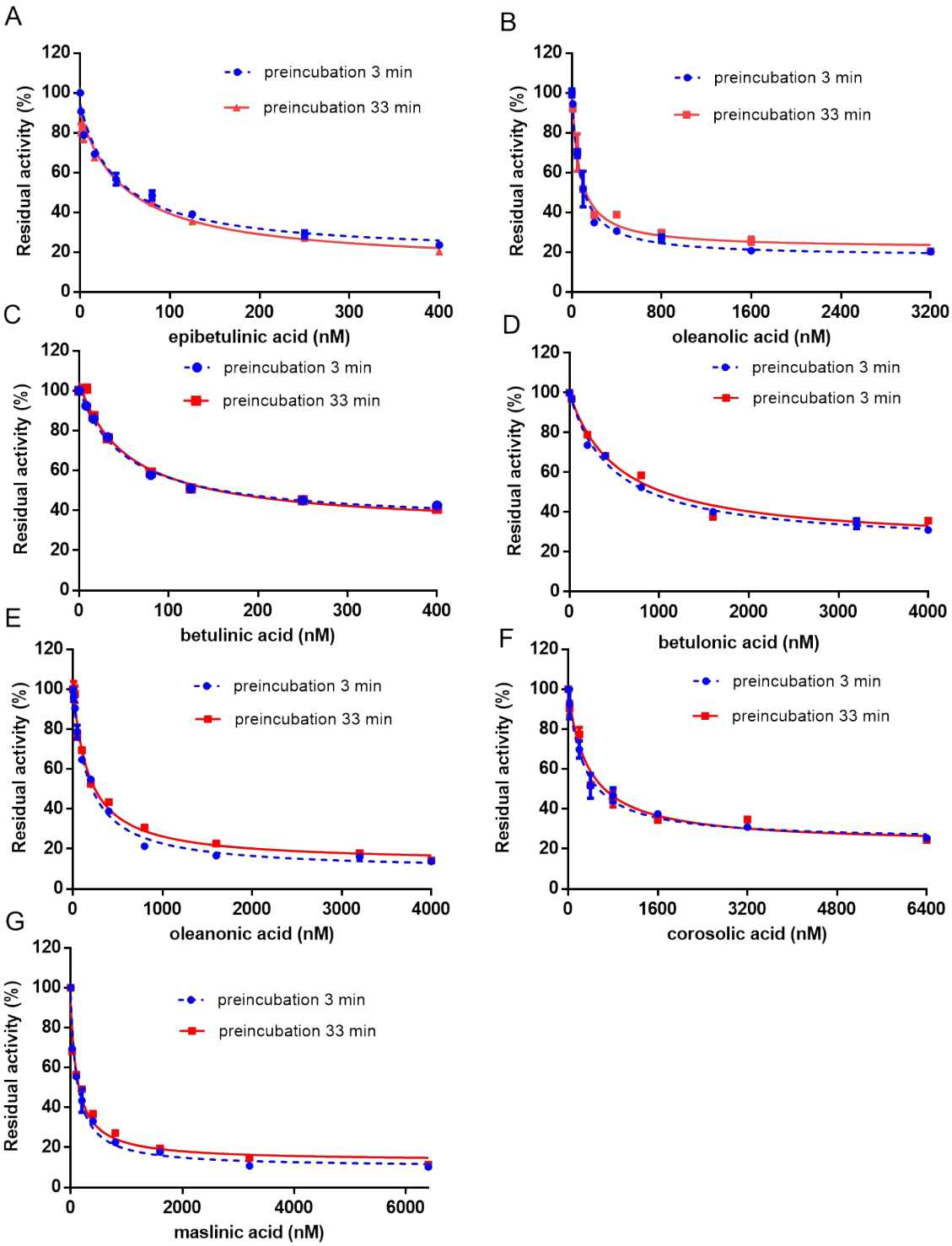

Fig. S18 Inhibition of hCES1A-mediated DME hydrolysis by epibetulinic acid (A), oleanolic acid (B), betulinic acid (C), betulonic acid (D), oleanonic acid (E), corosolic acid (F) or maslinic acid (G) with different preincubation times. All data were shown as mean ± SD.

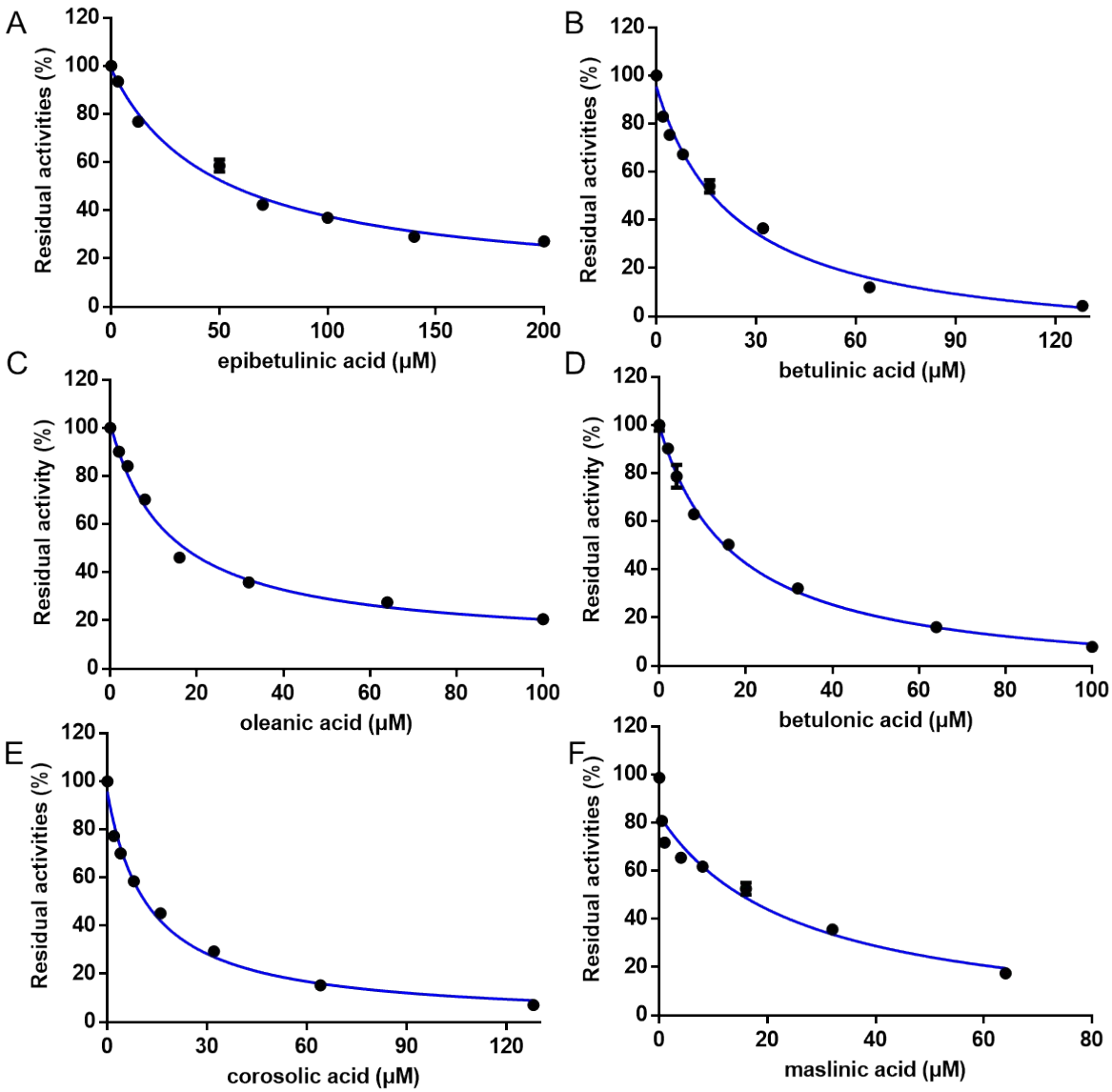

Fig. S19 Dose-inhibition curves of epibetulinic acid (A), betulinic acid (B), oleanonic acid (C), betulonic acid (D), corosolic acid (E) or maslinic acid (F) against hCES2A-mediated NCEN hydrolysis. All data were shown as mean ± SD.

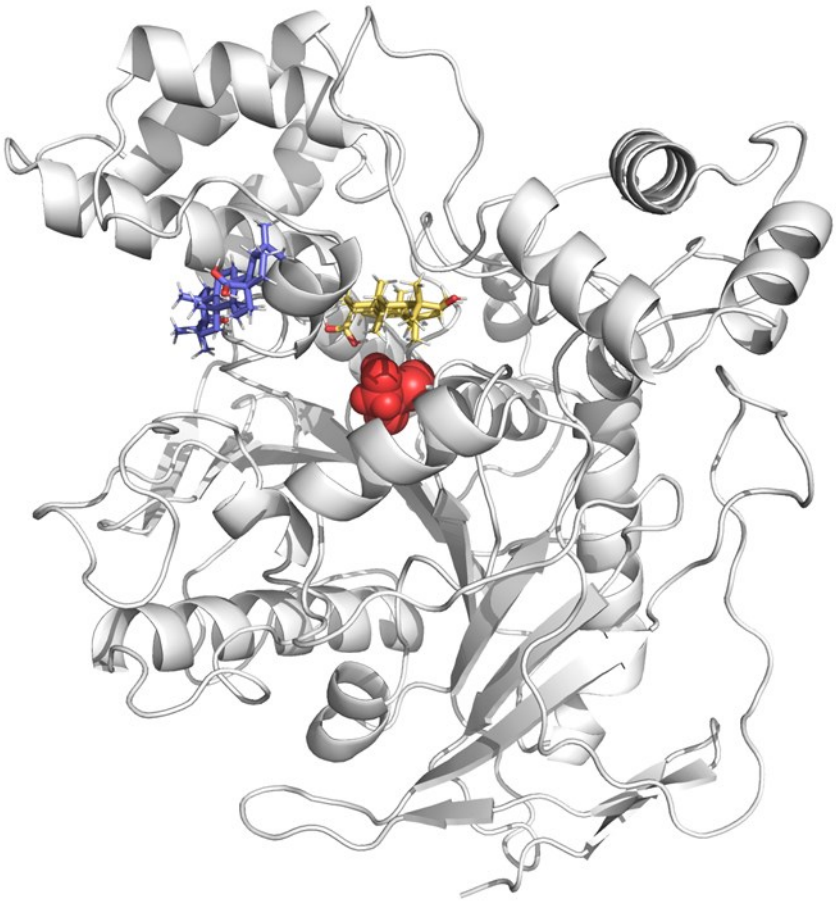

Fig. S20 Molecular docking simulations of epibetulinic acid into the crystal structure of hCES1A1 (PDB ID: 1MX5) by Autodock Vina. Two different binding modes with the lowest binding energy was presented in Site A (yellow) with -9.5 kcal/mol and Site B (blue) with -7.3 kcal/mol. The active site Ser221 was presented as red spheres.

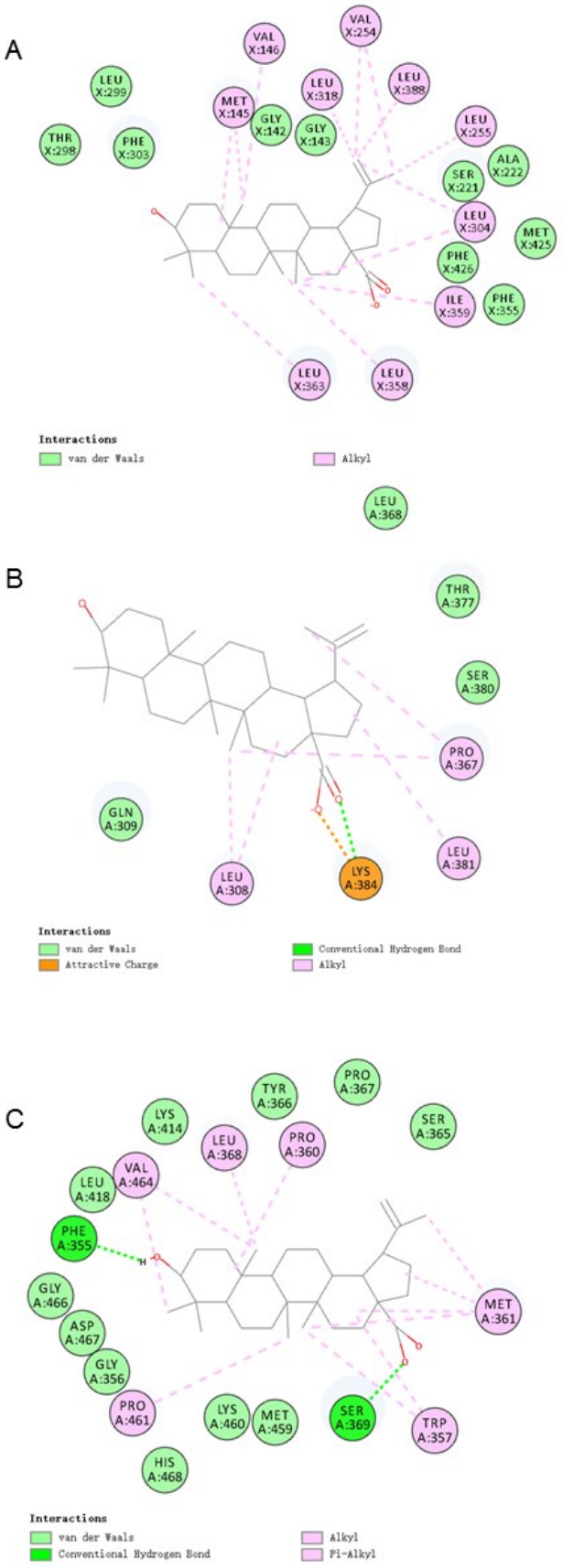

Fig. S21 The detailed 2D interactions between epibetulinic acid and the surrounding amino acids in Site I (A), Site II (B) and Site III (C) of hCES1A.

Table S1 Identification and characterization of major constituents with potent hCES1A inhibitory effect in styrax by using LC-TOF-MS/MS.

Peak	t_R（min）	Ionization	Observed *m/z*	ppm	Formula	Identification	Fragment ions
1	20.26	$[M+H]^+$	473.3641	2.5	$C_{30}H_{48}O_4$	Maslinic acid	473.3096, 437.3401, 391.3361, 203.1797
2	20.59	$[M+H]^+$	471.3482	1.9	$C_{30}H_{46}O_4$	3-oxo-12α-hydroxy-olean-28,13β-olide or isomer	471.3475, 453.3363, 435.3254, 343.2256
3	21.57	$[M+H-H_2O]^+$	455.3528	1.4	$C_{30}H_{48}O_4$	Corosolic acid	455.3536, 409.3477, 391.3358, 203.1805
4	22.56	$[M+H-H_2O]^+$	455.3531	1.6	$C_{30}H_{48}O_4$	3α, 25-Dihydroxy-olean-12-en-28-oic acid	455.3554, 437.3441, 391.3374, 203.1805
5	22.81	/	381.1856	/	$C_{18}H_{16}O_2$	Cinnamyl cinnamate	381.3467, 155.0856, 129.0700, 117.0700
6	23.36	$[M+H]^+$	267.1391	2.8	$C_{18}H_{18}O_2$	3-phenylpropyl cinnamate	149.0593, 131.0489, 119.0581, 103.0541
7	25.34	$[M+H]^+$	471.3478	2.4	$C_{30}H_{46}O_4$	3-oxo-12α-hydroxy-olean-28,13β-olide or isomer	471.3464, 435.3241, 389.3182, 187.1474
8	26.02	$[M+H]^+$	471.3473	2.1	$C_{30}H_{46}O_4$	3-oxo-12α-hydroxy-olean-28,13β-olide or isomer	471.3479, 435.3255, 425.3393, 389.3214
9	26.20	$[M+H]^+$	457.3683	1.7	$C_{30}H_{48}O_3$	Epibetulinic acid	457.3302, 393.3549, 249.1864 191.1809
10	26.95	$[M+H-H_2O]^+$	439.3580	2.1	$C_{30}H_{48}O_3$	Betulinic acid	439.3568, 421.3433, 393.3541, 249.1849
11	27.30	$[M+H-H_2O]^+$	439.3594	2.4	$C_{30}H_{48}O_3$	Oleanolic acid	439.3554, 393.3489, 203.1786
12	27.52	$[M+H]^+$	515.3744	1.9	$C_{32}H_{50}O_5$	3α-acetoxy-25-hydroxy-olean-12-en-28-oic acid	515.3716, 437.3419, 391.3367, 203.1797
13	28.97	$[M+H]^+$	455.3528	2.3	$C_{30}H_{46}O_3$	Betulonic acid	455.3548, 437.3429, 409.3478, 391.3378
14	29.41	$[M+H]^+$	455.3535	1.4	$C_{30}H_{46}O_3$	Oleanonic acid	455.3517, 437.3425, 409.3468, 391.3364

Table S2 The inhibition kinetic parameters of seven constituents from Styrax against hCES1A-mediated DME hydrolysis.

Inhibitor name	Inhibitor concentration (nM)	V_{max} (pmol/min/mg protein)	K_m (μM)	Linear fit equations	R^2
None	0	5.078	4.677	-	-
epibetulinic acid	16	5.088	5.040	Y=0.0184X+1.358	0.99
betulinic acid	32	4.788	6.312	Y=0.0179X+1.272	0.98
oleanolic acid	20	4.896	7.929	Y=0.0150X+1.575	0.98
betulonic acid	400	3.916	5.347	Y=0.0015X+1.323	0.99
oleanonic acid	80	4.130	6.501	Y=0.0067X+1.384	0.98
corosolic acid	800	3.315	2.302	Y=0.0005X+0.338	0.97
maslinic acid	100	3.410	4.310	Y=0.0021X+0.701	0.99