Supplementary Materials

for

Discovery and characterization of amentoflavone as a naturally occurring inhibitor against the bile salt hydrolase produced by *Lactobacillus salivarius*

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This file contains two supplementary tables and thirteen supplementary figures.

Table S1. The inhibitory effects of more than 100 kinds of natural products on lsBSH.

No.	Class	Compound	MW	Residual Activity (%)		
				1 μM	10 μM	100 μM
1	**Biflavones**	Amentoflavone	538.46	33.83	21.37	6.64
2		Hinokiflavone	538.46	78.45	55.99	32.09
3		Ginkgetin	566.51	70.43	52.24	26.52
4		Isoginkgetin	566.51	67.66	53.00	20.79
5		Bilobetin	552.48	77.40	52.68	26.02
6		Sciadopitysin	580.55	64.65	59.77	31.58
7	**Flavonoids**	Quercetin	302.24	91.96	83.73	44.13
8		Kaempferol	286.24	90.48	49.63	39.13
9		luteolin	286.24	78.34	67.12	11.20
10		Apigenin	270.24	85.94	65.82	46.85
11		Myricetin	318.24	102.49	88.39	68.05
12		Ampelopsin	320.25	88.15	85.03	73.58
13		Isorhamnetin	316.26	90.00	72.77	75.00
14		Genkwanin	284.26	98.38	109.26	116.02
15		Hesperetin	302.28	82.27	31.91	4.97
16		Pinocembrin	256.26	91.20	62.96	16.35
17		Alpinetin	270.28	87.87	74.98	51.01
18		Formononetin	268.27	112.73	110.56	93.93
19		Genistein	270.24	107.46	98.77	60.19
20		Naringenin	272.25	91.15	90.13	47.84

21	Oroxylin A	284.26	87.72	72.01	51.56
22	Baicalein	270.24	133.06	125.33	53.69
23	Liquiritigenin	256.25	128.83	115.75	93.07
24	Bavachin	324.37	91.18	85.44	57.15
25	Isobavachin	324.37	83.28	19.89	4.59
26	Silymarin	482.46	96.16	78.98	30.96
27	Licoflavonol	354.35	100.65	47.62	9.21
28	Isolicoflavonol	354.36	89.20	41.60	15.91
29	Isoxanthohumol	354.40	99.28	86.36	34.85
30	Biochanin A	284.26	104.87	99.08	79.69
31	Scutellarin	462.37	142.32	123.12	103.43
32	Liquiritin	418.39	120.64	123.33	117.70
33	Neoliquiritin	418.39	118.03	116.89	115.98
34	Liquiritin apioside	550.51	112.40	109.52	93.72
35	Tectorigenin	300.26	102.97	98.86	63.03
36	Neobavaisoflavone	322.36	97.34	49.60	6.38
37	Daidzein	254.24	113.14	110.27	99.91
38	Isoquercitrin	464.38	93.95	76.92	53.42
39	Naringin	580.53	95.45	91.02	99.55
40	Icariin	676.65	104.51	90.07	84.79
41	Calycosin-7-O-β-D-glucoside	446.40	115.31	104.57	97.32
42	Glycitin	446.41	98.37	103.69	102.69

43		Isoliquiritin	418.39	109.86	113.27	86.73
44		Neoisoliquiritin	418.39	116.22	115.98	102.87
45		Phloretin	274.27	88.64	95.23	86.00
46		Phlorizin	436.41	61.89	57.33	66.47
47	**Triterpenoids**	Glycyrrhizic acid	822.93	129.79	134.53	140.22
48		Glycyrrhetinic acid	470.68	110.49	109.94	66.35
49		Betulinic acid	456.70	108.12	145.23	72.82
50		Epibetulinic acid	456.71	108.25	99.26	137.97
51		Oleanolic acid	456.71	117.30	115.94	83.03
52		Oleanonic acid	454.68	121.46	87.80	28.99
53		Ursolic acid	456.70	69.40	46.85	48.23
54		Corosolic acid	472.71	118.91	141.61	76.20
55		Betulonic acid	454.69	138.32	143.78	136.79
56		Celastrol	450.61	127.98	98.69	71.87
57		Toosendanin	574.62	95.45	90.48	96.07
58		(20S)-Protopanaxdiol	460.00	79.10	67.37	39.87
59		20(R)-Protopanaxatriol	476.74	74.61	56.62	37.17
60		Alisol B	472.70	102.23	99.74	107.40
61		Alisol B 23-acetate	514.74	92.07	99.23	105.62
62	**Coumarins**	Isoglycyrol	366.36	103.94	101.98	108.33
63		Glycycoumarin	368.37	88.24	86.58	17.51
64		Praeruptorin A	386.40	73.77	26.62	3.13

65		Isofraxidin	222.19	90.13	83.87	46.64
66		Xanthotoxin	216.19	80.54	63.14	22.08
67		Xanthotoxol	202.16	74.81	73.58	40.32
68		Bergapten	216.19	66.50	32.96	14.07
69	**Alkaloids**	L-Phenylalanine	165.19	78.76	81.93	82.12
70		Berberine	336.37	98.34	91.94	83.38
71		Epiberberine	336.36	96.28	88.24	86.46
72		Coptisine	320.32	97.95	85.25	75.22
73		Jatrorrhizine	338.38	86.24	82.96	50.07
74		N, N-Dimethyl-L-proline	144.19	99.56	100.62	100.64
75		Palmatine	352.40	99.49	97.61	93.84
76	**Fatty acids**	Propionic acid	74.08	94.79	87.76	91.25
77		Butyric Acid	88.11	97.62	98.94	89.71
78		2-hydroxybutyric acid	104.10	82.58	96.59	92.25
79		Valeric acid	102.13	84.78	83.24	83.33
80		3-Methylvaleric acid	116.16	89.59	90.01	91.43
81		Octanoic acid	144.21	104.29	105.17	103.84
82		Lauric acid	200.31	106.26	106.54	91.05
83		Palmitic acid	256.42	109.74	107.39	98.32
84		Oleic acid	282.46	102.46	83.67	53.49
85		Linoleic acid	280.45	103.64	76.52	33.37
86		Arachidonic acid	304.47	106.26	52.34	5.78

87		Dehydroepiandrosterone	288.42	109.62	67.72	24.75
88	**Others**	Arctigenin	372.41	89.64	93.39	43.87
89		Magnolol	266.33	80.18	37.70	4.89
90		Honokiol	266.34	80.02	34.19	3.80
91		Caffeic acid	180.15	100.82	95.43	102.29
92		Phenylpyruvic Acid	164.16	94.07	81.44	32.88
93		D-3-Phenyllactic Acid	166.17	96.95	94.35	100.02
94		L-(-)-3-Phenyllactic acid	166.17	91.29	83.14	86.93
95		Bilobalide	326.30	107.10	105.91	100.11
96		Ginkgolide A	408.40	106.97	105.42	96.88
97		Ginkgolide B	424.40	102.60	105.51	98.36
98		Ginkgolide C	440.40	102.72	99.00	104.42
99		Salicylic acid	138.12	92.21	95.22	91.79
100		Gancaonin I	354.40	72.82	18.86	20.94
101		Phenylacetic acid	136.15	99.45	100.57	98.48
102		2-Hydroxyphenylacetic Acid	152.15	98.21	95.02	98.16
103		Resveratrol	228.24	78.39	76.50	67.37
104		Ginkgolic acid C13:0	320.47	81.99	23.48	-
105		Ginkgolic acid C17:1	374.56	78.84	9.13	-
106	**Positive inhibitor**	CAPE	284.31	100.67	69.41	39.62

Table S2. The results of CavityPlus calculation. 5y7p

No.	Pred.Avg pKd	Druggability
1	6.94	Strong
2	6.66	Medium

Pred.Avg pKd: The average value indicates the ligandability of a cavity binding site. A value less than 6.0 suggests that this site may not be a suitable ligand-binding site. Druggability indicates the possibility of a cavity binding site to be druggable or not.

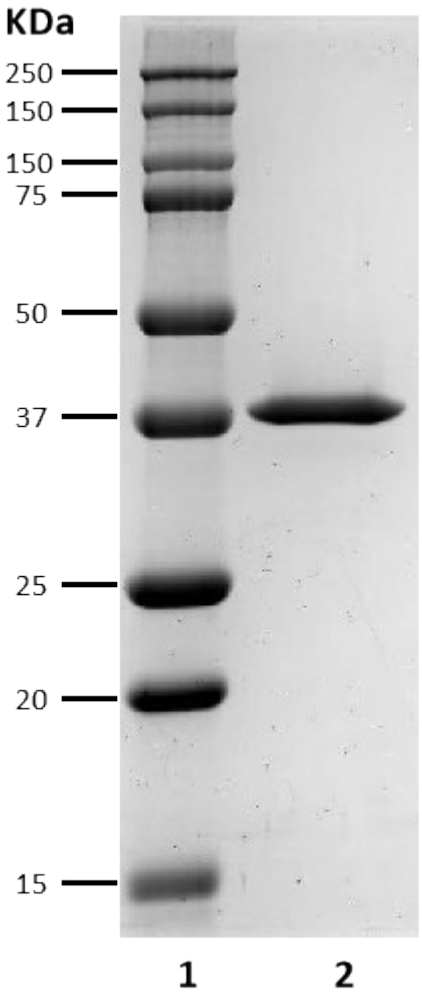

Fig. S1 SDS-PAGE analysis of the purification of lsBSH. (1. Marker; 2. Purified on a Superdex 200 10/300 GL column).

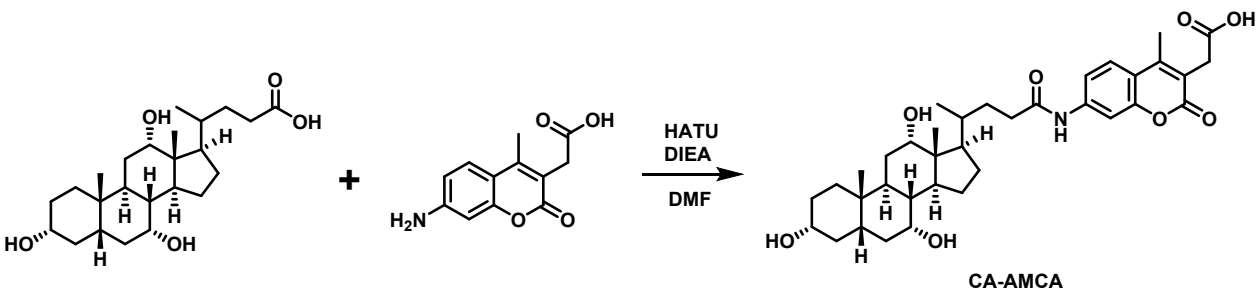

Fig S2 The synthetic procedure of CA-AMCA.

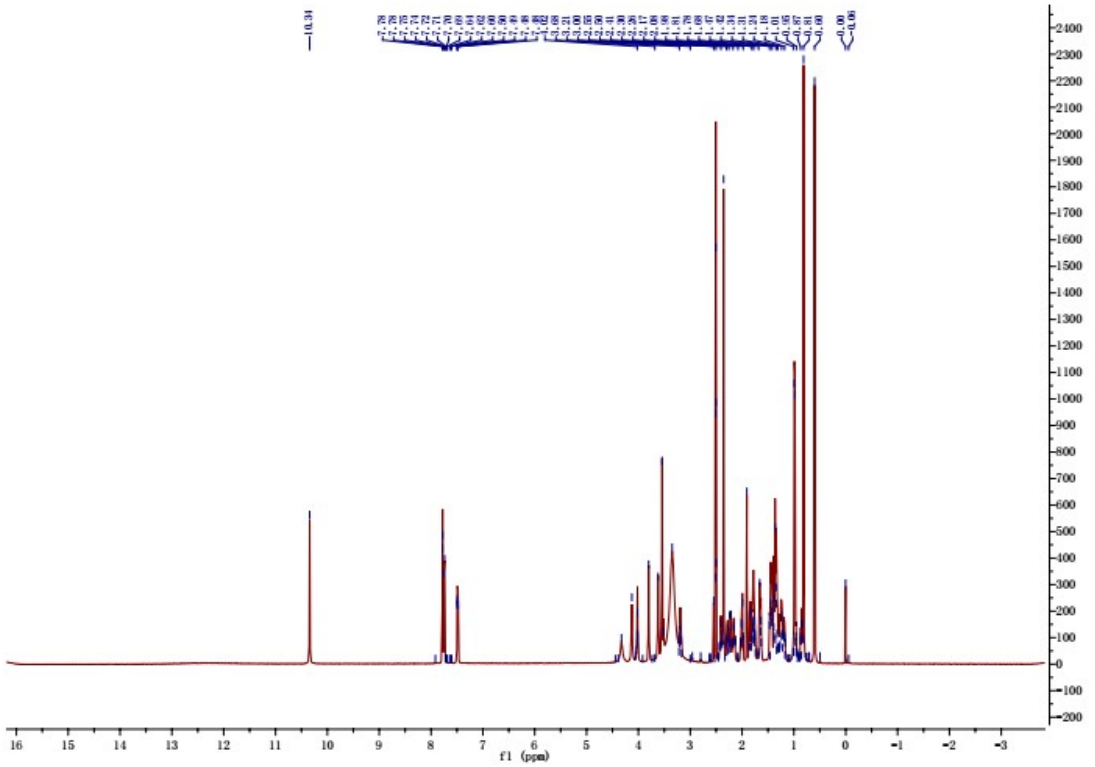

Fig.S3 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of CA-AMCA.

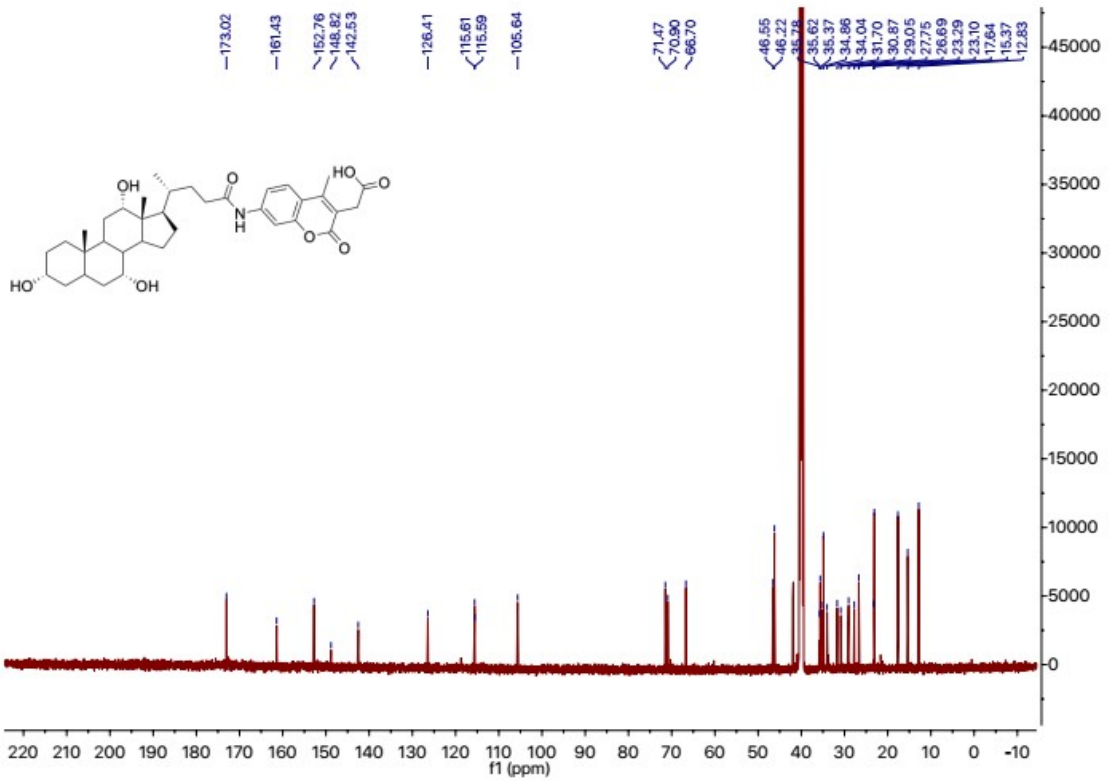

Fig.S4 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of CA-AMCA.

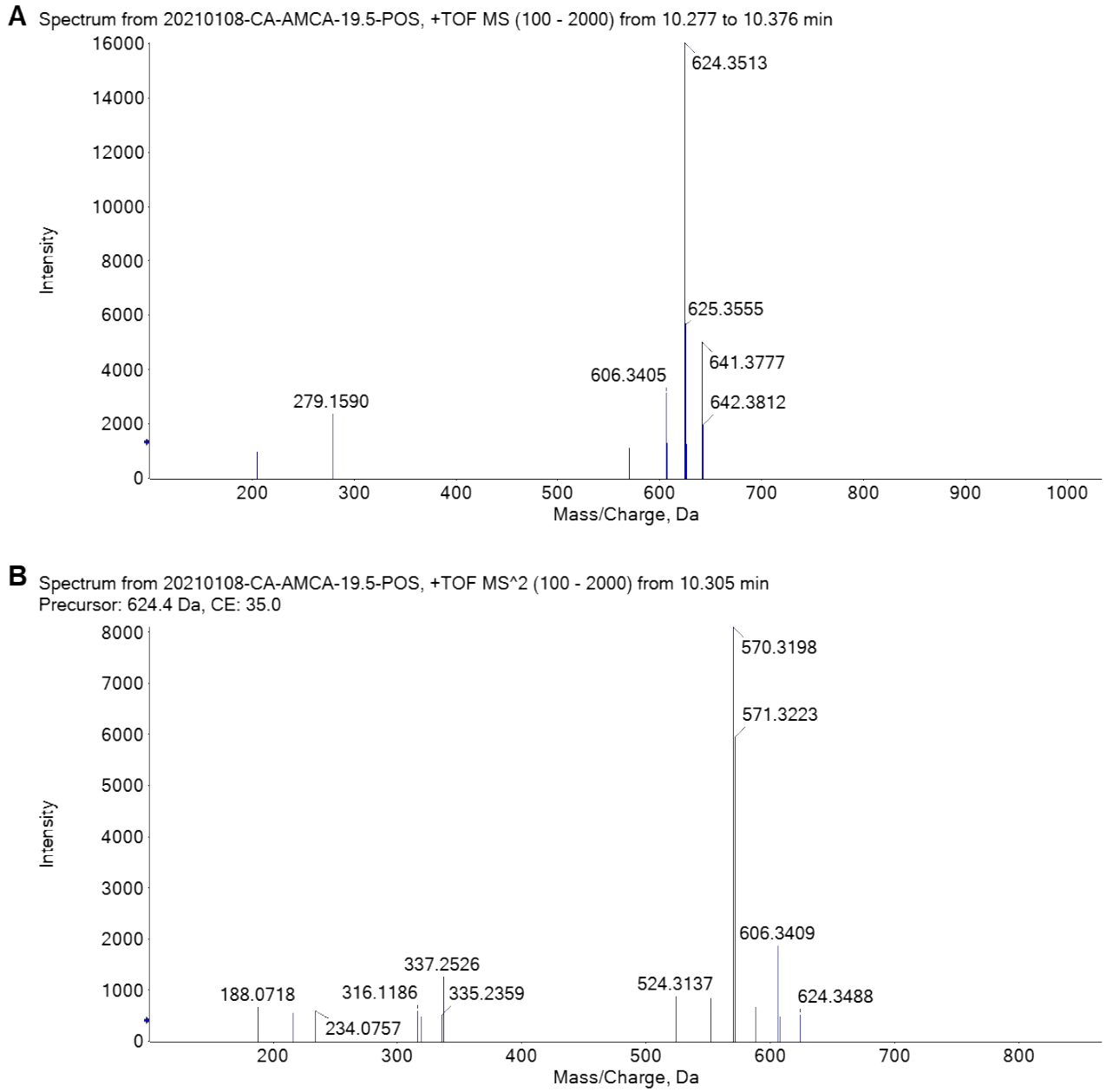

Fig. S5 MS^1 (A) and MS^2 (B) spectra of CA-AMCA.

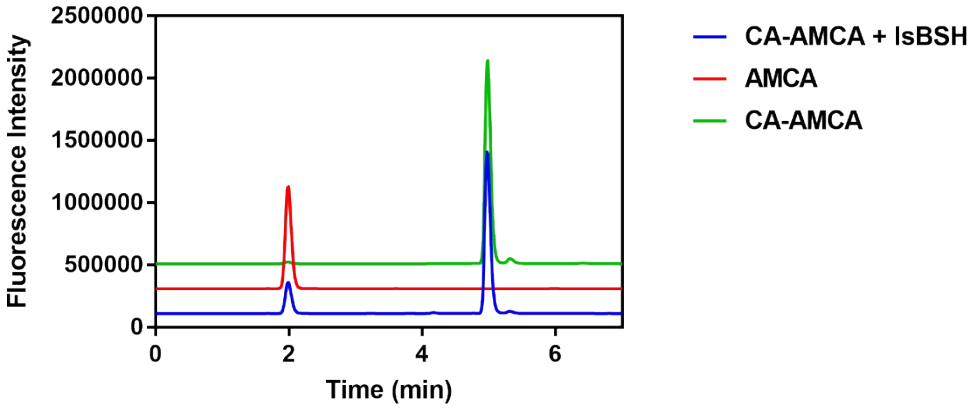

Fig. S6 Liquid chromatography-fluorescence detection (LC-FD) chromatograms of AMCA and CA-AMCA. (Green) CA-AMCA only, (Red) AMCA only, (Blue) CA-AMCA was co-incubated with lsBSH (2 μg/mL) at 37 ℃ for 30 min. The fluorescence signals of AMCA and CA-AMCA were recorded using excitation wavelength of 345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

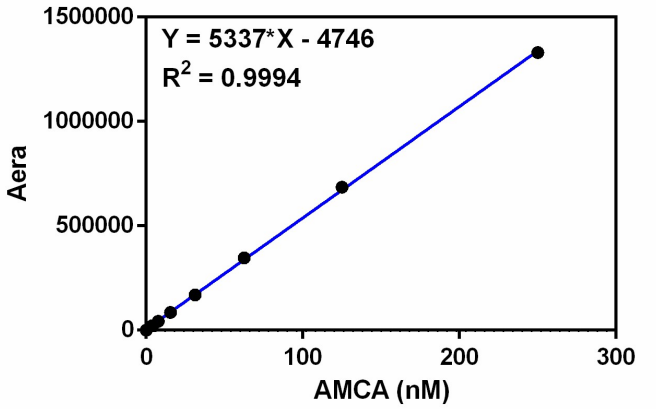

Fig. S7 The standard curve of AMCA (the hydrolytic metabolite of CA-AMCA).

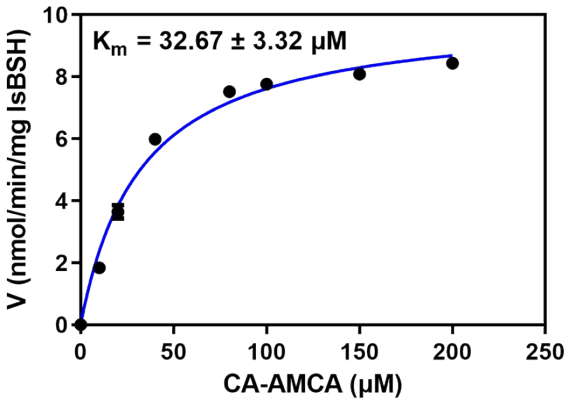

Fig. S8 Enzymatic kinetic plot of lsBSH-catalyzed CA-AMCA hydrolysis.

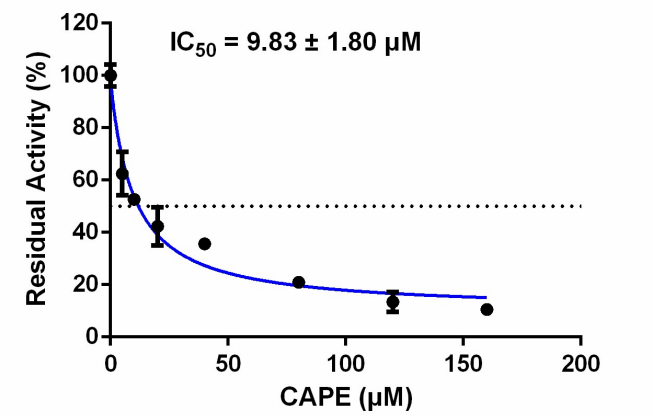

Fig. S9 The dose-inhibition curve of CAPE against lsBSH-catalyzed CA-AMCA hydrolysis.

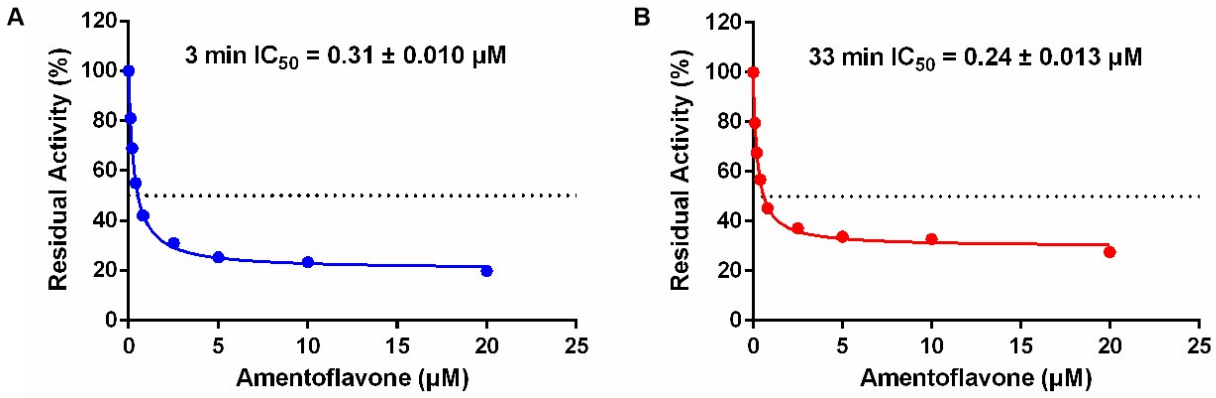

Fig. S10 The residual activity of lsBSH-catalyzed CA-AMCA hydrolysis in the presence of different concentrations of AMF at different pre-incubation times of 3 minutes (A) and 33 minutes (B). All data were shown as mean ± SD of triplicate determinations.

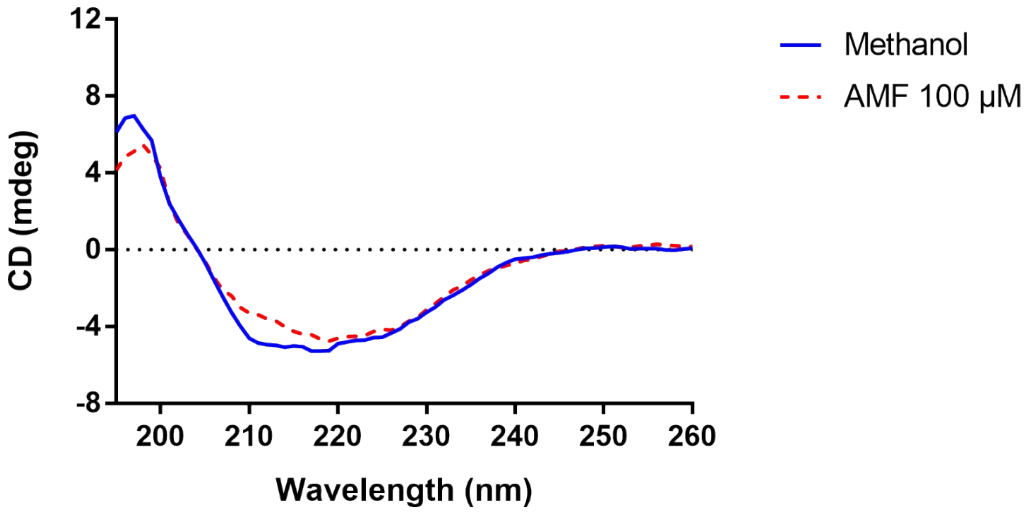

Fig. S11 Circular dichroism spectra of lsBSH and lsBSH-AMF complex.

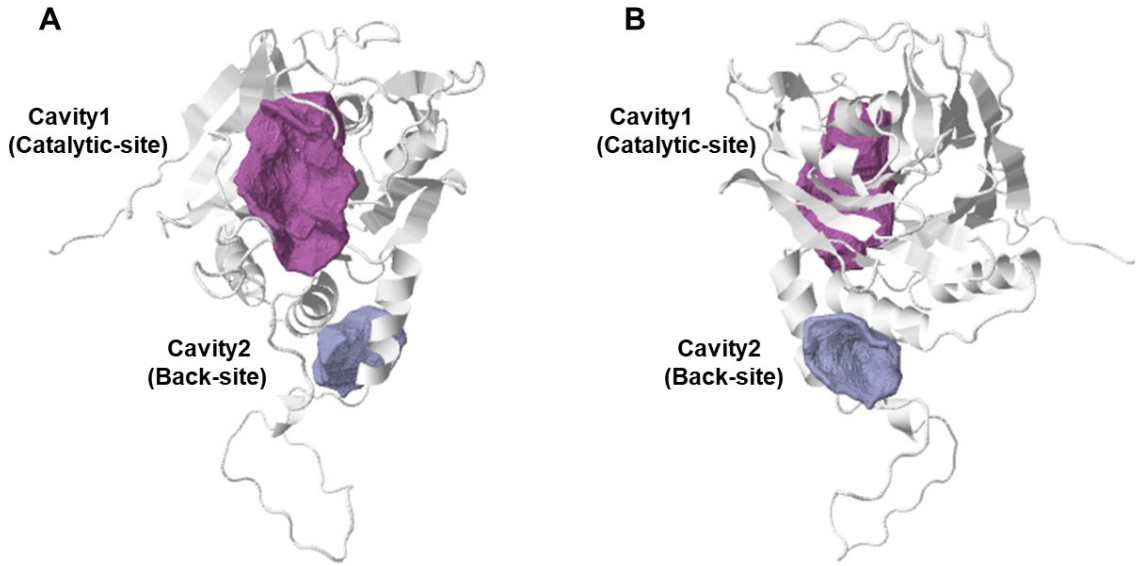

Fig. S12 Two possible ligand-binding pockets were predicted with CavityPlus. (A) Front view, (B) Back view.

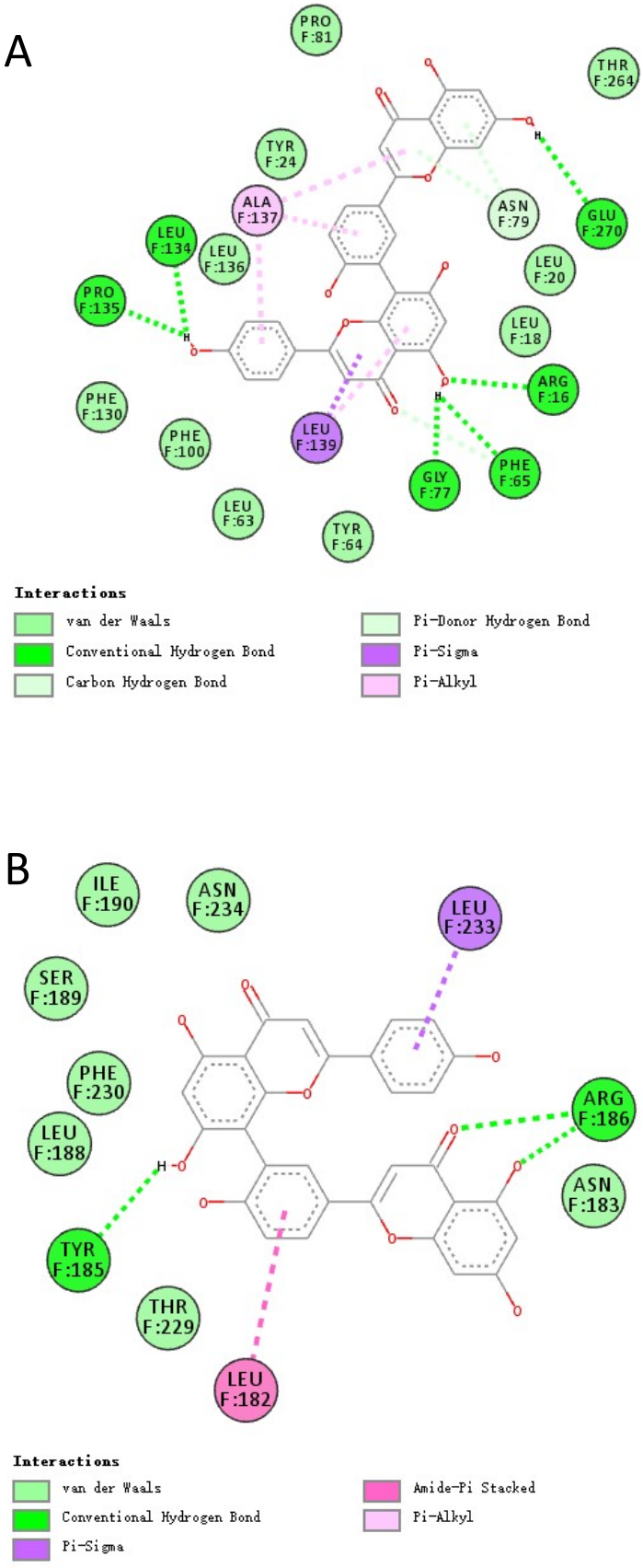

Fig. S13 2D interactions of AMF with lsBSH in the catalytic site (A) and back site (B).