# Synthesis and Antibacterial Activity Studies *in vitro* of Indirubin-3´-monoximes

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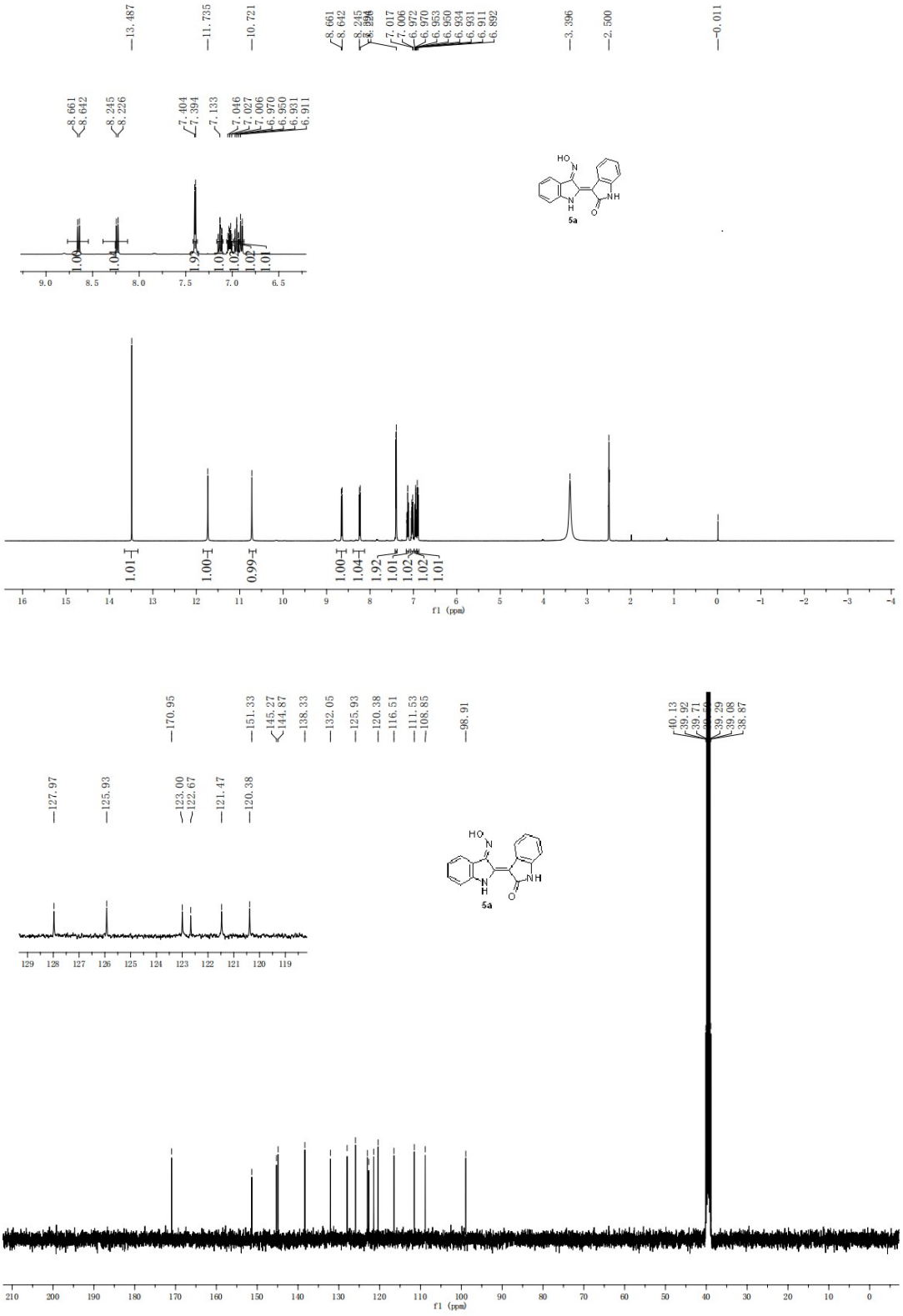
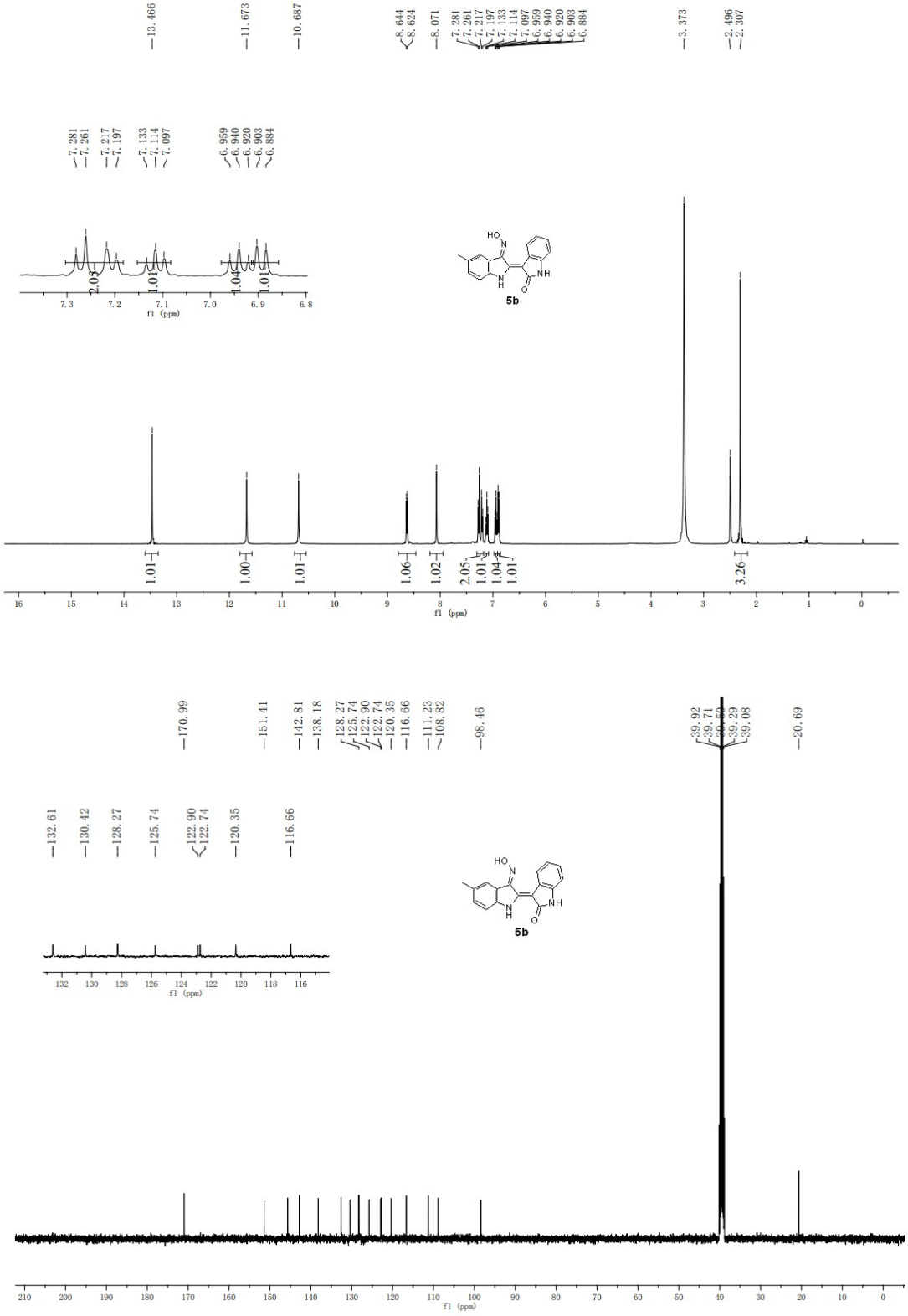
## NMR spectrogram

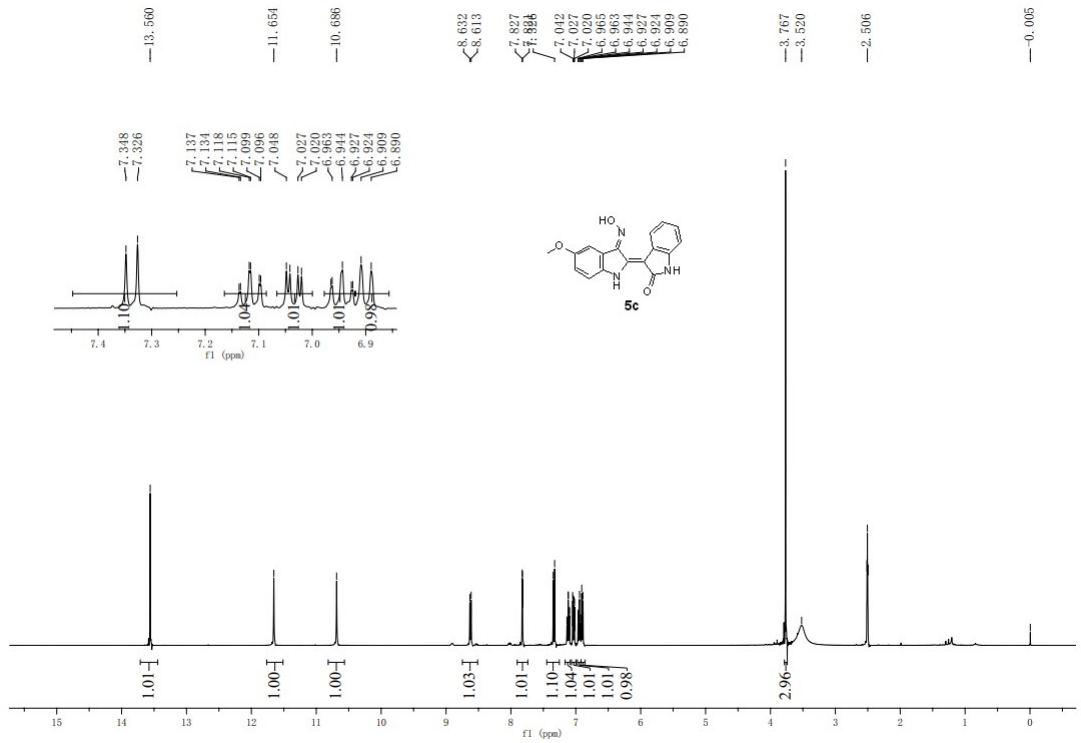
**(2*Z*, 3*E*)-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5a):**

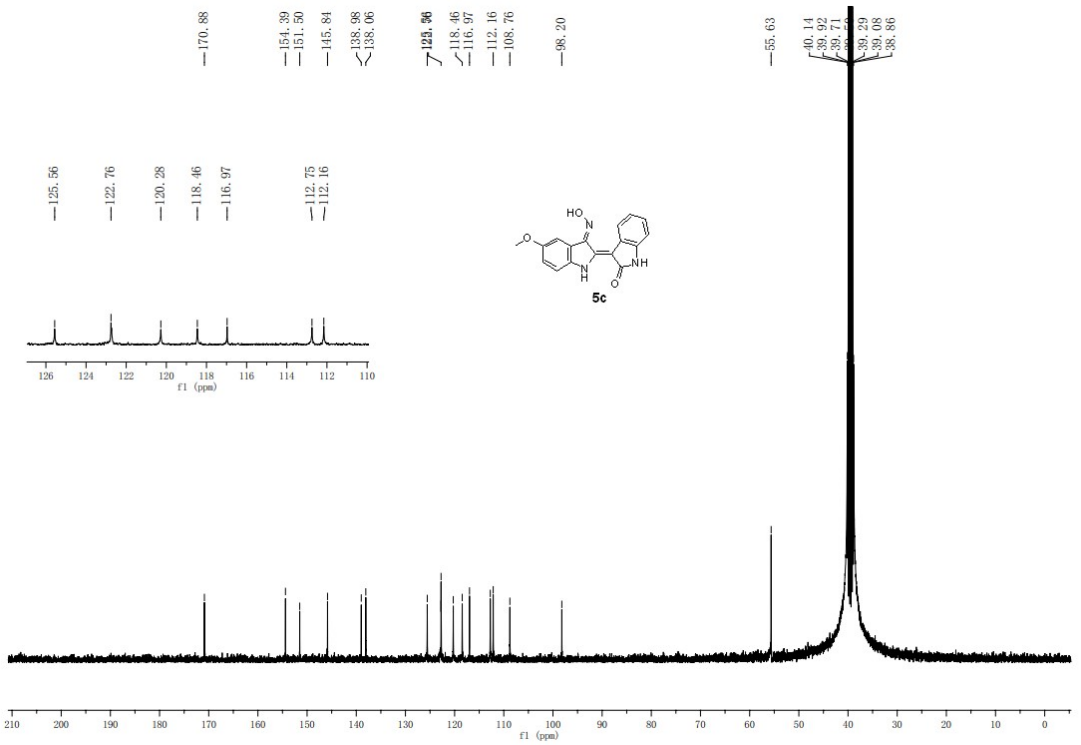


**(2*Z*, 3*E*)-3-(hydroxyimino)-5-methyl-[2, 3'-biindolinylidene]-2'-one (5b)**:

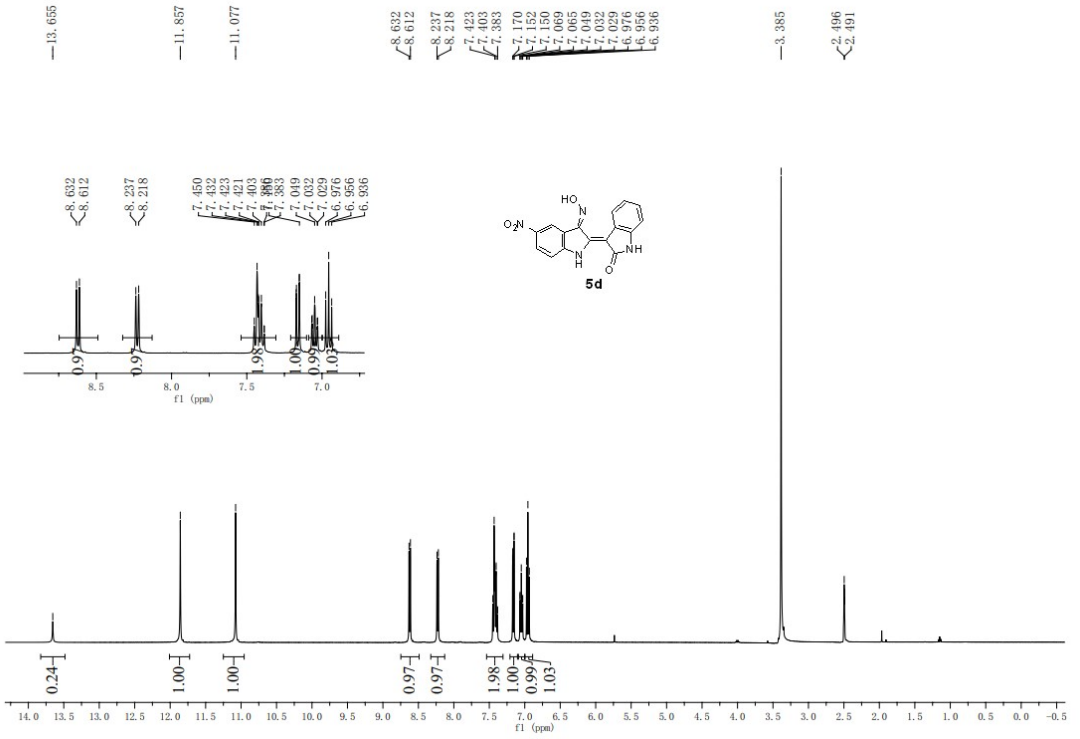


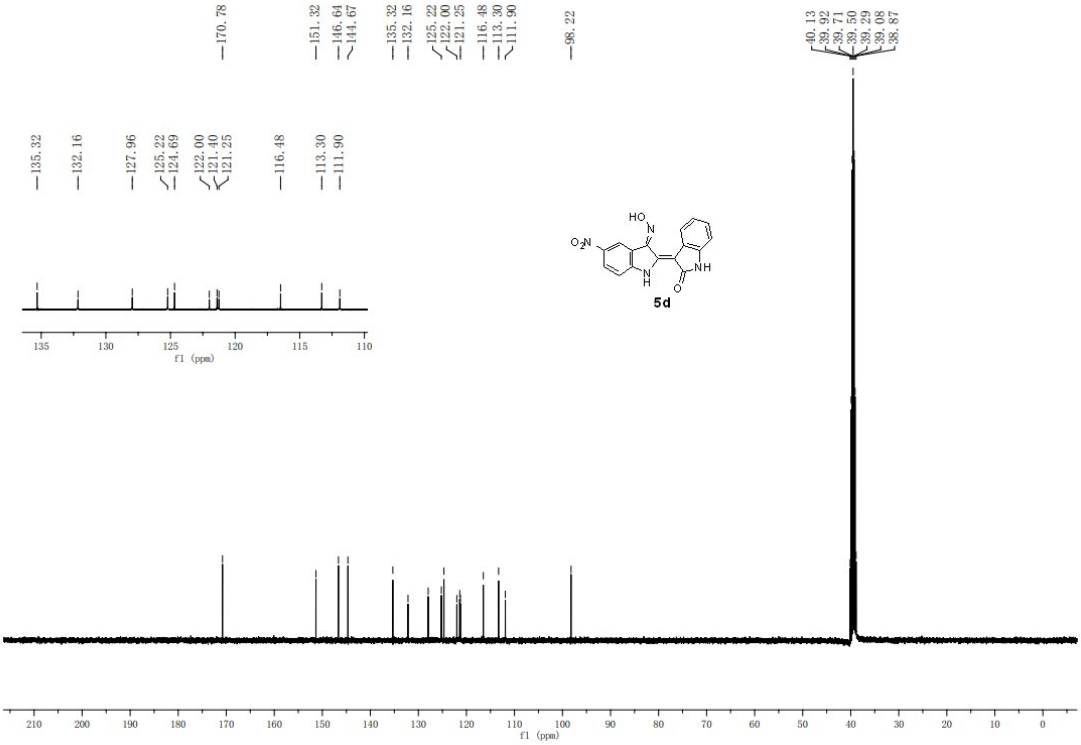
**(2*Z*, 3*E*)-3-(hydroxyimino)-5-methoxy-[2, 3'-biindolinylidene]-2'-one (5c):**



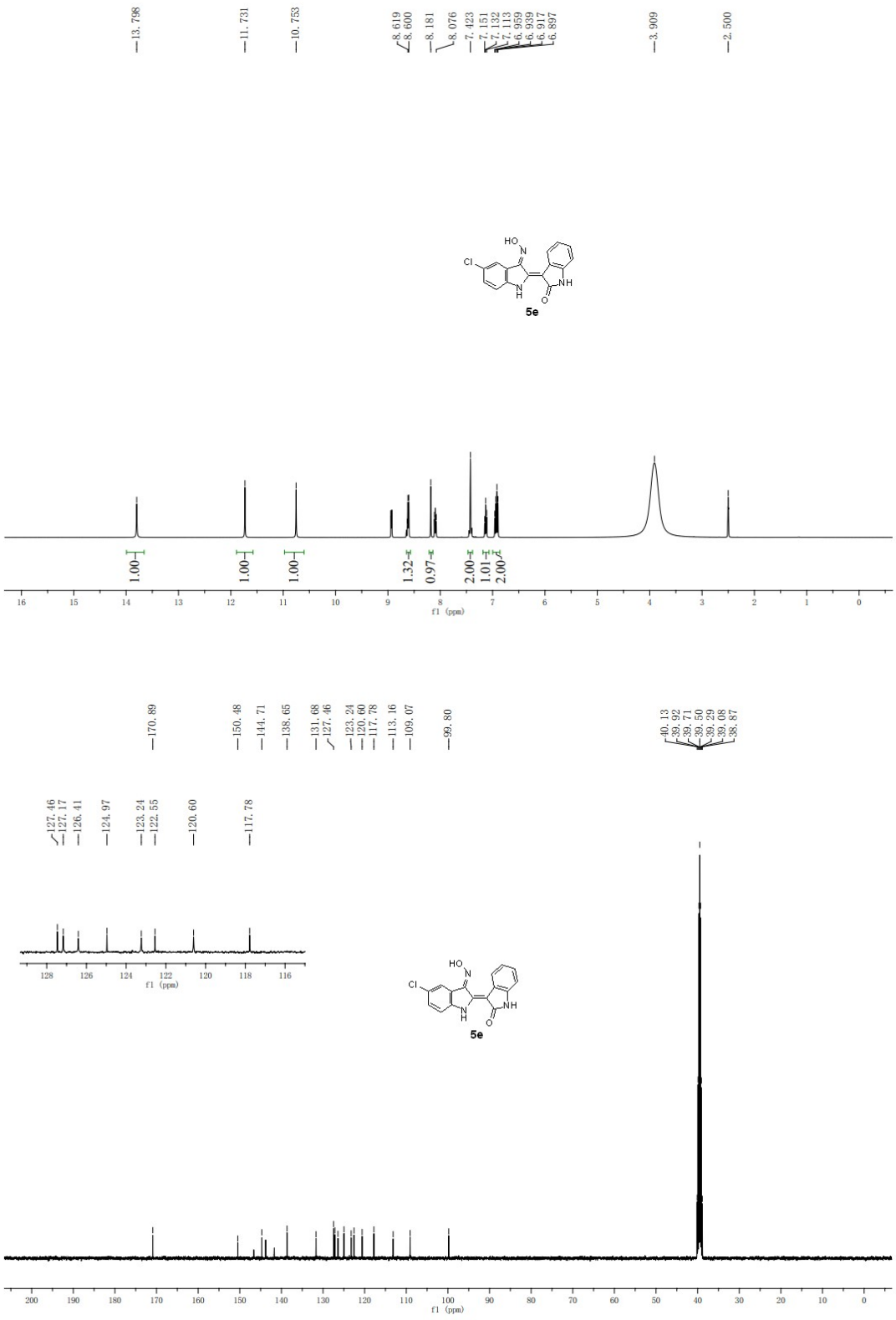



**(2*Z*, 3*E*)-3-(hydroxyimino)-5-nitro-[2, 3'-biindolinylidene]-2'-one (5d):**

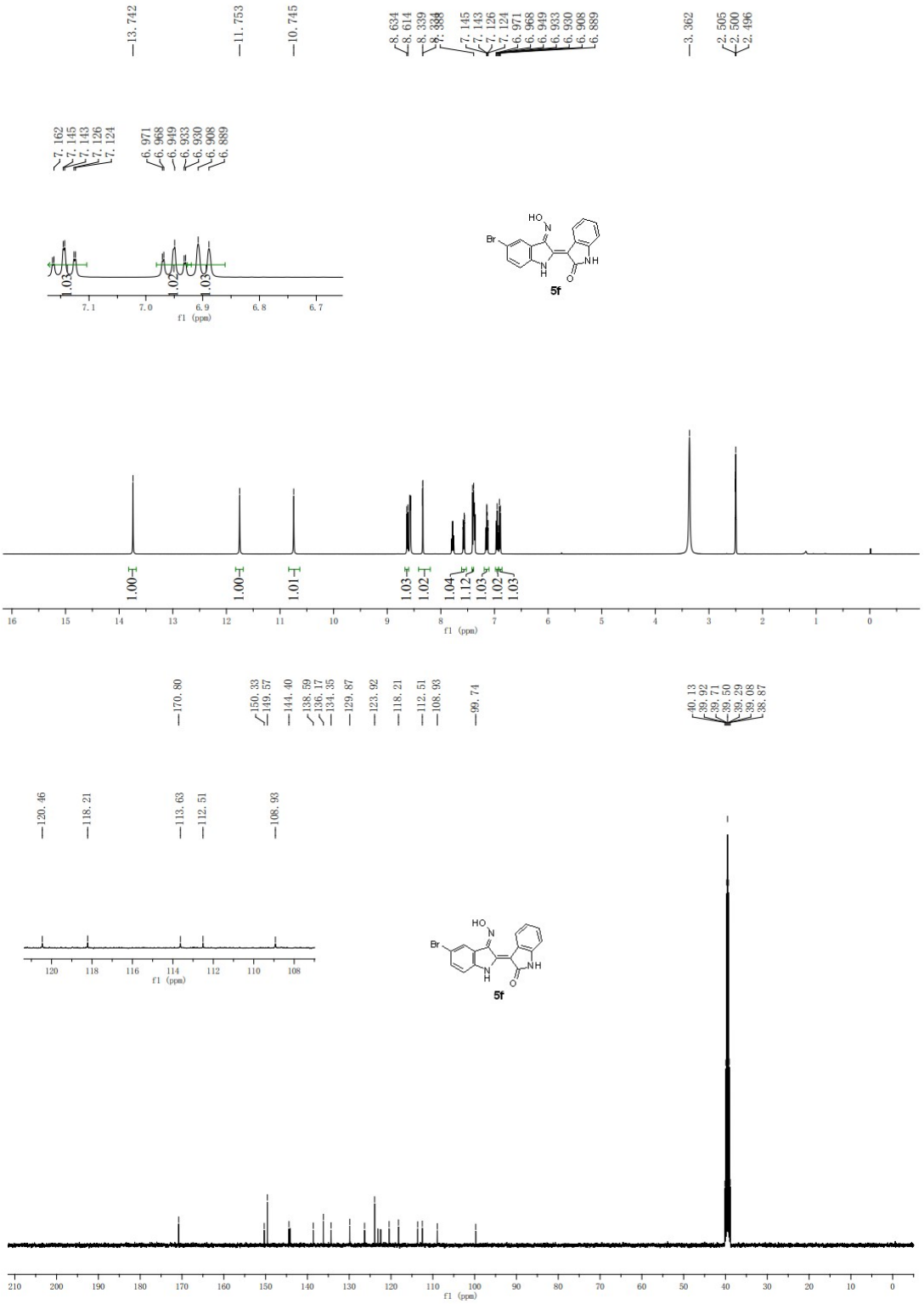


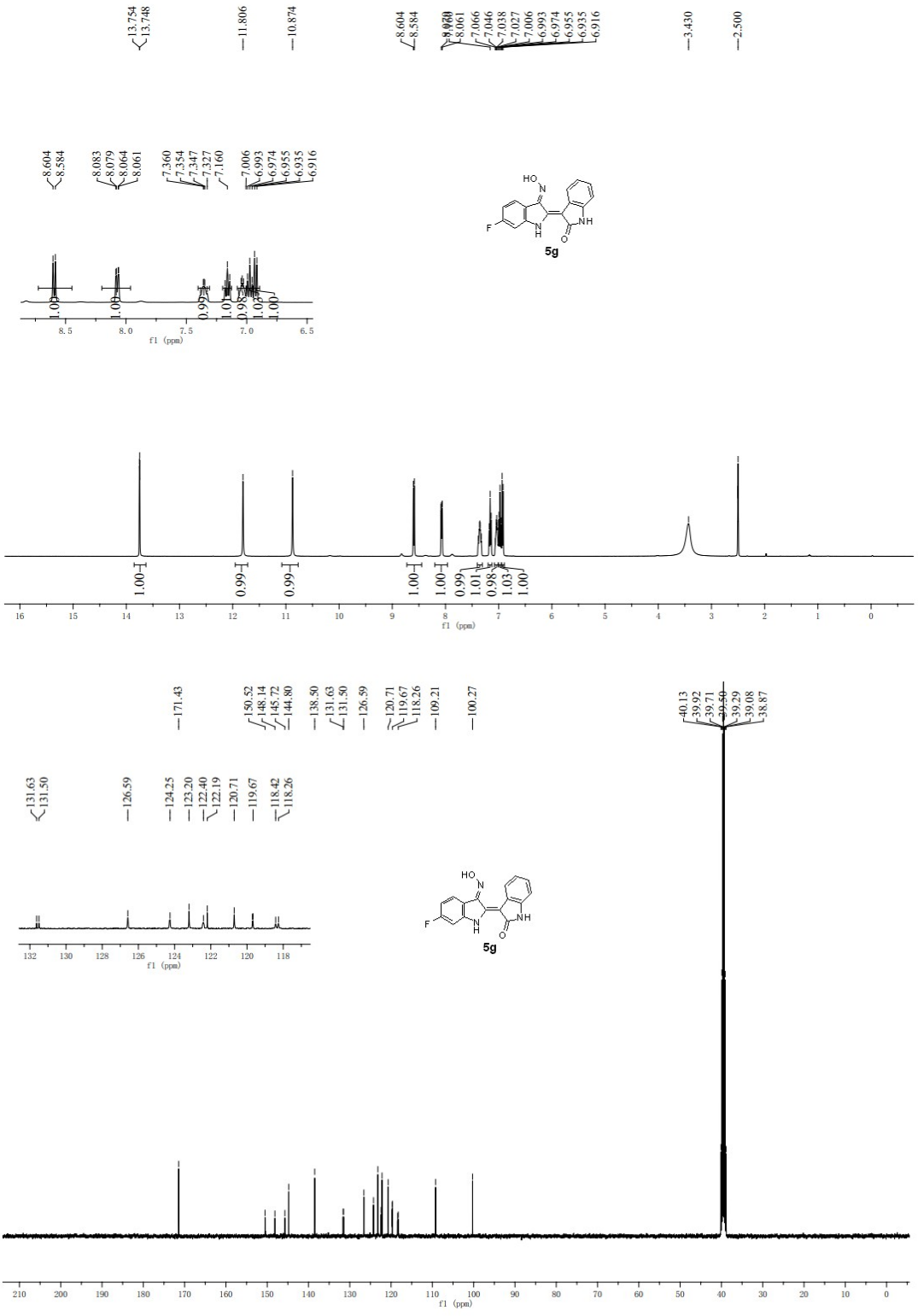
**(2*Z*, 3*E*)-5-chloro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5e):**



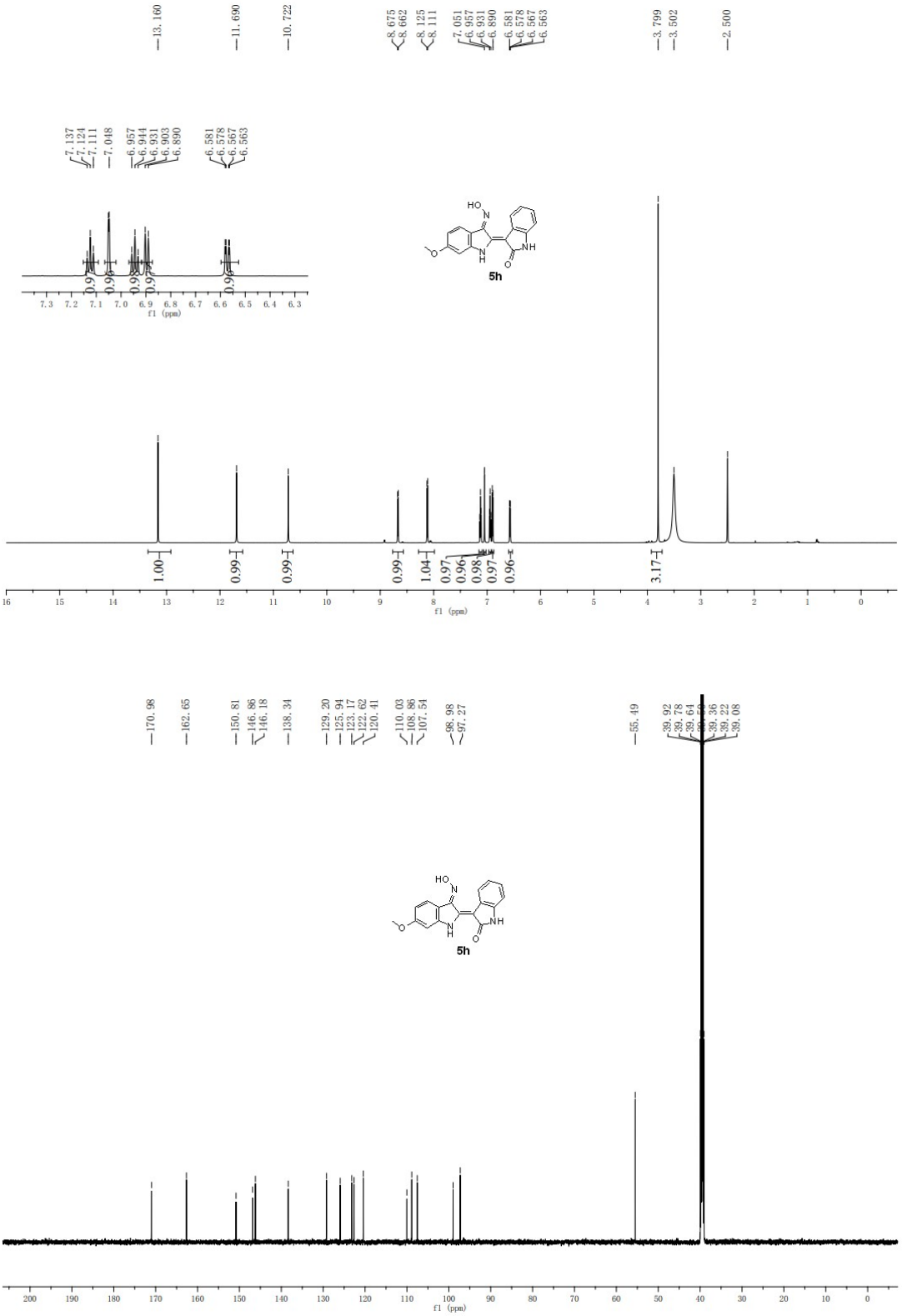
**(2*Z*, 3*E*)-5-bromo-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5f):**



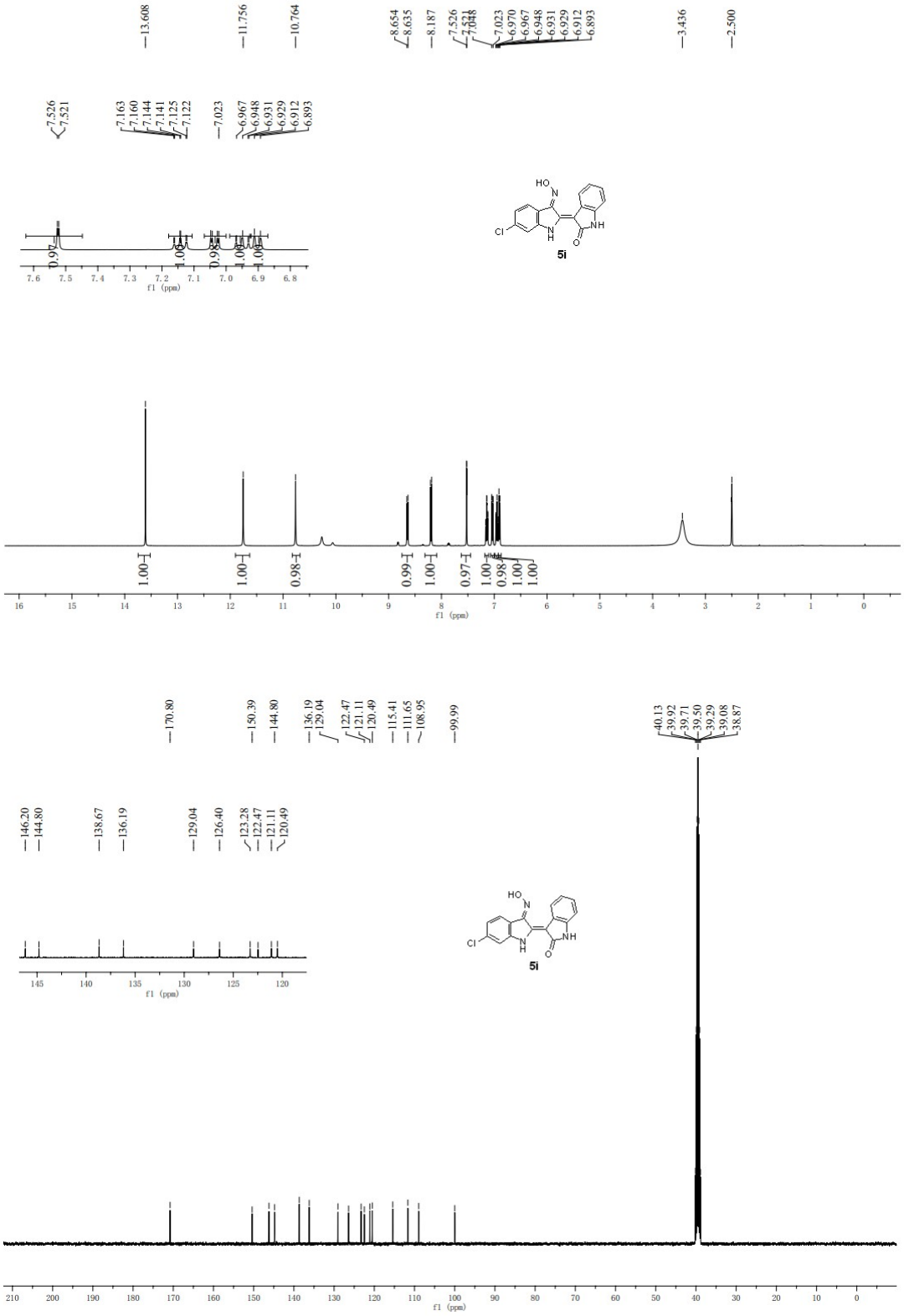
**(2*Z*, 3*E*)-6-fluoro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5g):**



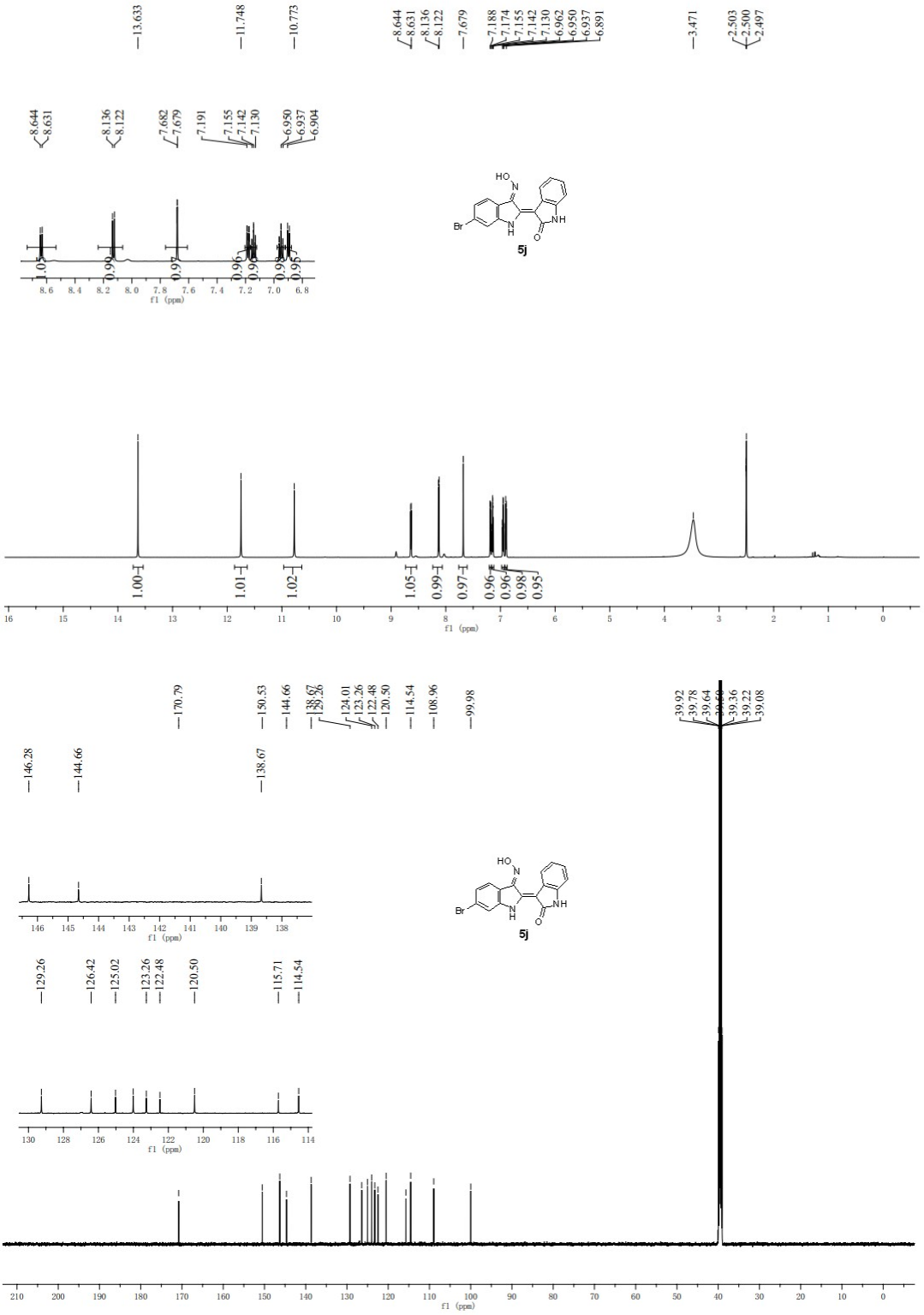
**(2*Z*, 3*E*)-3-(hydroxyimino)-6-methoxy-[2, 3'-biindolinylidene]-2'-one (5h):**



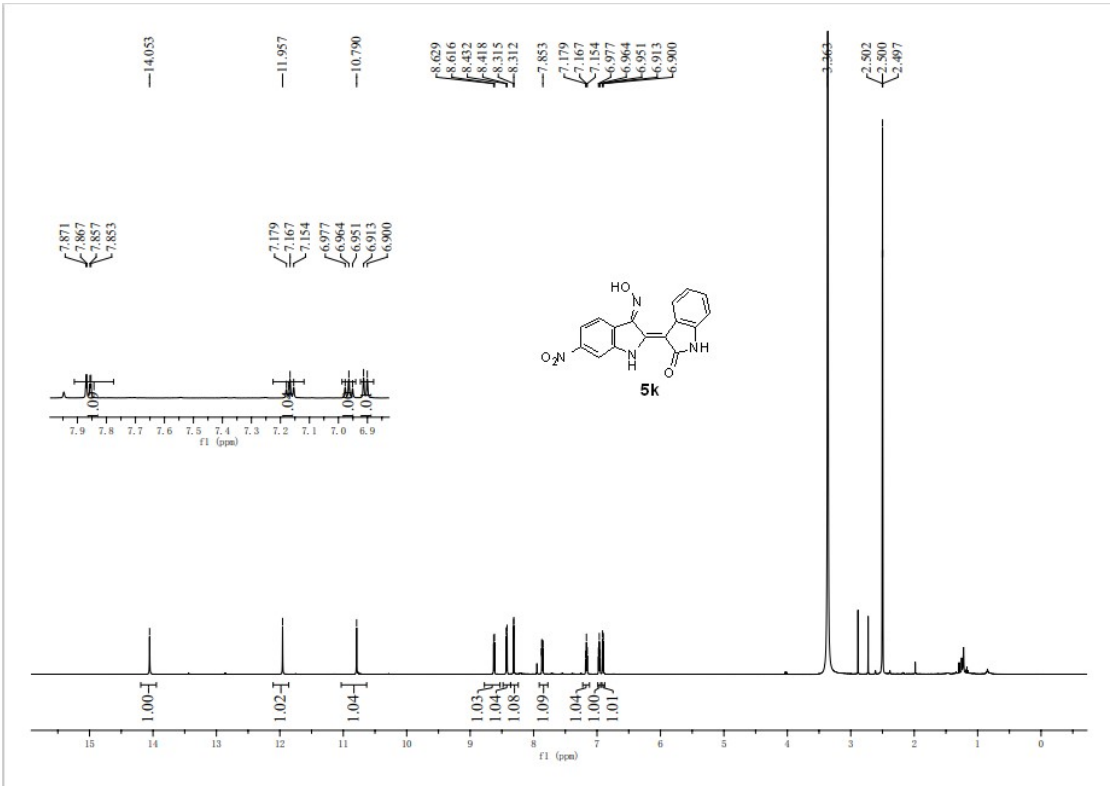
**(2*Z*, 3*E*)-6-chloro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5i):**

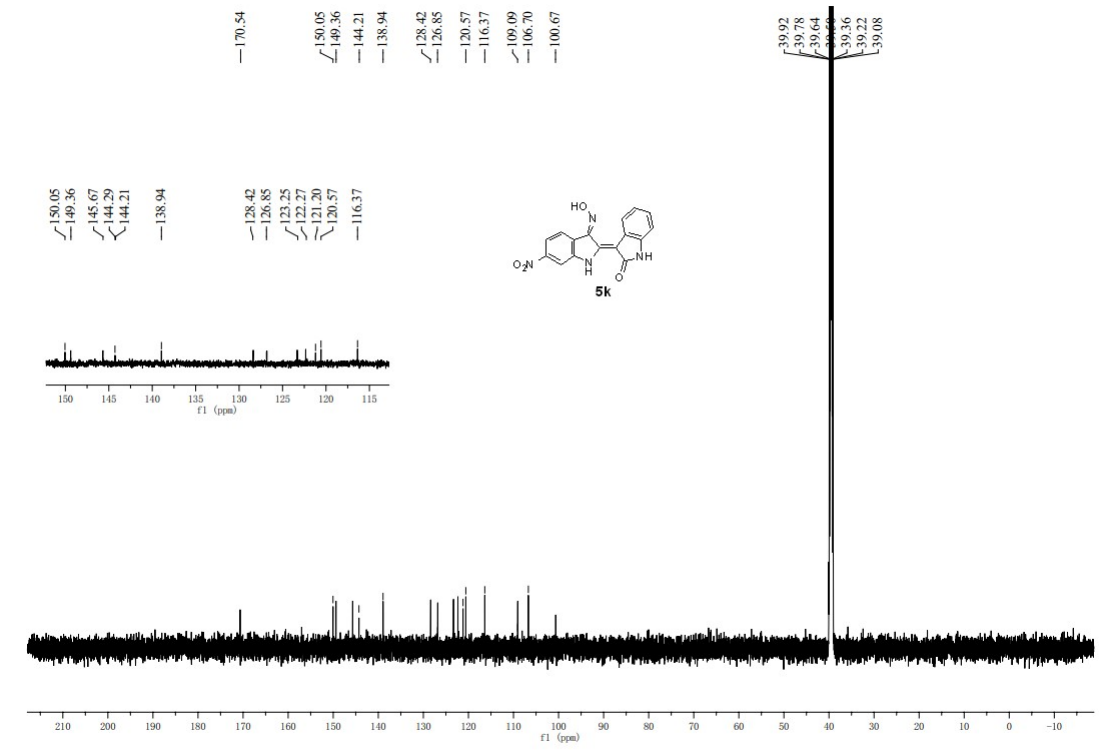


**(2*Z*, 3*E*)-6-bromo-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5j):**

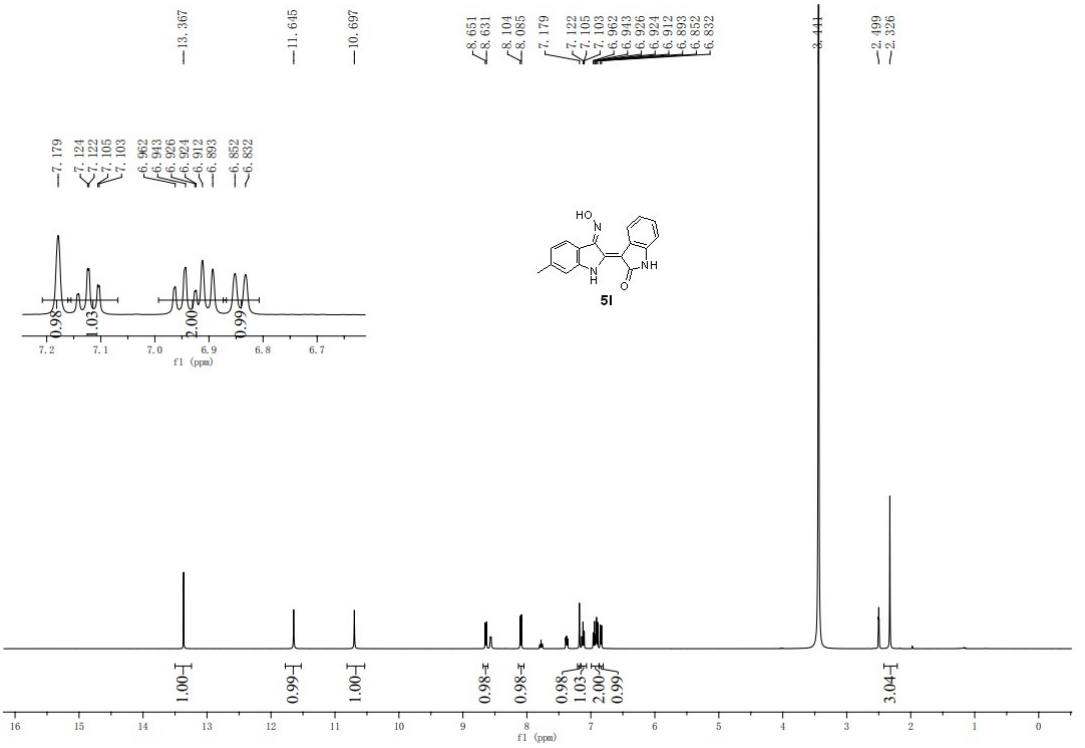


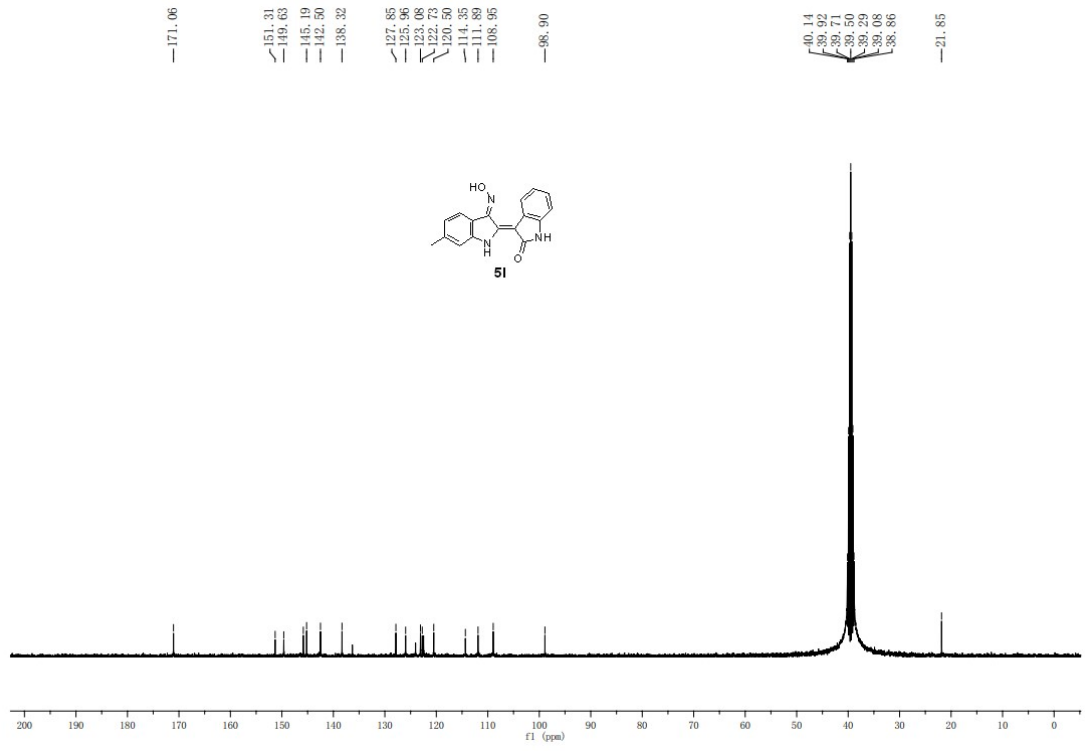
**(2*Z*, 3*E*)-3-(hydroxyimino)-6-nitro-[2, 3'-biindolinylidene]-2'-one (5k):**



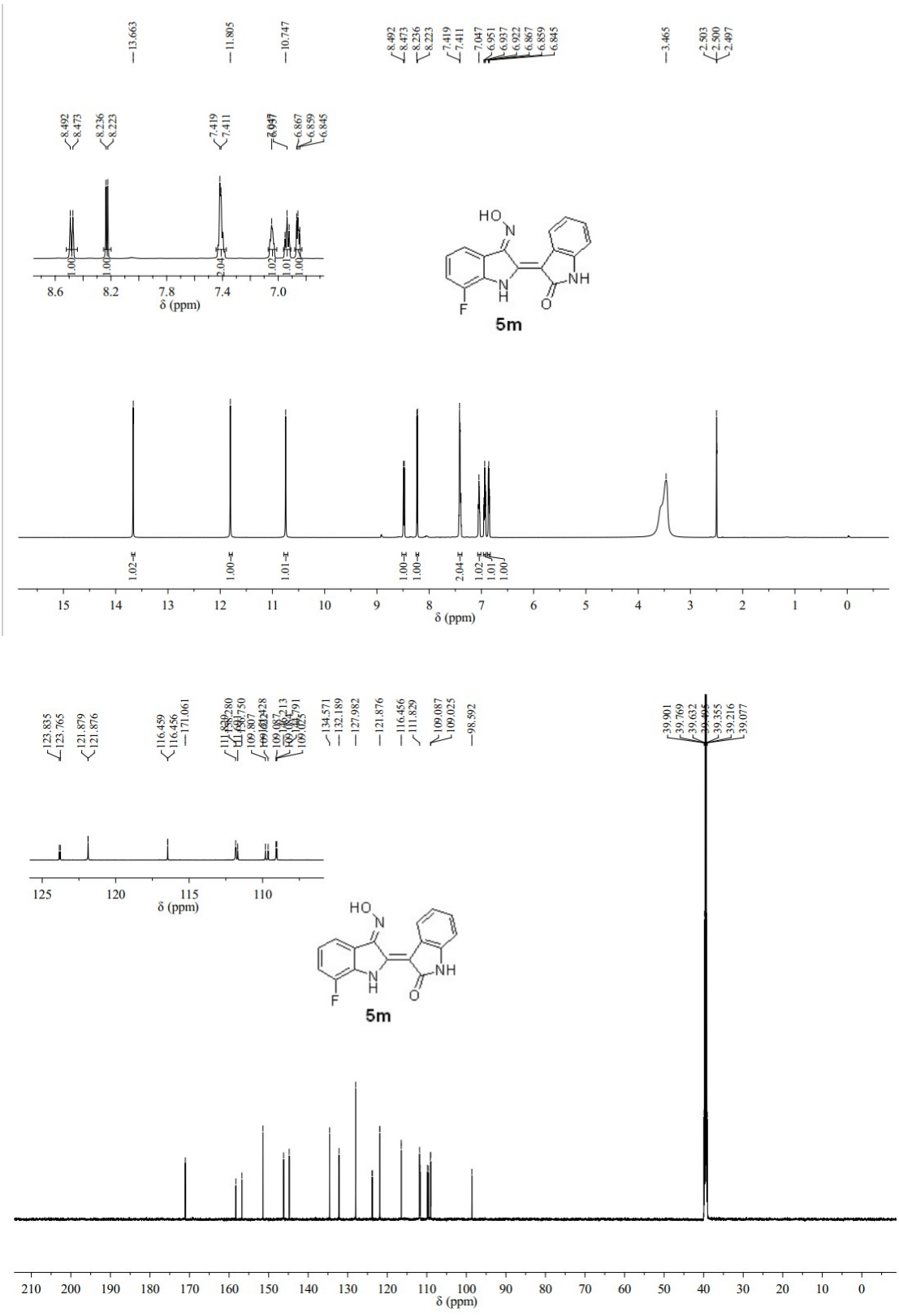



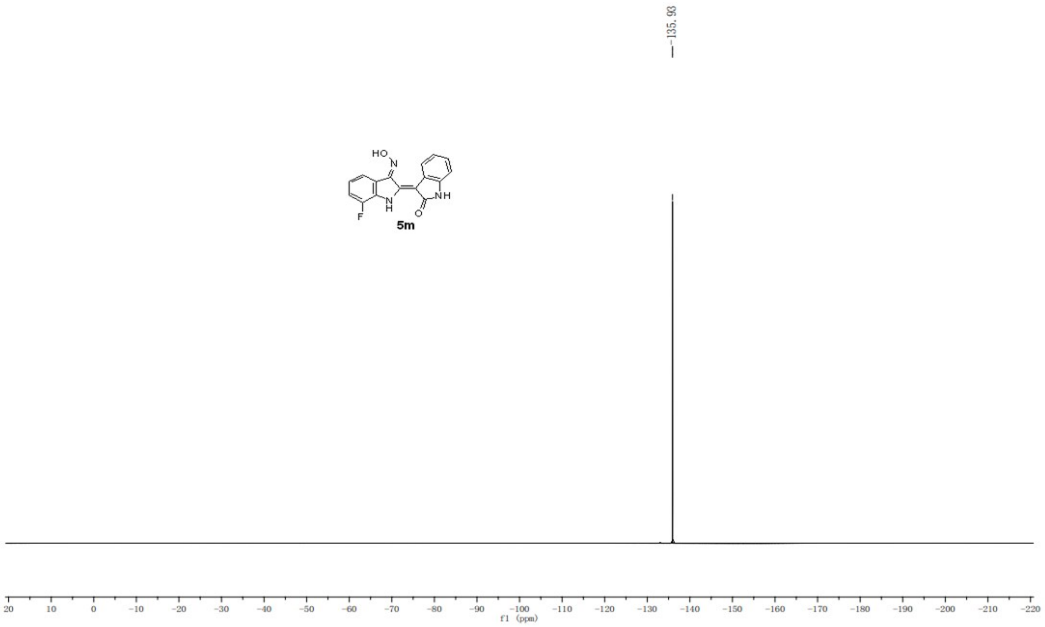
**(2*Z*, 3*E*)-3-(hydroxyimino)-6-methyl-[2, 3'-biindolinylidene]-2'-one (5l):**



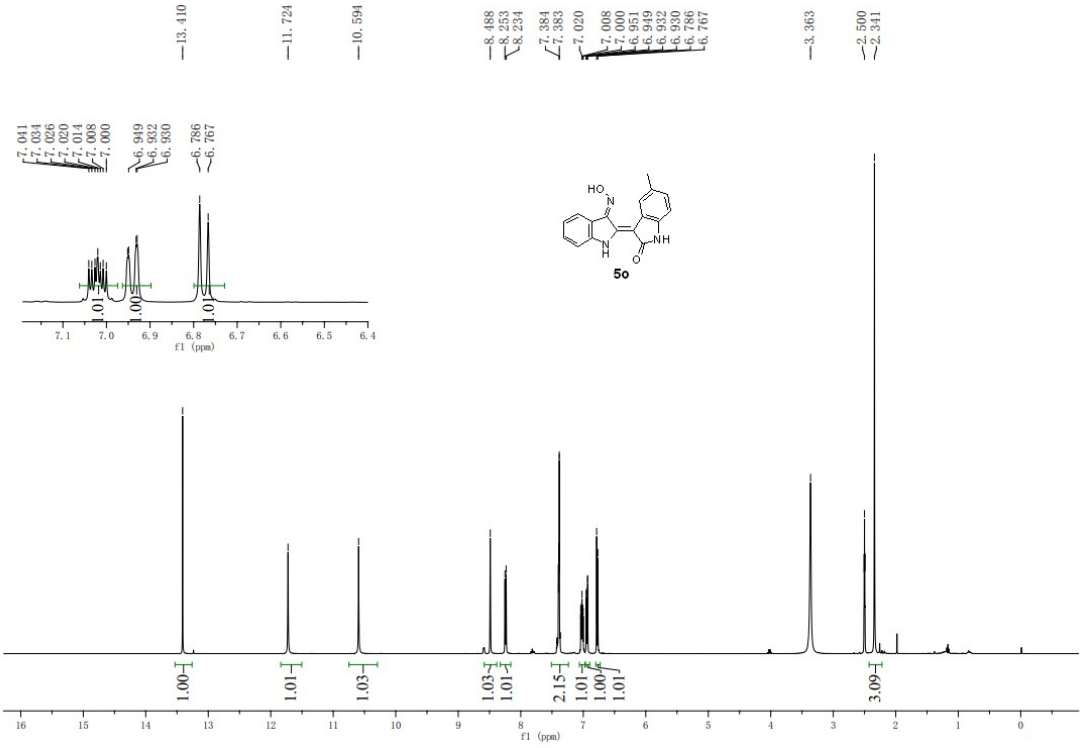



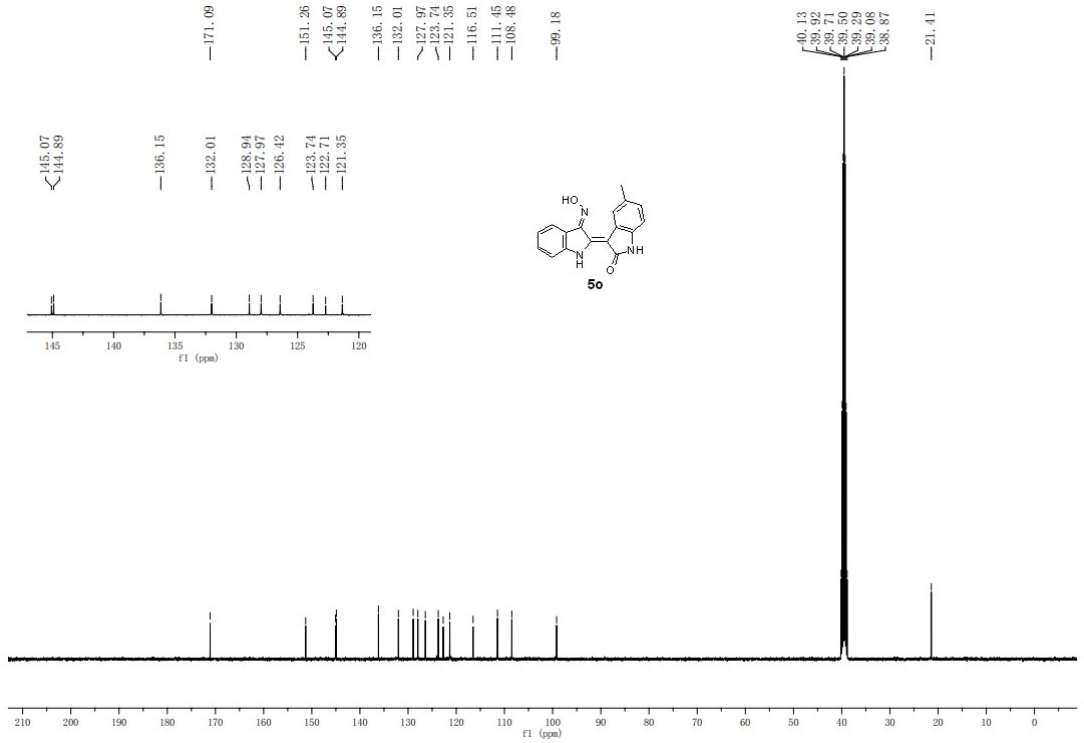
**(2*Z*, 3*E*)-7-fluoro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5m):**



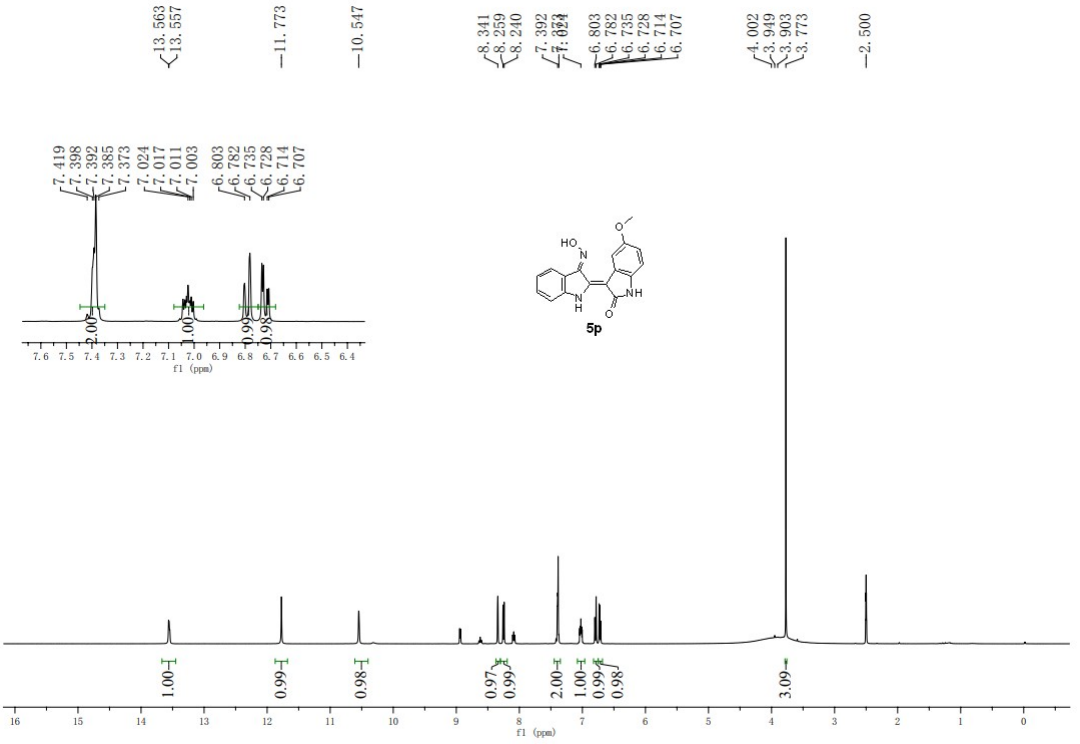

-135.93
HO
N
NH
F
O
5m
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
f1 (ppm)

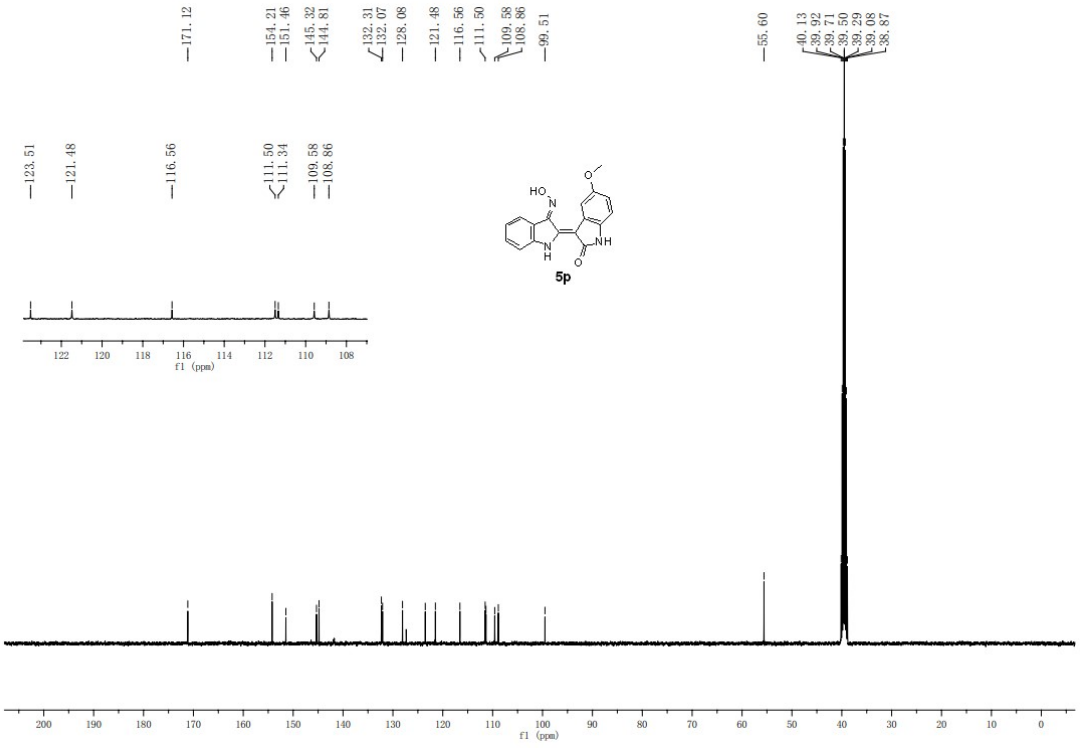
**(2*Z*, 3*E*)-3-(hydroxyimino)-5'-methyl-[2, 3'-biindolinylidene]-2'-one (5o):**



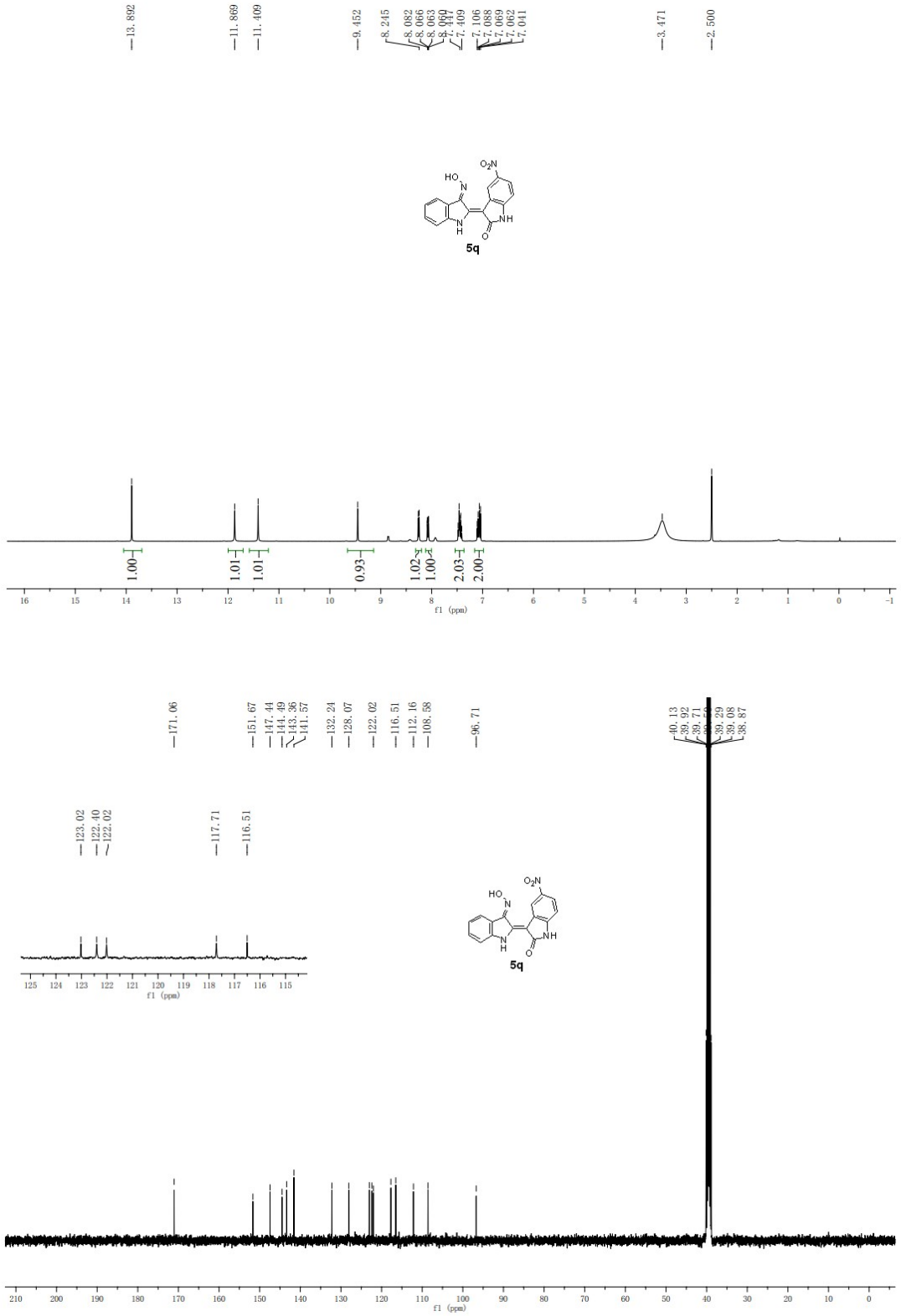



**(2*Z*, 3*E*)-3-(hydroxyimino)-5'-methoxy-[2, 3'-biindolinylidene]-2'-one (5p):**

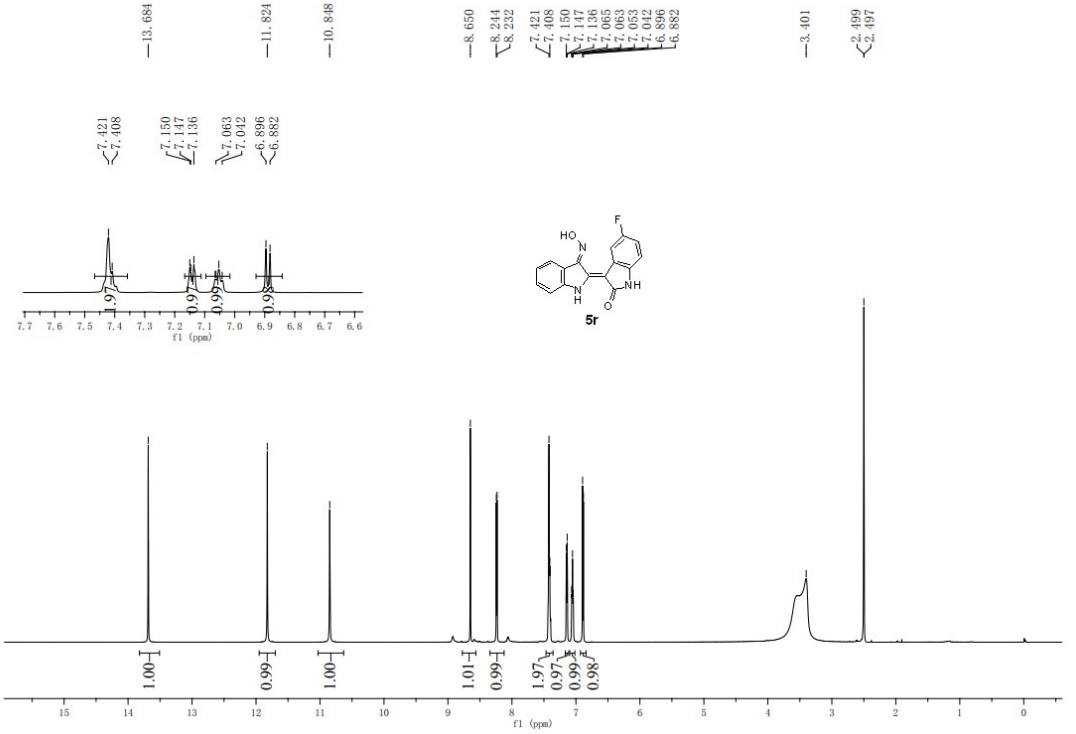


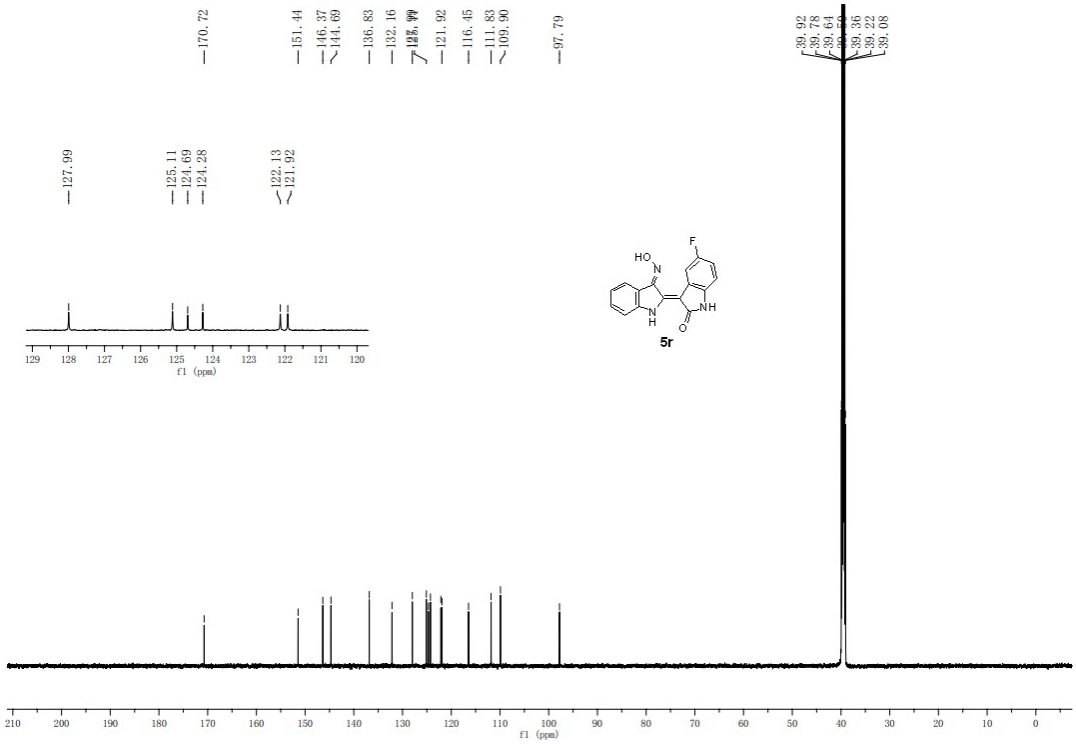



**(2*Z*, 3*E*)-3-(hydroxyimino)-5'-nitro-[2, 3'-biindolinylidene]-2'-one (5q):**

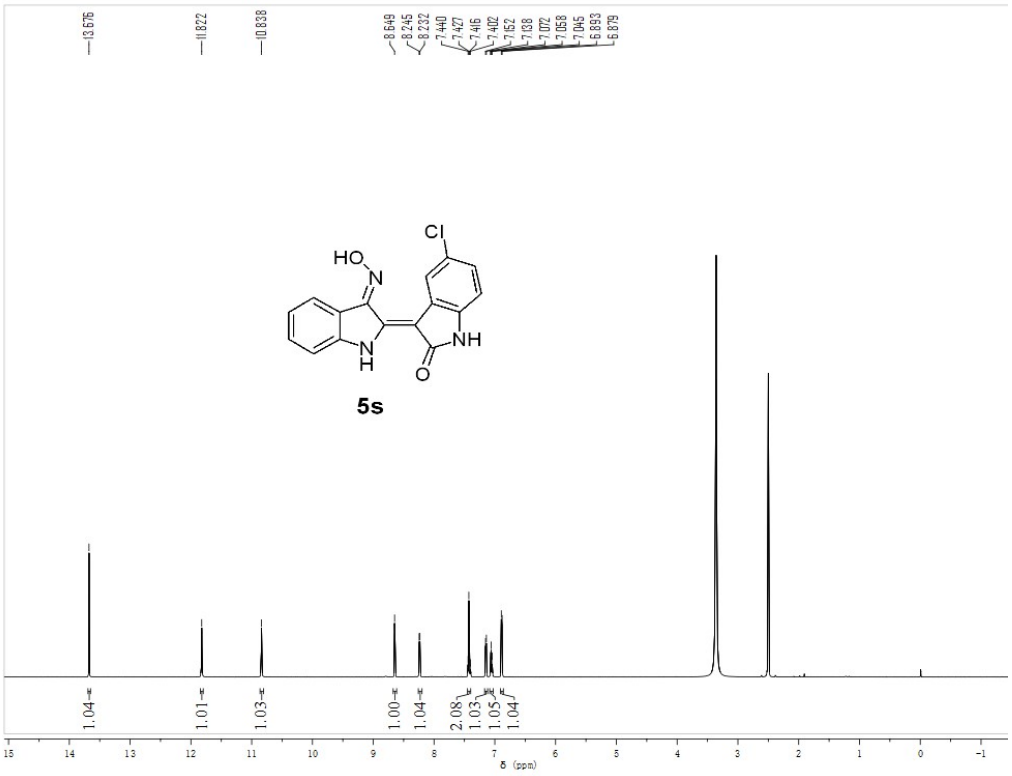


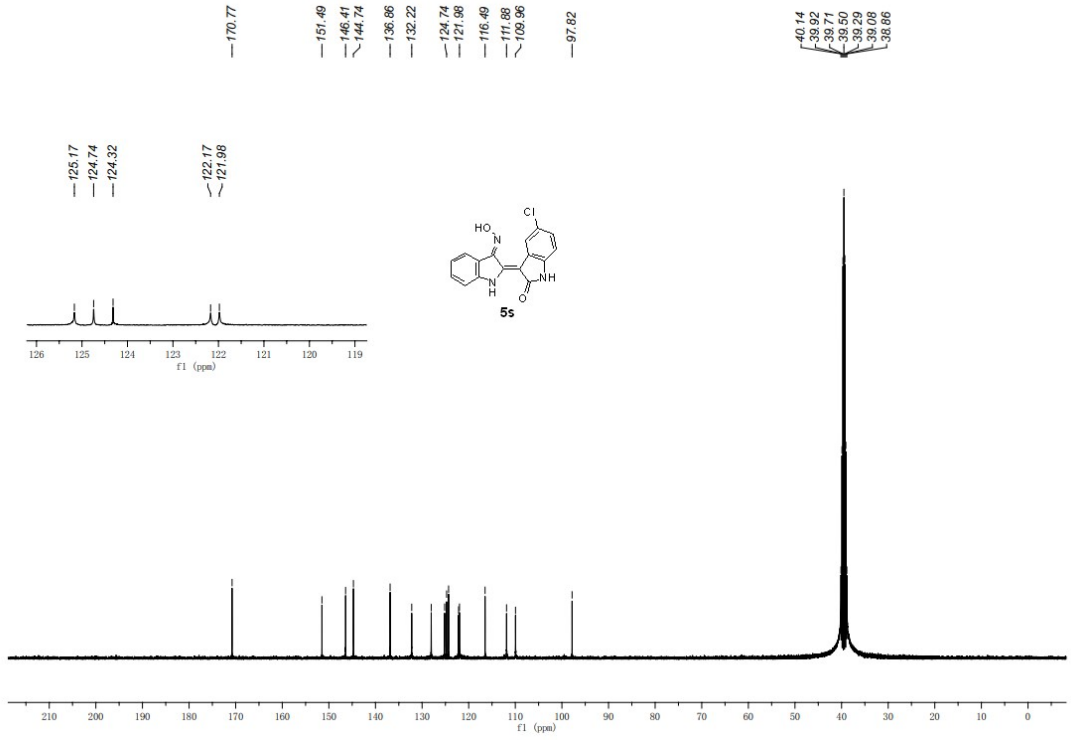
**(2*Z*, 3*E*)-5'-fluoro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5r):**



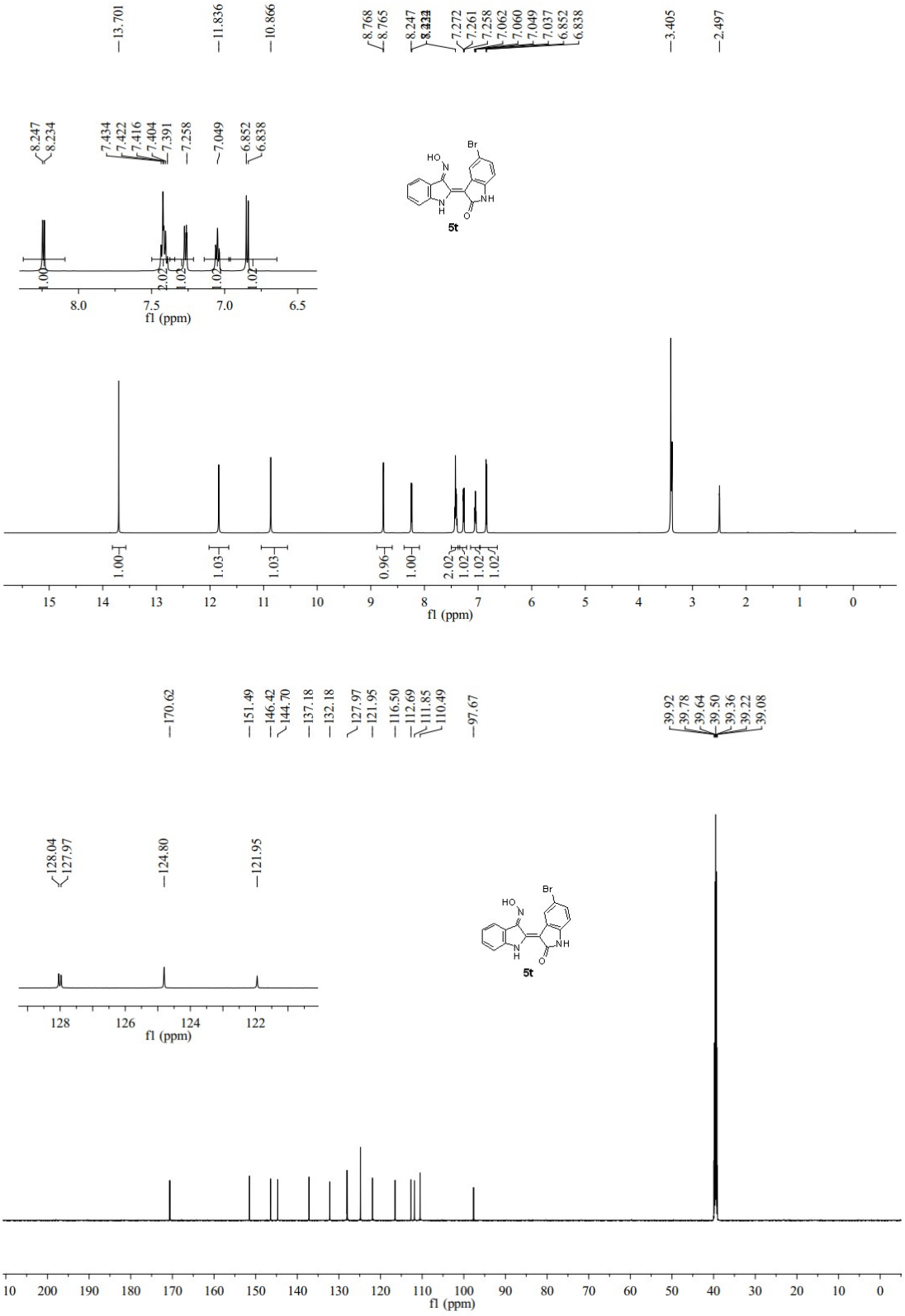



**(2*Z*, 3*E*)-5'-chloro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5s):**

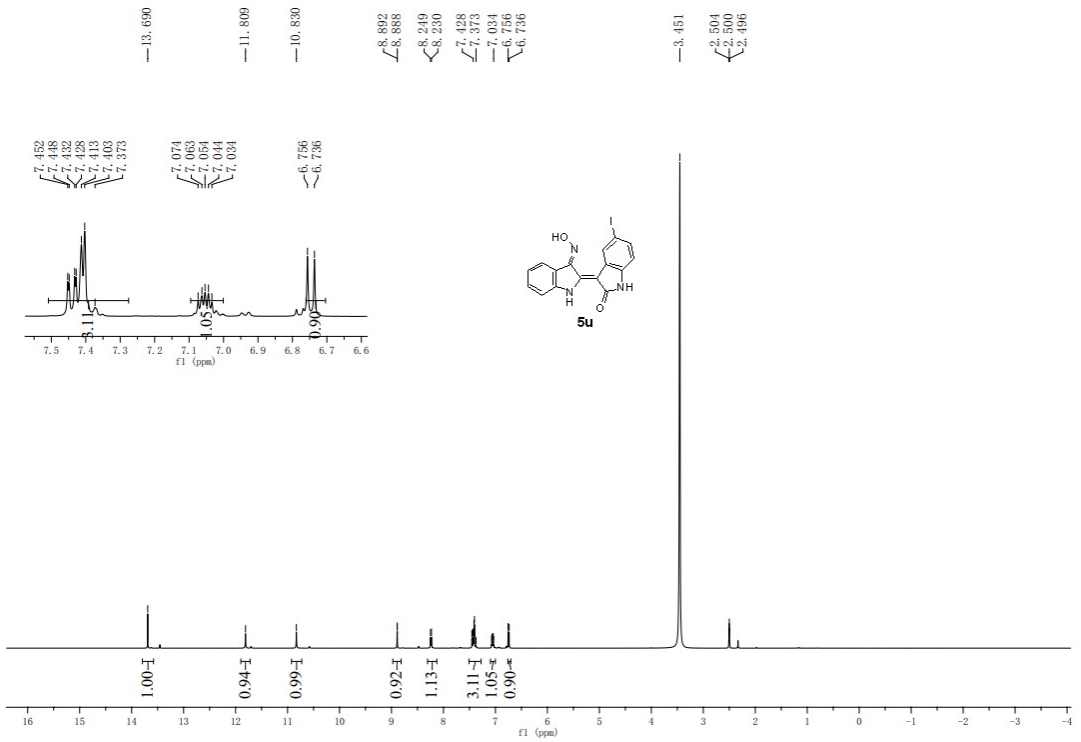


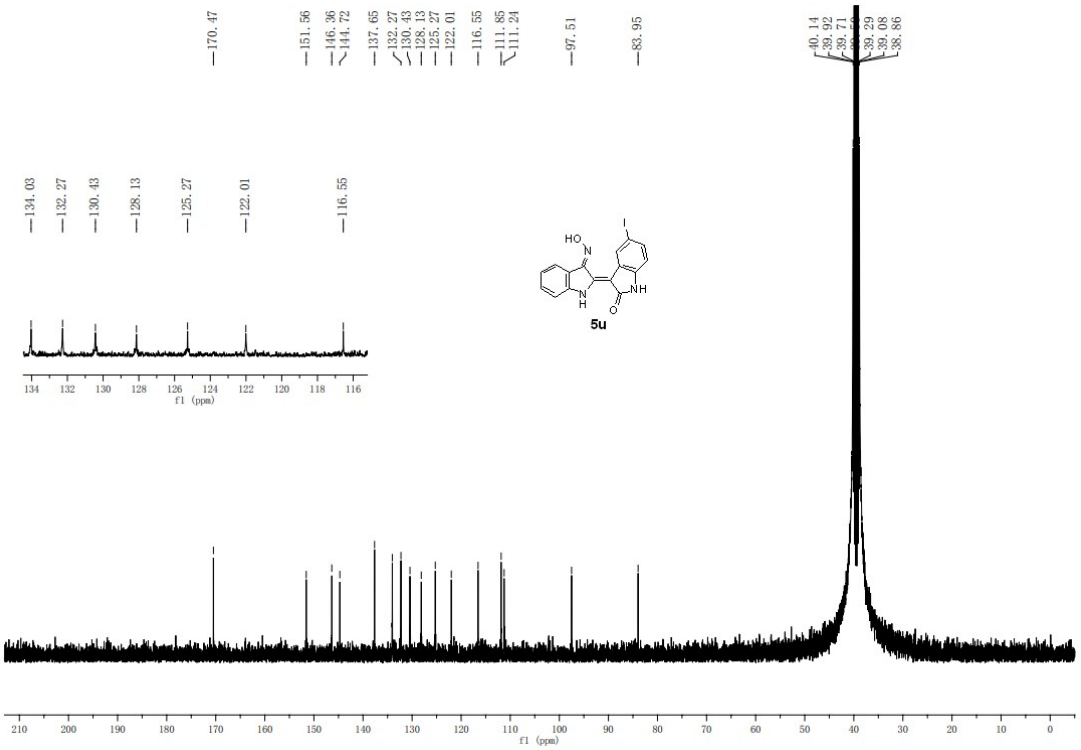



**(2*Z*, 3*E*)-5'-bromo-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5t):**

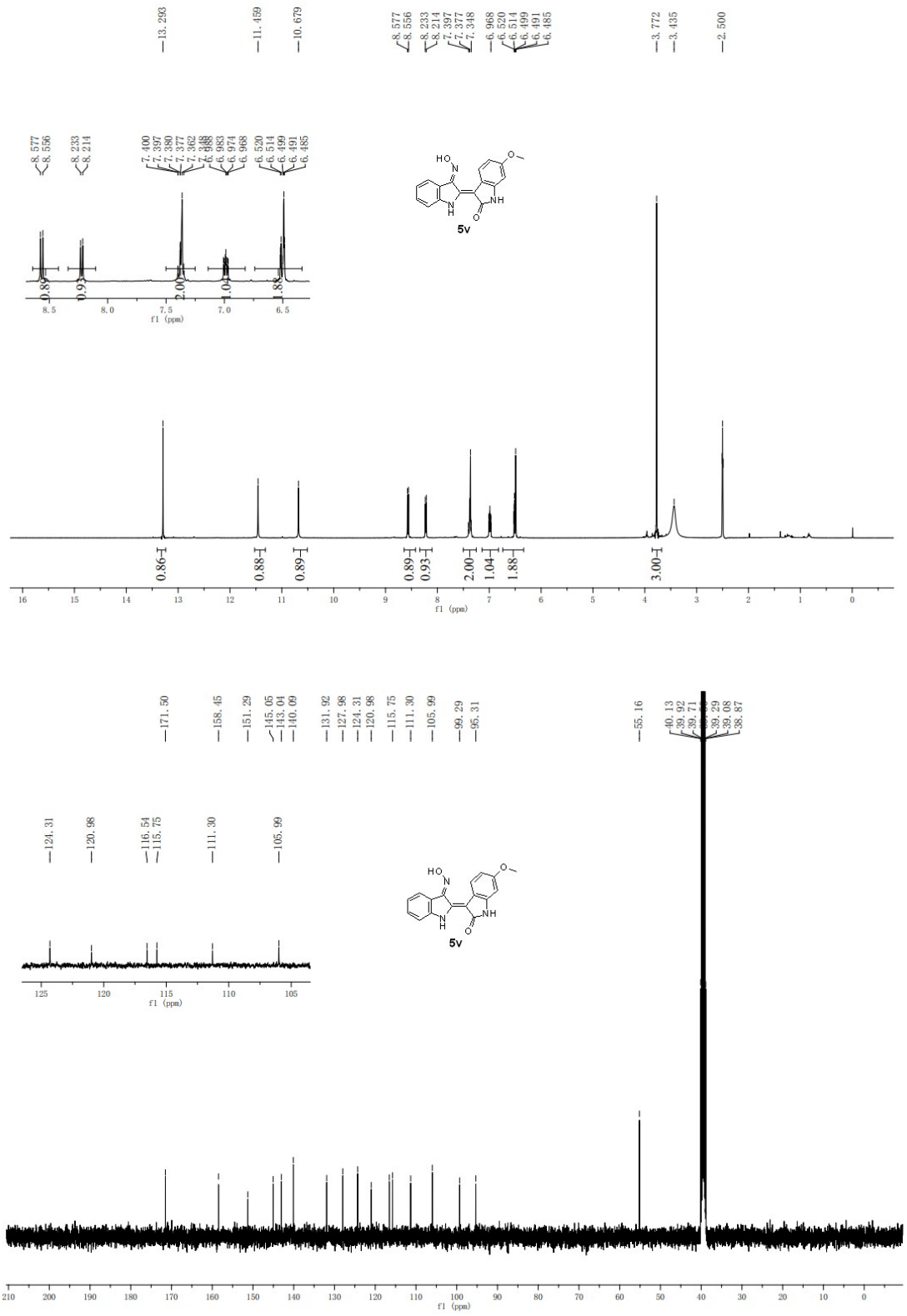


**(2*Z*, 3*E*)-3-(hydroxyimino)-5'-iodo-[2, 3'-biindolinylidene]-2'-one (5u):**

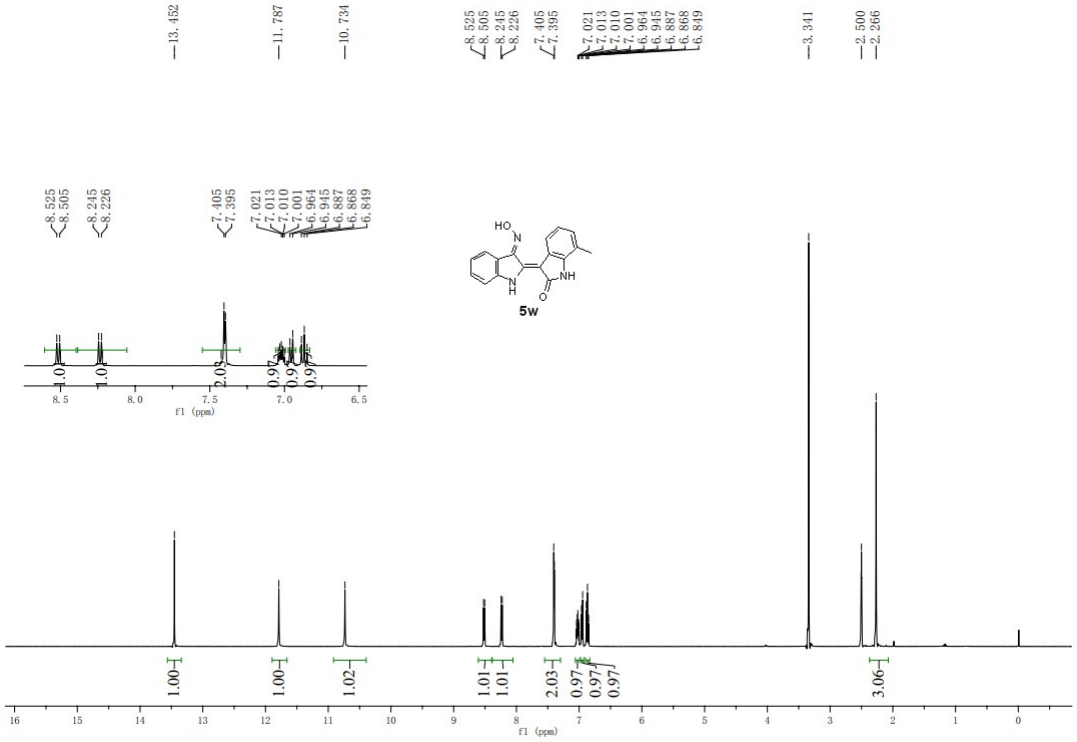


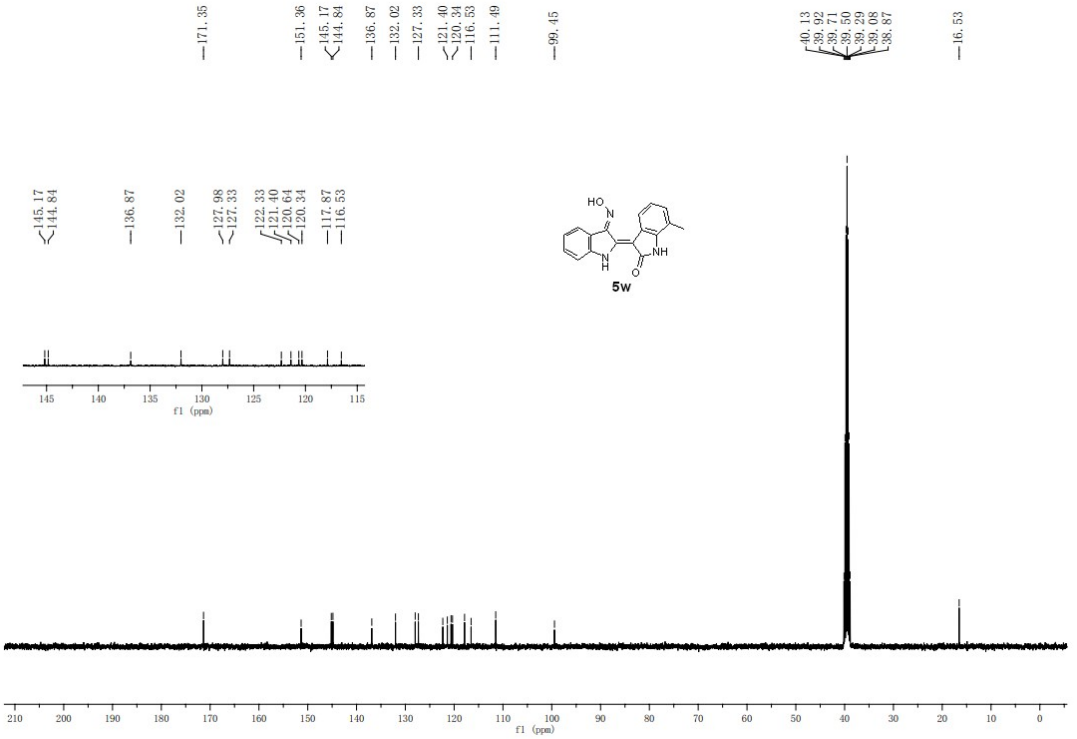



**(2*Z*, 3*E*)-3-(hydroxyimino)-6'-methoxy-[2, 3'-biindolinylidene]-2'-one (5v):**

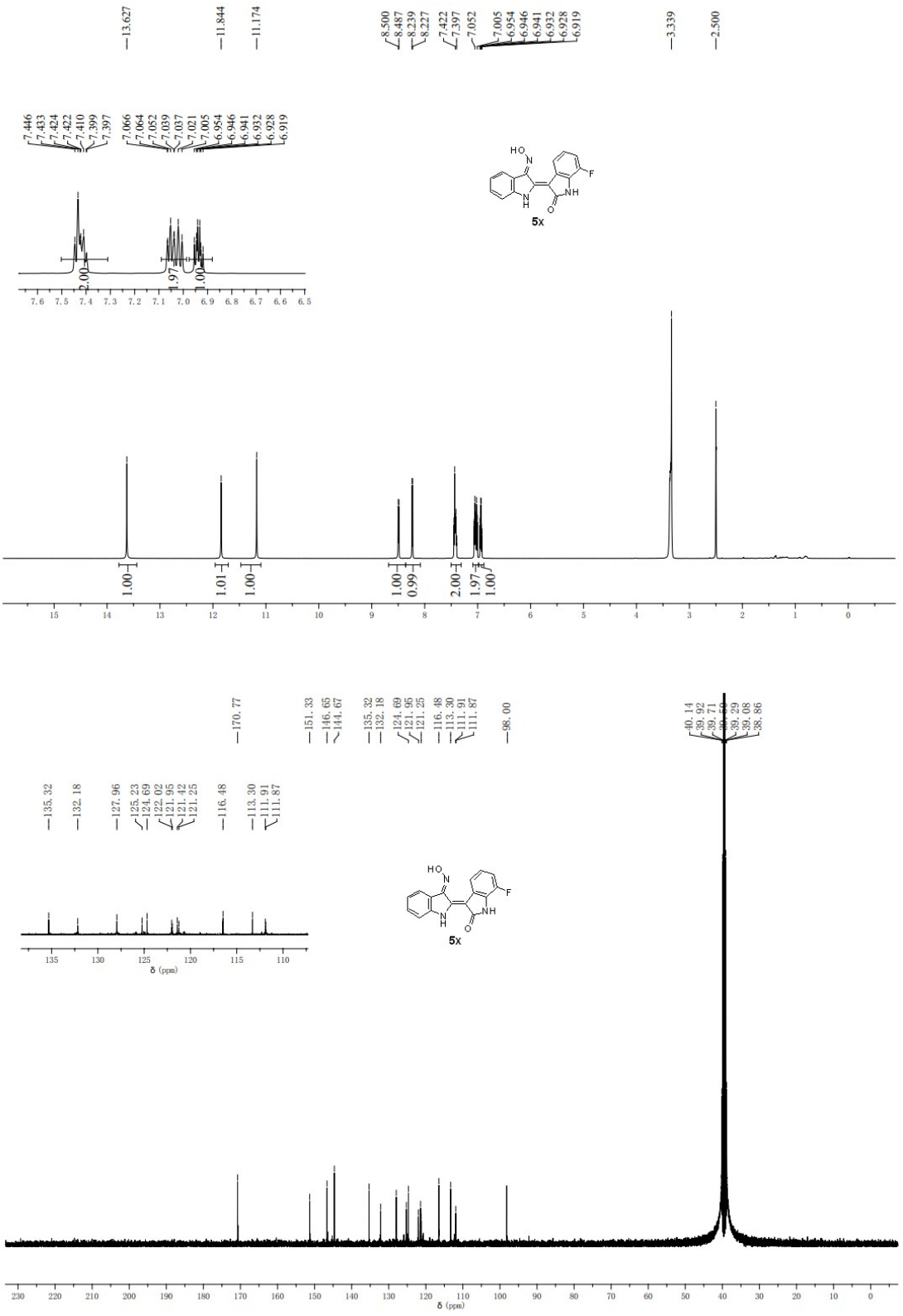


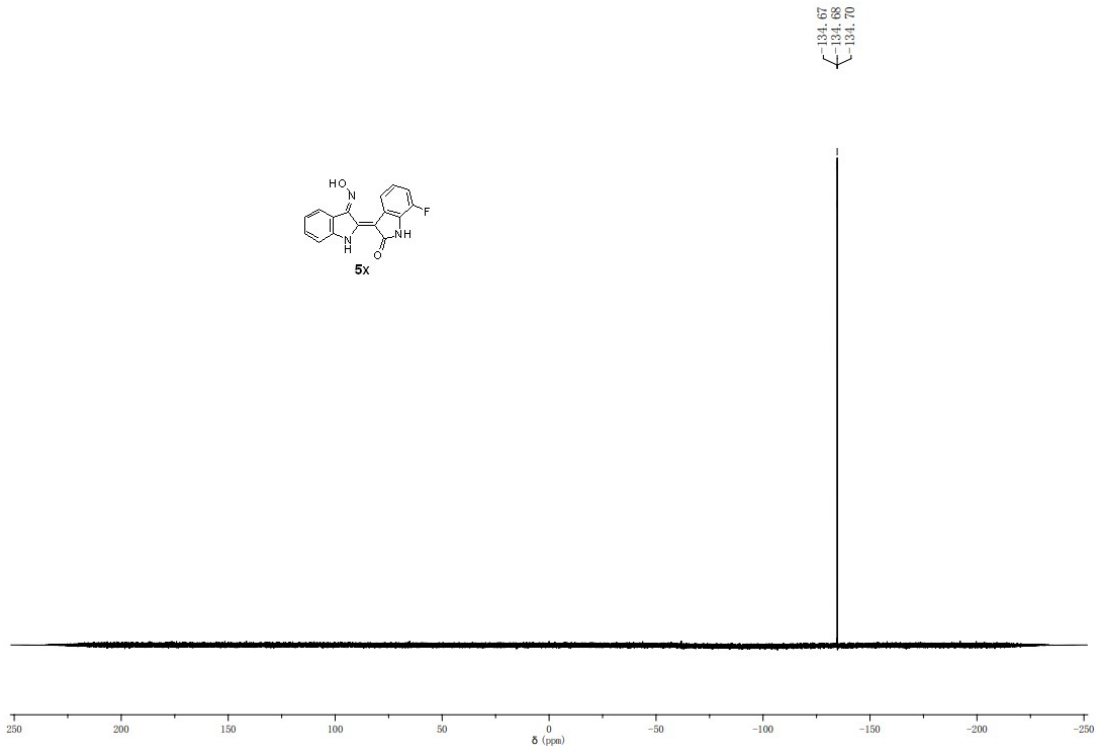
**(2*Z*, 3*E*)-3-(hydroxyimino)-7'-methyl-[2, 3'-biindolinylidene]-2'-one (5w):**



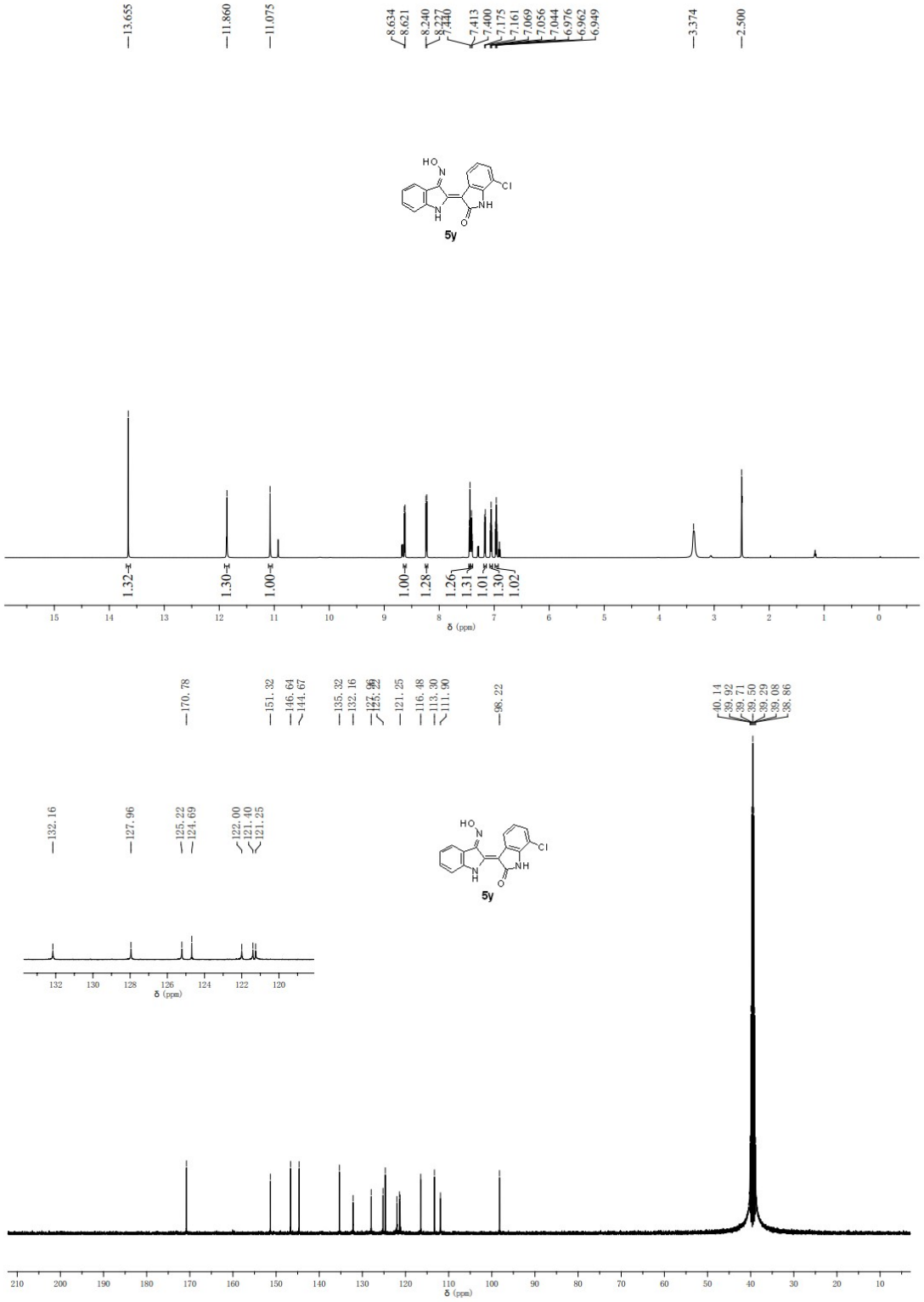



**(2*Z*, 3*E*)-7'-fluoro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5x):**

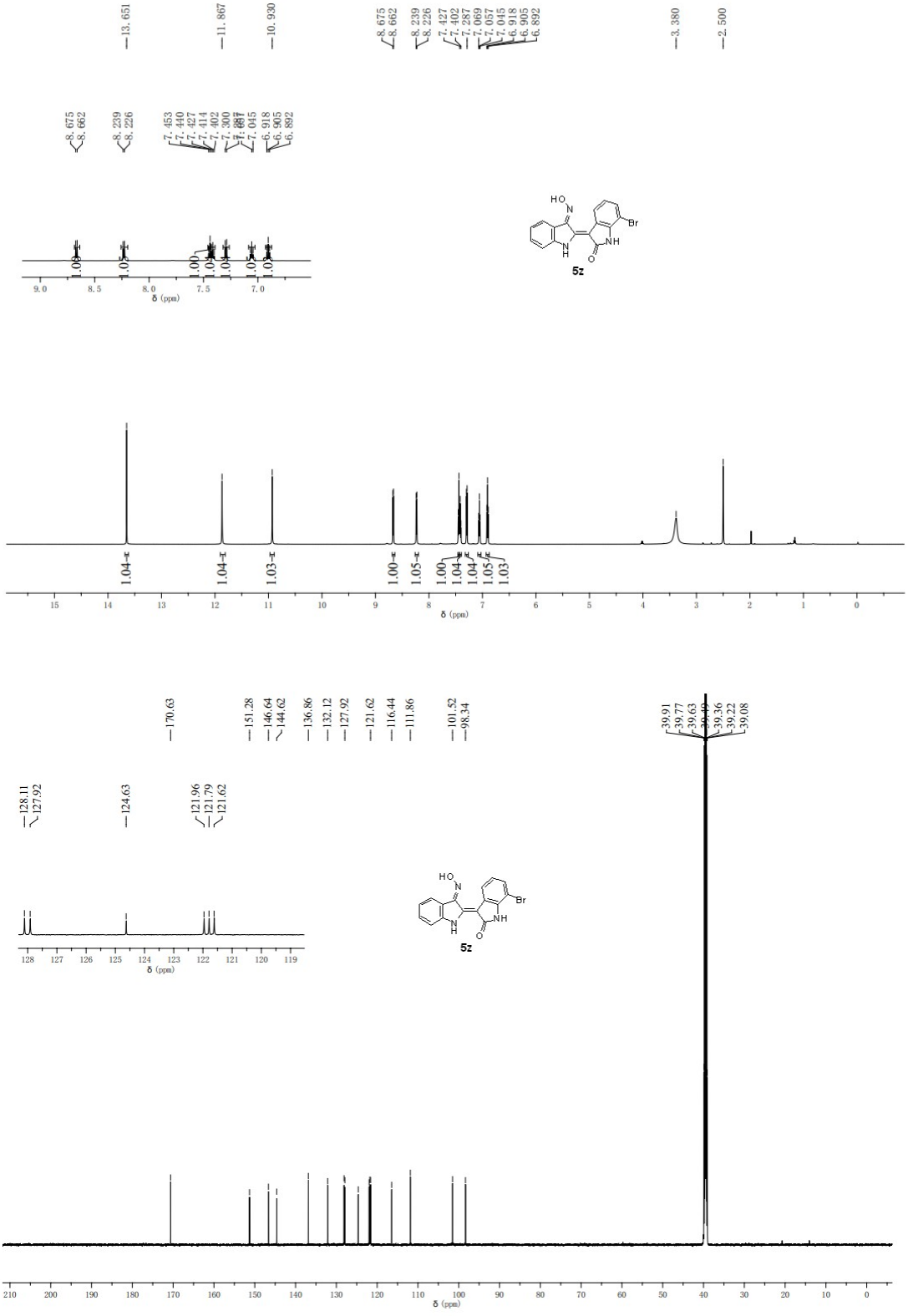



-134.67
-134.68
-134.70
HO
N
F
NH
NH
O
5x
250
200
150
100
50
0
δ (ppm)
-50
-100
-150
-200
-250

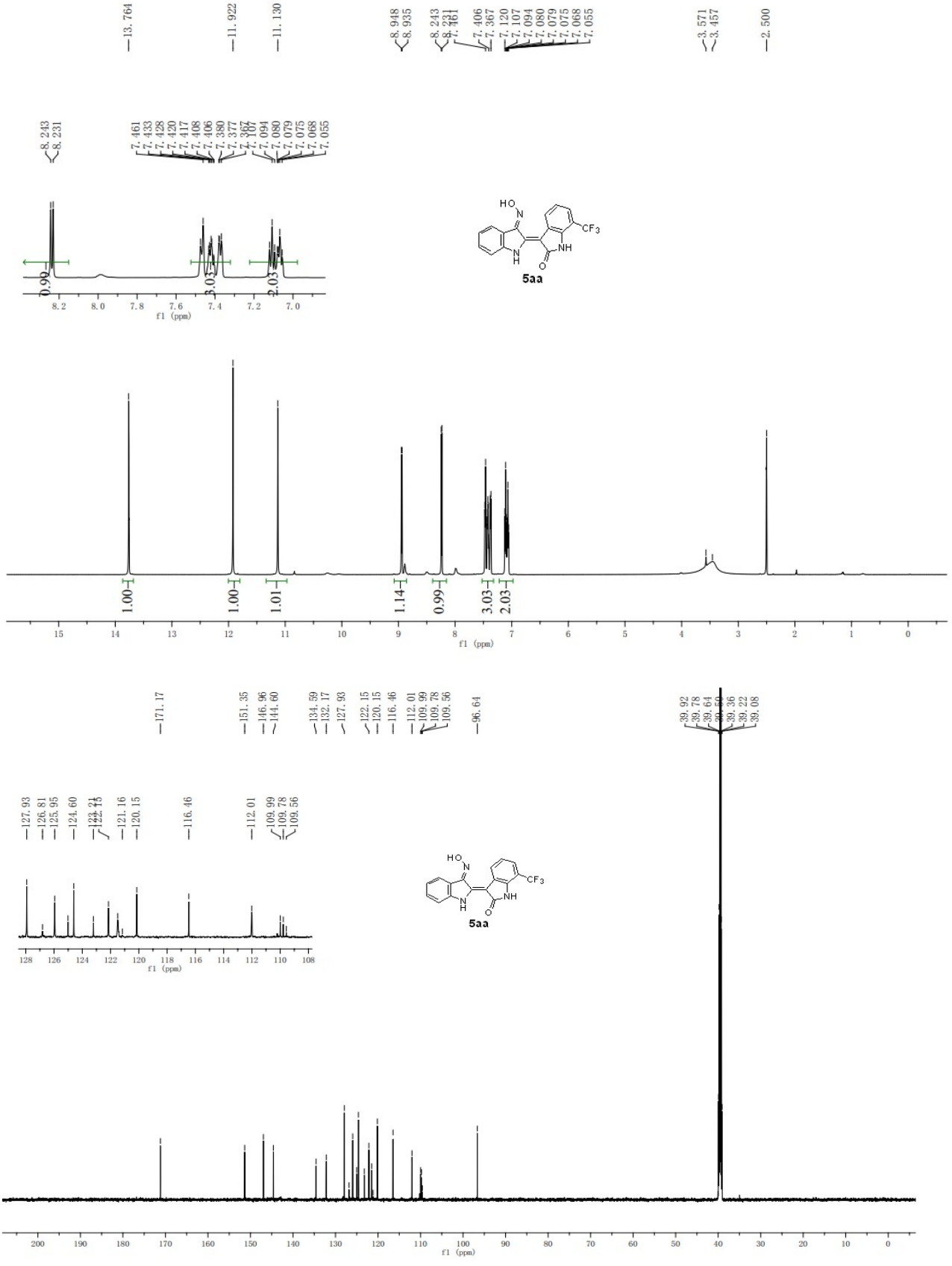
**(2*Z*, 3*E*)-7'-chloro-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5y):**

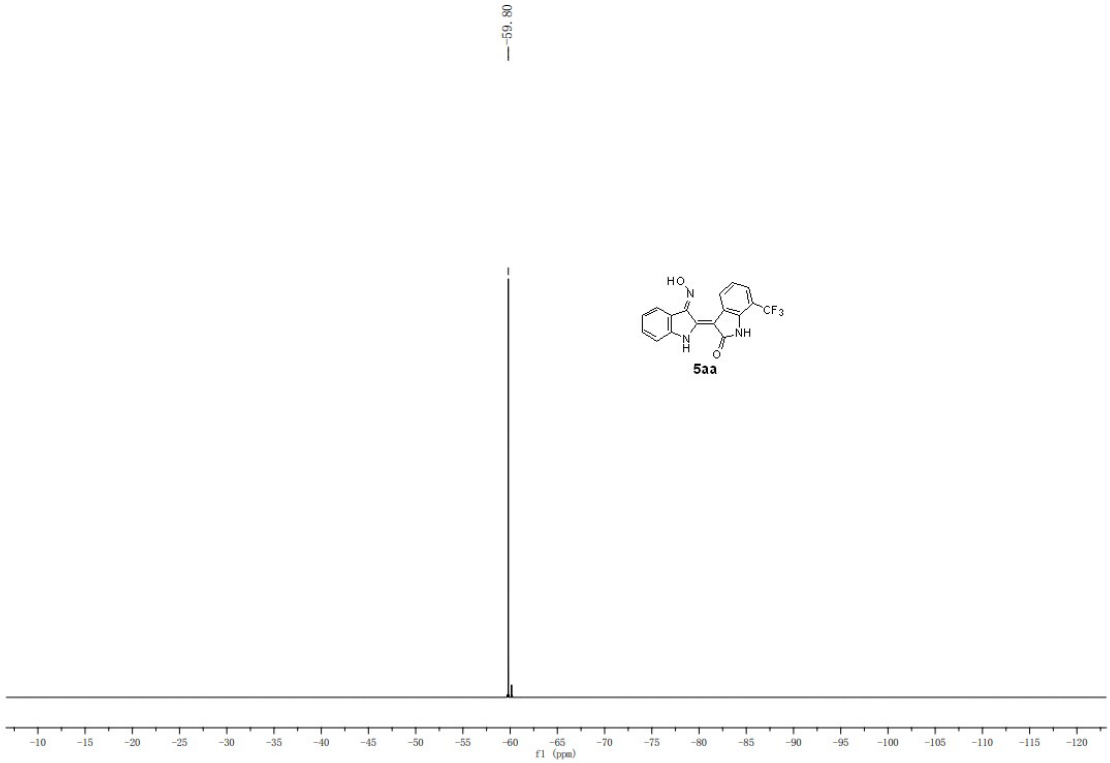


**(2*Z*, 3*E*)-7'-bromo-3-(hydroxyimino)-[2, 3'-biindolinylidene]-2'-one (5z):**

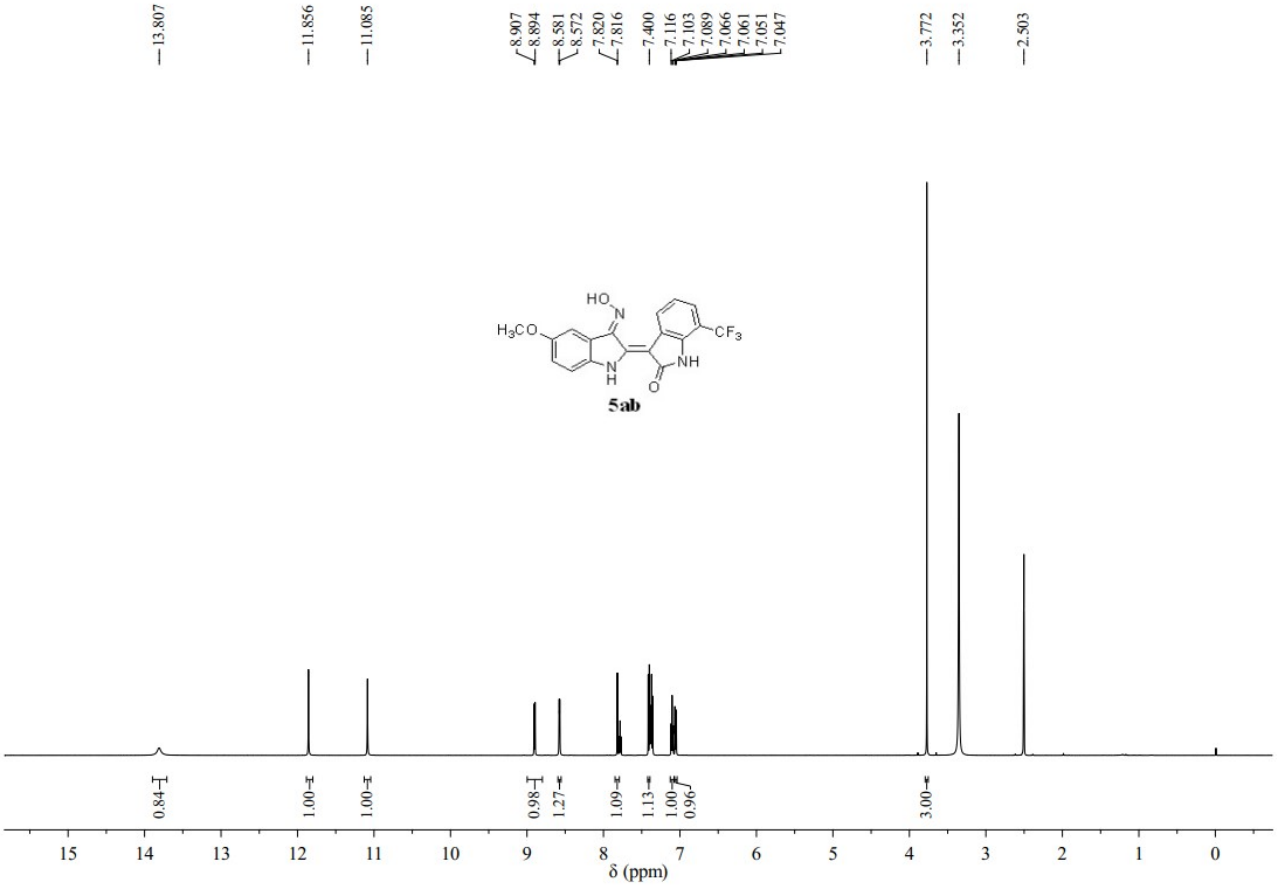


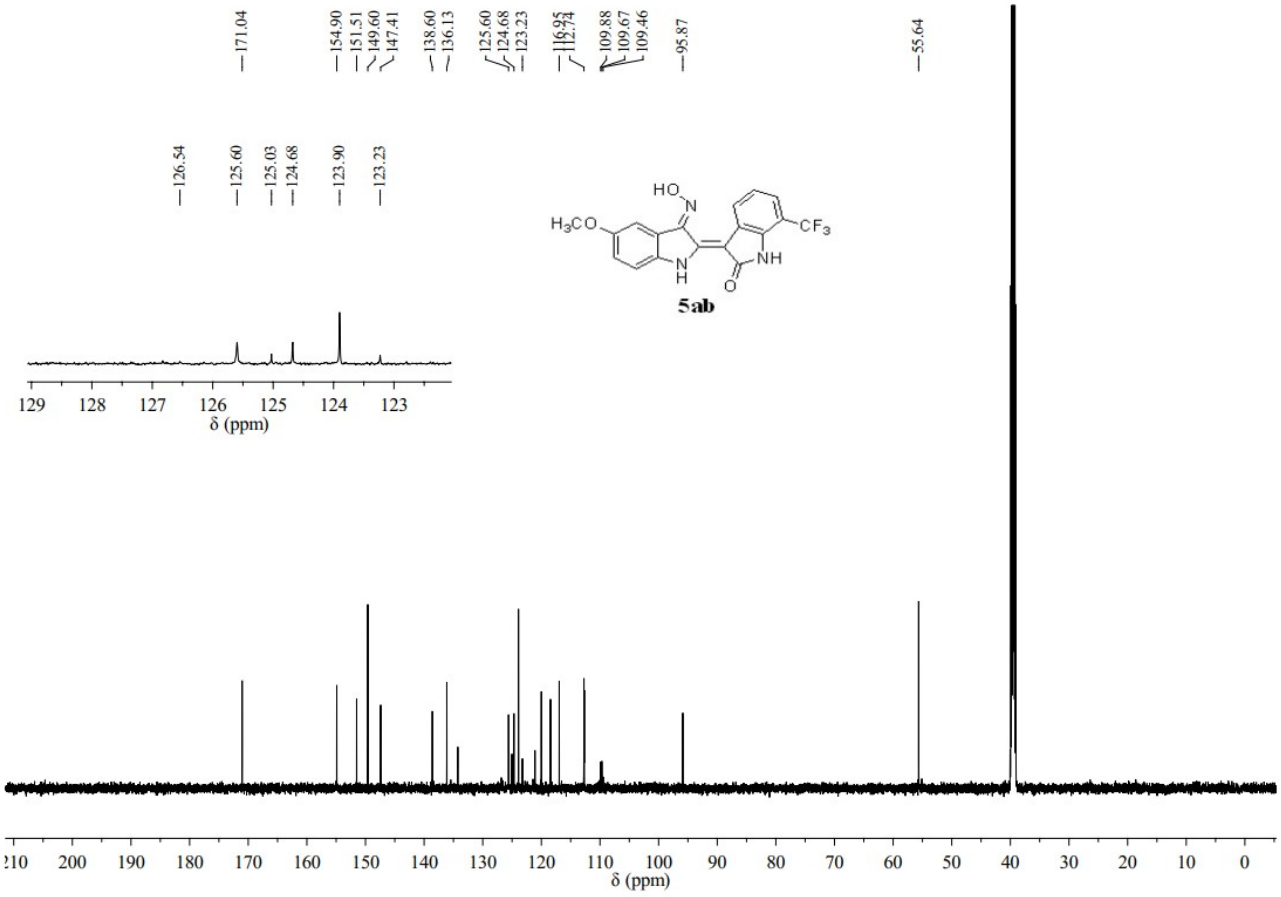
**(2*Z*, 3*E*)-3-(hydroxyimino)-7'-(trifluoromethyl)-[2, 3'-biindolinylidene]-2'-one (5aa)**:



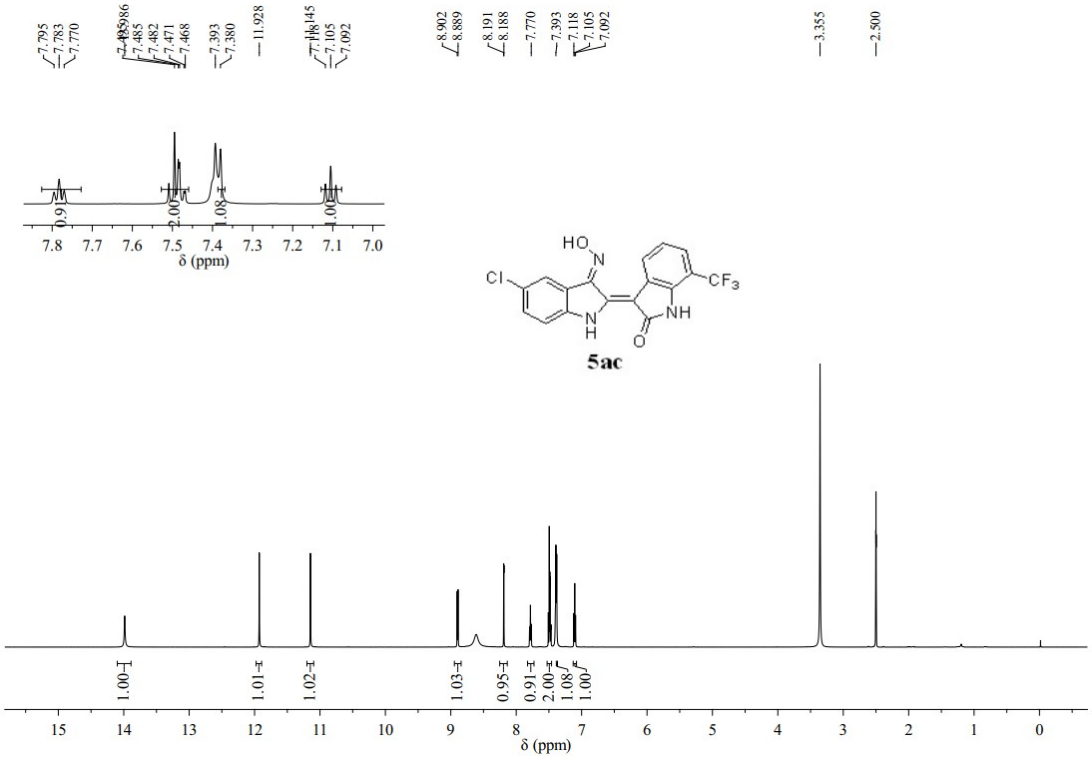


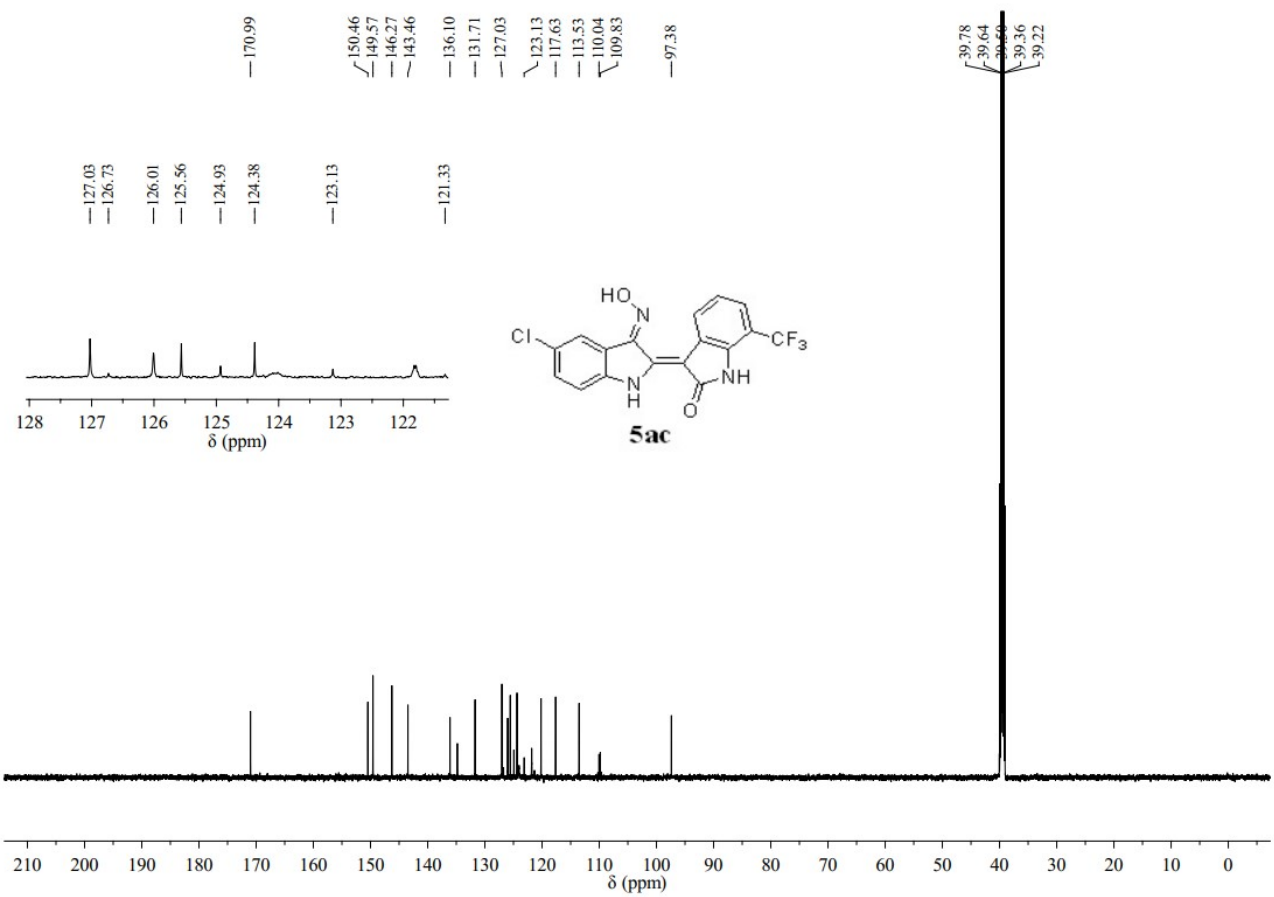

**(2*Z*,3*E*)-3-(hydroxyimino)-5-methoxy-7'-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (5ab)**



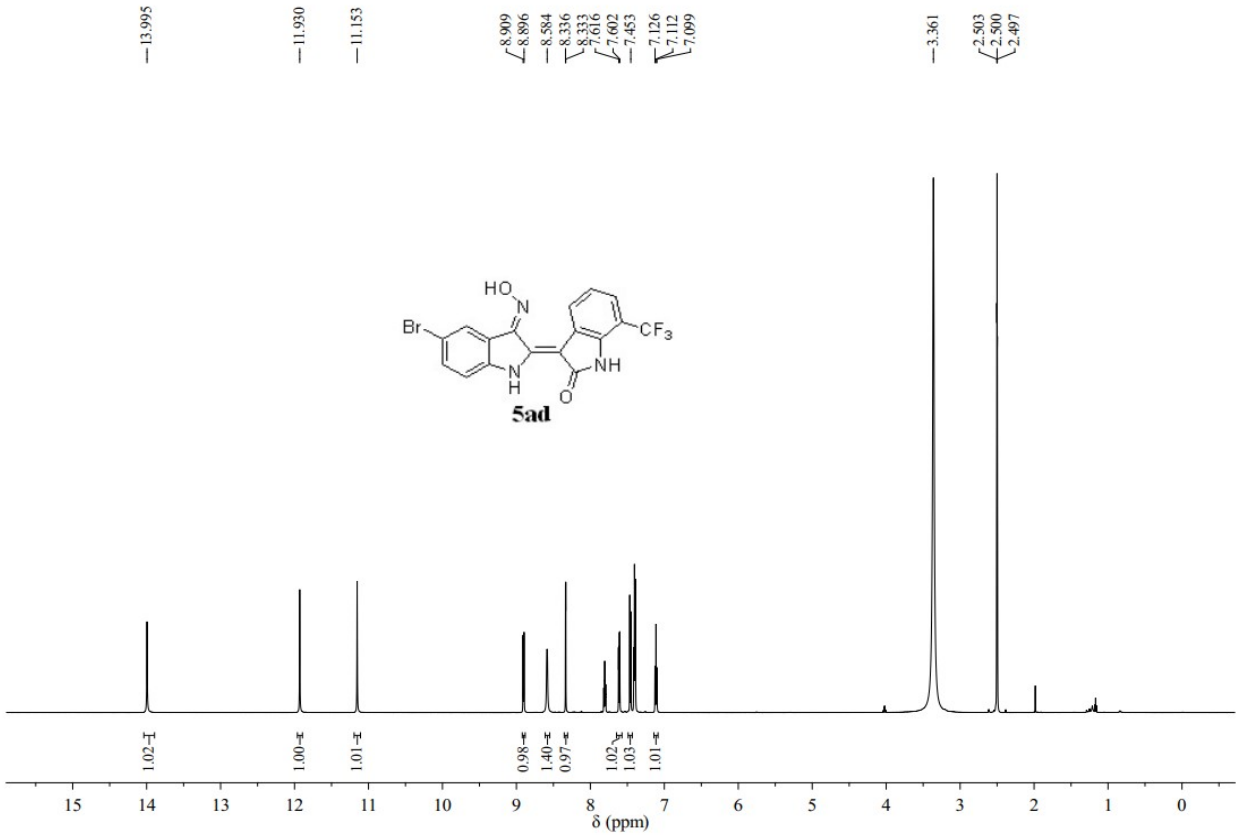


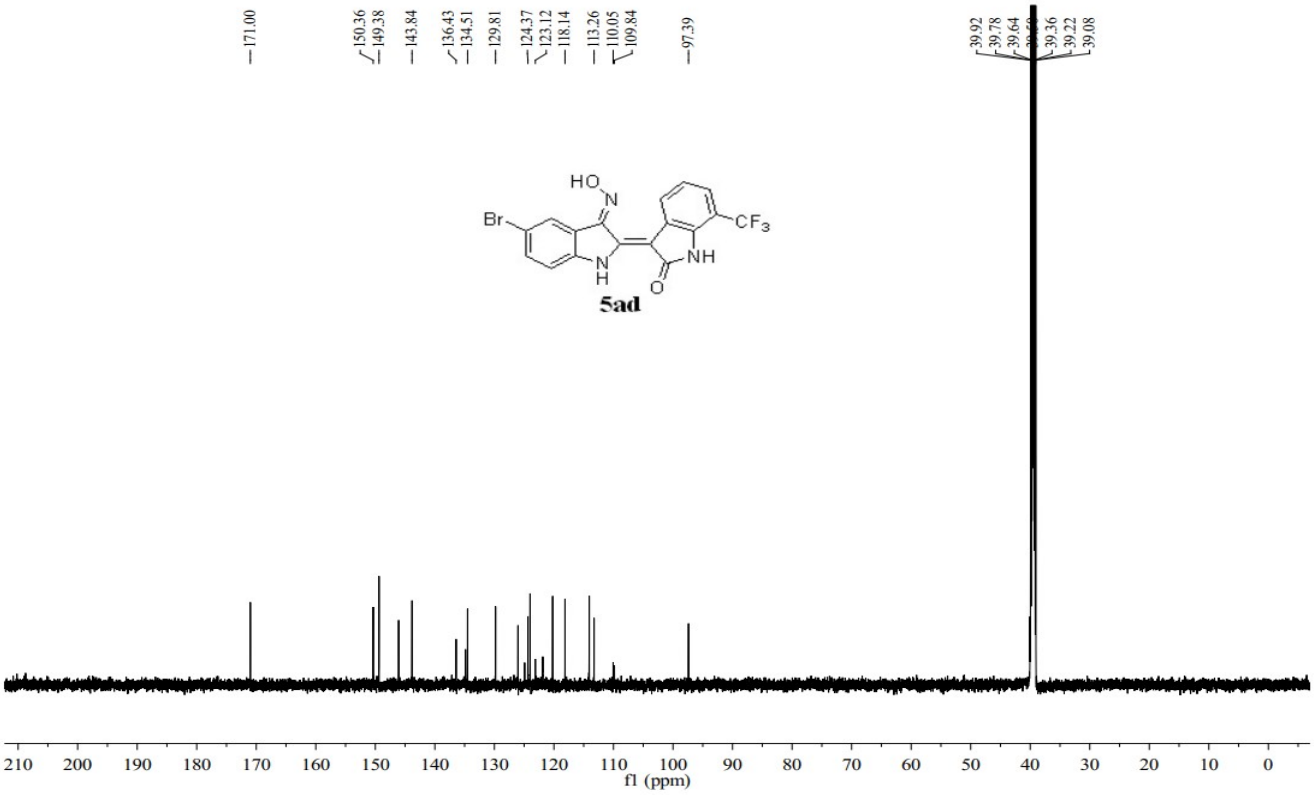

**(2*Z*,3*E*)-5-chloro-3-(hydroxyimino)-7'-(trifluoromethyl)-[2,3'-biindolinylidene]-2'-one (5ac)**



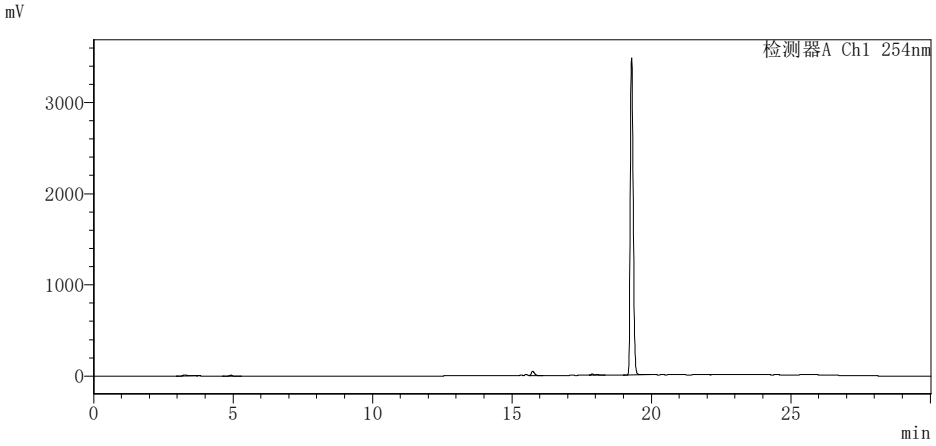



**(2*Z*, 3*E*)-5-bromo-3-(hydroxyimino)-7'-(trifluoromethyl)-[2, 3'-biindolinylidene]-2'-one (5ad)**



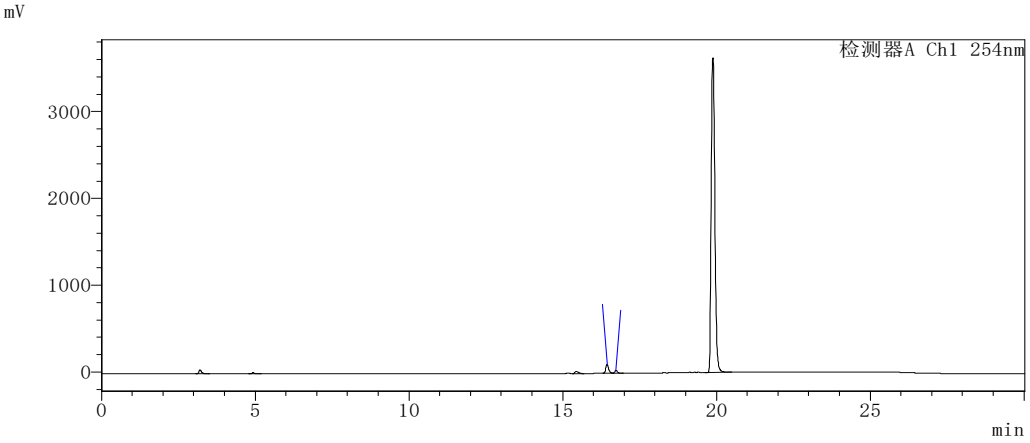

171.00
150.36
149.38
143.84
136.43
134.51
129.81
124.37
123.12
118.14
113.26
110.05
109.84
97.39
39.92
39.78
39.64
39.36
39.22
39.08
HO
N
Br
CF3
NH
N
H
O
5ad
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

**HPLC purity analysis for compound 5r using an InertSustain AQ-C18 5um 4.6× 250 mm Column （0.1%TFA/$CH_3CN$：0.1%TFA/dd $H_2O$ = 5:95, 1mL/min）**




| Peak | Retention Time (min) | Peak Area (mAU*s) | Peak Hight (mAU) | Peak Area (%) |
|---|---|---|---|---|
| 1 | 3.276 | 139911 | 12982 | 0.537 |
| 2 | 4.925 | 69591 | 12096 | 0.267 |
| 3 | 15.743 | 342766 | 45495 | 1.316 |
| 4 | 17.880 | 148921 | 14265 | 0.572 |
| 5 | 19.294 | 25353283 | 3478944 | 97.309 |
| total | | 26054473 | 3563781 | 100.000 |

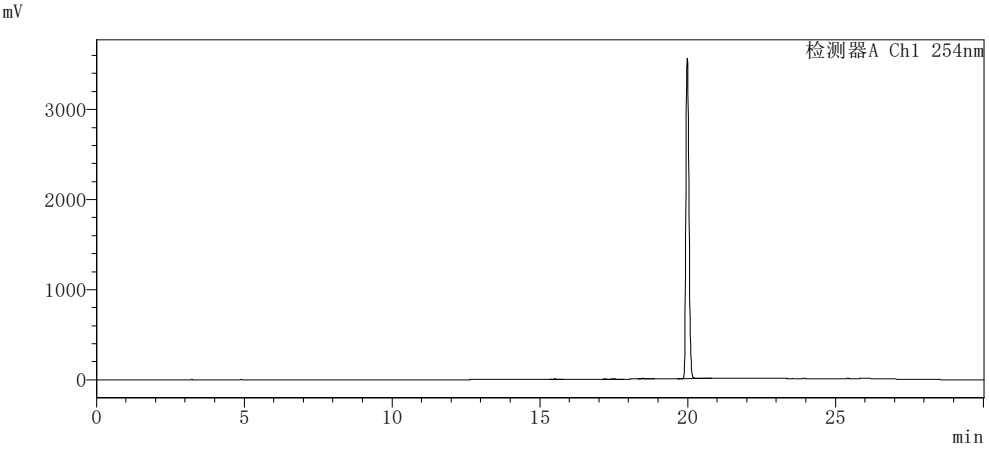
**HPLC purity analysis for compound 5s using an InertSustain AQ-C18 5um 4.6× 250 mm Column （0.1%TFA/$CH_3CN$：0.1%TFA/dd $H_2O$ = 5:95, 1mL/min）**




| Peak | Retention Time (min) | Peak Area (mAU*s) | Peak Hight (mAU) | Peak Area (%) |
|---|---|---|---|---|
| 1 | 3.205 | 269413 | 46130 | 0.860 |
| 2 | 4.932 | 62428 | 16190 | 0.199 |
| 3 | 15.435 | 182903 | 25459 | 0.584 |
| 4 | 16.442 | 653007 | 104053 | 2.084 |
| 5 | 16.733 | 164175 | 31511 | 0.524 |

| | | | | |
|---|---|---|---|---|
| 6 | 19.885 | 30003065 | 3625014 | 95.749 |
| total | | 31334991 | 3848358 | 100.000 |

**HPLC purity analysis for compound 5aa using an InertSustain AQ-C18 5um 4.6× 250 mm Column （0.1%TFA/$CH_3CN$：0.1%TFA/dd $H_2O$ = 5:95, 1mL/min）**




| Peak | Retention Time (min) | Peak Area (mAU*s) | Peak Hight (mAU) | Peak Area (%) |
|---|---|---|---|---|
| 1 | 15.513 | 29355 | 5559 | 0.114 |
| 2 | 17.472 | 45231 | 6842 | 0.175 |
| 3 | 18.489 | 40979 | 6439 | 0.159 |
| 4 | 19.990 | 25668572 | 3558511 | 99.552 |
| total | | 25784138 | 3577351 | 100.000 |

## Bacterial activity results

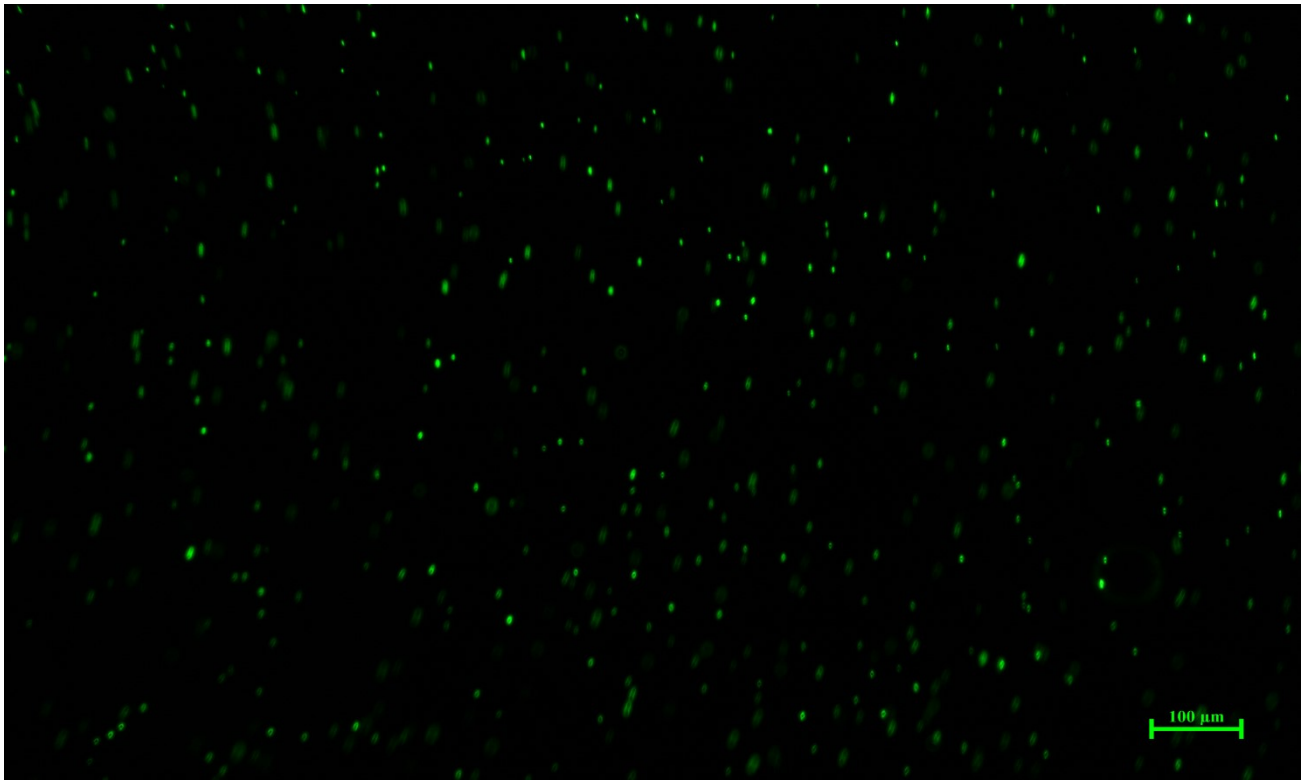
**Table S1.** In vitro antibacterial activity of indirubin-3'-monoximes used alone or in combination with Levofloxacin against *P. aeruginosa* ATCC9027 by the checkerboard microdilution assay.

| Compound | | | Lev | | FICI[a] | Interpretation |
|---|---|---|---|---|---|---|
| | alone | synergetic | alone | synergetic | | |
| Indirubin(**4a**) | 256 | 256 | 12.5 | 12.5 | 2 | Indifferent |
| **5r** | 256 | 256 | 12.5 | 6.25 | 1.5 | Indifferent |
| **5s** | 256 | 256 | 12.5 | 12.5 | 2 | Indifferent |
| **5aa** | 256 | 256 | 12.5 | 12.5 | 2 | Indifferent |

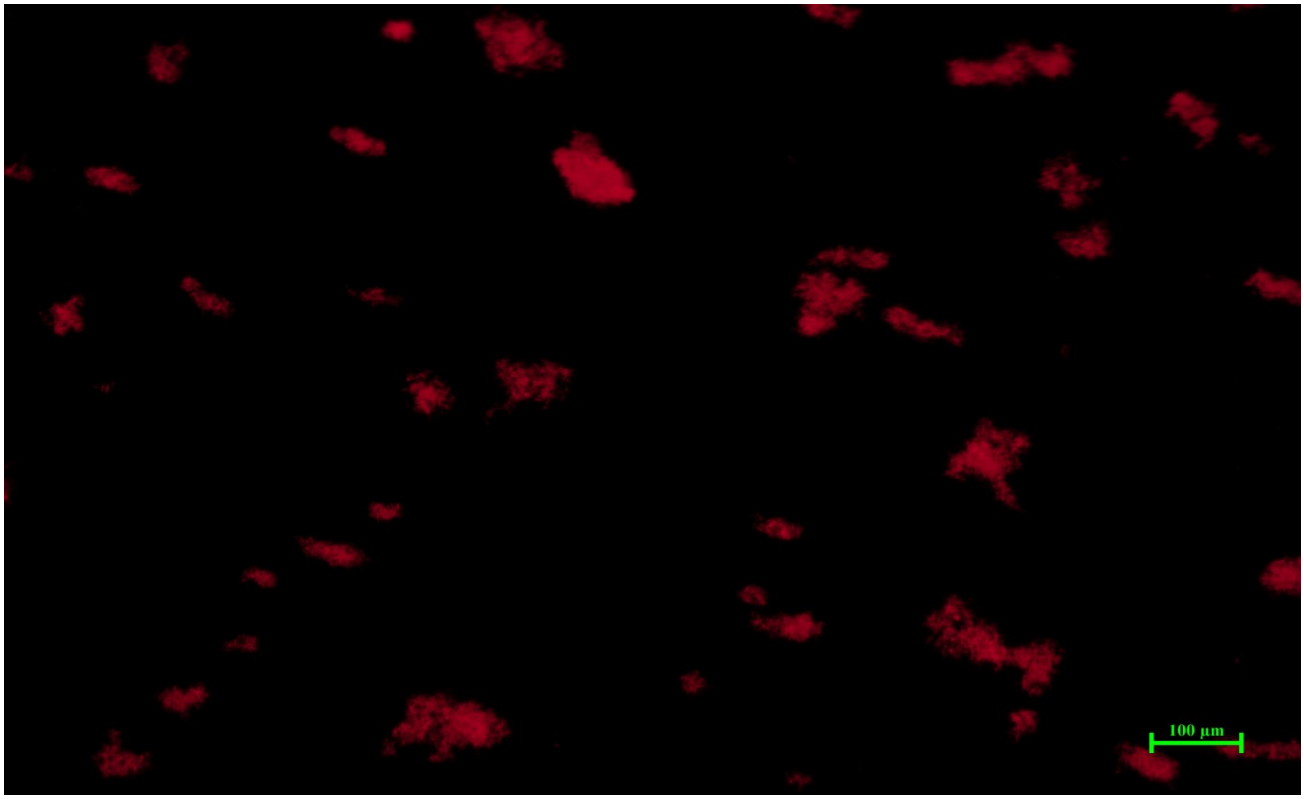
[a] The interaction as reflected by FICI values: synergistic (FICI ≤ 0.5), additive (0.5 < FICI ≤ 1), indifferent (1< FICI ≤ 4) and antagonistic (FICI > 4).

**Transmission electron micrographs (TEM) of *S. aureus* ATCC25923 cells (B: treated with 5aa at 12.8 $\mu$g/ mL for 16 h; A: blank control), (magnification, 15000).**

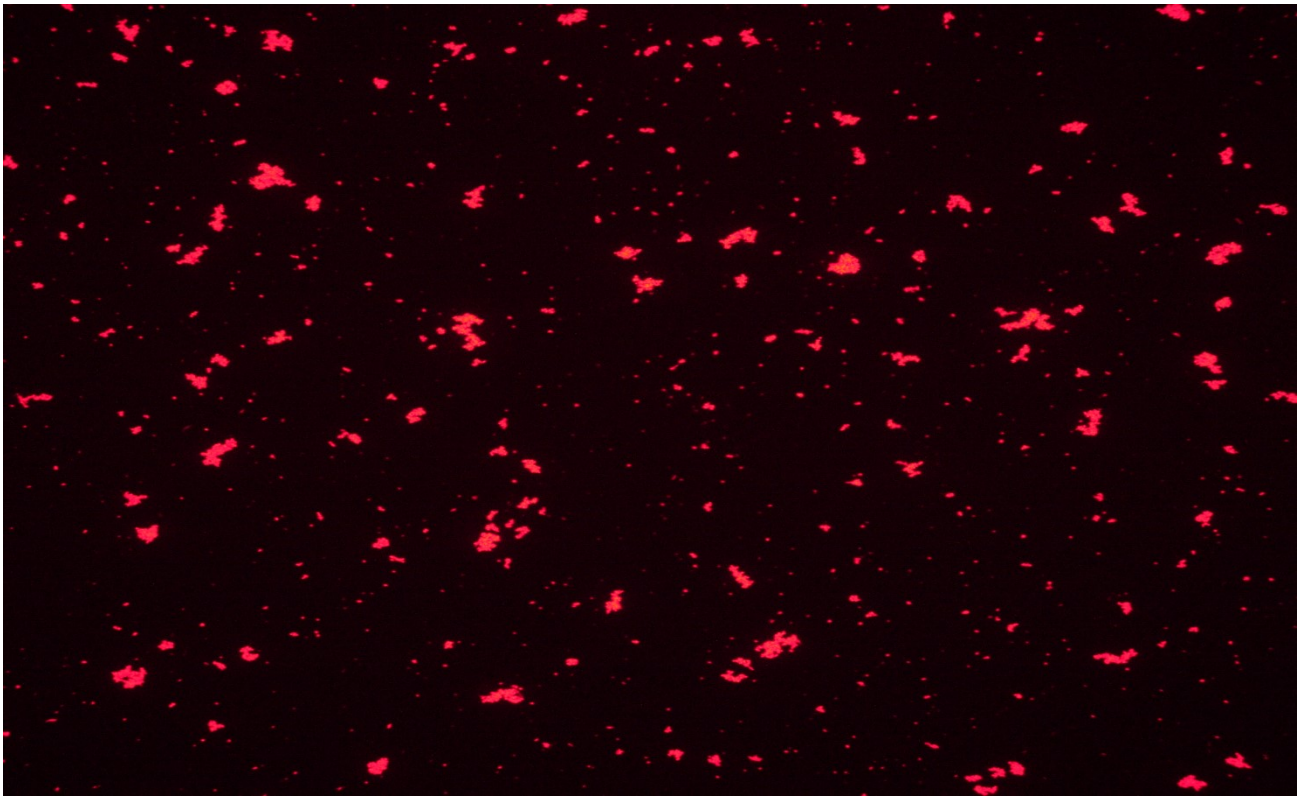
**Fluorescence micrographs of *S. aureus* ATCC 29213 cells.**



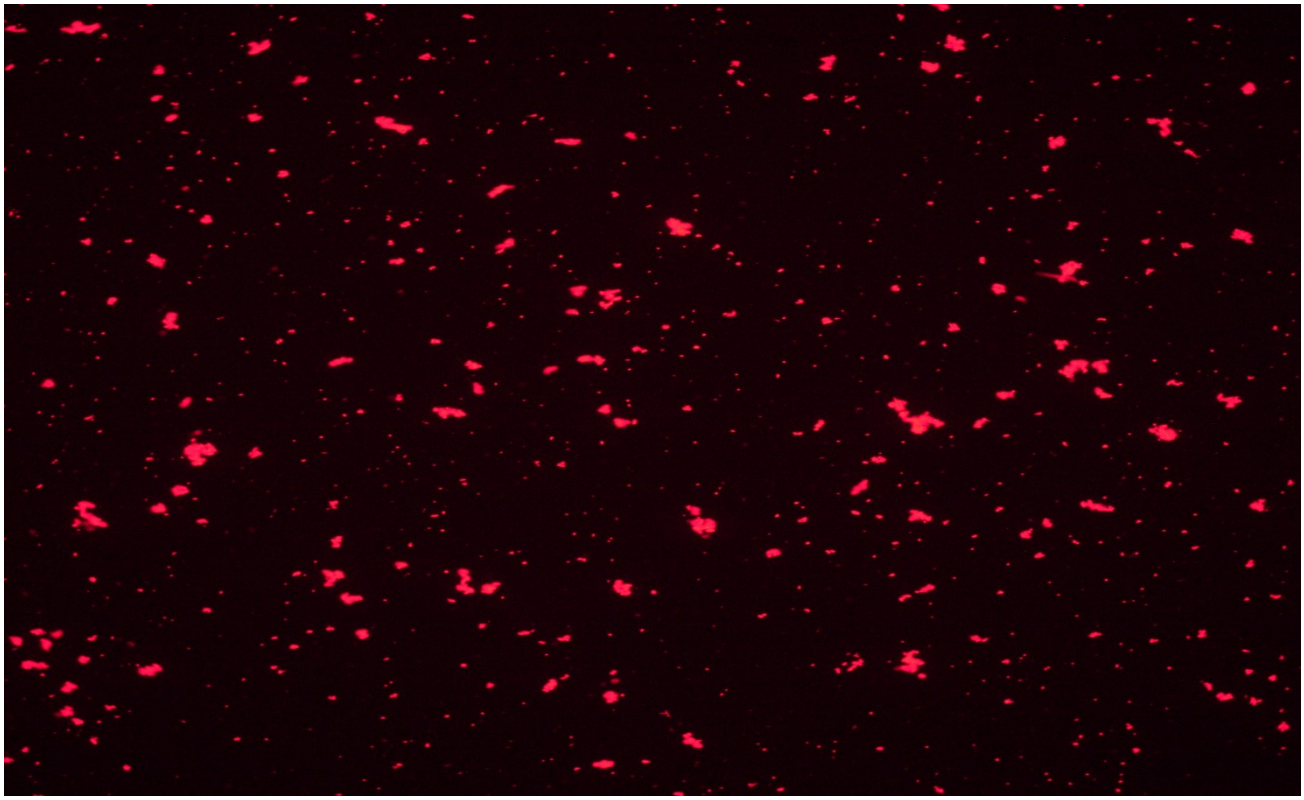

**treated with DMSO**




**treated with 5aa**

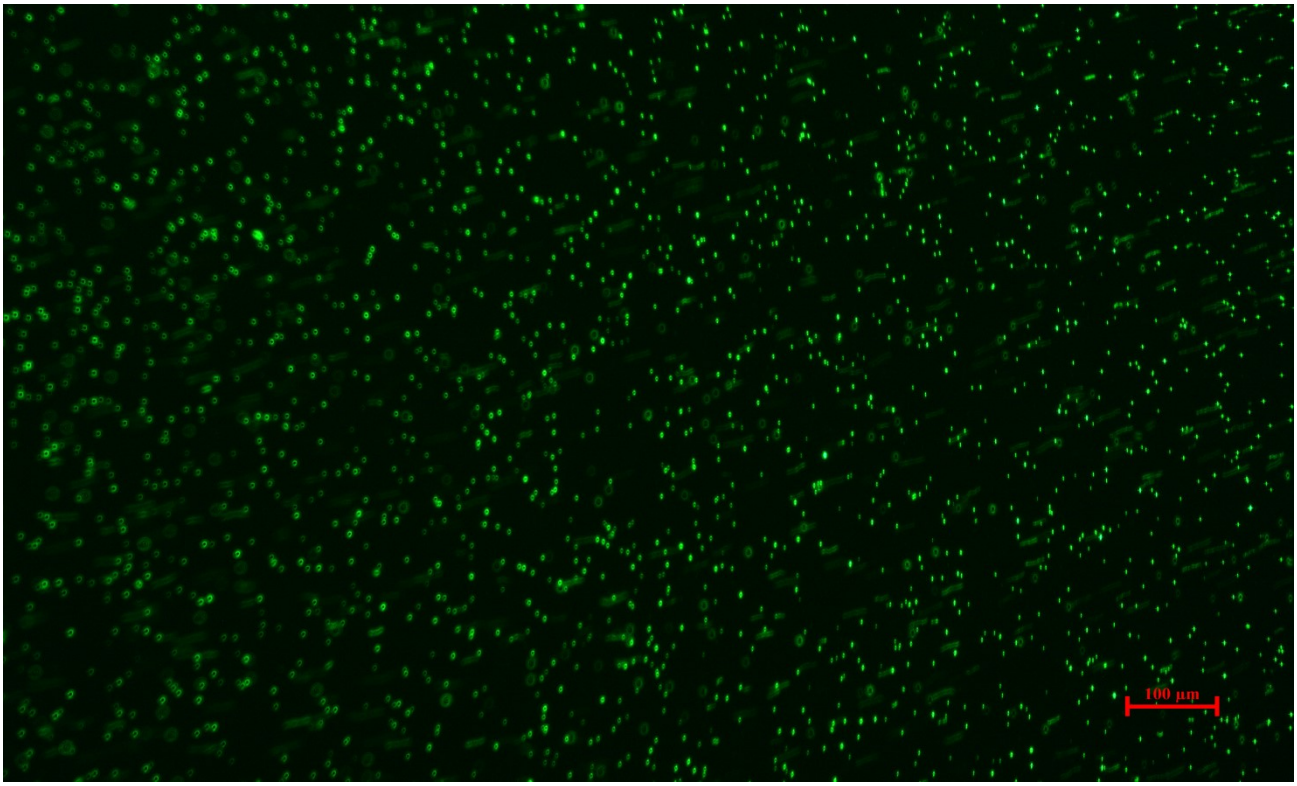


**treated with 5r**

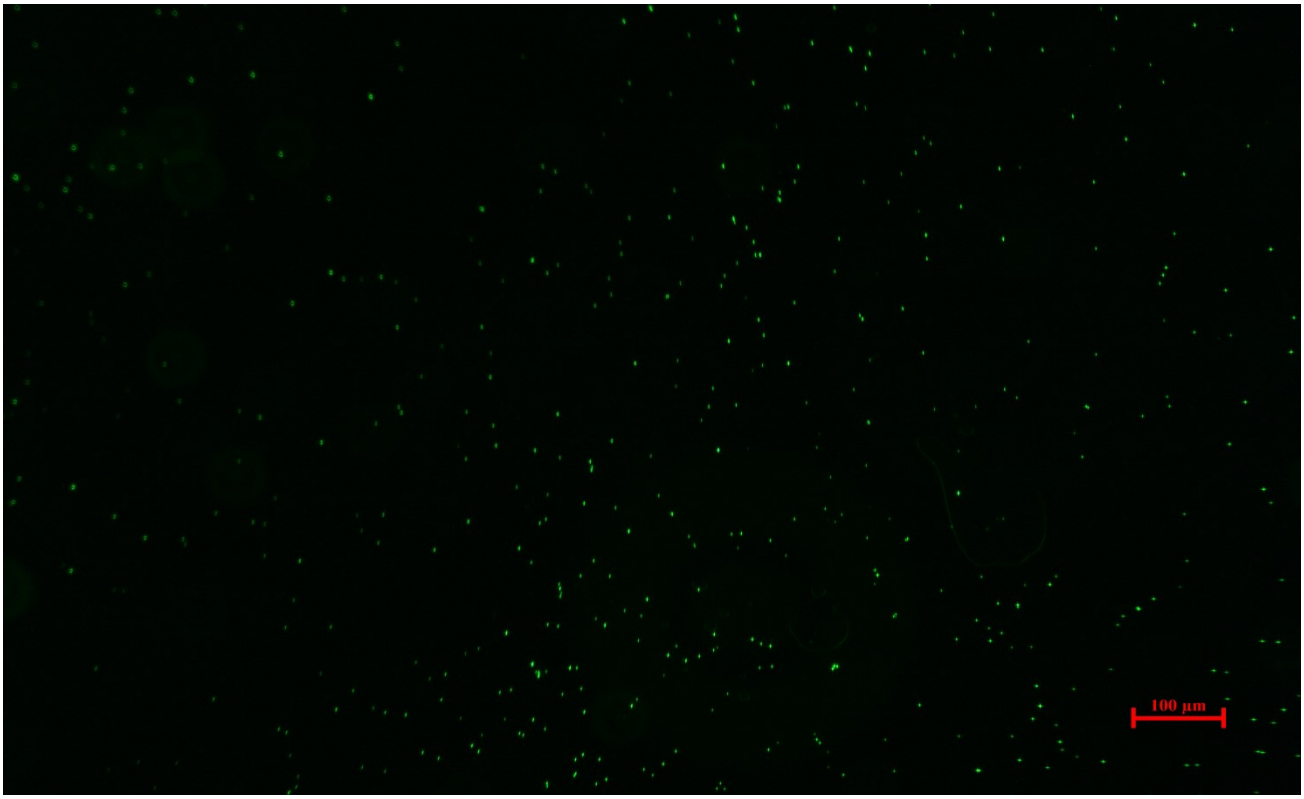


**treated with 5s**

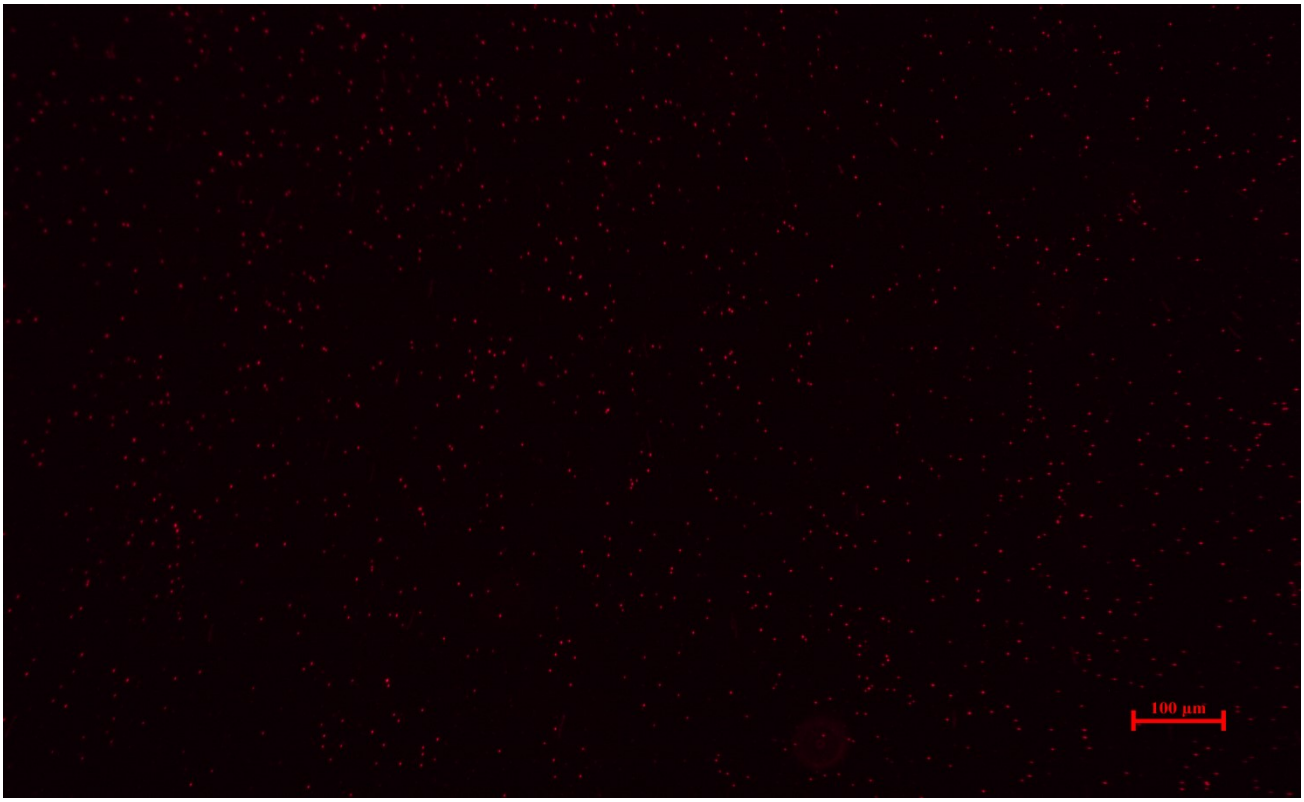
**Fluorescence micrographs of multi-drug resistant *S. aureus* 20151027077 cells.**



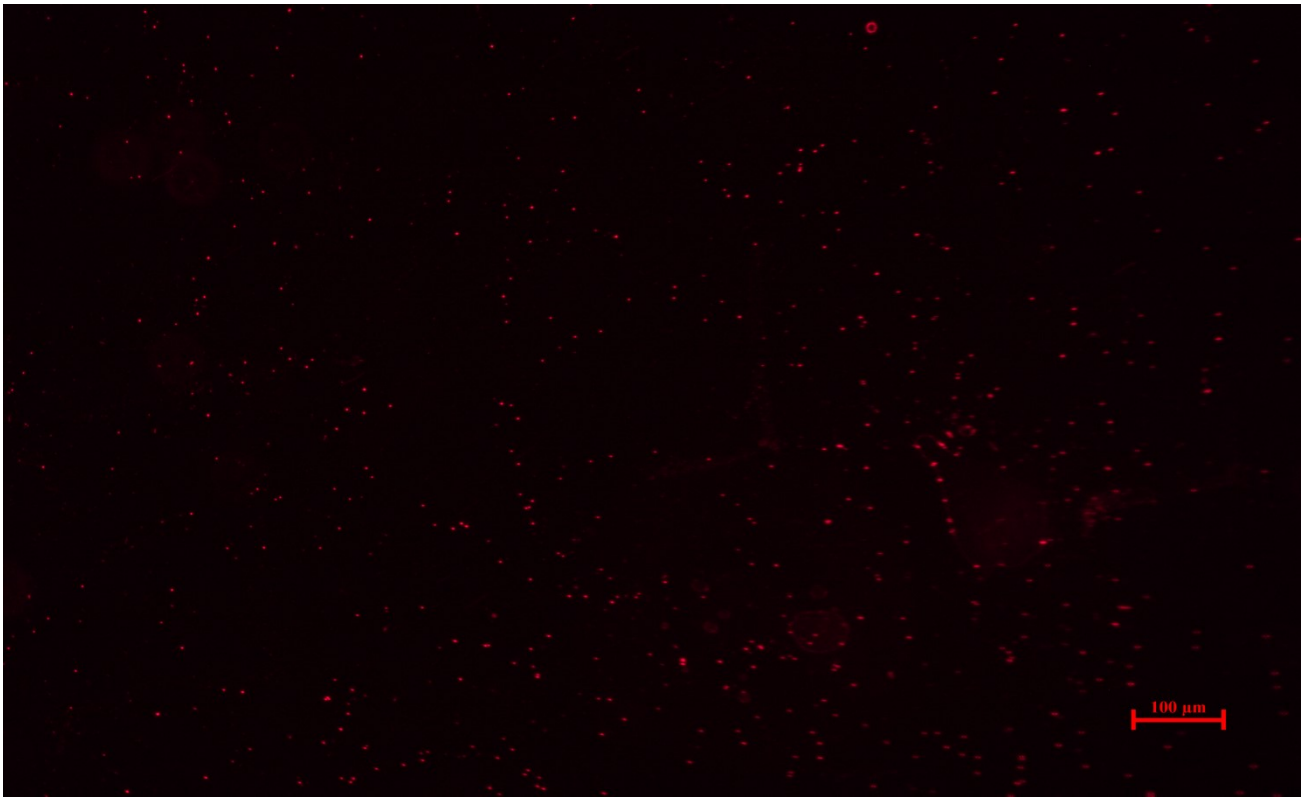

**treated with DMSO**




**treated with Levofloxacin**




**treated with 5aa**




**treated with 5aa and Levofloxacin**

**Histopathological section of mice liver tissue after skin irritation test treated with 12.8 μg/mL 5aa for 1-72 h.**