Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary data for:

Characterization of regio- and stereo-selective sulfation of bufadienolides: exploring the mechanism and providing insight into the structure-sulfation relationship by experimentation and molecular docking analysis

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Fig. S1 MS spectra of the sulfate metabolites of six bufadeinolides

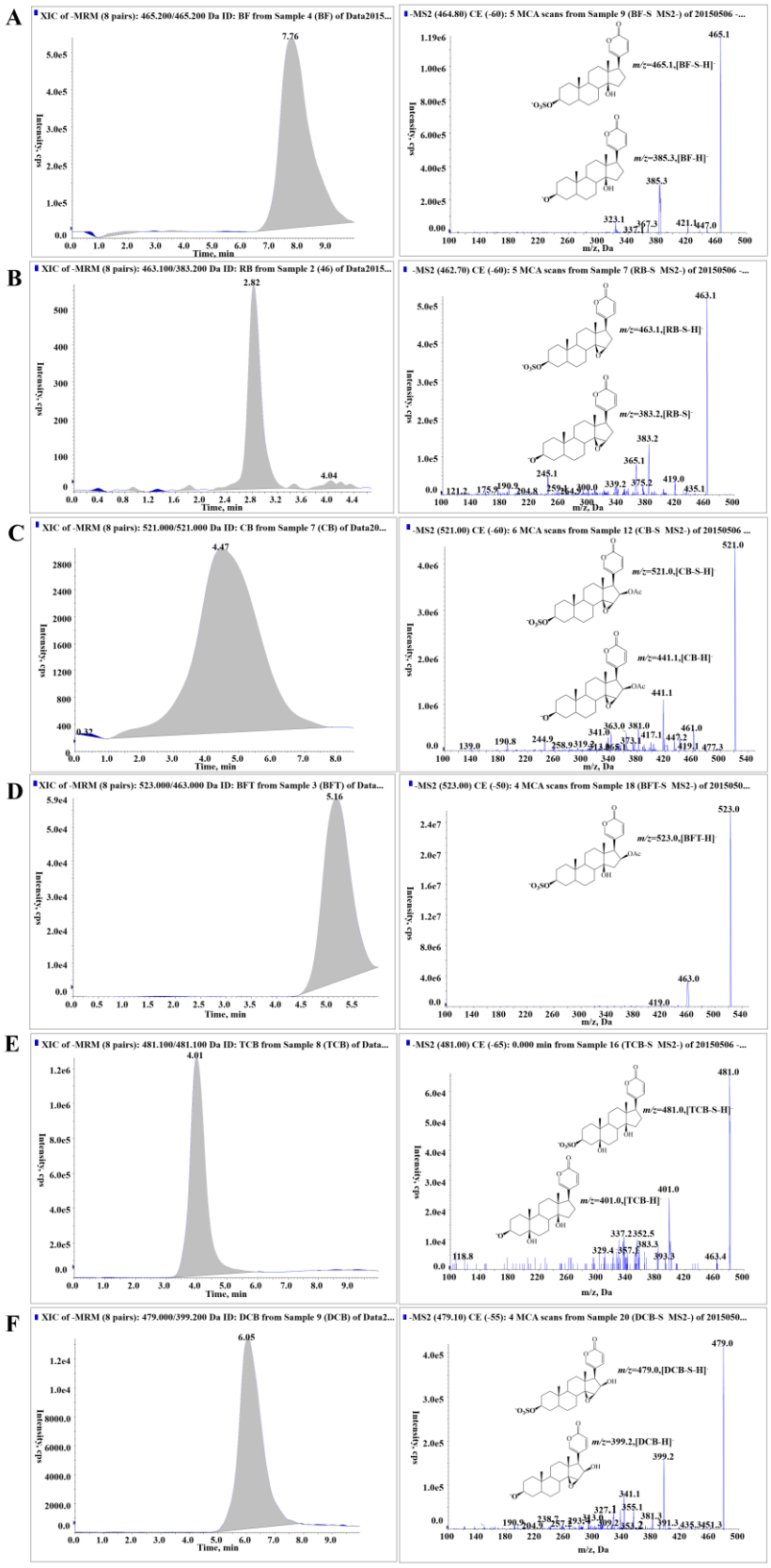

Fig.S2 Inhibition of DHEA on the sulfation of six bufadienolides in HLS9 and SULT2A1.

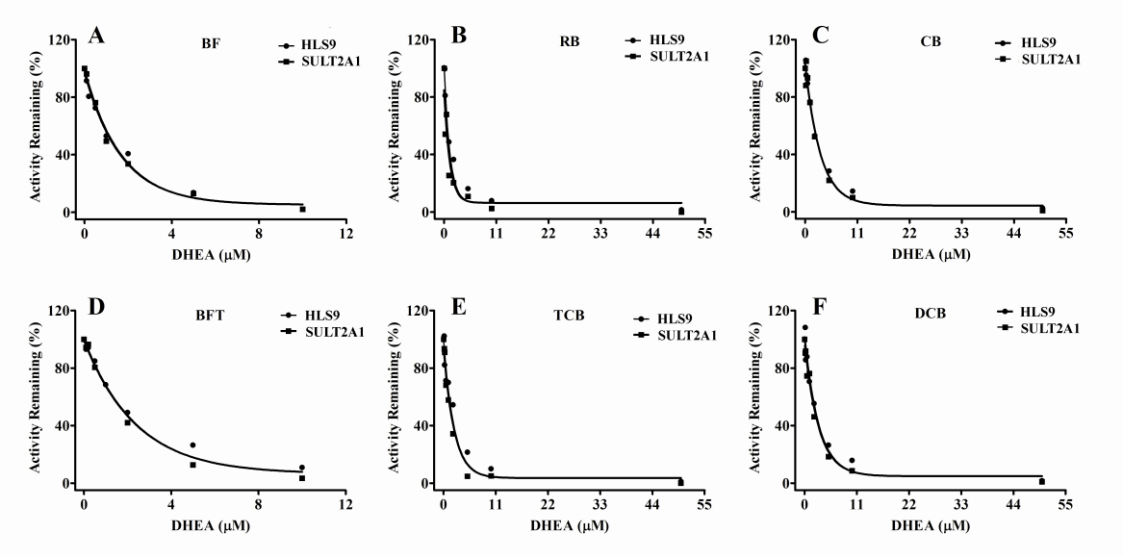

Fig.S3 Inhibitory effects of DHEA on the sulfation of BF and RB in liver S9 of different animal species including monkey, minipig, dog, rabbit, guinea pig, rat and mouse. The substrate concentrations were about K_m values of the six bufadienolides.

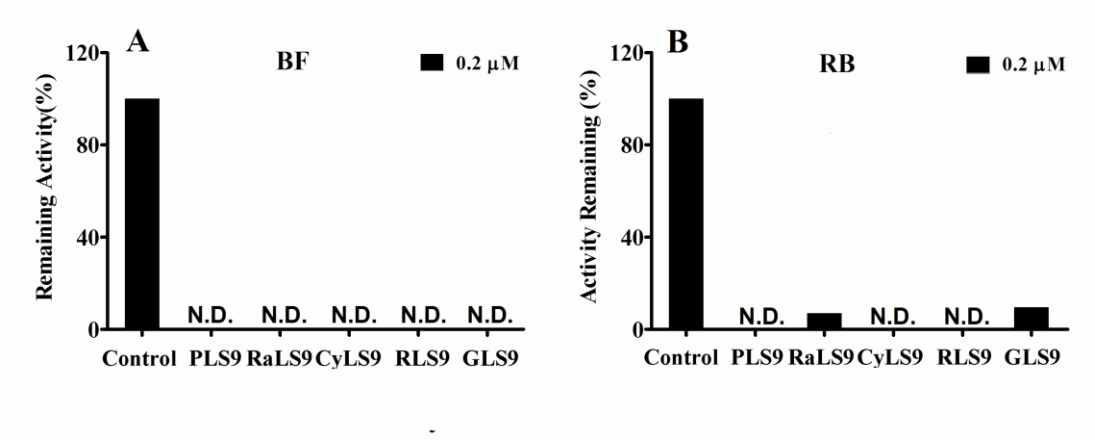

Fig.S4 The structure and K_m values of bufadienolide derivatives. K_m values (μM) are micromolar concentrations and colored blue.

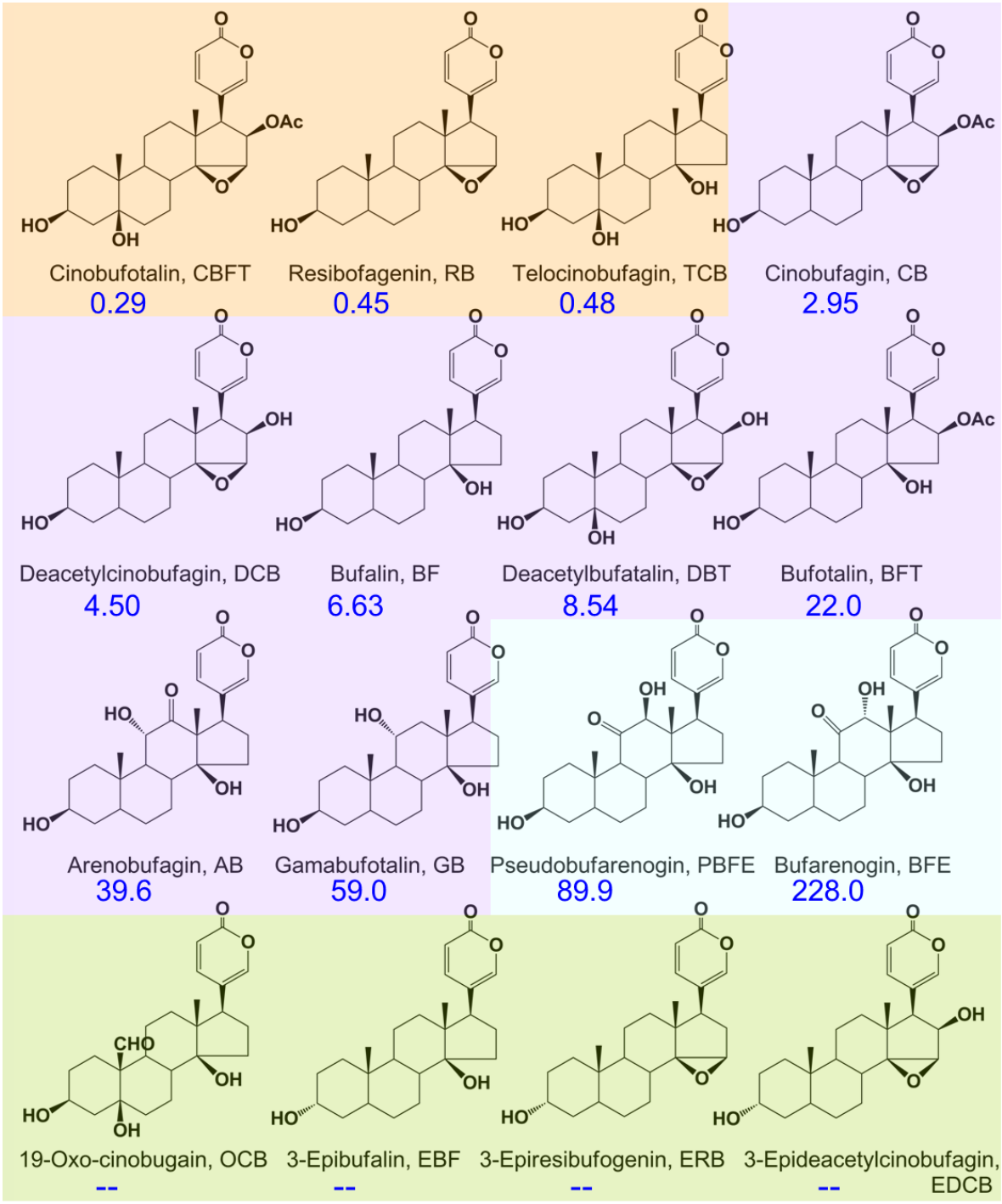

Fig.S5 Correlation analysis between the formation rates of six bufadienolides 3-O-sulfates with SULT2A1-catalyzed EHDA sulfation. Correlation coefficients were calculated, and statistical significance was set at $P < 0.05$.

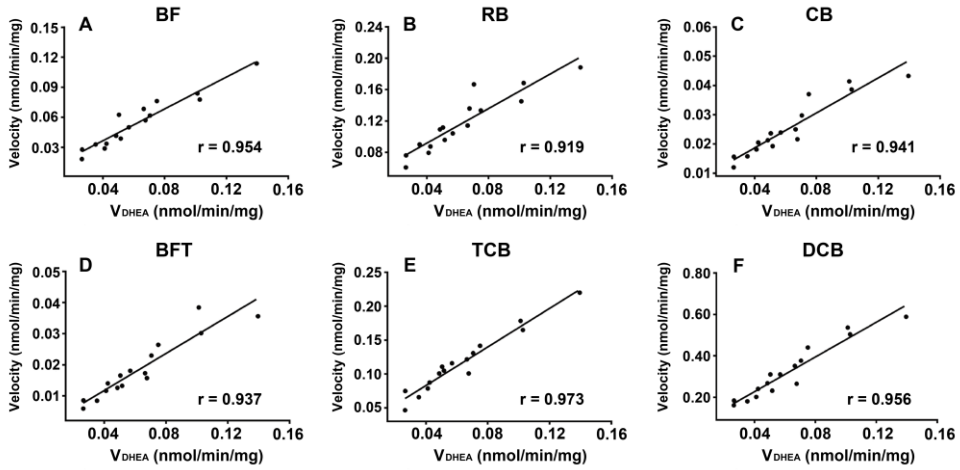

Fig. S6 The two binding orientation of the DHEA substrate in SULT2A1. (DHEA primary orientation, magenta; DHEA second binding orientation, green).

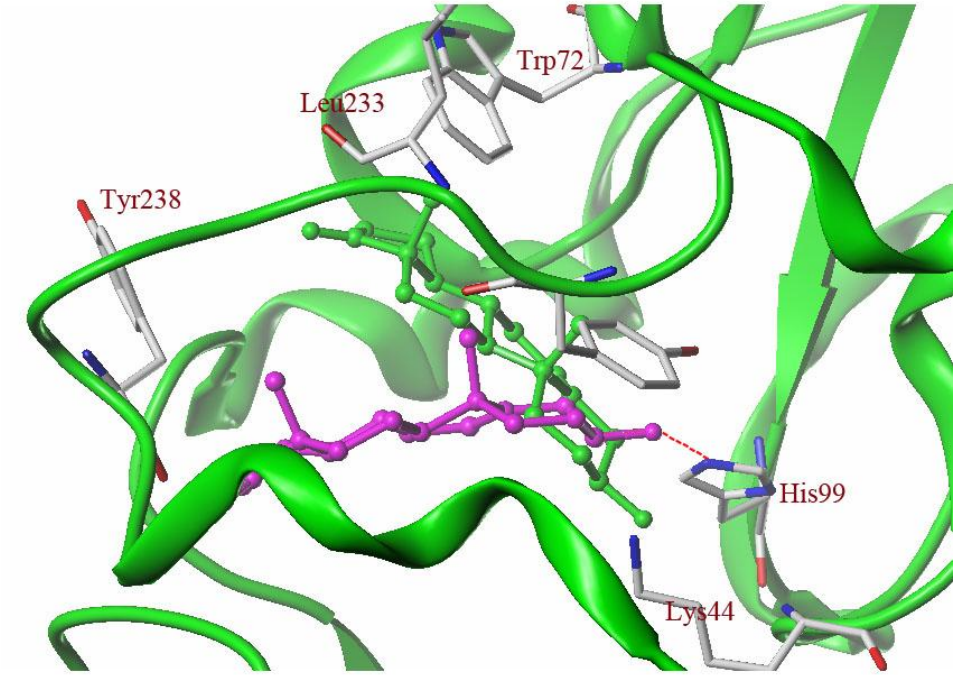

Fig. S7 The interaction binding modes comparison between bufadienolide derivatives and SULT2A1 (DHEA primary orientation, magenta; DHEA second binding orientation, green; bufadienolide derivatives, atom type).

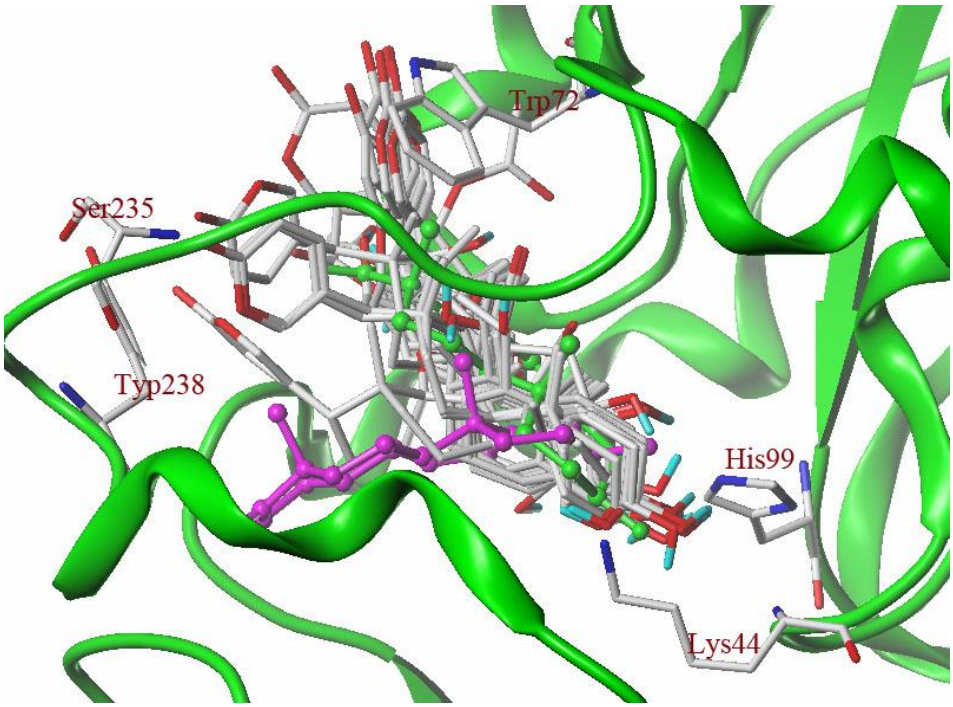

Table S1. ^{13}C-NMR (500 MHz, DMSO-d6) spectral data for bufadienolides 3-*O*-sulfates.

NO.	BF-S	RB-S	CB-S	BFT-S	DCB-S
1	30.1	31.8	30.8	30.1	30.8
2	28.9	27.4	25.4	26.3	29.9
3	71.5	73.9	71.4	71.5	71.5
4	32.0	35.1	32.6	30.9	32.7
5	36.2	38.1	36.1	36.2	36.2
6	26.4	25.5	25.4	25.3	31.1
7	21.1	22.1	20.1	20.7	20.5
8	41.2	33.5	32.6	41.3	39.5[a]
9	34.6	40.13	38.2	34.5	38.5
10	35.0	36.5	34.6	34.7	34.6
11	23.7	22.1	20.4	20.7	20.1
12	40.0	40.5	38.8	39.5[a]	39.5[a]
13	48.0	46.5	44.6	48.9	43.9
14	83.4	75.9	71.9	82.6	71.6
15	30.9	61.3	59.4	39.5[a]	60.4
16	28.4	32.8	74.5	73.8	71.5
17	50.2	48.1	49.0	55.9	49.3
18	16.6	18.4	16.9	16.6	17.0
19	25.3	22.5	23.5	23.6	23.6
20	122.6	123.9	116.0	117.2	117.4
21	149.0	152.4	152.2	151.4	151.4
22	147.2	149.3	148.5	150.1	149.5
23	114.1	116.0	112.8	111.6	112.2
24	161.3	162.9	160.8	161.1	161.2
25	--	--	169.3	--	--
26	--	--	20.5	--	--

[a] The signal was overlapped by other signals.

Table S2. Inhibitory effects of DHEA on the sulfation of six bufadienolides in HLS9 and SULT2A1. The substrate concentrations were about K_m values of the six bufadienolides.

Compounds	IC_{50} (μM)	
	HLS9	SULT2A1
BF	1.15	1.13
RB	1.02	0.37
CB	2.34	2.20
BFT	1.98	1.50
TCB	1.88	1.14
DCB	2.23	1.83

Table S3. Chemscore and apparent K_m values of bufadienolide derivatives

Compounds	***K*$_m$ (μM)**	**Chemscore**
CBFT	0.29	-36.68
RB	0.451	-32.26
TCB	0.48	-33.01
CB	2.95	-33.33
DCB	4.50	-31.35
BF	6.63	-31.12
DBT	8.54	-35.18
BFT	21.97	-31.33
AB	39.55	-31.26
GB	58.96	-29.98
PBFE	89.91	-28.96
BFE	228.0	-27.79
EDCB	--*	-32.34
ERB	--*	-32.29
EBF	--*	-31.12
OCB	--*	-30.47

*Not catalyzed by rhSULT2A1.