Supporting information for:

Cysteine-conjugated metabolite of ginger component [6]-shogaol serves as a carrier of [6]-shogaol in cancer cells and in mice

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RESULTS

Characterization of M19–M21.

In the extracted chromatogram of m/z 428 $[M + H]^+$, (M19, ketone-reduced product of M16 under positive mode), one new peak (RT: 19.27 min) was found in the urine samples collected from 6S and M2 treated mice (Figure S2). Its MS^2 spectrum showed a major product ion at m/z 410 (-18, dehydration), and 247 (loss of N-acetylcysteine of 410) as the major product ions. The MS^3 spectrum of its product ion *m/z* 247 showed product ions of m/z 149, and 163. Based on these fragments, a potential mass fragmentation pathway was proposed as shown in Figure S2. The structure of M19 was then tentatively identified as shown in Figure 1.

There was one major peak (M20) with molecular ion m/z 343 $[M + H]^+$. The molecular weight of M20 was 2 mass units higher than that of M17, indicating that M20 was the ketone-reduced product of M17. Its MS^2 spectrum showed product ions of m/z 325 (-18 Da, dehydrolyzation of M17) (Figure S2) and 261 (loss of HSOMe of 325). The MS^3 spectrum of its product ion *m/z* 261 showed product ions of m/z 149, and 163. Based on these fragments, a potential mass fragmentation pathway was proposed as shown in Figure S2. The structure of M20 was then tentatively identified as shown in Figure 1.

Similarly, there was one major peak (M21) with molecular ion m/z 329 $[M + H]^+$. The molecular weight of M21 was 14 Da lower than that of M20, indicating that M21 was mono-demethylated M20. This was further confirmed by observing m/z 311 $[M + H]^+$ (loss of one H_2O moiety from M20) and 247 (loss of HSOMe of 311) as its major product ions in the MS^2 spectrum of molecular ion m/z 329 (Figure S2). Since there was only one methyl group in M20, we then identified M21 as 3′-demethylated M20. The MS^3 spectrum (MS^3: m/z 247/329) of M21

showed m/z 149, and 163 as the major product ions, which further confirmed our above deduction (Figure S2).

Figure S1. Stability of M2 in H_2O (A), MeOH (B), PBS (C), and McCoy's 5A media (D).

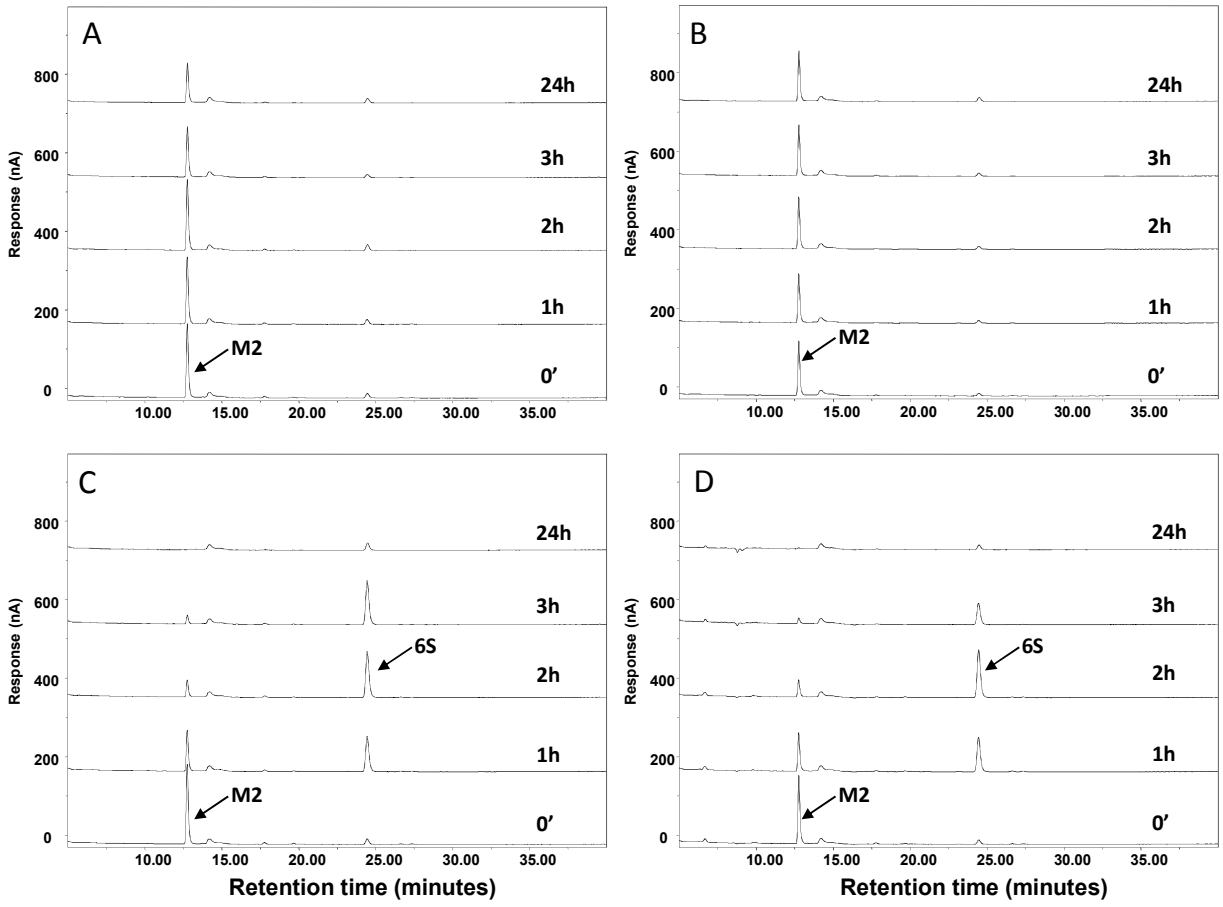

Figure S2. LC-MS^2 and MS^3 (positive) spectra of M19 (A), M20 (B), and M21 (C), and a potential fragmentation pathway of M19–M21 (D).

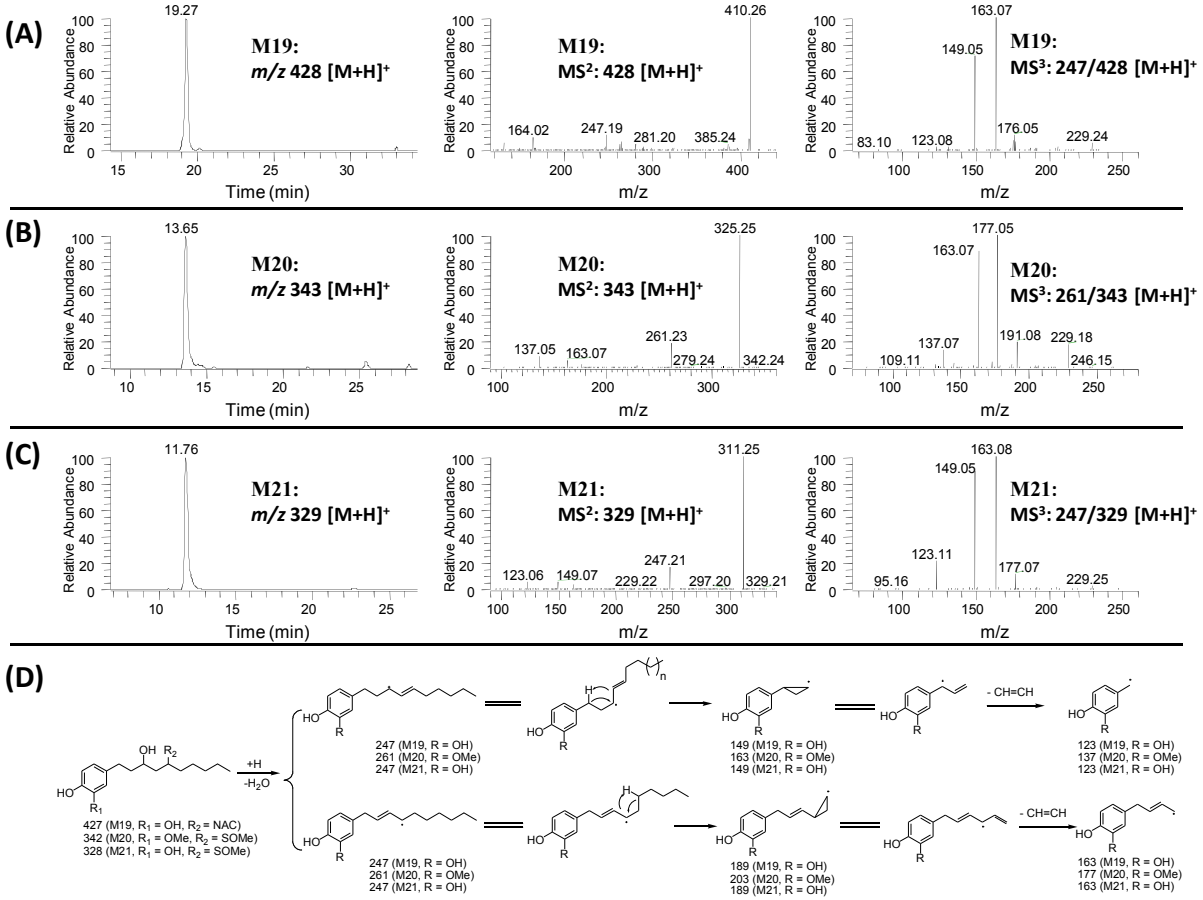

Figure S3. Ion chromatograms of the synthesized authentic M2 (A), and mouse urine (B) and feces (C) collected from M2 treated mice (200 mg/kg, oral gavage) obtained by positive APCI-MS interface extracted with m/z 327 $[M+H]^+$.