MICROWAVE-ASSISTED APPROACH TO NITROANILINE/AMINOPYRIDINE AMINE AND ITS INHIBITION ACTIVITY OF SEED GERMINATION

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1. General information

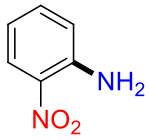
Solvents and reagents were commercially available and used without further purification. The concentration of water in the DMF was less than 50 ppm. Thin-layer chromatography(TLC) analysis was used to monitor reaction. Flash column chromatography was performed using silica gel(200-300 mesh). ^{1}H spectra were recorded in DMSO-d_6 or $CDCl_3$ on 600 or 400 MHz NMR spectrometers and resonances (•) are given in parts per million relative to tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (Hz) and integration. ^{13}C spectra were recorded in $CDCl_3$ or DMSO-d_6 on 100 or 150 MHz NMR spectrometers and resonances (•) are given in ppm. High resolution mass spectra (HRMS) were obtained by a TOF analyzer. Melting points were taken on a SGW X-4 melting point apparatus without correcting. The seed was cultivated in biochemical incubator SPX-250BSH. The version of microwave instruments was WX6000.

2. General procedure for the synthesis of compounds 3

A mixture of halogenated nitrobenzene/pyridine (**1**, 1.0 mmol), sulphamide (**2**, 1.3 mmol) and K_2CO_3 (2.5 mmol) were dissolved in DMF(6.0 mL) in a microwave tube. Then, the reaction mixture was irradiated in a microwave apparatus at 130 ℃ for 15 minutes. After the reaction mixture was cooled to ambient temperature, the product was concentrated, and the crude mixture was purified by column chromatography on silica gel to the desired products **3**.

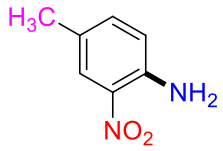
3. Analytical data of the products

2-nitroaniline (3a) [1,2]



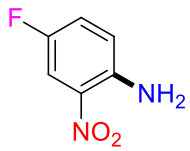
Yellow solid; mp 70-71 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (dd, J = 8.5, 1.5 Hz, 1H), 7.36 (ddd, J = 8.5, 7.0, 1.5 Hz, 1H), 6.81 (dd, J = 8.5, 1.5 Hz, 1H), 6.70 (ddd, J = 8.5, 4.5, 1.0 Hz, 1H), 6.07 (br, 2H).

4-methyl-2-nitroaniline (3b) [3]



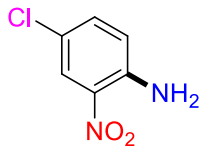
Yellow solid; mp 114-115 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.0, 1.0 Hz, 1H), 7.25 – 7.12 (m, 1H), 6.73 (d, J = 8.5 Hz, 1H), 5.93 (br, 2H), 2.27 (s, 3H).

4-fluoro-2-nitroaniline (3c) [4]



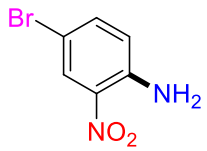
Yellow solid; mp 105-106 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (ddd, J = 9.0, 3.0, 0.5 Hz, 1H), 7.18 (ddd, J = 9.0, 7.0, 3.0 Hz, 1H), 6.81 (ddd, J = 9.0, 4.5, 0.5 Hz, 1H), 6.01 (br, 2H).

4-chloro-2-nitroaniline (3d) [4]



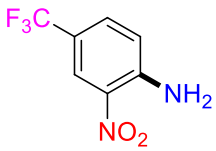
Yellow solid; mp 122-123 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 2.5 Hz, 1H), 7.32 (dd, J = 9.0, 2.5 Hz, 1H), 6.78 (d, J = 9.0 Hz, 1H), 6.09 (br, 2H).

4-bromo-2-nitroaniline (3e) [2]



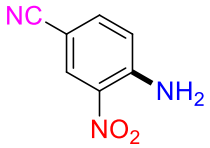
Yellow solid; mp 119-120 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 2.5 Hz, 1H), 7.43 (dd, J = 9.0, 2.5 Hz, 1H), 6.73 (d, J = 9.0 Hz, 1H), 6.10 (br, 2H).

2-nitro-4-(trifluoromethyl)aniline (3f) [3]



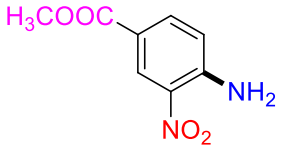
Yellow solid; mp 115-116 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, J = 1.0 Hz, 1H), 7.59-7.54 (m, 1H), 6.91 (d, J = 9.0 Hz, 1H), 6.37 (br, 2H).

4-amino-3-nitrobenzonitrile (3g) [5]



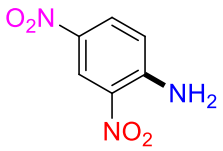
Yellow solid; mp 112-113 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (d, J = 2.0 Hz, 1H), 8.00 (br, 2H), 7.69 (dd, J = 9.0, 2.0 Hz, 1H), 7.13-7.07 (m, 1H).

methyl 4-amino-3-nitrobenzoate (3h) [2]



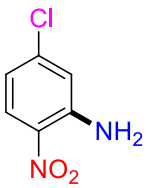
Yellow solid; mp 195-196 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (d, J = 2.0 Hz, 1H), 8.00 (br, 2H), 7.86 (dd, J = 9.0, 2.0 Hz, 1H), 7.06 (d, J = 9.0 Hz, 1H), 3.81 (s, 3H).

2,4-dinitroaniline (3i) [6]



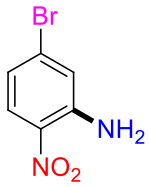
Yellow solid; mp 169-170 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (d, J = 2.5 Hz, 1H), 8.40 (br, 2H), 8.20-8.15 (m, 1H), 7.12 (d, J = 9.5 Hz, 1H).

5-chloro-2-nitroaniline (3j) [2]



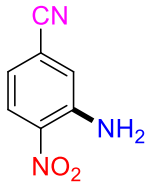
Yellow solid; mp 119-120 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, J = 9.5 Hz, 1H), 6.83 (t, J = 2.0 Hz, 1H), 6.67 (dd, J = 9.0, 2.0 Hz, 1H), 6.12 (br, 1H).

5-bromo-2-nitroaniline (3k) [2]



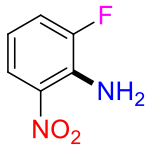
Yellow solid; mp 108-109 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 2.5 Hz, 1H), 7.43 (dd, J = 9.0, 2.5 Hz, 1H), 6.73 (d, J = 9.0 Hz, 1H), 6.08 (br, 1H).

3-amino-4-nitrobenzonitrile (3l) [3]



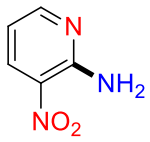
Yellow solid; mp 79-80 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, J = 9.0 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 6.92 (dd, J = 9.0, 2.0 Hz, 1H), 6.43 (br, 1H).

2-fluoro-6-nitroaniline (3m) [2]



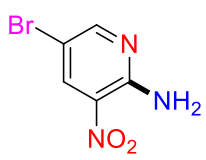
Yellow solid; mp 77-78 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dt, J = 9.0, 1.5 Hz, 1H), 7.23 (ddd, J = 10.5, 8.0, 1.5 Hz, 1H), 6.64 (ddd, J = 9.0, 8.0, 5.5 Hz, 1H), 6.11 (br, 2H).

3-nitropyridin-2-amine (3n) [6,7]



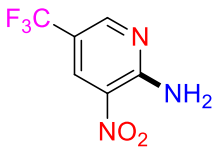
Yellow solid; mp 156-157 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 (m, 2H), 7.92 (br, 2H), 6.76 (dd, J = 8.5, 4.5 Hz, 1H).

5-bromo-3-nitropyridin-2-amine (3o) [7]



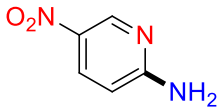
Yellow solid; mp 210-211 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (d, J = 2.5 Hz, 1H), 8.49 (d, J = 2.5 Hz, 1H), 8.09 (br, 2H).

3-nitro-5-(trifluoromethyl)pyridin-2-amine (3p) [1]



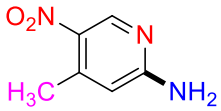
Yellow solid; mp 181-182 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 2.0 Hz), 8.60 (d, J = 2.0 Hz), 8.52-7.92 (br, 1H).

5-nitropyridin-2-amine (3q) [8]



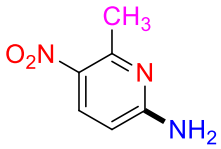
Yellow solid; mp 200-201 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.56 (br, 2H), 6.51 (d, *J* = 9.0 Hz, 1H).

4-methyl-5-nitropyridin-2-amine (3r) [8]



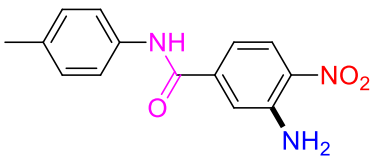
Yellow solid; mp 215-216 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (s, 1H), 7.31 (br, 2H), 6.32 (s, 1H), 2.46 (s, 3H).

6-methyl-5-nitropyridin-2-amine (3s) [1]



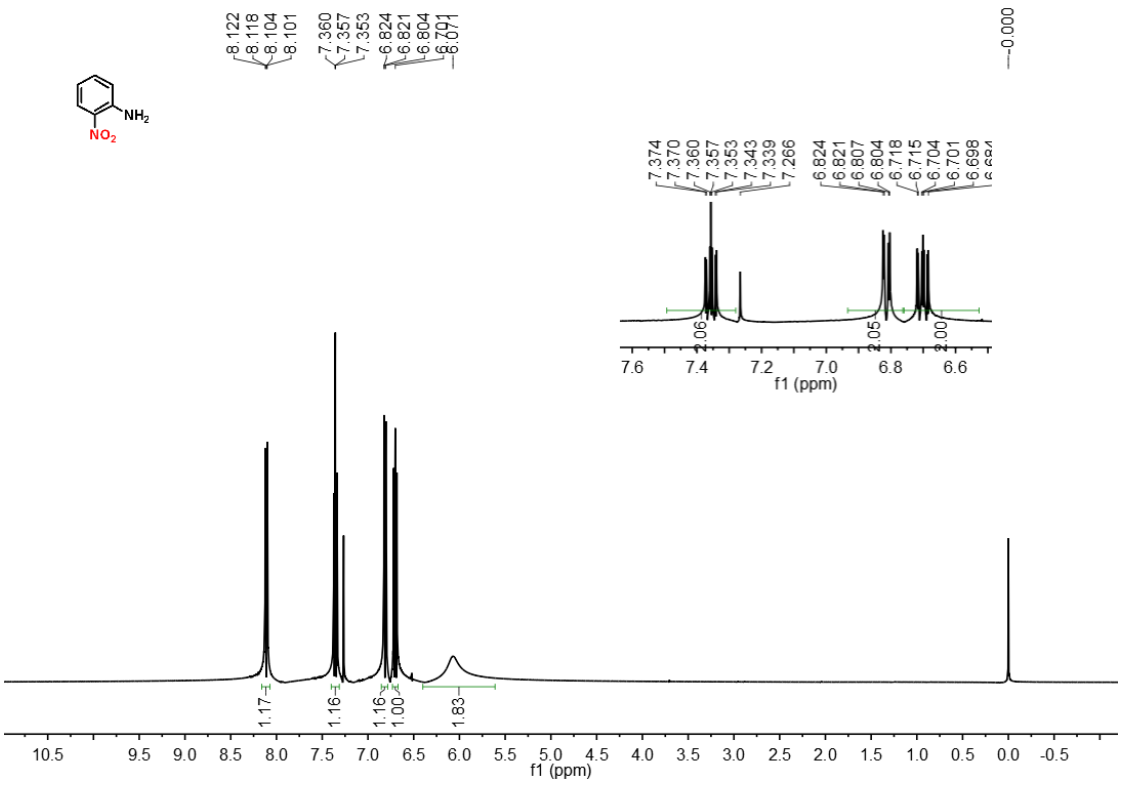
Yellow solid; mp 195-196 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ8.10 (d, *J* = 9.0 Hz, 1H), 7.37 (br, 2H), 6.38 (d, *J* = 9.0 Hz, 1H), 2.61 (s, 3H).

3-amino-4-nitro-*N*-(*p*-tolyl)benzamide **(3bb)**

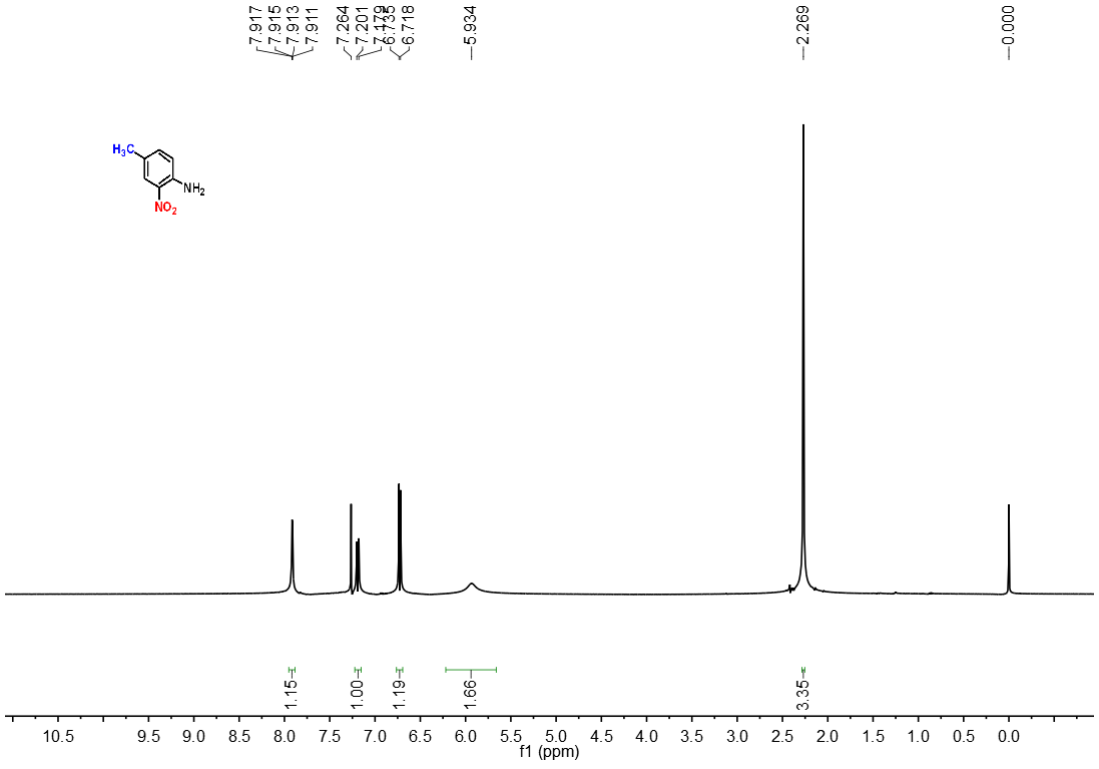


Yellow solid; mp 230-231 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.58 (br, 2H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.07 (dd, *J* = 9.0, 2.0 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.31, 145.80, 141.51, 136.26, 133.03, 131.21, 129.09, 125.83, 120.35, 119.12, 113.77,20.52. HRMS (ESI): m/z $[M+H]^+$ calcd for, $C_{14}H_{14}N_3O_3$: 272.1035 ; found: 272.1019. IR (cm^{-1}): 3550, 3496, 3417, 3381, 1647, 1630, 1596, 1531, 1489, 1403, 1332, 1249, 1171, 1066, 1019, 961, 883, 843, 806, 736, 703, 689, 664.

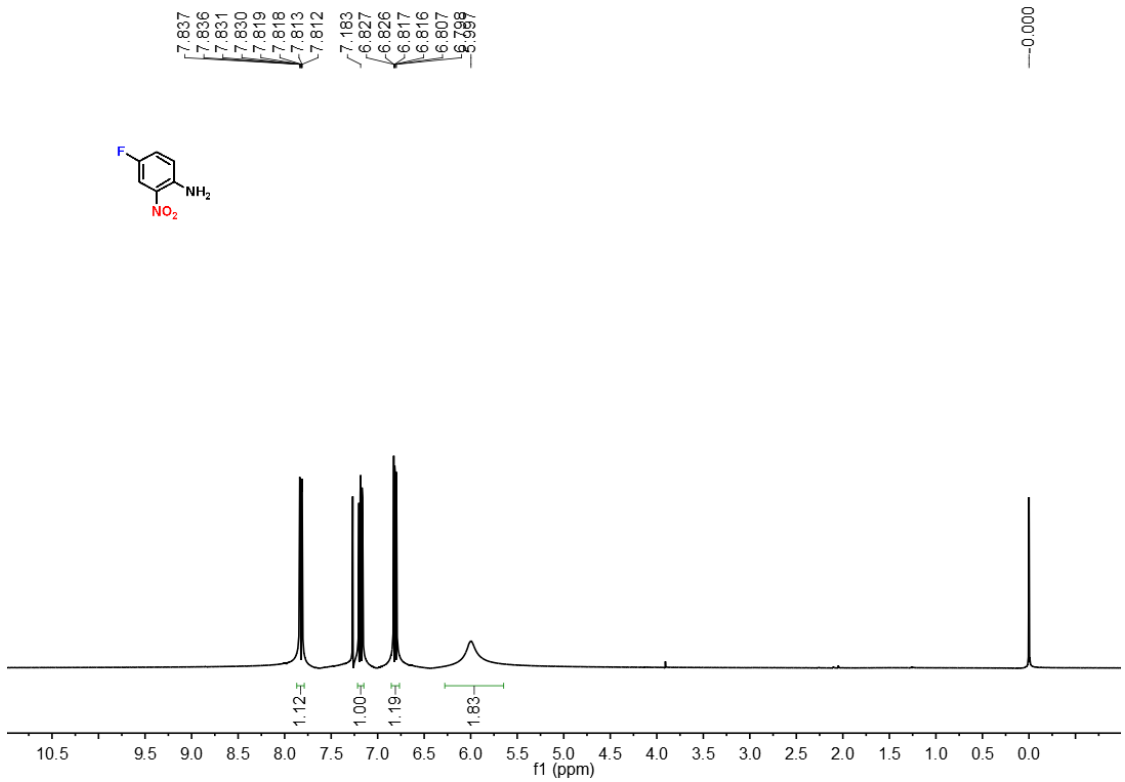
4. Copies of NMR Spectra



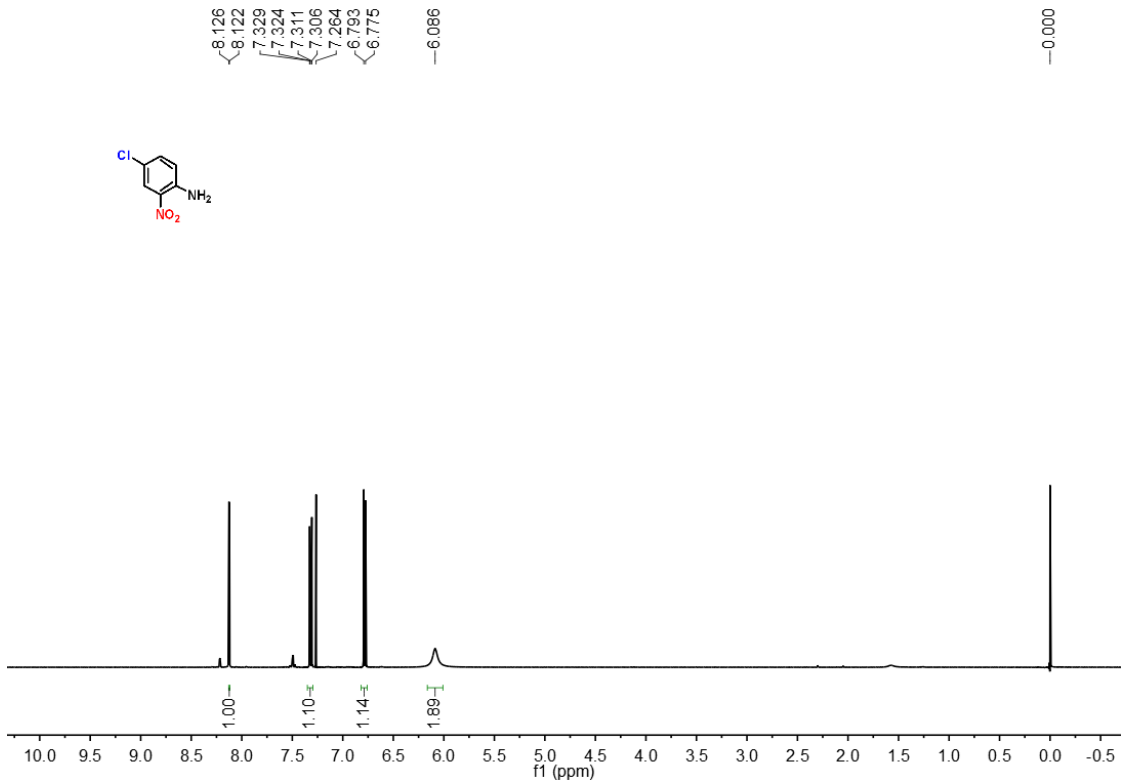
3**a**),[1]HNMR



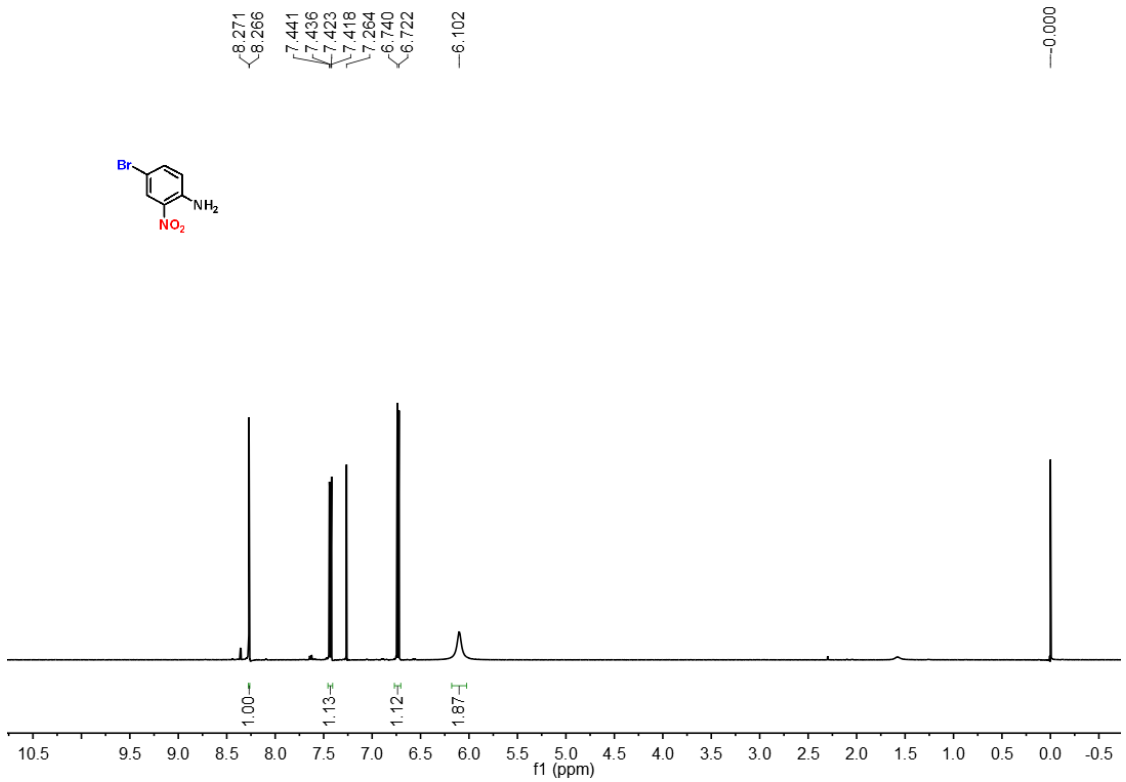
3**b**), [1]H NMR



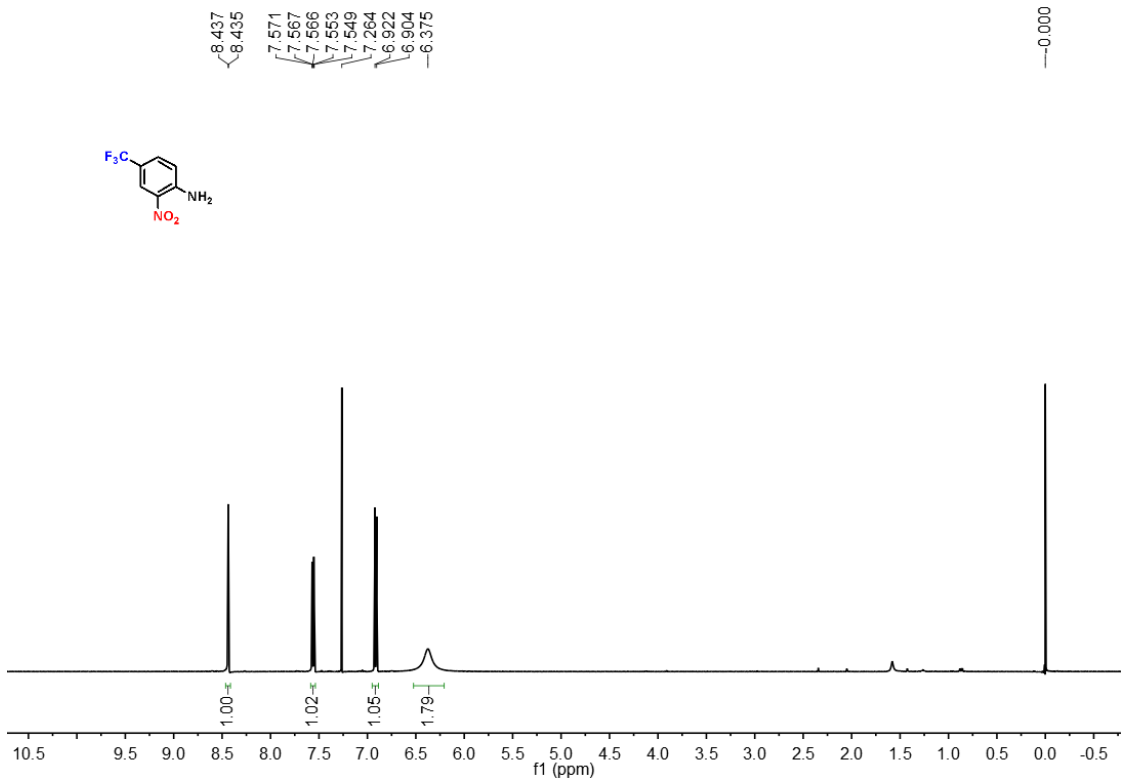
3**c**), ^{1}H NMR



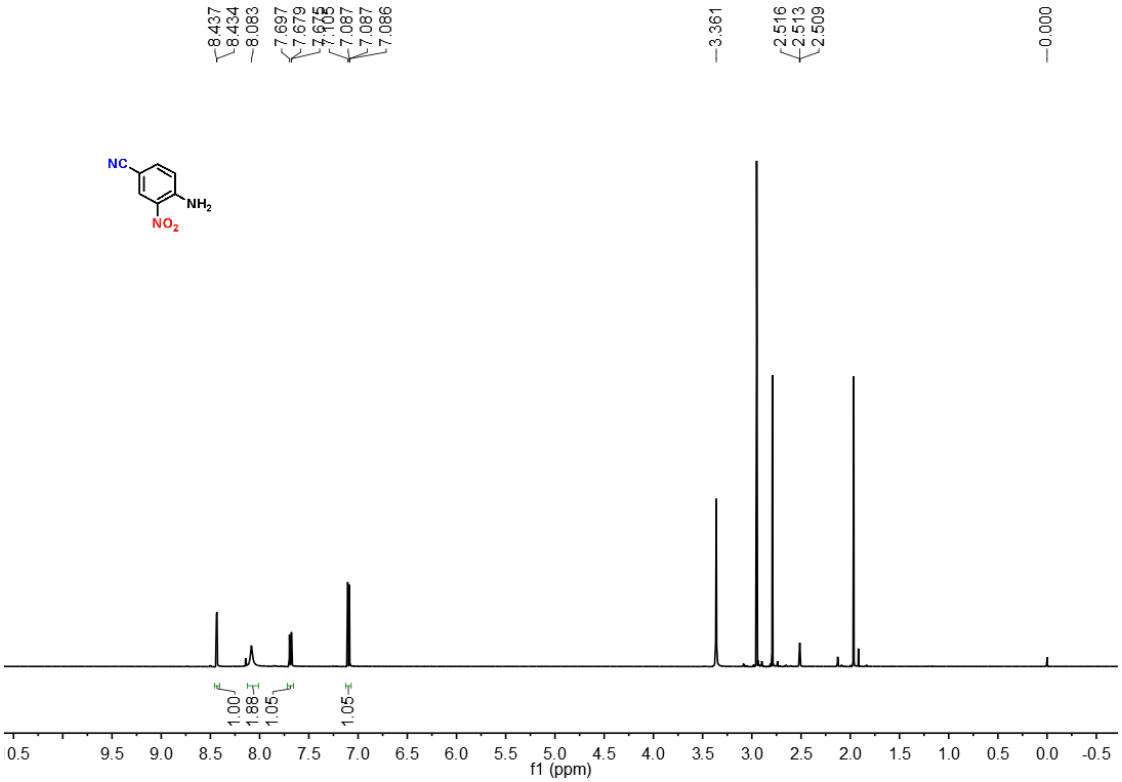
3**d**), ^{1}H NMR



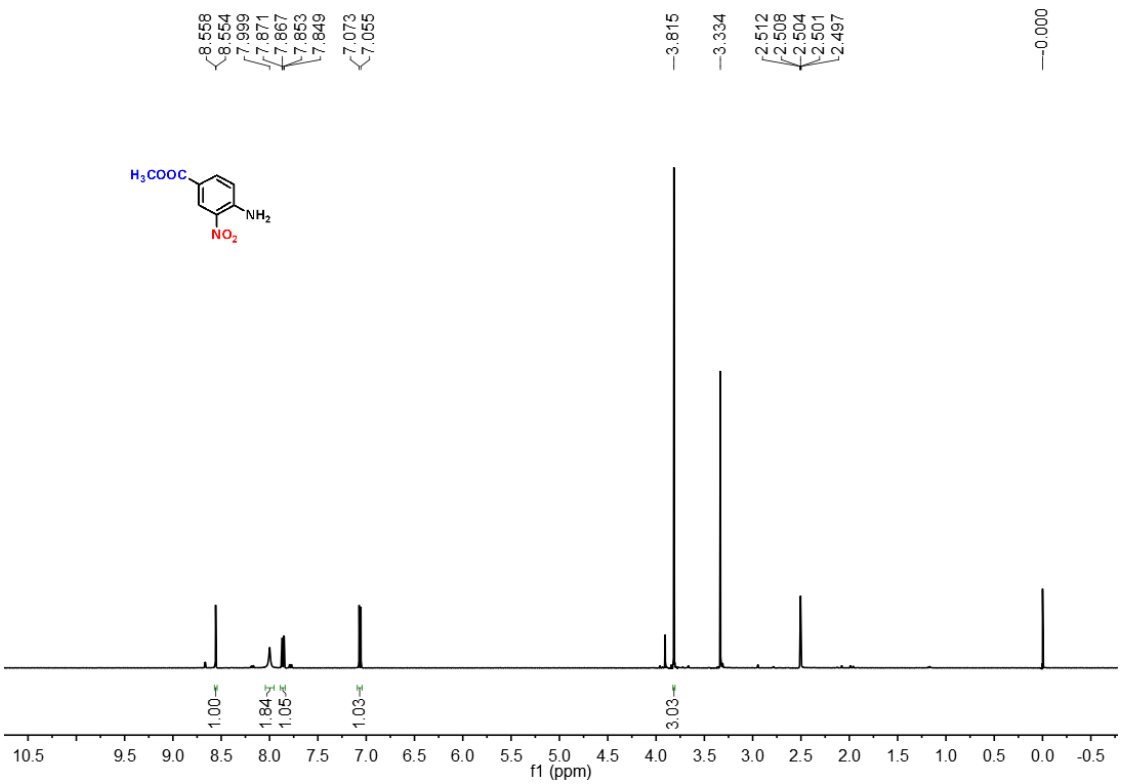
3**e**), ^{1}H NMR



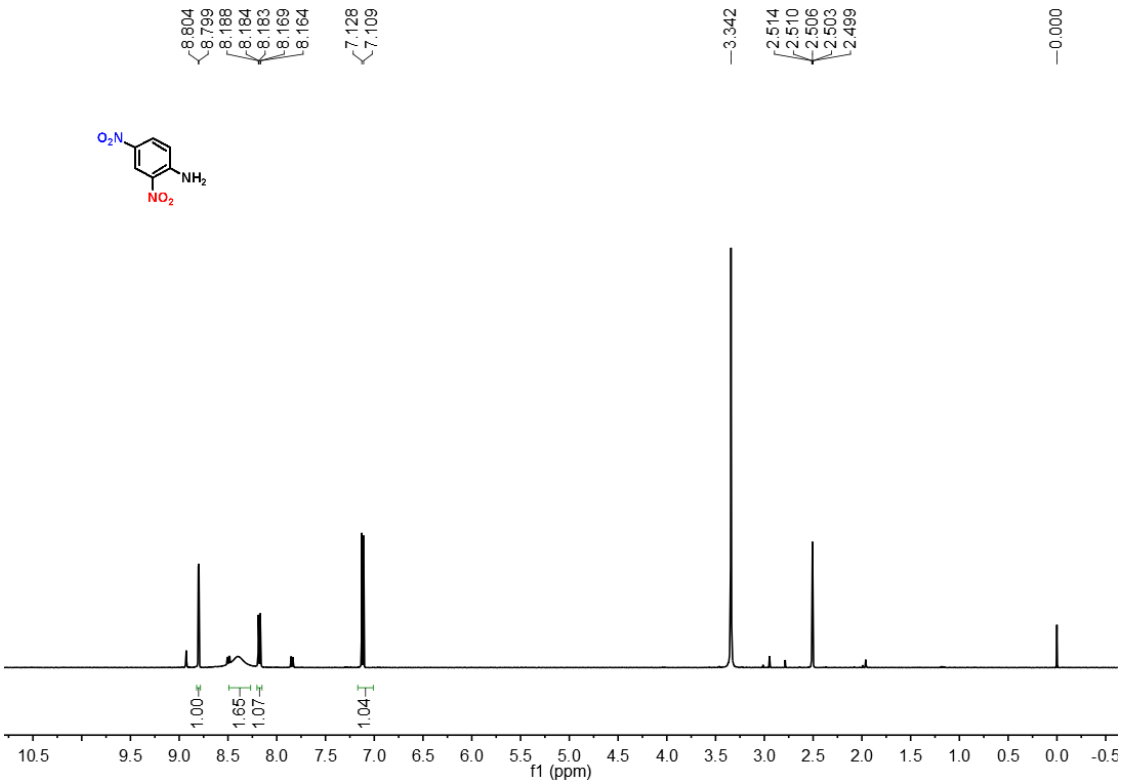
3**f**), ^{1}H NMR



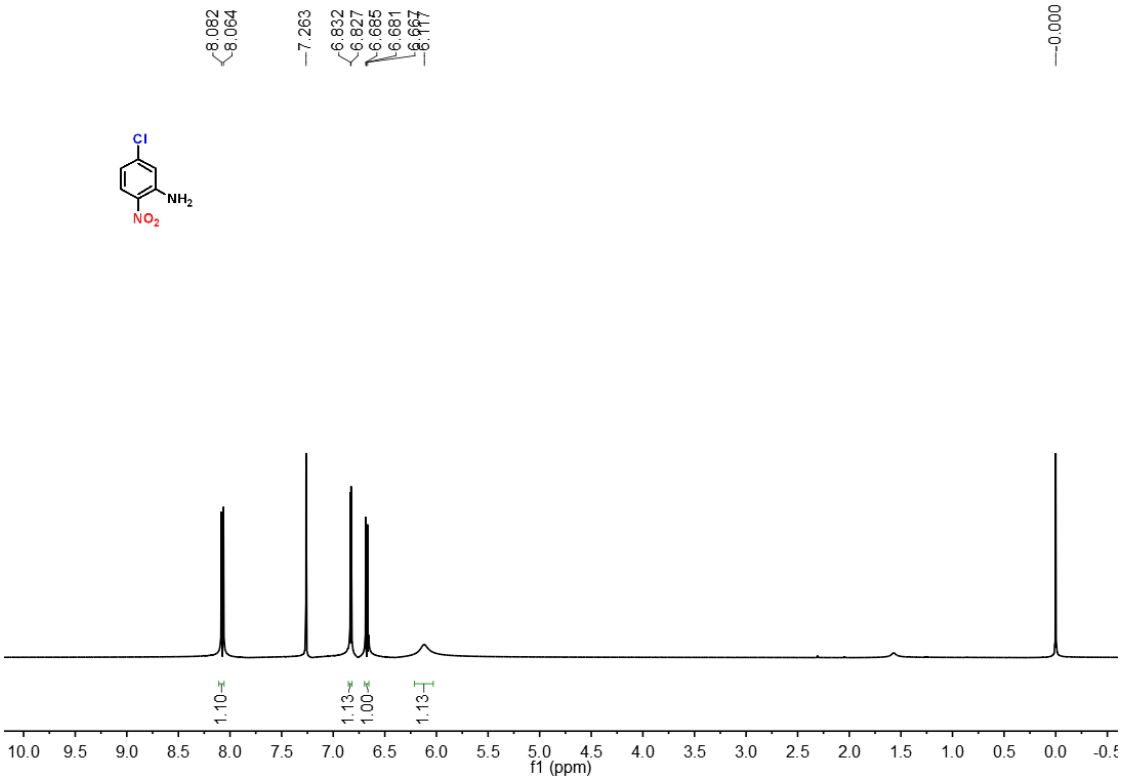
3**g**), ^{1}H NMR



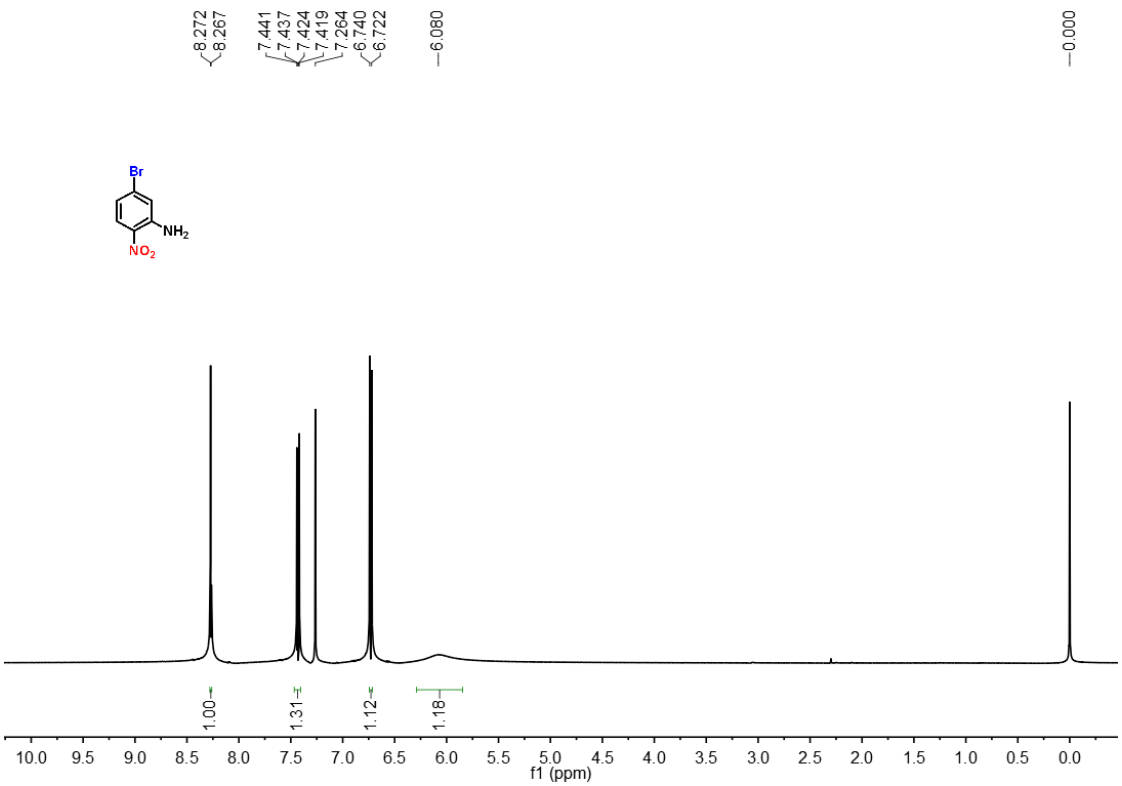
3**h**), ^{1}H NMR



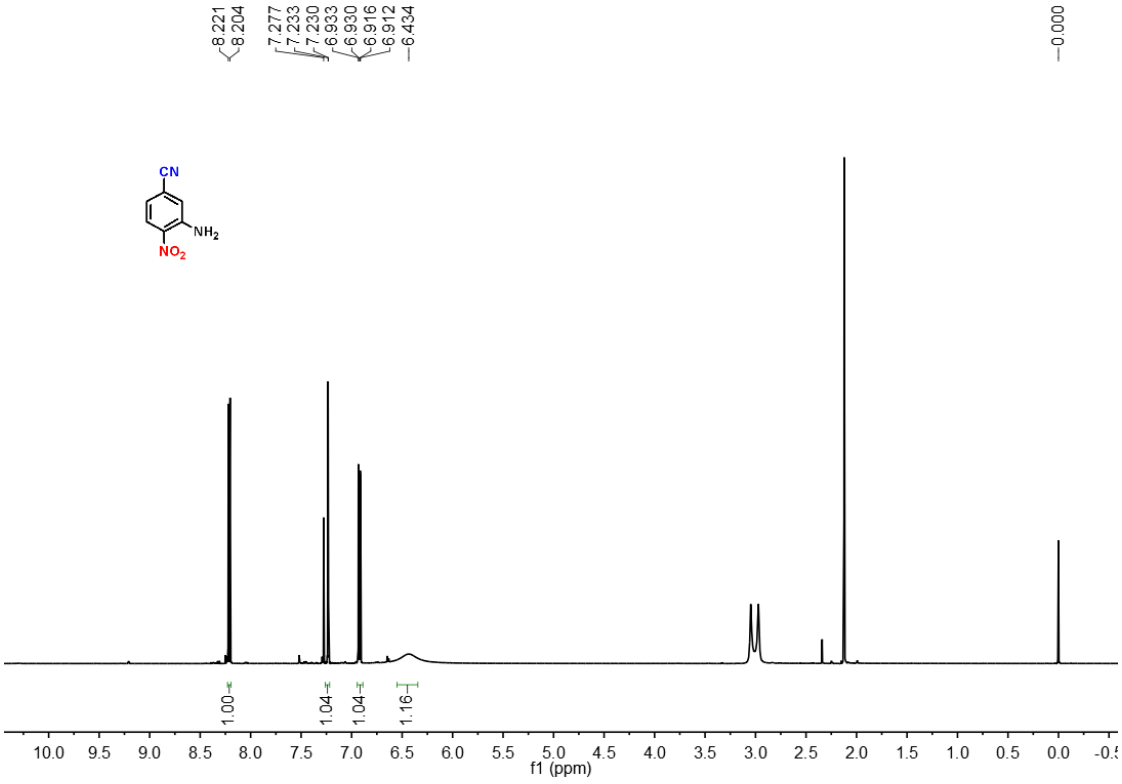
3**i**), ^{1}H NMR



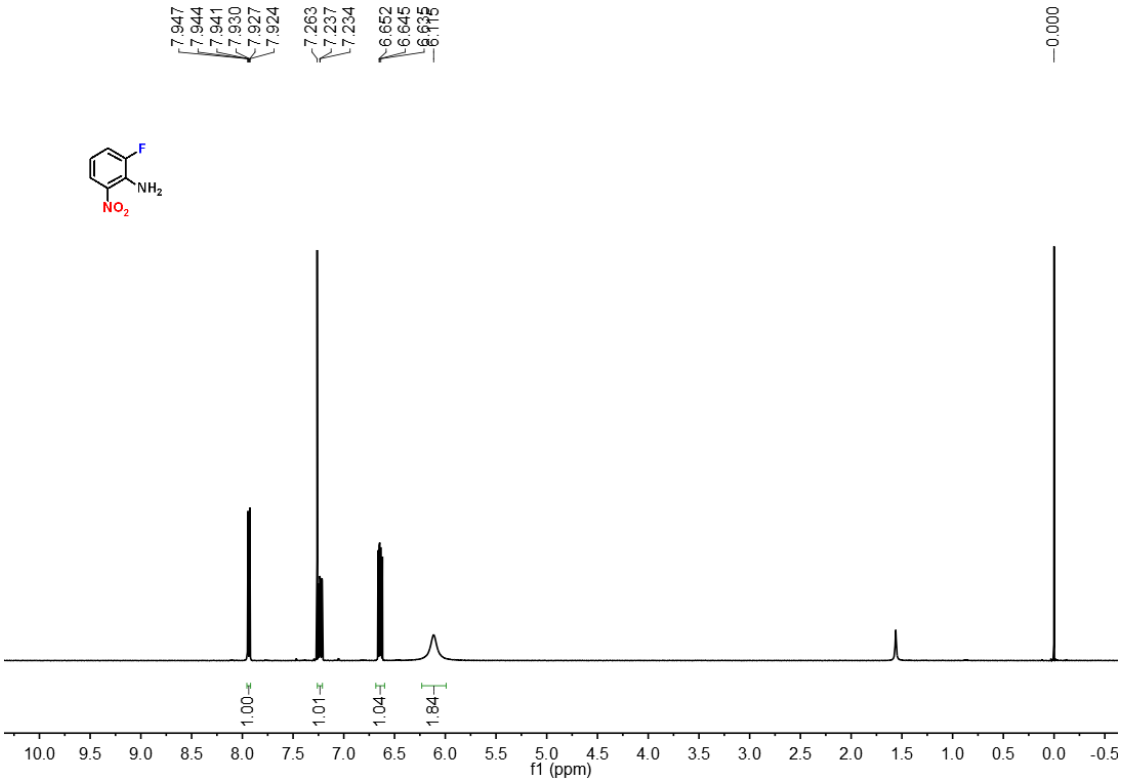
3**j**), ^{1}H NMR



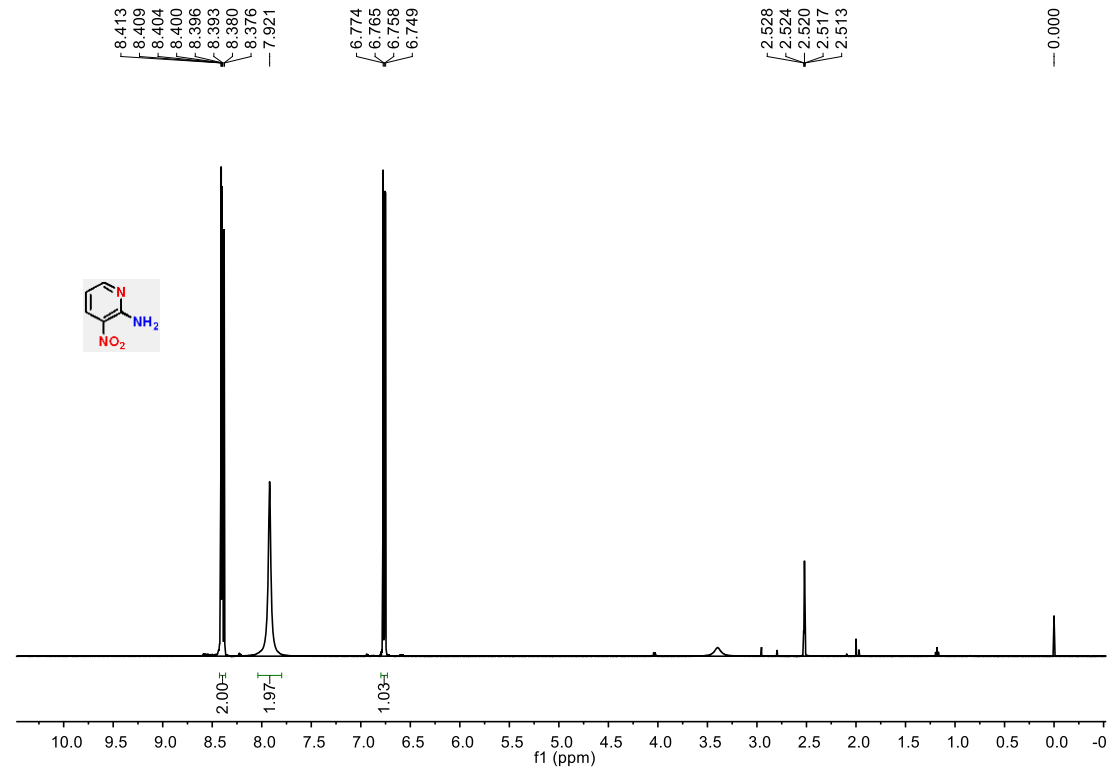
3**k**), ^{1}H NMR



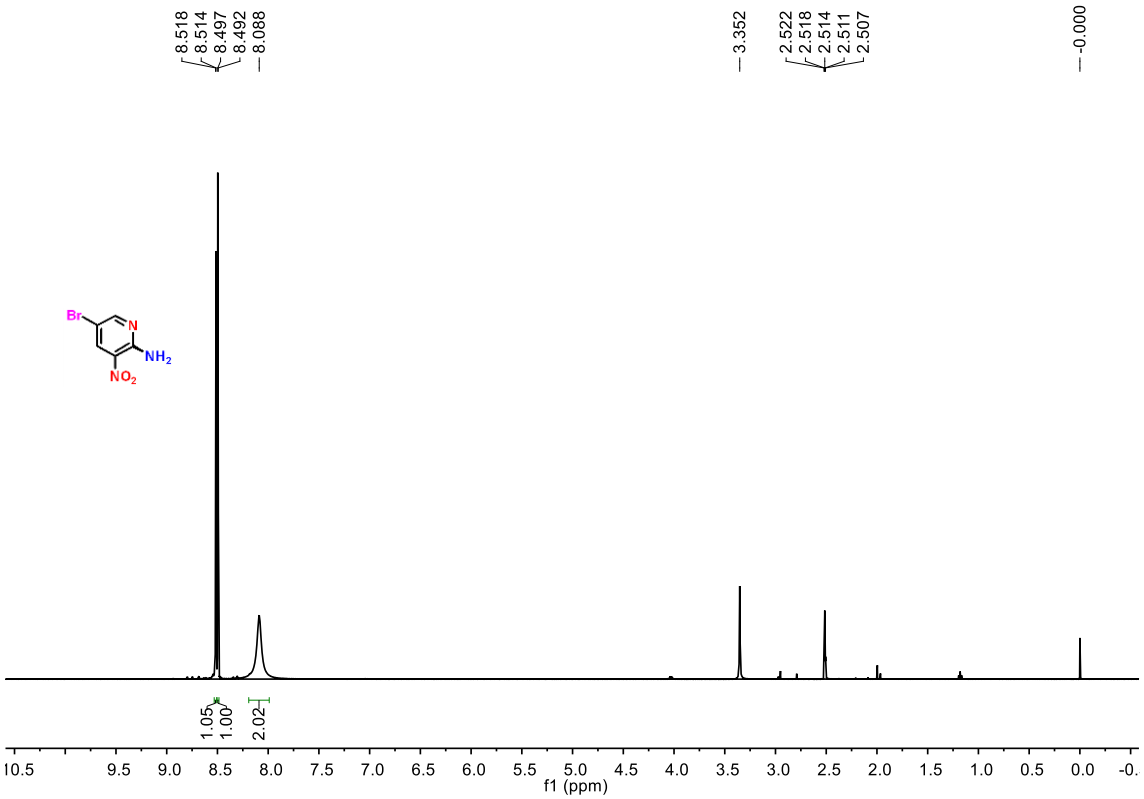
3**l**), ^{1}H NMR



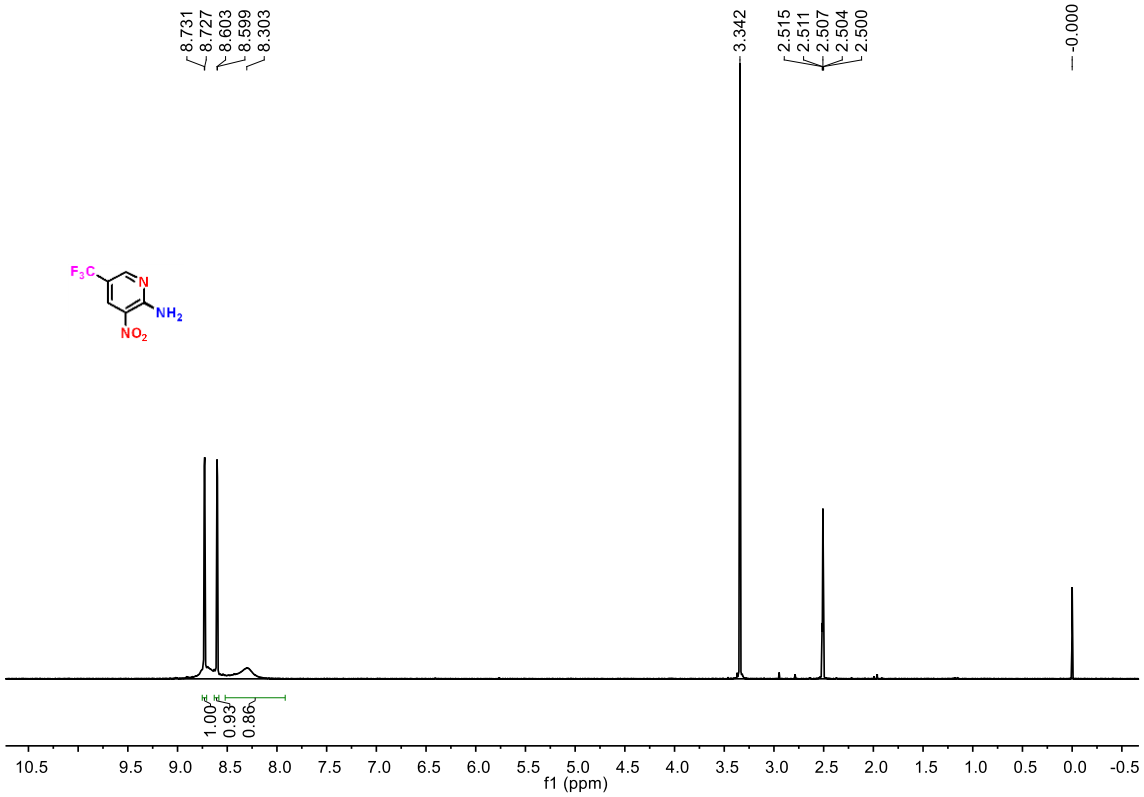
3**m**), ^{1}H NMR



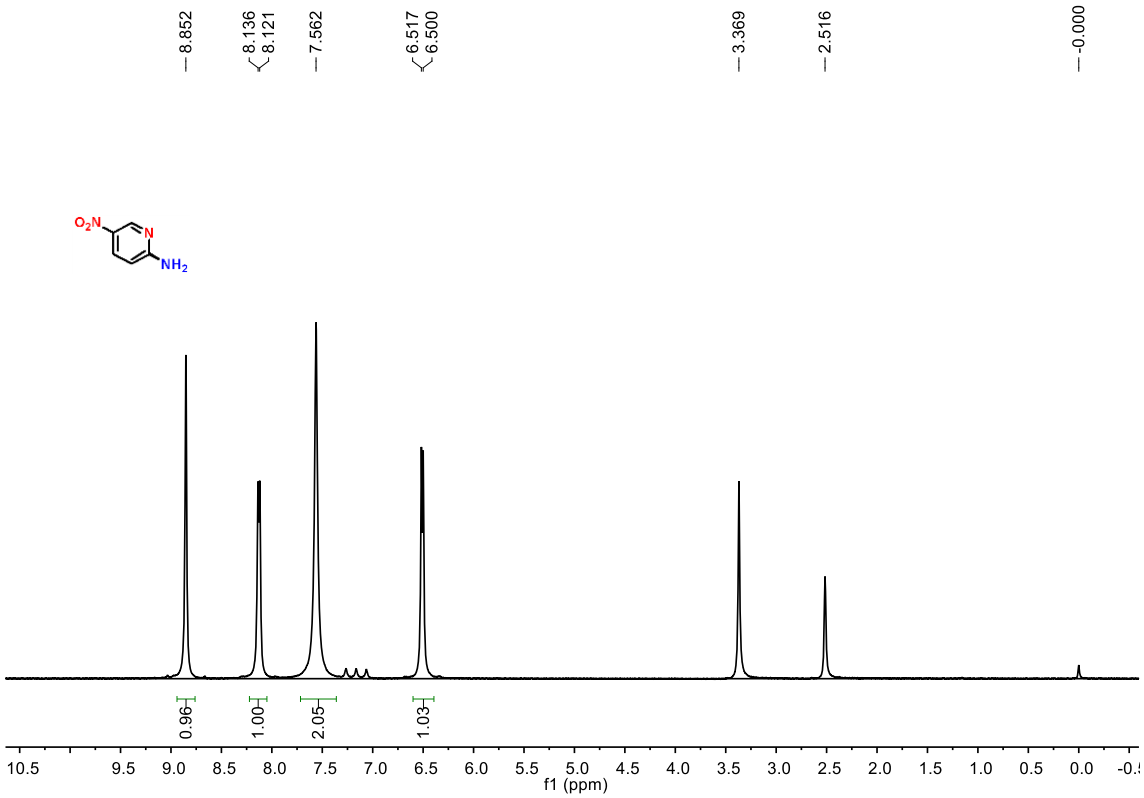
3n) , ^{1}H NMR



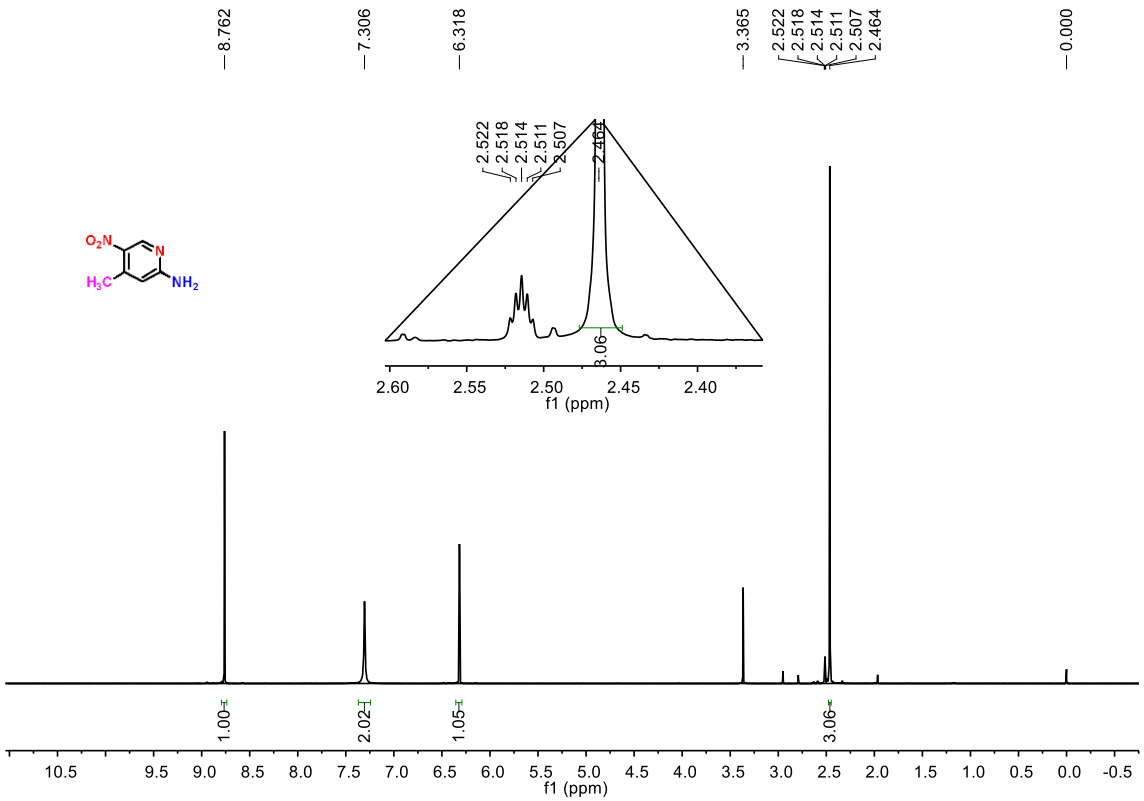
3o)，^{1}H NMR



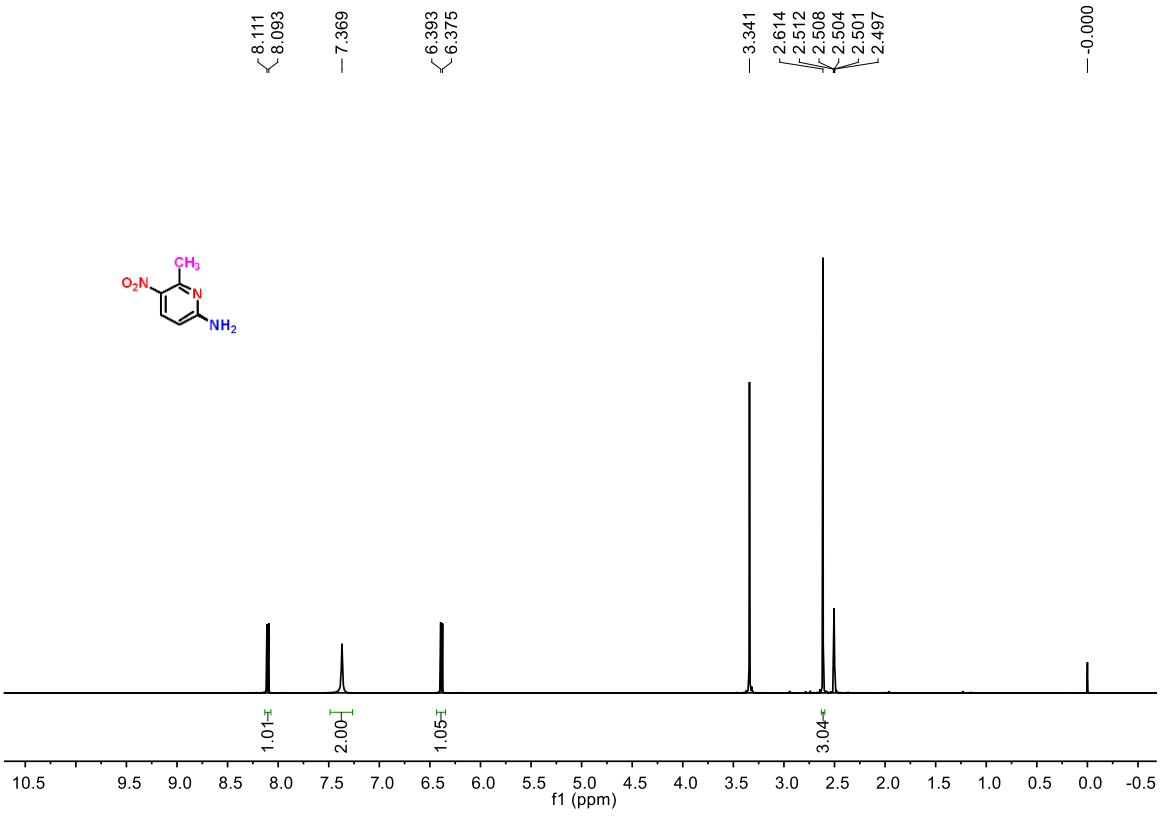
3p)，^{1}H NMR



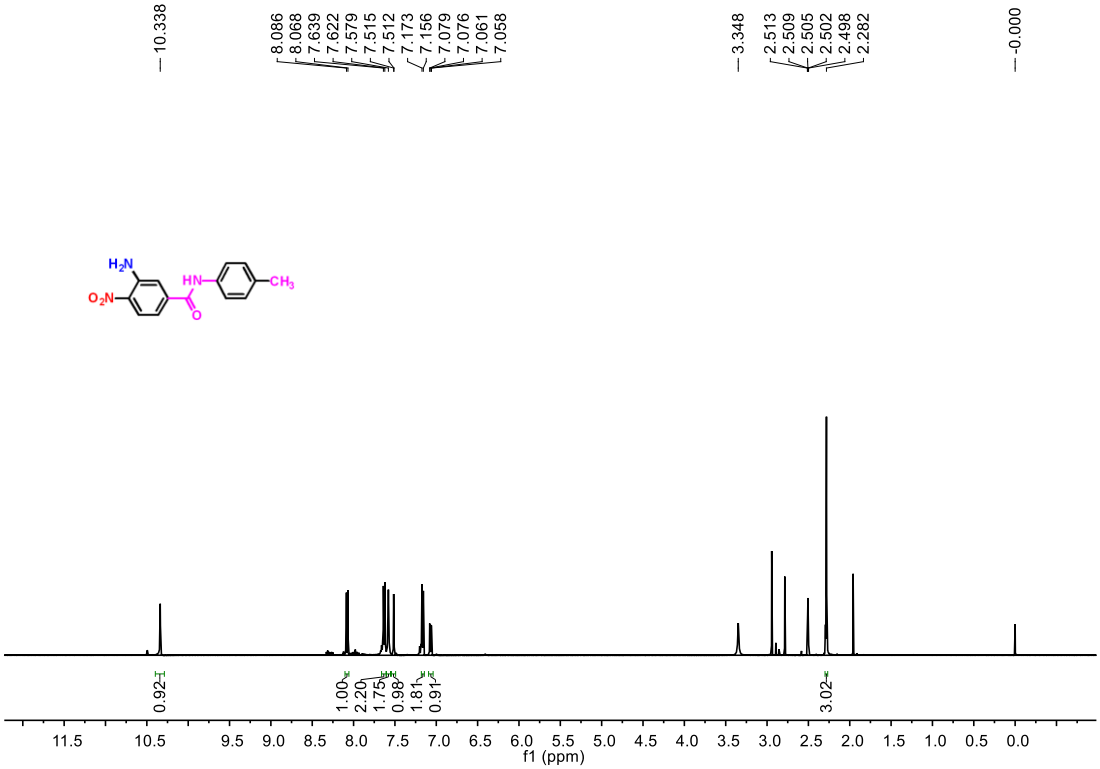
3q)，^{1}H NMR



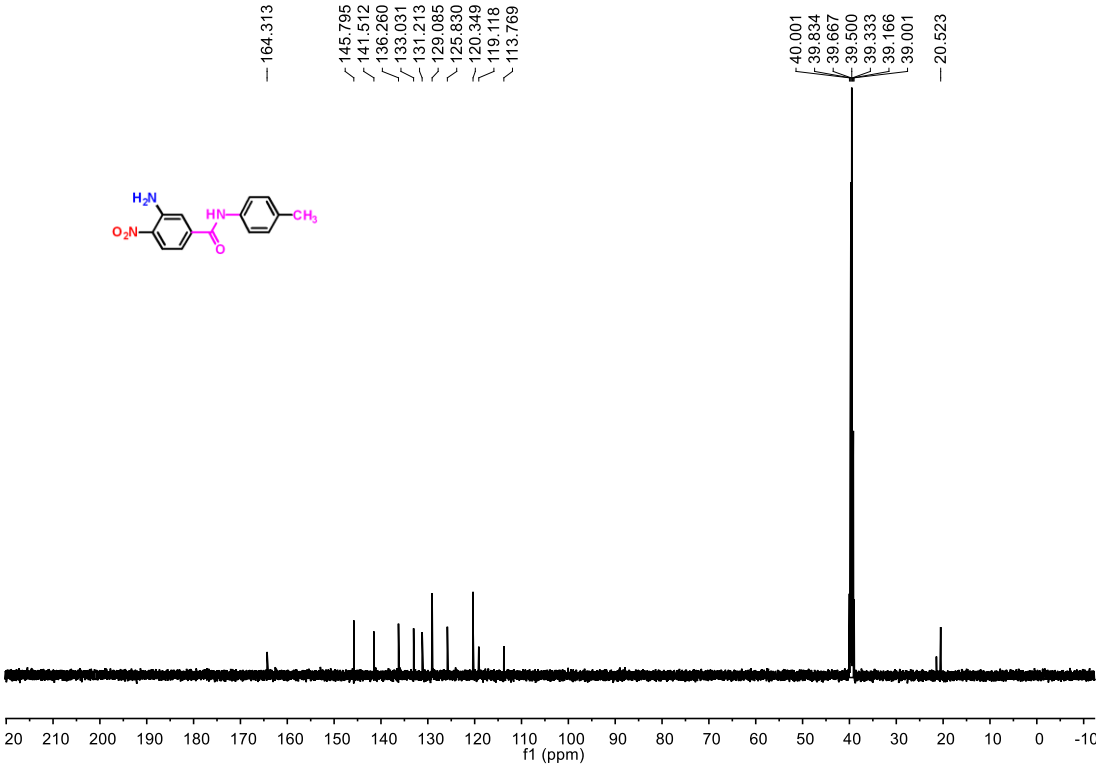
3r)，^{1}H NMR



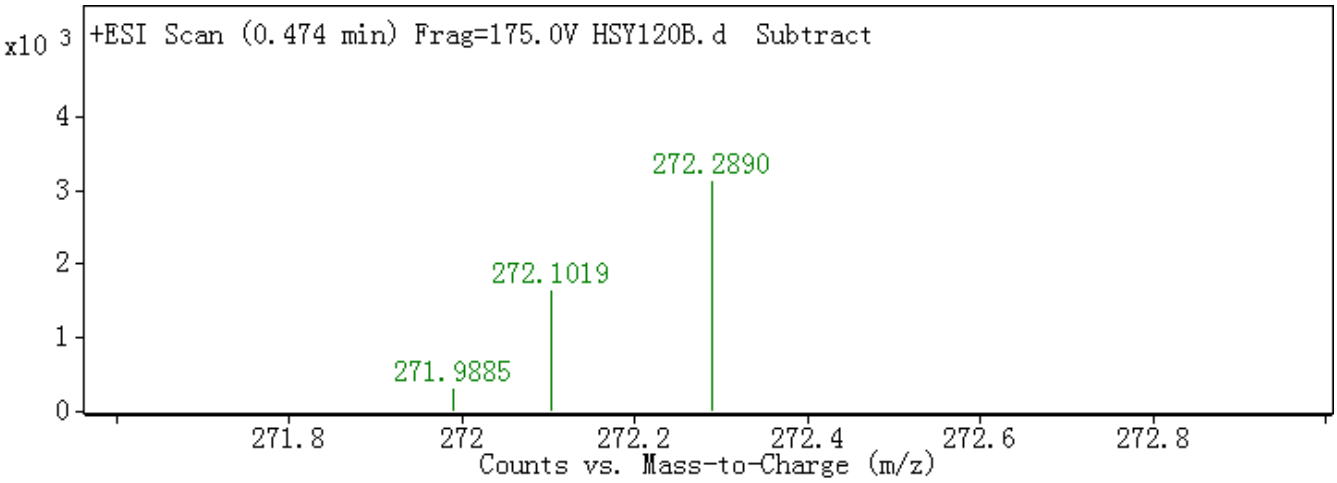
3s)，^{1}H NMR



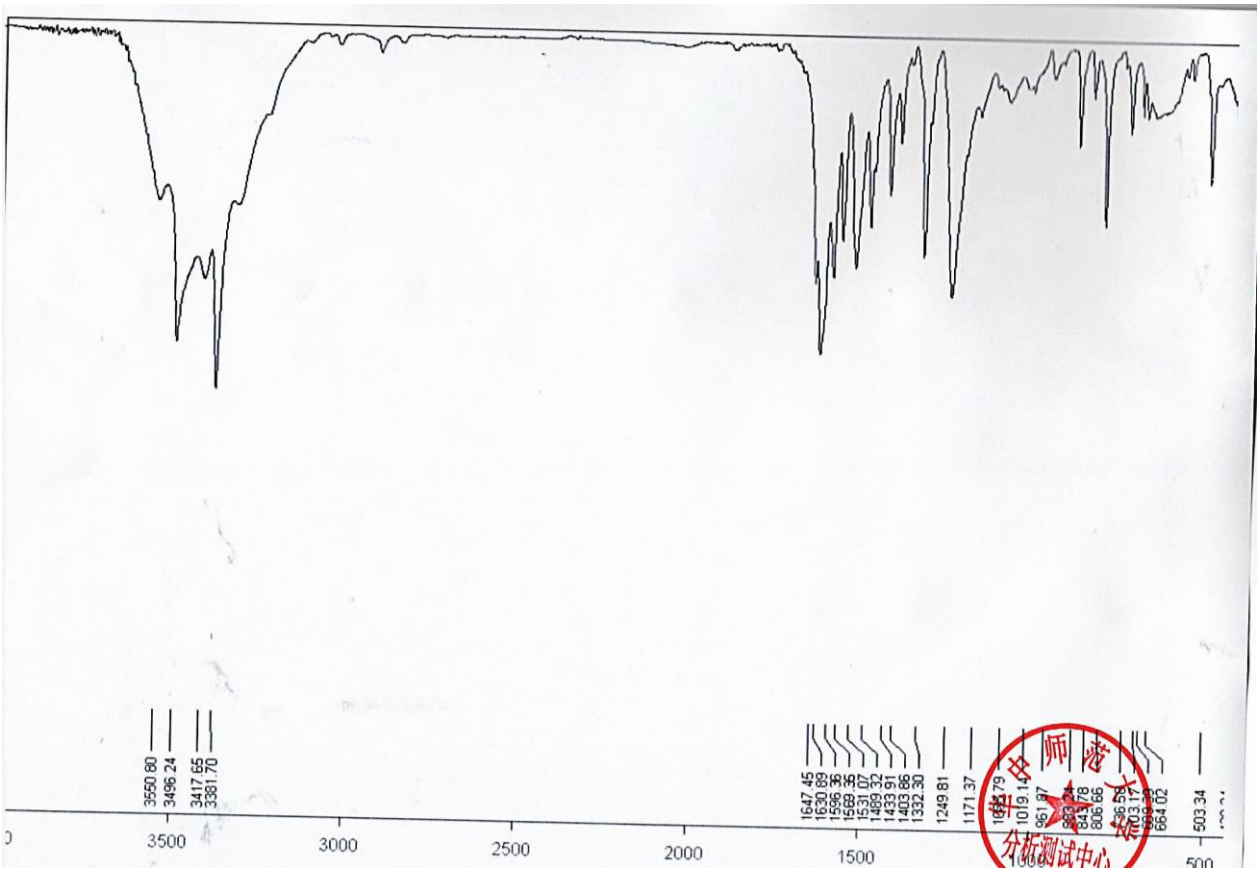
3bb)，^{1}H NMR



3bb)，^{13}C NMR



3bb)，HRMS (Error : -5.88 * 10^{-6})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1433.91
1403.86
1332.30
1249.81
1171.37
1019.14
961.87
806.66
664.02
503.34
3500
3000
2500
2000
1500

3bb)，FT-IR

5. XRD of compound 3c

Table 1. Crystal data and structure refinement for **3c**.

Identification code	**3c**	
Empirical formula	C6 H5 F N2 O2	
Formula weight	156.12	
Temperature	296(2) K	
Wavelength	0.71073	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a = 8.3170(11)	a= 90
	b = 12.3368(16)	b= 113.981(4)
	c = 7.1122(10)	g = 90
Volume	666.76(16) ^{3}	
Z	4	
Density (calculated)	1.555 Mg/m^3	
Absorption coefficient	0.137 mm^{-1}	
F(000)	320	
Crystal size	0.200 x 0.200 x 0.200 mm^3	
Theta range for data collection	2.680 to 25.037	
Index ranges	-9<=h<=8, -14<=k<=14, -7<=l<=8	
Reflections collected	9917	
Independent reflections	1169 [R(int) = 0.0528]	
Completeness to theta = 25.037?	99.1 %	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1169 / 0 / 100	
Goodness-of-fit on F^2	1.091	
Final R indices [I>2sigma(I)]	R1 = 0.0461, wR2 = 0.0886	
R indices (all data)	R1 = 0.0869, wR2 = 0.1062	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.123 and -0.191 e.?$^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (?^{2}x 10^3)for **3c**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
F(1)	2947(2)	2439(2)	6242(2)	89(1)
O(2)	9903(2)	3901(1)	7167(3)	77(1)
N(1)	8384(3)	3975(2)	7018(3)	57(1)
O(1)	7706(3)	4861(1)	7006(3)	94(1)
N(2)	9736(2)	1774(2)	7169(3)	64(1)
C(5)	7370(3)	3021(2)	6851(3)	40(1)
C(4)	8093(3)	1982(2)	6979(3)	43(1)
C(6)	5632(3)	3174(2)	6584(3)	47(1)
C(3)	6977(3)	1110(2)	6872(3)	54(1)
C(2)	5293(3)	1261(2)	6633(3)	60(1)
C(1)	4639(3)	2298(2)	6482(3)	53(1)

Table 3. Bond lengths and angles for **3c**.

F(1)-C(1)	1.358(2)
O(2)-N(1)	1.227(2)
N(1)-O(1)	1.228(2)
N(1)-C(5)	1.425(3)
N(2)-C(4)	1.342(3)
N(2)-H(2A)	0.8600
N(2)-H(2B)	0.8600
C(5)-C(6)	1.392(3)
C(5)-C(4)	1.404(3)
C(4)-C(3)	1.402(3)
C(6)-C(1)	1.344(3)
C(6)-H(6)	0.9300
C(3)-C(2)	1.354(3)
C(3)-H(3)	0.9300
C(2)-C(1)	1.378(3)
C(2)-H(2)	0.9300
O(2)-N(1)-O(1)	121.37(19)
O(2)-N(1)-C(5)	119.97(19)
O(1)-N(1)-C(5)	118.66(19)
C(4)-N(2)-H(2A)	120.0
C(4)-N(2)-H(2B)	120.0
H(2A)-N(2)-H(2B)	120.0
C(6)-C(5)-C(4)	121.75(19)
C(6)-C(5)-N(1)	116.53(19)
C(4)-C(5)-N(1)	121.72(18)
N(2)-C(4)-C(3)	118.9(2)
N(2)-C(4)-C(5)	124.94(19)
C(3)-C(4)-C(5)	116.14(19)
C(1)-C(6)-C(5)	118.7(2)
C(1)-C(6)-H(6)	120.7
C(5)-C(6)-H(6)	120.7
C(2)-C(3)-C(4)	122.0(2)
C(2)-C(3)-H(3)	119.0
C(4)-C(3)-H(3)	119.0
C(3)-C(2)-C(1)	119.6(2)
C(3)-C(2)-H(2)	120.2

C(1)-C(2)-H(2)	120.2
C(6)-C(1)-F(1)	119.1(2)
C(6)-C(1)-C(2)	121.9(2)
F(1)-C(1)-C(2)	119.0(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (?^{2}x 10^3) for **3c**.
The anisotropic displacement factor exponent takes the form: -2p^2[h^2 a*^{2}U^{11} + ... + 2 h k a* b* U^{12}]

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
F(1)	42(1)	138(2)	91(1)	6(1)	31(1)	-4(1)
O(2)	56(1)	73(1)	106(2)	-6(1)	39(1)	-20(1)
N(1)	59(1)	48(1)	68(1)	-7(1)	30(1)	-8(1)
O(1)	105(2)	37(1)	158(2)	-16(1)	71(2)	-7(1)
N(2)	51(1)	60(1)	83(2)	1(1)	29(1)	14(1)
C(5)	40(1)	37(1)	41(1)	-3(1)	16(1)	-5(1)
C(4)	42(1)	47(1)	39(1)	-1(1)	16(1)	1(1)
C(6)	44(1)	51(1)	46(1)	-1(1)	19(1)	6(1)
C(3)	66(2)	42(1)	53(2)	0(1)	25(1)	-1(1)
C(2)	68(2)	57(2)	53(2)	-2(1)	25(1)	-23(1)
C(1)	32(1)	81(2)	47(1)	1(1)	17(1)	-5(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (?^{2}x 10 ^{3}) for **3c**.

	x	y	z	U(eq)
H(2A)	10090	1115	7224	77
H(2B)	10436	2300	7237	77
H(6)	5167	3868	6479	56
H(3)	7407	407	6968	64
H(2)	4582	668	6572	71

Table 6. Torsion angles for **3c**.

O(2)-N(1)-C(5)-C(6)	-176.90(19)
O(1)-N(1)-C(5)-C(6)	3.0(3)
O(2)-N(1)-C(5)-C(4)	4.0(3)
O(1)-N(1)-C(5)-C(4)	-176.1(2)
C(6)-C(5)-C(4)-N(2)	177.8(2)
N(1)-C(5)-C(4)-N(2)	-3.2(3)
C(6)-C(5)-C(4)-C(3)	-1.5(3)
N(1)-C(5)-C(4)-C(3)	177.59(19)
C(4)-C(5)-C(6)-C(1)	1.1(3)
N(1)-C(5)-C(6)-C(1)	-178.01(19)
N(2)-C(4)-C(3)-C(2)	-178.5(2)
C(5)-C(4)-C(3)-C(2)	0.8(3)
C(4)-C(3)-C(2)-C(1)	0.2(3)
C(5)-C(6)-C(1)-F(1)	179.37(18)
C(5)-C(6)-C(1)-C(2)	0.0(3)
C(3)-C(2)-C(1)-C(6)	-0.7(4)
C(3)-C(2)-C(1)-F(1)	179.97(19)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for **3c**.

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)

6. Compound 3a-3s treatments inhibit seed germination

Plant materials and cultivating conditions: The soybean seeds were cultivated in culture dish in biochemical incubator,[9] and treated with 50 μmol/L. The number of seed germination was recorded every 24 hours.

Germination rate = (number of germination seed) ÷ (number of tested seed) * 100% (1)

Inhibition rate = [1- (Germination rate of experimental group treament with **3a-3s**) ÷

(Germination rate of control group treament with 0.5% DMSO)] * 100% (2)

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