Electronic Supplementary Material (ESI) for Analytical Methods

Supplementary Information

Experimental Procedures and Spectral Data

TanA: A fluorogenic probe for Thiaminase activity

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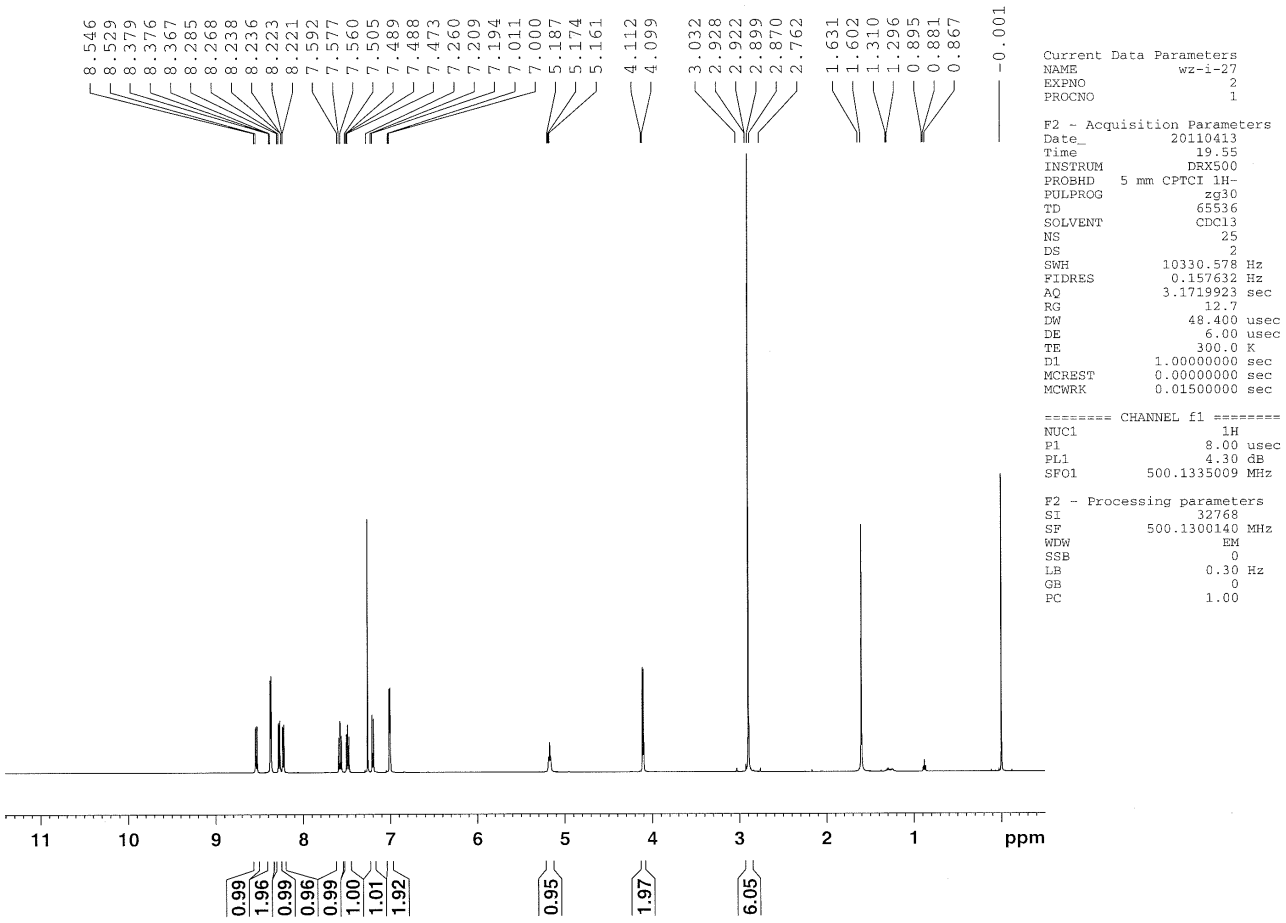
Preparation of compound 3:

4-aminomethylpyridine (112 ul, 1.11 mmol) was added into a solution of dansyl chloride (100 mg, 0.37 mmol) in dry CH_2Cl_2 (3 mL). The mixture was stirred overnight at room temperature, followed by dilution with water. The mixture was extracted with CH_2Cl_2 (3 x 10 ml) and the organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by chromatography on silica gel with methanol-methylene chloride (2:98) giving compound **3** (101 mg, 85%yield) as a white solid; mp 90-92°C; IR (film, cm^{-1}) ν 3431, 3078, 2939, 2828, 2780, 1314, 1139, 790; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.90 (s, 6H), 4.10 (d, *J*=6.5Hz, 2H), 5.17 (t, *J*= 6.5 Hz, 1H), 7.00 (d, *J*=5.5Hz, 2H), 7.20 (d, *J*=7.5Hz, 1H), 7.49 (t, *J*=8.0 Hz, 1H), 7.58 (t, *J*=8.5 Hz, 1H), 8.23 (dd, *J*=1.0, 7.5 Hz, 1H), 8.28 (d, *J*= 7.5 Hz, 1H), 8.37 (d, *J*=6Hz, 2H), 8.50 (d, *J*=8.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 45.3, 45.8, 115.2, 118.4, 122.2, 123.0, 128.5, 129.4, 129.7, 129.8, 130.7, 134.3, 145.7, 149.5 and 152.0; HRMS $[M+Na]^+$ calcd for $(C_{18}H_{19}N_3O_2SNa)^+$: 364.1090, found 364.1088.

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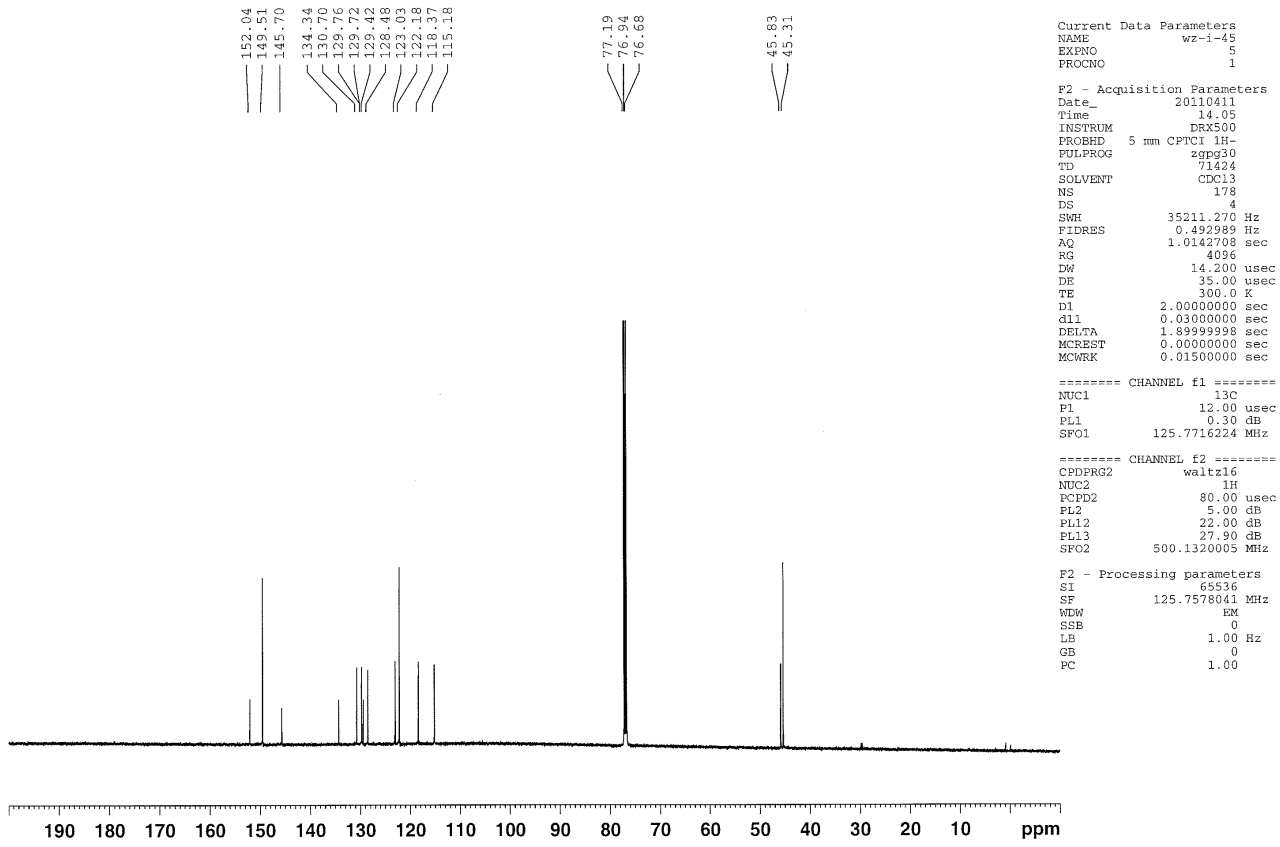
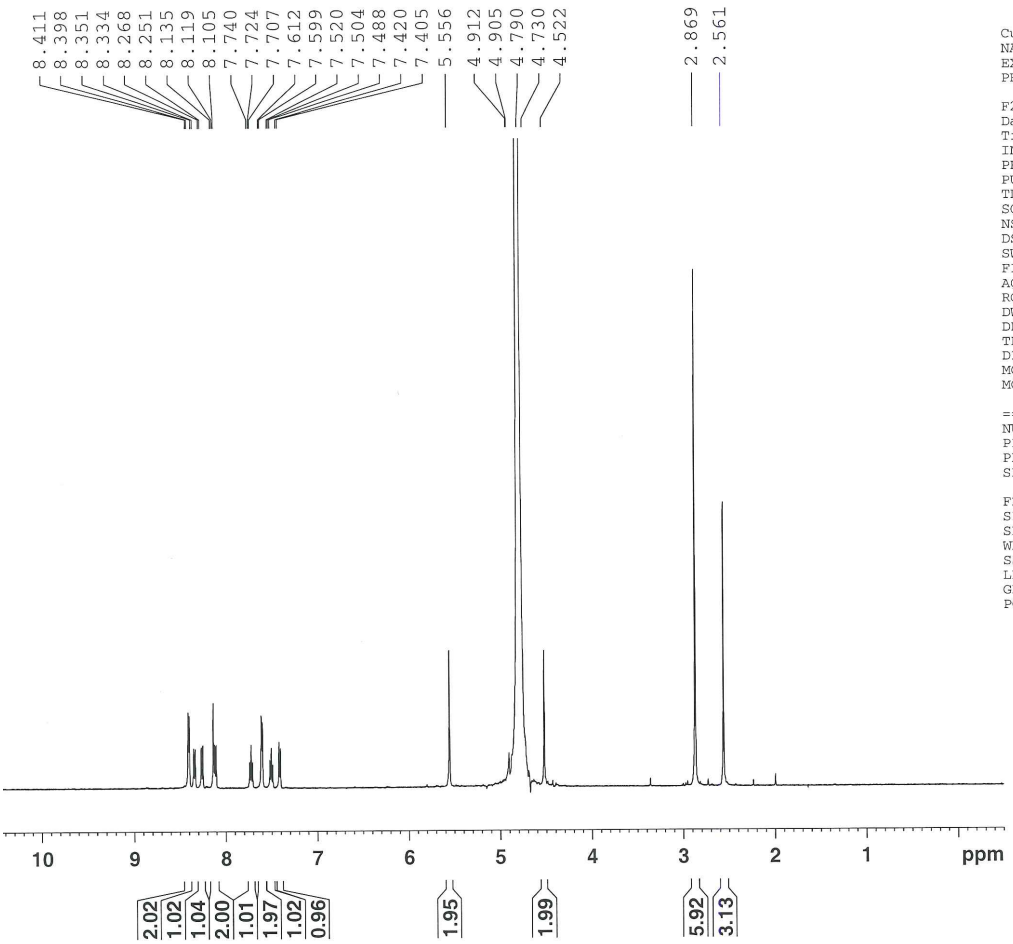


Preparation of TanA:

Compound **3** (72mg, 0.21mmol) was added to solution of 5-(bromomethyl)-2- methylpyrimidin-4-amonium bromide (20mg, 0.07mmol) in DMF (0.21 mL). The mixture was stirred under nitrogen overnight. CH_2Cl_2 was added to the DMF solution to give a yellow precipitate that was collected and washed with CH_2Cl_2 several times. The precipitate was dissolved in MeOH and allowed to evaporate slowly overnight. Crystals of pure TanA, as the HBr salt, were collected (37 mg, 84% yield) as a white solid; mp 208-210°C; IR (film, cm^{-1}) ν 3427, 1652, 1469, 1139, 1087; ^{1}H NMR (500MHz, D_2O) δ 2.56 (s, 3H), 2.87 (s, 6H), 4.52 (s, 2H), 5.56 (s, 2H), 7.41 (d, *J*=7.5 Hz, 1H), 7.50 (dd, *J*=8 Hz, 1H), 7.61 (d, *J*=6.5 Hz, 2H), 7.72 (dd, *J*=8 Hz, 1H), 8.11 (d, *J*=7 Hz, 1H), 8.14 (s, 1H), 8.26 (d, *J*=8.5 Hz, 1H), 8.34 (d, *J*=8.5 Hz, 1H), 8.40 (d, *J*=6.5 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 24.0, 47.4, 48.3, 58.9, 109.2, 120.1, 124.3, 127.7, 130.2, 131.3, 131.4, 131.5, 133.0, 136.5, 146.0, 148.8, 149.4, 162.2, 165.7, 166.7; HRMS $[M\text{-}HBr\text{-}Br^-]^+$ calcd for $(C_{24}H_{27}N_6O_2S)^+$: 463.1910, found 463.1911.

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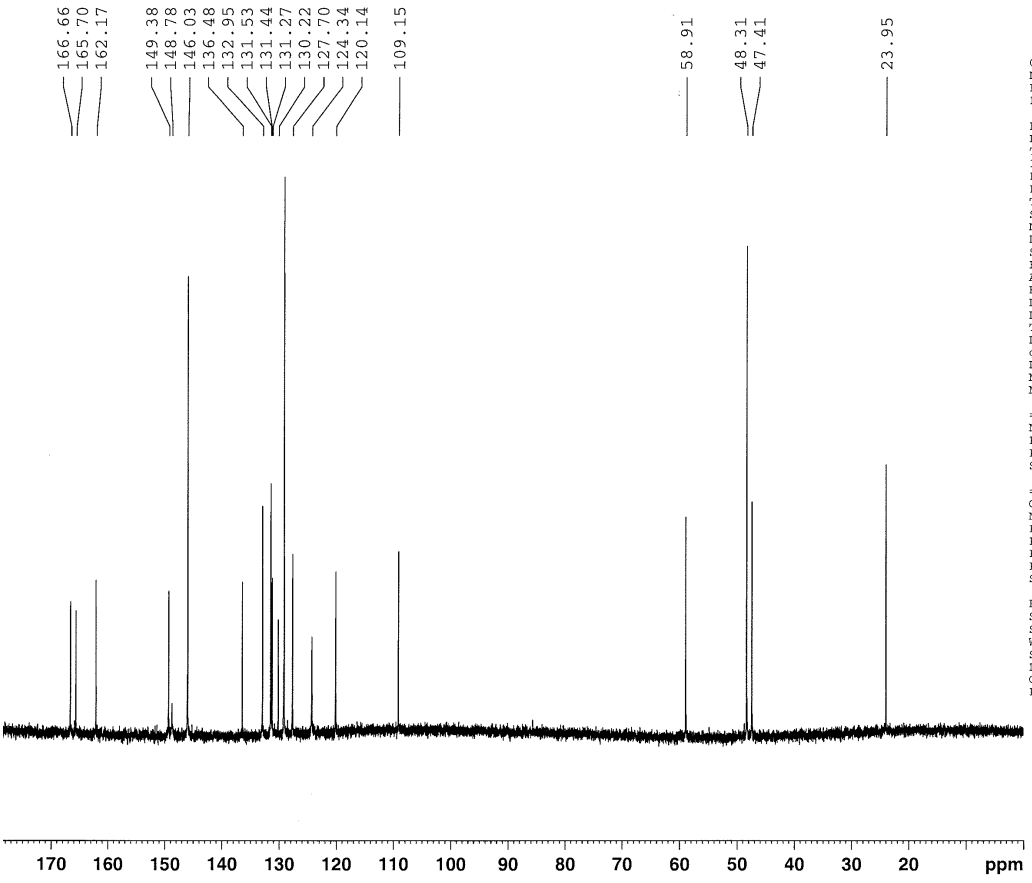


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PL12              22.00 dB
PL13              27.90 dB
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F2 - Processing parameters
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pKa determination

Compound **3** (10 µM in 20 mM citrate buffer) was placed in a 1.4 ml cuvette and the pH adjusted to 3.0. The fluorescence (λ_{ex} = 335 nm) was recorded on a Shimadzu RF-5301. The pH was adjusted by addition of a concentrated solution of NaOH and the fluorescence and pH recorded. The fluorescence at 575 nm was plotted vs. pH and fit to a standard pKa curve.

Bisulfite kinetics

TanA (8.8 µM in 0.8 mM citrate buffer) was placed in a 1.4 ml cuvette and the pH adjusted to 6.4. Sodium bisulfite was added to bring the final concentration of bisulfite up to 0.1mM and the fluorescence (λ_{ex} = 335 nm) was recorded at 5 minute intervals.

Thiaminase activity

Thiaminase activity was determined kinetically in dilutions of partially purified his(10)-tagged recombinant thiaminase I derived from *P. thiaminolyticus* strain 8118 (Honeyfield)[1] using probe TanA as substrate (in place of thiamine) and nicotinic acid as co-substrate at concentrations and pH employed in the standard radiometric assay[2]. Partially purified thiaminase I protein was diluted 1000, 2000, 4000 and 8000-fold in 0.1 M, pH 6.5, potassium phosphate buffer containing 0.1 M NaCl and 0.1 % (w/v) BSA (bovine serum albumin, globulin and protease free; Sigma-Aldrich, St. Louis, MO) to provide enzyme solutions containing 30, 60, 120, and 240 ng protein/assay. Aliquots of diluted enzyme (120 µL) were added to triplicate microwells each containing 108 µL of co-substrate, nicotinic acid (40 mM, pyridine-3-carboxylic acid, Sigma-Aldrich Corp., St. Louis, MO). The plate was covered with a lid, moved into the sample chamber of a Synergy 4 multi-mode plate reader (Millipore), shaken for 10 s and allowed to equilibrate at 28°C for 5 min. Following pre-incubation, the plate was ejected and 24 µL of probe TanA (75 µM) or buffer was added to appropriate wells, the plate returned to the sample chamber, and was shaken for 10 seconds to mix the contents. The emission of released compound **3** at 575 nm was measured at 2 minute intervals using xenon-flash lamp excitation at 350 nm. A dilution series of fluorescent compound **3** contained in separate microwells of the thiaminase assay plate was used to show that the emission was linearly related to solution concentration as pmol/assay (Figure S1); where the volume of solution/assay was 252 µL.

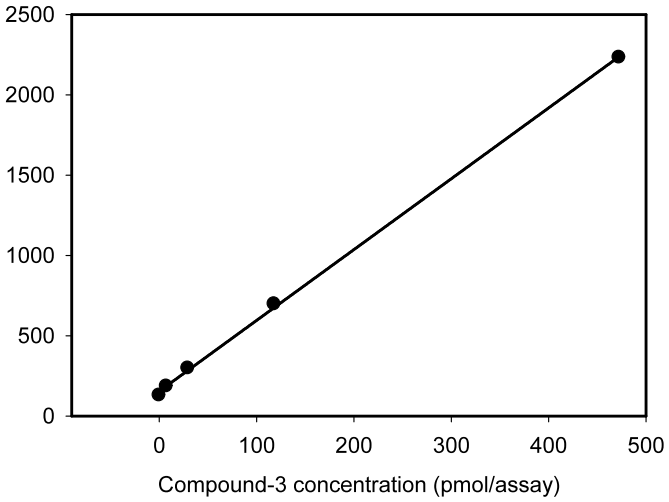

Figure S1. Fluorescent compound **3** emission was calibrated using a series of dilutions. The mean (n = 3) fluorescence emission at 575 nm, excited at 350 nm, for dilutions of free compound **3** in 0.1 M, pH 6.5, potassium phosphate buffer containing 0.1 M NaCl and 0.1 % (w/v) BSA was measured at 28 °C using a Synergy 4 multimode plate reader. Assay volumes were 252 µL/well. Fluorescence was linearly related to concentration, with F = 4.4102*[pmol compound-3/assay] + 154.58, R^2 = 0.9994.

References:

1. D. C. Honeyfield, J.P. Hinterkopf, S. B. Brown, *Transactions of the American Fisheries Society*, 2002, **131**, 171-175; C. A. Richter, A. N. Evans, M. K. Wright-Osment, J. L. Zajicek, S. A. Heppell, S. C. Riley, C. C. Krueger, D. E. Tillitt, *Canadian Journal of Fisheries and Aquatic Science*, 2012, **69**, 1-9.

2. J. L. Zajicek, D. E. Tillitt, D, C, Honeyfield, S. B. Brown, 2005, *Journal of Aquatic Animal Health*, **17**, 82-94.