

Design, Synthesis, and Biological Evaluation of HDAC Inhibitors Containing Natural Product-Inspired N-Linked 2-Acetylpyrrole Cap

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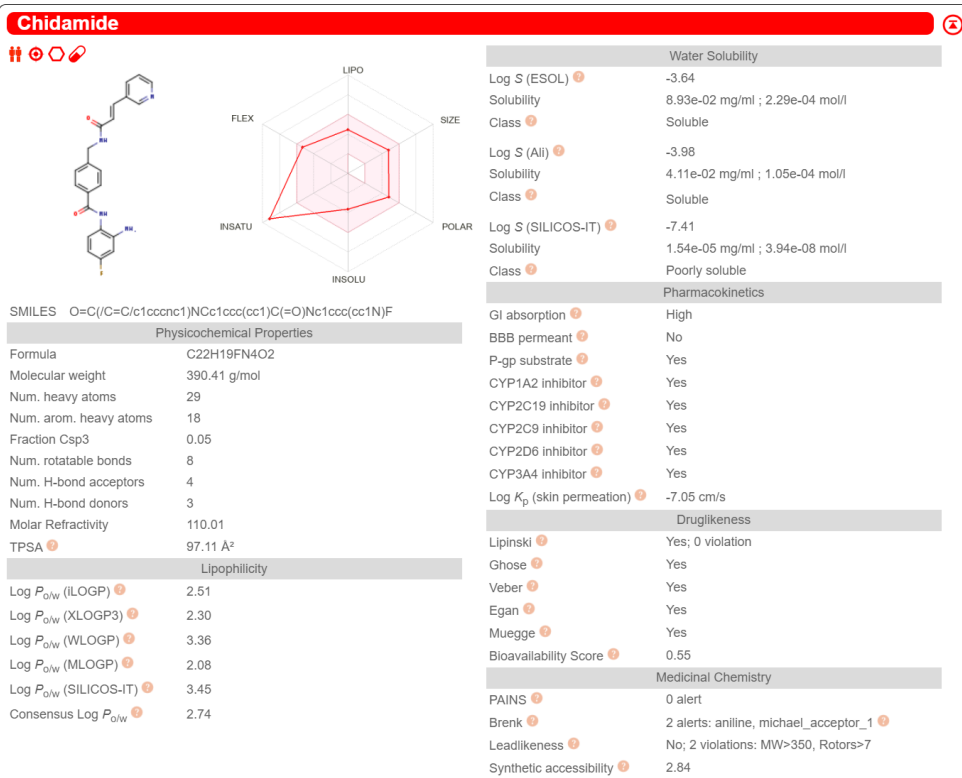
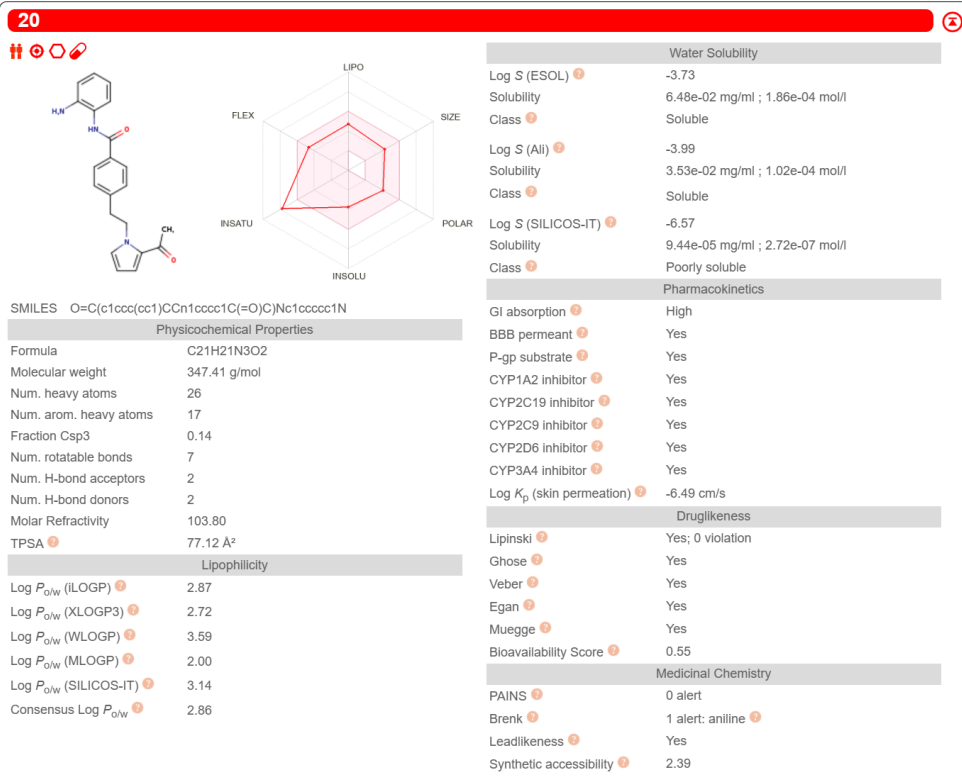


Figure S1. Physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry of **20** and chidamide predicted using SwissADME.

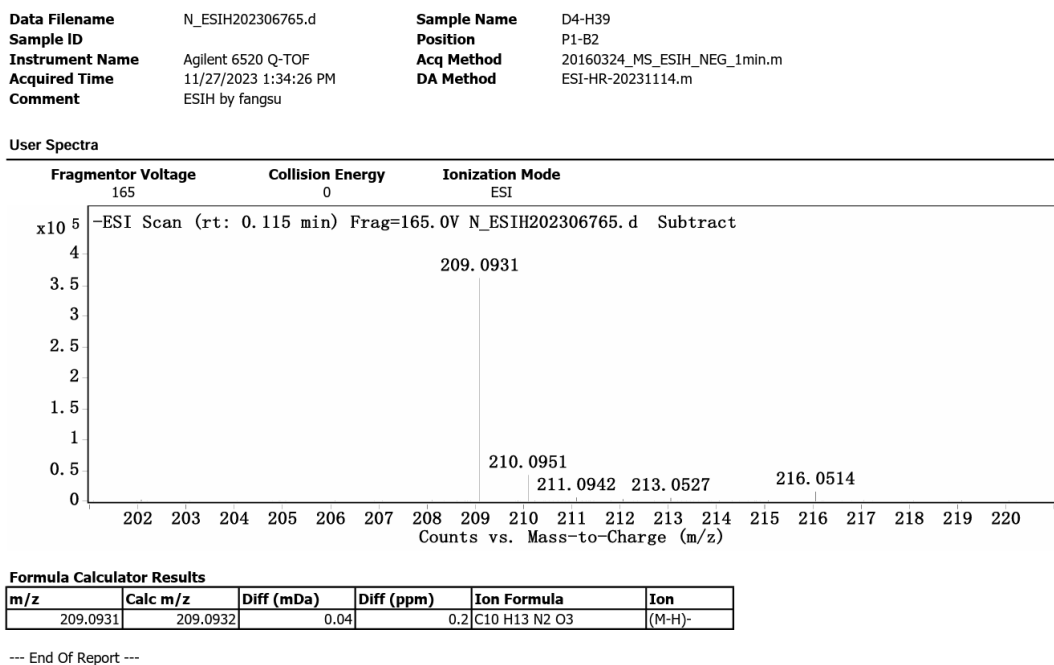


Figure S2. HRESIMS spectrum of compound **3a**.

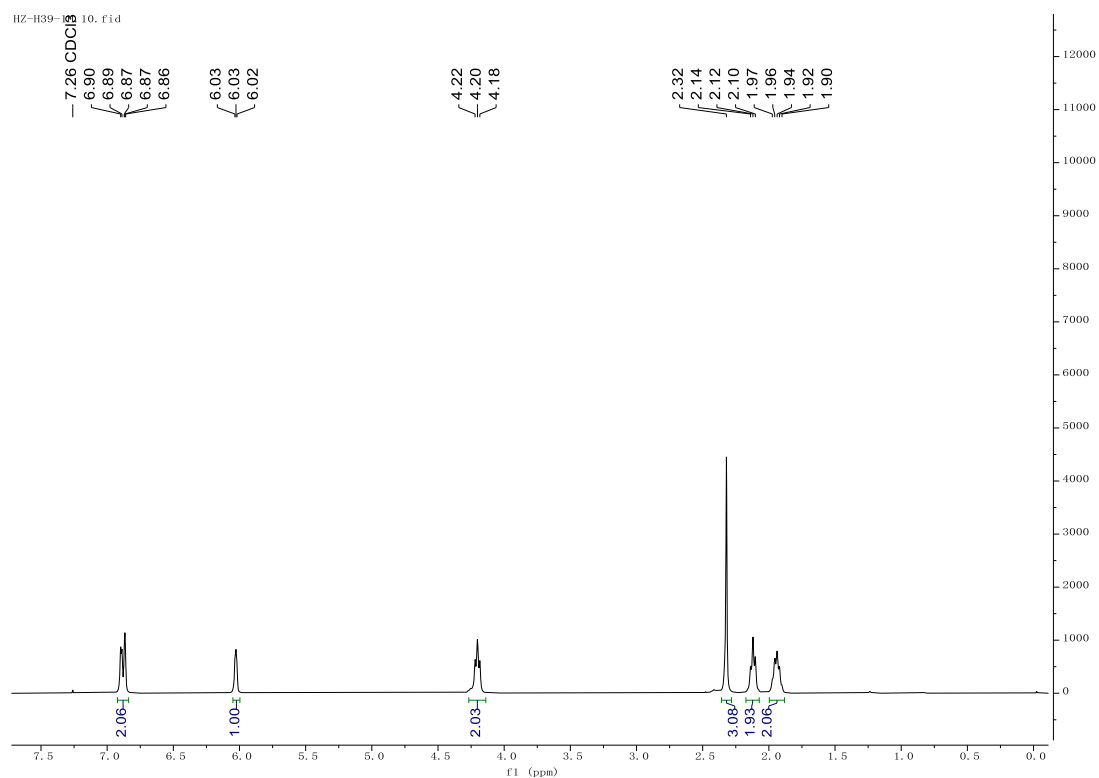


Figure S3. ^1H NMR spectrum of compound **3a**.

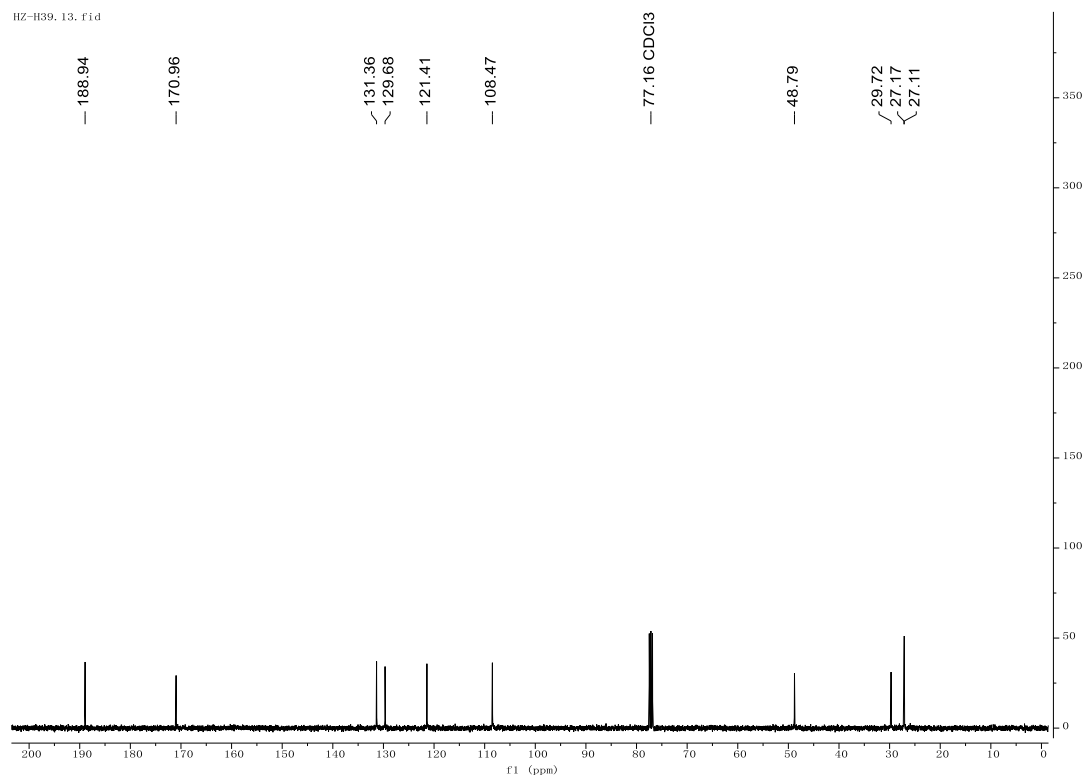
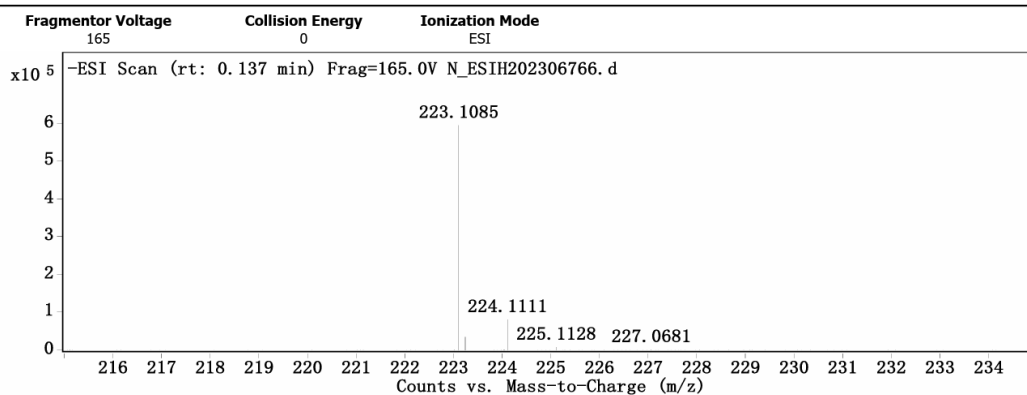


Figure S4. ^{13}C NMR spectrum of compound **3a**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306766.d | Sample Name | D4-H30 |
| Sample ID | | Position | P1-B3 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:35:41 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra

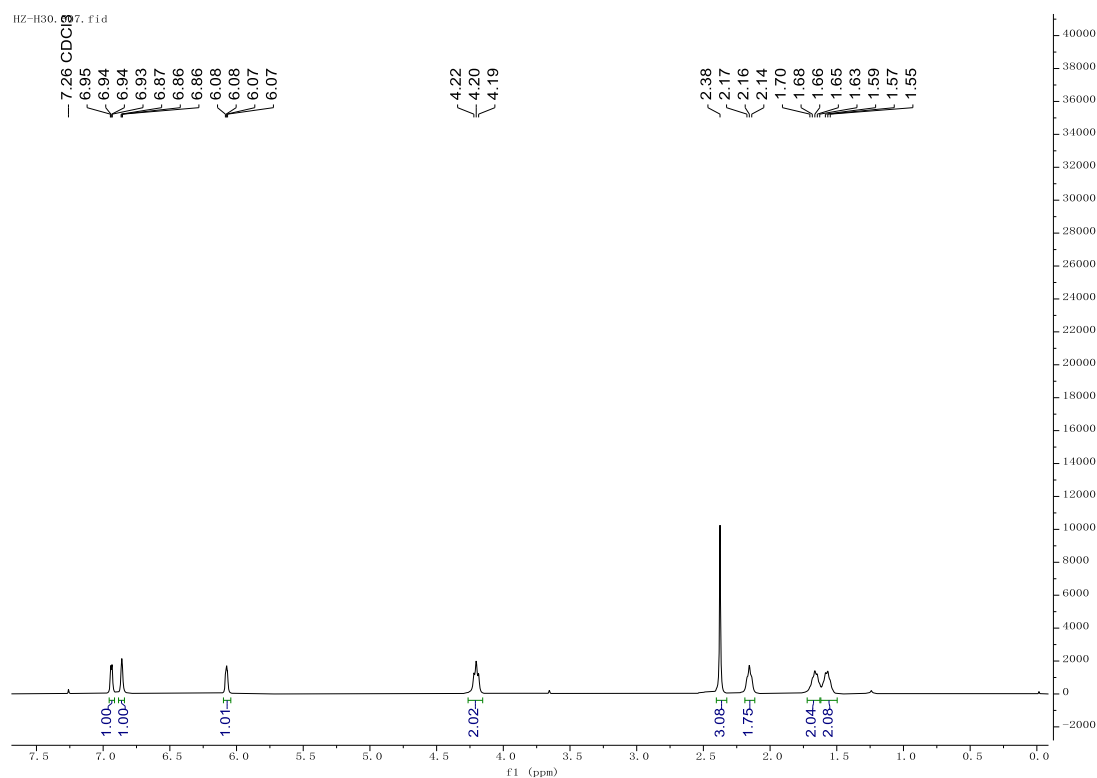


Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 223.1085 | 223.1088 | 0.33 | 1.47 | C ₁₁ H ₁₅ N ₂ O ₃ | (M-H) ⁻ |

--- End Of Report ---

Figure S5. HRESIMS spectrum of compound **3b**.



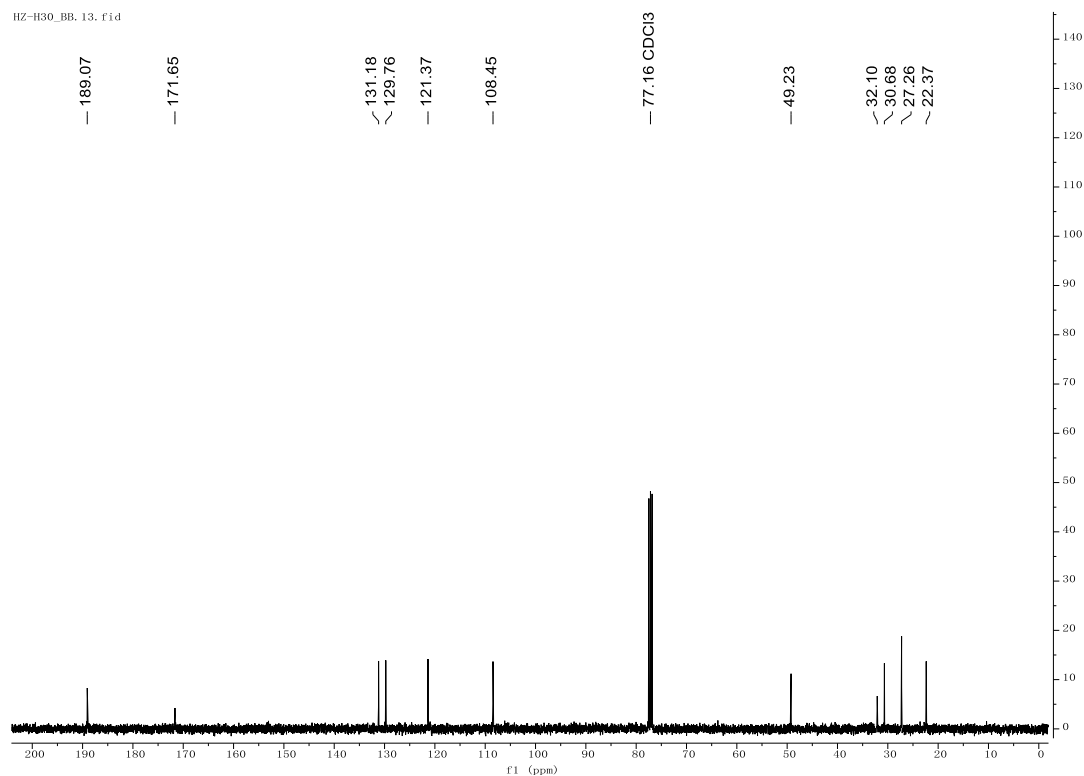
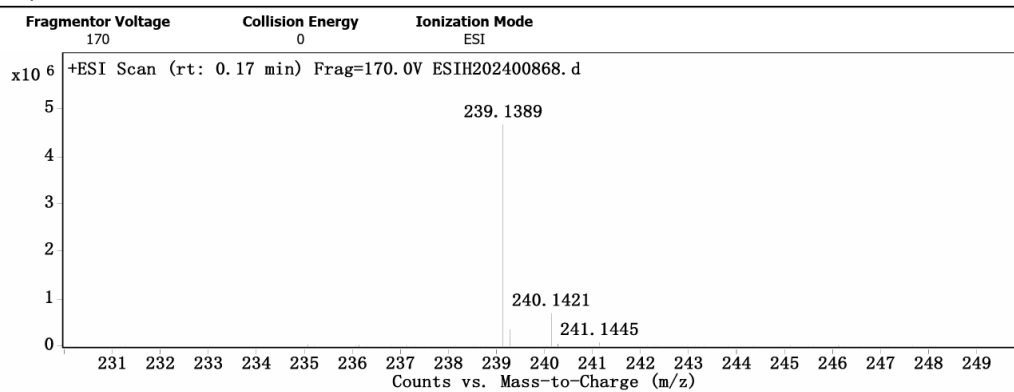


Figure S7. ¹³C NMR spectrum of compound **3b**.

| | | | |
|------------------------|----------------------|--------------------|-----------------------------|
| Data Filename | ESI202400868.d | Sample Name | D4-D16 |
| Sample ID | | Position | P1-C1 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160322_MS_ESIH_POS_1min.m |
| Acquired Time | 2/26/2024 4:17:44 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI202400868.d | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 239.1389 | 239.139 | 0.09 | 0.36 | C ₁₂ H ₁₉ N ₂ O ₃ | (M+H) ⁺ |

--- End Of Report ---

Figure S8. HRESIMS spectrum of compound **3c**.

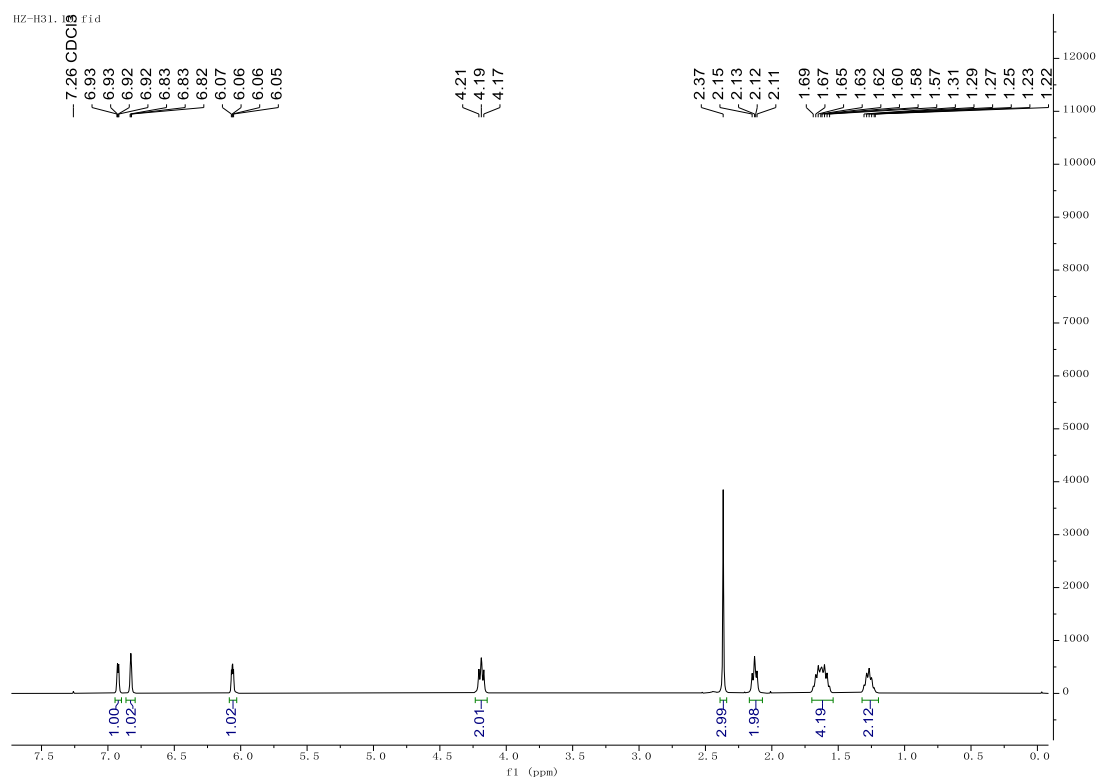


Figure S9. ^1H NMR spectrum of compound **3c**.

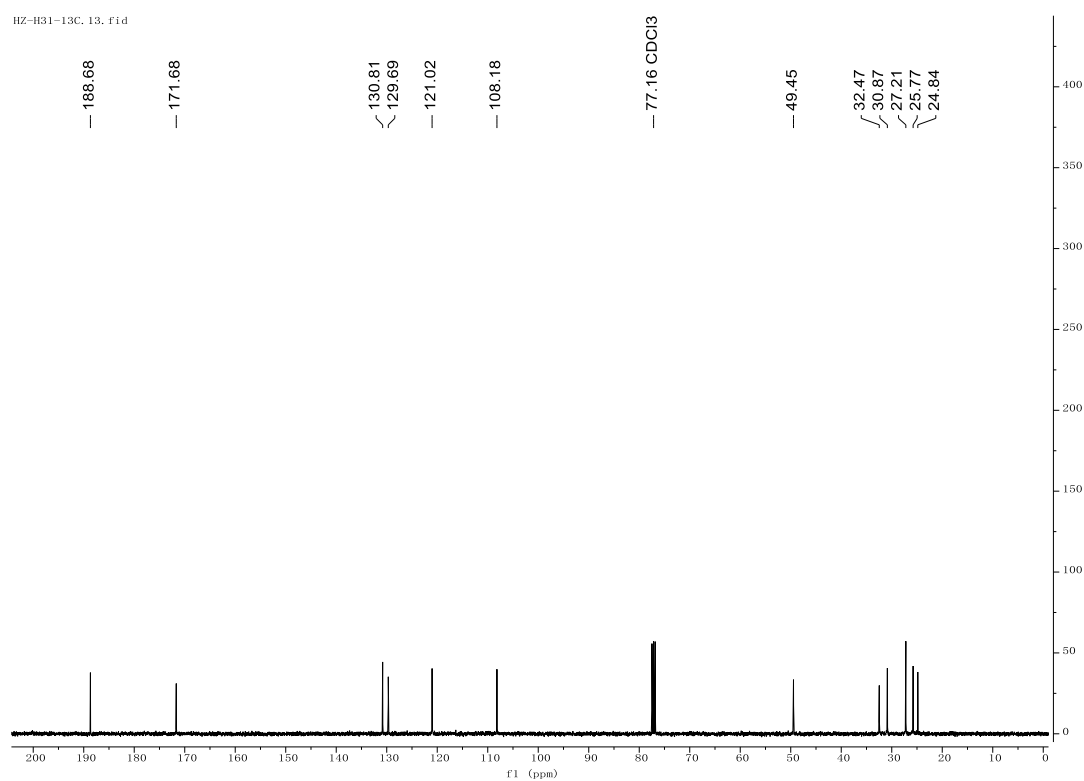
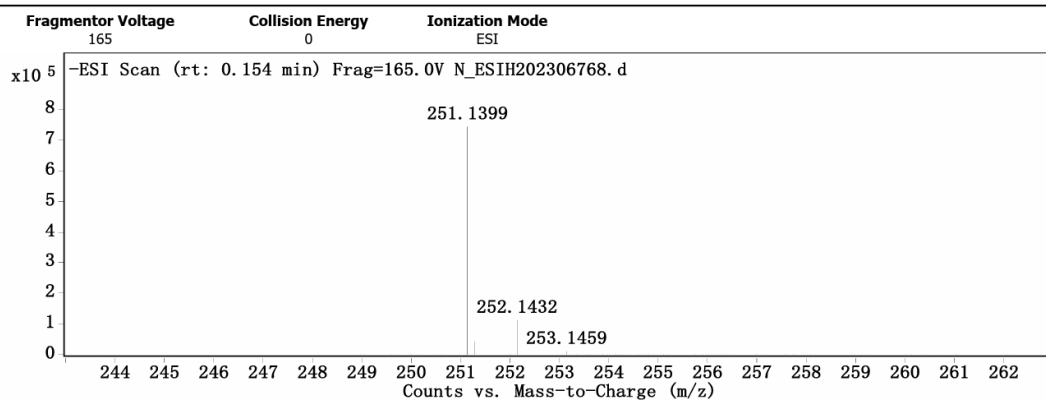


Figure S10. ^{13}C NMR spectrum of compound **3c**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306768.d | Sample Name | D4-H32 |
| Sample ID | | Position | P1-B5 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:38:12 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 251.1399 | 251.1401 | 0.19 | 0.77 | C ₁₃ H ₁₉ N ₂ O ₃ | (M-H) ⁻ |

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Figure S11. HRESIMS spectrum of compound **3d**.

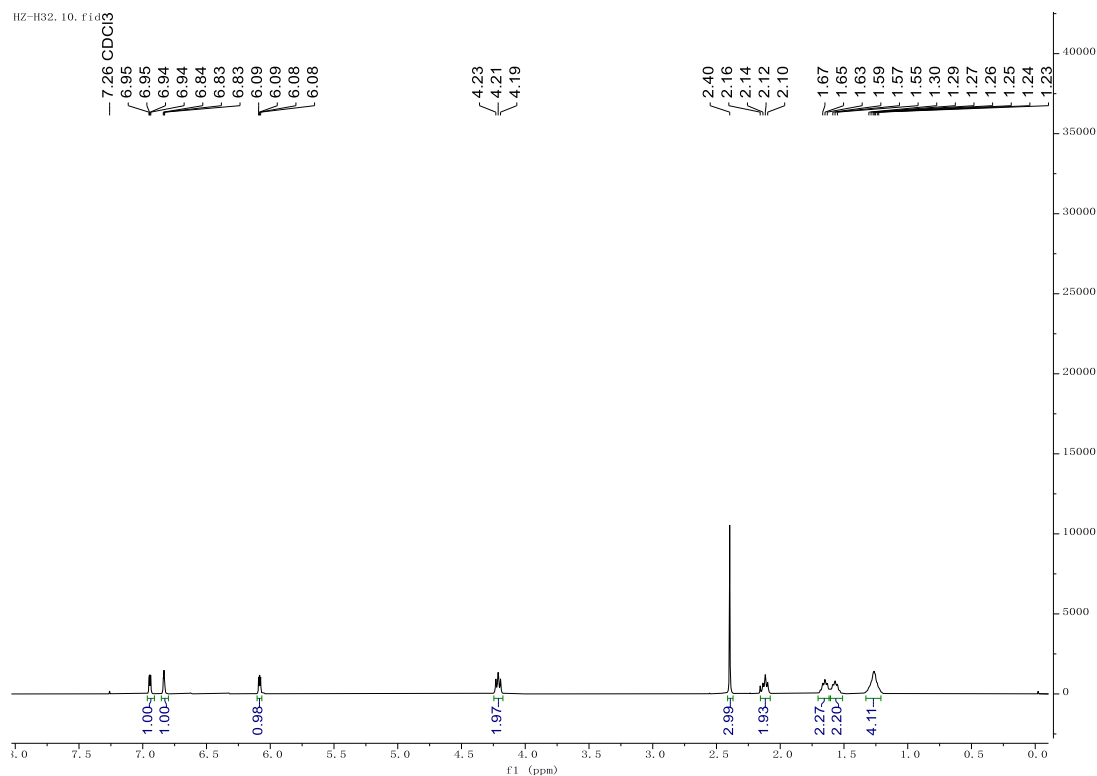


Figure S12. ¹H NMR spectrum of compound **3d**.

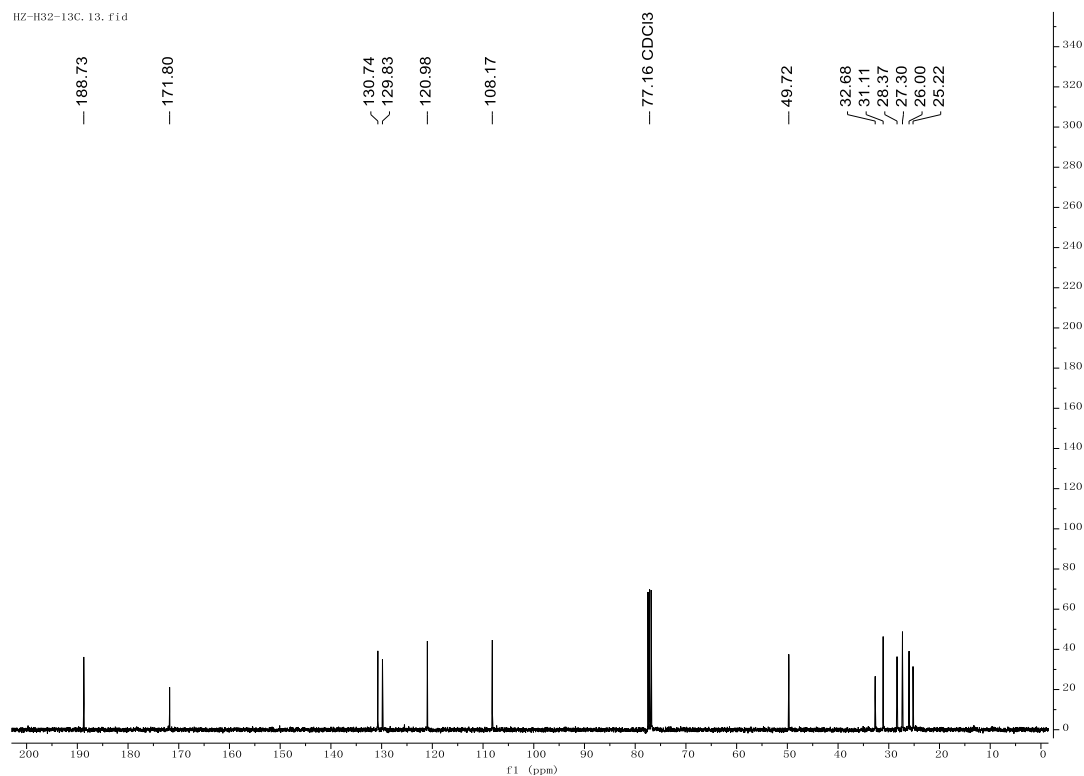
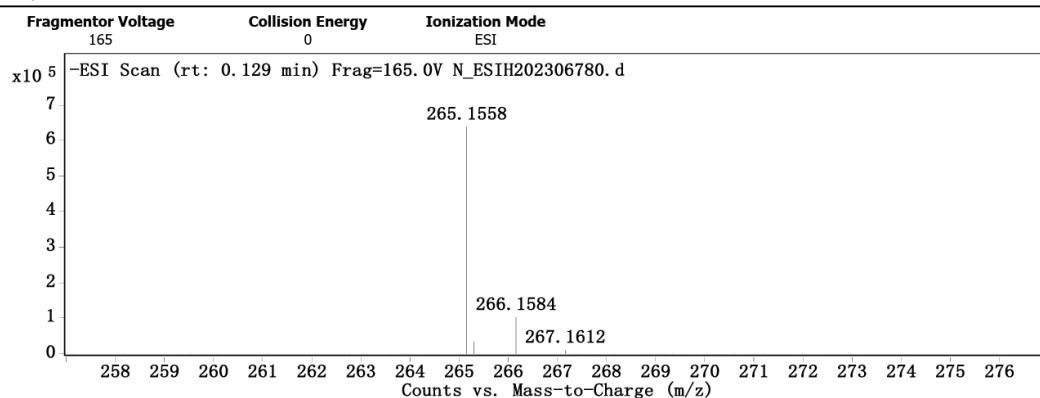


Figure S13. ¹³C NMR spectrum of compound **3d**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306780.d | Sample Name | D4-D18 |
| Sample ID | | Position | P1-C8 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:53:29 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsuo | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 265.1558 | 265.1558 | -0.02 | -0.08 | C14 H21 N2 O3 | (M-H)- |

--- End Of Report ---

Figure S14. HRESIMS spectrum of compound **3e**.

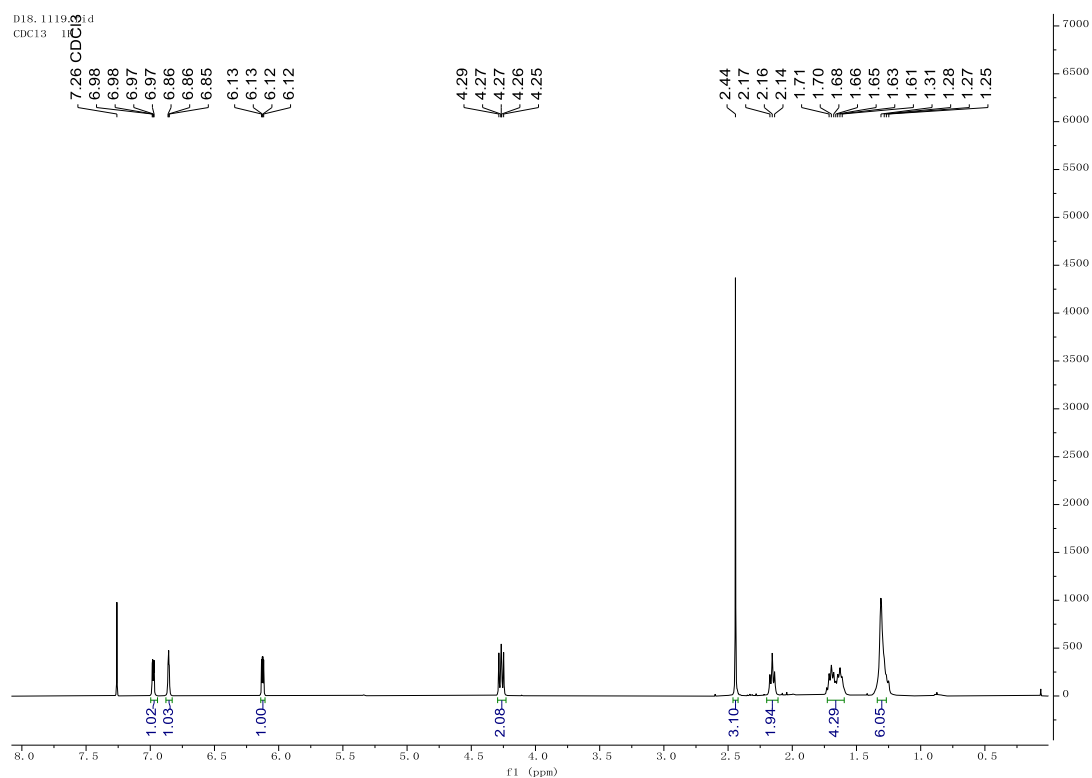


Figure S15. ^1H NMR spectrum of compound **3e**.

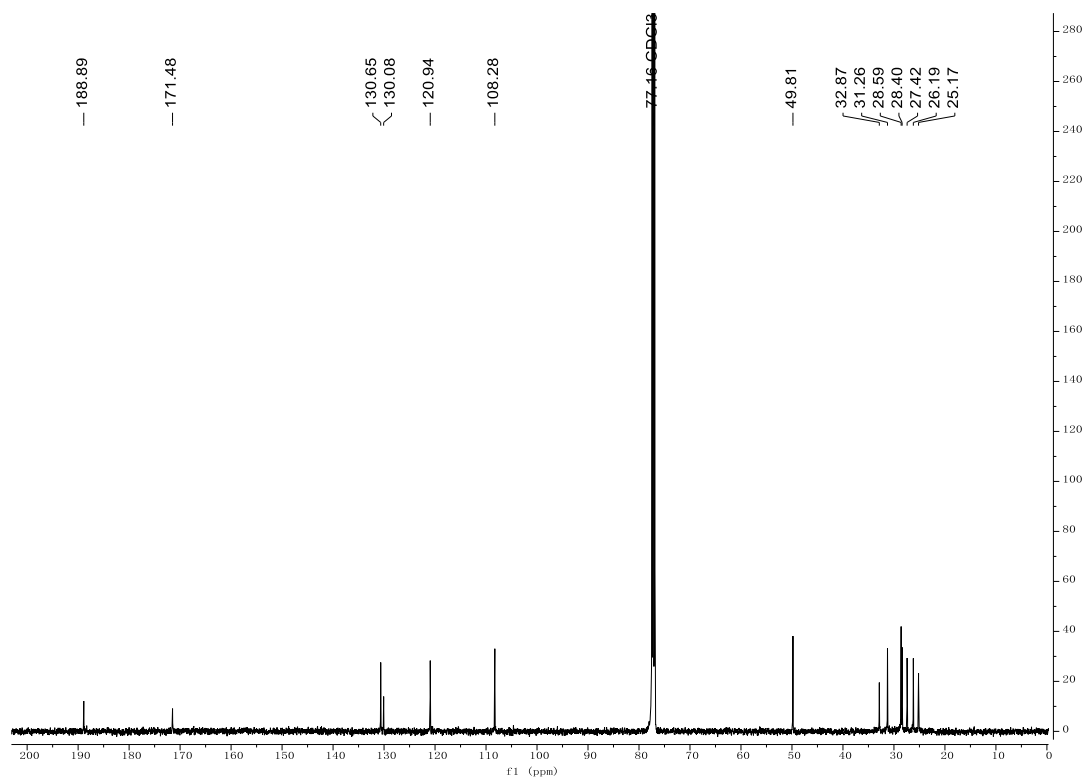
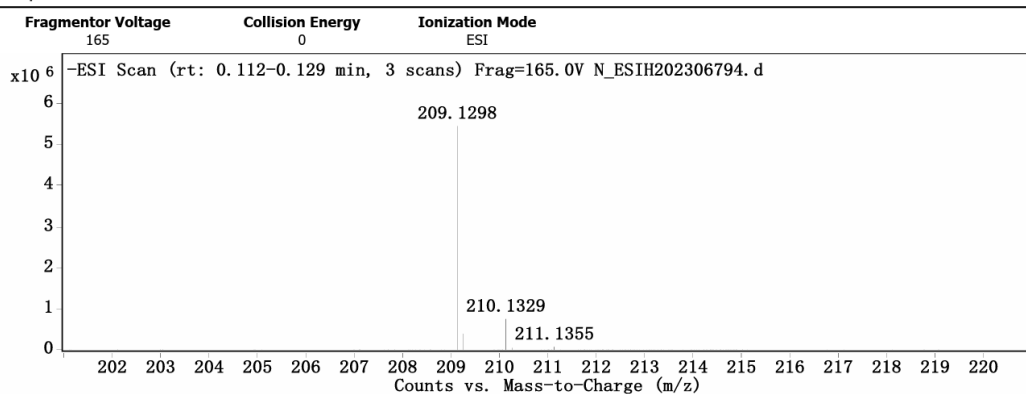


Figure S16. ^{13}C NMR spectrum of compound **3e**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306794.d | Sample Name | D4-D64 |
| Sample ID | | Position | P1-E4 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:11:17 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 209.1298 | 209.1296 | -0.28 | -1.33 | C11 H17 N2 O2 | (M-H)- |

--- End Of Report ---

Figure S17. HRESIMS spectrum of compound **5a**.

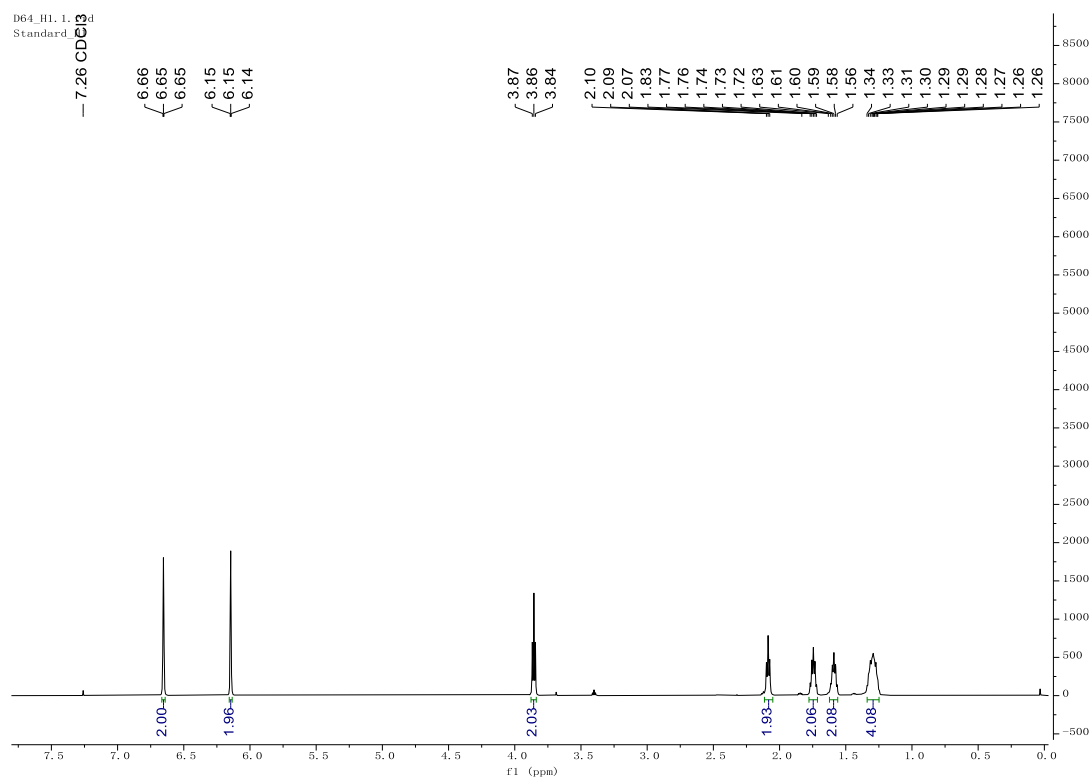


Figure S18. ^1H NMR spectrum of compound **5a**.

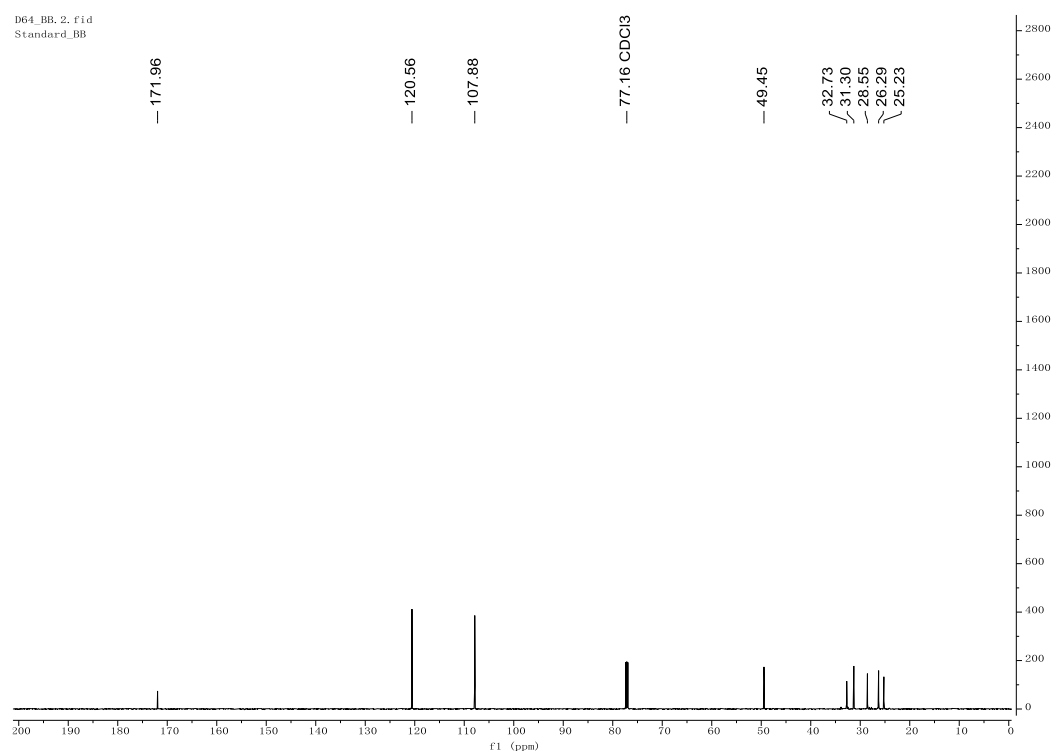
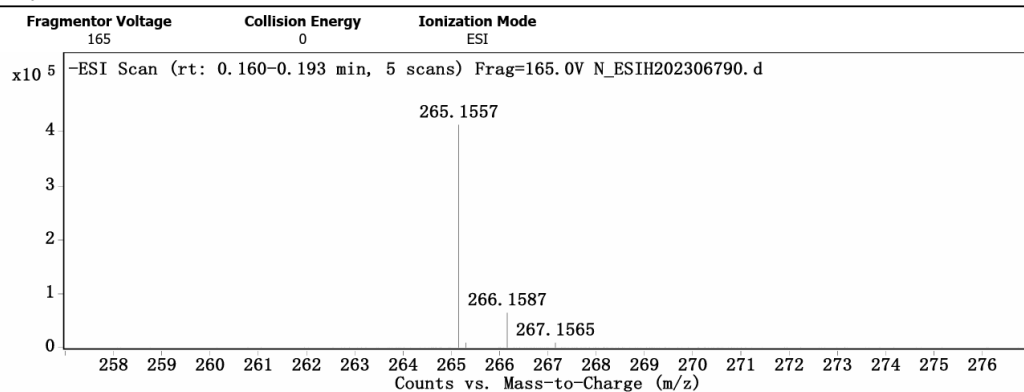


Figure S19. ^{13}C NMR spectrum of compound **5a**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306790.d | Sample Name | D4-D60 |
| Sample ID | | Position | P1-D9 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:06:10 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 265.1557 | 265.1558 | 0.04 | 0.16 | C ₁₄ H ₂₁ N ₂ O ₃ | (M-H) ⁻ |

--- End Of Report ---

Figure S20. HRESIMS spectrum of compound **5b**.

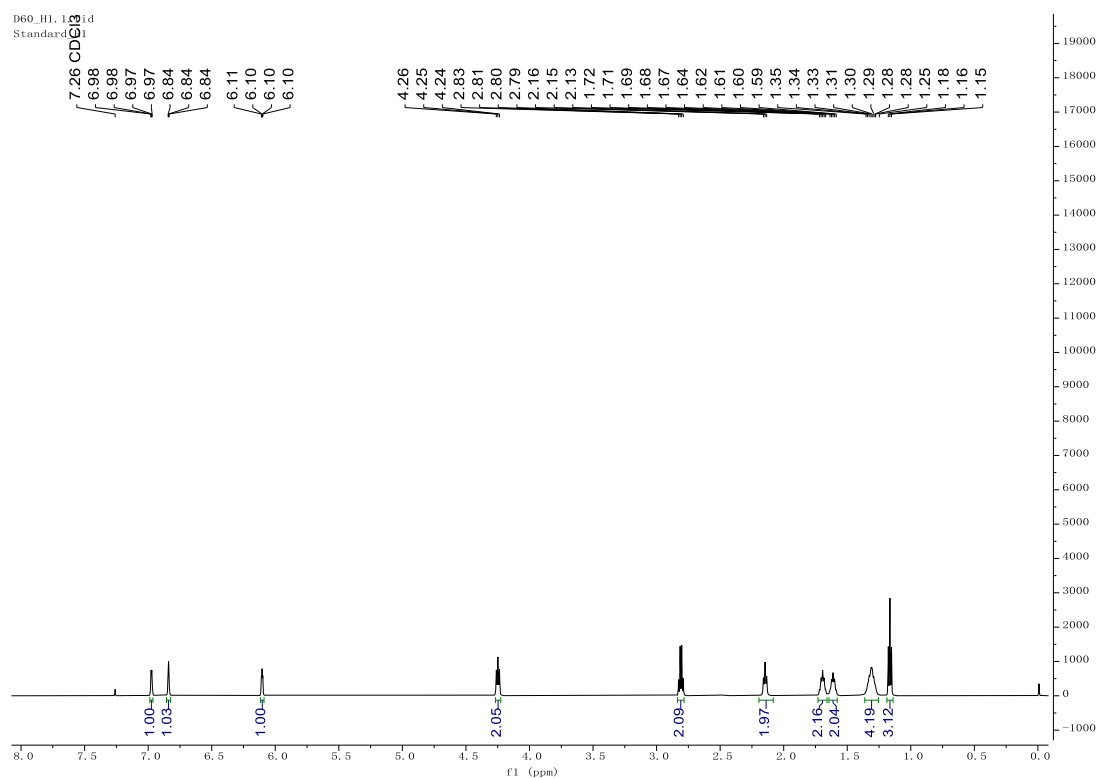


Figure S21. ^1H NMR spectrum of compound **5b**.

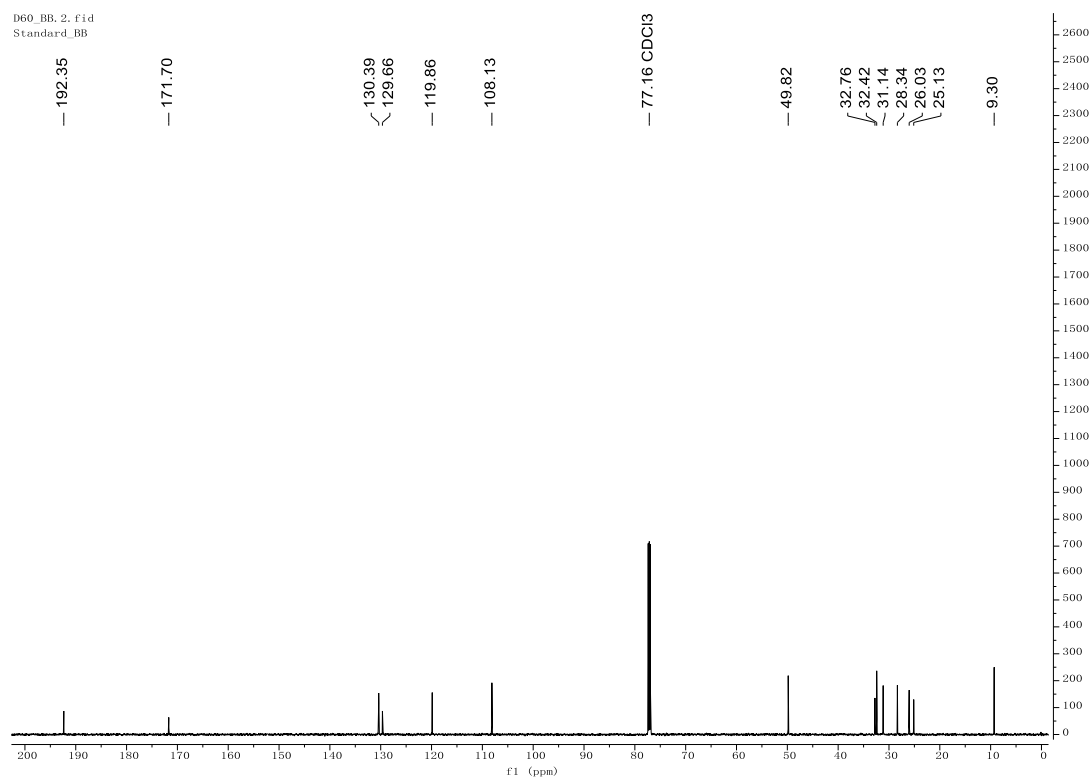
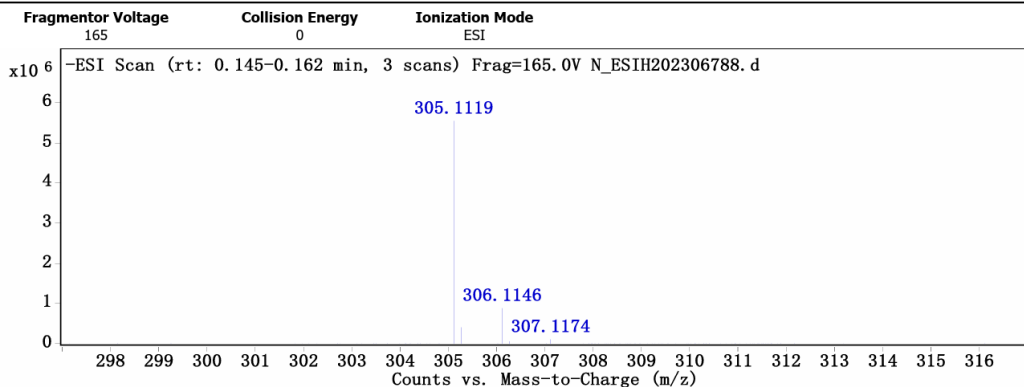


Figure S22. ^{13}C NMR spectrum of compound **5b**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306788.d | Sample Name | D4-D58 |
| Sample ID | | Position | P1-D7 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:03:38 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-H by fangsuo | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|------------------|--------------------|
| 305.1119 | 305.1119 | -0.05 | -0.15 | C13 H16 F3 N2 O3 | (M-H) ⁻ |

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Figure S23. HRESIMS spectrum of compound **5c**.

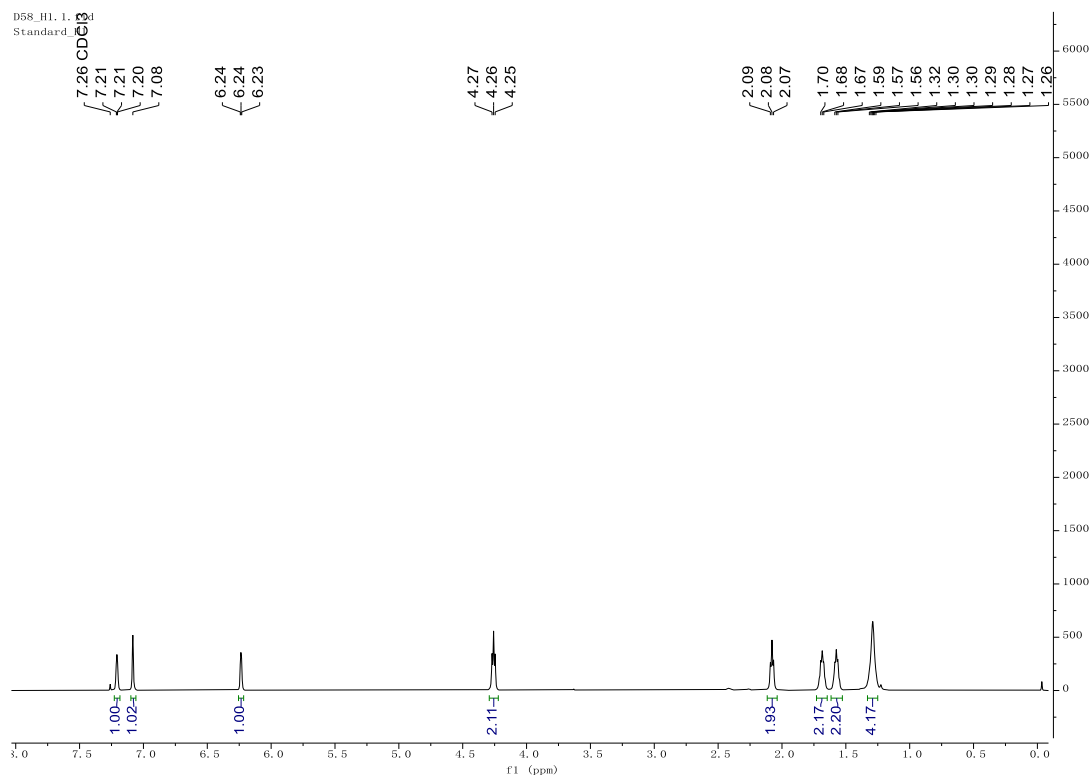


Figure S24. ¹H NMR spectrum of compound **5c**.

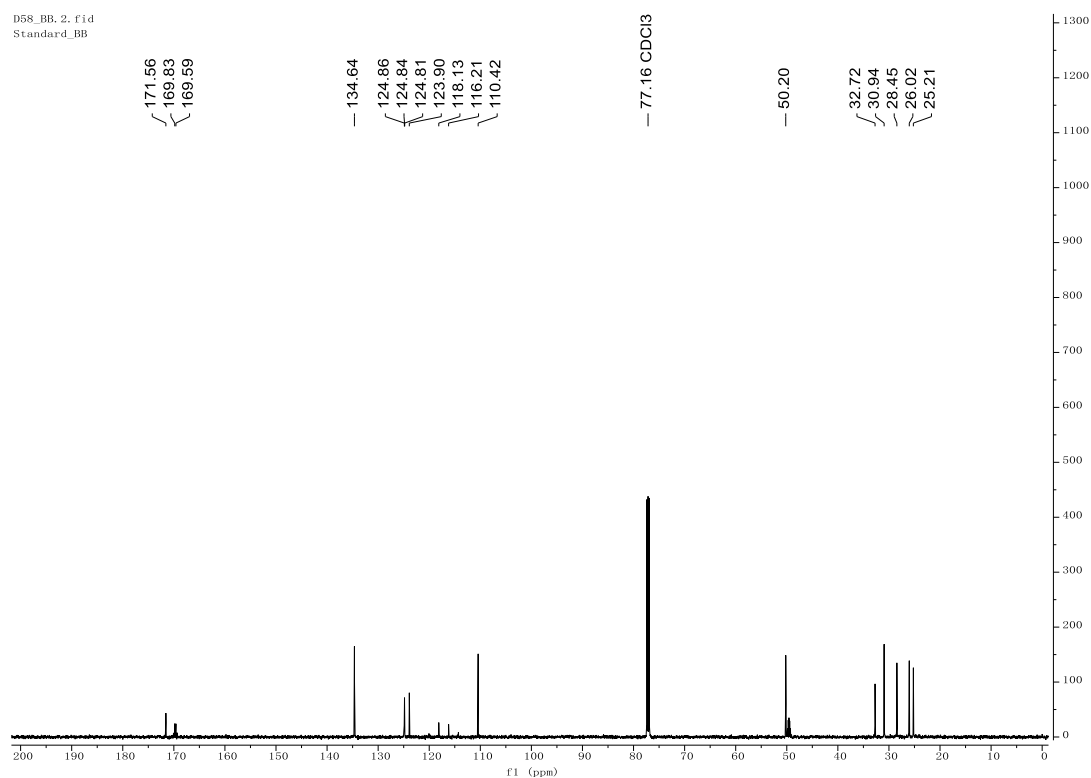
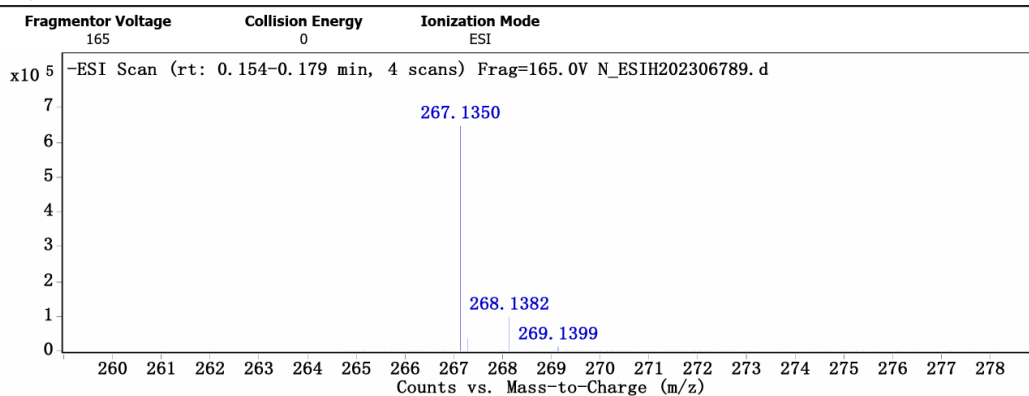


Figure S25. ^{13}C NMR spectrum of compound **5c**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306789.d | Sample Name | D4-D59 |
| Sample ID | | Position | P1-D8 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:04:54 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|---------|----------|------------|------------|---------------|--------------------|
| 267.135 | 267.135 | 0.07 | 0.27 | C13 H19 N2 O4 | (M-H) ⁻ |

--- End Of Report ---

Figure S26. HRESIMS spectrum of compound **5d**.

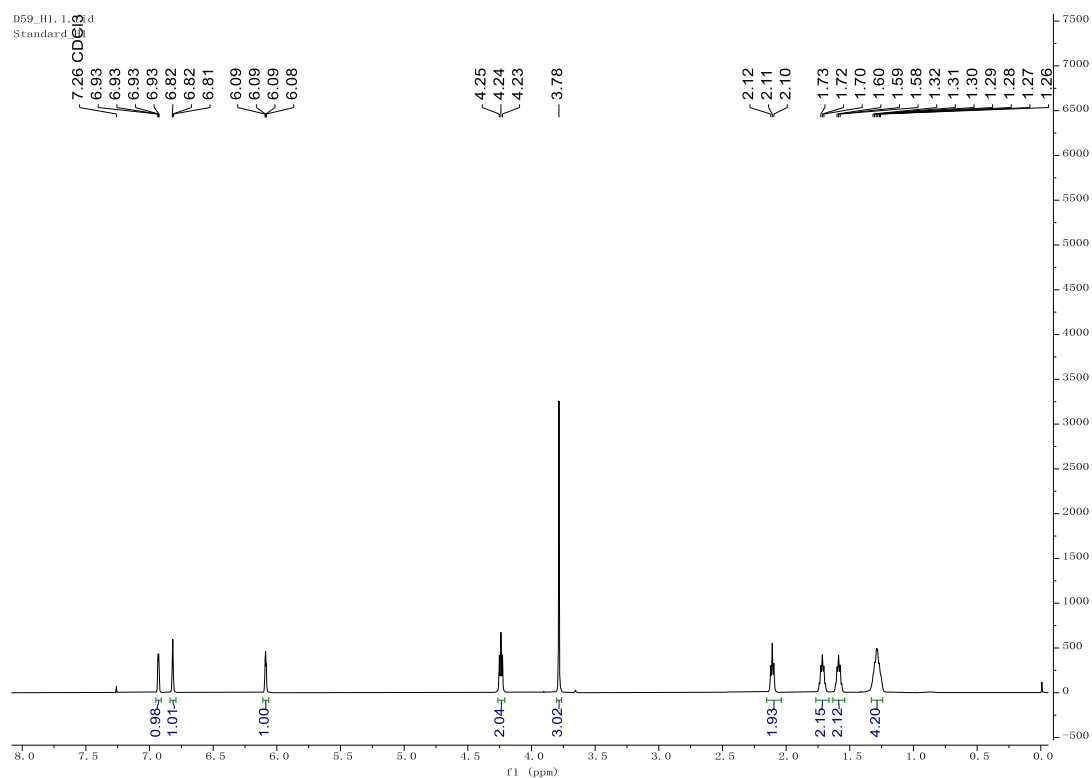


Figure S27. ^1H NMR spectrum of compound **5d**.

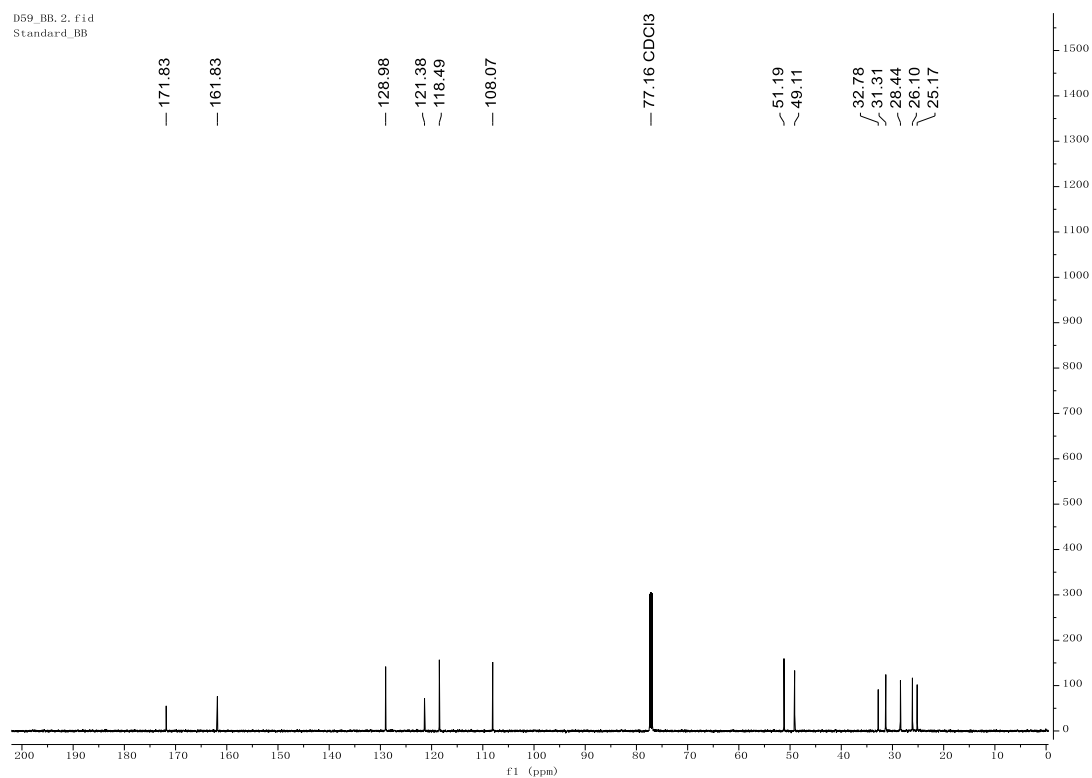
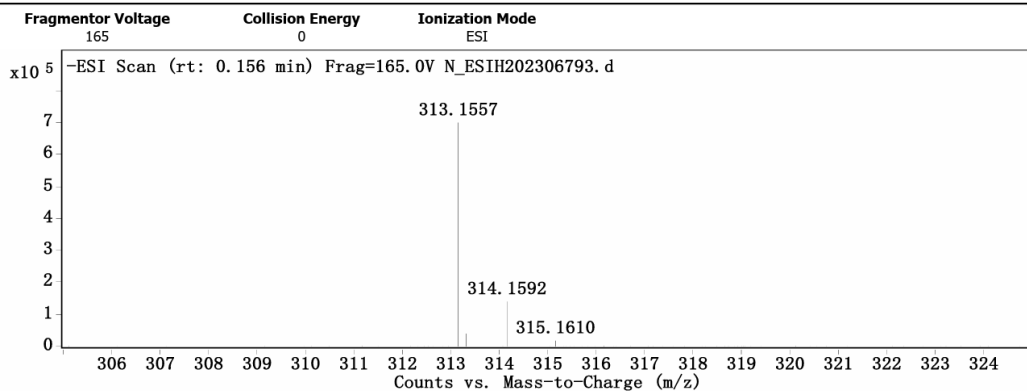


Figure S28. ^{13}C NMR spectrum of compound **5d**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306793.d | Sample Name | D4-D63 |
| Sample ID | | Position | P1-E3 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:10:00 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 313.1557 | 313.1558 | 0.04 | 0.13 | C18 H21 N2 O3 | (M-H)- |

--- End Of Report ---

Figure S29. HRESIMS spectrum of compound **5e**.

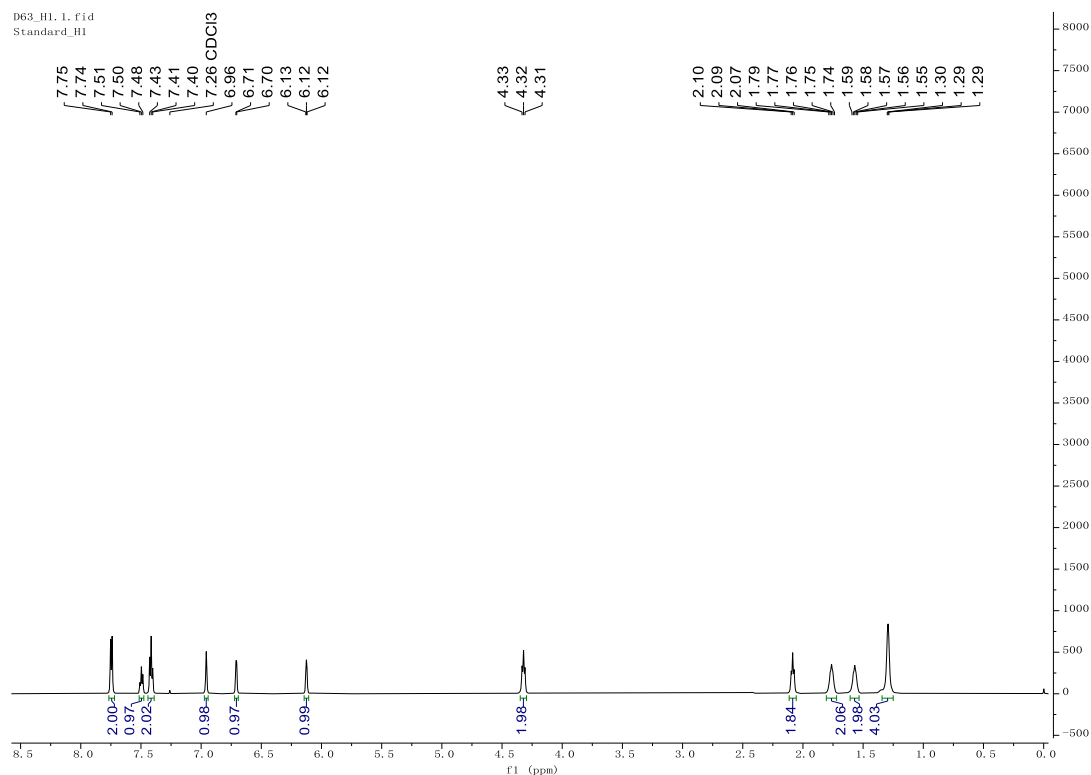


Figure S30. ^1H NMR spectrum of compound **5e**.

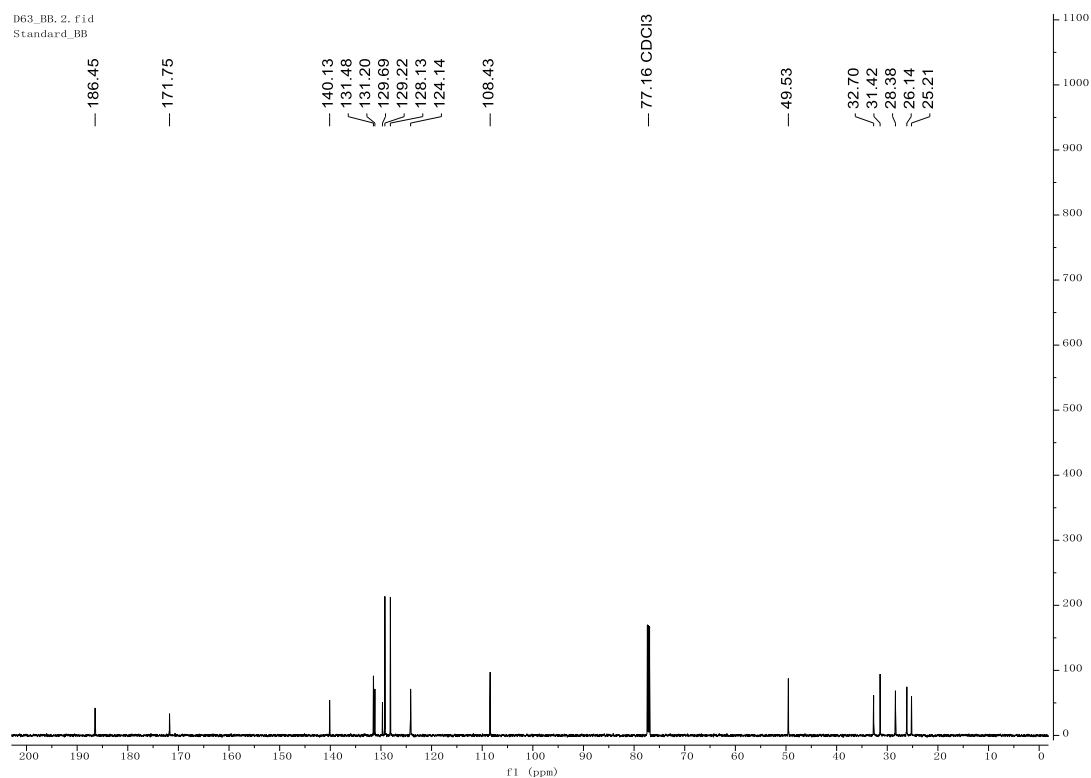
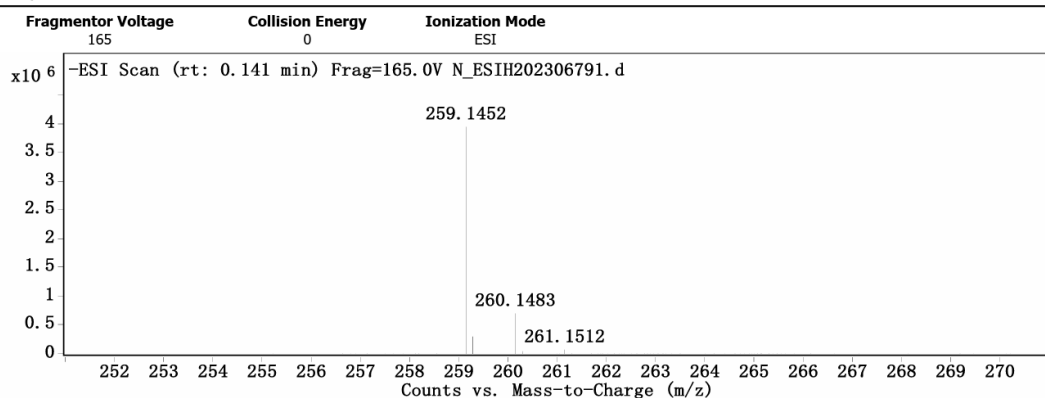


Figure S31. ¹³C NMR spectrum of compound **5e**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306791.d | Sample Name | D4-D61 |
| Sample ID | | Position | P1-E1 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:07:25 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------------------|
| 259.1452 | 259.1452 | 0.04 | 0.16 | C15 H19 N2 O2 | (M-H) ⁻ |

--- End Of Report ---

Figure S32. HRESIMS spectrum of compound **5f**.

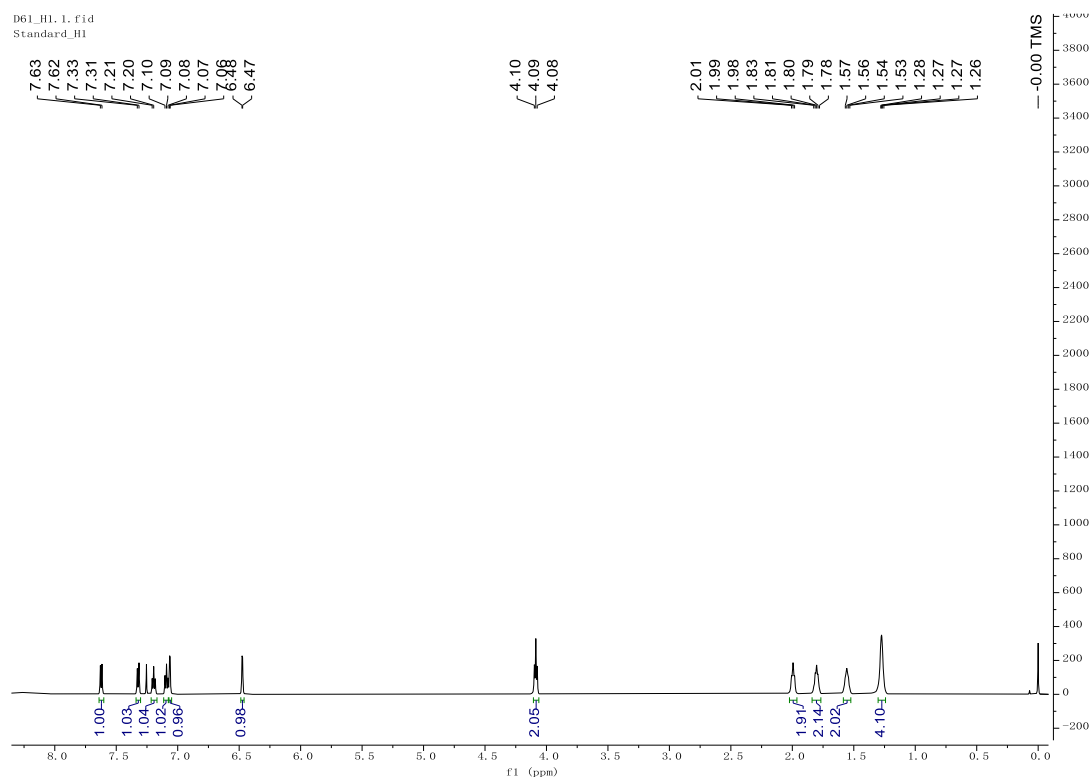


Figure S33. ^1H NMR spectrum of compound **5f**.

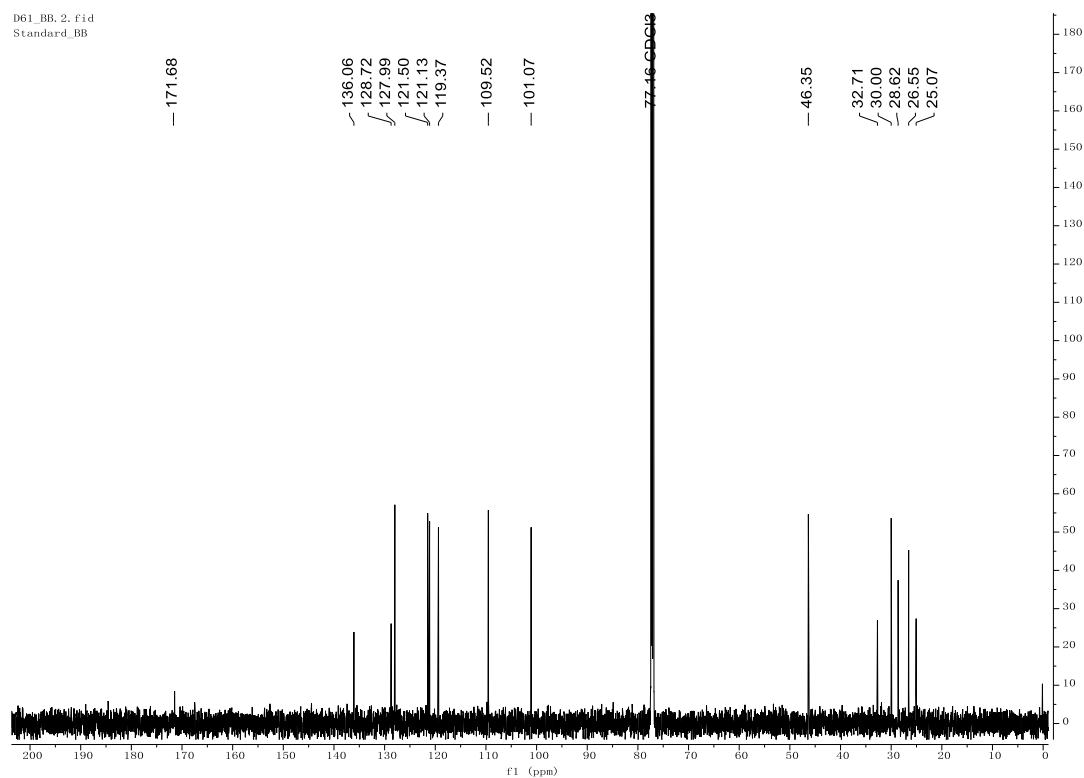
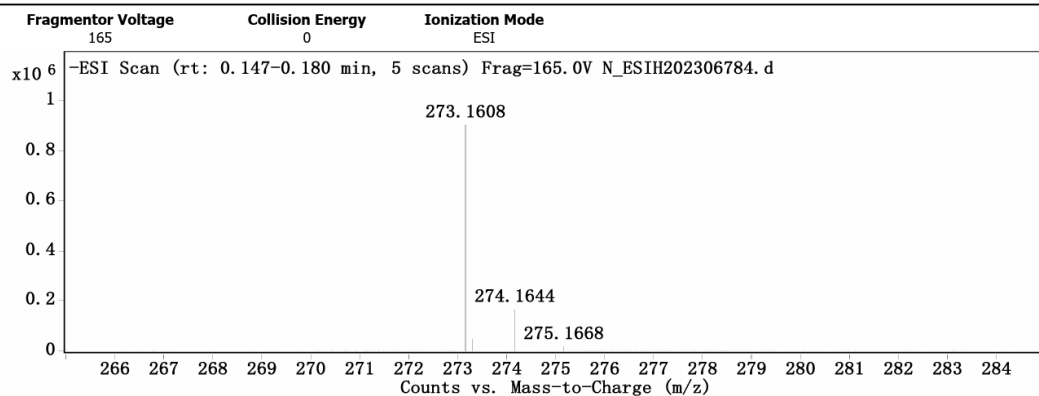


Figure S34. ^{13}C NMR spectrum of compound **5f**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306784.d | Sample Name | D4-D54 |
| Sample ID | | Position | P1-D3 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:58:36 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI/HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 273.1608 | 273.1609 | 0.08 | 0.3 | C16 H21 N2 O2 | (M-H)- |

--- End Of Report ---

Figure S35. HRESIMS spectrum of compound **5g**.

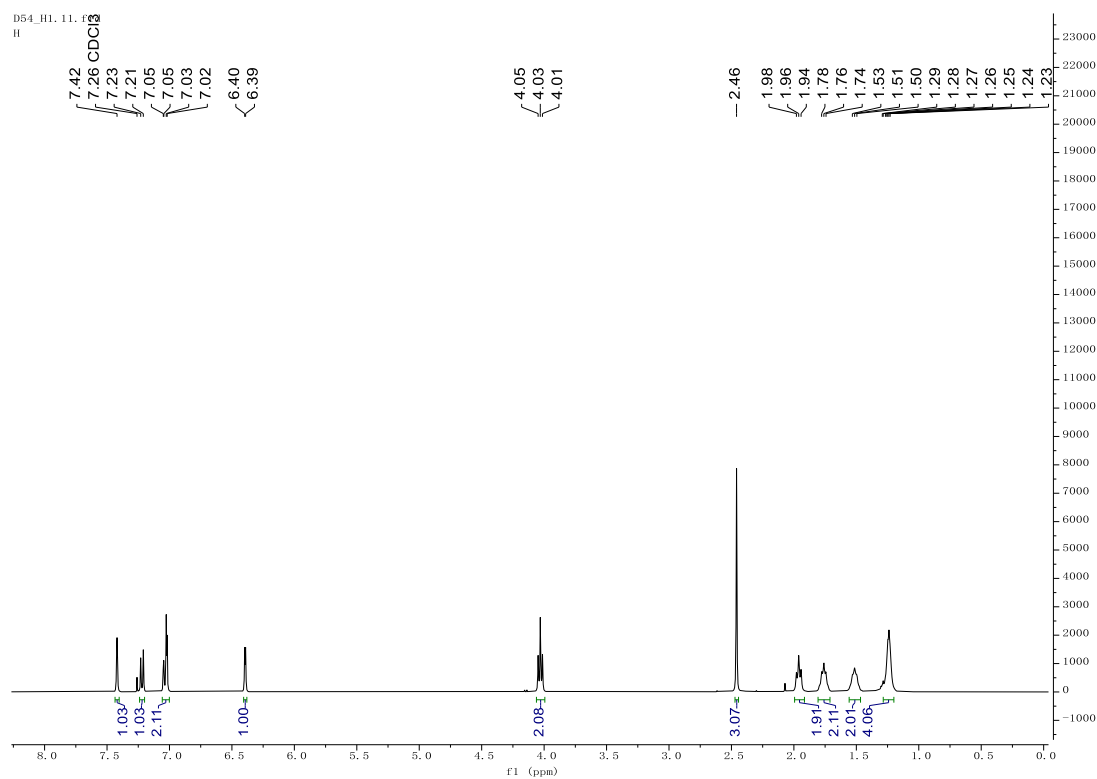


Figure S36. ^1H NMR spectrum of compound **5g**.

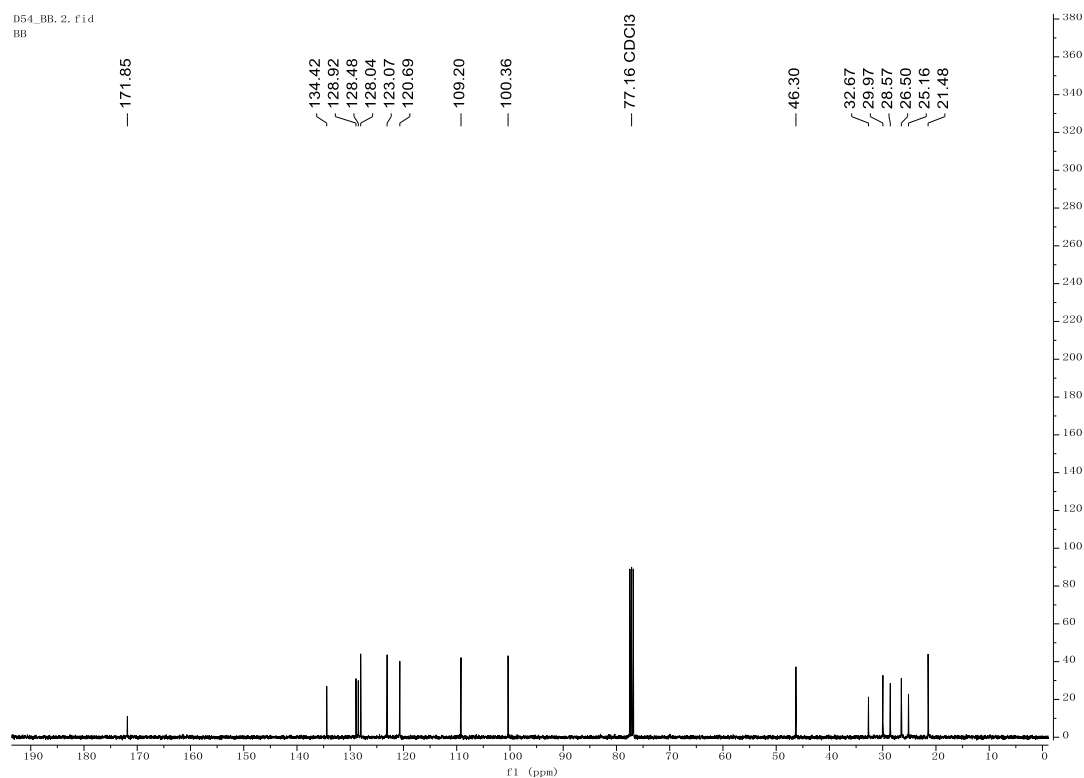
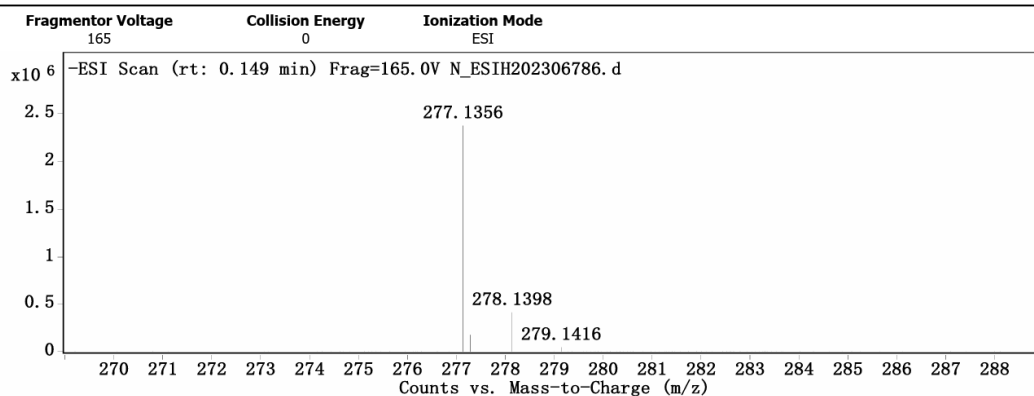


Figure S37. ¹³C NMR spectrum of compound **5g**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306786.d | Sample Name | D4-D56 |
| Sample ID | | Position | P1-D5 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:01:06 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|-----------------|--------------------|
| 277.1356 | 277.1358 | 0.17 | 0.61 | C15 H18 F N2 O2 | (M+H) ⁺ |

--- End Of Report ---

Figure S38. HRESIMS spectrum of compound **5h**.

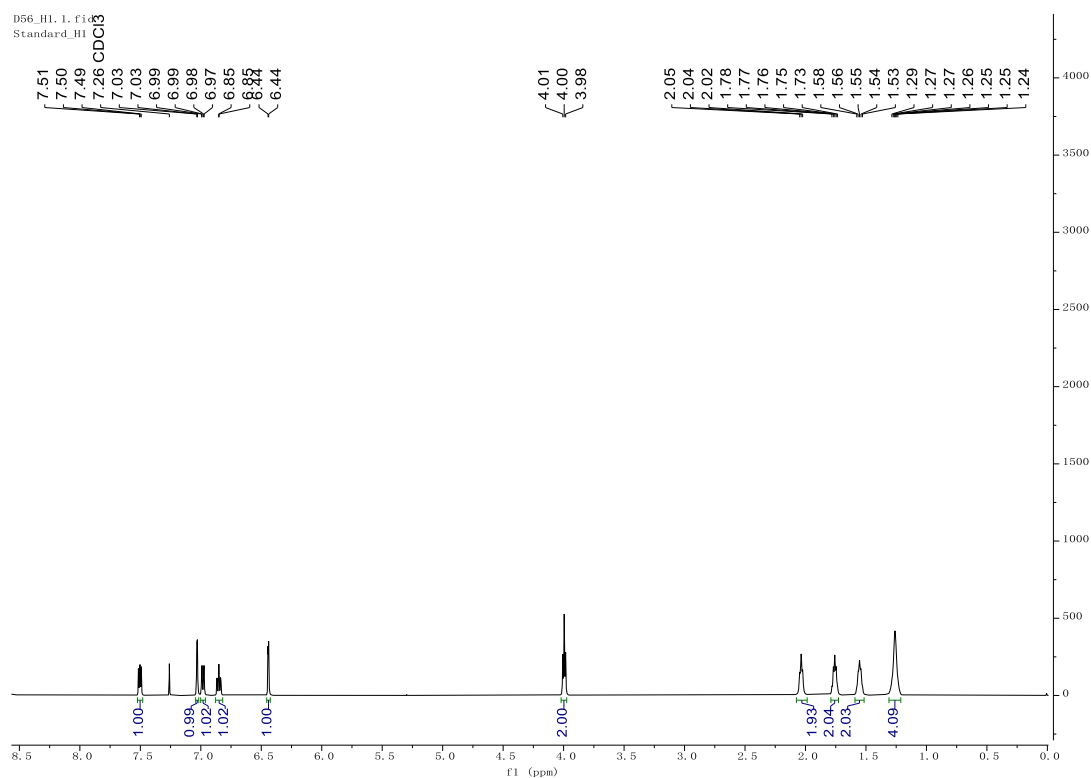


Figure S39. ^1H NMR spectrum of compound **5h**.

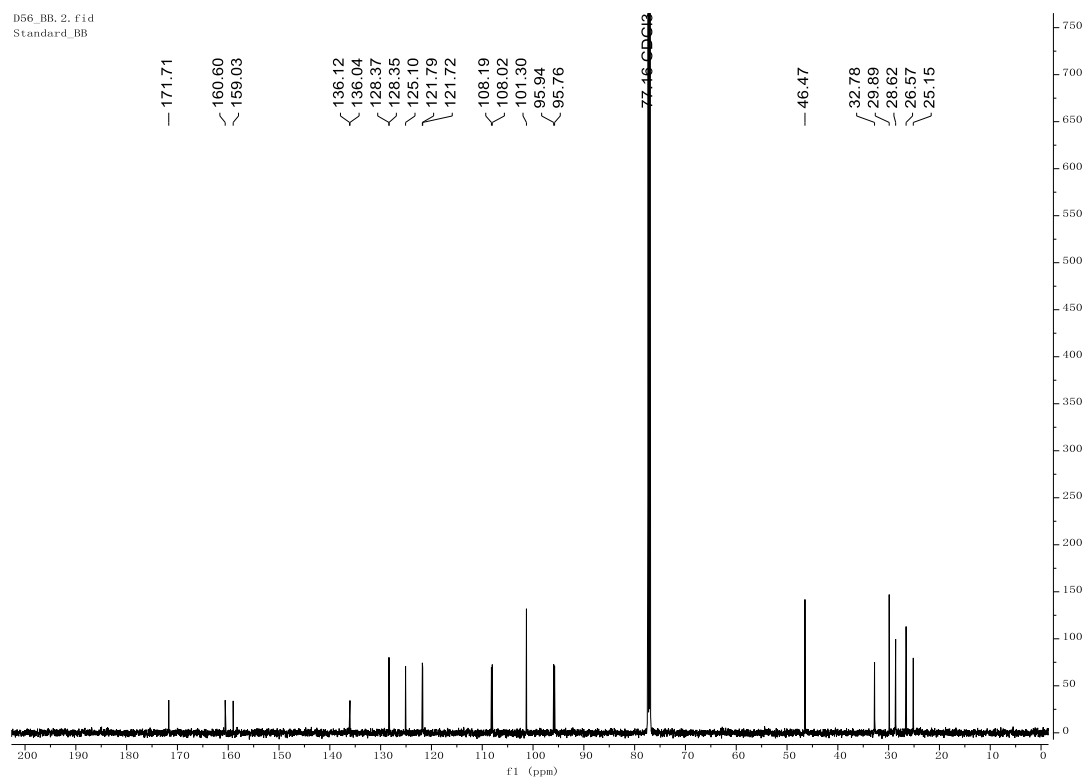
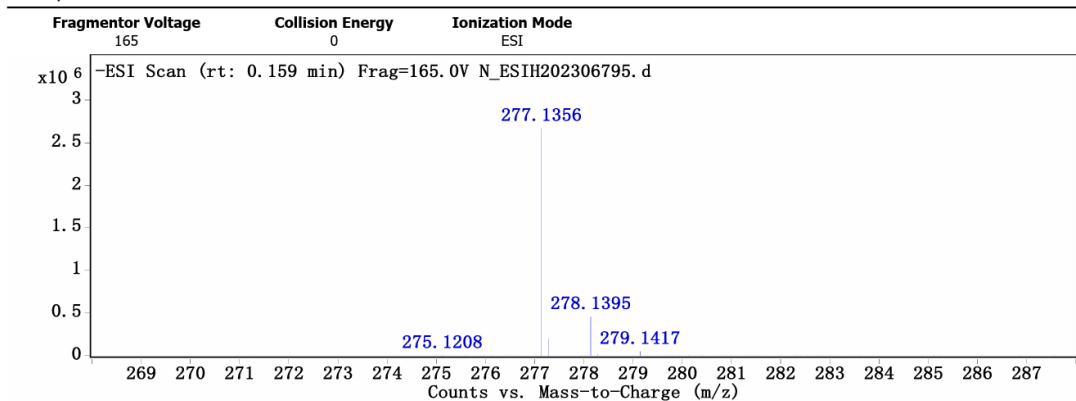


Figure S40. ^{13}C NMR spectrum of compound **5h**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306795.d | Sample Name | D4-D65 |
| Sample ID | | Position | P1-E5 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:12:33 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-HR by fangs | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|-----------------|--------------------|
| 277.1356 | 277.1358 | 0.23 | 0.81 | C15 H18 F N2 O2 | (M-H) ⁻ |

--- End Of Report ---

Figure S41. HRESIMS spectrum of compound **5i**.

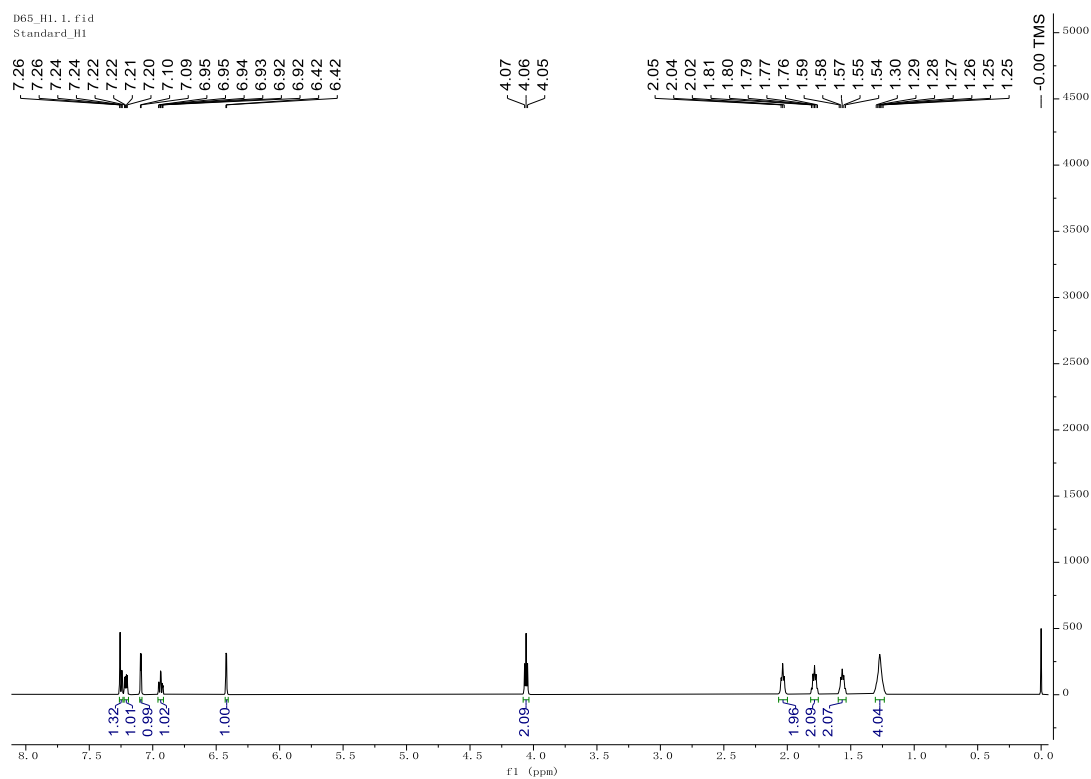


Figure S42. ¹H NMR spectrum of compound **5i**.

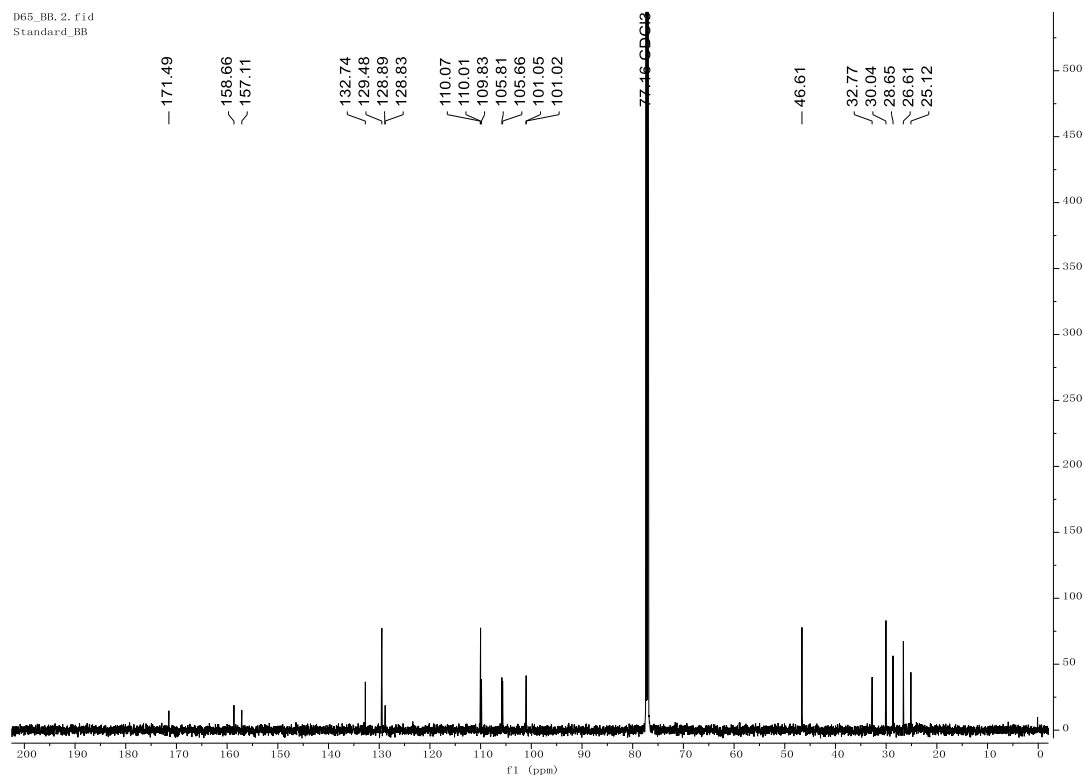
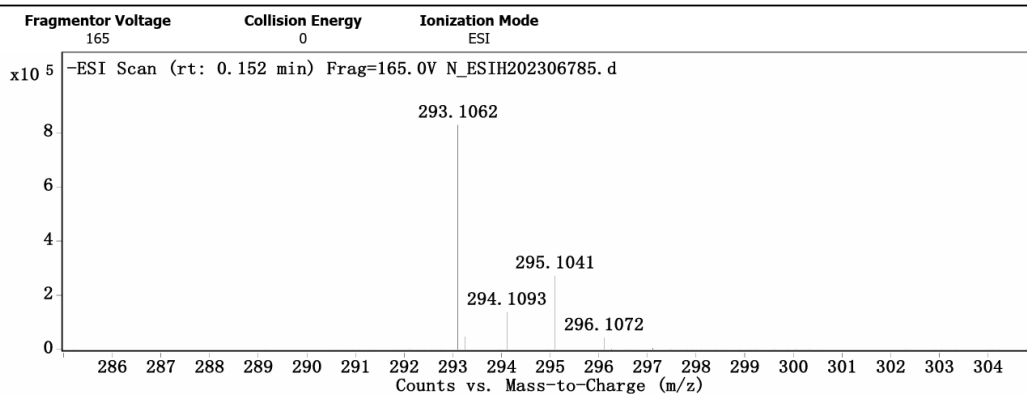


Figure S43. ¹³C NMR spectrum of compound **5i**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306785.d | Sample Name | D4-D55 |
| Sample ID | | Position | P1-D4 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:59:51 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-H by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|-----------------------------------------------------------------|--------------------|
| 293.1062 | 293.1062 | 0.04 | 0.12 | C ₁₅ H ₁₈ ClN ₂ O ₂ | (M-H) ⁻ |

--- End Of Report ---

Figure S44. HRESIMS spectrum of compound **5j**.

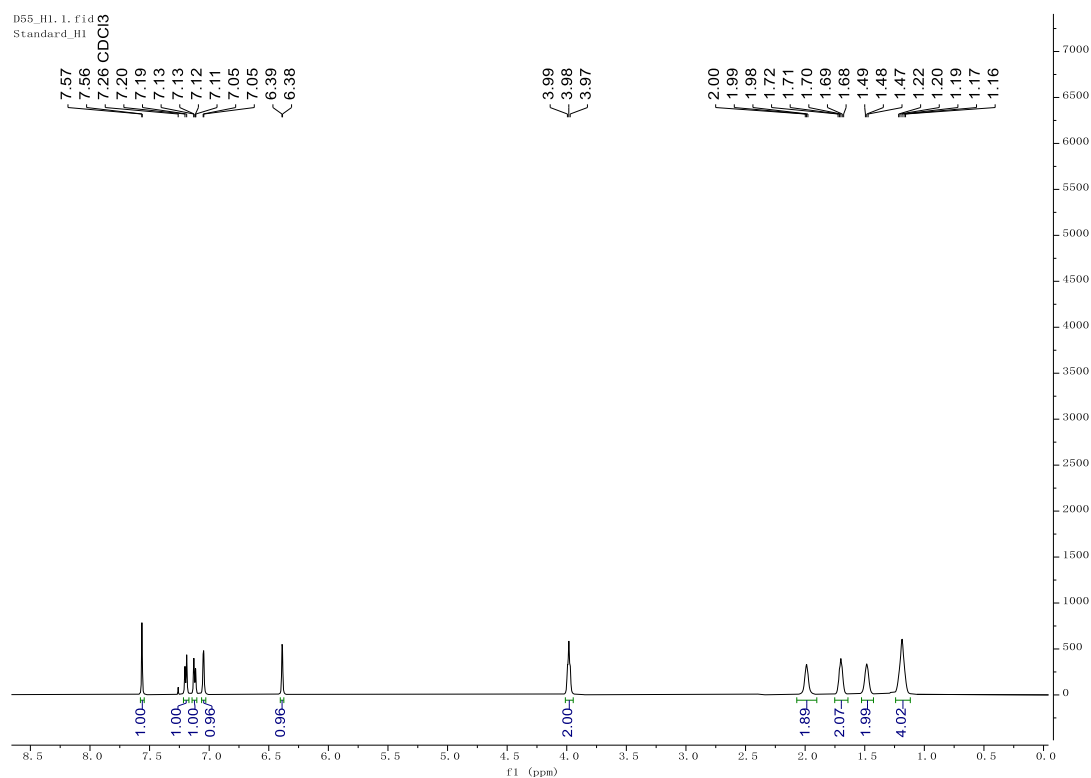


Figure S45. ^1H NMR spectrum of compound **5j**.

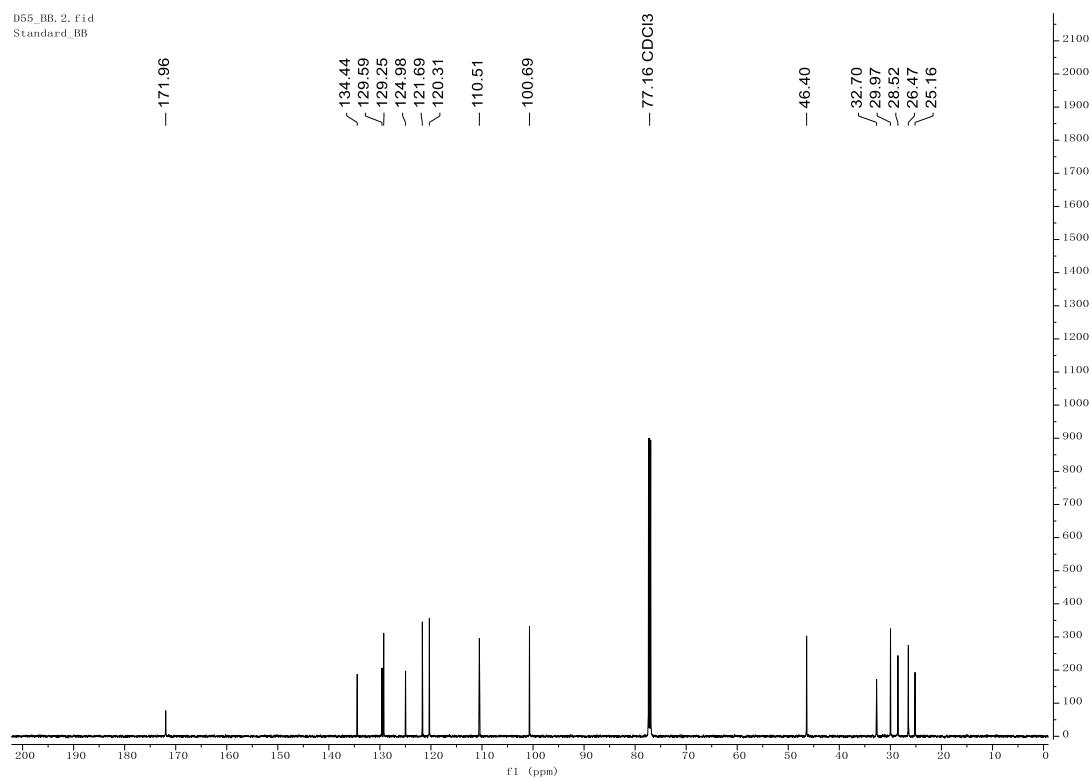
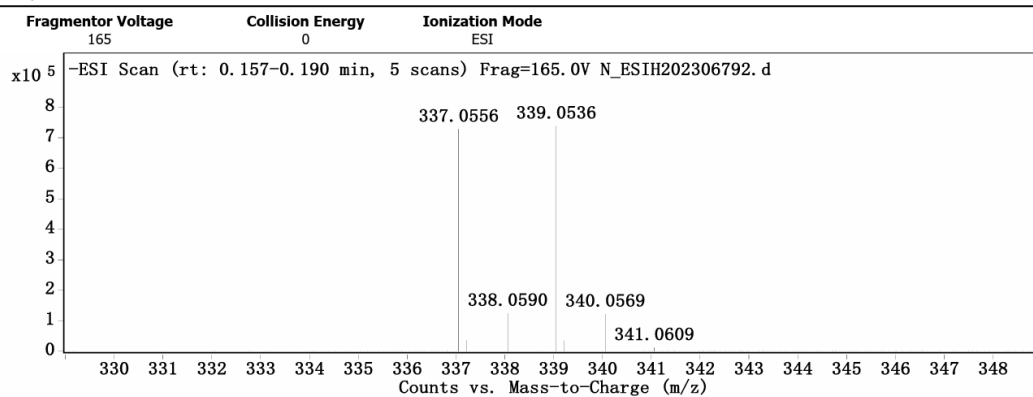


Figure S46. ^{13}C NMR spectrum of compound **5j**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306792.d | Sample Name | D4-D62 |
| Sample ID | | Position | P1-E2 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:08:42 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|------------------|--------|
| 337.0556 | 337.0557 | 0.13 | 0.39 | C15 H18 Br N2 O2 | (M-H)- |

--- End Of Report ---

Figure S47. HRESIMS spectrum of compound **5k**.

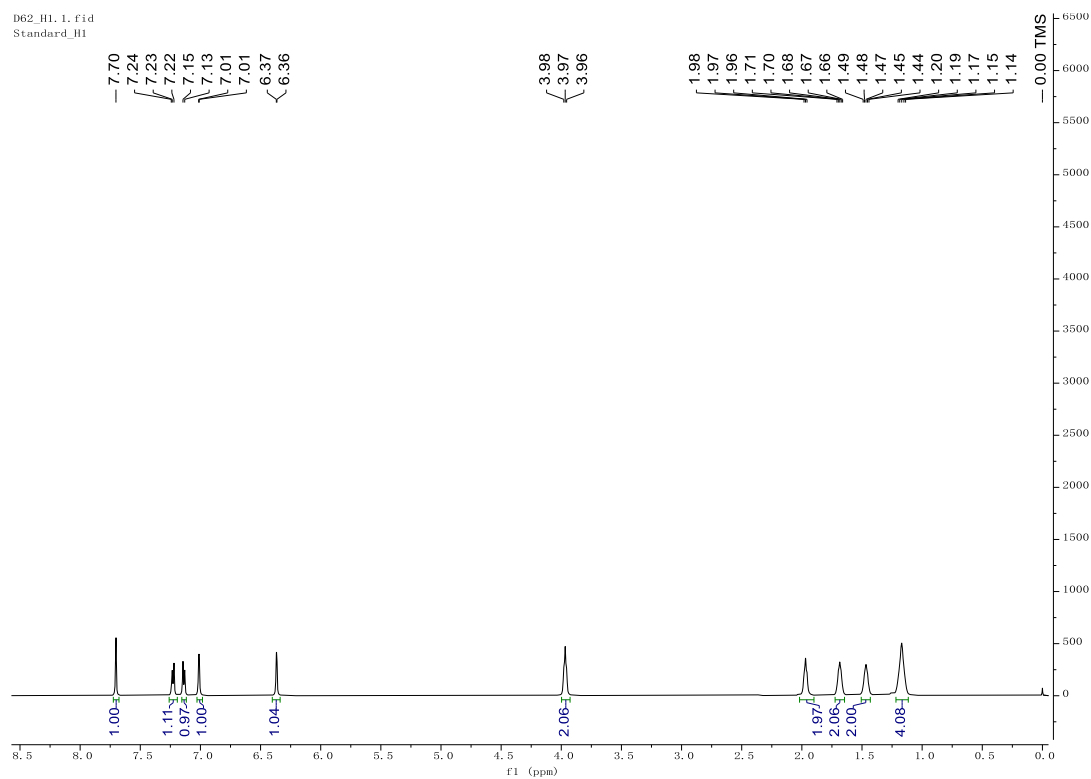


Figure S48. ¹H NMR spectrum of compound **5k**.

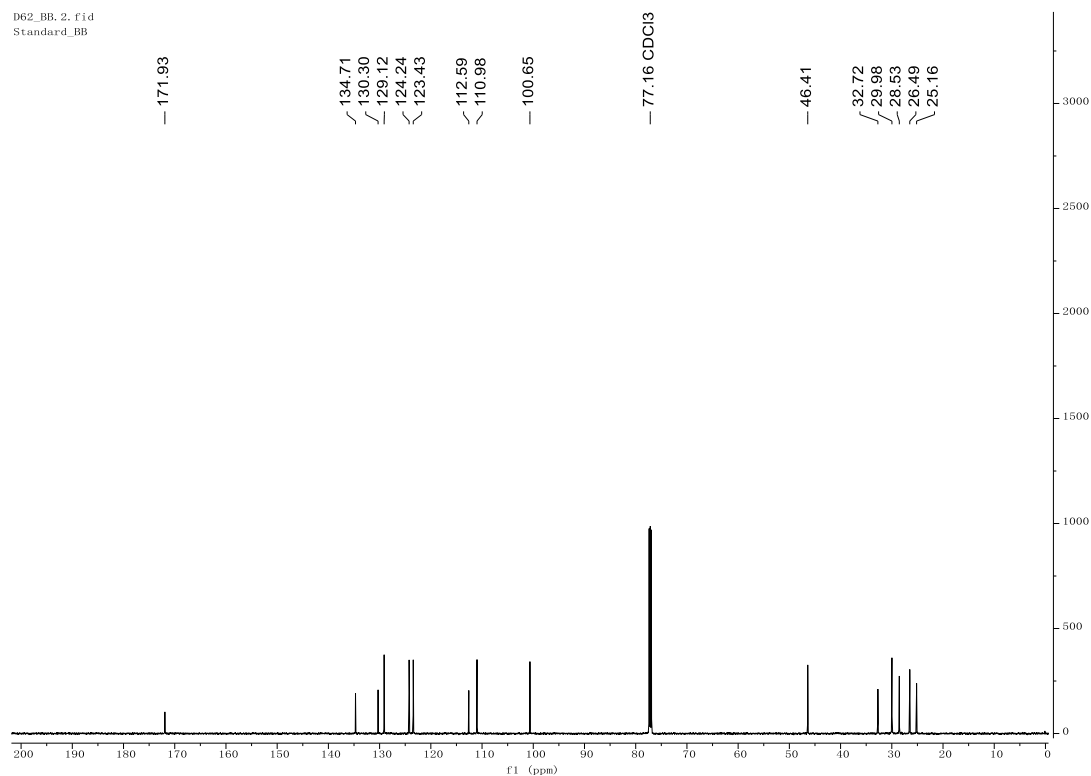
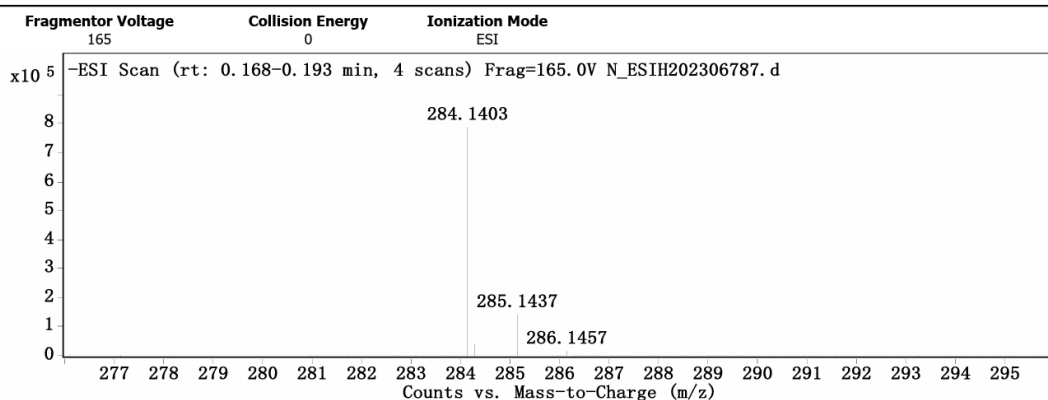


Figure S49. ¹³C NMR spectrum of compound **5k**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306787.d | Sample Name | D4-D57 |
| Sample ID | | Position | P1-D6 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 2:02:21 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI/HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 284.1403 | 284.1405 | 0.16 | 0.56 | C ₁₆ H ₁₈ N ₃ O ₂ | (M-H) ⁻ |

--- End Of Report ---

Figure S50. HRESIMS spectrum of compound **5l**.

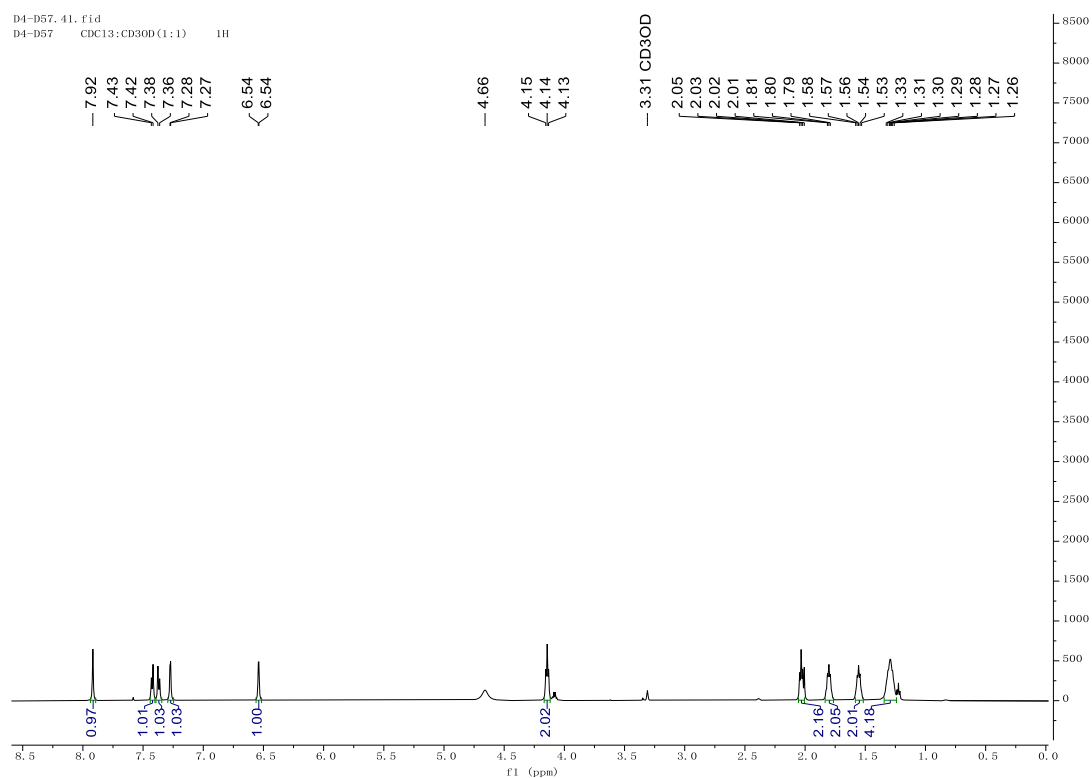


Figure S51. ^1H NMR spectrum of compound **5l**.

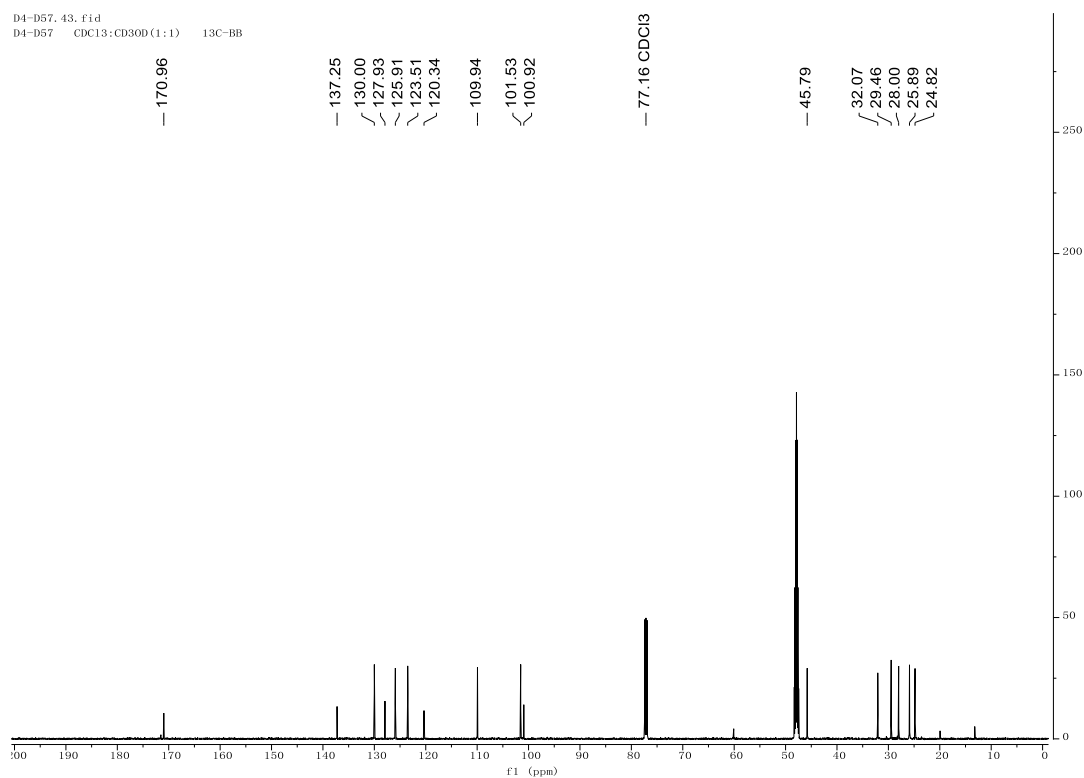
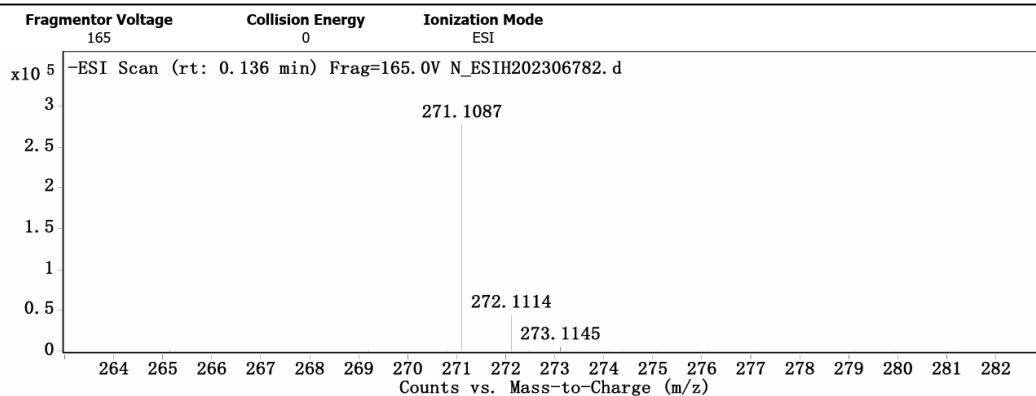


Figure S52. ^{13}C NMR spectrum of compound **5l**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306782.d | Sample Name | D4-D36 |
| Sample ID | | Position | P1-D1 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:56:03 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 271.1087 | 271.1088 | 0.16 | 0.58 | C15 H15 N2 O3 | (M-H)- |

--- End Of Report ---

Figure S53. HRESIMS spectrum of compound **11**.

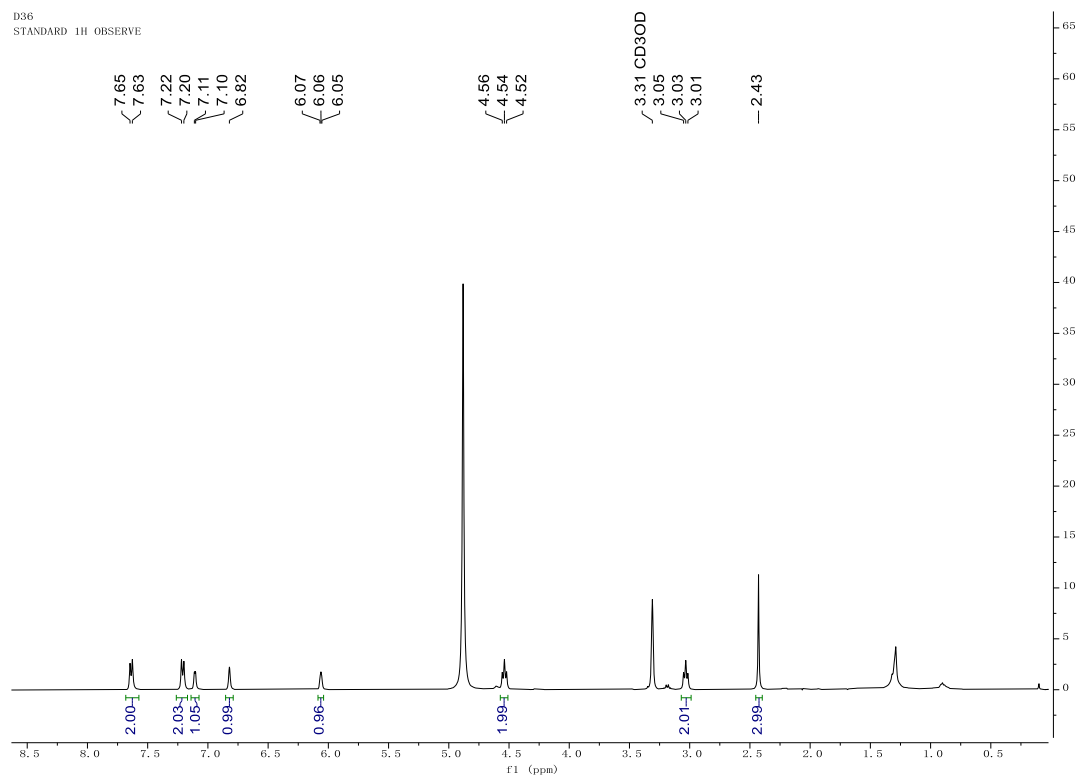


Figure S54. ^1H NMR spectrum of compound **11**.

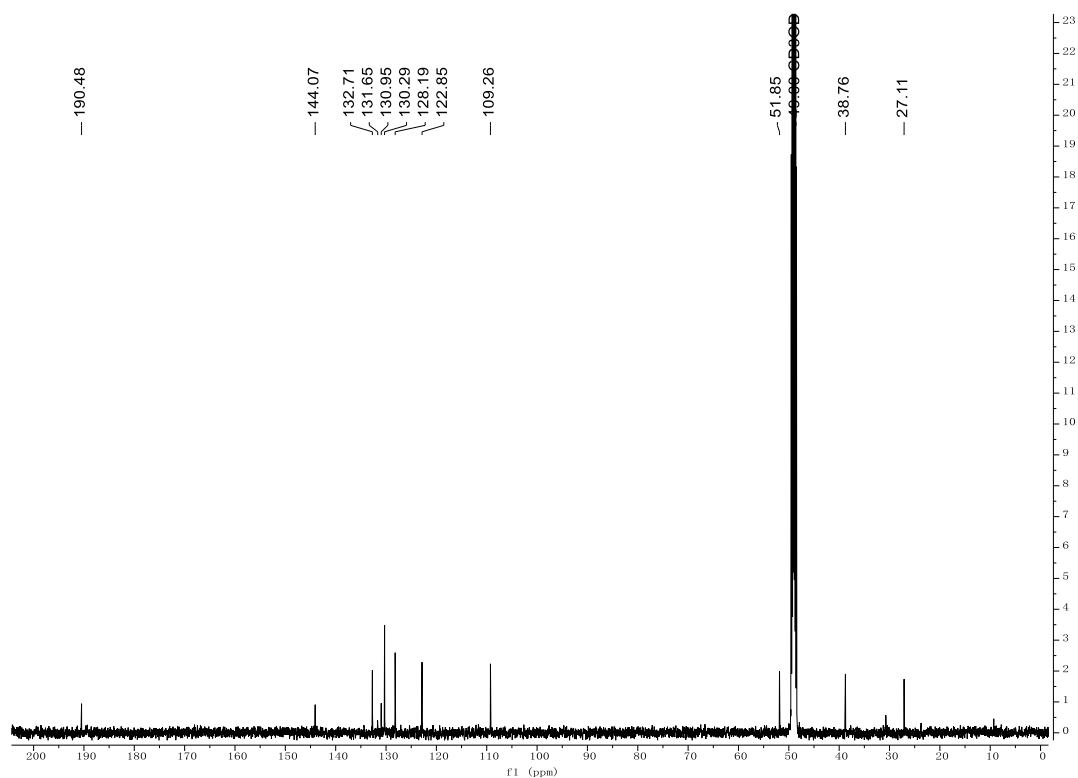
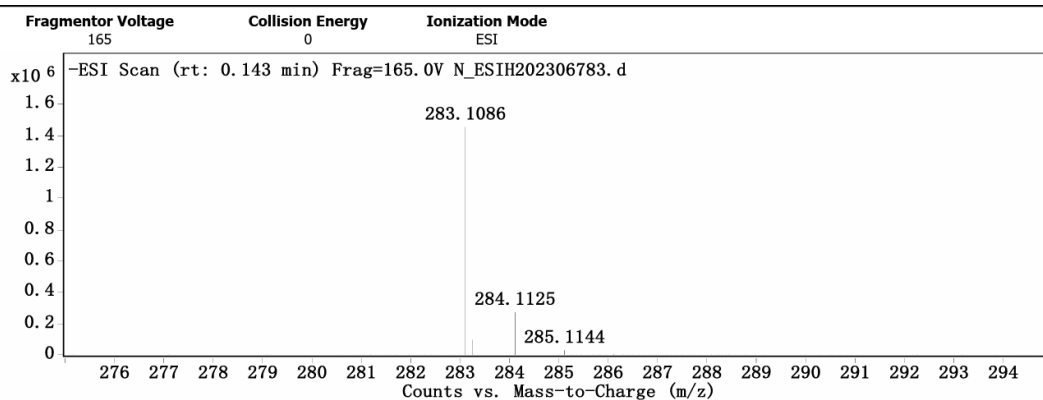


Figure S55. ^{13}C NMR spectrum of compound **11**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306783.d | Sample Name | D4-D53 |
| Sample ID | | Position | P1-D2 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:57:18 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 283.1086 | 283.1088 | 0.17 | 0.59 | C16 H15 N2 O3 | (M-H)- |

--- End Of Report ---

Figure S56. HRESIMS spectrum of compound **14**.

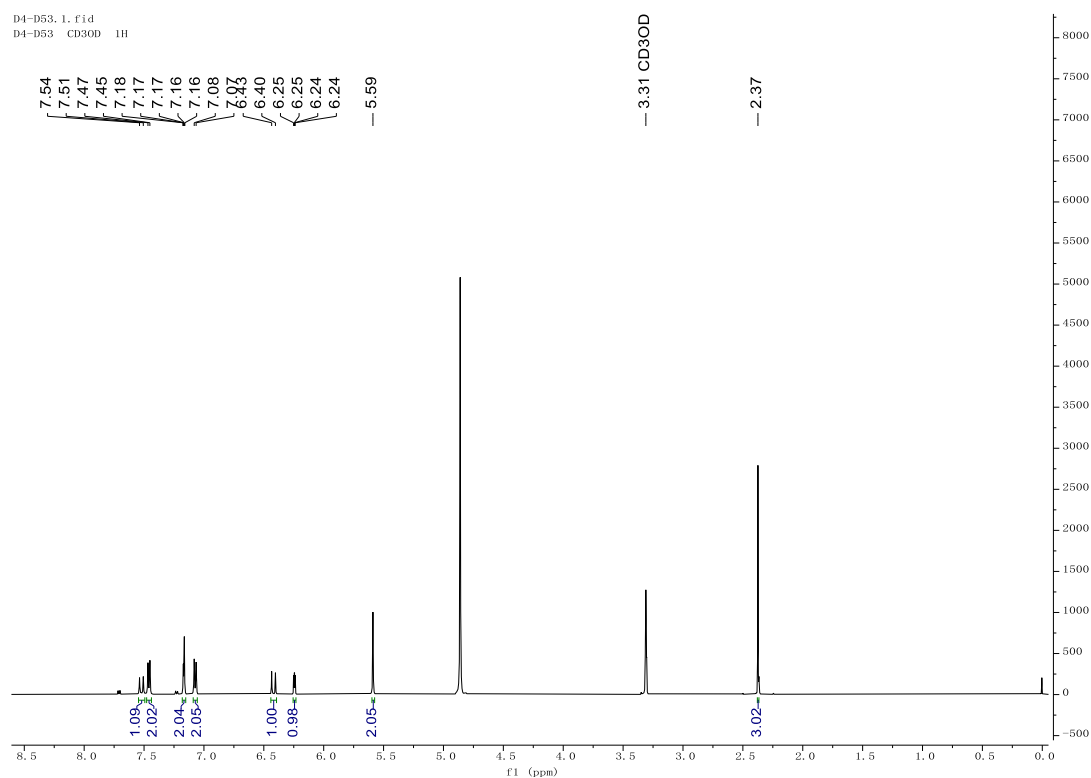


Figure S57. ^1H NMR spectrum of compound **14**.

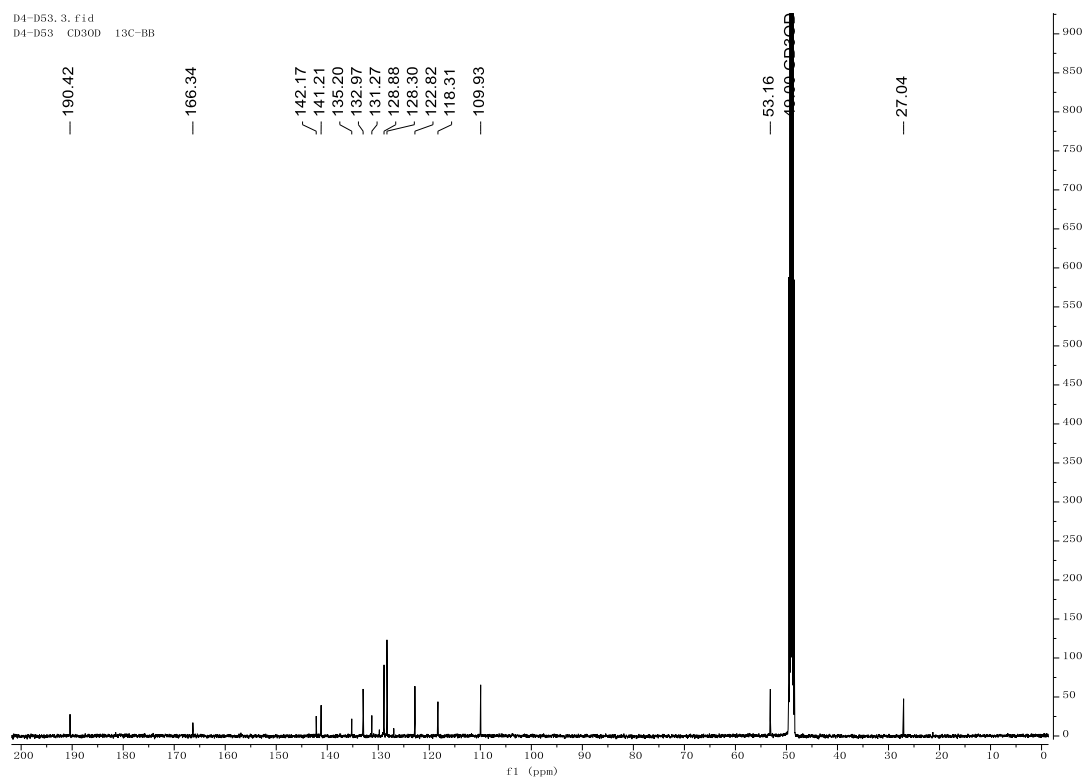
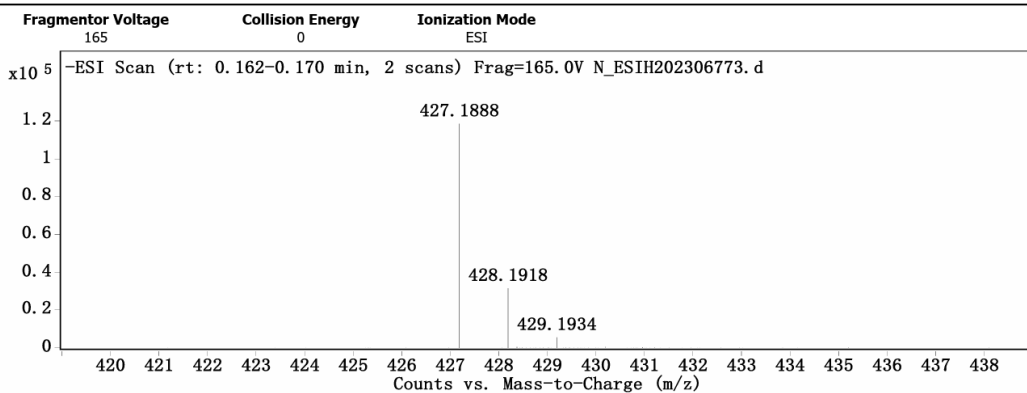


Figure S58. ^{13}C NMR spectrum of compound **14**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306773.d | Sample Name | D4-H21 |
| Sample ID | | Position | P1-C1 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:44:35 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESI-HR by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------|
| 427.1888 | 427.1888 | -0.03 | -0.08 | C24 H23 N6 O2 | (M-H)- |

--- End Of Report ---

Figure S59. HRESIMS spectrum of compound **19a**.

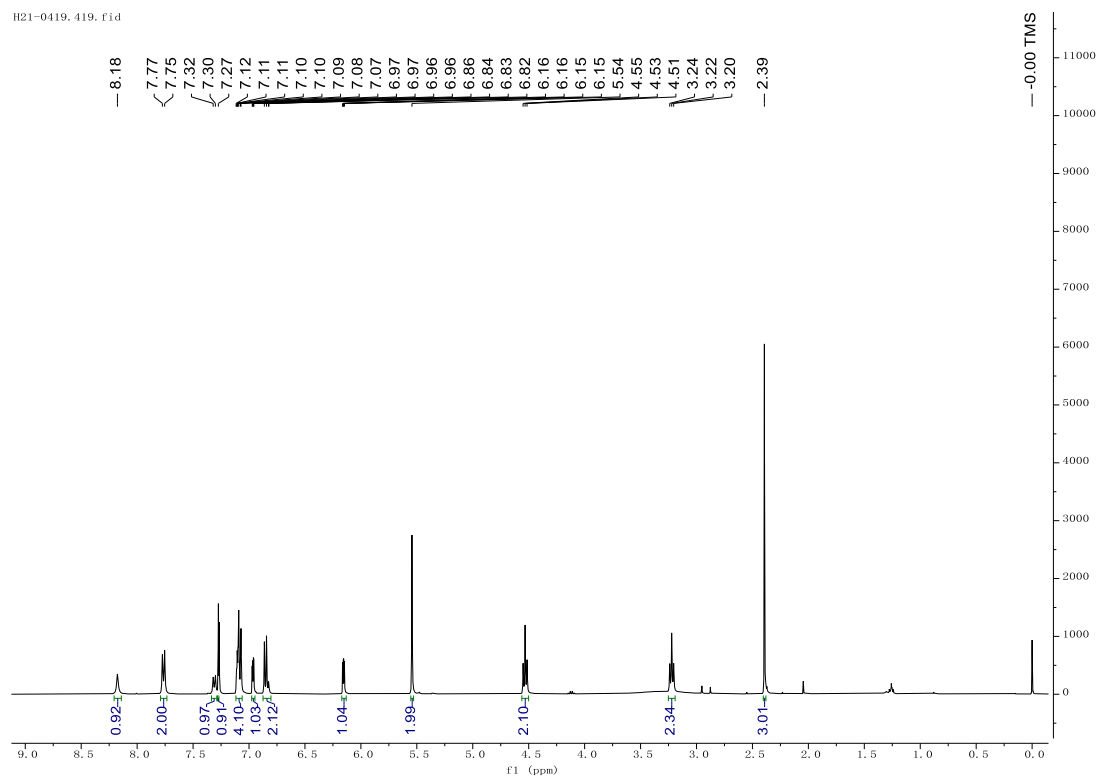


Figure S60. ^1H NMR spectrum of compound **19a**.

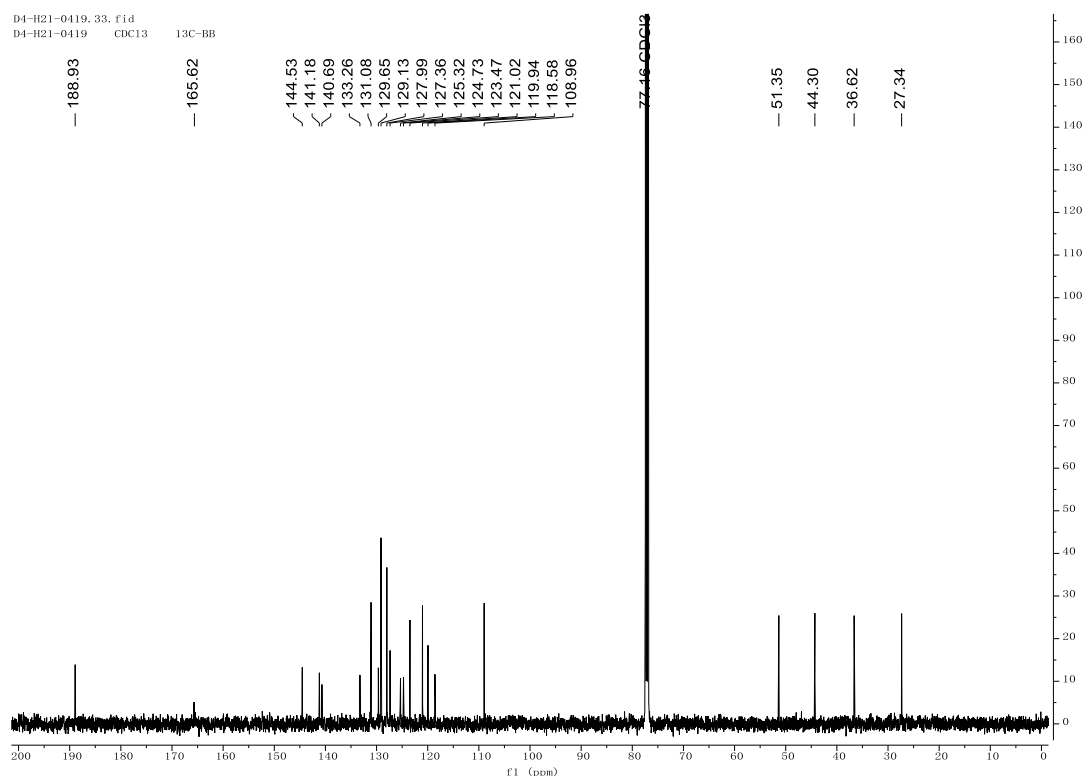
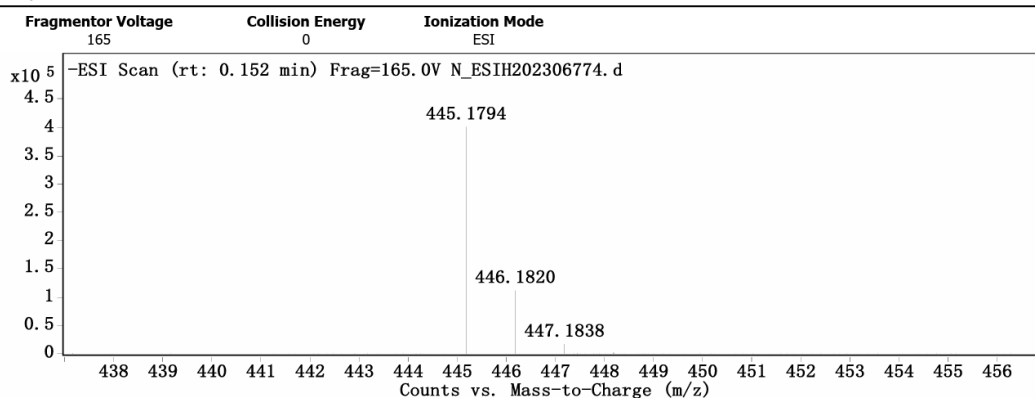


Figure S61. ¹³C NMR spectrum of compound **19a**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306774.d | Sample Name | D4-H40 |
| Sample ID | | Position | P1-C2 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:45:52 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|-----------------------------------------------------------------|--------------------|
| 445.1794 | 445.1794 | -0.07 | -0.15 | C ₂₄ H ₂₂ F N ₆ O ₂ | (M-H) ⁻ |

--- End Of Report ---

Figure S62. HRESIMS spectrum of compound **19b**.

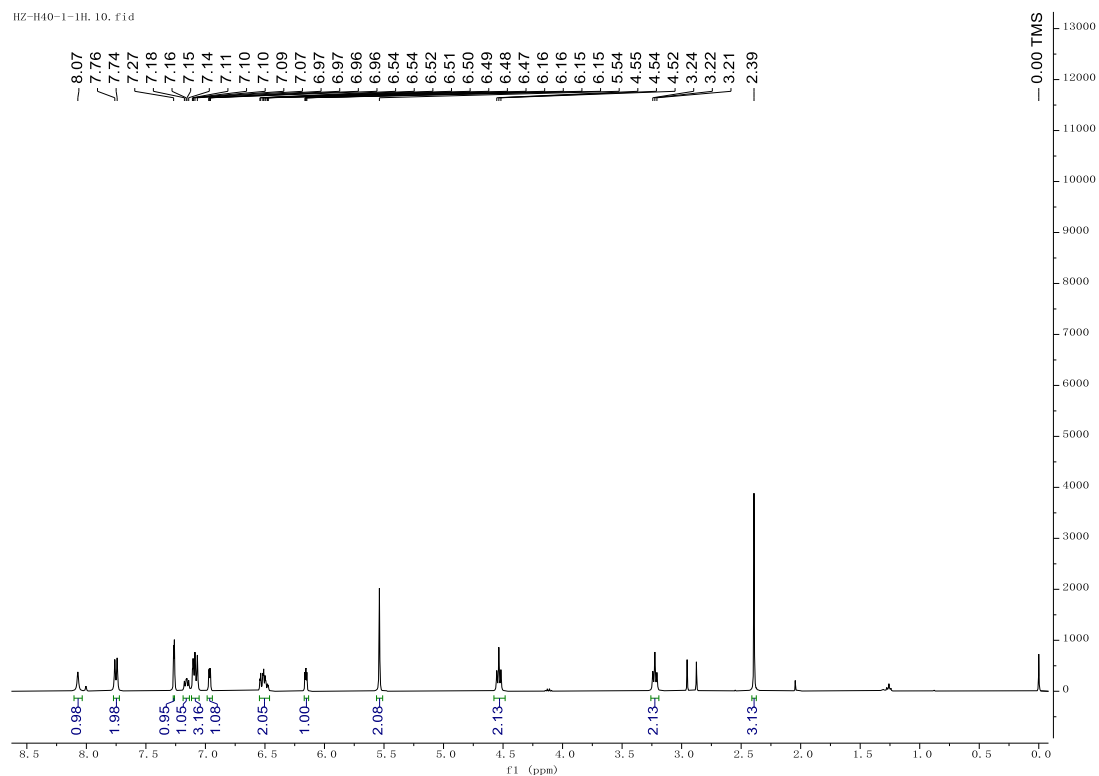


Figure S63. ^1H NMR spectrum of compound **19b**.

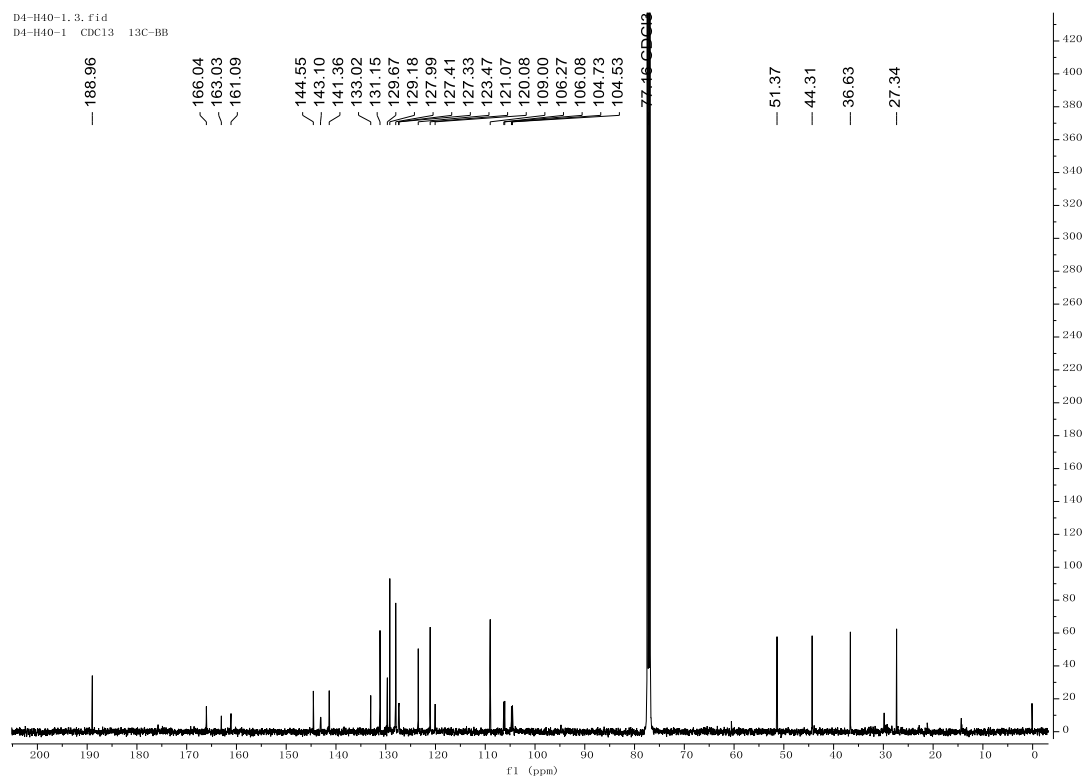
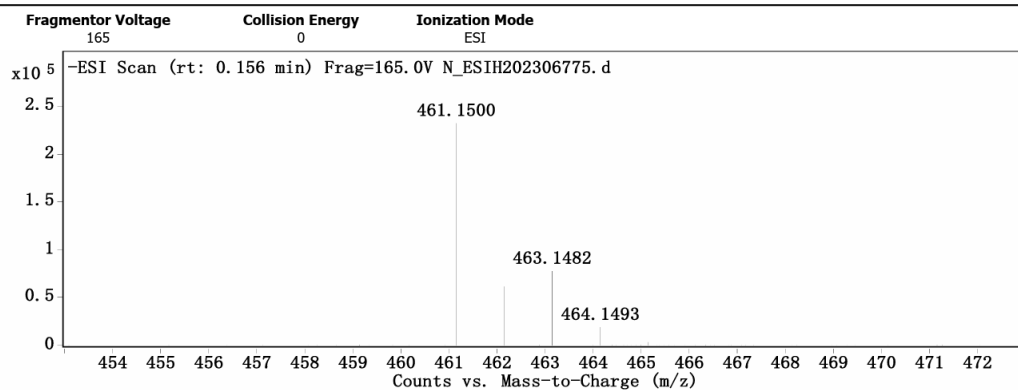


Figure S64. ^{13}C NMR spectrum of compound **19b**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306775.d | Sample Name | D4-H41 |
| Sample ID | | Position | P1-C3 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:47:10 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|--------|----------|------------|------------|------------------|--------|
| 461.15 | 461.1498 | -0.16 | -0.35 | C24 H22 Cl N6 O2 | (M-H)- |

--- End Of Report ---

Figure S65. HRESIMS spectrum of compound **19c**.

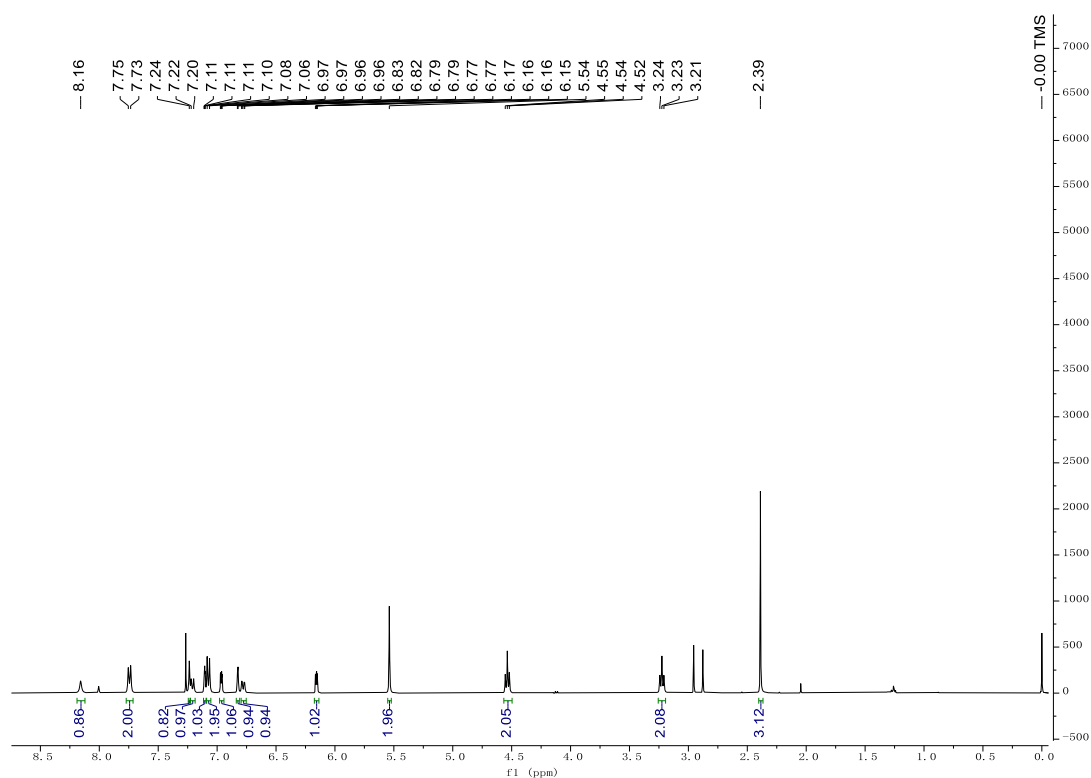


Figure S66. ¹H NMR spectrum of compound **19c**.

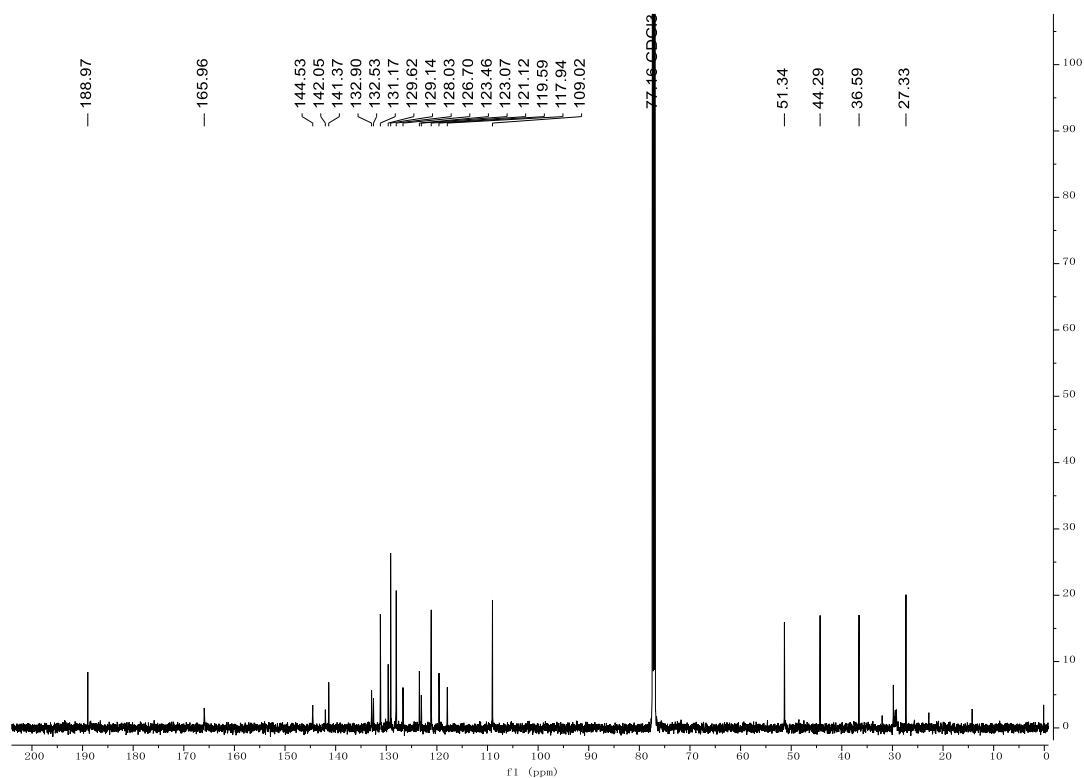
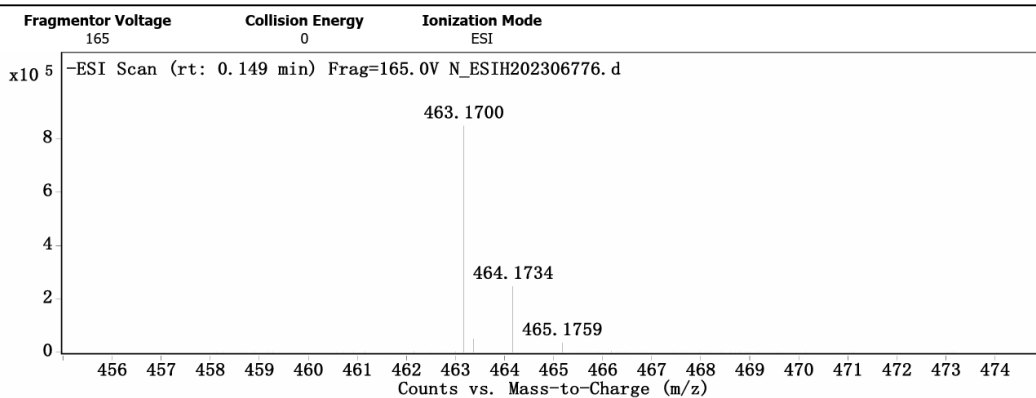


Figure S67. ^{13}C NMR spectrum of compound **19c**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306776.d | Sample Name | D4-H42 |
| Sample ID | | Position | P1-C4 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:48:28 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|--------|----------|------------|------------|------------------------------------------------------------------------------|--------------------|
| 463.17 | 463.17 | -0.03 | -0.06 | C ₂₄ H ₂₁ F ₂ N ₆ O ₂ | (M-H) ⁻ |

--- End Of Report ---

Figure S68. HRESIMS spectrum of compound **19d**.

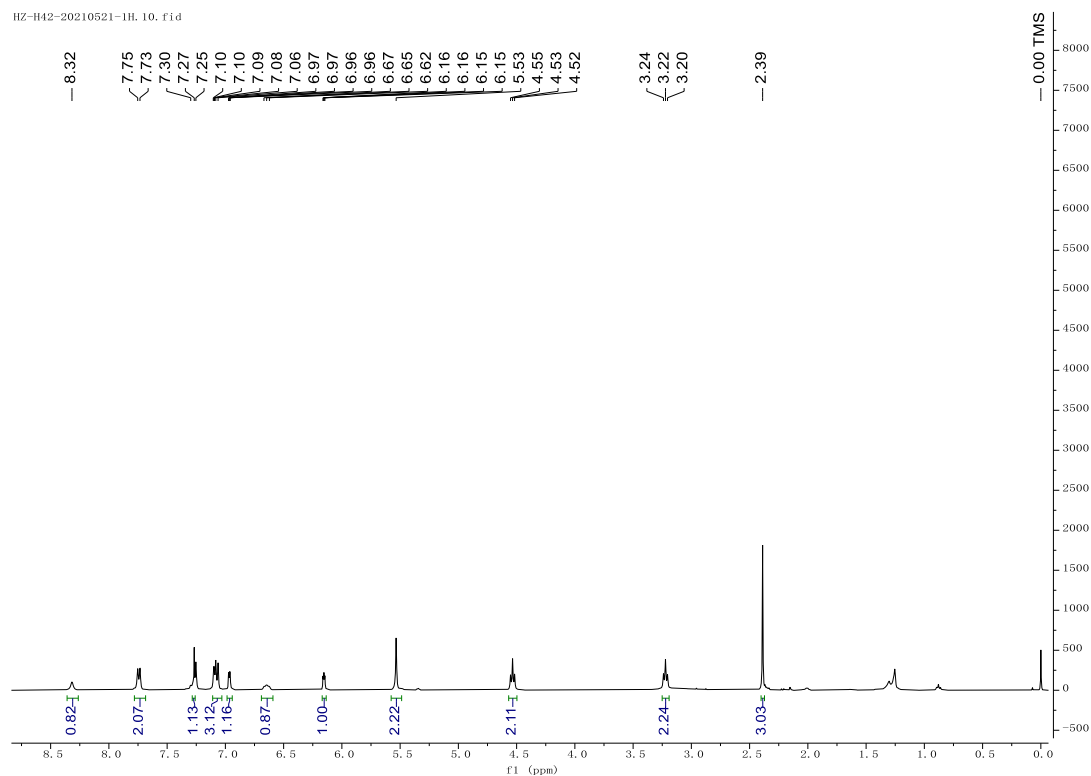


Figure S69. ^1H NMR spectrum of compound **19d**.

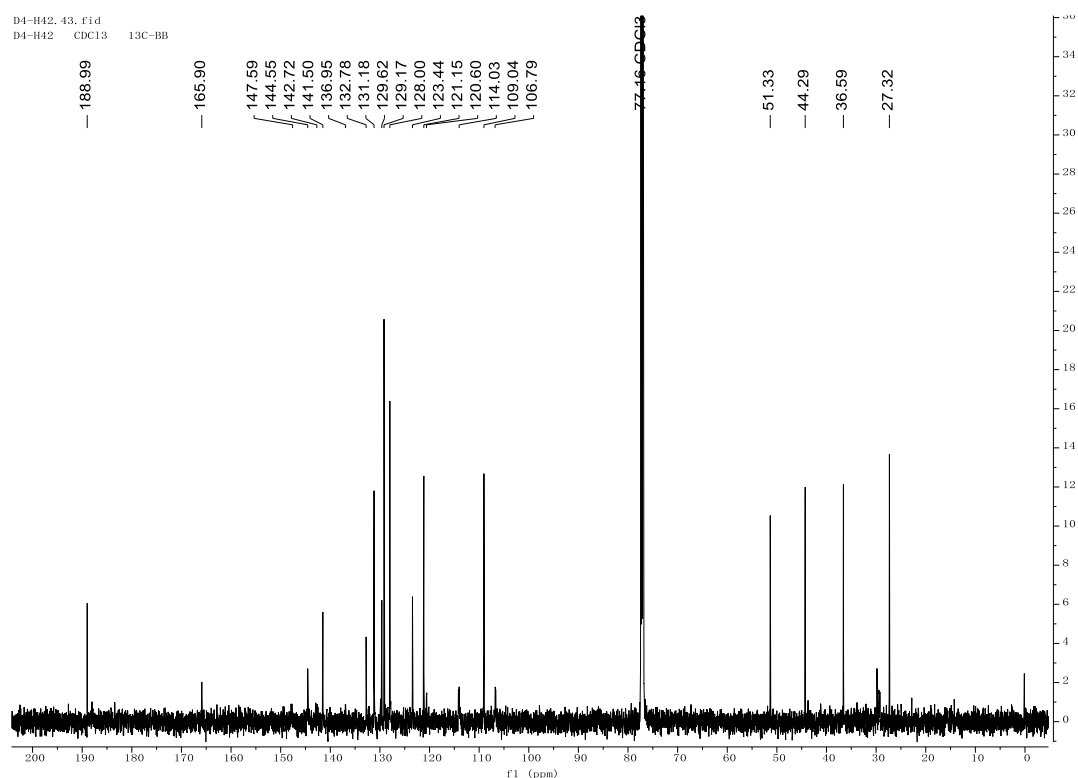
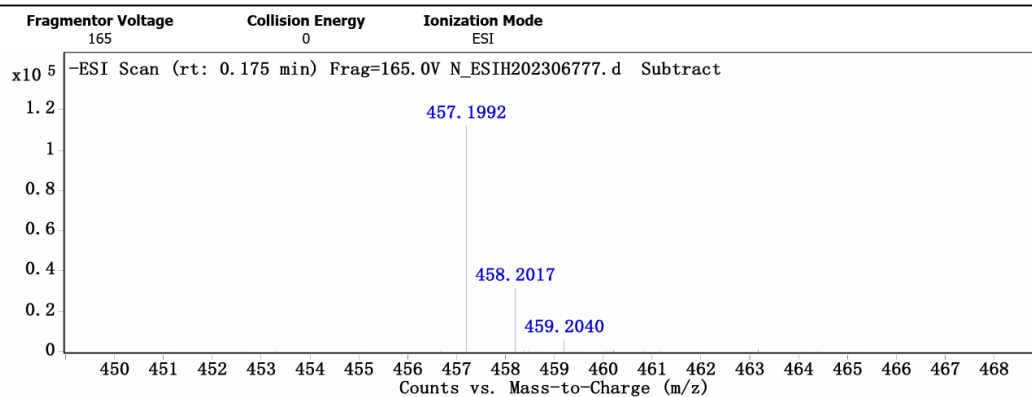


Figure S70. ^{13}C NMR spectrum of compound **19d**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306777.d | Sample Name | D4-H43 |
| Sample ID | | Position | P1-C5 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:49:42 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------|--------------------|
| 457.1992 | 457.1994 | 0.14 | 0.3 | C25 H25 N6 O3 | (M-H) ⁻ |

--- End Of Report ---

Figure S71. HRESIMS spectrum of compound **19e**.

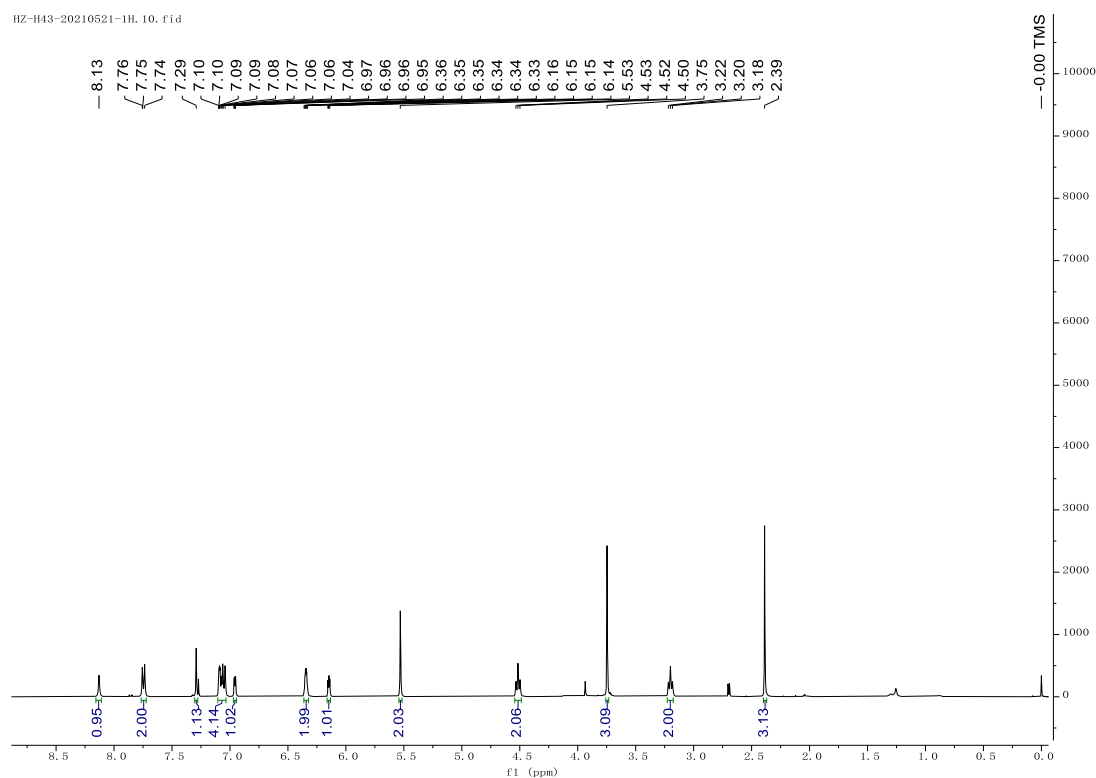


Figure S72. ¹H NMR spectrum of compound **19e**.

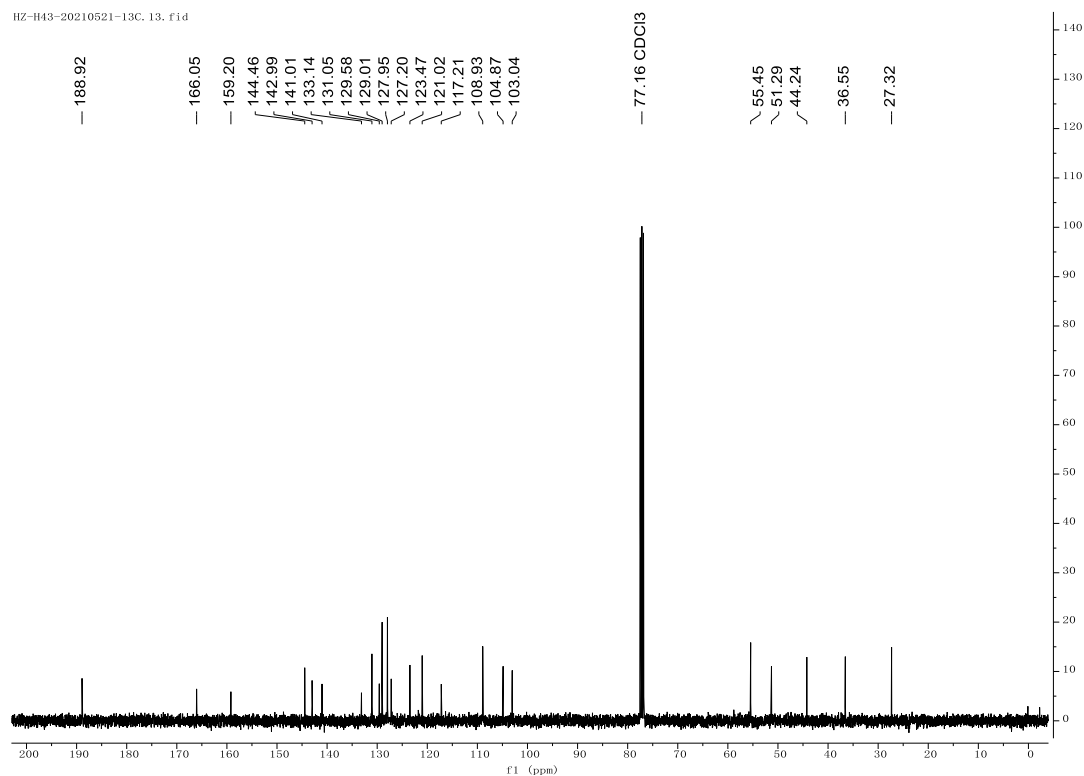
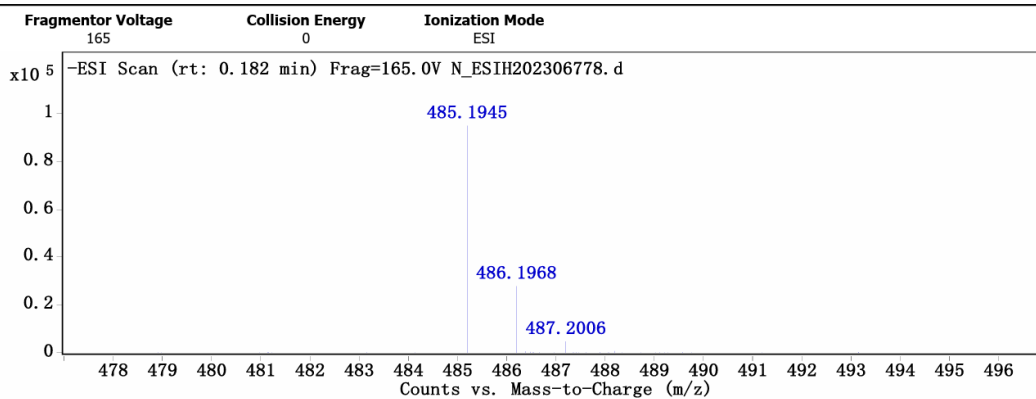


Figure S73. ¹³C NMR spectrum of compound **19e**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306778.d | Sample Name | D4-H44 |
| Sample ID | | Position | P1-C6 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:50:58 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|----------|----------|------------|------------|---------------------------------------------------------------|--------------------|
| 485.1945 | 485.1943 | -0.25 | -0.53 | C ₂₆ H ₂₅ N ₆ O ₄ | (M-H) ⁻ |

--- End Of Report ---

Figure S74. HRESIMS spectrum of compound **19f**.

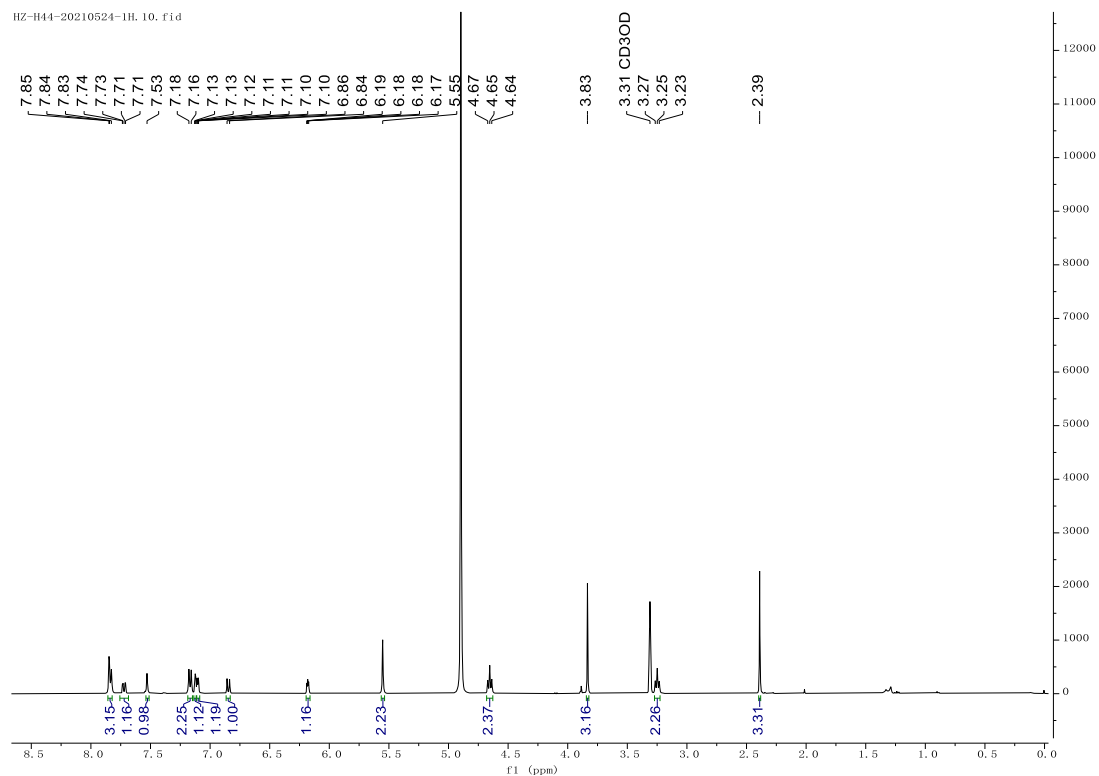


Figure S75. ^1H NMR spectrum of compound **19f**.

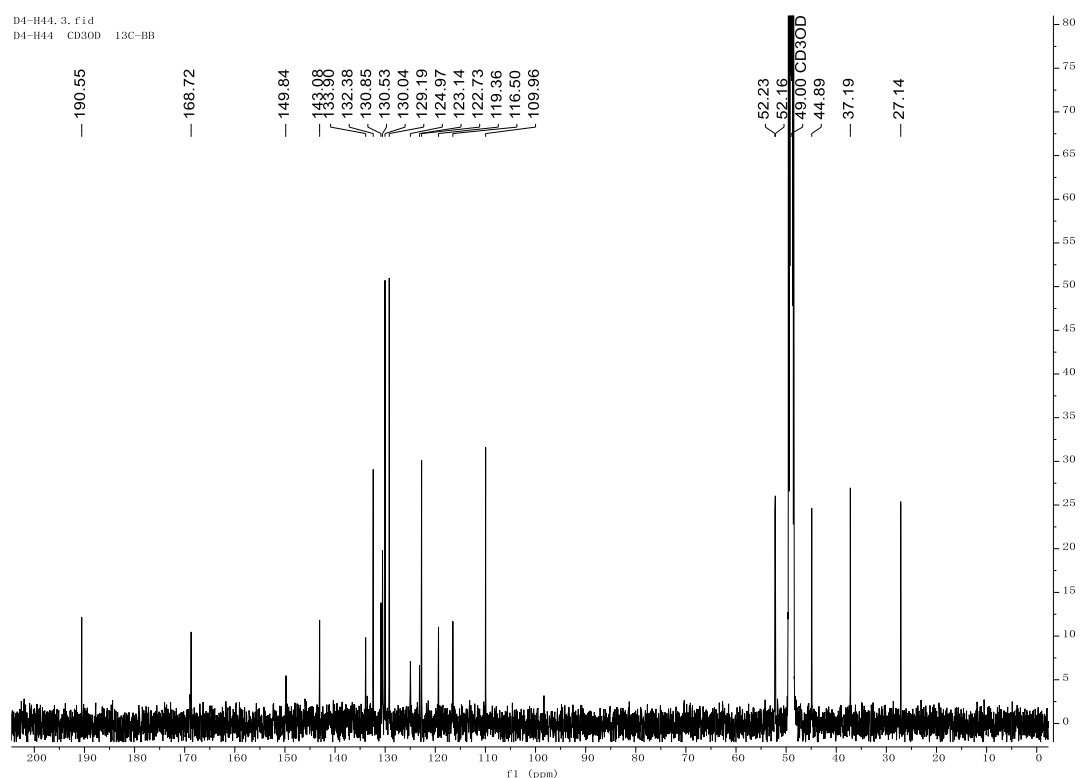
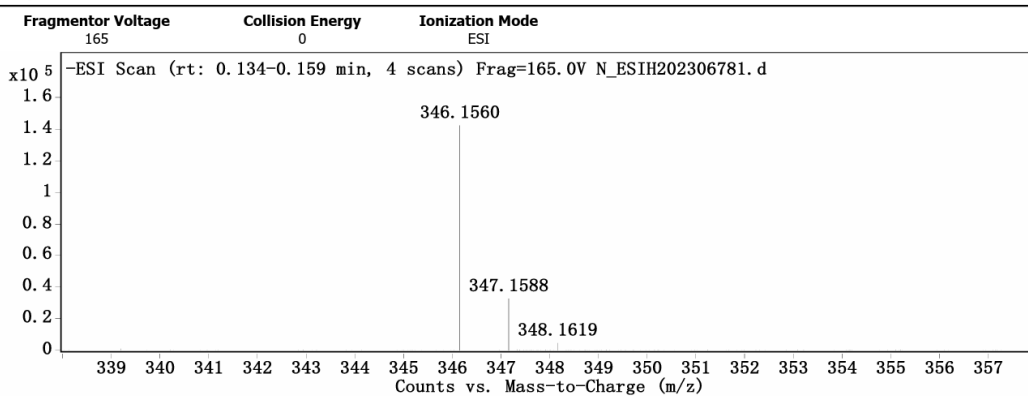


Figure S76. ^{13}C NMR spectrum of compound **19f**.

| | | | |
|------------------------|-----------------------|--------------------|-----------------------------|
| Data Filename | N_ESIH202306781.d | Sample Name | D4-D31 |
| Sample ID | | Position | P1-C9 |
| Instrument Name | Agilent 6520 Q-TOF | Acq Method | 20160324_MS_ESIH_NEG_1min.m |
| Acquired Time | 11/27/2023 1:54:47 PM | DA Method | ESI-HR-20231114.m |
| Comment | ESIH by fangsu | | |

User Spectra



Formula Calculator Results

| m/z | Calc m/z | Diff (mDa) | Diff (ppm) | Ion Formula | Ion |
|---------|----------|------------|------------|---------------|--------|
| 346.156 | 346.1561 | 0.08 | 0.24 | C21 H20 N3 O2 | (M-H)- |

--- End Of Report ---

Figure S77. HRESIMS spectrum of compound **20**.

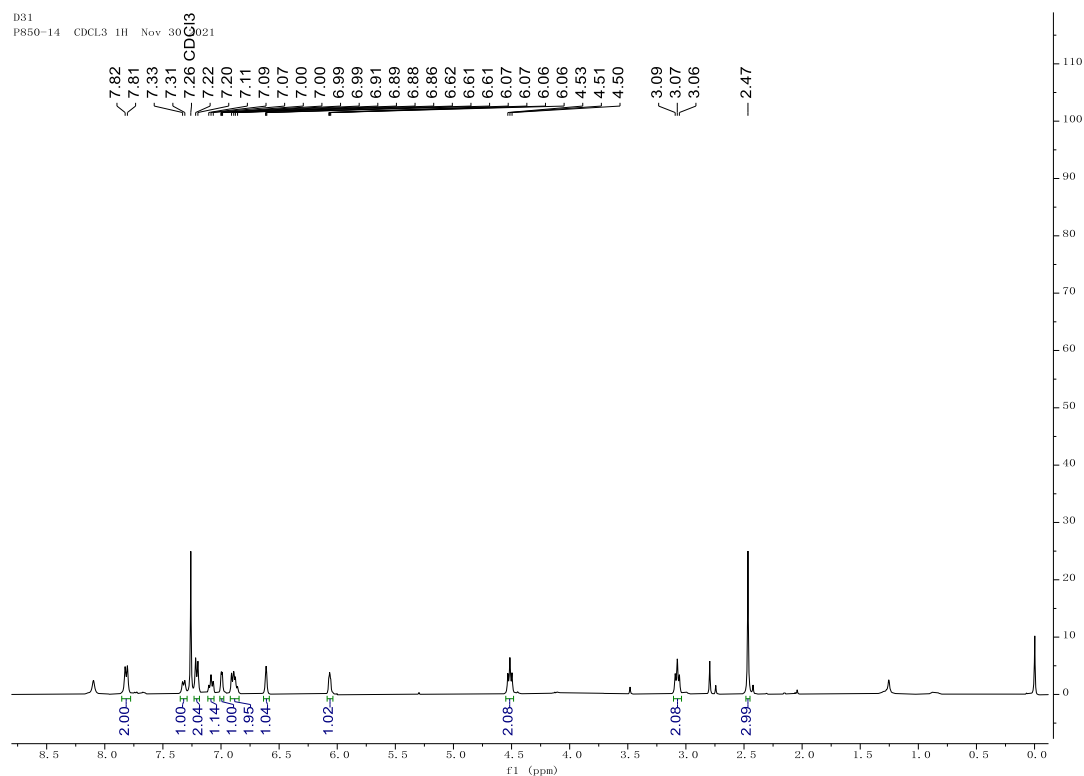


Figure S78. ^1H NMR spectrum of compound **20**.

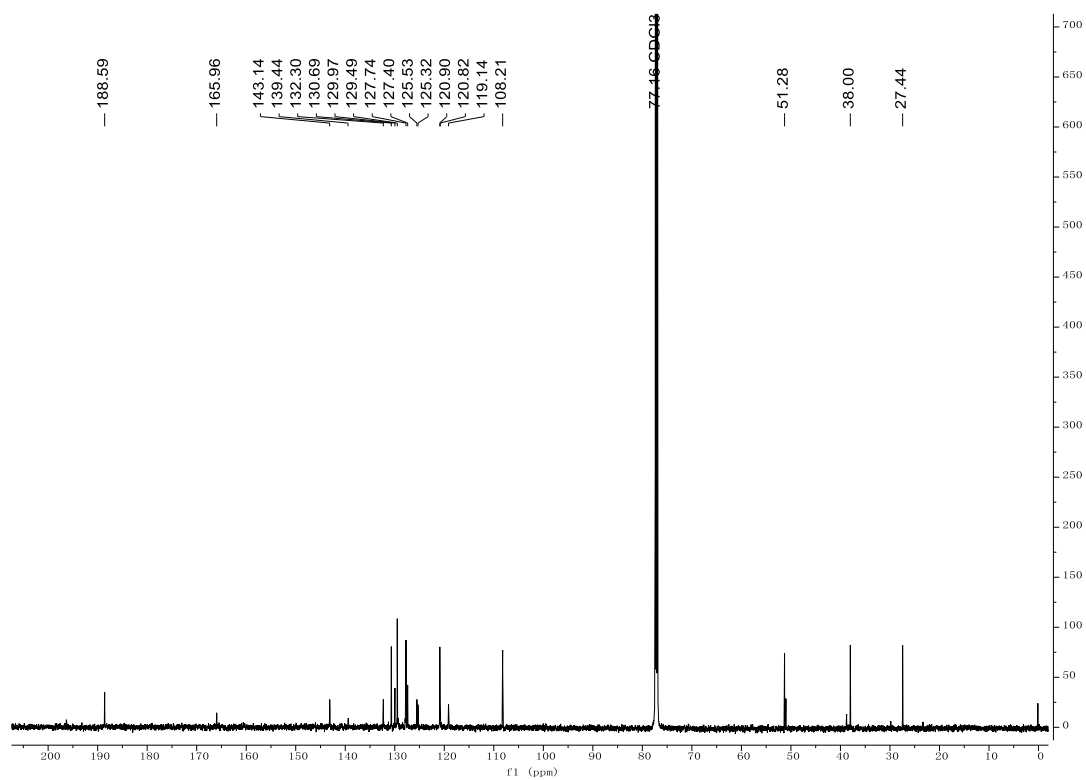


Figure S79. ¹³C NMR spectrum of compound 20.

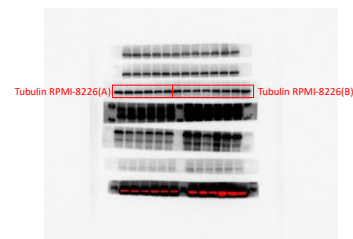
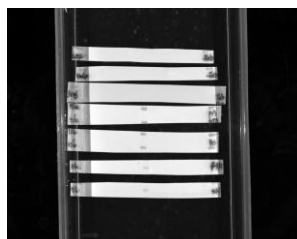
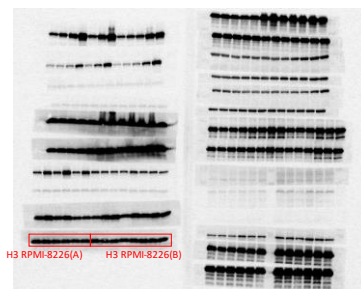
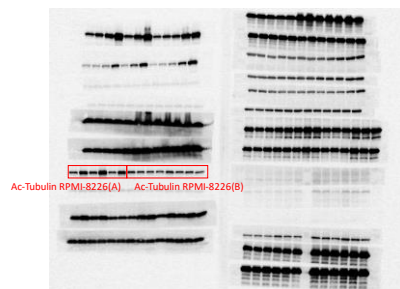
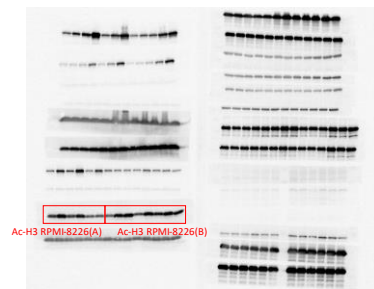
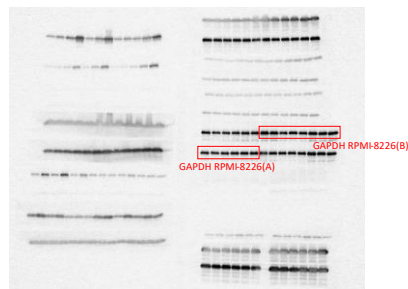


Figure S80. The whole un-cropped images of the original western blots in Figure 3.

| EV | Cell No. | Cell name | Locus names | | | | | | | | | Figure s |
|--------------|----------|-----------|-------------------------|--------------|--------------|-------------|------------|--------------|------------|------------|-------------|--------------|
| | | | D5S818 | D13S317 | D7S820 | D16S539 | VWA | TH01 | AM | TPOX | CSF1PO | |
| | | | <i>Query (RPM18226)</i> | <i>11,13</i> | <i>11,11</i> | <i>9,10</i> | <i>9,9</i> | <i>16,18</i> | <i>8,8</i> | <i>X,Y</i> | <i>8,11</i> | <i>12,12</i> |
| 1.00(3 6/36) | 248 | COLO-677 | 11,13 | 11,11 | 9,10 | 9,9 | 16,18 | 8,8 | X,Y | 8,11 | 12,12 | - |
| 1.00(3 6/36) | CCL-155 | RPMI 8226 | 11,13 | 11,11 | 9,10 | 9,9 | 16,18 | 8,8 | X,Y | 8,11 | 12,12 | - |
| 1.00(3 6/36) | IFO50013 | RPMI 8226 | 11,13 | 11,11 | 9,10 | 9,9 | 16,18 | 8,8 | X,Y | 8,11 | 12,12 | - |
| 1.00(3 6/36) | JCRB0034 | RPMI8226 | 11,13 | 11,11 | 9,10 | 9,9 | 16,18 | 8,8 | X,Y | 8,11 | 12,12 | - |

| EV | Cell No. | Cell name | Locus names | | | | | | | | | Figure s |
|--------------|----------|-----------|----------------------------|--------------|--------------|--------------|--------------|--------------|------------|------------|------------|-------------|
| | | | D5S818 | D13S317 | D7S820 | D16S539 | VWA | TH01 | AM | TPOX | CSF1PO | |
| | | | <i>Query (HCT-116Auc-)</i> | <i>10,11</i> | <i>10,12</i> | <i>11,12</i> | <i>11,13</i> | <i>17,22</i> | <i>8,9</i> | <i>X,Y</i> | <i>8,9</i> | <i>7,10</i> |
| 1.00(3 6/36) | CCL-247 | HCT 116 | 10,11 | 10,12 | 11,12 | 11,13 | 17,22 | 8,9 | X,Y | 8,9 | 7,10 | - |

| EV | Cell No. | Cell name | Locus names | | | | | | | | | Figures |
|--------------|----------|----------------|----------------------|--------------|-------------|--------------|--------------|--------------|------------|------------|-------------|--------------|
| | | | D5S818 | D13S317 | D7S820 | D16S539 | VWA | TH01 | AM | TPOX | CSF1PO | |
| | | | <i>Query (HL-60)</i> | <i>12,12</i> | <i>8,11</i> | <i>11,12</i> | <i>11,11</i> | <i>16,16</i> | <i>7,8</i> | <i>X,X</i> | <i>8,11</i> | <i>13,14</i> |
| 1.00(3 6/36) | 3 | HL-60 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | 139 | PLB-985 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | CCL-240 | HL-60 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | CRL-1964 | Clone 15 HL-60 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | CRL-2257 | HL-60/MX2 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | JCRB0085 | HL60 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | JCRB0163 | HL60(S) | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | RCB0041 | HL60 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |
| 1.00(3 6/36) | RCB1550 | HL-60-R2 | 12,12 | 8,11 | 11,12 | 11,11 | 16,16 | 7,8 | X,X | 8,11 | 13,14 | - |

Figure S81. The STR identification results of the three cells were correct.

Table S1. General information about the cells used in the experiments.

| Cell code | Cell line name | Cell source |
|-----------|-------------------------------------|-------------------------------------------------------------------------------------------|
| RPMI8226 | Human multiple myeloma cells | Nanjing Kebai Biotechnology Co., Ltd. |
| HL-60 | Human promyelocytic leukaemia cells | Chinese Academy of Sciences Committee Type Culture Collection cell bank (Shanghai, China) |
| HCT-116 | Human colon cancer cells | American Type Culture Collection |