

## Ring-Opened Aminothienopyridazines as Novel Tau Aggregation Inhibitors

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### General Experimental

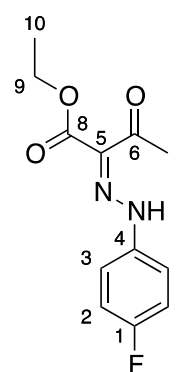
All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Diethylamine, cyclopropylamine and 4-fluoroaniline were distilled from calcium hydride. Anhydrous *N,N*-dimethylformamide, dichloromethane, tetrahydrofuran and toluene were obtained from a PureSolv MD 7 solvent purification system (Innovative Technology, Inc.). All other solvents and reagents were used as received from commercial sources. Microwave-assisted reactions were performed in sealed-tube systems using a Discover<sup>®</sup> S-Class (Ai Scientific CEM Corporation) instrument. Analytical thin-layer chromatography (TLC) was performed using Merck aluminium backed silica gel 60 F<sub>254</sub> (0.2 mm) plates which were visualised with shortwave (254 nm) ultraviolet light. Products were also visualised with potassium permanganate, vanillin, cerium molybdate, bromocresol green or ninhydrin stains. Flash column chromatography was performed using Grace DAVISIL 60 (230-400 mesh) silica gel, with the eluent mixture reported as the volume:volume ratio. Melting points were measured in open capillaries using a Stanford Research System OptiMelt Automated melting point apparatus and are uncorrected. Infrared absorption spectra were recorded on a Bruker ALPHA FT-IR spectrometer as a solid or a thin film from ethanol, and the data are reported as vibrational frequencies (cm<sup>-1</sup>). Nuclear magnetic resonance spectra were recorded at 300 K using a Bruker AVANCE DRX300 (300 MHz) spectrometer. <sup>1</sup>H chemical shifts are expressed as parts per million (ppm) with residual chloroform ( $\delta$  7.26) and dimethyl sulfoxide ( $\delta$  2.50) as reference and are reported as chemical shift ( $\delta$ ); relative integral; multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet); coupling constants (*J*) reported in Hz; assignment. <sup>13</sup>C chemical shifts are expressed as parts per million (ppm) with residual chloroform ( $\delta$  77.16) and dimethyl sulfoxide ( $\delta$  39.52) as reference and are reported as chemical shift ( $\delta$ ); multiplicity, coupling constants (*J*) reported in Hz; assignment. Proton decoupled <sup>19</sup>F chemical shifts are reported as parts per million (ppm). Low-resolution mass spectra (LRMS) were recorded using

electrospray ionisation (ESI) recorded on a Bruker AmaZon SL ion trap spectrometer. High resolution mass spectrometry was performed on a Bruker Apex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer equipped with an Apollo II ESI/MALDI dual source. Samples were run with syringe infusion at 150  $\mu\text{L/hr}$  on a Cole Palmer syringe pump into the electrospray ionisation (ESI) source. High performance liquid chromatography (HPLC) analysis of organic purity was conducted on a Waters Alliance 2695 instrument using a SunFire™ C18 column (5  $\mu\text{m}$ , 2.1 x 150 mm) and detected using a Waters 2996 photodiode array (PDA) detector set at 254 nm. Separation was achieved using water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.2 mL/min and a gradient of 0% B to 100% B over 30 minutes. HPLC data is reported as percentage purity and retention time (RT) in minutes.

### Synthesis of lead compound

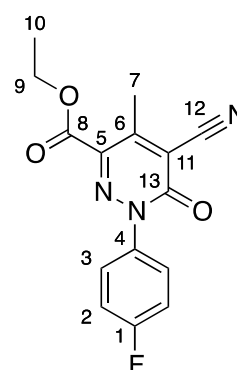
#### *Ethyl 2-(2-(4-fluorophenyl)hydrazono)-3-oxobutanoate S1*

To a solution of 4-fluoroaniline (860  $\mu\text{L}$ , 9.00 mmol) and concentrated hydrochloric acid (10.2 M, 2.70 mL, 27.6 mmol) in water (1.1 mL) and ethanol (1.1 mL) was added a solution of sodium nitrite (670 mg, 9.76 mmol) in water (3 mL), dropwise at 0  $^{\circ}\text{C}$ , and the mixture stirred for 30 min. A solution of sodium acetate (2.90 g, 35.5 mmol) in water (4.6 mL) and a solution of ethyl acetoacetate (1.17 g, 9.00 mmol) in ethanol (2.3 mL) were added, maintaining the temperature below 5  $^{\circ}\text{C}$ . The solution was stirred at 0  $^{\circ}\text{C}$  for 4 hours before water (15 mL) was added and the mixture extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were washed with brine, dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. Recrystallisation from ethanol afforded the desired hydrazone **2** (1.66 g, 77%) as a yellow crystalline solid, mp: 85.6-88.8  $^{\circ}\text{C}$  (Lit: 81-83  $^{\circ}\text{C}$ )<sup>11</sup>;  $R_f$ : 0.83 (1:3 ethyl acetate, hexane); IR ( $\nu_{\text{max}}$  (neat)): 3370, 2984, 2939, 1701, 1525, 1508, 1184, 1090, 834, 788  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.37 (3H, t,  $J = 7.2$  Hz, H10), 2.56 (3H, s, H7), 4.31 (2H, q,  $J = 7.2$  Hz, H9), 7.06 (2H, t,  $J = 8.5$  Hz, H3), 7.37 (2H, dd,  $J = 4.6$  and 8.8 Hz, H2), 14.83 (1H, bs, NH) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.4 (C10), 30.8 (C7), 61.0 (C9), 116.5 (d,  $J_{\text{CF}} = 23.2$  Hz, C3), 117.9 (d,  $J_{\text{CF}} = 8.2$  Hz, C2), 126.1 (C5), 138.1 (C4), 160.7 (d,  $J_{\text{CF}} = 245.5$  Hz, C1), 165.0 (C8), 197.2 (C6) ppm;  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -116.4 ppm; LRMS (+ESI)  $m/z$ : 275 ([M+Na]<sup>+</sup>, 100%), 527 ([2M+Na]<sup>+</sup>, 12%).



#### *Ethyl 5-cyano-1-(4-fluorophenyl)-4-methyl-6-oxo-1,6-dihydropyridazine-3-carboxylate S2*

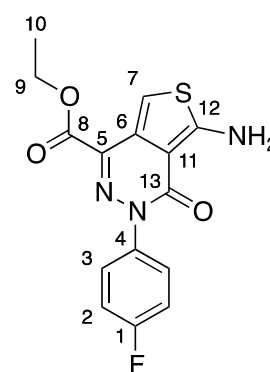
A mixture of hydrazone **S1** (1.00 g, 3.96 mmol), ethyl cyanoacetate (630  $\mu\text{L}$ , 5.94 mmol) and 4-aminobutyric acid (820 mg, 7.92 mmol) was heated at 160  $^{\circ}\text{C}$  with stirring for 2.5 hours. The reaction mixture was allowed to cool and the residue purified by flash column chromatography using ethyl acetate-hexane 1:3 as an eluent to afford the desired pyridazine **3** (540 mg, 47%) as an off-white solid, mp: 146.5-147.0  $^{\circ}\text{C}$  (Lit: 146-147  $^{\circ}\text{C}$ )<sup>11</sup>;  $R_f$ : 0.61 (1:3 ethyl acetate, hexane); IR ( $\nu_{\text{max}}$  (neat)): 3070, 2987, 2943, 2234, 1728,



1681, 1602, 1508, 1376, 1326, 1304, 1235, 1171, 1158, 1130, 1052, 836, 786, 761, 730  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.39 (3H, t,  $J = 7.2$  Hz, H10), 2.75 (3H, s, H7), 4.42 (2H, q,  $J = 7.1$  Hz, H9), 7.14-7.18 (2H, m, H3), 7.59-7.63 (2H, m, H2) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.2 (C7), 19.3 (C10), 62.9 (C9), 112.4 (C12), 116.0 (d,  $J_{\text{CF}} = 23.4$  Hz, C2), 116.1 (C11), 127.1 (d,  $J_{\text{CF}} = 9.1$  Hz, C3), 136.1 (C5), 137.4 (C4), 150.8 (C6), 162.0 (C8 and C13), 162.7 (d,  $J_{\text{CF}} = 250.1$  Hz, C1);  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -110.8 ppm; LRMS (+ESI)  $m/z$ : 324 ( $[\text{M}+\text{Na}]^+$ , 100%), 625 ( $[\text{2M}+\text{Na}]^+$ , 6%).

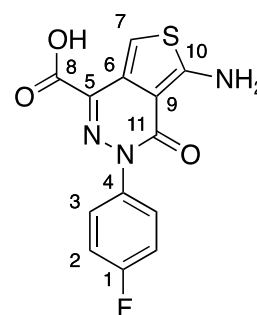
**Ethyl 5-amino-3-(4-fluorophenyl)-4-oxo-3,4-dihydrothieno[3,4-d]pyridazine-1-carboxylate S3**

A mixture of pyridazine **S2** (100 mg, 0.350 mmol), sulfur (17 mg, 0.520 mmol) and morpholine (60  $\mu\text{L}$ , 0.700 mmol) in ethanol (1.7 mL) was heated at 150  $^\circ\text{C}$  using microwave irradiation for 15 min. The reaction mixture was cooled to room temperature, the solvent removed by filtration and the crude solid purified by silica column chromatography using ethyl acetate-hexane 1:3 as an eluent to obtain the desired aminothienopyridazine **S3** (110 mg, 93%) as a dark yellow solid, mp: 182.1-184.2  $^\circ\text{C}$  (Lit: 178-180  $^\circ\text{C}$ )<sup>11</sup>;  $R_f$ : 0.50 (1:3 ethyl acetate, hexane); IR ( $\nu_{\text{max}}$  (neat)): 3424, 3396, 3320, 3172, 1726, 1704, 1603, 1505, 1370, 1321, 1241, 1188, 1149, 1132, 1079, 1020, 786, 767, 737, 694  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.40 (3H, t,  $J = 7.1$  Hz, H10), 4.43 (2H, q,  $J = 7.1$  Hz, H9), 7.12 (2H, t,  $J = 8.6$  Hz, H3), 7.22 (1H, s, H7), 7.51-7.58 (2H, m, H2) ppm, ( $\text{NH}_2$  peaks not observed);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.4 (C10), 62.2 (C9), 104.8 (C7), 106.9 (C6), 115.7 (d,  $J_{\text{CF}} = 22.9$  Hz, C2), 127.2 (C11), 127.9 (d,  $J_{\text{CF}} = 8.6$  Hz, C3), 133.9 (C5), 136.7 (C4), 159.6 (C12), 160.2 (C13), 161.7 (C8), 161.9 (d,  $J_{\text{CF}} = 247.9$  Hz, C1);  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -114.1 ppm; LRMS (+ESI)  $m/z$ : 356 ( $[\text{M}+\text{Na}]^+$ ).



**5-Amino-3-(4-fluorophenyl)-4-oxo-3,4-dihydrothieno[3,4-d]pyridazine-1-carboxylic acid S4**

Aminothienopyridazine **S3** (100 mg, 0.300 mmol) in tetrahydrofuran (3 mL) and water (2 mL) was treated with lithium hydroxide monohydrate (38 mg, 0.900 mmol). The mixture was stirred at room temperature for 18 hours. The reaction mixture was quenched with 1 M hydrochloric acid and the pH adjusted to 2. The resulting precipitate was collected to obtain the desired acid **5** (90 mg, 98%) as a brown solid, mp: 217.8-219.7  $^\circ\text{C}$ ;  $R_f$ : 0.32 (1:3 methanol, chloroform); IR ( $\nu_{\text{max}}$  (neat)): 3452, 3398, 3343, 3290, 3141, 1719, 1641, 1592, 1505, 1236, 1144, 829  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  7.12 (1H, s, H7), 7.30 (2H, t,  $J = 8.8$  Hz, H3), 7.54-7.59 (2H, m, H2) ppm, ( $\text{NH}_2$  and OH peaks not observed);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  103.7 (C7), 103.9 (C6), 115.2 (d,  $J_{\text{CF}} = 22.9$  Hz, C2), 126.3 (C9), 128.4 (d,  $J_{\text{CF}} = 8.7$  Hz, C3), 133.6 (C9), 137.1 (C4), 158.4 (C10), 160.7 (d,  $J_{\text{CF}} = 245.7$  Hz, C1), 163.2 (C11), 164.0 (C8);  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -114.8 ppm; LRMS (+ESI)  $m/z$ : 306 ( $[\text{M}+\text{H}]^+$ , 100%), 360 ( $[\text{M}+\text{2Na}-\text{H}]^+$ , 26%).



*5-Amino-N-cyclopropyl-3-(4-fluorophenyl)-4-oxo-3,4-dihydrothieno[3,4-d]pyridazine-1-carboxamide 1*

To a mixture of acid **S4** (100 mg, 0.328 mmol), cyclopropylamine (33  $\mu$ L, 0.473 mmol), and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (250 mg, 0.480 mmol) in anhydrous dimethyl sulfoxide (4.5 mL) was added *N,N*-diisopropylethylamine (87  $\mu$ L, 0.503 mmol) and the mixture stirred at room temperature for 4 hours. The reaction mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The organic fractions were combined, washed with water (3 x 20 mL) and brine (3 x 20 mL), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude residue was purified by silica column chromatography using ethyl acetate-hexane 1:3 as an eluent, followed by recrystallization from methanol, water, to obtain the title compound **1** (60 mg, 55%) as a yellow solid, mp: 212.1-214.2  $^{\circ}$ C;  $R_f$ : 0.51 (2:3 ethyl acetate, hexane); IR ( $\nu_{\max}$  (neat)): 3481, 3358, 3303, 3124, 1661, 1640, 1583, 1493, 1451, 1307, 1290, 1220, 783  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  0.57-0.64 (4H, m, H13), 2.75-2.82 (1H, m, H12), 7.19 (1H, s, H7), 7.22-7.32 (2H, m, H3), 7.58 (2H, s,  $\text{NH}_2$ ), 7.59-7.69 (2H, m, H2), 8.27 (1H, d,  $J = 3.9$  Hz, CONH) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  5.7 (C13), 22.6 (C12), 103.9 (C7), 104.3 (C6), 115.0 (d,  $J_{CF} = 22.8$  Hz, C2), 126.1 (C9), 128.2 (d,  $J_{CF} = 8.6$  Hz, C3), 136.1 (C5), 136.9 (C4), 158.4 (C10), 160.4 (d,  $J_{CF} = 243.9$  Hz, C1), 162.8 (C11), 163.8 (C8) ppm;  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ ):  $\delta$  -113.7; LRMS (+ESI)  $m/z$ : 367 ( $[\text{M}+\text{Na}]^+$ , 100%), 711 ( $[\text{2M}+\text{Na}]^+$  70%); HRMS (ESI) $^+$  Cald for  $\text{C}_{16}\text{H}_{13}\text{FN}_4\text{O}_2\text{S}$   $[\text{M}+\text{Na}]^+$ : 367.0636, found: 367.0636, HPLC: 97.9%, RT: 23.0 mins.

