Supplementary Materials

for

Discovery and characterization of naturally occurring chalcones as potent inhibitors against bile salt hydrolases

Chun-Yu Li[a], Hao-Nan Wang[a], Guang-Hao Zhu[a], Li-Lin Song[a,b], Xu-Dong Hou[c], Peng-Chao Huo[a], Jie Hou[c,]*, Guang-Bo Ge[a,]*

[a] Shanghai Frontiers Science Center for Chinese Medicine Chemical Biology; Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China.

[b] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116000, China.

[c] College of Basic Medical Sciences, Dalian Medical University, Dalian, 116044, China.

*Corresponding author.

E-mail address: geguangbo@dicp.ac.cn (G.B. Ge); houjie@dmu.edu.cn (J. Hou)

This file contains one supplementary table and fifteen supplementary figures.

Table S1. The inhibitory effects of more than 100 kinds of natural products on lsBSH. All data were shown as mean ± SD of triplicate determinations.

No.	Class	Compound	MW	Residual Activity (%)		
				1 μM	10 μM	100 μM
1	**Chalcones**	Licochalcone C	338.40	36.62	6.82	0.61
2		Isobavachalcone	324.37	49.90	9.81	8.55
3		Bavachalcone	324.37	62.21	17.69	10.50
4		4'-*O*-Methylbroussochalcone B	338.40	65.96	41.35	19.78
5		Licochalcone D	354.40	104.68	28.00	2.06
6		Licochalcone A	338.40	90.46	38.12	9.83
7		2',4'-Dihydroxy-6'-methoxy -3',5'-dimethylchalcone	298.34	86.08	46.85	23.99
8		Butein	272.25	96.28	74.51	29.73
9		Isoliquiritigenin	256.26	95.00	63.26	5.46
10		Naringenin chalcone	272.25	91.36	65.27	15.15
11		Echinatin	270.28	96.68	72.03	12.07
12		Licochalcone B	286.28	101.67	81.88	37.45
13		Flavokawain A	314.34	93.33	75.77	75.64
14		Isoliquiritin	418.40	109.86	113.27	86.73
15		Neoisoliquiritin	418.40	116.22	113.70	102.87
16	**Flavonoids**	Quercetin	302.24	91.96	83.73	44.13
17		Kaempferol	286.24	90.48	49.63	39.13
18		luteolin	286.24	78.34	67.12	11.20

19	Apigenin	270.24	85.94	65.82	46.85
20	Myricetin	318.24	102.49	88.39	68.05
21	Ampelopsin	320.25	88.15	85.03	73.58
22	Isorhamnetin	316.26	90.00	72.77	75.00
23	Genkwanin	284.26	98.38	109.26	116.02
24	Hesperetin	302.28	82.27	31.91	4.97
25	Pinocembrin	256.26	91.20	62.96	16.35
26	Alpinetin	270.28	87.87	74.98	51.01
27	Formononetin	268.27	112.73	110.56	93.93
28	Genistein	270.24	107.46	98.77	60.19
29	Naringenin	272.25	91.15	90.13	47.84
30	Oroxylin A	284.26	87.72	72.01	51.56
31	Baicalein	270.24	133.06	125.33	53.69
32	Liquiritigenin	256.25	128.83	115.75	93.07
33	Bavachin	324.37	91.18	85.44	57.15
34	Isobavachin	324.37	83.28	19.89	4.59
35	Silymarin	482.46	96.16	78.98	30.96
36	Licoflavonol	354.35	100.65	47.62	9.21
37	Isolicoflavonol	354.36	89.20	41.60	15.91
38	Isoxanthohumol	354.40	99.28	86.36	34.85
39	Biochanin A	284.26	104.87	99.08	79.69
40	Scutellarin	462.37	142.32	123.12	103.43

41		Liquiritin	418.39	120.64	123.33	117.70
42		Neoliquiritin	418.39	118.03	116.89	115.98
43		Liquiritin apioside	550.51	112.40	109.52	93.72
44		Tectorigenin	300.26	102.97	98.86	63.03
45		Neobavaisoflavone	322.36	97.34	49.60	6.38
46		Daidzein	254.24	113.14	110.27	99.91
47		Isoquercitrin	464.38	93.95	76.92	53.42
48		Naringin	580.53	95.45	91.02	99.55
49		Icariin	676.65	104.51	90.07	84.79
50		Calycosin-7-O-β-D-glucoside	446.40	115.31	104.57	97.32
51		Glycitin	446.41	98.37	103.69	102.69
52	**Triterpenoids**	Glycyrrhizic acid	822.93	129.79	134.53	140.22
53		Glycyrrhetinic acid	470.68	110.49	109.94	66.35
54		Betulinic acid	456.70	108.12	145.23	72.82
55		Epibetulinic acid	456.71	108.25	99.26	137.97
56		Oleanolic acid	456.71	117.30	115.94	83.03
57		Oleanonic acid	454.68	121.46	87.80	28.99
58		Ursolic acid	456.70	69.40	46.85	48.23
59		Corosolic acid	472.71	118.91	141.61	76.20
60		Betulonic acid	454.69	138.32	143.78	136.79
61		Celastrol	450.61	127.98	98.69	71.87
62		Toosendanin	574.62	95.45	90.48	96.07

63		(20S)-Protopanaxdiol	460.00	79.10	67.37	39.87
64		20(R)-Protopanaxatriol	476.74	74.61	56.62	37.17
65		Alisol B	472.70	102.23	99.74	107.40
66		Alisol B 23-acetate	514.74	92.07	99.23	105.62
67	**Coumarins**	Isoglycyrol	366.36	103.94	101.98	108.33
68		Glycycoumarin	368.37	88.24	86.58	17.51
69		Praeruptorin A	386.40	73.77	26.62	3.13
70		Isofraxidin	222.19	90.13	83.87	46.64
71		Xanthotoxin	216.19	80.54	63.14	22.08
72		Xanthotoxol	202.16	74.81	73.58	40.32
73		Bergapten	216.19	66.50	32.96	14.07
74	**Alkaloids**	L-Phenylalanine	165.19	78.76	81.93	82.12
75		Berberine	336.37	98.34	91.94	83.38
76		Epiberberine	336.36	96.28	88.24	86.46
77		Coptisine	320.32	97.95	85.25	75.22
78		Jatrorrhizine	338.38	86.24	82.96	50.07
79		N, N-Dimethyl-L-proline	144.19	99.56	100.62	100.64
80		Palmatine	352.40	99.49	97.61	93.84
81	**Fatty acids**	Propionic acid	74.08	94.79	87.76	91.25
82		Butyric Acid	88.11	97.62	98.94	89.71
83		2-hydroxybutyric acid	104.10	82.58	96.59	92.25
84		Valeric acid	102.13	84.78	83.24	83.33

85		3-Methylvaleric acid	116.16	89.59	90.01	91.43
86		Octanoic acid	144.21	104.29	105.17	103.84
87		Lauric acid	200.31	106.26	106.54	91.05
88		Palmitic acid	256.42	109.74	107.39	98.32
89		Oleic acid	282.46	102.46	83.67	53.49
90		Linoleic acid	280.45	103.64	76.52	33.37
91		Arachidonic acid	304.47	106.26	52.34	5.78
92		Dehydroepiandrosterone	288.42	109.62	67.72	24.75
93	**Others**	Arctigenin	372.41	89.64	93.39	43.87
94		Magnolol	266.33	80.18	37.70	4.89
95		Honokiol	266.34	80.02	34.19	3.80
96		Caffeic acid	180.15	100.82	95.43	102.29
97		Phenylpyruvic Acid	164.16	94.07	81.44	32.88
98		D-3-Phenyllactic Acid	166.17	96.95	94.35	100.02
99		L-(-)-3-Phenyllactic acid	166.17	91.29	83.14	86.93
100		Bilobalide	326.30	107.10	105.91	100.11
101		Ginkgolide A	408.40	106.97	105.42	96.88
102		Ginkgolide B	424.40	102.60	105.51	98.36
103		Ginkgolide C	440.40	102.72	99.00	104.42
104		Salicylic acid	138.12	92.21	95.22	91.79
105		Gancaonin I	354.40	72.82	18.86	20.94
106		Phenylacetic acid	136.15	99.45	100.57	98.48

107		2-Hydroxyphenylacetic Acid	152.15	98.21	95.02	98.16
108		Resveratrol	228.24	78.39	76.50	67.37
109		Ginkgolic acid C13:0	320.47	81.99	23.48	-
110		Ginkgolic acid C17:1	374.56	78.84	9.13	-
111	**Positive inhibitor**	CAPE	284.31	100.67	69.41	39.62

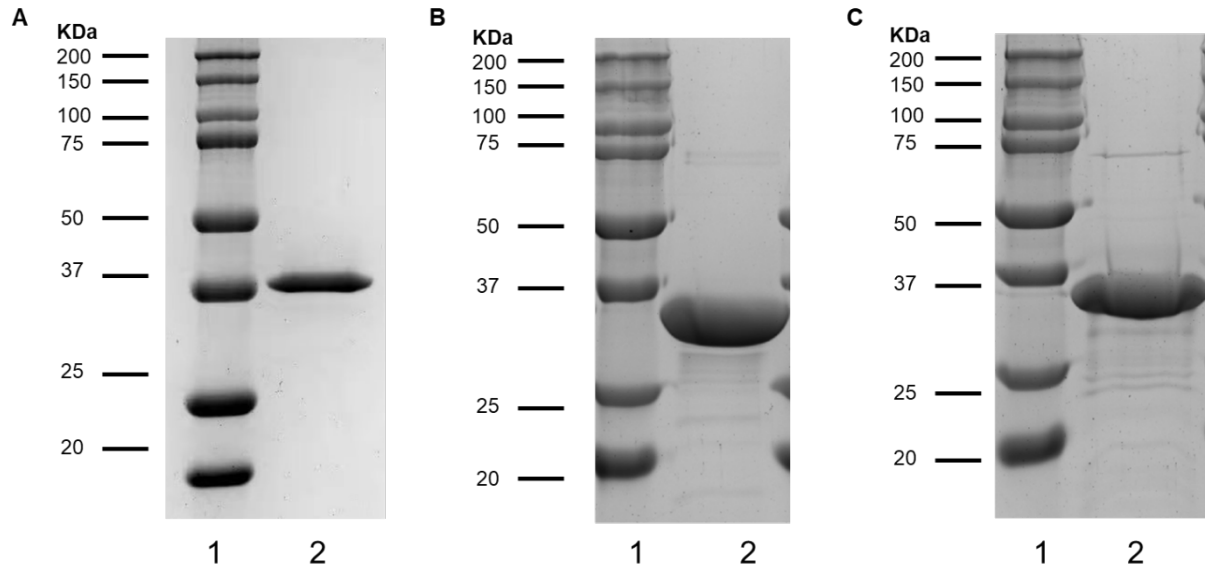

Fig. S1 SDS-PAGE analysis of lsBSH (A), btBSH (B)and efBSH (C). (1. Marker; 2. The enzyme purified on a Superdex 200 10/300 GL column).

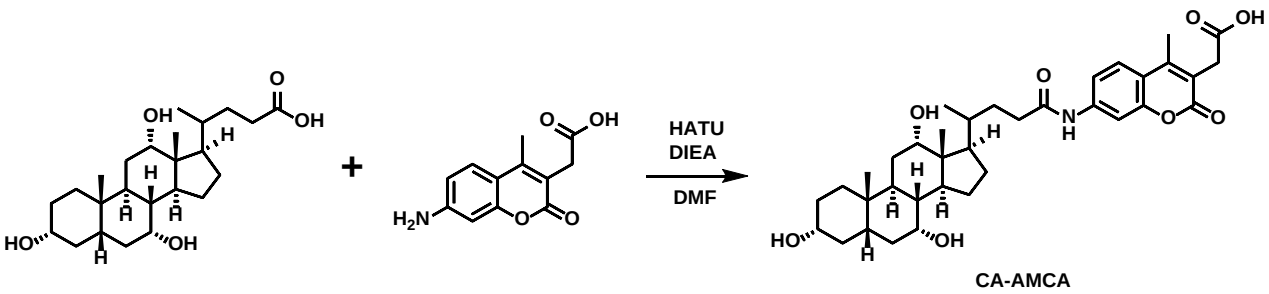

Fig S2 The synthetic procedure of CA-AMCA.

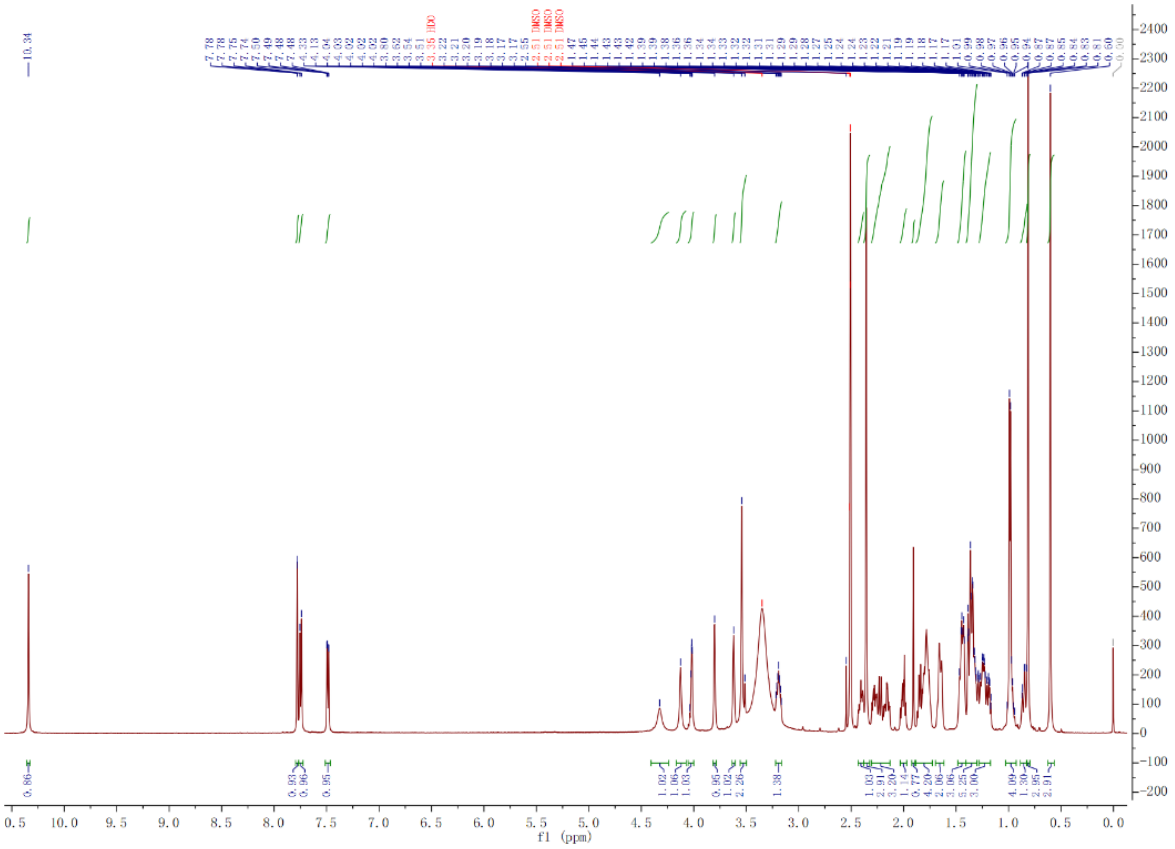

Fig.S3 ^{1}H NMR (600 MHz, DMSO-d6) spectrum of CA-AMCA.

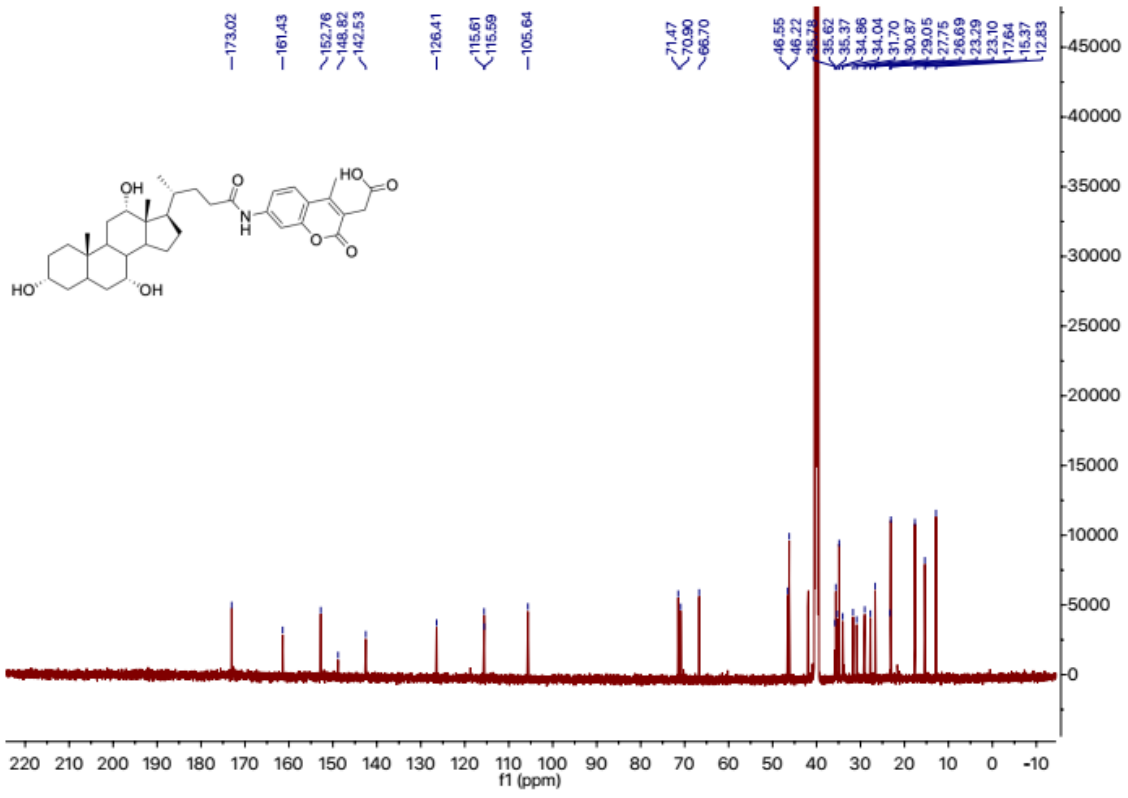

Fig.S4 ^{13}C NMR (150 MHz, DMSO-d6) spectrum of CA-AMCA.

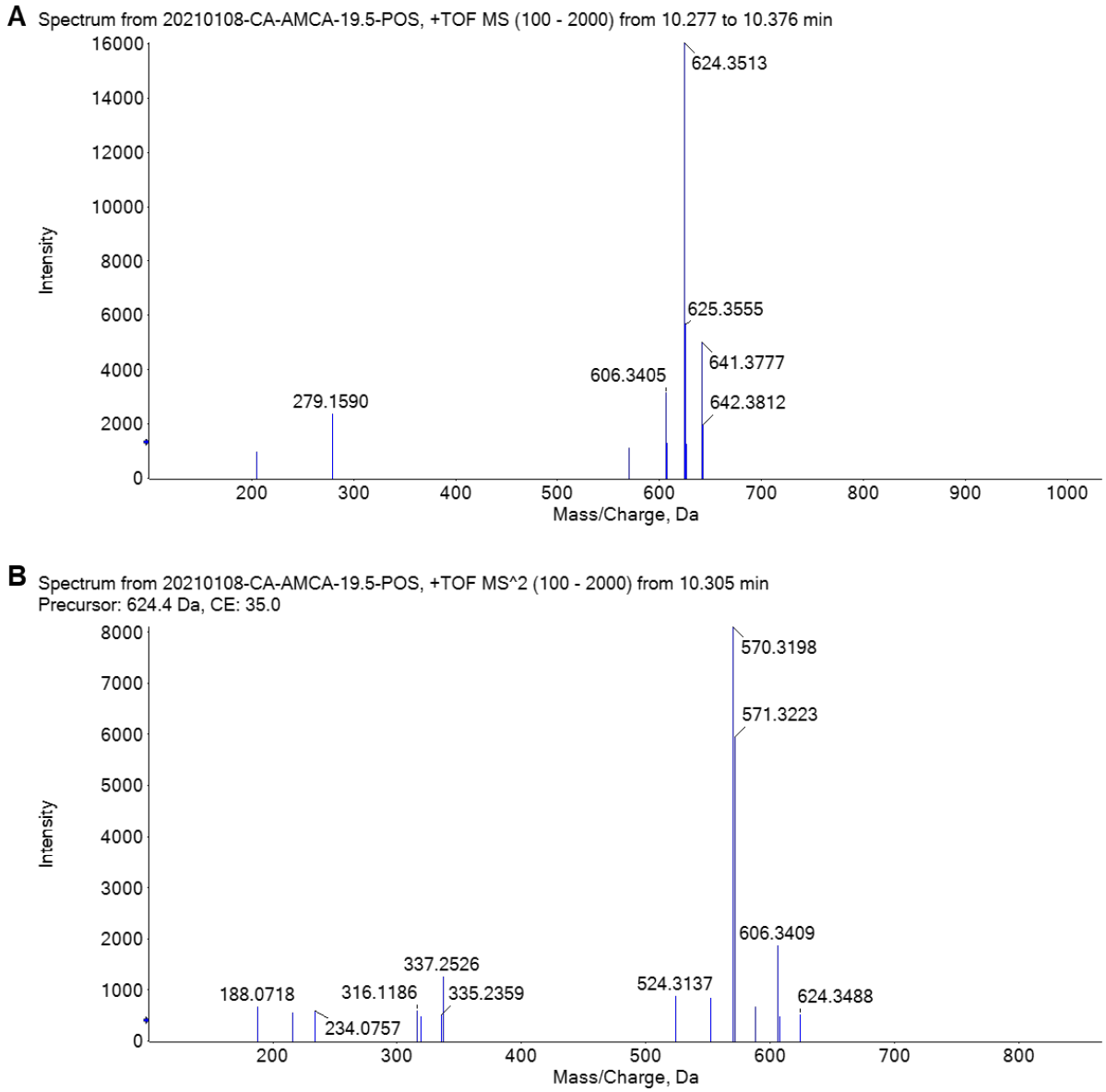

Fig. S5 MS^1 (A) and MS^2 (B) spectra of CA-AMCA.

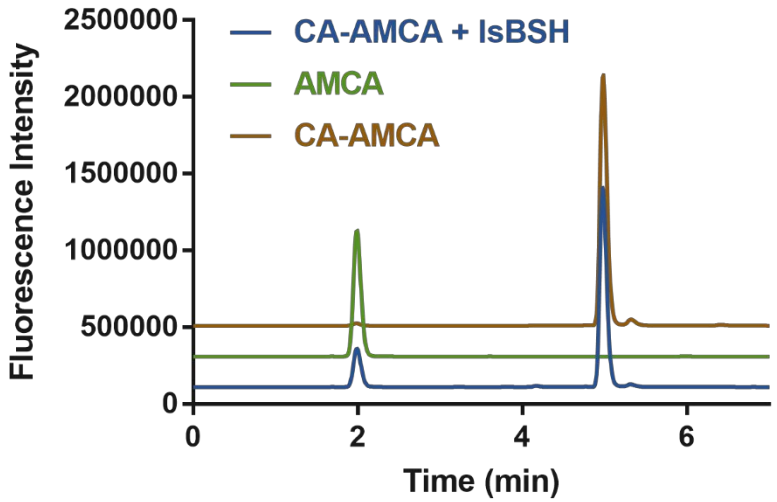

Fig. S6 Liquid chromatography-fluorescence detection (LC-FD) chromatograms of AMCA and CA-AMCA. (Brown) CA-AMCA only, (Green) AMCA only, (Blue) CA-AMCA was co-incubated with lsBSH (2 μg/mL) at 37 ℃ for 30 min. The fluorescence signals of AMCA and CA-AMCA were recorded using excitation wavelength of 345 nm and emission wavelength of 455 nm.

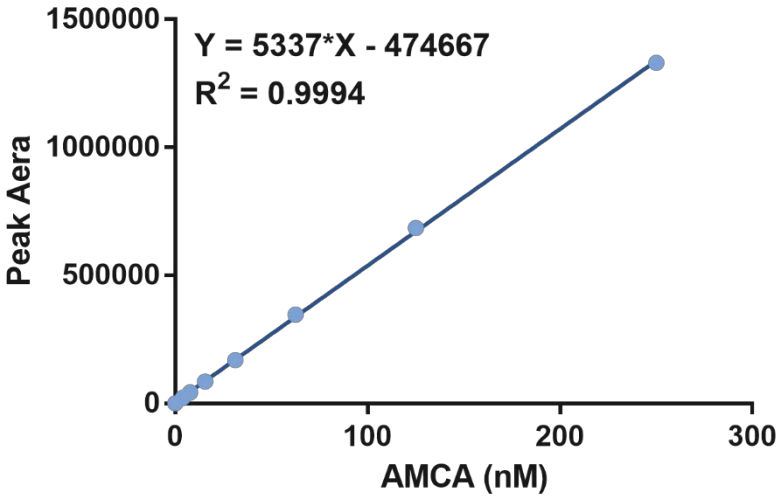

Fig. S7 The standard curve of AMCA (the hydrolytic metabolite of CA-AMCA). All data were shown as mean ± SD of triplicate determinations.

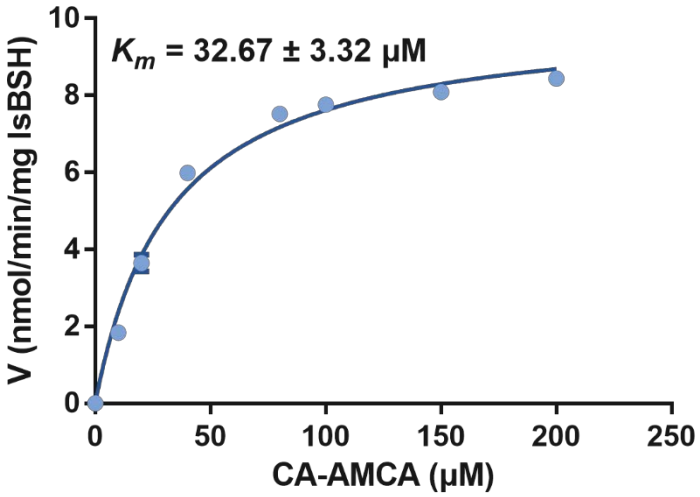

Fig. S8 Enzymatic kinetic plot of lsBSH-catalyzed CA-AMCA hydrolysis. All data were shown as mean ± SD of triplicate determinations.

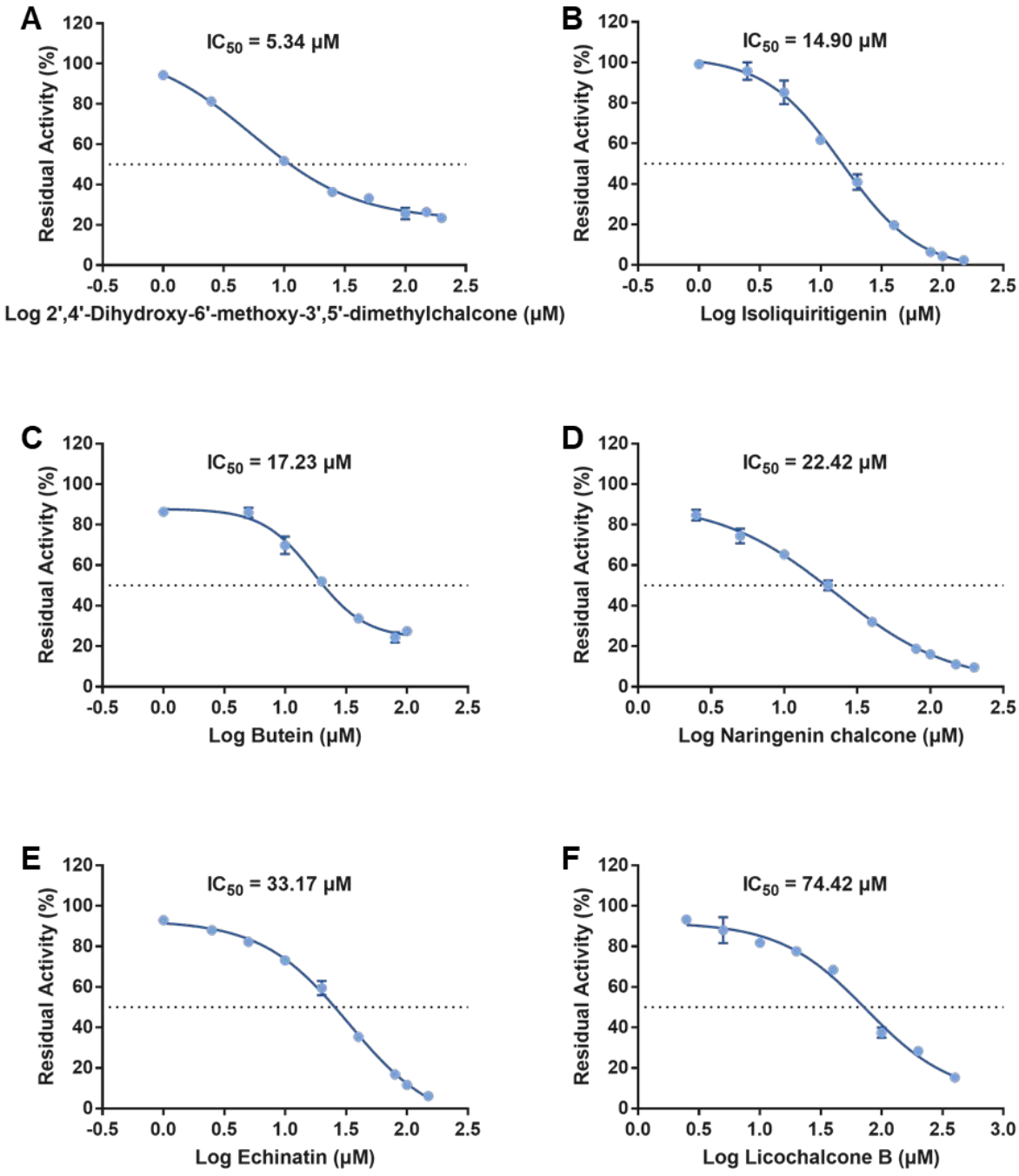

Fig. S9 The dose-inhibition curves of six natural chalcones (IC_{50} > 5 μM) against lsBSH-catalyzed CA-AMCA hydrolysis. All data were shown as mean ± SD of triplicate determinations.

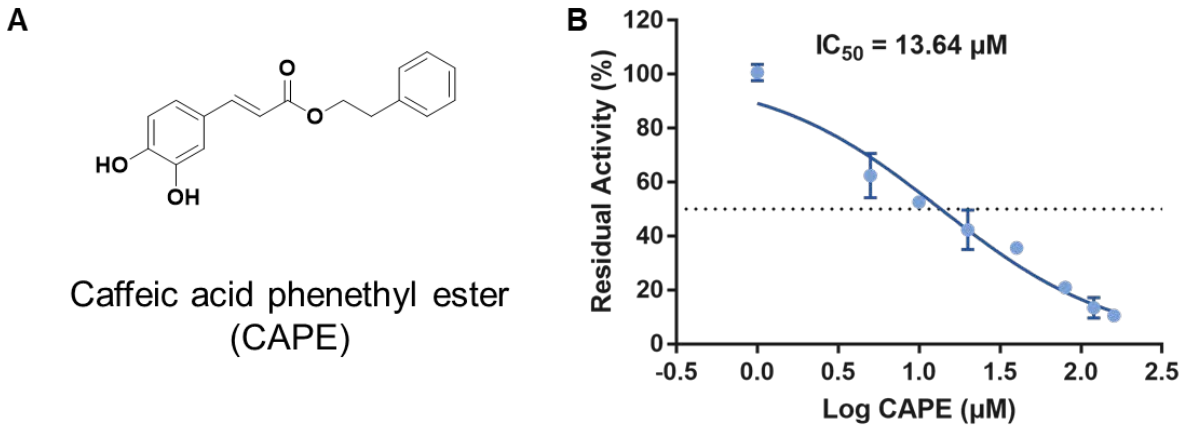

Fig. S10 (A) The name and structure of CAPE. (B)The dose-inhibition curve of CAPE against lsBSH-catalyzed CA-AMCA hydrolysis. All data were shown as mean ± SD of triplicate determinations.

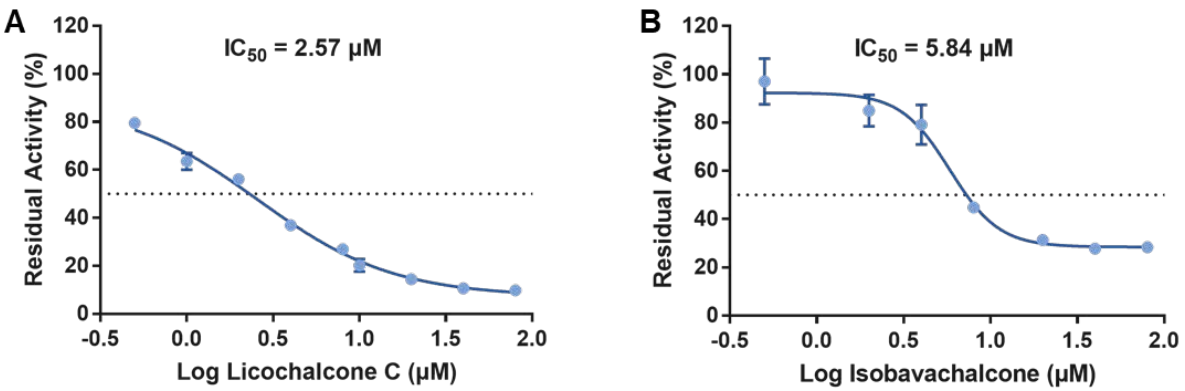

Fig. S11 The dose-inhibition curve of licochalcone C (A) and isobavachalcone (B) against lsBSH-catalyzed TCA hydrolysis. All data were shown as mean ± SD of triplicate determinations.

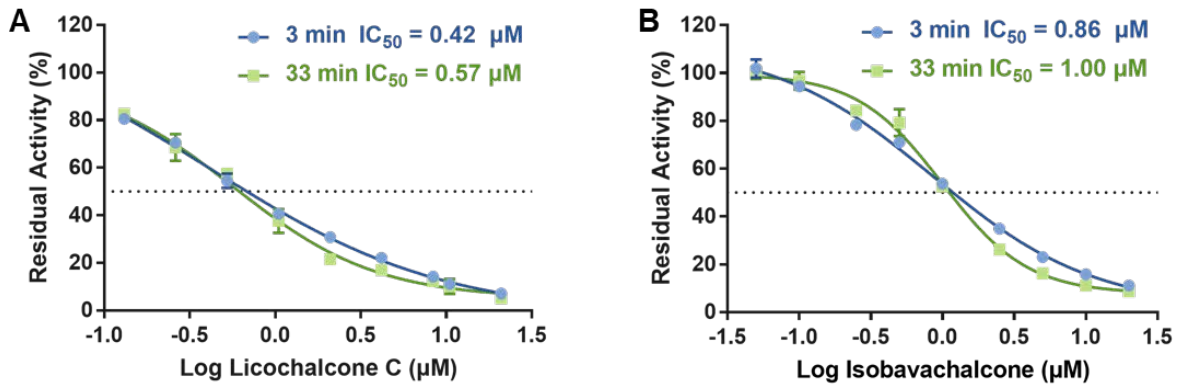

Fig. S12 The residual activity of lsBSH-catalyzed CA-AMCA hydrolysis in the presence of different concentrations of licochalcone C (A) and isobavachalcone (B) at different pre-incubation times of 3 min and 33 min. All data were shown as mean ± SD of triplicate determinations.

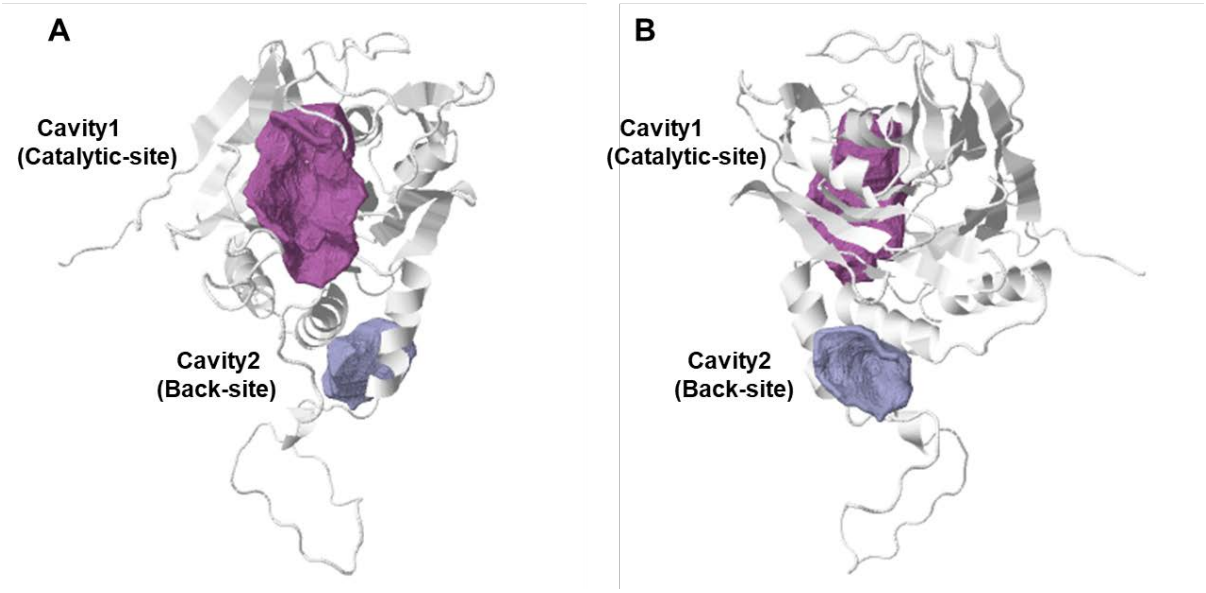

Fig. S13 Two possible ligand-binding pockets were predicted with CavityPlus. (A) Front view, (B) Back view.

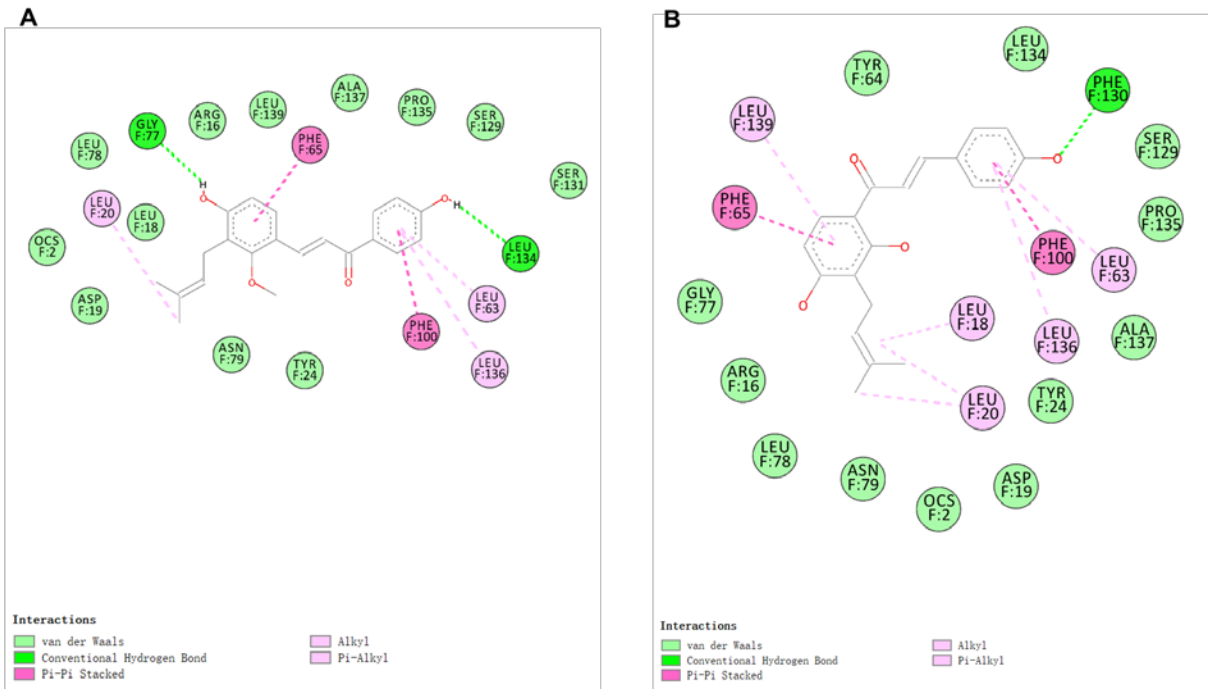

Fig. S14 The detailed 2D interactions of licochalcone C (A) and isobavachalcone (B) with lsBSH in the catalytic site.

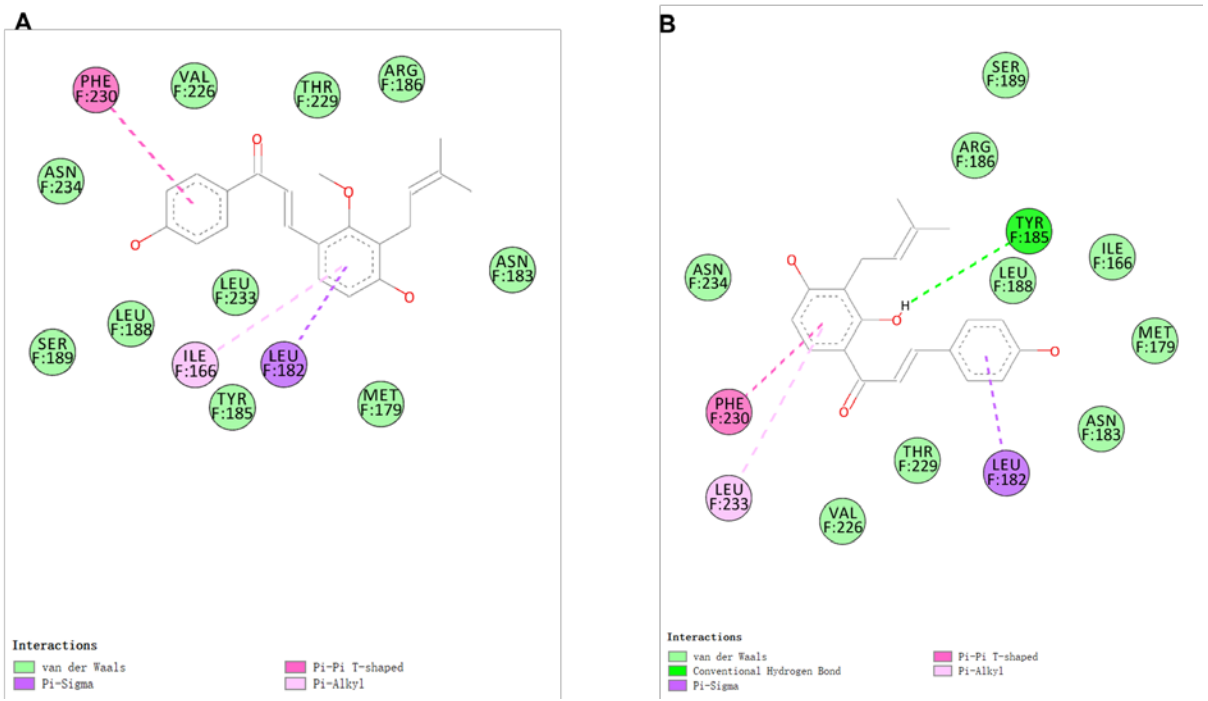

Fig. S15 The detailed 2D interactions of licochalcone C (A) and isobavachalcone (B) with lsBSH in the back site.