Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Materials

Novel type of AIE material as highly selective fluorenscent sensor for the detection of cysteine

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Content

Reagents and Measurement.

All chemicals purchased were used without purification. THF of analytical grade and de-ionized water were used throughout the experiment as solvents. ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) or 300 (300 MHz) spectrometer, using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectra were recorded on a Bruker Avance 100 (100 MHz) or 75 (75 MHz) spectrometer, using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. HEPES buffer solutions (pH 7.2) were prepared using 20 mM HEPES and the proper amount of aqueous sodium hydroxide using a pH meter. HRMS spectra were determined on a Q-TOF6510 spectrograph (Agilent). Single crystal X-ray diffraction were made on a Rigaku RAXIS-SPIDER IP diffractometer at 50 kV and 20 mA and data collection was performed at 293 K by using graphite-monochromated Mo-Kα radiation (λ =0.71073Å). The nanoparticle diameter was measured by the Zetasizer 2000 (Marven, UK). Fluorescent measurements were collected on a Hitachi F-4500 luminescence spectrophotometer with a 150 W Xe lamp. Calculations were conducted by using the Gaussian 03 program.

Preparation of Samples for Spectral Measurement

1×10^{-4}mol/L sample: 10 mM stock solution of 1 was prepared in THF. To a 100 mL volumetric flask was added 99 mL THF/water (1:99 ～ 9:1, v/v, containing 20 mM HEPES buffer, pH 7.2) and 1 mL stock solution of 1. Then the flask was treated in KQ-2000E ultrasonic cleaner for 10 minutes. 2 mL of the solution was transferred for fluorescence measurement.

1×10^{-5}mol/L probe sample: 1 mM stock solution of 1 was prepared in THF. To a 100 mL volumetric flask was added 99 mL water (containing 20 mM HEPES buffer, pH 7.2) and 1 mL stock solution of 1. Then the flask was treated in KQ-2000E ultrasonic cleaner for 10 minutes. 2 mL of the solution was transferred for fluorescence measurement.

Table 1 Energy in atomic units and Cartesian coordinate table for the optimized structure of **3a**.

Total energy (B3LYP/6-31G (d)) -1048.284285487932 a. u.

	X	Y	Z
C	-3.51688688	-0.14306498	0.59936053
C	-3.31518311	1.11796538	-0.17952228
C	-4.18630052	1.49710356	-1.12362036
C	-5.43596167	0.61019209	-1.29928149
C	-5.60308842	-0.49572571	-0.50326944
C	-2.02759091	1.81821271	0.28542743
H	-4.02643663	2.37075350	-1.72034418
H	-6.17092280	0.85747526	-2.03656243
H	-6.48286969	-1.09525401	-0.61027751
N	-4.57751548	-0.87223479	0.50283160
O	-1.49845816	2.78899888	-0.31554120
N	-1.48603360	1.21924236	1.54404530
S	-2.07892691	-0.40370177	1.58978103
C	-0.01707827	1.24888269	1.59086048
C	0.46792754	0.60127413	2.90119346
C	0.55061407	0.46761429	0.39130010
H	0.32074120	2.26324877	1.54797145
C	2.00683313	0.63232590	2.95023793
H	0.13010807	-0.41309195	2.94408249
H	0.07349196	1.14410347	3.73465424
C	2.08951966	0.49866606	0.44034457
H	0.21279460	-0.54675179	0.43418913
H	0.21362952	0.91757608	-0.51912606
C	2.57452546	-0.14894250	1.75067755
H	2.34381768	0.18236411	3.86066409
H	2.34465260	1.64669198	2.90734890
H	2.48395524	-0.04416328	-0.39311621

H	2.42733913	1.51303214	0.39745555
H	3.64376507	-0.12736757	1.78475391
H	2.23670600	-1.16330858	1.79356658

Energy in atomic units and Cartesian coordinate table for the optimized structure of **3b**.

Total energy (B3LYP/6-31G (d)) -1388.057352928338 a. u.

	X	Y	Z
C	-1.90478817	-0.86727886	0.34787140
C	-2.75566681	-0.66222072	1.56084475
C	-3.99766909	-1.15889728	1.62907363
C	-4.46085195	-2.01376041	0.43166170
C	-3.60729372	-2.22869910	-0.62183855
C	-1.98229608	0.15541962	2.60859038
H	-4.63247320	-0.97664203	2.47092086
H	-5.44233259	-2.43982198	0.42345926
H	-3.92214544	-2.84248107	-1.43978726
N	-2.25343295	-1.61841633	-0.64216628
O	-2.50481745	0.64848860	3.64175299
N	-0.53298360	0.28346745	2.26299608
S	-0.43679939	0.09268188	0.54775347
C	0.03096996	1.57354900	2.68554309
C	0.61270128	1.70220842	3.95398993
C	-0.01312563	2.67476733	1.81992439
C	1.15034007	2.93208526	4.35681676
H	0.64636871	0.86140395	4.61490924
C	0.52451163	3.90464462	2.22275187
C	1.10624636	4.03330303	3.49119725
H	-0.45729025	2.57653297	0.85143701
O	0.47951422	5.02833736	1.33946830
O	1.65485963	5.28827873	3.90224407
O	1.74394467	3.06337002	5.65114975

C	3.13735648	2.75229972	5.57028683
H	3.58152216	2.85053377	6.53877375
H	3.25894445	1.74777747	5.22236241
H	3.61422565	3.42582904	4.88921851
C	0.64068305	6.06379107	4.54639192
H	-0.16586766	6.23345599	3.86405053
H	0.27787351	5.53536687	5.40315158
H	1.05118389	7.00282883	4.85395842
C	1.47335294	4.87549818	0.32269655
H	2.44092585	4.81644129	0.77570313
H	1.28309098	3.97938782	-0.23019278
H	1.43968355	5.71630323	-0.33822192

Energy in atomic units and Cartesian coordinate table for the optimized structure of **3c**.

Total energy (B3LYP/6-31G (d)) -1272.435498058302 a. u.

	X	Y	Z
C	-3.12325031	-0.58674447	1.68687336
C	-3.85781751	0.65065611	2.09503814
C	-5.15786227	0.80990011	1.81497133
C	-5.86193152	-0.37871945	1.12897227
C	-5.15182767	-1.51882724	0.84519453
C	-2.90320576	1.60533336	2.83105218
H	-5.68094128	1.71102393	2.05841375
H	-6.90016165	-0.31597761	0.87789130
H	-5.64846579	-2.35111489	0.39182070
N	-3.70269027	-1.61119249	1.15707071
O	-3.18370796	2.79829397	3.11694388
N	-1.60764335	0.94509340	3.18025065
S	-1.41176521	-0.34315460	2.04457613
C	-0.47227682	1.87723278	3.12585383
C	-0.09388955	2.58828835	4.27265155

C	0.23171867	2.05481676	1.92719782
C	0.98849317	3.47692795	4.22079324
H	-0.63140560	2.45269890	5.18785207
C	1.31410118	2.94345660	1.87533942
C	1.69248841	3.65451222	3.02213712
H	1.27740156	4.01983309	5.09639929
H	1.85161920	3.07904371	0.96013970
H	2.51891361	4.33300591	2.98254296
C	-0.18409188	1.27343744	0.66698039
O	-1.26836277	1.54368864	0.08828230
O	0.66192434	0.24076730	0.15438998
C	0.49950608	0.15146393	-1.26354698
H	-0.52056239	-0.07692287	-1.49201967
H	0.76501170	1.08572657	-1.71250142
H	1.13253920	-0.62123331	-1.647093

Energy in atomic units and Cartesian coordinate table for the optimized structure of **3d**.

Total energy (B3LYP/6-31G (d)) -1198.224947316698 a. u.

	X	Y	Z
C	-3.74055523	1.13168446	0.98518023
C	-3.63564301	2.58003633	0.62654680
C	-4.41475392	3.11842386	-0.32057383
C	-5.46931738	2.18668826	-0.95215072
C	-5.56765665	0.88440752	-0.52904636
C	-2.55482261	3.25394537	1.48798907
H	-4.31437070	4.14092496	-0.61942949
H	-6.12338011	2.55461612	-1.71486367
H	-6.31640191	0.24716495	-0.95118666
N	-4.64673970	0.34782240	0.50525808
O	-2.10850731	4.41162355	1.27785947
N	-2.10675915	2.37751221	2.61379651

S	-2.43298217	0.75646497	2.11031646
C	0.28436573	1.78286741	2.27645494
C	1.64197778	1.95494639	2.57844884
C	2.03253353	2.90217262	3.53456109
C	1.06547688	3.67731644	4.18868298
C	-0.29213524	3.50523673	3.88668984
C	-0.68269071	2.55801325	2.93057476
H	4.12851521	2.48241614	3.33711421
H	-0.01383215	1.05964014	1.54644101
H	2.38034674	1.36310445	2.07901476
C	3.39014585	3.07425426	3.83655223
C	1.45603253	4.62454168	5.14479625
H	-1.03050447	4.09707610	4.38612657
C	2.81364485	4.79662327	5.44678745
C	3.78070153	4.02147974	4.79266526
H	0.71766358	5.21638371	5.64423023
H	3.11184276	5.51985082	6.17680110
H	4.81726856	4.15286690	5.02324322

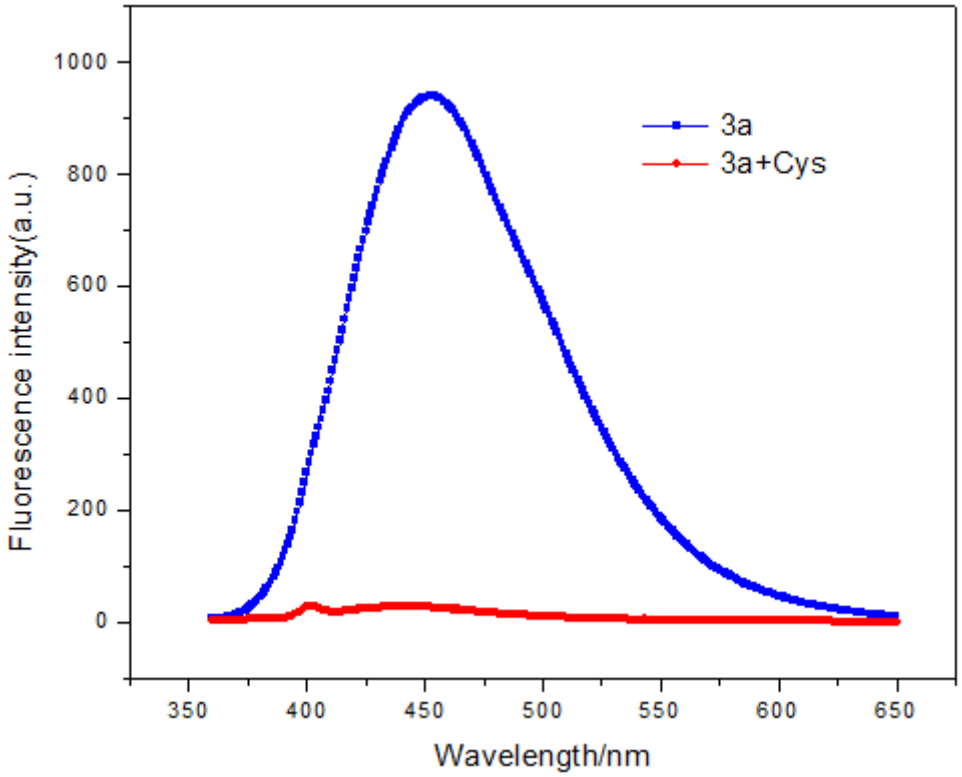


Figure 1. Fluorescence emission spectra (excitation at 330 nm) of 100 μM **3a** in THF/water (v/v, 1/99) mixtures buffered by 20 mM HEPES at pH 7.20 with 20 equiv Cys,

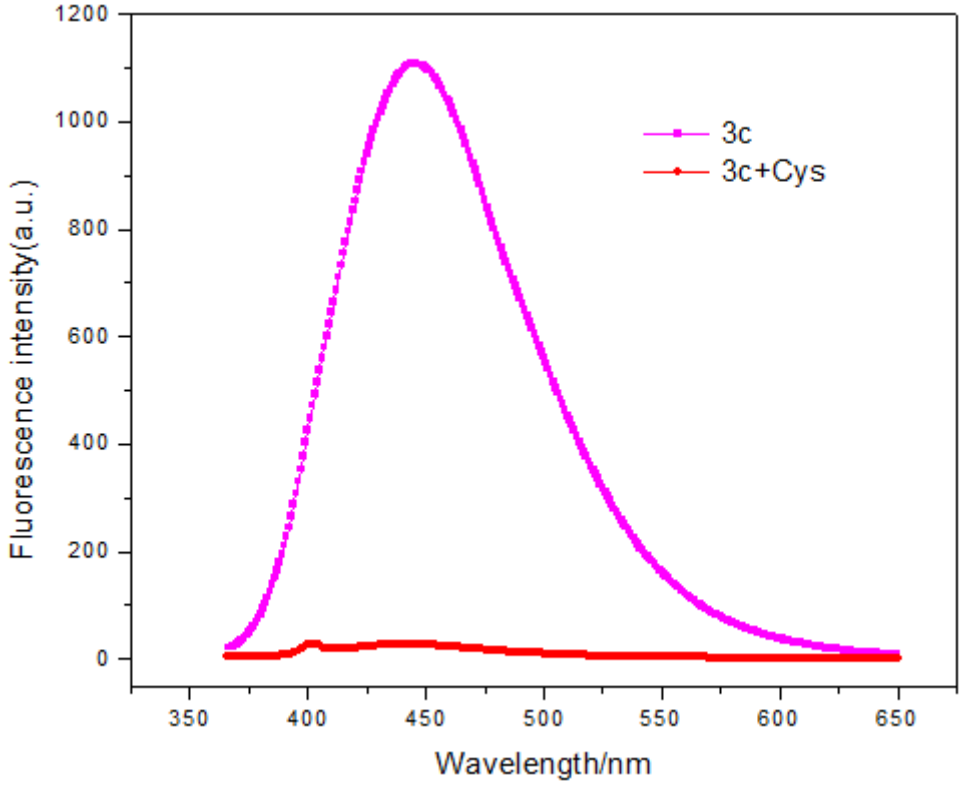


Figure 2 Fluorescence emission spectra (excitation at 330 nm) of 100 μM **3c** in THF/water (v/v, 1/99) mixtures buffered by 20 mM HEPES at pH 7.20 with 20 equiv Cys,

DLS (Dynamic Light Scattering) results

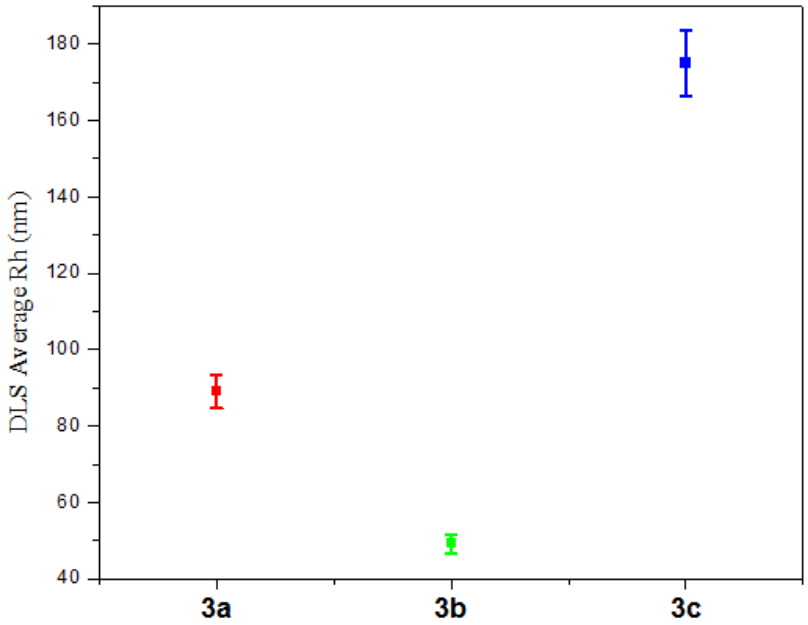


Figure 3 DLS Average Rh (nm) of **3a～c** (10 μM) in THF/ water mixtures (1/99, v/v).

CONFIGURATION

Light scattering instrument: DAWN HELEOS

Cell type: Scintillation vial

Laser wavelength: 658.0 nm

Calibration constant: 1.7375e-4 1/(V cm)

Replaced detector: 12

Temperature control: n/a

Temperature: n/a

QELS instrument:

Model: Wyatt QELS

Use temperature probe: yes

Solvent: water

Refractive index: 1.331

Viscosity: 8.9450e-3 g/(cm sec) (valid if QELS temperature not used)

PROCESSING

Processing time: Tuesday April 15, 2014 11:03 AM China Standard Time

Collection time: Tuesday April 15, 2014 10:18 AM China Standard Time

QELS delay time range: 2.00e-7 to 1.00 sec

QELS threshold values: 1.0 to 300.0 nm

Detectors used: 1 2 3 4 5 6 7 8 9 10 11 13 14 15 16 17 18

	Peak 1	Peak 2	Peak 3
Peak limits (min)	5.876 - 9.918	10.913 - 14.769	15.950 - 19.371
dn/dc (mL/g)	0.000	0.000	0.000
A_2 (mol mL/g²)	0.000	0.000	0.000

Model	Zimm	Zimm	Zimm
Fit degree	1	1	1
Concentration (g/mL)	0.00	0.00	0.00

RESULTS

	Peak 1	Peak 2	Peak 3
Average Mw (g/mol)	0.000	0.000	0.000
Average Rz (nm)	n/a	n/a	n/a
Average Rh (nm)	89.0(1%)	49.2(2%)	175.0(3%)
Average Dt (cm²/sec)	1.46e-8(1%)	4.95e-8(2%)	1.27e-8(3%)

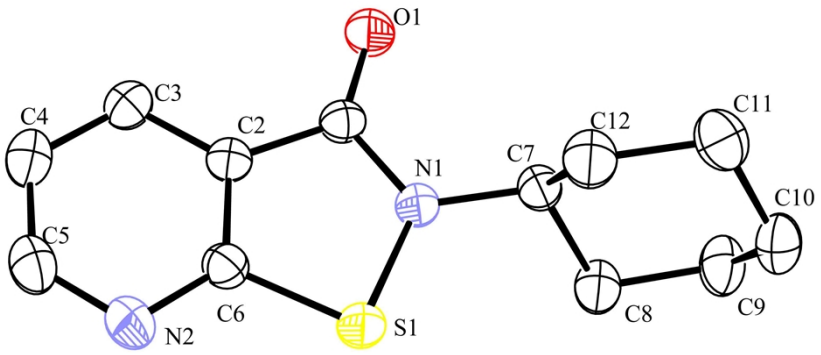
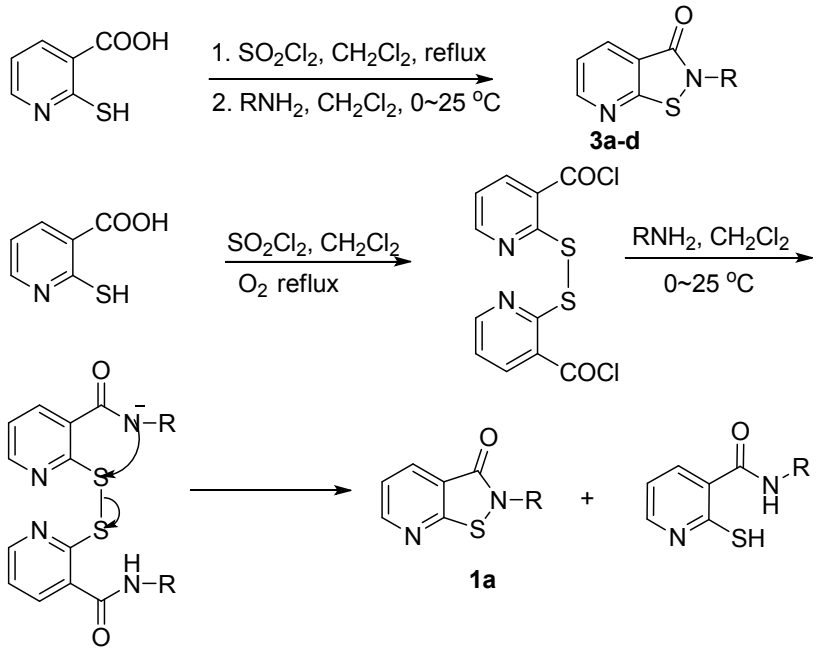


Figure 3. X-ray crystal structure of **3a**.

Table 2. Details of Crystal Data and Structure Refinement for Compound 3a

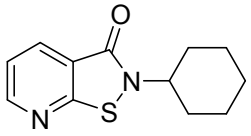
Chemical formula	$C_{12}H_{14}N_2OS$
Formula Mass	234.31
Crystal system	Monoclinic
a/Å	10.001(4)
b/Å	10.607(3)
c/Å	12.239(4)
α/°	76.315(16)
β/°	71.86(2)
γ/°	73.22(2)
Unit cell volume/Å^3	1165.8(7)
Temperature/K	296(2)
Space group	*Pca21*
No. of formula units per unit cell, Z	4
No. of reflections measured	10274
No. of independent reflections	3519
R_{int}	0.0339
Final R_1 values ($I > 2\sigma(I)$)	0.0394
Final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1128
Final R_1 values (all data)	0.0464
Final $wR(F^2)$ values (all data)	0.1201

General Experimental Procedure for the Synthesis of 2-cyclohexylisothiazolo[5,4-*b*]pyridin-3(2*H*)-one (3a). To a solution of 2-mercaptonicotinic acid (0.31 g, 20 mmol) in CH_2Cl_2 (20 mL) thionyl chloride (0.3 mL) was added, the solution was refluxed for 2.5 h (oil bath). The mixture was evaporated in vacuo, and the solvent and thionyl chloride were removed, yellow solid was obtained. The yellow solid was dissolved by CH_2Cl_2 (20mL), then cyclohexylamine (0.20 g, 2 mmol) dissolved in 10 mL CH_2Cl_2 was added dropwise to the ice bath solution. The mixture was stirred overnight, then H_2O (100 mL) was added and the mixture was filtered and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed by chilled water (3×100 mL), dried by Na_2SO_4, filtered, and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc = 5:1). Compound **3a** was obtained as white plate crystals (0.11 g, 47%).



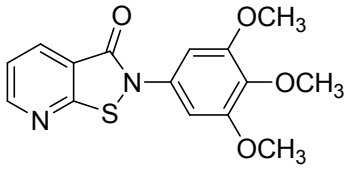
Scheme 1. Synthesis and reaction mechanism of fused isothiazolidin-3-one scaffolds.

2-cyclohexylisothiazolo[5,4-*b*]pyridin-3(2*H*)-one (3a): White solid (47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (dd, 1H, *J* = 4.5, 1.5 Hz), 8.29-8.26 (m, 1H), 7.37-7.32 (m, 1H), 4.69-4.59 (m, 1H), 2.07 (d, 2H, *J* = 11.4 Hz), 1.90 (d, 2H, *J* = 13.2 Hz), 1.74 (d, 1H, *J* = 13.2 Hz), 1.65-1.40 (m, 4H), 1.30-1.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.1, 162.4, 153.2, 134.7, 120.6, 120.3, 53.2, 32.9, 25.6, 25.2.. HRMS calcd for $C_{12}H_{14}N_2OS$, 235.0900; found, 235.1052.



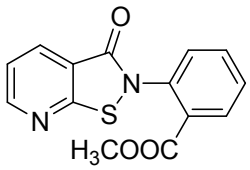
3a

2-(3,4,5-trimethoxyphenyl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one (3b): Yellow solid (46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (dd, 1H, *J* = 4.8, 1.5 Hz), 8.34 (dd, 1H, *J* = 7.8, 1.5 Hz), 7.42 (dd, 1H, *J* =8.0, 1.5 Hz), 6.91 (s, 1H), 3.90 (s, 6H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 161.9, 154.1, 153.6, 137.6, 135.2, 132.0, 121.1, 119.7, 103.1, 61.0, 56.3. HRMS calcd for $C_{15}H_{14}N_2O_4S$, 319.0708; found, 319.0751.



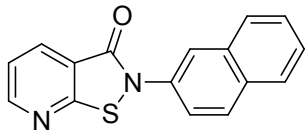
3b

methyl 2-(3-oxoisothiazolo[5,4-*b*]pyridin-2(3*H*)-yl)benzoate (3c): White solid (46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (dd, 1H, *J* = 7.6, 1.6 Hz), 8.38 (dd, 1H, *J* = 8.0, 1.6 Hz), 7.98 (dd, 1H, *J* =8.0, 1.6 Hz), 7.83-7.78 (m, 1H), 7.67-7.59 (m, 3H), 3.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 162.7, 161.9, 154.6, 135.3, 134.5, 133.6, 131.0, 129.7, 129.4, 129.2, 121.7, 117.9, 52.3. HRMS calcd for $C_{14}H_{10}N_2O_3S$, 287.0446; found, 287.0551.



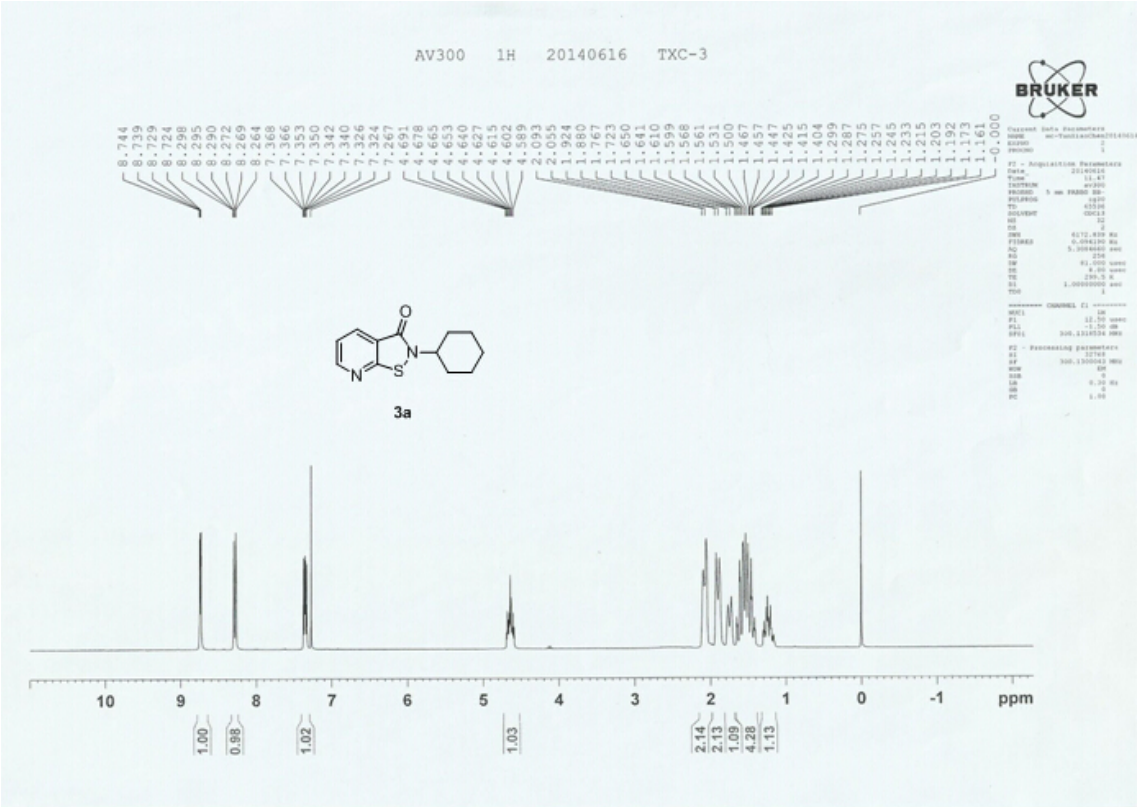
3c

2-(naphthalen-2-yl)isothiazolo[5,4-*b*]pyridin-3(2*H*)-one (3d): Colorless Oil (46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (dd, 1H, J = 4.6, 1.6 Hz), 8.45 (dd, 1H, J = 7.8, 1.5 Hz), 8.16-8.10 (m, 2H), 7.78 (d, 1H, J = 6.6 Hz), 7.70-7.56 (m, 5H), 3.31 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 162.7, 155.1, 135.9, 134.4, 132.4, 130.6, 129.0, 128.5, 128.0, 127.4, 126.2, 123.1, 122.3, 118.6. HRMS calcd for $C_{16}H_{10}N_2OS$, 279.0547; found, 279.0579.

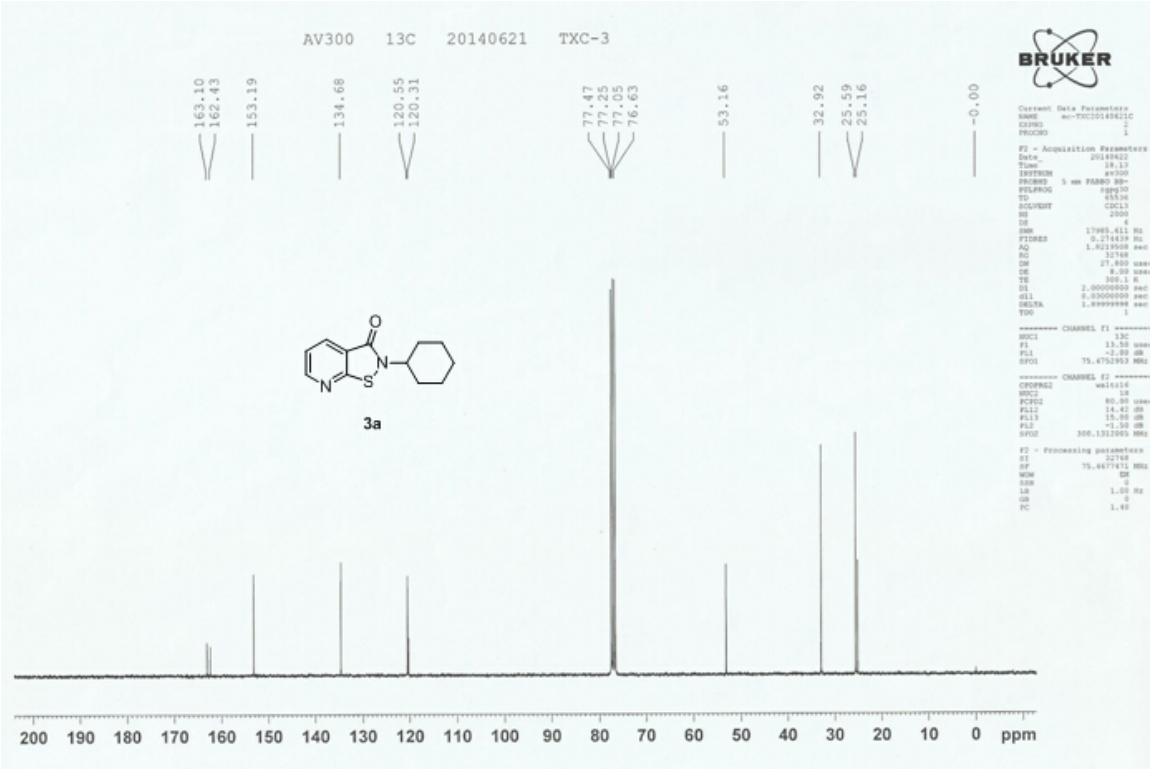


3d

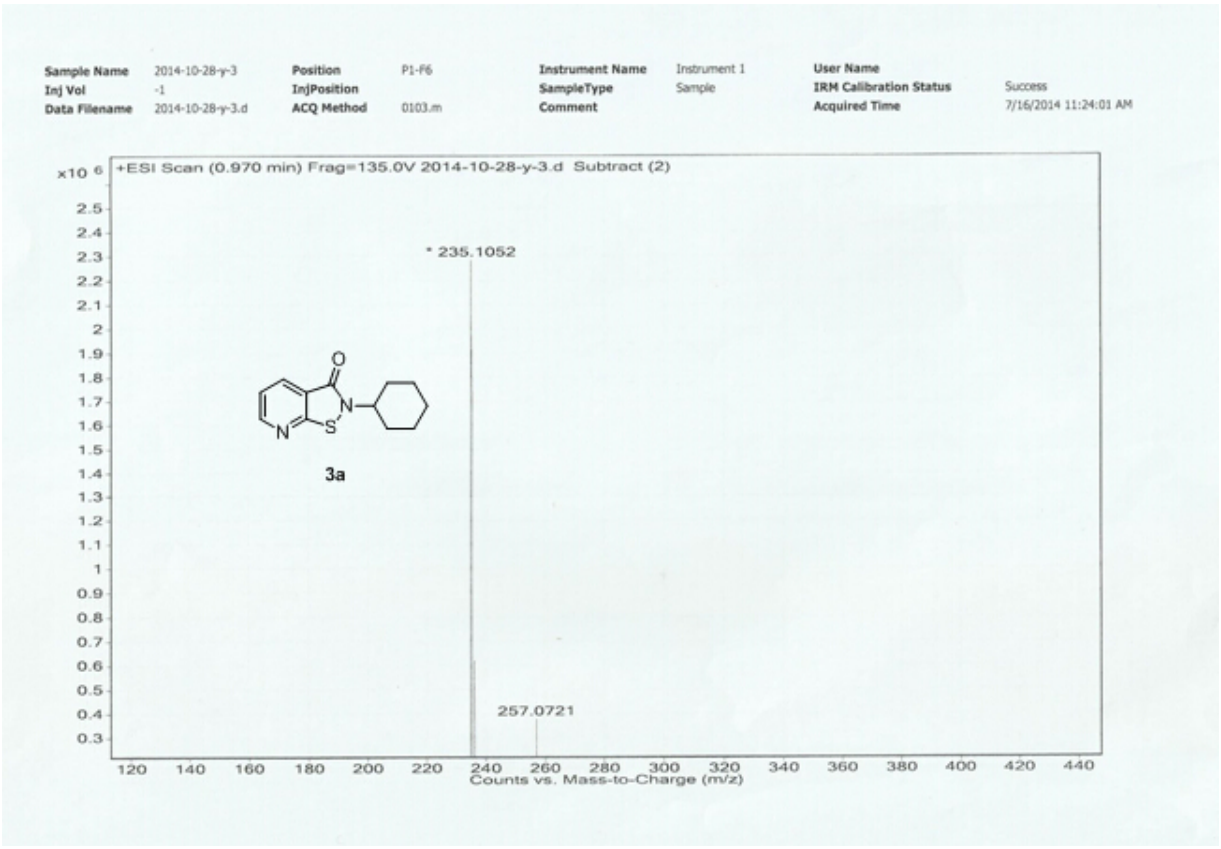
^{1}H NMR , ^{13}C NMR Spectra and Mass Spectrum of Compound 3a-3d



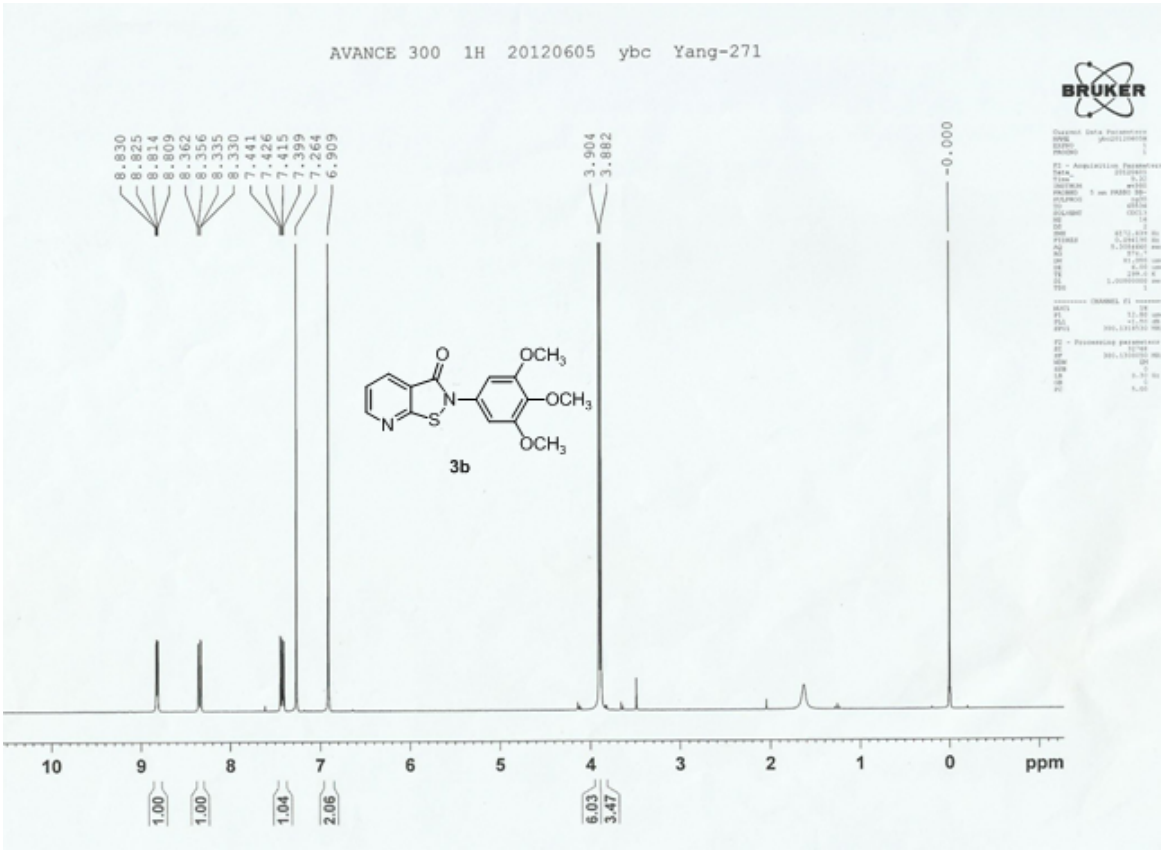
^{1}H NMR Spectra of Compound 3a in $CDCl_3$



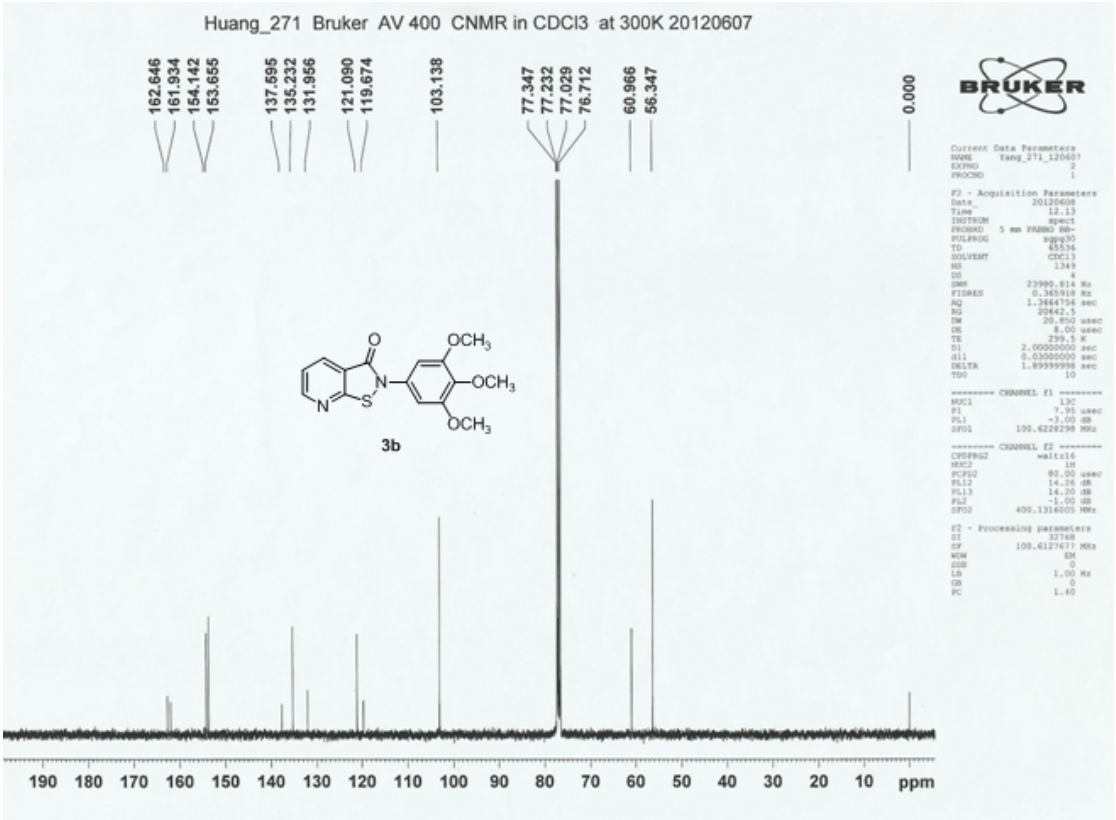
^{13}C NMR Spectra of Compound 3a in $CDCl_3$



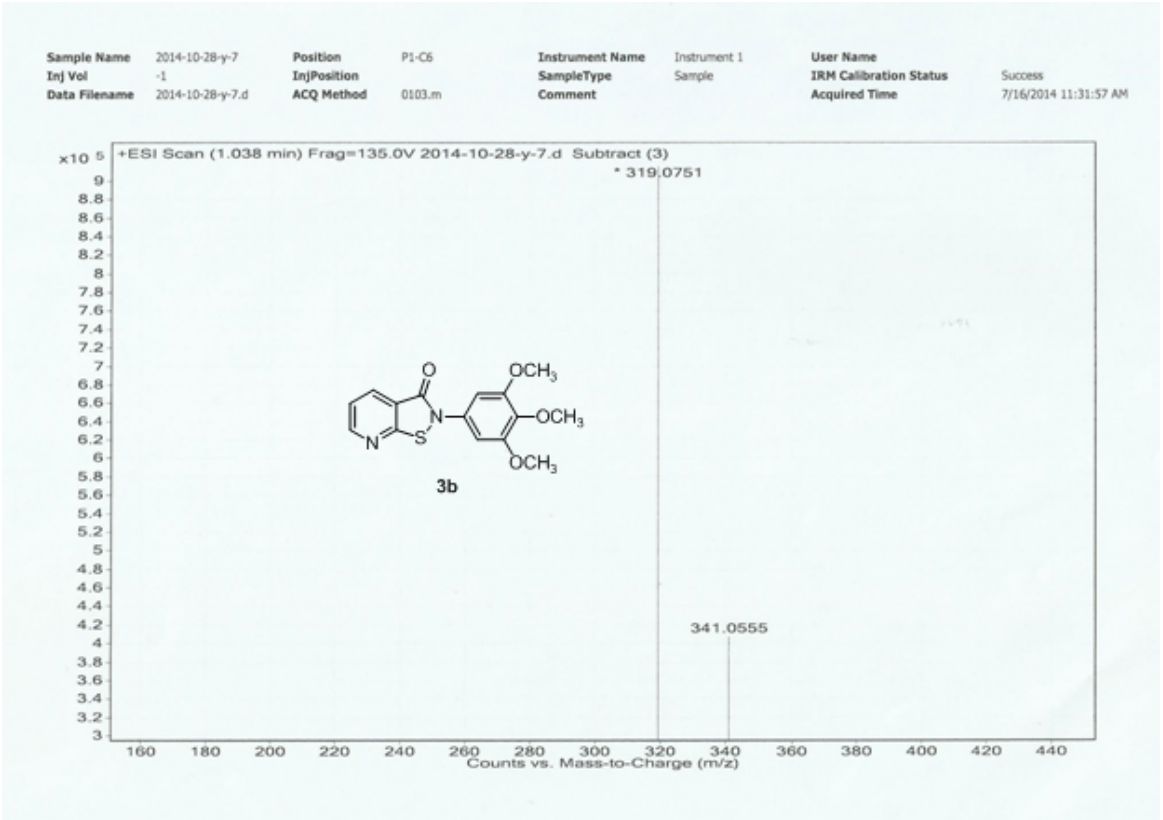
Mass Spectrum of Compound 3a



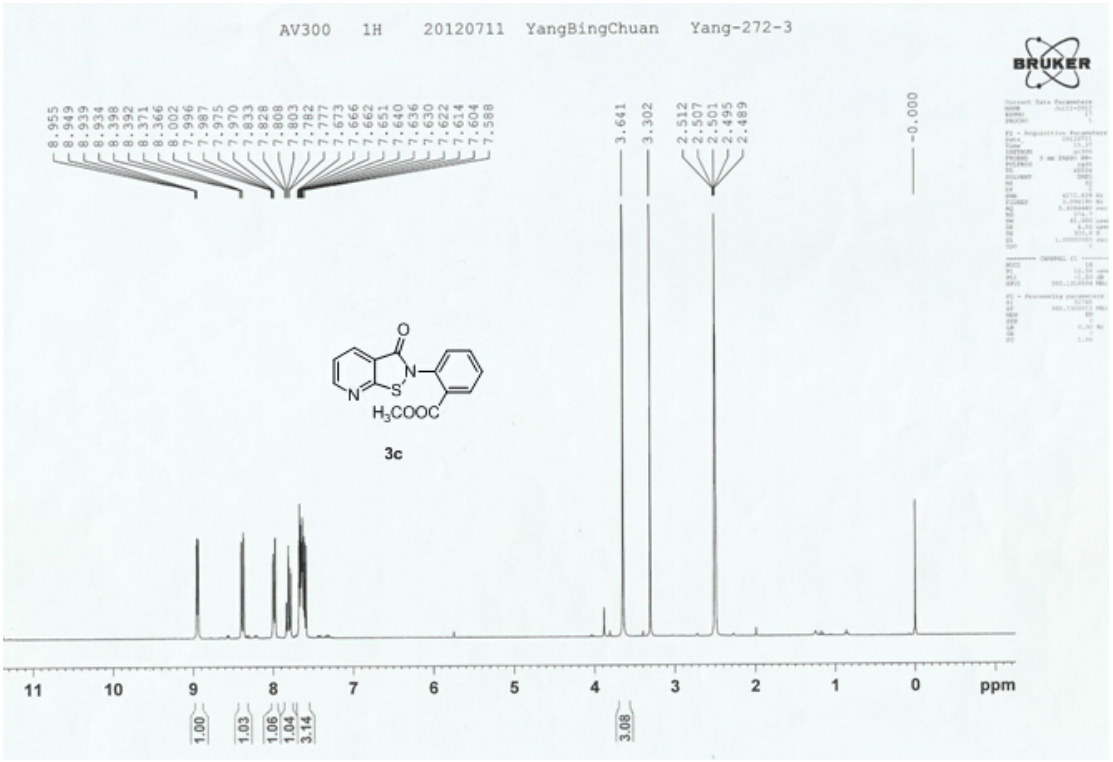
^{1}H NMR Spectra of Compound 3b in $CDCl_3$



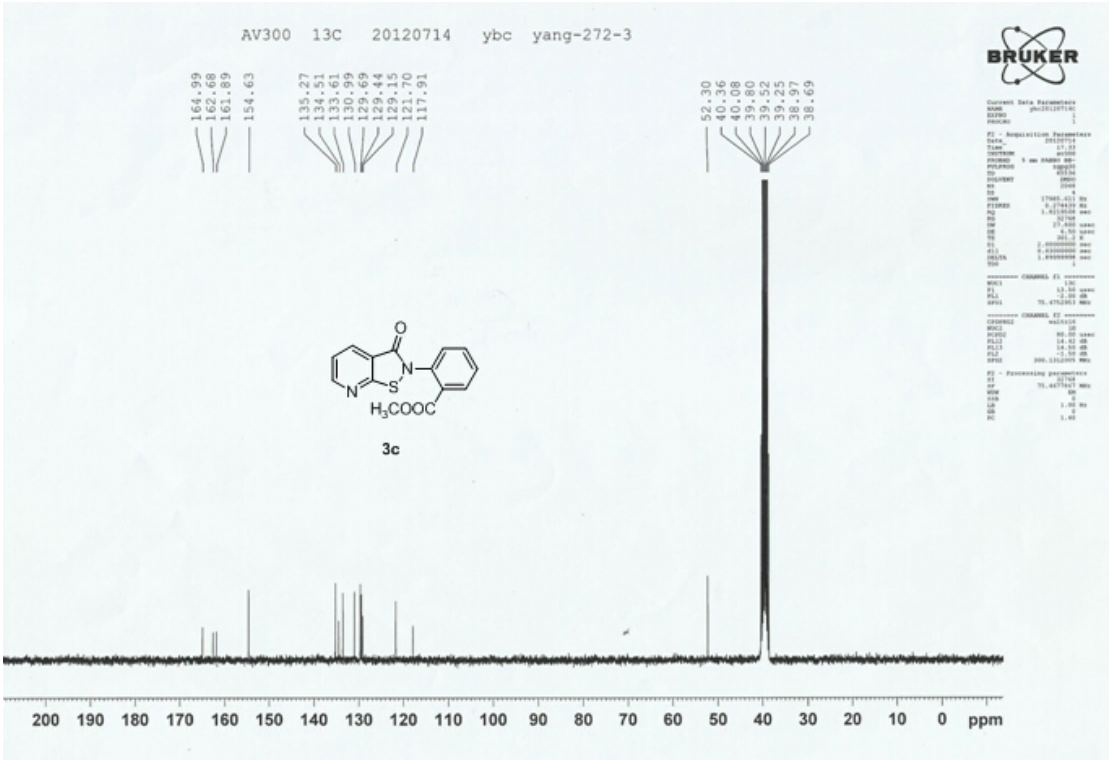
^{13}C NMR Spectra of Compound 3b in $CDCl_3$



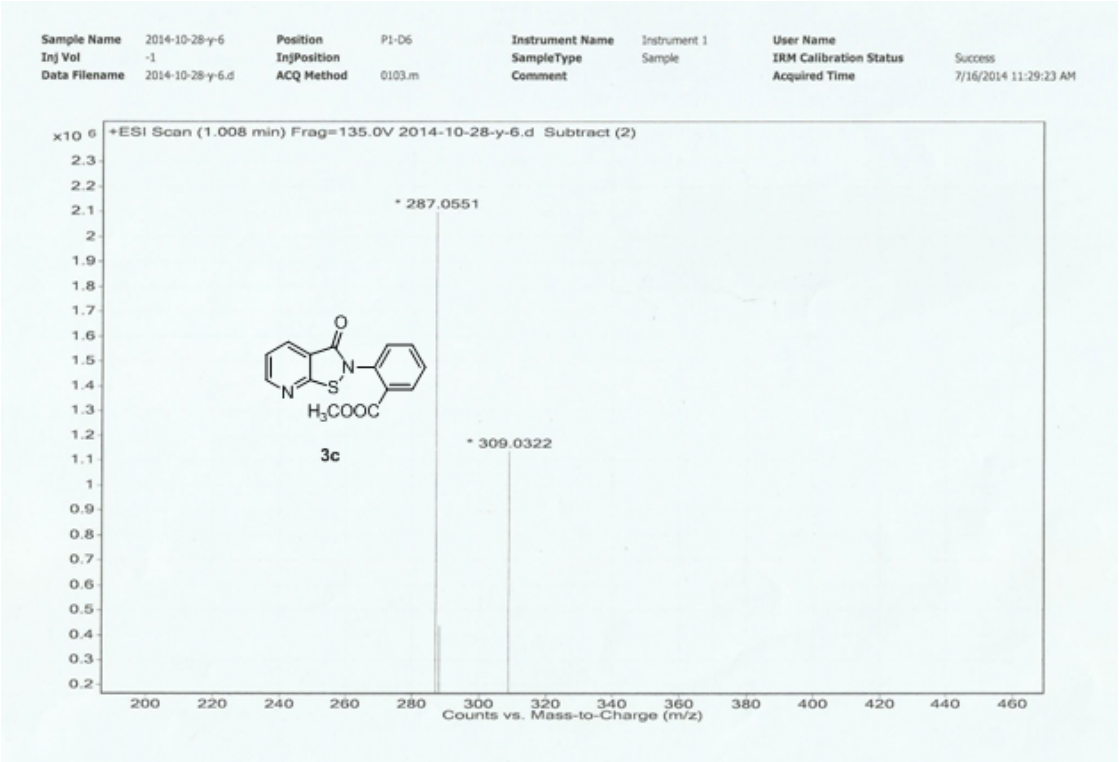
Mass Spectrum of Compound 3b



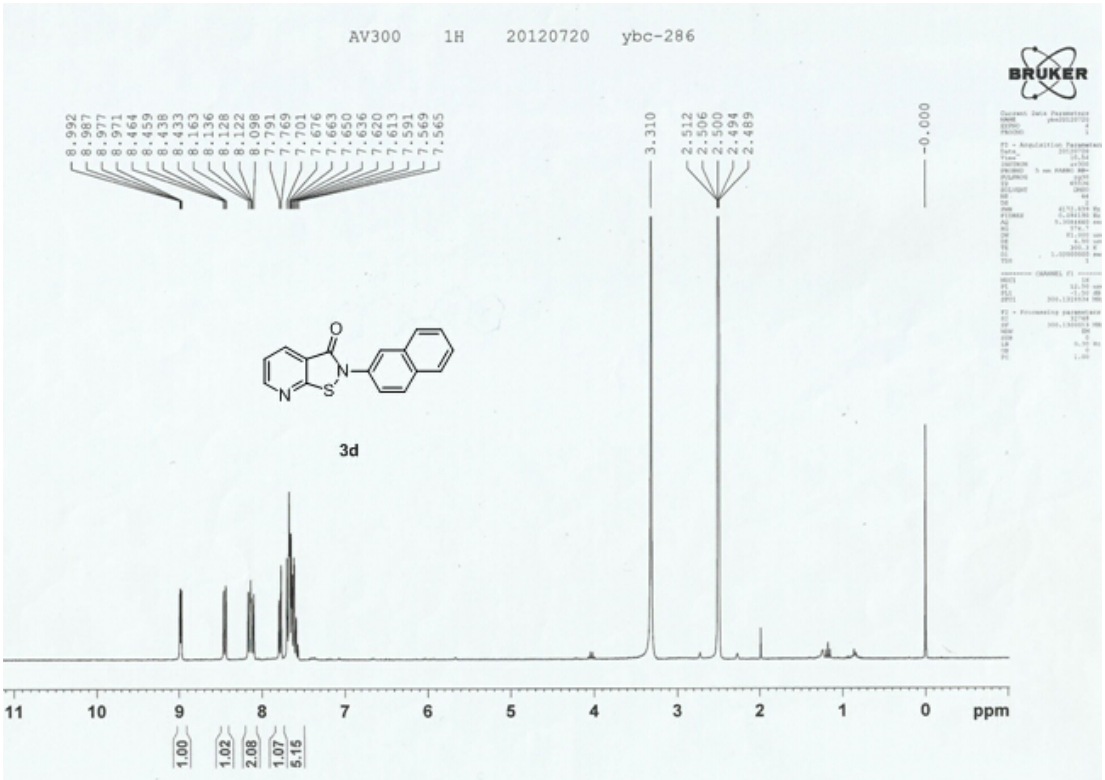
^{1}H NMR Spectra of Compound 3c in $CDCl_3$



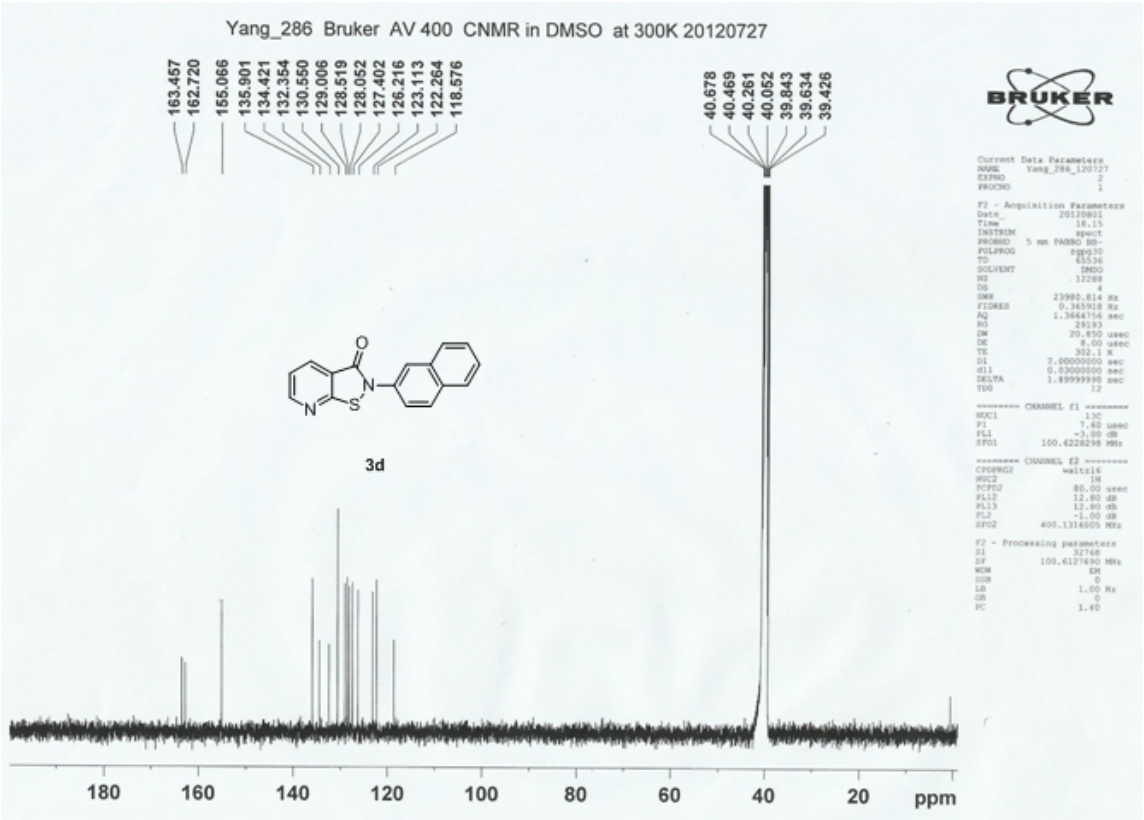
^{13}C NMR Spectra of Compound 3c in $CDCl_3$



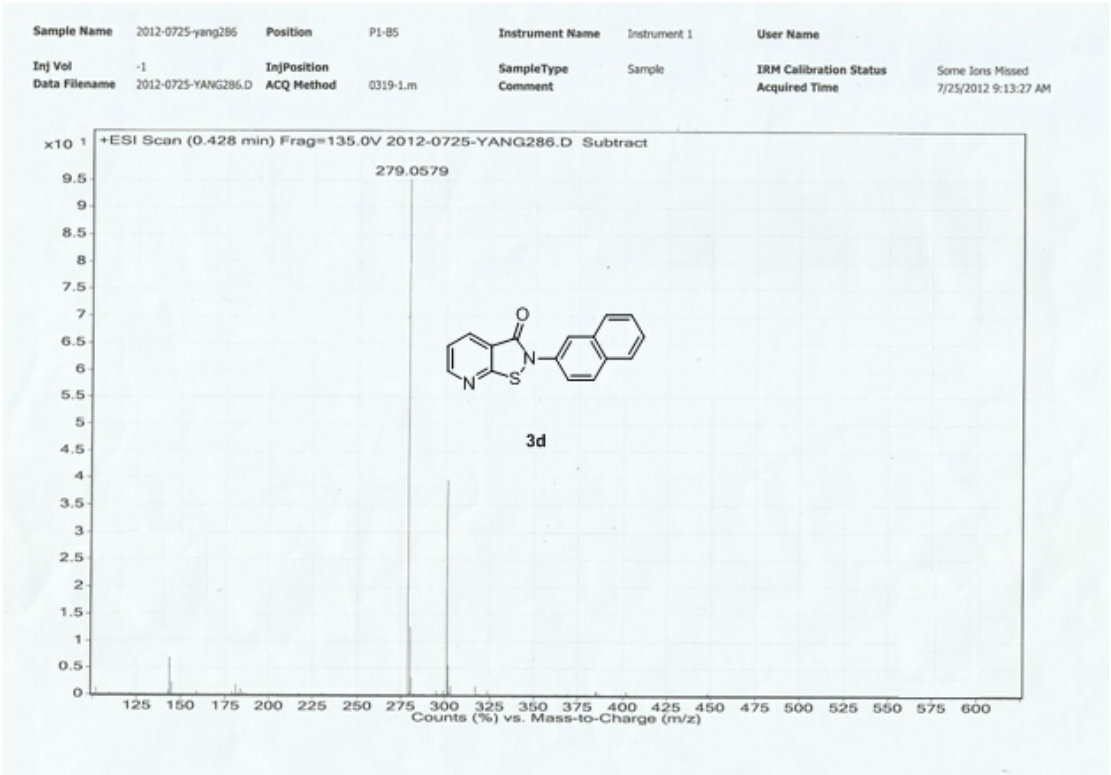
Mass Spectrum of Compound 3c



^{1}H NMR Spectra of Compound 3d in $CDCl_3$



^{13}C NMR Spectra of Compound 3d in $CDCl_3$



Mass Spectrum of Compound 3d