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Repositioning HIV-1 Integrase Inhibitors for Cancer Therapeutics: 1,6-Naphthyridine-7-carboxamide as a Promising Scaffold with Drug-like Properties

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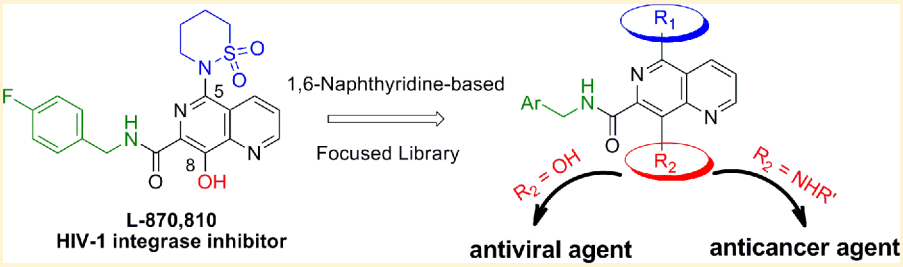

ABSTRACT: Among a large number of HIV-1 integrase (IN) inhibitors, the 8-hydroxy-[1,6]naphthyridines (i.e., L-870,810) were one of the promising class of antiretroviral drugs developed by Merck Laboratories. In spite of its remarkable potency and efficacy, unfortunately upon completion of phase I clinical studies, development of L-870,810 was halted. Because of its desirable pharmacological and pharmaceutical properties we were intrigued to design novel analogues of L-870,810 with goals to (1) improve upon limitations of naphthyridine-7-carboxamides as antiviral agents and (2) to reposition their use as innovative cytotoxic agents for cancer therapeutics. Herein, we report on the design and synthesis of a series of 1,6-naphthyridine-7-carboxamides with various substitutions at the 5- and 8-positions. All the new 5-substituted-8-hydroxy-[1,6]naphthyridines were potent IN inhibitors and the 5-substituted-8-amino-[1,6]naphthyridines were significantly cytotoxic. Further optimization of the 5,8-disubstituted-[1,6]naphthyridines with structural variation on 7-carboxamide delivered novel compounds with significant cytotoxicity in a panel of cancer cell lines and effective inhibition against select oncogenic kinases.

INTRODUCTION

Over the past several years, the concept of drug repositioning (aka drug repurposing, reprofiling, and indication switch: finding a new use for an existing drug) has gained considerable attention. This concept has also been considered for target repositioning, and it is expected to produce many more surprising new drugs for different diseases.[1,2] Drug repositioning is most effective when it is applied during all phases of clinical development. Historically, this approach was accomplished through serendipitous discoveries and unintended side effects, however, computational repositioning strategies are becoming increasingly popular because the benefit of such discoveries can be enormous.[3−5]

Quite often, when a drug fails early phase clinical development there is no further appetite to investigate potential off-targets and reasons for failure. In cases when a backup compound wins the competition for development, the earlier drugs are often dropped from the radar screen. One such agent, L-870,810, showed favorable pharmacological and pharmaceutical properties but was proven not to be an ideal candidate for antiviral drug development.[6,7] Because of our previous success in repositioning our earlier discovered hydrazide class of IN inhibitors[8,9] we hypothesized that 1,6-naphthyridine-7-carboxamides could serve as a promising new scaffold for further optimization into cytotoxic compounds useful as anticancer therapeutics (Figure 1). In fact, it was previously reported that the 1,6-naphthyridines inhibit FGFR-1 and VEGFR-2 kinases when substituted at positions 2 and 7 by amino and 3 by aryl groups, suggesting that various substitutions on this active scaffold could confer diverse biological activities.[10,11] Indeed, L-870,810 was used as an effective prototype to develop a series of

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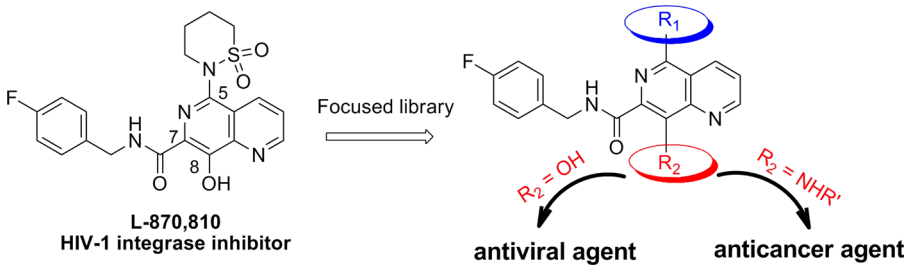

Figure 1. Repurposing HIV-1 IN inhibitors 5,8-substitutions of 1,6-naphthyridine-7-carboxamide into novel cytotoxic agents useful for preclinical development for cancer treatment.

structurally novel CGRP antagonists with good pharmacokinetic properties.[12]

HIV integrase (IN) is an attractive target for the development of anti-AIDS drugs because it is an essential enzyme for the replication of the virus and has no cellular counterparts.[13] IN catalyzes the integration of viral DNA into the host genome in a two-step process, 3′-processing and strand transfer. Among a large number of synthetic IN inhibitors,[14] aryl diketoacid and its bioisosteres are the most developed class that selectively inhibit the strand transfer step by metal chelation mechanism.[15] The 8-hydroxy-7-carboxy-1,6-naphthyridine serves as the bioisostere of aryl diketoacid with better bioavailability, affording potent in vivo antiviral potency.[7] Previous 1,6-naphthyridine-based SAR studies only focused on the substitutions at the 5- and 7-position with respect to the IN inhibition.[6,7,16−28] These later studies led to the discovery of highly potent IN inhibitors; however, to our knowledge none of these compounds were pursued as anticancer agents. Other earlier reports included the use of similar 1,6-naphthyridines as potent and selective inhibitors of human cytomegalovirus.[29−31] The above-mentioned antitumor 1,6-naphthyridines employed a different substitution pattern with ureido at the 2-position, aryl at the 3-position, and amino at the 7-position.

Herein, we designed, synthesized, and provided biological activities of a novel 1,6-naphthyridine-7-carboxamide-based focused library with variation on the 5- and 8-substituents (Figure 1). Our study shows that 1,6-naphthyridine scaffold is especially suitable for optimization of compounds that not only inhibit the catalytic activities of IN but also display significant cytotoxicity. This latter property was further pursued to develop novel agents useful for development as potential anticancer agents.

RESULTS AND DISCUSSION

Chemistry. An efficient synthesis to build the 1,6-naphthyridine-7-carboxyamide-based focused library with variation on 5- and 8-substitution was established by modifying the reaction conditions reported previously.[32] As depicted in Scheme 1, starting from quinolinic anhydride, treatment with refluxing isopropanol provided the mono acid **2**, which was converted to the corresponding acyl chloride **3** by refluxing with thionyl chloride. An optimization was made on this transformation due to the troublesome workup with a large amount of thionyl chloride and the hydrolysis of the acyl chloride **3** under moisture produced by the thionyl chloride. By employing dichloromethane as a solvent and thionyl chloride (1.5 equiv) as a reagent, we performed the transformation with ease and good yield. Consequently, refluxing the solution of acid **2** (1 equiv) and thionyl chloride (1.5 equiv) in dichloromethane with a catalytic amount of DMF smoothly afforded the acyl chloride **3** that was reduced to the alcohol **4** with sodium borohydride. Subsequent Mitsunobu reaction with the phenylsulfonamide of glycine methyl ester followed by a Dieckmann cyclization in the presence of sodium methoxide yielded the core structure 8-hydroxy-1,6-naphthyridine **6**. During the Mitsunobu reaction, the use of anhydrous $MgSO_4$ as an additive accelerated this reaction, probably due to the contribution to the anhydrous reaction condition that was beneficial for the subsequent cyclization reaction in a one-pot reaction. Further bromination at the 5-position and amidation at the 7-position afforded the key intermediate **I-1**, from which 5-amino substituted- and 8-amino substituted-1,6-naphthyridine-7-carboxamides were obtained via nucleophilic aromatic substitution by various amines. Specifically, **I-1** was heated to 135 °C with hexane-1,6-diamine or 2-(1*H*-indol-3-yl)-ethanamine in DMPU to afford **I-7** and **I-8**, respectively, and the direct refluxing of **I-1** in different amino alcohol provided **I-9**−**I-11**. The 5-sulfonamide substituted analogues (**I-4**−**I-6**) were synthesized by refluxing **I-1** in pyridine with different sulfonamides in the presence of cuprous oxide. In addition, structurally diverse analogues with variation on 8-amino substituent (**I-12**−**I-20**, **II-1**−**II-15**) were synthesized from the 8-mesylate or tosylate intermediate (**I-2** or **I-3**) by nucleophilic substitution of various amines in refluxing THF solution.

The 7-substituted analogues with 4-aminocyclohexylamino group held constant at the 8-position (**III-1**−**III-16**) were synthesized in a similar fashion by incorporating various amines into the 7-carboxamide position [**III-**(**1**−**16**)**-8**] followed by installation of the *trans*-cyclohexane-1,4-diamino at the 8-position. The 7-carboxylate substituted analog (**III-17**) was synthesized analogously by direct amination at the 8-position of the intermediate **10**. However, the 7,8-substituents interchanged analogue, **III-18**, was synthesized in a different reaction sequence, i.e., 8-nucleophilic aromatic substitution occurred prior to the 7-amidation because the cyclohexane-1,4-diamino group was suitable for introducing into the 7-carboxamide position at the final step of the synthesis.

New 1,6-Naphthyridine Derivatives are Potent IN Inhibitors and Display Significant Antiviral Activities. We first evaluated the IN inhibitory activity of these synthetic 5,8-disubstituted-1,6-naphthyridine derivatives. Interestingly, the substitutions at the 5- and 8-positions were found to play different roles in the bioactivity of the 1,6-naphthyridine chemotype. As shown in Table 1, the 5-substitution on the 8-hydroxy-1,6-naphthyridine-7-carboxamide just tuned the IN strand transfer activity (**I-1**−**I-11**), while the 8-substitution on the 1,6-naphthyridine-7-carboxamide was critical for retaining the IN inhibitory activity (compounds **I-12** to **I-20** vs **I-1** to **I-11**). It is clear that the presence of the hydroxyl group at the 8-position is essential for strand transfer activity, which is consistent with the original design of 8-hydroxy-1,6-naphthyridine-7-carboxamide scaffold as the bioisostere of the aryl diketoacid active structure.[28] The conversion of hydroxyl into methylsulfonate (**I-2**) or 4-methylbenzenesulfonate (**I-3**) resulted in a remarkable decrease in the potency of

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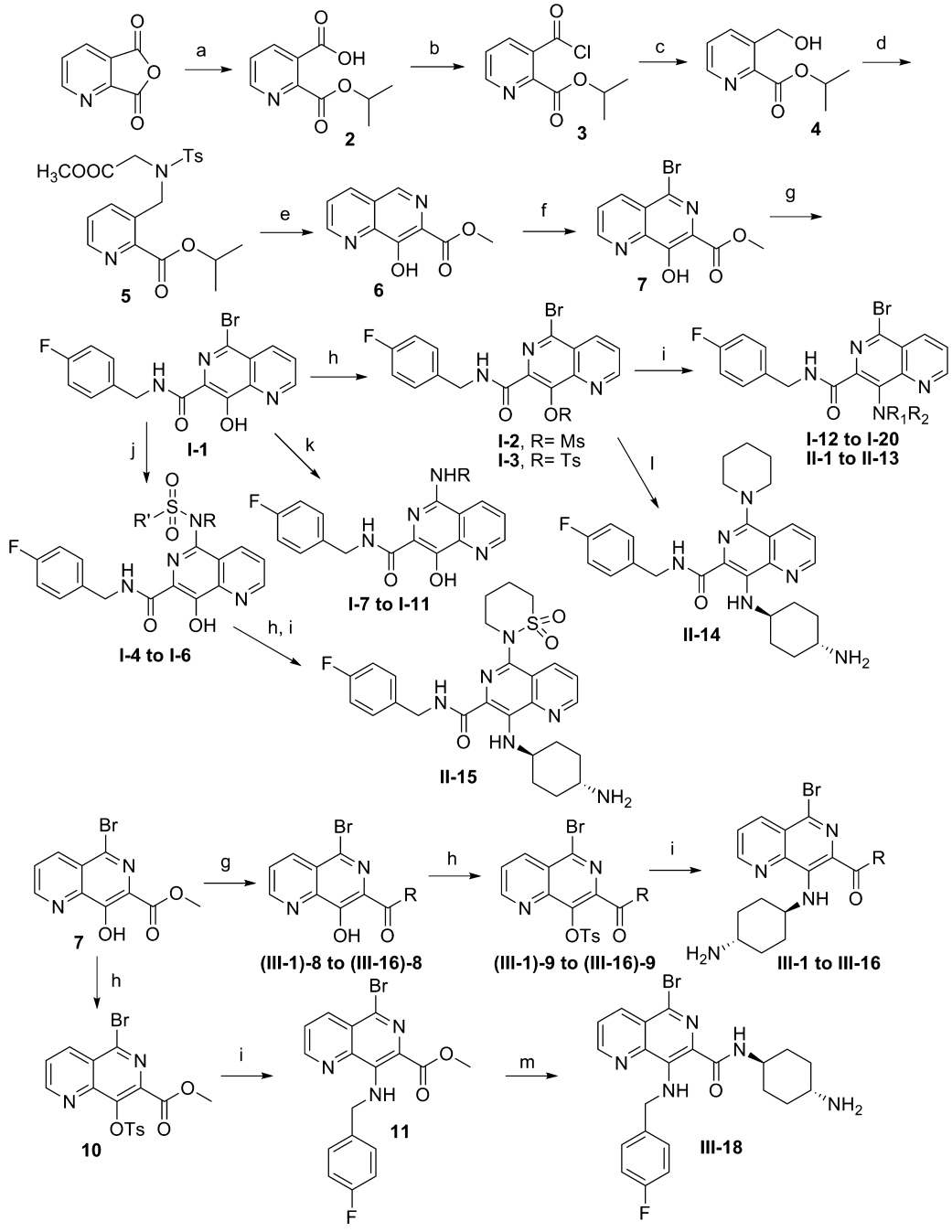
Scheme 1. General Synthetic Route toward the 5,8-Substituted 1,6-Naphthyridine-7-carboxamides[a]



[a]Reagents and conditions: (a) *i*-PrOH, reflux, 16 h, 78%; (b) $SOCl_2$ (1.5 equiv), CH_2Cl_2, cat. DMF, reflux, 3 h; (c) $NaBH_4$, THF, 0 °C, 40−50 min, overall yield 73% for two steps; (d) $TsNHCH_2COOCH_3$, DEAD, PPh_3, anhydrous $MgSO_4$, THF, 0 °C, 2 h; (e) NaOMe, CH_3OH, 0 °C to rt, 3 h, overall yield 65% for two steps; (f) NBS, CH_2Cl_2, rt, 1 h, 85%; (g) 4-fluoro-benzylamine or other amines, toluene, reflux, 20 h, 65−98%, or 1N LiOH, methanol, reflux, 5 h, then bis(trichloromethyl)carbonate, DIPEA, aniline, DMF, 0 °C, 2h (for the preparation of (**III-15**)-**8**); (h) TsCl or MsCl, TEA, CH_2Cl_2, 5 h, 90%; (i) NHR_1R_2, THF, reflux, 8 h, 50−95%; (j) $R'SO_2NRH$, pyridine, cuprous oxide, reflux, 16 h, 35−65%; (k) alcohol amine (neat), 130 °C, 9 h (for the preparation of **I-9**−**I-11**); RNH_2, DMPU, 135 °C, 48 h (for the preparation of **I-7**, **I-8**); (l) *tert*-butyl (1*r*,4*r*)-4-aminocyclohexylcarbamate, Et_3N, THF, reflux, 8 h, 70%; then piperidine, 110 °C, 48 h, 89%; finally, TFA, DCM, 2h, rt, 95%; (m)1N LiOH, THF, rt, 12 h; then Boc-protected amine, EDCI, HOBT, DIPEA, DCM, 3 h; finally, TFA, DCM, 2 h.

3′-processing and strand transfer. Furthermore, the replacement of the hydroxyl group with an amino substitution at the 8-position caused a substantial loss of IN inhibitory activity (**I-12**−**I-20**) that might be attributed to the loss of the chelation binding motif of the *β*-keto−enol, an essential pharmacophore for strand transfer inhibition.[33]

When the 8-hydroxy substituent was held constant, the amino substitution at the 5-position of 8-hydroxy-1,6-naphthyridine-7-carboxamide was generally beneficial for antiviral activity (Table 1, **I-4**−**I-11**), conferring submicromolar IC_{50} values in inhibiting the IN strand transfer reaction. Furthermore, all these 5-amino-substituted 8-hydroxy-1,6-naphthyridine-7-carboxamides displayed potent antiviral activity and a high therapeutic index. It is worth noting that the structurally simple 2-hydroxy ethyl amino turned out to be an optimal substituent for both

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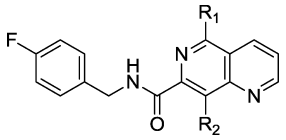
Table 1. Inhibition of HIV-1 Integrase Catalytic Activities and Antiviral Activities of the Lead Compounds



Compd.	R_1	R_2	HIV-1 Integrase inhibition		Antiviral activity		TI^d
			3'-Processing (IC_{50}: μM)[a]	Strand transfer (IC_{50}: μM)	EC_{50} (nM)[b]	CC_{50} (μM)[c]	
I-1	Br	OH	14 ± 6	0.5 ± 0.2	11085	80	7.2
I-2	Br	OMs	>>111	13 ± 3	11843	48	4.0
I-3	Br	OTs	>>111	31±7	5015	31	6.2
I-4 (L-870810)		OH	11 ± 6	0.3 ± 0.2	5	132	26400
I-5		OH	34 ± 15	0.7 ± 0.2	3	64	21333
I-6		OH	11 ± 1	0.7 ± 0.5	2	37	18500
I-7		OH	6 ± 2	0.2 ± 0.1	27	199	7370
I-8		OH	14	1	368	42	114
I-9		OH	9.5±1	0.3 ± 0.1	29	91	3138
I-10		OH	7.8±3	0.3 ± 0.1	23	107	4652
I-11		OH	6.9±1.4	0.2 ± 0.1	52	69	1327
I-12	Br	$-NHCH_3$	>100	>100	ND[e]	ND	ND
I-13	Br		>100	69 ± 8	48873	232	4.8
I-14	Br		>100	>100	ND	ND	ND
I-15	Br		>111	>111	ND	ND	ND
I-16	Br		>111	>111	ND	ND	ND
I-17	Br		>100	>100	ND	ND	ND
I-18	Br		>100	>100	ND	ND	ND
I-19	Br	 (dimer)	>100	>100	ND	ND	ND
I-20	Br		>100	>100	ND	ND	ND

[a] IC_{50}: the drug concentration that produced 50% inhibition of the enzyme function. [b] EC_{50}: effective concentration required to protect C8166 cells against the cytopathogenicity of HIV-1 by 50%. [c] CC_{50}: cytotoxic concentration required to reduce C8166 cell proliferation by 50% tested by MTT method. [d] TI: therapeutic index is a ratio of the CC_{50} value/EC_{50} value. [e] ND: not determined.

3′-processing and the strand transfer inhibition comparable with the sulfonamide substituent in L-870,810 (**I-9**–**I-11** vs **I-4**). Moreover, the chirality of the 2-methyl on the 1-hydroxypropan-2-yl-amino substituent had a subtle effect on the antiviral potency with *S*-configuration being favored (**I-10** vs **I-9**, **I-11**). However, the sulfonamide-substituted analogues displayed an overall superior antiviral effect in terms of the EC_{50} value and therapeutic index. Our investigation on the effect of the 5,8-substitution on the HIV-1 IN inhibitory activity of 1,6-naphthyridine shed new light on the design and understanding of the 1,6-naphthyridine-based IN inhibitors, which will be helpful for the evolution of this scaffold into clinically useful antiviral drugs.

Active Compounds Chelate Divalent Metals on the IN Active Site. Several active and inactive compounds were docked on the IN catalytic site using GOLD (Cambridge Crystallographic Data Center, Cambridge, UK) to explore mechanisms behind structure–activity relationships. Given the lack of a full length crystal structure of HIV-1 IN, a homology model was built based on the X-ray crystal structure of prototype foamy virus (PFV) intasome (PDB: 3OYA),[34,35] a retro-lentivirus belonging to the same viral genus as HIV. Binding modes of Raltegravir, **I-1**, **I-4** (L-970,810), **I-9**, **I-12**, and **I-14** are shown in Figure 2. Like raltegravir, deprotonated hydroxyl groups of active compounds, **I-1**, **I-4**, and **I-9**, formed strong metal chelating interactions with two Mg^{2+} ions along with Asp64, Asp116, and Glu152. Phenyl groups of these compounds formed stacking interactions with DC16 from DNA. Sulfonyl oxygen atoms of **I-4** forms H-bonds with Asn117. Thus, these compounds potentially displace the reactive 3′OH of DNA from the active site to cause deactivation of the intasome. Although **I-12** docked in a similar fashion as **I-1**, the *N*-methyl group of **I-12** created steric hindrance into the binding site to generate weaker interactions with Mg^{2+} ions. Another inactive compound, **I-14**, was docked completely in the reverse position. Substitution with benzyl on the NH group blocked chelating interaction with Mg^{2+} ions. These observations are further comfirmed by docking scores. The GOLD score of these active compounds are around 100, while that of inactive compounds are less than 70.

Repositioning of Novel Derivatives for Anticancer Properties. Because the 5,8-substitutions were shown to steer the biological profile of 1,6-naphthyridine-7-carboxamide scaffold, we were interested to test the 5,8-substituted 1,6-naphthyridine-7-carboxamides in a panel of cancer cell lines to better understand their biological activities. Interestingly, some of the 8-aliphatic diamino substituted analogues that were totally inactive in our IN assay displayed distinct cytotoxicity (Table 2, **I-18**, **I-20**). However, the 1,6-naphthyridine-7-carboxamides bearing aryl or alkyl substituted monoamino groups at the 8-position were not cytotoxic (i.e., **I-13**–**I-17**). On the other hand, the 5-sulfoxamide substituted 8-hydroxy-1,6-naphthyridines displayed low micromolar cytotoxicity against the tested tumor cell lines (i.e., **I-4**-**6**), whereas the 5-alkylamino substituted counterparts were inactive in inhibiting the cell growth even at the concentration of 20 μM (**I-8**-**11**). Because of these encouraging preliminary observations, further SAR and structural optimization were conducted around the 8-amino substituted-1,6-naphthyridine-7-carboxamide scaffold to select compounds for repurposing in our cancer program.

Our initial cytotoxicity data indicated that the diamino structure was an important functionality at the 8-position of 1,6-naphthyridine-7-carboxamide for maintaining antiproliferative activity (exemplified by **I-18**, **I-20** vs **I-12**–**I-17**). Therefore, during the lead optimization campaign, the open chain alkyl diamino, the aryl diamino, and the aliphatic cyclic diamino groups were examined as the 8-substituent in the second generation of the cytotoxic 1,6-naphthyridine series (Figure 3). As shown in Table 3, the aliphatic cyclic diamino structures generally exhibited significant antiproliferative activity against a panel of cancer cell lines with the 4-aminopiperidine and *trans*-1,4-diaminocyclohexane being of special interest (**II-10**, **II-13**). When the terminal amino group was protected by an acyl group (**II-3**, **II-5**, **II-9**, **II-11**),

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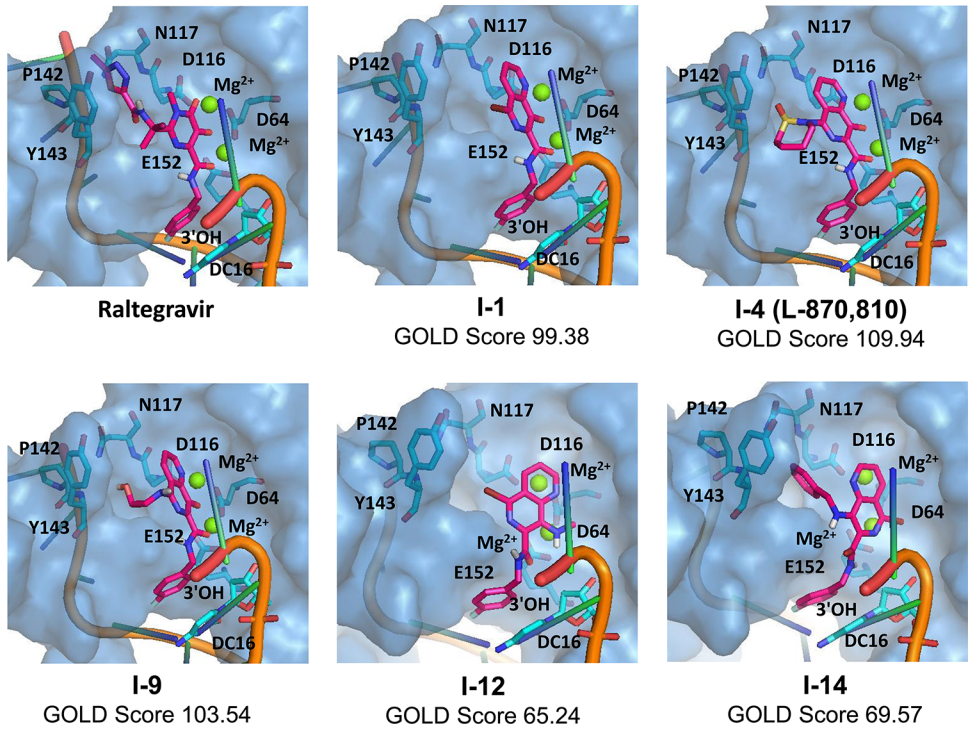

Figure 2. Binding modes of Raltegravir, **I-1**, **I-4**, **I-9**, **I-12**, and **I-14** on the modeled IN active site based on the X-ray crystal structure of prototype foamy virus (PFV) intasome (PDB: 3OYA).[34,35]

the loss of the free N−H resulted in decreased potency. This suggests that the amino substituent bearing a free terminal amino group at the 8-position is important for the cytotoxicity of the 1,6-naphthyridine scaffold. Setting *trans*-1,4-diaminocyclohexane as the privileged structure at the 8-position, the optimal substituent at 5-position was simply explored. The replacement of the original bromo group by a hydrogen (**II-1**), piperidine (**II-14**), or sultam (**II-15**) at the 5-position all led to a loss of cytotoxicity to a similar extent, although the amino substituent at the 5-position was beneficial for the IN inhibition. Therefore, the (1*r*,4*r*)-4-aminocyclohexylamino and the bromo groups were maintained at positions 8 and 5, respectively, for the following SAR study of the third generation series.

As antiproliferative agents, four representative compounds **I-18**, **II-6**, **II-10**, and **II-13**, were selected for colony formation assay. Encouragingly, these cellular active 8-amino-1,6-naphthyridine-7-carboxamides significantly inhibited the colony formation of highly invasive breast cancer cells MDA-MB-435 (Figure 4), consistent with their antiproliferative activity. Especially, the most potent compound **II-13** significantly blocked the colony formation of MDA-MB-435 cells in a dose-dependent manner, with a submicromolar IC_{50} value.

Starting from the evolved compound **II-13**, further structural optimization effort was focused on the 7-carboxamide substitution of the 1,6-naphthyridine framework while the (1*r*,4*r*)-4-aminocyclohexylamino moiety at the 8-position was held constant. As shown in Table 4, the third generation of cytotoxic 8-((4-aminocyclohexyl)amino)-1,6-naphthyridine-7-carboxamide series were synthesized and assessed with structural variation on the 7-carboxamide portion. The original 4-fluorobenzylamino group was examined with various substitutions on the phenyl ring or the methylene bridge (**III-1**−**III-10**). Furthermore, the effect of heteroaromatic or aliphatic cyclic amine or the methoxy replacement at 7-carboxamide (**III-11**−**III-13**, **III-16**, **III-17**) on cytotoxicity was investigated. The chain length of the arylamino moiety was investigated as well (i.e., **III-14**, **III-15** vs **II-13**). Interestingly, this scaffold well tolerated the structural variation on the arylamino group at the 7-carboxamide position. Except for the rigid (*S*)-1-(4-fluorophenyl)ethanamino, the furan-2-ylmethanamino, cyclohexanamino, and methoxy substitutions (**III-4**, **III-11**, **III-16**, **III-17**) that were detrimental to the antiproliferative activity, all other structural displacement exerted slight impact on the inhibitory potency against various tumor cell lines. The electron-donating group and halogen were favored substitutions on the benzyl ring. In addition, the interchange of 8-((4-aminocyclohexyl)amino and 7-*N*-(4-fluorobenzyl) carboxamide (**III-18** vs **II-13**) marginally decreased cytotoxicity. It is clear that *N*-(halogen substituted benzyl)carboxamide at 7-position is an important motif for cytotoxicity of 8-((4-aminocyclohexyl)amino-1,6-naphthyridine chemotype.

Select 8-Amino-1,6-naphthyridine-7-carboxamides Arrest Cells in G_0/G_1 and Show Multiple Kinase Inhibitory Activity. 8-Amino-1,6-naphthyridine-7-carboxamide represents a new class of antiproliferative agents and warrants further investigation. Compounds **II-6**, **II-10**, and **II-13** completely blocked colony formation of highly aggressive MDA-MB-435 cancer cells at 10 μM (Figure 4A). The IC_{50} value range for **II-13** in the same cell line was between 0.1 and 1 μM (Figure 4B). **I-18** blocked cells in G_0/G_1 as early as 12 h, and this block persisted till 48 h with 61% of cells in G_0/G_1 versus 36% in control cells (Figure 4C). Similarly, **II-13** arrested the cells in G_0/G_1 (Figure 4D).

So far, starting from L-870810, we established a focused library based on the 1,6-naphthyridine core structure with variations on 5,7,8-substitutions and discovered a new class of antiproliferative agents. Our initial target identification effort was directed at the tyrosine kinases important for cancer progression.

Some representative 5,8-substituted-1,6-naphthyridine-7-carboxamides that were active in our cytotoxicity assays were chosen to test their inhibitory activities against in-house tyrosine kinases including EGFR, erbB-2, c-Src, and KDR (Table 5).

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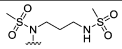
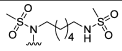
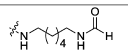
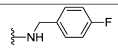
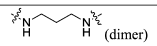
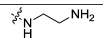
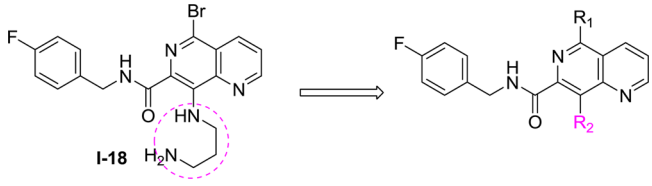
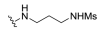
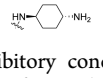
Table 2. Cytotoxicity of the First Generation 8-Substituted-1,6-naphthyridine-7-carboxamide Derivatives

Compd.	R_1	R_2	IC_{50} (μM)[a] MDA-MB-435	HCT116 $p53^{-/-}$	HCT116 $p53^{+/+}$	MCF-7
I-1	Br	OH	15 ± 5	>20	>20	ND[b]
I-2	Br	OMs	>20	>20	>20	ND
I-3	Br	OTs	>20	>20	>20	ND
I-4 (L-870810)		OH	6 ± 3	2	2	2
I-5		OH	>20	7 ± 1	15 ± 3	>20
I-6		OH	20	4 ± 2	3	7 ± 4
I-7		OH	16 ± 1	6 ± 3	4 ± 2	19 ± 1
I-8		OH	13	ND	ND	12
I-9		OH	>20	ND	ND	ND
I-10		OH	>20	ND	ND	ND
I-11		OH	>20	ND	ND	ND
I-12	Br	$-NHCH_3$	>20	>20	>20	ND
I-13	Br		>20	>20	>20	ND
I-14	Br		>20	>20	>20	ND
I-15	Br		>20	>20	>20	ND
I-16	Br		>20	>20	>20	ND
I-17	Br		>20	>20	>20	ND
I-18	Br		**5 ± 2**	**18**	**>20**	**6**
I-19	Br	 (dimer)	>20	>20	>20	ND
I-20	Br		**12 ± 5**	**>20**	**>20**	**6 ± 1**

[a]IC_{50}: Inhibitory concentrations at 50% in μM were measured by MTT assay after 72 h continuous drug exposure. Standard deviations are from three independent experiments run in triplicates. [b]ND: not determined.

Table 3. Cytotoxicity of the Second Generation 8-Amino Substituted-1,6-naphthyridine-7-carboxamide Derivatives with Variation on 8-Amino Substitution



Compd	R_1	R_2	IC_{50} (μM)[a] MDA-MB-435	HCT116 $p53^{-/-}$	HCT116 $p53^{+/+}$	MCF-7	SkBr-3	LNCaP	HT 29
I-18	Br		5 ± 2	18	>20	6	ND[b]	ND	ND
II-1	H		16	>10	>10	>10	>10	>10	>10
II-2	Br		~10	>10	>10	>10	1	5	>10
II-3	Br		11 ± 2	>10	9	>10	1	8	>10
II-4	Br		7 ± 1	>10	>10	7 ± 3	1	8	6 ± 2
II-5	Br		>20	>10	>10	2 ± 1	1	>10	>10
II-6	Br		6	>10	7	2 ± 1	3	8 ± 2	5 ± 2
II-7	Br		7 ± 1	7	~10	4 ± 2	4	7 ± 1	>10
II-8	Br		8 ± 1	>10	>10	8	>10	8 ± 1	>10
II-9	Br		20	9	>10	>10	1	>10	>10
II-10	Br		7 ± 1	6	9.2	3 ± 1	1	6	4 ± 1
II-11	Br		~10	>10	>10	>10	3	>10	>10
II-12	Br		8 ± 3	>10	>10	7 ± 1	6	4.5 ± 0.5	10
II-13	Br		6 ± 1	4	7	2 ± 1	2	5 ± 1	**<1**
II-14			5 ± 1	8.5 ± 2	8 ± 0.2	5	> 10	ND	ND
II-15			5	>10	>10	13	8.5	8 ± 0.5	>10

[a]IC_{50}: Inhibitory concentrations at 50% in μM were measured by MTT assay after 72 h continuous drug exposure. Standard deviations are from three independent experiments run in triplicate. Entries with two values are from two independent experiments run in triplicate. [b]ND: not determined.

The 8-hydroxy-5-amino substituted analogues (**I-4**, **I-7**) were inactive in the kinase assay, whereas some 8-((1,4)-*trans*-4-aminocyclohexyl)amino)-5-bromo-*N*-(substituted-benzyl)-1,6-naphthyridine-7-carboxamide displayed effective inhibition against the EGFR, c-Src, and/or KDR kinases at the concentration of 10 μM (**III-2**, **III-8**, **III-9**). Furthermore, the substitution at the 7-carboxamide was found to significantly affect

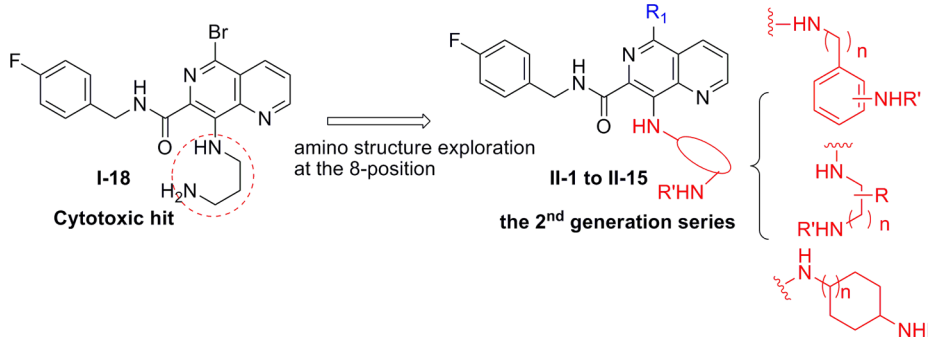


Figure 3. Structural optimization of hit compounds for their cytotoxic property with focus on amino substituent at the position 8.

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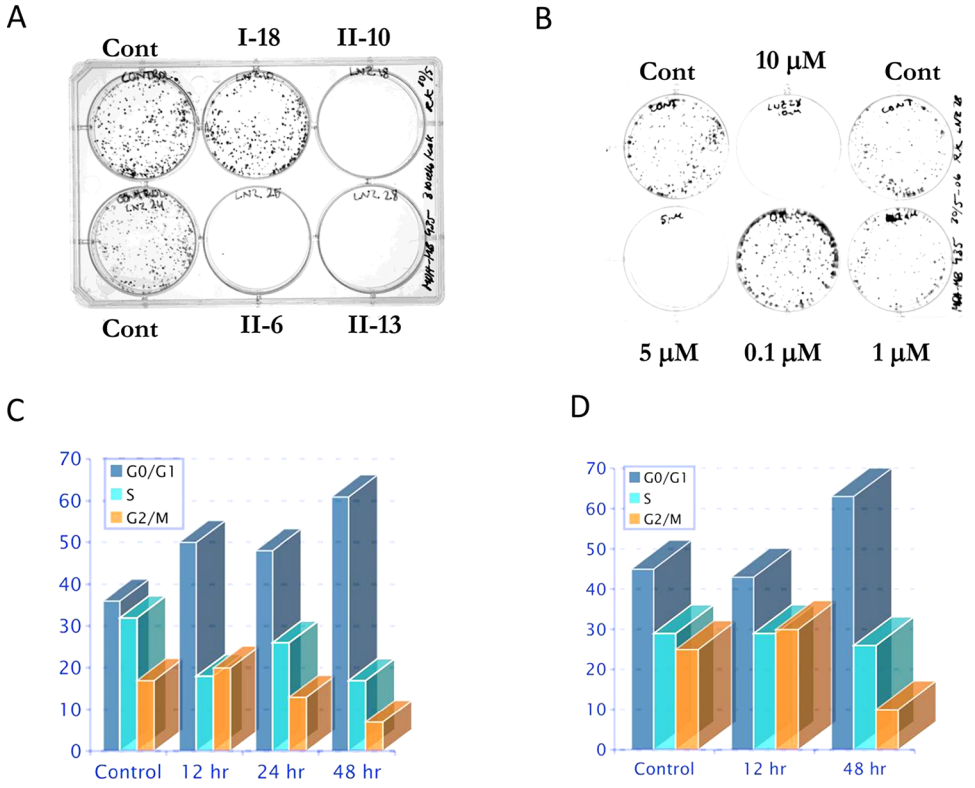

Figure 4. 8-Amino derivatives of 1,6-naphthyridine-7-carboxamide decrease cell proliferation and arrest cells in G_0/G_1 phase. Colony formation assay was performed using highly invasive MDA-MB-435 cells treated continuously for 7 days with **I-18**, **II-6**, **II-10**, and **II-13**, at 10 μM (A) and at various doses with **II-13** (B). DNA content was analyzed in propidium iodide stained MDA-MB-435. Cells were treated with 2 × IC_{50} values of **I-18** (C) and **II-13** (D) at indicated time. Dark-blue bars represent the G_0/G_1 fraction, light-cyan bars represent cells in S phase, and brown bars correspond to cells in the G_2/M phase of the cell cycle.

the kinase inhibitory activity although these 7-variously substituted analogues all displayed marked antiproliferative effect on the tumor cell lines tested. The phenethylamino or anilinyl or 1,4-*trans*-cyclohexyldiamino substitution at the 7-carboxamide portion resulted in no inhibition against these kinases (**III-14**, **III-15**, **III-18**). Therefore, the 8-((1,4)-*trans*-4-aminocyclohexyl)-amino)-5-bromo-*N*-(substituted-benzyl)-1,6-naphthyridine-7-carboxamides are a new class of pan-tyrosine kinase inhibitors. The chloro or alkoxy substituent at 4′- or 2′-position of the benzyl group was beneficial for the kinase inhibition, consistent with the antiproliferative activity in the tumor cells.

On the basis of the promising kinase inhibitory activity results, further kinase profiling assays were conducted against 31 oncology kinases (Figure 5).[36] Three compounds, **III-2**, **III-8**, and **III-9**, which were active in the preliminary tyrosine kinase screening, were selected for the kinase selectivity assay. Interestingly, the 2′-chloro and 2′,4′-dichloro substituted *N*-benzyl-1,6-naphthyridine-7-carboxamide derivatives (**III-8**, **III-9**) displayed significant inhibition against several oncogenic kinases such as IGF-1R, FGFR1, Aurora-A, LCK, and Abl(T315I) at 10 μM, whereas the 4′-methoxy substituted analogue (**III-2**) showed moderate inhibition against KDR, Met, FGFR1, Tie2, and IGF-1R kinases, as shown in Figure 5. Structural variation on the phenyl ring at 7-carboxamide was responsible for kinase selectivity and inhibitory potency. This suggests that the 8-((1,4)-*trans*-4-aminocyclohexyl)amino)-1,6-naphthyridine-7-carboxamide is an attractive scaffold for further structural optimization and development into targeted cancer therapeutics.

CONCLUSIONS

Starting from a well-known 8-hydroxy-[1,6]naphthyridine scaffold, we successfully identified novel IN inhibitors with significant antiviral activities. Furthermore, by varying the substituent at the 8-position, we showed for the first time that a subset of this class of compounds is quite useful for repurposing as innovative cytotoxic agents. The lead compounds disclosed in this study show significant cytotoxicity in a panel of cancer cell lines, arrest cells in G_0/G_1, and inhibit select kinases. Several of these compounds are interesting and warrant further preclinical development, and the results presented in this study validate our previous studies in repurposing select IN inhibitors for anticancer development. In-depth mechanistic studies are currently in progress in our laboratory and will be published in due course.

EXPERIMENTAL SECTION

Materials, Chemicals, and Enzymes. All compounds were dissolved in DMSO, and the stock solutions were stored at −20 °C. The γ-[^{32}P]ATP was purchased from either Amersham Biosciences or ICN. The expression system for the wild-type IN was a generous gift of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH (Bethesda, MD).

Preparation of Oligonucleotide Substrates. The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′, and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′, were purchased from Norris Cancer Center Microsequencing Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3′-processing and strand transfer using 5′-end-labeled substrates, 21top was 5′-end-labeled using T_4 polynucleotide kinase (Epicenter, Madison, WI) and γ-[^{32}P]ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated, and 21bot was added in 1.5 M

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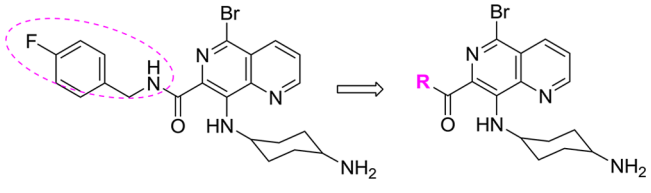
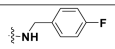
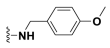
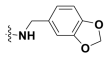
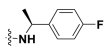
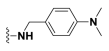
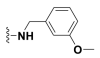
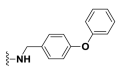
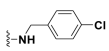
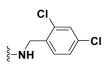
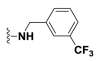
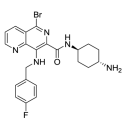
Table 4. Cytotoxicity of the Third Generation 8-Amino Substituted-1,6-naphthyridine-7-carboxamide Derivatives with Variation on the 7-Carboxamide Moiety



Comd.	R	IC$_{50}$ (μM)[a] MDA-MB 435	HEY	SkBr-3	HCT116 p53$^{+/+}$	MCF-7
II-13		6 ± 1	ND	2	7	ND[b]
III-1		7 ± 1	8	8	7 ± 1	ND
III-2		5 ± 1	4	5	3 ± 1	ND
III-3		7 ±1	4	6	7 ± 1	ND
III-4		>10	>10	9	>10	ND
III-5		4	5.3	ND	ND	5
III-6		7 ± 1	7	6	6 ± 1	ND
III-7		4	9	ND	ND	7.9
III-8		1 ± 0.2	1	2	4	ND
III-9		5.8 ± 0.2	5	6	6 ± 1	ND
III-10		4.2	12	ND	ND	15
III-11		>10	>10	10	>10	ND
III-12		4.1	11	ND	ND	7.3
III-13		4	>20	ND	ND	15
III-14		8	6	7	8.8	ND
III-15		1 ± 0.3	1	2	2	ND
III-16		>10	5.2	4.2	>10	5.2
III-17		>20	>20	ND	ND	>20
III-18		6.6 ± 0.4	7.1 ± 0.1	2.8 ± 0.5	6.4 ± 0.2	ND

[a]IC$_{50}$: inhibitory concentrations at 50% in μM were measured by MTT assay after 72 h continuous drug exposure. Standard deviations are from three independent experiments run in triplicate. [b]ND: not determined.

Table 5. Inhibition of EGFR, ErbB2, Src, and KDR Kinase Activities by Selected 5,8-Substituted-1,6-naphthyridine-7-carboxamide Derivatives

	% kinase inhibition at 10 μM for each compound[a]			
compd	EGFR	ErbB2	Src	KDR
I-4	1	8	13	ND[b]
I-7	30	11	23	ND
II-1	10	ND	15	10
II-4	0	ND	17	0
II-6	0	ND	18	0
II-7	7	1	7	8
II-10	5	1	9	0
II-12	0	ND	29	4
II-13	21	7	13	ND
III-1	20	0	ND	ND
III-2	**51**	**0**	**76**	**2**
III-3	0	0	ND	ND
III-6	22	0	ND	ND
III-8	**59**	**0**	**70**	**34**
III-9	**68**	**13**	**90**	**41**
III-14	0	0	ND	ND
III-15	0	0	ND	ND
III-18	5	3	0	ND
Lapatinib[c]	96	92		
PP2[c]			94	
SU11248[c]				76

[a]The inhibitory rate of tyrosine kinase in the presence of the fixed concentration of the drug was detected using enzyme-linked-immunosorbent assay (ELISA) in the presence of 10 μM of ATP. [b]ND: not determined. [c]The reference compound for the kinase assay: Lapatinib for EGFR and ErbB-2, PP2 for Src, SU11248 for KDR, tested at 10 μM.

excess. The mixture was heated at 95 °C, allowed to cool slowly to room temperature, and processed through a spin 25 minicolumn (USA Scientific, Ocala, FL) to separate annealed double-stranded oligonucleotide from unincorporated material.

IN Assays. To determine the extent of 3′-processing and strand transfer, wild-type IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. Then, a 20 nM concentration of the 5′-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional 1 h. Reactions were quenched by the addition of half the sample volume (8 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue). An aliquot was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate, pH 8.3, 2 mM EDTA, 20% acrylamide, and 8 M urea).

Gels were dried, exposed in a PhosphorImager cassette, and analyzed using a Typhoon 8610 variable mode imager (Amersham Biosciences, Piscataway, NJ) and quantitated using ImageQuant 5.2. The percent inhibition (% I) was calculated using the following equation

$$\%I = 100 \times [1 - (D - C)/(N - C)] \quad (1)$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC$_{50}$ values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain the concentration that produced 50% inhibition.

Antiviral Assay.[37] C8166 cells were maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (Gibco). The cells used in all experiments were in log-phase growth.

Syncytium Reduction Assay. In the presence of 100 μL of various concentrations of compounds, C8166 cells (4×10^5 mL^{-1}) were

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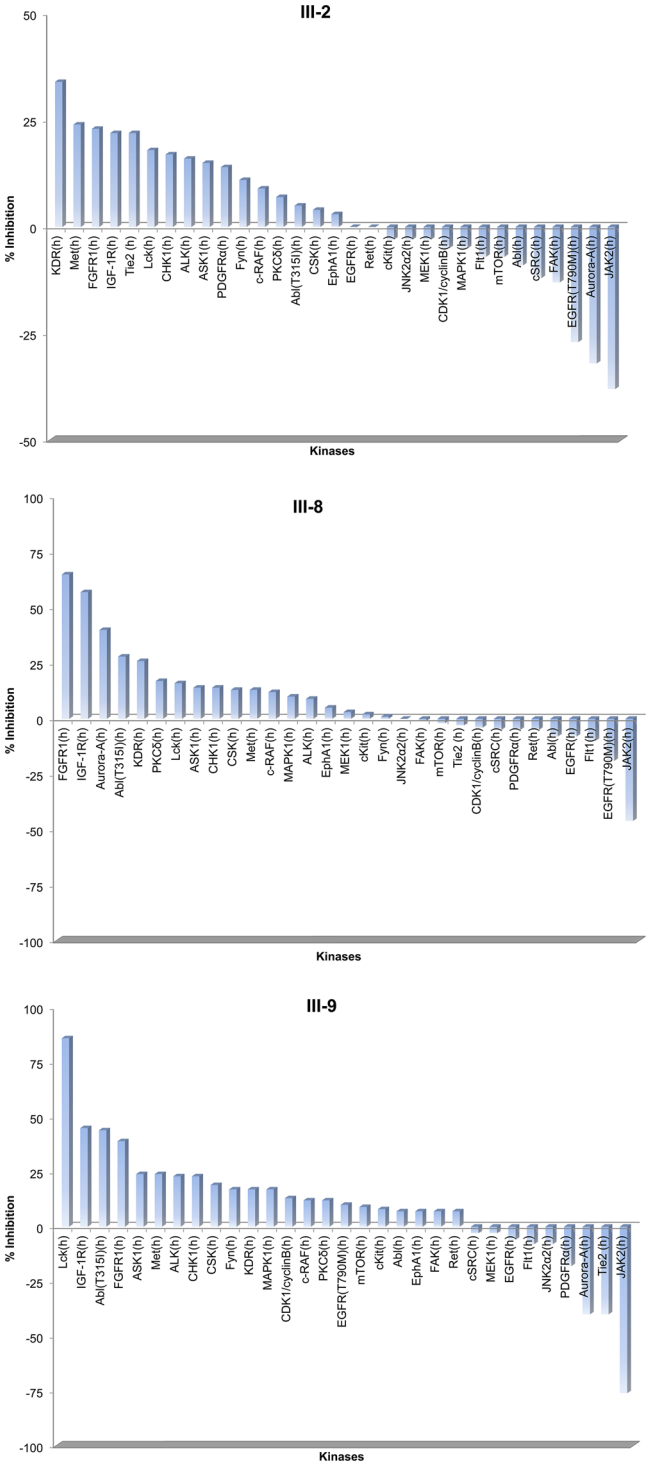

Figure 5. Inhibition rate of compounds **III-2**, **III-8**, and **III-9** against 31 oncogenic kinases at the drug concentration of 10 μM and at the K_m value for ATP for each kinase, by using KinaseProfiler techniques. All data are expressed as mean values of two independent experiments.

infected with HIV-1_{IIIB} at a multiplicity of infection (MOI) of 0.06. The final volume per well was 200 μL. AZT was used as a positive control. After 3 days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell) in each well under an inverted microscope. Percentage inhibition of syncytial cell number in treated culture to that in infected control culture and 50% effective concentration (EC_{50}) was calculated.

Cytotoxicity Assay. : The cellular toxicity of compounds on C8166 cells was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) method. Briefly, cells were seeded on a 96-well microplate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2 for 72 h. The supernatants were discarded, and MTT reagent (5 mg/mL in PBS) was added to each well and then incubated for 4 h and 100 μL of 50% DMF-20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek EL x 800 ELISA reader at 595/630 nm. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from dose−response curve. TI (therapeutic index) value is a ratio of the CC_{50} value/EC_{50} value.

Binding Mechanism Prediction. Given the lack of a full length crystal structure of HIV-1 IN, a homology model was built based on the X-ray crystal structure of prototype foamy virus (PFV) intasome (PDB: 3OYA),[34,35] a retro-lentivirus belonging to the same viral genus as HIV. Select compounds were docked in the catalytic binding site of the homology model of IN reported earlier[38] using GOLD (genetic optimization for ligand docking) software package, version 4.0 (Cambridge Crystallographic Data Centre, Cambridge, U.K.). GOLD uses a genetic algorithm (GA) to explore the conformational space of a compound inside the binding site of a protein.[39,40] The active site was defined as the collection of protein residues with a sphere of 15 Å radius. Before docking different possible stereoisomers, ionized forms and conformations of ligands were prepared by LigPrep (Schrodinger, LLC).[41] Ionization option, Epik,[42] was used to ionize ligands at pH 7.0 ± 0.2. Docking studies were performed using the standard default settings with 100 GA runs on each molecule. For each of the 100 independent GA runs, a maximum of 100000 operations were performed on a set of five groups with a population of 100 individuals. With respect to ligand flexibility special care has been taken by including options such as flipping of ring corners, amides, pyramidal nitrogens, secondary and tertiary amines, and rotation of carboxylate groups, as well as torsion angle distribution and postprocess rotatable bonds as default. The annealing parameters were used as default cutoff values of 3.0 Å for hydrogen bonds and 4.0 Å for van der Waals interactions. Hydrophobic fitting points were calculated to facilitate the correct starting orientation of the compound for docking by placing the hydrophobic atoms appropriately in the corresponding areas of the active site. When the top three solutions attained root-mean-square deviation (rmsd) values within 1.5 Å, docking was terminated. GOLD-Score, a scoring function of the software, is a dimensionless fitness value that takes into account the intra- and intermolecular hydrogen bonding interaction energy, van der Waals energy, and ligand torsion energy.[39,40]

Cell Culture. MDA-MB-435 and MCF7 breast cancer, LNCaP prostate cancer, and HT29 colon cancer cell lines were obtained from Dr. Alan Epstein (USC) or purchased from the American Type Cell Culture (Manassas, VA). HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ colon cancer cell lines were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). Cell lines were maintained in the appropriate growth media (DMEM for MDA-MB-435, MCF7 and LNCaP, RPMI for the HT29 and HCT116 cell lines) containing 10% heat-inactivated fetal bovine serum and supplemented with 2 mM L-gutamine at 37 °C in a humidified atmosphere of 5% CO_2. For subculture and experiments, cells were washed with 1× PBS, detached using 0.025% Trypsin-EDTA (Cellgro, Mediatech, Mannassas, VA), collected in growth media, and centrifuged. All experiments were performed in growth media using subconfluent cells in the exponential growth phase. For use in tissue culture experiments, compounds were prepared at 10 mM concentration in sterile dimethylsulfoxide (DMSO) (EMD Chemicals, Gibbstown, NJ) and stored at −20 °C when not in use.

Cytotoxicity Assay. Cytotoxicity was assessed by MTT assay as previously described.[43] Cells were seeded in 96-well tissue culture treated dishes and allowed to adhere overnight. Cells were subsequently treated with a continuous exposure to drugs for 72 h. An MTT solution was added to each well to give a final concentration of 0.3 mg/mL MTT. Cells were incubated with MTT for 3−4 h at 37 °C. After removal of the supernatant, DMSO was added and the absorbance was read at 570 nm. All assays were done in triplicate. The IC_{50} was then determined for each drug from a plot of log (drug concentration) versus percentage of cell kill.

dx.doi.org/10.1021/jm300667v | J. Med. Chem. 2012, 55, 9492−9509

Colony Formation Assay. Colony formation assay was performed as previously described to further assess drug toxicity.[44] Briefly, cells were seeded in 6-well tissue culture dishes at a density of 500 cells per well in growth media and allowed to adhere overnight. Cells were subsequently treated with varying concentrations of compound for 24 h. Following treatment, monolayers were washed with 1× PBS and incubated in growth media for a period of 7−10 days, allowing sufficient time for colonies to form in control wells. To visualize the extent of colony formation, cells were fixed and stained in a 2% solution of crystal violet containing 1% glutaraldehyde. Excess stain was removed through multiple washes in distilled water and allowed to air-dry. Stained plates were imaged using Quantity One software running on the VersaDoc imaging platform (BioRAD).

Cell Cycle Analysis. Cells were seeded in 100 mm tissue culture dishes at a density of 1×10^6 cells/plate in growth media and allowed to adhere overnight. The following day cells were treated with $2 \times IC_{50}$ concentrations of tested compounds or DMSO alone as vehicle control for 12−48 h. After treatment, cells were detached with trypsin, and both media and cells were collected by centrifugation. Cells were washed and resuspended in 1× PBS prior to fixation in ethanol overnight at −20 °C. Fixed cells were treated with 10 μg/mL RNase A (Sigma-Aldrich, St. Louis, MO) and stained in a 50 μg/mL solution of propidium iodide (Sigma-Aldrich, St. Louis, MO). DNA content was determined by flow cytometry using the BD LSR II (BD Biosciences, San Jose, CA) equipped with a 488 nM Sapphire argon-ion laser and PE emission detector.

Tyrosine Kinase Assay. The activity of tyrosine kinases were detected using enzyme-linked-immunosorbent assay (ELISA). Briefly, 20 μg/mL Poly(Glu,Tyr)$_{4:1}$ (Sigma, St. Louis, MO) was precoated in 96-well ELISA plates as substrate. Each well was treated with 50 μL of 10 μmol/L ATP solution, which was diluted in kinase reaction buffer (50 mM HEPES pH 7.4, 20 mM $MgCl_2$, 0.1 mM $MnCl_2$, 0.2 mM Na_3VO_4, 1 mM DTT). Then 1 μL of various concentrations of test compounds or reference compound dissolved in DMSO were added to each reaction well. Experiments at each concentration were performed in duplicate. The reaction was initiated by adding tyrosine kinase diluted in kinase reaction buffer. After incubation at 37 °C for 60 min, the wells were washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Next, 100 μL of anti-phosphotyrosine (PY99) antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) diluted in T-PBS containing 5 mg/mL BSA was added, and the plate was incubated at 37 °C for 30 min. After the plate was washed three times, 100 μL of horseradish peroxidase-conjugated goat antimouse IgG (1:2000, Calbiochem, SanDiego, CA) diluted in T-PBS containing 5 mg/mL BSA was added and the plate was incubated at 37 °C for 30 min. The plate was washed, and then 100 μL of citrate buffer (0.1 M, pH5.5) containing 0.03% H_2O_2 and 2 mg/mL *o*-phenylenediamine was added and samples were incubated at room temperature until color emerged. The reaction was terminated by adding 50 μL of 2 M H_2SO_4, and the plate was read using a multiwell spectrophotometer (VERSAmax, Molecular Devices, Sunnyvale, CA, USA) at 492 nm. The inhibitory rate (%) was calculated with the formula: $[1 - (A_{492}\ \text{treated}/A_{492}\ \text{control})] \times 100\%$.

General Synthetic Methods. Unless otherwise stated, the ^{1}H NMR spectra were recorded on a Varian 300 MHz or 400 MHz spectrometer and ^{13}C NMR spectra on a Varian Mercury 100 MHz spectrometer. The data are reported in parts per million relative to TMS and referenced to the solvent. Melting points (uncorrected) were determined on a Buchi-510 capillary apparatus. The MS and HRMS (ESI) spectra were obtained on an APEXIII 7.0 T FTMS mass spectrometer. Rotate power was detected by P-1030 (A012360639) automatic polarimeter. The flash column chromatography was performed on silica gel H (10−40 μm). Anhydrous solvents were obtained by standard procedures. The purity of all target compounds was ≥95% determined by elemental analysis or analytical HPLC. Elemental analyses were obtained using Elementar Vario EL II elemental analyzer. Analytical HPLC was performed on JASCO LC-1500 with Vydac C18 reversed-phase column (10 mm × 250 mm) and UV detector at 254 nm, using two solvent systems as indicated below.

Solvent system I: A, 0.05% TFA in water; B, methanol, gradient 30−90% of solvent B in A over 20 min at 2 mL/min;

Solvent system II: A, 0.05% TFA in water; C, 0.05% TFA in 90% acetonitrile in water, gradient 30−90% C in A over 20 min at 2 mL/min.

Pyridine-2,3-dicarboxylic Acid 2-Isopropyl Ester (2). A suspension of quinolinic anhydride (48.1 g, 0.32 mol) in 100 mL of 2-propanol was warmed to reflux for 16 h, cooled to room temperature, and concentrated in vacuo. The resulting yellow solid was recrystallized from ethyl acetate to give the desired monoester **2** as a white solid (52.7 g, 78.3%); mp 140−141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.87 (d, J = 4.2 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 7.55 (dd, J = 5.1, 8.1 Hz, 1 H), 5.36 (septet, J = 6.3 Hz, 1H), 1.41 (d, J = 6.3 Hz, 6H).

3-Hydroxymethyl-pyridine-2-carboxylic Acid Isopropyl Ester (4). To a solution of **2** (5.0 g, 23.9 mmol) in 25 mL of DCM was added thionyl chloride (2.5 mL, 35.85 mmol) and a catalytic amount of DMF (0.5 mL, 0.53 mmol). The suspension mixture was heated to reflux for 3 h and then concentrated in vacuum. The remaining thionyl chloride was removed by dissolving the residue in THF and then concentrating the resultant solution. This process was repeated twice. The resulting chloride was dissolved in 20 mL of THF and cooled to 0 °C. Sodium borohydride (1.2 g, 31.58 mmol) was added, and the reaction mixture was stirred at 0 °C for 40−50 min. The reaction solution was carefully poured into ice (foaming) and extracted with CH_2C1_2. The organic phase was dried over Na_2SO_4 and concentrated in vacuum. Purification by flash chromatography gave **4** as a yellow oil. (3.4 g, overall yield 73% for two steps). ^{1}H NMR ($CDC1_3$, 300 MHz) δ 8.69 (dd, J = 1.5, 4.7 Hz, 1H), 7.88 (dd, J = 1.5, 7.7 Hz, 1H), 7.46 (dd, J = 4.7, 7.8 Hz, 1 H), 5.35 (septet, J = 6.4 Hz, 1H), 4.81 (m, 2 H), 1.45 (d, J = 6.3 Hz, 6H). EI-MS m/z: 195 $(M)^+$.

Isopropyl 3-((*N*-(2-Methoxy-2-oxoethyl)-4-methylphenylsulfonamido)methyl)picolinate (5). Isopropyl 3-(hydroxymethyl) pyridine-2-carboxylate (1.734 g, 8.89 mmol), methyl 2-(tosylamino)acetate (2.163 g, 8.89 mmol), anhydrous $MgSO_4$ (1.066 g, 8.89 mmol), and triphenylphosphine (3.499 g, 13.338 mmol) were dissolved in dry THF (100 mL) and cooled to 0 °C under nitrogen. DEAD (2.165 mL, 13.338 mmol) in 10 mL of dry THF was added dropwise. The ice bath was removed, and the solution was stirred for 2 h at rt followed by concentration in vacuo. The red−orange oil was directly used for the next reaction without further purification.

Methyl 8-Hydroxy-1,6-naphthyridine-7-carboxylate (6). To the solution of the crude product **5** (8.89 mmol) in dry methanol (50 mL) at 0 °C was added sodium methoxide (1.681 g, 31.123 mmol) slowly. After addition, the reaction mixture was stirred at rt for 3 h. The solvent was removed in vacuo to obtain red−orange oil, which was partitioned between water (20 mL) and ethyl acetate (20 mL). The organic layer was back-extracted with saturated sodium bicarbonate solution. The pH of the aqueous layer was adjusted to 7, and then the aqueous layer was extracted with methylene chloride. The organic layer was dried over sodium sulfate. Purification by flash chromatography afforded **6** as an off-white solid. (1.19 g, 65% two steps); mp 179−180 °C. ^{1}H NMR ($CDC1_3$, 300 MHz): δ 11.79 (s, 1H), 9.20 (s, 1H), 8.85 (s, 1H), 8.32 (d, J = 8.2 Hz, 1H), 7.71 (dd, J = 4.1, 8.2 Hz, 1H), 4.12 (s, 3H).

Methyl 5-Bromo-8-hydroxy-1,6-naphthyridine-7-carboxylate (7). To a solution of **6** (34 mg, 0.167 mmol) in CH_2Cl_2 (1 mL) at room temperature was added NBS (30 mg, 0.167 mmol). The reaction mixture was stirred for 1 h. The solids were collected by filtration and dried in vacuo to afford **7** (30 mg, yield 64%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.26 (dd, J = 1.5, 4.2 Hz, 1H), 8.59 (dd, J = 1.6, 8.4 Hz, 1H), 8.00 (dd, J = 4.2, 8.4 Hz, 1 H), 3.94 (s, 3 H).

5-Bromo-8-hydroxy-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-1). A slurry of **7** (25 mg, 0.0883 mmol) and 4-fluorobenzylamine (12 mg, 0.0883 mmol) in toluene (2 mL) were heated at reflux for 20 h. Upon cooling to rt, the resulting solids were collected by filtration and washed with methanol (0.5 mL) to afford **I-1** as a white solid (yield 78%); mp 178−180 °C. ^{1}H NMR (300 MHz, $CDC1_3$): δ 13.31 (s, 1H), 9.20 (dd, J = 1.4, 4.2 Hz, 1H), 8.54 (dd, J = 1.4, 8.8 Hz, 1H), 8.17 (m, 1H), 7.74 (dd, J = 4.4, 8.6 Hz, 1H), 7.38 (dd, J = 5.4, 8.4 Hz, 2H), 7.07 (t, J = 8.4 Hz, 2H), 4.67 (d, J = 6.3 Hz, 2H). EI-MS m/z: 375 $(M)^+$, 377 $(M + 2)^+$. Anal. Calcd for

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$C_{16}H_{11}BrFN_3O_2$: C 51.08, H 2.95, N 11.17. Found: C 51.45, H 3.05, N 11.03.

Toluene-4-sulfonic Acid 5-Bromo-7-(4-fluoro-benzylcarbamoyl)-1,6-naphthyridin-8-yl Ester (I-3). *p*-Toluenesulfonyl chloride (268 mg,1.4 mmol) was added to a solution of **I-1** (283 mg, 0.75 mmol) and triethylamine (161.92 mg, 1.6 mmol) in chloroform (5 mL) over 10 min in 50 °C.The solution was stirred for 5 h and then washed by saturated aqueous NH_4Cl and brine and dried over Na_2SO_4, and the solution was removed under vacuum. Purification by flash chromatography (DCM:methanol = 40:1) to give **I-3** as a white solid. (360 mg, 91%); mp 152−154 °C. ^{1}H NMR ($CDCl_3$): δ 9.03 (dd, 1H, J = 1.5, 4.2 Hz), 8.58 (dd, 1H, J = 1.5, 8.7 Hz), 7.96 (m, 1H), 7.94 (d, 2H, J = 8.1 Hz), 7.69 (dd, 1H, J = 4.2, 8.7 Hz), 7.36 (m, 2H), 7.32 (d, 2H, J = 8.1 Hz), 7.04 (t, 2H, J = 8.7 Hz), 4.60 (d, J = 6.6 Hz, 2H), 2.47 (s, 3H). EI-MS m/z: 530 $(M + 1)^+$, 532 $(M + 3)^+$. Anal. Calcd for $C_{24}H_{20}BrFN_4O$: C 52.09, H 3.23, N 7.92. Found: C 51.94, H 3.17, N 7.94.

7-(4-Fluorobenzylcarbamoyl)-5-bromo-1,6-naphthyridin-8-yl Methanesulfonate (I-2). **I-2** was prepared according to the same procedure described for **I-3**. White solid, yield 83%; mp 150−152 °C. ^{1}H NMR (300 MHz, $CDC1_3$) δ 9.32 (d, J = 4.2 Hz, 1H), 8.62 (d, J = 8.7 Hz, 1H), 8.22 (m, 1H), 7.79 (dd, J = 4.2, 9.0 Hz, 1H), 7.38 (m, 2H), 7.05 (t, J = 8.4 Hz, 1H), 4.68 (d, J = 5.7 Hz, 2H), 3.70 (s, 1H). EI-MS m/z: 454 $(M + 1)^+$, 456 $(M + 3)^+$. Anal. Calcd. for $C_{17}H_{13}BrFN_3O_4S$·AcOEt: C 46.50, H 3.90, N 7.75. Found: C 47.44, H 3.75, N 8.10.

5-(1,1-Dioxido-1,2-thiazinan-2-yl)-*N*-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (I-4). **I-4** was prepared according to the same procedure described in patent WO 2002030931 A2, giving a yellow solid, yield 65%. ^{1}H NMR (300 MHz, $CDC1_3$) δ 13.34 (br, 1H), 9.19 (dd, J = 1.5, 4.2 Hz, 1H), 8.61 (d, J = 1.5, 8.7 Hz, 1H), 7.96 (m, 1H), 7.69 (dd, J = 4.8, 8.7 Hz, 1H), 7.37 (m, 2H), 7.07 (t, J = 8.6 Hz, 2H), 4.67 (m, 2H), 3.93 (m, 2H), 3.30 (t, J = 6.3 Hz, 2H), 2.45−2.36 (m, 2H), 2.18−2.15 (m, 1H), 1.85−1.82 (m, 1H). EI-MS m/z: 430 $(M)^+$. Anal. HPLC t_R = 17.04 min, 100% (solvent system I); t_R = 12.35 min, 99.45% (solvent system II).

***N*-(4-Fluorobenzyl)-8-hydroxy-5-(*N*-(3-(methylsulfonamido)-propyl)methylsulfonamido)-1,6-naphthyridine-7-carboxamid (I-5).** *N*-(3-Methanesulfonylamino-propyl)-methanesulfonamide (115 mg, 0.5 mmol) was added to a solution of **I-1** (187.5 mg, 0.5 mmol) and cuprous oxide (86 mg, 0.6 mmol) in pyridine (5 mL). After refluxing for 10 h, the solution was evaporated at vacuum, diluted with dichloromethane, and then filtered to remove the solids. The filtrate was stirred with a slurry of EDTA (400 mg) in water (50 mL). After 16 h, the dichloromethane extracts were dried over Na_2SO_4. After filtering, the solution was concentrated under vacuum. The residue was purified by flash chromatography on silica gel (DCM:methanol = 50:1) to give **I-5** as a yellow solid, yield 54%; mp 205−208 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 9.13 (d, J = 3.3 Hz, 1H), 8.80 (d, J = 7.8 Hz, 1H), 7.86 (dd, J = 3.6, 8.7 Hz, 1H), 7.44 (m, 2H), 7.07 (t, J = 9.0 Hz, 2H), 4.70 (s, 2H), 4.05 (m, 2H), 3.10 (m, 2H), 3.08 (s, 3H), 2.90 (s, 3H), 2.08−1.94 (m, 2H). EI-MS m/z: 525 $(M)^+$. Anal. HPLC t_R = 14.54 min, 100% (solvent system I); t_R = 10.84 min, 99.79% (solvent system II).

***N*-(4-Fluorobenzyl)-8-hydroxy-5-(*N*-(6-(methylsulfonamido)-hexyl)methylsulfonamido)-1,6-naphthyridine-7-carboxamide (I-6).** **I-6** was prepared according to the same procedure described for **I-5**, giving a yellow solid, yield 35%; mp 178−182 °C. ^{1}H NMR (300 MHz, $CDC1_3$) δ 13.51 (br, 1H), 9.20 (dd, J = 1.5, 4.2 Hz, 1H), 8.69 (d, J = 1.5, 8.7 Hz, 1H), 8.01 (t, J = 6.0 Hz, 1H), 7.73 (dd, J = 4.5, 8.4 Hz, 1H), 7.36 (m, 2H), 7.05 (t, J = 8.6 Hz, 2H), 4.69 (d, J = 6.0 Hz, 2H), 4.36 (m, 1H), 3.81 (t, J = 6.9 Hz, 2H), 3.10 (m, 2H), 3.01 (s, 3H), 2.91 (s, 3H), 1.5−1.2 (m, 8H). EI-MS m/z: 567 $(M)^+$. Anal. HPLC t_R = 16.117 min, 98.56% (solvent system I); t_R = 6.192 min, 96.20% (solvent system II).

***N*-(4-Fluorobenzyl)-5-((6-formamidohexyl)amino)-8-hydroxy-1,6-naphthyridine-7-carboxamide (I-7).** Hexane-1,3-diamine (167 mg, 1.44 mmol) was added to a solution of **I-1** (100 mg, 0.27 mmol) in DMPU (5 mL). After the solution was heated under 135 °C for 48 h, the solution was cooled to room temperature and the usual workup was performed. Purification by RP-HPLC (Vydac C18 column (10 mm × 250 mm), t_R = 19 min, gradient (water:nitrile = 70:30 to 35:65 over 30 min, with 0.05% TFA, UV detector, 254 nm) gave **I-7** as a red solid, yield 30%; mp 88−91 °C. ^{1}H NMR (300 MHz, $CDC1_3$) δ 12.31 (s, 1H), 9.06 (d, J = 4.2 Hz, 1H), 8.27 (m, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.15 (s, 1H), 7.51 (dd, J = 5.4, 8.6 Hz, 1H), 7.35 (m, 2H), 7.04 (t, J = 8.6 Hz, 2H), 4.65 (d, J = 6.2 Hz, 2H). EI-MS m/z: 439 $(M)^+$, ESI-MS m/z: 440 $(M + 1)^+$. Analytical HPLC t_R = 5.650 min, 100% (solvent system I); t_R = 13.500 min, 98.36% (solvent system II).

5-(2-(2*H*-Indol-3-yl)ethylamino)-*N*-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (I-8). 2-(1*H*-Indol-3-yl)-ethanamine (240 mg, 1.5 mmol) was added to a solution of **I-1** (112.5 mg, 0.3 mmol) in DMPU (5 mL). After heating at 135 °C for 48 h, the solution was cooled to room temperature, diluted with dichloromethane, and then washed by saturated aqueous NH_4Cl and brine. Usual workup followed by purification by flash chromatography on silica gel (DCM:methanol = 100:1) afforded **I-8** as a red solid, yield 38%; mp 101−103 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.35 (s, 1H), 9.05 (dd, 1H, J = 1.5, 4.2 Hz), 8.64 (s, 1H), 8.25 (m, 1H), 7.96 (d, 1H, J = 8.4 Hz), 7.60 (d, 1H, J = 7.8 Hz), 7.45−7.30 (m, 4H), 7.17−6.98 (m, 5H), 5.25 (brs, 1H), 3.79 (m, 2H), 3.16 (t, 2H, J = 6.9 Hz). EI-MS m/z: 455 $(M)^+$. Anal. HPLC t_R = 19.692 min, 100% (solvent system I); t_R = 13.667 min, 98.97% (solvent system II).

***N*-(4-Fluorobenzyl)-8-hydroxy-5-(2-hydroxyethylamino)-1,6-naphthyridine-7-carboxamide (I-9).** **I-1** (37.5 mg, 0.13 mmol) was added to 2-aminoethanol, then heated to 130 °C for 9 h, and the solution was cooled to room temperature, diluted with dichloromethane, and then washed by saturated aqueous NH_4Cl and brine and dried over Na_2SO_4, and then the solution was removed under vacuum. The residue was purified by RP-HPLC (Vydac C18 column (10 mm × 250 mm), R_t = 7.35 min, gradient (water:nitrile = 70:30 to 10:90 over 30 min, with 0.05% TFA, UV detector, 254 nm), giving **I-9** as a yellow solid, yield 51%; mp 121−124 °C. ^{1}H NMR (300 MHz, CD_3COCD_3) δ 9.08 (m, 1H), 8.72 (m, 1H), 7.67(m, 1H), 7.45 (m, 2H), 7.09 (t, J = 8.1 Hz, 2H), 4.63 (s, 2H), 3.68 (m, 2H), 3.58 (m, 2H). EI-MS m/z: 356 $(M)^+$. Anal. HPLC t_R = 14.483 min, 100% (solvent system I); t_R = 9.95 min, 97.43% (solvent system II).

(*S*)-*N*-(4-Fluorobenzyl)-8-hydroxy-5-((1-hydroxypropan-2-yl)amino)-1,6-naphthyridine-7-carboxamide (I-10). **I-10** was prepared according to the same procedure described for **I-9**, giving a yellow solid, yield 41%; mp 117−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (d, J = 7.5 Hz, 1H), 9.07 (d, J = 3.9 Hz, 1H), 8.13 (m, 1H), 7.73 (dd, J = 3.9, 7.5 Hz, 1H), 7.32 (m, 2H), 7.02 (t, J = 8.4 Hz, 2H), 4.64 (d, J = 5.7 Hz, 2H), 4.16 (m, 1H), 3.96 (d, J = 4.8 Hz, 2H), 1.39 (d, J = 6.0 Hz, 3H). EI-MS m/z: 370 $(M)^+$. HPLC t_R = 15.317 min, 100% (solvent system I); t_R = 10.258 min, 95.61% (solvent system II); $[\alpha]_D^{22}$ = +10.2° (c = 2.4200 g/100 mL, EtOH).

(*R*)-*N*-(4-Fluorobenzyl)-8-hydroxy-5-((1-hydroxypropan-2-yl)-amino)-1,6-naphthyridine-7-carboxamide (I-11). **I-11** was prepared according to the same procedure described for **I-9**, giving a yellow solid, yield 33%; mp 115−118 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (d, J = 7.5 Hz, 1H), 9.07 (d, J = 3.9 Hz, 1H), 8.13 (m, 1H), 7.73 (dd, J = 3.9, 7.5 Hz,1H), 7.32 (m, 2H), 7.02 (t, J = 8.4 Hz, 2H), 4.64 (d, J = 5.7 Hz, 2H), 4.16 (m, 1H), 3.96 (d, J = 4.8 Hz, 2H), 1.39 (d, J = 6.0 Hz, 3H). EI-MS m/z: 370 $(M)^+$. HPLC t_R = 15.808 min, 100% (solvent system I); t_R = 10.108 min, 98.94% (solvent system II); $[\alpha]_D^{22}$ = −20.1° (c = 2.1050 g/100 mL, EtOH).

5-Bromo-8-methylamino-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-12). Methylamine (5 mL) in alcohol was added to a solution of **I-3** (26 mg, 0.05 mmol) and triethylamine (15 mg, 0.15 mmol) in THF (5 mL). After refluxing for 8 h, the solution was evaporated at vacuum, and the residue was diluted with dichloromethane, then washed by saturated aqueous Na_2CO_3, saturated aqueous NH_4Cl, and brine, respectively. Usual workup and purification by flash chromatography on silica gel (petroleum:ethyl acetate = 8:1) gave **I-12** as a yellow solid, yield 52%; mp: 136−140 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.92 (d, 1H, J = 3 Hz), 8.40 (d, 1H, J = 8.4 Hz), 8.28 (m,1H), 7.57 (dd, 1H, J = 4.5,8.4 Hz), 7.34 (m, 2H), 7.03 (t, 2H, J = 8.5 Hz), 4.59 (d, 2H, J = 6.0 Hz), 3.61 (s, 3H). EI-MS m/z: 388 $(M - 1)^+$, 390 $(M + 1)^+$. Anal. HPLC t_R = 21.750 min, 100% (solvent system I); t_R = 16.758 min, 99.57% (solvent system II).

5-Bromo-8-(2-hydroxy-ethylamino)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-13). **I-13** was prepared according to the same procedure described for **I-12**, giving a yellow solid, yield 54%; mp 103−105 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.92 (d, 1H, J = 2.4 Hz), 8.46 (d, 1H, J = 8.4 Hz), 8.34 (m, 1H), 7.62 (dd, 1H, J = 4.5, 8.4 Hz), 7.34 (m, 2H), 7.03 (t, 2H, J = 8.5 Hz), 4.60 (d, 2H, J = 6.3 Hz), 4.23 (t, 2H, J = 4.8 Hz), 3.94 (t, 2H, J = 4.8 Hz). EI-MS m/z: 418 $(M)^+$, 420 $(M + 2)^+$. Anal. HPLC t_R = 19.575 min, 100% (solvent system I); t_R = 6.517 min, 100% (solvent system II).

8-Benzylamino-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-14). **I-14** was prepared according to the same procedure described for **I-12**, giving a green−yellow solid, yield 57%; mp 142−144 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.94 (d, 1H, J = 4.8 Hz), 8.41 (d, 1H, J = 8.1 Hz), 8.31 (m, 1H),7.59 (dd, 1H, J = 4.5, 8.7 Hz), 7.40−7.19 (m, 7H), 7.04 (t, 2H, J = 8.7 Hz), 5.40 (s, 2H), 4.60 (d, 2H, J = 6.3 Hz). Anal. HPLC t_R = 23.867 min, 100% (solvent system I); t_R = 19.550 min, 100% (solvent system II).

5-Bromo-8-((*S*)-1-phenyl-ethylamino)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-15). **I-15** was prepared according to the same procedure described for **I-12**, giving a yellow solid, yield 64%; mp 102−104 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.88 (d, 1H, J = 4.2 Hz), 8.33 (d, 1H, J = 8.4 Hz), 8.33 (m, 1H),7.51 (dd, 1H, J = 4.2, 8.4 Hz), 7.40−7.35 (m, 4H), 7.21 (t, 2H, J = 7.5 Hz), 7.12 (d, 1H, J = 7.2 Hz), 7.05 (t, 2H, J = 8.7 Hz), 6.45 (q, 1H, J = 6.9 Hz), 4.69−4.61 (m, 2H), 1.65 (d, 3H, J = 6.9 Hz). Anal. HPLC t_R = 23.800 min, 100% (solvent system I); t_R = 18.608 min, 96.93% (solvent system II).

5-Bromo-8-((*R*)-1-phenyl-ethylamino)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-16). **I-16** was prepared according to the same procedure described for **I-12**, giving a green−yellow solid, yield 55%; mp 107−108 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.88 (d, 1H, J = 4.2 Hz), 8.33 (d, 1H, J = 8.4 Hz), 8.33 (m, 1H),7.51 (dd, 1H, J = 4.2, 8.4 Hz), 7.40−7.35 (m, 4H), 7.21 (t, 2H, J = 7.5 Hz), 7.12 (d, 1H, J = 7.2 Hz), 7.05 (t, 2H, J = 8.7 Hz), 6.45 (q, 1H, J = 6.9 Hz), 4.69−4.61 (m,2H), 1.65 (d, 3H, J = 6.9 Hz). EI-MS m/z: 478 $(M)^+$, 480 $(M + 2)^+$. Anal. HPLC t_R = 23.983 min, 96.18% (solvent system I); t_R = 18.967 min, 100% (solvent system II).

5-Bromo-*N*-(4-fluorobenzyl)-8-((4-fluorobenzyl)amino)-1,6-naphthyridine-7-carboxamide (I-17). **I-17** was prepared according to the same procedure described for **I-12**, giving a yellow solid, yield 68%; mp 136−138 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.93 (dd, 1H, J = 1.5, 4.2 Hz), 8.42 (dd, 1H, J = 1.5, 8.4 Hz), 8.33 (m, 1H), 7.59 (dd, 1H, J = 4.2, 8.4 Hz), 7.38−7.32 (m, 4H), 7.04 (t, 2H, J = 8.4 Hz), 6.96 (t, 2H, J = 8.4 Hz), 5.35 (s, 2H), 4.60 (d, 2H, J = 6.6 Hz). EI-MS m/z: 482 $(M)^+$, 484 $(M + 2)^+$. Anal. HPLC t_R = 24.492 min, 100% (solvent system I); t_R = 19.458 min, 100% (solvent system II).

8-(3-Amino-propylamino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-18). Propane-1,3-diamine (22 mg, 0.3 mmol) was added to a solution of **I-3** (53 mg, 0.1 mmol) and triethylamine (30 mg, 0.3 mmol) in THF (5 mL). After refluxing for 8 h, the solution was evaporated at vacuum, and the residue was diluted with dichloromethane, washed by saturated aqueous Na_2CO_3, saturated aqueous NH_4Cl, and brine, and dried over Na_2SO_4. The filtrate was concentrated under vacuum. Purification by flash chromatography on basic aluminum oxide (DCM:methanol = 50:1) afforded **I-18** as a yellow solid, yield 58%; mp 78−80 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.60 (t, 1H, J = 5.4 Hz), 8.97 (d, 1H, J = 3 Hz), 8.42 (d, 1H, J = 8.4 Hz), 8.32 (t, 1H, J = 6.0 Hz), 7.60 (dd, 1H, J = 4.5, 8.4 Hz), 7.36 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 4.60 (d, 2H, J = 6.6 Hz), 4.19 (q, 2H, J = 6.3 Hz), 3.96 (t, 2H, J = 6.6 Hz), 1.93 (m, 2H). EI-MS m/z: 431$(M)^+$, 433 $(M + 2)^+$. Anal. HPLC t_R = 19.083 min, 100% (solvent system I); t_R = 12.928 min, 100% (solvent system II).

8,8′-(Propane-1,3-diylbis(azanediyl))bis(5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide) (I-19). **I-19** was prepared according to the same procedure described for **I-12**, giving a yellow solid, yield 75%; mp 169−171 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.72 (2H, brs), 8.82 (dd, 2H, J = 1.8, 4.2 Hz), 8.35 (dd, 2H, J = 1.8, 8.4 Hz), 8.22 (m, 2H), 7.50 (dd, 2H, J = 4.2, 8.4 Hz), 7.35 (m, 4H), 7.03 (t, 4H, J = 8.5 Hz), 4.57 (d, 4H, J = 6.0 Hz), 4.35 (t, 4H, J = 6.6 Hz), 2.15 (t, 2H, J = 6.6 Hz). EI-MS m/z: 788 $(M)^+$, 790 $(M + 2)^+$. Anal. HPLC t_R = 25.642 min, 95.27% (solvent system I); t_R = 5.933 min, 100% (solvent system II).

8-((2-Aminoethyl)amino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (I-20). **I-20** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 50%; mp 82−84 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.73 (m, 1H), 8.94 (d, 1H, J = 3 Hz), 8.40 (d, 1H, J = 8.4 Hz), 8.30 (m, 1H), 7.57 (dd, 1H, J = 4.5, 8.4 Hz), 7.33 (m, 2H), 7.02 (t, 2H, J = 8.4 Hz), 4.59 (d, 2H, J = 6.6 Hz), 4.19 (q, 2H, J = 6.3 Hz), 3.09 (t, 2H, J = 6.3 Hz). EI-MS m/z: 417 $(M)^+$, 419 $(M + 2)^+$. Anal. HPLC t_R = 19.475 min, 100% (solvent system I); t_R = 11.792 min, 99.29% (solvent system II).

8-((3-Aminopropyl)amino)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-1). **II-1** was prepared according to the same procedure described for **I-18**, giving a light-yellow solid, yield 73%; mp 62−64 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.59 (d, 1H, J = 7.5 Hz), 8.91 (dd, 1H, J = 1.5, 3.9 Hz), 8.62 (t, 1H, J = 6.3 Hz), 8.19 (s, 1H), 8.08 (dd, 1H, J = 1.5, 8.4 Hz), 7.48 (dd, 1H, J = 4.2, 8.4 Hz), 7.34 (m, 2H), 7.02 (t, 2H, J = 8.7 Hz), 4.60 (d, 2H, J = 6.3 Hz), 4.23 (q, 2H, J = 6.6 Hz), 2.91 (t, 2H, J = 6.6 Hz), 2.00 (s, 2H). EI-MS m/z: 353 $(M)^+$. Anal. HPLC t_R = 18.167 min, 100% (solvent system I); t_R = 12.742 min, 98.29% (solvent system II).

Methyl 2-((5-Bromo-7-((4-fluorobenzyl)carbamoyl)-1,6-naphthyridin-8-yl)amino)acetate (II-2). **II-2** was prepared according to the same procedure described for **I-18**, giving a green−yellow solid, yield 71%; mp 184−185 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.17 (br, 1H), 8.86 (dd, 1H, J = 1.5, 4.2 Hz), 8.43 (dd, 1H, J = 1.5, 8.4 Hz), 8.34 (t, 1H, J = 6.0 Hz), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.36 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 4.82 (s, 2H), 4.63 (d, 2H, J = 6.3 Hz), 3.73 (s, 3H). EI-MS m/z: 446 $(M)^+$, 448 $(M + 2)^+$. Anal. HPLC t_R = 21.025 min, 100% (solvent system I); t_R = 16.158 min, 100% (solvent system II).

5-Bromo-*N*-(4-fluorobenzyl)-8-((3-(methylsulfonamido)-propyl)amino)-1,6-naphthyridine-7-carboxamide (II-3). **II-3** was prepared according to the same procedure described for **I-18**, affording a yellow solid, yield 85%; mp 153−155 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.21 (m, 1H), 9.11 (dd, 1H, J = 1.8, 4.2 Hz), 8.53 (dd, 1H, J = 1.5, 8.4 Hz), 8.40 (t, 1H, J = 6.3 Hz), 7.68 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.05 (t, 2H, J = 8.7 Hz), 6.61 (t, 1H, J = 6.0 Hz), 4.61 (d, 2H, J = 6.0 Hz), 4.14 (m, 2H), 3.42 (q, 2H, J = 6.0 Hz), 2.91 (s, 3H), 2.01 (m, 2H). EI-MS m/z: 509 $(M)^+$, 511 $(M + 2)^+$. Anal. HPLC t_R = 20.833 min, 100% (solvent system I); t_R = 6.067 min, 99.46% (solvent system II).

5-Bromo-8-((3-(dimethylamino)propyl)amino)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-4). **II-4** was prepared according to the same procedure described for **I-18**, affording a light-yellow solid, yield 91%; mp 60−62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.74 (t, 1H, J = 6.6 Hz), 8.94 (dd, 1H, J = 1.8, 4.2 Hz), 8.42 (dd, 1H, J = 1.5, 8.4 Hz), 8.31 (t, 1H, J = 6.3 Hz), 7.68 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 4.61 (d, 2H, J = 6.0 Hz), 4.17 (q, 2H, J = 6.6 Hz), 2.54 (m, 2H), 1.96 (m, 2H). EI-MS m/z: 459 $(M)^+$, 461 $(M + 2)^+$. Anal. HPLC t_R = 18.867 min, 100% (solvent system I); t_R = 12.808 min, 100% (solvent system II).

8-((3-Benzamidopropyl)amino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-5). **II-5** was prepared according to the same procedure described for **I-18**, giving a brown solid, yield 57%; mp 144−146 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.63 (br, 1H), 8.83 (dd, 1H, J = 1.8, 4.2 Hz), 8.43 (dd, 1H, J = 1.5, 8.4 Hz), 8.34 (t, 1H, J = 6.3 Hz), 7.75 (d, 2H, J = 6.9 Hz), 7.57 (dd, 1H, J = 4.2, 8.4 Hz), 7.50−7.32 (m, 5H), 7.04 (t, 2H, J = 8.7 Hz), 6.68 (br, 1H), 4.59 (d, 2H, J = 6.6 Hz), 4.26 (t, 2H, J = 6.6 Hz), 3.66 (q, 2H, J = 6.3 Hz), 2.08 (m, 2H). EI-MS m/z: 535 $(M)^+$, 537 $(M + 2)^+$. Anal. HPLC t_R = 6.408 min, 100% (solvent system I); t_R = 6.208 min, 100% (solvent system II).

8-((6-Aminohexyl)amino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-6). **II-6** was prepared according to the same procedure described for **I-18**, giving a green−yellow solid, yield 48%; mp 108−112 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.65 (br, 1H), 8.91 (dd, 1H, J = 1.8, 4.2 Hz), 8.38 (dd, 1H, J = 1.5, 8.4 Hz), 8.30 (t, 1H, J = 6.3 Hz), 7.56 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.03 (t, 2H, J = 8.7 Hz), 4.60 (d, 2H, J = 6.6 Hz), 4.26 (m, 2H), 2.75 (m, 2H),

1.8−1.15 (m, 8H). EI-MS m/z: 473 $(M)^+$, 475 $(M + 2)^+$. Anal. HPLC t_R = 19.558 min, 100% (solvent system I); t_R = 13.508 min, 96.32% (solvent system II).

8-(4-Aminophenylamino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-7). **II-7** was prepared according to the same procedure described for **I-18**, giving a carmine solid, yield 46%; mp 190−192 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.64 (s, 1H), 8.73 (dd, 1H, J = 1.8, 4.2 Hz), 8.44 (dd, 1H, J = 1.5, 8.4 Hz), 8.38 (t, 1H, J = 6.3 Hz), 7.54 (dd, 1H, J = 4.2, 8.4 Hz), 7.36 (m, 2H), 7.03 (t, 2H, J = 8.7 Hz), 6.80 (d, 2H, J = 8.4 Hz), 6.56 (d, 2H, J = 8.4 Hz), 4.60 (d, 2H, J = 6.6 Hz). EI-MS m/z: 465 $(M)^+$, 467 $(M + 2)^+$. Anal. HPLC t_R = 16.067 min, 100% (solvent system I); t_R = 10.667 min, 99.18% (solvent system II).

8-(3-Aminophenylamino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-8). **II-8** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 44%; mp 167−169 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.54 (s, 1H), 8.85 (dd, 1H, J = 1.8, 4.2 Hz), 8.47 (dd, 1H, J = 1.5, 8.4 Hz), 8.38 (t, 1H, J = 6.3 Hz), 7.59 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 6.95 (t, 1H, J = 8.0 Hz), 6.36 (d, 1H, J = 8.0 Hz), 6.28 (m, 2H), 4.61 (d, 2H, J = 6.3 Hz), 3.56 (br, 2H). EI-MS m/z: 465 $(M)^+$, 467 $(M + 2)^+$. Analytical HPLC t_R = 16.292 min, 100% (solvent system I); t_R = 11.067 min, 100% (solvent system II).

***tert*-Butyl 4-((5-Bromo-7-((4-fluorobenzyl)carbamoyl)-1,6-naphthyridin-8-yl)amino)piperidine-1-carboxylate (II-9).** **II-9** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 75%; mp 134−137 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (dd, 1H, J = 1.5, 3.9 Hz), 8.44 (dd, 1H, J = 1.5, 8.4 Hz), 8.32 (t, 1H, J = 6.0 Hz), 7.61 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 5.18 (m, 1H), 4.60 (d, 2H, J = 6.0 Hz), 3.99 (m, 2H), 3.05 (m, 2H), 2.06 (m, 2H), 1.62 (m, 2H), 1.45 (s, 9H). EI-MS m/z: 557 $(M)^+$, 559 $(M + 2)^+$. Anal. HPLC t_R = 23.967 min, 100% (solvent system I); t_R = 19.517 min, 100% (solvent system II).

5-Bromo-*N*-(4-fluorobenzyl)-8-(piperidin-4-ylamino)-1,6-naphthyridine-7-carboxamide (II-10). **II-10** was prepared according to the same procedure described for **I-18**, giving a green−yellow solid, yield 46%; mp >200 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (dd, 1H, J = 2.1, 4.2 Hz), 8.46 (dd, 1H, J = 1.8, 8.4 Hz), 8.32 (t, 1H, J = 6.0 Hz), 7.63 (dd, 1H, J = 4.2, 8.4 Hz), 7.33 (m, 2H), 7.02 (t, 2H, J = 8.7 Hz), 5.27 (m, 1H), 4.56 (d, 2H, J = 6.0 Hz), 3.39 (m, 2H), 2.31 (m, 2H), 1.84 (m, 2H). EI-MS m/z: 457 $(M)^+$, 459 $(M + 2)^+$. Anal. HPLC t_R = 6.292 min, 100% (solvent system I); t_R = 12.658 min, 100% (solvent system II).

***tert*-Butyl 4-(5-Bromo-7-(4-fluorobenzylcarbamoyl)-1,6-naphthyridin-8-yl)piperazine-1-carboxylate (II-11).** **II-11** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 64%; mp 118−122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02 (dd, 1H, J = 1.5, 4.2 Hz), 8.47 (dd, 1H, J = 1.8, 8.4 Hz), 8.18 (t, 1H, J = 8.7 Hz), 7.59 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 6.99 (t, 2H, J = 8.7 Hz), 4.61 (d, 2H, J = 6.3 Hz), 3.59 (s, 4H), 3.47 (s, 4H), 1.49 (s, 9H). EI-MS m/z: 543 $(M)^+$, 545 $(M + 2)^+$. Anal. HPLC t_R = 20.483 min, 100% (solvent system I); t_R = 14.767 min, 99.35% (solvent system II).

5-Bromo-*N*-(4-fluorobenzyl)-8-(piperazin-1-yl)-1,6-naphthyridine-7-carboxamide (II-12). **II-12** was prepared according to the same procedure described for **I-18**, giving a brown solid, yield 38%; mp 191−195 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.07 (dd, 1H, J = 1.5, 4.2 Hz), 8.59 (t, 1H, J = 6.0 Hz), 8.53 (dd, 1H, J = 1.5, 8.4 Hz), 7.63 (dd, 1H, J = 4.2, 8.4 Hz), 7.39 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 4.66 (d, 2H, J = 6.3 Hz), 3.59 (t, 4H, J = 4.5 Hz), 3.13 (t, 4H, J = 4.5 Hz), 2.74 (br, 1H). EI-MS m/z: 443 $(M)^+$, 445 $(M + 2)^+$. Anal. HPLC t_R = 19.808 min, 100% (solvent system I); t_R = 12.050 min, 96.48% (solvent system II).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-13). **II-13** was prepared according to the same procedure described for **I-18**, giving a light-yellow solid, yield 81%; mp 147−152 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.63 (d, 1H, J = 7.5 Hz), 8.94 (dd, 1H, J = 1.5, 3.9 Hz), 8.40 (dd, 1H, J = 1.5, 8.4 Hz), 8.33 (t, 1H, J = 6.0 Hz), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.04 (t, 2H, J = 8.7 Hz), 4.97 (m, 1H), 4.60 (d, 2H, J = 6.0 Hz), 2.76 (m, 1H), 2.18 (m, 2H), 1.92 (m, 4H), 1.42 (m, 4H). EI-MS m/z: 471 $(M)^+$, 473 $(M + 2)^+$. HRMS calcd for $C_{22}H_{23}BrFN_5O$ $(M)^+$: 471.1070. Found: 471.1063. Anal. HPLC t_R = 5.325 min, 93.63% (solvent system I); t_R = 12.692 min, 99.60% (solvent system II).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-*N*-(4-fluorobenzyl)-5-(piperidin-1-yl)-1,6-naphthyridine-7-carboxamide (II-14). Following the procedure as the preparation of compound **I-18**, the reaction of *tert*-butyl ((1*r*,4*r*)-4-aminocyclohexyl)carbamate (65 mg, 0.3 mmol) with **I-3** (53 mg, 0.1 mmol) and triethylamine (30 mg, 0.3 mmol) gave *tert*-butyl ((1*r*,4*r*)-4-((5-bromo-7-((4-fluorobenzyl)-carbamoyl)-1,6-naphthyridin-8-yl)amino)cyclohexyl)carbamate as a yellow solid, yield 70%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.65 (d, 1H, J = 7.5 Hz), 8.93 (dd, 1H, J = 1.5, 3.9 Hz), 8.42 (dd, 1H, J = 1.5, 8.4 Hz), 8.30 (m, 1H), 7.59 (dd, 1H, J = 4.2, 8.4 Hz), 7.35 (m, 2H), 7.04 (t, 2H), 4.96 (m, 1H), 4.60 (d, 2H, J = 6.3 Hz), 4.38 (m, 1H), 3.48 (m, 1H), 2.20 (m, 2H), 2.06 (m, 2H), 1.45 (s, 9H), 1.32 (m, 4H). EI-MS m/z: 571 $(M)^+$, 573 $(M + 2)^+$.

tert-Butyl ((1*r*,4*r*)-4-((5-bromo-7-((4-fluorobenzyl)carbamoyl)-1,6-naphthyridin-8-yl)amino)cyclohexyl)carbamate (29 mg, 0.05 mmol) was added to a solution of piperidine (1 mL). After heating at 110 °C for 48 h, the solution was cooled to room temperature, diluted with dichloromethane, and then washed with saturated aqueous NH_4Cl and brine. Usual workup followed by purification by flash chromatography on silica gel (DCM:methanol = 100:1) afforded *tert*-butyl ((1*r*,4*r*)-4-((7-((4-fluorobenzyl)carbamoyl)-5-(piperidin-1-yl)-1,6-naphthyridin-8-yl)amino)cyclohexyl)carbamate as a yellow solid, yield 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (d, 1H, J = 1.5 Hz), 8.53 (m, 1H), 8.36 (dd, 1H, J = 1.5, 8.4 Hz), 7.47 (dd, 1H, J = 4.2, 8.4 Hz), 7.34 (m, 2H), 7.03 (m, 2H), 4.74 (m, 1H), 4.62 (d, 2H, J = 6.0 Hz), 4.30 (m, 1H), 3.74 (m, 1H), 3.46 (m, 1H), 3.11 (m, 4H), 2.14 (m, 2H), 1.84 (m, 4H), 1.69 (m, 4H), 1.44 (s, 9H), 1.45−1.23 (m, 4H).

The resultant *tert*-butyl ((1*r*,4*r*)-4-((7-((4-fluorobenzyl)carbamoyl)-5-(piperidin-1-yl)-1,6-naphthyridin-8-yl)amino)cyclohexyl)carbamate was deprotected in 20% TFA−DCM solution to give the target compound **II-14** as a yellow solid, yield 95%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (d, 1H, J = 2.7 Hz), 8.70 (m, 1H), 8.54 (m, 1H), 8.35 (d, 1H, J = 9.0 Hz), 7.46 (dd, 1H, J = 3.9, 8.1 Hz), 7.34 (m, 2H), 7.03 (m, 2H), 4.75 (m, 1H), 4.62 (d, 2H, J = 6.3 Hz), 3.11 (m, 4H), 2.75 (m, 1H), 2.13 (m, 2H), 2.05 (m, 3H), 1.72 (m, 5H), 1.45−1.23 (m, 4H). EI-MS m/z: 476 $(M)^+$. Anal. HPLC t_R = 13.843 min, 96.19% (solvent system I); t_R = 6.577 min, 99.29% (solvent system II).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-(1,1-dioxido-1,2-thiazinan-2-yl)-*N*-(4-fluorobenzyl)-1,6-naphthyridine-7-carboxamide (II-15). Following the procedure as the preparation of compound **I-3**, the treatment of **I-4** (43 mg, 0.1 mmol) with methylbenzenesulfonyl chloride (75 mg, 0.4 mmol) and triethylamine (40 mg, 0.4 mmol) gave 5-(1,1-dioxido-1,2-thiazinan-2-yl)-7-((4-fluorobenzyl)-carbamoyl)-1,6-naphthyridin-8-yl 4-methylbenzenesulfonate as a white solid, yield 82%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (d, 1H, J = 3.9 Hz), 8.60 (d, 1H, J = 8.4 Hz), 7.93 (d, 2H, J = 8.1 Hz), 7.59 (dd, 1H, J = 4.2, 8.7 Hz), 7.49 (m, 1H), 7.36 (m, 4H), 7.05 (t, 2H, J = 8.7 Hz), 4.63 (d, 2H, J = 6.0 Hz), 4.03 (m, 2H), 3.31 (m, 2H), 2.47 (s, 3H), 2.36 (m, 2H), 1.66 (m, 2H). EI-MS m/z: 584 $(M)^+$.

II-15 was prepared in a similar fashion as described for **I-18** from the precursor above, giving a yellow solid, yield 54%; mp 173−176 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.65 (d, 1H, J = 7.8 Hz), 8.92 (m, 1H), 8.48 (dd, 1H, J = 1.5, 8.7 Hz), 8.07 (m, 1H), 7.54 (dd, 1H, J = 4.2, 8.4 Hz), 7.34 (dd, 2H, J = 5.4, 8.4 Hz), 7.04 (t, 2H, J = 8.4 Hz), 5.00 (m, 1H), 4.56 (m, 2H), 3.92 (m, 1H), 3.74 (m, 1H), 3.24 (m, 2H), 2.75 (m, 1H), 2.40 (m, 1H), 2.20 (m, 4H), 1.90 (m, 2H), 1.65 (m, 2H), 1.43 (m, 4H). EI-MS m/z: 526 $(M)^+$. Anal. HPLC t_R = 17.875 min, 95.23% (solvent system I); t_R = 7.375 min, 100% (solvent system II).

***N*-Benzyl-5-bromo-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-1-8).** **III-1-8** was prepared in a similar fashion as described for **I-1**, giving a white solid (yield: 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (d, J = 4.2 Hz, 1H), 8.54 (d, J = 7.8 Hz, 1H), 8.21 (m, 1H), 7.74 (dd, J = 4.2, 7.8 Hz, 1H), 7.40−7.29 (m, 5H), 4.70 (d, J = 6.0 Hz, 2H).

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7-(Benzylcarbamoyl)-5-bromo-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-1-9). III-1-9 was prepared in a similar fashion as described for **I-3**, giving a white solid (yield: 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (dd, J = 1.5, 4.2 Hz, 1H), 8.56 (d, J = 1.8, 7.8 Hz, 1H), 7.93 (m, 3H), 7.68 (dd, J = 4.5, 8.7 Hz, 1H), 7.38−7.30 (m, 7H), 4.62 (d, J = 6.0 Hz, 2H), 2.46 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-*N*-benzyl-5-bromo-1,6-naphthyridine-7-carboxamide (III-1). III-1 was prepared in a similar fashion as described for **I-18**, giving a yellow solid, yield 56%; mp 123−126 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (d, 1H, J = 7.5 Hz), 8.94 (dd, 1H, J = 1.5, 3.9 Hz), 8.41 (d, 1H, J = 8.4 Hz), 8.31 (m, 1H), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.37−7.27 (m, 5H), 4.97 (m, 1H), 4.63 (d, 2H, J = 6.0 Hz), 2.76 (m, 1H), 2.18 (m, 1H), 1.92 (m, 4H), 1.42 (m, 4H). EI-MS m/z: 453 $(M)^+$, 455 $(M + 2)^+$. Anal. HPLC t_R = 5.8 min, 100% (solvent system I); t_R = 8.79 min, 95.72% (solvent system II).

5-Bromo-8-hydroxy-*N*-(4-methoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-2-8). III-2-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (d, J = 4.2 Hz, 1H), 8.53 (d, J = 8.1 Hz, 1H), 8.12 (m, 1H), 7.72 (dd, J = 4.2, 7.8 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 6.91 (t, J = 8.7 Hz, 2H), 4.63 (d, J = 6.6 Hz, 2H), 3.80 (s, 3H). Anal. HPLC t_R = 22.47 min, 99.99% (solvent system I); t_R = 14.81 min, 99.81% (solvent system II).

5-Bromo-7-((4-methoxybenzyl)carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-2-9). III-2-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (dd, J = 1.5, 4.2 Hz, 1H), 8.52 (dd, J = 1.5, 8.7 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.87 (m, 1H), 7.65 (dd, J = 4.2, 8.4 Hz, 1H), 7.31−7.27 (m, 4H), 6.87 (d, J = 8.4 Hz, 2H), 4.52 (d, J = 5.7 Hz, 2H), 3.78 (s, 3H), 2.44 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(4-methoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-2). III-2 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 78%; mp 96−99 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.64 (d, 1H, J = 8.1 Hz), 8.93 (dd, 1H, J = 1.2, 4.2 Hz), 8.40 (dd, 1H, J = 1.2, 8.4 Hz), 8.23 (m, 1H), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.30 (d, 2H, J = 8.4 Hz), 6.89 (d, 2H, J = 8.4 Hz), 4.95 (m, 1H), 4.56 (d, 2H, J = 6.3 Hz), 3.80 (s, 3H), 2.75 (m, 1H), 2.63 (m, 1H), 2.18 (m, 2H), 1.85 (m, 4H), 1.34 (m, 4H). EI-MS m/z: 483 $(M)^+$, 485 $(M + 2)^+$. Anal. HPLC t_R = 22.367 min, 97.28% (solvent system I); t_R = 13.950 min, 97.37% (solvent system II).

***N*-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-5-bromo-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-3-8).** III-3-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.32 (s, 1H), 9.20 (dd, J = 1.5, 3.9 Hz, 1H), 8.54 (dd, J = 1.2, 8.4 Hz, 1H), 8.11 (m, 1H), 7.72 (dd, J = 4.2, 8.4 Hz, 1H), 6.89−6.79 (m, 3H), 5.97 (s, 2H), 4.60 (d, J = 6.3 Hz, 2H).

7-((Benzo[*d*][1,3]dioxol-5-ylmethyl)carbamoyl)-5-bromo-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-3-9). III-3-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (dd, J = 1.2, 4.2 Hz, 1H), 8.54 (dd, J = 1.5, 8.7 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.87 (m, 1H), 7.67 (dd, J = 4.2, 8.4 Hz, 1H), 7.33 (d, J = 8.1 Hz, 2H), 6.86−6.75 (m, 3H), 5.94 (s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 2.46 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-*N*-(benzo[*d*][1,3]-dioxol-5-ylmethyl)-5-bromo-1,6-naphthyridine-7-carboxamide (III-3). III-3 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 43%; mp 131−134 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (d, 1H, J = 8.4 Hz), 8.93 (dd, 1H, J = 1.2, 4.2 Hz), 8.40 (dd, 1H, J = 1.2, 8.4 Hz), 8.23 (m, 1H), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 6.87−6.76 (m, 3H), 5.94 (s, 2H), 4.95 (m, 1H), 4.53 (d, 2H, J = 6.0 Hz), 2.75 (m, 1H), 2.18 (m, 2H), 1.92 (m, 4H), 1.34 (m, 4H). EI-MS m/z: 401 $(M)^+$, 403 $(M + 2)^+$. Anal. HPLC t_R = 14.43 min, 100% (solvent system I); t_R = 5.54 min, 100% (solvent system II).

(*S*)-5-Bromo-*N*-(1-(4-fluorophenyl)ethyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-4-8). III-4-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.30 (s, 1H), 9.17 (dd, J = 1.5, 4.2 Hz, 1H), 8.53 (dd, J = 1.5, 8.4 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.71 (dd, J = 4.2, 8.4 Hz, 1H), 7.40 (m, 2H), 7.06 (d, J = 8.7 Hz, 2H), 5.30 (m, 1H), 1.67 (d, J = 6.9 Hz, 3H).

(*S*)-5-Bromo-7-((1-(4-fluorophenyl)ethyl)carbamoyl)-1,6-naphthyridin-8-yl 4-methylbenzenesulfonate (III-4-9). III-4-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (dd, J = 1.2, 4.2 Hz, 1H), 8.56 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.84 (m, 1H), 7.67 (dd, J = 4.5, 8.7 Hz, 1H), 7.40 (m, 2H), 7.28 (d, J = 8.7 Hz, 2H), 5.26 (m, 1H), 2.45 (s, 3H), 1.60 (d, J = 6.9 Hz, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-((*S*)-1-(4-fluorophenyl)ethyl)-1,6-naphthyridine-7-carboxamide (III-4). III-4 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 66%; mp 129−131 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.57 (d, 1H, J = 7.5 Hz), 8.93 (dd, 1H, J = 1.5, 3.9 Hz), 8.41 (dd, 1H, J = 1.5, 8.4 Hz), 8.21 (m, 1H), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.37 (m, 2H), 7.01 (t, 2H, J = 8.7 Hz), 5.24 (m, 1H), 4.97 (m, 1H), 4.93 (m, 1H), 2.76 (m, 1H), 2.18 (m, 2H), 1.92 (m, 2H), 1.60 (d, 3H, J = 6.9 Hz), 1.42 (m, 4H). EI-MS m/z: 485 $(M)^+$, 487 $(M + 2)^+$. Anal. HPLC t_R = 14.31 min, 94.70% (solvent system I); t_R = 8.33 min, 100% (solvent system II).

5-Bromo-8-hydroxy-*N*-(4-(dimethylamino)benzyl)-1,6-naphthyridine-7-carboxamide (III-5-8). III-5-8 was prepared according to the same procedure described for **I-1**, giving the desired crude product as a brown solid (yield: 85%) that was used for the next step directly.

5-Bromo-7-(4-(dimethylamino)benzylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-5-9). III-5-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.03 (d, J = 4.5 Hz, 1H), 8.55 (d, J = 6.9 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.76 (m, 1H), 7.67 (dd, J = 4.5, 6.9 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.25 (m, 2H), 6.74 (d, J = 9 Hz, 2H), 4.49 (d, J = 5.7 Hz, 2H), 2.95 (s, 6H), 2.47 (s, 3H).

8-((1*r*,4*r*)-4-Aminocyclohexylamino)-*N*-(4-(dimethylamino)-benzyl)-5-bromo-1,6-naphthyridine-7-carboxamide (III-5). III-5 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 62%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.72 (d, J = 8.1 Hz, 1H), 8.95 (d, 1H, J = 2.4 Hz), 8.39 (d, 1H, J = 8.7 Hz), 8.20 (t, J = 5.7 Hz, 1H), 7.56 (dd, 1H, J = 2.4, 8.7 Hz), 7.24−7.27 (m, 2H), 6.73 (d, 2H, J = 8.7 Hz), 5.03 (m, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.24 (m, 1H), 2.94 (s, 6H), 2.29 (br s, 4H), 1.77 (m, 2H), 1.47 (m, 2H). MS-EI m/z: 496 $(M)^+$, 498 $(M + 2)^+$. Anal. Calcd for $C_{24}H_{29}BrN_6O\cdot 1/4CF_3COOH\cdot 3H_2O$: C 50.74, H 6.13, N 14.49. Found: C 50.71, H 6.22, N 14.22.

5-Bromo-8-hydroxy-*N*-(3-methoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-6-8). III-6-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (dd, J = 1.5, 4.2 Hz, 1H), 8.48 (dd, J = 1.5, 8.4 Hz, 1H), 8.26 (m, 1H), 7.68 (dd, J = 4.2, 8.4 Hz, 1H), 7.23 (m, 2H), 6.84 (m, 2H), 4.64 (d, J = 6.0 Hz, 2H), 3.78 (s, 3H).

5-Bromo-7-((3-methoxybenzyl)carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-6-9). III-6-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (dd, J = 1.5, 4.2 Hz, 1H), 8.50 (dd, J = 1.8, 7.5 Hz, 1H), 7.94 (m, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.64 (dd, J = 4.2, 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 2H), 7.25 (m, 1H), 6.92 (m, 2H), 6.80 (m, 1H), 4.68 (d, J = 6.0 Hz, 2H), 3.78 (s, 3H), 2.42 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(3-methoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-6). III-6 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 44%; mp 84−87 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (d, 1H, J = 8.4 Hz), 8.92 (dd, 1H, J = 1.5, 4.2 Hz), 8.39 (dd, 1H, J = 1.5, 8.4 Hz), 8.31 (m, 1H), 7.56 (dd, 1H, J = 4.2, 8.4 Hz), 7.30 (d, 2H, J = 8.4 Hz), 6.89 (d, 2H, J = 8.4 Hz), 4.95 (m, 1H), 4.56 (d, 2H, J = 6.3 Hz), 3.80 (s, 3H), 2.75 (m, 1H), 2.63 (m, 1H), 2.18 (m, 2H), 1.85 (m, 4H), 1.34 (m, 4H). EI-MS m/z: 483 $(M)^+$, 485 $(M + 2)^+$. Anal. HPLC t_R = 5.71 min, 100% (solvent system I); t_R = 8.80 min, 99.27% (solvent system II).

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5-Bromo-8-hydroxy-*N*-(4-phenoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-7-8). III-7-8 was prepared according to the same procedure described for **I-1**, giving the crude product as a brown solid (yield: 90%) that was used for the next step directly.

5-Bromo-7-(4-phenoxybenzylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-7-9). III-7-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 20%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.03 (d, J = 4.2 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 7.93 (m, 3H), 7.68 (dd, J = 4.2, 8.4 Hz, 1H), 7.31−7.37 (m, 6H), 7.10 (t, J = 6 Hz, 1H), 6.98−7.03 (m, 4H), 4.60 (d, J = 6 Hz, 2H), 2.47 (s, 3H).

8-((1*r*,4*r*)-4-Aminocyclohexylamino)-5-bromo-*N*-(4-phenoxybenzyl)-1,6-naphthyridine-7-carboxamide (III-7). III-7 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 61%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.65 (d, J = 7.8 Hz, 1H), 8.94 (d, J = 3.9 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 8.31 (t, J = 6 Hz, 1H), 7.59 (dd, J = 3.9, 8.7 Hz, 1H), 7.26−7.36 (m, 5H), 7.10 (t, J = 6.9 Hz, 1H), 6.98−7.03 (m, 3H), 4.99 (m, 1H), 4.61 (d, J = 6 Hz, 2H), 2.77 (m, 1H), 2.18 (d, J = 12.9 Hz, 2H), 1.91 (d, J = 12.3 Hz, 2H), 1.25−1.49 (m, 4H). MS-EI m/z: 545 $(M)^+$, 547 $(M + 2)^+$. HRMS calcd for $C_{28}H_{28}BrN_5O_2$ $(M)^+$, 545.1426; found, 545.1427. Anal. Calcd for $C_{28}H_{28}BrN_5O_2$: C 61.58, H 5.13, N 12.71. Found: C 61.54, H 5.16, N 12.82.

5-Bromo-*N*-(4-chlorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-8-8). III-8-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (d, J = 3.9 Hz, 1H), 8.54 (d, J = 9 Hz, 1H), 8.19 (m, 1H), 7.74 (dd, J = 4.2, 8.4 Hz, 1H), 7.35−7.23 (m, 8H), 4.67 (d, J = 6.3 Hz, 2H).

5-Bromo-7-((4-chlorobenzyl)carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-8-9). III-8-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 38%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (dd, J = 1.5, 4.2 Hz, 1H), 8.56 (dd, J = 1.5, 8.7 Hz, 1H), 7.98 (m, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.68 (dd, J = 4.2, 8.7 Hz, 1H), 7.32−7.30 (m, 6H), 4.61 (d, J = 6.0 Hz, 2H), 2.46 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(4-chlorobenzyl)-1,6-naphthyridine-7-carboxamide (III-8). III-8 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 58.5%; mp 128−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.60 (d, 1H, J = 8.1 Hz), 9.30 (dd, 1H, J = 1.5, 4.2 Hz), 8.40 (dd, 1H, J = 4.2, 8.7 Hz), 8.33 (m, 1H), 7.58 (dd, 1H, J = 4.2, 8.4 Hz), 7.30 (s, 4H), 4.96 (m, 1H), 4.59 (d, 2H, J = 6.0 Hz), 2.77 (m, 1H), 2.17 (m, 2H), 1.94 (m, 2H), 1.43−1.24 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.83 (s), 150.97 (s), 150.64 (s), 144.90 (s), 144.58 (s), 137.01 (s), 136.54 (s), 133.15 (s), 128.98 (s), 128.93 (s), 128.82 (s), 126.71 (s), 124.89 (s), 124.59 (s), 124.46 (s), 123.69 (s), 52.57 (s), 50.38 (s), 42.35 (s), 32.34 (s), 30.28 (s). EI-MS m/z: 487 $(M)^+$, 489 $(M + 2)^+$. HR-EIMS calcd for $C_{22}H_{23}N_5OClBr$, 487.0775; found, 487.0774. Anal. HPLC t_R = 30.39 min, 95.29% (solvent system I); t_R = 20.43 min, 100% (solvent system II).

5-Bromo-*N*-(2,4-dichlorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-9-8). III-9-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (d, J = 4.2 Hz, 1H), 8.55 (d, J = 7.8 Hz, 1H), 8.27 (m, 1H), 7.75 (dd, J = 4.2, 8.4 Hz, 1H), 7.42 (m, 3H), 4.76 (d, J = 6.3 Hz, 2H).

5-Bromo-7-((2,4-dichlorobenzyl)carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-9-9). III-9-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (dd, J = 1.5, 4.2 Hz, 1H), 8.57 (dd, J = 1.8, 8.7 Hz, 1H), 8.08 (m, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.68 (dd, J = 4.2, 8.7 Hz, 1H), 7.42 (m, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.24 (m, 1H), 4.67 (d, J = 6.3 Hz, 2H), 2.47 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(2,4-dichlorobenzyl)-1,6-naphthyridine-7-carboxamide (III-9). III-9 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 80.3%; mp 72−74 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.55 (d, 1H, J = 7.8 Hz), 8.94 (dd, 1H, J = 1.5, 3.9 Hz), 8.41 (d, 1H, J = 7.2 Hz), 8.40 (m, 1H), 7.59 (dd, 1H, J = 1.5, 8.7 Hz), 7.37 (s, 2H), 7.23 (m, 1H), 4.96 (m, 1H), 4.67 (d, 2H, J = 6.0 Hz), 2.76 (m, 1H), 2.18 (m, 2H), 1.87 (m, 2H), 1.37 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.96 (s), 150.83 (s), 144.97 (s), 144.80 (s), 136.50 (s), 134.56 (s), 134.03 (s), 133.68 (s), 129.98 (s), 129.34 (s), 127.29 (s), 126.79 (s), 124.64 (s), 124.57 (s), 123.51 (s), 53.16 (s), 50.20 (s), 40.42 (s), 32.79 (s), 32.69 (s). EI-MS m/z: 521 $(M)^+$. HR-EIMS calcd for $C_{22}H_{22}N_5OCl_2Br$, 521.0385; found, 521.0388. Anal. HPLC t_R = 29.56 min, 94.27% (solvent system I); t_R = 23.65 min, 95.94% (solvent system II).

5-Bromo-*N*-(3-(trifluoromethyl)benzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-10-8). III-10-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (d, J = 1.5, 4.2 Hz, 1H), 8.55 (d, J = 1.5, 7.8 Hz, 1H), 8.25 (m, 1H), 7.74 (dd, J = 4.5, 8.7 Hz, 1H), 7.64−7.44 (m, 4H), 4.76 (d, J = 6.6 Hz, 2H).

5-Bromo-7-((3-(trifluoromethyl))carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-10-9). III-10-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (dd, J = 1.5, 4.2 Hz, 1H), 8.57 (dd, J = 1.5, 8.4 Hz, 1H), 8.06 (m, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.69 (dd, J = 4.2, 8.4 Hz, 1H), 7.71−7.48 (m, 4H), 7.31 (d, J = 8.1 Hz, 2H), 4.70 (d, J = 6.6 Hz, 2H), 2.47 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(3-(trifluoromethyl)benzyl)-1,6-naphthyridine-7-carboxamide (III-10). III-10 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 74%; mp 94−96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.59 (d, 1H, J = 8.1 Hz), 8.59 (s, 1H), 8.42 (m, 2H), 7.61−7.44 (m, 4H), 4.97 (m, 1H), 4.69 (d, 2H, J = 6.0 Hz), 2.78 (m, 1H), 2.17 (m, 2H), 2.00−1.83 (m, 4H), 1.43−1.25 (m, 4H). EI-MS m/z: 521 $(M)^+$, 523 $(M + 2)^+$. Anal. HPLC t_R = 13.11 min, 100% (solvent system I); t_R = 9.94 min, 95.0% (solvent system II).

5-Bromo-*N*-(furan-2-ylmethyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (III-11-8). III-11-8 was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.20 (brs, 1H), 9.19 (dd, J = 1.5, 4.2 Hz, 1H), 8.54 (dd, J = 1.5, 8.4 Hz, 1H), 8.13 (m, 1H), 7.73 (dd, J = 4.2, 8.4 Hz, 1H), 7.42 (m, 1H), 6.37 (m, 2H), 4.70 (d, J = 6.0 Hz, 2H). EI-MS m/z: 347 $(M)^+$, 349 $(M + 2)^+$.

5-Bromo-7-((furan-2-ylmethyl)carbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-11-9). III-11-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (dd, J = 1.5, 4.2 Hz, 1H), 8.56 (dd, J = 1.5, 8.7 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.89 (m, 1H), 7.68 (dd, J = 4.2, 8.7 Hz, 1H), 7.40 (m, 1H), 7.32 (d, J = 8.1 Hz, 2H), 6.33 (m, 2H), 4.58 (d, J = 6.0 Hz, 2H), 2.47 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(furan-2-ylmethyl)-1,6-naphthyridine-7-carboxamide (III-11). III-11 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 46%; mp 96−98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.56 (d, 1H, J = 8.1 Hz), 8.90 (dd, 1H, J = 1.5, 3.9 Hz), 8.37 (d, 1H, J = 8.4 Hz), 8.23 (m, 1H), 7.54 (dd, 1H, J = 4.2, 8.4 Hz), 7.36 (s, 1H), 6.30 (m, 2H), 4.93 (m, 1H), 4.57 (d, 2H, J = 6.0 Hz), 2.71 (m, 1H), 2.18 (m, 2H), 1.92 (m, 4H), 1.42 (m, 4H). EI-MS m/z: 443 $(M)^+$, 445 $(M + 2)^+$. Anal. HPLC t_R = 5.91 min, 100% (solvent system I); t_R = 6.55 min, 100% (solvent system II).

5-Bromo-8-hydroxy-*N*-(thiophen-2-ylmethyl)-1,6-naphthyridine-7-carboxamide (III-12-8). III-12-8 was prepared according to the same procedure described for **I-1**, giving the desired crude product as a brown solid (yield: 98%) that was carried onto the next step directly.

5-Bromo-7-(thiophen-2-ylmethylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-12-9). III-12-9 was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (d, J = 2.4 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.68 (dd, J = 2.4, 8.7 Hz, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.25−7.27 (m, 1H), 7.07 (m, 1H), 6.99 (m, 1H), 4.77 (d, J = 4.5 Hz, 2H), 2.48 (s, 3H).

8-((1*r*,4*r*)-4-Aminocyclohexylamino)-5-bromo-*N*-(thiophen-2-ylmethyl)-1,6-naphthyridine-7-carboxamide (III-12). III-12 was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 43%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.60

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(d, J = 7.8 Hz, 1H), 8.95 (d, J = 4.5 Hz, 1H), 8.42 (d, J = 8.4 Hz, 1H), 8.32 (t, J = 6 Hz, 1H), 7.59 (dd, J = 4.5, 8.4 Hz, 1H), 7.23−7.25 (m, 1H), 7.07 (m, 1H), 6.98 (m, 1H), 4.98 (m, 1H), 4.80 (d, J = 6 Hz, 2H), 2.81 (m, 1H), 2.19 (d, J = 11.4 Hz, 2H), 1.94 (d, J = 12.3 Hz, 2H), 1.31−1.49 (m, 4H). MS-EI m/z: 459 $(M)^+$, 461 $(M + 2)^+$. Anal. Calcd for $C_{20}H_{22}BrN_5O\cdot 1/2H_2O$: C 51.68, H 5.08, N 14.70. Found: C 51.71, H 4.85, N 14.46.

5-Bromo-8-hydroxy-*N*-(pyridin-2-ylmethyl)-1,6-naphthyridine-7-carboxamide (III-13-8). **III-13-8** was prepared according to the same procedure described for **I-1**, giving the crude product as a brown solid (yield: 90%) that was used for the next step directly.

5-Bromo-7-(pyridin-2-ylmethylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-13-9). **III-13-9** was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (d, J = 4.2 Hz, 1H), 8.57−8.60 (m, 2H), 8.51 (t, J = 5.7 Hz, 1H), 7.90 (d, J = 8.1 Hz, 2H), 7.68 (m, 1H), 7.36 (d, 1H), 7.29 (m, 2H), 7.21 (m, 1H), 4.69 (d, J = 5.7 Hz, 2H), 2.43(s, 3H).

8-((1*r*,4*r*)-4-Aminocyclohexylamino)-5-bromo-*N*-(pyridin-2-ylmethyl)-1,6-naphthyridine-7-carboxamide (III-13). **III-13** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.6 (d, J = 8.4 Hz, 1H), 8.94 (d, J = 3.9 Hz, 1H), 8.73 (t, J = 6 Hz, 1H), 8.61 (d, J = 4.8 Hz, 1H), 8.43 (d, J = 10.2 Hz, 1H), 7.68 (t, 1H), 7.58 (dd, J = 3.9, 10.2 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.2 (t, 2H), 4.95 (m, 1H), 4.77 (d, J = 6 Hz, 2H), 2.74 (m, 1H), 2.16 (d, J = 13.5 Hz, 2H), 1.9 (d, J = 12.6 Hz, 2H), 1.23−1.43 (m, 4H). MS-EI m/z: 454 $(M)^+$, 456 $(M + 2)^+$. Anal. Calcd for $C_{21}H_{23}BrN_6O\cdot 1/2CF_3COOH\cdot CH_3OH$: C 50.74, H 5.09, N 15.44. Found: C 50.81, H 4.95, N 15.29.

5-Bromo-8-hydroxy-*N*-phenethyl-1,6-naphthyridine-7-carboxamide (III-14-8). **III-14-8** was prepared according to the same procedure described for **I-1**, giving a white solid (yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.18 (dd, J = 1.5, 4.2 Hz, 1H), 8.53 (dd, J = 1.5, 8.4 Hz, 1H), 7.93 (m, 1H), 7.71 (dd, J = 4.2, 8.4 Hz, 1H), 7.73−7.18 (m, 10H), 3.75 (q, J = 6.9 Hz, 2H), 2.98 (m, 4H), 2.76 (m, 2H). EI-MS m/z: 371 $(M)^+$, 373 $(M + 2)^+$.

5-Bromo-7-(phenethylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-14-9). **III-14-9** was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (d, J = 3.6 Hz, 1H), 8.51 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 7.8 Hz, 2H), 7.65 (dd, J = 4.2, 8.4 Hz, 1H), 7.64 (m, 1H), 7.35−7.21 (m, 7H), 3.63 (q, J = 6.9 Hz, 2H), 2.90 (t, J = 8.1 Hz, 2H), 2.44 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-phenethyl-1,6-naphthyridine-7-carboxamide (III-14). **III-14** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 41%; mp 64−65 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.60 (d, 1H, J = 8.4 Hz), 8.93 (d, 1H, J = 3.9 Hz), 8.40 (d, 1H, J = 8.4 Hz), 8.11 (m, 1H), 7.57 (dd, 1H, J = 4.2, 8.4 Hz), 7.36−7.24 (m, 5H), 4.93 (m, 1H), 3.65 (m, 2H), 2.95 (m, 2H), 2.75 (m, 1H), 2.18 (m, 2H), 1.94 (m, 2H), 1.42 (m, 4H). EI-MS m/z: 467 $(M)^+$, 469 $(M + 2)^+$. Anal. HPLC t_R = 15.05 min, 97.78% (solvent system I); t_R = 8.30 min, 99.26% (solvent system II).

5-Bromo-8-hydroxy-*N*-phenyl-1,6-naphthyridine-7-carboxamide (III-15-8). Compound 7 (150 mg, 0.735 mmol) was dissolved in 1N LiOH (2 mL) and MeOH (4 mL). The solution was heated to reflux for 5 h. After cooling to rt, the pH of the reaction mixture was adjusted to 3 with 1N HCl solution. The mixture was filtered to give crude product as a tan solid 0.116 g (yield: 81%). ^{1}H NMR (300 MHz, CD_3OD): δ 9.21 (s, 1H), 8.57 (d, 1H, J = 7.8 Hz), 7.97 (s, 1H). EI-MS m/z: 269 $(M)^+$.

Triphosgene (150 mg, 0.735 mmol) was added to DMF solution of 5-bromo-8-hydroxy-1,6-naphthyridine-7-carboxylic acid (45 mg, 0.234 mmol) and DIPEA (0.16 mL, 0.398 mmol) at 0 °C. The solution was stirred for 60 min at rt. And then excess aniline was added. After the reaction was completed, the mixture was diluted with DCM. The organic layer was washed with saturated NaCl solution. The organic layer was dried over Na_2SO_4 and evaporated to yield crude **III-15-8**, which was used for the next step directly.

5-Bromo-7-(phenylcarbamoyl)-1,6-naphthyridin-8-yl-4-methylbenzenesulfonate (III-15-9). **III-15-9** was prepared according to the same procedure described for **I-3**, giving a white solid (overall yield: 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.36 (s, 1H), 9.14 (dd, J = 1.5, 4.2 Hz, 1H), 8.59 (dd, J = 1.8, 8.4 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.73 (dd, J = 4.2, 8.4 Hz, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.34 (m, 4H), 7.13 (m, 1H), 2.35 (s, 3H).

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-phenyl-1,6-naphthyridine-7-carboxamide (III-15). **III-15** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 48%; mp 208−210 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 10.12 (s, 1H), 9.02 (d, 1H, J = 4.2 Hz), 8.55 (d, 1H, J = 8.7 Hz), 7.77 (dd, 1H, J = 4.2, 8.7 Hz), 7.71 (m, 2H), 7.31 (m, 2H), 7.14 (m, 1H), 4.98 (m, 1H), 3.18 (m, 1H), 2.33 (m, 2H), 2.11 (m, 2H), 1.53 (m, 4H). EI-MS m/z: 439 $(M)^+$, 441 $(M + 2)^+$. Anal. HPLC t_R = 15.07 min, 97.83% (solvent system I); t_R = 9.25 min, 94.67% (solvent system II).

5-Bromo-8-hydroxy-*N*-cyclohexyl-1,6-naphthyridine-7-carboxamide (III-16-8). **III-16-8** was prepared according to the same procedure described for **I-1**, giving the crude product as a brown solid (yield: 75%) that was used for the next step directly.

5-Bromo-7-(cyclohexylcarbamoyl)-1,6-naphthyridin-8-yl 4-Methylbenzenesulfonate (III-16-9). **III-16-9** was prepared according to the same procedure described for **I-3**, giving the desired crude product as a brown solid (yield: 40%) that was carried onto the next step directly.

8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-cyclohexyl-1,6-naphthyridine-7-carboxamide (III-16). **III-16** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 61%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.68 (d, 1H, J = 7.8 Hz), 8.93 (d, 1H, J = 2.7 Hz), 8.41 (d, 1H, J = 8.4 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.57 (dd, 1H, J = 3.9, 8.4 Hz), 4.92 (m, 1H), 3.87 (m, 1H), 2.73 (m, 1H), 2.16 (m, 2H), 2.03 (m, 2H), 1.90 (m, 2H), 1.81 (m, 3H), 1.45−1.26 (m, 9H). EI-MS m/z: 445 $(M)^+$, 447 $(M + 2)^+$. Anal. HPLC t_R = 15.46 min, 95.0% (solvent system I); t_R = 10.92 min, 97.8% (solvent system II).

Methyl 8-(((1*r*,4*r*)-4-Aminocyclohexyl)amino)-5-bromo-1,6-naphthyridine-7-carboxylate (III-17). Following the procedure as compound **I-18**, *tert*-butyl ((1*r*,4*r*)-4-aminocyclohexyl)carbamate (65 mg, 0.3 mmol), **10** (44 mg, 0.1 mmol), and triethylamine (30 mg, 0.3 mmol) gave methyl 5-bromo-8-(((1*r*,4*r*)-4-((*tert*-butoxycarbonyl)-amino)cyclohexyl)amino)-1,6-naphthyridine-7-carboxylate as a yellow solid, yield 70%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.95 (dd, 1H, J = 1.5, 4.2 Hz), 8.88 (d, 1H, J = 7.5 Hz), 8.47 (dd, 1H, J = 1.5, 8.4 Hz), 7.64 (dd, 1H, J = 4.2, 8.4 Hz), 4.92 (m, 1H), 4.42 (m, 1H), 3.97 (s, 3H), 3.47 (m, 1H), 2.20 (m, 2H), 2.05 (m, 2H), 1.45 (s, 9H), 1.43−1.23 (m, 4H). EI-MS m/z: 478 $(M)^+$, 480 $(M + 2)^+$.

The resultant methyl 5-bromo-8-(((1*r*,4*r*)-4-((*tert*-butoxycarbonyl)-amino)cyclohexyl)amino)-1,6-naphthyridine-7-carboxylate was deprotected in 20% TFA−DCM solution to give the target compound **III-17** as a yellow solid, yield 70%; mp 178−182 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 9.01 (dd, 1H, J = 1.5, 4.2 Hz), 8.49 (dd, 1H, J = 1.5, 8.4 Hz), 7.78 (dd, 1H, J = 4.2, 8.4 Hz), 4.94 (m, 1H), 3.93 (s, 3H), 3.19 (m, 1H), 2.31 (m, 2H), 2.13 (m, 2H), 1.68−1.39 (m, 4H). EI-MS m/z: 378 $(M)^+$, 380 $(M + 2)^+$. Anal. HPLC t_R = 10.366 min, 100% (solvent system I); t_R = 5.847 min, 100% (solvent system II).

Methyl 5-Bromo-8-(tosyloxy)-1,6-naphthyridine-7-carboxylate (10). The intermediate **10** was prepared according to the same procedure described for **I-3**, giving a white solid (yield: 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (dd, J = 1.5, 4.2 Hz, 1H), 8.60 (dd, J = 1.5, 8.7 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.72 (dd, J = 4.2, 8.4 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 3.83 (s, 3H), 2.47 (s, 3H). EI-MS m/z: 436 $(M)^+$.

Methyl 5-Bromo-8-((4-fluorobenzyl)amino)-1,6-naphthyridine-7-carboxylate (11). Compound **11** was prepared according to the same procedure described for **I-18**, giving a yellow solid, yield 61%. ^{1}H NMR (300 MHz, CD_3OD): δ 9.15 (m, 1H), 8.93 (dd, 1H, J = 1.5, 4.2 Hz), 8.48 (dd, 1H, J = 1.8, 8.4 Hz), 7.64 (dd, 1H, J = 4.2, 8.7 Hz), 7.37−7.32 (m, 2H), 7.02 (m, 2H), 5.30 (d, 2H, J = 6.0 Hz), 3.97 (s, 3H).

***N*-((1*r*,4*r*)-4-Aminocyclohexyl)-5-bromo-8-((4-fluorobenzyl)-amino)-1,6-naphthyridine-7-carboxamide (III-18).** Compound **11** was dissolved in 1N LiOH (2 mL) and THF (2 mL). The solution

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was stirred under nitrogen at rt. After the reaction was completed, the reaction mixture was concentrated. The residue was then partitioned between H_2O and DCM. The pH of the H_2O phase was adjusted to 3 and was extracted with DCM 3 times. The DCM layer was dried over Na_2SO_4 and evaporated to give crude product as a tan solid, yield 92%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.37 (m, 1H), 8.96 (dd, 1H, J = 1.5, 4.2 Hz), 8.48 (dd, 1H, J = 1.8, 8.4 Hz), 7.67 (dd, 1H, J = 4.5, 8.4 Hz), 7.35 (m, 2H), 6.99 (m, 2H), 5.42 (d, 2H, J = 5.7 Hz).

The resulting 5-bromo-8-((4-fluorobenzyl)amino)-1,6-naphthyridine-7-carboxylic acid (37.6 mg, 0.1 mmol), *tert*-butyl ((1*r*,4*r*)-4-aminocyclohexyl)carbamate (33.6 mg, 0.15 mmol), EDCI (40 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol), and DIPEA (26 mg, 0.2 mmol) were dissolved in dry DCM (15 mL), and the solution was stirred for 3 h at rt. After the reaction was completed, the mixture was diluted with DCM. The organic layer was washed with saturated $NaHCO_3$ solution and saturated NaCl solution. Usual workup and purification by column chromatography on silica gel eluting with PE:EtOAc (4:1) gave a yellow solid (50 mg, yield 95%); mp 189−191 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.90 (dd, 1H, J = 1.5, 3.9 Hz), 8.42 (dd, 1H, J = 1.5, 8.4 Hz), 7.81 (m, 1H), 7.57 (dd, 1H, J = 4.2, 8.4 Hz), 7.37 (m, 2H), 6.97 (m, 2H), 5.33 (s, 2H), 4.40 (m, 1H), 3.80 (m, 1H), 3.48 (m, 2H), 2.06 (m, 4H), 1.45 (s, 9H), 1.36 (m, 4H).

The resultant *tert*-butyl ((1*r*,4*r*)-4-(5-bromo-8-((4-fluorobenzyl)-amino)-1,6-naphthyridine-7-carboxamido)cyclohexyl)carbamate was deprotected in 20% TFA−DCM solution to give the target compound **III-18** as a yellow solid, yield 95%; mp 210−213 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (d, 1H, J = 3 Hz), 8.47 (d, 1H, J = 8.7 Hz), 8.25 (d, 1H, J = 7.8 Hz), 7.86 (dd, 1H, J = 4.2, 8.4 Hz), 7.40 (m, 2H), 7.15 (m, 2H), 5.26 (s, 2H), 3.72 (m, 2H), 2.99 (m, 2H), 2.73 (m, 1H), 1.93 (m, 4H), 1.48 (m, 4H). EI-MS m/z: 471 $(M)^+$, 473 $(M + 2)^+$. Anal. HPLC t_R = 26.14 min, 100% (solvent system I); t_R = 29.26 min, 100% (solvent system II).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AIDS, acquired immune deficiency syndrome; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; EDTA, ethylenediaminetetraacetic acid; EGFR, epidermal growth factor receptor; ErbB2, human epidermal growth factor receptor 2; HIV-1, human immunodeficiency virus type 1; HOBt, hydroxybenzotriazole; IC_{50}, half-maximal inhibitory concentration; IN, HIV-1 integrase; KDR, kinase insert domain containing receptor; NBS, *N*-bromosuccinimide; SAR, structure−activity relationship; c-Src, cellular proto-oncogene tyrosine kinase; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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