Assessment of a bifendate derivative bearing 6,7-dihydro-dibenzo[3,c,e]azepine scaffold as a potential anti-metastatic agent

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1. Chemical structure of compound 2H7

![Chemical structure of compound 2H7](image)

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

2. NMR spectra of 2H7

![NMR spectra of 2H7](image)
**Figure S2.** Spectra of compound 2H7

**1H 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$_2$O-, $J = 1.2$ Hz), 6.12 (d, 2H, -OCH$_2$O-, $J = 1.2$ Hz), 6.56 (s, 2H, 2 × Ar-H), 6.87 (s, 2H, 2 × Ar-H), 11.17 (s, 1H, -CONH-). **13C NMR** (CDCl$_3$, 100 MHz, δ ppm): 33.3, 50.2, 54.0 (2C, 2 × ArCH$_2$N), 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$_2$O-), 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**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RF&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2H7 + adriamycin</td>
<td>3.21 ± 1.22</td>
<td>7.44</td>
</tr>
<tr>
<td>verapamil + adriamycin</td>
<td>14.42 ± 0.89</td>
<td>1.66</td>
</tr>
<tr>
<td>adriamycin</td>
<td>23.89 ± 1.65</td>
<td>/</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<sup>b</sup> RF: Reversal fold (RF) refers to fold-change in drug sensitivity. RF = (IC<sub>50</sub> of adriamycin without target compound)/(IC<sub>50</sub> of adriamycin with target compound).

As shown in Table 1, 2H7 displayed the potent chemo-sensitizing effect with a significantly decreased IC<sub>50</sub> 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.