Electronic Supporting Information

Antibacterial activity evaluation and mode of action study of novel thiazole-quinolinium derivatives

Ying Li[a,1], Ning Sun[a,b,c,1,*], Hooi-Leng Ser[a,d,1], Wei Long[a], Yanan Li[e], Cuicui Chen[a], Boxin Zheng[a], Xuanhe Huang[a], Zhihua Liu[b,*], Yu-Jing Lu[a,*]

[a] School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, PR China;

[b] The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou 510700, PR China;

[c] The State Key Laboratory of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China;

[d] Novel Bacteria and Drug Discovery (NBDD) Research Group, Microbiome and Bioresource Research Strength, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia;

[e] Department of Pharmacy, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, 519000, P. R. China.

[1]These authors contributed equally to this work.

[*]Corresponding author:

Dr. Ning. SUN, Email: ning.sun@connect.polyu.hk

Dr. Zhihua LIU, Email: liuzhihualzh@hotmail.com

Dr. Yu-Jin LU, Email: luyj@gdut.edu.cn

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1. Visualization of bacterial morphology

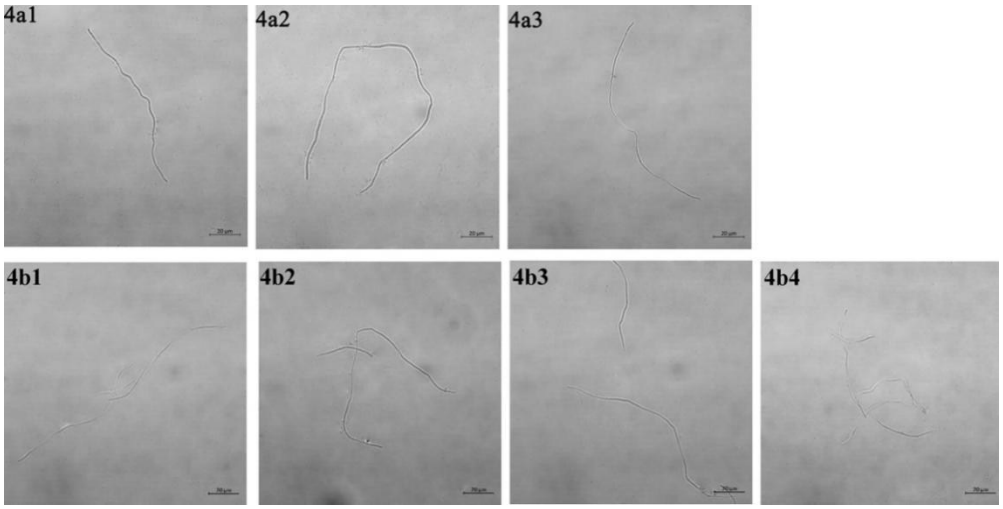


Fig. S1. Morphology analysis of *B. subtilis* 168. *Cells* were grown in the presence of **4a1**-**4a3** and **4b1**-**4b4**. Scale bar=20 μm.

2. Visualization of bacterial cell membrane

The perturbation of the membrane structure can also promote cell lysis or even trigger cell death. Compounds **4a1**, **4a4**, **4b1** and **4b4**, which possess strong antibacterial activity and cell division inhibitory effect, were subjected additional morphological studies to investigate their effects on cell membrane structures by using *B. subtilis* cells as a model. A commercial membrane stain FM 4-64 was used for imaging. From **Fig. S2** and **Fig. S3**, it revealed that the compounds did not cause any significant changes on the cell membrane of *B. subtilis* cells.

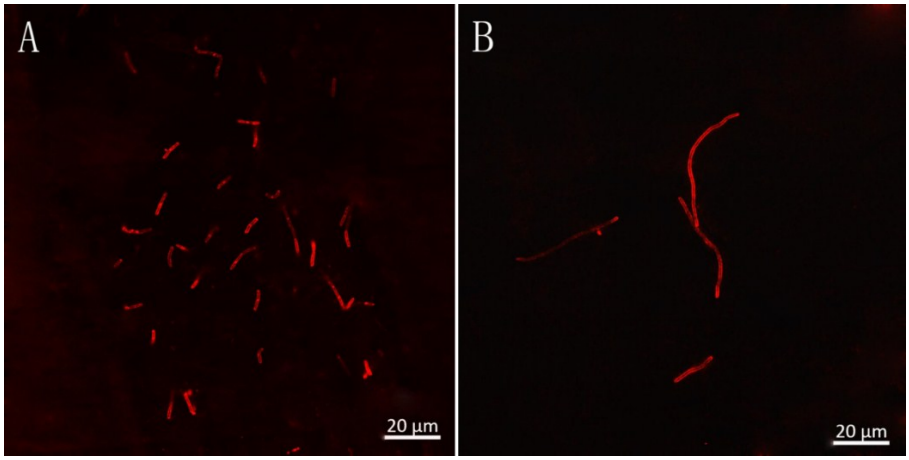


Fig. S2. Visualization of cell membrane of *B. subtilis* stained with red fluorescent dye FM4-64 in the absence (A) and presence of **4a4** (B). Scale bar=20 μm.

Fig. S3. Visualization of cell membrane of *B. subtilis* stained with red fluorescent dye FM4-64 in the presence of **4a1**, **4b1**, **4b4**. Scale bar=20 μm.

3. Light-scattering assay of 4b4, 4e1 and 4e3

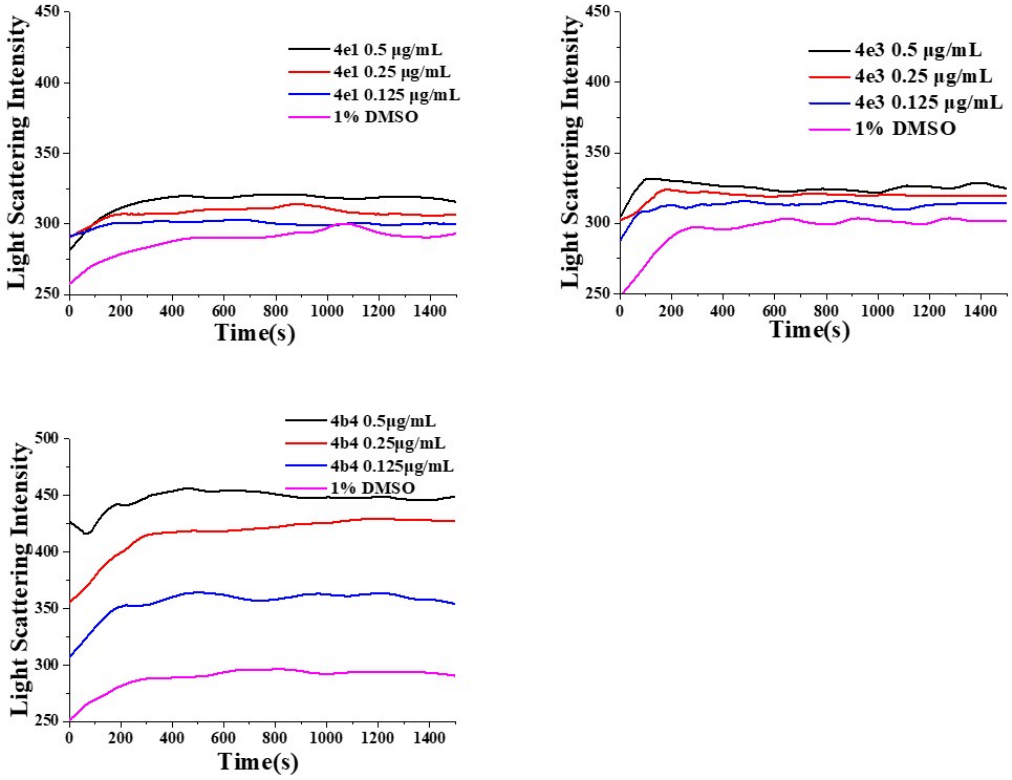


Fig. S4. Effect of **4e1**, **4e3** and **4b4** on the polymerization of FtsZ at a concentration of 0.125-0.5 μg/mL.

4. GTPase activity assay of 4a4 and 4b4

The dynamic assembly of FtsZ is strictly regulated by its GTPase activity [1, 2]. Two compounds, **4a4** and **4b4** were selected to explore their potential in disrupting GTPase activity of FtsZ. The results showed that these compounds did not have any significant effect on the GTPase activity of *Sa*FtsZ (**Fig. S5**). As a matter of fact, same phenomenon also occurs in the reported FtsZ-inhibitor 2,6-difluoro-3-aminobenzamide derivative [3] and a conversion product of PC190723 [4]; these compounds were reported to bind to the interdomain cleft of FtsZ without interfering GTPase activity.

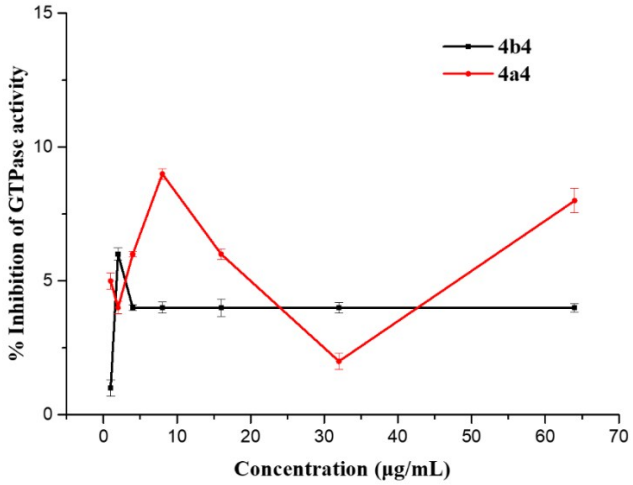


Fig. S5. Inhibition of GTPase activity of FtsZ by compounds **4a4** and **4b4.**

5. Visualization of Z-ring in bacterial cells

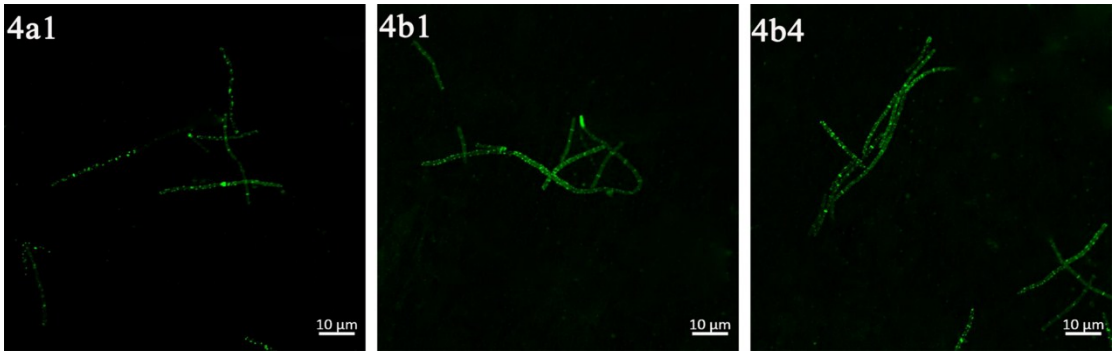


Fig. S6. The perturbation of the cytokinetic Z-ring in *B. subtilis*. Cells of *B. subtilis* were grown in the presence of **4a1**, **4b1**, **4b4**. Scale bar=10 μm.

6. Hemolytic activity of 4a4 and 4b4

Hemolytic activity of compounds **4a4** and **4b4** was conducted using human erythrocytes. While hemolysis rate of more than 5% indicates break down of erythrocytes [5], results showed that these compounds did not reflect significant hemolysis effect. The hemolysis rates of **4a4** and **4b4** at 32×MIC (MICs for *S. aureus* ATCC 29213 were 1 μg/mL and 2 μg/mL, respectively) were lower than 5%, and in the previous reports [6], the cells treated by Triton X-100 (0.002 to 1%) were completely hemolyzed under the same conditions, suggesting that compounds **4a4** and **4b4** did not display cytotoxicity against human erythrocytes.

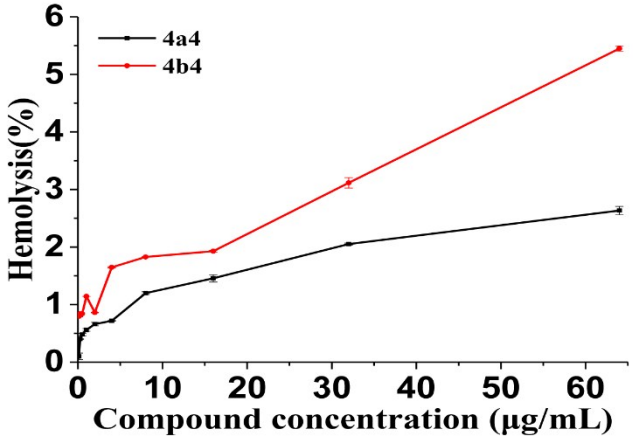


Fig. S7. Hemolytic activity of compound **4a4** and **4b4**. Human erythrocytes were treated with compounds **4a4** and **4b4** (0.125~64 μg/mL).

7. Drug resistance study of 4a4 and 4b4

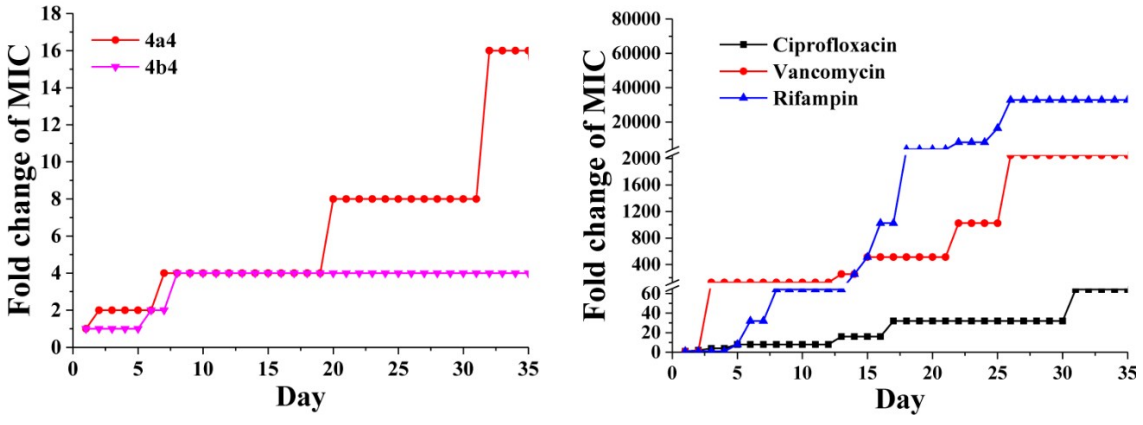


Fig. S8. Bacterial resistance study of compound **4a4** and **4b4** against *B. subtilis* 168.

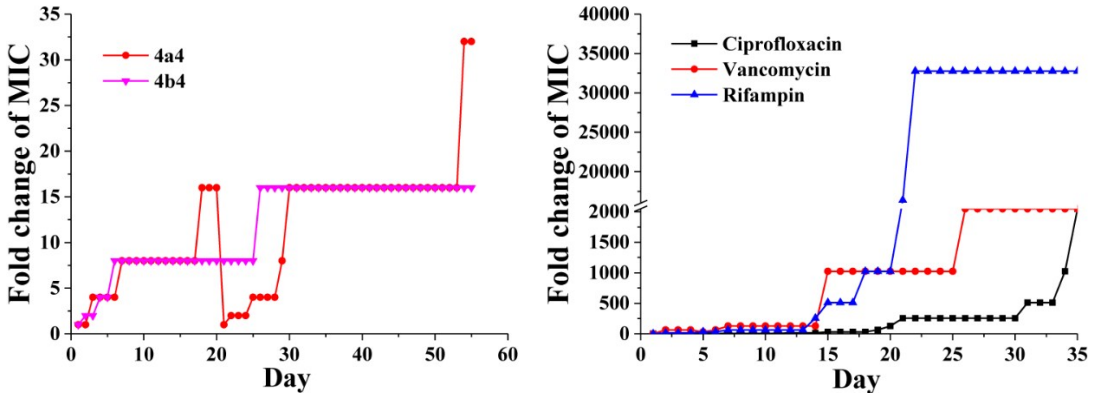


Fig. S9. Bacterial resistance study of compound **4a4** and **4b4** against *E. coli ATCC* 25922.

8. Molecular modeling studies of 4a4 and 4e1 with FtsZ protein

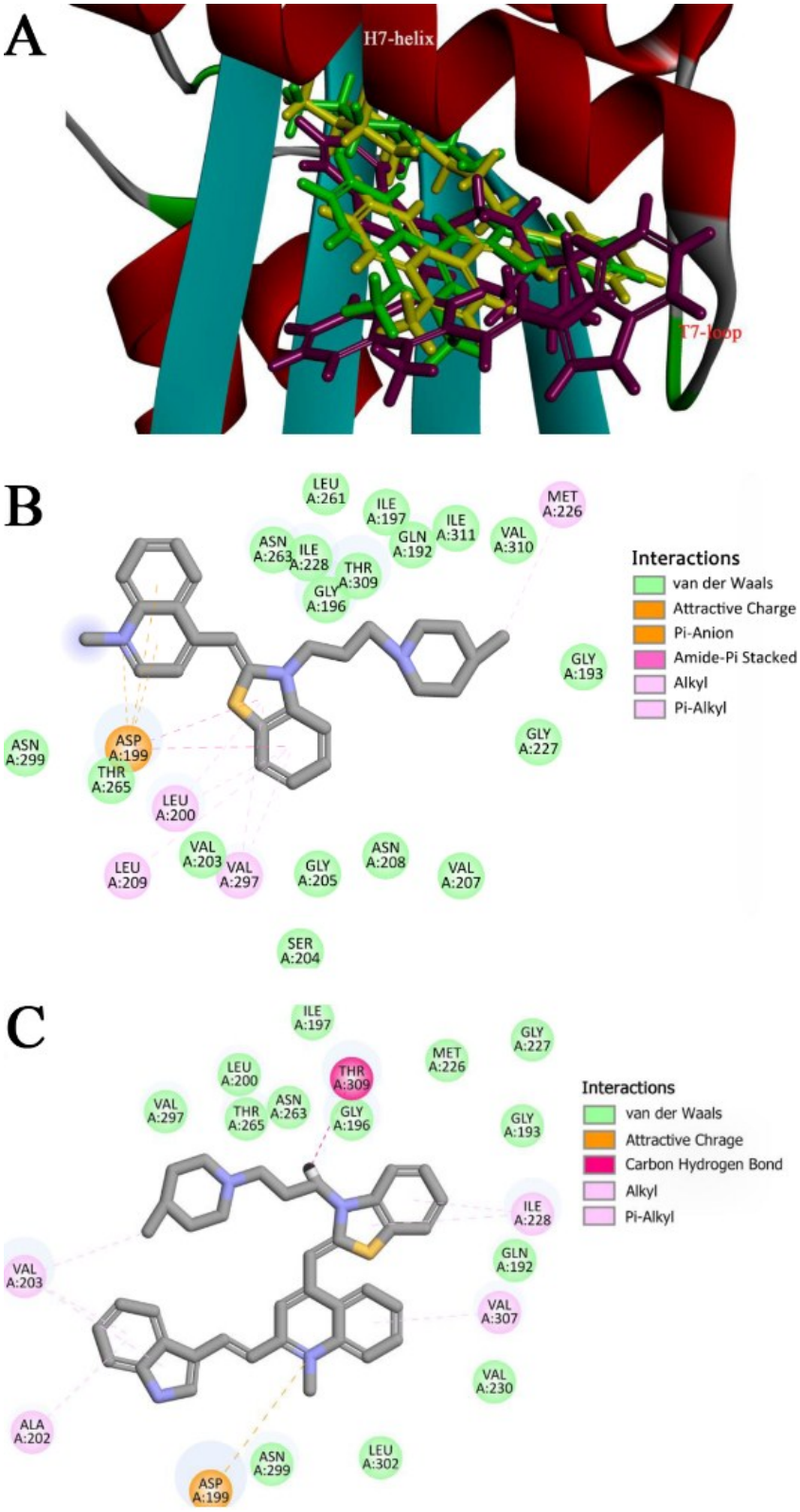


Fig. S10. (A) Molecular modeling studies of **4a4** (green), **4b4** (yellow) and **4e1** (purple) with FtsZ protein; (B) Predicted interactions between **4b4** and the amino acids of FtsZ; (C) Predicted interactions between **4e1** and the amino acids of FtsZ.

9. ^{1}H NMR, ^{13}C NMR and HRMS spectra of compounds 4a1, 4a3-4a4, 4b3-4b4 and 4c1-4e4

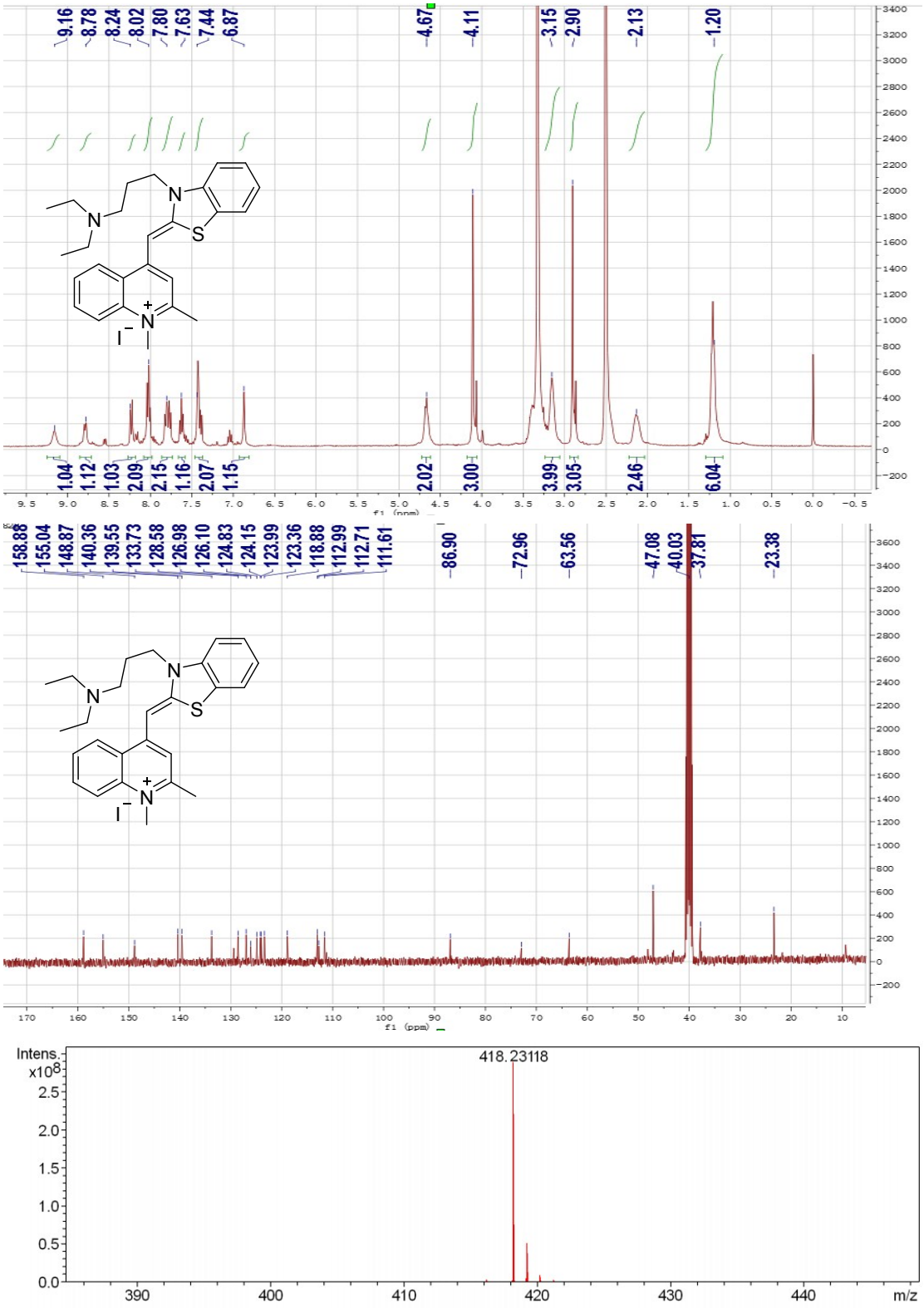


Fig.S11. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4a1.**

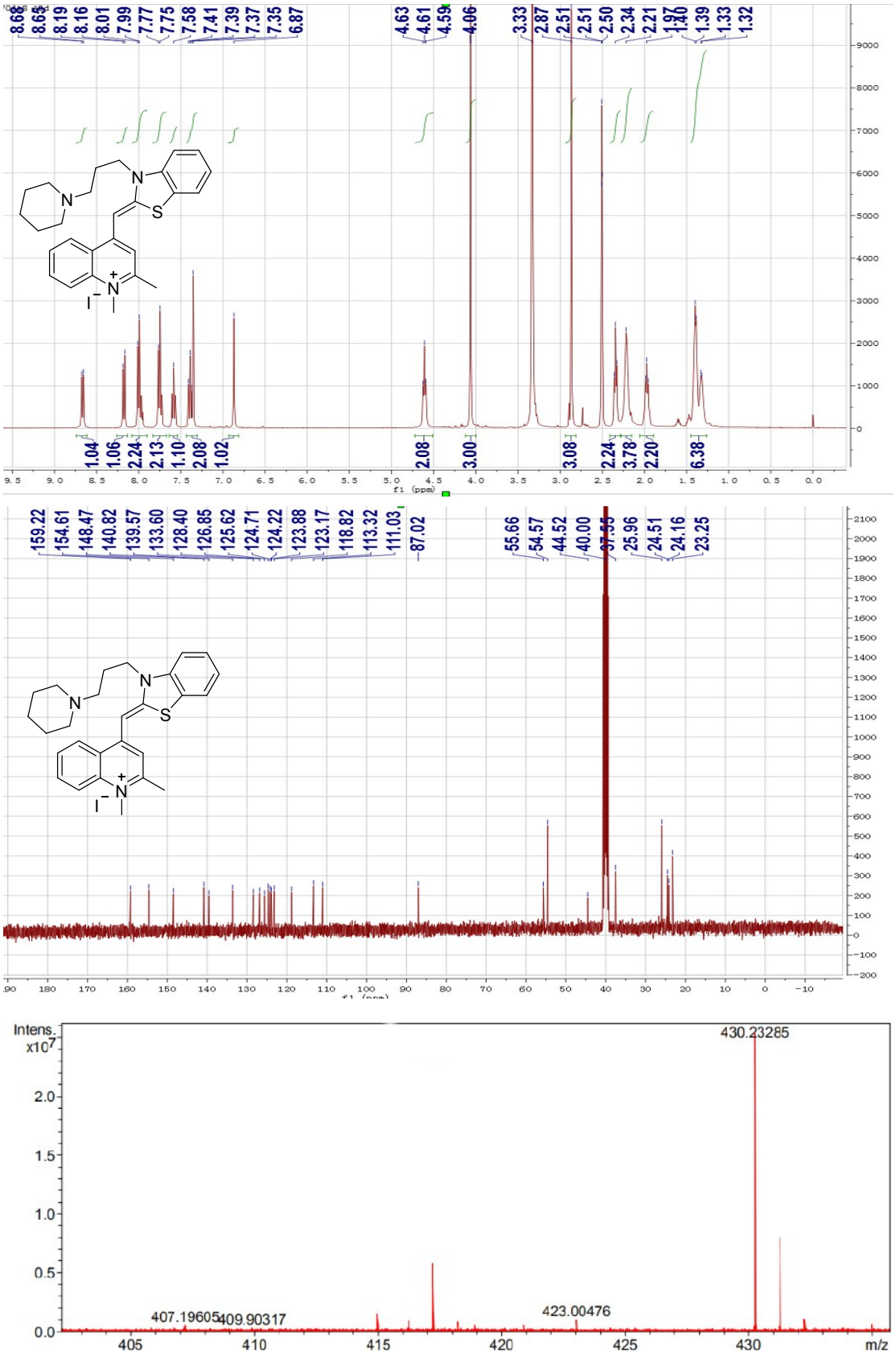


Fig.S12. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4a3.**

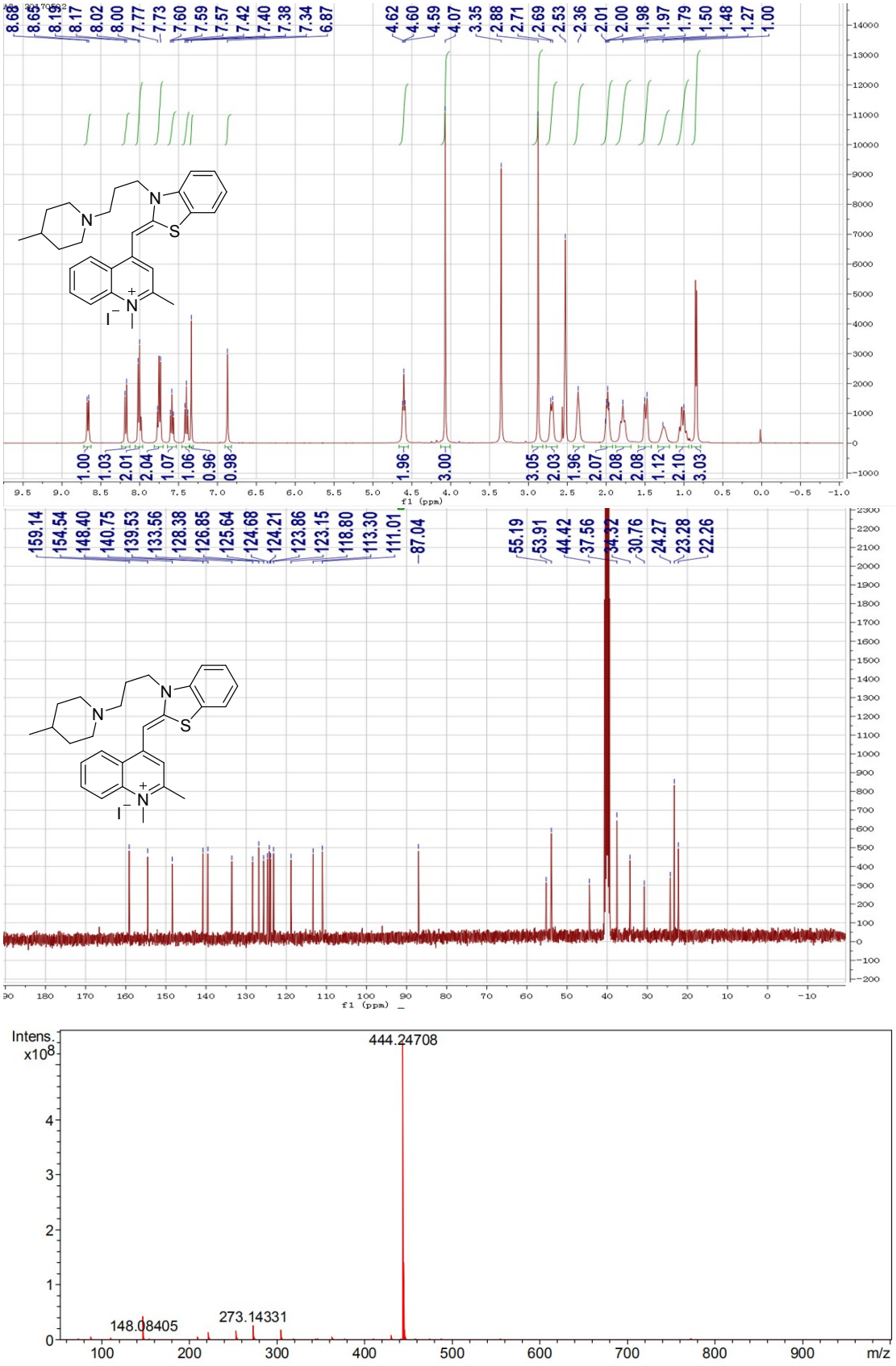


Fig.S13. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4a4.**

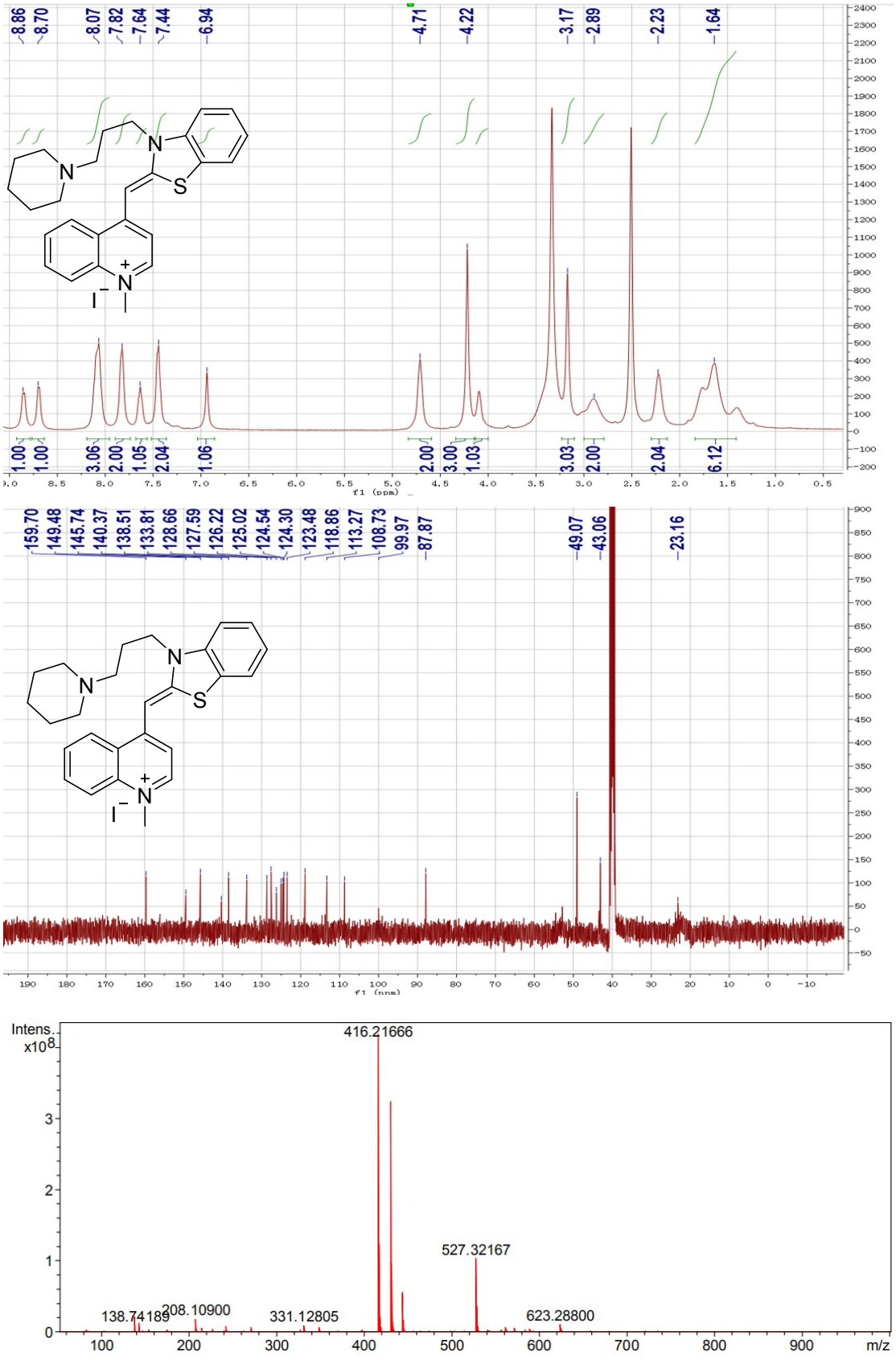


Fig.S14. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of

compound **4b3.**

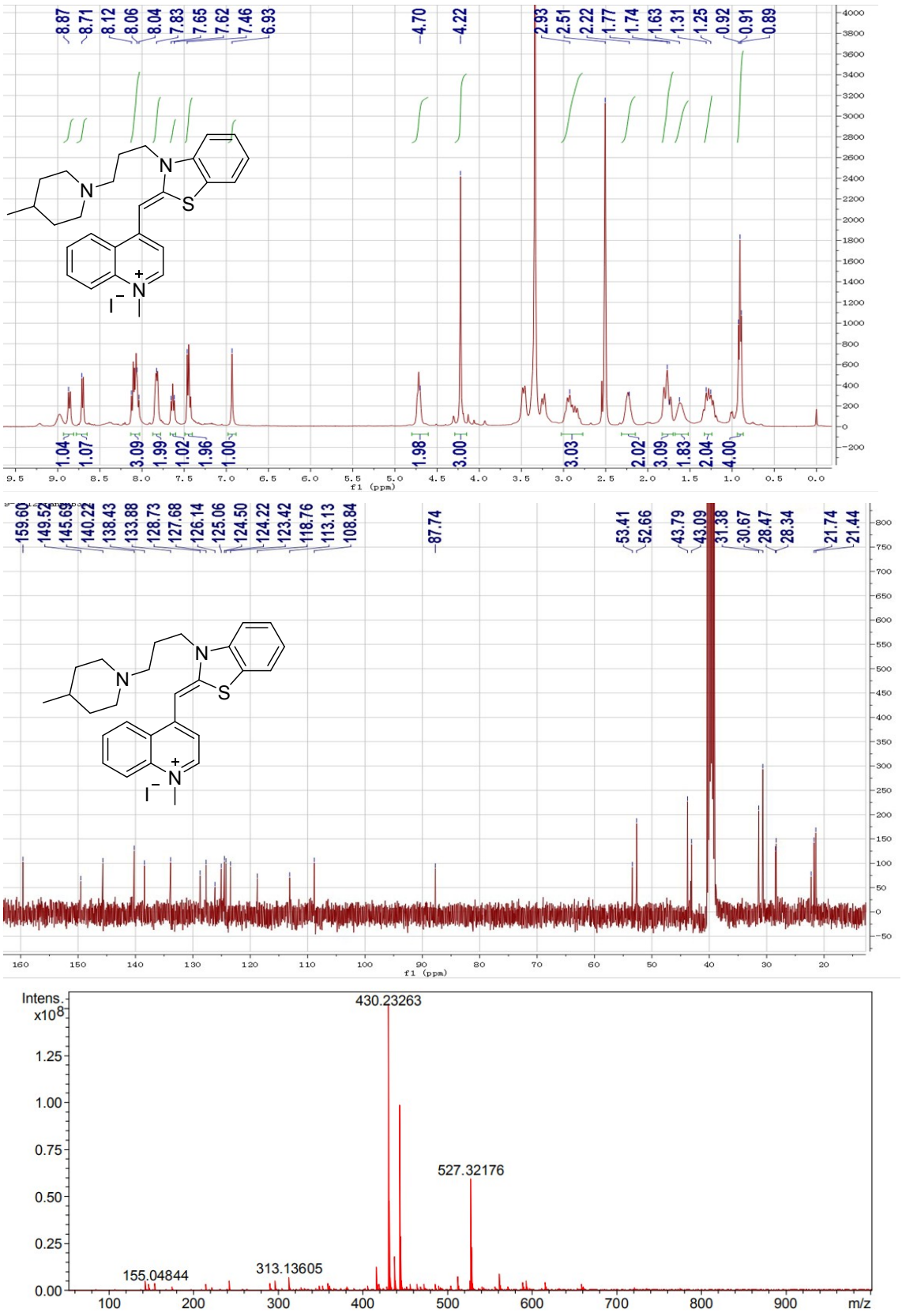
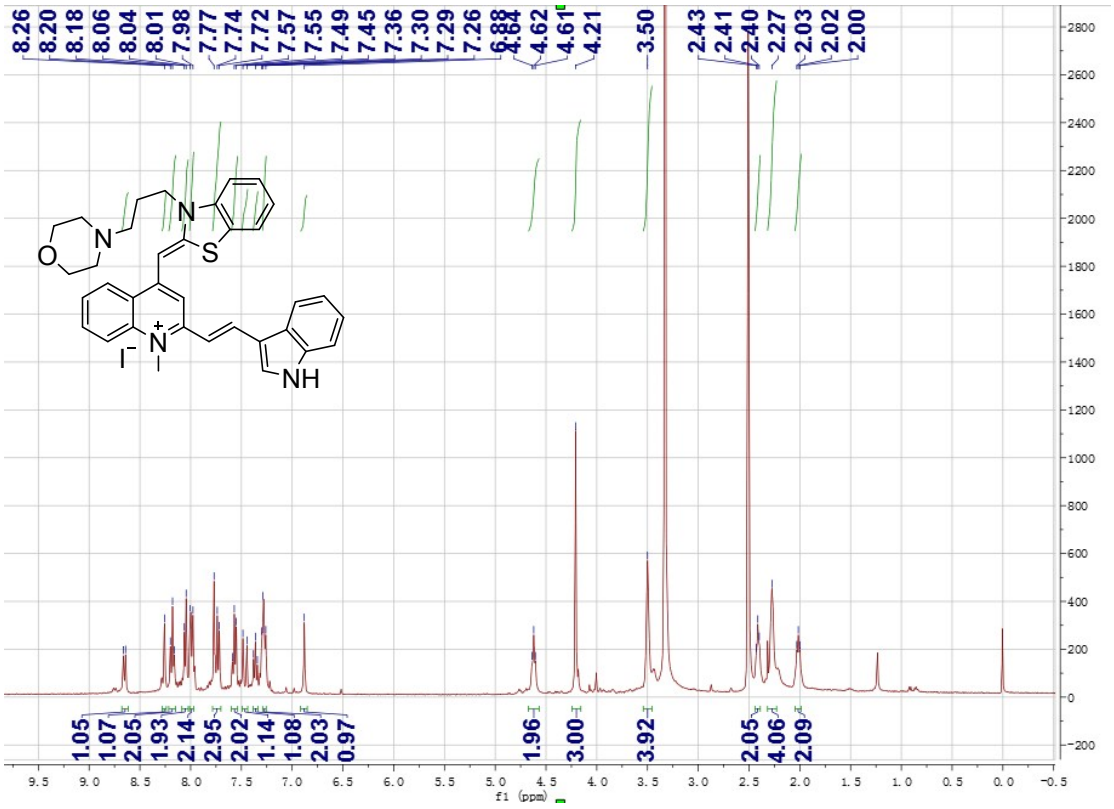
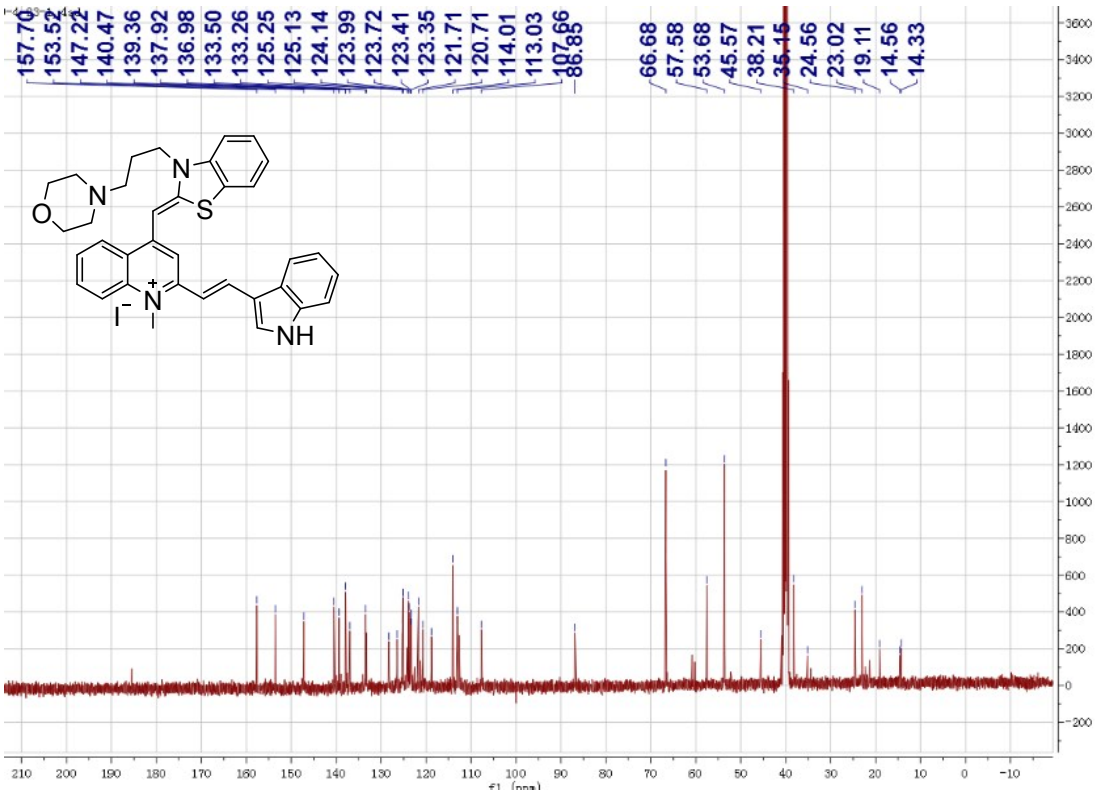


Fig.S15. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of

compound **4b4.**





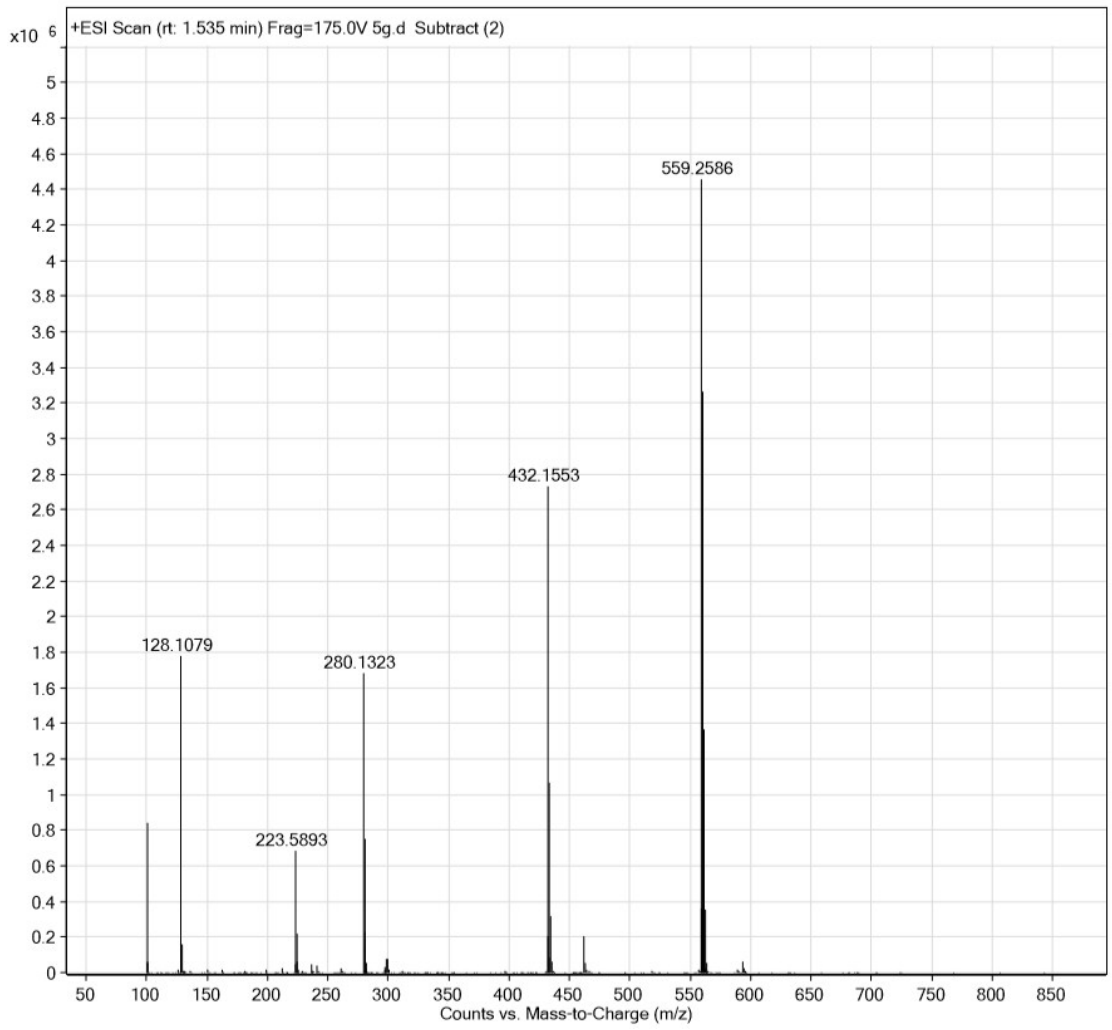
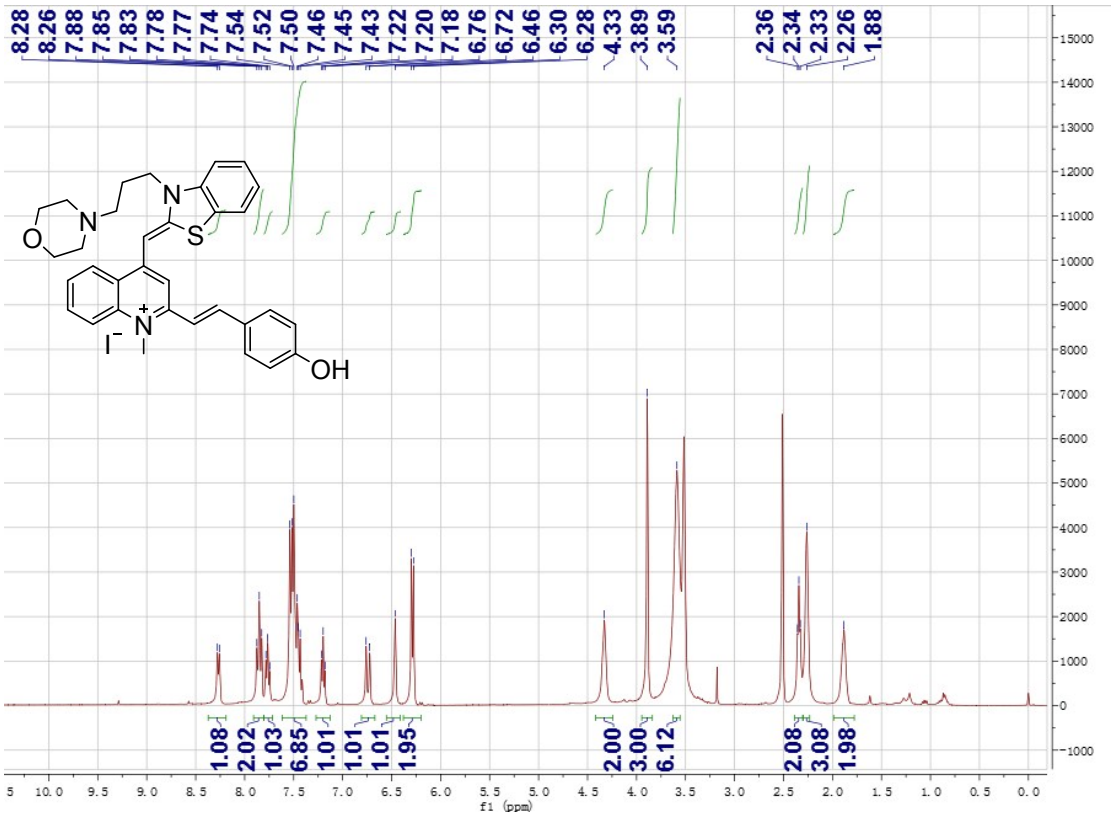


Fig.S16. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4c1.**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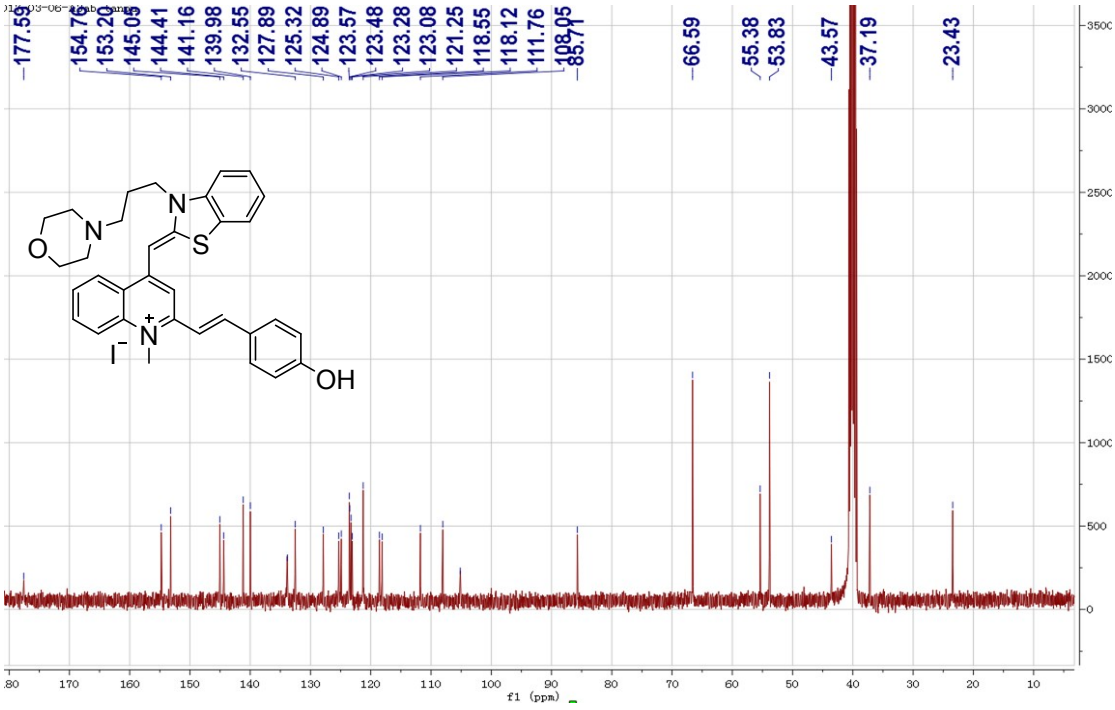
8.26
7.88
7.85
7.83
7.78
7.77
7.74
7.54
7.52
7.50
7.46
7.45
7.43
7.22
7.20
7.18
6.76
6.72
6.46
6.30
6.28
4.33
3.89
3.59
2.36
2.34
2.33
2.26
1.88
1.08
2.02
1.03
6.85
1.01
1.01
1.01
1.95
2.00
3.00
6.12
2.08
3.08
1.98
f1 (ppm)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154.76
153.20
145.05
144.41
141.16
139.98
132.55
127.89
125.32
124.89
123.57
123.48
123.28
123.08
121.25
118.55
118.12
111.76
108.05
85.71
66.59
55.38
53.83
43.57
37.19
23.43
f1 (ppm)

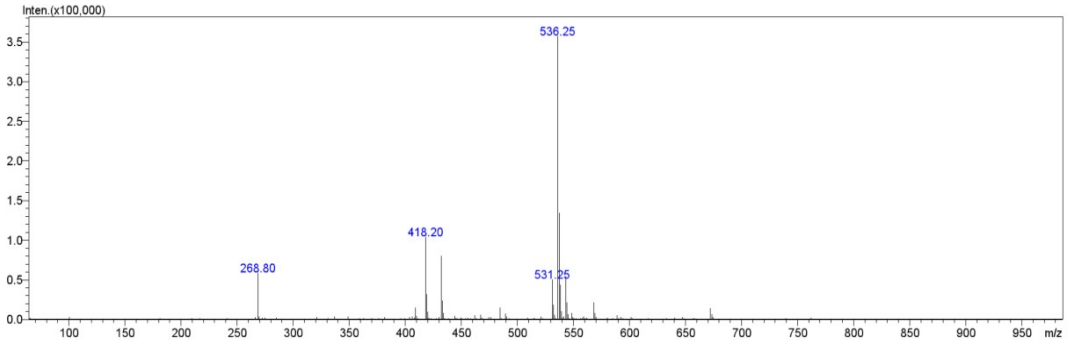
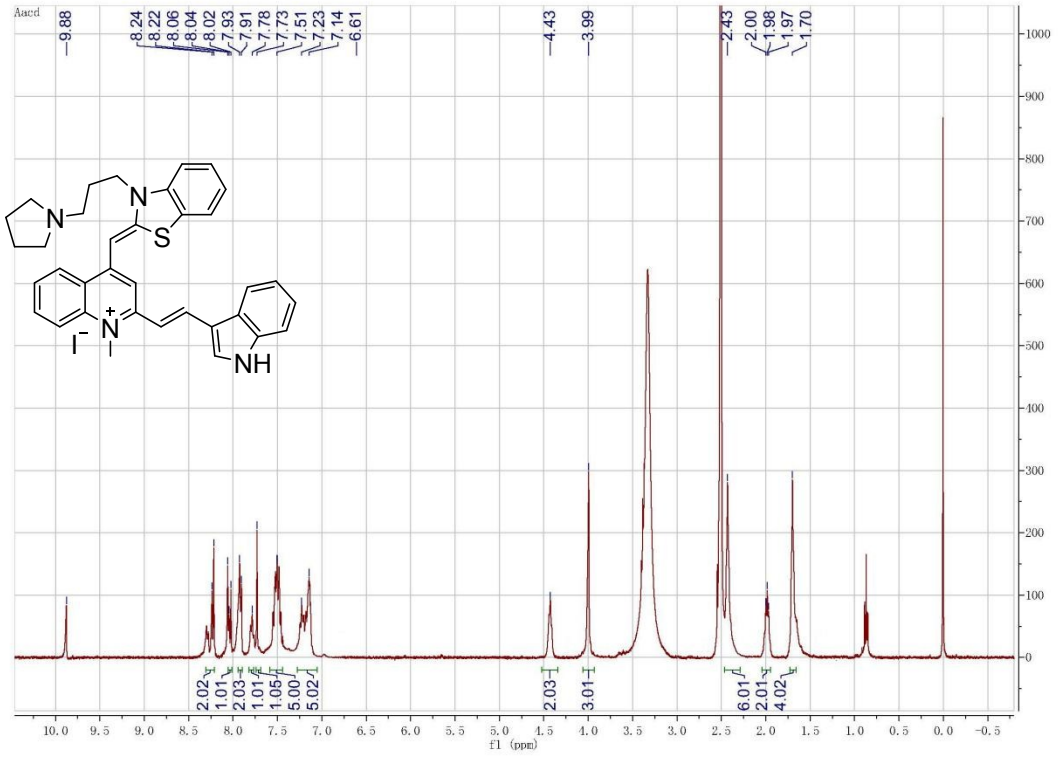


Fig.S17. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and ESI-MS spectra of compound **4c2.**



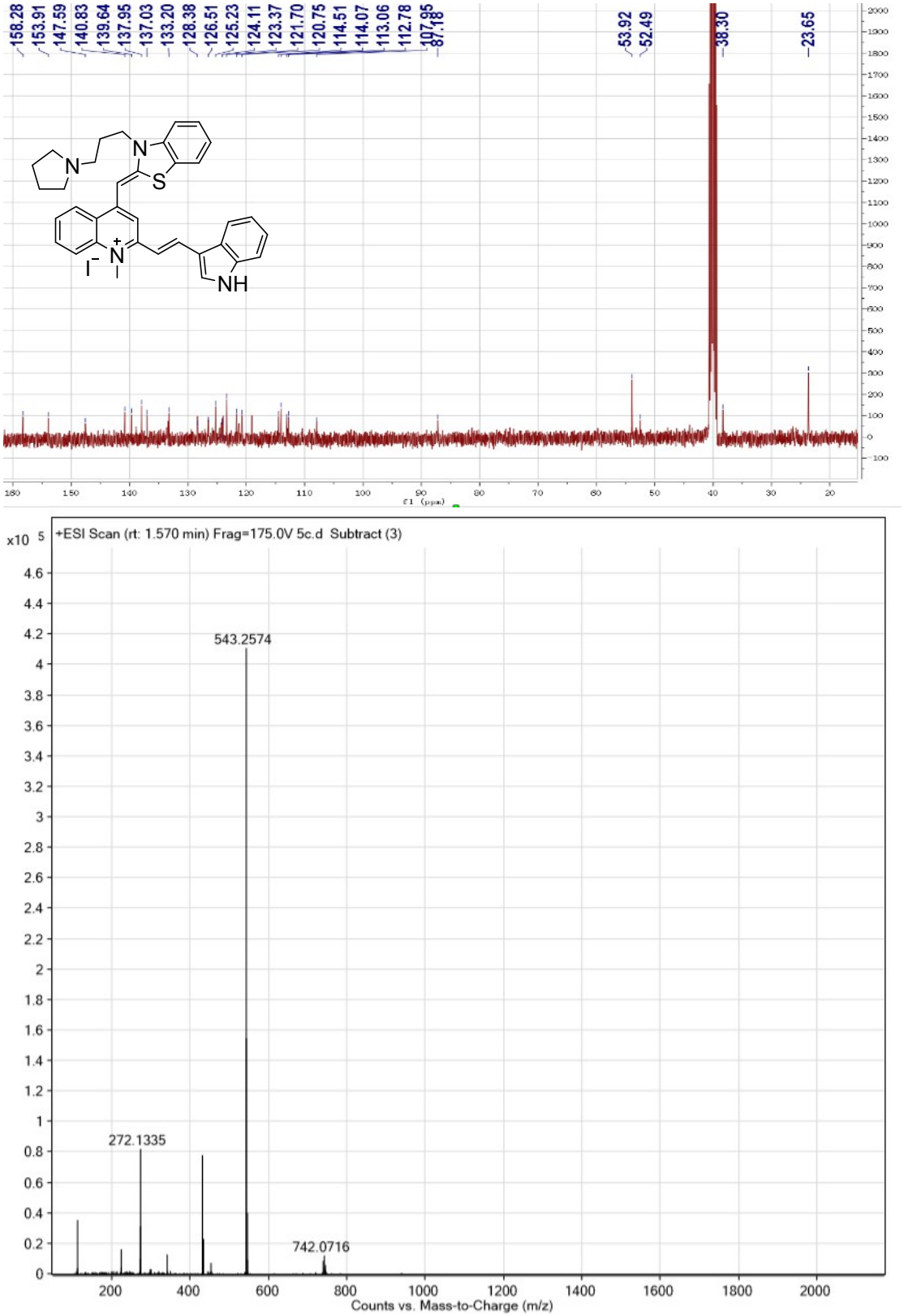
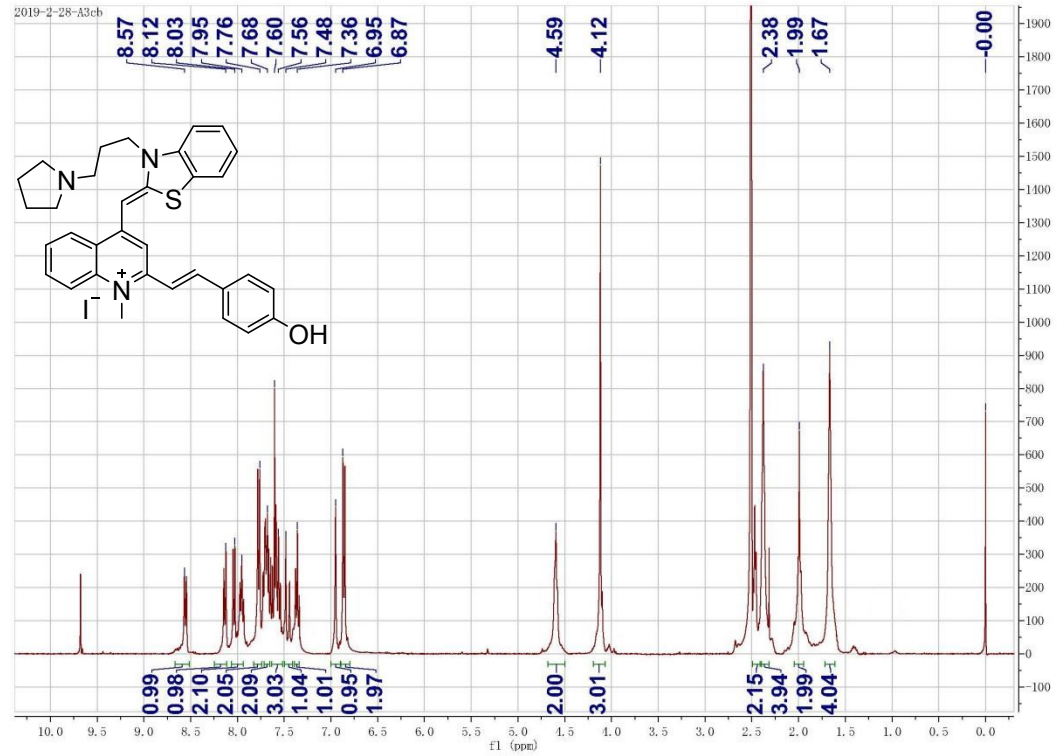


Fig.S18. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4d1.**


2019-2-28-A3cb
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8.03
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7.76
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7.60
7.56
7.48
7.36
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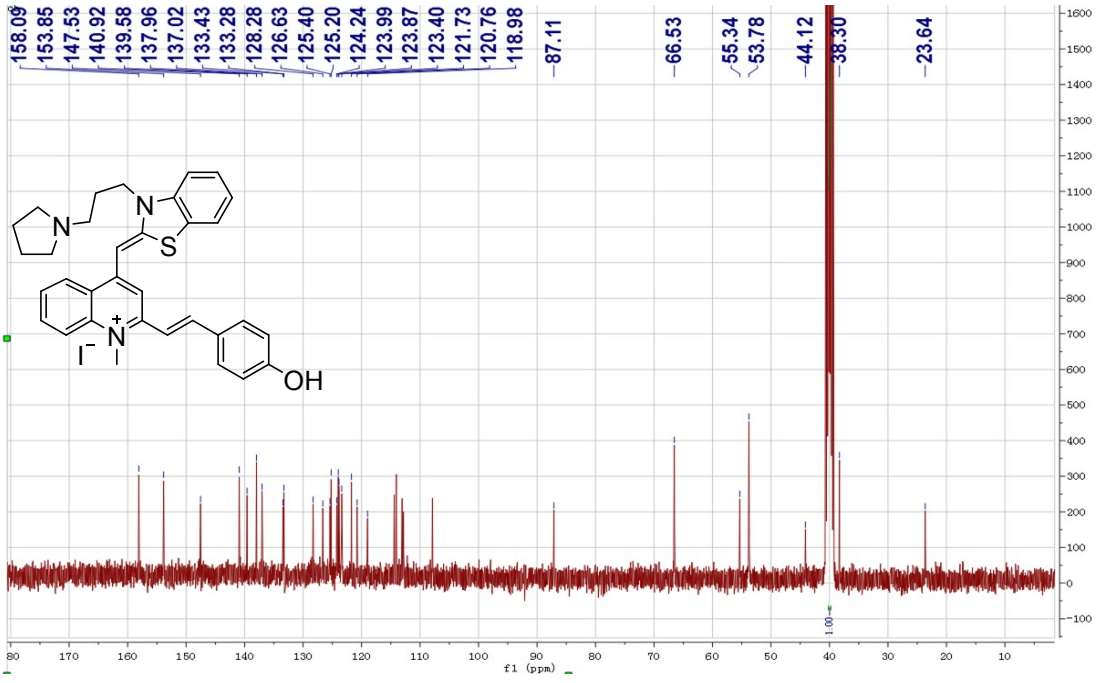
6.87
4.59
4.12
2.38
1.99
1.67
0.00
0.99
0.98
2.10
2.05
2.09
3.03
1.04
1.01
0.95
1.97
2.00
3.01
2.15
3.94
1.99
4.04
f1 (ppm)


158.09
153.85
147.53
140.92
139.58
137.96
137.02
133.43
133.28
128.28
126.63
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125.20
124.24
123.99
123.87
123.40
121.73
120.76
118.98
87.11
66.53
55.34
53.78
44.12
38.30
23.64
f1 (ppm)

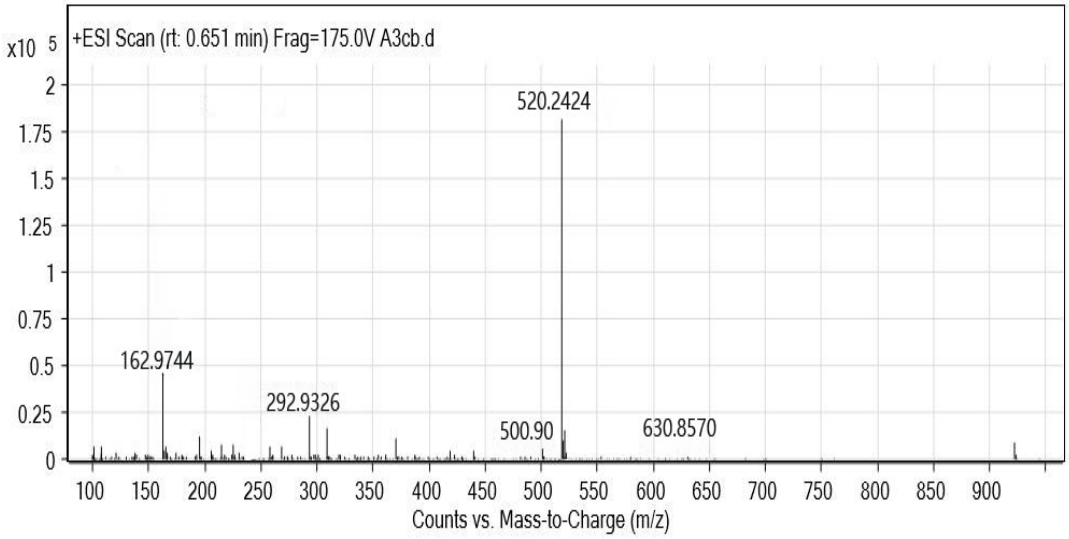
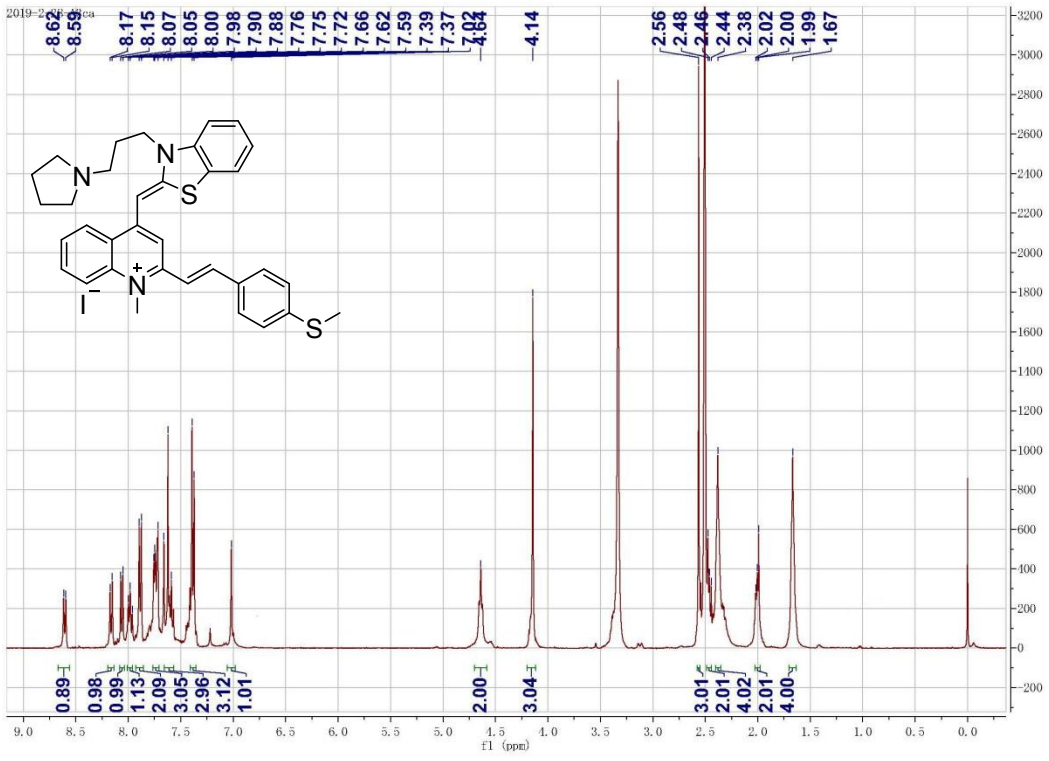


Fig.S19. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4d2.**



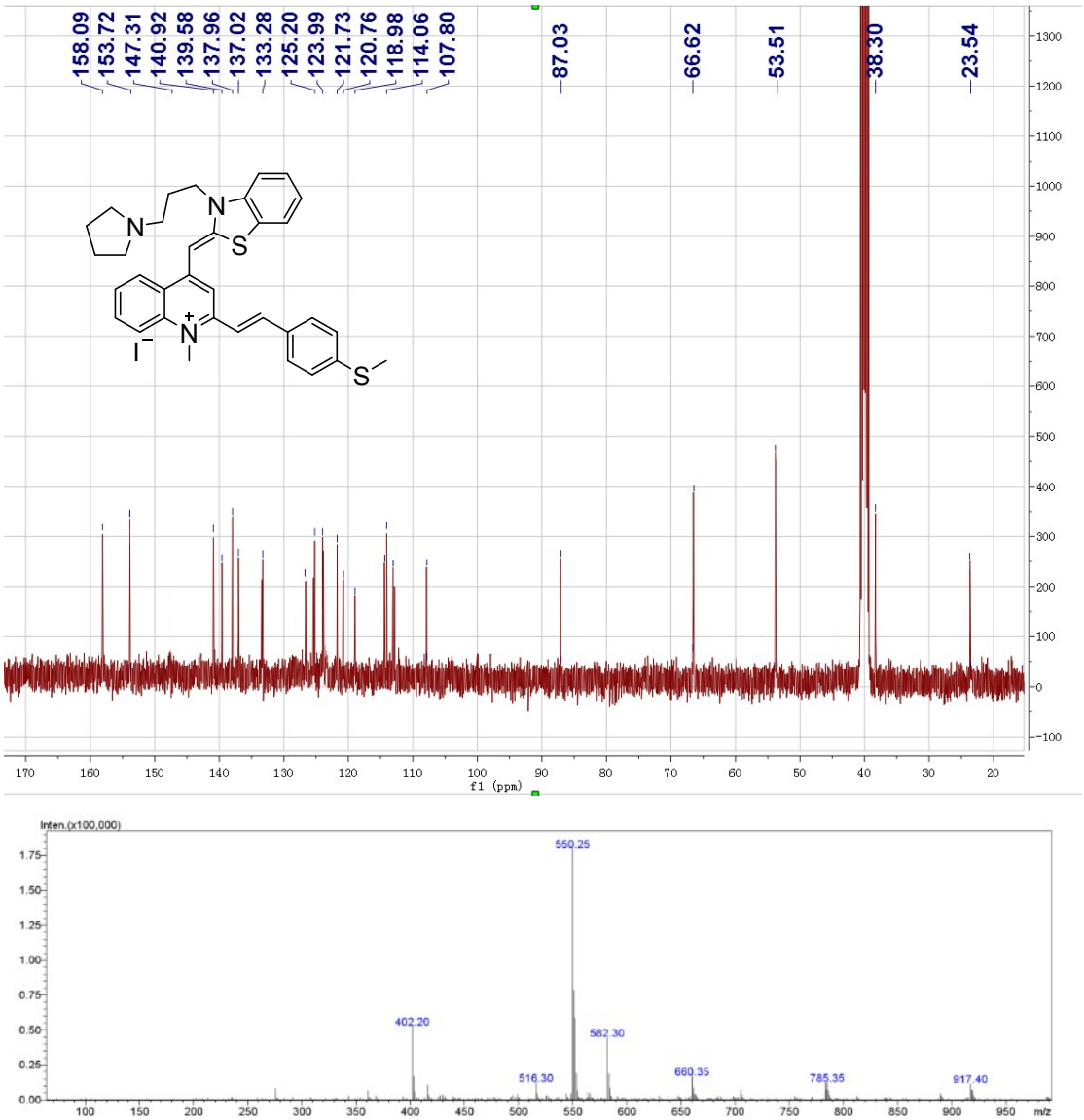
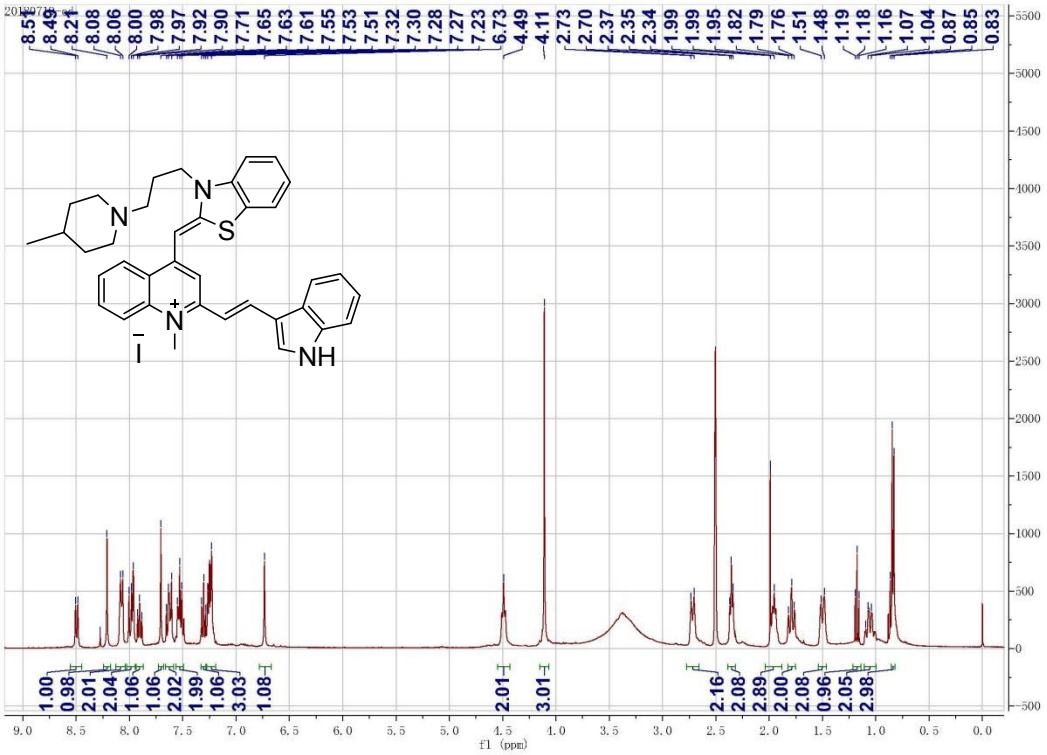
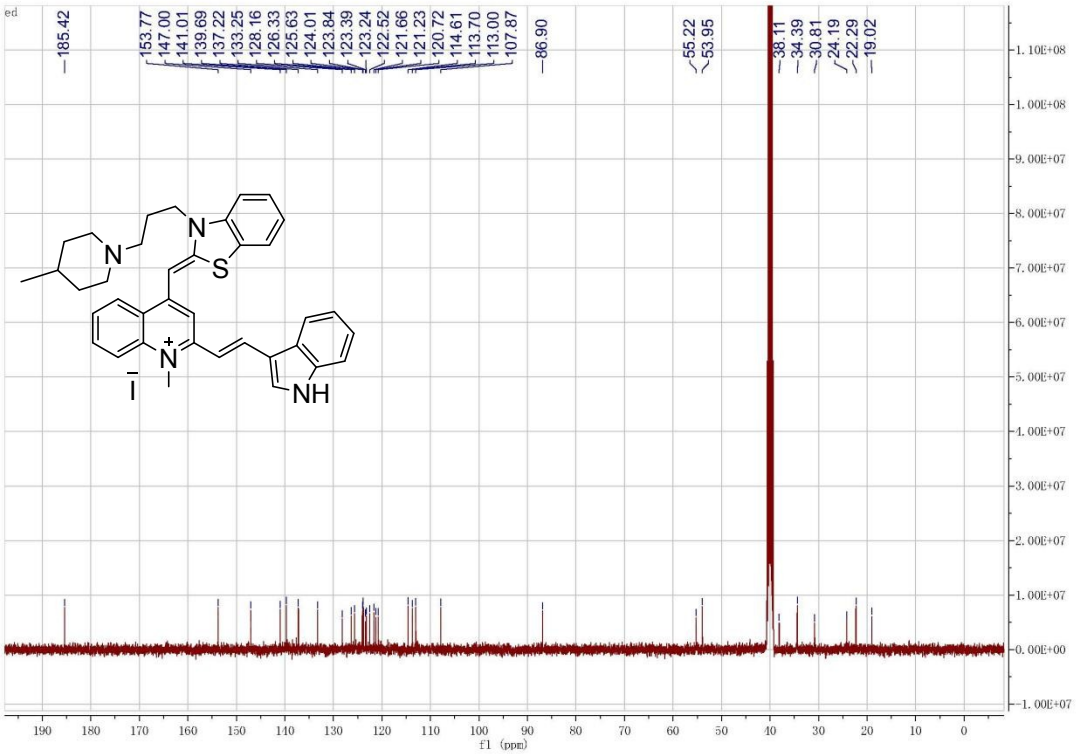


Fig.S20. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and ESI-MS spectra of compound **4d3.**


8.57 8.49 8.21 8.08 8.06 8.00 7.98 7.97 7.92 7.90 7.71 7.65 7.63 7.61 7.55 7.53 7.51 7.32 7.30 7.28 7.27 7.23 6.73 4.49 4.11 2.73 2.70 2.37 2.35 2.34 1.99 1.99 1.95 1.82 1.79 1.76 1.51 1.48 1.19 1.18 1.16 1.07 1.04 0.87 0.85 0.83
1.00 0.98 2.01 2.04 1.06 1.06 2.02 1.99 1.06 3.03 1.08 2.01 3.01 2.16 2.08 2.89 2.00 2.08 0.96 2.05 2.98
f1 (ppm)


185.42 153.77 147.00 141.01 139.69 137.22 133.25 128.16 126.33 125.63 124.01 123.84 123.39 123.24 122.52 121.66 121.23 120.72 114.61 113.70 113.00 107.87 86.90 55.22 53.95 38.11 34.39 30.81 24.19 22.29 19.02
f1 (ppm)

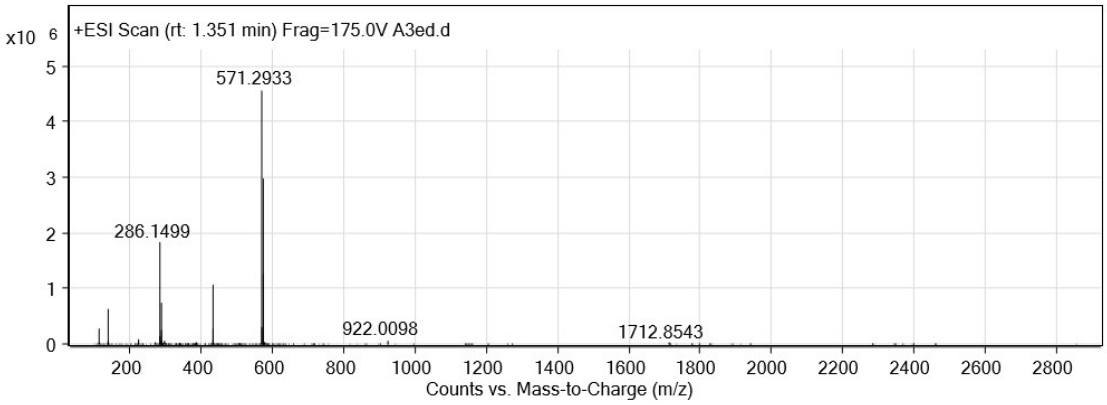
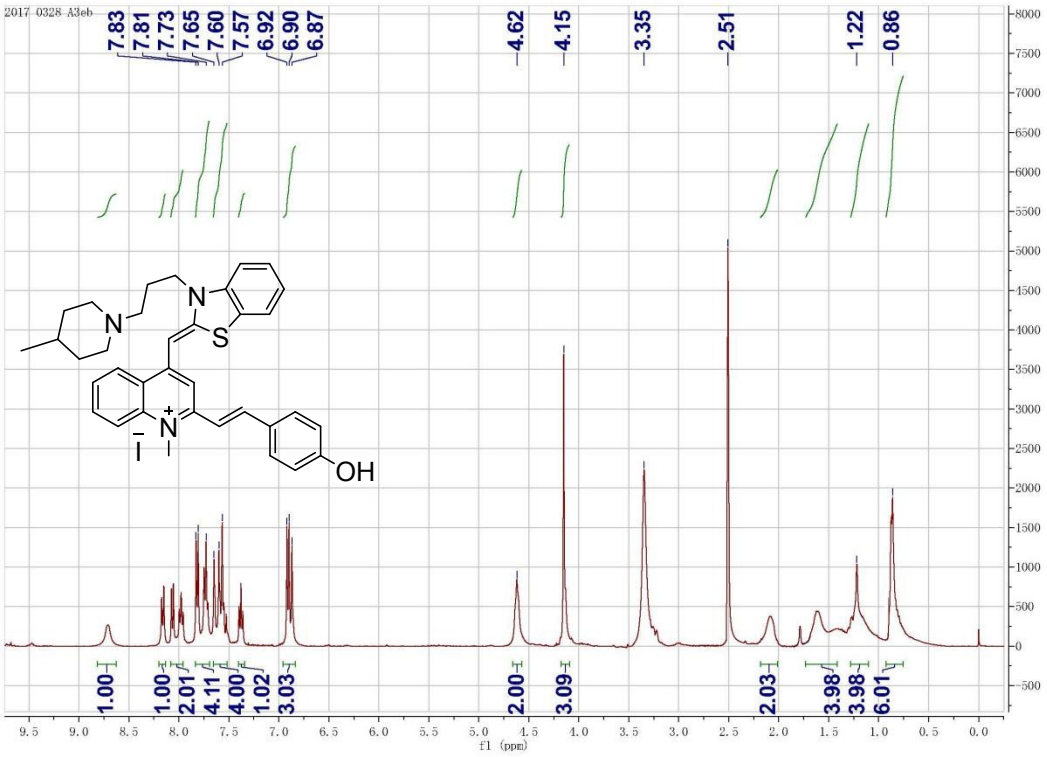


Fig.S21. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4e1.**



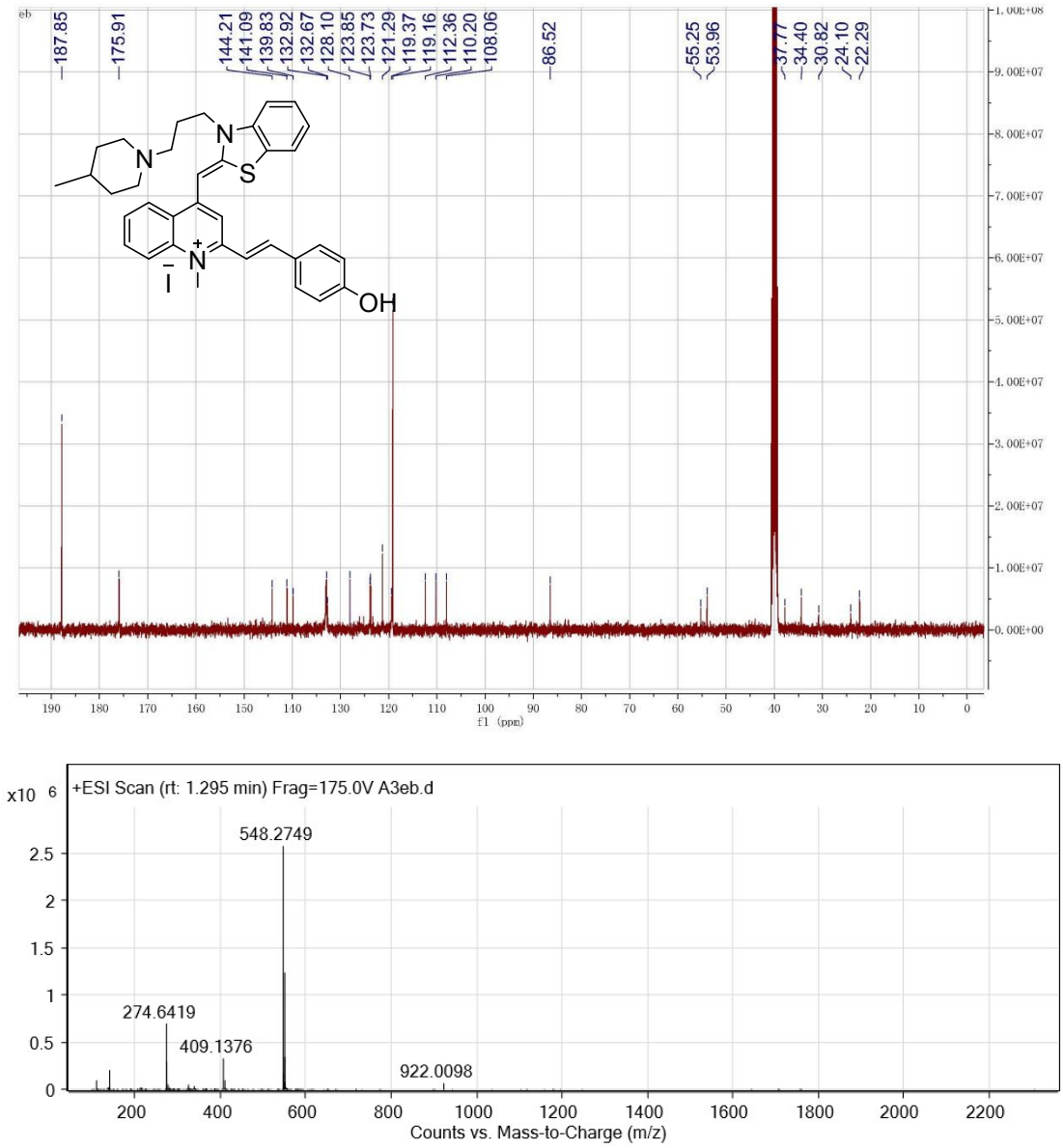
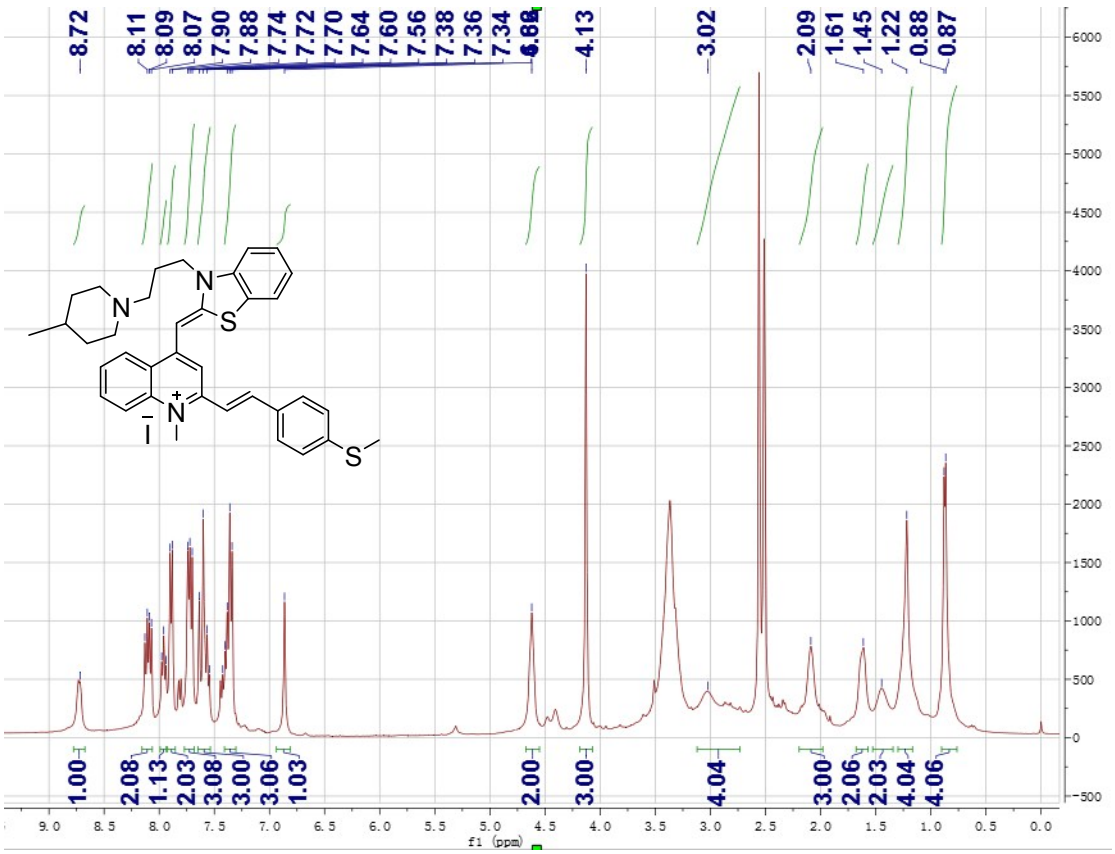


Fig.S22. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4e2.**


8.72
8.11
8.09
8.07
7.90
7.88
7.74
7.72
7.70
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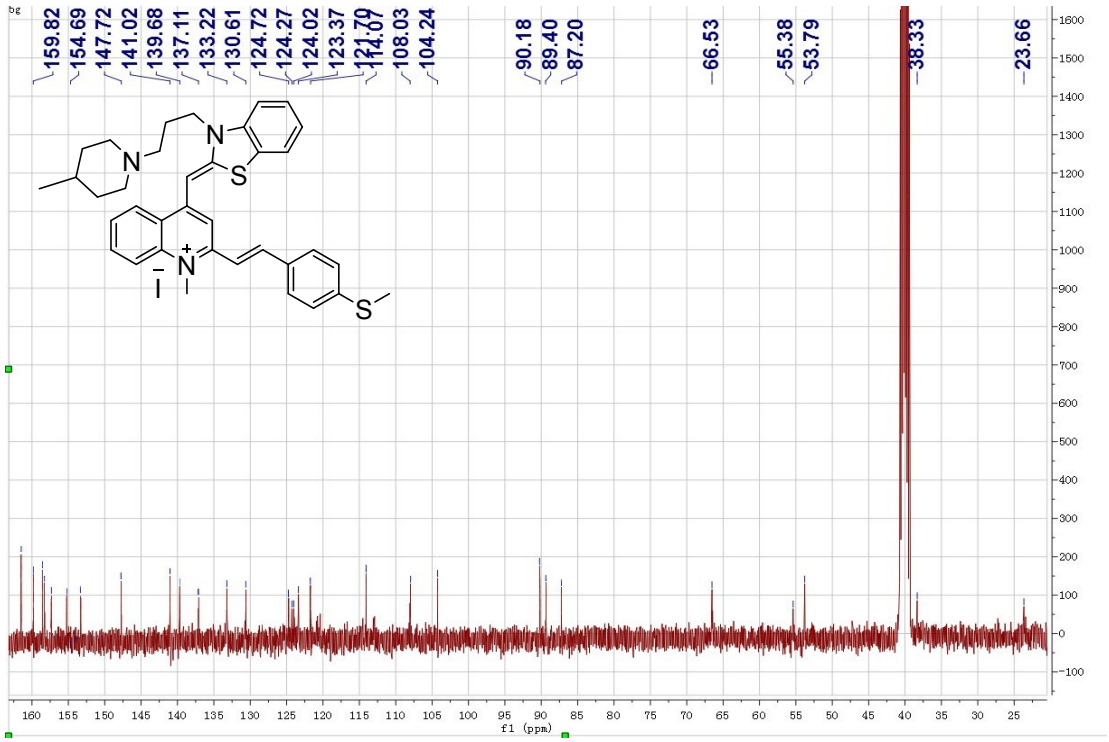
7.38
7.36
7.34
4.13
3.02
2.09
1.61
1.45
1.22
0.88
0.87
1.00
2.08
1.13
2.03
3.08
3.00
3.06
1.03
2.00
3.00
4.04
3.00
2.06
2.03
4.04
4.06
f1 (ppm)


159.82
154.69
147.72
141.02
139.68
137.11
133.22
130.61
124.72
124.27
124.02
123.37
121.70
114.07
108.03
104.24
90.18
89.40
87.20
66.53
55.38
53.79
38.33
23.66
f1 (ppm)

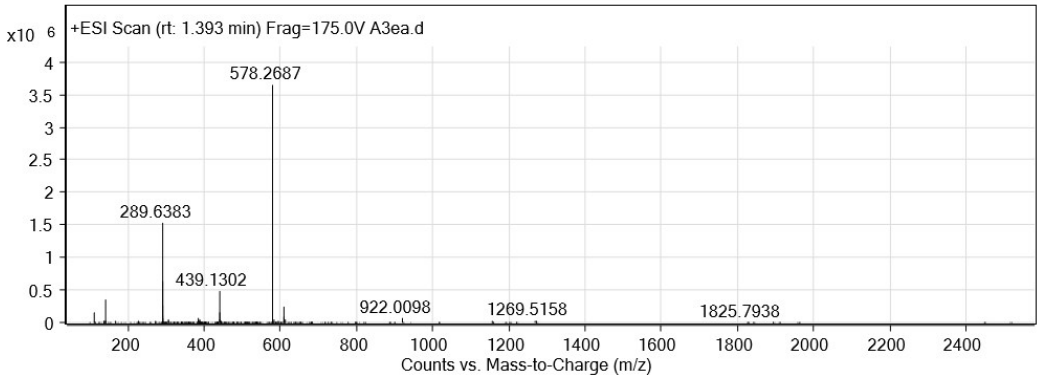
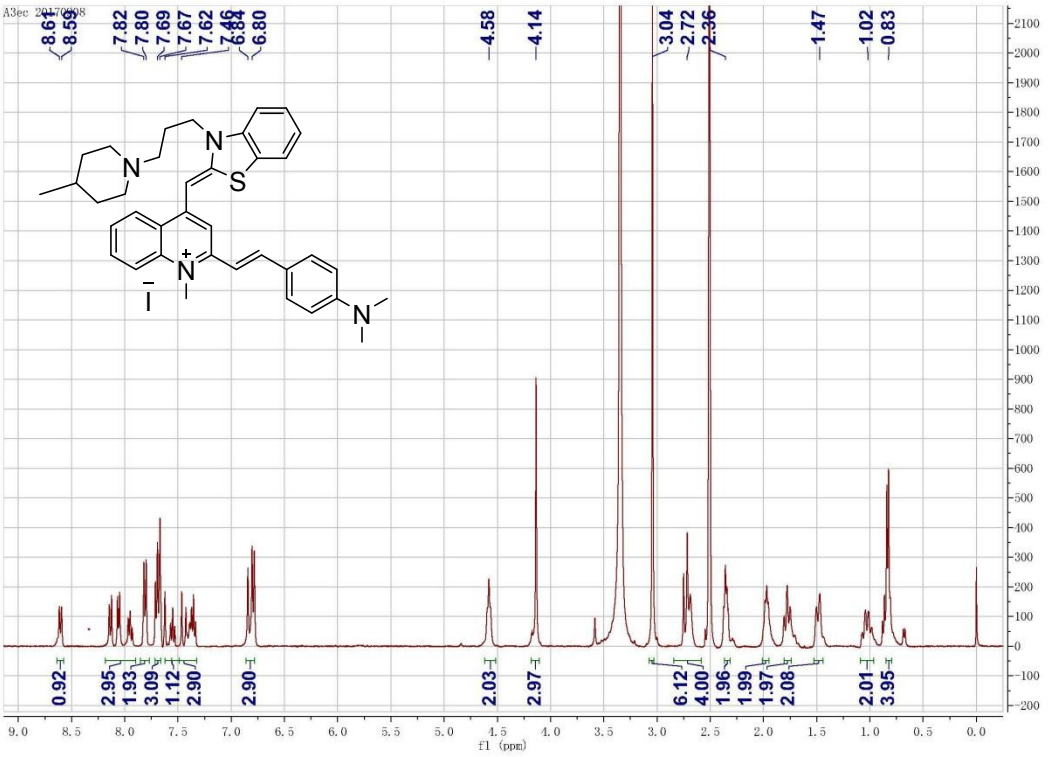


Fig.S23. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4e3.**



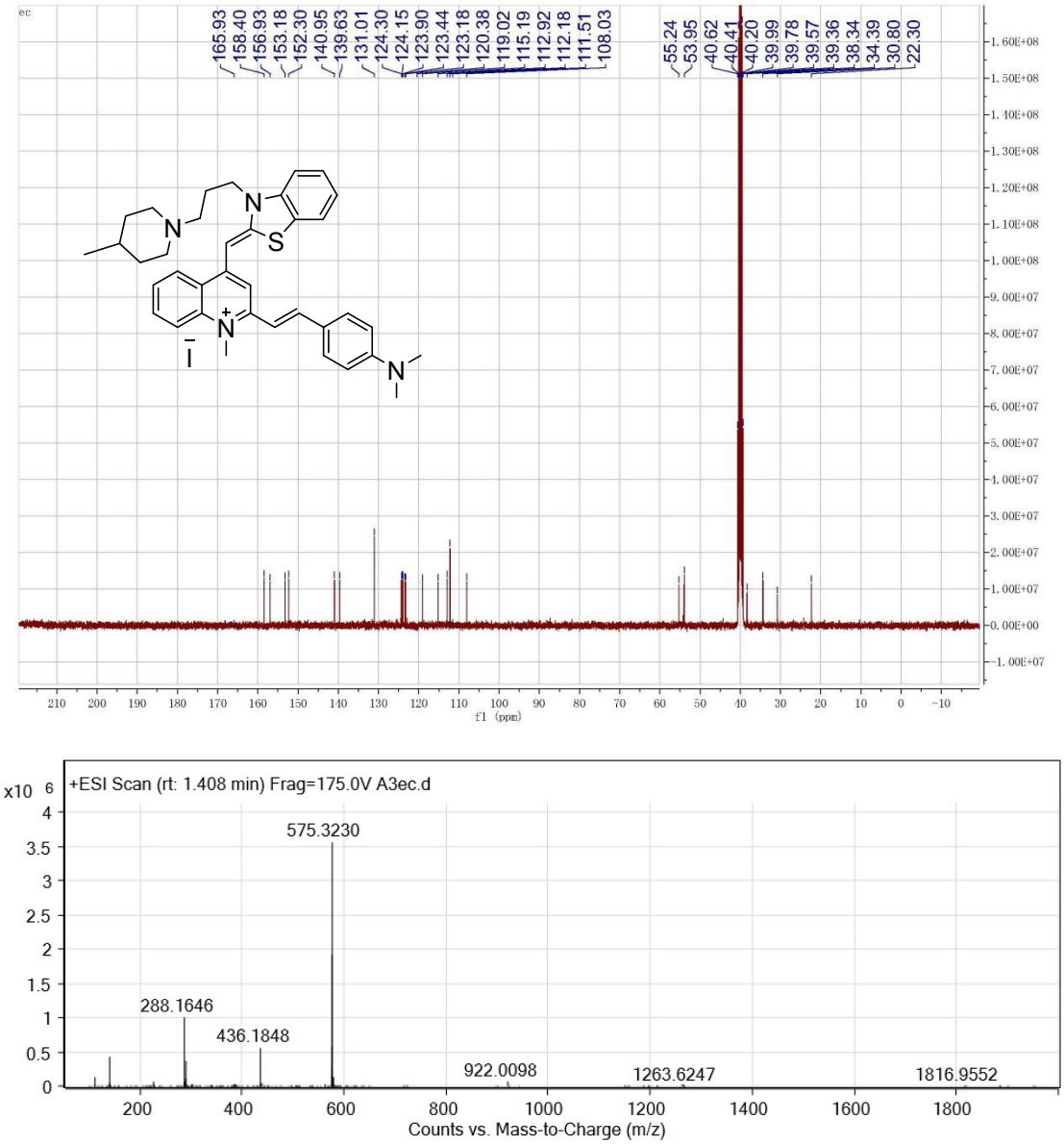


Fig.S24. ^{1}H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and HRMS spectra of compound **4e4.**

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