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Design, Synthesis, and Anaplastic Lymphoma Kinase (ALK) Inhibitory Activity for a Novel Series of 2,4,8,22-Tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]-docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene Macrocycles

Henry J. Breslin,* Brandon M. Lane, Gregory R. Ott, Arup K. Ghose, Thelma S. Angeles, Mark S. Albom, Mangeng Cheng, Weihua Wan, R. Curtis Haltiwanger, Kevin J. Wells-Knecht, and Bruce D. Dorsey

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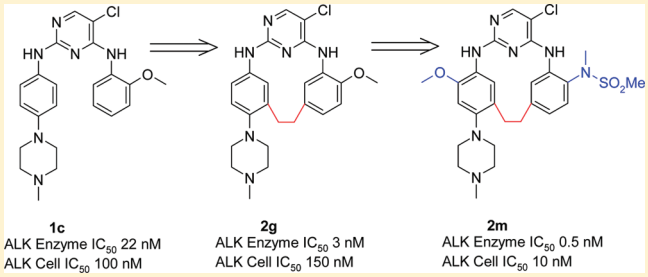
ABSTRACT: A novel set of 2,4,8,22-tetraazatetracyclo-[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene macrocycles were prepared as potential anaplastic lymphoma kinase (ALK) inhibitors, designed to rigidly lock an energy-minimized bioactive conformation of the diaminopyrimidine (DAP) scaffold, a well-documented kinase platform. From 13 analogues prepared, macrocycle **2m** showed the most promising in vitro ALK enzymatic (IC$_{50}$ = 0.5 nM) and cellular (IC$_{50}$ = 10 nM) activities. In addition, macrocycle **2m** exhibited a favorable kinase selectivity preference for inhibition of ALK relative to the highly homologous insulin receptor (IR) kinase (IR/ALK ratio of 173). The inclusive in vitro biological results for this set of macrocycles validate this scaffold as a viable kinase template and further corroborate recent DAP/ALK solid state studies indicating that the inverted "U" shaped conformation of the acyclic DAPs is a preferred bioactive conformation.



INTRODUCTION

Cancer, a group of diseases characterized by uncontrolled growth and spread of abnormal cells, was the second leading cause of deaths in the U.S. in 2010, accounting for one of every four mortalities, as reported by the American Cancer Society.[1] In recent years progress has been made in understanding the biomolecular etiologies contributing to certain cancers, resulting in successful targeted treatments for some of these better characterized cancers.[2] One such vein of targeted cancer treatment is inhibition of various aberrant up-regulated and unregulated kinases, which have been shown to be intimately tied to certain cancers.[3] Kinases are a group of over 500 different proteins that are typically responsible for strategic internal regulation of normal cellular signaling pathways, via phosphorylation of key cellular substrates.[4] Kinases are subgrouped, dependent on which amino acid of the key cellular substrate is phosphorylated, via catalytic transfer of a phosphate group from adenosine triphosphate (ATP). One major subgroup of the kinase family is the receptor tyrosine kinases. One particular tyrosine kinase that has gained increasing attention as a therapeutic target is analplastic lymphoma kinase (ALK), a member of the insulin receptor (IR) superfamily.[5] The chimeric protein nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) has been identified in about 60−70% of cases of anaplastic large cell lymphoma (ALCL), a non-Hodgkin's lymphoma cancer arising most often in children and young adults, with constitutively active ALK kinase activity directly associated with the pathogenesis of disease.[6] Other cancers that have been linked with translocated ALK proteins that have led to oncogenic signaling include non-small-cell lung cancer (NSCLC) (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase, EML4-ALK)[7] and inflammatory myofibroblastic tumors (tropomyosin 3-anaplastic lymphoma kinase, TPM3-ALK).[8] Promisingly, a number of ATP-competitive ALK inhibitors have demonstrated oncolytic activity against ALK-positive cancer cells in vitro and in vivo. This has led to several ALK inhibitors advancing to the level of clinical studies, including crizotinib[9] (Figure 1), which recently gained FDA approval for ALK-positive cancers.

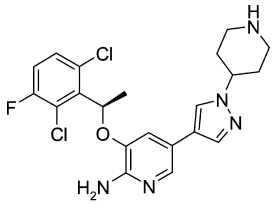


Figure 1.

2,4-Diaminopyrimidines (DAPs) have exhibited potent enzymatic inhibition of ALK and have also shown significant antiproliferative activity for overexpressed ALK-positive cancer cells both in vitro and in vivo.[10] Compound **1a**, a representative DAP ALK inhibitor, was evaluated via small-molecule X-ray crystallography and found to possess an inverted "U" shaped conformation for the three core aryl groups, as depicted in Figure 2. We questioned if locking appropriately substituted

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DAPs in this particular conformation (Figure 3) would yield macrocycles with enhanced ALK inhibitory activities and/or

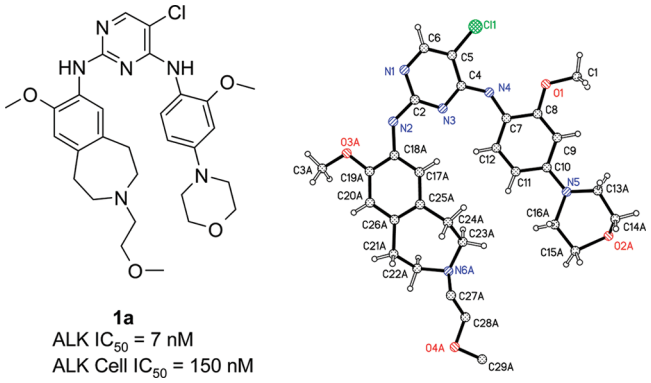

Figure 2.

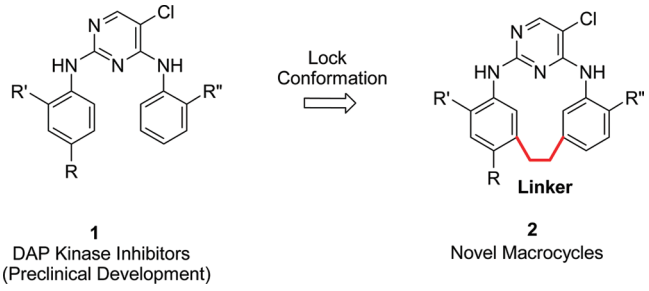

Figure 3.

kinase selectivity profiles. A few recent overviews of varied macrocyclic efforts directed at a panorama of biomolecular targets share some other potential advantages, as well as hurdles, that have been established for this type of strategic endeavor.[11] Prior to initiating our efforts, a literature search revealed that a Janssen group had reported preparing DAP macrocycles.[12] However, their macrocycles had extended linkers presenting flexible conformations that were not necessarily focused specifically toward ALK. Following are our experimental results appraising rigidly constrained, suitably functionalized DAP macrocycles that were designed purposely as ALK inhibitors.

RESULTS

To initially assess that locking the conformation of the DAPs proposed in Figure 3 might prove biologically favorable, we evaluated the potential overlap of an energy minimized macrocyclic motif relative to its respective energy minimized acyclic parent DAP via molecular modeling. Analogue **1c** was chosen as a preferable acyclic DAP for the modeling study because it was a minimally substituted DAP that possessed favorable ALK activity, as identified through routine screening. Consistent with the X-ray conformation for the aryl groups of DAP **1a** (Figure 2), the computational evaluation of the energy minimized structure of DAP **1c** displayed the three aryl groups aligned in a similar inverted "U" shaped conformation (Figure 4). Tying the pendent phenyl groups of **1c** with an ethylene linker yielded macrocycle **2g**, whose energy minimized conformation overlapped nicely with DAP **1c**, with the root-mean-square deviation of all the common non-hydrogen atoms in their

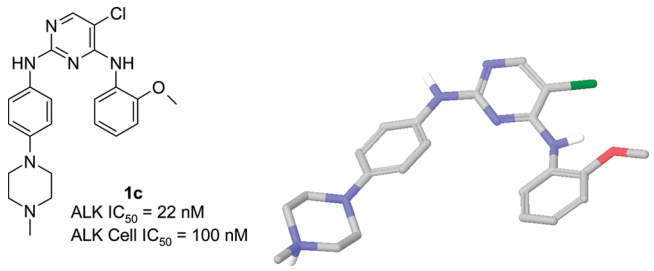

Figure 4. Calculated minimum energy conformation of **1c**.

minimum energy conformations at 1.29 Å (Figure 5). These encouraging modeling results added confidence that the proposed macrocycles **2** would show promise as ALK inhibitors. Subsequent to these modeling experiments, solid state crystal structures of apo and ligand bound structures (including DAP bound) of ALK were reported in the literature and showed similar conformational arrangement of the DAP's three aryl groups.[13]

Scheme 1 comprehensively outlines the routes used to prepare macrocycles **2a**–**m**. A minimally substituted macrocycle (**2a**) was prepared to validate the synthetic sequence. 3-Bromoaniline (**3a**) was reacted with 2,4,5-trichloropyrimidine (**4**) to yield intermediate **5a**, which was coupled with 3-vinylaniline (**6a**) to give acyclic DAP **7a**. This set the stage for macrocyclization by employing the Heck reaction between the vinyl (W) and bromo (Y) coupling partners, which yielded **8a** in 65% yield using microwave heating (120 °C for 30 min). Pt-catalyzed hydrogenation of **8a** gave the desired macrocycle **2a**. In tandem with the reduction of the olefin, some dechlorination of the substituted pyrimidine was observed, but this side product was readily separated via chromatography. Noteworthy, during the course of our work, an alternative synthesis of the minimally substituted macrocycle **8a** was reported in the patent literature, via a ring closing olefin metathesis reaction, albeit in a modest 9% yield.[14]

Encouraged by our preparation of core structure **2a**, we embarked on synthesizing macrocycles **2b**–**m** (Table 1), decorating the core macrocycle **2a** with key appendages that have been shown to enhance ALK activity. These synthetic efforts were initially focused on introducing varied R groups on **2a**. Prior to the synthesis of R group substituted macrocycles **2b**–**d**, substituted anilines **6c** and **6d** were prepared as outlined in Scheme 2. Intermediate **5b** was generated in a similar manner as **5a**. Then anilines **6b**–**d** were reacted with **5b** to form **7b**–**d** under identical reaction conditions used to construct DAP **7a**. Comparing the cyclization step for macrocycles **8b**–**d** relative to macrocycle **8a** demonstrated that the Heck macrocyclization reaction proceeded equally well regardless of whether the vinyl and bromide coupling partners were interchanged as W or Y (Scheme 1). Final saturated targets **2b**–**d** were generated cleanly by the selective diimide olefin reductions of **8b**–**d**. The diimide reduction circumvented potential dechlorination issues, as had been observed in the Pt catalyzed reduction of **2a**. As the related chloro moiety in acyclic DAPs has proven biologically favorable, we preferred to maintain the 6-Cl substituent as a constant on key analogues **2**; therefore, the diimide reduction was employed for all subsequent analogues.

Initial R″ substituted analogue **2e** was prepared following the general conditions used to generate **2a**. It was envisioned that the R,R″ disubstituted analogues **2f** and **2g** would be synthesized following the general procedures of analogues **2b**–**d**.

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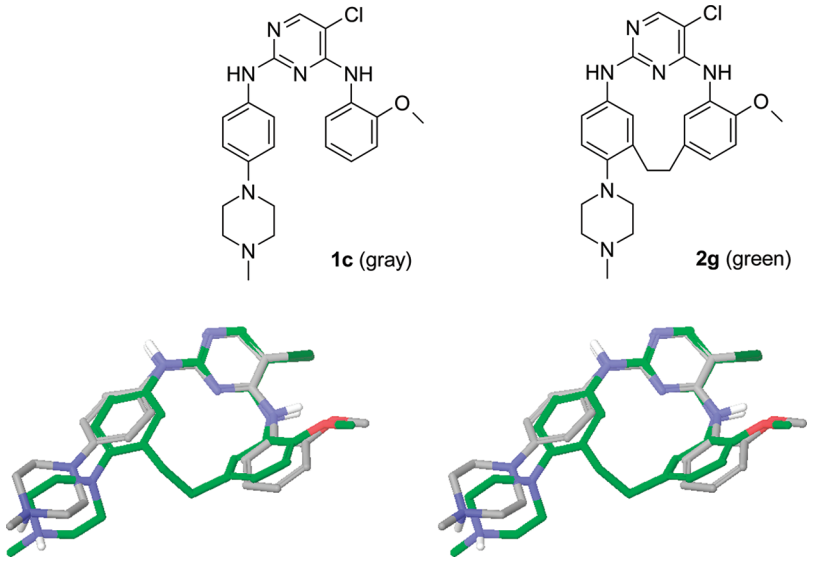
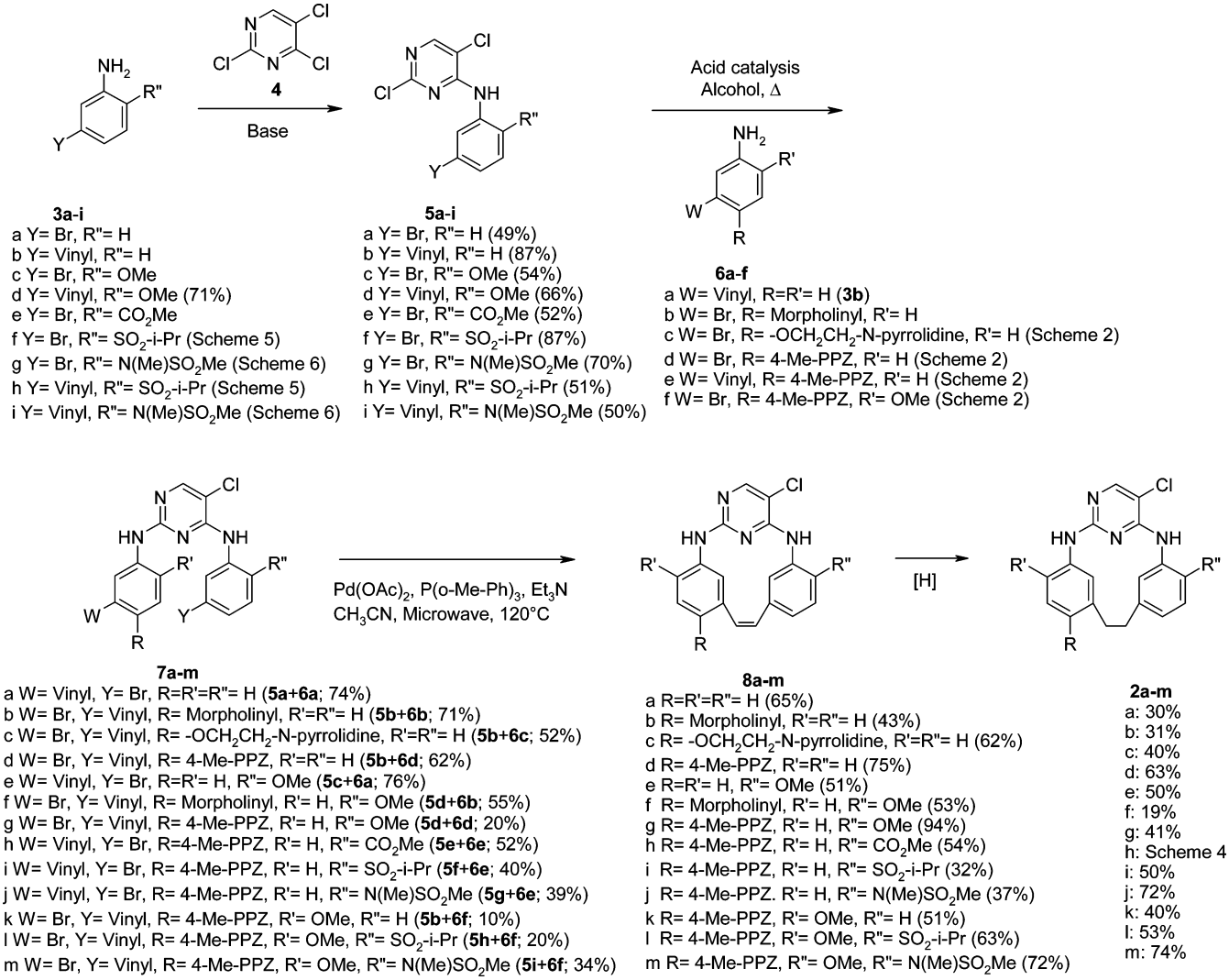


Figure 5. Stereoview of superimposed, minimum-energy calculated conformations of **1c** and **2g**.

Scheme 1. Method A



3a-i
a Y= Br, R"= H
b Y= Vinyl, R"= H
c Y= Br, R"= OMe
d Y= Vinyl, R"= OMe (71%)
e Y= Br, R"= CO_2Me
f Y= Br, R"= SO_2-i-Pr (Scheme 5)
g Y= Br, R"= N(Me)SO_2Me (Scheme 6)
h Y= Vinyl, R"= SO_2-i-Pr (Scheme 5)
i Y= Vinyl, R"= N(Me)SO_2Me (Scheme 6)

5a-i
a Y= Br, R"= H (49%)
b Y= Vinyl, R"= H (87%)
c Y= Br, R"= OMe (54%)
d Y= Vinyl, R"= OMe (66%)
e Y= Br, R"= CO_2Me (52%)
f Y= Br, R"= SO_2-i-Pr (87%)
g Y= Br, R"= N(Me)SO_2Me (70%)
h Y= Vinyl, R"= SO_2-i-Pr (51%)
i Y= Vinyl, R"= N(Me)SO_2Me (50%)

6a-f
a W= Vinyl, R=R'= H (**3b**)
b W= Br, R= Morpholinyl, R'= H
c W= Br, R= -OCH_2CH_2-N-pyrrolidine, R'= H (Scheme 2)
d W= Br, R= 4-Me-PPZ, R'= H (Scheme 2)
e W= Vinyl, R= 4-Me-PPZ, R'= H (Scheme 2)
f W= Br, R= 4-Me-PPZ, R'= OMe (Scheme 2)

7a-m
a W= Vinyl, Y= Br, R=R'=R"= H (**5a+6a**; 74%)
b W= Br, Y= Vinyl, R= Morpholinyl, R'=R"= H (**5b+6b**; 71%)
c W= Br, Y= Vinyl, R= -OCH_2CH_2-N-pyrrolidine, R'=R"= H (**5b+6c**; 52%)
d W= Br, Y= Vinyl, R= 4-Me-PPZ, R'=R"= H (**5b+6d**; 62%)
e W= Vinyl, Y= Br, R=R'= H, R"= OMe (**5c+6a**; 76%)
f W= Br, Y= Vinyl, R= Morpholinyl, R'= H, R"= OMe (**5d+6b**; 55%)
g W= Br, Y= Vinyl, R= 4-Me-PPZ, R'= H, R"= OMe (**5d+6d**; 20%)
h W= Vinyl, Y= Br, R=4-Me-PPZ, R'= H, R"= CO_2Me (**5e+6e**; 52%)
i W= Vinyl, Y= Br, R= 4-Me-PPZ, R'= H, R"= SO_2-i-Pr (**5f+6e**; 40%)
j W= Vinyl, Y= Br, R= 4-Me-PPZ, R'= H, R"= N(Me)SO_2Me (**5g+6e**; 39%)
k W= Br, Y= Vinyl, R= 4-Me-PPZ, R'= OMe, R"= H (**5b+6f**; 10%)
l W= Br, Y= Vinyl, R= 4-Me-PPZ, R'= OMe, R"= SO_2-i-Pr (**5h+6f**; 20%)
m W= Br, Y= Vinyl, R= 4-Me-PPZ, R'= OMe, R"= N(Me)SO_2Me (**5i+6f**; 34%)

8a-m
a R=R'=R"= H (65%)
b R= Morpholinyl, R'=R"= H (43%)
c R= -OCH_2CH_2-N-pyrrolidine, R'=R"= H (62%)
d R= 4-Me-PPZ, R'=R"= H (75%)
e R=R'= H, R"= OMe (51%)
f R= Morpholinyl, R'= H, R"= OMe (53%)
g R= 4-Me-PPZ, R'= H, R"= OMe (94%)
h R= 4-Me-PPZ, R'= H, R"= CO_2Me (54%)
i R= 4-Me-PPZ, R'= H, R"= SO_2-i-Pr (32%)
j R= 4-Me-PPZ. R'= H, R"= N(Me)SO_2Me (37%)
k R= 4-Me-PPZ, R'= OMe, R"= H (51%)
l R= 4-Me-PPZ, R'= OMe, R"= SO_2-i-Pr (63%)
m R= 4-Me-PPZ, R"= OMe, R"= N(Me)SO_2Me (72%)

2a-m
a: 30%
b: 31%
c: 40%
d: 63%
e: 50%
f: 19%
g: 41%
h: Scheme 4
i: 50%
j: 72%
k: 40%
l: 53%
m: 74%

Following this plan required the preparation of precursor 2-methoxy-5-vinylphenylamine (**3d**, method A), which was converted to intermediate **5d** as expected (Scheme 1). However, initial attempts to prepare DAP **7f** using the

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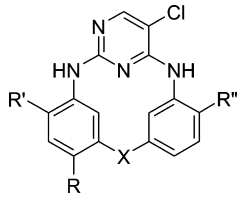
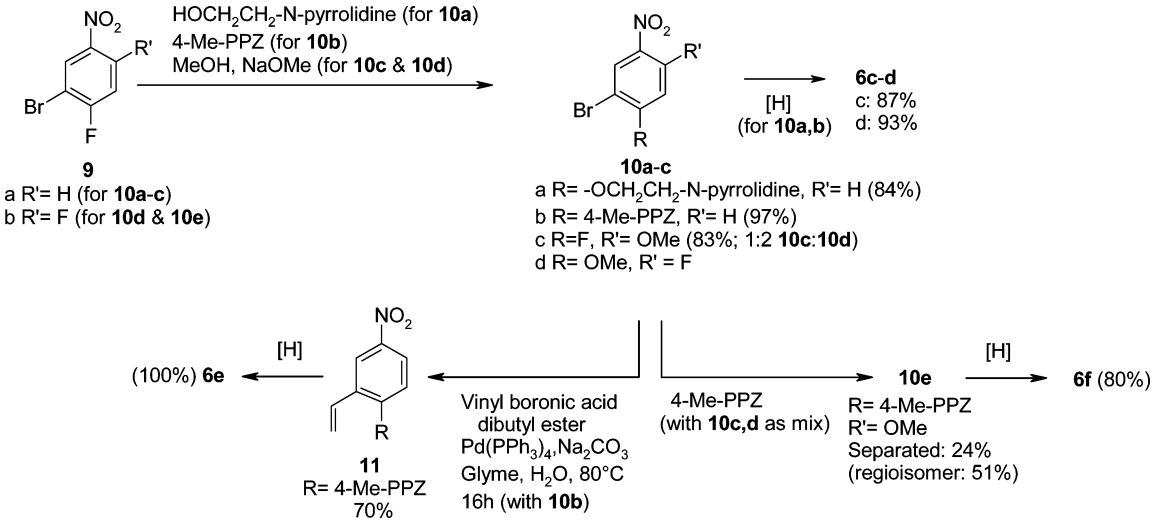
Table 1



compd	X	R	R′	R″	ALK enzyme IC_{50} [a] (nM)	NPM-ALK cell IC_{50} [b] (nM)	IR/ALK [c]	synthesis method
1b		4-Me-piperazinyl	H	H	67 ± 13	NT	1.3	F
1c		4-Me-piperazinyl	H	-OMe	22 ± 7	100	3.6	F
2a	$-CH_2CH_2-$	H	H	H	>10000	NT	ND	A
2b	$-CH_2CH_2-$	1-morpholinyl	H	H	>10000	NT	ND	A
2c	$-CH_2CH_2-$	$-OCH_2CH_2$-N-pyrrolodine	H	H	283 ± 121	NT	ND	B, A
2d	$-CH_2CH_2-$	4-Me-piperazinyl	H	H	92 ± 10	NT	2.8	B, A
2e	$-CH_2CH_2-$	H	H	-OMe	360 ± 80	NT	>27	A
2f	$-CH_2CH_2-$	1-morpholinyl	H	-OMe	>1000	NT	ND	A
2g	$-CH_2CH_2-$	4-Me-piperazinyl	H	-OMe	3.1 ± 0.7	150	67	B, A
2h	$-CH_2CH_2-$	4-Me-piperazinyl	H	-CONHMe	35 ± 16	1200	3.7	B, A, C
2i	$-CH_2CH_2-$	4-Me-piperazinyl	H	$-SO_2$-*i*-Pr	3.7 ± 0.5	70	10	D, B, A
2j	$-CH_2CH_2-$	4-Me-piperazinyl	H	$-N(Me)SO_2Me$	3.4 ± 0.8	120	75	E, B, A
2k	$-CH_2CH_2-$	4-Me-piperazinyl	-OMe	H	5 ± 2	800	58	B, A
2l	$-CH_2CH_2-$	4-Me-piperazinyl	-OMe	$-SO_2$-*i*-Pr	1.5 ± 0.1	30	19	D, B, A
2m	$-CH_2CH_2-$	4-Me-piperazinyl	-OMe	$-N(Me)SO_2Me$	0.51 ± 0.02	10	173	E, B, A
8d	-CH═CH-	4-Me-piperazinyl	H	H	392 ± 149	NT	4	B, A
8g	-CH═CH-	4-Me-piperazinyl	H	-OMe	259 ± 74	NT	>11	B, A
8m	-CH═CH-	4-Me-piperazinyl	-OMe	$-N(Me)SO_2Me$	7.0 ± 0.8	120	>140	E, B, A

[a]Reported as the average ± the standard deviation of three or more determinations. [b]Reported as the average of two determinations. NT = not tested. [c](IR enzyme IC_{50})/(ALK enzyme IC_{50}). ND = not determined.

Scheme 2. Method B



conditions to synthesize **7b–d** were unsuccessful. With 2-methoxyethanol as the solvent for **7f**, acid catalyzed attack by the solvent's hydroxyl moiety at the benzylic position of the olefin yielded its respective ether (**7n**) as a major side product (Scheme 3). Changing the protic solvent to the more encumbered *t*-BuOH thwarted this undesirable reaction and led to clean conversions for both **7f** and **7g**, which were carried on to give **2f** and **2g**.

Toward the preparation of target **2h**, it was found that the initial intermediate **5e** was prepared most efficiently by running the reaction neat using **4** as both reactant and solvent. Subsequent chemistries from **5e** en route to **8h** proceeded smoothly following Scheme 1. Amide **2h** was prepared over three steps from ester **8h** (Scheme 4). Intermediate **8n**, generated by acid hydrolysis, was subjected to an amine coupling reaction, and the resulting amide **8o** was reduced to give **2h**.

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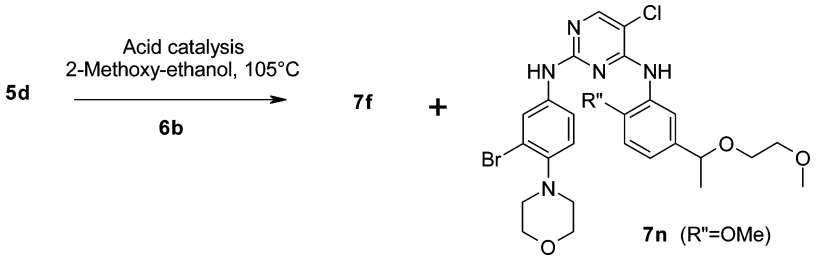
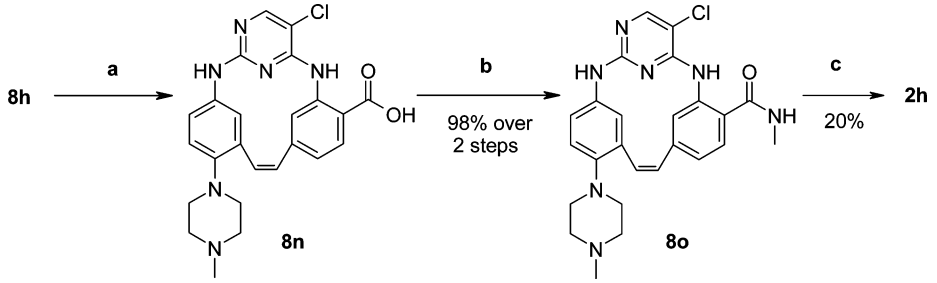
Scheme 3



Scheme 4. Method C[a]



[a](a) LiOH, H_2O, MeOH; (b) EDAC, HOBt, H_2NMe; (c) diimide.

Target **2k** was also prepared following the general protocol of Scheme 1. The syntheses of the last targeted group of macrocycles (**2i**, **2j**, **2l**, **2m**) were initiated with the preparations of anilines **3f–i** (Schemes 5 and 6). Anilines **3f–i** were treated with **4** under basic conditions to yield respective pyrimidines **5f–i**, all in reasonable yields. Subsequently, **5f–i** provided **2i**, **2j**, **2l**, and **2m** following Scheme 1.

The acyclic DAP analogues **1b** and **1c** were prepared as outlined in Scheme 7, following similar synthetic methods used for intermediates of the macrocycles illustrated in Scheme 1. Stepwise addition of the required anilines to **4** proceeded smoothly over two steps to provide the regiocontrolled products.

The general protocol to evaluate biological promise for the newly prepared compounds was to initially screen each for ALK enzyme inhibitory activity and subsequently select only the more potent enzyme inhibitors (ALK IC_{50} < 50 nM) for testing in the secondary ALK cellular assay, measuring inhibition of NPM-ALK phosphorylation. The compiled biological results for all target structures are outlined in Table 1.

With ALK being a member of the IR tyrosine kinase superfamily and recognizing that the IR has a vital role with a normal physiological function, a preferred ALK inhibitor would be devoid of IR kinase inhibition. This reasoning is supported by preclinical in vivo studies where it has been shown that inhibition of the IR resulted in variability of glucose homeostasis.[15] Therefore, an IR kinase enzyme assay was chosen as a primary kinase selectivity counterscreen within the testing paradigm for the targeted macrocycles. For simplicity of viewing the relative IR/ALK kinase selectivity profile, the related IR results listed in Table 1 are shown as a relative ratio to the reported ALK activity.

DISCUSSION AND CONCLUSIONS

The initial synthetic efforts were focused on confirming the feasibility of the planned synthetic route to generate macrocycles **2**, which was validated by the successful preparation of initial analogue **2a**. Not surprisingly, the simplified macrocycle **2a** did not demonstrate ALK inhibition at the highest testing concentration (ALK IC_{50} > 10 μM). However, from subsequent analogues prepared with singly R appended substitution on **2**, favorable ALK enzyme activity was observed; **2d** (R = 4-Me-piperazinyl; ALK IC_{50} = 92 nM) was more potent than **2c** (R = -OCH_2CH_2-N-pyrrolidine; ALK IC_{50} = 283 nM), with **2b** (R = 1-morpholinyl; ALK IC_{50} > 10 μM) proving inactive. Noteworthy, the activity of macrocycle **2d** (ALK IC_{50} = 92 ± 10 nM) was comparable to the activity of its parent DAP **1b** (ALK IC_{50} = 67 ± 13 nM). This was the first supportive experimental evidence that macrocycles **2** were constrained in a biologically desired conformation.

SAR evaluation of the R″ appended groups on **2** began with replacing hydrogen (**2a**) (ALK IC_{50} > 10 μM) with -OMe (**2e**) (ALK IC_{50} = 360 nM). The significant increase in ALK potency of **2e** was further enhanced ∼100-fold when the R = H of **2e** was replaced with R = 4-Me-piperazinyl (**2g**) (ALK IC_{50} = 3.1 nM). Analogue **2g** was the macrocycle that overlapped nicely with its DAP acyclic parent **1c** via molecular modeling (Figure 5), so it was interesting to find that **2g** proved greater than 5-fold more potent than **1c** in the ALK enzymatic assay. Along with improved target potency, **2g** showed an improved IR/ALK selectivity relative to **1c** (67× and 3.6×, respectively), suggesting that the conformationally constrained macrocycle avoids a preferred binding to IR. Unfortunately, the boost in enzymatic inhibition of macrocycle **2g** relative to DAP parent **1c** did not translate in the cellular assay, with both compounds displaying comparable activity (**2g**, cell IC_{50} = 150 nM; **1c**, cell IC_{50} = 100 nM). However, the fact that **2g** exhibited cellular activity was encouraging, as it was the first macrocycle screened in the cellular secondary assay and further validated the macrocycles. The lack of any observed ALK activity for macrocycle **2f** was surprising (ALK IC_{50} > 1000 nM), based on the positive results for analogues **2e** and **2g**, suggesting that the morpholine interacts with ALK differently from the piperazine moiety.

Having found that the most potent macrocycles shared the commonality of R as a 4-Me-piperazinyl moiety, additional SAR

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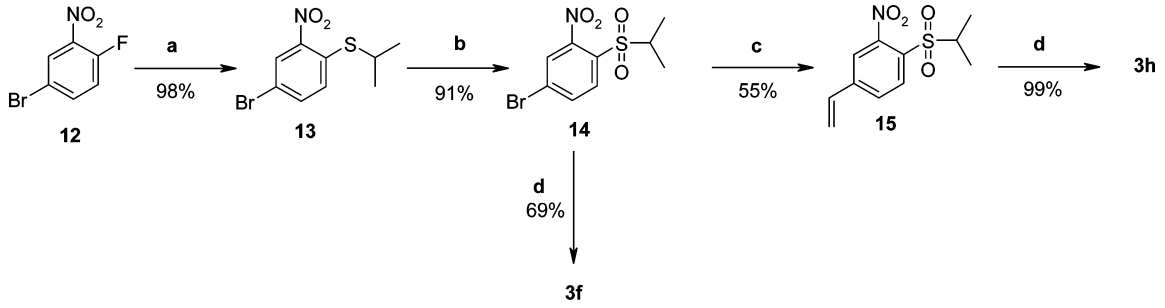
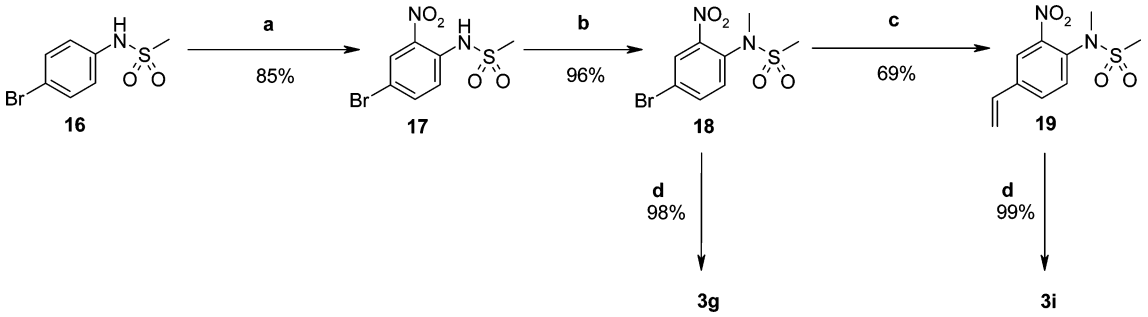
Scheme 5. Method D[a]



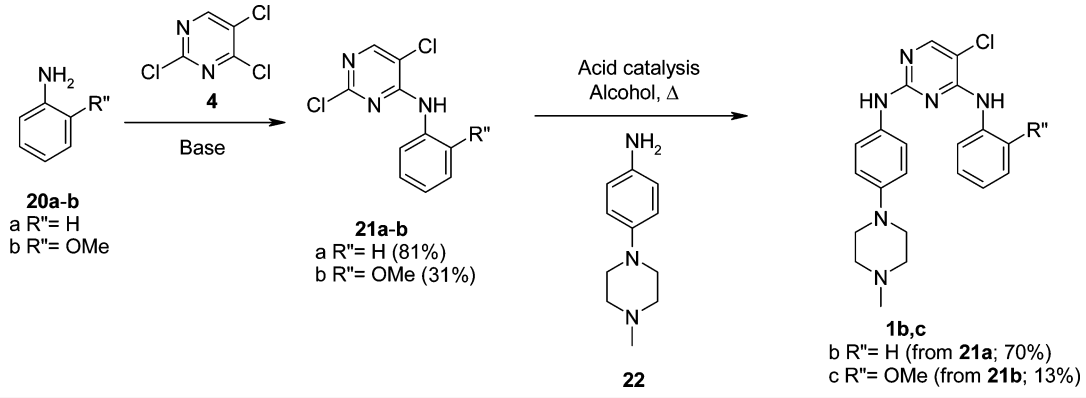
[a](a) 2-Propanethiol, EtOH, 50 °C, 72 h; (b) mCPBA, CH_2Cl_2, rt, 16 h; (c) vinylboronic acid dibutyl ester, $Pd(PPh_3)_4$, Na_2CO_3, glyme, H_2O, 80 °C; (d) Fe, AcOH, THF, 35 °C, 16 h.

Scheme 6. Method E[a]



[a](a) $(CF_3CO)_2O$, KNO_3, CH_3CN; (b) MeI, K_2CO_3, DMF; (c) vinylboronic acid dibutyl ester, $Pd(PPh_3)_4$, Na_2CO_3, glyme, H_2O, 90 °C, 16 h; (d) Fe, AcOH, THF, 35 °C, 16 h.

Scheme 7. Method F



studies on R′ and R″ were pursued holding R constant. Three additional R″ substituted macrocycles prepared for comparison to the methoxy-substituted analogue **2g** (ALK IC_{50} = 3.1 nM; cell IC_{50} = 150 nM) resulted in two comparably potent ALK inhibitors, sulfone **2i** (R″ = -SO_2-*i*-Pr; ALK IC_{50} = 3.7 nM; cell IC_{50} = 70 nM) and sulfonamide **2j** (R″ = -N(Me)SO_2Me; ALK IC_{50} = 3.4 nM; cell IC_{50} = 120 nM), with amide **2h** (R″= -CONHMe; ALK IC_{50} = 35 nM; cell IC_{50} = 1200 nM) proving about 10-fold less active. Also encouraging for sulfonamide **2j** was its favorable 75-fold IR/ALK selectivity ratio, while sulfone **2i** proved less selective with only a 10-fold IR/ALK ratio.

Having identified R,R″ substituted macrocycles exhibiting fairly robust ALK inhibition, we next turned our attention to the R′ substituent. The R′ substituent as a methoxy moiety has been recognized as a key ALK selectivity and potency determinant for DAPs.[10] This extrapolation to macrocycles **2** proved fruitful, as exemplified by comparing the activities of **2k** (ALK IC_{50} = 5 nM) to **2d** (ALK IC_{50} = 92 nM). The most promising compound from this exercise, and for the series as a whole, was sulfonamide **2m**, with subnanomolar ALK enzyme inhibition (IC_{50} = 0.51 nM) and low nanomolar ALK cellular activity (cell IC_{50} = 10 nM). Compound **2m** had concurrent improvements in both enzymatic (7-fold) and cellular (12-fold) activities when compared to its direct R′ desmethoxy analogue **2j** (IC_{50} = 3.4 nM; cell IC_{50} = 120 nM); these analogues proved the most definitive comparators from this series in showing overall potency improvement contributed by the R′ = methoxy group. Analogue **2m** also displayed the best overall kinase selectivity for the series

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with an IR/ALK ratio of 173, again illustrating the effectiveness of the R′ = methoxy.

Following the publication of apo and ligand bound crystal structures of ALK, an additional modeling study demonstrated a complementary fit of **2m** in the ALK binding pocket, supported by two favorable hydrogen bonding interactions at the hinge (Met-1199) and one with the salt-bridge Lys-1150 (Figure 6). Noteworthy, the binding mode of **2m** was also consistent with the DAP bound ALK structure (2XB7) in the Protein Data Bank (PDB).

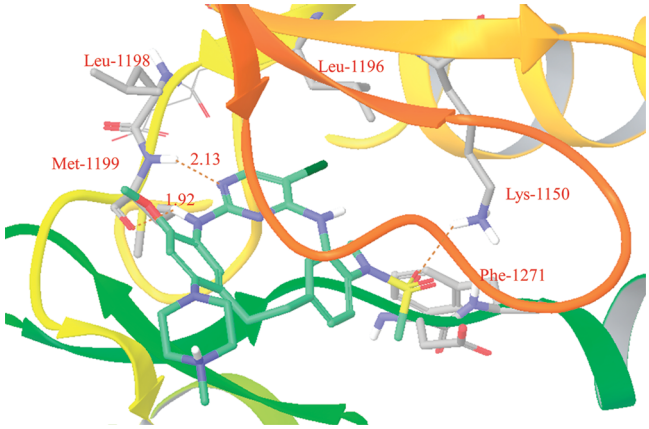

Figure 6. Glide/XP generated binding mode of compound **2m** docked in the ALK binding pocket, with the ALK structure retrieved from the PDB (PDB code 3LCT). Only the key residues of the ALK protein were made visible for clarity.

The last significant SAR observation was that olefin linked analogues **8** proved considerably less potent when compared to their respective ethylene linked macrocycles **2** [**8d** (ALK IC_{50} = 392 nM) relative to **2d** (ALK IC_{50} = 92 nM), **8g** (ALK IC_{50} = 259 nM) relative to **2g** (ALK IC_{50} = 3 nM), **8m** (ALK IC_{50} = 7 nM, cell IC_{50} = 120 nM) relative to **2m** (ALK IC_{50} = 0.5 nM, cell IC_{50} = 10 nM)]. A possible explanation for the loss of activity for analogues **8** may pertain to the olefinic sp^2 carbons adding further constraint to the macrocycle, leading to a less desirable conformer.

In conclusion, the synthetic and medicinal chemistry objectives outlined for macrocycles **2** were accomplished successfully. We demonstrated that conformationally constrained macrocycles **2** could be synthesized efficiently as inverted "U" shaped conformer mimics of DAPs **1** by employing a Heck reaction for the macrocyclization followed by selective diimide olefin reductions (Scheme 1). Subsequent comparison of ALK biological results for DAPs **1b** and **1c** relative to their respective constrained macrocycles **2d** and **2g** showed ALK activities to be fairly consistent, with a potential indication of improved kinase selectivity for the macrocycles. The similarity of biological activities between these directly related constrained macrocycles with their acyclic DAP parents is also good complementary evidence to the recently published DAP/ALK solid state cocrystal findings that an inverted "U" shaped conformer of DAPs is a preferred biologically active conformation. Finally, macrocycle **2m** was identified as an extremely potent ALK inhibitor with a favorable kinase selectivity profile relative to IR and proved to have the most promising overall profile from our initial set of 13 macrocycles prepared.

In addition to these findings demonstrating that macrocycles **2** are a useful template as an ALK scaffold, one may further speculate that macrocycles **2** might prove worthwhile as a scaffold for other therapeutic targets where encouraging activities have been found for DAPs, be it in the kinase area or otherwise.

EXPERIMENTAL SECTION

Chemistry. Table 1 lists all final products **2** and identifies the respective synthetic method(s) followed for their preparations. All final products **2** were characterized by 400 MHz ^{1}H NMR (Bruker Avance, in the solvent indicated, with tetramethylsilane as an internal standard), LC/MS (Bruker Esquire 2000 mass spectrometer with the Agilent 1100 HPLC equipped with an Agilent Eclipse XDB-C8, 2 mm × 30 mm 3.5 μm rt column, flow rate of 1.0 mL/min, and a solvent mixture of 10% (0.1% formic acid/water)/90% (acetonitrile/0.1% formic acid)), and HPLC (>98% purity at 215 and 254 nm by two methods, (a) Zorbax RX-C8, 5 mm × 150 mm column, eluting with a mixture of acetonitrile and water containing 0.1% trifluoroacetic acid with a gradient of 10−100% run over 5 min and (b) Agilent Eclipse XD8-C8, 4.6 mm × 150 mm column, eluting with a mixture of acetonitrile and water containing 0.1% trifluoroacetic acid with a gradient of 10−100% run over 20 min). Also, final products **2d**, **2f**, **2g**, **2i**, **2j**, **2l**, and **2m** were evaluated by high resolution mass spectrum (HRMS) analyses (Waters Synapt G2 Q-TOF mass spectrometer using leucine−enkephalin as a lock-mass standard). These HRMS were carried out internally at Cephalon. Reported melting points were taken on a Mel-Temp apparatus and are uncorrected. All spectral analysis results are included in the Experimental Section.

^{1}H NMR data of key intermediates were also obtained from the 400 MHz NMR instrument and are included in the Experimental Section. When indicated, compounds assayed by thin-layer chromatography were evaluated on Whatman MK6F (1 in. × 3 in. × 250 μm) silica gel plates.

A CEM Discover unit was used for microwave assisted reactions.

For purifications, automated normal phase column chromatography was performed on a CombiFlash Companion (ISCO, Inc.) using eluent solvent systems as defined for specific compounds. Reverse phase preparative HPLC was performed on a Gilson GX-281 equipped with Gilson 333 and 334 pumps using a Phenomenex 00F-4454-00-AX Gemini-NX 5 μm C18 column with mixtures of acetonitrile and water, both containing 0.1% trifluoroacetic acid, as the eluent. All reagents were commercially available unless otherwise specified, and all reactions were run under an inert atmosphere of Ar or N_2 unless otherwise specified.

Molecular Modeling. *Conformational Analysis.* Most of the computational work was done using the Schrodinger/Maestro molecular modeling package (Maestro, version 9.1.107, Schrodinger, LLC, New York, NY):

(1) Build 3D structures using LigPrep.
(2) Load all ligands in the Project Table, and select all.
(3) Set MacroModel Conformational Search Panel for Project Table (selected entries, using the OPLS_2005 force field and water as the implicit solvent, minimization method Polak−Ribiere conjugate gradient (PRCG), maximum iteration of 500, convergence gradient of 0.05, and 1000 steps of mixed torsion low mode sampling).

Docking Study. The essential steps in the docking experiment are summarized below:

(1) Prepare an ALK structure from the Protein Data Bank (PDB) (PDB code 3LCT)[16] using Maestro protein preparation workflow.
(2) Use the lowest energy conformation of the ligands for the docking study.
(3) Use Glide/XP flexible docking and keep the top 10 binding poses.
(4) Select the binding mode using our knowledge based approach.[17]

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Method A. *2-Methoxy-5-vinylphenylamine (**3d**).* To a room temperature (rt) mixture of 5-bromo-2-methoxyphenylamine (**3c**) (610 mg, 3.0 mmol), potassium carbonate (K_2CO_3) (1.73 g, 12.5 mmol), and tetrakis(triphenylphsosphine)palladium(0) ($Pd(PPh_3)_4$) (600 mg, 0.5 mmol) in 1,2-dimethoxyethane (18 mL) and water (3 mL) was added vinylboronic acid dibutyl ester (2.37 mL, 10.8 mmol). The mixture was then warmed to 90 °C for 16 h. The resulting mixture was concentrated under reduced pressure, and the residue was acidified with aqueous 1 N HCl. Attempted extraction with ethyl ether (Et_2O) or ethyl acetate (EtOAc) yielded a solid between the biphasic mix, which was filtered off. The aqueous phase of the filtrate was neutralized while cooling with 30% NaOH, and the resulting mixture was washed with ethyl acetate (EtOAc) (2×). The latter EtOAc extracts were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 320 mg (71%) of **3d** as an oil, which was used for subsequent steps without further manipulation. ^{1}H NMR (DMSO-d_6) δ 6.79–6.78 (d, 1H), 6.77–6.75 (d, 1H), 6.64–6.61 (m, 1H), 6.58–6.50 (m, 1H), 5.53–5.49 (d, 1H), 5.05–5.02 (d, 1H), 4.91 (bs, 2H), 3.75 (s, 3H).

*(3-Bromophenyl)-(2,5-dichloropyrimidin-4-yl)amine (**5a**).* 3-Bromoaniline (**3a**) (790 mg, 4.6 mmol), 2,4,5-trichloropyrimidine (**4**) (840 mg, 4.58 mmol), and K_2CO_3 (1.90 g, 14 mmol) in *N*,*N*-dimethylformamide (DMF) (20 mL) was warmed to 80 °C for 16 h. The mixture was cooled to rt and then diluted with water (220 mL). The resulting solid was filtered and rinsed with a small amount of water. After air drying, the solid was triturated with ice cold acetonitrile (CH_3CN) (10 mL), filtered, and rinsed with a small amount of ice cold CH_3CN to yield 720 mg (49%) of desired **5a** as a white solid, mp 146–148 °C. LC: 98%. LC/MS: M + H = 320.0. ^{1}H NMR (DMSO-d_6) δ 9.60 (s, 1H), 8.43 (s, 1H), 7.89–7.88 (m, 1H), 7.68–7.64 (m, 1H), 7.37–7.36 (m, 2H).

*(2,5-Dichloropyrimidin-4-yl)-3-vinylphenyl)amine (**5b**).* Compound **5b** was prepared in a similar manner as **5a** after substituting 3-vinylphenylamine (**3b**) for **3a**, substituting *N*,*N*-diisopropylethylamine (EtN(*i*-Pr)$_2$) for K_2CO_3, and altering the reaction temperature from 80 °C to rt. Workup varied in that after 16 h the mixture was diluted with water, which yielded an oil that crystallized to a solid. The solid was filtered, triturated with water, and refiltered to yield desired **5b** in 87% yield as a white solid, mp 87.5–90 °C. LC: 97%. LC/MS: M + H = 266.0. ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H), 7.69 (s, 1H), 7.59–7.56 (m, 1H), 7.40–7.37 (m, 1H), 7.28–7.26 (m, 2H), 6.79–6.71 (m, 1H), 5.85–5.80 (d, *J* = 17.7 Hz, 1H), 5.35–5.33 (d, *J* = 10.9 Hz, 1H).

*(5-Bromo-2-methoxyphenyl)-(2,5-dichloropyrimidin-4-yl)amine (**5c**).* Compound **5c** was prepared in a similar manner as **5a** after substituting 5-bromo-2-methoxyphenylamine (**3c**) for **3a** and altering the reaction time from 16 to 3 h. Workup varied in that after 16 h the resulting solid in the reaction mixture was filtered, rinsed with DMF, then water. The resulting solid was triturated with CH_3CN, filtered, and air-dried to yield desired **5c** in 54% yield as a white solid, mp 205–208 °C. LC: 99%. LC/MS: M + H = 349.9. ^{1}H NMR (DMSO-d_6) δ 9.00 (s, 1H), 8.38 (s, 1H), 7.81–7.80 (d, *J* = 2.48 Hz, 1H), 7.43–7.41 (m, 1H), 7.12–7.10 (d, *J* = 8.85 Hz, 1H), 3.82 (s, 3H).

*(2,5-Dichloropyrimidin-4-yl)-(2-methoxy-5-vinylphenyl)amine (**5d**).* Compounds **3d** (970 mg, 6.5 mmol), **4** (1.2 g, 6.5 mmol), and EtN(*i*-Pr)$_2$ (1.2 mL, 7.2 mmol) were combined in DMF (9 mL) at rt and stirred for 72 h. The reaction mixture was then diluted with ice cold water (100 mL). The resulting solid was filtered and rinsed liberally with water. The solid was further purified by trituration with methanol (2×) to yield desired **5d** in 66% yield as an off white solid. LC: 97%. LC/MS: M + H = 296.1. ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H), 8.36 (s, 1H), 7.70 (s, 1H), 7.36–7.35 (d, 1H), 7.13–7.11 (d, 1H), 6.73–6.66 (m, 1H), 5.72–5.68 (d, 1H), 5.19–5.17 (d, 1H), 3.82 (s, 3H).

*4-Bromo-2-(2,5-dichloropyrimidin-4-ylamino)benzoic acid methyl ester (**5e**).* Compound **5e** was prepared in a similar manner as **5b** after substituting 2-amino-4-bromobenzoic acid methyl ester (**3e**) for **3b**. In addition, rather than use of DMF as solvent, **4** (10 equiv) was used as the solvent as well as reactant, and the mixture was warmed to 120 °C rather than 80 °C for 16 h. The workup also varied in that the excess **4** was removed under high vacuum, and the residue was then treated with 1:1 MeOH/water. The resulting solid was filtered, retriturated with 1:1 MeOH/water, and refiltered, and the resulting solid was triturated in MeOH at 50 °C. After the mixture was filtered, the solid was rinsed liberally with MeOH. After air drying, **5e** was recovered in 52% yield as a brown solid. LC/MS: M + H = 377.9. ^{1}H NMR (DMSO-d_6) δ 11.25 (s, 1H), 8.84–8.83 (d, *J* = 1.89 Hz, 1H), 8.60 (s, 1H), 7.97–7.95 (d, *J* = 8.52 Hz, 1H), 7.50–7.47 (m, 1H), 3.90 (s, 3H).

*[5-Bromo-2-(propane-2-sulfonyl)phenyl]-(2,5-dichloropyrimidin-4-yl)amine (**5f**).* A mixture of **3f** (310 mg, 1.11 mmol) and **4** (153 μL, 1.34 mmol) in DMF (2 mL) at 0 °C was treated with neat NaH (89 mg, 2.2 mmol, 60% oil dispersion) over 30 s. After an hour at 0 °C, additional **4** (150 μL, 1.30 mmol) was added followed by neat NaH (160 mg, 4 mmol, 60% oil dispersion). After another hour the mixture was treated with saturated aqueous NH_4Cl, then diluted with water. The resulting brownish solid was filtered and washed liberally with water. The solid was then rinsed with ice cold CH_3CN (3 mL). The resulting 415 mg (87%) of tannish solid **5f** was used for subsequent steps without further manipulation. LC: 100%. LC/MS: M + H = 425.9. ^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H), 8.68 (bs, 1H), 8.60 (s, 1H), 7.82–7.79 (d, *J* = 8.49 Hz, 1H), 7.69–7.67 (d, *J* = 7.89 Hz, 1H), 3.59–3.56 (m, 1H), 1.18–1.17 (d, *J* = 7.76 Hz, 6 H).

*N-[4-Bromo-2-(2,5-dichloropyrimidin-4-ylamino)phenyl]-N-methylmethanesulfonamide (**5g**).* Compounds **3g** (550 mg, 2.0 mmol) and **4** (678 μL, 5.9 mmol) were combined with EtN(*i*-Pr)$_2$ (940 μL, 5.4 mmol) in *N*-methylpyrrolidinone (5 mL) and warmed to 100 °C for 2 days. The resulting mixture was concentrated under high vacuum and the residue treated with 1:1 H_2O/CH_3CN (8 mL). The resulting solid was filtered and rinsed with ice cold 1:1 H_2O/CH_3CN (2 mL), then with MeOH, yielding 587 mg (70%) of desired **5g** as a tannish solid, which was used for subsequent steps without further manipulation. LC: 95%. LC/MS: M + H = 426.9. ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H), 8.49 (s, 1H), 8.11 (s, 1H), 7.65–7.63 (d, *J* = 8.57 Hz, 1H), 7.56–7.54 (d, *J* = 8.50 Hz, 1H), 3.18 (s, 3H), 3.06 (s, 3H).

*(2,5-Dichloropyrimidin-4-yl)-[2-(propane-2-sulfonyl)-5-vinylphenyl]amine (**5h**).* Compound **5h** was prepared in a similar manner as **5e** after substituting **3h** for **3e**. The workup varied in that after concentration of the reaction mixture the residue was partitioned between $CHCl_3$ (2×) and water. The organic phase was dried over Na_2SO_4, filtered, and the crude product in the filtrate was adsorbed directly onto silica gel. The crude material was purified via normal phase chromatography (EtOAc/hexane eluent) to give desired **5h** in 51% yield as a yellow solid. LC: 100%. LC/MS: M + H = 372.1. ^{1}H NMR (DMSO-d_6) δ 9.79 (s, 1H), 8.55–8.32 (d, 1H), 7.85–7.83 (bs, 1H), 6.89–6.82 (m, 1H), 6.07–6.03 (d, 1H), 5.57–5.54 (d, 1H), 3.52 (bm, 1H), 1.17–1.15 (d, 6H).

*N-[2-(2,5-Dichloropyrimidin-4-ylamino)-4-vinylphenyl]-N-methylmethanesulfonamide (**5i**).* Compound **5i** was prepared in a similar manner as **5e** after substituting **3i** for **3e**. The workup varied in that after concentration of the reaction mixture the residue was partitioned between EtOAc and water. The organic phase was dried over Na_2SO_4, filtered, and the crude product in the filtrate was adsorbed directly onto silica gel. The crude material was purified via normal phase chromatography (EtOAc/hexane eluent) to give desired **5i** in 50% yield as a yellow solid. LC: 93%. LC/MS: M + H = 373.2. ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H, exchangeable), 8.45 (s, 1H), 7.93 (s, 1H), 7.64–7.62 (d, 1H), 7.47–7.45 (d, 1H), 6.82–6.75 (m, 1H), 5.91–5.87 (d, 1H), 5.40–5.37 (d, 1H), 3.17 (s, 3H), 3.04 (s, 3H).

*N(4)-(3-Bromophenyl)-5-chloro-N(2)-(3-vinylphenyl)pyrimidine-2,4-diamine (**7a**).* **5a** (318 mg, 1.0 mmol), 3-vinylphenylamine (**6a**) (119 mg, 1.0 mmol), and 4.0 M HCl in dioxane (275 μL, 1.10 mmol) were combined in 2-methoxyethanol (10 mL) and warmed to 110 °C for 16 h. The resulting solution was concentrated under reduced pressure, and the residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was triturated with CH_3CN, filtered, and rinsed with a small amount of CH_3CN to yield 297 mg (74%) of desired **7a** as an off white solid, mp 142–145 °C. LC: 96%. LC/MS: M + H = 402.9. ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 7.84 (s, 1H), 7.57–7.55 (d, *J* = 8.33 Hz, 1H), 7.51–7.49

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(m, 2H), 7.34−7.28 (m, 2H), 7.24−7.20 (m, 1H), 7.15−7.13 (d, J = 7.60 Hz, 1H), 7.07 (bs, 1H), 7.01 (bs, 1H), 6.71−6.64 (m, 1H), 5.72−5.67 (d, J = 17.69 Hz, 1H), 5.26−5.23 (d, J = 11.11 Hz, 1H).

*N(2)-(3-Bromo-4-morpholin-4-ylphenyl)-5-chloro-N(4)-3-vinylphenyl)pyrimidine-2,4-diamine (**7b**).* Compound **7b** was prepared in a similar manner as **7a** after substituting **5b** for **5a** and 3-bromo-4-morpholin-4-ylphenylamine (**6b**) for **6a**. Also, EtOAc was used for the extractive workup rather than $CHCl_3$. The desired product **7b** was isolated in 71% as a white solid, mp 142−144 °C. LC: 91%. LC/MS: M + H = 406.1. ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 1H), 8.86 (s, 1H), 8.16 (s, 1H), 7.85 (s, 1H), 7.70 (s, 1H), 7.63−7.60 (m, 2H), 7.38−7.34 (t, 1H), 7.28−7.26 (d, 1H), 6.99−6.96 (d, 1H), 6.76−6.68 (m, 1H), 5.81−5.77 (d, 1H), 5.27−5.24 (d, 1H), 3.73−3.71 (m, 4H), 2.88−2.86 (m, 4H).

*N(2)-[3-Bromo-4-(2-pyrrolidin-1-ylethoxy)phenyl]-5-chloro-N(4)-(3-vinylphenyl)pyrimidine-2,4-diamine (**7c**).* Compound **7c** was prepared in a similar manner as **7b** after substituting **6c** for **6b** and substituting methanesulfonic acid for 4.0 M HCl in dioxane. Also, $CHCl_3$ was used for the extractive workup rather than EtOAc. The desired product **7c** was isolated in 52% as a white solid. LC/MS: M + H = 515.4

*N(2)-[3-Bromo-4-(4-methylpiperain-1-yl)phenyl]-5-chloro-N(4)-(3-vinylphenyl)pyrimidine-2,4-diamine (**7d**).* Compound **7d** was prepared in a similar manner as **7b** after substituting **6d** for **6b**. Also, $CHCl_3$ was used for the extractive workup rather than EtOAc. The desired product **7d** was isolated in 62% as a tan solid, mp 149−153 °C. LC: 93%. LC/MS: M + H = 501.0. ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 1H), 8.85 (s, 1H), 8.16 (s, 1H), 7.83 (s, 1H), 7.70 (s, 1H), 7.61−7.58 (m, 2H), 7.37−7.33 (t, 1H), 7.28−7.26 (d, 1H), 6.97−6.95 (d, 1H), 6.75−6.68 (m, 1H), 5.81−5.77 (d, 1H), 5.27−5.24 (d, 1H), 2.87 (s, 4H), 2.46 (s, 4H), 2.23 (s, 3H).

*N(4)-(5-Bromo-2-methoxyphenyl]-5-chloro-N(2)-(3-vinylphenyl)-pyrimidine-2,4-diamine (**7e**).* Compound **7e** was prepared in a similar manner as **7a** after substituting **5c** for **5a** and heating the mixture for 8 h rather than 16 h. The desired product **7e** was isolated in 76% as a white solid, mp 183−185 °C. LC: 97%. LC/MS: M + H = 432.9. ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1H), 8.26 (s, 1H), 8.19 (s, 1H), 8.09 (bs, 1H), 7.56−7.54 (m, 2H), 7.36−7.33 (m, 1H), 7.22−7.18 (m, 1H), 7.11−7.08 (d, J = 8.78 Hz, 1H), 7.06−7.04 (d, J = 7.71 Hz, 1H), 6.61−6.54 (m, 1H), 5.68−5.64 (d, J = 17.55 Hz, 1H), 5.22−5.19 (d, J = 10.96 Hz, 1H), 3.83 (s, 3H).

*N(2)-[3-Bromo-4-morpholin-4-ylphenyl]-5-chloro-N(4)-(2-methoxy-5-vinylphenyl)pyrimidine-2,4-diamine (**7f**).* Compound **7f** was prepared in a similar manner as **7b** after substituting **5d** for **5b** and after altering the solvent system from 2-methoxyethanol to a 1:1 mixture of *tert*-butyl alcohol/1,2-dimethoxyethane (120 mL of solvent for a mmol reaction). The reaction mixture was warmed for 6 days at 85 °C, followed by the standard workup using EtOAc for the extractive workup. Some desired **7f** crystallized relatively pure from EtOAc on concentrating the filtrate, amd the remainder was purified via normal phase chromatography to yield the desired product **7f** in 55% overall yield as a yellow tinted solid, mp 186−190 °C. LC: 100%. LC/MS: M + H = 518.0. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 1H), 8.23 (s, 1H), 8.16 (s, 1H), 7.95 (s, 1H), 7.8 (s, 1H), 7.58−7.57 (m, 1H), 7.33−7.32 (m, 1H), 7.13−7.11 (d, 1H), 6.96−6.94 (d, 1H), 6.7−6.6 (m, 1H), 5.67−5.62 (d, 1H), 5.13−5.10 (d, 1H), 3.85 (s, 3H), 3.74−3.71 (m, 4H), 2.88−2.85 (m, 4H).

*N(2)-[3-Bromo-4-(4-methylpiperazin-1-yl)phenyl]-5-chloro-N(4)-(2-methoxy-5-vinylphenyl)pyrimidine-2,4-diamine (**7g**).* Compound **7g** was prepared in a similar manner as **7f** after substituting **6d** for **6b**. After the reaction mixture was concentrated, the crude mix was purified straightaway via preparative HPLC. The purest fractions were then partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give desired **7g** in 20% yield. This material was used without further manipulation for subsequent cyclization to **8g**. LC: 93%. LC/MS: M + H = 531.0.

*4-Bromo-2-(5-chloro-2-[4-(4-methylpiperazin-1-yl)-3-vinylphenylamino]pyrimidin-4-ylamino)benzoic Acid Methyl Ester (**7h**).* Compound **7h** was prepared in a similar manner as **7a** after substituting **5e** for **5a** and after substituting **6e** for **6a**. Also, methanesulfonic acid was used rather than 4.0 M HCl in dioxane. The desired product **7h** was isolated in 52% as a tan solid and was used for subsequent cyclization to **8h** without further manipulation. LC: 98%. LC/MS: M + H = 559.0. ^{1}H NMR (DMSO-d_6) δ 11.04 (s, 1H), 9.49 (s, 1H), 8.98 (s, 1H), 8.30 (s, 1H), 7.95−7.93 (d, J = 8.56 Hz, 1H), 7.60−7.56 (m, 2H), 7.35−7.32 (d, J = 10.16 Hz, 1H), 7.07−7.05 (d, J = 8.57 Hz, 1H), 7.01−6.93 (m, 1H), 5.58−5.53 (d, J = 17.37 Hz, 1H), 5.21−5.18 (d, J = 11.81 Hz, 1H), 3.91 (s, 3H), 2.84 (s, 4H), 2.5 (bs, 4H + DMSO), 2.23 (s, 3H).

*N(4)-[5-Bromo-2-(propane-2-sulfonyl)phenyl]-5-chloro-N(2)-[4-(4-methylpiperazin-1-yl)-3-vinylphenyl]pyrimidine-2,4-diamine (**7i**).* Compounds **5f** (150 mg, 0.35 mmol) and **6e** (77 mg, 0.35 mmol) and methanesulfonic acid (33 μL, 0.50 mmol) were combined in 2-methoxyethanol (4 mL) and warmed to 110 °C for 3 h. The resulting solution was concentrated under reduced pressure and the residue partitioned between $CHCl_3$ (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The 270 mg of remaining brown oil was dissolved in CH_3CN (3 mL). The solid which crystallized was filtered and rinsed with a small amount of ice cold CH_3CN to yield 90 mg (40%) of desired **7i** as a light brown solid, which was used without further manipulation for its subsequent cyclization to **8i**. LC: 92%. LC/MS: M + H = 607.0.

*N-(4-Bromo-2-{5-chloro-2-[4-(4-methylpiperazin-1-yl)-3-vinylphenylamino]pyrimidin-4-ylamino}phenyl)-N-methylmethanesulfonamide (**7j**).* Compounds **5g** (213 mg, 0.50 mmol) and **6e** (109 mg, 0.50 mmol) and methanesulfonic acid (46 μL, 0.71 mmol) were combined in 2-methoxyethanol (6 mL) and warmed to 110 °C for 4 h. The resulting solution was concentrated under reduced pressure and the residue partitioned between EtOAc (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The remaining brown oil was dissolved in CH_3CN (3 mL). The solid which crystallized was filtered and rinsed with a small amount of ice cold CH_3CN to yield 118 mg (39%) of desired **7j** as an off white solid, which was used without further manipulation for its subsequent cyclization to **8j**. LC: 92%. LC/MS: M + H = 608.1. ^{1}H NMR (DMSO-d_6) δ 9.40 (s, 1H), 8.45 (s, 1H), 8.39 (s, 1H), 8.23 (s, 1H), 7.60−7.58 (m, 3H), 7.41−7.39 (d, J = 8.60 Hz, 1H), 7.05−7.03 (d, J = 8.32 Hz, 1H), 7.00−6.92 (m, 1H), 5.55−5.51 (d, J = 17.81 Hz, 1H), 5.21−5.18 (d, J = 11.24 Hz, 1H), 3.19 (s, 3H), 3.12 (s, 3H), 2.83 (bs, 4H), 2.50 (bs, 4H + DMSO), 2.24 (s, 3H).

*N(2)-[5-Bromo-2-methoxy-4-(4-methylpiperazin-1-yl)phenyl]-5-chloro-N(4)-(3-vinylphenyl)pyrimidine-2,4-diamine (**7k**).* Compound **7k** was prepared in a similar manner as **7a** after substituting **5b** for **5a**, substituting **6f** for **6a**, and heating the mixture for 8 h rather than 16 h. The workup also varied in that after concentrating the reaction mixture, the crude mix was purified straightaway via preparative HPLC. The purest fractions were then partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give desired **7k** as an oil in 10% yield. This material was used without further manipulation for its subsequent cyclization to **8k**. LC: 95%. LC/MS: M + H = 530.9.

*N(2)-[5-Bromo-2-methoxy-4-(4-methylpiperazin-1-yl)phenyl]-5-chloro-N(4)-[2-(propane-2-sulfonyl)-5-vinylphenyl]pyrimidine-2,4-diamine (**7l**).* Compounds **5h** (200 mg, 0.54 mmol) and **6f** (177 mg, 0.59 mmol) and methanesulfonic acid (45.3 μL, 0.70 mmol) were combined in 2-methoxyethanol (3 mL) and warmed to 105 °C for 3 h. The resulting solution was concentrated under reduced pressure, dissolved in DMSO, and purified via preparative HPLC. The purest fractions of desired **7l** were combined and partitioned between EtOAc (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The 215 mg of yellow residue was triturated with ice cold CH_3CN, filtered, and rinsed with a small amount of ice cold CH_3CN to yield 70 mg (20%) of desired **7l** as an off white solid, which was used without further manipulation for its subsequent cyclization to **8l**. LC: 99%. LC/MS: M + H = 637.1. ^{1}H NMR (DMSO-d_6) δ 9.48 (s, 1H, exchangeable), 8.53 (s, 1H), 8.46 (s, 1H, exchangeable), 8.32 (s, 1H), 7.78−7.76 (m, 2H), 7.49−7.47 (d, J = 7.64 Hz, 1H), 6.80 (s, 1H),

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6.67−6.57 (m, 1H), 5.87−5.83 (d, J = 17.72 Hz, 1H), 5.40−5.37 (d, J = 10.85 Hz, 1H), 3.78 (s, 3H), 3.46−3.41 (m, 1H), 2.97 (s, 4H), 2.5 (s, 4H + DMSO), 2.24 (s, 3H), 1.18−1.16 (d, J = 6.68 Hz, 6H).

*N-(2-{2-[5-Bromo-2-methoxy-4-(4-methylpiperazin-1-yl)-phenylamino]-5-chloropyrimidin-4-ylamino}-4-vinylphenyl)-N-methylmethanesulfonamide (**7m**).* Compounds **5i** (192 mg, 0.52 mmol) and **6f** (170 mg, 0.57 mmol) and methanesulfonic acid (43.4 μL, 0.67 mmol) were combined in 2-methoxyethanol (3 mL) and warmed to 105 °C for 4 h. The resulting solution was concentrated under reduced pressure, dissolved in DMSO, and purified via preparative HPLC. The purest fractions of desired **7m** were combined and partitioned between EtOAc (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The 270 mg of yellow residue was triturated with ice cold CH_3CN, filtered, and rinsed with a small amount of ice cold CH_3CN to yield 110 mg (34%) of desired **7m** as an off white solid, which was used without further manipulation for its subsequent cyclization to **8m**. LC: 98%. LC/MS: M + H = 638.0. ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H, exchangeable), 8.23 (s, 1H, exchangeable), 8.21 (s, 1H), 8.18 (s, 1H), 7.82 (s, 1H), 7.58−7.56 (d, J = 8.25 Hz, 1H), 7.36−7.34 (d, J = 8.04 Hz, 1H), 6.81 (s, 1H), 6.61−6.54 (m, 1H), 5.77−5.72 (d, J = 17.69 Hz, 1H), 5.27−5.25 (d, J = 10.77 Hz, 1H), 3.79 (s, 3H), 3.18 (s, 3H), 3.10 (s, 3H), 2.98 (s, 4H), 2.5 (s, 4H + DMSO), 2.24 (s, 3H).

*N(2)-(3-Bromo-4-morpholin-4-ylphenyl)-5-chloro-N(4)-{2-methoxy-5-[1-(2-methoxyethoxy)ethyl]phenyl}pyrimidine-2,4-diamine (**7n**).* **7n** is the side product that was isolated from initial reaction toward **7f** when 2-methoxyethanol was used as solvent. LC: 95%. LC/MS: M + H = 594.1. ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H), 8.49 (s, 1H), 8.15 (s, 1H), 7.70−7.68 (m, 2H), 7.52−7.49 (m, 1H), 7.19−7.16 (m, 1H), 7.13−7.11 (d, J = 8.56 Hz, 1H), 6.98−6.95 (d, J = 8.80 Hz, 1H), 4.40−4.36 (m, 1H), 3.80 (s, 3H), 3.73−3.71 (m, 4H), 3.38−3.30 (m, 4H), 3.18 (s, 3H), 2.87−2.85 (m, 4H), 1.31−1.30 (d, J = 6.41 Hz, 3H).

*(14Z)-6-Chloro-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]-docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene (**8a**).* Palladium acetate ($Pd(OAc)_2$) (40 mg, 0.2 mmol), tri-*o*-tolylphosphine (240 mg, 0.79 mmol), and DAP **7a** (150 mg, 0.37 mmol) were combined with CH_3CN (4 mL) and triethylamine (Et_3N) (300 μL, 2 mmol). The mixture was treated under microwave irradiation at 120 °C for 30 min. After the mixture was cooled, the resulting solid was filtered and rinsed with ice cold CH_3CN to yield 78 mg (65%) of desired **8a** as an off white solid, mp >250 °C. TLC: 25% EtOAc/hexane R_f = 0.25; 5:1 CH_2Cl_2/MeOH R_f = 0.85. LC: 96%. LC/MS: M + H = 321.2. ^{1}H NMR (DMSO-d_6) δ 9.37 (s, 1H, exchangeable), 9.12 (s, 1H, exchangeable), 8.94 (s, 1H), 8.65 (s, 1H), 8.15 (s, 1H), 7.32−7.28 (m, 1H), 7.22−7.19 (m, 2H), 7.04 (bm, 2H), 6.93−6.91 (d, J = 7.6 Hz, 1H), 6.64−6.55 (m, 2H).

*(14Z)-6-Chloro-17-(morpholin-4-yl)-2,4,8,22-tetraazatetracyclo-[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene (**8b**).* Compound **8b** was prepared in a similar manner as **8a** after substituting **7b** for **7a**. In addition, after the CH_3CN trituration, the sample was also triturated with ethyl ether (Et_2O). The desired product **8b** was isolated in 43% yield as an off white solid, mp 267−270 °C. LC: 95%. LC/MS: M + H = 406.1. ^{1}H NMR (DMSO-d_6) δ 9.18 (s, 1H), 9.03 (s, 1H), 8.65 (s, 1H), 8.44−8.43 (d, 1H), 8.10 (s, 1H), 7.27−7.23 (t, 1H), 7.18−7.16 (d, 1H), 7.01−6.97 (m, 2H), 6.95−6.93 (d, 1H), 6.85−6.82 (d, 1H), 6.72−6.68 (d, 1H), 3.76−3.75 (m, 4H), 2.84 (m, 4H).

*(14Z)-6-Chloro-17-[2-(pyrrolidin-1-yl)ethoxy]-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,14,16,18-decaene (**8c**).* Compound **8c** was prepared in a similar manner as **8a** after substituting **7c** for **7a**. The desired product **8c** was isolated in 62% yield as a copper colored solid. LC: 100%. LC/MS: M + H = 434.1. ^{1}H NMR (DMSO-d_6) δ 9.7 (bs, 1H), 9.24 (s, 1H), 9.11 (s, 1H), 8.63 (s, 1H), 8.51 (d, 1H), 8.12 (s, 1H), 7.29−7.25 (t, 1H), 7.21−7.19 (d, 1H), 7.04−6.99 (m, 2H), 6.96−6.94 (d, 1H), 6.89−6.86 (d, 1H), 6.73−6.70 (d, 1H), 4.28−4.26 (m, 2H), 3.64−3.56 (bm, 2H + water), 3.18 (m, 2H), 2.07 (bm, 2H), 1.91−1.89 (m, 2H).

*(14Z)-6-Chloro-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,14,16,18-decaene (**8d**).* Compound **8d** was prepared in a similar manner as **8a** after substituting **7d** for **7a**. The desired product **8d** was isolated in 75% yield as an off white solid. LC: 92%, LC/MS: M + H = 419.1.

A 25 mg sample of the 92% pure material was further purified via preparative HPLC to return 17 mg of **8d** as a TFA salt white lyophylate, which was the sample used for biological testing. LC: 100%. LC/MS: M + H = 419.1. ^{1}H NMR (DMSO-d_6) δ 9.58 (bs, 1H), 9.29 (s, 1H), 9.12 (s, 1H), 8.61 (s, 1H), 8.45−8.44 (d, 1H), 8.12 (s, 1H), 7.29−7.25 (t, 1H), 7.20−7.18 (d, 1H), 7.02−6.97 (m, 3H), 6.83−6.80 (d, 1H), 6.75−6.72 (d, 1H), 3.74 (bs, H_2O), 3.54−3.51 (d, 2H), 3.29−3.22 (m, 4H), 2.93−2.89 (m, 5H).

*(14Z)-10-Methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]-docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene (**8e**).* Compound **8e** was prepared in a similar manner as **8a** after substituting **7e** for **7a**. The workup varied slightly in that the initial solid collected was dissolved in tetrahydrofuran (THF), filtered, and the filtrate was concentrated under reduced pressure. The resulting solid was triturated in CH_3CN, filtered, and rinsed with CH_3CN followed by Et_2O. The desired product **8e** was isolated in 51% yield as a tannish solid, mp 273−275 °C. LC: 99%. LC/MS: M + H = 351.1. ^{1}H NMR (DMSO-d_6) δ 9.49 (s, 1H), 8.99 (s, 1H), 8.80 (bs, 1H), 8.21 (s, 1H), 7.83 (s, 1H), 7.25−7.21 (m, 1H), 7.08−7.04 (m, 3H), 6.94−6.92 (d, J = 7.37 Hz, 1H), 6.55−6.44 (m, 2H), 3.91 (s, 3H).

*(14Z)-6-Chloro-10-methoxy-17-(morpholin-4-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,14,16,18-decaene (**8f**).* Compound **8f** was prepared in a similar manner as **8a** after substituting **7f** for **7a**. The desired product **8f** was isolated in 53% yield as a gray solid. LC: 87%. LC/MS: M + H = 436.2.

*(14Z)-6-Chloro-10-methoxy-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,14,16,18-decaene (**8g**).* Compound **8g** was prepared in a similar manner as **8a** after substituting **7g** for **7a**. The desired product **8g** was isolated in 94% yield as an off white solid. LC: 94%, M + H = 449.1.

A small sample of the 94% pure material was further purified via preparative HPLC to return pure **8g** as a TFA salt white lyophylate, which was the sample used for biological testing. LC: 99%. LC/MS: M + H = 449.1. ^{1}H NMR (DMSO-d_6) δ 9.6 (bs, 1H), 9.38 (s, 1H), 9.04−9.03 (d, 1H), 8.41−8.40 (d, 1H), 8.19 (d, 1H), 7.84 (s, 1H), 7.11−7.01 (m, 4H), 6.68−6.60 (m, 2H), 3.90 (s, 3H), 3.5−3.2 (bm, 6H + H_2O), 2.96−2.89 (m, 5H).

*Methyl (14Z)-6-Chloro-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,14,16,18-decaene-10-carboxylate (**8h**).* Compound **8h** was prepared in a similar manner as **8a** after substituting **7h** for **7a**. The desired product **8h** was isolated in 54% yield as a bronze solid. LC: 100%. LC/MS: M + H = 477.1. ^{1}H NMR (DMSO-d_6) δ 10.72 (s, 1H), 9.59 (s, 1H), 9.42 (s, 1H), 8.39 (s, 1H), 8.23 (s, 1H), 7.97−7.95 (d, 1H), 7.13−7.06 (m, 2H), 7.01−6.99 (d, 1H), 6.94−6.90 (d, 1H), 6.68−6.65 (d, 1H), 3.91 (s, 3H), 2.86 (s, 4H), 2.50 (s, 4H + DMSO), 2.25 (s, 3H).

*(14Z)-6-Chloro-10-(propane-2-sulfonyl)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,14,16,18-decaene Trifluoroacetate (1:1) (**8i**).* $Pd(OAc)_2$ (48 mg, 0.21 mmol), tri-*o*-tolylphosphine (275 mg, 0.90 mmol), and DAP **7i** (160 mg, 0.26 mmol) were combined with CH_3CN (4 mL) and Et_3N (250 μL, 1.8 mmol). The mixture was treated under microwave irradiation at 120 °C for 120 min. After cooling, the resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The concentrated residue was partitioned between 1 N HCl and EtOAc (2×). The aqueous phase was cooled and neutralized with KOH solution until ~pH 7 and then diluted with saturated aqueous $NaHCO_3$. The resulting basic solution was extracted with $CHCl_3$ (2×). The combined $CHCl_3$ extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via preparative HPLC and the purest fractions were combined and lyophilized to yield desired **8i** as its TFA salt in 32% yield. This material was carried on for subsequent conversion to **2i** without further manipulation. LC: 100%. LC/MS: M + H = 525.1. ^{1}H NMR (DMSO-d_6) δ 9.65 (bs, 1H), 9.55 (s, 1H), 9.48 (s, 1H),

9.26–9.25 (d, 1H), 8.46–8.45 (d, 1H), 8.28 (s, 1H), 7.77–7.75 (d, 1H), 7.33–7.31 (m, 1H), 7.12–7.05 (m, 2H), 7.02–6.98 (d, 1H), 6.77–6.74 (d, 1H), 4.1 (bs, H_2O), 3.54–3.51 (d, 2H), 3.42–3.38 (m, 1H), 3.30–3.22 (m, 4H), 2.96–2.89 (m, 5H), 1.17–1.15 (d, 6H).

*(14Z)-6-Chloro-10-(2-methanesulfonylmethylamino)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]-docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene Trifluoroacetate (1:1) (**8j**).* $Pd(OAc)_2$ (24 mg, 0.10 mmol), tri-*o*-tolylphosphine (140 mg, 0.45 mmol), and DAP **7j** (80 mg, 0.13 mmol) were combined with CH_3CN (2 mL) and Et_3N (120 μL, 0.9 mmol). The mixture was treated under microwave irradiation at 120 °C for 45 min. By LC analysis, the reaction had not proceeded to completion, so the reaction mixture was filtered and the solution recharged with second portions of $Pd(OAc)_2$ (35 mg, 0.16 mmol), tri-*o*-tolylphosphine (170 mg, 0.56 mmol), and Et_3N (160 μL, 1.1 mmol). The mixture was again treated under microwave irradiation at 120 °C for 120 min. After cooling, the resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The concentrated residue was partitioned between 1 N HCl (1.4 mL) and EtOAc (2×). The aqueous phase was purified directly via preparative HPLC and the purest fractions were combined and lyophilized to yield desired **8j** as its TFA salt in 37% yield. This material was carried on for subsequent conversion to **2j** without further manipulation. LC: 100%. LC/MS: M + H = 526.1. ^{1}H NMR (DMSO-d_6) δ 9.80 (bs, 1H), 9.42 (s, 1H), 8.89 (s, 1H), 8.42 (s, 1H), 8.23 (s, 1H), 8.20 (s, 1H), 7.58–7.56 (d, 1H), 7.20–7.18 (d, 1H), 7.07–7.01 (m, 2H), 6.88–6.84 (d, 1H), 6.74–6.71 (d, 1H), 3.53–3.51 (d, 2H), 3.35–3.21 (m, 7H), 3.09 (s, 3H), 2.97–2.91 (m, 2H), 2.89 (s, 3H).

*(14Z)-6-Chloro-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,14,16,18-decaene (**8k**).* Compound **8k** was prepared in a similar manner as **8a** after substituting **7k** for **7a**. The workup varied slightly in that no product precipitated from the reaction mixture; therefore, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between 1 N HCl and EtOAc (2×). The aqueous phase was neutralized and extracted with $CHCl_3$ (3×). The $CHCl_3$ extracts were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude **8k** in 51% yield, which was carried on for subsequent reduction toward **2k** without further manipulation. LC/MS: M + H = 449.0.

*(14Z)-6-Chloro-10-(propane-2-sulfonyl)-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]-docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene (**8l**).* $Pd(OAc)_2$ (6.5 mg, 0.03 mmol) was dissolved in CH_3CN (3 mL). Then tri-*o*-tolylphosphine (55 mg, 0.18 mmol) was added and the mixture was stirred at rt for 15 min under Ar. DAP **7l** (60 mg, 0.094 mmol) and Et_3N (92 μL, 0.66 mmol) were added to the resulting reaction mixture. The mixture was treated under microwave irradiation at 120 °C for 15 min. After cooling, the resulting solution was filtered through a Phenomenex Strat-X-C cation catch and release resin, which was then rinsed twice with CH_3CN followed by MeOH. The resin cartridge was transferred to a new filter flask, and the resin was rinsed twice with 2 M NH_3 in MeOH. The latter methanol solution was concentrated under reduced pressure to give a yellow residue. This material was treated with CH_3CN (2 mL) to yield a yellow solid, which was filtered and rinsed with a small amount of ice cold CH_3CN to give 33 mg (63%) of desired **8l** as a yellow solid. LC: 97%. LC/MS: M + H = 555.1. ^{1}H NMR (DMSO-d_6) δ 9.49 (s, 1H, exchangeable), 9.27 (s, 1H), 8.42 (s, 1H), 8.25 (s, 1H), 8.16 (s, 1H, exchangeable), 7.74–7.72 (d, *J* = 8.21 Hz, 1H), 7.28–7.26 (d, 8.00 Hz, 1H), 6.92–6.89 (d, *J* = 13.08 Hz, 1H), 6.72 (s, 1H), 6.62–6.58 (d, *J* = 13.20 Hz, 1H), 3.87 (s, 3H), 3.40–3.37 (m, 1H), 2.92 (s, 4H), 2.54 (s, 4H), 2.25 (s, 3H), 1.17–1.15 (d, *J* = 6.76 Hz, 6H).

*(14Z)-6-Chloro-10-(2-methanesulfonylmethylamino)-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo-[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,14,16,18-decaene (**8m**).* $Pd(OAc)_2$ (6.5 mg, 0.03 mmol) was dissolved in CH_3CN (3 mL). Then tri-*o*-tolylphosphine (55 mg, 0.18 mmol) was added and the mixture was stirred at rt for 15 min under Ar. DAP **7m** (90 mg, 0.14 mmol) and Et_3N (300 μL, 2 mmol) were added to the resulting reaction mixture. The mixture was treated under microwave irradiation at 120 °C for 15 min. After cooling, the resulting solution was filtered through a Phenomenex Strat-X-C cation catch and release resin, which was then rinsed twice with CH_3CN followed by MeOH. The resin cartridge was transferred to a new filter flask, and the resin was rinsed twice with 2 M NH_3 in MeOH. The latter methanol solution was concentrated under reduced pressure to give a yellow residue. This material was treated with CH_3CN (2 mL) to yield a yellow solid, which was filtered and rinsed with a small amount of ice cold CH_3CN to give 57 mg (72%) of desired **8m** as a yellow solid, mp 278–280 °C. LC: 97%. LC/MS: M + H = 556.1. ^{1}H NMR (DMSO-d_6) δ 8.90 (s, 1H), 8.37 (s, 1H), 8.21 (s, 1H), 8.18 (s, 1H, exchangeable), 8.02 (s, 1H, exchangeable), 7.57–7.55 (d, *J* = 8.25 Hz, 1H), 7.15–7.13 (d, *J* = 8.08 Hz, 1H), 6.78–6.75 (d, *J* = 13.0 Hz, 1H), 6.99 (s, 1H), 6.58–6.55 (d, *J* = 13.1 Hz, 1H), 3.85 (s, 3H), 3.21 (s, 3H), 3.08 (s, 3H), 2.92 (bm, 4H), 2.5 (4H + DMSO), 2.25 (s, 3H).

*6-Chloro-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1-(20),3(22),4,6,9(21),10,12,16,18-nonaene (**2a**).* Compound **8a** (50 mg, 0.2 mmol) was combined with platinum dioxide (PtO_2) (10 mg, 0.04 mmol) in THF (6 mL), and the mixture was blanketed under H_2 at atmospheric pressure. After being stirred at rt for 5 days the mixture was carefully filtered, rinsed with a small amount of THF, and the filtrate was subsequently concentrated. The residue was purified via preparative HPLC to yield after lyophylization 16 mg (30%) of desired **2a** as a white lyophylate. LC: 99%. LC/MS: M + H = 323.2. ^{1}H NMR (DMSO-d_6) δ 9.27 (s, 1H), 9.03 (s, 1H), 8.15 (s, 1H), 7.98 (s, 1H), 7.81 (s, 1H), 7.27–7.23 (m, 1H), 7.14–7.10 (m, 2H), 7.02–7.00 (d, *J* = 8.6 Hz, 1 H), 6.94–6.92 (d, *J* = 9.1 Hz, 1H), 6.82–6.80 (d, *J* = 7.4 Hz, 1H), 2.90 (s, 4H).

*6-Chloro-17-(morpholin-4-yl)-2,4,8,22-tetraazatetracyclo-[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene (**2b**).* At rt, dipotassium azodicarboxylate[18] (784 mg, 4.0 mmol) was added to a solution of **8b** (70 mg, 0.172 mmol) in pyridine (10 mL). The resulting mixture was treated with acetic acid (AcOH) (0.56 mL, 9.8 mmol). After 3 days the reaction mixture was treated with additional portions of dipotassium azodicarboxylate (330 mg, 1.7 mmol) and AcOH (0.28 mL, 4.9 mmol) and warmed to 35 °C for 2 days (LC supported reaction, >95% complete). The mixture was then combined with water, and the resulting solid was collected. This solid was dissolved in DMSO and purified via preparative HPLC to yield 28 mg (31%) of desired **2b** as a white lyophylate. LC: 100%. LC/MS: M + H = 408.2. ^{1}H NMR (DMSO-d_6) δ 9.47 (bs, 2H), 8.16 (s, 1H), 7.95–7.94 (d, 1H), 7.80 (s, 1H), 7.25–7.21 (t, 1H), 7.05–7.00 (m, 3H), 6.91–6.88 (m, 1H), 3.76–3.74 (m, 4H), 3.02–2.96 (m, 4H), 2.78–2.77 (m, 4H).

*6-Chloro-17-[2-(pyrrolidin-1-yl)ethoxy]-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2c**).* Compound **2c** was prepared in a similar manner as **2b** after substituting **8c** for **8b**. The workup varied slightly in that after the addition of water no solid precipitated, so the aqueous solution was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via preparative HPLC. The desired product **2c** was isolated as a TFA salt, white lyophylate in 40% yield. LC: 100%. LC/MS: M + H = 436.1. ^{1}H NMR (DMSO-d_6) δ 9.7 (bs, 1H), 9.16 (s, 1H), 9.05 (s, 1H), 8.09 (s, 1H), 7.82 (s, 1H), 7.80 (s, 1H), 7.23–7.19 (t, 1H), 7.07–7.05 (d, 1H), 6.97–6.95 (d, 1H), 6.88 (m, 2H), 4.25–4.23 (m, 2H), 3.64 (bs, 2H + H_2O), 3.20 (m, 2H), 2.91 (s, 4H), 2.08 (m, 2H), 1.91 (m, 2H).

*6-Chloro-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2d**).* Compound **2d** was prepared in a similar manner as **2b** after substituting **8d** for **8b**. The workup followed was akin to that for **2c**. The desired product **2d** was isolated in 63% yield as a TFA salt, white lyophylate. LC: 99%, M + H = 421.0. ^{1}H NMR (DMSO-d_6) δ 9.7 (bs, 1H), 9.40 (s, 1H), 9.27 (s, 1H), 8.14 (s, 1H), 8.03–8.02 (d, 1H), 7.82 (s, 1H), 7.26–7.22 (t, 1H), 7.07–7.05 (d, 1H), 7.02–7.01 (d, 1H), 7.00 (s, 1H), 6.93–6.90 (m, 1H), 3.52–3.49 (d, 2H), 3.26–3.2 (m, 2H), 3.09–3.05 (d, 2H), 2.99–2.90 (m, 9H). HRMS calcd for $C_{23}H_{25}ClN_6$ (MH^+): 421.1907. Found: 421.1913.

*10-Methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene (**2e**).* Compound **2e** was

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prepared in a similar manner as **2a** after substituting **8e** for **8a**. The crude product was dissolved in a small amount of warmed DMSO and purified via preparative HPLC. The purest fractions were combined and partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was triturated with CH_3CN, filtered, and rinsed with ice cold CH_3CN to give desired product **2e** in 50% yield as a white solid, mp 252−254 °C. LC: 98%. LC/MS: M + H = 353.2. ^{1}H NMR (DMSO-d_6) δ 9.33 (s, 1H), 8.15 (s, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.81 (s, 1H), 7.17−7.13 (t, 3H), 7.00 (s, 2H), 6.95−6.93 (d, 1H), 6.87−6.84 (d, 1H), 3.85 (s, 3H), 2.82 (s, 4H).

*6-Chloro-10-methoxy-17-(morpholin-4-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2f**).* Compound **2f** was prepared in a similar manner as **2b** after substituting **8f** for **8b**. The desired product **2f** was isolated in 19% yield as a TFA salt, white lyophylate. LC: 99%. LC/MS: M + H = 438.2. ^{1}H NMR (DMSO-d_6) δ 9.38 (bs, 1H), 8.20 (bs, 1H), 8.16 (s, 1H), 7.93 (s, 1H), 7.87−7.86 (d, 1H), 7.05−6.90 (m, 4H), 3.84 (s, 3H), 3.76−3.74 (m, 4H), 3.5 (bs, H_2O), 2.96−2.89 (M, 4H), 2.80−2.78 (m, 4H). HRMS calcd for $C_{23}H_{24}ClN_5O_2$ (MH^+): 438.1697. Found: 438.1698.

*6-Chloro-10-methoxy-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2g**).* Compound **2g** was prepared in a similar manner as **2b** after substituting **8g** for **8b**. The workup followed was akin to that for **2c**. The desired product **2g** was isolated in 41% yield as a white lyophylate. LC: 99%. LC/MS: M + H = 451.2. ^{1}H NMR (DMSO-d_6) δ 9.6 (bs, 1H), 9.38 (s, 1H), 8.15 (s, 1H), 8.04 (bs, 1H), 7.95 (bs, 1H), 7.92−7.91 (d, 1H), 7.06−7.03 (d, 1H), 6.98−6.92 (m, 3H), 3.83 (s, 3H), 3.61 (bs, H_2O), 3.53−3.50 (d, 2H), 3.25−3.22 (m, 2H), 3.10−3.07 (d, 2H), 2.99−2.96 (d, 2H), 2.92−2.87 (m, 7H). HRMS calcd for $C_{24}H_{27}ClN_6O$ (MH^+): 451.2013. Found: 451.2015.

*6-Chloro-10-(propane-2-sulfonyl)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,16,18-nonaene (**2i**).* Compound **2i** was prepared in a similar manner as **2b** after substituting **8i** for **8b**. The workup followed was akin to that for **2c** except that after the extractive workup the resulting crude material was not purified via preparative HPLC but rather via crystallization from CH_3CN. The desired product **2i** was isolated in 50% yield as a yellow tinted solid, mp >250 °C. LC: 98%. LC/MS: M + H = 527.2. ^{1}H NMR (DMSO-d_6) δ 9.47 (s, 1H), 9.27 (s, 1H), 8.36−8.35 (d, 1H), 8.25 (s, 1H), 7.99−7.97 (d, 1H), 7.71−7.69 (d, 1H), 7.28−7.26 (m, 1H), 7.04−7.02 (d, 1H), 6.95−6.92 (m, 1H), 3.31 (bs, 1H + H_2O), 3.04−2.98 (m, 4H), 2.81−2.79 (m, 4H), 2.5 (bs, 4H + DMSO), 2.25 (s, 3H), 1.14−1.12 (d, 6H). HRMS calcd for $C_{26}H_{31}ClN_6O_2S$ (MH^+): 527.1996. Found: 527.2007.

*6-Chloro-10-(2-methanesulfonylmethylamino)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2j**).* Compound **2j** was prepared in a similar manner as **2b** after substituting **8j** for **8b**. The workup followed was akin to that for **2c**. The desired product **2j** was isolated in 72% yield as a white lyophylate. LC: 100%. LC/MS: M + H = 528.1. ^{1}H NMR (DMSO-d_6) δ 9.58 (bs, 1H), 9.38 (s, 1H), 8.21 (s, 1H), 8.18 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 7.53−7.51 (d, J = 8.09 Hz, 1H), 7.14−7.12 (d, J = 8.12 Hz, 1H), 7.06−7.04 (d, J = 8.43 Hz, 1H), 6.96−6.94 (d, J = 8.48 Hz, 1H), 3.53−3.50 (d, 2H), 3.25−3.22 (m, 2H), 3.19 (s, 3H), 3.11−2.91 (m, 14H). HRMS calcd for $C_{25}H_{30}ClN_7O_2S$ (MH^+): 528.1948. Found: 528.1951.

*6-Chloro-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2k**).* Compound **2k** was prepared in a similar manner as **2b** after substituting **8k** for **8b**. The workup followed was akin to that for **2c**. The desired product **2k** was isolated in 40% yield as a TFA salt, white lyophylate. LC: 99%. LC/MS: M + H = 451.1. ^{1}H NMR (DMSO-d_6) δ 9.7 (bs, 1H), 9.36 (s, 1H), 8.14 (s, 1H), 8.12 (s, 1H), 7.90 (s, 1H), 7.76 (s, 1H), 7.25−7.21 (t, 1H), 7.05−7.03 (d, 1H), 7.00−6.98 (d, 1H), 6.69 (s, 1H), 3.81 (s, 3H), 3.70 (bs, water), 3.53 (d, 2H), 3.27−3.24 (m, 2H), 3.14−3.11 (d, 2H), 3.01−2.98 (d, 2H), 2.94 (s, 3H), 2.92−2.91 (d, 4H).

*6-Chloro-10-(propane-2-sulfonyl)-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1-(20),3(22),4,6,9(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2l**).* Compound **2l** was prepared in a similar manner as **2b** after substituting **8l** for **8b**. The workup followed was akin to that for **2c**. The desired product **2l** was isolated in 53% yield as a white lyophylate. LC: 99%. LC/MS: M + H = 557.1. ^{1}H NMR (DMSO-d_6) δ 9.71 (bs, 1H, exchangeable), 9.30 (s, 1H, exchangeable), 8.38 (bs, 2H, 1 exchangeable), 7.86 (s, 1H), 7.70−7.68 (d, J = 8.17 Hz, 1H), 7.22−7.20 (d, J = 8.16 Hz, 1H), 6.73 (s, 1H), 3.81 (s, 3H), 3.54−3.51 (d, 2H), 3.40−3.33 (m, 1H), 3.29−3.21 (m, 2H), 3.19−3.15 (d, 2H), 3.05−2.91 (m, 9H), 1.13−1.11 (d, J = 6.77 Hz, 6H). HRMS calcd for $C_{27}H_{33}ClN_6O_3S$ (MH^+): 557.2102. Found: 557.2101.

*6-Chloro-10-(2-methanesulfonylmethylamino)-17-(4-methylpiperazin-1-yl)-19-methoxy-2,4,8,22-tetraazatetracyclo-[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2m**).* Compound **2m** was prepared in a similar manner as **2b** after substituting **8m** for **8b**. The workup followed was akin to that for **2c**. The desired product **2m** was isolated in 74% yield as a white lyophylate. LC: 99%. LC/MS: M + H = 558.0. ^{1}H NMR (DMSO-d_6) δ 9.83 (bs, 1H, exchangeable), 8.32 (s, 1H, exchangeable), 8.25 (s, 1H, exchangeable), 8.19 (s, 1H), 8.04 (s, 1H), 7.85 (s, 1H), 7.51−7.49 (d, J = 8.21 Hz, 1H), 7.11−7.09 (d, J = 8.20 Hz, 1H), 6.72 (s, 1H), 3.81 (s, 3H), 3.54−3.51 (d, 2H), 3.28−3.21 (m, 2H), 3.18−3.14 (m, 5H), 3.04−2.92 (m, 12H). HRMS calcd for $C_{26}H_{32}ClN_7O_3S$ (MH^+): 558.2054. Found: 558.2050.

Method B. *1-[3-(2-Bromo-4-nitrophenoxy)propyl]pyrrolidine (**10a**).* A mixture of 2-bromo-1-fluoro-4-nitrobenzene (1.10 g, 5.0 mmol), *N*-β-hydroxyethylpyrrolidine (6.0 mL, 52 mmol), and K_2CO_3 (1.38 g, 10 mmol) was warmed to 100 °C for 24 h. The mixture was cooled to rt and diluted with water. The resulting solid was filtered, rinsed with water, and air-dried to yield 1.32 g (84%) of **10a** as a copper colored solid. LC: 93%. ^{1}H NMR (DMSO-d_6) δ 8.43−8.42 (d, 1H), 8.28−8.25 (m, 1H), 7.36−7.34 (d, 1H), 4.36−4.31 (t, 2H), 2.88−2.86 (t, 2H), 2.58−2.55 (m, 4H), 1.70−1.67 (m, 4H).

*1-(2-Bromo-4-nitrophenyl)-4-methylpiperazine (**10b**).* Compound **10b** was prepared in a similar manner as **10a** after substituting 1-methylpiperazine for *N*-β-hydroxyethylpyrrolidine. In addition, no K_2CO_3 was used, and the reaction was run at 90 °C rather than 100 °C. The desired product **10b** was isolated in 97% yield as a yellow solid. LC: 98%. ^{1}H NMR (DMSO-d_6) δ 8.38−8.37 (d, 1H), 8.21−8.18 (m, 1H), 7.29−7.27 (d, 1H), 3.18−3.16 (bm, 4H), 2.50 (s, 4H + DMSO), 2.25 (s, 3H).

*1-Bromo-2-fluoro-4-methoxy-5-nitrobenzene (**10c**) and 1-Bromo-4-fluoro-2-methoxy-5-nitrobenzene (**10d**).* 1-Bromo-2,4-difluoro-5-nitrobenzene (960 mg, 4.0 mmol) was dissolved in MeOH (20 mL). The mixture was cooled to 0 °C and treated with 0.5 M sodium methoxide in MeOH solution (8.1 mL, 4 mmol). After 2 h the reaction solution was concentrated under reduced pressure and the residue was partitioned between EtOAc and water. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 833 mg (83%) of yellowish oil. ^{1}H NMR spectral analysis supported that the product was a mixture of **10c** and **10d** (~1:2 ratio). The assignments of the respective regioisomers were determined based on further analysis of a purified **10e** analogue, generated from the subsequent step to this reaction. ^{1}H NMR (DMSO-d_6) δ 8.41−8.39 (d, 0.63H), 8.36−8.34 (d, 0.26H), 7.57−7.54 (d, 0.30H), 7.45−7.42 (d, 0.70H), 4.01 (s, 2.2H), 3.95 (s, 0.9H).

*1-(2-Bromo-5-methoxy-4-nitrophenyl)-4-methylpiperazine (**10e**).* The 833 mg mixture of **10c** and **10d** was treated at rt with 1-methylpiperazine (10 mL, 100 mmol). After 3 h the mixture was diluted with water and then extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield 1.13 g of oil. This material was purified via preparative HPLC to separate **10e** from its respective regioisomer. The purifed fractions of **10e** from the HPLC were partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 0.26 g (24%) of desired **10e** as an oil, which crystallized upon sitting. Free basing of the purified regioisomer of **10e**, i.e., 1-(4-bromo-5-methoxy-2-nitrophenyl)-4-methylpiperazine,

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resulted in a return of 0.56 g (51%) of this side product. Preliminary analysis of **10e**: LC, 100%. LC/MS: M + H = 330.0.

*1-Methyl-4-(4-nitro-2-vinylphenyl)piperazine (**11**).* Compound **11** was prepared in a similar manner as **3d** after substituting **10b** for **3c**. In addition, the reaction was only heated for 5 h. The workup varied in that after concentration of the reaction mixture under reduced pressure, it was partitioned between EtOAc and 1 N HCl. The aqueous phase was basified with 8 N KOH while cooling. The resulting solid was filtered off, rinsed with ice cold water, and air-dried yielding **11** in 70% yield as a yellow solid. LC: 98%. LC/MS M + H = 248.1, ^{1}H NMR (DMSO-d_6) δ 8.24−8.23 (d, 1H), 8.12−8.09 (m, 1H), 7.18−7.16 (d, 1H), 6.82−6.75 (m, 1H), 5.97−5.93 (d, 1H), 5.46−5.43 (d, 1H), 3.08−3.06 (m, 4H), 2.50 (s, 4H + DMSO), 2.25 (s, 3H).

*3-Bromo-4-(2-pyrrolidin-1-ylethoxy)phenylamine (**6c**).* Compound **10a** (0.74 g, 2.3 mmol) was combined with Raney nickel (120 mg) in ethanol (EtOH) (50 mL), and the mixture was warmed to reflux. Hydrazine hydrate (1.0 mL, 2 mmol) was added dropwise to the refluxing solution, and the elevated temperature was maintained for 1 h. After cooling, the reaction mixture was filtered and the filtrate concentrated under reduced pressure to yield 0.58 g (87%) of **6c** as a brown oil, which was used without further manipulation. LC/MS: M + H = 285.3. ^{1}H NMR ($CDCl_3$) δ 7.6.92−6.91 (d, 1H, J = 2.74 Hz), 6.79−6.77 (d, 1H, J = 8.64 Hz), 6.60−6.57 (m, 1H), 4.10−4.07 (t, 2H), 3.48 (bs, 2H), 2.94−2.91 (t, 2H), 2.68−2.65 (m, 4H), 1.84−1.80 (m, 4H).

*3-Bromo-4-(4-methylpiperazin-1-yl)phenylamine (**6d**).* Compound **6d** was prepared in a similar manner as **6c** after substituting **10b** for **10a**. The crude material was triturated with water to give **6d** as a light brown solid in 93% yield, which was used without further manipulation. ^{1}H NMR (DMSO-d_6) δ 6.90−6.88 (d, 1H), 6.82−6.81 (d, 1H), 6.54−6.51 (m, 1H), 5.03 (s, 2H), 2.79−2.77 (m, 4H), 2.43 (bm, 2H), 2.21 (s, 3H).

*4-(4-Methylpiperazin-1-yl)-3-vinylphenylamine (**6e**).* Iron (2.93 g, 52 mmol) was added at rt to a stirring solution of **11** (1.95 g, 7.9 mmol) in THF (30 mL) and acetic acid (60 mL). The mixture was warmed to 35 °C and stirred for 16 h. The mixture was then cooled to rt, filtered, and the resulting solution was concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 1.71 g (100%) of **6e** as a yellow tinted oil, which crystallized upon sitting. This material was used for subsequent steps without further manipulation. LC/MS: M + H = 218.1. ^{1}H NMR (DMSO-d_6) δ 7.04−6.96 (m, 1H), 6.82−6.80 (d, 1H), 6.75−6.74 (d, 1H), 6.50−6.48 (m, 1H), 5.59−5.54 (m, 1H), 5.17−5.14 (m, 1H), 4.77 (s, 2H), 2.27−2.70 (m, 4H), 2.43 (s, 4H), 2.20 (s, 3H).

*5-Bromo-2-methoxy-4-(4-methylpiperazin-1-yl)phenylamine (**6f**).* Compound **6f** was prepared in a similar manner as **6c** after substituting **10e** for **10a**. After workup a mass return of 100% was returned, but ^{1}H NMR and LC/MS confirmed the crude to be 80% **6f**, with a 20% impurity of the des-Br analogue of **6f**. This crude material was used for subsequent reactions without further manipulation. It was also found that **6f** could be prepared pure in 96% yield cleanly by following the procedure akin to **6e** after substituting **10e** for **11**. LC/MS: M + H = 302.0. ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 1H), 6.62 (s, 1H), 3.85 (s, 3H), 3.66 (s, 2H), 2.99 (bs, 4H), 2.61−2.59 (bm, 4H), 2.38 (s, 3H).

Method C. *(14Z)-6-Chloro-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3(22),4,6,9-(21),10,12,14,16,18-decaene-10-carboxylic Acid (**8n**).* A solution of LiOH (11 mg, 0.46 mmol) in water (0.6 mL) was added to a solution of 8 h (29 mg, 0.06 mmol) in MeOH (6 mL), and the resulting mixture was warmed to 65 °C for 16 h. After cooling to 0 °C, the mixture was treated with 1 N HCl (0.46 mL, 0.46 mmol), then concentrated under reduced pressure and used directly for the subsequent reaction toward **8o** without further manipulation. LC: 100%. LC/MS: M + H = 463.1.

*(14Z)-6-Chloro-10-(N-methylcarboxamido)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,14,16,18-decaene (**8o**).* *N*-(3-Dimethylaminopropyl)-*N*′ethylcarbodiimide hydrochloride (150 mg, 0.8 mmol) was added to a rt mixture of **8n** (28 mg, 0.06 mmol), 1-hydroxybenzotriazole (11 mg, 0.084 mmol), methylammonium chloride (100 mg, 1 mmol), and triethylamine (100 μL, 0.7 mmol). After 7 days the reaction mixture was partitioned between EtOAc (2×) and saturated aqueous $NaHCO_3$. The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 28 mg (98%) of **8o**, which was used toward **2h** without further manipulation. LC: 98%. LC/MS: M + H = 476.2.

A small sample of the 98% pure material was further purified via preparative HPLC to return pure **8o** as a TFA salt white lyophylate. LC: 100%. LC/MS: M + H = 476.1. ^{1}H NMR (DMSO-d_6) δ 11.55 (s, 1H), 9.55 (bs, 1H), 9.40 (s, 1H), 9.39 (s, 1H), 8.76−8.74 (m, 1H), 8.46−8.45 (d, 1H), 8.19 (s, 1H), 7.77−7.75 (d, 1H), 7.13−7.04 (m, 3H), 6.91−6.87 (d, 1H), 6.70−6.66 (d, 1H), 3.5 (d, 2H), 3.35−3.2 (bm, 4H + H_2O), 2.95−2.89 (m, 5H), 2.81−2.80 (d, 3H).

*6-Chloro-10-(N-methylcarboxamido)-17-(4-methylpiperazin-1-yl)-2,4,8,22-tetraazatetracyclo[14.3.1.1^{3,7}.1^{9,13}]docosa-1(20),3-(22),4,6,9(21),10,12,16,18-nonaene Trifluoroacetate (1:1) (**2h**).* Compound **2h** was prepared in a similar manner as **2b** after substituting **8o** for **8b**. The workup followed was akin to that for **2c**. The desired product **2h** was isolated in 20% yield as a TFA salt, white lyophylate. LC: 100%. LC/MS: M + H = 478.2. ^{1}H NMR (DMSO-d_6) δ 11.2 (s, 1H), 9.61 (bs, 1H), 9.38 (s, 1H), 8.66−8.65 (m, 1H), 8.40 (s, 1H), 8.18 (s, 1H), 8.08 (s, 1H), 7.70−7.67 (d, 1H), 7.08−6.96 (m, 3H), 3.53 (d, 2H), 3.26−3.23 (m, 2H), 3.12−3.09 (d, 2H), 3.0−2.93 (m, 6H), 2.91 (d, 3H), 2.79 (d, 3H).

Method D. *4-Bromo-1-isopropylsulfanyl-2-nitrobenzene (**13**).* 2-Propanethiol (0.76 mL, 8 mmol) was added to a rt solution of 4-bromo-1-fluoro-2-nitrobenzene (**12**) (1.10 g, 5.0 mmol) in EtOH (5 mL), and the resulting solution was warmed at 50 °C for 4 days. After cooling to rt, the solution was treated with water (15 mL). The resulting yellow solid was filtered and rinsed liberally with water. After air drying there remained 1.35 g (98%) of desired **13** as a yellow solid. This material was used for the subsequent step without further manipulation. LC: 96%. ^{1}H NMR (DMSO-d_6) δ 8.30−8.29 (d, 1H), 7.90−7.87 (m, 1H), 7.67−7.64 (d, 1H), 3.76−3.73 (m, 1H), 1.30−1.28 (d, 6H).

*4-Bromo-2-nitro-1-(propane-2-sulfonyl)benzene (**14**).* *m*-Chloroperbenzoic acid (1.18 g, 6.84 mmol) was added neat to a 0 °C solution of **13** (0.55 g, 2.0 mmol) in CH_2Cl_2 (15 mL). The reaction was warmed to rt and stirred for 16 h. The reaction solution was concentrated under reduced pressure, and the residual white solid was treated with saturated aqueous $NaHCO_3$ (40 mL). After being stirred for 10 min, the mixture was filtered and the resulting solid was washed liberally with water. After air drying there remained 0.56 g (91%) of desired **14** as a white solid. This material was used for the subsequent step without further manipulation. LC: 95%. ^{1}H NMR (DMSO-d_6) δ 8.49−8.48 (d, 1H), 8.19−8.16 (m, 1H), 7.97−7.95 (d, 1H), 3.77−3.73 (m, 1H), 1.27−1.26 (d, 6H).

*5-Bromo-2(propane-2-sulfonyl)phenylamine (**3f**).* Iron (710 mg, 13 mmol) was added at rt to a stirring solution of **14** (539 mg, 1.75 mmol) in THF (10 mL) and acetic acid (14 mL). The mixture was warmed to 35 °C and stirred for 2 h. The mixture was then cooled to rt, filtered, and the resulting solution was concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ (2×) and saturated aqueous $NaHCO_3$. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 334 mg (69%) of **3f** as a clear oil, which crystallized to a white solid upon sitting. This material was used for its subsequent step without further manipulation. LC: 97%. LC/MS: M + H = 279.8.

*2-Nitro-1-(propane-2-sulfonyl)-4-vinylbenzene (**15**).* Compound **15** was prepared in a similar manner as **3d** after substituting **14** for **3a**. The workup varied in that after concentration of the reaction mixture, the residue was partitioned between EtOAc (2×) and water. The combined organic phases were dried over $MgSO_4$, filtered, and the filtrate was adsorbed directly onto silica gel under reduced pressure. The residue was purified via normal phase chromatography (EtOAc/hexane eluent) to give desired **15** in 55% yield. LC: 99%. ^{1}H NMR (DMSO-d_6)

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δ 8.26 (s, 1H), 8.02−7.98 (m, 2H), 6.95−6.87 (m, 1H), 6.27−6.23 (d, 1H), 5.67−5.64 (d, 1H), 3.78−3.70 (m, 1H), 1.28−1.26 (d, 6H).

*2-(Propane-2-sulfonyl)-5-vinylphenylamine (**3h**).* Compound **3h** was prepared in a similar manner as **6e** after substituting **15** for **11**. The workup was similar except that EtOAc was used for extractions rather than $CHCl_3$. Compound **3h** was recovered in 99% yield as a brown oil and used directly after workup for subsequent steps without further manipulation. LC/MS: M + H = 226.0 . ^{1}H NMR ($CDCl_3$) δ 7.62−7.60 (d, 1H), 6.89−6.87 (d, 1H), 6.72 (s, 1H), 6.68−6.61 (m, 1H), 5.86−5.82 (d, 1H), 5.42−5.39 (d, 1H), 5.08 (bs, 2H), 3.37−3.30 (m, 1H), 1.34−1.32 (d, 6H).

Method E. *N-(4-Bromo-2-nitrophenyl)methanesulfonamide (**17**).* Potassium nitrate (0.34 g, 3.36 mmol) was added neat to a 0 °C solution of *N*-(4-bromophenyl)methanesulfonamide (**16**) (0.75 g, 3.0 mmol) in CH_3CN (20 mL) and trifluoroacetic anhydride (2.6 mL). After 2 h the 0 °C reaction mixture was slowly treated with saturated aqueous $NaHCO_3$ until the reaction mixture was basic. The resulting solid was filtered and rinsed liberally with ice cold water. After air drying there remained 0.75 g (85%) of desired **17** as a yellow solid, which was used for the subsequent step without further manipulation. LC: 98%. ^{1}H NMR (DMSO-d_6) δ 9.88 (s, 1H), 8.23 (s, 1H), 7.95−7.93 (d, 1H), 7.59−7.57 (d, 1H), 3.152 (s, 3H).

*N-(4-Bromo-2-nitrophenyl)-N-methylmethanesulfonamide (**18**).* Methyl iodide (430 μL, 6.9 mmol) was added to a mixture of **17** (0.68 g, 2.3 mmol) and K_2CO_3 (1.3 g, 9.2 mmol) in DMF (10 mL). After 16 h the reaction mixture was treated with water (20 mL). The resulting solid was filtered, rinsed liberally with water, and air-dried to yield 0.68 g (96%) of desired **18** as a white solid, which was used for the subsequent step without further manipulation. LC: 100%. ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H), 8.02−8.00 (d, 1H), 7.80−7.77 (d, 1H), 3.26 (s, 3H), 3.06 (s, 3H).

*N-(2-Amino-4-bromophenyl)-N-methylmethanesulfonamide (**3g**).* Compound **3g** was prepared in a similar manner as **3f** after substituting **18** for **11**. The workup was similar except that EtOAc was used for extractions rather than $CHCl_3$. Compound **3g** was recovered in 98% yield as a clear oil and used directly for subsequent steps without further manipulation. LC: 93%. ^{1}H NMR (DMSO-d_6) δ 7.14−7.12 (d, 1H), 6.92 (s, 1H), 6.69−6.67 (d, 1H), 5.43 (s, 2H), 3.05 (s, 6H).

*N-Methyl-N-(2-nitro-4-vinylphenyl)methanesulfonamide (**19**).* Compound **19** was prepared in a similar manner as **3d** after substituting **18** for **3a**. The workup varied in that after concentration of the reaction mixture, the residue was partitioned between EtOAc (2×) and water. The combined organic phases were dried over $MgSO_4$, filtered, and the crude product in the filtrate was adsorbed directly onto silica gel under reduced pressure. The residue was purified via normal phase chromatography (EtOAc/hexane eluent) to give desired **19** in 69% yield. LC: 98%. LC/MS: M + 23 = 279.0.

*N-(2-Amino-4-vinylphenyl)-N-methylmethanesulfonamide (**3i**).* Compound **3i** was prepared in a similar manner as **6e** after substituting **19** for **11**. The workup was similar except that EtOAc was used for extractions rather than $CHCl_3$. Compound **3i** was recovered in 99% yield as a brown oil and used directly after workup for subsequent steps without further manipulation. LC: 97%. LC/MS: M + 23 = 249.0. ^{1}H NMR ($CDCl_3$) δ 7.11−7.09 (d, 1H), 6.85−6.83 (m, 2H), 6.66−6.59 (m, 1H), 5.74−5.70 (d, 1H), 5.29−5.27 (d, 1H), 4.24 (bs, 2H), 3.25 (s, 3H), 2.98 (s, 3H).

Method F. *(2,5-Dichloropyrimidin-4-yl)phenylamine (**21a**).* Compound **21a** was prepared in a similar manner as **5a** after substituting aniline (**20a**) for **3a**, substituting EtN(*i*-Pr)$_2$ for K_2CO_3, and altering the reaction temperature from 80 °C to rt. Workup varied in that after 16 h the reaction was diluted with water, which yielded an oil that crystallized to a solid. The solid was filtered and rinsed liberally with water to yield desired **21a** in 81% yield as a white solid. LC: 99%. LC/MS: M + H = 240.0. ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 7.64−7.61 (m, 2H), 7.43−7.38 (m, 2H), 7.25 (bs, 1H), 7.23−7.16 (m, 1H).

*(2,5-Dichloropyrimidin-4-yl)-(2-methoxyphenyl)amine (**21b**).* Compound **21b** was prepared in a similar manner as **5a** after substituting *o*-anisidine (**20b**) for **3a**. Workup varied in that after 16 h the reaction was diluted with water, which yielded an oil that crystallized to a solid. The solid was filtered, then triturated with MeOH, refiltered, and rinsed with a small amount of MeOH to yield desired **21b** in 31% yield as a white solid. LC: 100%. LC/MS: M + H = 270.0. ^{1}H NMR ($CDCl_3$) δ 8.52−8.49 (m, 1H), 8.19 (s, 1H), 8.14 (bs, 1H), 7.15−7.03 (m, 2H), 6.96−6.94 (m, 1H), 3.96 (s, 3H).

*5-Chloro-N(2)-[4-(4-methylpiperazin-1-yl)phenyl]-N(4)-phenylpyrimidine-2,4-diamine Trifluoroacetate (1:1) (**1b**).* Compound **1b** was prepared in a similar manner as **7a** after substituting **21a** for **5a** and 4-(4-methylpiperazin-1-yl)phenylamine (**22**) for **6a**. Also, the mixture was heated for 48 h rather than 16 h. After workup the crude product was isolated as a purple tinted solid. This material was purified via preparative reverse phase HPLC to yield the desired product **1b** as a TFA salt in 71% as a white lyophylate. LC: 100%. LC/MS: M + H = 395.0. ^{1}H NMR (DMSO-d_6) δ 9.79 (bs, 1H), 9.37 (s, 1H), 8.98 (s, 1H), 8.15 (s, 1H), 7.68−7.66 (d, *J* = 7.90 Hz, 2H), 7.48−7.46 (d, *J* = 8.92 Hz, 2H), 7.38−7.34 (m, 2H), 7.17−7.13 (m, 1H), 6.88−6.86 (d, *J* = 9.00 Hz, 2H), 3.74−3.71 (m, 2H), 3.54−3.51 (m, 2H), 3.18 (bm, 2H), 2.92−2.87 (m, 5H).

*5-Chloro-N(4)-(2-methoxyphenyl)-N(2)-[4-(4-methylpiperazin-1-yl)phenyl]pyrimidine-2,4-diamine Fumarate (1:1) (**1c**).* Compounds **21b** (81 mg, 0.30 mmol) and **22** (57 mg, 0.30 mmol) and 10-camphorsulfonic acid (77 mg, 0.33 mmol) were combined in isopropanol (3 mL) with two drops of water, and the entire mixture was warmed under microwave conditions at 110 °C for 1 h. An attempt to partition the reaction mixture between EtOAc and saturated aqueous $NaHCO_3$ led to a solid precipitating. This solid was filtered and rinsed with a small amount of water to yield 40 mg of crude product after drying. The crude product was dissolved in a small amount of warm methanol and combined with a warm ethanol (1 mL) solution of fumaric acid (12 mg, 0.10 mmol). The resulting warm solution was filtered, and on cooling a solid precipitated. The solid was filtered, rinsed with a small amount of ice cold ethanol, and air-dried to yield 17 mg (13%) of **1c** as a slightly purple tinted solid, mp 242−244 °C. LC: 100%. LC/MS: M + H = 425.1. ^{1}H NMR (DMSO-d_6) δ 9.13 (s, 1H), 8.10−8.07 (m, 3H), 7.42−7.40 (d, *J* = 8.59 Hz, 2H), 7.15−7.13 (m, 2H), 6.96 (m, 1H), 6.82−6.79 (d, *J* = 8.85 Hz, 2H), 6.60 (s, 2H), 3.86 (s, 3H), 3.32 (bs, 4H + H_2O), 3.06 (bs, 4H), 2.26 (s, 3H).

Biology. Kinase Enzyme and Cellular Assays and Related Determinations of Binding and Inhibitory Activity. *ALK Assay.* Compounds were tested for their ability to inhibit the kinase activity of baculovirus-expressed human ALK cytoplasmic domain using a modification of the assay protocol reported for trkA.[19] Phosphorylation of the substrate, phospholipase C-γ (PLC-γ) generated as a fusion protein with glutathione *S*-transferase (GST),[20] was detected with a europium-labeled anti-phosphotyrosine antibody and measured by time-resolved fluorescence (TRF). Briefly, each 96-well plate was coated with 100 μL/well of 10 μg/mL substrate solution (recombinant GST-PLCγ) in Tris-buffered saline (TBS). The ALK assay mixture (total volume of 100 μL/well) consisting of 20 mM HEPES (pH 7.2), 1 μM ATP, 5 mM $MnCl_2$, 0.1% BSA, and test compound (diluted in DMSO, 2.5% DMSO final in assay) was then added to the assay plate. Enzyme (50 ng/mL ALK) was added, and the reaction was allowed to proceed at 37 °C for 15 min. Detection of the phosphorylated product was performed by adding 100 μL/well Eu-N1 labeled PT66 antibody diluted 1:5000 in 0.25% BSA in TBS containing 0.1% Tween-20 (TBS-T). Incubation at 37 °C then proceeded for 1 h, followed by addition of 100 μL of enhancement solution. The plate was gently agitated, and after 30 min, the fluorescence of the resulting solution was measured using the PerkinElmer EnVision 2102 (or 2104) multilabel plate reader (PerkinElmer, Waltham, MA). Data analysis was performed using ActivityBase (IDBS, Guilford, U.K.). IC_{50} values were calculated by plotting percent inhibition versus $\log_{10}$ of the concentration of compound and fitting to the nonlinear regression sigmoidal dose−response (variable slope) equation in XLFit (IDBS, Guilford, U.K.).

IR Kinase Assay. The IR kinase assay was performed using the TRF assay as described above for ALK. The nucleotide substrate ATP was used at 20 μM, and recombinant human baculovirus-expressed IR cytoplasmic domain was added to the assay at a final concentration of 20 ng/mL. In place of Eu-N1 labeled PT66 antibody, Eu-N1 labeled

PY100 antibody (diluted 1:10000 in antibody dilution buffer) was utilized for detection.

NPM-ALK Cellular Assay. The cellular IC_{50} values of NPM-ALK tyrosine phosphoryation for compounds were generated with a sandwich ELISA assay. The 96-well FluoroNunc Maxisorp white plates (Nunc, no. 437796) were incubated with Goat anti-mouse IgG (Southern Biotech, catalog no. 1070-01) at 1:200 dilution (495 ng/well, 100 μL/well) in 0.1 M sodium bicarbonate for 1.5 h at 25 °C. After being washed with TBS containing 0.05% Tween (TBST), the plates were incubated with mouse ALK antibody (Zymed, catalog no. 35-4300) at 1:500 dilution (100 ng/well, 100 μL/well) in Superblock buffer (Pierce, catalog no. 37545) for 1.5 h at 25 °C. After being washed with TBST, the plates were incubated in Superblock buffer (200 μL/well) for 1 h or overnight at at 4 °C. NPM-ALK positive ALCL Sup-M2 cells seeded in complete RPMI medium at 1×10^5 cells/well (100 μL) in 96-well V-bottom plates (Costar, plate no. 3896 and lid no. 3930) were treated with CEP compounds for 2−3 h. The plates were centrifuged at 233*g* (Allegra X-15R centrifuge with SX4750 rotor, Beckman Coulter) for 5 min and washed with PBS two times (200 μL/well). An amount of 100 μL of cell lysis buffer [10 mM Tris, pH 7.5, 1% Triton X-100, 50 mM sodium chloride, 20 mM sodium fluoride, 2 mM sodium pyrophosphate, 0.1% BSA, plus freshly prepared 1 mM activated sodium vanadate, 1 mM DTT, and 1 mM PMSF and the protease inhibitor cocktail III (1:100 dilution, catalog no. 539134, Calbiochem, La Jolla, CA)] was added into each well. After being kept on a microplate shaker at a speed of 400 for 10 min at 4 °C, the plates were centrifuged at 2450*g* (Allegra X-15R centrifuge with SX4750 rotor, Beckman Coulter) for 10 min at 4 °C. An amount of 40 μL of the cell lysates was transferred into the precoated FluoroNunc Maxisorp 96-well white plates, and the plates were incubated overnight at 4 °C. After being washed with TBST, the plates were incubated with phospho-ALK antibody (Cell Signaling Technology, catalog no. 3341) at 1:750 (100 μL/well) dilution in Superblock for 1 h at 37 °C. After being washed with TBST, the plates were incubated with alkaline phosphatase labeled goat anti-rabbit antibody (Southern Biotech, catalog no. 4050-04) at 1:2000 (100 μL/well) dilution in TBS/2% BSA for 1 h at 37 °C. After being washed with TBST and then TBS, the plates were incubated with the substrate 4-methylumbelliferyl phosphate (0.02 mg/mL, 100 μL/well) dissolved in DEA/$MgCl_2$ buffer for 20 min at 37 °C. The plates were read with a CytoFluor at excitation filter of 360 nm, emission filter of 460 nm, and gain of 65.

Data analysis was performed using ActivityBase (IDBS, Guilford, U.K.), and the IC_{50} values were calculated by plotting percent inhibition versus $\log_{10}$ of the concentration of compound.

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ABBREVIATIONS USED

ATP, adenosine triphosphate; ALK, analplastic lymphoma kinase; IR, insulin receptor; NPM-ALK, nucleophosmin-anaplastic lymphoma kinase; NSCLC, non-small-cell lung cancer; EML4-ALK, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; TPM3-ALK, tropomyosin 3-anaplastic lymphoma kinase; DAP, 2,4-diaminopyrimidine; PPZ, piperazine; PDB, Protein Data Bank; HRMS, high resolution mass spectrum; PRCG, Polak−Ribiere conjugate gradient; PLC-γ, phospholipase C-γ; GST, glutathione *S*-transferase; TRF, time-resolved fluorescence; TBS-T, Tween-20; rt, room temperature

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