Supplementary Materials: Chemical Identity of Interaction of Protein with Reactive Metabolite of Diosbulbin B In Vitro and In Vivo

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Figure S1. DIOB was oxidized by DMDO, and the resulting mixture was reacted with Cys and then with BBA, followed by LC-MS/MS analysis. **a**: Extracted ion chromatograms of MRM scans of m/z 631 \rightarrow 169 (orange) and 633 \rightarrow 171 (green). **b**: MS/MS spectrum of synthetic pyrrole **6**.



Figure S2. DIOB was oxidized by DMDO, and the resulting mixture was reacted with Lys and then with BBM, followed by LC-MS/MS analysis. **a**: Extracted ion chromatograms of MRM scans of m/z 673 \rightarrow 169 (orange) and 675 \rightarrow 171 (green). **b**: MS/MS spectrum of synthetic pyrrole **10**.



Figure S3. DIOB was oxidized by DMDO, and the resulting mixture was reacted with microsomal protein and then with BBA, followed by complete proteolytic digestion and LC-MS/MS analysis. **a**: Extracted ion chromatograms of MRM scans of m/z 631 \rightarrow 169 (orange) and 633 \rightarrow 171 (green). **b**: MS/MS spectrum of synthetic pyrrole **6**.



Figure S4. DIOB was oxidized by DMDO, and the resulting mixture was reacted with microsomal protein and then with BBM, followed by complete proteolytic digestion and LC-MS/MS analysis. **a**: Extracted ion chromatograms of MRM scans of m/z 673 \rightarrow 169 (orange) and 675 \rightarrow 171 (green). **b**: MS/MS spectrum of synthetic pyrrole **10**.



Figure S5. DIOB was oxidized by DMDO, and the resulting mixture was reacted with Cys and Lys, followed by LC-MS/MS analysis. **a**: Extracted ion chromatogram of MRM scan of m/z 592 \rightarrow 503. **b**: MS/MS spectrum of synthetic pyrrole **12**.

а

b

2.0e6 1.9el 1.8e£

1.7e6 1.6e6 1.5e6 1.4e6 1 3e6

1.2e6

1.0e6 9.0e5 ŝ

8.0e5

7.0e5

6.0e5

5.0e5

4.0e5

3.0e5 2.0e5 1.0e5 0.0

ntensity, 1.1e6

4.2et 4.0e6 3.8e6 3.6e6 3.2e6 3.0e6 2.8e6 2.6e6 2.4e6 2.2e6 ntensity, cps 2.0e6 1.8e6 1.6e6 1.4e6 1.2e6 1.0e6 8.0e5 6.0e5 4.0e5 2.0e5



440.1

'5

529.2

592.2

600

Figure S6. DIOB was oxidized by DMDO, and the resulting mixture was reacted with microsomal protein, followed by complete proteolytic digestion and LC-MS/MS analysis. a: Extracted ion chromatogram of MRM scan of m/z 592 \rightarrow 503. **b**: MS/MS spectrum of synthetic pyrrole **12**.

350 m/z, Da

346.0 ₃₇₄

234.0

249.0