Supporting Information

Synthesis, Structure Activity Relationship and Mechanistic Studies of Aminoquinazolinones Displaying Antimycobacterial Activity

Jessica N. Akester[a], Paul Njaria[b], Aloysius Nchinda[a], Claire Le Manach[a], Alissa Myrick[a,c,†], Vinayak Singh[a,c,h], Nina Lawrence[d], Mathew Njoroge[d], Dale Taylor[d], Atica Moosa[e], Anthony J. Smith[f], Elizabeth J. Brooks[f], Anne J. Lenaerts[f], Gregory T. Robertson[f], Thomas R. Ioerger[g], Rudolf Mueller[a] and Kelly Chibale [a,b,c,h]*

[a] Drug Discovery and Development Centre (H3D), Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[b] Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[c] Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

[d] H3D, Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Observatory, 7925, South Africa

[e] MRC/NHLS/UCT Molecular Mycobacteriology Research Unit, Department of Pathology, University of Cape Town, Rondebosch 7701, South Africa

[f]Mycobacteria Research Laboratories, Department of Microbiology, Immunology, and Pathology, Colorado State University, 200 West Lake Street, Fort Collins, Colorado 80523, United States

[g] Department of Computer Science, Texas A&M University, College Station, Texas 77843-3112, United States

[h] South African Medical Research Council Drug Discovery and Development Research Unit, Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[†]*Deceased. This paper is dedicated to Alissa Myrick.*

*Corresponding author: Kelly.chibale@uct.ac.za

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1. Synthesis and characterization of all intermediates and final compounds

General Methods. All reagents and solvents were obtained from commercial sources and used as received. All dry solvents were obtained from an SP-1 standalone solvent purification system from LC Technology Solutions Inc. Reactions were monitored by thin layer chromatography (TLC) using Merck TLC silica gel 60 F_{254} aluminium-backed precoated plates and were visualized by ultraviolet light at 254 nm. Purification of compounds was carried out by either column chromatography on silica gel 60 (Fluka), particle size 0.063−0.2 mm (70−230 mesh ASTM), as the stationary phase, or by Waters' HPLC using X-bridge C18 5µm column (4.6 x 150 mm); organic phase: 10 mM Ammonium acetate (pH 3.7) in HPLC grade methanol, aqueous phase: 10 mM Ammonium acetate (pH 3.7) in HPLC grade water; flow rate = 15.00 mL/min; detector: photodiode array (PDA). All target compounds and intermediates were characterised by ^{1}HNMR, ^{13}CNMR and MS. NMR spectra were recorded on either a Varian Mercury-300 (^{1}H 300.1 MHz, ^{13}C 75.5 MHz) or Bruker-400 (^{1}H 400.2 MHz, ^{13}C 100.6 MHz) instrument using $CDCl_3$, MeOH-d_4 and DMSO-d_6 as solvents. The ^{1}H NMR data are reported as follows: chemical shift in parts per million (δ) downfield of tetramethylsilane (TMS), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd =doublets of doublets, dt = triplets of doublets and m = multiplet), coupling constant (Hz), and integrated value. The ^{13}C NMR spectra were measured with complete proton decoupling. LC–MS/MS analysis was performed using an Agilent® 1260 Infinity Binary Pump, Agilent® 1260 Infinity Diode Array Detector (DAD), Agilent® 1290 Infinity Column Compartment, Agilent® 1260 Infinity Standard Autosampler, and a Agilent® 6120 Quadrupole (Single) MS, with APCI/ESI multimode ionisation source. The purities were determined by Agilent® LCMS/MS or Waters' HPLC using X-bridge C18 5µm column (4.6 x 150 mm); organic phase: 10 mM Ammonium acetate (pH 3.7) in HPLC grade methanol, aqueous phase: 10 mM Ammonium acetate (pH 3.7)

in HPLC grade water; flow rate = 1.20 mL/min; detector: photodiode array (PDA). The purities of all compounds were found to be > 95 %.

Scheme 2

General Procedure for the Synthesis of Intermediates 3a-d

A solution of 2-amino-5-iodobenzoic acid (1 equiv) or 2-amino-4-bromobenzoic acid (1 equiv) and the appropriate aryl isothiocyanate (1 equiv) in anhydrous 1,4-dioxane (4 mL/mmol) was treated with triethylamine (1.5 equiv). The solution was heated under refluxed for 4 hours, cooled to room temperature, and the solids that formed were filtered. The solids were resuspended in diethyl ether (Et_2O) and filtered again to afford the desired 2-thioxo-quinazolinone-based intermediates Aa-e.

6-Iodo-2-thioxo-3-(4-(trifluoromethyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one ***(3a).*** White solid (7.84 g, 91%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.20 (d, *J* = 1.9 Hz, 1H), 8.10 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.14, 159.02, 144. 21, 143.32, 139.64, 135.69, 130.72 (2C), 126.60 (2C), 126.56, 118.77, 118.54, 88.18, 66.84. LC-MS (ESI): found *m/z* = 448.9 $[M+H]^+$, (calcd for $C_{15}H_8F_3IN_2OS$: 447.0); HPLC purity 99%.

6-Iodo-2-thioxo-3-(3-(trifluoromethyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one ***(3b).*** Yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (br s, 1H), 8.20 (d, J = 2.0 Hz, 1H), 8.10 (dd, J = 8.6, 2.0 Hz, 1H), 7.83–7.70 (m, 3H), 7.64 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H). LC-MS (ESI): found *m/z* = 448.9 $[M+H]^+$, (calcd for $C_{15}H_8F_3IN_2OS$: 447.9); HPLC purity 99%.

6-Iodo-3-phenyl-2-thioxo-2,3-dihydroquinazolin-4(1H)-one ***(3c).*** Yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (br s, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.08 (dd, J = 8.6, 2.0 Hz, 1H), 7.51–7.45 (m, 2H), 7.44–7.39 (m, 1H), 7.30–7.23 (m, 3H). LC-MS (ESI): found *m/z* = 380.9 $[M+H]^+$, (calcd for $C_{14}H_9IN_2OS$: 379.9); HPLC purity 99%.

6-Iodo-2-thioxo-3-(p-tolyl)-2,3-dihydroquinazolin-4(1H)-one ***(3d).*** Yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (br s, 1H), 8.18 (d, J = 2.0 Hz, 1H), 8.07 (dd, J = 8.6, 2.0 Hz, 1H), 7.28 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.6 Hz, 1H), 7.13 (d, J = 8.2 Hz, 2H), 2.38 (s, 3H). LC-MS (ESI): found *m/z* = 395.0 $[M+H]^+$, (calcd for $C_{15}H_{11}IN_2OS$: 394.0); HPLC purity 99%.

7-bromo-2-thioxo-3-(4-(trifluoromethyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one ***(3e).*** A solution of 2-amino-4-bromobenzoic acid (2.5 g, 11.57 mmol, 1 equiv) and 1-isothiocyanato-4-(trifluoromethyl)benzene (2.351 g, 11.57 mmol, 1 equiv) in Dioxane (0.3M) was added triethylamine (2.419 ml, 17.36 mmol, 1.5 equiv) and the resulting reaction mixture heated under reflux at a temperature of 115˚C for 6 hours. Once complete, the reaction mixture was cooled to room temperature, concentrated in vacuo, resuspended in Et2O and the resulting precipitate was filtered to afford intermediate Ae (4g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.87 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 1.6 Hz, 1H), 7.19 (dd, J = 8.5, 1.8 Hz, 3H). LC-MS (ESI): found *m/z* = 400.0, 402.0 (1:1) $[M-H]^+$, (calcd for $C_{15}H_8F_3BrN_2OS$: 399.9); HPLC purity 97%.

General Procedure for the Synthesis of Intermediate 4

PCl_5 (1.75 equiv) was added in one portion to a mixture of intermediate **3** (1 equiv) in $POCl_3$ (25 equiv) at room temperature. The mixture was stirred for 15 minutes at room temperature and was subsequently heated to 110°C for 14 hours under nitrogen. The reaction was cooled to room temperature, concentrated *in vacuo* and then diluted with ethyl acetate to be added portionwise to stirred ice – saturated sodium bicarbonate and stirred until all solids dissolved. The organic layer was then separated, washed with brine, dried over magnesium sulphate (Mg_2SO_4) and concentrated under reduced pressure. The resulting residue was triturated in Et_2O and filtered to obtain **4a-e.**

2-Chloro-6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(4a).*** Off-white solid (6.26 g, 79%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, *J* = 1.8 Hz, 1H), 8.22 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 150.20, 143.82, 139.91, 135.86, 135.42, 130.71 (2C), 130.44, 129.22, 127.13 (2C), 126.41, 118.23, 116.99, 85.69. LC-MS (ESI): found *m/z* = 450.9 $[M+H]^+$, (calcd for $C_{15}H_7ClF_3IN_2O$: 449.9); HPLC purity 90%.

2-Chloro-6-iodo-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(4b).*** Yield 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, J = 2.1 Hz, 1H), 8.22 (dd, J = 8.5, 2.1 Hz, 1H), 8.06 (s, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.91–7.80 (m, 2H), 7.52 (d, J = 8.5 Hz, 1H). LC-MS (ESI): found *m/z* = 450.9 $[M+H]^+$, (calcd for $C_{15}H_7ClF_3IN_2O$: 449.9); HPLC purity 98%.

2-Chloro-6-iodo-3-phenylquinazolin-4(3H)-one ***(4c).*** Yield 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 2.1 Hz, 1H), 7.99 (dd, J = 8.6, 2.1 Hz, 1H), 7.50–7.46 (m, 2H), 7.46–7.40 (m, 1H), 7.35–7.29 (m, 2H), 7.07 (d, J = 8.6 Hz, 1H). LC-MS (ESI): found *m/z* = 382.9 $[M+H]^+$, (calcd for $C_{14}H_8ClIN_2O$: 381.9); HPLC purity 97%.

2-Chloro-6-iodo-3-(p-tolyl)quinazolin-4(3H)-one ***(4d)***. Yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 2.1 Hz, 1H), 8.19 (dd, J = 8.5, 2.1 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.36 (s, 4H), 2.41 (s, 3H). LC-MS (ESI): found *m/z* = 397.0 $[M+H]^+$, (calcd for $C_{15}H_{10}ClIN_2O$: 396.0); HPLC purity 98%.

2-Chloro-7-bromo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(4e)***. Yield 66%. ^{1}H NMR (300 MHz, CDCl3) δ 8.16 – 8.11 (m, 1H), 7.93 – 7.91 (m, 1H), 7.86 (dd, J = 8.9, 0.6 Hz, 2H), 7.69 (dd, J = 8.5, 1.9 Hz, 1H), 7.47 (dd, J = 8.9, 0.7 Hz, 2H). LC-MS (ESI): found *m/z* = 401.0 $[M-H]^+$, (calcd for $C_{15}H_7BrClF_3N_2O$: 401.9); HPLC purity 97%.

General Procedure for the Synthesis of Intermediate 5

4-Methoxybenzylamine (1.3 equiv) and *N,N*-diisopropylethylamine (DIPEA) (2 equiv) were dissolved in DMF (0.5M) and the appropriate intermediate **4a-e** (1.0 equiv) was added portionwise. The reaction was stirred for 4 hours at 80°C and allowed to cool to room temperature. The solvent was concentrated under reduced pressure and diluted with EtOAc. The organic phase was washed with 5% lithium chloride (3 x 20mL), brine (20mL), dried over $Mg2SO_4$ and concentrated *in* vacuo to afford the desired intermediate **5a-e**.

6-Iodo-2-((4-methoxybenzyl)amino)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(5a).*** Yield 83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15 (d, *J* = 2.0 Hz, 1H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.87 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.68 (t, *J* = 5.9 Hz, 1H), 4.44 (d, *J* = 5.9 Hz, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.00, 158.55, 150.59, 149.58, 143.08, 139.05, 134.95, 132.01, 130.97 (2C), 130.70, 130.14 (q, *J* = 32.0 Hz, 1C, $\underline{C}$-CF_3), 128.88 (2C), 124.7 (q, *J* = 272.5 Hz, 1C, $\underline{C}F_3$), 127.75 (2C), 127.60, 119.49, 114.00 (2C), 84.74, 44.18. LC-MS (ESI): found *m/z* = 552.0 $[M+H]^+$, (calcd for $C_{23}H_{17}F_3IN_3O_2$: 551.0); HPLC purity 96%.

6-Iodo-2-((4-methoxybenzyl)amino)-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(5b)***. Yield 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 2.1 Hz, 1H), 7.95–7.79 (m, 4H), 7.74 (d, J = 8.7 Hz, 1H), 7.25 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 8.6 Hz, 1H), 6.85 (d, J = 8.7 Hz, 2H), 6.69 (t, J = 5.8 Hz, 1H), 4.49 (d, J = 5.8 Hz, 2H), 3.71 (s, 3H). LC-MS (ESI): found *m/z* = 552.0 $[M+H]^+$, (calcd for $C_{23}H_{17}F_3IN_3O_2$: 551.0); HPLC purity 98%.

6-Iodo-2-((4-methoxybenzyl)amino)-3-phenylquinazolin-4(3H)-one ***(5c)***. Yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 2.2 Hz, 1H), 7.86 (dd, J = 8.6, 2.2 Hz, 1H), 7.64–7.58 (m, 2H), 7.58–7.53 (m, 1H), 7.41–7.36 (m, 2H), 7.23 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 8.6 Hz, 1H),

6.84 (d, J = 8.8 Hz, 2H), 6.41 (t, J = 5.9 Hz, 1H), 4.44 (d, J = 5.9 Hz, 2H), 3.71 (s, 3H). LC-MS (ESI): found *m/z* = 484.0 $[M+H]^+$, (calcd for $C_{22}H_{18}IN_3O_2$: 483.0); HPLC purity 98%.

6-Iodo-2-((4-methoxybenzyl)amino)-3-(p-tolyl)quinazolin-4(3H)-one ***(5d)***. Yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 2.2 Hz, 1H), 7.85 (dd, J = 8.6, 2.2 Hz, 1H), 7.40 (d, J = 8.6 Hz, 2H), 7.27–7.21 (m, 4H), 7.10 (d, J = 8.6 Hz, 1H), 6.84 (d, J = 8.6 Hz, 2H), 6.40 (t, J = 5.9 Hz, 1H), 4.43 (d, J = 5.9 Hz, 2H), 3.71 (s, 3H), 2.42 (s, 3H). LC-MS (ESI): found *m/z* = 498.1 $[M+H]^+$, (calcd for $C_{23}H_{20}IN_3O_2$: 497.1); HPLC purity 99%.

7-Bromo-2-((4-methoxybenzyl)amino)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(5e)***. Yield 75%. LC-MS (ESI): found *m/z* = 503.0, 505.0 (1:1) $[M+H]^+$, (calcd for $C_{23}H_{17}BrF_3N_3O_2$: 503.0); HPLC purity 93%.

General Procedure for the Synthesis of Intermediate 6

Intermediate **5a-e** (1.0 equiv) was dissolved in TFA (64 equiv) and refluxed at 80°C for 48 hours. The reaction was cooled to room temperature and the solvent was concentrated. The residue was resuspended in DCM and added portionwise to a stirred ice – saturated sodium bicarbonate solution. The organic phase was separated, the aqueous phase was extracted with DCM and the combined organic phases were diluted with Et_2O to a 1:1 ratio DCM/Et_2O. The solids were filtered, washed with Et_2O and dried under vacuum to afford the aminoquinazolinone intermediates **6a-e.**

2-Amino-6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(6a)***. Yield 87%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (d, *J* = 2.0 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 2H), 7.87 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 8.7 Hz, 1H), 6.60 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.06, 152.21, 150.20, 143.04, 139.51, 135.01, 130.59 (2C), 130.03,

127.59, 127.55 (2C), 126.93, 119.24, 84.35. LC-MS (ESI): found *m/z* = 432.0 $[M+H]^+$, (calcd for $C_{15}H_9F_3IN_3O$: 431.0); HPLC purity 98%.

2-Amino-6-iodo-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(6b)***. Yield 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 2.1 Hz, 1H), 7.91–7.83 (m, 3H), 7.80 (t, J = 7.9 Hz, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.07 (d, J = 8.7 Hz, 1H), 6.59 (br s, 2H). LC-MS (ESI): found *m/z* = 432.0 $[M+H]^+$, (calcd for $C_{15}H_9IN_3O$: 431.0); HPLC purity 98%.

2-Amino-6-iodo-3-phenylquinazolin-4(3H)-one ***(6c)***. Yield 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 2.2 Hz, 1H), 7.86 (dd, J = 8.7, 2.2 Hz, 1H), 7.62–7.55 (m, 2H), 7.55–7.49 (m, 1H), 7.41–7.32 (m, 2H), 7.07 (d, J = 8.7 Hz, 1H), 6.39 (s, 2H). LC-MS (ESI): found *m/z* = 364.0 $[M+H]^+$, (calcd for $C_{14}H_{10}IN_3O$: 363.0); HPLC purity 97%.

2-Amino-6-iodo-3-(p-tolyl)quinazolin-4(3H)-one ***(6d)***. Yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 2.2 Hz, 1H), 7.84 (dd, J = 8.7, 2.2 Hz, 1H), 7.37 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.7 Hz, 1H), 6.37 (br s, 2H), 2.40 (s, 3H). LC-MS (ESI): found *m/z* = 378.0 $[M+H]^+$, (calcd for $C_{15}H_{12}IN_3O$: 377.0); HPLC purity 99%.

2-Amino-7-bromo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one ***(6e)***. Yield 80%. LC-MS (ESI): found *m/z* = 385.0, 387.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_9BrF_3N_3O$: 382.99); HPLC purity 97%.

General Protocol for the Suzuki-Coupling Reactions 1, 2, 7 – 36

To a solution of the relevant intermediate **6a-e** (1 equiv) and the appropriate arylboronic acid or ester (1.5 equiv) in anhydrous 1,4-dioxane (2mL) and flushed with nitrogen. Bis(triphenylphosphine) palladium(II) dichloride, $PdCl_2(PPh_3)_2$, (0.1 equiv) was added to the solution, cesium carbonate or potassium carbonate (3 equiv) was dissolved in water (0.5mL) and subsequently added to the reaction mixture. The solution was heated at 80°C until TLC

monitoring showed completion (1-5 hours), allowed to cool to room temperature and diluted with EtOAc to be filtered through celite. The organic phase was washed with water (2 x 20mL) and brine (20mL), dried over Mg_2SO_4 and concentrated under reduced pressure. The resulting crude residue was purified by silica-gel flash chromatography eluting a gradient of either 0 – 100% EtOAc in hexane or 0 – 10% MeOH in DCM. Combined pure fractions were concentrated *in vacuo*, triturated with Et_2O, filtered and dried under vacuum to give the relevant target compounds.

2-Amino-6-(3-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(1).** Yield 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 2.1 Hz, 1H), 8.18 (t, J= 1.7 Hz, 1H), 8.11 (ddd, J = 7.8, 1.8, 1.1 Hz, 1H), 8.09 (dd, J = 8.6, 2.4 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.88 (ddd, J = 7.8, 1.8, 1.0 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.4 Hz, 1H), 6.57 (br s, 2H), 3.29 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.24, 152.18, 150.88, 142.21, 140.96, 139.69, 133.66, 131.86, 131.66, 130.64 (2C), 130.17 (q, J = 32.0 Hz, 1C, C-CF_3), 127.61, 127.58 (2C), 125.83, 125.41, 124.96, 124.87, 124.54 (q, J = 272.5 Hz, 1C, CF_3), 117.40, 43.95. LC-MS (ESI): found *m/z* = 460.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_3S$: 459.09); HPLC purity 98%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(2)**. Yield 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 2.2 Hz, 1H), 8.03 (dd, J = 8.6, 2.2 Hz, 1H), 7.98 (t, J = 2.2 Hz, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.86 (dt, J = 6.6, 2.2 Hz, 1H), 7.70–7.62 (m, 4H), 7.37 (d, J = 8.6 Hz, 1H), 6.55 (br s, 2H), 2.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.28, 152.08, 150.68, 147.89, 140.82, 139.73, 133.64, 132.55, 130.66 (2C), 130.45, 130.14 (q, J = 32.0 Hz, 1C, C-CF3), 128.86, 127.61, 127.57, 125.34, 124.75, 124.7 (q, J = 272.48 Hz, 1C, CF3), 122.64, 121.49, 117.37, 43.74. LC-MS (ESI): found *m/z* = 444.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_2S$: 443.1); HPLC purity 99%.

2-Amino-6-(2-methyl-5-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(7)**. Yield 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, J = 8.2 Hz, 2H), 7.88 (d, J = 2.3 Hz, 1H), 7.83 (dd, J = 8.0, 2.1 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.70 (dd, J = 8.5, 2.3 Hz, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 6.56 (s, 2H), 3.25 (s, 3H), 2.37 (s, 3H). LC-MS (ESI): found *m/z* = 474.0 $[M+H]^+$, (calcd for $C_{23}H_{18}F_3N_3O_3S$: 473.10); HPLC purity 97%.

2-Amino-6-(3-(cyclopropylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(8).** Yield 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 2.3 Hz, 1H), 8.14 (t, J = 1.7 Hz, 1H), 8.10 – 8.06 (m, 1H), 8.05 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.87 (dt, J = 7.8, 1.1 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.6 Hz, 1H), 6.60 (s, 2H), 3.01 (m, 1H), 1.21 – 1.15 (m, 2H), 1.10 – 1.02 (m, 2H). LC-MS (ESI): found *m/z* = 486.1 $[M+H]^+$, (calcd for $C_{24}H_{18}F_3N_3O_3S$, 485.10); HPLC purity 98%.

3-(2-Amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)benzamide **(9)**. Yield 25%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (d, J = 2.2 Hz, 1H), 8.20 (s, 1H), 8.16 (s, 2H), 8.03 (dd, J = 8.6, 2.3 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.86 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.55 (t, J = 6.5 Hz, 1H), 7.36 (d, J = 4.3 Hz, 1H), 6.55 (s, 2H). LC-MS (ESI): found *m/z* = 425.1 $[M+H]^+$, (calcd for $C_{22}H_{15}F_3N_4O_2$, 424.11); HPLC purity 98%.

3-(2-Amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N,N-dimethylbenzamide **(10)**. Yield 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (d, J = 2.3 Hz, 1H), 8.00 (dd, J = 8.6, 2.4 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.77 (ddd, J = 7.9, 1.9, 1.1 Hz, 1H), 7.68 (t, J = 2.0 Hz, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.54 (t, J = 7.7 Hz, 1H), 7.37 (dt, J = 7.6, 1.2 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 6.54 (s, 2H), 3.01 (s, 3H), 2.97 (s, 3H). LC-MS (ESI): found *m/z* = 453.1 $[M+H]^+$, (calcd for $C_{24}H_{19}F_3N_4O_2$: 452.15); HPLC purity 96%.

3-(2-Amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N-methyl benzamide **(11).** Yield 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (q, J = 3.9 Hz, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.16 (t, J = 1.8 Hz, 1H), 8.02 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.2 Hz, 2H), 7.84 (m, 1H), 7.82 (m, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.56 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.55 (s, 2H), 2.83 (d, J = 4.5 Hz, 3H). LC-MS (ESI): found *m/z* = 439.1 $[M+H]^+$, (calcd for $C_{23}H_{17}F_3N_4O_2$, 438.13); HPLC purity 99%.

5-(2-Amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N-methyl nicotinamide **(12).** Yield 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, J = 2.2 Hz, 1H), 8.96 (d, J = 2.0 Hz, 1H), 8.76 (q, J = 5.8 Hz, 1H), 8.47 (t, J = 2.2 Hz, 1H), 8.28 (d, J = 2.3 Hz, 1H), 8.08 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.71 – 7.65 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.6 Hz, 1H), 6.61 (s, 2H), 2.85 (d, J = 4.5 Hz, 3H). LC-MS (ESI): found *m/z* = 440.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_5O_2$, 439.13); HPLC purity 98%.

3-(2-amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N-(2-(dimethylamino)ethyl)benzamide **(13)**. Yield 73%. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.75 (t, J = 5.4 Hz, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.17 (t, J = 2.3 Hz, 1H), 8.02 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.91 – 7.82 (m, 2H), 7.67 (d, J = 8.2 Hz, 2H), 7.60 (t, J = 7.7 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 6.55 (s, 2H), 3.56 (dt, J = 13.9, 6.0 Hz, 2H), 3.05 (m, 2H), 2.68 (s, 6H). LC-MS (ESI): found *m/z* = 496.2 $[M+H]^+$, (calcd for $C_{26}H_{24}F_3N_5O_2$: 495.1); HPLC purity 98%.

3-(2-amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N-(2-hydroxyethyl)benzamide **(14)**. Yield 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (t, J = 5.5 Hz, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.18 (t, J = 1.7 Hz, 1H), 8.03 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.84 (ddd, J = 7.9, 2.9, 1.7 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.56 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.54 (s, 2H), 4.72 (t, J = 5.6 Hz, 1H), 3.55 (q, J = 6.0 Hz,

2H), 3.37 (q, J = 6.0 Hz, 2H). LC-MS (ESI): found *m/z* = 469.1 $[M+H]^+$, (calcd for $C_{24}H_{19}F_3N_4O_3$: 468.1); HPLC purity 98%.

3-(2-amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)-N-(2-morpholinoethyl)benzamide **(15)**. Yield 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.22 (d, J = 2.3 Hz, 1H), 8.15 (s, 1H), 8.03 (dd, J = 8.6, 2.3 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 7.84 (dd, J = 13.4, 7.8 Hz, 2H), 7.67 (d, J = 8.2 Hz, 2H), 7.57 (t, J = 7.7 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 6.55 (s, 2H), 3.69 – 3.55 (m, 8H), 3.46 (d, J = 7.2 Hz, 2H), 3.15 (d, J = 7.3 Hz, 2H). LC-MS (ESI): found *m/z* = 538.2 $[M+H]^+$, (calcd for $C_{28}H_{26}F_3N_5O_3$: 537.2); HPLC purity 98%.

2-Amino-6-(3-(morpholine-4-carbonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(16)**. Yield 87%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (d, *J* = 2.2 Hz, 1H), 8.00 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 2H), 7.79 (dt, *J* = 7.8, 1.1 Hz, 1H), 7.70 (t, *J* = 1.5 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.38 (dt, *J* = 6.4, 1.3 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 6.56 (s, 2H), 3.62 (m, 4H), 3.47 (m, 4H). LC-MS (ESI): found *m/z* = 495.1 $[M+H]^+$, (calcd for $C_{26}H_{21}F_3N_4O_3$: 494.16); HPLC purity 98%.

2-Amino-6-(5-(morpholine-4-carbonyl)pyridin-3-yl)-3-(4-(trifluoromethyl)phenyl) quinazolin-4(3H)-one **(17).** Yield 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, *J* = 2.3 Hz, 1H), 8.59 (d, *J* = 2.0 Hz, 1H), 8.23 (d, *J* = 2.3 Hz, 1H), 8.14 (t, *J* = 2.1 Hz, 1H), 8.06 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.39 (d, *J* = 8.6 Hz, 1H), 6.61 (s, 2H), 3.65 (m, 4H), 3.43 (m, 4H). LC-MS (ESI): found *m/z* = 496.1 $[M+H]^+$, (calcd for $C_{25}H_{20}F_3N_5O_3$: 495.15); HPLC purity 96%.

2-Amino-6-(3-(thiomorpholine-4-carbonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(18)**. Yield 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 1.9 Hz, 1H), 8.01 (dd, J = 8.6, 2.4 Hz, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.78 (dt, J = 7.9, 1.5 Hz, 1H), 7.70 (t, J = 1.9

Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.55 (t, J = 7.8 Hz, 1H), 7.37 (d, J = 8.9 Hz, 1H), 7.36 (dt, J = 7.6, 1.3 Hz, 1H), 6.55 (s, 2H), 3.74 (m, 4H), 2.67 (m, 4H). LC-MS (ESI): found *m/z* = 511.1 $[M+H]^+$, (calcd for $C_{26}H_{21}F_3N_4O_2S$: 510.13); HPLC purity 97%.

2-Amino-6-(3-(4-methylpiperazine-1-carbonyl)phenyl)-3-(4-(trifluoromethyl)phenyl) quinazolin -4(3H)-one **(19)**. Yield 32%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15 (d, J = 2.3 Hz, 1H), 8.00 (dd, J = 8.6, 2.4 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.78 (dt, J = 8.0, 1.2 Hz, 1H), 7.63 – 7.70 (m, 3H), 7.54 (t, J = 7.7 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.35 (dt, J = 7.6, 1.3 Hz, 1H), 6.56 (s, 2H), 3.51 (m, 4H), 2.34 (m, 4H), 2.20 (s, 3H). LC-MS (ESI): found *m/z* = 508.2 $[M+H]^+$, (calcd for $C_{27}H_{24}F_3N_5O_2$: 507.19); HPLC purity 99%.

3-(2-amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-6-yl)benzoic acid **(20)**. Yield 11%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01(br s, 1H), 8.22 (s, 1H), 8.17 (d, J = 2.2 Hz, 1H), 8.01 (dd, J = 8.6, 2.3 Hz, 1H), 7.94 (t, J = 8.5 Hz, 4H), 7.67 (d, J = 8.2 Hz, 2H), 7.59 (t, J = 7.8 Hz, 2H), 7.38 (d, J = 8.6 Hz, 1H), 6.55 (s, 2H). LC-MS (ESI): found *m/z* = 426.1 $[M+H]^+$, (calcd for $C_{22}H_{14}F_3N_3O_3$: 425.1); HPLC purity 96%.

2-amino-6-(pyrimidin-5-yl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(21)**. Yield 83%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.16 (s, 1H), 9.14 (s, 2H), 8.26 (d, J = 2.3 Hz, 1H), 8.05 (dd, J = 8.6, 2.3 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.40 (d, J =8.6 Hz, 1H), 6.52 (br s, 2H). LC-MS (ESI): found *m/z* = 384.1 $[M+H]^+$, (calcd for $C_{19}H_{12}F_3N_5O$: 383.10); HPLC purity 99%.

2-amino-6-(pyridin-3-yl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(22)**. Yield 74%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.56 (d, J = 4.7 Hz, 1H), 8.18 (d, J = 2.3 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.02 (dd, J = 8.6, 2.2 Hz, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.3 Hz, 2H), 7.49 (dd, J = 7.9, 4.7 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.61 (br s, 2H). LC-MS (ESI): found *m/z* = 383.1 $[M+H]^+$, (calcd for $C_{20}H_{13}F_3N_4O$: 382.10); HPLC purity 98%.

2-amino-6-(5-methylpyridin-3-yl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(23).** Yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.39 (s, 1H), 8.16 (d, J = 2.3 Hz, 1H), 7.99 (dd, J = 8.6, 2.3 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.91 (s, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.6 Hz, 1H), 6.56 (br s, 2H), 2.37 (s, 3H). LC-MS (ESI): found *m/z* = 397.1 $[M+H]^+$, (calcd for $C_{21}H_{15}F_3N_4O$: 396.10); HPLC purity 97%.

2-amino-6-(5-methoxypyridin-3-yl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(24)**. Yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 2.7 Hz, 1H), 8.18 (d, J = 2.3 Hz, 1H), 8.01 (dd, J = 8.6, 2.3 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.62 (dd, J = 2.7, 1.9 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 6.57 (br s, 2H), 3.92 (s, 3H). LC-MS (ESI): found *m/z* = 413.1 $[M+H]^+$, (calcd for $C_{21}H_{15}F_3N_4O_2$: 412.11); HPLC purity 99%.

2-amino-6-(3-methoxyphenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(25)**. Yield 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, J = 2.3 Hz, 1H), 7.96 (dd, J = 8.6, 2.4 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.1 Hz, 2H), 7.38 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 8.6 Hz, 1H), 7.24 (ddd, J = 7.7, 1.7, 0.9 Hz, 1H), 7.18 (t, J = 1.8 Hz, 1H), 6.93 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H), 6.51 (br s, 2H), 3.83 (s, 3H). LC-MS (ESI): found *m/z* = 412.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_2$: 411.12); HPLC purity 98%.

2-amino-6-(3-(tert-butyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(26)**. Yield 49%. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.11 (d, J = 2.1 Hz, 1H), 7.95 (dd, J = 8.5, 2.5 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.70–7.60 (m, 3H), 7.51– 7.44 (m, 1H), 7.41–7.37 (m, 2H), 7.35 (d, J = 8.5 Hz, 1H), 6.49 (br s, 2H), 1.34 (s, 9H). LC-MS (ESI): found *m/z* = 438.2 $[M+H]^+$, (calcd for $C_{25}H_{22}F_3N_3O$: 437.2); HPLC purity 98%.

2-amino-6-(3-(dimethylamino)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(27)**. Yield 55%. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.08 (d, J = 2.3 Hz, 1H), 7.97–7.88 (m,

3H), 7.63 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.6 Hz, 1H), 7.25 (t, J = 8.0 Hz, 1H), 6.96–6.87 (m, 2H), 6.71 (ddd, J = 8.4, 2.3, 1.0 Hz, 1H), 6.47 (br s, 2H), 2.95 (s, 6H). LC-MS (ESI): found *m/z* = 425.2 $[M+H]^+$, (calcd for $C_{23}H_{19}F_3N_4O$: 424.15); HPLC purity 99%.

2-amino-6-(3-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(28)**. Yield 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.19 (d, J = 2.2 Hz, 1H), 8.05 (dd, J = 8.7, 2.2 Hz, 4H), 7.99 – 7.93 (m, 2H), 7.69 (dd, J = 20.5, 6.5 Hz, 2H), 7.38 (d, J = 8.6 Hz, 1H), 6.58 (s, 2H). LC-MS (ESI): found *m/z* = 450.1 $[M+H]^+$, (calcd for $C_{22}H_{13}F_6N_3O$: 449.1); HPLC purity 98%.

2-Amino-6-(3-chlorophenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(29)**. Yield 67%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J =2.3 Hz, 1H), 7.98 (dd, J = 8.6, 2.4 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.72 (t, J = 1.9 Hz, 1H), 7.67 (ddd, J = 7.7, 1.8, 1.1 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.49 (t, J = 7.9 Hz, 1H), 7.40 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 6.55 (br s, 2H). LC-MS (ESI): found *m/z* = 416.1 $[M+H]^+$, (calcd for $C_{21}H_{13}ClF_3N_3O$: 415.09); HPLC purity 98%.

2-amino-7-(3-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(30)**. Yield 82%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (dt, J = 11.5, 1.7 Hz, 1H), 8.21 – 8.11 (m, 2H), 8.07 – 7.93 (m, 3H), 7.89 – 7.70 (m, 3H), 7.67 (d, J = 8.1 Hz, 1H), 7.53 (dd, J = 8.3, 1.8 Hz, 1H), 6.52 (s, 2H)., 2.76 (s, 3H). LC-MS (ESI): found *m/z* = 460.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_3S$: 459.1); HPLC purity 98%.

2-amino-7-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(31)**. Yield 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 – 7.98 (m, 2H), 7.98 – 7.91 (m, 3H), 7.80 – 7.70 (m, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 1.5 Hz, 1H), 7.50 (dd, J = 8.3, 1.8 Hz, 1H), 6.49 (s, 2H), 2.85 (s, 3H). LC-MS (ESI): found *m/z* = 444.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_2S$: 443.1); HPLC purity 98%.

2-amino-7-(3-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (**32).** Yield 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 7.7 Hz, 1H), 8.05 (s, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.79 (dt, J = 15.4, 7.8 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 1.6 Hz, 1H), 7.52 (dd, J = 8.3, 1.8 Hz, 1H), 6.56 (s, 2H). LC-MS (ESI): found *m/z* = 450.1 $[M+H]^+$, (calcd for $C_{22}H_{18}F_6N_3O$: 449.1); HPLC purity 99%.

3-(2-amino-4-oxo-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-7-yl)benzamide (**33**). Yield 10%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.17 (s, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 12.4, 8.0 Hz, 4H), 7.66 (d, J = 8.2 Hz, 2H), 7.60 (dd, J = 10.1, 4.8 Hz, 2H), 7.51 (dd, J = 8.3, 1.8 Hz, 1H), 7.43 (s, 1H), 6.47 (s, 2H). LC-MS (ESI): found *m/z* = 425.1 $[M+H]^+$, (calcd for $C_{22}H_{15}F_3N_4O_2$: 424.1)); HPLC purity 97%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(34)**. Yield 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.3 Hz, 1H), 8.03 (dd, J = 8.6, 2.4 Hz, 1H), 7.98 (s, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.88–7.84 (m, 2H), 7.82 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.68 (t, J = 6.1 Hz, 1H), 7.67 (s, 1H), 7.38 (d, J = 8.6 Hz, 1H), 6.57 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 444.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_2S$: 443.09); HPLC purity 96%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-phenylquinazolin-4(3H)-one **(35)**. Yield 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 1.8 Hz, 1H), 8.03 (dd, J = 8.5, 1.8 Hz, 1H), 7.98 (s, 1H), 7.90–7.82 (m, 1H), 7.73–7.51 (m, 5H), 7.46–7.31 (m, 3H), 6.39 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 376.1 $[M+H]^+$, (calcd for $C_{21}H_{17}N_3O_2S$, 375.10); HPLC purity 95%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(p-tolyl)quinazolin-4(3H)-one **(36)**. Yield 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.3 Hz, 1H), 8.02 (dd, J = 8.6, 2.4 Hz, 1H), 7.98 (dd, J = 2.2, 1.3 Hz, 1H), 7.87 (dt, J = 6.6, 2.2 Hz, 1H), 7.70–7.63 (m, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.37 (d, J = 8.8 Hz, 1H), 7.26 (d, J = 8.3 Hz, 2H), 6.36 (br s, 2H), 2.82 (s, 3H), 2.42 (s,

3H). LC-MS (ESI): found *m/z* = 390.1 $[M+H]^+$, (calcd for $C_{22}H_{19}N_3O_2S$: 389.12); HPLC purity 97%.

Scheme 2

6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(37).** To a solution of 2-amino-5-iodobenzoic acid (330mg, 1.25 mmol, 1 equiv) and 4-(trifluoromethyl)aniline (200mg, 1.25 mmol, 1 equiv) in Dioxane (30 ml) was added triethoxymethane (2g, 12.5 mmol, 10 equiv) and the resulting reaction mixture heated in a sealed tube at a temperature of 100˚C. After 16 hours, a precipitate is formed. The reaction mixture was cooled to room temperature at 25˚C, the precipitate was filtered, washed with Et_2O (10ml) and dried to afford a light yellow solid (150mg, 0.36 mmol, 29%). LC-MS (ESI): found *m/z* = 417.0 $[M+H]^+$, (calcd for $C_{15}H_8F_3IN_2O$: 416.0); HPLC purity 80%.

6-(3-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(38).** 6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (150mg, 0.3 6mmol, 1 equiv), (3-(methylsulfonyl)phenyl)boronic acid (78mg, 0.39 mmol, 1.1 equiv) and PdCl2(dppf) (30 mg, 0.041 mmol, 0.11 equiv) were dissolved in Dioxane (3 mL). Thereafter, Cs_2CO_3 (230mg, 0.706 mmol, 2 equiv) and water (0.3 mL) were added. The mixture was heated in a pressure tube to 85˚C for 2 hours. The cooled mixture was diluted with 50 ml NaHCO3 solution and extracted with EtOAc (2 x 50 ml). The organic phases were dried over Na_2SO_4 and evaporated. Flash chromatography eluting a gradient of 30 – 50% EtOAc in DCM and evaporation of the product fractions yielded a white solid (80mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.63 (1H, d, J = 3.0 Hz); 8.30-8.28 (1H, m); 8.18 (1H, s); 8.21 (1H, dd, J = 3.0 and 9.0 Hz); 8.04 -7.99 (2H, m); 7.93 (1H, d, J = 9.0 Hz); 7.88 (2H, d, J = 9.0 Hz); 7.77-7.71 (1H, m); 7.64 (2H, d, J = 9.0 Hz); 3.16 (3H, s). LC-MS (ESI): found *m/z* = 445.1 $[M+H]^+$, (calcd $C_{22}H_{15}F_3N_2O_3S$, 444.08); HPLC purity 100%.

Scheme 3

6-iodo-2-(methylamino)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(39a)**. 2-chloro-6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (intermediate Ba) (200 mg, 0.44 mmol, 1.0 equiv) was dissolved in THF (2mL) and a 33% solution of methylamine in ethanol was added and the mixture was heated in a pressure tube to 60˚C for 3 hours. Once cooled, 10ml water was added and this mixture was extracted with ethyl acetate (2 x 50 ml). The organic layers were dried over Na_2SO_4 and evaporated to afford an oily residue (240 mg, 121%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.44 (1H, d, J = 3.0 Hz); 7.93 (1H, dd, J = 3.0 & 9.0 Hz), 7.92 (2H, d, J = 9.0 Hz); 7.49 (2H, J = 9.0 Hz); 7.46 (1H, d, J = 9.0 Hz); 3.13-3.07 (3H, m). LC-MS (ESI): found *m/z* = 446.0 $[M+H]^+$, (calcd $C_{16}H_{11}F_3IN_3O$, 444.99).

2-(dimethylamino)-6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(39b)**. 2-chloro-6-iodo-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (Intermediate Ba) (220mg, 0.488 mmol, 1 equiv) was dissolved in DMF (5 mL), dimethylamine hydrochloride (350mg, 4.8 mmol, 10 equiv) and 1 ml triethylamine were heated in a pressure tube to 80˚C for 3 hours. To the cooled mixture was added 50 ml water, and this mixture was extracted with ethyl acetate (2 x 50 ml). The organic layers were dried over Na_2SO_4 and evaporated to afford a yellow solid (270mg, 120%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.36 (1H, d, J = 3.0 Hz); 7.83 (1H, dd, J = 3.0 & 9.0 Hz), 7.69 (2H, d, J = 9.0 Hz); 7.42 (2H, J = 9.0 Hz); 7.20 (1H, d, J = 9.0 Hz); 2.61 (6H, s). LC-MS (ESI): found *m/z* = 460.0 $[M+H]^+$, (calcd $C_{17}H_{13}F_3IN_3O$: 459.0); HPLC purity 98%.

General Procedure for the Suzuki-Coupling Reaction 40 and 43

Intermediate **39a** (120mg, 0.22 mmol, 1.0 equiv), the relevant boronic acid (56mg, 0.28 mmol, 1.3 equiv) and PdCl2(dppf) (20mg, 0.027 mmol, 0.12 equiv) were dissolved in Dioxane (2 mL). Thereafter, cesium carbonate (230mg, 0.71 mmol, 3 equiv) and water (0.3mL) were added. The mixture was heated in a pressure tube to 80˚C for 3.5 hours. Once complete, the

mixture was cooled, diluted with water and extracted with EtOAc (2 x 50mL). The organic layers were dried over Na2SO4 and evaporated. Flash chromatography eluting a gradient of 20 – 40% THF in DCM or 40 – 80% EtOAc in hexane to afford the target compounds 40 - 43.

2-(methylamino)-6-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(40).** Yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.22 (1H, d, J = 3.0 Hz); 8.06 (1H, dd, J = 3.0 & 9.0 Hz); 8.00-7.94 (3H, m); 7.90-7.86 (1H, m); 7.70-7.68 (4H, m); 7.47 (1H, d, J = 9.0 Hz); 6.08-6.02 (1H, m); 2.81 (6H, "s"). LC-MS (ESI): found *m/z* = 458.1 $[M+H]^+$, (calcd $C_{23}H_{18}F_3N_3O_2S$, 457.11); HPLC purity 99%.

2-(methylamino)-6-(3-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(41).** Yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.40 (1H, d, J = 3.0 hz); 8.24-8.22 (1H, m); 7.98-7.90 (5H, m); 7.72-7.65 (2H, m); 7.53 (2H, d, J = 9.0 Hz); 4.13 (1H, sb); 3.13 (3H, s); 3.08 (3H, d, J = 6.0 Hz). LC-MS (ESI): found *m/z* = 474.1 $[M+H]^+$, (calcd $C_{23}H_{18}F_3N_3O_3S$: 473.10); HPLC purity 98%.

2-(dimethylamino)-6-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(42).** Yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.40 (1H, d, J = 3.0 Hz); 8.00 (1H, dd, J = 3.0 & 9.0 Hz); 7.97-7.93 (1H, m); 7.84-7.62 (7H, m); 7.56 (1H, d, J = 9.0 Hz); 2.81 (3H, s); 2.78 (6H, s). LC-MS (ESI): found *m/z* = 472.1 $[M+H]^+$, (calcd $C_{24}H_{20}F_3N_3O_2S$, 471.12); HPLC purity 98%.

2-(dimethylamino)-6-(3-(methylsulfonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(43).** Yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.41 (1H, d, J = 3.0 hz); 8.24-8.22 (1H, m); 7.99-7.93 (3H, m); 7.81 (2H, d, J = 9.0 Hz); 7.72-7.65 (2H, m); 7.57 (2H, d, J = 9.0 Hz); 3.13 (3H, s); 2.75 (6H, s). LC-MS (ESI): found *m/z* = 488.1 $[M+H]^+$, (calcd for $C_{24}H_{20}F_3N_3O_3S$, 487.11); HPLC purity 98%.

Scheme 4

Methyl 5-bromo-2-(3-(ethoxycarbonyl)thioureido)benzoate **(44).** Methyl 2-amino-5-bromobenzoate (3.58g, 15.5 mmol, 1 equiv) was dissolved in acetonitrile (10 mL) and the yellow solution was treated with ethoxycarbonyl isothiocyanate (2.2mL, 18.6 mmol, 1.2 Eq). After stirring for 10 minutes at room temperature, the precipitate that formed was filtered, the resulting mother liquor was concentrated and solids refiltered to afford methyl 5-bromo-2-(3-(ethoxycarbonyl)thioureido)benzoate as a white solid (5g, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.61 (s, 1H), 8.47 (d, J = 8.9 Hz, 1H), 8.18 (s, 1H), 8.16 (d, J = 2.4 Hz, 1H), 7.68 (dd, J = 8.8, 2.5 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.97 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 178.10, 165.46, 151.67, 137.95, 135.35, 133.42, 127.86, 123.54, 118.74, 63.07, 52.81, 14.21. LC-MS (ESI): found *m/z* = 360.9, 362.9 (1:1) $[M+H]^+$, (calcd for $C_{12}H_{13}BrN_2O_4S$: 359.98); HPLC purity 99%.

General Procedure for the Synthesis of Intermediates 45a-i

Intermediate **44** (1 equiv) was dissolved in DCM or DMF and the appropriate aniline (1 equiv) and EDCI (2 equiv) were successively added to the solution. The solution was allowed to stir at room temperature for 18 hours. Once complete, DMF solutions were diluted with EtOAc and washed with 5% LiCl (3 x 20mL), thereafter the organic layer was washed with 1% of 1M HCl (2 x 20mL), water, brine (20mL) and dried over magnesium sulphate. The solution was filtered and the solvent concentrated under reduced pressure to furnish intermediates **45a-i**.

Ethyl(6-bromo-4-oxo-3-(6-(trifluoromethyl)pyridin-3-yl)-3,4-dihydroquinazolin-2-yl)carbamate **(45a).** Yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 1.9 Hz, 1H), 8.34 (d, J = 2.2 Hz, 1H), 7.90–7.79 (m, 3H), 7.19 (d, J = 8.6 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 4570, 459.0 (1:1) $[M+H]^+$, (calcd for $C_{17}H_{12}BrF_3N_4O_3$: 456.0); HPLC purity 97%.

Ethyl (6-bromo-4-oxo-3-(2-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)carbamate **(45b).** Yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, J = 2.3 Hz, 1H), 7.85 (d, J = 8.0, 1H), 7.83 (dd, J = 8.7, 2.3 Hz, 1H), 7.75 (td, J = 7.7, 1.0 Hz, 1H), 7.63 (tt, J = 7.8, 1.0 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.18 (d, J = 8.7 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 1.21 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 456.0, 458.0 (1:1) $[M+H]^+$, (calcd for $C_{18}H_{13}BrF_3N_3O_3$: 455.0); HPLC purity 90%.

Ethyl (6-bromo-3-(4-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **45c.** Yield 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, J = 2.3 Hz, 1H), 7.82 (dd, J = 8.6, 2.3 Hz, 1H), 7.29–7.22 (m, 4H), 7.20 (d, J = 8.6 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 406.0, 408.0 (1:1) $[M+H]^+$, (calcd for $C_{17}H_{13}BrFN_3O_3$: 405.0); HPLC purity 98%.

Ethyl (6-bromo-3-(2-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **45d.** Yield 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 2.2 Hz, 1H), 7.83 (dd, J = 8.6, 2.3 Hz, 1H), 7.55–7.43 (m, 1H), 7.33–7.23 (m, 3H), 7.19 (d, J = 8.6 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 406.0, 408.0 (1:1) $[M+H]^+$, (calcd for $C_{17}H_{13}BrFN_3O_3$: 405.0); HPLC purity 95%.

Ethyl (6-bromo-3-(4-(methylsulfonyl)phenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **(45e).** Yield 81%. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.09 (d, J = 2.7 Hz, 1H), 8.07 (d, J = 8.7 Hz, 2H), 7.98 (dd, J = 8.8, 2.3 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 3.98 (q, J = 7.1 Hz, 2H), 3.32 (s, 3H). LC-MS (ESI): found *m/z* = 466.0, 468.0 (1:1) $[M+H]^+$, (calcd for $C_{18}H_{16}BrN_3O_5S$: 465.0); HPLC purity 96%.

Ethyl (6-bromo-3-(4-hydroxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **(45f).** Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 2.3 Hz, 1H), 7.84 (dd, J = 8.7, 2.3 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 4.19 (q, J = 7.1

Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 404.0, 406.0 (1:1) $[M+H]^+$, (calcd for $C_{17}H_{14}BrN_3O_4$: 403.0); HPLC purity 96%.

Ethyl (6-bromo-3-(4-methoxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **(45g).** Yield 94%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.3 Hz, 1H), 7.80 (dd, J = 8.6, 2.3 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.17 (d, J = 9.1 Hz, 2H), 7.05 (d, J = 9.1 Hz, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.88 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 418.0, 420.0 (1:1) $[M+H]^+$, (calcd for $C_{18}H_{16}BrN_3O_4$: 417.0); HPLC purity 99%.

Ethyl (6-bromo-3-(4-cyanophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **(45h).** Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.2 Hz, 1H), 7.84 (dd, J = 8.6, 2.3 Hz, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.6 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 413.0, 415.0 (1:1) $[M+H]^+$, (calcd for $C_{18}H_{13}BrN_4O_3$: 412.0); HPLC purity 96%.

Ethyl (6-bromo-3-(3-cyanophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)carbamate **(45i).** Yield 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.3 Hz, 1H), 7.85 (dd, J = 8.6, 2.3 Hz, 1H), 7.77 (dt, J = 7.8, 1.3 Hz, 1H), 7.66 (t, J = 8.1 Hz, 1H), 7.58 (t, J = 2.0 Hz, 1H), 7.52 (ddd, J = 8.0, 2.1, 1.1 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H). LC-MS (ESI): found *m/z* = 413.0, 415.0 (1:1) $[M+H]^+$, (calcd for $C_{18}H_{13}BrN_4O_3$: 412.0); HPLC purity 99%.

General Procedure for the Synthesis of Intermediates 46a-i

The 2-carbamate-based intermediate **45a–i** (1 equiv) was dissolved in TFA (20 equiv). This solution was heated under microwave conditions at 110˚C for 20 min. After cooling to room temperature and evaporating the solvent, the residue was dissolved in DCM/MeOH (1:1). Amberlyst® A21 free base was then added to this solution, stirred at ambient temperature for 45 min, followed by filtration and rinsing of the resin with MeOH. The filtrate was then

concentrated under reduced pressure to give a residue that was triturated in Et_2O, filtered, and dried to afford the desired intermediate **46a-i.**

2-amino-6-bromo-3-(6-(trifluoromethyl)pyridin-3-yl)quinazolin-4(3H)-one **(46a).** Yield 64%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.83 (d, J = 2.3 Hz, 1H), 8.22 (dd, J = 7.7, 2.3 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 7.97 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.5 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 6.80 (br s, 2H). LC-MS (ESI): found *m/z* = 385.0, 387.0 (1:1) $[M+H]^+$, (calcd for $C_{14}H_8BrF_3N_4O$: 384.0); HPLC purity 99%.

2-amino-6-bromo-3-(2-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(46b).** Yield 71%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.95 (d, J = 2.5 Hz, 1H), 7.95–7.93 (m, 1H), 7.89 (td, J = 7.7, 0.9 Hz, 1H), 7.81–7.77 (m, 1H), 7.75 (dd, J = 8.8, 2.5 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.66 (br s, 2H). LC-MS (ESI): found *m/z* = 384.0, 386.0 (1:1) $[M+H]^+$, (calcd for $C_{14}H_9BrF_3N_3O$: 383.0); HPLC purity 97%.

2-amino-6-bromo-3-(4-fluorophenyl)quinazolin-4(3H)-one **(46c).** Yield 77%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.95 (d, J = 2.4 Hz, 1H), 7.73 (dd, J = 8.8, 2.5 Hz, 1H), 7.48–7.35 (m, 4H), 7.20 (d, J = 8.8 Hz, 1H), 6.52 (br s, 2H). LC-MS (ESI): found *m/z* = 334.0, 336.0 (1:1) $[M+H]^+$, (calcd for $C_{14}H_9BrFN_3O$: 333.0); HPLC purity 99%.

2-amino-6-bromo-3-(2-fluorophenyl)quinazolin-4(3H)-one **(46d).** Yield 73%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.97 (d, J = 2.5 Hz, 1H), 7.76 (dd, J = 8.8, 2.5 Hz, 1H), 7.60 (dddd, J = 8.4, 7.2, 5.2, 1.8 Hz, 1H), 7.52 (td, J = 7.8, 1.7 Hz, 1H), 7.47 (ddd, J = 9.8, 8.4, 1.2 Hz, 1H), 7.40 (td, J = 7.7, s1.3 Hz, 1H), 7.22 (d, J = 8.8 Hz, 1H), 6.73 (br s, 2H). LC-MS (ESI): found *m/z* = 334.0, 336.0 (1:1) $[M+H]^+$, (calcd for $C_{14}H_9BrFN_3O$: 333.0); HPLC purity 99%.

2-amino-6-bromo-3-(4-(methylsulfonyl)phenyl)quinazolin-4(3H)-one **(46e).** Yield 77%. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.15 (d, J = 8.7 Hz, 2H), 8.03 (d, J = 2.3 Hz, 1H), 7.88 (dd, J =

8.8, 2.4 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.48 (br s, 2H), 7.33 (d, J = 8.8 Hz, 1H), 3.30 (s, 3H). LC-MS (ESI): found *m/z* = 394.0, 396.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_{12}BrN_3O_3S$: 393.0); HPLC purity 99%.

2-amino-6-bromo-3-(4-hydroxyphenyl)quinazolin-4(3H)-one **(46f).** Yield 58%. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.81 (br s, 1H), 7.94 (d, J = 2.5 Hz, 1H), 7.71 (dd, J = 8.8, 2.5 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H), 7.13 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.36 (br s, 2H). LC-MS (ESI): found *m/z* = 332.0, 334.0 (1:1) $[M+H]^+$, (calcd for $C_{14}H_{10}BrN_3O_2$: 331.0); HPLC purity 99%.

2-amino-6-bromo-3-(4-methoxyphenyl)quinazolin-4(3H)-one **(46g).** Yield 80%. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.95 (d, J = 2.4 Hz, 1H), 7.72 (dd, J = 8.8, 2.5 Hz, 1H), 7.28 (d, J = 8.9 Hz, 2H), 7.19 (d, J = 8.8 Hz, 1H), 7.11 (d, J = 8.9 Hz, 2H), 6.41 (br s, 2H), 3.84 (s, 3H). LC-MS (ESI): found *m/z* = 346.0, 348.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_{12}BrN_3O_2$: 345.0); HPLC purity 99%.

4-(2-amino-6-bromo-4-oxoquinazolin-3(4H)-yl)benzonitrile **(46h).** Yield 54%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 2.5 Hz, 1H), 7.74 (dd, J = 8.8, 2.5 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 8.8 Hz, 1H), 6.59 (br s, 2H). LC-MS (ESI): found *m/z* = 341.0, 343.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_9BrN_4O$: 340.0); HPLC purity 99%.

3-(2-amino-6-bromo-4-oxoquinazolin-3(4H)-yl)benzonitrile **(46i).** Yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (br s, 2H), 8.10 (d, J = 2.1 Hz, 1H), 8.09 (t, J = 1.6 Hz, 1H), 8.06–8.00 (m, 2H), 7.92–7.82 (m, 2H), 7.57 (d, J = 8.7 Hz, 1H). LC-MS (ESI): found *m/z* = 341.0, 343.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_9BrN_4O$: 340.0); HPLC purity 98%.

General Procedure for the Synthesis of Intermediates 46j-l

To a solution of intermediate **44** (500mg, 1.384 mmol, 1 equiv) in DMF (10mL) was added the appropriate amine (1.5 equiv) and EDCI (530mg, 2.77 mmol, 2 equiv). The resulting mixture was stirred at 35˚C for 8 hours. Thereafter, the solution was transferred to a pressure tube and heated to 100˚C for 16 hours. The reaction was cooled to room temperature, diluted with EtOAc (50mL) and water (40mL), the organic layers separated and the aqueous phase extracted with EtOAc (2 x 50mL). The combined organic layers were washed with saturated $NaHCO_3$ (20mL) and LiCl (3 x 20mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo to afford intermediate **46j-l**.

2-amino-6-bromo-3-(4,4-difluorocyclohexyl)quinazolin-4(3H)-one **(46j).** Yield 35%. LC-MS (APCI): found *m/z* = 356.0, 358.0 (1:1) $[M+H]^-$, (calcd for $C_{14}H_{14}BrF_2N_3O$: 357.0); HPLC purity 74%.

2-amino-6-bromo-3-(4-(trifluoromethyl)cyclohexyl)quinazolin-4(3H)-one **(46k).** Yield 80%. LC-MS (ESI): found *m/z* = 391.0, 393.0 (1:1) $[M+H]^+$, (calcd for $C_{15}H_{15}BrF_3N_3O$: 389.0); HPLC purity 80%.

2-amino-6-bromo-3-(4-(trifluoromethyl)benzyl)quinazolin-4(3H)-one **(46l) (7188/58 intermediate).** Yield 98%. LC-MS (ESI): found *m/z* = 398.0, 400.0 (1:1) $[M+H]^+$, (calcd for $C_{16}H_{11}BrF_3N_3O$: 397.0); HPLC purity 98%.

General Procedure for the Synthesis of Final Compounds 47 – 58

3-(methylsulfinyl)phenyl) boronic acid (1.2 equiv) and the corresponding 2-amino-6-bromoquinazolinone- based intermediate **46a-l** (1 equiv) were dissolved in DMF (5 mL). The mixture was flushed with nitrogen gas for about 10 min at room temperature, after which $Pd(PPh_3)_2Cl_2$ (0.05 equiv) and K_2CO_3 (3 equiv) were added successively, and the mixture heated at 100 °C for 1 to 4 h. After cooling to room temperature, the mixture was diluted with

EtOAc (10 mL) and water (30 mL), and the extracted with EtOAc (3 x 20 mL). The combined organic fractions were filtered through a pad of celite, washed with 5% LiCl (3 x 30 mL), brine (2 x 30 mL), and dried over anhydrous MgSO4. The solvents were then removed in vacuo to yield a residue that was purified by flash chromatography eluting a gradient of 0 – 15% MeOH in DCM. Product fractions were combined and the solvents were rotary evaporated to give a solid that was then triturated with Et_2O, filtered, or recrystallized in an appropriate solvent (ethanol was used in most cases), and dried to furnish the desired final target compounds.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(6-(trifluoromethyl)pyridin-3-yl)quinazolin-4(3H)-one **(47).** Yield 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 2.3 Hz, 1H), 8.25 (dd, J = 8.3, 2.3 Hz, 1H), 8.22 (d, J = 2.3 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 8.05 (dd, J = 8.6, 2.4 Hz, 1H), 7.98 (dd, J = 2.2, 1.2 Hz, 1H), 7.91–7.84 (m, 1H), 7.71–7.64 (m, 2H), 7.39 (d, J = 8.6 Hz, 1H), 6.78 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 445.1 $[M+H]^+$, (calcd for $C_{21}H_{15}F_3N_4O_2S$: 444.09); HPLC purity 99%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(2-(trifluoromethyl)phenyl)quinazolin-4(3H)-one **(48).** Yield 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 2.4 Hz, 1H), 8.04 (dd, J = 8.6, 2.4 Hz, 1H), 8.00–7.94 (m, 2H), 7.91 (dd, J = 7.7, 0.9 Hz, 1H), 7.89–7.86 (m, 1H), 7.82–7.75 (m, 1H), 7.69–7.62 (m, 3H), 7.38 (d, J = 8.6 Hz, 1H), 6.61 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 444.1 $[M+H]^+$, (calcd for $C_{22}H_{16}F_3N_3O_2S$: 443.09); HPLC purity 97%.

2-amino-3-(4-fluorophenyl)-6-(3-(methylsulfinyl)phenyl)quinazolin-4(3H)-one **(49).** Yield 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.2 Hz, 1H), 8.02 (dd, J = 8.6, 2.4 Hz, 1H), 7.98 (s, 1H), 7.86 (dt, J = 6.7, 2.1 Hz, 1H), 7.71–7.63 (m, 2H), 7.50–7.38 (m, 4H), 7.37 (d, J = 8.6 Hz, 1H), 6.49 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 394.1 $[M+H]^+$, (calcd for $C_{21}H_{16}FN_3O_2S$: 393.09); HPLC purity 98%.

2-amino-3-(2-fluorophenyl)-6-(3-(methylsulfinyl)phenyl)quinazolin-4(3H)-one **(50).** Yield 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 2.3 Hz, 1H), 8.05 (dd, J = 8.6, 2.4 Hz, 1H), 7.99 (s, 1H), 7.88 (ddd, J = 6.4, 2.6, 2.0 Hz, 1H), 7.71–7.65 (m, 2H), 7.64–7.58 (m, 1H), 7.54 (td, J = 7.8, 1.7 Hz, 1H), 7.48 (ddd, J = 9.8, 8.4, 1.2 Hz, 1H), 7.44–7.37 (m, 2H), 6.68 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 394.1 $[M+H]^+$, (calcd for $C_{21}H_{16}FN_3O_2S$: 393.09); HPLC purity 99%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(4-(methylsulfonyl)phenyl)quinazolin-4(3H)-one **(51).** Yield 20%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.2 Hz, 1H), 8.13 (d, J = 8.5 Hz, 2H), 8.04 (dd, J = 8.6, 2.3 Hz, 1H), 7.98 (s, 1H), 7.87 (dt, J = 7.4, 1.9 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.69–7.63 (m, 2H), 7.39 (d, J = 8.6 Hz, 1H), 6.57 (br s, 2H), 3.31 (s, 3H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 454.1 $[M+H]^+$, (calcd for $C_{22}H_{19}N_3O_4S_2$: 453.08); HPLC purity 99%.

2-amino-3-(4-hydroxyphenyl)-6-(3-(methylsulfinyl)phenyl)quinazolin-4(3H)-one **(52).** Yield 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (br s, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.01 (dd, J = 8.6, 2.3 Hz, 1H), 7.97 (s, 1H), 7.86 (dt, J = 6.8, 2.0 Hz, 1H), 7.72–7.62 (m, 2H), 7.35 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.33 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 392.1 $[M+H]^+$, (calcd for $C_{21}H_{17}N_3O_3S$: 391.1); HPLC purity 99%.

2-amino-3-(4-methoxyphenyl)-6-(3-(methylsulfinyl)phenyl)quinazolin-4(3H)-one **(53).** Yield 81%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.3 Hz, 1H), 8.01 (dd, J = 8.6, 2.3 Hz, 1H), 7.98 (s, 1H), 7.87 (dt, J = 6.7, 2.0 Hz, 1H), 7.71–7.63 (m, 2H), 7.36 (d, J = 8.6 Hz, 1H), 7.30 (d, J = 8.9 Hz, 2H), 7.12 (d, J = 8.9 Hz, 2H), 6.38 (br s, 2H), 3.85 (s, 3H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 406.1 $[M+H]^+$, (calcd for $C_{22}H_{19}N_3O_3S$, 405.11); HPLC purity 97%.

4-(2-amino-6-(3-(methylsulfinyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzonitrile **(54).** Yield 32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.2 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 8.03

(dd, J = 8.6, 2.4 Hz, 1H), 7.98 (s, 1H), 7.86 (dt, J = 6.7, 2.1 Hz, 1H), 7.71–7.63 (m, 4H), 7.37 (d, J = 8.6 Hz, 1H), 6.57 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 401.1 $[M+H]^+$, (calcd for $C_{22}H_{16}N_4O_2S$: 400.10); HPLC purity 98%.

3-(2-amino-6-(3-(methylsulfinyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzonitrile **(55).** Yield 38%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, J = 2.2 Hz, 1H), 8.07–7.99 (m, 3H), 7.98 (s, 1H), 7.87 (dt, J = 6.6, 2.1 Hz, 1H), 7.81–7.75 (m, 2H), 7.71–7.63 (m, 2H), 7.38 (d, J = 8.6 Hz, 1H), 6.60 (br s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 401.1 $[M+H]^+$, (calcd for $C_{22}H_{16}N_4O_2S$: 400.10); HPLC purity 99%.

2-amino-3-(4,4-difluorocyclohexyl)-6-(3-(methylsulfinyl)phenyl)quinazolin-4(3H)-one **(56).** Yield 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 2.2 Hz, 1H), 7.96 (dd, J = 8.4, 2.5 Hz, 2H), 7.85 (dt, J = 6.7, 2.1 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.27 (d, J = 8.5 Hz, 1H), 7.14 (s, 2H), 4.38 – 4.12 (m, 1H), 2.98 – 2.80 (m, 2H), 2.21 – 1.91 (m, 4H), 1.77 (d, J = 12.3 Hz, 2H). LC-MS (ESI): found *m/z* = 418.1 $[M+H]^+$, (calcd for $C_{21}H_{21}F_2N_3O_2S$: 417.1); HPLC purity 94%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)cyclohexyl)quinazolin-4(3H)-one **(57).** Yield 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 2.2 Hz, 1H), 7.95 (dd, J = 8.6, 2.3 Hz, 2H), 7.85 (dt, J = 6.7, 2.1 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.27 (d, J = 8.6 Hz, 1H), 7.08 (s, 2H), 4.29 (s, 1H), 2.82 (s, 3H), 2.73 – 2.60 (m, 2H), 2.34 (s, 1H), 1.98 (d, J = 11.3 Hz, 2H), 1.79 (d, J = 11.0 Hz, 2H), 1.62 – 1.47 (m, 2H). LC-MS (ESI): found *m/z* = 450.1 $[M+H]^+$, (calcd for $C_{22}H_{22}F_3N_3O_2S$: 449.1); HPLC purity 99%.

2-amino-6-(3-(methylsulfinyl)phenyl)-3-(4-(trifluoromethyl)benzyl)quinazolin-4(3H)-one **(58).** Yield 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 2.0 Hz, 1H), 8.02 (dd, J = 8.6, 2.4 Hz, 1H), 7.99 (dd, J = 2.2, 1.3 Hz, 1H), 7.90 – 7.85 (m, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.67 (dd, J = 4.7, 2.3 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.7 Hz, 1H), 7.14 (s, 2H), 5.42

(s, 2H), 2.82 (s, 3H). LC-MS (ESI): found *m/z* = 458.1 $[M+H]^+$, (calcd for $C_{23}H_{18}F_3N_3O_2S$: 457.1); HPLC purity 99%.

2. GAST/Fe MIC_{90} Determinations

The broth microdilution method[1,2] allows a range of antibiotic concentrations to be tested on a single 96-well microtitre plate in order to determine the minimum inhibitory concentration (MIC). Briefly, a 10 mL culture of *Mycobacterium tuberculosis* (H37RvMa)[3] is grown to an OD600 of 0.6 - 0.7. The culture is then diluted 1:100 in GAST/Fe medium. In a 96-well microtitre plate, 50 μL of GAST/Fe medium is added to all wells from Rows 2-12. The compounds to be tested are added to Row 1 in duplicate, at a final concentration of 640 μM (stocks are made up to a concentration of 12.8 mM in DMSO and diluted to 640 μM in GAST/Fe medium). A two-fold serial dilution is prepared, by transferring 50 μL of the liquid in Row 1 to Row 2 and aspirating to mix. 50 μL of the liquid in Row 2 is then transferred to Row 3 and aspirated, and so on. This procedure is repeated until Row 12 is reached, from which 50 μL of the liquid is discarded so as to bring the final volume in all wells to 50 μL. Finally, 50 μL of the 1:100 diluted *M. tuberculosis* culture is added to all wells in Rows 2-12. Cells are not added to Row 1, as this serves as a contamination control. Controls include media only, 5% DMSO, Rifampicin and Kanamycin. The microtitre plate is stored is secondary container and incubated at 37°C with humidifier to prevent evaporation of liquid. The lowest concentration of drug that inhibits growth of more than 90% of the bacterial population is considered to be the MIC_{90}. MIC_{90} values are scored visually at 7-days and 14-days post inoculation, and digital images captured and stored.

3. *In vitro* ADME assays

3.1. Kinetic solubility

The kinetic solubility assay was performed using a miniaturised shake flask method. 10 mM stock solutions of each of the test compounds were used to prepare calibration standards (10-220μM) in DMSO. The same 10mM stock solutions were accurately dispensed in duplicate into 96-well plates and the DMSO dried down (MiVac GeneVac, 90 min, 37 ˚C). Thereafter, the samples were reconstituted (200 μM) in phosphate buffered saline (pH 7.4) and shaken (20 hours, 25 °C). The solutions were filtered and analysed by means of HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector). Best fit calibration curves were constructed using the calibration standards, which were used to determine the aqueous samples' solubility.[4]

3.2. Microsomal stability

The metabolic stability assay was performed in duplicate in a 96-well micro titre plate. The test compounds (0.1 μM) were incubated individually in mouse, rat and pooled human liver microsomes ((final protein concentration of 0.4 mg/mL; XenoTech, Lenexa, KS), suspended in 0.1M phosphate buffer (pH 7.4) at 37 °C for predetermined time points, in the presence and absence of the cofactor NADPH (1 mM). Reactions were quenched by adding ice cold acetonitrile containing internal standard (carbamazepine, 0.0236 μg/mL). The samples were centrifuged and test compound in the supernatant were analysed by means of LC-MS/MS (Agilent Rapid Resolution HPLC, AB SCIEX 4500 MS). The relative loss of parent compound over time was monitored and plots (concentration vs. time) were prepared per compound to determine the first order rate constant for compound depletion. This was in turn used to calculate half-life, *in vitro* intrinsic clearance and in vivo hepatic extraction ratio. Metabolite searches were not conducted during the metabolic stability assay.[5]

3.3. Cytotoxicity

Compounds were screened for in vitro cytotoxicity against VERO (kidney epithelial cells extracted from an African green monkey) or Chinese Hamster Ovarian (CHO) mammalian celllines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay. The MTT-assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays.[5] The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The tetrazolium ring is cleaved in active mitochondria. Thus only viable cells are able to reduce the water-soluble yellow coloured MTT to water-insoluble purple coloured formazan. Formazan crystals are dissolved in DMSO. The test samples were tested in triplicate on one occasion. The test samples were prepared to a 20 mg/mL stock solution in 100% DMSO. Stock solutions were stored at -20˚C. Further dilutions were prepared in complete medium on the day of the experiment. Samples were tested as a suspension if not completely dissolved. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 µg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/mL. The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.'

4. *In vivo* studies

4.1. *In vivo* pharmacokinetic studies

All studies and procedures were conducted with prior approval of the animal ethics committee of the University of Cape Town (approval numbers 013/028 and 014/028) in accordance with

the South African National Standard (SANS 10386:008) for the Care and Use of Animals for Scientific Purposes,[6] and guidelines from the Department of Health.[7]

The pharmacokinetic parameters of the compounds were determined by dosing the compound to C57/BL6 mice through oral (n = 3 mice) and intravenous (n = 3 mice) administration. For oral administration, the compounds were formulated in 0.5% hydroxypropylmethyl cellulose (HPMC) in Phosphate buffered saline pH 7.4. The intravenous doses were administered in dimethylacetamide-polyethylene glycol 400-polypropylene glycol (DMA-PEG400-PPG) (10:30:60) Blood samples were collected via tail bleeding at predetermined time points and stored at -80°C until liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

The whole-blood concentrations of the compounds were determined using a quantitative LC-MS/MS method. Sample preparation was achieved with a protein precipitation extraction method, using ice-cold acetonitrile.

After centrifugation, the supernatant was transferred to 96-well plates for analysis. LC-MS/MS was carried out on an AB Sciex 4000 QTrap coupled to an Agilent 1200 high-performance liquid chromatograph (HPLC). Separation was achieved on a Kinetex PFP column column, using 0.1% formic acid in water as the aqueous mobile phase and 0.1% formic acid in acetonitrile as the organic mobile phase. The analytical limit of quantitation (LOQ) was 1 ng/ml. LC-MS/MS method accuracy and precision were 15% (nom) for the standards and quality controls.

Concentration-versus-time data were used to derive the PK parameters using noncompartmental analysis on PK Solutions 2.0 (Summit Research Services, Montrose, CO, USA).

4.2. Murine model of acute TB infection

All infections were performed at Colorado State University in a certified ABSL3 facility in accordance to guidelines of the Colorado State University Institutional Animal Care and Use Committee. Six- to 8-week-old female specific-pathogen-free BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were infected with *M. tuberculosis* Erdman pFCA-LuxAB (Erdman-Lux) expressing luciferase[8] via a low-dose aerosol exposure in a Glas-Col aerosol generation device (Glas-Col Inc., Terre Haute, IN).[9] Each treatment group consisted of six mice. Treatment was started 7 d post-aerosol infection and continued for 12 consecutive days. Drugs were administered by oral gavage in a 0.2 mL volume. Compound **2** was formulated in 0.5% (w/v) carboxymethylcellulose (sigma). Rifampin and ethambutol were prepared in sterile water and administered daily by oral gavage at 10 mg/kg and 100 mg/kg, respectively. For endpoint analysis, mice were euthanized three days following the end of treatment and lungs were collected. The left lung lobe (1/3rd of the lung by weight) was homogenized for enumeration of relative light units (RLU) and CFU by plating dilutions of the organ homogenates on Middlebrook 7H11 medium supplemented 10% (v/v) OADC, 0.03 mg/mL cycloheximide and 0.05 mg/mL carbenicillin. The data were expressed as mean log10 CFU ± the standard error of the mean (SEM) for each group. Statistical analysis was by one analysis of variance with Dunnet's post-test to control for multiple comparisons (SigmaPlot, San Jose, CA). Values were considered significant at the 95% confidence level.

Test bleeds via the submandibular route using a GoldenRod lancet were performed for 'in-study' PK analysis (both Cmax/Cmin) during last week of treatment from n of 3 mice. Samples were collected in K3EDTA tubes, kept on ice during collection, and then centrifuged at 6,000×g for 10 minutes at 4°C within 1 hour of collection. The plasma samples were frozen at -80°C for shipment on dry ice to for analytical assessment. Table S1 below summarises *in vivo*

concentrations at 1 and 24 hr from the in-study PK, compared to those measured in healthy mice.

Table S1. In study PK results measuring the concentration of parent compound **2** and its metabolite **1** at 1 and 24 hours

Dose	Time Point	Plasma concentration in infected mice (μM)		Plasma concentration in healthy mice (μM)	
		6132/2	**1842/ 1**	**6132/2**	**1842/1**
100 mg/kg	1 hr	21	33	21	16
100 mg/kg	24 hr	2.5	32	1.3	18
200 mg/kg	1 hr	34	55	31	20.4
200 mg/kg	24 hr	5.8	50	5.1	52.3

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