Supplementary Information

Microbial Transformation of Pimavanserin by *Cunninghamella blakesleeana* AS 3.970

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The SIR chromatograms of the microbially transformed samples of pimavanserin and the strain control samples are shown in Figures S1 and S12, respectively. Compared with the strain control sample, 10 transformation metabolites were found. The possible structures of these transformation metabolites were analyzed by comprehensive profiling of chromatographic and MS data (see Tables S1).

Table S1. MS data and chromatographic retention time of microbial transformation metabolites of pimavanserin.

Metabolite No.	$[M+H]^+$	m/z			T_R(min)
M0	428	223	98		9.71
M1	444	223	98		5.61
M2	460	223	98		4.59
M3	372	223	98		4.50
M4	320	163	115	107	6.45
M5	266	223	98		2.92
M6	430	223	98		9.74
M7	446	223	98		5.62
M8	442	223	98		9.41
M9	458	223	98		5.41
M10	410	205	98		9.57

A. Strain control group; B. Pimavanserin transformation group.

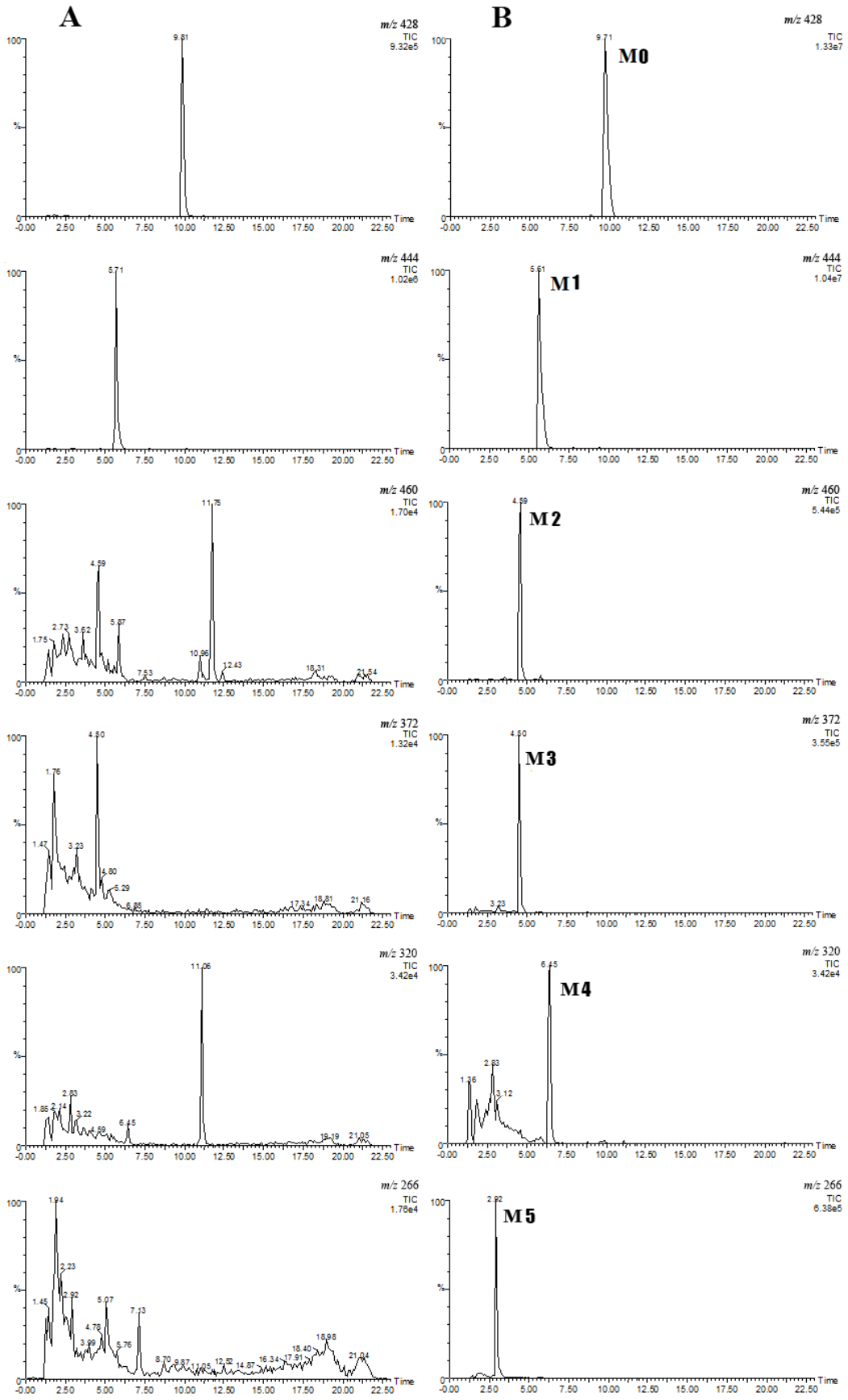

Figure S1. SIR chromatograms of pimavanserin and its transformation metabolites (M1–M5).

The pimavanserin M0quasi-molecular ion $[M+H]^+$ was *m/z* 428 and its fragment ions *m/z* were 223, 163, and 98, respectively, which did not change its molecular weight compared with the original pimavanserin , thus proving that the compound is the prototype pimavanserin .

The mass spectra of metabolite M1 are shown in Figure S2.

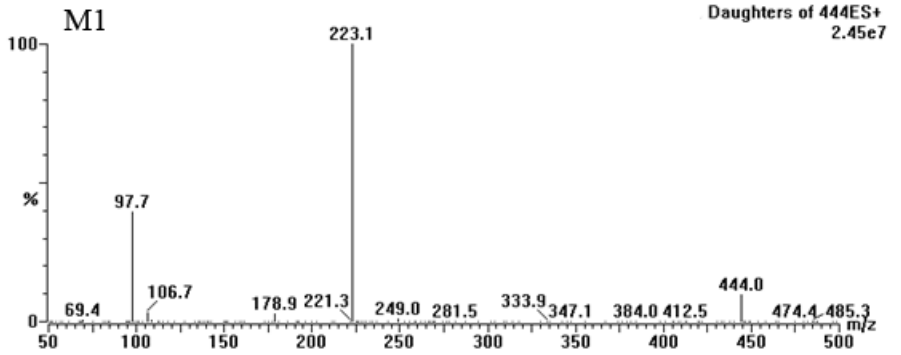

Figure S2. MS/MS spectrum of $[M+H]^+$ of M1.

From Figure S2, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m/z* 444, which is 16 more molecular weight compared to pimavanserin, and this compound may be the hydroxylated product of pimavanserin. Its fragment ions are mainly *m/z* 98 and *m/z* 223, indicating no structural changes in the 4-fluorobenzyl and 1-methylpiperidin-4-yl fractions, thus inferring the M1 structure as in Figure S3.

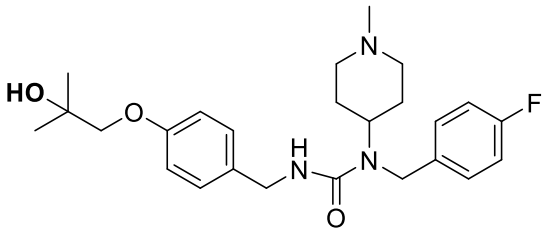

Figure S3. Predictive structure of M1.

The mass spectra of metabolite M2 are shown in Figure S4.

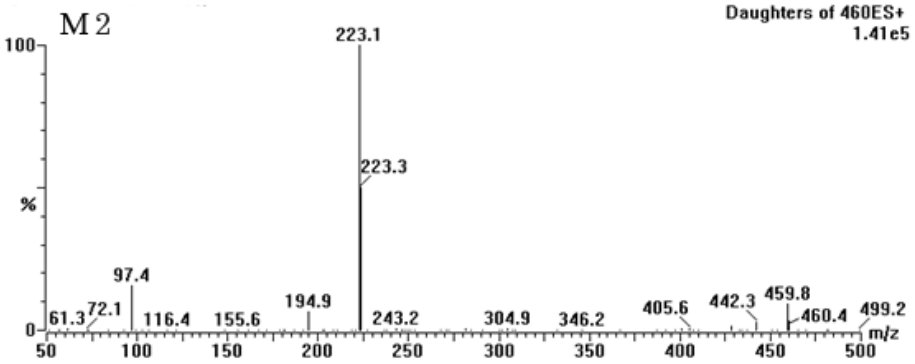

Figure S4. MS/MS spectrum of $[M+H]^+$ of M2.

From Figure S4, it can be seen that the quasi-molecular ion $[M+H]^+$ is *m/z* 460, which is 32 more molecular weight than that of pimavanserin , and the compound may be a dihydroxylated product of pimavanserin . Its fragment ions are mainly *m/z* 98 and *m/z*

223, indicating that no structural changes occurred in the 4-fluorobenzyl and 1-methylpiperidin-4-yl fractions, and, therefore, the inferred M2 structure is shown in Figure S5.

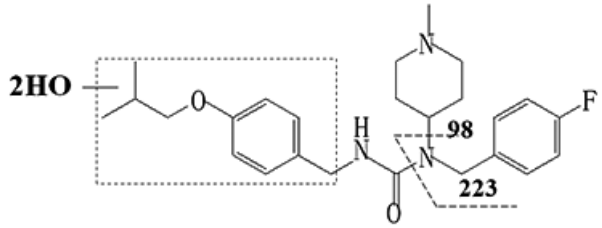

Figure S5. Predictive structure of M2.

The mass spectra of metabolite M3 are shown in Figure S6.

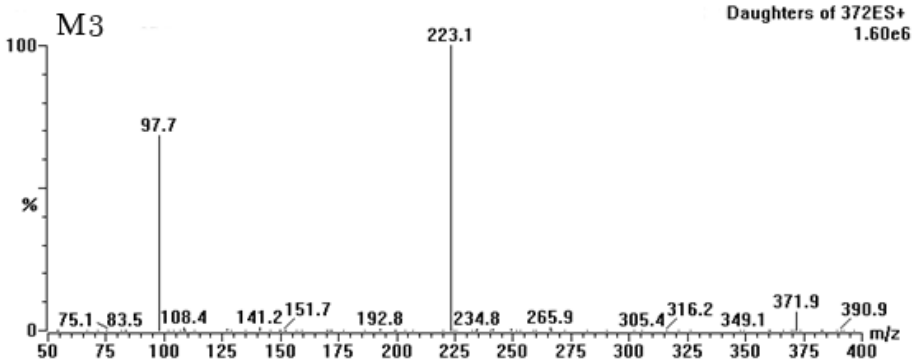

Figure S6. MS/MS spectrum of $[M+H]^+$ of M3.

From Figure S6, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 372, which is 56 less molecular weight than that of pimavanserin , and this compound may be a deisobutyl product of pimavanserin. Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating no structural changes in the 4-fluorobenzyl and 1-methylpiperidin-4-yl fractions, and therefore, the inferred M3 structure is shown in Figure S7.

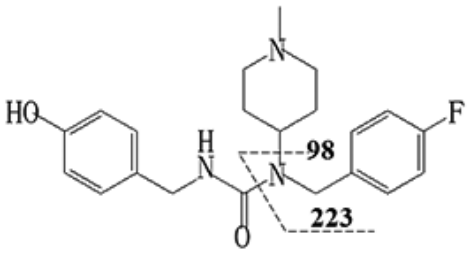

Figure S7. Predictive structure of M3.

The mass spectra of metabolite M4 are shown in Figure S8.

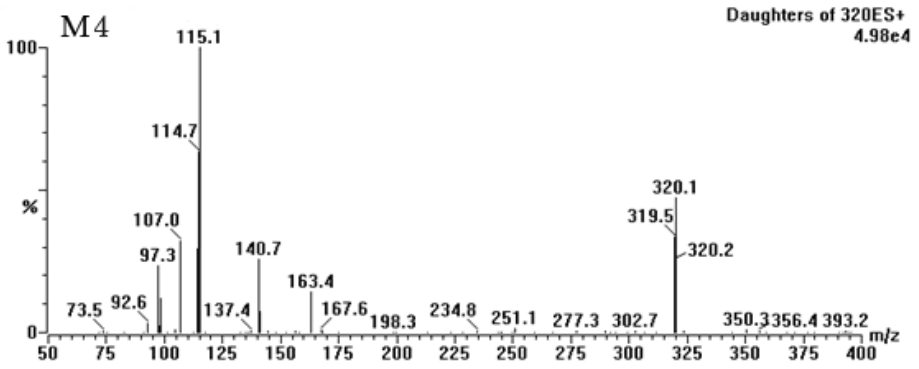

Figure S8. MS/MS spectrum of $[M+H]^+$ of M4.

From Figure S8, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 320, which is 108 less molecular weight than that of pimavanserin , and this compound may be a defluorobenzyl product of pimavanserin. The fragment ions are mainly *m*/*z* 98 and *m*/*z* 115, and the *m*/*z* 115 fragment ion is 1-methylpiperidin-4-amine ion, therefore, the inferred structure of M4 is shown in Figure S9.

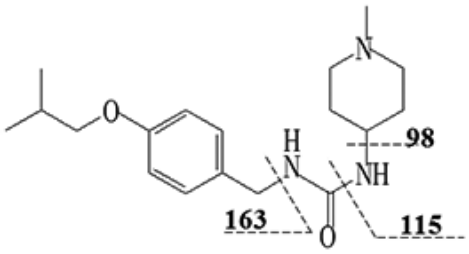

Figure S9. Predictive structure of M4.

The mass spectra of metabolite M5 are shown in Figure S10.

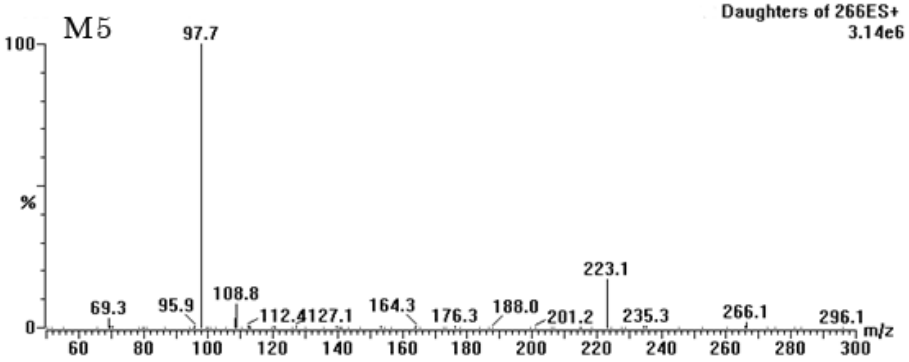

Figure 10. MS/MS spectrum of $[M+H]^+$ of M5.

From Figure S10, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 266, which is 162 less molecular weight compared to pimavanserin , and this compound may be an 4-isobutoxybenzyl missing product from pimavanserin. Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating that no structural changes occurred in the 4-fluorobenzyl and 1-methylpiperidin-4-yl fractions, and therefore, the structure of M5 is deduced as in Figure S11.

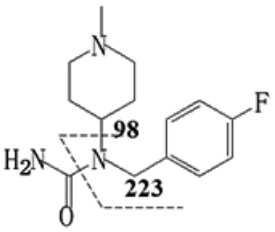

Figure S11. Predictive structure of M5.

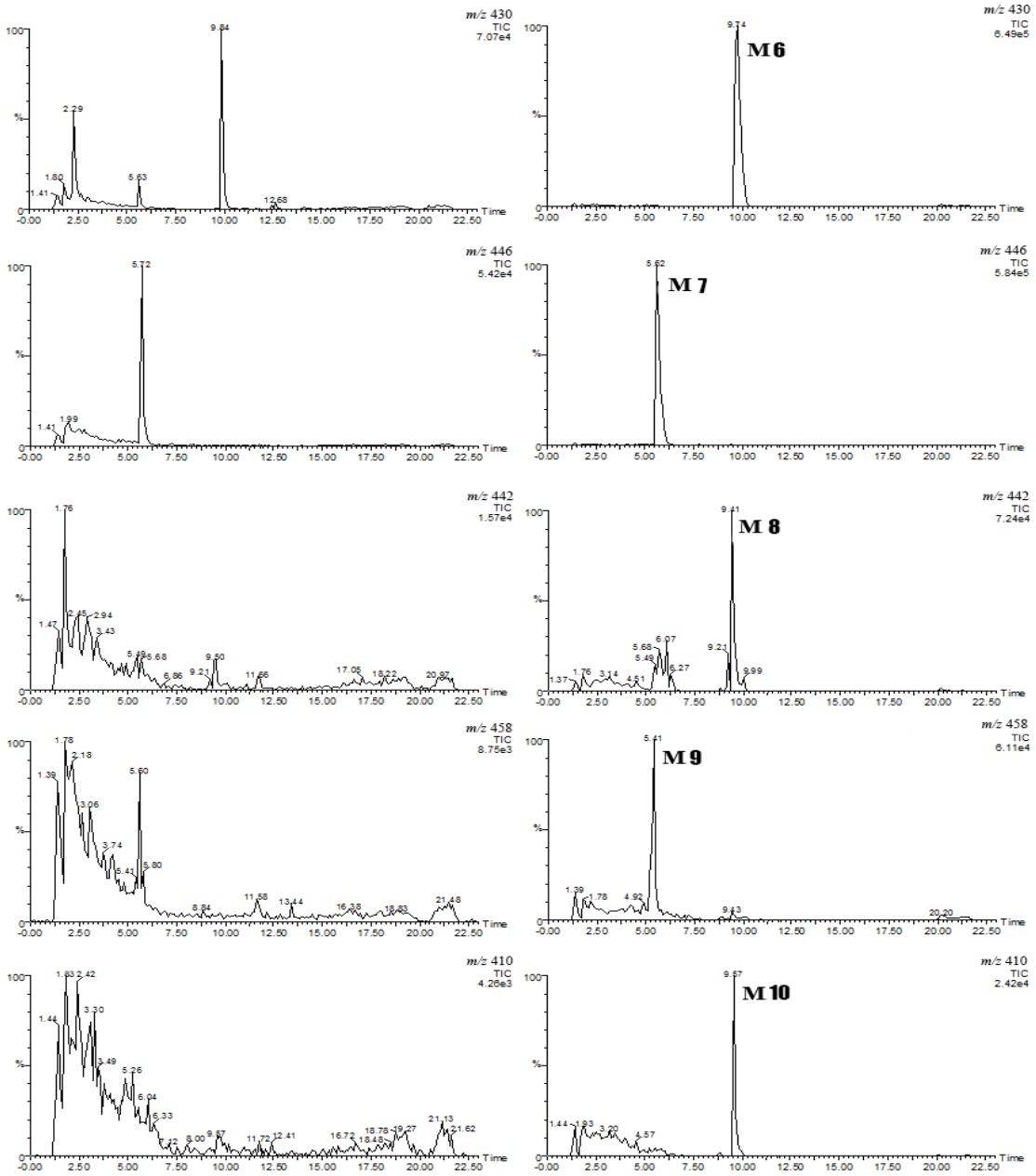

Figure S12. SIR chromatograms of pimavanserin and its transformation metabolites (M6-M10).

The mass spectra of metabolite M6 are shown in Figure S13.

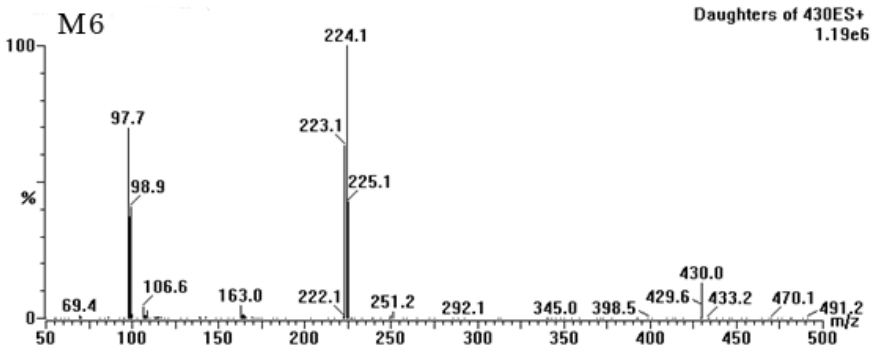

Figure S13. MS/MS spectrum of $[M+H]^+$ of M6.

From Figure S13, it can be seen that the quasi-molecular ion $[M+H]^+$ is *m*/*z* 430, which is 2 more molecular weight than that of pimavanserin , and this compound may be a pimavanserin derivative with one hydroxyl introduced as well as one methyl removed (2=16-14). Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating that no structural changes occurred in the 4-fluorobenzyl and 1-methylpiperidin-4-yl fractions, and therefore, the structure of M6 is deduced as in Figure S14.

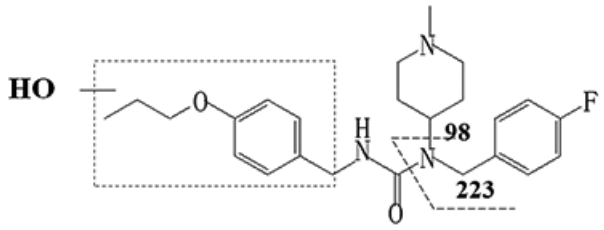

Figure S14. Predictive structure of M6.

The mass spectra of metabolite M7 are shown in Figure S15.

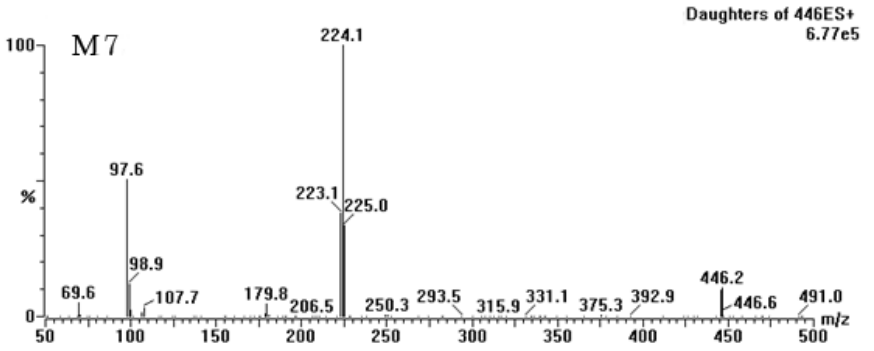

Figure S15. MS/MS spectrum of $[M+H]^+$ of M7.

From Figure S15, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 446, which is 18 more molecular weight than that of pimavanserin. This compound may be a pimavanserin derivative with two hydroxyl groups introduced as well as one methyl removed (18=32-14). Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating no structural changes in the 4-fluorobenzyl and 1-methylpiperidin-4-yl parts, and therefore, the structure of M7 is inferred as in Figure S16.

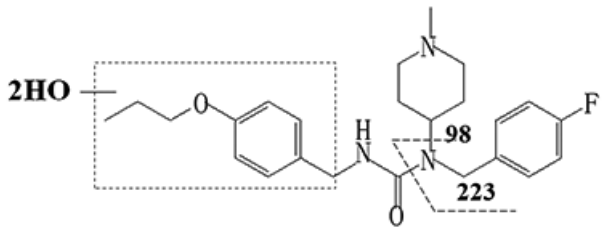

Figure S16. Predictive structure of M7.

The mass spectra of metabolite M8 are shown in Figure S17.

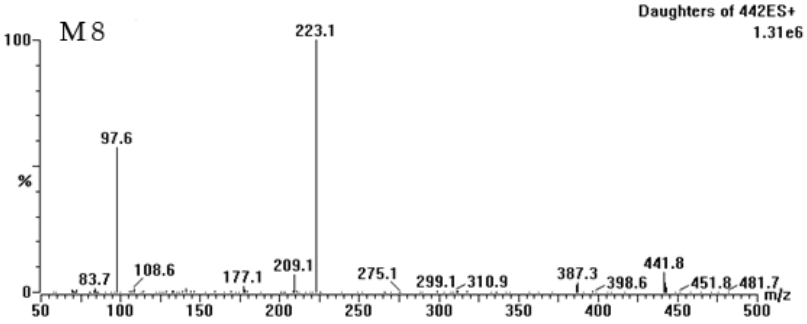

Figure 17. MS/MS spectrum of $[M+H]^+$ of M8.

From Figure S17, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 442, which has 14 more molecular weight than that of pimavanserin , and this compound may be a pimavanserin derivative with one methyl substituted. Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating no structural changes in the 4-fluorobenzyl and 1-methylpiperidin-4-yl portions, and therefore, the structure of M8 is inferred as in Figure S18.

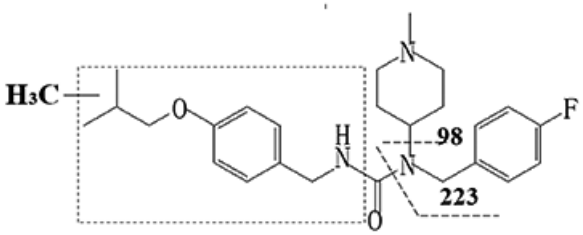

Figure S18. Predictive structure of M8.

The mass spectra of metabolite M9 are shown in Figure S19.

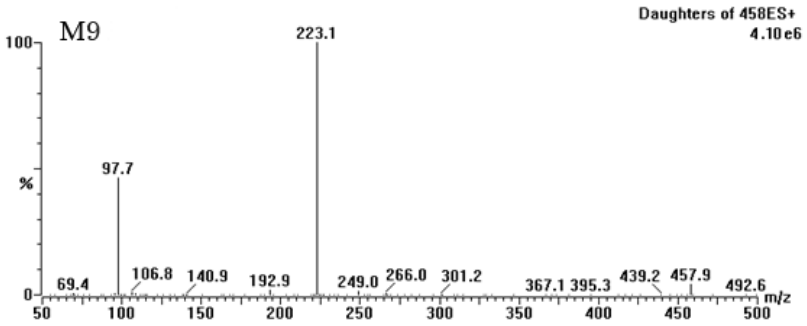

Figure S19. MS/MS spectrum of $[M+H]^+$ of M9.

From Figure S19, it can be seen that the quasi-molecular ion $[M+H]^+$ of this compound is *m*/*z* 458, which is 30 more molecular weight than that of pimavanserin, and this compound may be a pimavanserin derivative with one methoxy substituted. Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 223, indicating no structural changes in the 4-fluorobenzyl and 1-methylpiperidin-4-yl parts, and therefore, the structure of M9 is deduced as in Figure S20.

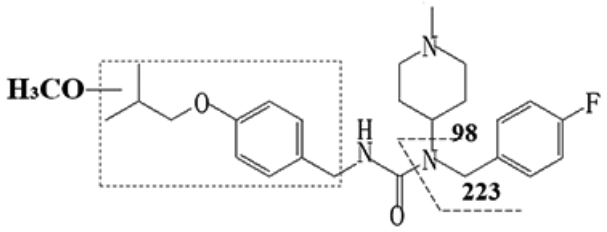

Figure S20. Predictive structure of M9.

The mass spectra of metabolite M10 are shown in Figure S21.

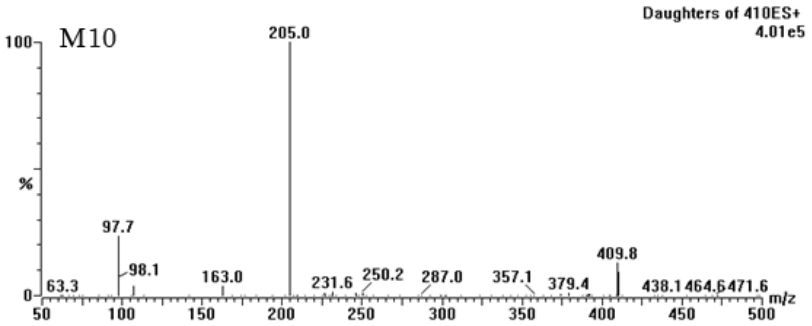

Figure S21. MS/MS spectrum of $[M+H]^+$ of M10.

From Figure S21, it can be seen that the quasi-molecular ion $[M+H]^+$ of the compound is *m*/*z* 410, which is 18 less molecular weight than that of pimavanserin, and the compound may be a pimavanserin derivative with one fluorine removed. Its fragment ions are mainly *m*/*z* 98 and *m*/*z* 205, and the *m*/*z* 205 fragment ion is the 1-methylpiperidin-4-yl bound to benzyl portion, therefore, the structure of M10 is inferred as in Figure S22.

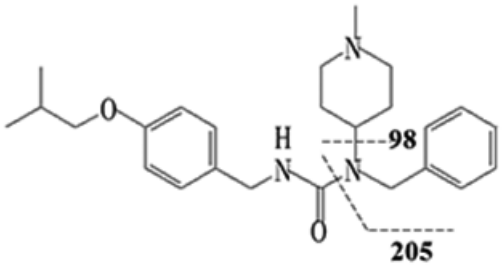

Figure S22. Predictive structure of M10.