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Discovery of Kinase Spectrum Selective Macrocycle (16*E*)-14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (SB1317/TG02), a Potent Inhibitor of Cyclin Dependent Kinases (CDKs), Janus Kinase 2 (JAK2), and Fms-like Tyrosine Kinase-3 (FLT3) for the Treatment of Cancer

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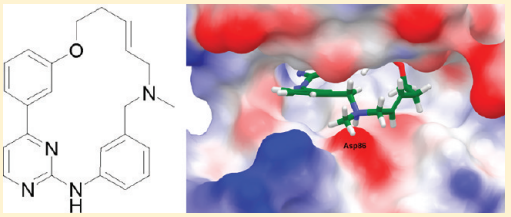
Supporting Information

ABSTRACT: Herein, we describe the design, synthesis, and SAR of a series of unique small molecule macrocycles that show spectrum selective kinase inhibition of CDKs, JAK2, and FLT3. The most promising leads were assessed in vitro for their inhibition of cancer cell proliferation, solubility, CYP450 inhibition, and microsomal stability. This screening cascade revealed **26h** as a preferred compound with target IC_{50} of 13, 73, and 56 nM for CDK2, JAK2 and FLT3, respectively. Pharmacokinetic (PK) studies of **26h** in preclinical species showed good oral exposures. Oral efficacy was observed in colon (HCT-116) and lymphoma (Ramos) xenograft studies, in line with the observed PK/PD correlation. **26h** (SB1317/TG02) was progressed into development in 2010 and is currently undergoing phase 1 clinical trials in advanced leukemias and multiple myeloma.



INTRODUCTION

Recent advances in the understanding of the molecular etiologies of cancer have engendered hopes of new targeted therapies, but to date, these have yielded limited success with only incremental and short-lived benefits being seen with single agent targeted therapy.[1] Blocking more than one aberrant pathway in cancer cells is the mainstay of current standard of care, usually with combinations of chemotherapies that increasingly include targeted agents such as kinase inhibitors.[2,3] Blockade of multiple pathways with multiple targeted therapies is one of the most rapidly developing areas in cancer therapy.[1,4] For example, multikinase inhibitors such as sorafenib,[5] approved for renal cell (RCC) and hepatocellular carcinomas (HCC), and sunitinib,[6] approved for RCC and gastrointestinal stromal tumors (GIST), are effective antiangiogenic and antitumor agents and owe their efficacy to blocking multiple signaling pathways. More recently, compounds combining inhibition of two independent pathways, one of which is a non-kinase, within a single molecular entity have entered the clinic.[7]

Cyclin-dependent kinases (CDKs) are serine−threonine kinases that play important roles in cell cycle control (CDKs 1, 2, 4, and 6), transcription initiation (CDKs 7 and 9) and neuronal function (CDK5).[8] Aberrations in the cell cycle CDKs and their cyclin partners have been observed in various tumor types, including those of the breast, colon, liver, and brain.[9] The simultaneous inhibition of CDKs 1, 2, and 9 has recently been shown to enhance apoptotic killing of lung cancer (H1299) and osteosarcoma cells (U2OS) compared with inhibition of a single CDK alone.[10] Small molecule CDK inhibitors have been in clinical testing for a number of years for hematological malignancies and solid tumors (for example, seliciclib/CYC202 (**1**)[11] and AT7519 (**2**),[12] Figure 1) but have shown limited efficacy as single agents and often require intravenous administration because of their poor oral pharmacokinetics.[13] To address the deficiencies that have hampered development of CDK inhibitors, we set out to identify an orally available inhibitor of a new structural class meeting a specific target product profile: inhibition of at least CDKs 1, 2, and 9, an acceptable safety profile, and physicochemical properties suitable for oral dosing.

JAK kinases (JAK1, JAK2, JAK3, and Tyk2) are a family of intracellular nonreceptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway.[14] Mutations in JAK2 have been associated with myeloproliferative neoplasms (MPNs) including polycythemia vera, essential thrombocythemia, and myelofibrosis as well as a wide range of leukemias and lymphomas.[15−17] JAK2 inhibitors such as

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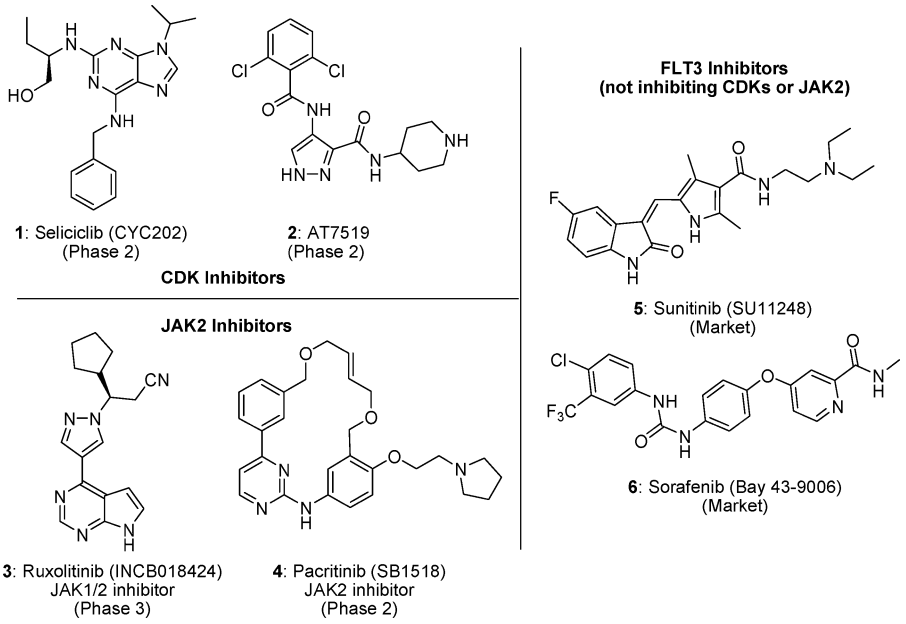

Figure 1. Advanced CDK, JAK2, and FLT3 kinase inhibitors in clinical trials.

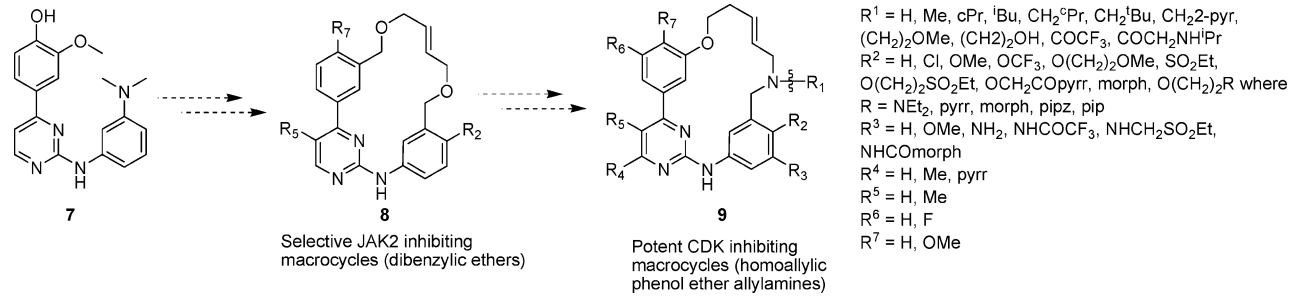

Figure 2. Evolution of CDK inhibitor motif by modulating the macrocycle linking moiety.

ruxolitinib/INCB018424 (**3**)[18] and pacritinib/SB1518 (**4**)[19] are in advanced clinical development for the treatment of myelofibrosis.[20−22]

Class III receptor tyrosine kinase (RTK), fms-like receptor tyrosine kinase 3 (FLT3), plays an important role in the maintenance, growth, and development of hematopoietic and nonhemotopotietic cells.[23] Overexpression and activating mutations of the RTKs are known to be involved in the pathophysiology of diverse human cancers. Mutations of the FLT3 receptor can lead to the development of leukemia.[24] Internal tandem duplications of FLT3 (FLT3-ITD) are the most common mutations associated with acute myelogenous leukemia (AML) and are a prognostic indicator associated with adverse disease outcome.[25] Examples of FLT3 kinase inhibitors, which do not inhibit CDKs or JAKs (but do inhibit many other kinases), are the marketed compounds sunitinib/SU11248 (**5**)[26] and sorafenib/Bay 43-9006 (**6**).[27]

We have recently reported the discovery of pacritinib, a unique macrocyclic JAK2/FLT3 dual inhibitor, as a therapy for myelofibrosis and lymphoma.[28] Herein we describe the work carried out to identify and develop a separate series of macrocycles targeting CDKs, JAK2, and FLT3. Although we were aiming to develop compounds with this inhibition profile, we did not know what level of inhibition of each enzyme would ultimately be required; hence, this work describes efforts toward a target profile of isolated enzyme IC_{50} of less than 50 nM for each target enzyme, CDK2, JAK2 and FLT3, a potentially compelling profile for the treatment of advanced cancers.

Complex natural products with macrocyclic features have always served as a prime source of inspiration for the synthetic organic chemist and continue to do so to this day. Almost limitless structural variations provide diverse functionality and stereochemical complexity in a conformationally preorganized ring structure. From a medicinal chemist's perspective, this can result in high affinity and selectivity for biological targets while preserving sufficient bioavailability to reach intracellular locations. Despite these valuable characteristics and the proven success of macrocycle drugs derived from natural products, this structural class has been historically poorly explored in small molecule drug discovery.[29] This is in part due to concerns about synthetic intractability and nondruglike properties, but recently, there has been greater interest in small molecule synthetic macrocycles as not just viable but attractive druglike molecules against a wide variety of targets.[30] We have demonstrated success with small macrocycles in the area of JAK2 inhibitors with the recent disclosure of the aforementioned pacritinib.[28] Using computational modeling, we developed CDK active macrocycles **9** bearing a basic center that "switched on" activity against CDKs (Figure 2) while maintaining JAK2 and FLT3 inhibition. Optimization of these multiple target activities, aiming at IC_{50} in isolated ATPase assays of less than 50 nM while achieving a balance of druglike features, will thus be described.

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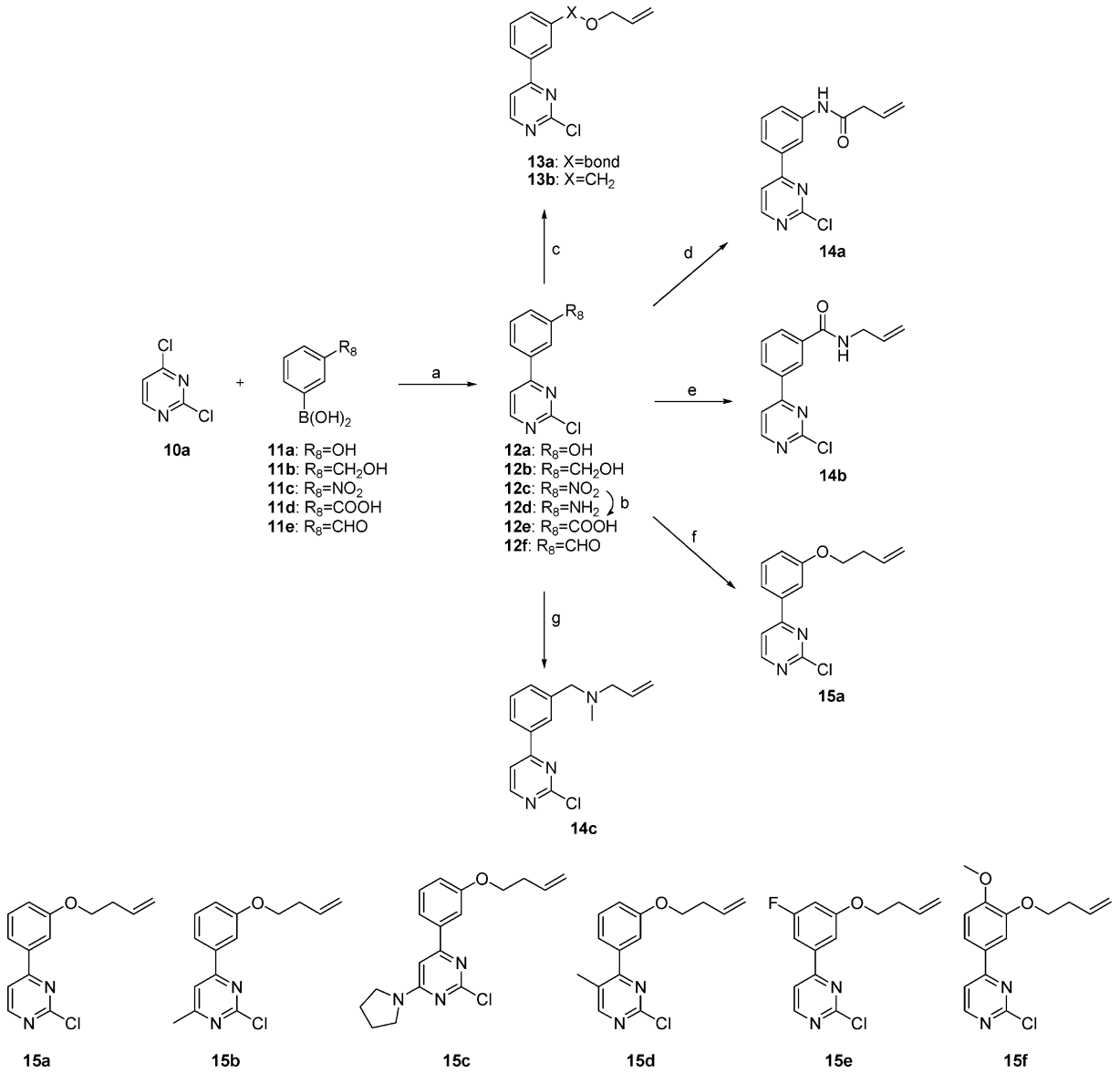
Scheme 1[a]



[a]Reactions and conditions: (a) tetrakis(triphenylphosphine)palladium(0), Na_2CO_3, 1,2-dimethoxyethane, water, 80 °C, 53−91%; (b) $SnCl_2·H_2O$, CH_2Cl_2/MeOH (1:1), room temp, 56−98%; (c) **12a** or **12b**, allyl bromide, KOH, $TBAHSO_4$, CH_2Cl_2, room temp, 55−59%; (d) **12d**, vinylacetic acid, HOBt, EDCI, CH_2Cl_2, room temp, 77%; (e) **12e**, allylamine, HOBt, EDCI, CH_2Cl_2, room temp, 59%. (f) **12a**, 4-bromobut-1-ene, Cs_2CO_3, DMF, 40 °C, 75%; (g) **12f**, *N*-allylmethylamine or allylamine, $Na(OAc)_3BH$, CH_2Cl_2, room temp, 88−95%.

CHEMISTRY

Synthesis of the macrocycles was achieved by coupling of the two halves of the molecules via acidic displacement between chloropyrimidines (left-hand fragment) and anilines (right-hand fragment) followed by ring-closing metathesis of the resulting dienes, employing either Grubbs second generation catalyst or Zhan-1B catalyst[31,32] with trifluoroacetic acid as an additive. Scheme 1 illustrates the synthetic routes for intermediates **14** and **15** and Scheme 2 describes compounds **26a−j**.

The left-hand fragments were constructed by Suzuki coupling of commercially available 2,4-dichloropyrimidine (**10a**) and boronic acids **11a−e**, affording the corresponding biaryls **12a−f** (Scheme 1). Alkylation of phenol **12a** and primary alcohol **12b** were achieved under phase transfer conditions with allyl bromide, giving allyl ethers **13a,b**. Nitro **12c** was reduced to free amine **12d** with $SnCl_2·H_2O$ and reacted with vinylacetic acid to form amide **14a**. The reverse amide **14b** was acquired from **12e** using allylamine under standard amide coupling conditions. Alkylation of phenol **12a** via cesium carbonate assisted displacement with 4-bromobut-1-ene furnished homoallyl ether **15a**. Last, reductive amination of aldehyde **12f** with *N*-allylmethylamine afforded **14c** with excellent yield. Left hand fragments **15b−f** were obtained in the same way as **15a**, starting from the corresponding commercially available starting materials.

Because of their diversity, right-hand fragments were prepared using a wide variety of methods, as illustrated in Schemes 2 and 3. Amidation proceeded with good yield between commercially available aniline **16a** and pentenoic acid to give **18a** (Scheme 2). Benzaldehyde **16b** reacted with the corresponding allylamines via reductive amination, affording

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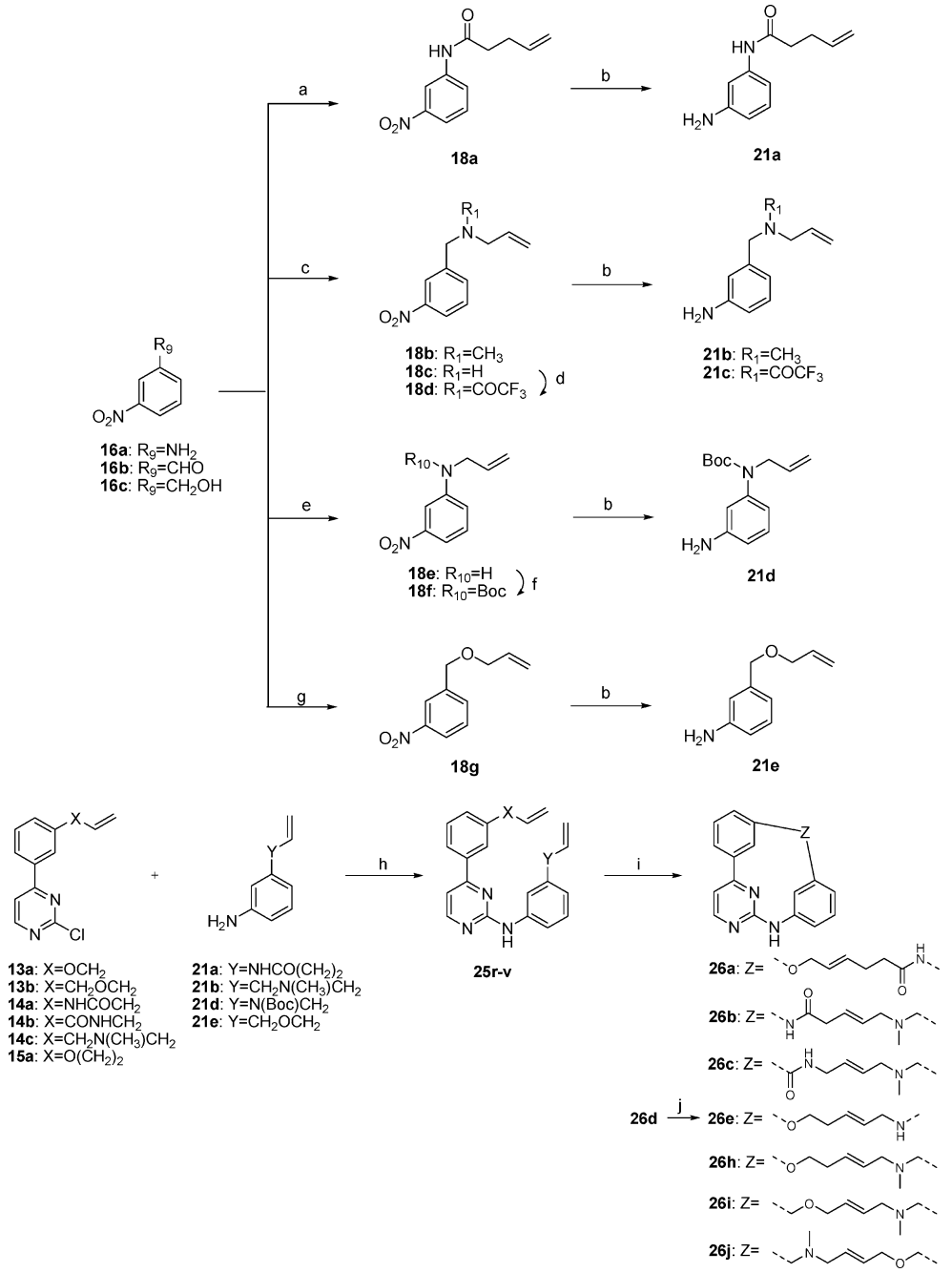
Scheme 2[a]



[a]Reactions and conditions: (a) **16a**, 4-pentenoic acid, HOBt, EDCI, CH_2Cl_2, room temp, 58%; (b) $SnCl_2{\cdot}H_2O$, CH_2Cl_2/MeOH (1:1), room temp, 56−98%; (c) **16b**, *N*-allylmethylamine or allylamine, $Na(OAc)_3BH$, CH_2Cl_2, room temp, 88−95%; (d) $(CF_3CO)_2O$, Et_3N, THF, room temp, 99%; (e) **16a**, allyl bromide, Et_3N, CH_2Cl_2, room temp, 66%; (f) $(Boc)_2O$, NaOH, THF, room temp, 86%; (g)**16c**, allyl bromide, KOH, $TBAHSO_4$, CH_2Cl_2, room temp, 78%; (h) 4 M HCl, *n*-butanol, 80−100 °C, 59−92%; (i) Grubbs second generation catalyst, TFA, CH_2Cl_2, 40−45 °C, 64−89%; (j) 4 M HCl, MeOH/CH_2Cl_2, 40 °C, 79%.

18b−c in excellent yields. Secondary amine **18c** was capped with trifluoroacetic anhydride to give **18d** in quantitative yield. Aniline **16a** was alkylated with allyl bromide, producing **18e**, which was then capped with a Boc group to give **18f**. Last, the nitro groups of all the above-described intermediates were reduced by the aforementioned $SnCl_2{\cdot}H_2O$ conditions, giving key anilines **21a−e** in moderate to excellent yields.

Couping of the left- and right-hand fragments proceeded efficiently in hot butanol to afford **26a−j** in moderate to good yields. The resulting dienes were macrocyclized as described above with Grubb's second generation catalyst. RCM of compounds with basic centers proceeds best in the presence of an acid as an additive; hence, we attempted the reaction with TFA (4−5 equiv) and were delighted to find that the desired products had formed with good yield.[33,34] Deprotection of **26d** was achieved with hydrochloric acid in methanol.

Schemes 3 and 4 illustrate the synthetic routes employed to obtain the diverse right-hand fragments **21f−o** and **22a−n**. Similar to the synthesis of aniline **21b**, anilines **21f−h** were obtained via reductive amination and nitro group reduction,

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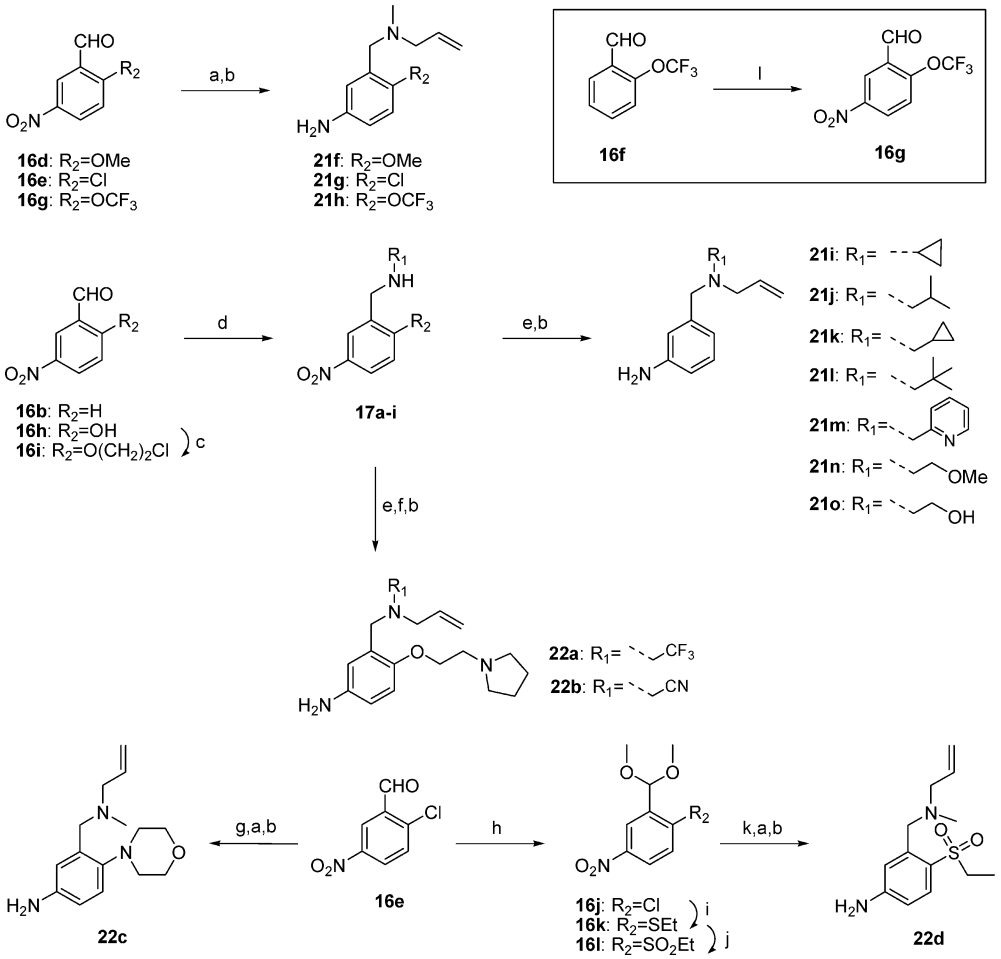
Scheme 3[a]



[a]Reactions and conditions: (a) *N*-allylmethylamine, $Na(OAc)_3BH$, CH_2Cl_2, room temp, 75%, quantitative; (b) Fe powder, NH_4Cl, EtOH, water, 80 °C, 81–93% or $SnCl_2·H_2O$, CH_2Cl_2/MeOH (1:1), room temp, 52–97%; (c) **16h** or **16c**, 1,2-dichloroethane, K_2CO_3, DMF, 100 °C, 64–72%; (d) amine, $Na(OAc)_3BH_3$, CH_2Cl_2, room temp, 68–88%; (e) allyl bromide, K_2CO_3, DMF, 70 °C, 48–91%; (f) amine, DMA, 90 °C, 75–90%; (g) morpholine, ACN, room temp, 83%; (h) NH_4Cl, MeOH, 60 °C, 95%; (i) ethanethiol, K_2CO_3, DMF, 70 °C, 86–97%; (j) MCPBA, CH_2Cl_2, room temp, 46%; (k) TFA/H_2O (1:1), CH_2Cl_2, room temp, quantitative; (l) conc HNO_3, conc H_2SO_4, 0 °C, 80%.

starting from commercially available **16d–f**. Nitro **16g** was prepared from **16f** via standard nitration conditions.

Diversity at the R_1 position was installed by reductive amination of **16b** and **16i** with the desired primary amines giving the corresponding secondary amines **17a–i**. Alkylation with allyl bromide followed by nitro group reduction gave anilines **21i–o**. Trisubstituted anilines **22a,b**, with a side chain at the R_2 position, were formed via a two-step procedure first by displacement of the terminal chloride with amine followed by the nitro reduction. The R_2 substituent can be further diversified from **16e** to give morpholine **22c** and ethylsulfonylaniline **22d**. More examples of anilines with group R_{12} were prepared as **22h–m**. The nitrophenol aldehydes **16h** and **16o** were alkylated cleanly with 1,2-dichloroethane in the presence of base to afford **16i** and **16u** in moderate to good yields. The displacement of the chloride of **16i** with ethanethiol under basic conditions furnished **16t**, which along with **16i** and **16u** was subjected to reductive amination followed by displacement of the terminal chloride with secondary and cyclic amines. Finally, the reduction of the aromatic nitro functions afforded anilines **22h–m** in good yields. The aniline **22e** was obtained from **16t** as described previously. The anilines **22f** and **22g** were derived from **16h** as follows. The alkylation of phenol **16h** with 2-bromoethyl methyl ether followed by reductive amination with *N*-methylamine and subsequent reduction of the aromatic nitro function afforded **22f**, while the aniline **22g** was obtained by alkylation with methyl bromoacetate to afford **16q**, which on lithium hydroxide hydrolysis furnished acid **16r**. Finally, the amide formation was accomplished with pyrrolidine to **16s** under standard amide coupling conditions. Scheme 4 describes the synthesis to obtain a 1,3,5-trisubstituted right-hand aniline. Starting with trifluoroacetylation of aniline **16v** to give **16w** followed by ester reduction using DIBAL gave the aldehyde which was reductively aminated with *N*-allylmethylamine followed by nitro group reduction, affording aniline **22n**.

Coupling of the left- and right-hand sides followed by RCM was carried out, as described above for Scheme 2, to give final products **26k–q** and **27a–q** (Scheme 5). Following macrocyclization, some of the macrocycles were further modified. Trifluoroacetyl, a group necessary to protect the linker amine for efficient ring closing metathesis, was deprotected under mild basic conditions to give the corresponding **26g** and **27s**. Amidation of **26g** with bromoacetyl bromide followed by

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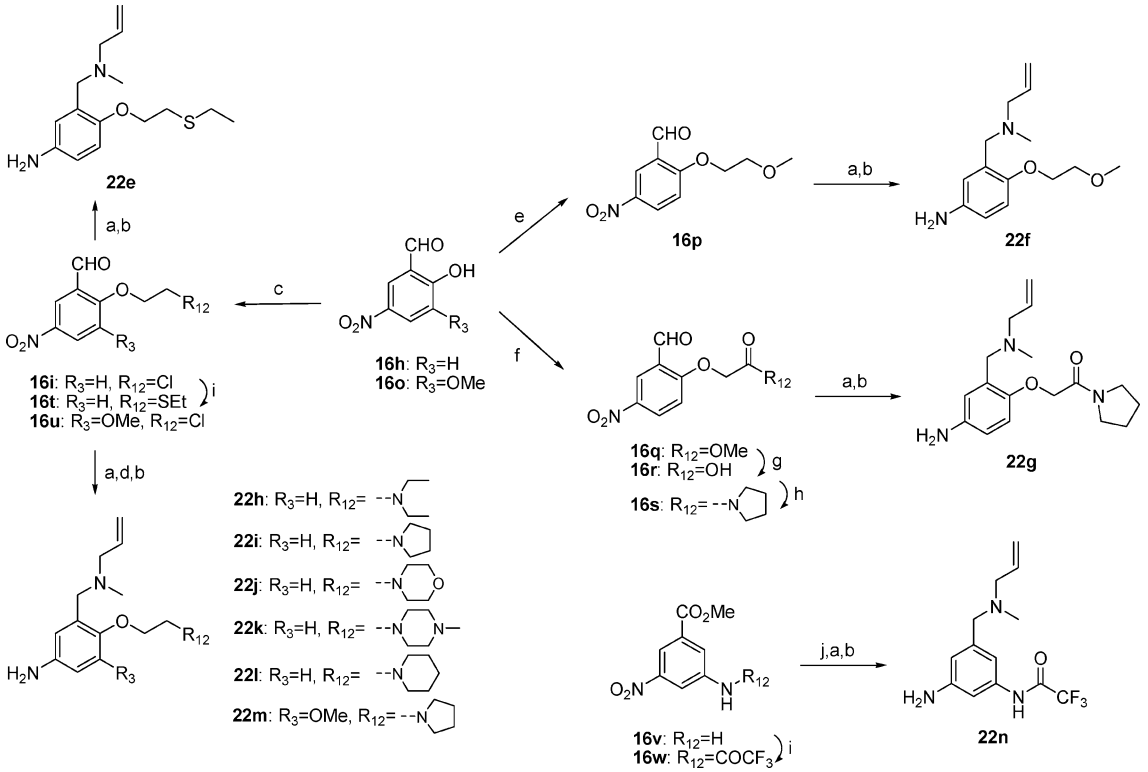
Scheme 4[a]



[a]Reactions and conditions: (a) *N*-allylmethylamine, $Na(OAc)_3BH$, CH_2Cl_2, room temp, 75%, quantitative; (b) Fe powder, NH_4Cl, EtOH, water, 80 °C, 81–93% or $SnCl_2{\cdot}H_2O$, CH_2Cl_2/MeOH (1:1), room temp, 52–97%; (c) **16h** or **16c**, 1,2-dichloroethane, K_2CO_3, DMF, 100 °C, 64–72%; (d) amine, DMA, 90 °C, 75–90%; (e) 2-bromoethyl methyl ether, K_2CO_3, DMF, 80 °C, 86%; (f) methyl bromoacetate, K_2CO_3, ACN, room temp, 87%; (g) LiOH, THF/H_2O (1:1), room temp, quantitative; (h) pyrrolidine, HOBt, EDCI, CH_2Cl_2, room temp, quantitative; (i) $(CF_3CO)_2O$, Et_3N, THF, room temp, quantitative; (j) DIBAL, CH_2Cl_2, −78 °C, 48%.

displacement with isopropylamine afforded **26r** in a one-pot reaction. Macrocycle **27s** was further derivatized with ethanesulfonyl chloride and 4-morpholinecarbonyl chloride in separate reactions to produce corresponding **27t–u** in moderate yield. Macrocycles **28a–e** were synthesized from diversified left-hand fragments **15b–f** using Grubbs second generation catalyst as described previously.

RESULTS AND DISCUSSION

Selection of the Preferred Linker. In the early stages of our kinase inhibitor research we prepared various macrocyclic linking moieties to explore the optimal choice for both our JAK2 program[28] and CDK program described herein. In conjunction with JAK2 and FLT3 we selected CDK2 as the primary biochemical ATPase assay for evaluation of compounds. Previously we have described dioxygen linkers with only moderately polar character. Given the acidic residue Asp86/Asp698 at the entrance to the CDK2/Flt3 active site, we explored nitrogen and amide containing groups in a screen for new interactions. The equivalent residue in JAK2 is Ser936. We observed less active compounds with amide containing linkers; for example, moderate activity against all enzymes was found for phenol ether amide **26a** but amides **26b** and **26c** with basic centers were quite weak (IC_{50} > 10 μM) against the enzymes described in Table 1. More flexible and shorter **26e** was quite active against CDK2 but not for JAK2 or FLT3. However, the one carbon longer **26g** and its *N*-methyl analogue **26h** were potent in the 100 nM range against all enzymes. When docked into CDK2, the more constrained amides were higher in conformational energy and/or had unfavorable electrostatic interactions with the protein. Intriguingly, when the phenol ether of **26h** is modified to benzyl ether **26i**, at least 1 order of magnitude in potency is lost even though the overall length of the linker is maintained. Some recovery against CDK2 and JAK2 is seen when the linker of **26i** is reversed to give **26j**, probably through a hydrogen bond with Asp145. Hence, if the basic nitrogen is correctly orientated in the allylic/benzylic position of the linker, it confers good potency most likely through a salt bridge interaction with Asp86 (see Figure 3). When docked into CDK2, **26i** is also forming a salt bridge to Asp86 but the benzylic ether oxygen is clashing with the backbone carbonyl of Gln131, offering a possible explanation for the 50-fold drop in potency compared to **26h**. When docked into FLT3, **26h** adopts a slightly different preferred conformation in order to achieve the key interaction with Asp698. A few low energy conformations of **26h** docked well into all three proteins, and the differences in the putative bioactive conformations seem to be due to subtle changes in the conformation of the active site rather than homology. However, JAK2 has a serine in place of aspartic acid; hence, the basic nitrogen of **26h** probably makes a hydrogen bond interaction with Ser936 (Figure 4) when docked into JAK2. On the basis of this SAR, we prioritized the promising **26h** linker for further studies.

Optimization of the Linker Nitrogen Substituent. We next turned our attention to the linker nitrogen substituent. Modeling showed that substituents of three or four atoms could be accommodated in this part of the active site. However, only

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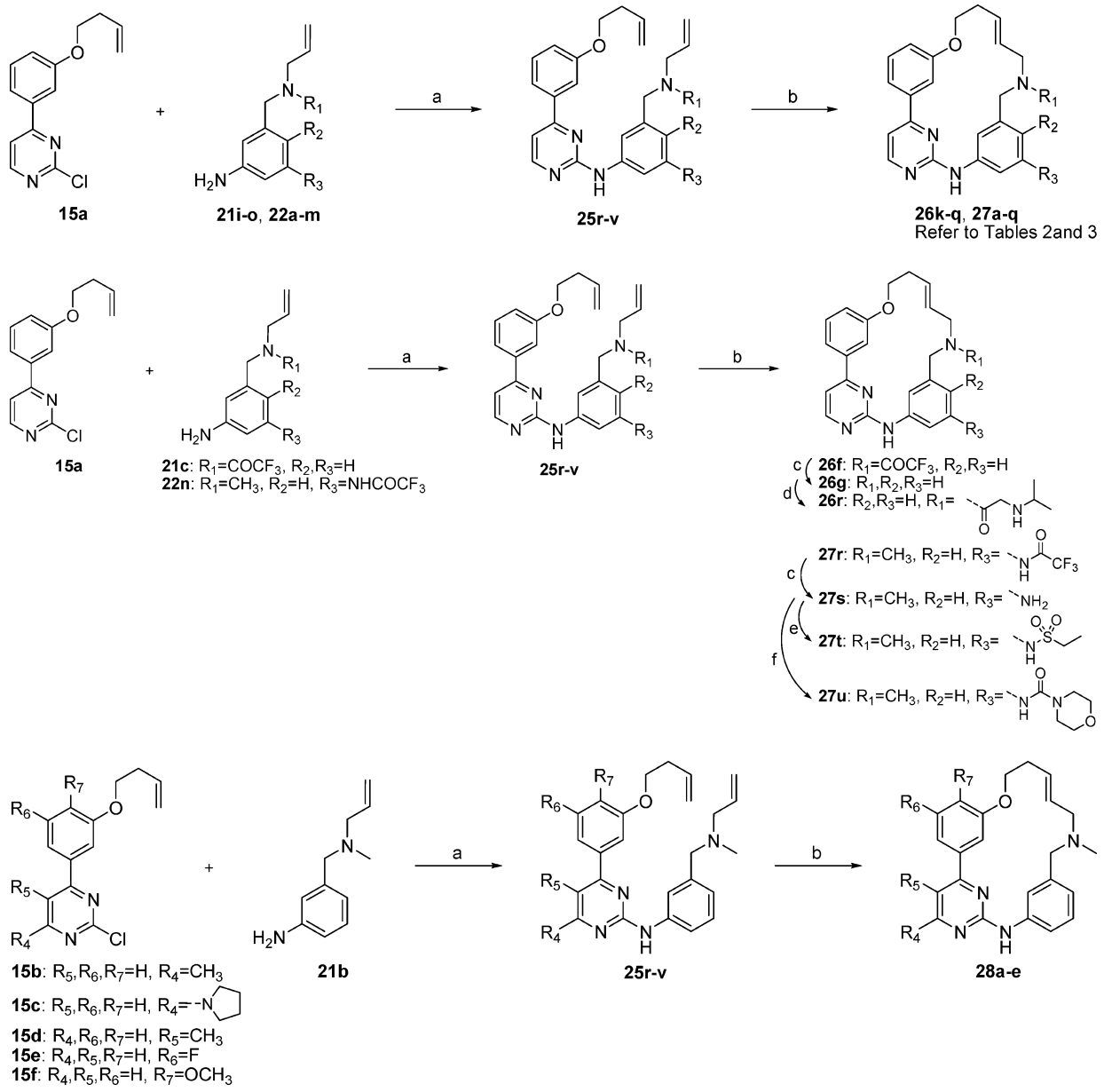
Scheme 5[a]



[a]Reactions and conditions: (a) 4 M HCl, *n*-butanol, 80−100 °C, 49−97%; (b) Grubbs second generation catalyst, TFA, CH_2Cl_2, 40−45 °C, 42−89%; (c) K_2CO_3, H_2O, MeOH, room temp, 48−58%; (d) (i) bromoacetyl bromide, CH_2Cl_2, room temp; (ii) isopropylamine, DIEA, CH_2Cl_2, room temp, two steps 39%; (e) ethanesulfonyl chloride, Et_3N, CH_2Cl_2, room temp, 40%; (f) 4-morpholinecarbonyl chloride, Et_3N, CH_2Cl_2, room temp, 48%.

the smallest lipophilic substituents (**26h**, **26k** and compare with **26g**) were potent against all enzymes (Table 2). All enzymes are similarly sensitive to steric effects (**26l**, **26m**), with activity being completely lost with the very bulky *tert*-butyl **26n** (see Figures 3 and 5 where docking of **26h** against CDK2 shows little available space particularly for bulky substituents). Increases in log *P* and reduction in solubility were undesirable trends in this series. Moderate activity was regained with the more planar pyridyl **26o** and longer methoxyethyl **26p**, but these groups were still over an order of magnitude less active than **26h**. Introduction of hydrogen-bonding functionality regained some CDK2 potency as seen with **26q**, but this was not observed with **26r**, the latter being less flexible and *α*-branched. However, the activity of **26r** could be considered surprising when compared with electron-withdrawing trifluoroacyl **26f**. All activity is abolished with **26f**, presumably through destruction of the salt bridge interaction with Asp86 coupled with unfavorable steric clashes with the enzyme. This surprising activity of **26r** is likely due to the hydrogen bond/salt bridge interaction with Asp86 being achieved through the basic center of the isopropylaminoacyl group (see Figure S1, Supporting Information). Despite the striking SAR, none of these compounds were close to the activity of the highly potent simpler analogues. Hence, we were persuaded by the data to avoid adding unnecessary molecular weight to this part of the molecule.

Other examples of electron-withdrawing N-substituents were prepared but with an additional R_2 substituent on the phenyl

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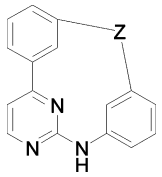
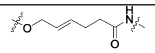
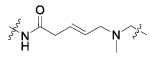
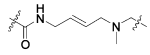
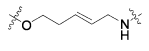
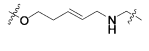
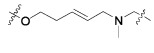
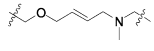
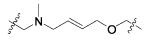
Table 1. SAR of Diverse Linkers



Cpd[a]	-Z-	CDK2 IC_{50} (μM)[b]	JAK2 IC_{50} (μM)[b]	FLT3 IC_{50} (μM)[b]	cLogP[c]
26a		2.6±1.6	1.75±0.21	1.02±0.40	3.3
26b		4.6±1.5	>10	2.75± 0.35	3.0
26c		>10	>10	>10	3.0
26e		0.15±0.015	3.3±0.092	2.9±1.2	3.8
26g		0.021±0.012	0.15±0.083	0.11±0.038	3.8
26h		0.013±0.004	0.073±0.017	0.056±0.026	4.1
26i		0.66±0.19	1.7±0.14	0.21±0.022	3.7
26j		0.099±0.016	0.23±0.0071	0.31±0.035	3.6

[a]All compounds were isolated as 95:5 trans/cis mixtures. [b]All IC_{50} values are the mean ± SD (of at least two independent experiments). [c]cLogP was calculated using QikProp.[35]

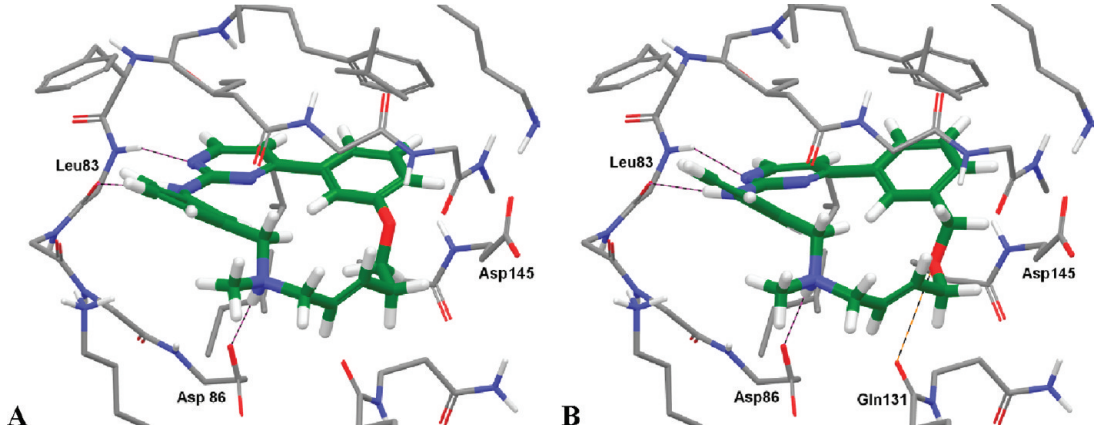


Figure 3. Macrocycles docked into CDK2. The CDK2 ATP-binding site is shown in thin tube with gray carbon. Inhibitors are shown in thick tube with green carbon. (A) In addition to the hydrogen bonds to the hinge residue Leu83, **26h** is forming a salt bridge to Asp86. (B) **26i** is also forming a salt bridge to Asp86, but the ether oxygen is clashing with the backbone carbonyl of Gln131, resulting in a lower IC_{50}.

ring (Table 3, **27l–m**, pK_a of the basic nitrogen of approximately 6 for both compounds). Nevertheless, a comparison between these two compounds is instructive: trifluoroethyl **27l** suffers complete loss of all CDK2 activity but a less significant impact on either JAK2 or FLT3, losing only 3- to 4-fold; cyanomethyl **27m** retains CDK2 potency (compared to **27k**) despite being similar electronically to the trifluoroethyl. Cyano is a much smaller group than trifluoromethyl, supporting the hypothesis that a small substituent is preferred for CDK2 potency. For optimum CDK2 activity in this series it is vital to preserve the strength of the Asp86 salt bridge by retaining basicity of the nitrogen center of at least pK_a = 9 (see electrostatic surface in Figure 5).

Exploration of Aromatic Substituents at R_2 and R_3. With the optimal *N*-methyl installed in the linker, various side chains were studied at the R_2 position. Lipophilic groups such as methoxy **27a**, chloro **27b**, trifluoromethoxy **27c**, and methoxyethoxy **27d** were slightly less active against all enzymes when compared to **26h** (Table 3). However, **27b** does retain a profile very similar to that of **26h**, but the cLogP is higher. In an effort to reduce cLogP and increase solubility while maintaining potency, more polar substituents were installed such as sulfones **27e** and **27g** and amide **27h**. Unfortunately these compounds all lost activity against either CDK2 or FLT3. The branched amine substituents, such as morpholine **27i**, were tolerated quite well against JAK2 and FLT3 but lost over 5-fold activity against CDK2. Efforts then turned to basic side chains installed

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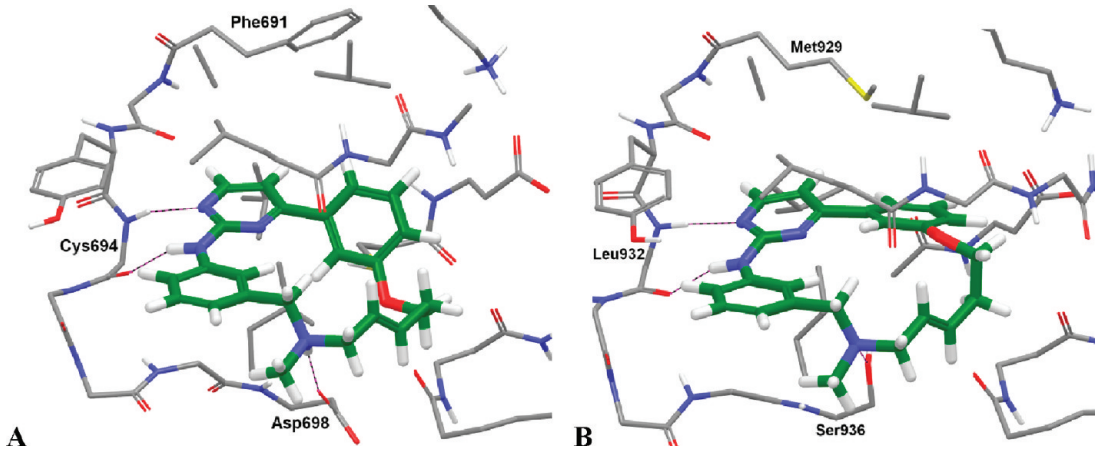
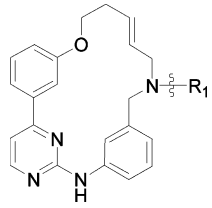


Figure 4. (A) **26h** docked into FLT3. The predicted bioactive conformation of the macrocycle is different from that docked into CDK2. However, the interactions with the protein are conserved with two hydrogen bonds formed to the hinge backbone and a salt bridge to Asp698. (B) **26h** docked into JAK2. The conformation of the macrocycle is different from that docked into CDK2 and FLT3. The two hydrogen bonds formed to the hinge backbone are conserved, but instead of the salt bridge interaction there is a hydrogen bond to Ser936.

Table 2. SAR Study of the Amine Substituent



Cpd[a]	R_1	CDK2 IC_{50} (μM)[b]	JAK2 IC_{50} (μM)[b]	FLT3 IC_{50} (μM)[b]	cLogP[c]	Solubility μg/ml[d]
26g	H	0.021±0.012	0.15±0.083	0.11±0.038	3.8	14.7
26h	CH_3	0.013±0.004	0.073±0.017	0.056±0.026	4.1	71.8
26k		0.036±0.017	0.090±0.020	0.042±0.006	4.6	16.9
26l		0.20±0.00	1.5±0.0	0.19±0.007	5.0	13.2
26m		0.094±0.001	1.5± 0.071	0.27±0.092	4.9	10.6
26n		>10	>10	>10	5.3	<10
26o		0.48±0.064	1.35±0.071	1.45±0.21	5.2	ND[e]
26p		0.41±0.064	1.6±0.71	0.49±0.042	4.2	ND[e]
26q		0.10±0.001	0.72±0.16	0.35±0.007	3.3	ND[e]
26f		>10	>10	>10	4.4	ND[e]
26r		0.53±0.092	0.12±0.00	0.14±0.062	3.6	105.1

[a]All compounds were isolated as 95:5 trans/cis mixtures. [b]All IC_{50} values are the mean ± SD (of at least two independent experiments). [c]cLogP was calculated using QikProp.[35] [d]High throughput solubility in PBS buffered at pH 7.0. [e]Denotes not tested.

on an ethoxy linker, which we had found to be productive in our selective JAK2 program.[28] Compounds **27j** and **27k** had good JAK2 and FLT3 activity, but CDK2 activity was reduced by over an order of magnitude when compared to **26h**. Unfortunately, this trend continued with analogues **27n**, **27o**, and **27p**. Furthermore, we determined the PK profile of **27j** which was only 5% bioavailable with a very high volume of distribution (see Table S1 and Figure S4). It is most probable that other very close derivatives with two basic centers and a high cLogP (**27j−q**) would exhibit a similar undesirable profile. Our attentions then turned to the neighboring position on the aromatic ring, R_3. Combination of R_2 and R_3 was explored with **27q**, which retained potency for JAK2 and FLT3 but dramatically lost activity against CDK2, probably as a result

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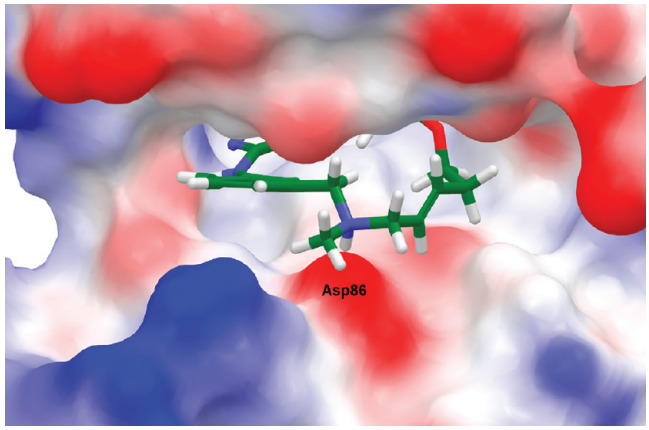


Figure 5. 26h docked into CDK2 which is shown with an electrostatic surface. The methyl substituent on the basic nitrogen is just the right size. A slightly larger substituent like cyclopropyl may be accommodated but will clash slightly with the protein. Larger or more bulky substituents cannot be docked without compromising the salt bridge to Asp86.

of a repulsive interaction with the carbonyl of His84 (this interaction is not seen in docking studies of JAK2 and FLT3). Hence mono-R_3 substitutions were explored in further detail. Very active compounds were found when aniline substituents were installed: **27r**, **27s**, and **27t** were all slightly more active than **26h** with IC_{50} below 10 nM. Potencies for JAK2 and FLT3 were generally also good for these compounds. An additional hydrogen bond to His84 at the end of the hinge region is most likely responsible for the high CDK2 activity (Figure 6). Compound **27u** was the exception, being over 10-fold less potent against CDK2 probably because of the inflexible urea group not being able to adopt a suitable conformation to maintain the hydrogen bond donor to His84. Despite these data, we felt that these compounds did not offer enough gains in potency for the increase in molecular weight; hence, the lower molecular weight **26h** was favored.

Exploration of Biaryl Substitutions (Table 4). A small number of compounds confirmed that substitution at R_4 was not tolerated by any of the target enzymes (**28a** and **28b**). Methyl substitution at R_5 (**28c**) was expected to boost JAK2 potency[28] but surprisingly reduced potency slightly for JAK2 and by an order of magnitude for CDK2. One possible explanation for this is the twist induced in the biaryl system, predicted by conformational analysis, reducing interactions between the phenyl ring and Phe80 (Figure 7). On the other hand, fluoro substitution at R_6 (**28d**) did produce a CDK2 and FLT3 potent compound (**28d** CDK2 IC_{50} = 9 nM and FLT3 IC_{50} = 22 nM), with only a slight reduction in JAK2 potency. When docked into CDK2/FLT3, the fluoro of **28d** is pointing toward the backbone NH of Asp145/Asp829. In JAK2 the peptide bond involving the homologous backbone NH is rotated 180° compared to CDK2. So when docked into JAK2, the fluoro is pointing toward the backbone carbonyl of Gly993. Substitution at R_7 was explored with a methoxy group (**28e**), but this resulted in dramatic loss of CDK2 potency and considerable loss of JAK2 and FLT3 activity. This was not surprising because of the likely close interactions between the methoxy and Lys33 and Asp145 in that part of the binding site (Figure 7). The phenyl ring ortho substituents were not studied because of the anticipated twist imparted to the biaryl system, which we envisioned to be unfavorable for our desired target activity in earlier analogues. Although only a cursory survey with a limited group of compounds, these data give little justification for further studies of biaryl substitutents

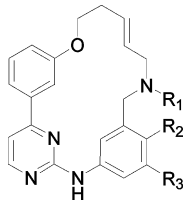
Further in Vitro Profiling of Preferred Compounds. Selected compounds with broad spectrum potency (IC_{50} < 100 nM) across the three target enzymes were profiled further to determine their in vitro ADME properties and cell proliferation inhibition in a range of solid and hematological cell lines (Table 5). HL-60 cells (acute myeloid leukemia), a cell line known to be sensitive to CDK inhibitors, were used to initially screen compounds. Most compounds were then assessed for their human liver microsomal (HLM) stability. The most potent CDK2 inhibitor, **27r**, was also the most potent in HL-60 cells with a IC_{50} for proliferation of 6 nM. Such high cellular activity could reflect the combinatorial strategy of inhibiting several key signaling pathways at the same time. This compound was only moderately stable in HLM ($t_{1/2}$ = 21 min) and did not meet our target profile for a once per day oral therapy and so was not profiled further. A similar set of data revealed **27i** and **27t** to be potent in cells but not satisfactorily stable enough in HLM. HL-60 is a FLT3-driven cell line; hence, the lower HL-60 IC_{50} for **27t** compared to **27r** is most likely related to its reduced FLT3 activity. The least active compound in the HL-60 cell proliferation assay was the *N*-cyclopropyl derivative **26k** with an IC_{50} of 1.38 μM, but this compound did have good stability in HLM and mouse liver microsomes (MLM); hence, it was profiled in a range of other cell lines. Unfortunately its poor cell potency was a general feature, perhaps because of its low solubility, and it was not progressed further. Potent CDK2 inhibitor **27b** was disappointing in terms of cell proliferation activity in three cell lines despite its good enzyme activity. The high cLogP of 4.5 indicates that there may be a solubility problem, but this was not studied further. The remaining compounds, **26g** and **26h**, were potent against all the cell lines tested including solid tumor cell lines such as colon (HCT-116, COLO205) and prostate (DU145). In general both compounds had a broadly similar pharmacological profile but **26h** was always more active in cells, particularly the prostate cancer cell line DU145 where there was a 5-fold difference between the compounds. Higher cellular potency of **26h** is probably due to its generally higher potency against the enzymes but could also be due to better solubility and permeability. Furthermore, **26g** was significantly less soluble than **26h**. Taken together, these data support **26h** as the preferred compound, and as such, it was selected for advanced profiling.

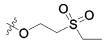
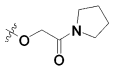
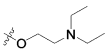
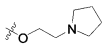
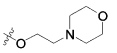
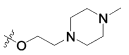
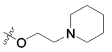
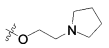
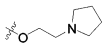
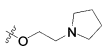
Intracellular Pharmacodynamic Marker Studies and Kinase Profiling of 26h. Intracellular pharmacodynamic marker studies showed that **26h** potently inhibited the CDK2 biomarker pRb (phospho-Rb, retinoblastoma tumor suppressor protein) in HCT-116 (Figure 8). Effects could be detected at the 40 nM with the protein phosphorylation being completely inhibited at 200 nM. Similar studies in leukemic cell lines[36] showed that **26h** was also potent against pRb in MV4-11 cells (IC_{50} = 0.13 μM) and also inhibited pFLT3 and pSTAT5 in the same cell line.

Extensive biological characterization, including kinase profiling, intracellular mechanistic studies, and antiproliferative effects on a wide range of leukemic cell lines, was reported elsewhere.[36] These data show that **26h** has a highly novel kinase inhibitory spectrum inhibiting 17 kinases from a panel of 63, 11 of which are CDK/JAK/FLT family members. The others, Lck, Fyn, Fms, TYRO3, ERK5, and p38δ, are implicated in inflammatory and proliferative processes, and further

dx.doi.org/10.1021/jm201112g | J. Med. Chem. 2012, 55, 169–196

Table 3. SAR Study of Amine and Aromatic Ring Substitutions



Cpd[a]	R_1	R_2	R_3	CDK2 IC_{50} (μM)[b]	JAK2 IC_{50} (μM)[b]	FLT3 IC_{50} (μM)[b]	cLogP[c]	Solubility μg/ml[d]
26h	Me	H	H	0.013±0.004	0.073±0.017	0.056±0.026	4.1	71.8
27a	Me	OCH_3	H	0.077±0.028	0.28±0.014	0.066±0.003	4.3	129.1
27b	Me	Cl	H	0.024±0.002	0.082±0.0028	0.077±0.005	4.5	ND[e]
27c	Me	OCF_3	H	0.057±0.006	0.15±0.021	0.16±0.042	5.2	ND[e]
27d	Me		H	0.11±0.015	0.062±0.010	0.025±0.005	4.3	ND[e]
27e	Me		H	0.022±0.003	0.10±0.001	0.44±0.11	3.1	ND[e]
27g	Me		H	0.17±0.061	0.11±0.022	0.093±0.022	3.7	127.2
27h	Me		H	0.39±0.064	0.14±0.019	0.15±0.15	3.6	ND[e]
27i	Me		H	0.074±0.006	0.049±0.019	0.071±0.035	4.1	147.0
27j	Me		H	0.30±0.17	0.048±0.004	0.030±0.007	4.6	150.4
27k	Me		H	0.25±0.11	0.056±0.024	0.035±0.000	4.5	149.9
27n	Me		H	0.30±0.15	0.12±0.014	0.04±0.005	3.8	153.9
27o	Me		H	0.90±0.28	0.22±0.042	0.25±0.042	3.2	ND[e]
27p	Me		H	0.26±0.057	0.062±0.00	0.045±0.004	4.7	145.9
27l			H	>10	0.22±0.028	0.11±0.028	4.9	ND[e]
27m			H	0.31±0.13	0.15±0.007	0.029±0.008	3.7	ND[e]
27q	Me		OMe	6.7±2.4	0.21±0.014	0.098±0.017	4.6	ND[e]
27r	Me	H		0.008±0.000	0.078±0.23	0.023±0.014	4.2	ND[e]
27s	Me	H	NH_2	0.007±0.000	0.050±0.012	0.019±0.010	3.1	ND[e]
27t	Me	H		0.008±0.000	0.018±0.001	0.14±0035	3.0	82.4
27u	Me	H		0.087±0.012	0.044±0.002	0.037±0.000	3.5	ND[e]

[a]All compounds were isolated as 95:5 trans/cis mixtures. [b]All IC_{50} values are the mean ± SD (of at least two independent experiments). [c]cLogP was calculated using QikProp.[35] [d]High throughput solubility in PBS buffered at pH 7.0. [e]Denotes not tested.

biological studies are underway to better understand these activities in an in vivo setting.

Extensive ADME Profiling of 26h. In the Caco-2 bidirectional permeability assays, the permeability (P_{app}) of **26h** in the apical to basolateral ($P_{app,A\rightarrow B}$) direction and in the

dx.doi.org/10.1021/jm201112g | J. Med. Chem. 2012, 55, 169−196

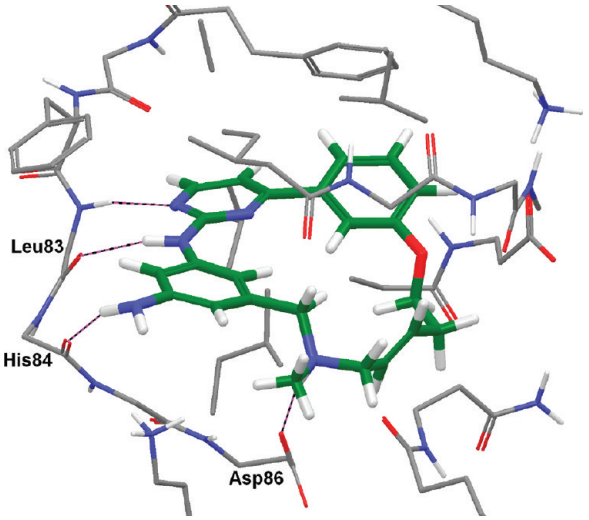


Figure 6. 27s docked into CDK2. The aniline nitrogen is forming an additional hydrogen bond to the backbone carbonyl of His84 at the end of the kinase hinge region. This is probably not a strong hydrogen bond as evident from the increase in activity of only 2-fold over **26h**.

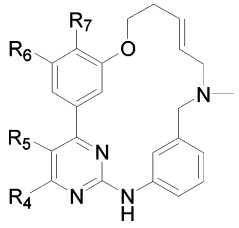
basolateral to apical ($P_{app,B\rightarrow A}$) direction was 28.0 × 10^{-6} and 27.4 x10^{-6} cm/s, respectively. The efflux ratio, defined as the ratio of $P_{app,\ B\rightarrow A}$ to $P_{app,A\rightarrow B}$, was less than 3 (1.0), indicating that **26h** was not a substrate for efflux by intestinal P-gp transporters, suggestive of high intestinal absorption in humans (Table 6). In human liver microsomes (HLM) **26h** was found to be stable with a half-life of 45 min, was moderately stable in DLM ($t_{1/2}$ = 33 min), and was quite rapidly cleared in MLM ($t_{1/2}$ = 12 min) and in RLM ($t_{1/2}$ = 11 min). Human CYP1A2, 3A4, 2C9, and 2C19 isoforms were not inhibited by **26h** at the highest tested concentration of 25 μM, but the compound inhibited CYP2D6 with IC_{50} = 0.95 μM, approximately at the plasma C_{max} observed at the maximum tolerated dose. Compound **26h** was highly bound to plasma proteins in human, mouse, and dog plasma with PPB ranging between 99.4% to 99.9%.

Pharmacokinetics of 26h in Mice. The PK properties of **26h** in mice are summarized in Table 7. **26h** showed high systemic clearance relative to liver blood flow and high volume of distribution at steady state, with a terminal half-life of ~5.0 h. It showed rapid absorption (t_{max} = 0.5 h) and a mean C_{max} and AUC of 1029 ng/mL and 2523 ng·h/mL, respectively, with a mean terminal half-life of 6.1 h following a single oral dose of 75 mg/kg. It showed an acceptable oral bioavailability of 24%. The exposures achieved in mice at the 75 mg/kg dose far exceeded the enzyme inhibiton (CDK2 IC_{50} = 0.013 μM, JAK2 IC_{50} = 0.073 μM, and FLT3 IC_{50} = 0.056 μM) and cell proliferation concentrations in HCT-116 (IC_{50} = 0.079 μM) and HL-60 (IC_{50} = 0.059 μM), correlating with the observed efficacy of **26h** in preclinical pharmacology models at similar doses.[36]

Efficacy of 26h in Xenograft Models. On the basis of its efficacy on a broad spectrum of tumor cell lines and good oral bioavailability, **26h** was selected for evaluation in human tumor xenograft mouse models. Two models were selected based on their relevance in cancer: HCT-116 colon cancer and Ramos B-cell lymphoma. Prior to conducting both experiments, dosing regimes in each model were explored and optimal schedules selected for each model that would be tolerated for the duration of the experiment.[36]

In the colon cancer model, HCT-116 cells were injected subcutaneously and tumors were established with mean group sizes of approximately 100 mm^3. Treatment with **26h** at doses of 50 and 75 mg/kg po 3 times per week on a Monday, Wednesday, Friday schedule was started 8 days after cell inoculation for 15 days. Treatment with **26h** at 75 mg/kg po q.d. 3×/week significantly inhibited the growth of tumors with

Table 4. SAR Study of Aromatic Ring Substitutions



Cpd[a]	R_4	R_5	R_6	R_7	CDK2 IC_{50} (μM)[b]	JAK2 IC_{50} (μM)[b]	FLT3 IC_{50} (μM)[b]	cLogP[c]
26h	H	H	H	H	0.013±0.004	0.073±0.017	0.056±0.026	4.1
28a	CH_3	H	H	H	4.7±0.21	>10	1.7±0.78	4.5
28b		H	H	H	>10	>10	>10	5.4
28c	H	CH_3	H	H	0.15±0.021	0.18±0.035	0.022±0.009	4.4
28d	H	H	F	H	0.009±0.001	0.16±0.007	0.030±0.006	4.4
28e	H	H	H	OMe	2.3±0.21	0.51±0.014	0.31±0.086	4.1

[a]All compounds were isolated as 95:5 trans/cis mixtures. [b]All IC_{50} values are the mean ± SD of at least two independent experiments. [c]cLogP was calculated using QikProp.[35]

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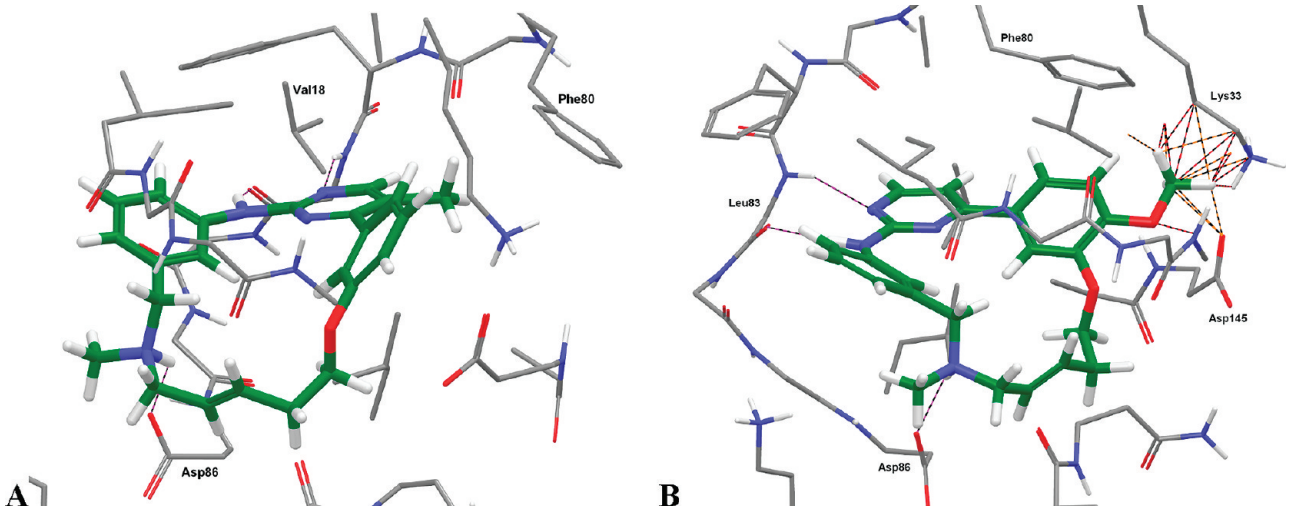

Figure 7. (A) **28c** docked into CDK2. In the putative bioactive conformation, the methyl group interacts favorably with Phe80. However, the methyl group induces a twist in the A and C rings, so they are less coplanar, shifting the A-ring slightly away from Phe80 and thus preventing the A-ring from interacting with Phe80. (B) **28e** docked into CDK2. The orange-black dashed lines indicate clashes between protein and ligand atoms. The methoxy substituent clashes with the side chains of Lys33 and Asp145. These are flexible residues and may move to accommodate the substituents. However, this is energetically unfavorable, as it will break the salt bridge between the two residues.

Table 5. In Vitro Profiling of Preferred Compounds

	IC_{50} (μM)[a]				$t_{1/2}$ (min)		IC_{50} (μM)[a]				
compd	CDK2	JAK2	FLT3	solubility (μg/mL)[b]	HLM	MLM	HL-60	HCT-116	RAMOS	COLO205	DU145
26g	0.021	0.15	0.11	14.7	48	10	0.089	0.135	ND[c]	0.12	0.71
26h	0.013	0.073	0.056	71.8	45	12	0.059	0.079	0.033	0.072	0.14
26k	0.036	0.090	0.042	16.6	>60	>60	1.38	1.6	ND[c]	3.6	3.0
27b	0.024	0.082	0.077	ND[c]	ND[c]	ND[c]	0.34	0.49	ND[c]	0.43	ND[c]
27i	0.074	0.049	0.071	147.0	21	ND[c]	0.076	0.41	ND[c]	ND[c]	ND[c]
27t	0.008	0.018	0.14	82.4	30	7	0.13	ND[c]	ND[c]	ND[c]	ND[c]
27r	0.008	0.050	0.019	ND[c]	21	ND[c]	0.006	ND[c]	ND[c]	ND[c]	ND[c]

[a]All IC_{50} values are the mean of at least two independent experiments. [b]High throughput solubility in PBS at pH 7.0. [c]ND denotes not tested.

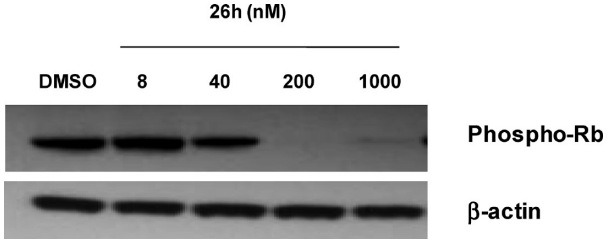

Figure 8. HCT-116 cells were treated separately with the indicated concentrations of **26h** for 24 h prior to denaturing lysis. An amount of 30 μg of lysate from each treatment was resolved on 10% SDS−PAGE, transferred onto PVDF membrane, and probed with antibodies against phospho-Rb and β-actin.

a mean TGI of 82%, while the lower dose of 50 mg/kg po 3×/week was marginally effective (Figure 9).

In the lymphoma model Ramos cells were injected subcutaneously and tumors were established with mean group sizes of approximately 200 mm^3. Two different dosing regimens of **26h** were explored in this model: 75 mg/kg po q.d. on a 2 days on and 5 days off schedule and 15 mg/kg ip q.d. on a 5 days on 5 days off schedule were started 12 days after cell inoculation for 15 days. There were two vehicle control groups that received either MC/Tween or DMA/CRE (see Experimental Section for details). The treatment groups were compared with the corresponding vehicle control groups for assessment of percentage TGI. Treatment with **26h** using either regime significantly inhibited the growth of tumors with mean TGIs of 42% and 63% for the oral and ip delivery

Table 6. Physicochemical Properties and in Vitro ADME of 26h

property	value
molecular wt	372.47
no. of HDB	1
no. of HBA	4
cLogP[a]	4.1
PSA ($Å^2$)[a]	43
permeability[b] ($P_{app,A\rightarrow B}$, $\times 10^{-6}$ cm/s)	28 (efflux ratio of 1.0)
metabolic stability ($t_{1/2}$, min)	
HLM	45
MLM	12
RLM	11
DLM	33
human CYP inhibition IC_{50} (μM)	>25,[c] 0.95[d]
plasma protein binding (%)[e]	
human	99.9
mouse	99.4
dog	99.9

[a]cLogP and PSA were calculated using QikProp.[35] [b]Caco-2 bidirectional permeability assay. [c]CYP3A4, 1A2, 2C9, 2C19. [d]CYP2D6. [e]Equilibrium dialysis assay in plasma at 1000 ng/mL.

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Table 7. Pharmacokinetics of 26h in Mice

(A) Intravenous Administration				
dose (mg/kg)	Cl (L h^{-1} kg^{-1})	V_{ss} (L/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)
5.0	6.6	23	4.6	752

(B) Oral Administration[a]					
dose (mg/kg)	t_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	F (%)
75	0.5	1029	6.1	2523	24

[a]In preliminary PK studies, **26h** showed oral bioavailability (F) of 4% and 37% in rats and dogs, respectively.

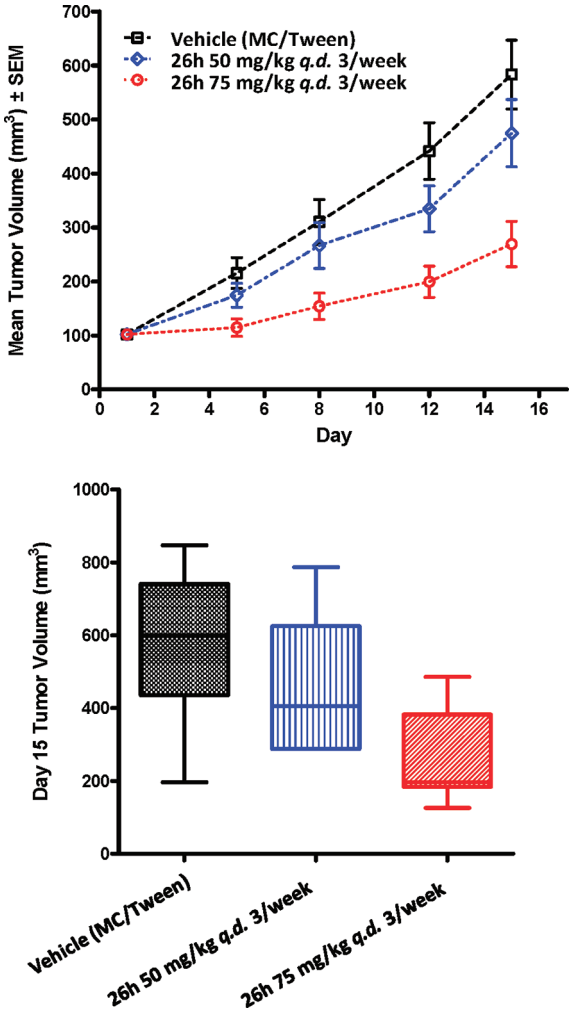

Figure 9. 26h is efficacious in a murine sc xenograft model of human colon cancer (HCT-116). Tumors were established in female BALB/c nude mice by sc implantation of 5×10^6 HCT-116 tumor cells. Tumor burden (volume, mm^3) was measured twice a week by $(w^2l)/2$, where w is the width and l is the length in mm of an HCT-116 carcinoma. Treatment started on day 1 when the group mean tumor volume reached 105 mm^3 and treatment ended on day 15.

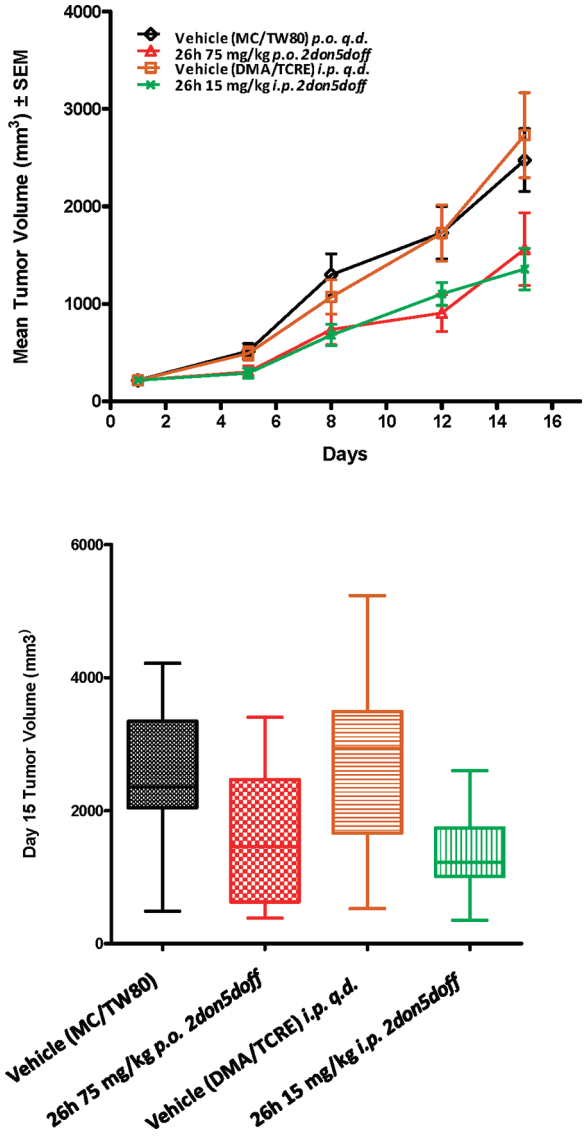

Figure 10. 26h is efficacious in a sc xenograft model of human B-cell lymphoma (Ramos). Ramos tumors were established in female BALB/c nude mice by sc implantation of 7×10^6 Ramos cells. Treatment with **26h** and vehicle (MC/Tween for po or DMA/CRE for ip) was initiated on day 1 and continued until day 15. The tumor volumes from the 15 mg/kg ip group was significantly different compared to the ip vehicle control group on day 15. Sample size, n = 8–10 mice per group.

methods, respectively (Figure 10). Given the encouraging TGIs observed with both oral and ip dosing schedules in these challenging models, **26h** was selected for further preclinical development.[36]

CONCLUSIONS

We have described the discovery of a series of small molecule macrocycles as potent inhibitors of CDKs, JAK2, and FLT3, a spectrum selective profile not previously reported. Application of a hypothesis of conformational constraint generated macrocycles that were synthesized using a RCM strategy. Screening of initial compounds in functional biochemical assays against CDK2, JAK2, and FLT3 kinases allowed selection of a preferred linker moiety containing a phenolic ether, trans double bond, and allylic/benzylic *N*-methyl group. SAR and broader in vitro profiling, particularly cellular assays, identified **26h**, a small molecule kinase inhibitor with a distinct kinase inhibitory spectrum, as the preferred lead candidate. Further evaluation revealed excellent pharmacokinetic properties of **26h** and dose-dependent efficacy in mouse models of cancer including a HCT-116 model of colon cancer and a Ramos model of lymphoma. On the basis of its favorable pharmaceutical and pharmacological profile, **26h** (SB1317/

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TG02) was advanced into development and is currently being evaluated in phase 1 clinical trials in leukemia patients.

EXPERIMENTAL SECTION

Chemistry. Solvents and reagents were used directly from the suppliers without further purification and were of the highest generally available quality. Workup for chemical reactions was typically done by diluting the reaction mixture or residue with the reaction solvent or extraction solvent and then washing with the indicated aqueous solution(s). Product solutions were dried over anhydrous sodium sulfate prior to filtration, and the solvents were removed under reduced pressure using a rotary evaporator. All compounds were purified by either flash or reverse phase chromatography. Flash column chromatography was conducted using silica gel 60 (Merck KGaA, 0.040−0.063 mm, 230−400 mesh ASTM). Reverse-phase preparative high performance liquid chromatography (rpHPLC) was operated on a Phenomenex column (Luna 5 μm C18 (2) 100A 150 mm × 21.2 mm) with adjustable solvent gradients, usually 5−95% acetonitrile in water + 0.1% trifluoroacetic acid (TFA) with a run time of 18 min at a flow rate of 20 mL/min, and was used for routine purification. Fractions containing the desired product were lyophilized or evaporated to dryness under vacuum to provide the dry compound or evaporated to remove volatile organic solvents and then extracted with a suitable organic solvent (ethyl acetate or dichloromethane were commonly used; if necessary, the pH of the aqueous solution was adjusted in order to get free base, acid, or the neutral compound).

The preliminary purity and identity of all compounds were assessed after purification by tandem HPLC/mass spectrometry (LC/MS) analyses on a Waters Micromass ZQ mass spectrometer in electrospray ionization (ESI) positive mode after separation on a Waters 2795 separations module. The HPLC separations were performed on a Phenomenex column (Luna 5 μm C18 (2) 100A 50 mm × 2.00 mm) with a flow rate of 0.8 mL/min and a 4 min gradient of x − 100% acetonitrile in water + 0.05% TFA (x = 5 or 30 or 50), using a Waters 2996 photodiode array detector. Purity and identity were assessed on the integrated UV chromatogram (220−400 nm) and the mass spectrum (homogeneity of the product peak and its fragmentation(s)).

The final purity was determined using a Waters 2695 separations module on a Waters Xterra RP18 3.5 μm, 4.6 mm × 20 mm IS column with a flow rate of 2.0 mL/min, gradient 5−65% B over 4 min, then 65−95% B over 1 min, and 95% B for an additional 0.1 min (solvent A, H_2O with 0.1% TFA; solvent B, acetonitrile with 0.1% TFA), and a Waters 2996 photodiode array detector. For compounds not suitable for the above HPLC methods either because of polarity or poor peak separation, a longer column (Phenomenex Gemini 5 μm C18, 110A, 4.6 mm × 150 mm) together with a flow rate of 1.0−1.2 mL/min and a 15 min gradient of 5−95% acetonitrile in water + 0.1% TFA was used for purity determination. Purity was >95% for all test compounds except those indicated in their respective synthesis descriptions.

All the 1D and 2D NMR experiments for ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) nuclei were performed on a Bruker AVANCE-400 digital NMR spectrometer. ^{1}H−^{1}H and ^{1}H−^{13}C experiments (COSY, HMQC, HSQC, and HMBC) were run with Z-gradient selection. NMR spectra are reported in ppm with reference to an internal tetramethylsilane standard (0.00 ppm for ^{1}H and ^{13}C) or solvent peak(s) of $CDCl_3$ (7.26 and 77.1 ppm) or CD_3OD (3.31 and 49.0 ppm) or DMSO-d_6 (2.50 and 39.5 ppm). Other NMR solvents were used as needed. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broadened, dd = doublet of doublets, dt = doublet of triplets, and bs = broadened singlet. Coupling constants, when given, are reported in hertz.

All melting points are uncorrected. Elemental analyses of CHN were performed on a Perkin-Elmer 2400 CHN/CHNS elemental analyzer. HRMS data were obtained from a Bruker microTOF-Q II with direct injection.

2,4-Dichloro-6-pyrrolidin-1-ylpyrimidine (10d). To a mixture of 2,4,6-trichloropyrimidine (1.83 g, 10.0 mmol) in THF (50 mL) at −20 °C was added pyrrolidine (0.83 mL, 10.0 mmol) dropwise over a period of 30 min. The resulting mixture was allowed to warm to room temperature over 1 h. The reaction mixture was concentrated under reduced pressure, and CH_2Cl_2 (50 mL) was added. It was washed with 1 N NaOH, water, dried over Na_2SO_4, and concentrated under reduced pressure to obtain the crude residue. The crude material was purified by column chromatography (EtOAc/hexane, 1:8) to afford **10d** (1.15 g, 53%). LC/MS (ESI positive mode) m/z 218 ($[M + H]^+$) $C_8H_9Cl_2N_3$.

3-(2-Chloropyrimidine-4-yl)phenol (12a). To a degassed (argon) solution of 2,4-dichloropyrimidine (10 g, 67.1 mmol) and 3-hydroxyphenylboronic acid (11.1 g, 80.5 mmol) in 1,2-dimethoxy ethane (150 mL) were added aqueous $NaHCO_3$ (10.6 g in 15 mL H_2O) and freshly prepared $Pd(PPh_3)_4$ (7.7 g, 6.6 mmol). The reaction mixture was heated to 80 °C for 7 h. It was cooled to room temperature and quenched with saturated NH_4Cl solution. The reaction mass was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to obtain the crude residue. The crude material was purified by column chromatography (EtOAc/hexane, 1:4) to afford **12a** (12.20 g, 88%) as an off white solid. LC/MS (ESI positive mode) m/z 207 ($[M + H]^+$) $C_{10}H_7ClN_2O$; ^{1}H NMR ($CDCl_3$) δ 9.71 (br, 1H), 8.71 (d, 1H), 7.98 d, 1H), 7.49−7.54 (m, 2H), 7.30 (t, 1H), 6.94 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ 166.7, 161.58, 160.95, 158.54, 136.30, 130.77, 119.6, 118.6, 117.5, 116.5, 114.2. Anal. Calcd for $C_{10}H_7ClN_2O$: C, 58.13; H, 3.14; N, 13.56; Cl, 17.16. Found: C, 58.22; H, 3.33; N, 13.32; Cl, 13.45. IR (KBr pellet): 3173, 1536, 1360, 789, 625, 1082, 791 cm^{-1}.

[3-(2-Chloropyrimidine-4-yl)phenyl]methanol (12b). The synthesis for this intermediate was prepared using a different Suzuki coupling procedure compared to **12a**. To a degassed (nitrogen) solution of **10a** (50 g, 335 mmol) and 3-(hydroxymethyl)-phenylboronic acid **11b** (48.5 g, 318 mmol) in THF (500 mL, 10 vol) were added saturated Na_2CO_3 (88.6 g, 838 mmol), palladium acetate [$Pd(OAc)_2$] (0.15 g, 0.67 mmol), and triphenylphosphine (0.35 g, 1.34 mmol). The resultant mixture was stirred at 70 °C for 6 h and filtered. Water was added to the filtrate, and it was extracted with EtOAc (3 × 250 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and filtered. The filtrate was treated with activated charcoal, and the mixture was filtered. The filtrate was concentrated under reduced pressure, and the crude product was slurried in isopropyl alcohol (300 mL). The mixture was then filtered and the solid was slurried in cold n-heptane and was again filtered to furnish **12b** (42.8 g, 61%). This material was taken up for the next step without any purification. LC/MS (ESI positive mode) m/z 221 ($[M + H]^+$) $C_{11}H_9ClN_2O$; ^{1}H NMR (DMSO-d_6) δ 8.81 (d, 1H), 8.15 (br, 1H), 8.10 (d, 1H), 8.04 (dt, 1H), 7.57−7.54 (m, 1H), 4.63 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 166.7, 161.4, 160.8, 143.9, 134.7, 130.5, 129.4, 126.1, 125.4, 116.4, 62.9.

Following a procedure similar to that for **12a**, the following intermediates were synthesized.

2-Chloro-4-(3-nitrophenyl)pyrimidine (12c). The title compound was synthesized from **10a** and 3-nitrophenylboronic acid **11c** (91%). LC/MS (ESI positive mode) m/z 236 ($[M + H]^+$) $C_{10}H_6ClN_3O_2$; ^{1}H NMR ($CDCl_3$) δ 8.94 (t, 1H), 8.76 (d, 1H), 8.48 (qd, 1H), 8.40 (m), 7.77−7.70 (m, 2H).

3-(2-Chloropyrimidin-4-yl)benzoic Acid (12e). The title compound was synthesized from **10a** and 3-carboxyphenylboronic acid **11d** (63%). LC/MS (ESI positive mode) m/z 235 ($[M + H]^+$) $C_{11}H_7ClN_2O_2$; ^{1}H NMR (MeOD-d_4) δ 8.66 (s, 1H), 8.62 (d, 1H), 8.29 (d, 1H), 8.17 (d, 1H), 7.69 (d, 1H), 7.55 (t, 1H).

3-(2-Chloropyrimidin-4-yl)benzaldehyde (12f). The title compound was synthesized from **10a** and 3-carbonylphenylboronic acid **11e** (60%). LC/MS (ESI positive mode) m/z 219 ($[M + H]^+$) $C_{11}H_7ClN_2O$.

3-(2-Chloro-6-methylpyrimidin-4-yl)phenol (12g). The title compound was synthesized from 2,4-dichloro-6-methylpyrimidine **10b** and 3-hydroxyphenylboronic acid **11a** (81%). LC/MS (ESI positive mode) m/z 221 ($[M + H]^+$) $C_{11}H_9ClN_2O$.

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3-(2-Chloro-6-pyrrolidin-1-ylpyrimidin-4-yl)phenol (12h). The title compound was synthesized from **10d** and 3-hydroxyphenylboronic acid **11a** (56%). LC/MS (ESI positive mode) m/z 276 ($[M + H]^+$) $C_{14}H_{14}ClN_3O$.

3-(2-Chloro-5-methylpyrimidin-4-yl)phenol (12i). The title compound was synthesized from 2,4-dichloro-5-methylpyrimidine **10e** and 3-hydroxyphenylboronic acid **11a** (66%). LC/MS (ESI positive mode) m/z 221 ($[M + H]^+$) $C_{11}H_9ClN_2O$.

3-(2-Chloropyrimidin-4-yl)-5-fluorophenol (12j). The title compound was synthesized from **10a** and 3-fluoro-5-hydroxyphenylboronic acid **11f** (53%). LC/MS (ESI positive mode) m/z 225 ($[M + H]^+$) $C_{10}H_6ClFN_2O$.

Acetic Acid 5-(2-Chloropyrimidin-4-yl)-2-methoxyphenyl Ester (12k). The title compound was synthesized from **10a** and 3-acetoxy-4-methoxyphenylboronic acid pinacol ester **11g** (74%). LC/MS (ESI positive mode) m/z 279 ($[M + H]^+$) $C_{13}H_{11}ClN_2O_3$.

3-(2-Chloropyrimidin-4-yl)phenylamine (12d). To a solution of **12c** (0.3 g, 1.07 mmol) in CH_2Cl_2/MeOH (1:1, 10 mL), cooled to 0 °C, $SnCl_2{\cdot}H_2O$ (1.15 g, 5.00 mmol) was added. The reaction mixture was gradually raised to room temperature and stirred for 7 h. After completion of the reaction, the solvent from the reaction mixture was removed under reduced pressure and the residue was taken into saturated Na_2CO_3 and CH_2Cl_2 (20 mL). The reaction mixture was filtered through filter paper. The organic layer was separated from the filtrate, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford **12d** (0.2 g, 91%) as a thick syrup which was used for the next step without further purification. LC/MS (ESI positive mode) m/z 206 ($[M + H]^+$) $C_{10}H_8ClN_3$.

5-(2-Chloropyrimidin-4-yl)-2-methoxyphenol (12l). To a solution of **12k** (0.27 g, 0.96 mmol) in THF (2.5 mL) was added LiOH (0.07 g, 2.88 mmol, dissolved in 2.5 mL of H_2O). The reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, 1 M HCl added to acidify the mixture and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford **12l** (0.22 g, 97%) as a pale orange solid which was used for the next step without further purification. LC/MS (ESI positive mode) m/z 237 ($[M + H]^+$) $C_{11}H_9ClN_2O_2$; ^{1}H NMR ($CDCl_3$) δ 8.56 (d, 1H), 7.71 (dd, 1H), 7.54 (d, 1H), 6.95 (d, 1H), 3.98 (s, 3H).

4-(3-Allyloxymethylphenyl)-2-chloropyrimidine (13b). A mixture of **12b** (20 g, 90.6 mmol), KOH (23.8 g, 172 mmol), and tetrabutylammonium hydrogen sulfate (1.5 g, 4.53 mmol) in allyl bromide (80 mL, 4 vol) was stirred at room temperature for 12 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with water, brine, dried (Na_2SO_4), and concentrated under reduced pressure to obtain the crude residue which was purified by column chromatography (using 15% EtOAc in hexane) to furnish **13b** (13 g, 55%) as a pale yellow oil. LC/MS (ESI positive mode) m/z 261 ($[M + H]^+$) $C_{14}H_{13}ClN_2O$; ^{1}H NMR ($CDCl_3$) δ 8.62 (d, 1H), 8.06 (br, 1H), 8.00 (dt, 1H), 7.66 (d, 1H), 7.54–7.46 (m, 2H), 6.02–5.93 (m, 1H), 5.27 (ddq, 2H), 4.60 (s, 2H), 4.08 (dq, 2H); ^{13}C NMR ($CDCl_3$) δ 167.1, 161.9, 159.9, 139.7, 135.3, 134.6, 131.2, 129.7, 129.3, 126.6, 117.5, 115.4, 71.7, 71.6; IR (KBr pellet): 1568, 1535, 1344, 1186, 1079 cm^{-1}.

Following a procedure similar to that of **13b**, the following intermediates were synthesized.

4-(3-Allyloxyphenyl)-2-chloropyrimidine (13a). The title compound was synthesized from **12a** and allyl bromide (59%). LC/MS (ESI positive mode) m/z 247 ($[M + H]^+$) $C_{13}H_{11}ClN_2O$.

1-Allyloxymethyl-3-nitrobenzene (18g). The title compound was synthesized from 3-nitrobenzyl alcohol **16c** and allyl bromide (78%). LC/MS (ESI positive mode) m/z 194 ($[M + H]^+$) $C_{10}H_{11}NO_3$; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H), 8.18 (dd, 1H), 7.73 (dd, 1H), 7.57 (t, 1H), 6.01 (m, 1H), 5.38 (m, 1H), 5.29 (m, 1H), 4.65 (s, 2H), 4.13 (dt, 2H).

But-3-enoic Acid [3-(2-Chloropyrimidin-4-yl)phenyl]amide (14a). To a mixture of **12e** (0.2 g, 0.852 mmol) and vinylacetic acid (81 μL, 0.942 mmol) in CH_2Cl_2 (4 mL) at room temperature were added HOBt (0.184 g, 1.36 mmol) and EDCI (0.26 g, 1.36 mmol). The resulting mixture was stirred for 4 h. The reaction mixture was cooled to 0 °C and quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 thrice, and the combined organic extracts were washed with saturated $NaHCO_3$ followed by brine, dried over Na_2SO_4, and concentrated under reduced pressure to give **14a** (0.18 g, 77%) which was taken for the next step without further purification. LC/MS (ESI positive mode) m/z 274 ($[M + H]^+$) $C_{14}H_{12}ClN_3O$.

Following a procedure similar to that of **14a**, the following intermediates were synthesized.

***N*-Allyl-3-(2-chloropyrimidin-4-yl)benzamide (14b).** The title compound was synthesized from **12e** and allylamine (59%). LC/MS (ESI positive mode) m/z 274 ($[M + H]^+$) $C_{14}H_{12}ClN_3O$; ^{1}H NMR ($CDCl_3$) δ 8.69 (d, 1H), 8.51 (t, 1H), 8.24 (m, 2H), 7.96 (m, 1H), 7.72 (d, 1H), 7.61 (t, 1H), 6.02–5.89 (m, 1H), 5.37–5.21 (m, 2H), 4.14 (m, 2H).

Pent-4-enoic Acid (3-Nitrophenyl)amide (18a). The title compound was synthesized from 3-nitroaniline **16a** and pent-4-enoic acid (yield, 58%). LC/MS (ESI positive mode) m/z 221 ($[M + H]^+$) $C_{11}H_{12}N_2O_3$.

4-(3-Allyloxyphenyl)-2-chloropyrimidine (15a). To a solution of **12a** (8 g, 38.8 mmol) in anhydrous DMF (40 mL), 4-bromo-1-butene (23.6 mL, 233 mmol) was added slowly followed by cesium carbonate (57 g, 174.7 mmol). The reaction mixture was heated to 40 °C for 12 h. The mixture was brought to room temperature and quenched with water (140 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 75 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane, 1:19) to afford **15a** (7.5 g, 75%) as a white solid. LC/MS (ESI positive mode) m/z 261 ($[M + H]^+$) $C_{13}H_{11}ClN_2O$; ^{1}H NMR ($CDCl_3$) δ 8.63 (d, 1H), 7.64–7.67 (m, 2H), 7.62 (d, 1H), 7.41 (dd, 1H), 5.90–5.94 (m, 1H), 5.15–5.22 (m, 1H), 4.12 (t, 1H), 2.59 (p, 1H); ^{13}C NMR ($CDCl_3$) δ 167.0, 161.8, 159.8, 159.6,136.5, 134.3, 130.2, 119.8, 118.3, 117.2, 115.3, 113.4, 67.5,33.6. Anal. Calcd for $C_{14}H_{13}ClN_2O$: C, 64.49; H, 5.03; N, 10.74; Cl, 13.60. Found: C, 64.73; H, 5.07; N, 10.54; Cl, 13.34. IR (KBr pellet): 1573, 1455, 1351, 1223, 1183, 1037, 784 cm^{-1}.

Following a procedure similar to that of **15a**, the following intermediates were synthesized.

4-(3-But-3-enyloxyphenyl)-2-chloro-6-methylpyrimidine (15b). The title compound was synthesized from **12g** and 4-bromo-1-butene (70%). LC/MS (ESI positive mode) m/z 275 ($[M + H]^+$) $C_{15}H_{15}ClN_2O$.

4-(3-But-3-enyloxyphenyl)-2-chloro-6-pyrrolidin-1-ylpyrimidine (15c). The title compound was synthesized from **12h** and 4-bromo-1-butene (41%). LC/MS (ESI positive mode) m/z 330 ($[M + H]^+$) $C_{18}H_{20}ClN_3O$.

4-(3-But-3-enyloxyphenyl)-2-chloro-5-methylpyrimidine (15d). The title compound was synthesized from **12i** and 4-bromo-1-butene (56%). LC/MS (ESI positive mode) m/z 275 ($[M + H]^+$) $C_{15}H_{15}ClN_2O$.

4-(3-But-3-enyloxy-5-fluorophenyl)-2-chloropyrimidine (15e). The title compound was synthesized from **12j** and 4-bromo-1-butene (65%). LC/MS (ESI positive mode) m/z 279 ($[M + H]^+$) $C_{14}H_{12}ClFN_2O$.

4-(3-But-3-enyloxy-4-methoxyphenyl)-2-chloropyrimidine (15f). The title compound was synthesized from **12l** and 4-bromo-1-butene (45%). LC/MS (ESI positive mode) m/z 291 ($[M + H]^+$) $C1_5H_{15}ClN_2O_2$; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H), 8.13 (m, 2H), 7.60 (d, 1H), 6.97 (d, 1H), 6.04–5.97 (m, 1H), 5.38–5.22 (m, 2H), 4.60 (s, 2H), 4.14–4.12 (m, 2H), 3.91 (s, 3H).

5-Nitro-2-trifluoromethoxybenzaldehyde (16g). To a cooled solution of 2-trifluoromethoxybenzaldehyde **16f** (2.58 g, 13.6 mmol) in concentrated H_2SO_4 (8.1 mL) at 0 °C was added concentrated HNO_3 (1.7 mL) dropwise, and reaction mixture was stirred for 2 h. Cold water was added, and resulting mixture was filtered. The product **16g** was obtained as an off-white solid (2.55 g, 80%) and was taken to the next step without further purification. LC/MS (ESI positive mode) m/z 236 ($[M + H]^+$) $C_8H_4F_3NO_4$.

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Allylmethyl-(3 nitrobenzyl)amine (18b). To a solution of 3-nitrobenzaldehyde **16b** (20 g, 132.4 mmol) in CH_2Cl_2 (500 mL) was added *N*-allylmethylamine (18.8 mL, 198.6 mmol), and the resulting mixture was stirred at room temperature for 12 h after which $Na(OAc)_3BH$ (56.13 g, 265 mmol) was added portionwise and the mixture stirred for an additional 4 h. The reaction mixture was then quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude material which was purified by column chromatography (EtOAc/hexane, 1:19) to afford **18b** (25.9 g, 95%) as a pale yellow syrup. LC/MS (ESI positive mode) *m/z* 207 ($[M + H]^+$) $C_{11}H_{14}N_2O_2$; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1H), 8.11 (dd, 1H), 7.68 (dd, 1H), 7.48 (t, 1H), 5.85−5.94 (m, 1H), 5.20 (t, 1H), 3.60 (s, 2H), 3.07 (dd, 1H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 148.4, 141.5, 135.20, 134.9, 129.1, 123.7, 122.1, 118.1, 60.52, 60.50. Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 62.31; H, 6.69; N, 13.25. IR (KBr pellet): 2787, 1528, 1349, 924, 733 cm^{-1}.

Following a procedure similar to that of **18b**, the following intermediates were synthesized.

***N*-Allyl-3-(2-chloropyrimidin-4-yl)benzamide (14c).** The title compound was synthesized from **12f** and *N*-allylmethylamine (60%). LC/MS (ESI positive mode) *m/z* 274 ($[M + H]^+$) $C_{11}H_{14}N_2O_2$.

Allyl-(3-nitrobenzyl)amine (18c). The title compound was synthesized from **10b** and allylamine (88%). LC/MS (ESI positive mode) *m/z* 193 ($[M + H]^+$) $C_{10}H_{12}N_2O_2$; ^1H NMR ($CDCl_3$) δ 8.20 (s, 1H), 8.07 (d, 1H), 7.68 (d, 1H), 7.47 (t, 1H), 5.93−5.87 (m, 1H), 5.22−5.11 (m, 2H), 3.88 (s, 2H), 3.28−3.26 (m, 2H).

***N*-Allyl-2,2,2-trifluoro-*N*-(3-nitrobenzyl)acetamide (18d).** To a solution of **18c** (0.673 g, 3.51 mmol) and trifluoroacetic anhydride (0.98 mL, 7.00 mmol) in THF (10 mL) was added triethylamine (0.98 mL, 7.00 mmol) dropwise, and the resulting mixture was stirred at room temperature for 1 h. Aqueous $NaHCO_3$ (20 mL) was added and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give **18d** (1 g, 99%) as an orange liquid. This material was taken up for the next step without further purification. LC/MS (ESI positive mode) *m/z* 289 ($[M + H]^+$) $C_{12}H_{11}F_3N_2O_3$; ^1H NMR ($CDCl_3$) δ 8.19 (m, 1H), 8.09 (s, 1H), 7.62−7.55 (m, 2H), 5.81−5.75 (m, 1H), 5.38−5.26 (m, 2H), 4.70 (s, 2H), 4.01−3.96 (m, 2H).

3-Nitro-5-(2,2,2-trifluoroacetylamino)benzoic Acid Methyl Ester (16w). Following a procedure similar to that of **18d**, the title compound was synthesized from 3-amino-5-nitrobenzoic acid methyl ester **16v** (yield, quantitative). LC/MS (ESI positive mode) *m/z* 293 ($[M + H]^+$) $C_{10}H_7F_3N_2O_5$.

Allyl-(3-nitrophenyl)amine (18e). To a solution of 3-nitroaniline **16a** (1 g, 7.24 mmol) and allyl bromide (0.86 mL, 7.60 mmol) in CH_2Cl_2 (20 mL) was added triethylamine (2.0 mL, 14.5 mmol), and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was cooled to 0 °C and quenched with water. The product was extracted with CH_2Cl_2 thrice, and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to furnish an oil, which was purified by column (EtOAc/hexane) to obtain 0.89 g of **18e** (66%). LC/MS (ESI positive mode) *m/z* 179 ($[M + H]^+$) $C_9H_{10}N_2O_2$.

Allyl-(3-nitrophenyl)carbamic Acid *tert*-Butyl Ester (18f). To a solution of **18e** (0.3 g, 1.68 mmol), di-*tert*-butyl dicarbonate (0.55 g, 2.52 mmol), and 4-(dimethylamino)pyridine (10 mg, 0.084 mmol) in THF (5 mL) was added triethylamine (0.33 mL, 2.36 mmol), and the resulting mixture was stirred at room temperature for 4 h. Upon completion, THF was removed under reduced pressure and water (20 mL) was added. The product was extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give **18f** (0.4 g, 86%) as a colorless liquid. This material was taken up for the next step without further purification. LC/MS (ESI positive mode) *m/z* 279 ($[M + H]^+$) $C_{14}H_{18}N_2O_4$.

1-Chloro-2-dimethoxymethyl-4-nitrobenzene (16j). To a solution of 2-chloro-5-nitrobenzaldehyde **16e** (1.85 g, 1.00 mmol) in MeOH (50 mL) was added ammonium chloride (0.2 g, 3.67 mmol), and the resulting mixture was refluxed for 7 h. Upon completion, the reaction mixture was concentrated under reduced pressure to give an oil, which was dissolved in CH_2Cl_2. It was washed with water once, dried over Na_2SO_4, and concentrated under reduced pressure to furnish colorless oil **16j** (2.2 g, 95%). LC/MS (ESI positive mode) *m/z* 232 ($[M + H]^+$) $C_9H_{10}ClNO_4$.

2-Dimethoxymethyl-1-ethylsulfanyl-4-nitrobenzene (16k). To a solution of **16j** (2.0 g, 8.63 mmol), K_2CO_3 (2.39 g, 17.3 mmol) in DMF (50 mL) was added ethanethiol (0.64 mL, 8.63 mmol), and the resulting mixture was refluxed for 7 h. Upon completion, the reaction mixture was concentrated under reduced pressure to give thick syrup, which was dissolved in CH_2Cl_2. It was washed with water once, dried over Na_2SO_4, and concentrated under reduced pressure to furnish colorless oil **16k** (1.9 g, 86%). LC/MS (ESI positive mode) *m/z* 258 ($[M + H]^+$) $C_{11}H_{15}NO_4S$.

2-Dimethoxymethyl-1-ethanesulfonyl-4-nitrobenzene (16l). To a cooled, degassed (N_2) solution of **16k** (1 g, 3.88 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added 3-chloroperbenzoic acid (1.34 g, 7.76 mmol), and the resulting mixture was stirred at room temperature overnight. The reaction mixture was cooled to 0 °C and quenched with water. The product was extracted with CH_2Cl_2 thrice, and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to furnish an oil, which was purified by column chromatography (EtOAc/hexane) to obtain 0.52 g of **16l** (46%). LC/MS (ESI positive mode) *m/z* 290 ($[M + H]^+$) $C_{11}H_{15}NO_6S$.

2-Ethanesulfonyl-5-nitrobenzaldehyde (16m). To a cooled solution of **16l** (0.5 g, 1.73 mmol) in water (10 mL) at 0 °C was added 50% TFA/CH_2Cl_2 (10 mL), and the resulting mixture was stirred at room temperature for 1 day. The organic layer was separated, and the aqueous layer was basified using saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 twice. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to furnish **16m** (0.42 g, quantitative) as an off-white solid. LC/MS (ESI positive mode) *m/z* 244 ($[M + H]^+$) $C_9H_9NO_5S$.

2-Morpholin-4-yl-5-nitrobenzaldehyde (16n). To a mixture of 2-chloro-5-nitrobenzaldehyde **16e** (2 g, 10.8 mmol) in ACN (40 mL, 20 vol) was added morpholine (3.5 mL, 32.4 mmol), and the resulting mixture was stirred at room temperature overnight. The reaction mixture was cooled to room temperature, and water (60 mL) was added to quench the reaction. The aqueous layer was extracted thrice with EtOAc (30 mL each), and the combined organic extract was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Product **16n** (2.11 g, 83%) was taken forward for the next step without any purification. LC/MS (ESI positive mode) *m/z* 237 ($[M + H]^+$) $C_{11}H_{12}N_2O_4$.

2-(2-Chloroethoxy)-5-nitrobenzaldehyde (16i). To a mixture of 2-hydroxy-4-nitrobenzaldehyde **16h** (50 g, 299 mmol) and 1,2-dichloroethane (300 mL, 6 vol) in DMF (600 mL, 12 vol)) was added K_2CO_3 (62.5 g, 450 mmol), and the resulting mixture was stirred at 100−105 °C for 6 h. The reaction mixture was quenched with water (500 mL), and the product was extracted four times with CH_2Cl_2 (200 mL each). The combined organic extracts were washed with water (500 mL), brine, dried (Na_2SO_4), and concentrated under reduced pressure, and hexane (500 mL) was added to the crude product. The resulting mixture was stirred for 30 min and evaporated to dryness under reduced pressure followed by hexane slurry. The slurry with cold hexane (500 mL) procedure was repeated, and the yellow solid was collected by filtration. Product **15e** (49.3 g, 72%) was taken forward to the next step without any purification. LC/MS (ESI positive mode) *m/z* 230 ($[M + H]^+$) $C_9H_8ClNO_4$; ^1H NMR ($CDCl_3$) δ 10.52 (s, 1H), 8.71 (d, 1H), 8.45 (dd, 1H), 7.14 (d, 1H), 4.51 (t, 2H), 3.96 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 187.3, 164.2, 142.1, 130.6, 125.0, 124.6, 113.2, 69.4, 41.3.

2-(2-Chloroethoxy)-3-methoxy-5-nitrobenzaldehyde (16u). Following a procedure similar to that of **16i**, the title compound was synthesized from 2-hydroxy-3-methoxy-5-nitro-benzaldehyde **16o** and 1,2-dichloroethane (64%). LC/MS (ESI positive mode) *m/z* 260 ($[M + H]^+$) $C_{10}H_{10}ClNO_5$.

2-(2-Methoxyethoxy)-5-nitrobenzaldehyde (16p). To a mixture of 2-hydroxy-5-nitrobenzaldehyde **16h** (5 g, 29.9 mmol) and 1-

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bromo-2-methoxyethane (5.6 mL, 59.8 mmol) in DMF (50 mL, 10 vol) was added K_2CO_3 (6.25 g, 45.0 mmol), and the resulting mixture was stirred at 100−105 °C for 6 h. The reaction mixture was cooled to 0 °C and quenched with water. The product was extracted with CH_2Cl_2 thrice and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to furnish an oil, which was purified by column (EtOAc/hexane) to obtain 5.8 g of **16p** (86%). LC/MS (ESI positive mode) m/z 226 ($[M + H]^+$) $C_{10}H_{11}NO_5$.

(2-Formyl-4-nitrophenoxy)acetic Acid Methyl Ester (16q). To a mixture of 2-hydroxy-5-nitrobenzaldehyde **16h** (0.5 g, 2.99 mmol) and methyl bromoacetate (1.4 mL, 14.9 mmol) in DMF (15 mL) was added K_2CO_3 (0.83 g, 5.98 mmol), and the resulting mixture was stirred at 40 °C for 20 h. The reaction mixture was cooled to 0 °C and quenched with water. The product was extracted with CH_2Cl_2 thrice and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to furnish an oil, which was purified by column (EtOAc/hexane) to obtain 0.62 g of **16q** (87%). LC/MS (ESI positive mode) m/z 240 ($[M + H]^+$) $C_{10}H_9NO_6$.

(2-Formyl-4-nitrophenoxy)acetic Acid (16r). Following a procedure similar to that of **12l**, the title compound was synthesized from **16q** (yield, quantitative). LC/MS (ESI positive mode) m/z 226 ($[M + H]^+$) $C_9H_7NO_6$.

5-Nitro-2-(2-oxo-2-pyrrolidin-1-ylethoxy)benzaldehyde (16s). Following a procedure similar to that of **14a**, the title compound was synthesized from **16r** (yield, quantitative). LC/MS (ESI positive mode) m/z 279 ($[M + H]^+$) $C_{13}H_{14}N_2O_5$.

2-(2-Ethylsulfanylethoxy)-5-nitrobenzaldehyde (16t). Following a procedure similar to that of **16k**, the title compound was synthesized from **16i** and ethanethiol (97%). LC/MS (ESI positive mode) m/z 256 ($[M + H]^+$) $C_{11}H_{13}NO_4S$.

2,2,2-Trifluoro-*N*-(3-formyl-5-nitrophenyl)acetamide (16x). To a cooled mixture of **16u** (1.4 g, 4.73 mmol) in CH_2Cl_2 (6 mL, 10 vol) at 0 °C was added 1.0 M DIBAL solution (4 mL, 4.00 mmol) dropwise. The resulting mixture was stirred for 1 h and allowed to warm to room temperature. Water was added, and product was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to obtain the crude residue which was purified by column chromatography (using 35% EtOAc in hexane) to furnish **11g** (230 mg, 48%) as a colorless oil. LC/MS (ESI positive mode) m/z 263 ($[M + H]^+$) $C_9H_5F_3N_2O_4$.

Cyclopropylmethyl-(3-nitrobenzyl)amine (17c). To a mixture of 3-nitrobenzaldehyde **16b** (1.2 g, 7.94 mmol) in CH_2Cl_2 (12 mL) was added aminomethylcyclopropane (1.35 mL, 15.6 mmol), and the resulting mixture was stirred at room temperature for 4 h after which $Na(OAc)_3BH$ (3.3 g, 15.6 mmol) was added portionwise and the mixture stirred for additional 4 h. The reaction mixture was then quenched with water (20 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford **17c** (1.4 g, 86%) as a pale yellow syrup. LC/MS (ESI positive mode) m/z 207 ($[M + H]^+$) $C_{11}H_{14}N_2O_2$.

Following a procedure similar to that of **17c**, the following intermediates were synthesized.

Cyclopropyl-(3-nitrobenzyl)amine (17a). The title compound was synthesized from **16b** and cyclopropylamine (72%). LC/MS (ESI positive mode) m/z 193 ($[M + H]^+$) $C_{10}H_{12}N_2O_2$; ^1H NMR ($CDCl_3$) δ 10.20 (br, 1H), 8.32 (s, 1H), 8.11 (d, 1H), 8.01 (d, 1H), 7.50 (t, 1H), 4.20 (s, 2H), 2.40−2.37 (m, 1H), 1.07 (m, 2H), 0.66 (m, 2H).

Isobutyl-(3-nitrobenzyl)amine (17b). The title compound was synthesized from **16b** and isobutylamine (79%). LC/MS (ESI positive mode) m/z 209 ($[M + H]^+$) $C_{11}H_{16}N_2O_2$.

(2,2-Dimethylpropyl)-(3-nitrobenzyl)amine (17d). The title compound was synthesized from **16b** and 2,2-dimethylpropan-1-amine (68%). LC/MS (ESI positive mode) m/z 223 ($[M + H]^+$) $C_{12}H_{18}N_2O_2$.

(3-Nitrobenzyl)pyridin-2-ylmethylamine (17e). The title compound was synthesized from **16b** and 2-(aminomethyl)pyridine (83%). LC/MS (ESI positive mode) m/z 244 ($[M + H]^+$) $C_{13}H_{13}N_3O_2$.

(2-Methoxyethyl)-(3-nitrobenzyl)amine (17f). The title compound was synthesized from **16b** and 2-methoxyethylamine (88%). LC/MS (ESI positive mode) m/z 211 ($[M + H]^+$) $C_{10}H_{14}N_2O_3$.

2-(3-Nitrobenzylamino)ethanol (17g). The title compound was synthesized from **16b** and 2-aminoethanol (75%). LC/MS (ESI positive mode) m/z 197 ($[M + H]^+$) $C_9H_{12}N_2O_3$; ^1H NMR ($CDCl_3$) δ 8.19 (s, 1H), 8.10 (d, 1H), 7.64 (dd, 1H), 7.51−7.44 (m, 1H), 3.90 (s, 2H), 3.67 (t, 2H), 2.76 (t, 2H).

[2-(2-Chloroethoxy)-5-nitrobenzyl]-(2,2,2-trifluoroethyl)-amine (17h). The title compound was synthesized from **16b** and 2,2,2-trifluoroethylamine (79%). LC/MS (ESI positive mode) m/z 313 ($[M + H]^+$) $C_{11}H_{12}ClF_3N_2O_3$.

[2-(2-Chloroethoxy)-5-nitrobenzylamino]acetonitrile (17i). The title compound was synthesized from **16b** and 2-amino-acetonitrile (75%). LC/MS (ESI positive mode) m/z 270 ($[M + H]^+$) $C_{11}H_{12}ClN_3O_3$.

Following a procedure similar to that of **18b**, the following intermediates were synthesized.

Allyl-(2-methoxy-5-nitrobenzyl)methylamine (18h). The title compound was synthesized from 2-methoxy-5-nitrobenzaldehyde **16d** and *N*-allylmethylamine (95%). LC/MS (ESI positive mode) m/z 237 ($[M + H]^+$) $C_{12}H_{16}N_2O_3$.

Allyl-(2-chloro-5-nitrobenzyl)methylamine (18i). The title compound was synthesized from 2-chloro-5-nitrobenzaldehyde **16e** and *N*-allylmethylamine (86%). LC/MS (ESI positive mode) m/z 241 ($[M + H]^+$) $C_{11}H_{13}ClN_2O_2$.

Allylmethyl-(5-nitro-2-trifluoromethoxybenzyl)amine (18j). The title compound was synthesized from **16g** and *N*-allylmethyl-amine (91%). LC/MS (ESI positive mode) m/z 291 ($[M + H]^+$) $C_{12}H_{13}F_3N_2O_3$.

Allyl-[2-(2-methoxyethoxy)-5-nitrobenzyl]methylamine (18k). The title compound was synthesized from **16p** and *N*-allylmethylamine (92%). LC/MS (ESI positive mode) m/z 281 ($[M + H]^+$) $C_{14}H_20N_2O_4$.

Allyl-(2-ethanesulfonyl-5-nitrobenzyl)methylamine (18l). The title compound was synthesized from **16m** and *N*-allylmethyl-amine (78%). LC/MS (ESI positive mode) m/z 299 ($[M + H]^+$) $C_{13}H_{18}N_2O_4S$.

Allylmethyl-(2-morpholin-4-yl-5-nitrobenzyl)amine (18m). The title compound was synthesized from **16n** and *N*-allylmethyl-amine (yield, quantitative). LC/MS (ESI positive mode) m/z 292 ($[M + H]^+$) $C_{15}H_{21}N_3O_3$.

Allyl-[2-(2-ethylsulfanylethoxy)-5-nitrobenzyl]methylamine (18n). The title compound was synthesized from **16t** and *N*-allylmethylamine (93%). LC/MS (ESI positive mode) m/z 311 ($[M + H]^+$) $C_{15}H_{22}N_2O_3S$.

2-{2-[(Allylmethylamino)methyl]-4-nitrophenoxy}-1-pyrrolidin-1-ylethanone (18o). The title compound was synthesized from **16s** and *N*-allylmethylamine (92%). LC/MS (ESI positive mode) m/z 335 ($[M + H]^+$) $C_{17}H_{23}N_3O_4$.

Allyl-[2-(2-chloroethoxy)-5-nitrobenzyl]methylamine (18p). The title compound was synthesized from **16i** and *N*-allylmethylamine (99%). LC/MS (ESI positive mode) m/z 285 ($[M + H]^+$) $C_{13}H_{17}ClN_2O_3$.

Allyl-[2-(2-chloroethoxy)-3-methoxy-5-nitrobenzyl]-methylamine (18q). The title compound was synthesized from **16u** and *N*-allylmethylamine (84%). LC/MS (ESI positive mode) m/z 315 ($[M + H]^+$) $C_{14}H_{19}ClN_2O_4$.

***N*-{3-[(Allylmethylamino)methyl]-5-nitrophenyl}-2,2,2-trifluoroacetamide (18r).** The title compound was synthesized from **16x** and *N*-allylmethylamine (96%). LC/MS (ESI positive mode) m/z 318 ($[M + H]^+$) $C_{13}H_{14}F_3N_3O_3$.

Allylcyclopropyl-(3-nitrobenzyl)amine (19a). To a stirring solution of **17a** (0.18 g, 0.938 mmol) and K_2CO_3 (0.39 g, 2.81 mmol) in DMF (2 mL) was added allyl bromide (0.2 mL, 2.25 mmol) dropwise, and the resulting mixture was stirred at 70 °C for 3 h. The reaction mixture was then brought to room temperature, quenched with water (15 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The

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combined organic extracts were washed with water, brine, dried (Na_2SO_4), and concentrated under reduced pressure to afford the crude **19a** (0.12 g, 86%) as a yellow syrup. This intermediate was taken forward to the next step without any purification. LC/MS (ESI positive mode) m/z 233 ($[M + H]^+$) $C_{13}H_{16}N_2O_2$.

Following a procedure similar to that of **19a**, the following intermediates were synthesized.

Allylisobutyl-(3-nitrobenzyl)amine (19b). The title compound was synthesized from **17b** and allyl bromide (83%). LC/MS (ESI positive mode) m/z 249 ($[M + H]^+$) $C_{14}H_{20}N_2O_2$.

Allylcyclopropylmethyl-(3-nitrobenzyl)amine (19c). The title compound was synthesized from **17c** and allyl bromide (80%). LC/MS (ESI positive mode) m/z 245 ($[M + H]^+$) $C_{14}H_{18}N_2O_2$; ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H), 8.08 (dd, 1H), 7.72 (d, 1H), 7.47 (t, 1H), 5.94−5.84 (m, 1H), 5.22−5.15 (m, 2H), 3.76 (s, 2H), 3.22 (d, 2H), 2.37 (d, 2H), 0.88−0.85 (m, 1H), 0.51−0.47 (m, 2H), 0.09−0.06 (m, 2H).

Allyl-(2,2-dimethylpropyl)-(3-nitrobenzyl)amine (19d). The title compound was synthesized from **17d** and allyl bromide (91%). LC/MS (ESI positive mode) m/z 263 ($[M + H]^+$) $C_{15}H_{22}N_2O_2$.

Allyl-(3-nitrobenzyl)pyridin-2-ylmethylamine (19e). The title compound was synthesized from **17e** and allyl bromide (48%). LC/MS (ESI positive mode) m/z 284 ($[M + H]^+$) $C_{16}H_{17}N_3O_2$.

Allyl-(2-methoxyethyl)-(3-nitrobenzyl)amine (19f). The title compound was synthesized from **17f** and allyl bromide (77%). LC/MS (ESI positive mode) m/z 251 ($[M + H]^+$) $C_{13}H_{18}N_2O_3$.

2-[Allyl-(3-nitrobenzyl)amino]ethanol (19g). The title compound was synthesized from **17g** and allyl bromide (72%). LC/MS (ESI positive mode) m/z 237 ($[M + H]^+$) $C_{12}H_{16}N_2O_3$.

Allyl-[2-(2-chloroethoxy)-5-nitrobenzyl]-(2,2,2-trifluoroethyl)amine (19h). The title compound was synthesized from **17h** and allyl bromide (57%). LC/MS (ESI positive mode) m/z 353 ($[M + H]^+$) $C_{14}H_{16}ClF_3N_2O_3$.

{Allyl-[2-(2-chloroethoxy)-5-nitrobenzyl]amino}acetonitrile (19i). The title compound was synthesized from **17i** and allyl bromide (64%). LC/MS (ESI positive mode) m/z 310 ($[M + H]^+$) $C_{14}H_{16}ClN_3O_3$.

Allyl-[2-(2-diethylaminoethoxy)-5-nitrobenzyl]methylamine (19j). To a solution of **18p** (2 g, 7.02 mmol) in *N*,*N*-dimethylacetamide (10 mL) was added dimethylamine (4 mL, 2 vol), and the resulting mixture was stirred at 90 °C for 20 h. The reaction mixture was brought to room temperature and quenched with water (20 mL) and extracted in ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water, brine, dried (Na_2SO_4), and concentrated under reduced pressure to afford the crude **19j** (1.85 g, 82%) as a thick syrup. This intermediate was taken forward to the next step without any purification. LC/MS (ESI positive mode) m/z 322 ($[M + H]^+$) $C_{17}H_{27}N_3O_3$.

Following a procedure similar to that of **19j**, the following intermediates were synthesized.

Allylmethyl-[5-nitro-2-(2-pyrrolidin-1-ylethoxy)benzyl]-amine (19k). The title compound was synthesized from **18p** and pyrrolidine (75%). LC/MS (ESI positive mode) m/z 320 ($[M + H]^+$) $C_{17}H_{25}N_3O_3$.

Allyl-[5-nitro-2-(2-pyrrolidin-1-ylethoxy)benzyl]-(2,2,2-trifluoroethyl)amine (19l). The title compound was synthesized from **19h** and pyrrolidine (79%). LC/MS (ESI positive mode) m/z 388 ($[M + H]^+$) $C_{18}H_{24}F_3N_3O_3$.

{Allyl-[5-nitro-2-(2-pyrrolidin-1-ylethoxy)benzyl]amino}-acetonitrile (19m). The title compound was synthesized from **19i** and pyrrolidine (90%). LC/MS (ESI positive mode) m/z 345 ($[M + H]^+$) $C_{18}H_{24}N_4O_3$.

Allylmethyl-[2-(2-morpholin-4-ylethoxy)-5-nitrobenzyl]-amine (19n). The title compound was synthesized from **18p** and morpholine (84%). LC/MS (ESI positive mode) m/z 336 ($[M + H]^+$) $C_{17}H_{25}N_3O_4$.

Allylmethyl-{2-[2-(4-methylpiperazin-1-yl)ethoxy]-5-nitrobenzyl}amine (19o). The title compound was synthesized from **18p** and *N*-methylpiperazine (88%). LC/MS (ESI positive mode) m/z 349 ($[M + H]^+$) $C_{18}H_{28}N_4O_3$.

Allylmethyl-[5-nitro-2-(2-piperidin-1-ylethoxy)benzyl]amine (19p). The title compound was synthesized from **18p** and piperidine (78%). LC/MS (ESI positive mode) m/z 334 ($[M + H]^+$) $C_{18}H_{27}N_3O_3$.

Allyl-[3-methoxy-5-nitro-2-(2-pyrrolidin-1-ylethoxy)benzyl]-methylamine (19q). The title compound was synthesized from **18q** and pyrrolidine (78%). LC/MS (ESI positive mode) m/z 350 ($[M + H]^+$) $C_{18}H_{27}N_3O_4$.

Following a procedure similar to that of **12d**, the following intermediates were synthesized.

Pent-4-enoic Acid (3-Aminophenyl)amide (21a). The title compound was synthesized from **18a** (96%). LC/MS (ESI positive mode) m/z 191 ($[M + H]^+$) $C_{11}H_{14}N_2O$.

3-[(Allylmethylamino)methyl]phenylamine (21b). The title compound was synthesized from **18b** (98%). LC/MS (ESI positive mode) m/z 177 ($[M + H]^+$) $C_{11}H_{16}N_2$. ^{1}H NMR ($CDCl_3$) δ 7.09 (t, 1H), 6.57−6.69 (m, 2H), 6.55 (dd, 1H), 5.85−5.95 (m, 1H), 5.12−5.17 (m, 2H), 3.67 (br, 1H), 3.39 (s, 2H), 3.02 (d, 2H), 2.18 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 146.4, 140.3, 136.0, 129.1, 119.4, 117.4, 115.7, 113.8, 61.7, 60.6, 42.2. Anal. Calcd for $C_{11}H_{16}N_2$: C, 74.96; H, 9.15; N, 15.89. Found: C, 74.34; H, 9.04; N, 15.68. IR (KBr pellet): 3346, 3210, 2782, 1605, 1460, 1294, 921, 785, 696 cm^{-1}.

Allyl-(3-aminophenyl)carbamic Acid *tert*-Butyl Ester (21c). The title compound was synthesized from **18f** (56%). LC/MS (ESI positive mode) m/z 249 ($[M + H]^+$) $C_{14}H_{20}N_2O_2$.

***N*-Allyl-*N*-(3-aminobenzyl)-2,2,2-trifluoroacetamide (21d).** The title compound was synthesized from **18d** (78%). LC/MS (ESI positive mode) m/z 259 ($[M + H]^+$) $C_{12}H_{13}F_3N_2O$.

3-[(Allylmethylamino)methyl]-4-methoxyphenylamine (21f). The title compound was synthesized from **18h** (63%). LC/MS (ESI positive mode) m/z 207 ($[M + H]^+$) $C_{12}H_{18}N_2O$.

3-[(Allylmethylamino)methyl]-4-chlorophenylamine (21g). The title compound was synthesized from **18i** (95%). LC/MS (ESI positive mode) m/z 211 ($[M + H]^+$) $C_{11}H_{15}ClN_2$.

3-[(Allylmethylamino)methyl]-4-trifluoromethoxyphenylamine (21h). The title compound was synthesized from **18j** (77%). LC/MS (ESI positive mode) m/z 261 ($[M + H]^+$) $C_{12}H_{15}F_3N_2O$.

3-[(Allylcyclopropylamino)methyl]phenylamine (21i). The title compound was synthesized from **19a** (88%). LC/MS (ESI positive mode) m/z 203 ($[M + H]^+$) $C_{13}H_{18}N_2$; ^{1}H NMR ($CDCl_3$) δ 7.09 (t, 1H), 6.69 (m, 1H), 6.59 (dd, 1H), 6.04−5.87 (m, 1H), 5.22−5.13 (m, 2H), 3.73 (s, 2H), 3.25 (d, 2H), 1.90 (br s, 1H), 0.59−0.40 (m, 4H).

3-[(Allylisobutylamino)methyl]phenylamine (21j). The title compound was synthesized from **19b** (65%). LC/MS (ESI positive mode) m/z 219 ($[M + H]^+$) $C_{14}H_{22}N_2$.

3-[(Allylcyclopropylmethylamino)methyl]phenylamine (21k). The title compound was synthesized from **19c** (90%). LC/MS (ESI positive mode) m/z 217 ($[M + H]^+$) $C_{14}H_{20}N_2$.

3-{[Allyl-(2,2-dimethylpropyl)amino]methyl}phenylamine (21l). The title compound was synthesized from **19d** (61%). LC/MS (ESI positive mode) m/z 233 ($[M + H]^+$) $C_{15}H_{24}N_2$.

3-[(Allylpyridin-2-ylmethylamino)methyl]phenylamine (21m). The title compound was synthesized from **19e** (97%). LC/MS (ESI positive mode) m/z 254 ($[M + H]^+$) $C_{16}H_{19}N_3$.

3-{[Allyl-(2-methoxyethyl)amino]methyl}phenylamine (21n). The title compound was synthesized from **19f** (64%). LC/MS (ESI positive mode) m/z 221 ($[M + H]^+$) $C_{13}H_{20}N_2O$.

2-[Allyl-(3-aminobenzyl)amino]ethanol (21o). The title compound was synthesized from **19g** (76%). LC/MS (ESI positive mode) m/z 207 ($[M + H]^+$) $C_{12}H_{18}N_2O$.

3-[(Allylmethylamino)methyl]-4-(2-ethylsulfanylethoxy)-phenylamine (22e). The title compound was synthesized from **18n** (91%). LC/MS (ESI positive mode) m/z 281 ($[M + H]^+$) $C_{15}H_{24}N_2OS$; ^{1}H NMR ($CDCl_3$) δ 6.77 (d, 1H), 6.69 (d, 1H), 6.54 (dd, 1H), 5.98−5.88 (m, 1H), 5.24−5.12 (m, 2H), 4.05 (t, 2H), 3.48 (s, 2H), 3.07 (d, 2H), 2.87 (t, 2H), 2.62 (q, 2H), 2.22 (s, 3H), 1.25 (t, 3H).

3-[(Allylmethylamino)methyl]-4-(2-methoxyethoxy)-phenylamine (22f). The title compound was synthesized from **18k**

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(64%). LC/MS (ESI positive mode) m/z 251 ($[M + H]^+$) $C_{14}H_{22}N_2O_2$.

3-[(Allylmethylamino)methyl]-4-(2-diethylaminoethoxy)-phenylamine (22h). The title compound was synthesized from **19j** (59%). LC/MS (ESI positive mode) m/z 292 ($[M + H]^+$) $C_{17}H_{29}N_3O$.

3-[(Allylmethylamino)methyl]-4-(2-pyrrolidin-1-ylethoxy)-phenylamine (22i). The title compound was synthesized from **19k** (88%). LC/MS (ESI positive mode) m/z 290 ($[M + H]^+$) $C_{17}H_{27}N_3O$.

3-[(Allylmethylamino)methyl]-4-(2-morpholin-4-ylethoxy)-phenylamine (22j). The title compound was synthesized from **19i** (54%). LC/MS (ESI positive mode) m/z 306 ($[M + H]^+$) $C_{17}H_{27}N_3O_2$.

3-[(Allylmethylamino)methyl]-4-[2-(4-methylpiperazin-1-yl)-ethoxy]phenylamine (22k). The title compound was synthesized from **19i** (77%). LC/MS (ESI positive mode) m/z 319 ($[M + H]^+$) $C_{18}H_{30}N_4O$.

3-[(Allylmethylamino)methyl]-4-(2-piperidin-1-ylethoxy)-phenylamine (22l). The title compound was synthesized from **19p** (85%). LC/MS (ESI positive mode) m/z 304 ($[M + H]^+$) $C_{18}H_{29}N_3O$.

3-[(Allylmethylamino)methyl]-5-methoxy-4-(2-pyrrolidin-1-ylethoxy)phenylamine (22m). The title compound was synthesized from **19q** (63%). LC/MS (ESI positive mode) m/z 288 ($[M + H]^+$) $C_{18}H_{29}N_3O_2$.

***N*-{3-[(Allylmethylamino)methyl]-5-aminophenyl}-2,2,2-trifluoroacetamide (22n).** The title compound was synthesized from **18r** (52%). LC/MS (ESI positive mode) m/z 320 ($[M + H]^+$) $C_{13}H_{16}F_3N_3O$; ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.10 (t, 1H), 6.67 (s, 1H), 6.53 (s, 1H), 5.93−5.86 (m, 1H), 5.30−5.15 (m, 2H), 3.86 (br s, 2H), 3.38 (s, 2H), 3.03 (d, 2H), 2.18 (s, 3H).

3-[(Allylmethylamino)methyl]-4-morpholin-4-ylphenylamine (22c). Compound **18m** (2.0 g, 6.86 mmol) was taken in EtOH (20 mL, 10 vol), and fine Fe powder (1.15 g, 20.6 mmol) was added at 50−55 °C followed by NH_4Cl solution (1.76 g, 32 mmol in 2 mL of water). The reaction mixture was refluxed for 4 h, and EtOH was removed from the reaction mixture under reduced pressure. The residue was basified with $NaHCO_3$ solution (pH 7−8) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to furnish **22c** (1.67 g, 93%) as a brown oil. This material was taken up for the next step without any purification. LC/MS (ESI positive mode) m/z 262 ($[M + H]^+$) $C_{15}H_{23}N_3O$.

Following a procedure similar to that of **22c**, the following intermediates were synthesized.

3-Allyloxymethylphenylamine (21e). The title compound was synthesized from **18g** (91%). LC/MS (ESI positive mode) m/z 164 ($[M + H]^+$) $C_{10}H_{13}NO$.

3-{[Allyl-(2,2,2-trifluoroethyl)amino]methyl}-4-(2-pyrrolidin-1-ylethoxy)phenylamine (22a). The title compound was synthesized from **19h** (87%). LC/MS (ESI positive mode) m/z 358 ($[M + H]^+$) $C_{18}H_{26}F_3N_3O$.

{Allyl-[5-amino-2-(2-pyrrolidin-1-ylethoxy)benzyl]amino}-acetonitrile (22b). The title compound was synthesized from **19i** (92%). LC/MS (ESI positive mode) m/z 315 ($[M + H]^+$) $C_{18}H_{26}N_4O$.

3-[(Allylmethylamino)methyl]-4-ethanesulfonylphenylamine (22d). The title compound was synthesized from **18l** (81%). LC/MS (ESI positive mode) m/z 267 ($[M + H]^+$) $C_{13}H_{20}N_2O_2S$.

2-{2-[(Allylmethylamino)methyl]-4-aminophenoxy}-1-pyrrolidin-1-ylethanone (22g). The title compound was synthesized from **18o** (86%). LC/MS (ESI positive mode) m/z 262 ($[M + H]^+$) $C_{17}H_{25}N_3O_2$.

{3-[(Alkylmethylamino)methyl]phenyl}-[4-(3-allyloxyphenyl)pyrimidine-2-yl]amine (23f). To a solution of **15a** (4.22 g, 24 mmol) and **18b** (5.2 g, 20 mmol) in *n*-butanol (70 mL) was added concentrated HCl (3 mL), and the resulting mixture was heated to 90−100 °C for 12 h. The reaction mixture was brought to room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL). Saturated $NaHCO_3$ was added, and pH was adjusted to 7−8. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and purified by column chromatography (1:3 EtOAc/hexane) to furnish **23f** as a white solid (7.4 g, 92%). LC/MS (ESI positive mode) m/z 401 ($[M + H]^+$); ^{1}H NMR ($CDCl_3$) δ 8.46 (d, 1H), 7.69−7.60 (m, 4H), 7.49 (s, 1H), 7.37 (t, 1H), 7.29 (t, 1H), 7.13 (d, 1H), 7.04−6.99 (m, 2H), 5.95−5.87 (m, 2H), 5.22−5.13 (m, 4H), 4.10 (t, 2H), 3.51 (s, 2H), 3.05 (d, 2H), 2.57 (m, 2H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.7, 160.3, 159.4, 158.6, 140.0, 139.7, 138.6, 136.0, 134.4, 129.8, 128.7, 123.2, 119.9, 119.6, 118.0, 117.5, 117.3, 117.2,113.1, 108.4, 67.4, 61.8, 60.6, 42.2, 33.7. Anal. Calcd for $C_{25}H_{28}N_4O$: C, 74.97; H, 7.05; N, 13.99. Found: C, 74.80; H, 7.15; N, 13.86. IR (KBr pellet): 3271, 3070, 2776, 1572, 1433, 1295, 913, 780, 627 cm^{-1}.

Following a procedure similar to that of **23f**, the following intermediates were synthesized.

Pent-4-enoic Acid {3-[4-(3-Allyloxyphenyl)pyrimidin-2-ylamino]phenyl}amide (23a). The title compound was synthesized from **13a** and **21a** (76%). LC/MS (ESI positive mode) m/z 401 ($[M + H]^+$) $C_{24}H_{24}N_4O_2$.

But-3-enoic Acid [3-(2-{3-[(Allylmethylamino)methyl]-phenylamino}pyrimidin-4-yl)phenyl]amide (23b). The title compound was synthesized from **14a** and **21b** (83%). LC/MS (ESI positive mode) m/z 414 ($[M + H]^+$) $C_{25}H_{27}N_5O$.

***N*-Allyl-3-(2-{3-[(allylmethylamino)methyl]phenylamino}-pyrimidin-4-yl)benzamide (23c).** The title compound was synthesized from **14b** and **21b** (91%). LC/MS (ESI positive mode) m/z 414 ($[M + H]^+$) $C_{25}H_{27}N_5O$.

Allyl-{3-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]-phenyl}carbamic Acid *tert*-Butyl Ester (23d). The title compound was synthesized from **15a** and **21c** (59%). LC/MS (ESI positive mode) m/z 473 ($[M + H]^+$) $C_{28}H_{32}N_4O_3$.

***N*-Allyl-*N*-{3-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]benzyl}-2,2,2-trifluoroacetamide (23e).** The title compound was synthesized from **15a** and **21d** (87%). LC/MS (ESI positive mode) m/z 483 ($[M + H]^+$) $C_{26}H_{25}F_3N_4O_2$; ^{1}H NMR ($CDCl_3$) δ 8.41 (t, 1H), 7.71−7.59 (m, 4H), 7.44 (m, 1H), 7.36 (m, 1H), 7.22 (m, 1H), 7.11 (m, 1H), 6.98 (dd, 1H), 5.96−5.89 (m, 1H), 5.78−5.73 (m, 1H), 5.33−5.07 (m, 4H), 4.66 (s, 2H), 4.13 (t, 2H), 3.94 (br, 2H), 2.59 (q, 2H).

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-allyloxymethylphenyl)pyrimidin-2-yl]amine (23g). The title compound was synthesized from **13b** and **21b** (87%). LC/MS (ESI positive mode) m/z 401 ($[M + H]^+$) $C_{25}H_{28}N_4O$.

(4-{3-[(Allylmethylamino)methyl]phenyl}pyrimidin-2-yl)-(3-allyloxymethylphenyl)amine (23h). The title compound was synthesized from **14c** and **21e** (71%). LC/MS (ESI positive mode) m/z 401 ($[M + H]^+$) $C_{25}H_{28}N_4O$.

{3-[(Allylcyclopropylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (18a). The title compound was synthesized from **15a** and **21i** (57%). LC/MS (ESI positive mode) m/z 427 ($[M + H]^+$) $C_{27}H_{30}N_4O$; ^{1}H NMR ($CDCl_3$) δ 11.73 (br, 1H), 8.29 (d, 1H), 8.02 (d, 1H), 7.80 (s, 1H), 7.64 (m, 2H), 7.58−7.46 (m, 2H), 7.38 (m, 1H), 7.32 (d, 1H), 7.18 (d, 1H), 6.20−6.10 (m, 1H), 5.97−5.83 (m, 1H), 5.57−5.53 (m, 2H), 5.29−5.13 (m, 2H), 4.33 (s, 2H), 4.10 (t, 2H), 2.62−2.57 (m, 2H), 2.34−2.29 (m, 1H), 0.83 (m, 4H).

{3-[(Allylisobutylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (24b). The title compound was synthesized from **15a** and **21j** (64%). LC/MS (ESI positive mode) m/z 443 ($[M + H]^+$) $C_{28}H_{34}N_4O$.

{3-[(Allylcyclopropylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (24c). The title compound was synthesized from **15a** and **21k** (56%). LC/MS (ESI positive mode) m/z 441 ($[M + H]^+$) $C_{28}H_{32}N_4O$.

(3-{[Allyl-(2,2-dimethylpropyl)amino]methyl}phenyl)-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (24d). The title compound was synthesized from **15a** and **21l** (96%). LC/MS (ESI positive mode) m/z 457 ($[M + H]^+$) $C_{29}H_{36}N_4O$.

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{3-[(Allylpyridin-2-ylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (24e). The title compound was synthesized from **15a** and **21m** (49%). LC/MS (ESI positive mode) m/z 478 ($[M + H]^+$) $C_{30}H_{31}N_5O$.

(3-{[Allyl-(2-methoxyethyl)amino]methyl}phenyl)-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (24f). The title compound was synthesized from **15a** and **21n** (77%). LC/MS (ESI positive mode) m/z 445 ($[M + H]^+$) $C_{27}H_{32}N_4O_2$.

2-(Allyl-{3-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]-benzyl}amino)ethanol (24g). The title compound was synthesized from **15a** and **21o** (85%). LC/MS (ESI positive mode) m/z 431 ($[M + H]^+$) $C_{26}H_{30}N_4O_2$.

{3-[(Allylmethylamino)methyl]-4-methoxyphenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25a). The title compound was synthesized from **15a** and **21f** (76%). LC/MS (ESI positive mode) m/z 431 ($[M + H]^+$) $C_{26}H_{30}N_4O_2$.

{3-[(Allylmethylamino)methyl]-4-chlorophenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25b). The title compound was synthesized from **15a** and **21g** (86%). LC/MS (ESI positive mode) m/z 435 ($[M + H]^+$) $C_{25}H_{27}ClN_4O$.

{3-[(Allylmethylamino)methyl]-4-trifluoromethoxyphenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25c). The title compound was synthesized from **15a** and **21h** (71%). LC/MS (ESI positive mode) m/z 485 ($[M + H]^+$) $C_{26}H_{27}F_3N_4O_2$.

[3-[(Allylmethylamino)methyl]-4-(2-methoxyethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25d). The title compound was synthesized from **15a** and **22f** (73%). LC/MS (ESI positive mode) m/z 475 ($[M + H]^+$) $C_{28}H_{34}N_4O_3$.

{3-[(Allylmethylamino)methyl]-4-ethanesulfonylphenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25e). The title compound was synthesized from **15a** and **22d** (88%). LC/MS (ESI positive mode) m/z 492 ($[M + H]^+$) $C_{27}H_{32}N_4O_3S$.

[3-[(Allylmethylamino)methyl]-4-(2-ethylsulfanylethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25f). The title compound was synthesized from **15a** and **22e** (67%). LC/MS (ESI positive mode) m/z 505 ($[M + H]^+$) $C_{29}H_{36}N_4O_2S$; ^1H NMR ($CDCl_3$) δ 8.42 (d, 1H), 7.64−7.62 (m, 3H), 7.54 (d, 1H), 7.38 (t, 1H), 7.10−7.08 (m, 2H), 7.06−7.04 (m, 1H), 6.87 (d, 1H), 6.04−5.88 (m, 2H), 5.22−5.12 (m, 4H), 4.15 (t, 2H), 4.08 (t, 2H), 3.57 (s, 2H), 3.11 (d, 2H), 2.93 (t, 2H), 2.66 (t, 2H), 2.62−2.58 (m, 2H), 2.26 (s, 3H), 1.31 (t, 3H).

2-{2-[(Allylmethylamino)methyl]-4-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]phenoxy}-1-pyrrolidin-1-ylethanone (25g). The title compound was synthesized from **15a** and **2g** (82%). LC/MS (ESI positive mode) m/z 528 ($[M + H]^+$) $C_{31}H_{37}N_5O_3$.

{3-[(Allylmethylamino)methyl]-4-morpholin-4-ylphenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25h). The title compound was synthesized from **15a** and **22c** (63%). LC/MS (ESI positive mode) m/z 586 ($[M + H]^+$) $C_{29}H_{35}N_5O_2$.

[3-[(Allylmethylamino)methyl]-4-(2-diethylaminoethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25i). The title compound was synthesized from **15a** and **22h** (97%). LC/MS (ESI positive mode) m/z 516 ($[M + H]^+$) $C_{31}H_{41}N_5O_2$.

[3-[(Allylmethylamino)methyl]-4-(2-pyrrolidin-1-ylethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25j). The title compound was synthesized from **15a** and **22i** (77%). LC/MS (ESI positive mode) m/z 514 ($[M + H]^+$) $C_{31}H_{39}N_5O_2$.

[3-{[Allyl-(2,2,2-trifluoroethyl)amino]methyl}-4-(2-pyrrolidin-1-ylethoxy)phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25k). The title compound was synthesized from **15a** and **22a** (67%). LC/MS (ESI positive mode) m/z 582 ($[M + H]^+$) $C_{32}H_{38}F_3N_5O_2$.

{Allyl-[5-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]-2-(2-pyrrolidin-1-ylethoxy)benzyl]amino}acetonitrile (25l). The title compound was synthesized from **15a** and **22b** (71%). LC/MS (ESI positive mode) m/z 539 ($[M + H]^+$) $C_{32}H_{38}N_6O_2$.

[3-[(Allylmethylamino)methyl]-4-(2-morpholin-4-ylethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25m). The title compound was synthesized from **15a** and **22j** (88%). LC/MS (ESI positive mode) m/z 530 ($[M + H]^+$) $C_{31}H_{39}N_5O_3$.

{3-[(Allylmethylamino)methyl]-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]-amine (25n). The title compound was synthesized from **15a** and **22k** (90%). LC/MS (ESI positive mode) m/z 543 ($[M + H]^+$) $C_{32}H_{42}N_6O_2$.

[3-[(Allylmethylamino)methyl]-4-(2-piperidin-1-ylethoxy)-phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]amine (25o). The title compound was synthesized from **15a** and **22l** (93%). LC/MS (ESI positive mode) m/z 528 ($[M + H]^+$) $C_{32}H_{41}N_5O_2$.

[3-[(Allylmethylamino)methyl]-5-methoxy-4-(2-pyrrolidin-1-ylethoxy)phenyl]-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-yl]-amine (25p). The title compound was synthesized from **15a** and **22m** (68%). LC/MS (ESI positive mode) m/z 544 ($[M + H]^+$) $C_{32}H_{41}N_5O_3$.

***N*-{3-[(Allylmethylamino)methyl]-5-[4-(3-but-3-enyloxyphenyl)pyrimidin-2-ylamino]phenyl}-2,2,2-trifluoroacetamide (25q).** The title compound was synthesized from **15a** and **22n** (89%). LC/MS (ESI positive mode) m/z 512 ($[M + H]^+$) $C_{27}H_{28}F_3N_5O_2$.

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)-6-methylpyrimidin-2-yl]amine (25r). The title compound was synthesized from **15b** and **21b** (92%). LC/MS (ESI positive mode) m/z 415 ($[M + H]^+$) $C_{26}H_{30}N_4O$.

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)-6-pyrrolidin-1-ylpyrimidin-2-yl]amine (25s). The title compound was synthesized from **15c** and **21b** (90%). LC/MS (ESI positive mode) m/z 470 ($[M + H]^+$) $C_{29}H_{35}N_5O$.

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxyphenyl)-5-methylpyrimidin-2-yl]amine (25t). The title compound was synthesized from **15d** and **21b** (68%). LC/MS (ESI positive mode) m/z 415 ($[M + H]^+$) $C_{26}H_{30}N_4O$.

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxy-5-fluorophenyl)pyrimidin-2-yl]amine (25u). The title compound was synthesized from **15e** and **21b** (66%). LC/MS (ESI positive mode) m/z 419 ($[M + H]^+$) $C_{25}H_{27}FN_4O$.

{3-[(Allylmethylamino)methyl]phenyl}-[4-(3-but-3-enyloxy-4-methoxyphenyl)pyrimidin-2-yl]amine (25v). The title compound was synthesized from **15f** and **21b** (51%). LC/MS (ESI positive mode) m/z 431 ($[M + H]^+$) $C_{26}H_{30}N_4O_2$.

(16*E*)-14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (26h). Diene **23f** (1.0 g, 2.5 mmol) was dissolved in CH_2Cl_2 (1 L). The reaction appartus was flushed with N_2 vigorously. The reaction mixture was heated to 45 °C for 1 h. The apparatus was flushed with N_2, and TFA (1 mL) was added maintaining the temperature at 45 °C. The mixture was then flushed with nitrogen gas for an additional 15 min, and Grubbs second generation catalyst (0.21 g, 0.25 mmol), predissolved in CH_2Cl_2 (10 mL), was added to the reaction mixture. The mixture was stirred at 45 °C for 5 h. Solvent was removed from the reaction mixture under reduced pressure. The crude residue was purified by column chromatography (EtOAc/MeOH) to furnish 1.2 g of the crude product which was recrystallized with EtOAc/hexane to give 950 mg of the desired product. This material was dissolved in CH_2Cl_2 (20 mL) and washed with saturated $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure to obtain (827 mg, 86%) of **26h** as free base. The free amine was dissolved in 1 N HCl (20 mL). The resulting mixture was filtered through a filter paper and lyophilized to afford **26h** as 2HCl monohydrate salt (900 mg, 78%) as a light yellow solid. LC/MS (ESI positive mode) m/z 373 ($[M + H]^+$); ^1H NMR ($CDCl_3$) δ 10.6 (br, 1H), 9.9 (s,1H), 8.80 (s, 1H), 8.51 (d, 1H), 7.81 (s, 1H), 7.57 (d, 1H),7.40 (m, 2H), 7.29 (d, 1H), 7.23 (m, 2H), 7.16 (d, 1H), 6.08 (m, 1H), 5.74 (m, 1H), 4.50 (m, 1H), 4.17−4.07 (m, 2H), 3.93−3.67 (m, 3H), 2.51 (m, 2H), 2.42 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.1, 160.2, 159.2, 141.6, 140.8, 138.6, 131.1, 130.7, 129.6, 124.0, 121.0,120.4, 119.7, 116.5, 115.1,108.5, 65.3, 57.7,57.5, 37.4, 32.4. Anal. Calcd for $C_{23}H_{28}Cl_2N_4O_2$ (2HCl·H_2O salt): C, 59.61; H, 6.09; N, 12.09; Cl, 15.30. Found: C, 59.87; H, 5.82; N, 12.28; Cl, 15.50. IR (KBr pellet): 3474, 3058, 1633, 1591, 1459, 1281, 1213, 1801, 1043, 793, 692 cm^{-1}.

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Following a procedure similar to that of **26h**, the following products were synthesized but isolated as free base.

(17*E*)-20-Oxa-5,7,13,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,17,21,23-decaen-14-one (26a). The title compound was synthesized from **23a** (73%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 373 ([M + H]$^+$) $C_{22}H_{20}N_4O_2$; ^{1}H NMR ($CDCl_3$) δ 11.23 (s, 1H), 8.34 (m, 1H), 8.13 (d, 1H), 7.71 (m, 1H), 7.50 (d, 1H), 7.41 (t, 1H), 7.24−7.34 (m, 4H), 7.14 (s, 1H), 7.02 (m, 1H), 5.75−5.87 (m, 2H), 4.61 (d, 2H), 2.45 (s, 4H).

(16*E*)-14-Methyl-5,7,14,20,26-pentaazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene-19-one (26b). The title compound was synthesized from **23b** (81%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 386 ([M + H]$^+$) $C_{23}H_{23}N_5O$; ^{1}H NMR ($CDCl_3$) δ 8.55−8.54 (m, 1H), 8.27 (s, 1H), 8.03−7.97 (m, 2H), 7.54−7.52 (m, 1H), 7.38−7.35 (m, 1H), 7.31 (s, 1H), 7.25−7.10 (m, 3H), 6.77−6.75 (m, 1H), 5.85−5.69 (m, 2H), 5.08 (s, 2H), 4.03−3.95 (m, 4H), 3.48 (s, 3H).

(16*E*)-14-Methyl-5,7,14,19,26-pentaazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene-20-one (26c). The title compound was synthesized from **23c** (82%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 386 ([M + H]$^+$) $C_{23}H_{23}N_5O$; ^{1}H NMR ($CDCl_3$) δ 8.55−8.54 (m, 1H), 8.27 (s, 1H), 8.03−7.97 (m, 2H), 7.54−7.52 (m, 1H), 7.38−7.35 (m, 1H), 7.31 (s, 1H), 7.25−7.10 (m, 3H), 6.77−6.75 (m, 1H), 5.85−5.69 (m, 2H), 5.08 (s, 2H), 4.03−3.95 (m, 4H), 3.48 (s, 3H).

1-(20-Oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1-(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaen-14-yl)-2,2,2-trifluoroethanone (26f). The title compound was synthesized from **23e** (73%, 90% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 455 ([M + H]$^+$) $C_{24}H_{21}F_3N_4O_2$; ^{1}H NMR ($CDCl_3$) δ 8.85 (d, 1H), 8.38 (d, 1H), 7.99−7.94 (m, 1H), 7.52−7.34 (m, 4H), 7.27 (d, 1H), 7.03−6.92 (m, 2H), 5.79−5.46 (m, 2H), 4.80 (s, 2H), 4.20 (t, 2H), 4.09−4.02 (m, 2H), 2.54−2.50 (m, 2H).

14-Methyl-19-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (26i). The title compound was synthesized from **23g** (74%, 15:85 cis/trans ratio by ^{1}H NMR). LC/MS (ESI positive mode) m/z 373 ([M + H]$^+$) $C_{23}H_{24}N_4O$; ^{1}H NMR (MeOD-d_4) δ 8.66−8.85 (m, 1H), 8.46 (d, 1H), 8.31 (s, 1H), 7.92 (d, 1H), 7.55−7.52 (m, 1H), 7.51−7.50 (m, 1H), 7.39−7.34 (m, 2H), 7.21−7.18 (m, 1H), 7.09−7.08 (m, 1H), 6.22 (td, 1H), 5.91−5.84 (m, 1H, CH), 4.61−4.58 (m, 2H), 4.15 (s, 2H), 3.94−3.86 (m, 2H), 2.73 (s, 2H), 2.59 (s, 3H).

(16*E*)-19-Methyl-14-oxa-5,7,19,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (26j). The title compound was synthesized from **23h** (64%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 373 ([M + H]$^+$) $C_{23}H_{24}N_4O$; ^{1}H NMR (MeOD-d_4) δ 8.57−8.43 (m, 3H), 8.22 (d, 1H), 7.76−7.74 (m, 1H), 7.68 (t, 1H), 7.48 (d, 1H), 7.34 (t, 1 H), 7.16 (d, 1H), 7.08 (dt, 1H), 6.25 (dt, 1H), 6.03 (dt, 1H), 4.81 (s, 2H), 4.29−4.26 (m, 2H), 3.82−3.79 (m, 2H), 2.91 (s, 3H).

(16*E*)-14-Cyclopropyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (26k). The title compound was synthesized from **24a** (49%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 399 ([M + H]$^+$) $C_{25}H_{26}N_4O$; ^{1}H NMR (MeOD-d_4) δ 9.05 (s, 1H), 8.44 (br s, 1H), 7.90 (br s, 1H), 7.52 (d, 1H), 7.43 (t, 1H), 7.38 (t, 1H), 7.31 (d, 1H), 7.22 (dd, 1H), 7.10−7.16 (m, 2H), 6.21 (dt, 1H), 5.80 (dt, 1H), 4.60 (br s, 1H), 3.90−4.19 (5H, m), 2.80 (m, 1H), 2.65−2.66 (m, 2H), 0.51−0.60 (m, 3H), 0.58−0.55 (br s, 1H).

(16*E*)-14-Isobutyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (26l). The title compound was synthesized from **24b** (53%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 415 ([M + H]$^+$) $C_{26}H_{30}N_4O$; ^{1}H NMR (MeOD-d_4) δ 8.95 (br s, 1H), 8.42 (d, 1H), 7.99 (t, 1H), 7.47 (t, 1H), 7.38 (t, 1H), 7.35 (t, 1H), 7.28 (d, 1H), 7.16 (dd, 1H), 7.11 (d, 1H), 7.08 (dd, 2H), 6.15 (dt, 1H), 5.85 (dt, 1H), 4.58 (d, 1H), 4.22−4.26 (m, 1H), 4.11 (d, 1H), 3.86−4.05 (m, 3H), 3.86−4.05 (m, 3H), 3.02−3.07 (m, 1H), 2.60−2.73 (m, 3H), 1.78−1.98 (m, 1H), 0.60 (d, 3H), 0.51 (d, 3H).

(16*E*)-14-(Cyclopropylmethyl)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (26m). The title compound was synthesized from **24c** (52%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 413 ([M + H]$^+$) $C_{26}H_{28}N_4O$; ^{1}H NMR ($CDCl_3$) δ 12.00 (s, 1H), 8.80 (s, 1H), 8.30 (br s, 1H), 7.93 (d, 1H), 7.38−7.59 (m, 6H), 7.17−7.21 (m, 1H), 6.12 (dt, 1H), 5.89 (dt, 1H), 4.79 (d, 1H), 4.22−4.27 (m, 3H), 3.94−4.00 (m, 1H), 3.86 (d, 1H), 2.68−2.86 (m, 4H), 1.05 (m, 1H), 0.64−0.72 (m, 2H), 0.19−0.23 (m, 2H).

(16*E*)-14-(2,2-Dimethylpropyl)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (26n). The title compound was synthesized from **24d** (75%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 429 ([M + H]$^+$) $C_{27}H_{32}N_4O$; ^{1}H NMR ($CDCl_3$) δ 8.79 (s, 1H), 8.39 (d, 1H), 7.86 (d, 1H), 7.68 (d, 1H), 7.45−7.47 (m, 2H), 7.42 (t, 1H), 7.27 (d, 1H), 7.14 (d, 1H), 7.05−7.08 (m, 1H), 6.02 (dt, 1H), 5.87 (dt, 1H), 4.87−4.91 (m, 1H), 4.50−4.51 (m, 1H), 4.27−4.31 (m, 1H), 4.04−4.12 (m, 1H), 3.80−3.85 (m, 2H), 2.97 (d, 2H), 2.65−2.70 (m, 2H), 0.66 (s, 9H).

14-(Pyridin-2-ylmethyl)-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (26o). The title compound was synthesized from **24e** (42%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 450 ([M + H]$^+$) $C_{28}H_{27}N_5O$; ^{1}H NMR ($CDCl_3$) δ 11.9 (s, 1H), 8.90 (s, 1H), 8.59 (d, 1H), 8.27 (d, 1H), 7.99 (s, 1H), 7.67 (t, 1H), 7.66 (d, 1H), 7.36−7.57 (m, 7H, m), 7.19−7.21 (m, 1H), 6.07 (dt, 1H), 5.92 (dt, 1H), 4.55 (s, 2H), 4.31 (s, 2H), 3.88−3.89 (m, 2H), 2.64 (d, 2H), 2.64 (t, 2H).

(16*E*)-14-(2-Methoxyethyl)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (26p). The title compound was synthesized from **24f** (77%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 417 ([M + H]$^+$) $C_{25}H_{28}N_4O_2$; ^{1}H NMR (MeOD-d_4) δ 8.71 (s, 1H), 8.36 (d, 1H), 7.88 (s, 1H), 7.43 (d, 1H), 7.31 (t, 1H), 7.19−7.15 (m, 2H), 7.02 (dd, 1H), 6.96 (t, 2H), 5.76−5.80 (m, 1H), 5.59−5.66 (m, 1H), 4.09 (t, 2H), 3.85 (s, 2H), 3.42 (d, 2H), 3.30−3.28 (m, 2H), 3.25, (s, 3H), 2.44, (br s, 2H), 2.43 (d, 2H).

2-(20-Oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1-(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaen-14-yl)ethanol (26q). The title compound was synthesized from **24g** (63%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 403 ([M + H]$^+$) $C_{24}H_{26}N_4O_2$; ^{1}H NMR (MeOD-d_4) δ 9.09 (s, 1H), 8.41 (d, 1H), 7.91 (s, 1H), 7.48−7.53 (m, 1H), 7.28−7.40 (m, 3H), 7.08−7.15 (m, 3H), 6.13−6.20 (m, 1H), 5.77−5.82 (m, 1H), 4.12−4.00 (m, 2H), 4.00−3.98 (m, 2H), 2.59−2.61 (m, 2H), 1.20−1.28 (m, 4H), 0.80−0.87 (m, 2H).

(16*E*)-11-Methoxy-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27a). The title compound was synthesized from **25a** (71%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 403 ([M + H]$^+$) $C_{24}H_{26}N_4O_2$; ^{1}H NMR ($CDCl_3$) δ 12.19 (s, 1H), 8.72 (d, 1H), 8.33 (d, 1H), 7.93 (s, 1H), 7.56−7.60 (m, 2H), 7.51 (dd, 1H), 7.38 (d, 1H), 7.22−7.24 (m, 1H), 7.03 (d, 1H), 4.18−4.33 (m, 6H), 2.74 (q, 2H), 2.68 (s, 3H), 2.14 (s, 3H).

(16*E*)-11-Chloro-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27b). The title compound was synthesized from **25b** (48%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 407 ([M + H]$^+$) $C_{23}H_{23}ClN_4O$; ^{1}H NMR (DMSO-d_6) δ 10.15 (s, 1H), 9.73 (br s, 1H), 9.10 (d, 1H), 8.63 (d, 1H), 7.90 (s, 1H), 7.69 (d, 1H), 7.50−7.55 (m, 2H), 7.39 (dd, 1H), 7.27 (dd, 1H), 6.12−6.23 (m, 1H), 5.67−5.78 (m, 1H), 4.59 (s, 2H), 4.08−4.27 (m, 4H), 3.85−3.89 (m, 2H), 2.59 (s, 3H).

(16*E*)-14-Methyl-11-trifluoromethoxy-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27c). The title compound was synthesized from **25c** (53%, >95% trans by ^{1}H NMR). LC/MS

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(ESI positive mode) m/z 457 ([M + H]$^+$) $C_{24}H_{23}F_3N_4O_2$; ^{1}H NMR (DMSO-d_6) δ 10.18 (s, 1H), 9.85 (br s, 1H), 9.13 (m, 1H), 8.63 (d, 1H), 7.90 (s, 1H), 7.69 (d, 1H), 7.55 (d, 1H), 7.52 (t, 1H), 7.46 (br s, 1H), 7.29 (dd, 1H), 6.12−6.23 (m, 1H), 5.67−5.78 (m, 1H), 3.95−4.59 (m, 6H), 3.85−3.89 (m, 2H), 2.59 (s, 3H).

(16*E*)-11-(2-Methoxyethoxy)-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27d). The title compound was synthesized from **25d** (56%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 447 ([M + H]$^+$) $C_{26}H_{30}N_4O_3$; ^{1}H NMR (DMSO-d_6) δ 9.48 (s, 1H), 8.52 (d, 1H), 8.50 (d, 1H), 7.87 (t, 1H), 7.64 (dd, 1H), 7.47 (t, 1H), 7.35 (d, 1H), 7.24 (dd, 1H), 7.04 (dd, 1H), 6.93 (d, 1H), 5.64−5.71 (m, 1H), 5.44−5.51 (m, 1H), 4.25 (t, 2H), 4.05−4.08 (m, 2H), 3.65−3.68 (m, 2H), 3.48 (s, 2H), 3.34 (s, 3H), 3.00 (d, 2H), 2.37−2.40 (m, 2H), 1.98 (s, 3H).

(16*E*)-11-Ethylsulfonyl-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27e). The title compound was synthesized from **25e** (68%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 465 ([M + H]$^+$) $C_{25}H_{28}N_4O_3S$; ^{1}H NMR (MeOD-d_4) δ 9.51 (d, 1H), 8.62 (d, 1H), 8.05−7.97 (m, 2H), 7.65−7.63 (m, 1H), 7.54−7.52 (m, 2H), 7.52−7.49 (m, 2H), 7.23−7.21 (m, 1H), 6.31−6.24 (m, 1H), 6.02−5.94 (m, 1H), 4.43−4.04 (m, 8H), 3.38−3.32 (m, 2H), 2.74 (s, 3H), 1.33 (t, 3H).

2-(14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaen-11-yl)oxy-1-(pyrrolidin-1-yl)ethanone (27h). The title compound was synthesized from **25g** (59%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 500 ([M + H]$^+$) $C_{29}H_{33}N_5O_3$; ^{1}H NMR (MeOD-d_4) δ 8.82 (d, 1H), 8.39 (d, 1H), 7.87−7.86 (m, 1H), 7.51−7.47 (m, 1H), 7.39 (t, 1H), 7.33 (d, 1H), 7.18 (dd, 1H), 7.14−7.09 (m, 2H), 6.18−6.10 (m 1H), 6.18−6.10 (m, 1H), 5.91−5.82 (m, 1H), 4.24−4.20 (m, 1H), 4.09−3.92 (m, 3H), 3.84−3.78 (m, 1H), 2.55 (s, 3H), 1.98−1.91 (m, 4H), 1.84−1.79 (m, 3H).

(16*E*)-11-(4-Morpholino)-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27i). The title compound was synthesized from **25h** (88%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 458 ([M + H]$^+$) $C_{27}H_{31}N_5O_2$; ^{1}H NMR ($CDCl_3$) δ 10.83 (s, 1H), 9.04 (br s, 1H), 8.34 (d, 1H), 8.06 (t, 1H), 7.56−7.59 (m, 1H), 7.53 (t, 1H), 7.35−7.38 (m, 2H), 7.25 (s, 1H), 7.19 (dd, 1H), 6.10−6.17 (m, 1H), 5.91−5.96 (m, 1H), 4.27−4.30 (m, 2H), 4.03−4.04 (m, 2H), 3.84−3.94 (m, 6H), 3.00 (m, 2H), 2.90 (m, 2H), 2.69 (m, 2H), 2.64 (s, 3H).

(16*E*)-11-(2-(Diethylamino)ethoxy)-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1-(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27j). The title compound was synthesized from **25i** (50%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 488 ([M + H]$^+$) $C_{29}H_{37}N_5O_2$; ^{1}H NMR ($CDCl_3$) δ 12.03 (s, 1H), 8.79 (d, 1H), 8.29 (d, 1H), 7.92−7.93 (br s, 1H), 7.56−7.60 (m, 2H), 7.49 (dd, 1H), 7.39 (d, 1H), 7.19−7.22 (m, 1H), 6.98 (d, 1H), 6.07−6.14 (m, 1H), 5.88−5.95 (m, 1H), 4.42 (br s, 2H), 4.34−4.38 (m, 2H), 3.90−4.09 (m, 2H), 3.28−3.58 (m, 6H), 2.74−2.85 (m, 2H), 2.66 (s, 3H), 1.47 (t, 3H), 1.42 (t, 3H).

(16*E*)-14-Methyl-11-(2-(pyrrolidin-1-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1-(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27k). The title compound was synthesized from **25j** (67%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 486 ([M + H]$^+$) $C_{29}H_{35}N_5O_2$; ^{1}H NMR ($CDCl_3$) δ 11.94 (s, 1H), 8.73 (d, 1H), 8.26 (d, 1H), 7.85−7.86 (br s, 1H), 7.50−7.52 (m, 2H), 7.41 (dd, 1H), 7.34 (d, 1H), 7.13−7.16 (m, 1H), 6.92 (d, 1H), 6.04−6.09 (m, 1H), 5.82−5.89 (m, 1H), 4.28−4.37 (m, 4H), 3.92−4.06. (m, 4H), 3.05 (br s, 2H), 2.65−2.74 (m, 2H), 2.60 (s, 3H), 2.20−2.24 (m, 4H), 2.12−2.14 (m, 4H).

(16*E*)-11-(2-(Pyrrolidin-1-yl)ethoxy)-14-(2,2,2-trifluoroethyl)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]-heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27l). The title compound was synthesized from **25k** (51%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 554 ([M + H]$^+$) $C_{30}H_{34}F_3N_5O_2$; ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1H), 8.30 (d, 1H), 7.92 (s, 1H), 7.57−7.54 (m, 2H), 7.40−7.36 (m, 2H), 7.24(d, 1H), 6.95 (d, 1H), 6.03−5.89 (m, 1H), 5.83−5.74 (m, 1H), 4.43 (m, 2H), 4.28−4.25 (m, 4H), 4.01 (m, 2H), 3.77 (d, 2H), 3.70 (m, 2H), 3.38 (m, 2H), 3.15−3.07 (m, 2H), 2.63−2.61 (m, 2H), 2.24−2.20 (m, 4H).

11-(2-(Pyrrolidin-1-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaen-14-acetonitrile (27m). The title compound was synthesized from **25l** (62%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 511 ([M + H]$^+$) $C_{30}H_{34}N_6O_2$; ^{1}H NMR ($CDCl_3$) δ 8.44 (d, 1H), 8.30 (d, 1H), 7.84 (s, 1H), 7.50 (d, 1H), 7.45 (t, 1H), 7.27 (d, 1H), 7.16 (d, 1H), 7.11 (d, 1H), 5.81 (dt, 1H), 5.52 (dt, 1H), 4.36 (t, 2H), 4.27 (t, 2H), 3.85−3.62 (m, 8H), 3.33 (s, 2H), 3.24 (d, 2H), 2.11−2.15 (m, 4H).

14-Methyl-11-(2-(morpholin-4-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27n). The title compound was synthesized from **25m** (66%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 502 ([M + H]$^+$) $C_{29}H_{35}N_5O_3$; ^{1}H NMR ($CDCl_3$) δ 12.05 (s, 1H), 8.66 (d, 1H), 8.22 (d, 1H), 7.78 (s, 1H), 7.45−7.47 (m, 2H), 6.38 (dd, 1H), 7.29 (d, 1H), 7.08−7.11 (m, 1H), 6.83 (d, 1H), 5.98−6.05 (m, 1H), 5.72−5.78 (m, 1H), 4.33−4.39 (m, 2H), 4.19−4.25 (m, 2H), 3.96−4.00 (m, 6H,), 3.54−3.81 (m, 6H), 2.99−3.01 (m, 2H), 2.63−2.68 (m, 2H), 2.53 (s, 3H).

14-Methyl-11-(2-(4-methylpiperazin-1-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1-(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27o). The title compound was synthesized from **25n** (50%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 515 ([M + H]$^+$) $C_{30}H_{38}N_6O_2$; ^{1}H NMR ($CDCl_3$) δ 11.99 (s, 1H), 8.73 (d, 1H), 8.27 (d, 1H), 7.86−7.87 (br s, 1H), 7.52−7.56 (m, 2H), 7.47 (dd, 1H), 7.35 (d, 1H), 7.16−7.19 (m, 1H), 6.96 (d, 1H), 6.05−6.11 (m, 1H), 5.82−5.88 (m, 1H), 4.28−4.36 (m, 4H), 3.71−4.03. (m, 12H), 3.05 (br s, 2H), 2.91 (s, 3H), 2.60 (s, 3H), 2.05 (s, 2H).

14-Methyl-11-(2-(piperidin-1-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (27p). The title compound was synthesized from **25o** (62%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 500 ([M + H]$^+$) $C_{30}H_{37}N_5O_2$; ^{1}H NMR ($CDCl_3$) δ 12.04 (s, 1H), 8.72 (d, 1H), 8.25 (d, 1H), 7.82 (br s, 1H), 7.51−7.52 (m, 2H), 7.41 (dd, 1H), 7.34 (d, 1H), 7.14−7.18 (m, 1H), 6.94 (d, 1H), 6.03−6.10 (m, 1H), 5.80−5.87 (m, 1H), 4.95 (d, 1H), 4.22−4.33 (m, 4H), 4.02−4.06 (m, 1H), 3.59−4.06 (m, 6H), 2.93 (br s, 2H), 2.65−2.77 (m, 2H, CH2), 2.59 (s, 3H), 1.83−2.01 (m, 6H).

10-Methoxy-14-methyl-11-(2-(pyrrolidin-1-yl)ethoxy)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]-heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27q). The title compound was synthesized from **25p** (44%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 516 ([M + H]$^+$) $C_{30}H_{37}N_5O_3$; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, 1H), 8.49 (br s, 1H), 7.93 (s, 1H), 7.52 (d, 1H), 7.41 (t, 1H), 7.23 (d, 1H), 7.13 (dd, 1H), 6.97 (d, 1H), 6.18 (dt, 1H), 5.77 (m, 1H), 4.32−4.24 (m, 4H), 4.05 (d, 2H), 3.90 (s, 2H), 3.85−3.81 (m, 4H), 3.65 (t, 2H), 3.26−3.20 (m, 2H), 3.25 (s, 3H), 2.61 (s, 3H), 2.16−2.05 (m, 4H).

***N*-(14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaen-10-yl)-2,2,2-trifluoroacetamide (27r).** The title compound was synthesized from **25q** (68%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 483 ([M + H]$^+$) $C_{25}H_{24}F_3N_5O_2$; ^{1}H NMR (MeOD-d_4) δ 8.98 (s, 1H), 8.51 (d, 1H), 7.98 (s, 1H), 7.59−7.57 (m, 1H), 7.51−7.47 (m, 3H), 7.41−7.39 (m, 1H), 7.20−7.18 (m, 1H), 6.22 (dt, 1H), 5.83 (dt, 1H), 4.85−4.35 (m, 2H), 4.34−4.09 (m, 2H), 4.07−3.79 (m, 2H), 2.74−2.64 (m, 2H), 2.69 (s, 3H)

(16*E*)-4,14-Dimethyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (28a). The title compound was synthesized from **25r** (56%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) m/z 387 ([M + H]$^+$) $C_{24}H_{26}N_4O$; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 1H), 8.60 (s, 1H), 8.42 (s, 1H), 7.92 (t, 1H), 7.64 (d, 1H), 7.46 (t, 1H), 7.34 (s, 1H), 7.16−7.22 (m, 2H), 7.05 (d, 1H), 6.86 (d, 1H, 5.48−5.74 (m, 2H), 4.20 (t, 2H), 3.48 (s, 2H), 3.06 (d, 2H), 2.45−2.46 (m, 2H), 2.42 (s, 3H), 2.01 (s, 3H).

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(16*E*)-14-Methyl-4-(pyrrolidin-1-yl)-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (28b). The title compound was synthesized from **25s** (49%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) *m/z* 442 ([M + H]$^+$) $C_{27}H_{31}N_5O$; ^{1}H NMR ($CDCl_3$) δ 12.6 (s, 1H), 8.73 (s, 1H), 7.86−7.91 (m, 2H), 7.41−7.48 (m, 2H), 7.34−7.35 (m, 1H), 7.10 (d, 1H), 6.45 (s, 1H), 6.03−6.18 (m, 1H), 5.86−5.92 (m, 1H), 4.13−4.30 (m, 4H), 3.83−3.84 (m, 2H), 3.68 (s, 2H), 2.74 (m, 2H), 2.56 (s, 2H), 2.21 (s, 3H), 1.63 (m, 4H).

(16*E*)-3,14-Dimethyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaene (28c). The title compound was synthesized from **25t** (54%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) *m/z* 387 ([M + H]$^+$) $C_{24}H_{26}N_4O$; ^{1}H NMR (DMSO-d_6) δ 9.52 (s, 1H), 8.63 (br s, 1H), 8.42 (s, 1H), 7.51 (t, 1H), 7.44 (t, 1H), 7.32−7.37 (m, 1H), 7.13−7.19 (m, 2H), 7.02−7.08 (m, 1H), 6.82 (d, 1H), 5.64−5.72 (m, 1H), 5.62−5.71 (m, 1H), 5.51−5.55 (m, 1H), 4.20 (t, 2H), 3.41 (s, 2H), 3.07 (d, 2H), 3.34 (s, 3H), 1.98 (s, 3H).

(16*E*)-23-Fluoro-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (28d). The title compound was synthesized from **25u** (51%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) *m/z* 391 ([M + H]$^+$) $C_{23}H_{23}FN_4O$; ^{1}H NMR ($CDCl_3$) δ 9.78 (s, 1H), 8.85 (s, 1H), 8.45 (m, 1H), 7.82 (s, 1H), 7.48−7.50 (m, 2H), 7.21−7.29 (m, 3H), 6.87 (td, 1H), 6.06−6.13 (m, 1H), 5.85−5.93 (m, 1H), 4.16−4.27 (m, 4H), 3.75−3.85 (m, 2H), 2.74−2.75 (m, 2H), 2.62 (s, 3H).

(16*E*)-22-Methoxy-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaene (28e). The title compound was synthesized from **25v** (44%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) *m/z* 403 ([M + H]$^+$) $C_{24}H_{26}N_4O_2$; ^{1}H NMR ($CDCl_3$) δ 11.8 (s, 1H), 8.41−8.44 (m, 2H), 8.26 (br s, 1H), 7.95 (d, 1H), 7.65 (br s, 1H), 7.52−7.54 (m, 1H), 7.45 (d, 1H), 7.45 (d, 1H), 7.31−7.34 (m, 1H), 7.07 (d, 1H), 6.18−6.24 (m, 2H), 4.67 (s, 2H), 4.08−4.27 (m, 4H), 3.99 (s, 3H), 3.93−4.04 (m, 1H), 3.65 (br s, 1H), 2.84 (s, 3H).

19-Oxa-5,7,13,25-tetraazatetracyclo[18.3.1.1(2,6).1(8,12)]-hexacosa-1(24),2(25),3,5,8(26),9,11,15,20,22-decaene (26e). To a solution of **26d** (12 mg, 0.027 mmol) in MeOH/CH_2Cl_2 (0.2:1, 2 mL) was added 4 M HCl (0.5 mL), and the resulting mixture was heated at 40 °C for 4 h. It was concentrated under reduced pressure and purified by preparative HPLC to furnish **26e** (7 mg, 79%, >95% trans by ^{1}H NMR) as a yellow solid. LC/MS (ESI positive mode) *m/z* 346 ([M + H]$^+$) $C_{21}H_{20}N_4O$; ^{1}H NMR (MeOD-d_4) δ 8.58−8.57 (m, 2H), 8.52 (d, 1H), 8.02 (t, 1H), 7.74−7.72 (m, 1H), 7.56−7.41 (m, 3H), 7.30−7.27 (m, 2H), 7.13 (dd, 1H), 6.18 (td, 1H), 5.86 (td, 1H), 4.32−4.30 (m, 2H), 4.12 (d, 2H), 2.39−2.34 (m, 2H).

20-Oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]-heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (26g). To a mixture of **26f** (45 mg, 0.100 mmol) in 5.5% H_2O/MeOH (3 mL) was added K_2CO_3 (69 mg, 0.500 mmol), and the resulting mixture was stirred at 90 °C for 6 h. It was concentrated under reduced pressure and purified by preparative HPLC to furnish **26g** (17 mg, 48%, >95% trans by ^{1}H NMR) as a pale yellow solid. LC/MS (ESI positive mode) *m/z* 360 ([M + H]$^+$) $C_{22}H_{22}N_4O$; ^{1}H NMR (MeOD-d_4) δ 8.99 (br s, 1H), 8.44 (d, 1H), 8.44 (d, 1H), 8.01 (t, 1H), 7.51 (dd, 1H), 7.42 (t, 1H), 7.24−7.31 (m, 2H), 7.24−7.31 (m, 2H), 7.13 (dd, 1H), 7.02−7.06 (m, 2H), 5.85 (dt, 1H), 5.67 (dt, 1H), 4.19 (t, 2H), 3.93 (s, 2H), 3.48 (d, 2H), 2.53−2.57 (m, 2H).

(16*E*)-14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaen-10-amine (27s). Following a procedure similar to that of **26g**, the title compound was synthesized from **27r** (58%, >95% trans by ^{1}H NMR). LC/MS (ESI positive mode) *m/z* 388 ([M + H]$^+$) $C_{23}H_{25}N_5O$; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, 1H), 8.17 (s, 1H), 8.10 (t, 1H), 7.48−7.43 (m, 1H), 7.38 (t, 1H), 7.16 (s, 1H), 7.04−7.01 (m, 1H), 6.50 (s, 1H), 6.16 (t, 1H), 5.78− 5.69 (m, 2H), 4.19 (t, 2H), 3.62−3.59 (m, 2H), 3.29−3.27 (m, 2H), 2.91 (q, 2H), 2.53 (q, 2H), 2.24 (s, 3H).

2-(Isopropylamino)-1-(20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8-(27),9,11,16,21,23-decaen-14-yl)ethanone (26r). To a solution of **26g** (5 mg, 0.0168 mmol) and bromoacetyl bromide (1.5 μL, 0.0168 mmol) in CH_2Cl_2 (1 mL) was added diisopropylethylamine (3 μL, 0.0168 mmol), and the resulting solution was stirred at room temperature for 4 h. Isopropylamine (5 μL) was added, and resulting mixture was stirred for another 4 h. It was concentrated under reduced pressure and purified by preparative HPLC to furnish **26r** (3 mg, 39%, >95% trans by ^{1}H NMR) as a pale yellow solid. LC/MS (ESI positive mode) *m/z* 458 ([M + H]$^+$) $C_{27}H_{31}N_5O_2$; ^{1}H NMR (MeOD-d_4) δ 8.60 (s, 1H), 8.48 (d, 1H), 7.97 (d, 1H), 7.62 (m, 1H), 7.46 (t, 1H), 7.35 (m, 1H), 7.28 (t, 1H), 7.24 (dd, 1H), 7.06 (t, 1H), 6.86 (d, 1H), 5.78−5.71 (m, 1H), 5.52−5.42 (m, 1H), 4.23 (t, 2H), 4.18−4.10 (m, 5H), 3.92 (s, 2H), 2.52 (br, 2H), 1.39 (d, 6H).

(16*E*)-11-(2-(Ethylsulfonyl)ethoxy)-14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1-(25),2(26),3,5,8(27),9,11,16,21,23-decaene (27g). To a solution of **27f** (20 mg, 0.042 mmol) in acetic acid (2 mL) at 0 °C was added 50% hydrogen peroxide solution (0.1 mL), and the resulting mixture was stirred at room temperature for 7 h. It was concentrated under reduced pressure and purified by preparative HPLC to furnish **27g** (4 mg, 19%, >95% trans by ^{1}H NMR) as a yellow solid. LC/MS (ESI positive mode) *m/z* 509 ([M + H]$^+$) $C_{27}H_{32}N_4O_4S$; ^{1}H NMR (MeOD-d_4) δ 8.66 (d, 1H), 8.41 (d, 1H), 7.97 (t, 1H), 7.52 (d, 1H), 7.42 (t, 1H), 7.26 (d, 1H,), 7.09−7.14 (m, 2H), 7.03 (d, 1H), 5.88 (dt, 1H), 5.72 (dt, 1H, CH), 4.49 (t, 2H), 4.20 (t, 2H), 3.91 (brs, 2H), 3.64 (t, 2H), 3.46−3.48 (m, 2H), 3.25−3.28 (m, 2H), 2.53−2.57 (m, 2H), 2.28 (s, 3H), 1.42 (t, 3H).

***N*-(Ethylsulfonylmethyl)(14-methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2-(26),3,5,8(27),9,11,16,21,23-decaen-10-yl)amine (27t).** To a solution of **27s** (9 mg, 0.021 mmol) and ethanesulfonyl chloride (10 μL) in CH_2Cl_2 (1 mL) was added triethylamine (10 μL), and the resulting solution was stirred at room temperature for 4 h. It was concentrated under reduced pressure and purified by preparative HPLC to furnish **27t** (4 mg, 40%, >95% trans by ^{1}H NMR) as a pale yellow solid. LC/MS (ESI positive mode) *m/z* 480 ([M + H]$^+$) $C_{26}H_{31}N_5O_3S$; ^{1}H NMR (MeOD-d_4) δ 8.47 (s, 1H), 8.39 (d, 1H), 8.01 (s, 1H), 7.41−7.39 (m, 1H), 7.34 (t, 1H), 7.13 (d, 1H), 7.01−6.98 (m, 1H), 7.86−7.83 (m, 2H), 5.68−5.65 (m, 2H), 4.14 (t, 2H), 3.60−3.55 (m, 2H), 3.39 (s, 2H), 3.17−3.13 (m, 2H), 3.07 (q, 2H), 2.54−2.52 (m, 2H), 2.28 (s, 3H), 1.28 (t, 3H).

***N*-(14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo[19.3.1.1-(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-dec-aen-10-yl)morpholine-4-carboxamide (27u).** Following a procedure similar to that of **27t**, the title compound was synthesized from **27s** and Grubbs second generation catalyst. LC/MS (ESI positive mode) *m/z* 501 ([M + H]$^+$) $C_{28}H_{32}N_6O_3$; ^{1}H NMR (MeOD-d_4) δ 8.81 (s, 1H), 8.50 (d, 1H), 8.02 (s, 1H), 7.58−7.56 (m, 1H), 7.48 (t, 1H), 7.38 (d, 1H), 7.22−7.16 (m, 1H), 6.92 (s, 1H), 6.89−6.85 (m, 1H), 6.26−6.18 (m, 1H), 5.90−5.83 (m, 1H), 4.56 (t, 2H), 4.38−4.30 (m, 2H), 4.19−4.05 (m, 2H), 3.73−3.70 (m, 2H), 3.56−3.53 (m, 2H), 2.70 (s, 3H), 2.24−2.02 (m, 4H).

Enzyme Assays. The recombinant enzymes (CDK2/cyclin A, JAK2, and FLT3) were purchased from Invitrogen (catalog numbers PV 3267, 4210, and 3182, respectively). All assays were carried out in 384-well white microtiter plates using the PKLight assay system from Cambrex. This assay platform is a luminometric assay for the detection of ATP in the reaction using a luciferase-coupled reaction. The compounds were tested at eight concentrations prepared from 3- or 4-fold serial dilution starting at 10 μM. For CDK2/cyclin A assay, the reaction mixture consisted of the following components in 25 μL of assay buffer (50 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 5 mM BGP, 1 mM DTT, 0.1 mM sodium orthovanadate), 1.4 μg/mL of CDK2/cyclin A complex, 0.5 μM RbING substrate (Invitrogen, catalog number PV2939), and 0.5 μM ATP. The mixture was incubated at room temperature for 2 h. Then 13 μL of PKLight ATP detection reagent was added and the mixture was incubated for 10 min. Luminescence signals were detected on a multilabel plate reader (Victor^2 V 1420, Perkin-Elmer). The other kinase assays were similar,

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with the following differences in reagents: For FLT3 assays, the mixture contained 2.0 μg/mL FLT3 enzyme, 5 μM poly(Glu,Tyr) substrate (Sigma, catalog number P0275), and 4 μM ATP. For JAK2 assays, the reaction contained 0.35 μg/mL JAK2 enzyme, 10 μM poly(Glu,Ala,Tyr) substrate (Sigma, catalog number P3899), and 0.15 μM ATP. The analytical software Prism 5.0 (GraphPad Software Pte Ltd.) was used to generate IC_{50} values from the data.

Cell Proliferation Assays. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured according to the recommended guidelines. For proliferation assays in 96-well plates, 20 000 cells were seeded in 100 μL of medium and treated the following day with compounds (in triplicate) at concentrations up to 10 μM for 48 h. Cell viability was monitored using the CellTiter-96 Aqueous One solution cell proliferation assay (Promega, Madison, WI). Dose−response curves were plotted to determine IC_{50} values for the compounds using the XL-fit software (IDBS Ltd., Alameda, CA).

Cell Pharmacodynamic Assay. HCT-116 cells (2×10^5 in 5 mL of McCoy's medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum) were seeded in 60 mm dishes 16−24 h before drug treatment. Each dish was treated separately with different concentrations of **26h** or DMSO for 24 h prior to lysis using a modified radioimmunoprecipitation buffer (50 mM Tris-HCl, 150 mM NaCl, 1% sodium deoxycholate, 0.25 mM EDTA (pH 8.0), 1% Triton X-100, 0.2% NaF, and protease inhibitor cocktail (Sigma catalog number P8340). Proteins were measured using the Bradford assay, and 30 μg of lysate from each treatment was resolved on 10% SDS−PAGE and transferred onto PVDF membrane. Western blot analyses using antibodies against phospho-Rb (catalog number 9308, Cell Signaling Technology, Danvers, MA) and β-actin (catalog number A2066, Sigma-Aldrich, St. Louis, MO) were performed using dilutions recommended by suppliers. Signals were detected using autoradiography with Pierce ECL Western blotting substrate (Thermo Fisher Scientific, Rockford, IL).

High Throughput Solubility Assay. This assay measures the solubility of a compound in PBS in a high throughput mode. The assay was done using 96-well semitransparent PP microplates with V-shaped bottom (Greiner Bio-One) and 96-well UV transparent microplates (Greiner Bio-One). Compound solutions (250 μM) were prepared in 10 mM phosphate buffer (pH 7.0) containing 20% DMSO in a total volume of 0.2 mL. Plates were placed on a shaker set at 600 rpm for 1.5 h, following which the plates were allowed to stand for 2 h at room temperature. The plates were centrifuged at 1500*g* for 15 min. The supernatants were transferred to a UV transparent microplate and analyzed by UV spectrophotometry at the appropriate absorption maxima. The concentration of the compound in the supernatant was quantified using a calibration curve. For calculated solubilities of 250 ± 30 μM, solubilities are reported as >250 μM (>150 μg/mL).

Metabolic Stability in Liver Microsomes. Compounds (5 μM) were incubated with MLM (mouse liver microsomes), RLM (rat liver microsomes), DLM (dog liver microsomes), and HLM (human liver microsomes) (final microsomal concentration of ∼0.87 mg/mL) in a reaction mix containing 50 mM potassium phosphate buffer (pH 7.4) and NADPH regeneration system, at 37 °C, in a total reaction volume of 1 mL. Reactions were terminated at 0, 15, 30, 45, and 60 min of incubation with a chilled mixture of acetonitrile and DMSO (80:20). The mixture was vortexed for 5 min, centrifuged at 13 200 rpm for 15 min at 4 °C, and the supernatants were analyzed by LC−MS/MS. Stability was assessed by plotting the percent of parent compound remaining against time on a log−linear scale, and half-life was estimated from the linear portion of the log−linear curve using the first order equation $t_{1/2} = 0.693/k$, where k is the slope of the curve (equal to the first order elimination rate constant).

Human in Vitro CYP450 Inhibition Assay. **26h** was incubated (at concentrations of 0.05, 0.25, 0.5, 2.5, 5, 25 μM in DMSO; final DMSO concentration of 0.35%) with human liver microsomes (0.25 mg/mL for CYP1A and CYP3A4, 0.5 mg/mL for CYP2C19 and CYP2D6, 1 mg/mL for CYP2C9) and NADPH (1 mM) in the presence of the probe substrate ethoxyresorufin (0.5 μM) for 5 min (CYP1A), tolbutamide (120 μM) for 60 min (CYP2C9), mephenytoin (25 μM) for 60 min (CYP2C19), dextromethorphan (5 μM) for 30 min (CYP2D6), and midazolam (2.5 μM) for 5 min (CYP3A4) at 37 °C. The selective inhibitors α-naphthoflavone, sulfaphenazole, tranylcypromine, quinidine, and ketoconazole were used as positive controls for CYP1A, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 inhibitor, respectively. For CYP1A, the reactions were terminated by the addition of methanol, and the formation of the metabolite, resorufin, was monitored by fluorescence (excitation wavelength of 535 nm, emission wavelength of 595 nm). For the CYP2C9, CYP2C19, CYP2D6, and CYP3A4 incubations, the reactions were terminated by the addition of methanol containing an internal standard. The samples were centrifuged, and the supernatants were combined for the simultaneous analysis of 4-hydroxytolbutamide, 4-hydroxymephenytoin, dextrorphan, 1-hydroxymidazolam, and the internal standard by LC−MS/MS. Formic acid in deionized water (final concentration of 0.1%) was added to the final sample prior to analysis. A decrease in the formation of the metabolites compared to vehicle control was used to calculate an IC_{50} value (test compound concentration that produces 50% inhibition).

Caco-2 Bidirectional Permeability Assay. **26h** at 5 μM in Hank's balanced salt solution (HBSS), final DMSO concentration less than 1%, was placed in 21−28 day confluent monolayer cells in Transwell assay plates. Both apical and basolateral sides were maintained at pH 7.4. When dosed on the apical side, the permeability in the A → B direction was assessed, and when dosed on the basolateral side, the B → A direction was assessed. Both apical and basolateral sides were sampled at 2 h. The concentration of **26h** was determined by LC/MS using a four-point calibration curve. Atenolol ($P_{app} < 0.5 \times 10^{-6}$ cm/s), propranolol (15×10^{-6} cm/s < $P_{app} < 25 \times 10^{-6}$ cm/s), Lucifer yellow ($P_{app} > 0.4 \times 10^{-6}$ cm/s), and digoxin (efflux ratio of >3) were used in the quality control of the monolayer batch. The integrity of the monolayer was determined by measuring the pre-experiment TEER (between 450 and 650 Ωcm^2) and using Lucifer yellow (efflux of ≤0.5%). The efflux ratio was defined as the ratio of $P_{app,B\rightarrow A}$ to $P_{app,A\rightarrow B}$.

In Vitro Plasma Protein Binding. Equilibrium dialysis was performed in a Micro-Equilibrium Dialyzer (Harvard Apparatus) with a chamber volume of 500 μL (each compartment with a volume of 250 μL). The semipermeable membrane used was rinsed with Milli-Q water and soaked for 10 min in PBS. **26h** was added to plasma (from mouse, dog, and humans) to obtain a final concentration of 1000 ng/mL. The spiked plasma was vortexed, and 250 μL was aliquoted into one chamber of the dialyzer cell. The other chamber was filled with 250 μL of PBS buffer. The assembled cell was placed into a water bath at 37 °C, and dialysis was performed for 4 h. Following dialysis, 50 μL of PBS dialyzed samples containing free **26h** was transferred into 2 mL Eppendorf tubes in triplicate for extraction. Samples were extracted with 1500 μL of MTBE (methyl *tert*-butyl ether) for 30 min using a mixer at motor speed setting 60 with pulsing. After 30 min, the sample tubes were centrifuged at 4 °C for 10 min at 13 000 rpm in a microcentrifuge. The supernatant (1400 μL) was transferred into fresh 2 mL Eppendorf tubes and dried in a SpeedVac at 43 °C for 35 min. The dried samples were reconstituted with 100 μL of methanol/Milli-Q H_2O (60:40) and analyzed by LC−MS/MS.

Pharmacokinetics. Male BALB/c mice (aged ∼10−12 weeks and weighing 17−22 g), male Beagle dogs (∼6−7 months of age, weighing 10−14 kg), and male Wistar rats (aged 6−8 weeks, weighing 239−249 g) were used in this study. All the animal studies were performed as per approved internal protocols for animal care and use. The oral doses for mice, dogs, and rats were 75, 10, and 10 mg/kg, respectively. The doses were administered by gavage as suspensions (0.5% methylcellulose and 0.1% Tween 80) to mice and rats, and as gelatin capsules (12 Torpac) to dogs. Following oral dosing serial blood samples were collected (cardiac puncture in mice, jugular vein in dogs, and superior vena cava in rats) at different time points (0−24 h) in tubes containing K_3EDTA as anticoagulant, centrifuged, and plasma was separated and stored at −70 °C until analysis. Plasma samples were processed and analyzed by LC−MS/MS. Pharmacokinetic parameters were estimated by noncompartmental methods using WinNonlin (version 5.1, Pharsight, CA).

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Materials and Methods for HCT-116 and Ramos Studies. *1. Mice/Husbandry.* Female BALB/c nude mice (ARC, West Australia), 10−12 weeks of age, were fed with sterilized tap water (ad libitum water) and irradiated standard rodent diet consisting of 19% protein, 5% fat, and 5% fiber. Mice were housed in individual ventilated cages on a 12 h light cycle at 21−22 °C and 40−60% humidity. The use of animals in the Biological Resource Centre (BRC), Biopolis, Singapore, is compliant with the recommendations of the Guide for Care and Use of Laboratory Animals with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care. The animal care and use program, 050076 at BRC, is Institutional Animal Care and Use Committee (IACUC) accredited.

2. Tumor Implantation. 2.1. For HCT-116 Study. Mice were implanted subcutaneously in the right flank with 5×10^6 cells of HCT-116 human colon carcinoma. Each tumor was monitored twice per week and subsequently daily as the neoplasms reached the desired size of approximately 100 mm^3. At day 8, when the tumors attained a calculated tumor volume between 75 to 144 mm^3, the animals were pair-matched and distributed randomly into various treatment groups (the mean tumor volume in each group was 105 mm^3). Estimated tumor volume was calculated using the formula

$$\text{tumor volume}(\text{mm}^3) = (w^2 l)/2$$

where w is the width and l is the length in mm of an HCT-116 carcinoma.

2.2. For Ramos Study. Mice were implanted subcutaneously in the right flank with 7×10^6 cells of Ramos (ATCC No. CRL-9591) cells (100 μL). The tumor size was monitored twice per week and subsequently daily as the neoplasms reached the desired size, approximately 200 mm^3. On day 12, when the tumors attained a volume of between 75 and 405 mm^3, the animal were pair-matched and distributed randomly into various treatment groups (the mean tumor volume in each group was 216 mm^3). Estimated tumor volume was calculated using the formula

$$\text{tumor volume}(\text{mm}^3) = (w^2 l)/2$$

where w is the width and l is the length in mm of a Ramos tumor.

3. Drug. **26h** hydrochloride was synthesized at S*BIO PTE LTD and dissolved in 0.5% methyl cellulose/0.1% Tween 80 (MC/Tween) for oral (po) dosing or in 10% dimethylacetamide (DMA) and 10% Cremophor (DMA/CRE) for ip dosing. Dosing solutions were prepared weekly in a feeding volume of 10 mL per kilogram body weight and stored at 4 °C.

4. Treatment Plan. 4.1. For HCT-116 Study. On day 1, HCT-116-bearing nude mice were pair-matched and placed into 3 groups of 9−10 animals each. Treatment with all drugs was initiated on day 1. The test compound, **26h**, was administered po at the following dosing schedules: 50 and 75 mg/kg × 3 times a week (Monday, Wednesday, and Friday; 3/w). There was a vehicle control group that received vehicle (MC/Tween) on the same schedule. The study was terminated on day 15.

4.2. For Ramos Study. **26h** was administered once daily at doses of 75 mg/kg po q.d. 2d_on−5d_off or 15 mg/kg ip q.d. 5d_on−5d_off. There were two vehicle control groups that received either MC/Tween or DMA/CRE. The treatment groups were compared with the corresponding vehicle control group for the percentage of the tumor growth inhibition (% TGI). The treatment was terminated after 14 days of dosing.

5. End Points.Tumor Growth Inhibition. Activity was assessed by the tumor growth inhibition (TGI) method in which treatment-effected decreases in tumor volume on day 15 of various treatment groups were compared to the vehicle group. The percent tumor growth inhibition (% TGI) was calculated as follows:

$$\%\,\text{TGI} = (\text{Cday\_a} - \text{Tday\_a})/(\text{Cday\_a} - \text{Cday\_1}) \times 100$$

where Cday_1 is the median tumor volume for control group (vehicle) on day 1, Cday_a is the median tumor volume for control group (vehicle) on day a, and Tday_a is median tumor volume for treatment group on day a.

6. Statistics. 6.1. For HCT-116 study. The two-way ANOVA followed by Bonferroni posttests was used to determine the statistical significance of median tumor volume between groups. Statistical analyses were conducted at a p level of 0.05. Prism (GraphPad), version 3, was used for all statistical analyses and graphic presentations.

6.2. For Ramos study. The Mann−Whitney U test was used to determine the statistical significance of median tumor volume between a treatment group and the vehicle control group. Statistical analyses were conducted at a p level of 0.05. Prism (GraphPad), version 3, was used for all statistical analyses and graphic presentations.

Computational. The molecules were built using Maestro, version 8.0.308, or converted to 3D structures from the 2D structure using LigPrep, version 2.1.207.[35] Basic amines were protonated as in aqueous solution at physiological pH. The conformational space was searched using the Monte Carlo multiple minima (MCMM) method as implemented in MacroModel, version 9.5.207.[35] All heavy atoms and hydrogens on heteroatoms were included in the test for duplicate conformations. All rotatable single bonds were included in the conformational search. All aliphatic rings were ring-opened, and quaternary atoms were allowed to invert. Each search was continued until the global energy minima were found at least 3 times. The energy minimizations were carried out using the truncated Newton conjugate gradient algorithm (TNCG) and the OPLS-AA force field as implemented in MacroModel.[37] Default parameters were used. The conformational searches were done for aqueous solution using the generalized Born/solvent accessible surface (GB/SA) continuum solvation model.[38]

The CDK2 (PDB entry 1AQ1),[39] and Flt3 (PDB entry 1RJB)[40] X-ray structures were downloaded from the Protein Data Bank (PDB[41]). The protein structures were prepared using the protein preparation wizard in Maestro with standard settings. Grids were generated using Glide, version 4.5.208, following the standard procedure recommended by Schrödinger.[35] The conformational ensembles were docked flexibly using Glide with standard settings in both standard and extra precision mode. Only poses with low energy conformations and good hydrogen bond geometries were considered. cLogP values were calculated using QikProp, version 3.0.207, with standard settings.[35]

■ ASSOCIATED CONTENT

Supporting Information

Explanation for the potency of **26r** against CDK2, structures of the RCM catalysts employed and the iv/po PK of **27j** in mice. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AML, acute myeloid leukemia; CDK, cyclin-dependent kinase; CYP, cytochrome P450; ERK5, extracellular signal-regulated kinase-5; ET, essential thrombocythemia; FLT3, fms-like receptor tyrosine kinase 3; JAK2, Janus kinase 2; MF, myelofibrosis; MPN, myeloproliferative neoplasms; PV, polycythemia vera; RCM, ring closing metathesis; RTKs, receptor

tyrosine kinases; STAT, signal transducer and activator of transcription; TGI, tumor growth inhibition

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