Supporting Information

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Photoresponsive prodrug for regulated inhibition of indoleamine 2,3-dioxygenase 1 enzyme activity

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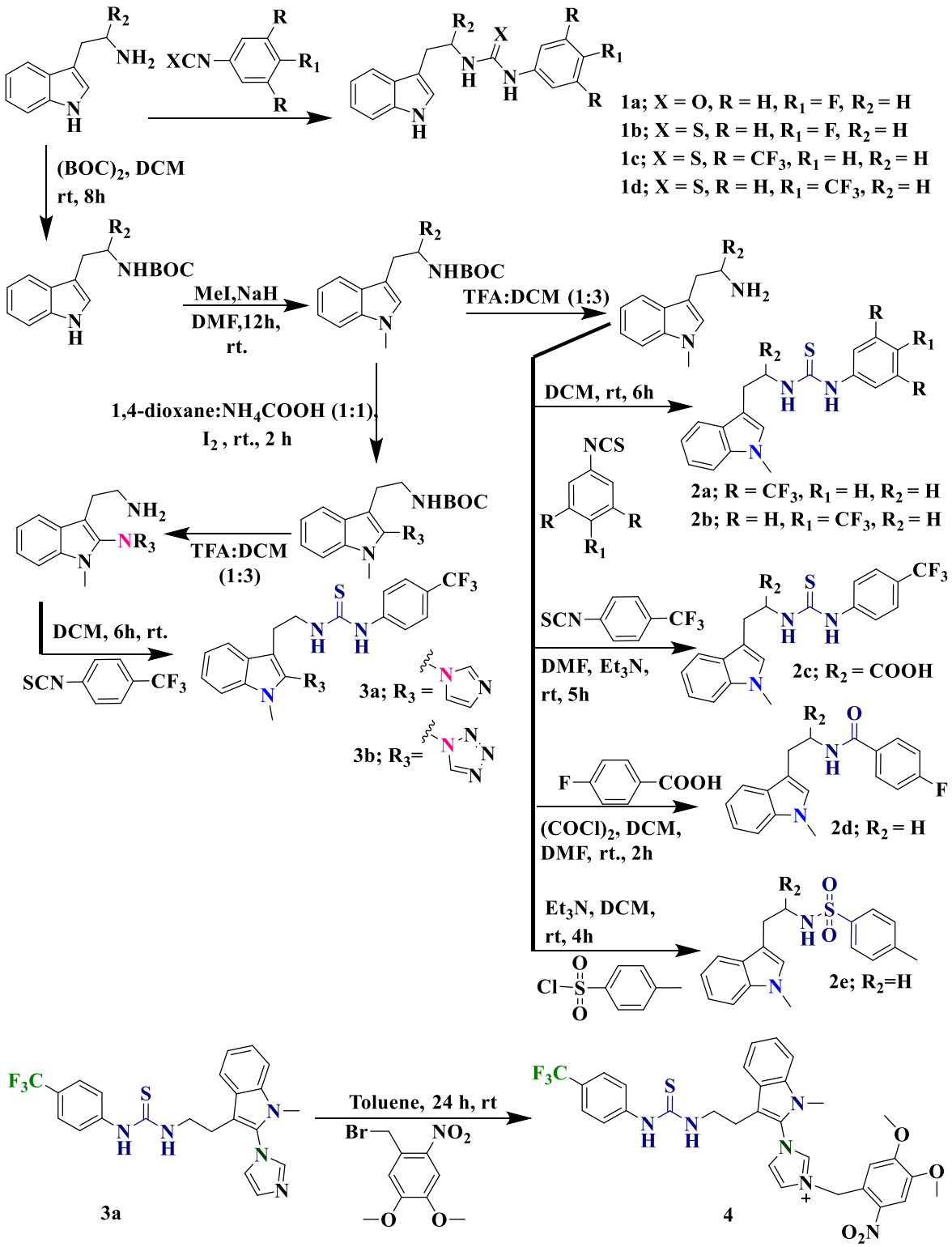


Scheme S1: Synthetic routes to various indole derivatives from tryptamine and tryptophan.

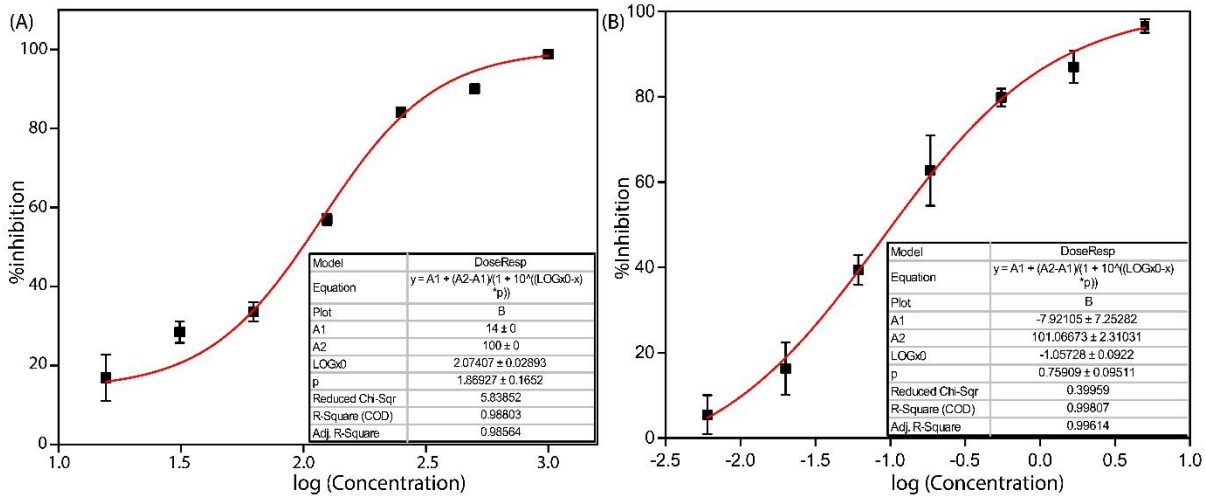


Fig. S1. Plots of concentration of *N*-formyl kynurenine in the presence of different concentrations of (A) compound **3a** and (B) epacadostat.

Table S1. Inhibitory activity of the **3a** against purified human TDO enzyme.

Compounds	% TDO Inhibition		
	25 µM	50 µM	100 µM
3a	4 ± 0.5	17 ± 0.4	48 ± 1

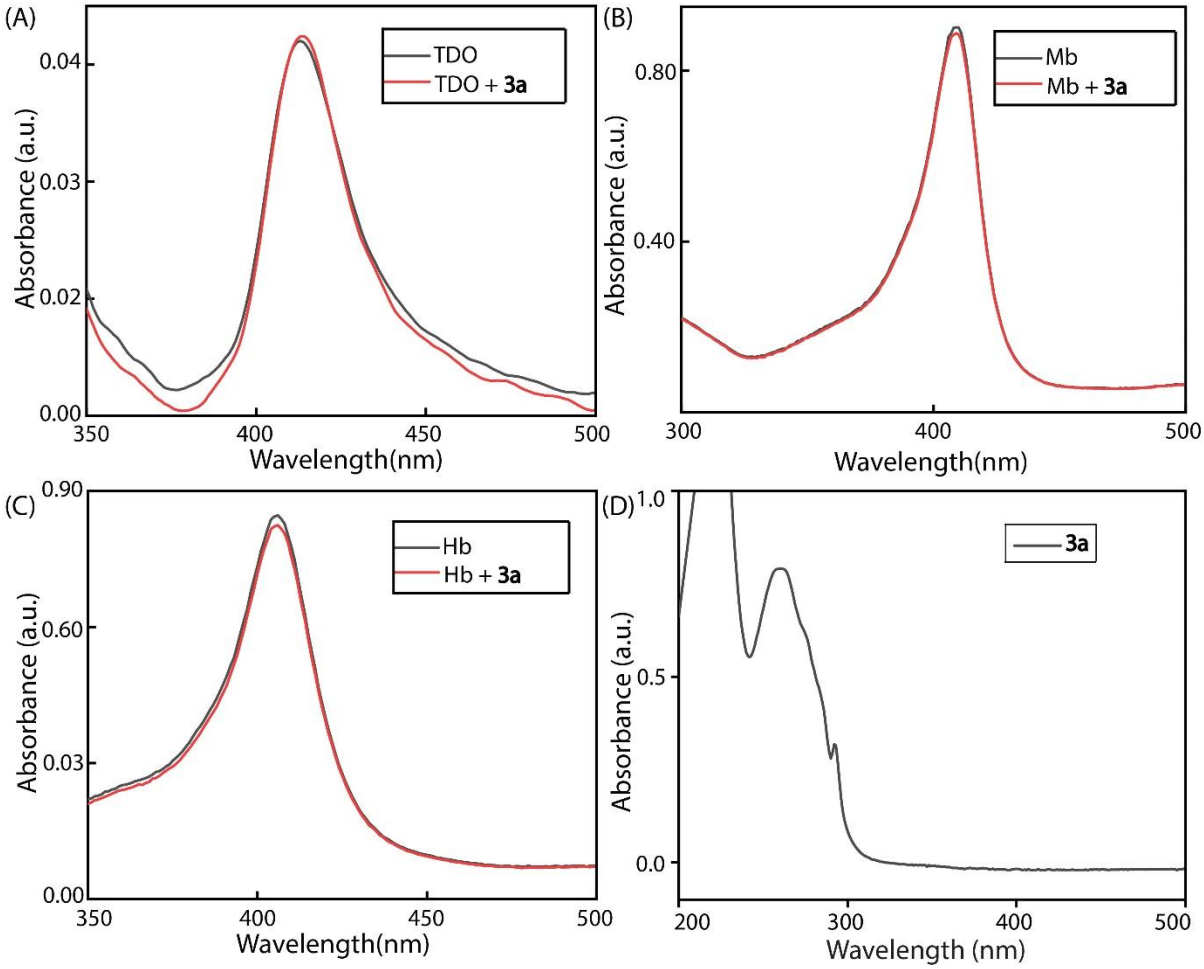


Fig. S2. UV-Vis spectra of the (A) TDO, (B) myoglobin, and (C) hemoglobin proteins (200 nM) in the absence and presence of compound **3a** (5 μM) in 100 mM KPB buffer at pH 6.5 after 60 min of incubation at 37 °C. (D) UV-Vis spectra of compound **3a**.

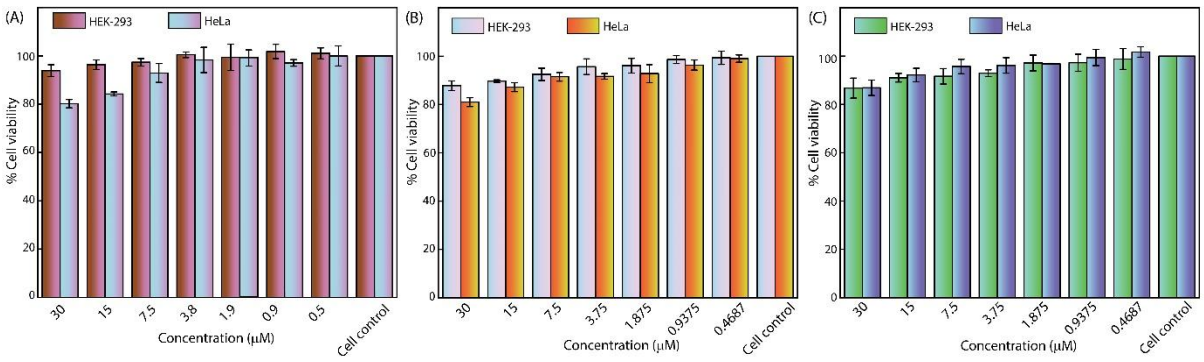


Fig. S3. Viability of HEK-293 and HeLa cells in the presence of (A) compound **3a**, (B) epacadostat and (C) indoximod.

Table S2. Characteristic peaks from the UV-Vis measurements of the IDO1 enzyme in the absence and presence of compound **3a**.

Sl. No.	Compound	λ_{max} (nm) Fe^{3+} state binding	λ_{max} (nm) Fe^{2+} state binding
1	Only IDO1	410, 527, 557, 568	425, 535, 561
2	IDO1 + **3a**	414, 537, 562, 571	421, 539, 573

Enzyme concentration: 650 nM, compound **3a** concentration 10 µM

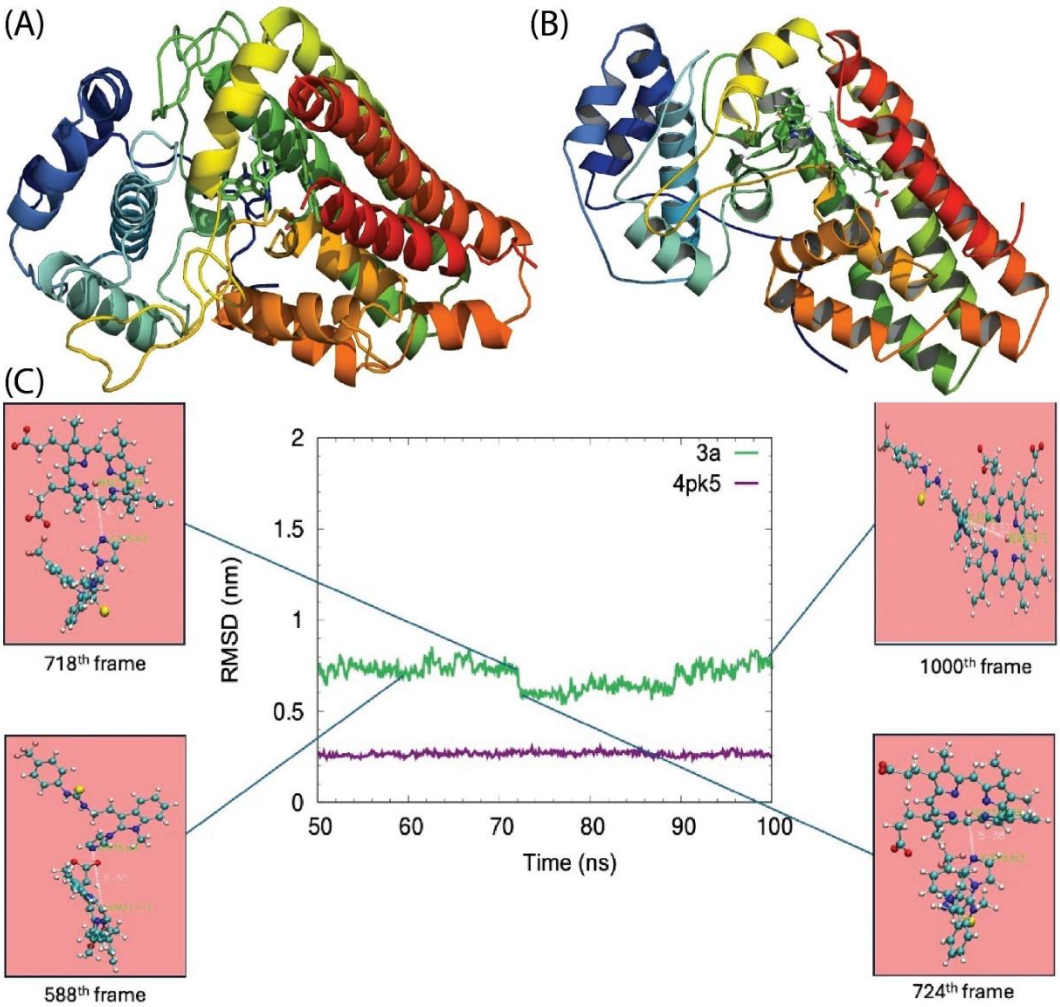


Fig. S4. The mode of interaction of compound **3a** with the protein 4PK5 in the presence of the heme group after (A) molecular docking and (B) MD simulation, respectively. Root means square displacement (RMSD) of protein (protein ID: 4PK5; purple) and the ligand (compound **3a**; green) at different time frames.

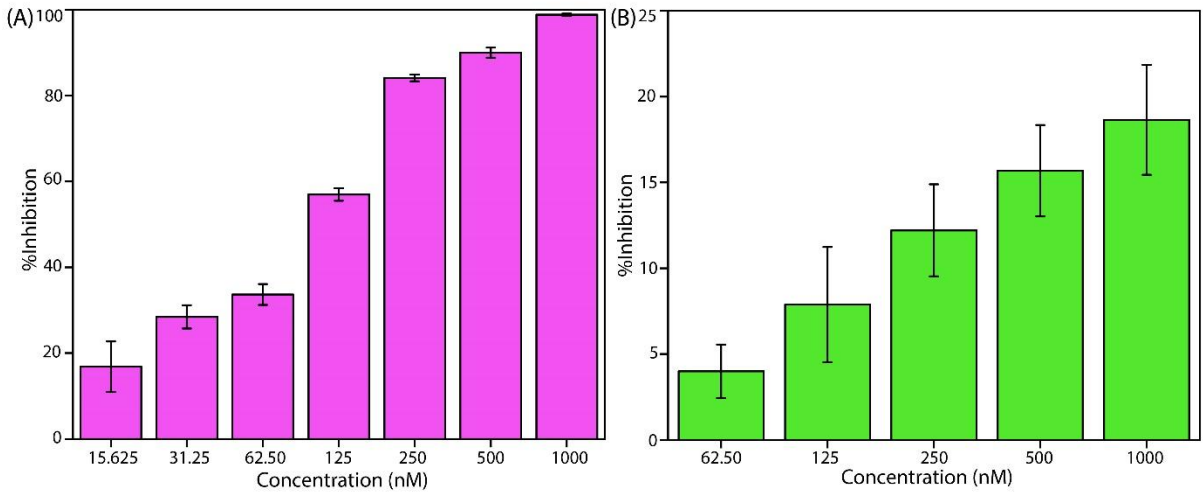


Fig. S5. Variation of %IDO1 inhibition in the presence of different concentrations for (A) compound **3a** and (B) compound **4** in the presence of IDO1 enzyme (650 nM).

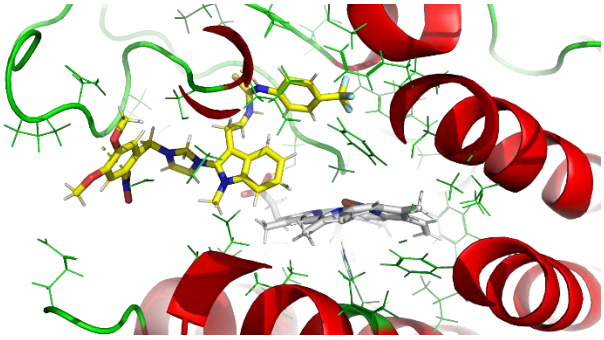


Fig. S6. Probable mode of interaction of the compound **4** with the active site of IDO1 enzyme (4PK5).

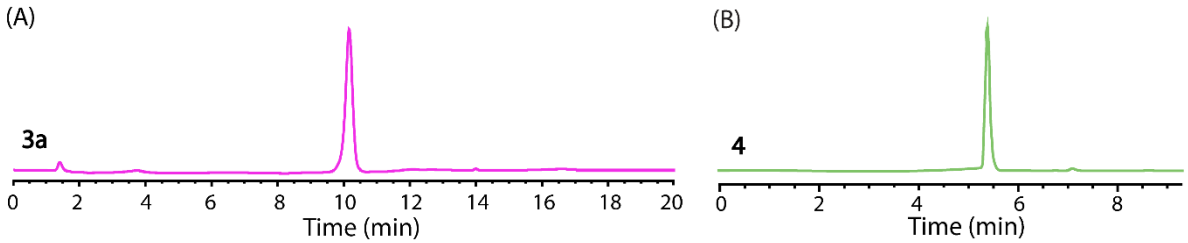


Fig. S7. HPLC traces of (A) compound **3a** and (B) compound **4**.

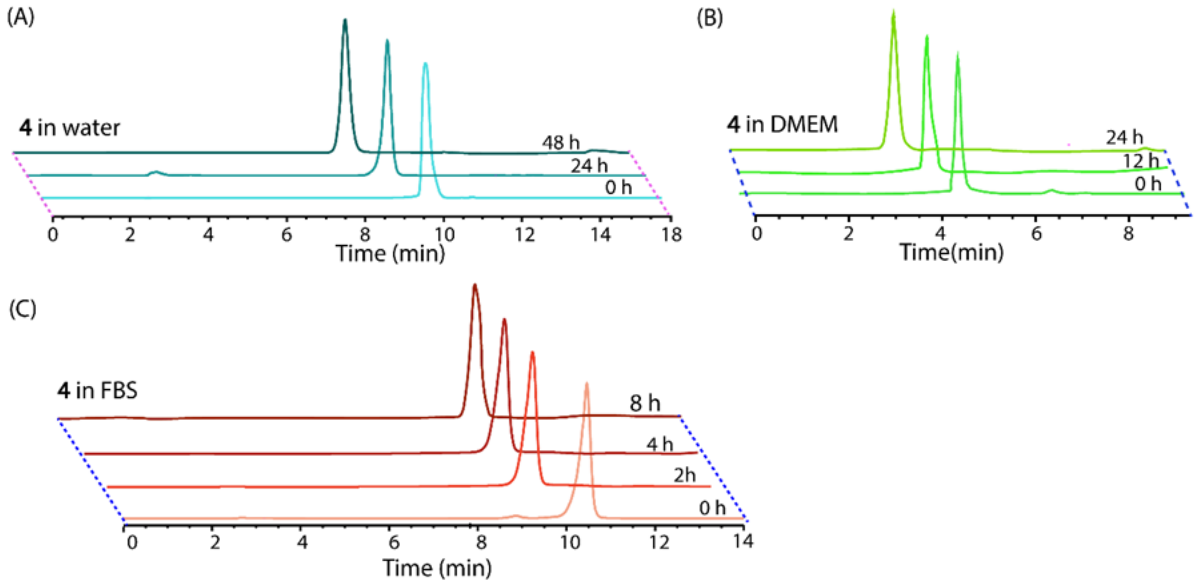


Fig. S8. HPLC analysis to investigate the stability of the prodrug **4** at different time intervals in (A) water, (B) DMEM, and (C) FBS.

Rate of photolysis and apparent quantum yield calculation:

The photolysis rate of the prodrug was observed to be 4.69×10^{-9} mol/s by HPLC analysis. Approximately 90% photolysis was observed by irradiating 18W 400nm light for 30 min to compound **4**. The apparent quantum yield was found to be 7.82×10^{-5}. From the HRMS analysis, we can assume that 4,5-dimethoxy-2-nitrosobenzaldehyde is the potential side product apart from **3a** (HRMS calcd. for $C_9H_9NO_4$ $(M+H)^+$: 196.060, obtained: 196.0598).

$$Photolysis\ rate = \frac{Moles\ reacted}{Time}$$

$$= 4.69 \times 10^{-9}\ \text{mol/s}$$

The apparent quantum yield (Φ) of a photochemical reaction is defined as the number of molecules reacting per photon absorbed. In this experiment, the photolysis of a compound with a molar mass of 639 g/mol was carried out under 400 nm light with an intensity of 18 W for 30 minutes (1800 seconds). A 90% conversion was observed using a sample mass of 6 mg.

$$\Phi = \frac{moles\ of\ compound\ reacted}{moles\ of\ photons} = 7.82 \times 10^{-5}$$

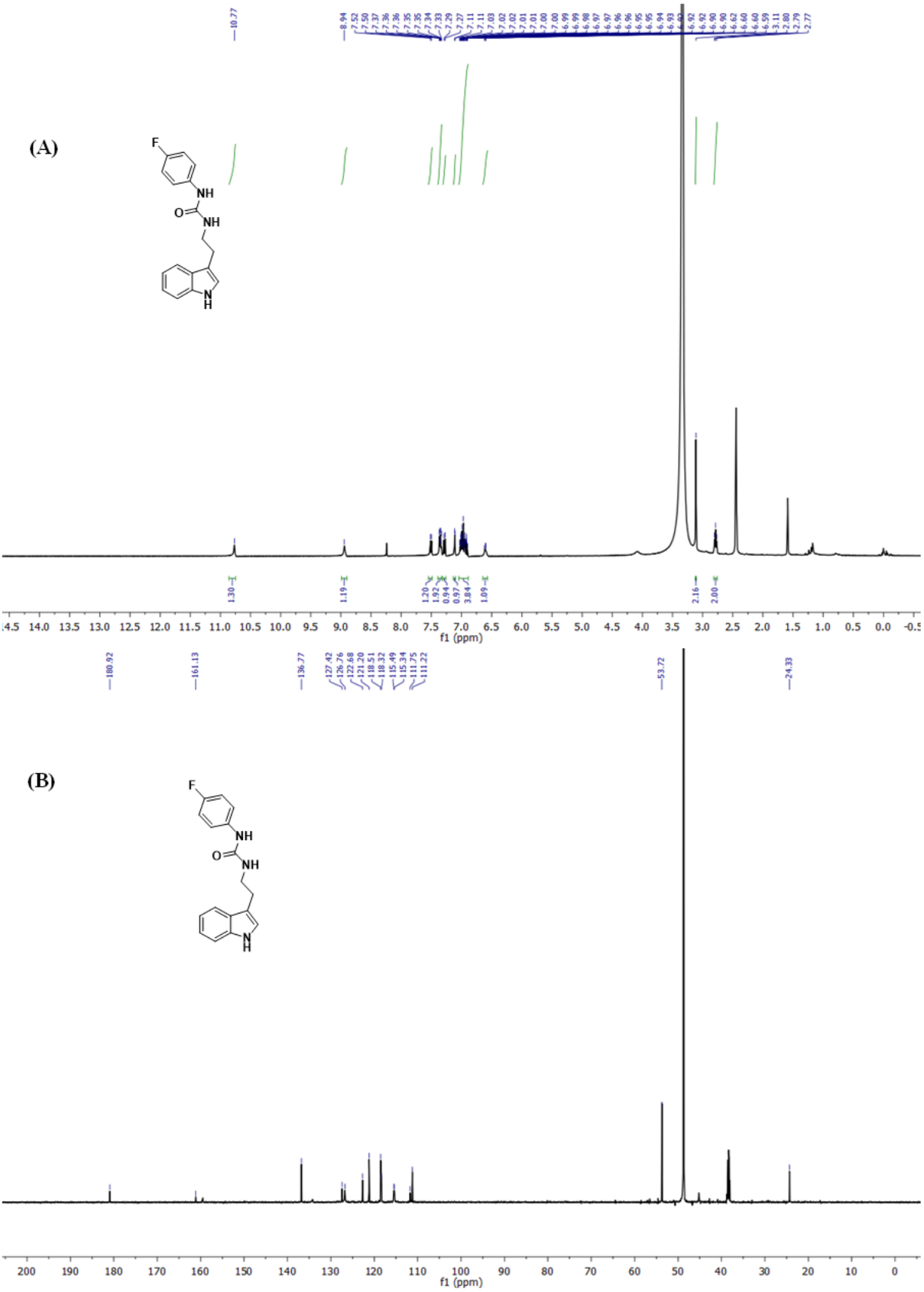


Fig. S9. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1*H*-indol-3-yl)ethyl)-3-(4-fluorophenyl)urea (**1a**) in DMSO-d_6 solvent.

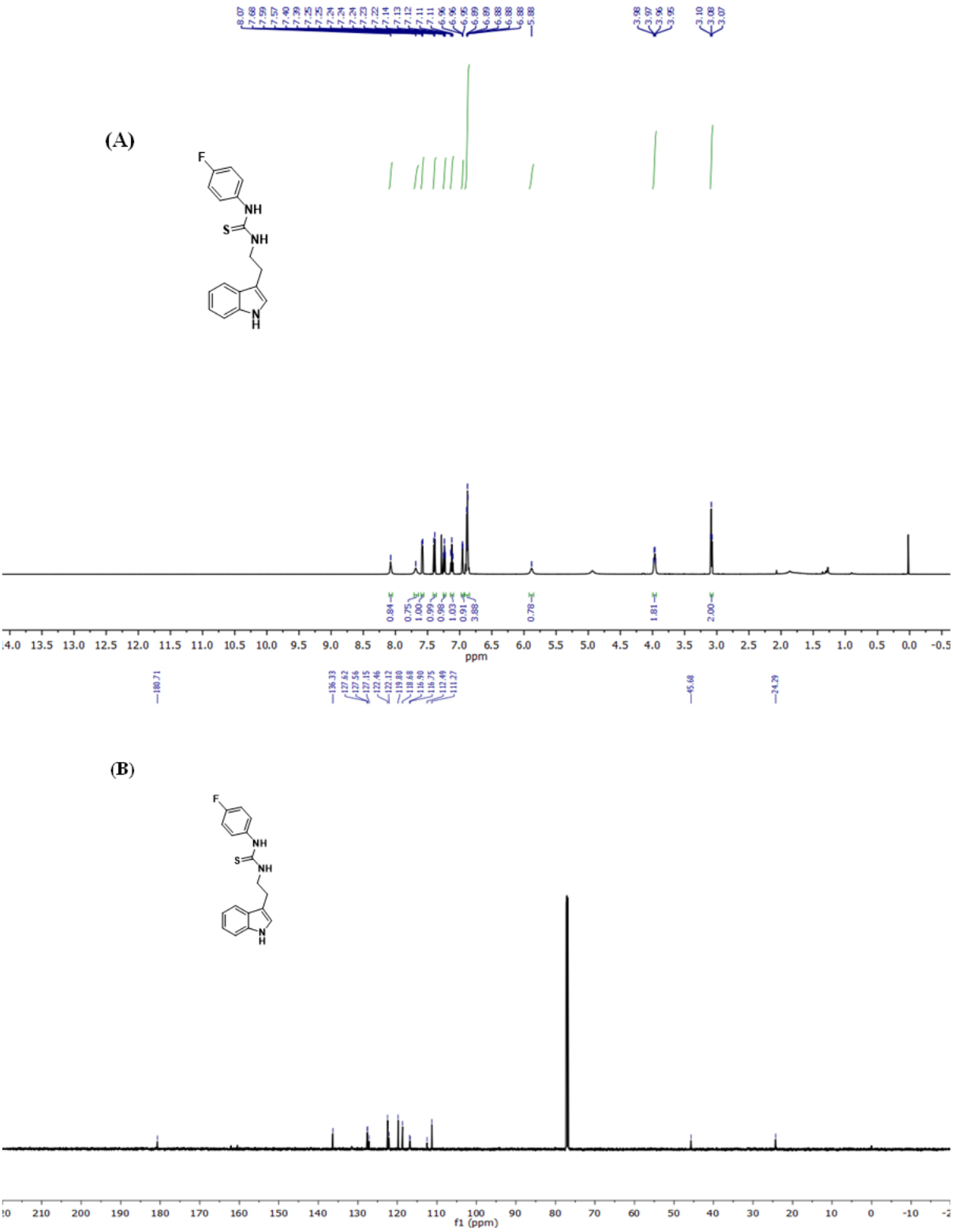


Fig. S10. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1*H*-indol-3-yl)ethyl)-3-(4-fluorophenyl)thiourea (**1b**) in $CDCl_3$ solvent.

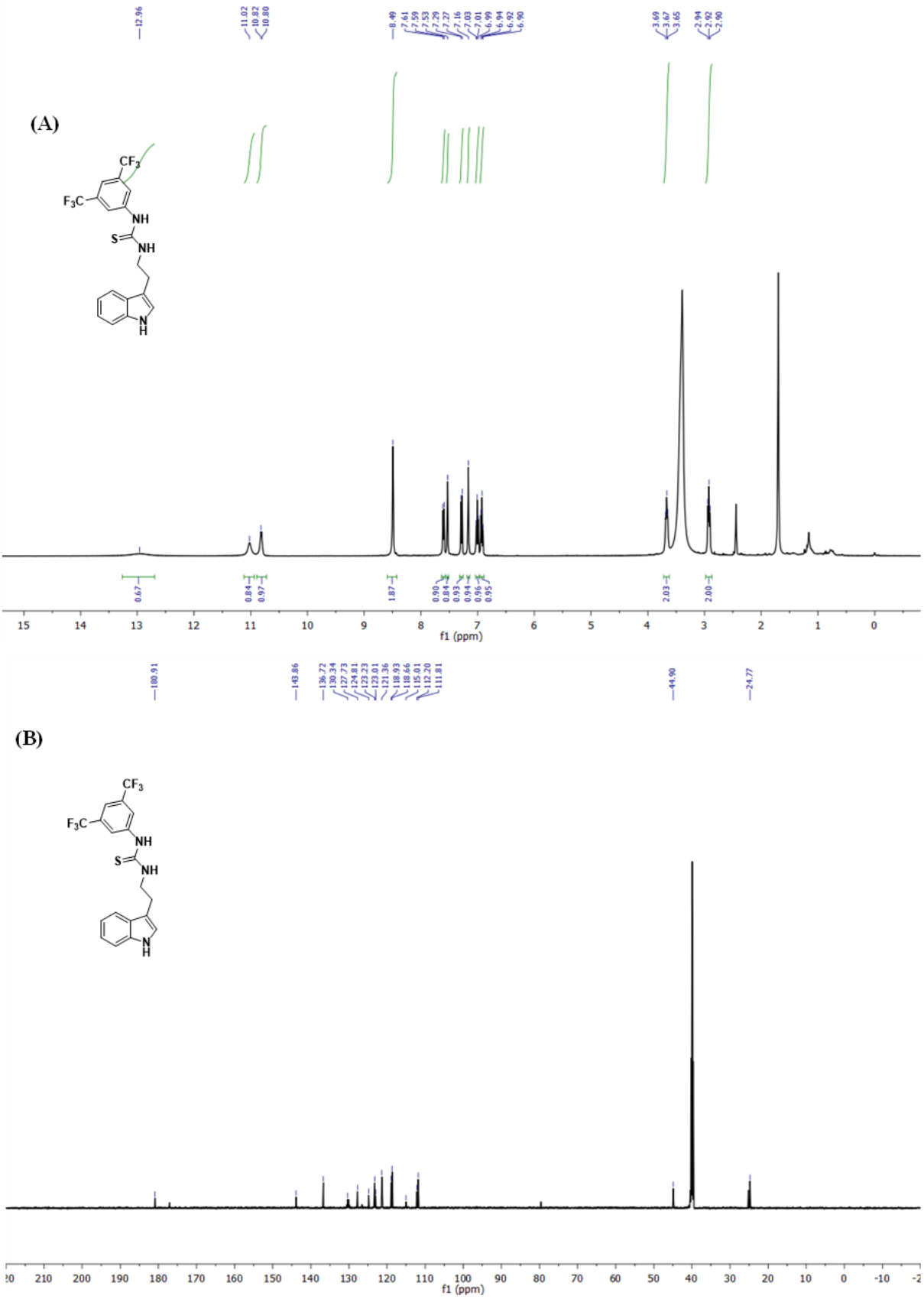


Fig. S11. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1*H*-indol-3-yl)ethyl)-3-(3,5-bis(trifluoromethyl)phenyl)thiourea (**1c**) in DMSO-d_6 solvent.

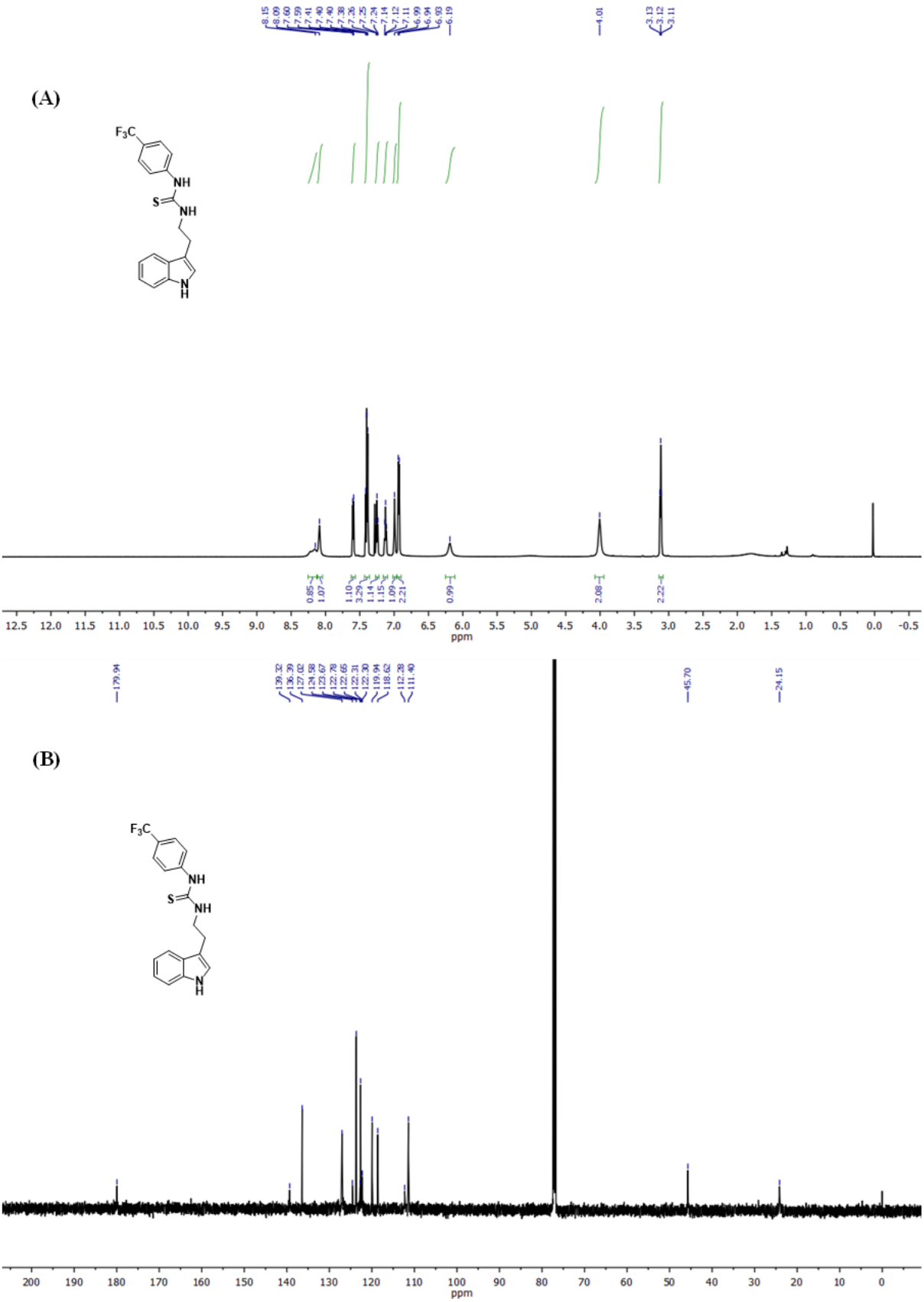


Fig. S12. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1*H*-indol-3-yl)ethyl)-3-(4-(trifluoromethyl)phenyl)thiourea (**1d**) in $CDCl_3$ solvent.

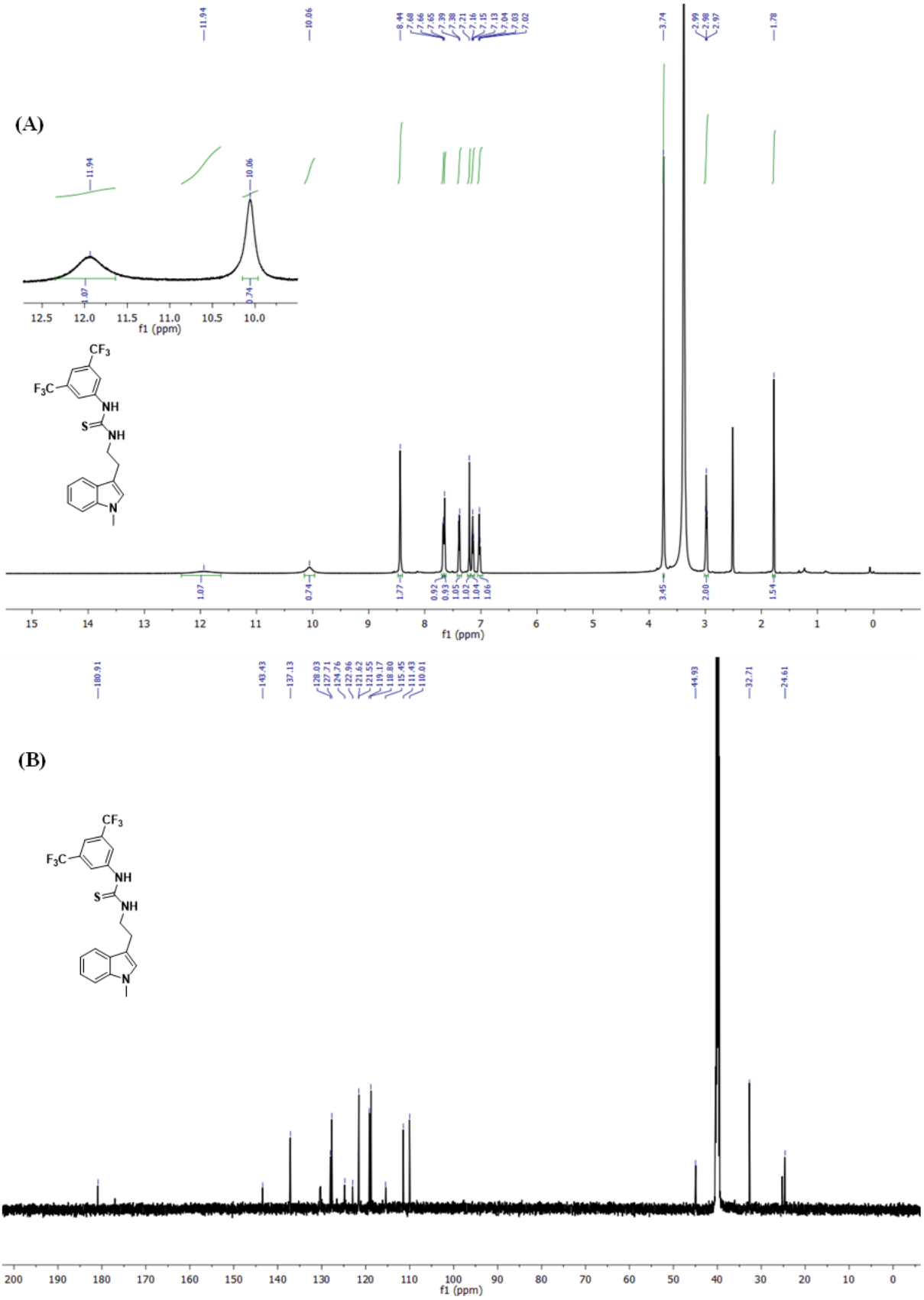


Fig. S13. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(3,5-bis(trifluoromethyl)phenyl)-3-(2-(1-methyl-1*H*-indol-3-yl)ethyl)thiourea (**2a**) in DMSO-d_6 solvent.

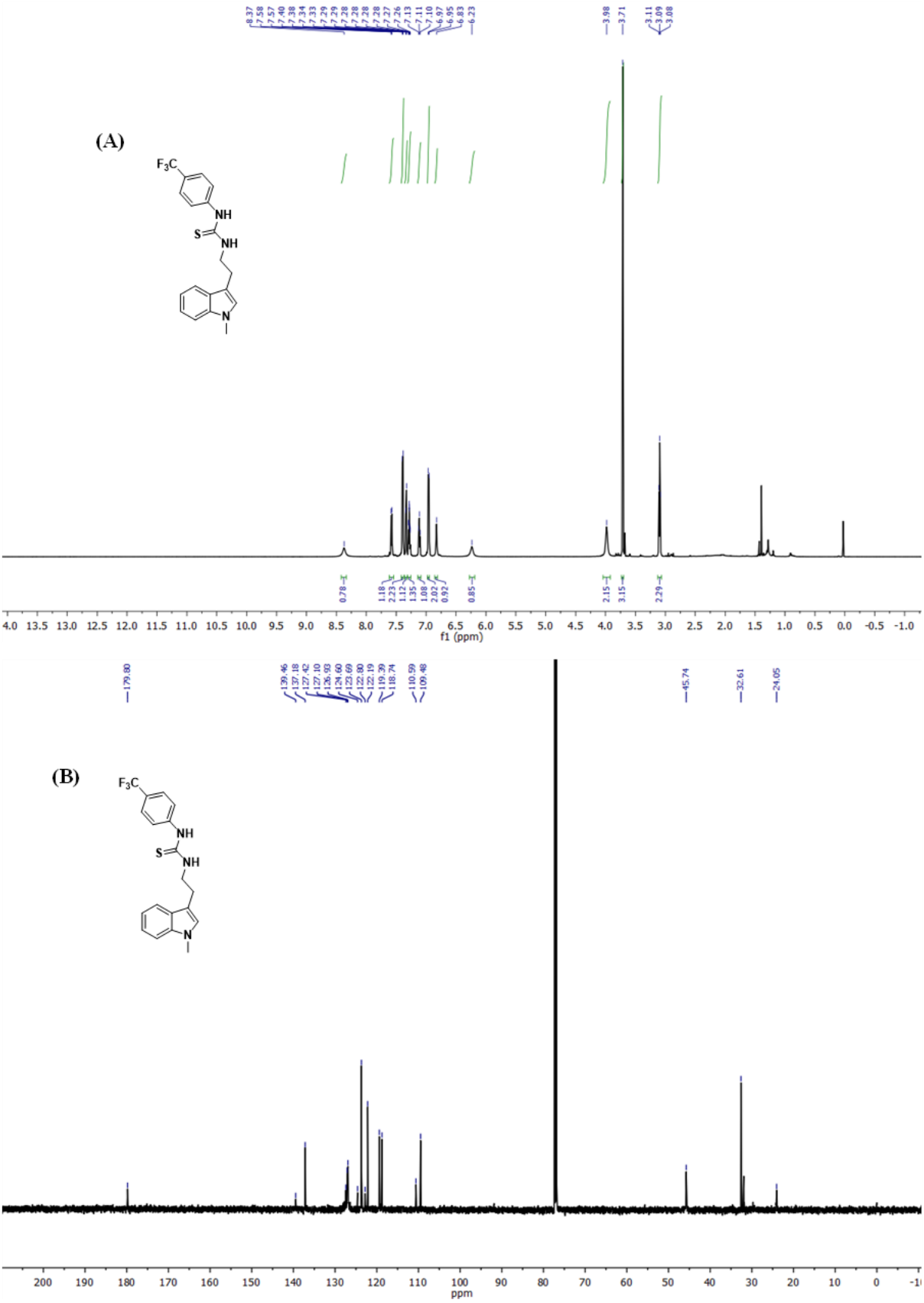


Fig. S14. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1-methyl-1*H*-indol-3-yl)ethyl)-3-(4-(trifluoromethyl)phenyl)thiourea (**2b**) in $CDCl_3$ solvent.

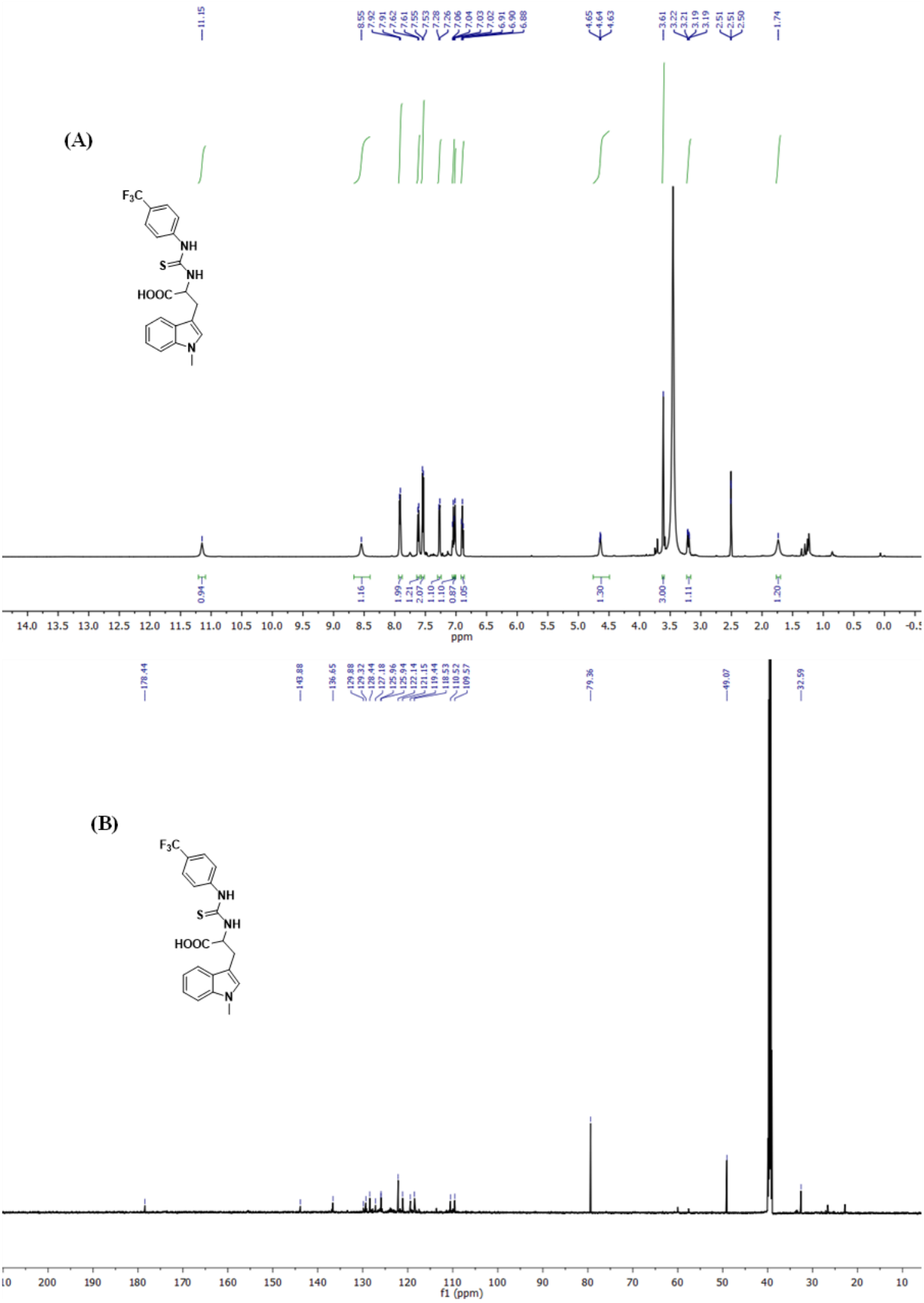


Fig. S15. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of ((4-(trifluoromethyl)phenyl)carbamothioyl)tryptophan (**2c**) in DMSO-d_6 solvent.

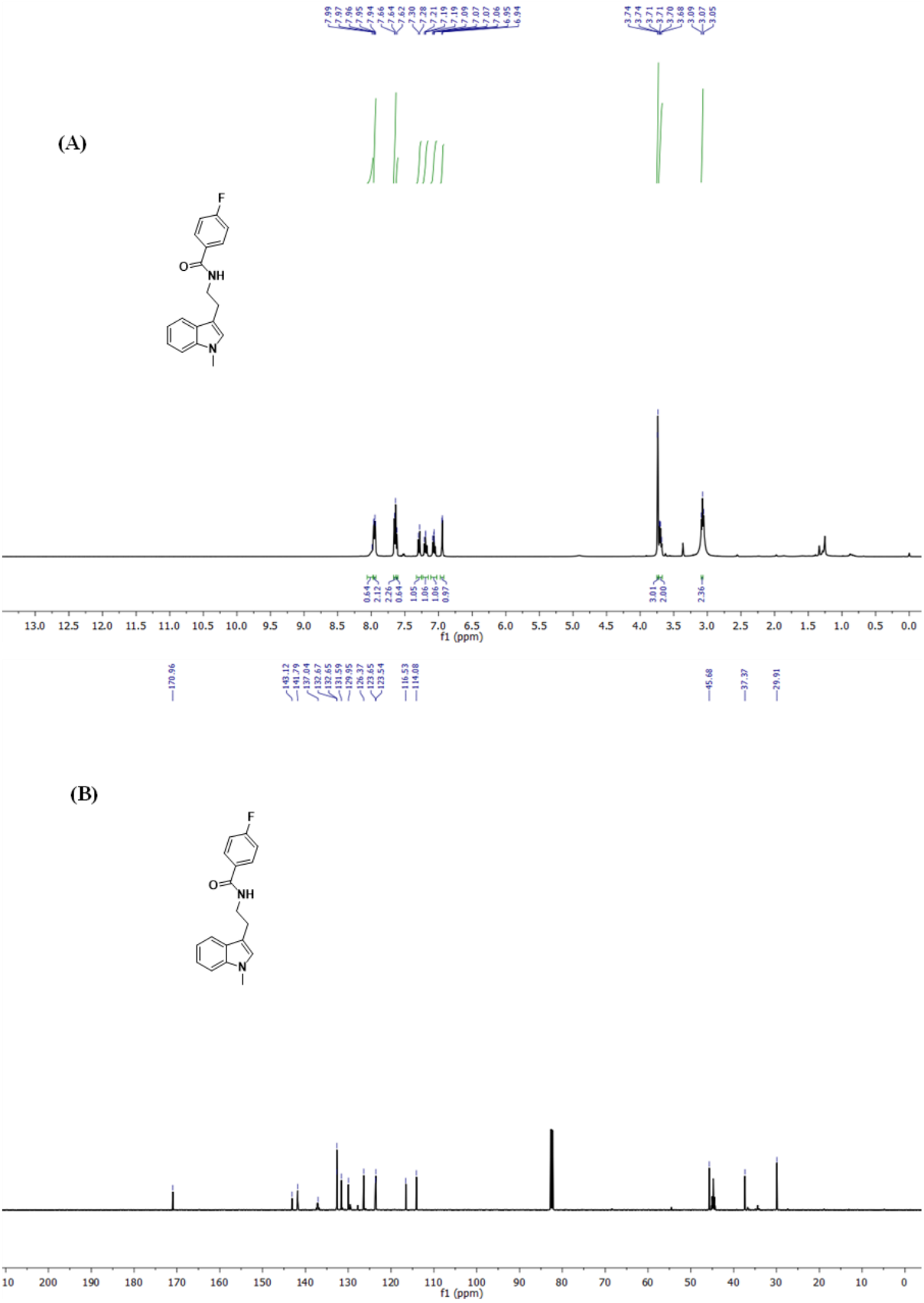


Fig. S16. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 4-fluoro-*N*-(2-(1-methyl-1*H*-indol-3-yl)ethyl)benzamide (**2d**) in $CDCl_3$ solvent.

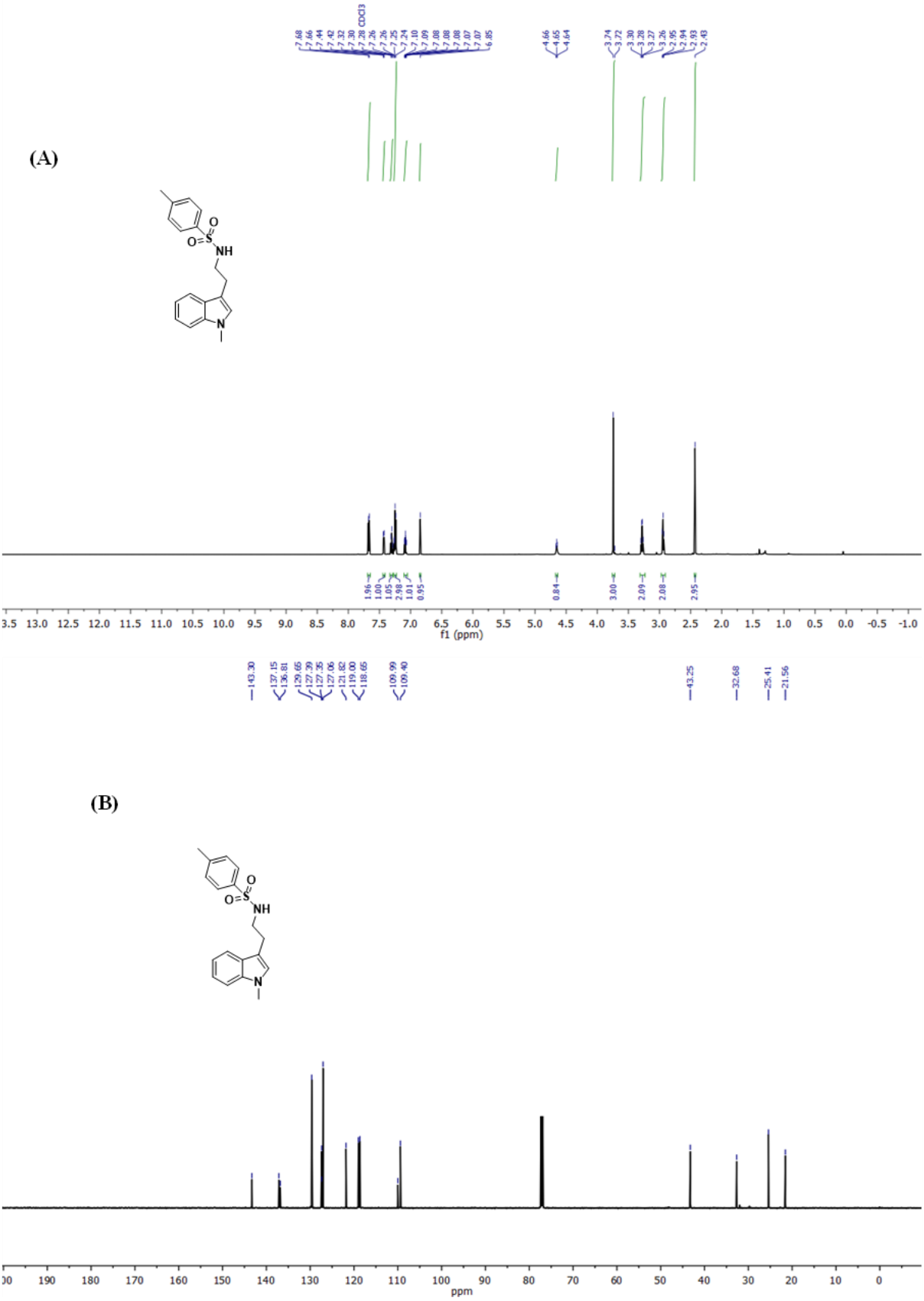
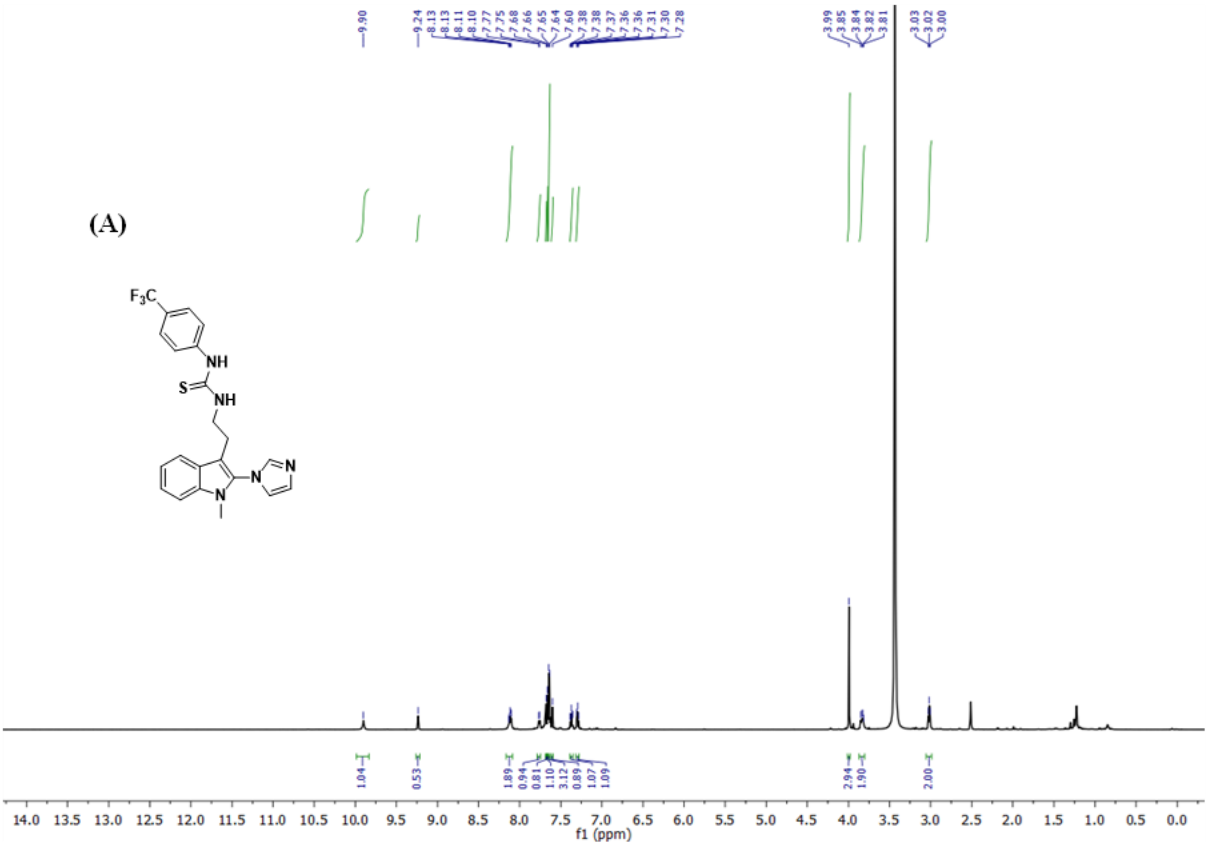
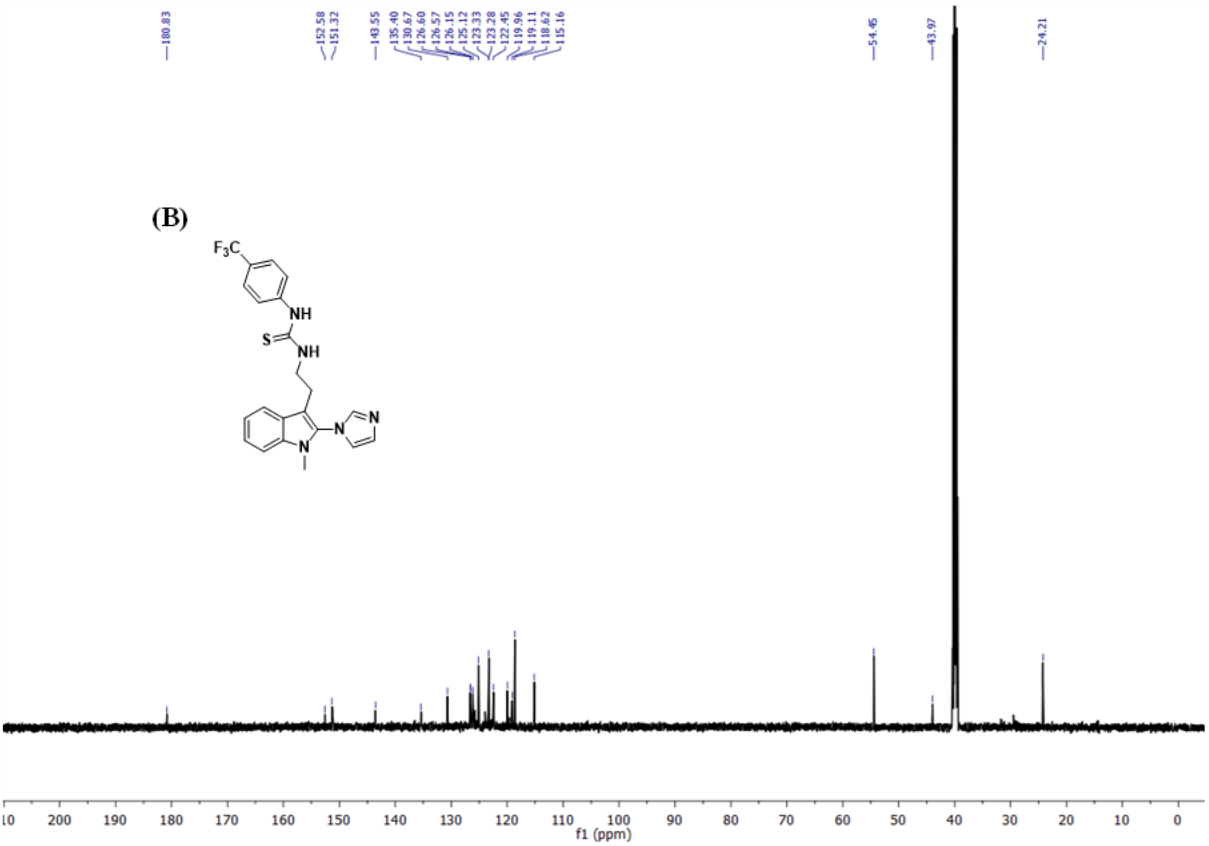


Fig. S17. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 4-methyl-*N*-(2-(1-methyl-1*H*-indol-3-yl)ethyl)benzenesulfonamide (**2e**) in $CDCl_3$ solvent.


(A)
F3C
NH
S
NH
N
N
9.90
9.24
8.13
8.13
8.11
8.10
7.77
7.75
7.68
7.66
7.65
7.64
7.60
7.38
7.38
7.37
7.36
7.36
7.31
7.30
7.28
3.99
3.85
3.84
3.82
3.81
3.03
3.02
3.00
1.04
0.53
1.89
0.94
0.81
1.10
3.12
0.89
1.07
1.09
2.94
1.90
2.00
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


(B)
F3C
NH
S
NH
N
N
180.83
152.58
151.32
143.55
135.40
130.67
126.60
126.57
126.15
125.12
123.33
123.28
122.45
119.96
119.11
118.62
115.16
54.46
43.97
24.21
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

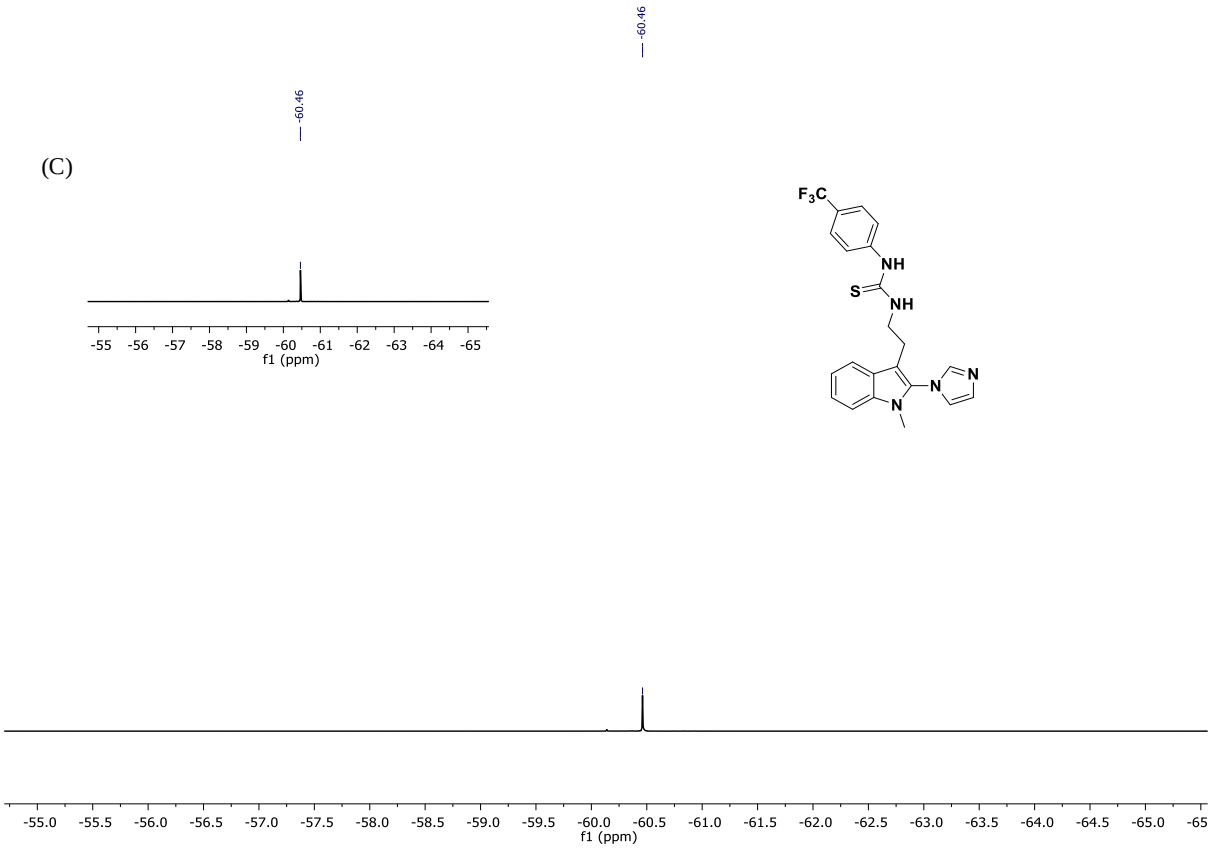


Fig. S18. (A) ^{1}H NMR and (B) ^{13}C NMR and (C) ^{19}F NMR spectra of 1-(2-(2-(1*H*-imidazol-1-yl)-1-methyl-1*H*-indol-3-yl)ethyl)-3-(4-(trifluoromethyl)phenyl)thiourea (**3a**) in DMSO-d_6 solvent.

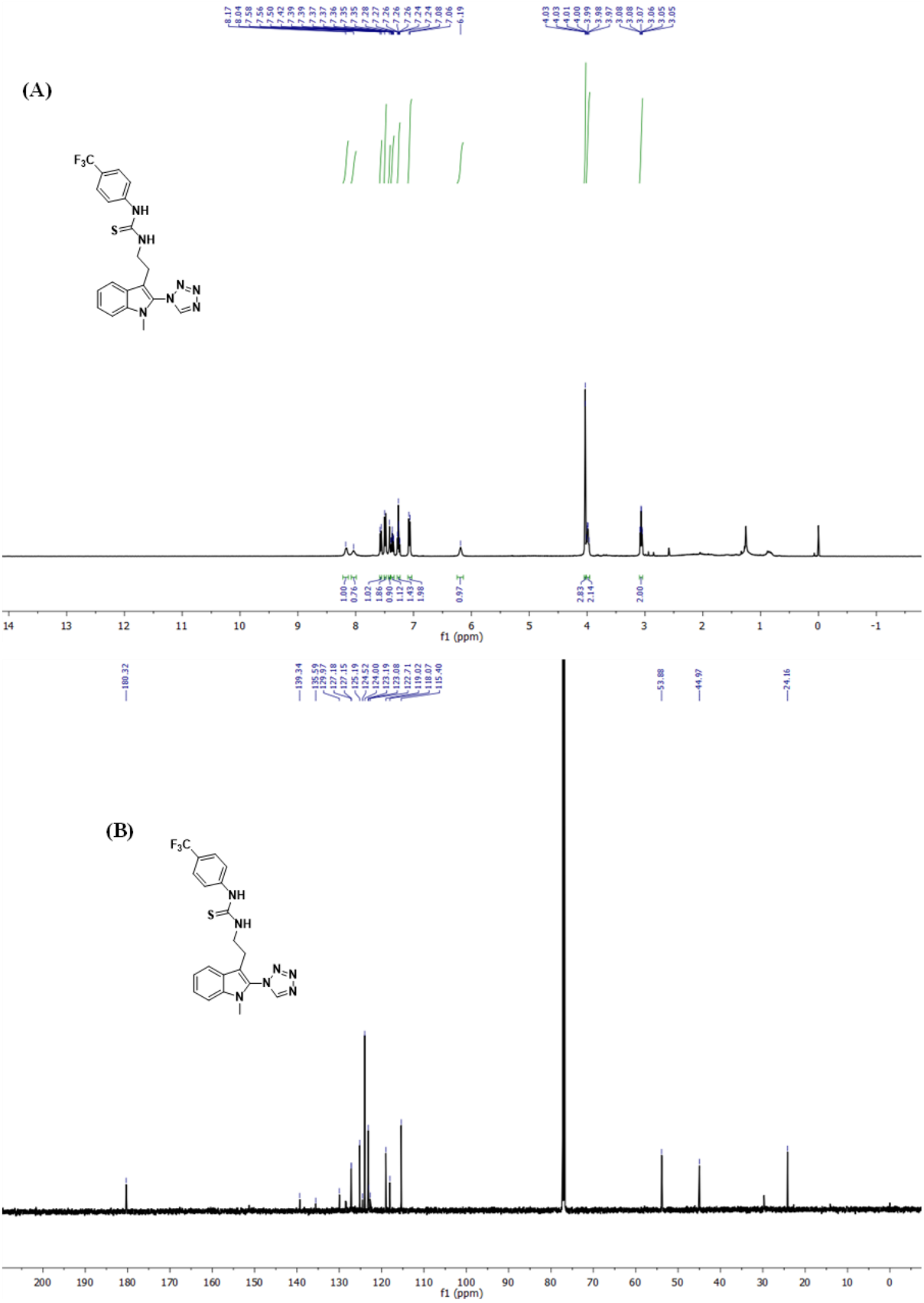
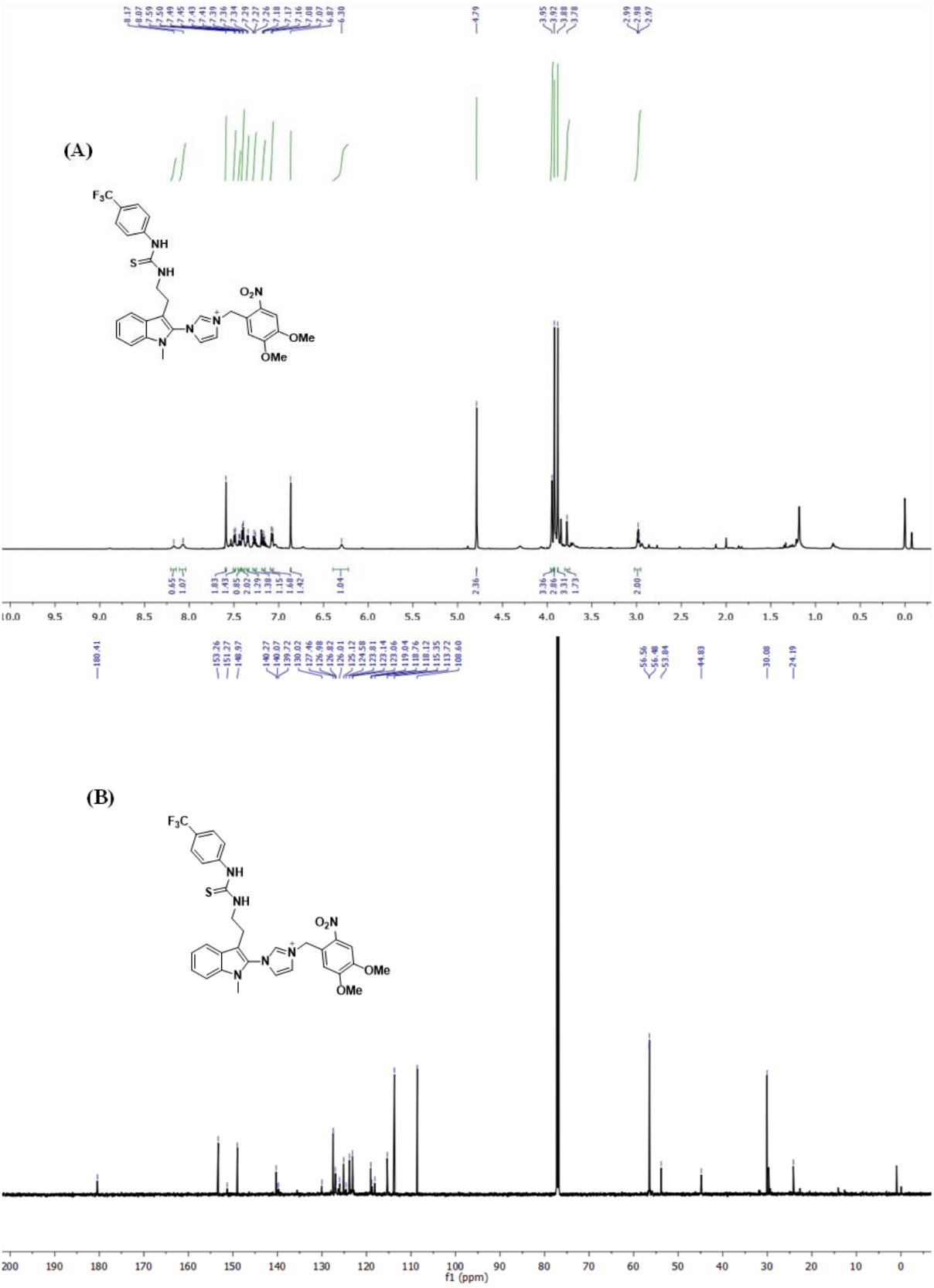


Fig. S19. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of 1-(2-(1-methyl-2-(1*H*-tetrazol-1-yl)-1*H*-indol-3-yl)ethyl)-3-(4-(trifluoromethyl)phenyl)thiourea (**3b**) in DMSO-d_6 solvent.


(A)
F3C
NH
S
NH
O2N
OMe
OMe
(B)
F3C
NH
S
NH
O2N
OMe
OMe
f1 (ppm)

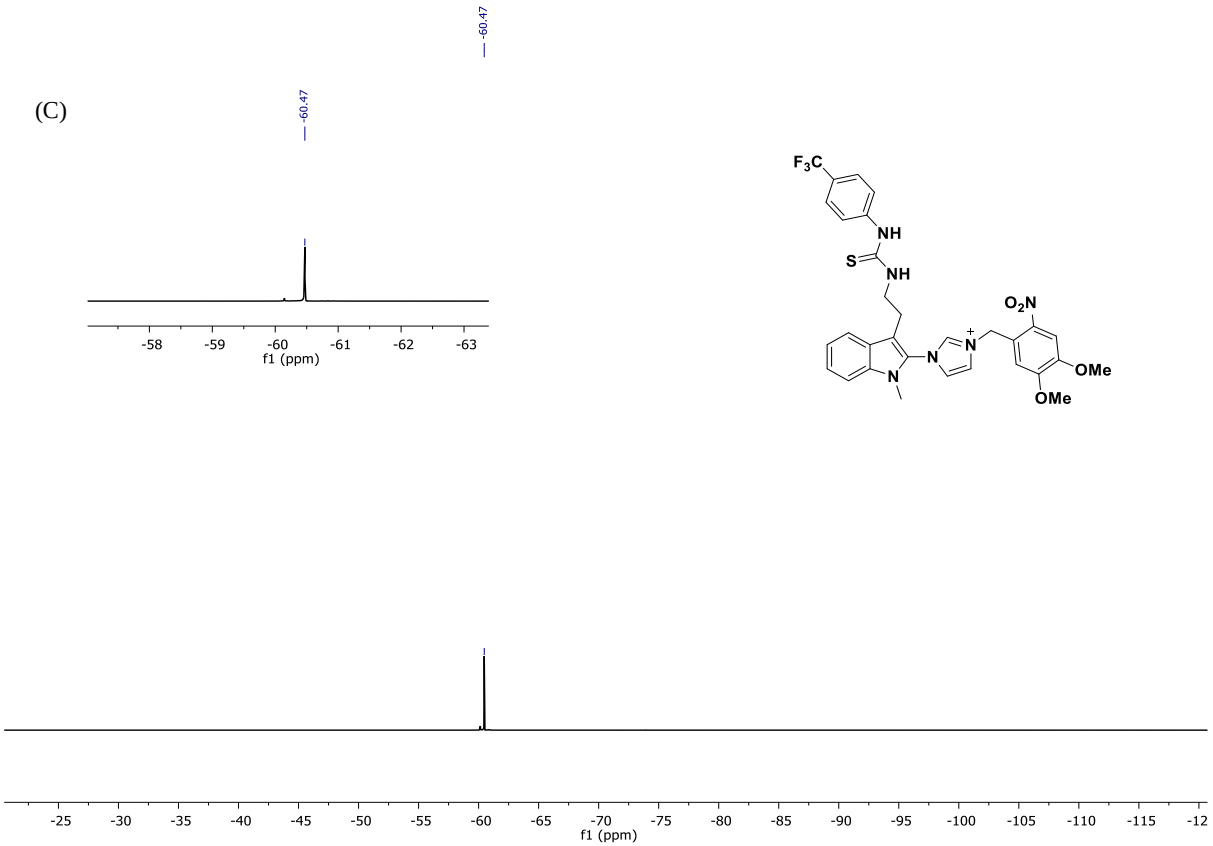


Fig. S20. (A) ^{1}H NMR and (B) ^{13}C NMR and (C) ^{19}F NMR spectra of 3-(4,5-dimethoxy-2-nitrobenzyl)-1-(1-methyl-3-(2-(3-(4-(trifluoromethyl)phenyl)thioureido)ethyl)-1*H*-indol-2-yl)-1*H*-imidazol-3-ium (**4**) in $CDCl_3$ solvent.