Supporting Information for

Original article

Identification of Novel 1,2,3-triazole Isatin Derivatives as Potent SARS-CoV-2 3CLpro Inhibitors via Click-chemistry-based Rapid Screening

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Table S1. Anti-SARS-CoV-2 activity and cytotoxicity of the tested compounds.

Compounds	D1N8	D1N18	D1N52	L-26
EC_{50} (µM) [a]	NA[c]	NA	NA	NA
CC_{50} (µM) [b]	> 20	> 20	> 20	< 2.2

[a] EC_{50}: concentration of compound required to achieve 50% protection of Vero E6 cell cultures against SARS-CoV-2-induced cytopathicity.

[b] CC_{50}: concentration required to reduce the viability of mock-infected cell cultures (cytotoxicity, CC) by 50%.

[c] NA: no anti-SARS-CoV-2 activity at the test concentration.

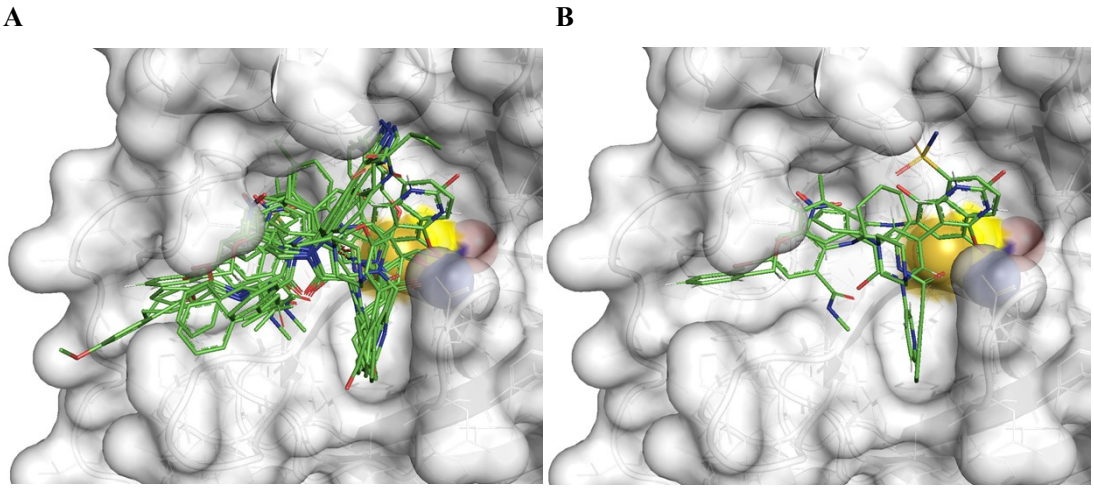


Figure S1. (**a**) Representation of the arrangement of selected covalent and noncovalent inhibitors bound to the binding pocket of the SARS-CoV-2 $3CL^{pro}$ enzyme (ligands taken from X-ray structures with PDB ID 7EN8, 7EN9, 7LMD, 7LME, 7LMF, 7LTJ, 7LTN, 7M8P, 7TEK, 7TEL, 7V1T, 7WO1, 7WO2, 7WO3, 7WOF, and 7WOH). (**b**) Representation of the binding mode of ligands taken from X-ray structures with PDB ID 7EN8, 7M8P and 7V1T. The shape and size of the binding pocket is shown by the surface of the enzyme (using the X-ray crystallographic structure with PDB ID 7EN8), and the reactive Cys is highlighted in yellow. The superposition of the X-ray ligands (shown as sticks with carbon atoms colored in green) fills the distinct subpockets that can be identified in the binding site.

A **B**

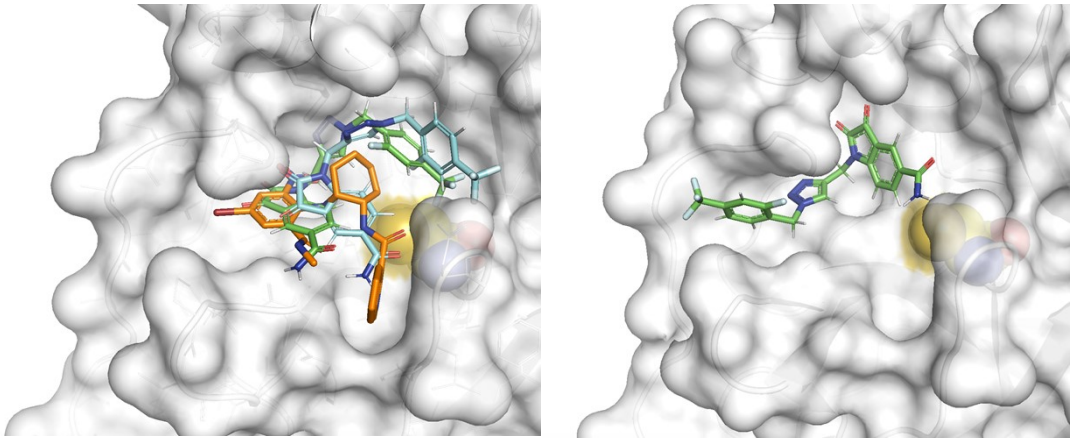
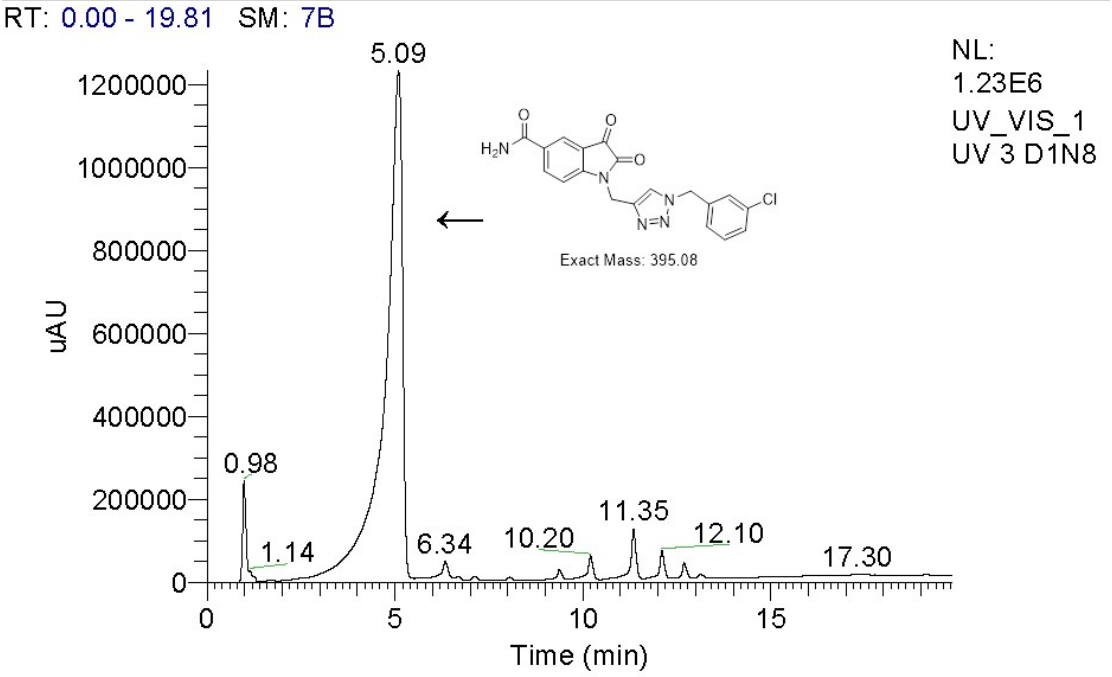
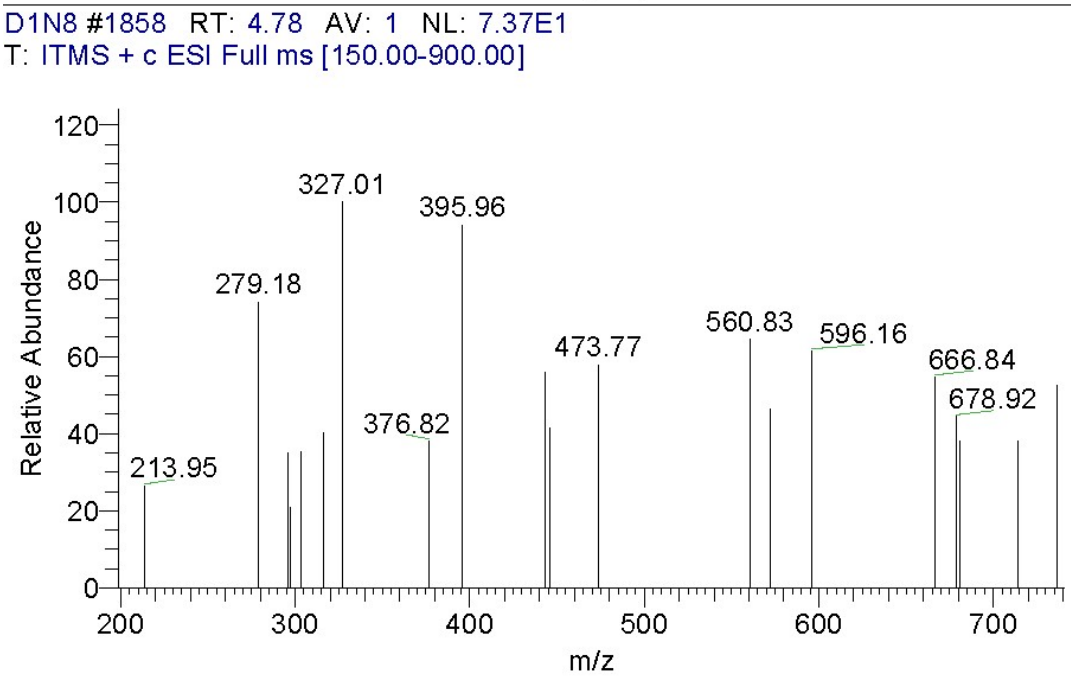


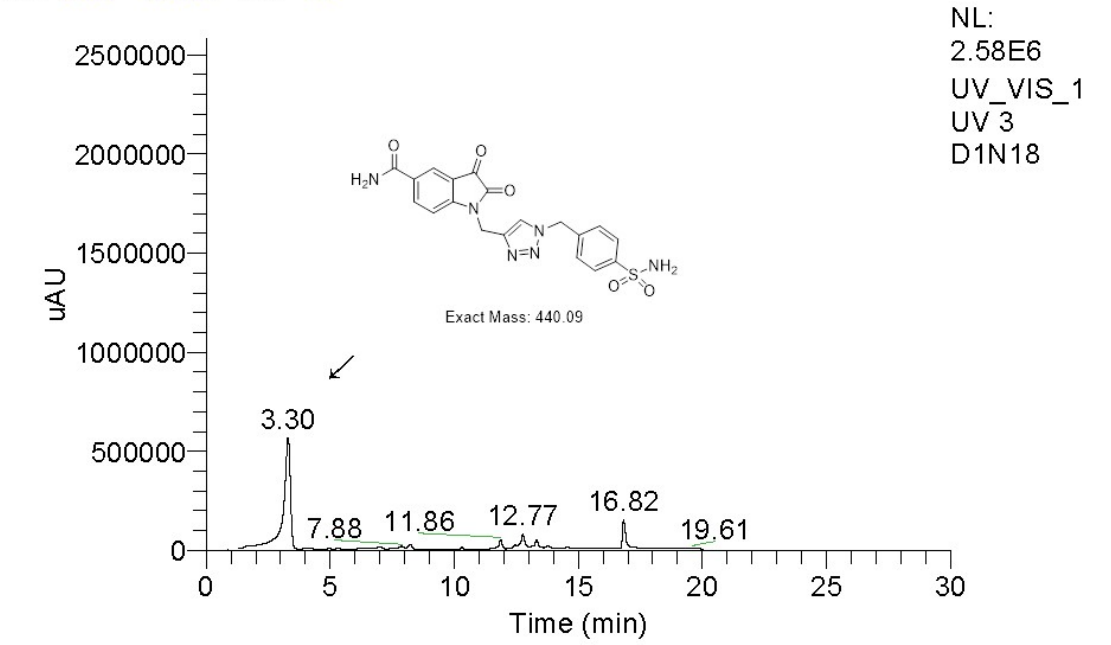
Figure S2. (**a**) Representation of the doc ked poses of **D1N52** in the binding pocket of 7EN8 (sticks with carbon atoms in green) and 7V1T (sticks with carbon atom in cyan). The X-ray ligand bound to 7EN8 is shown as sticks with carbon atoms in orange. (**b**) Representation of the binding mode of D1N52 in the binding pocket of 7M8P (sticks with carbon atoms in green). The shape and size of the binding pocket is shown by the surface of the enzyme, and the reactive Cys is highlighted in yellow.

LC-MS results of crude products D1N8, D1N18 and D1N52.

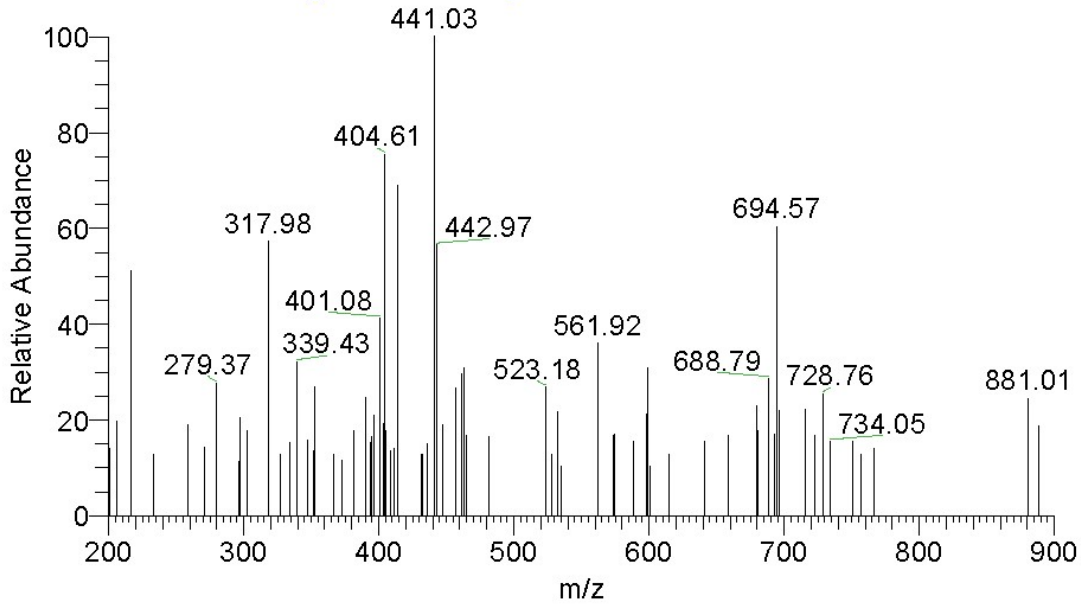


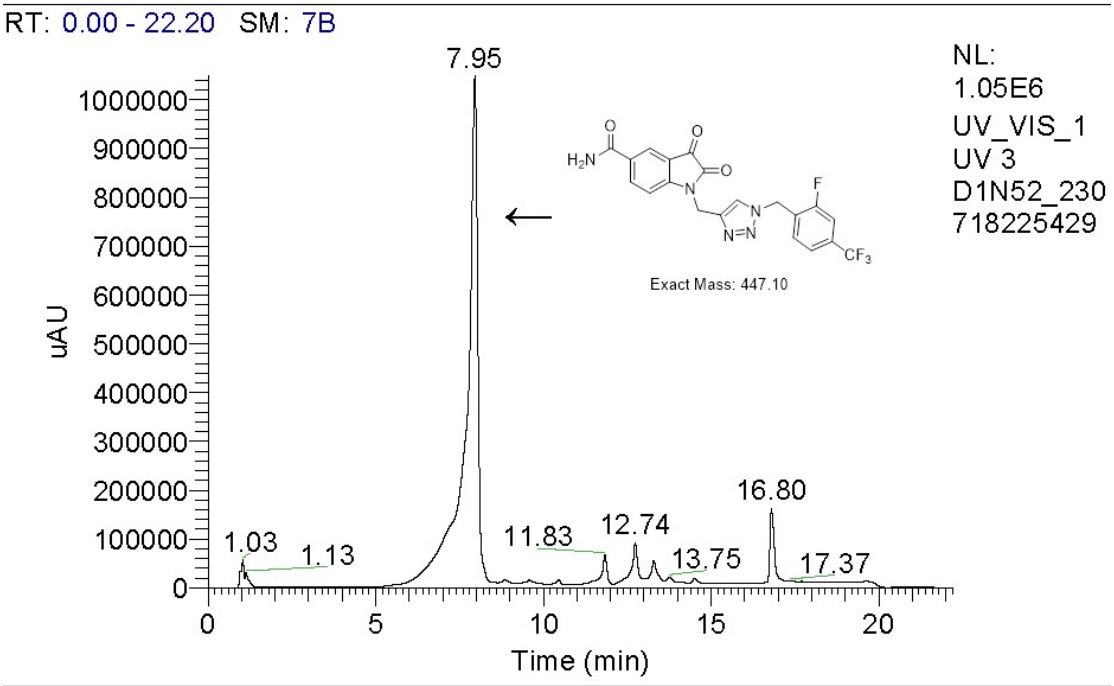


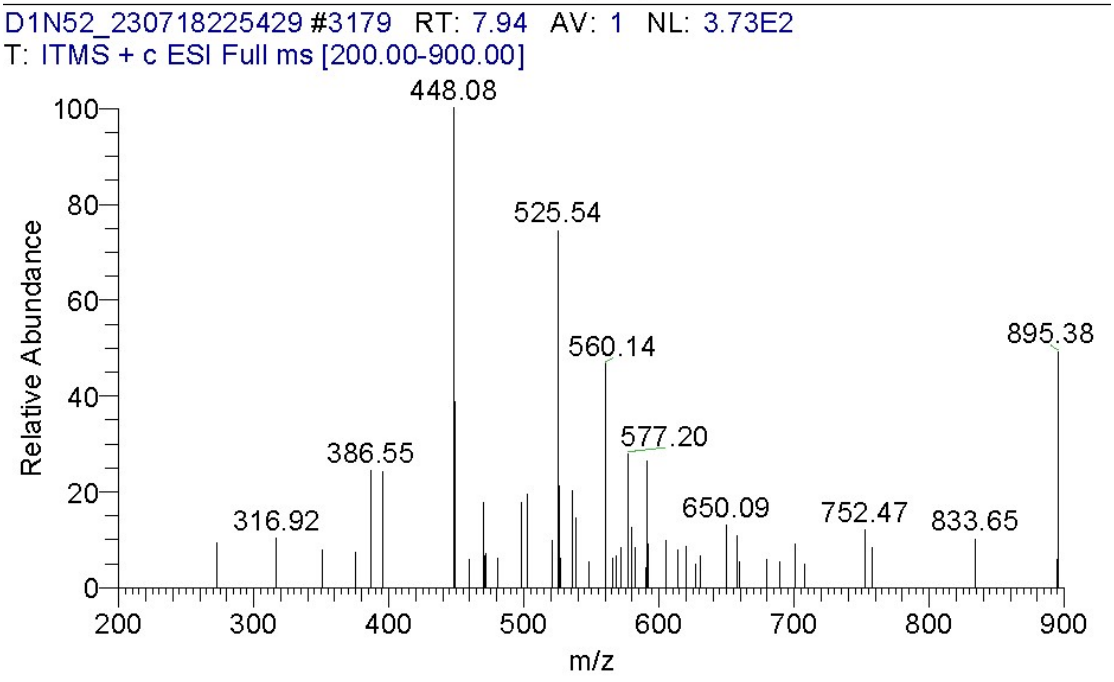
RT: 0.00 - 30.00 SM: 7B



D1N18 #1257 RT: 3.14 AV: 1 NL: 1.73E2
T: ITMS + c ESI Full ms [200.00-900.00]

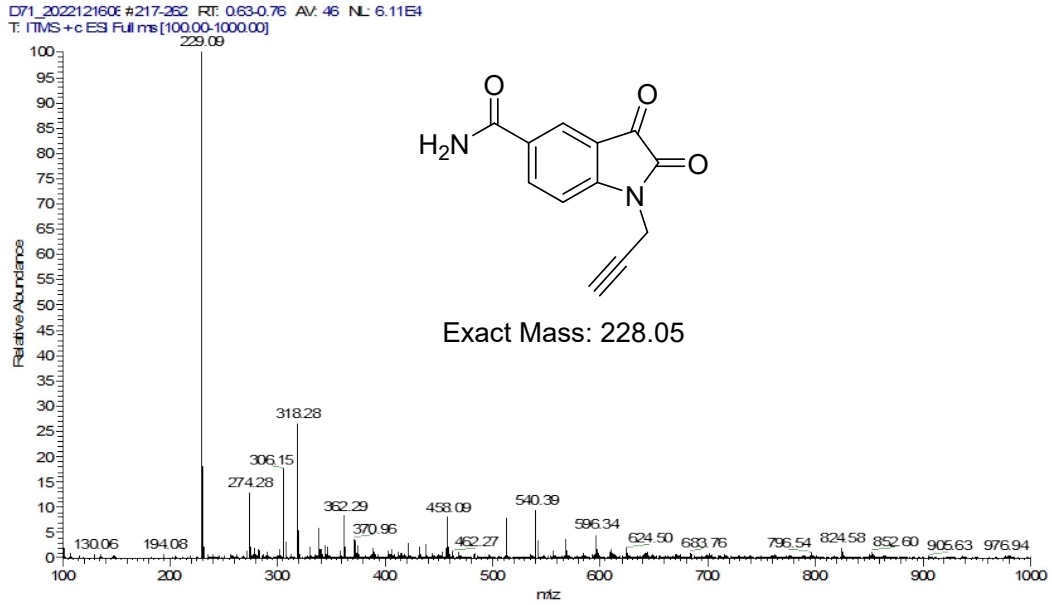



RT: 0.00 - 22.20 SM: 7B
7.95
NL:
1.05E6
UV_VIS_1
UV 3
D1N52_230
718225429
Exact Mass: 447.10
uAU
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
1.03
1.13
11.83
12.74
13.75
16.80
17.37
0
5
10
15
20
Time (min)

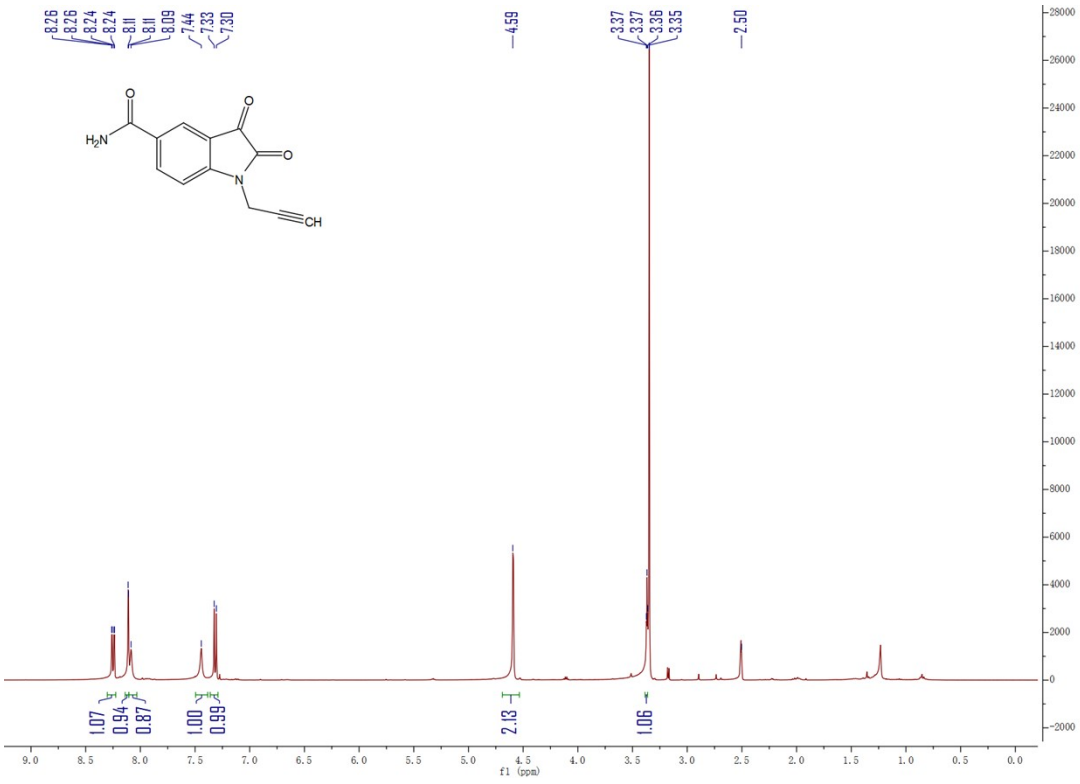

D1N52_230718225429 #3179 RT: 7.94 AV: 1 NL: 3.73E2
T: ITMS + c ESI Full ms [200.00-900.00]
448.08
525.54
560.14
895.38
577.20
386.55
650.09
752.47
833.65
316.92
Relative Abundance
100
80
60
40
20
0
200
300
400
500
600
700
800
900
m/z

Original spectra of compounds.

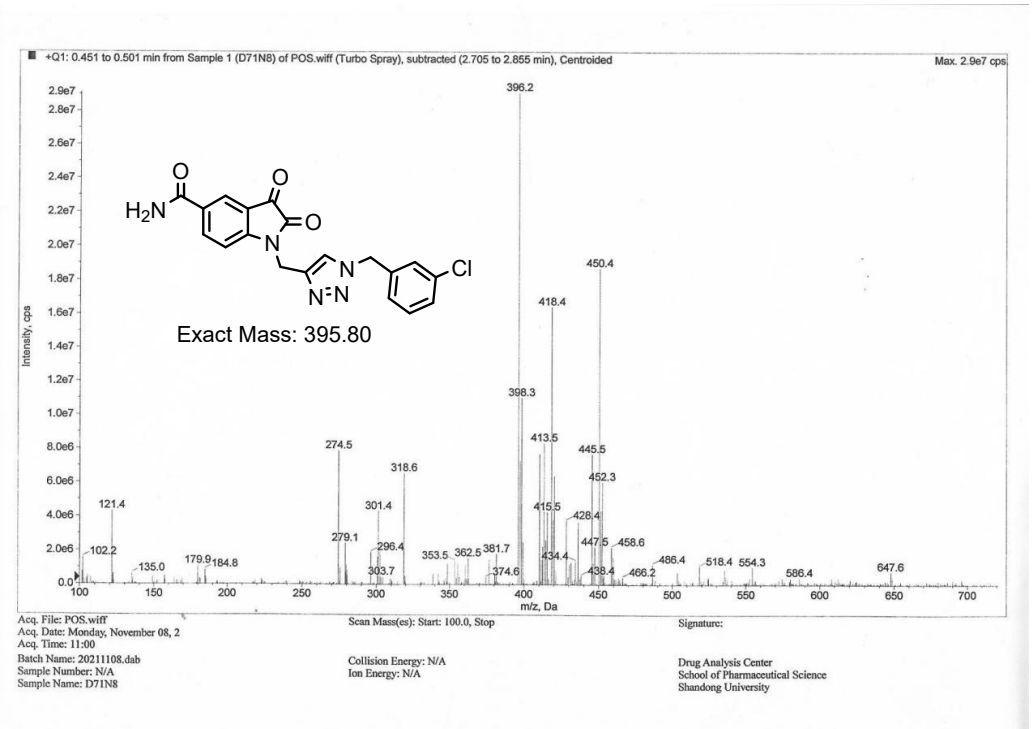
Compound **D1**

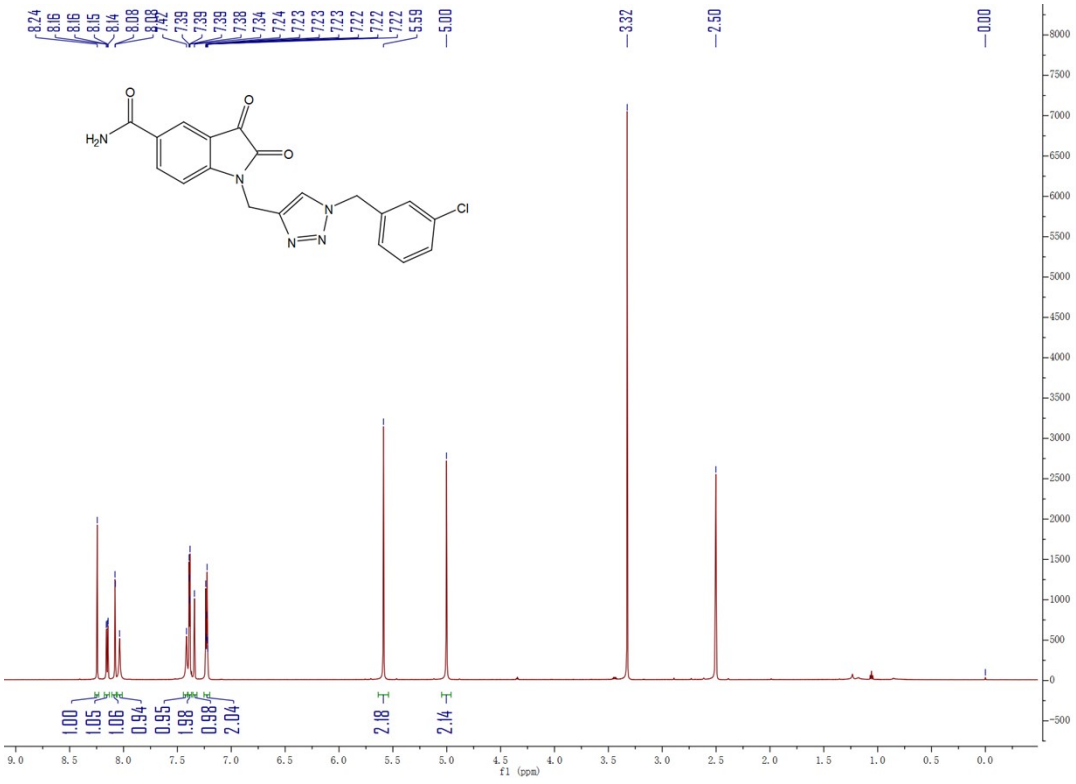


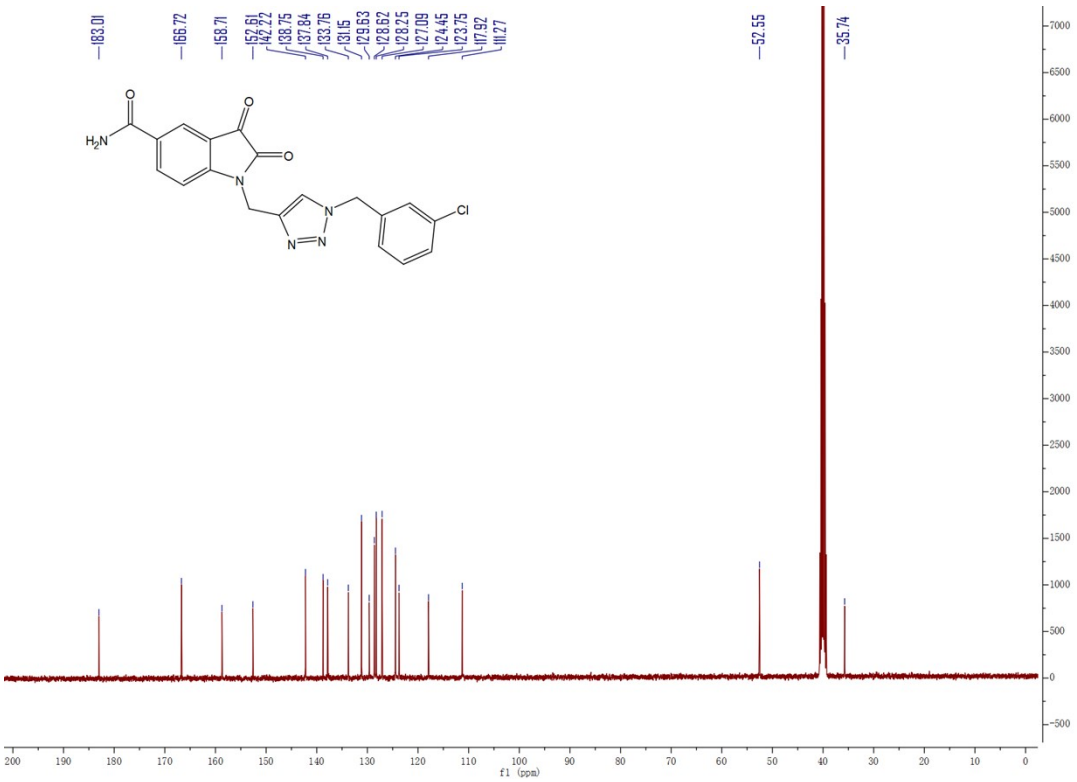
Exact Mass: 228.05



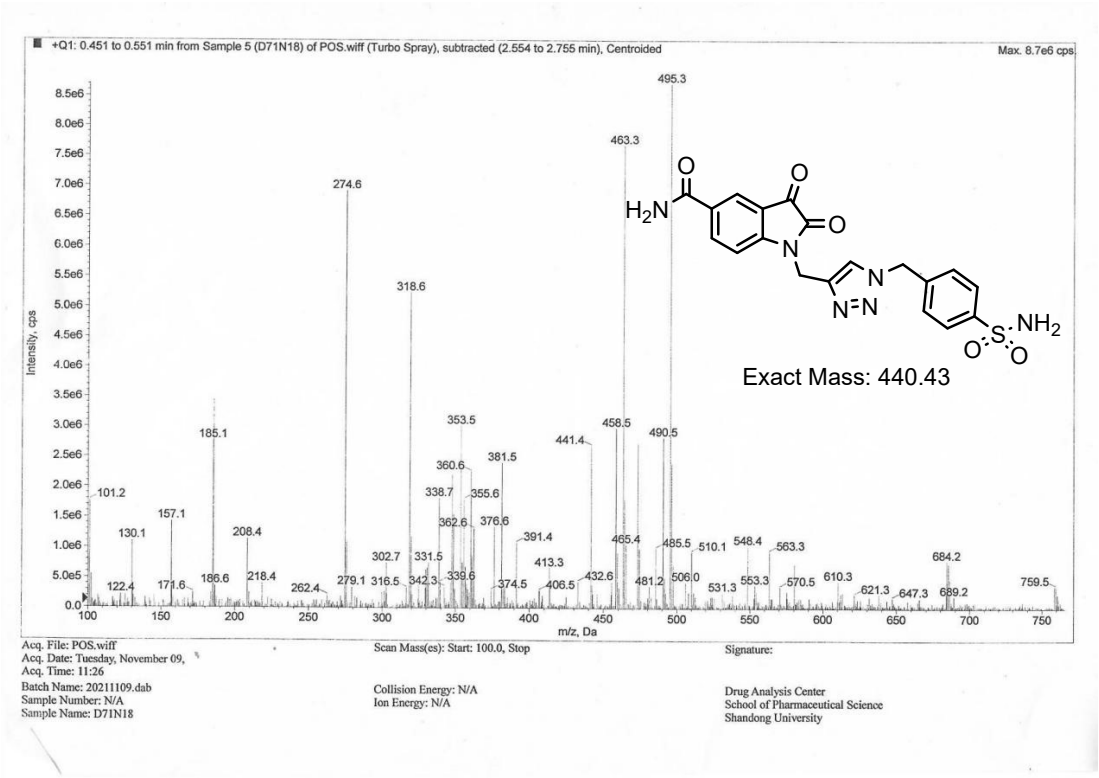
Compound **D1N8**

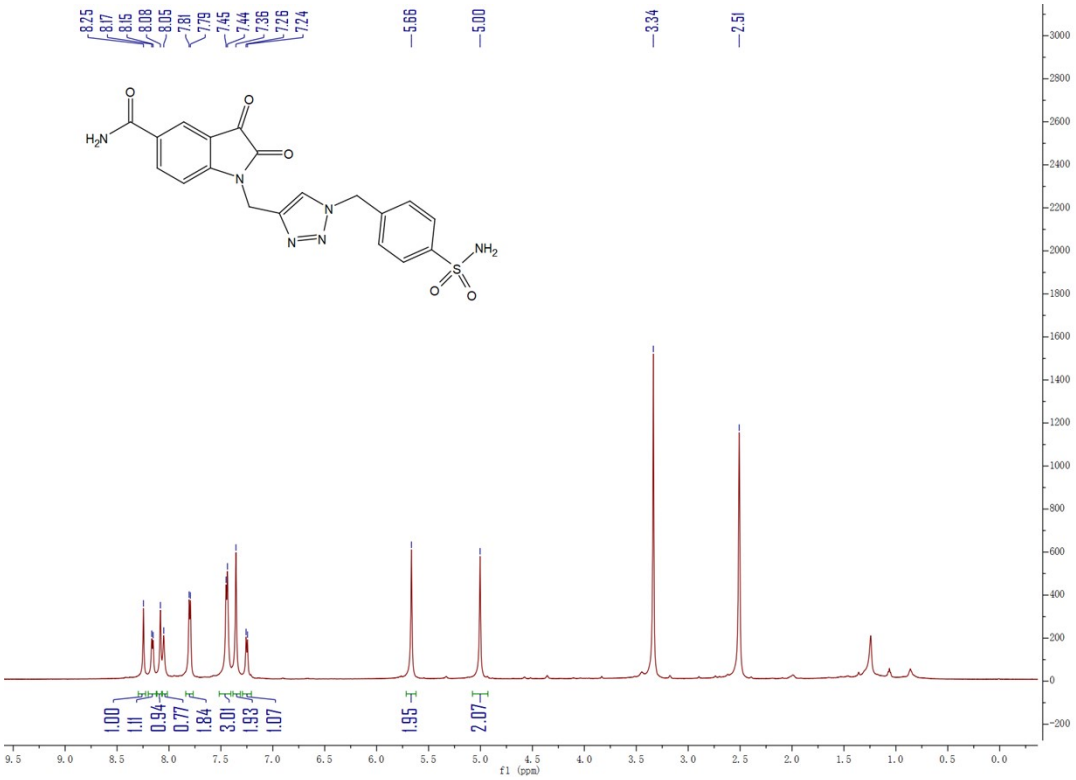


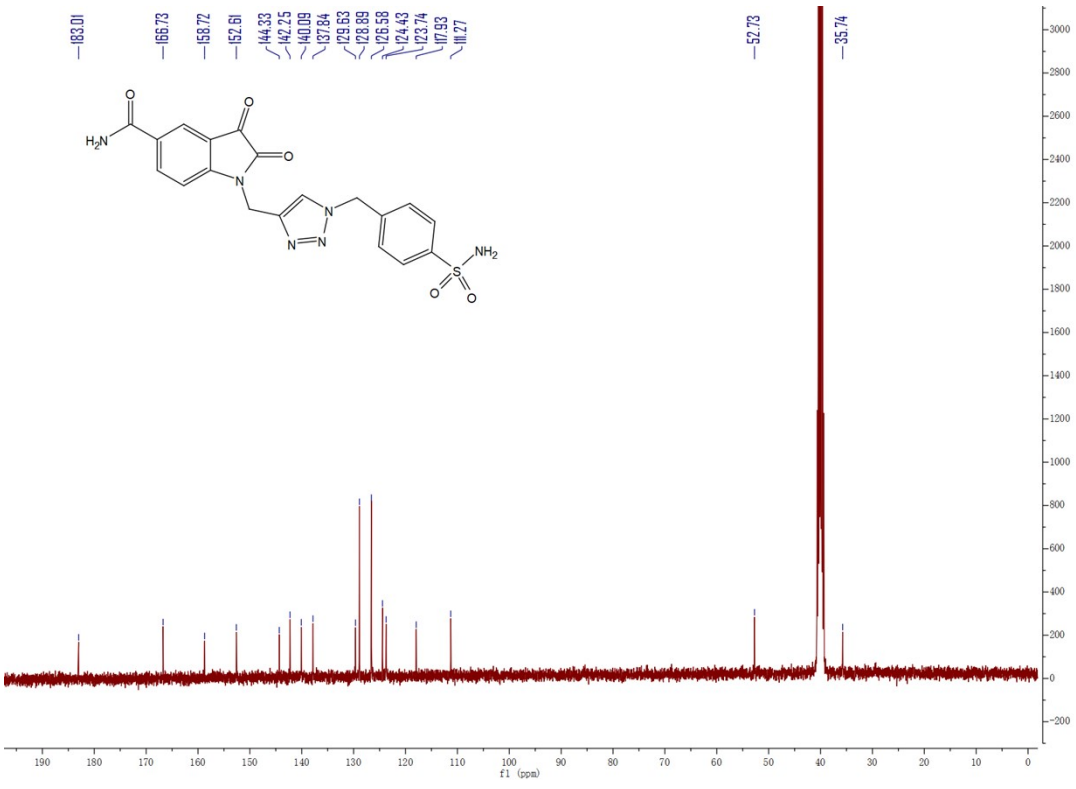




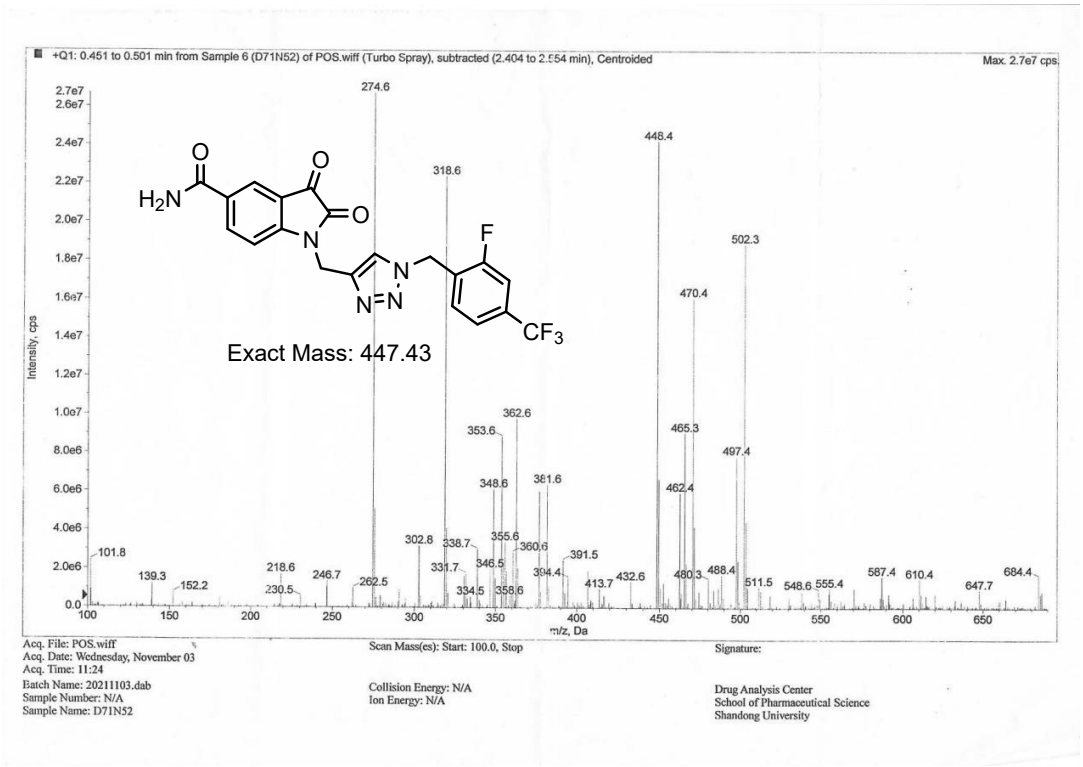
Compound **D1N18**

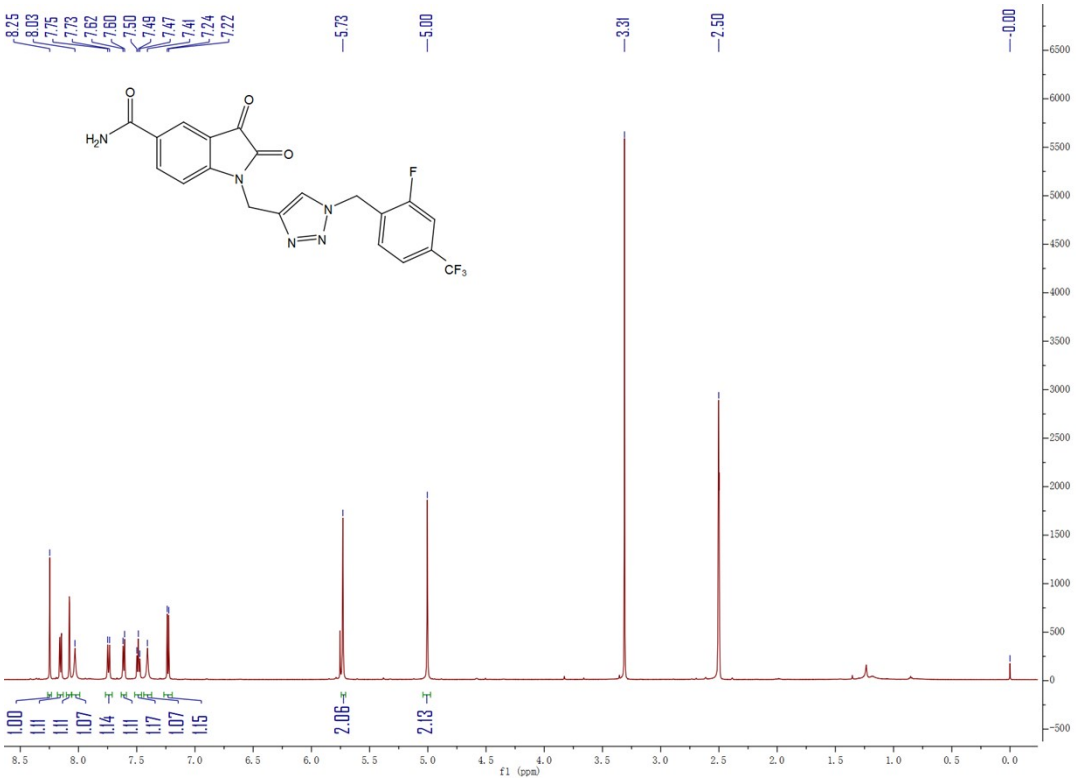


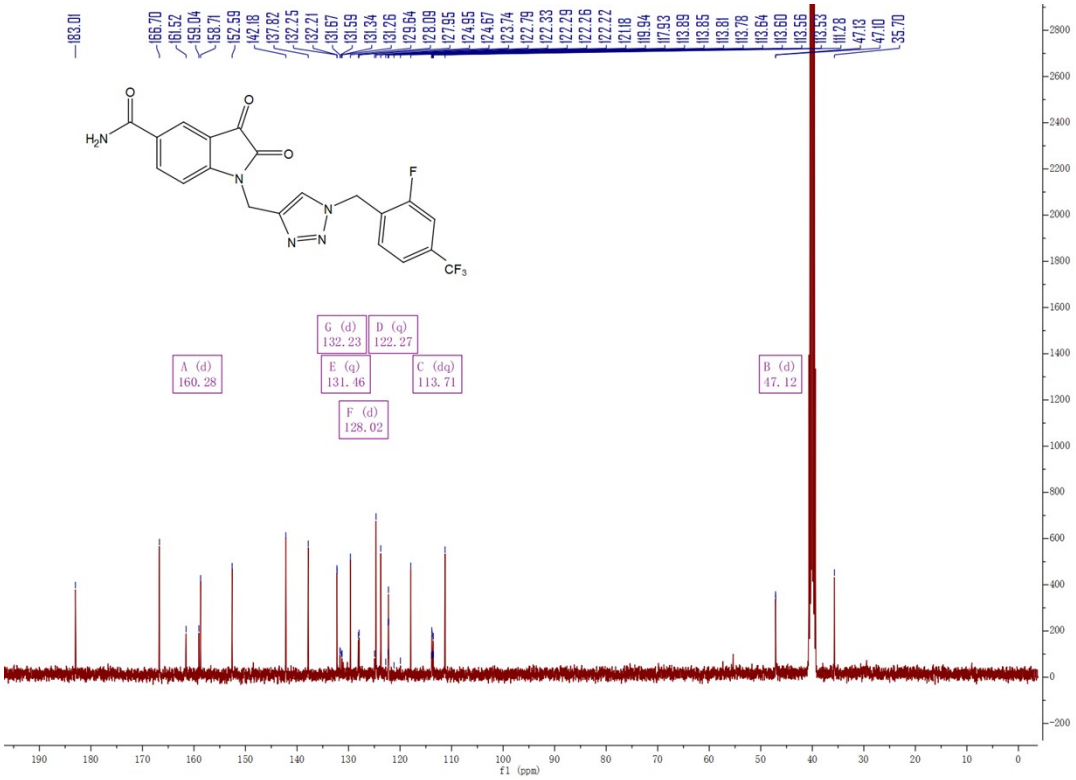

8.25
8.17
8.15
8.08
8.05
7.81
7.79
7.45
7.44
7.36
7.26
7.24
5.66
5.00
3.34
2.51
1.00
1.11
0.94
0.77
1.84
3.01
1.93
1.07
1.95
2.07
f1 (ppm)


183.01
166.73
158.72
152.61
144.33
142.25
140.09
137.84
129.63
128.89
126.58
124.43
123.74
117.93
111.27
52.73
35.74
f1 (ppm)

Compound **D1N52**






183.01
166.70
161.52
159.04
158.71
152.59
142.18
137.82
132.25
132.21
131.67
131.59
131.34
131.26
129.64
128.09
127.95
124.95
124.67
123.74
122.79
122.33
122.29
122.26
122.22
121.18
119.94
117.93
113.89
113.85
113.81
113.78
113.64
113.60
113.56
113.53
111.28
47.13
47.10
35.70
O
O
O
H_2N
N
N
N
F
CF_3
A (d)
160.28
G (d)
132.23
D (q)
122.27
E (q)
131.46
C (dq)
113.71
F (d)
128.02
B (d)
47.12
f1 (ppm)