Supporting Information

Synthesis and evaluation of 5-fluoro-2-aryl-oxazolo[5,4-*b*]pyridines as β-amyloid PET ligands and the identification of MK-3328

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All animal studies were performed according to the NIH Guide for the Care and Use of Laboratory Animals, and experimental protocols were reviewed by the Merck Animal Care and Use Committee.

AD brain homogenate binding assay: Postmortem frozen human brain samples from donors with clinical diagnosis of Alzheimer's diseases (AD) or normal control subjects (non-AD) were purchased from Analytical Biological Services Inc., at 701-4 Cornell Business Park, Wilmington, DE 19801. Brain homogenates of frontal cortex were prepared, divided into aliquots and stored at -70°C prior to use.

[^{3}H]-DMAB was synthesized at a specific activity of ~80 Ci/mmol. The final concentration of radioligand for tissue homogenate binding assay was 1.5nM. Brain homogenates were diluted with phosphate buffered saline (PBS) to 0.4mg/mL from original 10mg/mL volume and 200µl was used in assay for a final concentration of 50µg/assay tube. Unlabeled test compounds were dissolved in dimethylsulfoxide (DMSO) at 1mM. Dilution of test compound to various concentrations was made with PBS containing 2% DMSO. Total binding was defined in the absence of competing compound, and non-displaceable binding was determined in the presence of 1µM unlabeled self block. Compound dilutions (10X) were added into the assay tube (25µL each / per tube, separately) containing 200µL brain homogenate dilution, and the tubes were pre-incubated at room temperature for 10 minutes. Then radioligand

dilutions (10X) were added into the assay tube (25µL each / per tube, separately) to a final volume of 250µL per tube. Incubation was carried out at room temperature (25ºC) for 90 minutes, and then the assay samples were filtered onto GF/C filters using Skatron 12 well harvester, washing on setting 5 – 5 – 5 (~ 3x2ml) ice cold buffer (PBS, pH 7.4). GF/C filter papers for the Skatron harvester were pre-soaked in 0.1% BSA for 1 hour at room temperature before use. Filters were punched into scintillation vials and counted in 2mL Ultima Gold on Perkin Elmer Tri-Carb 2900TR for 1 minute. The data analysis was done with Prism software. All assays were done in triplicate, and in the laboratory designated for studies using human tissues.

***In vitro* autoradiography:** Postmortem frozen human brain samples from donors with clinical diagnosis of Alzheimer's diseases (AD) or normal control subjects (non-AD) were purchased from a commercial source. Frozen brain slices (20µm thickness) were prepared using a cryostat (Leica CM3050) and kept in sequential order. The tissue slices were placed on Superfrost Plus glass slides (Cat.# 5075-FR, Brain Research Laboratories, USA), dried at room temperature, and stored in a slide box at -70ºC before use. The final concentration of radioligand for in vitro autoradiography was 1.0nM. On the day of a binding experiment, adjacent slices were selected from each brain region of interest for *in vitro* autoradiographic study, and were designated as total binding and non-specific binding (NSB). These slices were thawed at room temperature for 15 minutes in a biosafety hood. Total binding of radioligand in brain slices was defined in the absence of competitor, and non-specific binding (NSB) was determined in the presence of competitor (1.0µM unlabeled compound). The brain slides were first pre-incubated at room temperature for twenty minutes in PBS buffer, pH 7.4. The slices were then transferred to fresh buffer containing radioligand or radioligand plus competitor as described above, and incubated at room temperature for ninety minutes. Incubation was terminated by washing the slices three times in ice cold (4ºC) wash buffer (PBS, pH 7.4) with each wash lasting three minutes. After washing, the slices were briefly rinsed in ice cold (4ºC) deionized water, and then dried completely by an air blower at room temperature. The slices were placed against Fuji Phosphor Image Plates (TR25, Fuji) in a sealed cassette for exposure at room temperature. After one week exposure, the plates were scanned in Fuji BAS 5000 Scanner, and the scanned images were analyzed using MCID 7.0 software. [^{3}H]-microscales (Amersham Biosciences, GE), were used

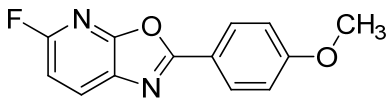
for quantification of radioligand binding density. All the slice binding assays were done in the laboratory designated for studies using human tissues.

Synthetic Procedures and Characterization

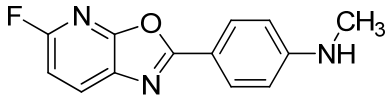
HRMS measurements were acquired by use of a Bruker Daltonics 7T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Samples were dissolved in acetonitrile:water:acetic acid [50:50:0.1%(v/v)], and ionized by use of electrospray ionization (ESI). External calibration was accomplished with oligomers of polypropylene glycol (PPG, average molecular weight 1000 Da). ^{1}H-NMR data were collected on a Varian 500 MHz instrument equipped with a Protasis flow NMR probe. Mass-guided purifications were accomplished on Agilent 1100 hardware utilizing Penomenex Luna 5 micron C18 columns (2 cm x 5 cm), eluting at 25 mL / min with a custom 8 minute focused gradient containing acetonitrile and water with 0.1% TFA.

General procedure for the synthesis of 13, 14a, 14b, 15a, 15b, 16a, 16b, 18–24:

To a stirred suspension of each carboxylic acid (0.27 mmol) in 3 mL CH_2Cl_2 (3 mL) in uncapped microwave reaction tubes was added 1-chloro-N,N-2-trimethylpropenylamine (150 uL, 1.13 mmol). After stirring for 30 minutes, the reaction mixtures were concentrated *in vacuo* before the addition of pyridine (1 mL) followed by 2,6-difluoro-pyridin-3-ylamine (50 mg, 0.38 mmol) dissolved in pyridine (1 mL). After an additional 30 minutes, the reaction mixtures were again concentrated in vacuo. DMF was added (2 mL) along with Cs_2CO_3 (376 mg, 1.15 mmol) and the reaction mixtures were then heated by microwave to 165 °C for 10 minutes each. After cooling, the crude reaction mixtures were filtered through Celite and subjected to mass-guided reverse phase HPLC purification (5%-35% isolated yields). All tested compounds were >95% pure as assessed by LCMS and ^{1}H NMR. Unless otherwise specified, Boc protecting groups were removed as a consequence of the microwave heating step.

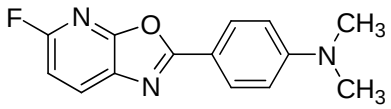


5-Fluoro-2-(4-methoxy-phenyl)-oxazolo[5,4-*b*]pyridine 13. ^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.39 (1 H, dd, J = 8.40, 7.09 Hz), 8.14 (2 H, d, J = 8.63 Hz), 7.28 (1 H, d, J = 8.40 Hz), 7.18 (2 H, d, J = 8.61 Hz), 3.88 (3 H, s). HRMS m/z 245.0722 ($C_{13}H_9FN_2O_2$ + H^+ requires 245.0721).



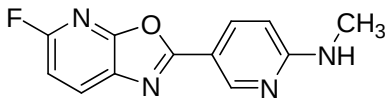
[4-(5-Fluoro-oxazolo[5,4-*b*]pyridin-2-yl)-phenyl]-methyl-amine 14a.

Synthesized using 4-(*tert*-butoxycarbonyl-methyl-amino)-benzoic acid. ^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.26 (1 H, t, J = 7.72 Hz), 7.91 (2 H, d, J = 8.55 Hz), 7.20 (1 H, d, J = 8.36 Hz), 6.70 (2 H, d, J = 8.55 Hz), 2.78 (3 H, d, J = 4.17 Hz). HRMS m/z 244.0878 ($C_{13}H_{10}FN_3O$ + H^+ requires 244.0881).



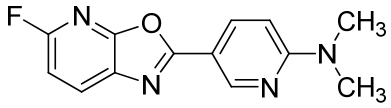
[4-(5-Fluoro-oxazolo[5,4-*b*]pyridin-2-yl)-phenyl]-dimethyl-amine 14b.

^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.28 (1 H, t, J = 7.75 Hz), 7.98 (2 H, d, J = 8.59 Hz), 7.22 (1 H, d, J = 8.39 Hz), 6.87 (3 H, d, J = 8.64 Hz), 3.05 (7 H, s). HRMS m/z 258.1039 ($C_{14}H_{12}FN_3O$ + H^+ requires 258.1037).



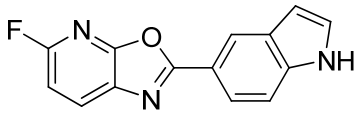
[5-(5-Fluoro-oxazolo[5,4-*b*]pyridin-2-yl)-pyridin-2-yl]-methyl-amine 15a.

Synthesized using 6-(*tert*-Butoxycarbonyl-methyl-amino)-nicotinic acid. ^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.80 (1 H, d, J = 2.33 Hz), 8.31 (1 H, t, J = 7.73 Hz), 8.05 (1 H, d, J = 8.77 Hz), 7.50 (1 H, s), 7.23 (1 H, d, J = 8.39 Hz), 6.63 (1 H, d, J = 8.90 Hz), 2.88 (3 H, d, J = 4.78 Hz). HRMS m/z 245.0829 ($C_{12}H_9FN_4O + H^+$ requires 245.0833).



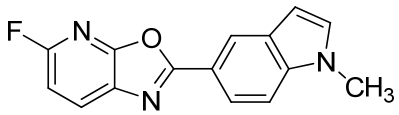
[5-(5-Fluoro-oxazolo[5,4-*b*]pyridin-2-yl)-pyridin-2-yl]-dimethyl-amine 15b.

^{1}H NMR δ (ppm)(DMSO-d$_6$): 8.87 (1 H, d, J = 2.40 Hz), 8.32 (1 H, dd, J = 8.38, 7.11 Hz), 8.16 (1 H, dd, J = 9.03, 2.47 Hz), 7.25 (1 H, d, J = 8.37 Hz), 6.84 (1 H, d, J = 9.09 Hz), 3.16 (4 H, s). HRMS m/z 259.0985 ($C_{13}H_{11}FN_4O + H^+$ requires 259.0990).



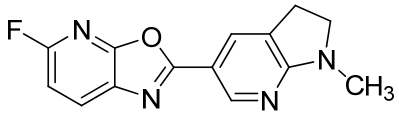
5-Fluoro-2-(1*H*-indol-5-yl)-oxazolo[5,4-*b*]pyridine 16a.

Synthesized using *N*-Boc-indole-5-carboxylic acid. ^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 11.57 (1 H, s), 8.46 (1 H, s), 8.38 (1 H, dd, J = 8.40, 7.11 Hz), 7.95 (1 H, dd, J = 8.50, 1.69 Hz), 7.61 (1 H, d, J = 8.55 Hz), 7.53–7.51 (1 H, m), 7.27 (1 H, d, J = 8.40 Hz), 6.66 (1 H, s). HRMS m/z 254.0722 ($C_{14}H_8FN_3O + H^+$ requires 254.0724).



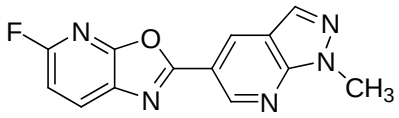
5-Fluoro-2-(1-methyl-1*H*-indol-5-yl)-oxazolo[5,4-*b*]pyridine 16b.

^{1}H NMR δ (ppm)(DMSO-d$_6$): 8.52 (1 H, d, J = 1.62 Hz), 8.45 (1 H, dd, J = 8.38, 7.12 Hz), 8.06 (1 H, dd, J = 8.65, 1.69 Hz), 7.75 (1 H, d, J = 8.67 Hz), 7.57 (1 H, d, J = 3.13 Hz), 7.33 (1 H, d, J = 8.38 Hz), 6.73 (1 H, d, J = 3.13 Hz), 3.94 (3 H, s). HRMS m/z 268.0878 ($C_{15}H_{10}FN_3O + H^+$ requires 268.0881).



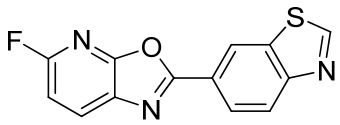
5-Fluoro-2-(1-methyl-2,3-dihydro-1*H*-indol-5-yl)-oxazolo[5,4-*b*]pyridine 18.

^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.61 (1 H, s), 8.29 (1 H, dd, J = 8.37, 7.11 Hz), 7.82 (1 H, d, J = 2.01 Hz), 7.23 (1 H, d, J = 8.39 Hz), 3.64 (2 H, t, J = 8.40 Hz), 3.07 (2 H, t, J = 8.41 Hz), 2.98 (3 H, s). HRMS m/z 271.0995 ($C_{14}H_{11}FN_4O + H^+$ requires 271.0990).



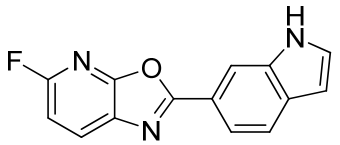
5-Fluoro-2-(1-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)-oxazolo[5,4-*b*]pyridine 19.

^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 9.34 (1 H, d, J = 2.03 Hz), 9.07 (1 H, d, J = 2.04 Hz), 8.49 (1 H, dd, J = 8.40, 7.06 Hz), 8.38 (1 H, s), 7.34 (1 H, d, J = 8.42 Hz), 4.14 (3 H, s). HRMS m/z 270.0786 ($C_{13}H_8FN_5O + H^+$ requires 270.0786).



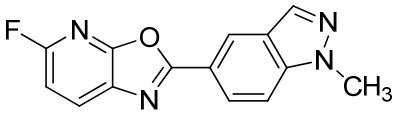
2-Benzothiazol-6-yl-5-fluoro-oxazolo[5,4-*b*]pyridine 20.

^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 9.61 (1 H, s), 9.11 (1 H, d, J = 1.63 Hz), 8.50 (1 H, dd, J = 8.42, 7.10 Hz), 8.35 (1 H, dd, J = 8.61, 1.73 Hz), 8.31 (1 H, d, J = 8.57 Hz), 7.35 (1 H, d, J = 8.43 Hz). HRMS m/z 272.0290 ($C_{13}H_6FN_3OS + H^+$ requires 272.0288).



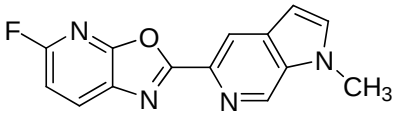
5-Fluoro-2-(1*H*-indol-6-yl)-oxazolo[5,4-*b*]pyridine 21.

Synthesized using *N*-Boc-indole-6-carboxylic acid. ^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 11.57 (1 H, s), 8.46 (1 H, s), 8.38 (1 H, dd, J = 8.40, 7.11 Hz), 7.95 (1 H, dd, J = 8.50, 1.69 Hz), 7.61 (1 H, d, J = 8.55 Hz), 7.53–7.51 (1 H, m), 7.27 (1 H, d, J = 8.40 Hz), 6.66 (1 H, m). HRMS m/z 254.0729 ($C_{14}H_8FN_3O + H^+$ requires 254.0724).



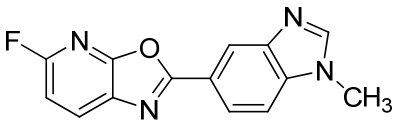
5-Fluoro-2-(1-methyl-1*H*-indazol-5-yl)-oxazolo[5,4-*b*]pyridine 22.

^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.68 (1 H, s), 8.43 (1 H, dd, J = 8.35, 7.05 Hz), 8.30 (1 H, s), 8.20 (1 H, dd, J = 8.88, 1.60 Hz), 7.89 (1 H, d, J = 8.88 Hz), 7.30 (1 H, d, J = 8.43 Hz), 4.13 (3 H, s). HRMS m/z 254.0729 ($C_{14}H_8FN_3O + H^+$ requires 254.0724).



5-Fluoro-2-(1-methyl-1*H*-pyrrolo[2,3-*c*]pyridin-5-yl)-oxazolo[5,4-*b*]pyridine 23.

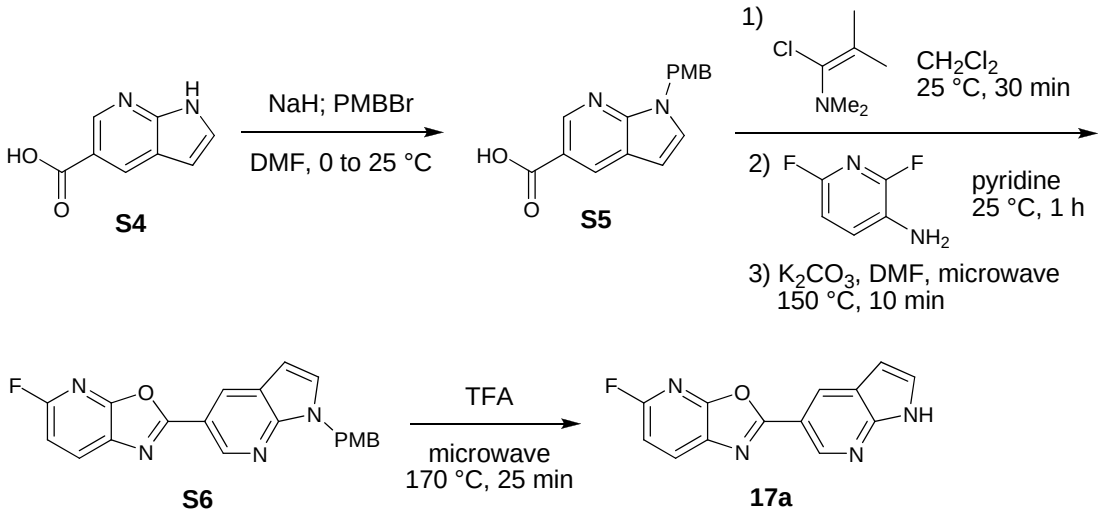
^{1}H NMR δ (ppm)(500 MHz, DMSO-d$_6$): 8.68 (1 H, s), 8.43 (1 H, dd, J = 8.39, 7.12 Hz), 8.30 (1 H, s), 8.20 (1 H, dd, J = 8.86, 1.59 Hz), 7.89 (1 H, d, J = 8.87 Hz), 7.30 (1 H, d, J = 8.42 Hz), 4.12 (3 H, s). HRMS m/z 269.0834 ($C_{14}H_9FN_4O + H^+$ requires 269.0833).



5-Fluoro-2-(1-methyl-1*H*-benzoimidazol-5-yl)-oxazolo[5,4-*b*]pyridine 24.

^{1}H NMR δ (ppm)(DMSO-d$_6$): 8.46–8.36 (3 H, m), 8.13 (1 H, dd, J = 8.49, 1.61 Hz), 7.82 (1 H, d, J = 8.52 Hz), 7.29 (1 H, d, J = 8.39 Hz), 3.91 (3 H, s). HRMS m/z 269.0833 ($C_{14}H_9FN_4O + H^+$ requires 269.0833).

Synthesis of unlabled 17a:



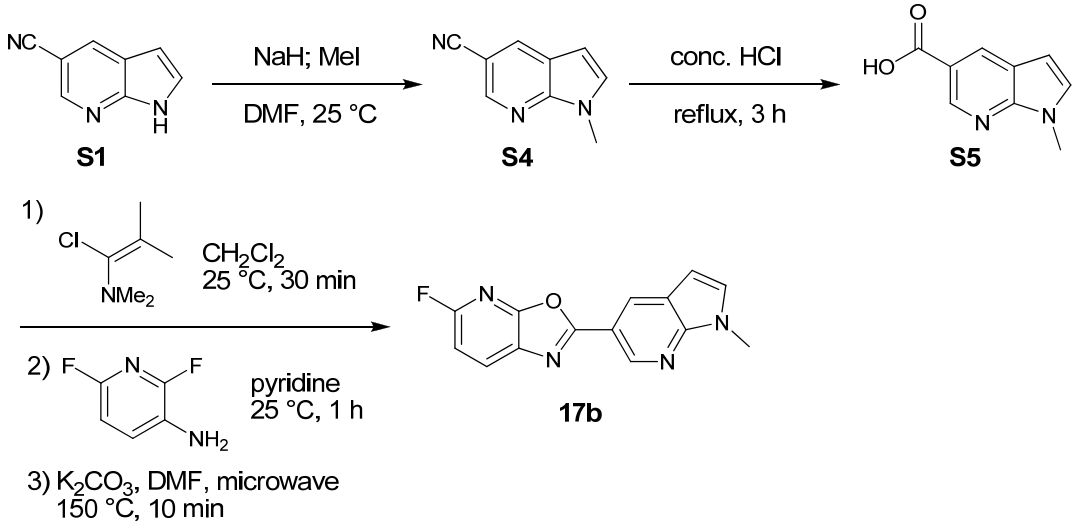
1-(4-Methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid S5. To a stirred cooled 0 °C suspension of NaH (272 mg, 6.81 mmol) in DMF (23 mL) was added 1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid methyl ester (400 mg, 2.27 mmol). After 5 min, PMBBr (548 mg, 2.72 mmol) and KI (377 mg, 2.27 mmol) were added and the reaction mixture was allowed to warm to room temperature and stir overnight. The following day, water was added to quench the remaining NaH and after 30 minutes of stirring, the aqueous mixture was washed with EtOAc. The aqueous phase was collected and carefully acidified (pH ~3) before extraction with EtOAc. The combined organics were dried and evaporated to afford 1-(4-methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid (160 mg, 0.57 mmol, 25% yield) which was used without additional purification. ES MS ($M+H^+$) = 283.

5-Fluoro-2-[1-(4-methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl]-oxazolo[5,4-*b*]pyridine S6. To a stirred solution of 1-(4-methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid **S5** (47 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) was added 1-chloro-N,N-2-trimethylpropenylamine (45 µL, 0.34 mmol). After 30 minutse, the reaction mixture was concentrated and the resulting residue was dissolved in pyridine (2 mL) before 2,6-difluoro-pyridin-3-ylamine (20 mg, 0.15 mmol) was added in one portion. After an additional 30 minutes, the reaction mixture was again concentrated to dryness affording a residue, to which was added DMF (2 mL) and K_2CO_3 (64 mg,

0.46 mmol). The resulting mixture was heated by microwave to 150 °C for 10 min, after which the resulting mixture was filtered and concentrated, affording 5-fluoro-2-[1-(4-methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl]-oxazolo[5,4-*b*]pyridine as a crude residue which was subsequently used without further purification. ES MS ($M+H^+$) = 375.

5-Fluoro-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)-oxazolo[5,4-*b*]pyridine 7a. Crude 5-fluoro-2-[1-(4-methoxy-benzyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl]-oxazolo[5,4-*b*]pyridine **S6** was dissolved in TFA (0.5 mL) and heated by microwave to 170 °C for 25 min. The volatiles were then removed *in vacuo* and the resulting residue was purified by reverse phase HPLC (acetonitrile/water gradient containing 0.1% TFA) to afford 5-fluoro-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)-oxazolo[5,4-*b*]pyridine (2.5 mg, 9.8 μmol, 6% yield). ES MS ($M+H^+$) = 255; ^{1}H NMR δ (ppm)(DMSO-d_6): 12.18 (1 H, s), 9.04 (1 H, d, J = 2.10 Hz), 8.76 (1 H, d, J = 2.06 Hz), 8.44 (1 H, dd, J = 8.39, 7.10 Hz), 7.67 (1 H, t, J = 2.75 Hz), 7.31 (1 H, d, J = 8.40 Hz), 6.68 (1 H, d, J = 3.43 Hz); HRMS m/z 255.0675 ($C_{13}H_7FN_4O + H^+$ requires 255.0677).

Synthesis of unlabeled 17b:



1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile S2. To a stirred solution of 1*H*-Pyrrolo[2,3-*b*]pyridine-5-carbonitrile **S1** (2.88 g, 20.1 mmol) in DMF (40 mL) was added 60% NaH (2.41 g, 60.4 mmol). After 20 minutes, iodomethane was added in one portion (6.3 mL,

101 mmol) and the resulting mixture was stirred overnight. The following day, water was carefully added drop-wise to quench the remaining NaH before additional water was added (50 mL) causing precipitation of the product. Filtration and drying in vacuo afforded **S2** (3.16 g, 20.1 mmol, 100% yield) which was subsequently used without further purification. ES MS ($M+H^+$) = 158.

1-Methyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile S3. 1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile **S2** (3.16 g, 20.1 mmol) was dissolved in concentrated aqueous HCl (15 mL) and refluxed for 3 h. After cooling, the mixture was evaporated *in vacuo* affording **S3** (3.54 g, 20.1 mmol, 100% yield) which was used subsequently without further purification. ES MS ($M+H^+$) = 177.

5-Fluoro-2-(1-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)-oxazolo[5,4-*b*]pyridine 17b. To a suspension of 1-methyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid (68 mg, 0.38 mmol) in CH_2Cl_2 (2 mL) was added 1-chloro-N,N-2-trimethylpropenylamine (50 µL, 0.38 mmol). Following formation of the resulting acid chloride, the reaction mixture was concentrated affording a residue that was dissolved in pyridine (2 mL) before 2,6-difluoro-pyridin-3-ylamine (50 mg, 0.38 mmol) was added in one portion. After an additional 30 minutes the reaction mixture was concentrated to dryness affording a residue, to which was added DMF (2 mL) and K_2CO_3 (53 mg, 0.38 mmol). The resulting mixture was heated by microwave to 150 °C for 10 min, after which the resulting mixture was filtered and concentrated affording a residue which was purified by reverse phase HPLC (acetonitrile/water gradient containing 0.1% TFA) affording **17b** (13.1 mg, 0.049 mmol, 13% yield). ES MS ($M+H^+$) = 269; ^{1}H NMR δ (ppm)(DMSO-d_6): 9.07 (1 H, d, J = 2.12 Hz), 8.75 (1 H, d, J = 2.13 Hz), 8.42 (1 H, t, J = 7.74 Hz), 7.70 (1 H, d, J = 3.52 Hz), 7.29 (1 H, d, J = 8.41 Hz), 6.69 (1 H, d, J = 3.52 Hz), 3.89 (3 H, s); HRMS m/z 269.0831 ($C_{14}H_9FN_4O + H^+$ requires 269.0833).