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Evaluation of Quinazoline analogues as Glucocerebrosidase Inhibitors with Chaperone activity

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Abstract

Gaucher disease is a Lysosomal Storage Disorder (LSD) caused by deficiency in the enzyme glucocerebrosidase (GC). Small molecule chaperones of protein folding and translocation have been proposed as a promising therapeutic approach to this LSD. Most small molecule chaperones described in the literature contain an iminosugar scaffold. Here we present the discovery and evaluation of a new series of GC inhibitors with a quinazoline core. We demonstrate that this series can improve the translocation of GC to the lysosome in patient-derived cells. To optimize this chemical series, systematic synthetic modifications were performed and the SAR was evaluated and compared using three different readouts of compound activity – enzymatic inhibition, enzyme thermostabilization, and lysosomal translocation of GC.

1. Introduction

Gaucher disease, the most common of the lipidoses, is an autosomal recessive disorder resulting from mutations in the enzyme glucocerebrosidase (EC 3.2.1.45)¹. The function of glucocerebrosidase (GC) is to hydrolyze beta glycosidic linkages of glucocerebrosides, also called glucosylceramides, in the lysosome². These glycosphingolipids are cell membrane components that maintain the stability of the lipid bilayer, function as cellular recognition elements and play an important role in cellular adherence³. There are more than 200 recognized mutations in the glucocerebrosidase gene⁴. Although many GC mutants are still functional,⁵ many affect translocation to the lysosome and results in protein premature degradation in the ER. The inability of GC protein to reach the lysosome produces accumulation of glucosylceramides in the lysosome causing tissue-specific lysosomal enlargement, characteristic of the disease. Currently, the major FDA approved medication for the treatment of Gaucher disease is the infusion of recombinant human enzyme as enzymatic replacement therapy (ERT). Although ERT successfully reverses some of the disease manifestation, the limited tissue distribution of the infused enzyme to the CNS and lungs, and its high cost require the need for improvement⁶.

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Supporting Information Available The data from the primary screening as well as all the inhibitory curves of every final compound in all the described assays are available on line (PubChem AID's: 2101, 2590, 2592, 2588, 2595, 2597, 2596, 2577, 2578, 2587, 2589). Additional images of our translocation experiment with our best inhibitors are available free of charge at <http://pubs.acs.org>.

A proposed alternate therapeutic strategy is the use of small molecular chaperones to restore the cellular function of the mutant enzyme. Small molecules that bind the mutant protein can facilitate its proper folding and increase the translocation of the mutant enzyme to the lysosome⁷⁻⁸. Several iminosugar inhibitors of glycosidases have been reported to have chaperone activity⁹⁻²⁰. For GC, two iminosugars have been clinically evaluated, eliglustat (bis{*N*-[(1*R*,2*R*)-2-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-hydroxy-1-(pyrrolidin-1-ylmethyl)ethyl]octanamide} (2*R*,3*R*)-2,3-dihydroxybutanedioate, Genz-112638),²¹ currently in clinical trials and isofagomine (3,4-Piperidinediol, 5-(hydroxymethyl)-, (3*R*,4*R*,5*R*)-, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1), Afegostat, **1**)²² whose development was recently halted during phase II testing.

Because iminosugar inhibitors work by mimicking the transition state of the glycosidic cleavage, they tend to be poorly selective¹³. Alternative scaffolds with chaperone activity are quite desirable. In addition, it is important that the compound inhibitory potency is not the primary determinant of therapeutic potential, because native substrates need to be able to displace the inhibitor after translocation to the lysosome.^{2,8,23} Thus, molecules that have moved to clinical testing are not the most potent inhibitors known.²⁴ A goal of the current study was to develop a non-iminosugar series with a favorable balance between inhibitory potency and chaperone activity.

In the cell, GC activity is modulated through the binding of an allosteric activator, Saposin C²⁵. In isolation, the addition of a bile salt is required to induce GC activity²⁶. A series of GC inhibitors identified by screening with purified enzyme were found to have reduced or absent activity when tested in tissue homogenate assays. We speculate that this difference in activity is due to nonspecific protein binding and/or to GC conformational differences between the conformation induced by detergent and that induced by Saposin C. In addition, activity differences are observed between wildtype and mutant enzyme. As 70 % of Gaucher patients carry the N370S mutation, we focused our efforts on the use of spleen²⁷ homogenate homozygous for the N370S mutation for screening for GC inhibitors and activators. GC specific activity was evaluated using 4-methylumbelliferone β -D-glucopyranoside. Upon hydrolysis, the blue dye 4-methylumbelliferone (4-MU) is liberated, producing a fluorescent emission at 440 nm when excited at 370 nm. Active compounds were then further characterized in several additional assays to confirm specificity, rule out artifacts, and most importantly, characterize chaperone activity.

2. Results

In a screen of 326,770 compounds,^{26,28,37} we found several series of GC inhibitors. Among them, several quinazoline analogues were confirmed. Additional evaluation of the series was carried out by purchasing available analogues from commercial sources. Table 1 shows the inhibitory activity of several of the commercially-available analogues in the primary screening assay. All were inactive against the related sugar hydrolases α -glucosidase and α -galactosidase at a 50 μ M concentration, and none exhibited auto-fluorescence. In addition, these compounds had very similar activity against GC as determined using an alternate resorufin-based substrate thereby ruling out nonspecific effects on the fluorescent reporter. However, all of the compounds were much less potent in purified enzyme assays of GC activity (Figure 1). This is in contrast to isofagomine **1**, which shows similar activity in tissue homogenate and purified enzyme assays. The difference may reflect the lack of exogenous activating cofactors in the purified enzyme assay. Importantly, this hampers our ability to measure the effect of these compounds on glucosylceramide cleavage, whose activity we are only able to measure using purified enzyme preparations. To demonstrate chaperone activity, we measured the capacity of hit compound **14** to increase the translocation of GC to the lysosome^{8,16,29,30}. In this experiment, wildtype and mutant

fibroblasts were incubated for five days with compound **14**, at a range of concentrations from 1 nM to 50 μ M, followed by cell fixation and staining with a selective fluorescent GC antibody. Compounds able to promote trafficking from the ER to the lysosome increased the fluorescent lysosomal signal. DMSO and isofagomine were used as negative and positive controls. Figure 3 shows the increment of signal in several Gaucher cell lines that resulted from treatment with our lead compound, confirming the chaperone capacity of the series.

With this data in hand, we embarked on systematic SAR modifications. Scheme 1 shows the synthesis strategy for some of the modifications at the quinazoline core³¹. Commercially available Boc protected piperazine **15** was sulphonated, using the sulphonyl chloride **16** in the presence of a suitable base such as Diisopropylethylamine, followed by a quantitative deprotection of the Boc functional group to yield intermediate **17**. The next step selectively displaced chloro at the core ring **18** to produce compound **19**, followed by a Suzuki cross-coupling reaction with an aromatic or heteroaromatic boronic acid **20** to produce the final compound **21**. Application of this synthetic strategy allowed us to synthesize analogues having quinazoline, pyrimidine and isoquinoline cores.

The synthesis of analogues with a purine core, Scheme 2, required the selective protection of the ring NH with methyl pivalate, followed by cross coupling and deprotection. Intriguingly, numerous attempts to carry out the Suzuki reaction with free purine cores or using a Boc protecting group, failed to yield the coupling product.

To evaluate SAR on the sulphonamide portion of the molecule, we modified the synthetic procedure that allowed us to introduce variation at the last step of the synthesis. Scheme 3 shows how sulphonylation, carboxylic coupling and alkylation of the key intermediate **25**, provides a variety of final compounds.

We also synthesized a number of analogues testing the flexibility of the diamine linker as well as the activity of some piperazine bioisosteres attached at position four of the quinazoline ring. Scheme 4, 5 and 6 show some of these alternative strategies.

To produce analogues with aromatic and heteroaromatic modifications at the 2 position we utilized Scheme 7, which details the production of the organotin **55** as a convenient intermediate for last step diversification using Stille coupling. 2,4 quinazoline dione, **52**, was converted to 2,4-dibromoquinazoline **53** with phosphorus oxybromide. Selective halogen displacement with substituted piperidine **17a** and Lithium halogen exchange followed by reaction with tributyl tin chloride yielded compound **55**. Cross coupling between **55** and heteroaromatic halogens yielded final compounds **56**.

Additional modifications at the same position 2 were obtained as shown in Scheme 8. Cross-coupling with Zinc cyanide catalyzed by Palladium yielded intermediate **57**. Then, hydrolysis of the nitrile functional group with aqueous HCl produced the amide **58**. Alternatively, hydrolysis with HCl gas in the presence of MeOH yields the methyl ester **59**. Additionally, reaction with methylmagnesium bromide provides the ketone **60**.

Tables 2-6 show the capacity of all final compounds to inhibit the hydrolysis of 4-methylumbelliferone β -D-glucopyranoside in N370S tissue homogenate. In the context of our assay, AC50 corresponds with half maximal activity concentration, either inhibitory or activating. As a positive control, we measured the activity of isofagomine in the same assay (AC50 = 0.080 μ M).

Figure 4 compares the compound profiles of isofagomine and one of the more potent analogs against the hydrolysis of 4-methylumbelliferone β -D-glucopyranoside and a

fluorescent glucosylceramide by wildtype and mutant N370S GC using tissue homogenate and isolated enzyme.

We also measured the capacity of several compounds from this series to protect the GC protein from thermal denaturation by using the fluorescence thermal shift techniques, also referred to differential scanning fluorimetry. Figure 5 shows that several compounds in the series have a strong ability to protect the GC enzyme from denaturation, with some concentration-dependent shifts in the transition temperature (T_m) exceeding 15 °C.

Last, we evaluated the chaperone capacity of some of our best compounds by measuring the increase in ER-lysosome GC translocation using human fibroblasts from both normal individuals and Gaucher patients. Figure 6 shows the results after five day incubation and treatment with a fluorescent GC antibody.

3. Discussion

One of the main problems facing rare and neglected disease research is the development of relevant assays for HTS and SAR studies. The field of lysosomal storage disorders is characteristic of this problem. While some small molecule chaperones, like the iminosugars, have progressed into clinical testing, the clinical development of most compounds has been challenging due to their poor selectivity and the difficulty in resolving the therapeutic index between enzymatic inhibition and pharmacologic chaperoning. Ideally, a good small molecule chaperone would not impact the hydrolytic capacity of the enzyme, however, all previously advanced series have been inhibitors. This is likely a consequence of the type of assay chosen for HTS and reflects the ready availability of the purified enzyme for high-throughput screening and the ease of implementation of fluorogenic screening assays. In our hands, we have observed that several series found in purified enzyme assay reduced or lost their activity in the native environment of tissue homogenate. This might be due to the absence of necessary cofactor subunits in the purified enzyme. We also have observed that some series with inhibitory capacity in tissue homogenate assays do not show enhanced activity with isolated enzyme, probably also due to differences in the enzymatic conformational states induced by different assay conditions. Purified enzyme is likely insensitive to activators because of artificial activation by the bile salt in the assay buffer³². Figure 1 shows that the series described in this paper display N370S inhibitory activity only in tissue homogenate conditions, and therefore HTS using purified enzyme would have failed to discover this series.

The capacity of a molecule to inhibit a hydrolytic enzyme does not directly correlate with its capacity to induce proper folding and chaperone activity. For this reason, before the initiation of our SAR studies we decided to evaluate the chaperone capacity of our series. The increase in GC translocation to the lysosome can be measured in patient-derived fibroblasts (Figure 3), but this remains a labor-intensive, low-throughput assay, unsuitable for broad SAR analysis. As surrogate assay, we used the primary screening assay, hydrolyzing an artificial pro-fluorescent substrate in the context of tissue homogenate. Increases in inhibition should correlate with increases in binding and render this assay a reasonable surrogate. Another surrogate for chaperone activity is to measure the capacity of compounds to protect the protein from denaturation regardless of their ability to inhibit its enzymatic activity. We have used both of these assays to guide our SAR with the aim of obtaining compounds with a greater binding capacity that should translate into better chaperone activity.

It should be noted that the kinetics of the hydrolytic reaction not only depends on the conditions of the assay (purified enzyme or homogenate) and enzyme (wildtype or mutant)

but also on the nature of the enzymatic substrate. We have previously observed³³ important differences between the hydrolysis of natural and artificial substrates. To better characterize the potential of the present compounds to inhibit the hydrolysis of the natural substrate, glucosylceramides, in native enzyme preparation (e.g. tissue homogenate), we developed and validated an LC-MS method to follow the hydrolysis of a native substrate tagged with a fluorescent dye, Figure 2.

SAR of this series demonstrates that there are strict requirements for maintaining activity. Tables 2 and 3 show the activity of analogues with sulfonamide aromatic modifications. In general, potency changes up to 25-fold between aromatic substituents. Analyzing derivatives with a single substitution it can be observed that substituents in para, and especially in ortho position tend to provide better activity, with the para methyl analogue being our most potent compound (**26ad**) with an IC₅₀ of 320 nM. As a group, there are no large differences between the activity provided by halogen, electron withdrawing and electron donating functional groups. Regarding bicyclic rings, combination of electrodonating groups in meta and para positions (Table 3, compounds **26bs**, **26bt** and **26by**) provide the most potent compounds.

We also studied modifications in the piperidine linker. Elimination of the aromatic ring of the sulphonamide is detrimental to the activity of the molecule (Table 4, compounds **26cb**, **26cc** to **26cd**). Replacement of the sulphonamide by an amide group also reduces the activity (Table 4, compounds **29** and **30**). This reduction is even greater when the sulphone is eliminated and a benzyl substituent is directly attached to the piperidine ring (Table 4, compound **28**). Analogues **51** and **38** show that both nitrogens of the piperidine ring play an important role in maintaining the activity, with the one next to the quinazoline ring being especially important. The angle (Table 4, compound **35c**) and rigidity (Table 4, compounds **35a** and **35b**) of the linker is also important for activity.

The next modifications studied entailed the replacement of the aromatic substituent at the two position of the quinazoline ring. Table 5 shows that the complete elimination of the substitution (compound **62**) reduces the activity by more than 60 times, and replacing it with non-aromatic functional groups, compounds **59**, **60**, **58**, **57** and **61**, greatly reduce the activity. Introduction of an unsubstituted phenyl ring at two position (compounds **62** and **21aa**) increase the activity of the molecule, although all analogues with substituents in this phenyl ring abolish the activity (compounds **56l**, **56m**, **56n** and **56o**). Replacement of the phenyl aromatic ring by a six-member heteroaromatic ring, encompassing a nitrogen reduces or abolishes the activity (compare compounds **21aa** with **56f**, **56g**, **56h**, **56i**, **56j** and **56k**). The introduction of a five member heteroaromatic ring with an unsubstituted 2-thiophene displayed the best activity (compound **26ad**), followed by 3-furan (compound **56e**) and 4-thiazolyl (compound **56c**). In addition, a 1-benzimidazole substitution (compound **63**) also provides better activity than a plain phenyl ring (compound **21aa**).

The last modifications studied were analogues of the quinazoline core. Replacement of the quinazoline core by a purine results in a loss of activity (Table 6, compounds **21bb** and **21bc**). Elimination of the quinazoline nitrogens also impacts the activity, reducing (compounds **71**) or completely abolishing it (compound **70**). The introduction of one additional nitrogen within position 5 or 8 of the quinazoline ring (compounds **72** and **73**) reduced at least the activity by at least 10 times. Replacement of the quinazoline ring by a unsubstituted pyrimidine ring (compound **74**) reduced the activity by 32 times, although the reduction was only by only 10 times if the pyrimidine was bearing methyl groups in positions 5 and 6 (compound **76**). Lastly, introduction of a chloro substituent in position 7 of the quinazoline ring completely abolished the activity (compounds **77** to **84**).

In summary, SAR of this series demonstrates a very narrow functional modification, with the need to keep most of the functionalities of the molecule, such as the quinazoline core, the piperidine ring, the sulphonamide substitution and the thiophene heteroaromatic ring at two positions to avoid elimination or drastic reduction of activity. Even so, we were able to increase the potency of the molecule to the desired range. Our most potent non-iminosugar compound displays an IC_{50} in the “blue” GC enzyme assay, Figure 2, of 320 nM, while the iminosugar isofagomine has an IC_{50} of 80 nM.

In addition, we used HPLC to track by the hydrolysis of a native substrate tagged with a fluorophore³⁴. As validation of this method, Figure 4A, we first compared the IC_{50} values of isofagomine using LC-MS or a ViewLux fluorescent reader when the tissue homogenate hydrolysis of 4-methylumbelliferone β -D-glucopyranoside was measured. Although each molecule was able to inhibit the hydrolysis of the ceramide, in Figure 4B, it can be seen that the series displayed better IC_{50} 's for the hydrolysis of the 4-methylumbelliferone β -D-glucopyranoside than for the hydrolysis of the ceramide. This was also characteristic of isofagomine, but, in general, the inhibitory capacity of our series is smaller both in terms of IC_{50} 's as well as maximum inhibition of the enzyme.

We also evaluated the selectivity of our inhibitors toward other lysosomal glycosidases. None of the compounds in our series showed any capacity to modulate the activity of acid α glucosidase and α galactosidase, thus ruling them out as promiscuous agents.

One means to measure the capacity of a small molecule to bind and stabilize a protein is by evaluating its ability to change the transition temperature (T_m) during thermal denaturation experiments. Previous authors³⁵ have shown that small molecule chaperones are able to thermostabilize mutant proteins. Figure 5 shows the impact of our best inhibitors in raising the melting point of glucocerebrosidase. Our best inhibitors are able to produce an extraordinary shift in the temperature of denaturation of GC (see Table 8 for details in T_m shift at each concentration for each compound), with much greater values than those observed for isofagomine or DNJ. Reports have shown that the increment in melting point often correlates directly with the compound's binding affinity³⁶ and, therefore this experiment, in conjunction with the previous one, demonstrates that our best inhibitors have a lower inhibitory capacity than isofagomine, but a greater protein stabilizing capacity and binding affinity.

To demonstrate that this binding affinity translates into chaperone capacity, we evaluated the ability of our best compounds to increase the translocation of GC. Wildtype and mutant fibroblast were incubated for five days with compounds **14**, **26ad**, **26ai**, **26bs** and **62** in a range of concentrations from 1 nM to 25 μ M, using isofagomine as a positive control. At the end of the experiment, cells were fixed and the GC concentration evaluated using a selective fluorescently labeled antibody. Co-localization of GC in the lysosome was evaluated using confocal microscopy. Figure 6 shows that upon treatment with our compounds there was a clear increase of the amount of GC localized to the lysosome.

In conclusion, we present the discovery, SAR study and biological evaluation of a new non-iminosugar GC inhibitory series with chaperone activity. SAR optimization yielded compounds at an IC_{50} of 320 nM in our homogenate tissue assay. The compounds have a reduced capacity to inhibit the hydrolysis of a ceramide natural-like substrate, and they were selective versus other glycosidases. Thermal denaturation assays further demonstrated the capacity of our series to stabilize the GC structure. Lastly, these compounds increased the ER-lysosomal trafficking of several GC mutants. Additional metabolic optimization studies, pharmacokinetics and *in vivo* evaluation are currently under way, to advance the development of this series as a potential therapeutic modality.

4. Experimental Section

4.1 Chemistry

The reagents and solvents were used as commercial anhydrous grade without further purification.

Compounds **2**, **3**, **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **14**, **21aa**, **21ab**, **21ac**, **21ad**, **21ae**, **21af**, **21ag**, **21ah**, **21ai**, **21aj**, **21ak**, **21al**, **21am**, **21an**, **21ao**, **21ap**, **21aq**, **21ar**, **21as**, **21at**, **21au**, **21av**, **21aw**, **21ax**, **21ay**, **21az**, **21ba**, **26aa**, **26al**, **26am**, **26au**, **26av**, **26aw**, **26ax**, **26zy**, **26az**, **26bc**, **26bd**, **26bf**, **26bg**, **26bh**, **26bi**, **25bl** and **26br** were purchase from Enamine.

Compounds **56e**, **56l**, **56m**, **56n**, **56o**, **61**, **75**, **77**, **78**, **79**, **84**, **80**, **81**, **82**, **83** and **85** were purchase from AMRI. Beside the certificate of analysis provided by those companies, we performed quality control analysis using LC-MS system. All of them showed purity greater than 95%.

Column chromatography was carried out over silica gel (100–200 mesh). ¹H NMR spectra were recorded with a Bruker 400 MHz spectrometer from solutions in CDCl₃ and DMSO-*d*₆. Chemical shifts in ¹H NMR spectra are reported in parts per million (ppm, δ) downfield from the internal standard Me₄Si (TMS, δ = 0 ppm). Molecular weight confirmation was performed using an Agilent Time-Of-Flight Mass Spectrometer (TOF, Agilent Technologies, Santa Clara, CA). A 3 minute gradient from 4 to 100% Acetonitrile (0.1% formic acid) in water (0.1% formic acid) was used with a 4 minute run time at a flow rate of 1 mL/min. A Zorbax SB-C18 column (3.5 micron, 2.1 $\times$ 30 mm) was used at a temperature of 50°C. Confirmation of molecular formula was confirmed using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).

Preparation of 1-Tosylpiperazine (17a)—*N,N*-Diisopropylethylamine (28.5 mL, 167.52 mmol) and *p*-toluenesulfonylchloride **16a** (11.27 g, 59.13 mmol) were added to a stirring solution of *N*-Boc-piperizine **15** (10.0 g, 53.75 mmol) in NMP (80 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (150 mL) and extracted with EtOAc (3 $\times$ 50 mL). The combined organic layer was washed with water (3 $\times$ 50 mL) and brine (3 $\times$ 50 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by trituration with hexanes and dried under vacuum to afford the intermediate Boc-protected sulphonamidopiperizine (18.0 g, 96%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 2.97–2.90 (m, 8H), 2.43 (s, 3H), 1.45 (s, 9H); MS (ESI) *m/z* 341 [C₁₆H₂₄N₂O₄S + H]⁺.

Hydrochloric acid in 1,4-dioxane (20%, 30 mL) was added to a stirring solution of the above Boc-protected sulphonamidopiperizine (18.0 g, 60.60 mmol) in CH₂Cl₂ (50 mL) at 0 °C. After stirring for 16 h at room temperature, the precipitated solids were filtered off, and the filter cake was dissolved in water (50 mL). The resulting aqueous solution was washed with CH₂Cl₂ (2 $\times$ 20 mL), cooled to 0 °C, and basified to pH 12 with a 6 N NaOH solution. The resulting aqueous solution was extracted with CH₂Cl₂ (2 $\times$ 30 mL) and the combined organic layers were dried over Na₂SO₄, filtered, and the filtrate was concentrated under reduced pressure to afford amine **17a** (11.0 g, 84%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 2.97–2.90 (m, 8H), 2.43 (s, 3H); MS (ESI) *m/z* 241 [C₁₁H₁₆N₂O₂S + H]⁺.

General Procedure for the Synthesis of Core 19: Displacement of Halide on the Heterocyclic 18: *N,N*-Diisopropylethylamine (11.2 mL, 64.40 mmol) and amine **17** (8.00 g, 33.20 mmol) were added to a stirring solution of the heterocyclic halide **18** (36.5 mmol) in NMP (50 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was

diluted with water (100 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (3 × 50 mL) and brine (3 × 50 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by trituration with hexanes and dried under vacuum to afford heterocycle **19**.

2-chloro-4-(4-tosylpiperazin-1-yl)quinazoline (19a)—63% yield; ¹H NMR (400 MHz, CDCl₃) δ 7.81 (d, *J* = 8.8 Hz, 1H), 7.75–7.66 (m, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 3.95 (t, *J* = 4.8 Hz, 4H), 3.20 (t, *J* = 4.8 Hz, 4H), 2.45 (s, 3H); MS (ESI) *m/z* 403 [C₁₉H₁₉ClN₄O₂S]⁺.

2-chloro-4-(4-tosylpiperazin-1-yl)pyrido[2,3-d]pyrimidine (19d)—82% yield; ¹H NMR (400 MHz, CDCl₃) δ 7.84–7.32 (m, 5H), 7.47–7.40 (m, 3H), 4.51 (d, *J* = 13.2 Hz, 2H), 3.26–3.11 (m, 3H), 2.48 (s, 3H), 2.18 (d, *J* = 10.8 Hz, 2H), 2.05–1.94 (m, 2H); MS (ESI) *m/z* 402 [C₂₀H₂₀ClN₃O₂S]⁺.

2-chloro-4-(4-tosylpiperazin-1-yl)pyrido[3,2-d]pyrimidine (19e)—61% yield; ¹H NMR (400 MHz, CDCl₃) δ 8.66 (d, *J* = 1.6 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.62–7.60 (m, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 4.77–4.66 (m, 4H), 3.20 (t, *J* = 5.2 Hz, 4H), 2.41 (s, 3H); MS (ESI) *m/z* 404 [C₁₈H₁₈ClN₅O₂S]⁺.

2-chloro-4-(4-tosylpiperazin-1-yl)pyrimidine (19f)—58% yield; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.05 (d, *J* = 4.0 Hz, 1H), 7.61 (t, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 1H), 3.70 (s, 4H), 2.93 (t, *J* = 4.0 Hz, 4H), 2.38 (s, 3H); MS (ESI) *m/z* 353 [C₁₅H₁₇ClN₄O₂S]⁺.

2-chloro-4,5-dimethyl-6-(4-tosylpiperazin-1-yl)pyrimidine (19g)—63% yield; ¹H NMR (400 MHz, CDCl₃) δ 7.66 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 3.46 (t, *J* = 4.8 Hz, 4H), 3.12 (t, *J* = 4.8 Hz, 4H), 2.45 (s, 3H), 2.36 (s, 3H), 2.04 (s, 3H); MS (ESI) *m/z* 381 [C₁₇H₂₁ClN₄O₂S]⁺.

3-Chloro-1-(4-tosylpiperazin-1-yl) isoquinoline (19h)—41% yield; MS (ESI) *m/z* 402 [C₂₀H₂₀ClN₃O₂S + H]⁺.

2-(Thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)quinoline (71)—Prepared from 4-chloro-2-(thiophen-2-yl)quinoline²⁹ according to the same general procedure for synthesis of compounds **18**; 30% yield; ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.78–7.74 (m, 3H), 7.70 (d, *J* = 4.8 Hz, 1H), 7.64–7.60 (m, 1H), 7.46 (d, *J* = 6 Hz, 1H), 7.43–7.36 (m, 3H), 7.24 (s, 1H), 7.17–7.15 (m, 1H), 3.35 (s, 8 H), 2.49 (s, 3H); MS (ESI) *m/z* 450 [C₂₄H₂₃N₃O₂S₂ + H]⁺.

General Procedure for the Suzuki Coupling: The boronic acid **20** (2.48 mmol) and K₂CO₃ (2.48 mmol) were added to a stirring solution of 2-chloroheterocycle **19** (1.24 mmol) in 1,4-dioxane and water (10 mL:1 mL). The reaction mixture was purged with argon gas for 20 min and Pd(PPh₃)₄ (0.12 mmol) was added. The reaction mixture was heated at reflux temperature for 16 h under an argon atmosphere. The reaction mixture was cooled to room temperature, diluted with water (30 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (3 × 20 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography to afford **21** as an oil.

2-(thiophen-3-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (26ad)—25% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.27 (s, 1H), 7.92–7.90 (m, 2H), 7.75–7.68 (m, 4H), 7.40–7.34 (m, 4H), 3.89 (s, 4H), 3.26 (s, 4H), 2.43 (s, 3H), MS (ESI) m/z 449 $[\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(5-methylthiophen-2-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56a)—25% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.86 (d, $J = 8.0$ Hz, 1H), 7.80 (d, $J = 4.0$ Hz, 1H), 7.72–7.66 (m, 4H), 7.33 (t, $J = 6.0$ Hz, 1H), 3.89 (t, $J = 8.0$ Hz, 4H), 3.24 (t, $J = 4.0$ Hz, 4H), 2.55 (s, 3H), 2.43 (s, 3H); MS (ESI) m/z 465 $[\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(pyridin-3-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56f)—25% yield; ^1H NMR (400 MHz, CDCl_3) δ 9.66 (s, 1H), 8.75–8.69 (m, 2H), 7.97 (d, $J = 8.4$ Hz, 1H), 7.89–7.68 (m, 4H), 7.46–7.40 (m, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 3.96 (t, $J = 4.4$ Hz, 4H), 3.26 (t, $J = 4.4$ Hz, 4H), 2.42 (s, 3H); MS (ESI) m/z 446 $[\text{C}_{24}\text{H}_{23}\text{N}_5\text{O}_2\text{S} + \text{H}]^+$.

2-(pyridin-4-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (55g)—18% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.75 (d, $J = 5.2$ Hz, 2H), 8.31 (d, $J = 5.6$ Hz, 2H), 7.99 (d, $J = 8.4$ Hz, 1H), 7.81–7.75 (m, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.47 (t, $J = 7.6$ Hz, 1H), 7.34 (d, $J = 8.4$ Hz, 2H), 3.95 (t, $J = 4.4$ Hz, 4H), 3.27 (t, $J = 4.8$ Hz, 4H), 2.42 (s, 3H); MS (ESI) m/z 446 $[\text{C}_{24}\text{H}_{23}\text{N}_5\text{O}_2\text{S} + \text{H}]^+$.

Preparation of 3-(Thiophen-2-yl)-1-(4-tosylpiperazin-1-yl)isoquinoline (70)—12% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.86 (d, $J = 8.0$ Hz, 1H), 7.72 (d, $J = 8.0$ Hz, 3H), 7.61–7.53 (m, 3H), 7.40–7.33 (m, 4H), 7.10 (t, $J = 4.0$ Hz, 1H), 3.59 (t, $J = 4.0$ Hz, 4H), 3.30 (t, $J = 4.0$ Hz, 3H), 2.44 (s, 3H); MS (ESI) m/z 450 $[\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrido[3,2-d]pyrimidine (72)—18% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.61–8.59 (m, 1H), 8.09–8.07 (m, 1H), 7.96 (d, $J = 2.4$ Hz, 1H), 7.66 (d, $J = 8.0$ Hz, 2H), 7.58–7.54 (m, 1H), 7.45–7.44 (m, 1H), 7.30 (d, $J = 8.0$ Hz, 2H), 7.14–7.12 (m, 1H), 4.64 (br s, 4H), 3.22 (t, $J = 4.8$ Hz, 4H), 2.39 (s, 3H); MS (ESI) m/z 452 $[\text{C}_{22}\text{H}_{21}\text{N}_5\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrido[2,3-d]pyrimidine (73)—60% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.99 (d, $J = 2.8$ Hz, 1H), 8.13–8.06 (m, 2H), 7.66 (d, $J = 8.0$ Hz, 2H), 7.50 (d, $J = 4.8$ Hz, 1H), 7.34–7.28 (m, 3H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.96 (t, $J = 4.8$ Hz, 4H), 3.23 (t, $J = 4.8$ Hz, 4H), 2.41 (s, 3H); MS (ESI) m/z 452 $[\text{C}_{22}\text{H}_{21}\text{N}_5\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrimidine (74)—40% yield; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.17 (d, $J = 8.0$ Hz, 1H), 7.80 (d, $J = 3.2$ Hz, 1H), 7.64–7.61 (m, 3H), 7.42 (d, $J = 8.0$ Hz, 2H), 7.12 (t, $J = 4.0$ Hz, 1H), 6.64 (d, $J = 8.0$ Hz, 1H), 3.77 (br s, 4H), 2.94 (t, $J = 4.6$ Hz, 4H), 2.36 (s, 3H), MS (ESI) m/z 400 $[\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4,5-dimethyl-2-(thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)pyrimidine (76)—21% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.85 (d, $J = 3.2$ Hz, 1H), 7.68 (d, $J = 8.4$ Hz, 2H), 7.39–7.33 (m, 3H), 7.09 (t, $J = 4$ Hz, 1H), 3.46 (t, $J = 4.8$ Hz, 4H), 3.16 (t, $J = 4.0$ Hz, 4H), 2.43 (s, 3H), 2.41 (s, 3H), 2.08 (s, 3H); MS (ESI) m/z 429 $[\text{C}_{21}\text{H}_{24}\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

Preparation of 2-Chloro-6-(4-tosylpiperazin-1-yl)-9H-purine (19b)—*N,N*-Diisopropylethylamine (0.92 mL, 3.28 mmol) and piperazine **17a** (761 mg, 3.174 mmol) were added to a stirring solution of dichloride **18b** (500 mg, 2.64 mmol) in NMP (6 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 $\times$ 15 mL). The combined organic layers were washed

with water (3 × 10 mL) and brine (3 × 10 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The resulting solids were washed with methanol to afford purine **19b** (600 mg, 51%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 13.21 (br s, 1H), 8.13 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.33 (br s, 4H), 2.99 (s, 4H), 2.49 (s, 3H); MS (ESI) *m/z* 393 [C₁₆H₁₇N₆O₂S]⁺.

Preparation of [2-Chloro-6-(4-tosylpiperazin-1-yl)-9H-purin-9-yl]methyl pivalate (19c)—Chloromethylpivalate (**22**, 0.1 mL, 0.56 mmol) and K₂CO₃ (43 mg, 0.36 mmol) were added to a stirring solution of purine **19b** (100 mg, 0.25 mmol) in DMF (2 mL) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water (3 × 5 mL) and brine (3 × 5 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by trituration with CH₂Cl₂/hexanes to afford pivalate **19c** (110 mg, 63%) as an off-white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.22 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 5.99 (s, 2H), 4.40 (br s, 2H), 3.90 (br s, 2H), 2.96 (s, 4H), 2.43 (s, 9H), 2.30 (s, 3H); MS (ESI) *m/z* 507 [C₂₂H₂₇ClN₆O₄S]⁺.

Preparation of [2-(Thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)-9H-purin-9-yl]methyl Pivalate (21bb)—2-Thiophene boronic acid (**20a**, 75 mg, 0.59 mmol) and Cs₂CO₃ (256 mg, 0.78 mmol) were added to a stirring solution of chloride **19c** (200 mg, 0.39 mmol) in DMF (6 mL). The reaction mixture was purged with argon for 20 min and Pd(PPh₃)₄ (46 mg, 0.03 mmol) was added. The reaction mixture was heated under microwave conditions for 1 h (temp = 100 °C, pressure = 200 psi, power = 150 watts). After cooling to room temperature, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (3 × 10 mL) and brine (3 × 10 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (20% EtOAc in hexanes) to afford purine **21bb** (70 mg, 35%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.92 (s, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 4.0 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.12 (s, 2H), 4.45 (br s, 4H), 3.16 (t, *J* = 4.0 Hz, 4H), 2.39 (s, 3H), 1.16 (s, 9H); MS (ESI) *m/z* 555 [C₂₆H₃₀N₆O₄S₂ + H]⁺.

Preparation of 2-(Thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)-9H-purine (21bc)—A 3 N sodium hydroxide solution (0.2 mL) was added to a stirring solution of pivalate **21bb** (100 mg, 0.18 mmol) in a mixture of THF/MeOH (2 mL:1 mL) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (1% MeOH in CH₂Cl₂) to afford purine **21bc** (55 mg, 45%) as an off-white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.11 (s, 1H), 8.10 (s, 1H), 7.76 (d, *J* = 3.2 Hz, 1H), 7.63–7.58 (m, 3H), 7.39 (d, *J* = 8 Hz, 2H), 7.11 (t, *J* = 4.4 Hz, 1H), 4.36 (br s, 4H), 2.99 (s, 4H), 2.33 (s, 3H); MS (ESI) *m/z* 441 [C₂₀H₂₀N₆O₂S₂ + H]⁺.

Preparation of tert-Butyl 4-(2-chloroquinazolin-4-yl)piperazine-1-carboxylate (23)—*N,N*-Diisopropylethylamine (5.2 mL, 30.14 mmol) and Boc-piperazine **15** (3.08 g, 16.87 mmol) were added to a stirring solution of 2,4-dichloroquinazoline (**18a**, 3.00 g, 15.07 mmol) in NMP (30 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was diluted with ice-cold water (50 mL) and the resulting precipitate was filtered. The filter cake was washed with water (3 × 20 mL) and dried to afford 2-chloroquinazoline **23** (4.50 g, 63%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.85 (t, *J* = 8.0 Hz, 2H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 3.85–3.84 (m, 4H), 3.66 (t, *J* = 4.4 Hz, 4H), 1.50 (s, 9H); MS (ESI) *m/z* 349 [C₁₇H₂₁ClN₄O₂ + H]⁺.

Preparation of tert-Butyl 4-[2-(thiophen-2-yl)quinazolin-4-yl]piperazine-1-carboxylate (24)—2-Thiophene boronic acid **20a** (4.39 g, 34.38 mmol) and K_2CO_3 (9.50 g, 68.76 mmol) were added to a stirring solution of chloroquinazoline **23** (8.00 g, 22.92 mmol) in 1,4-dioxane and water (110 mL/15 mL). The reaction mixture was purged with argon gas for 20 min and $Pd(PPh_3)_2Cl_2$ (1.60 gm, 2.29 mmol) was added. The reaction mixture was heated at reflux for 16 h under an argon atmosphere. The reaction mixture was cooled to room temperature, diluted with water (100 mL), and extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were washed with water (3 $\times$ 100 mL), dried over Na_2SO_4 , filtered, and the filtrate was concentrated under reduced pressure. The resulting crude residue was purified by silica-gel column chromatography (20% EtOAc in hexanes) to afford thiophene **24** (6.00 g, 65%) as an off-white solid: 1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 4.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 4.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.15 (t, J = 4.0 Hz, 2H), 3.79 (br s, 4H), 3.68 (br s, 4H), 1.50 (s, 9H); MS (ESI) m/z 397 [$C_{21}H_{24}N_4O_2S + H$] $^+$.

Preparation of 4-(Piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (25)—Hydrochloric acid (20% in 1,4-dioxane, 6 mL) was added to a stirring solution of carbamate **24** (3.50 g, 8.83 mmol) in CH_2Cl_2 (20 mL) at 0 $^\circ C$. After stirring for 16 h at room temperature, the precipitated solids were filtered and the filter cake was dissolved in water (30 mL). The resulting aqueous solution was washed with CH_2Cl_2 (2 $\times$ 30 mL), cooled to 0 $^\circ C$, and basified to pH 12 with a saturated aqueous $NaHCO_3$ solution. The aqueous solution was then extracted with CH_2Cl_2 (2 $\times$ 30 mL) and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to afford the secondary amine **25** (1.80 g, 62%) as an off-white solid: 1H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.90–7.84 (m, 2H), 7.69 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 4.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.16 (d, J = 4.0 Hz, 1H), 3.81 (s, 4H), 3.70 (s, 1H), 3.11 (s, 4H); MS (ESI) m/z 297 [$C_{16}H_{16}N_4S + H$] $^+$.

General Procedure for the Synthesis of Sulfonamide Analogues (26)—*N,N*-Diisopropylethylamine (1.34 mmol) and a sulfonyl chloride (0.74 mmol) were added to a stirring solution of amine **25** (0.67 mmol) in NMP (3 mL) at 0 $^\circ C$. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 $\times$ 5 mL). The combined organic layers were washed with water (3 $\times$ 10 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography to afford sulphonamide **26** as an off-white solid.

4-(4-(4-chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (10)—75% yield; 1H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0 Hz, 2H), 7.80–7.69 (m, 7H), 7.46–7.43 (m, 1H), 7.17 (t, J = 4.4 Hz, 1H), 3.82 (br s, 4H), 3.20 (br s, 4H); MS (ESI) m/z 471 [$C_{22}H_{19}ClN_4O_2S_2$] $^+$.

4-(4-(2-methylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ab)—29% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.97–7.90 (m, 2H), 7.79 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 6.0 Hz, 1H), 7.51–7.45 (m, 2H), 7.42–7.34 (m, 3H), 7.15 (t, J = 4.0 Hz, 1H), 3.87 (t, J = 6.0 Hz, 4H), 3.45 (t, J = 6.0 Hz, 4H), 2.69 (s, 3H); MS (ESI) m/z 451 [$C_{23}H_{22}N_4O_2S_2 + H$] $^+$.

4-(4-(3-methylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ac)—29% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 2.8 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.75–7.69 (m, 2H), 7.61 (s, 2H), 7.46–7.16 (m, 4H), 7.15 (t, J = 4.4 Hz, 1H),

3.92 (t, $J = 4.4$ Hz, 4H), 3.28 (t, $J = 4.8$ Hz, 4H), 2.44 (s, 3H); MS (ESI) m/z 451 [$C_{23}H_{22}N_4O_2S_2 + H$] $^+$.

4-(4-(4-methylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ad)—70% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, $J = 4.0$ Hz, 1H), 7.88 (d, $J = 8.0$ Hz, 1H), 7.74–7.67 (m, 4H), 7.45 (d, $J = 4.8$ Hz, 1H), 7.39–7.33 (m, 3H), 7.15 (t, $J = 4.0$ Hz), 3.91 (t, $J = 8.0$ Hz, 4H), 3.25 (t, $J = 8.0$ Hz, 4H), 2.41 (s, 3H); MS (ESI) m/z 451 [$C_{23}H_{22}N_4O_2S_2 + H$] $^+$.

4-(4-(3,5-dimethylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ae)—64% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, $J = 3.2$ Hz, 1H), 7.89 (d, $J = 8.4$ Hz, 1H), 7.76–7.69 (m, 2H), 7.46 (d, $J = 4.8$ Hz, 1H), 7.40–7.36 (m, 3H), 7.22 (s, 1H), 7.15 (t, $J = 4.4$ Hz, 1H), 3.92 (t, $J = 4.8$ Hz, 4H), 3.26 (t, $J = 4.4$ Hz, 4H), 2.39 (s, 6H); MS (ESI) m/z 465 [$C_{24}H_{24}N_4O_2S_2 + H$] $^+$.

4-(4-(2,4-dimethylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26af)—62% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, $J = 3.2$ Hz, 1H), 7.89 (d, $J = 8.4$ Hz, 1H), 7.76–7.69 (m, 2H), 7.46 (d, $J = 4.8$ Hz, 1H), 7.40–7.362 (m, 3H), 7.22 (s, 1H), 7.15 (t, $J = 4.4$ Hz, 1H), 3.92 (t, $J = 4.8$ Hz, 4H), 3.26 (t, $J = 4.4$ Hz, 4H), 2.39 (s, 6H); MS (ESI) m/z 465 [$C_{24}H_{24}N_4O_2S_2 + H$] $^+$.

4-(4-(3-tert-butylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ag)—66% yield; 1H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, $J = 4.0$ Hz, 1H), 7.89 (d, $J = 8.0$ Hz, 1H), 7.79 (s, 1H), 7.75–7.68 (m, 2H), 7.62 (t, $J = 8.0$ Hz, 2H), 7.49–7.44 (m, 2H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.14 (d, $J = 4.0$ Hz, 1H), 3.92 (t, $J = 4.0$ Hz, 4H), 3.26 (t, $J = 4.0$ Hz, 4H), 1.34 (s, 9H); MS (ESI) m/z 481 [$C_{26}H_{28}N_4O_2S_2 + H$] $^+$.

4-(4-(4-tert-butylphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ah)—63% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, $J = 4.0$ Hz, 1H), 7.89 (d, $J = 8.0$ Hz, 1H), 7.75–7.68 (m, 4H), 7.55 (d, $J = 8.0$ Hz, 2H), 7.46 (d, $J = 4.0$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 1H), 7.27 (s, 1H), 7.14 (t, $J = 4.0$ Hz, 1H), 3.91 (t, $J = 4.0$ Hz, 4H), 3.28 (t, $J = 4.0$ Hz, 4H), 1.33 (s, 9H); MS (ESI) m/z 481 [$C_{26}H_{28}N_4O_2S_2 + H$] $^+$.

4-(4-(2-methoxyphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ai)—63% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, $J = 4.0$ Hz, 1H), 7.94–7.89 (m, 2H), 7.79 (d, $J = 4.0$ Hz, 1H), 7.71 (t, $J = 8.0$ Hz, 1H), 7.53 (t, $J = 8.0$ Hz, 1H), 7.45 (d, $J = 4.0$ Hz, 1H), 7.39 (t, $J = 8.0$ Hz, 1H), 7.14 (t, $J = 4.0$ Hz, 1H), 7.07–7.00 (m, 2H), 3.92 (s, 3H), 3.90 (t, $J = 4.0$ Hz, 4H), 3.49 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 467 [$C_{23}H_{22}N_4O_3S_2 + H$] $^+$.

4-(4-(3-methoxyphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26aj)—38% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.75–7.69 (m, 2H), 7.48–7.44 (m, 2H), 7.38 (t, $J = 6.0$ Hz, 2H), 7.30 (s, 1H), 7.16–7.12 (m, 2H), 3.92 (s, 4H), 3.86 (s, 3H), 3.29 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 467 [$C_{23}H_{22}N_4O_3S_2 + H$] $^+$.

4-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ak)—57% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.01 (br s, 1H), 7.90 (d, $J = 4.0$ Hz, 1H), 7.75–7.69 (m, 4H), 7.46 (d, $J = 4.0$ Hz, 1H), 7.37 (t, $J = 6.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 7.01 (d, $J = 4.0$ Hz, 1H), 3.92 (s, 4H), 3.86 (s, 3H), 3.25 (t, $J = 6.0$ Hz, 4H); MS (ESI) m/z 467 [$C_{23}H_{22}N_4O_3S_2 + H$] $^+$.

4-(4-(2-fluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26an)—71% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 3.2$ Hz, 1H), 7.91–7.88 (m, 2H), 7.77 (d, $J = 8.0$ Hz, 1H), 7.72 (t, $J = 7.6$ Hz, 1H), 7.62–7.57 (m, 1H), 7.45 (d, $J = 4.8$ Hz, 1H), 7.39 (t, $J = 7.6$ Hz, 1H), 7.33–7.24 (m, 2H), 7.15 (t, $J = 4.4$ Hz, 1H), 3.91 (t, $J = 4.8$ Hz, 4H), 3.47 (t, $J = 4.4$ Hz, 4H); MS (ESI) m/z 455 $[\text{C}_{22}\text{H}_{19}\text{FN}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(3-fluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ao)—70% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.00 (d, $J = 3.2$ Hz, 1H), 7.90 (d, $J = 8.0$ Hz, 1H), 7.75–7.69 (m, 2H), 7.61–7.51 (m, 3H), 7.46 (d, $J = 4.8$ Hz, 1H), 7.40–7.27 (m, 2H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.91 (t, $J = 4.4$ Hz, 4H), 3.30 (t, $J = 4.4$ Hz, 4H); MS (ESI) m/z 455 $[\text{C}_{22}\text{H}_{19}\text{FN}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(4-fluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ap)—67% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 4.0$ Hz, 1H), 7.90 (d, $J = 8.0$ Hz, 1H), 7.84–7.81 (m, 2H), 7.75–7.69 (m, 2H), 7.46 (t, $J = 4.0$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 1H), 7.27–7.22 (m, 2H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.91 (t, $J = 4.0$ Hz, 4H), 3.27 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 455 $[\text{C}_{22}\text{H}_{19}\text{FN}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(2,4-difluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26aq)—68% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.02 (d, $J = 4.0$ Hz, 1H), 7.94–7.88 (m, 2H), 7.78 (d, $J = 8.0$ Hz, 1H), 7.72 (t, $J = 8.0$ Hz, 1H), 7.45 (d, $J = 4.0$ Hz, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 7.05–6.95 (m, 2H), 3.92 (t, $J = 4.0$ Hz, 4H), 3.45 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 473 $[\text{C}_{22}\text{H}_{18}\text{F}_2\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(2-chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26ar)—67% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (d, $J = 4.0$ Hz, 1H), 8.01 (d, $J = 2.8$ Hz, 1H), 7.91 (d, $J = 8.4$ Hz), 7.80 (d, $J = 8.0$ Hz, 1H), 7.72 (t, $J = 7.6$ Hz, 1H), 7.56–7.38 (m, 5H), 7.15 (t, $J = 4$ Hz, 1H), 3.89 (t, $J = 4.4$ Hz, 4H), 3.57 (t, $J = 4.8$ Hz, 4H); MS (ESI) m/z 471 $[\text{C}_{22}\text{H}_{19}\text{ClN}_4\text{O}_2\text{S}_2]^+$.

4-(4-(3-chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26as)—30% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (br s, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.80 (s, 1H), 7.76–7.68 (m, 3H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.53–7.46 (m, 2H), 7.39 (t, $J = 8.0$ Hz, 1H), 7.15 (br s, 1H), 3.92 (br s, 4H), 3.30 (br s, 4H); MS (ESI) m/z 471 $[\text{C}_{22}\text{H}_{19}\text{ClN}_4\text{O}_2\text{S}_2]^+$.

4-(4-(3,5-dichlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26at)—59% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 3.6$ Hz, 1H), 7.92 (d, $J = 8.4$ Hz, 1H), 7.77–7.70 (m, 2H), 7.67 (s, 2H), 7.59 (s, 1H), 7.46 (d, $J = 5.2$ Hz, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 7.15 (t, $J = 4.4$ Hz, 1H), 3.92 (t, $J = 4.8$ Hz, 4H), 3.32 (t, $J = 4.4$ Hz, 4H); MS (ESI) m/z 506 $[\text{C}_{22}\text{H}_{18}\text{Cl}_2\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

1-(2-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)phenyl)ethanone (26ba)—60% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 3.2$ Hz, 1H), 7.90 (d, $J = 8.4$ Hz, 2H), 7.73–7.69 (m, 2H), 7.62 (t, $J = 7.2$ Hz, 1H), 7.54 (t, $J = 7.6$ Hz, 1H), 7.45 (d, $J = 5.2$ Hz, 1H), 7.40–7.32 (m, 2H), 7.14 (t, $J = 4.4$ Hz, 1H), 3.87 (t, $J = 4.4$ Hz, 4H), 3.40 (t, $J = 4.8$ Hz, 4H), 2.47 (s, 3H); MS (ESI) m/z 479 $[\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_3\text{S}_2 + \text{H}]^+$.

1-(3-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)phenyl)ethanone (26bb)—65% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 3.2$ Hz, 1H), 7.90 (d, $J = 8.4$ Hz, 2H), 7.73–7.69 (m, 2H), 7.62 (t, $J = 7.2$ Hz, 1H),

7.54 (t, $J = 7.6$ Hz, 1H), 7.45 (d, $J = 5.2$ Hz, 1H), 7.40–7.32 (m, 2H), 7.14 (t, $J = 4.4$ Hz, 1H), 3.87 (t, $J = 4.4$ Hz, 4H), 3.40 (t, $J = 4.8$ Hz, 4H), 2.47 (s, 3H); MS (ESI) m/z 479 $[\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_3\text{S}_2 + \text{H}]^+$.

ethyl 2-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzoate (26be)—45% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, $J = 3.2$ Hz, 1H), 7.89 (t, $J = 8.8$ Hz, 2H), 7.77 (d, $J = 8.4$ Hz, 1H), 7.71 (t, $J = 7.2$ Hz, 1H), 7.62 (t, $J = 6.8$ Hz, 2H), 7.51 (d, $J = 8.4$ Hz, 1H), 7.45 (d, $J = 4.8$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 4.44 (q, $J = 7.2$ Hz, 2H), 3.90 (t, $J = 4.4$ Hz, 4H), 3.48 (t, $J = 4.4$ Hz, 4H), 1.42 (t, $J = 7.2$ Hz, 3H); MS (ESI) m/z 509 $[\text{C}_{25}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_2 + \text{H}]^+$.

2-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzonitrile (26bj)—75% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (d, $J = 8.0$ Hz, 1H), 8.01 (d, $J = 3.6$ Hz, 1H), 7.91 (d, $J = 8.0$ Hz, 2H), 7.81–7.71 (m, 4H), 7.46–7.27 (m, 2H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.93 (t, $J = 4.8$ Hz, 4H), 3.53 (t, $J = 4.8$ Hz, 4H); MS (ESI) m/z 462 $[\text{C}_{23}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2 + \text{H}]^+$.

3-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzonitrile (26bk)—70% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.10 (s, 1H), 8.03–8.01 (m, 2H), 7.90 (t, $J = 8.0$ Hz, 2H), 7.76–7.69 (m, 3H), 7.47 (d, $J = 5.2$ Hz, 1H), 7.42–7.37 (m, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.93 (t, $J = 4.8$ Hz, 4H), 3.31 (t, $J = 4.8$ Hz, 4H); MS (ESI) m/z 462 $[\text{C}_{23}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(pyridin-3-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bm)—55% yield; ^1H NMR (400 MHz, CDCl_3) δ 9.10 (s, 1H), 8.86 (s, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 8.01 (d, $J = 2.4$ Hz, 1H), 7.91 (d, $J = 8.4$ Hz, 1H), 7.74–7.70 (m, 2H), 7.53–7.50 (m, 1H), 7.46 (d, $J = 4.8$ Hz, 1H), 7.39 (t, $J = 7.6$ Hz, 1H), 7.15 (t, $J = 4.4$ Hz, 1H), 3.92 (t, $J = 4.8$ Hz, 4H), 3.33 (t, $J = 4.4$ Hz, 4H); MS (ESI) m/z 438 $[\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2 + \text{H}]^+$.

2-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)thiazole (26bn)—19% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (dd, $J = 6.0$ Hz, 2H), 7.92 (d, $J = 8.0$ Hz, 1H), 7.79 (d, $J = 8.0$ Hz, 1H), 7.73 (t, $J = 8.0$ Hz, 1H), 7.66 (d, $J = 4.0$ Hz, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.41 (t, $J = 8.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.94 (t, $J = 4.0$ Hz, 4H), 3.60 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 444 $[\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2\text{S}_3 + \text{H}]^+$.

2-(thiophen-2-yl)-4-(4-(thiophen-2-ylsulfonyl)piperazin-1-yl)quinazoline (26bo)—38% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (s, 1H), 7.96 (s, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.77–7.70 (m, 2H), 7.47–7.46 (m, 2H), 7.39 (t, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 4.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.95 (s, 4H), 3.33 (s, 4H); MS (ESI) m/z 443 $[\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_2\text{S}_3 + \text{H}]^+$.

2-(thiophen-2-yl)-4-(4-(thiophen-3-ylsulfonyl)piperazin-1-yl)quinazoline (26bp)—80% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (s, 1H), 7.96 (s, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.77–7.70 (m, 2H), 7.47–7.46 (m, 2H), 7.39 (t, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 4.0$ Hz, 1H), 7.15 (t, $J = 4.0$ Hz, 1H), 3.93 (s, 4H), 3.31 (s, 4H); MS (ESI) m/z 443 $[\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_2\text{S}_3 + \text{H}]^+$.

4-(4-(benzylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bq)—50% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.03 (d, $J = 4.0$ Hz, 1H), 7.92 (d, $J = 8.0$ Hz, 1H), 7.76–7.71 (m, 2H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.42–7.38 (m, 6H), 7.17 (d, $J = 8.0$ Hz, 1H), 4.28 (s, 2H), 3.78 (t, $J = 4.0$ Hz, 4H), 3.34 (t, $J = 4.0$ Hz, 4H); MS (ESI) m/z 451 $[\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_2\text{S}_2 + \text{H}]^+$.

4-(4-(2,3-dihydrobenzo[b][1,4]dioxin-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bs)—58% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, J = 4.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.76–7.69 (m, 2H), 7.46 (d, J = 4.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.32 (s, 1H), 7.31 (s, 1H), 7.15 (t, J = 4.0 Hz, 1H), 6.98 (d, J = 8 Hz, 1H), 4.30 (br s, 4H), 3.91 (t, J = 6.0 Hz, 4H), 3.25 (t, J = 6.0 Hz, 4H); MS (ESI) m/z 495 [$\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_2 + \text{H}$] $^+$.

4-methyl-7-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (26bt)—14% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.77–7.68 (m, 2H), 7.46 (d, J = 4.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.15 (s, 1H), 7.08 (d, J = 8.0 Hz, 1H), 6.98 (s, 1H), 6.84 (d, J = 8.0 Hz, 1H), 4.32 (s, 2H), 3.92 (s, 4H), 3.30–3.26 (m, 6H), 2.93 (s, 3H); MS (ESI) m/z 508 [$\text{C}_{25}\text{H}_{25}\text{N}_5\text{O}_3\text{S}_2 + \text{H}$] $^+$.

4-(4-(2,2-dimethylchroman-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bu)—34% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, J = 4.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.77–7.69 (m, 2H), 7.51 (d, J = 4.0 Hz, 2H), 7.45 (d, J = 4.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 4.0 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 3.92 (t, J = 4.0 Hz, 4H), 3.25 (t, J = 4.0 Hz, 4H), 2.82 (t, J = 4.0 Hz, 2H), 1.83 (t, J = 4.0 Hz, 2H), 1.34 (s, 6H); MS (ESI) m/z 521 [$\text{C}_{27}\text{H}_{28}\text{N}_4\text{O}_3\text{S}_2 + \text{H}$] $^+$.

4-(4-(quinolin-3-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bv)—34% yield; ^1H NMR (400 MHz, CDCl_3) δ 9.23 (s, 1H), 8.66 (s, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 10.8 Hz, 2H), 7.93–7.87 (m, 2H), 7.73–7.68 (m, 3H), 7.43 (d, J = 5.2 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.12 (t, J = 4.0 Hz, 1H), 3.92 (t, J = 4.4 Hz, 4H), 3.40 (t, J = 4.4 Hz, 4H); MS (ESI) m/z 487 [$\text{C}_{25}\text{H}_{21}\text{N}_5\text{O}_2\text{S}_2 + \text{H}$] $^+$.

4-(4-(5-chloronaphthalen-2-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bw)—63% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.44 (d, J = 8.0 Hz, 1H), 8.39 (s, 1H), 7.98 (s, 1H), 7.93–7.86 (m, 3H), 7.75–7.66 (m, 3H), 7.54 (t, J = 6.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 1H), 7.35 (t, J = 4.0 Hz, 1H), 7.12 (t, J = 4.0 Hz, 1H), 3.91 (t, J = 4.0 Hz, 4H), 3.35 (t, J = 4.0 Hz, 4H); MS (ESI) m/z 521 [$\text{C}_{26}\text{H}_{21}\text{ClN}_4\text{O}_2\text{S}_2$] $^+$.

4-(4-(naphthalen-1-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bx)—21% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.80 (d, J = 4.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.96–7.87 (m, 3H), 7.67–7.72 (m, 3H), 7.57–7.62 (m, 2H), 7.42 (d, J = 4.0 Hz, 1H), 7.35 (t, J = 6.0 Hz, 1H), 7.12 (s, 1H), 3.84 (s, 4H), 3.46 (s, 4H); MS (ESI) m/z 487 [$\text{C}_{26}\text{H}_{22}\text{N}_4\text{O}_2\text{S}_2 + \text{H}$] $^+$.

4-(4-(benzo[d][1,3]dioxol-5-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26by)—16% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, J = 4.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.76–7.69 (m, 2H), 7.46 (d, J = 8.0 Hz, 1H), 7.40–7.35 (m, 2H), 7.21 (s, 1H), 7.15 (d, J = 4.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.08 (s, 2H), 3.92 (t, J = 4.0 Hz, 4H), 3.25 (t, J = 4.0 Hz, 4H); MS (ESI) m/z 481 [$\text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_4\text{S}_2 + \text{H}$] $^+$.

4-(4-(quinolin-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bz)—46% yield; ^1H NMR (400 MHz, CDCl_3) δ 9.08 (d, J = 4.0 Hz, 1H), 8.38 (s, 1H), 8.32–8.26 (m, 2H), 8.04 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 4.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.72–7.67 (m, 2H), 7.44 (d, J = 4.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.12 (t, J = 4.0 Hz, 1H), 3.92 (t, J = 4.0 Hz, 4H), 3.37 (t, J = 4.0 Hz, 4H); MS (ESI) m/z 488 [$\text{C}_{25}\text{H}_{21}\text{N}_5\text{O}_2\text{S}_2 + \text{H}$] $^+$.

6-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzo[d]thiazole (26ca)—63% yield; ^1H NMR (400 MHz, CDCl_3) δ 9.21 (s, 1H), 8.48 (s, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 4.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.72–7.67 (m, 2H), 7.44 (t, J = 4.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.13 (t, J = 4.0 Hz, 1H), 3.91 (t, J = 4.0 Hz, 4H), 3.33 (t, J = 4.0 Hz, 4H); MS (ESI) m/z 494 [$\text{C}_{23}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_3 + \text{H}$] $^+$.

4-(4-(methylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26cb)—32% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.05 (d, J = 4.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.48–7.41 (m, 2H), 7.17 (d, J = 4.0 Hz, 1H), 3.96 (t, J = 4.0 Hz, 4H), 3.47 (t, J = 4.0 Hz, 4H) 2.84 (s, 3H); MS (ESI) m/z 375 [$\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_2\text{S}_2 + \text{H}$] $^+$.

4-(4-(isopropylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26cc)—40% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.05 (d, J = 4.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 8.0 Hz, 1H), 7.47–7.40 (m, 2H), 7.16 (t, J = 4.0 Hz, 1H), 3.89 (t, J = 4.0 Hz, 4H), 3.61 (t, J = 4.0 Hz, 4H) 3.30–3.20 (m, 1H) 1.40 (s, 3H), 1.38 (s, 6H); MS (ESI) m/z 403 [$\text{C}_{29}\text{H}_{22}\text{N}_4\text{O}_2\text{S}_2 + \text{H}$] $^+$.

Preparation of 2-(thiophen-2-yl)-4-(4-(trifluoromethylsulfonyl)piperazin-1-yl)quinazoline (26cd)—Triflic anhydride (0.33 mL, 2.02 mmol) was added to a suspension of amine **24** (500 mg, 1.68 mmol) in CH_2Cl_2 (10 mL) and saturated aqueous NaHCO_3 (0.5 mL) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and was washed with water (3×10 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford triflamide **25-56** (420 mg, 58%) as an off-white solid: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.06–8.04 (m, 1H), 7.97–7.95 (m, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.78–7.74 (m, 1H), 7.49–7.47 (m, 1H), 7.45–7.43 (m, 1H), 7.18–7.16 (m, 1H), 3.91–3.76 (m, 8H); MS (ESI) m/z 429 [$\text{C}_{17}\text{H}_{15}\text{F}_3\text{N}_4\text{O}_2\text{S}_2 + \text{H}$] $^+$.

General Procedure for Preparation of Piperazine Amide Analogues (29 and 30)

—An aromatic carboxylic acid (0.18 mmol), HATU (0.25 mmol), and DIPEA (0.25 mmol) were added to a stirring solution of amine **25** (0.16 mmol) in DMF (10 mL). After stirring for 4 h at room temperature, the reaction mixture was diluted with EtOAc (20 mL). The organic layer was washed with water (2×10 mL) followed by brine (2×10 mL). The organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (40% EtOAc in hexanes) to afford amide **29** or **30** as an off-white solid.

Phenyl{4-[2-(thiophen-2-yl)quinazolin-4-yl]piperazin-1-yl}methanone (29)—68% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.04 (d, J = 2.8 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.46–7.40 (m, 7H), 7.15 (t, J = 7.2 Hz, 1H), 4.02–3.73 (m, 8H); MS (ESI) m/z 401 [$\text{C}_{23}\text{H}_{20}\text{N}_4\text{OS} + \text{H}$] $^+$.

(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl){4-[2-(thiophen-2-yl)quinazolin-4-yl]piperazin-1-yl}methanone (30)—91% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.05 (d, J = 3.2 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 8.0 Hz, 1H), 7.47–7.40 (m, 2H), 7.16 (t, J = 4.0 Hz, 1H), 7.03–6.87 (m, 3H), 4.30 (s, 4H), 3.86 (br s, 8H); MS (ESI) m/z 458 [$\text{C}_{25}\text{H}_{22}\text{N}_4\text{O}_3\text{S} + \text{H}$] $^+$.

Preparation of 6-(Bromomethyl)-2,3-dihydrobenzo[b][1,4]dioxine (27)—A solution of 2,3-dihydrobenzo[b][1,4]dioxine-6-carboxylic acid (500 mg, 2.75 mmol) in THF (5 mL) was added dropwise to a suspension of LAH (315 mg, 8.32 mmol) in THF (5 mL) at

–10 °C under a nitrogen atmosphere. After stirring for 4 h at room temperature, the reaction mixture was cooled to –10 °C and slowly quenched with THF/water (5 mL:5 mL). The reaction mixture was extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (20% EtOAc in hexanes) to afford (2,3-dihydrobenzo[b][1,4]dioxin-6-yl)methanol (300 mg, 65%) as a thick colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 6.90–6.79 (m, 3H), 4.56 (s, 2H), 4.25 (s, 4H); MS (ESI) *m/z* 167 [C₉H₁₀O₃ + H]⁺.

Phosphorous tribromide (0.13 mL, 1.44 mmol) was added dropwise to an ice-cold solution of the above alcohol (400 mg, 1.02 mmol) in CH₂Cl₂ (5 mL) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was quenched with an aqueous NaHCO₃ solution (4 mL). The organic layer was separated, and washed with water (3 × 10 mL) and brine (3 × 10 mL). The organic layer was dried over Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure to afford benzylbromide **27** (300 mg, crude). The product was characterized by MS analysis and subjected to the next step without further purification: MS (ESI) *m/z* 227 [C₉H₉BrO₂ + H]⁺.

Preparation of 4-{4-[(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)methyl]piperazin-1-yl}-2-(thiophen-2-yl)quinazoline (28**)**—Amine **24** (210 mg, 0.71 mmol) was added to a solution of bromide **27** (180 mg, 0.79 mmol) in DMF (2 mL) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layer was washed with water (3 × 10 mL) and brine (3 × 10 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (30% EtOAc in hexanes) to afford tertiary amine **28** (50 mg, 15%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.03 (s, 1H), 7.89–7.84 (m, 2H), 7.69 (br s, 1H), 7.44–7.36 (m, 2H), 7.14 (s, 1H), 6.90–6.83 (m, 3H), 4.27 (s, 4H), 3.86 (s, 4H), 3.50 (s, 2H), 2.67 (s, 4H); MS (ESI) *m/z* 445 [C₂₅H₂₄N₄O₂S + H]⁺.

General Procedure for Preparation of Boc-protected N,N-

Dimethylalkyldiamines—A solution of (Boc)₂O (22.68 mmol) in CH₂Cl₂ (100 mL) was added dropwise to a stirring solution of *N,N*-dimethylalkyldiamine (56.70 mmol) in CH₂Cl₂ (150 mL) at 0 °C over a period of 60 min. After stirring for 18 h at room temperature, the reaction mixture was washed with an aqueous NaHCO₃ solution (100 mL) and the organic layer was acidified with 10% AcOH to pH 3. The aqueous layer was separated and basified with 6 N aqueous NaOH solution to pH 12. The aqueous layer was extracted with CH₂Cl₂ (2 × 100 mL) and the combined organic layer was dried over Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure to afford the mono-Boc-protected *N,N*-dimethylalkyldiamines.

tert-Butyl Methyl[2-(methylamino)ethyl]carbamate—24% yield; ¹H NMR (400 MHz, CDCl₃) δ 3.33 (br s, 2H), 2.88 (s, 3H), 2.73 (t, *J* = 6.0 Hz, 2H), 2.45 (s, 3H), 1.46 (s, 9H); MS (ESI) *m/z* 189 [C₉H₂₀N₂O₂ + H]⁺.

tert-Butyl Methyl[3-(methylamino)propyl]carbamate—17% yield; ¹H NMR (400 MHz, CDCl₃) δ 9.86 (s, 1H), 9.67 (s, 1H), 8.89 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.18 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H); MS (ESI) *m/z* 326 [C₁₇H₁₂ClN₃O₂ + H]⁺.

Preparation of tert-Butyl 2-[(2-Chloroquinazolin-4-yl)

(methyl)amino]ethyl(methyl) Carbamate (32a**)**—Utilizing the general procedure outlined for synthesis of **19** derivatives provided a 35% yield; ¹H NMR (400 MHz, CDCl₃) δ

8.09 (d, $J = 8.0$ Hz, 1H), 7.76–7.68 (m, 2H), 7.38 (d, $J = 6.0$ Hz, 1H), 3.91 (s, 2H), 3.62–3.54 (m, 5H), 2.96 (m, 3H) 1.31 (s, 9H); MS(ESI) m/z 351 [$C_{17}H_{23}ClN_4O_2 + H$] $^+$.

Preparation of tert-Butyl 3-[(2-chloroquinazolin-4-yl)

(methyl)amino]propyl(methyl) Carbamate (32b)—Utilizing the general procedure outlined for synthesis of **19** derivatives provided a 66% yield; 1H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, $J = 8.0$ Hz, 1H), 7.76 (d, $J = 8.0$ Hz, 1H), 7.68 (t, $J = 8.0$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 1H), 3.75 (t, $J = 8.0$ Hz, 2H), 3.42 (s, 3H), 3.34 (br s, 2H), 2.90 (s, 3H), 2.05–2.01 (m, 3H), 1.44 (s, 9H); MS (ESI) m/z 365 [$C_{18}H_{25}ClN_4O_2 + H$] $^+$.

Preparation of tert-Butyl 4-(2-chloroquinazolin-4-yl)-1,4-diazepane-1-

carboxylate (32c)—Utilizing the general procedure outlined for synthesis of **19** derivatives provided 1.10 g, 70% yield: 1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, $J = 8.4$ Hz, 1H), 7.79–7.68 (m, 2H), 7.41–7.36 (m, 1H), 4.13–4.07 (m, 2H), 3.99–3.98 (m, 2H), 3.72 (br s, 2H), 3.56–3.47 (m, 2H), 2.16–2.04 (m, 2H), 1.41 (s, 9H); MS (ESI) m/z 363 [$C_{18}H_{23}ClN_4O_2 + H$] $^+$.

Preparation of tert-Butyl Methyl(2-{methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}ethyl)carbamate (33a)

—Utilizing the general procedure outlined for synthesis of **21** derivatives provided 600 mg, 30% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 2H), 7.86 (br s, 1H), 7.68–7.67 (m, 1H), 7.43 (d, $J = 4.4$ Hz, 1H), 7.34 (d, $J = 6.0$ Hz, 1H), 7.15 (t, $J = 4.4$ Hz, 1H), 4.06 (br s, 1H), 3.95 (br s, 1H), 3.69 (s, 2H), 3.53 (d, $J = 10.4$ Hz, 3H), 3.01–2.93 (m, 3H), 1.39 (s, 9H); MS (ESI) m/z 399 [$C_{21}H_{26}N_4O_2S + H$] $^+$.

Preparation of tert-Butyl Methyl(3-{methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}propyl)carbamate (33b)

—Utilizing the general procedure outlined for synthesis of **21** derivatives provided 1.0 g, 54% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, $J = 4.0$ Hz, 1H), 7.97 (d, $J = 8.0$ Hz, 1H), 7.86 (d, $J = 8.0$ Hz, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 7.43 (d, $J = 4.0$ Hz, 1H), 7.34 (t, $J = 4.0$ Hz, 1H), 7.14 (t, $J = 4.0$ Hz, 1H), 3.81 (t, $J = 8.0$ Hz, 2H), 3.38 (s, 3H), 3.36 (br s, 2H), 2.91 (s, 3H), 2.11–2.07 (m, 2H), 1.43 (s, 9H); MS (ESI) m/z 413 [$C_{22}H_{28}N_4O_2S + H$] $^+$; 600 mg (54%) yield.

Preparation of tert-Butyl 4-[2-(thiophen-2-yl)quinazolin-4-yl]-1,4-diazepane-1-carboxylate (33c)

—Utilizing the general procedure outlined for synthesis of **21** derivatives provided 1.00 g, 80% yield 1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, $J = 2.8$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 7.86 (d, $J = 8.4$ Hz, 1H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.42 (d, $J = 4.8$ Hz, 1H), 7.32–7.27 (m, 1H), 7.13 (t, $J = 4.0$ Hz, 1H), 4.06–4.05 (m, 4H), 3.23 (t, $J = 5.2$ Hz, 2H), 2.97 (t, $J = 5.6$ Hz, 2H), 2.10 (t, $J = 5.2$ Hz, 2H), 1.41 (s, 9H); MS (ESI) m/z 411 [$C_{22}H_{26}N_4O_2S + H$] $^+$.

General Procedure for Preparation of Flexible Diaminolinker quinazoline analogues (34)

—A 20% hydrochloric acid solution in 1,4-dioxane (3 mL) was added to a stirring solution of Boc-protected amine (1.21 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After stirring for 16 h at room temperature, the precipitated solids were filtered off and the filter cake was dissolved in water (10 mL). The resulting aqueous solution was washed with CH_2Cl_2 (2 $\times$ 10 mL), cooled to 0 °C, basified with a 6 N NaOH solution to pH 12, and extracted with CH_2Cl_2 (2 $\times$ 10 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford **34** as an oil.

N¹,N²-Dimethyl-N1-[2-(thiophen-2-yl)quinazolin-4-yl]ethane-1,2-diamine (34a)

—300 mg, 80% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.07–8.02 (m, 2H), 7.86 (d, $J = 8.4$ Hz, 1H), 7.67 (t, $J = 7.6$ Hz, 1H), 7.43 (t, $J = 4.8$ Hz, 1H), 7.34 (t, $J = 8.0$ Hz, 1H), 7.15 (t, J

= 8.0 Hz, 1H), 3.95 (t, J = 6.4 Hz, 2H), 3.47 (s, 3H), 3.08 (t, J = 6.4 Hz, 2H), 2.53 (s, 3H); ESI m/z 299 [$C_{16}H_{18}N_4S + H$] $^+$.

N¹,N³-Dimethyl-N¹-[2-(thiophen-2-yl)quinazolin-4-yl]propane-1,3-diamine (34b)—300 mg, 79% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.02–7.98 (m, 2H), 7.86 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.43 (d, J = 4.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 4.0 Hz, 1H), 3.88 (t, J = 8.0 Hz, 2H), 3.70 (s, 3H), 2.69 (t, J = 8.0 Hz, 2H), 2.43 (s, 3H), 2.08–2.00 (s, 2H); MS (ESI) m/z 313 [$C_{17}H_{20}N_4S + H$] $^+$.

4-(1,4-Diazepan-1-yl)-2-(thiophen-2-yl)quinazoline (34c)—500 mg, 60% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 2.8 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 4.8 Hz, 1H), 7.32–7.27 (m, 1H), 7.13 (t, J = 4.0 Hz, 1H), 4.06–4.05 (m, 4H), 3.23 (t, J = 5.2 Hz, 2H), 2.97 (t, J = 5.6 Hz, 2H), 2.10 (t, J = 5.2 Hz, 2H); MS (ESI) m/z 311 [$C_{17}H_{18}N_4S + H$] $^+$.

Preparation of N,4-Dimethyl-N-(2-{methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}ethyl)benzenesulfonamide (35a)—Utilizing the general procedure outlined for synthesis of **32** derivatives provided 135 mg, 55% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 3.6 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.70–7.66 (m, 3H), 7.41 (d, J = 4.8 Hz, 1H), 7.36 (t, J = 8.0 Hz, 2H), 7.25 (s, 1H), 7.13 (t, J = 4.0 Hz, 1H), 4.07 (t, J = 6.8 Hz, 2H), 3.58 (s, 3H), 3.49 (t, J = 6.8 Hz, 2H), 2.90 (s, 3H), 2.39 (s, 3H); ESI m/z 453 [$C_{23}H_{24}N_4O_2S_2 + H$] $^+$.

Preparation of N,4-Dimethyl-N-(3-{methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}propyl)benzenesulfonamide (35b)—Utilizing the general procedure outlined for synthesis of **32** derivatives provided 220 mg, 73% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.63 (d, J = 8 Hz, 2H), 7.42 (d, J = 5.2 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.27 (s, 2H), 7.14 (t, J = 8.0 Hz, 1H), 3.89 (t, J = 8.0 Hz, 2H), 3.50 (s, 3H), 3.13 (t, J = 8.0 Hz, 2H), 3.09 (s, 3H), 2.40 (s, 3H), 2.14–2.07 (m, 2H); MS (ESI) m/z 467 [$C_{24}H_{26}N_4O_2S_2 + H$] $^+$.

Preparation of 2-(Thiophen-2-yl)-4-(4-tosyl-1,4-diazepan-1-yl)quinazoline (35c)—Utilizing the general procedure outlined for synthesis of **32** derivatives provided 355 mg, 74% yield; 1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2.8 Hz, 1H), 7.85 (t, J = 14.4 Hz, 2H), 7.68 (t, J = 7.2 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 4.4 Hz, 1H), 7.34–7.32 (m, 1H), 7.14 (t, J = 4.4 Hz, 1H), 7.08 (d, J = 8.0 Hz, 2H), 4.14 (t, J = 4.8 Hz, 2H), 4.04 (t, J = 6.0 Hz, 2H), 3.70 (t, J = 5.2 Hz, 2H), 3.40 (t, J = 6.0 Hz, 2H), 2.29 (s, 3H), 2.17 (t, J = 5.6 Hz, 2H); MS (ESI) m/z 465 [$C_{24}H_{24}N_4O_2S_2 + H$] $^+$.

Preparation of 2-Chloro-4-(4-tosylpiperidin-1-yl)quinazoline (36)—Utilizing the general procedure outlined for synthesis of **19** derivatives provided 600 mg, 82% yield; 1H NMR (400 MHz, $CDCl_3$) δ 7.84–7.32 (m, 5H), 7.47–7.40 (m, 3H), 4.51 (d, J = 13.2 Hz, 2H), 3.26–3.11 (m, 3H), 2.48 (s, 3H), 2.18 (d, J = 10.8 Hz, 2H), 2.05–1.94 (m, 2H); MS (ESI) m/z 402 [$C_{20}H_{20}ClN_3O_2S$] $^+$.

Preparation of 2-(2-Chloroquinazolin-4-ylamino)ethanol (37)—Utilizing the general procedure outlined for synthesis of **19** derivatives provided 2.70 g, 48% yield; 1H NMR (400 MHz, $DMSO-d_6$) δ 8.73 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 7.77 (t, J = 7.2 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 4.83 (t, J = 4.2 Hz, 1H), 3.64–3.56 (m, 4H); MS (ESI) m/z 223 [$C_{10}H_{10}ClN_3O$] $^+$.

Preparation of 2-(Thiophen-2-yl)-4-(4-tosylpiperidin-1-yl)quinazoline (38)—

Utilizing the general procedure outlined for synthesis of **21** derivatives provided 220 mg, 66% yield: ^1H NMR (400 MHz, CDCl_3) δ 8.01 (d, J = 3.6 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.81–7.77 (m, 3H), 7.71 (t, J = 7.6 Hz, 1H), 7.45 (d, J = 4.8 Hz, 1H), 7.41–7.38 (m, 3H), 7.14 (t, J = 4.4 Hz, 1H), 4.48 (d, J = 13.6 Hz, 2H), 3.26–3.08 (m, 3H), 2.46 (s, 3H), 2.19 (d, J = 11.2 Hz, 2H), 2.08–1.98 (m, 2H); MS (ESI) m/z 450 [$\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_2\text{S}_2 + \text{H}$] $^+$.

Preparation of 2-[2-(Thiophen-2-yl)quinazolin-4-ylamino]ethanol (39)—Utilizing the general procedure outlined for synthesis of **21** derivatives provided 320 mg, 53% yield; ^1H NMR (400 MHz, CDCl_3) δ 8.32 (s, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.66 (t, J = 4.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.16 (t, J = 4.0 Hz, 1H), 4.81 (t, J = 4.0 Hz, 1H), 3.70 (d, J = 4.0 Hz, 4H); MS (ESI) m/z 372 [$\text{C}_{14}\text{H}_{13}\text{N}_3\text{OS} + \text{H}$] $^+$.

Preparation of 2-[2-(Thiophen-2-yl)quinazolin-4-ylamino]ethyl Pivalate (40)—

Cesium carbonate (670 mg, 2.06 mmol) was added to a stirring suspension of alcohol **39** (280 mg, 1.03 mmol) in NMP (5 mL) at room temperature. After stirring for 30 min, pivaloyl chloride (0.14 mL, 1.13 mmol) was added and the stirring was continued for 16 h at room temperature. The reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layer was washed with a saturated NaHCO_3 solution (3 $\times$ 20 mL) and water (3 $\times$ 20 mL), dried over Na_2SO_4 , and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel silica-gel column chromatography (12% EtOAc in hexanes) to afford pivalate **40** (50 mg, 14%) as an oil: ^1H NMR (400 MHz, CDCl_3) δ 8.06 (d, J = 4.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.73–7.64 (m, 2H), 7.45–7.39 (m, 2H), 7.15 (t, J = 4.0 Hz, 1H), 6.23 (br s, 1H), 4.49 (t, J = 8.0 Hz, 2H), 4.06–4.02 (m, 2H), 1.21 (s, 9H); MS (ESI) m/z 356 [$\text{C}_{19}\text{H}_{21}\text{N}_3\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 2-{Methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}ethyl Pivalate (41)—

Amine **40** (400 mg, 1.12 mmol) was added to a suspension of NaH (60% in mineral oil, 88 mg, 2.24 mmol) in DMF (8 mL) at 0 $^\circ\text{C}$. After stirring for 15 min at 0 $^\circ\text{C}$, CH_3I (0.20 mL, 3.38 mmol) was added. After stirring for 6 h at room temperature under a nitrogen atmosphere, the reaction mixture was cooled to 0 $^\circ\text{C}$, quenched with a saturated solution of NH_4Cl (10 mL), and extracted with EtOAc (3 $\times$ 10 mL). The combined organic layer was washed with water (3 $\times$ 20 mL) and brine (3 $\times$ 20 mL), dried over Na_2SO_4 , and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel silica-gel column chromatography (12% EtOAc in hexanes) to afford the *N*-methylated derivative **41** (150 mg, 35%) as an oil: ^1H NMR (400 MHz, CDCl_3) δ 8.03 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 1H), 7.68 (t, J = 8.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 4.0 Hz, 1H), 4.54 (t, J = 8.0 Hz, 2H), 4.11 (t, J = 5.6 Hz, 2H), 3.53 (s, 3H), 1.15 (s, 9H); MS (ESI) m/z 370 [$\text{C}_{20}\text{H}_{23}\text{N}_3\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 2-{Methyl[2-(thiophen-2-yl)quinazolin-4-yl]amino}ethanol (42)—

A solution of 3 N NaOH (0.12 mL) was added to a stirring solution of pivalate **41** (120 mg, 0.32 mmol) in a mixture of THF/ MeOH (2 mL:1 mL) at room temperature. After stirring for 6 h at room temperature, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by trituration with CH_2Cl_2 /hexanes to afford *N*-methylaminoethanol derivative **42** (40 mg, 55%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 8.07 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 4.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.44 (d, J = 4.0 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 4.0 Hz, 1H), 5.01 (br s, 1H), 4.08 (d, J = 8.0 Hz, 2H), 4.01 (d, J = 8.0 Hz, 2H), 3.53 (s, 3H); MS (ESI) m/z 286 [$\text{C}_{15}\text{H}_{15}\text{N}_3\text{OS} + \text{H}$] $^+$.

Preparation of N-(2-Carbamoylphenyl)thiophene-2-carboxamide (45)—Oxalyl chloride (2 mL, 18.87 mmol) was added to a stirring suspension of acid **43** (2.00 g, 15.74 mmol) in CH₂Cl₂ (40 mL) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (40 mL) and 2-aminobenzamide (**44**, 2.50 g, 18.87 mmol) was added. After stirring for 4 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in CH₂Cl₂ (30 mL); the organic layer was washed with water (3 × 10 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure to afford amide **45** (3.00 g, 78%) as an off-white solid. The product was characterized by MS analysis and subjected to the next step without further purification: MS (ESI) *m/z* 247 [C₁₂H₁₀N₂O₂S + H]⁺.

Preparation of 2-(Thiophen-2-yl)quinazolin-4-ol (46)—A solution of 6 N NaOH (10 mL) was added to a suspension of amide **45** (1.0 g, 4.05 mmol) in ethanol (20 mL) and the reaction mixture was heated at reflux temperature for 2 h. After this time, the reaction was cooled and concentrated under reduced pressure. The resulting residue was diluted with water and acidified with a saturated citric acid solution. The resulting precipitate was filtered off; the filter cake was washed with cold acetone (20 mL), and dried under reduced pressure to afford quinazilone **46** (800 mg, 85%) as an off-white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.65 (s, 1H), 8.22 (d, *J* = 3.6 Hz, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 4.8 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.22 (t, *J* = 4.4 Hz, 1H); MS (ESI) *m/z* 229 [C₁₂H₈N₂OS + H]⁺.

Preparation of 4-Chloro-2-(thiophen-2-yl)quinazoline (47)—*N,N*-Dimethylaniline (4 mL) was added to a suspension of quinazilone **46** (800 mg, 3.50 mmol) in phosphorous oxychloride (12 mL) at room temperature. The reaction mixture was heated at reflux temperature for 4 h. After this time, the reaction mixture was cooled to 0 °C and the ice cold reaction mixture was added slowly to crushed ice with continuous stirring. The aqueous layer was extracted with CH₂Cl₂ (3 × 10 mL). The combined organic layer was washed with an aqueous NaHCO₃ solution (2 × 10 mL) and water (3 × 10 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (10% EtOAc in hexanes) to afford chloroquinazoline **47** (700 mg, 81%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.23–8.21 (m, 1H), 8.16–8.15 (m, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.93–7.89 (m, 1H), 7.65–7.61 (m, 1H), 7.55–7.54 (m, 1H), 7.27–7.23 (m, 1H).

Preparation of tert-Butyl 4-[2-(thiophen-2-yl)quinazolin-4-yl]-5,6-dihydropyridine-1(2H)-carboxylate (49)—Boronate ester **48** (1.00 g, 3.41 mmol) and K₂CO₃ (785 mg, 5.69 mmol) were added to a stirring solution of chloroquinazoline **47** (700 mg, 2.84 mmol) in DMF (15 mL). The reaction mixture was purged with argon gas for 20 min and Pd(PPh₃)₄ (328 mg, 0.28 mmol) was added. The reaction mixture was heated at 100 °C for 4 h under an argon atmosphere. After this time, the reaction was cooled to room temperature, diluted with water (30 mL), and extracted with EtOAc (3 × 15 mL). The combined organic layer was washed with water (3 × 30 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (10% EtOAc in hexanes) to afford dihydropyridine **49** (450 mg, 40%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.14–8.13 (m, 2H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.86–7.82 (m, 1H), 7.58–7.49 (m, 2H), 7.18 (t, *J* = 4 Hz, 1H), 5.12–5.02 (m, 1H), 4.42 (br s, 1H), 4.15–4.08 (m, 1H), 3.79 (br s, 1H), 2.52–2.51 (m, 1H), 2.36–2.24 (m, 2H), 1.54 (s, 9H); MS (ESI) *m/z* 394 [C₂₂H₂₃N₃O₂S + H]⁺.

Preparation of tert-Butyl 4-[2-(thiophen-2-yl)quinazolin-4-yl]piperidine-1-carboxylate (50)—Ammonium formate (40 mg, 0.63 mmol) and Pd/C (35 mg) were added to a stirring solution of dihydropyridine **49** (50 mg, 0.12 mmol) in ethanol (2 mL). The reaction mixture was heated at reflux temperature for 48 h under a nitrogen atmosphere, cooled, and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and the crude residue was purified by silica-gel column chromatography (5% EtOAc in hexanes) to afford piperidine **50** (8 mg, 16%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 8.16–8.15 (m, 1H), 8.10 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.86–7.82 (m, 1H), 7.57–7.49 (m, 2H), 7.19–7.17 (m, 1H), 4.31 (br s, 2H), 3.71–3.65 (m, 1H), 3.04–2.99 (m, 2H), 2.17–1.96 (m, 4H), 1.52 (s, 9H); MS (ESI) m/z 396 [$\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 2-(Thiophen-2-yl)-4-(1-tosylpiperidin-4-yl)quinazoline (51)—Trifluoroacetic acid (0.3 mL) was added to an ice-cold solution of Boc-protected piperidine **50** (90 mg, 0.22 mmol) in CH_2Cl_2 at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was concentrated under reduced pressure. The crude residue was washed with MTBE, characterized by MS analysis, and subjected to the next step without further purification: MS (ESI) m/z 296 [$\text{C}_{17}\text{H}_{17}\text{N}_3\text{S} - \text{TFA}$] $^+$.

N,N-Diisopropylethylamine (0.17 mL, 1.02 mmol) and tosyl chloride (70 mg, 0.36 mmol) were added to a stirring solution of the TFA salt of the piperidine (90 mg, 0.34 mmol) in NMP (2 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 $\times$ 5 mL). The combined organic layer was washed with water (3 $\times$ 10 mL) and brine (3 $\times$ 10 mL), dried over Na_2SO_4 , and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (30% EtOAc in hexanes) to afford sulphonamide **51** (80 mg, 58%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 8.10–8.09 (m, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.83–7.79 (m, 1H), 7.75–7.73 (m, 2H), 7.51–7.51 (m, 2H), 7.38 (d, J = 7.6 Hz, 2H), 7.19–7.17 (m, 1H), 3.98–3.95 (m, 2H), 3.55–3.49 (m, 1H), 2.69–2.62 (m, 2H), 2.47 (s, 3H), 2.32–2.07 (m, 2H), 2.04–2.03 (m, 2H); MS (ESI) m/z 450 [$\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_2\text{S}_2 + \text{H}$] $^+$.

Preparation of 2,4-Dibromoquinazoline (53)—*N,N*-Dimethylaniline (4.5 mL, 12.34 mmol) was added to a suspension of quinazolinone **52** (10.0 g, 61.72 mmol) in phosphoryltribromide (125 g, 407.4 mmol) at room temperature. The reaction mixture was heated at 110 °C for 4 h. The reaction was cooled to 0 °C and the ice-cold reaction mixture was added to crushed ice with continuous stirring. The aqueous layer was extracted with CH_2Cl_2 . The combined organic layers were washed with H_2O (3 $\times$ 50 mL) and saturated aqueous NaHCO_3 solution (3 $\times$ 50 mL), dried over Na_2SO_4 , filtered, and the filtrate was concentrated under reduced pressure to afford 2,4-dibromoquinazoline (**53**, 12.0 g, 68%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.13–7.09 (m, 1H), 6.79–6.72 (m, 2H), 6.60–6.57 (m, 1H).

Preparation of 2-Bromo-4-(4-tosylpiperazin-1-yl) Quinazoline (54)—*N,N*-Diisopropylethylamine (13.36 mL, 76.64 mmol) and piperazine **17a** (9.10 g, 38.32 mmol) were added to a stirring solution of dibromide **53** (10.0 gm, 34.84 mmol) in NMP (70 mL) at 0 °C. After stirring for 3 h at room temperature the reaction mixture was diluted with water (200 mL) and extracted with EtOAc (3 $\times$ 50 mL). The combined organic layers were washed with water (3 $\times$ 50 mL) and brine (3 $\times$ 50 mL), dried over Na_2SO_4 , filtered, and the filtrate was concentrated under reduced pressure. The crude residue was purified by trituration with hexanes and dried under vacuum to afford 2-bromoquinazoline **54** (12.0 g, 82%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.81 (d, J = 8.8 Hz, 1H), 7.75–7.66 (m, 2H),

7.67 (d, $J = 8.0$ Hz, 2H), 7.44 (t, $J = 7.6$ Hz, 1H), 7.36 (d, $J = 8.0$ Hz, 2H), 3.95 (t, $J = 4.8$ Hz, 4H), 3.20 (t, $J = 4.8$ Hz, 4H), 2.45 (s, 3H); MS (ESI) m/z 447 [$C_{19}H_{19}BrN_4O_2S$] $^+$.

General Procedure for Stille Coupling: The heterocyclic stannane (0.33 mmol), K_2CO_3 (0.22 mmol), and tetraethylammoniumchloride (0.22 mmol) were added to a stirring solution of bromide **54** (0.22 mmol) in DMF (3 mL). The reaction mixture was purged with argon gas for 30 min and then heated at 110 °C for 6 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, diluted with water (10 mL), and extracted with EtOAc (3×5 mL). The combined organics were washed with water (3×10 mL), dried over Na_2SO_4 , and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography.

5-(4-(4-tosylpiperazin-1-yl)quinazolin-2-yl)thiazole (56b)—50% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.68 (s, 1H), 7.89 (d, $J = 8.4$ Hz, 1H), 7.70–7.67 (m, 4H), 7.42 (t, $J = 7.2$ Hz, 1H), 7.35 (d, $J = 8.0$ Hz, 2H), 3.93 (t, $J = 4.4$ Hz, 4H), 3.25 (t, $J = 4.4$ Hz, 4H), 2.43 (s, 3H); MS (ESI) m/z 452 [$C_{22}H_{22}N_5O_2S_2 + H$] $^+$.

4-(4-(4-tosylpiperazin-1-yl)quinazolin-2-yl)thiazole (56c)—38% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.33 (s, 1H), 8.11 (d, $J = 8.4$ Hz, 1H), 7.78–7.68 (m, 4H), 7.45–7.34 (m, 3H), 3.91 (s, 4H), 3.27 (s, 4H), 2.44 (s, 3H); MS (ESI) m/z 452 [$C_{22}H_{22}N_5O_2S_2 + H$] $^+$.

2-(4-(4-tosylpiperazin-1-yl)quinazolin-2-yl)oxazole (56d)—10% yield; 1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, $J = 8.8$ Hz, 1H), 7.86 (s, 1H), 7.81–7.77 (m, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.50 (t, $J = 7.6$ Hz, 1H), 7.40 (s, 1H), 7.35 (d, $J = 8.0$ Hz, 2H), 3.99 (t, $J = 8.0$ Hz, 4H), 3.27 (t, $J = 4.8$ Hz, 4H), 2.43 (s, 3H); MS (ESI) m/z 435 [$C_{22}H_{21}N_5O_3S + H$] $^+$.

2-(pyridazin-4-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56h)—16% yield; 1H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H), 9.34 (d, $J = 4.0$ Hz, 1H), 8.44 (s, 1H), 8.00 (d, $J = 8.0$ Hz, 1H), 7.82 (d, $J = 7.6$ Hz, 2H), 7.69 (d, $J = 7.6$ Hz, 2H), 7.52 (t, $J = 7.2$ Hz, 1H), 7.35 (d, $J = 7.2$ Hz, 2H), 3.99 (s, 4H), 3.27 (s, 4H), 2.42 (s, 3H); MS (ESI) m/z 447 [$C_{23}H_{22}N_6O_2S + H$] $^+$.

2-(pyrazin-2-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56i)—21% yield; 1H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.80 (s, 1H), 8.67 (s, 1H), 8.17 (d, $J = 8.4$ Hz, 1H), 7.84–7.80 (m, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.51 (t, $J = 7.2$ Hz, 1H), 7.34 (d, $J = 7.6$ Hz, 2H), 4.00–3.99 (m, 4H), 3.27 (s, 4H), 2.42 (s, 3H); MS (ESI) m/z 447 [$C_{23}H_{22}N_6O_2S + H$] $^+$.

2-(pyrimidin-2-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56j)—29% yield; 1H NMR (400 MHz, $CDCl_3$) δ 9.01 (d, $J = 4.8$ Hz, 2H), 8.19 (d, $J = 8.0$ Hz, 1H), 7.83–7.77 (m, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.51 (t, $J = 7.6$ Hz, 1H), 7.41 (t, $J = 4.8$ Hz, 1H), 7.35 (d, $J = 7.6$ Hz, 2H), 4.00 (t, $J = 4.8$ Hz, 4H), 3.27 (t, $J = 4.4$ Hz, 4H), 2.44 (s, 3H); MS (ESI) m/z 447 [$C_{23}H_{22}N_6O_2S + H$] $^+$.

Preparation of 4-(4-Tosylpiperazin-1-yl)-2-(tributylstannyl) Quinazoline (55)—*n*-Butyl lithium (1.2 mL, 2.46 mmol) was added dropwise to a stirring solution of bromide **54** (1.00 g, 2.23 mmol) in THF at –78 °C. After stirring for 30 min under an argon atmosphere, tributylchlorostannane (0.8 mL, 2.99 mmol) was added dropwise to the reaction mixture at –78 °C and stirring was continued for 16 h at room temperature. After this time, the reaction mixture was quenched with water and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed with water (2×20 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The

crude residue was purified by silica-gel silica-gel column chromatography (10–15% EtOAc in hexanes) to afford stannane **55** (420 mg, 29%) as an oil: ^1H NMR (400 MHz, CDCl_3) δ 7.90–7.88 (m, 1H), 7.71–7.66 (m, 4H), 7.40–7.34 (m, 3H), 3.81 (br s, 4H), 3.19 (br s, 4H), 2.44 (s, 3H), 1.67–1.52 (m, 6H), 1.38–1.21 (m, 7H), 1.16–1.08 (m, 6H), 0.89–0.86 (m, 8H).

Preparation of 2-(Pyrimidin-5-yl)-4-(4-tosylpiperazin-1-yl) Quinazoline (56k)—5-

Bromopyrimidine (110 mg, 0.68 mmol), CsF (205 mg, 1.36 mmol), and CuI (13 mg, 0.10 mmol) were added to a stirring solution of stannane **55** (450 mg, 0.68 mmol) in DMF (5 mL). The reaction mixture was purged with nitrogen for 30 min followed by the addition of $\text{Pd}(\text{PPh}_3)_4$ (50 mg, 0.06 mmol). The reaction mixture was heated at 110 °C for 16 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, diluted with water (40 mL), and extracted with EtOAc (3 $\times$ 30 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel silica-gel column chromatography (40% EtOAc in hexanes) followed by trituration with CH_2Cl_2 /hexanes to afford quinazoline **56k** (24 mg, 9%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 9.72 (s, 2H), 9.30 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.82–7.77 (m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.48 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 3.98 (t, J = 4.8 Hz, 4H), 3.26 (t, J = 4.4 Hz, 4H), 2.42 (s, 3H); MS (ESI) m/z 447 [$\text{C}_{23}\text{H}_{22}\text{N}_6\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 4-(4-Tosylpiperazin-1-yl)quinazoline (62)—Bis(pinacolato)diboron (236 mg, 0.93 mmol) and KOAc (183 mg, 1.86 mmol) were added to a stirring solution of chloroquinazoline **19a** (250 mg, 0.62 mmol) in DMF (8 mL). The reaction mixture was purged with argon gas for 30 min and $\text{Pd}(\text{dppf})_2\text{Cl}_2$ (125 mg, 0.15 mmol) was added. The reaction mixture was heated at 100 °C for 18 h under an argon atmosphere. After this time, the reaction mixture was cooled to room temperature, diluted with ice-cold water (30 mL), and extracted with EtOAc (2 $\times$ 10 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (50% EtOAc in hexanes) to afford quinazoline **62** (80 mg, 34%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 8.71 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.78–7.72 (m, 2H), 7.67 (d, J = 8.0 Hz, 2H), 7.46 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 8.0 Hz, 2H), 3.87 (t, J = 4.4 Hz, 4H), 3.21 (t, J = 4.8 Hz, 4H), 2.47 (s, 3H); MS (ESI) m/z 411 [$\text{C}_{22}\text{H}_{26}\text{N}_4\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 4-(4-Tosylpiperazin-1-yl)quinazoline-2-carbonitrile (57)—Zinc(II) cyanide (350 mg, 2.98 mmol) and dppf (250 mg, 0.48 mmol) were added to a stirring solution of chloroquinazoline **19a** (1.00 g, 2.48 mmol) in NMP (30 mL). The reaction mixture was purged with argon for 30 min and $\text{Pd}_2(\text{dba})_3$ (220 mg, 0.24 mmol) was added. The reaction mixture was heated at 90 °C for 18 h under an argon atmosphere. The reaction mixture was cooled to room temperature, diluted with water (30 mL), and extracted with EtOAc (3 $\times$ 15 mL). The combined organic layer was filtered through a cotton plug and the filtrate was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (30% EtOAc in hexanes) to afford nitrile **57** (300 mg, 30%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.94 (d, J = 8.0 Hz, 1H), 7.85–7.80 (m, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.59 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 4.00 (br s, 4H), 3.18 (br s, 4H), 2.45 (s, 3H); MS (ESI) m/z 394 [$\text{C}_{20}\text{H}_{19}\text{N}_5\text{O}_2\text{S} + \text{H}$] $^+$.

Preparation of 4-(4-Tosylpiperazin-1-yl)quinazoline-2-carboxamide (58)—

Concentrated hydrochloric acid (10 mL) was added to nitrile **57** (100 mg, 0.25 mmol) and the reaction was heated at 50 °C for 16 h. The reaction mixture was cooled and neutralized with a 6 N solution of NaOH to pH 7 and extracted with CH_2Cl_2 (2 $\times$ 25 mL). The

combined organic layers were dried over Na₂SO₄ and filtered. The filtrate was concentrated under reduced pressure to afford amide **58** (60 mg, 43%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, *J* = 8.4 Hz, 1H), 7.82–7.81 (m, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 2H), 5.85 (br s, 2H), 3.95 (br s, 4H), 3.25 (br s, 4H), 2.45 (s, 3H); MS (ESI) *m/z* 412 [C₂₀H₂₁N₅O₃S + H]⁺.

Preparation of Methyl 4-(4-tosylpiperazin-1-yl) Quinazoline-2-carboxylate (59)

—Dry HCl gas was bubbled through a stirring solution of nitrile **57** (200 mg, 0.50 mmol) in methanol (10 mL) for 30 min. After stirring for 6 h at room temperature, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (20% EtOAc in hexanes) to afford ester **59** (150 mg, 67%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, *J* = 8 Hz, 1H), 7.80 (t, *J* = 8 Hz, 2H), 7.67 (d, *J* = 8 Hz, 2H), 7.55 (t, *J* = 8 Hz, 1H), 7.35 (d, *J* = 8 Hz, 2H), 4.03 (s, 3H), 3.99 (t, *J* = 4.8 Hz, 4H), 3.22 (t, *J* = 4.8 Hz, 3H), 2.44 (s, 3H), MS (ESI) *m/z* 427 [C₂₁H₂₂N₄O₄S + H]⁺.

Preparation of 1-[4-(4-Tosylpiperazin-1-yl)quinazolin-2-yl]ethanone (60)—

Methyl magnesium bromide (1.64 mmol) was added to a stirring solution of nitrile **57** (0.74 mmol) in THF (15 mL) at 0 °C. After stirring for 5 h at room temperature under a nitrogen atmosphere, the reaction mixture was quenched with water (20 mL). Solvents were removed under reduced pressure. The resulting residue was diluted with EtOAc (20 mL) and washed with water (2 × 10 mL) followed by brine (2 × 10 mL). The organic layer was separated, dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (30% EtOAc in hexanes) to afford ketone **59** (45 mg, 15%) as an off-white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, *J* = 8.0 Hz, 1H), 7.81 (t, *J* = 6.0 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 3.96 (t, *J* = 4.0 Hz, 4H), 3.23 (t, *J* = 4.0 Hz, 4H), 2.75 (s, 3H), 2.44 (s, 3H); MS (ESI) *m/z* 410 [C₂₁H₂₂N₄O₃S + H]⁺.

General Procedure for the Substitution Reaction: A mixture of **19a** (0.49 mmol) and the NH-heterocycle (0.54 mmol) in acetonitrile (1.5 mL) was heated at reflux for 18 h. The resulting precipitate was filtered off and the filter cake was dissolved in 20% MeOH in CH₂Cl₂ (6 mL). The organic layer was washed with an aqueous solution of NaHCO₃ (5 mL), dried over Na₂SO₄, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by preparative TLC plate (1% MeOH in CHCl₃) to afford heterocyclic derivatives.

Preparation of 2-(1H-Benzo[d]imidazol-1-yl)-4-(4-tosylpiperazin-1-

yl)quinazoline (63)—60 mg, 25% yield; ¹H NMR (400 MHz, CDCl₃) δ 9.06 (s, 1H), 8.71 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.86–7.76 (m, 3H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.46–7.34 (m, 5H), 4.01 (t, *J* = 4.4 Hz, 4H), 3.92 (t, *J* = 4.8 Hz, 4H), 2.42 (s, 3H); MS (ESI) *m/z* 485 [C₂₆H₂₄N₆O₂S + H]⁺.

Preparation of 5-[4-(4-Tosylpiperazin-1-yl)quinazolin-2-yl]thiophene-2-carbaldehyde (64)—

Utilizing the general procedure outlined for synthesis of **21** derivatives provided aldehyde **64** which was immediately utilized due to instability: MS (ESI) *m/z* 479 [C₂₄H₂₂N₄O₃S₂ + H]⁺.

Preparation of {5-[4-(4-Tosylpiperazin-1-yl)quinazolin-2-yl]thiophen-2-yl}methanol (65)—

Sodium borohydride (165 mg, 4.42 mmol) was added portion wise to a stirring ice-cold solution of aldehyde **64** (850 mg, 1.77 mmol) in THF/MeOH (1:1, 20 mL) at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was concentrated

under reduced pressure. The crude residue was dissolved in water (15 mL) and the aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (40% EtOAc in hexanes) to afford alcohol **65** (350 mg, 47%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.90–7.85 (m, 2H), 7.69–7.67 (m, 4H), 7.38–7.33 (m, 3H), 7.03 (d, $J = 3.6$ Hz, 1H), 4.87 (s, 2H), 3.90 (t, $J = 4.8$ Hz, 4H), 3.24 (t, $J = 4.8$ Hz, 4H), 2.43 (s, 3H), 2.11 (br s, 1H); MS (ESI) m/z 481 $[\text{C}_{24}\text{H}_{24}\text{N}_4\text{O}_3\text{S}_2 + \text{H}]^+$.

General Procedure for Coupling Reaction: Triethylamine (1.24 mmol) was added to a stirring solution of alcohol **65** (0.41 mmol) in CH_2Cl_2 (3.0 mL) at 0°C followed by the slow addition of MsCl (0.83 mmol). After stirring for 4 h at room temperature, the reaction mixture was cooled to 0°C and amine **66** (4.16 mmol) was added. After stirring for 18 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with water (3×10 mL), dried over Na_2SO_4 , and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica-gel column chromatography (50% EtOAc in hexanes) to afford the tertiary amines.

tert-Butyl 4-((5-[4-(4-tosylpiperazin-1-yl)quinazolin-2-yl]thiophen-2-yl)methyl)piperazine-1-carboxylate (67)—15 mg, 11% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.88–7.85 (m, 2H), 7.73–7.67 (m, 4H), 7.38–7.34 (m, 3H), 6.96 (d, $J = 2.8$ Hz, 1H), 3.90 (t, $J = 4.4$ Hz, 4H), 3.48 (br s, 4H), 3.25 (t, $J = 4.4$ Hz, 4H), 2.50 (br s, 4H), 2.43 (s, 3H), 1.46 (s, 9H); MS (ESI) m/z 649 $[\text{C}_{33}\text{H}_{40}\text{N}_4\text{S}_2]^+$.

4-((5-(4-(4-tosylpiperazin-1-yl)quinazolin-2-yl)thiophen-2-yl)methyl)morpholine (68)—45 mg, 19% yield; ^1H NMR (400 MHz, CDCl_3) δ 7.88–7.84 (m, 2H), 7.73–7.67 (m, 4H), 7.38–7.34 (m, 3H), 6.96 (d, $J = 3.6$ Hz, 1H), 3.89 (t, $J = 4.8$ Hz, 4H), 3.75–3.74 (m, 6H), 3.25 (t, $J = 4.8$ Hz, 4H), 2.55 (br s, 4H), 2.43 (s, 3H); MS (ESI) m/z 550 $[\text{C}_{28}\text{H}_{31}\text{N}_5\text{O}_3\text{S}_2]^+$.

Preparation of 2-[5-(Piperazin-1-ylmethyl)thiophen-2-yl]-4-(4-tosylpiperazin-1-yl)quinazoline (69)—A 20% hydrochloric acid solution in 1,4-dioxane (0.1 mL) was added to a stirring solution of carbamate **67** (45 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) at 0°C . After stirring for 2 h at room temperature, the reaction mixture was diluted with water and the aqueous layer was washed with CH_2Cl_2 (2×5 mL). The aqueous layer was basified with a saturated aqueous NaHCO_3 solution to pH 12 and extracted with CH_2Cl_2 (2×5 mL). The combined organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford secondary amine **69** (25 mg, 66%) as an off-white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.87–7.84 (m, 2H), 7.73–7.68 (m, 4H), 7.37–7.34 (m, 3H), 6.95 (d, $J = 3.6$ Hz, 1H), 3.89–3.88 (m, 4H), 3.74 (s, 2H), 3.25 (br s, 4H), 2.94 (t, $J = 4.8$ Hz, 4H), 2.53 (br s, 4H), 2.43 (s, 3H); MS (ESI) m/z 549 $[\text{C}_{28}\text{H}_{32}\text{N}_6\text{O}_2\text{S}_2 + \text{H}]^+$.

4.2 Biology

4.2.1 Biological experiments—The recombinant wildtype enzyme, Cerezyme[®], was obtained from Genzyme Corporation (Cambridge, MA). N370S recombinant glucocerebrosidase was a gift from Dr. Tim Edmunds at Genzyme. Patients' spleens were obtained from splenectomies with informed consent under an NIH-IRB approved clinical protocol. Control spleens were obtained under an NIH protocol. 4-methylumbelliferyl- β -D-glucopyranoside (4MU- β -glc), a blue fluorogenic substrate, resorufin β -D-glucopyranoside (res- β -glc), a red fluorogenic substrate, sodium taurocholate, and the buffer components were purchased from Sigma-Aldrich (St. Louis, MO). Isofagomine and N-nonyl-

deoxynojirimycin (NN-DNJ) were purchased from Toronto Research Biochemicals (Ontario, Canada).

The human spleen tissue was homogenized using a food blender at the maximal speed for 5 minutes, followed by 10 passes in a motor-driven 50 ml glass-Teflon homogenizer. The homogenate was centrifuged at 1000×g for 10 min. The supernatant was then filtered using a 40 µm filter and aliquots of resultant spleen homogenate were frozen at -80°C until use.

The assay buffer was composed of 50 mM citric acid, (titrated with K₂PO₄ to make different pH solutions, and 0.01% Tween-20. The spleen homogenate assays used buffer at pH 5, assays with recombinant wildtype enzyme used buffer at pH 5.9, and assays with recombinant N370S/N370S enzyme used buffer at pH 7. The buffer was stored at 4°C for up to 6 months. A solution of 1 M NaOH, 1M glycine at pH 10 was used as the stop solution for the blue substrate assay. 1 M TRIS-HCl at pH 8.0 was used as the stop solution for the red substrate assay.

4.2.2 Enzyme assay in 1536-well plate format—In black 1536-well plates, a BioRAPTR FRD™ Microfluidic Workstation (Beckman Coulter, Inc. Fullerton, CA) was used to dispense 2 µl of the enzyme solutions into 1536-well plates, and an automated pin-tool station (Kalypsys, San Diego, CA) was used to transfer 23 nl/well of compound to the assay plate. After 5 min incubation at room temperature, the enzyme reaction was initiated by the addition of 2 µl/well substrate. After 45 min incubation at 37°C, the reaction was terminated by the addition of 2 µl/well stop solution. The fluorescence was then measured in the Viewlux, a CCD-based plate reader (Perkin Elmer, Waltham, MA), with a 365 nm excitation and 440 nm emission for the blue substrate, and 573 nm excitation and 610 nm emission for the red substrate. 27 µg/well of spleen homogenate was used as the enzyme solution. The final concentrations of the blue substrate and red substrate were 1 mM and 15 µM, respectively.

4.2.3 Thermodenaturation experiment—This assay measures the change in the melting temperature of the recombinant wildtype GC in the presence of different concentrations of the inhibitors. A mixture of wtGC and SYPRO Orange (5000X stock concentration, Invitrogen, Carlsbad, CA) was added to a 96-well skirted thin-wall PCR plate (Bio-Rad, Hercules, CA) with a final concentration of 2 µM and 5X, respectively. wtGC and SYPRO Orange were diluted in 150 mM phosphate/citrate buffer at pH 4.8. A 12-point DMSO dilution series was made separately in a 96-well polypropylene plate (Thermo Fisher Scientific, Hudson, NH) for all analogs, whose final concentrations ranged from 0.1 – 600 µM (400 µM for NCGC00182292-02). One microliter of each dilution point of each compound was transferred to the aforementioned wtGC-SYPRO Orange mixture, with a final DMSO concentration of 2% (1 µl in 50 µl final volume). DMSO alone was also transferred to the PCR plate for each dilution series as a control sample. The plate was immediately centrifuged at 1000 rpm for 10 s and subsequently sealed with Optical-Quality Sealing Tape (Bio-Rad). The plate was then heated using an iQ5 real-time PCR detection system (Bio-Rad) from 20 to 95°C with an increment of 1°C and a ramping rate of 0.1°C/s. SYPRO Orange fluorescence was monitored by a CCD camera using excitation and emission wavelengths of 490 and 575 nm, respectively. Protein melting temperature (T_m) was obtained through an EXCEL-based DSF worksheet (provided by Structure Genomics Consortium, <ftp://ftp.sgc.ox.ac.uk/pub/biophysics>, [Niesen, 2007 #1]) and GraphPad Prism 4 (GraphPad Software, Inc., La Jolla, CA). The T_m of wtGC was found to follow a logarithmic dose-dependent trend when denaturation was performed in the presence of isofagomine or selected compounds.

4.2.4 LC-MS hydrolysis experiment—This assay uses liquid chromatography linked to a mass spectrometer to assess the ability of glucocerebrosidase in the spleen homogenate to cleave its natural substrate (glucosylceramide). The substrate has a fluorescent tag, which allows the cleavage to be measured, however is not believed to have a role in the enzymatic reaction. This assay most closely reflects the physiological condition in the body.

Chromatography was performed using an Agilent HPLC. The Agilent 1200 LC was equipped with a quaternary pump, a G1315 diode array detector, and a G1321 Fluorescent Detector. A 4.6 × 250 mm Agilent Eclipse Plus C18 (5 micron) at ambient temperature was used at a flow rate of 1.8 mL/Min with a gradient of 85/15 (methanol/0.1% formic acid in water) to 100 methanol over 10 minutes. Compounds were monitored using fluorescence detection with an excitation wavelength of 505 nanometers and emission wavelength at 540 nanometers. We verified that the mass of the fluorescent peaks match with the expected ones for the substrate and product of the reaction.

4.2.5 Immunocytochemistry and Laser Scanning Confocal Microscopy—

Primary dermal fibroblasts derived from skin biopsies from two previously described N370S/N370S Gaucher patients (Goker-Alpan et al, 2008) and a control were seeded in Lab-Tek 4 chamber slides (Fisher Scientific, Pittsburgh, PA). After compound treatment, fibroblasts were fixed in 3% paraformaldehyde. The cells were permeabilized with 0.1 % Triton-X for 10 min. and blocked in PBS containing 0.1% saponin, 100 μM glycine, 0.1% BSA and 2% donkey serum followed by incubation with mouse monoclonal anti-LAMP1 or LAMP-2 (1:100, Developmental Studies Hybridoma bank, University of Iowa, Iowa City, IA) and the rabbit polyclonal anti-GCase R386 antibody (1:500); The cells were washed and incubated with secondary donkey anti-mouse or anti-rabbit antibodies conjugated to ALEXA-488 or ALEXA-555, respectively (Invitrogen, Carlsbad, CA), washed again, and mounted in VectaShield with DAPI (Vector Laboratories, Burlingame, CA).

Cells were imaged with a Zeiss 510 META confocal laser-scanning microscope (Carl Zeiss, Microimaging Inc., Germany) using an Argon (458, 477, 488, 514 nm) 30 mW laser, a HeNe (543 nm) 1 mW laser, and a laser diode (405 nm). Low and high magnification images were acquired using a Plan-Apochromat 20X/0.75 objective and a Plan-Apochromat 100x/1.4 oil DIC objective, respectively. Images were taken with the same laser settings and all the images shown are collapsed z-stacks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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 38. Reference about the Amplex red method.

List of abbreviations

4MU	4-methylumbellifereone
Boc	tert-Butyloxycarbonyl
CNS	Central Nervous System

DMSO	Dimethylsulphoxide
DNJ	Deoxynojirimycin
ER	Endoplasmic Reticulum
ERT	Enzymatic Replacement Therapy
FDA	Food and Drug Administration
GC	Glucocerebrosidase
HPLC	High Performance Liquid Chromatography
HTS	High Throughput Screening
IC50	Half Maximal Inhibitory Concentration
LC-MS	Liquid, Chromatography-Mass Spectrum
LSD	Lysosomal Storage Disorder
SRA	Structure Activity Relationship

Figure 1.

Evaluation of the hydrolytic capacity of GC in the presence of compound **10**. Activity of Compound **10** was representative of all the analogs in this series; they are selective inhibitors of GC, but their activity in purified GC assays without exogenous cofactors is greatly diminished. The inhibitory capacity was analyzed by measuring the fluorescent signal of 4-methylumbelliferone using ViewLux.

Figure 2.

Principles of enzyme reactions and product spectrums of two GC enzyme assays. (a) The “Blue” GC enzyme assay. The pro-fluorescent substrate 4MU-β-Glc is hydrolyzed to form two products, glucose and 4MU with an excitation peak of 365 nm and an emission peak of 440 nm. This assay is used for the primary screen. (b) The “Red” GC enzyme assay. The pro-fluorescent substrate Res-β-glucopyranoside is hydrolyzed to form two products, glucose and resorufin with an excitation peak of 573 nm and an emission peak of 590 nm. (c) The “fluorescent ceramide” GC enzyme assay. The glucosyl ceramide fluorescent substrate is hydrolyzed to form two products, glucose and fluorescent ceramide. Both substrate and product are fluorescent and its ratio was detected with an excitation peak of 505 nm and an emission peak of 540 nm upon separation by HPLC. (d) The “natural ceramide” GC enzyme assay. The glucosyl ceramide natural substrate is hydrolyzed to form two products, glucose and ceramide. Then the glucose was coupled with the Amplex Red glucose oxidase assay³⁸ for final detection.

Figure 3. Chaperone activity of compound **14**, and Isogagomine using wildtype and homozygous, mutant N370S GC fibroblasts. Two genotypes of fibroblasts, fibroblasts homozygous for wildtype GC (top) and fibroblasts homozygous for N370S GC (bottom) were stained both with a Cy3-labeled antibody for GC protein content (first row) and a FITC-labeled antibody specific for lysosomal compartments (LAMP1; second row) after treatment with (1) DMSO vehicle, (2) 10 uM Isogagomine, (3) 10uM compound **14**. Both compounds show increased lysosomal GC protein after treatment.

A)

B)

C)

Figure 4.

ViewLux and LC-MS analysis of the capacity of some our best compounds to inhibit of the hydrolysis of 4-methylumbelliferone β -D-glucopyranoside (4MU) and a fluorescent glucosylceramide (FlourGC) carried out by wt GC or by N370S mutant variant using tissue homogenate. ViewLux experiments were run in triplicate and mean and standard deviation values are plotted.

A) Inhibitory curves of isofagomine **1** in tissue homogenate.

B) Inhibitory curves of **26ad** in tissue homogenate.

C) LC-MS chromatogram analysis of the hydrolysis of fluorescent glucosylceramide carried out by tissue homogenate wt GC in the presence of no inhibitor (A) 12.5 μ M of **26ad** (B) or 12.5 μ M of isofagomine **1** (C). The first eluting peak corresponds with the starting material (Figure 2C) and the second one with the product of the reaction.

A)

B)

Figure 5.

Capacity of select compounds to protect wildtype glucocerebrosidase from thermal denaturation (increase in T_m relative to a protein-only control shown)

A) Thermal melt curves of selected compounds

B) Structure of analyzed inhibitors.

Figure 6. Chaperone activity of some of our compounds using a GC fluorescent antibody (red), a lysosome marker (LAMP2, green) and a nucleus marker (DAPI, blue). Active GC tranlocators increase the colocalization of GC and LAMP2 antibodies, visually increasing the yellow color.

Scheme 1.
General strategy for modification in the functional core

Scheme 2.
Synthesis of purine analogues.

Scheme 3.
 Synthesis of analogues with modification of the aromatic sulphonamide

Scheme 4.
Synthesis of analogues with a flexible linker

Scheme 5.

Synthesis of additional analogues with a modifications in position four.

Scheme 6.

Synthesis of analogues having a substituted piperidine in position four.

Scheme 7.

Synthesis of analogues with modifications at 2 position.

Scheme 8.
additional modifications at the 2 position

Scheme 9.
modification at the thiophene ring.

Table 1

of 4-methylumbelliferone β -D-glucopyranoside in N370S tissue homogenate followed by

R2	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
Inactive	9		1.27
Inactive	10		1.27

R2	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)	R2
	Inactive	11		25.29	
	79.97	12		25.29

N370S AC₅₀ (μM)

R2

R1

Compound number

N370S AC₅₀ (μM)

R2

6.35

13

63.52

R2	N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
	63.52	14			1.59
	79.97				

Table 2

370S tissue homogenate followed by ViewLux of analogues with

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Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21ak		25.29	21au		20.08

Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21al		12.67	21av		31.83

N370S AC₅₀ (μM)

100.67

R1

Compound number

21aw

N370S AC₅₀ (μM)

7.99

R1

Compound number

21am

Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21an		31.83	21ax		15.85

Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21ao		31.83	21ay		12.67

N370S AC₅₀ (μM)

25.29

R1

Compound number

N370S AC₅₀ (μM)

25.29

R1

Compound number

21ap

21az

Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21aq		20.08	12		6.35
21ar		25.29	21ba		12.67

Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
21as		31.83			
21at		40.07			

370S tissue homogenate followed by ViewLux of analogues with

Compound number	R1	Chemical structure	N370S AC ₅₀ (μM)	Compound number	R1	Chemical structure	N370S AC ₅₀ (μM)
9			1.27	26bl			2.53
26at			2.52	26bm			4.00

4)	Compound number	R1			N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)	N370S AC ₅₀ (μM)
	26au				2.52	26bn		5.04	
	26av				3.18	26bo		1.00	

4)	Compound number	R1			Compound number	R1	N370S AC ₅₀ (μM)	N370S AC ₅₀ (μM)	26bp	26bq	3.18	1.27
	26aw						1.26					
	26ax						0.63

4)	Compound number	R1			N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)	N370S AC ₅₀ (μM)
	26ay				7.99	13		1.59	
	26az				4.00	26br		1.01	
	26ba				2.53	26bs		0.50	

4)	Compound number	R1		N370S AC ₅₀ (μM)	Compound number	R1		N370S AC ₅₀ (μM)
	26bb			2.53	26bt			0.50
	26bc			2.53	26bu			1.59

4)	Compound number	R1		N370S AC ₅₀ (μM)	Compound number	R1		N370S AC ₅₀ (μM)
	26bd			5.04	26bv			1.26

4)	Compound number	R1	N370S AC ₅₀ (μM)	Compound number	R1	N370S AC ₅₀ (μM)
	26be		0.32	26bw		2.53

<p>4) </p>	<p>Compound number</p>	<p>R1</p>	<p>26bf</p>	<p>0.80</p>	<p>26bx</p>	<p>Compound number</p>	<p>R1</p>	<p>1.01</p>	<p>N370S AC₅₀ (μM)</p>

4)	Compound number	R1		N370S AC ₅₀ (μM)	Compound number	R1		N370S AC ₅₀ (μM)
	26bh			3.18	26bz			1.26
	26bi			4.01	26ca			1.01
	26bj			1.59				
	26bk			2.35				

Table 4

g the hydrolysis of 4-methylumbelliferone β -D-glucopyranoside in N370S tissue homogenate followed by ViewLux of analogues with at the linker.

R1	R2	N370S AC50 (μ M)	Compound number	N370S AC50 (μ M)
0.32	26cd	50.45
2.52	26cc	10.07

R1	R2	N370S AC50 (μM)	Compound number	R1	R2	N370S AC50 (μM)
2.52	29			2.00
50.45	30			3.18

R1	R2	N370S AC50 (μM)	Compound number	R1	R2	N370S AC50 (μM)
		1.00	28			79.98
		5.04	51			Inactive

R1	N370S AC50 (μ M)	Compound number	R1	R2	N370S AC50 (μ M)
<p>Chemical structure of a piperazine derivative. The piperazine ring is shown with a substituent R_1 at the 4-position and a tert-butyl ester group at the 2-position.</p>	50.45	38	<p>Chemical structure of a piperazine derivative. The piperazine ring is shown with a substituent R_1 at the 4-position and a 4-methylphenyl sulfonamide group at the 2-position.</p>	<p>Chemical structure of a thienyl group.</p>	6.35

R1	R2	N370S AC50 (μM)	Compound number	R1	R2	N370S AC50 (μM)
		79.98	35c			6.35
		15.96				

Table 5

mogenate followed by ViewLux of analogues with

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N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
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20.58

56j

Inactive

N370S AC₅₀ (μM)

Inactive

R2

Compound number

56k

N370S AC₅₀ (μM)

0.32

R1

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N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
3.18	63			2.53

N370S AC₅₀ (μM)

R2

Compound number

R1

N370S AC₅₀ (μM)

12.67

561

40.09

N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
12.67	21aa			12.67

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N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
79.97	56m			Inactive

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N370S AC₅₀ (μM)

R2

Compound number R1

4.00 56n

Inactive

N370S AC₅₀ (μM)

R2

Compound number

N370S AC₅₀ (μM)

25.29

560

Inactive

N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
1.27	59			15.95

N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
20.09	60			100.67

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N370S AC₅₀ (μM)

R2

Compound number R1

25.29

58

Inactive

N370S AC ₅₀ (μM)	Compound number	R1	R2	N370S AC ₅₀ (μM)
200.86	57			Inactive

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N370S AC₅₀ (μM)

R2

N370S AC₅₀ (μM) Compound number R1

40.09

61

Inactive

Molecular core	R1	R2	N370S AC50 (μM)
Inactive

Molecular core	R1	R2	N370S AC50 (μM)
r				Inactive

Inactive

Molecular core	R1	R2	N370S AC50 (μM)
a				Inactive
b				Inactive

Molecular core	N370S ACS0 (μM)	R2	R1
Inactive

Inactive

Table 7

HRMS found values for all final compounds.

Compound number	Formula	Calculated for Formula + H ⁺	HRMS found	Compound number	Formula	Calculated for Formula + H ⁺	HRMS found
2	C17H18N4S	311.1331	311.1325	26bf	C25H24N4O4S2	509.1318	509.1327
3	C18H20N4S	325.1487	325.1482	26bg	C22H19N5O4S2	482.0957	482.0957
4	C19H22N4S	339.1644	339.1637	26bh	C22H19N5O4S2	482.0957	482.0957
5	C18H18N4OS	339.12797	339.1275	26bi	C22H19N5O4S2	482.0957	482.0951
6	C22H19FN4S	391.13927	391.1388	26bj	C23H19N5O2S2	462.1059	462.1054
7	C22H19N4FS	391.1393	391.1388	26bk	C23H19N5O2S2	462.1059	462.1056
6	C22H20N4OS	389.1436	389.1431	26bl	C23H19N5O2S2	462.1059	462.1055
8	C23H22N4OS	403.1593	403.1586	26bm	C21H19N5O2S2	438.1059	438.1056
9	C22H20N4O2S2	437.1106	437.1107	26bn	C19H17N5O2S3	444.0623	444.0620
10	C22H19N4O2S2Cl	471.0716	471.0719	26bo	C20H18N4O2S3	443.0670	443.0665
11	C25H23N5O2S	458.1651	458.1645	26bp	C20H18N4O2S3	443.0670	443.0670
12	C25H23N5O4S	490.1549	490.1542	26bq	C23H22N4O2S2	451.1263	451.1262
13	C26H24N4O4S	489.1597	489.1592	26br	C26H26N4O2S2	491.1576	491.1579
14	C26H22N4O2S2	487.1263	487.1255	26bs	C24H22N4O4S2	495.1161	495.1157
21aa	C25H24N4O2S	445.1698	445.1701	26bt	C25H25N5O3S2	508.1477	508.1476
21ab	C28H30N4O2S	487.2168	487.2168	26bu	C27H28N4O3S2	521.1681	521.1682
21ac	C25H24N4O3S	461.1648	461.1642	25bv	C25H21N5O2S2	488.1215	488.1211
21ad	C26H26N4O3S	475.1804	475.1804	26bw	C26H21N4O2S2Cl	521.0873	521.0871
21ae	C25H21N4O3F3S	515.1365	515.1362	26bx	C26H22N4O2S2	487.1263	487.1259
21af	C28H30N4O4S	519.2066	519.2067	26by	C23H20N4O4S2	481.1005	481.1001
21ag	C24H21FN4O2S	449.1448	449.1450	26bz	C25H21N5O2S2	488.1215	488.1209
21ah	C24H21N4O2S2Cl	465.1152	465.1154	26ca	C23H19N5O2S3	494.0779	494.0777
21ai	C24H21N4O2S2Cl	465.1152	465.1151	26cb	C17H18N4O2S2	375.0950	375.0944
21aj	C24H21N4O2S2Cl	465.1152	465.1150	26cc	C19H22N4O2S2	403.1263	403.1262
21ak	C24H21N4O2S2Br	509.0647	511.0629	26cd	C17H15N4O2F3S2	429.0667	429.0671
21al	C24H21N4O2S2Br	509.0647	511.0628	28	C25H24N4O2S	445.1698	445.1690
21am	C25H21N4O2F3S	499.1416	499.1421	29	C23H20N4O4S	401.1436	401.1436

Compound number	Formula	Calculated for Formula + H ⁺	HRMS found	Compound number	Formula	Calculated for Formula + H ⁺	HRMS found
21an	C25H21N4O2F3S	499.1416	499.1420	30	C25H22N4O3S	459.1491	459.1487
21ao	C25H21N4O2F3S	499.1416	499.1422	33a	C21H26N4O2S	399.1855	399.1853
21ap	C27H26N4O4S	503.1753	503.1749	35a	C23H24N4O2S2	453.1419	453.1414
21aq	C26H24N4O3S	473.1648	473.1652	35b	C24H26N4O2S2	467.1576	467.1571
21ar	C24H23N5O4S2	510.1270	510.1271	35c	C24H24N4O2S2	465.1419	465.1420
21as	C25H21N5O2S	456.1494	456.1492	38	C24H23N3O2S2	450.1310	450.1308
21at	C25H21N5O2S	456.1494	456.1492	39	C19H21N3O2S	356.1433	356.1431
21au	C24H21N5O4S	476.1393	476.1385	51	C24H23N3O2S2	450.1310	450.1303
21av	C24H21N5O4S	476.1393	476.1395	56a	C24H24N4O2S2	465.1419	465.1416
21aw	C24H21N5O4S	476.1393	476.1395	56b	C22H21N5O2S2	452.1215	452.1204
21ax	C28H24N4O2S	481.1698	481.1698	56c	C22H21N5O2S2	452.1215	452.1211
21ay	C28H28N4O2S	485.2011	485.2019	56d	C22H21N5O3S	436.1444	436.1433
21az	C27H26N4O2S	471.1855	471.1856	56e	C23H22N4O3S	435.1491	435.1491
21ba	C26H24N4O2S	457.1698	457.1695	56f	C24H23N5O2S	446.1651	446.1642
21bb	C26H30N6O4S2	555.1848	555.1848	56g	C24H23N5O2S	446.1651	446.1639
21bc	C20H20N6O2S2	441.1168	441.1167	56h	C23H22N6O2S	447.1603	447.1585
24	C21H24N4O2S	397.1698	397.1694	56i	C23H22N6O2S	447.1603	447.1596
26aa	C22H20N4O2S2	437.1106	437.1103	56j	C23H22N6O2S	447.1603	447.1597
26ab	C23H22N4O2S2	451.1263	451.1258	56k	C23H22N6O2S	447.1603	447.1589
26ac	C23H22N4O2S2	451.1263	451.1255	56l	C26H26N4O3S	475.1804	475.1798
26ad	C23H22N4O2S2	451.1263	451.1261	56m	C24H20N4O2FSBr	527.0553	529.0529
26ae	C24H24N4O2S2	465.1419	465.1418	56n	C24H20N4O2SClBr	543.0257	545.0226
26af	C24H24N4O2S2	465.1419	465.1415	56o	C24H20N4O2SCl2	499.0763	499.0757
26ag	C26H28N4O2S2	493.1732	493.1736	57	C20H19N5O2S	394.1338	394.1322
26ah	C26H28N4O2S2	493.1732	493.1729	59	C20H21N5O3S	412.1444	412.1424
26ai	C23H22N4O3S2	467.1212	467.1209	60	C21H22N4O4S	427.1440	427.1432
26aj	C23H22N4O3S2	467.1212	467.1209	61	C21H22N4O3S	411.1491	411.1484
26ak	C23H22N4O3S2	467.1212	467.1208	62	C21H22N4O2S	395.1542	395.1537
26al	C23H19N4O3F3S2	521.0929	521.0935	63	C19H20N4O2S	369.1385	369.1384

Compound number	Formula	Calculated for Formula + H ⁺	HRMS found	Compound number	Formula	Calculated for Formula + H ⁺	HRMS found
26am	C24H24N4O4S2	497.1318	497.1320	68	C26H24N6O2S	485.1760	485.1758
26an	C22H19FN4O2S2	455.1012	455.1010	69	C28H31N5O3S2	550.1947	550.1946
26ao	C22H19N4O2FS2	455.1012	455.1012	70	C28H32N6O2S2	549.2106	549.2101
26ap	C22H19N4O2FS2	455.1012	455.1015	71	C24H23N3O2S2	450.1310	450.1311
26aq	C22H18N4O2F2S2	473.0918	473.0914	72	C24H23N3O2S2	450.1310	450.1312
26ar	C22H19N4O2S2Cl	471.0716	471.0712	73	C22H21N5O2S2	452.1215	452.1217
26as	C22H19N4O2S2Cl	471.0716	471.0715	74	C22H21N5O2S2	452.1215	452.1219
26at	C22H18N4O2S2Cl2	505.0327	505.0329	75	C19H20N4O2S2	401.1106	401.1110
26au	C22H19N4O2S2Br	515.0211	517.0190	76	C18H18N4O2S2	387.0950	387.0944
26av	C22H19N4O2S2Br	515.0211	517.0186	77	C21H24N4O2S2	429.1419	429.1423
26aw	C22H19N4O2S2Br	515.0211	517.0191	78	C22H18N4O2FS2Cl	489.0622	489.0619
26ax	C23H19N4O2F3S2	505.0980	505.0975	79	C23H18N4O2F3S2C	539.0590	539.0586
26ay	C23H19N4O2F3S2	505.0980	505.0976	80	C24H20N4O2FSCl	483.1058	483.1054
26az	C23H19N4O2F3S2	505.0980	505.0981	81	C25H22N4O2FSCl	497.1214	497.1213
26ba	C24H22N4O3S2	479.1212	479.1199	82	C24H19N4O2FSCl2	517.0668	517.0667
26bb	C24H22N4O3S2	479.1212	479.1213	83	C24H19N4O2F2SCl	501.0964	501.0962
26bc	C24H22N4O3S2	479.1212	479.1219	84	C25H22ClFN4O2S	497.1214	497.1219
27bd	C22H21N5O4S3	516.0834	516.0830	85	C24H20N4O2FSCl	483.1058	483.1055
26de	C25H24N4O4S2	509.1318	509.1320	86	C20H18N4O3S	395.1178	395.1173

Table 8

ΔT_m tabulated vs. compound concentration. Empty cells indicate undetectable melt transition.

Concentration (μ M)	26ad	14	26bs	26ai	62	DNJ 87	Isogomine 1
600	*		24.3	*			
500	*		23.4	7.69			8.75
400	*	19.94	22.81	8.09			9.56
300		19.82	22.8	7.48			8.98
200	11.02	17.31	20.73	6.86	-0.3		8.25
100	9.89	14.51	19.27	3.97	0.42		7.94
50	5.18	10.8	13.35	-0.76	0.43	2.58	6.72
25	0.84	4.35	11.67	-0.01	0.29	1.79	5.5
10	-1.4	1.6	5.39		0.88	0.84	
1	-0.09	-0.19	0.24	0.55	-0.26	-0.2	1.16
0.1	0.03	-1.33	0.37	0.6	0.5	0.72	0.4
0	0.28	-1.27	0.07	0.59	0.5	0.09	-0.12

* indicates that compound was observed to have precipitated out of solution.