

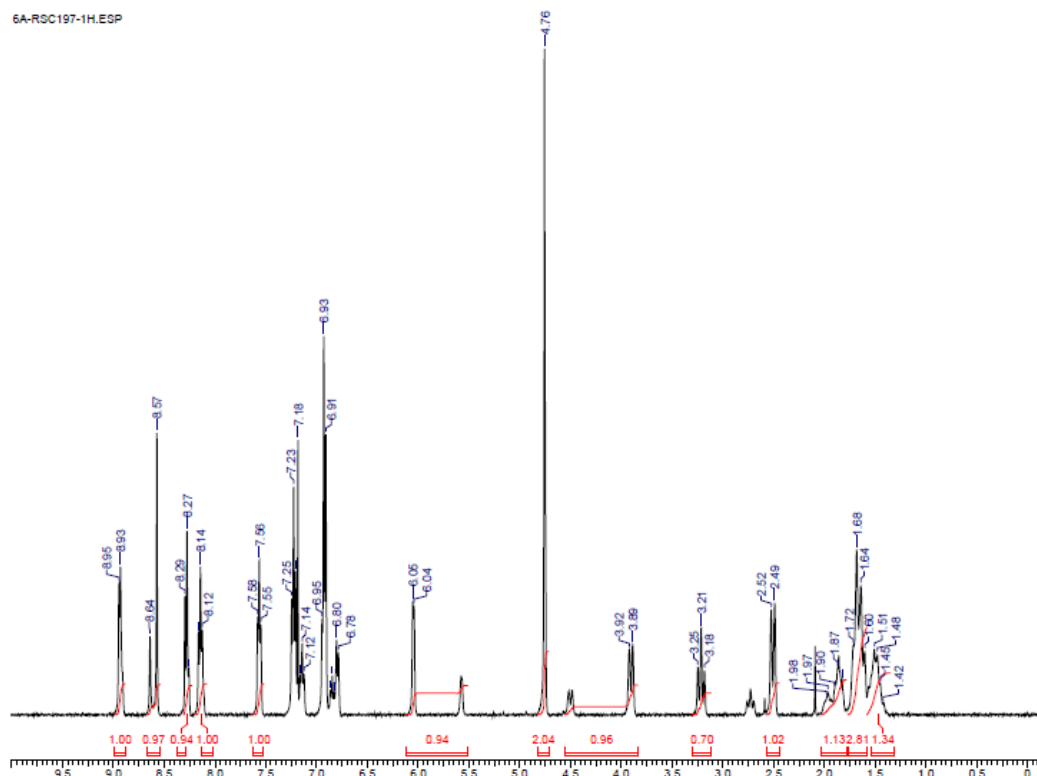
Supporting Information

Small molecule inhibitors of metabolic enzymes repurposed as a new class of anthelmintics

Rahul Tyagi^{1#}, Amarendar Reddy Maddirala^{2#}, Mostafa Elfawal^{3#}, Chelsea Fischer⁴, Bruce A. Rosa¹, Xin Gao¹, Ryan Chugani², Mingzhou Zhou², Jon Helander², Paul Brindley⁶, Chih-Chung Tseng⁷, Iain R. Greig⁷, Judy Sakanari⁴, Scott A. Wildman⁵, Raffi Aroian^{3*}, James W. Janetka^{2*} and Makedonka Mitreva^{1,8*}

Table of Content

Details	Page number
Figures S1-S26: 1H-NMR & LCMS spectral data for compounds 6(a-o) , 10(a-b) , 17(a-b) , 23(a-c) & 24(a-c)	S2-S27
Figure S27: Time- and species- dependence of the IC ₅₀ values. A) <i>Brugia pahangi</i> IC ₅₀ values for seven compounds over three days of exposure; B) <i>Trichuris muris</i> and <i>B. pahangi</i> IC ₅₀ values for five compounds at day 2.	S28
Figures S28-S44: Bioaccumulation analysis figures	S29-S56
Figure S45. RNAseq based gene expression profile in different tissues of adult intestinal nematode <i>Ascaris suum</i> shows increased expression of CPT1/2 in ovary compared to the uterus in female worms and the seminal vesicle and testis in male worms. Gene expression values used are from our earlier report, Rosa et al, 2014.(Rosa, Jasmer et al. 2014).	S57



LCMS of compound 6a

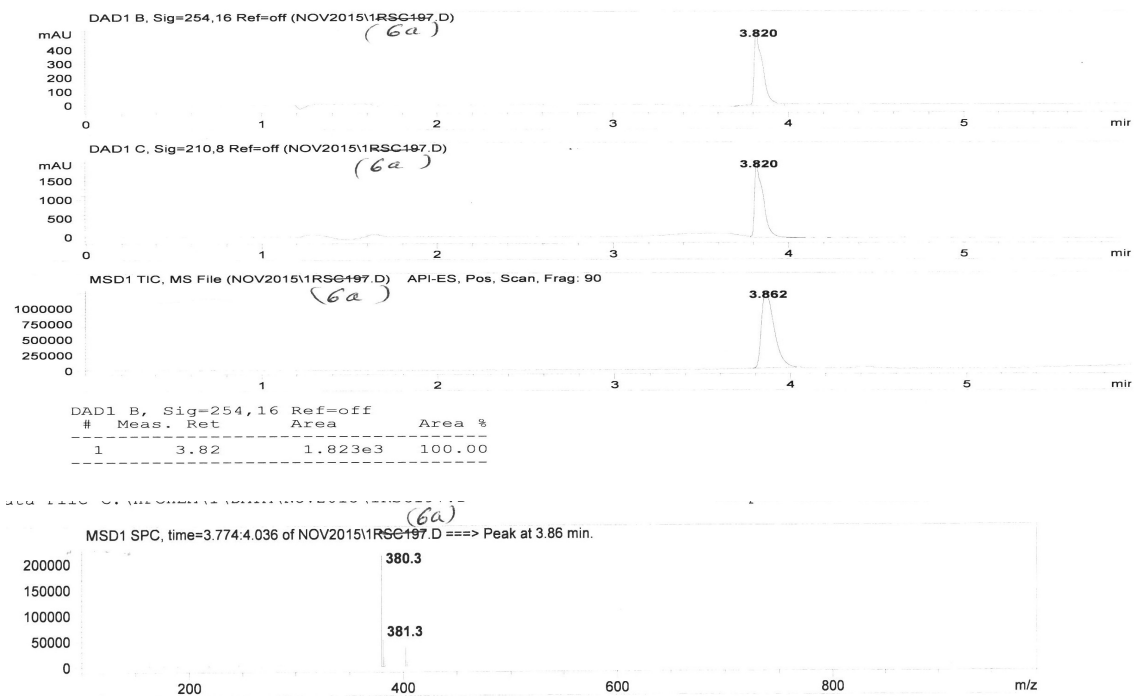
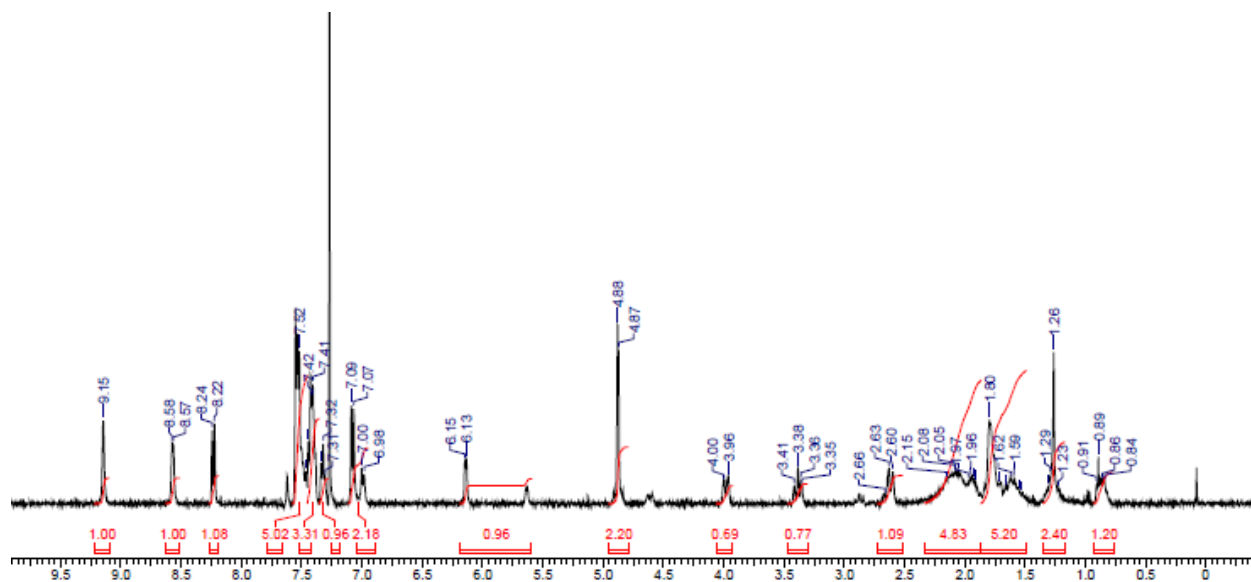


Figure S1: ^1H NMR of compound 6a



LCMS of compound **6b**

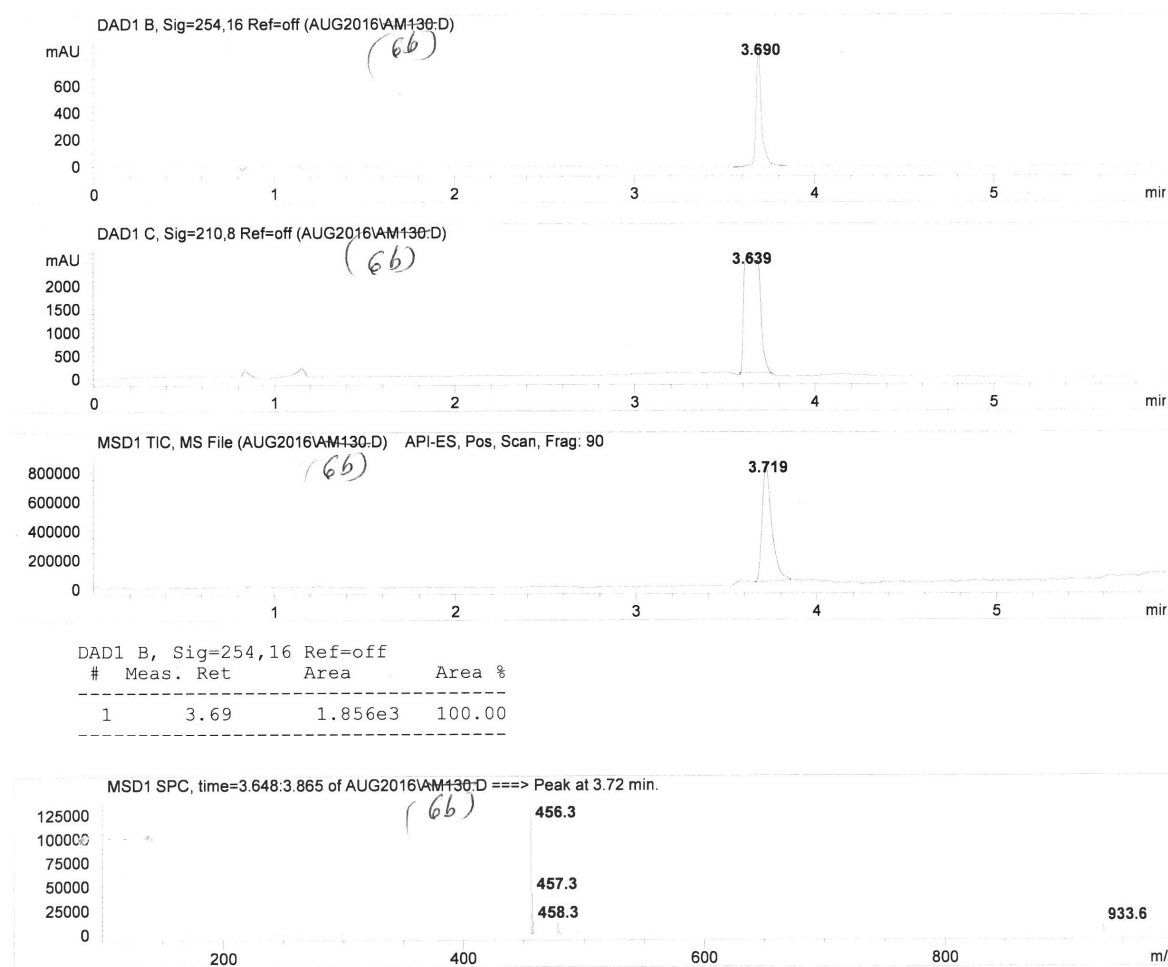
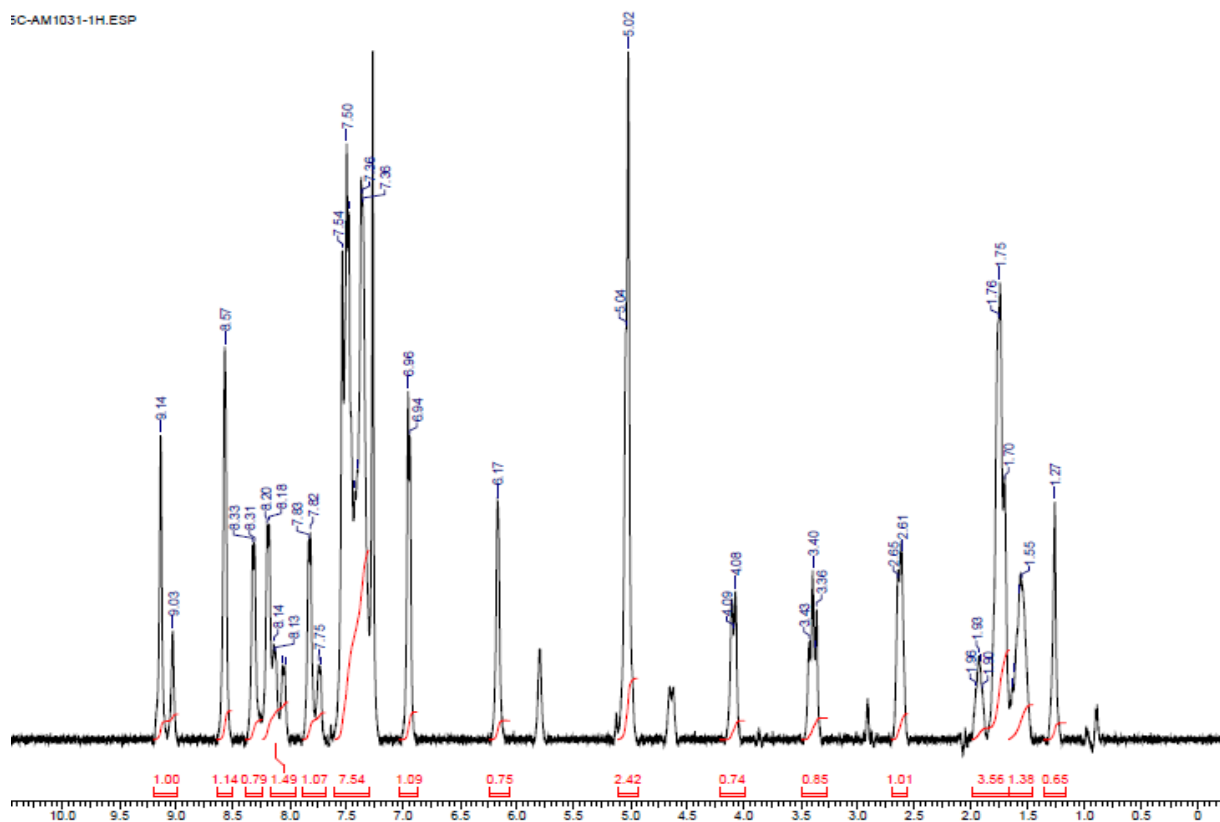
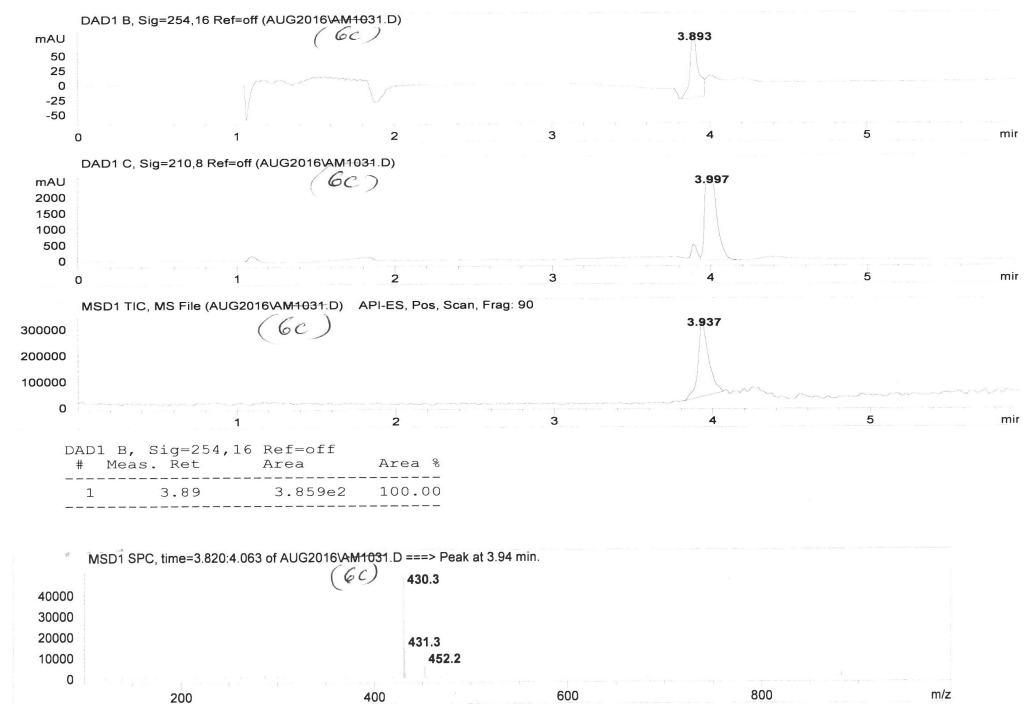
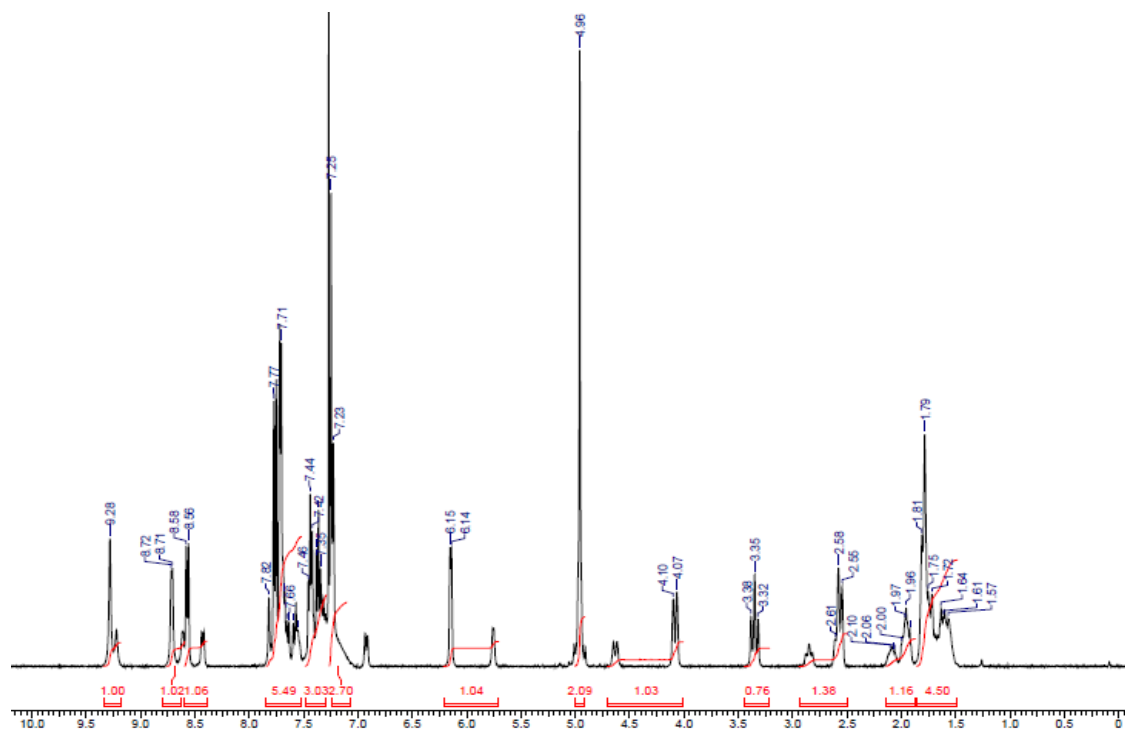


Figure S2: ^1H NMR of compound **6b**



LCMS of compound 6c

Figure S3: ^1H NMR of compound 6c



LCMS of compound **6d**

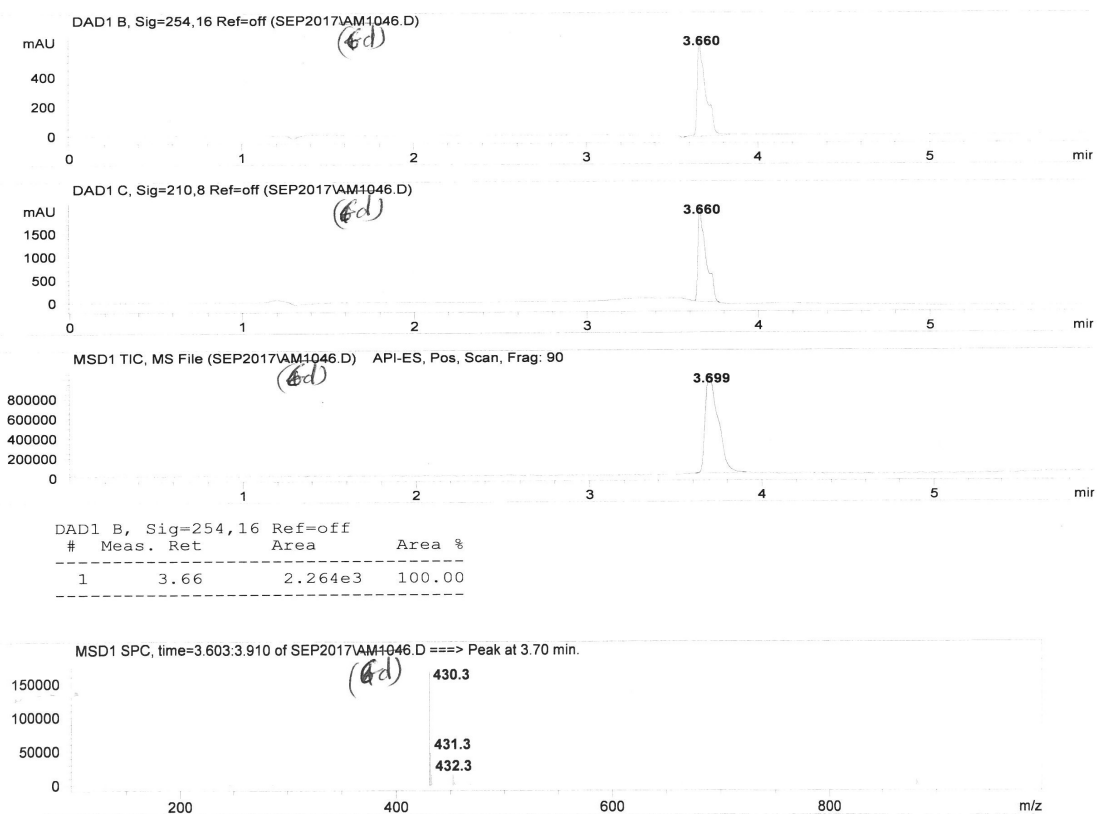
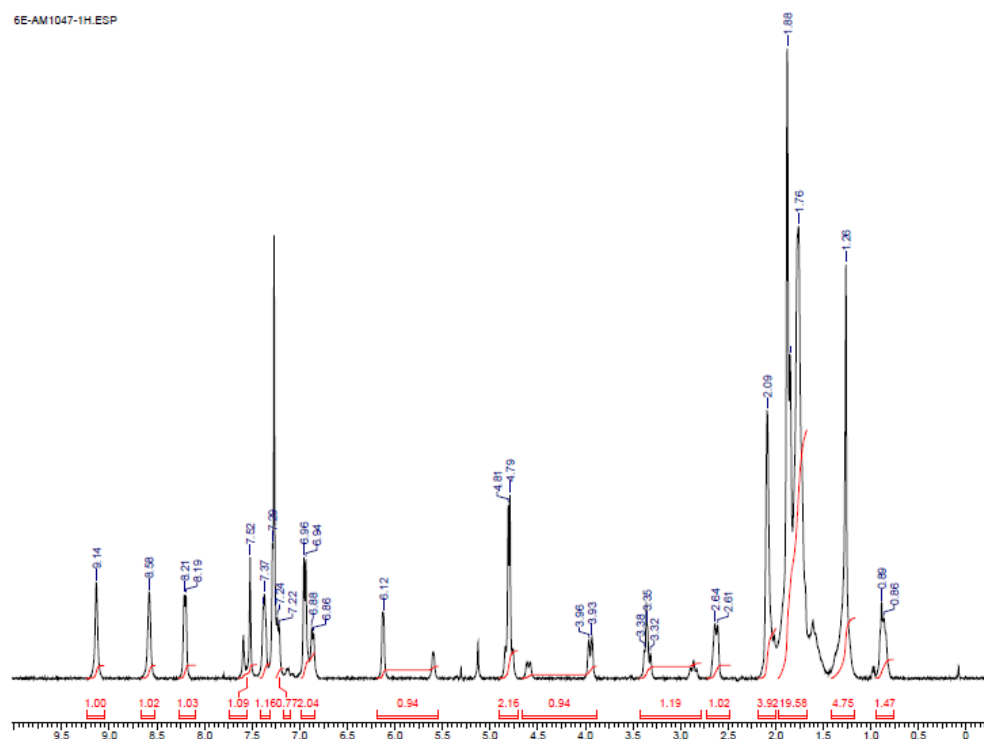


Figure S4: ^1H NMR of compound **6d**



LCMS of compound 6e

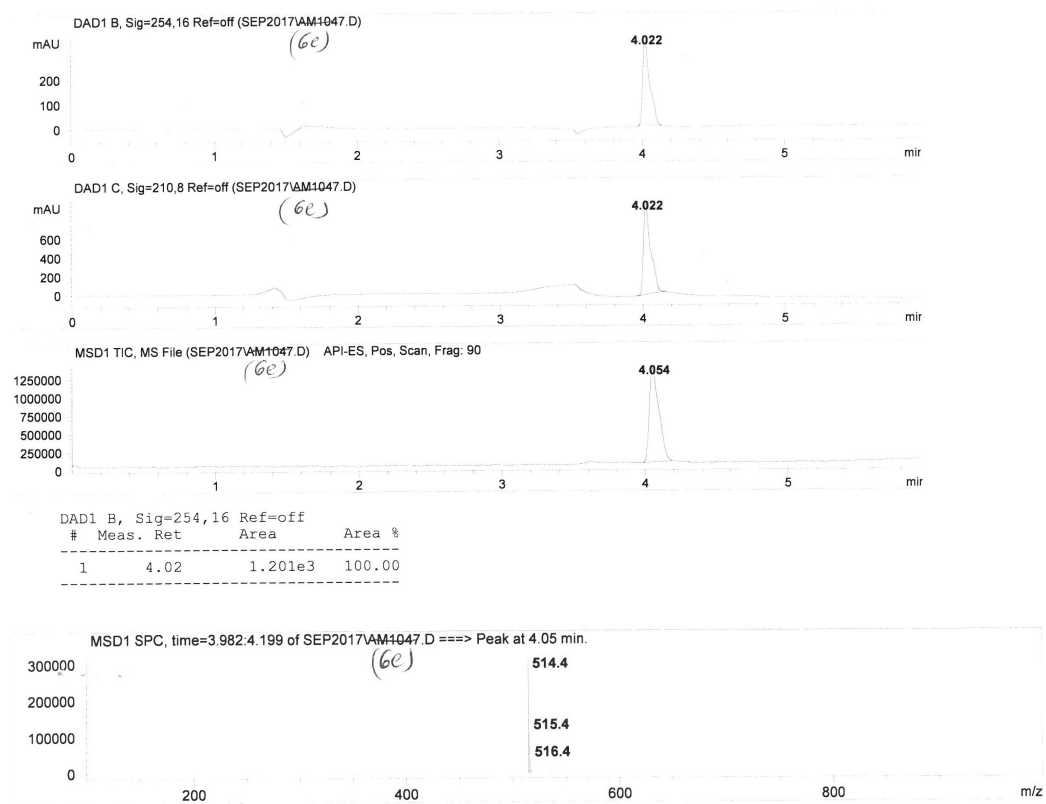
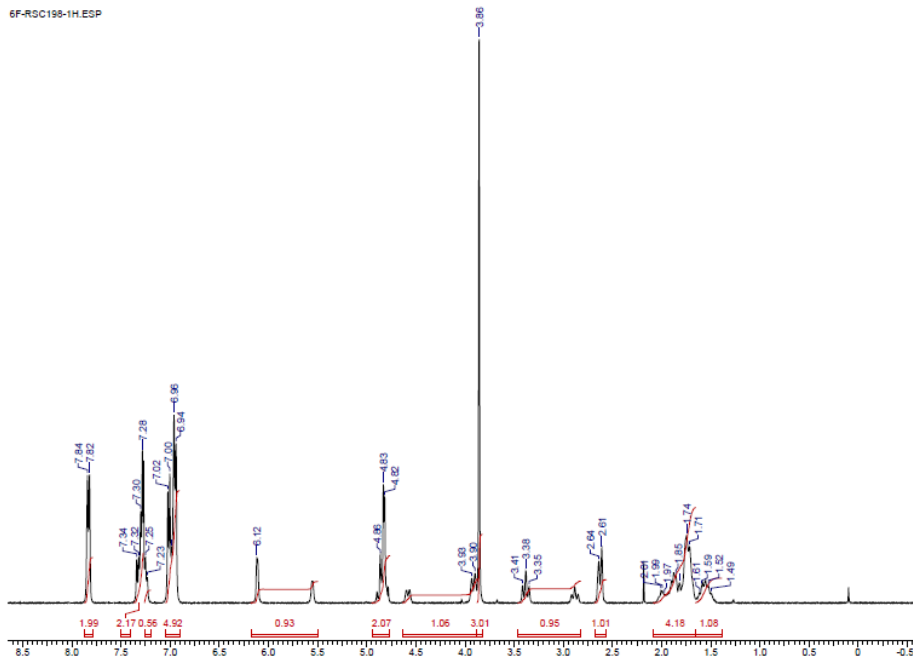


Figure S5: ¹H NMR of compound 6e

6F-RSC198-1H-ESP



LCMS of compound 6f

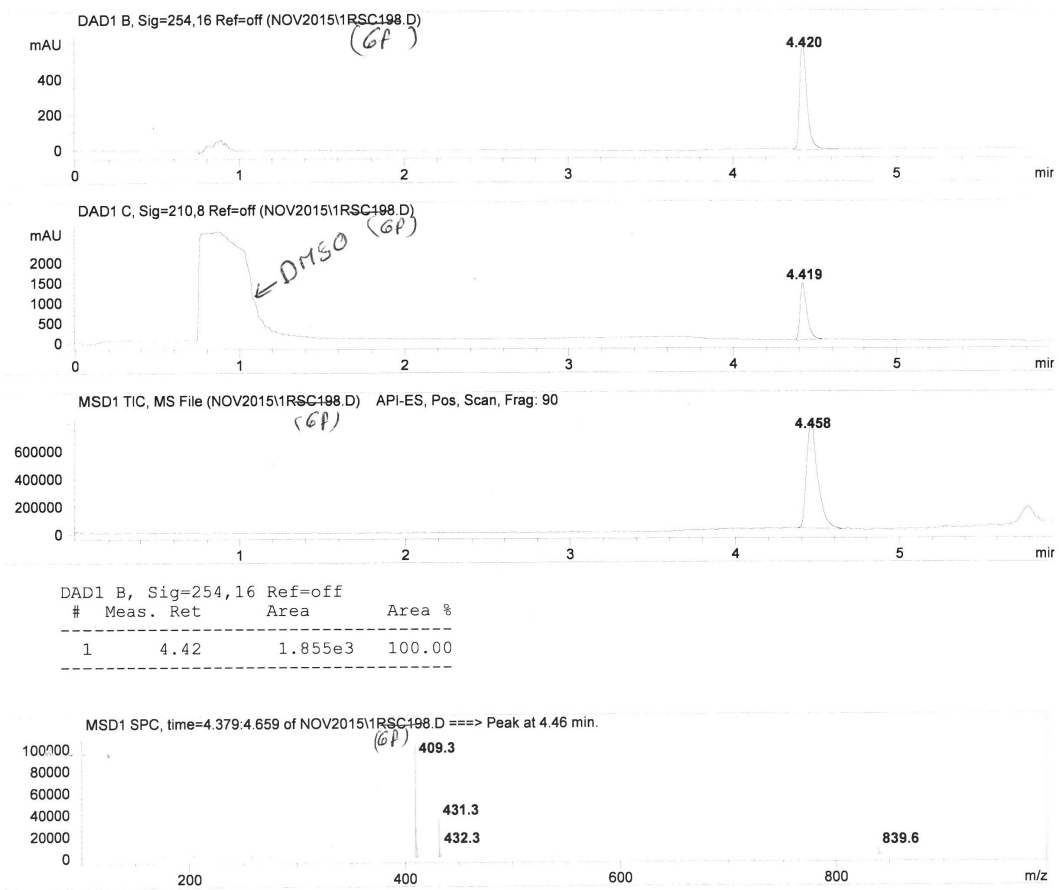
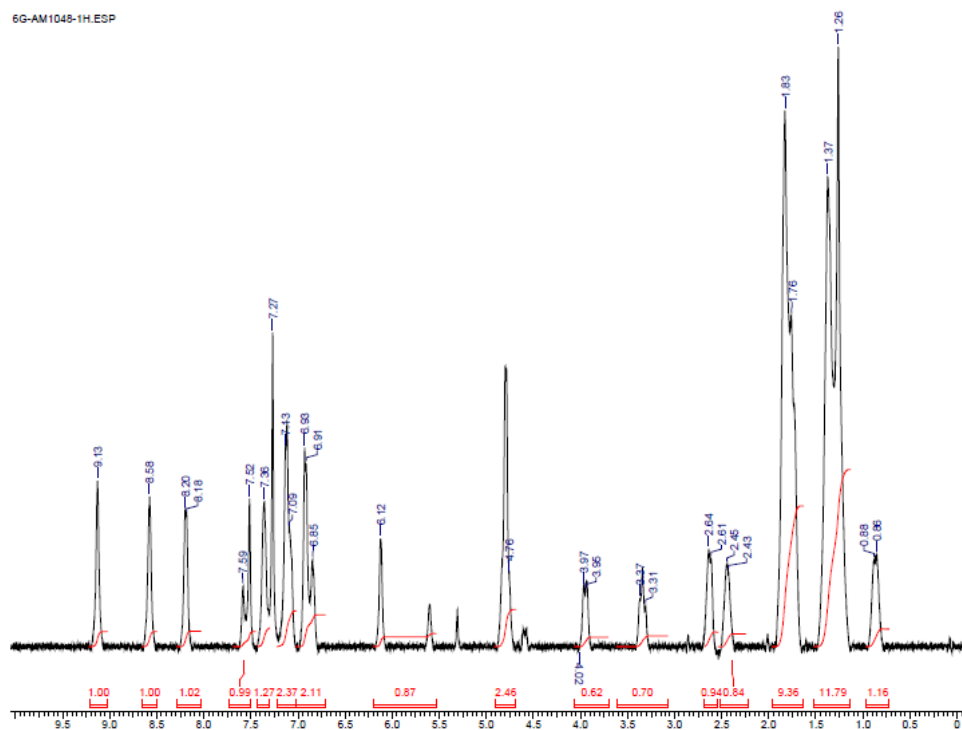


Figure S6: ¹H NMR of compound 6f



LCMS of compound **6g**

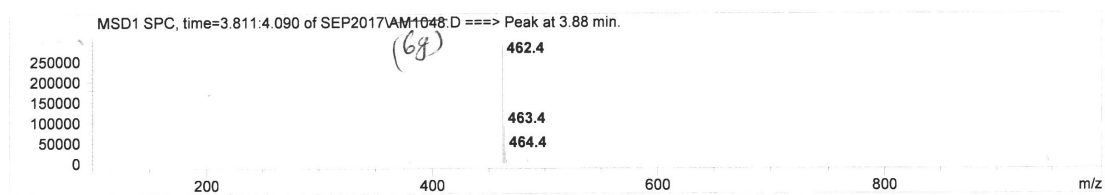
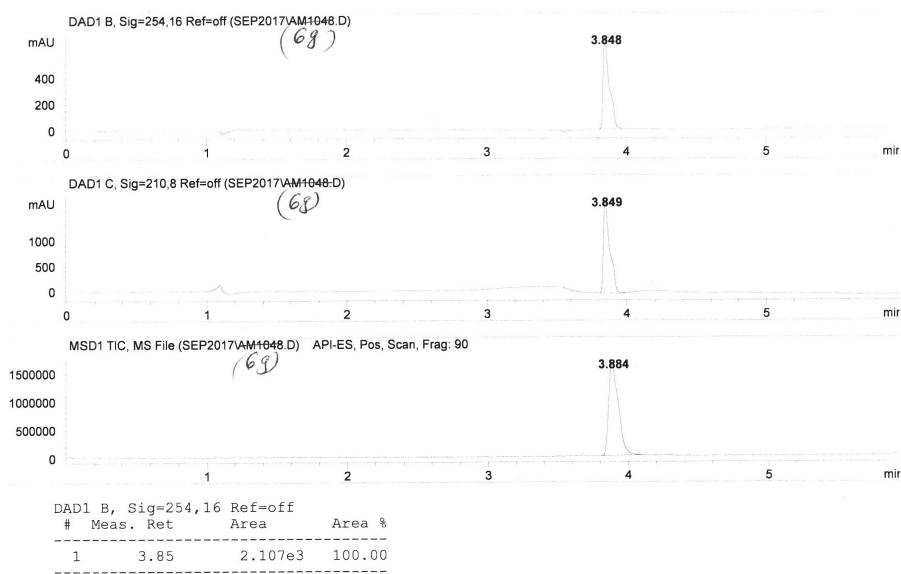
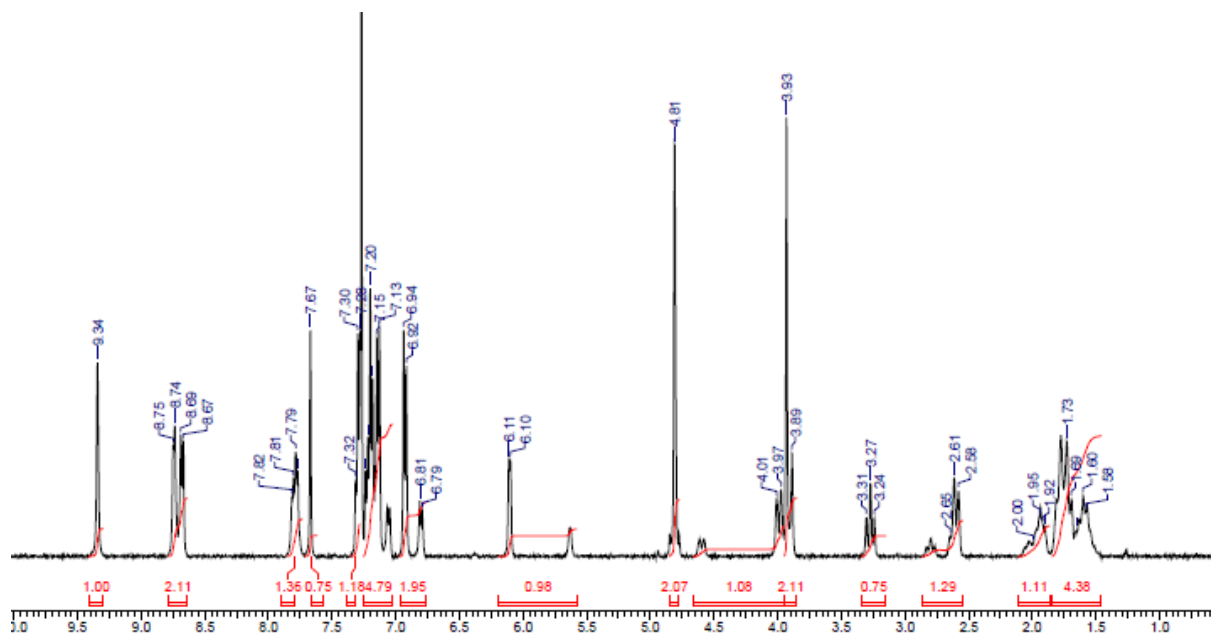


Figure S7: ^1H NMR of compound **6g**



LCMS of compound **6h**

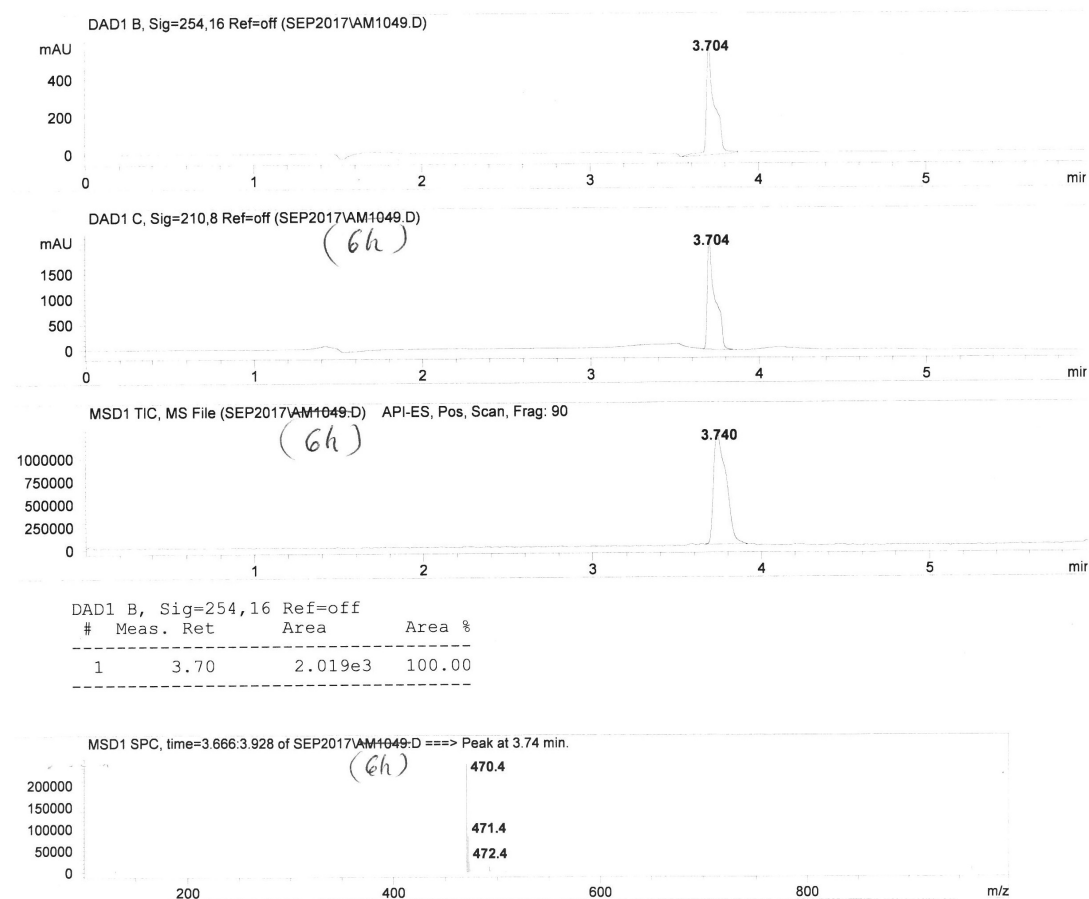
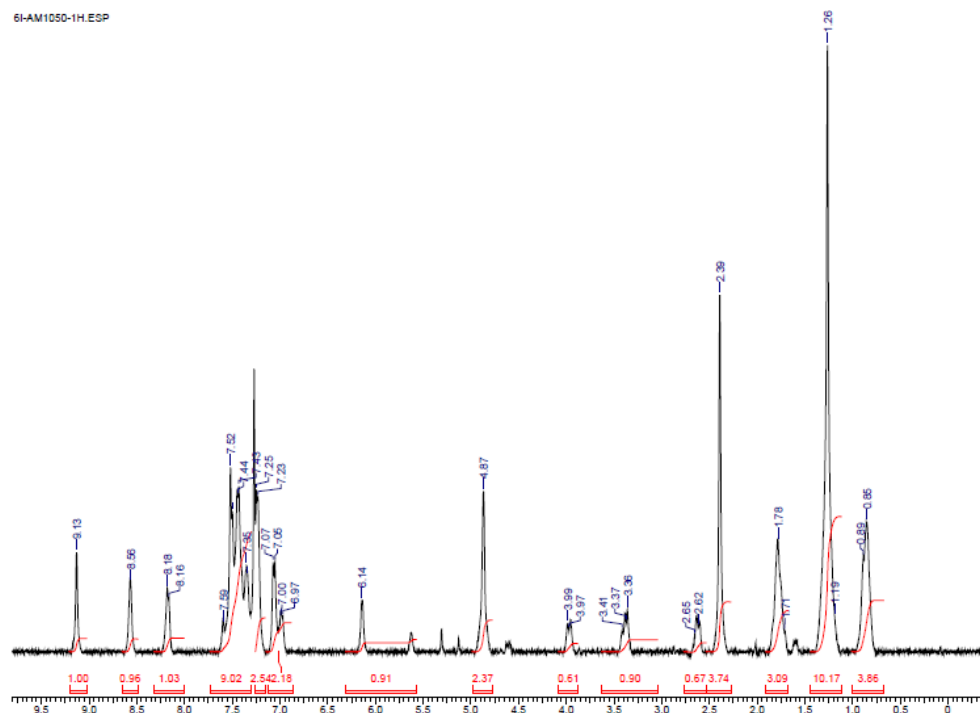


Figure S8: ^1H NMR of compound **6h**



LCMS of compound 6i

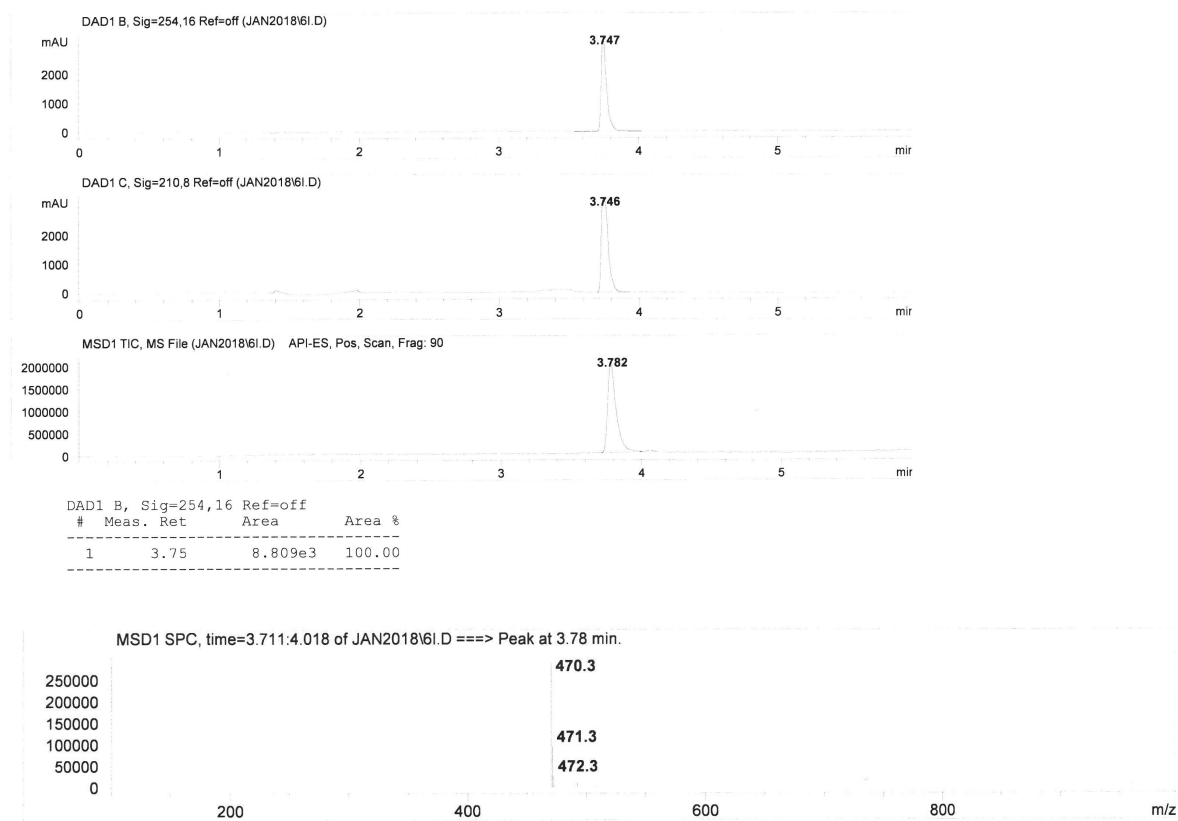
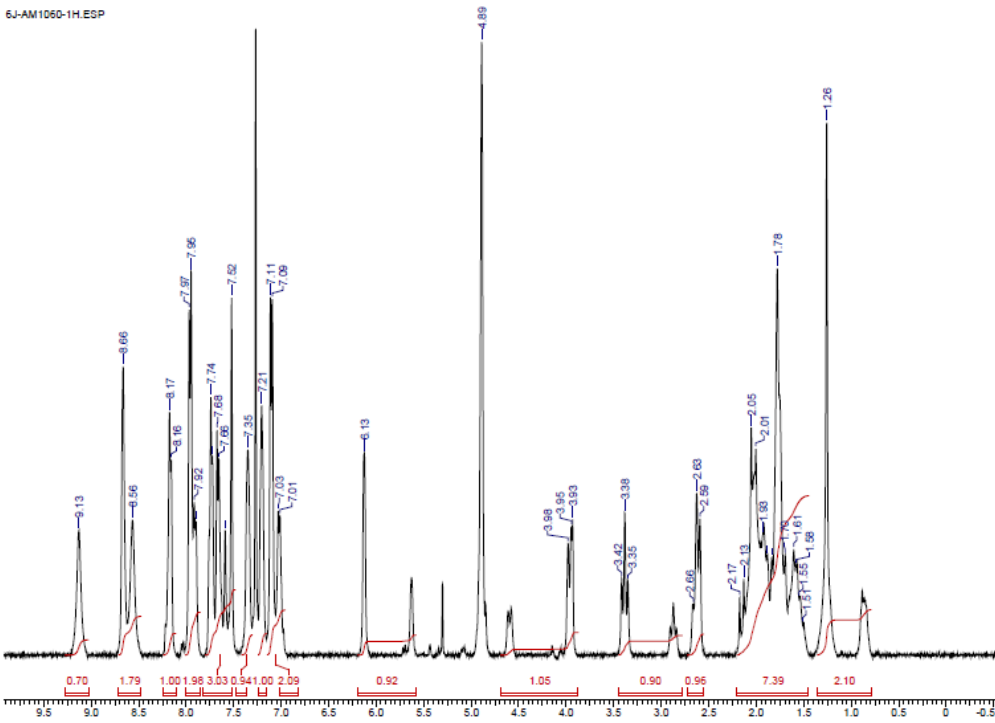


Figure S9: ^1H NMR of compound 6i



LCMS of compound 6j

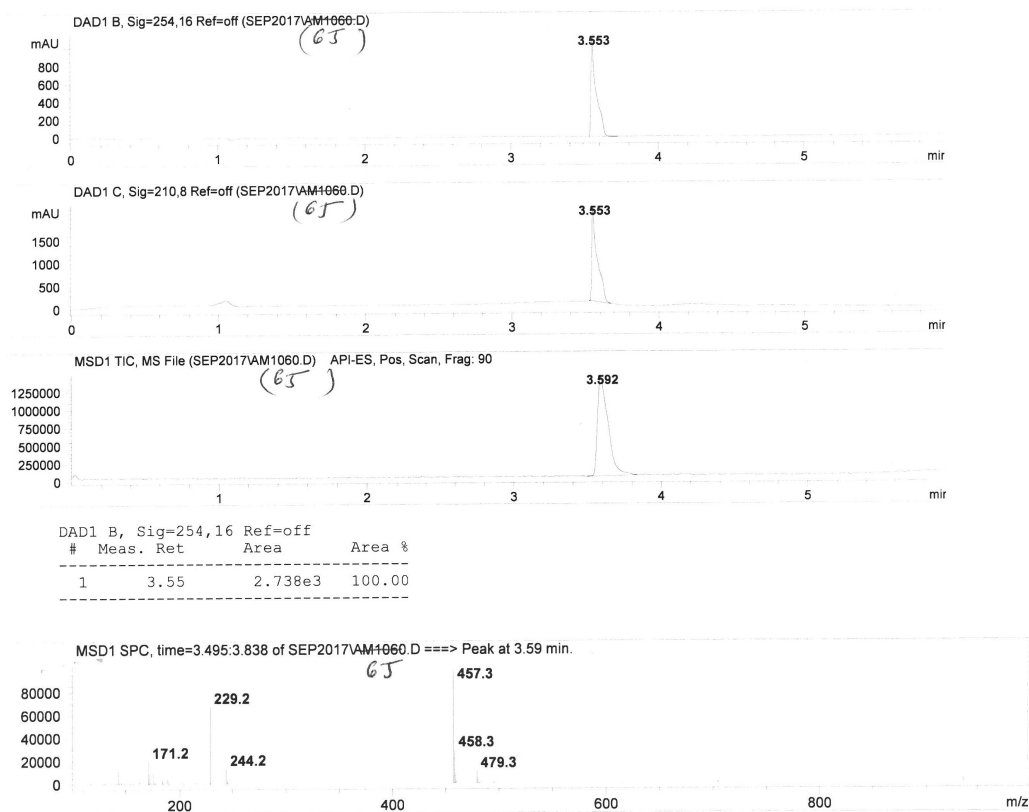
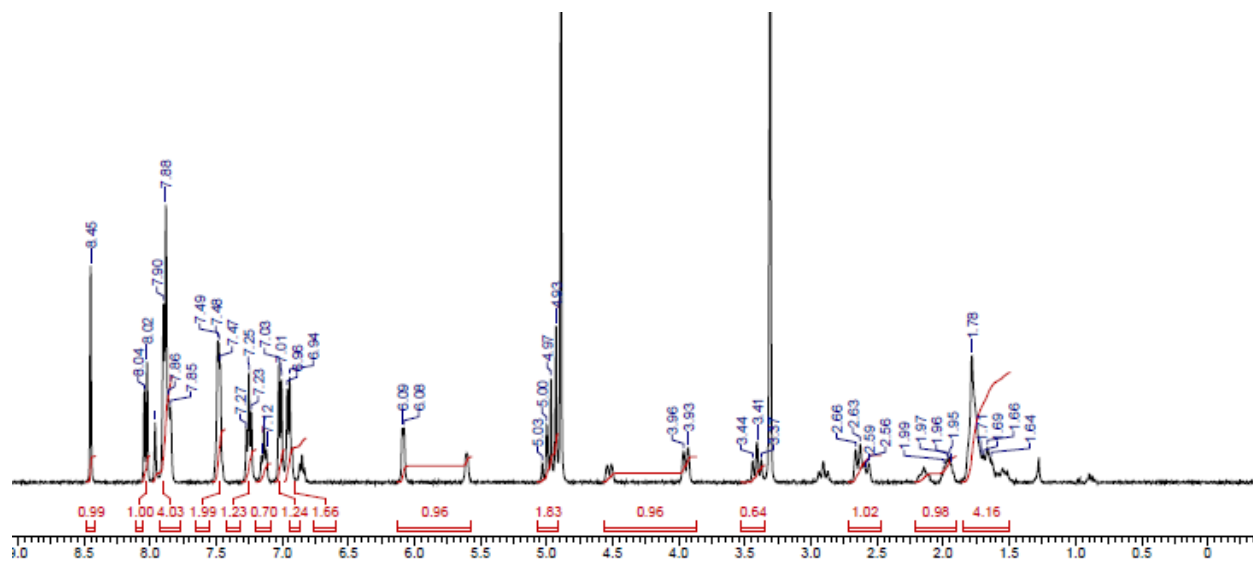


Figure S10: ¹H NMR of compound 6j



LCMS of compound **6k**

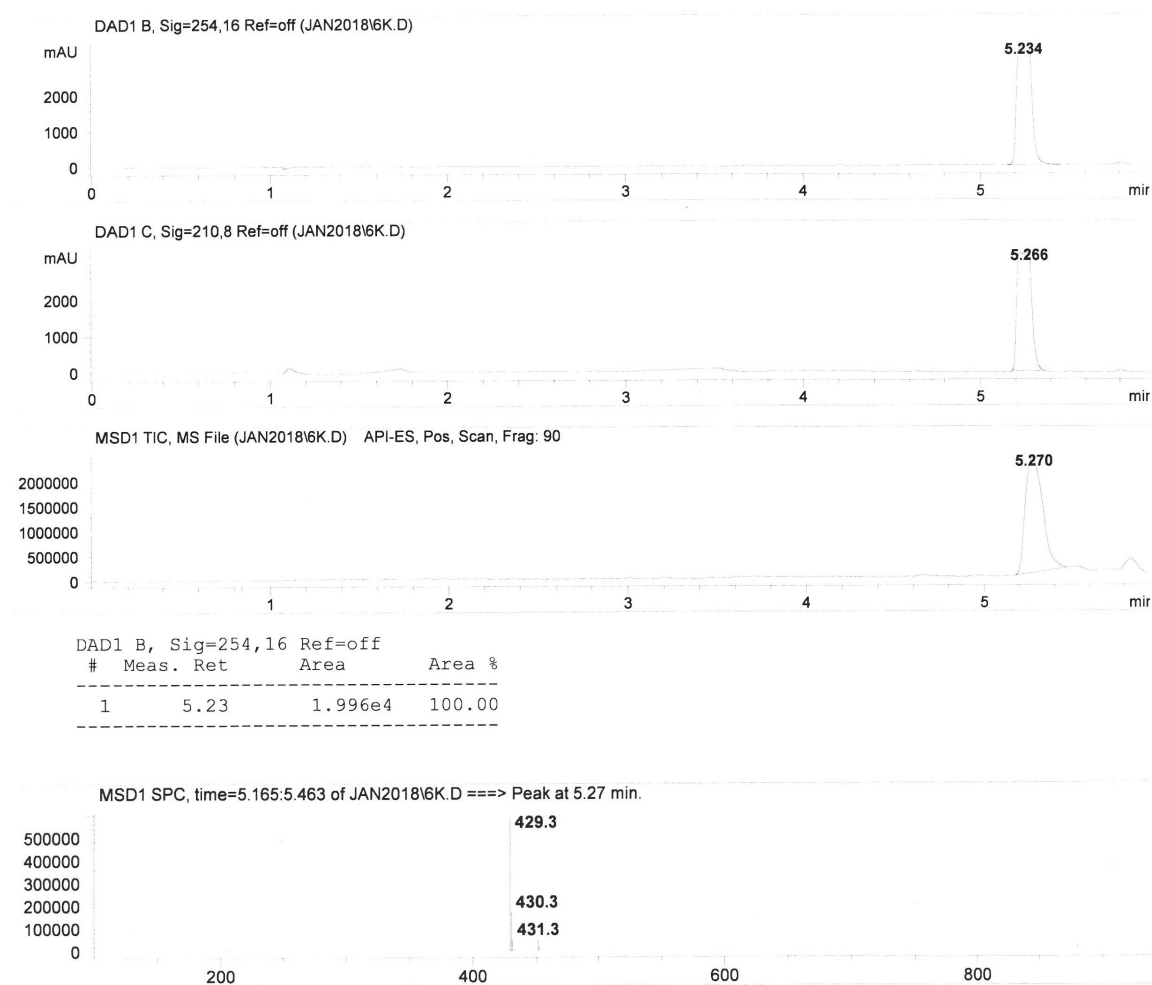
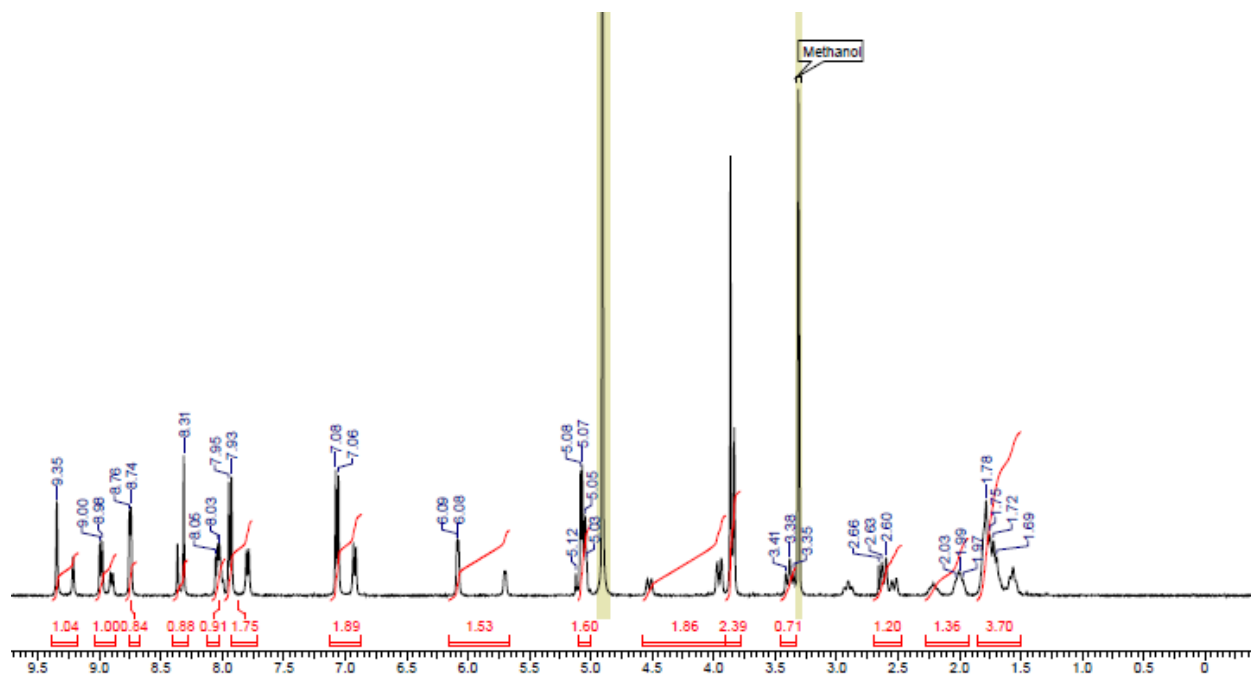


Figure S11: ^1H NMR of compound **6k**



LCMS of compound **6l**

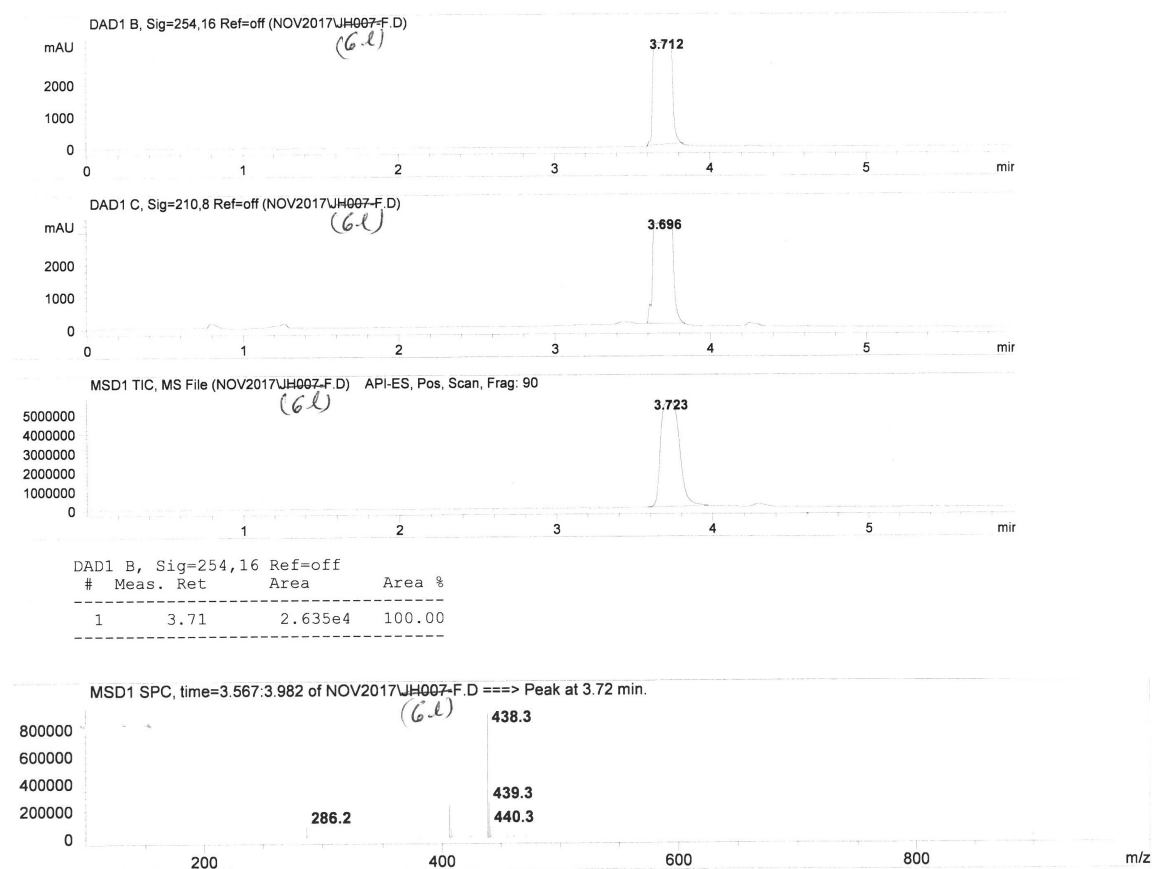
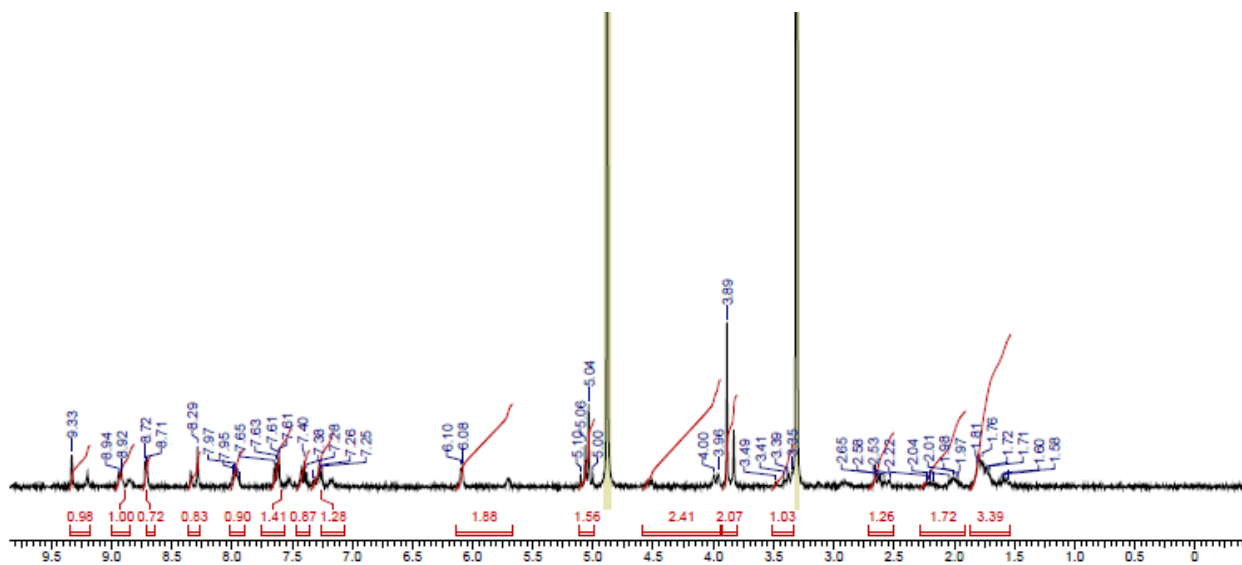


Figure S12: ^1H NMR of compound **6l**



LCMS of compound **6m**

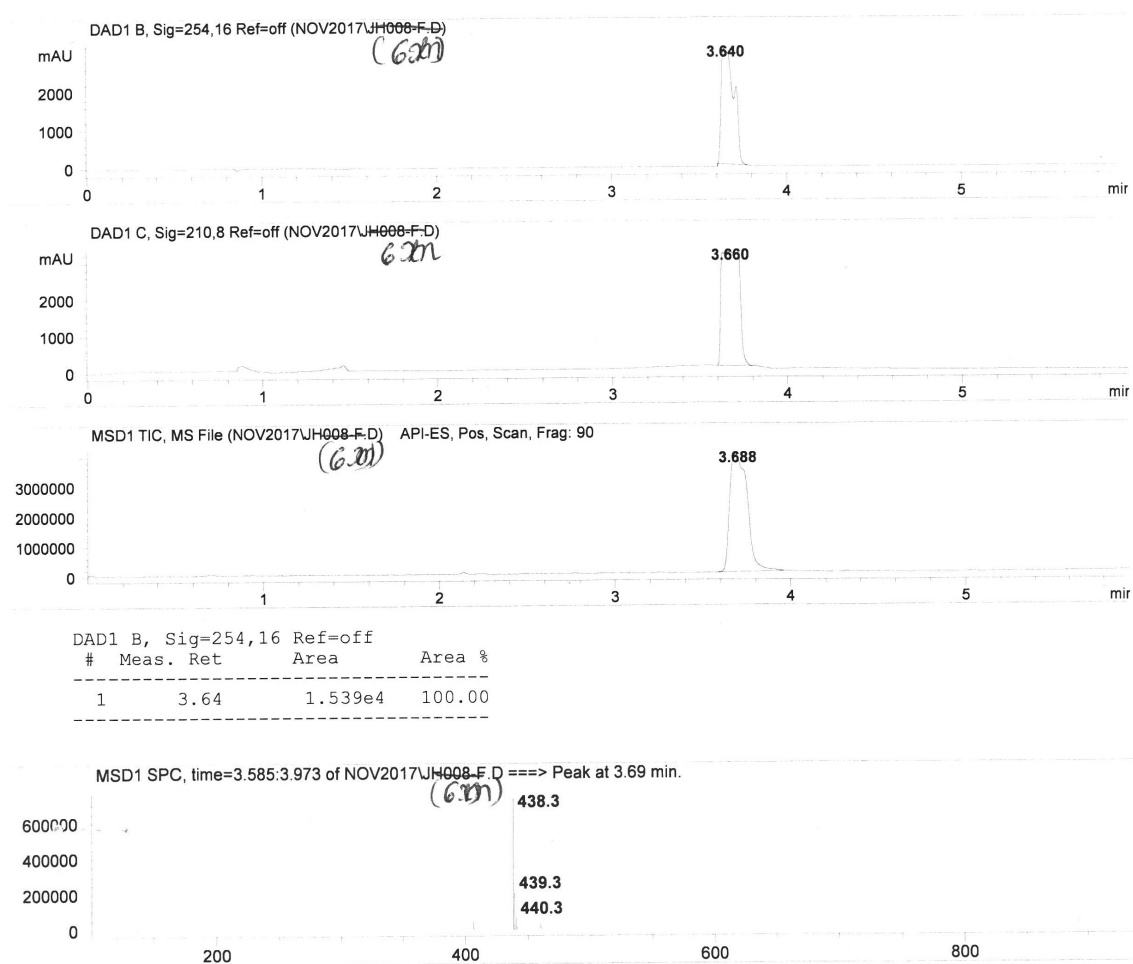
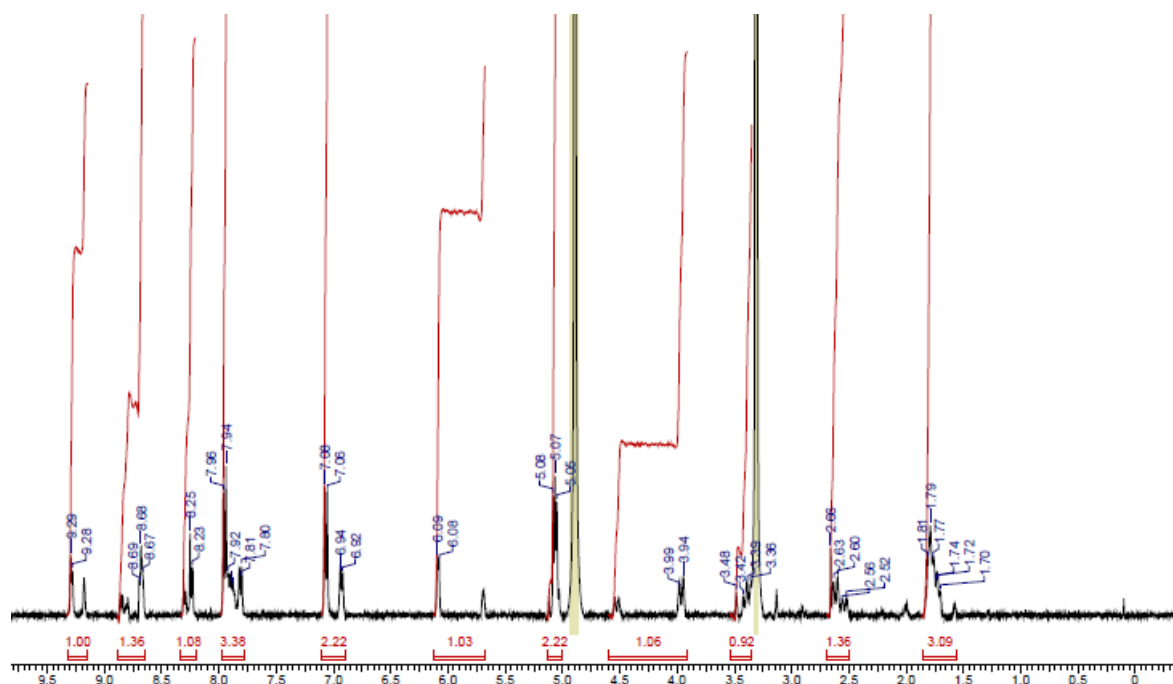


Figure S13: ^1H NMR of compound **6m**



LCMS of compound **6n**

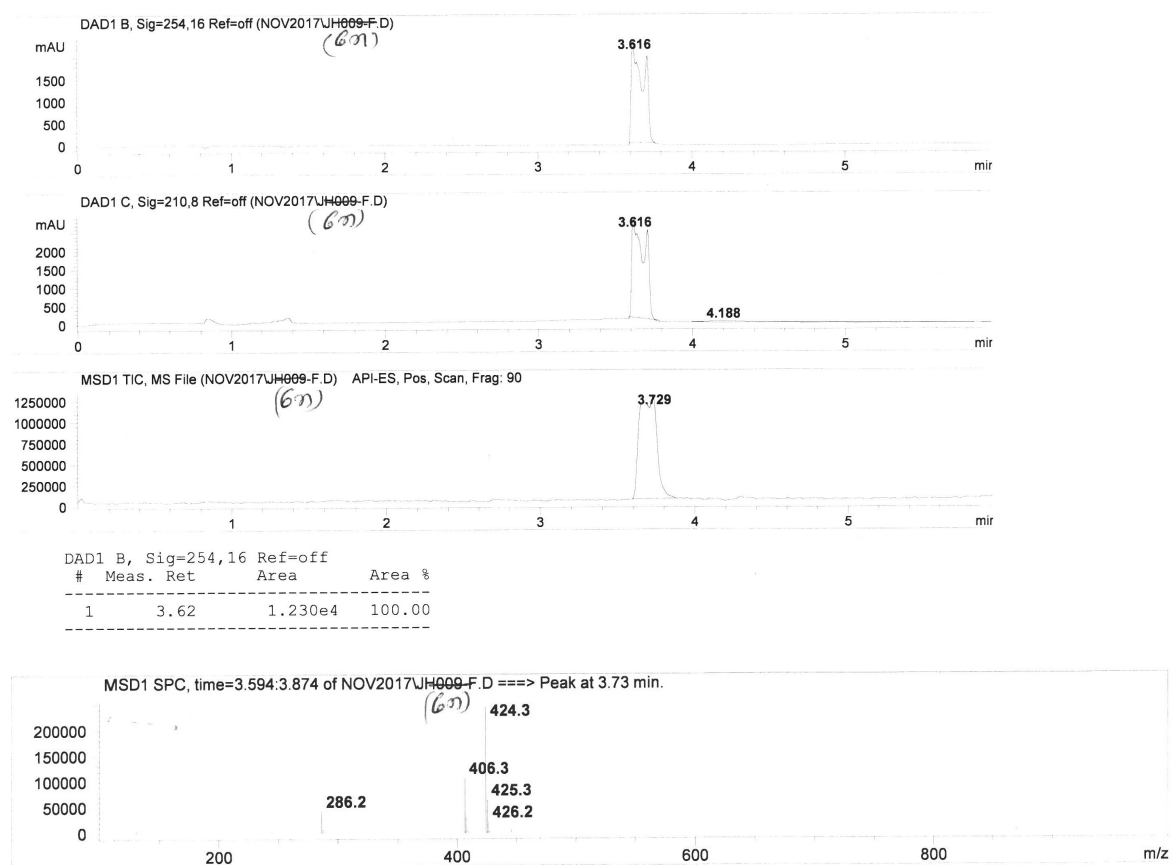
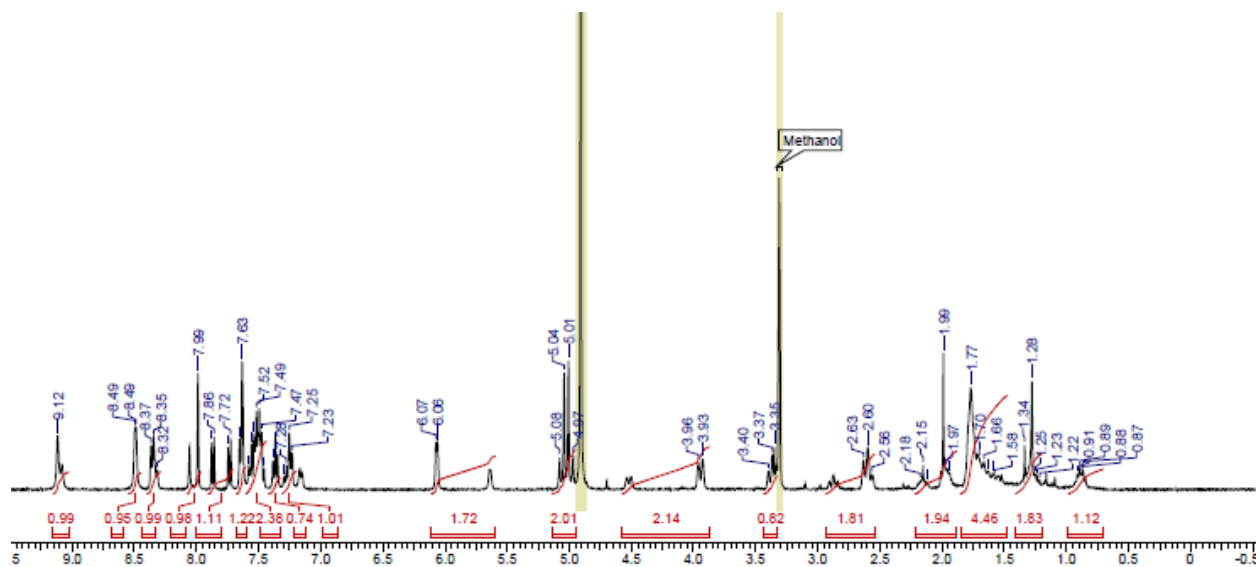


Figure S14: ^1H NMR of compound **6n**



LCMS of compound **60**

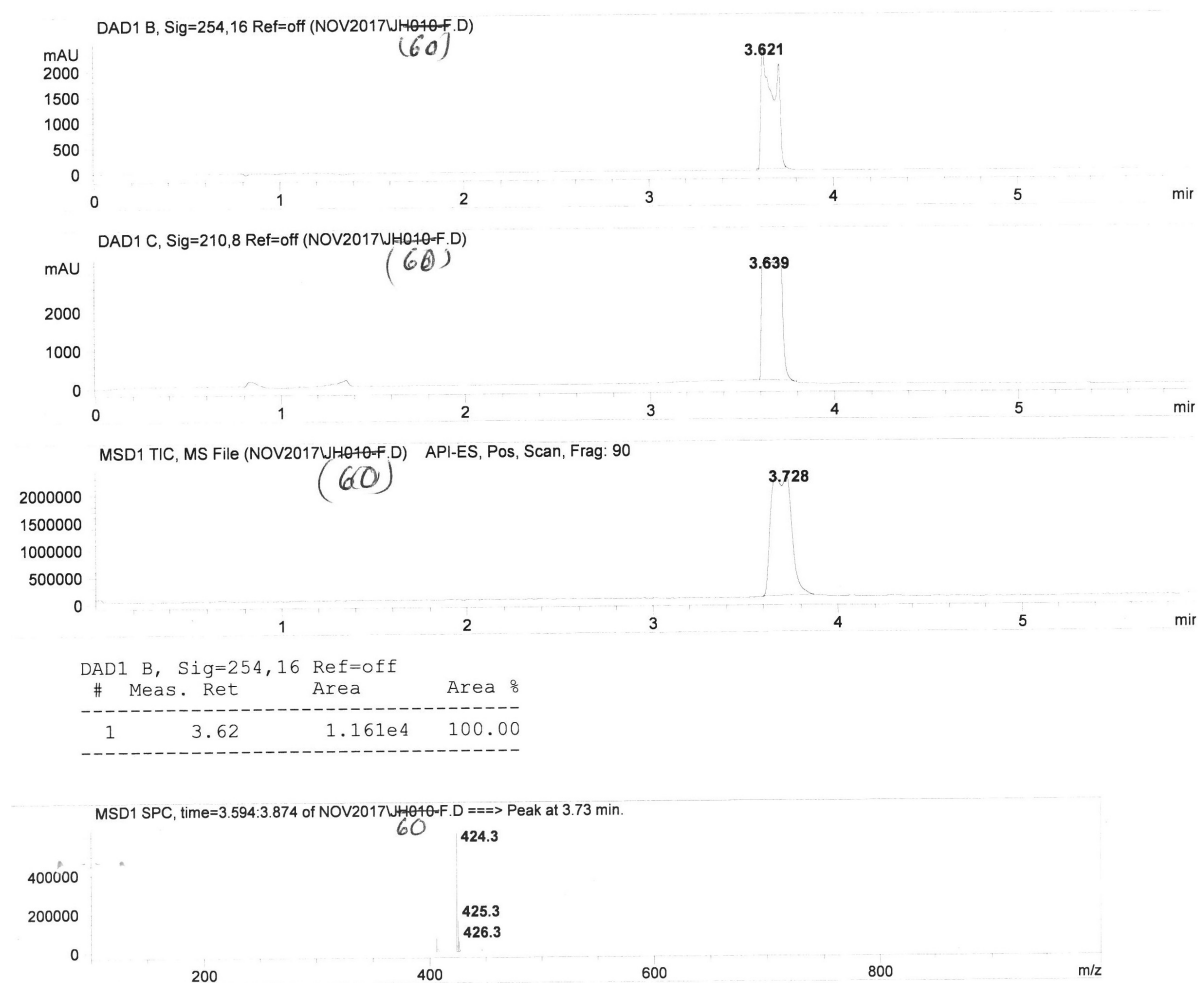
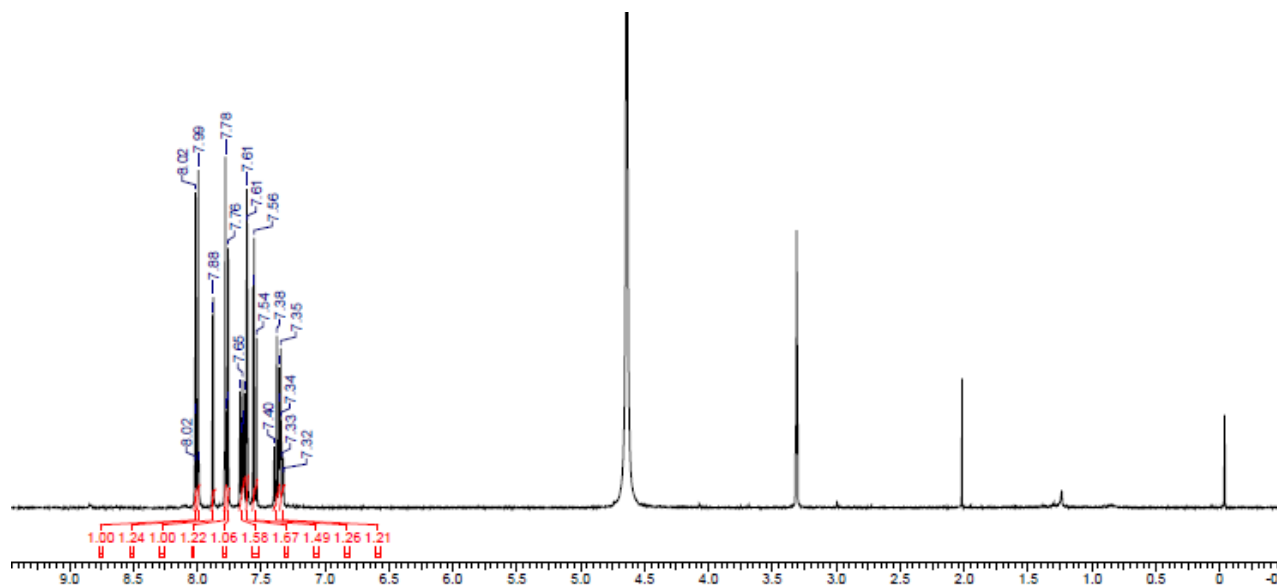


Figure S15: ^1H NMR of compound **60**



LCMS of compound 10a

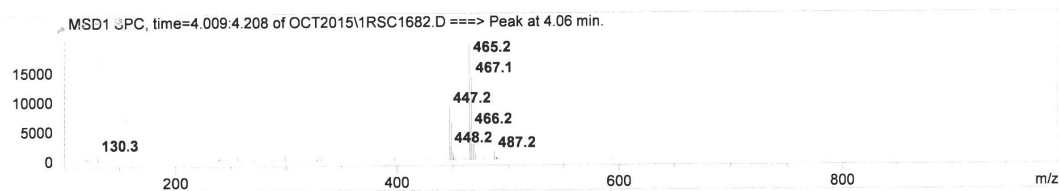
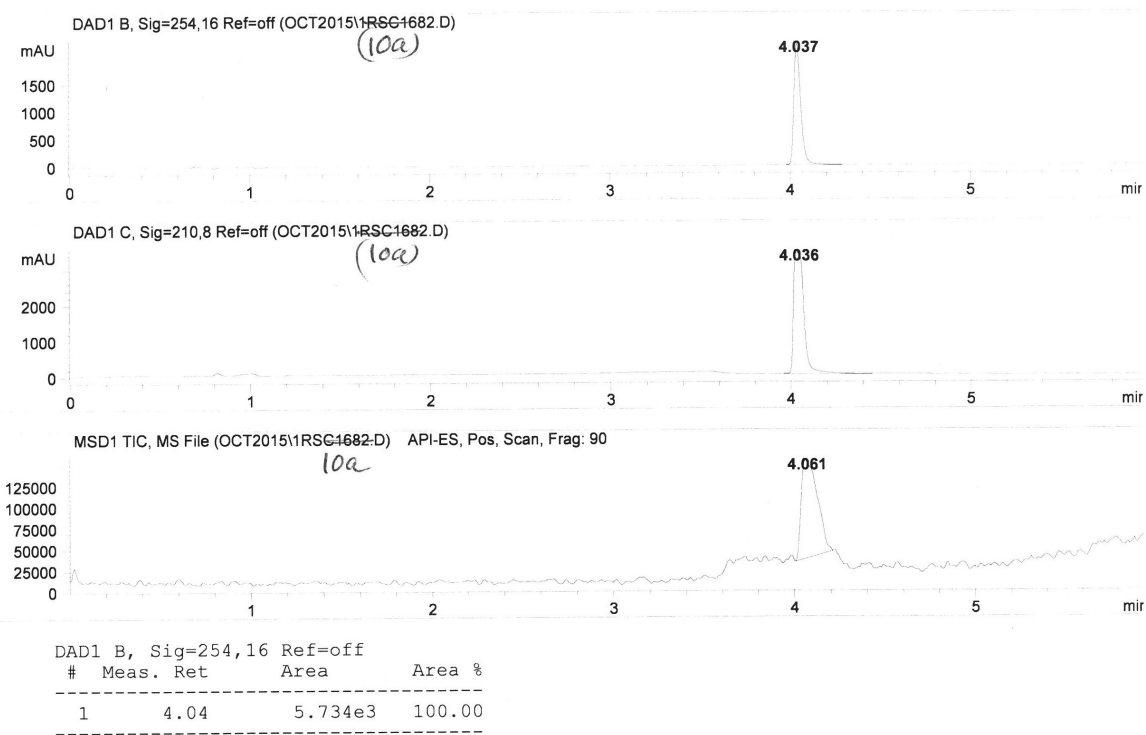
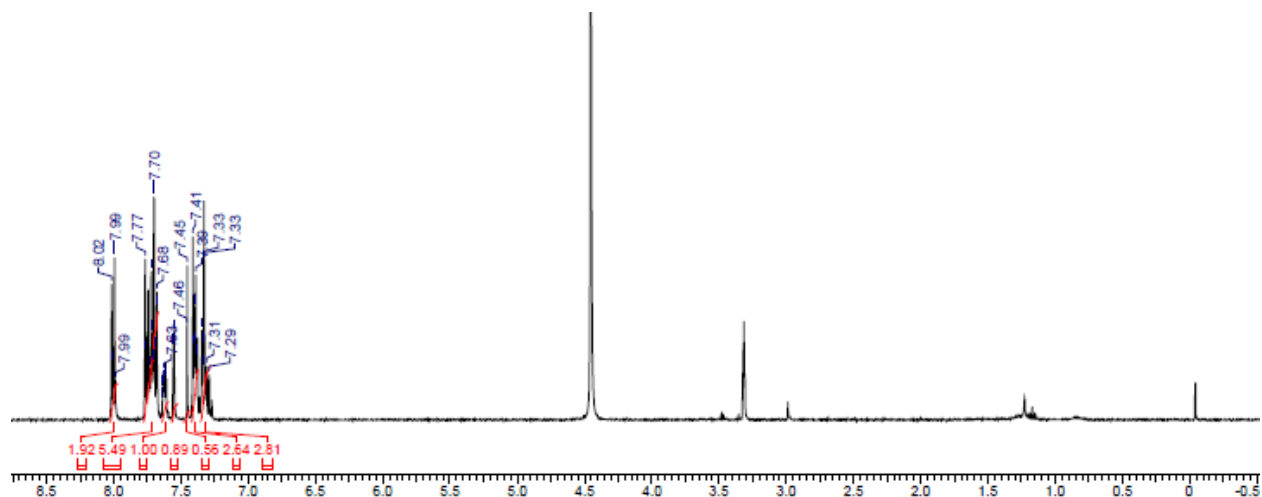


Figure S16: ^1H NMR of compound 10a



LCMS of compound **10b**

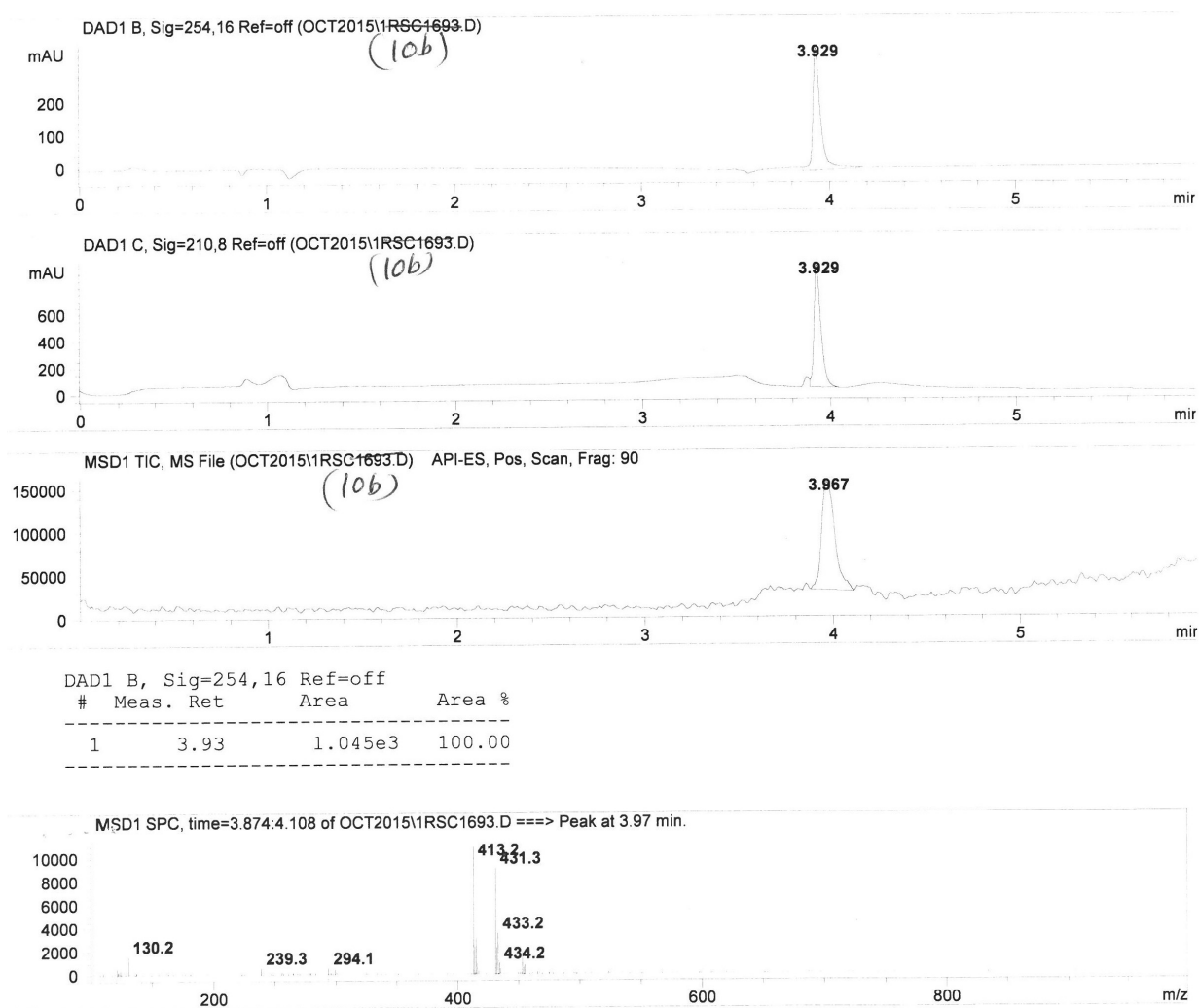
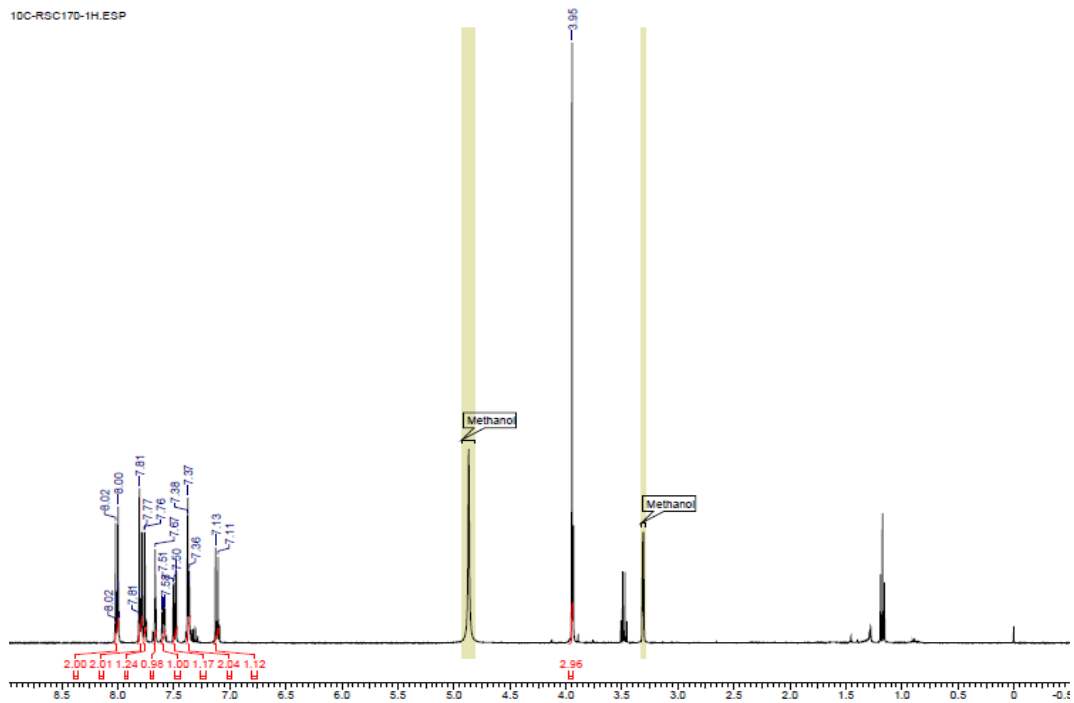


Figure S17: ^1H NMR of compound **10b**



LCMS of compound 10c

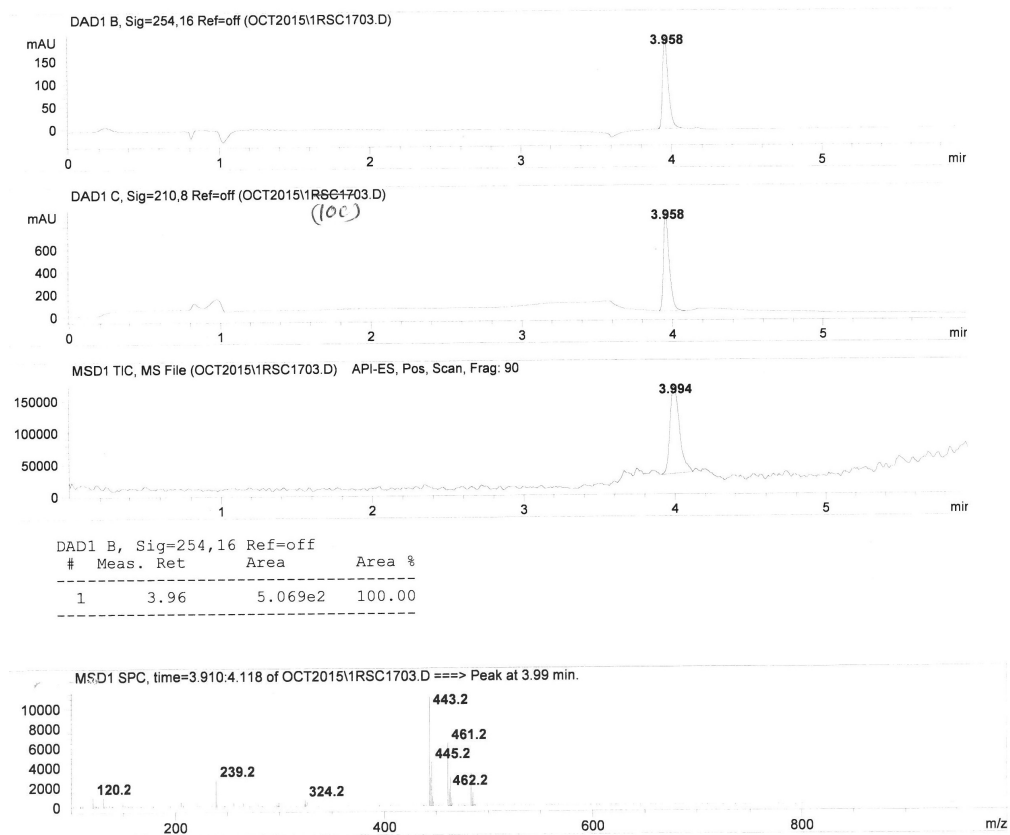
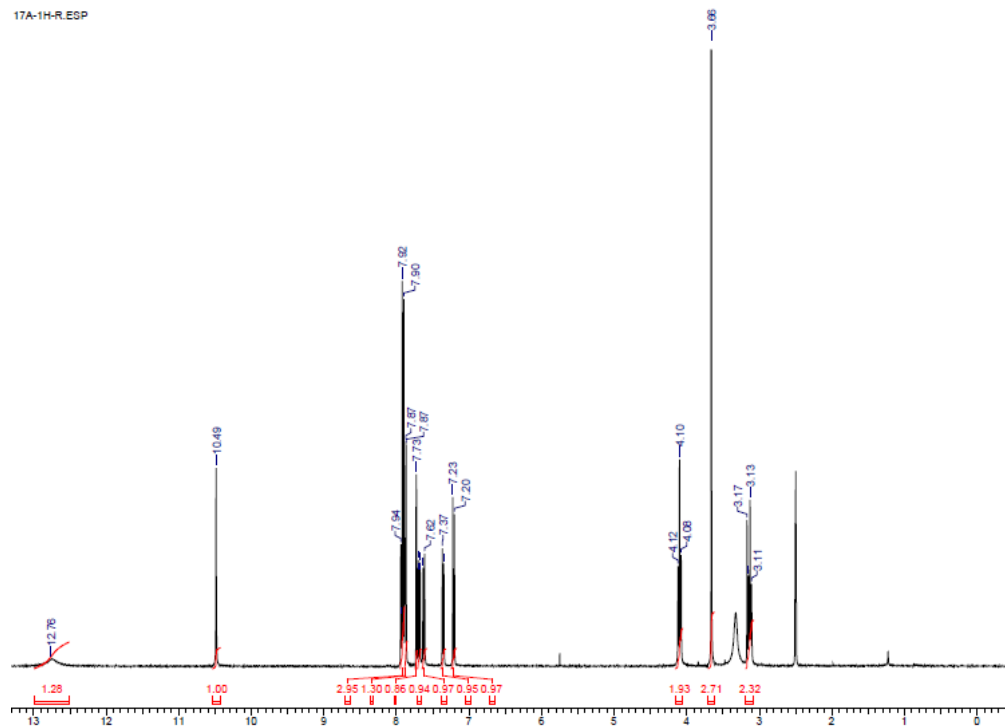


Figure S18: ^1H NMR of compound 10c



LCMS of compound 17a

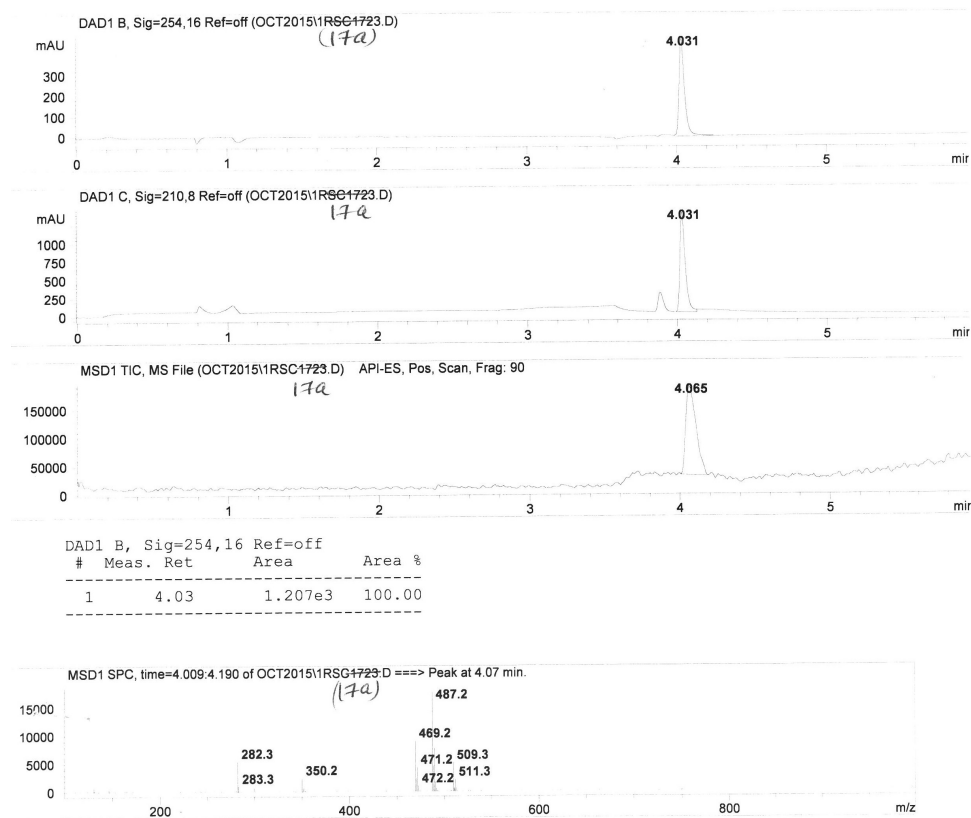
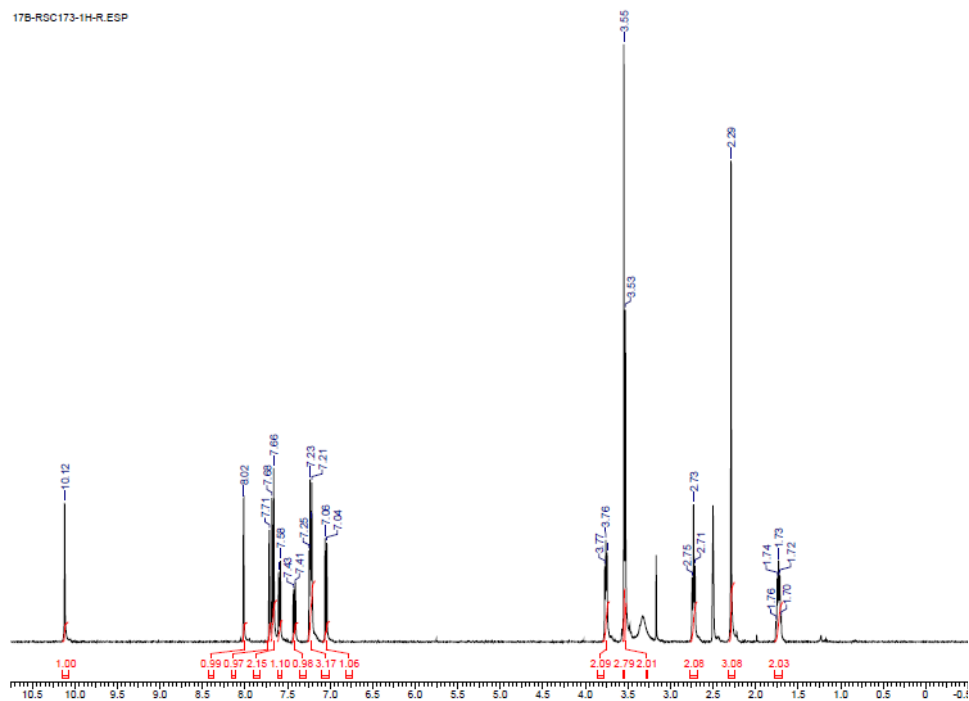


Figure S19: ^1H NMR of compound 17a



LCMS of compound **17b**

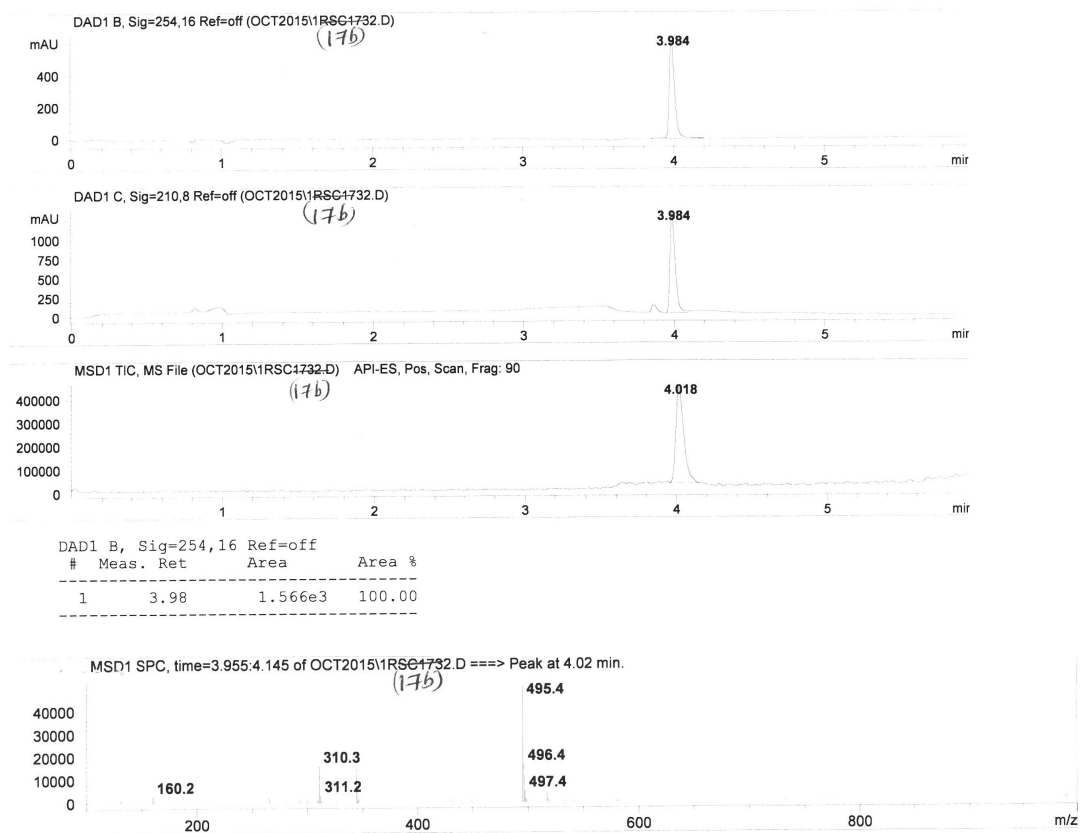
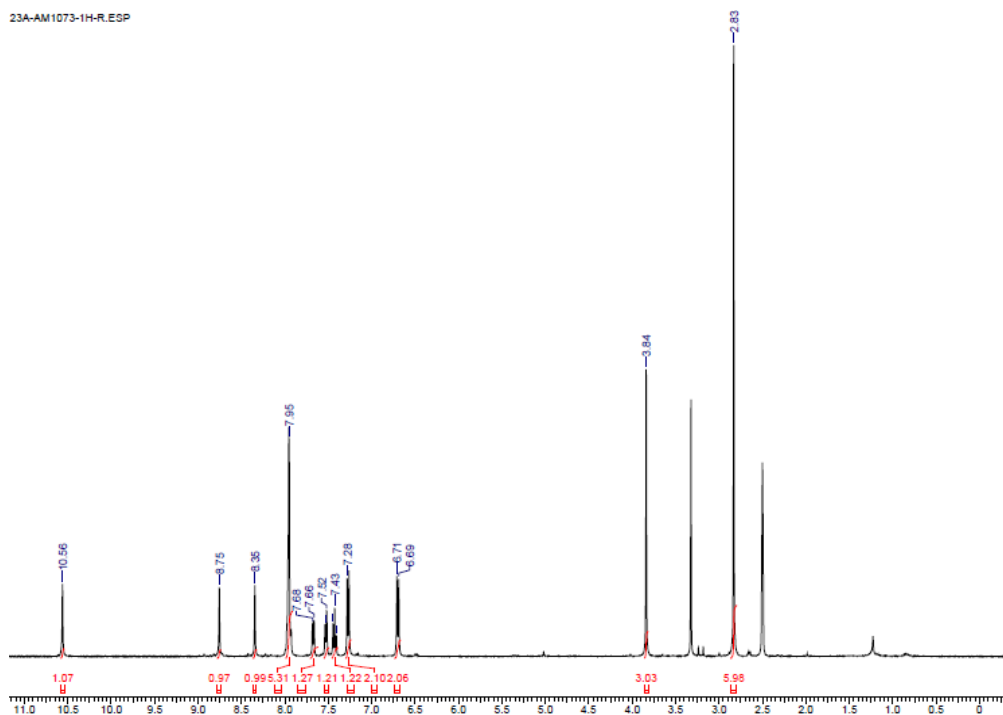


Figure S20: ^1H NMR of compound **17b**



LCMS of compound **23a**

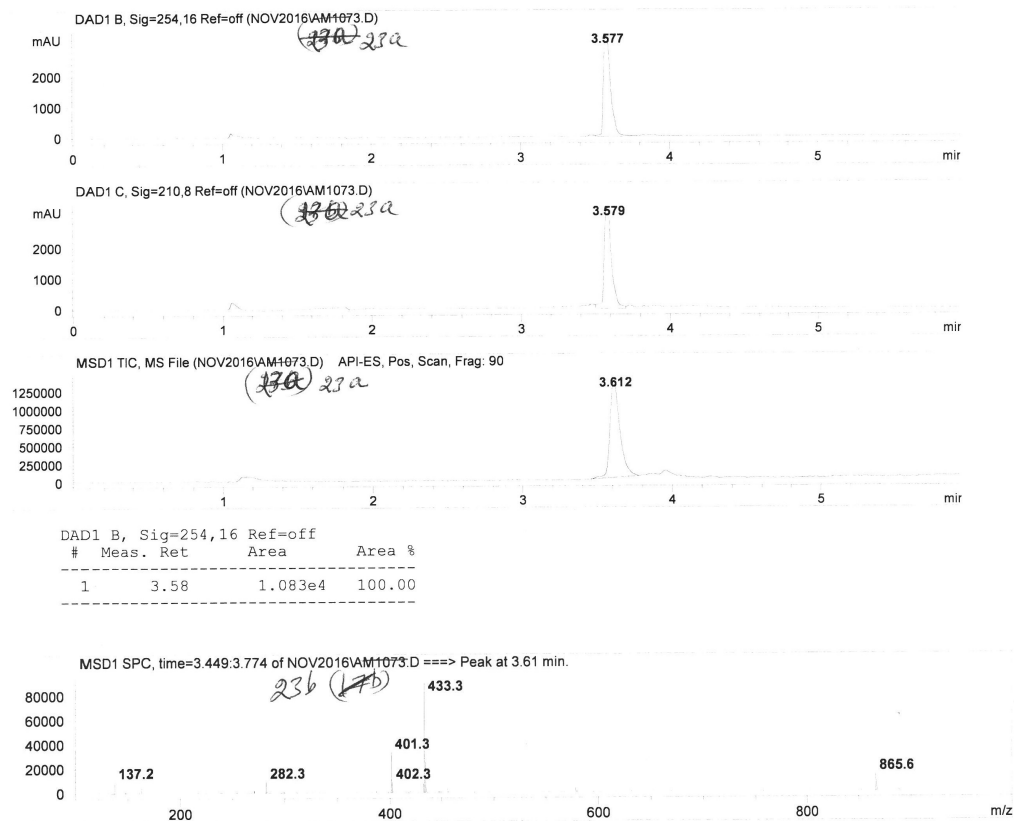
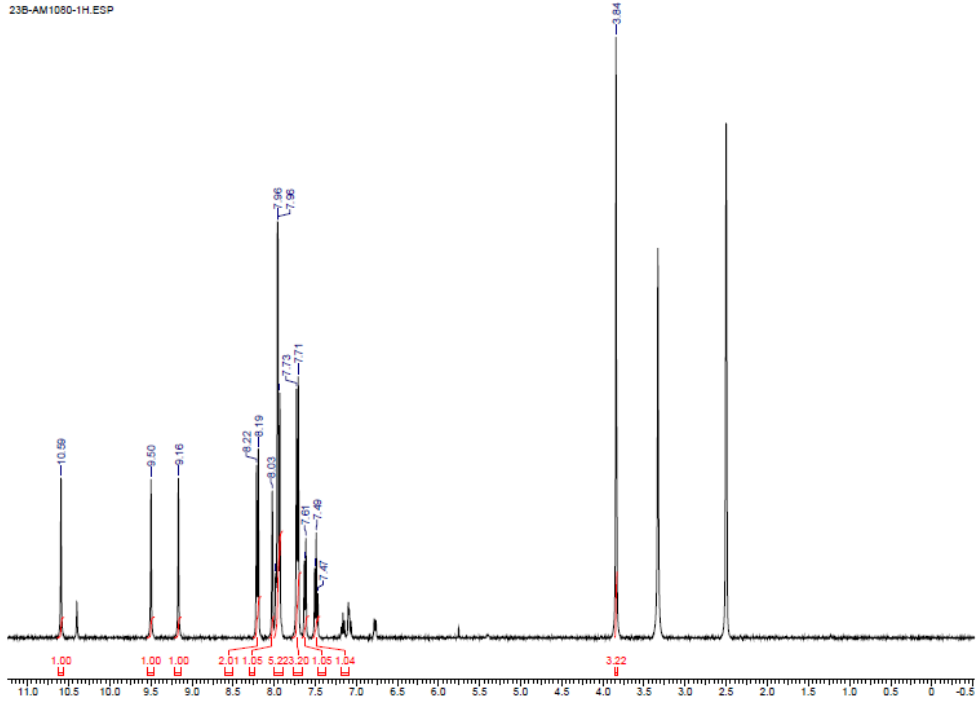


Figure S21: ^1H NMR of compound **23a**



LCMS of compound **23b**

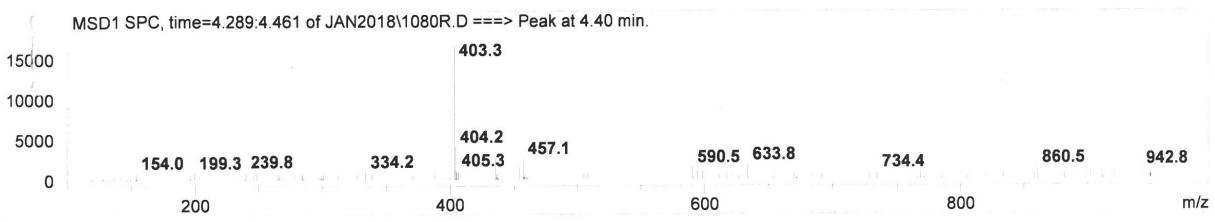
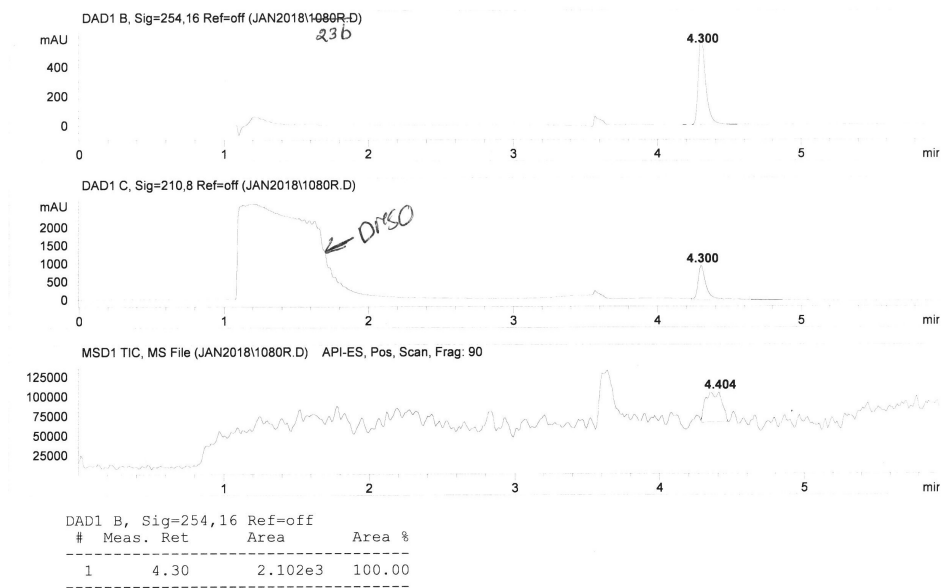
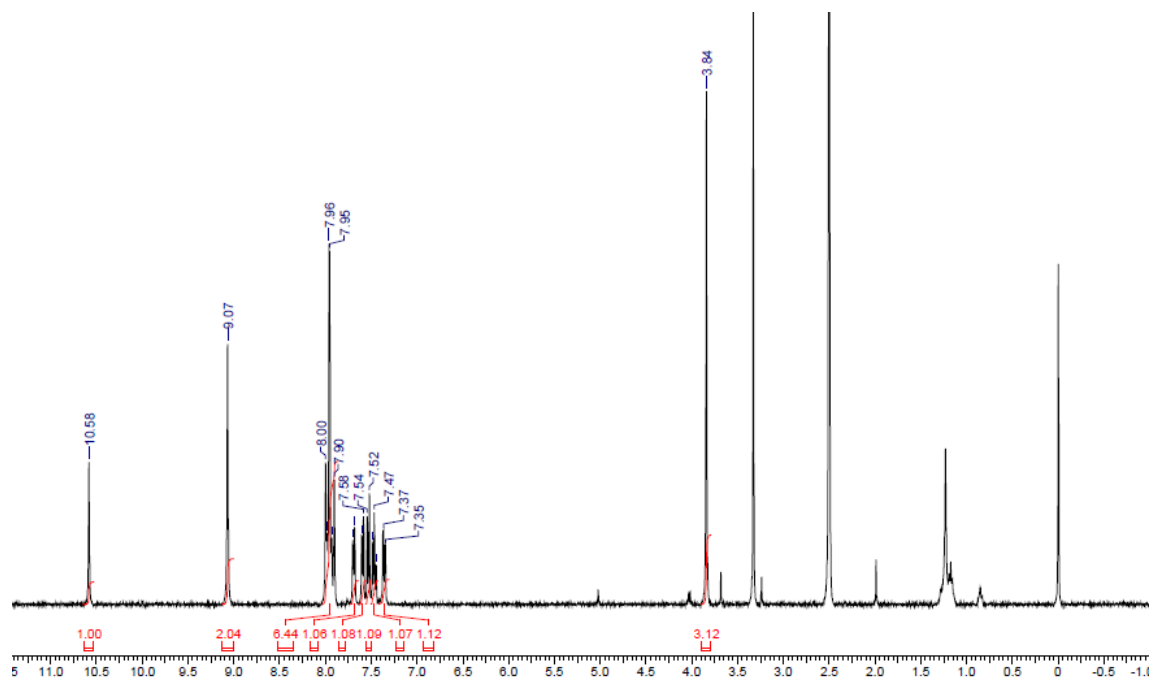


Figure S22: ¹H NMR of compound **23b**



LCMS of compound **23c**

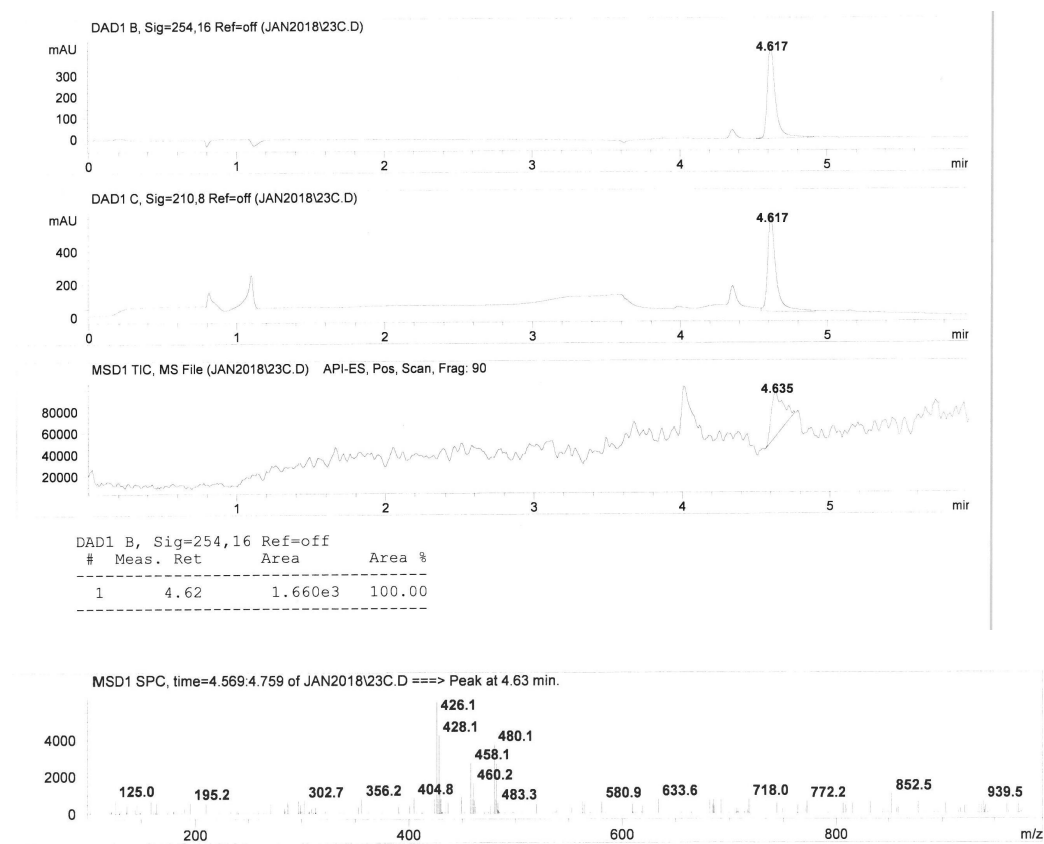
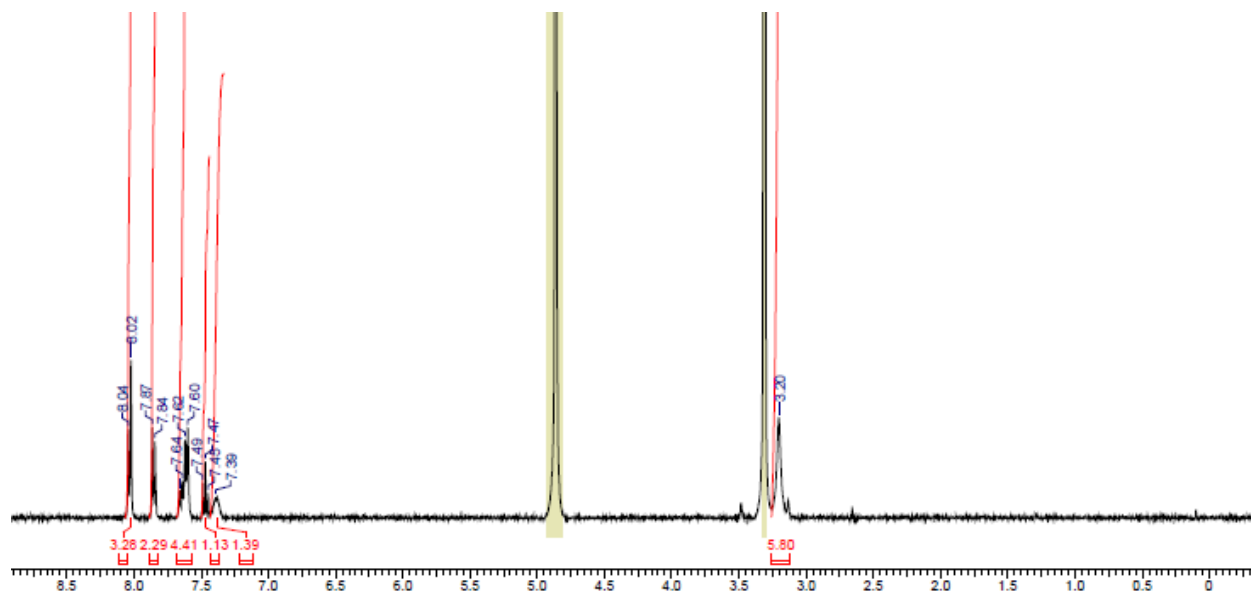


Figure S23: ^1H NMR of compound **23c**



LCMS of compound **24a**

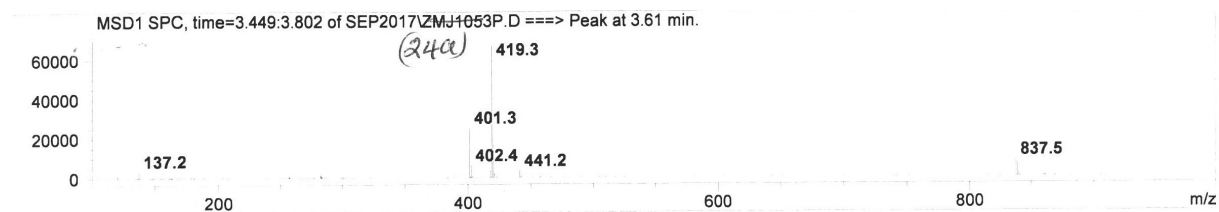
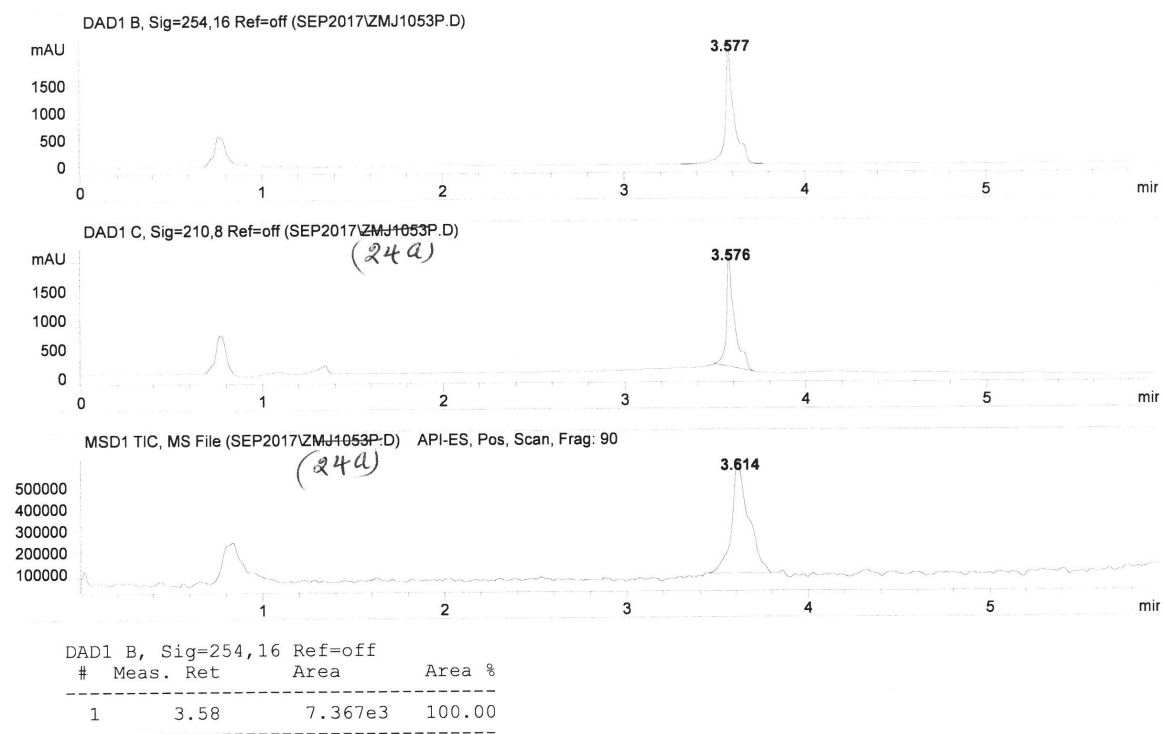
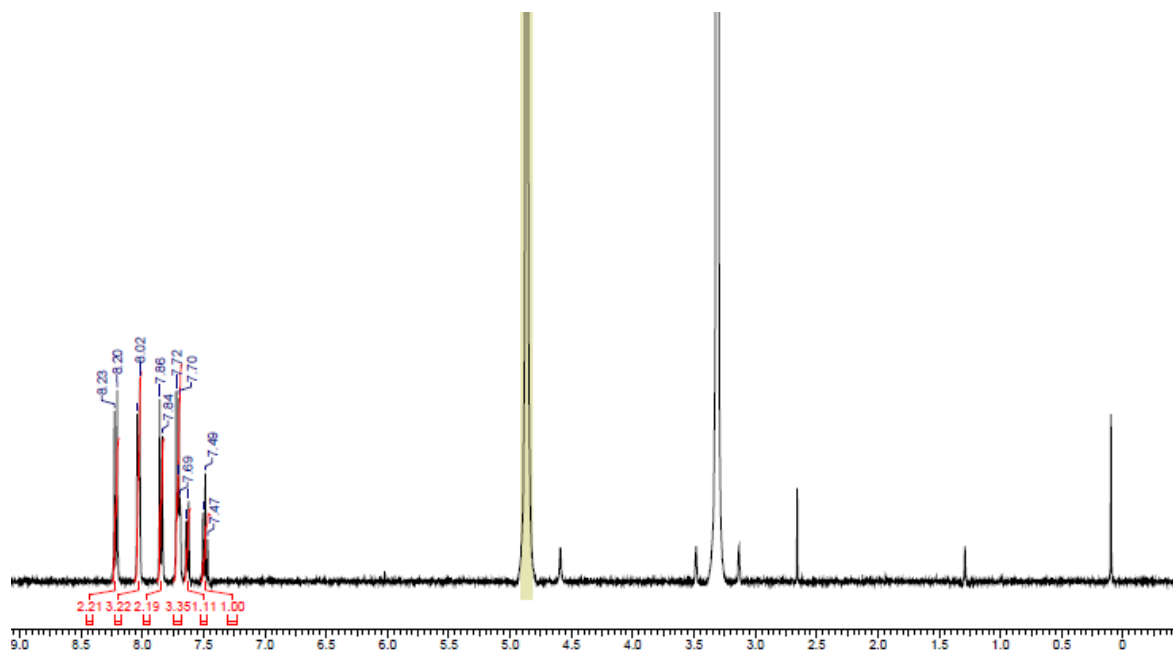
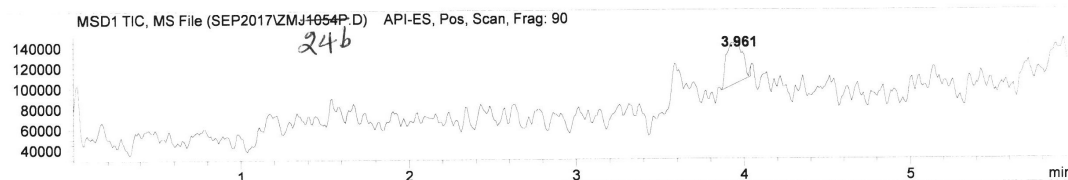
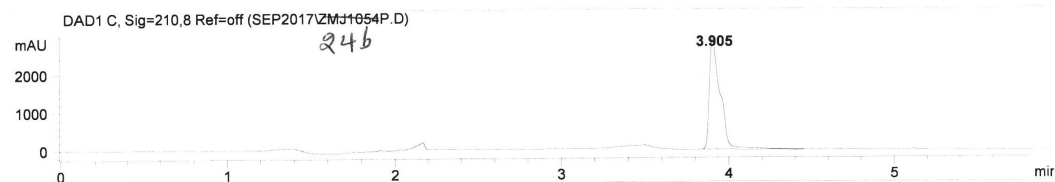
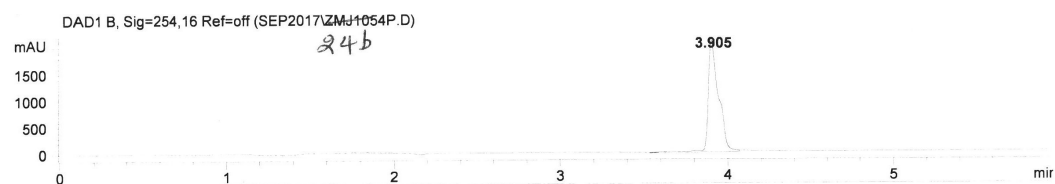


Figure S24: ^1H NMR of compound **24a**



LCMS of compound **24b**



DAD1 B, Sig=254,16 Ref=off

#	Meas. Ret	Area	Area %
1	3.90	8.977e3	100.00

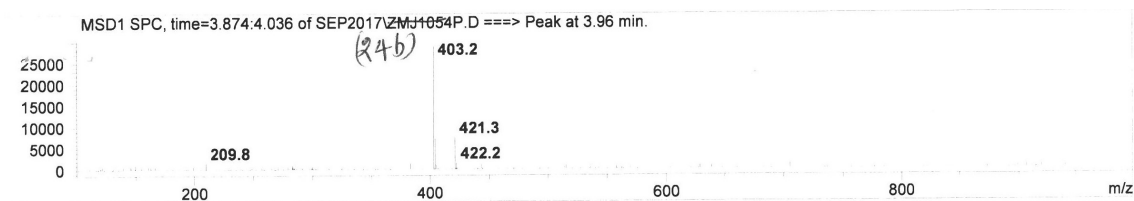
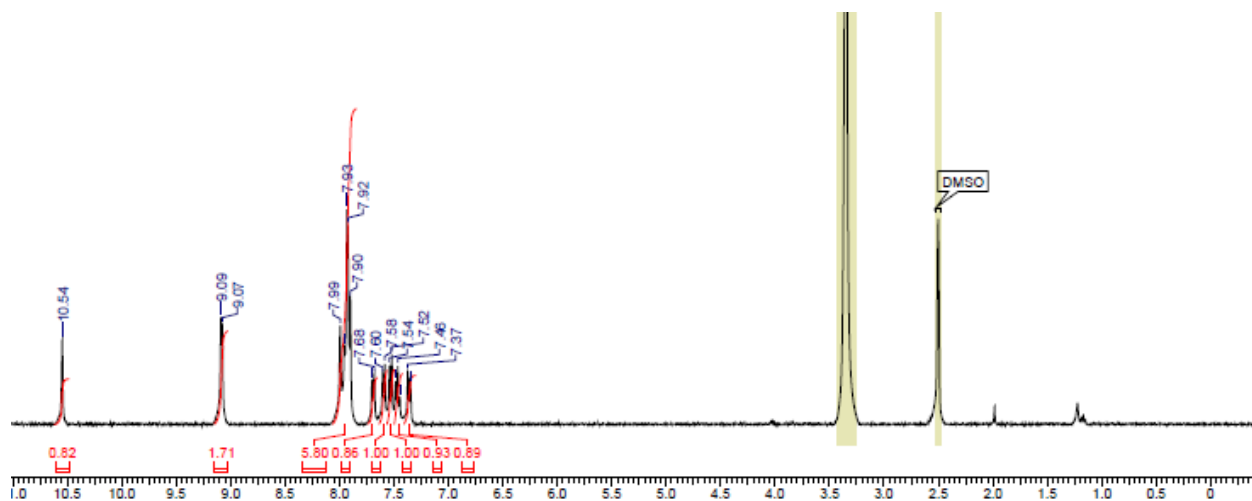


Figure S25: ^1H NMR of compound **24b**



LCMS of compound 24c

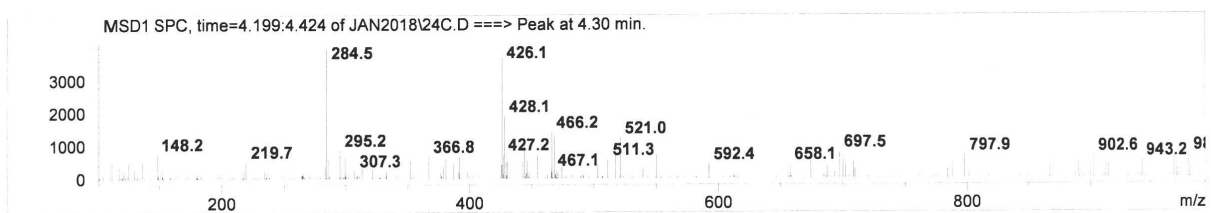
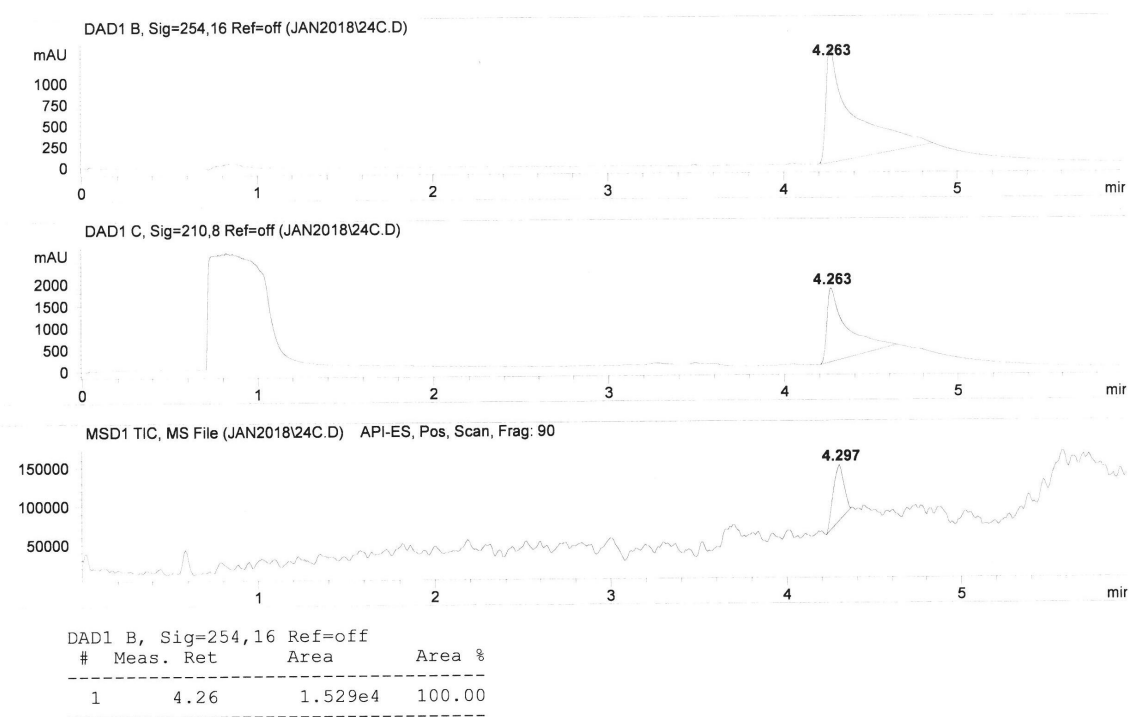


Figure S26: ^1H NMR of compound 24c

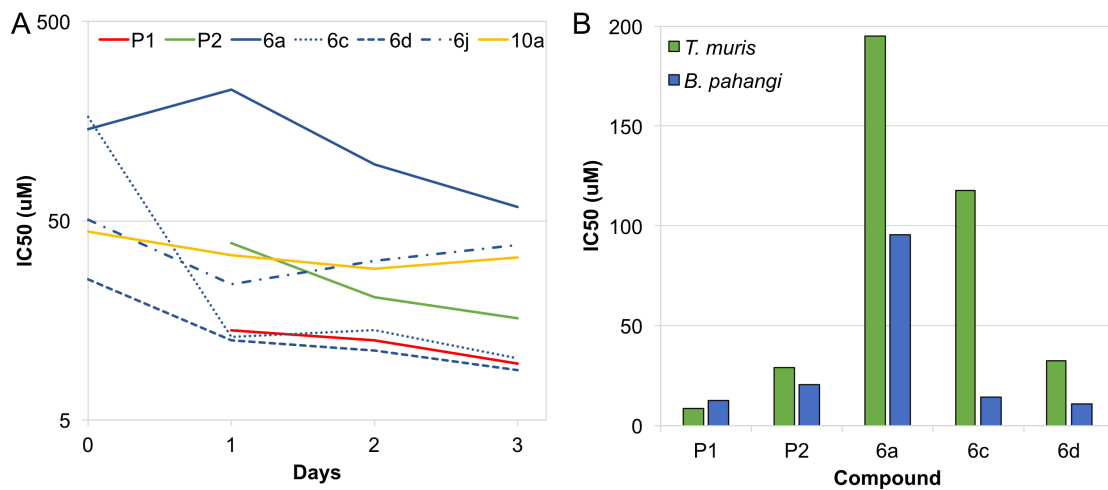


Figure S27: Time- and species- dependence of the IC₅₀ values. A) *Brugia pahangi* IC₅₀ values for seven compounds over three days of exposure; B) *Trichuris muris* and *B. pahangi* IC₅₀ values for five compounds at day 2.

Bioaccumulation Analysis Figures

Samples are from either *B. pahangi* or *T. muris* as indicated in upper right hand corner. Figure number and compound names are in upper right hand corner, as well as protein concentration of the sample. Molecular structure, formula, and exact mass are located at the bottom of each figure.

Mass spectrograms contain four panels (except DMSO):

- A) Absorbance of 254 nm UV
- B) Absorbance of 210 nm UV
- C) Total ion chromatogram
- D) Extracted ion chromatogram of $[M+H]^+$ for test compound (except DMSO)

When compound identified to be present, the MS time scan follows. In some cases the compound detection is below the limit of detection and not included.

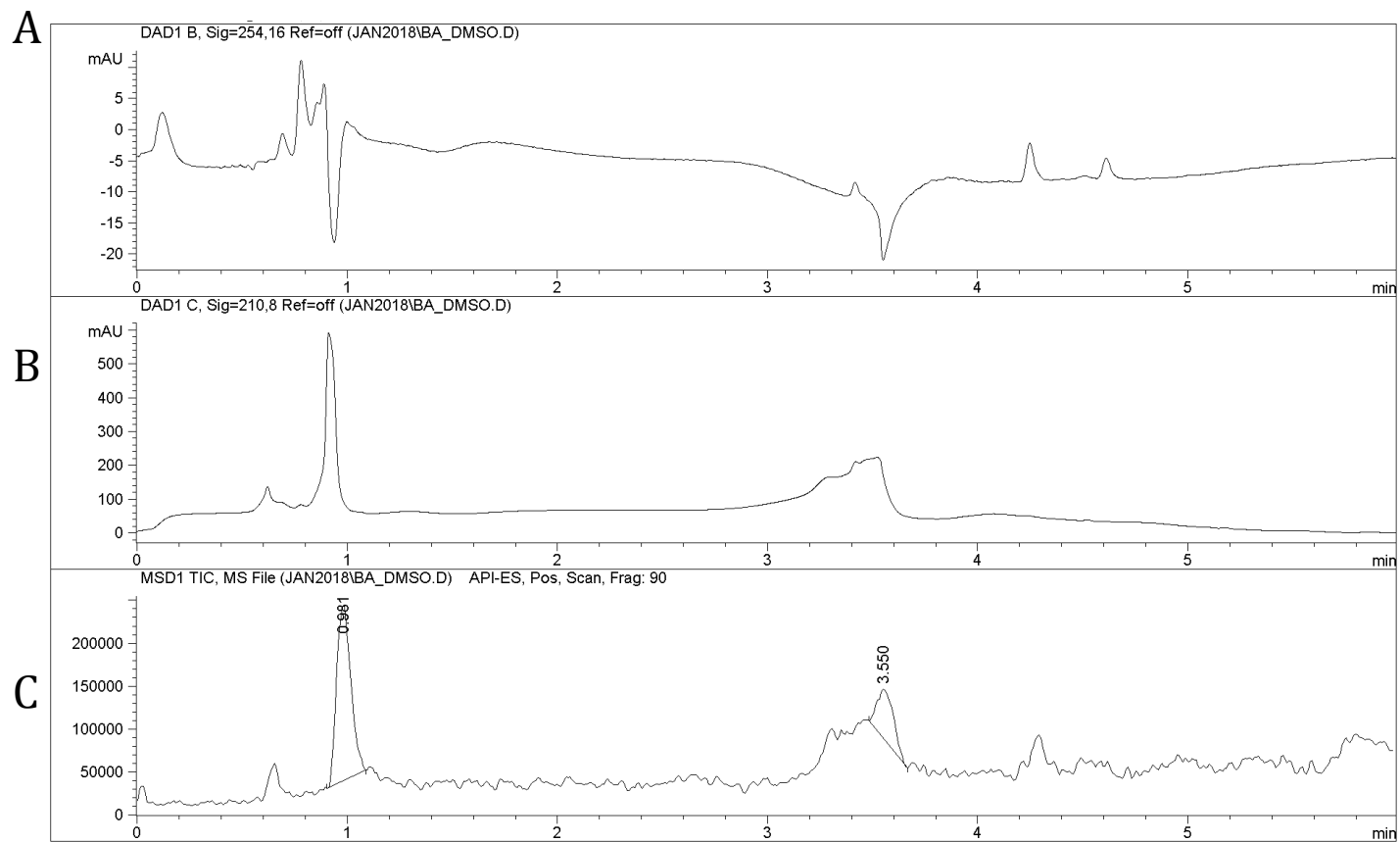


Figure S28:

B. pahangi DMSO
4.52 mg/mL [protein]
8 worms per sample

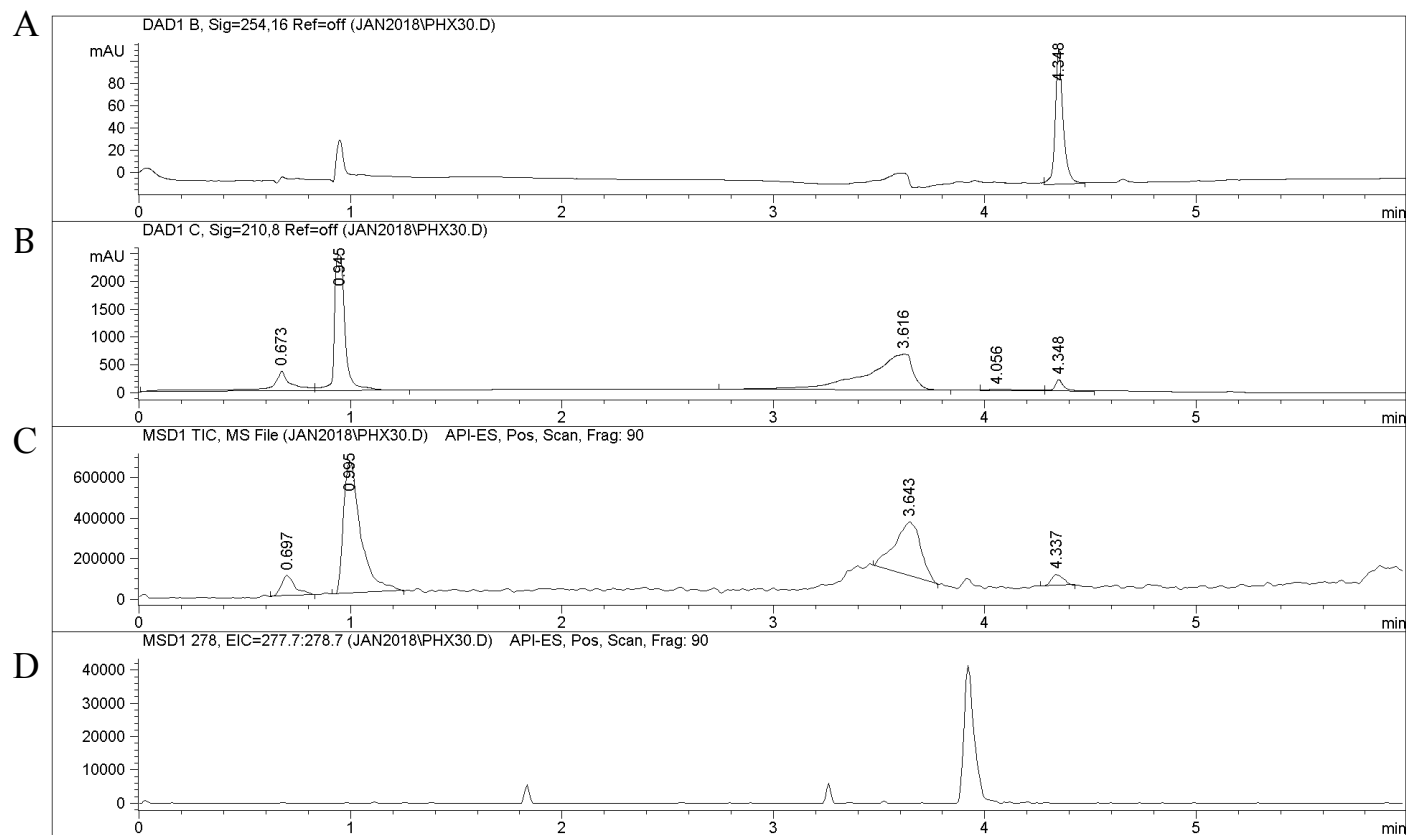
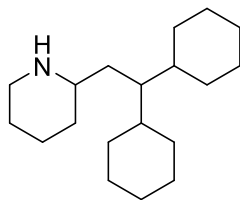
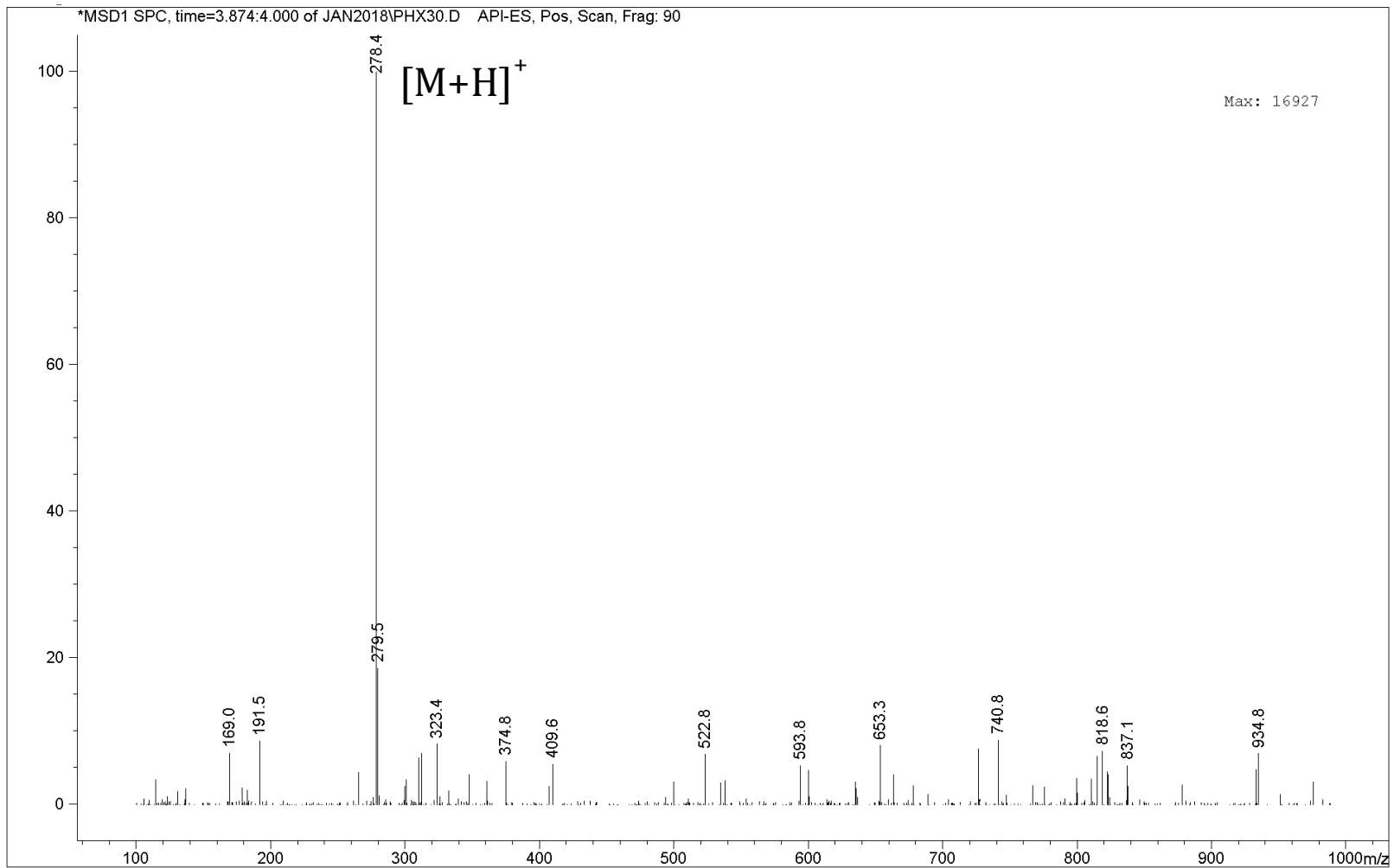


Figure S29:
B. pahangi
Compound P1
 1.14 mg/mL [protein]



Chemical Formula: C₁₉H₃₅N
 Exact Mass: 277.28

*MSD1 SPC, time=3.874:4.000 of JAN2018\PHX30.D API-ES, Pos, Scan, Frag: 90



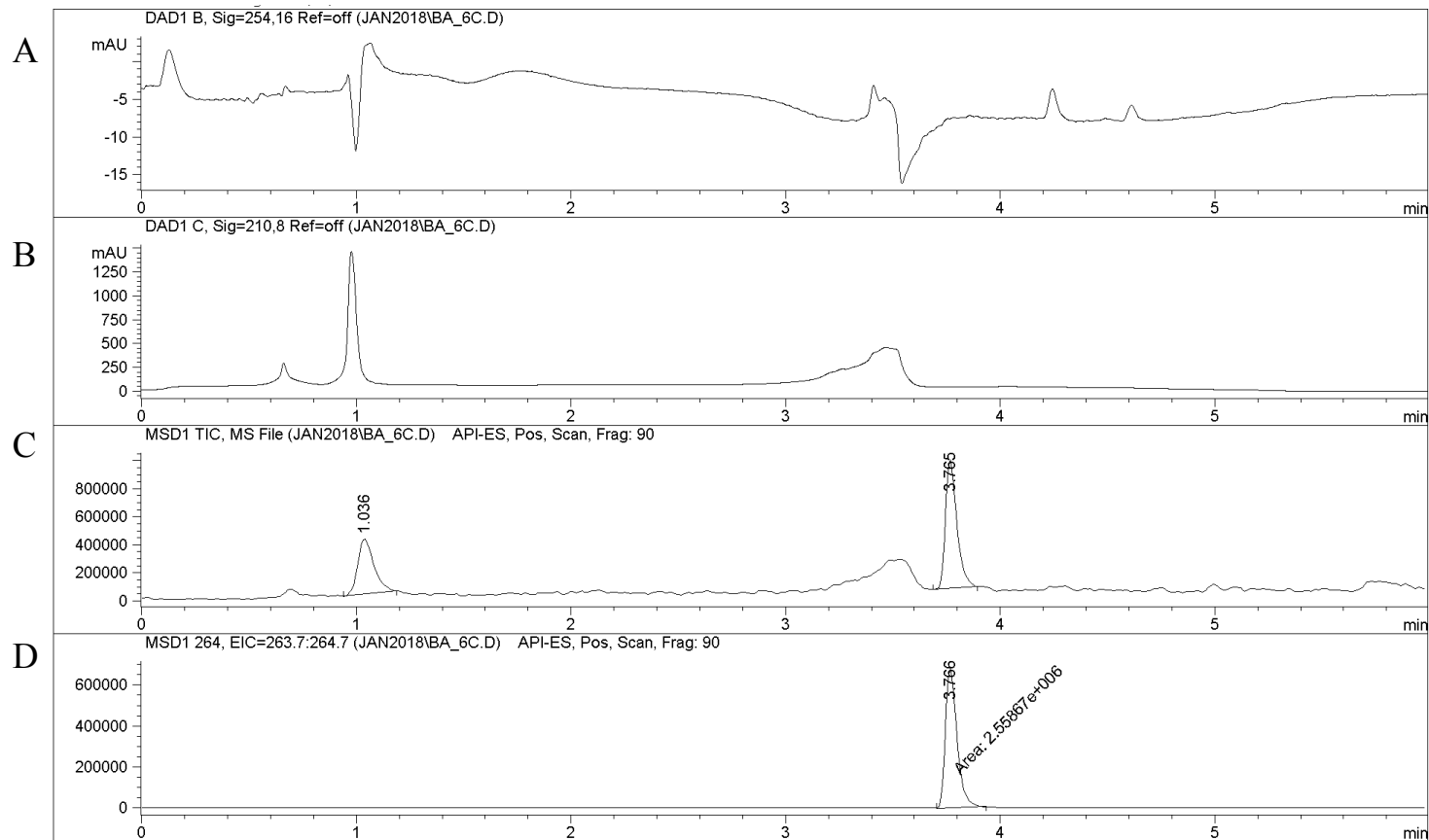
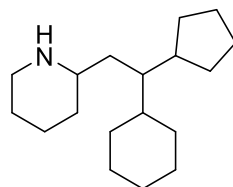
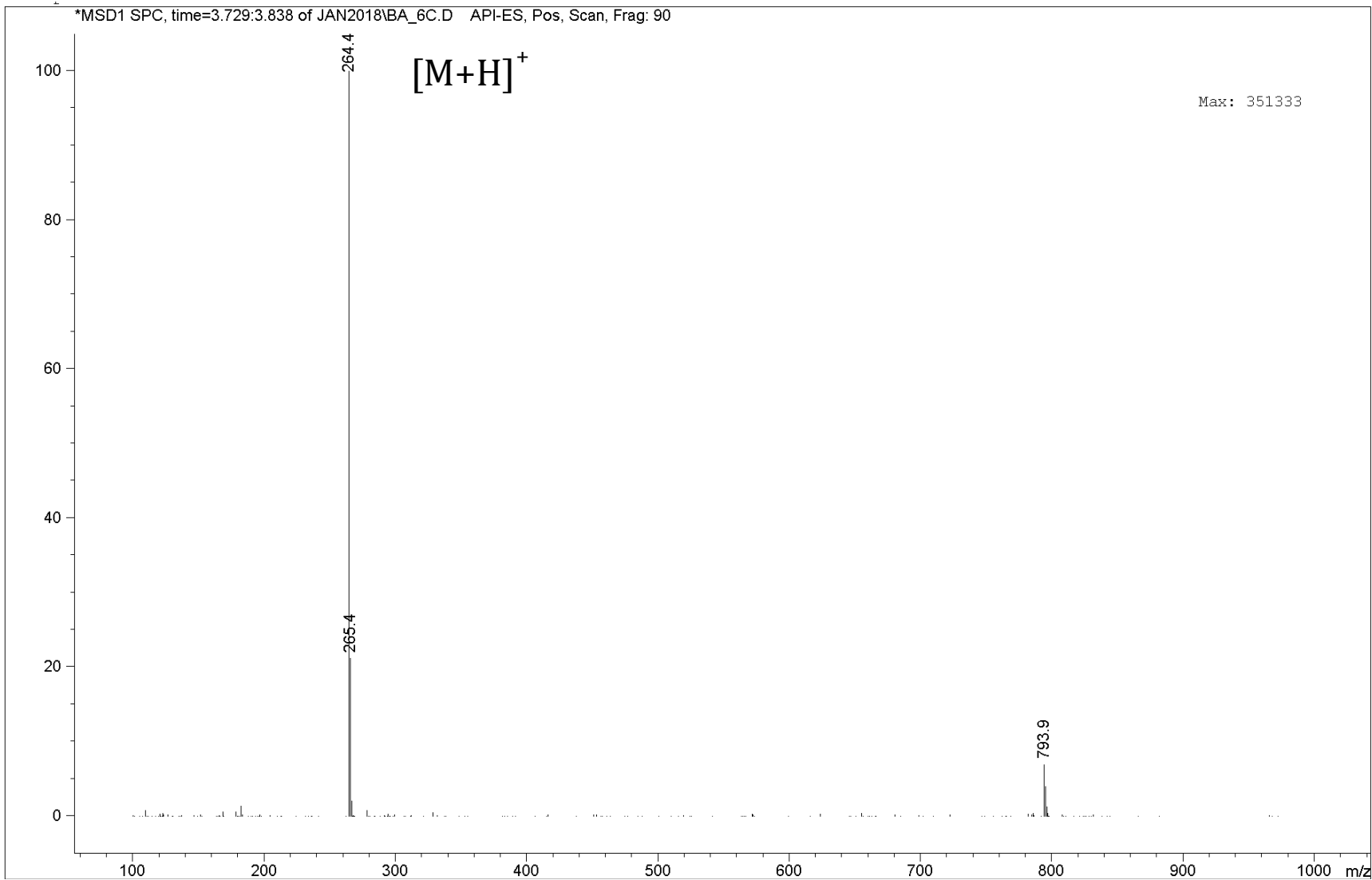


Figure S30:
B. pahangi
Compound P1a
 4.06 mg/mL [protein]



Chemical Formula: C₁₈H₃₃N
 Exact Mass: 263.26
 S33

*MSD1 SPC, time=3.729:3.838 of JAN2018\BA_6C.D API-ES, Pos, Scan, Frag: 90



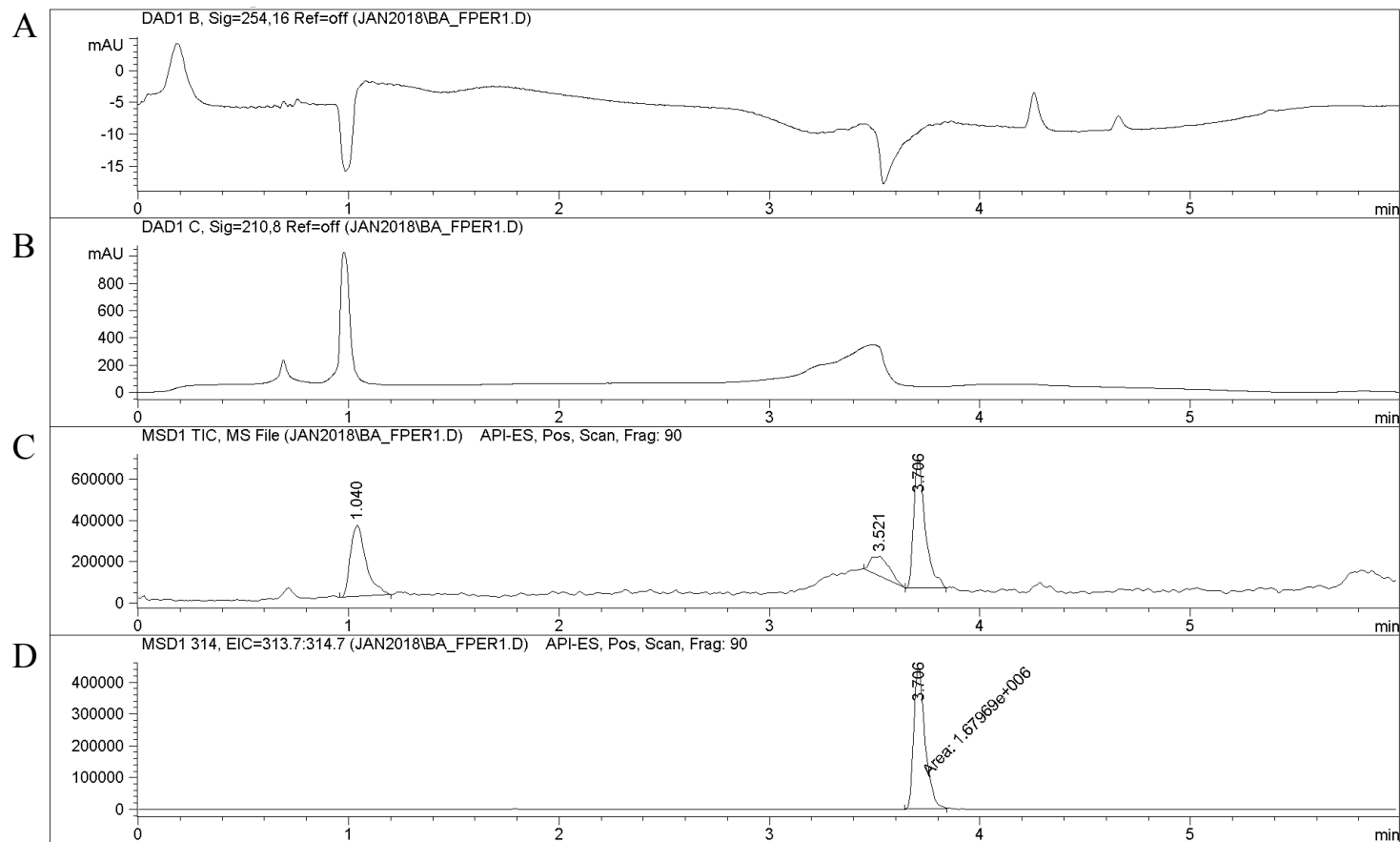
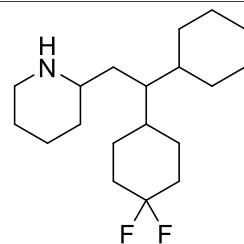


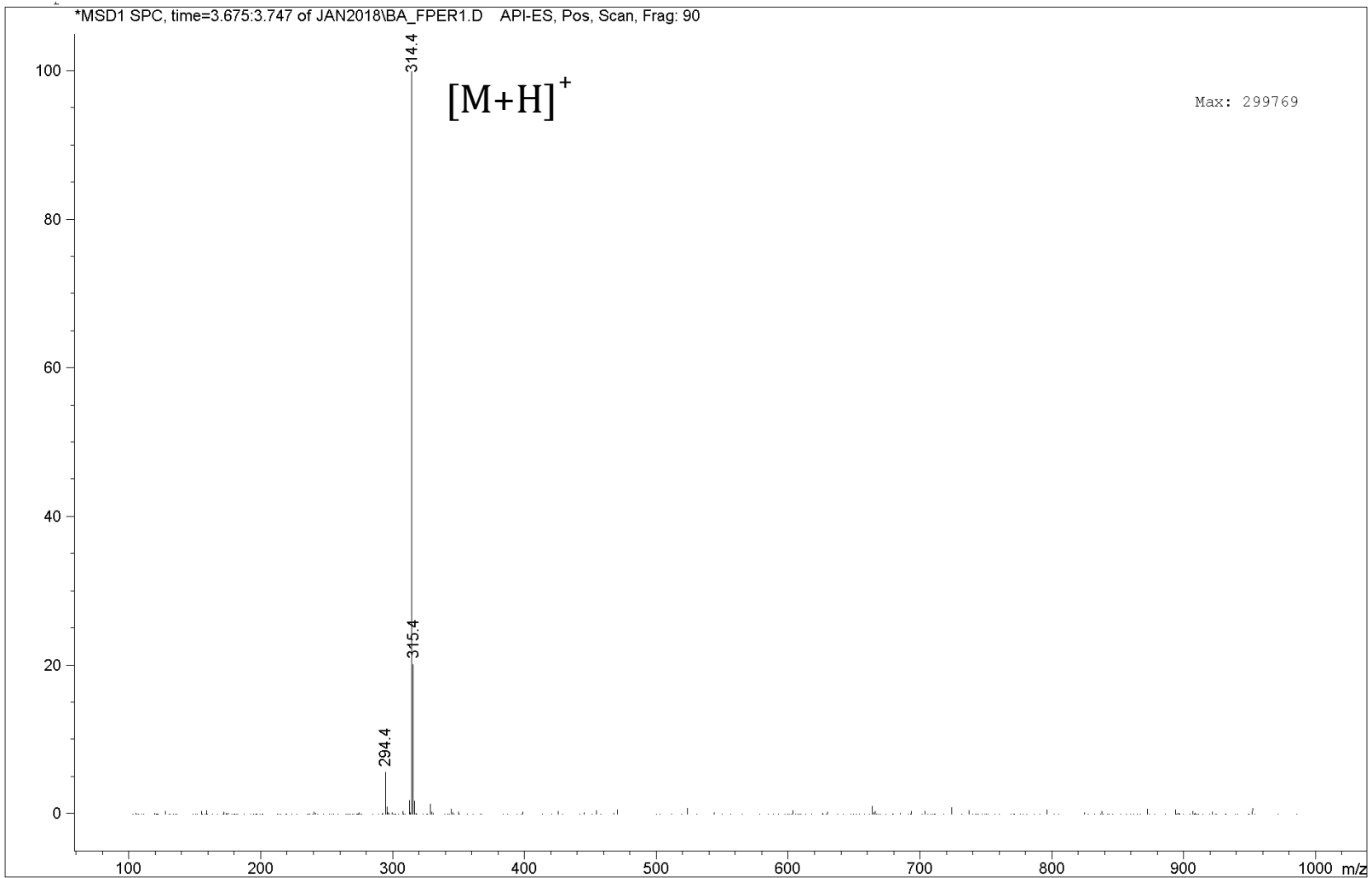
Figure S31:
B. Pahangi
Compound P1b
 3.05 mg/mL [protein]
 8 worms per sample



Chemical Formula: C₁₉H₃₃F₂N

Exact Mass: 313.26

*MSD1 SPC, time=3.675:3.747 of JAN2018\BA_FPER1.D API-ES, Pos, Scan, Frag: 90



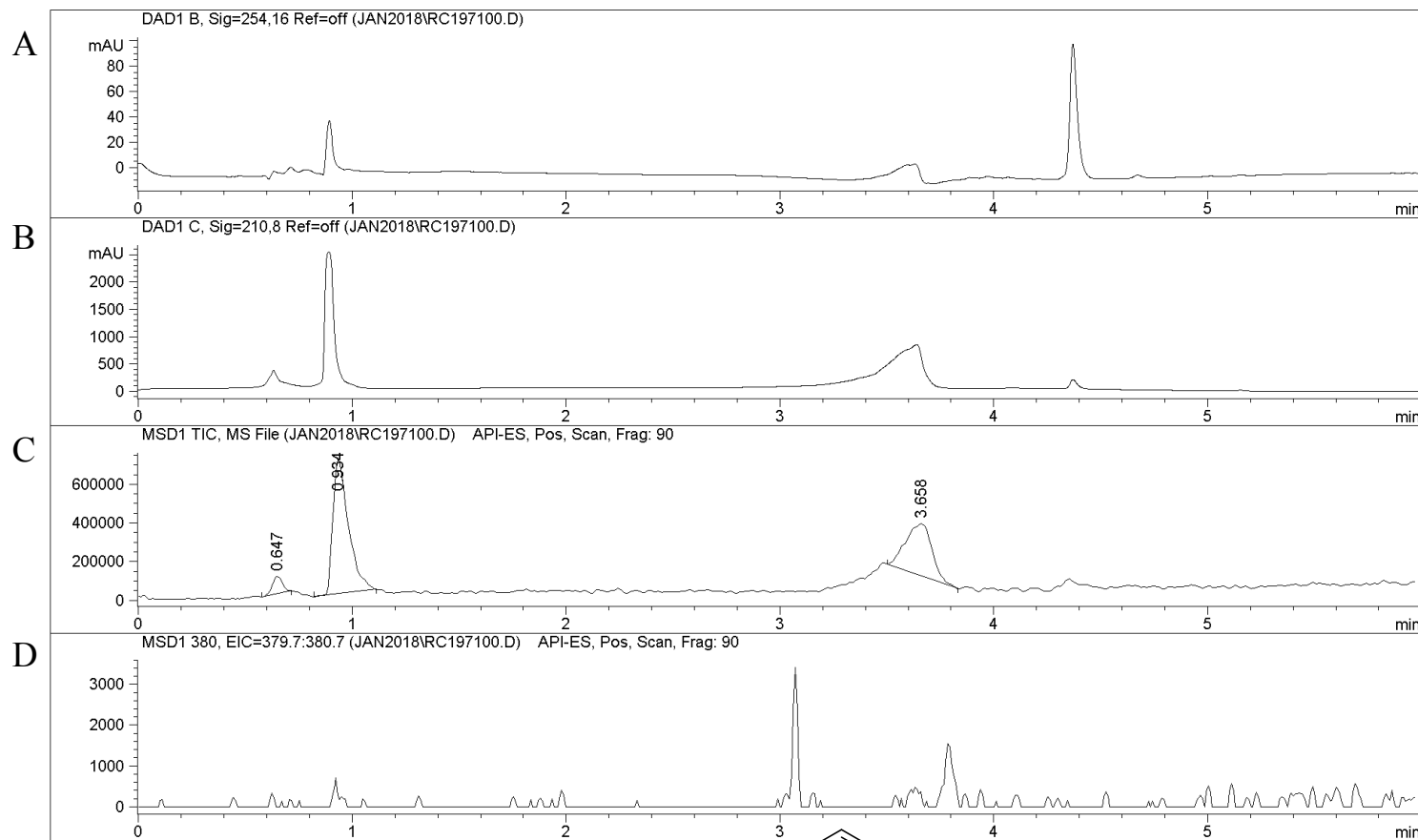
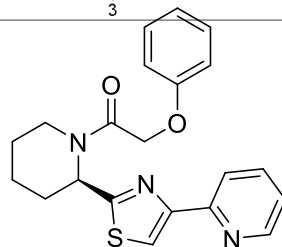


Figure S32:
B. Pahangi
Compound 6a
 1.37 mg/mL [protein]



Chemical Formula: C₂₁H₂₁N₃O₂S

Exact Mass: 379.14

S37

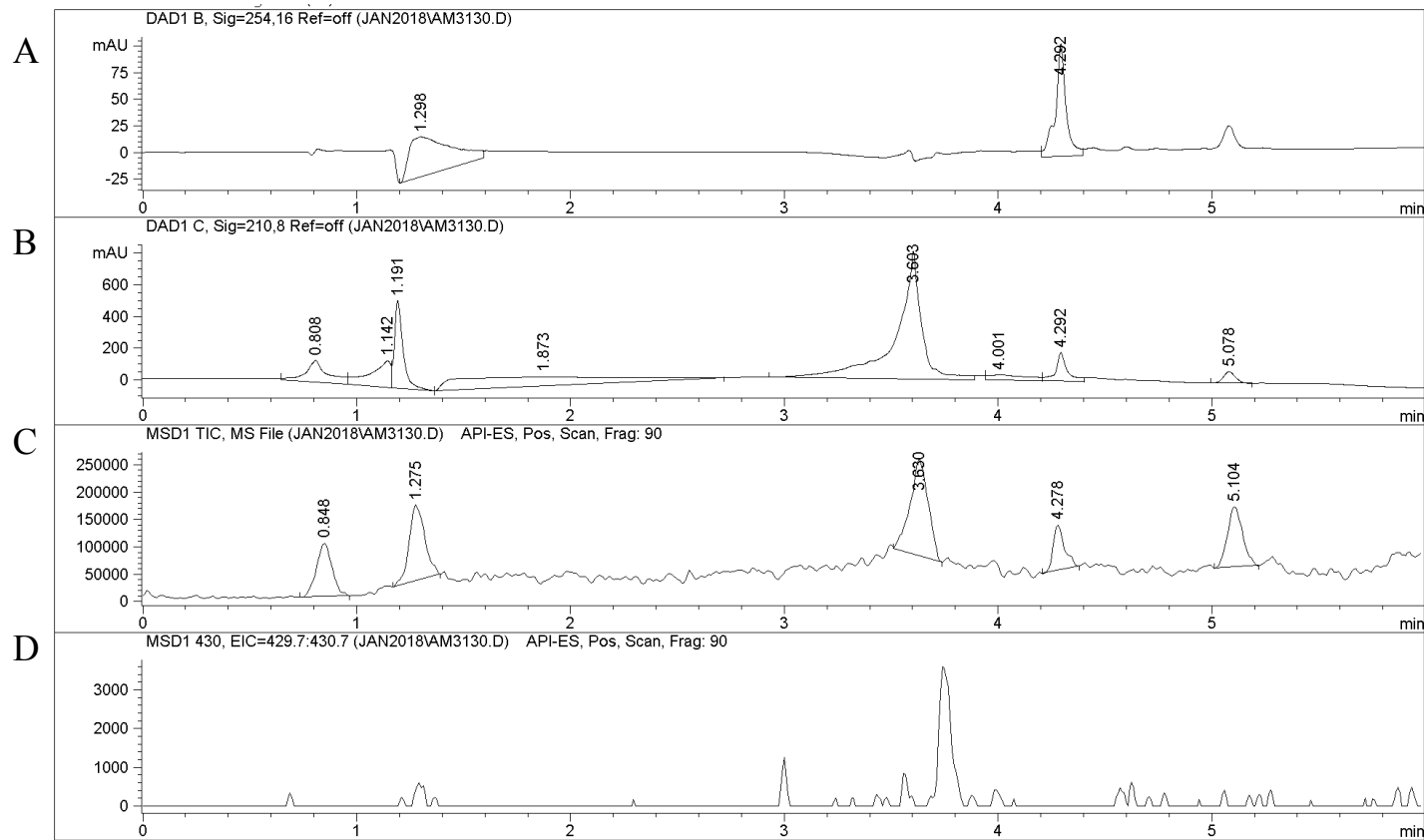
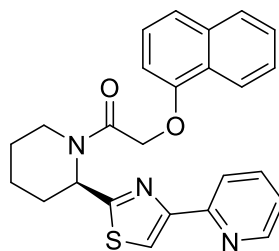


Figure S33:
B. Pahangi
Compound 6c
 1.14 mg/mL [protein]
 3 worms per sample



Chemical Formula: C₂₅H₂₃N₃O₂S

Exact Mass: 429.15

S38

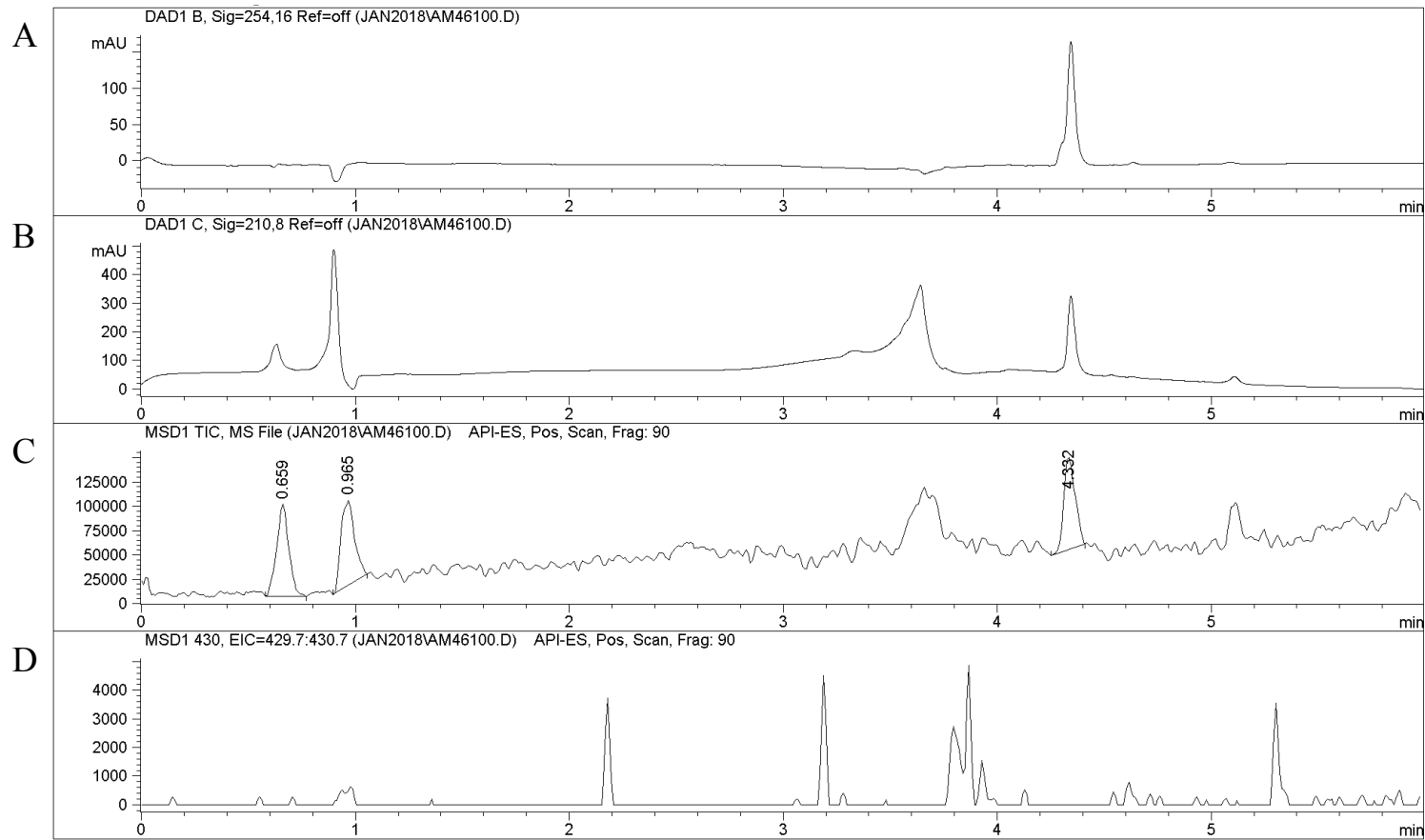
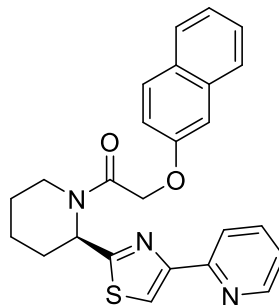


Figure S34:
B. Pahangi
Compound 6d
 0.97 mg/mL [protein]
 3 worms per sample



S39

Chemical Formula: C₂₅H₂₃N₃O₂S

Exact Mass: 429.15

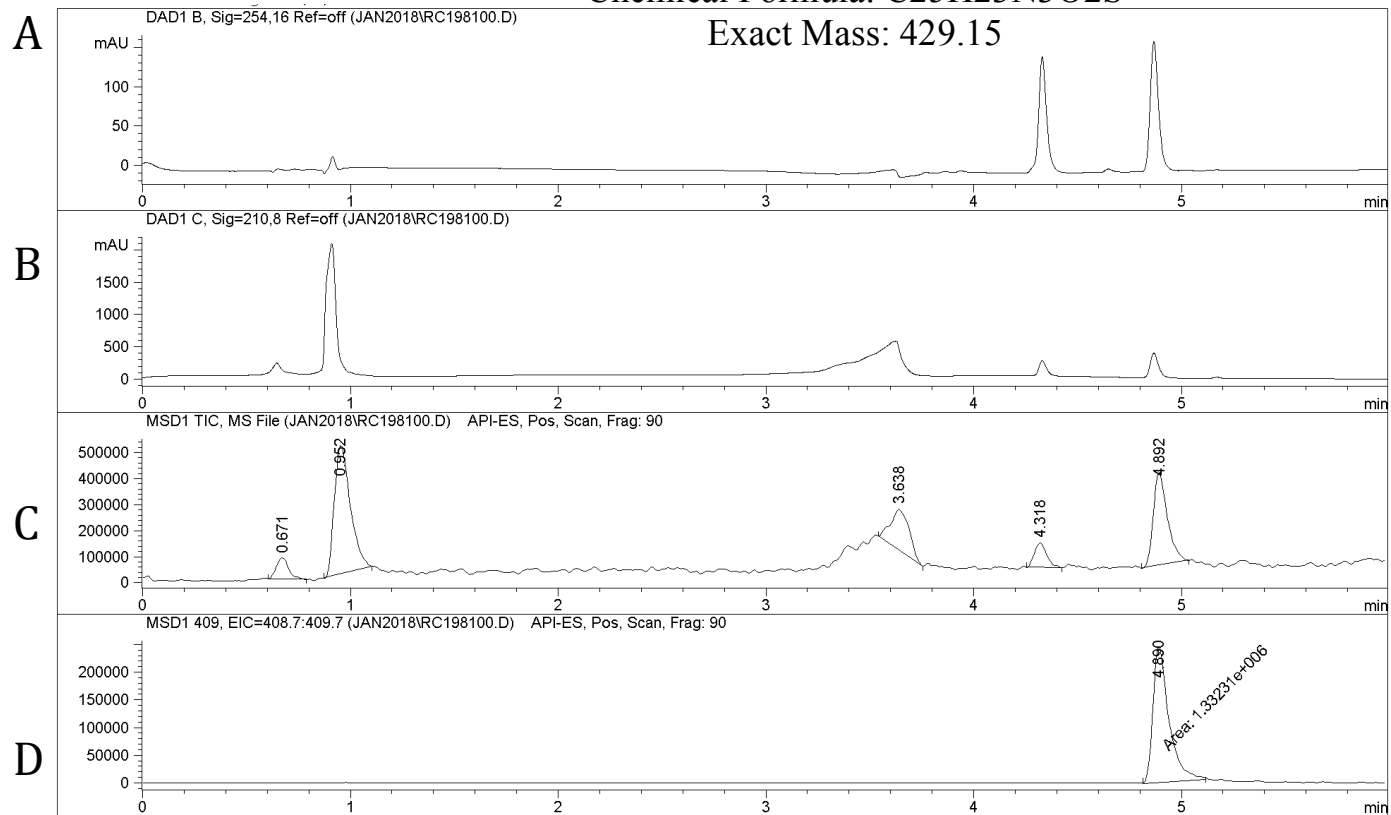
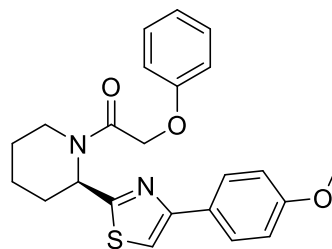


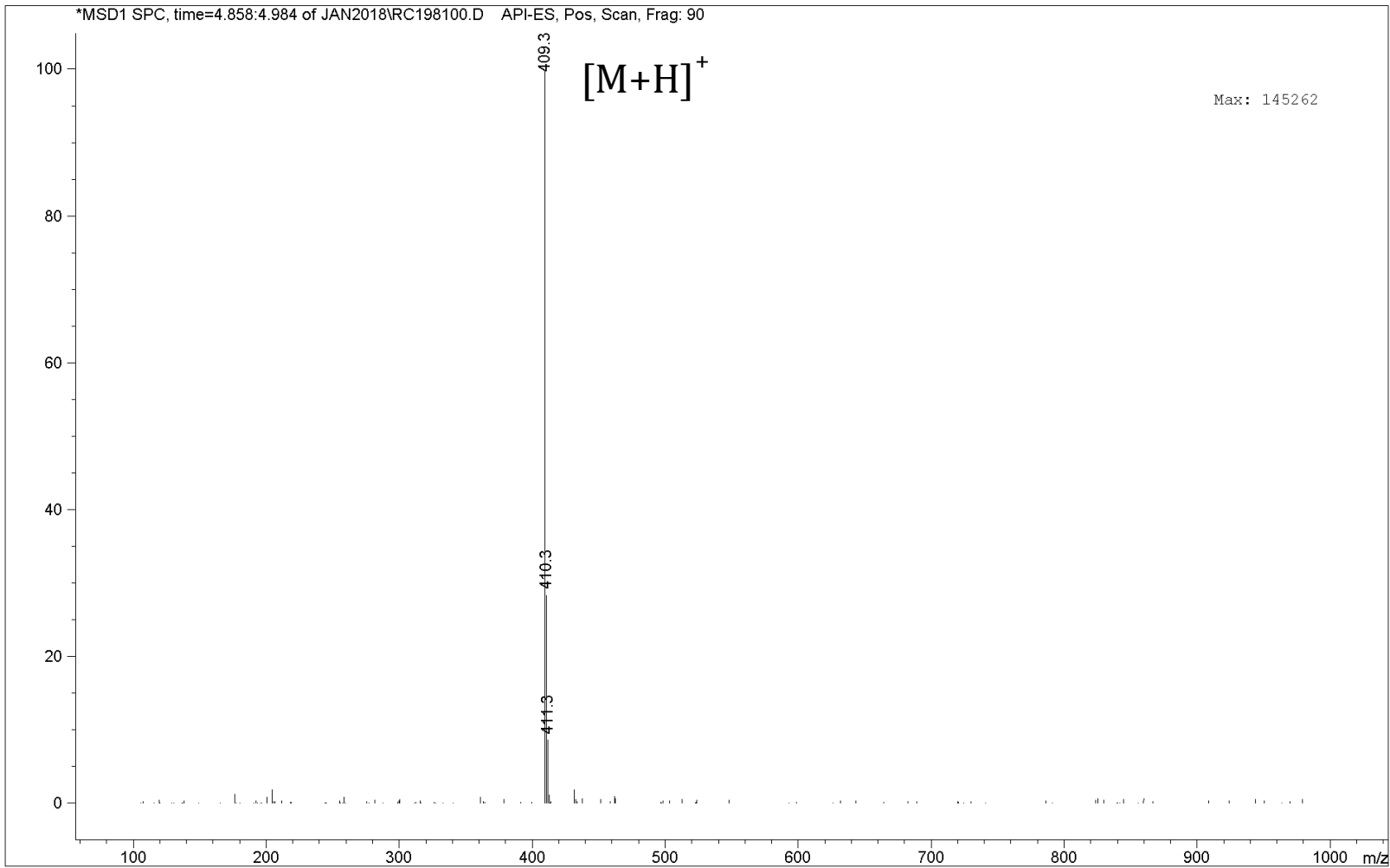
Figure S35:
B. Pahangi
Compound 6f



Chemical Formula: C₂₃H₂₄N₂O₃S

Exact Mass: 408.15

*MSD1 SPC, time=4.858:4.984 of JAN2018\RC198100.D API-ES, Pos, Scan, Frag: 90



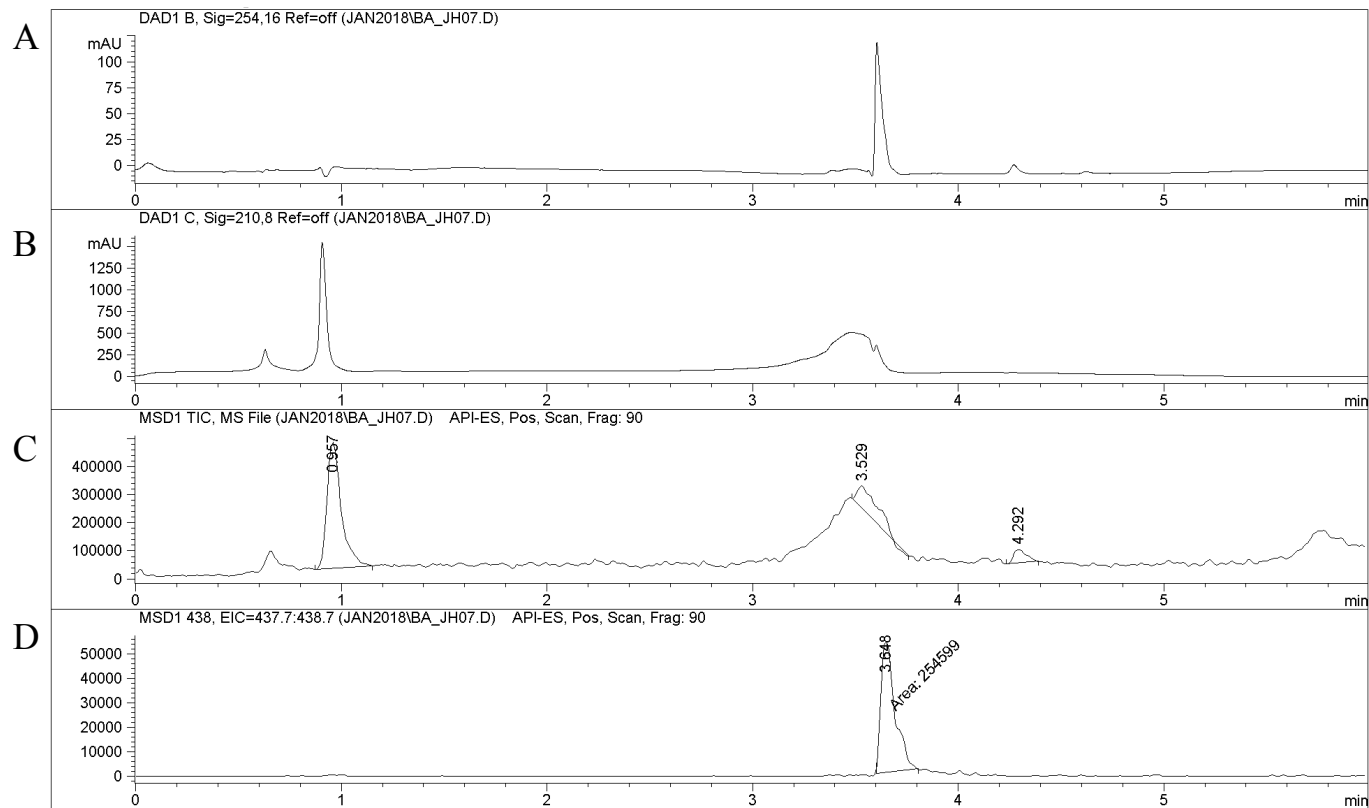
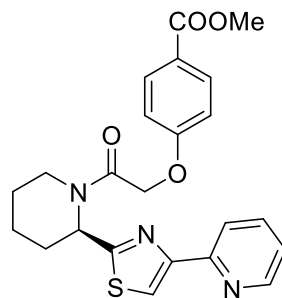


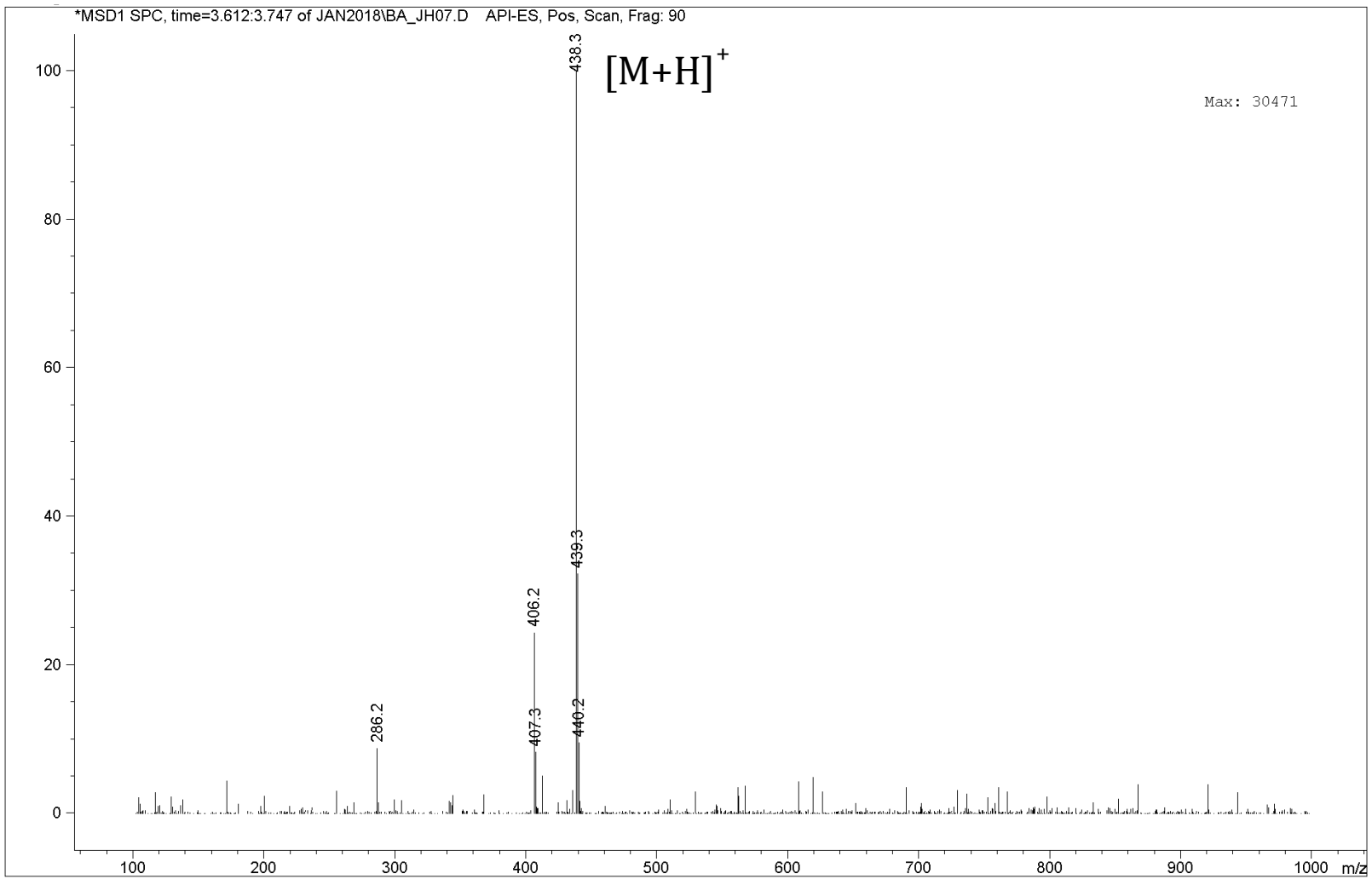
Figure S36:
B. Pahangi
Compound 6l
 2.32 mg/mL [protein]
 8 worms per sample



Chemical Formula: $C_{23}H_{23}N_3O_4S$

Exact Mass: 437.14
 S42

*MSD1 SPC, time=3.612:3.747 of JAN2018\BA_JH07.D API-ES, Pos, Scan, Frag: 90



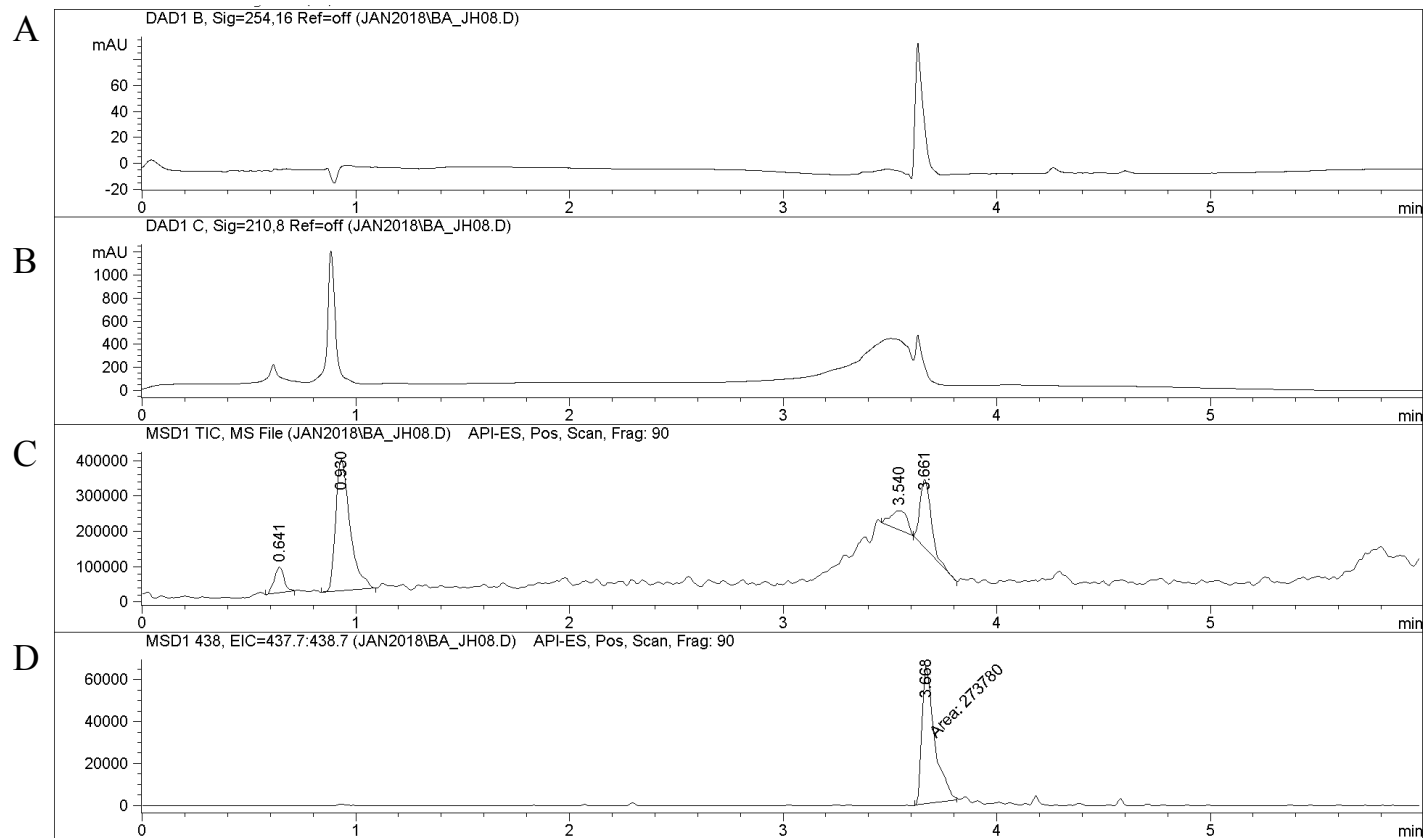
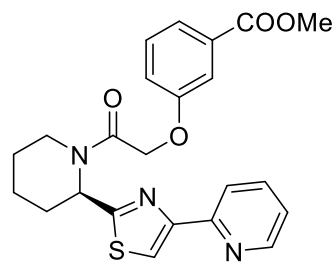


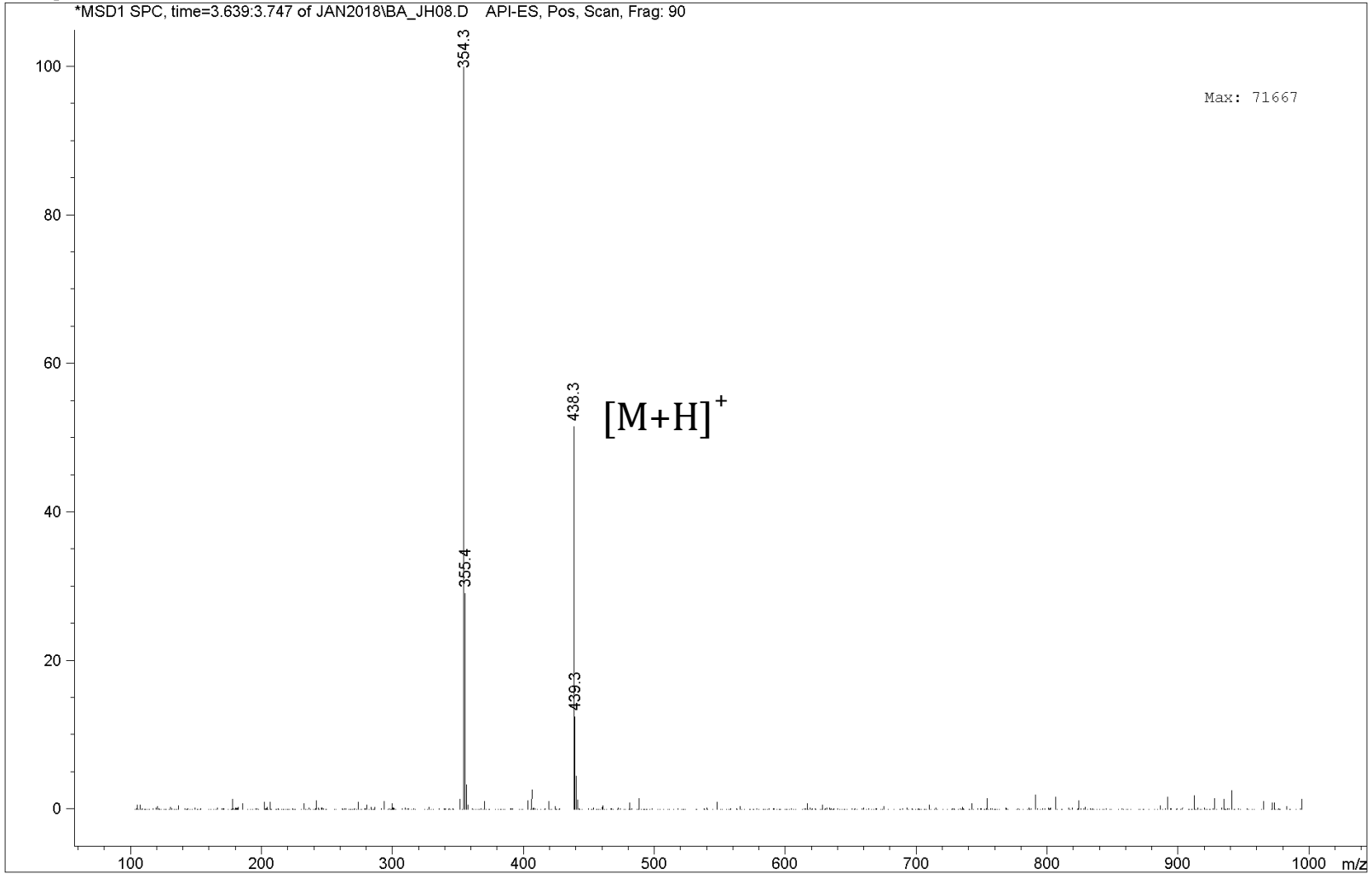
Figure S37:
B. Pahangi
Compound 6m
 2.75 mg/mL [protein]
 8 worms per sample



Chemical Formula: $C_{23}H_{23}N_3O_4S$

Exact Mass: 437.14

*MSD1 SPC, time=3.639:3.747 of JAN2018\BA_JH08.D API-ES, Pos, Scan, Frag: 90



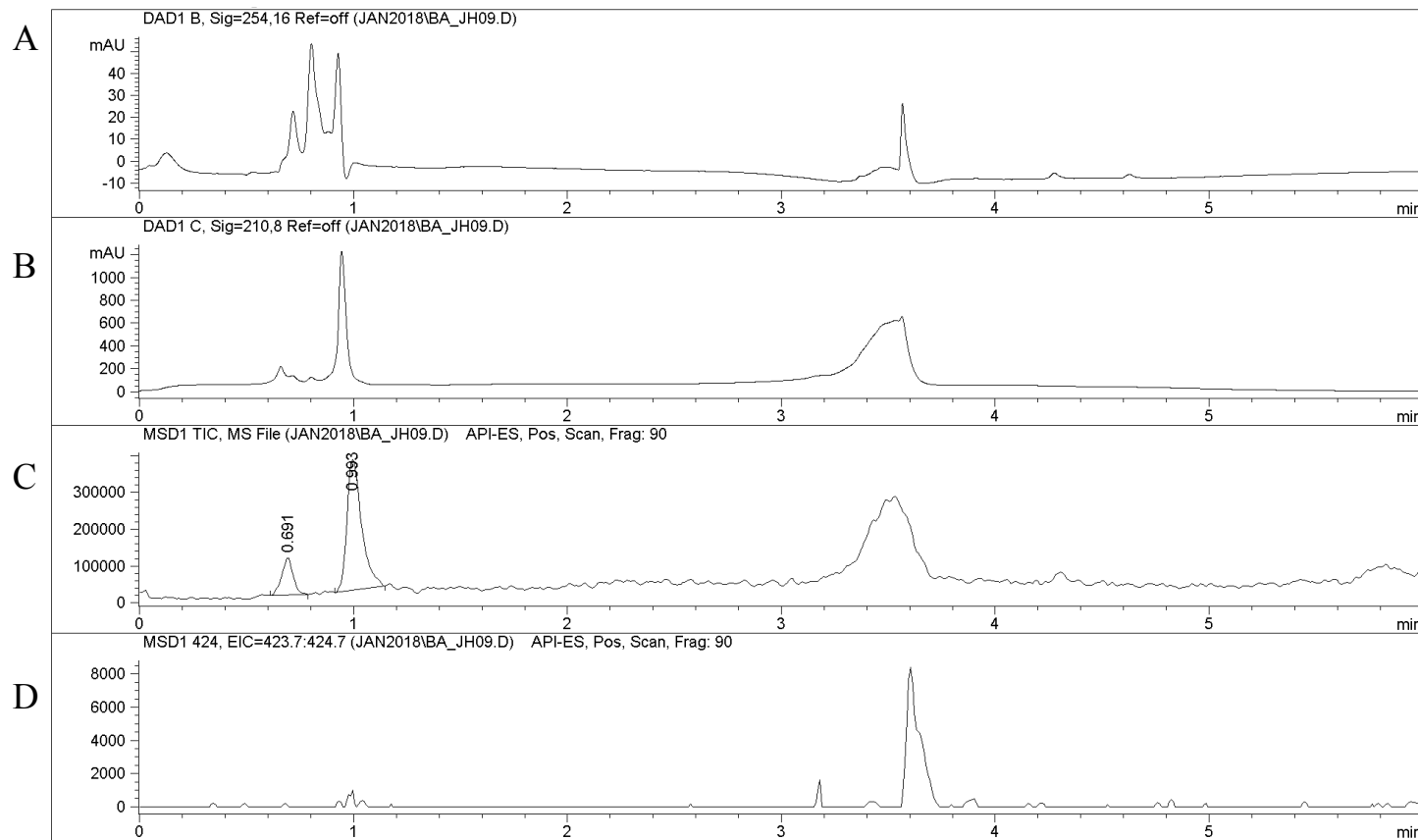
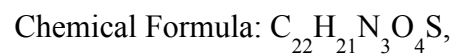
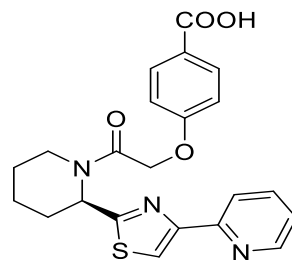
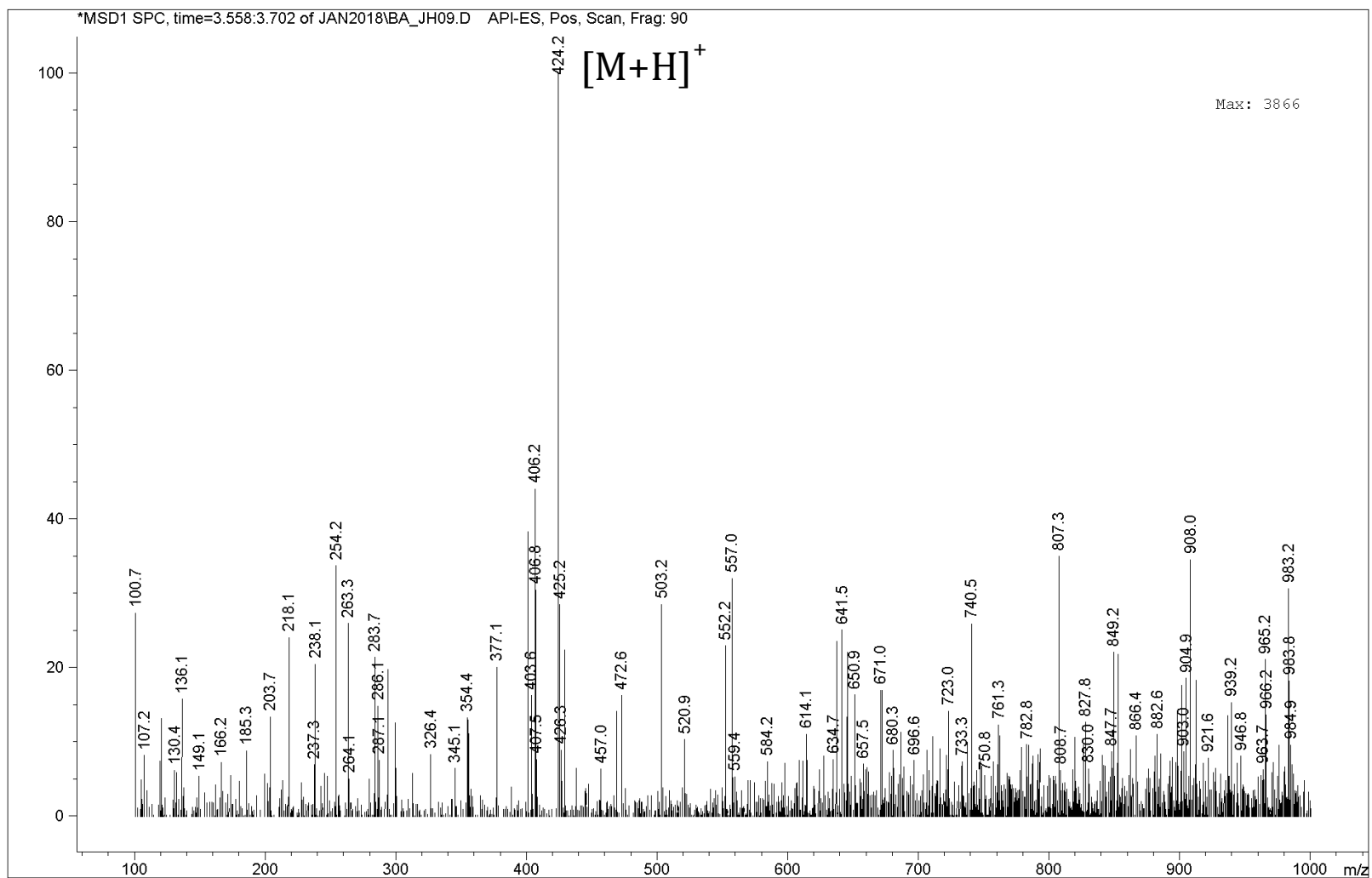


Figure S38:
B. Pahangi
Compound 6n
 5.47 mg/mL [protein]



Exact Mass: 423.13



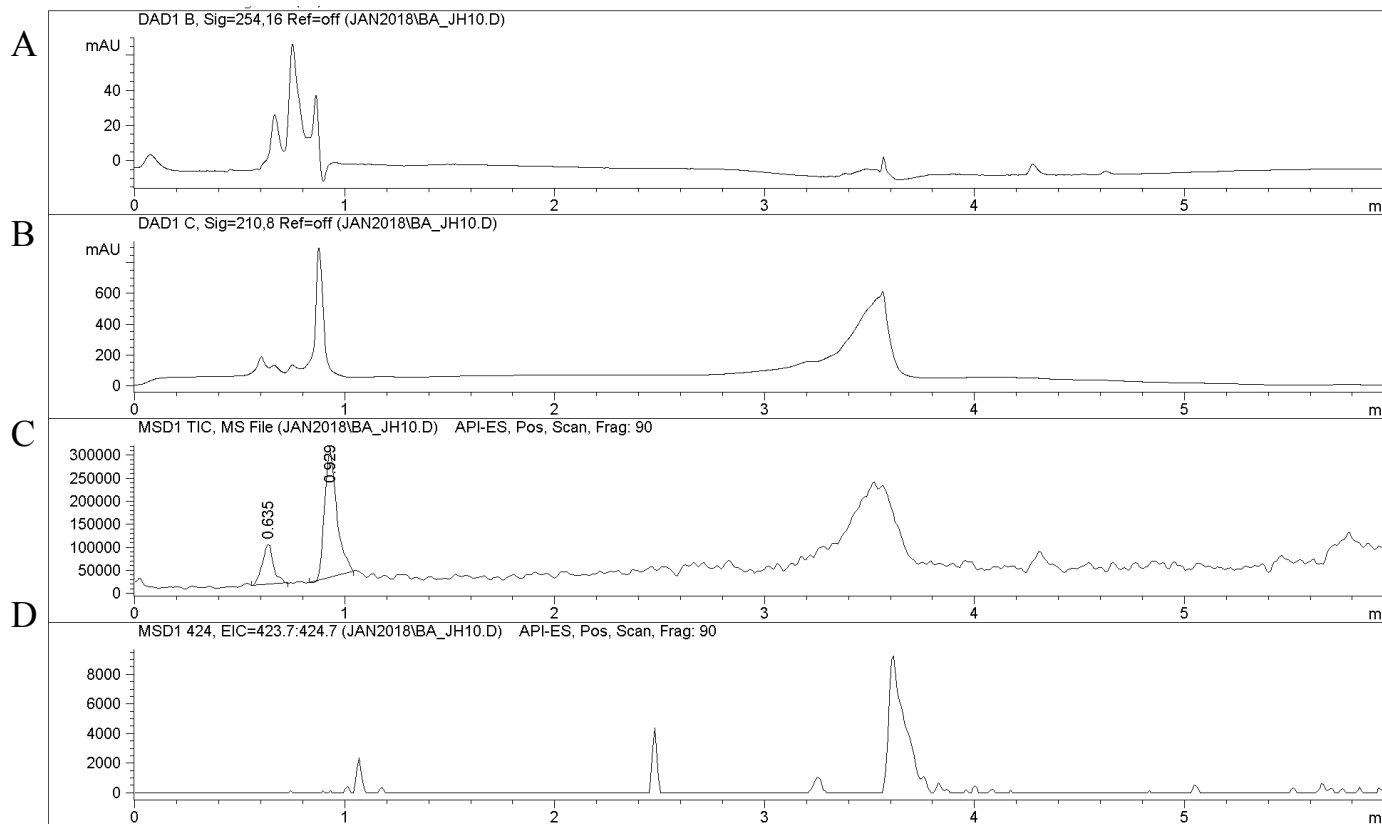
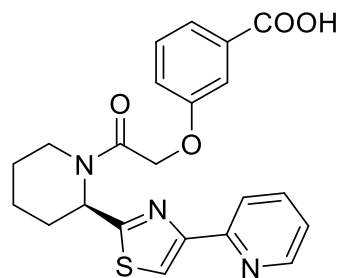
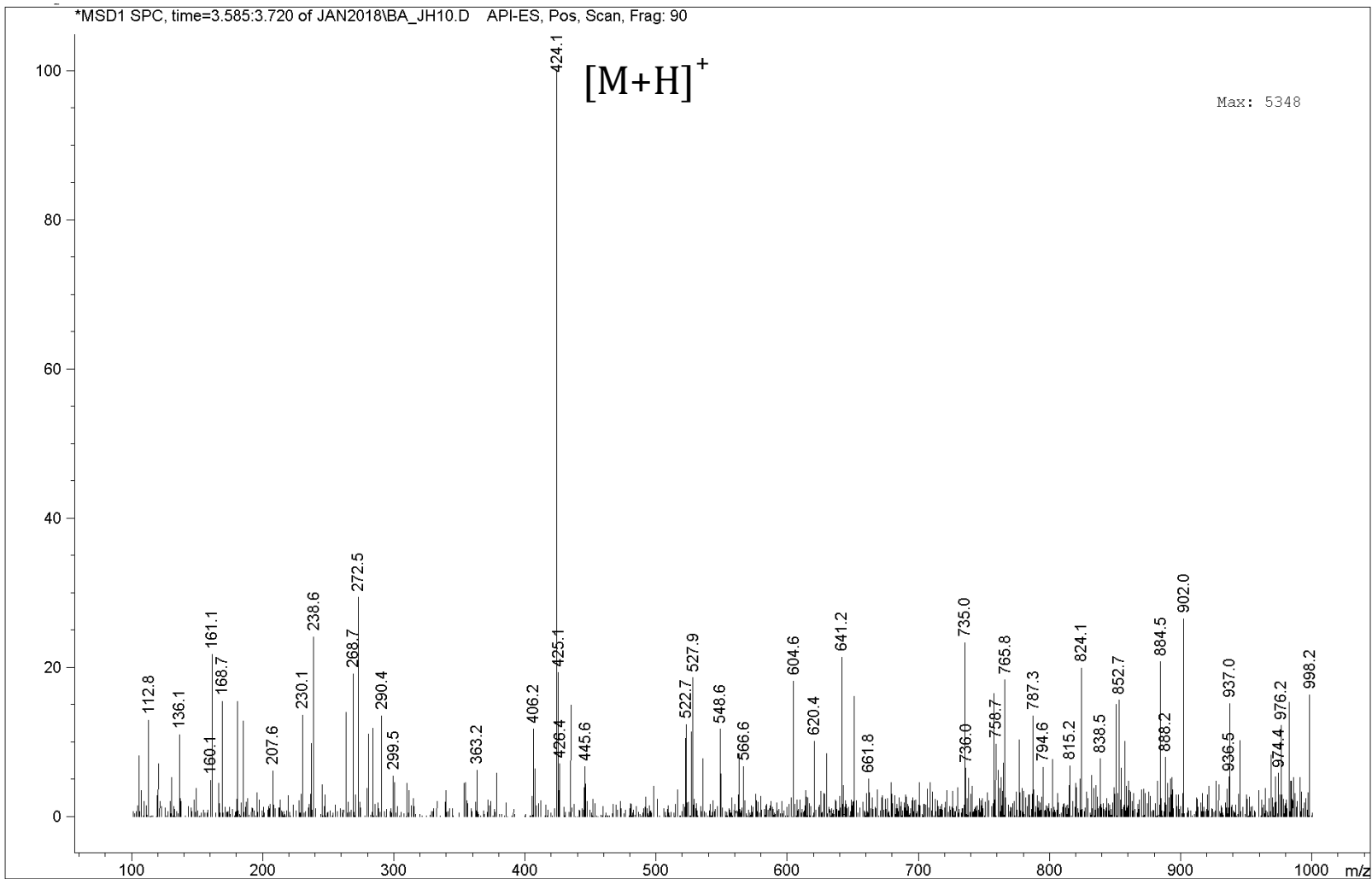


Figure S39:
B. Pahangi
Compound 60
 3.33 mg/mL [protein]



Chemical Formula: $C_{22}H_{21}N_3O_4S$
 Exact Mass: 423.13
 S48

*MSD1 SPC, time=3.585:3.720 of JAN2018\BA_JH10.D API-ES, Pos, Scan, Frag: 90



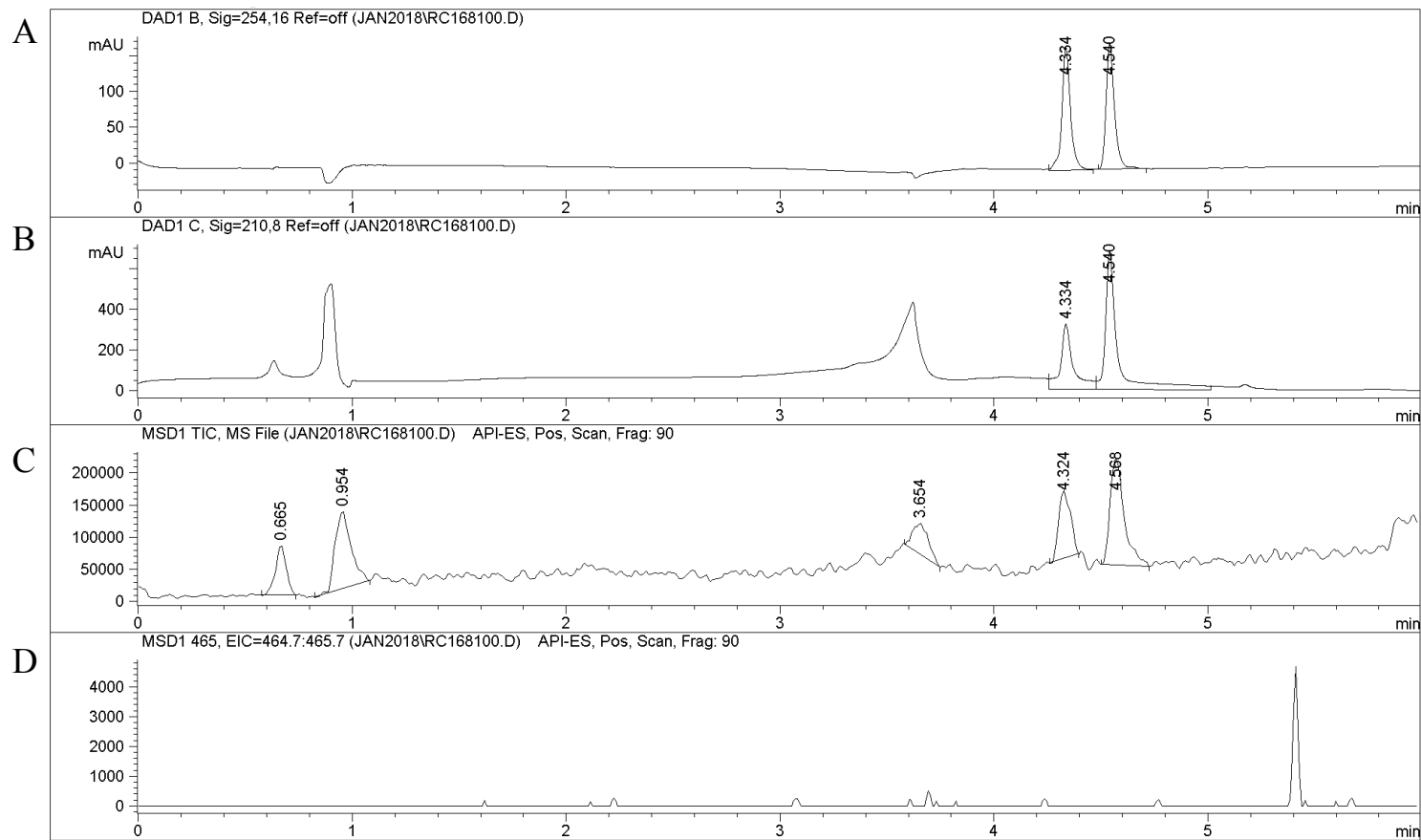
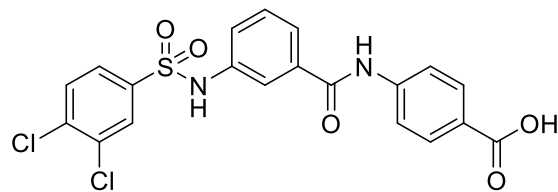


Figure S40:
B. Pahangi
Compound 10a
 1.16 mg/mL [protein]



Chemical Formula: C₂₀H₁₄Cl₂N₂O₅S

Exact Mass: 464.00

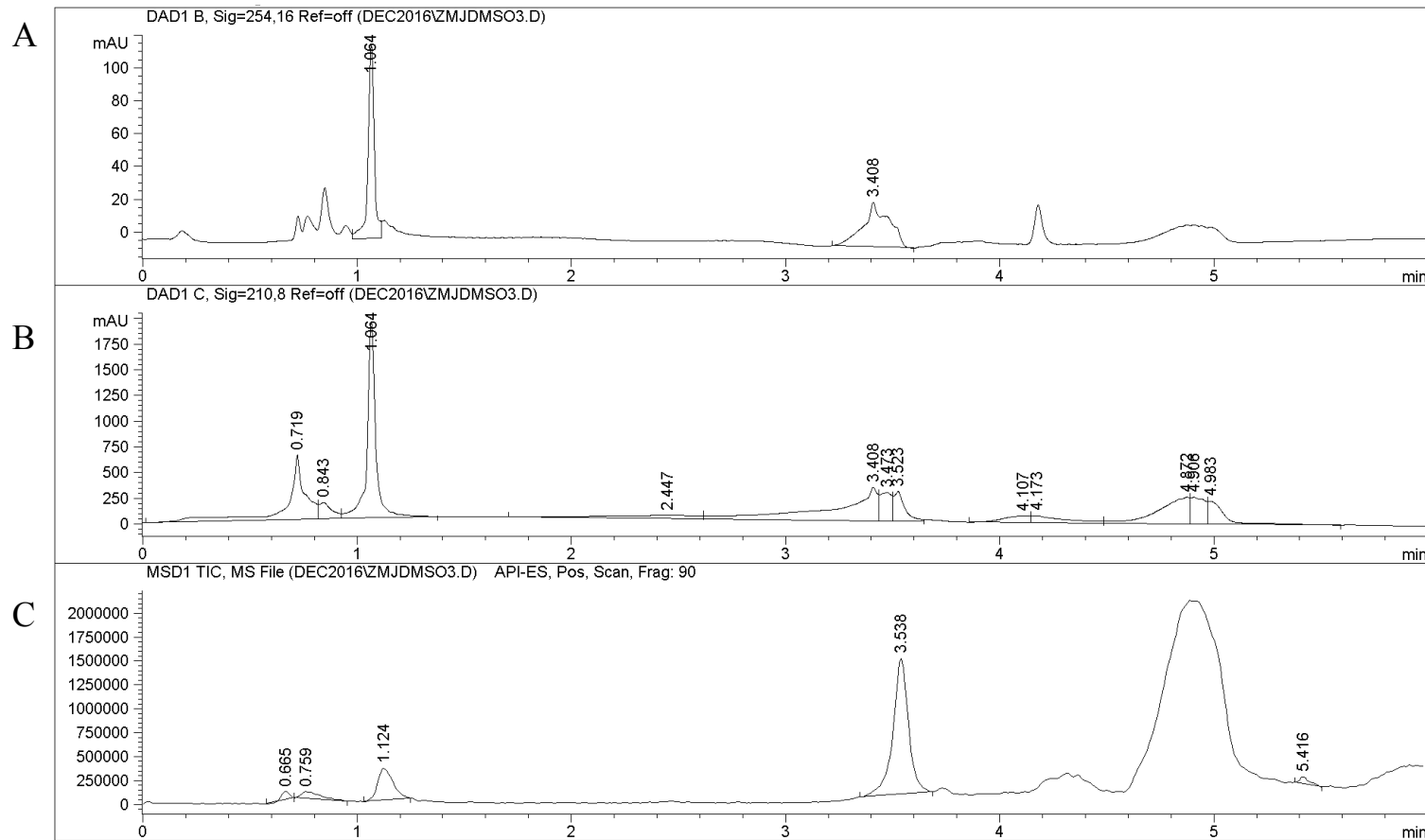


Figure S41:
T. muris
DMSO
 10.1 mg/mL [protein]

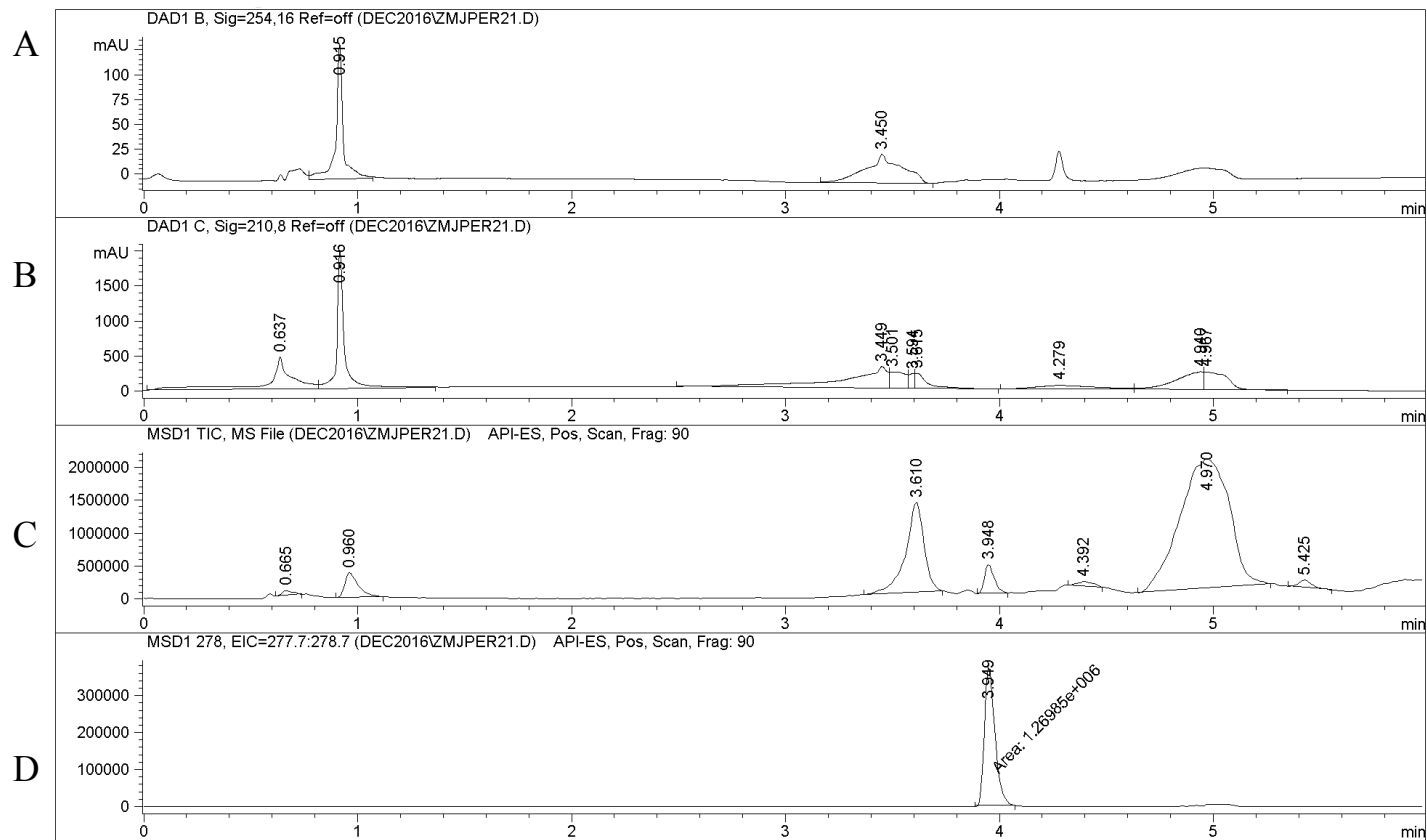
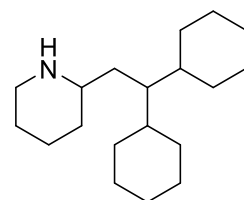
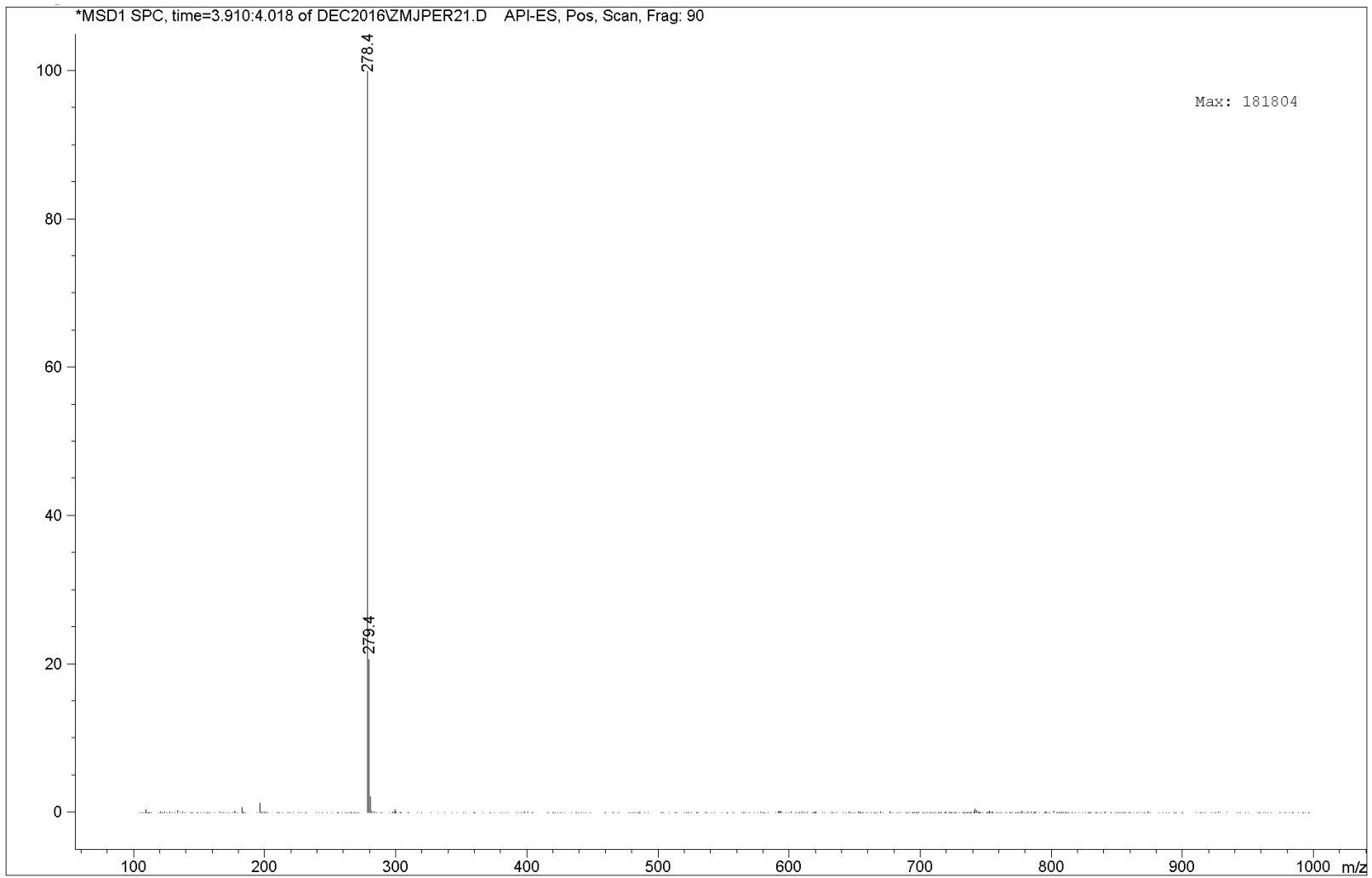


Figure S42:
T. muris
Compound P1
 10.25 mg/mL [protein]



Chemical Formula: C₁₉H₃₅N
 Exact Mass: 277.28

*MSD1 SPC, time=3.910:4.018 of DEC2016\ZMJPER21.D API-ES, Pos, Scan, Frag: 90



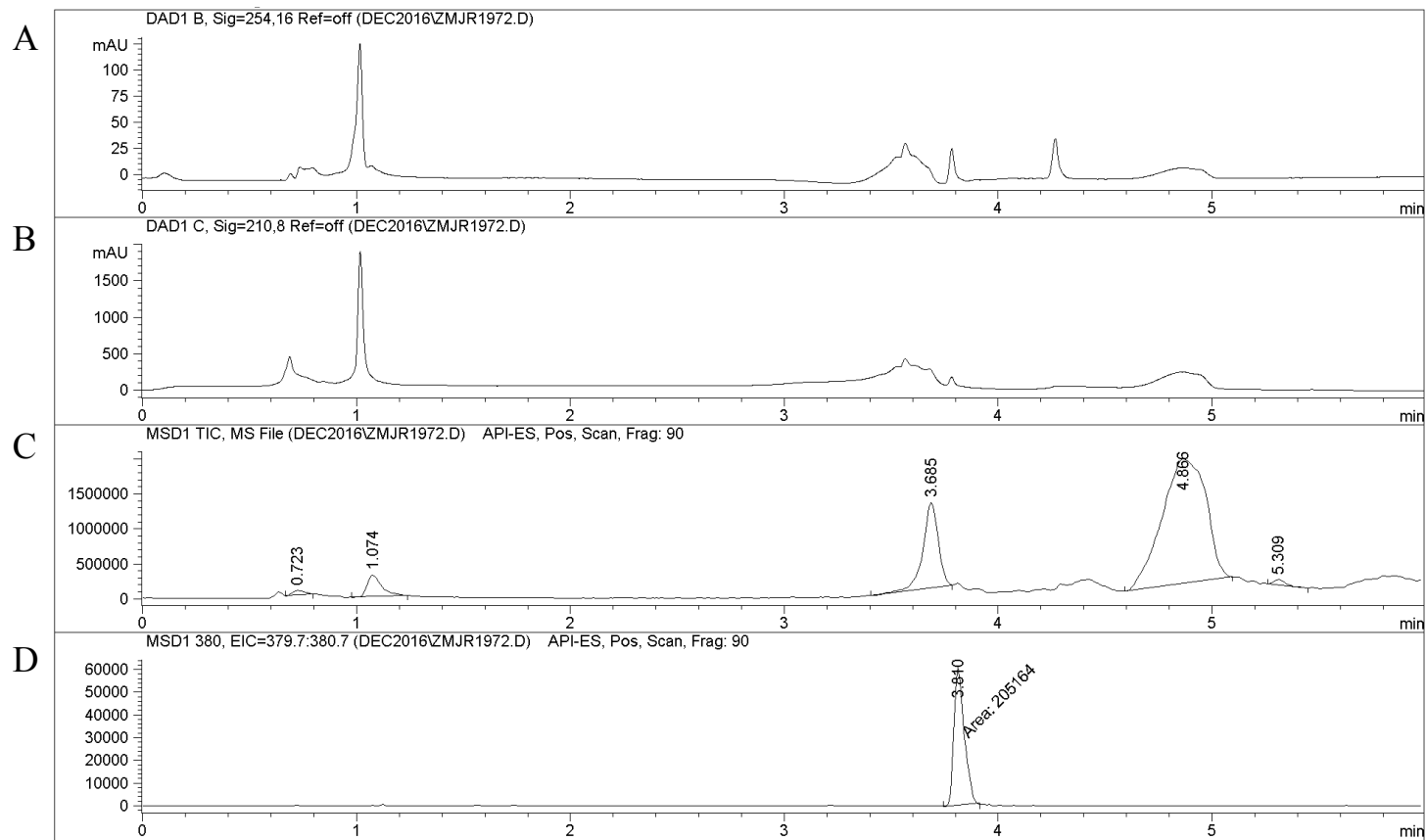
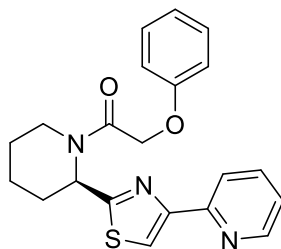


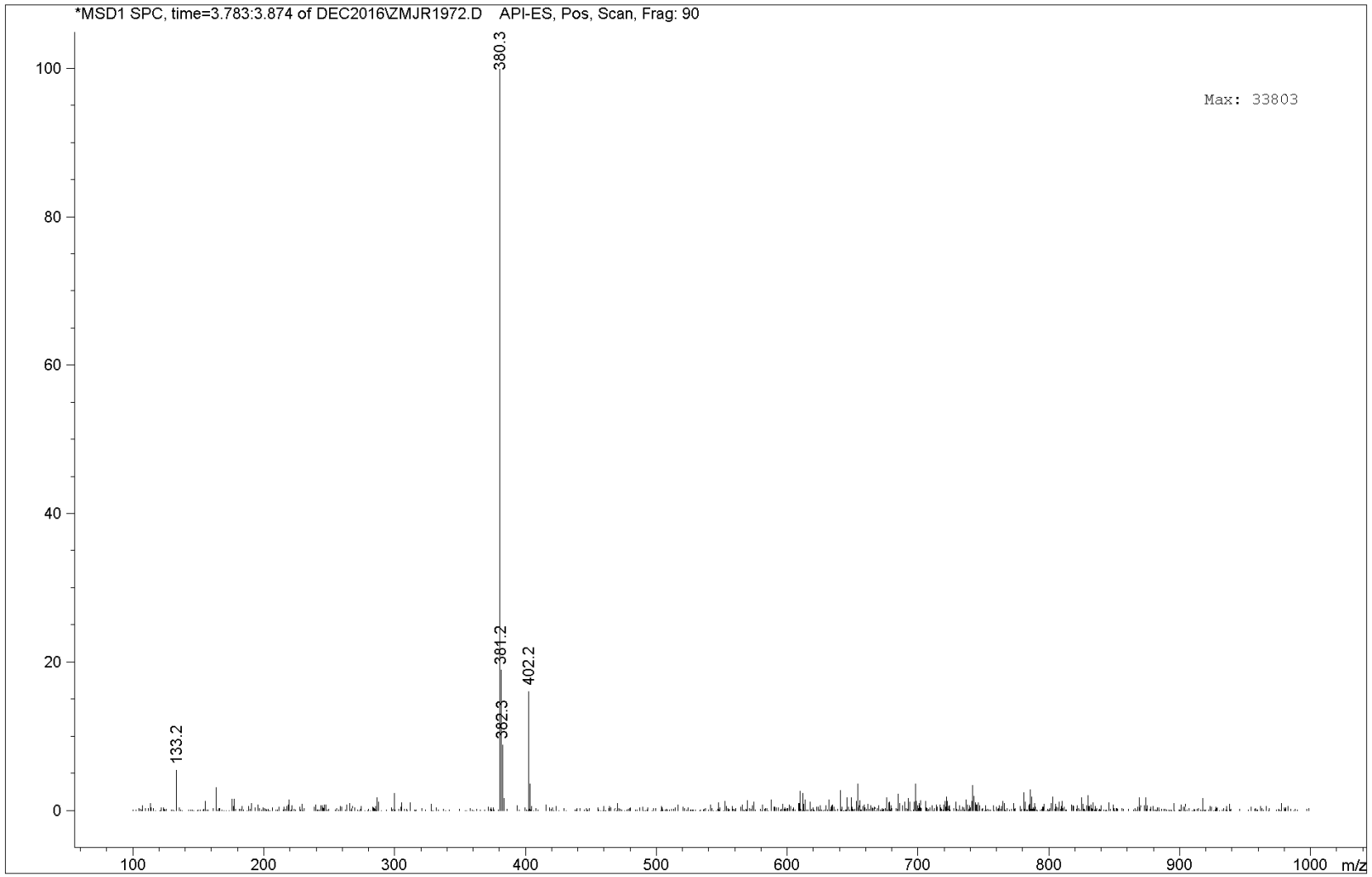
Figure S43:
T. muris
Compound 6a
 11.85 mg/mL [protein]



Chemical Formula: C₂₁H₂₁N₃O₂S

Exact Mass: 379.14

S54



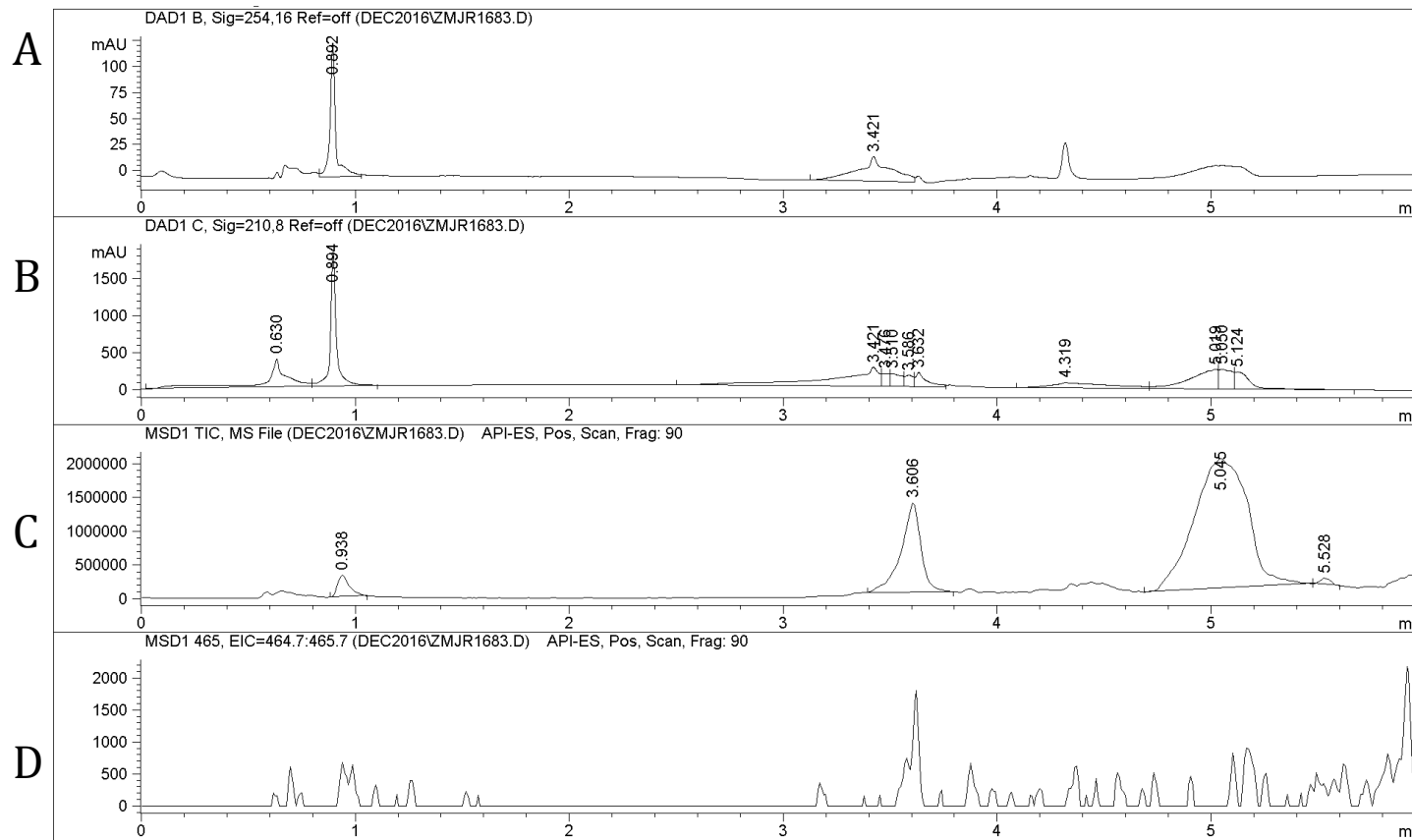
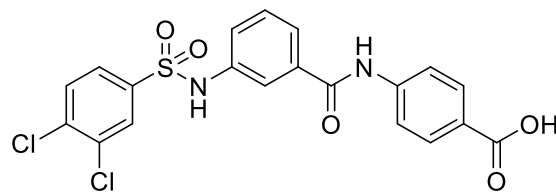


Figure S44:
T. muris
Compound 10a
 8.58 mg/mL [protein]



Chemical Formula: C₂₀H₁₄Cl₂N₂O₅S

Exact Mass: 464.00

S56

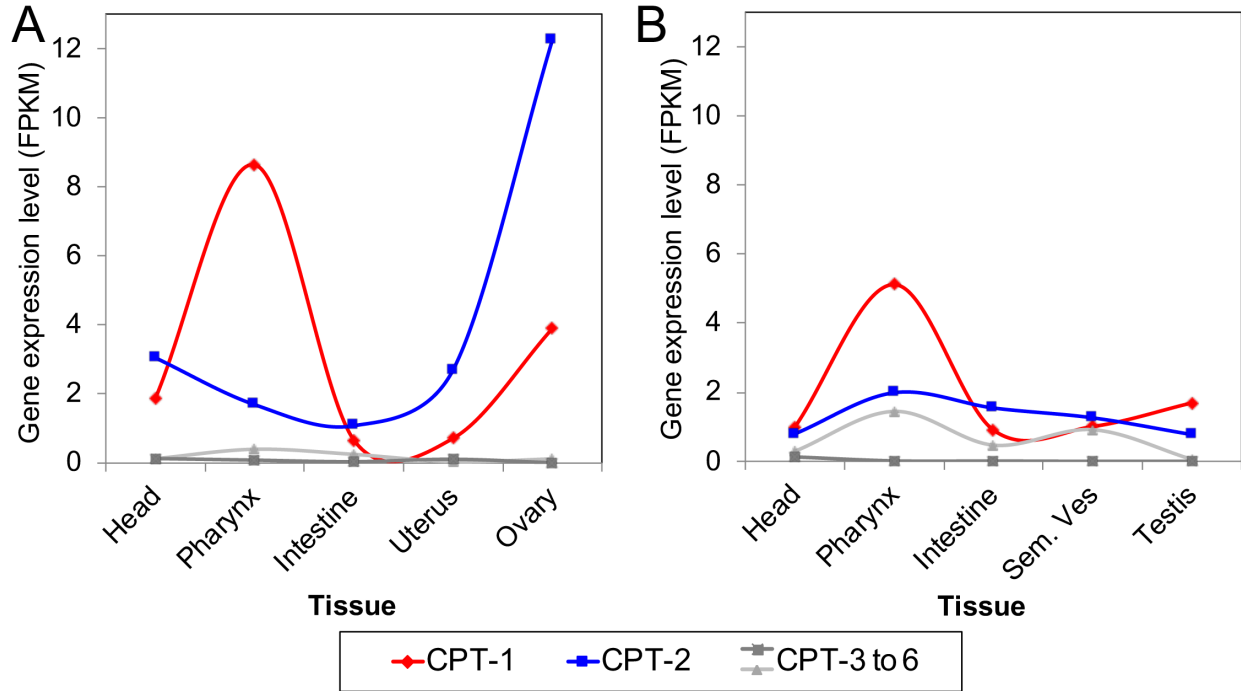


Figure S45. RNAseq based gene expression profile in different tissues of adult intestinal nematode *Ascaris suum* shows increased expression of CPT1/2 in ovary compared to the uterus in female worms and the seminal vesicle and testis in male worms. Gene expression values used are from our earlier report, Rosa et al, 2014.(Rosa, Jasmer et al. 2014).

Rosa, B. A., et al. (2014). "Genome-wide tissue-specific gene expression, co-expression and regulation of co-expressed genes in adult nematode *Ascaris suum*." *PLoS Negl Trop Dis* **8**(2): e2678.