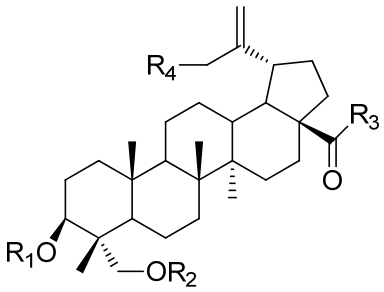
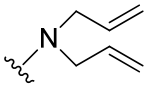
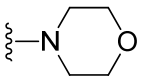
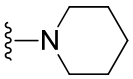
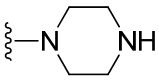
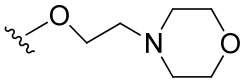
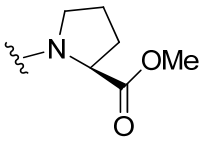
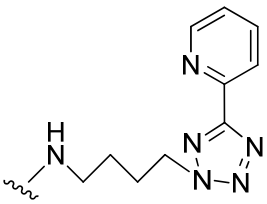
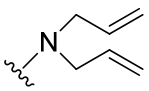
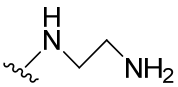
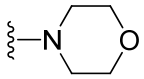
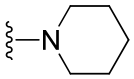
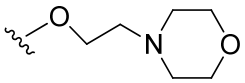
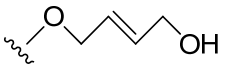
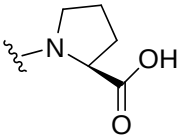
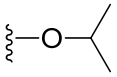
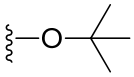
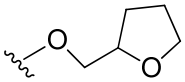
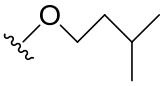
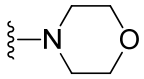
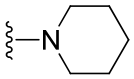
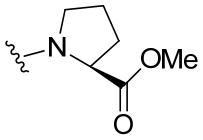
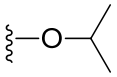
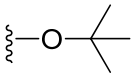
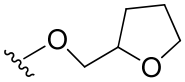
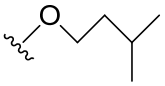
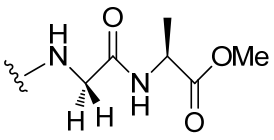
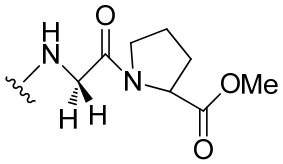
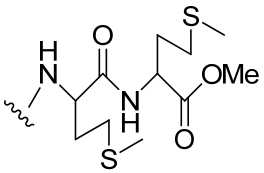
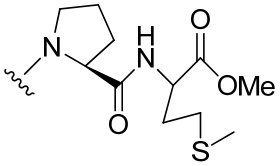
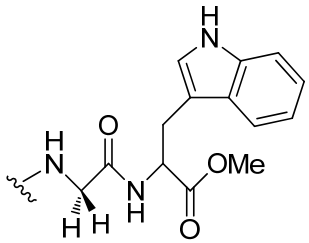
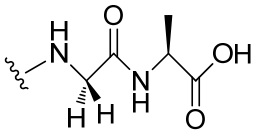
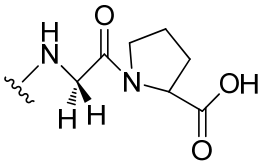
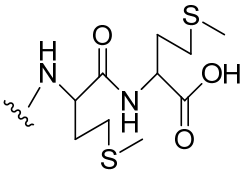
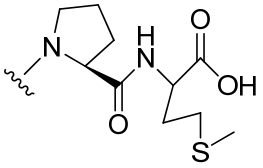
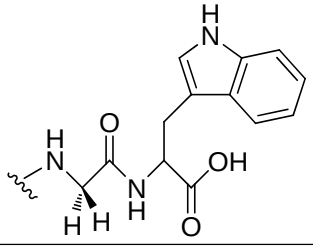
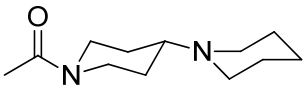
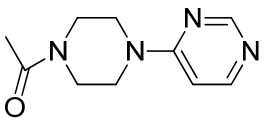
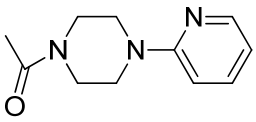
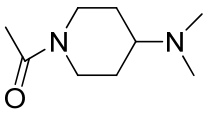
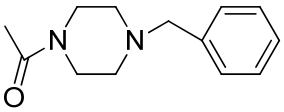
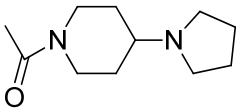
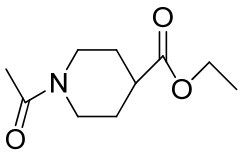
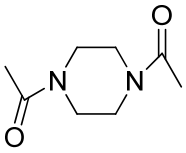
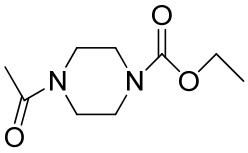
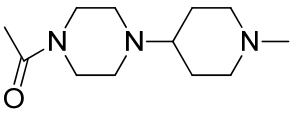
Supplementary Table 1 MDR reversal activities of 23-HBA derivatives in HepG2/ADM cells



Compd.[a] No.	Substituent				HepG2/ADM Inhibition rate (%)[b]		
	R1	R2	R3	R4	Compd.	DOX	Combined Treatment
23-HBA	H	H	OH	H	9.78 ± 3.54	14.72±2.93	28.78±3.33
1	CH_3CO	CH_3CO		H	4.36 ±1.51	12.41±0.19	13.93±5.47
2	CH_3CO	CH_3CO		H	18.5 ± 4.36	14.18±2.90	29.79±4.47
3	CH_3CO	CH_3CO		H	7.44 ± 0.46	12.51±0.11	8.9±4.22
4	CH_3CO	CH_3CO		H	3.74 ± 1.20	12.51±0.11	10.49±3.88
5	CH_3CO	CH_3CO		H	1.02 ± 0.23	10.61±6.56	21.03±6.11
6	CH_3CO	CH_3CO		H	0.56 ± 0.12	13.86±6.23	26.68±6.65
7	CH_3CO	CH_3CO		H	12.27 ± 6.43	12.38±6.63	25.91±11.23
8	H	H		H	8.60±2.12	12.38±6.63	72.43±6.63
9	H	H		H	3.76 ± 1.05	9.24±3.47	16.89±5.56
10	H	H		H	21.71 ± 7.38	5.03±1.45	59.88±4.86

11	H	H		H	8.39 ± 4.23	9.24±4.21	32.34±3.45
12	H	H		H	6.84 ± 2.54	8.62±3.24	23.37±5.68
13	H	H		H	11.93 ± 1.62	11.42±2.84	29.84±7.95
14	H	H		H	9.94 ± 3.80	10.93±4.47	19.98±5.44
15	H	H		H	1.57 ± 0.45	15.15±2.94	23.64±5.86
16	CH_3CO	CH_3CO		OH	8.35 ± 1.62	11.83±2.12	36.54±9.67
17	CH_3CO	CH_3CO		OH	1.61 ± 0.57	11.83±2.12	36.52±5.45
18	CH_3CO	CH_3CO		OH	6.17 ± 1.98	11.83±2.12	55.47±4.69
19	CH_3CO	CH_3CO		OH	10.79 ± 0.24	11.83±2.12	20.22±2.89
20	CH_3CO	CH_3CO		OH	13.03 ± 1.89	11.83±2.12	70.88 ± 2.12
21	CH_3CO	CH_3CO		OH	13.94 ± 1.31	11.83±2.12	29.26±6.22
22	CH_3CO	CH_3CO		OH	17.67 ± 2.79	11.52±4.67	59.59±5.76
23	H	H		OH	6.88 ± 2.25	16.66±2.31	32.25±2.07
24	H	H		OH	4.59 ± 1.25	16.66±2.31	34.14±3.79
25	H	H		OH	9.44 ± 4.54	16.66±2.31	25.11±5.34
26	H	H		OH	11.81± 4.32	16.66±2.31	33.27±7.09
27	H	H		H	4.56 ± 3.21	11.98±3.31	36.38±7.38

28	H	H		H	9.11 ± 1.26	11.98±3.31	62.21±2.76
29	H	H		H	9.62 ± 4.65	11.98±3.31	13.78±6.41
30	H	H		H	6.03 ± 2.83	11.98±3.31	27.89±10.74
31	H	H		H	3.72 ± 0.82	10.74±1.44	21.67±8.70
32	H	H		H	8.82 ± 4.32	13.34±5.10	37.65±12.32
33	H	H		H	1.20 ± 0.83	13.34±5.10	17.41±7.81
34	H	H		H	5.24 ± 3.12	13.34±5.10	14.93±1.52
35	H	H		H	2.31 ± 1.29	13.34±5.10	14.1±5.74
36	H	H		H	6.58 ± 0.93	12.43±3.32	15.66±7.49

37[c]	H		OH	H	8.93 ± 1.84	14.72±2.93	80.69±1.81
38	H		OH	H	0.51±0.11	10.05±2.31	12.65±2.32
39	H		OH	H	1.13±0.25	10.05±2.31	7.68±1.45
40	H		OH	H	2.58±0.18	10.05±2.31	22.34±4.38
41	H		OH	H	0.92±0.11	10.05±2.31	11.62±3.56
42	H		OH	H	15.40±4.22	18.24±5.42	25.35±6.33
43	H		OH	H	2.24±1.01	18.24±5.42	22.19±4.26
44	H		OH	H	0.79±0.11	18.24±5.42	35.68±8.52
45	H		OH	H	10.65±4.21	18.24±5.42	34.28±7.99
46	H		OH	H	8.57±3.24	14.34±4.21	10.89±2.65
Verapamil					2.11 ± 0.79	13.63±2.28	75.77±7.52

[a] Synthesis and chemical structure of 23-HBA derivatives were reported previously (31).

[b] MDR reversal effects of 23-HBA derivatives in ABCB1-overexpressing HepG2/ADM cell line. Cells were treated with 23-HBA derivative (5 μmol/L), DOX (10 μmol/L) or both 23-HBA derivative (5 μmol/L) and DOX (10 μmol/L) for 72 h. The growth inhibitory rates of HepG2/ADM cells were determined by MTT assay. Verapamil (5 μmol/L) was used as a positive control inhibitor of ABCB1. Data are shown as mean ± SD of six replicates from three independent experiments.

[c] Compound **37** (BBA) was identified as the agent with highest activity to reverse drug resistance without cytotoxicity by itself.

Table 2 The reversal effect of BBA on ABCB1-mediated resistance in the tumor xenograft model

Day	Tumor volume (mm^3)[a]			
	Control	BBA	Paclitaxel	BBA+Paclitaxel
0	18.64 ± 7.260	9.440 ± 9.830	9.090 ± 7.720	11.15 ± 9.27
3	28.32 ± 19.49	16.61 ± 25.15	16.36 ± 17.55	21.35 ± 18.94
6	112.5 ± 69.78	57.79 ± 47.31	42.64 ± 50.02	61.26 ± 64.02
9	328.6 ± 206.0	268.8 ± 204.7	189.7 ± 137.7	90.69 ± 68.75
12	938.5 ± 661.6	741.9 ± 526.2	461.5 ± 365.5	213.6 ± 189.4
15	1548 ± 904.8	1491 ± 1042	817.0 ± 688.3	381.5 ± 320.5
18	2581 ± 1125	2403 ± 1225	1287 ± 925.1*	681.4 ± 552.6#

[a] MDR reversal effect of BBA on tumor xenograft model. 1×10^7 KB-C2 cells were implanted s.c. under the shoulder of the nude mice. (a) Control (saline, q3d × 6, i.p.); (b) Paclitaxel (q3d × 6, i.p., 18 mg/kg); (c) BBA (q3d × 6, p.o., 15 mg/kg); (d) BBA (q3d × 6, p.o., 15 mg/kg) and Paclitaxel (q3d × 6, i.p., 18 mg/kg) (BBA was given 2 h before paclitaxel was injected). The mice tumor volumes were recorded every 3 days. Data are shown as mean ± SD (n = 6). *, $P < 0.05$ versus the control group; #, $P < 0.05$ versus paclitaxel alone group.

Supplementary Figure Captions 1-3

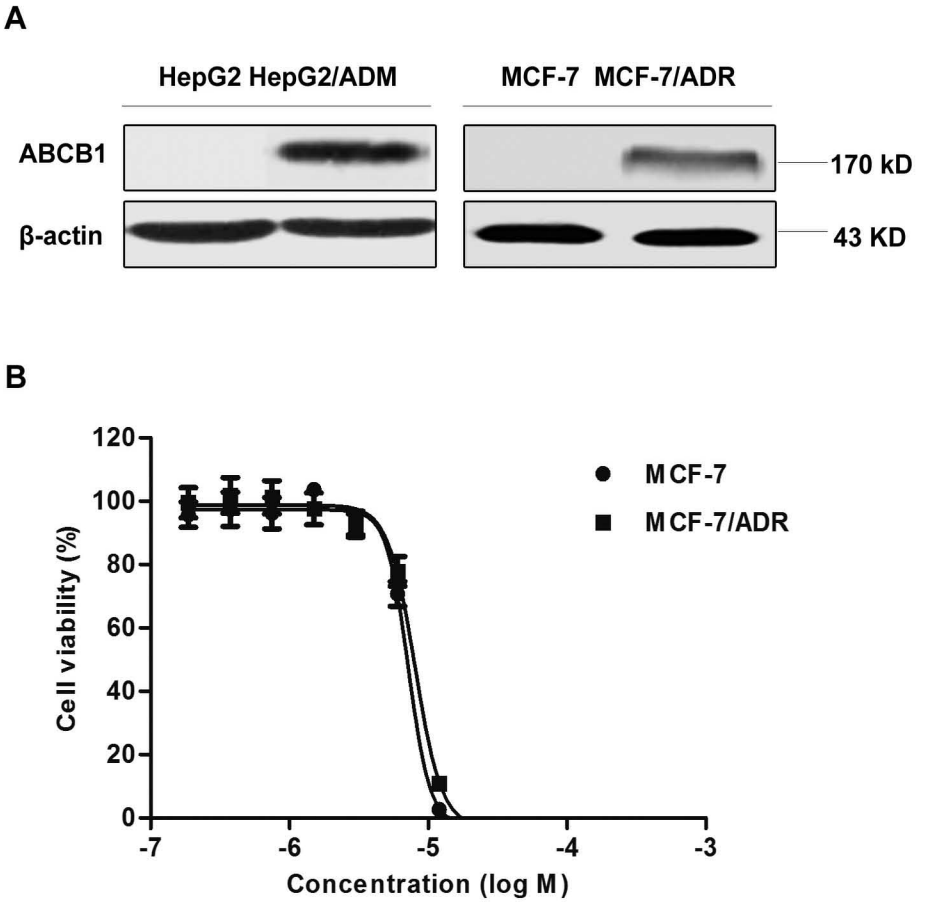
Figure S1 (A) The expression level of ABCB1 on HepG2/ADM, MCF-7/ADR and their parental cells detected by Western blot. Representative bands of three independent experiments were shown. (B) Cytotoxic effect of BBA on MCF-7/ADR and its parental cells detected by MTT assay. The mean values are plotted and the error bars depict standard of deviation from at least three independent experiments performed in six replicates.

Figure S2 BBA inhibited the function of ABCB1, but not the expression level of ABCB1 in MCF-7/ADR cells. (A) The effect of BBA on the accumulation of DOX in MCF-7/ADR cells detected by laser scanning confocal microscope. Original amplification: 63; bar: 10 μm. (B) The accumulation of DOX (left) and rhodamine123 (right) in MCF-7/ADR cells were analyzed by flow cytometry. (C) The effect of BBA on the efflux of rhodamine123 in MCF-7/ADR cells. The mean values are plotted and the error bars depict standard of deviation from at least three independent experiments performed in triplicates. Effect of BBA on mRNA (D) and protein (E) expression levels of ABCB1 in MCF-7/ADR cell detected by reverse transcription PCR and Western blot, respectively.

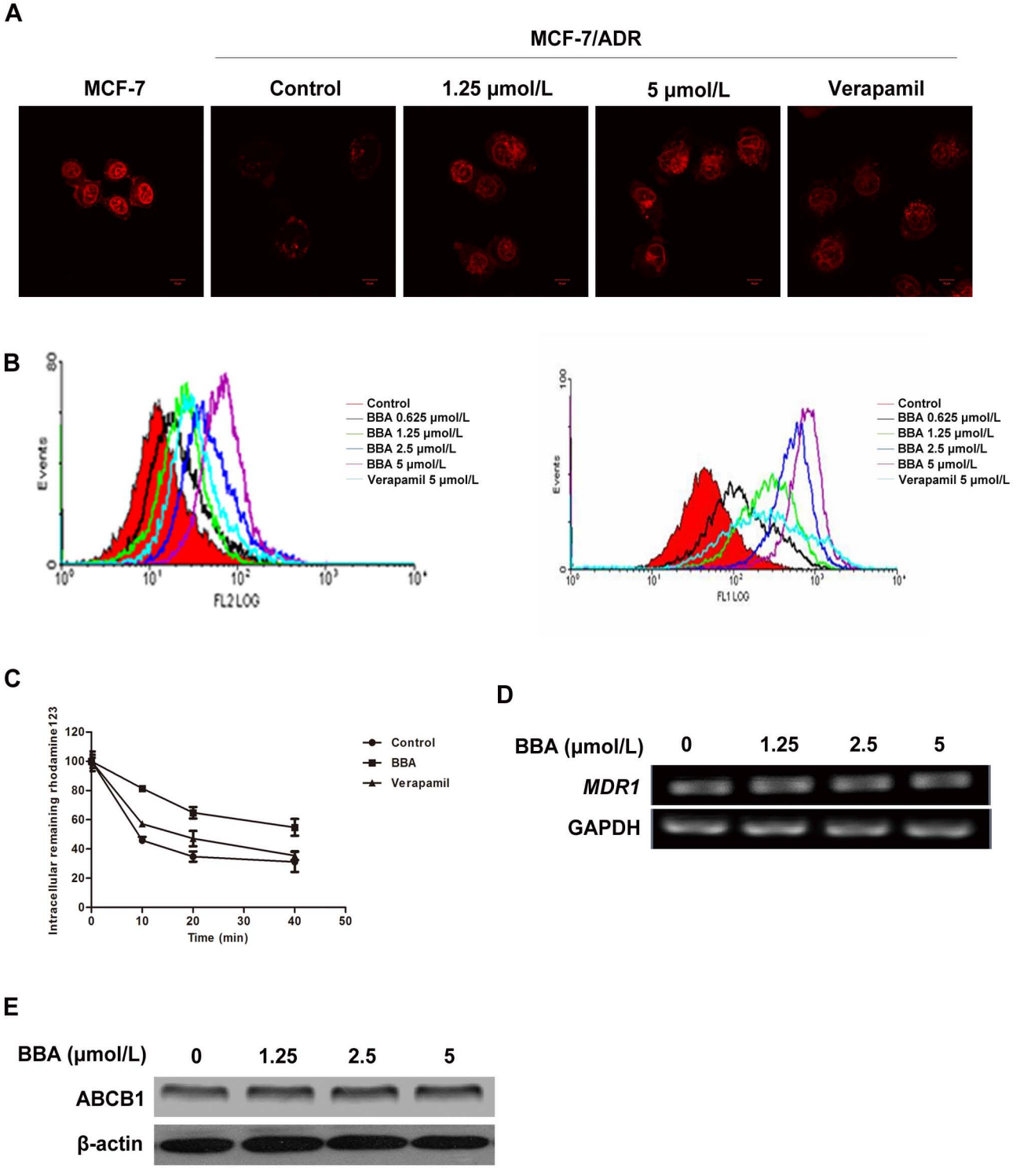
Figure S3 BBA inhibited the phosphorylation of AKT and ERK1/2 in HepG2/ADM cells while not affecting the phosphorylation of AKT and ERK in HepG2 cell line. AKT, p-AKT^{Thr308}, p-AKT^{Ser473}, ERK, p-ERK^{Tyr204} and Bcl-2 expression levels in

HepG2 and HepG2/ADM cells were analyzed by Western blot. β-actin was used as the internal control. Representative bands of three independent experiments were shown.

Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3

