Inhibition of the histone demethylase JMJD2E by 3-substituted pyridine 2,4-dicarboxylates

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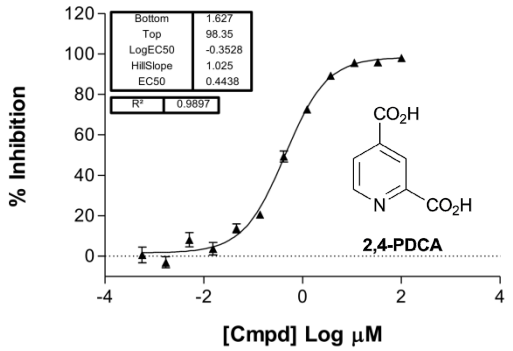
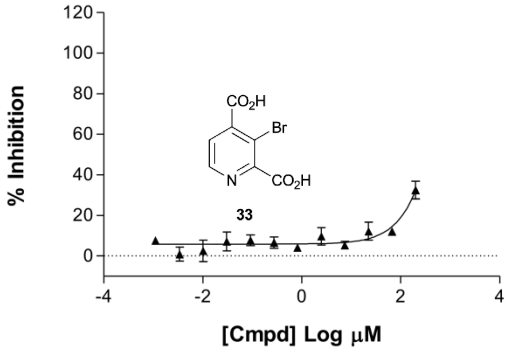
Contents

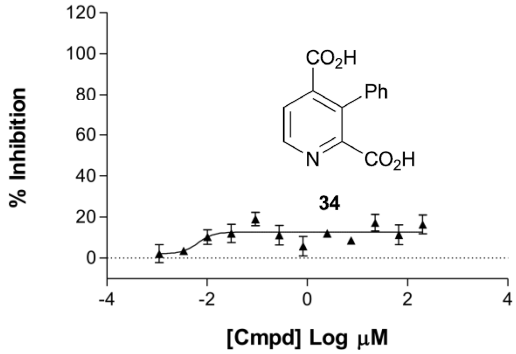
Formaldehyde dehydrogenase-based coupled demethylation assays

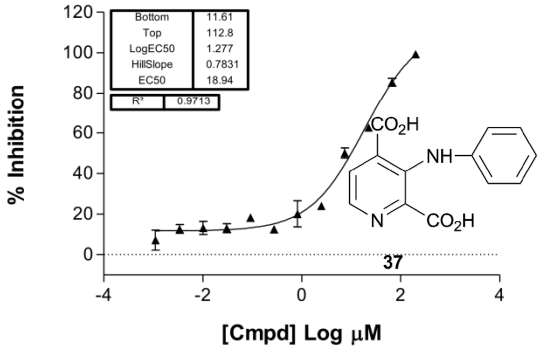
IC_{50} values for synthesised compounds were determined using a modified version of a reported[1] coupled demethylation assay. Compounds were dissolved in DMSO or DMSO/H_2O (1:1, v/v) at stock concentrations of 10 mM or 1 mM depending on solubility; assay mixtures had a final DMSO concentration of 1%. The peptide substrate sequence used was a modified fragment of histone H3, ARK(me_3)STGGK-NH_2 (Peptide Protein Research Ltd., Fareham, UK). FDH was cloned as a 1320 bp fragment in pNIC28-Bsa4, expressed in an *E. coli* Rosetta strain and purified by immobilized metal affinity chromatography to yield an *N*-terminally hexahistidine-tagged protein. Fluorescence measurements were performed in triplicate using black 384-well microplates with a non-binding surface (Corning Inc., Cat. No. 3655). Incubations contained NAD^+ (0.25 mM), ascorbate (1 mM), ferrous ammonium sulfate (10 μM), peptide substrate (25 μM) sequence from Peptide Protein Research, Hampshire, UK), 2OG (50 μM), FDH (50 μM) and JMJD2E (400 nM) in HEPES buffer (50 mM, pH 7.5) supplemented with Tween®-20 (0.01%) in a total volume of 80 μL. Reactions were performed without preincubation and at room temperature. Note that IC_{50} values are dependent on the concentration of JMJD2E. The fluorescence increase was measured using an OmegaStar Plus fluorescence microplate reader (BMG Labtech) with excitation and emission wavelengths of 355 and 460 nM, respectively. Percent inhibition was calculated based on the endpoint readings 30 minutes after addition of substrate. Positive control reactions for complete inhibition contained no JMJD2E, and negative control reactions (no inhibition) contained JMJD2E in the absence of inhibitors. The percentage inhibition was plotted against the logarithm of the compound concentration using GraphPad Prism 5.0™ and fitted with a sigmoidal dose-response model.

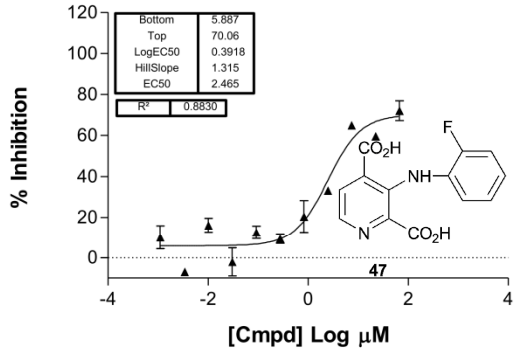
IC_{50} determinations against JMJD2E for selected compounds

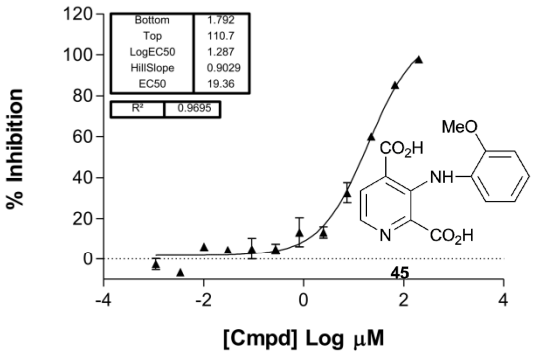


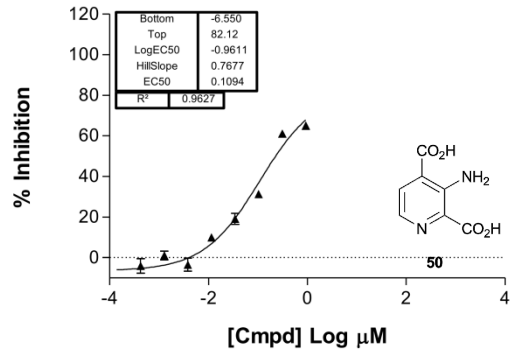


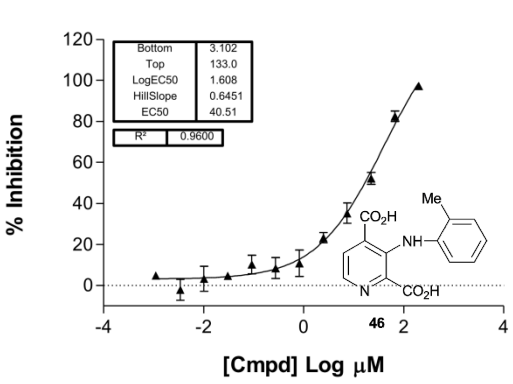








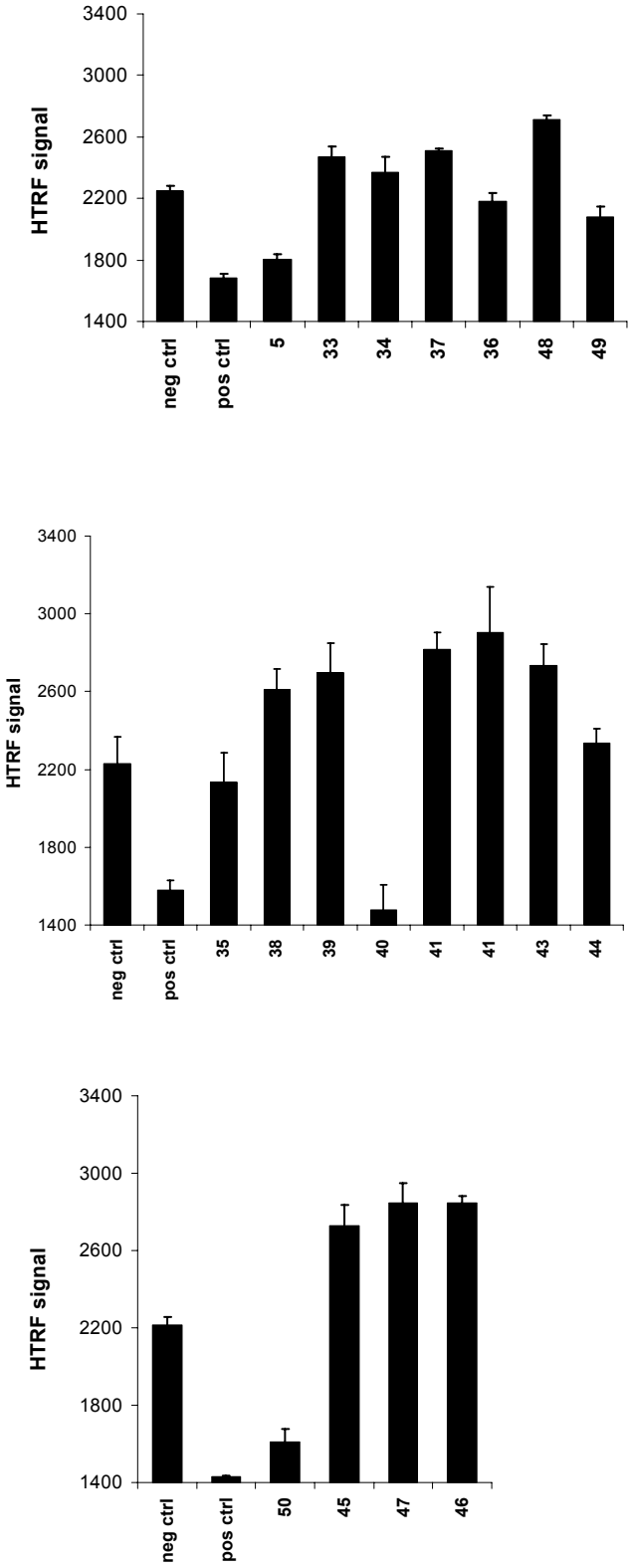




Homogeneous time-resolved fluorescence (HTRF)-based PHD2 inhibition assays

Compounds were tested for inhibition of the human 2OG oxygenase PHD2 (final concentration 20 nM) with the compounds at a final concentration of 400 μM. The assay was carried out essentially as described.[2] Inhibition of PHD2 activity was analyzed by determining the binding of biotinylated HIF-1$\alpha_{556-574}$ (hypoxia-inducible factor 1α) peptides to the VCB (von Hippel Lindau protein, Elongin C and B) complex. The ternary VCB complex was produced as described,[3] purified by successive GST (glutathione-*S*-transferase) and gel filtration chromatography, thrombin cleavage, and further GST and gel filtration chromatography. VCB was labeled with a Eu^{3+}-cryptate linker (LANCE Eu-W1024-ITC, PerkinElmer; ~6 Eu per VCB). Inhibition assays were performed in 50 μl total volume. For single-point inhibition tests, compounds were tested at 400 μM final concentration; reaction mixtures contained 20 nM PHD2, 300 nM HIF-1$\alpha_{556-574}$, 750 nM 2OG, 100 μM Fe(II) and 2 mM ascorbate in HTRF buffer (50 mM Tris pH 7.5, 100 mM NaCl, 0.05% purified BSA, 0.05% Tween-20). Reactions were incubated with shaking at 25 °C for 1 h and quenched by supplementing with 150 mM succinate. Negative controls (corresponding to zero inhibition) contained all components except inhibitors, whereas positive controls (corresponding to full inhibition) contained all components except PHD2.

The quenched reaction (containing 100 nM of biotinylated peptide) was allowed to reach binding equilibrium with streptavidin-allophycocyanin (SA-APC, 75 nM) and Eu^{3+}-VCB (0.9 nM) at 20 μl total volume in black 384-well clear-bottom microplates by shaking for five minutes before analysis using three repeats per well on an Envision Multilabel plate reader (PerkinElmer) at 25 ºC. The readout (HTRF signal) is the ratio of the 665 nm and 615 nm emission signals resulting from the FRET excitation of SA-APC at 615 nm and the laser excitation of Eu^{3+}-VCB at 320 nm, respectively, multiplied by 10,000.



Supporting Figure 1. Inhibition of PHD2 by the 3-substituted 2,4-PDCA analogues. Compounds were screened against PDH2 (20 nM) at a compound concentration of 400 μM. Error bars are standard deviations from 3 replicate measurements.

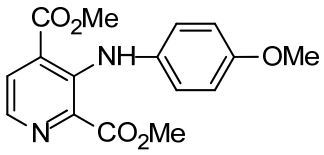
Synthesis

General

All reactions involving moisture sensitive reagents were carried out under a nitrogen atmosphere. Oven-dried glassware was used throughout. Cooling was performed in ice-water baths (0 °C) or dry ice-acetone baths (-78 °C). Anhydrous solvents were obtained from solvent stills and were activated by passing over a short column of activated alumina. Reagents were obtained from Acros, Aldrich, Avocado, Fluka, or Lancaster fine chemical suppliers and used as supplied. Thin layer chromatography (TLC) was performed on Merck DC-Kieselgel 60 F_{254} 0.2 mm precoated plates with fluorescence indicator. Visualization of spots was achieved using UV light (254 nm) and by developing in a basic solution of $KMnO_4$ or a 3% ninhydrin solution, followed by heating. Chromatography was performed using a Biotage SP4 chromatography system, using prepacked Biotage KP-SIL SNAP columns, or by filtration through silica purchased from VWR (40−63 μm particle size). ^{1}H NMR spectra were recorded on a Bruker AC200 (200 MHz), Bruker AV400 (400 MHz) or Bruker Avance AV500 (500 MHz) spectrometer and referenced to residual solvent peaks. Chemical shifts are quoted in parts per million (ppm). Assignments were made on the basis of chemical shifts, coupling constants (*J*), ^{13}C, DEPT, COSY, HMQC data and comparison with spectra of related compounds. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), and brs (broad singlet). Coupling constants are given in Hz and are reported to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer at 100 MHz or a Bruker Avance AV500 spectrometer (fitted with an inverse cryoprobe for ^{13}C observation) at 125 MHz and referenced to $CDCl_3$ or DMSO-d_6. ^{19}F NMR spectra were recorded on a Bruker AV400 spectrometer at 377 MHz. Infra-red (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer as KBr disks or films on sodium chloride plates (from CH_2Cl_2). Selected absorption maxima (ν_{max}) are given in wavenumbers (cm^{-1}) and are uncorrected. High resolution mass spectra (HRMS) were recorded under the conditions of electrospray ionization on a Bruker MicroTOF. Melting points were recorded on a Leica VMTG heated-stage microscope melting point apparatus. Elemental analyses were performed at London Metropolitan University, London, U.K. All compounds used in inhibition assays were prepared as described in the example procedures in the main text or in the following Supporting Information and were analytically pure as determined by CHN microanalysis or analytical HPLC (>95% pure).

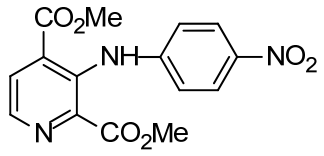
Detailed synthetic procedures

Dimethyl 3-(4-methoxyphenylamino)pyridine-2,4-dicarboxylate 19



A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), *p*-anisidine (107 mg, 0.88 mmol, 1.2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 0.014 mmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 0.044 mmol, 6 mol%) in anhydrous toluene (5 cm^3) was heated to 150 °C for 3 h in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **19** (165 mg, 71%) as a yellow oil; ν_{max} (film)/cm^{-1} 2951, 1732, 1692, 1512, 1287, 1242, 1196 and 1164; δ_H (400 MHz; $CDCl_3$) 3.52 (3 H, s, OC*H*$_3$), 3.79 (3 H, s, OC*H*$_3$), 3.82 (3 H, s, OC*H*$_3$), 6.84 (2 H, d, *J* 9.0 Hz, Ar*H*), 6.99 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.60 (1 H, d, *J* 4.5 Hz, py*H*), 8.17 (1 H, d, *J* 4.5 Hz, py*H*) and 9.50 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.3, 52.7, 55.5, 114.6, 122.5, 125.9, 127.6, 135.0, 138.2, 142.5, 156.5, 166.9 and 167.3; *m/z* (ESI+) 339.0950 ($M + Na^+$. $C_{16}H_{16}N_2NaO_5$ requires 339.0951).

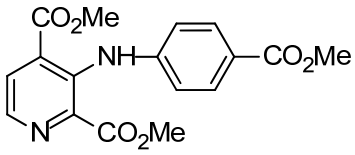
Dimethyl 3-(4-nitrophenylamino)pyridine-2,4-dicarboxylate 20



A stirred suspension of 3-bromopyridine **14** (150 mg, 0.55 mmol, 1 eq.), 4-nitroaniline (91 mg, 0.66 mmol, 1.2 eq.), Cs_2CO_3 (250 mg, 0.77 mmol, 1.4 eq.), $Pd_2(dba)_3$ (10 mg, 0.011 mmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (19 mg, 0.033 mmol, 6 mol%) in anhydrous toluene (3 cm^3) was heated to 150 °C for 6 h in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **20** (81 mg, 45%) as a brown solid (Found: C, 51.37; H, 2.92; N, 13.76. $C_{13}H_9N_3O_6$

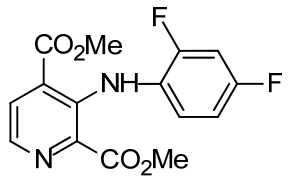
requires C, 51.49; H, 2.99; N, 13.86%); mp 139-142 ºC; ν_{max} (KBr disk)/cm^{-1} 3439, 3066, 1740, 1722, 1704, 1596, 1502 and 1335; δ_H (400 MHz; $CDCl_3$) 3.67 (3 H, s, OC*H*$_3$), 3.89 (3 H, s, OC*H*$_3$), 7.00 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.83 (1 H, d, *J* 5.0 Hz, py*H*), 8.16 (2 H, d, *J* 9.0 Hz, Ar*H*), 8.46 (1 H, d, *J* 5.0 Hz, py*H*) and 9.70 (1 H, s, N*H*); δ_C (125 MHz; $CDCl_3$) 52.9, 53.2, 116.7, 125.7, 127.7, 128.8, 138.30 and 138.32, 142.18 and 142.34, 148.4, 165.8 and 166.6; *m/z* (ESI+) 354.0694 ($M + Na^+$. $C_{15}H_{13}N_3NaO_6$ requires 354.0697).

Dimethyl 3-(4-(methoxycarbonyl)phenylamino)pyridine-2,4-dicarboxylate 21



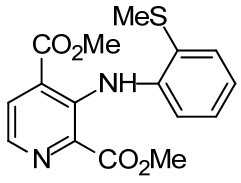
A stirred suspension of 3-bromopyridine **14** (250 mg, 0.91 mmol, 1 eq.), methyl 4-aminobenzoate (193 mg, 1.28 mmol, 1.4 eq.), Cs_2CO_3 (416 mg, 1.28 mmol, 1.4 eq.), $Pd_2(dba)_3$ (17 mg, 0.018 mmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (32 mg, 0.055 mmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 1 hr in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **21** (166 mg, 53%) as an orange solid; mp 115-117 ºC; ν_{max} (KBr disk)/cm^{-1} 3326, 2954, 1732, 1712, 1606, 1584, 1437, 1414, 1286, 1194 and 1170; δ_H (400 MHz; $CDCl_3$) 3.54 (3 H, s, OC*H*$_3$), 3.82 (3 H, s, OC*H*$_3$), 3.86 (3 H, s, OC*H*$_3$), 6.97 (2 H, d, *J* 8.5 Hz, Ar*H*), 7.72 (1 H, d, *J* 4.5 Hz, py*H*), 7.93 (2 H, d, *J* 8.5 Hz, Ar*H*), 8.33 (1 H, d, *J* 4.5 Hz, py*H*) and 9.62 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 51.9, 52.5, 52.9, 117.3, 124.4, 127.6, 127.8, 131.2, 137.1, 139.5, 140.7, 146.4, 166.1, 166.4 and 166.8; *m/z* (ESI+) 367.0902 ($M + Na^+$. $C_{17}H_{16}N_2NaO_6$ requires 367.0901).

Dimethyl 3-(2,4-difluorophenylamino)pyridine-2,4-dicarboxylate 22



A stirred suspension of 3-bromopyridine **14** (250 mg, 0.91 mmol, 1 eq.), 2,4-difluoroaniline (130 μL, 1.28 mmol, 1.4 eq.), Cs_2CO_3 (416 mg, 1.28 mmol, 1.4 eq.), $Pd_2(dba)_3$ (17 mg, 0.018 mmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (32 mg, 0.055 mmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 1 h in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **22** (182 mg, 62%) as a yellow solid; mp 137-140 ºC; ν_{max} (KBr disk)/cm^{-1} 3303, 3081, 3063, 2951, 1724, 1690, 1516, 1435, 1296, 1241, 1197, 1169; δ_H (500 MHz; $CDCl_3$) 3.60 (3 H, s, OC*H*$_3$), 3.85 (3 H, s, OC*H*$_3$), 6.77-6.83 (1 H, m, Ar*H*), 6.88-6.94 (1 H, m, Ar*H*), 6.98-7.05 (1 H, m, Ar*H*), 7.66 (1 H, d, *J* 4.5 Hz, py*H*), 8.26 (1 H, d, *J* 4.5 Hz, py*H*) and 9.37 (1 H, s, N*H*); δ_C (125 MHz; $CDCl_3$) 52.5, 52.9, 104.9, 111.1, 122.5, 126.2, 126.3, 127.5, 135.7, 139.4, 141.6, 155.7, 159.1, 166.4 and 167.1; *m/z* (ESI+) 345.0652 (M + Na^+. $C_{15}H_{12}F_2N_2NaO_4$ requires 345.0657).

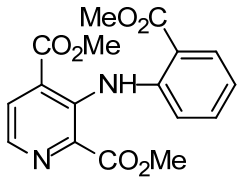
Dimethyl 3-(2-(methylthio)phenylamino)pyridine-2,4-dicarboxylate 23



A stirred suspension of 3-bromopyridine **14** (250 mg, 0.91 mmol, 1 eq.), 2-methylthioaniline (229 μL, 1.82 mmol, 2 eq.), Cs_2CO_3 (416 mg, 1.28 mmol, 1.4 eq.), $Pd_2(dba)_3$ (16 mg, 18 μmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (32 mg, 55 μmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 125 °C for 90 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **23** (259 mg, 85%) as a deep yellow oil; ν_{max} (film)/cm^{-1} 3288, 2951, 1733, 1698, 1583,

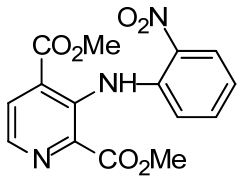
1502, 1438, 1286, 1197 and 1166; δ_H (500 MHz; $CDCl_3$) 2.46 (3 H, s, SCH_3), 3.47 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3), 6.85 (1 H, d, *J* 7.5 Hz, Ar*H*), 6.94-7.09 (1 H, m, Ar*H*), 6.99 (1 H, d, *J* 8.5 Hz, Ar*H*), 7.30 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.64 (1 H, d, *J* 4.5 Hz, py*H*), 8.21 (1 H, d, *J* 4.5 Hz, py*H*) and 9.60 (1 H, s, N*H*); δ_C (126 MHz; $CDCl_3$) 15.7, 52.3, 52.8, 116.8, 124.1, 126.3, 126.8, 127.6, 128.9, 130.2, 136.2, 139.3, 140.7, 141.0, 166.4 and 166.8; *m/z* (ESI+) 355.0719 ($M + Na^+$. $C_{16}H_{16}N_2NaO_4S$ requires 355.0723).

Dimethyl 3-(2-(methoxycarbonyl)phenylamino)pyridine-2,4-dicarboxylate 24



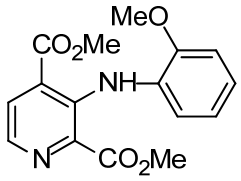
A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), methyl anthranilate (190 µL, 1.46 mmol, 2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 14 µmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 µmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 15 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **24** (151 mg, 60%) as a yellow oil; ν_{max} (film)/cm^{-1} 2952, 1733, 1704, 1584, 1504, 1450, 1436, 1252, 1195 and 1165; δ_H (400 MHz; $CDCl_3$) 3.54 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 6.85-6.94 (2 H, m, Ar*H*), 7.26 (1 H, t, *J* 7.5 Hz, Ar*H*), 7.71 (1 H, d, *J* 5.0 Hz, py*H*), 7.94 (1 H, dd, *J* 7.5, 1.0 Hz, Ar*H*), 8.33 (1 H, d, *J* 5.0 Hz, py*H*) and 11.02 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.2, 52.4, 52.8, 114.4, 117.0, 120.7, 127.2, 129.4, 131.6, 133.4, 138.1, 139.6, 141.1, 144.7, 165.9, 166.0 and 167.5; *m/z* 367.0894 ($M + Na^+$. $C_{17}H_{16}N_2NaO_6$ requires 367.0901).

Dimethyl 3-(2-nitrophenylamino)pyridine-2,4-dicarboxylate 25



A stirred suspension of 3-bromopyridine **14** (250 mg, 0.91 mmol, 1 eq.), 2-nitroaniline (252 mg, 1.82 mmol, 2 eq.), Cs_2CO_3 (416 mg, 1.28 mmol, 1.4 eq.), $Pd_2(dba)_3$ (16 mg, 18 μmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 μmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 10 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **25** (158 mg, 65%) as a yellow solid; mp 146-150 ºC; ν_{max} (KBr disk)/cm^{-1} 3422, 3295, 1719, 1700, 1498, 1434, 1274, 1197 and 1166; δ_H (400 MHz; $CDCl_3$) 3.60 (3 H, s, OC*H*$_3$), 3.79 (3 H, s, OC*H*$_3$), 6.88-7.02 (2 H, m, Ar*H*), 7.36 (1 H, t, *J* 7.0 Hz, Ar*H*), 7.79 (1 H, d, *J* 5.0 Hz, py*H*), 8.11 (1 H, dd, *J* 8.5, 1.5 Hz, Ar*H*), 8.45 (1 H, d, *J* 5.0 Hz, py*H*) and 10.83 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.8, 53.1, 116.6, 121.1, 126.5, 127.4, 130.4, 134.8, 136.9, 138.0, 139.4, 140.6, 143.0, 165.5 and 165.8; *m/z* (ESI+) 354.0701 ($M + Na^+$. $C_{15}H_{13}N_3NaO_6$ requires 354.0697).

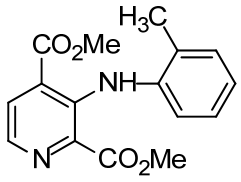
Dimethyl 3-(2-methoxyphenylamino)pyridine-2,4-dicarboxylate 26



A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), *o*-anisidine (165 μL, 1.46 mmol, 2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 15 μmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 μmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 10 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **26** (169 mg, 73%) as a yellow oil; ν_{max} (film)/cm^{-1} 3319, 2951, 1733, 1698, 1598, 1560, 1512, 1437, 1287

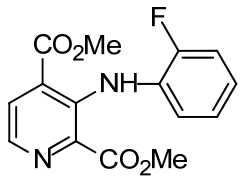
and 1244; δ_H (400 MHz; $CDCl_3$) 3.49 (3 H, s, OC*H*$_3$), 3.80 (3 H, s, OC*H*$_3$), 3.87 (3 H, s, OC*H*$_3$), 6.82 (1 H, t, *J* 7.5 Hz, Ar*H*), 6.87-6.96 (2 H, m, Ar*H*), 7.02 (1 H, Ar*H*), 7.63 (1 H, d, *J* 4.5 Hz, py*H*), 8.19 (1 H, d, *J* 4.5 Hz, py*H*) and 9.44 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.2, 52.7, 55.7, 111.3, 118.0, 120.5, 124.2, 126.6, 127.4, 130.9, 135.9, 138.7, 141.3, 151.2, 166.6 and 167.0; *m/z* (ESI+) 339.0948 ($M + Na^+$. $C_{16}H_{16}N_2NaO_5$ requires 339.0951).

Dimethyl 3-(*o*-tolylamino)pyridine-2,4-dicarboxylate 27



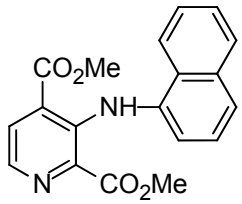
A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), *o*-toluidine (156 µL, 1.46 mmol, 2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 15 µmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 µmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 10 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **27** (126 mg, 58%) as a yellow solid. mp 110-112 ºC**;** ν_{max} (KBr disk)/cm^{-1} 3297, 2945, 1726, 1681, 1585, 1502, 1433, 1366, 1276, 1194 and 1166; δ_H (400 MHz; $CDCl_3$) 2.37 (3 H, s, C*H*$_3$), 3.44 (3 H, s, OC*H*$_3$), 3.80 (3 H, s, OC*H*$_3$), 6.90 (1 H, d, *J* 8.0 Hz, Ar*H*), 7.02 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.08 (1 H, t, *J* 7.0 Hz, Ar*H*), 7.24 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.61 (1 H, d, *J* 4.5 Hz, py*H*), 8.19 (1 H, d, *J* 4.5 Hz, py*H*) and 9.37 (1 H, s, N*H*); δ_C (100 MHz, $CDCl_3$) 18.0, 52.1, 52.7, 118.9, 124.5, 126.1, 126.6, 127.5, 131.0, 131.3, 135.2, 138.4, 140.5, 142.4, 166.8 and 167.3; *m/z* (ESI+) 323.1000 ($M + Na^+$. $C_{16}H_{16}N_2NaO_4$ requires 323.1002).

Dimethyl 3-(2-fluorophenylamino)pyridine-2,4-dicarboxylate 28



A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), 2-fluoroaniline (141 µL, 1.46 mmol, 2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 15 µmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 µmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 15 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **28** (169 mg, 76%) as a yellow oil; ν_{max} (film)/cm^{-1} 3302, 2952, 1733, 1698, 1513, 1288, 1197 and 1167; δ_H (400 MHz; $CDCl_3$) 3.53 (3 H, s, OC*H*$_3$), 3.82 (3 H, s, OC*H*$_3$), 6.97-7.05 (3 H, m, Ar*H*), 7.08-7.16 (1 H, m, Ar*H*), 7.67 (1 H, d, *J* 4.5 Hz, py*H*), 8.26 (1 H, d, *J* 4.5 Hz, py*H*) and 9.44 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.4, 52.8, 116.3, 120.3, 124.3, 124.7, 126.8, 127.6, 130.0 136.1, 139.6, 141.1, 155.2, 166.5 and 167.0; δ_F (377 MHz; $CDCl_3$) -125.6; *m/z* (ESI+) 327.0752 ($M + Na^+$. $C_{15}H_{13}FN_2NaO_4$ requires 327.0752).

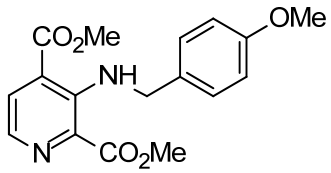
Dimethyl 3-(naphthalen-1-ylamino)pyridine-2,4-dicarboxylate 29



A stirred suspension of 3-bromopyridine **14** (200 mg, 0.73 mmol, 1 eq.), 1-naphthylamine (209 mg, 1.46 mmol, 2 eq.), Cs_2CO_3 (333 mg, 1.02 mmol, 1.4 eq.), $Pd_2(dba)_3$ (13 mg, 15 µmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (25 mg, 44 µmol, 6 mol%) in anhydrous toluene (4 cm^3) was heated to 150 °C for 15 min in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **29** (171 mg, 70%) as a deep yellow oil. ν_{max} (KBr disk)/cm^{-1} 3433, 3304, 3046, 3005, 2949, 1730, 1711, 1692, 1584, 1475, 1458, 1443, 1430, 1282, 1198 and 1169; δ_H (400 MHz;

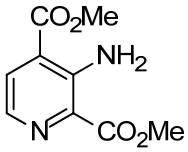
$CDCl_3$) 3.09 (3 H, s, OCH_3), 3.68 (3 H, s, OCH_3), 7.07 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.29 (1 H, t, *J* 8.0 Hz, Ar*H*), 7.53 (2 H, td, *J* 8.0, 1.5 Hz, Ar*H*), 7.56-7.60 (1 H, m, Ar*H*), 7.60 (1 H, d, *J* 5.0 Hz, py*H*), 7.83 (1 H, d, *J* 7.5 Hz, Ar*H*), 8.21 (1 H, d, *J* 5.0 Hz, py*H*), 8.25 (1 H, d, *J* 8.5 Hz, Ar*H*) and 9.87 (1 H, s, N*H*); δ_C (100 MHz; $CDCl_3$) 52.0, 52.7, 115.5, 122.1, 124.9, 125.4, 126.5, 126.7, 127.7, 128.2, 128.5, 134.5, 135.5, 138.5, 138.9, 143.0, 166.8 and 167.5; *m/z* (ESI+) 359.0995 ($M + Na^+$. $C_{19}H_{16}N_2NaO_4$ requires 359.1002).

Dimethyl 3-(4-methoxybenzylamino)pyridine-2,4-dicarboxylate 31



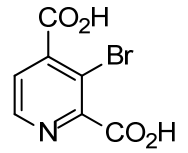
A stirred suspension of 3-bromopyridine **14** (500 mg, 1.82 mmol, 1 eq.), 4-methoxybenzylamine (290 μL, 2.19 mmol, 1.2 eq.), Cs_2CO_3 (830 mg, 2.55 mmol, 1.4 eq.), $Pd_2(dba)_3$ (30 mg, 0.037 mmol, 2 mol%) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (60 mg, 0.109 mmol, 6 mol%) in anhydrous toluene (15 cm^3) was heated to 110 °C for 12 h in a Biotage Initiator microwave oven. The reaction mixture was directly purified by automated flash column chromatography to afford **31** (418 mg, 69%) as a yellow solid (Found: C, 61.84; H, 5.46; N, 8.39. $C_{17}H_{18}N_2O_5$ requires C, 61.81; H, 5.49; N, 8.48%); mp 60-64 ºC; ν_{max} (film)/cm^{-1} 3329, 2953, 1726, 1697, 1513, 1439, 1295, 1249, 1194 and 1164; δ_H (400 MHz; $CDCl_3$) 3.79 (s, 3 H, OCH_3), 3.89 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.17 (2 H, s, NHCH_2), 6.86 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.20 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.62 (1 H, d, *J* 5.0 Hz, py*H*) and 8.03 (1 H, d, *J* 5.0 Hz, py*H*); δ_C (100 MHz; $CDCl_3$) 50.4, 52.6, 52.8, 55.2, 114.1, 122.7, 127.5, 129.1, 129.9, 133.3, 135.9, 145.4, 159.1, 167.3 and 167.6; *m/z* (ESI+) 353.1103 ($M + Na^+$. $C_{17}H_{18}N_2NaO_5$ requires 353.1108).

Dimethyl 3-aminopyridine-2,4-dicarboxylate 32



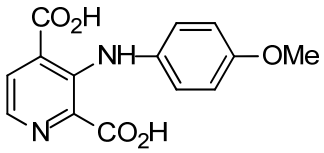
To a stirred solution of dimethyl ester **31** (146 mg, 0.44 mmol, 1 eq.) in CH_2Cl_2 (1 cm^3) was added dropwise CF_3CO_2H (1 cm^3) while cooling in an ice bath. The resulting solution was stirred overnight. Volatiles were removed *in vacuo* and the residue purified by flash column chromatography (Biotage KP-SIL SNAP 25 g cartridge, eluting with EtOAc/hexane) to afford **32** (83 mg, 89%) as a pale yellow solid; mp 98-102 ºC; ν_{max} (film)/cm^{-1} 3449, 3340, 2957, 1714, 1606, 1563, 1462, 1439, 1337 and 1303; δ_H (400 MHz; $CDCl_3$) 3.93 (3 H, s, OC*H*$_3$), 3.97 (3 H, s, OC*H*$_3$), 7.90 (1 H, d, *J* 5.0 Hz, py*H*), 7.96 (2 H, brs, N*H*$_2$) and 8.03 (1 H, d, *J* 5.0 Hz, py*H*); δ_C (100 MHz; $CDCl_3$) 52.5, 52.6, 119.1, 128.9, 129.6, 134.9, 148.1, 166.7 and 166.9; *m/z* (ESI+) 233.0534 (M + Na^+. $C_9H_{10}N_2NaO_4$ requires 233.0533).

3-Bromopyridine-2,4-dicarboxylic acid 33



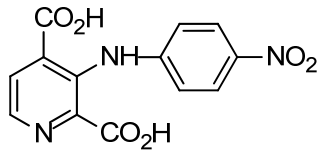
A solution of dimethyl ester **14** (100 mg, 0.36 mmol, 1 eq.) in MeOH (3 cm^3) was treated with a solution of NaOH (88 mg, 2.16 mmol, 6 eq.) in water (3 cm^3) and stirred for 6 h at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water, acidified with conc. HCl and extracted repeatedly with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **33** (74 mg, 82%) as a pale pink solid (Found: C, 34.09; H, 1.56; N, 5.65. $C_7H_4BrNO_4$ requires C, 34.17; H, 1.64; N, 5.69%); mp 244-246 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3259, 2919, 2851, 1744, 1707, 1578 and 1355; δ_H (400 MHz; DMSO-d_6) 7.72 (1 H, d, *J* 5.0 Hz, py*H*), 8.65 (1 H, d, *J* 5.0 Hz, py*H*) and 14.02 (2 H, brs, CO_2H); δ_C (125 MHz; DMSO-d_6) 112.3, 123.9, 143.7, 148.5, 153.9, 166.1 and 166.8; *m/z* (ESI-) 243.9249 (M – H^+. $C_7H_3BrNO_4$ requires 243.9251);

3-(4-Methoxyphenylamino)pyridine-2,4-dicarboxylic acid 38



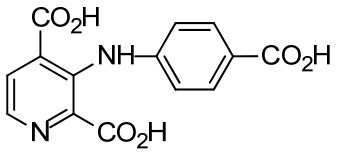
A solution of dimethyl ester **19** (110 mg, 0.35 mmol, 1 eq.) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water and acidified with conc. HCl. The precipitated product was filtered off and recrystallised from water/MeOH to afford **38** (85 mg, 85%) as a deep red solid (Found: C, 58.20; H, 4.14; N, 9.80. $C_{14}H_{12}N_2O_5$ requires C, 58.33; H, 4.20; N, 9.72%); mp >200 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3444, 3016, 1702, 1640, 1522, 1446, 1237 and 1154; δ_H (400 MHz; DMSO-d_6) 3.71 (3 H, s, OCH_3), 6.82 (2 H, d, *J* 9.0 Hz, Ar*H*), 6.95 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.69 (1 H, d, *J* 5.0 Hz, py*H*), 8.11 (1 H, d, *J* 5.0 Hz, py*H*) and 9.48 (1 H, brs, N*H*); δ_C (100 MHz; DMSO-d_6) 55.2, 114.3, 121.8, 127.1, 127.3, 135.5, 137.1, 137.8, 141.0, 155.6, 167.5 and 167.8; *m/z* (ESI-) 287.0666 ($M - H^+$. $C_{14}H_{11}N_2O_5$ requires 287.0673).

3-(4-Nitrophenylamino)pyridine-2,4-dicarboxylic acid 39



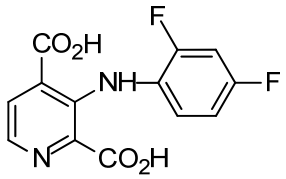
A solution of dimethyl ester **20** (60 mg, 0.181 mmol, 1 eq.) in MeOH (1 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water and acidified with conc. HCl. The precipitated product was filtered off and dried under high vacuum to afford **39** (30 mg, 55%) as a yellow solid; mp >160 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3159, 2991, 1722, 1658, 1596, 1527, 1502, 1469, 1340, 1288 and 1260; δ_H (500 MHz; DMSO-d_6) 6.77 (2 H, d, *J* 9.0 Hz, Ar*H*), 7.86 (1 H, d, *J* 5.0 Hz, py*H*), 8.04 (2 H, d, *J* 9.0 Hz, Ar*H*), 8.59 (1 H, d, *J* 5.0 Hz, py*H*), 9.34 (1 H, brs, N*H*) and 13.58 (brs, CO_2H); δ_C (125 MHz; DMSO-d_6) 114.0, 125.7, 125.9, 132.5, 136.7, 138.7, 145.9, 148.1, 152.1, 166.2 and 166.8; *m/z* (ESI-) 302.0420 ($M - H^+$. $C_{13}H_8N_3O_6$ requires 302.0419).

3-(4-Carboxyphenylamino)pyridine-2,4-dicarboxylic acid 40



A solution of dimethyl ester **21** (166 mg, 0.166 mmol, 1 eq.) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water, acidified with conc. HCl and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **40** (92 mg, 63%) as a yellow solid; mp >200 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3471, 2968, 1716, 1668, 1604, 1526 and 1277; δ_H (400 MHz; DMSO-d_6) 6.85 (2 H, d, *J* 8.5 Hz, Ar*H*), 7.74 (2 H, d, *J* 8.5 Hz, Ar*H*), 7.81 (1 H, d, *J* 5.0 Hz, py*H*), 8.41 (1 H, d, *J* 5.0 Hz, py*H*), 9.21 (1 H, br s, N*H*) and 12.78 (br s, CO_2H); δ_C (100 MHz; DMSO-d_6) 52.5, 116.2, 121.8, 127.2, 131.5, 134.3, 136.1, 144.0, 145.1, 149.7, 166.8, 167.5 and 168.0; *m/z* (ESI-) 301.0486 ($M - H^+$. $C_{14}H_9N_2O_6$ requires 301.0466).

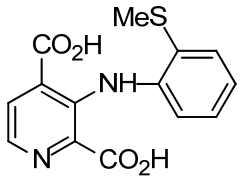
3-(2,4-Difluorophenylamino)pyridine-2,4-dicarboxylic acid 41



A solution of dimethyl ester **22** (60 mg, 0.186 mmol) in MeOH (1 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water and acidified with conc. HCl. The precipitated product was filtered off and dried under high vacuum to afford **41** (40 mg, 73%) as an orange solid **(**Found: C, 52.97; H, 2.66; N, 9.43. $C_{13}H_8F_2N_2O_4$ requires C, 53.07; H, 2.74; N, 9.52%); mp >130 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3651, 2926, 1725, 1657, 1524, 1262, 1148 and 1098; δ_H (500 MHz; DMSO-d_6) 6.96 (1 H, td, *J* 8.5, 1.5 Hz, Ar*H*), 7.10 (1 H, td, *J* 9.0, 6.0 Hz, Ar*H*), 7.28 (1 H, ddd, *J* 11.5, 8.5, 2.5 Hz, Ar*H*), 7.75 (1 H, d, *J* 4.5 Hz, py*H*), 8.21 (1 H, d, *J* 4.5 Hz, py*H*) and 9.39 (1 H, br s, N*H*); δ_C (125 MHz; DMSO-d_6) 104.5, 111.2, 122.8, 126.9, 127.0 and 127.6, 137.8, 139.1

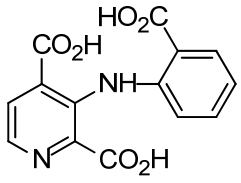
and 140.1, 154.9, 158.0, 167.3 and 167.7; *m/z* (FI+) 294.0473 (M^+. $C_{13}H_8N_2F_2O_4$ requires 294.0452).

3-(2-(Methylthio)phenylamino)pyridine-2,4-dicarboxylic acid 42



A solution of dimethyl ester **23** (229 mg, 0.688 mmol, 1 eq.) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **42** (160 mg, 76%) as a brown solid (Found: C, 55.16; H, 3.97; N, 9.15. $C_{14}H_{12}N_2O_4S$ requires C, 55.25; H, 3.97; N, 9.21%); mp >140 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3426, 3089, 1731, 1574, 1523, 1472 and 1257; δ_H (400 MHz; DMSO-d_6) 2.43 (3 H, s, C*H*$_3$), 6.82-6.87 (1 H, d, *J* 7.0 Hz, ArC*H*), 6.96-7.08 (2 H, broad, ArC*H*), 7.31-7.36 (1 H, d, *J* 7.0 Hz, ArC*H*), 7.76 (1 H, d, *J* 4.5 Hz, py*H*) and 8.24 (1 H, d, *J* 4.5 Hz, py*H*); δ_C (400 MHz; DMSO-d_6) 16.1, 116.8, 124.1, 127.2, 127.9, 129.1, 129.4, 129.8, 139.2, 140.0, 140.3, 142.0, 168.0 and 168.4.

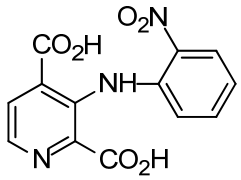
3-(2-Carboxyphenylamino)pyridine-2,4-dicarboxylic acid 43



A solution of dimethyl ester **24** (101 mg, 0.293 mmol) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to

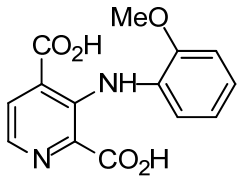
afford **43** (80 mg, 90%) as an orange solid (Found: C, 55.55; H, 3.34; N, 9.20. $C_{14}H_{10}N_2O_6$ requires C, 55.63; H, 3.33; N, 9.27%); mp >160 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3426, 1704, 1579, 1519, 1452 and 1249; δ_H (100 MHz; DMSO-d_6) 6.72-6.79 (1 H, broad, ArC*H*), 6.83-6.89 (broad, 1H, ArC*H*), 7.27-7.34 (1 H, broad, ArC*H*), 7.77-7.82 (1 H, broad, py*H*), 7.83-7.89 (1 H, broad, ArC*H*), 8.36-8.46 (1 H, broad, py*H*) and 10.45-10.55 (1 H, broad, N*H*); δ_C (100 MHz; DMSO-d_6) 114.8, 116.4, 120.3, 127.1, 132.4, 134.0, 134.4, 136.0, 143.5, 144.8, 146.8, 167.3, 167.7 and 169.7.

3-(2-Nitrophenylamino)pyridine-2,4-dicarboxylic acid 44



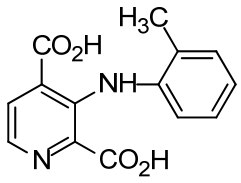
A solution of dimethyl ester **25** (132 mg, 0.398 mmol, 1 eq.) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **44** (105 mg, 87%) as a yellow solid (Found: C, 51.38; H, 3.08; N, 13.78. $C_{13}H_9N_3O_6$ requires C, 51.49; H, 2.99; N, 13.86); mp >175 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3436, 2924, 1708, 1609, 1576, 1499 and 1446; δ_H (400 MHz; DMSO-d_6) 6.99 (1 H, d, *J* 8.5 Hz, Ar*H*), 7.03 (1 H, t, *J* 7.5 Hz, Ar*H*), 7.51 (1 H, t, *J* 7.5 Hz, Ar*H*), 7.89 (1 H, d, *J* 4.5 Hz, py*H*), 8.11 (1 H, d, *J* 8.5 Hz, Ar*H*), 8.50 (1 H, d, *J* 4.5 Hz, py*H*), 10.55 (1 H, br s, N*H*) and 13.71 (2 H, br s, CO_2H); δ_C (126 MHz; DMSO-d_6) 117.4, 120.6, 126.0, 126.7, 132.8, 135.1, 135.5, 136.7, 140.2, 143.3, 143.7, 166.4 and 166.9.

3-(2-Methoxyphenylamino)pyridine-2,4-dicarboxylic acid 45



A solution of dimethyl ester **26** (169 mg, 0.534 mmol) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **45** (100 mg, 65%) as an orange solid (Found: C, 58.45; H, 4.27; N, 9.65. $C_{14}H_{12}N_2O_5$ requires C, 58.33; H, 4.20; N 9.72%); mp >155 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3424, 2995, 1708, 1670, 1599, 1531, 1494 and 1459; δ_H (500 MHz; DMSO-d_6) 3.81 (3 H, s, OCH_3), 6.79 (1 H, t, *J* 7.5 Hz, Ar*H*), 6.87 (1 H, d, *J* 6.5 Hz, Ar*H*), 6.95 (1 H, t, *J* 7.5 Hz, Ar*H*), 6.98-7.03 (1 H, m, Ar*H*), 7.76 (1 H, d, *J* 4.5 Hz, py*H*), 8.19 (1 H, d, *J* 4.5 Hz, py*H*) and 9.46 (1 H, br s, N*H*); δ_C (126 MHz; DMSO-d_6) 55.6, 111.6, 115.9, 120.2, 122.9, 126.9, 128.4, 131.1, 137.8, 138.5, 139.2, 149.9, 167.0 and 167.4.

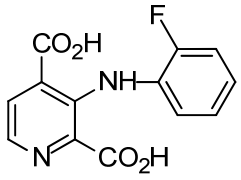
3-(*o*-Tolylamino)pyridine-2,4-dicarboxylic acid 46



A solution of dimethyl ester **27** (106 mg, 0.353 mmol) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **46** (70 mg, 73%) as an orange solid (Found: C, 61.62; H, 4.37; N, 10.38. $C_{14}H_{12}N_2O_4$ requires C, 61.76; H, 4.44; N, 10.29%); mp >135 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3426, 2924, 1725, 1647, 1584, 1523, 1461 and 1254; δ_H (500 MHz; DMSO-d_6) 2.26 (3 H, s, CH_3),

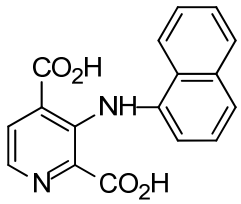
6.85 (1 H, d, *J* 8.0 Hz, Ar*H*), 6.95 (1 H, t, *J* 7.0 Hz, Ar*H*), 7.04 (1 H, t, *J* 7.5 Hz, Ar*H*), 7.21 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.74 (1 H, d, *J* 4.5 Hz, py*H*), 8.18 (1 H, d, *J* 4.5 Hz, py*H*), 9.37 (1 H, br s, N*H*) and 13.36 (br s, CO_2H); δ_C (126 MHz; DMSO-d_6) 17.7, 117.8, 123.4, 126.3, 127.0, 127.7, 129.6, 130.6, 137.5, 138.4, 140.7, 141.0, 167.3 and 167.7.

3-(2-Fluorophenylamino)pyridine-2,4-dicarboxylic acid 47



A solution of dimethyl ester **28** (149 mg, 0.49 mmol, 1 eq.) in MeOH (4 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 1 h at room temperature. The reaction mixture was diluted with MeOH (5 cm^3), filtered and concentrated to ~1 cm^3 final volume *in vacuo*. The remainder was acidified with conc. HCl, diluted with brine (5 cm^3) and extracted with EtOAc (3 × 5 cm^3). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford **47** (72 mg, 53%) as an orange solid (Found: C, 56.43; H, 3.22; N, 10.07. $C_{13}H_9FN_2O_4$ requires C, 56.53; H, 3.28; N, 10.14%); mp >160 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3430, 2927, 1719, 1532, 1465, 1292, 1249, 1153 and 1105; δ_H (400 MHz; DMSO-d_6) 6.94-7.29 (4 H, m, Ar*H*), 7.79 (1 H, d, *J* 5.0 Hz, py*H*), 8.26 (1 H, d, *J* 5.0 Hz, py*H*), 9.45 (1 H, br s, N*H*) and 13.58 (br s, CO_2H); δ_C (125 MHz; DMSO-d_6) 15.8, 119.3, 123.4, 124.4, 127.0, 128.5, 128.6, 138.8, 139.7, 153.1, 155.0, 167.1 and 167.6; δ_F (377 MHz; DMSO-d_6) -127.75.

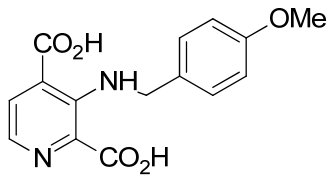
3-(Naphthalen-1-ylamino)pyridine-2,4-dicarboxylic acid 48



A solution of dimethyl ester **29** (114 mg, 0.339 mmol, 1 eq.) in MeOH (1 cm^3) was treated with a solution of NaOH (120 mg, 3.0 mmol, 8.9 eq.) in water (1 cm^3) and stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and acidified with

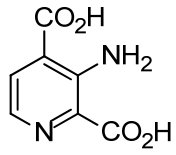
conc. HCl. The precipitate was collected by filtration, washed with cold water and ether and dried under high vacuum to afford **48** (60 mg, 57%) as an orange solid (Found: C, 66.29; H, 4.02; N, 9.04. $C_{17}H_{12}N_2O_4$ requires C, 66.23; H, 3.92; N, 9.09%); mp >180 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3436, 3094, 2988, 1727, 1646, 1581, 1522, 1476, 1388, 1288 and 1151; δ_H (500 MHz; DMSO-d_6) 7.02 (1 H, d, *J* 7.5 Hz, Ar*H*), 7.33 (1 H, t, *J* 7.5 Hz, Ar*H*), 7.54-7.66 (3 H, m, Ar*H*), 7.80 (1 H, d, *J* 4.5 Hz, py*H*), 7.89-7.97 (1 H, m, Ar*H*), 8.18 (1 H, dd, *J* 7.5, 2.0 Hz, Ar*H*), 8.27 (1 H, d, *J* 4.5 Hz, py*H*), 9.93 (1 H, br s, N*H*) and 13.46 (br s, CO_2H); δ_C (126 MHz; DMSO-d_6) 113.1, 121.8, 123.2, 125.6, 126.1, 126.5, 127.0, 127.1, 128.1, 128.7, 134.1, 138.5, 139.0, 139.2, 140.6, 167.4 and 167.8.

3-(4-Methoxybenzylamino)pyridine-2,4-dicarboxylic acid 49



A solution of dimethyl ester **31** (130 mg, 0.39 mmol, 1 eq.) in MeOH (1 cm^3) was treated with a solution of NaOH (200 mg, 5 mmol, 12.7 eq.) in water (1 cm^3). Additional MeOH was added until a clear, yellow solution resulted. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*. The residue was treated with water (5 cm^3), filtered and the filtrate acidified with conc. HCl. The resulting precipitate was isolated by filtration, dissolved in MeOH/CH_2Cl_2 and dried under high vacuum to afford **49** (114 mg, 96%) as a yellow solid; mp >110 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3431, 3189, 1712, 1659, 1614, 1531, 1515, 1461, 1252, 1153, 1104 and 1031; δ_H (400 MHz; DMSO-d_6) 3.72 (3 H, s, OCH_3), 4.21 (2 H, s, NHCH_2), 6.90 (2 H, d, *J* 8.0 Hz, ArC*H*), 7.24 (2 H, d, *J* 8.0 Hz, ArC*H*), 7.68 (1 H, d, *J* 4.5 Hz, py*H*), 7.94 (1 H, d, *J* 4.5 Hz, py*H*) and 8.50 (br s, N*H*); δ_C (100 MHz; DMSO-d_6) 49.2, 55.1, 114.1, 124.2, 127.2, 129.3, 130.3, 134.5, 135.0, 144.4, 158.8, 168.3 and 168.5; *m/z* (ESI-) 301.0834 (M – H$^+$. $C_{15}H_{13}N_2O_5$ requires 301.0830).

3-Aminopyridine-2,4-dicarboxylic acid 50



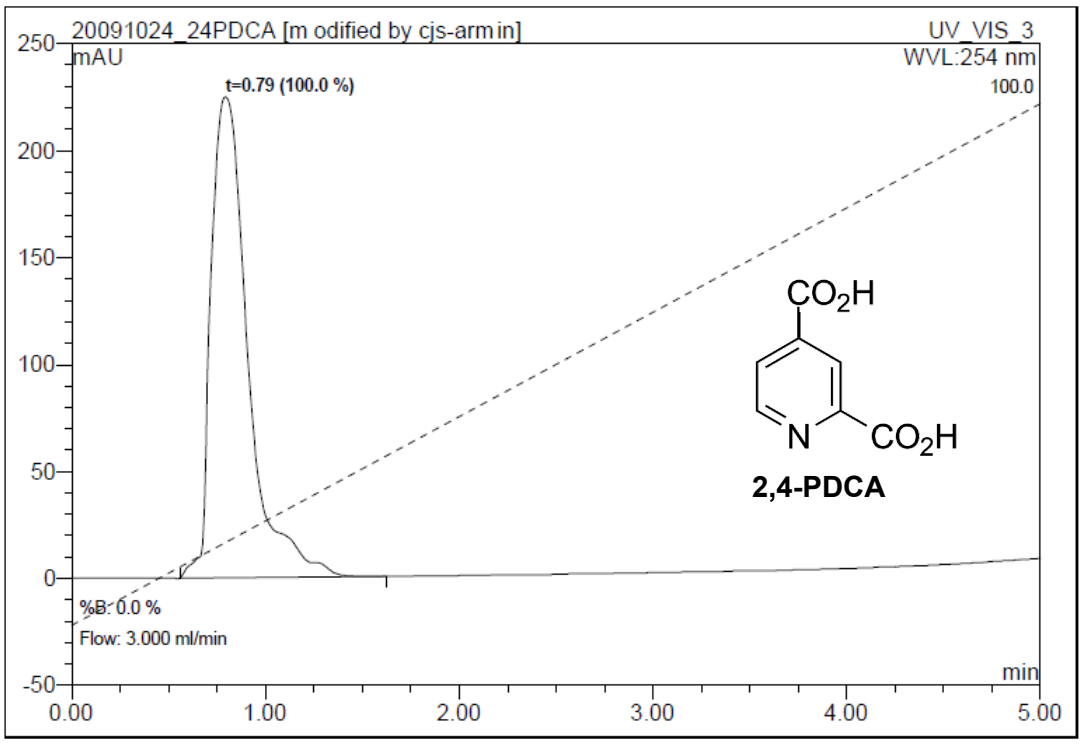
A solution of dimethyl ester **32** (50 mg, 0.24 mmol, 1 eq.) in MeOH (1 cm^3) was treated with aq. NaOH (20% w/v, 1 cm^3) and stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo*, the residue dissolved in the minimum required amount of water and neutralised with conc. HCl. The solution was cooled to 4 °C, the precipitate was isolated by filtration, resuspended in MeOH/CH_2Cl_2, concentrated *in vacuo* and dried in high vacuum to afford **50** (29 mg, 67%) as a pale yellow solid (Found: C, 46.06; H, 3.23; N, 15.28. $C_7H_6N_2O_4$ requires C, 46.16; H, 3.32; N, 15.38%); mp >250 ºC (decomp.); ν_{max} (KBr disk)/cm^{-1} 3421, 3316, 1692, 1585, 1537, 1302, 1256, 1162 and 1101; δ_H (500 MHz; DMSO-d_6) 7.85 (2 H, apparent s, py*H*); δ_C (126 MHz; $CDCl_3$) 128.2, 134.1, 135.0, 138.6, 152.8, 172.9, 173.2; *m/z* (ESI-) 181.0246 ($M - H^+$. $C_7H_5N_2O_4$ requires 181.0255).

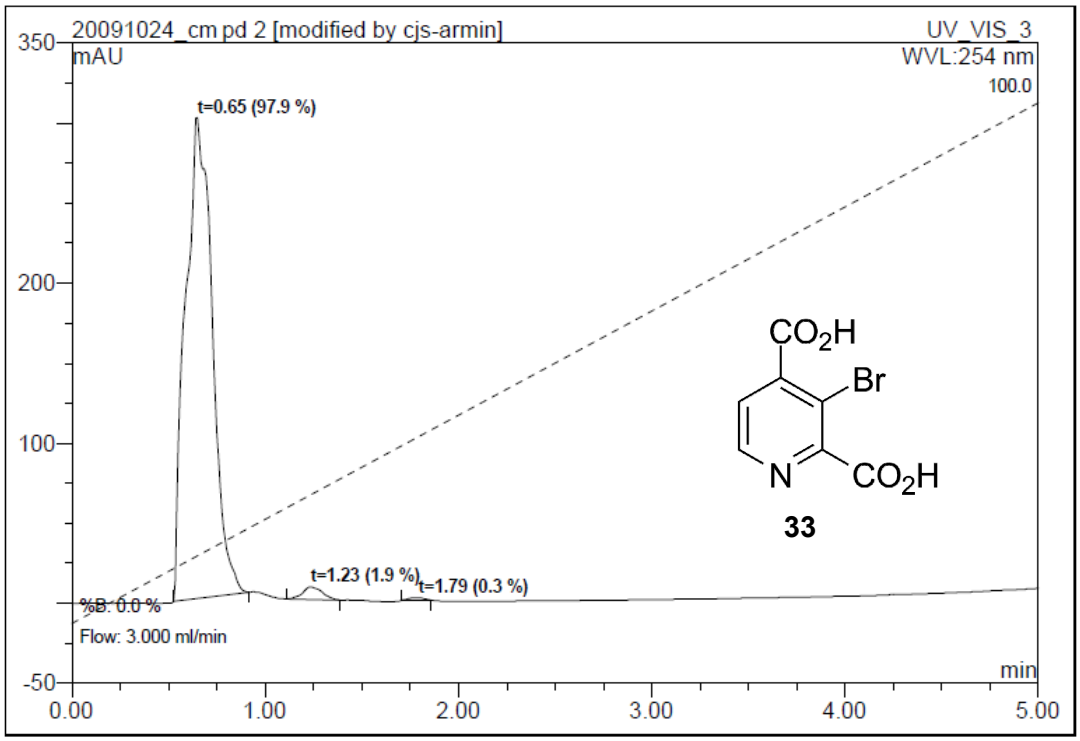
Analytical HPLC analysis for tested compounds

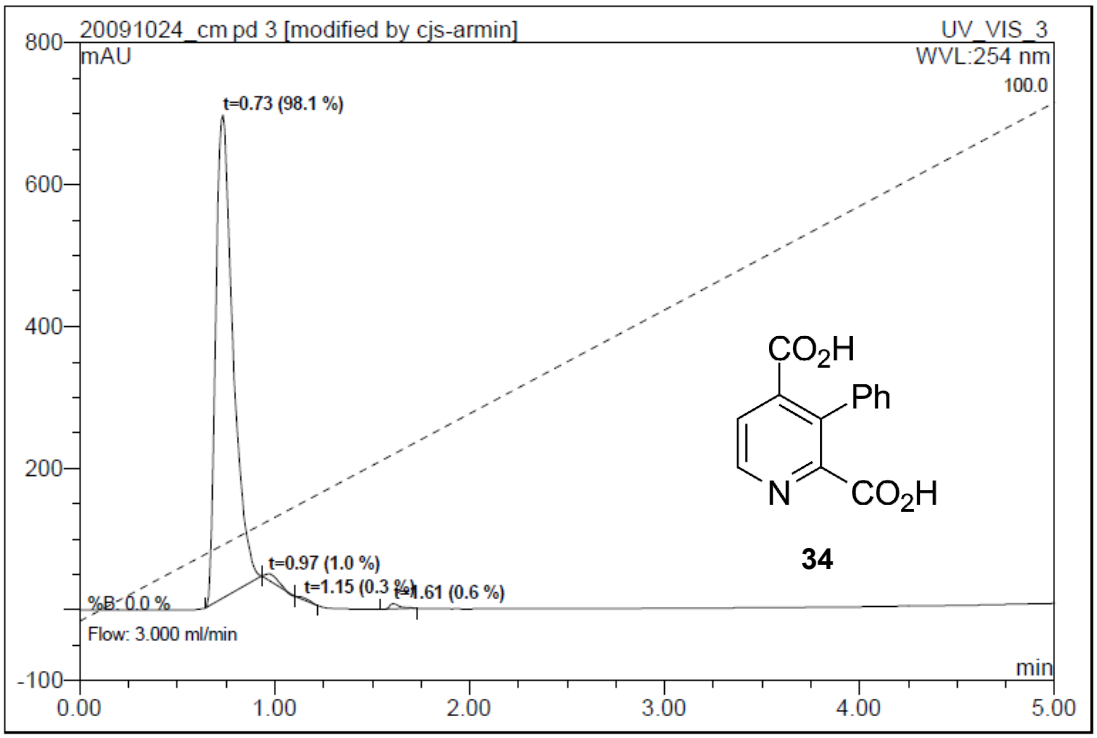
Supporting Table 1**: % purity of tested compounds.** % purity was determined using a Dionex Ultimate 3000 analytical HPLC system, with a Phenomenex Onyx Monolithic C_{18} analytical HPLC column (100x4.6 mm), injection volume 20 µL and a gradient of H_2O/MeCN (0.1% CF_3CO_2H) at a flow rate of 3 mL/min. The reported purities (in %) refer to the relative area under the curve (AUC) of the major peak as determined by integration of the UV absorbance traces at 254 nm.

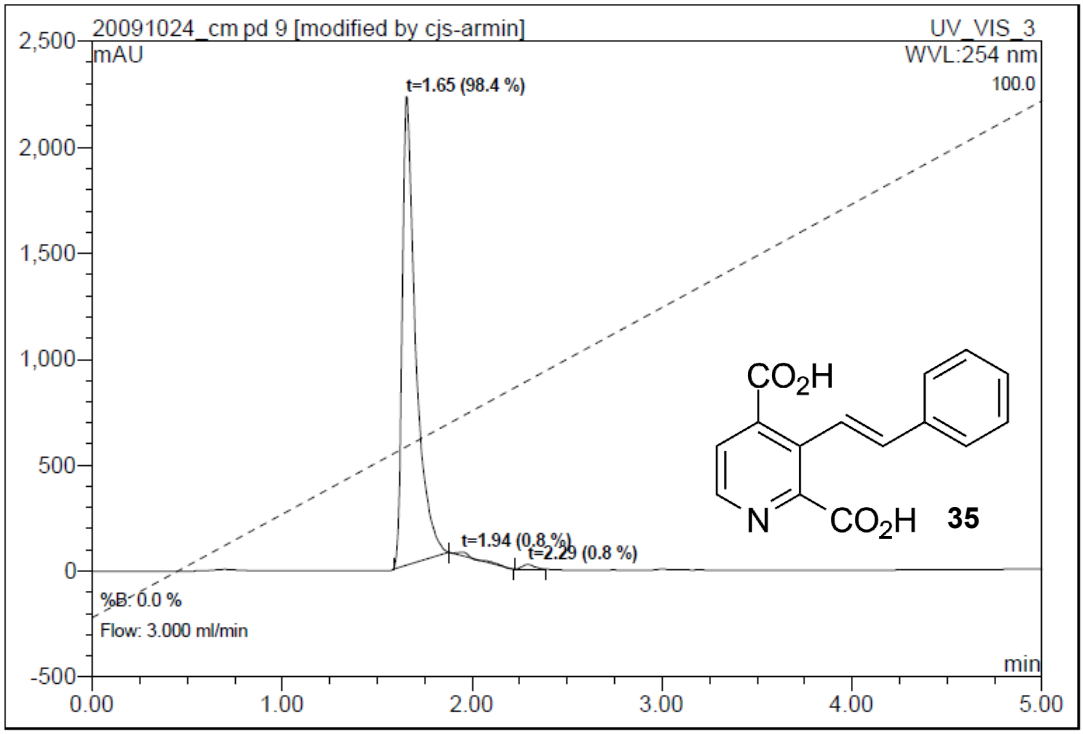
Compound	% purity by analytical HPLC
24PDCA	>99%
33	97.9%
34	98.1%
35	98.4%
36	99.7%
37	98.5%
38	97.4%
39	99.9%
40	95.8%
41	98.6%
42	96.6%
43	98.3%
44	98.8%
45	98.0%
46	96.8%
47	96.6%
48	96.9%
49	96.1%
50	98.3%

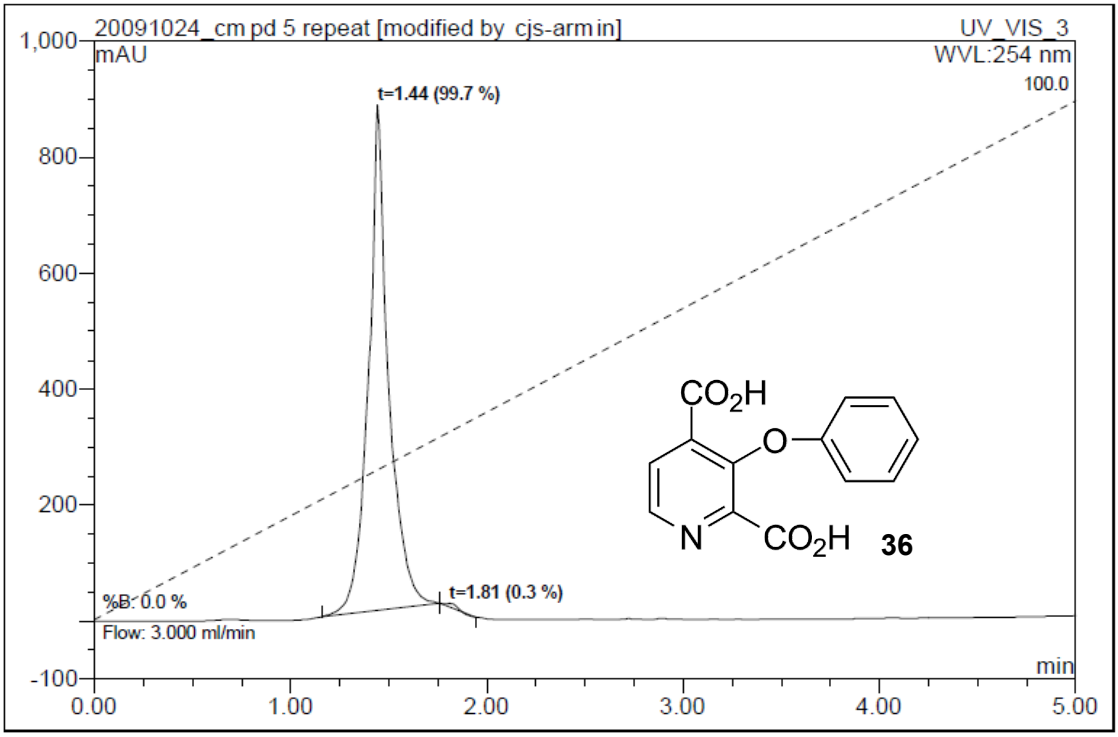
HPLC chromatograms for compounds 33-50.






20091024_cm pd 3 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
800
600
400
200
-100
t=0.73 (98.1 %)
t=0.97 (1.0 %)
t=1.15 (0.3 %)
t=1.61 (0.6 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
Ph
N
CO2H
34


20091024_cm pd 9 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
2,500
2,000
1,500
1,000
500
0
-500
t=1.65 (98.4 %)
t=1.94 (0.8 %)
t=2.29 (0.8 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
N
CO2H
35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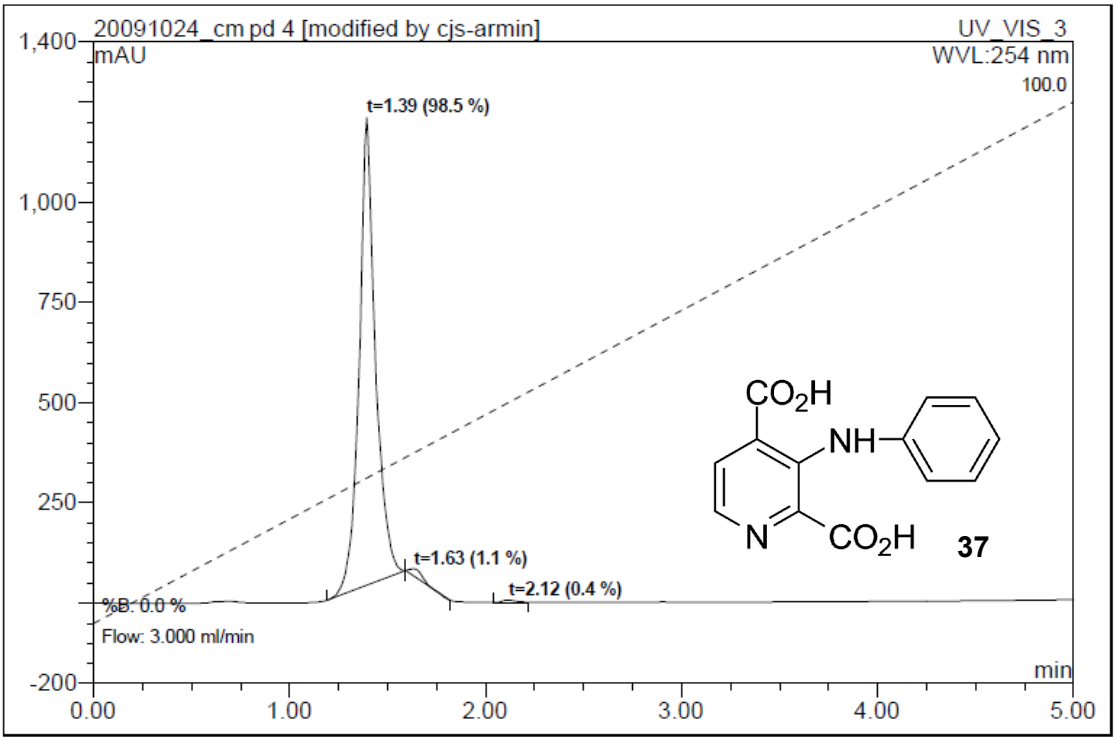
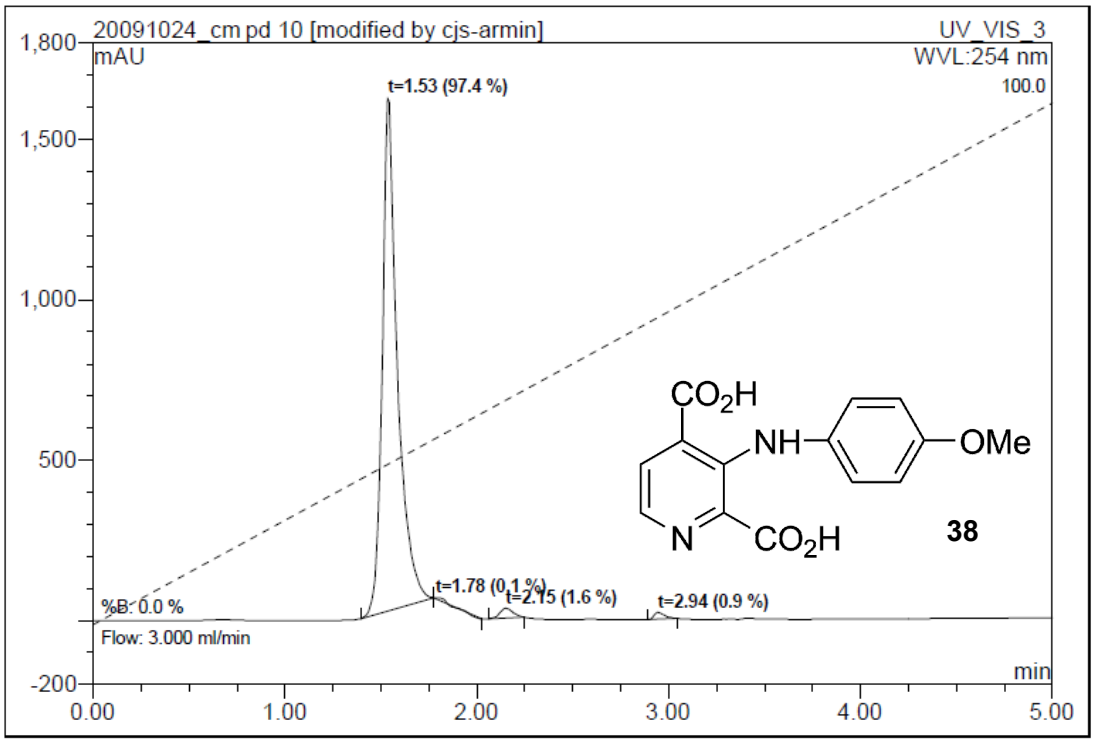
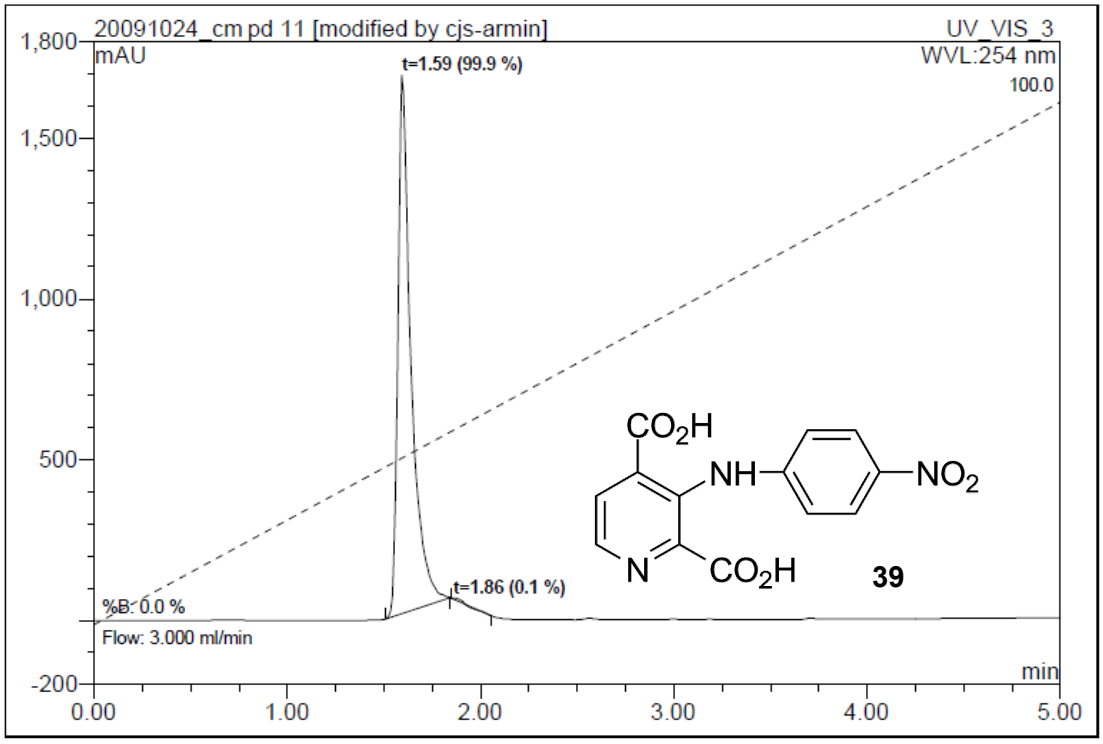
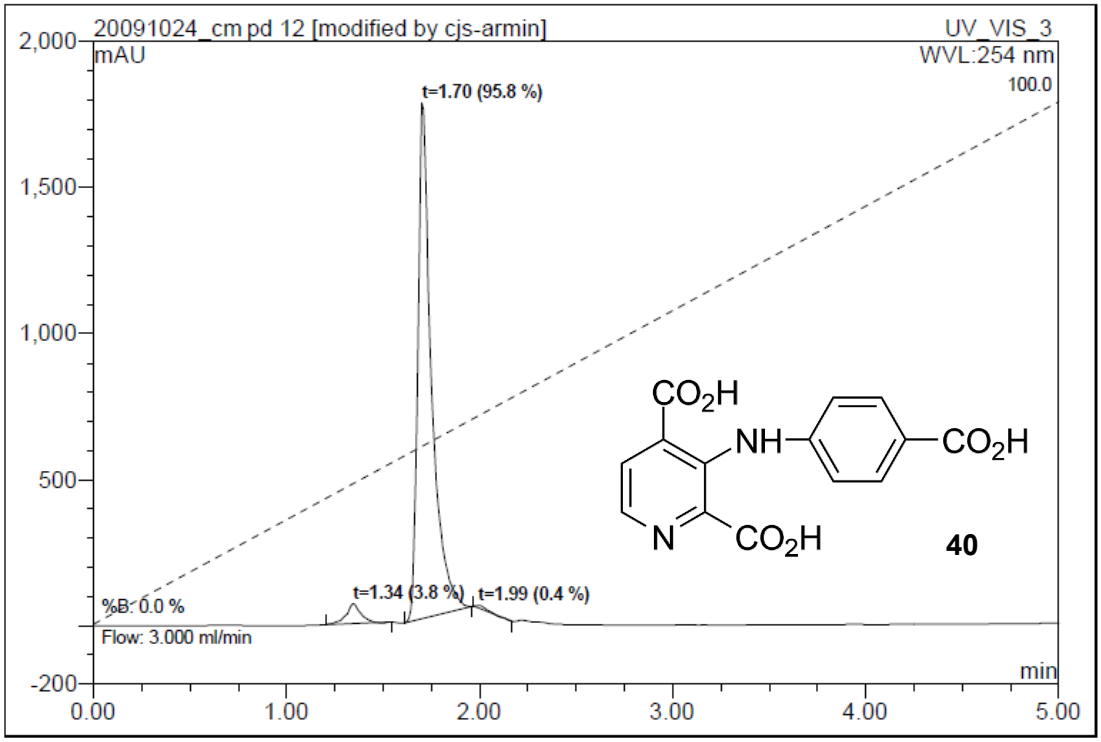
UV_VIS_3
WVL:254 nm
100.0
mAU
1,000
800
600
400
200
-100
t=1.44 (99.7 %)
t=1.81 (0.3 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
O
N
CO2H
36


20091024_cm pd 4 [modified by cjs-armin]
UV_VIS_3
WVL:254 nm
100.0
mAU
1,400
1,000
750
500
250
-200
t=1.39 (98.5 %)
t=1.63 (1.1 %)
t=2.12 (0.4 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
N
CO2H
37


20091024_cm pd 10 [modified by cjs-armin]
UV_VIS_3
WVL:254 nm
100.0
mAU
1,800
1,500
1,000
500
-200
t=1.53 (97.4 %)
t=1.78 (0.1 %)
t=2.15 (1.6 %)
t=2.94 (0.9 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
OMe
N
CO2H
38


20091024_cm pd 11 [modified by cjs-armin]
UV_VIS_3
WVL:254 nm
100.0
mAU
1,800
1,500
1,000
500
-200
t=1.59 (99.9 %)
t=1.86 (0.1 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
NO2
N
CO2H
39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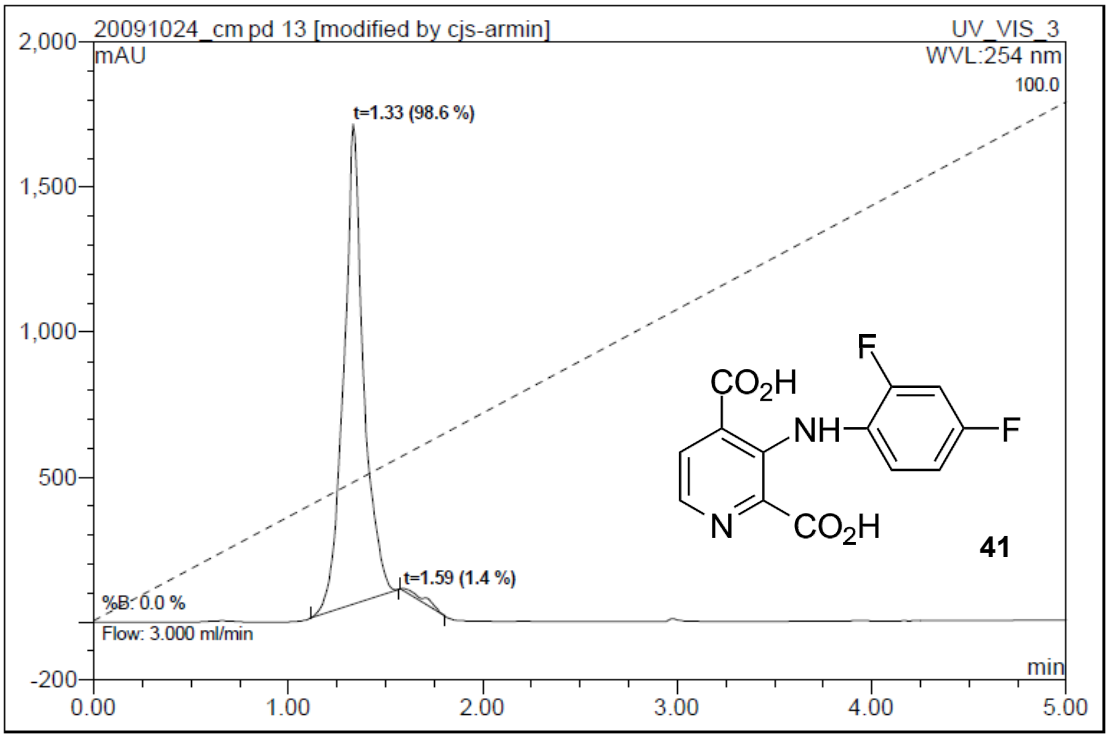
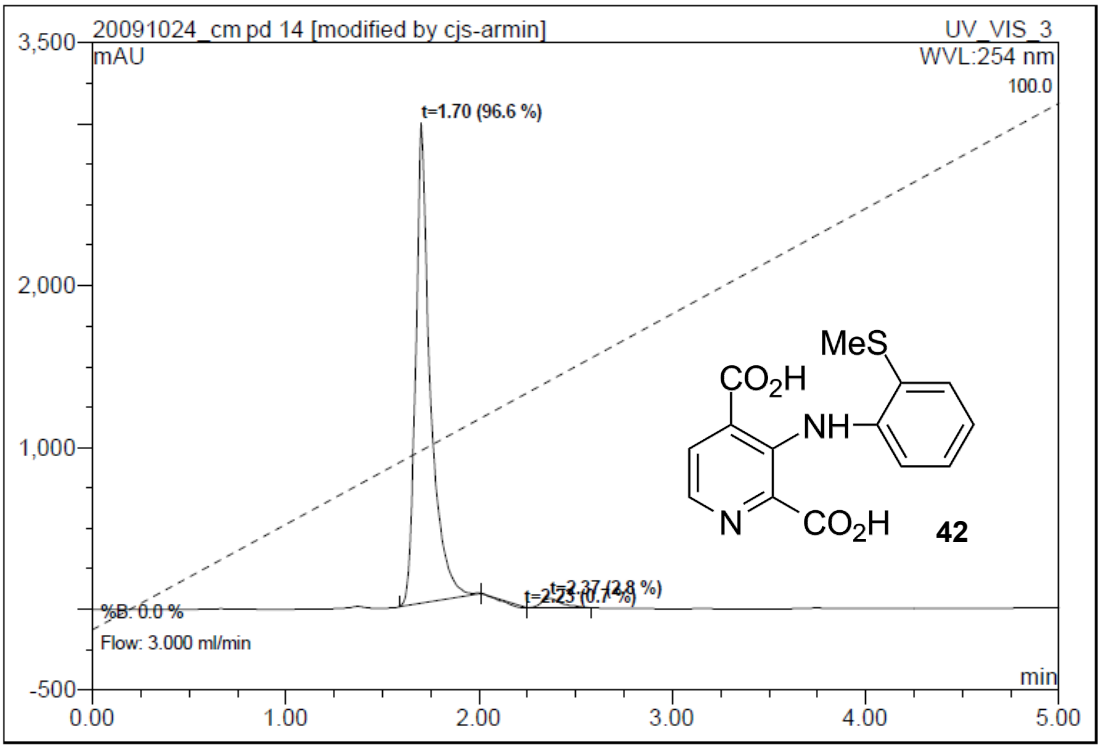
UV_VIS_3
WVL:254 nm
100.0
mAU
2,000
1,500
1,000
500
-200
t=1.70 (95.8 %)
t=1.34 (3.8 %)
t=1.99 (0.4 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
CO2H
N
CO2H
40


20091024_cm pd 13 [modified by cjs-armin]
UV_VIS_3
WVL:254 nm
100.0
mAU
2,000
1,500
1,000
500
-200
t=1.33 (98.6 %)
t=1.59 (1.4 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
F
CO2H
NH
F
N
CO2H
41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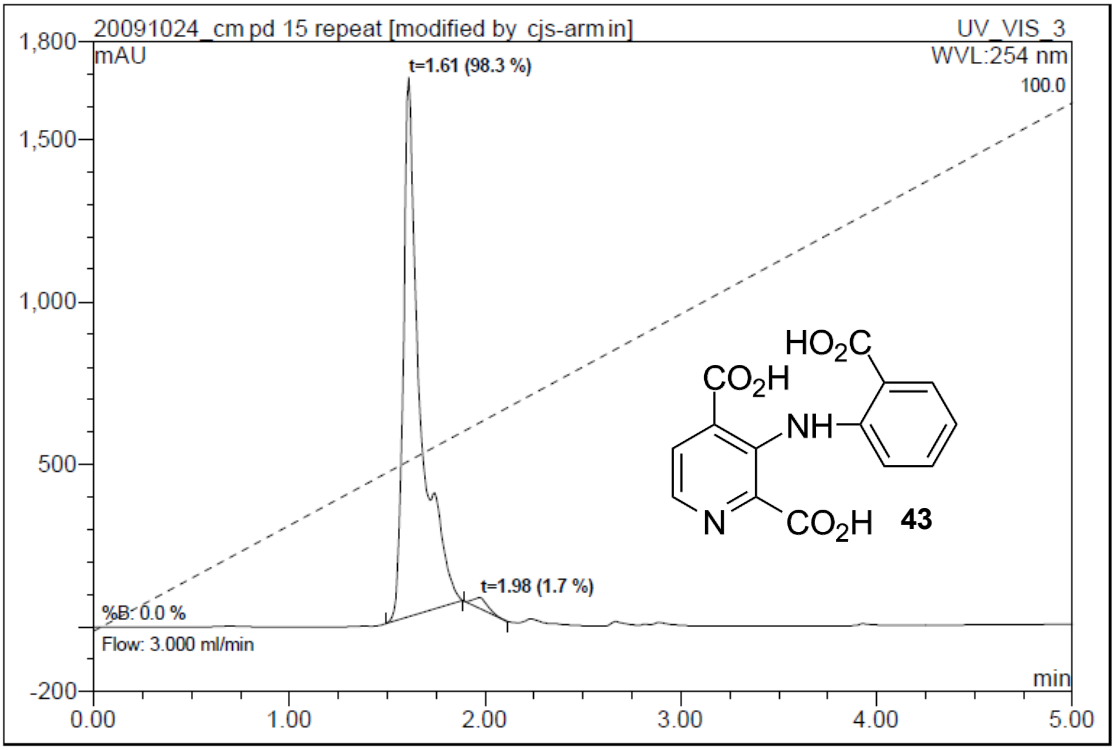
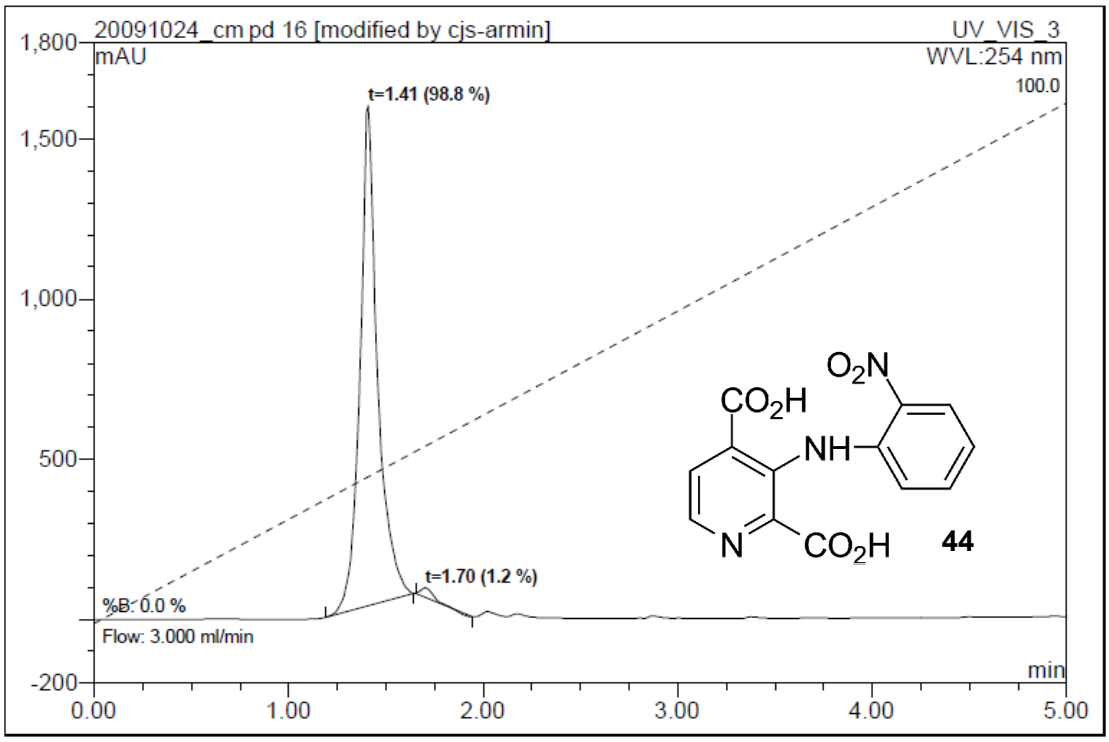
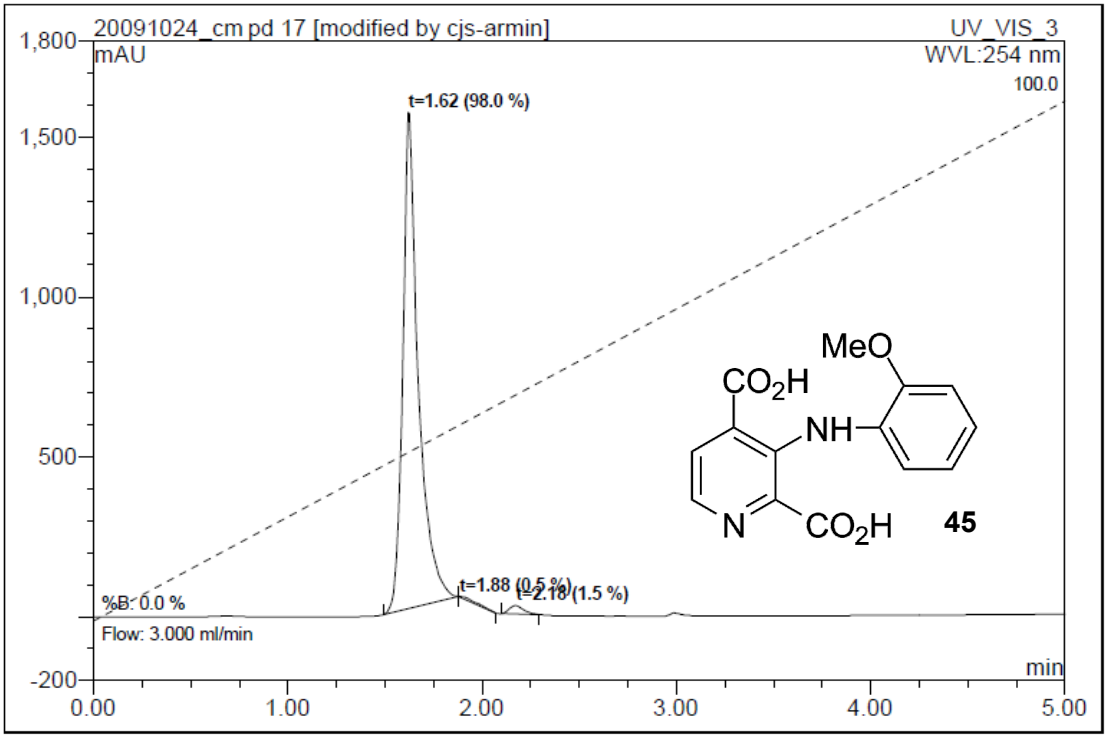
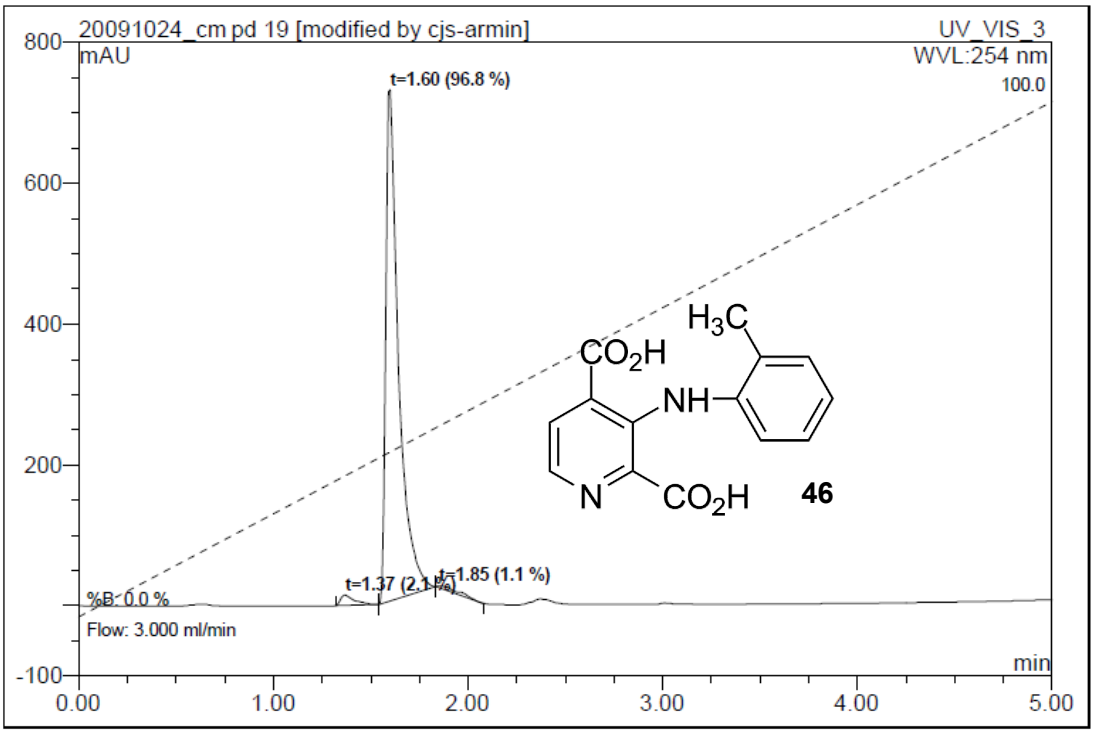
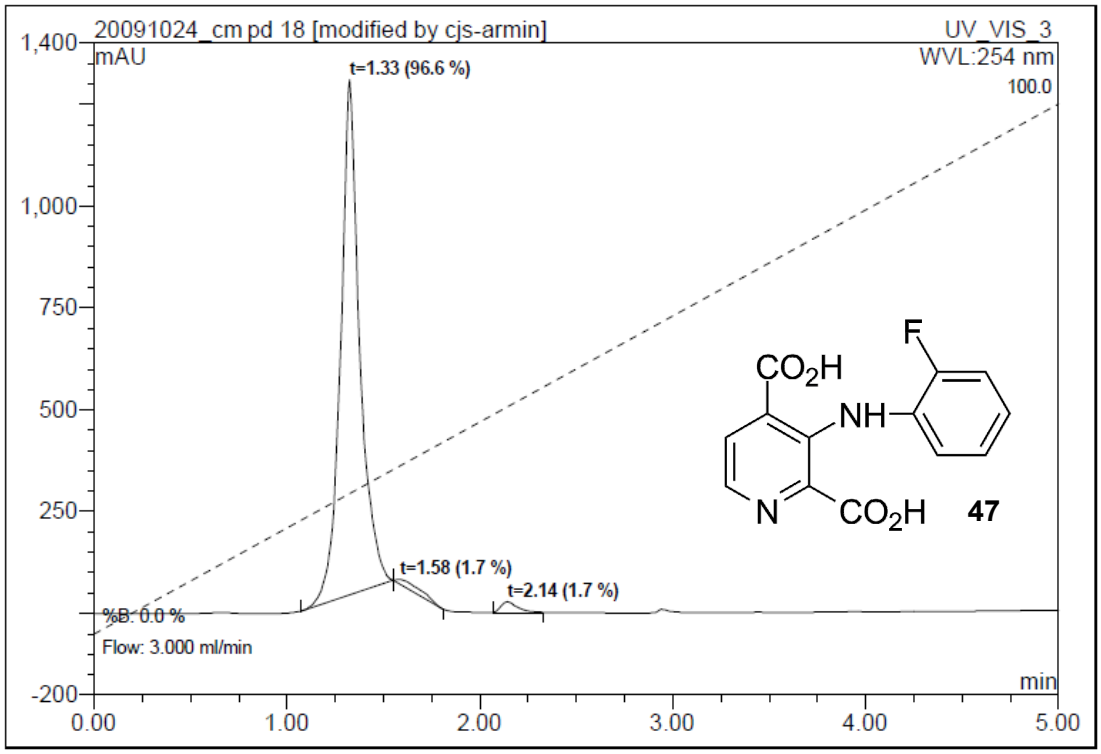
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.70 (96.6 %)
MeS
CO2H
NH
N
CO2H
42
%B: 0.0 %
Flow: 3.000 ml/min
min
3,500
2,000
1,000
-500
0.00
1.00
2.00
3.00
4.00
5.00

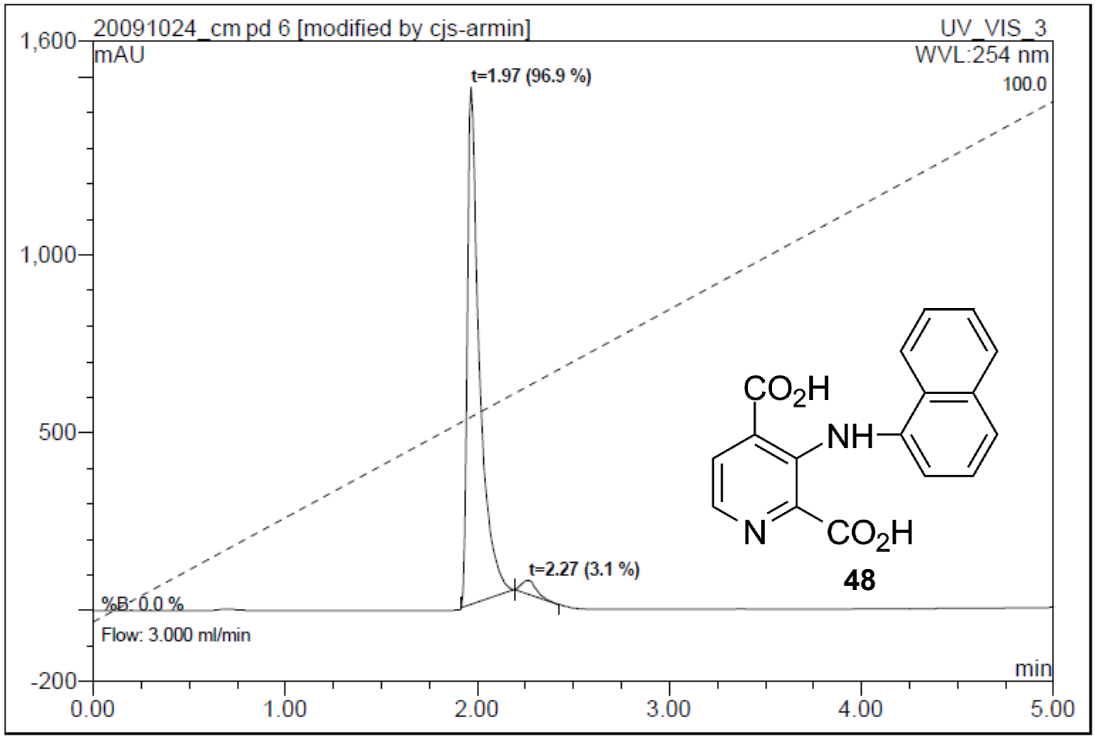

20091024_cm pd 15 repeat [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.61 (98.3 %)
HO2C
CO2H
NH
N
CO2H
43
t=1.98 (1.7 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,800
1,500
1,000
500
-200
0.00
1.00
2.00
3.00
4.00
5.00

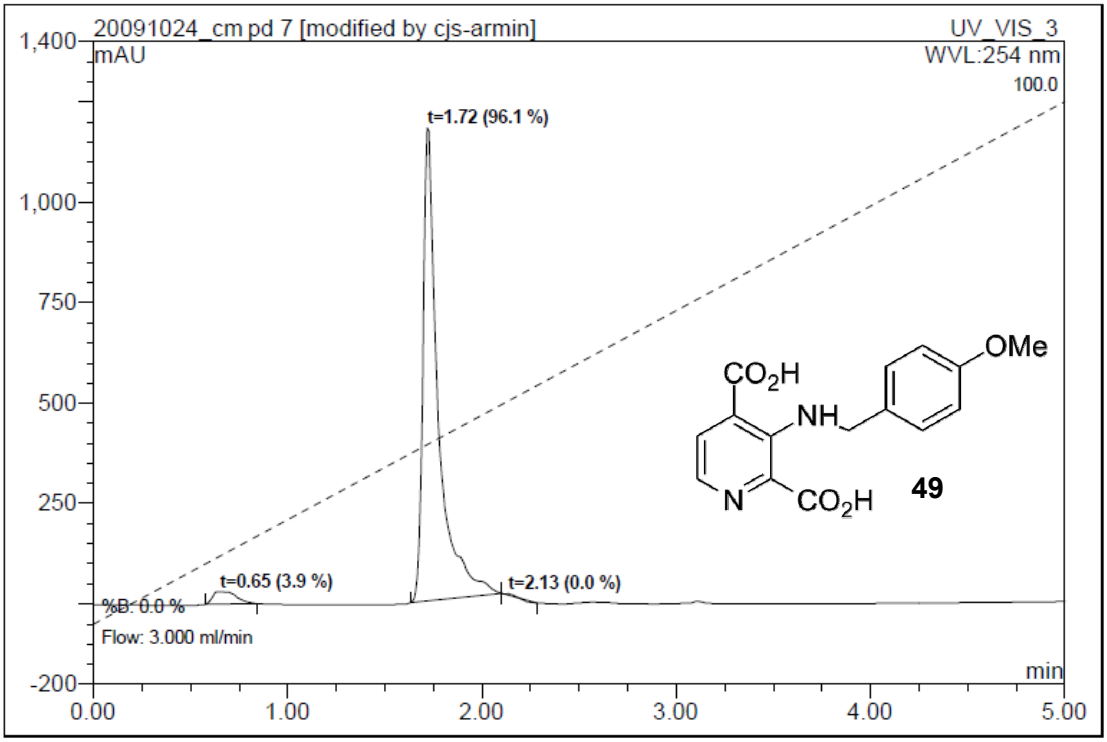

20091024_cm pd 16 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.41 (98.8 %)
t=1.70 (1.2 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,800
1,500
1,000
500
-200
0.00
1.00
2.00
3.00
4.00
5.00
O2N
CO2H
NH
N
CO2H
44

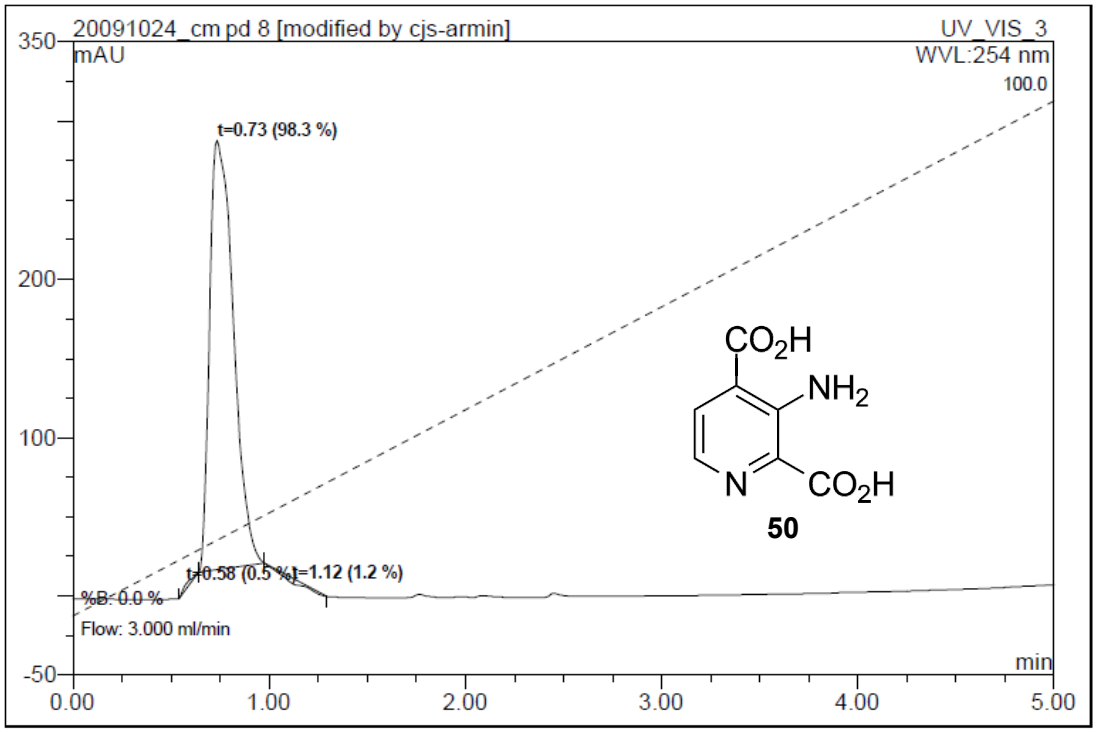

20091024_cm pd 17 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.62 (98.0 %)
t=1.88 (0.5 %)
t=2.18 (1.5 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,800
1,500
1,000
500
-200
0.00
1.00
2.00
3.00
4.00
5.00
MeO
CO2H
NH
N
CO2H
45


20091024_cm pd 19 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.60 (96.8 %)
t=1.37 (2.1 %)
t=1.85 (1.1 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
800
600
400
200
-100
0.00
1.00
2.00
3.00
4.00
5.00
H3C
CO2H
NH
N
CO2H
46


20091024_cm pd 18 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.33 (96.6 %)
t=1.58 (1.7 %)
t=2.14 (1.7 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,400
1,000
750
500
250
-200
0.00
1.00
2.00
3.00
4.00
5.00
F
CO2H
NH
N
CO2H
47


20091024_cm pd 6 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.97 (96.9 %)
t=2.27 (3.1 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,600
1,000
500
-200
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
N
CO2H
48


20091024_cm pd 7 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
t=1.72 (96.1 %)
t=0.65 (3.9 %)
t=2.13 (0.0 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
1,400
1,000
750
500
250
-200
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH
N
CO2H
OMe
49


20091024_cm pd 8 [modified by cjs-armin]
UV_VIS_3
mAU
WVL:254 nm
100.0
350
200
100
-50
t=0.73 (98.3 %)
t=0.58 (0.5 %)
t=1.12 (1.2 %)
%B: 0.0 %
Flow: 3.000 ml/min
min
0.00
1.00
2.00
3.00
4.00
5.00
CO2H
NH2
N
CO2H
50

References for Supporting Information

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