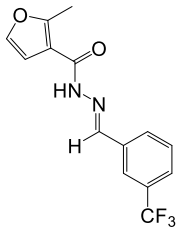
Discovery and antibacterial study of potential PPK1 inhibitors against uropathogenic *E. coli*

Supplemental materials



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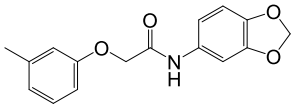

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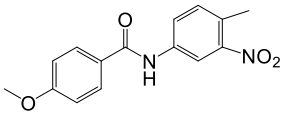

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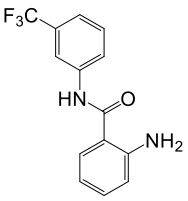

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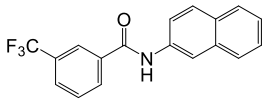

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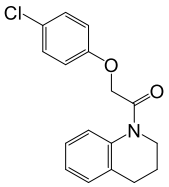

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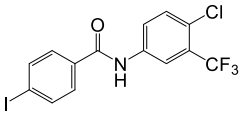

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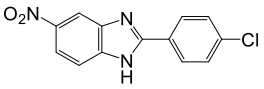

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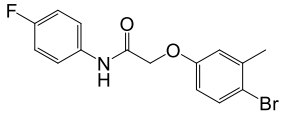

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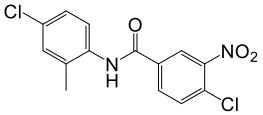

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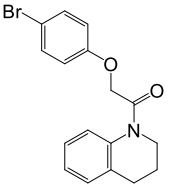

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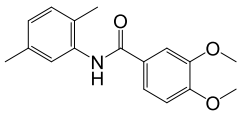

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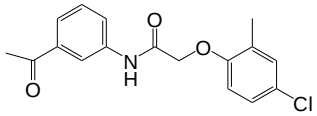

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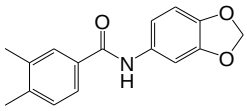

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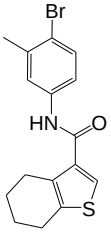

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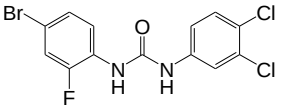

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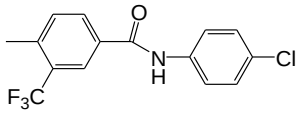

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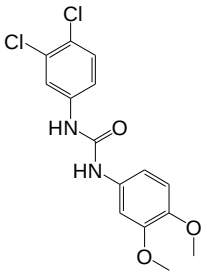

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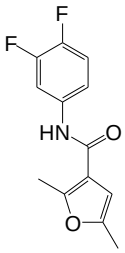

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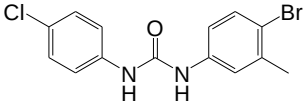

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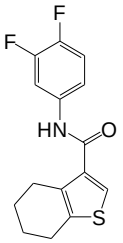

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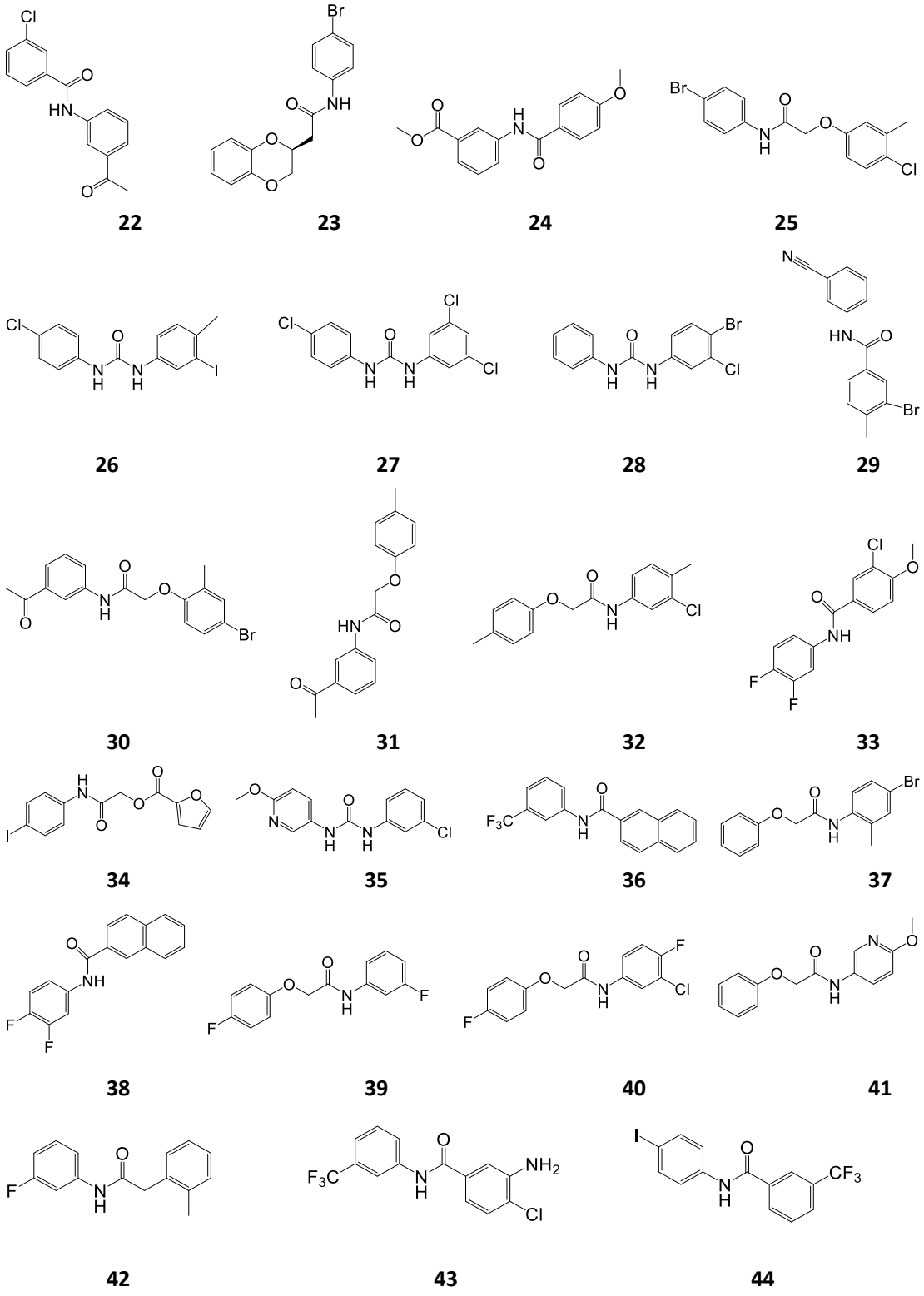


Fig S1. 44 compounds selected after the virtual screening

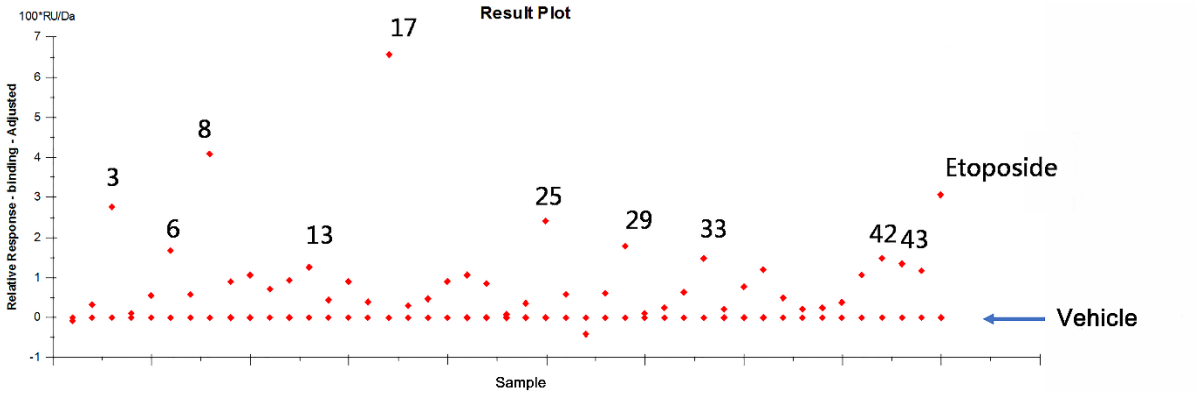


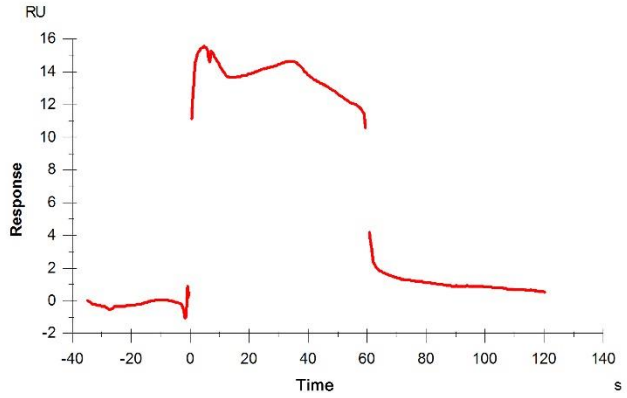
Fig S2. Relative binding responses of 44 compounds and etoposide with PPK1.

Table S1. Relative binding responses of 44 compounds and etoposide with PPK1.

Compound	Relative Biding Response （100RU/Da）
17	6.555
8	4.073
Etoposide	3.06
3	2.745
25	2.389
29	1.766
6	1.673
33	1.483
42	1.482
43	1.323
13	1.253
36	1.198
44	1.165
10	1.072
21	1.069
41	1.064
12	0.9229
15	0.9108
9	0.8927
20	0.8844
22	0.8409
35	0.7663
11	0.7053
32	0.6143
28	0.5949
7	0.5767
26	0.5627
5	0.5362

37	0.4769
19	0.4519
14	0.4355
16	0.3937
40	0.392
24	0.3437
2	0.3333
18	0.298
31	0.2458
39	0.2351
38	0.2263
34	0.225
30	0.1171
4	0.1059
23	0.06621
1	-0.07901
27	-0.4218

A



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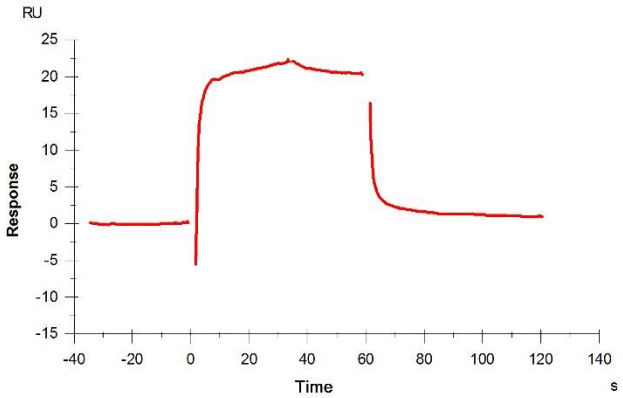

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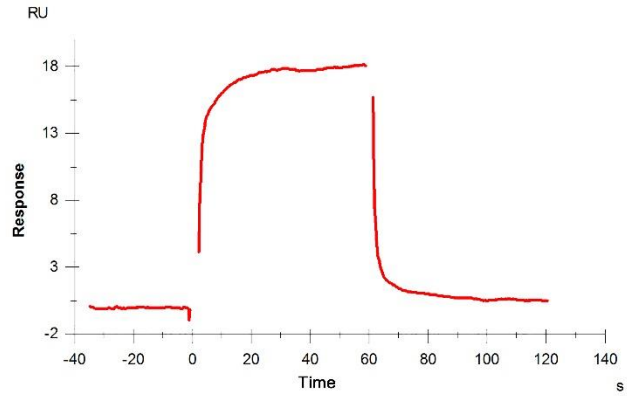


Fig S3. Sensorgrams of 8 (A), 17 (B) and etoposide (C) with PPK1.

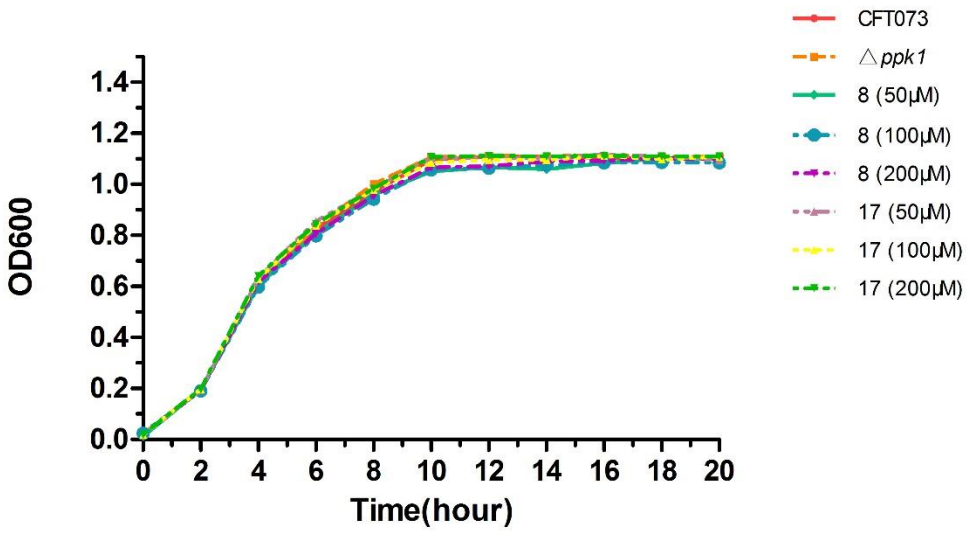
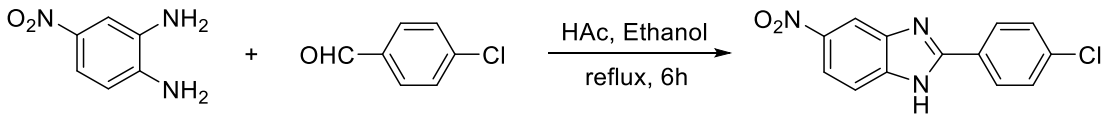


Fig S4. Growth curves of uropathogenic *E. coli* in the absence or presence of **8** and **17**.

Preparation and characterization of **8** and **17**

Compounds synthesis:

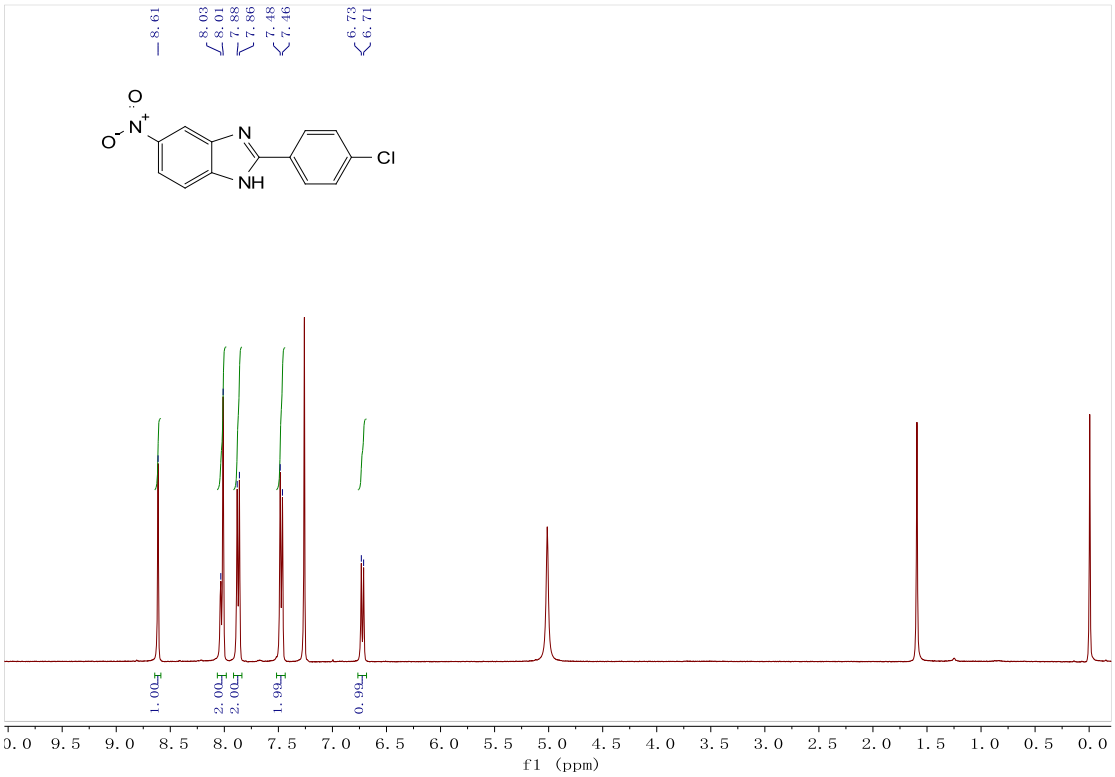


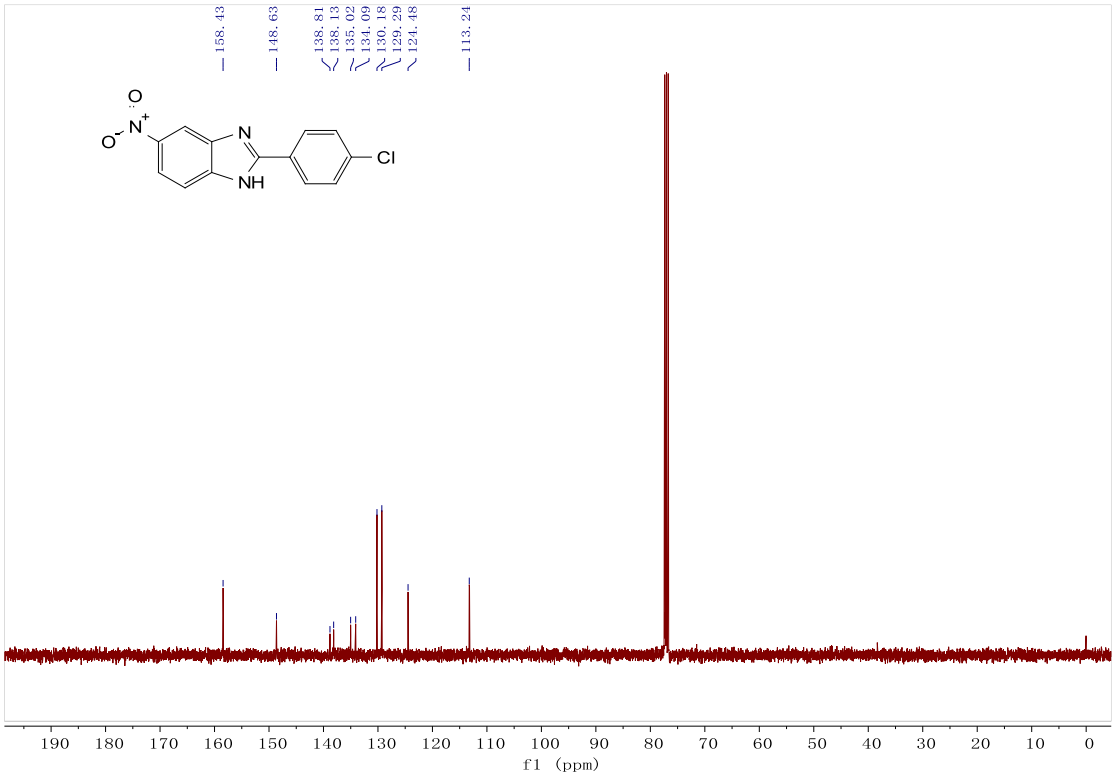
Scheme 2. Synthesis of compound **8**.

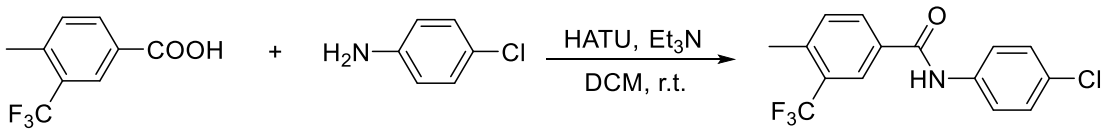
Experimental procedure: 2-(4-chlorophenyl)-5-nitro-1H-benzo[d]imidazole***(8)***. To the ethanol (10 mL) solvent added 4-nitrobenzene-1,2-diamine (155 mg, 1 mmol) and 4-chlorobenzaldehyde (140 mg, 1 mmol). Several drops of glacial HAc were added before the mixture was heated under 80℃ for 6h. Then the solvent was evaporated under reduced pressure. The remaining residue was extracted with EtOAc, and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography eluting with PE/EA (3/1) to afford the off-white solid compound, 250

mg. Yield: 80%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.87 (d, J = 8.2 Hz, 2H), 7.47 (d, *J* = 8.3 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.4, 148.6, 138.8, 138.1, 135.0, 134.1, 130.2, 129.3, 124.5, 113.2. ESI-MS^{+}: 273.08.

^{1}H NMR, ^{13}C NMR





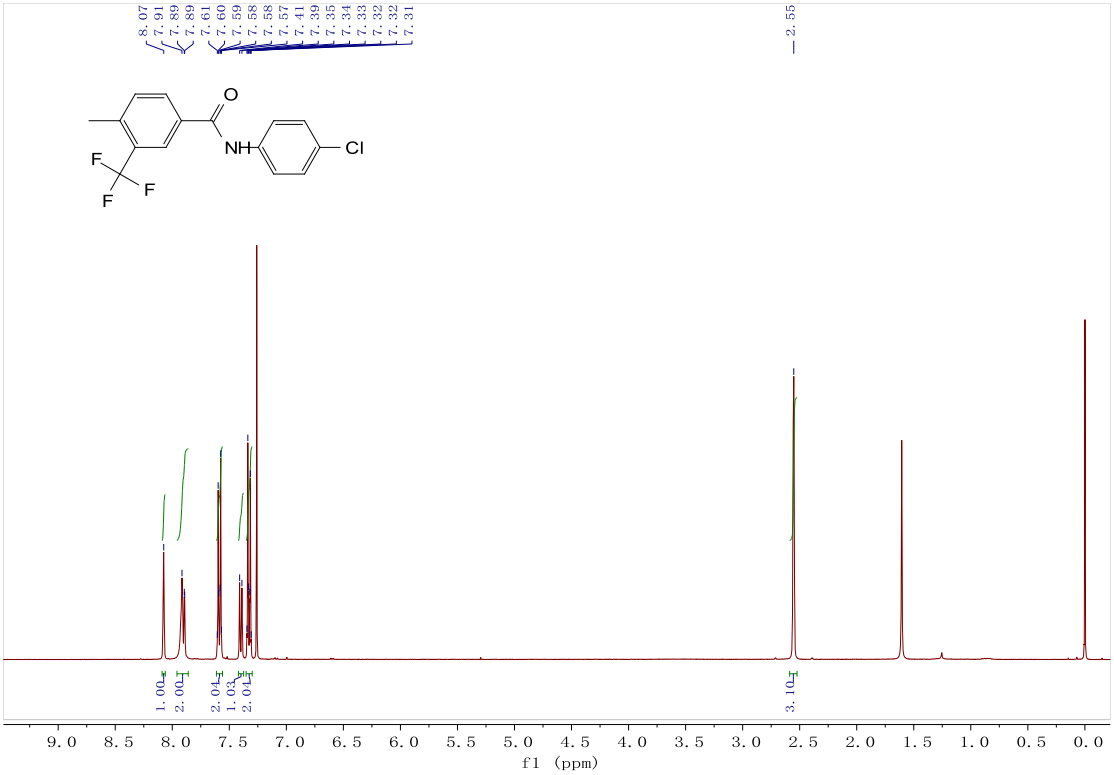


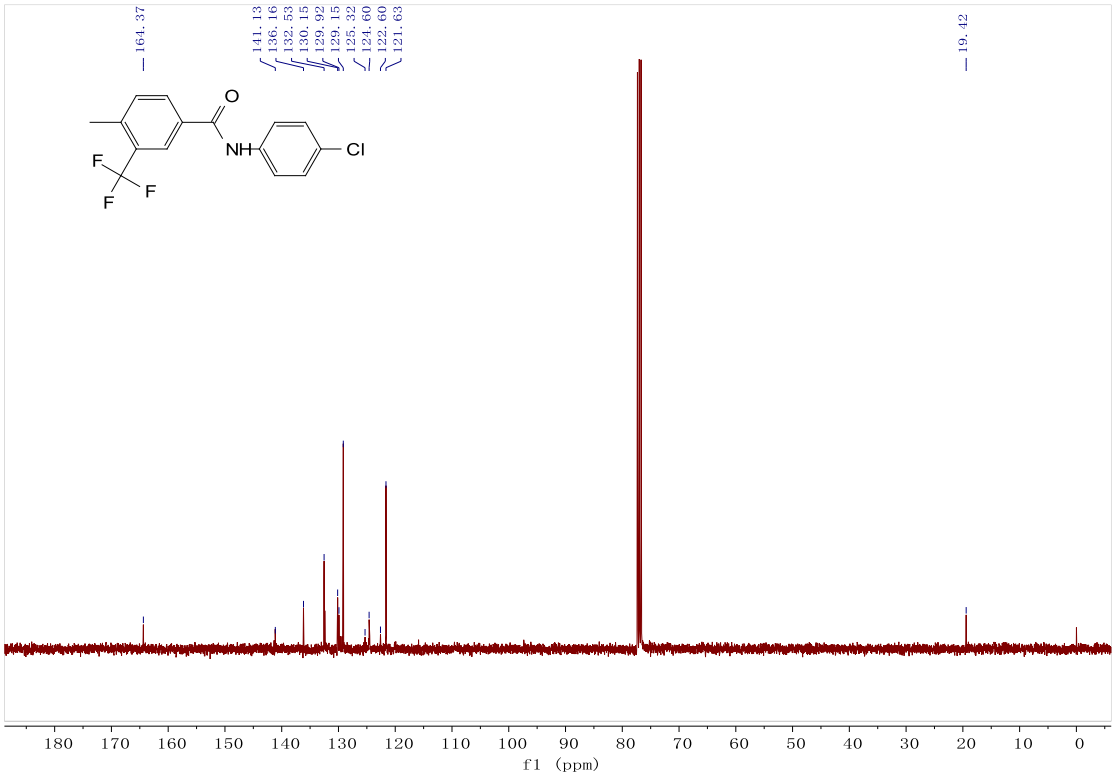
Scheme 1. Synthesis of compound **17**.

Experimental procedure: N-(4-chlorophenyl)-4-methyl-3-(trifluoromethyl)benzamide ***(17)***. A mixture of 4-methyl-3-(trifluoromethyl)benzoic acid (103 mg, 0.5 mmol) and HATU (247 mg, 0.65 mmol) in DCM was stirred in the room temperature for 5 min, and to the solvent 4-chloroaniline (77 mg, 0.6 mmol) and several drops of Et_3N were then added. The final mixture was reacted overnight before the solvent was removed under reduced pressure. The residue was extracted with EtOAc, and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography

eluting with PE/EA (4/1) to afford the colored solid compound, 140 mg. Yield: 90%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (s, 1H), 7.96 – 7.86 (m, 2H), 7.61 – 7.56 (m, 2H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.30 (m, 2H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.4, 141.1, 136.2, 132.5, 130.2, 129.9, 129.2, 125.3, 124.6, 122.6, 121.6. ESI-MS^{+}: 314.15.

^{1}H NMR, ^{13}C NMR

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121.63
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NH
Cl
F
F
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f1 (ppm)