Article

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KEYWORDS: Nicotinamide adenine dinucleotide, NAD, CD38, pyridine nucleotides, nicotinamide, NAADP, NADH, nicotinamide mononucleotide

Abstract

A series of thiazoloquin(az)olinones were synthesized and found to have potent inhibitory activity against CD38. Several of these compounds were also shown to have good pharmacokinetic properties and demonstrated the ability to elevate NAD levels in plasma, liver and muscle tissue. In particular, compound **78c** was given to diet induced obese (DIO) C57Bl6 mice elevating NAD > 5-fold in liver and > 1.2-fold in muscle versus control animals at a 2 hour time point. The compounds described herein possess the most potent CD38 inhibitory activity of any small molecules described in the literature to date. The inhibitors should allow for a more detailed assessment of how NAD elevation via CD38 inhibition affects physiology in NAD deficient states.

Introduction

Nicotinamide adenine dinucleotide (NAD) is a biochemical found in all cells that was first discovered over 80 years ago. Cellular NAD is produced by either the de novo synthesis pathway from tryptophan or by a salvage synthesis pathway from precursors such as nicotinic acid (niacin) and nicotinamide, both of which are obtained from dietary sources.[1] NAD and its related pyridine nucleotides NADH, nicotinamide adenine dinucleotide phosphate (NADP), and NADPH have been recognized as the major redox carriers in all organisms. These pyridine dinucleotides determine the cytosolic and mitochondrial redox state and are key participants monitoring the metabolic status of the cell. NAD and NADH act as hydride accepting and donating cofactors for metabolic enzymes involved in glycolysis, the tricarboxylic acid cycle, and in electron transport. By transfering reducing equivalents generated from catabolic processes into the de novo synthesis of new biomolecules, pyridine nucleotides are central regulators of sugar, fat and protein metabolism[2-5]

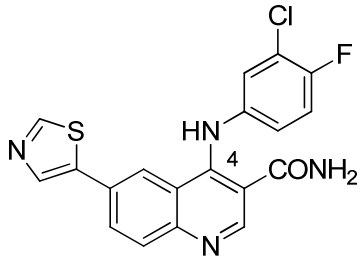
Recent research demonstrates that NAD is also a substrate for various enzymes, where it is consumed in the process of donating ADP ribose to acceptor molecules. Major consumers of NAD

include the ADP ribosyl transferases (i.e. PARP and ART family of enzymes),[6] the sirtuins (Sirt1-7),[7] and the ADP ribosyl cyclases/hydrolases (CD38/CD157).[8] These enzymes are involved in pathways that can regulate calcium signaling, gene transcription, DNA repair, cell survival, energy metabolism, and oxidative stress. Thus, NAD and its phosphorylated relatives NADP and nicotinic acid adenine dinucleotide phosphate (NAADP), both of which are derived from NAD, also act as signaling molecules.[9] NAD levels are subject to circadian rhythms, with daily oscillations that tie cellular metabolism to chromatin remodeling and gene transcription.[10] It is known that exercise and caloric restriction elevate NAD levels while aging and obesity decrease cellular NAD levels.[11,12] Restoring NAD levels in disease states that consume significant amounts of NAD will likely have medical benefits as the cell strives to survive and maintain its energy status during redox stress.

Increasing steady-state cellular levels of NAD can be achieved by two fundamental approaches: upregulating production of NAD or downregulating its consumption. CD38 is one such consumer of NAD. Also known as ADP ribosyl cyclase, CD38 is a type II membrane-anchored enzyme. It efficiently catalyzes the breakdown of NAD to nicotinamide and ADPR and hydrolyzes NAADP to adenosine-5'-O-diphosphoribose phosphate (ADPRP). CD38 can also act as a cyclase converting NAD to cADPR, although it is 100-fold less efficient as a cyclase than as a hydrolase. CD38 was first characterized as a surface antigen on immune cells and is broadly distributed throughout most tissues in the body. It exists on the plasma membrane and on the membranes of intracellular organelles such as the nucleus and mitochondria.[13] As predicted from its function as a NAD glycohydrolase, CD38 knock-out (KO) mice have elevated NAD levels relative to wild-type controls.[14] These animals are protected against weight gain upon being fed a high fat diet. They also have an increase in energy expenditure. A genetic linkage has been shown in humans between chromosome 4 near marker D4S403 where the CD38 gene is located and metabolic syndrome.[15] This data collectively suggest diseases linked to metabolic syndrome may be possible targets of CD38 inhibitors. Diseases where CD38 is over expressed, or where cellular NAD levels are depressed or desynchronized, could also be viable intervention venues.

There have been several reports in the literature of small molecule inhibitors of CD38.[16] However, many of these compounds possess moderate to weak inhibitory activity against the enzyme (IC50's > 1 μM) and/or have limited selectivity. Arguably, more potent and selective inhibitors are needed to better understand the biology around inhibition of CD38 and what, if any, potential uses these inhibitors might possess with regards to treating various human diseases tied into NAD biology.

Our initial efforts to identify novel potent inhibitors of CD38 started with internal screening campaigns of pre-existing compound collections, which produced a number of novel hits. An attractive exemplar from these screening efforts was compound **1** demonstrating a human IC50 = 1.21 μM (Figure 1).



1

Figure 1. CD38 thiazole screening hit.

Based on the modeling, the synthetic work was initially directed towards the bicyclic core and substitution at the 4-position of compound **1**. The modeling studies suggested that the primary carboxamide at the 3-position was detrimental in terms of potency due to interactions with W176. When compound **2** was synthesized to test this hypothesis the potency did improve by ~ 4-fold (IC50 = 298 nM) against the human enzyme (Figure 2).

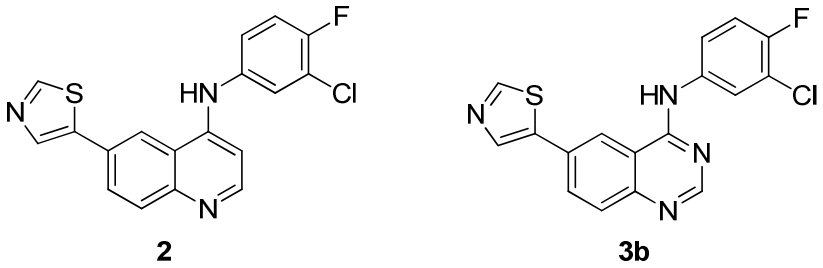


Figure 2. Thiazoloquinoline and thiazoloquinazoline CD38 inhibitors.

The corresponding quinazoline **3b** (Figure 2) was synthesized as well and found to have similar potency to the original hit compound **1** (IC50 = 1.20 μM), which is a 4-fold loss in CD38 potency relative to **2**; however we felt this to be an acceptable trade-off given the improved synthetic tractability of the quinazoline versus the quinoline for rapid SAR expansion. As the SAR became more refined, additional synthetic work was done on a quinolin-2-one core, which arose from the SAR established on the quinazoline template.

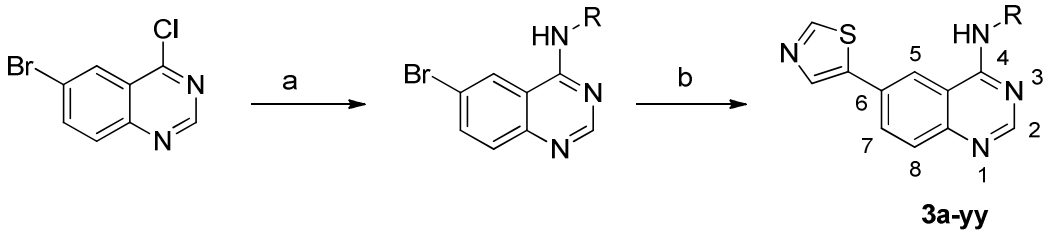
Described herein are our efforts to identify, synthesize and biologically evaluate a series of novel potent small molecule inhibitors of CD38 that arose from screening hit **1**. Several of these inhibitors were shown to elevate NAD in various tissues upon oral administration. The identification of these inhibitors should allow for a more thorough understanding of CD38 pharmacology in animals and how elevating this important cofactor, in NAD deficit animal models, affects whole body physiology.

Chemistry

The following chemistry sections describe the compounds that were made as part of a thorough investigation of the SAR around the original screening hit **1**. The biochemical data for these compounds is described in the Results and Discussion section. As mentioned earlier, to more rapidly probe the SAR around compound **1**, chemistry focused on final targets that incorporated a quinazoline ring in place of the quinoline. Synthetic work started on C4 substituted analogs containing amino substituted aryl, heteroaryl

or saturated 6-thiazoloquinazolines via a two step sequence whereby 6-bromo-4-chloroquinazoline was treated with the requisite aniline or amine followed by a palladium mediated Stille coupling with commercially available 5-tributylstannylthiazole (Scheme 1).

Scheme 1. Synthesis of 4-amino substituted thiazoloquinazolines.[a]

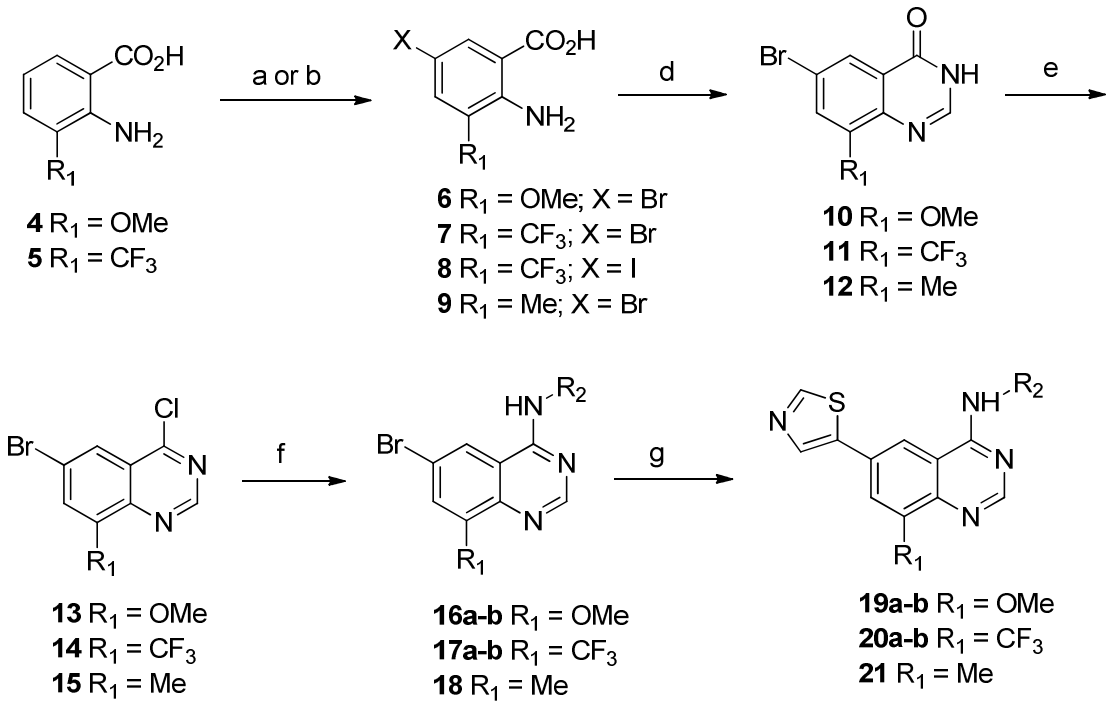


3a-yy

[a]Reagents and conditions: (a) RNH_2 (see Tables 1 and 2 for specifics with regards to what R groups were incorporated into the final compounds **3a-yy**), rt, CH_3CN; (b) CuI, K_2CO_3, rt or heating to 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF.

Chemistry was then expanded to investigate C8 and/or C2 substituted quinazolin(on)e analogs. The C8 analogs started with either the 3-methoxy or 3-trifluoromethylanthranilic acids **4** and **5**, halogenation generated the respective 5-bromo or 5-iodoanthranilic derivatives **6**, **7** or **8**. The quinazolin-4-one ring was synthesized from these intermediates or from the commercially available 3-methyl-5-bromoanthranilic acid **9** via heating with formamide to 165 °C yielding compounds **10-12** (anthranilic acid **8** was used to synthesize analogs found in Scheme 4). Treatment of the quinazolin-4-ones with thionyl chloride under thermal conditions generated the 4-chloroquinazolines **13-15** in good yields. The last two steps were performed under the same conditions as described in Scheme 1 to provide the desired final targets (**19a-b**, **20a-b** and **21**).

Scheme 2. Synthesis of C8 substituted quinazolines.[a]

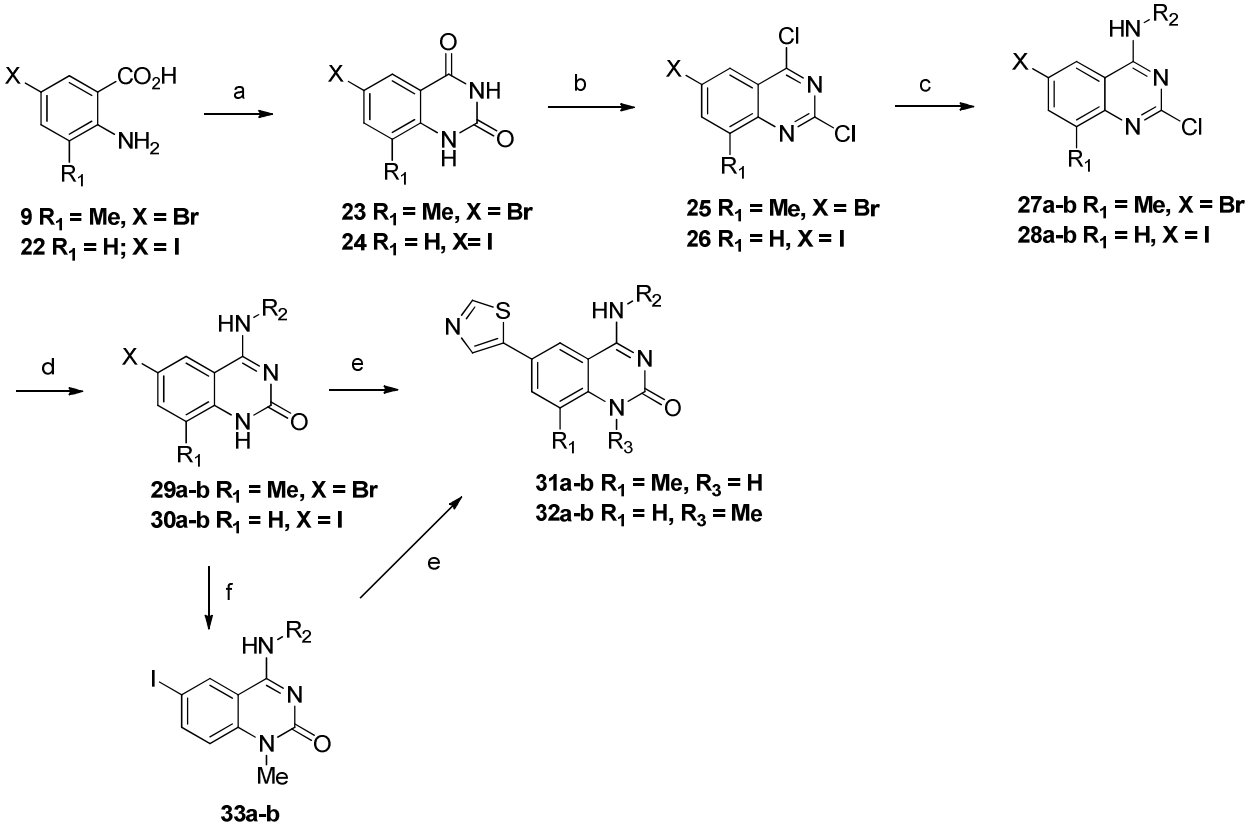


[a]Reagents and conditions: (a) when R_1 = OMe, Br_2, $CHCl_3$, 0 °C to rt; (b) when R_1 = CF_3, 48% HBr, H_2O, 30% H_2O_2, 75 °C or NIS, rt, DMF; (d) $HCONH_2$, 165 °C; (e) $SOCl_2$, $DMF_{(cat)}$; (f) R_2NH_2 (R_2 equals 3-Cl-4-F-Ph or trans 4-CONHMe-cyclohexyl), rt, CH_3CN; (g) CuI, K_2CO_3, rt or heating to 100-140 °C, $PdCl_2$·dppf·CH_2Cl_2, 5-tributylstannyl thiazole, DMF.

The chemistry then targeted C2 substituted compounds (several of these final targets also incorporated C8 functionality as well) on the quinazolin(on)e ring. It was found that incorporation of a primary amino moiety at that position provided potent CD38 inhibitors (both mouse and human), although many of these compounds suffered from either poor solubility and/or permeability (data not shown). A more productive functional group turned out to be the primary hydroxyl residue (drawn as the keto tautomer in Scheme 3). The synthesis to get into these compounds started with either 5-bromo-3-methylanthranilic acid **9** or 5-iodoanthranilic acid **22** (commercial) which upon melting with urea provided the 2,4-quinazolinones **23** or **24**. Chlorination with $POCl_3$ then cleanly provided the 2,4-dichloroquinazolines **25** and **26**. Reaction of these intermediates with a substituted aniline or saturated amine generated the desired 4-amino-2-chloroquinazolines **27a-b** and **28a-b**. Hydrolysis with acetic acid at 90 °C, provided compounds **29a-b** or **30a-b**. These compounds could then be converted to the final

product using the same coupling conditions as previously described to give **31a-b** or **32a-b**. Compound **30** could also be N-alkylated before the thiazole was installed to generate compounds **33a-b** followed by thiazole incorporation (Scheme 3). Unfortunately, attempted N-alkylation of compound **29** provided predominantly the O-alkylated product presumably due to the steric crowding imparted by the C8 methyl group. An alternative route into these compounds was devised (Scheme 5).

Scheme 3. 2-Quinazolinone synthesis.[a]

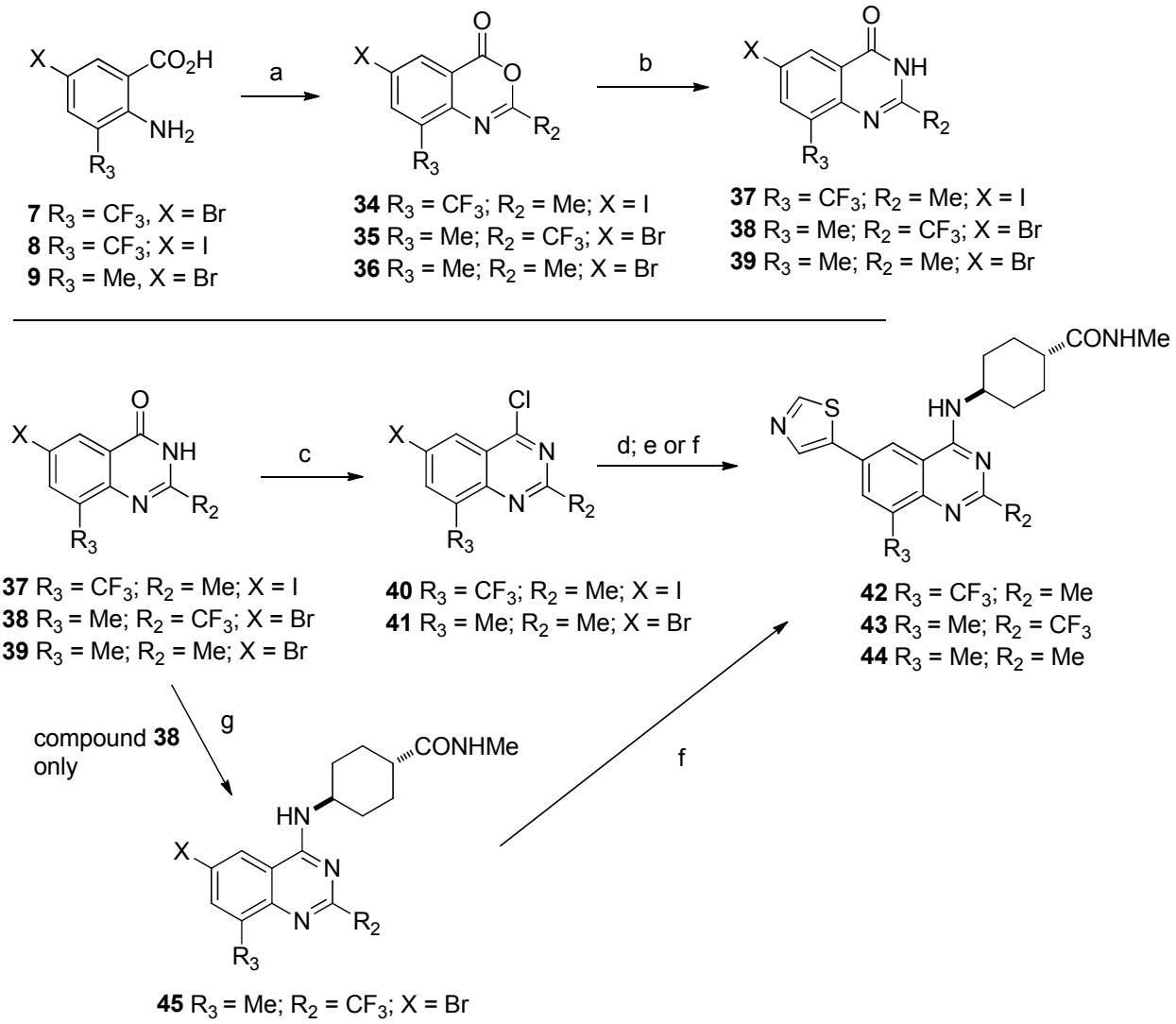


[a]Reagents and conditions: (a) urea, melt (b) $POCl_3$, 105 °C; (c) iPr_2NEt, R_2NH_2 (see Table 3 for the specific R_2 groups that were incoroporated into the final targets), iPrOH; (d) HOAc, 90 °C; (e) CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (f) NaH, MeI, DMF.

The remaining C2 and C8 substituted quinazolines were synthesized as outlined in Scheme 4. The starting materials for all of these were the 3-substituted anthranilic acids **7-9**. Treatment of these acids

with either acetic or trifluoroacetic anhydride yielded the corresponding 2-trifluoromethyl or 2-methyl-4H-benzo[d][1,3]oxazin-4-ones **34-36**. Heating these intermediates in the presence of either NH_4OAc or NH_4OH, afforded the 2-substituted quinazolin-4-ones **37-39** in good yield. The C4 amine could then be incorporated either by chlorination ($POCl_3$), followed by thermal addition of the desired amine (step d) or via a direct amine coupling reaction (CH_3CN, DBU, pyBOP, 75 °C – step g), to afford the 4-aminoquinazolines (it should be noted that for these final targets the trans 4-amino-N-methylcyclohexylcarboxamide was used as the amine based on potent biochemical data for a close analog compound **3oo** shown in Table 2). The final step was a palladium mediated coupling of either 5-tributylstannylthiazole or 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] with the 6-bromo or 6-iodo-4-aminoquinazoline to give the final targets **42-44**.

Scheme 4. C2 and C8 substituted thiazoloquinazoline synthetic routes.

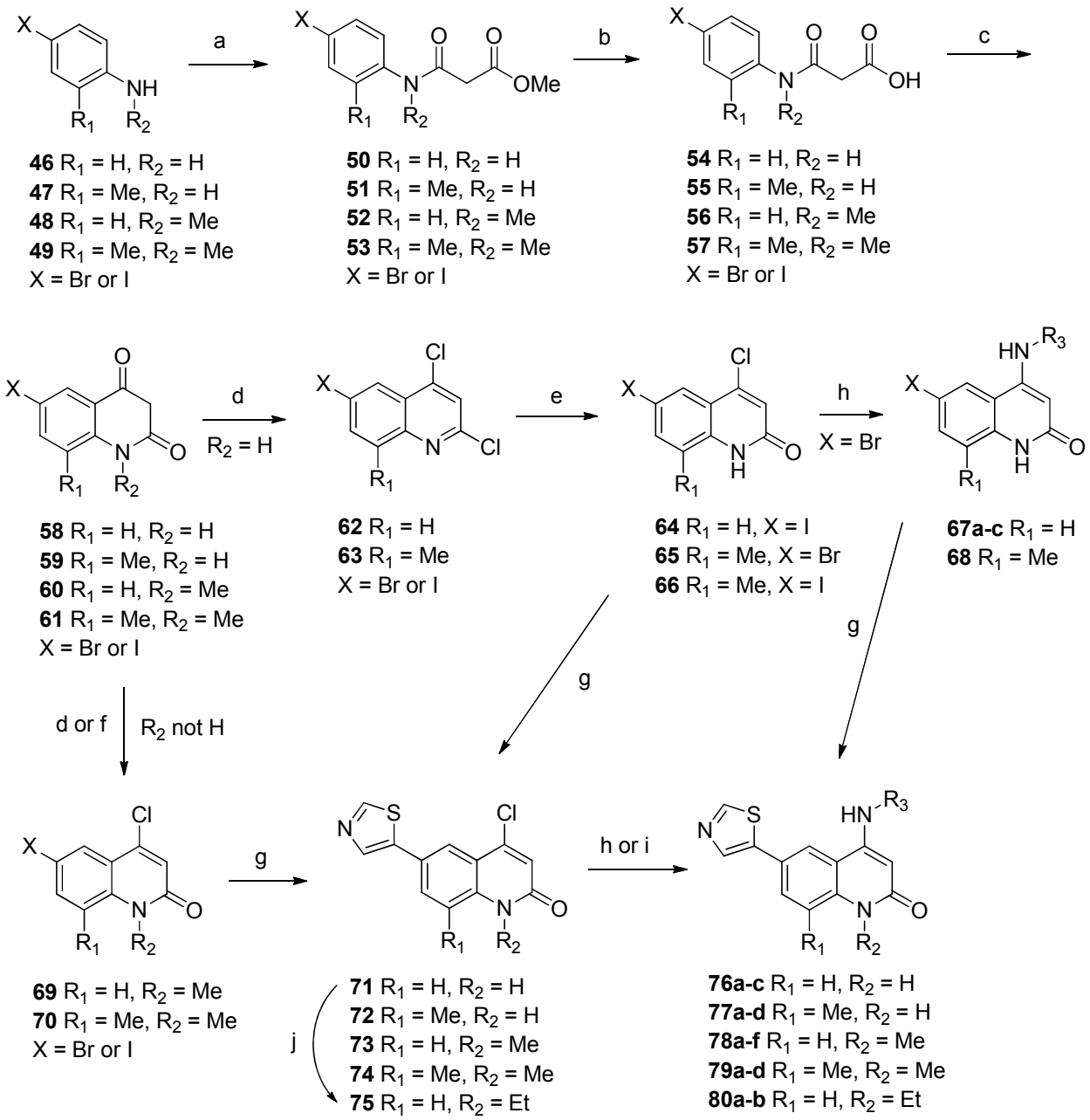


[a]Reagents and conditions: (a) Ac_2O or TFAA, reflux; (b) NH_4OAc or NH_4OH, 150 °C; (c) $POCl_3$, toluene, iPr_2NEt; (d) trans 4-NH_2-cyclohexyl-CONHMe, iPr_2NEt, CH_3CN, 70 °C; (e) CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (f) CuI, CsF, $(Ph_3P)_4Pd$, 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole, DMF, 90 °C; (g) DBU, pyBOP, trans 4-NH_2-cyclohexyl-CONHMe, CH_3CN.

The final synthetic efforts were directed towards the thiazoloquinolin-2-ones. The quinolin-2-one core was synthesized from appropriately substituted and halogenated anilines. Acylation of the starting aniline with methyl malonylchloride provided the carboxamides **50-53**, which upon hydrolysis generated the corresponding carboxylic acids **54-57**. The acids were cyclized under thermal acidic conditions affording the quinolin-2,4-diones **58-61**, The 2,4-diones were then converted to the 2,4-

dichloroquinazolines **62-63** upon treatment with $POCl_3$ when R_2 equaled H. Selective hydrolysis (HCl, CH_3CN, 80 °C) then provides the 4-chloroquinolin-2-ones **64-66**. The C4 amine was then attached under thermal conditions (step h) followed by incorporation of the 6-thiazole moiety as before (step g) generating final targets **76a-c** and **77a-d**. It should be noted that incorporation of the amine (step h) via this route was limited to the thermal conditions due to chemoselective issues between the C4 chloro moiety and the 6-halo substituent under the palladium catalyzed conditions used in step i. Alternatively, the last two steps could be switched (step g first followed by either step h or i). This sequence then allowed the amine to be attached either under thermal or palladium catalyzed conditions. When the quinoline-2,4-diones were N-substituted (compounds **60-61**), they could be converted directly to the 4-chloroquinolin-2-ones **69-70** upon treatment with either PCl_3 or $POCl_3$. The 4-chloroquinolin-2-ones were then reacted under the aforementioned conditions (step g) generating the 6-thiazolo-4-chloroquinolin-2-ones **73-**74. Compound **75** was synthesized from compound **71** upon treatment with EtI, NaH, DMF – step j. The two synthetic pathways (NH and N-substituted) merged at this point and were converted to final products, **78a-f**, **79a-d** and **80a-b** (Scheme 5). In some cases compounds, where R_1 equals H, could be directly alkylated to provide their N1 alkylated products (the aforementioned compound **75** is one example of this chemistry) prior to attachment of the thiazole functionality at C6. As mentioned earlier, when a substituent larger than hydrogen was placed at C8 the O-alkylated product predominated. Therefore, for the final targets where C8 was not hydrogen, the desired N-alkyl group was installed on the starting aniline before cyclization.

Scheme 5. Synthetic route into the 6-thiazoloquinazolin-2-ones.



[a]Reagents and conditions: (a) methylmalonyl chloride, TEA, EtOAc; (b) THF, MeOH, 2.0 N NaOH; (c) CH_3SO_3H, P_2O_5, 70 °C; (d) $POCl_3$, 100 °C; (e) $HCl_{(conc)}$, 80 °C, CH_3CN or MsOH, dioxane, H_2O, 105 °C; (f) PCl_3, 80 °C; (g) CuI, CsF, $(Ph_3P)_4Pd$, 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole, DMF, 90 °C or CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (h) CH_3CN, R_3NH_2 (see Table 4 for the specific R_3 groups that were incorporated into the final compounds), 150-160 °C, NMP; (i) R_3NH_2 (see Table 4 for the specific R_3 groups that were incorporated into the final compounds), Pd_2dba_3, BrettPhos, NaOtBu, 110 °C; dioxane; (j) EtI, NaH, DMF

Results and Discussion

As we were unable to obtain a liganded crystal structure within this class of compounds with either the human or mouse enzyme under numerous conditions, a binding model was constructed utilizing a crystal structure of hCD38 E226Q mutant bound with nicotinamide mononucleotide (NMN) (code: 4OGW).[18] For our modeling and docking calculations, the hCD38 structure was prepared by systematically adding hydrogens to both the protein and NMN using a standard in-house procedure.[19] Previously, we collected and reported CD38 biochemical and kinetic evidence on compounds similar to this series[20] suggesting that the nitrogen of the thiazole ring may be involved in a reversible, short-lived catalytic reaction with substrate intermediates produced by CD38. Therefore, we modeled the ribose substrate intermediate in the hCD38 binding site by removing the nicotinamide ring from crystallographically bound NMN. With a model of this hCD38/ribose intermediate complex in hand; the thiazole ring of an initial 3D model of compound **1** was pre-orientated in the hCD38 catalytic site such that the nitrogen of that ring was in close proximity to the 1'-position of the ribose-5-phosphate intermediate. The initial structure of **3b** was generated using the CONCORD program.[21] After orientating the compound, an in-house docking procedure, MVP, was used to model **1** in the active site of hCD38 in the presence of the ribose-5-phosphate intermediate.[19]

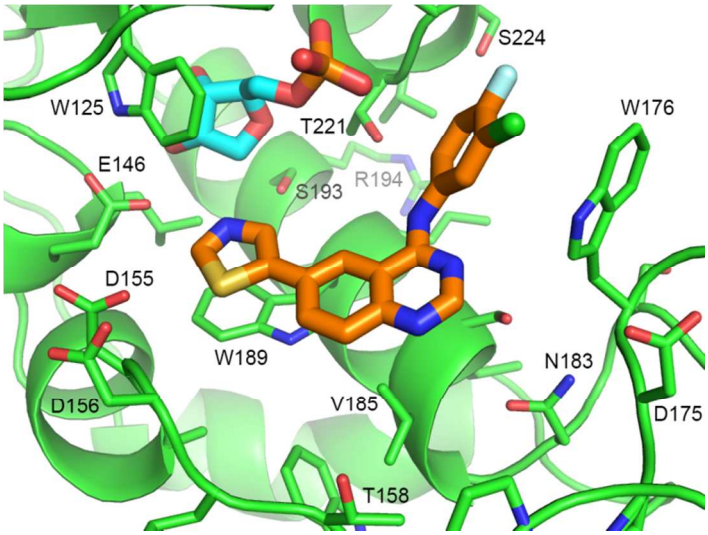
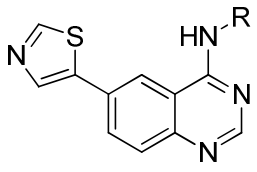


Figure 3. Docking model of **3b** (in orange) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose substrate intermediate is shown in cyan.

This modeling suggests that the majority of the binding affinity of this compound may be due to a π-π stacking interaction between the thiazolophenyl ring of the bicyclic core and the indole of W189 (Figure 2). Although not explicitly evident in our binding model, T221 may form a hydrogen bond with the 4-amino nitrogen, providing additional binding affinity as several compounds that removed this hydrogen through the addition of a methyl substituent on this nitrogen were significantly less active, as were oxygen and sulfur heteroatom replacements (data not shown). This model of hCD38 E226Q bound with a ribose-5-phosphate intermediate was utilized for further docking studies to facilitate inhibitor design, improve potency and better interpret the SAR of synthesized compounds.

Mouse enzymatic data was collected in parallel with the human data in an effort to identify an inhibitor that could be used for both acute and chronic rodent efficacy studies. Based on the KO data mentioned earlier, the mouse was considered a good pre-clinical species with which to conduct target validation studies. The biochemical assays were the initial filtering mechanism by which compounds were progressed with a goal of identifying inhibitors which had IC50's < 100 nM, although inhibitors which had potency less than this were still considered if they possessed other properties condusive to in vivo efficacy studies (e.g. good physiochemical properties and/or good selectivity profiles).

Table 1.



compound	R	human IC50 (μM)	std dev[a] (μM)	mouse IC50 (μM)	std dev[a] (μM)

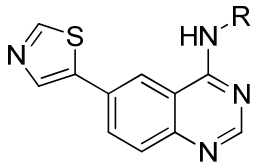
1	--	1.21	0.29	0.205	0.22
2	--	0.298[b]	0.037	0.019	0.004
3a	Ph	0.84	0.34	0.046	0.078
3b	3-Cl-4-F-Ph	1.20	0.38	0.032	0.010
3c	4-Cl-Ph	1.32	0.27	0.026	0.005
3d	3-Cl-Ph	0.70	0.14	0.019	0.005
3e	2-Cl-Ph	3.61	0.63	0.058	0.025
3f	4-F-Ph	2.33	0.76	0.031	0.010
3g	3-F-Ph	0.97	0.12	0.031	0.012
3h	2-F-Ph	4.68	0.88	0.045	0.010
3i	4-Me-Ph	1.23	0.15	0.022	0.009
3j	3-Me-Ph	1.16	0.33	0.017	0.006
3k	2-Me-Ph	12.8	3.19	0.065	0.022
3l	3-CF_3-Ph	2.15	0.4	0.042	0.020
3m	4-SO_2NH_2-Ph	0.70	0.05	0.027	0.005
3n	4-$NHSO_2Me$-Ph	0.97	0.09	0.050	0.012
3o	3-SO_2Me-Ph	2.69	1.27	0.031	0.017
3p	4-$CONH_2$-Ph	0.56	0.16	0.024	0.009
3q	4-(5-oxazole)-Ph	0.84	0.32	0.013	0.003
3r	4-(1-imidazole)-Ph	0.46	0.08	0.026	0.004
3s	4-(N-γ-lactam)-Ph	1.46	0.24	0.026	0.006
3t	2-pyridyl	12.3	2.85	0.097	0.034
3u	3-pyridyl	3.94	0.41	0.059	0.010
3v	4-pyridyl	0.95	0.28	0.024	0.005

[a]The standard deviation was determined where the $N \geq 4$. [b]$N = 3$.

Table 1 outlines the initial exploration of the C4 position of the thiazoloquinazolines, where the focus was probing anilines. The SAR for this set of compounds shows that none have equivalent human potency when compared to compound **2**, although two compounds were within a 2-fold difference (compounds **3p** and **3r**). In fact, the potency against the human enzyme stayed fairly flat with respect to compound **3b**, with the exception of compound **3t** where there was ~10-fold difference. It was also noteworthy that many compounds were close in activity to phenyl derivative **3a**. The pyridyl analogs **3t-v** showed that as the nitrogen atom moved from the 2 to the 4-position potency against the human enzyme increased. The docking models support this finding as the nitrogen of the 4-pyridyl compound was much more solvent exposed than either the 2- or 3-pyridyl analogs. Another finding was that as the substituent on the aromatic ring was moved to the ortho position from the para position the potency decreased (see compounds **3c** versus **3e**, **3f** versus **3h** and **3i** versus **3k**). According to the modeling results, the ortho substituents result in a non-planar conformation of the anilino ring which leads to a detrimental interaction between the ortho group and the hydrophilic cleft near T221 of the hCD38 protein. Interestingly, the meta substituted compounds **3d** and **3g** were found to be more potent that their para counterparts against the human enzyme. It was also noted that the protein tolerated more elaborate functionality as evidenced by compounds **3m-s**. Modeling suggests that functionalty can extend towards solvent coming off the 4-position perhaps explaining why such a diverse set of functional groups appear to be tolerated. All of the compounds had IC50's ≤ 0.050 μM, other than **3e**, **3k**, **3t** and **3u** against the mouse enzyme. The difference between the mouse and human potency could be explained by the fact that the human enzyme assay used for our high throughput biochemical screening contained non-glycosylated protein; whereas the mouse enzyme was fully glycosylated (there is 75% identity between the human mouse protein sequences as calculated based on an alignment of the primary full-length amino acid sequences of human and mouse CD38 downloaded from UNIPROT (http://www.uniprot.org)). The alignment was calculated using standard procedures from the aforementioned MVP program.[19] It is unclear why the glycosylation state of the enzyme provides such a wide potency difference between the two biochemical assays. One hypothesis is that the non-glycosylated human enzyme has more hydrolytic

activity with the exchange reaction product. Not having the glycosyl residues on the protein opens it up and allows water into the active site producing less of the exchange reaction product leading to less inhibition. However, we were able to obtain human kinetic data from fully glycosylated human protein albeit in a non-high throughput assay on select compounds (Table 7).

Table 2. Thiazoloquinazoline biochemical data.



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1	--	1.21	0.29	0.205	0.22
2	--	0.298[b]	0.037	0.019	0.004
3w	cyclopropyl	7.83	2.82	0.144	0.075
3x	cyclobutyl	1.93	0.19	0.031	0.014
3y	cyclopentyl	0.281	0.083	0.012	0.003
3z	cyclohexyl	0.132	0.020	0.013	0.002
3aa	2-tetrafuranyl	1.68	0.44	0.086	0.022
3bb	4-pyranyl	1.06	0.37	0.051	0.027
3cc	3-pyranyl	1.03	0.16	0.045	0.026
3dd	4-piperidinyl	> 30.2	0	0.275	0.094
3ee	3-piperidinyl	> 30.2	0	0.296	0.180
3ff	4-piperidinyl-N-formamide	0.149	0.036	0.034	0.005
3gg	4-thiopyranyl dioxide	1.22	0.21	0.085	0.007
3hh	4-methylcyclohexyl	0.117	0.027	0.007	0.002
3ii	trans-2-methylcyclohyhexyl	0.140	0.011	0.010	0.003

3jj	cis-2-methylcyclohexyl	0.049	0.006	0.012	0.002
3kk	3-methylcyclohexyl	0.142	0.013	0.015	0.005
3ll	4-hydroxycyclohexyl	0.406	0.112	0.025	0.015
3mm	trans-4-$CONH_2$-cyclohexyl	0.078	0.026	0.009	0.002
3nn	trans-3-$CONH_2$-cyclohexyl	0.971	0.172	0.037	0.004
3oo	trans 4-CONHEt-cyclohexyl	0.092	0.027	0.012	0.002
3pp	cis 4-CO_2H-cyclohexyl	> 30.2	0	0.459	0.094
3qq	trans 4-CO_2H-cyclohexyl	0.498	0.149	0.040	0.015
3rr	trans -4-SO_2Me-cyclohexyl	0.959	0.155	0.135	0.036
3ss	cis-4-(N-γ-lactam)cyclohexyl	8.54	1.35	1.11	0.20
3tt	trans-4-(N-γ-lactam)cyclohexyl	1.96	0.57	0.077	0.024
3uu	ethyl	11.4	4.12	0.145	0.046
3vv	isopropyl	3.35	0.30	0.075	0.023
3ww	t-butyl	2.3	0.42	0.066	0.022
3xx	2-hydroxyethyl	23.4[c]	4.78	0.189	0.060
3yy	2-methoxyethyl	> 30.2	0	0.298	0.089

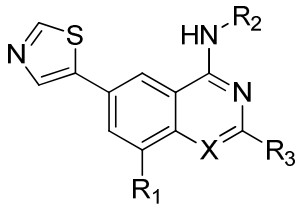
[a]During curve fitting if the desired IC50 value was outside the test concentration range, ActivityBase XE reports the value with a < or > modifier which was not included in the statistics calculation. The IC50 and standard deviation were determined where the N ≥ 4. [b]N = 3. [c]N = 3. Here there were 2 values reported by ActivityBase XE as > 30.2 μM.

A structure-activity relationship for analogs in which the C4 position was an aliphatic amine is outlined in Table 2. In general, these compounds achieved equivalent if not superior potency to compound **3b**. In fact, compounds **3y-z**, **3ff**, **3hh-kk**, **3mm** and **3oo** had potency equivalent or better than compound **2**. Many of these compounds were very hydrophobic in nature. Interestingly, the urea or carboxamides (**3ff**, **3mm** and **3oo**) showed very good potency as well. The trans/cis stereochemistry also appeared to be important on the 4-position of the cyclohexyl ring as demonstrated by comparing compounds **3pp** to **3qq** and **3ss** to **3tt**, although when the substituent was at the 2-position and

hydrophobic, (e.g. **3ii** and **3jj**) the same trend no longer held. The primary amide functionality found in compound **3mm**, which proved to be one of the most potent inhibitors within this series, was consisitent with the SAR from the aromatic series (Table 1, **3p**). In fact, our docking models of these 4-amido substituted compounds suggest that this primary amide may be more solvent exposed than others and could potentially hydrogen bond with the alcohol side chain of S224 in the hCD38 protein, a residue near its surface and important for binding some substrates. The directionality of the amide was also important based on a > 10-fold potency difference between compounds **3mm** and **3nn**. The heterocyclic compounds exhibited mixed potency. Compounds **3aa-cc** and **3gg** possessed potency similar to compound **3b**, however, the piperidines, **3dd** and **3ee**, were completely inactive against the human enzyme. This result suggests that CD38 does not tolerate a positive charge in the space with which these rings reside; interestingly our model did not predict this result. All non-cyclic amines turned out to be less potent than **3b**. All of the saturated compounds, with the exception of **3ss**, were found to have IC50's < 0.5 μM against the mouse enzyme with many showing low nanomolar potency. As mentioned earlier, a potent mouse inhibitor was necessary in order to conduct in vivo efficacy studies. Many of these inhibitors achieved the desired potency (IC50 < 100 nM).

Having established some SAR at the 4-position on the quinazoline ring, it was expanded to explore both the C2 and C8 positions. The biochemical data for these compounds is shown in Table 3.

Table 3. C2 and C8 substituted thiazoloquinazoli(no)ne biochemical data.



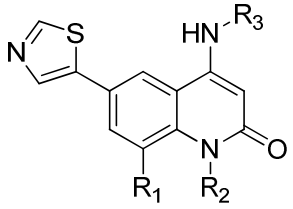
compound	R_1	R_2	R_3	X	human IC50 (μM)[a]	std dev (μM)[a]	mouse IC50 (μM)[a]	std dev (μM)[a]
19a	OMe	3-Cl-4-F-Ph	H	N	4.71	1.48	0.009	0.004
19b	OMe	4-$CONH_2$-Ph	H	N	1.16[b]	0.22	0.004[c]	0.003
20a	CF_3	3-Cl-4-F-Ph	H	N	1.59	0.24	0.139	0.048
20b	CF_3	4-$CONH_2$-Ph	H	N	0.263	0.114	0.008	0.003
21	Me	3-Cl-4-F-Ph	H	N	0.206	0.036	0.010	0.002
31a	Me	3-Cl-4-F-Ph	OH	N	0.028	0.009	0.004	0.0008
31b	Me	trans 4-MeO-cyclohexyl	OH	N	0.026	0.007	0.001	0.0003
32a	H	trans 4-OCH_2CH_2OMe-cyclohexyl	OH[d]	NMe	0.064	0.012	0.008	0.0002
32b	H	trans 4-MeO-cyclohexyl	OH[d]	NMe	0.053	0.013	0.008	0.001
42	CF_3	trans 4-CONHMe-cyclohexyl	Me	N	0.042	0.006	0.072	0.025
43	Me	trans 4-CONHMe-cyclohexyl	CF_3	N	0.245	0.083	0.037	0.013
44	Me	trans 4-CONHMe-cyclohexyl	Me	N	0.050	0.003	0.007	0.002

[a]During curve fitting if the derived IC50 value was outside the test concentration range, ActivityBase XE reports the value with a < or > modifier which was not included in the statistics calculation. The standard deviation was determined where the N ≥ 4. [b]N = 2. [c]N = 3. [d]Drawn as a hydroxyl residue, but in the keto form in the final product due to the N1 methyl substituent.

The anilines/amines, which provided some of the more potent inhibitors in the earlier examples described in Tables 1 and 2 or those that were were deemed to have more desirable physiochemical properties were used to assess how substitution at C2 and/or C8 impacted the potency against the human

enzyme. Compounds **19a** and **b**, where R_1 = OMe, both had IC50's greater than compounds **3b** and **3p** respectively. However, compounds **20a** and **b**, where R_1 = CF_3, had potencies either similar to or better than their comparator compounds **3b** and **3p**. Compound **21**, containing the C8 methyl, was the most potent C8 substituent examined within the 3-Cl-4-F-phenyl aniline series with an IC50 = 206 nM (> 5-fold potency increase versus **3b**). Compounds **31a-b** and **32a-b**, containing the C2 hydroxy substituent (drawn as the enol tautomer for clarity), dramatically increased the human potency with compound **31a** demonstrating a > 40-fold improvement versus compound **3b**. The remaining compounds in Table 3 (**42-44**), all had human IC50's ≤ 245 nM. The placement of a trifluoromethyl or methyl substituent at C8, compounds **42** and **44**, provided potent inhibitors (≥ 24-fold increase versus **3b** against the human enzyme). Interestingly, compound **43,** where the CF_3 moiety was placed at the C2 position, lost ~ 5-fold in potency when compared to compounds **42** and **44**.

Table 4. 6-Thiazoloquinolin-2-one biochemical data.



compound	R_1	R_2	R_3	human IC_{50} (nM)[a]	std dev (nM)[a]	mouse IC_{50} (nM)[a]	std dev (nM)[a]
76a	H	H	trans 4-CONHMe-cyclohexyl	3.4	0.9	0.9	0.19
77a	Me	H	trans 4-CONHMe-cyclohexyl	1.4[b]	0.53	< 0.18[c]	0
78a	H	Me	trans 4-CONHMe-cyclohexyl	4.8	2.5	1.1	0.52
79a	Me	Me	trans 4-CONHMe-cyclohexyl	5.3	2.6	0.78	0.42
80a	H	Et	trans 4-CONHMe-	58	34	6.1	4.0

			cyclohexyl				
76b	H	H	4-pyranyl	61	16	11	4.2
77b	Me	H	4-pyranyl	55	16	4.5	1.5
78b	H	Me	4-pyranyl	89	22	15	7.2
79b	Me	Me	4-pyranyl	52	15	6	3.7
76c	H	H	trans 4-OCH_2CH_2OMe-cyclohexyl	6	1.4	1.3	0.47
77c	Me	H	trans 4-OCH_2CH_2OMe-cyclohexyl	3.9	2.1	0.62[d]	0.46
78c	H	Me	trans 4-OCH_2CH_2OMe-cyclohexyl	7.3	1.8	1.9	0.68
79c	Me	Me	trans 4-OCH_2CH_2OMe-cyclohexyl	10	2.9	1.6	0.38
80b	H	Et	trans 4-OCH_2CH_2OMe-cyclohexyl	54	15	5.2	1.8
77d	Me	H	trans 4-OMe-cyclohexyl	3.8	1.3	0.65[d]	0.47
78d	H	Me	trans 4-OMe-cyclohexyl	13	2.1	1.6	0.49
79d	Me	Me	trans 4-OMe-cyclohexyl	15[e]	7.1	1.4[f]	0.38
78e	H	Me	trans 4-OH-cyclohexyl	14	5.6	2.5	1.4
78f	H	Me	H	10773	1836	163	28.1

[a]During curve fitting if the derived IC_{50} value was outside the test concentration range, ActivityBase XE reports the value with a < or > modifier which was not included in the statistics calculation. The standard deviation was determined where the $N \geq 4$. [b]N = 7 and 2 additional values were reported by ActivityBase XE as < 0.51 nM. [c]N = 7 and 2 additional values not included in the stats reported by ActivityBase XE as 0.18 and 0.36 nM. [d]Here there were 2 values reported by ActivityBase XE as < 0.18 nM. [e]1 value was reported by ActivityBase XE as < 1.51 nM. [f]Here there was 1 value reported by ActivityBase as < 0.18 nM.

We felt that compound **31a** now had the requisite potency at both human and mouse enzymes, particularly given the C4 substituent was not optimal based on our SAR to date. However, **31a** did not have good physicochemical properties, suffering from poor solubility and permeability (see Table 5 for specifics). Thus we sought to incorporate substituents that maintained potency while improving the physiochemical properties necessary for conducting in vivo studies. In particular, we identified four amines which we found to possess not only very good inhibitory properties, but good drug-like properties as well. Although many aromatic amines showed very good potency, none had sufficiently good physiochemical properties needed for in vivo studies. All of the 6-thiazoloquinolin-2-ones shown in Table 4 demonstrated very good potency against both the human and mouse enzymes with the exception of compound **78f**, which clearly shows the importance of the substitution off the amine at C4. In terms of the R_2 substituent, both hydrogen and methyl proved to be equipotent at CD38, although the methylated compounds were in general better physiochemically. For example, compounds **76a** and **76c** had poorer permeability and solubility than their methylated analogs **78a** and **78c** (see Table 5). When R_2 was ethyl, potency against the human enzyme was reduced when compared to the NH compound (see **80a** versus **76a** and **80b** versus **76c**). The C2 and C8 methyl analogs were also very potent against both enzymes (see compounds **79a-d**).

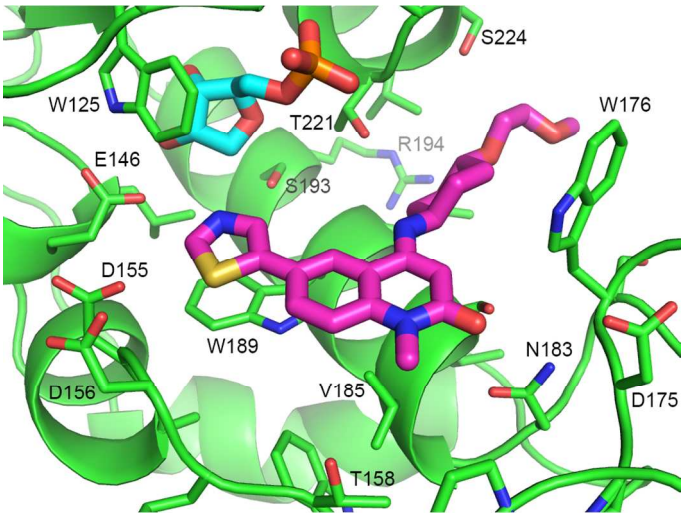


Figure 4. Docking model of **78c** (in magenta) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose-5-phosphate substrate intermediate is shown in cyan.

As was found from earlier compounds, the trans 4-N-methylcarboxamide cyclohexylamine containing compounds **76a-79a** and **80a** provided very potent inhibitors of both human and mouse enzyme (IC50's ≤ 58 nM versus human and ≤ 6.1 nM versus mouse). Several of the trans 4-alkoxy cyclohexylethers were found to provide potent inhibitors as well, with compounds **77c-d** showing IC50's < 4 nM on the human enzyme. The trans 4-hydroxycyclohexylamine analog **78e** was similar in potency to compounds **78c-d**, demonstrating that the additional functionality was not necessary for binding affinity. The C4 pyranyl amines **76b-79b**, although providing potent inhibitors, were in general less potent than either the ether or carboxamide C4 amines. The docking of compound **78c** (Figure 4) suggests that some of the additional binding affinity seen with compounds having the 4-methoxyethoxyl moiety may arise from increased hydrophobic interactions between this C4 group and W176. The 2-keto residue's ability to form a hydrogen bond with the side chain amide of N183 appears unlikely as the distance is too far (4.8 and 4.7 angstroms to the N183 amide and carboxy groups, respectively), the angle is bad, and the amide group of N183 is tied up in a critical loop-stabilizing hydrogen bond with a backbone residue (C180) and thus, is not available for interacting with the inhibitor. As our goal was to identify a molecule which was suitable for in vivo PK studies, those compounds with sufficient potency for physiochemical property profiling had their cell permeability, solubility and hydrophobicity measured. These data are shown in Table 5.

Table 5. Measured physiochemical properties on a select group 6-thiazoloquin(az)olin-2-ones.

compound	permeability (nm/sec)[a]	CLND (μM)[a]	ChromLogD pH = 7.4[a]
31a	ND	11	4.19
32a	30	≥ 429	2.47

32b	47	≥ 410	2.51
76a	< 3.0	74	1.45
76c	52	39	2.40
77d	202[b]	36[b]	2.85[c]
78a	9.9	361	2.03
78b	200	350	2.37
78c	162[d]	≥ 364[d]	3.22[e]
79a	21	339	2.34
79c	195[f]	≥ 323[b]	3.54[f]
79d	300[f]	≥ 340[f]	3.47[f]

[a]N = 1. [b]N = 4. [c]N = 3. [d]N = 6. [e]N = 5. [f]N = 2.

The criteria for progression of compounds into PK studies were as follows: permeability (Madin-Darby canine kidney (MDCK) cells) ≥ 100 nm/sec, solubility (measured by chemiluminescent nitrogen detection (CLND)) ≥ 100 μM and ChromLogD values < 4.0. All of the compounds in Table 5 met the ChromLogD criteria (compound **31a** was inserted as a comparator and did not meet these criteria as indicated earlier). However, compounds **32a-b**, **76a** and **c**, **77d**, **78a** and **79a** failed to meet either the permeability and/or solubility filters prescribed. The remaining four compounds **78b**, **78c**, **79c**, and **79d** passed all three criteria and thus were evaluated in mouse PK studies. The key in vivo data are shown in Table 6.

Table 6. Mouse in vivo PK data for compounds **78b**, **78c**, **79c** and **79d**.

compound	**dose (mg/kg)**	**route**	**Cl (mL/min/kg)**	**Vdss (L/kg)**	**t½ (hr)**	**%F**	**Cmax (ng/mL)**	**DNAUC (ng/hr/mL)**
78b	30	po[a]			ND	ND	ND	[c]
	1	iv[b]	44.7	1.2	1.6		1110	371

78c	30	po			3.5	87	10800	685
	2	iv	21	1.3	5.3		2980	779
79c	10	po			1.3	46	3810	930
	1	iv	8.9	0.32	0.46		2500	2030
79d	30	po			3.2	89	6250	443
	2	iv	33.4	1.1	1.2		1480	497

[a] p.o. formulation conditions: 2% DMSO:5% solutol in 93% (20%SBE-CD, CB pH 3.0); [b]i.v. formulation conditions: 10% DMSO:10% PEG400:80% (20% SBE-CD, citrate buffered pH 6.0); [c]A DNAUC could not be calculated as the compound exceeded the analytical range.

The PK data for the four compounds showed that compounds **78b** and **79d** had clearances that were in the moderate to high range, and thus were not chosen for the in vivo studies. Compounds **78c** and **79c** had better clearance values with, **79c** being the best. However, **78c** had a higher volume of distribution at steady state (Vdss) (1.3) and a longer terminal half-life (both i.v. and p.o.) than **79c**. Based on its overall profile, compound **78c** was selected to carry out in vivo studies. In an effort to maximize the oral exposure of **78c**, a number of formulations were evaluated and it was found that a 5% solutol:95% (20% SBE-CD, citrate buffered pH 3.0) vehicle increased the dose normalized area under the curve (DNAUC) from 685 ng/hr/mL to 10525 ng/hr/mL (> 15-fold increase). Also, human wild type (WT) enzymatic data was collected in an attempt to better define the binding affinity of these compounds versus the earlier reported biochemical data in Table 4. As can be seen in Table 7, compounds **78b**, **78c**, **79c** and **79d** all demonstrated low to subnanomolar Ki's against the fully glycosylated protein.

Table 7. Potency data for compounds **78b**, **78c**, **79c** and **79d** against both the biochemical and wild type (WT) human CD38 enzyme.

compound	**human IC_{50} (nM)[a]**	**human Ki WT enzyme (nM)[b]**
78b	89	14

78c	7.3	0.3
79c	10	0.7
79d	15	0.45

[a]Values from Table 4. [b]N = 1.

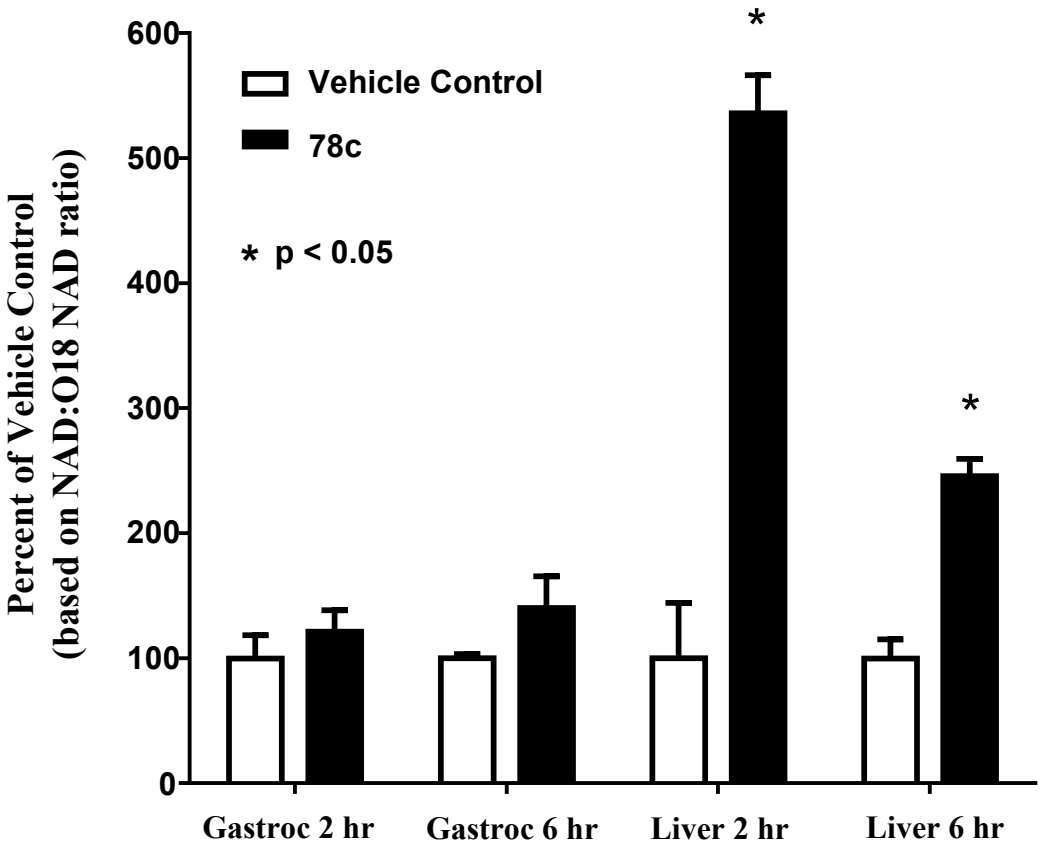
Because we did not have a robust cellular assay that allowed determination of CD38-mediated increases in intracellular NAD, we developed an in vivo-based method for determining changes in the levels of NAD in various tissues. The diet induced obese (DIO) mouse (60% high fat diet) was utilized because this model demonstrated a deficit in steady-state NAD levels relative to lean controls. Thus an in vivo study was performed with compound **78c** looking at changes in NAD levels versus control animals in liver and muscle (gastrocnemius) tissue at 2 and 6 hours after a 30 mg/kg dose. As can be seen in Figure 5, NAD levels were significantly increased at both the 2 and 6 hour time points in liver (536% and 246% respectively over control animals). The NAD levels were also elevated in gastrocnemius muscle, although to a smaller extent (121% and 141% respectively versus lean controls). The lower NAD levels in muscle could be attributed to lower drug levels in this tissue versus liver (data not shown). The NAD levels returned to controls once drug levels dropped below the IC50 (appproximately 12-15 h in liver and 8-10 h in muscle – data not shown). This study validates the hypothesis that inhibition of CD38 leads to modulation of NAD, the endogeneous substrate, in an in vivo setting. Having demonstrated that CD38 inhibition can elevate NAD levels in vivo, compound **78c** should provide a valuable tool for evaluation of CD38-mediated pharmacology in animal models of disease in which a NAD deficiency exists.

Figure 5. NAD levels in liver and gastrocnemius tissue versus lean controls after treatment with compound **78c**.

Tissue	**2 hr post-dose**			**6 hr post-dose**		
	Vehicle	**78c (30 mg/kg)**	**p-value**	**Vehicle**	**78c (30 mg/kg)**	**p-value**

Gastrocnemius	100 ± 18	121 ± 17	0.2122	100 ± 3	141 ± 25	0.0520
Liver	100 ± 44	536 ± 31	0.0001	100 ± 15	246 ± 14	0.0003

Values presented as percent of vehicle control ± standard deviation.



Conclusion

In summary, we were able to identify a series of 6-thiazoloquin(az)olin-2-one-based CD38 inhibitors with heretofore unprecedented potency through detailed SAR around screening hit **1**. In particular, compound **78c** was found to not only be a very potent inhibitor, but also had suitable physiochemical properties to serve as a robust tool for in vivo studies. In addition, we demonstrated that compound **78c** was able to elevate NAD in both liver and gastrocnemius tissue when dosed orally in a

DIO mouse. The identification and development of these potent small molecule inhibitors will enable a detailed analysis of how elevating NAD in NAD deficit states affects whole body physiology and more importantly whether this approach may be a viable strategy to treat diseases where elevation of NAD may provide therapeutic benefit.

Experimental Section

All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. The following solvents and reagents have been abbreviated: tetrahydrofuran (THF), diethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (CH_2Cl_2 or DCM), trifluoroacetic acid (TFA), formic acid (FA), N,N-dimethylformamide (DMF), methanol (MeOH), 1,2-dimethoxyethane (DME), N-methylpyrrolidine (NMP), acetonitrile (CH_3CN or ACN or MeCN), chloroform ($CHCl_3$), phosphorous oxychloride ($POCl_3$), magnesium sulfate ($MgSO_4$), triethylamine (Et_3N), 2-propanol (iPrOH), N,N-diisopropylethylamine (iPr_2NEt), sodium hydroxide (NaOH), t-butylmethylether (TBME), acetic acid (AcOH or HOAc), ethanol (EtOH), di-t-butyldicarbonate (BOC_2O), sodium sulfate (Na_2SO_4), N,N-dimethylacetamide (DMA), sodium bicarbonate ($NaHCO_3$), potassium carbonate (K_2CO_3), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), azobisisobutyronitrile (AIBN), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), dithiothreitol (DTT). All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (60F-254, E. Merck) and visualized with UV light, cerium molybdate, or 5% phosphomolybdic acid in 95% ethanol. Final compounds were typically purified either by flash chromatography on silica gel (E. Merck 40-63 mm), radial chromatography on a Chromatotron using prepared silica gel plates, on a Biotage Horizon or ISCO Combiflash pump and fraction collection system utilizing prepacked silica gel.

Analytical purity was assessed by reversed phase ultra-performance liquid chromatography (RP-UPLC) on a Waters SQD. The UPLC analysis was conducted utilizing a Phenomenex Kinetex 1.7 µm 2.1 x 50 mm XB-C18 column at 40 °C. The gradient employed was: mobile phase A: water + 0.1% v/v formic acid mobile phase B: CH_3CN + 0.1% v/v formic acid. All compounds were found to be ≥ 95% pure unless otherwise indicated. Analytical data are reported as retention time (*t*R) in minutes and % purity. ^{1}H NMR spectra were recorded on either a Varian UnityPlus-400 MHz or a Bruker Avance III 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; or br, broad. Low-resolution mass spectra (MS) were recorded on a Waters SQD. The UPLC analysis was conducted utilizing a Phenomenex Kinetex 1.7 µm 2.1 x 50mm XB-C18 column at 40 °C. The gradient employed was: mobile phase A: water + 0.2% v/v formic acid mobile phase B: CH_3CN + 0.15% v/v FA. High-resolution MS were recorded on a Waters (Micromass) LCT time-of-flight mass spectrometer. Mass spectra were obtained under electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or fast atom bombardment (FAB) methods. Combustion analysis for compound **78c** was performed by Robertson Microlit Laboratories, Inc. (Ledgewood, NJ).

6-Bromo-N-(3-chloro-4-fluorophenyl)-4-quinolinamine. A small microwave vial containing 6-bromo-4-chloroquinoline (149 mg, 0.61 mmol) and 3-chloro-4-fluoroaniline (98 mg, 0.68 mmol) in 4 mL of EtOH had one drop of concentrated HCl added to it. The solution was then heated in the microwave for 30 min at 150 ºC. The crude LCMS showed the major product to be the desired one. Another reaction was set up this time containing 500 mg of the starting quinoline and run as before (in 14 mL of EtOH). The two reactions were combined taken up in a CH_2Cl_2 and a minimal amount of MeOH. The organics were washed with saturated $NaHCO_3$ followed by drying over $MgSO_4$. The solvent was removed in vacuo and the residual solid triterated with Et_2O. The solid was then collected via vacuum filtration yielding 600 mg (1.71 mmol) of 6-bromo-N-(3-chloro-4-fluorophenyl)-4-quinolinamine as a beige solid. ^{1}H NMR

(DMSO-d_6): 400 MHz δ 9.11 (s, 1H), 8.61 (s, 1H), 8.52 (d, *J* = 4.9 Hz, 1H), 7.83 (s, 2H), 7.52 - 7.59 (m, 1H), 7.42 - 7.51 (m, 1H), 7.32 - 7.42 (m, 1H), 6.95 (d, *J* = 4.9 Hz, 1H). Low-resolution MS (ES^+) *m/e* 351 & 353 (MH^+).

N-(3-Chloro-4-fluorophenyl)-6-(1,3-thiazol-5-yl)-4-quinolinamine (2). A DMF (6 mL) solution containing 6-bromo-N-(3-chloro-4-fluorophenyl)-4-quinolinamine (327 mg, 0.93 mmol), K_2CO_3 (129 mg, 0.93 mmol), tetraethylammonium chloride hydrate (171 mg, 0.93 mmol), bistriphenylphosphine palladium (II) dichloride (131 mg, 0.19 mmol, 20 mole%) and 5-(tributylstannanyl)-1,3-thiazole (522 mg, 1.40 mmol) was heated to 100 ºC for 1 h. The dark solution was taken up in EtOAc and washed with water (2x) followed by filtering though a bed of celite. The celite was rinsed with EtOAc and then the organics were dried over $MgSO_4$. The solvent removed in vacuo and the residual yellow oil was dissolved in MeOH/CH_2Cl_2 and purified on the Biotage (5-20% MeOH/CH_2Cl_2) yielding a yellow oil which by LCMS wasn't clean. The solid was taken up in MeOH and a minimum amount of DMF and further purified on the Agilent semi-prep HPLC yielding 276 mg (0.59 mmol) of N-(3-chloro-4-fluorophenyl)-6-(1,3-thiazol-5-yl)-4-quinolinamine as a yellow solid. ^1H NMR (DMSO-d_6): 400 MHz δ 10.9 (br. s., 1H), 9.25 (s, 1H), 8.92 (d, *J* = 1.2 Hz, 1H), 8.58 (s, 1H), 8.56 (s, 1H), 8.43 (dd, *J* = 8.8, 1.4 Hz, 1H), 8.07 (d, *J* = 8.8 Hz, 1H), 7.84 (dd, *J* = 6.6, 2.3 Hz, 1H), 7.64 - 7.74 (m, 1H), 7.57 (ddd, *J* = 8.6, 4.2, 2.6 Hz, 1H), 6.87 (d, *J* = 7.0 Hz, 1H). Low-resolution MS (ES^+) *m/e* 356 (MH^+). RP-HPLC, *t*R = 0.54 min, 100% purity.

6-Bromo-N-phenylquinazolin-4-amine. A mixture of 6-bromo-4-chloroquinazoline (1.5 g, 4.07 mmol), aniline (0.80 g, 8.59 mmol) and acetonitrile (40 mL) was stirred under N_2 atmosphere overnight. The resulting solid was filtered and washed with a small amount of acetonitrile to afford 6-bromo-N-phenylquinazolin-4-amine (1.05 g, 3.01 mmol) as yellow solid. Low-resolution MS (ES^+) *m/e* 300 & 302 (MH^+).

N-phenyl-6-(thiazol-5-yl)quinazolin-4-amine (3a). To a suspension of 6-bromo-N-phenylquinazolin-4-amine (100 mg, 0.33 mmol) and K_2CO_3 (92 mg, 0.67 mmol) in DMF (4 mL) was added $PdCl_2(dppf)•CH_2Cl_2$ (54.4 mg, 0.067 mmol) and copper(I) iodide (12.7 mg, 0.067 mmol). The mixture was stirred at room temperature for 5 min and then 5-tributylstannylthiazole (249 mg, 0.67 mmol) was added. The reaction mixture was stirred under N_2 atmosphere at 100 °C overnight. After cooling, 10 mL of H_2O was added and the solution was filtered. The filtrate was extracted with DCM (3 x 10 mL) and the combined organic layers were collected and washed with H_2O (2 x 10 mL). After drying over Na_2SO_4 the solvent was removed in vacuo to afford crude product. The crude product was purified on the Gilson GX-281. The title compound was collected and concentrated in vacuo to afford N-phenyl-6-(thiazol-5-yl)quinazolin-4-amine (32 mg, 0.10 mmol) as white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.98 (s, 1H), 9.19 (s, 1H), 8.83 (d, *J* = 1.5 Hz, 1H), 8.58 (s, 1H), 8.51 (s, 1H), 8.21(dd, *J* = 8.8, 1.8 Hz, 1H), 7.84 (dd, *J* = 8.0, 6.0 Hz, 3H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.14 - 7.23 (m, 1H). HRMS: $C_{17}H_{12}N_4S$ requires M+H at *m/z* 305.0861; found, 305.0855. RP-HPLC, *t*R = 0.85 min, 100% purity.

Compounds **3b-yy** were prepared in a similar manner to **3a**. The experimentals and analytical data can be found in the supporting information.

2-Amino-5-bromo-3-methoxybenzoic acid (6). A $CHCl_3$ solution (60 mL) containing 2-amino-3-(methyloxy)benzoic acid (2.0 g, 12.0 mmol) cooled to 0 ºC was added a $CHCl_3$ solution (5 mL) containing bromine (2.01 g, 12.6 mmol) dropwise. The heterogeneous solution was slowly allowed to warm to rt overnight at which time the solution was concentrated. The solid was triterated with Et_2O and collected via vacuum filtration yielding 3.78 g (11.6 mmol) of 2-amino-5-bromo-3-(methyloxy)benzoic acid hydrobromide as a tan solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 7.41 (d, *J* = 1.95 Hz, 1H), 7.07 (d, *J* = 1.76 Hz, 1H), 3.83 (s, 3H) (aniline and acid H's not seen). Low-resolution MS (ES^+) *m/e* 246 & 248 (MH^+).

2-Amino-5-bromo-3-(trifluoromethyl)benzoic acid (7). A solution of 2-amino-3-(trifluoromethyl)benzoic acid (4.96 g, 24.2 mmol) in 50 mL of H_2O and 5.7 mL of 48% hydrobromic acid heated to 75 ºC had 2.5 mL of 30% hydrogen peroxide added to it dropwise. The resulting heterogeneous solution was vigorously stirred and heated for an additional 2 h. Upon cooling the solid was collected via vacuum filtration and washed with Et_2O. The material dissolved in the Et_2O. Therefore it was taken up in additional Et_2O, washed with H_2O followed by drying over $MgSO_4$ yielding 7.0 g (19.2 mmol) of 2-amino-5-bromo-3-(trifluoromethyl)benzoic acid as a light yellow powder. ^{1}H NMR (DMSO-d_6): 400 MHz δ 13.5 (br. s., 1H), 8.08 (d, J = 2.3 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.18 (br. s., 2H).

2-Amino-5-iodo-3-(trifluoromethyl)benzoic acid (8). 2-Amino-3-(trifluoromethyl)benzoic acid (1.0 g, 4.87 mmol) was stirring in DMF (5 mL) at rt under N_2 then NIS (1.1 g, 4.89 mmol) in DMF (5 mL) was added dropwise. After addition the reaction was allowed to stir at same temperature for 3 h. The solution was concentrated under reduced pressure to half the volume then poured slowly over ice-water with vigorous stirring. The ice-water warmed to rt and the precipitate collected by filtration. The precipitate was washed with water and then diethyl ether. The filtrate was concentrated under reduced pressure to an orange solid that was 85% desired 2-amino-5-iodo-3-(trifluoromethyl)benzoic acid (1.35 g, 4.08 mmol). Low-resolution MS (ES^+) *m/e* 332 (MH^+).

6-Bromo-8-methoxyquinazolin-4(3H)-one (10). A formamide solution (50 mL) containing compound **6** (3.76 g, 11.5 mmol) was heated to 165 ºC for 24 h. Upon cooling the solution was poured onto crushed ice. The precipitate was collected via vacuum filtration and then washed successively with H_2O and Et_2O followed by drying under high vacuum affording 2.73 g (10.7 mmol) of 6-bromo-8-(methyloxy)-4(1H)-quinazolinone as a gray solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 8.07 (s, 1H), 7.74 (d, J = 1.56 Hz, 1H), 7.49 (d, J = 1.56 Hz, 1H), 3.92 (s, 3H), 3.33 (br. s., 1H). Low-resolution MS (ES^+) *m/e* 255 & 257 (MH^+).

6-Bromo-8-(trifluoromethyl)-4(1H)-quinazolinone (11). A formamide solution (130 mL) containing compound **7** (7.0 g, 19.2 mmol) was heated to 165 ºC overnight. Upon cooling the solution was poured onto crushed ice. The precipitate was collected via vacuum filtration and then washed successively with H_2O and Et_2O followed by drying under high vacuum affording a sticky tan solid. The solid was taken up in MeOH and Et_2O followed by washing with H_2O. The organic layer was separated and the aqueous layer extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and the solvent removed in vacuo yielding 3.97 g (13.6 mmol) of 6-bromo-8-(trifluoromethyl)-4(1H)-quinazolinone as a yellow solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.8 (br. s., 1H), 8.46 (d, J = 2.1 Hz, 1H), 8.30 (s, 2H). Low-resolution MS (ES^+) *m/e* 293 & 295 (MH^+).

6-Bromo-8-methyl-4(1H)-quinazolinone (12). A formamide solution (140 mL) containing 2-amino-5-bromo-3-methylbenzoic acid (9.90 g, 43.0 mmol) was heated to 165 ºC for 24 h. Upon cooling the solution was poured onto crushed ice. The precipitate was collected via vacuum filtration and then washed successively with H_2O and Et_2O followed by drying under high vacuum affording 9.29 g (38.9 mmol) of 6-bromo-8-methyl-4(1H)-quinazolinone as a reddish-gray solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.4 (br. s., 1H), 8.15 (s, 1H), 8.01 (d, J = 2.0 Hz, 1H), 7.85 (s, 1H), 2.50 (s, 3H). Low-resolution MS (ES^+) *m/e* 239 & 241 (MH^+).

6-Bromo-4-chloro-8-methoxyquinazoline (13). A flask containing compound **10** (2.73 g, 10.7 mmol) in 25 mL of thionyl chloride had a few drops of DMF added to it. The solution was then heated to reflux for 5 h and upon cooling the bulk of the thionyl chloride was removed in vacuo. The residue was taken up in $CHCl_3$ and then poured onto crushed ice. Additional $CHCl_3$ was added and the organic layer separated followed by drying over $MgSO_4$. The solvent was removed in vacuo yielding 1.24 g (4.53 mmol) of 6-bromo-4-chloro-8-(methyloxy)quinazoline as a light yellow solid. ^{1}H NMR (DMSO-d_6): MHz δ 8.25 (s, 1H), 7.75 (d, J = 1.95 Hz, 1H), 7.55 (d, J = 1.76 Hz, 1H), 3.94 (s, 3H) Low-resolution MS (ES^+) *m/e* 269 & 271 (MH^+), MeOH adduct.

6-Bromo-4-chloro-8-(trifluoromethyl)quinazoline (14). A flask containing compound **11** (3.96 g, 13.5 mmol) in 50 mL of thionyl chloride had a few drops of DMF added to it. The solution was then heated to reflux for 7 h and upon cooling the thionyl chloride was removed in vacuo. The solid residue was taken up in Et_2O and the solid collected via vacuum filtration. After rinsing the solid with additional Et_2O it was pumped on under high vacuum yielding 4.24 g (13.6 mmol) of 6-bromo-4-chloro-8-(trifluoromethyl)quinazoline as a yellow solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.29 (s, 1H), 8.75 (d, *J* = 1.8 Hz, 1H), 8.66 (s, 1H). Low-resolution MS (ES^+) *m/e* 311 & 313 (MH^+).

6-Bromo-4-chloro-8-methylquinazoline (15). A flask containing compound **12** (1.0 g, 4.18 mmol) in 5 mL of thionyl chloride had a few drops of DMF added to it. The solution was then heated to reflux for 7 h and upon cooling the bulk of the thionyl chloride was removed in vacuo. The residue was taken up in $CHCl_3$ and then poured onto crushed ice containing saturated $NaHCO_3$. The heteroegenous solution was filtered and then the layers separated. The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo yielding 521 mg (2.02 mmol) of 6-bromo-4-chloro-8-methylquinazoline as a grayish solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.17 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 2.71 (s, 3H).

6-Bromo-N-(3-chloro-4-fluorophenyl)-8-methoxyquinazolin-4-amine (16a). An CH_3CN solution (15 mL) containing compound **13** (450 mg, 1.64 mmol) and 3-chloro-4-fluoroaniline (287 mg, 1.97 mmol) was stirred at rt for 2 h at which time additional CH_3CN was added (10 mL due to heterogenity of the solution) along with additional aniline. The slurry was stirred over three days (not necessary) at which time Et_2O was added and the solid collected via vaccum filtration. The collected solid was rinsed thoroughly with Et_2O yielding 606 mg (1.58 mmol) of 6-bromo-N-(3-chloro-4-fluorophenyl)-8-(methyloxy)-4-quinazolinamine as an off-white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.7 (br. s., 1H), 8.84 (s, 1H), 8.80 (s, 1H), 8.07 (dd, *J* = 2.44, 6.73 Hz, 1H), 7.83 (s, 1H), 7.77 (ddd, *J* = 2.63, 4.19, 8.78 Hz, 1H), 7.56 (t, *J* = 9.07 Hz, 1H), 3.84-4.27 (m, 3H). Low-resolution MS (ES^+) *m/e* 382 & 384 (MH^+).

4-{[6-Bromo-8-(methyloxy)-4-quinazolinyl]amino}benzamide (16b). The entitled compound was made in a similar manner as described for compound **16a**. ^{1}H NMR (DMSO-d_6): 400 MHz δ: 11.6 (br. s., 1H), 8.84 (s, 1H), 8.79 (s, 1H), 8.05 (br. s., 1H), 7.98 (d, *J* = 8.6 Hz, 2H), 7.82 - 7.89 (m, 3H), 7.44 (br. s., 1H), 4.10 (s, 3H). Low-resolution MS (ES^+) *m/e* 373 & 375 (MH^+).

4-{[6-Bromo-8-(trifluoromethyl)-4-quinazolinyl]amino}benzamide (17b). An CH_3CN solution (20 mL) containing compound **14** (547 mg, 1.76 mmol) had 4-aminobenzamide (287 mg, 2.11 mmol) added to it at rt. The solution was stirred at rt for 3 days. The heterogeneous solution was poured onto crushed ice and the resulting precipitate collected via vacuum filtration. The solid was rinsed with Et_2O yielding 294 mg (0.72 mmol) of 4-{[6-bromo-8-(trifluoromethyl)-4-quinazolinyl]amino}benzamide as a grayish-white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.2 (s, 1H), 9.19 (d, *J* = 1.0 Hz, 1H), 8.79 (s, 1H), 8.38 (s, 1H), 7.90 - 7.98 (m, 5H), 7.33 (br. s., 1H). Low-resolution MS (ES^+) *m/e* 411 & 413 (MH^+).

6-Bromo-N-(3-chloro-4-fluorophenyl)-8-(trifluoromethyl)-4-quinazolinamine (17a). The entitled compound was made in a similar manner as described for compound **17b**. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.2 (s, 1H), 9.09 (d, *J* = 1.17 Hz, 1H), 8.76 (s, 1H), 8.36 (s, 1H), 8.15 (dd, *J* = 2.54, 6.83 Hz, 1H), 7.80 (ddd, *J* = 2.83, 4.05, 8.93 Hz, 1H), 7.46 (t, *J* = 9.07 Hz, 1H). Low-resolution MS (ES^+) *m/e* 420 & 422 (MH^+).

6-Bromo-N-(3-chloro-4-fluorophenyl)-8-methyl-4-quinazolinamine (18). An CH_3CN solution (10 mL) containing compound **15** (198 mg, 0.77 mmol) and 3-chloro-4-fluoroaniline (134 mg, 0.92 mmol) was stirred at rt overnight. The heterogeneous solution was taken up in Et_2O and the solid collected via vacuum filtration. The solid was rinsed thoroughly with Et_2O followed by drying under high vacuum yielding 253 mg (0.69 mmol) of 6-bromo-N-(3-chloro-4-fluorophenyl)-8-methyl-4-quinazolinamine as a beige powder. ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.6 (br. s., 1H), 9.10 (br. s., 1H), 8.87 (s, 1H), 8.11 (br. s., 2H), 7.73 - 7.88 (m, 7.54 (t, *J* = 9.1 Hz, 1H), 2.63 (s, 3H). Low-resolution MS (ES^+) *m/e* 366 & 368 (MH^+).

N-(3-Chloro-4-fluorophenyl)-8-methoxy-6-(thiazol-5-yl)quinazolin-4-amine (19a). A DMF (12 mL) solution containing compound **16a** (348 mg, 0.91 mmol), potassium carbonate (126 mg, 0.91 mmol), tetraethylammonium chloride hydrate (167 mg, 0.91 mmol), bistriphenylphosphine palladium (II) dichloride (128 mg, 0.18 mmol, 20 mole%) and 5-tributylstannanyl thiazole (442 mg, 1.18 mmol) was heated to 100 ºC for 2.5 h. The dark solution was taken up in EtOAc and water added. The resulting mulsion was filtered through a bed of celite. The celite was rinsed with EtOAc and then the organic layer separated followed by another aqueous wash. The organics were dried over $MgSO_4$ and the solvent removed in vacuo. The residual dark oil was purified on the Biotage (initially 60-100% EtOAc/hexanes followed by 5-10% MeOH/CH_2Cl_2) yielding a yellow oil which still wasn't clean. Therefore it was purified on the Agilent semi-prep HPLC affording 21 mg (0.05 mmol) of N-(3-chloro-4-fluorophenyl)-8-(methyloxy)-6-(1,3-thiazol-5-yl)-4-quinazolinamine as a light yellow solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.92 (s, 1H), 9.19 (s, 1H), 8.59 (s, 1H), 8.55 (s, 1H), 8.28 (s, 1H), 8.14 (dd, *J* = 2.34, 6.83 Hz, 1H), 7.75-7.90 (m, 1H), 7.63 (s, 1H), 7.48 (t, *J* = 9.07 Hz, 1H), 4.04 (s, 3H). HRMS: $C_{18}H_{12}ClFN_4OS$ requires M+H at *m/z* 387.0482; found, 387.0472. RP-HPLC, *t*R = 1.19 min, 93% purity.

4-{[8-(Methyloxy)-6-(1,3-thiazol-5-yl)-4-quinazolinyl]amino}benzamide (19b). The entitled compound was made in a similar manner as described for compound **19a**. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.4 (br. s., 1H), 9.35 (br. s., 1H), 8.81 (br. s., 1H), 8.69 (br. s., 2H), 8.09 (br. s., 5H), 7.74 (br. s., 1H), 7.43 (br. s., 1H), 4.21 (br. s., 3H). Low-resolution MS (ES^+) *m/e* 378 (MH^+).

4-{[6-(1,3-Thiazol-5-yl)-8-(trifluoromethyl)-4-quinazolinyl]amino}benzamide (20b). A DMF solution (12 mL) containing **17b** (294 mg, 0.72 mmol), copper (I) iodide (14 mg, 0.072 mmol, 10 mole%) and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (117 mg, 0.14 mmol, 20 mole%) had 5-tributylstannanyl thiazole (401 mg, 1.07 mmol) added to it. The solution was then heated to 100 ºC for 2 h. The organics were taken up in $CHCl_3$ upon cooling and washed with H_2O. The aqueous layer was extracted with $CHCl_3$ and then the combined organic layers were dried over $MgSO_4$. The solution was passed though a small bed of celite with the celite being rinsed with 10%

MeOH/CH_2Cl_2. The black solution was concentrated and then taken up in a minimal amount of MeOH. The solution was poured onto crushed ice and the resulting solution filtered. The tarry residue was triturated with Et_2O yielding a brown solid which by LCMS looked good. However, the ^{1}H NMR showed impurities. Therefore the solid was taken up in a minimal amount of DMF and MeOH and purified on the Agilent semi-prep HPLC yielding 21 mg (0.051 mmol) of 4-{[6-(1,3-thiazol-5-yl)-8-(trifluoromethyl)-4-quinazolinyl]amino}benzamide as a beige solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.3 (br. s., 1H), 9.24 (s, 1H), 9.05 (s, 1H), 8.73 (s, 1H), 8.63 (s, 1H), 8.53 (s, 1H), 7.85 - 8.01 (m, 5H), 7.32 (br. s., 1H). HRMS: $C_{19}H_{12}F_3N_5OS$ requires M+H at *m/z* 416.0793; found, 416.0788. RP-HPLC, *t*R = 1.41 min, 100% purity.

N-(3-Chloro-4-fluorophenyl)-6-(1,3-thiazol-5-yl)-8-(trifluoromethyl)-4-quinazolinamine (20a). The entitled compound was made in a similar manner as described for compound **20b**. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.3 (s, 1H), 9.25 (s, 1H), 8.98 (d, *J* = 0.78 Hz, 1H), 8.72 (s, 1H), 8.62 (s, 1H), 8.53 (s, 1H), 8.12 (dd, *J* = 2.54, 6.83 Hz, 1H), 7.79 (ddd, *J* = 2.73, 4.15, 8.93 Hz, 1H), 7.50 (t, *J* = 9.07 Hz, 1H). HRMS: $C_{18}H_9ClF_4N_4S$ requires M+H at *m/z* 425.0251; found, 425.0239. RP-HPLC, *t*R = 2.29 min, 99% purity.

N-(3-Chloro-4-fluorophenyl)-8-methyl-6-(1,3-thiazol-5-yl)-4-quinazolinamine (21). A DMF solution (7 mL) containing compound **18** (235 mg, 0.64 mmol), 5-tributylstannanyl) thiazole (360 mg, 0.96 mmol), tetraethyl ammonium chloride hydrate (118 mg, 0.64 mmol), K_2CO_3 (89 mg, 0.64 mmol) and bis(triphenylphosphine)palladium(II) chloride (90 mg, 0.13 mmol, 20 mole%) was heated to 100 ºC for 1 h. Upon cooling the organics were taken up in EtOAc and washed with H_2O (2x) followed by drying over $MgSO_4$. The solvent was removed in vacuo and the residue was purified on the Biotage (60-100% EtOAc/hexanes) yielding a yellow solid which by analytical HPLC still contained impurities. Therefore the solid was taken up in a minimal amount of DMF and purified on the Agilent semi-prep HPLC yielding 55 mg (0.15 mmol) of N-(3-chloro-4-fluorophenyl)-8-methyl-6-(1,3-thiazol-5-yl)-4-quinazolinamine as a light yellow solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.95 (s, 1H), 9.18 (s, 1H), 8.66

(s, 1H), 8.60 (s, 1H), 8.47 (s, 1H), 8.16 (dd, *J* = 6.6, 2.1 Hz, 1H), 8.11 (s, 1H), 7.75 - 7.89 (m, 1H), 7.48 (t, *J* = 9.1 Hz, 1H), 2.65 (s, 3H). HRMS: $C_{18}H_{12}ClFN_4S$ requires M+H at *m/z* 371.0533; found, 371.0522. RP-HPLC, *t*R = 1.43 min, 100% purity.

6-Bromo-8-methyl-2,4(1H,3H)-quinazolinedione (23). 2-Amino-5-bromo-3-methylbenzoic acid (1.0 g, 4.35 mmol) and urea (1.5 g, 25.0 mmol) in a pressure tube were heated to melt temperature (~150 °C). At 1 h, the LCMS showed the reaction to be a mixture of the desired product and the intermediate 2-amino-5-bromo-3-methylbenzamide. Heating was continued until only product was seen. Upon cooling to rt, the reaction mixture was washed with water to afford 6-bromo-8-methyl-2,4(1H,3H)-quinazolinedione (1.1 g, 4.3 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.5 (br. s., 1H), 10.5 (br.s., 1H), 7.82 (s, 1H), 7.69 (s, 1H), 2.33 (s, 3H). Low-resolution MS (ES^+) *m/e* 255 & 257 (MH^+).

4-Hydroxy-6-iodoquinazolin-2(1H)-one (24). 2-Amino-5-iodobenzoic acid (1.0 g, 3.80 mmol) and urea (2.0 g, 33.3 mmol) were heated neat to 150 °C overnight in a sealed tube. The reaction was monitored by LCMS, added more urea (1.0 g, 16.6 mmol) and heated to same temperature for another 8 h. The reaction was cooled to rt and the solid was broken up and diluted with water. The suspension was filtered to collect a brown solid as 4-hydroxy-6-iodoquinazolin-2(1H)-one (1.12 g, 3.88 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 5.38 (br. s., 1H), 6.98 (d, *J* = 8.53 Hz, 1H), 7.92 (dd, *J* = 8.66, 2.13 Hz, 1H), 8.11 (d, *J* = 2.01 Hz, 1H), 11.3 (d, *J* = 6.27 Hz, 1H). Low-resolution MS (ES^+) *m/e* 289 (MH^+).

6-Bromo-2,4-dichloro-8-methylquinazoline (25). Compound **23** (630 mg, 2.47 mmol) was suspended in $POCl_3$ (10 mL, 107 mmol) and heated to 105 °C. The mixture was stirred overnight at which time it was cooled to rt and poured slowly onto ice. The precipitate was collected by filtration and washed with water affording 6-bromo-2,4-dichloro-8-methylquinazoline as a dull yellowish solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 8.28 (s, 1H), 8.22 (s, 1H), 2.64 (s, 3H), 2.33 (s, 1H). Low-resolution MS (ES^+) *m/e* 291 & 293 (MH^+).

2,4-Dichloro-6-iodoquinazoline (26). Compound **24** (1.0 g, 3.47 mmol) was suspended in $POCl_3$ (5 mL, 53.6 mmol) and heated to 90 °C for 3 h. The reaction was cooled to rt and diluted with toluene (4 mL) and iPr_2NEt (4 mL, 22.9 mmol) was slowly added. After addition, the solution was heated to 90 °C overnight, cooled to rt and concentrated under reduced pressure. The slurry was concentrated onto silica and purified by silica gel chromatography eluting with a gradient from 0-5% DCM/MeOH yielding an orange-tan solid as 2,4-dichloro-6-iodoquinazoline (700 mg, 2.15 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 7.64 (d, *J* = 8.78 Hz, 1H), 8.16 (dd, *J* = 8.91, 1.63 Hz, 1H), 8.56 (d, *J* = 1.76 Hz, 1H).

6-Bromo-2-chloro-N-(3-chloro-4-fluorophenyl)-8-methyl-4-quinazolinamine (27a). An iPrOH (20 mL) suspension of compound **25** (557 mg, 1.91 mmol), 3-chloro-4-fluoroaniline (278 mg, 1.91 mmol) and iPr_2NEt (247 mg, 1.91mmol) was stirred at rt overnight. The unreacted starting material was filtered off and the filtrate was concentrated in vacuo to afford a yellow solid that was a 1:1 mixture of the desired product and the bis-replacement side product. This crude product was carried onto the next step without further purification. Low-resolution MS (ES^+) *m/e* 402 & 404 (MH^+) and 509 & 511 (MH^+) (for bis-replacement side product).

6-Bromo-2-chloro-N-((1s,4s)-4-methoxycyclohexyl)-8-methylquinazolin-4-amine (27b). Compound **25** (0.15 g, 0.514 mmol), (1r,4r)-4-methoxycyclohexanamine hydrochloride (0.102 g, 0.617 mmol) and iPr_2NEt (0.2 mL, 1.14 mmol) in acetonitrile (1.5 mL) was heated to 70 °C for 3 h. The reaction was cooled to rt and diluted with ethyl acetate, washed with water (2x) followed by brine (1x). The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The material was purified by silica gel chromatography via the ISCO Combiflash eluting with a gradient from 25-50% EtOAc/hexanes yielding a yellow solid as 6-bromo-2-chloro-N-((1s,4s)-4-methoxycyclohexyl)-8-methylquinazolin-4-amine (192 mg, 0.499 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 1.29 - 1.41 (m, 2H), 1.43 - 1.55 (m, 2H), 2.13 (br. s., 2H), 2.26 (br. s., 2H), 2.63 (s, 3H), 3.17 - 3.27 (m, 1H), 3.39 (s, 3H), 4.19

- 4.33 (m, 1H), 5.48 (d, *J* = 7.53 Hz, 1H), 7.60 (s, 1H), 7.67 (s, 1H). Low-resolution MS (ES^+) *m/e* 386 & 388 (MH^+).

2-Chloro-6-iodo-N-((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)quinazolin-4-amine (28a). Compound **26** (0.34 g, 1.05 mmol), (1r,4r)-4-(2-methoxyethoxy)cyclohexanamine (see the supporting information for the preparation of this intermediate) (0.218 g, 1.26 mmol) and iPr_2NEt (0.275 mL, 1.58 mmol) in acetonitrile (3 mL) was heated to 70 °C and stirred for 3 h. Upon cooling to rt and the solution was diluted with EtOAc, washed with water (2x) and brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure to a afford a golden solid as 2-chloro-6-iodo-N-((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)quinazolin-4-amine (511 mg, 1.107 mmol). ^1H NMR ($CDCl_3$): 400 MHz δ 1.29 - 1.43 (m, 2H), 1.44 - 1.55 (m, 2H), 2.12 (d, *J* = 11.3 Hz, 2H), 2.22 (d, *J* = 10.3 Hz, 2H), 3.27 - 3.37 (m, 1H), 3.38 - 3.42 (m, 3H), 3.55 - 3.59 (m, 2H), 3.62 - 3.67 (m, 2H), 4.24 (m, 1H), 5.93 (d, *J* = 7.78 Hz, 1H), 7.45 (d, *J* = 8.78 Hz, 1H), 7.92 (dd, *J* = 8.78, 1.51 Hz, 1H), 8.07 (d, *J* = 1.76 Hz, 1H). Low-resolution MS (ES^+) *m/e* 461 (MH^+)

2-Chloro-6-iodo-N-((1s,4s)-4-methoxycyclohexyl)quinazolin-4-amine (28b). Compound **26** (0.34 g, 1.05 mmol), (1r,4r)-4-methoxycyclohexanamine hydrochloride (0.208 g, 1.26 mmol) and iPr_2NEt (0.5 mL, 2.86 mmol) in acetonitrile (3 mL) was heated to 70 °C for 3 h. Upon cooling to rt the solution was diluted with EtOAc, washed with water (2x) and brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure to yield a golden solid as 2-chloro-6-iodo-N-((1s,4s)-4-methoxycyclohexyl)quinazolin-4-amine (382 mg, 0.915 mmol). ^1H NMR ($CDCl_3$): 400 MHz δ 1.35-1.51 (m, 4H), 2.11-2.19 (m, 2H), 2.25 (d, *J* = 12.0 Hz, 2H), 3.18-3.26 (m, 1H), 3.39 (s, 3H), 4.24 - 4.33 (m, 1H), 5.67 (d, *J* = 7.28 Hz, 1H), 7.49 (d, *J* = 8.78 Hz, 1H), 7.96 (dd, *J* = 8.78, 1.76 Hz, 1H), 8.00 (d, *J* = 1.51 Hz, 1H). Low-resolution MS (ES^+) *m/e* 418 (MH^+).

6-Bromo-4-((3-chloro-4-fluorophenyl)amino)-8-methylquinazolin-2(1H)-one (29a). A flask containing compound **27a** (110 mg, 0.28 mmol) in 3 mL of HOAc was heated to 90 ºC overnight at which the heterogeneous solution was taken up in H_2O and the solid collected via vacuum filtration. The solid was rinsed with H_2O and a mixture of hexanes/Et_2O yielding 76 mg (0.20 mmol) of 6-bromo-4-((3-chloro-4-fluorophenyl)amino)-8-methylquinazolin-2(1H)-one as a white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.5 (br. s., 1H), 9.79 (br. s., 1H), 8.42 (br. s., 1H), 8.19 (br. s., 1H), 7.79 (br. s., 1H), 7.67 (br. s., 1H), 7.44 (br. s., 1H), 2.50 (br. s., 3H). Low-resolution MS (ES^+) *m/e* 382 & 384 (MH^+).

6-Bromo-4-(((1s,4s)-4-methoxycyclohexyl)amino)-8-methylquinazolin-2(1H)-one (29b). Compound **27b** (0.19 g, 0.494 mmol) was stirring in acetic acid (4.5 mL) at 90 °C overnight. The reaction was cooled to rt and poured over ice-water. The ice-water warmed to rt and set for 3 h, a precipitate formed and was collected by filtration. The precipitate was washed with water and then dried in oven at 60 °C for 1 h providing a white solid as 6-bromo-4-(((1s,4s)-4-methoxycyclohexyl)amino)-8-methylquinazolin-2(1H)-one (144 mg, 0.393 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.13 - 1.30 (m, 2H), 1.34 - 1.50 (m, 2H), 1.90 - 1.97 (m, 2H), 2.02 - 2.12 (m, 2H), 2.31 (s, 3H), 3.08 - 3.19 (m, 1H), 3.26 (s, 3H), 4.07 (m, 1H), 7.57 (s, 1H), 7.93 (d, *J* =7.53 Hz, 1H), 8.23 (d, *J* = 1.25 Hz, 1H), 9.97 (s, 1H). Low-resolution MS (ES^+) *m/e* 367 & 369 (MH^+).

6-Iodo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)quinazolin-2-ol (30a). Compound **28a** (0.5 g, 1.08 mmol) was stirred in acetic acid (10.8 mL) and heated to 90 °C for 3 h. Upon cooling to rt the solution was poured over ice and let sit overnight. No precipitate was observed, therefore the organics extracted with EtOAc (2x). The combined organics were washed with 1.0 N aqueous NaOH, water and brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The golden oil was washed with MeOH and a solid formed. The solution was concentrated under reduced pressure to a pale yellow solid as 6-iodo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)quinazolin-2-ol (344 mg, 0.776 mmol). ^{1}H NMR (DMSO-d_6): 400

MHz δ 1.17 - 1.31 (m, 2H), 1.35 - 1.49 (m, 2H), 1.94 (br. s., 2H), 2.04 (d, *J* = 10.0 Hz, 2H), 3.22 - 3.28 (m, 4H), 3.40 - 3.47 (m, 2H), 3.54 - 3.59 (m, 2H), 4.03 - 4.11 (m, 1H), 6.93 (d, *J* = 8.78 Hz, 1H), 7.81 (dd, *J* = 8.53, 1.51 Hz, 1H), 7.92 (d, *J* = 7.78 Hz, 1H), 8.48 (d, *J* = 1.25 Hz, 1H), 10.7 (s, 1H). Low-resolution MS (ES^+) *m/e* 444 (MH^+).

6-Iodo-4-(((1s,4s)-4-methoxycyclohexyl)amino)quinazolin-2-ol (30b). Compound **28b** (0.382 g, 0.915 mmol) was stirred in acetic acid (9 mL) at 90 °C for 3 h. Upon cooling to rt it was poured over ice and let sit overnight. No precipitate was observed, therefore the organics were extracted with EtOAc (2x). The combined layers were washed with 1.0 N aqueous NaOH, water and brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The golden oil was washed with MeOH and a solid formed which was concentrated under reduced pressure to afford a pale yellow solid as 6-iodo-4-(((1s,4s)-4-methoxycyclohexyl)amino)quinazolin-2-ol (0.303 g, 0.759 mmol). ^{1}H NMR (METHANOL-d_4): 400 MHz δ 1.29-1.40 (m, 2H), 1.42-1.53 (m, 2H), 2.05 - 2.15 (m, 4H), 3.21 - 3.27 (m, 1H), 3.36 (s, 3H), 4.22 - 4.30 (m, 1H), 6.98 (d, *J* = 8.53 Hz, 1H), 7.83 (dd, *J* = 8.78, 1.76 Hz, 1H), 8.40 (d, *J* = 1.76 Hz, 1H). Low-resolution MS (ES^+) *m/e* 399 (MH^+).

4-((3-Chloro-4-fluorophenyl)amino)-8-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (31a). A DMF solution (11 mL) containing compound **27a** (370 mg, 0.97 mmol), copper (I) iodide (18 mg, 0.10 mmol, 10 mole%) and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (158 mg, 0.19 mmol, 20 mole%) had 5-tributylstannanyl thiazole (543 mg, 1.45 mmol) added to it. The solution was evacuated under vacuum and then placed under a nitrogen atmosphere. After being heated to 110 ºC for 2 h, the organics were taken up in EtOAc and washed with H_2O (2x). The heterogeneous solution that remained was filtered under vacuum and the resulting gray solid turned out to be desired product. It was taken up in MeOH and DMF and filtered though a fritted plug of celite. The filtrate did show product, however some of the initial solid did not dissolve. It was treated with DMF/MeOH and filtered into a new flask. The solvent was concentrated and treated with H_2O. The

precipitated solid was collected via vacuum filtration, washed with Et_2O and pumped on under high vacuum yielding 129 mg (0.33 mmol) of 4-((3-chloro-4-fluorophenyl)amino)-8-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one as a light tan solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.5 (br. s., 1H), 9.88 (br. s., 1H), 9.10 (br. s., 1H), 8.41 (br. s., 1H), 8.32 (br. s., 1H), 8.16 (br. s., 1H), 7.85 (br. s., 1H), 7.78 (br. s., 1H), 7.48 (br. s., 1H), 2.50 (br. s., 3H). Low-resolution MS (ES^+) *m/e* 373 (MH^+). RP-HPLC, *t*R = 1.54 min, 95% purity.

4-(((1s,4s)-4-Methoxycyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (31b). Compound **29b** (0.14 g, 0.382 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole (reference 17) (0.161 g, 0.764 mmol), CsF (0.145 g, 0.956 mmol), and copper(I) iodide (0.015 g, 0.076 mmol) in DMF (3.8 mL) was degassed for ~ 5 min before the addition of tetrakis (0.022 g, 0.019 mmol). The reaction was heated to 90 °C, stirred overnight and then concentrated under reduced pressure to remove DMF. The residue was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and the residue was triturated with DCM:MeOH (10:1) to recover a yellow solid as 4-(((1s,4s)-4-methoxycyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (25 mg, 0.064 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.24 (d, *J* = 12.8 Hz, 2H), 1.40 - 1.55 (m, 2H), 1.96 (br. s., 2H), 2.08 (br. s., 3H), 2.37 (s, 3H), 3.11 - 3.22 (m, 1H), 3.27 (s, 3H), 4.08 - 4.22 (m, 1H), 7.74 (s, 1H), 7.98 (d, 1H), 8.22 (s, 1H), 8.28 (s, 1H), 9.08 (br. s., 1H), 10.0 (s, 1H). HRMS: $C_{19}H_{22}N_4O_2S$ requires M+H at *m/z* 371.1541; found, 371.1535.

6-Iodo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-1-methylquinazolin-2(1H)-one (33a). Compound **30a** (0.20 g, 0.451 mmol) was stirring in DMF (4.5 mL) when a 60% dispersion in mineral oil of NaH (0.020 g, 0.496 mmol) was added at 0 °C. After addition, the reaction was allowed to stir at same temperature for ~ 10 min before the addition of iodomethane (0.03 mL, 0.480 mmol). The reaction stirred at same temperature for ~ 20 min before warming to rt where it stirred for 30 min. The reaction was quenched with water and extracted with EtOAc. The organics were washed with 1.0 N

aqueous NaOH, water (2x) and brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure affording 6-iodo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-1-methylquinazolin-2(1H)-one (0.165 g, 0.361 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.17 - 1.33 (m, 2H), 1.36 - 1.48 (m, 2H), 1.92 (d, *J* = 10.9 Hz, 2H), 2.00 - 2.08 (m, 2H), 3.21 - 3.29 (m, 4H), 3.40 (s, 3H), 3.43 (dd, *J* = 5.86, 3.91 Hz, 2H), 3.52 - 3.58 (m, 2H), 4.06 (m, 1H), 7.15 (d, *J* = 8.98 Hz, 1H), 7.94 (dd, *J* = 8.89, 1.86 Hz, 1H), 7.99 (d, *J* = 7.62 Hz, 1H), 8.54 (d, *J* = 1.76 Hz, 1H). Low-resolution MS (ES^+) *m/e* 458 (MH^+).

6-Iodo-4-(((1s,4s)-4-methoxycyclohexyl)amino)-1-methylquinazolin-2(1H)-one (33b). Compound **30b** (0.10 g, 0.250 mmol) was stirring in DMF (1.5 mL) in an ice-bath, then a 60% dispersion in mineral oil of NaH (10.0 mg, 0.250 mmol) was added portionwise. The reaction was allowed to stir at same temperature for ~ 10 min before iodomethane (0.014 mL, 0.225 mmol) was added. The reaction stirred in ice-bath for an additional 20 min before warming to rt where stirring continued for another 1 h. The solution was poured over ice-water and let set overnight. A yellow solid was observed which was collected by filtration, washed with water and EtOAc yielding a golden solid as 6-iodo-4-(((1s,4s)-4-methoxycyclohexyl)amino)-1-methylquinazolin-2(1H)-one (81.4 mg, 0.158 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.16 - 1.29 (m, 2H), 1.36 - 1.49 (m, 2H), 1.88 - 1.98 (m, 2H), 2.02 - 2.10 (m, 2H), 3.14 (tt, *J* = 10.6, 3.95 Hz, 1H), 3.26 (s, 3H), 3.40 (s, 3H), 4.02 - 4.15 (m, 1H), 7.15 (d, *J* = 9.03 Hz, 1H), 7.91 - 8.00 (m, 2H), 8.53 (d, *J* = 2.01 Hz, 1H). Low-resolution MS (ES^+) *m/e* 414 (MH^+).

4-(((1s,4s)-4-(2-Methoxyethoxy)cyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (32a). Compound **33a** (0.1 g, 0.219 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.092 g, 0.437 mmol), cesium fluoride (0.083 g, 0.547 mmol), and copper(I) iodide (8.33 mg, 0.044 mmol) in DMF (2.18 mL) was degassed for ~ 10 min before the addition of tetrakis (0.025 g, 0.022 mmol). The reaction was allowed to stir at 90 °C overnight before cooling to rt and concentrating under reduced pressure. The residue was taken up in DCM/MeOH and filtered through a pad of celite.

The filtrate was concentrated under reduced pressure and the resulting material was purified silica gel chromatography eluting with a gradient from 2-10% MeOH/DCM yielding a yellow solid as 4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (58.7 mg, 0.142 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 1.38 (t, *J* = 9.54 Hz, 4H), 2.04 (br. s., 2H), 2.14 (br. s., 2H), 3.21 - 3.31 (m, 1H,) 3.38 (s, 3H), 3.51 - 3.55 (m, 2H), 3.57 - 3.62 (m, 2H), 3.64 (s, 3H), 4.27 - 4.38 (m, 1H), 7.26 - 7.31 (m, 1H), 7.81 (dd, *J* = 8.78, 1.51 Hz, 1H), 8.00 (s, 1H), 8.13 (s, 1H), 8.76 (s, 1H). HRMS: $C_{21}H_{26}N_4O_3S$ requires M+H at *m/z* 415.1804; found, 415.1793. RP-HPLC, *t*R = 1.03 min, 95% purity.

4-(((1s,4s)-4-Methoxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (32b). Compound **33b** (0.10 g, 0.242 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.102 g, 0.484 mmol), cesium fluoride (0.092 g, 0.605 mmol), and copper(I) iodide (9.2 mg, 0.048 mmol) in DMF (2.5 mL) was degassed for ~ 10 min before the addition of tetrakis (0.028 g, 0.024 mmol). The reaction was allowed to stir at 90 °C overnight before cooling to rt and concentrating under reduced pressure. The residue was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and the resulting material was purified by reverse phase C18 HPLC eluting with a gradient from 5-99% CH_3CN/water/0.1% NH_4OH yielding a pale solid as 4-(((1s,4s)-4-methoxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinazolin-2(1H)-one (16 mg, 0.041 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 1.35 - 1.51 (m, 4H), 2.08 - 2.17 (m, 2H), 2.23 (br. s., 2H), 3.15 - 3.25 (m, 1H), 3.37 (s, 3H), 3.66 (s, 3H), 4.41 (br. s., 1H), 7.31 (d, *J* = 8.79 Hz, 1H), 7.77 - 7.82 (m, 1H), 7.84 (dd, *J* = 8.79, 1.76 Hz, 1H), 8.14 (br. s., 1H), 8.82 (br. s., 1H). HRMS: $C_{19}H_{22}N_4O_2S$ requires M+H at *m/z* 371.1541; found, 371.1523. RP-HPLC, *t*R = 1.01 min, 98% purity.

6-Iodo-2-methyl-8-(trifluoromethyl)quinazolin-4-ol (37). Compound **8** (1.35 g, 4.08 mmol) in acetic anhydride (5 mL, 53.0 mmol) neat was heated to 110 °C for 5 h. The reaction was concentrated under reduced pressure to a brown solid. To crude (6-iodo-2-methyl-8-(trifluoromethyl)-4H-benzo[d][1,3]oxazin-4-one) **34** was added ammonium acetate (0.534 g, 6.93 mmol) and DMA (8 mL).

The reaction was heated to 150 ºC for 5 h before cooling to rt. The reaction solution was diluted with water and a tan precipitate formed. It was collected by filtration and washed with water and then diethyl ether. It was dried in an oven at 60 ºC for 30 min to recover a tan solid as 6-iodo-2-methyl-8-(trifluoromethyl)quinazolin-4-ol (1.0 g, 2.82 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 2.37 (s, 3H) 8.33 (s, 1H) 8.58 (d, *J* = 1.76 Hz, 1H) 12.6 (br. s., 1H). Low-resolution MS (ES^+) *m/e* 354 (MH^+).

6-Bromo-8-methyl-2-(trifluoromethyl)-4H-benzo[d][1,3]oxazin-4-one (35). A stirred solution of 2-amino-5-bromo-3-methylbenzoic acid (2.3 g, 10 mmol) in 20 mL TFAA refluxed for 2 h. The mixture was then allowed to cool to room temperature. The solid was filtered and dried on the vacuum pump for 3 h providing 6-bromo-8-methyl-2-(trifluoromethyl)-4H-benzo[d][1,3]oxazin-4-one (1.80 g). ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.26 (s, 1H), 7.91 (s, 1H), 2.61 (s, 3H).

6-Bromo-8-methyl-2-(trifluoromethyl)quinazolin-4-(3H)-one (38). Ammonium acetate (0.75 g, 9.73 mmol) was added to a stirred solution of compound **35** (1.75 g, 5.68 mmol) in 5 mL DMA. The solution was warmed to 160 °C for 30 min, cooled to room temperature and poured into 50 mL stirred ice-water. The suspension was stirred for 15 min and filtered. The cake was washed with water, then dried to obtain 6-bromo-8-methyl-2-(trifluoromethyl)-quinazolin-4-(3H)-one (1.72 g) as a white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 13.8 (s,br, 1H), 8.10 (s, 1H), 8.01 (s, 1H), 2.55 (s, 3H). Low-resolution MS (ES^-) *m/e* 307 (MH^-).

4-Chloro-6-iodo-2-methyl-8-(trifluoromethyl)quinazoline (40). Compound **37** (0.30 g, 0.85 mmol) was stirred in toluene (1.5 mL) then $POCl_3$ (0.32 mL, 3.43 mmol) was added followed by the cautious addition of iPr_2NEt (0.30 mL, 1.72 mmol). The reaction was then heated to 90 °C for 5 h. Upon cooling to rt, the solution was concentrated under reduced pressure. The solid was taken up in DCM and concentrated again. The material was then purified by silica gel chromatography via ISCO Combiflash eluting with a gradient from 0-5% MeOH/DCM yielding 4-chloro-6-iodo-2-methyl-8-

(trifluoromethyl)quinazoline (271 mg, 0.727 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 2.88 (s, 3H) 8.46 (s, 1H) 8.81 (d, J = 1.76 Hz, 1H). Low-resolution MS (ES^+) *m/e* 368 (MH^+).

6-Bromo-4-chloro-2,8-dimethylquinazoline (41). 6-Bromo-2,8-dimethylquinazolin-4-ol (1.0 g, 3.95 mmol) **39** (prepared in a similar manner from compound **9** as what was described for compound **35** and **38** whereby acetic anhydride was used rather than TFAA in the first step) and $POCl_3$ (1.5 mL, 16.1 mmol) were stirring in toluene (3 mL) and iPr_2NEt (1.4 mL, 8.02 mmol) was slowly added. After addition the reaction was warmed to 90 °C and stirred for 3 h before cooling to rt then concentrating under reduced pressure. The material was purified by silica gel chromatography eluting with a gradient from 0-10% MeOH/DCM providing as a white solid 6-bromo-4-chloro-2,8-dimethylquinazoline (933 mg, 3.44 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 2.74 (s, 3H) 2.85 (s, 3H) 7.84 (s, 1H) 8.23 (d, J = 1.76 Hz, 1H). Low-resolution MS (ES^+) *m/e* 272 & 274 (MH^+).

(1s,4s)-N-Methyl-4-((2-methyl-6-(thiazol-5-yl)-8-(trifluoromethyl)quinazolin-4-yl)amino)cyclohexanecarboxamide (42). Compound **40** (0.12 g, 0.32 mmol), (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (see the supporting information for the preparation of this intermediate) (0.06 g, 0.39 mmol) and iPr_2NEt (0.10 mL, 0.57 mmol) in CH_3CN (2 mL) stirred at 70 °C for 3 h. Upon cooling to rt the solution was diluted with EtOAc and washed with water (2x) followed by brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure providing a pale solid as (1s,4s)-4-((6-iodo-2-methyl-8-(trifluoromethyl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (0.143 g, 0.290 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.33-1.59 (m, 3H) 1.80 (br. s., 1H) 2.00 (d, J = 10.3 Hz, 1H) 2.10-2.16 (m, 1H) 2.45 (s, 2H) 2.57 (d, J = 4.52 Hz, 2H) 4.11-4.26 (m, 1H) 7.68 (d, J = 4.52 Hz, 1H) 8.14 (d, J = 7.53 Hz, 1H) 8.25 (s, 1H) 8.97 (s, 1H). Low-resolution MS (ES^+) *m/e* 493 (MH^+). (1s,4s)-4-((6-Iodo-2-methyl-8-(trifluoromethyl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (0.12 g, 0.24 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.103 g, 0.49 mmol), cesium fluoride (0.093 g, 0.61 mmol) and copper(I) iodide (9.29 mg, 0.05 mmol) in DMF (2.5 mL) was degassed for ~ 3 min before tetrakis (0.028 g, 0.02

mmol) was added. The reaction was heated to 100 °C for 2 h then cooled to rt and concentrated under reduced pressure. The residue was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography via an ISCO Combiflash eluting with 2-10% MeOH:DCM yielding a yellow solid as (1s,4s)-N-methyl-4-((2-methyl-6-(thiazol-5-yl)-8-(trifluoromethyl)quinazolin-4-yl)amino)cyclohexanecarboxamide (56.8 mg, 0.125 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.39-1.59 (m, 4H) 1.82 (br. s., 2H) 2.01-2.06 (m, 2H) 2.07 (s, 3H) 2.14 (br. s., 1H) 2.58 (d, *J* = 4.52 Hz, 3H) 4.25 (br. s., 1H) 7.70 (d, *J* = 4.52 Hz, 1H) 8.21 (d, *J* = 7.53 Hz, 1H) 8.33 (s, 1H) 8.51 (s, 1H) 8.76 (s, 1H) 9.19 (s, 1H). HRMS: $C_{21}H_{22}F_3N_5OS$ requires M+H at *m/z* 450.1575; found, 450.1577. RP-HPLC, *t*R = 2.01 min, 99% purity.

(1s,4s)-4-((6-Bromo-8-methyl-2-(trifluoromethyl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (45). Compound **38** (0.30 g, 0.98 mmol) was stirring in acetonitrile (4.5 mL) then DBU (0.3 mL, 1.99 mmol) was added followed by PyBOP (0.763 g, 1.47 mmol). The reaction was allowed to stir at rt for ~ 10 min before the addition of (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (0.305 g, 1.95 mmol). The reaction was allowed to stir at same temperature overnight, then diluted with EtOAc and washed with water (2x) followed by brine. The organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography via the ISCO Combiflash eluting with a gradient from 15-30% EtOAc/hexanes providing (1s,4s)-4-((6-bromo-8-methyl-2-(trifluoromethyl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (384 mg, 0.561 mmol) as a solid that was carried forward. Low-resolution MS (ES^+) *m/e* 445 & 447 (MH^+).

(1s,4s)-N-Methyl-4-((8-methyl-6-(thiazol-5-yl)-2-(trifluoromethyl)quinazolin-4-yl)amino)cyclohexanecarboxamide (43). Compound **45** (0.38 g, 0.853 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.360 g, 1.71 mmol), cesium fluoride (0.324 g, 2.134 mmol) and copper(I) iodide (0.033 g, 0.171 mmol) in DMF (8.5 mL) was degassed for ~ 5 min before the addition of tetrakis (0.099 g, 0.085 mmol). The reaction was then heated to 90 °C overnight, cooled to rt and

concentrated under reduced pressure. The residue was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was dried under reduced pressure, then triturated with DCM/MeOH. A copper colored solid was collected by filtration to yield (1s,4s)-N-methyl-4-((8-methyl-6-(thiazol-5-yl)-2-(trifluoromethyl)quinazolin-4-yl)amino)cyclohexanecarboxamide (46 mg, 0.097 mmol). The filtrate was dried onto silica and purified by silica gel chromatography eluting with a gradient from 2-5% MeOH. The recovered material was re-purified by reverse phase C13 Agilent HPLC eluting with a 10-100% CH_3CN/water/0.1%TFA gradient. The fractions recovered were diluted with EtOAc and extracted with sat'd $NaHCO_3$ followed by brine. The organics were dried over sodium sulfate to yield a yellow solid as (1s,4s)-N-methyl-4-((8-methyl-6-(thiazol-5-yl)-2-(trifluoromethyl)quinazolin-4-yl)amino)cyclohexanecarboxamide (31.7 mg, 0.070 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.41-1.60 (m, 4H) 1.81-1.90 (m, 2H) 2.07 (br. s., 2H) 2.10-2.19 (m, 1H) 2.58 (d, *J* = 4.52 Hz, 3H) 2.62 (s, 3H) 4.13-4.26 (m, 1H) 7.67-7.74 (m, 1H) 8.11 (s, 1H) 8.45-8.53 (m, 3H) 9.18 (s, 1H). HRMS: $C_{21}H_{22}F_3N_5OS$ requires M+H at *m/z* 450.1575; found, 450.1567. RP-HPLC, *t*R = 1.12 min, 99% purity.

(1s,4s)-4-((2,8-Dimethyl-6-(thiazol-5-yl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (44). Compound **41** (0.5 g, 1.84 mmol), (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (0.575 g, 3.68 mmol) and iPr_2NEt (0.482 mL, 2.76 mmol) in CH_3CN (8 mL) was stirred as a suspension to 70 °C overnight. The solution was cooled to rt and diluted with EtOAc and the resulting solid collected by filtration. The tan solid was washed with ethyl acetate and dried under vacuum to yield (1s,4s)-4-((6-bromo-2,8-dimethylquinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (0.495 g, 1.26 mmol). The filtrate was extracted with water (2x) followed by brine and the organics were dried over sodium sulfate, filtered and concentrated under reduced pressure to a pale solid as (1s,4s)-4-((6-bromo-2,8-dimethylquinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (480 mg, 1.10 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.33-1.56 (m, 4 H) 1.79 (br. s., 2 H) 1.94-2.02 (m, 2 H) 2.06-2.16 (m, 1 H) 2.45 (s, 3 H) 2.57 (d, *J* = 4.52 Hz, 3 H) 3.17 (d, *J* = 5.27 Hz, 3 H) 4.11-4.22 (m, 1 H) 7.68 (d, *J* = 4.52 Hz, 1 H) 7.71 (s, 1 H) 7.81 (br. s., 1 H) 8.37 (s,

1 H). Low-resolution MS (ES^+) *m/e* 393 & 395 (MH^+). (1s,4s)-4-((6-Bromo-2,8-dimethylquinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (0.20 g, 0.51 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.216 g, 1.02 mmol), CsF (0.19 g, 1.28 mmol), and copper(I) iodide (0.019 g, 0.10 mmol) in DMF (5 mL) was degassed for ~ 5 min. before the addition of tetrakis (30 mg, 0.026 mmol). The reaction was heated to 90 °C and stirred overnight. The reaction was concentrated under reduced pressure to remove DMF. The residue was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and purified by prep HPLC (15-55% CH_3CN/water/0.1% FA) providing a pale solid as (1s,4s)-4-((2,8-dimethyl-6-(thiazol-5-yl)quinazolin-4-yl)amino)-N-methylcyclohexanecarboxamide (69 mg, 0.171 mmol). ^{1}H NMR (METHANOL-d_4): 400 MHz δ 1.50-1.62 (m, 2H) 1.63-1.76 (m, 2H) 1.95 (br. s., 2H) 2.16 (d, *J* = 10.2 Hz, 2H) 2.20-2.29 (m, 1H) 2.66 (d, *J* = 4.30 Hz, 6H) 2.71-2.77 (m, 3H) 4.39-4.50 (m, 1H) 7.82 (d, *J* = 5.08 Hz, 1H) 8.31-8.39 (m, 1H) 8.43 (s, 1H) 9.07 (br. s., 1H). HRMS: $C_{21}H_{25}N_5OS$ requires M+H at *m/z* 396.1858; found, 396.1857.

4-Iodo-N-methylaniline (48). A 2 L JLR reactor was charged with N-methylaniline (154 g, 1437 mmol), and DMF (1.45 L) and the jacket cooled to 0 °C. To this was added N-iodosuccinimide (323 g, 1.44 mol) portionwise over 30 min starting at 15 °C such that the temp fluctuated +/- 2 °C. The reaction mixture stirred at ambient temperature for 30 min. The reactor jacket was cooled to 10 °C and water (1.5 L) was slowly added maintaining a temperature < 30 °C over the course of the addition and the reactor contents transferred to a 6 L JLR. The organics were extracted four times with heptane (4 x 2 L). The combined organics were washed with 10% $Na_2S_2O_3$ (2.5 L) and then dried ($MgSO_4$), filtered and concentrated to give an amber oil (300 g, 1.29 mol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 7.39 - 7.48 (m, 2H), 6.34 - 6.45 (m, 2H), 3.56 - 3.93 (m, 1H), 2.81 (s, 3H).

4-Iodo-N,2-dimethylaniline (49). N,2-Dimethylaniline (66 g, 542 mmol) was added dropwise over 10 min to a stirred mixture of N-iodosuccinimide (134 g, 596 mmol) and DMSO (1.3 L) at 15-17 °C and stirring was continued for 1 h. Ice water (800 mL) was added during which time the reaction temperature increased to 29 °C. Heptane (800 mL) was added and the dark reaction mixture was stirred

rapidly for 30 min. The layers were separated and the aqueous phase was extracted with heptane (2 x 1 L). The combined heptane layers were washed once with water, dried over $MgSO_4$, filtered and concentrated to yield 4-iodo-N,2-dimethylaniline (113.3 g, 459 mmol) as a dark oil. ^{1}H NMR (DMSO-d_6): 400 MHz δ 6.29 (d, *J* = 8 Hz, 1H), 7.23 (s, 1H), 6.28 (d, *J* = 8 Hz, 1H), 5.19 (br s, 1H), 2.69 (s, 3H), 2.02 (s, 3H).

Methyl 3-((4-iodophenyl)amino)-3-oxopropanoate (50). A 500 mL 4-neck flask was charged with a solution of 4-iodoaniline (25.7 g, 117 mmol) in EtOAc (250 mL) along with Et_3N (21.2 mL, 152 mmol). The mixture was cooled to an internal temperature of 10 °C (ice bath) and the neat methyl 3-chloro-3-oxopropanoate (15.75 mL, 147 mmol) was added over 15 min maintaining the internal temperature below 15 °C. Following the addition, the mixture was stirred for 15 min. HPLC showed no remaining starting material. The reaction mixture was poured into ice cold water and stirred for 15 min and the layers separated. The organics were washed with $NaHCO_3$ and then dried ($MgSO_4$), filtered and concentrated to half the volume, chased with heptane (250 mL) and the slurry aged in an ice bath for 15 min and then filtered to give methyl 3-((4-iodophenyl)amino)-3-oxopropanoate as a lavender solid (29.8 g, 93.4 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 9.14 (br. s., 1H), 7.53 (d, *J* = 8.78 Hz, 2H), 7.25 (d, *J* = 8.78 Hz, 2H), 3.71 (s, 3H), 3.38 (s, 2H).

Methyl 3-((4-iodo-2-methylphenyl)amino)-3-oxopropanoate (51). A stirred mixture of 4-iodo-2-methylaniline (253.4 g, 1.09 mol), EtOAc (2.5 L) and Et_3N (183 mL, 1.31 mol) was cooled to 7 °C internal temperature. Methyl malonyl chloride (179.2 g, 1.31 mol) in EtOAc (130 mL) was added dropwise over 25 min maintaining the internal temperature between 10-15 °C. The reaction mixture (a partial slurry) was stirred at 5 °C for 1 h and was then quenched slowly with water (3 L) over 20 min while maintaining the internal temperature below 13 °C. The dark mixture was allowed to warm to ambient temperature and NaCl (300 g), water (3 L), and EtOAc (3 L) were added. The bottom aqueous phase was removed and the organic layer was washed with 5% $NaHCO_3$ (4 L). The organic layer was filtered and diluted with CH_2Cl_2 (500 mL). The solution was stirred over $MgSO_4$ and decolorizing

charcoal, filtered and concentrated by rotovap chasing twice with TBME. The resulting TBME slurry was filtered and the filter cake was washed with 1:1 TBME/heptane to afford methyl 3-((4-iodo-2-methylphenyl)amino)-3-oxopropanoate as a beige solid. A second crop of product was obtained from the filtrate (combined yield: 280 g, 840 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.55 (s, 1H), 7.60 (d, J = 2 Hz, 1H), 7.51 (dd, J = 8.4, 2.0 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 3.66 (s, 3H), 3.51 (s, 2H), 2.17 (s, 3H).

Methyl 3-((4-iodophenyl)(methyl)amino)-3-oxopropanoate (52). A 3 L reaction vessel equipped with overhead stirrer was charged with 4-iodo-N-methylaniline **48** (95.4 g, 409 mmol), Et_3N (74.2 mL, 532 mmol) and ethyl acetate (800 mL). The solution was placed in an ice/water bath and neat methyl malonyl chloride (52.8 mL, 491 mmol) was added over 20 min maintaining an internal temperature less than 10 °C. Following the addition, the mixture was stirred for 15 min. The reaction mixture was poured into ice cold water and stirred for 15 min and the layers separated. The organics were washed with $NaHCO_3$ and then dried ($MgSO_4$), filtered and concentrated to an oil. No further purification was done and the material was taken into the next reaction (~ 97% purity) (136 g, 396 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 7.75 (d, J = 8.53 Hz, 2H), 6.92 - 7.06 (m, 2H), 3.67 (s, 3H), 3.27 (s, 3H), 3.21 (s, 2H).

3-((4-Iodophenyl)amino)-3-oxopropanoic acid (54). A 500 mL 4-neck flask equipped with overhead stirring was charged with the methyl 3-((4-iodophenyl)amino)-3-oxopropanoate **50** (20.0 g, 62.7 mmol), THF (200 mL) and methanol (50 mL) and the solution was cooled to -5 °C. 2.0 N NaOH (50 mL) was added dropwise over 50 min maintaining an internal temperature of 5-7 °C. The reaction was stirred at 10 °C for 30 min and then cooled once again to -5 °C and 6.0 N HCl (20 mL) was added over 15 min. The reaction was warmed to 20 °C and then the reaction mixture was concentrated under reduced pressure removing 200 mL of solvent and chasing with water (100 mL). The resulting slurry was then filtered, washed with water twice and the resulting filter cake dried for 2 h to give 3-((4-iodophenyl)amino)-3-oxopropanoic acid as a lavender solid (18.45 g, 59.9 mmol). ^{1}H NMR (DMSO-d_6):

400 MHz δ 12.6 (br. s., 1H), 10.2 (s, 1H), 7.64 (d, *J* = 8.78 Hz, 2H), 7.42 (d, *J* = 8.78 Hz, 2H), 3.34 (s, 2H).

3-((4-Iodo-2-methylphenyl)amino)-3-oxopropanoic acid (55). A solution of methyl 3-((4-iodo-2-methylphenyl)amino)-3-oxopropanoate **51** (256 g, 768 mmol) in THF (3.5 L) and MeOH (1.0 L) was cooled to -3 °C and a 2.0 M solution of NaOH (1 L) was added dropwise over 50 min maintaining the internal temperature between 5 and -2 °C. The reaction mixture was stirred at 0 °C for 2.5 h. The reaction mixture was acidified by dropwise addition of 6.0 N HCl (350 mL, 2.1 mol) over 10 min maintaining the internal temperature below 7 °C. The resulting solution was concentrated until most of the THF/MeOH was removed. The resulting solids were collected by filtration and washed with water. The filter cake was dried at rt under vacuum for 2 days to afford 3-((4-iodo-2-methylphenyl)amino)-3-oxopropanoic acid (241 g, 755 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.6 (br s, 1H), 9.54 (s, 1H), 7.59 (d, *J* = 1.5 Hz, 1H), 7.50 (dd, *J* = 8, 1.5 Hz, 1H), 7.30 (d, *J* = 8 Hz), 3.40 (s, 2H), 2.18 (s, 3H).

3-((4-Iodophenyl)(methyl)amino)-3-oxopropanoic acid (56). A 3 L 3-neck flask equipped with overhead stirring was charged with the methyl 3-((4-iodophenyl)(methyl)amino)-3-oxopropanoate **52** (136 g, 408 mmol), THF (1.0 L) and methanol (250 mL) and the solution was cooled to -5 °C. 50% w/w NaOH (49.0 g, 612 mmol) (diluted with water (250 mL)) was added dropwise over 40 min maintaining an internal temperature of 5-7 °C. The reaction was stirred at 10 °C for 30 min and then cooled once again to -5 °C and 6.0 N HCl (100 mL) was added over 15 min. The reaction warmed to 20 °C and then the reaction mixture was concentrated under reduced pressure removing 1 L of solvent and chasing with water (500 mL). The resulting slurry was then filtered, washed with water twice and the resulting filter cake dried overnight to give an off-white solid (120 g, 376 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.5 (br. s., 1H), 7.80 (d, *J* = 8.03 Hz, 2H), 7.15 (d, *J* = 8.03 Hz, 2H), 2.99 - 3.32 (m, 5 H).

3-((4-Iodo-2-methylphenyl)(methyl)amino)-3-oxopropanoic acid (57). A solution of methyl malonyl chloride (120.4 g, 882 mmol) in EtOAc (50 mL) was added dropwise over 30 min to a stirred

solution of 4-iodo-N,2-dimethylaniline **49** (172 g, 696 mmol) and Et_3N (121 mL) in EtOAc (2.5 L) while maintaining the reaction temperature below 10 °C. The reaction mixture was stirred for 1 h at 0-10 °C and quenched with water (2 L). The layers were separated and the organic phase was washed once with 10% Na_2CO_3 (2 L), stirred over $MgSO_4$ and decolorizing charcoal and then filtered through celite. The filtrate was concentrated by rotovap chasing with THF to remove most of the EtOAc. This afforded the crude methyl 3-((4-iodo-2-methylphenyl)(methyl)amino)-3-oxopropanoate **53** as a dark amber oil. This material was dissolved in THF (1.5 L) and MeOH (600 mL). The THF/MeOH solution was cooled to -5 °C and a 3.6 M solution of NaOH in water (600 mL, 2.16 mol) was added dropwise over 30 min maintaining the reaction temperature between -5 °C and 1 °C. The reaction mixture was stirred an additional 30 min at -5 °C. 6.0 N HCl (380 mL, 2.28 mol) was added dropwise over 30 min maintaining the reaction temperature below 5 °C. The reaction mixture was allowed to warm to ambient temperature and concentrated by rotovap. The remaining material was partitioned between water (1 L) and TBME (2 L). The layers were separated and the aqueous layer was further extracted with EtOAc (2 L) and DCM (2 L). The combined organic layers were concentrated by rotovap to afford a dark purple oil which was triturated with hexanes to afford, after filtration and drying, 3-((4-iodo-2-methylphenyl)(methyl)amino)-3-oxopropanoic acid (177 g, 531 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.5 (br s, 1H), 7.77 (s, 1H), 7.63 (d, J = 8 Hz, 1H), 7.04 (d, J = 8 Hz, 1H), 3.05 (s, 3H), 2.94 (s, 2H), 2.18 (s, 3H). Low-resolution MS (ES^+) *m/e* 334 (MH^+).

6-Iodoquinoline-2,4(1H,3H)-dione (58). A 250 mL 3-neck flask equipped with mechanical stirring was charged with the 3-((4-iodophenyl)amino)-3-oxopropanoic acid **54** (18 g, 59.0 mmol) and methanesulfonic acid (75 mL, 1.16 mol) and the internal temperature was brought to 50 °C. In 3 portions of 5-6 g each, phosphorus pentoxide (16.75 g, 118 mmol) was carefully added to the reaction mixture. The temperature was then brought to 70-72 °C and the mixture stirred for 120 min until the HPLC showed complete reaction. Ice cold water was added slowly at 5 °C maintaining an internal temperature < 15 °C over the course of the addition - the first 15 mL was added over 15 min, the remaining 60 mL was

added over another 15 min and the resulting mixture aged for another 20 min in an ice bath then filtered and washed once more with ice cold water and dried for 1 h. The solid was suspended in water on the funnel and the pH adjusted to 7-8 with the addition of 60 mL of 1.0 N NaOH. The filter cake was dried overnight to remove any excess water using a 65 °C vacuum oven with a nitrogen purge to give a nice colorless solid (16.53 g, 57.0 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.2 (br. s., 2H), 7.99 (d, J = 1.76 Hz, 1H), 7.72 (dd, J = 8.66, 1.88 Hz, 1H), 7.03 (d, J = 8.53 Hz, 1H), 5.68 (s, 1H).

6-Iodo-8-methylquinoline-2,4-diol (59). 3-((4-Iodo-2-methylphenyl)amino)-3-oxopropanoic acid **55** (240.6 g, 754 mmol) was added over 10 min to neat methanesulfonic acid (1.1 L) at 45 °C rinsing with methanesulfonic acid (500 mL). Phosphorus pentoxide (214 g, 1.51 mol) was added portionwise; the reaction mixture was stirred for 20 min and then heated at 78-79 °C internal temperature for 1.25 h. The reaction mixture was cooled to rt. Water (5.0 L) was added slowly over 40 min maintaining the reaction temperature between 30-45 °C with external cooling. The reaction mixture was stirred at rt for 3 days and the solids were collected by filtration. The filter cake was stirred with water (2.0 L) and the slurry was adjusted to pH 9 with 50% NaOH solution. The solids were collected by filtration and the filter cake was washed three times with water and then washed with MeCN. The filter cake was allowed to air dry on the filter funnel for 2 days to afford 6-iodo-8-methylquinoline-2,4-diol as a pale violet powder (208 g, 692 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 10.3 (br s, 1H), 7.95 (d, J = 1.5 Hz, 1H), 7.62 (d, J = 1.5 Hz, 1H), 5.67 (s, 1H), 3.50 (br s, 1H), 2.35 (s, 3H).

4-Hydroxy-6-iodo-1-methylquinolin-2(1H)-one (60). A 2 L 3-neck flask equipped with overhead stirring was charged with the 3-((4-iodophenyl)(methyl)amino)-3-oxopropanoic acid **56** (120 g, 376 mmol), and methanesulfonic acid (430 mL, 6622 mmol) and the solution was heated to 50 °C. Phosphorus pentoxide (107 g, 752 mmol) was added portionwise over 20 min maintaining an internal temperature of 50-60 °C. The reaction temperature was then brought to 70-72 °C and the mixture stirred for 120 min. Ice cold water was added slowly at 5 °C maintaining an internal temperature < 15 °C over the course of the addition - the first 130 mL was added over 30 min, the remaining 300 mL was added

over another 25 min and the resulting mixture aged for another 20 min in an ice bath then filtered and washed once more with ice cold water and dried for 2 h. The solid was suspended in water in a beaker and the pH adjusted to 7-8 with the addition of 600 mL of 1.0 N NaOH. The solid was filtered off and the filter cake was dried overnight to remove any excess water using a 65 °C vacuum oven with a nitrogen purge to give the title compound as a nice white solid (105 g, 349 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 8.12 (d, J = 2.26 Hz, 1H), 7.88 (dd, J = 8.91, 2.13 Hz, 1H), 7.28 (d, J = 8.78 Hz, 1H), 5.85 (s, 1H), 3.48 (s, 4H).

4-Hydroxy-6-iodo-1,8-dimethylquinolin-2(1H)-one (61). P_2O_5 (215 g, 1.52 mol) was added portionwise at ambient temperature to a stirred mixture of 3-((4-iodo-2-methylphenyl)(methyl)amino)-3-oxopropanoic acid **57** (174 g, 522 mmol) and methanesulfonic acid (865 mL). The reaction temperature rose to 45 °C during the addition. The reaction mixture was heated gradually to 103 °C over 45 min and maintained at this temperature for 3 h. The mixture was cooled to below 40 °C and poured slowly onto crushed ice (ca. 8 L) with occasional stirring. The mixture was aged for 3 days at ambient temperature. The mixture was filtered and the wet filter cake was diluted with water (1 L) and the slurry was adjusted to pH 7 with 50% NaOH and filtration was continued. The filter cake was washed twice with water and twice with cold (-40 °C) CH_3CN. The solid material was triturated with boiling CH_3CN (400 mL), diluted with an equal volume of water and filtered, rinsing with CH_3CN. The material was dried in a vacuum oven overnight at 50 °C to afford 4-hydroxy-6-iodo-1,8-dimethylquinolin-2(1H)-one (96 g, 305 mmol) as a light beige solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.5 (br s, 1H), 7.99 (s, 1H), 7.71 (s, 1H), 5.85 (s, 1H), 3.61 (s, 3H), 2.63 (s, 3H). Low-resolution MS (ES^+) *m/e* 316 (MH^+).

2,4-Dichloro-6-iodoquinoline (62). A 250 mL 3-neck flask equipped with overhead stirrer, and a 1.0 N NaOH scrubber, was charged with 6-iodoquinoline-2,4(1H,3H)-dione **58** (15 g, 52.3 mmol) and $POCl_3$ (45 mL, 483 mmol). The stirred mixture was heated to 100 °C internal temperature using a 130 °C sand bath during which the suspension never completely went into solution. The reaction was maintained at this temperature for 2 h until about 98% complete by HPLC. The sand bath was removed and once the

temperature was 60 °C 100 mL of acetonitrile was added and then the suspension was poured carefully over 350 mL of crushed ice with stirring and then filtered to give 2,4-dichloro-6-iodoquinoline as an off-white solid (16.4 g, 50.6 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 8.48 (d, *J* = 1.76 Hz, 10H), 8.16 (dd, *J* = 8.78, 2.01 Hz, 1H), 7.96 (s, 1H), 7.76 (d, *J* = 8.78 Hz, 1H).

2,4-Dichloro-6-iodo-8-methylquinoline (63). 6-Iodo-8-methylquinoline-2,4-diol **59** (100 g, 332 mmol) was added portionwise over 20 min to neat $POCl_3$ (300 mL) with stirring during which time the reaction temperature rose to 48 °C. The stirred reaction mixture was heated slowly over 40 min to 100 °C and maintained at this temperature for 1.5 h. The mixture was cooled to 30 °C, diluted with MeCN (300 mL) and poured cautiously onto crushed ice (5 L) with rapid stirring. Following the quench, the ice cold mixture was aged for 45 min and then filtered. The filter cake was washed with water and MeCN and dried to afford 2,4-dichloro-6-iodo-8-methylquinoline (104 g, 307 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.42 (d, *J* = 1Hz, 1H), 7.92 (d, *J* = 1Hz, 1H), 7.50 (s, 1H), 2.72 (s, 3H).

4-Chloro-6-iodoquinolin-2(1H)-one (64). A 1 L 4-neck flask equipped with overhead stirring was charged with 2,4-dichloro-6-iodoquinoline **62** (30 g, 93 mmol), conc. HCl (100 mL, 1200 mmol), and acetonitrile (250 mL). The reaction mixture was heated to 80 °C for 20 h and then the reaction was cooled and aged in an ice bath for 30 min and filtered. The cake was washed with water (2x 100 mL) and then dried yielding 4-chloro-6-iodoquinolin-2(1H)-one (19.46 g, 63.7 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.1 (br. s., 1H), 8.03 (d, *J* = 1.76 Hz, 1H), 7.85 (dd, *J* = 8.66, 1.88 Hz, 1H), 7.13 (d, *J* = 8.53 Hz, 1H), 6.80 (s, 1H). **Note:** the analogous 6-bromo-4-chloroquinolin-2(1H)-one was made in a similar manner and its experimentals are included in the supporting information.

6-Bromo-4-chloro-8-methylquinolin-2(1H)-one (65). 6-Bromo-2,4-dichloro-8-methylquinoline (synthesized in an analogous manner as compound **63** starting with 5-bromo-2-methylanthranilic acid) (2.78 g, 9.55 mmol) was stirred in 4.0 M HCl in 1,4-dioxane (25 mL) and 6.0 M aqueous HCl (25 mL, 150 mmol) and then heated to 90 °C. The LCMS showed the reaction to be complete, thus cooled to rt

and stirred overnight. A precipitate crashed out and was collected via filtration, washed with water and Et_2O. A brown solid was recovered affording 6-bromo-4-chloro-8-methylquinolin-2-ol (1.94 g, 7.12 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 2.44 (s, 3H), 6.90 (s, 1H), 7.68 (d, J = 1.51 Hz, 1H), 7.83 (d, J = 2.01 Hz, 1H), 11.3 (br. s.,1H). Low-resolution MS (ES^+) *m/e* 274 & 276 (MH^+).

4-Chloro-6-iodo-8-methylquinolin-2(1H)-one (66). Methanesulfonic acid (600 mL) was added over 40 min at rt to a stirred mixture of 2,4-dichloro-6-iodo-8-methylquinoline **63** (103.7 g, 307 mmol), 1,4- dioxane (600 mL) and water (300 mL). The internal temperature rose to 62 °C during the addition. The reaction mixture was then heated to 106 °C internal temperature over 1 h. Caution: at this point, HCl evolution became quite vigorous and the heating mantel was dropped from the vessel. Once HCl gas evolution had subsided, heating was continued at 106 °C for 2 h. The reaction mixture was cooled to 50 °C and poured onto 3 L of crushed ice. The resulting thick slurry was aged for 1 h and filtered rinsing with water. The filter cake was air dried overnight and then slurried with water and adjusted to pH 13 with 50% NaOH. The slurry was filtered, washed with water and MeCN, and dried to afford 4-chloro-6-iodo-8-methylquinolin-2(1H)-one (90 g, 282 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.2 (br s, 1H), 7.98 (br s, 1H), 7.81 (br s, 1H), 6.87 (s, 1H), 2.41 (s, 3H).

(1s,4s)-4-((6-Bromo-2-oxo-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (67a). 6-Bromo-4-chloroquinolin-2(1H)-one (see supporting information for the synthesis of this intermediate) **65** (0.5 g, 1.93 mmol), (1s,4s)-4-amino-N-methylcyclohexanecarboxamide (0.589 g, 3.77 mmol), and iPr_2NEt (1.0 mL, 5.73 mmol) in NMP (5.5 mL) was microwaved at 150 °C for 8 h. The solution was diluted with EtOAc and washed with water (2x). A solid formed between the two layers and was collected by vacuum filtration. The material recovered was a 60% product with starting material and was set aside. The organic phase was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with a gradient from 2-10% MeOH/DCM yielding a tan

solid as (1s,4s)-4-((6-bromo-2-oxo-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (230 mg, 0.602 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.29 - 1.40 (m, 2H), 1.47 - 1.59 (m, 2H), 1.77 (d, 2H), 1.98 - 2.05 (m, 2H), 2.09 - 2.14 (m, 1H), 2.57 (d, 3H), 4.07 (q, *J* = 5.27 Hz, 1H), 5.34 (s, 1H), 6.56 (d, *J* = 7.53 Hz, 1H), 7.15 (d, *J* = 8.78 Hz, 1H), 7.57 (dd, *J* = 8.66, 2.13 Hz, 1H), 7.66 (br. s., 1H), 8.28 (d, *J* = 2.01 Hz, 1H), 10.8 (s, 1H). Low-resolution MS (ES^+) *m/e* 380 & 382 (MH^+).

6-Bromo-4-((tetrahydro-2H-pyran-4-yl)amino)quinolin-2(1H)-one (67b). 6-Bromo-4-chloroquinolin-2(1H)-one (see supporting information for the synthesis of this intermediate) (0.10 g, 0.387 mmol), tetrahydro-2H-pyran-4-amine (0.078 g, 0.774 mmol), and iPr_2NEt (0.05 mL, 0.29 mmol) in NMP (1.25 mL) was heated in the microwave at 150 °C for 6 h. The solution was diluted with EtOAc and washed with water (2x) and then brine. The combined aqueous fractions were back extracted with EtOAc. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with a gradient from 2-10% MeOH/DCM affording a brown solid as 6-bromo-4-((tetrahydro-2H-pyran-4-yl)amino)quinolin-2(1H)-one (77.7 mg, 0.240 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.53 - 1.66 (m, 2H), 1.90 (dd, *J* = 12.7, 1.88 Hz, 2H), 3.41 - 3.52 (m, 2H), 3.62 (dd, *J* = 7.03, 3.76 Hz, 1H), 3.90 (dd, *J* = 11.8, 1.76 Hz, 2H), 6.62 (d, 1H), 7.17 (d, *J* = 8.78 Hz, 1H), 7.58 (dd, *J* = 8.66, 2.13 Hz, 1H), 8.30 (d, *J* = 2.01 Hz, 1H). Low-resolution MS (ES^+) *m/e* 325 & 327 (MH^+).

6-Bromo-4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)quinolin-2(1H)-one (67c). 6-Bromo-4-chloroquinolin-2(1H)-one (see supporting information for the synthesis of this intermediate) (0.20 g, 0.774 mmol), (1r,4r)-4-(2-methoxyethoxy)cyclohexanamine (0.201 g, 1.16 mmol), and iPr_2NEt (0.405 mL, 2.32 mmol) in NMP (2.2 mL) was microwaved at 160 °C for 6 h. The reaction was diluted with EtOAc and washed with water (2x), and then brine. The combined aqueous fractions were back extracted with EtOAc. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The material was purified by silica gel chromatography by eluting with a gradient from 2-10% MeOH/DCM yielding as a tan solid as 6-bromo-4-(((1r,4r)-4-(2-

methoxyethoxy)cyclohexyl)amino)quinolin-2(1H)-one (0.166 g, 0.42 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.25 - 1.44 (m, 6H), 2.00 (br. s., 6H), 3.17 (d, *J* = 5.27 Hz, 5H), 3.36 (br. s., 2H), 3.40 - 3.45 (m, 3H), 3.51 - 3.58 (m, 3H), 4.07 (q, 2H), 5.34 (s, 2H), 6.55 (d, *J* = 7.53 Hz, 1H), 7.15 (d, *J* = 8.78 Hz, 1H), 7.57 (dd, *J* = 8.78, 2.01 Hz, 1H), 8.28 (d, *J* = 1.76 Hz, 1H), 10.82 (s, 1H). Low-resolution MS (ES^+) *m/e* 395 & 397 (MH^+).

6-Bromo-4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-8-methylquinolin-2(1H)-one (68). 6-Bromo-4-chloro-8-methylquinolin-2(1H)-one **65** (0.20 g, 0.734 mmol), (1r,4r)-4-(2-methoxyethoxy)cyclohexanamine (see supporting information for the preparation of this intermediate) (0.191 g, 1.10 mmol), and iPr_2NEt (0.38 mL, 2.20 mmol) in NMP (2.1 mL) was microwaved at 160 °C for 6 h. The reaction was diluted with DCM and washed with water (2x) then brine. The combined aqueous fractions were back extracted with DCM. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The material was purified by silica gel chromatography by eluting with a gradient from 2-10% MeOH/DCM. Recovered a tan solid as 6-bromo-4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-8-methylquinolin-2(1H)-one (166 mg, 0.406 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 1.28 - 1.40 (m, 2H), 1.41 - 1.53 (m, 2H), 2.13 - 2.18 (m, 2H), 2.21 - 2.29 (m, 2H), 2.44 (s, 3H), 3.32 - 3.47 (m, 4H), 3.54 - 3.58 (m, 2H), 3.65 (dd, *J* = 5.77, 3.76 Hz, 2H), 4.76 (br. s., 1H), 5.70 (s, 1H), 7.48 (s, 2H). Low-resolution MS (ES^+) *m/e* 411 & 413 (MH^+).

4-Chloro-6-iodo-1-methylquinolin-2(1H)-one (69). A 2 L 3-neck flask equipped with overhead stirrer, and a 5.0 N NaOH scrubber with backflow flask in place, was charged with 6-iodo-1-methylquinoline-2,4(1H,3H)-dione **60** (102 g, 339 mmol) and phosphoryl trichloride (400 mL, 4.30 mol). The stirred mixture was heated to 80 °C internal temperature using a 100 °C sand bath with everything into solution. The reaction was maintained at this temperature for 30 min until complete by HPLC. The sand bath was removed and once the temperature was 50-60 °C 800 mL of acetonitrile was added and then the solution was poured carefully over 7.50 L of crushed ice with stirring for several hours and then

filtered to give an off-white solid. The filtrate contained an appreciable amount of product so its volume was reduced to half and the precipitate filtered again. The combined cake was dried overnight in a vacuum oven w/ nitrogen purge at 65 °C to give the title compound as an off-white solid (88 g, 275 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 8.15 (d, J = 2.01 Hz, 1H), 8.00 (dd, J = 8.78, 2.01 Hz, 1H), 7.43 (d, J = 8.78 Hz, 1H), 6.97 (s, 1H), 3.57 (s, 3H).

4-Chloro-6-iodo-1,8-dimethylquinolin-2(1H)-one (70). 4-Hydroxy-6-iodo-1,8-dimethylquinolin-2(1H)-one **61** (104 g, 330 mmol) was added portionwise with stirring to $POCl_3$ (500 mL, 5.36 mol). The resulting mixture was heated to 89 °C and maintained under these conditions for 30 min. The mixture was cooled to 50 °C and concentrated. The dark oil was poured onto 2 L of crushed ice rinsing with MeCN. The mixture was neutralized with K_3PO_4 and extracted with CH_2Cl_2 (3 x 800 mL) and EtOAc (1 x 500 mL). The combined organic layers were dried over $MgSO_4$ and filtered through a 200 g plug of silica gel washing with EtOAc. The filtrate was concentrated and triturated with hexanes to afford 4-chloro-6-iodo-1,8-dimethylquinolin-2(1H)-one as a beige solid which was collected by filtration and dried (67 g, 201 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.19 (s, 1H), 7.72 (s, 1H), 6.92 (s, 1H), 3.78 (s, 3H), 2.68 (s, 3H). Low-resolution MS (ES^+) *m/e* 334 (MH^+).

4-Chloro-6-(thiazol-5-yl)quinolin-2(1H)-one (71). A 500 mL 3-neck flask equipped with overhead stirrer was charged with 4-chloro-6-iodoquinolin-2(1H)-one **64** (17 g, 55.6 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (2.0 g, 2.45 mmol), copper(I) iodide (1.06 g, 5.56 mmol) and DMA (150 mL). After 10 min of stirring at 50 °C, 5-tributylstannyl thiazole (23.0 g, 61.5 mmol) was added over 5 min and the reaction mixture heated to 60 °C - note: after 10 min the mixture became a homogeneous solution and then 15 min later the solution began to cloud up and become heterogeneous once again. The reaction was complete by the 2 h when 60 mL of EtOAc was added and the mixture filtered over celite and to the filtrate was added 150 mL of water. The solid precipitant was collected on a filter and dried, then washed with 2:1 heptane/EtOAc (4 x 100 mL) to remove any remaining stannane by-products. A final hot trituration with isopropyl acetate (150 mL) and filtering removed more color to give the title compound as

an off-white solid (13.35 g, 47.8 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 12.2 (br. s., 1H), 9.16 (br. s., 1H), 8.39 (br. s., 1H), 7.91 - 8.03 (m, 2H), 7.44 (d, J = 8.28 Hz, 1 H), 6.88 (s, 1H). Low-resolution MS (ES^+) *m/e* 263 (MH^+).

4-Chloro-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (72). A stirred mixture of 4-chloro-6-iodo-8-methylquinolin-2(1H)-one **66** (46.3 g, 145 mmol) and DMA (625 mL) was charged with $PdCl_2$(dppf)-CH_2Cl_2 adduct (8.87 g, 10.87 mmol) and CuI (2.76 g, 14.49 mmol) and heated to 112 °C. 5-tributylstannyl thiazole (70.5 g, 188 mmol) was added dropwise over 2 min rinsing with a small volume of DMA. The reaction mixture was stirred for 15 min at 115 °C at which point Darco G-60 (7 g) was added. The mixture was stirred hot for a few minutes and then hot filtered through a 1 inch plug of celite rinsing with a small volume of DMA and then with MeCN. The filtrate was aged for 30 min at rt and then cooled to 0 °C. The resulting precipitate was collected by filtration, washed with MeCN and heptane and dried to afford 4-chloro-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (19 g, 68.7 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 11.3 (s, 1H), 9.21 (br s, 1H), 8.41 (br s, 1H), 7.88 (s, 1H), 7.88 (s, 1H), 6.91 (s, 1H), 2.50 (s, 3H).

4-Chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (73). A 250 mL reaction vessel equipped with mechanical stirring was charged with 4-chloro-6-iodo-1-methylquinolin-2(1H)-one **69** (11 g, 34.4 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (0.703 g, 0.861 mmol), copper(I) iodide (0.328 g, 1.72 mmol) and potassium carbonate (9.52 g, 68.9 mmol) in EtOH (100 mL) and water (10 mL). The reaction mixture was heated to 60 °C and the 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (7.27 g, 34.4 mmol) was added. The reaction was checked by HPLC at 10 min - 80% complete. To the mixture 1 g of additional boronate ester was added and after 15 min the reaction was complete and cooled to 18 °C and filtered. The cake was washed with equal volume of EtOH, then twice with 100 mL water w/ 7% NH_4OH and the cake was dried 72 h in a vacuum oven w/ nitrogen purge at 65 °C to give 4-chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (9.2 g, 32.2 mmol) as an off-white tannish solid (9.2 g, 32.2 mmol). ^{1}H

NMR (DMSO-d_6): 400 MHz δ 9.12 (s, 1H), 8.40 (s, 1H), 7.94 - 8.08 (m, 2H), 7.65 (d, *J* = 8.78 Hz, 1H), 6.97 (s, 1 H), 3.61 (s, 3H). Low-resolution MS (ES^+) *m/e* 277 (MH^+).

4-Chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (74). A stirred mixture of 4-chloro-6-iodo-1,8-dimethylquinolin-2(1H)-one **70** (10 g, 30 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (857 mg, 1.05 mmol), CuI (400 mg, 2.1 mmol), K_2CO_3 (5.18 g, 37.5 mmol) and EtOH (110 mL) was heated to 50 °C and degassed with N_2. Water (7 mL) was added and heating was continued to 62 °C at which point 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] was added portionwise over 2-3 min. Additional 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole (2.12 g, 1.00 mmol) was added in three portions. The warm mixture was filtered and the filtrate was cooled to rt and then cooled in a dry ice/acetone bath. The solids were collected by filtration. The filter cake was washed with cold (-78 °C) EtOH. The filter cake was slurried with water, filtered and dried in a vacuum oven at 70 °C to afford 4-chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (7.77 g, 26.7 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.11 (s, 1H), 8.37 (s, 1H), 7.89 (s, 1H), 7.87 (s, 1H), 6.95 (s, 1H), 3.70 (s, 3H), 2.74 (s, 3H). Low-resolution MS (ES^+) *m/e* 291 (MH^+).

4-Chloro-1-ethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (75). To a DMF (25 mL) solution containing 4-chloro-6-(thiazol-5-yl)quinolin-2(1H)-one **71** (1.0 g, 3.81 mmol) cooled to 0 °C was added sodium hydride (183 mg, 4.57 mmol, 60% dispersion in mineral oil) followed by iodoethane (0.338 mL, 4.19 mmol). The resulting solution was stirred for 15 min at 0 °C and then warmed to rt. After 1 h, the LCMS showed primarily starting material. Therefore additional NaH and iodoethane were added and the mixture was left stirring overnight. The reaction was quenched with water and the organics taken up in EtOAc. The organic layer was washed twice with H_2O followed by drying over sodium sulfate. The solvent was removed in vacuo and the residual solid was purified on the ISCO (0-10% MeOH/DCM) yielding 4-chloro-1-ethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (381 mg, 1.31 mmol) as a tan solid. ^{1}H NMR ($CDCl_3$) δ: 8.82 (s, 1H), 8.20 (d, *J* = 1.61 Hz, 1H), 8.15 (s, 1H), 7.85 (dd, *J* = 8.65, 1.81 Hz, 1H),

7.47 (d, *J* = 8.85 Hz, 1H), 6.95 (s, 1H), 4.38 (q, *J* = 7.24 Hz, 2H), 1.39 (t, *J* = 7.04 Hz, 3H). LCMS: (ESI) M+H = 291.

(1s,4s)-N-Methyl-4-((2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide (76a). (1s,4s)-4-((6-Bromo-2-oxo-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide **67a** (0.10 g, 0.264 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.112 g, 0.529 mmol), CsF (0.100 g, 0.661 mmol), and copper(I) iodide (10.0 mg, 0.053 mmol) in DMF (2.5 mL) was degassed for ~ 5 min before the addition of tetrakis(bistriphenylphosphine)palladium(0) (0.031 g, 0.026 mmol). The reaction was then heated to 90 °C and allowed to stir for 3 h. Upon cooling to rt the solution was diluted with MeOH and concentrated on the Biotage V10. The solid was taken up in DCM/MeOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and purfied by reverse phase HPLC (15-55% CH_3CN/water/0.1% formic acid) providing a tan solid as (1s,4s)-N-methyl-4-((2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide (10 mg, 0.026 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.32 - 1.46 (m, 2H), 1.48 - 1.62 (m, 2H), 1.76 - 1.86 (m, 2H), 2.03 - 2.17 (m, 4H), 2.57 (d, *J* = 4.52 Hz, 3H), 5.36 (s, 1H), 6.62 (d, *J* = 7.53 Hz, 1H), 7.26 (d, *J* = 8.53 Hz, 1H), 7.65 - 7.77 (m, 2H), 8.27 (d, *J* = 1.51 Hz, 1H), 8.31 (s, 1H), 9.06 (s, 1H), 10.8 (s, 1H). HRMS: $C_{20}H_{22}N_4O_2S$ requires M+H at *m/z* 383.1541; found, 383.1542.

4-((Tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (76b). 6-Bromo-4-((tetrahydro-2H-pyran-4-yl)amino)quinolin-2(1H)-one **67b** (0.075 g, 0.232 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.098 g, 0.464 mmol), CsF (0.088 g, 0.580 mmol), and copper(I) iodide (8.8 mg, 0.046 mmol) in DMF (2.3 mL) was degassed for ~ 5 min. before the addition of tetrakis (0.027 g, 0.023 mmol). The reaction was heated to 90 °C for 2 h, cooled to rt and concentrated under reduced pressure on the Biotoge V10. The material was taken up in MeOH/DCM and filtered through a pad of celite. The filtrate was concentrated, taken up in DMSO/MeOH and purified by reverse

phase HPLC (15-55% CH_3CN/H_2O/0.1% formic acid) providing a white solid as 4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (5.3 mg, 0.016 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.56 - 1.70 (m, 3H), 1.94 (dd, *J* = 12.4, 2.13 Hz, 2H), 3.43 - 3.52 (m, 2H), 3.68 (br. s., 1H), 3.91 (d, 2H), 5.46 (s, 1H), 6.68 (d, *J* = 7.53 Hz, 1H), 7.27 (d, *J* = 8.53 Hz, 1H), 7.73 (dd, *J* = 8.53, 1.76 Hz, 1H), 8.26 - 8.34 (m, 3H), 9.06 (s, 1H), 10.9 (s, 1H). HRMS: $C_{17}H_{17}N_3O_2S$ requires M+H at *m/z* 328.1119; found, 328.1110.

4-(((1s,4s)-4-(2-Methoxyethoxy)cyclohexyl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (76c). 6-Bromo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)quinolin-2(1H)-one **67c** (0.16 g, 0.405 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.171 g, 0.810 mmol), CsF (0.154 g, 1.01 mmol), and copper(I) iodide (0.015 g, 0.081 mmol) in DMF (4.05 mL) was degassed for ~ 5 min before tetrakis (0.047 g, 0.040 mmol) was added. The reaction was heated to 90 °C for 2 h, before cooling to rt and concentrating under reduced pressure via the Biotage V10. The residue was taken up in MeOH and filtered through a pad of celite. The filtrate was purified by reverse phase HPLC (10-100% CH_3CN/H_2O/0.1% formic acid) yielding a white solid as 4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (19.5 mg, 0.048 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.27 - 1.50 (m, 4H), 1.99 - 2.06 (m, 4H), 3.17 (d, *J* = 5.27 Hz, 3H), 3.36 - 3.42 (m, 1H), 3.41 - 3.46 (m, 2H), 3.53 - 3.59 (m, 2H), 4.07 (q, *J* = 5.19 Hz, 1H). 5.37 (s, 1H), 6.60 (d, *J* = 7.53 Hz, 1H), 7.26 (d, *J* = 8.53 Hz, 1H), 7.72 (dd, *J* = 8.53, 1.51 Hz, 1H), 8.26 (d, *J* = 1.51 Hz, 1H), 8.30 (s, 1H), 9.06 (s, 1H), 10.8 (s, 1H). HRMS: $C_{21}H_{25}N_3O_3S$ requires M+H at *m/z* 400.1695; found, 400.1684. RP-HPLC, *t*R = 1.21 min, 98% purity.

(**1r,4r)-N-Methyl-4-((8-methyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide (77a)**. 4-Chloro-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **72** (0.15 g, 0.542 mmol), (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (prepared as described above)

(0.127 g, 0.813 mmol), and iPr_2NEt (0.284 mL, 1.63 mmol) in NMP (1.5 mL) was microwaved at 160 °C for 6 h. The material was purified by reverse phase HPLC (5-50% CH_3CN/water/0.1% formic acid) providing a tan solid as (1r,4r)-N-methyl-4-((8-methyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide (20.5 mg, 0.051 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.32 - 1.47 (m, 2H), 1.47 - 1.62 (m, 2H), 1.76 - 1.84 (m, 2H), 2.08 - 2.17 (m, 2H), 2.41 (s, 3H), 2.57 (d, *J* = 4.69 Hz, 3H), 3.35 - 3.40 (m, 2H), 5.39 (s, 1H), 6.63 (d, *J* = 7.81 Hz, 1H), 7.62 (s, 1H), 7.72 (d, *J* = 4.49 Hz, 1H), 8.14 (d, *J* = 1.37 Hz, 1H), 8.32 (s, 1H), 9.06 (s, 1H), 9.93 (br. s., 1H), 9.06 (s, 1H) 9.93 (br. s., 1H). HRMS: $C_{21}H_{24}N_4O_2S$ requires M+H at *m/z* 397.1698; found, 397.1683. RP-HPLC, *t*R = 1.08 min, 95% purity.

8-Methyl-4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (77b). 4-Chloro-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **72** (0.30 g, 1.08 mmol), tetrahydro-2H-pyran-4-amine (0.14 g, 1.41 mmol) and iPr_2NEt (0.3 mL, 1.72 mmol) in CH_3CN (1.7 mL) and NMP (1.7 mL) was microwaved at 180 °C for 6 h. Upon cooling, the solution was diluted with DCM and the resulting precipitate collected by filtration. The material was purified by reverse phase HPLC eluting with a gradient from 10-90% CH_3CN/water/0.2% NH_4OH yielding a pale yellow solid as 8-methyl-4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (80.9 mg, 0.235 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.57 - 1.71 (m, 2H), 1.91 - 1.98 (m, 2H), 2.42 (s, 3H), 3.48 (t, *J* = 11.17 Hz, 2H), 3.62 - 3.73 (m, 1H), 3.92 (m., 2H), 5.49 (s, 1H), 6.67 (d, *J* = 7.53 Hz, 1H), 7.62 (s, 1H), 8.14 (s, 1H), 8.32 (s, 1H), 9.06 (s, 1H), 9.93 (s, 1H). Low-resolution MS (ES^+) *m/e* 324 (MH^+).

4-(((1s,4s)-4-(2-Methoxyethoxy)cyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (77c). 6-Bromo-4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-8-methylquinolin-2(1H)-one **68c** (0.16 g, 0.391 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole[17] (0.165 g, 0.782 mmol), CsF (0.148 g, 0.977 mmol), and copper(I) iodide (0.015 g, 0.078 mmol) in DMF (3.9 mL) was degassed for ~ 5 min before tetrakis (0.045 g, 0.039 mmol) was added. The reaction was heated to 90 °C for 2 h

before cooling to rt and concentrating via the Biotage V10. The residue was taken up in MeOH and filtered through a pad of celite. The filtrate was purified by C18 reverse phase HPLC (30-70% CH_3CN/H_2O/0.1% formic acid) providing a white solid as 4-(((1s,4s)-4-(2-methoxyethoxy)cyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (22.6 mg, 0.054 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.89 (s, 1H), 9.05 (s, 1H), 8.31 (s, 1H), 8.13 (s, 1H), 7.61 (s, 1H), 6.60 (d, *J* = 7.53 Hz, 1H), 5.41 (s, 1H), 3.56 (dd, *J* = 5.77, 4.0 Hz, 2H), 3.42 - 3.46 (m, 2H), 3.38 (m, *J* = 5.21, 5.21, 1.63 Hz, 2H), 3.26 (s, 3H), 2.42 (s, 3H), 2.04 (m, 4H), 1.31 - 1.45 (m, 4H). HRMS: $C_{22}H_{27}N_3O_3S$ requires M+H at *m/z* 414.1851; found, 414.1837. RP-HPLC, *t*R = 1.33 min, 96% purity.

4-(((1s,4s)-4-Methoxycyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (77d). 4-Chloro-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **72** (0.50 g, 1.81 mmol), (1r,4r)-4-methoxycyclohexanamine (0.449 g, 2.71 mmol) and iPr_2NEt (0.95 mL, 5.44 mmol) in NMP (2.5 mL) and CH_3CN (2.5 mL) were microwaved at 180 °C for 10 h. The solution was diluted with EtOAc and a tan precipitate was collected. The solid was taken up in DMSO and NMP via heat which showed 30% starting material, thus purified by reverse phase C18 HPLC (15-45% CH_3CN/water/0.2% NH_4OH). The fractions recovered were concentrated under reduced pressure to provide a pale tan solid as 4-(((1s,4s)-4-methoxycyclohexyl)amino)-8-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (138 mg, 0.362 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.24 - 1.51 (m, 4H), 1.98 - 2.11 (m, 4H), 2.42 (s, 3H), 3.19 (br. s., 1H), 3.25 - 3.29 (m, 3H), 3.35 - 3.47 (m, 1H), 5.40 (s, 1H), 6.61 (d, *J* = 7.53 Hz, 1H), 7.61 (s, 1H), 8.13 (s, 1H), 8.31 (s, 1H), 9.05 (s, 1H), 9.88 (br. s., 1H). HRMS: $C_{20}H_{23}N_3O_2S$ requires M+H at *m/z* 370.1589; found, 370.1575. RP-HPLC, *t*R = 1.32 min, 100% purity.

(1r,4r)-N-Methyl-4-((1-methyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide (78a). A dioxane solution (10 mL) containing 4-chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **73** (245 mg, 0.88 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (106 mg, 0.22 mmol), Pd_2dba_3 (81 mg, 0.089 mmol, 10 mole%), sodium tert-

butoxide (298 mg, 3.10 mmol) and (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (207 mg, 1.33 mmol) was evacuated with N_2. The solution was then heated to 110 °C on the microwave for 1 h. The dark heterogeneous solution was taken up in MeOH and filtered through celite. The celite was rinsed with CH_2Cl_2 and the filtrate concentrated. The residue was purified on the ISCO Combiflash (0-20% MeOH/CH_2Cl_2) yielding 42 mg (0.11 mmol) of (1r,4r)-N-methyl-4-((1-methyl2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)cyclohexanecarboxamide as a light orange solid. ^{1}H NMR (MeOH-d_4): 400 MHz δ 8.97 (br. s., 1H), 8.33 (br. s., 1H), 8.28 (br. s., 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 6.73 (br. s., 1H), 5.68 (s, 1H), 3.63 (s, 3H), 3.44 (br. s., 1H), 2.71 (br. s., 3H), 2.10 - 2.35 (m, 3H), 1.93 (d, *J* = 12.1 Hz, 2H), 1.56 - 1.74 (m, 2H), 1.39 - 1.55 (m, 2H). HRMS: $C_{21}H_{24}N_4O_2S$ requires M+H at *m/z* 397.1698; found, 397.1682. RP-HPLC, *t*R = 1.12 min, 99% purity.

1-Methyl-4-((tetrahydro-2Hpyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (78b). A dioxane solution (7 mL) containing 4-chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **73** (153 mg, 0.55 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (66 mg, 0.14 mmol), Pd_2dba_3 (51 mg, 0.055 mmol, 10 mole%), sodium tert-butoxide (186 mg, 1.94 mmol) and tetrahydro-2H-pyran-4-amine (84 mg, 0.83 mmol) was evacuated with N_2. The solution was then heated to 100 °C on the microwave for 1 h. The dark heterogeneous solution was taken up in MeOH and filtered through celite. The celite was rinsed with MeOH and the filtrate concentrated. The residue was purified on the ISCO Combiflash (5-20% MeOH/CH_2Cl_2) yielding 35 mg (0.09 mmol) of 1-methyl-4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one as a light orange solid. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.80 (s, 1H), 8.10 (s, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.66 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 5.83 (s, 1H), 4.72 (d, *J* = 5.9 Hz, 1H), 4.06 (d, *J* = 11.3 Hz, 2H), 3.69 (s, 3H), 3.50 - 3.61 (m, 2H), 2.15 (d, *J* = 11.9 Hz, 2H), 1.65 (br. s., 2H). HRMS: $C_{18}H_{19}N_3O_2S$ requires M+H at *m/z* 342.1276; found, 342.1266. RP-HPLC, *t*R = 1.16 min, 95% purity.

4-(((1r,4r)-4-(2-Methoxyethoxy)cyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (78c). 4-Chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **73** (0.14 g, 0.506 mmol), (1r,4r)-4-(2-

methoxyethoxy)cyclohexanamine (see supporting information for the preparation of this intermediate) (0.175 g, 1.01 mmol), and iPr_2NEt (0.25 mL, 1.43 mmol) in CH_3CN (1.0 mL) and NMP (1 mL) were microwaved at 180 °C for 10 h. The solution was diluted with EtOAc and washed with water (2x) and brine. The combined organics were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was chomatographed eluting with a gradient from 2-10% MeOH/DCM. A brown oil was recovered as 4(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (87.3 mg, 0.201 mmol). ^{1}H NMR ($CDCl_3$): 400 MHz δ 1.28 - 1.48 (m, 4H), 2.11 (d, *J* = 11.5 Hz, 2H), 2.21 (d, *J* = 11.7 Hz, 2H), 3.26 - 3.37 (m, 2H), 3.38 (s, 3H), 3.51 - 3.54 (m, 2H), 3.60 - 3.62 (m, 2H), 3.63 (s, 3H), 4.98 (d, *J* = 6.83 Hz, 1H), 5.74 (s, 1H), 7.35 (d, *J* = 8.79 Hz, 1H), 7.69 (dd, *J* = 8.69, 1.86 Hz, 1H), 7.73 (d, *J* = 1.76 Hz, 1H), 8.07 (s, 1H), 8.74 (br. s., 1H). ^{13}C NMR ($CDCl_3$): 100 MHz δ 164.7, 153.5, 149.7, 141.7, 140.5, 139.9, 131.0, 125.6, 120.6, 117.2, 117.0, 95.1, 79.0, 73.7, 69.1, 60.6, 52.5, 31.9, 31.7, 30.5. HRMS: $C_{22}H_{27}N_3O_3S$ requires M+H at *m/z* 414.1851; found, 414.1837. Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N, S (Karl Fischer physicochemical analysis shows 0.37% H_2O). RP-HPLC, *t*R = 1.39 min, 100.0% purity.

4-(((1r,4r)-4-Methoxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (78d). A dioxane solution (8 mL) containing 4-chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **73** (151 mg, 0.55 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (65 mg, 0.14 mmol), Pd_2dba_3 (50 mg, 0.055 mmol, 10 mole%), sodium tert-butoxide (184 mg, 1.91 mmol) and (1r,4r)-4-methoxycyclohexanamine hydrochloride (136 mg, 0.82 mmol) was evacuated then filled with N_2 two times and sealed in a 20 mL microwave vial. The solution was then heated on the microwave to 110 °C for 1 h and upon cooling the dark solution was filtered through celite. The celite was rinsed with CH_2Cl_2 and the filtrate concentrated. The residue was purified on the ISCO Combiflash (0-15% MeOH/CH_2Cl_2) yielding 22 mg (0.058 mmol) of 4-(((1r,4r)-4-methoxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one as a orange solid. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.76 (s, 1H), 8.06 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.61 (br. s., 1H), 7.37 (d, *J* = 8.6 Hz, 1H), 5.78 (s, 1H), 4.64 (d, *J* = 6.3 Hz, 1H), 3.65 (s,

3H), 3.36 (s, 4H), 3.20 (br. s., 1H), 2.25 (d, *J* = 7.4 Hz, 2H), 2.13 (d, *J* = 7.8 Hz, 2H), 1.36 (q, *J* = 8.4 Hz, 4H). HRMS: $C_{20}H_{23}N_3O_2S$ requires M+H at *m/z* 370.1589; found, 370.1585. RP-HPLC, *t*R = 1.38 min, 98% purity.

4-(((1s,4s)-4-Hydroxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one trifluoroacetic acid salt (78e). 4-Chloro-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one **73** (170 mg, 0.614 mmol), (1r,4r)-4-aminocyclohexanol (186 mg, 1.23 mmol), and iPr_2NEt (0.215 mL, 1.23 mmol) in CH_3CN (1 mL) and NMP (1.0 mL) was microwaved at 180 °C for 10 h to roughly 70% completion. The solution was concentrated under reduced pressure and purified by reverse phase C18 HPLC eluting with a gradient from 1-99% CH_3CN/water/0.1% TFA providing a solid as 4-(((1s,4s)-4-hydroxycyclohexyl)amino)-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one trifluoroacetic acid salt (187 mg, 0.370 mmol). ^{1}H NMR (DMSO-d_6): 400 MHz δ 1.27-1.51 (m, 4H), 1.85-1.93 (m, 2H), 2.0 (d, *J* = 12.0 Hz, 2H), 3.32-3.40 (m, 1H), 3.42 - 3.51 (m, 1H), 3.52 (s, 3H), 5.54 (s, 1H), 6.67 (br. s., 1H), 7.49 (d, *J* = 8.78 Hz, 1H), 7.85 (dd, *J* = 8.66, 1.88 Hz, 1H), 8.33 (d, *J* = 1.51 Hz, 1H), 8.38 (s, 1H), 9.09 (s, 1H). HRMS: $C_{19}H_{21}N_3O_2S$ requires M+H at *m/z* 356.1432; found, 356.1427. RP-HPLC, *t*R = 1.10 min, 99% purity.

4-Amino-1-methyl-6-(thiazol-5-yl)quinolin-2(1H)-one (78f). This compound was obtained as a by-product of an attempted addition of an amine into compound **73**. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.06 (s, 1H), 8.34 (s, 1 H), 8.25 (d, *J* = 1.95 Hz, 1H), 7.85 (dd, *J* = 8.79, 1.95 Hz, 1H), 7.45 (d, *J* = 8.79 Hz, 1H), 6.68 (br. s, 2H), 5.59 (s, 1H), 3.49 (s, 3H). HRMS: $C_{13}H_{11}N_3OS$ requires M+H at *m/z* 258.0701; found, 258.0693. RP-HPLC, *t*R = 0.89 min, 98% purity.

(1r,4r)-4-((1,8-Dimethyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (79a). To a microwave vial containing 4-chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **74** (200 mg, 0.688 mmol), (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (140 mg, 0.894 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-

[1,1'-biphenyl]-2-yl)phosphine (92 mg, 0.172 mmol), 4A activated molecular sieves (600 mgs), sodium tert-butoxide (132 mg, 1.376 mmol) and tris(dibenzylideneacetone)dipalladium(0) (31.5 mg, 0.034 mmol) was added 1,4-dioxane (7 mL). The mixture was purged with N_2 before sealing and heating in an oil bath at 110 °C for 3 h. After cooling to rt, the mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in DCM and water, partitioned and then the aqueous layer extracted with DCM. The combined organic layers were washed with brine and dried over sodium sulfate. After filtration, the filtrate was concentrated in vacuo and the residue was loaded onto the ISCO Combiflash for purification eluting with 0-25% MeOH/DCM affording (1r,4r)-4-((1,8-dimethyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (40 mg, 0.097 mmol) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.77 (s, 1H), 8.11 (s, 1H), 7.57 (s, 1H), 7.53 (s, 1H), 5.88 (s, 1H), 5.66 (br. s., 1H), 3.72 (s, 3H), 3.26 - 3.46 (m, 1H), 2.81 (d, *J* = 4.69 Hz, 3H), 2.69 (s, 3H), 2.29 (d, *J* = 10.6 Hz, 2H), 2.05 - 2.21 (m, 1H), 1.93 - 2.06 (m, 2H), 1.52 - 1.71 (m, 2H), 1.24 - 1.45 (m, 2H). HRMS: $C_{22}H_{26}N_4O_2S$ requires M+H at *m/z* 411.1854; found, 411.1850. RP-HPLC, *t*R = 1.21 min, 95% purity.

1,8-Dimethyl-4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (79b). To a microwave vial containing 4-chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **74** (200 mg, 0.688 mmol), tetrahydro-2H-pyran-4-amine (90 mg, 0.894 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (92 mg, 0.172 mmol), 4Å activated molecular sieves (600 mgs), sodium tert-butoxide (132 mg, 1.38 mmol) and tris(dibenzylideneacetone)dipalladium (0) (31.5 mg, 0.034 mmol) was added 1,4-dioxane (7 mL). The mixture was purged with N_2 before sealing and heating in an oil bath at 110 °C for 3 h. After cooling to rt, the mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in MeOH and loaded onto a semi-prep HPLC for purification. The desired fractions were collected and concentrated and made basic with solid $NaHCO_3$. The organics were extracted with DCM (2x). The combined organic layers were washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated in vacuo affording 1,8-

dimethyl-4-((tetrahydro-2H-pyran-4-yl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (52 mg, 0.146 mmol) as an off white solid. ^{1}H NMR (DMSO-d_6): 400 MHz δ 9.02 (s, 1H), 8.31 (s, 2H), 8.09 (s, 2H), 7.61 (s, 1H), 6.45 (br. s., 1H), 5.59 (s, 1H), 3.91 (d, *J* = 10.4 Hz, 2H), 3.61 - 3.73 (m, 1H), 3.47 (t, *J* =11.1 Hz, 2H), 3.02 (s, 3H), 2.64 (s, 3H), 1.45 - 2.05 (m, 4H). HRMS: $C_{19}H_{21}N_3O_2S$ requires M+H at *m/z* 356.1432; found, 356.1426. RP-HPLC, *t*R = 1.24 min, 100% purity.

4-(((1r,4r)-4-(2-Methoxyethoxy)cyclohexyl)amino)-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (79c). To a microwave vial containing 4-chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **74** (120 mg, 0.413 mmol), (1r,4r)-4-(2-methoxyethoxy)cyclohexanamine (86 mg, 0.495 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (55.4 mg, 0.103 mmol), 4Å activated molecular sieves (1 g), sodium tert-butoxide (99 mg, 1.03 mmol), $Pd_2(dba)_3$ (18.9 mg, 0.021 mmol) was added 1,4-dioxane (7 mL). The mixture was purged with N_2 before sealing and heating in an oil bath at 110 °C for 2 h. After cooling to rt, the mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in MeOH and loaded onto a semi-prep HPLC for purification. The pure fractions were concentrated in vacuo and solid $NaHCO_3$ added to make it basic. The organics were extracted with DCM (2x). The combined organics were washed with brine and dried over sodium sulfate. Upon filtration, the filtrate was concentrated in vacuo affording 4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (66.3 mg, 0.155 mmol) as a white foam. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.74 (s, 1H), 8.04 (s, 1H), 7.49 (s, 1H), 7.43 (s, 1H), 5.75 (s, 1H), 4.63 (d, *J* = 6.64 Hz, 1H), 3.69 (s, 3H), 3.59 - 3.65 (m, 2H), 3.50 - 3.57 (m, 2H), 3.38 (s, 3H), 3.24 - 3.37 (m, 1H), 2.67 (s, 3H), 1.98 - 2.32 (m, 4H), 1.18 - 1.51 (m, 4H). HRMS: $C_{23}H_{29}N_3O_3S$ requires M+H at *m/z* 428.2008; found, 428.2001. RP-HPLC, *t*R = 1.45 min, 99% purity.

4-(((1r,4r)-4-Methoxycyclohexyl)amino)-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (79d). To a microwave vial containing 4-chloro-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **74** (250 mg, 0.86 mmol), (1r,4r)-4-methoxycyclohexanamine hydrochloride (185 mg, 1.12 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (115 mg, 0.215 mmol),

4Å activated molecular sieves (600 mg), sodium tert-butoxide (207 mg, 2.15 mmol) and tris(dibenzylideneacetone)dipalladium(0) (39.4 mg, 0.043 mmol) was added 1,4-dioxane (9 mL). The reaction mixture was purged with N_2 before sealing and heating in an oil bath at 110 °C for 3 h. Upon cooling to rt, the mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in MeOH and loaded onto a semi-prep HPLC for purification. The desired fractions were collected and removed concentrated in vacuo. The residue was taken up in DCM and basified with sat'd $NaHCO_3$. After partition, the aqueous layer was extracted with DCM. The combined organic layers were washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated in vacuo affording 4-(((1r,4r)-4-methoxycyclohexyl)amino)-1,8-dimethyl-6-(thiazol-5-yl)quinolin-2(1H)-one (135 mg, 0.35 mmol), as a pale yellow solid. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.74 (s, 1H), 8.05 (s, 1H), 7.50 (s, 2H), 5.76 (s, 1H), 4.78 (s, 1H), 3.69 (s, 3H), 3.40 - 3.53 (m, 1H), 3.35 (s, 3H), 3.19 (s, 1H), 2.67 (s, 3H), 1.86 - 2.48 (m, 4H), 1.35 (m., 4H). HRMS: $C_{21}H_{25}N_3O_2S$ requires M+H at *m/z* 384.1745; found, 384.1736. RP-HPLC, *t*R = 1.44 min, 99% purity.

(1r,4r)-4-((1-Ethyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (80a). To a microwave vial containing 4-chloro-1-ethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **75** (see supporting information for the preparation of this intermediate) (120 mg, 0.413 mmol), (1r,4r)-4-amino-N-methylcyclohexanecarboxamide (84 mg, 0.537 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (55.4 mg, 0.103 mmol), 4Å activated molecular sieves (600 mg), sodium tert-butoxide (79 mg, 0.825 mmol) and tris(dibenzylideneacetone)dipalladium(0) (18.9 mg, 0.021 mmol) was added 1,4-dioxane (7 mL). The mixture was purged with N_2 before sealing and heating in an oil bath at 110 °C for 3 h. After cooling to rt, the mixture was filtered through celite and the filtrate was concentrated in vacuo. The residue was dissolved in MeOH and loaded onto a semi-prep HPLC for purification. The desired fractions were collected and concentrated in vacuo and solid $NaHCO_3$ was added to make the solution slightly basic. The organics were extracted with DCM (2x). A fluffy solid was seen between the layers and it was collected

by filtration providing the desired product (1r,4r)-4-((1-ethyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (59.4 mg, 0.145 mmol) as an off white solid. The combined organic layers were washed with brine, dried over sodium sulfate and then filtered. The filtrate was concentrated in vacuo affording (1r,4r)-4-((1-ethyl-2-oxo-6-(thiazol-5-yl)-1,2-dihydroquinolin-4-yl)amino)-N-methylcyclohexanecarboxamide (28.7 mg, 0.070 mmol) as an off white solid. ^{1}H NMR (MeOH-d_4): 400 MHz δ 8.96 (s, 1H), 8.33 (d, *J* = 1.76 Hz, 1H), 8.27 (s, 1H), 7.85 (dd, *J* = 8.89, 1.86 Hz, 1H), 7.58 (d, *J* = 8.98 Hz, 1H), 5.67 (s, 1H), 4.29 (q, *J* = 6.83 Hz, 2H), 3.35 - 3.52 (m, 1H), 2.70 (s, 3H), 2.05 - 2.36 (m, 3H), 1.93 (d, *J* = 12.5 Hz, 2H), 1.53 - 1.75 (m, 2H), 1.34 - 1.55 (m, 2H), 1.27 (t, *J* = 6.93 Hz, 3H). HRMS: $C_{22}H_{26}N_4O_2S$ requires M+H at *m/z* 411.1854; found, 411.1844. RP-HPLC, *t*R = 1.23 min, 94% purity.

1-Ethyl-4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (80b). To a microwave vial containing 4-chloro-1-ethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **75** (150 mg, 0.516 mmol), and (1r,4r)-4-(2-methoxyethoxy)cyclohexanamine (107 mg, 0.619 mmol), 1,4-dioxane (9 mL) was added 4Å molecular sieves, dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (69.2 mg, 0.129 mmol), sodium tert-butoxide (124 mg, 1.29 mmol), and $Pd_2(dba)_3$ (23.6 mg, 0.026 mmol). The solution was shaken and then degassed with N_2. The vessel was sealed and heated in an oil bath at 110 °C for 2 h. Upon cooling to rt, the mixture was filtered through celite and washed with MeOH. The filtrate was concentrated in vacuo and the residue was purified on an ISCO Combiflash eluting with 0-20% MeOH/DCM affording 1-ethyl-4-(((1r,4r)-4-(2-methoxyethoxy)cyclohexyl)amino)-6-(thiazol-5-yl)quinolin-2(1H)-one (77 mg, 0.180 mmol) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): 400 MHz δ 8.74 (s, 1H), 8.05 (s, 1H), 7.70 (dd, *J* = 8.79, 1.95 Hz, 1H), 7.64 (d, *J* = 1.95 Hz, 1H), 7.38 (d, *J* = 8.79 Hz, 1H), 5.76 (s, 1H), 4.48 - 4.78 (m, 1H), 4.30 (q, *J* = 7.04 Hz, 2H), 3.57 - 3.71 (m, 2H), 3.48 - 3.56 (m, 2H), 3.38 (s, 3H), 3.25 - 3.37 (m, 2H), 2.02 - 2.32 (m, 4H), 1.25 - 1.48 (m, 7H). HRMS: $C_{23}H_{29}N_3O_3S$ requires M+H at *m/z* 428.2008; found, 428.2007. RP-HPLC, *t*R = 1.48 min, 95% purity.

Biochemical assay details for the pIC50 determinations against the human and mouse CD38 enzymes. CD38 inhibitors were tested for their capacity to inhibit human CD38 enzyme activity in a colorimetric based assay[20]. The extracellular domain of human CD38 was expressed in *Pichia Pastoris* and purified to homogeneity. The enzyme activity assay was performed in a low-volume 384-well plate in a total volume of 20 µL. A range of concentrations of test compound in 200 nL DMSO was delivered into the assay plate wells. Columns 6 and 18 of the plate contained DMSO with no compound and served as the high signal and low signal controls (no CD38 added), respectively. All additions of assay reagents to the plate were done using a Multidrop Combi, and the plate was shaken 3-5 seconds after each addition. CD38 (0.8 nM) was incubated with test compound in 10 µL containing 100 mM HEPES, pH 7.4, 4 mM EDTA, and 1 mM CHAPS for 30 minutes prior to initiation of the reaction. The reaction was initiated by a 10 µL addition containing 5 mM sodium acetate, pH 4.5, 1 mM CHAPS, 200 µM NAD and 500 µM GW323424X. The solutions for each of the two additions were prepared fresh each day from concentrated stocks of the individual components. The final concentrations in the assay were 50 mM HEPES, 2 mM EDTA, 1 mM CHAPS, and 2.5 mM sodium acetate, 100 uM NAD, 250 uM GW323434X, and 0.4 nM CD38. GW323434X is a 4-pyridynal compound that acts as a nucleophile that participates in the base exchange reaction with the nicotinamide on NAD to form a novel dinucleotide that absorbs at 405 nm. Catalytic formation of this novel chromophore was followed in an Envision microplate reader by reading absorbance at two time points, typically 30 minutes apart within the first 45 minutes of the reaction. These time points were established empirically to ensure the rates determined were in a linear range of product formation. Data analysis was performed in the following way using ActivityBase XE (Abase XE). The data from the 15 and 45 minute reads was processed by performing a subtraction function of 45 minute read value minus 15 minute read value for each plate well. The resulting values for non-control wells were converted to % inhibition using the formula 100*((U-C1)/(C2-C1)) where U is the value of the test well, C1 is the average of the values of the high signal (column 6) control wells, and C2 is the average of the values of the low signal (column 18) control wells. Percent inhibition (y) was plotted versus inhibitor concentration (x), and curve fitting was performed with the following four parameter

equation: y = A+((B-A)/(1+(10^x/10^C)^D)), where A is the minimum response, B is the maximum response, C is the $\log_{10}$IC50, and D is the Hill slope. The results for each compound were recorded as pIC50 values (-C in the above equation). For the data presented in this manuscript, the pIC50 values were converted to molar IC50 values according to the equation IC50 = 10^-pIC50. Statistics were performed on the IC50 values.

The recombinant extracellular domain of mouse CD38 was expressed in CHO CGE cells and purified to homogeneity. The pIC50 values for the inhibitors against mouse CD38 were generated using the enzyme in a fluorescence based assay in which the enzyme reaction occurred in a 10 uL volume in a low-volume 384-well assay plate. The assay quantitated CD38 catalyzed NAD hydrolysis over 45 minutes of reaction time in which the rate was linear. A range of concentrations of test compound in 100 nL DMSO was delivered into the assay plate wells. Columns 6 and 18 of the plate contained DMSO and served as the low signal and high signal controls, respectively. Column 18 contained a potent mouse CD38 inhibitor to define the high signal (no enzyme activity) control. Additions to the plate other than compound were done using a Multidrop Combi, and the plate was shaken 3-5 seconds after each addition. CD38 (0.45 nM) was incubated with test compound in 5 uL containing 20 mM HEPES, pH 7.2, 1 mM EDTA, 1 mM CHAPS for 30 minutes prior to initiation of the reaction. The reaction was initiated by a 5 uL addition containing 20 mM HEPES, pH 7.2, 1 mM EDTA, 1 mM CHAPS, and 60 uM NAD. The final concentrations in the assay were 20 mM HEPES, pH 7.2, 1 mM EDTA, 1 mM CHAPS, 30 μM NAD, and 0.225 nM mouse CD38. After the reaction time, the amount of NAD remaining was quatitated by converting it to NADH using alcohol dehydrogenase (ADH). The ADH was added in 5 μL containing 9U/mL ADH, 90 mM sodium pyrophosphate, pH 8.8, 90 mM ethanol, 1 mM EDTA, and 1 mM CHAPS. The alcohol dehydrogenase reaction was stopped by the addition of 5 μL of 1M HEPES, pH 7.0, 1.0 mM EDTA, and 1 mM CHAPS containing 0.8M dithiothreitol (DTT), and the NADH fluorescence was measured in an Envision plate reader (340 nm excitation, 460 nm emission). The solutions for each of the four additions were prepared fresh each day from concentrated stocks of the individual components,

except the DTT which was prepared fresh daily from solid. In this assay an increase in enzyme activity results in a decreased measured fluorescent signal. Each compound plate was run in duplicate with (plate A) and without (plate B) ADH. Data were acquired by reading plates in pairs and subtracting the values for plate B from plate A to obtain "corrected" data (accounts for intrinsic fluorescence from test compound). Using Abase XE, "corrected" fluorescence signals for non-control wells are converted to percent inhibition values using the formula 100-100*((U-C2)/(C1-C2)) where U is the "corrected" fluorescence signal value of the test well, C1 is the average of the "corrected" fluorescence values of the low signal (column 6; full CD38 enzyme activity) control wells, and C2 is the average of the "corrected" fluorescence values of the high signal (column 18; 100% inhibited CD38 enzyme activity) control wells. Percent inhibition data were fit using the four parameter curve fit equation described above. For the data presented in this manuscript, the pIC50 values were converted to molar IC50 values according to the equation IC50 = 10^-pIC50. Statistics were performed on the IC50 values.

Assay details for Ki determinations with the human wild-type CD38 enzyme. The CD38-catalyzed hydrolysis of NAD resulted in a decrease in absorbance at 280 nm (using $\Delta\varepsilon_{280}$ = -1.2 mM^{-1} cm^{-1} for NAD). The fully glycosylated human recombinant enzyme used for Ki determination was purchased from R&D Systems (Minneapolis, MN, catalog number 2404-AC, 95% purity). The enzyme was diluted 1:500 into standard buffer (Hepes (K+) pH 7.0) and the reaction was initiated using 100 mM NAD. Progress curves were fitted using a mixed inhibition model (Dynafit, Waltham MA) to determine Ki's for individual compounds.

NAD tissue extraction and analysis procedure. DIO (diet induced obese) mice were dosed at 30 mg/kg in a formulation of 2% DMSO: 5% solutol: 93% SBE-CD in pH 3 citrate buffer. Blood and tissues were collected 2 and 6 h post dose with blood diluted 1:1 with 5% EDTA in water, mixed and frozen on dry ice. Gastrocnemius muscle were collected with the soleus removed, wet weight recorded and tissue placed in Sarsted tube and snap frozen in liquid nitrogen. Liver samples were sliced down to

150-200 mgs with the wet weights recorded and tissues placed in a tube and snap frozen in liquid nitrogen.

Samples were stored at -80 °C with sample preparation performed immediately after removal from freezer due to instability of NAD in matrixes at room temperature. Metal bead lysing matrix was added to each tube along with a 4 fold dilution of the sample with 80:20 acetonitrile:water containing a CD38 inhibitor and O_{18}NAD. Samples were homogenized on a MP FastPrep-24 at 6 m/sec for 60 seconds with additional cycles for gastroc homogenization. The homogenate was centrifuged at 13,000 rpm for 5 minutes with the supernatant transferred to 96 well plate and diluted 1:10 with water. Analysis of the CD38 inhibitors was performed by injection of 10 µL onto an Acuity UPLC T3 column (98% water/1% formic acid/2% methanol). The sample was held for 30 seconds followed by a 30 second gradient to 5% water with formic acid holding for another 30 seconds while monitoring the 444 – 258 transition and the appropriate transition of the CD38 internal standard. Analysis of NAD was performed by injecting 10 µL on a Zorbax Hillic Plus column on an Agilent 1290 HPLC and a Sciex API4000 Mass Spectrometer monitoring the 664-428 transition for NAD and 668-136 for the O_{18}NAD internal standard. The LC separation was achieved with mobile phase A - water with 0.1% ammonium acetate and mobile phase B - acetonitrile w/ 0.1% formic acid using the following gradient:

Step	total time (min)	flow rate (µl/min)	A (%)	B (%)
0	0	600	2	98
1	0.5	600	2	98
2	2.5	600	80	20
3	2.9	600	80	20
4	3	600	2	98

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

ASSOCIATED CONTENT

Supporting Information

The experimentals and analytical data for compounds **3a–yy,** 6-bromo-4-chloroquinolin-2(1H)-one, (1r,4r)-4-amino-N-methylcyclohexanecarboxamide, trans-4-(2-methoxyethoxy)cyclohexanamine and 4-chloro-1-ethyl-6-(thiazol-5-yl)quinolin-2(1H)-one **75** can be found in this section. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure Legends.

Figure 1. CD38 thiazole screening hit.

Figure 2. Thiazoloquinoline and thiazoloquinazoline CD38 inhibitors.

Figure 3. Docking model of **3b** (in orange) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose substrate intermediate is shown in cyan.

Figure 4. Docking model of **78c** (in magenta) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose-5-phosphate substrate intermediate is shown in cyan.

Scheme Legends

Scheme 1

[a]Reagents and conditions: (a) RNH_2 (see Tables 1 and 2 for specifics with regards to what R groups were incorporated into the final compounds **3a-yy**), rt, CH_3CN; (b) CuI, K_2CO_3, rt or heating to 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF.

Scheme 2

[a]Reagents and conditions: (a) when R_1 = OMe, Br_2, $CHCl_3$, 0 °C to rt; (b) when R_1 = CF_3, 48% HBr, H_2O, 30% H_2O_2, 75 °C or NIS, rt, DMF; (d) $HCONH_2$, 165 °C; (e) $SOCl_2$, $DMF_{(cat)}$; (f) R_2NH_2 (R_2 equals 3-Cl-4-F-Ph or trans 4-CONHMe-cyclohexyl), rt, CH_3CN; (g) CuI, K_2CO_3, rt or heating to 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF.

Scheme 3

[a]Reagents and conditions: (a) urea, melt (b) $POCl_3$, 105 °C; (c) iPr_2NEt, R_2NH_2 (see Table 3 for the specific R_2 groups that were incoroporated into the final targets), iPrOH; (d) HOAc, 90 °C; (e) CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (f) NaH, MeI, DMF.

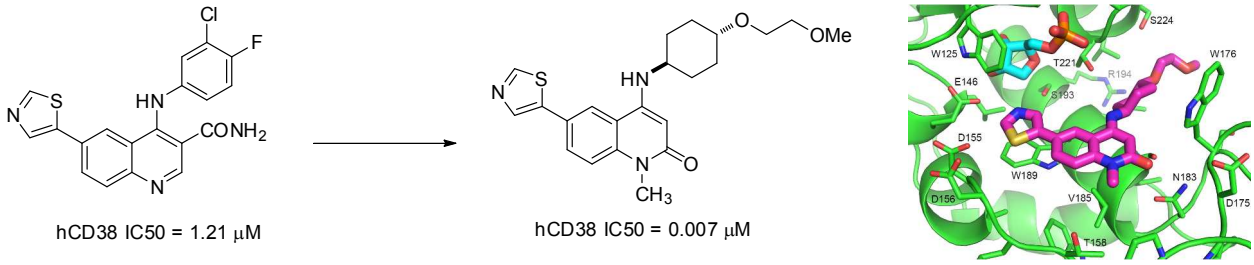
Scheme 4

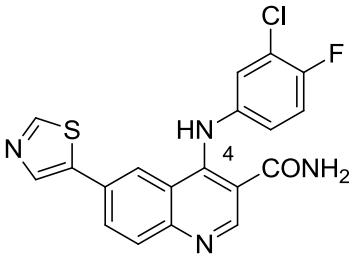
[a]Reagents and conditions: (a) Ac_2O or TFAA, reflux; (b) NH_4OAc or NH_4OH, 150 °C; (c) $POCl_3$, toluene, iPr_2NEt; (d) trans 4-NH_2-cyclohexyl-CONHMe, iPr_2NEt, CH_3CN, 70 °C; (e) CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (f) CuI, CsF, $(Ph_3P)_4Pd$, 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole, DMF, 90 °C; (g) DBU, pyBOP, trans 4-NH_2-cyclohexyl-CONHMe, CH_3CN.

Scheme 5

[a]Reagents and conditions: (a) methylmalonyl chloride, TEA, EtOAc; (b) THF, MeOH, 2.0 N NaOH; (c) CH_3SO_3H, P_2O_5, 70 °C; (d) $POCl_3$, 100 °C; (e) $HCl_{(conc)}$, 80 °C, CH_3CN or MsOH, dioxane, H_2O, 105 °C; (f) PCl_3, 80 °C (g) CuI, CsF, $(Ph_3P)_4Pd$, 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole, DMF, 90 °C; or CuI, K_2CO_3, 100-140 °C, $PdCl_2 \cdot dppf \cdot CH_2Cl_2$, 5-tributylstannyl thiazole, DMF; (h) CH_3CN, R_3NH_2 (see Table 4 for the specific R_3 groups that were incorporated into the final compounds), 150-160 °C, NMP; (i) R_3NH_2 (see Table 4 for the specific R_3 groups that were incorporated into the final compounds), Pd_2dba_3, BrettPhos, NaOtBu, 110 °C; dioxane; (j) EtI, NaH, DMF

Table of Contents graphic





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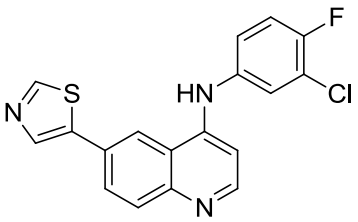

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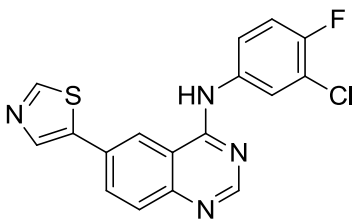

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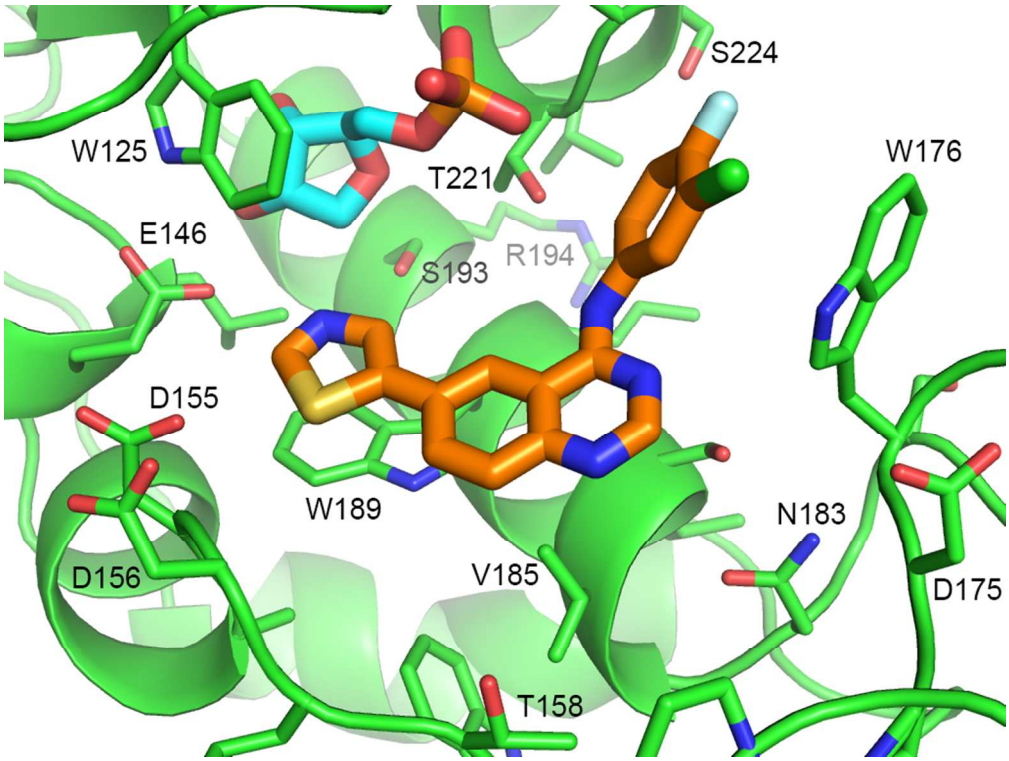


Figure 3. Docking model of **3b** (in orange) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose substrate intermediate is shown in cyan. 361x270mm (72 x 72 DPI)

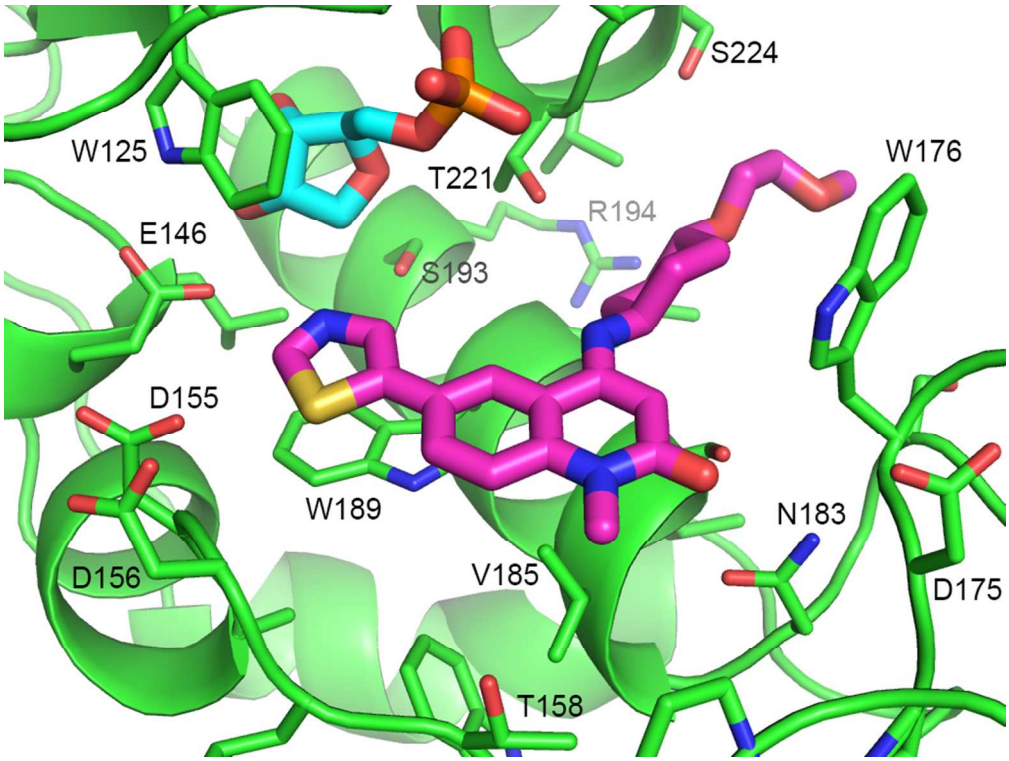
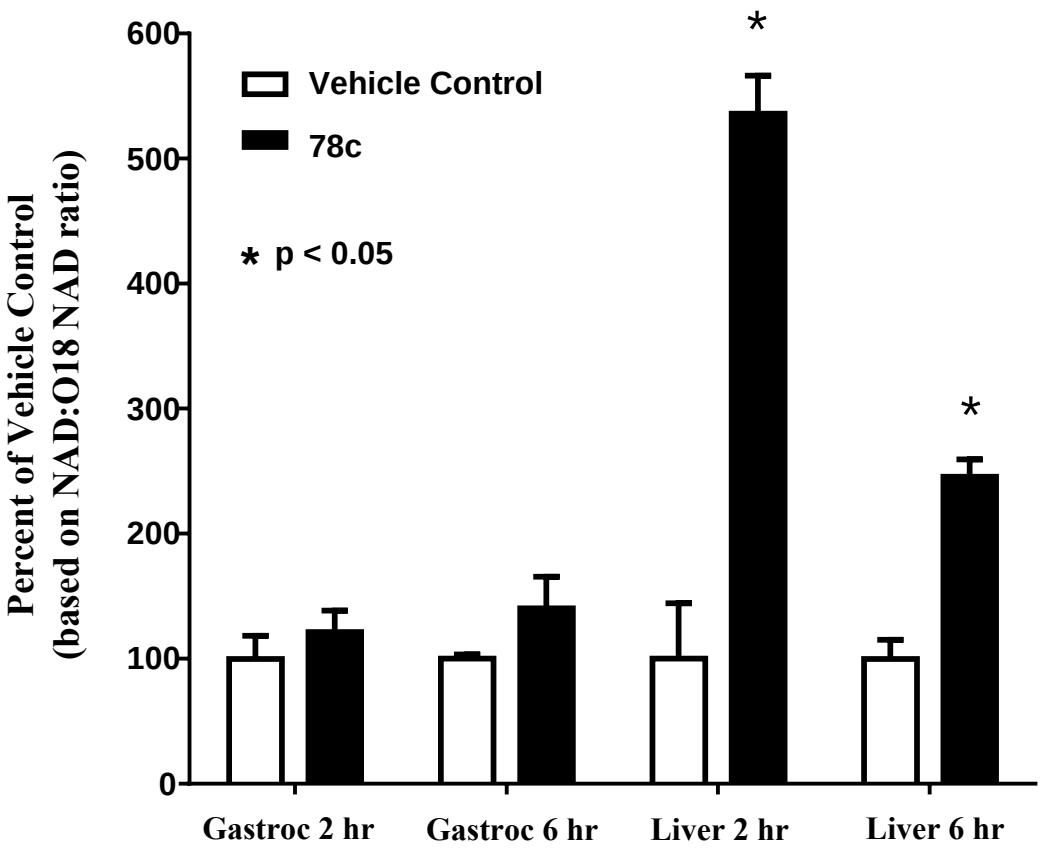
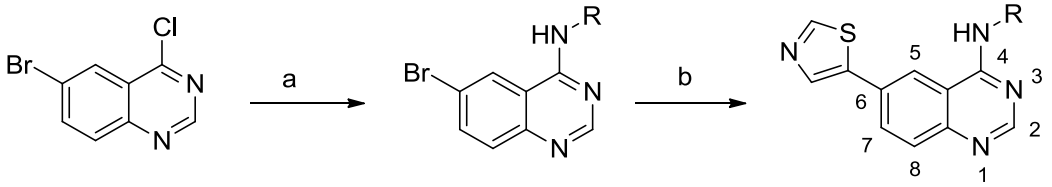


Figure 4. Docking model of **78c** (in magenta) in the hCD38 E226Q mutant protein (in green) bound with ribose-5-phosphate (PDB code: 4OGW). The modeled ribose-5-phosphate substrate intermediate is shown in cyan.
361x270mm (72 x 72 DPI)

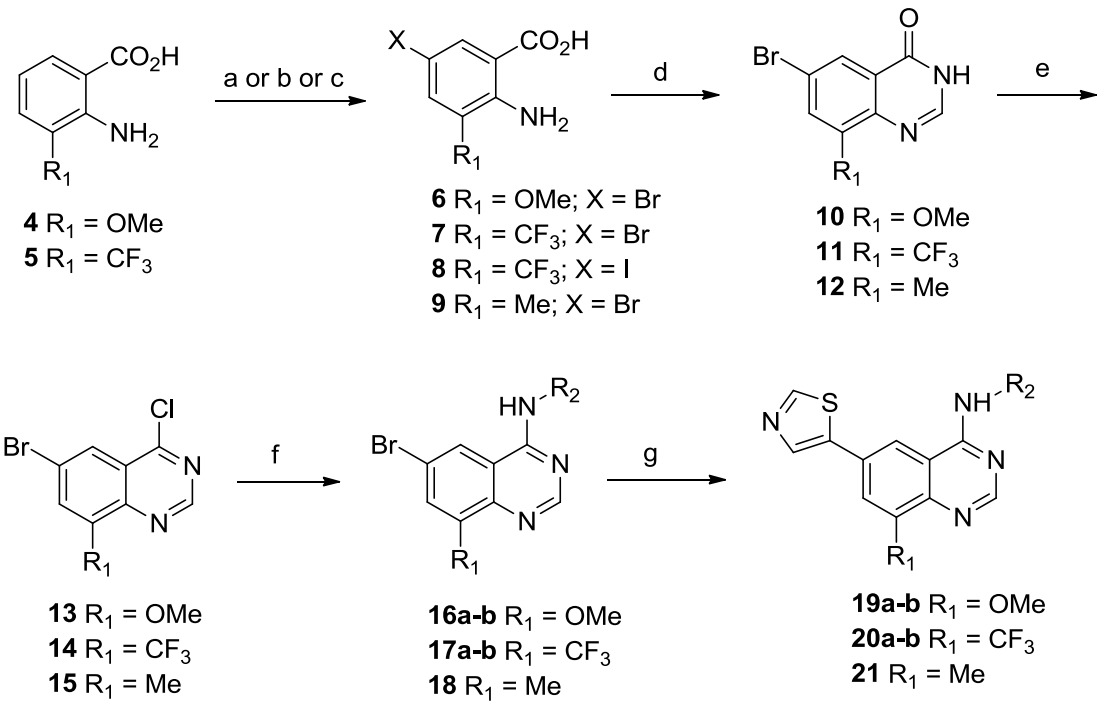
Tissue	2 hr post-dose			6 hr post-dose		
	Vehicle	78c (30 mg/kg)	p-value	Vehicle	78c (30 mg/kg)	p-value
Gastrocnemius	100 ± 18	121 ± 17	0.2122	100 ± 3	141 ± 25	0.0520
Liver	100 ± 44	536 ± 31	0.0001	100 ± 15	246 ± 14	0.0003

Values presented as percent of vehicle control ± standard deviation.





3a-yy



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5 R_1 = CF_3

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7 R_1 = CF_3; X = Br
8 R_1 = CF_3; X = I
9 R_1 = Me; X = Br

10 R_1 = OMe
11 R_1 = CF_3
12 R_1 = Me

13 R_1 = OMe
14 R_1 = CF_3
15 R_1 = Me

16a-b R_1 = OMe
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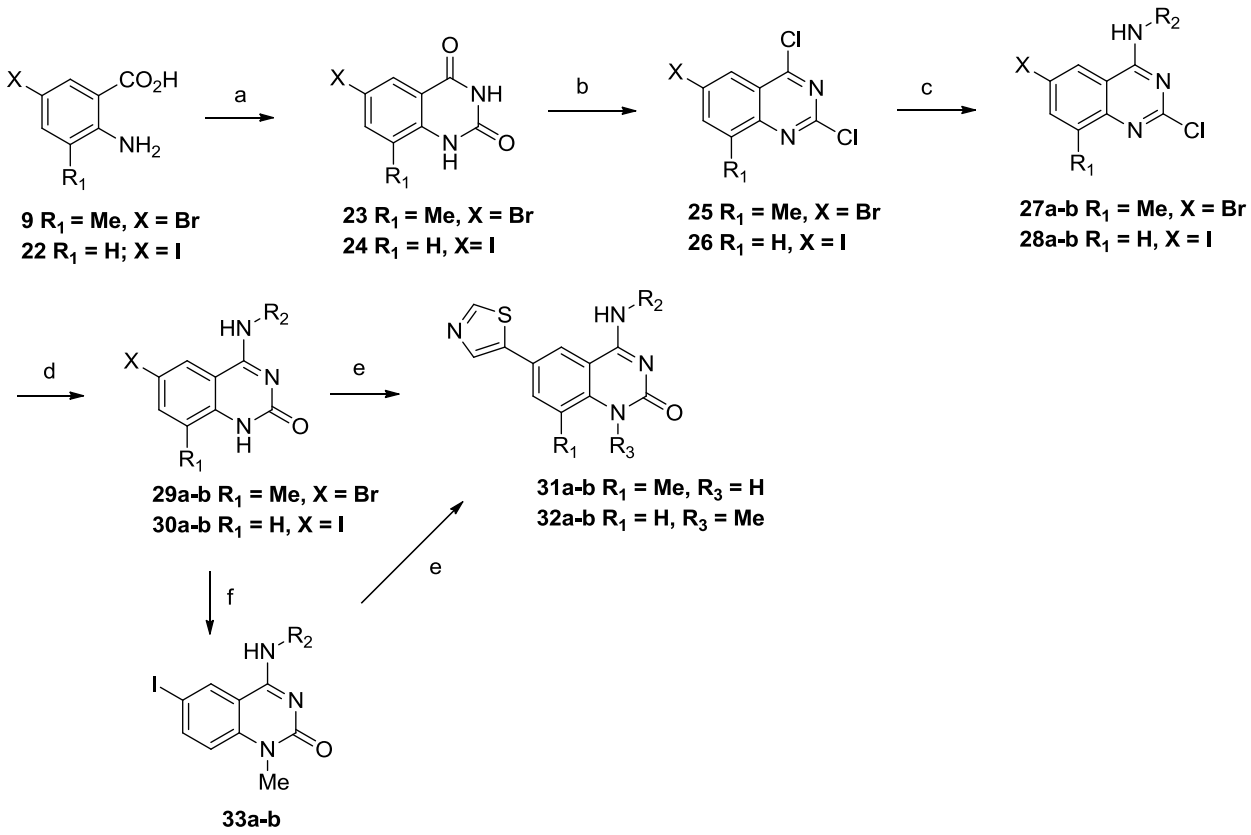
18 R_1 = Me

19a-b R_1 = OMe
20a-b R_1 = CF_3
21 R_1 = Me



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22 R_1 = H; X = I

23 R_1 = Me, X = Br
24 R_1 = H, X= I

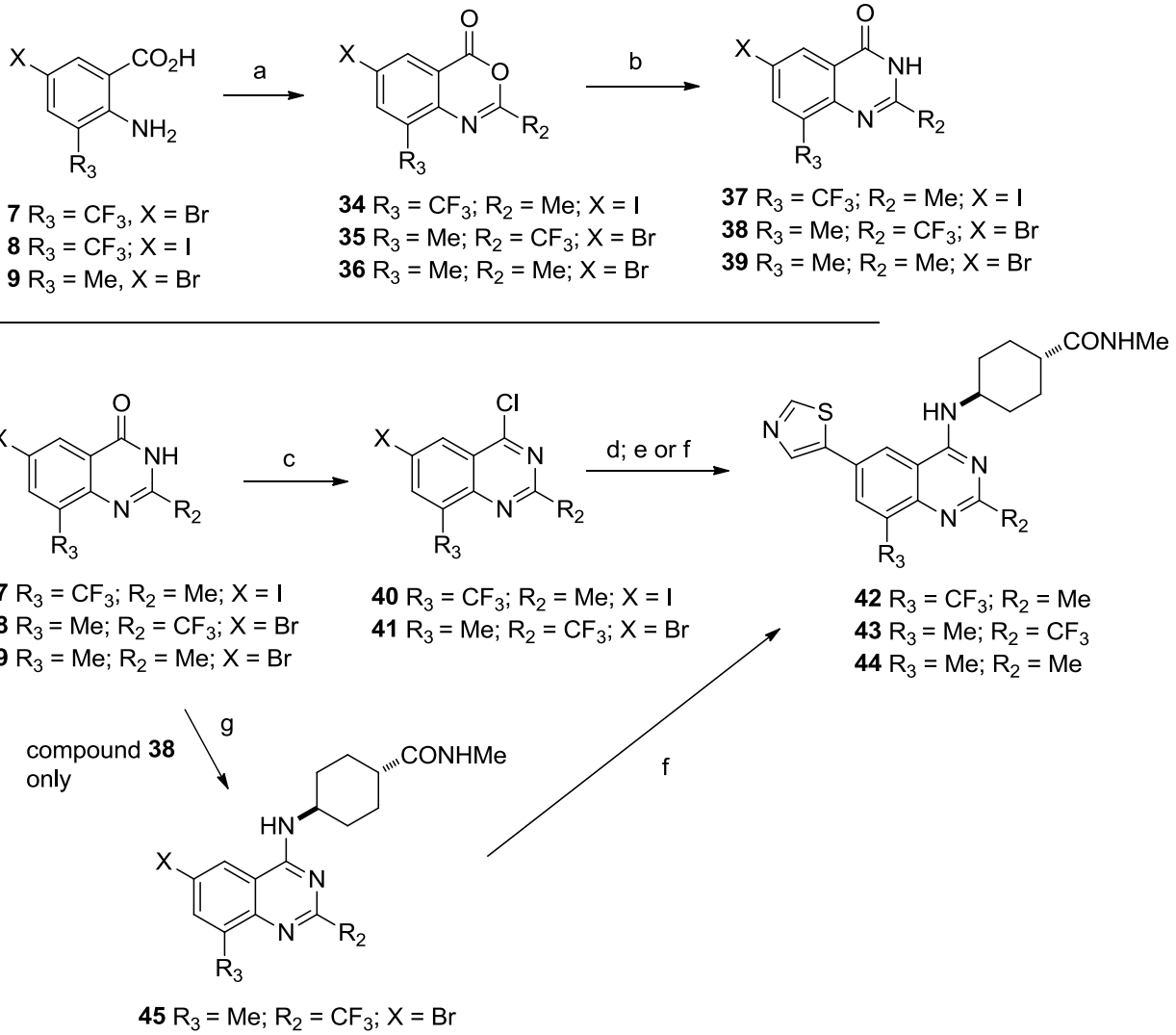
25 R_1 = Me, X = Br
26 R_1 = H, X = I

27a-b R_1 = Me, X = Br
28a-b R_1 = H, X = I

29a-b R_1 = Me, X = Br
30a-b R_1 = H, X = I

31a-b R_1 = Me, R_3 = H
32a-b R_1 = H, R_3 = Me

33a-b



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8 $R_3 = CF_3$; X = I
9 R_3 = Me, X = Br

34 $R_3 = CF_3$; R_2 = Me; X = I
35 R_3 = Me; $R_2 = CF_3$; X = Br
36 R_3 = Me; R_2 = Me; X = Br

37 $R_3 = CF_3$; R_2 = Me; X = I
38 R_3 = Me; $R_2 = CF_3$; X = Br
39 R_3 = Me; R_2 = Me; X = Br

37 $R_3 = CF_3$; R_2 = Me; X = I
38 R_3 = Me; $R_2 = CF_3$; X = Br
39 R_3 = Me; R_2 = Me; X = Br

40 $R_3 = CF_3$; R_2 = Me; X = I
41 R_3 = Me; $R_2 = CF_3$; X = Br

42 $R_3 = CF_3$; R_2 = Me
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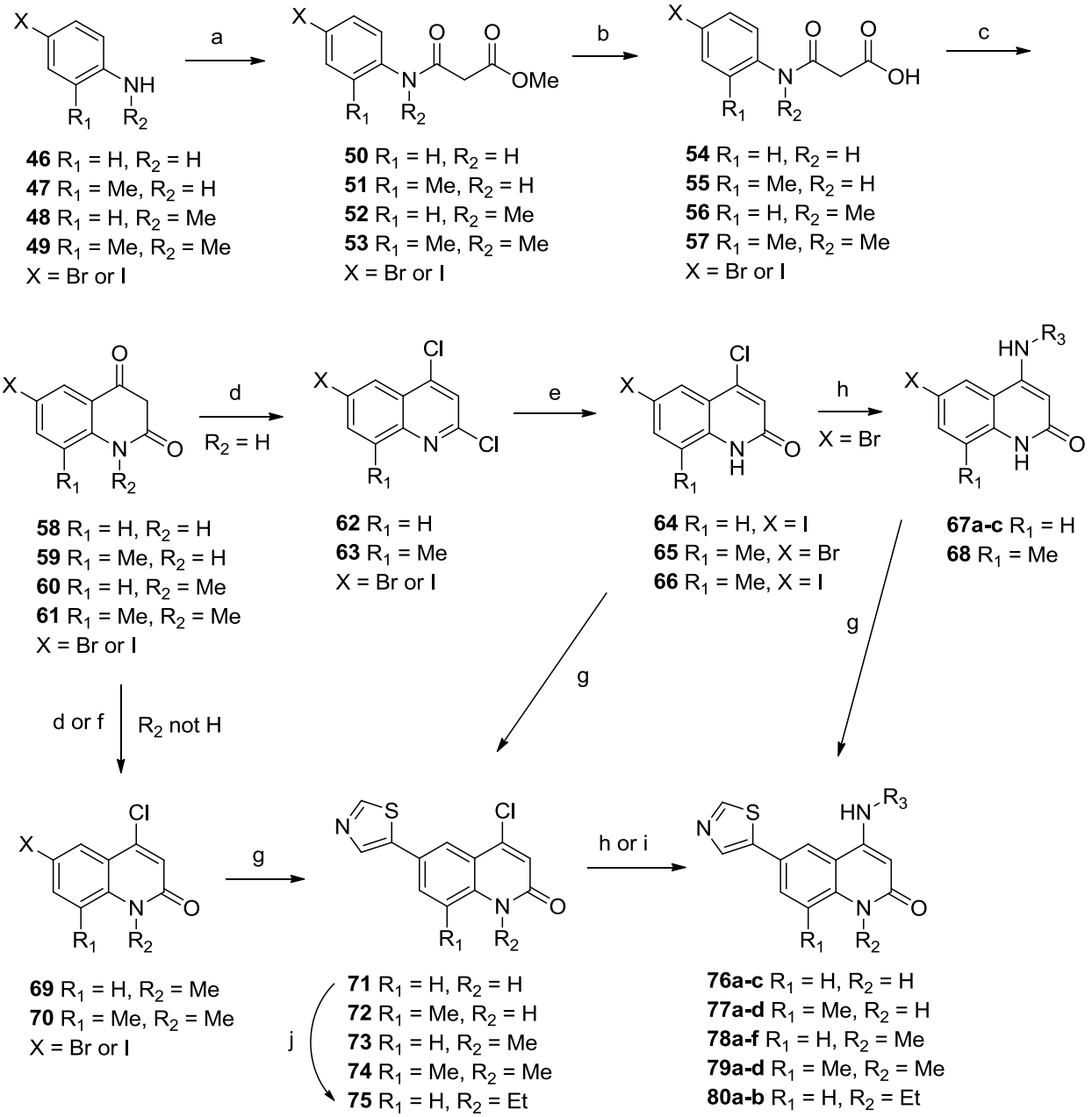
44 R_3 = Me; R_2 = Me

45 R_3 = Me; $R_2 = CF_3$; X = Br



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49 R_1 = Me, R_2 = Me
X = Br or I

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52 R_1 = H, R_2 = Me
53 R_1 = Me, R_2 = Me
X = Br or I

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X = Br or I

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61 R_1 = Me, R_2 = Me
X = Br or I

62 R_1 = H
63 R_1 = Me
X = Br or I

64 R_1 = H, X = I
65 R_1 = Me, X = Br
66 R_1 = Me, X = I

67a-c R_1 = H
68 R_1 = Me

69 R_1 = H, R_2 = Me
70 R_1 = Me, R_2 = Me
X = Br or I

71 R_1 = H, R_2 = H
72 R_1 = Me, R_2 = H
73 R_1 = H, R_2 = Me
74 R_1 = Me, R_2 = Me
75 R_1 = H, R_2 = Et

76a-c R_1 = H, R_2 = H
77a-d R_1 = Me, R_2 = H
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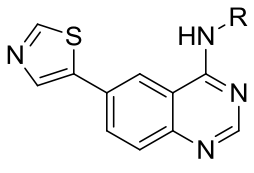
80a-b R_1 = H, R_2 = Et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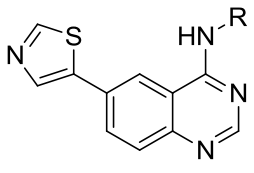
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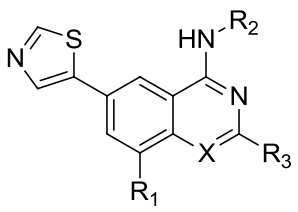
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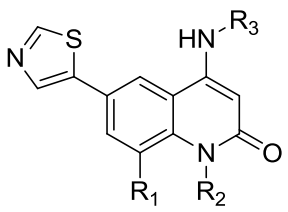
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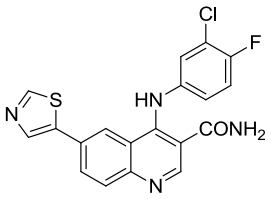

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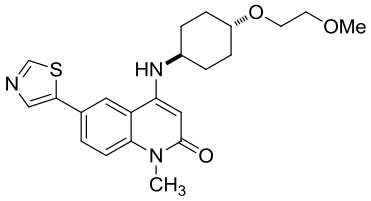

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