

Supplementary Material

S1. Materials and Methods

S1.1. Culture medium

Potato dextrose liquid, 200 g L⁻¹ potato extract and 20 g L⁻¹ glucose, adjusted the pH to 4.5.

Potato dextrose agar (PDA), 200 g L⁻¹ potato extract, 20 g L⁻¹ glucose and 20 g L⁻¹ agar, adjusted the pH to 4.5.

Liquid enzyme production medium, the optimized concentrations of 10 g·L⁻¹ glucose, 0.2 g·L⁻¹ ammonium tartrate and 1 mmol·L⁻¹ MnSO₄ were used, 2.0 g KH₂PO₄, 0.1 g CaCl₂·2H₂O, 0.5 g MgSO₄·7H₂O, 1.0 g Tween80, 1.5 mL veratryl alcohol, 1 mg vitamin B1, 0.2 g yeast extract, 70 mL trace-element solution (1.5 g nitrilotriacetate, 1.46 g MgSO₄·7H₂O, 1.0 g NaCl, 0.1 g FeSO₄·7H₂O, 0.1 g CoSO₄, 0.1 g ZnSO₄·7H₂O, 0.01 g CuSO₄, 0.01 g AlK(SO₄)·12H₂O, 0.01 g H₂BO₃, 0.01 g NaMoO₄·2H₂O, 1 L water), 1L water (Tien and Kirk, 1988).

S1.2. Enzyme Activity Assay

The enzyme activity of MnP was calculated by equation S1

$$\text{MnP activity (U L}^{-1}\text{)} = \frac{\Delta A_{238} \times v_0}{\xi \times v} \times n \quad (\text{S1})$$

ΔA_{238} is the absorbance change value of sample per minute, v_0 is the total volume of the reaction system (1 mL), v is the solution volume of MnP (0.1 mL), ξ is the coefficient (6, 500 L mol⁻¹·cm⁻¹), n is the dilution rate.

S1.3. Modeling

The transformation kinetics of tetracycline by MnP was calculated by the

Michaelis-Menten model (S2).

$$v = \frac{v_{max} \times S}{K_m + S} \quad (S2)$$

v is the initial transformation rate ($\text{mg L}^{-1} \text{ min}^{-1}$), v_{max} is the maximum initial transformation rate ($\text{mg L}^{-1} \text{ min}^{-1}$), K_m (mg L^{-1}) is half saturation constant, and S is the initial concentration of tetracycline (mg L^{-1}). Non-linear regression analysis was conducted using the software Origin 9.0.

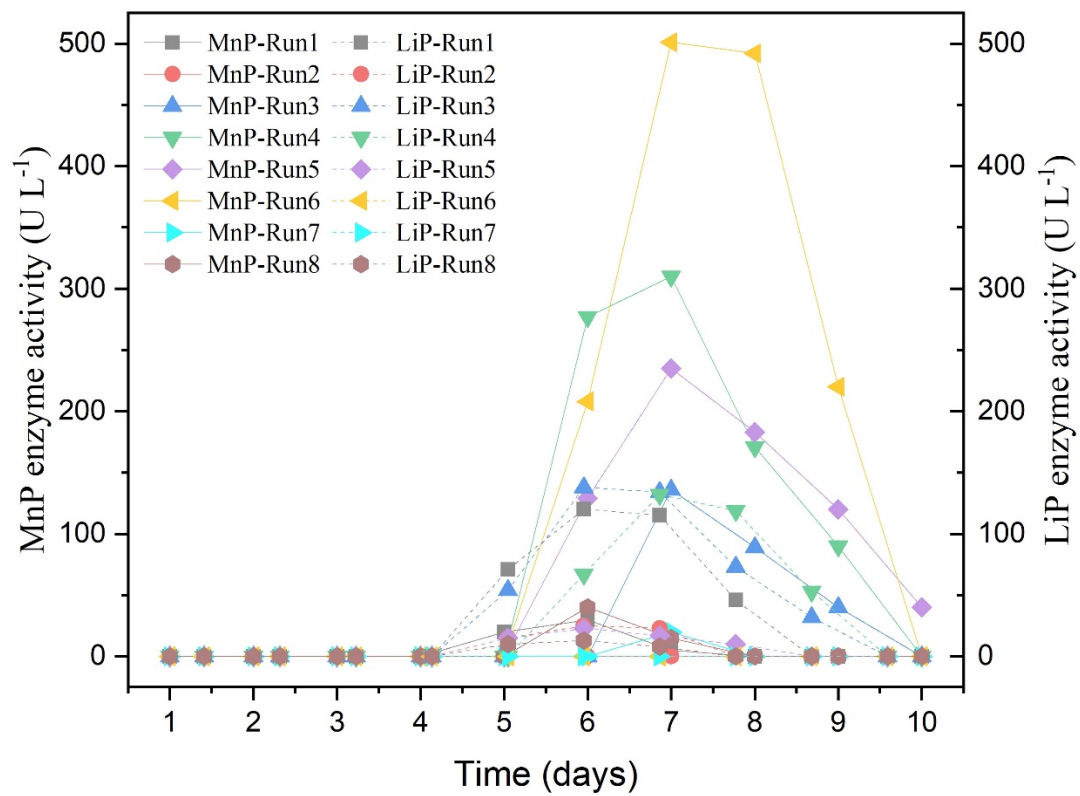


Figure S1. Expression of MnP and LiP in different runs of orthogonal experiment.

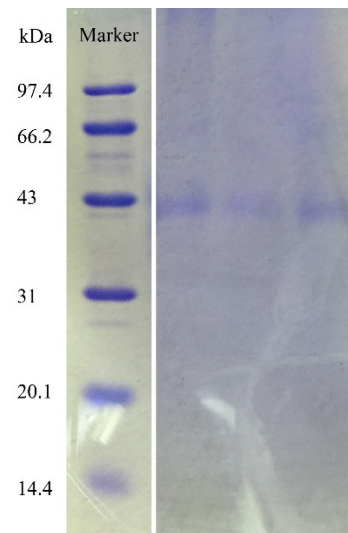


Figure S2. Electrophoretic analysis (SDS-PAGE) of purified MnP, which were stained with coomassie blue.

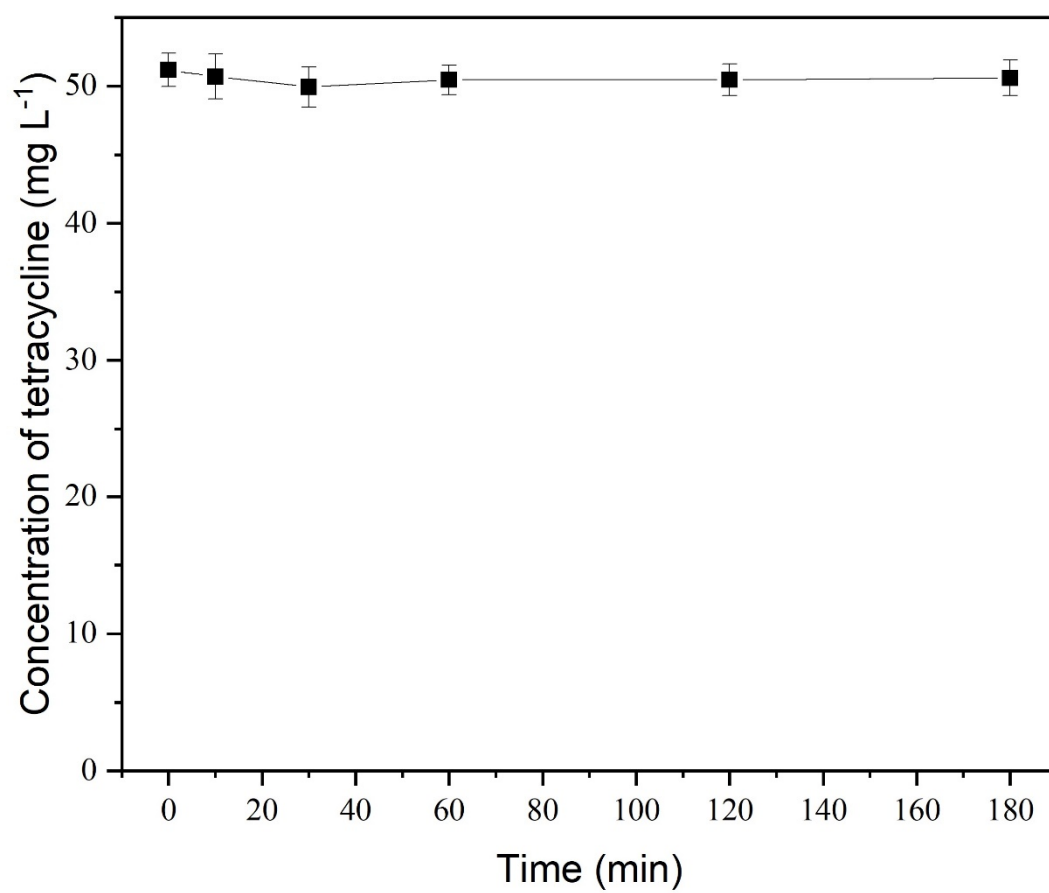


Figure S3. Treatment of tetracycline with H₂O₂ alone.

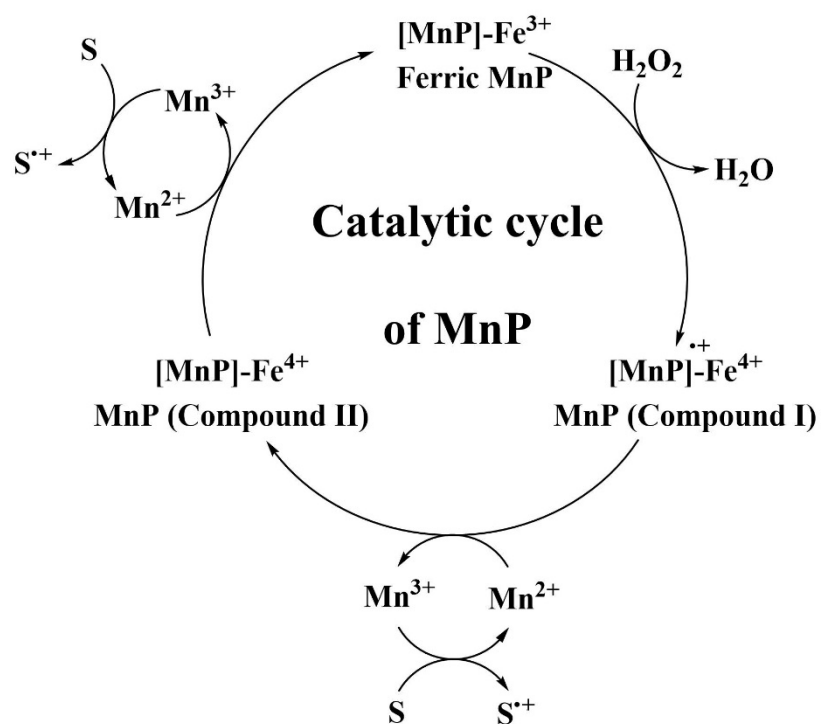


Figure S4. Mechanism of the catalytic cycle of MnP (Rahul et al., 2017).

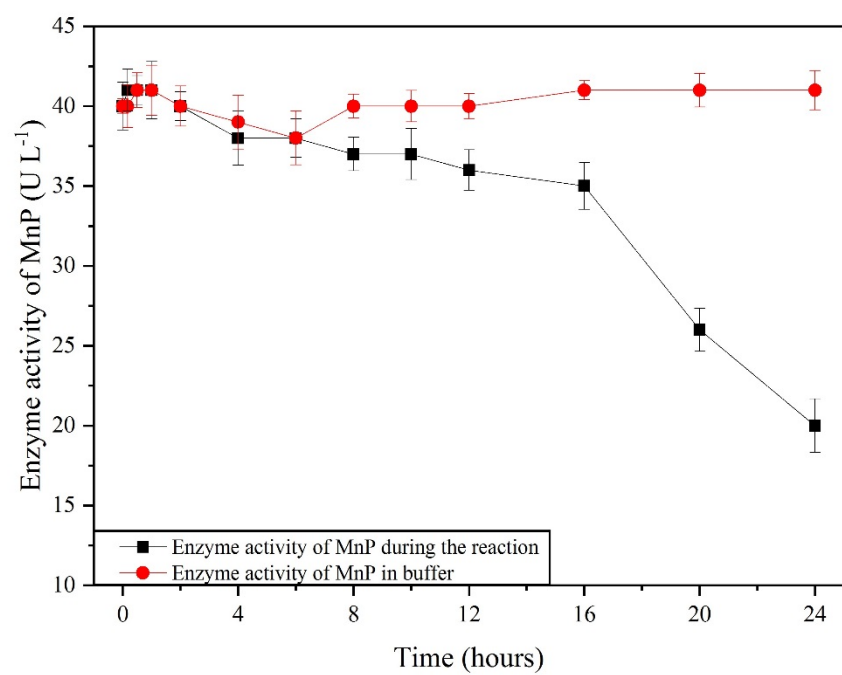


Figure S5. MnP enzyme activity changes in the catalytic system and buffer.

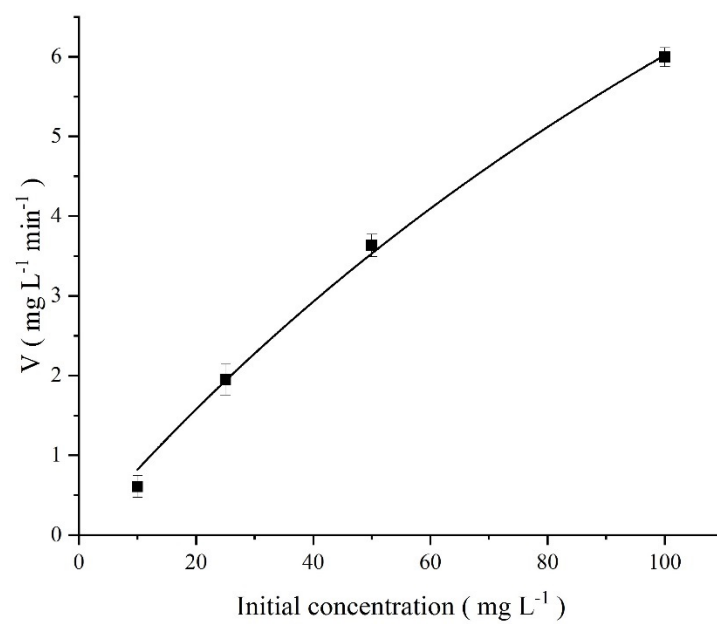
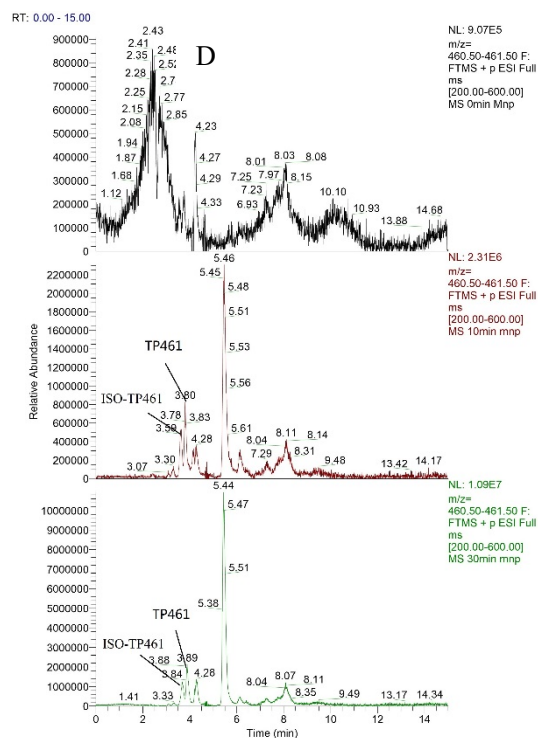
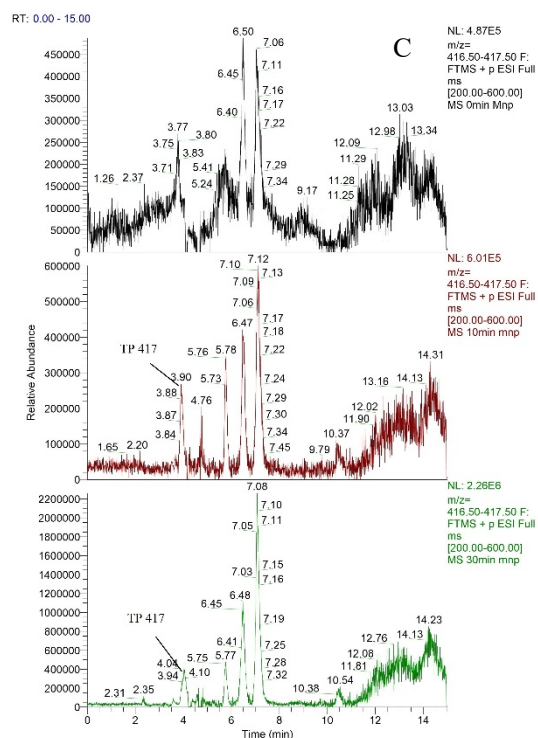
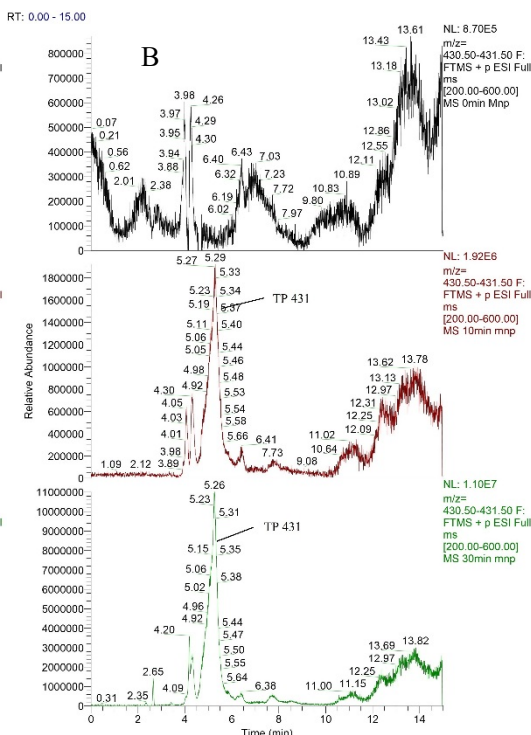
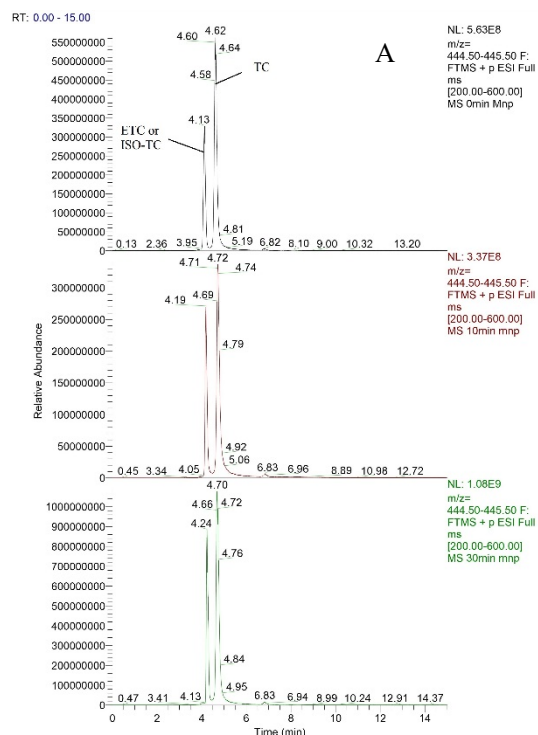


Figure S6. Simulation of transformation kinetics of tetracycline by Michaelis-Menten model in MnP system. The value of R^2 is 0.9953.



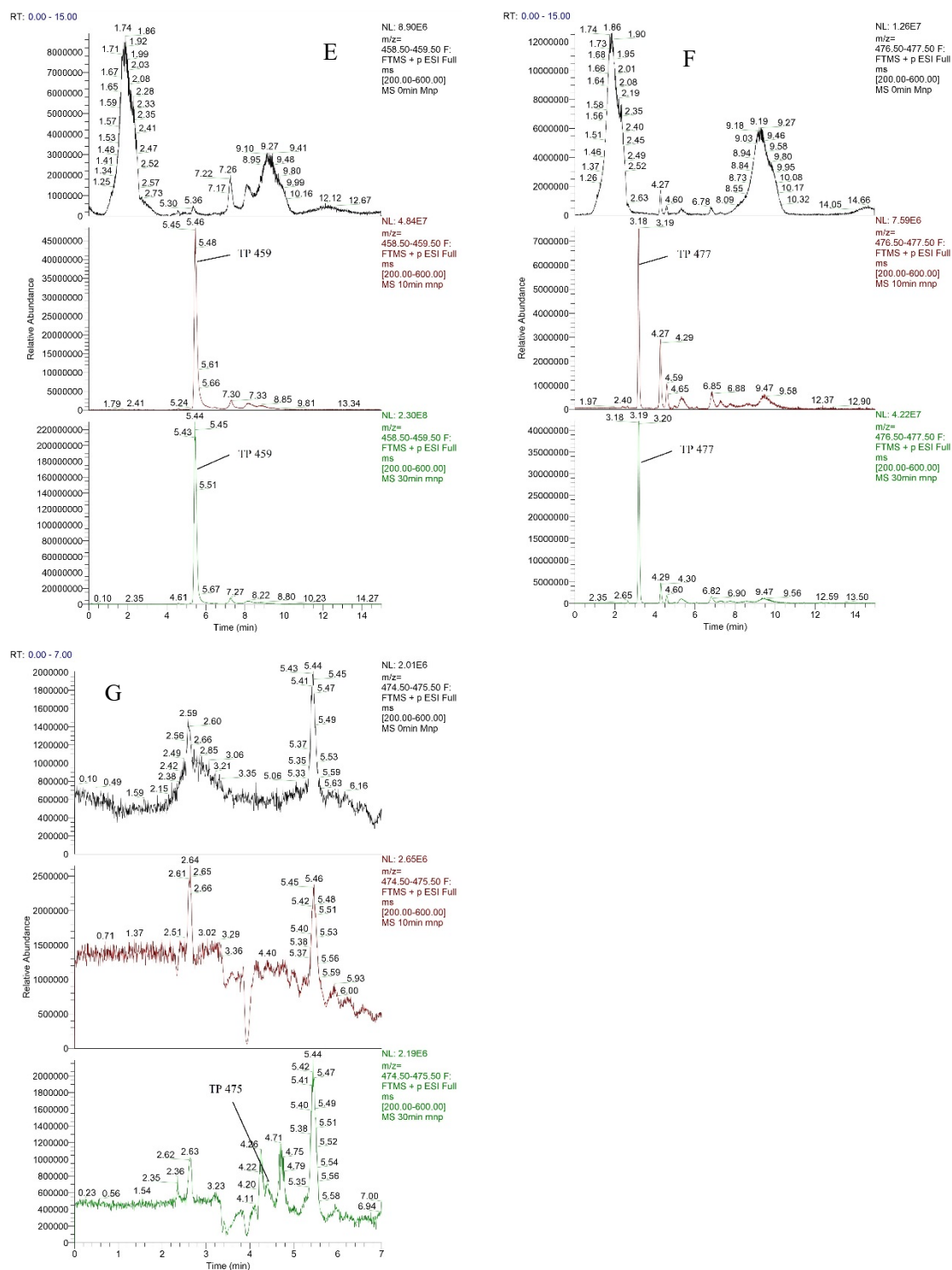


Figure S7. Extracted ion chromatograms at M/Z 445 (A), M/Z 431 (B), M/Z 417 (C), M/Z 461 (D), M/Z 459 (E), M/Z 477 (F) and M/Z 475 (G). Within each panel, the ion chromatograms from top to bottom represent the initial tetracycline parent compound sampled after 0 min, 10 min, and 30 min. The peaks were identified using mass spectrometry

30min Mnp #842 RT: 4.70 AV: 1 NL: 1.01E9
T: FTMS + p ESI Full ms [200.00-600.00]

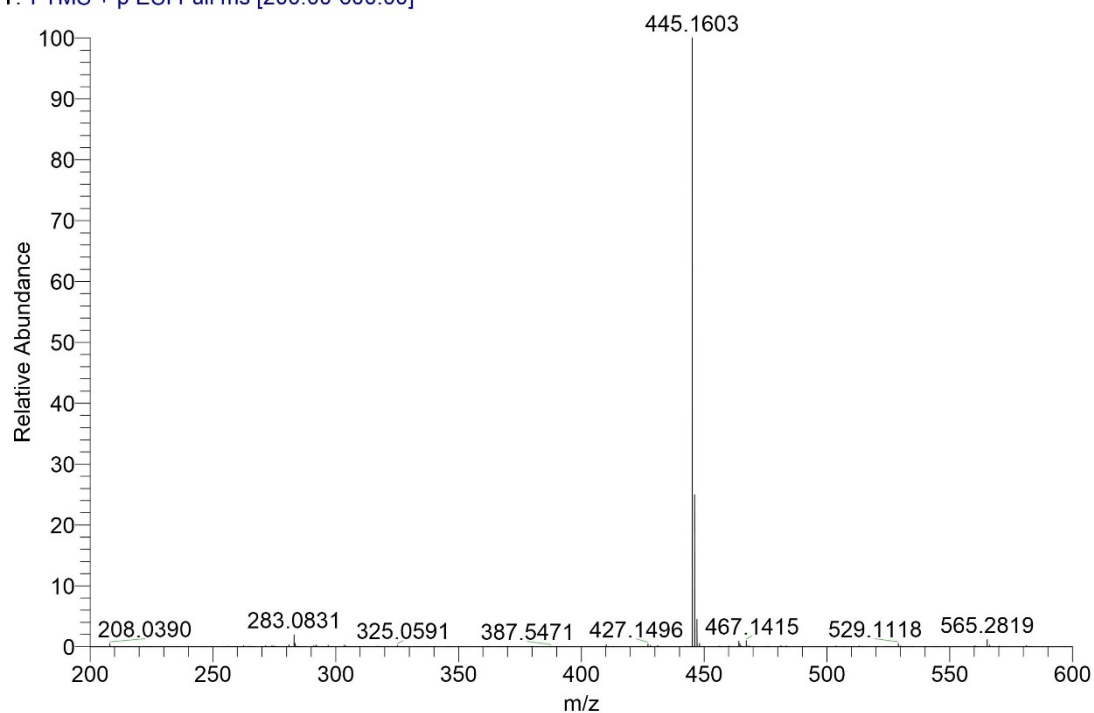


Figure S8. Mass spectrum of tetracycline.

30min Mnp #754 RT: 4.24 AV: 1 NL: 8.25E8
T: FTMS + p ESI Full ms [200.00-600.00]

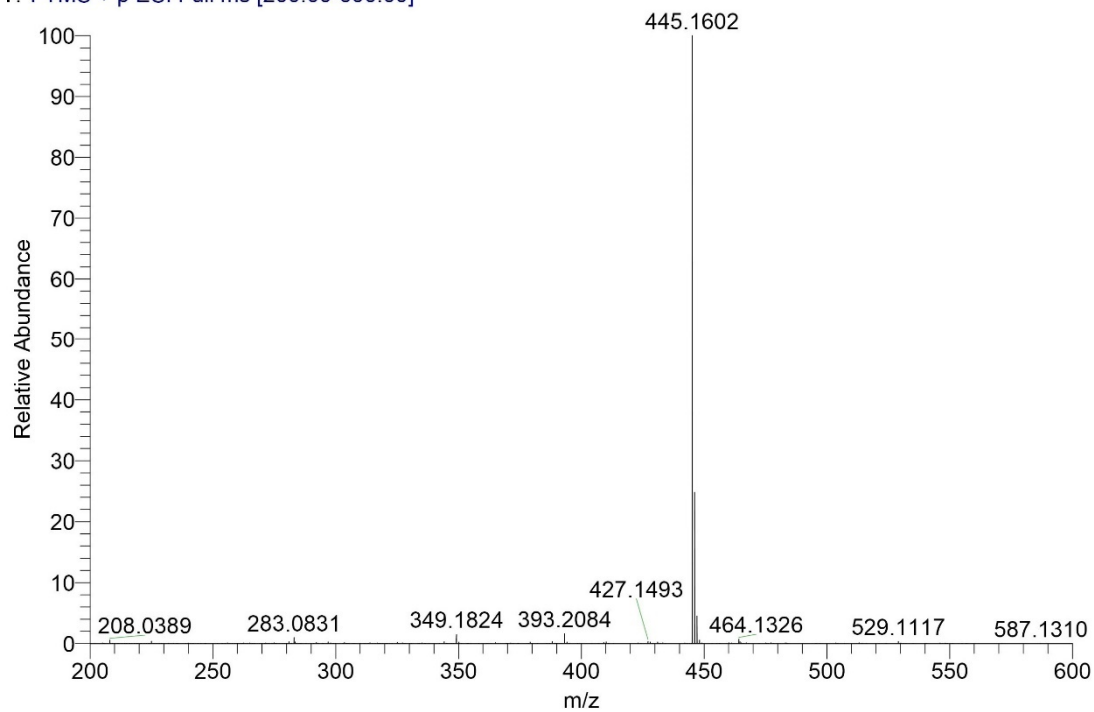


Figure S9. Mass spectrum of ETC or ISO-TC.

30min Mnp #948 RT: 5.26 AV: 1 NL: 1.05E7
T: FTMS + p ESI Full ms [200.00-600.00]

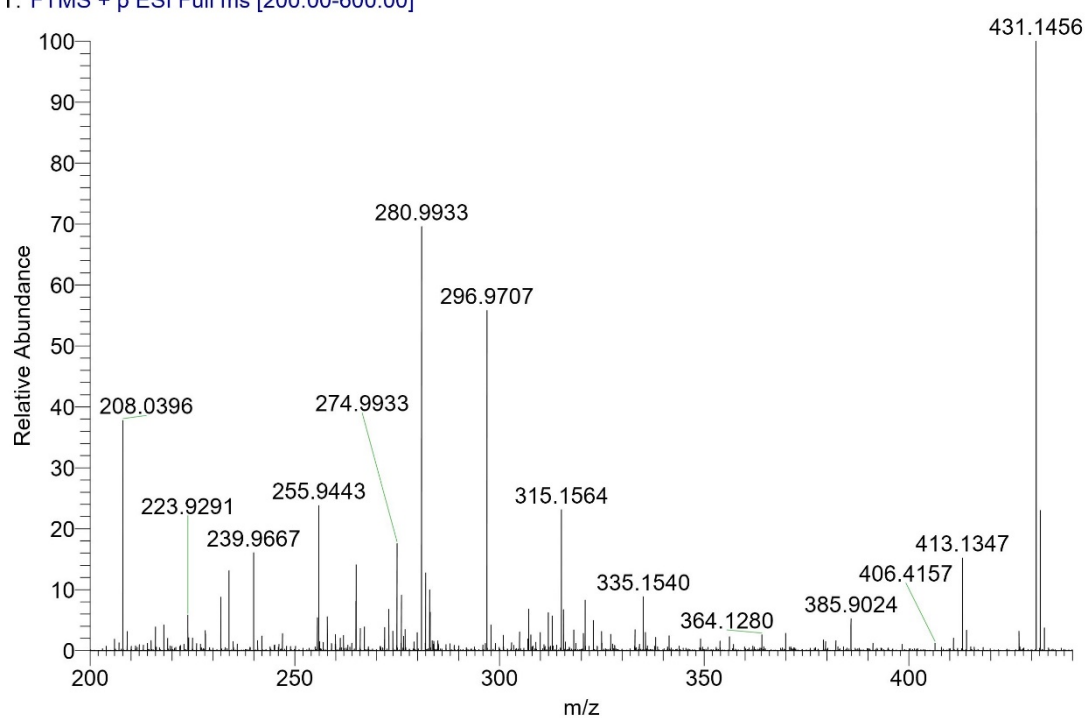


Figure S10. Mass spectrum of TP431.

10min mnp #695 RT: 3.91 AV: 1 NL: 1.48E6
T: FTMS + p ESI Full ms [200.00-600.00]

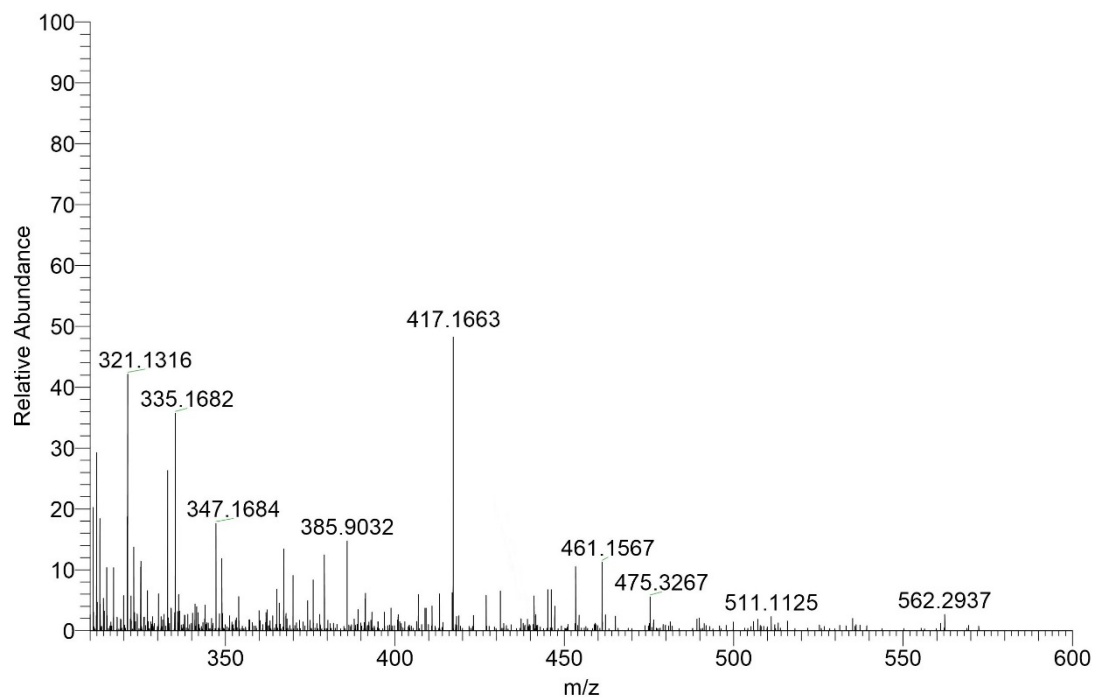


Figure S11. Mass spectrum of TP417.

30min Mnp #654 RT: 3.68 AV: 1 NL: 3.18E6
T: FTMS + p ESI Full ms [200.00-600.00]

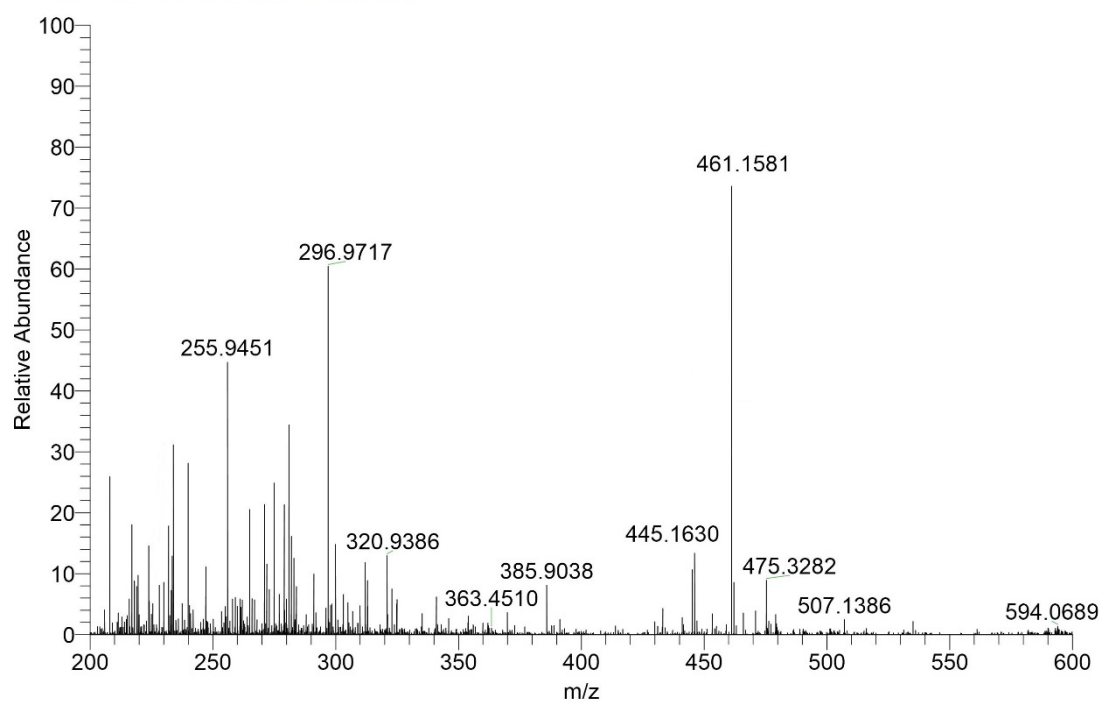


Figure S12. Mass spectrum of TP461.

30min Mnp #690 RT: 3.89 AV: 1 NL: 5.03E6
T: FTMS + p ESI Full ms [200.00-600.00]

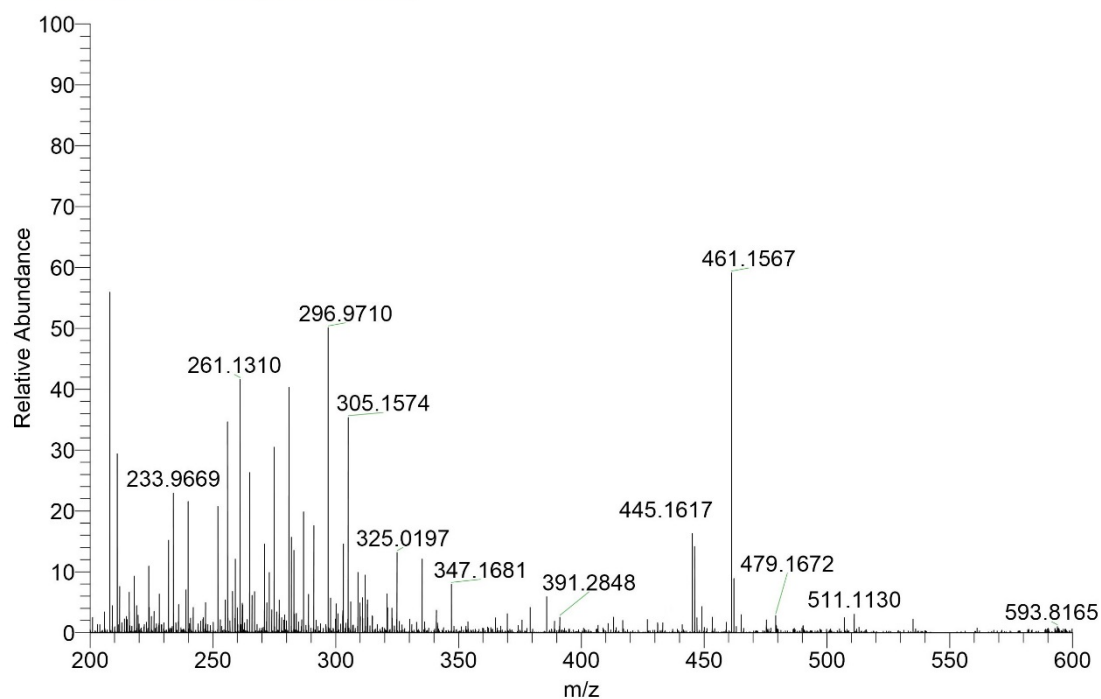


Figure S13. Mass spectrum of ISO-TP461.

30min Mnp #984 RT: 5.45 AV: 1 NL: 2.12E8
T: FTMS + p ESI Full ms [200.00-600.00]

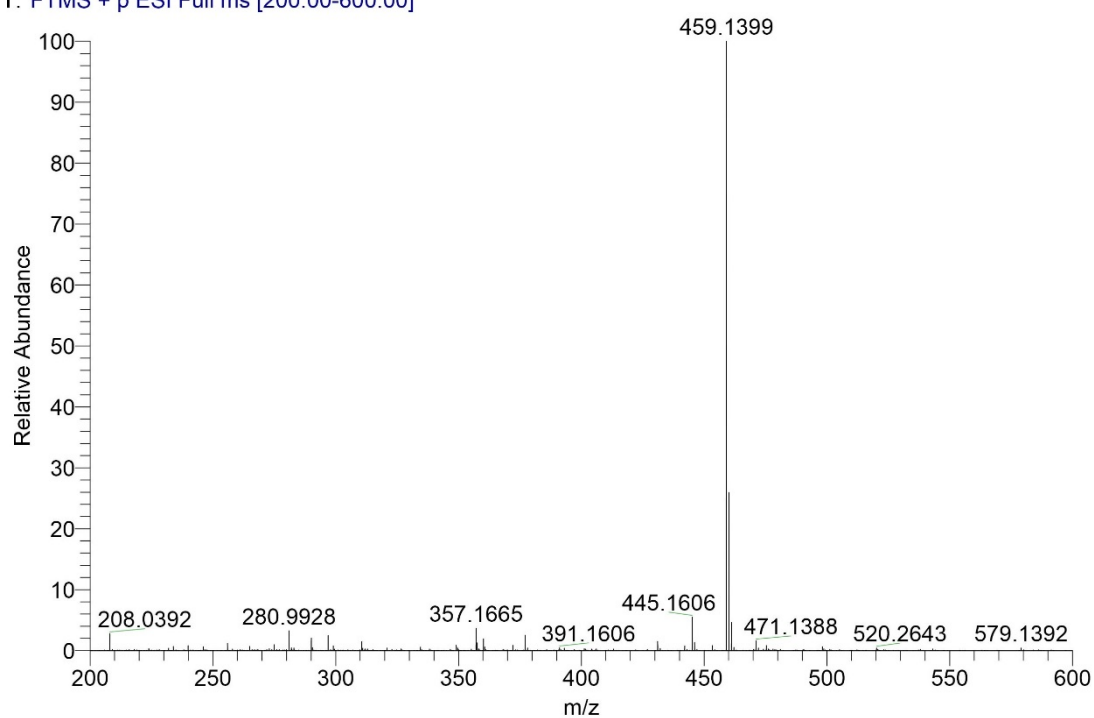


Figure S14. Mass spectrum of TP459.

30min Mnp #568 RT: 3.19 AV: 1 NL: 4.14E7
T: FTMS + p ESI Full ms [200.00-600.00]

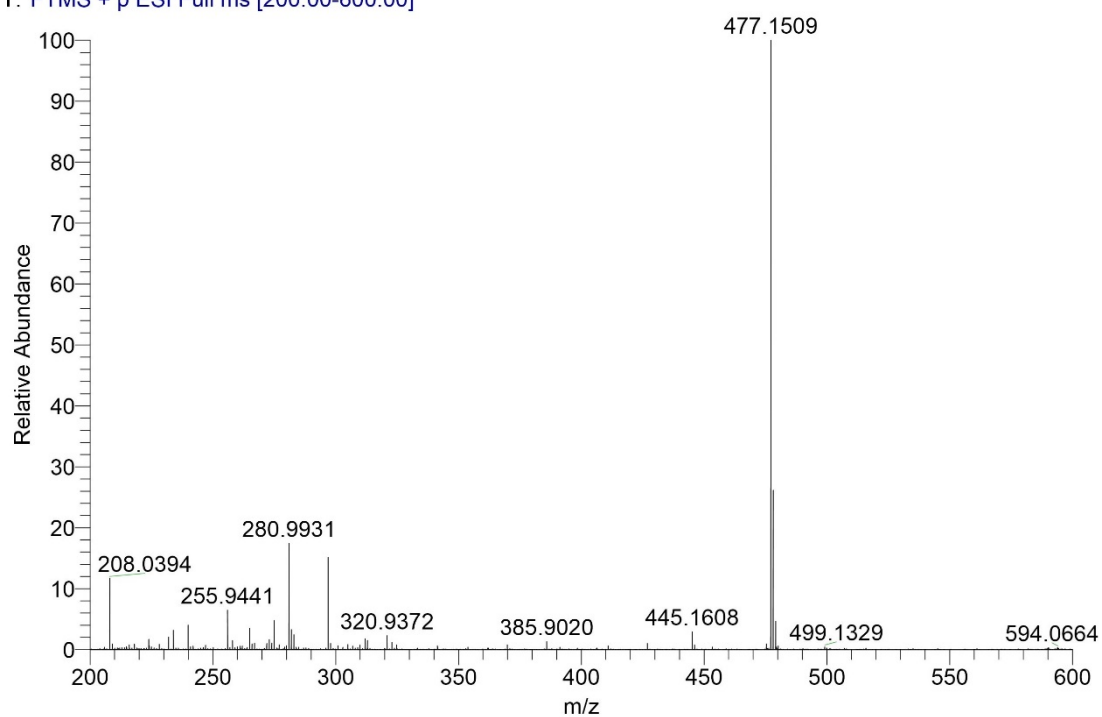


Figure S15. Mass spectrum of TP477.

30min Mnp #784 RT: 4.39 AV: 1 NL: 2.92E6
T: FTMS + p ESI Full ms [200.00-600.00]

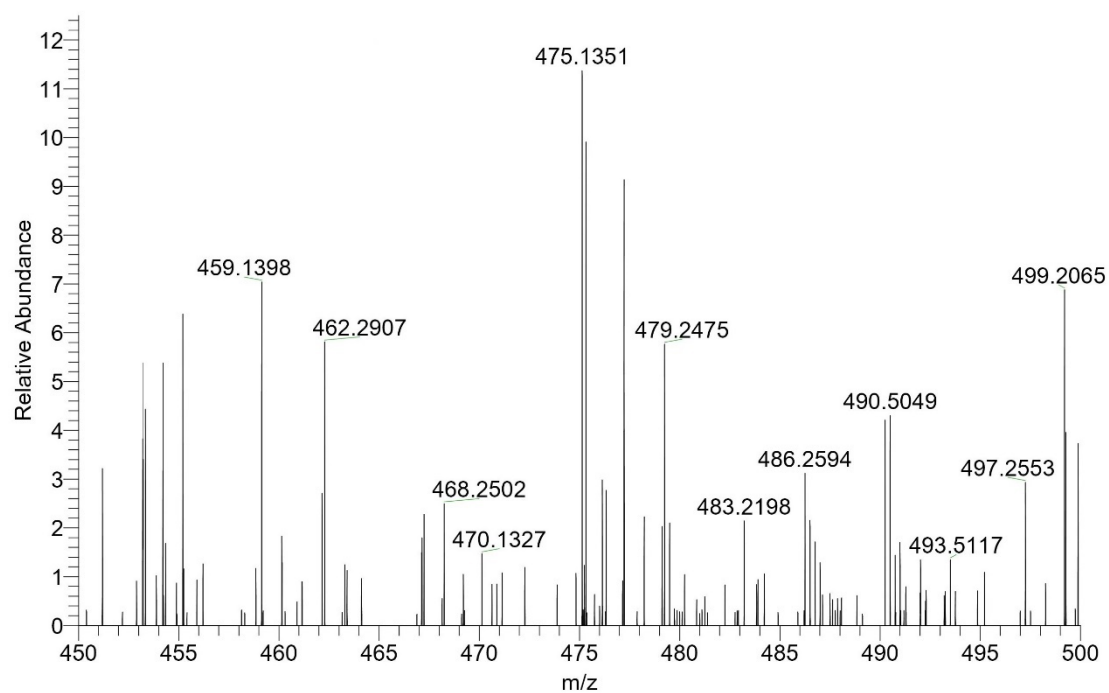


Figure S16. Mass spectrum of TP475.

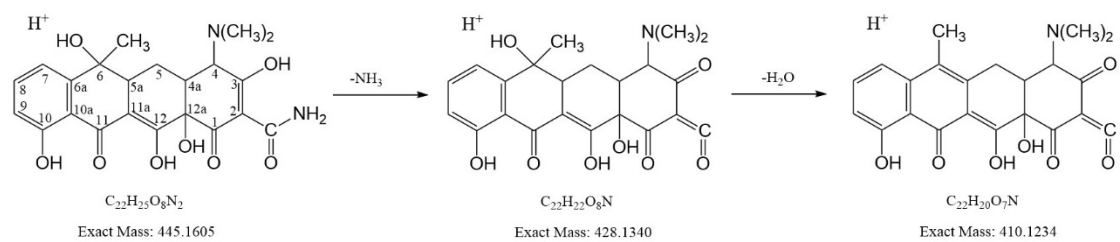
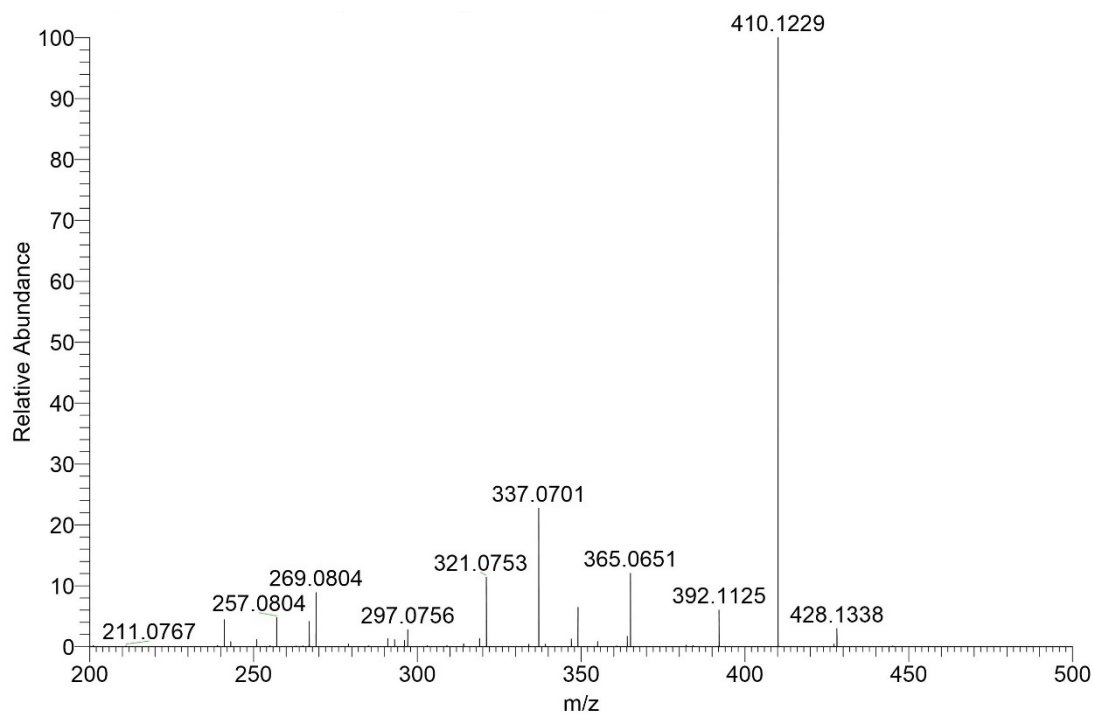


Figure S17. The MS2 fragmentation profile and proposed fragmentation pattern of tetracycline.

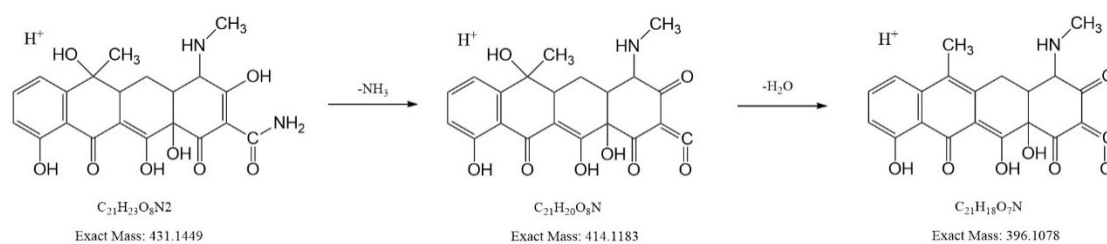
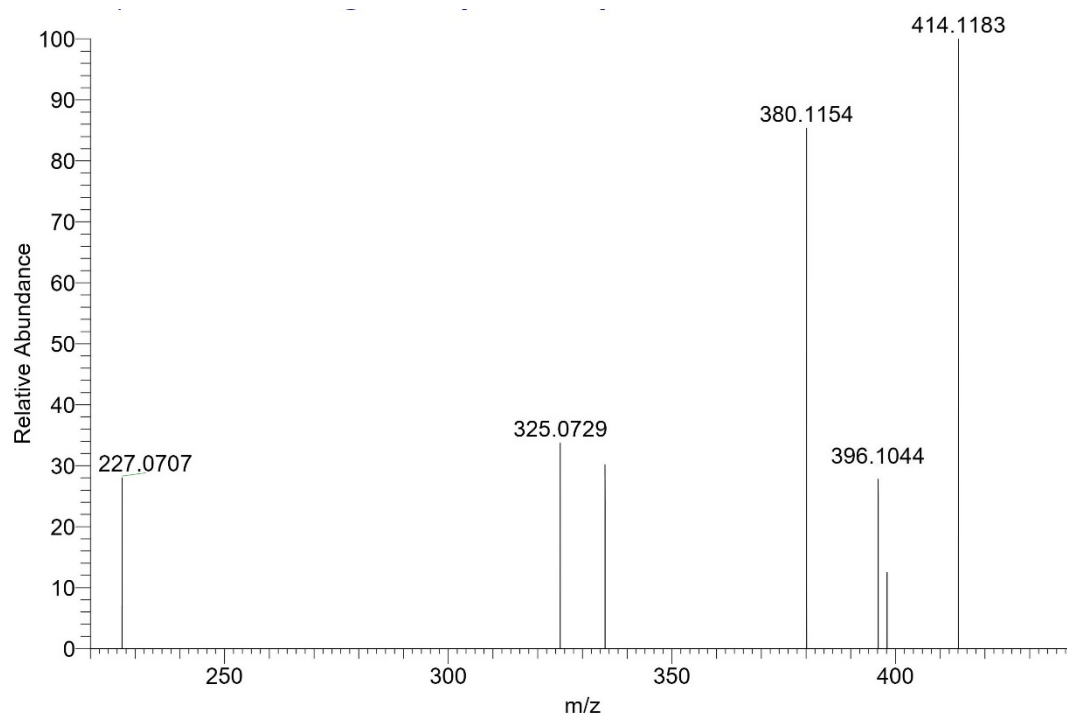


Figure S18. The MS2 fragmentation profile and proposed fragmentation pattern of TP431.

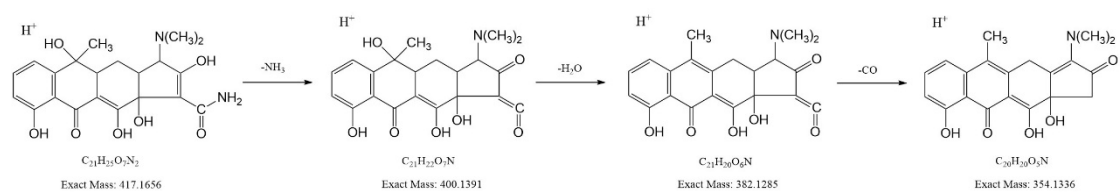
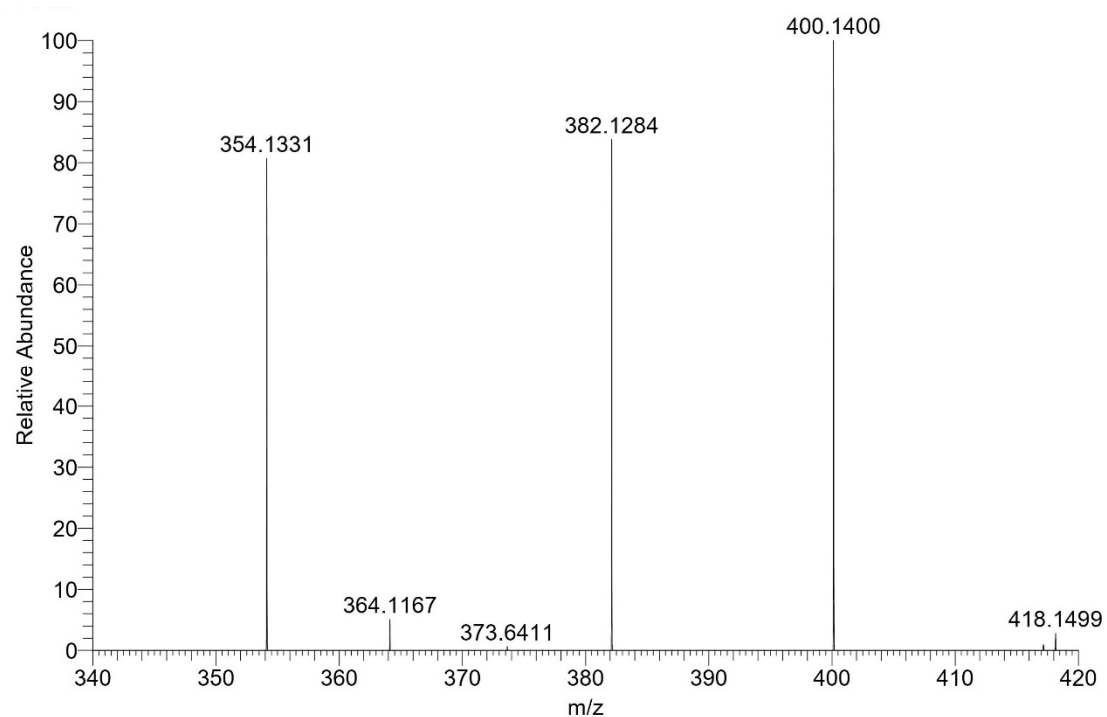


Figure S19. The MS2 fragmentation profile and proposed fragmentation pattern of TP417.

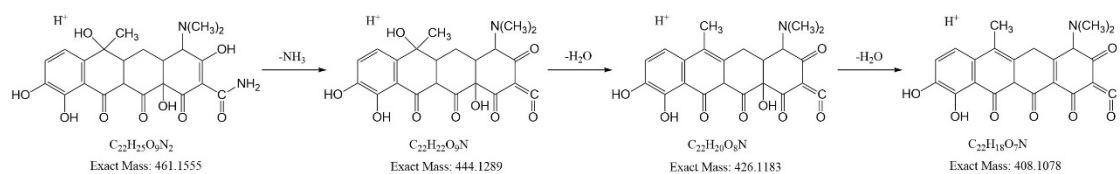
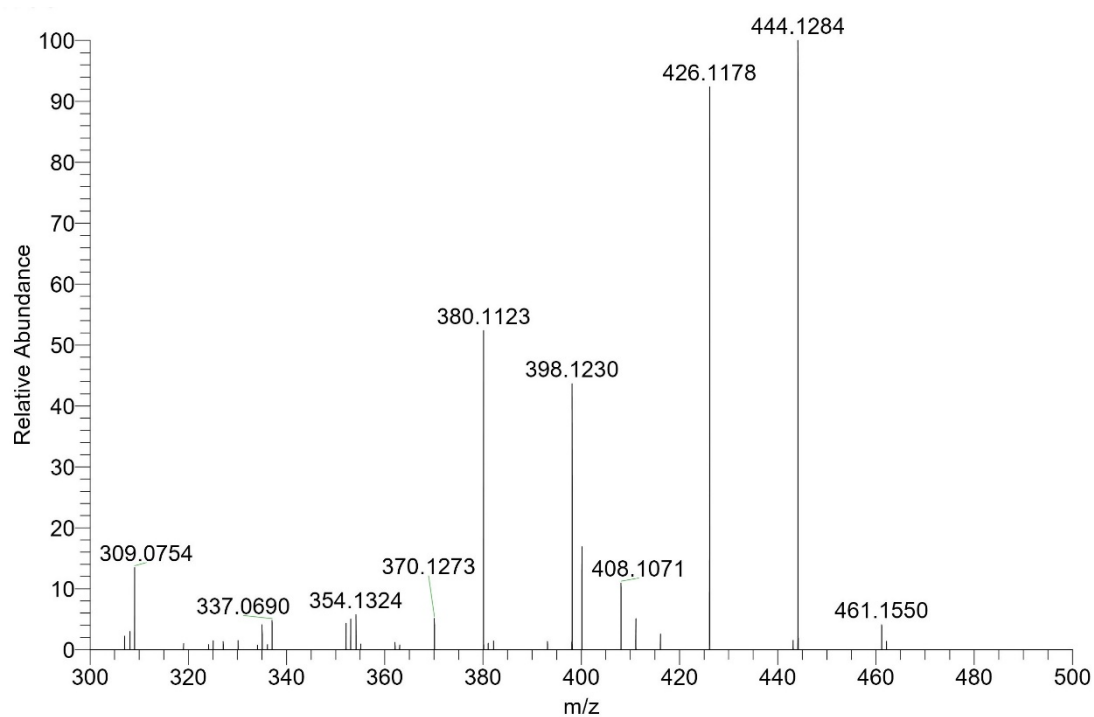


Figure S20. The MS2 fragmentation profile and proposed fragmentation pattern of TP461.

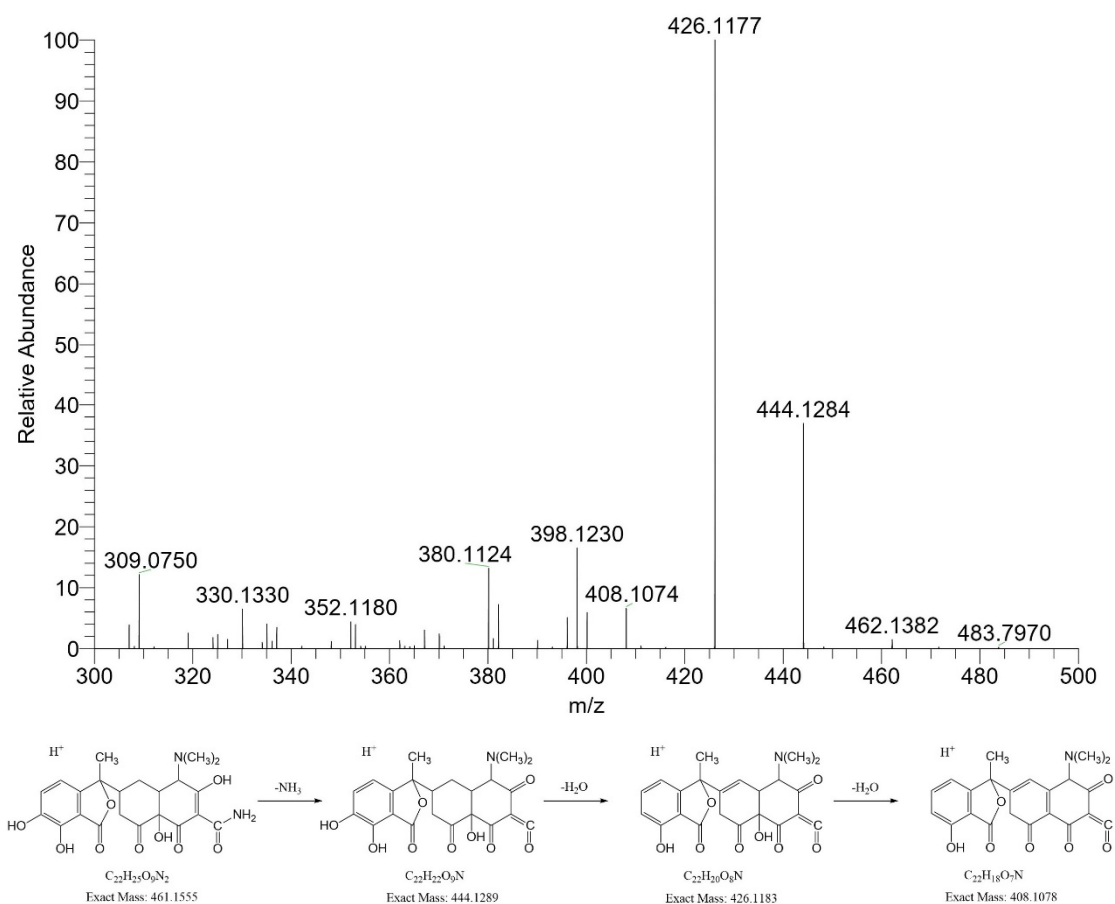


Figure S21. The MS2 fragmentation profile and proposed fragmentation pattern of ISO-TP461.

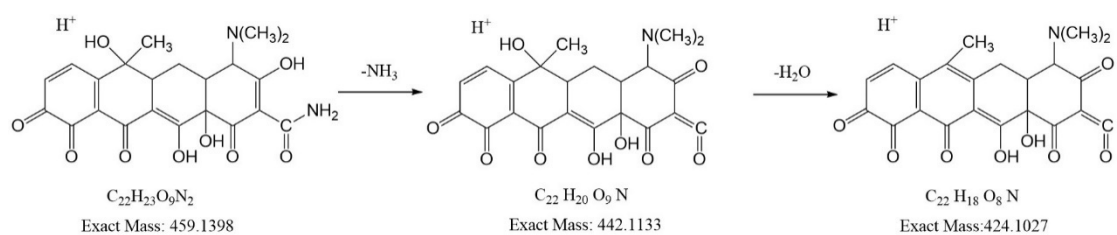
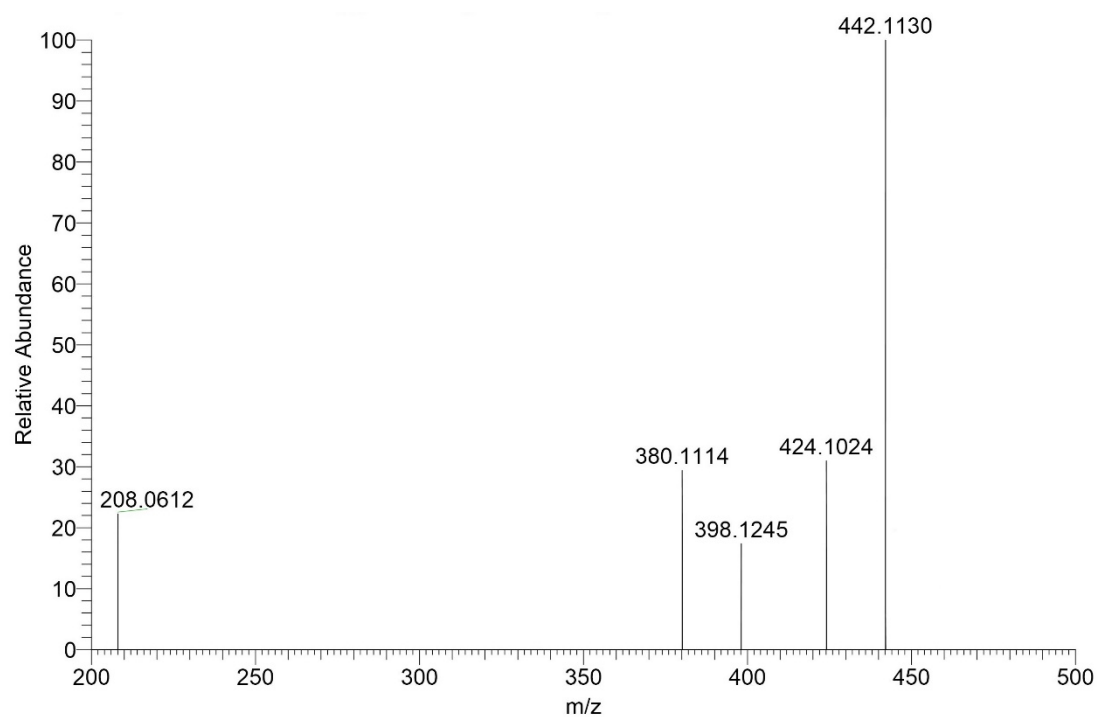


Figure S22. The MS2 fragmentation profile and proposed fragmentation pattern of TP459.

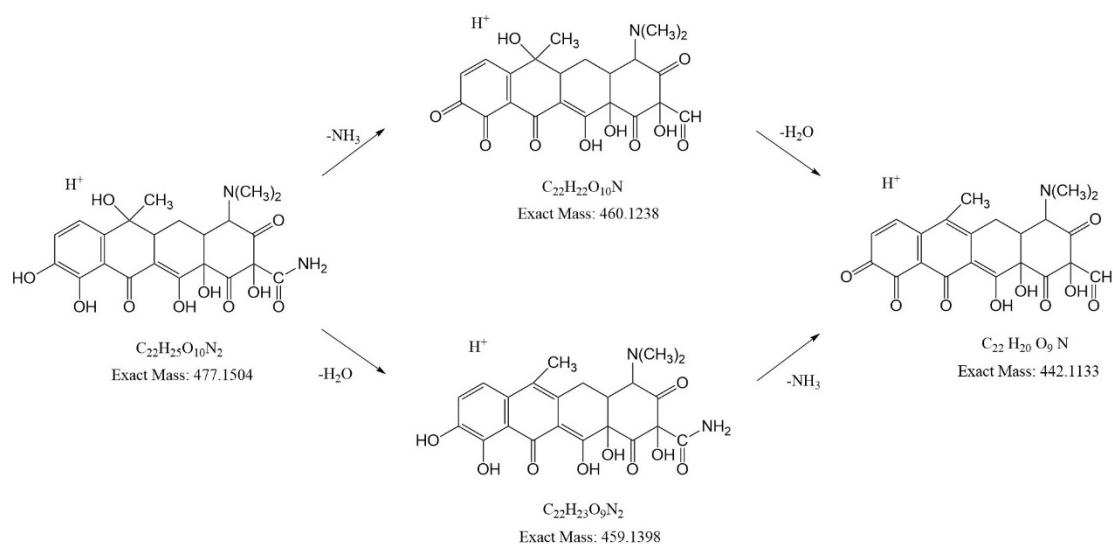
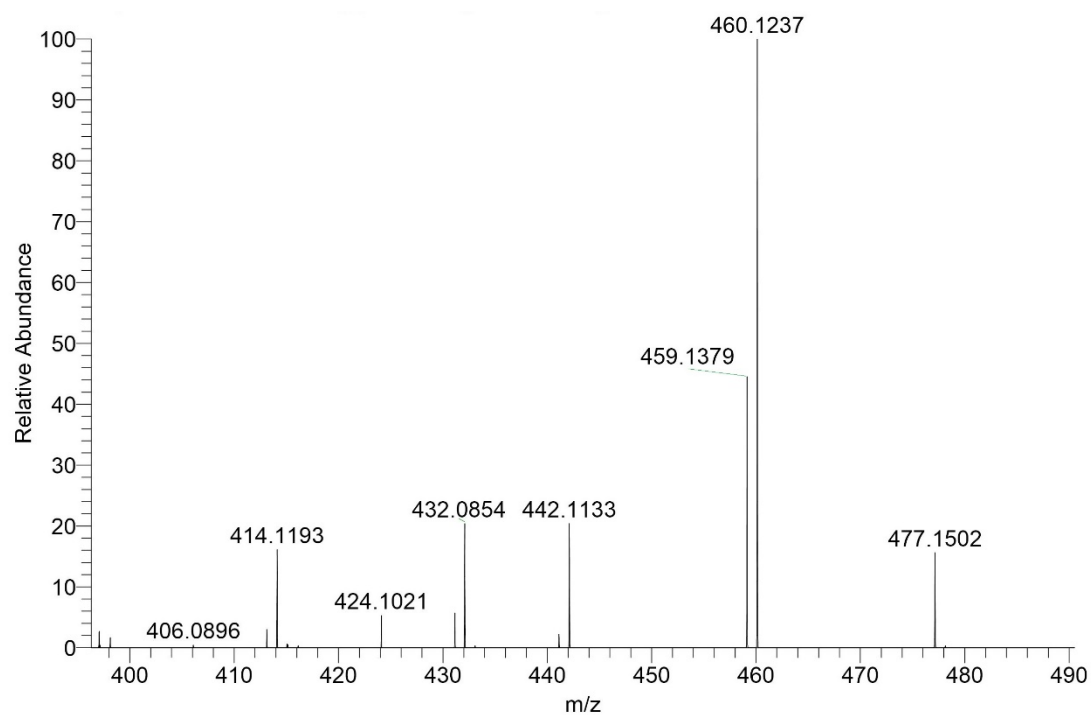


Figure S23. The MS2 fragmentation profile and proposed fragmentation pattern of TP477.

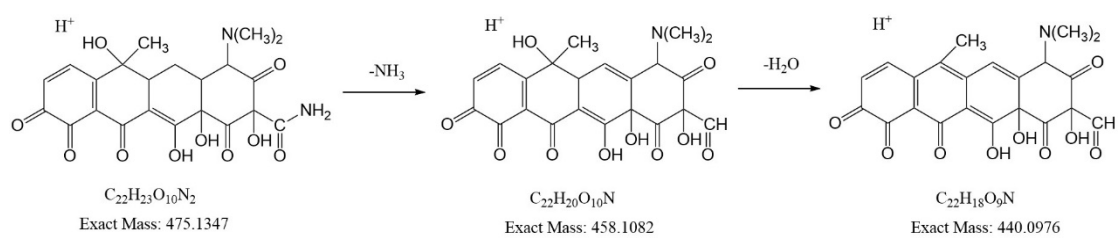
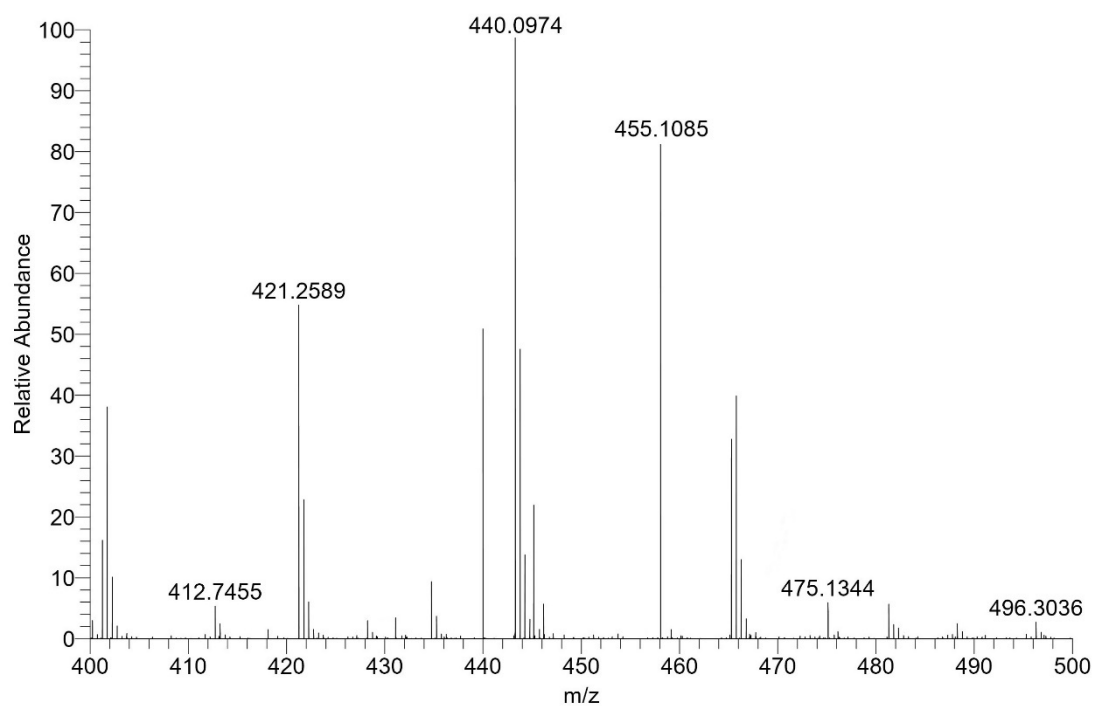


Figure S24. The MS2 fragmentation profile and proposed fragmentation pattern of TP475.

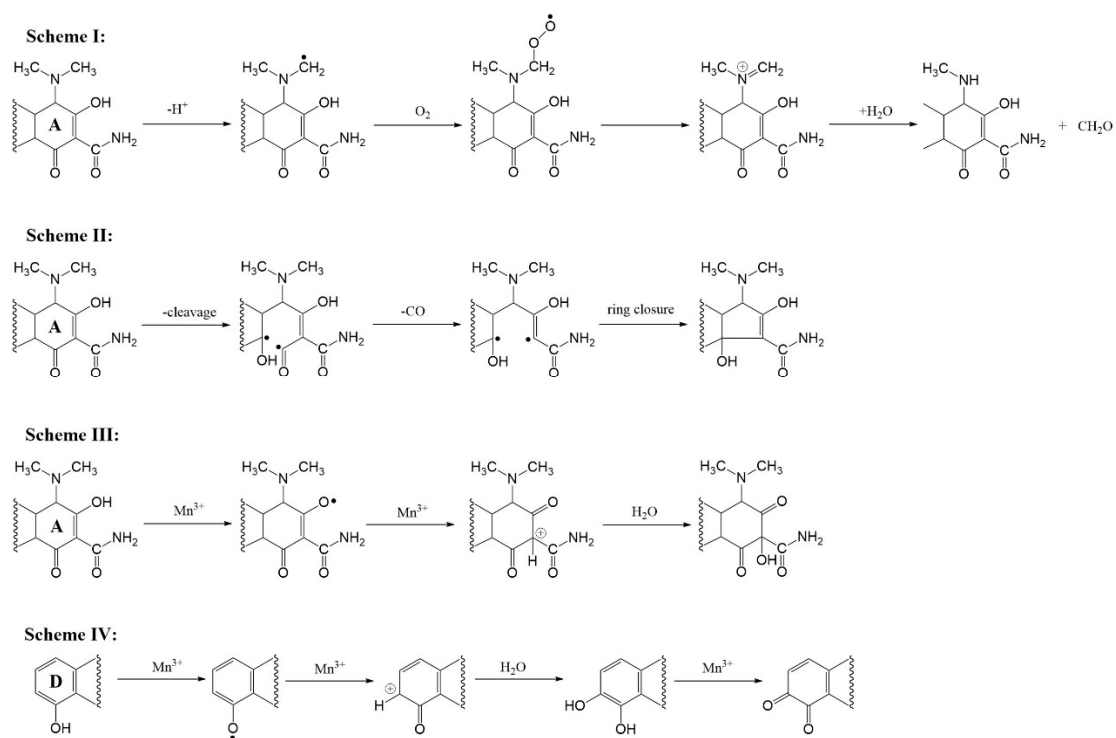


Figure. S25. Mechanistic steps Scheme I Scheme II Scheme III Scheme IV.

Table.S1 Factors and corresponding levels in $L_8 (2^3)$ orthogonal experimental design.

level	Glucose/ $\text{g}\cdot\text{L}^{-1}$ (X1)	Ammonium tartrate/ $\text{g}\cdot\text{L}^{-1}$ (X2)	$\text{MnSO}_4/\text{mmol}\cdot\text{L}^{-1}$ (X3)
1	10	1.62	1.00
2	2	0.20	0.02

Table.S2 Matrix layout of the $L_8 (2^3)$ orthogonal array design.

Experiment No.	Glucose ($\text{g}\cdot\text{L}^{-1}$)	Ammonium tartrate ($\text{g}\cdot\text{L}^{-1}$)	MnSO_4 ($\text{mmol}\cdot\text{L}^{-1}$)
1	2	1.62	0.02
2	10	1.62	0.02
3	2	0.20	0.02
4	10	0.20	0.02
5	2	0.20	1.00
6	10	0.20	1.00
7	10	1.62	1.00
8	2	1.62	1.00

Reference:

Rahul, D., Aditi, K., Divyashri, B., Ali, M., Amitava, M., Ram, M., Pavel, F., 2017. Enzymatic Degradation of Lignin in Soil: A Review. Sustainability 9, 1163.

Tien, M., Kirk, T.K., 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods in Enzymology. Academic Press, pp. 238-249.