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Evaluation of Quinazoline Analogues as Glucocerebrosidase Inhibitors with Chaperone Activity

Juan J. Marugan,*[,†] Wei Zheng,[†] Omid Motabar,[†,‡] Noel Southall,[†] Ehud Goldin,[‡] Wendy Westbroek,[‡] Barbara K. Stubblefield,[‡] Ellen Sidransky,[‡] Ronald A. Aungst,[§] Wendy A. Lea,[†] Anton Simeonov,[†] William Leister,[†] and Christopher P. Austin[†]

[†]*NIH Chemical Genomic Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland, United States,* [‡]*Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, United States, and* [§]*Albany Molecular Research Institute, 30 Corporate Circle, Albany, New York 12203, United States*

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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[a]) is to hydrolyze β-glycosidic linkages of glucocerebrosides, also called glucosylceramides, in the lysosome.[2] 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 adherence.[3]

There are more than 200 recognized mutations in the glucocerebrosidase gene.[4] 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 improvement.[6]

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 lysosome.[7,8] Several iminosugar inhibitors of glycosidases have been reported to have chaperone activity.[9−20] 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 selective.[13] Alternative scaffolds with chaperone activity are quite desirable. In addition, it is important that the compound inhibitory potency is not the primary determinent 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 noniminosugar 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 C.[25] In isolation, the addition of a bile salt is required to induce GC activity.[26] 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 wild-type and mutant enzymes. As 70% of Gaucher patients carry the N370S mutation, we focused our efforts on the use of spleen[27] homogenate

*To whom correspondence should be addressed. Phone: 301-217-9198. Fax: 301-217-5736. E-mail: maruganj@mail.nih.gov.

[a] Abbreviations: 4MU, 4-methylumbellifereone; Boc, *tert*-butyloxycarbonyl; CNS, central nervous system; DMSO, dimethylsulfoxide; DNJ, deoxynojirimycin; ER, endoplasmic reticulum; ERT, enzymatic replacement therapy; FDA, Food and Drug Adminstration; GC, glucocerebrosidase; HPLC, high performance liquid chromatography; HTS, high throughput screening; IC_{50}, half maximal inhibitory concentration; LC-MS, liquid chromatography−mass spectrometry; LSD, lysosomal storage disorder; SRA, structure−activity relationship.

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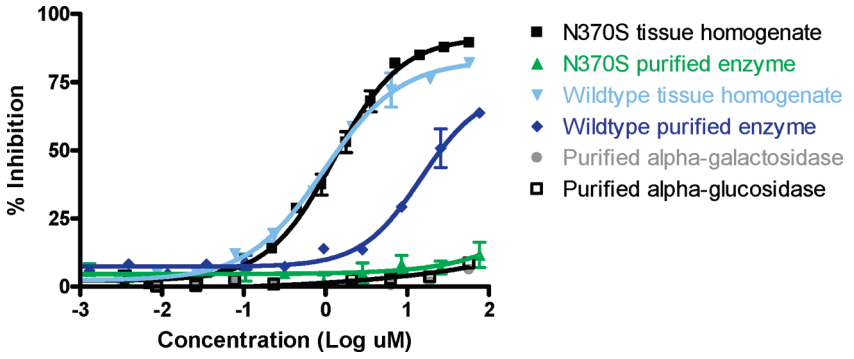
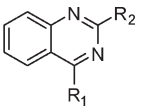
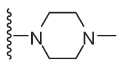
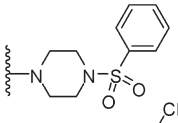
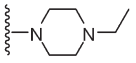
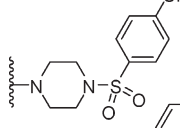
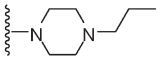
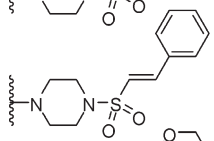
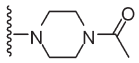
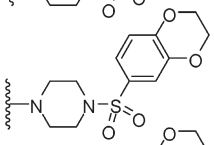
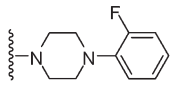
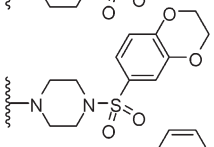
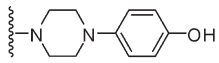
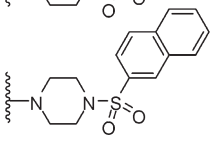
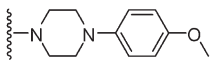


Figure 1. Evaluation of the hydrolytic capacity of GC in the presence of compound **10**. Activity of compound **10** was representative of all the analogues in this series; they are selective inhibitors of GC, but their activity in purified GC assays without exogenous cofactors is greatly diminished. The inhibitory capacity was analyzed by mesuaring the fluorescent signal of 4-methylumbelliferone using ViewLux.

Table 1. SAR of Commercial Quinazolines for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux



Compound number	R1	R2	N370S AC_{50} (μM)	Compound number	R1	R2	N370S AC_{50} (μM)
2			Inactive	9			1.27
3			Inactive	10			1.27
4			Inactive	11			25.29
5			79.97	12			25.29
6			63.52	13			6.35
7			63.52	14			1.59
8			79.97				

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 326770 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 μM

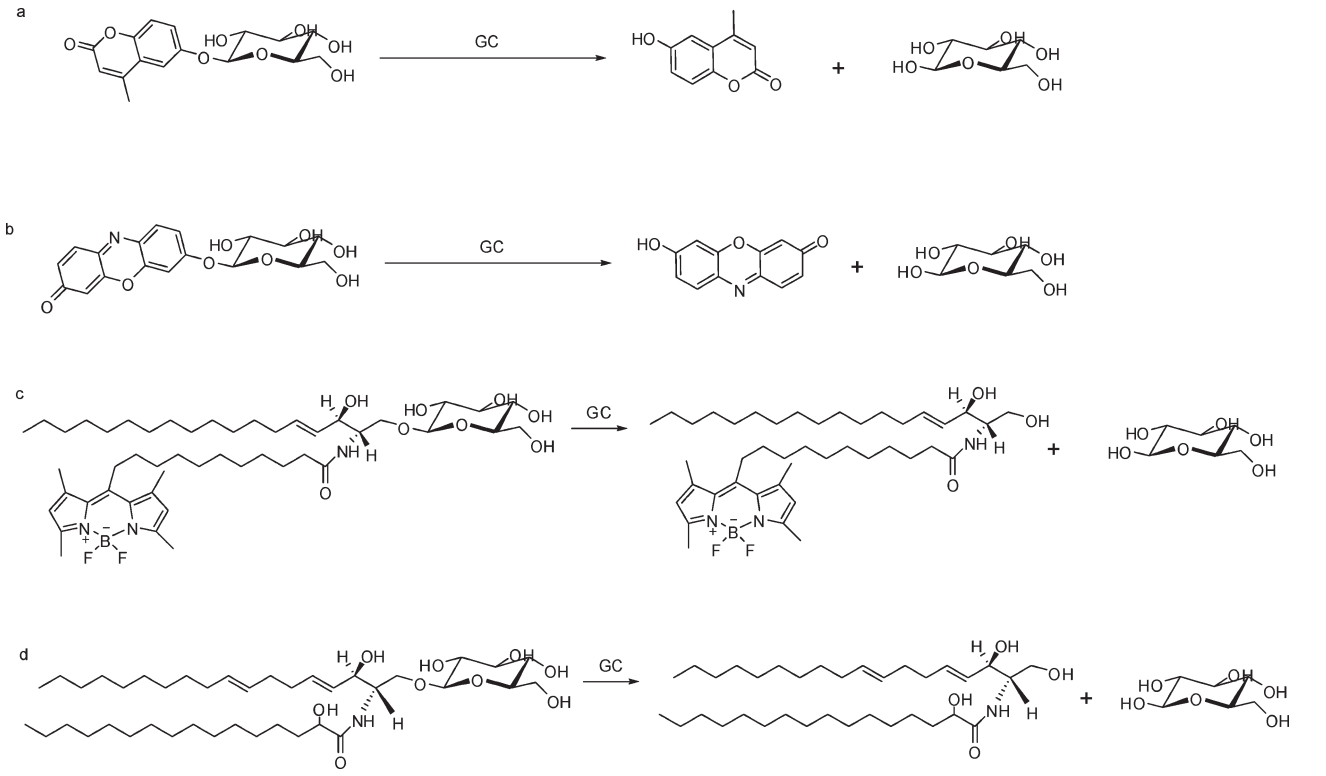


Figure 2. Principles of enzyme reactions and product spectra of two GC enzyme assays. (a) The "Blue" GC enzyme assay. The pro-fluorescent substrate 4MU-β-Glc is hydrolyzed to form two products, glucose and 4MU, with an excitation peak of 365 nm and an emission peak of 440 nm. This assay is used for the 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 assay[38] for final detection.

concentration, and none exhibited autofluorescence. 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, wild-type and mutant fibroblasts were incubated for five days with compound **14** at a range of concentrations from 1 nM to 50 μM, 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 use 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 core.[31] Commercially available Boc protected piperazine **15** was sulphonated, using

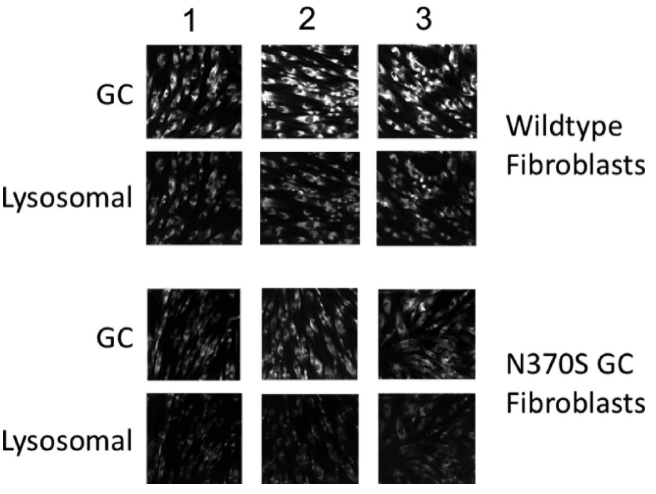
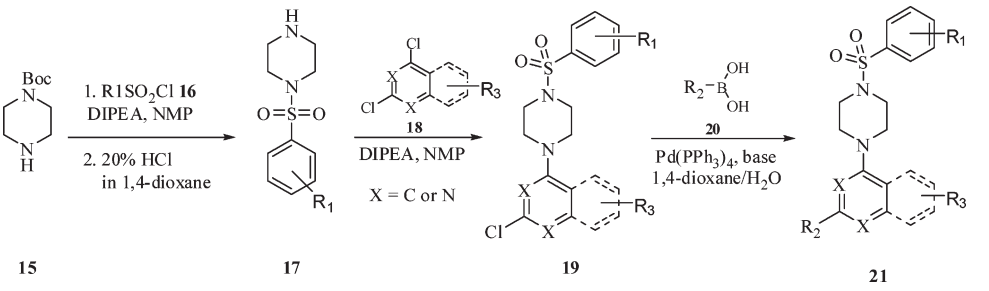


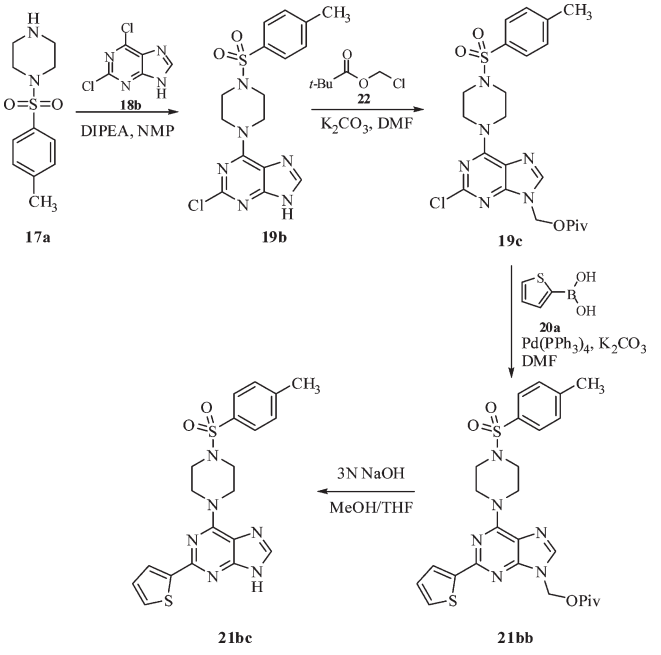
Figure 3. Chaperone activity of compound **14**, and isofagomine using wild-type and homozygous, mutant N370S GC fibroblasts. Two genotypes of fibroblasts, fibroblasts homozygous for wild-type GC (top) and fibroblasts homozygous for N370S GC (bottom) were stained both with a Cy3-labeled antibody for 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 Isofagomine, (3) 10 μM compound **14**. Both compounds show increased lysosomal GC protein after treatment.

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

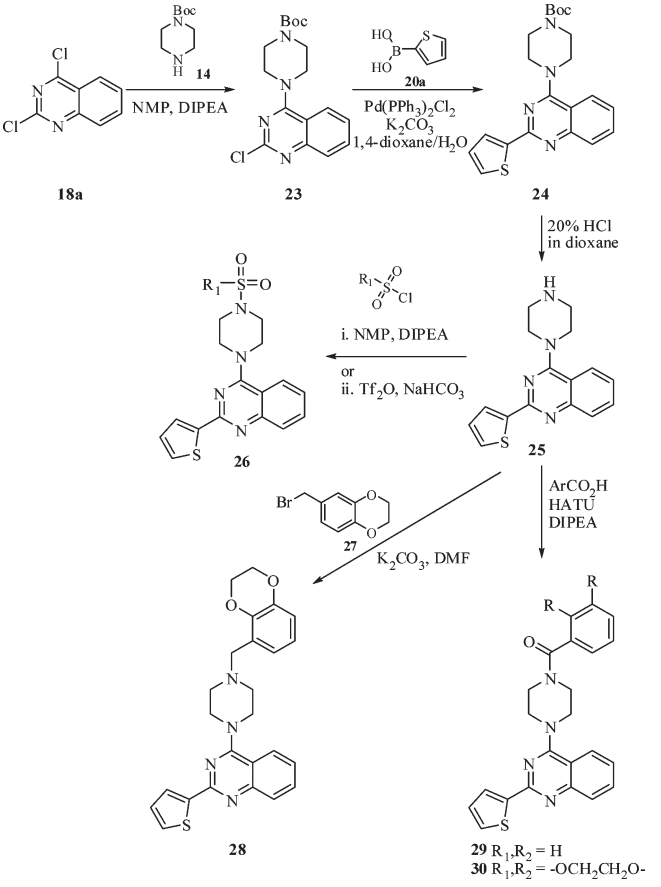
Scheme 1. General Strategy for Modification in the Functional Core



Scheme 2. Synthesis of Purine Analogues



Scheme 3. Synthesis of Analogues with Modification of the Aromatic Sulphonamide



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.

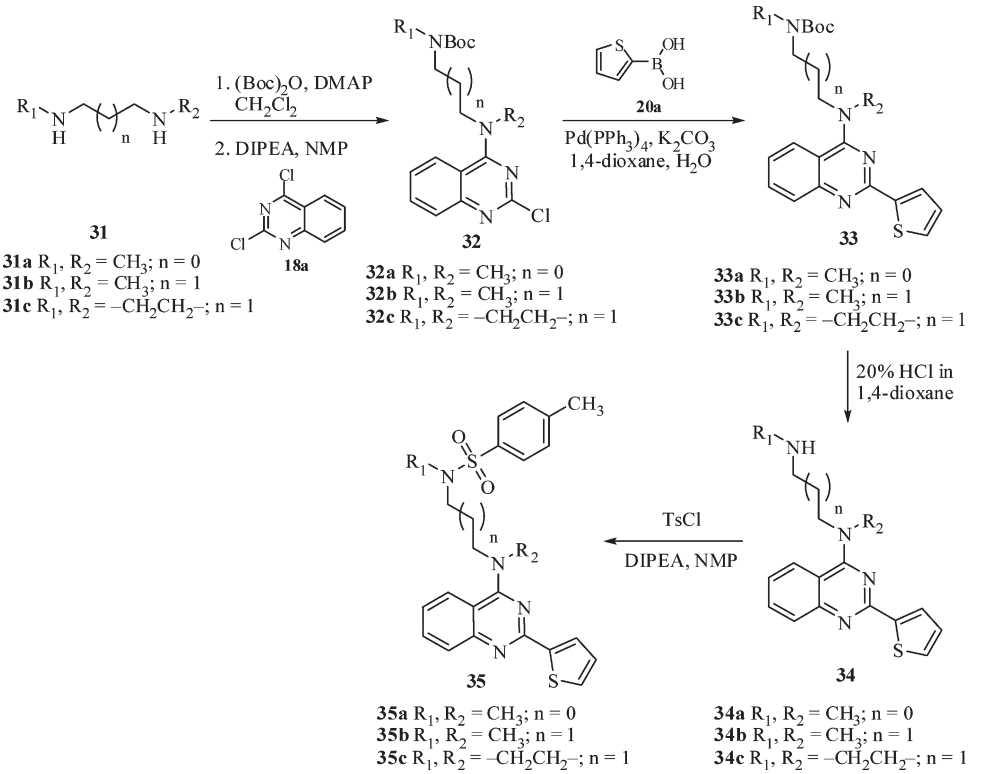
We also synthesized a number of analogues, testing the flexibility of the diamine linker as well as the activity of some piperazine bioisosteres attached at position four of the quinazoline ring. Schemes 4, 5, and 6 show some of these alternative strategies.

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 follow by reaction with tributyl tin chloride yielded compound **55**. Cross coupling between **55** and heteroaromatic halogens yielded final compounds **56**.

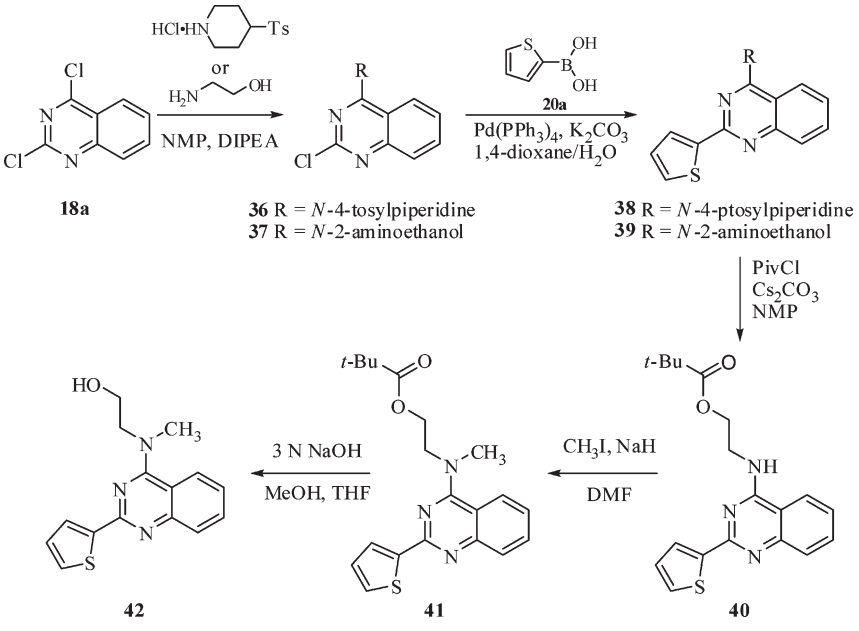
Additional modifications at the same position 2 were obtained as shown in Scheme 8. Cross-coupling with zinc cyanide catalyzed by palladium yielded intermediate **57**. Then, hydrolysis of the nitrile functional group with aqueous HCl produced the amide **58**. Alternatively, hydrolysis with HCl gas in the presence of MeOH yielded the methyl ester **59**. Additionally, reaction with methylmagnesium bromide provided the ketone **60**. Last, Scheme 9 disclosed modifications in position 2 trying to increase the solubility of the molecule.

Tables 2−6 show the capacity of all final compounds to inhibit the hydrolysis of 4-methylumbelliferone β-D-glucopyranoside

Scheme 4. Synthesis of Analogues with a Flexible Linker



Scheme 5. Synthesis of Additional Analogues with a Modifications in Position 4



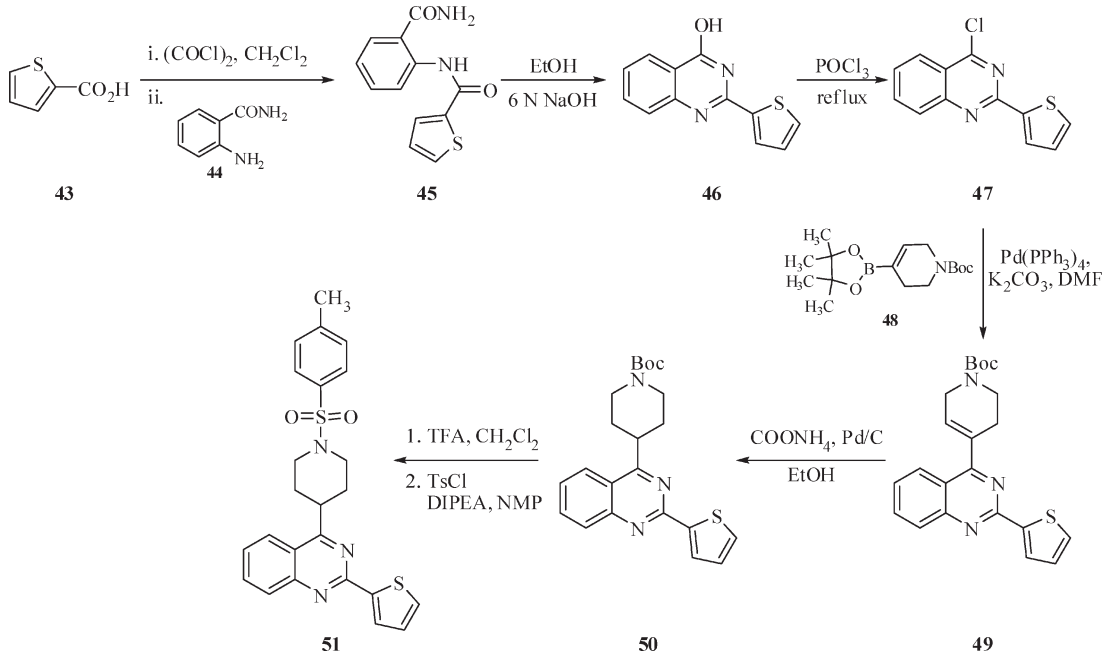
in N370S tissue homogenate. In the context of our assay, AC_{50} corresponds with half maximal activity concentration, either inhibitory or activating. As a positive control, we measured the activity of isofagomine in the same assay (AC_{50} = 0.080 μM).

Figure 4 compares the compound profiles of isofagomine and one of the more potent analogues against the hydrolysis of 4-methylumbelliferone β-D-glucopyranoside and a fluorescent glucosylceramide by wild-type and mutant N370S GC using tissue homogenate and isolated enzyme.

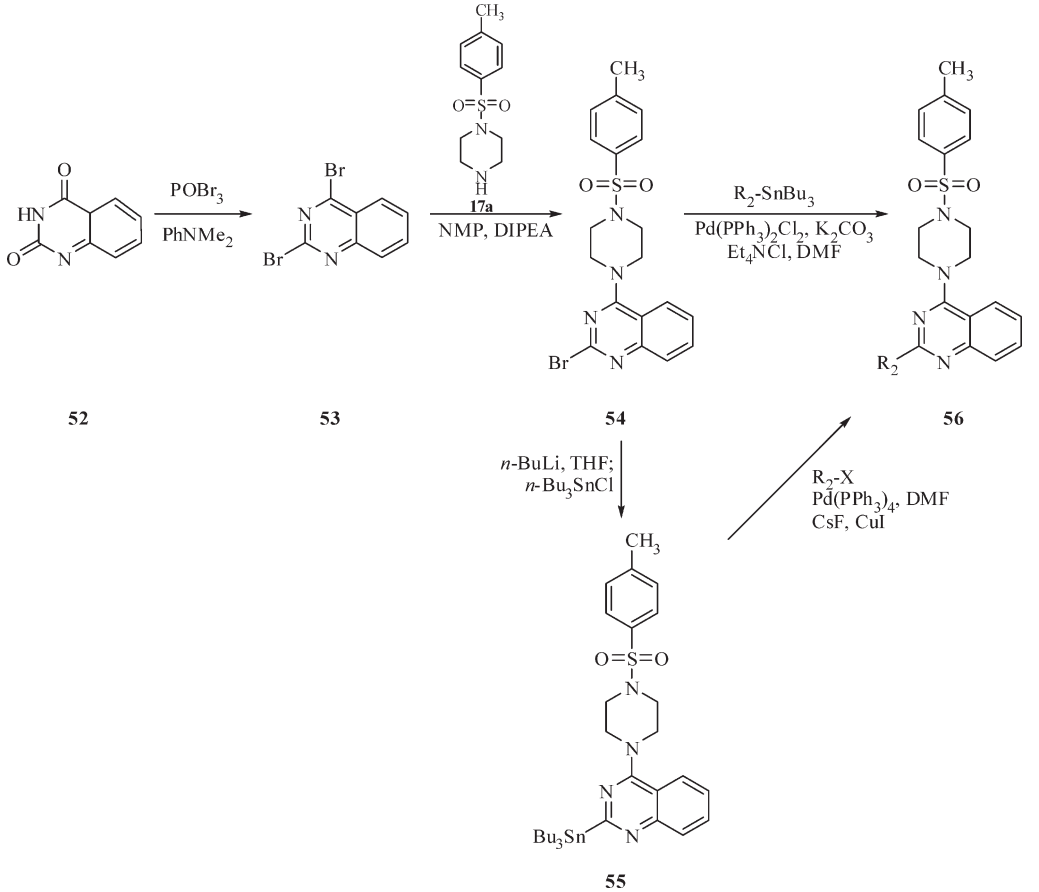
We also measured the capacity of several compounds from this series to protect the GC protein from thermal denaturation by using the fluorescence thermal shift techniques, also referred to as differential scanning fluorometry. Figure 5 shows that several compounds in the series have a strong ability 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.

Scheme 6. Synthesis of Analogues Having a Substituted Piperidine in Position 4



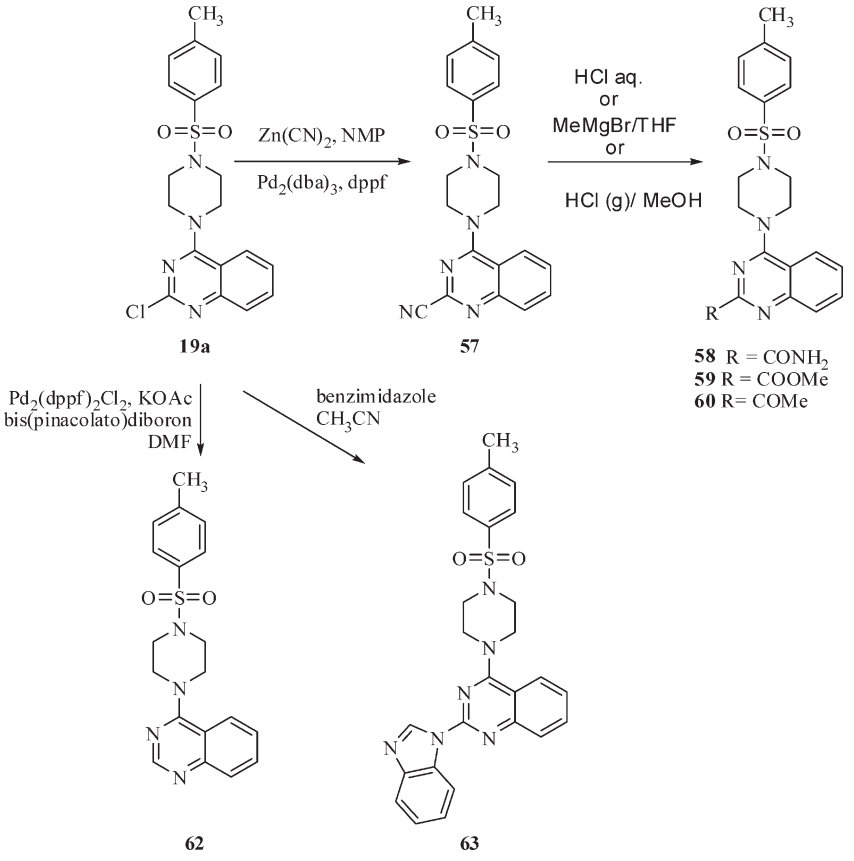
Scheme 7. Synthesis of Analogues with Modifications at 2 Position



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

Scheme 8. Additional Modifications at the 2 Position



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 buffer.[32] 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.

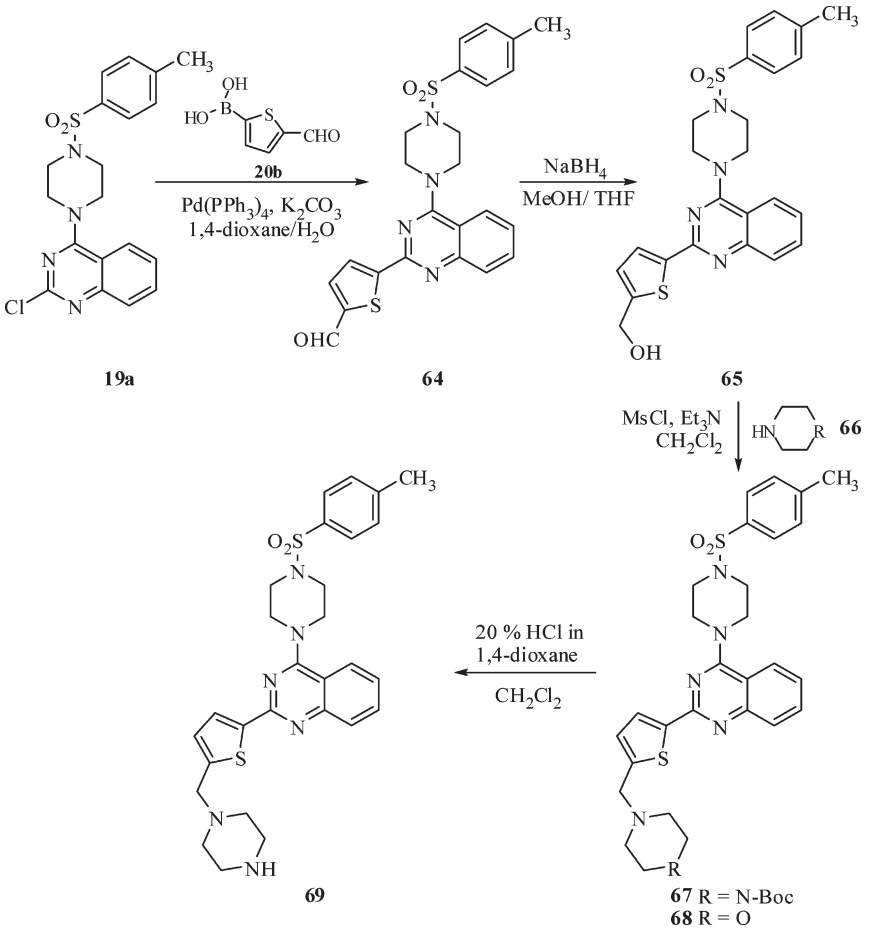
The capacity of a molecule to inhibit a hydrolytic enzyme does not directly correlate with its capacity to induce proper folding and chaperone activity. For this reason, before the initiation of our SAR studies, we decided to evaluate the chaperone capacity of our series. The increase in GC translocation 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 (wild-type or mutant) but also on the nature of the enzymatic substrate. We have previously observed[33] 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. In analyzing derivatives with a single substitution, it can be observed that substituents in para, and especially in ortho, positions tend to provide better activity, with the para methyl analogue being our most potent compound (**26ad**) with an IC_{50} 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**,

Scheme 9. Modification at the Thiophene Ring



26cc, **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.

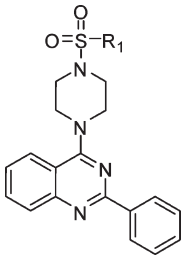
The next modifications studied entailed the replacement of the aromatic substituent at the two position of the quinazoline ring. Table 5 shows that the complete elimination of the substitution (compound **62**) reduces the activity by more than 60 times and replacing it with nonaromatic functional groups, 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-membered 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-membered 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-benzoimidazole substitution (compound **63**) also provides better activity than a plain phenyl ring (compound **21aa**).

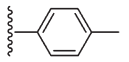
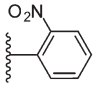
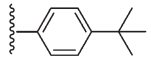
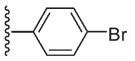
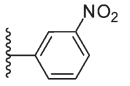
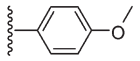
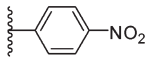
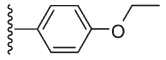
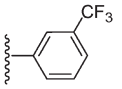
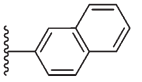
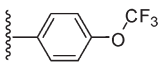
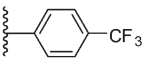
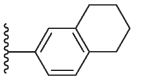
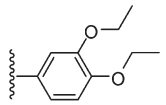
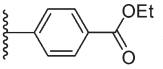
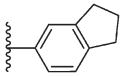
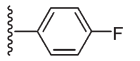
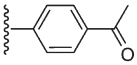
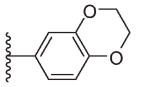
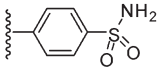
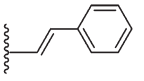
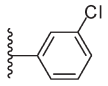
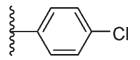
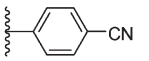
The last modifications studied were analogues of the quinazoline core. Replacement of the quinazoline core by a purine results in a loss of activity (Table 6, compounds **21bb** and **21bc**). Elimination of the quinazoline nitrogens also impacts the activity, reducing (compounds **71**) or completely 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 by only 10 times if the pyrimidine was bearing methyl groups in positions 5 and 6 (compound **76**). Last, introduction of a chloro substituent in position 7 of the quinazoline ring completely abolished the activity (compounds **77**−**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 us the quinazoline core, the piperidine ring, the sulphonamide substitution, and the thiophene hetearomatic ring at 2 position 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 noniminosugar 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 fluorophore.[34] 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

Table 2. SAR for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux of Analogues with Sulfonamide Aromatic Modifications Having a Phenyl at 2 Position



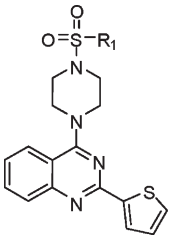
Compound number	R1	N370S AC_{50} (μM)	Compound number	R1	N370S AC_{50} (μM)	Compound number	R1	N370S AC_{50} (μM)
21aa		12.67	**21ak**		25.29	**21au**		20.08
21ab		31.83	**21al**		12.67	**21av**		31.83
21ac		12.67	**21am**		7.99	**21aw**		100.67
21ad		12.67	**21an**		31.83	**21ax**		15.85
21ae		31.83	**21ao**		31.83	**21ay**		12.67
21af		15.95	**21ap**		25.29	**21az**		25.29
21ag		20.08	**21aq**		20.08	**12**		6.35
21ah		50.45	**21ar**		25.29	**21ba**		12.67
21ai		15.95	**21as**		31.83			
21aj		12.67	**21at**		40.07			

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 term 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 authors[35] 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 affinity[36] 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.

Table 3. SAR for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux of Analogues with Sulfonamide Aromatic Modifications Having a Thiophene at 2 Position



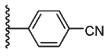
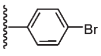
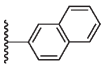
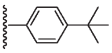
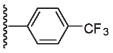
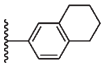
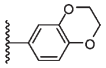
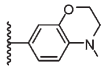
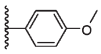
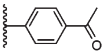
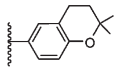
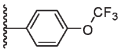
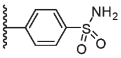
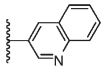
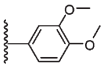
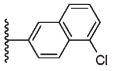
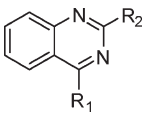
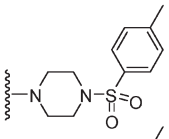
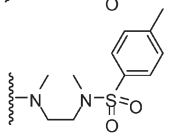
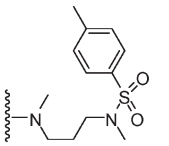
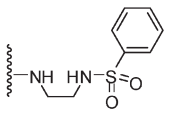
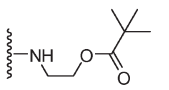
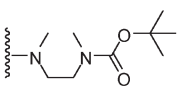
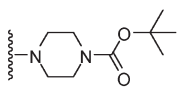
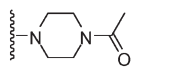
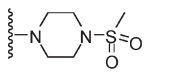
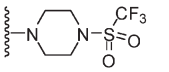
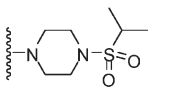
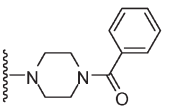
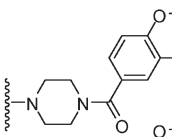
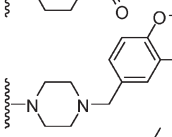
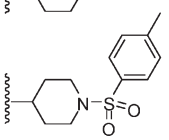
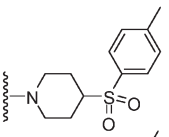
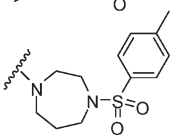
Compound number	R1	N370S AC_{50} (μM)	Compound number	R1	N370S AC_{50} (μM)	Compound number	R1	N370S AC_{50} (μM)
26aa		1.26	**9**		1.27	**26bl**		2.53
26ab		3.18	**26at**		2.52	**26bm**		4.00
26ac		2.52	**26au**		2.52	**26bn**		5.04
26ad		0.32	**26av**		3.18	**26bo**		1.00
26ae		0.80	**26aw**		1.26	**26bp**		1.27
26af		1.59	**26ax**		0.63	**26bq**		3.18
26ag		2.00	**26ay**		7.99	**13**		1.59
26ah		2.00	**26az**		4.00	**26br**		1.01
26ai		0.40	**26ba**		2.53	**26bs**		0.50
26aj		1.00	**26bb**		2.53	**26bt**		0.50
26ak		1.00	**26bc**		2.53	**26bu**		1.59
26al		3.18	**26bd**		5.04	**26bv**		1.26
26am		2.00	**26be**		0.32	**26bw**		2.53
26an		1.27	**26bf**		0.80	**26bx**		1.01
26ao		1.27	**26bg**		1.27	**26by**		0.50
26ap		1.00	**26bh**		3.18	**26bz**		1.26
26aq		1.59	**26bi**		4.01	**26ca**		1.01
26ar		1.27	**26bj**		1.59			
26as		2.01	**26bk**		2.35			

Table 4. SAR for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux of Analogues with the Modifications at the Linker



Compound number	R1	R2	N370S AC50 (μM)
26ad			0.32
35a			2.52
35b			2.52
85			50.45
39			1.00
33a			5.04
23			50.45
4			79.98
26cb			15.96
26cd			50.45
26cc			10.07
29			2.00
30			3.18
28			79.98
51			Inactive
38			6.35
35c			6.35

To demonstrate that this binding affinity translates into chaperone capacity, we evaluated the ability of our best compounds to increase the translocation of GC. Wild-type 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 μM, 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.

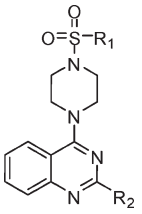
Conclusion

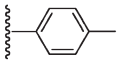
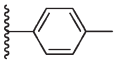
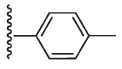
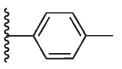
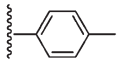
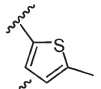
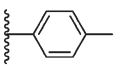
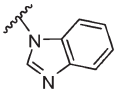
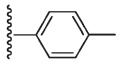
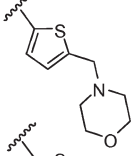
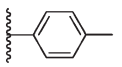
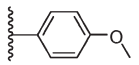
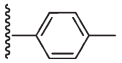
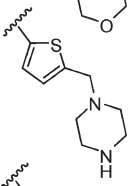
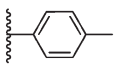
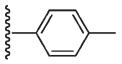
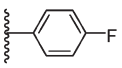
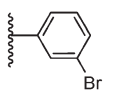
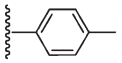
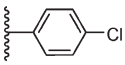
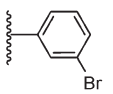
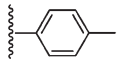
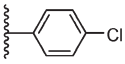
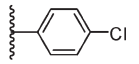
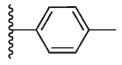
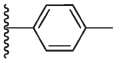
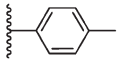
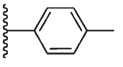
In conclusion, we present the discovery, SAR study, and biological evaluation of a new non-iminosugar GC inhibitory series with chaperone activity. SAR optimization yielded compounds at an IC_{50} of 320 nM in our homogenate tissue assay. The compounds have a reduced capacity to inhibit the hydrolysis 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. Last, these compounds increased the ER-lysosomal trafficking of several GC mutants. Additional metabolic optimization studies, pharmacokinetics, and in vivo evaluation are currently underway to advance the development of this series as a potential therapeutic modality.

5. Experimental Section

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

Table 5. SAR for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux of Analogues with Modification at the 2 Position of the Quinazoline Core

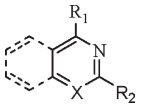


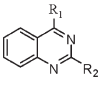
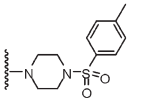
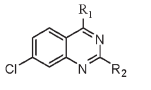
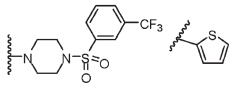
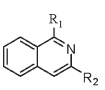
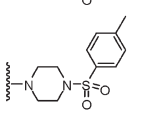
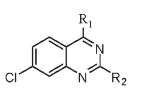
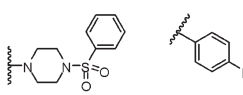
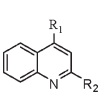
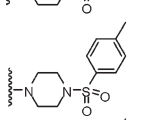
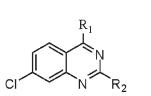
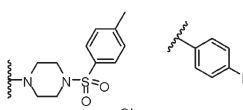
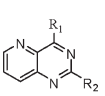
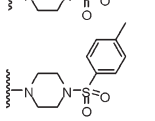
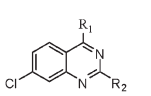
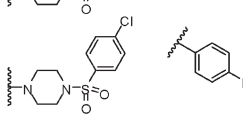
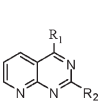
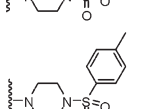
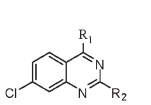
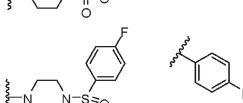
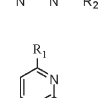
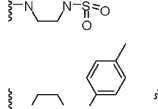
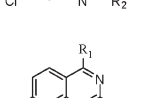
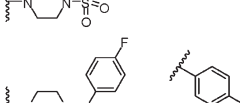
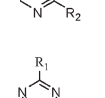
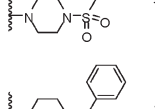
Compound number	R1	R2	N370S AC_{50} (μM)	Compound number	R1	R2	N370S AC_{50} (μM)
62		H	20.58	**56j**			Inactive
26ad			0.32	**56k**			Inactive
56a			3.18	**63**			2.53
68			12.67	**56l**			40.09
69			12.67	**21aa**			12.67
56b			79.97	**56m**			Inactive
56c			4.00	**56n**			Inactive
56d			25.29	**56o**			Inactive
56e			1.27	**59**			15.95
56f			20.09	**60**			100.67
56g			25.29	**58**			Inactive
56h			200.86	**57**			Inactive
56i			Inactive	**61**			40.09

Compounds **2**−**8**, **10**−**14**, **21aa**−**21az**, **21ba**, **26aa**, **26al**, **26am**, **26au**−**26az**, **26bc**, **26bd**, **26bf**−**26bi**, **25bl**, and **26br** were purchased from Enamine. Compounds **56e**, **56l**−**56o**, **61**, **75**, and **77**−**85** were purchased from AMRI. Besides the certificate of analysis provided by those companies, we performed quality control analysis using a LC-MS system. All of them showed purity greater than 95%.

Column chromatography was carried out over silica gel (100−200 mesh). ^{1}H NMR spectra were recorded with a Bruker

Table 6. SAR for Inhibiting the Hydrolysis of 4-Methylumbelliferone β-D-Glucopyranoside in N370S Tissue Homogenate Followed by ViewLux of Analogues with Modifications at the Molecular Core



Compound number	Molecular core	R1	R2	N370S AC50 (μM)	Compound number	Molecular core	R1	R2	N370S AC50 (μM)
26ad				0.32	78				Inactive
70				Inactive	79				Inactive
71				20.08	80				Inactive
72				4.00	81				Inactive
73				3.18	82				Inactive
74				10.31	83				Inactive
75				Inactive	84				Inactive
76				3.18	21bc				Inactive
77				Inactive	21bb				Inactive

400 MHz spectrometer from solutions in $CDCl_3$ and DMSO-d_6. Chemical shifts in ^{1}H NMR spectra are reported in parts per million (ppm, δ) downfield from the internal standard Me_4Si (TMS, $\delta = 0$ ppm). Molecular weight confirmation was performed using an Agilent time-of-flight mass spectrometer (TOF, Agilent Technologies, Santa Clara, CA). A 3 min gradient from 4 to 100% acetonitrile (0.1% formic acid) in water (0.1% formic acid) was used with a 4 min run time at a flow rate of 1 mL/min. A Zorbax SB-C18 column (3.5 μm, 2.1 mm × 30 mm) was used at a temperature of 50 °C. Confirmation of molecular formula (Table 7) was done 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 × 50 mL). The combined organic layer was washed with water (3 × 50 mL) and brine (3 × 50 mL), dried over Na_2SO_4, 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{16}H_{24}N_2O_4S + H]^+$.

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 CH_2Cl_2 (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 CH_2Cl_2 (2 × 20 mL), cooled to 0 °C, and basified to pH 12 with a 6 N NaOH solution. The resulting aqueous solution was extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic layers were dried over Na_2SO_4 and then filtered, and the filtrate was concentrated under reduced pressure to afford amine **17a** (11.0 g, 84%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{11}H_{16}N_2O_2S + H]^+$.

General Procedure for the Synthesis of Core 19: Displacement of Halide on the Heterocyclic 18. *N*,*N*-Diisopropylethylamine

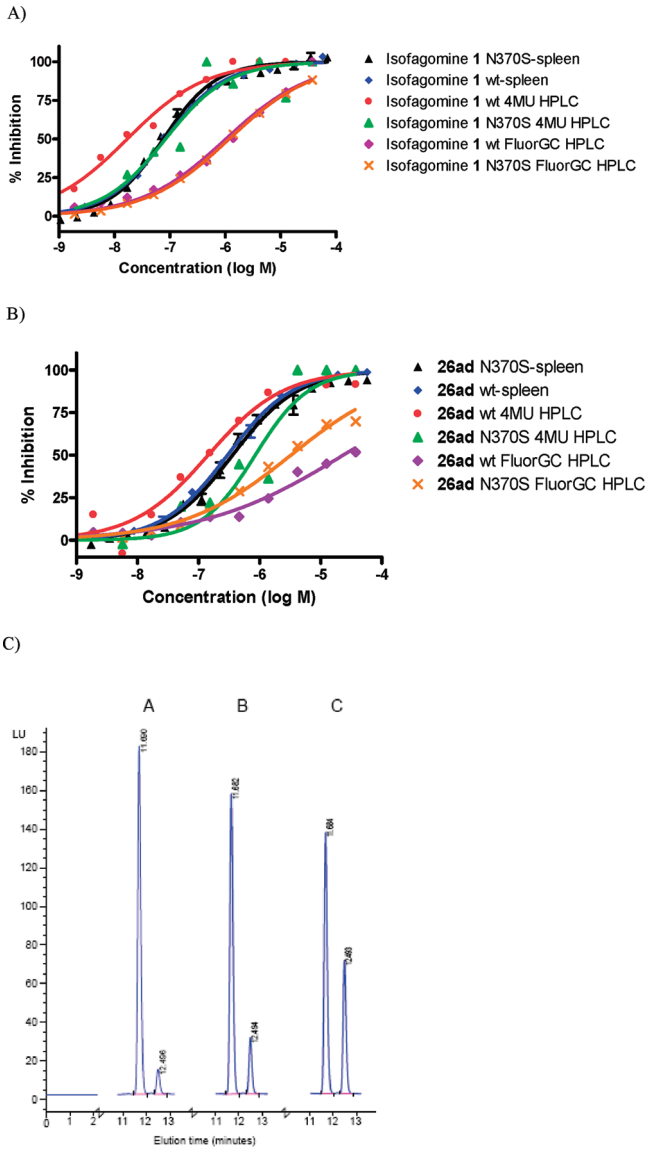


Figure 4. ViewLux and LC-MS analysis of the capacity of some our best compounds to inhibit of the hydrolysis of 4-methylumbelliferone β-D-glucopyranoside (4MU) and a fluorescent glucosylceramide (FlourGC) carried out by wt GC or by N370S mutant variant using tissue homogenate. ViewLux experiments were 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 μM of **26ad** (B) or 12.5 μM of isofagomine **1** (C). The first eluding peak corresponds with the starting material (Figure 2C) and the second one with the product of the reaction.

(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 Na_2SO_4, 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). Yield 63%. ^{1}H 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

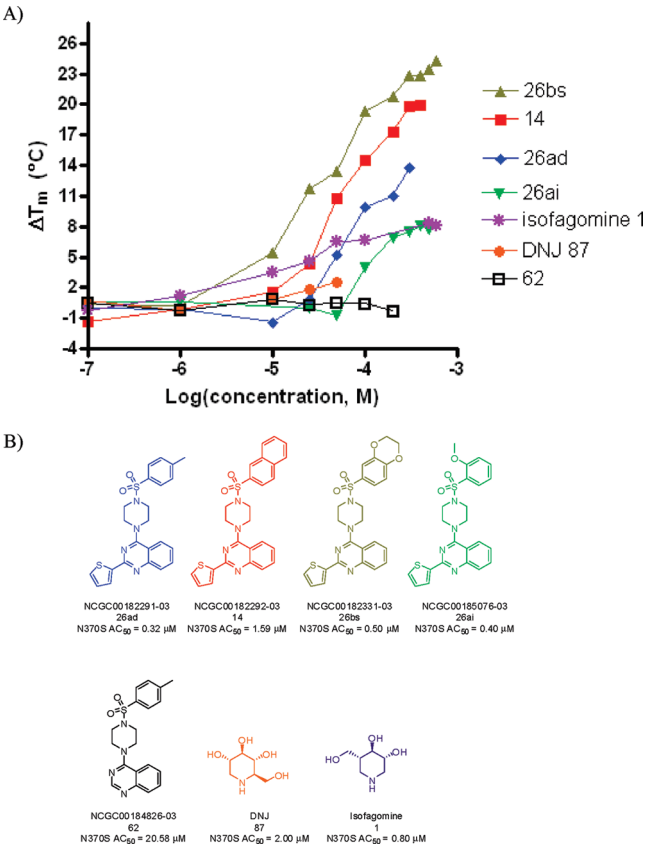


Figure 5. Capacity of select compounds to protect wild-type glucocerebrosidase from thermal denaturation (increase in T_m relative to a protein-only control shown). (A) Thermal melt curves of selected compounds. (B) Structure of analyzed inhibitors.

(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 $[C_{19}H_{19}ClN_4O_2S]^+$.

2-Chloro-4-(4-tosylpiperazin-1-yl)pyrido[2,3-*d*]pyrimidine (19d). Yield 82%. ^{1}H 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 $[C_{20}H_{20}ClN_3O_2S]^+$.

2-Chloro-4-(4-tosylpiperazin-1-yl)pyrido[3,2-*d*]pyrimidine (19e). Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{18}H_{18}ClN_5O_2S]^+$.

2-Chloro-4-(4-tosylpiperazin-1-yl)pyrimidine (19f). Yield 58%. ^{1}H 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 $[C_{15}H_{17}ClN_4O_2S]^+$.

2-Chloro-4,5-dimethyl-6-(4-tosylpiperazin-1-yl)pyrimidine (19g). Yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{17}H_{21}ClN_4O_2S]^+$.

3-Chloro-1-(4-tosylpiperazin-1-yl) isoquinoline (19h). Yield 41% yield ; MS (ESI) m/z 402 $[C_{20}H_{20}ClN_3O_2S + H]^+$.

2-(Thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)quinoline (71). Prepared from 4-chloro-2-(thiophen-2-yl)quinoline[29] according to the same general procedure for synthesis of compounds **18**; 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{24}H_{23}N_3O_2S_2 + H]^+$.

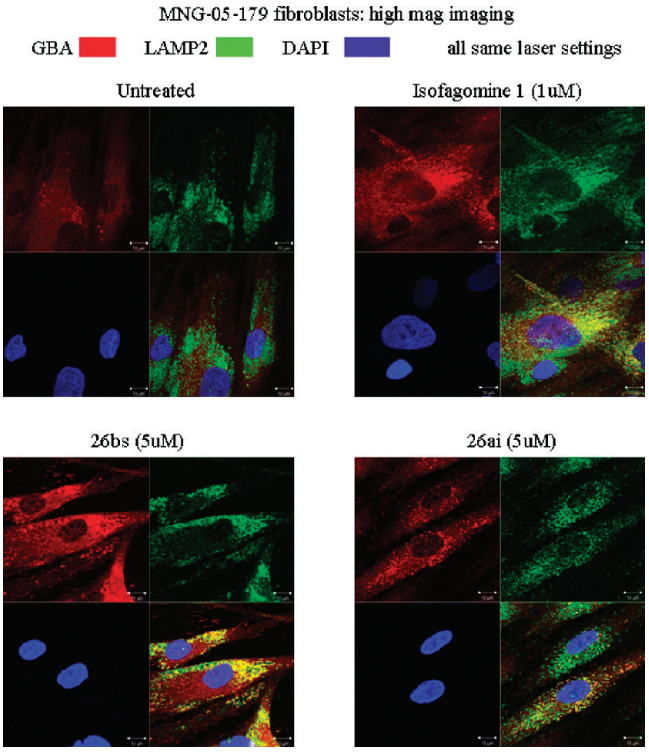


Figure 6. Chaperone activity of some of our compounds using a GC fluorescent antibody (red), a lisosome marker (LAMP2, green), and a nucleus marker (DAPI, blue). Active GC tranlocators increase the colocalization of GC and LAMP2 antibodies, visually increasing the yellow color.

General Procedure for the Suzuki Coupling. The boronic acid **20** (2.48 mmol) and K_2CO_3 (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 $Pd(PPh_3)_4$ (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 Na_2SO_4, 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). Yield 25%. ^{1}H 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 $[C_{24}H_{23}N_3O_2S_2 + H]^+$.

2-(5-Methylthiophen-2-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56a). Yield 25%. ^{1}H 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 $[C_{24}H_{24}N_4O_2S_2 + H]^+$.

2-(Pyridin-3-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (56f). Yield 25%. ^{1}H 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 $[C_{24}H_{23}N_5O_2S + H]^+$.

2-(Pyridin-4-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (55g). Yield 18%. ^{1}H 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 $[C_{24}H_{23}N_5O_2S + H]^+$.

Preparation of 3-(Thiophen-2-yl)-1-(4-tosylpiperazin-1-yl)isoquinoline (70). Yield 12%. ^{1}H 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 $[C_{24}H_{23}N_3O_2S_2 + H]^+$.

2-(Thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrido[3,2-*d*]pyrimidine (72). Yield 18%. ^{1}H 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 $[C_{22}H_{21}N_5O_2S_2 + H]^+$.

2-(Thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrido[2,3-*d*]pyrimidine (73). Yield 60%. ^{1}H 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 $[C_{22}H_{21}N_5O_2S_2 + H]^+$.

2-(Thiophen-2-yl)-4-(4-tosylpiperazin-1-yl)pyrimidine (74). Yield 40%. ^{1}H NMR (400 MHz, 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 $[C_{19}H_{20}N_4O_2S_2 + H]^+$.

4,5-Dimethyl-2-(thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)pyrimidine (76). Yield 21%. ^{1}H 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 $[C_{21}H_{24}N_4O_2S_2 + H]^+$.

Preparation of 2-Chloro-6-(4-tosylpiperazin-1-yl)-9*H*-purine (19b). *N*,*N*-Diisopropylethylamine (0.92 mL, 3.28 mmol) and piperizine **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 × 15 mL). The combined organic layers were washed with water (3 × 10 mL) and brine (3 × 10 mL), dried over Na_2SO_4, 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{16}H_{17}N_6O_2S]^+$.

Preparation of [2-Chloro-6-(4-tosylpiperazin-1-yl)-9*H*-purin-9-yl]methyl Pivalate (19c). Chloromethylpivalate (**22**, 0.1 mL, 0.56 mmol) and K_2CO_3 (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 Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by trituration with CH_2Cl_2/hexanes to afford pivalate **19c** (110 mg, 63%) as an off-white solid. ^{1}H 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 $[C_{22}H_{27}ClN_6O_4S]^+$.

Preparation of [2-(Thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)-9*H*-purin-9-yl]methyl Pivalate (21bb). 2-Thiophene boronic acid (**20a**, 75 mg, 0.59 mmol) and Cs_2CO_3 (256 mg, 078 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 $Pd(PPh_3)_4$ (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 W). 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 Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was

Table 7. HRMS Found Values for All Final Compounds

compd	formula	calcd for formula + H^+	HRMS found	compd	formula	calcd for formula + H^+	HRMS found
2	$C_{17}H_{18}N_4S$	311.1331	311.1325	**26bf**	$C_{25}H_{24}N_4O_4S_2$	509.1318	509.1327
3	$C_{18}H_{20}N_4S$	325.1487	325.1487	**26bg**	$C_{22}H_{19}N_5O_4S_2$	482.0957	482.0957
4	$C_{19}H_{22}N_4S$	339.1644	339.1637	**26bh**	$C_{22}H_{19}N_5O_4S_2$	482.0957	482.0957
5	$C_{18}H_{18}N_4OS$	339.12797	339.1275	**26bi**	$C_{22}H_{19}N_5O_4S_2$	482.0957	482.0951
6	$C_{22}H_{19}FN_4S$	391.13927	391.1388	**26bj**	$C_{23}H_{19}N_5O_2S_2$	462.1059	462.1054
7	$C_{22}H_{19}N_4FS$	391.1393	391.1388	**26bk**	$C_{23}H_{19}N_5O_2S_2$	462.1059	462.1056
6	$C_{22}H_{20}N_4OS$	389.1436	389.1431	**26bl**	$C_{23}H_{19}N_5O_2S_2$	462.1059	462.1055
8	$C_{23}H_{22}N_4OS$	403.1593	403.1586	**26bm**	$C_{21}H_{19}N_5O_2S_2$	438.1059	438.1056
9	$C_{22}H_{20}N_4O_2S_2$	437.1106	437.1106	**26bn**	$C_{19}H_{17}N_5O_2S_3$	444.0623	444.0620
10	$C_{22}H_{19}N_4O_2S_2Cl$	471.0716	471.0719	**26bo**	$C_{20}H_{18}N_4O_2S_3$	443.0670	443.0665
11	$C_{25}H_{23}N_5O_2S$	458.1651	458.1645	**26bp**	$C_{20}H_{18}N_4O_2S_3$	443.0670	443.0670
12	$C_{25}H_{23}N_5O_4S$	490.1549	490.1542	**26bq**	$C_{23}H_{22}N_4O_2S_2$	451.1263	451.1262
13	$C_{26}H_{24}N_4O_4S$	489.1597	489.1592	**26br**	$C_{26}H_{26}N_4O_2S_2$	491.1576	491.1579
14	$C_{26}H_{22}N_4O_2S_2$	487.1263	487.1255	**26bs**	$C_{24}H_{22}N_4O_4S_2$	495.1161	495.1157
21aa	$C_{25}H_{24}N_4O_2S$	445.1698	445.1701	**26bt**	$C_{25}H_{25}N_5O_3S_2$	508.1477	508.1476
21ab	$C_{28}H_{30}N_4O_2S$	487.2168	487.2168	**26bu**	$C_{27}H_{28}N_4O_3S_2$	521.1681	521.1682
21ac	$C_{25}H_{24}N_4O_3S$	461.1648	461.1642	**25bv**	$C_{25}H_{21}N_5O_2S_2$	488.1215	488.1211
21ad	$C_{26}H_{26}N_4O_3S$	475.1804	475.1804	**26bw**	$C_{26}H_{21}N_4O_2S_2Cl$	521.0873	521.0871
21ae	$C_{25}H_{21}N_4O_3F_3S$	515.1365	515.1362	**26bx**	$C_{26}H_{22}N_4O_2S_2$	487.1263	487.1259
21af	$C_{28}H_{30}N_4O_4S$	519.2066	519.2067	**26by**	$C_{23}H_{20}N_4O_4S_2$	481.1005	481.1001
21an	$C_{24}H_{21}FN_4O_2S$	449.1448	449.1450	**26bz**	$C_{25}H_{21}N_5O_2S_2$	488.1215	488.1209
21ah	$C_{24}H_{21}N_4O_2SCl$	465.1152	465.1154	**26ca**	$C_{23}H_{19}N_5O_2S_3$	494.0779	494.0777
21ai	$C_{24}H_{21}N_4O_2SCl$	465.1152	465.1151	**26cb**	$C_{17}H_{18}N_4O_2S_2$	375.0950	375.0944
21aj	$C_{24}H_{21}N_4O_2SCl$	465.1152	465.1150	**26cc**	$C_{19}H_{22}N_4O_2S_2$	403.1263	403.1262
21ak	$C_{24}H_{21}N_4O_2SBr$	509.0647	511.0629	**26 cd**	$C_{17}H_{15}N_4O_2F_3S_2$	429.0667	429.0671
21al	$C_{24}H_{21}N_4O_2SBr$	509.0647	511.0628	**28**	$C_{25}H_{24}N_4O_2S$	445.1698	445.1690
21am	$C_{25}H_{21}N_4O_2F_3S$	499.1416	499.1421	**29**	$C_{23}H_{20}N_4OS$	401.1436	401.1436
21ao	$C_{25}H_{21}N_4O_2F_3S$	499.1416	499.1422	**33a**	$C_{21}H_{26}N_4O_2S$	399.1855	399.1853
21ap	$C_{27}H_{26}N_4O_4S$	503.1753	503.1749	**35a**	$C_{23}H_{24}N_4O_2S_2$	453.1419	453.1414
21aq	$C_{26}H_{24}N_4O_3S$	473.1648	473.1652	**35b**	$C_{24}H_{26}N_4O_2S_2$	467.1576	467.1571
21ar	$C_{24}H_{23}N_5O_4S_2$	510.1270	510.1271	**35c**	$C_{24}H_{24}N_4O_2S_2$	465.1419	465.1420
21as	$C_{25}H_{21}N_5O_2S$	456.1494	456.1492	**38**	$C_{24}H_{23}N_3O_2S_2$	450.1310	450.1308
21at	$C_{25}H_{21}N_5O_2S$	456.1494	456.1492	**39**	$C_{19}H_{21}N_3O_2S$	356.1433	356.1431
21au	$C_{24}H_{21}N_5O_4S$	476.1393	476.1385	**51**	$C_{24}H_{23}N_3O_2S_2$	450.1310	450.1303
21av	$C_{24}H_{21}N_5O_4S$	476.1393	476.1395	**56a**	$C_{24}H_{24}N_4O_2S_2$	465.1419	465.1416
21aw	$C_{24}H_{21}N_5O_4S$	476.1393	476.1395	**56b**	$C_{22}H_{21}N_5O_2S_2$	452.1215	452.1204
21ax	$C_{28}H_{24}N_4O_2S$	481.1698	481.1698	**56c**	$C_{22}H_{21}N_5O_2S_2$	452.1215	452.1211
21ay	$C_{28}H_{28}N_4O_2S$	485.2011	485.2019	**56d**	$C_{22}H_{21}N_5O_3S$	436.1444	436.1433
21az	$C_{27}H_{26}N_4O_2S$	471.1855	471.1856	**56e**	$C_{23}H_{22}N_4O_3S$	435.1491	435.1491
21ba	$C_{26}H_{24}N_4O_2S$	457.1698	457.1695	**56f**	$C_{24}H_{23}N_5O_2S$	446.1651	446.1642
21bb	$C_{26}H_{30}N_6O_4S_2$	555.1848	555.1848	**56g**	$C_{24}H_{23}N_5O_2S$	446.1651	446.1639
21bc	$C_{20}H_{20}N_6O_2S_2$	441.1168	441.1167	**56h**	$C_{23}H_{22}N_6O_2S$	447.1603	447.1585
24	$C_{21}H_{24}N_4O_2S$	397.1698	397.1694	**56i**	$C_{23}H_{22}N_6O_2S$	447.1603	447.1596
26aa	$C_{22}H_{20}N_4O_2S_2$	437.1106	437.1103	**56i**	$C_{23}H_{22}N_6O_2S$	437.1103	447.1597
26ab	$C_{23}H_{22}N_4O_2S_2$	451.1263	451.1258	**56k**	$C_{23}H_{22}N_6O_2S$	447.1603	447.1589
26ac	$C_{23}H_{22}N_4O_2S_2$	451.1263	451.1255	**56l**	$C_{26}H_{26}N_4O_3S$	475.1804	475.1798
26ad	$C_{23}H_{22}N_4O_2S_2$	451.1263	451.1261	**56m**	$C_{24}H_{20}N_4O_2FSBr$	527.0553	529.0529
26ae	$C_{24}H_{24}N_4O_2S_2$	465.1419	465.1418	**56n**	$C_{24}H_{20}N_4O_2SClBr$	543.0257	545.0226
26af	$C_{24}H_{24}N_4O_2S_2$	465.1419	465.1415	**560**	$C_{24}H_{20}N_4O_2SCl_2$	499.0763	499.0757
26ag	$C_{26}H_{28}N_4O_2S_2$	493.1732	493.1736	**57**	$C_{20}H_{19}N_5O_2S$	394.1338	394.1322
26ah	$C_{26}H_{28}N_4O_2S_2$	493.1732	493.1729	**59**	$C_{20}H_{21}N_5O_3S$	412.1444	412.1424
26ai	$C_{23}H_{22}N_4O_3S_2$	467.1212	467.1209	**60**	$C_{21}H_{22}N_4O_4S$	427.1440	427.1432
26ai	$C_{23}H_{22}N_4O_3S_2$	467.1212	467.1209	**61**	$C_{21}H_{22}N_4O_3S$	411.1491	411.1484
26ak	$C_{23}H_{22}N_4O_3S_2$	467.1212	467.1208	**62**	$C_{21}H_{22}N_4O_2S$	395.1542	395.1537
26al	$C_{23}H_{19}N_4O_3F_3S_2$	521.0929	521.0935	**63**	$C_{19}H_{20}N_4O_2S$	369.1385	369.1384
26am	$C_{24}H_{24}N_4O_4S_2$	497.1318	497.1320	**68**	$C_{26}H_{24}N_6O_2S$	485.1760	485.1758
26an	$C_{22}H_{19}FN_4O_2S_2$	455.1012	455.1010	**69**	$C_{28}H_{31}N_5O_3S_2$	550.1947	550.1946
26ao	$C_{22}H_{19}N_4O_2FS_2$	455.1012	455.1012	**70**	$C_{28}H_{32}N_6O_2S_2$	549.2106	549.2101
26ap	$C_{22}H_{19}N_4O_2FS_2$	455.1012	455.1015	**71**	$C_{24}H_{23}N_3O_2S_2$	450.1310	450.1311
26aq	$C_{22}H_{18}N_4O_2F_2S_2$	473.0918	473.0914	**72**	$C_{24}H_{23}N_3O_2S_2$	450.1310	450.1312
26ar	$C_{22}H_{19}N_4O_2S_2Cl$	471.0716	471.0712	**73**	$C_{22}H_{21}N_5O_2S_2$	452.1215	452.1217
26as	$C_{22}H_{19}N_4O_2S_2Cl$	471.0716	471.0715	**74**	$C_{22}H_{21}N_5O_2S_2$	452.1215	452.1219
26at	$C_{22}H_{18}N_4O_2S_2Cl_2$	505.0327	505.0329	**75**	$C_{19}H_{20}N_4O_2S_2$	401.1106	401.1110
26au	$C_{22}H_{19}N_4O_2S_2Br$	515.0211	517.0190	**76**	$C_{18}H_{18}N_4O_2S_2$	387.0950	387.0944
26av	$C_{22}H_{19}N_4O_2S_2Br$	515.0211	517.0186	**77**	$C_{21}H_{24}N_4O_2S_2$	429.1419	429.1423
26aw	$C_{22}H_{19}N_4O_2S_2Br$	515.0211	517.0191	**78**	$C_{22}H_{18}N_4O_2FS_2Cl$	489.0622	489.0619
26ax	$C_{23}H_{19}N_4O_2F_3S_2$	505.0980	505.0975	**79**	$C_{23}H_{18}N_4O_2F_3S_2C$	539.0590	539.0586

Table 7. Continued

compd	formula	calcd for formula + H^+	HRMS found	compd	formula	calcd for formula + H^+	HRMS found
26ay	$C_{23}H_{19}N_4O_2F_3S_2$	505.0980	505.0976	**80**	$C_{24}H_{20}N_4O_2FSCl$	483.1058	483.1054
26az	$C_{23}H_{19}N_4O_2F_3S_2$	505.0980	505.0981	**81**	$C_{25}H_{22}N_4O_2FSCl$	497.1214	497.1213
26ba	$C_{24}H_{22}N_4O_3S_2$	479.1212	479.1199	**82**	$C_{24}H_{19}N_4O_2FSCl_2$	517.0668	517.0667
26bb	$C_{24}H_{22}N_4O_3S_2$	479.1212	479.1213	**83**	$C_{24}H_{19}N_4O_2F_2SCl$	501.0964	501.0962
26bc	$C_{24}H_{22}N_4O_3S_2$	479.1212	479.1219	**84**	$C_{25}H_{22}ClFN_4O_2S$	479.1214	497.1219
27bd	$C_{22}H_{21}N_5O_4S_3$	516.0834	516.0830	**85**	$C_{24}H_{20}N_4O_2FSCl$	483.1058	483.1055
26de	$C_{25}H_{24}N_4O_4S_2$	509.1318	509.1320	**86**	$C_{20}H_{18}N_4O_3S$	395.1178	395.1173

Table 8. ΔT_m Tabulated vs Compound Concentration[a]

concentration (μM)	**26ad**	**14**	**26bs**	**26ai**	62	DNJ 87	isofagomine 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

[a] Empty cells indicate undetectable melt transition. * indicates that compound was observed to have precipitated out of solution.

purified by silica-gel column chromatography (20% EtOAc in hexanes) to afford purine **21bb** (70 mg, 35%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{26}H_{30}N_6O_4S_2 + H]^+$.

Preparation of 2-(Thiophen-2-yl)-6-(4-tosylpiperazin-1-yl)-9*H*-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 CH_2Cl_2) to afford purine **21bc** (55 mg, 45%) as an off-white solid. ^{1}H 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 $[C_{20}H_{20}N_6O_2S_2 + H]^+$.

Preparation of *tert*-Butyl 4-(2-Chloroquinazolin-4-yl)piperazine-1-carboxylate (23). *N*,*N*-Diisopropylethylamine (5.2 mL, 30.14 mmol) and Boc-piperzine **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. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{17}H_{21}ClN_4O_2 + H]^+$.

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 g, 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 × 30 mL). The combined organic layers were washed with water (3 × 100 mL), dried over Na_2SO_4 and then 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. ^{1}H 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 °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 × 30 mL), cooled to 0 °C, and basified to pH 12 with a saturated aqueous $NaHCO_3$ solution. The aqueous solution was then extracted with CH_2Cl_2 (2 × 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. ^{1}H 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 °C. After stirring for 3 h at room temperature, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water (3 × 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 anoff-white solid.

4-(4-(4-Chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (10). Yield 75%. ^{1}H 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). Yield 29%. ^{1}H 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). Yield 29%. ^{1}H 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). Yield 70%. ^{1}H 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). Yield 64%. ^{1}H 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). Yield 62%. ^{1}H 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). Yield 66%. ^{1}H 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). Yield 63%. ^{1}H 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). Yield 63%. ^{1}H 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). Yield 38%. ^{1}H 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). Yield 57%. ^{1}H 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). Yield 71%. ^{1}H 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 $[C_{22}H_{19}FN_4O_2S_2 + H]^+$.

4-(4-(3-Fluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26ao). Yield 70%. ^{1}H 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 $[C_{22}H_{19}FN_4O_2S_2 + H]^+$.

4-(4-(4-Fluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26ap). Yield 67%. ^{1}H 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 $[C_{22}H_{19}FN_4O_2S_2 + H]^+$.

4-(4-(2,4-Difluorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26aq). Yield 68%. ^{1}H 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 $[C_{22}H_{18}F_2N_4O_2S_2 + H]^+$.

4-(4-(2-Chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26ar). Yield 67%. ^{1}H 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 $[C_{22}H_{19}ClN_4O_2S_2]^+$.

4-(4-(3-Chlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26as). Yield 30%. ^{1}H 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 $[C_{22}H_{19}ClN_4O_2S_2]^+$.

4-(4-(3,5-Dichlorophenylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26at). Yield 59%. ^{1}H 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 $[C_{22}H_{18}Cl_2N_4O_2S_2 + H]^+$.

1-(2-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)phenyl)ethanone (26ba). Yield 60%. ^{1}H 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 $[C_{24}H_{22}N_4O_3S_2 + H]^+$.

1-(3-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)phenyl)ethanone (26bb). Yield 65%. ^{1}H 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 $[C_{24}H_{22}N_4O_3S_2 + H]^+$.

Ethyl 2-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzoate (26be). Yield 45%. ^{1}H 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 MS (ESI) m/z 509 $[C_{25}H_{24}N_4O_4S_2 + H]^+$.

2-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)benzonitrile (26bj). Yield 75%. ^{1}H 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 $[C_{23}H_{19}N_5O_2S_2 + H]^+$.

3-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)-benzonitrile (26bk). Yield 70%. ^{1}H 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 = 4H), 3.31 (t, J = 4.8 Hz, 4H). MS (ESI) m/z MS (ESI) m/z 462 $[C_{23}H_{19}N_5O_2S_2 + H]^+$.

4-(4-(Pyridin-3-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26bm). Yield 55%. ^{1}H 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 $[C_{21}H_{19}N_5O_2S_2 + H]^+$.

2-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)-thiazole (26bn). Yield 19%. ^{1}H 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 $[C_{19}H_{17}N_5O_2S_3 + H]^+$.

2-(Thiophen-2-yl)-4-(4-(thiophen-2-ylsulfonyl)piperazin-1-yl)-quinazoline (26bo). Yield 38%. ^{1}H 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 $[C_{20}H_{18}N_4O_2S_3 + H]^+$.

2-(Thiophen-2-yl)-4-(4-(thiophen-3-ylsulfonyl)piperazin-1-yl)-quinazoline (26bp). Yield 80%. ^{1}H 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 $[C_{20}H_{18}N_4O_2S_3 + H]^+$.

4-(4-Benzylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bq). Yield 50%. ^{1}H 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 $[C_{23}H_{22}N_4O_2S_2 + H]^+$.

4-(4-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bs). Yield 58%. ^{1}H 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 $[C_{24}H_{22}N_4O_4S_2 + H]^+$.

4-Methyl-7-(4-(2-(thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (26bt). Yield 14%. ^{1}H 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 $[C_{25}H_{25}N_5O_3S_2 + H]^+$.

4-(4-(2,2-Dimethylchroman-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bu). Yield 34%. ^{1}H 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 $[C_{27}H_{28}N_4O_3S_2 + H]^+$.

4-(4-(Quinolin-3-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26bv). Yield 34%. ^{1}H 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 $[C_{25}H_{21}N_5O_2S_2 + H]^+$.

4-(4-(5-Chloronaphthalen-2-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26bw). Yield 63%. ^{1}H 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 $[C_{26}H_{21}ClN_4O_2S_2]^+$.

4-(4-(Naphthalen-1-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26bx). Yield 21%. ^{1}H 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 $[C_{26}H_{22}N_4O_2S_2 + H]^+$.

4-(4-(Benzo[*d*][1,3]dioxol-5-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26by). Yield 16%. ^{1}H 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 $[C_{23}H_{20}N_4O_4S_2 + H]^+$.

4-(4-(Quinolin-6-ylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)-quinazoline (26bz). Yield 46%. ^{1}H 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 $[C_{25}H_{21}N_5O_2S_2 + H]^+$.

6-(4-(2-(Thiophen-2-yl)quinazolin-4-yl)piperazin-1-ylsulfonyl)-benzo[*d*]thiazole (26ca). Yield 63%. ^{1}H 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 $[C_{23}H_{19}N_5O_2S_3 + H]^+$.

4-(4-(Methylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26cb). Yield 32%. ^{1}H 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 $[C_{17}H_{18}N_4O_2S_2 + H]^+$.

4-(4-(Isopropylsulfonyl)piperazin-1-yl)-2-(thiophen-2-yl)quinazoline (26cc). Yield 40%. ^{1}H 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 $[C_{29}H_{22}N_4O_2S_2 + H]^+$.

Preparation of 2-(Thiophen-2-yl)-4-(4-(trifluoromethylsulfonyl)-piperazin-1-yl)quinazoline (26 cd). 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 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. ^{1}H NMR (400 MHz, 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 $[C_{17}H_{15}F_3N_4O_2S_2 + H]^+$.

General Procedure for Preparation of Piperizine 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). Yield 68%. ^{1}H 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 $[C_{23}H_{20}N_4OS + H]^+$.

(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl){4-[2-(thiophen-2-yl)-quinazolin-4-yl]piperazin-1-yl}methanone (30). Yield 91%. ^{1}H 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 $[C_{25}H_{22}N_4O_3S + H]^+$.

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 Na_2SO_4, 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. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90−6.79 (m, 3H), 4.56 (s, 2H), 4.25 (s, 4H). MS (ESI) m/z 167 $[C_9H_{10}O_3 + H]^+$.

Phosphorus 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 CH_2Cl_2 (5 mL) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was quenched with an aqueous $NaHCO_3$ 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 Na_2SO_4 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 $[C_9H_9BrO_2 + H]^+$.

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 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 tertiary amine **28** (50 mg, 15%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{25}H_{24}N_4O_2S + H]^+$.

General Procedure for Preparation of Boc-Protected *N,N*-Dimethylalkyldiamines. A solution of $(Boc)_2O$ (22.68 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a stirring solution of *N*, *N*-dimethylalkyldiamine (56.70 mmol) in CH_2Cl_2 (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 $NaHCO_3$ 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 CH_2Cl_2 (2 × 100 mL), and the combined organic layer was dried over Na_2SO_4 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.** Yield 24%. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_9H_{20}N_20_2 + H]^+$.

***tert*-Butyl Methyl[3-(methylamino)propyl]carbamate.** Yield 17%. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{17}H_{12}ClN_3O_2 + H]^+$.

Preparation of *tert*-Butyl 2-[(2-Chloroquinazolin-4-yl)(methyl)-amino]ethyl(methyl) Carbamate (32a). Utilizing the general procedure outlined for syntheis of **19** derivatives provided a 35% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 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 syntheis of **19** derivatives provided a 66% yield. ^{1}H 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 syntheis of **19** derivatives provided 1.10 g, 70% yield. ^{1}H 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 $[C_{18}H_{23}ClN_4O_2]^+$.

Preparation of *tert*-Butyl Methyl(2-{methyl[2-(thiophen-2-yl)-quinazolin-4-yl]amino}ethyl)carbamate (33a). Utilizing the general procedure outlined for syntheis of **21** derivatives provided 600 mg, 30% yield. ^{1}H 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 syntheis of **21** derivatives provided 1.0 g, 54% yield. ^{1}H 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 syntheis of **21** derivatives provided 1.00 g, 80% yield. ^{1}H 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 × 10 mL), cooled to 0 °C, basified with a 6 N NaOH solution to pH 12, and extracted with CH_2Cl_2 (2 × 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.

***N*^{1},*N*^{2}-Dimethyl-*N*1-[2-(thiophen-2-yl)quinazolin-4-yl]ethane-1,2-diamine (34a).** Yield 80%, 300 mg. ^{1}H 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 $[C_{16}H_{18}N_4S + H]^+$.

***N*^{1},*N*^{3}-Dimethyl-*N*1-[2-(thiophen-2-yl)quinazolin-4-yl]propane-1,3-diamine (34b).** Yield 79%, 300 mg. ^{1}H 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). Yield 60%, 500 mg. ^{1}H 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 syntheis of **32** derivatives provided 135 mg, 55% yield. ^{1}H 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 syntheis of **32** derivatives provided 220 mg, 73% yield. ^{1}H 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 syntheis of **32** derivatives provided 355 mg, 74% yield. ^{1}H 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 syntheis of **19** derivatives provided 600 mg, 82% yield. ^{1}H 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 syntheis of **19** derivatives provided 2.70 g, 48% yield. ^{1}H 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 syntheis of **21** derivatives provided 220 mg, 66% yield. ^{1}H 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 $[C_{24}H_{23}N_3O_2S_2 + H]^+$.

Preparation of 2-[2-(Thiophen-2-yl)quinazolin-4-ylamino]ethanol (39). Utilizing the general procedure outlined for syntheis of **21** derivatives provided 320 mg, 53% yield. ^{1}H 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 $[C_{14}H_{13}N_3OS + H]^+$.

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 × 20 mL). The combined organic layer was washed with a saturated $NaHCO_3$ solution (3 × 20 mL) and water (3 × 20 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 (12% EtOAc in hexanes) to afford pivalate **40** (50 mg, 14%) as an oil. ^{1}H 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 $[C_{19}H_{21}N_3O_2S + H]^+$.

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 °C. After stirring for 15 min at 0 °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 °C, quenched with a saturated solution of NH_4Cl (10 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (3 × 20 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 (12% EtOAc in hexanes) to afford the *N*-methylated derivative **41** (150 mg, 35%) as an oil. ^{1}H 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 $[C_{20}H_{23}N_3O_2S + H]^+$.

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. ^{1}H 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 $[C_{15}H_{15}N_3OS + H]^+$.

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 CH_2Cl_2 (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 CH_2Cl_2 (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 CH_2Cl_2 (30 mL); the organic layer was washed with water (3 × 10 mL), dried over Na_2SO_2, 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 $[C_{12}H_{10}N_2O_2S + H]^+$.

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. ^{1}H 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 $[C_{12}H_8N_2OS + H]^+$.

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 phosphorus 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 CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with an aqueous $NaHCO_3$ solution (2 × 10 mL) and water (3 × 10 mL), dried over Na_2SO_2, 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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(2*H*)-carboxylate (49). Boronate ester **48** (1.00 g, 3.41 mmol) and K_2CO_3 (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 $Pd(PPh_3)_4$ (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 Na_2SO_4, 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{22}H_{23}N_3O_2S + H]^+$.

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. ^{1}H 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 $[C_{22}H_{25}N_3O_2S + H]^+$.

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 $[C_{17}H_{17}N_3S - TFA]^+$.

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 × 5 mL). The combined organic layer was washed with water (3 × 10 mL) and brine (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 (30% EtOAc in hexanes) to afford sulphonamide **51** (80 mg, 58%) as an off-white solid. ^{1}H 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 $[C_{21}H_{23}N_3O_2S_2 + H]^+$.

Preparation of 2,4-Dibromoquinazoline (53). *N*,*N*-Dimethylaniline (4.5 mL, 12.34 mmol) was added to a suspension of quinazolindione **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 × 50 mL) and saturated aqueous $NaHCO_3$ solution (3 × 50 mL), dried over Na_2SO_4, and filtered, and the filtrate was concentrated under reduced pressure to afford 2,4-dibromoquinazoline (**53**, 12.0 g, 68%) as an off-white solid. ^{1}H 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 piperizine **17a** (9.10 g, 38.32 mmol) were added to a stirring solution of dibromide **53** (10.0 g, 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 × 50 mL). The combined organic layers were washed with water (3 × 50 mL) and brine (3 × 50 mL), dried over Na_2SO_4, and 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. ^{1}H 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). Yield 50%. ^{1}H 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). Yield 38%. ^{1}H 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). Yield 10%. ^{1}H 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). Yield 16%. ^{1}H 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). Yield 21%. ^{1}H 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). Yield 29%. ^{1}H 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, 4 Hz), 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 column chromatography (10−15% EtOAc in hexanes) to afford stannane **55** (420 mg, 29%) as an oil. ^{1}H 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 $Pd(PPh_3)_4$ (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 × 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 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. ^{1}H 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 $[C_{23}H_{22}N_6O_2S + H]^+$.

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 $Pd(dppf)_2Cl_2$ (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 × 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. ^{1}H 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 $[C_{22}H_{26}N_4O_2S + H]^+$.

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 $Pd_2(dba)_3$ (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 × 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. ^{1}H 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 $[C_{20}H_{19}N_5O_2S + H]^+$.

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 × 25 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford amide **58** (60 mg, 43%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{20}H_{21}N_5O_3S + H]^+$.

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. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{21}H_{22}N_4O_4S + H]^+$.

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 nitile **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 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 ketone **59** (45 mg, 15%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{21}H_{22}N_4O_3S + H]^+$.

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 CH_2Cl_2 (6 mL). The organic layer was washed with an aqueous solution of $NaHCO_3$ (5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by preparative TLC plate (1% MeOH in $CHCl_3$) to afford heterocyclic derivatives.

Preparation of 2-(1*H*-Benzo[*d*]imidazol-1-yl)-4-(4-tosylpiperazin-1-yl)quinazoline (63). Yield 25%, 60 mg. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[C_{26}H_{24}N_6O_2S + H]^+$.

Preparation of 5-[4-(4-Tosylpiperazin-1-yl)quinazolin-2-yl]-thiophene-2-carbaldehyde (64). Utilizing the general procedure outlined for syntheis of **21** derivatives provided aldehyde **64** which was immediately utilized due to instability. MS (ESI) m/z 479 $[C_{24}H_{22}N_4O_3S_2 + H]^+$.

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. ^{1}H 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 $[C_{24}H_{24}N_4O_3S_2 + H]^+$.

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).** Yield 11%, 15 mg. ^{1}H 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 $[C_{33}H_{40}N_4S_2]^+$.

4-((5-(4-(4-Tosylpiperazin-1-yl)quinazolin-2-yl)thiophen-2-yl)-methyl)morpholine (68). Yield 19%, 45 mg. ^{1}H 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 $[C_{28}H_{31}N_5O_3S_2]^+$.

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. ^{1}H 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 $[C_{28}H_{32}N_6O_2S_2 + H]^+$.

5.2. Biology. 5.2.1. Biological Experiments. The recombinant wild-type 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 min, followed by 10 passes in a motor-driven 50 mL glass-Teflon homogenizer. The homogenate was centrifuged at 1000g 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 K_2PO_4 to make different pH solutions) and 0.01% Tween-20. The spleen homogenate assays used buffer at pH 5, assays with recombinant wild-type 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, 1 M glycine at pH 10 was used as the stop solution for the blue substrate assay. One M TRIS-HCl at pH 8.0 was used as the stop solution for the red substrate assay.

5.2.2. 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 of 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. Then 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.

5.2.3. Thermodenaturation Experiment. This assay measures the change in the melting temperature of the recombinant wild-type GC in the presence of different concentrations of the inhibitors. A mixture of wtGC and SYPRO Orange (5000× 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 5×, 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 analogues, whose final concentrations ranged from 0.1 to 600 μM (400 μM for NCGC00182292−02). One μL 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 no. 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.

5.2.4. 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 mm × 250 mm Agilent Eclipse Plus C18 (5 μm) 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 min. Compounds were monitored using fluorescence detection with an excitation wavelength of 505 nm and emission wavelength at 540 nm. We verified that the mass of the fluorescent peaks matched with the expected ones for the substrate and product of the reaction.

5.2.5. Immunocytochemistry and Laser Scanning Confocal Microscopy. Primary dermal fibroblasts derived from skin biopsies from two previously described N370S/N370S Gaucher patients[39] 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 permeabelized 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 antimouse or antirabbit 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 20×/0.75 objective and a Plan-Apochromat 100×/1.4 oil DIC objective, respectively. Images were taken with the same laser settings, and all the images shown are collapsed *z*-stacks.

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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). Addional images of our translocation experiment with our best inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

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