Supporting Information

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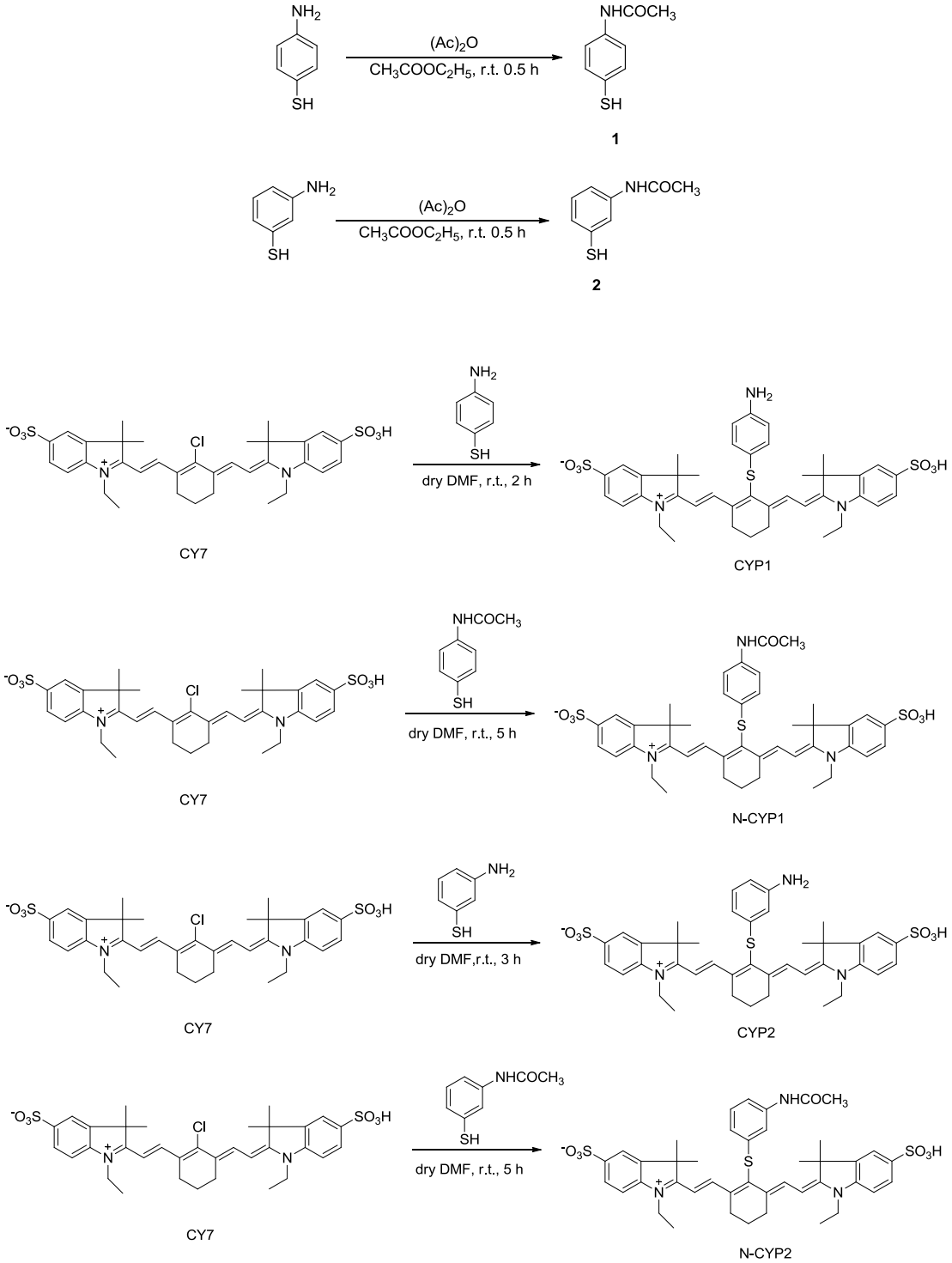
1. Materials and general methods.

All chemical reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel plates. Column chromatography was performed using silica gel (Hailang, Qingdao) 200-300 mesh. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, CD_3OD, TMS as internal standard). The mass spectra were collected at the Mass Instrumentation Facility of the Analysis and Research Center of ECUST.

2. **Synthesis.**



Scheme S1 Synthesis of corresponding thiophenols, CYPs and metabolite products N-CYPs.

Compound **1**[1]**.**

To a solution of 4-aminobenzenethiol (1.00 g, 7.99 mmol) in ethyl acetate (20 mL) was added acidic anhydride (0.89 g, 8.79 mmol) at 0 ^{o}C, and slowly warmed up to room temperature while stirring for 0.5 h. The reaction mixture was diluted with ethyl acetate, and washed with 1N HCl and brine solution. The organic layer was dried over

sodium sulfate, and the filtrate was concentrated and purified by silica column chromatography (PE: EA = 1:1). Yield: 1.12 g (83.9 %). ^{1}H NMR(400 MHz, $CDCl_3$): δ (ppm) 2.17 (s, 3H); 3.44 (s, 1H); 7.25 (d, *J* = 8.0 Hz, 2H); 7.40 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) : δ (ppm) 24.5, 120.7 (2C), 125.3, 130.7, 136.1, 168.4. MS (ESI) calcd for $C_8H_{10}NOS$ $[M+H]^+$: 168.0, found: 168.0. Ref[2]: ^{1}H NMR (300 Hz, $CDCl_3$): 2.16 (s, 3H), 3.43 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.7 Hz, 2H).

Compound **2.**

To a solution of 3-aminobenzenethiol (1.00 g, 7.99 mmol) in ethyl acetate (20 mL) was added acidic anhydride (0.89 g, 8.79 mmol) at 0 ^{o}C, and slowly warmed up to room temperature while stirring for 0.5 h. The reaction mixture was diluted with ethyl acetate, and washed with 1N HCl and brine solution. The organic layer was dried over sodium sulfate, and the filtrate was concentrated and purified by silica column chromatography (PE : EA = 1 : 1).Yield: 1.18 g (88.3 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.18 (s, 3H); 3.48 (s, 1H); 7.01 (d, *J* = 8.0 Hz, 1H); 7.17 (t, *J* = 8.0 Hz, 1H); 7.23 (d, *J* = 8.0 Hz, 1H); 7.57 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) : δ (ppm) 24.3, 117.5, 120.8, 125.0, 129.5, 131.7, 138.8, 169.7. MS (ESI) calcd for $C_8H_{10}NOS$ $[M+H]^+$: 168.0, found: 168.0.

CYPs and N-CYPs were prepared by the following same procedure[3].

All the glassware was dried at 120 ^{o}C for 2 hours. A 2-neck, 25 mL flask was loaded with CYP7 (100 mg, 0.15 mmol) and corresponding thiophenol (0.45 mmol) and then the vessel was purged with dry argon. After anhydrous DMF (5 ml) added by injector, the mixture was stirred under argon at room temperature in the dark for 1-5 hours, then cooled and poured into 200 ml of ether with violent stirring. The obtained solid was precipitated, filtered and dried under vacuum, then purified by column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 5 : 1) .

Synthesis of **CYP1.**

Yield: CYP1, 48.0 mg (40.1 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.28 (t, *J* = 6.8 Hz, 6H), 1.55 (s, 12H), 1.85-1.86 (m, 2H), 2.69-2.70(m, 4H), 4.19-4.21 (m, 4H), 5.17(s, 2H), 6.30 (d, *J* = 14.4 Hz, 2H), 6.52 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.73 (s, 2H), 8.73 (d, *J* = 14.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) : δ (ppm) 12.6, 21.0, 26.3, 27.7, 49.2, 102.0, 110.6, 115.5, 120.3, 121.2, 126.7, 129.0, 133.9, 141.0, 142.1, 145.9, 145.9, 148.3, 153.4, 172.0. HRMS(ESI): calculated $C_{40}H_{45}N_3O_6S_3$ $[M-H]^-$ 758.2392, measured: 759.2382.

Synthesis of **N-CYP1.**

Yield: N-CYP1, 38.0 mg (31.8 %). ^{1}H NMR(400 MHz, CD_3OD): δ (ppm) 1.38 (t, *J* = 6.8 Hz, 6H), 1.53 (s, 12H), 2.00-2.05 (m, 5H), 2.79 (t, *J* = 5.4 Hz, 4H), 4.19 (q, *J* = 6.8 Hz, 4H), 6.34 (d, *J* = 14.0 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.87-7.85 (m, 4H), 8.82 (d, *J* = 14.0 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 12.5, 22.2, 23.8, 27.3, 28.1, 40.5, 50.6, 102.9, 111.5, 121.5, 122.3, 127.9, 128.2, 128.3, 133.2, 135.8, 138.3, 142.7, 143.5, 144.5, 148.1, 157.8, 171.6, 174.2. HRMS(ESI): calculated $C_{42}H_{47}N_3O_7S_3$ $[M-H]^-$ 800.2498, measured: 800.2485.

Synthesis of **CYP2.**

Yield: CYP2, 49.0 mg (41.2 %). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.38 (t, *J* = 7.2 Hz, 6H), 1.54 (s, 12H), 2.00-2.02 (m, 2H), 2.77 (t, *J* = 5.6 Hz, 4H), 4.19 (q, *J* = 7.2 Hz, 4H), 6.32 (d, *J* = 14.0 Hz, 2H), 6.44 (d, *J* = 8.0 Hz, 1H), 6.52 (d, *J* = 8.0 Hz, 1H), 6.62 (s, 1H), 6.98 (t, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.85-7.87 (m, 4H), 8.83 (d, *J* = 14.0 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) : δ (ppm) 12.5, 22.2, 27.3, 28.1, 40.5, 50.6, 102.8, 111.4, 113.6, 114.1, 116.4, 121.5, 128.1, 131.2, 135.9, 138.9, 142.8, 143.4, 144.6, 148.4, 150.5, 154.5, 174.2. HRMS(ESI): calculated $C_{40}H_{45}N_3O_6S_3$ $[M-H]^-$ 758.2392, measured: 759.2376.

Synthesis of **N-CYP2.**

Yield: N-CYP2, 38.0 mg (31.8 %). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 1.39 (t, *J* = 7.2 Hz, 6H), 1.53 (s, 12H), 2.04-2.07 (m, 2H), 2.11 (s, 3H), 2.81 (t, *J* = 6.0 Hz, 4H), 4.21 (q, *J* = 7.2 Hz, 4H), 6.36 (d, *J* = 14.0 Hz, 2H), 7.03 (d, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.79 (s, 1H), 7.86-7.88 (m, 4H), 8.82 (d, *J* = 14.0 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) : δ (ppm) 11.0, 20.7, 22.6, 25.9, 26.6, 39.1, 48.5, 49.1, 101.6, 110.0, 116.6, 116.9, 120.0, 121.2, 126.7, 129.5, 134.6, 137.8, 140.0, 141.3, 142.0, 143.1, 146.6, 151.8, 170.3, 172.7. HRMS(ESI): calculated $C_{42}H_{47}N_3O_7S_3$ $[M-H]^-$ 800.2498, measured: 800.2482.

3. Spectroscopic materials and methods.

Double distilled water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in 0.05 M Tris-HCl buffer (containing 1% DMSO unless special instructions) (pH = 7.5 at 37 ºC). All pH measurements were made with a Sartorius basic pH-Meter PB-10. Absorption spectra were recorded using a Varian Cary100 Bio UV-Visible spectrophotometer. Fluorescence spectra were recorded using a Varian Cary Eclipse scanning spectrofluorometer equipped with a Xenon flash lamp. Samples for absorption and fluorescence measurements were contained in 1 cm×1 cm quartz cuvettes (3.5 mL volume) or 40 μL Fluor Micro Cell (Varian Cary FLR). All cell images were taken Olympus IX 51 with Xenon lamp and Olympus digital camera.

4. N-acetyltransferase activity assay[4] and corresponding research**.**

NAT2 and NAT1 activity experiments were performed in 40 μL Fluor Micro Cell. Recombinant forms of human *NAT2* (2.5 mg/mL in Tris-HCl buffer solution, pH = 7.5, Sigma-Aldrich) and *NAT1* (2.5 mg/mL in Tris-HCl buffer solution, pH = 7.5, BD Biosciences) expressed from infected BTI-TN-5B1-4 insect cells. Stock solutions of CYP1 and N-CYP1 were prepared in pure DMSO (10^{-3} M) and diluted with enzyme assay buffer (0.05 mM acetyl CoA, Tris-HCl, pH 7.5). Concentrations of CYPs were all 10 μM. The final enzyme concentrations were respectively 2.5, 5.0, 10.0, 15.0, 20 μg/mL. All the reactions performed at 37 ºC. Fluorescence measurements were taken at 10 min intervals from 0 to 120 min on a Varian Cary Eclipse scanning spectrofluorometer with excitation at 780 nm and emission intensity collected from 795 nm to 880 nm. The curves were slightly smoothed.

At first, the response of CYP1 to acetyl-CoA was investigated. As shown in figure S1, addition of acetyl-CoA did not cause obvious increase of fluorescence. Hence, the acetyl-CoA wound not interfere the detection of NAT2.

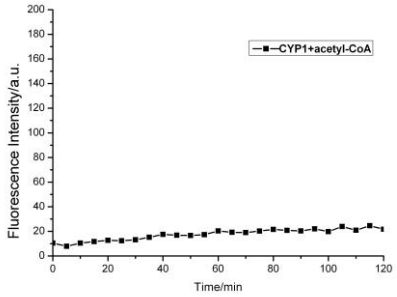


Figure. S1 The response of CYP1 (10 μM) to Acetyl CoA. The reaction was performed at 37 ^{o}C in buffer (0.05 M Tris-HCl, pH = 7.5, with 1% DMSO, and 0.05 mM acetyl-CoA). The excitation wavelength was 780 nm. The fluorescence emission was at 812 nm. Silt (10, 10).

The detection limit (CDL) was calculated by IUPAC assay. CDL = $3sbm^{-1}$(sb is the ration signal and noise, m is the slope of linear eqution). The sb was detection by N-CYP1 at 10 μM. The standard deviation sb was 2.1 a.u.. According the linear equation (Y = 20.62+6.02X) of CYP1 to NAT2 concentration (figure S2), the slope was 6.02. Hence, the detection limit was 1.0 μg/mL.

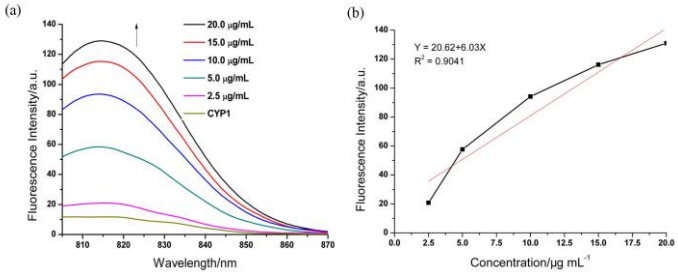
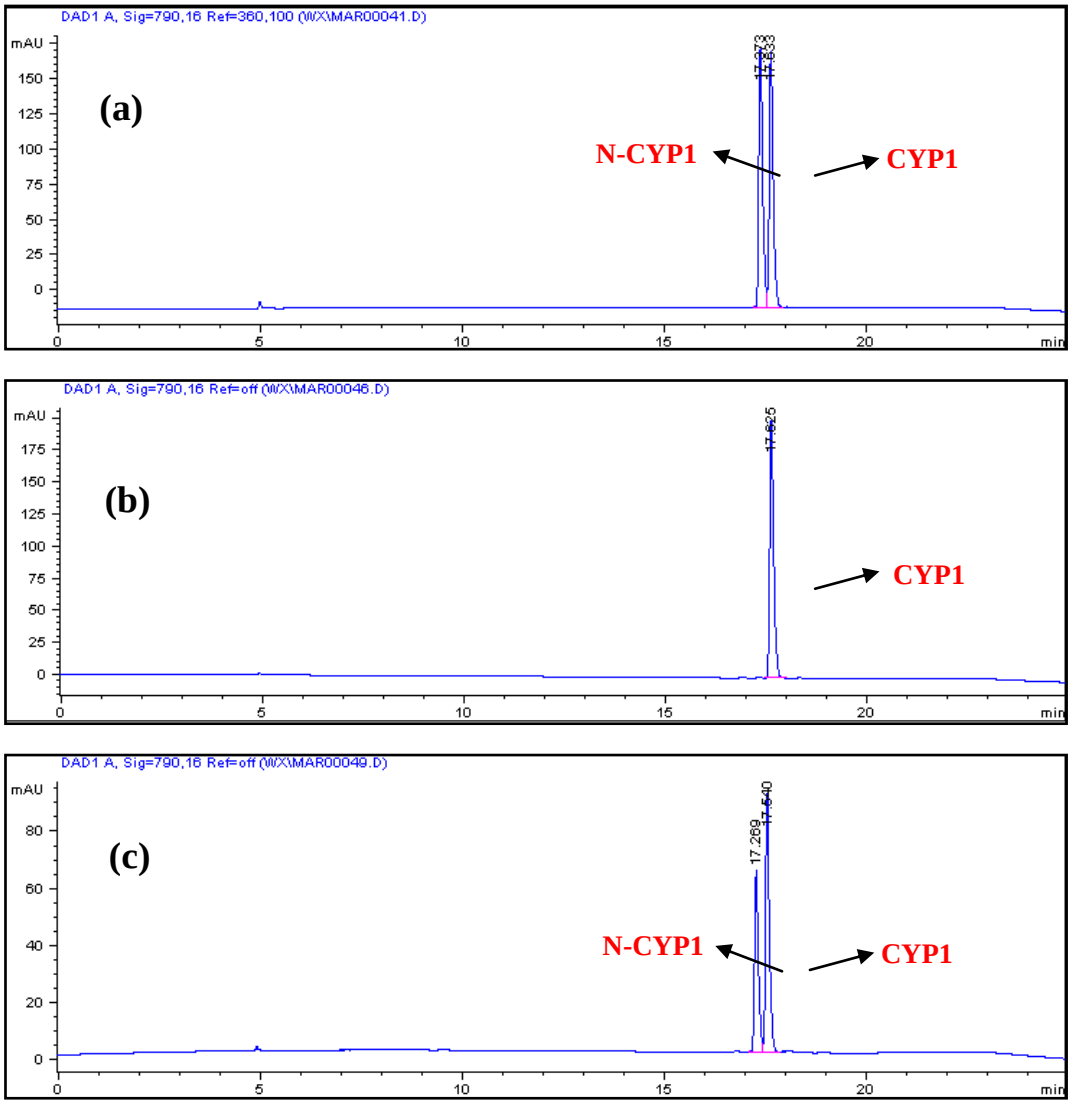


Figure. S2 Quantitation of NAT2 cytosol by CYP1 (10 μM). The reaction was performed at 37 ^{o}C in buffer (0.05 M Tris-HCl, pH = 7.5, with 1% DMSO, and 0.05 mM acetyl-CoA). The excitation wavelength was 780 nm. The fluorescence emission was at 812 nm. Silt (10, 10).

5. HPLC for N-acetyltransferase 2 metabolism assay.

HPLC was performed using a ZoRBAX XDB-C18 column (Analytical 4.6 × 250 mm 5-micrm, Agilent) with a HP 1100 system. The HPLC mobile phase was a mixture of acetonitrile and buffer (acetic acid and ammonium acetate pH = 6.0). HPLC conditions were as follows: 0-16 min, a gradient of 0-40% acetonitrile; 16-20 min, 40% acetonitrile; 20-25 min, a gradient of 40-100% acetonitrile. The flow rate was 1 mL/min, and detected by UV (790 nm). The reaction solution of CYP1 (10 μM) and Acetyl CoA (0.05 mM), NAT2 (10 μg/mL) was incubated with or without irreversible inhibitor Iodoacetamide (10 μM, J&K Chemica) for 30 min.



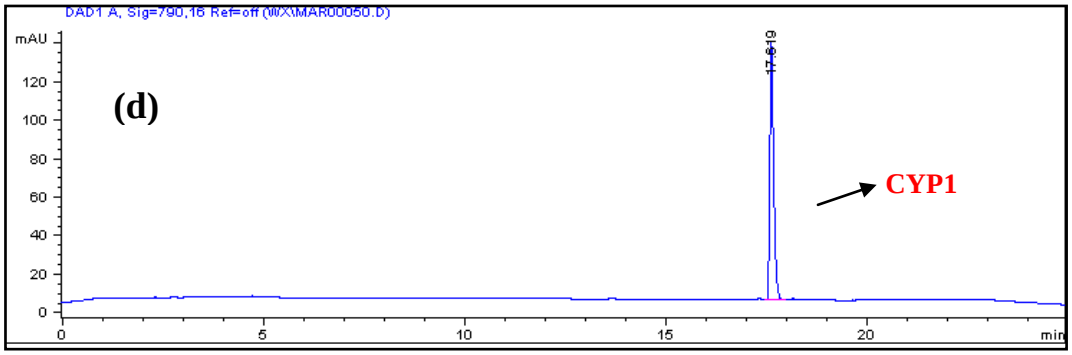


Figure. S3 HPLC profiles (detected by UV at 790 nm) of a) CYP1 (10 μM) and N-CYP1 (10 μM) or b) CYP1 (10 μM); c) and d), NTA2 solution (10 μg/ mL) incubating with Acetyl CoA (0.05 mM) and CYP1 (10 μM) without / with irreversible inhibitor Iodoacetamide for 30 min, respectively.

6. NAT2 activity assay in tissue homogenate samples.

An ICR mouse around 30 g was anaesthetized by intraperitoneal injection (with 1% pentobarbital sodium 60 mg/kg). After transcardial perfusion with saline, the tissues from liver, Kidney, Heart, Spleen, Brain, lung, Testicle, tongue and muscle were dissected out. Then the tissues were homogenized in phosphate buffered saline (PBS, pH 7.4) by a homogenizer (10^4 rpm for 10 min) on ice bath. The homogenates were centrifuged (1.3×10^4 rpm, 15 min) at 4 ^oC[5, 6]. The supernatant was taken for testing NAT2 activity. The concentration of CYP1 was also 10 μM. The hepatic homogenate were diluted serially at the concentration of 2.5, 5.0, 10.0, 15.0 and 20.0 mg/mL to determine the concentration response of tissue NAT2 on CYP1. Then we chose the concentration of 10.0 mg/mL to examine the time response within 2 h. To compare NAT2 activity levels in different tissues, 10.0 mg/mL homogenates were incubated with CYP1 for 30 min.

7. N-acetyltransferase 2 fluorescence imaging assay *in vivo*.

Male ICR mice weighing 25-30 g, supplied by Shanghai Laboratory Animal Research Center, were housed at 24 ^{o}C with a 12 h light-dark cycle. Mice were allowed free access to food and water. All experimental procedures carried out in this study were performed in accordance with the guidelines of Care and Use of Laboratory Animals of China for animal experimentation.

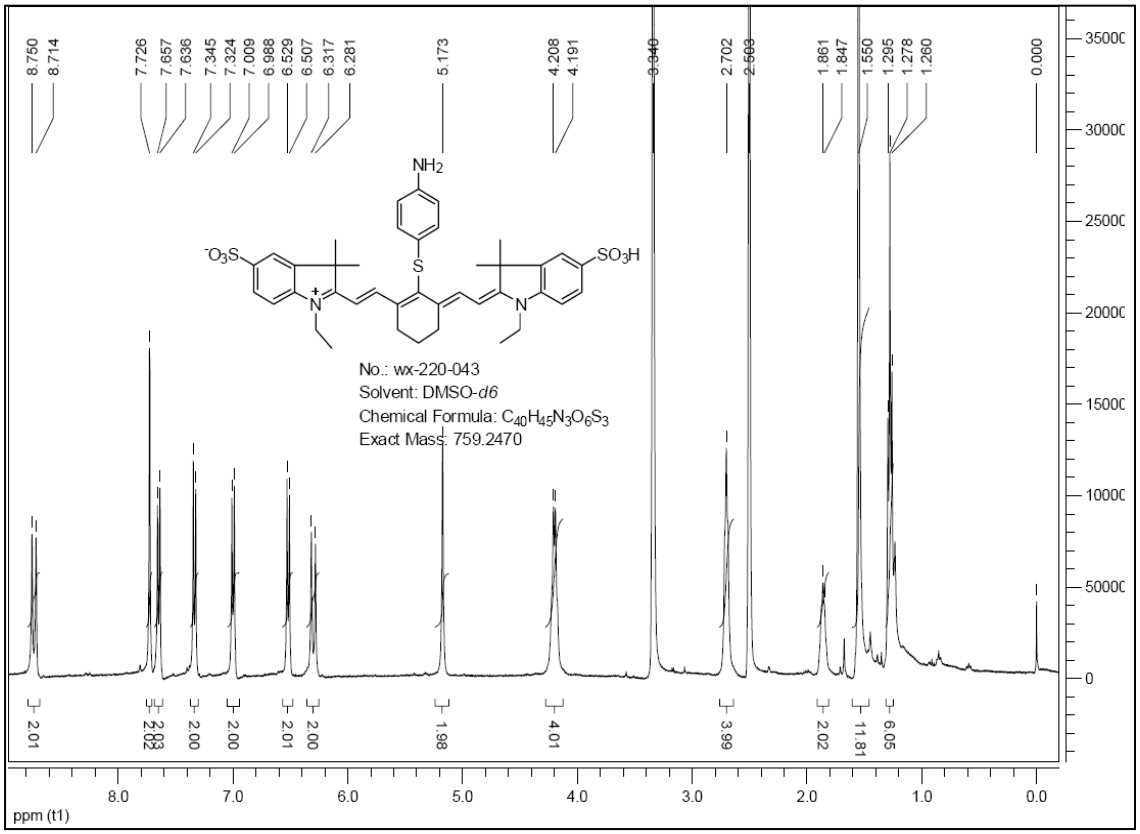
We examined imaging at the whole-body level. CYP1 probe (1.5 mg/kg) was administered to mice by tail intravenous injection. NIRF image were obtained using Kodak In-Vivo Multispectral Imaging System with a 770 nm filter for excitation and a 830 nm filter for emission. Imaging was performed 10 min after injection of CYP1 probe. During the imaging process, mice were kept on the imaging stage under anesthesia (1% pentobarbital sodium, 60 mg/kg) [7, 8]. Carestream MI SE was used for image analysis.

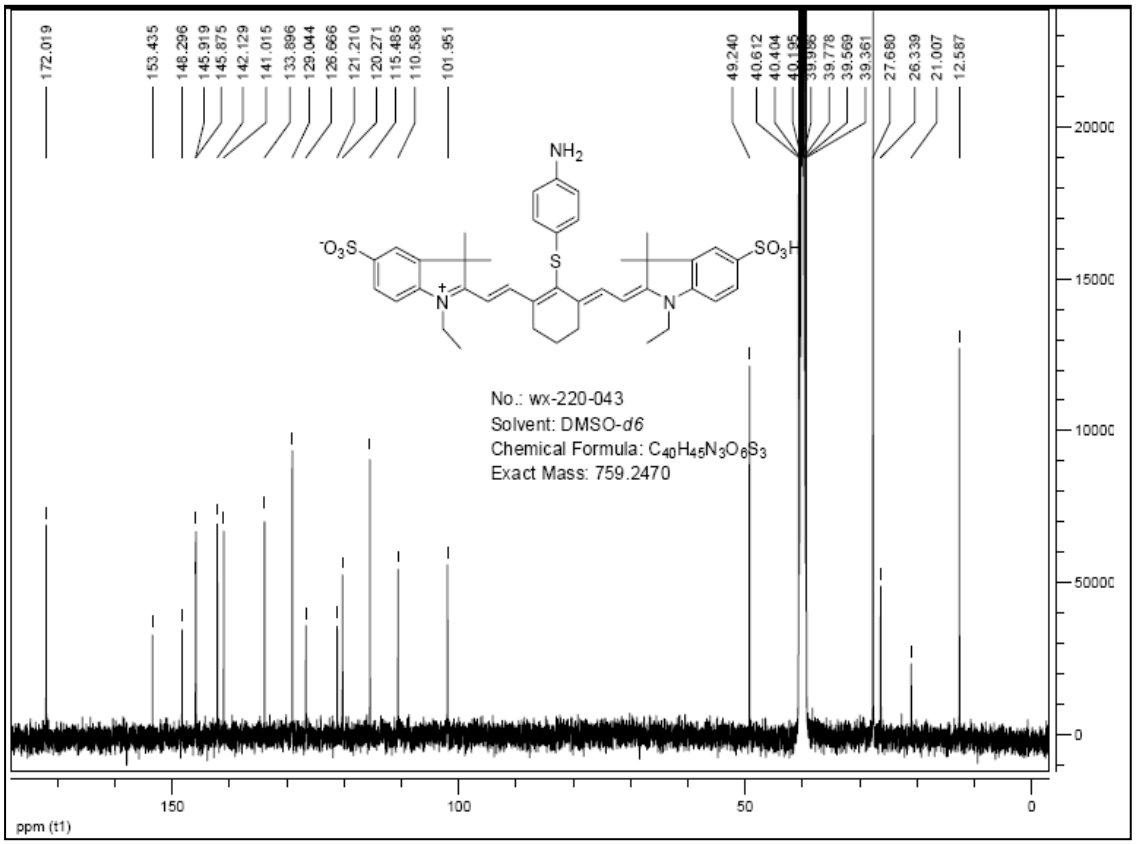
8. Cytotoxicity of CYP1 and N-CYP1 to normal cell line.

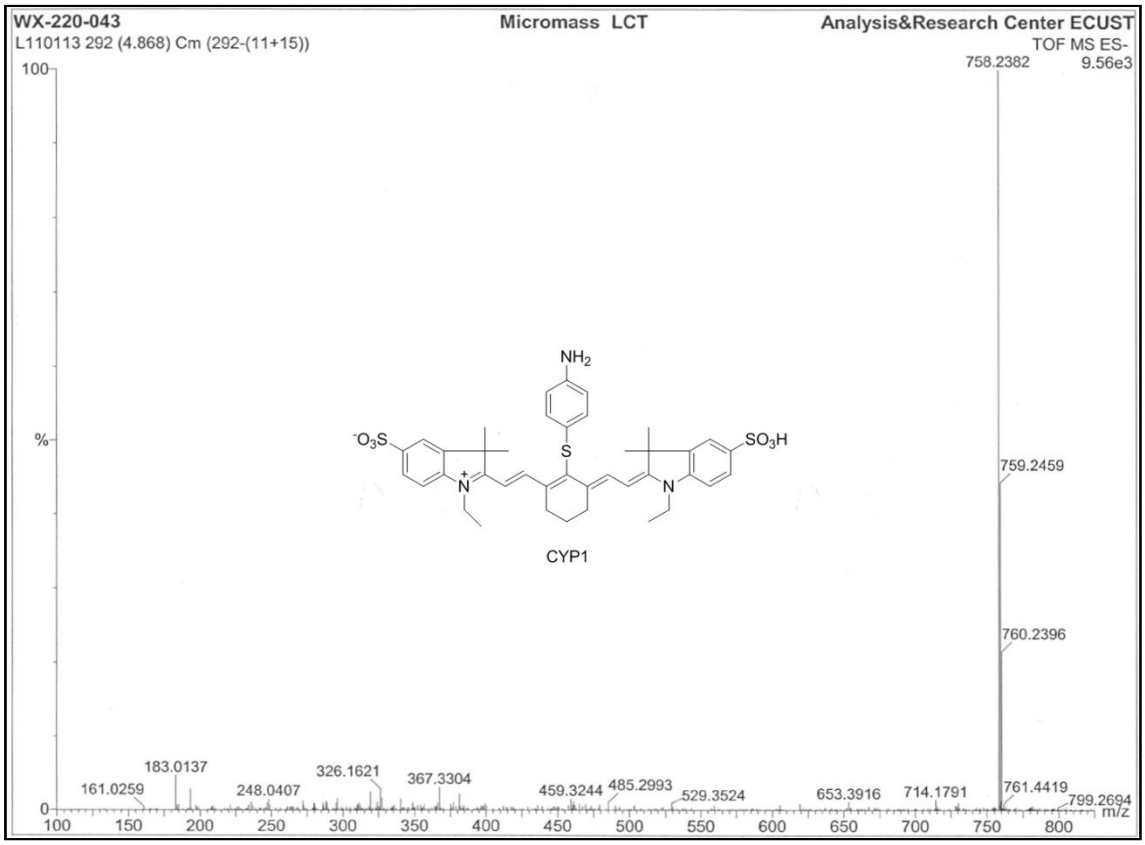
The IC_{50} of CYP1 and N-CYP1 were both more than 100 μM. Hence, the probe and the products were almost nontoxic to the normal cell line.

MTT Assay: WI-38 cells (human lung fibroblast cell line), purchased from the American Type Culture Collection(ATCC), were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. Cells were seeded into 96 wells plates at a density of 1×10^5 per mL in Minimum Essential Medium(MEM), supplemented with 10% (v/v) heat –inactivated fatal bovine serum, 2 mM L-glutamine. Experiments were carried out 24 h after cells were seeded.CYP1/N-CYP1 was freshly prepared in dimethyl sulfoxide (DMSO) and diluted in serum-free MEM prior to each experiment. Cells were exposed to CYP1 and cultured continuously for 24 h. Cell survival was evaluated by the ability to reduce 3-[4,5-dimethylthylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), an indication of metabolic activity. 20 μL MTT stock solution (5 mg/ml) dissolved in PBS was added into each well and incubated for another 4 h at 37 ^oC. Finally, the medium was replaced by 100 μL dimethyl sulfoxide (DMSO). The amount of MTT formazan was quantified by determining the absorbance at 490 nm. All data were expressed as a percent of control value and mean ± SEM of three independent experiments. Statistical analyses were performed with one-way ANOVA followed by a post hoc LSD test.

9. The characterization data of all compounds.



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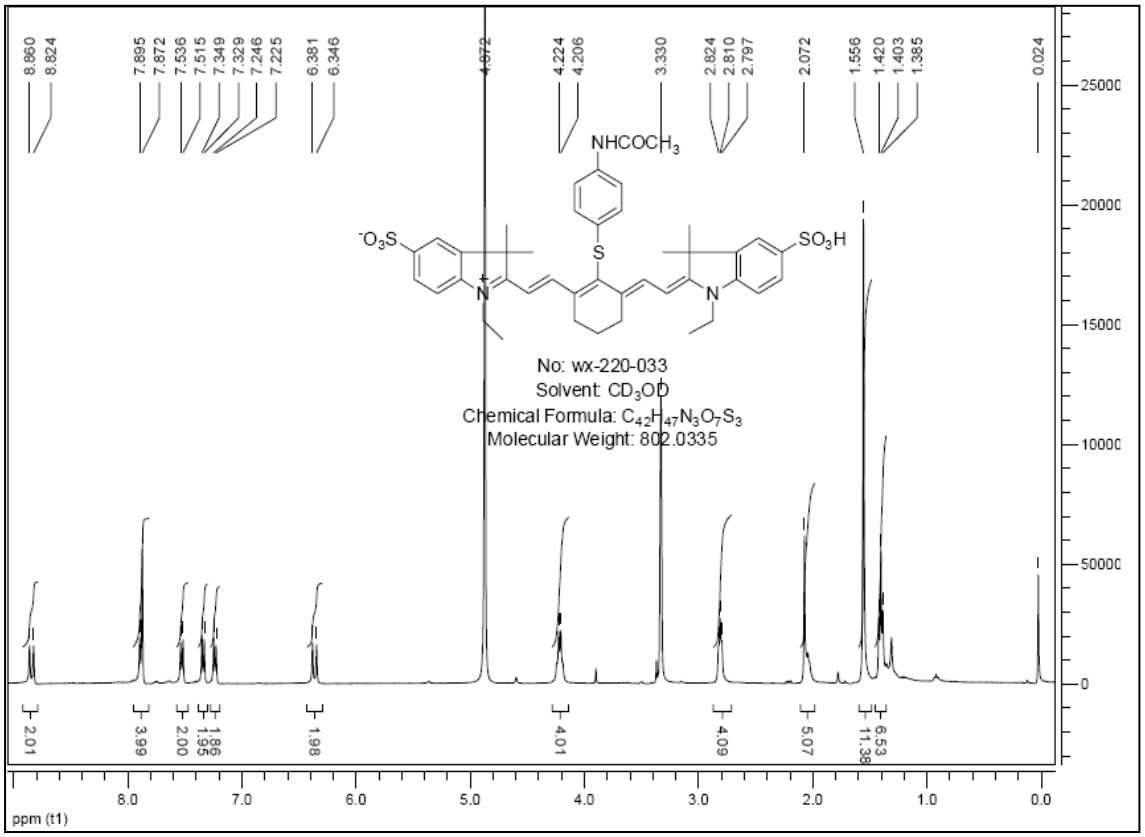
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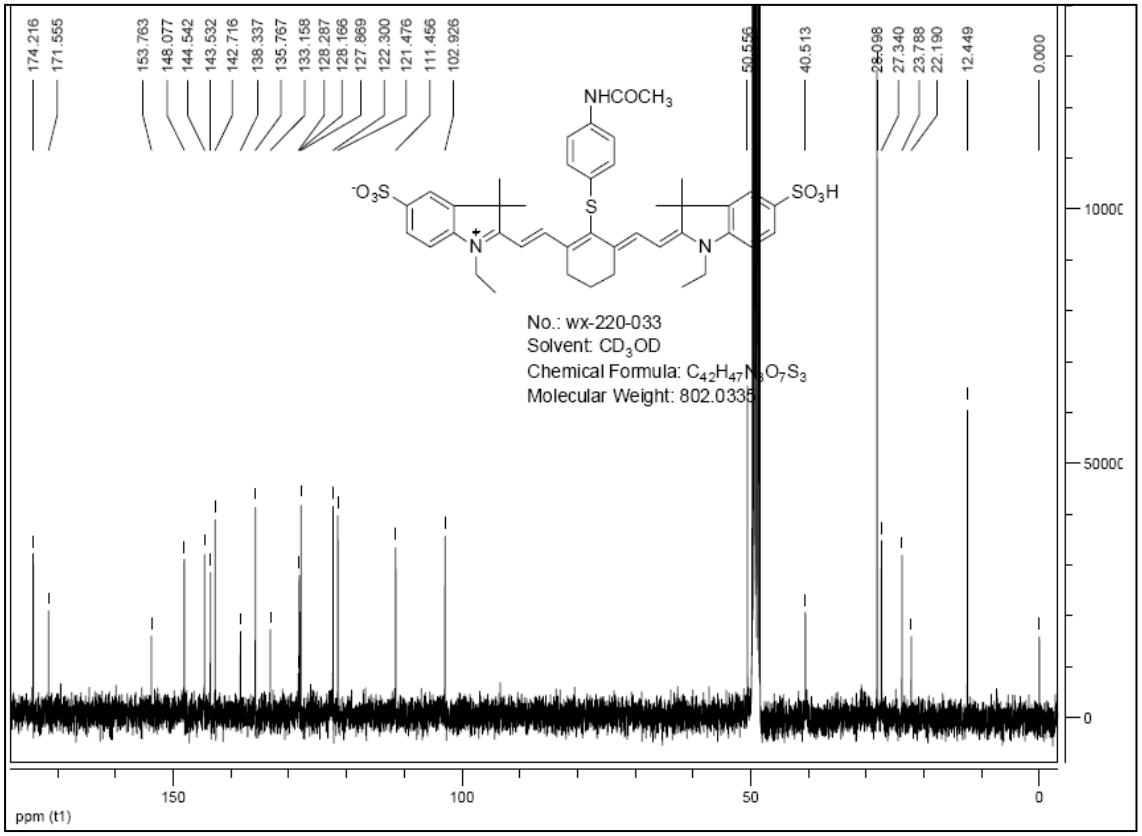
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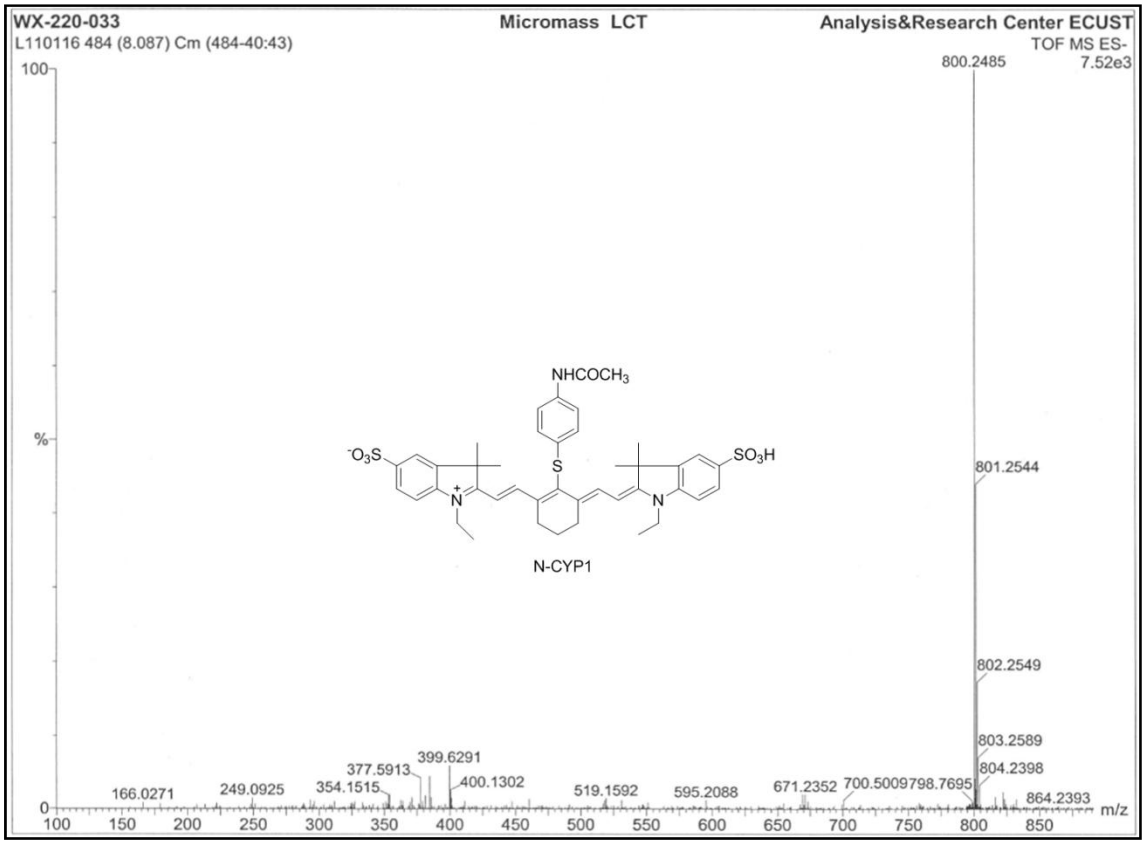
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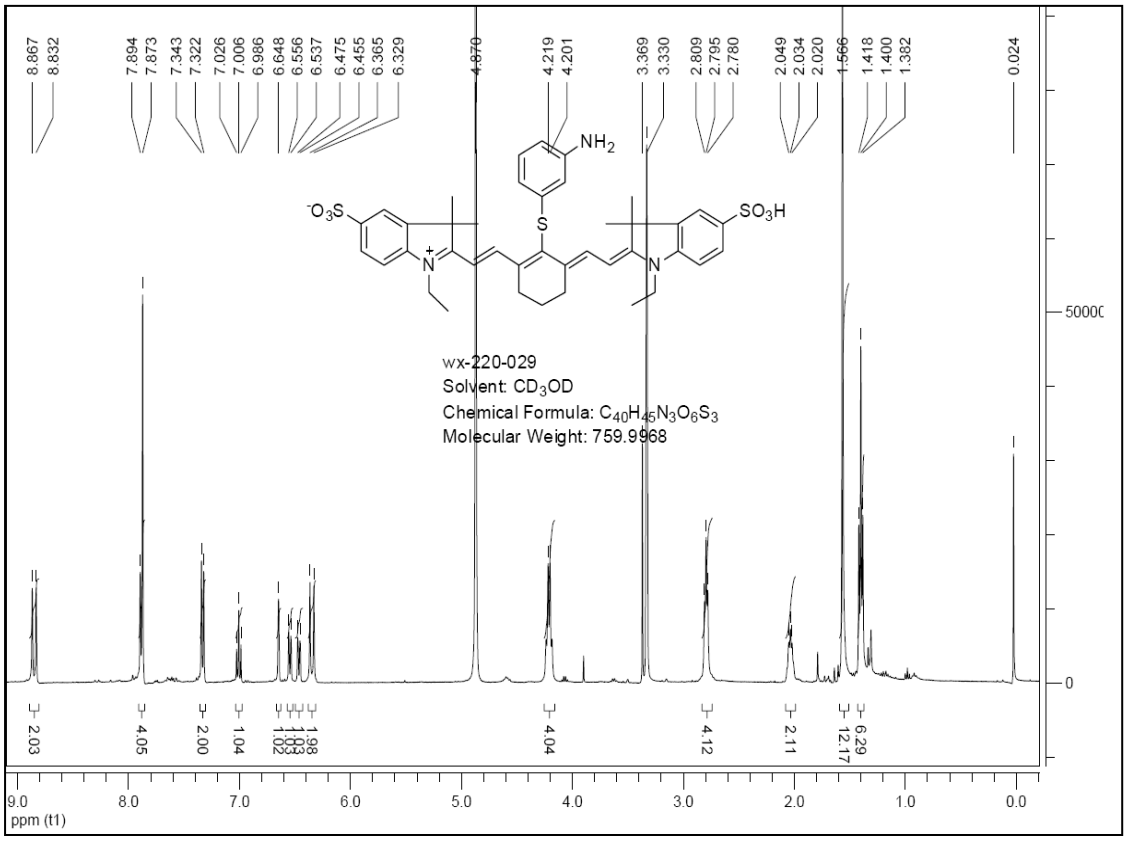
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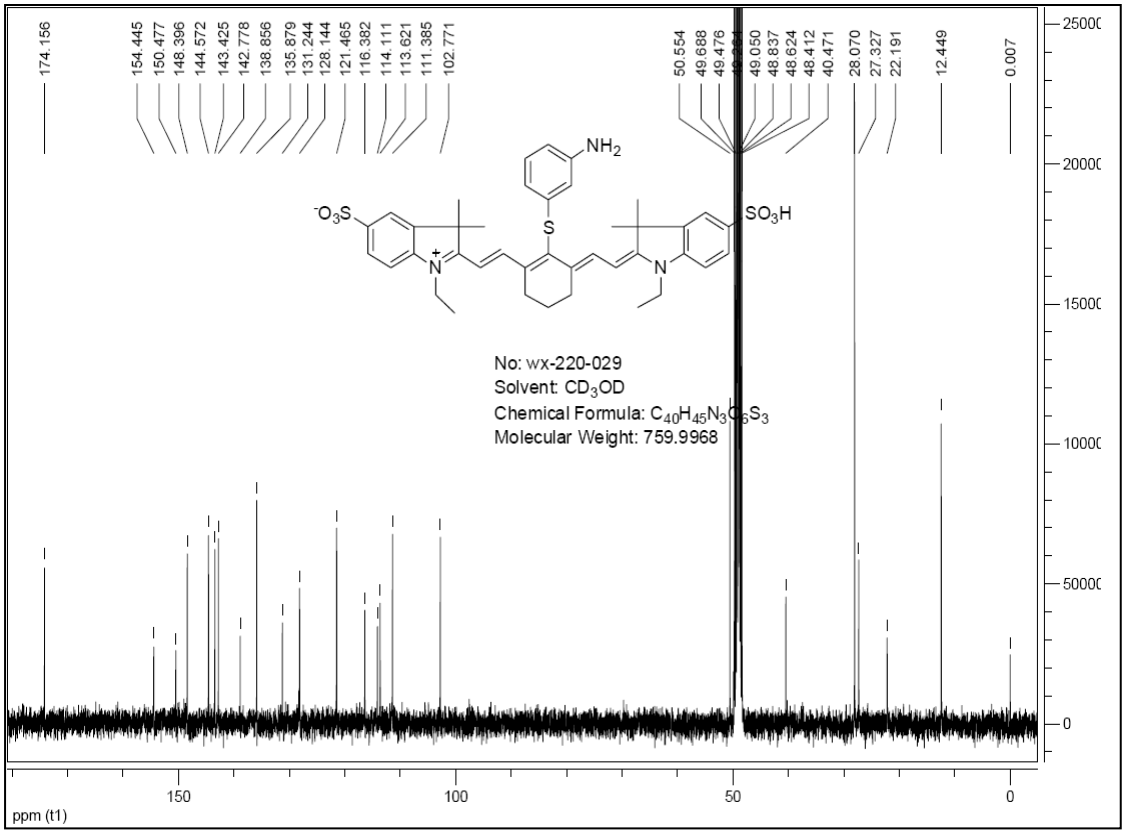
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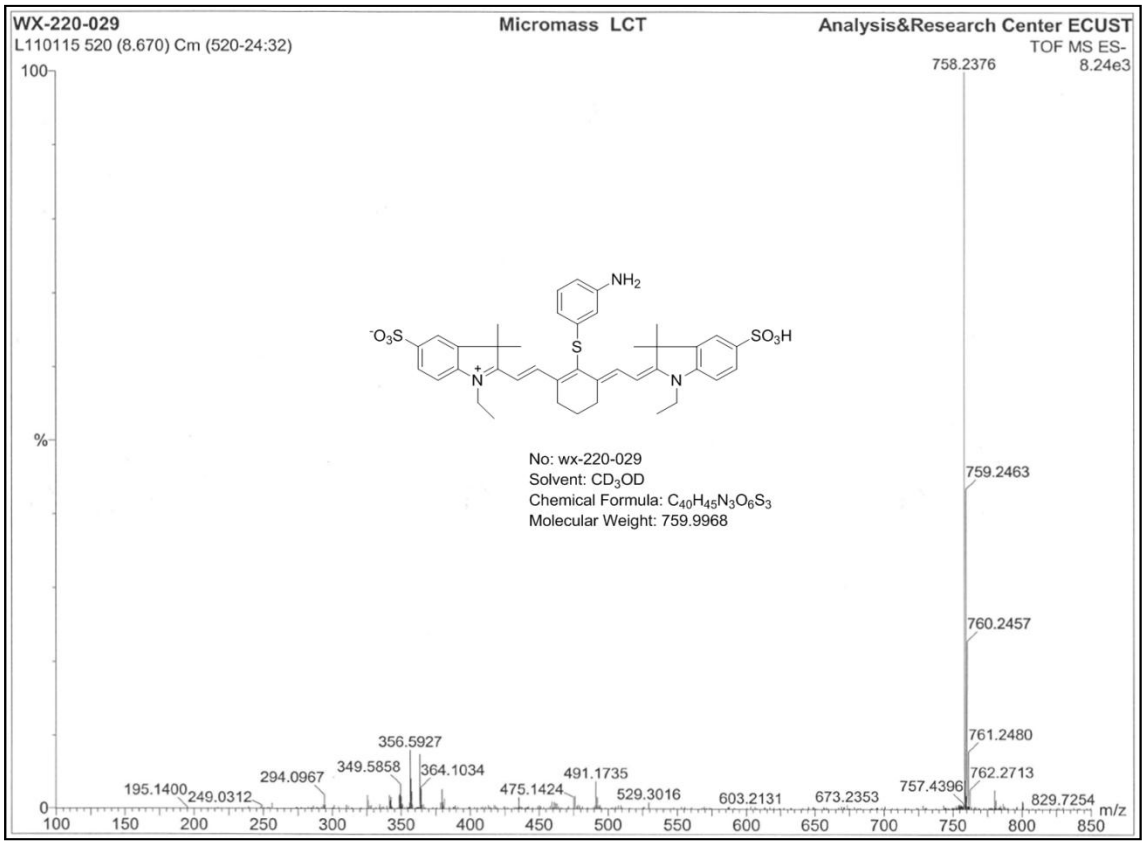
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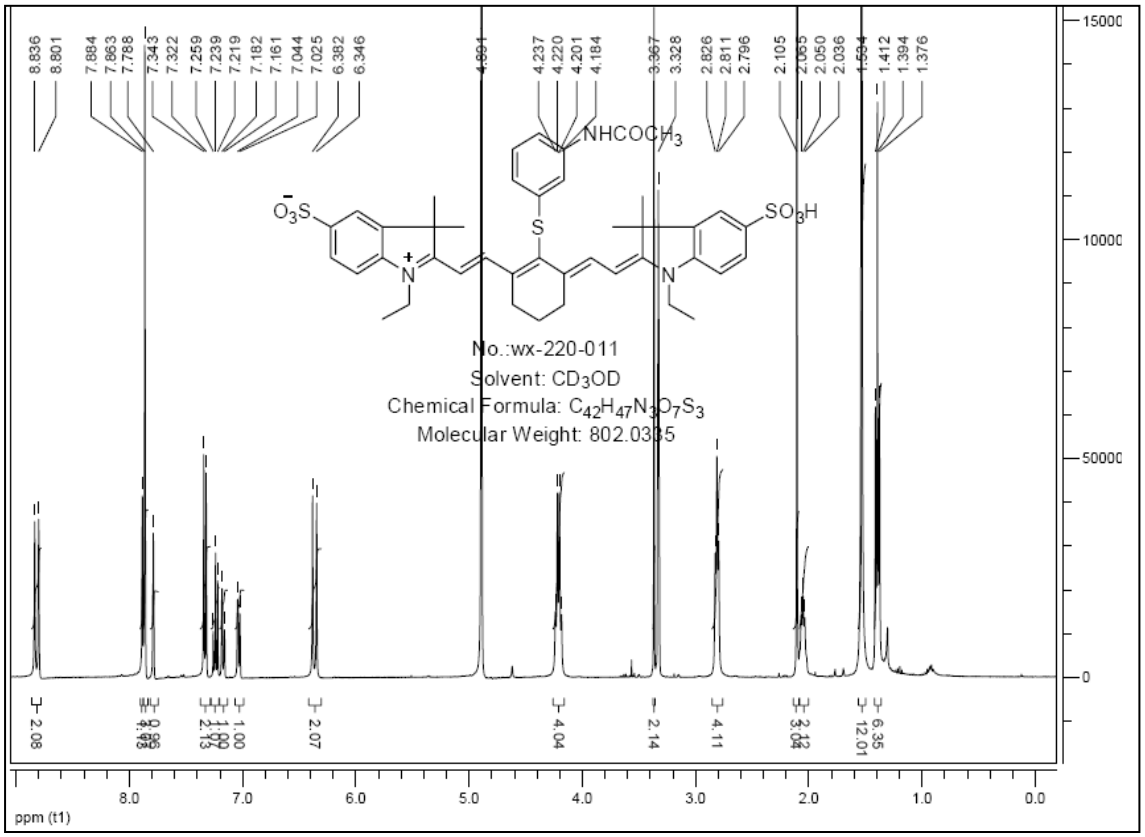
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8.801
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7.863
7.788
7.343
7.322
7.259
7.239
7.219
7.182
7.161
7.044
7.025
6.382
6.346
4.881
4.237
4.220
4.201
4.184
3.367
3.328
2.826
2.811
2.796
2.105
2.065
2.050
2.036
1.534
1.412
1.394
1.376
NHCOCH3
O3S
SO3H
S
N
No.:wx-220-011
Solvent: CD3OD
Chemical Formula: C42H47N3O7S3
Molecular Weight: 802.0335
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10000
50000
0
2.08
0.96
2.13
1.00
2.07
4.04
2.14
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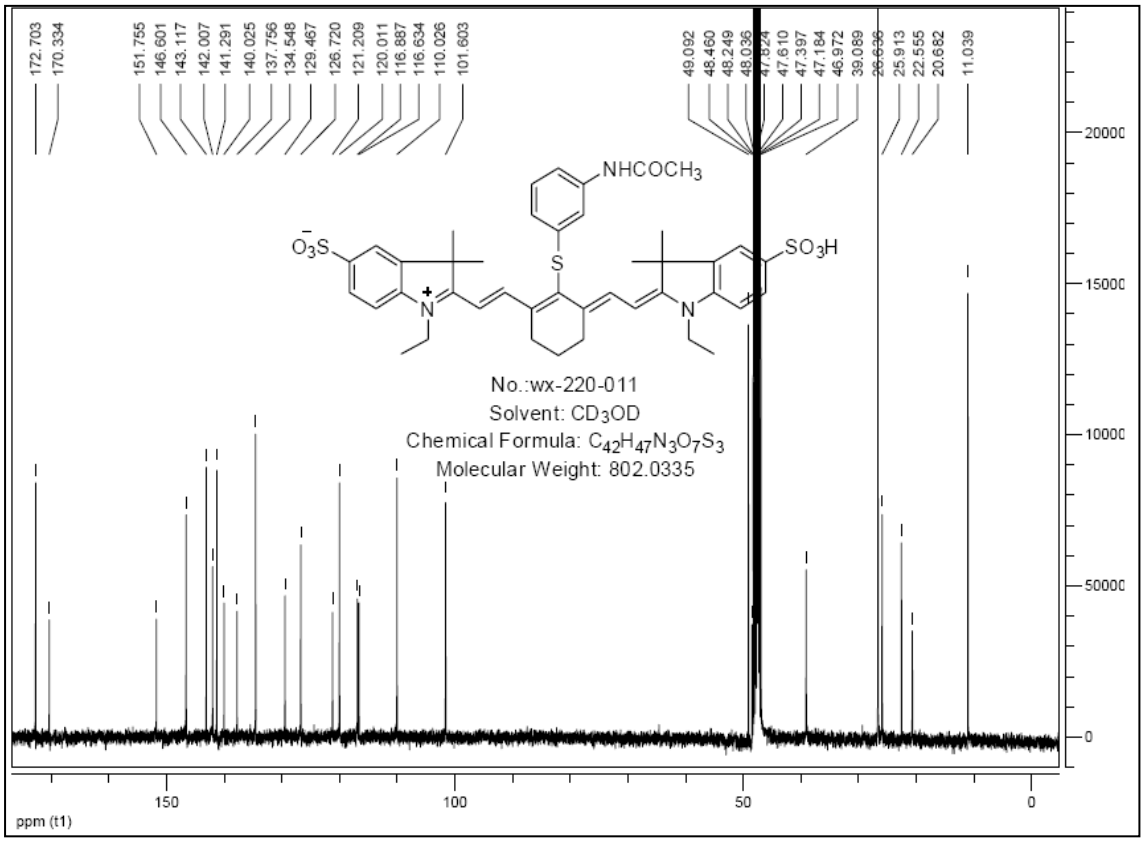
12.01
6.35
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
ppm (t1)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170.334
151.755
146.601
143.117
142.007
141.291
140.025
137.756
134.548
129.467
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121.209
120.011
116.887
116.634
110.026
101.603
49.092
48.460
48.249
48.036
47.824
47.610
47.397
47.184
46.972
39.089
26.636
25.913
22.555
20.682
11.039
NHCOCH3
O3S
SO3H
S
N
N
No.:wx-220-011
Solvent: CD3OD
Chemical Formula: C42H47N3O7S3
Molecular Weight: 802.0335
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50000
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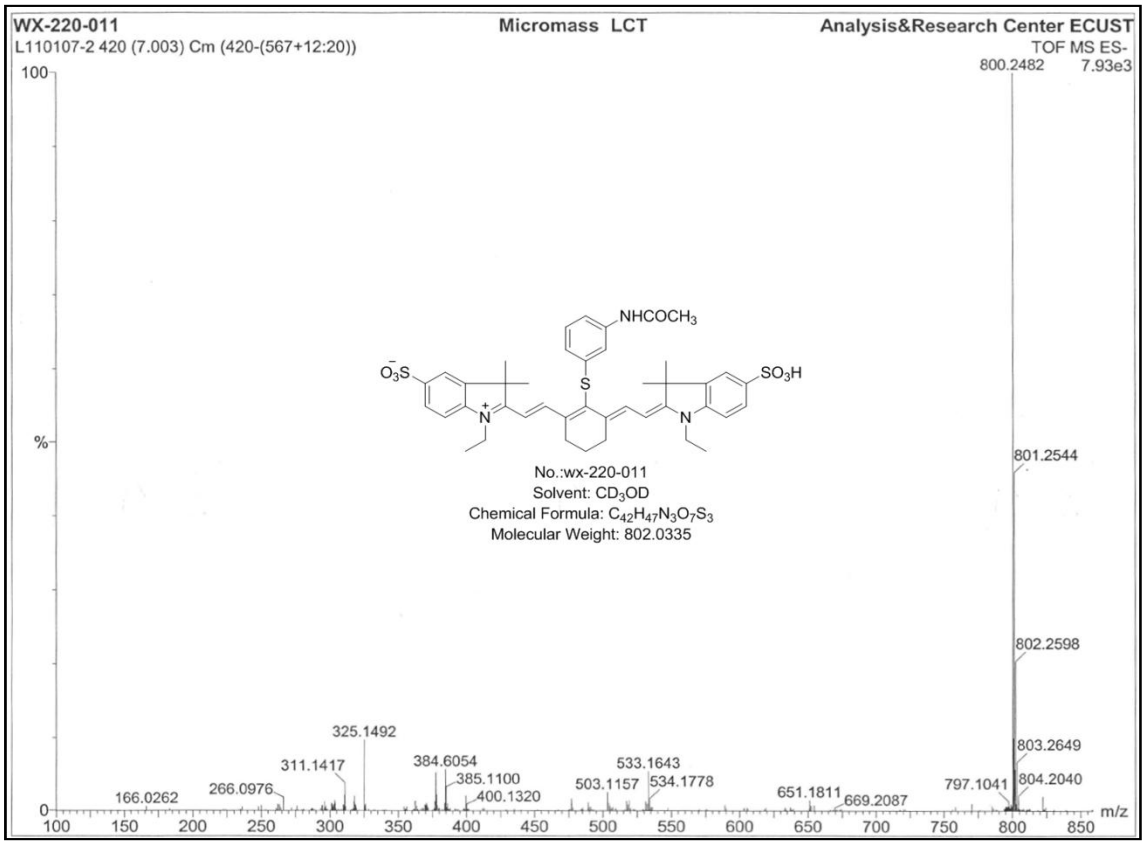
50
0
ppm (t1)


WX-220-011
Micromass LCT
Analysis&Research Center ECUST
L110107-2 420 (7.003) Cm (420-(567+12:20))
TOF MS ES-
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7.93e3
100
%
0
NHCOCH3
O3S
SO3H
S
N
N
No.:wx-220-011
Solvent: CD3OD
Chemical Formula: C42H47N3O7S3
Molecular Weight: 802.0335
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669.2087
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
m/z

10. References

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