Supporting Information

Dual Stage Picolinic Acid Derived Inhibitors of *Toxoplasma gondii*

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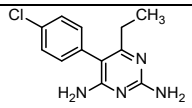
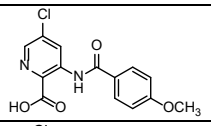
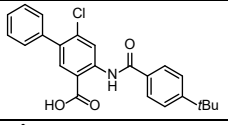
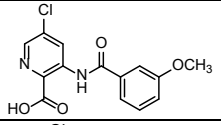
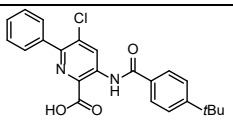
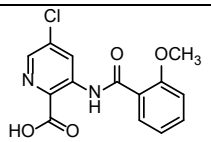
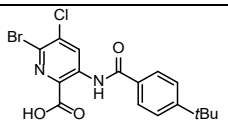
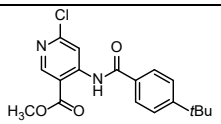
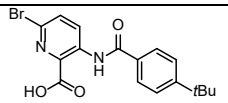
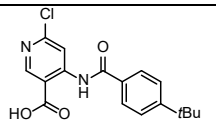
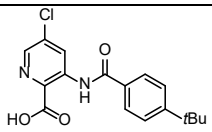
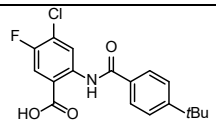
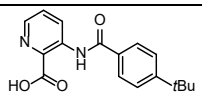
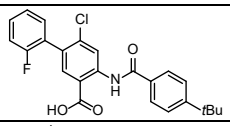
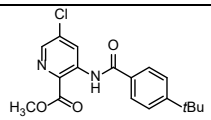
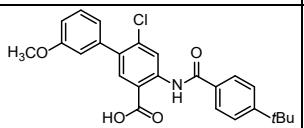
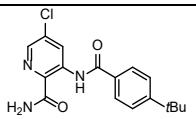
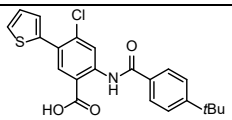
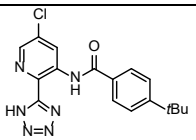
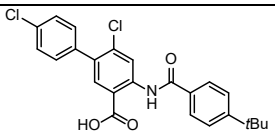
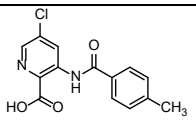
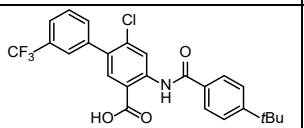
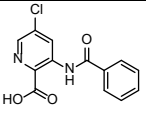
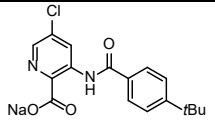
Table 1S. Percent growth inhibition in *T. gondii* by compounds **1-23** at 10 μM

cmpnd	structure	tachy-zoites	brady-zoites	cmpnd	structure	tachy-zoites	brady-zoites
pyri-meth-amine		98.0%	0.0%	**12**		39.7%	84.3%
1		99.6%	96.7%	**13**		89.1%	84.0%
2		99.6%	92.1%	**14**		54.4%	43.6%
3		92.4%	78.1%	**15**		89.0%	7.6%
4		72.2%	70.9%	**16**		52.1%	35.9%
5		99.0%	98.8%	**17**		81.0%	89.1%
6		95.4%	94.4%	**18**		92.0%	94.7%
7		97.4%	98.9%	**19**		91.7%	98.4%
8		51.7%	43.7%	**20**		96.9%	95.9%
9		93.3%	95.5%	**21**		94.1%	98.7%
10		79.5%	82.8%	**22**		99.7%	96.4%
11		88.2%	87.9%	**23**		96.2%	97.0%

Animal Use and Care Statement: Mice were treated in compliance with the guidelines set by the Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin School of Medicine and Public Health (protocol #M005217).

Assay of compounds against *T. gondii* tachyzoites: The assay employed was modified from Pfefferkorn *et al*.[1] Confluent monolayers of Human Foreskin Fibroblast (HFF-1) were infected with 2 x 10^5 ME49 tachyzoites for 4 hours, then new media (DMEM; Gibco 21013024, plus 10% FBS; Gibco 26140079) was added containing different compounds to evaluate potential growth inhibition. In the negative control, only DMSO (Santa Cruz, 67-58-5) was added. As a positive control for parasite growth inhibition, pyrimethamine (Sigma, P7771) was used at 1μM. Parasites were grown for 48 hours, and then 1 μCi of [^{3}H] (Moraveck, MT 512) uracil was added in each well. After additional 24 hours, monolayers were quenched by adding 1 mL ice-cold 0.6 N (10%) Trichloroacetic acid (TCA; Merck, 100807) to the existing medium and incubate at 4 °C for 1 hour to fix the monolayer. TCA was removed and properly discarded, and the plates were washed with tap water for at least 4 hours. After the wash, it was added 500 mL of 1 M NaOH (Honeywell, 35256) per well, and the plates were gently shaken for one hour at room temperature. 4 ml of Scintillation fluid (RPI, 111167) was added to each vial. Then each vial received 400 mL of sample for each well, and after overnight incubation, [^{3}H] was measured.

Assay of compounds against *T. gondii* bradyzoites using an in vitro differentiation method: Tachyzoites were differentiated into bradyzoite for 4 days in RPMI (Gibco, 31800089) pH 8.1, as previously described.[2] After differentiation, the growth assay was performed similarly to the one described for tachyzoites; however, 10 μCi of [3H] uracil was used per well instead.

Assay of compounds against ex vivo harvested *T. gondii* bradyzoites from mice: C57BL/6J mice were intraperitoneally infected with 1,000 ME49 tachyzoites. At 30 days post-infection, or later, four mice were euthanized following ethical guidelines. Brains were removed, washed in cold PBS, and homogenized with a glass tissue grinder. The suspension was centrifuged at 400 x*g* for 10 minutes and the pellet suspended in 20% m/v Dextran (Average MW 150,000 Sigma). Bradyzoite cysts were pelleted and separated from brain material by centrifugation at 2,200 x*g* for 10 minutes. The pellet was washed in PBS, digested in 0.1 mg/mL pepsin (pH 2.1) for 5 minutes at 37 °C, and neutralized with an equal volume of 1% Sodium Carbonate (Sigma). Bradyzoites were centrifugated at 250 x*g* for 10 minutes, resuspended in switch medium, and added into the 96 well plates; 10,000 bradyzoites per well.

After 24 hours post HFF infection with bradyzoites, compound **23** at different concentrations (1x, 3x, or 6x EC_{50}) was added to the wells or DMSO as control. Three days post-treatment, cells were fixed in 3.7% formaldehyde in PBS for 20 minutes, permeabilized with 0.2% triton X-100 (Sigma)

in PBS at room temperature for 1 hour, and then blocked with 3% BSA in PBS at room temperature for 1 hour. Primary antibody was incubated at 4 °C overnight in 0.2% v/v Triton x-100 and 3% BSA in PBS (1:500 rabbit anti-BAG1, 1:500 mouse anti-SAG1). Wells were incubated with the specific secondary antibody (1:500 goat anti-rabbit Alexa Fluor 488 and 1:500 goat anti-mouse Alexa Fluor 594) at room temperature for 1 hour, then washed three times with PBS. Samples were imaged on Incucyte S3 (Sartorius). See **Fig. 1S** below.

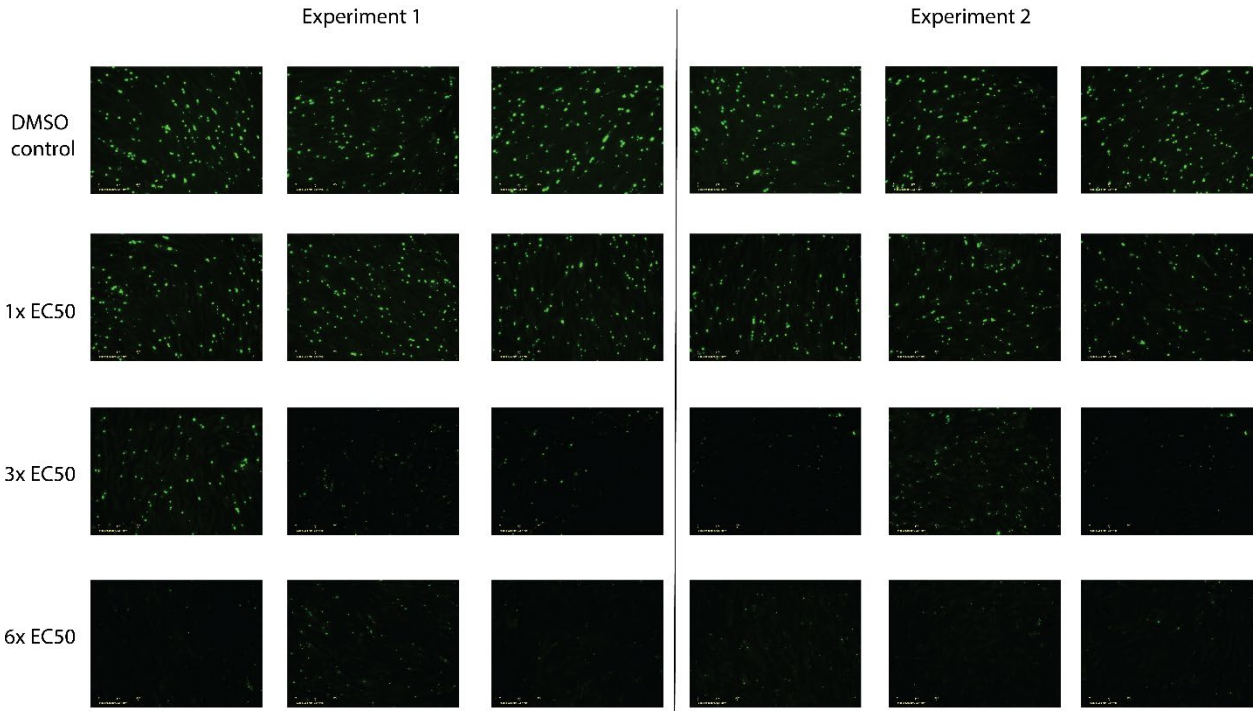


Figure 1S. Fluorescent imaging of two separate BAG1 labeling experiments, performed in triplicate, in the absence (DMSO control) and escalating concentrations of compound **23**. Only experiment 1 is included in the main manuscript for clarity. Labeled BAG1 shows up in green.

The fluorescent signal was quantified (represented by green intensity of labeled BAG1). Fluorescence per area (GCU x µm2/well) in which GFU is Total green Object Integrated Intensity (**Table 2S**).

Table 2S. Quantified fluorescence from Incucyte experiments shown in **Fig 1S**. Values reflect total well fluorescence per area (GCU x µm2/well).

	Experiment 1			Experiment 2			Average
	RUN1	RUN2	RUN3	RUN4	RUN5	RUN6	(RUNS 1-6)
DMSO	2.08E+07	1.50E+07	1.99E+07	1.33E+07	1.66E+07	1.20E+07	1.63E+07
1x EC_{50} of cmpd 23	1.32E+07	1.28E+07	6938311	8580216	7894094	6121916	9.25E+06
3x EC_{50} of cmpd 23	6905537	3443917	346996	651332.3	1259810	1901079	2.42E+06
6 EC_{50} of cmpd 23	480044.3	1191397	989763.3	630626	457900.5	417059	6.94E+05

$^{13}C_6$-Glucose Metabolomic experiment: Metabolomic analysis and [^{13}C]-glucose labeling of HFF cells infected with *T. gondii* was performed as previously described.[1] HFFs were grown to deep quiescence in 60 mm dishes in triplicate, then infected with 2 X 10^6 tachyzoites of *T. gondii* ME49 strain, or mock-infected with an equal volume of media. At 4 hours post-infection (HPI), compound 23 at 2X or 6X the IC_{50}, or an equal DMSO volume was added to the dishes. After an additional 5 hours incubation, the media was changed to glucose-free RPMI1640 supplemented to 1 g/L with [$^{13}C_6$] glucose (Sigma-Aldrich). After 10 minutes of labeling, dishes were washed 3x with ice-cold PBS, then quenched with 80:20 HPLC grade Methanol:Water and incubated on dry ice in a -80°C for 15 minutes. Plates were scraped, the solution washed twice, and spun at 2500 x g for 5 minutes at 4°C. Supernatants were combined, dried down under N_2 gas manifold, and resuspended in 100 µL HPLC grade water for analysis on a Thermo-Fisher Vanquish Horizon UHPLC joined by electrospray ionization (negative mode) to a hybrid quadrupole-Orbitrap high-resolution mass spectrometer (Q Exactive Orbitrap; Thermo Scientific). Chromatography was performed using a 100 mm X 2.1 mm X 1.7 µm BEH C18 column (Acquity) at 30°C. 20 µL of the sample was injected via an autosampler at 4° C, and flow rate was 200 µL/minute. Solvent A was 97:3 water/methanol with 9 mM Acetate and 10 mM tributylamine (TBA) with a pH of 8.2 (Sigma-Aldrich). Solvent B was 100% methanol with no TBA (Sigma-Aldrich). Products were eluted in 95% A/5% B for 2.5 minutes, then a gradient of 95% A/5% B to 5% A/95% B over 14.5 minutes, then held for an additional 2.5 minutes at 5%A/95%B. The gradient was returned to 95% A/5% B over 0.5 minutes, and held for 5 minutes to re-equilibrate the column. Data analysis was performed using the Metabolomics Analysis and Visualization Engine (MAVEN) software[2] and peaks for glucose-6-phosphate and fructose-6-phosphate were matched to known standards. The experiment was performed twice in triplicate.

Cytotoxicity assay of compounds in HFF cells: 1000 human foreskin fibroblasts cells per well were plated in a 384 well plate. The cells were treated with a single compound at eight concentrations in duplicate, for 72 hours. Following treatment, 20 uL of CellTiterGLO reagent was added per well and submitted to a plate reader to assess toxicity.

In vitro blood brain barrier parallel artificial membrane permeability assay (BBB PAMPA): A 96-well filter plate and the donor plate was used, along with the following reagents: porcine polar brain lipid (PBL), clonidine, theophylline, Verapamil, hydrocortisone, DMSO, PBS, Dodecane. Procedure: The 96-well filter plate (catalog no. MAIPN4550) and the donor plate (catalog no. MATRNPS50) were both purchased from Millipore. The porcine polar brain lipid (PBL) was purchased from Avanti Polar Lipids (catalog no. 141101P). Dodecane and DMSO were obtained from Sigma-Aldrich. Verapamil, clonidine, hydrocortisone and theophylline were purchased from Sigma-Aldrich and used as positive control. Tested compounds were dissolved in DMSO at 5 mg/mL as stock solutions, which were diluted in PBS to make a final solution (final concentration 25 μg/mL). The PBL was dissolved in dodecane as 20 mg/mL PBL solution. A 350 μL final solution was added to the donor well, the filter membrane of the filter plate was coated with 4 μL of PBL solution, and then the filter plate well was filled with 150 μL of PBS. The filter plate was carefully put on the donor plate to form a "sandwich" with tested compounds solution on the bottom, artificial lipid membrane in the middle, and the acceptor PBS on the top. The sandwich was incubated at room temperature for 18 h. The donor plate was removed after incubation. The solution from donor and filter wells were carefully transferred into the HPLC vials. The concentration ratios were determined by HPLC. Every sample was analyzed in triplicate. The P_e (cm/s) was obtained by the following equations:

$$P_e = -\frac{V_{dp}V_{fp}}{st(V_{dp} + V_{fp})}\ln\left(1 - \frac{[drug]_{fp}}{[drug]_{ref}}\right)$$

$$[drug]_{ref} = \frac{[drug]_{dp} \times 350 + [drug]_{fp} \times 150}{500}$$

where V_{dp} (mL) = volume of the donor plate (0.35 mL), V_{fp} (mL) = volume of the filter plate (0.15 mL), $[drug]_{fp}$ = compound concentration (area under the curve of the compound peak in the HPLC plot) of the filter plate, $[drug]_{dp}$ = compound concentration (area under the curve of the compound peak in the HPLC plot) of the donor plate, s (cm^2) = membrane area (0.28 cm^2), and t (s) = incubation time (64,800 s).

Reagents/supplies were sourced as follows:

reagent/supply	Vendor	Catalog No.
96-well filter plate	EMD Millipore	MATRNPS50
96-well donor plate	EMD Millipore	MAIPN4550
PBL	Avanti Polar Lipids	141101P
PBS	Thermal Fisher	10010-031
Dodecane	Sigma-Aldrich	297879-100ML
DMSO	Sigma-Aldrich	D8418-500ML
Verapamil hydrochloride	Sigma-Aldrich	PHR1131-1G

Clonidine hydrochloride	Sigma-Aldrich	C7897-100MG
Hydrocortisone	Sigma-Aldrich	H4001-1G
Theophylline	Sigma-Aldrich	T1633-50G

Kinetic Solubility assessment: *Media prep:* The phosphate buffer was prepared (pH at 7.4). The preparation of 50 mM NaH_2PO_4 in water (pH 4.50): 6.06 g of $NaH_2PO_4 \cdot 2H_2O$ was dissolved in 778 mL of water in a 1000 mL volumetric flask, and the measured pH was 4.50. Separately, 50 mM Na_2HPO_4 in water (pH 9.35) was prepared by dissolving 6.48 g of Na_2HPO_4 in 914 mL of water in a 1000 mL volumetric flask, and the pH measured was 9.35. The preparation of 50 mM phosphate buffer pH 7.4 was completed by adding 15 mL of 50 mM Na_2HPO_4 in water (pH 9.35) into a 50 mL tube, and then adjusting pH to 7.4 with 50 mM NaH_2PO_4 in water (pH 4.50). *Procedure:* Compound (10 mM in DMSO,10 µL) was added into lower chambers of a whatman miniuniprep vial (Miniuniprep (PTFE filter media with polypropylene housing, cat. no. UN203NPUORG, GE Halthcare Whatman), and then 490 µL of buffer at pH 7.4 was added into lower chamber of the whatman miniuniprep vial. The solubility sample was vortexed for at least 2 minutes. The miniuniprep vial was agitated on a barnstead shaker for 24 h at RT at the speed of 800 rpm, and then centrifuged 20 min (eg.4000 rpm). The miniuniprep was compressed to prepare the filtrate for injection into HPLC system to calculate the concentration with standard curve. The positive controls, amiodarone hydrochloride, carbamazepine, and chloramphenicol, were included in the analysis.

In vitro plasma stability assay: Pooled frozen plasma from a CD-1 mouse was thawed in a water bath at 37 ℃ prior to experiment. Plasma was centrifuged at 4000 rpm for 5 min and the clots were removed if any. The pH was adjusted to 7.4 ± 0.1 if required. Preparation of compound and positive control: 1 mM intermediate solution was prepared by diluting 10 µL of the stock solution with 90 µL DMSO; 1 mM intermediate of positive control Propantheline was prepared by diluting 10 µL of the stock solution with 90 µL ultrapure water. 100 µM dosing solution was prepared by diluting 10 µL of the intermediate solution (1 mM) with 90 µL DMSO. 98 µL of blank plasma was spiked with 2 µL of dosing solution (100 µM) to achieve 2 µM of the final concentration in duplicate and samples were Incubated at 37 ℃ in a water bath. At each time point (0,10, 30, 60 and 120 min), 400 µL of stop solution (200 ng/mL tolbutamide and 200 ng/mL Labetalol in 50% ACN/MeOH) was added to precipitate protein and mixed thoroughly. Centrifuged sample plates at 4,000 rpm for 10 min. An aliquot of supernatant (50 µL) was transferred from each well and mixed with 100 µL ultrapure water. The samples were agitated via shaker at 800 rpm for about 10 min before submitting to LC-MS/MS analysis. The % remaining of test compound after incubation in plasma was calculated using following equation: % Remaining= 100 x (PAR at appointed incubation time / PAR at T0 time) where PAR is the peak area ratio of analyte versus internal standard (IS). Propantheline bromide was used as reference compound in this assay.

In vitro mouse microsomal stability assay: Intermediate solution: 5 µL of compound stock solution (10 mM in dimethyl sulfoxide (DMSO)) was diluted with 495 µL of methanol (MeOH) (intermediate solution concentration: 100 µM, 99% MeOH). Working solution: 50 µL of compound intermediate solution (100 µM) were diluted with 450 µL of 100 mM potassium phosphate buffer (working solution concentration: 10 µM, 9.9% MeOH). NADPH Cofactor Preparation: NADPH powder: β-Nicotinamide adenine dinucleotide phosphate reduced form, tetrasodium salt; NADPH·4Na (Vendor: Chem-Impex International, Cat. No. 00616). The appropriate amount of NADPH powder was weighed and diluted into a 10 mM $MgCl_2$ solution (working solution concentration: 10 unit/mL; final concentration in reaction system: 1 unit/mL). Liver Microsomes Preparation: CD-1 Mouse (MLM, Cat No. BQM1000, Lot No. MIC255036 from Biopredic). The appropriate concentrations of microsome working solutions were prepared in 100 mM potassium phosphate buffer. Stop Solution Preparation: Cold (4°C) acetonitrile (ACN) containing 100 ng/mL tolbutamide and 100 ng/mL labetalol as internal standards (IS) was used as the stop solution. Using an Apricot automation workstation, 10 µL/well of compound working solution were added to all 96-well reaction plates except the blank (T0, T5, T10, T20, T30, T60, and NCF60). An Apricot automation workstation was used to add 80 µL/well of microsome solution to all reaction plates (Blank, T0, T5, T10, T20, T30, T60, and NCF60). All reaction plates containing mixtures of compound and microsomes were pre-incubated at 37 °C for 10 minutes. An Apricot automation workstation was used to add 10 µL/well of 100 mM potassium phosphate buffer to reaction plate NCF60. Reaction plate NCF60 was incubated at 37 °C, and timer 1 was started. After pre-incubation, an Apricot automation workstation was used to add 10 µL/well of NADPH regenerating system to every reaction plate except NCF60 (Blank, T0, T5, T10, T20, T30, and T60) to start the reaction. The reaction plates were incubated at 37°C, and timer 2 was started. An Apricot automation workstation was used to add 300 µL/well of stop solution to each reaction plate at its appropriate end time point to terminate the reaction. Each plate was sealed and shaken for 10 minutes. After shaking, each plate was centrifuged at 4000 rpm and 4°C for 20 minutes. During centrifugation, an Apricot automation workstation was used to add 300 µL/well of HPLC grade water to eight new 96-well plates. After centrifugation, an Apricot automation workstation was used to transfer 100 µL of supernatant from each reaction plate to its corresponding bioanaylsis plate. Each bioanalysis plate was sealed and shaken for 10 minutes prior to LC-MS/MS analysis. The equation of first order kinetics was used to calculate T1/2 and CLint(mic) (µL/min/mg). Controls used in the analysis included testosterone, diclofenac, and propafenone.

Snapshot pharmacokinetic analysis of compound 23: Six fed, male Balb/c Mouse and 6 in 1 internal standard (Labetalol & tolbutamide & Verapamil & dexamethasone & glyburide & Celecoxib 100 ng/mL for each) in acetonitrile were used for this study. Each mouse was administered a single IP dose of 15 mg/kg (formulation: 3 mg/ml in 12% SBE-β-CD, clear solution). Plasma samples were evaluated 30 and 120 min. Brain homogenate of each mouse was analyzed at 120 min. For plasma and brain homogenate samples: Brain homogenate was prepared by homogenizing brain with 5 volumes (w:v) of homogenizing solution (PBS(pH=7.4) buffer : MeOH (v:v, 2:1)). An aliquot of 20 µL sample, calibration standard, quality control, dilute quality control, single blank and double blank samples were added to the 1.5 mL tube; each sample (except

the double blank) was quenched with 200 μL IS solution respectively (double blank sample was quenched with 200 μL ACN), and then the mixture was vortex-mixed well (at least 15 s) with vortexer and centrifuged for 15 min at 12000 g, 4 ℃; 5 μL the supernatant were directly injected for LC-MS/MS analysis. Dilution factor: an aliquot of 2 μL sample was added with 18 μL blank plasma. Samples were analyzed by LC-MS/MS.

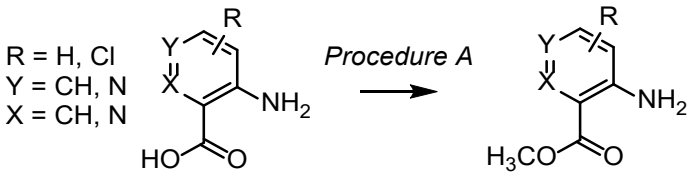
Synthetic procedures and characterization for compounds 1-23

General Information. Commercial reagents (3-amino-5-chloropicolinic acid, 3-aminopicolinic acid, 5-chloro-3-nitropicolinonitrile, 5-chloropyridin-3-amine, 4-amino-6-chloronicotinic acid, 2-amino-6-chloronicotinic acid, methyl 2-amino-5-bromo-4-chlorobenzoate, all boronic acids, and all benzoyl chlorides) and solvents were used as received. All thin layer chromatography was performed using precoated silica gel 60 F_{254} plates (EMD Millipore). Flash chromatography separations were carried out using a Teledyne ISCO CombiFlash Rf 200 purification system (MPLC) with silica gel columns. Purity of all final compounds was confirmed by HPLC/MS analysis on an Agilent 1290 LCMS and determined to be ≥ 95% AUC at 254 nm or 218 nm. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity-Inova 400 MHz NMR Spectrometer (operating at 400 and 101 MHz, respectively) or a Varian Unity-Inova 500 MHz NMR Spectrometer (operating at 500 and 126 MHz, respectively) or Bruker Ascend 400 MHz Spectrometer (operating at 400, 376, and 101 MHz, respectively) in $CDCl_3$, MeOD, or DMSO-d_6. The chemical shifts (δ) reported are given in parts per million (ppm). The signal splitting patterns were described as s = singlet, d = doublet, t = triplet, q = quartet, p = pentuplet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet, tt = triplet of triplet, ddd = doublet of doublet of doublet, br = broad and m = multiplet, with coupling constants (J) in hertz (Hz). The LC-MS analysis was performed on an Agilent 1290 Infinity II HPLC system with 1290 Infinity II Diode Array Detector and an Agilent 6120 Quadrupole LC-MS system. The analytical chromatography method utilized the following parameters: Poroshell 120 EC-C18, 1.9 μm column, UV detection wavelength = 254 nm, Flow rate = 1.0 mL/min, Gradient = 5-100% LC-MS grade Methanol over 4 min; The organic mobile phase and aqueous mobile phase contained 0.1% LC-MS grade formic acid. The purity analysis of some compounds was performed on Waters Prep LC-150 HPLC system with Waters 2545 Binary Gradient Module Pump, and Waters 2998 Photodiode Array Detector. The analytical chromatography method utilized the following parameters: XBridge® C18, 4.6 x 50 mm, 3.5 μm column; UV detection wavelength = 254 nm, Flow rate = 1.0 mL/min, Gradient = 5-100% HPLC grade Methanol over 10 min. The organic mobile phase and aqueous mobile phase contained 0.1% HPLC grade ammonium chloride. The mass spectrometer utilized the following parameters: an Agilent multimode source that simultaneously acquires ESI+/APCI+. High resolution mass spectra (HRMS) were performed by the Analytical Instrument Center at the School of Pharmacy on a Bruker MaXis 4G mass spectrometer. Chemical

nomenclature was generated using ChemBioDraw Ultra version 14.0. Calculated cLogD and cLogP values were obtained using MarvinSketch version 18.25, ChemAxon: Budapest, Hungary, 2018 or ACD/LogD version 8.07, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2004.

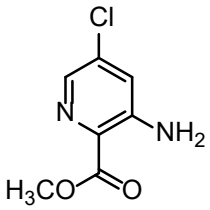
Syntheses of compounds **1** and **17-20** have been previously reported.[3] Intermediates **S1a-c** and **S2a-b** have been previously reported via alternative synthetic routes.[5-6] Spectral data for known compounds that were resynthesized matched reported values. All final compounds were assessed for purity by LCMS/UV and determined to be $\geq$ 95% prior to assessments.

General Procedure A. *Esterification of picolinic and nicotinic acids*.



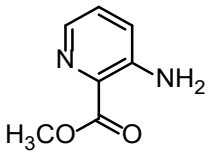
S1a-c

To a solution of the corresponding acid (1 equiv.) in 1:4 MeOH/CH_2Cl_2 (0.1M) was added dropwise a solution of TMS-diazomethane (2M in hexanes, 2 equiv.) and the reaction stirred at rt for 18 h. The reaction was then concentrated and purified as indicated to give the title compound.



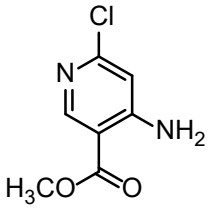
S1a

3-Amino-5-chloropicolinic acid (251 mg, 1.5 mmol) was subjected to procedure A, then purified by MPLC (4g SiO_2, 0-25% EtOAc, 30 mL/min) to give methyl 3-amino-5-chloropicolinate **S1a** as a yellow solid (149 mg, 0.8 mmol, 55% yield). Characterization matched literature report.[5]



S1b

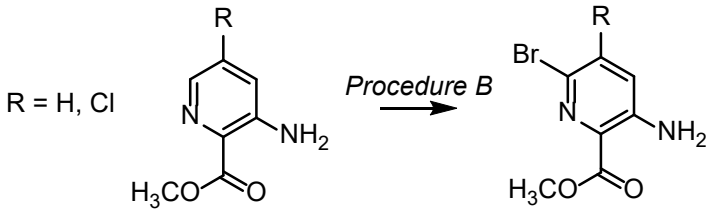
3-Aminopicolinic acid (499 mg, 3.6 mmol) was subjected to procedure A, then purified by MPLC (12g SiO_2, 0-100% EtOAc/Hexanes, 30 mL/min) to give methyl 3-aminopicolinate **S1b** as a white solid (232 mg, 1.5 mmol, 42% yield). Characterization matched literature report.[5]



S1c

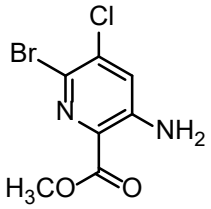
4-Amino-6-chloronicotinic acid was subjected to procedure A, then purified by MPLC (4g SiO_2, 0-25% EtOAc/Hexanes, 20 mL/min) to give methyl 4-amino-6-chloronicotinate **S1c** as a white solid (83 mg, 0.45 mmol, 76% yield). Characterization matched literature report.[6]

General Procedure B. *Bromination of methyl 3-amino picolinates*.



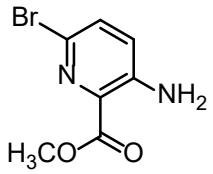
S1a-b **S2a-b**

To a suspension of the corresponding methyl 3-amino picolinate in water (0.5M) was added 2M H_2SO_4 (aq) (10 mL/mmoL, 20 equiv.) and this was stirred until the starting material was dissolved. To the reaction solution was then added 2.6M Br_2 (AcOH) solution dropwise (0.4 mL/mmoL, 1 equiv.), and the mixture stirred at rt for 18 h. The pH was then adjusted to 12 using 1M NaOH (aq) and the resulting precipitate collected by filtration.



S2a

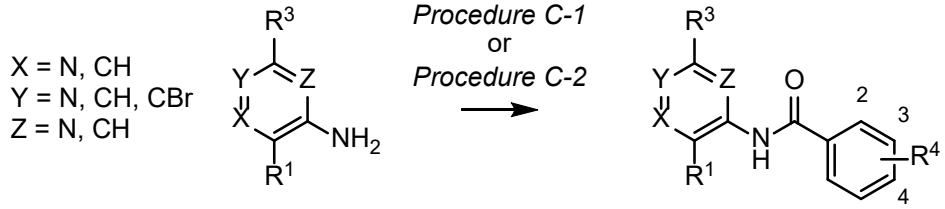
S1a (369 mg, 1.98 mmol) was subjected to general procedure B to give methyl 3-amino-6-bromo-5-chloropicolinate **S2a** as an off-white solid (268 mg, 1.0 mmol, 51% yield). Characterization matched literature report.[5]



S2b

S1b (23 mg, 0.15 mmol) was subjected to general procedure B, then purified by MPLC (4g SiO_2, 25% EtOAc/Hexanes, 20 mL/min) to give methyl 3-amino-6-bromopicolinate **S2b** as a white solid (91 mg, 0.40 mmol, 55% yield). Characterization matched literature report.[5]

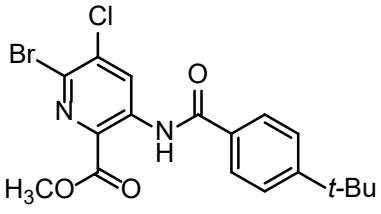
General Procedures C-1 and C-2. *Amide coupling of (hetero)aryl amine building blocks to give benzamido compounds*.



S3a-h, 7-9, 15-16

Procedure C-1: To a solution of the corresponding (hetero)aryl amine (1 equiv.) in CH_3CN (1M) was added the corresponding benzoyl chloride (1.1 equiv.), then Et_3N or iPr_2EtN as indicated (2.2 equiv.), and the reaction stirred at rt for 18 h. The reaction mixture was then diluted with EtOAc and washed with 1M HCl (aq) (2 x 10 mL), the organic layer separated, dried over $MgSO_4$ (s), concentrated, and purified as indicated to give the title compound.

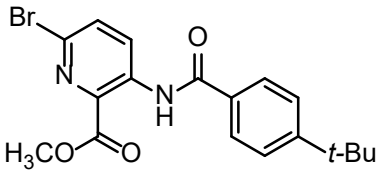
Procedure C-2: To a G10 microwave vial equipped with stir bar was added the corresponding (hetero)aryl amine (1 equiv.) in CH_3CN (0.06M) and the corresponding benzoyl chloride (1.1 equiv.). The vessel was sealed and heated to 150°C by microwave irradiation with stirring for 1 hour. The reaction mixture was then treated as indicated to give the title compound.



S3a

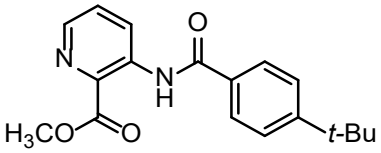
S2a (399 mg, 1.5 mmol) was subjected to procedure C-2 using 4-(*tert*-butyl)benzoyl chloride, then concentrated and purified by MPLC (12g SiO_2, 0-25% EtOAc, 30 mL/min) to give methyl 6-

bromo-3-(4-(tert-butyl)benzamido)-5-chloropicolinate **S3a** as a white solid (371 mg, 0.87 mmol, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.89 (s, 1H), 9.56 (s, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 4.06 (s, 3H), 1.37 (s, 9H).



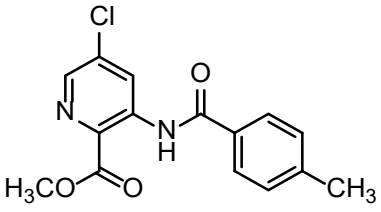
S3b

S2b (15 mg, 0.07 mmol) was subjected to procedure C-2 using 4-(*tert*-butyl)benzoyl chloride, then the reaction was diluted with CH_2Cl_2, washed with sat. $NaHCO_3$ (aq) (3 x 10 mL), brine (1 x 10 mL), separated and dried over $MgSO_4$ (s), then purified by MPLC (4g SiO_2, 0-10% EtOAc/Hexanes, 20 mL/min) to give methyl 6-bromo-3-(4-(tert-butyl)benzamido)picolinate **S3b** as a white solid (8 mg, 0.02 mmol, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.90 (s, 1H), 9.36 (d, J = 9.0 Hz, 1H), 7.96 (dd, J = 8.5, 1.6 Hz, 2H), 7.57-7.54 (m, 3H), 4.06 (s, 3H), 1.37 (s, 9H).



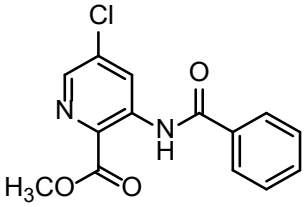
S3c

S1b (40 mg, 0.26 mmol) was subjected to procedure C-1 using 4-(*tert*-butyl)benzoyl chloride and *i*Pr_2EtN, then purified by MPLC (4g SiO_2, 0-25% EtOAc/Hexanes, 20 mL/min) to give methyl 3-(4-(tert-butyl)benzamido)picolinate **S3c** as a brown oil (73 mg, 0.23 mmol, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.87 (s, 1H), 9.27 (d, J = 8.6 Hz, 1H), 8.38 (d, J = 4.4 Hz, 1H), 7.92 (d, J = 7.0 Hz, 2H), 7.50 (d, J = 7.1 Hz, 2H), 7.47 (t, J = 4.5 Hz, 1H), 4.01 (s, 3H), 1.31 (s, 9H).



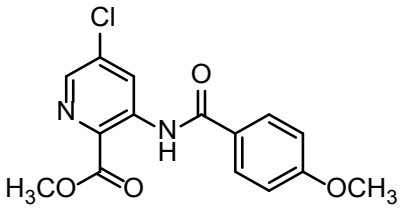
S3d

S1a (29 mg, 0.16 mmol) was subjected to procedure C-2 using 4-methylbenzoyl chloride. The reaction mixture was filtered to collect methyl 5-chloro-3-(4-methylbenzamido)picolinate **S3d** as a white solid (20 mg, 0.06 mmol, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.95 (s, 1H), 9.45 (d, J = 2.2 Hz, 1H), 8.38 (d, J = 2.2 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.08 (s, 3H), 2.45 (s, 3H).



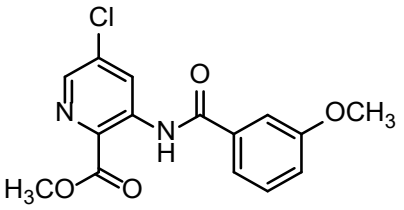
S3e

S1a (35 mg, 0.19 mmol) was subjected to procedure C-2 using benzoyl chloride. The reaction mixture was filtered to collect methyl 3-benzamido-5-chloropicolinate **S3e** as an off-white solid (25 mg, 0.09 mmol, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 9.46 (d, J = 2.2 Hz, 1H), 8.40 (d, J = 2.2 Hz, 1H), 8.05-8.03 (m, 2H), 7.62-7.54 (m, 3H), 4.08 (s, 3H).



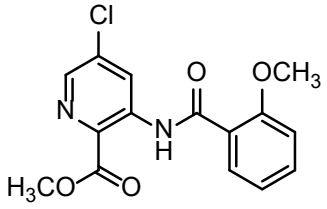
S3f

S1a (38 mg, 0.20 mmol) was subjected to procedure C-2 using 4-methoxybenzoyl chloride. The reaction mixture was filtered to collect methyl 5-chloro-3-(4-methoxybenzamido)picolinate **S3f** as a white solid (27 mg, 0.09 mmol, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.92 (s, 1H), 9.44 (d, J = 2.2 Hz, 1H), 8.37 (d, J = 2.2 Hz, 1H), 8.01 (d, J = 8.9 Hz, 2H), 7.04 (d, J = 9.0 Hz, 2H), 4.08 (s, 3H), 3.90 (s, 3H).



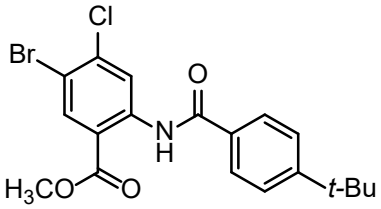
S3g

S1a (39 mg, 0.20 mmol) was subjected to procedure C-2 using 3-methoxybenzoyl chloride. The reaction mixture was filtered to collect a white solid, which was purified by MPLC (4g SiO_2, 0-50% EtOAc/Hexanes, 20 mL/min) to give methyl 5-chloro-3-(3-methoxybenzamido)picolinate **S3g** as a white solid (48 mg, 0.15 mmol, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (s, 1H), 9.44 (d, J = 2.2 Hz, 1H), 8.39 (d, J = 2.2 Hz, 1H), 7.60-7.58 (m, 2H), 7.46 (t, J = 8.2 Hz, 1H), 7.15 (ddd, J = 8.3, 2.4, 1.2 Hz, 1H), 4.08 (s, 3H), 3.91 (s, 3H).



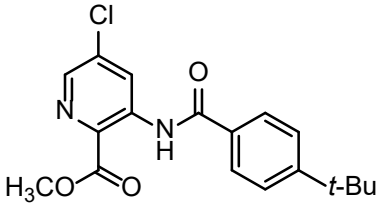
S3h

S1a was subjected to procedure C-2 using 2-methoxybenzoyl chloride. The reaction mixture was filtered to collect methyl 5-chloro-3-(2-methoxybenzamido)picolinate **S3h** as an off-white solid (18 mg, 0.06 mmol, 38% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.36 (s, 1H), 9.51 (d, J = 2.2 Hz, 1H), 8.36 (d, J = 2.2 Hz, 1H), 8.21 (dd, J = 7.9, 1.8 Hz, 1H), 7.54 (ddd, J = 8.8, 7.4, 1.9 Hz, 1H), 7.12 (dd, J = 8.0, 7.2 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 4.13 (s, 3H), 4.04 (s, 3H).



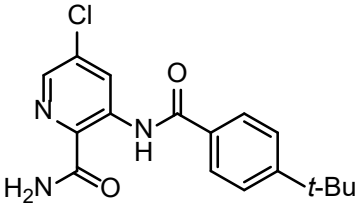
S3i

Methyl 2-amino-5-bromo-4-chlorobenzoate was subjected to general procedure C-2 using 4-(*tert*-butyl)benzoyl chloride, then filtered to give methyl 5-bromo-2-(4-(tert-butyl)benzamido)-4-chlorobenzoate **S3i** as a light brown solid (1.6 g, 3.8 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.94 (s, 1H), 9.20 (s, 1H), 8.29 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 3.98 (s, 3H), 1.36 (s, 7H).



Methyl 3-(4-(tert-butyl)benzamido)-5-chloropicolinate (**7**).

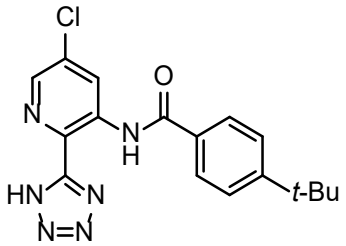
S1a (95 mg, 0.51 mmol) was subjected to procedure C-2 using 4-(*tert*-butyl)benzoyl chloride. The reaction mixture was filtered, and the filtrate concentrated and redissolved in CH_2Cl_2. The organic layer was washed with sat. $NaHCO_3$ (aq) (3 x 10 mL), brine (1 x 10 mL), separated and dried over $MgSO_4$ (s), then purified by MPLC (4g SiO_2, 0-50% EtOAc/Hexanes, 20 mL/min) to give **7** as a white solid (90 mg, 0.26 mmol, 51% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.97 (s, 1H), 9.46 (d, J = 2.2 Hz, 1H), 8.38 (d, J = 2.2 Hz, 1H), 7.97 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 4.08 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 166.4, 156.6, 142.5, 140.2, 137.2, 131.0, 129.8, 127.9, 127.5, 126.2, 53.6, 35.3, 31.3. HRMS (ESI-QTOF) m/z: $[M + H]^+$ Calcd for $C_{18}H_{20}ClN_2O_3^+$ 347.1157, 349.1133; Found: 347.1157, 349.1132. HPLC Purity ≥ 96%.



3-(4-(*tert*-Butyl)benzamido)-5-chloropicolinamide (**8**).

5-chloro-3-nitropicolinonitrile (187 mg, 1.0 mmol, 1 equiv.) was dissolved in MeOH (10 mL) before the addition of $FeCl_3$*$6H_2O$ (25 mg, 0.09 mmol, 0.09 equiv.) and activated charcoal (45 mg, 3.8 mmol, 3 equiv.). The reaction flask was fitted with reflux condenser, heated to reflux, and stirred 10 min before the dropwise addition of hydrazine monohydrate (0.2 mL, 4.1 mmol, 4 equiv.). The reaction was stirred at reflux for 1 hour, then cooled to rt, filtered through celite, concentrated, and purified by MPLC (4g SiO_2, 0-50% EtOAc/Hexanes, 20 mL/min) to give 3-amino-5-chloropicolinamide **S4a** as a white solid (76 mg, 0.44 mmol, 44% yield). Characterization matched literature report.[4]

S4a (66 mg, 0.43 mmol) was subjected to procedure C-2 using 4-(*tert*-butyl)benzoyl chloride. The reaction was filtered to give **8** as a green solid (85 mg, 0.27 mmol, 63% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.91 (s, 1H), 9.44 (d, J = 2.2 Hz, 1H), 8.20 (d, J = 1.9 Hz, 1H), 8.06 (br s, 1H), 7.98 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 5.71 (br s, 1H), 1.36 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.6, 166.6, 156.3, 141.0, 139.5, 136.5, 131.2, 130.8, 127.9, 127.5, 126.0, 35.2, 31.3. HRMS (ESI-QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{19}ClN_3O_2^+$ 332.1160, 334.1136; Found: 332.1156, 334.1137. HPLC Purity ≥ 98%.



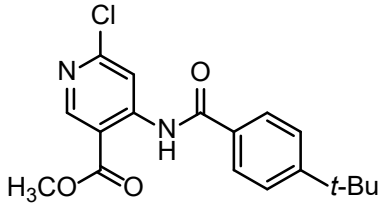
4-(*tert*-Butyl)-N-(5-chloro-2-(*1H*-tetrazol-5-yl)pyridin-3-yl)benzamide (**9**).

5-chloro-3-nitropicolinonitrile (1.8 g, 9.6 mmol, 1 equiv.) was dissolved in *n*-BuOH (25 mL) before the addition of zinc chloride (1.3 g, 9.9 mmol, 1 equiv.) and sodium azide (0.83 g, 13 mmol, 1.2 equiv.). The reaction mixture was heated to 110°C with vigorous stirring for 1.5 h, then cooled to rt and concentrated to a cream residue. The residue was redissolved in 1M NaOH (aq) (50 mL) and stirred 20 min before filtering. The filter cake was washed with water (2 x 25 mL). The resulting filtrate was acidified with concentrated HCl until a precipitate formed (pH 6), this was collected to give 5-chloro-3-nitro-2-(1H-tetrazol-5-yl)pyridine **S5a** as a brown solid (1.4 g, 6.1 mmol, 63% yield), used without purification.

S5a (223 mg, 0.99 mmol, 1 equiv.) was dissolved in MeOH (10 mL) before the addition of $FeCl_3$*$6H_2O$ (21 mg, 0.08 mmol, 0.07 equiv.) and activated charcoal (37 mg, 3.1 mmol, 3 equiv.).

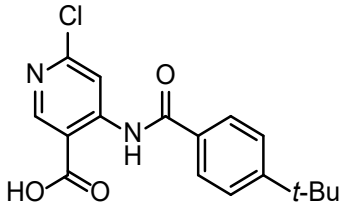
The mixture was heated to reflux and stirred 10 min before the dropwise addition of hydrazine monohydrate (0.2 mL, 4.1 mmol, 4 equiv.). The reaction was stirred at reflux for 4 hours, then cooled to rt, filtered through celite, concentrated, and triturated with MeOH to give 5-chloro-2-(1H-tetrazol-5-yl)pyridin-3-amine **S6a** as a green/off-white solid (67 mg, 0.34 mmol, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 2.0 Hz, 1H), 7.44 (d, J = 2.1 Hz, 1H), 7.01 (br s, 2H).

S6a (31 mg, 0.16 mmol) was subjected to procedure C-1 using 4-(*tert*-butyl)benzoyl chloride and *i*Pr_2EtN, then purified by MPLC (4g SiO_2, 0-10% EtOAc/Hexanes, 20 mL/min) to give **9** as an off-white solid (16 mg, 0.05 mmol, 30% yield). ^{1}H NMR (500 MHz, MeOD) δ 9.36 (d, J = 2.1 Hz, 1H), 8.47 (d, J = 2.2 Hz, 1H), 8.13 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.4 Hz, 2H), 1.40 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ ^{13}C NMR (MHz,) δ 168.0, 157.9, 156.0, 144.0, 137.2, 135.3, 132.0, 130.4, 128.7, 128.3, 127.1, 36.0, 31.5. HRMS (ESI-QTOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{18}ClN_6O^+$ 357.1225, 359.1201; Found: 357.1226, 359.1198. HPLC Purity ≥ 95%.



Methyl 4-(4-(tert-butyl)benzamido)-6-chloronicotinate (**15**).

S1c (48 mg, 0.25 mmol) was subjected to procedure C-1 using 4-(*tert*-butyl)benzoyl chloride and *i*Pr_2EtN, then purified by MPLC (4g SiO_2, 0-25% EtOAc/Hexanes, 20 mL/min) to give **15** as an off-white solid (83 mg, 0.24 mmol, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.09 (s, 1H), 8.98 (s, 1H), 8.94 (s, 1H), 7.97 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 4.02 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 166.2, 157.6, 156.9, 152.7, 149.6, 130.7, 127.6, 126.2, 113.8, 110.0, 53.1, 35.3, 31.3. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{18}H_{18}ClN_2O_3^-$ 345.1011, 349.0976; Found: 345.1009, 347.0983. HPLC Purity ≥ 98%.

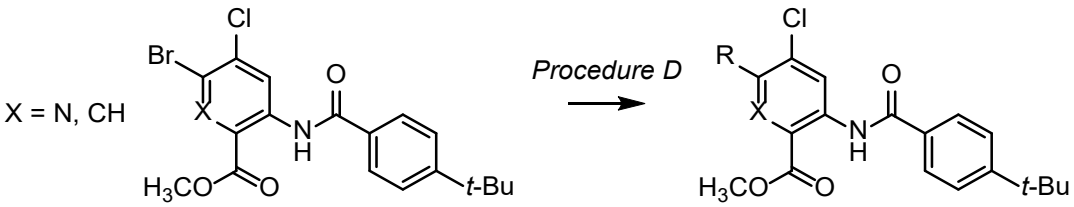


4-(4-(*tert*-Butyl)benzamido)-6-chloronicotinic acid (**16**).

4-Amino-6-chloronicotinic acid (25 mg, 0.15 mmol) was subjected to procedure C-1 using 4-(*tert*-butyl)benzoyl chloride and *i*Pr_2EtN, then purified by MPLC (4g SiO_2, 0-10% EtOAc/Hexanes, 20 mL/min) to give **16** as a white solid (9 mg, 0.03 mmol, 20% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ (exchangeable acid and amide protons were not observed) 9.20 (s, 1H), 8.24 (d, J = 8.3 Hz, 2H), 7.57 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (126 MHz,) δ 162.5, 158.6, 158.6,

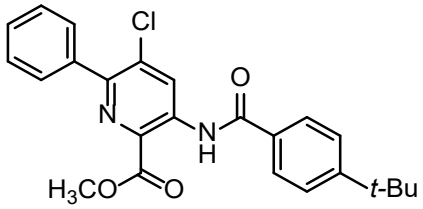
157.5, 154.8, 152.0, 129.3, 126.3, 126.2, 120.6, 112.0, 35.5, 31.2. HRMS (ESI-QTOF) m/z: [M - H]$^-$ Calcd for $C_{17}H_{16}ClN_2O_3^-$ 331.0855, 333.0820; Found: 331.0848, 333.0823. HPLC Purity ≥ 96%.

General Procedure D. *Suzuki coupling of (hetero)aryl benzamido bromide esters to give 6-substituted (hetero)aryl benzamido methyl esters*.



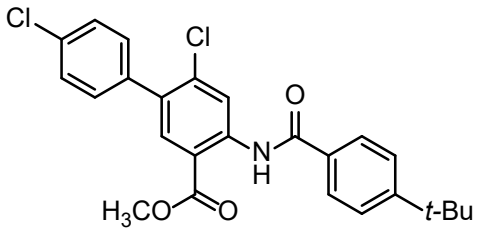
S3a, S3i **S7a-c**

To a G4 microwave vial equipped with stir bar was added the corresponding (hetero)aryl bromide (1 equiv.), corresponding boronic acid (1.2 equiv.), bis(triphenylphosphine)palladium dichloride (0.1 equiv.), and sodium carbonate (1.5 equiv.) before addition of a 10:1 (v/v) CH_3CN/H_2O solution (0.1M). The vessel was sealed and heated at 100-150°C by microwave irradiation with stirring for 30 min. Upon cooling to room temperature, the reaction was diluted with EtOAc (5 mL) and washed with water (2 x 5 mL) and brine (2 x 5 mL). The organic layer was separated and dried over MgSO4 (s), filtered, and concentrated. The resulting crude material was purified as indicated to give the title compound.



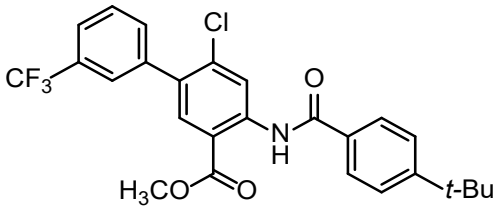
S7a

S3a (47 mg, 0.11 mmol) was subjected to procedure D using phenylboronic acid (20 mg, 0.16 mmol) at 100°C, then purified by (4g SiO_2, 0-5% EtOAc, 20 mL/min) to give methyl 3-(4-(tert-butyl)benzamido)-5-chloro-6-phenylpicolinate **S7a** as a white solid (31 mg, 0.07 mmol, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.95 (s, 1H), 9.54 (s, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.78-7.76 (m, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.49-7.41 (m, 3H), 4.04 (s, 3H), 1.38 (s, 9H).



S7b

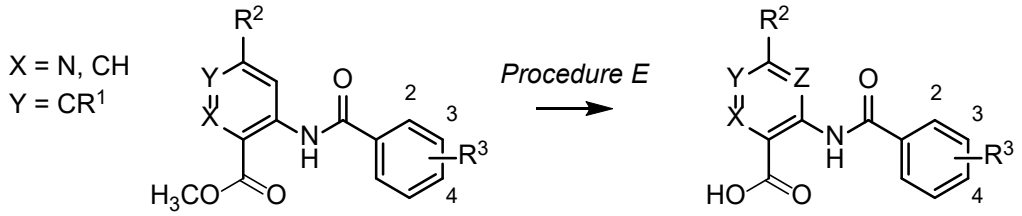
S3i (50 mg, 0.12 mmol) was subjected to procedure D using 4-chlorophenylboronic acid (20 mg, 0.14 mmol, 1.2 equiv.) at 150°C, then purified by MPLC (4g SiO_2, 0-2% EtOAc/Hexanes over 10 min, 20 mL/min) to give methyl 4-(4-(*tert*-butyl)benzamido)-4',6-dichloro-[1,1'-biphenyl]-3-carboxylate **S7b** (27 mg, 0.07 mmol, 49% yield). Used without analysis.



S7c

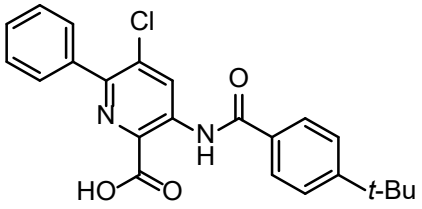
S3i (50 mg, 0.12 mmol) was subjected to procedure D using 3-(trifluoromethyl)phenylboronic acid (27 mg, 0.14 mmol, 1.2 equiv.) at 150°C, then purified by MPLC (4g SiO_2, 0-5% EtOAc/Hexanes over 10 min, 20 mL/min) to give methyl 4-(4-(tert-butyl)benzamido)-6-chloro-3'-trifluoromethyl-[1,1'-biphenyl]-3-carboxylate **S7c** (35 mg, 0.07 mmol, 61% yield). Used without analysis.

General Procedure E. *Hydrolysis of (hetero)aryl benzamido methyl esters to give (hetero)aryl benzamido acid compounds* (**2-6, 10-14, 21-23**).



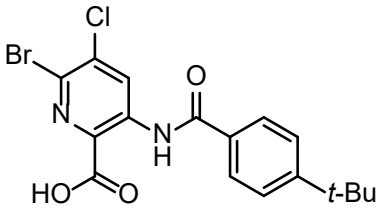
S3a-i, S7a-c, 7 **2-6, 10-14, 21-23**

To a 1-dram vial equipped with stir bar was added the corresponding methyl ester (1 equiv.) as a solution in THF (0.1M). 1.3M LiOH (aq) solution (7 equiv.) was added and the solution stirred at rt for 18 h. The reaction mixture was then acidified with 1M HCl (aq) to pH 2 and the resulting precipitate isolated by vacuum filtration to give the title compound.



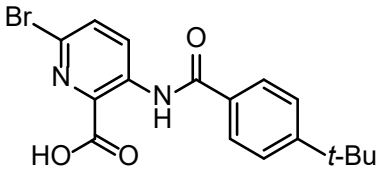
3-(4-(*tert*-Butyl)benzamido)-5-chloro-6-phenylpicolinic acid (**2**).

S7a (31 mg, 0.07 mmol) was subjected to procedure E to give **2** as a white solid (21 mg, 0.05 mmol, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (exchangeable acid proton was not observed) 11.95 (s, 1H), 9.22 (s, 1H), 7.98-7.83 (m, 2H), 7.73 (d, J = 7.1 Hz, 2H), 7.67-7.62 (m, 2H), 7.55-7.47 (m, 3H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.4, 165.3, 155.8, 149.0, 137.2, 136.8, 132.6, 132.5, 130.8, 129.3, 129.1, 128.9, 128.1, 127.1, 126.0, 34.9, 30.9. HRMS (ESI-QTOF) m/z: [M - H]$^-$ Calcd for $C_{23}H_{20}ClN_2O_3^-$ 407.1168, 409.1133; Found: 407.1156, 409.1131. HPLC Purity ≥ 98%.



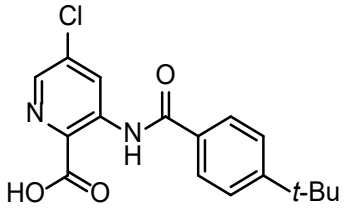
6-Bromo-3-(4-(*tert*-butyl)benzamido)-5-chloropicolinic acid (**3**).

S3a (50 mg, 0.12 mmol) was subjected to procedure E to give **3** as a white solid (16 mg, 0.04 mmol, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.87 (s, 1H), 9.67 (s, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 165.6, 157.1, 149.5, 138.9, 131.9, 131.7, 130.2, 127.6, 126.3, 35.3, 31.2. HRMS (ESI-QTOF) m/z: [M - H]$^-$ Calcd for $C_{17}H_{15}BrClN_2O_3^-$ 408.9955, 410.9932; Found: 408.9951, 410.9930. HPLC Purity ≥ 95%.



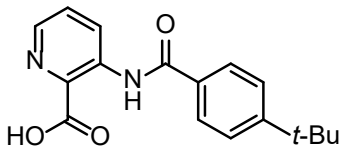
6-Bromo-3-(4-(*tert*-butyl)benzamido)picolinic acid (**4**).

S3b (8 mg, 0.02 mmol) was subjected to procedure E to give **4** as a white solid (6 mg, 0.02 mmol, 80% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ (exchangeable acid proton was not observed) 11.92 (s, 1H), 9.43 (d, J = 9.0 Hz, 1H), 7.97 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 156.8, 142.4, 139.2, 138.9, 132.4, 132.0, 130.8, 130.6, 127.6, 126.2, 35.3, 31.2. HRMS (ESI-QTOF) m/z: [M - H]$^-$ Calcd for $C_{17}H_{16}BrN_2O_3^-$ 375.0350, 377.0324; Found: 375.0344, 377.0325. HPLC Purity ≥ 97%.



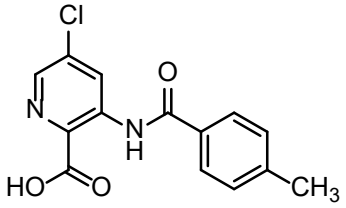
3-(4-(*tert*-Butyl)benzamido)-5-chloropicolinic acid (**5**).

7 (41 mg, 0.12 mmol) was subjected to general procedure E to give **5** as a white solid (36 mg, 0.11 mmol, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.94 (s, 1H), 9.52 (d, J = 2.1 Hz, 1H), 8.25 (d, J = 2.1 Hz, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 166.5, 156.9, 140.9, 139.9, 138.6, 130.5, 128.6, 128.0, 127.6, 126.2, 35.3, 31.3. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{17}H_{16}ClN_2O_3^-$ 331.0855, 333.0820; Found: 331.0844, 333.0816. HPLC Purity ≥ 95%.



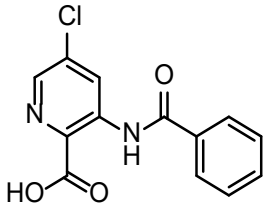
3-(4-(*tert*-Butyl)benzamido)picolinic acid (**6**).

S3c (36 mg, 0.12 mmol) was subjected to procedure E to give **6** as a white solid (26 mg, 0.09 mmol, 77% yield).^{1}H NMR (500 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.97 (s, 1H), 9.43 (d, J = 8.6 Hz, 1H), 8.31 (d, J = 4.4 Hz, 1H), 8.00 (d, J = 8.0 Hz, 2H), 7.65 (dd, J = 8.7, 4.5 Hz, 1H), 7.56 (d, J = 8.0 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0, 166.6, 156.5, 141.2, 139.8, 130.8, 130.04, 129.95, 129.8, 127.5, 126.1, 35.2, 31.2. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{17}H_{17}N_2O_3^-$ 297.1245; Found: 297.1241. HPLC Purity ≥ 97%.



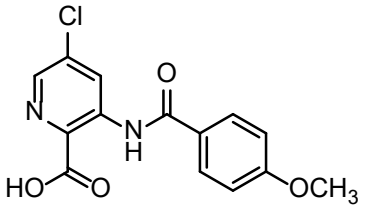
5-Chloro-3-(4-methylbenzamido)picolinic acid (**10**).

S3d (20 mg, 0.07 mmol) was subjected to procedure E to give **10** as a white solid (17 mg, 0.06 mmol, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.94 (s, 1H), 9.52 (d, J = 2.0 Hz, 1H), 8.25 (d, J = 2.1 Hz, 1H), 7.94 (d, J = 7.9 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 166.5, 143.9, 140.9, 139.9, 138.6, 130.5, 129.9, 128.6, 128.0, 127.7, 21.8. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{14}H_{10}ClN_2O_3^-$ 289.03854, 291.0350; Found: 289.0378, 291.0349. HPLC Purity ≥ 99%.



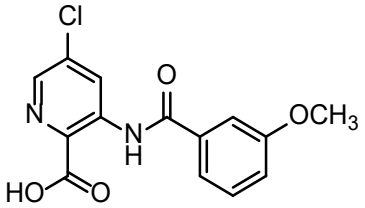
3-Benzamido-5-chloropicolinic acid (**11**).

S3e (18 mg, 0.06 mmol) was subjected to procedure E to give **11** as a white solid (16 mg, 0.06 mmol, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.99 (s, 1H), 9.52 (d, J = 2.0 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 8.11 – 7.97 (m, 2H), 7.76 – 7.45 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 166.5, 141.1, 139.8, 138.7, 133.3, 133.1, 129.2, 128.7, 128.1, 127.7. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{13}H_8ClN_2O_3^-$ 275.0229, 277.0194; Found: 275.0219, 277.0190. HPLC Purity ≥ 98%.



5-Chloro-3-(4-methoxybenzamido)picolinic acid (**12**).

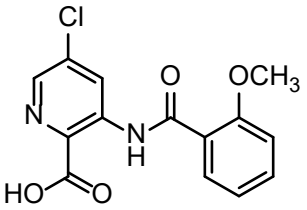
S3f (22 mg, 0.07 mmol) was subjected to procedure E to give **12** as a tan solid (14 mg, 0.05 mmol, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.89 (s, 1H), 9.50 (d, J = 2.1 Hz, 1H), 8.24 (d, J = 2.0 Hz, 1H), 8.02 (d, J = 8.9 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 166.0, 163.5, 140.8, 140.0, 138.6, 129.8, 128.5, 127.9, 125.6, 114.5, 55.7. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{14}H_{10}ClN_2O_4^-$ 305.0335, 307.0300; Found: 305.0328, 307.0300. HPLC Purity ≥ 95%.



5-Chloro-3-(3-methoxybenzamido)picolinic acid (**13**).

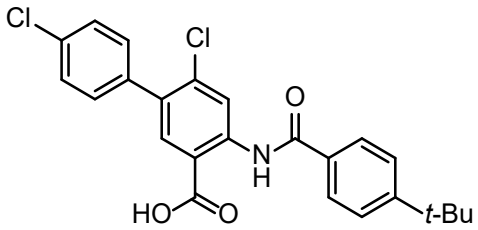
S3g (29 mg, 0.09 mmol) was subjected to procedure E to give **13** as an off-white solid (24 mg, 0.08 mmol, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (exchangeable acid proton was not observed) 11.98 (s, 1H), 9.50 (d, J = 2.0 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.15 (dd, J = 8.1, 2.5 Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 166.4, 160.3, 141.1, 139.7, 138.7, 134.7, 130.3, 128.6, 128.1, 119.7, 119.6, 112.5, 55.7.

HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{14}H_{10}ClN_2O_4^-$ 305.0335, 307.0300; Found: 305.0326, 307.0297. HPLC Purity ≥ 95%.



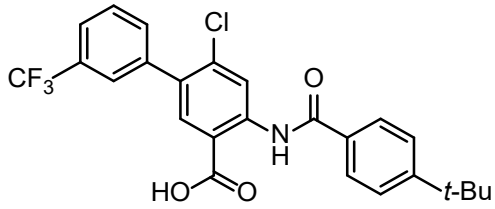
5-Chloro-3-(2-methoxybenzamido)picolinic acid (**14**).

S3h (18 mg, 0.06 mmol) was subjected to procedure E to give **14** as a white solid (13 mg, 0.04 mmol, 68% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.55 (s, 1H), 11.32 (br s, 1H), 9.67 (s, 1H), 8.23 (s, 1H), 8.20 (s, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 4.13 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.6, 165.2, 158.1, 140.5, 139.6, 138.1, 134.5, 132.8, 129.9, 128.8, 121.3, 121.1, 111.6, 55.8. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{14}H_{10}ClN_2O_4^-$ 305.0335, 307.0300; Found: 305.0322, 307.0293. HPLC Purity ≥ 98%.



4-(4-(*tert*-Butyl)benzamido)-4',6-dichloro-[1,1'-biphenyl]-3-carboxylic acid (**21**).

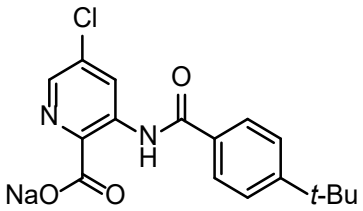
S7b (27 mg, 0.07 mmol) was subjected to procedure E to give **21** as an off-white solid (19 mg, 0.04 mmol, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (exchangeable acid proton was not observed) 12.23 (s, 1H), 8.97 (s, 1H), 8.01 (s, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.6 Hz, 2H), 7.58 – 7.48 (m, 4H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.1, 164.8, 155.6, 141.2, 136.7, 136.3, 133.5, 132.9, 132.7, 131.2, 131.1, 128.4, 127.0, 125.9, 120.4, 115.7, 34.8, 30.8. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{24}H_{20}Cl_2NO_3^-$ 440.0826, 442.0791; Found: 440.0818, 442.0791. HPLC Purity ≥ 96%.



4-(4-(*tert*-Butyl)benzamido)-6-chloro-3'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylic acid (**22**).

S7c (35 mg, 0.07 mmol) was subjected to procedure E to give **22** as an off-white solid (26 mg, 0.06 mmol, 86% yield). ^{1}H NMR (400 MHz, Acetone -d_6) δ (exchangeable acid proton was not observed) 12.36 (s, 1H), 9.23 (s, 1H), 8.21 (s, 1H), 7.99 (d, J = 8.3 Hz, 2H), 7.88 – 7.70 (m, 4H), 7.65 (d, J = 8.2 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, Acetone -d_6) δ 165.1, 165.0, 155.9,

142.6, 142.4, 139.2, 137.9, 133.9, 133.4, 132.7, 131.6 (d, J = 5.1 Hz), 130.1 (d, J = 32.2 Hz), 129.3, 127.2, 126.1 (q, J = 3.9 Hz), 125.9, 124.6 (q, J = 4.1 Hz), 124.3 (d, J = 271.6 Hz) 120.6 (d, J = 5.3 Hz), 34.7, 30.5. HRMS (ESI-QTOF) m/z: $[M - H]^-$ Calcd for $C_{25}H_{20}ClF_3NO_3^-$ 474.1089, 476.1054; Found: 474.1084, 476.1059. HPLC Purity ≥ 95%.



Sodium 3-(4-(*tert*-butyl)benzamido)-5-chloropicolinate (**23**).

To a solution of **7** (1.0 g, 2.9 mmol, 1 equiv.) in THF (25 mL) was added 1M NaOH (aq) (2.9 mL, 1 equiv.) and stirred at rt for 18 h. The reaction was then concentrated to give **23** as a white solid (1.0 g, 2.8 mmol, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.64 (s, 1H), 9.11 (d, J = 2.4 Hz, 1H), 8.21 (d, J = 2.3 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.6, 165.3, 155.0, 140.2, 140.1, 138.3, 131.7, 131.1, 127.1, 125.6, 125.1, 34.7, 30.9. HRMS (ESI-QTOF) m/z: $[M - Na]^-$ Calcd for $C_{17}H_{16}ClN_2O_3^-$ 331.0855, 333.0820; Found: 331.0846, 333.0818. HPLC Purity ≥ 96%.

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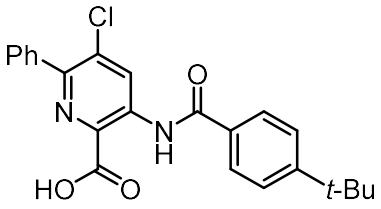
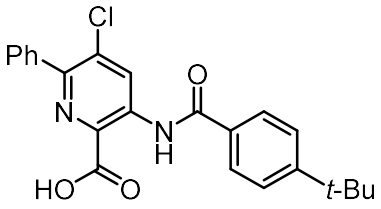
1H NMR (400 MHz, DMSO-d6)



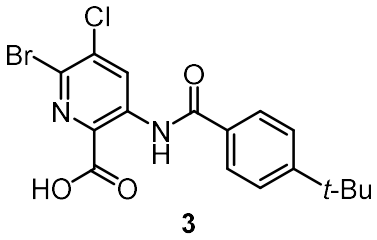
2

13C NMR (101 MHz, DMSO-d6)



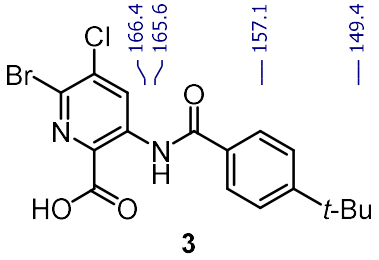
2

1H NMR (400 MHz, CDCl3)



3

13C NMR (126 MHz, CDCl3)



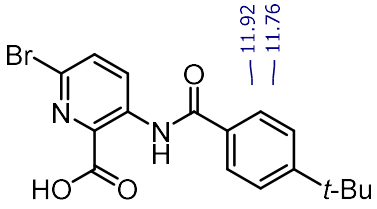
3

166.4
165.6
157.1
149.4
138.9
131.9
131.7
130.9
130.2
127.6
126.3
131.9
131.7
130.9
130.2
35.3
31.2
$CDCl_3$

133 132 131 130 129 128
f1 (ppm)

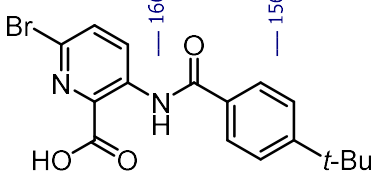
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

1H NMR (500 MHz, CDCl3)



4

13C NMR (126 MHz, CDCl3)

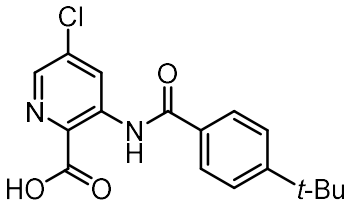


4

166.4
156.8
142.4
139.2
138.9
132.4
132.0
130.7
130.6
127.6
126.2
$CDCl_3$
77.2 $CDCl_3$
35.3
31.2

f1 (ppm)

1H NMR (500 MHz, CDCl3)



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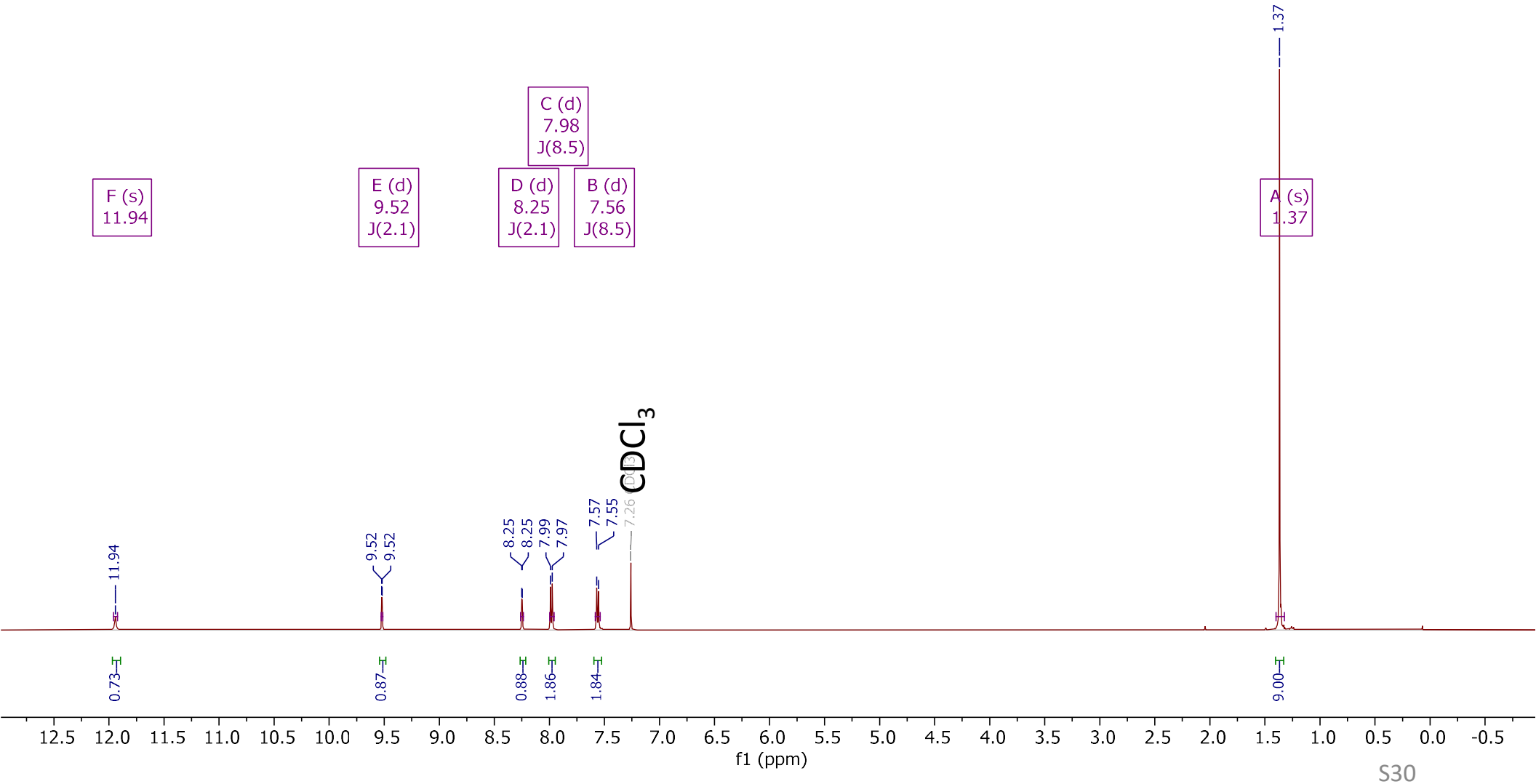
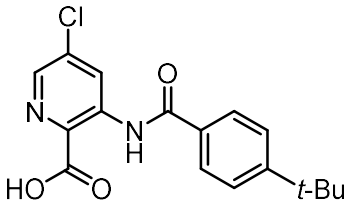


13C NMR (126 MHz, CDCl3)



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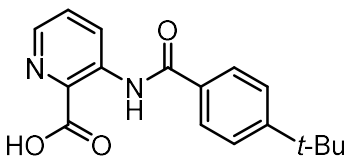
1H NMR (500 MHz, CDCl3)



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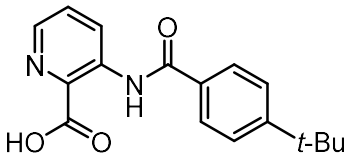
13C NMR (126 MHz, CDCl3)



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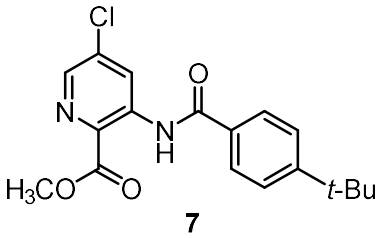
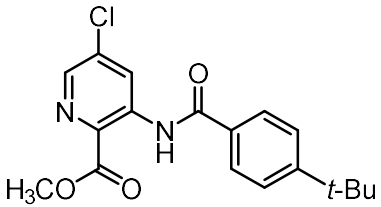
1H NMR (500 MHz, CDCl3)



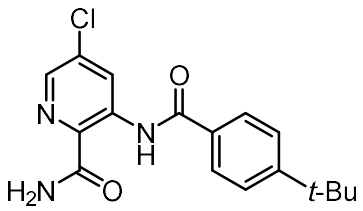
7

13C NMR (126 MHz, CDCl3)



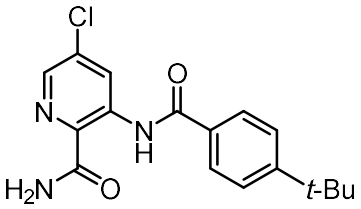
7

1H NMR (500 MHz, CDCl3)



8

13C NMR (126 MHz, CDCl3)



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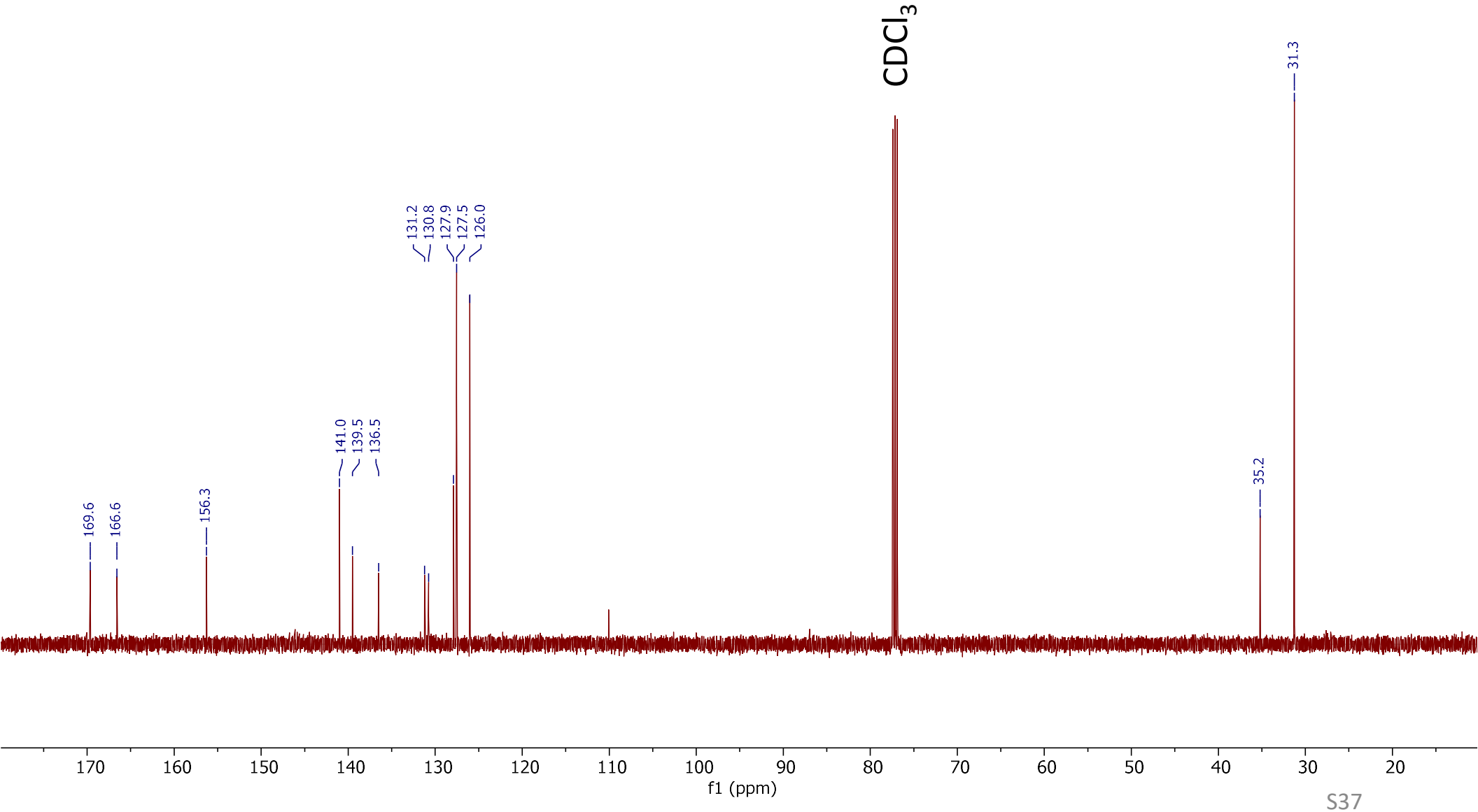

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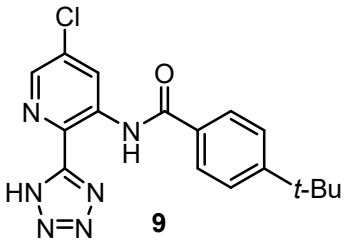
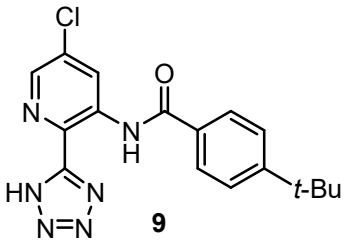


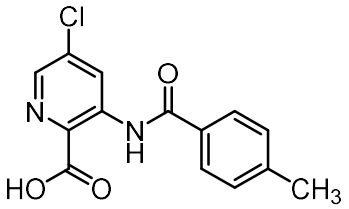
9

13C NMR (126 MHz, MeOD)



9

1H NMR (400 MHz, CDCl3)



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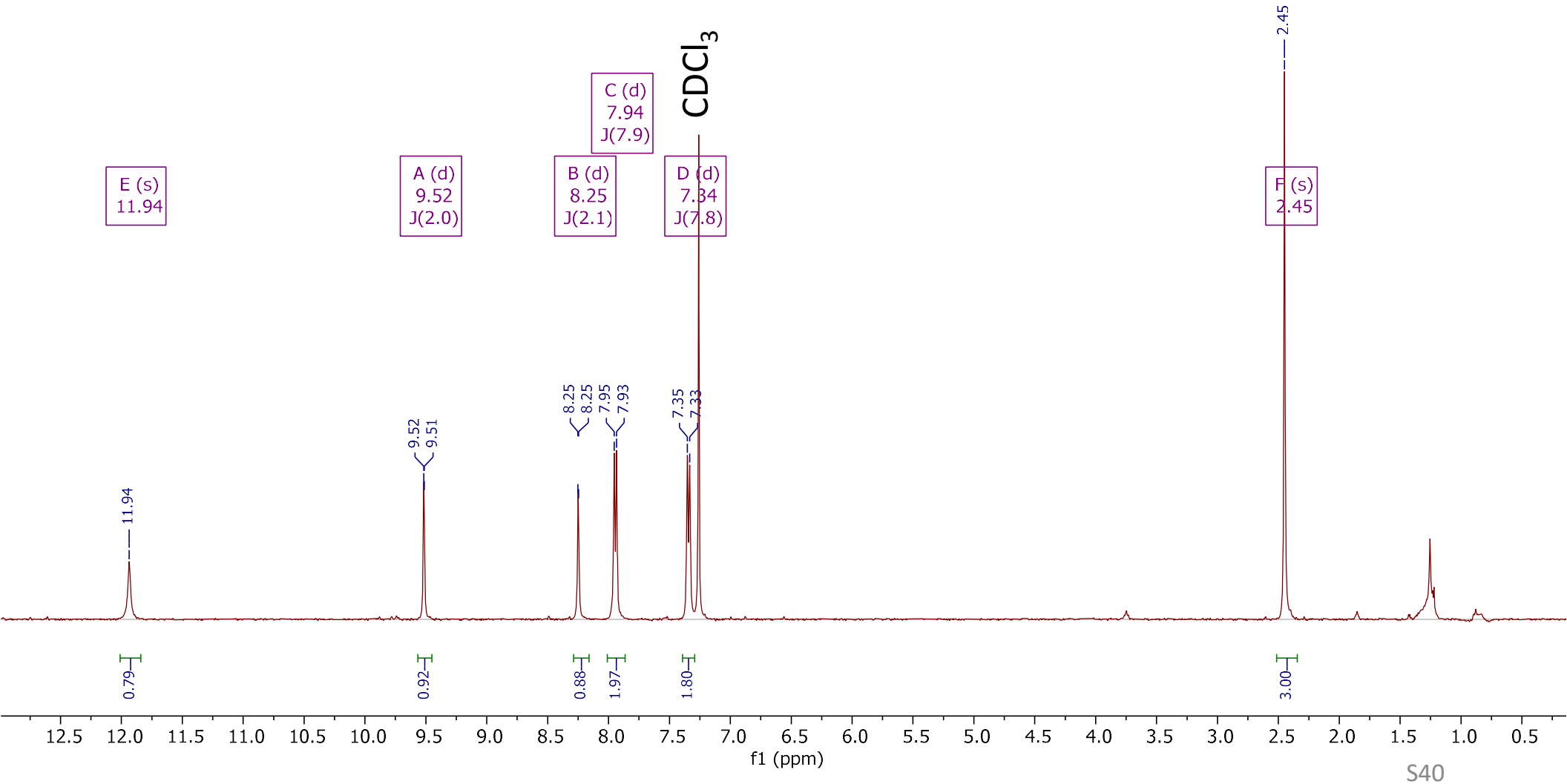
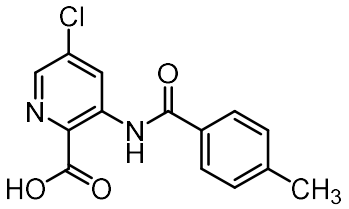


13C NMR (126 MHz, CDCl3)



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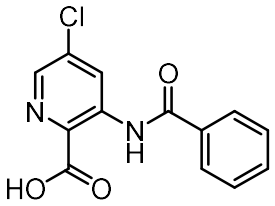
1H NMR (400 MHz, CDCl3)



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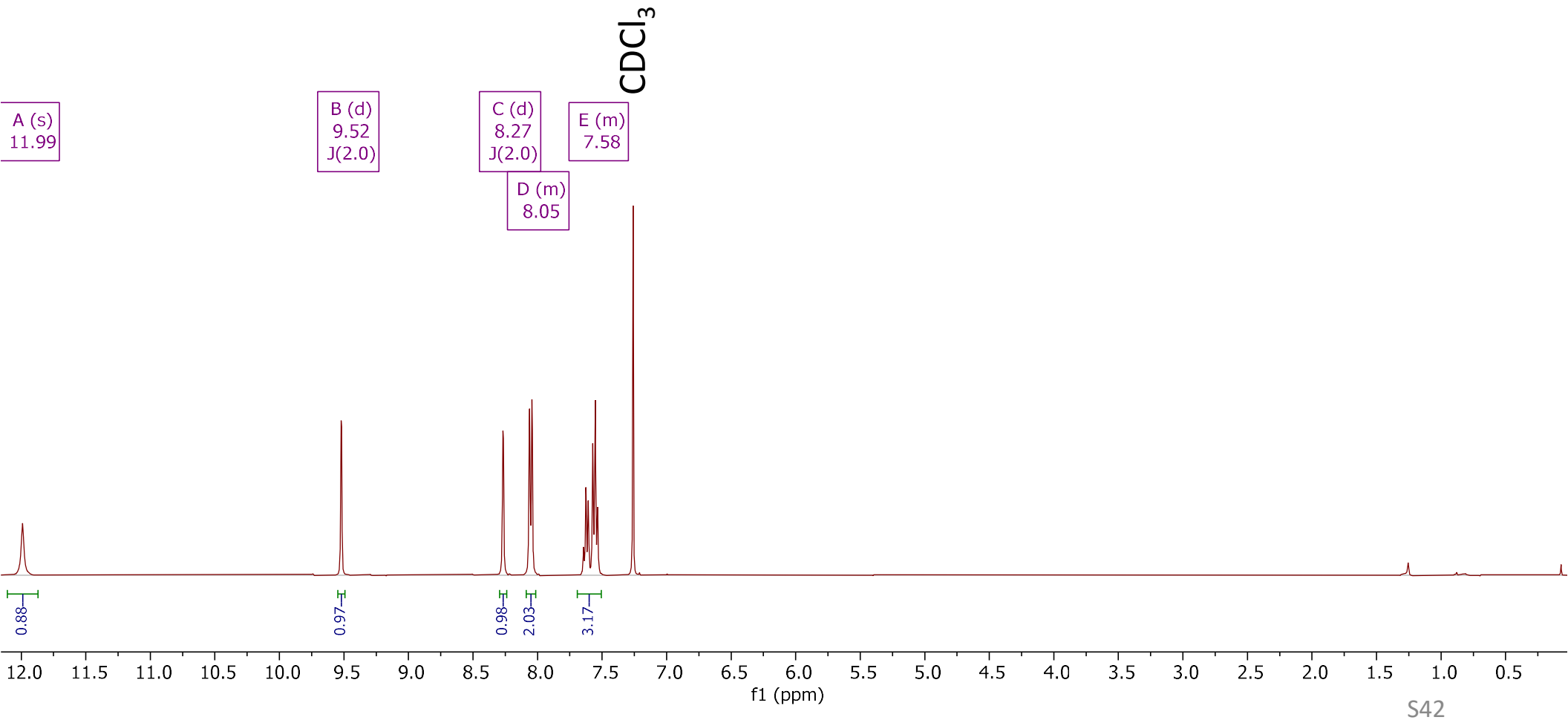
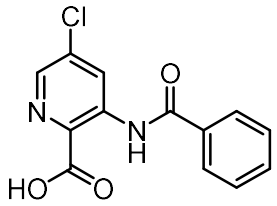


13C NMR (126 MHz, CDCl3)



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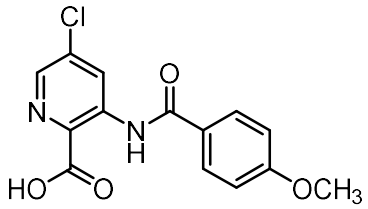
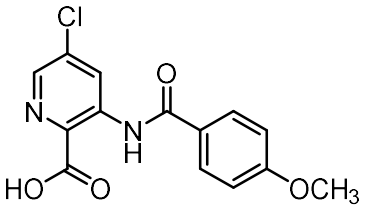
1H NMR (400 MHz, CDCl3)



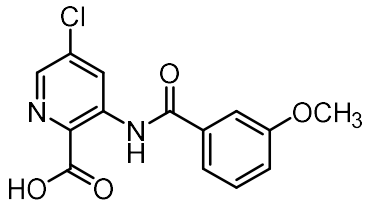
12

13C NMR (126 MHz, CDCl3)

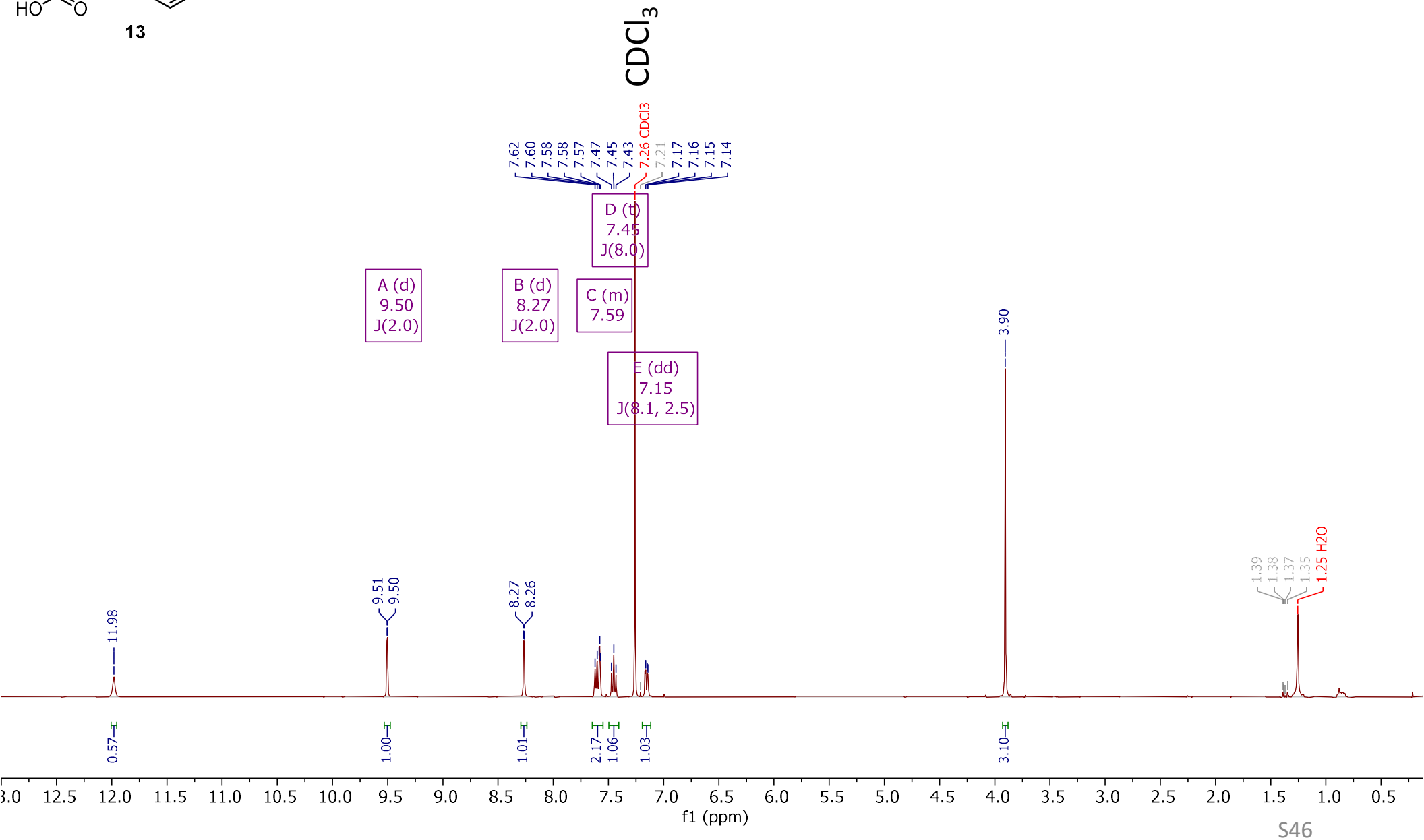


12

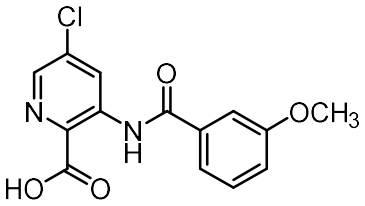
1H NMR (400 MHz, CDCl3)



13

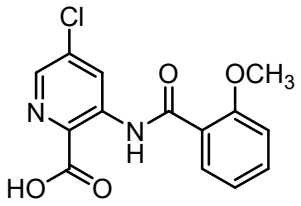


13C NMR (126 MHz, CDCl3)

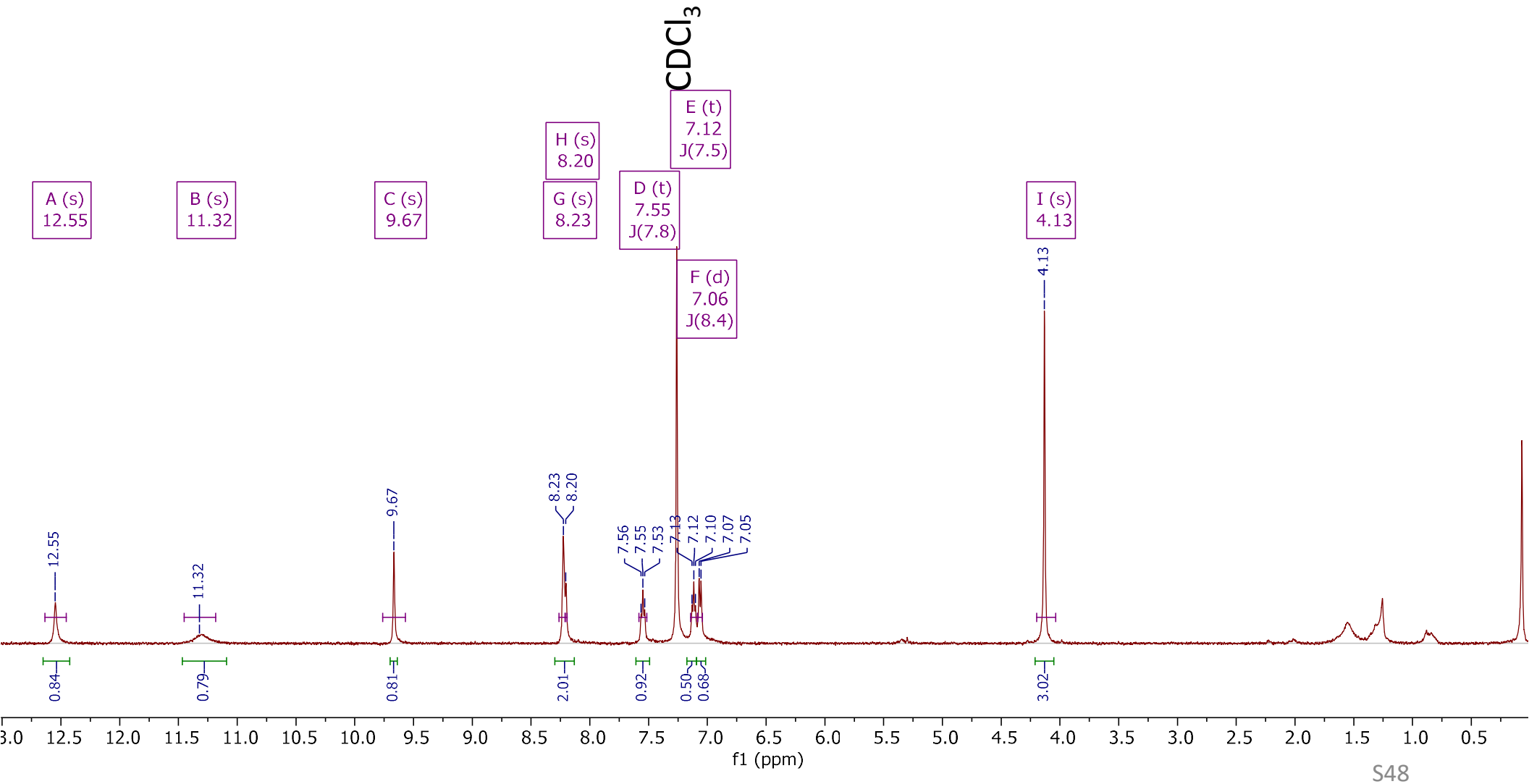


13

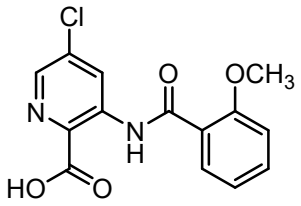
1H NMR (500 MHz, CDCl3)



14

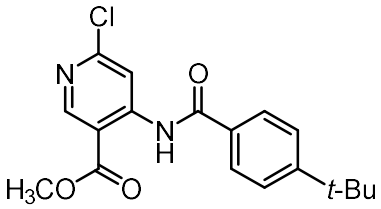


13C NMR (126 MHz, CDCl3)



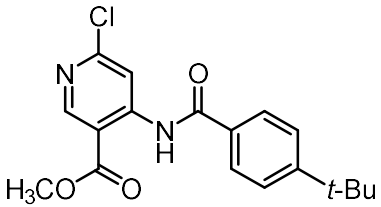
14

1H NMR (500 MHz, CDCl3)



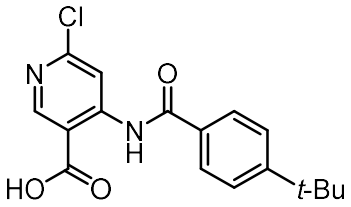
15

13C NMR (101 MHz, CDCl3)



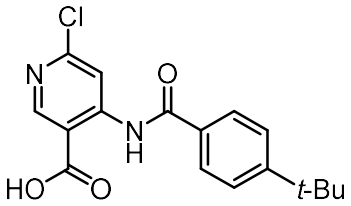
15

1H NMR (500 MHz, CDCl3)



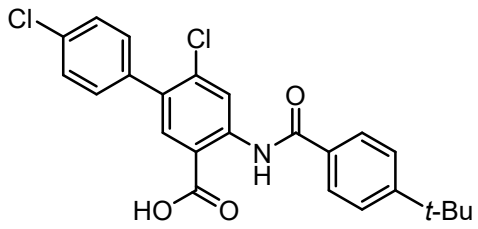
16

13C NMR (126 MHz, CDCl3)

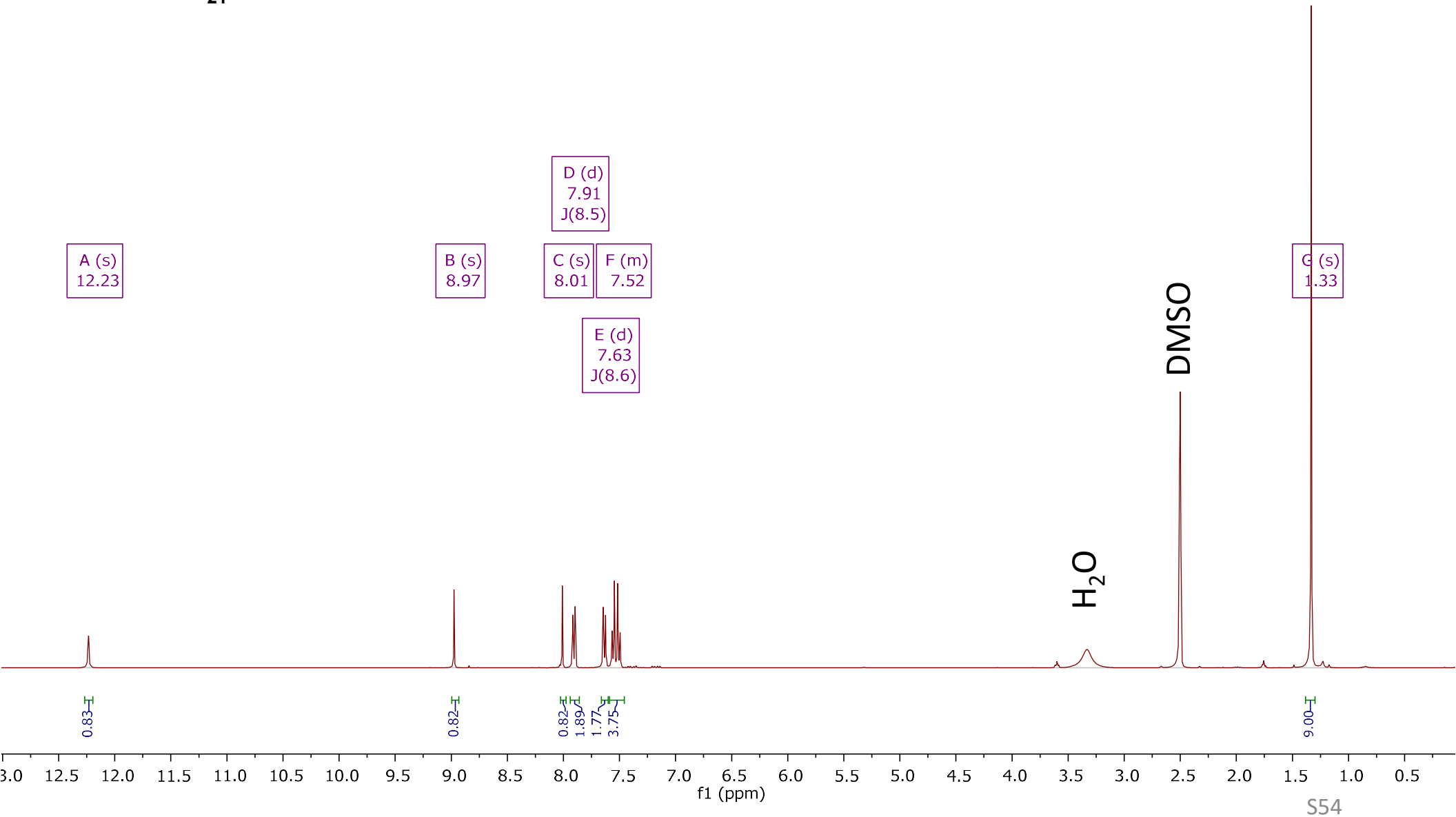


16

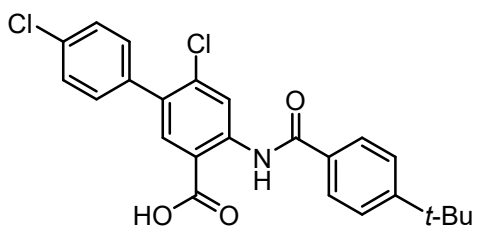
1H NMR (400 MHz, DMSO-d6)



21

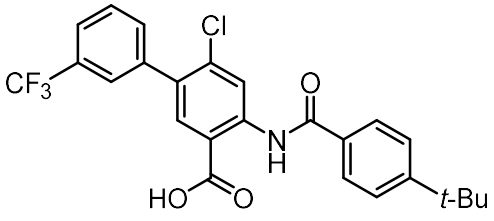


13C NMR (101 MHz, DMSO-d6)



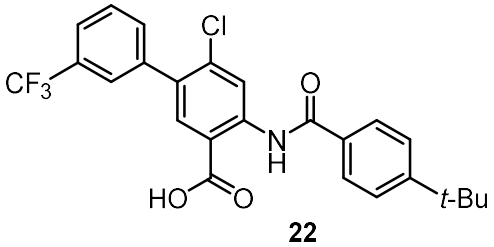
21

1H NMR (400 MHz, Acetone-d6)



22

13C NMR (101 MHz, Acetone-d6)



22

G (d) 124.3 J(271.6)

C (d) 130.1 J(32.2)

F (d) 120.6 J(5.3)

B (d) 131.6 J(5.1)

E (q) 124.6 J(4.1)

D (q) 126.1 J(3.9)

Acetone

30.5

34.7

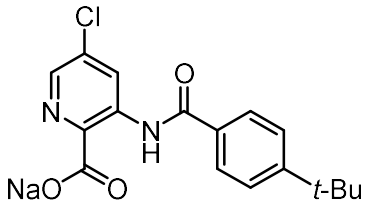
133.9 133.4 132.7 131.7 131.6 130.3 129.9 129.3 127.2 126.1 126.1 126.0 126.0 125.9 125.7 124.7 124.7 124.6 124.6 123.0 120.7 120.6

165.1 165.0 155.9 142.6 142.4 139.2 137.9

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

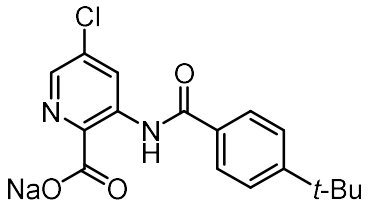
f1 (ppm)

1H NMR (400 MHz, DMSO-d6)



23

13C NMR (126 MHz, DMSO-d6)



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