SUPPLEMENTARY METHODS

Molecular modeling and computational studies. Molecular modeling was performed for the proposed analogs with the human SHMT2 crystal structure (PDB: 5V7I) (1) using the induced fit docking protocol of Maestro (2,3). The ligands were prepared using the Ligprep (4) application. The docking protocol was validated by re-docking the co-crystallized pyranopyrazole ligand (1) into the SHMT2 crystal structure with a RMSD of 0.15 Å. The centroid around the pyranopyrazole inhibitor in Chain B was defined as the inhibitor binding site. The OPLS_2005 force field was used and amino acid residues within 3 Å from the docked poses were optimized using prime refinement (5). The compounds were also docked into rabbit SHMT1 (PDB: 1LS3) (6) binding sites.

Enzyme expression and purification. N-terminal His-tagged β-glycinamide ribonucleotide (GAR) formyltransferase (GARFTase) (formyltransferase domain; residues 100-302) was expressed and purified as described previously (7). To express full-length human 5-aminoimidazole-4-carboxamide formyltransferse (AICARFTase)/IMP cyclohydrolase (ATIC) with an N-terminal, cleavable hexahistidine tag, ATIC cDNA was cloned into pHis-parallel via Gibson assembly (8). The resulting plasmid, pSD001, was transformed and expressed in Rosetta (DE3) pLysS cells. Cultures (1 L) were grown in LB media containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol at 37°C until OD_{600} reached 0.6. Expression was induced with the addition of 500 µM isopropyl *β*-D-1-thiogalactopyranoside and incubated at 20°C for 16-18 h. Cultures were pelleted and resuspended in 40 mL 25 mM Tris, pH 7.5, 300 mM NaCl, 5 mM *β*-mercaptoethanol (*β*-Me), 10 mM $CaCl_2$, 10 mM $MgCl_2$, 40 mg lysozyme, and 8 U DNAse I (Sigma) before cell lysis by emulsification. His-ATIC was purified from the lysate by immobilized metal affinity chromatography over a 4 mL nickel nitrilo-triacetic acid (Ni-NTA) column (Gold Biotechnology). The lysate was passed over the column, washed with 5 column volumes (CV) of 25 mM Tris, pH 7.5, 300 mM NaCl, 10 mM imidazole, and 5 mM *β*-Me (wash buffer) and 5 CV of the wash buffer with 25 mM imidazole. Protein was eluted with 5 CV (4 mL fractions) of elution buffer containing components of the wash buffer and 300 mM imidazole. Samples were further purified on ÄKTA FPLC (GE Healthcare) via Superdex 200 16/60 (GE Healthcare) column, equilibrated with 20 mM Tris, pH 7.5, 150 mM NaCl, 50 mM KCl, 5 mM EDTA, and 5 mM dithiothreitol. His-ATIC was stored at 150 µM at 4°C in this buffer for up to six months, or at 100 µM with 20% glycerol at -80°C for long-term storage.

Plasmids to express human cytosolic SHMT1 (residues 7-478, Uniprot ID P34896) and mitochondrial SHMT2 (residues 42-504, Uniprot ID 34897), both with cleavable N-terminal His_6-tags, were inserted into pHis-parallel and expressed in Rosetta™ (DE3)pLysS cells. Expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside when the OD_{600} reached 0.6, and induction continued at 20° C for 18-20 h before cell harvesting. Cells were lysed by emulsification and Ni-NTA chromatography (Gold Biotechnology) was used for initial purification, as described for His-ATIC. Pyridoxal 5'-phosphate (PLP) was added in 3-fold molar excess to SHMT samples and allowed to incubate for 24 h. Size exclusion chromatography was employed using a Superdex 200 16/60 column (GE Healthcare) while monitoring absorbance at 435 nm for final purification of proteins with PLP bound. SHMT enzymes were stored in 20 mM sodium phosphate buffer, pH 7.5, 100 mM potassium chloride, 0.2 mM EDTA, and 5 mM *β*-Me.

For MTHFD2 expression, cDNA encoding the bifunctional human mitochondrial MTHFD2 (residues 36-333, Uniprot ID P13995) was inserted into a pHis-parallel vector for expression in Rosetta™ (DE3)pLysS cells as a fusion protein with a cleavable N-terminal His_6-tag. After cell lysis by emulsification, Ni-NTA chromatography and size exclusion chromatography (Superdex 200 16/60 column) were used for purification, as described for His-ATIC. Purified His-MTHFD2 was stored in 50 mM Tris buffer, pH 7.5, 250 mM sodium chloride, 5% glycerol, and 0.5 mM tris(2-chloroethyl) phosphate.

All plasmid constructs were confirmed by DNA sequencing. The purities of purified enzyme preparations were checked by SDS gel electrophoresis (9).

In vitro enzymatic assays and K_i determinations. AICARFTase catalytic activity was measured by monitoring the formation of tetrahydrofolate (THF) spectrophotometrically from 10-formyl-THF in the presence of various concentrations of inhibitor (10). Assays included a final concentration of 50 µM 10-formyl-THF, 100 nM ATIC, and a range of inhibitors in 32.6 mM Tris-HCl pH 7.4, 25 mM KCl, and 5 mM *β*-Me. Reactions were pre-incubated at 25°C in a UV-transparent 96-well plate (Costar 3635) for 90 seconds, with measurements at 298 nm every 6 seconds. Reactions were then initiated by adding 10 µL 500 µM AICAR (ZMP) or buffer

(control wells) for a final reaction volume of 100 µL. Measurements were recorded in triplicate at 298 nm every 6 seconds over 10 min using a BioTek Synergy Neo2 Plate Reader. To determine the initial rate for each inhibitor concentration, absorbances of the preincubation period were averaged and subtracted from all measurements in that well. Initial rate changes in absorbance at 298 nm were determined for regions of linear absorbance increases in all replicates. Initial slopes were graphed against inhibitor concentrations and fit to a hyperbolic curve [$y = (-a*x/(IC_{50} + x)) + b$, where "a" is the amplitude and "b" is the y-intercept] to calculate the IC_{50} for each compound (GraphPad Prism 7.0). The K_i was then calculated from the IC_{50} [$K_i = IC_{50}/([S]/K_M+1)$], using K_m and substrate values for 10-formyl-THF. The calculated K_m for 10-formyl-THF with His-ATIC, determined as a function of initial velocity versus 10-formyl-THF concentration, was 100 µM.

In vitro enzymatic assays of GARFTase were carried out with His-GARFTase containing an N-terminal cleavable hexahistidine tag. GARFTase catalytic activity was measured by monitoring the formation of THF spectrophotometrically from 10-formyl-THF in the presence of a range of inhibitor concentrations. Assays included final concentrations of 40 µM 10-formyl-THF, 50 nM GARFTase, and a range of inhibitors in 0.1 M Hepes, pH 7.5 (11). Reactions were pre-incubated at 37°C in a UV-transparent 96-well plate (Costar) for 90 seconds, with measurements at 298 nm every 5 seconds. Reactions were then initiated by adding 10 µL 150 µM α,β-GAR or buffer (control wells) for a final reaction volume of 100 µL. Measurements were recorded at 298 nm every 5 seconds for 15 min using a BioTek Synergy Neo2 Plate Reader in triplicate. Procedures for data fitting and determinations of K_i values for His-GARFTase were as described for AICARFTase. The calculated K_m for 10-formyl-THF with His-GARFTase, determined as a function of initial velocity versus 10-formyl-THF concentration, was 84.8 µM.

In vitro activities of His-SHMT1 and His-SHMT2 were assayed by a coupled reaction with His-MTHFD2 in 200-fold molar excess, and NADH production was monitored by fluorescence at 470 nm with excitation at 360 nm (Synergy Neo2 Biotek plate reader) using a black well, black bottom 96-well plate (Corning #3916) in triplicate. The reaction volume was 100 µL with final concentrations of 50 nM SHMT enzyme, 10 µM MTHFD2, 50 µM THF, 2.5 mM NAD^+, and 20 mM L-serine. Serine was added to initiate the reaction and data were acquired every 19 seconds over 15 min. Linear initial velocities were determined and data fitting, IC_{50} and K_i calculations were performed as described for AICARFTase. The calculated K_m for THF with His-SMHT1 and His-SHMT2, determined as a function of initial velocity versus THF concentration, are 62.8 µM and 108 µM, respectively.

To confirm that MTHFD2 was not inhibited by the AGF molecules, MTHFD2 activity was evaluated with an NAD(P)H-Glo™ Detection System Kit (Promega, Ref G9061). Final concentrations for the reactions were 100 nM MTHFD2, 100 µM NAD^+, and 100 µM me-THF, with reaction initiation with NAD^+. Reactions were performed for 10 min at room temperature. To stop the reaction, the temperature was increased to 100 ºC for 30 min and 1 µL of 1 M hydrochloric acid was added. Thereafter, 1 µL of 1 M sodium hydroxide was added to neutralize the acid. From this reaction, 12.5 µL was transferred to each of 3 wells of a white 96-well plate (Corning #3917) containing 12.5 µL luciferase reagents (as specified by kit). Luminescence was allowed to develop in the dark for 55 min, and samples were read with a Synergy Neo2 Biotek plate reader using the Biotek Lum 1536 filter cube for 10 min. Luminescence data for the last 5 min were averaged for final endpoint measurements.

In vivo efficacy trials with MIA PaCa-2 pancreatic cancer xenografts. MIA PaCa-2 human pancreatic cancer tumor fragments (30-50 mg) were bilaterally implanted subcutaneously with a 12-gauge trocar in female NCr SCID mice (NCI Animal Production Program stock strain; Charles River Labs #561). The mice were 11 (early stage trial) and 10 (upstage) weeks old on day 0 (tumor implant) with average body weights of 20.2 and 19 g, respectively. For the efficacy trials, the mice were maintained on a folate-deficient diet from Envigo (TD.00434) or a folate–replete control diet from Lab Diet (#5021) starting 16 days before subcutaneous (SC) tumor implant. Mice were supplied with food and water *ad libitum*. Serum folate concentrations were monitored prior to tumor implant and post study by *Lactobacillus casei* bioassay (12). The mice in each group (folate-deficient and standard diet) were pooled before unselective distribution to each group's respective treatment and control arms. For the early stage trial, chemotherapy was initiated one day post-tumor implantation with **AGF347**; for the upstage trial, chemotherapy was initiated seven days post-tumor implantation (when tumors had grown to 100-150 mg) with **AGF347**. For both designs, dosing for **AGF347** was 15 mg/kg/injection every 2 days x 8 (total dose of 120 mg/kg); for GEM, dosing was 120 mg/kg/injection every 4 days x 4 (total dose of 480 mg/kg). Both drugs were administered intravenously (0.2 ml/injection). **AGF347**

compound was dissolved in 5% ethanol (v/v), 1% Tween-80 (v/v), and 0.06% $NaHCO_3$ and sterile USP H_2O; the GEM clinical stock was prepared with sterile USP 0.9% NaCl. The mice were weighed and their conditions assessed daily; the tumors were measured by caliper two-to-three times weekly. Mice were sacrificed when the cumulative tumor burden reached 5-10% body weight (1-2 g). Tumor volumes (mg) were estimated from two-dimensional measurements, where tumor mass (in mg) = $(a \times b^2)/2$, and a and b are the tumor length and width in mm, respectively. The tumor masses from both flanks of each mouse were added together, and the total mass per mouse was used for calculations of anti-tumor activity. Quantitative end-points include: (i) tumor growth delay [T-C, where T is the median time in days required for the treatment group tumors to reach a predetermined size (e.g., 1000 mg), and C is the median time in days for the control group tumors to reach the same size; tumor-free survivors are excluded from these calculations]; and (ii) gross $\log_{10}$ cell kill (LCK), determined by the formula LCK = (T-C; tumor growth delay in days)/3.32 x Td (tumor doubling time in days determined by growth plot). Qualitative analysis included determination of T/C values (in percent) on all days of tumor measurement using the median total tumor burden for treatment (T) and control (C) groups. The end point %T/C value for this study corresponds to the first measurement taken post last treatment (day 16 for early stage or day 21 for late stage), when control tumors were still in exponential growth phase (i.e., 500-1250 mg). The median value of each group was determined including zeros. The %T/C value is the inverse of tumor growth inhibition (TGI). Mouse body weight, percent weight loss and host recovery time (time in days for mice to regain starting weight from weight loss nadir) were used along with daily health monitoring to gauge drug effects and potential toxicity, and to determine the optimized highest non-toxic total dose schedules. Working definitions are as follows: partial remission (PR), treatment-induced tumor burden nadir < 50% of peak tumor burden after tumor engraftment (i.e. tumor burden > 200 mg); and complete response (CR), treatment-induced tumor burden nadir = 0 mg after tumor engraftment.

Cytochrome c oxidase assay. Additional tumor samples from the *in vivo* metabolomics arm were harvested to assess cytochrome *c* oxidase activity. Tumor samples were solubilized in HEPES solubilization buffer (10 mM HEPES pH 7.4, 40 mM KCl, 2 mM EGTA, 1 mM Na_3VO_4, 10 mM KF, 1 mM PMSF, 1 µM oligomycin, and 1% Tween-20), sonicated, and cell debris was removed by centrifugation. Protein concentration was determined by Folin phenol reagent (13) and samples were diluted in HEPES solubilization buffer (with 20 mM ascorbic acid added) to a final concentration of 2 mg/ml. Cytochrome *c* oxidase oxygen consumption was analyzed at 25ºC in a closed 200 µL chamber equipped with a micro Clark-type oxygen electrode (Oxygraph Plus System, Hansatech Instruments, Norfolk, UK). Baseline oxygen consumption was determined, after which 200 µM bovine heart cytochrome *c* (Sigma) was added to determine cytochrome *c* oxidase-specific oxygen consumption. Oxygen consumption was recorded and analyzed with the Oxygraph software. Cytochrome *c* oxidase protein levels were confirmed to be similar by electrophoresing equal amounts of protein (20 µg) from each sample on a 10% polyacrylamide gel with SDS (9), following protein transfer to polyvinylidene difluoride membranes (ThermoFisher) (14). To detect cytochrome *c* oxidase levels, membranes were incubated for 24 h with rabbit anti-COXIV primary antibody (Proteintech, Rosemont, IL - 11242-1-AP). Subsequently, membranes were incubated with IRDye800CW-conjugated goat anti-rabbit IgG secondary antibody (LICOR Biosciences, Omaha, NE) for 90 min and scanned with an Odyssey infrared imaging system (LICOR Biosciences).

Synthesis of AGF94 and 5-substituted pyrrolo[3,2-d]pyrimidine compounds*.* All evaporations were carried out at reduced pressure with a rotary evaporator. Analytical samples were dried *in vacuo* in a CHEM-DRY drying apparatus over P_2O_5 at 50 °C. Melting points were determined either using a MEL-TEMP, II melting point apparatus with FLUKE 51 K/J electronic thermometer or using an MPA100 OptiMelt automated melting point system and are uncorrected. Nuclear magnetic resonance (NMR) spectra for proton (^{1}H NMR) were recorded on the Bruker Avance II 400 (400 MHz) or Bruker Avance II 500 (500 MHz) NMR systems with TopSpin processing software. The chemical shift values (δ) are expressed in parts per million relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet; td, triplet of doublet; dt, doublet of triplet; quin, quintet; exch., exchangeable using D_2O. Thin-layer chromatography (TLC) was performed on Whatman® PE SIL G/UV254 flexible silica gel plates and the spots were visualized under 254 and 365 nm ultraviolet illumination. Proportions of solvents used for TLC are by volume. All analytical samples were homogeneous on TLC in at least two different solvent systems. Column chromatography was performed on silica gel (70 to 230 mesh, Fisher Scientific) column. Flash chromatography was carried out on the CombiFlash® *Rf* systems, model COMBIFLASH *RF*. Pre-packed

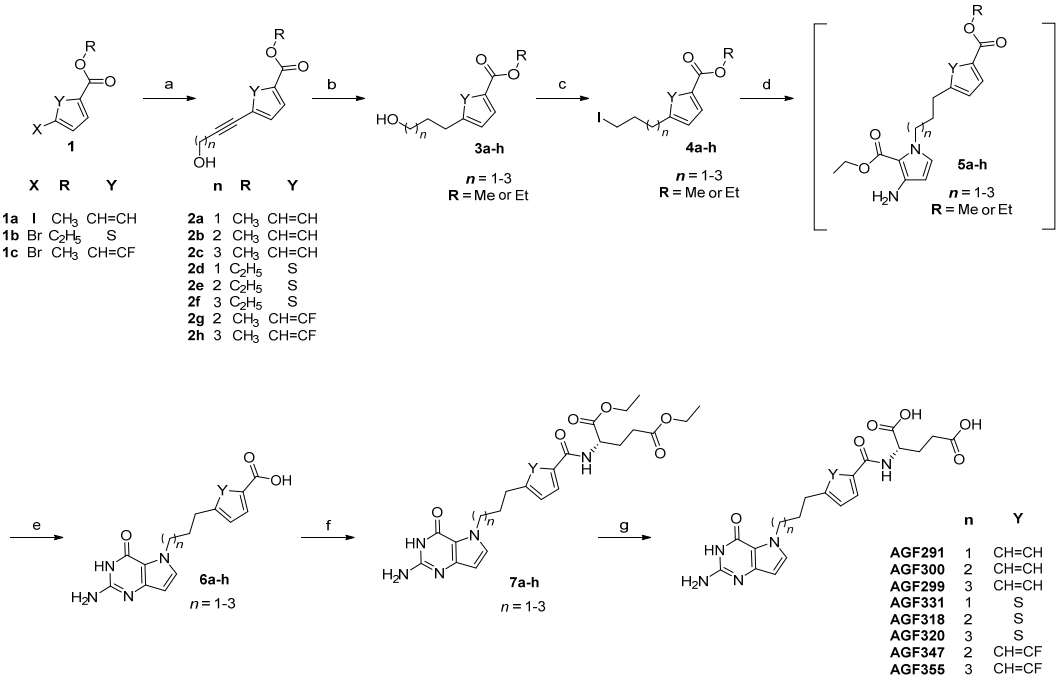
RediSep® *Rf* normal-phase flash columns (230 to 400 mesh) of diverse sizes were used. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within ± 0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples could not be prevented despite 24 to 48 h of drying *in vacuo* and were confirmed where possible by their presence in the ^{1}H NMR spectra. The HPLC was performed using UltiMate 3000 UHPLC+ system. Reverse phase HPLC was carried out XSelect CSH C18 XP, 130Å, 2.5 µm, 3 mm X 100 mm column. Solvent A: water with 0.1% TFA; Solvent B: acetonitrile. Mass spectrometry m/z determination was performed by an Advion Expression-S CMS (a single quadrupole compact MS) controlled by Advion Chems Express 4.0.13.8 software.

AGF 94 was synthesized as previously described (15).

Synthesis of the target compounds **AGF291, AGF299, AGF300, AGF318, AGF320, AGF331, AGF347** and **AGF355** started with a palladium-catalyzed Sonogashira coupling of 4-iodobenzoate methyl ester (**1a**) or 4-bromo-thiophene-2-carboxylic acid ethyl ester (**1b**) or methyl 4-bromo-2-fluorobenzoate (**1c**) with the appropriate alkyne alcohols to afford the appropriate 4-substituted alcohol benzoates **2a-h**. Catalytic hydrogenation afforded the saturated alcohols **3a-h** (11). The alcohols **3a-h** were converted to the mesylate derivatives using mesyl chloride and triethylamine base at 0 ºC (16). The mesylate derivatives were not purified and after workup were converted to their respective iodide **4a-h** using the Finkelstein reaction. The *N*-alkylation of iodides, **4a-h** using ethyl 3-amino-1*H*-pyrrole-2-carboxylate and sodium hydride under anhydrous conditions afforded the *N*-5 substituted pyrroles **5a-h** (17). This reaction was incomplete as observed on TLC. Longer reaction times resulted in decomposition of the product (TLC). The intermediates **5a-h** could not be isolated due to presence of multiple spots, even after repeated column chromatography. The crude *N*-substituted pyrroles **5a-h** were directly subjected to condensation with 1,3-bis(methoxycarbonyl)-2-methylthiopseudourea with 5 equivalents of acetic acid as catalyst and MeOH. The hydrolysis of the carbamate group formed was carried out *in situ* with aqueous sodium hydroxide at 55 °C to afforded the 2-amino-4-oxo-pyrrolo[3,2-*d*]pyrimidines **6a-h** (17). This hydrolysis required higher than room temperature. Performing the hydrolysis at room temperature causes the hydrolysis of the ester, but not the carbamate (as observed on the ^{1}H-NMR). However, temperatures greater than 70 ºC caused degradation of the starting material. The optimum temperature for hydrolysis of both ester and carbamate was found to be 55 ºC. Conversion of free acids **6a-h** to the corresponding L-glutamic acid diethyl esters **7a-h** involved conventional peptide coupling with L-glutamic acid diethyl ester hydrochloride using 2-chloro-4,6-dimethoxy-l,3,5-triazine followed by chromatographic purification to afford the coupled products (17). Hydrolysis of **7a-h** with aqueous NaOH at room temperature, followed by acidification with 1 N HCl in the cold, afforded the target compounds.

General procedure for synthesis of 2a-h. To a 20 mL vial for a microwave reaction were added a mixture of palladium chloride (71 mg, 0.40 mmol), triphenylphosphine (131 mg, 0.40 mmol), triethylamine (10.1 g, 100 mmol), methyl 4-iodobenzoate, **1a** (2.21 g, 8.43 mmol) or ethyl 5-bromothiophene-2-carboxylate **1b** (1.9 g, 8 mmol) or methyl 4-bromo-2-fluorobenzoate **1c** (2.5 g, 10.73 mmol), and anhydrous acetonitrile (10 mL). To the stirred mixture were added copper(I) iodide (304 mg, 1.60 mmol) and the appropriate alkyne alcohol (1.05 equiv), and the vial was sealed and maintained in the microwave reactor at 100 °C for 1 h. Silica gel (5 g) was added, and the solvent was evaporated under reduced pressure. The resulting plug was loaded on to a silica gel column (3.5 x 12 cm) and eluted with hexanes followed by 20% EtOAc in hexanes. The desired fractions (TLC) were collected, and the solvent was evaporated under reduced pressure to afford the target compounds.

Methyl 4-(3-hydroxyprop-1-yn-1-yl)benzoate (2a) Compound **2a** was synthesized using the general method described for the preparation of **2a-h** using prop-2-yn-1-ol (0.5 ml, 8 mmol), to give 1.3 g of **2a** as a yellow solid (1.36 g, 85%); TLC *Rf* = 0.16 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 8.06 – 7.87 (d, J = 8.4 Hz, 2 H, Ar), 7.55 (d, J = 8.4 Hz, 2 H, Ar), 4.30 (s, 1H, exch., -OH), 4.15 (s, 2 H, -CH_2-), 3.81 (s, 3 H, -OCH_3). The ^{1}H-NMR matched the ^{1}H-NMR reported in the literature (18).



Synthetic Scheme. a) $PdCl_2$, PPh_3, alcohol, TEA, CuI, acetonitrile, 1 h, 100 ^{0}C, microwave; b) H_2/Pd, Parr vessel, 14 h, r.t.; c) (i) TEA, mesyl chloride, DCM, 0 ^{0}C, 4 h; (ii) NaI, acetone, 8 h, reflux; d) ethyl 3-amino-1H-pyrrole-2-carboxylate, NaH, DMF, 4 h, r.t.; e) (i) 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea, MeOH, AcOH, r.t.,16 h; (ii) NaOMe, MeOH, 16 h, r.t.; (iii) 1 N NaOH, 55 ^{0}C, 3 h; f) L-glutamic acid diethyl ester hydrochloride, 2-chloro-4,6-dimethoxy-triazine, NMM, DMF, r.t., 12 h; g) 1N NaOH, r.t., 1 h

Methyl 4-(4-hydroxybut-1-yn-1-yl)benzoate (2b) Compound **2b** was synthesized using the general method described for the preparation of **2a-h**, using but-3-yn-1-ol (0.6 ml, 8 mmol), to give 1.2 g of **2b** as a yellow solid (1.53 g, 78%); TLC *Rf* = 0.16 (EtOAc:Hexane, 1:2); mp,(19) 92.3-94.6 ºC; ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.90 (d, J = 8.7 Hz, 2 H, Ar), 7.51 (d, J = 8.7 Hz, 2 H, Ar), 4.96 (s, 1 H, exch., -OH), 3.84 (s, 3 H, -OCH_3), 3.61 (m, 2 H, -CH_2-), 2.60 (t, J =6.0 Hz, 2 H, -CH_2-). The ^{1}H-NMR matched the ^{1}H-NMR reported in the literature (20).

Methyl 4-(5-hydroxypent-1-yn-1-yl)benzoate (2c) Compound **2c** was synthesized using the general method described for the preparation of **2a-h**, using pent-4-yn-1-ol (0.67 ml, 8 mmol), to give 1.34 g of **2c** as a yellow semi-solid (1.62 g, 88%); TLC *Rf* 0.16 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 6.50 (d, J = 8.4 Hz, 2 H, Ar), 6.37 (d, J = 8.4 Hz, 2 H, Ar), 5.15 (s, 1 H, exch., -OH), 3.61 (s, 3 H, -OCH_3), 3.11 (t, J = 4.9 Hz, 2 H, -CH_2-), 2.64 (t, J = 6.5 Hz, 2 H, -CH_2-), 1.83 – 1.67 (m, 2 H, -CH_2-). The ^{1}H-NMR matched the ^{1}H-NMR reported in the literature (21).

Ethyl 5-(3-hydroxyprop-1-yn-1-yl)thiophene-2-carboxylate (2d) Compound **2d** was synthesized using the general method described for the preparation of **2a-h**, using prop-2-yn-1-ol (0.5 ml, 8 mmol), to give 1.2 g of **2d** as a yellow semi-solid (70%); TLC *Rf* = 0.11 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.73 (d, J = 4.0 Hz, 1 H, Ar), 7.36 (d, J = 3.9 Hz, 1 H, Ar), 5.49 (t, J = 6.0 Hz, 1 H, -OH, exch.), 4.37 – 4.28 (m, 4 H, -OCH_2 and -CH_2-), 1.29 (t, J = 7.1 Hz, 3 H, -CH_3). This compound was used for the next reaction without further characterization.

Ethyl 5-(4-hydroxybut-1-yn-1-yl)thiophene-2-carboxylatee (2e) Compound **388** was synthesized using the general method described for the preparation of **2a-h**, using but-3-yn-1-ol (0.6 ml, 8 mmol), to give 1.1 g of **2e** as a yellow semi-solid (61%); TLC *Rf* = 0.11 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.70 (d, J = 4.0 Hz, 1 H, Ar), 7.28 (d, J = 4.0 Hz, 1 H, Ar), 4.96 (t, J = 5.6 Hz, 1 H, OH, exch.), 3.91-3.81 (m, 2 H, -OCH_2), 3.57 (t, J = 6.4 Hz, 2 H, -CH_2), 2.61 (t, J = 6.4 Hz, 2 H, -CH_2), 1.30 (t, J = 7.1 Hz, 3 H, -CH_3). The ^{1}H-NMR matches the ^{1}H-NMR reported previously (22).

Ethyl 5-(5-hydroxypent-1-yn-1-yl)thiophene-2-carboxylate (2f) Compound **2f** was synthesized using the general method described for the preparation **2a-h**, using pent-4-yn-1-ol (0.67 ml, 8 mmol), to give 1.3 g of **2f** as a yellow semi-solid (68%); TLC *Rf* = 0.11 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.63 (d, J= 3.8 Hz, 1 H, Ar), 6.95 (d, J = 3.8 Hz, 1 H, Ar), 4.44 (t, J = 7.5 Hz, exch., -OH), 4.26 (q, J = 7.0 Hz, 2 H, -OCH_2), 2.83 (t, J = 7.6 Hz, 2 H, -CH_2-), 1.66 (p, J = 6.5 Hz, 2 H, -CH_2-), 1.46 (p, J = 6.5 Hz, 2 H, -CH_2-), 1.29-1.24 (m, 3 H, -CH_3). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(4-hydroxybut-1-yn-1-yl)benzoate (2g) Compound **2g** was synthesized using the general method described for the preparation of **2a-h**, using but-3-yn-1-ol (0.6 ml, 8 mmol), to give 1.86 g of **2g** as a yellow semi solid (1.86 g, 78%); TLC *Rf* = 0.3 (EtOAc:Hexane, 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (t, J = 8.0 Hz, 1 H, Ar), 7.42 – 7.30 (m, 2 H, Ar), 4.98 (t, *J* = 5.6 Hz, 1 H, exch., -OH), 3.85 (s, 3 H, -OCH_3), 3.60 (td, *J* = 6.7, 5.6 Hz, 2 H, -CH_2-), 2.60 (t, *J* = 6.7 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(5-hydroxypent-1-yn-1-yl)benzoate (2h) Compound **2h** was synthesized using the general method described for the preparation of **2a-h**, using pent-4-yn-1-ol (1.5 ml, 16.01 mmol), to give 2.23 g of **2h** as a yellow semi solid (2.01g, 79%); TLC *Rf* 0.3 (EtOAc:Hexane, 1:1); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.85 (t, *J* = 7.9 Hz, 1 H, Ar), 7.38 (d, *J* = 11.6 Hz, 1 H, Ar), 7.33 (d, *J* = 8.1 Hz, 1 H, Ar), 6.67 (t, *J* = 3.0 Hz, 0H), 4.57 (t, *J* = 10.4 Hz, 1 H, exch., -OH), 3.85 (s, 3 H, -OCH_3), 3.52 (q, *J* = 5.8 Hz, 2 H, -CH_2-), 1.69 (q, *J* = 6.7 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

General procedure for synthesis of 3a-h. To a Parr flask was added **2a-h**, 10% palladium on activated carbon (50% w/w), and MeOH (100 mL). Hydrogenation was carried out at 55 psi of H_2 for 14 h. The reaction mixture was filtered through Celite, washed with MeOH (100 mL), and concentrated under reduced pressure to give a crude mixture containing **3a-h**. Without chromatographic separation, these compounds were used for the next reaction.

Methyl 4-(3-hydroxypropyl)benzoate (3a) Compound **371** was prepared using the general method described for the preparation of **3a-h**, from **2a** (1.45 g, 7.4 mmol) to give 1.2 g (98%) of **3a** as a clear oil; TLC *Rf* = 0.16 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) 7.93 (d, J = 8.0 Hz, 2 H, Ar), 7.55 (d, J = 7.9 Hz, 2 H, Ar), 5.43 (s, 1 H, exch., -OH), 3.85 (s, 3 H, -OCH_3), 3.29 (t, J = 7.8 Hz, 2 H, -CH_2-), 2.67 (t, J = 7.8 Hz, 2 H, -CH_2-), 1.72 (dt, J = 41.3, 7.4 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Methyl 4-(4-hydroxybutyl)benzoate (3b) Compound **3b** was prepared using the general method described for the preparation of **3a-h**, from **3a** (1.45 g, 7.4 mmol) to give 1.1 g (90%) of **3b** as a clear oil; TLC *Rf* = 0.16 (EtOAc:Hexane, 1:2); ^{1}H-NMR (500 MHz) (Me_2SO-d_6) δ 7.88 (d, J = 7.9 Hz, 2 H, Ar), 7.34 (d, J = 7.9 Hz, 2 H, Ar), 4.43 (s, 1 H, exch., -OH), 3.83 (s, 3 H, -OCH_3), 3.35 – 3.25 (m, 2 H, -CH_2-), 2.68 (q, J = 10.4, 7.9 Hz, 2 H, -CH_2-), 1.72 (dtd, J = 49.7, 17.2, 15.4, 9.9 Hz, 4 H, -CH_2-). This compound was used for the next reaction without further characterization.

Methyl 4-(5-hydroxypentyl)benzoate (3c) Compound **3c** was prepared using the general method described for the preparation of **3a-h**, from **2c** (1.45 g, 7.4 mmol) to give 1.2 g (98%) of **3c** as a clear oil; TLC *Rf* = 0.16 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.86 (d, J = 7.9 Hz, 2 H, Ar), 7.32 (d, J = 7.9 Hz, 2 H, Ar), 4.36 (s, 1 H, exch., -OH), 3.82 (s, 3 H, -OCH_3), 3.37 (t, J = 6.4 Hz, 2 H, -CH_2-), 2.62 (t, J = 7.6 Hz, 2 H, -CH_2-), 1.57 (p, J = 7.7 Hz, 2 H, -CH_2-), 1.43 (p, J = 6.6 Hz, 2 H, -CH_2-), 1.29 (ddt, J = 8.6, 6.5, 3.9 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Ethyl 5-(3-hydroxypropyl)thiophene-2-carboxylate (3d) Compound **3d** was prepared using the general method described for the preparation of **3a-h**, from **2d** (1.1 g, 5.23 mmol) to give 1.0 g (89%) of **3d** as a clear oil; TLC *Rf* 0.12 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.63 (t, J = 6.1 Hz, 1 H, Ar), 6.94 (d, J = 6.1 Hz, 1 H, Ar), 4.44 (t, J = 5.1 Hz, 1 H, exch., -OH), 4.26 (p, J = 8.3, 7.1 Hz, 2 H, -OCH_2-), 2.83 (t, J = 7.6 Hz, 2 H, -CH_2-), 1.66 (p, J = 7.6 Hz, 2 H, -CH_2-), 1.46 (p, J = 6.8 Hz, 2 H, -CH_2-), 1.27 (t, J = 7.0 Hz, 3 H, -CH_3). This compound was used for the next reaction without further characterization.

Ethyl 5-(4-hydroxybutyl)thiophene-2-carboxylate (3e) Compound **3e** was prepared using the general method described for the preparation of **3a-h**, from **2e** (1.2 g, 5.35 mmol) to give 1.0 g (82%) of **3e** as a clear oil; TLC *Rf* = 0.12 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.63 (d, J = 3.7 Hz, 1 H, Ar), 6.95 (d, J = 3.8 Hz, 1 H, Ar), 4.38 (s, 1 H, exch., -OH), 4.23-4.20 (m, 2 H, -OCH_2-), 2.84 (q, J = 9.6, 7.5 Hz, 2 H, -CH_2-), 1.63 (p, J = 7.5 Hz, 3 H, -CH_3), 1.56 – 1.39 (m, 2 H, -CH_2-), 1.24-1.50 (m, 4 H, -CH_2-). The ^{1}H-NMR matches ^{1}H-NMR of the reported compound (22).

Ethyl 5-(5-hydroxypentyl)thiophene-2-carboxylate (3f) Compound **3f** was prepared using the general method described for the preparation of **3a-h**, from **2f** (1.1 g, 4.62 mmol) to give 1.0 g (89%) of **3f** as a clear oil; TLC *Rf* = 0.12 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.63 (d, J = 3.7 Hz, 1 H, Ar), 6.95 (d, J = 3.8 Hz, 1 H, Ar), 4.36 (s, 1 H, exch., -OH), 4.25 (q, J = 7.0 Hz, 2 H, -OCH_2-), 3.38 (t, J = 6.3 Hz, 2 H, -CH_2-), 2.83 (t, J = 7.4 Hz, 2 H, -CH_2-), 1.63 (p, J = 7.5 Hz, 2 H, -CH_2-), 1.44 (p, J = 6.6 Hz, 2 H, -CH_2-), 1.39 – 1.30 (m, 2 H, -CH_2-), 1.28 (t, J = 7.0 Hz, 3 H, -CH_3). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(4-hydroxybutyl)benzoate (3g) Compound **3g** was prepared using the general method described for the preparation of **3a-h**, from **2g** (1.86 g, 8.4 mmol) to give 1.68 g (89%) of **3g** as a clear oil; TLC *Rf* = 0.3 (EtOAc:Hexane, 1:1); ^{1}H-NMR (500 MHz) (Me_2SO-d_6) δ 7.80 (t, *J* = 7.9 Hz, 1 H, Ar), 7.22 – 7.14 (m, 2 H, Ar), 4.39 (s, 1 H, exch., -OH), 3.84 (s, 3 H, -OCH_3), 3.40 (d, *J* = 11.7 Hz, 2 H, -CH_2-), 2.65 (t, *J* = 7.7 Hz, 2 H, -CH_2-), 1.66 – 1.55 (m, 2 H, -CH_2-), 1.42 (dt, *J* = 13.4, 6.5 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(5-hydroxypentyl)benzoate (3h) Compound **3h** was prepared using the general method described for the preparation of **3a-h**, from **2h** (2.23 g, 9.44 mmol) to give 2.07 g (91%) of **3h** as a clear oil; TLC *Rf* = 0.3 (EtOAc:Hexane, 1:1); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) 1H NMR (400 MHz, DMSO-d6) δ 7.80 (t, J = 7.9 Hz, 1 H, Ar), 7.24 – 7.13 (m, 2 H, Ar), 4.37 (s, 1 H, exch., -OH), 3.84 (s, 3 H, -OCH_3), 3.37 (t, J = 6.4 Hz, 2 H, -CH_2-), 2.69 – 2.60 (m, 2 H, -CH_2-), 1.58 (p, J = 7.6 Hz, 2 H, -CH_2-), 1.44 (dd, J = 14.2, 7.4 Hz, 2 H, -CH_2-), 1.35 – 1.23 (m, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

General procedure for synthesis of 4a-h. To the alcohols **3a-h**, was added triethylamine (1 equivalent) and dichloromethane (25 mL). The reaction was cooled to 0 ºC and purged with nitrogen gas. Under anhydrous conditions, methanesulfonyl chloride (1.05 equivalent) was added dropwise over 30 min. The reaction was stirred at room temperature for 2 h and the reaction was added to a 0.5M sodium bisulfite solution (25 mL). The water layer was washed thrice with dichloromethane (100 mL). The dichloromethane was evaporated to obtain a semi-solid product. To this intermediate in acetone, was added sodium iodide (1 equivalent) and the mixture was kept at reflux for 8 h. The reaction mixture was filtered. The filtrate was evaporated to obtain **4a-h**.

Methyl 4-(3-iodopropyl)benzoate (4a) Compound **4a** was prepared using the general method described for the preparation of **4a-h**, from **3a** (1 g, 4.5 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 0.9 g (72%) of **4a** as a clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.95 – 7.83 (d, J = 8.0 Hz, 2 H, Ar), 7.36 (d, J = 8.0 Hz, 2 H, Ar), 3.84 (s, 3 H, -OCH_3), 3.24 (t, J = 6.8 Hz, 2 H, -CH_2-), 2.74 (t, J = 7.5 Hz, 2 H, -CH_2-), 2.07 (p, J = 7.0 Hz, 2 H, -CH_2-).

This compound was used for the next reaction without further characterization.

Methyl 4-(4-iodobutyl)benzoate (4b) Compound **4b** was prepared using the general method described for the preparation of **4a-h**, from **3b** (1 g, 4.5 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 1.0 g (80%) of **4b** as a clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (500 MHz) (Me_2SO-d_6) δ 7.86 (d, J = 8.2 Hz, 2 H, Ar), 7.31 (d, J = 8.3 Hz, 2 H, Ar), 4.55 (t, J = 5.1 Hz, 2 H, -CH_2-), 3.82 (s, 3 H, -OCH_3), 3.41 (t, J = 6.4, 2 H, -CH_2-), 2.65 (t, J = 6.4, 2 H, -CH_2-), 1.78 – 1.66 (m, 2 H, -CH_2-), 1.47-1.40 (m, 2 H, -CH_2). This compound was used for the next reaction without further characterization.

Methyl 4-(5-iodopentyl)benzoate (4c) Compound **4c** was prepared using the general method described for the preparation of **4a-h**, from **3c** (1 g, 4.5 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and

triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 1.05 g (85%) as **4c** clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.87 (d, J = 8.2 Hz, 2 H, Ar), 7.31 (d, J = 8.3 Hz, 2 H, Ar), 3.82 (s, 3 H, -OCH_3), 3.23 (t, J = 6.9 Hz, 2 H, -CH_2-), 2.62 (t, J = 7.7 Hz, 2 H, -CH_2-), 1.76 (p, J = 7.0 Hz, 2 H, -CH_2-), 1.57 (tt, J = 9.2, 6.9 Hz, 2 H, -CH_2-), 1.40 – 1.29 (m, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Ethyl 5-(3-iodopropyl)thiophene-2-carboxylate (4d) Compound **4d** was prepared using the general method described for the preparation of **4a-h**, from **3d** (0.9 g, 4.5 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 0.85 g (61%) of **4d** as a clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.63 (t, J = 4.6 Hz, 1 H, Ar), 6.97 (d, J = 3.7 Hz, 1 H, Ar), 4.25 (q, J = 7.1 Hz, 2 H, -CH_2-), 3.26 (t, J = 6.8 Hz, 2 H, -CH_2-), 2.93 (q, J = 9.0, 7.4 Hz, 2 H, -CH_2-), 2.10 (p, J = 6.9 Hz, 2 H, -CH_2-), 1.28 (t, J = 7.1 Hz, 3 H, -CH_3). This compound was used for the next reaction without further characterization.

Ethyl 5-(4-iodobutyl)thiophene-2-carboxylate (4e) Compound **4e** was prepared using the general method described for the preparation of **4a-h**, from **3e** (0.95 g, 4.5 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 0.9 g (63%) of **4e** as a clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.64 (d, J = 3.8 Hz, 1 H, Ar), 6.97 (d, J = 3.8 Hz, 1 H, Ar), 4.26 (q, J = 7.1 Hz, 2 H, -CH_2-), 3.46 – 3.24 (m, 2 H, -CH_2-), 2.87 (t, J = 7.3 Hz, 2 H, -CH_2-), 1.93 – 1.64 (m, 4 H, -CH_2-), 1.28 (t, J = 7.1 Hz, 3 H, -OCH_3). This compound was used for the next reaction without further characterization.

Ethyl 5-(5-iodopentyl)thiophene-2-carboxylate (4f) Compound **395** was prepared using the general method described for the preparation of **4a-h**, from **3f** (1 g, 4.38 mmol), methanesulfonyl chloride (0.35 mL, 4.5 mmol) and triethylamine (0.62 mL, 4.5 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 0.95 g (64%) of **4f** as a clear oil; TLC *Rf* = 0.63 (EtOAc:Hexane, 1:2); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.60 (t, J = 4.6 Hz, 1 H, Ar), 7.21 (d, J = 3.7 Hz, 1 H, Ar), 4.22 (q, J = 7.1 Hz, 2 H, -CH_2-), 3.35 – 3.27 (m, 2 H, -CH_2-), 3.12 (tt, J = 9.3, 5.2 Hz, 2 H, -CH_2-), 2.57 (t, J = 6.8 Hz, 2 H, -CH_2-), 1.97 (q, J = 7.1 Hz, 2 H, -CH_2-), 1.29 – 1.12 (m, 5 H, -CH_2- and -CH_3). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(4-iodobutyl)benzoate (4g) Compound **4g** was prepared using the general method described for the preparation of **4a-h**, from **3g** (1.68 g, 7.51 mmol), methanesulfonyl chloride (0.77 mL, 9.99 mmol) and triethylamine (1.6 mL, 11.27 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 2.02 g (80%) of **4g** as a clear oil; TLC *Rf* = 0.8 (EtOAc:Hexane, 1:1); ^{1}H-NMR (500 MHz) (Me_2SO-d_6) δ 7.86 – 7.77 (m, 1 H, Ar), 7.25 – 7.13 (m, 2 H, Ar), 3.84 (s, 3 H, Ar), 3.32 – 3.25 (m, 2 H, -CH_2-), 2.67 (t, *J* = 7.4 Hz, 2 H, -CH_2-), 1.82 – 1.72 (m, 2 H, -CH_2-), 1.70 – 1.6 (m, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

Methyl 2-fluoro-4-(5-iodopentyl)benzoate (4h) Compound **4h** was prepared using the general method described for the preparation of **4a-h**, from **3h** (2.07 g, 8.62 mmol), methanesulfonyl chloride (0.96 mL, 12.34 mmol) and triethylamine (1.9 mL, 13.92 mmol) to form the intermediate. To this was added sodium iodide and the procedure was followed to give 2.47 g (82%) of **4h** as a clear oil; TLC *Rf* = 0.8 (EtOAc:Hexane, 1:1); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.81 (t, *J* = 7.9 Hz, 1 H, Ar), 7.30 – 7.18 (m, 2 H, Ar), 3.84 (s, 3 H, -OCH_3), 3.28 (t, *J* = 6.9 Hz, 2 H, -CH_2-), 2.66 (t, *J* = 7.6 Hz, 2 H, -CH_2-), 1.83 – 1.73 (m, 2 H, -CH_2-), 1.67 – 1.58 (m, 2 H, -CH_2-), 1.37 (q, *J* = 7.8 Hz, 2 H, -CH_2-). This compound was used for the next reaction without further characterization.

General procedure for synthesis of 6a-h. To a solution of ethyl 3-amino-1*H*-pyrrole-2-carboxylate hydrochloride (0.5 g, 3.24 mmol) in dry DMF (10 mL) was slowly added NaH (0.17 g, 7.1 mmol) under nitrogen at room temperature. The resulting mixture was stirred for about 15 min when there was no more gas evolved, and then the appropriate iodide (1 equivalent) was added. The reaction mixture was stirred at room temperature for 4 h, and DMF was evaporated at elevated temperature to offer a gummy residue, which was used for the next step without purification. The gummy residue was dissolved in MeOH (10 mL), and 1,3-bis(methoxycarbonyl)-2-methyl-2- thiopseudourea (0.7 g, 3.3 mmol) was added followed by AcOH (1.0 g, 15

mmol). The mixture was stirred at room temperature overnight and became a thick paste. NaOMe in MeOH (25%) (7 mL, 22 mmol) was added, and stirring was continued at room temperature overnight. The mixture was neutralized with AcOH, and the methanol was removed under reduced pressure. To the residue was added water (20 mL), and the pH was adjusted to 10–11 by adding $NH_3 \cdot H_2O$. The solid was collected by filtration and washed well with water. The resulting solid was added to 1 N NaOH (2 mL), and the mixture was heated at 55 °C for 3 h. The mixture was cooled and acidified using 1 N HCl. The precipitate was collected and dried overnight under reduced pressure to obtain **6a-h**.

General procedure for synthesis of 7a-h. To a solution of **6a-h** in anhydrous DMF (10 mL) was added N-methylmorpholine (73 mg, 0.72 mmol) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (127 mg, 0.72 mmol). The resulting mixture was stirred at room temperature for 2 h. To this mixture was added N-methylmorpholine (73 mg, 0.72 mmol) and L-glutamate diethyl ester hydrochloride (144 mg, 0.6 mmol). The reaction mixture was stirred for an additional 4 h at room temperature. Silica gel (400 mg) was then added, and the solvent was evaporated under reduced pressure. The resulting plug was loaded on to a silica gel column with 5% MeOH in $CHCl_3$ as the eluent. Fractions that showed the desired spot (TLC) were pooled and the solvent evaporated to dryness to afford compounds **7a-h**.

General method for synthesis of target compounds. To a solution of **7a-h**, was added 4 mL methanol and 2 mL of 1 N sodium hydroxide solution. The reaction mixture was stirred for 1 h at room temperature and the disappearance of the starting material was followed with TLC. The mixture was concentrated to 1 mL and acidified to pH 2-3 using 1 N HCl to obtain target compounds as precipitated residues on filtration.

Diethyl (4-(3-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)propyl) benzoyl-L-glutamate (7a) Using the general method for synthesis of compounds **6a-h**, **5a** (1.1 g, 3.62 mmol) was used to obtain **6a** (0.3 g, 30%) as a white solid. Compound **6a** was taken further, without any characterization, using the general method for synthesis of compounds **7a-h**, to obtain **7a** (0.18 g, 75%) as a greyish brown solid; TLC $Rf = 0.23$ ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.49 (s, 1 H, exch., -NH), 8.67 (d, J = 7.4 Hz, 1 H, exch., -NH), 7.80 (d, J = 8.0 Hz, 2 H, Ar), 7.29 (d, J = 8.0 Hz, 2 H, Ar), 7.21 (d, J = 2.6 Hz, 1 H, Ar), 5.91 (d, J = 2.7 Hz, 1 H, Ar), 5.81 (s, 2 H, exch., 2-NH_2), 4.42 (d, J = 7.4 Hz, 1 H, -CH), 4.30 – 4.20 (m, 2 H, -CH_2-), 4.17 – 3.94 (m, 4 H, -CH_2-), 2.65 – 2.54 (m, 2 H, -CH_2-), 2.44 (t, J = 7.4 Hz, 2 H, -CH_2-), 2.22 – 1.86 (m, 4 H, -CH_2-), 1.35 – 1.09 (m, 6 H, -CH_3). Anal. Calcd. $C_{25}H_{31}N_5O_6$: C, 60.35; H, 6.28; N, 14.08; O, 19.29. Found: C, 60.03; H, 6.17; N, 13.76.

Diethyl (4-(4-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)butyl) benzoyl)-L-glutamate (7b) Using the general method for synthesis of compounds **6a-h**, **5b** (1.1 g, 3.46 mmol) was used to obtain **6b** (0.25 g, 25%) as a white solid; TLC $Rf = 0.0$ ($MeOH:CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.50 (s, br, exch., -COOH), 7.81 (d, J = 7.9 Hz, 2 H, Ar), 7.21 (d, J = 8.0 Hz, 2 H, Ar), 7.18 (d, J = 2.9 Hz, 1 H, Ar), 5.92 (s, 2 H, exch., 2-NH_2), 5.87 (d, J = 2.7 Hz, 1 H, Ar), 4.25 (t, J = 6.8 Hz, 2 H, -CH_2-), 2.61 (t, J = 7.7 Hz, 2 H, -CH_2-), 1.73 (p, J = 7.8 Hz, 2 H, -CH_2-), 1.48 (p, J = 7.8 Hz, 2 H, -CH_2-). The melting point assessment suggested impurities and hence this compound was used for the next reaction without further characterization. Using the general method for synthesis of compounds **7a-h**, **6b** (0.15 g, 0.46 mmol) was used to obtain **7b** (0.1 g, 43%) as a brown solid; TLC $Rf = 0.23$ ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.44 (s, 1 H, exch., -NH), 8.64 (d, J = 7.4 Hz, 1 H, exch., -NH), 7.77 (d, J = 8.2 Hz, 2 H, Ar), 7.26 (d, J = 8.2 Hz, 2 H, Ar), 7.19 (d, J = 2.9 Hz, 1 H, Ar), 5.88 (s, J = 2.9 Hz, 1 H, Ar), 5.76 (s, 2 H, exch., 2-NH_2), 4.45-4.41 (m, 1 H, -CH), 4.25 (t, J = 6.8 Hz, 2 H, -CH_2-), 4.18 – 4.00 (m, 4 H, -CH_2-), 2.62 (t, J = 7.6 Hz, 2 H, -CH_2-), 2.44 (t, J = 7.5 Hz, 2 H, -CH_2-), 2.28 – 1.89 (m, 2 H, -CH_2-), 1.87 – 1.62 (m, 2 H, -CH_2-), 1.60 – 1.40 (m, 2 H, -CH_2-), 1.18 (dt, J = 9.9, 7.0 Hz, 6 H, -CH_3). Anal. Calcd. $C_{26}H_{33}N_5O_6$ 0.05 $CHCl_3$: C, 61.04; H, 6.50; N, 13.69; O, 18.76. Found: C, 60.57; H, 6.44; N, 13.28.

Diethyl (4-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl) benzoyl)-L-glutamate (7c) Using the general method for synthesis of compounds **6a-h**, **5c** (1.2 g, 3.61 mmol) was used to obtain **6c** (0.32 g, 29%) as a white solid; TLC $Rf = 0.0$ ($MeOH:CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 12.74 (s, 1 H, exch., -COOH), 8.14 (s, 2 H, exch., 2-NH_2), 7.83 (d, J = 7.9 Hz, 2 H, Ar), 7.45 (d, J = 2.9 Hz, 1 H, Ar), 7.28 (d, J = 8.0 Hz, 2 H, Ar), 6.13 (d, J = 2.9 Hz, 1 H, Ar), 4.29 (t, J = 6.9 Hz, 2 H, -CH_2-), 3.37 (t, J = 6.4 Hz, 2 H, -CH_2-), 2.64 (t, J = 7.8 Hz, 2 H, -CH_2-), 1.79 – 1.70 (m, 2 H, -CH_2-), 1.54 – 1.45 (m, 2 H, -CH_2-). The

melting point assessment suggested impurities and hence this compound was used for the next reaction without further characterization. Using the general method for synthesis of compounds **7a-h**, **6c** (0.15 g, 0.44 mmol) was used to obtain **7c** (0.11 g, 47.50%) as a grey solid TLC *Rf* = 0.23 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.45 (s, 1 H, exch., -NH), 8.66 (d, J = 7.6 Hz, 1 H, exch., -NH), 7.77 (d, J = 8.0 Hz, 2 H, Ar), 7.27 (d, J = 8.0 Hz, 2 H, Ar), 7.16 (d, J = 2.7 Hz, 1 H, Ar), 5.87 (d, J = 2.8 Hz, 1 H, -Ar), 5.75 (s, 2 H, exch., $-NH_2$), 4.41 (d, J = 13.0 Hz, 1 H, -CH), 4.19 (t, J = 6.9 Hz, 2 H, $-CH_2$-), 4.14 – 3.96 (m, 4 H, $-CH_2$), 2.59 (t, J = 7.6 Hz, 2 H, $-CH_2$-), 2.43 (t, J = 7.4 Hz, 2 H, $-CH_2$-), 2.20 – 1.92 (m, 2 H, $-CH_2$-), 1.81 – 1.65 (m, 2 H, $-CH_2$-), 1.63 – 1.51 (m, 2 H, $-CH_2$-), 1.22-1.11 (m, 8 H, $-CH_2$- and $-CH_3$). Anal. Calcd. for $C_{27}H_{35}N_5O_6$ 0.24 H_2O: C, 61.70; H, 6.71; N, 13.32. Found: C, 61.20; H, 6.763; N, 13.13.

Diethyl (5-(3-(2-amino-4-oxo-3,4-dihydro-5H-pyrrolo[3,2-*d*]pyrimidin-5-yl)propyl) thiophene-2-carbonyl)-L-glutamate (7d) Using the general method for synthesis of compounds **6a-h**, **5d** (1.0 g, 2.97 mmol) was used to obtain **6d** (0.18 g, 19%) as a white solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 11.22 (s, br, 1 H, exch., -COOH), 7.49 (d, J = 3.6 Hz, 1 H, Ar), 7.19 (d, J = 3.0 Hz, 1 H, Ar), 6.87 (d, J = 3.8 Hz, 1 H, Ar), 6.00 (s, 2g, exch., 2-NH_2), 5.94 (d, J = 3.0 Hz, 1 H, Ar), 4.25 (t, J = 6.8 Hz, 2 H, $-CH_2$-), 2.70 (t, J = 7.8 Hz, 2 H, $-CH_2$-), 2.06 (t, J = 7.6 Hz, 2 H, $-CH_2$-). The melting point assessment suggested impurities and hence this compound was used for the next reaction without further characterization. Using the general method for synthesis of compounds **7a-h**, **6d** (0.15 g, 0.47 mmol) was used to obtain **7d** (0.125 g, 53%) as a grey semi-solid; TLC *Rf* = 0.23 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.44 (s, 1 H, exch., -NH), 8.62 (d, J = 7.5 Hz, 1 H, exch., -NH), 7.69 (d, J = 3.8 Hz, 1 H, Ar), 7.20 (d, J = 2.9 Hz, 1 H, Ar), 6.91 (d, J = 3.8 Hz, 1 H, Ar), 5.91 (d, J = 2.9 Hz, 1 H, Ar), 5.77 (s, 2 H, exch., 2-NH_2), 4.38 (dt, J = 9.4, 5.9 Hz, 1 H, -CH), 4.27 (t, J = 6.8 Hz, 2 H, $-CH_2$-), 4.08 (dq, J = 23.8, 7.0 Hz, 4 H, $-CH_2$-), 2.72 (t, J = 7.9 Hz, 2 H, $-CH_2$-), 2.42 (t, J = 7.5 Hz, 2 H, $-CH_2$-),), 2.10 (q, J = 7.2 Hz, 2 H, $-CH_2$-), 1.97 (ddd, J = 16.7, 14.0, 7.6 Hz, 2 H, $-CH_2$-), 1.18 (dt, J = 9.0, 7.1 Hz, 6 H, $-CH_3$). This compound was used for the next reaction without further characterization.

Diethyl (5-(4-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)butyl) thiophene-2-carbonyl)-L-glutamate (7e) Using the general method for synthesis of compounds **6a-h**, **5e** (1.0 g, 2.97 mmol) was used to obtain **6e** (0.20 g, 20%) as a white solid. Using the general method for synthesis of compounds **7a-h**, **6e** (0.18 g, 0.54 mmol) was used to obtain **7e** (0.1 g, 37%) as a brown semi- solid; TLC *Rf* = 0.23 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.45 (s, 1 H, exch., -NH), 8.63 (d, J = 7.7 Hz, 1 H, exch., -NH), 7.67 (d, J = 3.8 Hz, 1 H, Ar), 7.20 (d, J = 2.5 Hz, 1 H, Ar), 6.85 (d, J = 3.9 Hz, 1 H, Ar), 5.88 (d, J = 2.9 Hz, 1 H, Ar), 5.76 (s, 2 H, exch., 2-NH_2), 4.3-4.45 (m, 1 H, -CH), 4.26 (t, J = 6.8 Hz, 2 H, $-CH_2$-), 4.07 (dq, J = 22.7, 7.2 Hz, 4 H, $-CH_2$-), 3.46 – 3.24 (m, 2 H, $-CH_2$-), 2.79 (t, J = 7.4 Hz, 2 H, $-CH_2$-), 2.42 (t, J = 7.4 Hz, 2 H, $-CH_2$-), 1.76 (t, J = 7.6 Hz, 2 H, $-CH_2$-), 1.51 (t, J = 7.6 Hz, 2 H, $-CH_2$-), 1.17 (dt, J = 9.5, 7.1 Hz, 6 H, $-CH_3$). This compound was used for the next reaction without further characterization.

Diethyl (5-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl) thiophene-2-carbonyl)-*L*-glutamate (7f) Using the general method for synthesis of compounds **6a-h**, **5f** (2.0 g, 5.74 mmol) was used to obtain **6f** (0.34 g, 30%) as a white solid. Using the general method for synthesis of compounds **7a-h**, **6f** (0.34g, 1.03 mmol) was used to obtain **7f** (0.11 g, 72 %) as a grey semi-solid; TLC *Rf* = 0.23 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 10.69 (s, 1H, exch., -NH-), 8.65 (d, J = 7.5 Hz, 1 H, exch., -NH), 7.69 (d, J = 3.8 Hz, 1 H, Ar), 7.16 (d, J = 2.9 Hz, 1 H, Ar), 6.85 (d, J = 3.7 Hz, 1 H, Ar), 6.12 – 5.71 (m, 3 H, Ar (1 H) and 2-NH_2 (2 H, exch.)), 4.41 (d, J = 5.6 Hz, 1 H, -CH), 4.21 (t, J = 6.8 Hz, 2 H, $-CH_2$-), 4.11 (q, J = 7.1 Hz, 2 H, $-CH_2$-), 4.04 (q, J = 7.1 Hz, 2 H, $-CH_2$-), 2.75 (t, J = 7.4 Hz, 2 H, $-CH_2$-), 2.42 (d, J = 7.5 Hz, 2 H, $-CH_2$-), 2.21 – 1.89 (m, 2 H, $-CH_2$-), 1.73 (t, J = 7.4 Hz, 2 H, $-CH_2$-), 1.60 (t, J = 7.6 Hz, 2 H, $-CH_2$-), 1.16 (m, 8 H, $-CH_2$- and $-CH_3$). This compound was used for the next reaction without further characterization.

Diethyl (4-(4-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)butyl)-2-fluorobenzoyl)-L-glutamate (7g) Using the general method for synthesis of compounds **6a-h**, crude **5g** (1.1 g) was used to obtain **6g** (0.25 g, 0.73 mmol) as a grey solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.76 (t, *J* = 8.1 Hz, 1 H, Ar), 7.23 (d, *J* = 2.9 Hz, 1 H, Ar), 7.16 – 7.05 (m, 2 H, Ar), 6.03 (s, 2g, exch., 2-NH_2), 5.91 (d, *J* = 2.8 Hz, 1 H, Ar), 4.25 (t, *J* = 6.8 Hz, 2 H, $-CH_2$-), 2.62 (t, *J* = 7.7 Hz, 2 H, $-CH_2$-), 1.72 (p, *J* = 6.9 Hz, 2 H, $-CH_2$-), 1.48 (qd, *J* = 9.3, 8.8, 6.3 Hz, 2 H, $-CH_2$-). The melting point assessment

suggested impurities and hence this compound was used for the next reaction without further characterization. Using the general method for synthesis of compounds **7a-h**, **6g** (0.15 g, 0.44 mmol) was used to obtain **7g** (0.1 g, 43%) as a brown semi solid; TLC *Rf* = 0.3 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (s, 1 H, exch., -NH), 7.55 – 7.45 (m, 2 H, Ar), 7.17 – 7.06 (m, 2 H, Ar), 6.15 (d, *J* = 2.8 Hz, 1 H, Ar), 4.42 (s, 1 H, -CH), 4.29 (t, *J* = 6.8 Hz, 2 H, -CH_2-), 4.17 – 4.01 (m, 4 H, -CH_2-), 4.05 – 3.93 (m, 2 H, -CH_2-), 3.66 (t, *J* = 12.4 Hz, 2 H, -CH_2-), 2.64 (t, *J* = 7.6 Hz, 2 H, -CH_2-), 1.74 (m, 2 H, -CH_2-), 1.51 (d, *J* = 6.8 Hz, 2 H, -CH_2-), 1.26 – 1.13 (m, 6 H, -CH_3). This compound was used for the next reaction without further characterization.

Diethyl (4-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl)-2-fluorobenzoyl)-L-glutamate (7h) Using the general method for synthesis of compounds **6a-h**, crude **5h** (0.93 g) was used to obtain **6h** (0.15 g, 0.42 mmol) as a white solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 7.70 (td, *J* = 8.2, 6.3 Hz, 1 H, Ar), 7.16 (d, *J* = 2.8 Hz, 1 H, Ar), 7.10 – 7.00 (m, 2 H, Ar), 5.94 (s, 2 H, exch., -NH_2), 5.86 (d, *J* = 2.8 Hz, 1 H, Ar), 4.23 – 4.14 (m, 2 H, -CH_2-), 2.60 (dt, *J* = 21.4, 7.8 Hz, 2 H, -CH_2-), 1.75 – 1.50 (m, 4 H, -CH_2-), 1.25 – 1.15 (m, 2g). The melting point assessment suggested impurities and hence this compound was used for the next reaction without further characterization. Using the general method for synthesis of compounds **7a-h**, **6h** (0.15 g, 0.4 mmol) was used to obtain **7h** (0.07 g, 32%) as a grey solid TLC *Rf* = 0.3 ($MeOH:CHCl_3:NH_4OH$, 1:10:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 11.17 (s, 1 H, exch., -NH), 8.57 (dd, *J* = 7.5, 2.0 Hz, 1 H, exch., -NH), 7.50 (t, *J* = 7.8 Hz, 1 H, Ar), 7.38 (d, *J* = 2.9 Hz, 1 H, Ar), 7.16 – 7.07 (m, 2 H, Ar), 6.07 (d, *J* = 2.8 Hz, 1 H, Ar), 4.43 (ddd, *J* = 9.5, 7.4, 5.1 Hz, 1 H, -CH), 4.24 (t, *J* = 7.1 Hz, 2 H, -CH_2-), 4.12 (qq, *J* = 7.0, 3.7 Hz, 2 H, -CH_2-), 4.08 – 4.03 (m, 2 H, -CH_2-), 2.61 (t, *J* = 7.7 Hz, 2 H, , -CH_2-), 2.46 – 2.40 (m, 2 H, -CH_2-), 2.09 (m, 2 H, -CH_2-), 1.75 (p, *J* = 7.3 Hz, 2 H), 1.57 (q, *J* = 7.6 Hz, 2g), 1.22 – 1.17 (m, 8 H, -CH_2- and -CH_3). This compound was used for the next reaction without further characterization.

(4-(3-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)propyl)benzoyl)-L-glutamic acid (AGF291) Using the general method for synthesis of target compounds, **7a** (0.10 g, 0.2 mmol) was used to obtain **AGF291** (0.06 g, 67%) as a white solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); mp, 71.8-80.0 ºC; ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 12.10-11.20 (s, br, 3 H, exch., -COOH and -NH), 8.59 – 8.25 (m, 1 H, exch., -NH), 7.75 (d, J = 7.9 Hz, 2 H, Ar), 7.27 (d, J = 7.7 Hz, 2 H, Ar), 7.19 (d, J = 2.6 Hz, 1 H, Ar), 6.44 (s, 2 H, exch., 2-NH_2), 5.89 (d, J = 2.7 Hz, 1 H, Ar), 4.32 – 4.19 (m, 3 H, -CH and -CH_2), 2.57 (t, J = 7.3 Hz, 2 H, -CH_2-), 2.34 – 2.11 (m, 2 H, -CH_2-), 2.11 – 1.98 (m, 2 H, -CH_2-), 2.01 – 1.83 (m, 2 H, -CH_2-). Anal. Calcd. for $C_{21}H_{23}N_5O_6$ 0.9 CH_3OH 0.8 HCl: C, 52.67; H, 5.53; N, 14.02; Found: C, 52.53; H, 5.63; N, 14.07; Cl, 1.80.

(4-(4-(2-amino-4-oxo-3,4-dihydro-5H-pyrrolo[3,2-d]pyrimidin-5-yl)butyl)benzoyl)-L-glutamic acid (AGF300) Using the general method for synthesis of target compounds, **7b** (0.10 g, 0.195 mmol) was used to obtain **AGF300** (0.056 g, 63%) as a white solid; TLC Rf = 0.0 (MeOH:CHCl3:HCl, 1:5:0.5); mp, 82.3-87.0 ºC; 1H-NMR (400 MHz) (Me2SO-d6) δ 11.80-11.00 (s, br, exch., 3 H, COOH and NH), 8.26 (s, 1 H, exch., -NH), 7.71 (d, J = 7.9 Hz, 2 H, Ar), 7.33 – 7.09 (m, 3 H, Ar), 6.14 (s, 2 H, exch., 2-NH2), 5.86 (d, J = 2.2 Hz, 1 H, Ar), 4.23 (m, 3 H, -CH- and -CH2-), 2.77 – 2.56 (m, 2 H, -CH_2-), 2.37 – 2.09 (m, 2 H, -CH_2-), 2.04 – 1.84 (m, 2 H, -CH_2-), 1.71 (m, 2 H, -CH_2-), 1.46 (d, *J* = 7.4 Hz, 2 H, -CH_2). Anal. Calcd. for $C_{22g25}N_5O_6$ 0.77 HCl: C, 54.64; H, 5.37; N, 14.48. Found: C, 54.71; H, 5.34; N, 14.28; Cl, 1.88.

(4-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl)benzoyl)-L-glutamic acid (AGF299) Using the general method for synthesis of target compounds, **7c** (0.10 g, 0.195 mmol) was used to obtain **AGF299** (0.050 g, 56%) as a white solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); mp, 82.3-84.8 ºC; ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 11.95 (s, 2 H, exch., -COOH), 8.54 (d, J = 7.7 Hz, exch., -NH), 7.79 (d, J = 7.8 Hz, 2 H, Ar), 7.40 – 7.00 (m, 3 H, Ar), 6.37 (s, 2 H, exch., 2-NH_2), 5.94 (d, J = 2.9 Hz, 1 H, Ar), 4.38 (d, J = 8.2 Hz, 1 H, -CH-), 4.21 (t, J = 7.0 Hz, 2 H, -CH_2-), 2.60 (t, J = 7.7 Hz, 2 H, -CH_2-), 2.36 (t, J = 7.4 Hz, 2 H, -CH_2-), 2.17 – 1.82 (m, 2 H, -CH_2-), 1.80-1.65 (m, 2 H, -CH_2-), 1.60-1.45 (m, 2 H, -CH_2-), 1.26-1.00 (m, 2 H, -CH_2-). Anal. Calcd. $C_{23g27}N_5O_6$ 1.08 H_2O: C, 56.50; H, 6.01; N, 14.23. Found: C, 56.49; H, 5.83; N, 14.28.

(5-(3-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)propyl) thiophene-2-carbonyl)-L-glutamic acid (AGF331) Using the general method for synthesis of target compounds, **7d** (0.10 g, 0.2 mmol) was used to obtain **AGF331** (0.054 g, 61%) as a white solid; TLC *Rf* = 0.0 ($MeOH:CHCl_3$:HCl, 1:5:0.5); mp, 150.3-154.3 ºC; ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 12.20-11.10 (s, br, exch., 3 H, COOH and NH), 8.49 (d, J =

7.8 Hz, 1 H, exch., -NH), 7.68 (d, J = 3.8 Hz, 1 H, Ar), 7.20 (d, J = 2.8 Hz, 1 H, Ar), 6.90 (d, J = 3.8 Hz, 1 H, Ar), 5.91 (d, J = 2.8 Hz, 1 H, Ar), 5.80 (s, 2 H, exch., 2-NH_2), 4.31 (dt, J = 28.9, 8.4 Hz, 3 H, -CH and -CH_2-), 2.72 (t, J = 7.7 Hz, 2 H, -CH_2-), 2.33 (t, J = 7.5 Hz, 2 H, -CH_2-), 2.08 (dq, J = 12.7, 6.5, 5.7 Hz, 2 H, -CH_2-), 1.91 (m, 2 H, -CH_2-). Anal. Calcd. for $C_{19}H_{21}N_5O_5S$ 0.8 H_2O: C, 49.41; H, 4.93; N, 15.16; S, 6.94. Found: C, 49.44; H, 4.84; N, 15.13; S, 6.84.

(5-(4-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)butyl)thiophene-2-carbonyl)-L-glutamic acid (AGF318) Using the general method for synthesis of target compounds, **7e** (0.10 g, 0.193 mmol) was used to obtain **AGF318** (0.045 g, 50%) as a white solid; mp, 148.3-150.2 ºC; TLC *Rf* = 0.0 (MeOH:$CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 11.94 (s, 3 H, exch., -COOH and NH), 8.54 (d, J = 7.8 Hz, 1 H, exch., -NH), 7.69 (d, J = 7.8 Hz, 1 H, Ar), 7.33 (d, J = 3.8 Hz, 1 H, Ar), 6.96 (s, 2 H, exch., 2-NH_2), 6.85 (d, J = 3.8 Hz, 1 H, Ar), 6.01 (d, J = 2.8 Hz, 1 H, Ar), 4.38 – 4.23 (m, 3 H, -CH- and -CH_2-), 2.79 (t, J = 7.6 Hz, 2 H, -CH_2-), 2.34 (t, J = 7.4 Hz, 2 H, -CH_2-), 2.14 – 1.92 (m, 2 H, -CH_2-), 1.76 (p, J = 6.9 Hz, 2 H, -CH_2-), 1.52 (p, J = 7.6 Hz, 2 H, -CH_2-). Anal. Calcd. for $C_{20}H_{23}N_5O_6S$ 0.58 HCl: C, 49.77; H, 4.92; N, 14.51; S, 6.64. Found: C, 49.80; H, 5.08; N, 14.51; S, 6.74; Cl, 2.26.

(5-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl)thiophene-2-carbonyl)-L-glutamic acid (AGF320) Using the general method for synthesis of target compounds, **7f** (0.10 g, 0.188 mmol) was used to obtain **AGF320** (0.072 g, 81%) as a white solid; mp, 73.4-78.7 ºC; TLC *Rf* = 0.0 (MeOH:$CHCl_3$:HCl, 1:5:0.5); ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 12.32 (s, 2g, exch., -COOH), 8.53 (d, J = 7.9 Hz, 1 H, exch., -NH), 7.69 (d, J = 3.8 Hz, 1 H, Ar), 7.34 (d, J = 7.9 Hz, 1 H, Ar), 7.09 (s, 2g, exch., 2-NH_2), 6.87 (d, J = 3.8 Hz, 1 H, Ar), 6.03 (d, J = 2.8 Hz, 1 H, Ar), 4.34 (d, J = 2.8 Hz, 1 H, -CH), 4.23 (t, J = 7 .0 Hz, 2 H, -CH_2-), 2.77 (t, J = 7.5 Hz, 2 H, -CH_2-), 2.34 (t, J = 7.5 Hz, 2 H, -CH_2-), 1.92 (d, J = 11.9 Hz, 2 H, -CH_2-), 1.74 (m, 2 H, -CH_2-), 1.61 (m, 2 H, -CH_2-), 1.25 (t, J = 7.5 Hz, 2 H, -CH_2-). Anal. Calcd. for $C_{21}H_{25}N_5O_6S$ 0.94 HCl: C, 49.48; H, 5.13; N, 13.74; S, 6.29. Found: C, 49.51; H, 5.21; N, 13.53; S, 6.31; Cl, 4.25.

(4-(4-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)butyl)-2-fluorobenzoyl)-L-glutamic acid (AGF347) Using the general method for synthesis of target compounds, **7g** (0.40 g, 0.845 mmol) was used to obtain **AGF347** (0.2 g, 56%) as a white solid; TLC Rf = 0.0 (MeOH:CHCl3:HCl, 1:5:0.5); mp, 161.6-164.4 ºC; 1H-NMR (500 MHz) (Me_2SO-d6) δ 8.43 (dd, *J* = 7.6, 2.6 Hz, 1 H, exch., -NH), 7.51 (t, *J* = 7.7 Hz, 1 H, Ar), 7.23 (d, *J* = 2.9 Hz, 1 H, Ar), 7.13 – 7.05 (m, 2 H, Ar), 6.2 (s, 2g, exch., –NH_2), 5.92 (d, *J* = 2.8 Hz, 1 H, Ar), 4.38 (ddd, *J* = 9.5, 7.5, 4.8 Hz, 1 H, -CH), 4.25 (t, *J* = 6.8 Hz, 2 H, -CH_2-), 2.61 (t, *J* = 7.7 Hz, 2 H, -CH_2-), 2.40 – 2.28 (m, 2 H, -CH_2-), 2.08 – 1.84 (m, 2 H, -CH_2-), 1.72 (p, *J* = 7.1 Hz, 2 H, -CH_2-), 1.48 (td, *J* = 8.5, 4.1 Hz, 2 H, , -CH_2-). Anal. Calcd. for $C_{22g24}FN_5O_6$ 0.58 HCl: C, 55.81; H, 5.11; N, 14.79; F,4.01 Found: C, 53.30; H, 5.15; N, 14.18; F, 3.81; Cl, 1.47.

(4-(5-(2-amino-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)pentyl)-2-fluorobenzoyl)-L-glutamic acid (AGF355) Using the general method for synthesis of target compounds, **7h** (0.05 g, 0.09 mmol) was used to obtain **AGF355** (0.02 g, 45%) as a grey solid; TLC *Rf* = 0.0 (MeOH:$CHCl_3$:HCl, 1:5:0.5); mp, 138.5-145.7 ºC; ^{1}H-NMR (400 MHz) (Me_2SO-d_6) δ 8.42 (d, *J* = 9.0 Hz, 1 H, exch., -NH), 7.52 (d, *J* = 15.6 Hz, 1 H, Ar), 7.19 (d, *J* = 2.8 Hz, 1 H, Ar), 7.11 (d, *J* = 25.0 Hz, 2 H, Ar), 5.94 (s, 2 H, exch., -NH_2), 5.89 (d, *J* = 2.8 Hz, 1 H, Ar), 4.39 (d, *J* = 21.9 Hz, 1 H, -CH), 4.20 (d, *J* = 14.1 Hz, 2g, -CH_2-), 2.60 (d, *J* = 15.7 Hz, 2g, -CH_2-), 2.38 – 2.32 (m, 2 H, -CH_2-), 2.12-1.91 (m, 2 H, -CH_2-), 1.73 (p, *J* = 7.2 Hz, 2 H, -CH_2-), 1.57 (p, *J* = 7.7 Hz, 2 H, -CH_2-), 1.23 (d, *J* = 51.8 Hz, 2 H, -CH_2-). MS calculated for $C_{21}H_{22}FN_5O_6$ $[M+H]^+$, 488.19. Found: 487.9. HPLC analysis: retention time, 12.75 min; peak area, 95.23%; eluent A, H_2O: eluent B, ACN; gradient elution (100% H_2O to 10% H_2O) over 45 min with flow rate of 0.3 mL/min and detection at 254 nm; column temperature, rt.

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