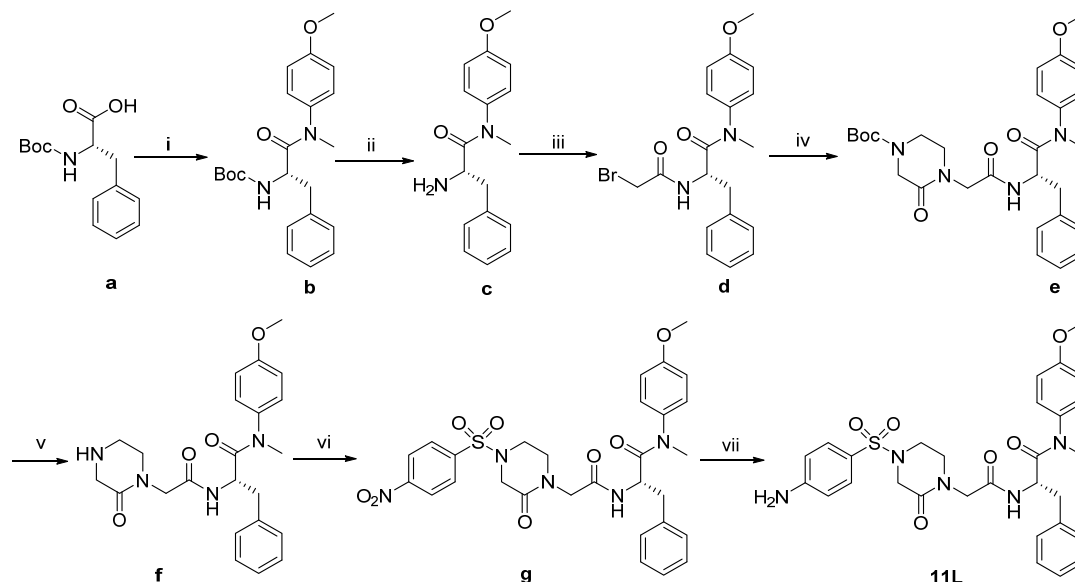


Supplementary Materials

1. The detailed information of 11L [1].

1.1 The synthetic route of 11L.



Scheme S1. The synthetic route of **11L**. Reagents and conditions: (i) 4-Methoxy-*N*-methylaniline, HATU, DIEA, 0°C to r.t.; (ii) TFA, DCM, r.t.; (iii) Bromoacetic acid, HATU, DIEA, 0°C to r.t.; (iv) 1-Boc-3-oxopiperazine, Cs₂CO₃, DMF, 45°C; (v) TFA, DCM, r.t.; (vi) TEA, DCM, r.t.; (vii) H₂, 10%Pd•C, DCM, r.t..

11L was prepared according to the general **Scheme S1**. Briefly, the commercially available (*tert*-butoxycarbonyl)-*L*-phenylalanine (**a**) was treated with 4-methoxy-*N*-methylaniline and 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro (HATU) in the presence of *N*, *N*-diisopropylethyl-amine (DIEA) and dichloromethane (DCM) to give **b**, followed by the removal of *tert*-butoxycarbonyl (Boc) protection using trifluoroacetic acid (TFA) to produce the free amine **c**. The acylation of **c** with bromoacetic acid in dichloromethane solution resulted in the key intermediate **d**. Further treatment of **d** with 1-Boc-3-oxopiperazine *via* a nucleophilic substitution (S_N2) reaction to produce intermediate **e**. Removal of the Boc protection of **e** yield the free amine **f**, which was reacted with *p*-nitro benzenesulfonyl chloride by an acylation reaction to give the compound **g**. The amino analogues **11L** was prepared by a hydrogenation reduction of the nitro group of **g**.

1.2 Spectral data of 11L.

(S)-2-(2-(4-((4-aminophenyl)sulfonyl)-2-oxopiperazin-1-yl)acetamido)-N-(4-methoxyphenyl)-N-methyl-3-phenylpropanamide (11L). White solid, yield: 61%. mp: 128-129°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.37 (d, *J* = 7.9 Hz, 1H, NH), 7.42 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.30 – 7.15 (m, 3H, Ph-H), 7.12 (d, *J* = 7.9 Hz, 2H, Ph-H), 6.98 (d, *J* = 8.8 Hz, 2H, Ph-H), 6.88 – 6.79 (m, 2H, Ph-H), 6.68 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.20 (s, 2H, NH₂), 4.44 (td, *J* = 8.8, 5.2 Hz, 1H, CH), 3.86 (s, 2H, piperazine-CH₂), 3.79 (s, 3H, OCH₃), 3.46 – 3.36 (m, 2H, piperazineCH₂), 3.23 – 3.12 (m, 2H, piperazine-CH₂), 3.10 (s, 3H, NCH₃), 3.07 – 2.95 (m, 2H, piperazine-CH₂), 2.84 (dd, *J* = 13.5, 4.8 Hz, 1H, PhCH), 2.63 (dd, *J* = 13.4, 9.5 Hz, 1H, PhCH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 171.38 (C=O), 167.38 (C=O), 163.98 (C=O), 158.99, 154.17, 137.91, 135.90, 130.31, 129.29, 129.14, 128.58, 126.88, 118.49, 115.14, 113.30, 55.88, 51.83, 49.30, 48.38, 46.94, 43.32, 37.77, 37.66. HRMS: *m/z* 580.2228 (M+1)⁺, 1181.4192 (2M+23)⁺. C₂₉H₃₃N₅O₆S [579.2152].

2. Chromatographic and Mass Spectrometric Methods of 7-EC

The UPLC-UV-HRMS analysis was performed on an ACQUITY UPLC system hyphenated to a Thermo Q-Exactive HF tandem mass spectrometer equipped with an electrospray ionization interface operating in positive ion mode.

Chromatographic Method: The chromatography was carried out on an ACQUITY UPLC HSS T3 column (2.1×100 mm, 1.8 μm; Waters Corporation, MA, USA) maintained at 40°C. Automatic injector temperature maintained at 4 °C. The mobile phase consisting of water containing 0.1 % formic acid (A) and acetonitrile solution containing 0.1 % formic acid (B) was delivered at a flow rate of 300 μL/min. The gradient procedures were optimized as follows: 2% B at 0–1 min, 2%–15% B at 1–8 min, 15%–40% B at 8–14 min, 40%–80% B at 14–16 min, 90% B at 16–18 min and finally 2% B at 18–20 min. The injection volume was 10 μL. UV detection was performed using a photodiode array detector, and wavelength was 190~500 nm.

Mass Spectrometric Method: The electrospray ionization source parameters were set as follows: capillary temperature, 375 °C; spray voltage, 3.5 kV; sheath gas flow rate, 40 L/h; auxiliary gas flow rate, 15 L/h; auxiliary gas heater temperature, 350°C; S-Lens voltage, 55 V. The data were acquired in the *m/z* range of 80–1200 Da (full mass scan) and 200-2000 Da

(MS/MS scan) in centroid mode. The collision energy was 10/15/25 V. The mass resolution for full mass scan and MS/MS scan was selected to be above 120000 and 30000, respectively. Collision-induced dissociation (CID) was used to obtain the MS/MS fragment of 7-EC and its metabolites. All the data acquisition was obtained by Xcalibur software (version 4.2; Thermo Fisher Scientific).

Table S1 Metabolism rate of 7-EC in HLMs (%)

Species	UV peak area in T0 (wavelength: 290-360 nm)	UV peak area in T60 (wavelength: 290-360 nm)	Metabolism rate (%)
Human	245954	0	100

Note: Metabolism rate (%) = (1 - UV peak area in T60/ UV peak area in T0)×100%

RT: 7.80–17.11

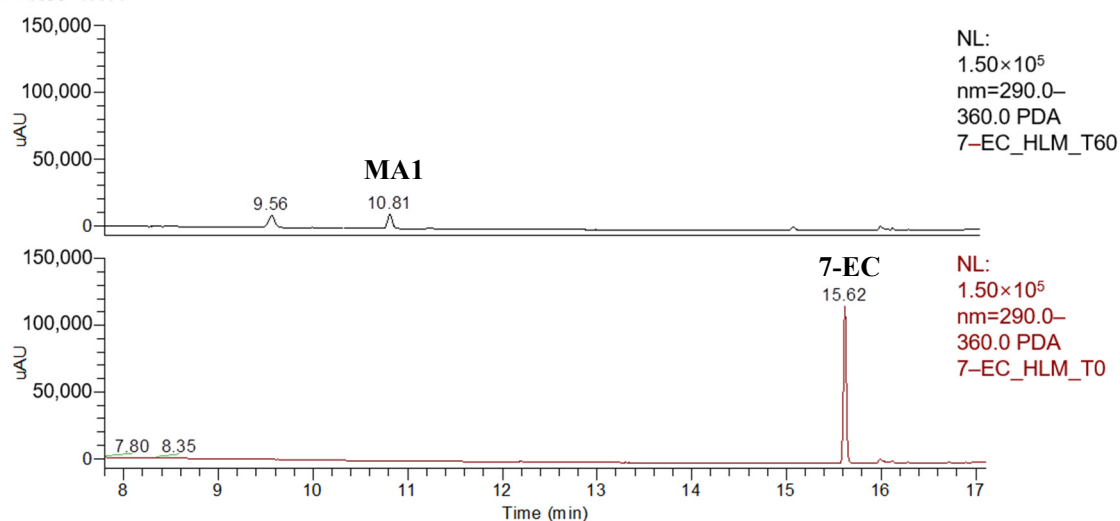
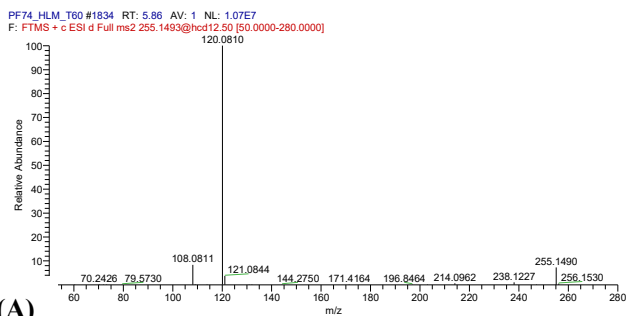
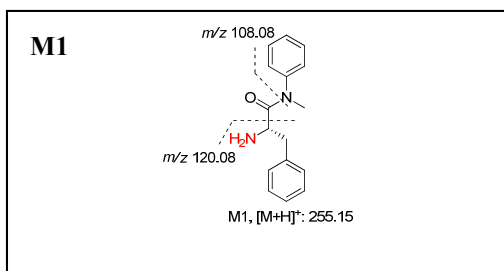
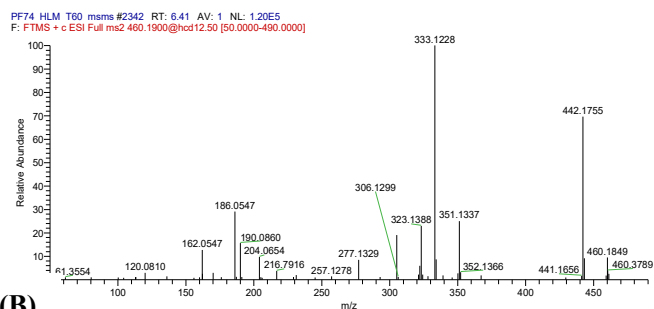
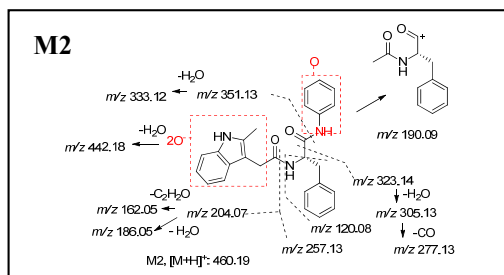


Figure S1 UPLC-UV chromatograms of 7-EC and its metabolites in HLMs (wavelength: 290 – 360 nm)

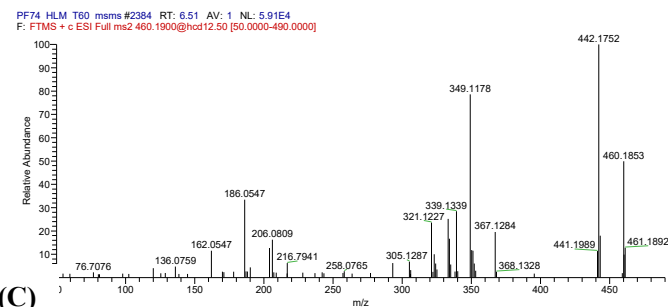
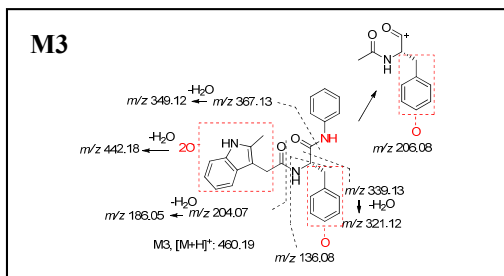
Note: MA1 was a deethyl metabolite.



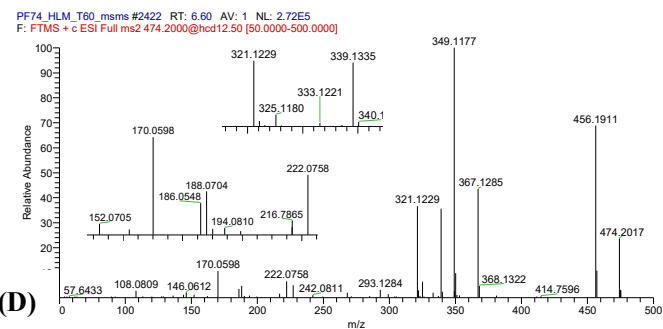
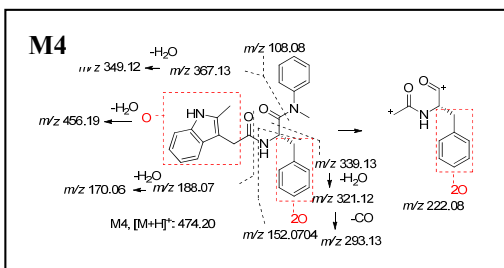
(A)



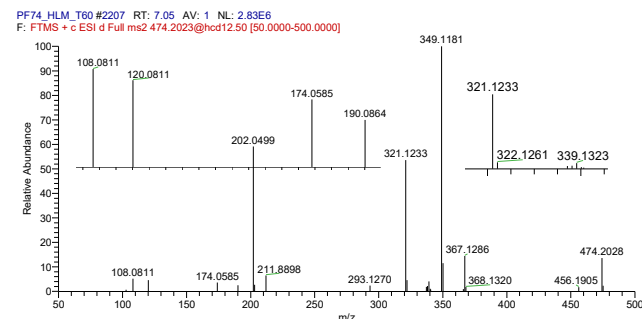
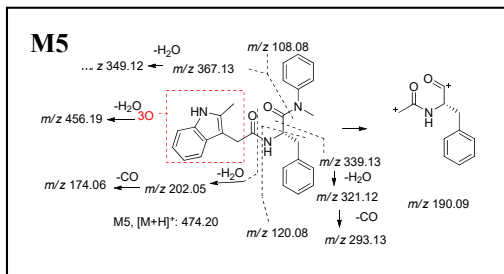
(B)



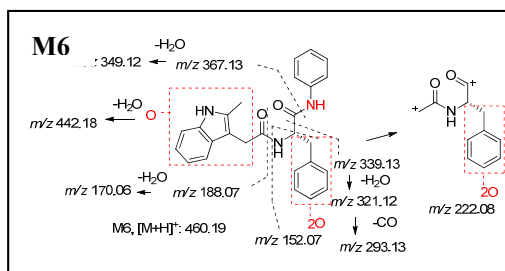
(C)



(D)

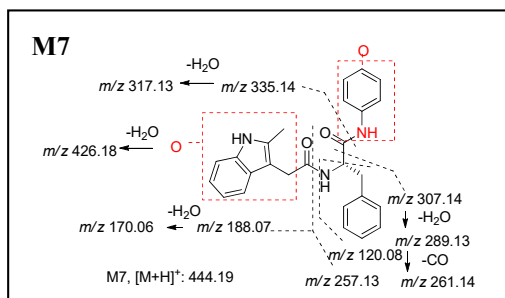
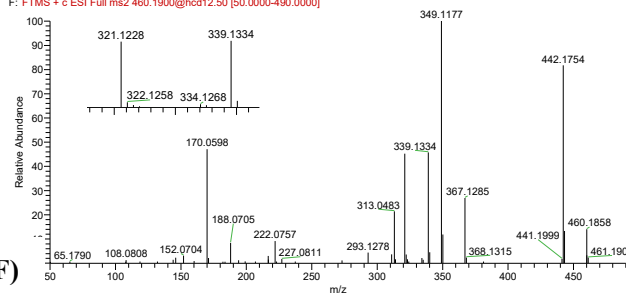


(E)



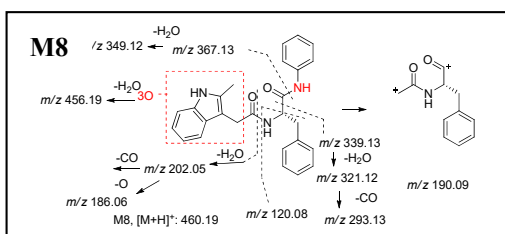
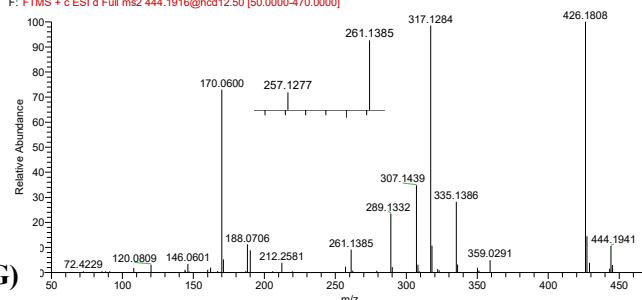
(F)

PF74_HLM_T60_msms #2566 RT: 6.95 AV: 1 NL: 3.89E5
F: FTMS + c ESI Full ms2 460.1900@hcd12.50 [50.0000-490.0000]



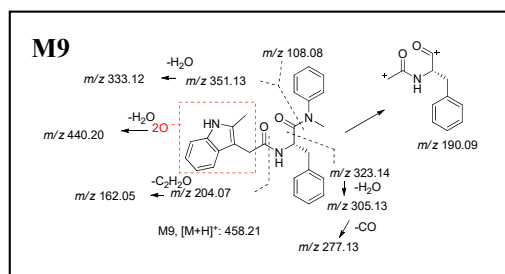
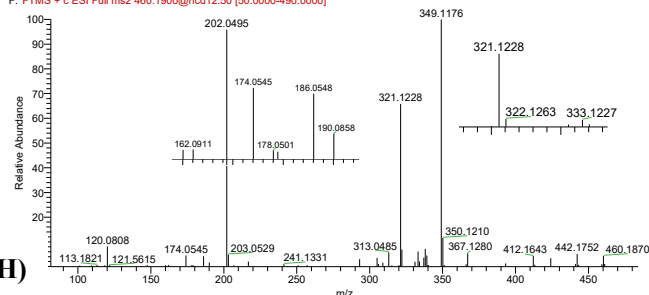
(G)

PF74_HLM_T60_msms #2221 RT: 7.08 AV: 1 NL: 5.48E5
F: FTMS + c ESI Full ms2 444.1916@hcd12.50 [50.0000-470.0000]



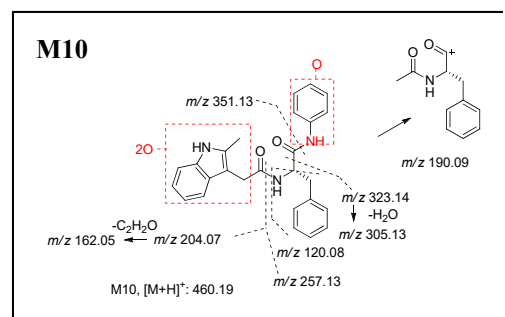
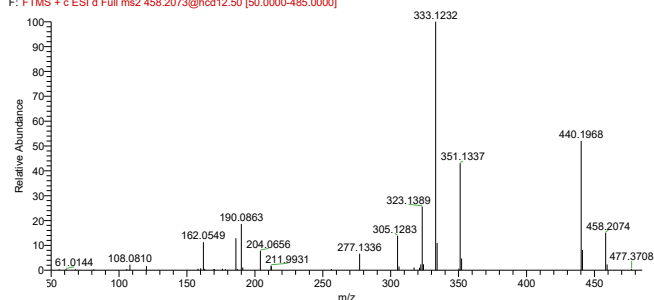
(H)

PF74_HLM_T60_msms #2654 RT: 7.15 AV: 1 NL: 5.42E5
F: FTMS + c ESI Full ms2 460.1900@hcd12.50 [50.0000-490.0000]



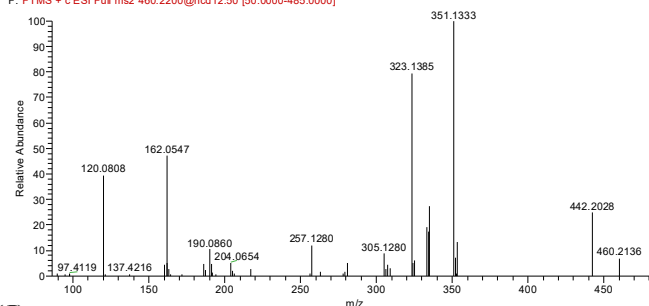
(I)

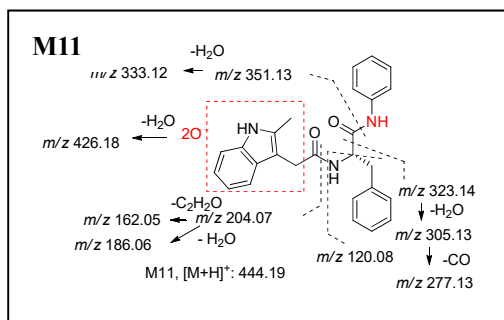
PF74_HLM_T60_msms #2327 RT: 7.37 AV: 1 NL: 2.36E6
F: FTMS + c ESI d Full ms2 458.2073@hcd12.50 [50.0000-485.0000]



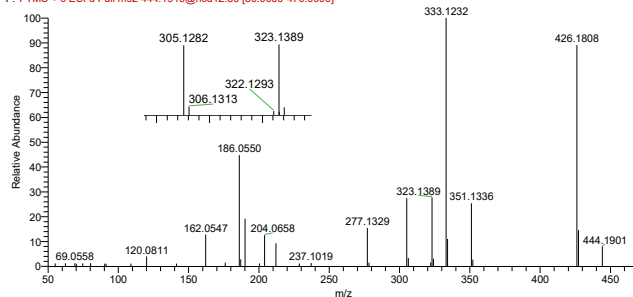
(J)

PF74_HLM_T60_msms #2826 RT: 7.34 AV: 1 NL: 3.21E5
F: FTMS + c ESI Full ms2 460.2200@hcd12.50 [50.0000-485.0000]

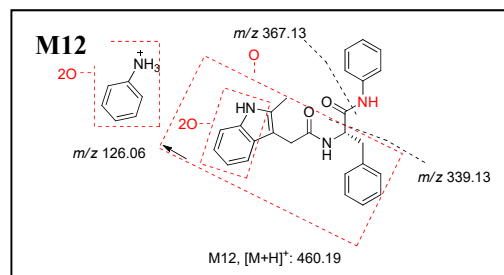




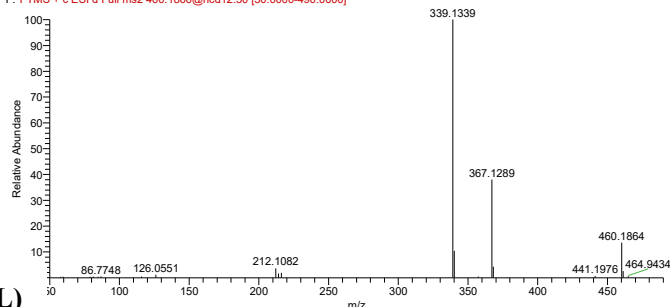
PF74_HLM_T60 #2398 RT: 7.56 AV: 1 NL: 3.24E6
F: FTMS + c ESI d Full ms2 444.1916@hcd12.50 [50.0000-470.0000]



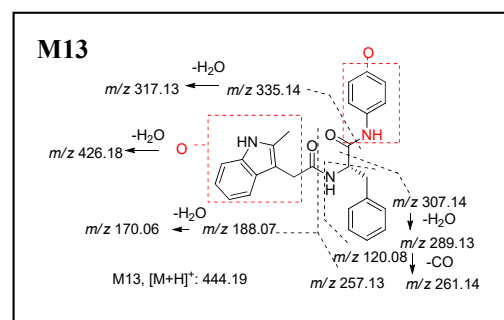
(K)



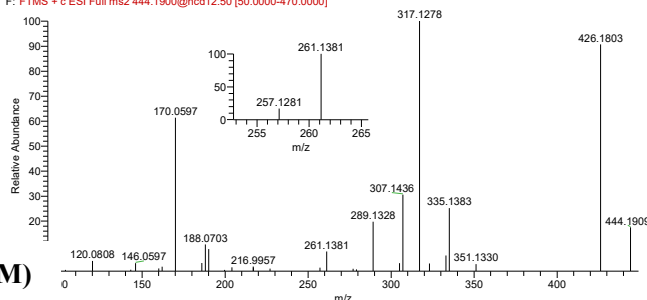
PF74_HLM_T60 #2436 RT: 7.66 AV: 1 NL: 1.20E6
F: FTMS + c ESI d Full ms2 460.1866@hcd12.50 [50.0000-490.0000]



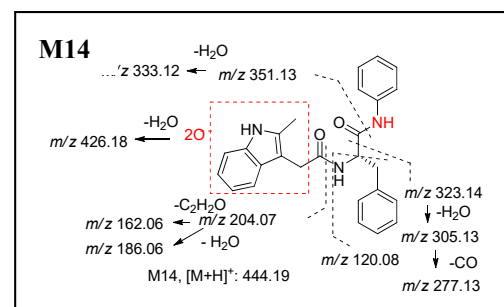
(L)



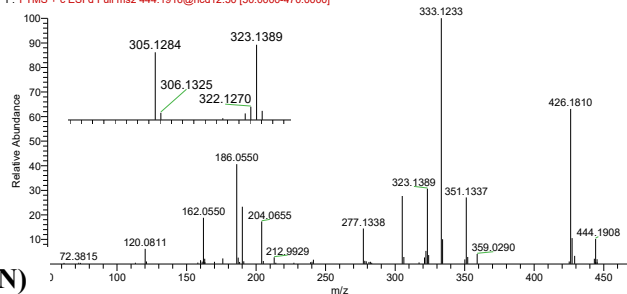
PF74_HLM_T60_msms2 #2920 RT: 7.56 AV: 1 NL: 6.79E5
F: FTMS + c ESI d Full ms2 444.1900@hcd12.50 [50.0000-470.0000]



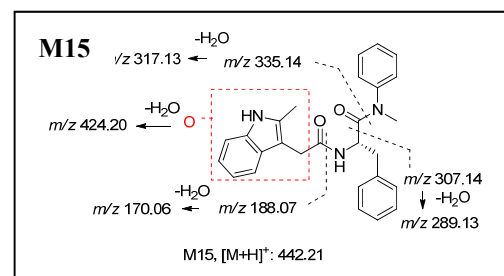
(M)



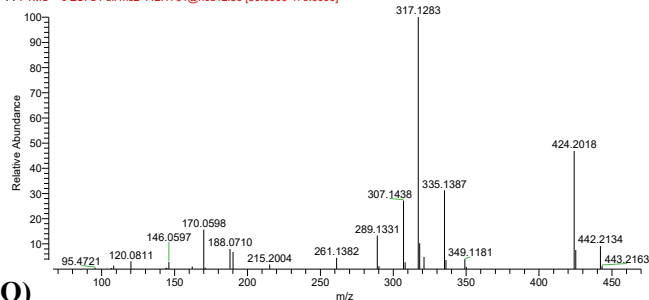
PF74_HLM_T60 #2471 RT: 7.76 AV: 1 NL: 5.03E5
F: FTMS + c ESI d Full ms2 444.1916@hcd12.50 [50.0000-470.0000]



(N)



PF74_HLM_T60 #2796 RT: 8.72 AV: 1 NL: 6.33E5
F: FTMS + c ESI d Full ms2 442.1761@hcd12.50 [50.0000-470.0000]



(O)

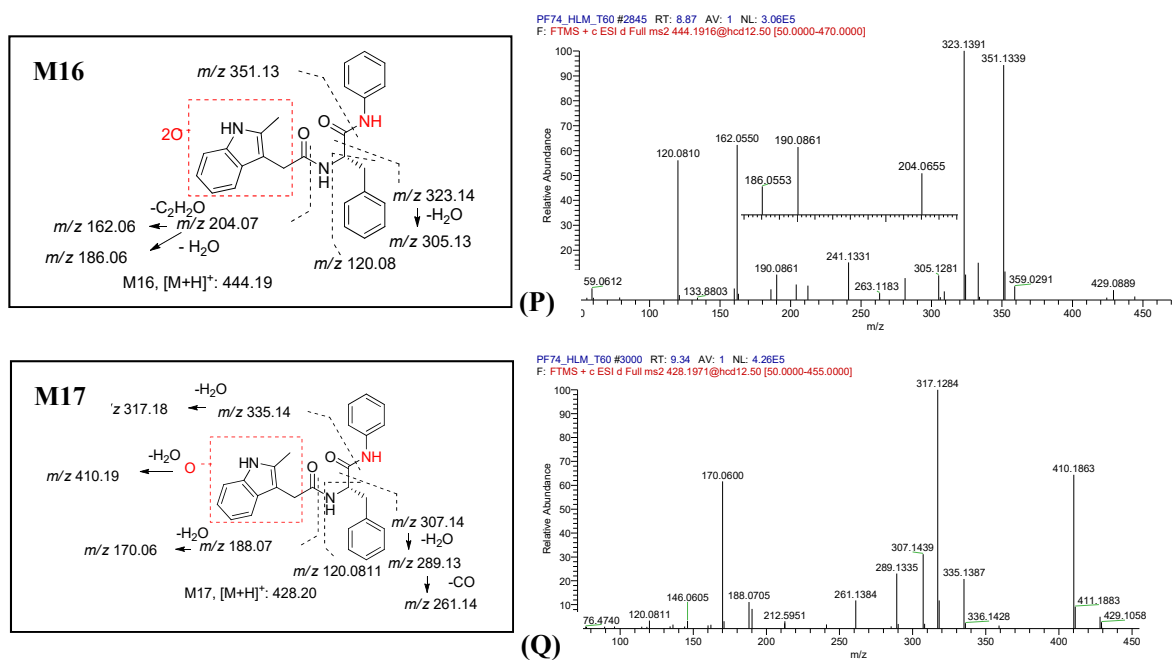
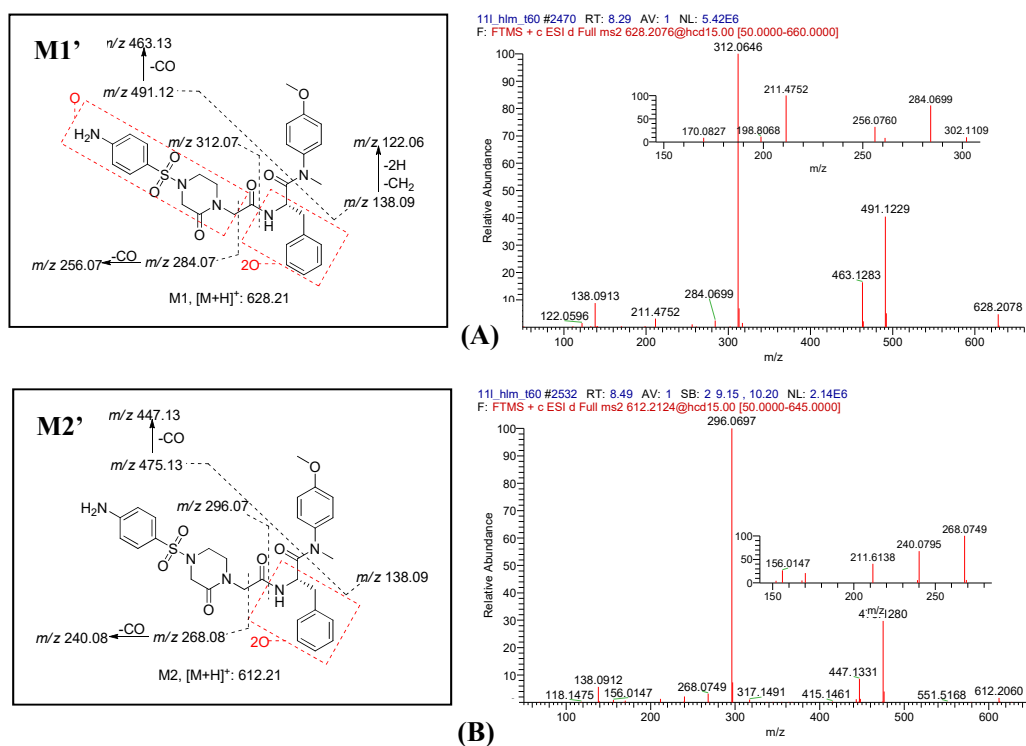
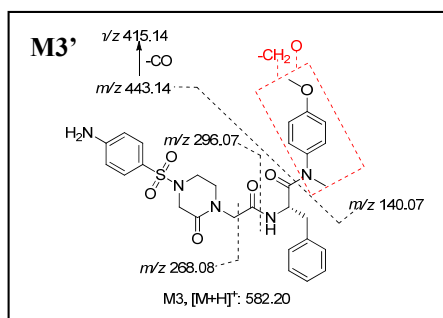
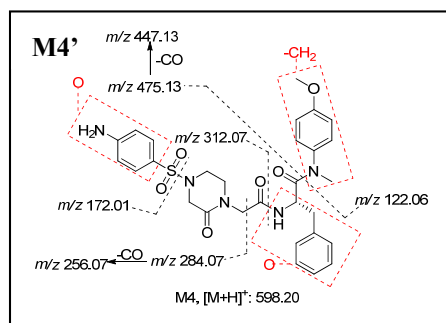
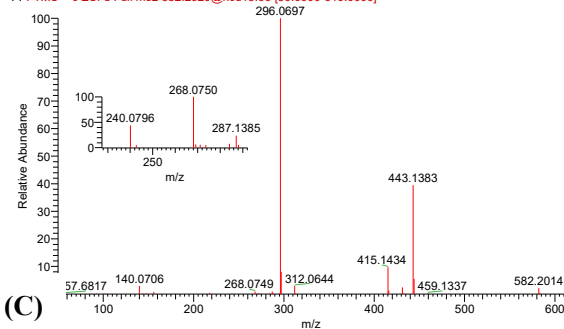


Figure S2. Collision induced dissociation (CID) spectra and proposed fragmentation patterns for metabolites of PF74.

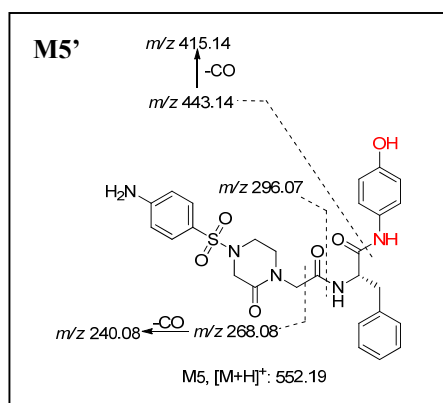
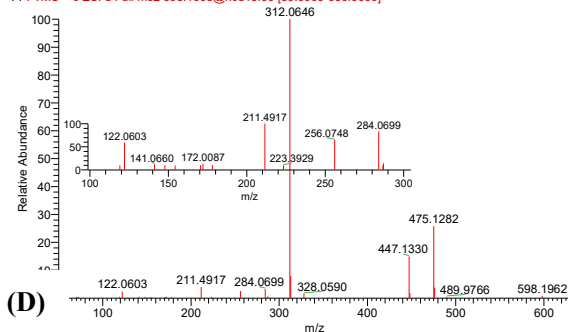




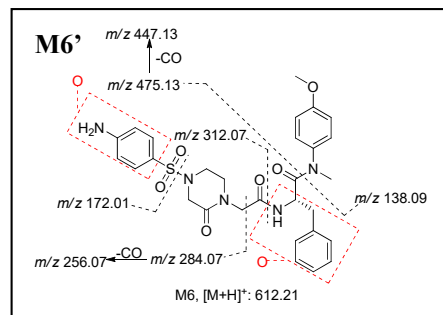
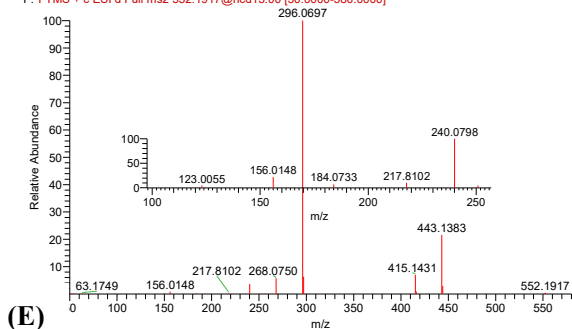
11i_hlm_t60 #2556 RT: 8.56 AV: 1 SB: 2 9.11, 13.71 NL: 1.53E6
F: FTMS + c ESI d Full ms2 582.2020@hcd15.00 [50.0000-610.0000]



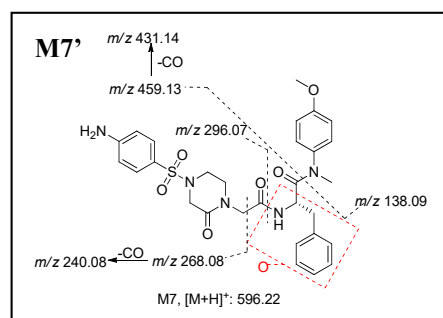
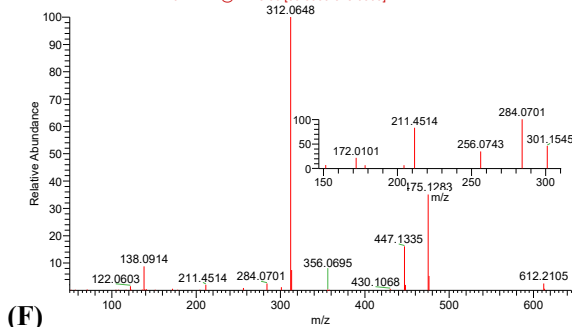
11i_hlm_t60 #2569 RT: 8.60 AV: 1 NL: 1.37E6
F: FTMS + c ESI d Full ms2 598.1968@hcd15.00 [50.0000-630.0000]



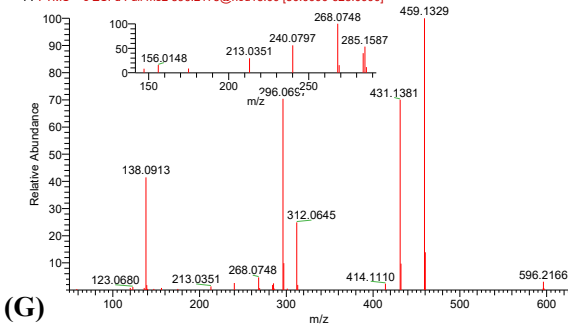
11i_hlm_t60 #2627 RT: 8.79 AV: 1 NL: 1.17E6
F: FTMS + c ESI d Full ms2 552.1917@hcd15.00 [50.0000-580.0000]

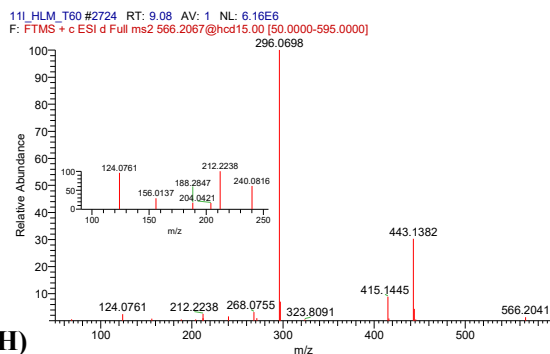
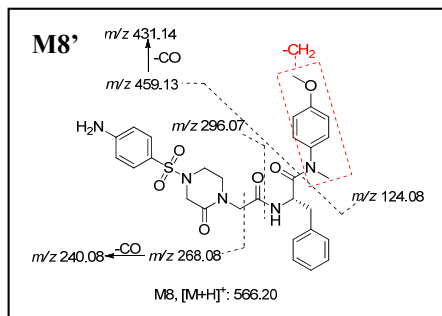


11i_hlm_t60 #2641 RT: 8.82 AV: 1 SB: 2 9.15, 10.20 NL: 3.75E6
F: FTMS + c ESI d Full ms2 612.2124@hcd15.00 [50.0000-645.0000]

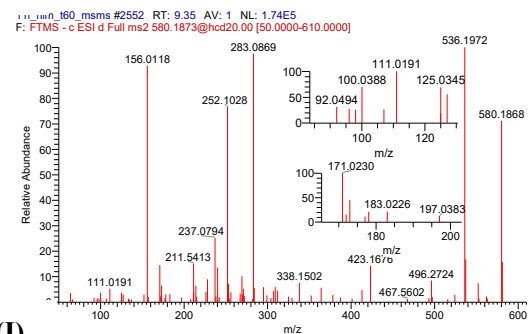
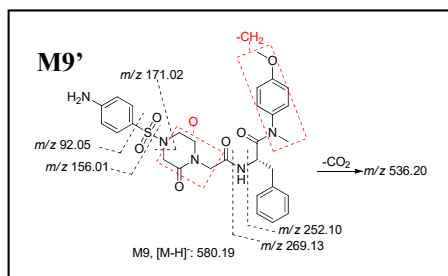


11i_hlm_t60 #2711 RT: 9.05 AV: 1 SB: 1 9.42, 10.46 NL: 5.35E5
F: FTMS + c ESI d Full ms2 596.2175@hcd15.00 [50.0000-625.0000]

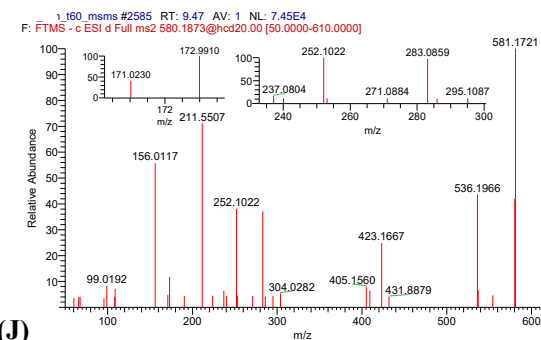
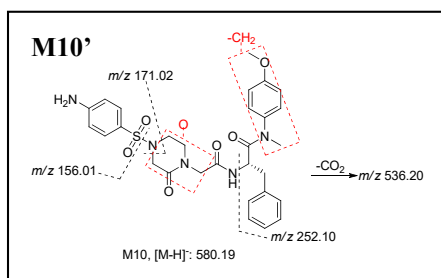




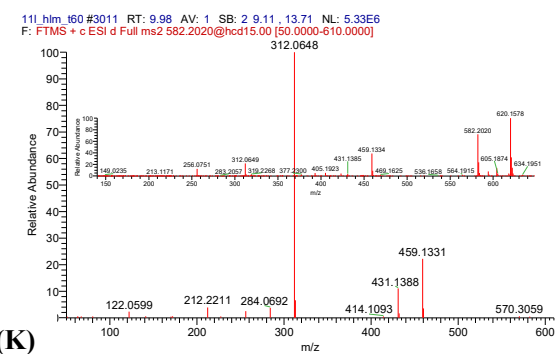
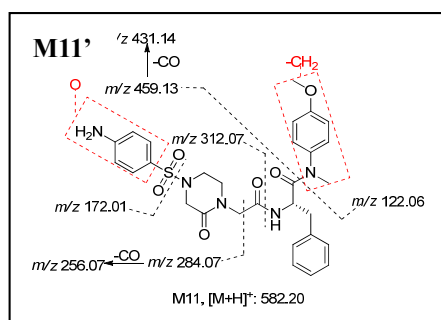
(H)



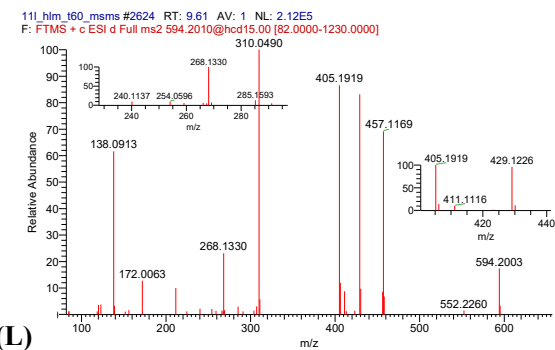
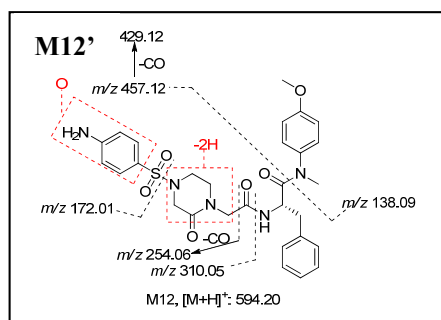
(I)



(J)



(K)



(L)

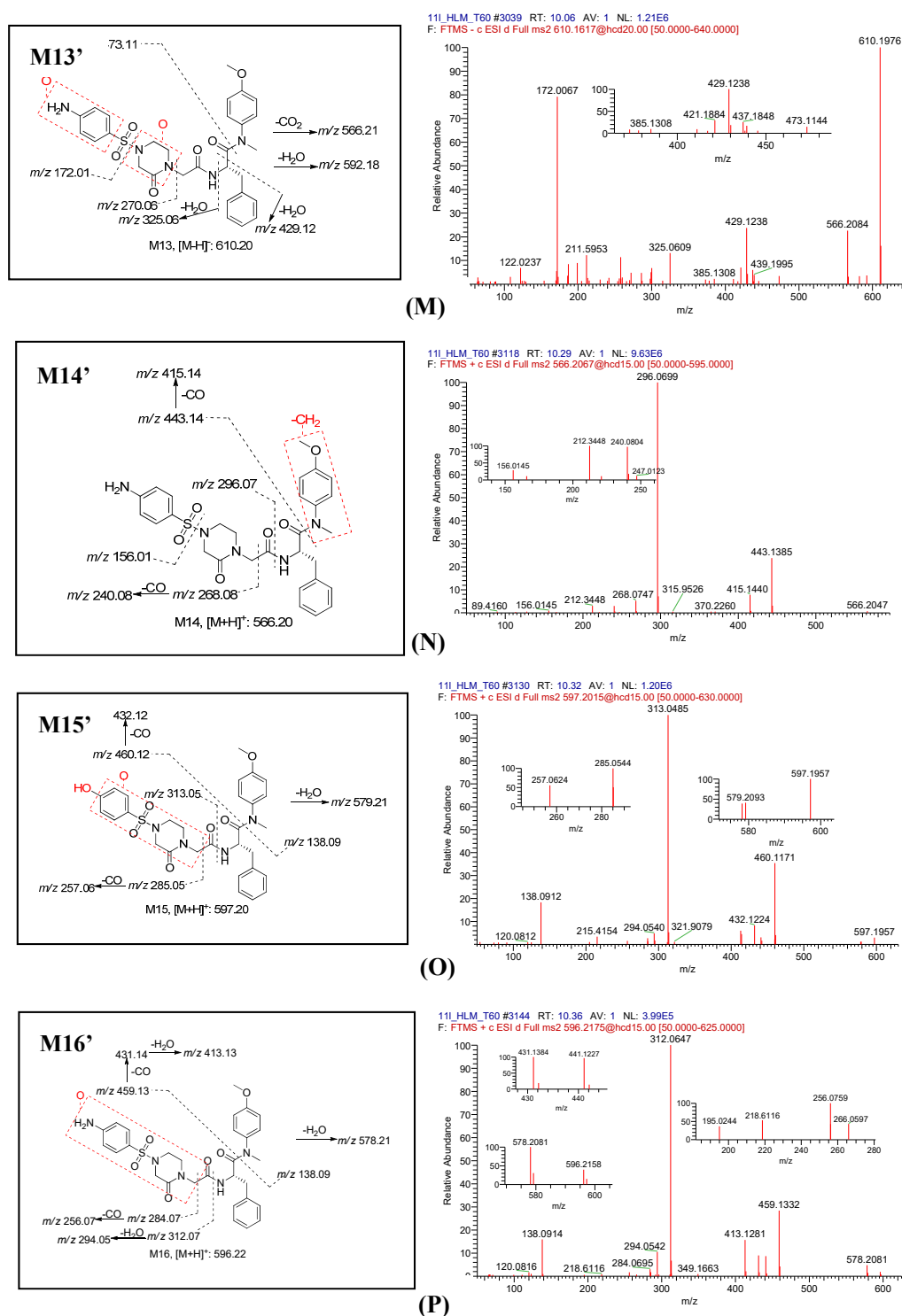


Figure S3. CID spectra and proposed fragmentation patterns for metabolites of **11L**.

Reference:

[1] Sun L, Dick A, Meuser ME, Huang T, Zalloum WA, Chen CH, Cherukupalli S, Xu S, Ding X, Gao P, Kang D, De Clercq E, Pannecouque C, Cocklin S, Lee KH, Liu X, Zhan P. Design, Synthesis, and Mechanism Study of Benzenesulfonamide-Containing Phenylalanine Derivatives as Novel HIV-1 Capsid Inhibitors with Improved Antiviral Activities. *J. Med. Chem.* **2020**, *63*, 4790-4810