Electronic Supplementary Material (ESI)

Assessment of a bifendate derivative bearing 6,7-dihydro-dibenzo[*c,e*]azepine scaffold as a potential anti-metastatic agent

Jingying Qiu[a, 1], Wang Chen[a, 1], Yanfei Jiang[a], Jing Chen[a], Yinpeng Zhang[a], Xiaoke Gu[a, *]

[a]*Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Jiangsu Center for the Collaboration and Innovation of Cancer Biotherapy, Xuzhou Medical University, Xuzhou 221004, People's Republic of China*

Table of concents:

1. Chemical structure of compound 2H7

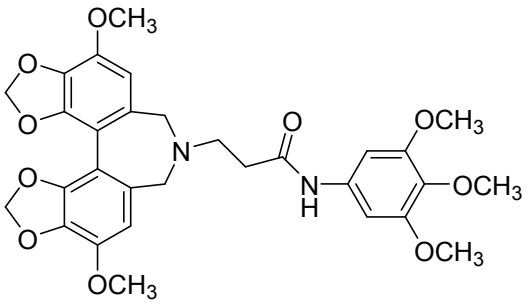
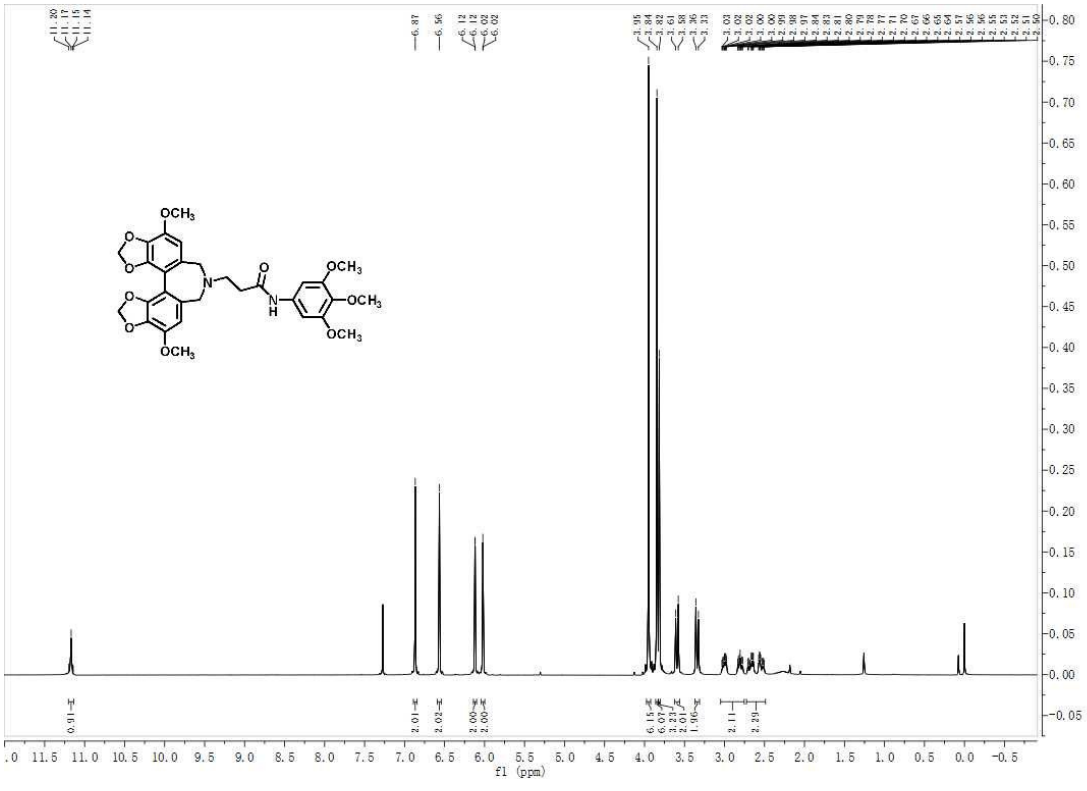


Figure S1. Chemical structure of compound **2H7**

2. NMR spectra of 2H7



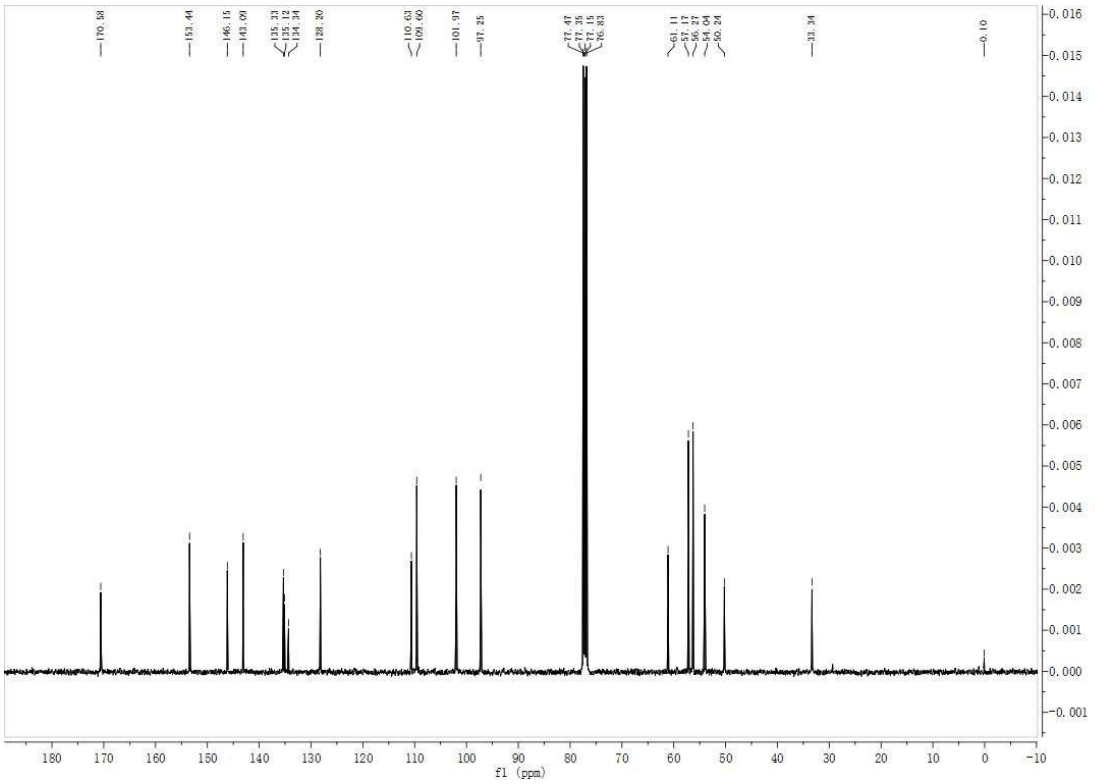


Figure S2. Spectra of compound **2H7**

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.50-2.71 (m, 2H), 2.77-3.03 (m, 2H), 3.34 (d, 2H, *J* = 12.8 Hz), 3.59 (d, 2H, *J* = 12.7 Hz), 3.82 (s, 3H, Ar-OCH_3), 3.84 (s, 6H, 2 × Ar-OCH_3), 3.95 (s, 6H, 2 ×Ar-OCH_3), 6.02 (d, 2H, -OCH_2O-, *J* = 1.2 Hz), 6.12 (d, 2H, -OCH_2O-, *J* = 1.2 Hz), 6.56 (s, 2H, 2 × Ar-H), 6.87 (s, 2H, 2 × Ar-H), 11.17 (s, 1H, -CONH-). **^{13}C NMR** ($CDCl_3$, 100 MHz, δ ppm): 33.3, 50.2, 54.0 (2C, 2 × $ArCH_2N$), 56.3 (2C, 2 × OCH_3), 57.2 (2C, 2 × OCH_3), 61.1, 97.3 (2C, 2 × Ar-C), 102.0 (2C, 2 × -OCH_2O-), 109.6 (2C, 2 × Ar-C), 110.6 (2C, 2 × Ar-C), 128.2 (2C, 2 × Ar-C), 134.3, 135.1, 135.3 (2C, 2 × Ar-C), 143.1 (2C, 2 × Ar-C), 146.2 (2C, 2 × Ar-C), 153.4 (2C, 2 × Ar-C), 170.6 (-CONH-).

3. In vitro anti-multidrug resistance (MDR) activity of 2H7 against K562/A02 cells

Table S1. Chemo-sensitizing effect of compound **2H7**

Compound	IC_{50} (μM) [a]	RF [b]
2H7 + adriamycin	3.21 ± 1.22	7.44
verapamil + adriamycin	14.42 ± 0.89	1.66
adriamycin	23.89 ± 1.65	/

[a] The cytotoxicity of adriamycin against K562/A02 cells in the presence or absence of the target compounds (2.0 μM) was evaluated by MTT assay. The classic P-gp inhibitor adriamycin was selected as the positive control.

[b] RF: Reversal fold (RF) refers to fold-change in drug sensitivity. RF = (IC_{50} of adriamycin without target compound)/(IC_{50} of adriamycin with target compound).

As shown in Table 1, **2H7** displayed the potent chemo-sensitizing effect with a significantly decreased IC_{50} of adriamycin (3.21 μM), and its reversal fold (RF) was 7.44.

4. In vitro anti-migration activity of 2H7 against MDA-MB-231 cells

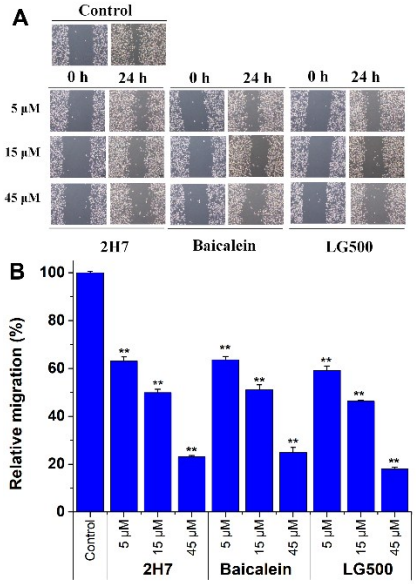


Figure S3. Inhibitory effect of **2H7** on MDA-MB-231 cells migration *in vitro* was determined by wound-healing assay. Baicalein and **LG500** were selected as positive control groups. Cell monolayer was wounded by a 200 μL pipette tip followed by treatment with **2H7**, Baicalein or **LG500** (5, 15 or 45 μM) for 24 h. (A) **2H7** inhibits migration of cells across the wounded space. Distance of the wound edge was measured before and after the treatment. Baicalein and **LG500** were selected as positive control group sets. Image magnification: × 100. (B) Quantification of the relative migration. Relative migration (%) were identified by dividing the migration distance of MDA-MB-231 cells treated with **2H7**, Baicalein or **LG500** by that of control group. $^{**}P < 0.01$ *vs*. control group.

Data showed that **2H7** displayed potent inhibitory effect on the migration of MDA-MB-231 cells, which was comparable with that of positive control Baicalein and **LG500**.