Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Identification of Thienopyridine Carboxamides as Selective Binders of HIV-1 Trans Activation Response (TAR) and Rev Response Element (RRE) RNAs

Xue-Dong Li,[a,b] Li Liu,[a,b] and Liang Cheng*[,a,b]

[a] *Beijing National Laboratory for Molecular Sciences (BNLMS), CAS Key Laboratory of Molecular Recognition and Function, CAS Research/Education Center for Excellence in Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*

[b] *University of Chinese Academy of Sciences, Beijing 100049, China*

chengl@iccas.ac.cn

Table of contents

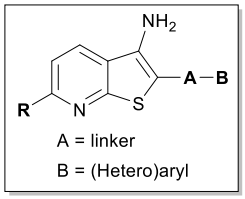
1. General information

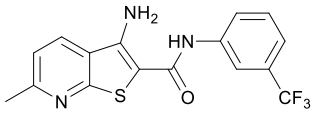
Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. Column chromatographic purification of products was carried out using silica gel (200~300 mesh). Tetrahydrofuran (THF), toluene and diethyl ether (Et_2O) were distilled from sodium/benzophenone. Dry dichloromethane (CH_2Cl_2) was distilled over calcium hydride. *N*,*N*-dimethylformate (DMF), dimethyl sulphoxide (DMSO) were dried over molecular sieves 3Å. All reactions were carried out under nitrogen atmosphere in oven-dried glassware.

IR spectra were recorded on a Thermofisher-Nicolet6700 FT-IR spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 with Bruker Avance-400 or 500 (500 MHz) spectrometer. C hemical shifts are given in ppm relative to tetramethylsilane (TMS). Multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m). High-resolution mass spectralanalysis (HRMS) data were measured on a Thermo Exactive by means of the ESI technique.

2. Synthesis details and characterization

2.1 A full list of all synthesized compounds

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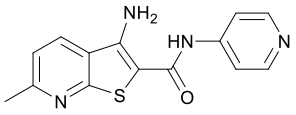
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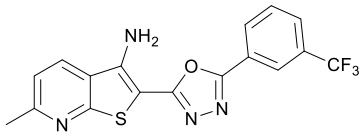
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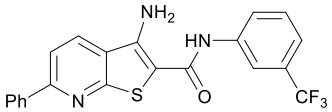
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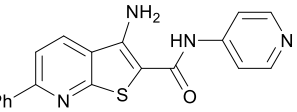
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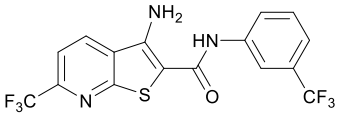
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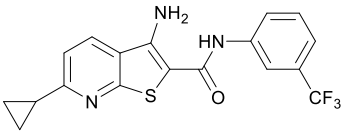
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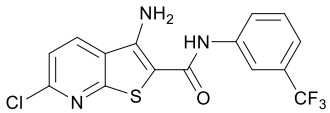
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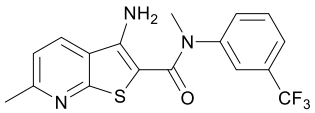
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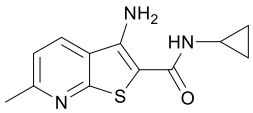
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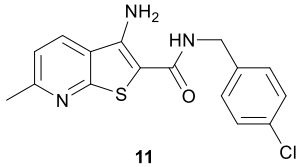
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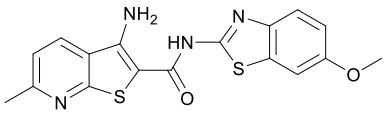
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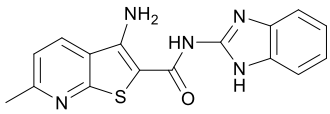
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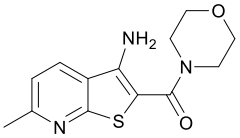
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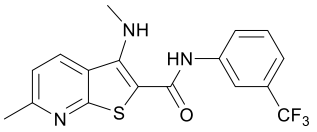
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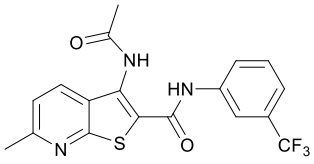
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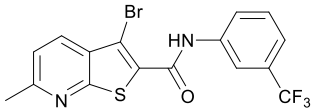
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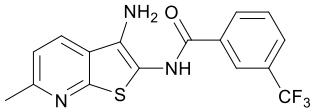
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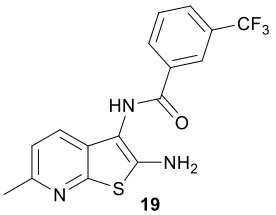
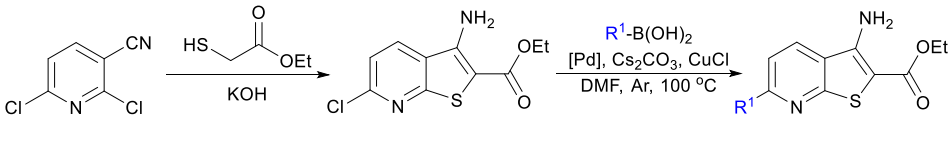

19

2.2 General procedure 1



4-5, 7-8, 10-14

3-amino-6-phenyl-N-(3-(trifluoromethyl)phenyl)thieno[2,3-b]pyridine-2-carboxamide 4

3-Cyano-2,6-dichloropyridine (3.0 mmol, 546 mg) and ethyl thioglycolate (3.0 mmol, 364 mg) were combined in *N*,*N*-dimethylformamide (2.5 mL) and the solution was cooled to 0 °C. To the reaction was added potassium hydroxide (5.4 mmol, 300 mg) and water (1.2 mL). After stirring at 0 °C for 1.5 hours, the reaction was diluted with water (10 mL) and the precipitate was collected by filtration. The product was recrystallized from ethyl acetate/hexane to give ethyl 3-amino-6-chlorothieno[2,3-*b*]pyridine-2-carboxylate as yellow soild (0.19 g, yield 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88-7.85 (d, *J* = 8.4 Hz, 1H), 7.32-7.30 (d, *J* = 8.4 Hz, 1H), 5.89 (s, 2H), 4.40-4.33 (q, *J* = 7.1 Hz, 2H), 1.42-1.37 (t, *J* = 7.1 Hz, 3H)[1].

Phenylboronic acid (3.6 mmol, 0.439 g), 6-chloro-3-aminothieno[2,3-*b*]pyridine (1.8 mmol, 154.1 mg), cesium carbonate (3.6 mmol, 1.173 g), $PdCl_2$dppf (0.18 mmol, 0.132 g), copper chloride (1.8 mmol, 0.178 g) were heated in *N*,*N*-dimethylformamide at 100 °C for 12 hours. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography to yield Ethyl 3-amino-6-phenylthieno[2,3-*b*]pyridine-2-carboxylate as pale yellow solid (0.266 g, yield 49.5%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63-8.60 (d, *J* = 8.6 Hz, 1H), 8.20-8.17 (dd, *J* = 7.9, 1.5 Hz, 2H), 8.09-8.06 (d, *J* = 8.6 Hz, 1H), 7.54-7.50 (m, 3H), 7.32 (s, 2H), 4.33-4.26 (q, *J* = 7.1 Hz, 2H), 1.34-1.29 (t, *J* = 7.1 Hz, 3H). HRMS (ESI): calcd for $C_{16}H_{15}N_2O_2S$ 299.0849 [M+H$^+$] Found: 299.0850.

To a solution of ethyl 3-amino-6-phenylthieno[2,3-*b*]pyridine-2-carboxylate (0.62 mmol, 0.186 g) in ethanol (3.5 mL) was added an aqueous solution of sodium hydroxide (3 M, 0.6 mL) and the mixture was heated to reflux for 2 hours. The solvent was removed *in vacuo* and the resulting residue dissolved in water (5 mL). Excess aqueous hydrochloric acid (1 M) was added which caused precipitation of the desired compound. Filtration of the precipitate gave 3-amino-6-phenylthieno[2,3-*b*]pyridine-2-carboxylic acid as a yellow solid (132 mg, yield 98%). HRMS (ESI): calcd for $C_{14}H_9N_2O_2S$ 269.0390 [M-H$^+$] Found: 269.0386.

To a solution of 3-Amino-6-phenylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.1 mmol, 27.0 mg) in *N*,*N*-dimethylformamide (0.5 mL) was added *N*,*N*-diisopropylethylamine (0.3 mmol, 38.8 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.13 mmol, 47.5 mg) under argon at room temperature and stirred for 5-10 mins. 3-(Trifluoromethyl)aniline (0.15 mmol, 24.2 mg) was subsequently added and the reaction mixture continued to stir at room temperature for another 16 hours. 1 mL of saturated sodium chloride solution is then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give the title compound 3-amino-6-phenyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **4** as a yellow solid (14 mg, yield 34%). m.p. 232-233 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.64-8.62 (d, *J* = 8.5 Hz, 1H), 8.24 (s, 1H), 8.23-8.19 (m, 2H), 8.11-8.09 (d, *J* = 8.5 Hz, 1H), 8.03-8.01 (d, *J* = 8.5 Hz, 1H), 7.59-7.50 (m, 6H), 7.43-7.42 (d, *J* = 7.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.1, 157.1, 147.6, 140.0, 132.1, 129.8, 129.6, 129.3, 129.1, 128.9, 128.8, 127.5, 127.1, 124.3, 121.0, 119.7, 119.5, 116.91, 116.88, 116.5. HRMS (ESI): calcd for $C_{21}H_{13}ON_3F_3S$ 412.0737 [M-H$^+$] Found: 412.0733. IR ν_{max} (KBr, cm^{-1}): 3459, 3260, 2921, 1622,

1534, 1437, 1109, 902, 748, 695.

3-amino-6-phenyl-*N*-(pyridin-4-yl)thieno[2,3-*b*]pyridine-2-carboxamide 5

To a solution of 3-amino-6-phenylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.2 mmol, 54.1 mg) in *N*,*N*-dimethylformamide (1.0 mL) was added *N*,*N*-diisopropylethylamine (0.6 mmol, 77.6 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.26 mmol, 98.9 mg) under argon at room temperature and stirred for 1 hour. 4-Aminopyridine (0.6 mmol, 56.5 mg) was subsequently added and the reaction mixture was stirred at 60 °C for another 24 hours. 1 mL of saturated sodium chloride solution was then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give the title compound 3-amino-6-phenyl-*N*-(pyridin-4-yl)thieno[2,3-*b*]pyridine-2-carboxamide **5** as a yellow solid (66 mg, yield 95%). m.p. 172-174 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.64-8.62 (d, J = 8.5 Hz, 1H), 8.44-8.43 (d, J = 5.0 Hz, 2H), 8.21-8.19 (d, J = 7.2 Hz, 2H), 8.11-8.09 (d, J = 8.5 Hz, 1H), 7.78-7.77 (d, J = 5.8 Hz, 2H), 7.60 (s, 2H), 7.58 – 7.49 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.9, 157.7, 150.5, 148.9, 146.6, 138.3, 132.7, 130.3, 129.4, 127.6, 125.1, 117.0, 114.8, 96.2, HRMS (ESI): calcd for $C_{19}H_{15}ON_4S$ 347.0961 [M+H$^+$] Found: 347.0965. IR ν_{max} (KBr, cm^{-1}): 3494, 3364, 2922, 1736, 1584, 1287, 1069, 828, 687, 560.

3-amino-6-cyclopropyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 7

A mixture of cyclopropylboronic acid (4 mmol, 0.344 g), 6-chloro-3-aminothieno[2,3-*b*]pyridine (2 mmol, 0.513 g), potassium phosphate (8 mmol, 1.698 g), $Pd(OAc)_2$ (0.2 mmol, 45.2 mg), tricyclohexyl phosphine (0.4 mmol,113.0 mg) in water-toluene mixture (2.1 mL, 1:20) was heated at 100 °C for 24 hours. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography to afford the ethyl 3-amino-6-cyclopropylthieno[2,3-*b*]pyridine-2-carboxylate as a yellow solid (349 mg, yield 66.5 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37-8.34 (d, *J* = 8.3 Hz, 1H), 7.36-7.33 (d, *J* = 8.4 Hz, 1H), 7.22 (s, 2H), 4.30-4.22 (q, *J* = 14.1, 7.0 Hz, 2H), 2.23-2.18 (m, 1H), 1.31-1.26 (t, *J* = 7.0 Hz, 3H), 1.06-1.01 (m, 4H). HRMS (ESI): calcd for $C_{13}H_{15}N_2O_2S$ 263.0849 [M+H$^+$] Found: 263.0850.

To a solution of ethyl 3-amino-6-cyclopropylthieno[2,3-*b*]pyridine-2-carboxylate (1.35 mmol, 0.354 g) in ethanol (6.5 mL) was added an aqueous solution of sodium hydroxide (3 M, 1.3 mL), and the mixture was refluxed for 6 hours. The solvent was removed *in vacuo* and the resulting residue was dissolved in water (10 mL). Excess aqueous hydrochloric acid (1 M) was added which caused precipitation of the desired 3-amino-6-cyclopropylthieno[2,3-*b*]pyridine-2-carboxylic acid as a yellow solid (297 mg, yield 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35-8.33 (d, *J* = 8.4 Hz, 1H), 7.35-7.32 (d, *J* = 8.4 Hz, 1H), 2.24-2.18 (m, 1H), 1.07-0.98 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2, 164.4, 159.7, 147.4, 131.0, 123.4, 117.6, 93.7, 17.2, 10.7. HRMS (ESI): calcd for $C_{11}H_9N_2O_2S$ 233.0390 [M-H$^+$] Found: 233.0385.

To a solution of 3-amino-6-cyclopropylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.4 mmol, 93.7 mg) in *N*,*N*-

dimethylformamide (1.5 mL) was added *N*,*N*-diisopropylethylamine (1.2 mmol, 155.1 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.48 mmol, 182.5 mg) under argon at room temperature and stirred for 5-10 mins. 3-(Trifluoromethyl)aniline (0.8 mmol, 128.9 mg) was subsequently added and the reaction mixture was stirred at 80 °C for another 20 hours.. 2 mL of saturated sodium chloride solution was then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give the title compound 3-amino-6-cyclopropyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **7** as a yellow solid (67.5 mg, yield 45%). m.p. 172-173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (s, 1H), 8.39-8.36 (d, *J* = 8.4 Hz, 1H), 8.21 (s, 1H), 8.01-7.98 (d, *J* = 8.3 Hz, 1H), 7.58-7.53 (t, *J* = 8.0 Hz, 1H), 7.46 (s, 2H), 7.41-7.34 (dd, *J* = 13.0, 8.1 Hz, 2H), 2.28-2.20 (m, 1H), 1.08-1.03 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.6, 164.2, 159.0, 147.9, 140.0 , 130.9, 129.6, 124.3, 123.5, 119.41, 119.38, 117.7, 116.9, 116.8, 94.5, 17.3, 10.8. HRMS (ESI): calcd for $C_{18}H_{13}F_3N_3OS$ 376.0737 [M-H$^+$] Found: 376.0735. IR ν_{max} (KBr, cm^{-1}): 3385, 3284, 2928, 1724, 1630, 1539, 1336, 1307, 1119, 805, 697, 587.

3-amino-6-chloro-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 8

To a solution of ethyl 3-amino-6-chlorothieno[2,3-*b*]pyridine-2-carboxylate (1 mmol, 0.257 g) in ethanol (5.5 mL) was added an aqueous solution of sodium hydroxide (3 M, 1.0 mL) and the mixture was refluxed for 2 hours. The solvent was removed *in vacuo* and the resulting residue was dissolved in water (5 mL). Excess aqueous hydrochloric acid (1 M) was added which caused precipitation of the desired compound. Filtration of the precipitate gave the desired 3-amino-6-chlorothieno[2,3-*b*]pyridine-2-carboxylic acid as earthy yellow solid (102.9 mg, yield 45%).

To a solution of 3-amino-6-chlorothieno[2,3-*b*]pyridine-2-carboxylic acid (0.4 mmol, 91.5 mg) in *N*,*N*-dimethylformamide (1.5 mL) was added *N*,*N*-diisopropylethylamine (1.2 mmol, 155.1 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.52 mmol, 197.7 mg) under argon at room temperature and stirred for 5-10 min. 3-(Trifluoromethyl)aniline (0.6 mmol, 96.7 mg) was added and the reaction mixture was stirred at room temperature for another 6 hours, then heated to 80 °C for 18 hours. 1 mL of saturated sodium chloride solution was then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give the title compound 3-amino-6-chloro-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **8** as a yellow solid (53 mg, yield 36%). m.p. 219-220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.60-8.58 (d, *J* = 8.4 Hz, 1H), 8.22 (s, 1H), 8.00-7.98 (d, *J* = 7.7 Hz, 1H), 7.61-7.56 (m, 4H), 7.43-7.42 (d, *J* = 7.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.8, 158.2, 150.6, 147.1, 139.8, 135.3, 134.2, 131.8, 129.6, 129.5, 129.3, 125.2, 125.1, 124.3, 124.1, 120.2, 119.7, 119.6, 117.2, 116.9, 96.2. HRMS (ESI): calcd for $C_{15}H_8ClF_3N_3OS$ 370.0034 [M-H$^+$] Found: 370.0026. IR ν_{max} (KBr, cm^{-1}): 3415, 3314, 2925, 1729, 1559, 1490, 1438, 1350, 1119, 796, 696, 615.

3-amino-*N*-cyclopropyl-6-methylthieno[2,3-*b*]pyridine-2-carboxamide 10

A mixture of methylboronic acid (8 mmol, 0.479 g), 6-chloro-3-aminothieno[2,3-*b*lpyridine (4 mmol, 1.027 g), cesium carbonate (8 mmol, 2.606 g), $PdCl_2$dppf (0.4 mmol, 0.293 g), copper chloride (4 mmol, 0.396 g) were heated in *N*,*N*-dimethylformamide at 100 °C for 12 hours. After cooling to room temperature the reaction mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography to afford ethyl 3-amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylate as a yellow solid (0.378 g, yield 40%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42-8.39 (d, *J* = 8.3 Hz, 1H), 7.34-7.31 (d, *J* = 8.3 Hz, 1H), 7.24 (s, 2H), 4.30-4.23 (q, *J* = 7.1 Hz, 2H), 2.58 (s, 3H), 1.32-1.27 (t, *J* = 7.1 Hz, 3H).

To a solution of ethyl 3-amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylate (9.9 mmol, 2.3 g) in methanol (15 mL) was added an aqueous solution of sodium hydroxide (3 M, 15 mL), and the mixture was stirred at 70 °C for 6 hours. The solvent was removed *in vacuo* and the resulting residue dissolved in water (10 mL). The pH was adjusted to 6 with aqueous hydrochloric acid (1 M) which caused precipitation of the desired compound. Filtration of the precipitate gave 3-amino-6-methylthieno[2,3-b]pyridine-2-carboxylic acid as earthy yellow solid (1.65 g, yield 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37-8.35 (d, *J* = 8.3 Hz, 1H), 7.31-7.28 (d, *J* = 8.3 Hz, 1H), 2.57 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.3, 159.8, 159.4, 147.5, 131.4, 123.6, 119.4, 94.1, 24.3.

3-Amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.4 mmol, 83.3 mg) was dissolved in dry *N*,*N*-dimethylformamide (1 mL) under argon atmosphere. Diisopropylethylamine (0.8 mmol, 143 µL) was added to the solution followed by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (0.44 mmol, 229 mg). The mixture was stirred for 10 min until completely dissolved. Cyclopropylamine (0.8 mmol, 55.5 µL) dissolved in dry *N*,*N*-dimethylformamide (0.5 mL) was slowly added to the mixture. The reaction mixture was stirred at room temperature under argon for another 3 hours. The reaction mixture was partitioned between ethyl acetate (10 mL) and water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL × 2). The combined ethyl acetate layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purified by column chromatography gave the 3-amino-*N*-cyclopropyl-6-methylthieno[2,3-*b*]pyridine-2-carboxamide **10** as yellow solid particles (95 mg, yield 96%). m.p. 226-227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29-8.27 (d, *J* = 8.3 Hz, 1H), 7.67 (s, 1H), 7.29-7.28 (d, *J* = 8.3 Hz, 1H), 7.15 (s, 2H), 2.77 (m, 1H), 2.57 (s, 3H), 0.65 - 0.57 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.4, 159.0, 158.1, 145.4, 130.7, 123.9, 119.3, 96.1, 24.2, 22.9, 5.8. HRMS (ESI): calcd for $C_{12}H_{13}N_3OS$ 246.0696 [M-H$^+$] Found: 246.0695. IR ν_{max} (KBr, cm^{-1}): 3426, 3311, 3007, 1702, 1603, 1498, 1299, 1074, 822, 689, 558.

3-amino-*N*-(4-chlorobenzyl)-6-methylthieno[2,3-*b*]pyridine-2-carboxamide 11

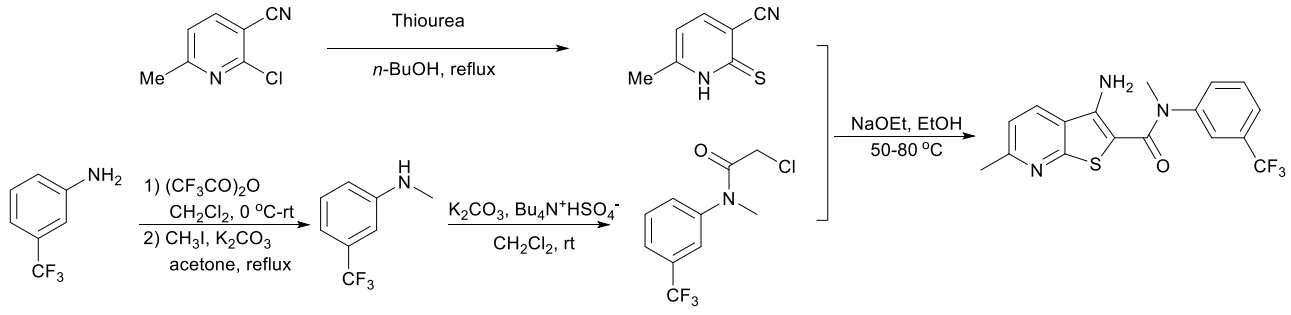
3-amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.5 mmol, 104.1 mg) were added to *N*,*N*-dimethylformamide (1.5 mL), followed by *N*,*N*-diisopropylethylamine (1.5 mmol, 193.9 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.55 mmol, 210.4 mg) under argon at room temperature and stirred for 30 min. 4-Chlorobenzylamine (0.75 mmol, 106.2 mg) was subsequently added and the reaction mixture stirred at 80 °C for another 20 hours. 2 mL of saturated sodium chloride solution was then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was

dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give the compound 3-amino-*N*-(4-chlorobenzyl)-6-methylthieno[2,3-*b*]pyridine-2-carboxamide **11** as a white flaky solid (45 mg, yield 27%). m.p. 179-180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32-8.30 (d, *J* = 8.2 Hz, 1H), 8.27-8.25 (t, *J* = 5.4 Hz, 1H), 7.39-7.30 (m, 5H), 7.17 (s, 2H), 4.39-4.38 (d, *J* = 5.6 Hz, 2H), 2.58 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.1, 159.1, 158.1, 145.8, 139.2, 131.2, 130.8, 129.2, 128.2, 119.4, 41.7, 24.2. HRMS (ESI): calcd for $C_{16}H_{15}ClN_3OS$ 332.0619 [M+H$^+$] Found: 332.0618. IR ν_{max} (KBr, cm^{-1}) 3420, 3310, 2923, 1608, 1544, 1299, 800, 691.

(3-amino-6-methylthieno[2,3-*b*]pyridin-2-yl)(morpholino)methanone 14

To a suspension solution of 3-amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.4 mmol, 83.3 mg) and morpholine (0.6 mmol, 52.3 mg) in dichloromethane (1.5 mL) was added 1-ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDCI) (0.6 mmol, 115.2 mg) and 1-hydroxybenzotriazole (0.48 mmol, 64.9 mg). The mixture was allowed to stir under room temperature for 12 hours until the starting materials were consumed, as determined by TLC. Then water was added to the reaction mixture and the organic phase was wash twice with diluted hydrochloric acid (1 N), saturated potassium carbonate solution and brine, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Purified by column chromatography gave (3-amino-6-methylthieno[2,3-*b*]pyridin-2-yl)(morpholino)methanone **14** as an orange-red crystal (87 mg, yield 78.5%). m.p. 176-177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31-8.29 (d, *J* = 8.3 Hz, 1H), 7.30-7.28 (d, *J* = 8.3 Hz, 1H), 6.48 (s, 2H), 3.64-3.63 (d, *J* = 4.9 Hz, 4H), 3.60-3.59 (d, *J* = 4.9 Hz, 4H), 2.56 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.4, 158.6, 158.3, 142.3, 130.7, 123.9, 119.3, 97.9, 66.2, 45.5, 24.1. HRMS (ESI): calcd for $C_{13}H_{14}N_3O_2S$ 276.0812 [M-H$^+$] Found: 276.0810. IR ν_{max} (KBr, cm^{-1}): 3447, 3348, 2920, 2852, 1725, 1417, 1275, 1104, 864, 739, 597.

2.3 General procedure 2



3-amino-*N*,6-dimethyl-N-(3-(trifluoromethyl)phenyl)thieno[2,3-b]pyridine-2-carboxamide 9

A dry 100 mL two necked flask was equipped with argon. The system was purged with argon and was charged with 3-(trifluoromethyl)aniline (6.4 mmol, 2.58 g) and dry dichloromethane (30 mL). The mixture was cooled to 0 °C with an ice bath and trifluoroacetic anhydride (48 mmol, 6.7 mL) was added with a syringe through the septum. Then the solution was allowed to warm to room temperature and was stirred for 20 min. The volatiles were evaporated *in*

vacuo and the residue was dissolved in acetone (30 mL). Anhydrous potassium carbonate (12.8 mmol, 4.42 g) was added followed by methyliodide (48 mmol, 3.0 mL). This mixture was refluxed for 2 hours then the precipitate was filtered off and the filtrate was concentrated *in vacuo*. Water (10 mL), methanol (50 mL), and potassium carbonate (2.2 g) were added; the mixture was stirred for 1 hour at room temperature. The bulk of the methanol was distilled off and the crude product was extracted with dichloromethane (60 mL), which was subsequently washed with water (20 mL × 2) and brine (20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to yield the compound *N*-methyl-3- (trifluoromethyl)aniline as a yellow oil (2.38 g, yield 85%). The product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.23 (dd, *J* = 9.8, 6.0 Hz, 1H), 6.93-6.92 (d, *J* = 7.6 Hz, 1H), 6.78 (s, 1H), 6.73-6.72 (d, *J* = 8.2 Hz, 1H), 3.88 (s, 1H), 2.85 (s, 3H)[2]. ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.5, 132.0, 131.8, 131.5, 131.28, 129.7, 127.8, 125.6, 123.5, 121.3, 115.59, 115.58, 113.71, 113.68, 113.65, 113.6, 108.49, 108.46, 108.43, 108.40, 30.6. HRMS (ESI): calcd for $C_8H_7NF_3$ 174.0536 [M-H$^+$] Found: 174.0531

3-(trifluoromethyl)-*N*-methylbenzenamine (5.71 mmol, 1 g) and 2-chloroacetyl chloride (23 mmol, 2.58 g) in 10 mL of dichloromethane was added a catalytic amount of tetrabutylammonium hydrosulfate, followed by a solution of potassium carbonate (112 mmol, 15 g) in 100 mL of water. The reaction mixture was stirred at room temperature for 40 min and the dichloromethane layer was collected and combined with another same scale reaction. The residue was purified through a silica gel column to give aimed product 2-chloro-*N*-methy *N*-[3-(trifluoromethyl)phenyl]acetamide as a yellowish oil (1.34 g, yield 93.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67-7.61 (dd, *J* = 13.9, 5.8 Hz, 2H), 7.57 (s, 1H), 7.53-7.50 (d, *J* = 7.5 Hz, 1H), 3.85 (s, 2H), 3.35 (s, 3H).

To a mixture of compound 2-chloro-*N*-methy *N*-[3-(trifluoromethyl)phenyl]acetamide (0.4 mmol, 100.7 mg) and 6-methyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (0.6 mmol, 90.1 mg) in 2 mL of ethanol was added sodium ethoxide (2.0 mmol) in 1 mL of ethanol. The reaction mixture was heated 80 °C for 6 hours and then the solvent was removed *in vacuo* and the resulting residue was dissolved in water, extracted with ethyl acetate. The organic layers were combined, dried over anhydrous sodium sulfate, concentrated and purified through a silica gel column to give the product 3-amino-*N*,6-dimethyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **9** (148 mg, yield 95%). m.p. 175-176 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76-7.75 (d, *J* = 8.3 Hz, 1H), 7.64-7.63 (d, *J* = 7.5 Hz, 1H), 7.59 (s, 1H), 7.54 - 7.48 (m, 2H), 7.10-7.08 (d, *J* = 8.3 Hz, 1H), 6.35 (s, 2H), 3.43 (s, 3H), 2.57 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 160.7, 160.0, 147.6, 144.0, 132.8, 132.7, 132.5, 132.3, 132.0, 130.5, 129.1 126.9, 126.1, 126.04, 126.02, 125.99, 125.4, 125.34, 125.31, 124.7, 122.5, 122.4, 119.3, 98.8, 39.2, 24.8. HRMS (ESI): calcd for $C_{17}H_{15}ON_3F_3S$ 366.0882 [M+H$^+$] Found: 366.0883. IR ν_{max} (KBr, cm^{-1}): 3401, 3265, 2924, 1591, 1489, 1296, 1275, 1127, 1069, 822, 696, 610.

3-amino-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)-6-methylthieno[2,3-*b*]pyridine-2-carboxamide 12

To a solution of 6-methoxybenzo[*d*]thiazol-2-amine (10 mmol, 1.80 g) in acetonitrile (100 mL) was added *N*,*N*-diisopropylethylamine (15mmol, 1.94 g). The mixture was cooled to 0 °C. The Chloroacetyl chloride (15 mmol, 1.70 g) was gradually added with stirring and cooling. After stirring for 2 hours at room temperature, volatiles were evaporated. The red-brown, oily residue was mixed with water (100 mL), and precipitates were collected by filtration. Precipitates were washed with water, hydrochloric acid (1 M) and dried to yield 2-chloro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide as cream-colored solid (1.28 g, yield 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.97

(s, 1H), 7.72-7.69 (d, *J* = 8.9 Hz, 1H), 7.30-7.30 (d, *J* = 2.4 Hz, 1H), 7.09-7.05 (dd, *J* = 8.9, 2.5 Hz, 1H), 4.29 (s, 2H), 3.88 (s, 3H)[3]. HRMS (ESI): calcd for $C_{10}H_9ClN_2O_2S$ 255.0001 [M-H$^+$] Found: 254.9993.

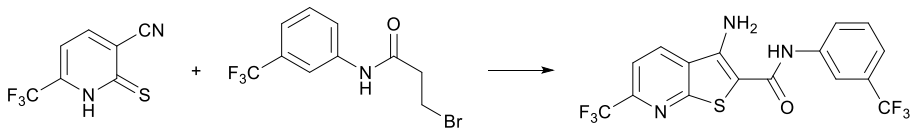
To a suspension of 2-sulfany6-(trifluoromethyl)pyridine-3-carbonitrile (1.0 mmol, 150 mg) in anhydrous ethanol (4 mL) was added 2-chloro-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (0.67 mmol, 172 mg) and sodium ethoxide (3.35 mmol, 228 mg). The mixture was heated to reflux for 16 hours. Upon cooling to room temperature and addition of water, yellow solid precipitated, which was filtered and washed with cold ethanol to afford 3-amino-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)-6-methylthieno[2,3-*b*]pyridine-2-carboxamide **12** as yellow solid (300 mg, yield 82%). m.p. 346-348 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16-8.14 (d, *J* = 8.2 Hz, 1H), 7.31-7.28 (d, *J* = 8.6 Hz, 1H), 7.23-7.19 (dd, *J* = 9.2, 5.4 Hz, 2H), 7.08 (s, 2H), 6.80-6.79 (dd, *J* = 8.6, 2.6 Hz, 1H), 3.76 (s, 3H), 2.56 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.7, 166.5, 158.9, 156.4, 153.8, 145.1, 140.7, 134.2, 129.9, 125.4, 118.3, 112.3, 109.1, 104.4, 55.5, 24.1. HRMS (ESI): calcd for $C_{17}H_{15}N_4O_2S_2$ 371.0631 [M+H$^+$] Found: 371.0630. IR ν_{max} (KBr, cm^{-1}): 3524, 3442, 2925, 1601, 1456, 1351, 1227, 1038, 812, 771, 643.

3-amino-*N*-(1*H*-benzo[*d*]imidazol-2-yl)-6-methylbenzo[*b*]thiophene-2-carboxamide 13

A solution of 2-aminobenzimidazole (3.75 mmol, 0.5 g) and triethylamine (3.75 mmol, 0.52 mL) in anhydrous tetrahydrofuran was stirred for 45 min. Subsequently, a solution of chloroacetyl chloride (3.75 mmol, 0.3 mL) in tetrahydrofuran was added drop wise to the reaction mixture under ice cold conditions using a dropping funnel and the contents were stirred further for 2 hours. Completion of the reaction was monitored by TLC. The slurry was filtered and the filtrate was evaporated under reduced pressure to obtain a solid residue which was purified by column chromatography on silica gel to afford *N*-(1*H*-benzo[*d*]imidazol-2-yl)-2-chloroacetamide as cream-colored solid (0.4 g, yield 51%). HRMS (ESI): calcd for $C_9H_9ClN_3O$ 210.0429 [M+H$^+$] Found: 210.0422.

To a suspension of 2-sulfany6-(trifluoromethyl)pyridine-3-carbonitrile (1.0 mmol, 150 mg) in anhydrous ethanol (4 mL) was added *N*-(1*H*-benzo[*d*]imidazol-2-yl)-2-chloroacetamide (0.67 mmol, 140 mg) and sodium ethoxide (3.35 mmol, 228 mg). The mixture was heated to reflux for 24 hours. Upon cooling to room temperature and addition of water, yellow solid precipitated, which was filtered and washed with cold ethanol and was purified by column chromatography on silica gel to afford 3-amino-*N*-(1*H*-benzo[*d*]imidazol-2-yl)-6-methylbenzo[*b*]thiophene-2-carboxamide **13** as Lemon yellow solid (0.145g, yield 45%). m.p. 281-282 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26-8.24 (d, *J* = 8.2 Hz, 1H), 7.37-7.35 (dd, *J* = 5.8, 3.2 Hz, 2H), 7.33-7.21 (m, 3H), 7.17-7.15 (dd, *J* = 5.8, 3.2 Hz, 2H), 2.57 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.2, 158.2, 152.5, 143.9, 130.6, 129.3, 124.6, 122.3, 118.8, 111.0, 24.19. HRMS (ESI): calcd for $C_{16}H_{14}N_5OS$ 324.0914 [M+H$^+$] Found: 324.0911. IR ν_{max} (KBr, cm^{-1}): 3336, 3258, 2923, 1621, 1567, 1327, 1273, 991, 821, 725, 688.

2.4 Procedure 3



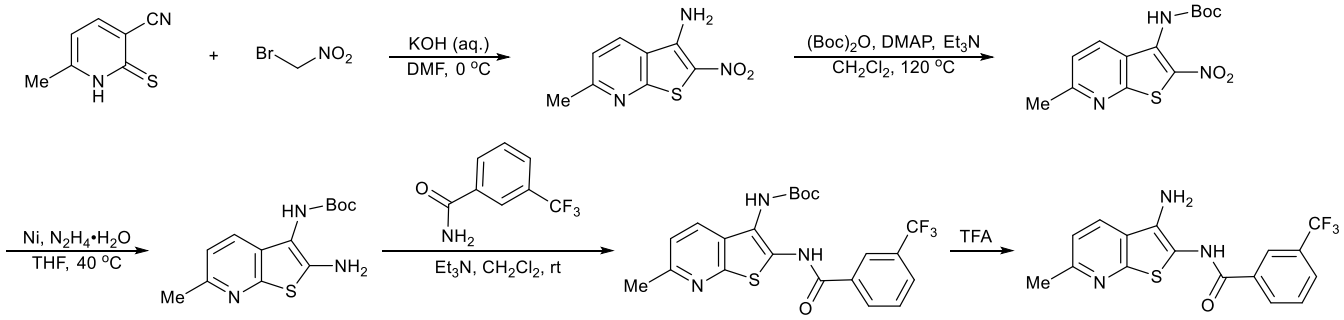
3-amino-6-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 6

To a mixture of 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (17.85 mmol, 3 g) and 2-cyanothioacetamide (26.8 mmol, 2.68 g) in ethanol (30 mL) was added *N*-methylmorpholine (2.5 mL) and refluxed for 24 h. The reaction mixture was evaporated *in vacuo* to afford the crude 2-sulfany6-(trifluoromethyl)pyridine-3-carbonitrile which was used in the next step without purification.

3-(Trifluoromethyl)aniline (20 mmol, 3.22 g) was added to a suspension of triethylamine (23 mmol, 3.2 mL) in dichloromethane (6 mL) at 0 °C followed by the addition of bromoacetyl bromide (22 mmol, 1.92 mL) in dichloromethane (14 mL). The mixture was stirred for 4 hours at ambient temperature, quenched with water (20 mL), the organic layer was washed twice with water (20 mL), dried over anhydrous sodium sulfate, filtered through a silica gel plug, concentrated under reduced pressure to afford the 3-bromo-*N*-(3-(trifluoromethyl)phenyl)propanamide (5.03 g, yield 85%).

To a slurry of 2-sulfany 6-(trifluoromethyl)pyridine-3-carbonitrile (0.5 mmol, 141 mg) in anhydrous ethanol (2.0 mL) was added 3-bromo-*N*-(3-(trifluoromethyl)phenyl)propanamide (0.6 mmol, 123 mg), followed by a solution of sodium ethoxide (2.5 mmol, 170.2 mg) in 1 mL of ethanol at room temperature under argon. The reaction was heated to reflux for 2 hours. The mixture was cooled to room temperature, diluted with ethyl acetate (5 mL) and washed with water (10 mL × 2) and brine (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (hexanes/ethyl acetate) on silica gel to give the product 3-amino-6-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **6** as a light-yellow solid (39 mg, yield 19%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.89-8.87 (d, J = 7.9 Hz, 1H), 8.27 (s, 1H), 8.05-8.04 (d, J = 7.5 Hz, 2H), 7.68-7.61 (m, 3H), 7.49-7.48 (d, J = 6.9 Hz, 1H)[4]. HRMS (ESI): calcd for $C_{16}H_8F_6N_3OS$ 404.0298 [M-H$^+$] Found: 404.0294.

2.5 Procedure 4



***N*-(3-amino-6-methylthieno[2,3-*b*]pyridin-2-yl)-3-(trifluoromethyl)benzamide 18**

A mixture of 2-chloro-6-methylnicotinonitrile (2.90 mmol, 440 mg) and thiourea (9.14 mmol, 700 mg) was heated at reflux in *n*-butanol (9.0 mL) for 4 hours. After cooling to room temperature, the yellow solution turned to a

suspension containing light yellow solids. The solids were collected by filtration, rinsed with *n*-butanol, and dried *in vacuo* to give 2-mercapto-6-methylnicotinonitrile as a yellow powder (2.85 g, yield 95%).

To a solution of 2-mercaptonicotinonitrile (13.3 mmol, 2 g) in *N*,*N*-dimethylformamide (35 mL) at 0 °C was added a solution of potassium hydroxide (40 mmol, 2.72 g) in water (10 mL). The mixture was stirred for 20 min, then bromonitromethane (26.6 mmol, 3.72 g) was added dropwise. The resulting mixture was stirred for 1 h at 0 °C, diluted with water (200 mL) and filtered. The filter cake was washed with water (20 mL x 3) to give 6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-amine as a yellow solid (1.3 g, yield 54%), which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (s, 2H), 8.59-8.57 (d, *J* = 7.0 Hz, 1H), 7.42-7.40 (d, *J* = 7.4 Hz, 1H), 2.60 (s, 3H)[5].

To a solution of 6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-amine (0.5 mmol, 104.6 mg) in dichloromethane (3.0 mL) at 0 °C was added dimethylaminopyridine (1.0 mmol, 122 mg), triethylamine (2.5 mmol, 0.35 mL), di-*tert*-butyl dicarbonate (5.0 mmol, 1.09 g, in 1.2 mL of dichloromethane). The reaction solution was heated to reflux for 12 hours, and the reaction was neutralized with saturated ammonium chloride solution, extracted with dichloromethane (20 mL), and washed with distilled water. The obtained organic layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure, and then purified by silica gel column chromatography to obtain the *tert*-butyl (6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-yl)carbamate as a yellow solid (80 mg, yield 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.39-8.36 (d, *J* = 8.5 Hz, 1H), 7.53-7.50 (d, *J* = 8.5 Hz, 1H), 2.65 (s, 3H), 1.49 (s, 9H).

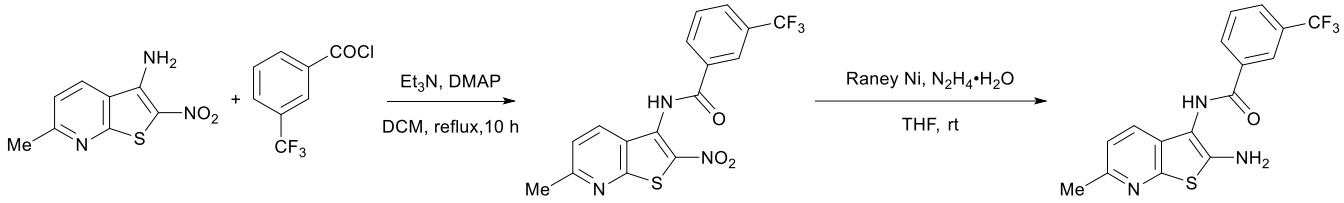
To a solution of *tert*-butyl (6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-yl)carbamate (0.226 mmol, 70 mg) in tetrahydrofuran (5 mL) was added hydrazine monohydrate (6.2 mmol, 0.3 mL) and Raney Ni (~0.5 mL) and then stirred at 40 °C for 3 hours. After heating at 60 °C until all hydrazine was quenched, the mixture was cooled to room temperature and then filtered. The filtrate was concentrated by rotary evaporation to remove the solvent, and the residue was purified by silica gel column chromatography to afford *tert*-butyl (2-amino-6-methylthieno[2,3-*b*]pyridin-3-yl)carbamate as a yellow powder (26 mg, yield 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.43 (d, *J* = 8.1 Hz, 1H), 7.07-7.05 (d, *J* = 8.1 Hz, 1H), 6.04 (s, 1H), 4.39 (s, 2H), 2.58 (s, 3H), 1.52 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7, 125.3, 119.9, 81.2, 28.4, 24.2.

To a solution of *tert*-butyl (2-amino-6-methylthieno[2,3-*b*]pyridin-3-yl)carbamate (0.2 mmol, 56 mg) in dichloromethane (0.5 mL) was added triethylamine (0.3 mmol, 30.4 mg) and 3-(trifluoromethyl)benzoyl chloride (0.24 mmol, 50.1 mg) dropwise. The reaction was stirred at 25 °C for 3 hours, diluted with dichloromethane, sodium hydroxide (1N) and saturated sodium chloride solution. The organic layer was separated, merged and dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to obtain *tert*-butyl (6-methyl-2-(3-(trifluoromethyl)benzamido)thieno[2,3-*b*]pyridin-3-yl)carbamate as a yellow solid (45 mg, yield 49.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.13 (s, 1H), 9.25 (s, 1H), 8.29-8.27 (d, *J* = 7.8 Hz, 2H), 8.04-8.01 (d, *J* = 7.7 Hz, 1H), 7.85-7.80 (t, *J* = 7.2 Hz, 2H), 7.31-7.28 (d, *J* = 8.2 Hz, 1H), 2.58 (s, 3H), 1.52 (s, 9H). HRMS (ESI): calcd for $C_{21}H_{21}F_3N_3O_3S$ 452.1250 [M+H$^+$] Found: 452.1253.

To a solution of *tert*-butyl (6-methyl-2-(3-(trifluoromethyl)benzamido)thieno[2,3-*b*]pyridin-3-yl)carbamate (0.09 mmol, 40 mg) in dichloromethane (1 mL) at 0 °C was added trifluoroacetic acid (1.0 mL). The mixture was stirred for 30 min

at room temperature, and was evaporated to dryness and partitioned between ethyl acetate and statured sodium bicarbonate. The organic layer was separated and dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography to afford *N*-(3-amino-6-methylthieno[2,3-*b*]pyridin-2-yl)-3-(trifluoromethyl)benzamide **18** as a pale-yellow solid (19 mg, yield 60 %). m.p. 197-198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.36 (s, 1H), 8.32-8.31 (d, *J* = 7.8 Hz, 1H), 8.08-8.06 (d, *J* = 8.1 Hz, 1H), 7.99-7.98 (d, *J* = 7.7 Hz, 1H), 7.82-7.79 (t, *J* = 7.8 Hz, 1H), 7.25-7.24 (d, *J* = 8.2 Hz, 1H), 5.37 (s, 2H), 2.56 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.3, 155.8, 154.5, 134.6, 132.0, 131.5, 129.7, 129.3, 129.0, 128.7, 128.3, 128.2, 127.7, 125.1, 124.6, 124.5, 124.5, 123.9, 122.9, 118.9, 108.3, 23.9. HRMS (ESI): calcd for $C_{16}H_{13}F_3N_3OS$ 352.0726 [M+H$^+$] Found: 352.0726. IR ν_{max} (KBr, cm^{-1}): 3370, 3227, 2923, 1656, 1507, 1334, 1257, 1166, 1124, 1072, 816, 696.

_N_-(2-amino-6-methylthieno[2,3-*b*]pyridin-3-yl)-3-(trifluoromethyl)benzamide 19



To a solution of 6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-amine (5 mmol, 1.04 g) in dichloromethane (5 mL) was added triethylamine (7.5 mmol, 0.505 g) and 3-(trifluoromethyl)benzoyl chloride (6 mmol, 1.25 g). The reaction was refluxed for 10 hours, diluted with dichloromethane, aqueous sodium hydroxide (1N) and then with saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to obtain *N*-(6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-yl)-3-(trifluoromethyl)benzamide as a brownish yellow solid (1.52 g, yield 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.09 (s, 1H), 8.64-8.61 (d, *J* = 8.7 Hz, 1H), 8.35 (s, 1H), 8.24-8.22 (d, *J* = 7.9 Hz, 1H), 7.95-7.92 (d, *J* = 7.8 Hz, 1H), 7.77-7.72 (t, *J* = 7.8 Hz, 1H), 7.33-7.30 (d, *J* = 8.7 Hz, 1H), 2.73 (s, 3H).

To a solution of *N*-(6-methyl-2-nitrothieno[2,3-*b*]pyridin-3-yl)-3-(trifluoromethyl)benzamide (1.2 mmol, 457.2 mg) in tetrahydrofuran (30 mL) was added hydrazine monohydrate (5.0 mmol, 251 mg) and Raney Ni (~1.0 mL) and then stirred at 40 °C for 3 hours. After heating at 60 °C until all hydrazine was quenched, the mixture was cooled to room temperature and then filtered. The filtrate was concentrated by rotary evaporation to remove the solvent, and the product as a yellow powder and purified by silica gel column chromatography to afford *N*-(2-amino-6-methylthieno[2,3-*b*]pyridin-3-yl)-3-(trifluoromethyl)benzamide **19** as a light brown powder (210 mg, yield 50%). m.p. 279-281 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.39 (s, 1H), 8.34-8.32 (d, *J* = 7.8 Hz, 1H), 7.97-7.95 (d, *J* = 7.7 Hz, 1H), 7.80-7.76 (t, *J* = 7.8 Hz, 1H), 7.36-7.34 (d, *J* = 8.0 Hz, 1H), 7.08-7.05 (d, *J* = 8.1 Hz, 1H), 6.12 (s, 2H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.1, 150.5, 150.1, 144.8, 135.3, 131.9, 129.5, 129.4, 129.2, 128.9, 127.9, 125.4, 125.1, 124.6, 124.5, 122.7, 119.3, 102.0, 23.5. HRMS (ESI): calcd for $C_{16}H_{13}F_3N_3OS$ 352.0726 [M+H$^+$] Found: 352.0729. IR ν_{max} (KBr, cm^{-1}): 3275, 3141, 2922, 2856, 1627, 1593, 1444, 1339, 1270, 1122, 1099, 822, 695.

2.6 3-bromo-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 17

To a mixture of *tert*-butyl nitrite (13 mmol, 1.56 mL) in acetonitrile (20 mL) was added ethyl 3-amino-6-methylthieno[2,3-*b*]pyridine-2-carboxylate (10 mmol, 2.36 g) and copper bromide (II) (11 mmol, 2.46 g) over 2 hours under water cooling and the mixture was stirred for 1 hour. To the reaction mixture, 1 N hydrochloric acid (35 mL) was added slowly, the resulting precipitate was collected by filtration and washed with water. The solid was dissolved in tetrahydrofuran and diluted with ethyl acetate. The solution was washed with brine and 1 N hydrochloric acid and dried over anhydrous sodium sulfate (solution A). The previously filtrate was extracted with ethyl acetate, the extract was washed with saturated brine and dried over anhydrous sodium sulfate. The residue was subjected to basic silica gel column chromatography (ethyl acetate) and the crude product was obtained. This was combined with solution A, again subjected to basic silica gel column chromatography (ethyl acetate), and crystallized from hexane to afford ethyl 3-bromo-6-methylthieno[2,3-*b*]pyridine-2-carboxylate as a pale yellow solid (2g, yield 66%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19-8.17 (d, *J* = 8.4 Hz, 1H), 7.54-7.51 (d, *J* = 8.4 Hz, 1H), 4.42-4.35 (q, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 1.38-1.33 (t, *J* = 7.1 Hz, 3H)[6]. HRMS (ESI): calcd for $C_{11}H_{11}BrNO_2S$ 299.9688 [M+H$^+$] Found: 299.9681.

To a solution of ethyl 3-bromo-6-methylthieno[2,3-*b*]pyridine-2-carboxylate in methanol was added an aqueous solution of sodium hydroxide (3 M, 15 mL), and the mixture was stirred at 70 °C for 6 hours. The solvent was removed *in vacuo* and the resulting residue was dissolved in water, the pH was adjusted to 6 with aqueous hydrochloric acid (1 M) was added which caused precipitation. Filtration of the precipitate gave 3-bromo-6-methylthieno[2,3-b]pyridine-2-carboxylic acid as a yellow solid (1.53 g, yield 84 %). HRMS (ESI): calcd for $C_9H_7BrNO_2S$ 271.9375 [M+H$^+$] Found: 271.9373.

3-Bromo-6-methylthieno[2,3-*b*]pyridine-2-carboxylic acid (0.5 mmol, 136 mg) was added to *N*,*N*-dimethylformamide (1.5 mL), followed by *N*,*N*-diisopropylethylamine (1.5 mmol, 193.9 mg) and 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (0.6 mmol, 228.1 mg) under argon at room temperature and stirred for 30 min. 3-(Trifluoromethyl)aniline (1.0 mmol, 161.1 mg) was subsequently added and the reaction mixture stirred at 40 °C for another 16 hours. 2 mL of saturated sodium chloride solution was then added and the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with hydrochloride acid (2N), water, sodium bicarbonate (5%) and saturated sodium chloride. The organic phases was dried over anhydrous sodium sulfate, filtered, and then concentrated *in vacuo* and purified by column chromatography to give 3-bromo-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **17** as a white solid (120 mg, yield 58%). m.p. 219-220 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.18-8.17 (d, J = 7.6 Hz, 2H), 7.98-7.96 (d, J = 8.0 Hz, 1H), 7.66-7.63 (t, J = 7.9 Hz, 1H), 7.55-7.52 (t, J = 7.3 Hz, 2H), 2.67 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.8, 159.6, 157.6, 138.9, 132.4, 131.3, 130.2, 129.7, 129.5, 129.23, 129.17, 125.1, 123.8, 122.1, 120.85, 120.83, 116.24, 116.21, 106.6, 24.2. HRMS (ESI): calcd for $C_{16}H_{11}BrF_3N_2OS$ 414.9722 [M+H$^+$] Found: 414.9726. IR ν_{max} (KBr, cm^{-1}): 3375, 2923, 1659, 1600, 1549, 1445, 1342, 1246, 1115, 900, 796, 741, 571.

2.7 6-methyl-3-(methylamino)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 15

Copper (II) acetate (1.0 mmol, 181.6 mg) was added to a solution of 3-amino-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **1** (0.3 mmol, 105.2 mg) and pyridine (1.2 mmol) in

dioxane (5 mL). The mixture was stirred for 15 min, methyl boronic acid (0.8 mmol, 49 mg) was added, and the reaction was refluxed until aniline was totally consumed[7] (TLC analysis, 24 hours). The reaction mixture was allowed to reach at room temperature, filtered through celite and the solvent was concentrated off. The residue was purified by column chromatography to give 6-methyl-3-(methylamino)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **15** as yellow solid (45 mg, yield 41%). m.p. 165-166 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.52-8.50 (d, *J* = 8.5 Hz, 1H), 8.20 (s, 2H), 7.96-7.95 (d, *J* = 8.2 Hz, 1H), 7.56-7.53 (t, *J* = 8.0 Hz, 1H), 7.40-7.39 (d, *J* = 7.7 Hz, 1H), 7.29-7.27 (d, *J* = 8.5 Hz, 1H), 3.27-3.26 (d, J = 5.3 Hz, 3H), 2.59 (s, 3H). HRMS (ESI): calcd for $C_{17}H_{13}F_3N_3OS$ 364.0737 [M-H$^+$] Found: 364.0730. IR ν_{max} (KBr, cm^{-1}): 3291, 2934, 1729, 1631, 1489, 1333, 1245, 1124, 804, 756, 696.

2.8 3-acetamido-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide 16

A sample of 3-amino-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-b]pyridine-2-carboxamide **1** (1.0 mmol, 351.4 mg) in acetic anhydride (4 mL) was heated at 60 °C on a steam bath for 1 hour, cooled to room temperature and poured into an ice/water mixture (40 g), and then neutralized with aqueous ammonia. The white solid product was collected as 3-acetamido-6-methyl-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*b*]pyridine-2-carboxamide **16** (0.256 g, yield 65%). m.p. 281-282 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 2H), 8.17-8.15 (m, 2H), 7.92-7.90 (d, *J* = 8.2 Hz, 1H), 7.63-7.59 (t, *J* = 8.0 Hz, 1H), 7.49-7.47 (d, *J* = 7.7 Hz, 1H), 7.42-7.40 (d, *J* = 8.4 Hz, 1H), 2.63 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8, 160.7, 158.6, 157.2, 139.4, 132.4, 129.9, 129.5, 129.23, 129.16, 126.5, 125.4, 124.2, 123.9, 122.7, 120.3, 116.4, 24.2, 23.0. HRMS (ESI): calcd for $C_{18}H_{15}F_3N_3O_2S$ 394.0832 [M+H$^+$] Found: 394.0836; calcd for C18H14F3N3NaO2S 416.0651 [M+Na$^+$] Found: 416.0655. IR ν_{max} (KBr, cm^{-1}): 3281, 3091, 1670, 1645, 1559, 1329, 1271, 1166, 1126, 829, 696, 657.

3. Fluorescence-based binding assay

Nucleic acids were purchased from Suzhou Biosyntech Co., Ltd with the following sequences:

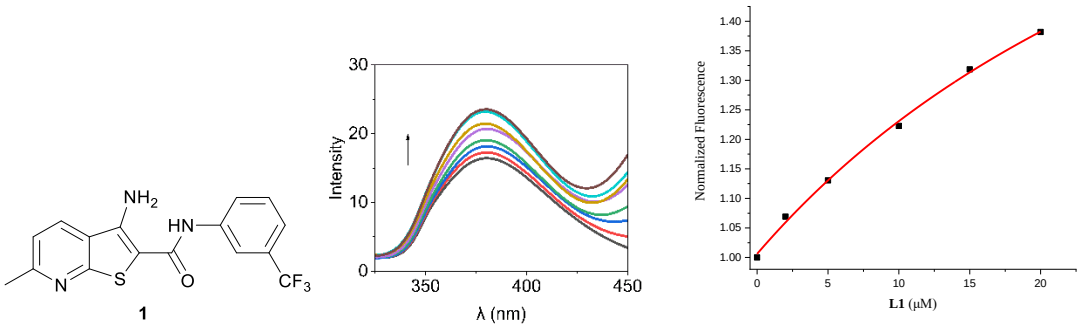
TAR RNA	5′-GGC AGA UCU GAG CCU GGG AGC UCU CUG CC -3′
2-AP TAR RNA	5′-GGC AGA UC(2-AP) GAG CCU GGG AGC UCU CUG CC -3′
RRE RNA	5′-GGU CUG GGC GCA GCG CAA GCU GAC GGU ACA GGC C-3′
2-AP RRE RNA	5′-GGU CUG GGC GCA GCG CAA GCU GAC GG(2-AP) ACA GGC C-3′

For the determination of binding constraints of each ligand with TAR RNA, 5OD of 2-AP TAR was dissolved in 190 µL of Na_2HPO_4-KH_2PO_4 buffer solution (pH 7.4 at 25 °C, containing 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) to a concentration of ~100 µM and annealed by heating to 95 °C for 5 min. Analogously, 5OD of 2-AP RRE was dissolved in 148 µL of Na_2HPO_4-KH_2PO_4 buffer solution (pH 7.4 at 25 °C, containing 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) to a concentration of ~100 µM and annealed by heating to 95 °C for 5 min. The mixture were allowed to cool to room temperature for at least 30 min. The aforementioned RNA solution was diluted to 10 µM with Na_2HPO_4-KH_2PO_4 buffer solution before use. 10 µL of each RNA solution (10 µM) was added to a centrifuge tube containing 180 µL of Na_2HPO_4-KH_2PO_4 buffer solution and 10 µL of the ligand in DMSO. The solution was incubated at 37 °C for 30 min. Fluorescence values were measured at an excitation wavelength of 310 nm and an emission wavelength of 320-600 nm using a fluorescence spectrophotometer Hitachi F7000, and values were normalized to free 2-AP TAR/RRE. Apparent K_d values were determined by nonlinear regression by fitting the measured fluorescence (Origin software).

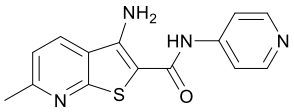
$$F = A \times [RNA] + B \times \frac{\left\{([RNA]+[\mathbf{L}]+K_d)-\sqrt{([RNA]+[\mathbf{L}]+K_d)^2-(4\times[RNA]\times[\mathbf{L}])}\right\}}{2} \qquad (1)$$

in which [RNA] is the concentration of RNA used (0.5 µM), [**L**] is the concentration of the ligand. A and B are weighting factors that were allowed to float during curve fitting.

TAR RNA-Ligands Binding Spectra



K_d = 46.2 ± 22.3 µM, R^2 = 0.998



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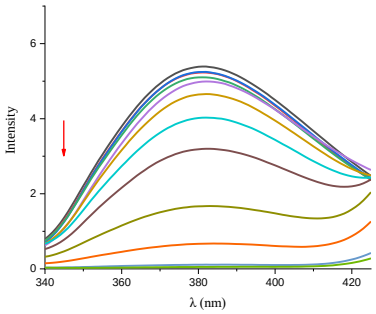
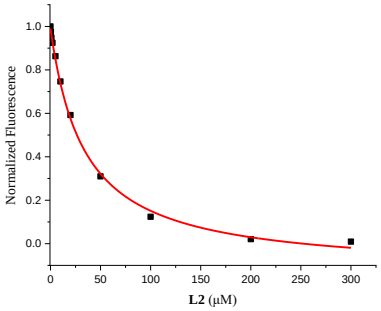
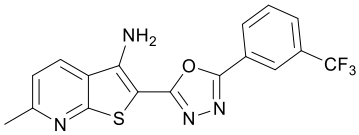




K_d = 33.5 ± 2.3 μM, R^2 = 0.998



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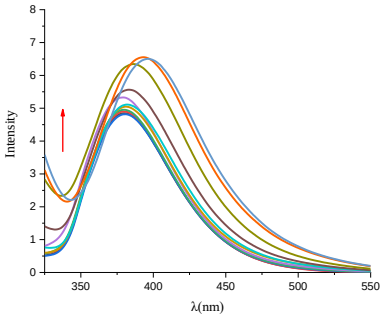
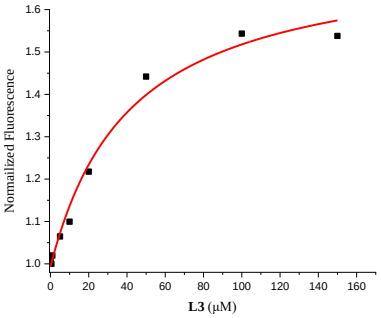
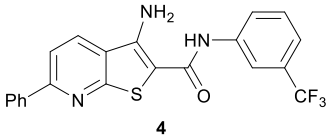




K_d = 40.8 ± 1.0 μM, R^2 = 0.987



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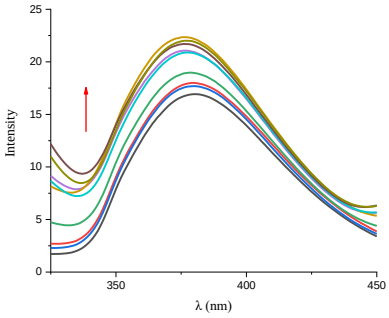
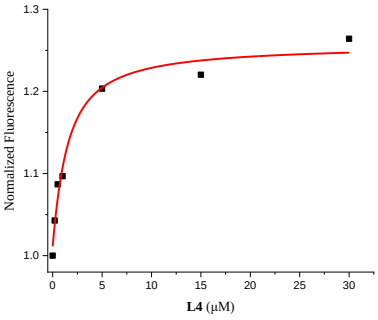
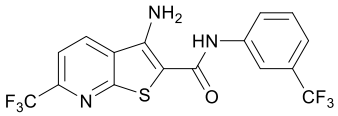




K_d = 1.3 ± 0.5 μM, R^2 = 0.980



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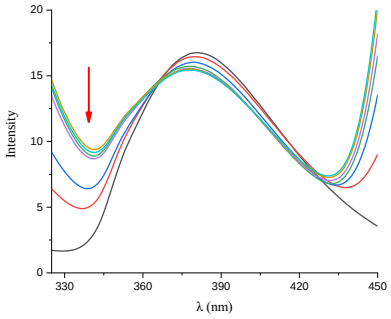
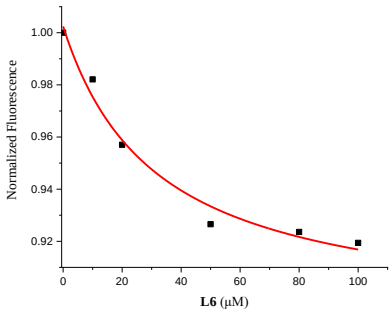
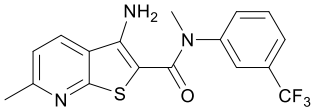




K_d = 25.7 ± 10.2 μM, R^2 = 0.981



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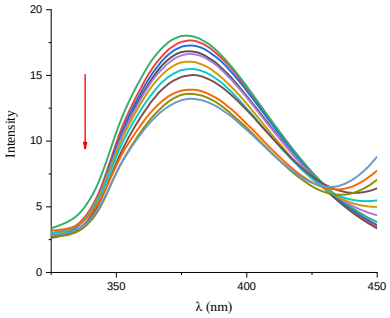
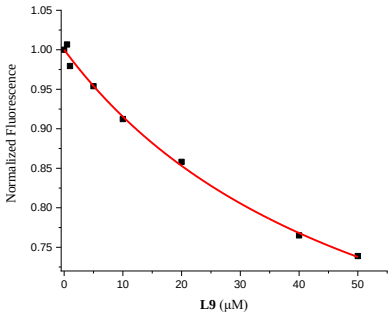
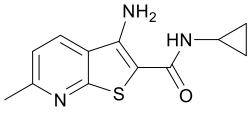




K_d = 46.0 ± 11.6 μM, R^2 = 0. 995



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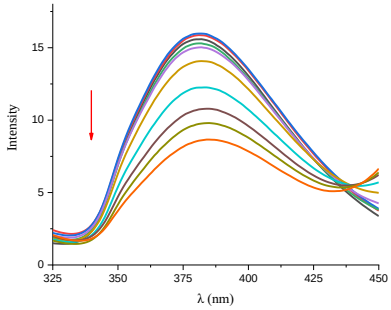
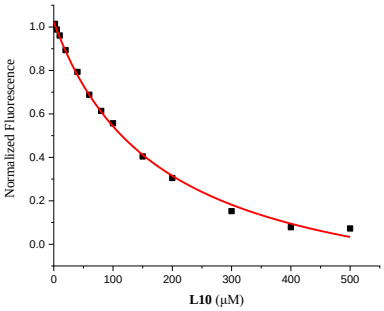
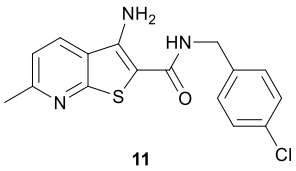




K_d = 180.2 ± 12.9 μM, R^2 = 0.978



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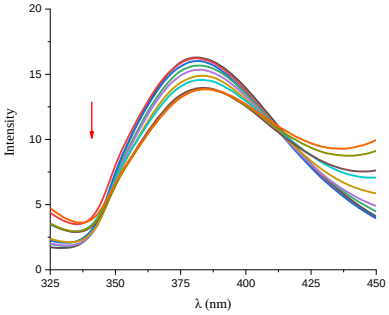
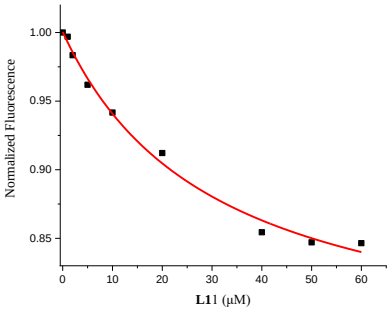
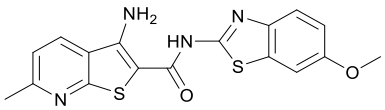




K_d = 30.1 ± 6.8 μM, R^2 = 0.993



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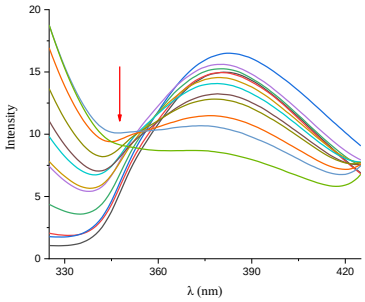
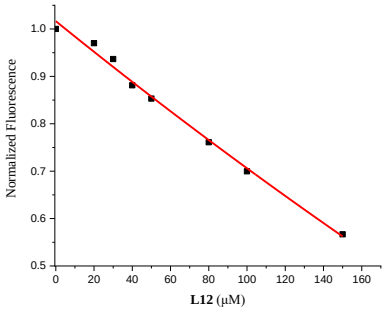
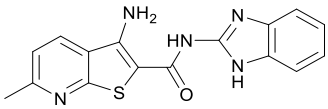




K_d = 1882 μM, R^2 = 0.993



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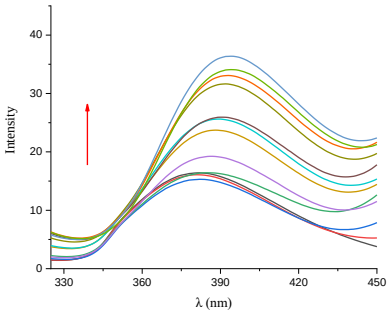
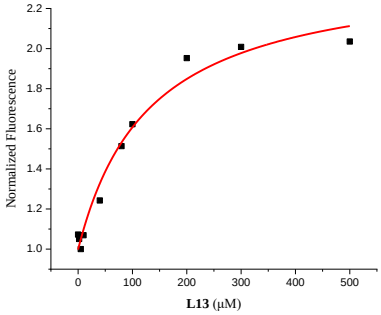
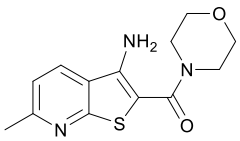




K_d = 130.1 ± 36.2 μM, R^2 = 0.978



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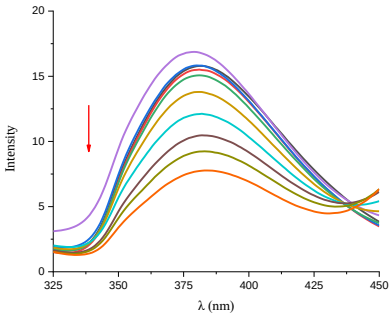
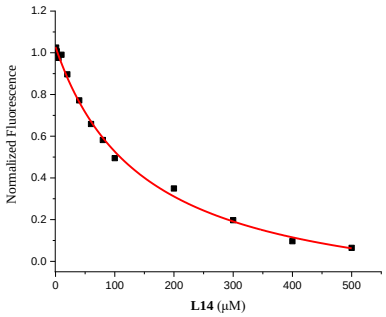
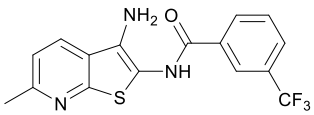




K_d = 150.1 ± 14.0 μM, R^2 = 0.996



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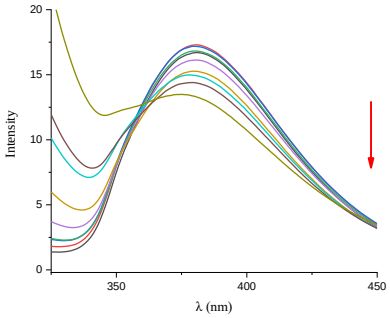
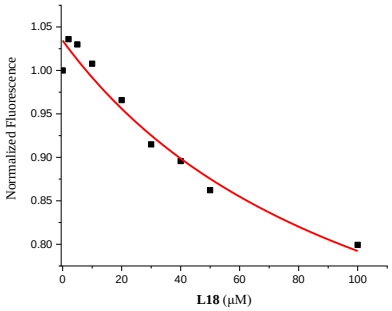
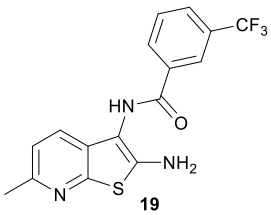




K_d = 108.8 ± 63.9 μM, R^2 = 0.958



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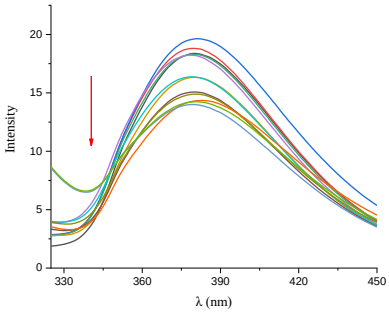
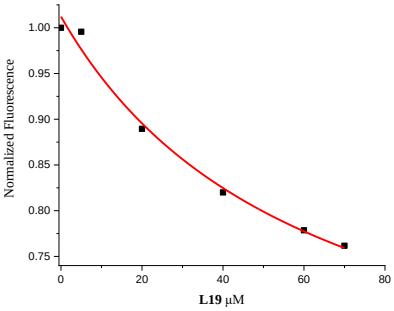
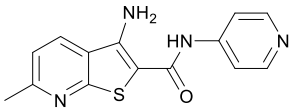




K_d = 45.0 ± 19.8 μM, R^2 = 0. 989

RRE RNA-Ligands Binding Spectra



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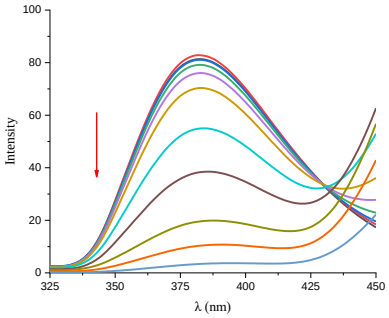
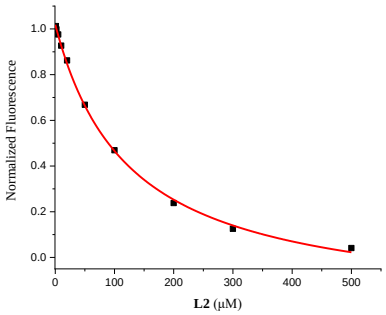
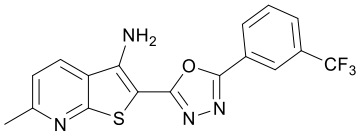




K_d = 125.0 ± 7.6 μM, R^2 = 0.999



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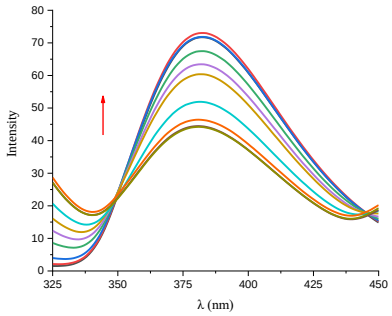
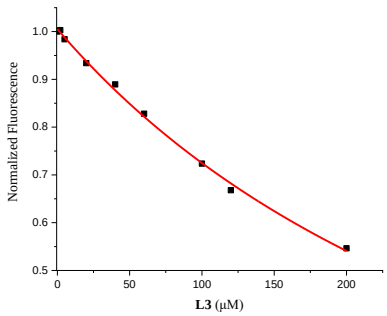
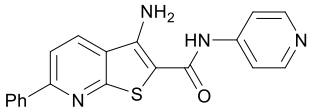




K_d = 450.1 ± 143.6 μM, R^2 = 0.998



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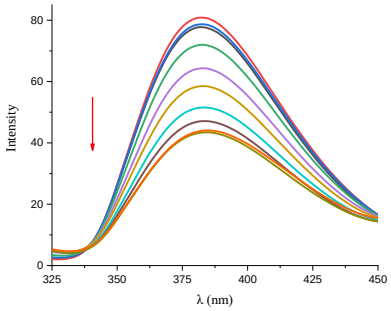
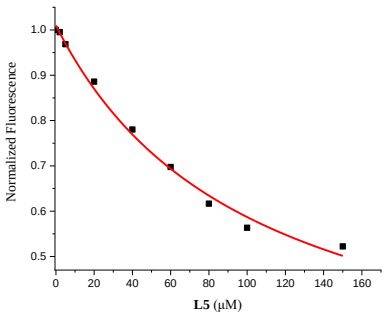
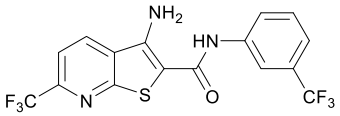




K_d = 101.4 ± 21.4 μM, R^2 = 0.994



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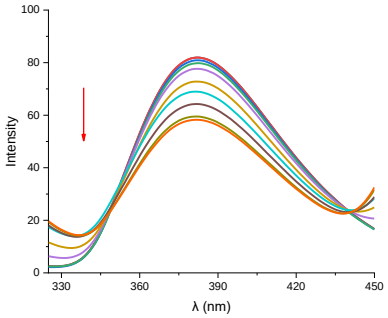
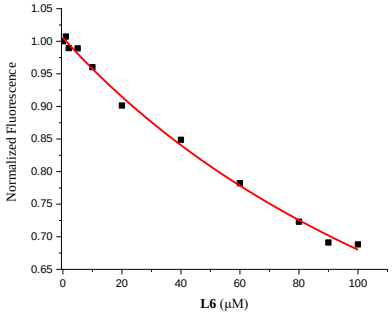
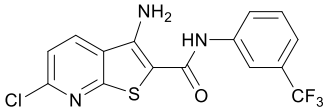




K_d = 184.8 ± 54.7 μM, R^2 = 0.996



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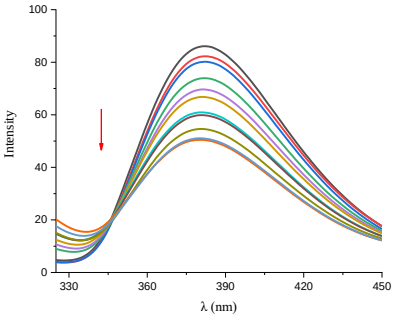
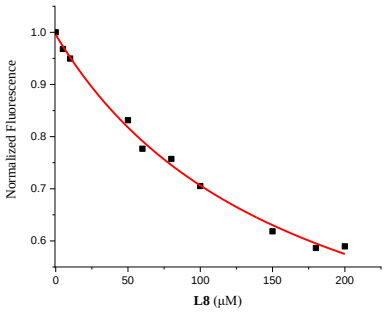
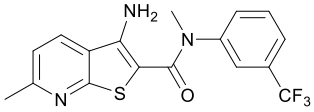




K_d = 166.0 ± 26.0 μM, R^2 = 0.995



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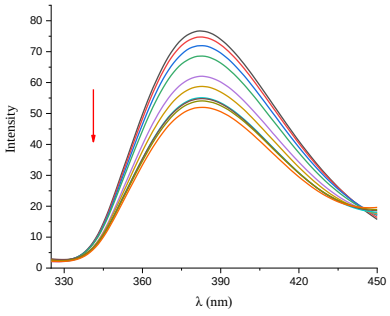
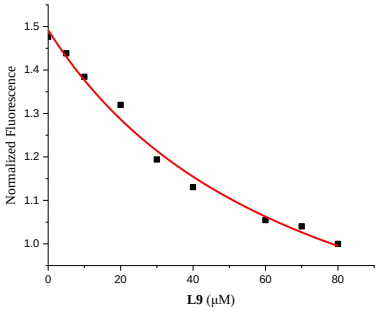
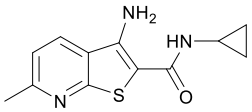




K_d = 71.1 ± 21.3 μM, R^2 = 0.990



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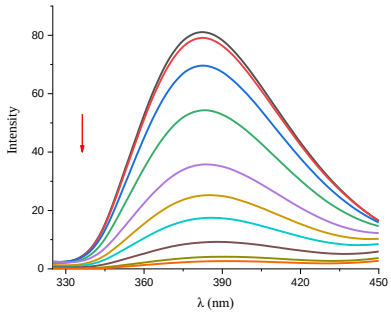
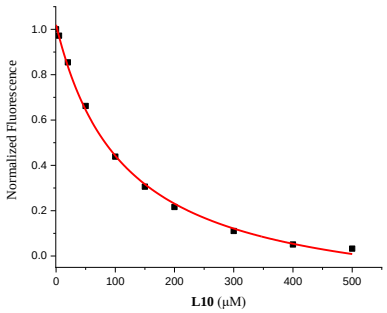
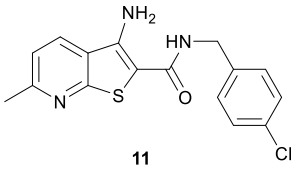




K_d = 90.7 ±10.3 μM, R^2 = 0.998



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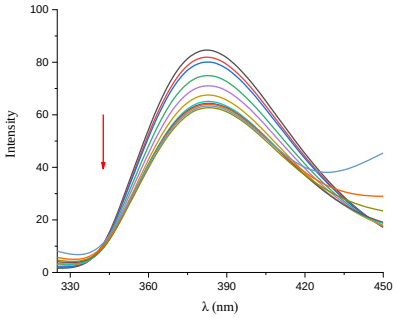
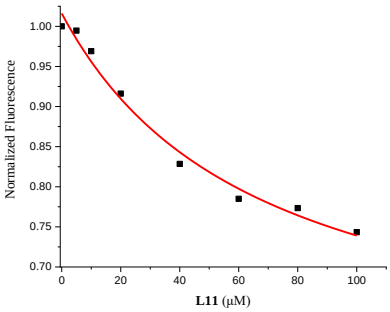
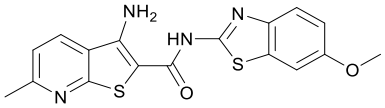




K_d = 71.8 ± 21.6 μM, R^2 = 0.987



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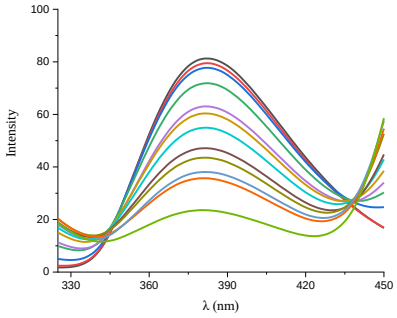
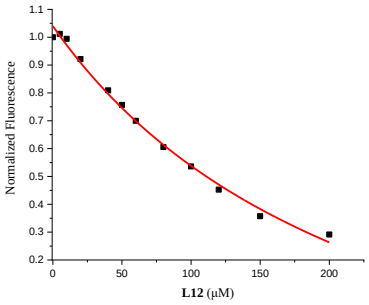
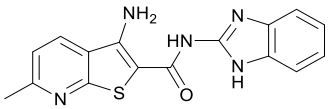




K_d = 240.9± 49.5 μM, R^2 = 0.994



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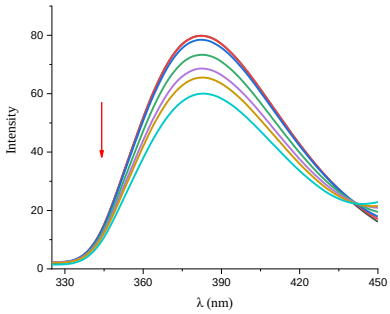
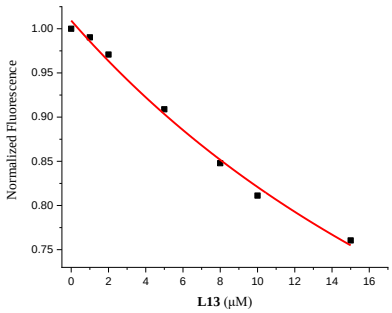
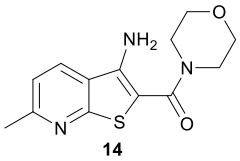




K_d = 34.5 ± 14.3 μM, R^2 = 0.994



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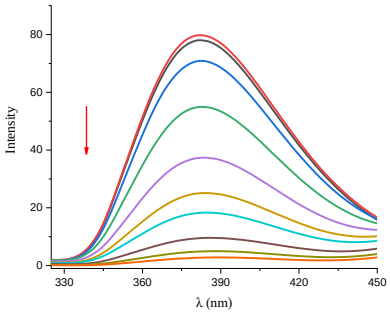
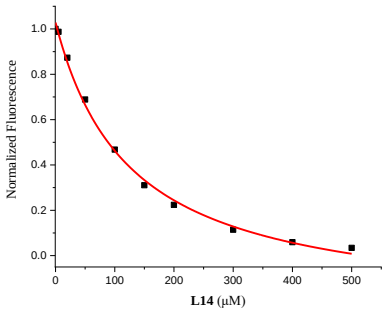
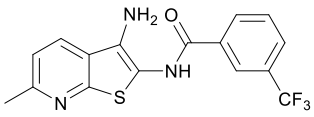




K_d = 125.2 ± 12.4 μM, R^2 = 0.997



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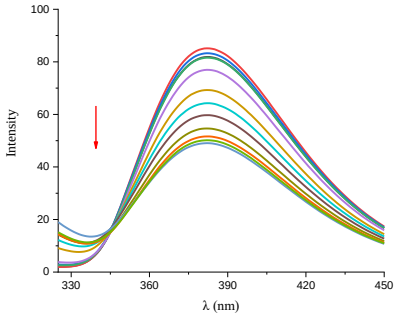
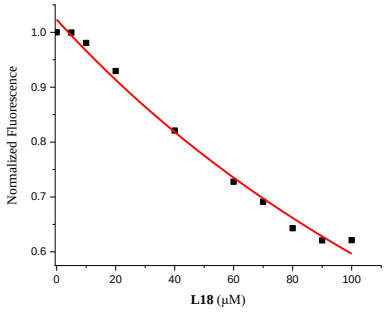
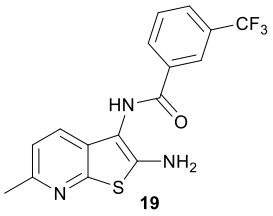




K_d = 259.3 ± 146.4 μM, R^2 = 0.991



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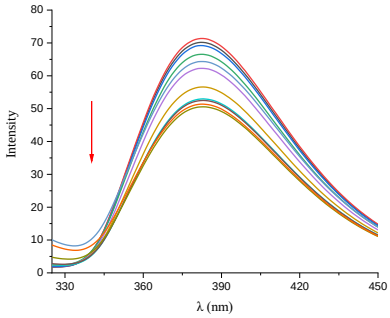
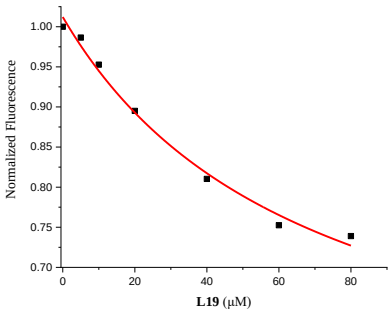
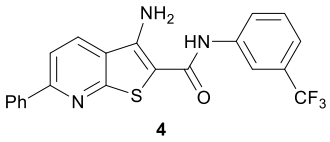




K_d = 66.8 ± 28.2 μM, R^2 = 0. 983

TAR RNA mutants-Ligands Binding Spectra

U23→C: 5’-GGC AGA CC(2-AP) GAG CCU GGG AGC UCU CUG CC



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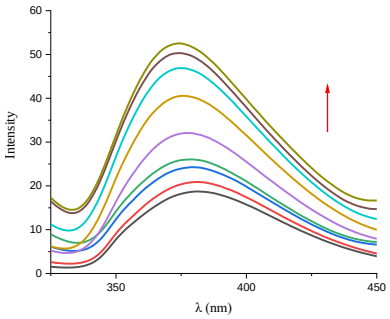
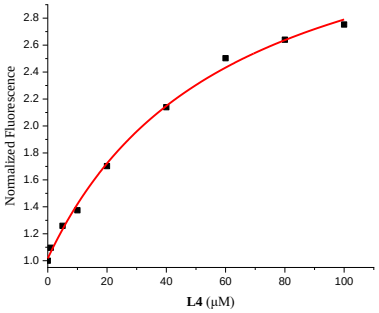
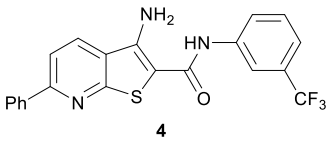




K_d = 60.33 ± 8.87 μM, R^2=0.997

G21C→AU: 5’-GGC AAA UC(2-AP) GAG CCU GGG AGC UCU UUG CC



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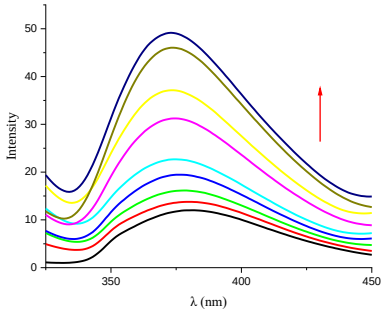
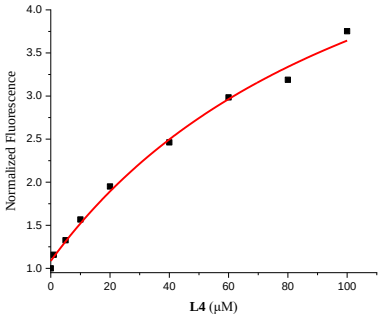
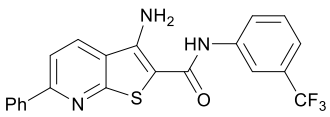




K_d = 117.81 ± 35.00 μM, R^2=0.994

G43C→AU: 5’-GGU AGA UC(2-AP) GAG CCU GGG AGC UCU CUA CC



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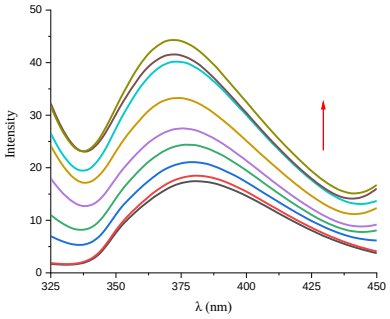
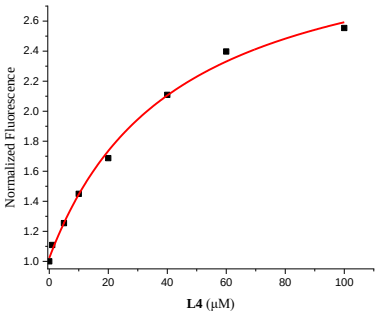
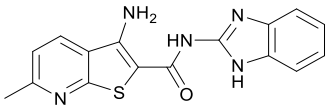




K_d = 42.50 ± 6.78 μM, R^2=0.996

RRE RNA mutants-Ligands Binding Spectra

G70C49→AU: 5’- GGU CUG GGU GCA GCG CAA GCU GAC AG(2-AP) ACA GGC C -3’



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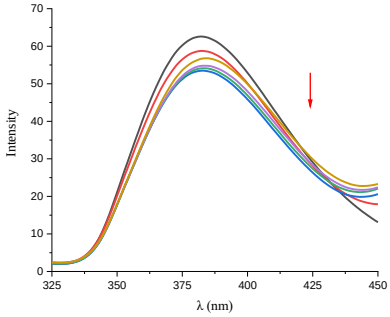
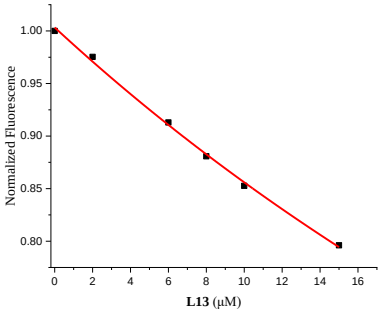
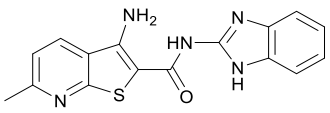




K_d = 75.76 ± 37.42 μM, R^2=0.998

C69G→UA: 5’-GGU CUG GGC ACA GCG CAA GCU GAU GG(2-AP) ACA GGC C -3’



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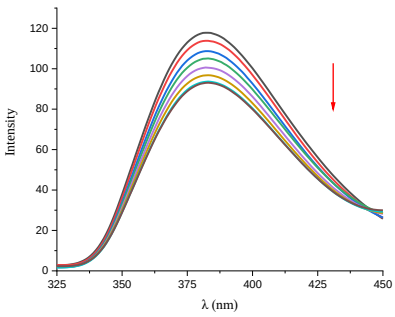
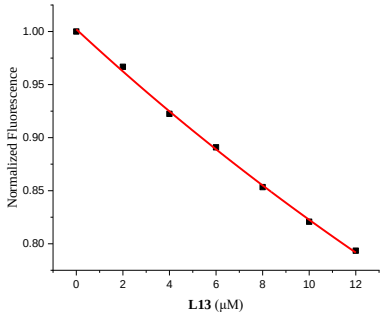
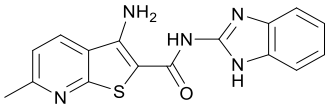




K_d = 73.26 ± 32.07 μM, R^2=0.999

G71G→AA: 5’-GGU CUG GAC GCA GCG CAA GCU GAC GA(2-AP) ACA GGC C



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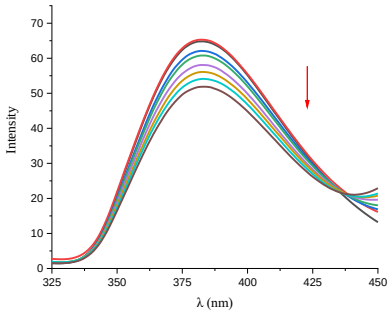
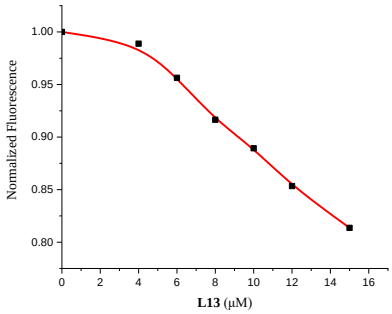
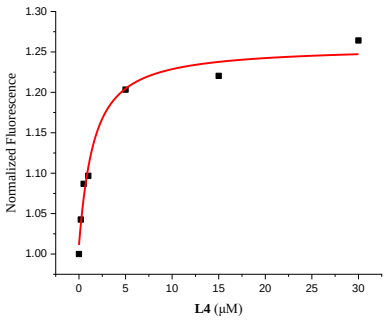




K_d > 1000 μM

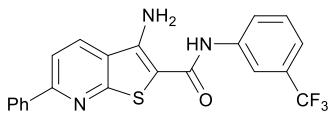
We assume compound **4** binds with TAR RNA in a 1:1 ratio. It turned out the titration data for **4** with TAR exhibited single inflection points characteristic of single-site binding (eq. (1)). However, the binding affinities deduced from eq (2), which was used to calculate multiple binding sites, were not reasonable[9-10]:

$$F = A \times [RNA] + B \times \frac{\left\{([RNA]+[\mathbf{L}]+K_d)-\sqrt{([RNA]+[\mathbf{L}]+K_d)^2-(4\times[RNA]\times[\mathbf{L}])}\right\}}{2} \qquad (1)$$



K_d = 1.25 ± 0.49 μM, R^2=0.980

$$F = A \times [RNA] + B \times \frac{\left\{([RNA]+[\mathbf{L}]+K_{d1})-\sqrt{([RNA]+[\mathbf{L}]+K_{d1})^2-(4\times[RNA]\times[\mathbf{L}])}\right\}}{2} + C \times \frac{\left\{([RNA]+[\mathbf{L}]+K_{d2})-\sqrt{([RNA]+[\mathbf{L}]+K_{d2})^2-(4\times[RNA]\times[\mathbf{L}])}\right\}}{2} \qquad (2)$$



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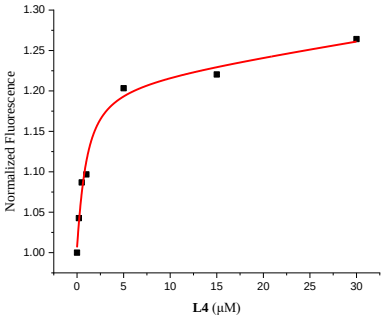


K_{d1} = 0.72 ± 0.48 μM, R^2=0.988

K_{d2} = 276843 ± 9.84E8 μM

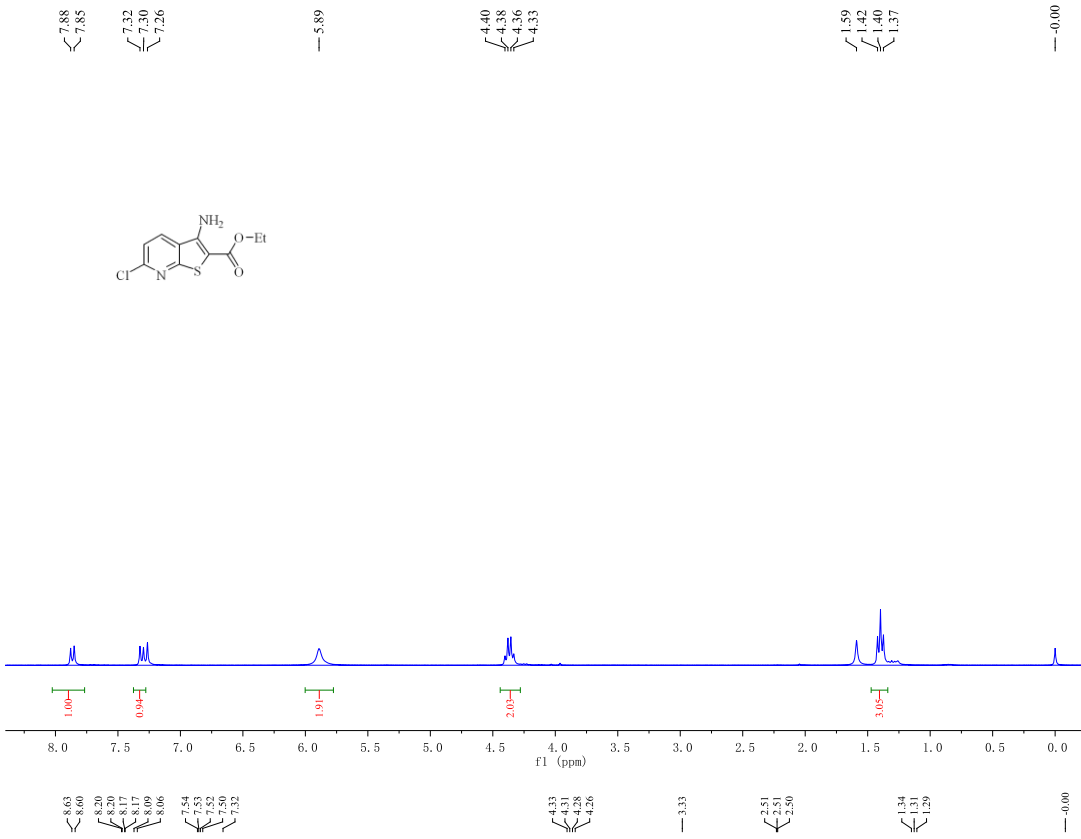
4. Docking details

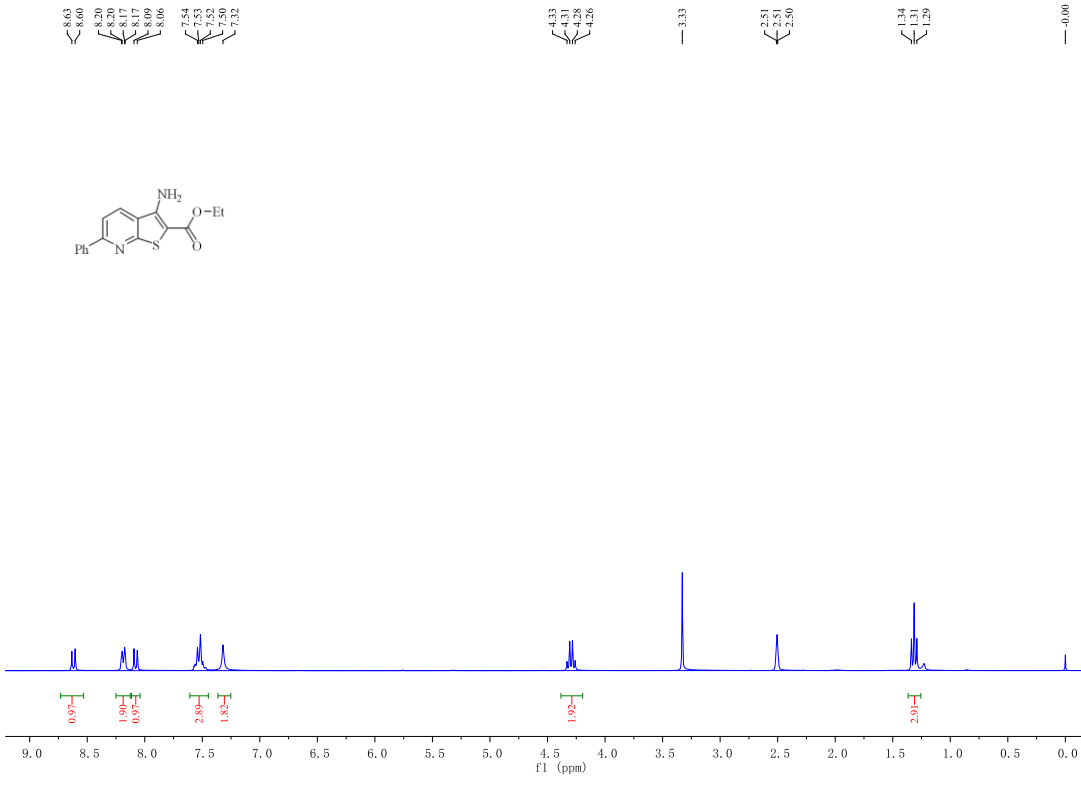
Docking studies were performed by using the program AutoDock 4.2 was used to dock thieno[2,3-b]pyridine-2-carboxamide complexes into TAR/RRE RNA. AutoDock 4.2 using the Lamarckian Genetic Algorithm (LGA) for the prediction of binding affinity and searching for the optimum binding site together with the AutoDock Tools (ADT) were employed to set up and perform blind docking calculations of the thieno[2,3-b]pyridine-2-carboxamide complexes[8]. The structure of TAR RNA with sequence GCCAGAUUUGAGCCUGGGAGCUCUCUGGC (PDB ID: 1QD3, a sequence used in oligonucleotide study) and RRE RNA with sequence chains A AACGGGGCGCAGAA, chains B UCUGACGGUACGUUU (PDB ID: 1CSL, a sequence used in oligonucleotide study)obtained from the Protein Data Bank (www.rcsb.org/pdb) was constructed using AutoDock 4.2 package to study the RNA-binding properties.

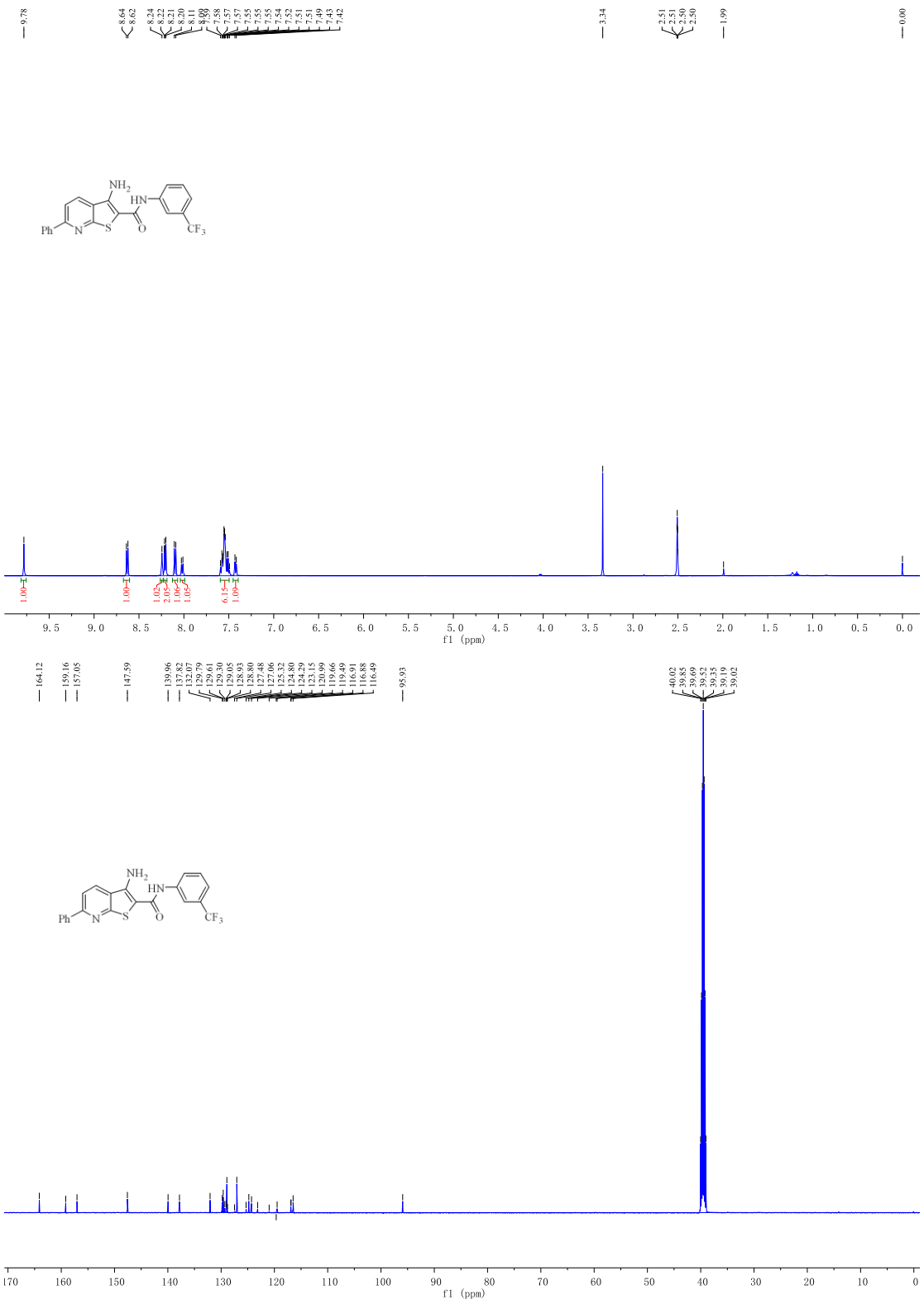
Thieno[2,3-b]pyridine-2-carboxamide complexes structures were simulated in chem3D professional; Cambridge software; using MM2 method (RMS gradient = 0.05 kcal/mol). The output files were subsequently minimized by Semi-empirical AM1 method (Convergence limit = 0.01; Iteration limit = 50; RMS gradient = 0.05 kcal/mol; Fletcher-Reevesoptimizer algorithm)[11].

The receptor (TAR/RRE RNA) and the ligands (thieno[2,3-b]pyridine-2-carboxamide complexes) files were prepared using AutoDock Tools. The heteroatoms including water molecules were deleted and polar hydrogen atoms and Kollman charges were added to the receptor molecule. All other bonds were allowed to be rotatable. In the docking analysis, the binding site was assigned across the binding site of Tat/Rev protein, which was enclosed in a box with the number of grid points in x × y × z directions, 66 × 66 × 66 and a grid spacing of 0.375 Å. Initially, AutoGrid was run to generate the grid map of various atoms of the ligands and receptor. After the completion of the grid map, AutoDock was run by using autodock parameters as follows: GA population size, 150; maximum number of energy evaluations 2500000; and the number of generations 27000. A total of 100-independent runs were carried out. A maximum of 100 conformers were considered for each molecule, and the root-meansquare (RMS) cluster tolerance was set to 2.0 Å in each run. All calculations were performed on an Intel Core i5 based machine running Windows as the operating system. For each of the docking cases, the lowest energy docked conformation, according to the Autodock scoring function, was selected as the binding mode. Visualization of the docked pose was done by using PyMOL (The PyMOL Molecular Graphics System, Version 2.0, Schrödinger, LLC) molecular graphics program.

5. NMR spectra



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8.62
8.24
8.22
8.21
8.20
8.11
8.09
7.59
7.58
7.57
7.57
7.55
7.55
7.55
7.54
7.52
7.51
7.51
7.49
7.43
7.42
3.34
2.51
2.51
2.50
2.50
1.99
0.00
NH2
HN
Ph
N
S
O
CF3
1.00
1.00
1.03
2.01
1.06
1.05
6.15
1.05
f1 (ppm)
164.12
159.16
157.05
147.59
139.96
137.82
132.07
129.79
129.61
129.30
129.05
128.93
128.80
127.48
127.06
125.32
124.80
124.29
123.15
120.99
119.66
119.49
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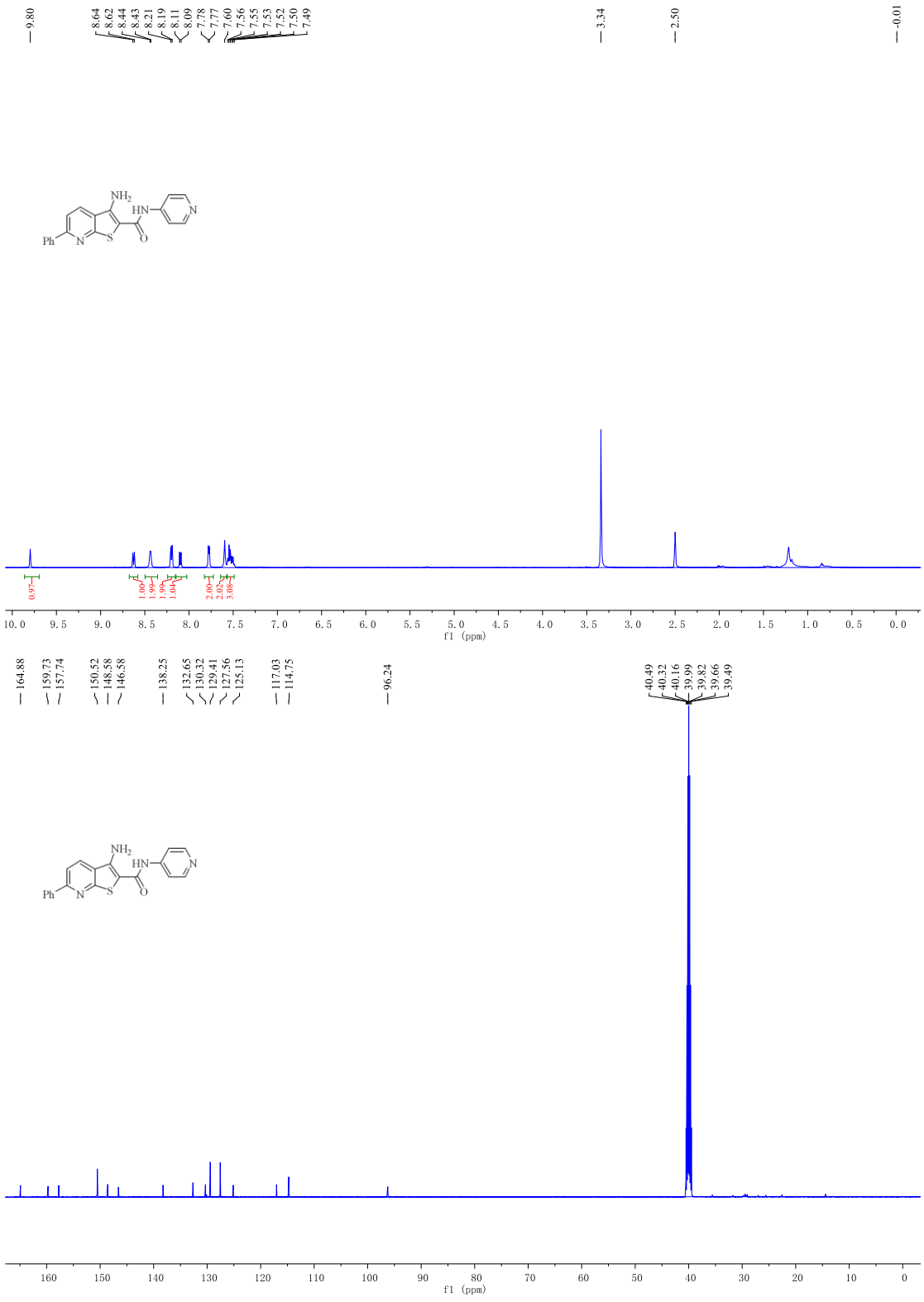
39.85
39.69
39.52
39.35
39.19
39.02
f1 (ppm)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8.62
8.44
8.43
8.21
8.19
8.11
8.09
7.78
7.77
7.60
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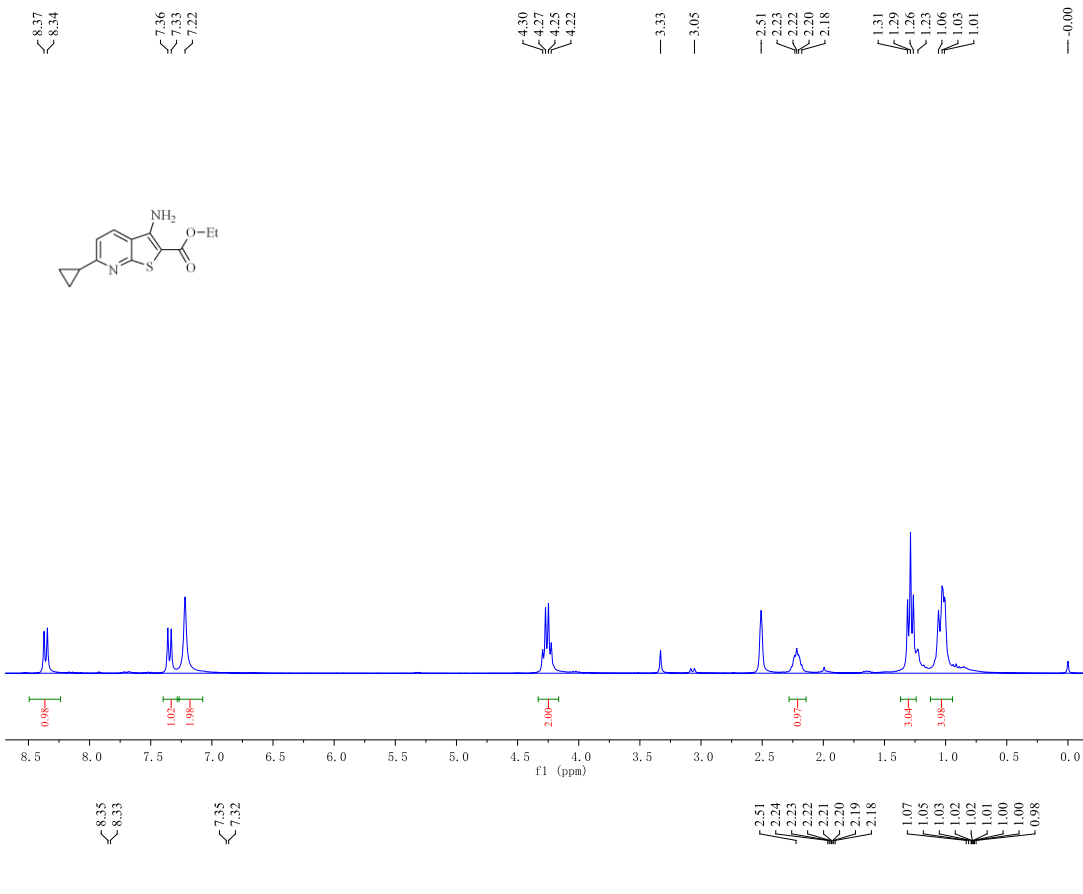
7.55
7.53
7.52
7.50
7.49
3.34
2.50
-0.01
NH2
HN
N
Ph
N
S
O
0.97
1.00
1.99
1.99
1.04
2.00
2.02
3.08
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
164.88
159.73
157.74
150.52
148.58
146.58
138.25
132.65
130.32
129.41
127.56
125.13
117.03
114.75
96.24
40.49
40.32
40.16
39.99
39.82
39.66
39.49
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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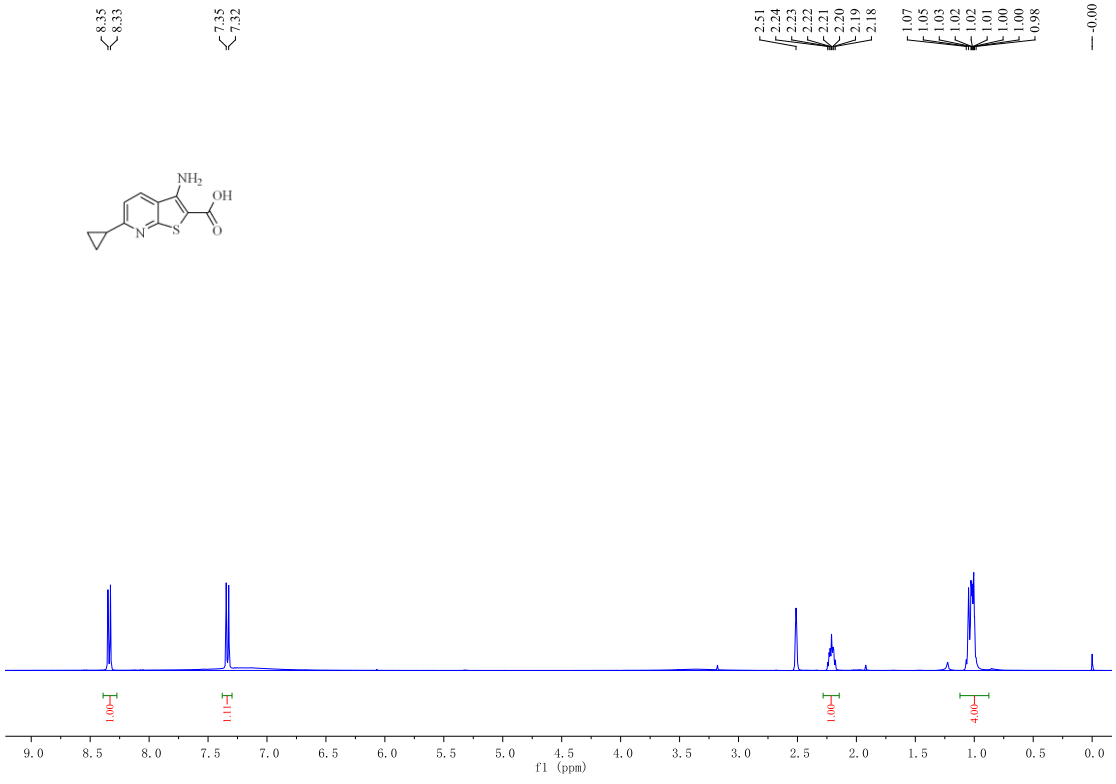
7.36
7.33
7.22
4.30
4.27
4.25
4.22
3.33
3.05
2.51
2.23
2.22
2.20
2.18
1.31
1.29
1.26
1.23
1.06
1.03
1.01
-0.00
NH2
O-Et
O
N
S
0.98
1.02
1.98
2.00
0.97
3.04
3.98
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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8.33
7.35
7.32
2.51
2.24
2.23
2.22
2.21
2.20
2.19
2.18
1.07
1.05
1.03
1.02
1.02
1.01
1.00
1.00
0.98
-0.00
NH2
OH
O
N
S
1.00
1.11
1.00
4.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.0
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2.5
2.0
1.5
1.0
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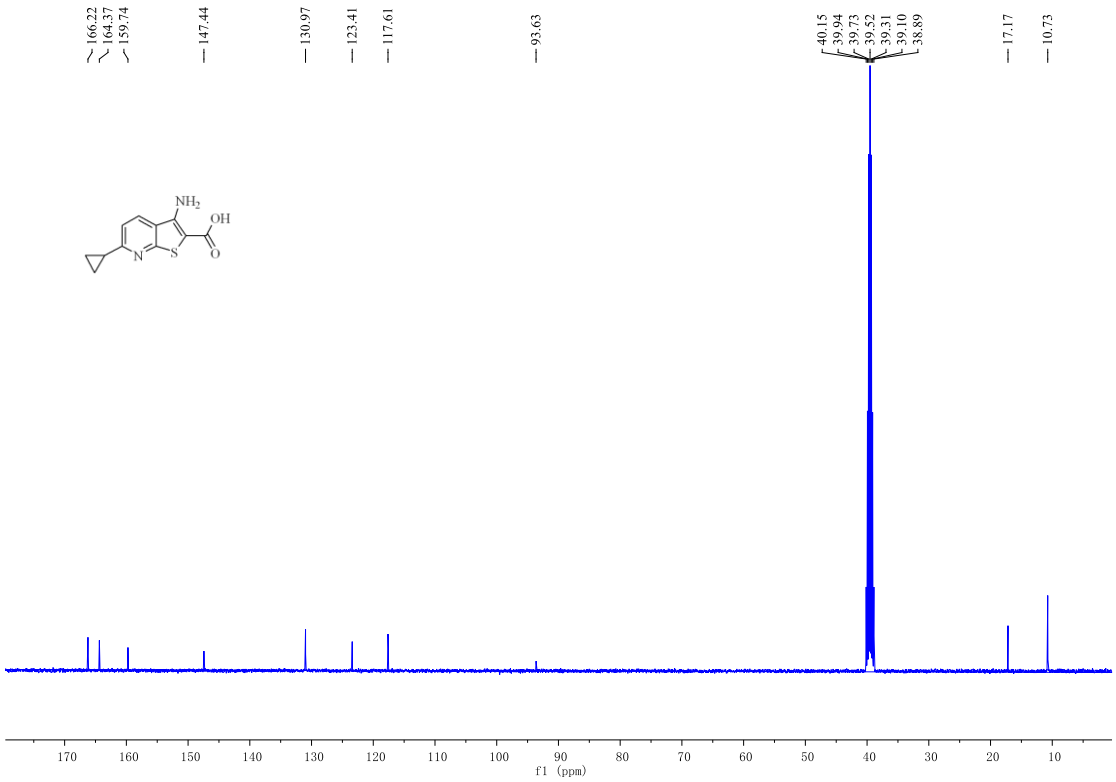
0.0
f1 (ppm)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130.97
123.41
117.61
93.63
40.15
39.94
39.73
39.52
39.31
39.10
38.89
17.17
10.73
NH2
OH
O
N
S
f1 (ppm)


9.64
8.37
8.35
8.21
7.99
7.98
7.57
7.56
7.54
7.45
7.41
7.40
7.37
7.35
3.83
3.33
2.51
2.50
2.27
2.26
2.25
2.24
2.23
2.22
1.26
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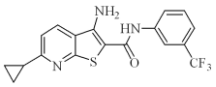
1.07
1.06
1.05
1.04
1.03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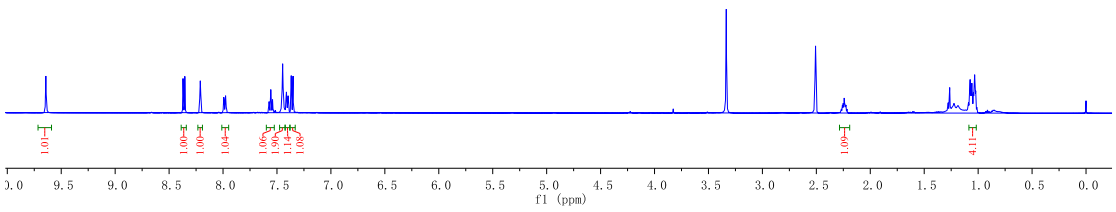
O
N
S
CF3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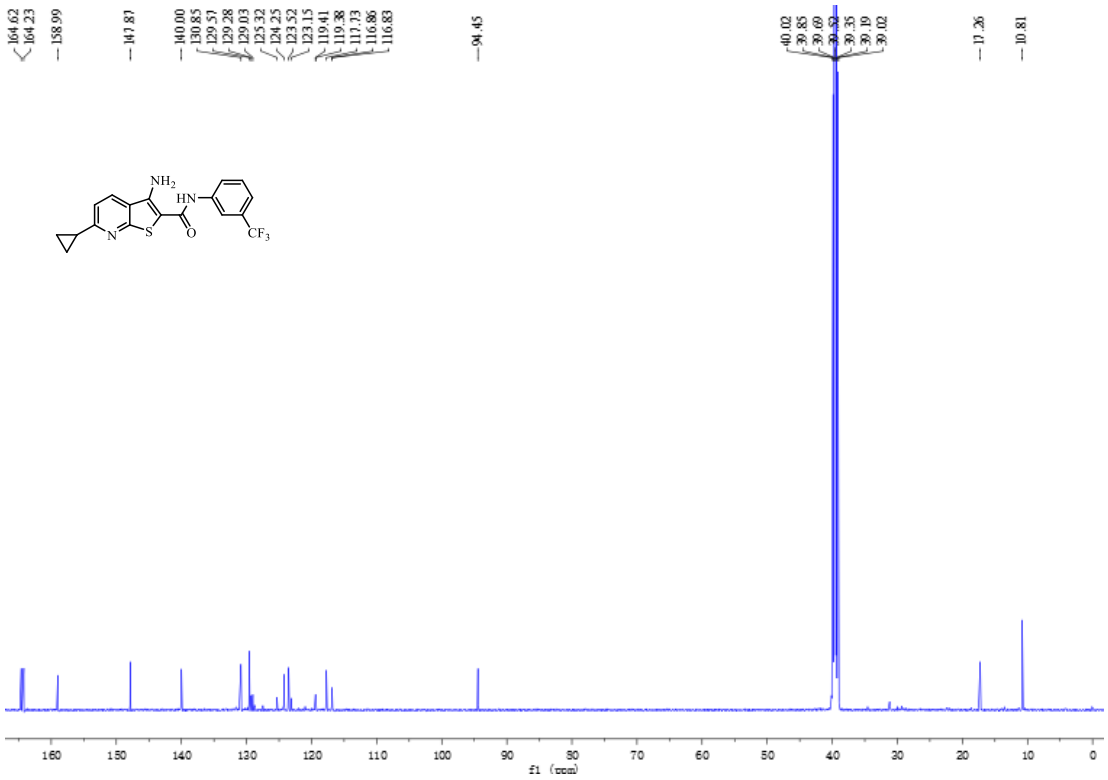
1.08
1.09
4.11
f1 (ppm)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125.32
124.25
123.52
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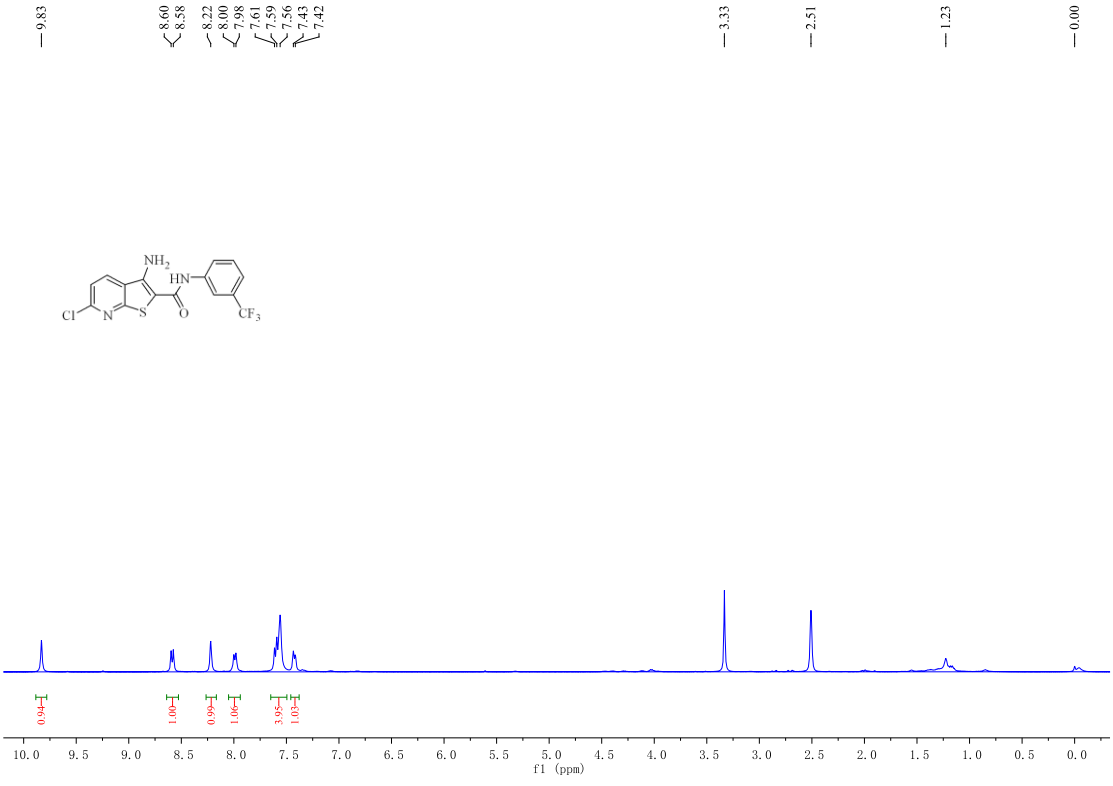
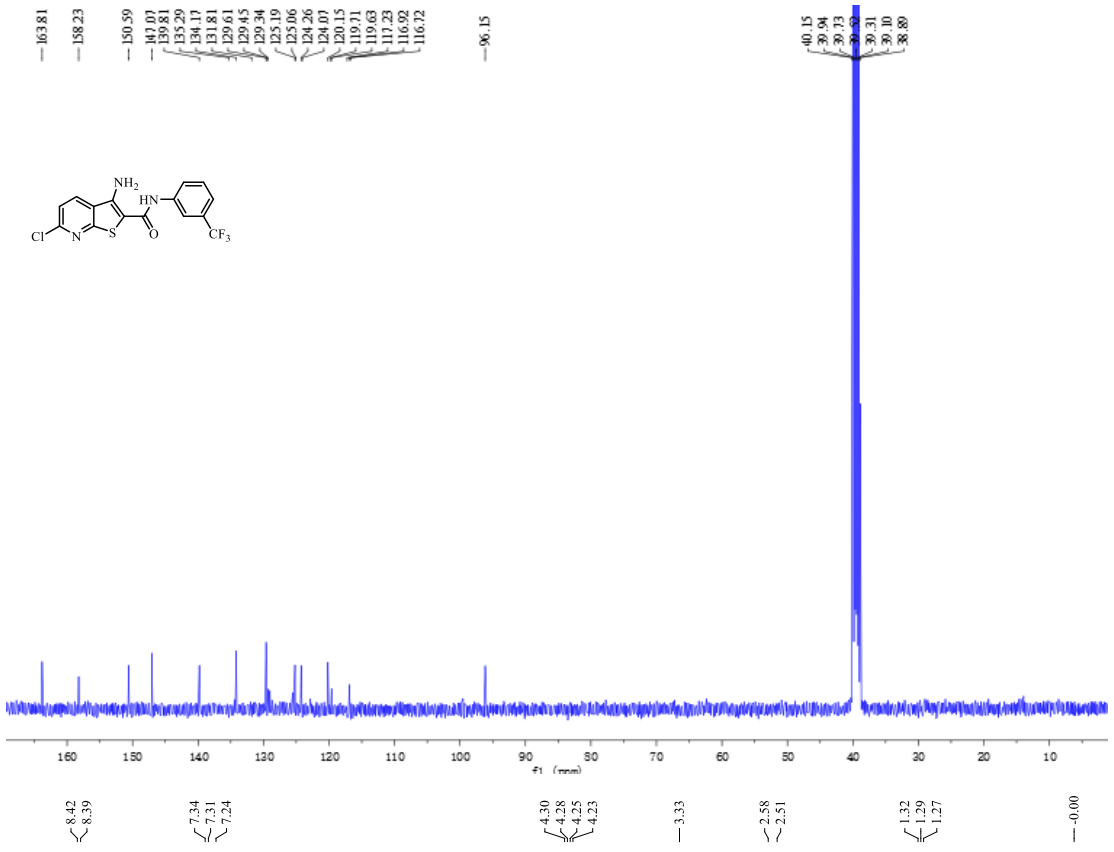
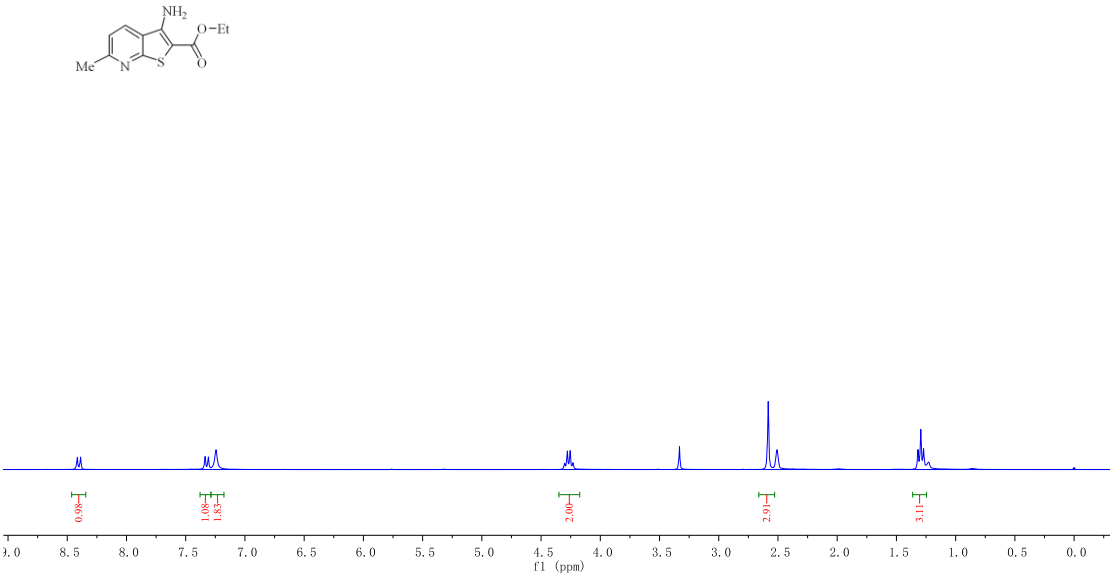
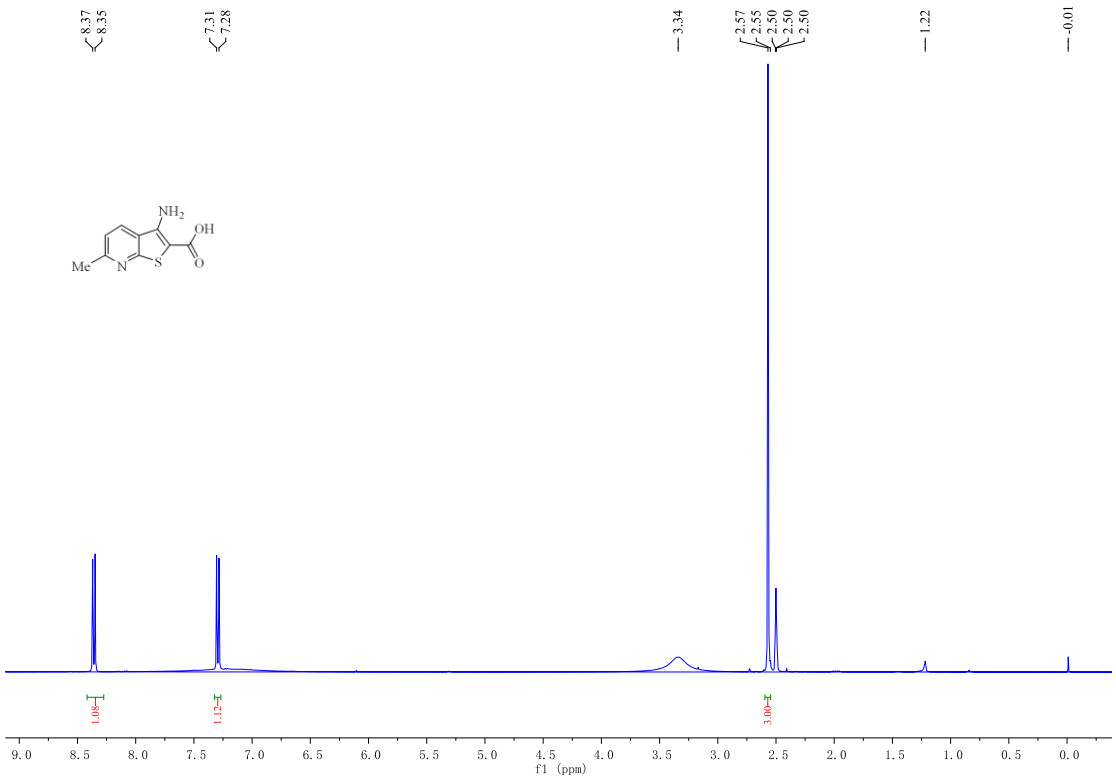
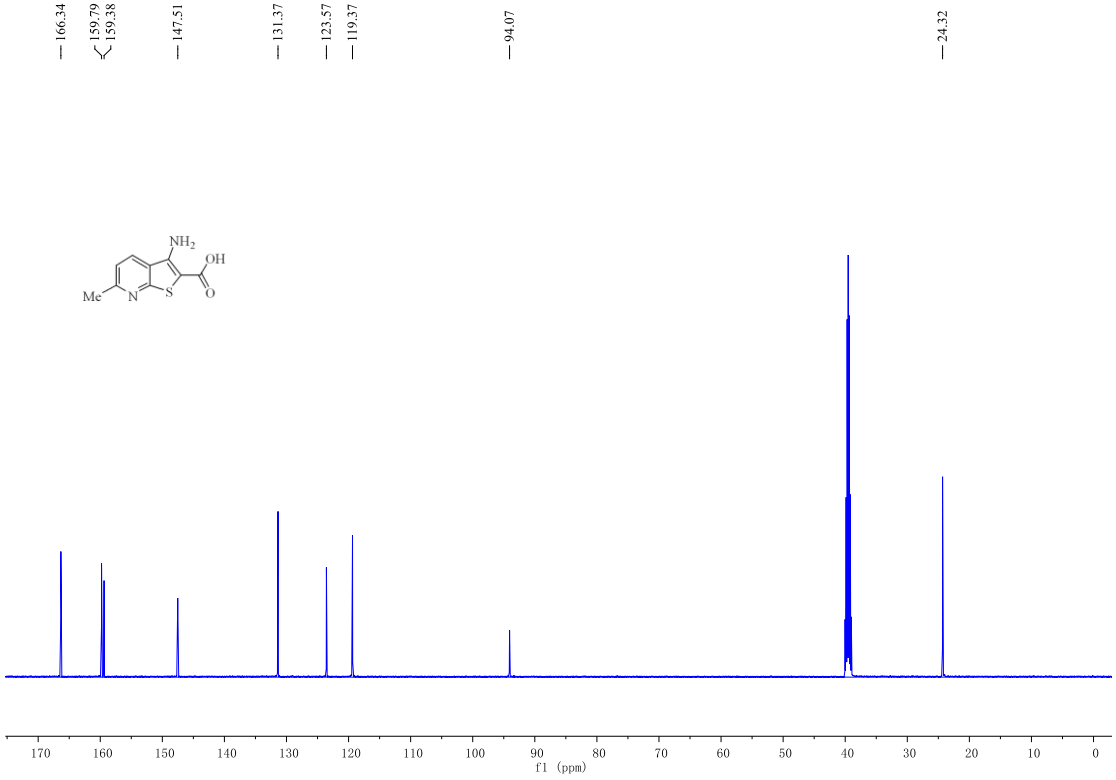
119.41
119.38
117.73
116.86
116.83
94.45
40.02
39.85
39.69
39.52
39.35
39.19
39.02
17.26
10.81
NH2
HN
O
N
S
CF3
f1 (ppm)

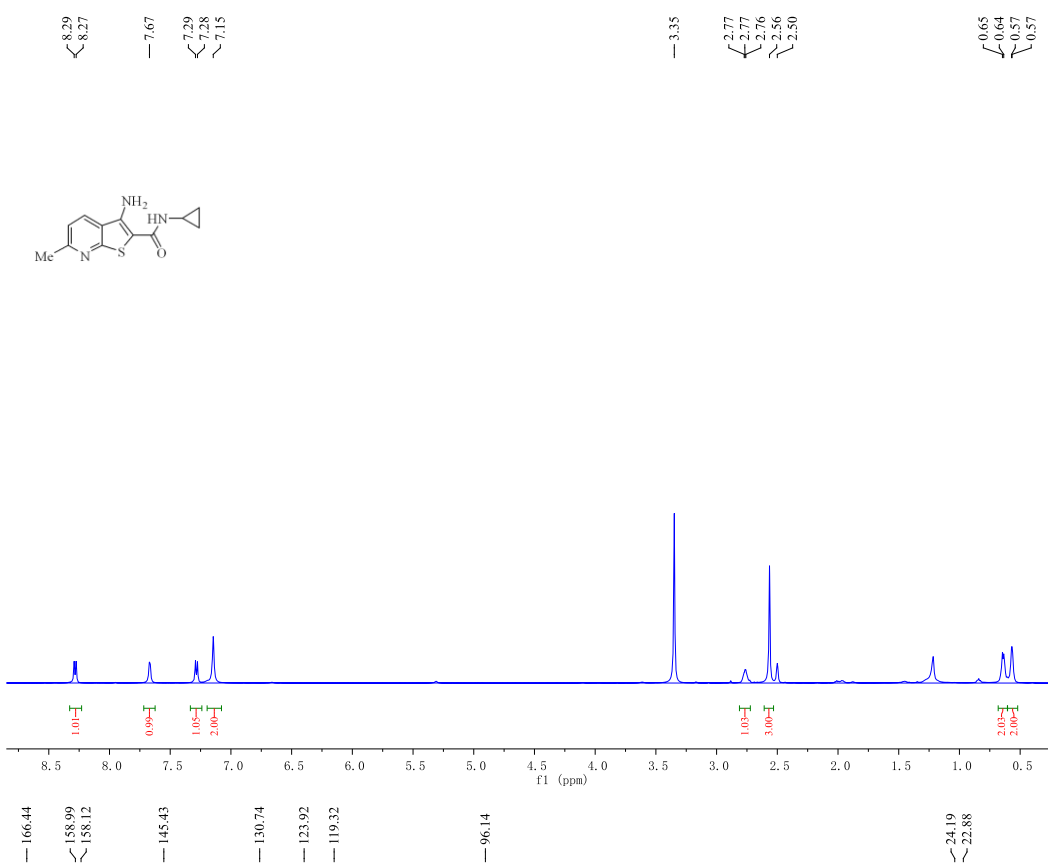

9.83
8.60
8.58
8.22
8.00
7.98
7.61
7.59
7.56
7.43
7.42
3.33
2.51
1.23
0.00
NH2
HN
O
N
S
Cl
CF3
0.94
1.00
0.99
1.06
3.95
1.03
f1 (ppm)

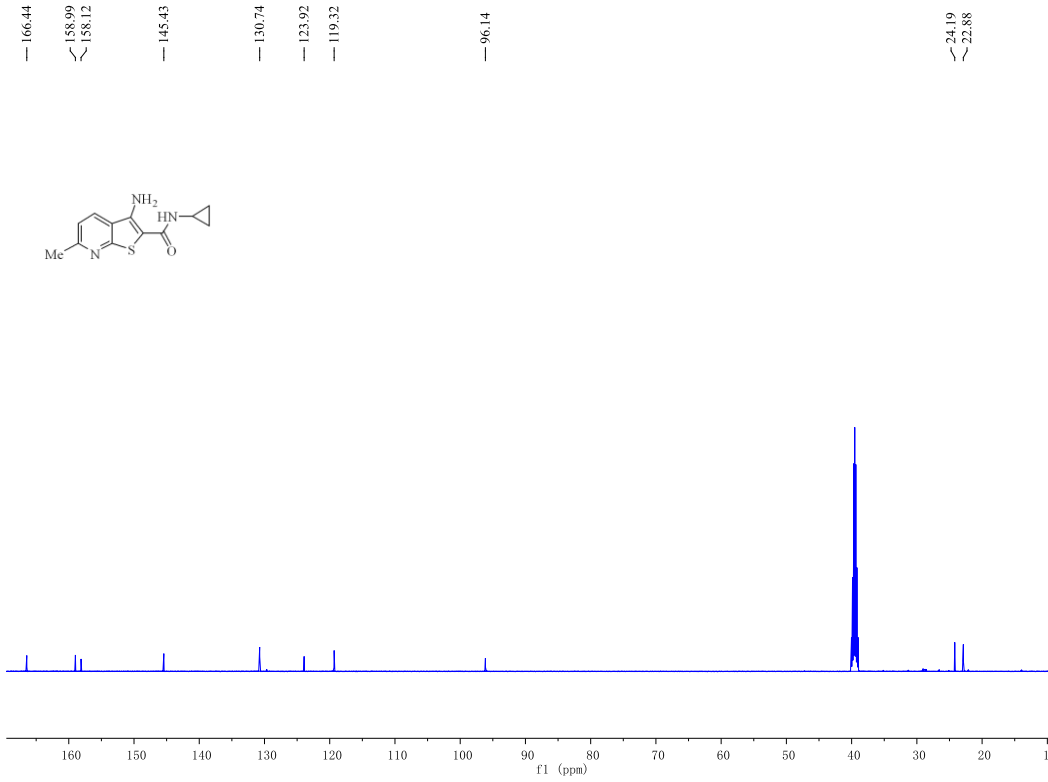

163.81
158.23
150.59
147.07
139.81
135.29
134.17
131.81
129.61
129.45
129.34
125.19
125.06
124.26
124.07
120.15
119.71
119.63
117.23
116.92
116.12
96.15
40.15
39.94
39.73
39.52
39.31
39.10
38.89
NH2
HN
Cl
N
S
O
CF3
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
8.42
8.39
7.34
7.31
7.24
4.30
4.28
4.25
4.23
3.33
2.58
2.51
1.32
1.29
1.27
0.00

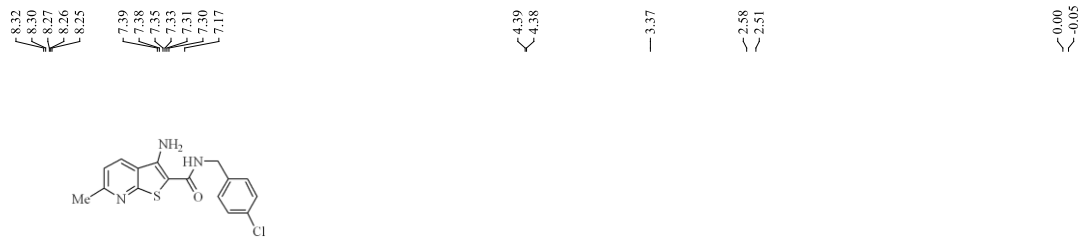

NH2
O
Et
Me
N
S
O
0.98
1.08
1.83
2.00
2.91
3.11
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

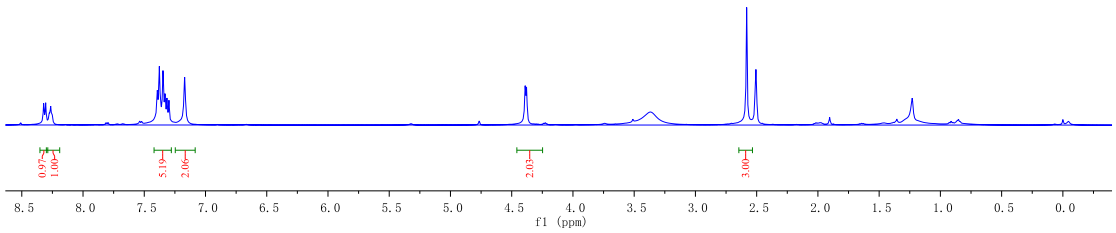

8.37
8.35
7.31
7.28
3.34
2.57
2.55
2.50
2.50
2.50
1.22
-0.01
NH2
OH
Me
N
S
O
1.08
1.12
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

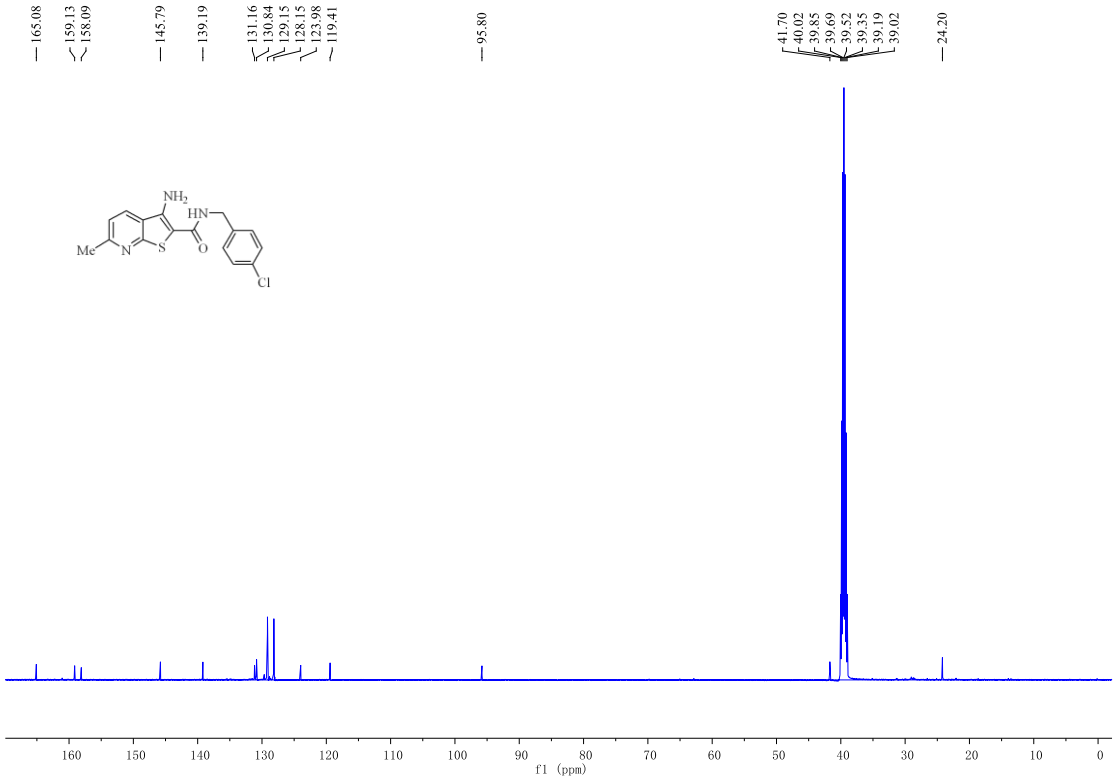

166.34
159.79
159.38
147.51
131.37
123.57
119.37
94.07
24.32
NH2
OH
Me
N
S
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

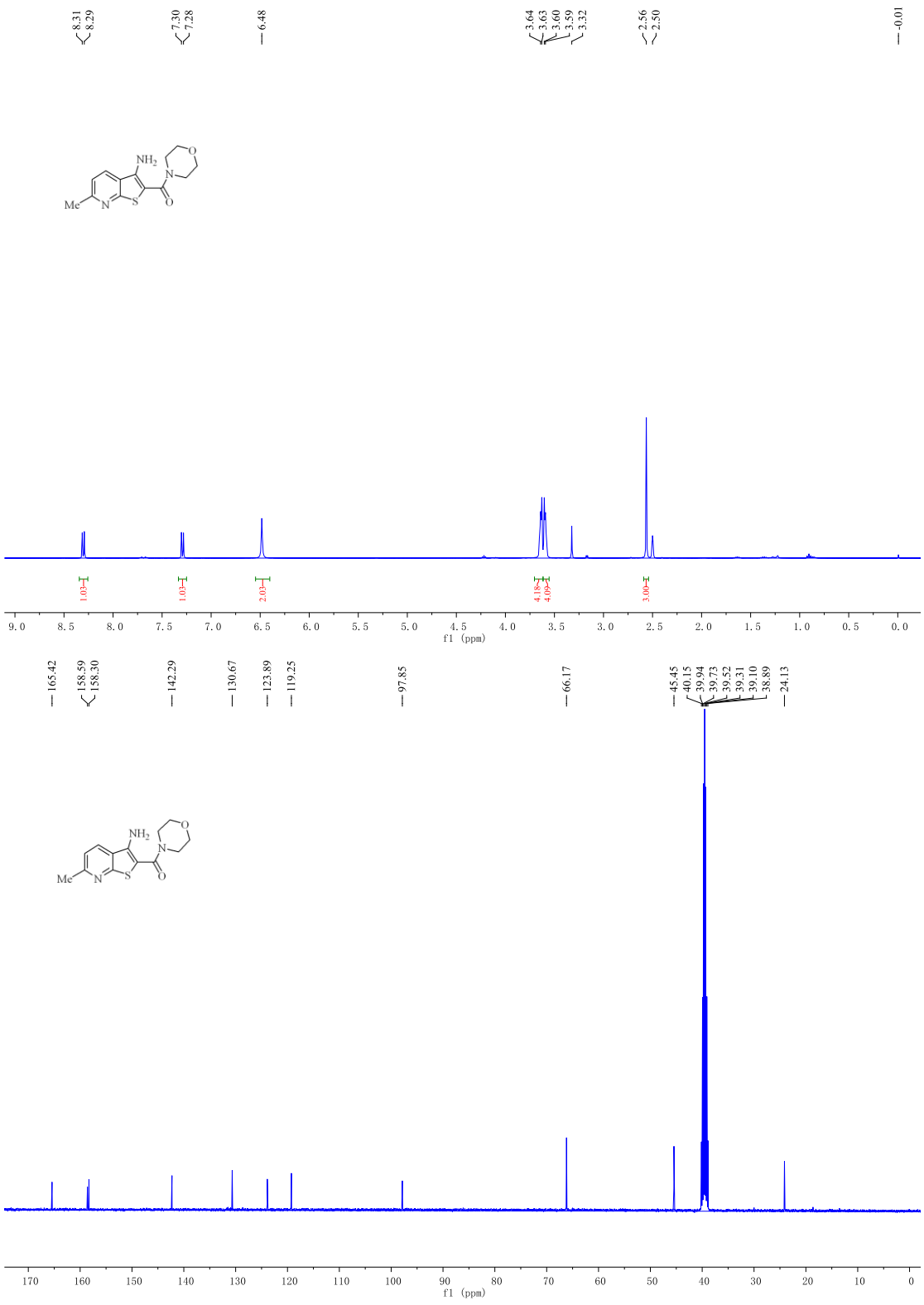

8.29
8.27
7.67
7.29
7.28
7.15
3.35
2.77
2.77
2.76
2.56
2.50
0.65
0.64
0.57
0.57
NH2
HN
Me
N
S
O
1.01
0.99
1.03
2.00
1.03
3.00
2.03
2.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

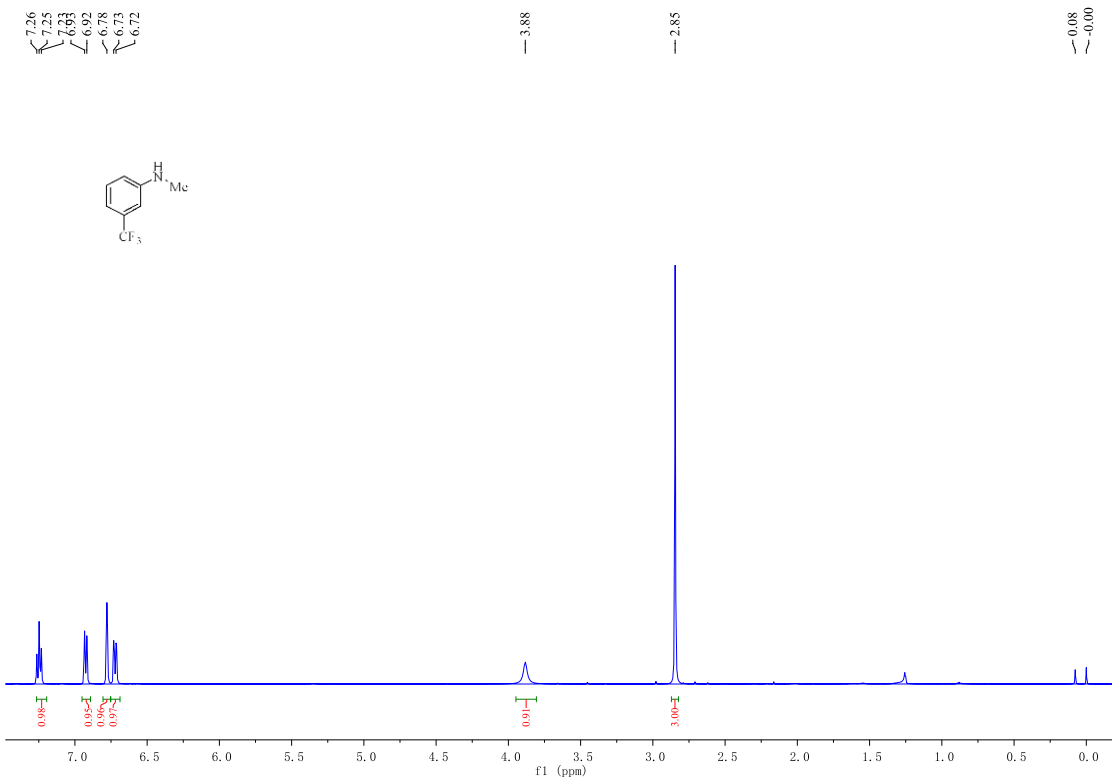

166.44
158.99
158.12
145.43
130.74
123.92
119.32
96.14
24.19
22.88
5.83
NH2
HN
Me
N
S
O
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

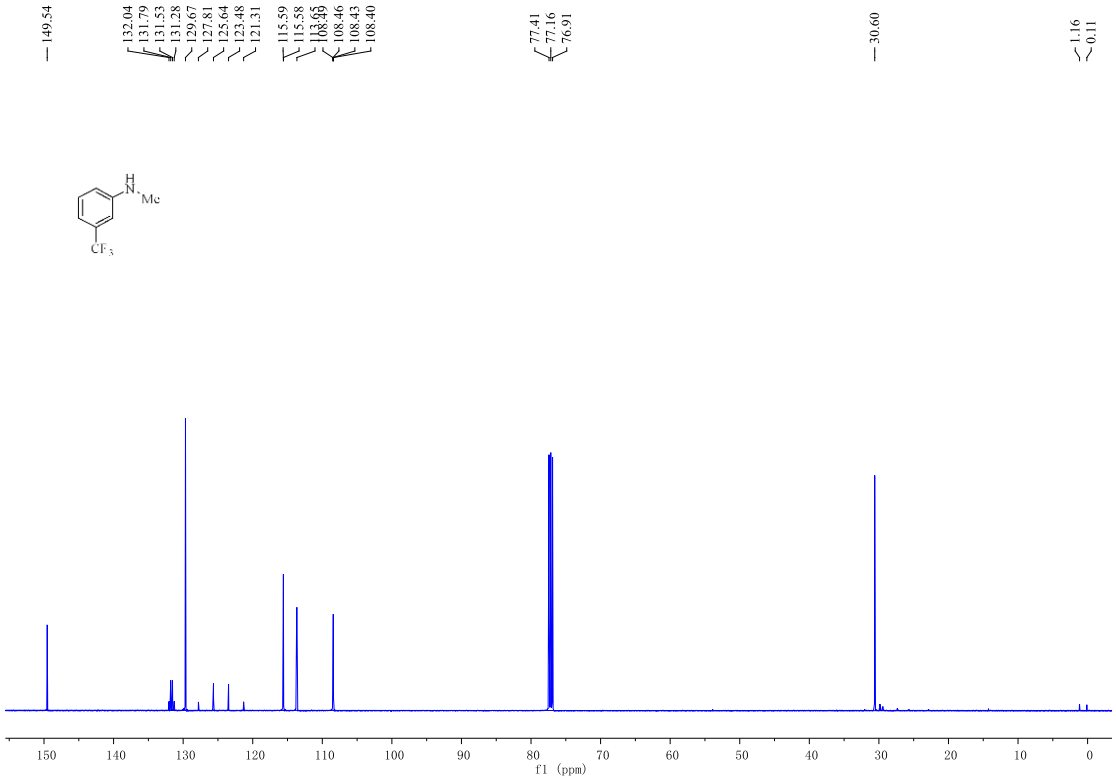

8.32
8.30
8.27
8.26
8.25
7.39
7.38
7.35
7.33
7.31
7.30
7.17
4.39
4.38
3.37
2.58
2.51
0.00
-0.05
NH2
HN
Me
N
S
O
Cl

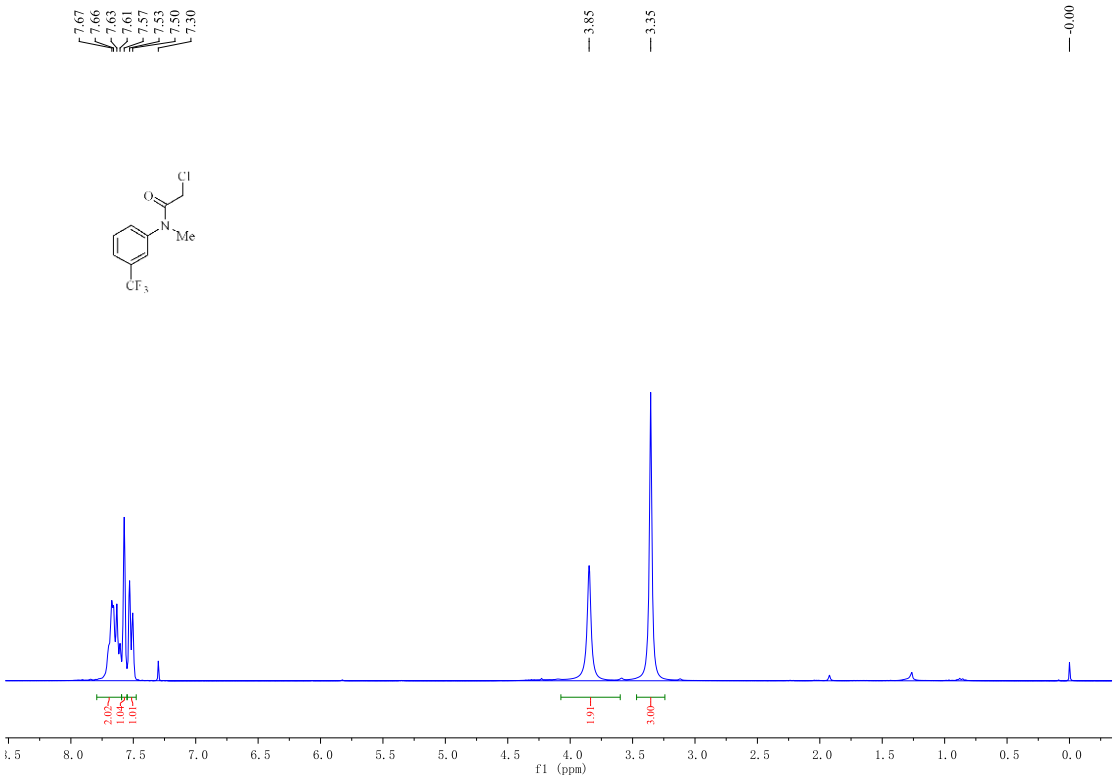

0.97
1.00
5.19
2.06
2.03
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

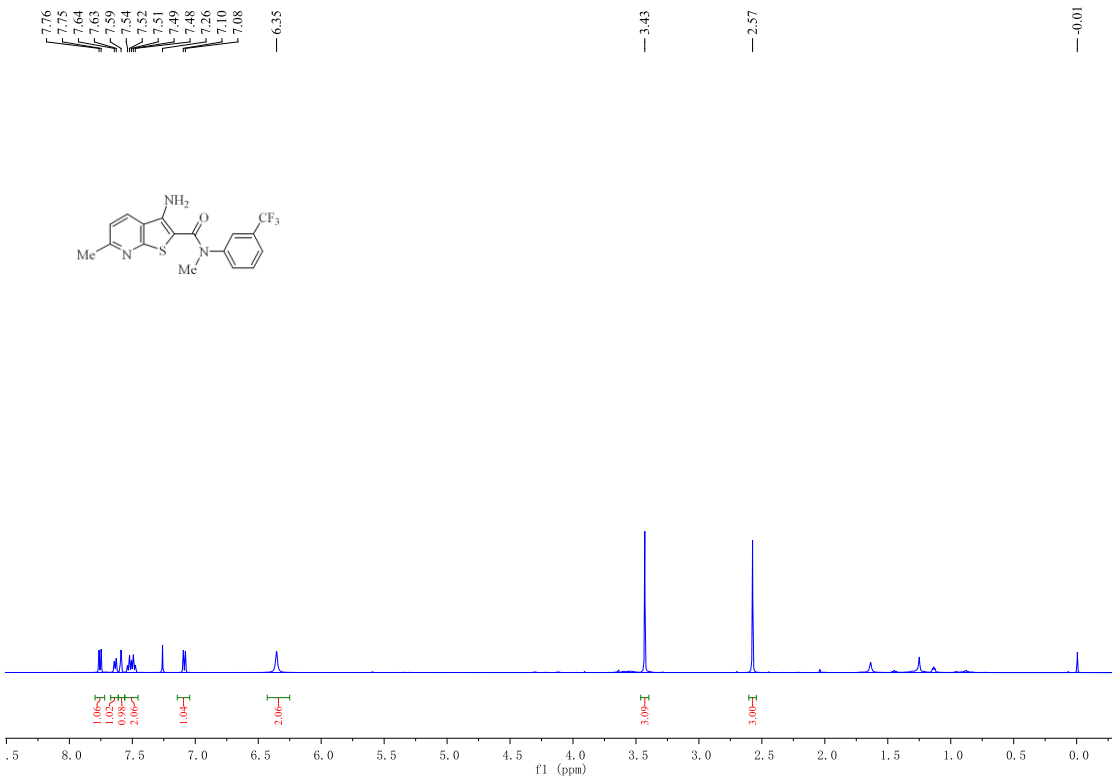

165.08
159.13
158.09
145.79
139.19
131.16
130.84
129.15
128.15
123.98
119.41
95.80
41.70
40.02
39.85
39.69
39.52
39.35
39.19
39.02
24.20
NH2
HN
Me
N
S
O
Cl
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

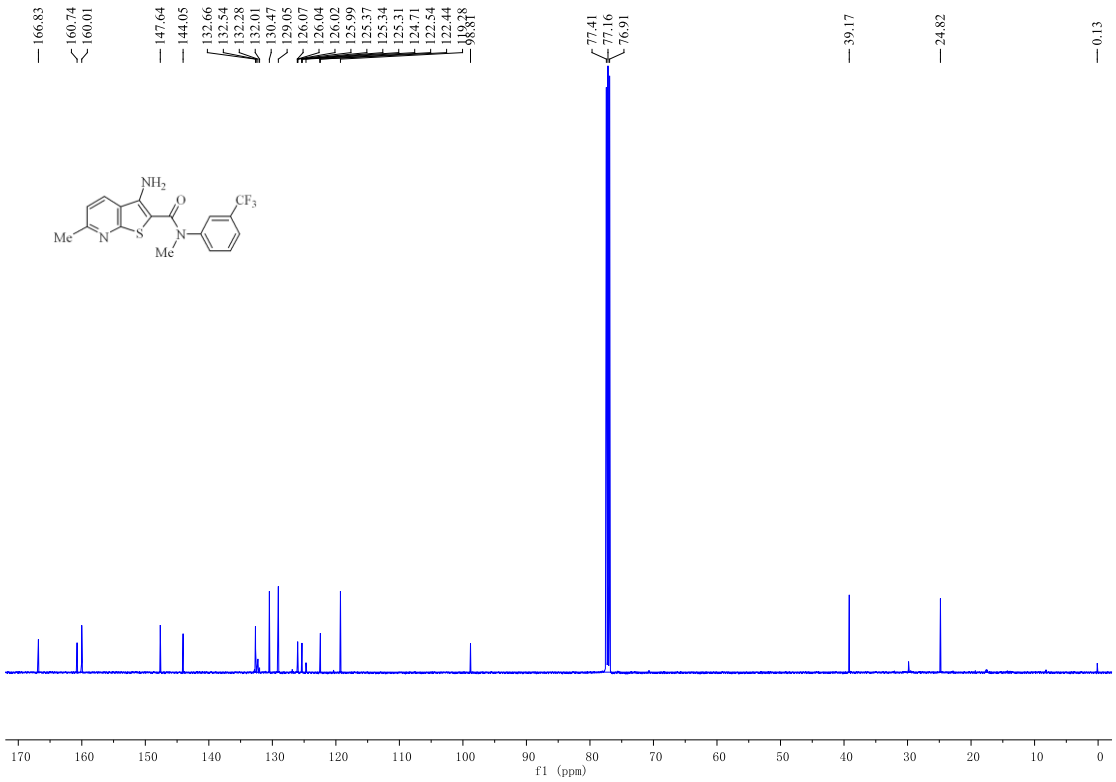

8.31
8.29
7.30
7.28
6.48
3.64
3.63
3.60
3.59
3.32
2.56
2.50
0.01
NH2
O
N
Me
S
O
1.03
1.03
2.03
4.18
4.09
3.00
f1 (ppm)
165.42
158.59
158.30
142.29
130.67
123.89
119.25
97.85
66.17
45.45
40.15
39.94
39.73
39.52
39.31
39.10
38.89
24.13
f1 (ppm)

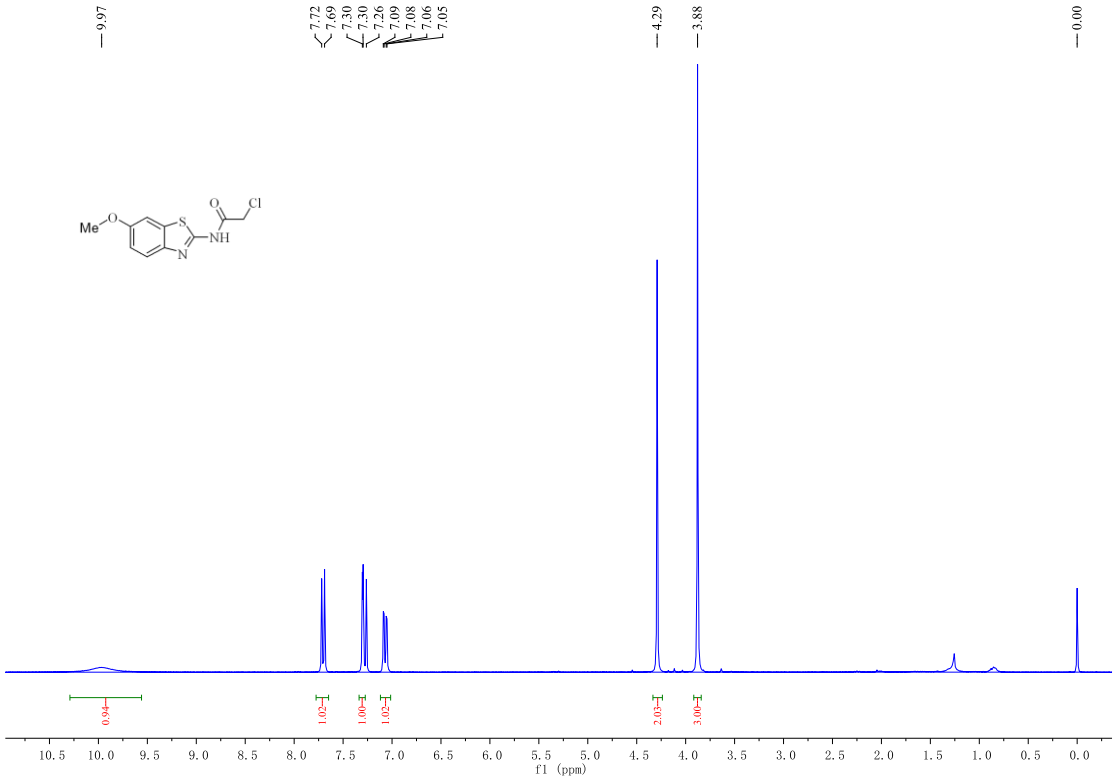

7.26
7.25
7.23
6.93
6.92
6.78
6.73
6.72
3.88
2.85
0.08
-0.00
H
N
Me
CF3
0.98
0.95
0.96
0.97
0.91
3.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

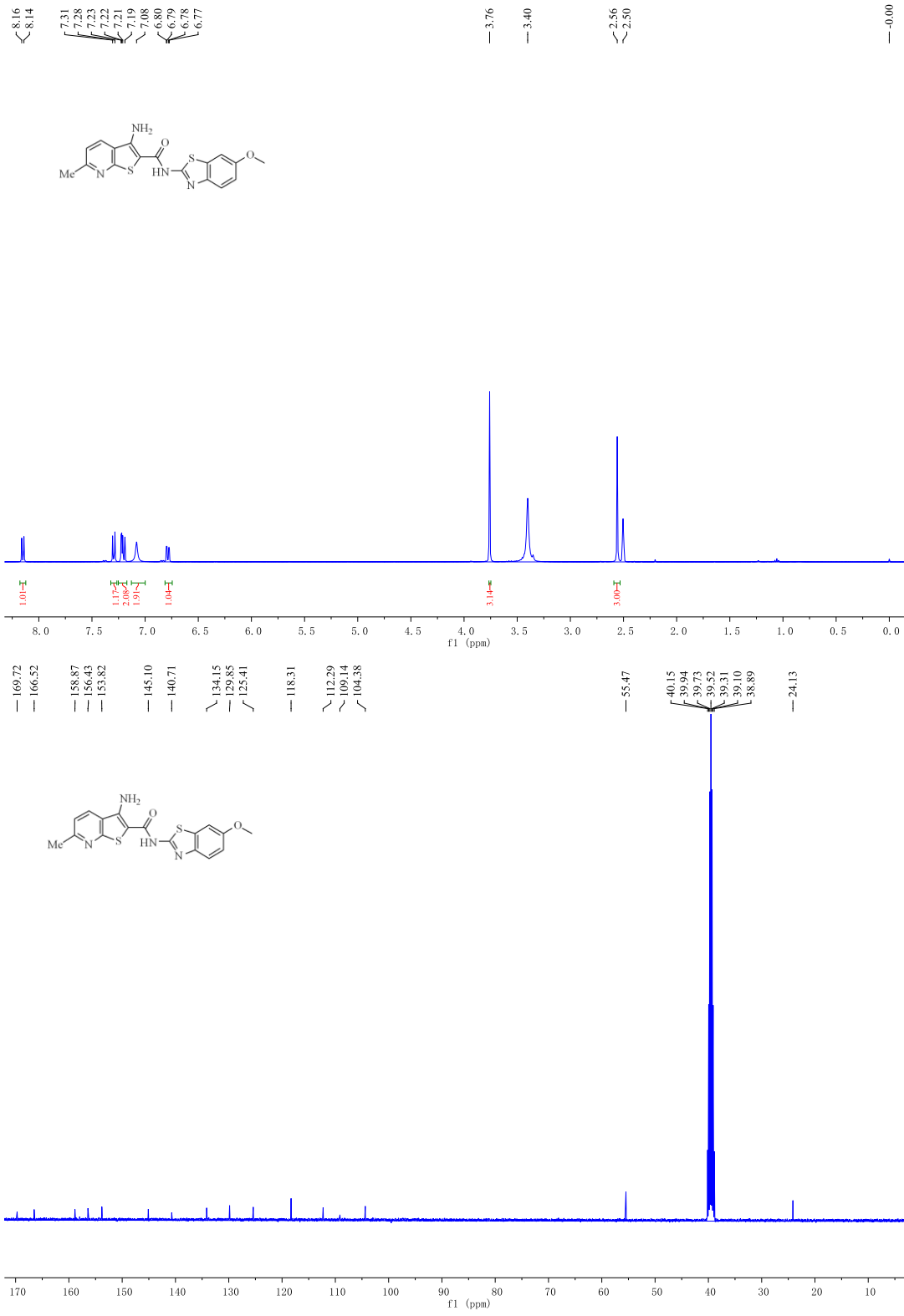

149.54
132.04
131.79
131.53
131.28
129.67
127.81
125.64
123.48
121.31
115.59
115.58
113.65
108.49
108.46
108.43
108.40
77.41
77.16
76.91
30.60
1.16
0.11
H
N
Me
CF3
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

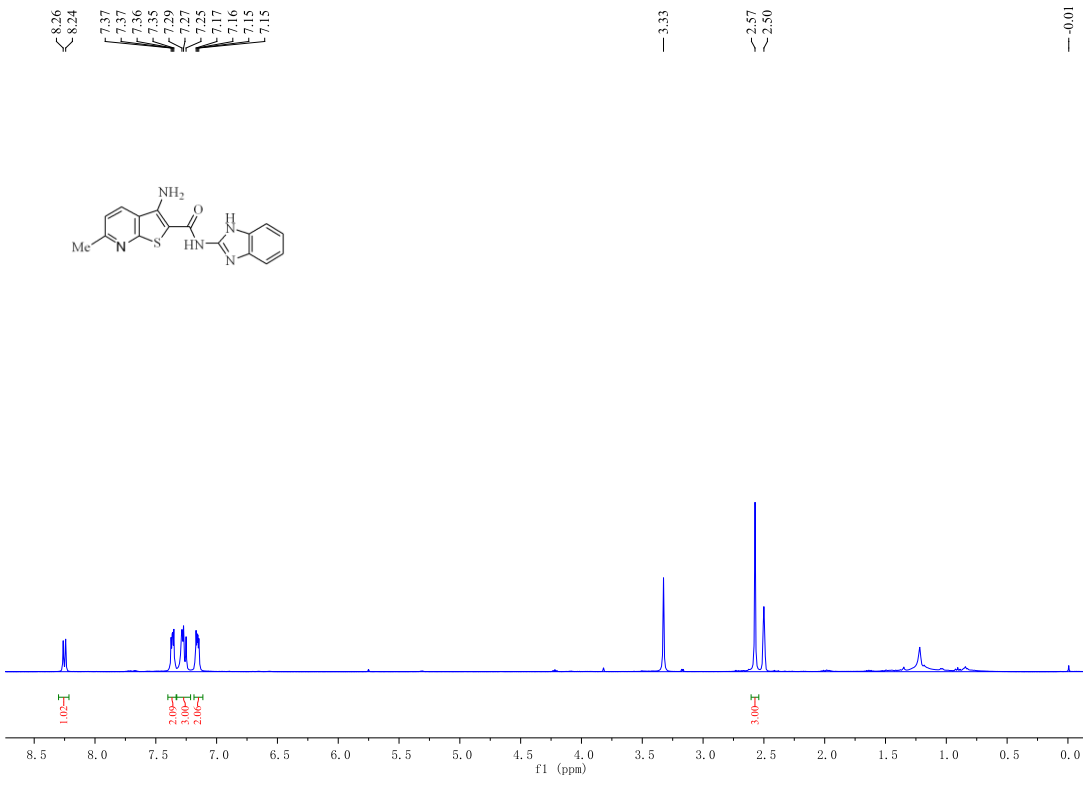

7.67
7.66
7.63
7.61
7.57
7.53
7.50
7.30
3.85
3.35
-0.00
Cl
O
N
Me
CF3
2.02
1.04
1.01
1.91
3.00
f1 (ppm)

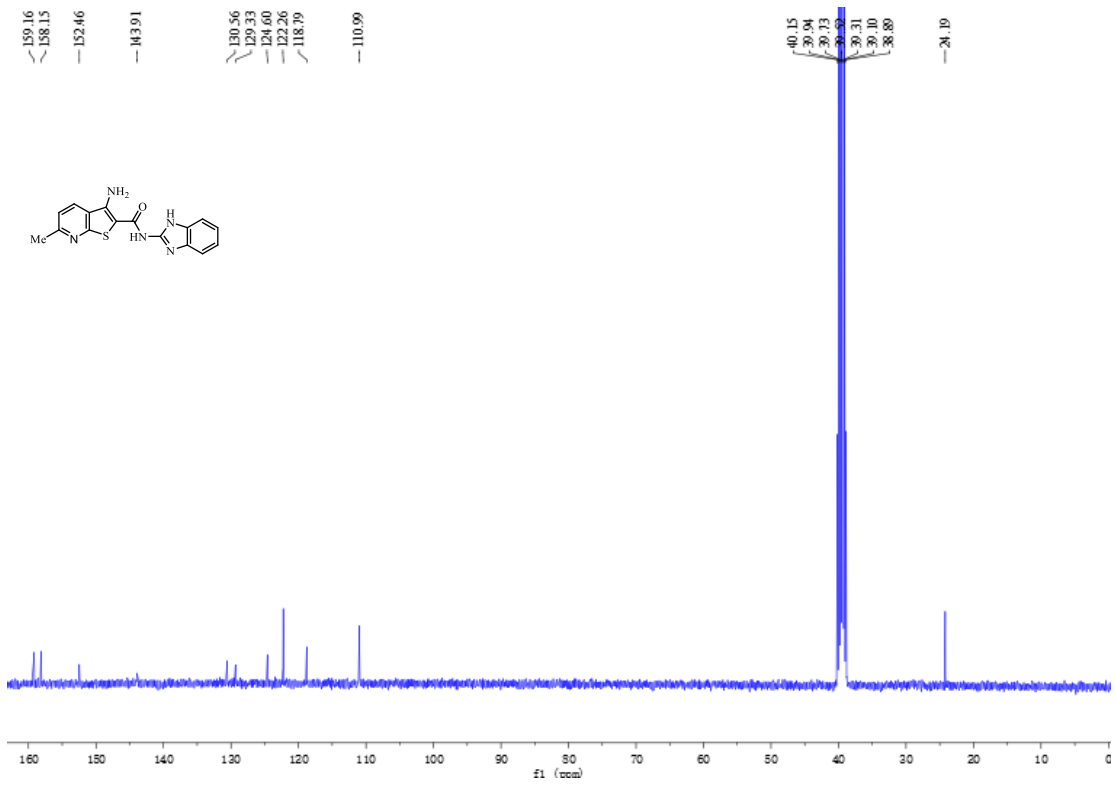

7.76
7.75
7.64
7.63
7.59
7.54
7.52
7.51
7.49
7.48
7.26
7.10
7.08
6.35
3.43
2.57
-0.01
NH2
O
CF3
Me
N
S
N
Me
1.06
1.02
0.98
2.06
1.04
2.06
3.09
3.00
f1 (ppm)

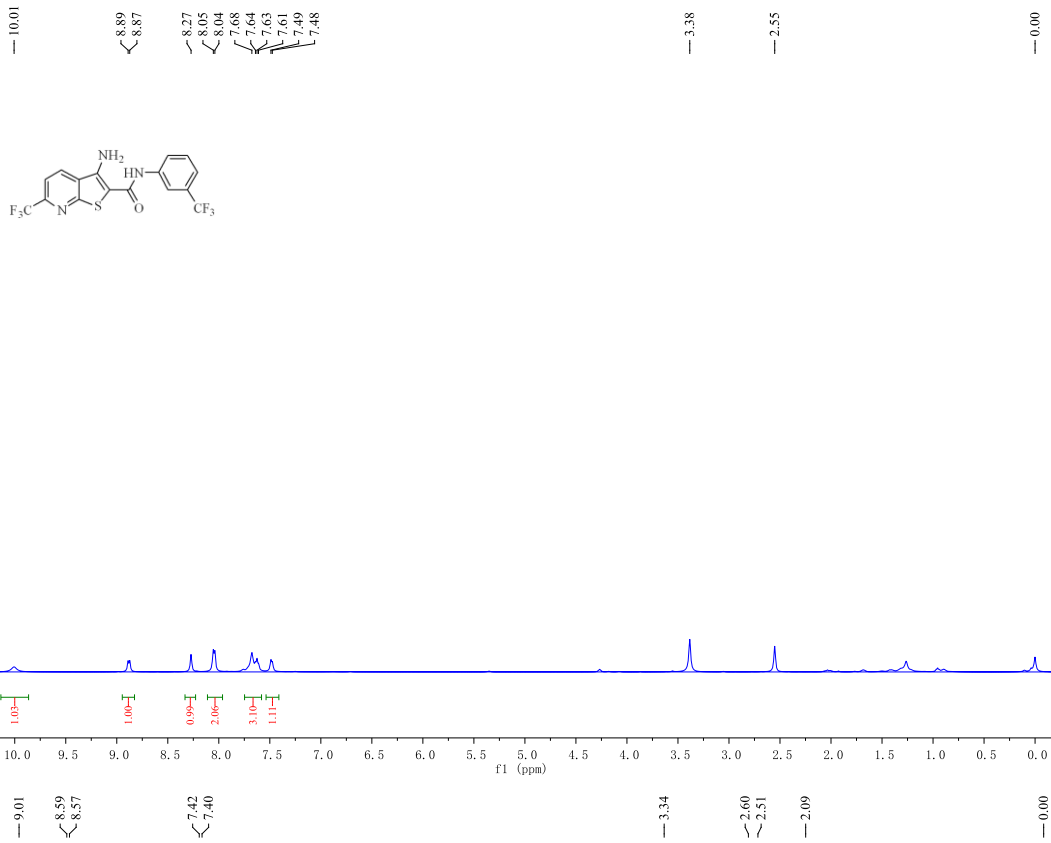

166.83
160.74
160.01
147.64
144.05
132.66
132.54
132.28
132.01
130.47
129.05
126.07
126.04
126.02
125.99
125.37
125.34
125.31
124.71
122.54
122.44
119.28
98.81
77.41
77.16
76.91
39.17
24.82
0.13
NH2
O
CF3
Me
N
S
N
Me
f1 (ppm)

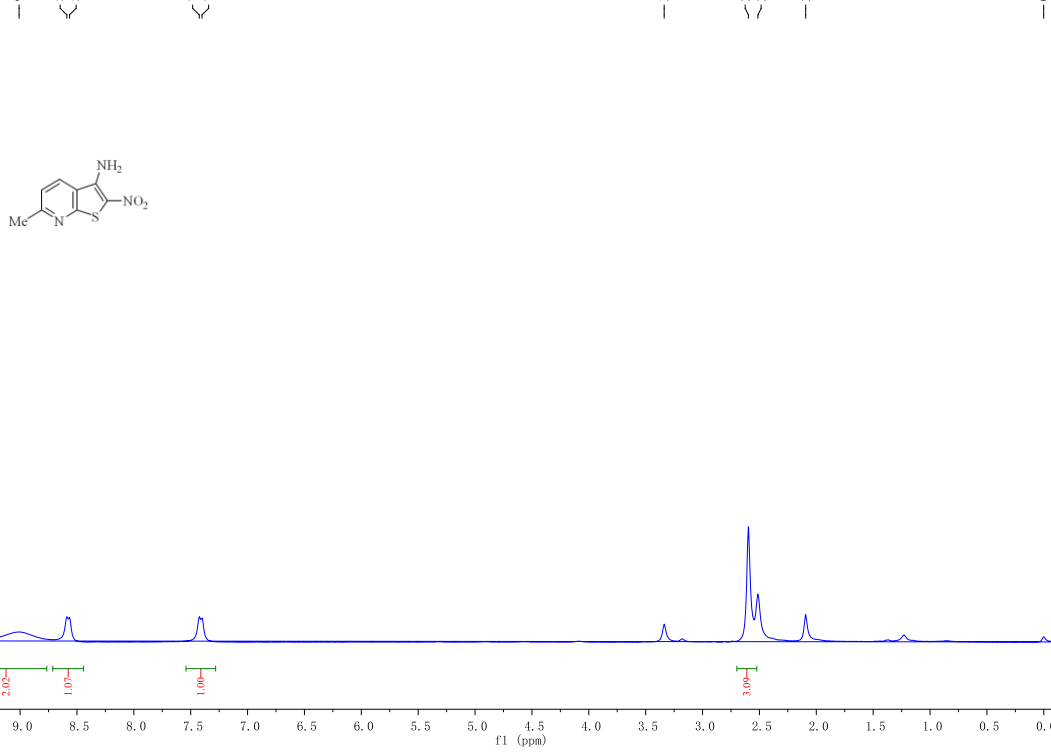

9.97
7.72
7.69
7.30
7.30
7.26
7.09
7.08
7.06
7.05
4.29
3.88
0.00
Me
O
S
N
NH
O
Cl
0.94
1.02
1.00
1.02
2.03
3.00
f1 (ppm)

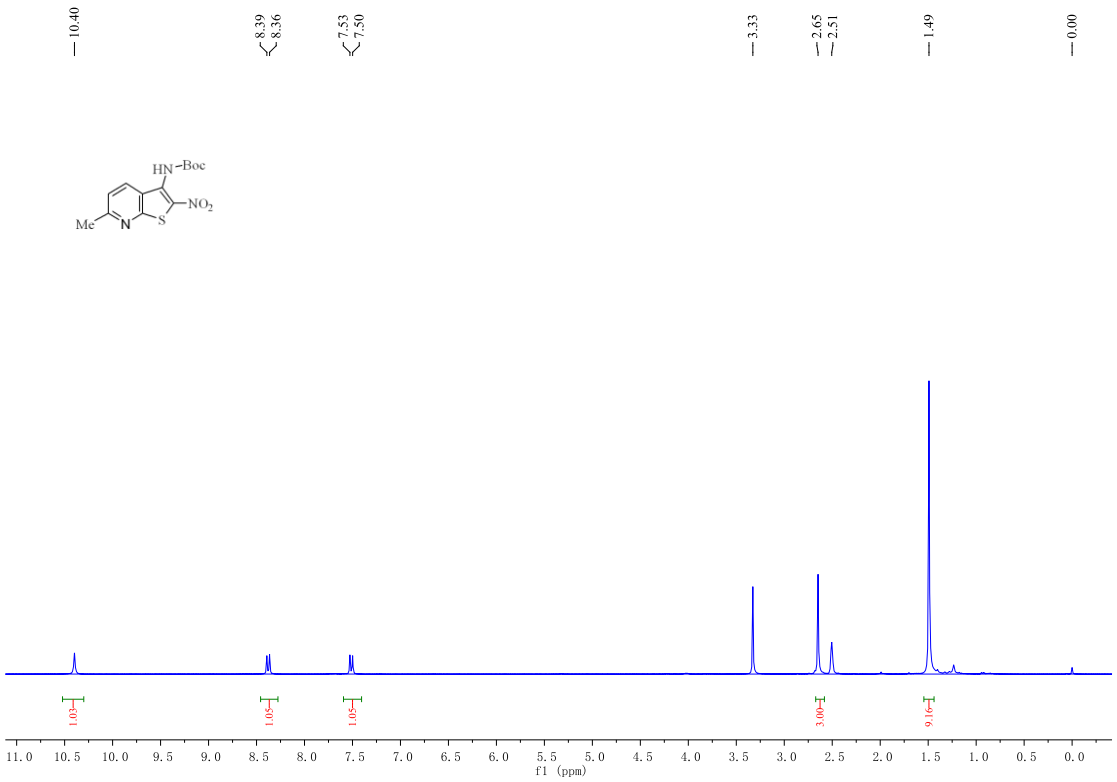

8.16
8.14
7.31
7.28
7.23
7.22
7.21
7.19
7.08
6.80
6.79
6.78
6.77
3.76
3.40
2.56
2.50
0.00
NH2
O
S
O
Me
N
S
HN
N
1.01
1.17
2.08
1.91
1.04
3.14
3.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
169.72
166.52
158.87
156.43
153.82
145.10
140.71
134.15
129.85
125.41
118.31
112.29
109.14
104.38
55.47
40.15
39.94
39.73
39.52
39.31
39.10
38.89
24.13
NH2
O
S
O
Me
N
S
HN
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

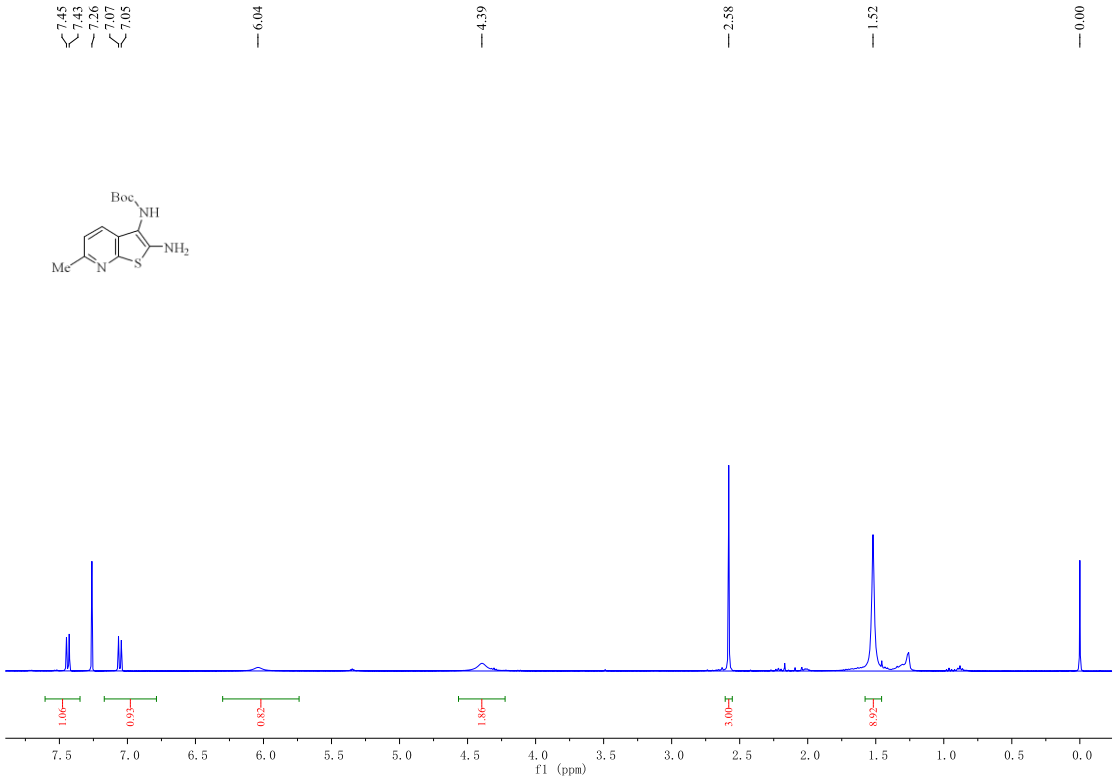

8.26
8.24
7.37
7.37
7.36
7.35
7.29
7.27
7.25
7.17
7.16
7.15
7.15
3.33
2.57
2.50
0.01
NH2
O
Me
N
S
HN
H
N
N
1.02
2.09
3.00
2.06
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

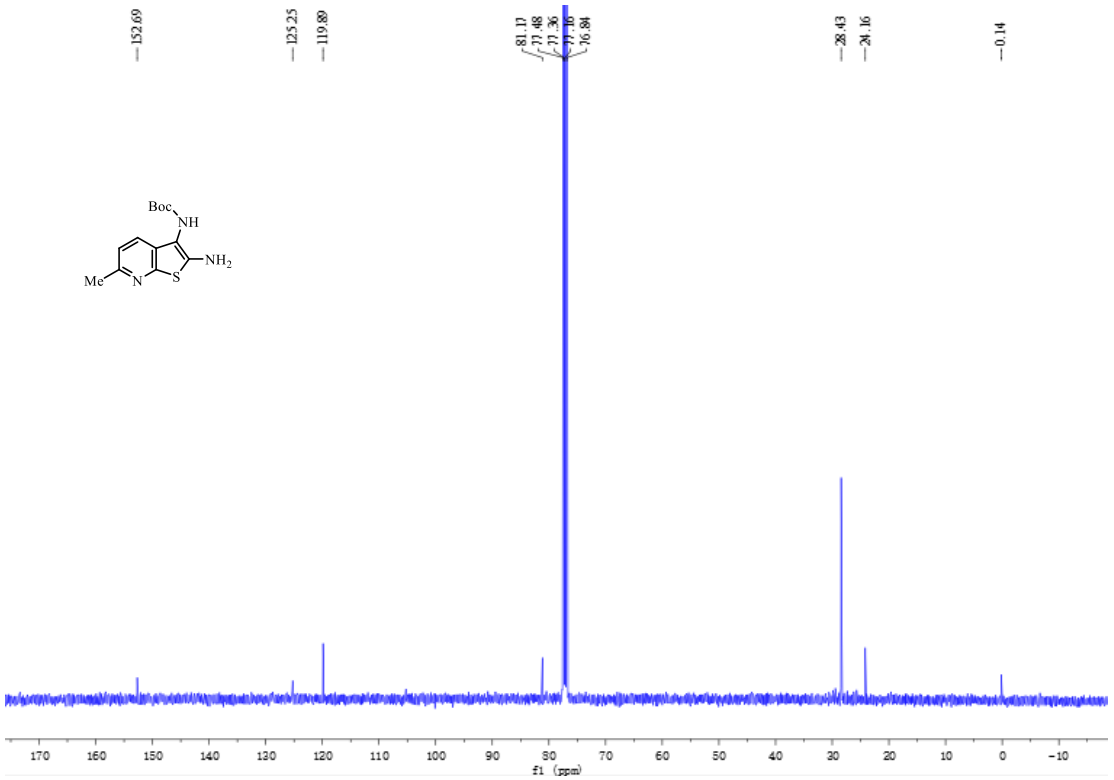

159.16
158.15
152.46
143.91
130.56
129.33
124.60
122.26
118.79
110.99
40.15
39.94
39.73
39.52
39.31
39.10
38.89
24.19
NH2
O
Me
N
S
HN
H
N
N
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

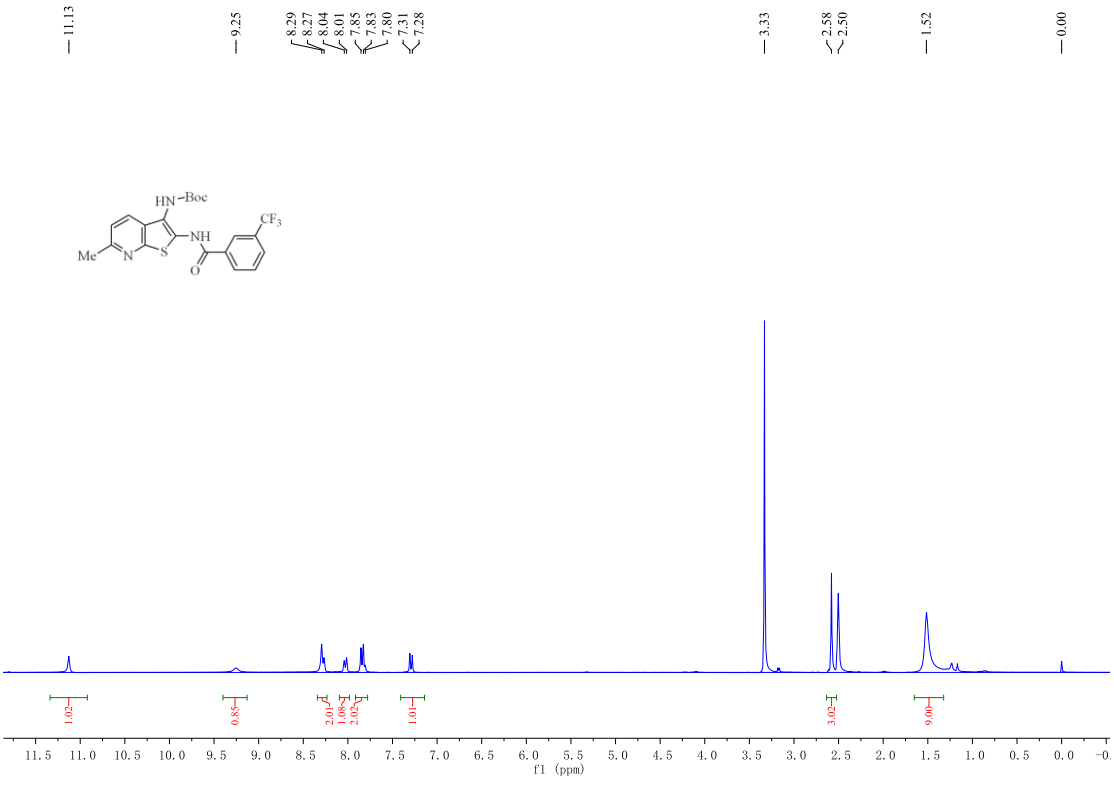

10.01
8.89
8.87
8.27
8.05
8.04
7.68
7.64
7.63
7.61
7.49
7.48
3.38
2.55
0.00
NH2
HN
F3C
N
S
O
CF3
1.03
1.00
0.99
2.06
3.10
1.11
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

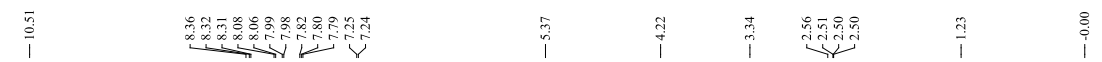

9.01
8.59
8.57
7.42
7.40
3.34
2.60
2.51
2.09
0.00
NH2
NO2
Me
N
S
2.02
1.07
1.00
3.09
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


10.40
8.39
8.36
7.53
7.50
3.33
2.65
2.51
1.49
0.00
HN-Boc
NO2
Me
N
S
1.03
1.05
1.05
3.00
9.16
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)


7.45
7.43
7.26
7.07
7.05
6.04
4.39
2.58
1.52
0.00
Boc
NH
NH2
Me
N
S
1.06
0.93
0.82
1.86
3.00
8.92
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)


152.69
125.25
119.89
81.17
77.48
77.36
77.16
76.84
28.43
24.16
0.14
Boc
NH
NH2
Me
N
S
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


11.13
9.25
8.29
8.27
8.04
8.01
7.85
7.83
7.80
7.31
7.28
3.33
2.58
2.50
1.52
0.00
HN
Boc
NH
CF3
Me
N
S
O
1.02
0.85
2.01
1.08
2.02
1.01
3.02
9.00
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)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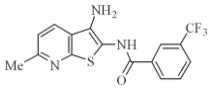
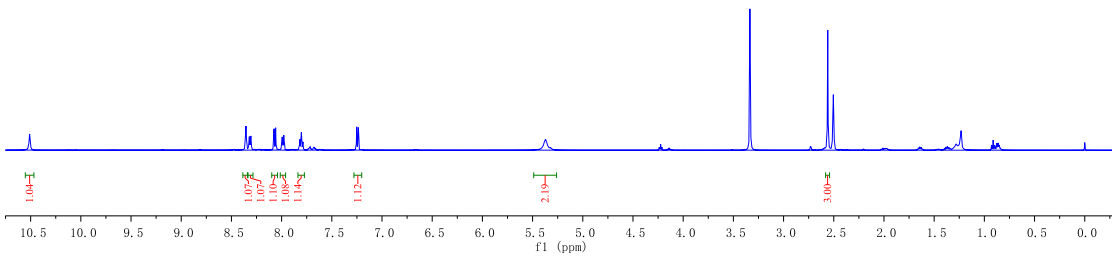
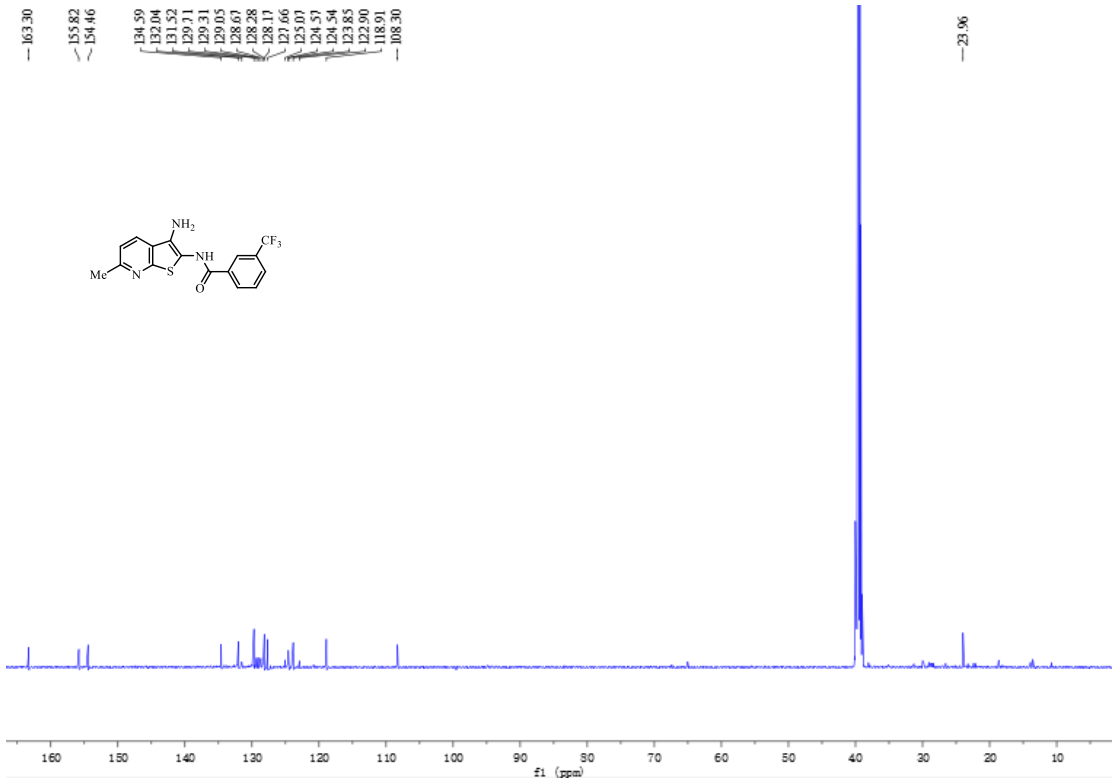
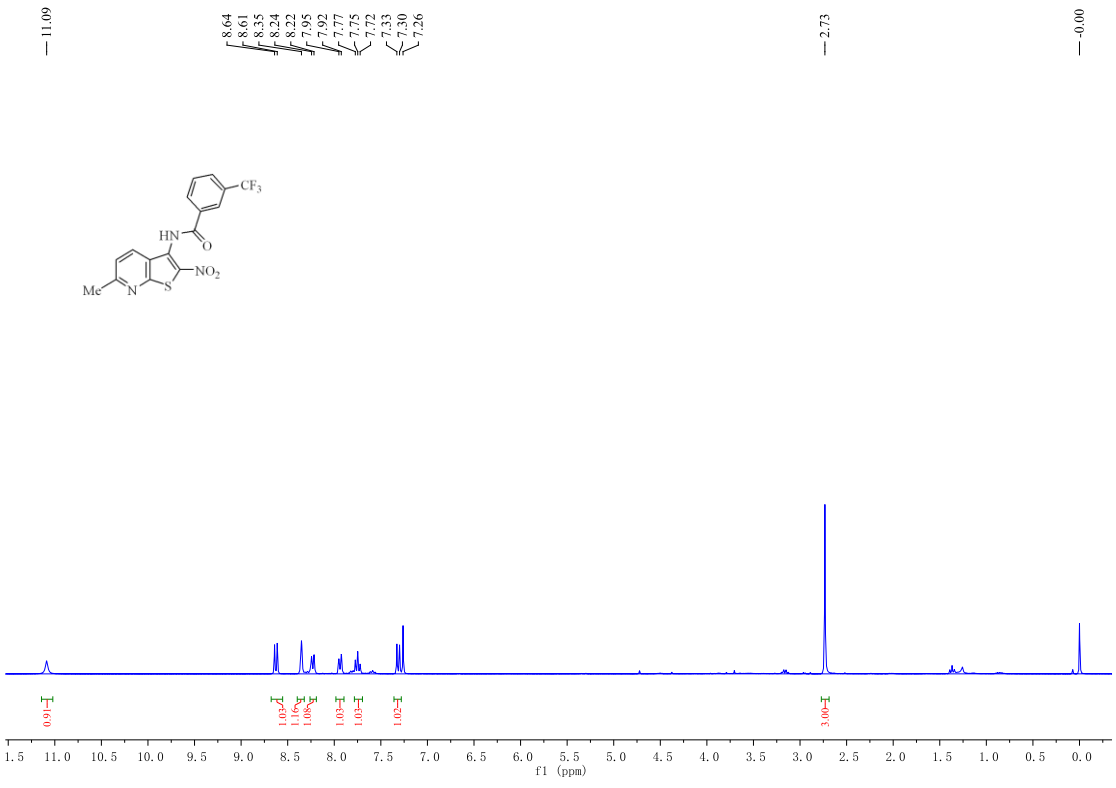
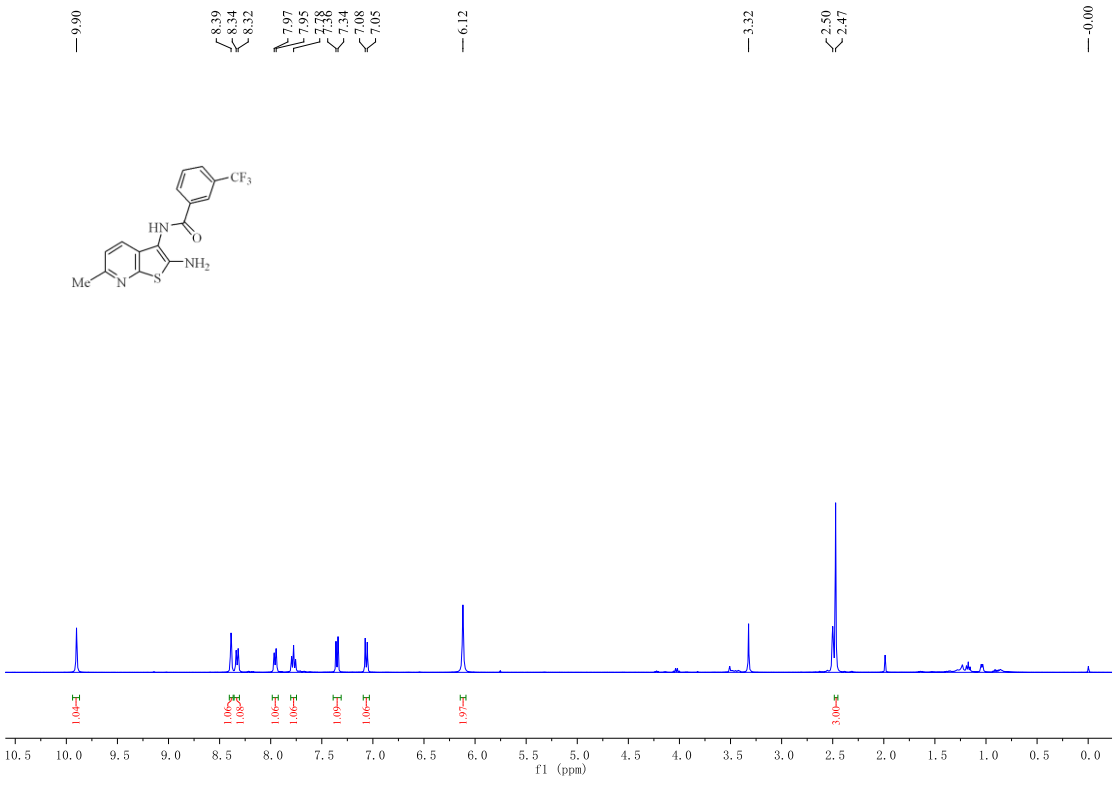
8.36
8.32
8.31
8.08
8.06
7.99
7.98
7.82
7.80
7.79
7.25
7.24
5.37
4.22
3.34
2.56
2.51
2.50
2.50
1.23
-0.00

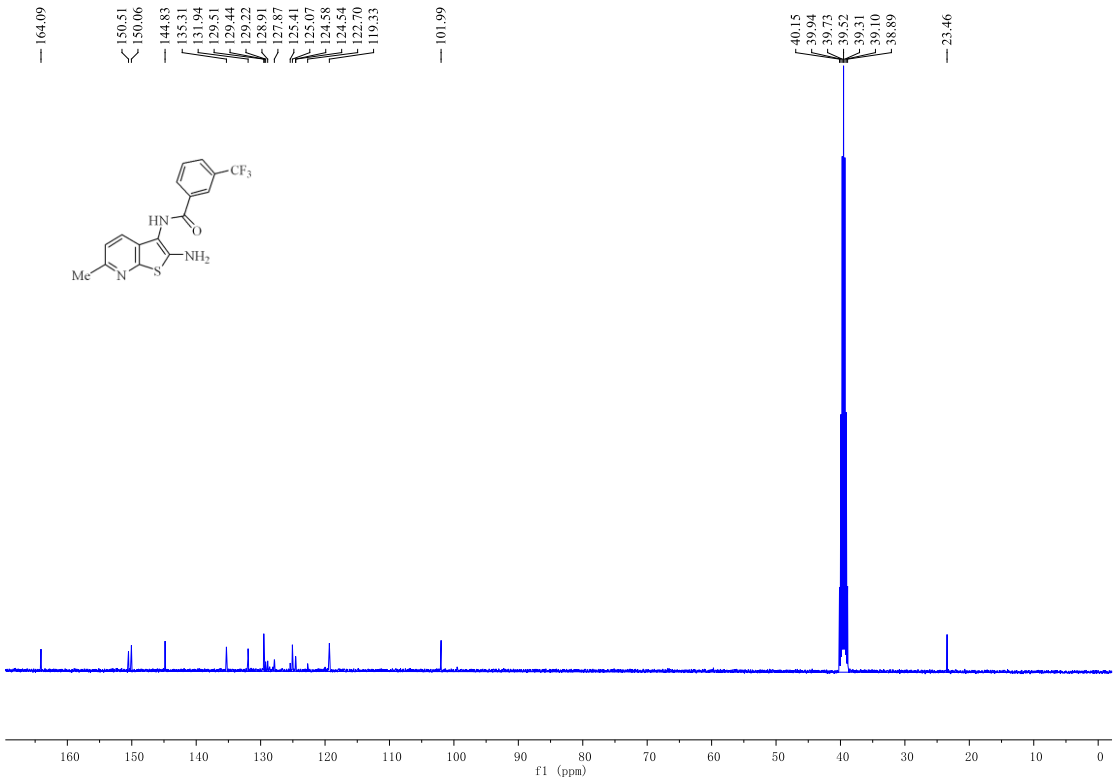

NH2
NH
CF3
Me
N
S
O

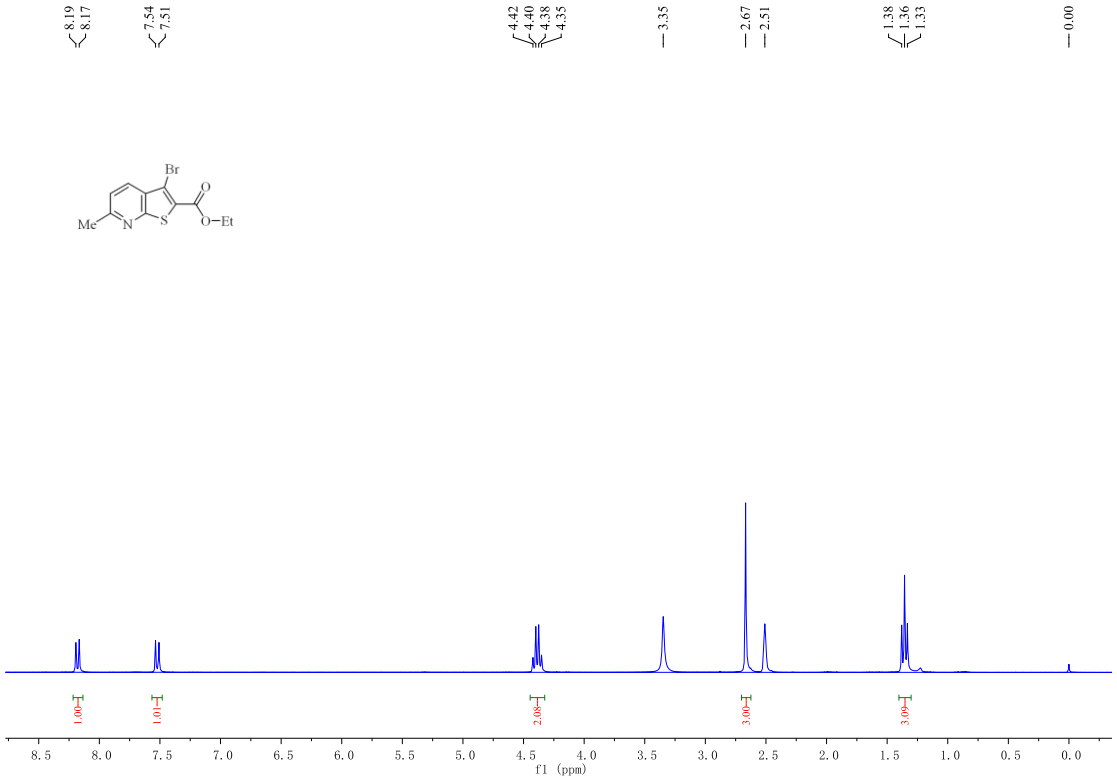

1.04
1.07
1.07
1.10
1.08
1.14
1.12
2.19
3.00
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

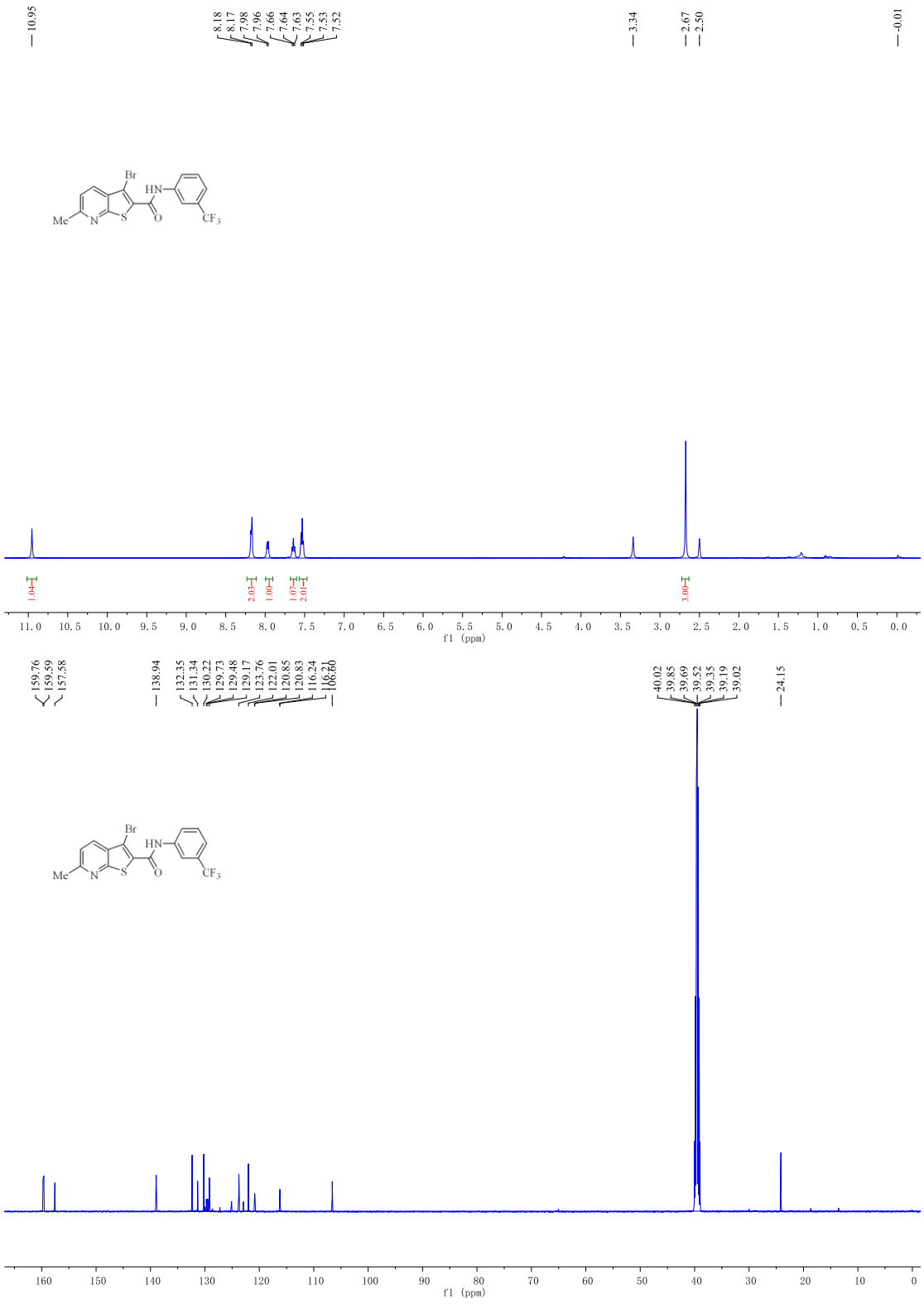

163.30
155.82
154.46
134.59
132.04
131.52
129.11
129.31
129.05
128.67
128.28
128.17
127.66
125.03
124.57
124.54
123.85
122.90
118.91
108.30
23.96
NH2
NH
CF3
Me
N
S
O
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

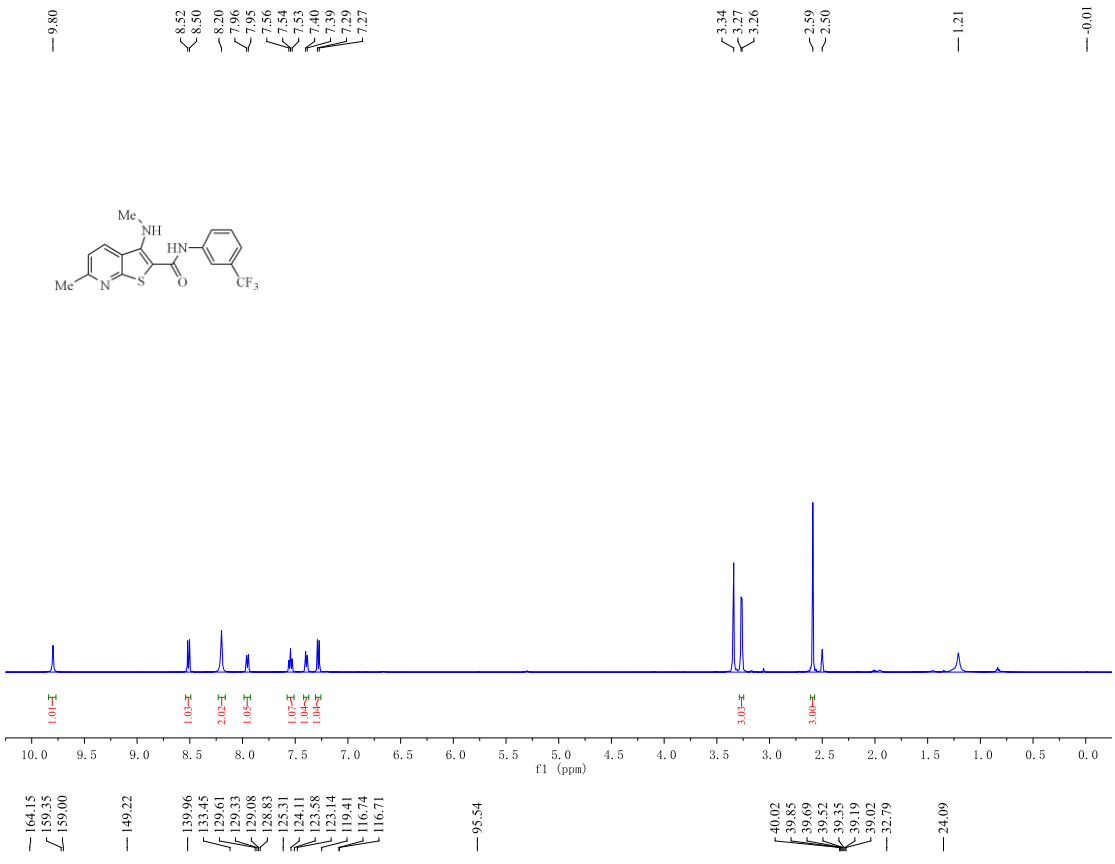

11.09
8.64
8.61
8.35
8.24
8.22
7.95
7.92
7.77
7.75
7.72
7.33
7.30
7.26
2.73
-0.00
CF3
HN
O
NO2
Me
N
S
0.91
1.03
1.16
1.08
1.03
1.03
1.02
3.00
f1 (ppm)

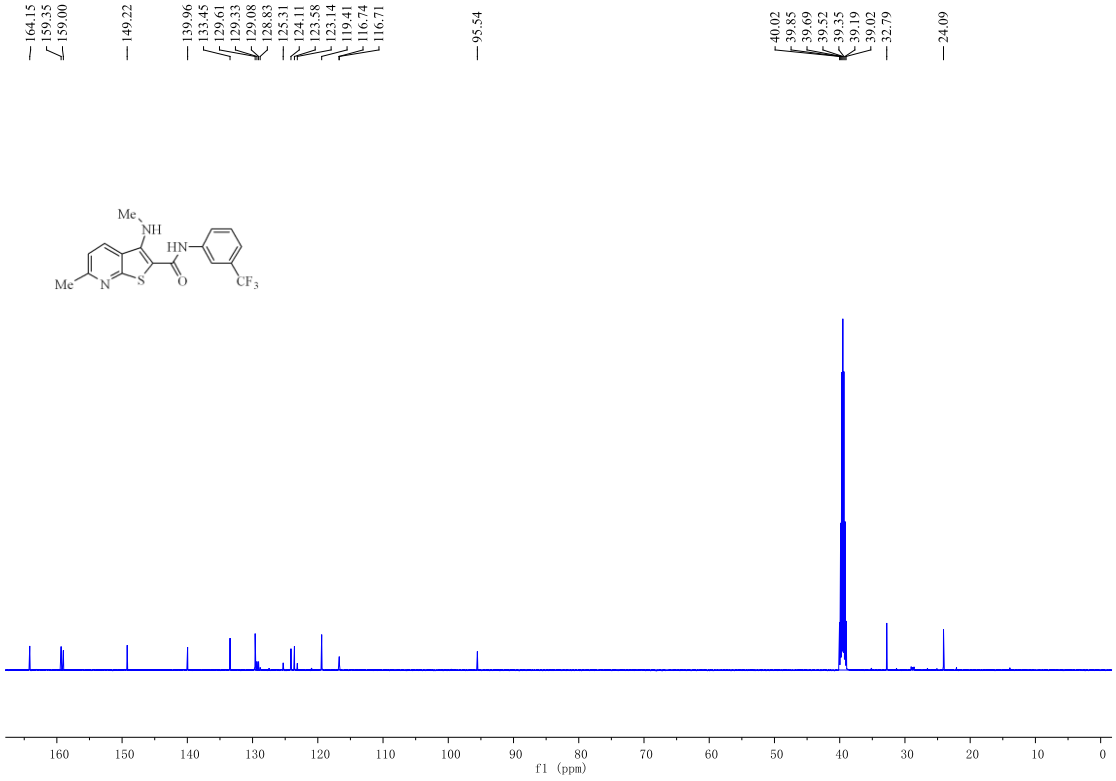

9.90
8.39
8.34
8.32
7.97
7.95
7.38
7.36
7.34
7.08
7.05
6.12
3.32
2.50
2.47
-0.00
CF3
HN
O
NH2
Me
N
S
1.04
1.06
1.08
1.06
1.06
1.09
1.06
1.97
3.00
f1 (ppm)

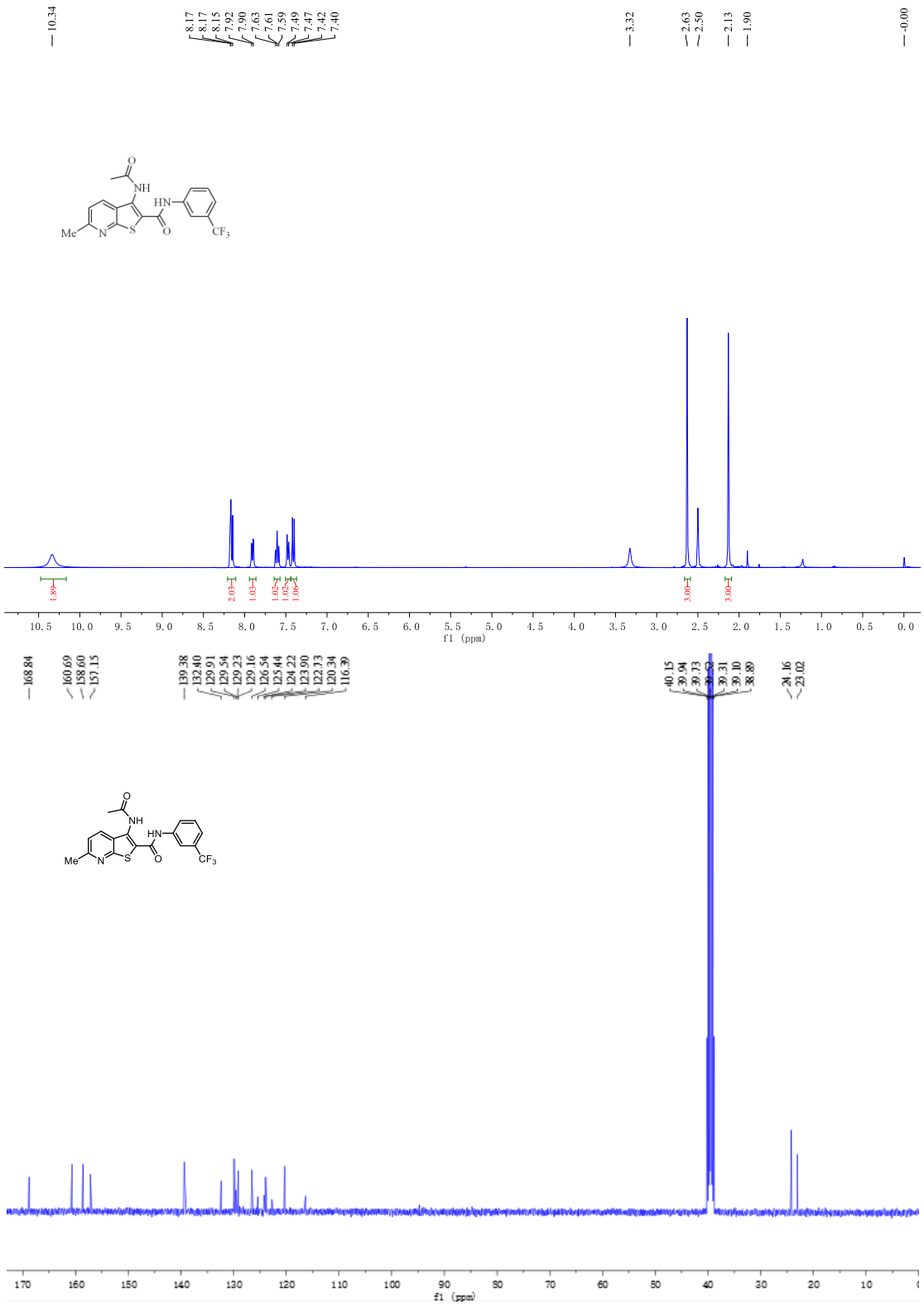

164.09
150.51
150.06
144.83
135.31
131.94
129.51
129.44
129.22
128.91
127.87
125.41
125.07
124.58
124.54
122.70
119.33
101.99
40.15
39.94
39.73
39.52
39.31
39.10
38.89
23.46
CF3
HN
O
NH2
Me
N
S
f1 (ppm)


8.19
8.17
7.54
7.51
4.42
4.40
4.38
4.35
3.35
2.67
2.51
1.38
1.36
1.33
0.00
Br
O
Me
N
S
O–Et
1.00
1.01
2.08
3.00
3.09
f1 (ppm)


10.95
8.18
8.17
7.98
7.96
7.66
7.64
7.63
7.55
7.53
7.52
3.34
2.67
2.50
-0.01
Br
HN
Me
N
S
O
CF3
1.04
2.03
1.00
1.07
2.01
3.00
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)
159.76
159.59
157.58
138.94
132.35
131.34
130.22
129.73
129.48
129.17
123.76
122.01
120.85
120.83
116.24
116.21
116.00
40.02
39.85
39.69
39.52
39.35
39.19
39.02
24.15
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


9.80
8.52
8.50
8.20
7.96
7.95
7.56
7.54
7.53
7.40
7.39
7.29
7.27
3.34
3.27
3.26
2.59
2.50
1.21
-0.01
Me
NH
HN
Me
N
S
O
CF3
1.01
1.03
2.02
1.06
1.07
1.04
1.04
3.03
3.00
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)


164.15
159.35
159.00
149.22
139.96
133.45
129.61
129.33
129.08
128.83
125.31
124.11
123.58
123.14
119.41
116.74
116.71
95.54
40.02
39.85
39.69
39.52
39.35
39.19
39.02
32.79
24.09
Me
NH
HN
Me
N
S
O
CF3
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


10.34
8.17
8.17
8.15
7.92
7.90
7.63
7.61
7.59
7.49
7.47
7.42
7.40
3.32
2.63
2.50
2.13
1.90
0.00
NH
HN
Me
N
S
O
O
CF3
1.89
2.03
1.03
1.02
1.02
1.06
3.00
3.00
f1 (ppm)
168.84
160.69
158.60
157.15
139.38
132.40
129.91
129.54
129.23
129.16
126.54
125.44
124.22
123.90
122.13
120.34
116.39
40.15
39.94
39.73
39.52
39.31
39.10
38.89
24.16
23.02
f1 (ppm)

6. References

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