Supplementary File

Benzocyclohexane Oxide Derivatives and Neolignans from *Piper betle* Inhibit Efflux-Related Resistance in *Staphylococcus aureus*

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Electronic Circular Dichroism (ECD) Spectra Calculations

Electronic circular dichroism spectra of all compounds were calculated and six calculated CD curves were obtained (Figure S1). The results revealed good agreement between the calculated and the experimental ECD curves, especially for the compounds (-)-acuminatin (**1**), (-)-denudatin B (**2**) and ellipeiopsol B (**5**). For (-)-acuminatin (**1**), both experimental and calculated CD curves (Figure 2 a1, a2) showed two positive Cotton effects (CE) (210 nm, 230 nm) and two negative CE (220 nm, 230 nm). For (-)-denudatin B (**2**), both curves showed three positive CE (234 nm, 273 nm and 313 nm) and three negative CE (208 nm, 248 nm and 288 nm). There was a similar CE consistency between the calculated and the experimental ECD curves for puberulin D (**3**), ferrudiol (**4**) and zeylenol (**6**) respectively. For ellipeiopsol B (**5**), both two curves showed one positive CE at 228 nm and two negative CE at 204 nm and 247 nm.

Legends of Tables and Figures

Table S1. The ^{1}H and ^{13}C NMR data (δ_H, δ_C in ppm,, *J* in Hz) in $CDCl_3$ for synergistic compounds **1 - 3** isolated from *Piper betle*.

Table S2. The ^{1}H and ^{13}C NMR data (δ_H, δ_C in ppm,, *J* in Hz) in $CDCl_3$ for synergistic compounds **4 - 6** isolated from *Piper betle*.

Figure S1. Curricular Dichrom (CD) spectrum of compounds **1** – **6**

S1A. Experimental CD curves of compound **1**

S1a. Calculated CD curves of compound **1**

S1B. Experimental CD curves of compound **2**

S1b. Calculated CD curves of compound **2**

S1C. Experimental CD curves of compound **3**

S1c. Calculated CD curves of compound **3**

S1D. Experimental CD curves of compound **4**

S1d. Calculated CD curves of compound **4**

S1E. Experimental CD curves of compound **5**

S1e. Calculated CD curves of compound **5**

S1F. Experimental CD curves of compound **6**

S1f. Calculated CD curves of compound **6**

Figure S2 A. The HPLC plot of compounds **1** – **3**

B. The HPLC plot of compounds **4** – **6**

Figure S3. The isobologram of Compounds **1** – **6** with Norfloxacin

An isobologram depicts the results of the checkerboard assay and the FICI values. The x axis of the isobologram represents compounds, the y axis norfloxacin. The MIC value

of compound is located on the x axis, and the MIC of norfloxacin on the y axis. The line connecting these two points represents the line of no interaction (line of indifference). Below the line of indifference we find the area of additive (4 > FICI > 0.5) and synergistic (FICI ≤ 0.5) effects. Above the line of indifference are the combinations with antagonistic (FICI ≥ 4) effects.

Figure S4. The cytotoxicity of the benzocyclohexane oxide derivatives against the HEK293T cells. Within the 1-100 μM (< 48.8 mg/L for compound **4,** < 38.4 mg/L for compound **5** and **6**) concentration range of all three compounds the viability of HEK293T was approximately 100%

Figure S5. The cytotoxicity of the mixture of compounds (**4** and **6**) and norfloxacin as well as norfloxacin alone against the HEK293T cells at concentration of 100 μM.

The results showed that the HEK293T cell viability is 50% at 100 μM of the mixtures (the concentration of compounds and norfloxacin is 100 μM respectively) (< 48.8 mg/L for compound **4,** < 38.4 mg/L for compound **6** or norfloxacin (100 μM) (31.9 mg/L) alone. Compounds alone has no cytotoxicity and didn't increase the toxity of norfloxacin compared with norfloxacin alone.

Table S1. The ^{1}H and ^{13}C NMR data (δ_H, δ_C in ppm, *J* in Hz) in $CDCl_3$ for synergistic compounds

1 - **3** isolated from *Piper betle*.

	(-)-Acuminatin (**1**)		(-)-Denudatin B (**2**)		Puberulin D (**3**)	
Position	H	C	H	C	H	C
1		132.6		129.6		135.2
2	6.98, m	109.3	6.78, s	109.4	6.82-6.69, m	108.1
3		149.0		149.7		147.9
4		149.0		149.3		146.3
5	6.86, d, 8.2	110.6	6.87, m	110.9	6.82-6.69, m	108.2
6	6.98, m	119.2	6.87, m	119.7	6.82-6.69, m	121.6
7	5.13, d, 9.4	93.7	5.35, d, 9.4	91.4	2.54, d, 8.1	53.5
8	3.48, m	45.6	2.18, m	49.7	2.54-2.39, m	48.5
9	1.40, 3H, d, 6.7	17.5	1.12, 3H, d, 7.0	6.8	0.98, d, 6.6	13.6
1'		132.2		143.0		53.8
2'	6.80, s	113.2		187.2	5.64, s	119.6
3'		144.1	5.82, s	102.8		151.5
4'		146.5		174.6		193.6
5'		133.1		77.7	2.99, s	61.3
6'	6.79, s	109.1	6.26, s	131.1	5.09, s	79.2
7'	6.38, d, 16.0	130.9	3.14, 2H, s	33.5	2.54-2.39, 2H, m	36.6
8'	6.13, m	123.5	5.88, m	135.1	5.86-5.70, m	133.9
9'	1.89, 3H, d, 6.3	18.4	5.14, 2H, m	117.3	5.15-5.10, 2H, m	118.3
3-OCH_3	3.90, 3H, s	55.9	3.89, 3H, s	55.9		
4-OCH_3	3.90, 3H, s	55.9	3.89, 3H, s	55.9		
3'-OCH_3	3.90, 3H, s	55.9				
5'-OCH_3			3.14, 3H, s	51.2		
OCH_2O					5.90, 2H, s	101.0
OCH_3					3.64, 3H, s	55.4
$OCOCH_3$					2.13, 3H, s	13.6
						193.6

Strong NOE correlation of (**1**): H-C(7)/H_3-C(9); Strong NOE correlation of (**2**): H-C(7)/H_3-C(9); H-C(7)/H_3-C(5'-OCH_3).

Key NOESY correlations of (**3**): H-C(2')/H_3-C(9); H-C(7)/H_3-C(9); H-C(5')/H_3-C(8).

Table S2. The ^{1}H and ^{13}C NMR data (δ_H, δ_C in ppm, *J* in Hz) in $CDCl_3$ for synergistic compounds **4 - 6** isolated from *Piper betle*.

	Ferrudiol (**4**)		Ellipeiopsol B (**5**)		Zeylenol (**6**)	
Position	H	C	H	C	H	C
1		76.0		75.8		75.9
2	4.31, d, 8.6	75.4	4.15, d, 7.0	74.6	4.23, d,6.3	70.8
3	5.85, m	76.8	5.87, m	73.7	5.70, m	74.3
4	6.00, d, 10.2	127.9	5.76, dt, 10.2, 2.0	124.9	5.87, dd, 10.2, 2.7	126.9
5	5.87, s	128.3	5.95, dt, 10.2, 2.0	132.3	6.02, m	129.5
6	5.85, m	72.8	4.46, m	72.4	4.31, d, 3.9	68.5
7	4.73-4.83, 2H, q, 11.7	62.7	4.89, d, 12.1; 4.73, d, 12.1	63.8	4.75, d, 12.1; 4.90, d, 12.1	66.7
1'		129.5		129.3		129.1
2'	7.99, d, 7.4	129.9	8.11-8.06, m	129.9	8.03, d, 7.0	129.8
3'	7.45-7.54, m	128.5	7.52-7.45, m	128.6	7.37, m	128.4
4'	7.45-7.54, m	133.6	7.64-7.59, m	133.6	7.56, m	133.4
5'	7.45-7.54, m	128.5	7.52-7.45, m	128.6	7.37, m	128.4
6'	7.99, d, 7.4	129.9	8.11-8.06, m	129.9	8.03, d, 7.0	129.8
7'		166.6		168.1		167.9
1"		129.4		129.4		129.4
2"	8.09, d, 7.4	130.0	8.11-8.06, m	129.8	7.97, d, 7.0	129.8
3"	7.33, q, 7.8	128.4	7.52-7.45, m	128.4	7.37, m	128.4
4"	7.45-7.54, m	133.4	7.64-7.59, m	133.4	7.56, m	133.5
5"	7.33, q, 7.8	128.4	7.52-7.45, m	128.4	7.37, m	128.4
6"	8.09, d, 7.4	130.0	8.11-8.06, m	129.8	7.97, d, 7.0	129.8
7"		166.5		166.6		167.2
1'"		129.0				
2'"	7.91, d, 7.4	129.6				
3'"	7.33, q, 7.8	128.3				
4'"	7.45-7.54, m	133.1				
5'"	7.33, q, 7.8	128.3				
6'"	7.91, d, 7.4	129.6				
7'"	4.31, d, 8.6	167.3				

Strong NOE correlation of (**4**): H-C(2)/H_3-C(6); Strong NOE correlation of (**5**): H-C(2)/H_3-C(6); Strong NOE correlation of (**6**): H-C(2)/H_3-C(7); H-C(3)/H_3-C(6).

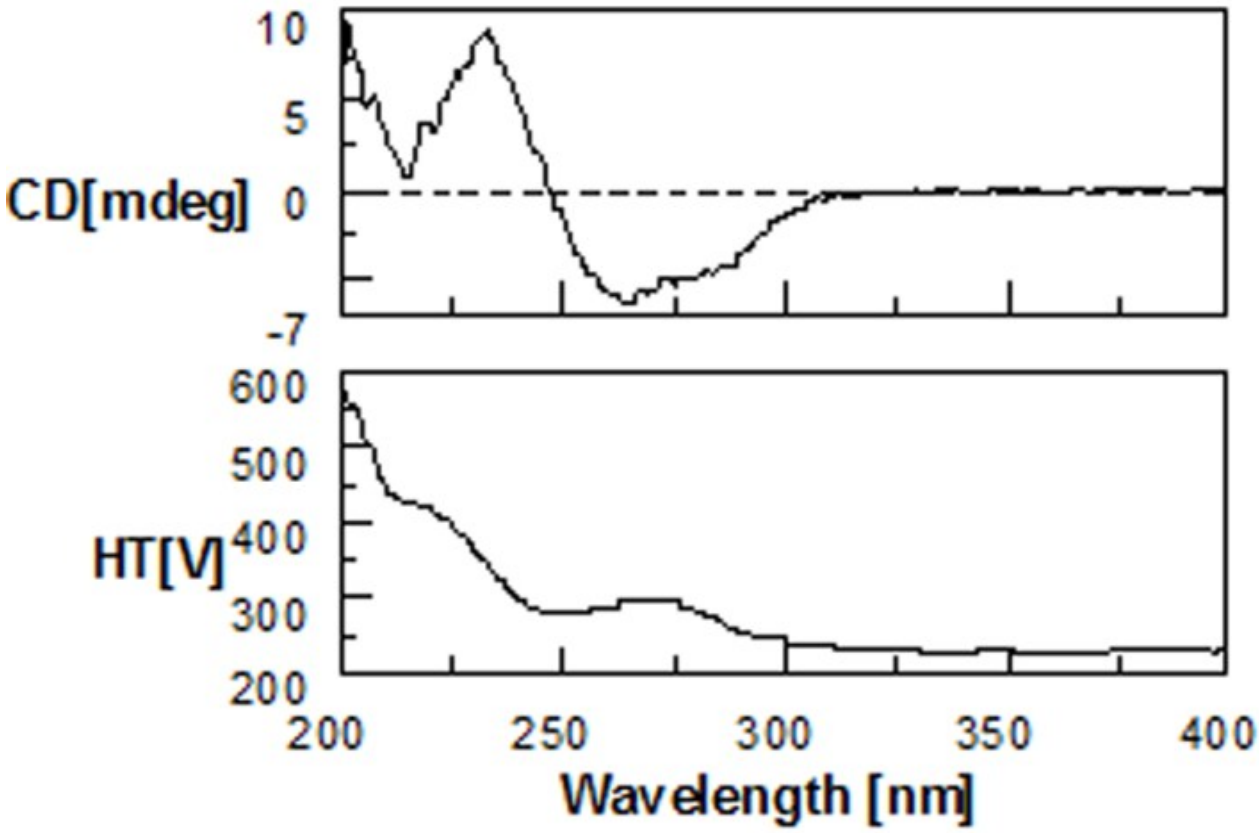


Figure S1A

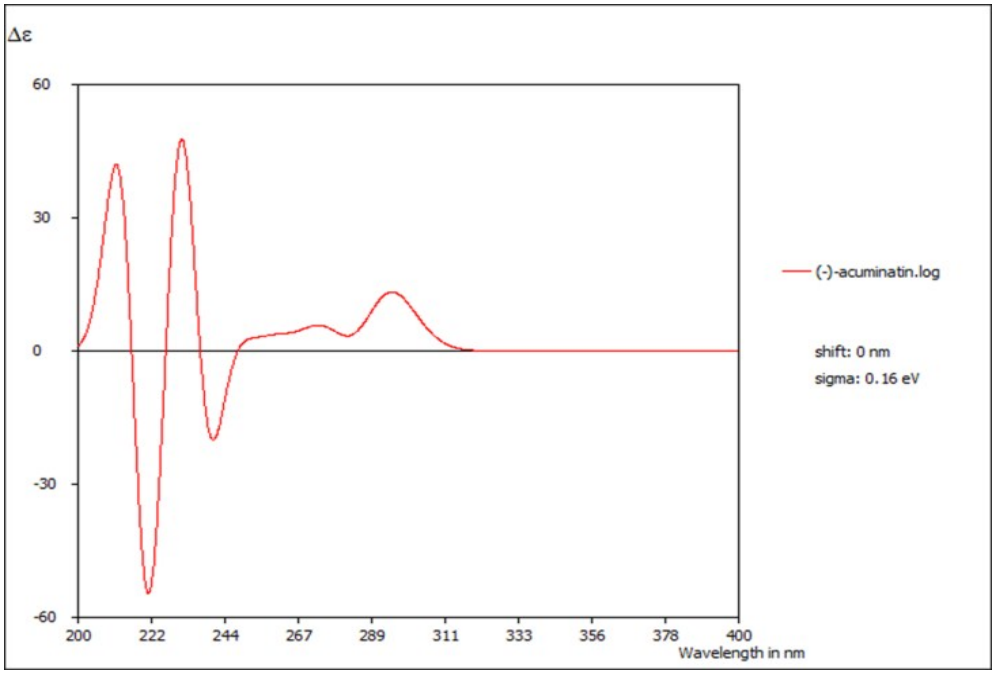


Figure S1a

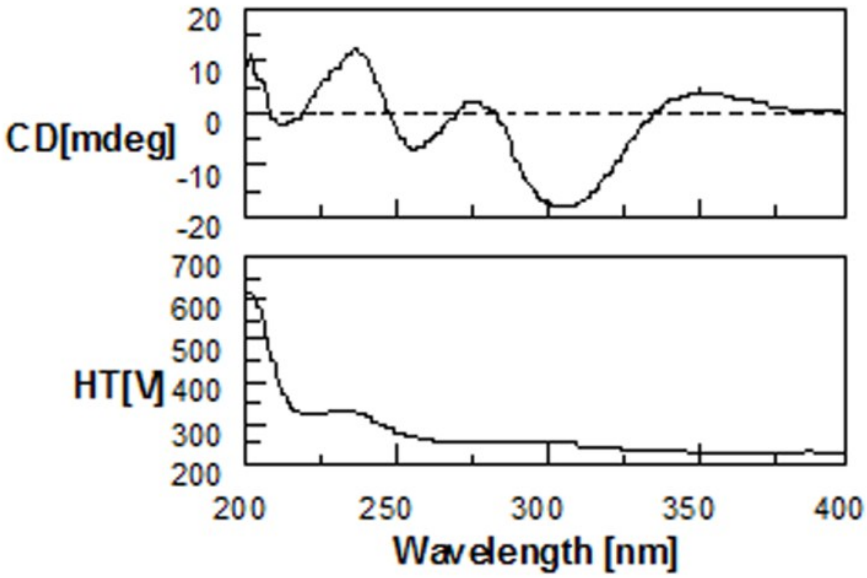
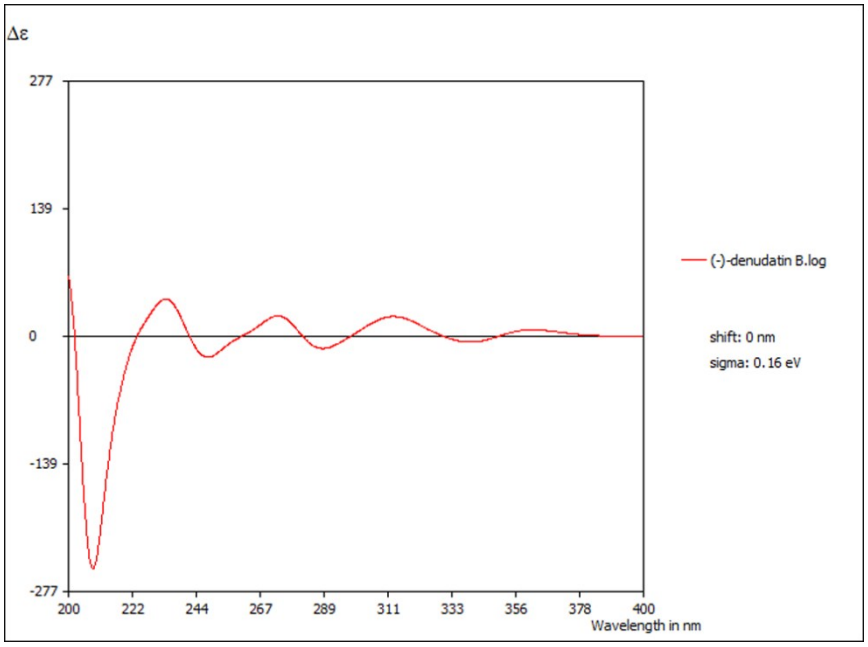


Figure S1B



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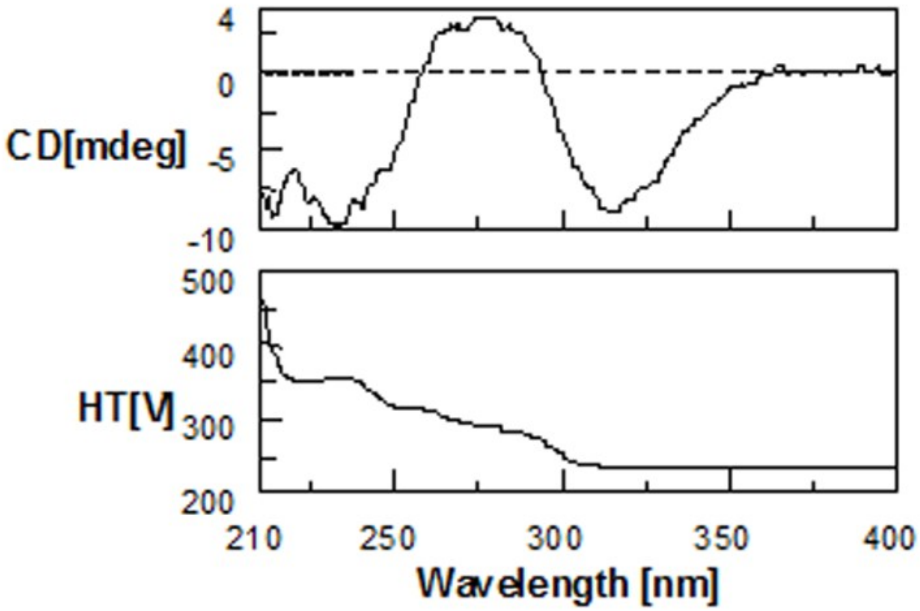

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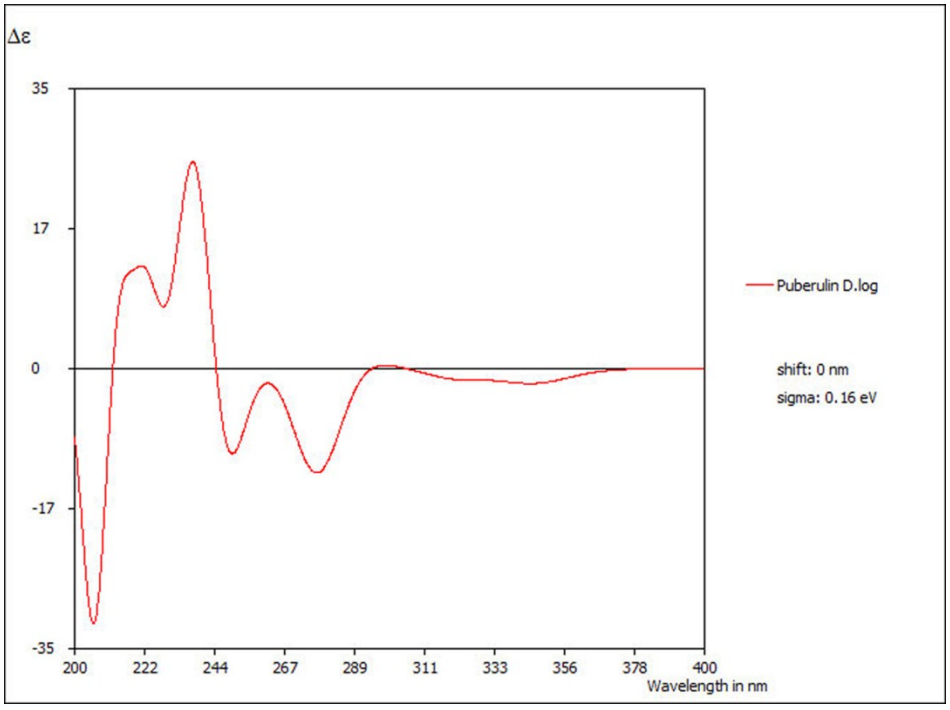

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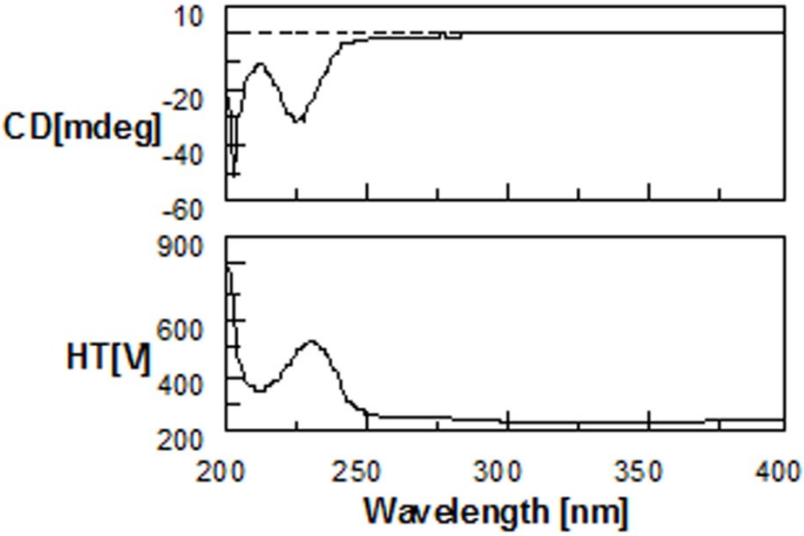

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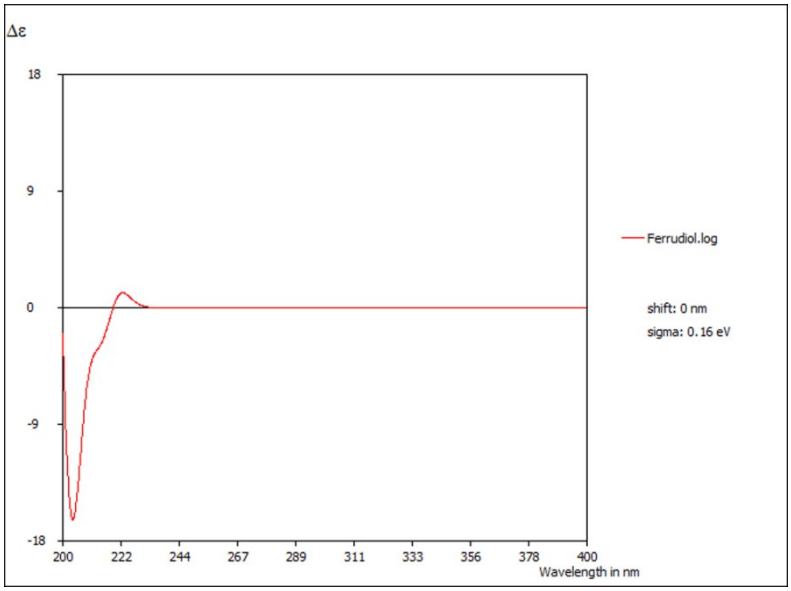

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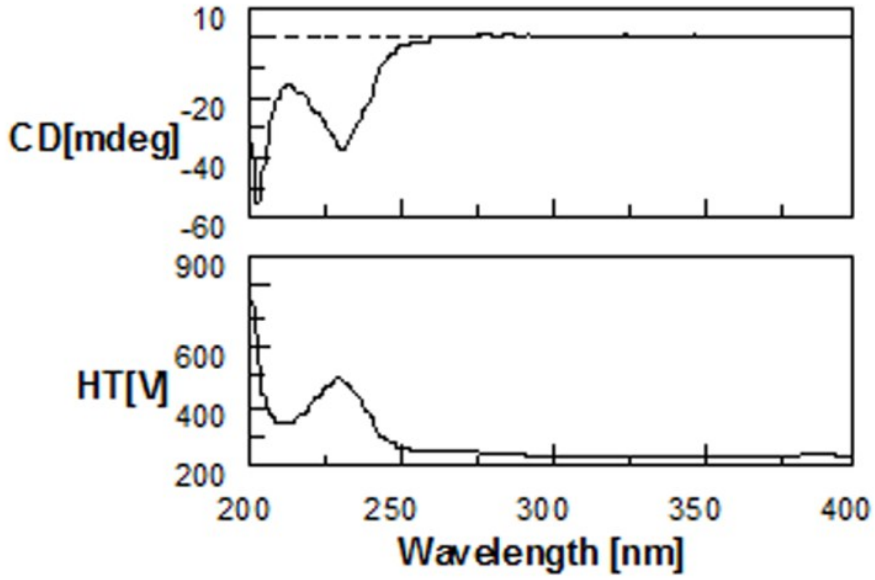

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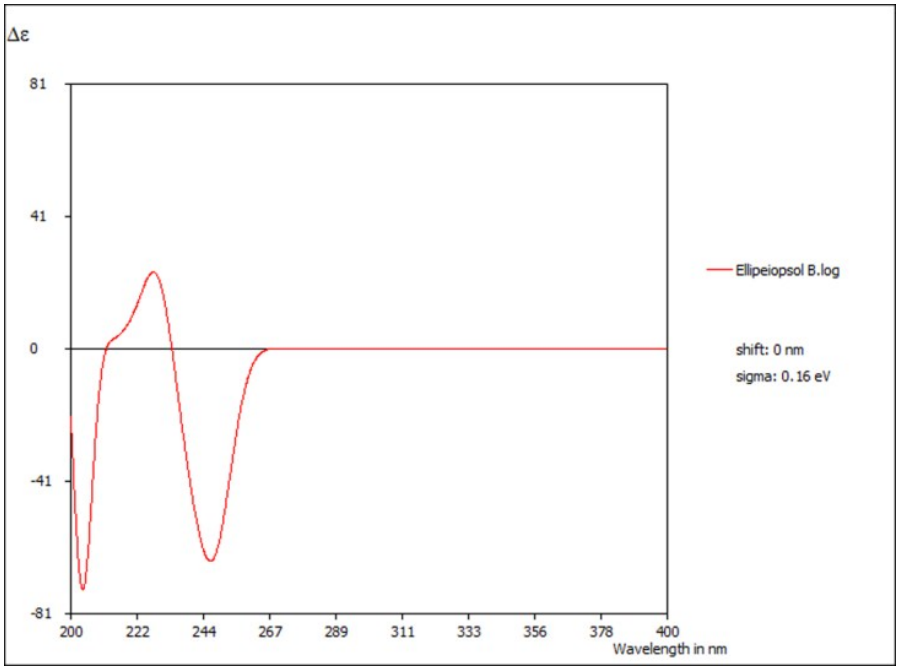

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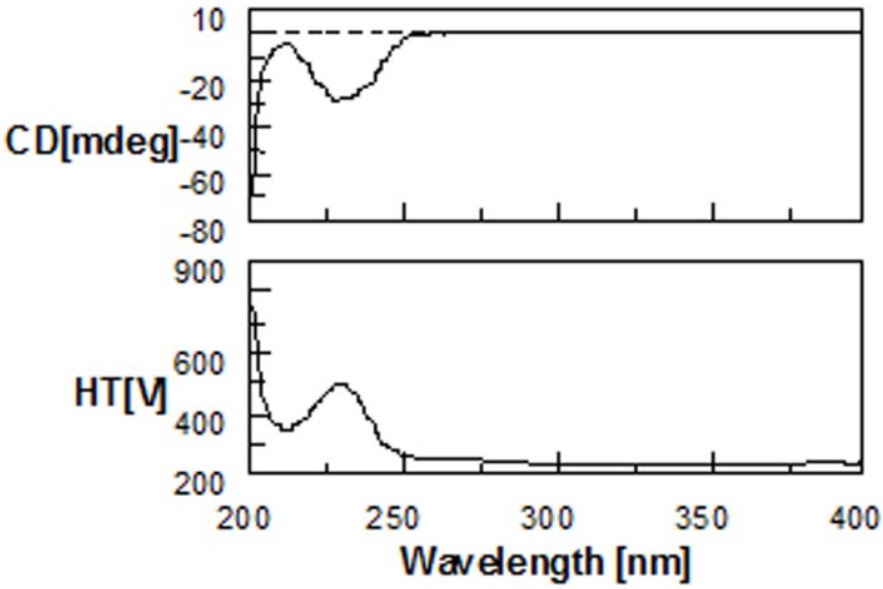

Figure S1F

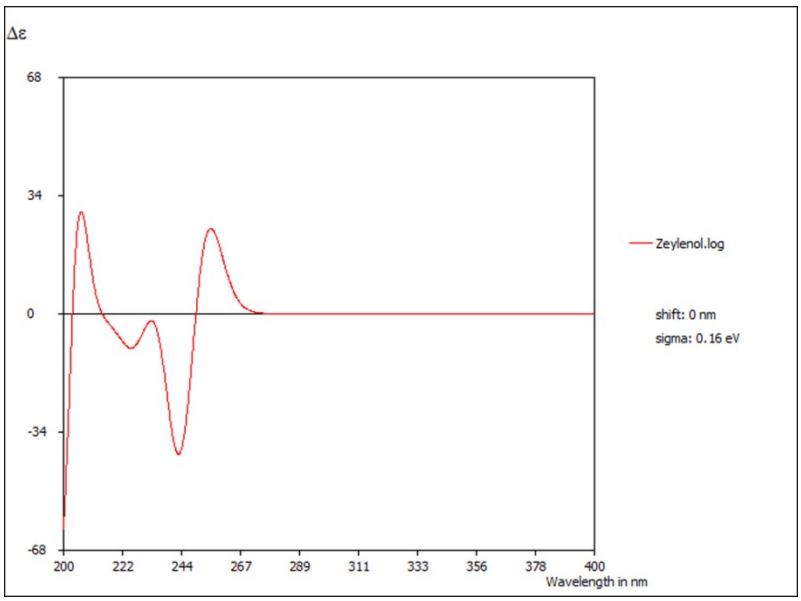

Figure S1f

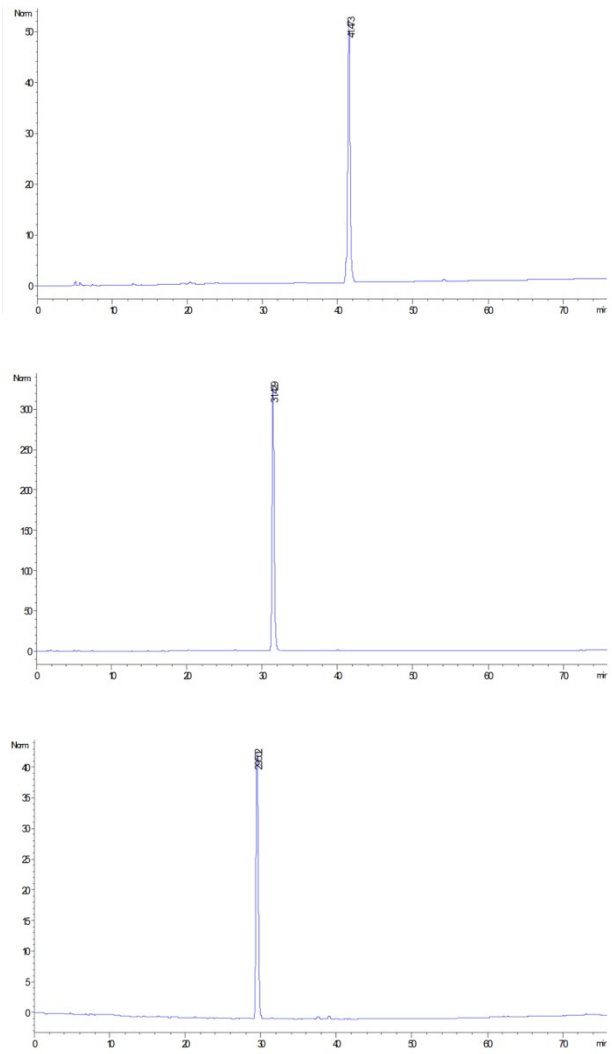

Figure S2A

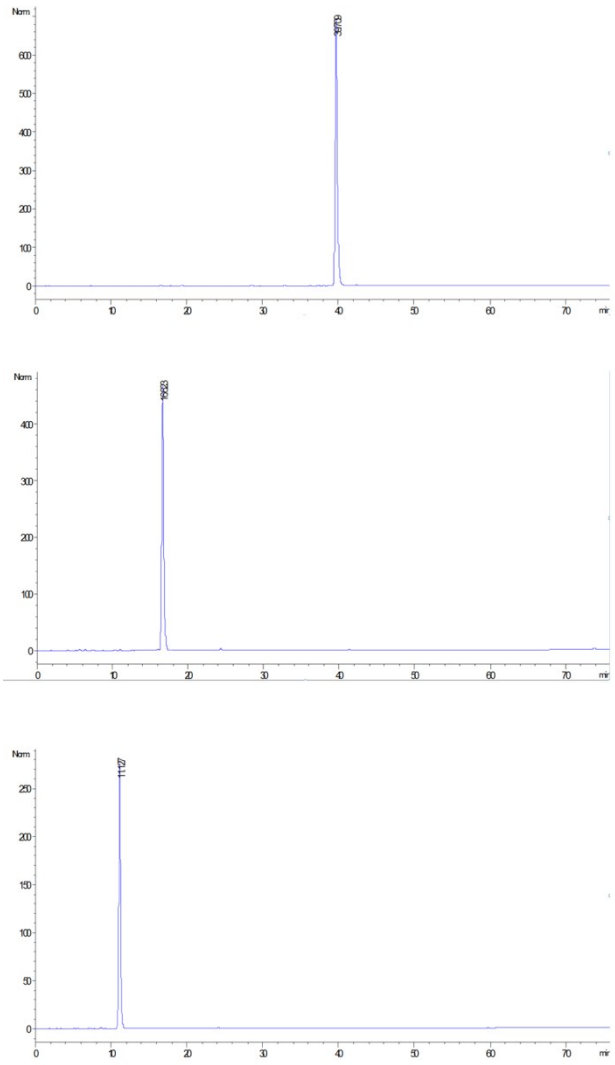
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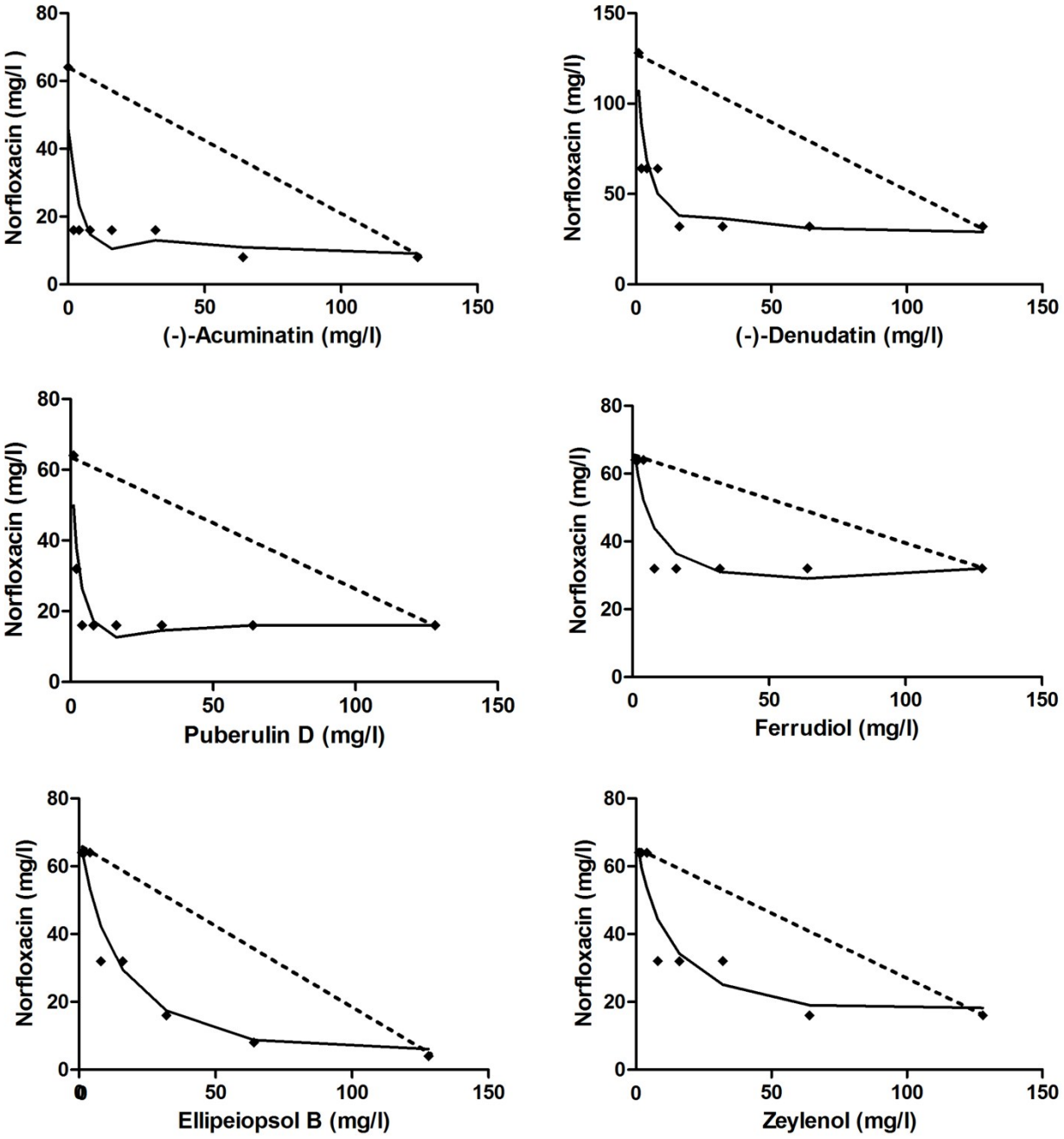
Figure S2B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Norfloxacin (mg/l)
(-)-Denudatin (mg/l)
Norfloxacin (mg/l)
Puberulin D (mg/l)
Norfloxacin (mg/l)
Ferrudiol (mg/l)
Norfloxacin (mg/l)
Ellipeiopsol B (mg/l)
Norfloxacin (mg/l)
Zeylenol (mg/l)

Figure S3

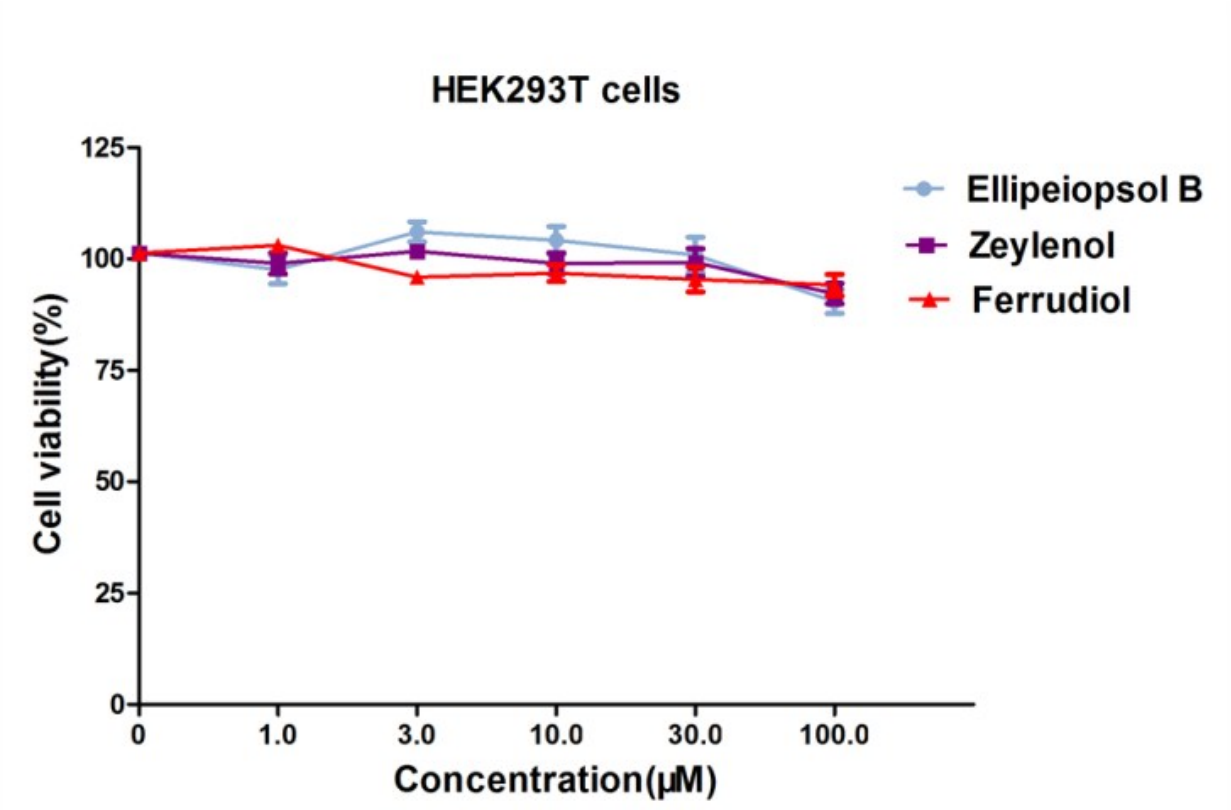

Figure S4

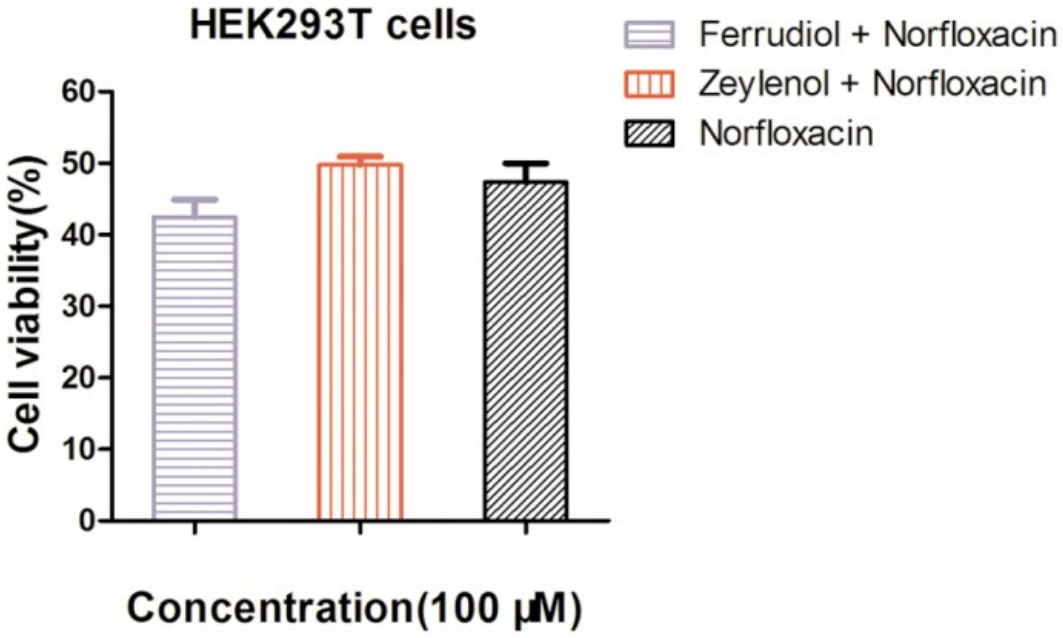

Figure S5