Supporting Information:

Modulating carnitine levels by targeting its biosynthesis pathway – selective inhibition of γ-butyrobetaine hydroxylase

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Contents

Carnitine biosynthesis pathway

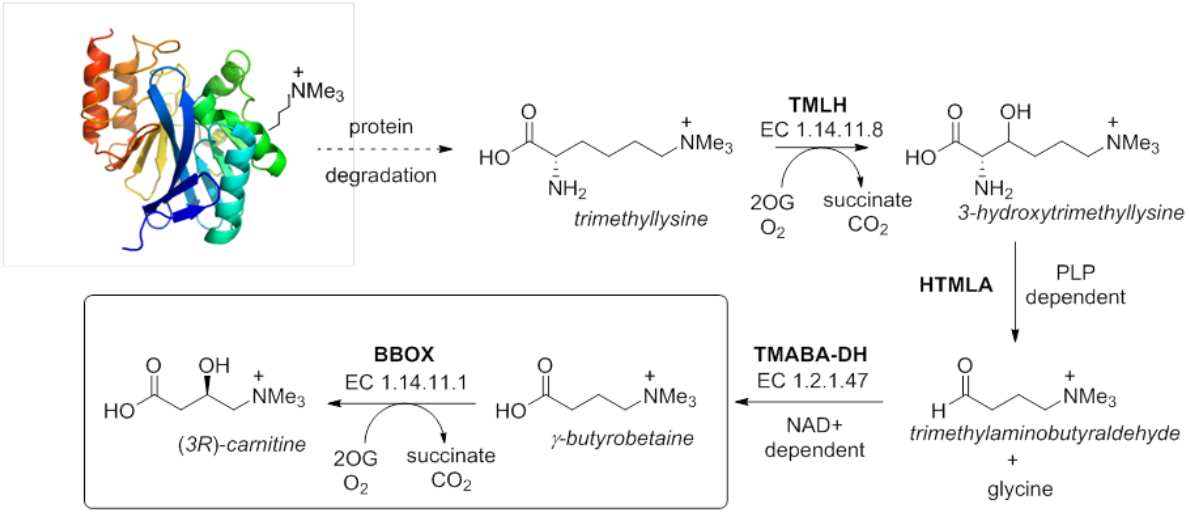


Figure S1 Carnitine biosynthesis as defined in humans. The final step comprising the stereospecific hydroxylation of γ-butyrobetaine (GBB) to give carnitine is catalysed by γ-butyrobetaine hydroxylase (BBOX).

Structures of carnitine, γ – butyrobetaine and mildronate

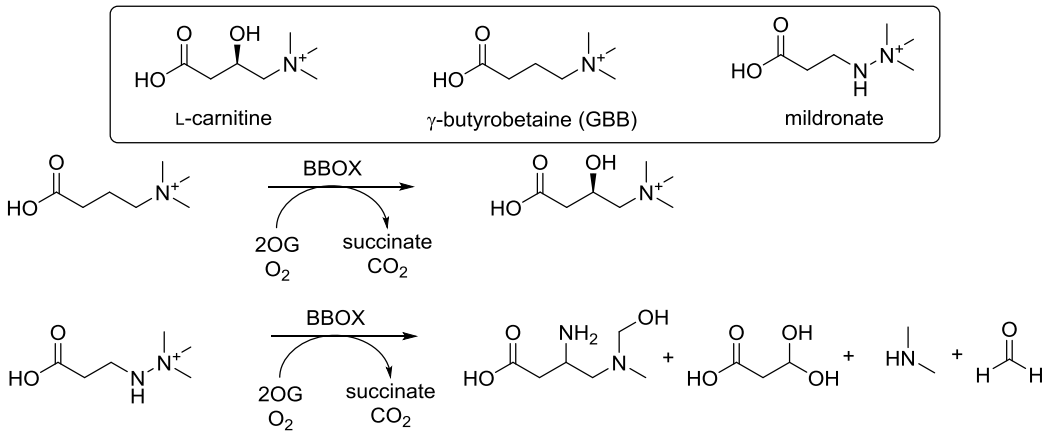


Figure S2 Structures of carnitine, γ-butyrobetaine and mildronate. Mildronate is a close structural mimic of γ-butyrobetaine (GBB) and carnitine. Both GBB and Mildronate are BBOX substrates. In the case of Mildronate multiple products of BBOX catalysis are observed.[2]

Fluoride release assay

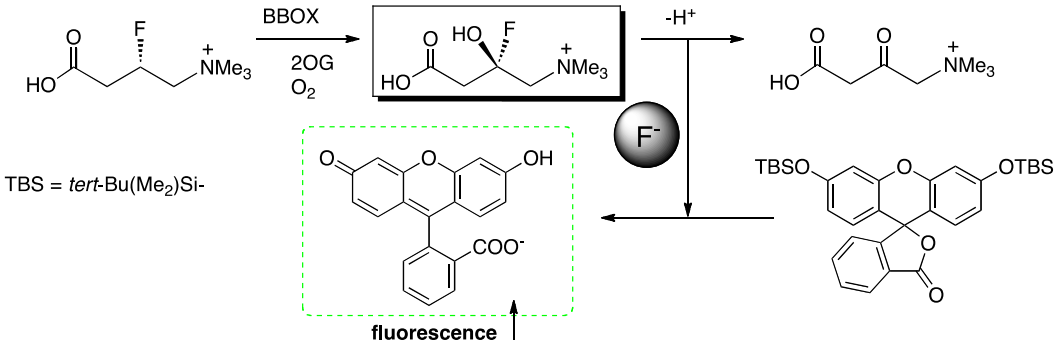


Figure S3 Assay based on BBOX catalysed hydroxylation of a fluorinated GBB analogue with subsequent fluoride ion release. Fluoride is detected using a *tert*-butyldimethylsilyl (TBS) protected fluorescein chemical probe.[1]

The fluorinated GBB analogue (GBBF) and TBS-protected fluorescein probe were synthesized as described[1]. Other reagents were from Sigma-Aldrich. Reactions were performed in a final volume of 10 µl (black 384-well plate, clear, flat bottom, Grenier BioOne). The following final concentrations of reagents were used: 40 µM GBBF (fluorinated analogue of GBB), 500 µM 2OG (disodium salt, Sigma-Aldrich), 250 µM ascorbate (potassium salt, Sigma-Aldrich), 160 mM KCl, 40 µM Fe (II) ($Fe(NH_4)_2(SO_4)_2$ salt, prepared fresh as a 100 mM solution in 20 mM HCl and added to reaction mixture just before start of the assay), BBOX 200 nM. Reactions were performed in 50 mM TRIS buffer pH 7.0, initiated by addition of BBOX to the mixture containing all cofactors and inhibitor and quenched after 10 min by addition of TBS-fluorescein probe in DMSO (40 µl, to a final concentration of 5 µM). The plate was then sealed and incubated for 60 min at room temperature prior to addition of 10 µl of 50 mM HEPES buffer pH 7.0. The resultant signal was read up to 5 min after addition of HEPES buffer using an EnVision Multilabel plate reader (Perkin Elmer) fitted with FITC FP 480/30 (480 nm, bandwidth 30 nm) and FITC FP 535/40 emission (535 nm, bandwidth 40 nm) filters. For each reaction, controls containing all reagents but without BBOX or without inhibitor were recorded. The normalised fluorescence signal was defined as the observed fluorescence signal minus the control signal. Percentage activity was calculated as a ratio of normalised signal for reaction containing inhibitor to reaction containing no inhibitor. Errors were calculated as standard deviations from four separate measurements. IC_{50} data were fitted using XLfit software (IDBS Solutions) using 4-parameter logistic model (sigmoidal dose-response with variable slope).

SAR studies – varying side chain in the lead scaffold

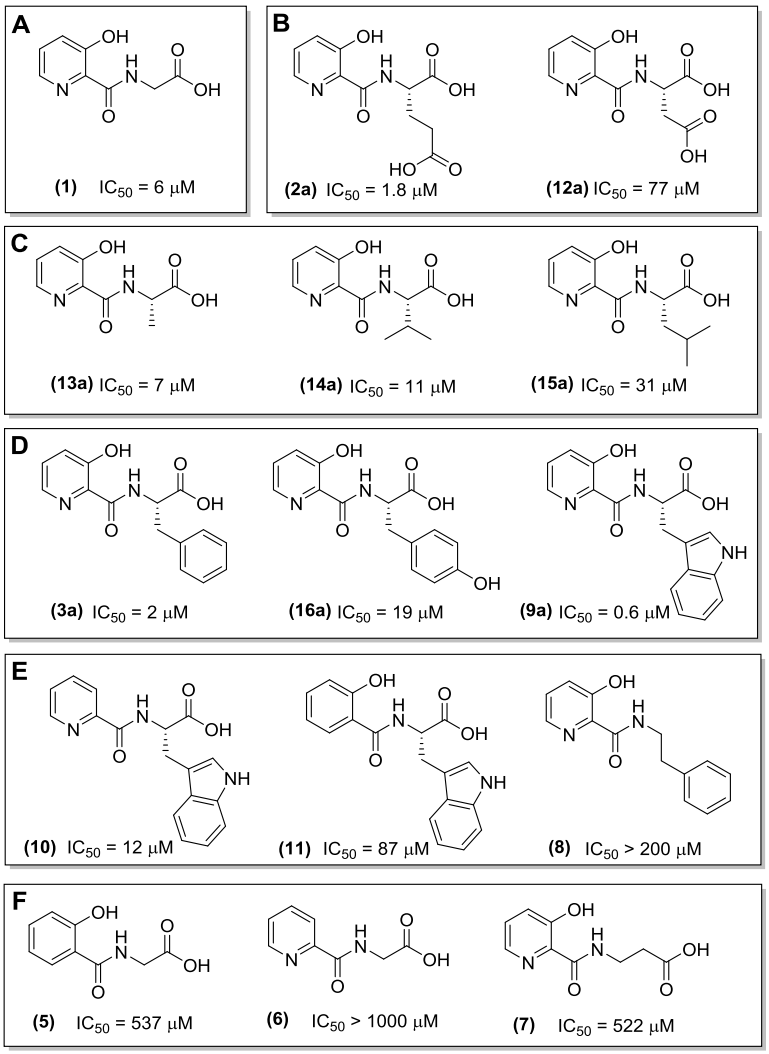


Figure S4 Effects of structural variations on inhibitor activity. A – Template inhibitor structure. B – The presence of a carboxylate side chain can improve potency, depending on the chain length. C – The tested alkyl side chains do not improve potency (**13a** and **14a**) and in case of the isobutyl side chain (**15a**) potency is lower than the template compound. D – Aromatic side chains can improve potency (**3a** and **9a**) or lead to decrease in activity (**16a**). E – Effects of the pyridine nitrogen, phenolic hydroxyl, and carboxylate on activity of compounds with aromatic side chain. F - Effects of pyridine nitrogen, phenolic hydroxyl and carboxylate on activity of the template compound.

SAR studies – side chains with (R) stereochemistry

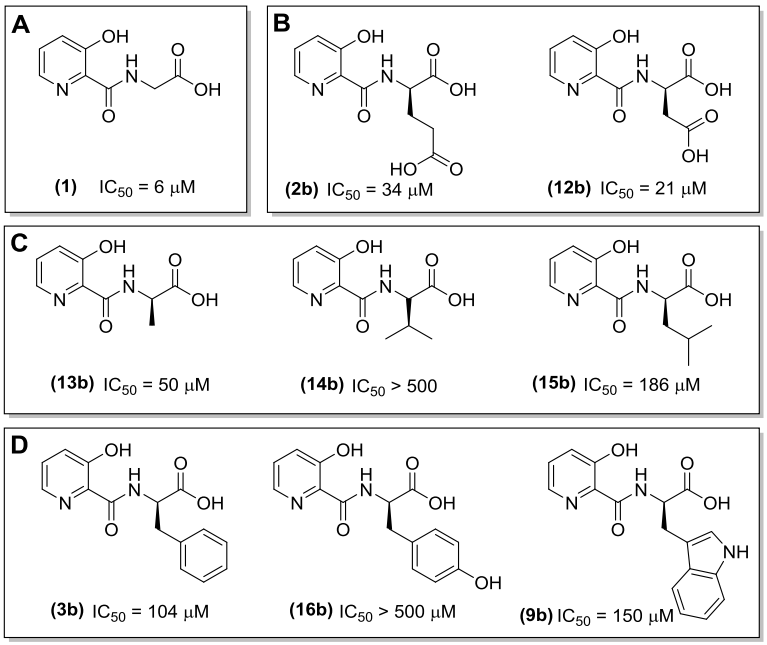


Figure S5 The activity of analogues with the (*R*)-stereocentre at C-α is lower than the (*S*) side chain enantiomers. Analogues with small (**13b**) or flexible (**2b**) side chain retain some activity.

Possible binding modes of 3 – hydroxyl pyridine scaffold

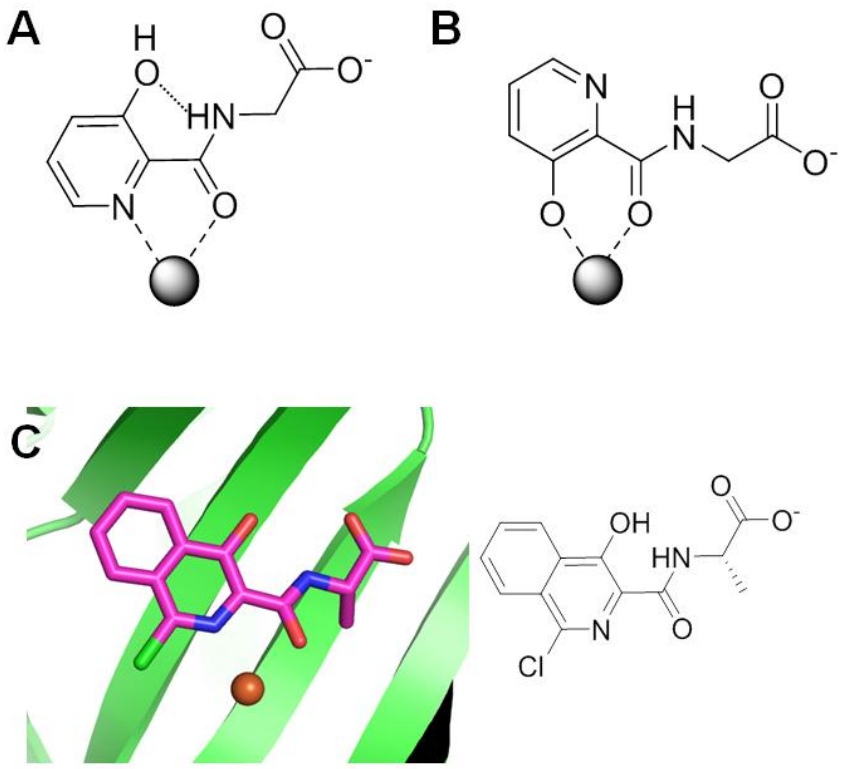


Figure S6 Possible binding modes of the 3-hydroxypyridine scaffold. C – Binding mode A was modelled based on a crystal structure of a related 2OG dependent Hypoxia-Inducible Factor Prolyl Hydroxylase 2 (PHD2/ EGLN1) in a complex with N-[(1-chloro-4-hydroxyisoquinolin-3-yl)carbonyl]alanine (PDB code: 4BQY).[3]

SAR – quinoline and isoquinoline series

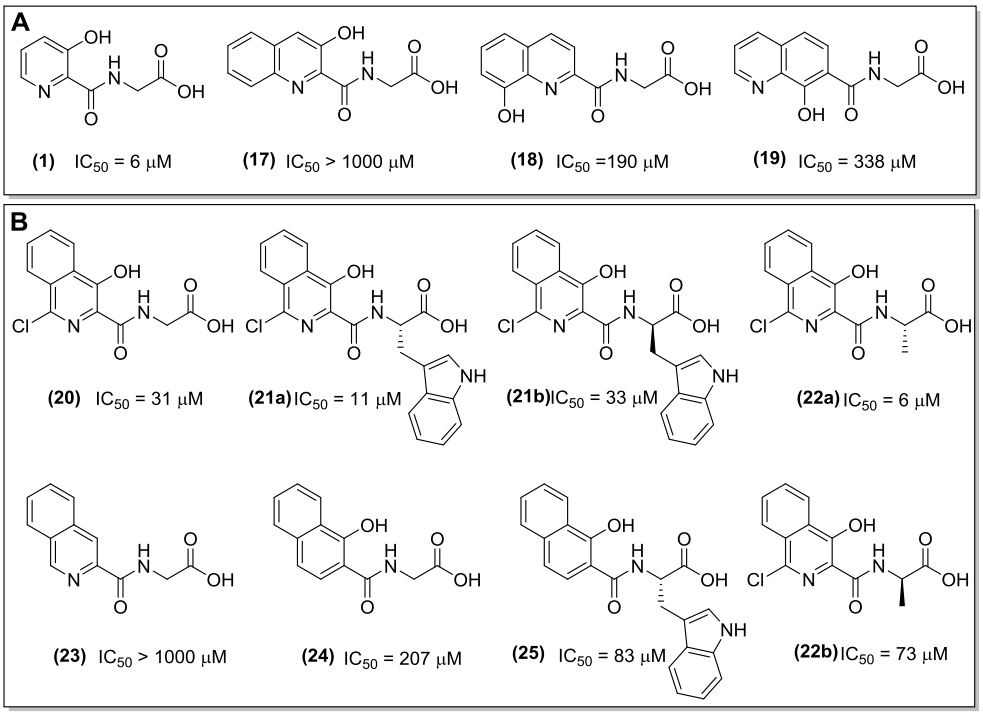
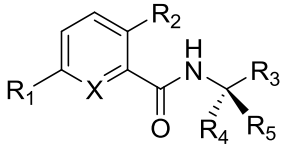


Figure S7 Effect of an additional aromatic ring on inhibitor potency. A – Analogues from the quinoline series were less potent than their pyridine analogues. B – Isoquinoline series analogues display relatively good inhibition with IC_{50} values in low micromolar region. In isoquinolines, as in the pyridines, removal of the pyridine-nitrogen (**24**) or hydroxyl (**23**) group leads to loss of potency. Unlike the pyridinyl-series, small side chains (**22a**) are preferred; however the (*S*)-stereochemistry was still favoured (**21a**, **22a**). Analogues with bulky aromatic side chains attached were less potent inhibitors and the difference between potency of (*S*)-analogue (**22a**) and (*R*)-analogue (**22b**) was less profound as in the case of methyl substituent.

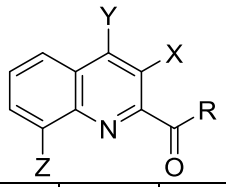
Summary of inhibition data

Table **S1A** Inhibition of BBOX by pyridine series

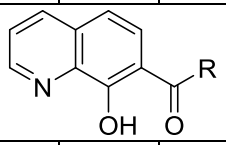


code	X	R_1	R_2	R_3	R_4	R_5	IC_{50} [µM]
1	N	H	OH	COOH	H	H	6.2±2.7
2a	N	H	OH	COOH	$(CH_2)_2COOH$	H	1.8±0.26
2b	N	H	OH	COOH	H	$(CH_2)_2COOH$	34.0±2.9
3a	N	H	OH	COOH	CH_2Ph	H	2.0±0.2
3b	N	H	OH	COOH	H	CH_2Ph	104±21
9a	N	H	OH	COOH	CH_2-(3-indole)	H	0.52±0.09
9b	N	H	OH	COOH	H	CH_2-(3-indole)	150±16
12a	N	H	OH	COOH	CH_2COOH	H	76.6±10.3
12b	N	H	OH	COOH	H	CH_2COOH	21.2±8.9
13a	N	H	OH	COOH	CH_3	H	7.4±1.4
13b	N	H	OH	COOH	H	CH_3	49.5±9.7
14a	N	H	OH	COOH	$CH(CH_3)_2$	H	10.7±1.5
14b	N	H	OH	COOH	H	$CH(CH_3)_2$	754±63
15a	N	H	OH	COOH	$CH_2CH(CH_3)_2$	H	31.0±9.3
15b	N	H	OH	COOH	H	$CH_2CH(CH_3)_2$	186±20
16a	N	H	OH	COOH	CH_2Ph(4-OH)	H	19.2±4.4
16b	N	H	OH	COOH	H	CH_2Ph(4-OH)	>1000
27	N	H	OH	COOH	CH_2-(4-imidazole)	H	126±10
5	H	H	OH	COOH	H	H	536.8±110.2
6	N	H	H	COOH	H	H	>1000
8	N	H	OH	H	CH_2Ph	H	>1000
10	N	H	H	COOH	CH_2-(3-indole)	H	12.5±3.1
11	H	H	OH	COOH	CH_2-(3-indole)	H	87.6±27.3
7	N	H	OH	CH_2COOH	H	H	522±61
35 (AR693B)	N	H	OH	COOH	CH_2SCH_2-(4-pyridin)	H	0.72±0.19
26	N	Cl	Cl	COOH	CH_2-(3-indole)	H	159±61
4 (AR692B)	N	H	OH	COOH	CH_2SCH_2-(2-pyridin)	H	0.47±0.06
36 (AR780)	N	H	OH	$COO(CH_2)_7CH_3$	CH_2SCH_2-(2-pyridin)	H	>1000
42 (AR692)	N	H	OH	$COOCH_3$	CH_2SCH_2-(2-pyridin)	H	>1000

Table **S1B** Inhibition of BBOX by quinoline series

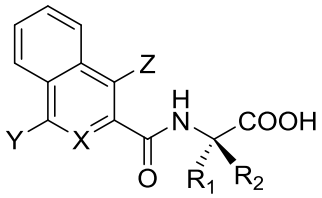


code	X	Y	Z	R	IC_{50} [μM]
17	OH	H	H	$NHCH_2COOH$	>1000
18	H	H	OH	$NHCH_2COOH$	190±15
28	OH	H	H	OH	152±40
37	H	H	OH	OH	15.8±1.4
39	H	OH	H	OH	>1000
40	OH	OH	H	OH	179.2±18



19	-	-	-	$NHCH_2COOH$	338±42
38	-	-	-	OH	149±12

Table **S1C** Inhibition of BBOX by isoquinoline series



code	X	Y	Z	R_1	R_2	IC_{50} [μM]
20	N	Cl	OH	H	H	30.2±6.4
21a	N	Cl	OH	CH_2-3-indole	H	11.4±0.84
21b	N	Cl	OH	H	CH_2-3-indole	33.4±4.5
22a	N	Cl	OH	CH_3	H	5.8±0.77
22b	N	Cl	OH	H	CH_3	72.6±11.6
29a	N	Cl	OH	$CH(CH_3)_2$	H	99.6±22.0
29b	N	Cl	OH	H	$CH(CH_3)_2$	115±39
30a	N	Cl	OH	$CH_2CH(CH_3)_2$	H	89.2±14.5
30b	N	Cl	OH	H	$CH_2CH(CH_3)_2$	49.1±7.9
31a	N	Cl	OH	CH_2Ph	H	13.6±0.77
31b	N	Cl	OH	H	CH_2Ph	62.6±11.6
32a	N	Cl	OH	$(CH_2)_2COOH$	H	418±96
32b	N	Cl	OH	H	$(CH_2)_2COOH$	380±35
33a	N	Cl	OH	CH_2COOH	H	459±82
33b	N	Cl	OH	H	CH_2COOH	628±102
23	N	H	H	H	H	>1000
24	H	H	OH	H	H	207±12
25	H	H	OH	CH_2-3-indole	H	83±24

As a negative control DMSO was used, positive control was mildronate, which under screening conditions had IC_{50} of 105±13 μM. All values were measured by fluoride release assay according to the procedure on page S4.

Characterization of the inhibitory properties of AR692B

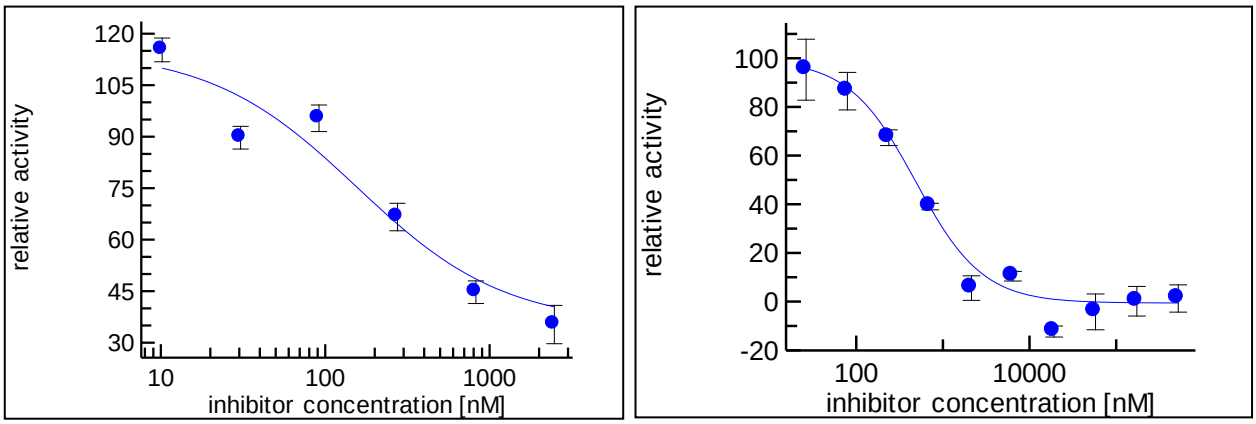


Figure S8 Dose – response curve for inhibition of BBOX by AR692B as measured by ^{1}H NMR assay (left) and the fluoride release assay (right). The fluoride release assay uses a fluorinated substrate analogue for BBOX (Fig. S3), which has a slightly higher K_M value than GBB (20 µM vs. 4 µM) and also leads to certain level of uncoupling between 2OG decarboxylation and substrate hydroxylation[1], so that higher quantities of 2OG are used (assays were performed at 500 µM 2OG concentration). Therefore, we also examined the kinetic properties of AR692B using an assay employing ^{1}H NMR, in which the native substrate (GBB) and 2OG at its K_M value[1] (150 µM) were used. With this assay we obtained an IC_{50} value of 153 nM.

IC_{50} measured with the NMR assay

IC_{50} = 153.2±46.5 nM; Fitted with 4 Parameter Logistic Model or Sigmoidal Dose-Response Mode (XLfit, IDBS Solutions), where A = 0.36, B = 1.16, C = IC_{50}, D= -0.96.

NMR assays conditions:

40 µM GBB, 150 µM 2OG, 500 µM ascorbate, 200 mM KCl, 50 µM Fe (II), BBOX 50 nM, TRIS d-11 50 mM pH 7.5, 10% D_2O, 0.01% NaN_3, final volume 160 µl. Error calculated as standard deviation from data derived from 8 different time points per reaction. NMR assay was performed on Bruker AVIII 700 with inverse TCI cryoprobe. All experiments were conducted at 298 K. MATCH NMR tubes (3 mm diameter, Bruker) were used in all experiments. The pulse tip-angle calibration using the single-pulse nutation method84 (Bruker pulsecal routine) was undertaken for each sample.

IC_{50} measured with the fluoride release assay

IC_{50} = 0.47±0.06 µM; Fitted with 4 Parameter Logistic Model or Sigmoidal Dose-Response Mode (XLfit, IDBS Solutions); where A = 0, B = 100, C = IC_{50}, D= -1.12.

Substrate competition experiments

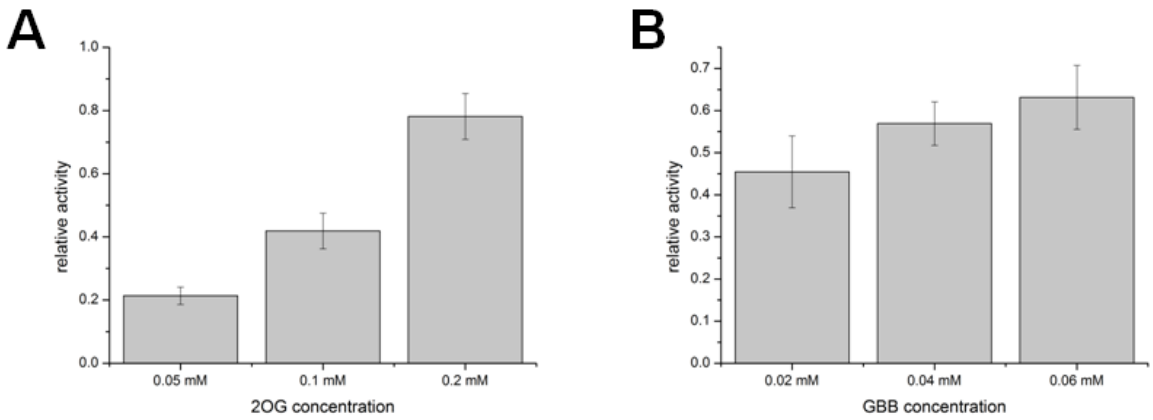


Figure S9 BBOX inhibition by AR692B is dependent on both 2OG and GBB concentrations. A – Increasing the 2OG concentration leads to an increase in BBOX activity (at constant inhibitor concentration). B – Increasing the GBB concentration leads to an increase in BBOX activity (at constant inhibitor concentration) Experiments were performed employing the NMR assay as described for IC_{50} measurements. Values are given as the ratio of carnitine formation in the reaction containing inhibitor to control reaction with no inhibitor added. The following conditions were used: 50 µM GBB (for 2OG competition experiment), 150 µM 2OG (for GBB competition experiment), 500 µM ascorbate, 200 mM KCl, 50 µM Fe (II), 400 nM AR692B, 50 nM BBOX, 50 mM TRIS d_{11} pH 7.5, 10% D_2O, 0.01% NaN_3.

BBOX fluorescence based binding assay

Binding assay was developed basing on BBOX intrinsic fluorescence quenching observed upon titration with 3-hydroxypyridine based inhibitors (Fig. S19). Method was optimised for use in 96-well plate format (Greiner). Fluorescence was measured using Pherastar FS plate reader (BMG Labtech) using following parameters: excitation 280 nm, emission 350 nm, 5 µM BBOX, 20 µM Fe(II) and variable concentration of tested compound. Assays were performed in 50 mM Tris buffer pH 7.5 containing 200 mM NaCl in total volume of 50 µL per well. The observed fluorescence signal was defined as measured fluorescence of protein-ligand complex minus signal of compound (Fig. S20).

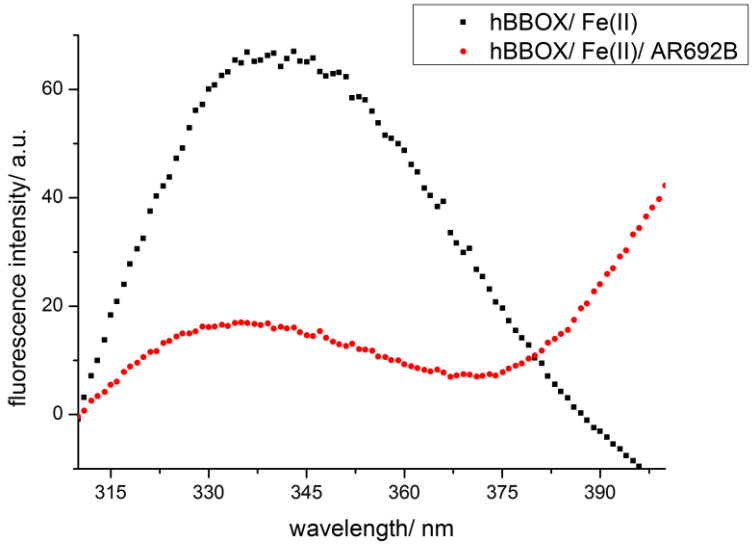


Figure S10 Fluorescence measurements. Fluorescence emission spectra of the BBOX/ Fe(II) complex compared to BBOX/ Fe(II)/ AR692B complex. Excitation 270 nm, emission 310-400 nm. Spectra were measured using 10 µM BBOX, 20 µM Fe(II) and 20 µM AR692B.

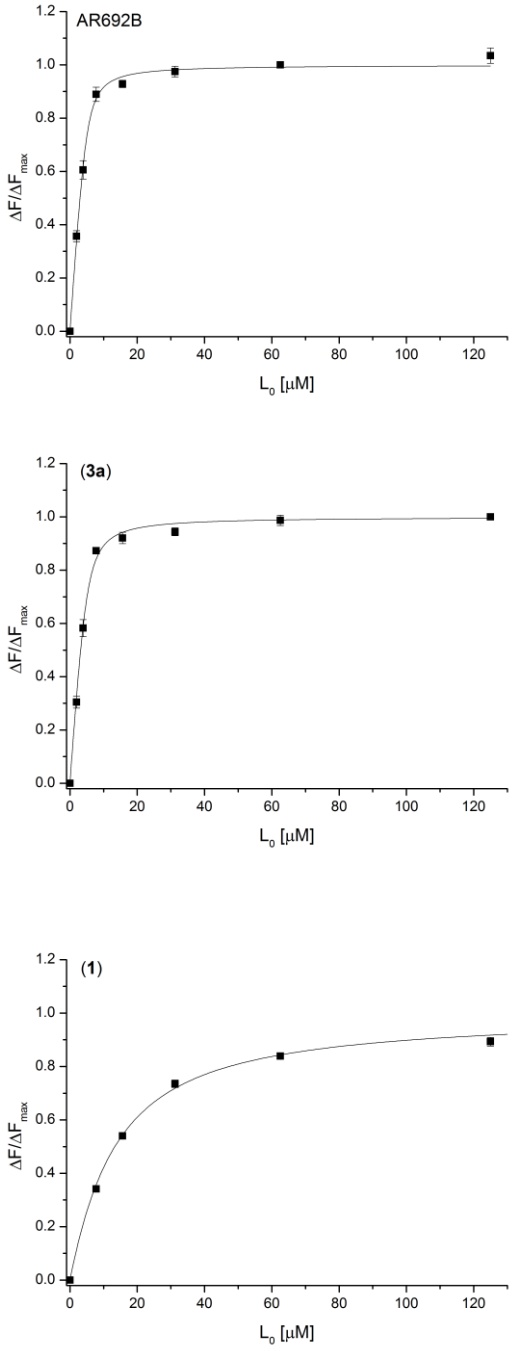


Figure S11 Binding curves of AR692B and related analogues to BBOX. BBOX (5 μM) was mixed with Fe(II) (40 μM) in 50 mM Tris buffer pH 7.5 and titrated with increasing concentration of inhibitor. Concentration of inhibitor was plotted against $\Delta F_{obs}/\Delta F_{max}$, where ΔF_{obs} is the observed decrease in fluorescence signal and ΔF_{max} is maximal measured decrease in fluorescence signal (decrease in fluorescence signal when the saturation was reached compared to initial fluorescence measurement for BBOX alone). Data were fitted with equation: $y = (([L_0]+[P_0]+K_D)-(([L_0]+[P_0]+K_D)^2-4[L_0][P_0])^{0.5})/(2[P_0])$, where $[L_0]$ is total concentration of ligand and $[P_0]$ is total concentration of the protein (OriginPro 8.5.1). Obtained K_D values were as follows: 0.50±0.07 μM (AR692B), 1.3±0.3 μM (**3a**) and 10.8±0.8 μM (**1**). Measurements were done in triplicates, error bars represent standard deviation.

Selectivity screen

Table S2: Inhibition of human 2OG dependent oxygenases by AR692B

Enzyme	Other nomenclature	Function	substrate	% inhibition at 100uM	±STDEV
JMJD2A	KDM4A	H3K9/36 demethylase	H3K9me3 a.a.7-14	9.4	7.0
PHF8	KDM7B	H3K9/27me2/1 demethylase	H3K9me2 a.a.1-28	13.9	1.7
FBXL11	KDM2A	H3K36me2/1 demethylase	H3K36me2 a.a. 31-43	0.00	0.01
JMJD1A	KDM3A	H3K9me2/1 demethylase	H3K9me2 a.a 1-28	-0.02	0.01
JMJD3	KDM6B	H3K27 demethylase	H3K27me3 a.a. 14-35	26.3	3.0
PHD2	EGLN1	HIF hydroxylase	CODD HIF1a 19-mer	72.5	5.0
FIH	HIF1AN	HIF hydroxylase	CADD HIF2a 36-mer	5.0	1.6

The enzyme activities of JMJD2A, JMJD1A, PHF8, FBXL11, JMJD3, FIH and PHD2 were measured by relative demethylation/hydroxylation of known substrate peptides using Matrix-assisted laser-desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS), Waters. The reaction mixture composition varied as optimised for each reaction (JMJD2A, FBXL11 – 1 µM enzyme, 10 µM peptide, 50 mM HEPES pH 7.5, 200 µM 2-oxyglutarate (disodium salt, Sigma), 10 µM $FeSO_4 \times 7$ H_2O, 100 µM sodium ascorbate (Sigma); JMJD1A, PHF8, JMJD3 with additional 150 mM NaCl; FIH and PHD2, in TRIS pH 7.5, 100mM NaCl). Enzyme, sodium ascorbate and Fe (II) were incubated with 100 µM AR692B for 15min before adding peptide and 2-oxoglutarate mix. Reaction was incubated for 20min at 25^{o}C, quenched with MeOH and spotted using α-Cyano-4-hydroxycinnamic acid (CHCA) matrix. Inhibition was calculated relative to the activity of a null-inhibitor control.

The positive control inhibitors used were IOX1 (5-Carboxy-8-Hydroxyquinoline), IC_{50} 1.7 µM (JMJD2A)[4], 12.6 µM (PHF8)[5], 14.3 µM (PHD2)[4], 20.5 µM (FIH)[4]; 2,4-PDCA (2,4-pyridine dicarboxylic acid) IC_{50} 4.1 µM (FBXL11)[5], 8 µM (JMJD1A)[5], 33 µM (JMJD3)[5]. The screening method and conditions were as reported[4-6].

Inhibition of PHD2 by AR692B

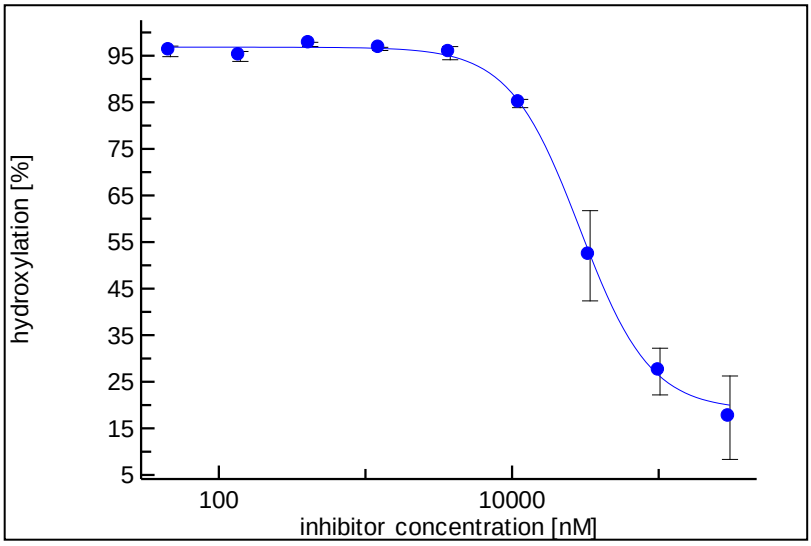


Figure S12 Dose – response curve for inhibition of PHD2 by AR692B as measured by the MALDI based assay.

IC_{50} = 28.4±8.0 µM; fitted with 4 Parameter Logistic Model or Sigmoidal Dose-Response Mode (XLfit, IDBS Solutions)fit = (A+((B-A)/(1+((C/x)^D)))), inv = (C/((((B-A)/(y-A))-1)^(1/D))), res = (y-fit), where A = 19.2, B = 77.9, C = IC_{50}, D= -1.81

Cell studies

Cell culture

HEK 293T cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Lonza/ BioWhittaker, supplemented with 4.5 g/L glucose) with a Penicillin/ Streptomycin antibiotic mixture (Sigma, 0.1 mg/mL Streptomycin and 100 units/mL Penicillin), 2 mM GlutaMAX (Gibco) and 10% FBS (PAA) in 10 cm sterile Petri dishes. When cells reached confluence, the media was removed and cells washed twice with PBS (Lonza). The pellet was resuspended in 6 mL of PBS and split into 6 new Petri dishes (1 mL of cell suspension each ($\sim 3\times10^6$)) with 10 mL of media containing desired additives. Cells were grown for 48h (37°C, 5% CO_2). Media was discarded and cells washed 3 times with 5 mL of PBS, cells were scraped and centrifuged for 5 min at 1000 rpm. The supernatant was removed and the pellet was frozen at -80°C. Before measurement the pellet was incubated on ice with 100 mL of PBS for 20 min. Cells were re-suspended in PBS by vortexing and lysed in sonication water bath (5 cycles of 1sec sonication-1 sec break) and spun down at 14800 rpm for 10 min. Supernatants were collected and transferred to MS vials for measurement.

MS method for cell analyses

Chromatographic separation of the carnitine biosynthesis metabolites was performed using mixed mode chromatography.Chromatographic separation was performed using an Aquity UPLC system (Waters). Column: PrimeSep 200 mixed mode, 2.1×250mm, particles 5µm (SIELC, Prospect Heights, US). Mobile phase: Solvent A – 9:1 H_2O-acetonitrile mixture, 0.05% formic acid, solvent B – 8:2 H_2O-acetonitrile mixture, 0.2% formic acid. Gradient: Linear gradient from 0% to 100% B in 25 min, column reconditioning: 25-26 min from 0% to 100% A, 26-30 min 100% A. Flow rate: 0.3 mL/min. Injection volume 10 µL. Detection was performed using a Waters Quattro Micro instrument (triple quadrupole MS, electrospray ionisation, positive ion mode). The single ion mode was used and scan mode was running in parallel as a control.

LC-MS based assay development

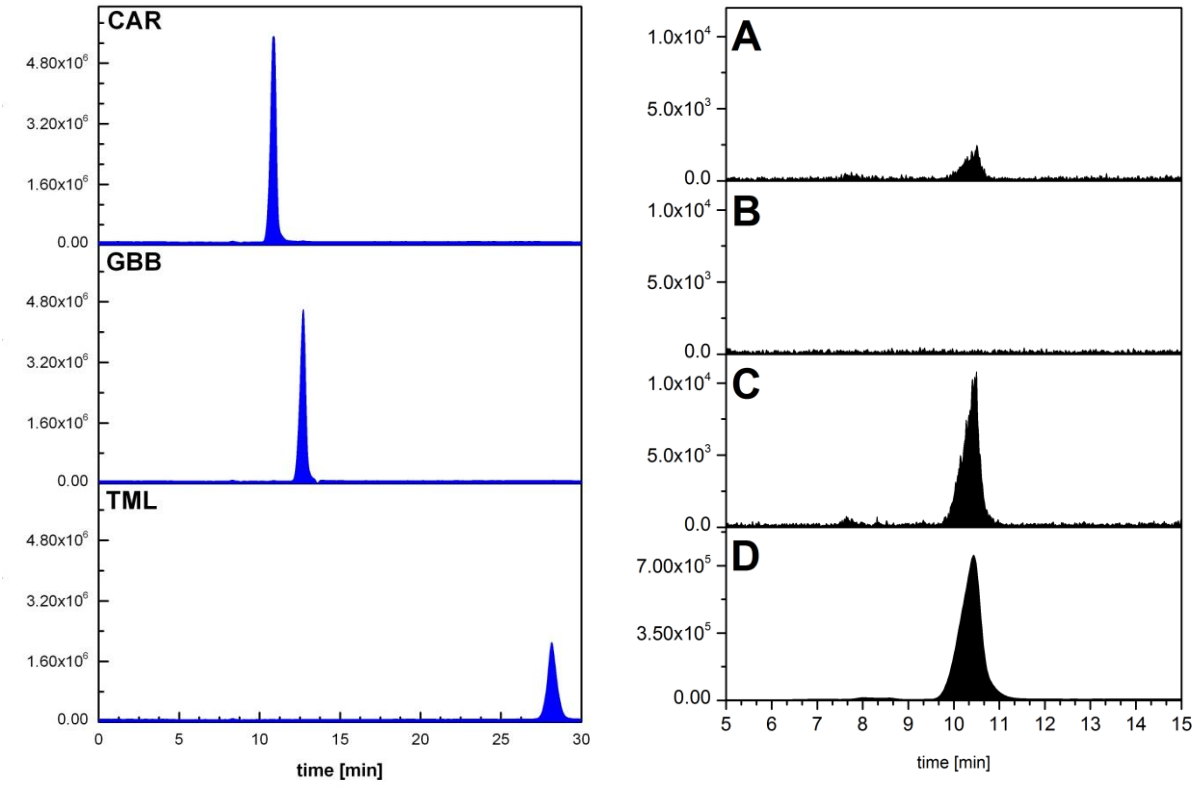


Figure S13 Left: Carnitine biosynthesis pathway standards analysed using the developed cell-assay conditions. The assay enables good separation of trimethyllysine, γ-butyrobetaine and carnitine. 100 µM standards: CAR – carnitine, GBB – γ-butyrobetaine, TML –*Nε*-trimethyllysine. Spectra were recorded in mass range 120-300 Da (scan mode), single ion mode used in parallel to monitor masses: 189.2 (TML), 162.2 (CAR), 146.2 (GBB). **Right:** Analysis of the carnitine content in HEK 293T cell lysates. A – cells incubated with 125 µM of mildronate B – cells incubated with 500 µM mildronate, C – control cell sample with no additives, D – carnitine standard.

Inhibition of carnitine biosynthesis by AR780

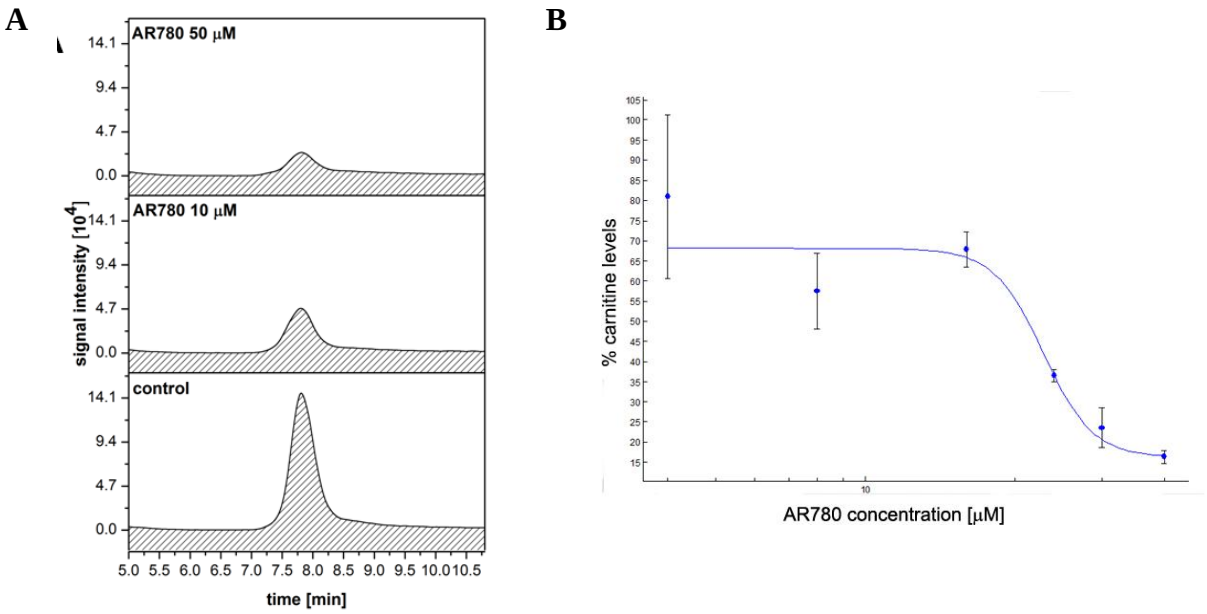


Figure S14. Inhibition of carnitine biosynthesis in HEK 293T cells. A – LC-MS chromatogram of carnitine peak in cells incubated with AR780 and control cells with no compound added. B – The dependency of carnitine levels on AR780 doses in cells (IC_{50} value of 22 μM). Spectra were recorded in the mass range 120-300 Da (scan mode), single ion mode was used in parallel to monitor masses: 189.2 (TML), 162.2 (CAR), 146.2 (GBB). Mildronate run as a control displayed 39±3% carnitine levels, when used at 50 μM. Each point represents an average of three independent cell samples, errors represent standard deviation.

Crystallography

Crystallization was performed in 24-well plates; hanging drops were equilibrated against 400 µl of reservoir solution. Drops consisted of 1 µl reservoir solution and 1 µl protein solution. The reservoir solution contained: 0.2 M ammonium citrate, 1 mM $NiSO_4$, 2% 1, 6-diaminohexane, 19% PEG 3350. Protein (20 mg/ml) was in TRIS buffer (50 mM pH 7.5) containing 200 mM NaCl and was supplemented with 5 mM AR692B (100 mM solution in TRIS 50 mM, pH adjusted to 7.0) prior to crystallization. Crystals formed after a week were used for micro seeding to yield bigger crystals which formed overnight. No crystals were observed in the presence of GBB. Crystals were flash cooled in liquid nitrogen using 25% glycerol in mother liquor as a cryoprotectant. A dataset for BBOX.Ni.AR692B complex was collected at the Diamond beamline I02 with a Pilatus 6M detector. Difficulty in data processing arose as there was a crack/warping in the crystal. Therefore, data were integrated and scaled using only the most intense spots setting a $I/\sigma I$ cut-off of 3.0 and using HKL2000 [7]. The structure was solved by molecular replacement using PHASER [8] (search model PDB ID: 3O2G). Parameter and topology files for AR692B were generated using PRODRG [9] for refinement in CNS [10]. Iterative cycles of model building in COOT [11] and slowcool-simulated annealing refinement using the maximum-likelihood function and bulk-solvent modeling in CNS proceeded until the R_{cryst}/R_{free} no longer decreased. PROCHECK [12] was used to monitor the geometric quality of the model between refinement cycles.

Table S3: Crystallographic data processing, refinement statistics of BBOX.Ni(II).AR692B complex.

Measurement	**BBOX.Ni(II).AR692B**
PDB acquisition code	4C8R
Data collection	
Space Group	$P2_12_12_1$
Cell dimensions a,b,c (Å)	195.744
	91.659
	167.132
Resolution (Å)	48.42 – 2.82 (2.90 – 2.82)*
No. of unique reflections	71043 (7115)*
Completeness (%)	97.8 (99.3)*
Redundancy	3.7 (3.5)*
R_{sym}**	0.203 (0.801)*
Mean I/σ(I)	4.0 (1.9)*
Wilson B value ($Å^2$)	58.9
Refinement	
R_{factor}	0.215
R_{free}	0.244
R.m.s. deviation	
Bond length, Å	0.009
Bond angle, °	1.3

*Highest resolution shell shown in parenthesis.

**$R_{sym} = \sum|I-\langle I\rangle|/\sum I$, where I is the intensity of an individual measurement and $\langle I\rangle$ is the average intensity from multiple observations.

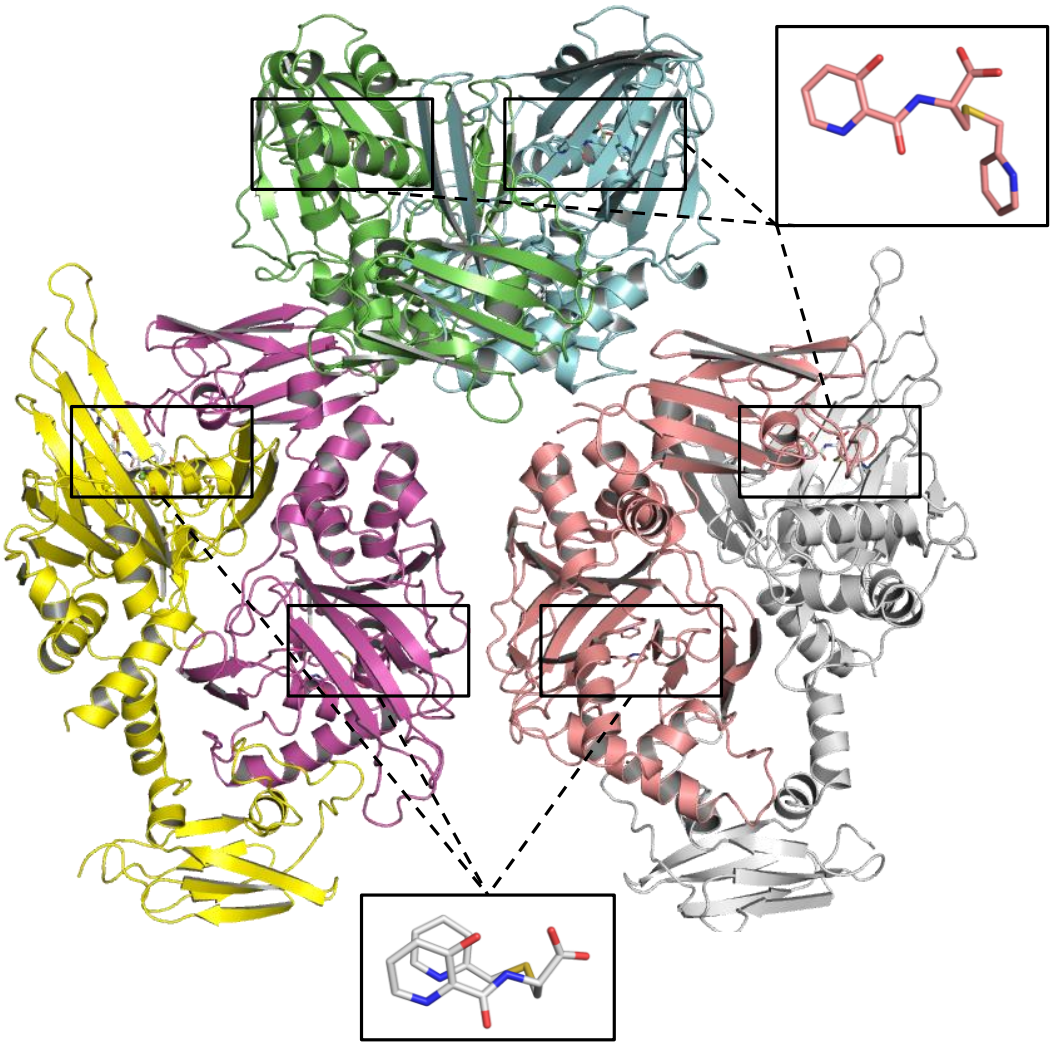


Figure S15 View from the crystal structure of the BBOX.Ni(II).AR692B complex (PDB code: 4C8R) showing the molecules in each asymmetric unit (6 molecules per asymmetric unit, organised as 3 dimers). The inhibitor in grey corresponds to binding mode I and pink corresponds to binding mode II.

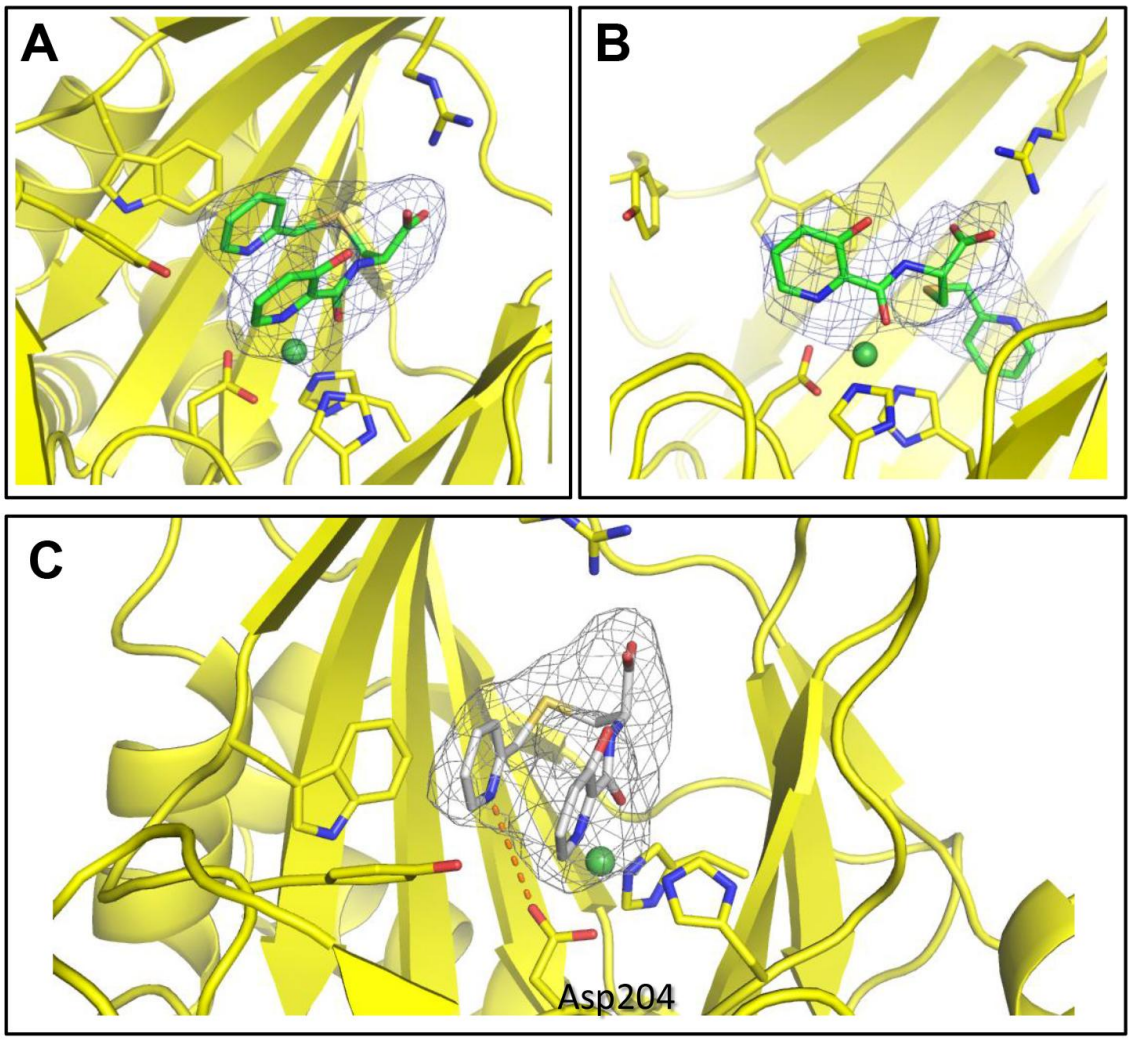


Figure S16. Electron density maps (OMIT Fo-Fc map contoured to 3σ) for ligand binding modes I (A) and II (B). For binding mode I we cannot rule out that the side chain pyridine nitrogen adopts the alternate conformation, in which the pyridine ring is rotatet by 180° (by rotation of S-CH_2-CH bond). The conformer with nitrogen pointing towards Asp204 was modelled; in this conformation side chain pyridine nitrogen is positioned to form a weak hydrogen bond with Asp204 carboxylate (C).

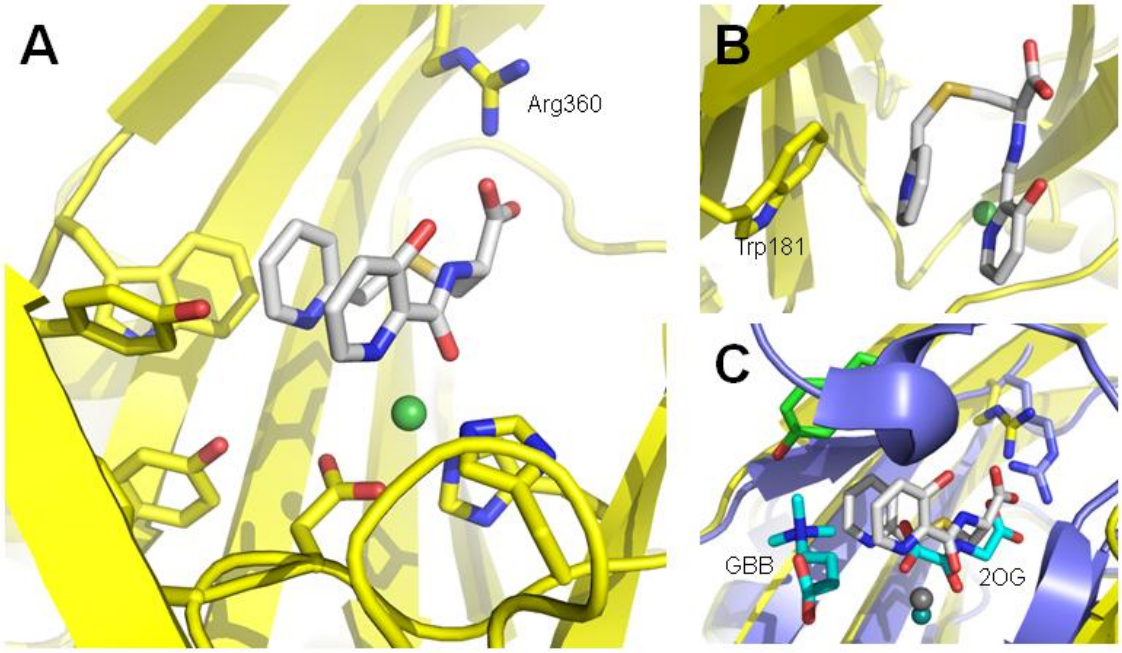


Figure S17 View of AR692B bound to the active site of BBOX – sandwich binding mode. A – The AR692B carboxylate interacts with Arg360, mimicking an interaction made by 2OG (Fig. **2A**). The nitrogen of the main core pyridine ring and the neighbouring carbonyl group bind to the metal in the active site. B – In one chain AR692B forms a sandwich type structure stabilized by apparent π–stacking interactions between pyridine rings of the inhibitor and Trp181. C – Overlay of BBOX crystal structure with GBB and *N*-oxalyl glycine bound (blue, PDB code: 3O2G) with AR692B bound structure (yellow, PDB code: 4C8R). AR692B occupies the 2OG binding pocket mimicking the metal binding mode and interactions with Arg360 of 2OG.

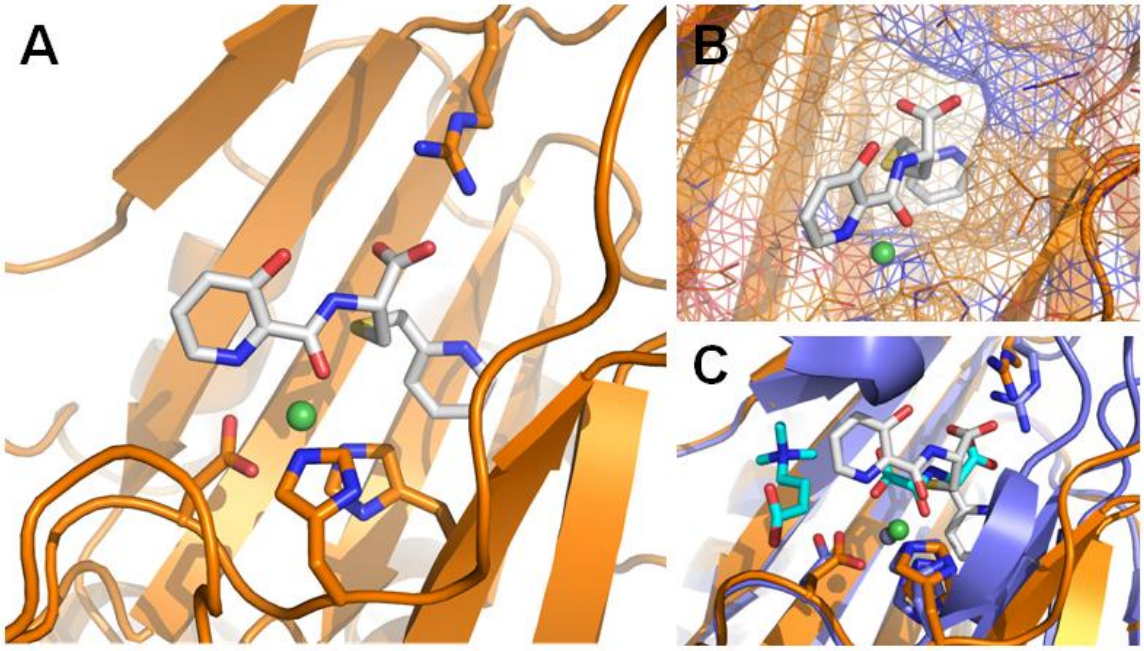


Figure S18 AR692B bound to the active site of BBOX – the second binding mode. A – Similarly to binding mode I (Fig. S11), in the binding mode II the inhibitor carboxylate interacts with Arg360; the main core pyridine nitrogen and the neighbouring carbonyl group chelate the active site metal. B – The pyridine side chain occupies a pocket near the 2OG binding site. C - Overlay of BBOX crystal structure with GBB and NOG bound (blue, PDB code: 3O2G) with obtained inhibitor structure (orange, PDB code: 4C8R).

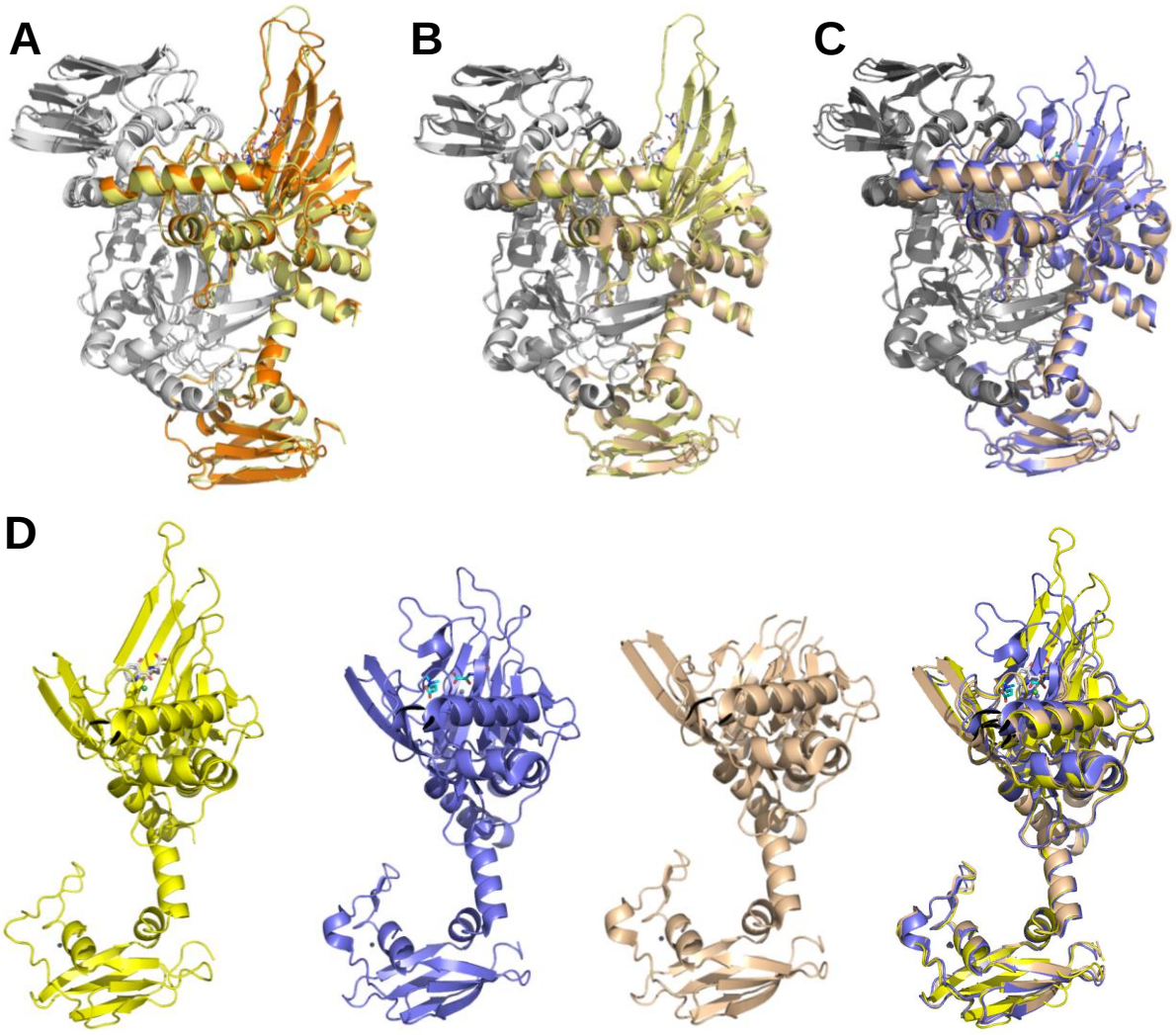


Figure S19 Structural differences in BBOX monomers observed on ligand binding. BBOX.Ni(II).AR692B – binding mode 1 (yellow, PDB code: 4C8R), BBOX.Ni(II).AR692B – binding mode 2 (orange, PDB code: 4C8R), BBOX – apo structure (pink, PDB code: 3N6W), BBOX.Ni(II).NOG.GBB (blue, PDB code: 3O2G). A-C: Overlay of BBOX crystal structures D – comparison of single monomers.

Regulation of carnitine levels by GBB

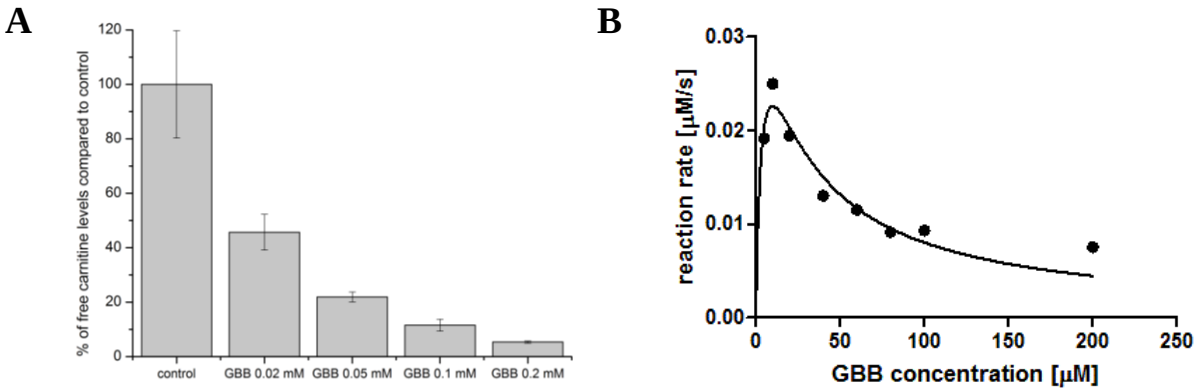


Figure S20 Inhibition of carnitine biosynthesis by GBB. Cells were cultured with additional GBB added to the media. Cells incubated with GBB have shown decrease in levels of free carnitine compared to control free carnitine levels (panel A). These observations are consistent with *in vitro* data showing an inhibitory effect of GBB on BBOX activity at concentrations above 20 µM (panel B)[2].

Synthesis

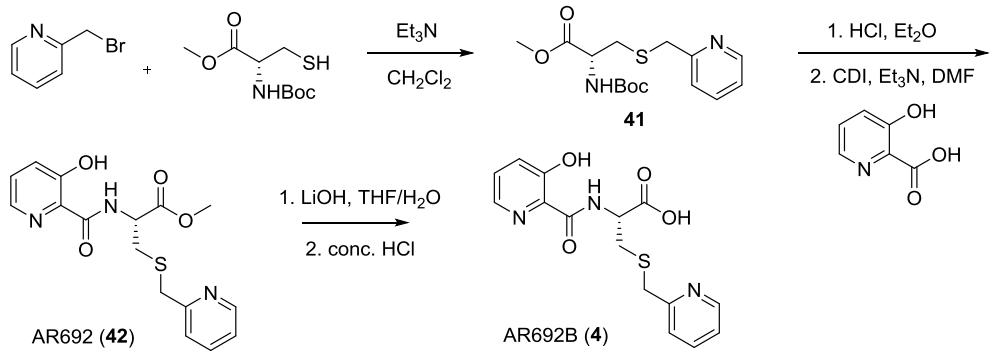
General experimental

Chemicals were purchased from Sigma-Aldrich and used without further purification. Solvents for chemical transformations, work-up and chromatography were purchased from Aldrich at HPLC grade, and used without further purification. Silica gel 60 F254 analytical thin layer chromatography (TLC) plates were from Merck (Darmstadt, Germany) and visualised under UV light, or with potassium permanganate stain. Chromatographic purifications were performed using prepacked SNAP columns on a Biotage SP1 Purification system (Uppsala, Sweden). Deuterated solvents were obtained from Sigma and Apollo Scientific Ltd. ^{1}H NMR spectra were recorded using Bruker AVANCE AV400 (400 MHz), Bruker AV 500 MHz with ^{13}C cryoprobe and variable temperature setup, Bruker AVIII 700 with inverse TCI cryoprobe or Bruker AVII 500 machines. Signal positions were recorded in δ ppm with the abbreviations br s., s, d, t, q, and m denoting broad singlet, singlet, doublet, triplet, quartet and multiplet respectively. All NMR chemical shifts were referenced to residual solvent peaks. Coupling constants, *J*, are registered in Hz to a resolution of 0.5 Hz. All compounds used in screening were more that 90% pure by ^{1}H NMR. High Resolution (HR) mass spectrometry data (m/z) were obtained from a Bruker MicroTOF instrument using an ESI source and Time of Flight (TOF) analyzer. Low Resolution (LR) mass spectrometry data (m/z) were obtained from a Waters LCT Premier instrument using an ESI source and Time of Flight (TOF) analyzer. Values are reported as ratio of mass to charge in Daltons. Melting points were obtained using a Leica VMTG heated-stage microscope or Stuart SMP-40 automatic melting point apparatus. Fourier transform Infrared (FT-IR) spectra were recorded on a Bruker Tensor 27 instrument. Optical rotations were recorded on a Perkin Elmer 241 Polarimeter.

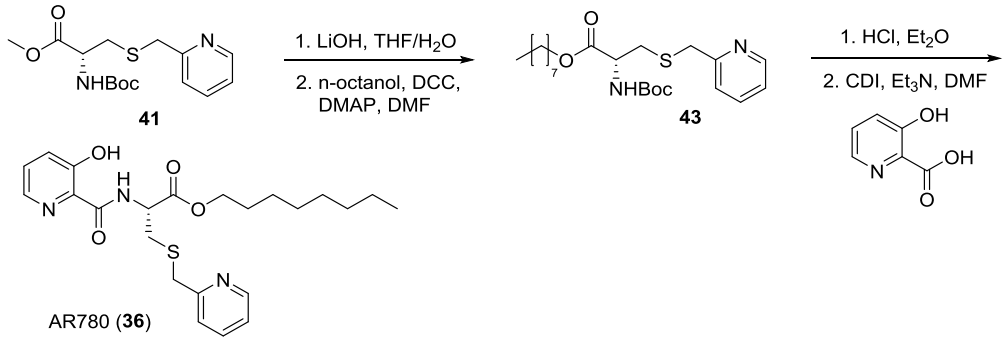
Abbreviations:

DCM – dichloromethane
cHex – cyclohexane
EtOAc – Ethyl acetate
DMF – dimethylformamide
CDI – carbonyldiimidazole
PyBOP - (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
THF – tetrahydrofuran
Et_2O – diethyl ether
Et_3N - trimethylamine

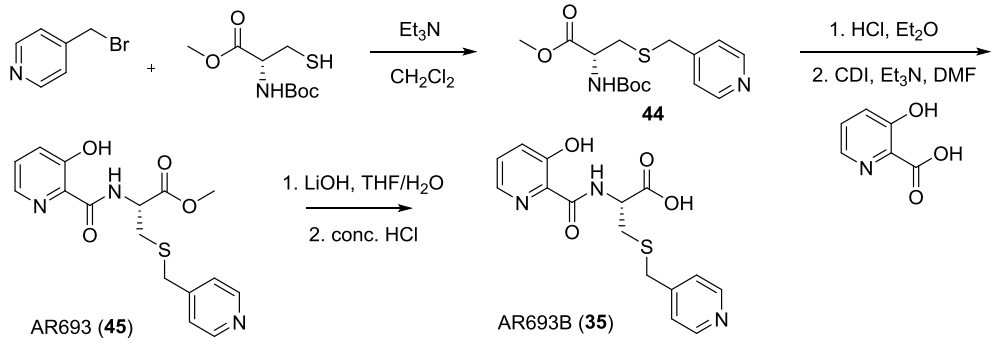
Synthesis of AR692B and derivatives for cell studies



Scheme S1: Synthesis of AR692B.

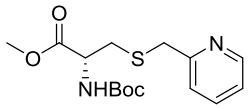


Scheme S2: Synthesis of AR780.



Scheme S3: Synthesis of AR693B

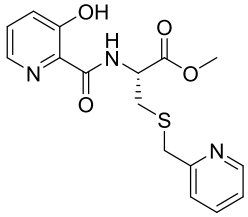
Methyl N-(tert-butoxycarbonyl)-S-(pyridin-2-ylmethyl)-L-cysteinate (41)[13]



Et_3N (1.69 mL, 12.15 mmol, 2.5 eq) was added dropwise to stirred solution of 2-(bromomethyl)pyridine hydrobromide (1.29 g, 5.10 mmol, 1.05 eq) in anhydrous DCM (10 mL). Methyl (tert-butoxycarbonyl)-L-cysteinate (1 mL, 4.86 mmol, 1 eq) was dissolved in 5 mL of DCM and added to 2-(bromomethyl)pyridine solution dropwise at 0 °C. The resultant mixture was stirred overnight at room temperature. The DCM was then evaporated *in vacuo* and remaining residue was subjected to column chromatography on silica (Biotage SNAP KP-SIL™ 50 g cartridge, cHex/EtOAc). Solvents were evaporated to yield the desired compound as a yellow oil (1.19 g, 3.65 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.49 - 8.58 (m, 1 H, ArH), 7.65 (td, *J*=8.0, 2.0 Hz, 1 H, ArH), 7.30 (d, *J*=8.0 Hz, 1 H, ArH), 7.17 (ddd, *J*=7.5, 5.0, 1.0 Hz, 1 H, ArH), 5.92 (d, *J*=7.5 Hz, 1 H, NH), 4.42 - 4.61 (m, 1 H, CH), 3.85 (s, 2 H, SCH_2), 3.71 (s, 3 H, OCH_3), 2.94 (d, *J*=5.3 Hz, 2 H, CH*CH*$_2$), 1.43 (s, 9 H, $C(CH_3)_3$) ppm, ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.6, 158.0, 155.4, 149.2, 137.0, 123.2, 122.2, 79.9, 53.6, 52.5, 38.2, 33.9, 28.3 ppm. $[\alpha]^{20}_D$ = -27.2 (c = 0.5 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{22}N_2NaO_4S$ [M+Na^+]: 349.1192, found: 349.1199. FT-IR ν_{max} (neat): 2978, 1747, 1712, 1159, 730 cm^{-1}.

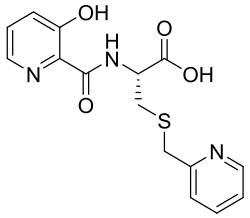
Methyl N-(3-hydroxypicolinoyl)-S-(pyridin-2-ylmethyl)-L-cysteinate (AR692, 42)



Methyl *N*-(tert-butoxycarbonyl)-*S*-(pyridin-2-ylmethyl)-L-cysteinate (2.35 g, 7.20 mmol, 1 eq) was treated with a 1M solution of HCl in Et_2O (20 mL) and stirred overnight at room temperature. The reaction mixture was evaporated *in vacuo* and dissolved in 5 mL of anhydrous DMF. Et_3N (1.5 mL, 10.8 mmol, 1.5 eq) was then added. The resultant solution was added to a mixture of commercially available 3-hydroxypicolinic acid (1.00 g, 7.20 mmol, 1 eq) stirred with CDI (1.40 g, 8.64 mmol, 1.2 eq) in anhydrous DMF (5 mL) for 15 min prior to addition of the amine solution in DMF. After stirring for 24 h at room temperature the DMF was evaporated *in vacuo* and the remaining residue was taken up in 20 mL DCM and washed 3 times with 10 mL of water. The organic layer was dried over $MgSO_4$, evaporated *in vacuo* and subjected to column chromatography on silica (Biotage SNAP KP-SIL™ 50 g cartridge, cHex/EtOAc). The solvent was evaporated to yield the desired compound as a yellow oil (1.12 g, 3.24 mmol, 45%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.76 (s, 1 H, OH), 8.81 (d, *J*=8.0 Hz, 1 H, NH), 8.47 - 8.59 (m, 1 H), 8.10 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.61 (td, *J*=7.5, 2.0 Hz, 1 H), 7.25 - 7.43 (m, 3 H), 7.14 (m, 1 H), 4.88 - 5.04 (m, 1 H, CH), 3.89 (d, *J*=2.8 Hz, 2 H, SCH_2), 3.79 (s, 3 H, OCH_3), 3.09 (m, 2 H, CHC*H*$_2$) ppm, ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.6, 168.7, 157.9, 157.8, 149.4, 139.8, 136.8, 131.1, 128.9, 126.0, 123.1, 122.1, 52.8, 51.7, 38.2, 33.3 ppm. $[\alpha]^{20}_D$ = -37.2 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{16}H_{17}N_3NaO_4S$ [M+Na^+]: 370.0832, found: 370.0836. FT-IR ν_{max} (neat): 3370, 2953, 1743, 1648, 1526, 1473, 701 cm^{-1}.

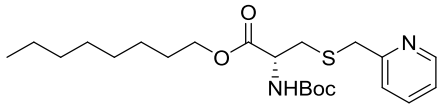
N-(3-hydroxypicolinoyl)-S-(pyridin-2-ylmethyl)-L-cysteine (AR692B, 4)



Methyl *N*-(3-hydroxypicolinoyl)-*S*-(pyridin-2-ylmethyl)-L-cysteinate (200 mg, 0.58 mmol) was stirred overnight with $LiOH{\cdot}H_2O$ (120 mg, 2.90 mmol, 5eq) in a mixture of THF (2 mL) and water (2 mL). The THF was evaporated *in vacuo* and the resultant aqueous solution was acidified with concentrated HCl. Upon acidification product precipitated as a white solid, which was filtered off and dried under vacuum to yield desired compound (153 mg, 0.46 mmol, 80%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.17 (br. s., 1 H, OH), 9.28 (d, *J*=8.0 Hz, 1 H, NH), 8.46 (dd, *J*=5.0, 1.0 Hz, 1 H), 8.21 (dd, *J*=4.5, 1.1 Hz, 1 H), 7.73 (td, *J*=7.5, 2.0 Hz, 1 H), 7.57 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.46 (dd, *J*=8.5, 1.0 Hz, 1 H), 7.39 (d, *J*=8.0 Hz, 1 H), 7.18 - 7.28 (m, 1 H), 4.64 - 4.83 (m, 1 H, CH), 3.86 (d, *J*=4.5 Hz, 2 H, SCH_2), 3.06 - 3.13 (m, 2 H, $CHCH_2$) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.8, 169.0, 158.7, 157.7, 149.4, 140.6, 137.4, 131.2, 130.0, 126.6, 123.5, 122.6, 51.7, 37.3, 32.4 ppm. Mp = 165-167 °C. $[\alpha]^{20}_D$ = -26.8 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{15}N_3NaO_4S$ [M+Na$^+$]: 356.0675, found: 356.0677. FT-IR νmax (neat): 3362, 1721, 1649, 1530, 1445, 702 cm^{-1}.

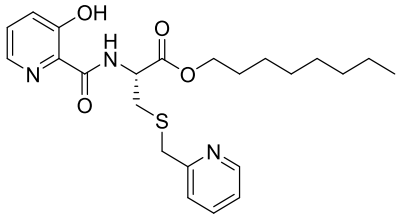
Octyl N-(tert-butoxycarbonyl)-S-(pyridin-2-ylmethyl)-L-cysteinate (43)



A solution of methyl *N*-(tert-butoxycarbonyl)-*S*-(pyridin-2-ylmethyl)-L-cysteinate (1.70 g, 5.45 mmol, 1 eq) in THF (10 mL) was treated with 20 mL of a 1M solution of LiOH in water and stirred overnight at room temperature. The THF was evaporated and resultant aqueous solution brought to pH 7.0 with concentrated HCl and extracted 3 times with 10 mL of DCM. The organic layer was dried over $MgSO_4$ and evaporated. Obtained residue was mixed with DCC (1.70 g, 8.17 mmol, 1.5 eq) and DMAP (200 mg, 1.64 mmol, 0.3 eq) in anhydrous DMF (15 mL) prior to the dropwise addition of n-octanol (4.3 mL, 27.3 mmol, 5eq). After 12 h the DMF was evaporated and remaining residue taken into DCM (20 mL) and washed with H_2O (3×10 mL). Organic layer was dried over $MgSO_4$, evaporated and subjected to column chromatography on silica (Biotage SNAP KP-SIL^{TM} 50 g cartridge, cHex/EtOAc). Solvents were evaporated to yield the desired compound as a yellow oil (1.30 g, 3.07 mmol, 56%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.54 - 8.66 (m, 1 H), 7.89 (t, *J*=7.0 Hz, 1 H), 7.54 (d, *J*=8.0 Hz, 1 H), 7.35 - 7.46 (m, 1 H), 5.80 (d, *J*=7.0 Hz, 1 H, NH), 4.53 (d, *J*=5.0 Hz, 1 H, CH), 4.15 (t, *J*=6.5 Hz, 2 H, OCH_2), 4.06 (d, *J*=3.5 Hz, 2 H, SCH_2), 2.94 - 3.09 (m, 2 H, $CHCH_2$), 1.61 - 1.68 (m, 2 H), 1.47 (s, 9 H, $C(CH_3)_3$), 1.26 - 1.36 (m, 10 H), 0.90 (t, *J*=6.1 Hz, 3 H, CH_2CH_3) ppm, ^{13}C NMR (126 MHz, $CDCl_3$) δ = 170.9, 156.8, 155.4, 145.7, 140.5, 125.0, 123.3, 80.1, 66.0, 53.7, 35.9, 34.4, 32.8, 31.8, 29.2, 28.5, 28.4, 25.8, 22.7, 14.1 ppm. $[\alpha]^{20}_D$ = -20.5 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{22}H_{36}N_2NaO_4S$ [M+Na$^+$]: 447.2288, found: 447.2283. FT-IR ν_{max} (neat): 2926, 2856, 1714, 1435, 1165, 748 cm^{-1}.

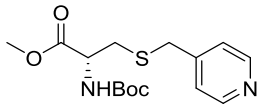
Octyl N-(3-hydroxypicolinoyl)-S-(pyridin-2-ylmethyl)-L-cysteinate (AR780, 36)



Octyl *N*-(tert-butoxycarbonyl)-*S*-(pyridin-2-ylmethyl)-L-cysteinate (0.93 g, 2.20 mmol, 1 eq) was treated with a 1M solution of HCl in Et_2O (10 mL) and stirred overnight at room temperature. The reaction mixture was evaporated *in vacuo* and dissolved in 7 mL of anhydrous DMF followed by addition of Et_3N (460 µL, 3.3 mmol, 1.5 eq). The resultant solution was added to the mixture of commercially available 3-hydroxypicolinic acid (370 mg, 2.64 mmol, 1.2 eq) stirred with CDI (430 mg, 2.64 mmol, 1.2 eq) in anhydrous DMF (7 mL) for 15 min prior to the addition of the amine solution in DMF. After stirring for 24h at room temperature DMF was evaporated *in vacuo* and remaining residue was taken up in DCM (20 mL) and washed with H_2O (3×10 mL). Organic layer was dried over $MgSO_4$, evaporated *in vacuo* and subjected to column chromatography on silica (Biotage SNAP KP-SIL™ 25 g cartridge, eluent system cHex/EtOAc). The solvent was evaporated to yield the desired compound as a yellow oil (290 mg, 0.66 mmol, 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.70 (s, 1 H, OH), 8.71 (d, *J*=8.5 Hz, 1 H, NH), 8.43 - 8.49 (m, 1 H), 8.03 (dd, *J*=4.0, 1.5 Hz, 1 H), 7.60 (td, *J*=7.5, 2.0 Hz, 1 H), 7.27 - 7.33 (m, 2 H), 7.23 (dd, *J*=8.5, 1.5 Hz, 1 H), 7.12 (ddd, *J*=7.5, 5.0, 0.5 Hz, 1 H), 4.87 (ddd, *J*=8.5, 6.5, 5.0 Hz, 1 H, CH), 4.12 (td, *J*=7.0, 1.0 Hz, 3 H, OCH_2), 3.88 (br. s, 2 H, SCH_2), 3.07 (dd, *J*=14.0, 5.0 Hz, 1 H, CHC*H*'H''), 3.00 (dd, *J*=14.0, 6.5 Hz, 1 H, CHCH'*H*''), 1.58 (quin, *J*=7.0 Hz, 2 H), 1.17 - 1.26 (m, 10 H), 0.77 - 0.85 (m, 3 H, CH_2CH_3) ppm, ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.1, 168.7, 157.8, 157.6, 148.4, 139.9, 137.6, 131.1, 128.9, 126.0, 123.6, 122.3, 66.2, 51.8, 37.6, 33.5, 31.7, 29.1, 28.5, 25.8, 22.6, 14.1 ppm. $[\alpha]^{20}_D$ = -38.1 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{23}H_{31}N_3NaO_4S$ [M+Na$^+$]: 468.1927, found: 468.1934. FT-IR ν_{max} (neat): 3372, 2957, 2926, 2854, 1739, 1649, 1570, 1470, 1179, 701 cm^{-1}.

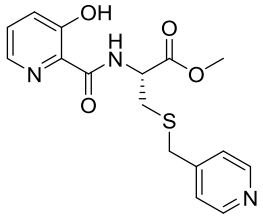
Methyl N-(tert-butoxycarbonyl)-S-(pyridin-4-ylmethyl)-L-cysteinate (44)[14]



Et_3N (1.69 mL, 12.15 mmol, 2.5 eq) was added dropwise to stirred solution of 2-(bromomethyl)pyridine hydrobromide (1.29 g, 5.10 mmol, 1.05 eq) in anhydrous DCM (10 mL). Methyl (tert-butoxycarbonyl)-L-cysteinate (1 mL, 4.86 mmol, 1 eq) was dissolved in 5 mL of DCM and added to 2-(bromomethyl)pyridine solution dropwise at 0 °C. The resultant mixture was stirred overnight at room temperature. DCM was evaporated *in vacuo* and remaining residue was subjected to column chromatography on silica (Biotage SNAP KP-SIL™ 50 g cartridge, cHex/EtOAc). The solvents were evaporated to yield the desired compound as a yellow oil (1.08 g, 3.32 mmol, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.55 (d, *J*=5.5 Hz, 2 H), 7.25 (d, *J*=5.5 Hz, 2 H), 5.32 (d, *J*=7.0 Hz, 1 H, NH), 4.46 - 4.62 (m, 1 H, CH), 3.75 (s, 3 H, OCH_3), 3.69 (s, 2 H, SCH_2), 2.89 (dd, *J*=14.0, 4.5 Hz, 1 H, CHC*H*'H''), 2.78 (dd, *J*=14.0, 5.5 Hz, 1 H, CHCH'*H*''), 1.46 (s, 9 H, $C(CH_3)_3$) ppm, ^{13}C NMR (126 MHz, $CDCl_3$) δ = 171.3, 155.2, 149.4, 148.2, 124.6, 80.5, 53.1, 52.8, 35.5, 33.9, 28.3 ppm. $[\alpha]^{20}_D$ = -34.0 (c = 0.3 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{22}N_2NaO_4S$ [M+Na$^+$]: 349.1192, found: 349.1193. FT-IR ν_{max} (neat): 3191, 2982, 1743, 1708, 1604, 1163, 740 cm^{-1}.

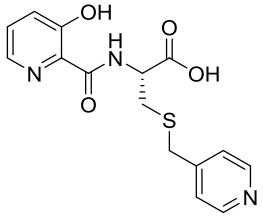
Methyl N-(3-hydroxypicolinoyl)-S-(pyridin-4-ylmethyl)-L-cysteinate (45)



Methyl *N*-(tert-butoxycarbonyl)-*S*-(pyridin-4-ylmethyl)-L-cysteinate (430 mg, 1.32 mmol, 1 eq) was treated with a 1M solution of HCl in diethyl ether (5 mL) and stirred overnight at room temperature. The reaction mixture was evaporated *in vacuo* and dissolved in 3 mL of the anhydrous DMF followed by the addition of Et_3N (275 µL, 1.98 mmol, 1.5 eq). The resultant solution was added to a mixture of commercially available 3-hydroxypicolinic acid (186 mg, 1.32 mmol, 1 eq) stirred with CDI (255 mg, 1.58 mmol, 1.2 eq) in anhydrous DMF (3 mL) for 15 min prior to addition of the amine solution in DMF. After stirring for 24h at room temperature DMF was evaporated *in vacuo* and remaining residue was taken up in 10 mL of DCM and washed 3 times with 5 mL of water. The organic layer was dried over $MgSO_4$, evaporated *in vacuo* and subjected to the column chromatography on silica (Biotage SNAP KP-SIL™ 25 g cartridge, eluent system cHex/EtOAc). Solvent was evaporated to yield the desired compound as a yellow oil (183 mg, 0.53 mmol, 40%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.66 (br. s., 1 H, OH), 8.66 (d, *J*=8.0 Hz, 1 H, NH), 8.53 - 8.59 (m, 2 H), 8.14 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.30 - 7.44 (m, 4 H), 4.92 - 5.02 (m, 1 H, CH), 3.82 (s, 3 H, OCH_3), 3.77 (s, 2 H, SCH_2), 2.92 - 3.10 (m, 2 H, CHC*H*$_2$) ppm, ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.3, 168.7, 157.9, 149.1, 147.8, 140.0, 130.8, 129.1, 126.2, 124.3, 53.0, 51.4, 35.5, 33.5 ppm. $[\alpha]^{20}_D$ = -33.4 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{16}H_{18}NO_4S$ [M+H$^+$]: 348.1013, found: 348.1005. FT-IR ν_{max} (neat): 3370, 3067, 2954, 1744, 1647, 1523, 701 cm^{-1}.

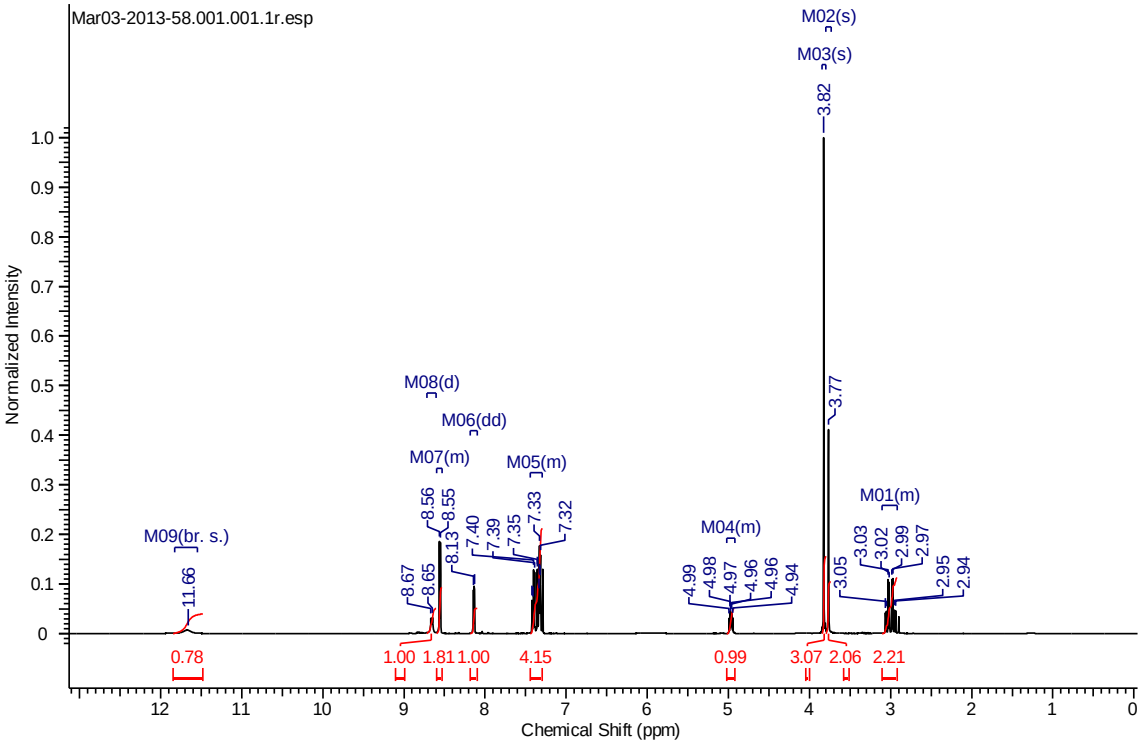
N-(3-hydroxypicolinoyl)-S-(pyridin-4-ylmethyl)-L-cysteine (AR693B, 35)

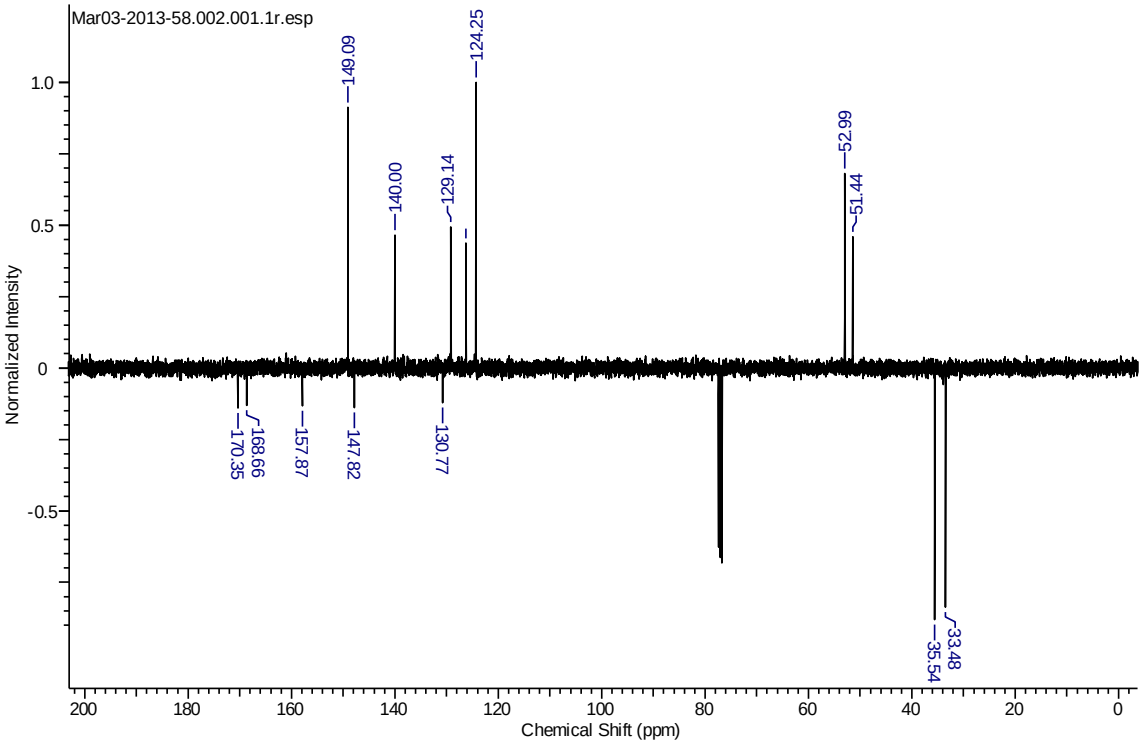


Methyl *N*-(3-hydroxypicolinoyl)-*S*-(pyridin-4-ylmethyl)-L-cysteinate (50 mg, 0.15 mmol) was stirred overnight with $LiOH{\cdot}H_2O$ (30 mg, 0.73 mmol, 5eq) in a mixture of THF (1 mL) and water (1 mL). The THF was evaporated *in vacuo* and the resultant aqueous solution was acidified with concentrated HCl. Upon acidification the product precipitated as a white solid, which was filtered off and dried under the vacuum to yield the desired compound (30 mg, 0.087 mmol, 58%).

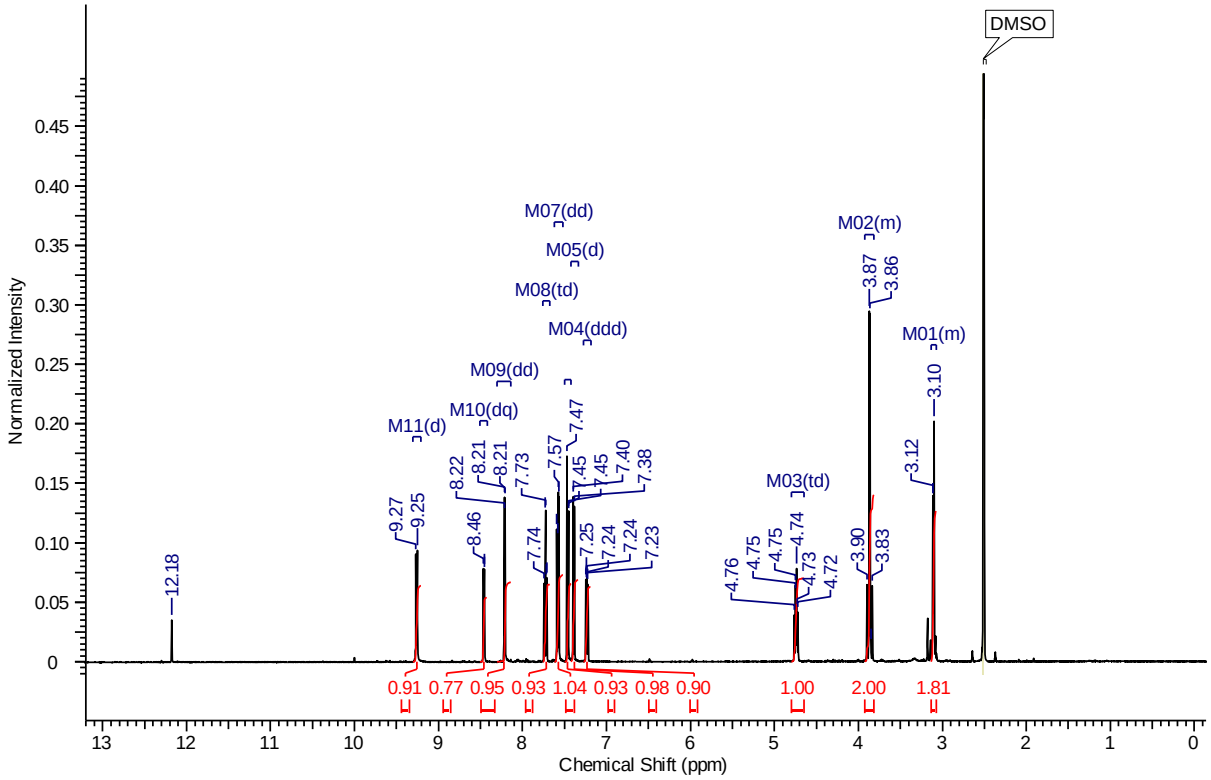
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 12.12 (br. s., 1 H, OH), 9.24 (d, *J*=8.5 Hz, 1 H, NH), 8.47 - 8.57 (m, 2 H), 8.20 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.57 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.45 (dd, *J*=8.5, 1.5 Hz, 1 H), 7.38 - 7.41 (m, 2 H), 4.67 (td, *J*=8.5, 4.5 Hz, 1 H, CH), 3.81 (br. s, 2 H, SCH_2), 2.95 - 3.08 (m, 2 H, CHC*H*$_2$) ppm, ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ = 171.6, 169.0, 157.7, 149.5, 149.1, 140.6, 131.1, 130.0, 126.6, 124.8, 51.7, 34.4, 32.4 ppm. Mp = 190-192 °C. $[\alpha]^{20}_D$ = -23.4 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{15}N_3NaO_4S$ [M+Na$^+$]: 356.0675, found: 356.0678. FT-IR ν_{max} (neat): 3368, 1645, 1515, 1448, 1298, 701 cm^{-1}.

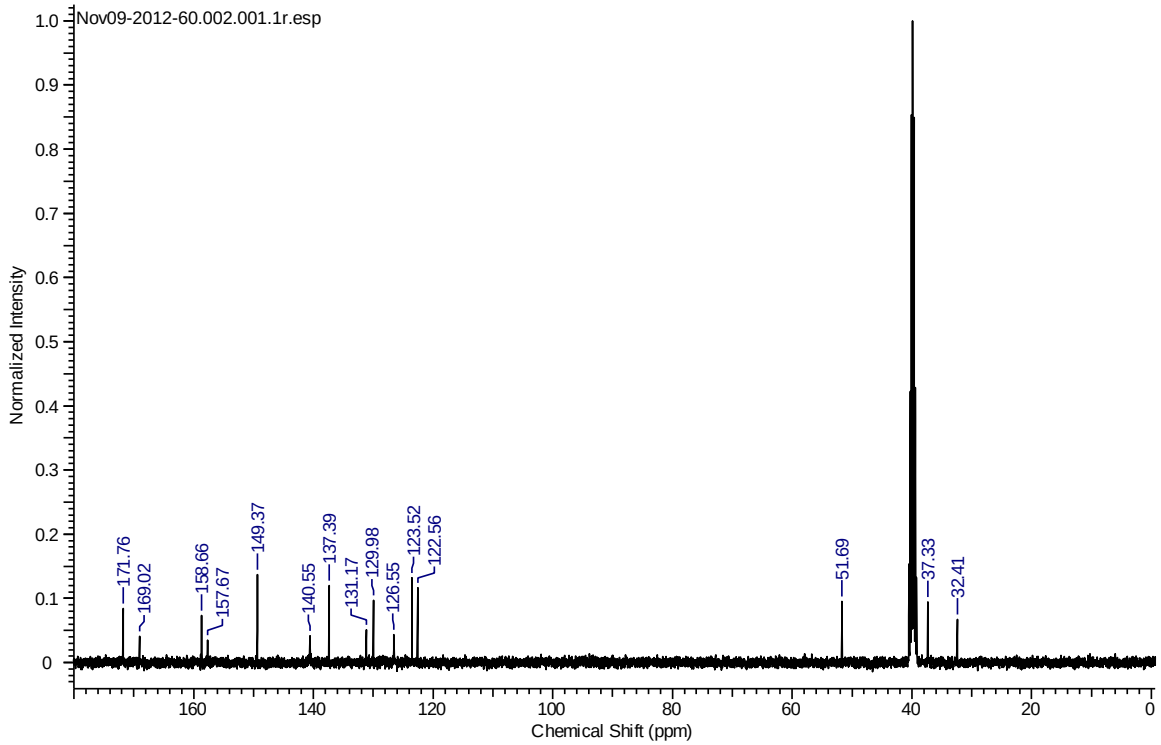
NMR spectra of AR692



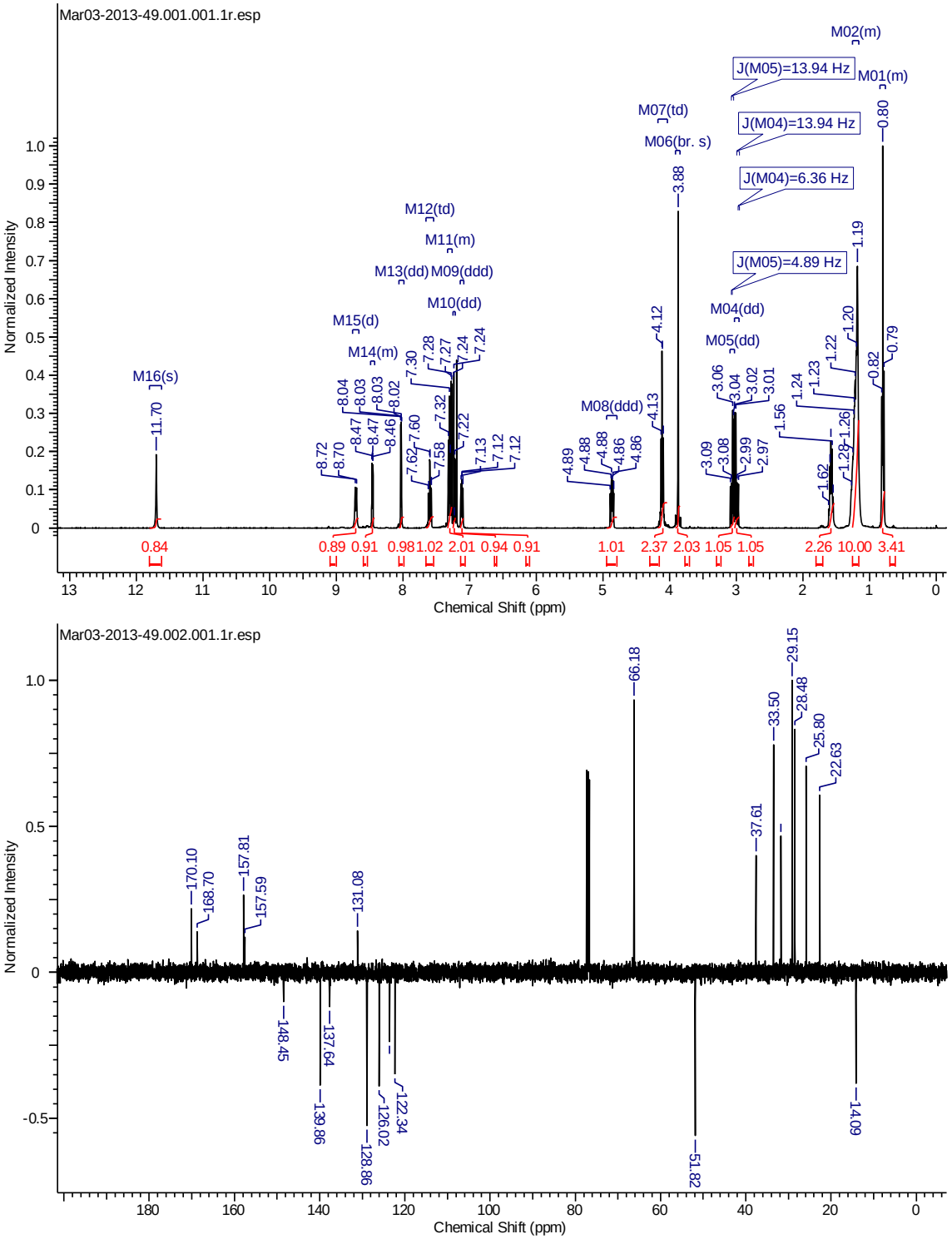


NMR spectra of AR692B

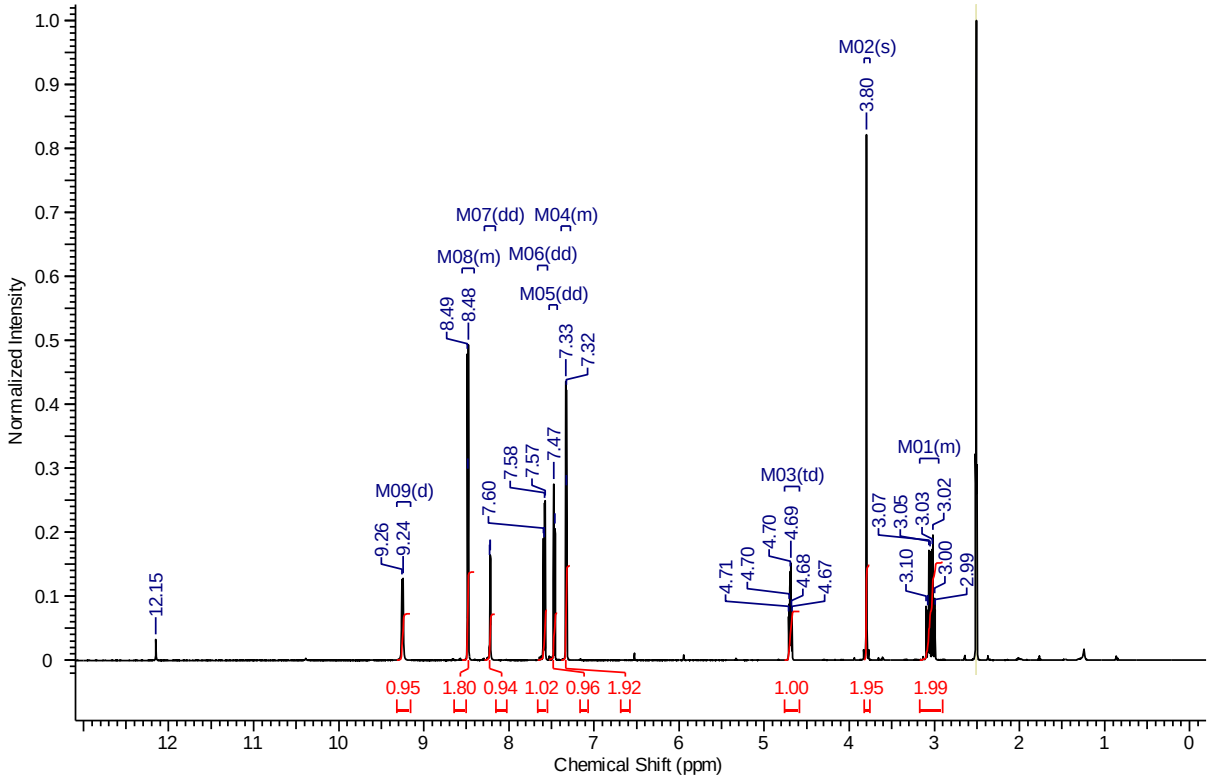


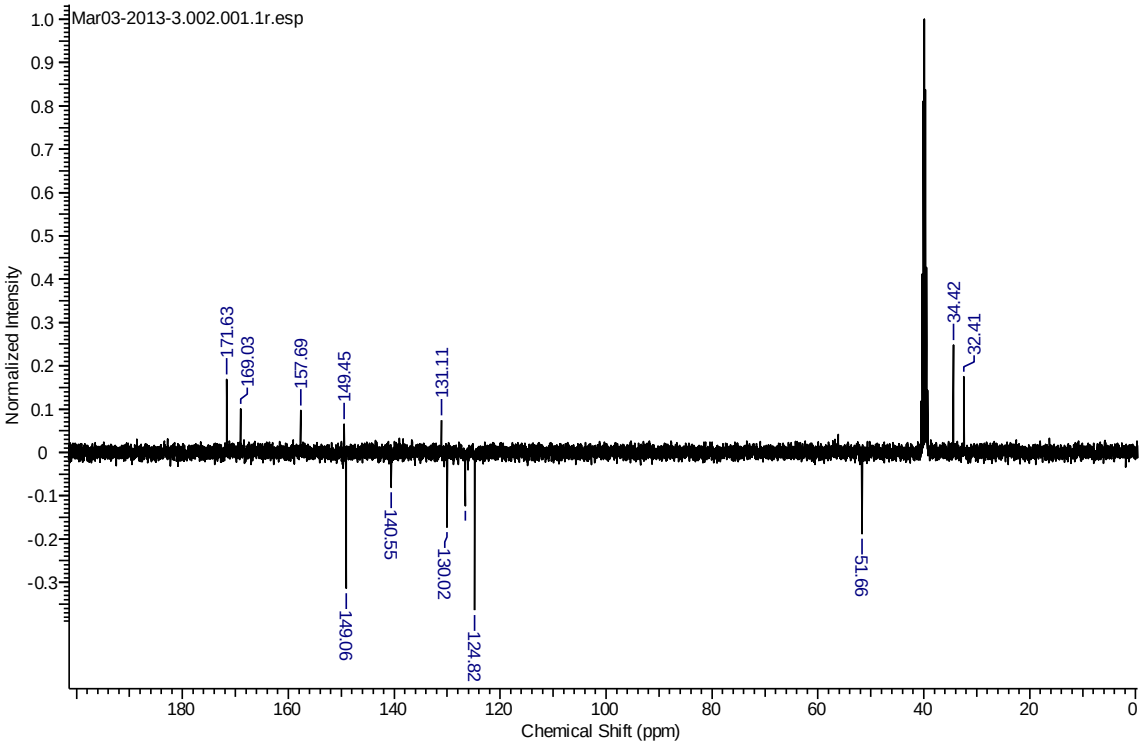


NMR spectra of AR780

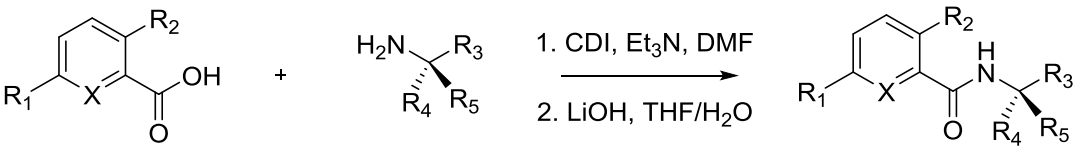


NMR spectra of AR693B





Synthesis of other 3-hydroxypicolinic acid derivatives



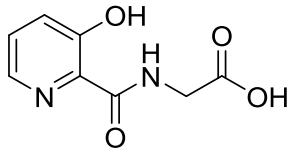
Scheme S4: Synthesis of 3-hydroxypicolinic acid derivatives.

code	X	R_1	R_2	R_3	R_4	R_5
1	N	H	OH	COOH	H	H
2a	N	H	OH	COOH	$(CH_2)_2COOH$	H
2b	N	H	OH	COOH	H	$(CH_2)_2COOH$
3a	N	H	OH	COOH	CH_2Ph	H
3b	N	H	OH	COOH	H	CH_2Ph
5	H	H	OH	COOH	H	H
6	N	H	H	COOH	H	H
7	N	H	OH	CH_2COOH	H	H
8	N	H	OH	H	CH_2Ph	H
9a	N	H	OH	COOH	CH_2-(3-indole)	H
9b	N	H	OH	COOH	H	CH_2-(3-indole)
10	N	H	H	COOH	CH_2-(3-indole)	H
11	H	H	OH	COOH	CH_2-(3-indole)	H
12a	N	H	OH	COOH	CH_2COOH	H
12b	N	H	OH	COOH	H	CH_2COOH
13a	N	H	OH	COOH	CH_3	H
13b	N	H	OH	COOH	H	CH_3
14a	N	H	OH	COOH	$CH(CH_3)_2$	H
14b	N	H	OH	COOH	H	$CH(CH_3)_2$
15a	N	H	OH	COOH	$CH_2CH(CH_3)_2$	H
15b	N	H	OH	COOH	H	$CH_2CH(CH_3)_2$
16a	N	H	OH	COOH	CH_2Ph(4-OH)	H
16b	N	H	OH	COOH	H	CH_2Ph(4-OH)
26	N	Cl	Cl	COOH	CH_2-(3-indole)	H
27	N	H	OH	COOH	CH_2-(4-imidazole)	H

General procedure for preparation of amino acids conjugates with 3-hydroxypicolinic acid

3-Hydroxypicolinic acid (300 mg, 2.16 mmol) and CDI (420 mg, 2.59 mmol, 1.2 eq) were stirred in anhydrous DMF (3 mL) for 10 min, prior to the addition of solution of amino acid methyl ester (2.59 mmol, 1.2 eq) and Et_3N (320 µL, 2.27 mmol, 1.05 eq) in DMF (3 mL). The resultant mixture was stirred overnight at room temperature. Upon completion of the reaction most of the DMF was evaporated *in vacuo* and the resultant residue was suspended in CH_2Cl_2 (10 mL) and washed with H_2O (3 x 5 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to the column chromatography (Biotage SNAP KP-SIL™ 25 g cartridge, eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 6 mL) and subsequently treated with LiOH · H_2O (450 mg, 10.8 mmol, 5 eq). The reaction was stirred at room temperature for 12 h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. If precipitate was formed it was filtered-off and dried *in vacuo* to yield the desired product. In case no precipitate was formed, the aqueous solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield the desired product.

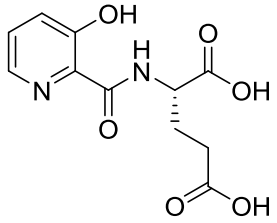
(3-Hydroxypicolinoyl)glycine (1) [15]



The desired compound (1) was obtained as a white solid (178 mg, 0.91 mmol, 42%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.28 (br. s., 1 H, OH), 9.33 (t, *J*=6.0 Hz, 1 H, NH), 8.18 (dd, *J*=4.3, 1.3 Hz, 1 H), 7.55 (dd, *J*=8.5, 4.4 Hz, 1 H), 7.43 (dd, *J*=8.5, 1.4 Hz, 1 H), 3.99 (d, *J*=6.1 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.4, 169.8, 158.0, 140.9, 131.8, 130.2, 126.9, 41.5 ppm. Mp = 136-139 °C (lit. 169-170°C) [15]. HRMS (ESI-TOF) calcd for $C_8H_8N_2NaO_4$ [M+Na^+]: 219.0376, found: 219.0366. FT-IR ν_{max} (neat): 3234, 3017, 1608, 1519, 1404, 1344, 816, 662 cm^{-1}.

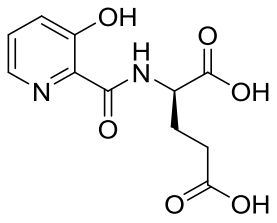
(3-Hydroxypicolinoyl)-L-glutamic acid (2a)



The desired compound (2a) was obtained as a pink solid (170 mg, 0.63 mmol, 24%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).

^{1}H NMR (400 MHz, CD_3OD) δ = 8.15 (d, *J*=4.5 Hz, 1 H), 7.46 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.36 (d, *J*=8.5 Hz, 1 H), 4.71 (dd, *J*=8.5, 4.5 Hz, 1 H, CH), 2.43 - 2.52 (m, 2 H, C*H*$_2$COOH), 2.31 - 2.43 (m, 1 H, CHC*H*'H''), 2.08 - 2.22 (m, 1 H, CHCH'*H*'') ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 175.3, 173.3, 169.3, 158.1, 140.0, 131.3, 129.2, 126.2, 51.6, 30.1, 27.1 ppm. Mp = 73-75 °C. $[\alpha]^{20}_D$ = +6.3 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{11}H_{12}N_2NaO_6$ [M+Na^+]: 291.0588, found: 291.0576. FT-IR ν_{max} (neat): 3366, 2961, 2931, 1733, 1647, 1527, 1239, 1149, 806, 700 cm^{-1}.

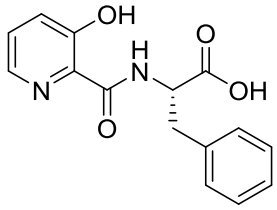
(3-Hydroxypicolinoyl)-D-glutamic acid (2b)



The desired compound (2b) was obtained as a pink solid (140 mg, 0.53 mmol, 20%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).

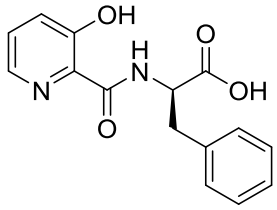
^{1}H NMR (400 MHz, CD_3OD) δ = 8.15 (d, *J*=4.5 Hz, 1 H), 7.46 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.36 (d, *J*=8.5 Hz, 1 H), 4.71 (dd, *J*=8.5, 4.5 Hz, 1 H, CH), 2.43 - 2.52 (m, 2 H, C*H*$_2$COOH), 2.31 - 2.43 (m, 1 H, CHC*H*'H''), 2.08 - 2.22 (m, 1 H, CHCH'*H*'') ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 175.3, 173.3, 169.3, 158.1, 140.0, 131.3, 129.2, 126.2, 51.6, 30.1, 27.1 ppm. Mp = 84-87 °C. $[\alpha]^{20}_D$ = -6.8 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{11}H_{12}N_2NaO_6$ [M+Na^+]: 291.0588, found: 291.0585. FT-IR ν_{max} (neat): 3363, 2961, 1715, 1645, 1526, 1448, 1187, 1119, 801 cm^{-1}.

(3-Hydroxypicolinoyl)-L-phenylalanine (3a)



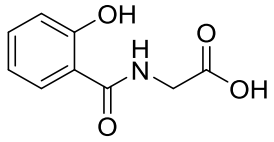
The desired compound (3a) was obtained as a white solid (277 mg, 0.97 mmol, 45%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.48 (br. s., 1 H, NH), 8.05 (br. s., 1 H), 7.10 - 7.40 (m, 8 H), 5.06 (q, *J*=7.0 Hz, 1 H, CH), 3.35 (dd, *J*=14.0, 5.5 Hz, 1 H, CHC*H*'H''), 3.24 (dd, *J*=14.0, 7.0 Hz, 1 H, CHCH'*H*'') ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.9, 168.5, 157.9, 139.8, 135.5, 130.7, 129.3, 129.0, 128.7, 127.3, 126.5, 53.0, 37.7 ppm. Mp = 116-118 °C. $[\alpha]^{20}_{D}$ = +25.6 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{14}N_2NaO_4$ [M+Na$^+$]: 309.0846, found: 309.0834. FT-IR ν_{max} (neat): 3344, 3027, 1727, 1635, 1272, 637 cm^{-1}.

(3-Hydroxypicolinoyl)-D-phenylalanine (3b)



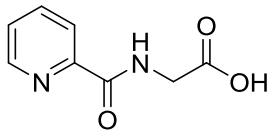
The desired compound (3b) was obtained as a white solid (246 mg, 0.86 mmol, 40%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.48 (br. s., 1 H, NH), 8.05 (br. s., 1 H), 7.10 - 7.40 (m, 8 H), 5.06 (q, *J*=7.0 Hz, 1 H, CH), 3.35 (dd, *J*=14.0, 5.5 Hz, 1 H, CHC*H*'H''), 3.24 (dd, *J*=14.0, 7.0 Hz, 1 H, CHCH'*H*'') ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.9, 168.5, 157.9, 139.8, 135.5, 130.7, 129.3, 129.0, 128.7, 127.3, 126.5, 53.0, 37.7 ppm. Mp = 123-126 °C. $[\alpha]^{20}_{D}$ = -23.2 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{14}N_2NaO_4$ [M+Na$^+$]: 309.0846, found: 309.0835. FT-IR ν_{max} (neat): 3345, 3032, 1727, 1635, 1265, 639 cm^{-1}.

(2-Hydroxybenzoyl)glycine (5)[16]



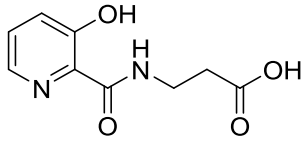
The desired compound (5) was obtained as a white solid (205 mg, 0.98 mmol, 45%) starting from salicylic acid (300 mg, 2.17 mmol) and following the general procedure.
^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.24 (s, 1 H, OH), 9.15 (t, *J*=5.5 Hz, 1 H, NH), 7.89 (dd, *J*=8.0, 1.5 Hz, 1 H), 7.42 (ddd, *J*=8.5, 7.5, 1.5 Hz, 1 H), 6.82 - 7.01 (m, 2 H), 4.00 (d, *J*=6.0 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.4, 169.2, 160.0, 134.3, 128.7, 119.3, 117.8, 115.8, 41.5 ppm. Mp = 165-167 °C (lit. 150-152 °C)[16]. HRMS (ESI-TOF) calcd for $C_9H_9NaNO_4$ [M+Na$^+$]: 218.0424, found: 218.0414. FT-IR ν_{max} (neat): 3389, 3345, 1705, 1607, 1546, 1234, 793 cm^{-1}.

Picolinoylglycine (6)[15]



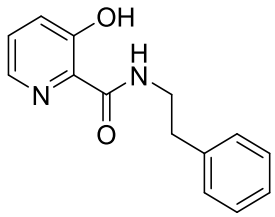
The desired compound (6) was obtained as a white solid (293 mg, 1.63 mmol, 40%) starting from picolinic acid (500 mg, 4.07 mmol) and following the general procedure.
^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.01 (t, *J*=6.0 Hz, 1 H, NH), 8.65 (d, *J*=4.5 Hz, 1 H), 7.95 - 8.07 (m, 2 H), 7.61 (ddd, *J*=7.0, 5.0, 1.5 Hz, 1 H), 3.99 (d, *J*=6.0 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.9, 165.0, 150.2, 149.4, 138.7, 127.6, 122.8, 41.8 ppm. Mp = 180-183 °C (lit. 119-121 °C)[15]. HRMS (ESI-TOF) calcd for $C_8H_8NaN_2O_3$ [M+Na$^+$]: 203.0427, found: 203.0427. FT-IR ν_{max} (neat): 3335, 2933, 2160, 1751, 1633, 1532, 1196, 671 cm^{-1}.

3-(3-Hydroxypicolinamido)propanoic acid (7)



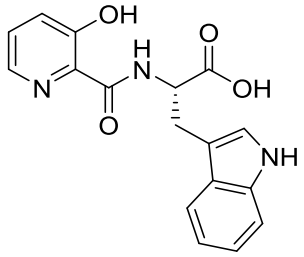
The desired compound (7) was obtained as a white solid (204 mg, 0.97 mmol, 45%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.12 (t, *J*=6.0 Hz, 1 H, NH), 8.16 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.54 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.44 (dd, *J*=8.5, 1.5 Hz, 1 H), 3.52 (td, *J*=7.0, 6.0 Hz, 2 H, NHC*H*$_2$), 2.56 (t, *J*=7.0 Hz, 2 H, CH_2CO_2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 173.7, 168.9, 158.0, 140.4, 131.8, 130.1, 127.2, 35.5, 34.3 ppm. Mp = 175-177 °C. HRMS (ESI-TOF) calcd for $C_9H_{10}N_2NaO_4$ [M+Na$^+$]: 233.0533, found: 233.0523. FT-IR ν_{max} (neat): 3354, 3107, 2933, 2166, 1713, 1664, 1397, 672 cm^{-1}.

3-Hydroxy-N-phenethylpicolinamide (8)



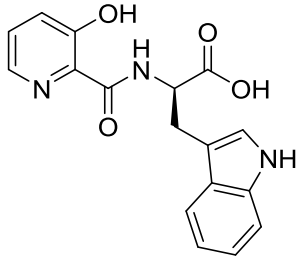
The desired compound (8) was obtained as yellow oil (290 mg, 1.19 mmol, 55%) by coupling 3-hydroxypicolinic acid (300 mg, 2.16 mmol) with 2-phenylethan-1-amine (300 µl, 2.38 mmol, 1.1eq) and following the general procedure.
^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.24 (s, 1 H, OH), 8.15 (br. s., 1 H, NH), 8.02 (dd, *J*=4.0, 2.0 Hz, 1 H), 7.21 - 7.39 (m, 7 H), 3.60 - 3.83 (m, 2 H, CH_2), 2.96 (t, *J*=7.3 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.8, 157.8, 139.5, 138.5, 131.5, 128.8, 128.7, 128.6, 126.6, 126.0, 40.3, 35.8 ppm. HRMS (ESI-TOF) calcd for $C_{14}H_{14}N_2NaO_2$ [M+Na$^+$]: 265.0947, found: 265.0949. FT-IR ν_{max} (neat): 3370, 3027, 1647, 1531, 1447, 1296, 808 cm^{-1}.

(3-Hydroxypicolinoyl)-L-tryptophan (9a)[15]



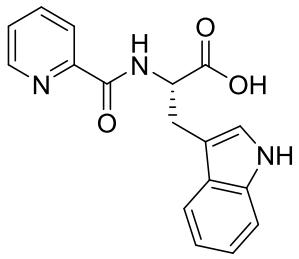
The desired compound (9a) was obtained as a yellow solid (295 mg, 0.91 mmol, 35%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, CD_3OD) δ = 8.28 (br. s., 1 H), 7.77 - 7.93 (m, 2 H), 7.53 (dd, *J*=7.5, 34.0 Hz, 1 H), 7.28 - 7.39 (m, 1 H), 7.11 - 7.18 (m, 1 H), 7.03 - 7.11 (m, 1 H), 6.88 - 7.00 (m, 1 H), 4.99 - 5.06 (m, 1 H, CH), 3.41 - 3.58 (m, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 172.1, 156.9, 137.0, 136.4, 132.5, 130.2, 127.6, 123.8, 121.6, 118.9, 118.1, 111.5, 108.7, 54.1, 27.3 ppm. Mp = 170-174 °C (lit. 109-111 °C)[15]. $[\alpha]^{20}_{D}$ = +7.6 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{17}H_{15}N_3NaO_4$ [M+Na$^+$]: 348.0955, found: 348.0947. FT-IR ν_{max} (neat): 3328, 1715, 1522, 1324, 1198, 779cm^{-1}.

(3-Hydroxypicolinoyl)-D-tryptophan (9b)



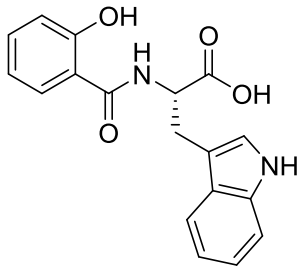
The desired compound (9b) was obtained as a yellow solid (287 mg, 0.89 mmol, 34%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, CD_3OD) δ = 8.28 (br. s., 1 H), 7.77 - 7.93 (m, 2 H), 7.53 (dd, *J*=7.5, 34.0 Hz, 1 H), 7.28 - 7.39 (m, 1 H), 7.11 - 7.18 (m, 1 H), 7.03 - 7.11 (m, 1 H), 6.88 - 7.00 (m, 1 H), 4.99 - 5.06 (m, 1 H, CH), 3.41 - 3.58 (m, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 172.1, 156.9, 137.0, 136.4, 132.5, 130.2, 127.6, 123.8, 121.6, 118.9, 118.1, 111.5, 108.7, 54.1, 27.3 ppm. Mp = 180-182 °C. $[\alpha]^{20}_{D}$ = -7.9 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{17}H_{16}N_3O_4$ [M+H$^+$]: 326.1135, found: 326.1128. FT-IR ν_{max} (neat): 3329, 1715, 1618, 1518, 1324, 1198, 778 cm^{-1}.

Picolinoyl-L-tryptophan (10)[17]



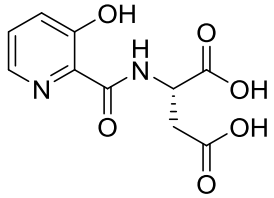
The desired compound (10)[17] was obtained as a white solid (478 mg, 1.55 mmol, 38%) starting from picolinic acid (500 mg, 4.07 mmol) and following the general procedure.
^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.88 (br. s., 1 H), 8.67 (d, *J*=8.0 Hz, 1 H), 8.60 (d, *J*=4.5 Hz, 1 H), 7.93 - 8.09 (m, 2 H), 7.56 - 7.63 (m, 1 H), 7.52 (d, *J*=8.0 Hz, 1 H), 7.32 (d, *J*=8.1 Hz, 1 H), 7.14 (d, *J*=2.0 Hz, 1 H), 7.05 (t, *J*=7.5 Hz, 1 H), 6.92 (t, *J*=7.5 Hz, 1 H), 4.73 - 4.84 (m, 1 H), 3.36 (d, *J*=6.0 Hz, 2 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 173.8, 164.2, 150.1, 149.4, 138.8, 137.0, 128.1, 127.7, 124.6, 122.7, 121.8, 119.2, 119.2, 112.3, 110.2, 53.7, 27.7 ppm. Mp = 144-146 °C. $[\alpha]^{20}_{D}$ = +10.2 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{17}H_{15}NaN_3O_3$ [M+Na$^+$]: 332.1006, found: 332.0993. FT-IR ν_{max} (neat): 3353, 1724, 1657, 1521, 1211, 741 cm^{-1}.

(2-Hydroxybenzoyl)-L-tryptophan (11)



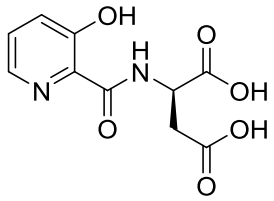
The desired compound (11)[17] was obtained as a white solid (339 mg, 1.05 mmol, 48%) starting from salicylic acid (300 mg, 2.17 mmol) and following the general procedure.
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.98 (br. s., 1 H, OH), 10.91 (d, *J*=1.5 Hz, 1 H, NH), 9.01 (d, *J*=7.5 Hz, 1 H, NH), 7.94 (dd, *J*=8.0, 1.5 Hz, 1 H), 7.57 (d, *J*=7.5 Hz, 1 H), 7.29 - 7.43 (m, 2 H), 7.18 (d, *J*=2.5 Hz, 1 H), 7.05 (td, *J*=7.5, 1.0 Hz, 1 H), 6.85 - 7.01 (m, 3 H), 4.63 - 4.84 (m, 1 H, CH), 3.31 (m, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.0, 168.4, 159.7, 137.0, 134.5, 129.8, 128.0, 124.5, 121.8, 119.7, 119.3, 119.0, 118.0, 116.8, 112.3, 110.6, 54.2, 27.6 ppm. Mp = 130-133 °C. $[\alpha]^{20}_{D}$ = -11.9 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{16}H_{18}NaN_2O_4$ [M+Na$^+$]: 347.1002, found: 347.0992. FT-IR ν_{max} (neat): 3395, 1718, 1597, 1490, 1215, 741 cm^{-1}.

(3-Hydroxypicolinoyl)-L-aspartic acid (12a)



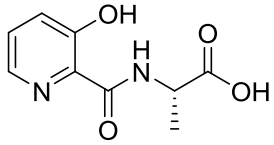
The desired compound (12a) was obtained as a pink solid (185 mg, 0.73 mmol, 28%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, CD_3OD) δ = 8.13 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.45 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.35 (d, *J*=8.5 Hz, 1 H), 4.99 (m, 1H, CH overlapped with H_2O, assigned from ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC spectra), 3.09 (dd, *J*=17.0, 5.5 Hz, 1 H, C*H*'H''), 2.98 (dd, *J*=17.0, 5.0 Hz, 1 H, CH'*H*'') ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 173.2, 172.6, 169.0, 158.0, 140.1, 131.3, 129.2, 126.1, 48.4, 35.8 ppm. Mp = 196-198 °C. $[\alpha]^{20}_{D}$ = +36.7 (c = 0.3 in MeOH). HRMS (ESI-TOF) calcd for $C_{10}H_{10}N_2NaO_6$ [M+Na^+]: 277.0431, found: 277.0426. FT-IR ν_{max} (neat): 3053, 1611, 1517, 1322, 1206, 802 cm^{-1}.

(3-Hydroxypicolinoyl)-D-aspartic acid (12b)



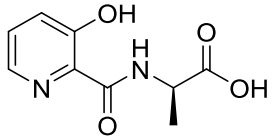
The desired compound (12b) was obtained as a pink solid (150 mg, 0.60 mmol, 23%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, CD_3OD) δ = 8.13 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.45 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.35 (d, *J*=8.5 Hz, 1 H), 4.99 (m, 1H, CH overlapped with H_2O, assigned from ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC spectra), 3.09 (dd, *J*=17.0, 5.5 Hz, 1 H, C*H*'H''), 2.98 (dd, *J*=17.0, 5.0 Hz, 1 H, CH'*H*'') ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 173.2, 172.6, 169.0, 158.0, 140.1, 131.3, 129.2, 126.1, 48.4, 35.8 ppm. Mp = 196-199 °C. $[\alpha]^{20}_{D}$ = -35.6 (c = 0.3 in MeOH). HRMS (ESI-TOF) calcd for $C_{10}H_{10}N_2NaO_6$ [M+Na^+]: 277.0431, found: 277.0424. FT-IR ν_{max} (neat): 3053, 1615, 1517, 1325, 1206, 803 cm^{-1}.

(3-Hydroxypicolinoyl)-L-alanine (13a)[15]



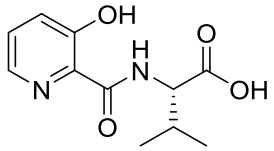
The desired compound (13a) was obtained as a white solid (190 mg, 0.91 mmol, 42%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.26 (s, 1 H, OH), 9.17 (d, *J*=7.5 Hz, 1 H, NH), 8.19 (dd, *J*=4.5, 1.0 Hz, 1 H), 7.55 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.43 (dd, *J*=8.5, 1.0 Hz, 1 H), 4.49 (quin, *J*=7.5×4 Hz, 1 H, CH), 1.45 (d, *J*=7.5 Hz, 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.1, 169.1, 158.1, 140.9, 131.7, 130.2, 126.9, 48.4, 17.8 ppm. Mp = 188-190 °C (187-189 °C)[15]. $[\alpha]^{20}_{D}$ = +24.4 (c = 0.5 in MeOH). HRMS (ESI-TOF) calcd for $C_9H_{10}N_2NaO_4$ [M+Na^+]: 233.0533, found: 233.0524. FT-IR ν_{max} (neat): 3354, 2947, 1729, 1596, 1187, 698 cm^{-1}.

(3-Hydroxypicolinoyl)-D-alanine (13b)



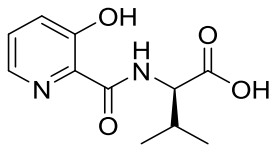
The desired compound (13b) was obtained as a white solid (170 mg, 0.81 mmol, 37%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.26 (s, 1 H, OH), 9.17 (d, *J*=7.5 Hz, 1 H, NH), 8.19 (dd, *J*=4.5, 1.0 Hz, 1 H), 7.55 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.43 (dd, *J*=8.5, 1.0 Hz, 1 H), 4.49 (quin, *J*=7.5×4 Hz, 1 H, CH), 1.45 (d, *J*=7.5 Hz, 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.1, 169.1, 158.1, 140.9, 131.7, 130.2, 126.9, 48.4, 17.8 ppm. Mp = 195-196 °C. $[\alpha]^{20}_{D}$ = -24.7 (c = 0.5 in MeOH). HRMS (ESI-TOF) calcd for $C_9H_{10}N_2NaO_4$ [M+Na$^+$]: 233.0533, found: 233.0522. FT-IR ν_{max} (neat): 3354, 2947, 1730, 1627, 1596, 1187, 701 cm^{-1}.

(3-Hydroxypicolinoyl)-L-valine (14a)



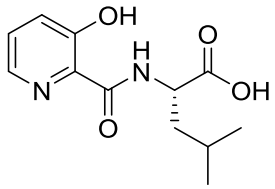
The desired compound (14a) was obtained as a white solid (180 mg, 0.76 mmol, 35%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.48 (br. s., 1 H, NH), 8.01 - 8.12 (m, 1 H), 7.23 - 7.41 (m, 2 H), 4.70 (dd, *J*=8.5, 4.5 Hz, 1 H, C*H*CO_2H), 2.32 - 2.49 (m, 1 H, C*H*$(CH_3)_2$), 1.05 (br. s., 3 H, CH_3), 1.03 (br. s., 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.2, 168.6, 157.9, 139.6, 130.8, 128.9, 126.5, 56.8, 31.1, 19.1, 17.7 ppm. Mp = 81-83 °C. $[\alpha]^{20}_{D}$ = +36.9 (c = 0.3 in MeOH). HRMS (ESI-TOF) calcd for $C_{11}H_{14}N_2NaO_4$ [M+Na$^+$]: 261.0846, found: 261.0844. FT-IR ν_{max} (neat): 3348, 2964, 1728, 1647, 1528, 1451, 805 cm^{-1}.

(3-Hydroxypicolinoyl)-D-valine (14b)



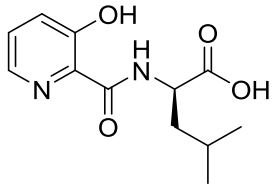
The desired compound (14b) was obtained as a white solid (196 mg, 0.83 mmol, 38%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.65 (br. s., 1 H, OH), 8.46 (d, *J*=8.0 Hz, 1 H, NH), 8.10 (dd, *J*=4.0, 1.0 Hz, 1 H), 7.37 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.32 (d, *J*=8.0 Hz, 1 H), 4.72 (dd, *J*=9.0, 5.0 Hz, 1 H, C*H*CO_2H), 2.40 (sptd, *J*=6.8×6, 5.5 Hz, 1 H, C*H*$(CH_3)_2$), 1.07 (d, *J*=7.0 Hz, 3 H, CH_3), 1.06 (d, *J*=7.0 Hz, 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.2, 168.6, 157.9, 139.6, 130.8, 128.9, 126.5, 56.8, 31.1, 19.1, 17.7 ppm. Mp = 73-77 °C. $[\alpha]^{20}_{D}$ = -36.5 (c = 0.3 in MeOH). HRMS (ESI-TOF) calcd for $C_{11}H_{14}N_2NaO_4$ [M+Na$^+$]: 261.0846, found: 261.0846. FT-IR ν_{max} (neat): 3378, 3340, 2965, 2874, 1740, 1647, 1528, 1451, 1339, 806 cm^{-1}.

(3-Hydroxypicolinoyl)-L-leucine (15a)



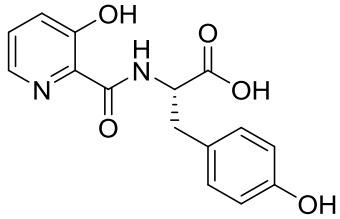
The desired compound (15a) was obtained as a white solid (230 mg, 0.91 mmol, 42%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.82 (br. s., 1 H, OH), 8.31 (d, *J*=8.5 Hz, 1 H, NH), 8.09 (d, *J*=4.5 Hz, 1 H), 7.35 - 7.40 (m, 1 H), 7.30 - 7.35 (m, 1 H), 4.70 - 4.89 (m, 1 H, C*H*COOH), 1.70 - 1.95 (m, 3 H, CH_2, CH(CH_3)$_2$), 1.01 (br. s, 3 H, CH_3), 0.99 (br. s, 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 177.4, 168.7, 157.9, 139.7, 130.9, 128.9, 126.3, 50.3, 41.0, 24.9, 22.8, 21.7 ppm. Mp = 93-95 °C. $[\alpha]^{20}_{D}$ = +7.4 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{12}H_{16}N_2NaO_4$ [M+Na^+]: 275.1002, found: 275.1003. FT-IR ν_{max} (neat): 3372, 2961, 1724, 1654, 1445, 1295, 632 cm^{-1}.

(3-Hydroxypicolinoyl)-D-leucine (15b)



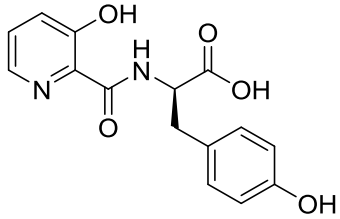
The desired compound (15b) was obtained as a white solid (200 mg, 0.79 mmol, 37%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.82 (br. s., 1 H, OH), 8.31 (d, *J*=8.5 Hz, 1 H, NH), 8.09 (d, *J*=4.5 Hz, 1 H), 7.35 - 7.40 (m, 1 H), 7.30 - 7.35 (m, 1 H), 4.70 - 4.89 (m, 1 H, C*H*COOH), 1.70 - 1.95 (m, 3 H, CH_2, CH(CH_3)$_2$), 1.01 (br. s, 3 H, CH_3), 0.99 (br. s, 3 H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 177.4, 168.7, 157.9, 139.7, 130.9, 128.9, 126.3, 50.3, 41.0, 24.9, 22.8, 21.7 ppm. Mp = 90-92 °C. $[\alpha]^{20}_{D}$ = -7.8 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{12}H_{16}N_2NaO_4$ [M+Na^+]: 275.1002, found: 275.0998. FT-IR ν_{max} (neat): 3372, 2961, 1724, 1654, 1445, 1295, 632 cm^{-1}.

(3-Hydroxypicolinoyl)-L-tyrosine (16a)



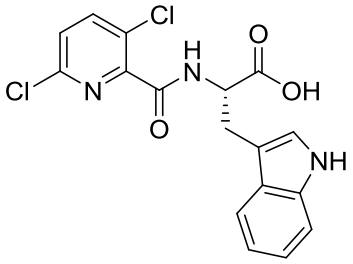
The desired compound (16a) was obtained as a white solid (294 mg, 0.97 mmol, 45%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, CD_3OD) δ = 8.10 (dd, *J*=4.5, 1.0 Hz, 1 H), 7.44 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.34 (dd, *J*=8.5, 1.0 Hz, 1 H), 7.05 (d, *J*=8.5 Hz, 2 H), 6.69 (d, *J*=8.5 Hz, 2 H), 4.84 (dd, *J*=7.0, 5.5 Hz, 1 H, CH), 3.23 (dd, *J*=14.0, 5.5 Hz, 1 H, C*H*'H''), 3.13 (dd, *J*=14.0, 7.0 Hz, 1 H, CH'*H*'') ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 173.1, 168.7, 158.0, 156.5, 140.0, 131.2, 130.4, 129.1, 127.4, 126.2, 115.3, 53.5, 36.6 ppm. Mp = 123-125 °C. $[\alpha]^{20}_{D}$ = +41.6 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{14}N_2NaO_5$ [M+Na$^+$]: 325.0795, found: 325.0782. FT-IR ν_{max} (neat): 3497, 2941, 1674, 1534, 1275, 1132, 836, 637 cm^{-1}.

(3-Hydroxypicolinoyl)-D-tyrosine (16b)



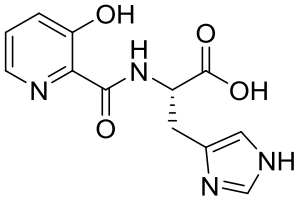
The desired compound (16b) was obtained as a white solid (294 mg, 0.97 mmol, 45%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.93 (d, *J*=8.5 Hz, 1 H, NH), 8.15 (dd, *J*=4.5, 1.5 Hz, 1 H), 7.52 (dd, *J*=8.5, 4.5 Hz, 1 H), 7.40 (dd, *J*=8.5, 1.0 Hz, 1 H), 7.01 (d, *J*=8.5 Hz, 2 H), 6.62 (d, *J*=8.5 Hz, 2 H), 4.57 - 4.72 (m, 1 H, CH), 3.04 - 3.15 (m, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 168.7, 157.6, 156.4, 140.5, 131.1, 130.5, 129.9, 127.6, 126.6, 115.6, 53.8, 35.7 ppm. Mp = 123-125 °C. $[\alpha]^{20}_{D}$ = -40.9 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{15}H_{14}N_2NaO_5$ [M+Na$^+$]: 325.0795, found: 325.0783. FT-IR ν_{max} (neat): 3488, 2941, 1674, 1276, 1132, 636 cm^{-1}.

(3,6-Dichloropicolinoyl)-L-tryptophan (26)



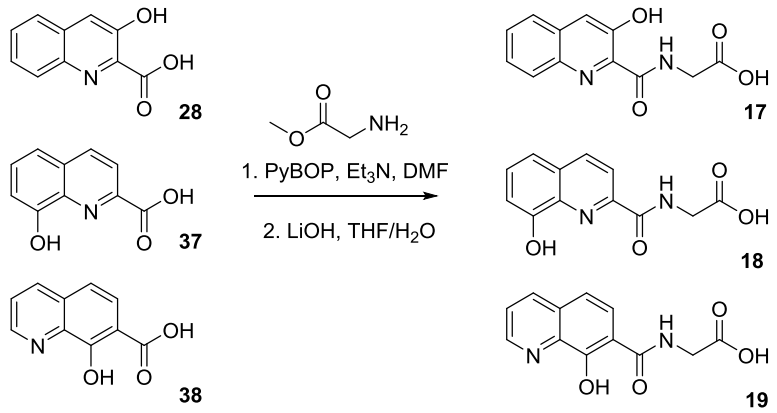
3,6-Dichloropicolinic acid (100 mg, 0.52 mmol) and CDI (102 mg, 0.63 mmol, 1.2 eq) were stirred in the anhydrous DMF (2 mL) for 10 min prior to the addition of a solution of tryptophan ethyl ester hydrochloride (168 mg, 0.63 mmol, 1.2 eq) and Et_3N (88 µL, 0.63 mmol, 1.2 eq) in DMF (3 mL). The resultant mixture was stirred overnight at room temperature. Upon completion of the reaction the DMF was evaporated *in vacuo* and the resultant residue was suspended in DCM (10 mL) and washed with H_2O (3 x 5 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to column chromatography (Biotage SNAP KP-SIL™ 10 g cartridge, eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 4 mL) and subsequently treated with LiOH · H_2O (109 mg, 2.6 mmol, 5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. The formed precipitate was filtered-off and dried *in vacuo* to yield the desired product (26) as a yellow solid (58 mg, 0.16 mmol, 30%). ^{1}H NMR (700 MHz, DMSO-d_6) δ = 10.87 (s, 1 H), 8.91 (d, *J*=8.0 Hz, 1 H, NH), 8.06 (d, *J*=8.5 Hz, 1 H), 7.66 (d, *J*=8.5 Hz, 1 H), 7.56 (d, *J*=7.5 Hz, 1 H), 7.33 (d, *J*=8.0 Hz, 1 H), 7.18 (d, *J*=2.0 Hz, 1 H), 7.07 (t, *J*=7.5 Hz, 1 H), 6.98 (t, *J*=7.5 Hz, 1 H), 4.67 (td, *J*=8.0, 5.1 Hz, 1 H, CH), 3.28 (dd, *J*=15.0, 5.0 Hz, 2 H, CH_2), 3.17 (dd, *J*=14.5, 8.3 Hz, 1 H, CH_2) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 173.1, 163.5, 150.9, 147.8, 142.4, 136.5, 128.4, 127.6, 127.3, 124.2, 121.4, 118.9, 118.6, 111.8, 109.9, 53.6, 27.3 ppm. Mp = 227-229 °C. $[\alpha]^{20}_D$ = -4.8 (c = 0.1 in MeOH). HRMS (ESI-TOF) calcd for $C_{17}H_{13}Cl_2NaN_3O_3$ [M+Na$^+$]: 400.0226, found: 400.0210. FT-IR ν_{max} (neat): 3392, 3378, 3064, 1715, 1661, 1507, 1280, 746 cm^{-1}.

(3-Hydroxypicolinoyl)-L-histidine (27)



The desired compound (27) was obtained as a white solid (250 mg, 0.91 mmol, 35%) from 3-hydroxypicolinic acid (300 mg, 2.16 mmol).
^{1}H NMR (400 MHz, D_2O) δ = 8.49 (s, 1 H), 8.23 (d, *J*=5.0 Hz, 1 H), 7.99 (d, *J*=9.0 Hz, 1 H), 7.85 (dd, *J*=9.0, 5.5 Hz, 1 H), 7.22 (s, 1 H), 4.92 (dd, *J*=7.5, 5.0 Hz, 1 H, CH), 3.39 (dd, *J*=15.5, 5.0 Hz, 1 H, C*H*'H''), 3.29 (dd, *J*=15.5, 7.5 Hz, 1 H, CH'*H*'') ppm. ^{13}C NMR (101 MHz, D_2O) δ = 173.2, 160.7, 157.5, 135.8, 134.9, 133.9, 130.8, 128.7, 127.8, 117.7, 52.9, 26.6 ppm. Mp = 222-225 °C. $[\alpha]^{20}_D$ = -9.6 (c = 0.2 in MeOH). HRMS (ESI-TOF) calcd for $C_{12}H_{12}N_4O_4$ [M+H$^+$]: 277.0931, found: 277.0928. FT-IR ν_{max} (neat): 3217, 2460, 1720, 1658, 1523, 810, 621 cm^{-1}.

Synthesis of quinoline derivatives

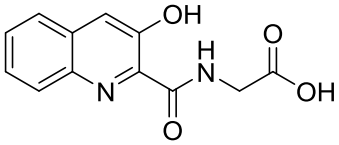


Scheme S5: Synthesis of quinoline based inhibitors.

General procedure for synthesis of glycine conjugates with quinoline derivatives:

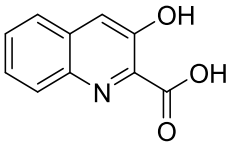
The quinoline analogue (300 mg, 1.59 mmol), glycine methyl ester hydrochloride (240 mg, 1.91 mmol, 1.2 eq), PyBOP (990 mg, 1.91 mmol, 1.2 eq) and Et_3N (553 µl, 3.98 mmol, 2.5 mmol) were dissolved in anhydrous DMF (10 mL) and subsequently stirred at room temperature for 24h. Upon the completion of the reaction the DMF was evaporated *in vacuo* and the resultant residue was suspended in DCM (30 mL) and washed with H_2O (2 x 15 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to the column chromatography (Biotage SNAP KP-SIL™ 25 g cartridge, eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and subsequently treated with LiOH · H_2O (5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the resultant aqueous solution was neutralized with conc. HCl. If a precipitate was formed it was filtered-off and dried *in vacuo* to yield the desired product. In case no precipitate was formed, the aqueous solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield the desired product.

(3-Hydroxyquinoline-2-carbonyl)glycine (17)[15]



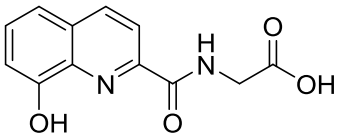
The desired compound (17)[15] was obtained as a yellow solid (150 mg, 0.60 mmol, 38%) starting from 3-hydroxyquinoline-2-carboxylic acid (300 mg, 1.59 mmol) and following the general procedure.
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.55 (t, *J*=5.0 Hz, 1 H, NH), 8.44 (d, *J*=8.5 Hz, 1 H), 7.92 (d, *J*=7.5 Hz, 1 H), 7.61 - 7.75 (m, 2 H), 7.51 (s, 1 H, OH), 4.17 (d, *J*=5.5 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 170.5, 164.9, 153.9, 136.7, 130.8, 130.4, 128.8, 127.6, 119.6, 111.1, 41.8 ppm. Mp = 218-220°C. HRMS (ESI-TOF) calcd for $C_{12}H_{10}NaN_2O_4$ [M+Na^+]: 269.0533, found: 269.0538. FT-IR ν_{max} (neat): 3346, 1712, 1651, 1332, 835 cm^{-1}.

3-Hydroxyquinoline-2-carboxylic acid (28) [18]



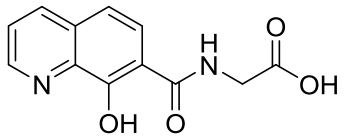
3-hydroxyquinoline-2-carboxylic acid (28) was synthesized according to the literature procedure and obtained as a brown solid[18].
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.91 (br. s., 1 H, OH), 8.43 - 8.50 (m, 1 H), 8.02 - 8.11 (m, 1 H), 7.77 - 7.84 (m, 3 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 165.5, 163.5, 151.5, 134.0, 130.9, 130.3, 129.9, 127.8, 118.6, 114.4 ppm. Mp = 155-157 °C. HRMS (EI/FI) calcd for $C_{10}H_7NO_3$ [M^+]: 189.0426, found: 189.0426. FT-IR ν_{max} (neat): 3374, 1655, 1510, 1448, 1229, 1141, 763 cm^{-1}.

(8-Hydroxyquinoline-2-carbonyl)glycine (18)[19]



The desired compound (18) was obtained as a yellow solid (160 mg, 0.66 mmol, 42%) starting from 8-hydroxyquinoline-2-carboxylic acid and following the general procedure.
^{1}H NMR (400 MHz, CD_3OD) δ = 8.42 (d, *J*=8.5 Hz, 1 H), 8.18 (d, *J*=8.5 Hz, 1 H), 7.55 (t, *J*=8.0 Hz, 1 H), 7.44 (dd, *J*=8.5, 1.0 Hz, 1 H), 7.18 (dd, *J*=7.5, 1.5 Hz, 1 H), 4.23 (s, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 172.1, 166.2, 154.0, 147.3, 137.9, 137.4, 130.5, 129.7, 118.9, 117.9, 111.7, 40.9 ppm. Mp = 236-238 °C (lit. 292 °C)[19]. HRMS (ESI-TOF) calcd for $C_{12}H_{10}NaN_2O_4$ [M+Na^+]: 269.0533, found: 269.0529. FT-IR ν_{max} (neat): 3344, 1726, 1656, 1504, 1210, 855 cm^{-1}.

(8-hydroxyquinoline-7-carbonyl)glycine (19)[20]

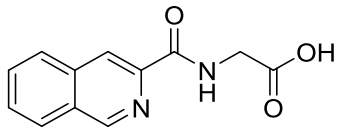


The desired compound (19)[20] was obtained as a yellow solid (160 mg, 0.66 mmol, 42%) starting from 8-hydroxyquinoline-7-carboxylic acid (Sigma-Aldrich) (300 mg, 1.59 mmol) and following the general procedure.

^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.17 (t, *J*=5.5 Hz, 1 H, NH), 8.92 (d, *J*=3.0 Hz, 1 H), 8.37 (d, *J*=8.0 Hz, 1 H), 8.00 (d, *J*=9.0 Hz, 1 H), 7.67 (dd, *J*=8.0, 4.0 Hz, 1 H), 7.45 (d, *J*=9.0 Hz, 1 H), 4.07 (d, *J*=5.0 Hz, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.5, 168.0, 156.4, 149.5, 139.3, 136.8, 131.1, 126.1, 124.1, 117.6, 113.2, 41.8 ppm. Mp = 224-226°C, HRMS (ESI-TOF) calcd for $C_{12}H_{10}NaN_2O_4$ [M+Na^+]: 269.0533, found: 269.0542. FT-IR ν_{max} (neat): 3350, 2160, 1695, 1632, 1543, 1300, 832 cm^{-1}.

Synthesis of other bicyclic derivatives

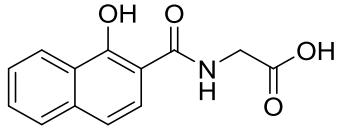
(Isoquinoline-3-carbonyl)glycine (23)[21]



Isoquinoline-3-carboxylic acid (500 mg, 2.89 mmol), glycine methyl ester hydrochloride (363 mg, 2.89 mmol, 1 eq), PyBOP (1.65 g, 3.18 mmol, 1.2 eq) and Et_3N (400 µL, 2.89 mmol, 1 eq) were dissolved in the anhydrous DMF (10 mL) and stirred at room temperature for 24h. Upon completion of the reaction the DMF was evaporated *in vacuo* and the resultant residue was suspended in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to the column chromatography (eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and treated with LiOH · H_2O (600 mg, 14.45 mmol, 5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. The precipitate was collected by filtration and dried *in vacuo* to yield the desired product (266 mg, 1.16 mmol, 40%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.42 (s, 1 H, H6), 9.14 (t, *J*=6.0 Hz, 1 H, NH), 8.58 (s1 H, H5), 8.27 (d, *J*=8.0 Hz, 1 H, H3), 8.22 (d, *J*=8.0 Hz, 1 H, H4) 7.90 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, H2) 7.83 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, H1) 4.05 (d, *J*=6.0 Hz, 2 H, H7', H7'')ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.7, 164.9, 152.1, 143.8, 135.8, 131.9, 129.8, 129.7, 128.5, 128.3, 120.3, 41.6 ppm. Mp = 208-210 °C (223-224 °C)[21]. HRMS (ESI-TOF) calcd for $C_{12}H_{10}N_2NaO_3$ [M+Na$^+$]: 253.0584, found: 253.0595, FT-IR ν_{max} (neat): 3378, 1733, 1631, 1531, 1233, 766 cm^{-1}.

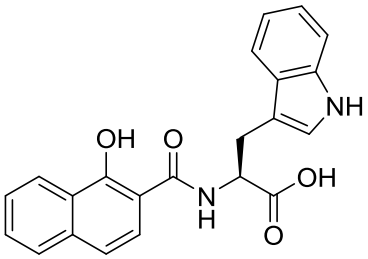
(1-Hydroxy-2-naphthoyl)glycine (24)



1-Hydroxy-2-naphthoic acid (500 mg, 2.66 mmol) was stirred with CDI (639 mg, 3.94 mmol, 1.5 eq) in the anhydrous DMF (5 mL) for 10 min prior to the addition of mixture of glycine methyl ester hydrochloride (400 mg, 3.18 mmol, 1.2 eq) and Et_3N (440 µL, 3.18 mmol, 1.2 eq). The resultant mixture was stirred at room temperature for 24h. Upon completion of the reaction DMF was evaporated *in vacuo* and the residue was suspended in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to the column chromatography (eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and treated with LiOH · H_2O (560 mg, 13.30 mmol, 5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. The precipitate was collected by filtration and dried *in vacuo* to yield the desired product as a solid (325 mg, 1.33 mmol, 50%).

^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.29 (br. s., 1 H, OH), 9.43 (t, *J*=6.0 Hz, 1 H, NH), 8.27 (d, *J*=8.5 Hz, 1 H, ArH), 7.84 - 7.95 (m, 2 H, ArH), 7.65 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, H4), 7.56 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, H3), 7.41 (d, *J*=8.5 Hz, 1 H, ArH), 4.01 (d, *J*=6.0 Hz, 2 H, H7', H7'') ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ = 171.4, 171.3, 160.1, 136.3, 129.5, 128.0, 126.4, 125.1, 123.5, 122.9, 118.3, 107.3, 41.5 ppm. Mp = 225-227 °C. HRMS (ESI-TOF) calcd for $C_{13}H_{10}N_2O_4$ [M-H$^+$]: 244.0615, found: 244.0615, FT-IR ν_{max} (neat): 3408, 1730, 1622, 1547, 1243, 760 cm^{-1}.

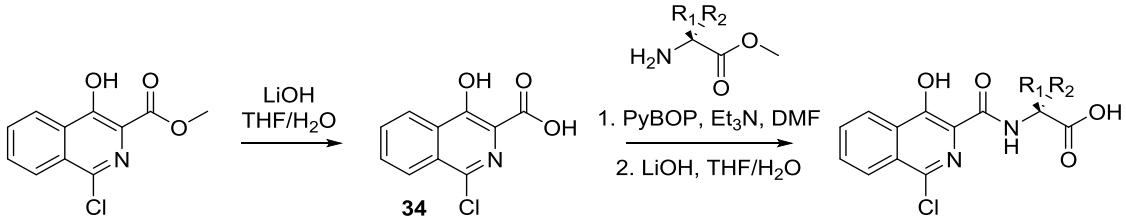
(1-Hydroxy-2-naphthoyl)-L-tryptophan (25)



1-Hydroxy-2-naphthoic acid (500 mg, 2.66 mmol) was stirred with CDI (639 mg, 3.94 mmol, 1.5 eq) in anhydrous DMF (5 mL) for 10 min prior to the addition of mixture of tryptophan methyl ester hydrochloride (847 mg, 3.18 mmol, 1.2 eq) and Et_3N (440 µL, 3.18 mmol, 1.2 eq). The resultant mixture was stirred at room temperature for 24h. Upon completion of the reaction DMF was evaporated *in vacuo* and the residue was suspended in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to the column chromatography (eluent system: cHex/EtOAc). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and treated with LiOH · H_2O (560 mg, 13.30 mmol, 5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. The precipitate was collected by filtration and dried *in vacuo* to yield the desired product as a solid (460 mg, 1.22 mmol, 46%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.13 (br. s., 1 H, OH), 10.87 (d, *J*=1.5 Hz, 1 H, NH), 9.17 (d, *J*=8.0 Hz, 1 H, NH), 8.22 (d, *J*=8.5 Hz, 1 H, ArH), 7.97 (d, *J*=9.0 Hz, 1 H, ArH), 7.87 (d, *J*=8.5 Hz, 1 H, ArH), 7.58 - 7.68 (m, 2 H, ArH), 7.54 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, ArH), 7.39 (d, *J*=9.0 Hz, 1 H, ArH), 7.33 (d, *J*=8.0 Hz, 1 H, ArH), 7.25 (d, *J*=2.0 Hz, 1 H, ArH), 7.06 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, ArH), 6.99 (ddd, *J*=8.0, 7.0, 1.0 Hz, 1 H, ArH), 4.75 (ddd, *J*=9.5, 7.5, 5.0 Hz, 1 H, CH), 3.27 - 3.42 (m, 2 H, CH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 173.5, 171.1, 160.0, 136.5, 136.3, 129.5, 127.9, 127.4, 126.4, 125.0, 124.1, 123.4, 123.2, 121.5, 119.0, 118.5, 118.2, 112.0, 110.6, 107.2, 54.1, 26.8 ppm. Mp = 230-232 °C, $[\alpha]^{20}_D$ = -41.3 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{22}H_{17}N_2O_4$ [M-H^+]: 373.1194, found: 373.1197, FT-IR ν_{max} (neat): 3345, 1708, 1620, 1527, 1283, 746 cm^{-1}.

Synthesis of 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid derivatives

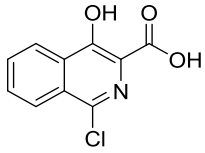


Scheme S6 Synthesis of isoquinoline based inhibitors.

code	R_1	R_2
20	H	H
21a	CH_2-3-indole	H
21b	H	CH_2-3-indole
22a	CH_3	H
22b	H	CH_3
29a	$CH(CH_3)_2$	H
29b	H	$CH(CH_3)_2$
30a	$CH_2CH(CH_3)_2$	H
30b	H	$CH_2CH(CH_3)_2$
31a	CH_2Ph	H
31b	H	CH_2Ph
32a	$(CH_2)_2COOH$	H
32b	H	$(CH_2)_2COOH$
33a	CH_2COOH	H
33b	H	CH_2COOH

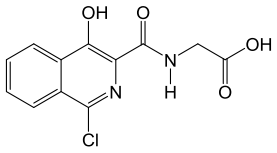
General procedure for synthesis of amino acids conjugates of 1-chloro-4-hydroxyisoquinolines:
1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol), amino acid methyl ester (1.2 eq), PyBOP (1.2 eq) and Et_3N (1.5 mmol) were dissolved in the anhydrous DMF (5 mL) and subsequently stirred at room temperature for 24h. Upon completion of the reaction the DMF was evaporated *in vacuo* and the resultant residue was suspended in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to column chromatography (Biotage SNAP KP-SIL^{TM} 25 g cartridge, eluent system: cHex/EtOAc, ratio for elution of each methyl ester is given along with characterization of final product). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and subsequently treated with LiOH · H_2O (5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. If precipitate was formed it was filtered-off and dried *in vacuo* to yield the desired product. In case that no precipitate was formed, the aqueous solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield desired product.

1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (34)[21]



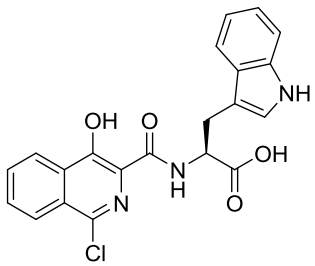
Commercially available methyl 1-chloro-4-hydroxyisoquinoline-3-carboxylate (5.0 g, 21.0 mmol) was dissolved in a mixture of H_2O and THF (1:1, 50 mL) and treated with lithium hydroxide (LiOH · H_2O, 4.42 g, 0.21 mol, 10 eq). The reaction was stirred at room temperature for 24h. The THF was evaporated and resulting water solution extracted with EtOAc (2 x 20 mL). The aqueous phase was acidified with conc. HCl (pH = 1) and subsequently extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated *in vacuo* to yield the desired compound as a white solid (4.47 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.31-8.36 (m, 1H, ArH), 8.21-8.25 (m, 1H, ArH), 7.96-8.01 (m, 2H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.5, 156.0, 138.8, 132.1, 131.8, 129.1, 128.8, 125.9, 123.2, 119.5 ppm. Mp = 202-205°C (194-196 °C)[21]. HRMS (ESI-TOF) calcd. for $C_{10}H_6ClNO_3$ [M+H$^+$]: 221.9963, found: 221.9958. FT-IR ν_{max} (neat): 2966, 1656, 1312, 1233, 768 cm^{-1}.

2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)acetic acid (20)[22]



The desired compound (20)[22] was obtained as a white solid (175 mg, 46%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.16 (t, *J*=6.0 Hz, 1 H, NH), 8.30 (m, 1 H, ArH) 8.24 (m, 1 H, ArH), 7.84 - 8.09 (m, 2 H, ArH) 4.02 (d, *J*=6.0 Hz, 2 H, CH_2) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.4, 169.6, 155.1, 139.4, 132.6, 132.5, 130.3, 129.3, 126.9, 123.8, 121.3, 41.7 ppm. Mp = 212-214 °C, HRMS (ESI-TOF) calcd for $C_{12}H_9ClN_2NaO_4$ [M+Na$^+$]: 303.0144, found: 303.0143, FT-IR ν_{max} (neat): 3402, 2915, 1712, 1641, 1354, 1255, 794 cm^{-1}.

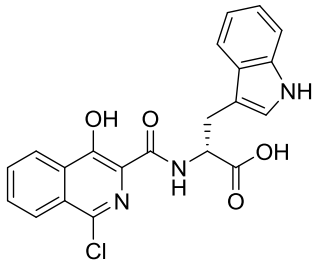
(S)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(1H-indol-3-yl)propanoic acid (21a)[23]



The desired compound (21a) was obtained as an off-white solid (232 mg, 42%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (s, 1H, OH), 10.93 (s, 1H, NH indole), 8.80 (d, *J* = 8.1 Hz, 1H, NH), 8.30 - 8.38 (m, 1H, ArH), 8.24 - 8.30 (m, 1H, ArH), 7.93 - 8.08 (m, 2H, ArH), 7.58 (d, *J* = 8.0 Hz, 1H, ArH), 7.33 (d, *J* = 8.0 Hz, 1H, ArH), 7.19 (d, *J* = 2.0 Hz, 1H, H7), 7.05 (t, *J* = 7.0 Hz, 1H, ArH), 6.95 (t, *J* = 7.5 Hz, 1H, ArH), 4.83 (td, *J* = 7.5, 5.0 Hz, 1H, H5), 3.46 (overlapping with water signal, 2H, H6, determined by HSQC) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.9, 168.7, 154.7, 139.0, 136.6, 132.3, 132.3,

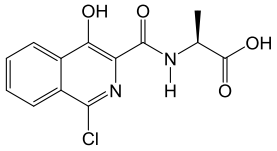
129.9, 129.0, 127.7, 126.6, 124.2, 123.5, 121.5, 120.8, 119.0, 118.7, 111.9, 109.8, 53.3, 26.9 ppm. Mp = 205-208 °C (lit. 205-208 °C)[23], $[\alpha]^{20}_{D}$ = -21.7 (c = 0.133 in DMSO). HRMS (ESI-TOF) calcd for $C_{21}H_{16}ClN_3NaO_4$ [M+Na$^+$]: 432.0722, found: 432.0737. FT-IR ν_{max} (neat): 3365, 1718, 1633, 1529, 1320, 768 cm^{-1}.

(R)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(1H-indol-3-yl)propanoic acid (21b)[23]



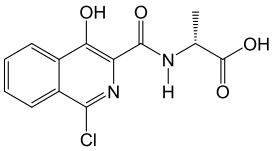
The desired compound (21b) was obtained as a white solid (249 mg, 45%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (s, 1H, OH), 10.93 (s, 1H, NH indole), 8.80 (d, *J* = 8.0 Hz, 1H, NH), 8.30 - 8.38 (m, 1H, ArH), 8.24 - 8.30 (m, 1H, ArH), 7.93 - 8.08 (m, 2H, ArH), 7.58 (d, *J* = 8.0 Hz, 1H, ArH), 7.33 (d, *J* = 8.0 Hz, 1H, ArH), 7.19 (d, *J* = 2.0 Hz, 1H, H7), 7.05 (t, *J* = 7.0 Hz, 1H, ArH), 6.95 (t, *J* = 7.5 Hz, 1H, ArH), 4.83 (td, *J* = 7.5, 5.0 Hz, 1H, H5), 3.46 (overlapped with water signal, 2H, H6, determined by HSQC) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.9, 168.7, 154.7, 139.0, 136.6, 132.3, 132.3, 129.9, 129.0, 127.7, 126.6, 124.2, 123.5, 121.5, 120.8, 119.0, 118.7, 111.9, 109.8, 53.3, 26.9 ppm. Mp = 212-214 °C (lit. 212-214 °C)[23], $[\alpha]^{20}_{D}$ = 24.9 (c = 0.088 in DMSO). HRMS (ESI-TOF) calcd for $C_{21}H_{16}ClN_3NaO_4$ [M+Na$^+$]: 432.0722, found: 432.0729. FT-IR ν_{max} (neat): 3362, 1634, 1529, 1319, 770 cm^{-1}.

(S)-2(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)propanoic acid (22a)[24]



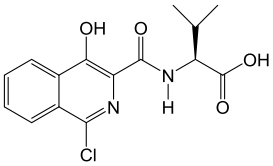
The desired compound (22a) was obtained as a white solid (145 mg, 38%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.15 (d, *J*=7.5 Hz, 1 H, NH), 8.39 - 8.45 (m, 1 H, ArH), 8.32 - 8.39 (m, 1 H, ArH), 8.02 - 8.10 (m, 2 H, ArH), 4.63 (quin, *J*=7.5 Hz, 1 H, CH), 1.55 (d, *J*=7.5 Hz, 3 H, CH_3) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 173.7, 168.7, 154.9, 139.0, 132.3, 132.2, 130.0, 129.0, 126.6, 123.5, 120.9, 48.1, 17.4 ppm. Mp =195-198 °C (lit. 178-180 °C)[24], $[\alpha]^{20}_{D}$ = -11.7 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{13}H_{10}ClN_2O_4$ [M-H$^+$]: 293.0335, found: 293.0327, FT-IR ν_{max} (neat): 3440, 3372, 1735, 1620, 1547, 1361, 1286, 770 cm^{-1}.

(R)-2(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)propanoic acid (22b)[24]



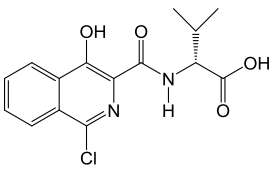
The desired compound (22b) was obtained as a white solid (148 mg, 40%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.15 (d, *J*=7.5 Hz, 1 H, NH), 8.39 - 8.45 (m, 1 H, ArH), 8.32 - 8.39 (m, 1 H, ArH), 8.02 - 8.10 (m, 2 H, ArH), 4.63 (quin, *J*=7.5 Hz, 1 H, CH), 1.55 (d, *J*=7.5 Hz, 3 H, CH_3) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 173.7, 168.7, 154.9, 139.0, 132.3, 132.2, 130.0, 129.0, 126.6, 123.5, 120.9, 48.1, 17.4 ppm. Mp =191-193 °C (lit. 116-118 °C)[24], $[\alpha]^{20}_{D}$ = +10.5 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{13}H_{10}ClN_2O_4$ [M-H$^+$]: 293.0335, found: 293.0329, FT-IR ν_{max} (neat): 3441, 3370, 1735, 1622, 1544, 1361, 1286, 772 cm^{-1}.

(S)-Methyl 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-3-methylbutanoic acid (29a)[25]



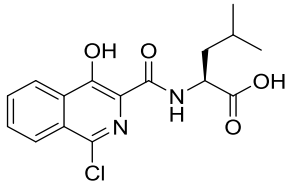
The desired compound (29a) was obtained as a white solid (148 mg, 34%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.55 (d, *J*=8.5 Hz, 1 H, NH), 8.40 (m, 1 H, ArH), 8.35 (m, 1 H, ArH), 8.06 (m, 2 H, ArH), 4.49 (dd, *J*=8.5, 5.5 Hz, 1 H, C*H*CO_2H), 2.38 (sptd, *J*=7.0×4, 5.5 Hz, 1 H, C*H*$(CH_3)_2$), 1.04 (d, *J*=7.0 Hz, 3 H, CH_3), 1.03 (d, *J*=7.0 Hz, 3 H, CH_3) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 168.7, 154.8, 139.2, 132.4, 132.3, 130.0, 129.1, 126.6, 123.5, 120.6, 57.6, 30.6, 19.5, 18.6 ppm. Mp =165-168°C (lit. 178-180 °C)[25], $[\alpha]^{20}_{D}$ = +16.7 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{15}ClNaN_2O_4$ [M+H$^+$]: 345.0613, found: 345.0611, FT-IR ν_{max} (neat): 3447, 3373, 2965, 1759, 1572, 1259, 776 cm^{-1}.

(R)-Methyl 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-3-methylbutanoic acid (29b)[25]



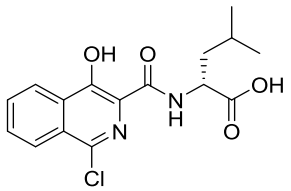
The desired compound (29b) was obtained as a white solid (165 mg, 38%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.55 (d, *J*=8.5 Hz, 1 H, NH), 8.40 (m, 1 H, ArH), 8.35 (m, 1 H, ArH), 8.06 (m, 2 H, ArH), 4.49 (dd, *J*=8.5, 5.5 Hz, 1 H, C*H*CO_2H), 2.38 (sptd, *J*=7.0×4, 5.5 Hz, 1 H, C*H*$(CH_3)_2$), 1.04 (d, *J*=7.0 Hz, 3 H, CH_3), 1.03 (d, *J*=7.0 Hz, 3 H, CH_3) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 168.7, 154.8, 139.2, 132.4, 132.3, 130.0, 129.1, 126.6, 123.5, 120.6, 57.6, 30.6, 19.5, 18.6 ppm. Mp =172-175°C (lit. 178-180 °C)[25], $[\alpha]^{20}_{D}$ = -18.1 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{15}ClNaN_2O_4$ [M+H$^+$]: 345.0613, found: 345.0614, FT-IR ν_{max} (neat): 3447, 3373, 2965, 1759, 1572, 1259, 776 cm^{-1}.

(S)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-4-methylpentanoic acid (30a)[23]



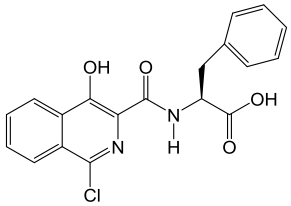
The desired compound (30a) was obtained as an off-white solid (160 mg, 35%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.67 (br. s., 1H, OH), 9.03 (d, *J* = 8.5 Hz, 1H, NH), 8.29 - 8.35 (m, 1H, ArH), 8.24 - 8.29 (m, 1H, ArH), 7.91 - 8.06 (m, 2H, ArH), 4.44 - 4.66 (m, 1H, H5), 1.96 (m, 1H, H6'), 1.54 - 1.77 (m, 2H, H6", H7), 0.91 (d, *J* = 6.0 Hz, 3H, H8), 0.92 (d, *J* = 6.0 Hz, 3H, H9) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.0, 169.7, 155.3, 139.4, 132.6, 132.6, 130.4, 129.4, 126.9, 123.8, 121.2, 51.1, 39.6 (overlapped with DMSO signal), 25.4, 23.8, 22.1 ppm. Mp = 158-160 °C (lit. 158-160 °C)[23], $[\alpha]^{20}_{D}$ = 13.4 (c = 0.104 in DMSO). HRMS (ESI-TOF) calcd for $C_{16}H_{17}ClN_2NaO_4$ [M+Na$^+$]: 359.0769, found: 359.0763. FT-IR ν_{max} (neat): 3380, 2966, 1656, 1312, 1233, 768 cm^{-1}.

(R)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)-4-methylpentanoic acid (30b)[23]



The desired compound (30b) was obtained as an off-white solid (146 mg, 32%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.67 (br. s., 1H, OH), 9.03 (d, *J* = 8.5 Hz, 1H, NH), 8.29 - 8.35 (m, 1H, ArH), 8.24 - 8.29 (m, 1H, ArH), 7.91 - 8.06 (m, 2H, ArH), 4.44 - 4.66 (m, 1H, H5), 1.96 (m, 1H, H6'), 1.54 - 1.77 (m, 2H, H6", H7), 0.91 (d, 3H, *J* = 6.0 Hz, H8), 0.92 (d, *J* = 6.0 Hz, 3H, H9) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.0, 169.7, 155.3, 139.4, 132.6, 132.6, 130.4, 129.4, 126.9, 123.8, 121.2, 51.1, 39.6 (overlapped with DMSO signal), 25.4, 23.8, 22.1 ppm. Mp = 157-159 °C (lit. 157-159 °C)[23], $[\alpha]^{20}_{D}$ = -12.6 (c = 0.095 in DMSO). HRMS (ESI-TOF) calcd for $C_{16}H_{17}ClN_2NaO_4$ [M+Na$^+$]: 359.0769, found: 359.0765. FT-IR ν_{max} (neat): 3379, 2968, 1656, 1312, 1233, 768 cm^{-1}.

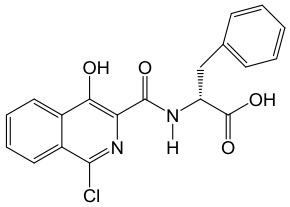
(S)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-phenylpropanoic acid (31a)[26]



The desired compound (31a) was obtained as a white solid (219 mg, 44%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.99 (d, *J*=8.5 Hz, 1 H, NH), 8.37 (m, 1 H, ArH), 8.32 (m, 1 H, ArH), 8.03 (m, 2 H, ArH), 7.32 (m, 4 H, PhH), 7.25 (m, 1 H, PhH), 4.86 (dt, *J*=8.5, 7.0 Hz, 1 H, CH), 3.35 (d, *J*=7.0 Hz, 2 H, CH_2Ph) ppm,

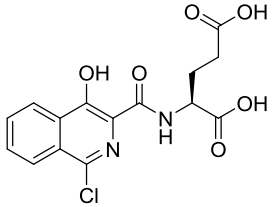
^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.5, 168.8, 154.8, 139.0, 137.8, 132.3, 132.3, 129.9, 129.6, 129.0, 128.8, 127.1, 126.6, 123.4, 120.7, 53.6, 36.4 ppm. Mp =180-183 °C (lit. 185-188 °C)[26], $[\alpha]^{20}_D$ = -31.1 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClNaN_2O_4$ [M+H$^+$]: 393.0613, found: 393.0610, FT-IR ν_{max} (neat): 3448, 3360, 2931, 1736, 1570, 1259, 773 cm^{-1}.

(R)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-phenylpropanoic acid (31b)[26]



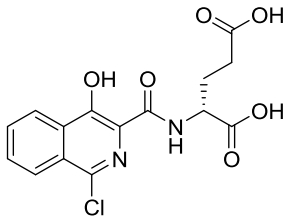
The desired compound (31b) was obtained as a white solid (205 mg, 41%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.99 (d, *J*=8.5 Hz, 1 H, NH), 8.37 (m, 1 H, ArH), 8.32 (m, 1 H, ArH), 8.03 (m, 2 H, ArH), 7.32 (m, 4 H, PhH), 7.25 (m, 1 H, PhH), 4.86 (dt, *J*=8.5, 7.0 Hz, 1 H, CH), 3.35 (d, *J*=7.0 Hz, 2 H, CH_2Ph) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.5, 168.8, 154.8, 139.0, 137.8, 132.3, 132.3, 129.9, 129.6, 129.0, 128.8, 127.1, 126.6, 123.4, 120.7, 53.6, 36.4 ppm. Mp =168-171 °C (lit. 184-186 °C)[26], $[\alpha]^{20}_D$ = +29.7 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClNaN_2O_4$ [M+H$^+$]: 393.0613, found: 393.0612, FT-IR ν_{max} (neat): 3449, 3359, 2931, 1736, 1574, 1260, 770 cm^{-1}.

(S)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)pentanedioic acid (32a)[23]



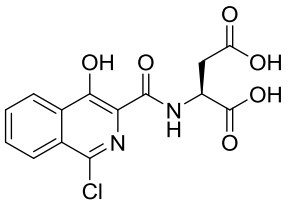
The desired compound (32a) was obtained as a cream white solid (133 mg, 28%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.63 (br. s., 1H, OH), 9.14 (d, *J* = 8.0 Hz, 1H, NH), 8.31 - 8.36 (m, 1H, ArH), 8.25 - 8.31 (m, 1H, ArH), 7.94 - 8.03 (m, 2H, ArH), 4.54 - 4.65 (m, 1H, H5), 2.37 (t, *J* = 7.5 Hz, 2H, H7), 2.07 - 2.31 (m, 2H, H6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.2, 172.8, 169.3, 154.9, 139.1, 132.0, 132.0, 130.0, 129.0, 126.4, 123.3, 120.2, 51.8, 30.7, 26.1 ppm. Mp = 128-130 °C (lit. 128-130 °C)[23], $[\alpha]^{20}_D$ = -6.4 (c = 0.125 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{13}ClN_2NaO_6$ [M+Na$^+$]: 375.0354, found: 375.0352, FT-IR ν_{max} (neat): 2923, 2853, 1709, 1528, 1319, 1214, 766 cm^{-1}.

(R)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)pentanedioic acid (32b)[23]



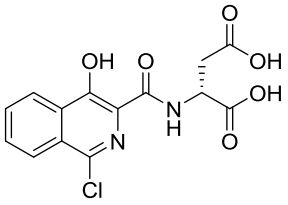
The desired compound (32b) was obtained as a cream white solid (166 mg, 35%) starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.63 (br. s., 1H, OH), 9.14 (d, *J* = 8.0 Hz, 1H, NH), 8.31 - 8.36 (m, 1H, ArH), 8.25 - 8.31 (m, 1H, ArH), 7.94 - 8.03 (m, 2H, ArH), 4.54 - 4.65 (m, 1H, H5), 2.37 (t, *J* = 7.5 Hz, 2H, H7), 2.07 - 2.31 (m, 2H, H6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.2, 172.8, 169.3, 154.9, 139.1, 132.0, 132.0, 130.0, 129.0, 126.4, 123.3, 120.2, 51.8, 30.7, 26.1 ppm. Mp = 135-138 °C (lit. 135-138 °C)[23], $[\alpha]^{20}_{D}$ = 6.2 (c = 0.127 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{13}ClN_2NaO_6$ [M+Na$^+$]: 375.0354, found: 375.0342. FT-IR ν_{max} (neat): 2923, 2853, 1709, 1527, 1319, 1214, 766 cm^{-1}.

(S)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)succinic acid (33a)[23]



The desired compound (33a) was obtained as a pink solid (186 mg, 41%), starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ =13.56 (br. s., 1H, OH), 9.16 (d, *J* = 8.5 Hz, 1H, NH), 8.22 - 8.43 (m, 2H, ArH), 7.95 - 8.13 (m, 2H, ArH), 4.78 - 5.05 (m, 1H, H5), 2.97 (d, *J* = 6.0 Hz, 2H, H6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 172.2, 168.7, 154.9, 139.1, 132.3, 132.3, 130.0, 129.1, 126.6, 123.5, 120.8, 48.8, 36.0 ppm. Mp = 240-243 °C (lit. 240-243 °C)[23], $[\alpha]^{20}_{D}$ = -14.2 (c = 0.098 in DMSO). HRMS (ESI-TOF) calcd for $C_{14}H_{11}ClN_2NaO_6$ [M+Na$^+$]: 337.0233, found 337.0247. FT-IR ν_{max} (neat): 3413, 2927, 1707, 1528, 1212, 767 cm^{-1}.

(R)-2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido)succinic acid (33b)[23]



The desired compound (33b) was obtained as a pink solid (177 mg, 39%), starting from 1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol).
^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.56 (br. s., 1H, OH), 9.16 (d, *J* = 8.5 Hz, 1H, NH), 8.22 - 8.43 (m, 2H, ArH), 7.95 - 8.13 (m, 2H, ArH), 4.78 - 5.05 (m, 1H, H5), 2.97 (d, *J* = 6.0 Hz, 2H, H6), ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 172.2, 168.7, 154.9, 139.1, 132.3, 132.3, 130.0, 129.1, 126.6, 123.5, 120.8, 48.8, 36.0 ppm. Mp = 239-241 °C (lit. 239-241 °C)[23], $[\alpha]^{20}_{D}$ = 13.8 (c = 0.102 in DMSO). HRMS (ESI-TOF) calcd for $C_{14}H_{11}ClN_2NaO_6$ [M+Na$^+$]: 337.0233, found: 337.0248, FT-IR ν_{max} (neat): 3357, 2926, 1704, 1526, 1194, 765 cm^{-1}.

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