Supporting Information

Title: Identification of *N*-(5-*tert*-Butylisoxazol-3-yl)-*N*'-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Dihydrochloride (AC220), a Uniquely Potent, Selective and Efficacious FMS-Like Tyrosine Kinase-3 (FLT3) Inhibitor

Authors: Qi Chao, Kelly G. Sprankle, Robert M. Grotzfeld, Andiliy G. Lai, Todd A. Carter, Anne Marie Velasco, Ruwanthi N. Gunawardane, Merryl D. Cramer, Michael F. Gardner, Joyce James, Patrick P. Zarrinkar, Hitesh K. Patel, Shripad S. Bhagwat*

Index to Supporting Information

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-[4-(imidazo[2,1-*b*][1,3]benzothiazol-2-yl)phenyl]urea (6a).** A mixture of 1,3-benzothiazol-2-amine (751 mg, 5 mmol) and 2-bromo-4'-nitroacetophenone (1.22g, 5 mmol) in ethanol was heated to reflux overnight. The solution was then allowed to stand at room temperature for 24 hours. The precipitate (**3a**) was collected by filtration, washed with methanol and dried under vacuum.

Compound (**3a**, 428 mg, 1.5 mmol) was suspended in isopropyl alcohol, and iron powder (419 mg, 7.5mmol) was added. The suspension was heated to reflux overnight with vigorous stirring. Completion of the reaction was confirmed by LCMS. Aqueous 1N HCl was added and the mixture was allowed to cool to room temperature. The precipitate was separated by filtration and washing with methanol. The filtrates were evaporated and azeotroped with toluene. To the resulting oil was added to cold saturated $NaHCO_3$ (20 mL) and the mixture was sonicated. The mixture was then concentrated and the residual solvent was azeotroped with toluene. The resulting residue was triturated with $CHCl_3$, and the solids were removed by filtration followed by washing with $CHCl_3$. The combined filtrates were concentrated and the residue was purified by silica gel chromatography (dichloromethane/5 % MeOH/0.5% Et_3N) to give intermediate **4a**.

A solution of **4a** (133 mg, 0.5 mmol) and compound **5** (83 mg, 0.5 mmol) in chloroform was heated at 90 °C for two hours. The reaction mixture was purified by silica gel chromatography (dichloromethane/MeOH) to give **6a** as solid. ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.9 (s, 1H), 8.7 (s, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.95 (d, J =

7.9 Hz, 1H), 7.8 (d, *J* = 8.3 Hz, 2H), 7.65 (m, 3H), 7.4 (t, *J* = 7.9 Hz, 1H), 6.55 (s, 1H), 1.3 (s, 9H); LC-MS (ESI) *m/z* 432 $(M+H)^+$.

2-(4-Aminophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-ol (4b). General Procedure F. A mixture of 2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-ol (**3b**) (3.50 g, 11.2 mmol) and $SnCl_2 \cdot H_2O$ (12.41 g, 55 mmol) in ethanol (80 mL) was heated at 90 °C overnight. Water (100 mL) and dichloromethane (500 mL) were added, and the mixture was neutralized with saturated $NaHCO_3$ solution. The separated aqueous phase was extracted with dichloromethane, and the combined organic extracts were concentrated to give 2-(4-aminophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-ol (**4b**) as a solid (1.821 g, 58%). ^{1}H NMR (DMSO-d_6) δ 9.83 (s, 1H), 8.32 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.34 (d, *J* = 1.2 Hz, 1H), 6.95 (dd, *J* = 8.6 and 1.2 Hz, 1H), 6.60 (d, *J* = 7.6 Hz, 2H), 4.09 (br, 2H).

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-[4-(7-hydroxyimidazo[2,1-*b*][1,3]benzothiazol-2-yl)phenyl]urea (6b).** Compound **6b** was synthesized from **4b** according to General Procedure D (1.103 g, 69%). ^{1}H NMR (DMSO-d_6) δ 10.1 (br, 1H), 9.65 (s, 1H), 9.17 (s, 1H), 8.68 (s, 1H), 7.76-7.84 (m, 3H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.01 (dd, *J* = 8.6 and 2.0 Hz, 1H), 6.53 (d, 1H), 1.30 (s, 9H); LC-MS (ESI) *m/z* 448 $(M+H)^+$.

6-(4-Methylpiperazin-1-yl)-1,3-benzothiazol-2-amine (2c). To a solution of 4-(4-methylpiperazin-1-yl)aniline (**10**) (2.00 g, 10.5 mmol) in acetic acid (30 mL) was added NH_4SCN (2.398 g, 31.5 mmol). After stirring for 30 minutes, bromine (1.68 g, 10.5 mmol) was added dropwise over 30 minutes. The reaction mixture was stirred at room temperature overnight, then heated at 90 ºC for 30 minutes. It was poured into ice and neutralized with saturated $NaHCO_3$ soluton to form a precipitate. Collection of the solids by filtration gave 6-(4-methylpiperazin-1-yl)-1,3-benzothiazol-2-amine (**2c**) as a brown solid (1.484 g, 57%). ^{1}H NMR ($CDCl_3$) δ 7.44 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.09 (dd, J = 8.8 and 2.4 Hz, 1H), 4.98 (br, 2H), 3.19 (t, J = 5.0 Hz, 2H), 2.61 (t, J = 5.0 Hz, 2H), 2.37 (s, 3H).

2-(4-Aminophenyl)-7-(4-methylpiperazin-1-yl)imidazo[2,1-*b*][1,3]benzothiazole (4c). Compound **4c** was synthesized from **2c** according to General Procedure A and General Procedure F. (154 mg, 7%). ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.45 (dd, J = 8.8 and 1.8 Hz, 1H), 7.18 (dd, J = 13.0 and 2.4 Hz, 1H), 7.00 (ddd, J = 13.0 and 8.8 and 2.4 Hz, 1H), 6.74 (d, J = 8.3 Hz, 2H), 4.98 (br, 2H), 3.23 (t, J = 4.8 Hz, 4H), 2.61 (t, J = 4.8 Hz, 4H), 2.37 (s, 3H).

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-{4-[7-(4-methylpiperazin-1-yl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea (6c).** Compound **6c** was synthesized from **4c** according to General Procedure D. (14 mg, 7%). ^{1}H NMR ($CDCl_3$) δ 9.3 (br, 1H), 7.81-7.85 (m, 3H), 7.57-7.65 (m, 3H), 7.47 (d, J = 8.8 Hz, 1H), 7.19 (d, J = 2.1 Hz, 1H), 7.04

(dd, J = 8.8 and 2.1 Hz, 1H), 5.85 (s, 1H), 3.25 (t, J = 4.7 Hz, 4H), 2.62 (t, J = 4.7 Hz, 4H), 2.38 (s, 3H), 1.36 (s, 9H); LC-MS (ESI) *m/z* 530 $(M+H)^+$

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (7). General Procedure G.** To a solution of *N*-(5-*tert*-butyl-isoxazol-3-yl)-*N'*-[4-(7-hydroxyimidazo[2,1-*b*][1,3]benzothiazol-2-yl)phenyl]urea **(6b)** (110 mg, 0.25 mmol), Ph_3P (79 mg, 0.3 mmol), and 2-morpholinoethanol (39 mg, 0.3 mmol) in THF was added dropwise a solution of diisopropyl azodicarboxylate (61 mg, 0.3 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature overnight, then purified by silica gel chromatography eluting with a mixture of MeOH/dichloromethane to give *N*-(5-*tert*-butyl-isoxazol-3-yl)-*N'*-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea (**7**) as the free base (30 mg, 21%).

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-{4-[7-(2-piperidin-1-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (8).** Compound **8** was synthesized from **6b** according to General Procedure G and General Procedure E. (118 mg, 40%). ^1H NMR (DMSO-d_6) δ 10.2 (br, 1H), 9.69 (s, 1H), 9.27 (s, 1H), 8.68 (s, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.77-7.79 (m, 3H), 7.54 (d, J = 8.7 Hz, 2H), 7.26 (dd, J = 8.9 and 2.4 Hz, 1H), 6.53 (s, 1H), 4.48 (t, J = 5.2 Hz, 2H), 3.52 (m, 4H), 3.02 (m, 2H), 1.7-1.8 (m, 5H), 1.4 (m, 1H), 1.30 (s, 9H); LC-MS (ESI) *m/z* 559 $(M+H)^+$.

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-{4-[7-(3-morpholin-4-yl-propoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (9).** Compound **9** was synthesized from **6b** according to General Procedure G and General Procedure E. (64 mg, 20%). ^{1}H NMR (DMSO-d_6) δ 10.2 (br, 1H), 9.60 (s, 1H), 9.04 (s, 1H), 8.64 (s, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.70 (s, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 8.1 Hz, 1H), 6.52 (s, 1H), 4.16 (t, J = 4.2 Hz, 2H), 3.99 (m, 4H), 3.33 (t, J = 4.1 Hz, 2H), 3.09 (m, 4H), 2.20 (m, 2H), 1.31 (s, 9H); LC-MS (ESI) *m/z* 575 $(M+H)^+$.

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-(4-{7-[3-(4-methylpiperazin-1-yl)propoxy] imidazo[2,1-*b*][1,3]benzothiazol -2-yl}phenyl)urea Hydrochloride (14).** Compound **14** was synthesized from **11** according to procedures for compound **15**. ^{1}H NMR (CD_3OD) δ8 (s, 1H); 7.7 (d, J = 8.5 Hz, 2H); 7.6 (d, J = 8.8 Hz, 1H); 7.5 (d, J = 8.5 Hz, 2H); 7.26 (d, J = 2.4 Hz, 1H), 6.9 (dd, J = 8.8 and 2.4 Hz, 1H); 6.4 (s, 1H); 3.9 (m, 1H); 3.3 (s, 3H); 2.5 (m, 8H); 2.3 (s, 3H); 1.8 (m, 2H); 1.3 (s, 9H); LC-MS (ESI) *m/z* 588 $(M+H)^+$.

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-(4-{7-[3-(4-methanesulfonyl-piperazin-1-yl)propoxy]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}phenyl)urea Hydrochloride (15).** To a mixture of compound **3b** (500 mg, 1.6 mmol) in DMF were added potassium carbonate (221 mg, 1.6 mmol) and 1-bromo-4-chloropropane (756 mg, 4.8 mmol), and the mixture was heated at 80 °C overnight, then concentrated to dryness. The residue was

purified by silica gel chromatography eluting with ethanol/hexane to give 7-(3-chloropropoxy)-2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazole **(11)** (440 mg, 85%).

To compound **11** (1.37 g, 3.5 mmol) in DMF were added tetrabutylammonium iodide (0.150 g) and 1-(methanesulfonyl)piperazine (1.20 g, 7.0 mmol), and the mixture was heated at 90°C overnight. After the reaction was allowed to go to completion, the mixture was diluted water, and filtered to give 7-[3-(4-methanesulfonylpiperazin-1-yl)propoxy]-2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazole **(12b)**.

To a suspension of **12b** in isopropyl alcohol (45 mL) were added 10% HCl (5 mL) and iron powder (1.82 g). The suspension was heated at reflux for 2 hours, then the mixture was filtered and washed with methanol and dichloromethane. The filtrate was concentrated, poured into saturated sodium bicarbonate and extracted three times with dichloromethane to give compound **13b** (1.00 g).

To a solution of **13b** (1.00 g, 2.6 mmol) in chloroform was added compound **5** (0.431 g, 2.6 mmol) and the mixture was heated at reflux for approximately 3 hours. The crude product was purified by silica gel chromatography eluting with a mixture of methanol/dichloromethane with 0.5% triethylamine to give compound **15** as the free base, which converted into its hydrochloride salt, *N*-(5-*tert*butyl-isoxazol-3-yl)-*N'*-(4-{7-[3-(4-methanesulfonyl-piperazin-1-yl)propoxy]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}phenyl)urea hydrochloride (**15**), according to Gerneral Procedure E,. ^{1}H NMR (DMSO-d_6) δ 10.33 (br, 1H), 9.8 (s, 1H); 9.5 (s, 1H); 8.8 (s, 1H); 8.0 (d, J = 8.8 Hz, 1H); 7.7 (m, 3H); 7.6 (d, J = 8.8 Hz, 2H); 7.2 (dd, J = 8.9 and 2.3 Hz, 1H); 6.5 (s, 1H); 4.3 (t, J

= 4.8 Hz, 2H); 3.7 (m, 5H); 3.4 (m, 4H); 3.2 (m, 1H); 3.0 (s, 3H); 2.3 (m, 2H); 1.3 (s, 9H); LC-MS (ESI) *m/z* 652 $(M+H)^+$.

2-(4-Nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-5-ol (17a). Compound **17a** was synthesized from **16a** according to General Procedure A. (4.203 g, 45%). ^{1}H NMR (DMSO-d_6) δ 11.1 (s, 1H), 8.79 (s, 1H), 8.25 (d, J = 8.9 Hz, 2H), 8.23 (d, J = 8.9 Hz, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.27 (t, J = 8.0 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H).

5-(2-Morpholin-4-yl-ethoxy)-2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazole (18a). Compound **18a** was synthesized from **17a** according to General Procedure B. (1.21 g, 89%). ^{1}H NMR (DMSO-d_6) δ 8.78 (s, 1H), 8.29 (d, J = 8.2 Hz, 2H), 8.17 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 4.43 (t, J = 5.6 Hz, 2H), 3.56 (t, J = 4.3 Hz, 4H), 3.34 (overlapping with solvent, 4H), 2.93 (t, J = 5.6 Hz, 2H).

2-(4-Aminophenyl)-5-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazole (19a). Compound **19a** was synthesized from **18a** according to General Procedure C. (1.071 g, 96%). ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1H), 7.52-7.55 (m, 3H), 7.33 (t, J = 8.1 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 6.62 (d, J = 8.5 Hz, 2H), 5.20 (s, 2H), 4.37 (t, J = 5.0 Hz, 2H), 3.59 (t, J = 4.5 Hz, 4H), 3.34 (overlapping with solvent, 4H), 2.89 (t, J = 5.0 Hz, 2H).

***N*-(5-*tert*-Butylisoxazol-3-yl)-*N'*-{4-[5-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (20a).** Compound **20a** was synthesized from **19a** according to General Procedure D and General Procedure E. (1.226 g, 76%). ^{1}H NMR (DMSO-d_6) δ 11.62 (br, 1H), 9.78 (br, 1H), 9.56 (br, 1H), 8.64 (s, 1H), 7.94 (d, J = 7.6 Hz, 2H), 7.70 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 7.6 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 6.54 (s, 1H), 4.79 (t, J = 4.1 Hz, 2H), 3.87 (m, 6H), 3.60 (m, 2H), 3.34 (m, 2H), 1.30 (s, 9H); LC-MS (ESI) m/z 561 $(M+H)^+$.

2-(4-Nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-6-ol (17b). Compound **17b** was synthesized from **16b** according to General Procedure A. (644 mg, 35%). ^{1}H NMR (DMSO-d_6) δ 10.3 (br, 1H), 9.02 (s, 1H), 8.31 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 8.7 Hz, 1H), 7.39 (d, J = 2.3 Hz, 1H), 6.92 (dd, J = 8.7 and 2.3 Hz, 1H).

6-(2-Morpholin-4-yl-ethoxy)-2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazole (18b). Compound **18b** was synthesized from **17b** according to General Procedure B. (688 mg, 79%). ^{1}H NMR (DMSO-d_6) δ 9.02 (s, 1H), 8.33 (d, J = 8.9 Hz, 2H), 8.07 (d, J = 8.9 Hz, 2H), 7.92 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 2.4 Hz, 1H), 7.08 (dd, J = 8.8 and 2.4 Hz, 1H), 4.22 (t, J = 5.7 Hz, 2H), 3.60 (t, J = 4.6 Hz, 4H), 3.33 (overlapping with solvent, 4H), 2.75 (t, J = 5.7 Hz, 2H).

2-(4-Aminophenyl)-6-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazole (19b). Compound **19b** was synthesized from **18b** according to

General Procedure C. (504 mg, 79%). ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.00 (dd, J = 8.8 and 2.4 Hz, 1H), 6.61 (d, J = 8.5 Hz, 2H), 5.20 (s, 2H), 4.20 (t, J = 5.7 Hz, 2H), 3.60 (t, J = 4.6 Hz, 4H), 3.33 (overlapping with solvent, 4H), 2.76 (t, J = 5.7 Hz, 2H).

***N*-(5-*tert*-Butylisoxazol-3-yl)-*N*′-{4-[6-(2-morpholin-4-yl-ethoxy)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (20b).** Compound **20b** was synthesized from **19b** according to General Procedure D and General Procedure E. (51 mg, 7%). ^{1}H NMR (DMSO-d_6) δ 11.1 (br, 1H), 9.69 (br, 1H), 9.28 (br, 1H), 8.71 (s, 1H), 7.97 (d, J = 8.9 Hz, 1H), 7.76-7.81 (m, 3H), 7.56 (d, J = 8.6 Hz, 2H), 7.13 (dd, J = 8.9 and 2.4 Hz, 1H), 6.53 (s, 1H), 4.56 (t, J = 4.0 Hz, 2H), 3.98 (m, 2H), 3.82 (m, 2H), 3.65 (m, 2H), 3.55 (m, 2H), 3.25 (m, 2H), 1.31 (s, 9H); LC-MS (ESI) *m/z* 561 $(M+H)^+$

Ethyl 2-(4-Nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-carboxylate (23a). General Procedure H. A mixture of ethyl 2-amino-1,3-benzothiazole-6-carboxylate (4.453 g, 20 mmol) and 2-bromo-4'-nitroacetophenone (5.369 g, 22 mmol) in 2-methoxy ethanol was stirred at 40°C for 24 hours, then the mixture was heated at 140 °C for 18 hours. After cooling to room temperature, a precipitate was formed. The precipitate was collected by filtration, washed with ethanol and ethyl ether, and dried under high vacuum to give ethyl 2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-carboxylate (**23a**) as a yellow solid (3.087 g, 52%). ^{1}H NMR (DMSO-d_6) δ 9.16 (s, 1H), 8.74 (d, J = 1.4 Hz,

1H), 8.33 (d, *J* = 10.4 Hz, 2H), 8.10-8.17 (m, 4H), 4.37 (q, *J* = 7.0 Hz, 2H), 1.36 (t, *J* = 7.0 Hz, 3H).

Ethyl 2-(4-Aminophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-carboxylate (24a) Compound **24a** was synthesized from **23a** according to General Procedure F. (1.518g, 75%). ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.52 (s, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 6.62 (d, *J* = 8.2 Hz, 2H), 5.24 (br, 2H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.36 (t, *J* = 7.1 Hz, 3H).

Ethyl 2-{4-[3-(5-*tert*-Butyl-isoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazole-7-carboxylate (25a). Compound **25a** was synthesized from **24a** according to General Procedure D. (2.245 g, 99%). ^{1}H NMR (DMSO-d_6) δ 9.60 (s, 1H), 9.03 (s, 1H), 8.78 (s, 1H), 8.71 (s, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 6.48 (s, 1H), 4.30 (q, *J* = 7.0 Hz, 2H), 1.32 (t, *J* = 7.0 Hz, 3H), 1.24 (s, 9H); LC-MS (ESI) *m/z* 504 $(M+H)^+$.

Sodium 2-{4-[3-(5-*tert*-Butyl-isoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazole-7-carboxylate (26a). General Procedure I. To a mixture of ethyl 2-{4-[3-(5-*tert*-butylisoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazole-7-carboxylate (**25a**) (1.79 g, 3.55 mmol) in THF (40 mL) and water (20 mL) was added LiOH·H_2O (755 mg, 18 mmol) and the mixture was stirred at room

temperature overnight. THF was evaporated. The remaining aqueous solution was neutralized with 10% HCl to give solid (1.636 g, 97%).

To the solid (150 mg) was added methanol (30 mL) followed by, NaOMe (1.2 equiv). After most of the methanol was evaporated, a solid was formed, which was collected by filtration, washed with methanol, and dried under high vacuum to give sodium 2-{4-[3-(5-*tert*-butylisoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazole-7-carboxylate (**26a**) as a solid (150 mg). ^{1}H NMR (DMSO-d_6) δ 8.67 (s, 1H), 8.47 (d, J = 1.2 Hz, 1H), 8.10 (dd, J = 8.3 and 1.2 Hz, 1H), 8.87 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 6.55 (s, 1H), 1.31 (s, 9H); LC-MS (ESI) *m/z* 476 $(M+H)^+$.

1-(5-*tert*-Butyl-isoxazol-3-yl)-3-{4-[7-(4-ethylpiperazine-1-carbonyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (27a). Compound **27a** was synthesized from **26a** according to General Procedure J and E. ^{1}H NMR (DMSO-d_6) δ 10.7 (br, 1H), 9.73 (s, 1H), 9.27 (s, 1H), 8.84 (s, 1H), 8.27 (s, 1H), 8.12 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.76 (d, J = 8.3 Hz, 1H), 7.64 (d, J = 8.5 Hz, 2H), 6.60 (s, 1H), 3.54 (m, 4H), 3.12-3.24 (m, 6H), 1.37 (s, 9H), 1.33 (t, J = 7.3 Hz, 3H); LC-MS (ESI) *m/z* 572 $(M+H)^+$.

Methyl 3-(2-Amino-1,3-benzothiazol-6-yl)propanoate (22b). To a solution of 3-(2-amino-1,3-benzothiazol-6-yl)propanoic acid (**21**) (13.42 g, 60.4 mmol) in methanol (150 mL) was added concentrated H_2SO_4 (2mL), and then the mixture was stirred at

room temperature overnight. After evaporation of most of the solvent, dichloromethane (200mL) was added and the mixture was neutralized with saturated $NaHCO_3$ solution. The separated aqueous phase was extracted with dichloromethane, and the combined organic phases were dried over $MgSO_4$ and concentrated to give methyl 3-(2-amino-1,3-benzothiazol-6-yl)propanoate (**22b**) as yellow solid (9.762 g, 68 %). ^{1}H NMR ($CDCl_3$) δ 7.47-7.43 (m, 2H), 7.15 (d, J = 8.2 Hz, 1H), 5.30 (br, 2H), 3.68 (s, 3H), 3.00 (t, J = 7.7 Hz, 2H), 2.17 (t, J = 7.7 Hz, 2H); LC-MS (ESI) *m/z* 237 $(M+H)^+$.

Methyl 3-[2-(4-Nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-yl]propanoate (23b) Compound **23b** was synthesized from **22b** according to General Procedure A. (6.015 g, 38 %). ^{1}H NMR (DMSO-d_6) δ 9.05 (s, 1H), 8.20 (d, J = 8.1 Hz, 2H), 8.11 (d, J = 8.1 Hz, 2H), 7.92 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 3.60 (s, 3H), 3.09 (t, J = 7.5 Hz, 2H), 2.68 (t, J = 7.5 Hz, 2H); LC-MS (ESI) *m/z* 382 $(M+H)^+$.

Ethyl 3-[2-(4-Aminophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-yl]propanoate (24b). Compound **24b** was synthesized from **23b** according to General Procedure F. (3.824 g, 66 %). ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1H), 7.61 (d, J = 8.5 Hz, 2H), 7.53 (s, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 8.5 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H), 3.73 (br, 2H), 3.06 (t, J = 7.6 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); LC-MS (ESI) *m/z* 366 $(M+H)^+$.

Ethyl 3-(2-{4-[3-(5-*tert*-Butyl-isoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazol-7-yl)propanoate (25b). Compound **25b** was synthesized from **24b** according to General Procedure D. (5.056 g, 91 %). ^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 8.89 (s, 1H), 8.13 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 1H), 7.69 (s, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.4 Hz, 1H), 6.53 (s, 1H), 4.13 (q, J = 7.1 Hz, 2H), 2.98 (t, J = 7.4 Hz, 2H), 2.70 (t, J = 7.4 Hz, 2H), 1.30 (s, 9H), 1.15 (t, J = 7.1 Hz, 3H); LC-MS (ESI) *m/z* 532 $(M+H)^+$.

Sodium 3-(2-{4-[3-(5-*tert*-Butyl-isoxazol-3-yl)ureido]phenyl}imidazo[2,1-*b*][1,3]benzothiazol-7-yl)propanoate (26b). Compound **26b** was synthesized from **25b** according to General Procedure I. (475 mg, 94%). ^{1}H NMR (DMSO-d_6) δ 12.2 (br, 1H), 11.2 (br, 1H), 8.58 (s, 1H), 8.51 (s, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.81 (s, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 6.49 (s, 1H), 2.9 (t, J = 6.0 Hz, 2H), 2.45 (t, J = 6.0 Hz, 2H), 1.29 (s, 9H).; LC-MS (ESI) *m/z* 504 $(M+H)^+$.

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-(4-{7-[3-(4-ethylpiperazin-1-yl)-3-oxo-propyl]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}phenyl)urea (27b). General Procedure J.** To a solution of **26b** (0.250 g, 0.5 mmol) in DMF (4 mL) at room temperature were added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.192 g, 1 mmol) and 1-hydroxybenzotriazole hydrate (0.135 g, 1 mmol) and the mixture was stirring for 1 hour, 1-ethylpiperazine (4 eq.) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with 40 mL of ice-water, and the precipitate was collected by filtration, washed with water and ethyl ether, and dried

under vacuum over P_2O_5 to afford the free base as a solid, which was converted to the corresponding HCl salt **27b** (232 mg, 73%) following the General Procedure E. ^{1}H NMR (DMSO-d_6) δ 10.9 (br, 1H), 9.69 (s, 1H), 9.41 (s, 1H), 8.71 (s, 1H), 7.89 (s, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.5 Hz, 1H), 6.47 (s, 1H), 4.40 (m, 1H), 4.0 (m, 1H), 3.30-3.41 (m, 4H), 3.03 (t, J = 5.6 Hz, 2H), 2.87-2.92 (m, 3H), 2.69-2.74 (m, 3H), 1.24 (s, 9H), 1.17 (t, J = 7.2 Hz, 3H); LC-MS (ESI) m/z 600 $(M+H)^+$.

***N*-(5-*tert*-Butylisoxazol-3-yl)-*N*′-{4-[7-(3-morpholino-4-yl-3-oxo-propyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea (27c).** Compound **27c** was synthesized from **26b** according to General Procedure J. (402 mg, 89%). ^{1}H NMR (DMSO-d_6) δ 9.56 (s, 1H), 8.90 (s, 1H), 8.66 (s, 1H), 7.89 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.5 Hz, 1H), 6.52 (s, 1H), 3.52 (m, 4H), 3.50 (m, 4H), 2.94 (t, J = 5.5 Hz, 2H), 2.70 (t, J = 5.5 Hz, 2H), 1.30 (s, 9H); LC-MS (ESI) m/z 573 $(M+H)^+$.

***N*-(5-*tert*-Butylisoxazol-3-yl)-*N*′-{4-[7-(3-morphlino-4-yl-propyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (28).** To a suspension of *N*-(5-*tert*-butyl-isoxazol-3-yl)-*N*′-{4-[7-(3-morpholino-4-yl-3-oxo-propyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea (**27c**) (380 mg, 0.66 mmol) in THF (10 mL) at room temperature was added 2.0 M solution of BH_3/Me_2S in THF (1.5 mL, 3 mmol). The mixture was heated at reflux overnight, then the reaction was quenched by dropwise

addition of 10% HCl. The mixture was basified with saturated $NaHCO_3$ and extracted with dichloromethane. The combined organic extracts were dried over $MgSO_4$ and concentrated. To the residue was added toluene (10 mL) and compound **5** (150 mg) and the mixture was heated at 110 ºC overnight. The reaction was diluted with CH_2Cl_2 and quenched with saturated $NaCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography on silica gel eluting with a 0-10% MeOH/CH_2Cl_2 gradient to give the product as a free base. The free base was converted to *N*-(5-*tert*-butylisoxazol-3-yl)-*N'*-{4-[7-(3-morphlino-4-yl-propyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea hydrochloride (**28**) as a solid (55 mg, 14%), according to General Procedure E. ^{1}H NMR (DMSO-d_6) δ 10.4 (br, 1H), 9.64 (br, 1H), 9.1 (br, 1H), 8.70 (s, 1H), 7.91-7.93 (m, 2H), 7.79 (d, J = 7.4 Hz, 2H), 7.54 (d, J = 7.4 Hz, 2H), 7.45 (d, J = 8.2 Hz, 1H), 6.53 (s, 1H), 3.93 (m, 2H), 3.73 (m, 4H), 3.09 (m, 4H), 2.78 (t, J = 6.5 Hz, 2H), 2.1 (m, 2H), 1.30 (s, 9H); LC-MS (ESI) *m/z* 559 $(M+H)^+$.

2-(4-Nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazol-7-carboxylic acid (30). Compound **30** was synthesized from 2-aminobenzo[d]thiazole-6-carboxylic acid (**29**) according to General Procedure H. (4.015 g, 29%). ^{1}H NMR (DMSO-d_6) δ 13.25 (br, 1H), 9.14 (s, 1H), 8.69 (d, J = 1.4 Hz, 1H), 8.33 (d, J = 8.5 Hz, J = 8.9 Hz, 2H), 8.07-8.17 (m, 4H).

7-[(4-Ethylpiperazin-1-yl)carbonyl]-2-(4-nitrophenyl)imidazo[2,1-*b*][1,3]benzothiazole (31). Compound **31** was synthesized from **30** according to General Procedure J. (984 mg, 75%). ^{1}H NMR (DMSO-d_6) δ 9.12 (s, 1H), 8.33 (d, J = 8.9 Hz, 2H), 8.17 (d, J = 1.4 Hz, 1H), 8.13 (d, J = 8.9 Hz, 2H), 8.06 (d, J = 8.3 Hz, 1H), 8.62 (dd, J = 8.3 and 1.4 Hz, 1H), 3.6 (br, 2H), 3.35 (overlapping with solvent, 2H), 2.3-2.4 (m, 6H), 1.01 (t, J = 7.1 Hz, 3H).

4-{7-[4-(Ethylpiperazin-1-yl)carbonyl]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}aniline (32) Compound **32** was synthesized from **31** according to General Procedure F. (511 mg, 56%). ^{1}H NMR (DMSO-d_6) δ 8.48 (s, 1H), 8.10 (s, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.52-7.57 (m, 3H), 6.61 (d, J = 8.3 Hz, 2H), 5.22 (br, 2H), 3.6 (br, 2H), 3.35 (overlapping with solvent, 2H), 2.33-2.40 (m, 6H), 1.01 (t, J = 7.1 Hz, 3H).

4-{7-[(4-Ethylpiperazin-1-yl)methyl]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}aniline (33). To a solution of 4-{7-[4-(ethylpiperazin-1-yl)carbonyl]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}aniline (**32**) (360 mg, 0.89 mmol) in THF (10 mL) at room temperature was added dropwise a 2.0 M solution of borane dimethyl sulfide complex in THF (2 mL, 4 mmol) and the reaction mixture was heated to reflux for 4 hours. To the mixture was then 10% HCl (15 mL) was added dropwise and was stirred at room temperature for 20 minutes. The reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with dichloromethane. The combined extracts were dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography eluting with

MeOH/dichloromethane to give 4-{7-[(4-ethylpiperazin-1-yl)methyl]imidazo[2,1-*b*][1,3]benzothiazol-2-yl}aniline (**33**) as a solid (246 mg, 71%). ^{1}H NMR ($CDCl_3$) δ 7.81 (s, 1H), 7.63-7.71 (m, 3H), 7.52 (d, J = 8.2 Hz, 1H), 7.36 (dd, J = 8.2 and 1.2 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 5.30 (br, 2H), 3.67 (s, 2H), 3.05 (m, 2H), 2.88 (m, 4H), 2.74 (m, 2H), 2.54 (m, 2H), 1.31 (t, J = 7.2 Hz, 3H).

***N*-(5-*tert*-Butyl-isoxazol-3-yl)-*N'*-{4-[7-(4-ethylpiperazin-1-ylmethyl)imidazo[2,1-*b*][1,3]benzothiazol-2-yl]phenyl}urea Hydrochloride (34).** Compound **34** was synthesized from **33** according to General Procedure D and General Procedure E. (63 mg, 17%). ^{1}H NMR (DMSO-d_6) δ 11.5 (br, 1H), 9.71 (s, 1H), 9.32 (s, 1H), 8.77 (s, 1H), 8.25 (s, 1H), 8.06 (d, J = 8.5 Hz, 1H), 7.82 (m, 3H), 7.56 (d, J = 8.6 Hz, 2H), 6.53 (s, 1H), 4.39 (s, 2H), 3.65 (m, 4H), 3.38 (m, 4H), 3.15 (m, 2H), 1.30 (s, 9H), 1.22 (t, 3H); LC-MS (ESI) *m/z* 558 $(M+H)^+$.