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Investigation on the 1,6-naphthyridine motif: discovery and SAR study of 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one-based c-Met kinase inhibitors

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The 1,6-naphthyridine motif is a multivalent scaffold in medicinal chemistry presenting various bioactivities when properly substituted. By incorporating a cyclic urea pharmacophore into the 1,6-naphthyridine framework through conformationally constraining the 7,8-positions, the resulting 1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one was identified as a new class of c-Met kinase inhibitor. A comprehensive SAR study indicated that an N-1 alkyl substituent bearing a terminal free amino group, a hydrophobic substituted benzyl group at the N-3 position and the tricyclic core were essential for retaining effective Met inhibition of the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one chemotype. Further introduction of a 4′-carboxamide phenoxy group at the C-5 position significantly improved the potency. The best c-Met kinase inhibitory activity was exemplified by **2t** with an IC_{50} = 2.6 μM, which also displayed effective inhibition against TPR-Met phosphorylation and the proliferation of the BaF3-TPR-Met cells at low micromolar concentrations.

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Introduction

Met is a receptor tyrosine kinase which is activated by its specific natural ligand hepatocyte growth factor (HGF, also known as scatter factor). Met signaling plays an important physiological role in embryogenesis and early development.[1] However, the dysregulation of c-Met in mature adult tissues through overexpression, gene amplification, mutation, a ligand-dependent autocrine/paracrine loop or interaction with other receptor families (heterodimerization/cross-talk) can lead to oncogenic transformation.[1–4] In fact, aberrant HGF/c-Met signaling is found in a variety of human cancers and typically associated with poor prognosis, aggressive disease, increased metastasis and shortened patient survival.[1–4] Furthermore, constitutive c-Met activation due to *MET* amplification was found to be linked to acquired resistance of lung cancers to epidermal growth factor receptor (EGFR) inhibitors.[5,6] Therefore, targeting the HGF/c-Met signaling pathway as a means of cancer therapy has become increasingly popular with a number of different therapeutic approaches undergoing clinical trials,[2,4,7] among which small molecule c-Met inhibitors constitute the largest effort within the pharmaceutical industry towards c-Met-based therapies.

Small molecule kinase inhibitors targeting the intracellular kinase domain are predicted to inhibit c-Met activity irrespective of the activation mechanism, while most of the other intervention strategies prevent ligand-mediated activation of the receptor, such as HGF/c-Met biological antagonists and antibodies. Therefore, small-molecule c-Met inhibitors could benefit a different, and potentially larger, cancer patient population. Numerous c-Met kinase inhibitors have been reported in the literature,[8–10] which have basically been categorized into two classes based on their chemotype and predicted binding mode in the c-Met kinase domain.[11] Class I inhibitors bind in a U-shaped conformation to the ATP-binding site at the entrance of the kinase pocket and wrap around Met1211, while class II inhibitors bind to c-Met with an extended conformation that stretches from the ATP-binding site, delineated by the kinase hinge to the deep hydrophobic Ile 1145 pocket near the C-helix region. Some representative chemical structures from each class are shown in Fig. 1. In general, class I inhibitors block c-Met kinase activity with high selectivity against other kinases, whereas a majority of class II molecules are multikinase inhibitors, bearing the general theme of a thiourea/urea or cyclized derivatives of the urea. Recent studies have suggested that certain mutations near the active site of c-Met may lead to resistance to class I inhibitors.[12,13] In this regard, class II inhibitors might be more effective against the

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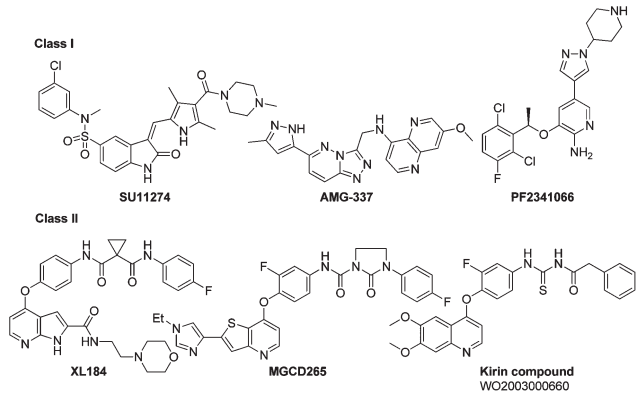
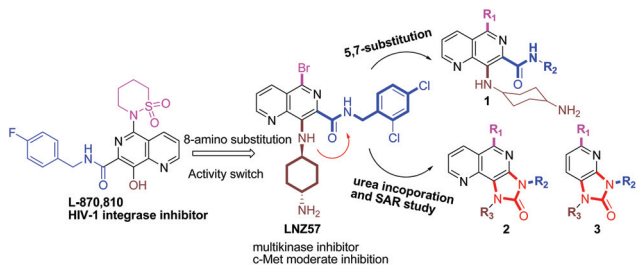


Fig. 1 Examples of class I and class II c-Met kinase inhibitors.

mutations that disrupt the class I binding mode since their binding interactions extend the entrance of c-Met active site. Moreover, the selectivity profiles of class II c-Met inhibitors can be significantly improved by structure-based optimization to avoid off-target side effects.[14]

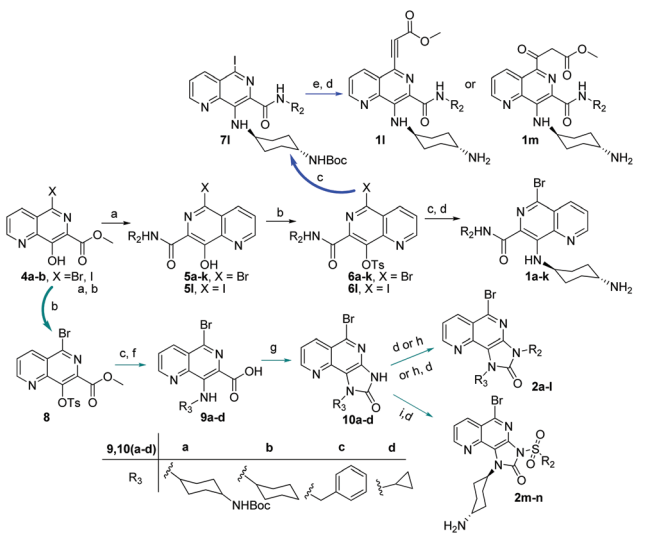
Our interest in the 1,6-naphthyridine-7-carboxamide motif was initiated by the HIV-1 integrase inhibitor L-870,810,[15] which entered clinical trials as an antiviral drug with favorable pharmacological and pharmaceutical properties but was terminated due to liver and kidney toxicity observed in dogs.[15,16] The drug repurposing study on L-870,810 with an amino substituent at the 8-position and proper benzyl substitution at the 7-position resulted in the discovery of novel antitumor agents.[17] The replacement of the hydroxyl group with a diamino structure at the 8-position was the key to the activity switch from HIV-1 inhibition to tumor cytotoxicity. During the target identification, a kinase profiling assay indicated that the 5,8-substituted-1,6-naphthyridine-7-carboxamide derivatives (lead compound **LNZ-57**, Scheme 1) have inhibitory activity on multiple kinases, including Lck, IGF-1R, Abl (T315I), FGFR, Met and ALK.[17] Furthermore, the 1,6-naphthyridines were reported to 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.[18,19] Therefore, we were intrigued to develop novel class II c-Met inhibitors based on the 1,6-naphthyrdine-7-carboxamide framework by varying the substitution at the 5,8-position and further introducing the pharmacophore urea group into the core structure by constraining the 7,8-positions of 1,6-naphthyridine into an imidazolidin-2-one ring (Scheme 1). Encouragingly, the resulting 1,3,5-trisubstituted-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-ones effectively inhibit the c-Met kinase as well as the phosphorylation of c-Met in TPR-Met-transformed BaF3 cell lines which stably express a constitutively active, ligand-independent, oncogenic form of c-Met. Furthermore, these new scaffold c-Met inhibitors significantly inhibit the proliferation of BaF3-TPR-Met cells at low micromolar concentrations. Herein we report the synthesis, structure–activity relationship study and biological evaluation of this new class of c-Met kinase inhibitors, which were predicted to function as class II inhibitors by molecular modeling. Based on this promising new chemotype c-Met inhibitor and its SAR information disclosed here, further effort to improve the potency and selectivity over other protein kinases is under way.



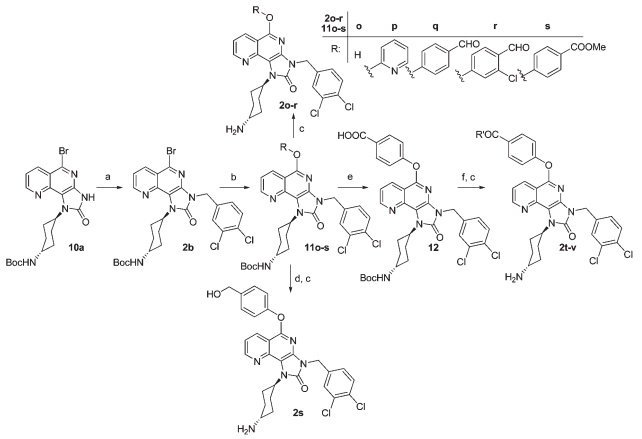
Scheme 1 Design of 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one-based c-Met inhibitors starting from the 1,6-naphthyridine-7-carboxamide scaffold.

Chemistry

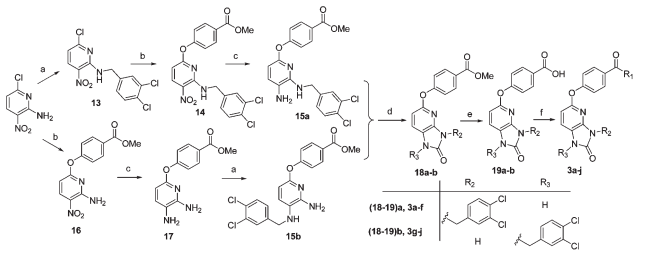
The synthesis of 5,7-substituted-1,6-naphthyridine-7-carboxamides (**1a–m**) and the conformationally constrained 1,3,5-substituted-1*H*-imidazo[4,5-*h*] [1,6]naphthyridin-2(3*H*)-one derivatives **2a–v** is depicted in Schemes 2 and 3. The key precursor, 5-halo-8-hydroxy-1,6-naphthyridine-7-carboxylate (**4a–b**) was prepared conveniently according to our modified methodology,[17] from which 7-various amido substituted-1,6-naphthyridines (**5a–l**) and 8-various amino substituted-1,6-naphthyridines (**9a–d**) were prepared *via* amidation and aromatic nucleophilic substitution, respectively (Scheme 2). The introduction of a



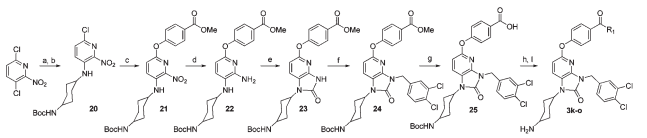
Scheme 2 *Reagents and conditions*: (a) NHR_2, toluene, reflux; (b) TsCl, TEA, CH_2Cl_2, reflux; (c) *tert*-butyl ((1*r*,4*r*)-4-aminocyclohexyl)carbamate, or NHR_3, THF, reflux; (d) TFA, CH_2Cl_2, rt, or TFA (trace H_2O), CH_2Cl_2, 30 °C (for the synthesis of **1m**); (e) methyl propiolate, $Pd(PPh_3)_2Cl_2$, CuI, K_2CO_3, THF, reflux; (f) LiOH, THF–H_2O, rt; (g) toluene, DPPA, TEA, reflux; (h) R_2Br, K_2CO_3, DMF, rt (for synthesis of **2b–e**) (note: condition d for **2a**; h, d for **2f–l**); (i) R_2 substituted sulfonyl chloride, TEA, THF, reflux.



Scheme 3 *Reagents and conditions*: (a) 3,4-dichlorobenzyl bromide, NaH, THF, rt; (b) ROH, K_2CO_3, DMF, 110 °C; (c) TFA, CH_2Cl_2, rt; (d) $NaBH_4$, THF, rt for 2 h; (e) LiOH, THF–H_2O, rt; (f) R′NH_2, BOP, DIEA, DCM, rt.



Scheme 4 *Reagents and conditions*: (a) 3,4-dichlorobenzyl bromide, NaH, THF, rt; (b) methyl 4-hydroxybenzoate, NaH, DMF, 80 °C; (c) $NiCl_2$, $NaBH_4$, 0 °C; (d) BTC, cat. DMF, THF, rt; (e) LiOH, THF–H_2O, rt; (f) NHR_1, EDCI, HOBt, CH_2Cl_2, rt.



Scheme 5 *Reagents and conditions*: (a) *trans*-1,4-diaminocyclohexane, TEA, MeCN, 40 °C; (b) $(Boc)_2O$, TEA, MeCN, 50 °C; (c) methyl 4-hydroxybenzoate, NaH, DMF, 80 °C; (d) $NiCl_2$, $NaBH_4$, 0 °C; (e) BTC, catalytic DMF, THF, rt; (f) 3,4-dichlorobenzyl bromide, NaH, THF, rt; (g) LiOH, THF–H_2O, rt; (h) NHR_1, EDCI, HOBt, CH_2Cl_2, rt; (i) TFA, CH_2Cl_2, rt.

propiolate group at position 5 (*i.e.* compound **1l**) was achieved by Sonogashira coupling of the 8-amino-5-iodo-1,6-naphthyridine-7-carboxamide (**7l**) with methyl propiolate. Treatment with trace water containing TFA at 30 °C easily converted the 5-propiolate 1,6-naphthyridine into the 5-β-keto propanoate counterpart (**1m**).

For the construction of the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one nucleus, we established an efficient methodology to constrain the 7-carboxyl and 8-amino groups at the 1,6-naphthyridine ring into 1*H*-imidazol-2(3*H*)-one as a cyclic urea derivative (Scheme 2). By employing a tandem Curtius rearrangement and intramolecular nucleophilic substitution strategy, the 8-amino substituted-1,6-naphthyridine-7-carboxylic acid (**9a–d**) was transformed into acyl azide by reacting with diphenylphosphoryl azide (DPPA) under basic conditions in refluxing toluene, which immediately underwent Curtius rearrangement to generate an isocyanate *in situ* followed by an attack of the neighboring 8-amino group to form the cyclic urea functionality (**10a–d**). Various substituents can be introduced into the 3-position on the 1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one scaffold by nucleophilic substitution (**2a–l**) or sulfonamidation reaction (**2m–n**).

The 5-substituted 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2-(3*H*)-one derivatives (**2o–v**) were synthesized from the key intermediate **10a** by Williamson etherification reaction (Scheme 3). The 5-bromo tricyclic urea **2b** was heated in DMF with substituted phenol and K_2CO_3 to afford 5-phenyloxy substituted 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-ones (**11o–s**). Then Boc cleavage of 1-(4-aminocyclohexyl) by TFA delivered the 5-substituted products **2o–r**. It is noteworthy that the 5-hydroxy substituted analog **11o** was accidentally obtained by the hydrolysis of intermediate **2b** under the Williamson ether synthesis conditions. Further transformation was performed to introduce more structural variation on the 5-phenoxyl ring to further explore the SAR of the 5-position, *e.g.* the reduction of the aldehyde (**2s**) and the hydrolysis of the ester followed by amidation affording the desired products (**2t–v**).

Since the 1,3,5-substituted-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one represents a new chemotype to inhibit c-Met kinase, an extensive SAR study was conducted to explore the pharmacophores and guide further structural optimization. The simplified core structure, 1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one was designed and three substitution patterns were examined while the privileged structure determined from 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one series was installed at the C-5 position (Schemes 4 and 5, Compounds **3a–o**).

As depicted in Schemes 4 and 5, the 1*H*-imidazo[4,5-*b*]-pyridin-2(3*H*)-one nucleus was constructed from commercially available 2-amino-6-chloro-3-nitropyridine or 3,6-dichloro-2-nitropyridine through triphosgene-mediated tandem reaction. Briefly, the nitro group on the pyridine ring was reduced into an amino group by $NiCl_2$–$NaBH_4$ in high yield. The resulting primary amine was treated with triphosgene [bis(trichloromethyl)carbonate, BTC) and a catalytic amount of DMF in THF to form the isocyanate followed by an intramolecular nucleophilic substitution to furnish the cyclic urea structure (**18a–b** and **23**). The 1,3,5-substituent was introduced by nucleophilic substitution and Williamson etherification reaction in a different sequence dependent on the substitution pattern. For example, 3,5-substituted 1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-ones (**18a**) was synthesized by a sequential nucleophilic displacement of the 2-amino-6-chloro-3-nitropyridine, Williamson ether synthesis and the formation of the cyclic urea moiety, whereas 1,5-substituted 1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-ones (**18b**) required Williamson ether synthesis prior to the nucleophilic substitution then the similar construction of the 1*H*-imidazol-2(3*H*)-one ring by reacting with triphosgene. Further saponification and coupling with different amines delivered

various 1,3,5-substituted-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one derivatives (**3a–j**).

For the synthesis of 4-(1-(4-aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-5-yloxy)-*N*-substituted benzamide series (**3k–o**), starting from the 3,6-dichloro-2-nitropyridine, nucleophilic displacement of the 3-chloro group by *trans*-1,4-diaminocyclohexane gave the 3-aminocyclohexyl substituted analog **20**, which underwent similar transformations as **13** to generate the target products (Scheme 5).

Results and discussion

SAR and lead generation

Initial efforts were directed at exploring the SAR of the 8-amino substituted-1,6-naphthyridine scaffold with structural variation on the 7-carboxamide portion. Encouragingly, these analogs were active in inhibiting the Met kinase in low to mid micromolar concentration range, representing a new chemotype of c-Met inhibitors. The variation on the C-7 substituent remarkably impacted the enzymatic activity, with a hydrophobic substituent being more favorable on the phenyl ring (exemplified by compound **1f**, IC_{50} = 6.1 μM, Table 1). A polar substituent such as an amino group was disfavored at the 4′-position of the 7-benzyl ring (**1g**). Accordingly, the replacement of the 7-phenyl ring by a heterocycle such as a pyridine, thiophene or indole ring resulted in a big loss of the Met inhibitory activity (**1h–j**), while the hydrophobic naphthalene displacement maintained a moderate inhibition effect (**1k**). Introduction of the propiolate group, as a Michael reaction acceptor, at the 5-position had no potentiating effect (**1l**), but its hydrolysis derivative β-ketoester showed an improved potency (**1m**, IC_{50} = 6.6 μM), indicating that substitution at the 5-position had a subtle effect on the c-Met inhibition thus this was an optimizable site.

In order to improve the c-Met potency of the 1,6-naphthyridine based inhibitors, we incorporated the urea pharmacophore, typically carried by the class II inhibitors, into the new scaffold. Interestingly, the conformationally constrained C-7 and C-8 analogs with a cyclic urea moiety turned out superior to the 8-amino-1,6-naphthyridine-7-carboxamides in inhibiting Met kinase and proliferation of BaF3-TPR-Met cells that contain an overexpression level of *Met* gene (Table 2, **2f** *vs.* **1c**). Structural variation at the N-1 position of the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one core by replacing the 4-aminocyclohexyl group with a cyclohexyl, benzyl, cyclopropyl or 4-(*tert*-butoxycarbonylamino)cyclohexyl group all led to inactivation (**2b–e**), indicating the critical role of the free amino substituent at the N-1 position in the Met kinase inhibition. Therefore, the 4-aminocyclohexyl moiety at the N-1 position was held constant as the substituent at the N-3 or C-5 position was explored to achieve a comprehensive SAR study and enhanced potency.

The N-3 unsubstituted analog **2a** was inactive in the enzymatic assay suggesting that the hydrophobic benzyl group was essential for the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one scaffold to achieve effective Met kinase inhibition. Consistent with the SAR established in the 1,6-naphthyridine-7-carboxamide series, a dramatic increase in enzyme potency was observed upon installation of the hydrophobic substituent at the 2, 3 or 4-position of the phenyl ring (Table 2, **2h–l** compared to **2g**). Consistently, the replacement of the benzyl moiety by a more polar sulfonyl substituent at the N-3 position impaired the Met inhibitory activity (**2m–n**), among which the relatively hydrophobic 3,4-dichlorobenzenesulfonyl group partly restored the activity (**2n**). Thus, the optimal 3,4-dichlorobenzyl moiety at the N-3 position was held constant as a further structural variation on the C-5 substitution was investigated to improve the potency.

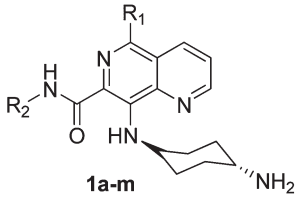
Gratifyingly, most of the 5-aryloxy substituted 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one derivatives gave an improved Met kinase inhibitory activity (**2p–v**), especially an additional introduction of the benzylamino or aliphatic cyclic amino group such as piperidin-1-yl or morpholino on the phenoxyl ring by a carboxamide bridge greatly advanced the enzyme inhibition (**2t**, IC_{50} = 2.6 μM; **2u**, IC_{50} = 2.8 μM; **2v**, IC_{50} = 6.1 μM). The hydrophobic structure attached to the 4′-carboxamide site of the 5-phenoxyl ring seemed beneficial for the activity. However, a formyl group substituted at the phenoxyl ring was unfavorable for the c-Met inhibition (**2q**, **2r**). Another unusual activity change was observed on the simple 5-hydroxy analog which displayed an equally potent inhibition against c-Met kinase (**2o**, IC_{50} = 3.5 μM) compared to the 5-phenoxyl substituted derivatives (**2t–v**). We proposed that the two structures might adopt a different conformation when binding to the ATP binding pocket since they bear different substitution.

Inspired by this observation, we envisioned that the bulky tricyclic core structure might be truncated into a bicyclic structure to gain deep access to the ATP binding pocket. On the other hand, for this new scaffold Met inhibitor, we need more SAR information to reveal the structural requirements. By installing the privileged structures as constant substituents at positions N-1, N-3 and C-5, three substitution patterns were examined. However, when 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one was truncated into 1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one, the Met inhibitory activity was generally sacrificed regardless of the substitution pattern, only the 1-(4-aminocyclohexyl)-3-(3,4-dichlorobenzyl)-substituted series stably exhibited a moderate level of enzyme inhibition at a concentration of 10 μM (Table 3). The negative results indicated that the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one framework played an important role in the binding to the c-Met kinase, probably being involved in the interaction with the residues of the kinase protein and orienting the essential substituents into an appropriate region in the binding pocket.

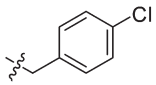
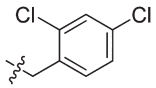
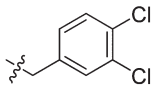
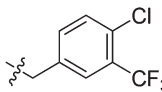
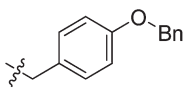
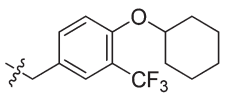
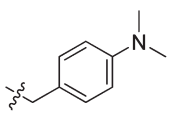
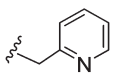
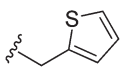
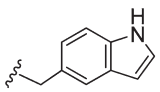
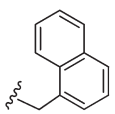
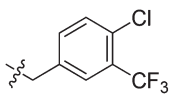
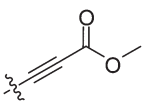
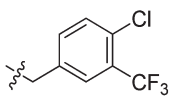
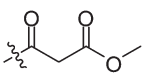
In vitro cell assays

Some of the more potent compounds that were identified in the enzyme assay were further tested for their ability to inhibit c-Met signaling in cellular settings. The TPR-Met-transformed BaF3 cells were selected for this purpose, which stably express

Table 1 c-Met enzymatic activity of 5,7-substituted-1,6-naphthyridine-7-carboxamides[a]



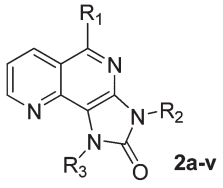
1a-m

Compd.	R_2	R_1	c-Met inhibition % at 10 μM	c-Met inhibition IC_{50} (μM)[b]
1a		Br	19.6	>10
1b		Br	24.0	>10
1c		Br	36.6	>10
1d		Br	45.4	>10
1e		Br	58.4	ND[c]
1f		Br	50.6	6.1 ± 0.51
1g		Br	25.6	>10
1h		Br	9.4	>10
1i		Br	24.9	>10
1j		Br	10.6	>10
1k		Br	42.8	>10
1l			34.6	>10
1m			54.6	6.6 ± 1.9

[a] See the Experimental section for assay details. [b] IC_{50}'s were calculated by the Logit method from the results of at least two independent tests with six concentrations each and expressed as means ± SD. [c] Not determined.

a constitutively active, ligand-independent, oncogenic form of c-Met.[20,21] Since activation of c-Met ultimately results in cell proliferation, we first evaluated the inhibitory effect of these c-Met kinase inhibitors on the growth of BaF3-TPR-Met cell

Table 2 c-Met enzymatic activity of 1,3,5-substituted-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one derivatives[a]



2a-v

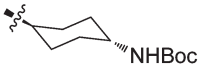
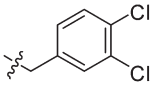
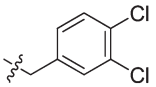
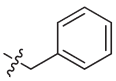
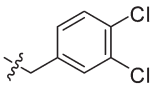
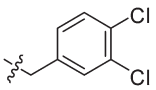
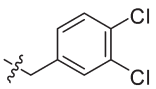
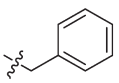
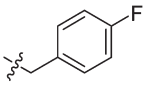
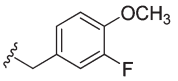
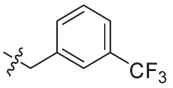
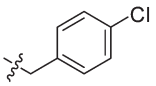
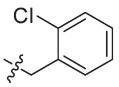
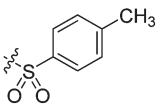
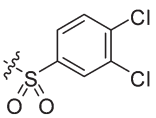
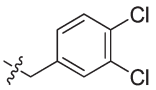
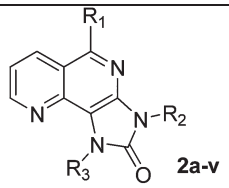
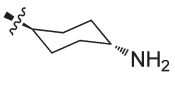
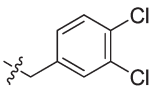
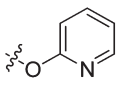
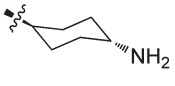
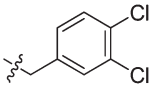
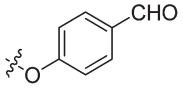
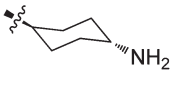
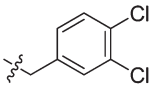
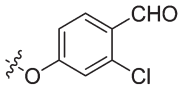
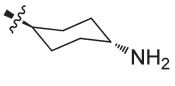
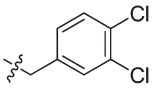
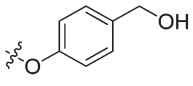
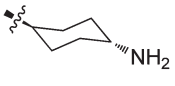
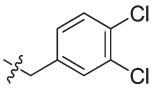
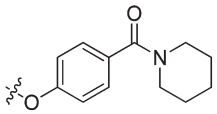
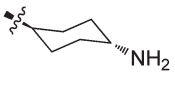
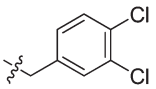
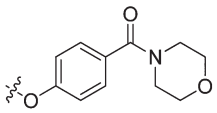
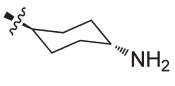
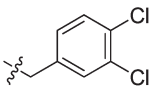
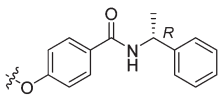
Compd.	R_3	R_2	R_1	c-Met inhibition % at 10 μM	c-Met inhibition IC_{50} (μM)[b]
2a		H	Br	0.0	—
2b			Br	0.0	—
2c			Br	0	—
2d			Br	0	—
2e			Br	0	—
2f			Br	55.6	8.7 ± 0.1
2g			Br	0.0	—
2h			Br	23.0	>10
2i			Br	21.7	>10
2j			Br	16.1	>10
2k			Br	52.3	ND[c]
2l			Br	30.7	>10
2m			Br	6.5	—
2n			Br	40.6	>10
2o			OH	82.4	3.5 ± 2.2

Table 2 *(Contd.)*



2a-v

Compd.	R_3	R_2	R_1	c-Met inhibition % at 10 μM	c-Met inhibition IC_{50} (μM)[b]
2p				54.3	12.7 ± 2.9
2q				30.3	>10
2r				20.4	>10
2s				65	7.7 ± 1.5
2t				64.2	2.6 ± 0.5
2u				74.7	2.8 ± 1.1
2v				57	6.1 ± 1.2

[a] See the Experimental section for assay details. [b] IC_{50}'s were calculated by the Logit method from the results of at least two independent tests with six concentrations each and expressed as means ± SD. [c] Not determined.

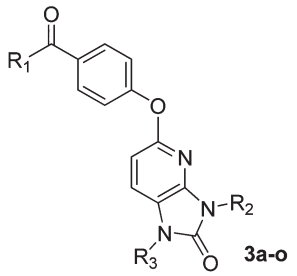
lines. As shown in Table 4, the 1,3,5-trisubstituted-1*H*-imidazo-[4,5-*h*][1,6]naphthyridin-2(3*H*)-one derivatives (**2o–p**, **2s–v**) significantly inhibited the cell growth at 10 μM, while only compound **2t** retained good inhibitory effect at 1 μM concentration. Of note, the trends observed for enzyme potency for these compounds correlated with those that were observed for their cellular activities. Obviously, the urea-containing 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one, especially bearing a 5-phenoxy substituent, is superior to the 1,6-naphthyridine-7-carboxamide in cellular potency.

Then some selected compounds with representative structures (**1m**, **1f**, **2t**, **2v**) were further assessed for their ability to inhibit the phosphorylation of c-Met receptor in TPR-Met-transformed BaF3 cells. The phosphorylation levels of the Met protein determined by Western blotting are illustrated in Fig. 2. Significantly, the 1,3,5-trisubstituted 1*H*-imidazo[4,5-*h*]-[1,6]naphthyridin-2(3*H*)-one derivative **2t** effectively inhibited the TPR-Met phosphorylation in BaF3-TPR-Met cells in a dose-dependent manner, consistent with the enzyme inhibitory activity and almost consistent with the antiproliferative activity in BaF3 cells. However, the 2-methylbenzyl analog **2v** displayed a marginal inhibitory effect on TPR-Met phosphorylation. As a comparison, the open frame 5,7,8-substituted 1,6-naphthyridine-7-carboxamide derivatives **1m** and **1f** lacked activity against the phosphorylation of c-Met protein even at a concentration of 10 μM, in agreement with the cell growth assay. Consequently, compound **2t** stood out as a promising lead for further structural optimization to gain improved potency and selectivity.

Kinase selectivity of the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2-(3*H*)-one scaffold

Since our initial investigation on the 1,6-naphthyridine-7-carboxamide structure disclosed its multiple kinase inhibitory property, we were interested in examining the kinase selectivity of the newly developed 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2-(3*H*)-one scaffold. The lead compound **2t** was selected for kinase profiling against c-Met family member, Ron, along with

Table 3 c-Met enzymatic activity of 1,3,5-substituted-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one derivatives[a]



3a-o

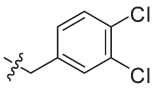
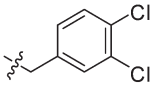
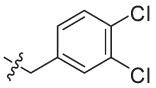
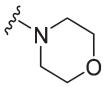
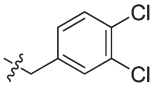
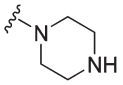
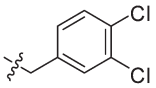
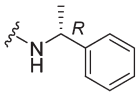
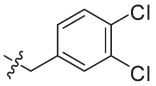
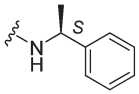
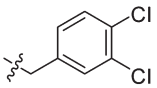
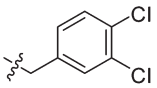
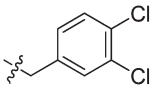
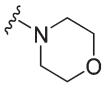
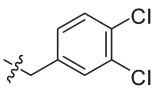
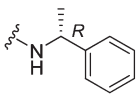
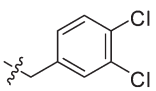
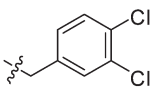
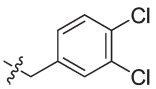
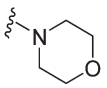
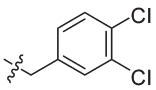
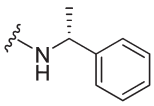
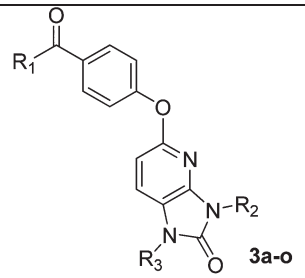
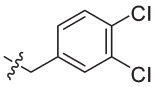
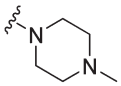
Compd.	R_3	R_2	R_1	c-Met inhibition % at 10 μM
3a	H			27.7
3b	H			0
3c	H			0
3d	H			21.9
3e	H			17.2
3f	H			13.6
3g		H		8.7
3h		H		22.3
3i		H		4.3
3j		H		11
3k			OMe	40.0
3l				33.6
3m				26.8
3n				23.0

Table 3 *(Contd.)*



3a-o

Compd.	R_3	R_2	R_1	c-Met inhibition % at 10 μM
3o				29.8

[a] See the Experimental section for assay details.

Table 4 Inhibitory effect of selected compounds on BaF3 cell proliferation[a]

	Inhibition rate (%)		
Compd	10 μM	1 μM	0.1 μM
1m	20.7	5.7	6.6
	22.6	2.6	3.6
1f	10.1	2.3	2.8
	8.0	−0.5	−0.2
2f	−1.3	1.9	19.3
	12.1	0.4	0.4
2o	82.9	−4.9	−4.6
	86.4	−5.5	−4.0
2p	70.5	−2.9	−3.8
	69.8	−2.7	−3.4
2s	90.5	0.8	−3.2
	90.2	4.2	−0.8
2t	**85.9**	**63.1**	−5.0
	85.7	**64.8**	−2.2
2u	78.5	−1.5	−8.5
	79.5	−6.2	−6.4
2v	67.4	1.2	−6.0
	68.3	5.8	−2.6

[a] The inhibition ratio (%) of cell proliferation was calculated using the following equation: [1 − (A570/A570 control)] × 100%.

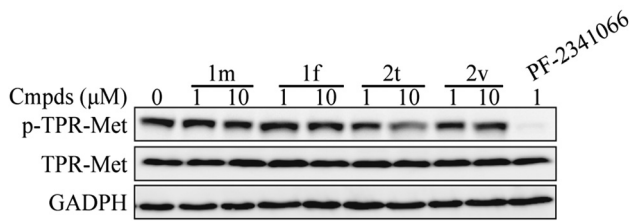


Fig. 2 Effects of selected compounds on TPR-Met phosphorylation in BaF3-TPR-Met cells.

14 other oncogenic tyrosine kinases including Tyro3, Mer, IGF1R, PDGFR-α, PDGFR-β, FGFR1, ErbB4, Flt-1, KDR, c-Kit, RET, EGFR, ABL, and EPH-A2 (Table 5). As shown in Table 5, besides being an effective c-Met inhibitor, **2t** also displayed more than 70% inhibition for RON, PDGFR-α, FGFR1, ErbB4, Flt-1, KDR, c-Kit, RET, and EPH-A2 kinase at a concentration of 10 μM. This result demonstrated the 1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one scaffold was a multi-target kinase inhibitor, which is consistent with the observation that **2t** exhibited a more potent antiproliferative effect with a sub-micromolar IC_{50} value than its c-Met kinase enzyme as well as Met phosphorylation inhibitory activity. However, **2t** still displayed good selectivity over some other kinases such as Mer and IGF1R; moreover, its kinase profile has changed distinctly compared to 1,6-naphthyridine-7-carboxamide chemotype,[17]

Table 5 Effect of **2t** on a panel of RTKs at 10 μM concentration[a]

RTK	Inhibition rate (%)
Ron	77.3
Tyro3	57.2
Mer	29.5
IGF1R	24.5
PDGFR-α	81.6
PDGFR-β	54.2
FGFR1	79.6
ErbB4	74.4
Flt-1	72.8
KDR	84.0
c-Kit	75.7
RET	83.7
EGFR	63.3
ABL	63.2
EPH-A2	86.4

[a] The kinase profiling assay was conducted as described in the Experimental section by using ELISA kinase assay. Each reference compound and its inhibition rate for every kinase at a concentration of 1 μM was indicated below: PF0234106 for RON (91.9%); XL880 for Tyro3 (80.8%) and Mer (59.1%); CCA9 for IGF1R (90.8%); Su11248 for PDGFR-α (98.1%), PDGFR-β (96.4%), Flt-1 (83.2%), KDR (88.1%), c-Kit (96.5%) and RET (78.9%); BIBW2992 for ErbB4 (96.8%) and EGFR (97.5%); Dasatinib for ABL (89.4%) and EPH-A2 (96.7%).

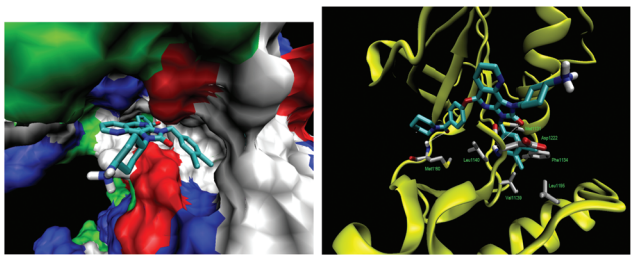


Fig. 3 A proposed binding mode of the lead compound **2t** to the Met kinase domain, based on the X-ray co-crystal structure of c-Met in complex with a small molecule inhibitor.[22]

implying the great potential and promise of the lead structure to be evolved into selective c-Met kinase inhibitors by further structural optimization.

Binding mode predicted by molecular modeling

Based on the X-ray co-crystal structure of the class II c-Met inhibitor bound to the Met kinase domain (PDB: 3C1X),[22] we established the binding mode of lead compound **2t** in the Met kinase domain by autodocking. As demonstrated by Fig. 3, the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one based c-Met inhibitor **2t** adopted an extended conformation in the ATP-binding site of the enzyme with the activation loop in an inactive, DFG-out conformation. However, the heterotricyclic core resides in the middle of the channel between the hinge region and the DFG motif, due to the bulky size, thus losing the key hydrogen bonding interactions with Met1160. That might be the major reason why the kinase inhibitory activity of this chemotype fell in the low micromolar range. However, the carbonyl oxygen in the 1*H*-imidazole ring formed a hydrogen bond with the NH of Asp1222, and the 3-(3,4-dichlorobenzyl) and 1-(4-aminocyclohexyl) groups were favorably positioned into the hydrophobic pocket and solvent area, respectively. Moreover, the 5-(4-(piperidine-1-carbonyl)phenoxy) moiety engages in the hydrophobic interaction within the hinge region.

Conclusions

Starting from the active scaffold of 1,6-naphthyridine, the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one based c-Met inhibitors were designed by incorporation of the urea pharmacophore. A systematic SAR study revealed the privileged structures at the 1,3,5-positions and emphasized the important role of the tricyclic core structure. This new chemotype effectively inhibits the c-Met kinase as well as the phosphorylation of TPR-Met in the TPR-Met-transformed BaF3 cell line which contains an amplified *MET* gene locus in a dose-dependent manner. More significantly, these new scaffold c-Met inhibitors effectively inhibit the proliferation of BaF3-TPR-Met cells at low to sub-micromolar concentrations. Molecular modeling predicted the 1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one based Met inhibitors functioned as class II inhibitor with multiple kinase inhibition, providing a promising starting point for lead optimization and further development of new structure targeted cancer therapeutics.

Experimental section

Chemistry

Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and used without further purification. Dry solvents were purified and stored according to standard procedures. TLC was performed on F254 plates of 0.25 mm thickness. Products were visualised under UV light (254 nM) and/or stained with iodine, potassium permanganate or phosphomolybdic acid solutions followed by heating. Standard silica gel (SiO_2) column chromatography was employed as a method of purification using the indicated solvent mixtures. ^{1}H NMR spectra were recorded at Varian Mercury-400 or 300 MHz and ^{13}C NMR spectra at Varian Mercury-100 MHz. Mass spectra were recorded using a Finnigan MAT-95 instrument for EI and Finnigan LCQ Deca for ESI. Elemental analysis were performed on a CE 1106 elemental analyzer.

General procedure for the preparation of 8-amino-5-bromo-1,6-naphthyridine-7-carboxamide compounds (1a–1k). Method A. Taking the synthesis of **1d** as an example. A solution of (4-chloro-3-(trifluoromethyl))methanamine (86 mg, 0.41 mmol) and methyl 5-bromo-8-hydroxy-1,6-naphthyridine-7-carboxylate (**4a**) (97 mg, 0.34 mmol) in toluene (2 mL) was heated to reflux for 20 h. Upon cooling to room temperature, the precipitate was collected by filtration and washed with small amount of toluene to afford **5d** as a white solid (116 mg, yield 74.1%), which was used for the next step directly.

The intermediate **5d** (116 mg, 0.25 mmol) was dissolved in dry DCM (5 mL) under nitrogen. Methylbenzenesulfonyl chloride (95 mg, 0.5 mmol) and TEA (0.1 mL) were added. The resulting solution was heated to 40 °C and stirred for 12 h. The reaction mixture was then partitioned between water and dichloromethane. The aqueous layer was then extracted with dichloromethane (20 mL × 3). The combined organic extracts were washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with PE : EtOAc (1 : 1) to give **6d** as a white solid (75 mg, yield 48.5%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.01 (d, J = 4.2 Hz, 1H), 8.57 (d, J = 8.1 Hz, 1H), 8.09 (t, J = 6.3 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.67–7.7 (m, 2H), 7.47–7.56 (m, 2H), 7.31 (d, 2H), 4.68 (d, J = 6.3 Hz, 2H), 2.46 (s, 3H).

The precursor **6d** (75 mg, 0.12 mmol) was dissolved in dry THF (10 mL) under nitrogen. (1*r*,4*r*)-Cyclohexane-1,4-diamine (65 mg, 0.57 mmol) and TEA (0.1 mL) were added, and the reaction mixture was heated to 70 °C and stirred for 12 h. After the reaction was completed, the reaction solution was concentrated. The residue was diluted with dichloromethane (20 mL). Usual work-up with the organic phase and purification by column chromatography on silica gel eluting with PE : EtOAc (1 : 1) afforded target compound **1d** as a light yellow solid

(40 mg, yield 59%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.54 (d, J = 8.1 Hz, 1H), 8.95 (d, J = 3.9 Hz, 1H), 8.41 (m, 2H), 7.68 (s, 1H), 7.6 (dd, J = 8.1, 3.9 Hz, 1H), 7.48 (m, 2H), 4.97 (m, 1H), 4.64 (d, J = 6.9 Hz, 2H), 2.74 (m, 1H), 2.17 (d, J = 14.4 Hz, 2H), 1.9 (d, J = 12 Hz, 2H), 1.26–1.43 (m, 4H); MS-EI m/z: 555 $(M)^+$, 557 $(M + 2)^+$; Anal. calcd for $C_{23}H_{22}BrClF_3N_5O{\cdot}1/4CH_3OH$: C 49.44, H 4.10, N 12.40, found: C 49.74, H 4.32, N 12.06%.

Compounds **1a**, **1b**, **1g**, **1h** and **1i** were prepared according to the same procedure reported previously.[17]

8-(((1*R*,4*R*)-4-Aminocyclohexyl)amino)-5-bromo-*N*-(3,4-dichlorobenzyl)-1,6-naphthyridine-7-carboxamide (1c). **1c** was prepared analogously using general method A, giving a light yellow solid, yield 73.4%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.56 (d, 1H, J = 8.1 Hz), 8.94 (d, 1H, J = 3.9 Hz), 8.42–8.35 (m, 3H), 7.59 (dd, 1H, J = 4.2, 8.7 Hz), 7.44–7.38 (m, 2H), 7.20 (m, 1H), 4.96 (m, 1H), 4.57 (d, 2H, J = 6.0 Hz), 2.76 (m, 1H), 2.18 (m, 2H), 1.91 (m, 2H), 1.37 (m, 4H); EI-MS m/z: 521 $(M)^+$, 523 $(M + 2)^+$.

8-((1*R*,4*R*)-4-Aminocyclohexylamino)-5-bromo-*N*-(4-(benzyloxy)benzyl)-1,6-naphthyridine-7-carboxamide (1e). **1e** was prepared analogously using general method A, giving a light yellow solid, yield 56.2%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.66 (d, J = 8.1 Hz, 1H), 8.94 (d, 1H, J = 4.2 Hz), 8.41 (d, 1H, J = 10.2 Hz), 8.25 (t, J = 6.3 Hz, 1H), 7.58 (dd, 1H, J = 4.2, 10.2 Hz), 7.3–7.45 (m, 9H), 6.96 (d, 2H, J = 8.7 Hz), 5.06 (s, 1H), 4.94 (m, 1H), 4.56 (d, J = 6.3 Hz, 2H), 2.76 (m, 1H), 2.17 (d, 2H, J = 12.3 Hz), 1.9 (d, 2H, J = 13.5 Hz), 1.39 (m, 4H); MS-EI m/z: 559 $(M)^+$; HR-EIMS calcd for $C_{29}H_{30}BrN_5O_2$: 559.1583, found: 559.1588.

8-((1*R*,4*R*)-4-Aminocyclohexylamino)-5-bromo-*N*-(4-(cyclohexyloxy)-3-(trifluoromethyl)benzyl)-1,6-naphthyridine-7-carboxamide (1f). **1f** was prepared analogously using general method A, giving a light yellow solid, yield 61.8%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.61 (d, J = 8.4 Hz, 1H), 8.95 (d, J = 2.7 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 8.29 (t, J = 6.3 Hz, 1H), 7.59 (dd, J = 2.7, 8.7 Hz, 1H), 7.55 (s, 1H), 7.46 (d, J = 6.3 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 4.98 (m, 1H), 4.57 (d, J = 6.3 Hz, 2H), 4.39 (m, 1H), 2.83 (m, 1H), 2.19 (m, 2H), 1.86–1.97 (m, 5H), 1.51–1.54 (m, 3H), 1.25–1.45 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7, 155.2, 150.6, 145.0, 144.9, 136.3, 132.3, 129.7, 126.7, 126.6, 124.4, 124.3, 123.6, 114.3, 75.7, 53.7, 50.0, 42.1, 34.8, 33.2, 31.2, 25.5, 23.0; MS-ESI m/z: 620 $(M + H)^+$, 622 $(M + 2 + H)^+$; Anal. calcd for $C_{29}H_{33}BrF_3N_5O_2$: C 56.13, H 5.36, N 11.29, found: C 55.84, H 5.37, N 11.20%.

8-((1*R*,4*R*)-4-Aminocyclohexylamino)-5-bromo-*N*-((1*H*-indol-5-yl)methyl)-1,6-naphthyridine-7-carboxamide (1j). **1j** was prepared analogously using general method A, giving a light yellow solid, yield 36.3%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.7 (d, J = 9 Hz, 1H), 8.94 (d, J = 3.9 Hz, 1H), 8.41 (d, J = 7.8 Hz, 1H), 8.29 (t, J = 5.1 Hz, 1H), 8.19 (m, 1H), 7.66 (s, 1H), 7.57 (dd, J = 3.9, 7.8 Hz, 1H), 7.4 (d, J = 7.8 Hz, 1H), 7.23–7.26 (m, 1H), 6.55 (m, 1H), 4.97 (m, 1H), 4.73 (d, J = 5.1 Hz, 2H), 2.8 (m, 1H), 2.2 (d, J = 14.7 Hz, 2H), 1.95 (d, J = 12.9 Hz, 2H), 1.36–1.46 (m, 4H); MS-EI m/z: 492 $(M)^+$, 494 $(M + 2)^+$; Anal. Calcd for $C_{21}H_{23}BrN_6O{\cdot}2/5CF_3COOH{\cdot}3/4CH_3OH$: C 54.50, H 5.08, N 14.93, found: C 54.56, H 4.84, N 14.66%.

8-((1*R*,4*R*)-4-Aminocyclohexylamino)-5-bromo-*N*-(naphthalen-1-ylmethyl)-1,6-naphthyridine-7-carboxamide (1k). **1k** was prepared analogously using general method A, giving a light yellow solid, yield 30.5%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.69 (d, J = 8.1 Hz, 1H), 8.95 (d, J = 4.2 Hz, 1H), 8.4 (d, J = 8.7 Hz, 1H), 8.29 (t, J = 6 Hz, 1H), 8.12 (d, J = 7.5 Hz, 1H), 7.82–7.91 (m, 2H), 7.44–7.6 (m, 5H), 5.10 (d, J = 6 Hz, 2H), 4.98 (m, 1H), 2.82 (m, 1H), 2.21 (d, J = 12 Hz, 2H), 1.96 (d, J = 11.4 Hz, 2H), 1.29–1.52 (m, 4H); MS-EI m/z: 503 $(M)^+$, 505 $(M + 2)^+$; Anal. Calcd for $C_{26}H_{26}BrN_5O$: C 61.91, H 5.20, N 13.88, found: C 61.80, H 5.55, N 13.73%.

General procedure for the preparation of 5-propiolate ester or β-keto ester-1,6-naphthyridine-7-carboxamide compounds (1l–1m). **Method B. 7l** was prepared analogously following Method A, giving a light yellow solid, yield 74.2%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.58 (d, J = 8.1 Hz, 1H), 8.88 (d, J = 4.8 Hz, 1H), 8.41 (t, J = 6.6 Hz, 1H), 8.25 (d, J = 9.9 Hz, 1H), 7.68 (s, 1H), 7.58 (dd, J = 4.8, 9.9 Hz, 1H), 7.46–7.52 (m, 2H), 4.96 (m, 1H), 4.65 (d, J = 6.6 Hz, 2H), 4.39 (br s, 1H), 3.48 (m, 1H), 2.18 (d, J = 12.6 Hz, 2H), 2.06 (d, J = 13.8 Hz, 2H), 1.44 (s, 9H), 1.23–1.38 (m, 4H).

7l (0.104 g, 0.15 mmol), $Pd(PPh_3)_2Cl_2$ (4 mg), CuI (2 mg), and anhydrous potassium carbonate (50 mg, 0.36 mmol) were dissolved in dry THF (3 mL) under nitrogen. Methyl propiolate (60 μL) was added and the reaction mixture was heated to 65 °C and stirred for 12 h. After the reaction was completed, the reaction solution was concentrated. The residue was dissolved in dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with PE : EtOAc (1 : 1) to give 5-propiolate ester compound as a light yellow solid 76 mg, yield 77.8%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.35 (d, J = 8.4 Hz, 1H), 8.93 (d, J = 4.2 Hz, 1H), 8.67 (t, J = 6.6 Hz, 1H), 8.51 (d, J = 8.1 Hz, 1H), 7.68 (s, 1H), 7.60 (dd, J = 4.2, 8.1 Hz, 1H), 7.46–7.52 (m, 2H), 5.11 (m, 1H), 4.63 (d, J = 6.6 Hz, 2H), 4.42 (br s, 1H), 3.88 (s, 3H), 3.49 (m, 1H), 2.24 (d, J = 10.2 Hz, 2H), 2.09 (d, J = 13.8 Hz, 2H), 1.45 (s, 9H), 1.25–1.38 (m, 4H).

The compound obtained from the last step was dissolved in dry DCM (2 mL) and cooled to 0 °C under nitrogen. TFA (0.2 mL) was added dropwise over 3 min. The ice bath was removed and the reaction solution was allowed to warm slowly to room temperature. After the reaction was completed, the reaction mixture was diluted dichloromethane (20 mL). The DCM layer was washed with saturated $NaHCO_3$ solution (20 mL × 2) and saturated NaCl solution (20 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with CH_2Cl_2 : CH_3OH (10 : 1) to give **1l** as a light yellow solid 15 mg, yield: 59.7%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.32 (d, J = 8.4 Hz, 1H), 8.94 (d, J = 6 Hz, 1H), 8.68 (t, J = 6 Hz, 1H), 8.52 (d, J = 6.3 Hz, 1H), 7.69 (s, 1H), 7.60 (dd, J = 6, 6.3 Hz, 1H), 7.46–7.53 (m, 2H), 5.14 (m, 1H), 4.63 (d, J = 6 Hz, 2H), 3.88 (s, 3H), 2.81 (m, 1H), 2.24 (d, J = 12.3 Hz, 2H), 1.96 (d, J = 13.5 Hz, 2H), 1.33–1.51 (m, 4H); MS-EI m/z: 559 $(M)^+$, 561 $(M + 2)^+$; Anal. calcd for $C_{27}H_{25}ClF_3N_5O_3{\cdot}1/3H_2O$: C 57.30, H 4.57, N 12.37, found: C 57.55, H 4.78, N 12.07%.

But if the deprotecting condition was changed to be stirred at 30 °C, **1m** was produced as a tan solid, yield 84.9%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.72 (d, J = 8.4 Hz, 1H), 9.68 (d, J = 8.7 Hz, 1H), 8.91 (d, J = 3.9 Hz, 1H), 8.83 (t, J = 6.9 Hz, 1H), 7.80 (s, 1H), 7.56–7.63 (m, 2H), 7.48 (d, J = 8.4 Hz, 2H), 5.20 (m, 1H), 4.66 (d, J = 6.9 Hz, 2H), 3.92 (s, 2H), 3.54 (s, 3H), 2.83 (m, 1H), 2.25 (d, J = 11.1 Hz, 2H), 1.98 (d, J = 11.4 Hz, 2H), 1.38–1.49 (m, 4H); MS-EI m/z: 577 $(M)^+$, 579 $(M + 2)^+$; Anal. calcd for $C_{27}H_{27}ClF_3N_5O_4 \cdot 1/10CF_3COOH \cdot 4/5H_2O$: C 54.11, H 4.79, N 11.60, found: C 54.32, H 5.06, N 11.24%.

Methyl 5-bromo-8-(tosyloxy)-1,6-naphthyridine-7-carboxylate (8). Following the procedure as compound **6a**, methylbenzenesulfonyl chloride (0.302 g, 1.58 mmol), methyl 5-bromo-8-hydroxy-1,6-naphthyridine-7-carboxylate (**4a**) (0.120 g, 0.42 mmol), TEA (0.5 mL), gave compound **8a** as a brown solid 140 mg (yield: 76.2%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (d, J = 4.2 Hz, 1H), 8.59 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.70 (dd, J = 4.2, 7.8 Hz, 1H), 7.34 (d, J = 8.1 Hz, 2H), 3.83 (s, 3H), 2.47 (s, 3H).

5-Bromo-8-(((1*R*,4*R*)-4-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)-1,6-naphthyridine-7-carboxylic acid (9a). Following the procedure as compound **1a**, *tert*-butyl (1*r*,4*r*)-4-aminocyclohexylcarbamate (0.208 g, 0.97 mmol), compound **8** (142 mg, 0.32 mmol), TEA (0.15 mL), gave a light yellow solid 63 mg (yield: 41.1%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (d, J = 3.9 Hz, 1H), 8.88 (br, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.64 (dd, J = 3.9, 8.4 Hz, 1H), 4.93 (m, 1H), 4.40 (br, 1H), 3.98 (s, 3H), 3.5 (m, 1H), 2.20 (d, J = 11.4 Hz, 2H), 2.08 (d, J = 13.5 Hz, 2H), 1.45 (s, 9H), 1.25–1.42 (m, 4H).

The compound obtained from the last step (0.052 g, 0.11 mmol) was dissolved in 1 N LiOH (2 mL) and THF (2 mL). The solution was stirred under nitrogen at rt. After the reaction was complete, the reaction mixture was concentrated. The residue was then partitioned between H_2O (10 mL) and dichloromethane (10 mL). The pH of the H_2O phase was adjusted to 3 and was extracted with DCM (10 mL × 2). The DCM layer was dried over Na_2SO_4 and evaporated to give crude **9a** as a tan solid 0.051 g (yield: 99.6%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.75 (br s, 1H), 8.99 (d, J = 5.7 Hz, 1H), 8.49 (d, J = 10.2 Hz, 1H), 7.68 (dd, J = 5.7, 10.2 Hz, 1H), 5.03 (m, 1H), 4.40 (br s, 1H), 3.49 (m, 1H), 2.20 (d, J = 13.8 Hz, 2H), 2.09 (d, J = 13.2 Hz, 2H), 1.45 (s, 9H), 1.25–1.41 (m, 4H); MS-EI m/z: 464 $(M)^+$, 466 $(M + 2)^+$.

tert-Butyl ((1*R*,4*R*)-4-(5-bromo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-1-yl)cyclohexyl)carbamate (10a). **9a** (108 mg, 0.23 mmol), DPPA (0.06 ml, 0.25 mmol), and TEA (0.05 ml) were dissolved in anhydrous toluene (2 mL). The reaction mixture was heated to 90 °C. After the reaction was completed, the mixture was filtered to give crude **10a** as a brown–yellow solid 89 mg (yield: 83.9%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (d, J = 3.9 Hz, 1H), 8.58 (d, J = 8.7 Hz, 1H), 7.47 (dd, J = 3.9, 8.7 Hz, 1H), 4.47 (br s, 1H), 2.19 (d, J = 12.3 Hz, 2H), 1.87 (d, J = 11.7 Hz, 2H), 1.48 (s, 9H), 1.30–1.42 (m, 4H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 154.9, 152.8, 139.6, 137.4, 133.3, 121.3, 119.3, 115.7, 77.5, 48.3, 32.0, 28.3, 28.0; MS-EI m/z: 461 $(M)^+$, 463 $(M + 2)^+$.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one (2a). **11a** (75 mg, 0.16 mmol) was deprotected in 20% TFA–DCM solution (4 ml) to give **2a** as a light yellow solid 45 mg (yield: 77.6%): ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.07 (d, J = 3.0 Hz, 1H), 8.56 (d, J = 6.9 Hz, 1H), 7.54 (dd, J = 3.0, 6.9 Hz, 1H), 5.25 (br s, 1H), 2.85 (t, 1H), 2.66 (d, J = 12 Hz, 2H), 1.96 (d, J = 11.1 Hz, 2H), 1.74 (d, J = 10.2 Hz, 2H), 1.31 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO): δ 154.1, 140.1, 135.2, 120.2, 116.7, 49.2, 34.4, 29.9; MS-ESI m/z: 362 $(M + H)^+$; HR-ESI MS calcd for $C_{15}H_{16}BrN_5O_3$ $(M + H)^+$: 362.0616, found: 362.0620.

General procedure for the preparation of 1-substituted-3-(3,4-dichlorobenzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one compounds (2b–2e). **Method C. 2b. 10a** (91 mg, 0.2 mmol), anhydrous K_2CO_3 (55 mg, 0.4 mmol) and 4-(bromomethyl)-1,2-dichlorobenzene (0.05 mL, 0.3 mmol) were added to DMF (2 mL). The mixture was stirred at room temperature for 12 h. After the reaction was completed, the reaction mixture was diluted with dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with PE : EtOAc (2 : 1) to give compound **2b** as a white solid 113 mg (yield: 90.9%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02 (d, J = 3.9 Hz, 1H), 8.57 (d, J = 9.0 Hz, 1H), 7.62 (s, 1H), 7.44 (dd, J = 3.9, 9.0 Hz, 1H), 7.38 (s, 2H), 5.18 (s, 2H), 4.43 (br s, 1H), 3.71 (br s, 1H), 2.88 (br s, 1H), 2.17 (d, J = 12.6 Hz, 2H), 1.85 (d, J = 11.4 Hz, 2H), 1.47 (s, 9H), 1.21–1.40 (m, 4H); MS-ESI m/z: 622$(M + H)^+$.

5-Bromo-1-cyclohexyl-3-(3,4-dichlorobenzyl)-1*H*-imidazo[4,5-*h*]-[1,6]naphthyridin-2(3*H*)-one (2c). **2c** was prepared analogously with general method C, giving a light yellow solid, yield 82.3%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (d, J = 4.2 Hz, 1H), 8.54 (d, J = 8.7 Hz, 1H), 7.62 (s, 1H), 7.42 (dd, J = 4.2, 8.7 Hz, 1H), 7. 38 (d, J = 0.9 Hz, 2H), 5.17 (s, 2H), 2.64 (m, 1H), 1.89 (m, 4H), 1.71 (m, 2H), 1.46 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.7, 152.5, 139.0, 138.6, 137.8, 136.6, 134.5, 132.6, 131.9, 130.6, 130.6, 128.1, 120.6, 42.7, 31.2, 29.9, 26.1, 25.2; MS-EI m/z 505 $(M)^+$; HR-EI MS calcd for $C_{22}H_{19}BrCl_2N_4O$ $(M)^+$: 505.2225, found: 505.2221.

1-Benzyl-5-bromo-3-(3,4-dichlorobenzyl)-1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one (2d). **2d** was prepared similar to the general method C, giving a light yellow solid, yield 72.3%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (d, J = 4.2 Hz, 1H), 8.52 (d, J = 8.7 Hz, 1H), 7.56 (m, 3H), 7.42 (dd, J = 4.2, 8.7 Hz, 1H), 7.37 (m, 2H), 5.78 (s, 2H), 5.21 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.1, 153.1, 138.7, 138.6, 137.7, 137.4, 136.4, 135.5, 134.7, 132.6, 132.1, 130.6, 130.5, 128.4, 128.0, 127.7, 121.0, 120.7, 115.5, 46.3, 42.9; MS-EI m/z 513 $(M)^+$; HR-EI MS calcd for $C_{23}H_{15}BrCl_2N_4O$ $(M)^+$: 513.2015, found: 513.2010.

5-Bromo-1-cyclopropyl-3-(3,4-dichlorobenzyl)-1*H*-imidazo[4,5-*h*]-[1,6]naphthyridin-2(3*H*)-one (2e). **2e** was prepared according to the procedure described in the general method C, giving a light yellow solid, yield 86.3%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02 (d, J = 4.2 Hz, 1H), 8.57 (d, J = 8.4 Hz, 1H), 7.64 (s, 1H), 7.45 (dd, J = 4.2, 8.4 Hz, 1H), 7.37 (d, J = 1.2 Hz, 2H), 5.16 (s,

2H), 3.49 (m, 1 H), 1.56 (m, 2 H), 1.25 (m, 2 H); MS-EI *m/z*: 464 $(M)^+$; HR-EI MS calcd for $C_{19}H_{13}BrCl_2N_4O$ $(M)^+$: 463.9645, found: 463.9640.

General procedure for the preparation of 1-((1*R*,4*R*)-4-aminocyclohexyl)-3-(substituted-benzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one compounds (2f–2l). **Method D. 2f. 2b** (91 mg, 0.15 mmol) was deprotected in 20% TFA–DCM solution (4 ml) to give **14b** as a light yellow solid 55 mg (yield: 70.6%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.97 (d, J = 2.7 Hz, 1H), 8.56 (d, J = 10.5 Hz, 1H), 7.61 (s, 1H), 7.43 (dd, J = 2.7, 10.5 Hz, 1H), 7.38 (s, 2H), 5.17 (s, 2H), 2.97 (t, 1H), 2.74 (m, 2H), 2.03 (d, J = 12.0 Hz, 2H), 1.89 (d, J = 16.2 Hz, 2H), 1.35–1.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.8, 152.4, 138.9, 137.9, 136.4, 134.7, 132.5, 131.9, 130.5, 128.1, 120.7, 120.6, 49.4, 42.6, 35.5, 28.2; MS-EI *m/z*: 519 $(M)^+$, 521 $(M + 2)^+$; HR-EI MS calcd for $C_{22}H_{20}BrCl_2N_5O$ $(M)^+$: 519.0228, found: 519.0229; Anal. calcd for $C_{22}H_{21}BrFN_5O{\cdot}1/4CF_3COOH$: C 49.16, H 3.71, N 12.74, found: C 49.28, H 3.75, N 12.77%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-benzyl-5-bromo-1*H*-imidazo[4,5-*h*]-[1,6]naphthyridin-2(3*H*)-one (2g). **2g** was prepared as described above in the general method D, giving a light yellow solid, yield 44.8%: ^{1}H NMR (300 MHz, CD_3OD): δ 9.06 (d, J = 3.6 Hz, 1H), 8.68 (d, J = 8.4 Hz, 1H), 7.60 (m, 1H), 7.43 (d, J = 6.9 Hz, 2H), 7.32 (m, 3H), 5.24 (s, 2H), 2.84 (m, 2H), 2.21 (d, J = 14.7 Hz, 2H), 2.02 (d, J = 8.1 Hz, 2H), 1.59–1.72 (m, 2H); MS-EI *m/z*: 451 $(M)^+$, 453 $(M + 2)^+$.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(4-fluorobenzyl)-5-bromo-1*H*-imidazo-[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2h). **2h** was prepared as described above in the general method D, giving a light yellow solid, yield 61.4%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (d, J = 3.9 Hz, 1H), 8.56 (d, J = 7.5 Hz, 1H), 7.53 (t, J = 9.0 Hz, 2H), 7.42 (dd, J = 3.9, 7.5 Hz, 1H), 6.99 (t, J = 8.4 Hz, 2H), 5.20 (s, 2H), 2.96 (t, 1H), 2.75 (m, 2H), 2.03 (d, J = 13.2 Hz, 2H), 1.87 (d, J = 9.9 Hz, 2H), 1.34–1.46 (m, 2H); MS-ESI *m/z*: 470 $(M + H)^+$; Anal. calcd for $C_{22}H_{21}BrFN_5O{\cdot}1/4CF_3COOH$: C 54.17, H 4.29, N 14.04, found: C 54.20, H 4.32, N 14.03%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3-fluoro-4-methoxybenzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2i). **2i** was prepared as described above in the general method D, giving a light yellow solid, yield 82.6%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (d, J = 3.9 Hz, 1H), 8.55 (d, J = 8.7 Hz, 1H), 7.42 (dd, J = 3.9, 8.7 Hz, 1H), 7.29 (d, J = 3.9 Hz, 1H), 7.26 (m, 1H), 6.90 (t, J = 8.7 Hz, 1H), 5.15 (s, 2H), 3.84 (s, 3H), 2.94 (t, J = 11.1 Hz, 1H), 2.74 (m, 2H), 2.00 (d, J = 12.0 Hz, 2H), 1.86 (d, J = 11.1 Hz, 2H), 1.31–1.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.7, 153.3, 150.8, 147.3, 147.2, 139.2, 138.4, 137.9, 134.6, 129.4, 129.3, 124.7, 120.6, 120.5, 116.6, 116.4, 113.2, 56.1, 49.5, 42.9, 35.8, 28.3; MS-ESI *m/z*: 500 $(M + H)^+$; Anal. calcd for $C_{23}H_{23}BrFN_5O_2{\cdot}3/2H_2O$: C 52.38, H 4.97, N 13.28, found: C 52.42, H 4.73, N 13.06%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3-(trifluoromethyl)benzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2j). **2j** was prepared as described above in the general method D, giving a light yellow solid, yield 85.4%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (d, J = 2.7 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 7.82 (s, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.46 (m, 2H), 5.28 (s, 2H), 2.98 (t, J = 10.2 Hz, 1H), 2.76 (m, 2H), 2.04 (d, J = 11.7 Hz, 2H), 1.88 (d, J = 13.8 Hz, 2H), 1.36–1.48 (m, 2H); MS-EI *m/z*: 519 $(M)^+$, 521 $(M + 2)^+$; HR-EI MS calcd for $C_{23}H_{21}BrF_3N_5O$ $(M)^+$: 519.0882, found: 519.0878; Anal. calcd for $C_{23}H_{21}BrF_3N_5O{\cdot}1/6CF_3COOH$: C 51.96, H 3.96, N 12.98, found: C 51.98, H 3.88, N 12.93%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(4-chlorobenzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2k). **2k** was prepared as described above in the general method D, giving a light yellow solid, yield 60%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.97 (d, J = 3.9 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.44 (dd, J = 3.9, 8.7 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 5.20 (s, 2H), 3.00 (t, J = 11.7 Hz, 1H), 2.75 (m, 2H), 2.05 (d, J = 13.2 Hz, 2H), 1.88 (d, J = 12.9 Hz, 2H), 1.38–1.50 (m, 2H); MS-ESI *m/z*: 488 $(M + H)^+$; HR-ESI MS calcd for $C_{22}H_{21}BrClN_5O$ $(M + Na)^+$: 508.0516, found: 508.0503; Anal. calcd for $C_{22}H_{21}BrClN_5O{\cdot}1/6CF_3COOH$: C 53.03, H 4.22, N 13.85, found: C 53.19, H 4.25, N 13.76%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(2-chlorobenzyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2l). **2l** was prepared as described above in the general method D, giving a light yellow solid, yield 48.6%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.00 (d, J = 2.7 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 7.38–7.47 (m, 2H), 7.13–7.23 (m, 2H), 7.06 (d, J = 6.0 Hz, 1H), 5.39 (s, 2H), 3.03 (t, J = 12.6 Hz, 1H), 2.81 (m, 2H), 2.09 (d, J = 10.8 Hz, 2H), 1.93 (d, J = 10.5 Hz, 2H), 1.42–1.54 (m, 2H); MS-ESI *m/z*: 488 $(M + H)^+$; Anal. calcd for $C_{22}H_{21}BrClN_5O{\cdot}1/3CF_3COOH$: C 51.88, H 4.10, N 13.34, found: C 52.10, H 4.36, N 13.17%.

General procedure for the preparation of 1-((1*R*,4*R*)-4-aminocyclohexyl)-3-(substituted-sulfonyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]-naphthyridin-2(3*H*)-one compounds (2m–2n). **Method E. 2m**. A solution of **10a** (69 mg, 0.15 mmol), TsCl (71 mg, 0.3 mmol) and TEA (0.05 ml) in DCM (4 mL) was refluxed for 12 h. After the reaction was complete, the reaction mixture was diluted with dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with PE : EtOAc (4 : 1) to give a light yellow solid 50 mg (yield: 54.1%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02 (d, J = 3.3 Hz, 1H), 8.59 (d, J = 8.7 Hz, 1H), 8.21 (d, J = 8.1 Hz, 2H), 7.52 (dd, J = 3.3, 8.7 Hz, 1H), 7.36 (d, J = 8.4 Hz, 2H), 4.42 (br s, 1H), 3.64 (br s, 1H), 2.76 (m, 2H), 2.42 (s, 3H), 2.14 (d, J = 12.9 Hz, 2H), 1.80 (d, J = 12.9 Hz, 2H), 1.46 (s, 9H), 1.25–1.40 (m, 2H); MS-ESI *m/z*: 618 $(M + H)^+$.

The light yellow solid (50 mg, 0.08 mmol) was deprotected in 20% TFA–DCM solution (2 ml) to give **2m** as a light yellow solid 32 mg (yield: 77.5%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.99 (d, J = 2.4 Hz, 1H), 8.58 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.1 Hz, 2H), 7.52 (dd, J = 2.4, 8.4 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 2.97 (t, J = 12.3 Hz, 1H), 2.66 (m, 2H), 2.05 (d, J = 10.5 Hz, 2H), 1.83 (d, J = 11.4 Hz, 2H), 1.35–1.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.0, 146.0, 137.8, 135.1, 129.8, 128.6, 122.1, 49.4, 34.6, 27.6, 21.7; MS-ESI *m/z*: 518 $(M + H)^+$; Anal. calcd for $C_{22}H_{22}BrFN_5O_3S{\cdot}1/4CF_3COOH$: C 49.59, H 4.12, N 12.85, found: C 49.48, H 4.10, N 12.84%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-((3,4-dichlorophenyl)sulfonyl)-5-bromo-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2n). **2n** was prepared as described above in the general method E, giving a light yellow solid, yield 43.8%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.01 (d, J = 4.2 Hz, 1H), 8.61 (d, J = 8.7 Hz, 1H), 8.46 (d, J = 2.1 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.55 (dd, J = 4.2, 8.7 Hz, 1H), 2.94 (m, 1H), 2.64 (m, 2H), 2.03 (d, J = 12.3 Hz, 2H), 1.88 (m, 2H), 1.33–1.45 (m, 2H); MS-ESI m/z: 572 $(M + H)^+$; Anal. calcd for $C_{21}H_{18}BrCl_2N_5O_3S{\cdot}2/H_2O{\cdot}1/10CF_3COOH$: C 43.17, H 3.23, N 11.87, found: C 43.43, H 3.52, N 11.61%.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-hydroxy-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2o). **2f** (90 mg, 0.15 mmol) and K_2CO_3 (55 mg, 0.4 mmol) were added to DMF (2 mL). The mixture was stirred at 120 °C. After the reaction was complete, the reaction mixture was diluted with dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel to give a white solid 110 mg, then deprotected in 20% TFA–DCM solution (4 ml) to give **2o** as a light yellow solid 39 mg (yield: 56.7% in tow steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (d, J = 2.5 Hz, 1H), 8.52 (d, J = 8.8 Hz, 1H), 7.52 (d, J = 1.9 Hz, 1H), 7.41 (dd, J = 8.7, 4.2 Hz, 1H), 7.33–7.26 (m, 2H), 5.09 (s, 2H), 3.31–3.17 (m, 1H), 2.86–2.62 (m, 2H), 2.29–2.01 (m, 2H), 1.95–1.79 (m, 2H), 1.72–1.47 (m, 2H); MS-EI m/z: 457 $(M)^+$; HR-EI MS calcd for $C_{22}H_{21}Cl_2N_5O_2$ $(M)^+$: 457.1072, found: 457.1040.

General procedure for the preparation of 1,3,5-trisubstituted-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one compounds (2p–2v). **Method F. 2p**: A solution of **2f** (91 mg, 0.15 mmol), anhydrous K_2CO_3 (55 mg, 0.4 mmol) and 2-hydroxypyridine (0.05 mL, 0.3 mmol) in DMF (2 mL) was stirred at 110 °C for 12 h. After the reaction was complete, the reaction mixture was diluted with dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL × 1). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel to give a white solid 113 mg, then deprotected in 20% TFA–DCM solution (4 ml) to give **2p** as a light yellow solid 62 mg (overall yield of 77.6% for two steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.07 (d, J = 4.2 Hz, 1H), 8.82 (d, J = 8.6 Hz, 1H), 8.57 (d, J = 2.5 Hz, 1H), 8.53 (d, J = 4.8 Hz, 1H), 7.78 (d, J = 7.0 Hz, 1H), 7.63–7.51 (m, 2H), 7.38 (d, J = 8.3 Hz, 1H), 7.35 (d, J = 1.9 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 4.91 (s, 2H), 2.96–2.80 (m, 2H), 2.31–2.18 (m, 2H), 2.07–1.96 (m, 2H), 1.78–1.58 (m, 2H); MS-EI m/z: 534 $(M)^+$; HR-EI MS calcd for $C_{27}H_{24}Cl_2N_6O_2$ $(M)^+$: 534.1342, found: 534.1356.

4-((1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-5-yl)oxy)benzaldehyde (2q). **2q** was prepared as described above in the general method E, giving a light yellow solid, yield 63.4%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.04 (s, 1H), 9.07 (d, J = 4.3 Hz, 1H), 8.80 (d, J = 8.7 Hz, 1H), 8.05 (d, J = 8.4 Hz, 2H), 7.90 (s, 1H), 7.57–7.53 (m, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.37 (s, 1H), 7.13 (d, J = 11.3 Hz, 1H), 4.94 (s, 2H), 3.00–2.80 (m, 2H), 2.30–2.16 (m, 2H), 2.12–1.96 (m, 2H), 1.77–1.59 (m, 2H); MS-EI m/z: 561 $(M)^+$; HR-EI MS calcd for $C_{29}H_{25}Cl_2N_5O_3$ $(M)^+$: 561.2353, found: 561.2359.

4-((1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-5-yl)oxy)-2-chlorobenzaldehyde (2r). **2r** was prepared as described above in the general method E, giving a light yellow solid, yield 65.5%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.04 (s, 1H), 9.12–9.03 (m, 1H), 8.74 (d, J = 8.5 Hz, 1H), 8.15–8.05 (m, 1H), 7.24–7.20 (m, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.30–7.27 (m, 1H), 7.14–7.03 (m, 1H), 4.86 (s, 2H), 4.57–4.34 (m, 2H), 3.90–3.60 (m, 2H), 3.02–2.85 (m, 2H), 2.26–2.06 (m, 2H), 1.43–1.37 (m, 2H); MS-EI m/z: 595 $(M)^+$; HR-EI MS calcd for $C_{29}H_{24}Cl_3N_5O_3$ $(M)^+$: 595.1047, found: 595.1042.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(hydroxymethyl)phenoxy)-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2s). **2s** was prepared as described above in the general method E, giving a light yellow solid, yield 72.6%: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.03 (d, J = 4.0 Hz, 1H), 8.78 (d, J = 8.5 Hz, 1H), 7.86 (s, 1H), 7.50 (d, J = 7.8 Hz, 3H), 7.42–7.35 (m, 1H), 7.21 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.2 Hz, 1H), 4.88 (s, 2H), 4.70 (s, 2H), 3.03–2.94 (m, 1H), 2.84–2.75 (m, 1H), 2.11–2.04 (m, 2H), 1.93–1.88 (m, 2H), 1.55–1.36 (m, 4H); MS-EI m/z: 563 $(M)^+$; HR-EI MS calcd for $C_{29}H_{27}Cl_2N_5O_3$ $(M)^+$: 563.1491, found: 563.1495.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(piperidine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2t). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.12 (d, J = 5.5 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 7.60 (d, J = 8.5 Hz, 3H), 7.47 (d, J = 5.8 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 9.5 Hz, 1H), 4.98 (s, 2H), 3.80–3.75 (m, 1H), 3.56–3.46 (m, 1H), 3.03–2.82 (m, 3H), 2.31–2.22 (m, 2H), 2.11–2.00 (m, 2H), 1.84–1.68 (m, 6H), 1.68–1.50 (m, 4H), 1.40–1.26 (m, 4H); MS-EI m/z: 644 $(M)^+$; HR-EI MS calcd for $C_{34}H_{34}Cl_2N_6O_3$ $(M)^+$: 644.2069, found: 644.2072.

1-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(morpholine-4-carbonyl)phenoxy)-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-2(3*H*)-one (2u). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (d, J = 5.7 Hz, 1H), 8.61 (d, J = 8.2 Hz, 1H), 7.64 (s, 1H), 7.56–7.49 (m, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.40–7.35 (m, 1H), 5.15 (s, 2H), 4.63 (t, J = 6.2 Hz, 2H), 3.78 (t, J = 6.3 Hz, 2H), 3.69–3.50 (m, 4H), 3.18 (dd, J = 14.6, 7.3 Hz, 4H), 2.19 (s, 1H), 2.12–2.05 (m, 2H), 1.98–1.94 (m, 1H), 1.32–1.25 (m, 4H), 1.20–1.11 (m, 4H); MS-EI m/z: 646 $(M)^+$; HR-EI MS calcd for $C_{33}H_{32}Cl_2N_6O_4$ $(M)^+$: 646.1860, found: 646.1862.

(*R*)-4-((1*R*,4*R*)-4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*h*][1,6]naphthyridin-5-yloxy)-*N*-(1-phenylethyl)benzamide (2v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.958 (d, J = 3.9, 1 H), 8.557 (d, J = 8.4, 1 H), 7.584 (m, 3 H), 7.425 (dd, J = 3.9, 8.4, 1 H), 7.368 (m, 2 H), 7.132 (m, 1 H), 5.183 (s, 2 H), 2.142 (d, J = 7.2, 3 H); MS-EI m/z: 680 $(M)^+$; HR-EI MS calcd for $C_{37}H_{34}Cl_2N_6O_3$ $(M)^+$: 680.2069, found: 680.2071.

General procedure for the preparation of 1*H*-imidazo[4,5-*b*]-pyridin-2(3*H*)-one compounds (3a–3j). Method G. 6-Chloro-*N*-(3,4-dichlorobenzyl)-3-nitropyridin-2-amine (13). To a solution of

2-amino-6-chloro-3-nitropyridine (500 mg, 2.9 mmol) and 4-(bromomethyl)-1,2-dichlorobenzene (0.5 mL, 3.0 mmol) in anhydrous DMF (5 mL) was added a 60% mineral oil dispersion of sodium hydride (412.5 mg, 3.0 mmol) in several portions. The mixture was stirred at room temperature for 6 h. After the reaction was complete, the reaction mixture was diluted with dichloromethane (20 mL). The DCM layer was washed with saturated NH_4Cl solution (20 mL × 2) and saturated NaCl solution (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with PE : EtOAc to give compound **13** as a light yellow solid 789 mg (yield: 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.18 (d, J = 8.2 Hz, 1H), 6.81 (d, J = 7.7 Hz, 1H), 6.52 (d, J = 7.7 Hz, 1H), 4.53 (s, 2H); EI MS m/z 331 [M^+].

Methyl 4-((6-((3,4-dichlorobenzyl)amino)-5-nitropyridin-2-yl)oxy)-benzoate (14). A solution of **13** (150 mg, 0.45 mmol), anhydrous K_2CO_3 (165 mg, 1.2 mmol) and methyl 4-hydroxybenzoate (137 mg, 0.9 mmol) in DMF (2 mL) was stirred at 80 °C for 12 h. After the reaction was completed, the reaction mixture was diluted with EtOAc (80 mL). Usual work-up with the organic layer and purification of the residue by column chromatography on silica gel gave a white solid 210.6 mg, (yield: 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.29 (d, J = 8.6 Hz, 1H), 8.01 (d, J = 8.8 Hz, 2H), 7.59–7.51 (m, 2H), 7.23 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.6 Hz, 1H), 5.36 (s, 2H), 3.87 (s, 3H); EI MS m/z 447 [M^+].

Methyl 4-((5-amino-6-((3,4-dichlorobenzyl)amino)pyridin-2-yl)-oxy)benzoate (15a). To a solution of **14** (200 mg, 0.45 mmol) and $NiCl_2{\cdot}6H_2O$ (124 mg, 0.90 mmol) in MeOH (5 mL) was added $NaBH_4$ (34.2 mg, 0.90 mmol) slowly at 0 °C. The mixture was stirred at room temperature for 1 h. After the reaction was completed, the reaction mixture was diluted with EtOAc (80 mL). Usual work-up and purification by column chromatography on silica gel afforded a white solid 171.1 mg, (yield: 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 2H), 7.57–7.48 (m, 2H), 7.46 (s, 1H), 7.19 (d, J = 8.6 Hz, 1H), 7.06 (d, J = 8.3 Hz, 2H), 6.75 (d, J = 8.1 Hz, 1H), 4.88 (s, 2H); EI MS m/z 417 [M^+].

Methyl 4-((3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo-[4,5-*b*]pyridin-5-yl)oxy)benzoate (18a). To a solution of **15a** (200 mg, 0.48 mmol) and a catalytic amount of DMF (50 μL) in THF (5 mL) was added triphosgene (60 mg, 0.20 mmol) slowly at rt. The mixture was stirred at room temperature for 10 min. After the reaction was complete, the reaction mixture was diluted with EtOAc (80 mL). Usual work-up and purification by column chromatography on silica gel gave a white solid 208.3 mg (yield: 98%): ^{1}H NMR (300 MHz, $CDCl_3$) 8.28 (s, 1H), 8.09 (d, J = 15.0 Hz, 1H), 7.81–7.71 (m, 2H), 7.65 (d, J = 15.0 Hz, 1H), 7.40 (d, J = 3.1 Hz, 1H), 7.20 (dd, J = 14.9, 3.0 Hz, 1H), 7.03–6.93 (m, 2H), 6.58 (d, J = 15.0 Hz, 1H), 4.70 (s, 2H), 3.90 (s, 3H); EI MS m/z 443 [M^+].

4-((3-(3,4-Dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]-pyridin-5-yl)oxy)benzoic acid (19a). The solution of **18a** (200 mg, 0.45 mmol) and LiOH (184.5 mg, 4.5 mmol) in THF–H_2O (2 : 1, 10 mL) was stirred at rt for 12 h. Then the reaction mixture was quenched with aqueous NH_4Cl (50 mL) and extracted with EtOAc (3 × 20 mL). The extracts were washed with H_2O (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel to give a white solid 185.3 mg (yield: 96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.09 (d, J = 15.0 Hz, 1H), 7.97–7.89 (m, 2H), 7.65 (d, J = 15.0 Hz, 1H), 7.40 (d, J = 3.1 Hz, 1H), 7.20 (dd, J = 15.0, 3.1 Hz, 1H), 7.11–7.04 (m, 2H), 6.58 (d, J = 15.0 Hz, 1H), 4.70 (s, 2H); EI MS m/z 429 [M^+].

N-Cyclopropyl-4-((3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-5-yl)oxy)benzamide (3a). To a solution of **19a** (60 mg, 0.14 mmol) and cyclopropylamine (15.9 mg, 0.28 mmol) in DCM (5 mL) was added EDCI (43.4 mg, 0.28 mmol) and HOBt (137 mg, 0.28 mmol). The mixture was stirred at rt for 4 h. After the reaction was complete, the reaction mixture was diluted with EtOAc (80 mL). Usual work-up of the organic layer and purification of the residue by column chromatography on silica gel gave a white solid 56.5 mg (yield: 86%): ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, J = 3.2 Hz, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.2 Hz, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 7.8, 1.8 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.0 Hz, 1H), 4.86 (s, 2H), 2.87–2.78 (m, 1H), 0.69 (dt, J = 7.1, 4.7 Hz, 2H), 0.56 (dt, J = 7.1, 4.7 Hz, 2H). MS-ESI m/z: 467.3 $(M)^-$; HR-ESI MS calcd for $C_{23}H_{18}Cl_2N_4O_3Na$ $(M + Na)^+$: 491.0654, found: 491.0678.

3-(3,4-Dichlorobenzyl)-5-(4-(piperidine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3b). White solid, yield 87.5%: ^{1}H NMR (300 MHz, DMSO) δ 11.26 (s, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.50 (d, J = 1.7 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 6.71 (dd, J = 8.1, 0.7 Hz, 1H), 4.89 (s, 2H), 1.67–1.59 (m, 2H), 1.56–1.46 (m, 3H); ESI MS m/z 497.3 [M] $^+$; HR-ESI MS calcd for $C_{25}H_{23}Cl_2N_4O_3$ $(M + H)^+$: 497.1674, found: 497.1682.

3-(3,4-Dichlorobenzyl)-5-(4-(morpholine-4-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3c). White solid, yield 84.7%: ^{1}H NMR (300 MHz, DMSO) δ 11.26 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 1.9 Hz, 1H), 7.47–7.39 (m, 2H), 7.18 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 4.88 (s, 2H), 3.66–3.53 (m, 4H), 3.34–3.26 (m, 4H); ESI MS m/z 499.3 [M]$^+$. HR-ESI MS calcd for $C_{24}H_{21}Cl_2N_4O_4$ $(M + H)^+$: 499.1801, found: 499.1822.

3-(3,4-Dichlorobenzyl)-5-(4-(piperazine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3d). White solid, yield 84.7%: ^{1}H NMR (300 MHz, DMSO) δ 11.26 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 1.9 Hz, 1H), 7.47–7.39 (m, 2H), 7.18 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 4.88 (s, 2H), 3.66–3.53 (m, 4H), 3.34–3.26 (m, 4H); ESI MS m/z 498.2 [M^+]. HR-ESI MS calcd for $C_{24}H_{22}Cl_2N_5O_3$ $(M + H)^+$: 498.1100, found: 498.1076.

(*R*)-4-((3-(3,4-Dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]-pyridin-5-yl)oxy)-*N*-(1-phenylethyl)benzamide (3e). White solid, yield 88.6%: ^{1}H NMR (300 MHz, DMSO) δ 8.76 (s, 1H), 7.98–7.88 (m, 2H), 7.56–7.31 (m, 2H), 7.20 (s, 2H), 7.07 (dd, J = 13.6, 9.2 Hz, 1H), 6.75–6.64 (m, 1H), 5.17 (t, J = 10.4 Hz, 1H), 4.86 (d, J = 9.6 Hz, 2H), 1.47 (t, J = 10.4 Hz, 3H); ESI MS m/z

531.4 $[M]^-$. HR-ESI MS calcd for $C_{28}H_{22}Cl_2N_4O_3Na$ $(M + Na)^+$: 555.0967, found: 555.0972.

(*S*)-4-((3-(3,4-Dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]-pyridin-5-yl)oxy)-*N*-(1-phenylethyl)benzamide (3f). White solid, yield 84.6%: ^{1}H NMR (300 MHz, DMSO) δ 9.34 (d, J = 7.7 Hz, 1H), 8.50 (d, J = 8.8 Hz, 2H), 8.12 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 8.2 Hz, 2H), 7.96 (d, J = 7.4 Hz, 2H), 7.89 (t, J = 7.4 Hz, 2H), 7.78 (dd, J = 13.7, 7.7 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.1 Hz, 1H), 5.80–5.70 (m, 1H), 5.44 (s, 2H), 2.05 (d, J = 7.0 Hz, 3H); ESI MS m/z 531.3 $[M]^-$. HR-ESI MS calcd for $C_{28}H_{22}Cl_2N_4O_3Na$ $(M + Na)^+$: 555.0967, found: 555.0970.

N-Cyclopropyl-4-((1-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-5-yl)oxy)benzamide (3g). White solid, yield 87.3%: ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, J = 4.1 Hz, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.31 (dd, J = 8.3, 2.1 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 8.2 Hz, 1H), 5.03 (s, 2H), 2.86–2.78 (m, 1H), 0.68 (dt, J = 7.1, 4.7 Hz, 2H), 0.55 (dt, J = 7.1, 4.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 166.77, 157.77, 156.58, 153.62, 141.50, 137.94, 131.17, 130.90, 130.23, 129.63, 129.61, 128.93, 127.82, 120.52, 118.96, 118.14, 104.12, 42.26, 23.00, 5.74. MS-ESI m/z: 467.2 $(M)^-$; HR-ESI MS calcd for $C_{23}H_{18}Cl_2N_4O_3Na$ $(M + Na)^+$: 491.0654, found: 491.0662.

1-(3,4-Dichlorobenzyl)-5-(4-(piperidine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3h). White solid, yield 92.3%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.42 (t, J = 8.5 Hz, 4H), 7.18 (d, J = 8.3 Hz, 1H), 7.08 (dd, J = 8.2, 3.5 Hz, 3H), 6.60 (d, J = 8.3 Hz, 1H), 5.01 (s, 2H), 3.85–3.61 (m, 2H), 3.52–3.30 (m, 2H), 1.77–1.62 (m, 4H), 1.62–1.46 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.10, 157.82, 156.31, 153.88, 140.78, 135.56, 133.08, 132.32, 131.27, 130.93, 129.35, 128.73, 126.81, 119.99, 119.61, 118.12, 104.80, 48.98, 48.96, 43.54, 26.46, 25.54, 24.41. ESI MS m/z 497.3 $[M]^+$; HR-ESI MS calcd for $C_{25}H_{23}Cl_2N_4O_3$ $(M + H)^+$: 497.1674, found: 497.1680.

1-(3,4-Dichlorobenzyl)-5-(4-(morpholine-4-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3i). White solid, yield 92.3%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 7.44–7.35 (m, 4H), 7.16 (d, J = 8.2 Hz, 1H), 7.07 (d, J = 8.4 Hz, 3H), 6.60 (d, J = 8.3 Hz, 1H), 4.99 (s, 2H), 3.79–3.65 (m, 4H), 3.65–3.44 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.97, 157.65, 156.67, 153.93, 140.89, 135.60, 133.10, 132.34, 130.95, 130.49, 129.36, 129.05, 126.83, 120.12, 119.66, 118.05, 104.90, 66.82, 43.55. MS-ESI m/z: 499.3 $(M)^+$; HR-ESI MS calcd for $C_{24}H_{20}Cl_2N_4O_4Na$ $(M + Na)^+$: 521.0757, found: 521.0759.

(*R*)-4-((1-(3,4-Dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]-pyridin-5-yl)oxy)-*N*-(1-phenylethyl)benzamide (3j). White solid, yield 88.3%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.40–7.36 (m, 2H), 7.35–7.28 (m, 3H), 7.21 (t, J = 7.2 Hz, 1H), 7.10 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 8.2 Hz, 1H), 5.21–5.12 (m, 1H), 5.03 (s, 2H), 1.47 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.83, 157.79, 156.65, 153.62, 144.93, 141.49, 137.93, 131.18, 130.90, 130.25, 129.76, 129.61, 129.17, 128.18, 127.82, 126.52, 125.98, 120.49, 119.05, 118.15, 104.06, 48.40, 42.27, 22.24. ESI MS m/z 531.3 $[M]^-$. HR-ESI MS calcd for $C_{28}H_{22}Cl_2N_4O_3Na$ $(M + Na)^+$: 555.0967, found: 555.0961.

Methyl 4-((1-(4-aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-5-yl)oxy)benzoate (3k). White solid, yield 78.3%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 7.14–7.11 (m, 1H), 7.08 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.3 Hz, 1H), 6.55 (d, J = 8.3 Hz, 1H), 4.97 (s, 2H), 4.42–4.26 (m, 1H), 3.85 (s, 3H), 3.05–2.92 (m, 1H), 2.46–2.26 (m, 2H), 2.20–2.05 (m, 2H), 1.90–1.77 (m, 2H), 1.67–1.45 (m, 2H). ESI MS m/z 541.2 $[M]^+$. HR-ESI MS calcd for $C_{27}H_{27}Cl_2N_4O_4$ $(M)^+$: 541.0363, found: 541.0371.

1-(4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(piperidine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3l). White solid, yield 82%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.41 (m, 1H), 7.40 (d, J = 8.3 Hz, 1H), 7.38 (d, J = 2.0 Hz, 2H), 7.15–7.09 (m, 3H), 7.02 (d, J = 8.3 Hz, 1H), 6.58 (d, J = 8.3 Hz, 1H), 4.96 (s, 2H), 4.35–4.21 (m, 1H), 3.66 (s, 3H), 3.44–3.30 (m, 2H), 2.79–2.63 (m, 1H), 2.32–2.15 (m, 2H), 2.12–1.98 (m, 2H), 1.81–1.72 (m, 2H), 1.72–1.60 (m, 4H), 1.60–1.47 (m, 4H). ESI MS m/z 594.3 $[M]^+$. HR-ESI MS calcd for $C_{31}H_{34}Cl_2N_5O_3$ $(M)^+$: 594.1033, found: 541.1041.

1-(4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(morpholine-4-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3m). White solid, yield 91%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J = 8.5 Hz, 2H), 7.42–7.35 (m, 2H), 7.17–7.09 (m, 3H), 7.02 (d, J = 8.3 Hz, 1H), 6.58 (d, J = 8.3 Hz, 1H), 4.96 (s, 2H), 4.35–4.18 (m, 1H), 3.79–3.62 (m, 4H), 3.49–3.35 (m, 4H), 2.76–2.60 (m, 1H), 2.32–2.12 (m, 2H), 2.09–1.92 (m, 2H), 1.84–1.67 (m, 2H), 1.62–1.41 (m, 2H). ESI MS m/z 596.3 $[M]^+$. HR-ESI MS calcd for $C_{30}H_{32}Cl_2N_5O_4$ $(M)^+$: 596.1831, found: 596.1843.

(*R*)-4-((1-(4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-5-yl)oxy)-*N*-(1-phenylethyl)benzamide (3n). White solid, yield 86%: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H), 7.47–7.38 (m, 4H), 7.36–7.31 (m, 2H), 7.27–7.16 (m, 1H), 7.16–7.11 (m, 2H), 7.11–7.05 (m, 1H), 6.55 (d, J = 8.2 Hz, 1H), 5.35–5.25 (m, 1H), 4.98 (s, 2H), 4.40–4.26 (m, 1H), 2.82–2.70 (m, 1H), 2.44–2.26 (m, 2H), 2.19–2.05 (m, 2H), 1.89–1.74 (m, 2H), 1.57 (s, 3H), 1.54–1.47 (m, 2H). ESI MS m/z 630.3 $[M]^+$. HR-ESI MS calcd for $C_{34}H_{34}Cl_2N_5O_3$ $(M)^+$: 630.2039, found: 630.2008.

1-(4-Aminocyclohexyl)-3-(3,4-dichlorobenzyl)-5-(4-(4-methylpiperazine-1-carbonyl)phenoxy)-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (3o). White solid, yield 92%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.2 Hz, 2H), 7.44–7.36 (m, 2H), 7.19–7.10 (m, 3H), 7.05 (d, J = 8.4 Hz, 1H), 6.61 (d, J = 8.3 Hz, 1H), 4.96 (s, 2H), 4.30–4.18 (m, 1H), 4.02–3.67 (m, 4H), 3.20–3.02 (m, 4H), 2.73 (s, 3H), 2.69–2.63 (m, 1H), 2.26–2.12 (m, 2H), 2.09–1.99 (m, 2H), 1.82–1.71 (m, 2H), 1.58–1.44 (m, 2H). ESI MS m/z 609.4 $[M]^+$. HR-ESI MS calcd for $C_{31}H_{35}Cl_2N_6O_3$ $(M)^+$: 609.2148, found: 609.2133.

Biological evaluations

ELISA kinase assay. The kinase domain of c-Met was expressed using the Bac-to-Bac™ baculovirus expression system (Invitrogen, Carlsbad, CA, USA) and purified on Ni-NTA columns (QIAGEN Inc., Valencia, CA, USA). The activity of

c-Met tyrosine kinase was detected using enzyme-linked-immunosorbent assay (ELISA). Briefly, 20 μg mL^{-1} Poly(Glu, $Tyr)_{4:1}$ (Sigma, St. Louis, MO) was pre-coated in 96-well ELISA plates as substrate. Each well was treated with 50 μL of 10 μmol L^{-1} 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 tested 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 the addition of purified tyrosine kinase proteins diluted in 40 μL of 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 anti-phosphotyrosine (PY99) antibody (1 : 1000, Santa Cruz Biotechnology, Santa Cruz, CA) diluted in T-PBS containing 5 mg mL^{-1} BSA was added and the plate was incubated at 37 °C for 30 min. After the plate was washed three times, 100 μL horseradish peroxidase-conjugated goat anti-mouse IgG (1 : 2000, Calbiochem, San Diego, CA) diluted in T-PBS containing 5 mg mL^{-1} BSA was added and the plate was incubated at 37 °C for 30 min. The plate was washed, then 100 μL citrate buffer (0.1 M, pH 5.5) containing 0.03% H_2O_2 and 2 mg mL^{-1} *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: $[1 - (A_{492}\ \text{treated}/A_{492}\ \text{control})] \times 100\%$. IC_{50} values were calculated from the inhibitory curves.

Cell proliferation assay. Cells were seeded in 96-well tissue culture plates. On the next day, cells were exposed to various concentrations of compounds and further cultured for 72 h. Finally, cell proliferation was determined using methyl thiazolyl tetrazolium (MTT) assay.

Western blot analysis. Cells were cultured under regular growth conditions to exponential growth phase. Then the cells were treated with the indicated concentration of compounds for 4 h at 37 °C and lysed in 1 × SDS sample buffer. Those cell lysates were subsequently resolved on 10% SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were probed with anti-phosphoc-Met (Y1234/1235, Cell Signaling Technology) (1 : 1000), anti-c-Met (C12, SantaCruz Biotechnology) (1 : 500), anti-GAPDH (KangChen bio-tech) (1 : 10 000) antibody, then subsequently with anti-rabbit or anti-mouse IgG horseradish peroxidase (1 : 2000). Immunoreactive proteins were detected using an enhanced chemiluminescence detection reagent.

Binding mechanism prediction

Inhibitor **2t** is docked to the active site of c-Met with autodock 4.0[23] by using the genetic search algorithm. The Met protein is extracted from the crystal structure of c-Met complexed with an inhibitor[24] available from the Protein Data Bank (PDB code: 3C1X). The numbers of grid points in xyz are set to 54, 54, 54 with the spacing value equivalent to 0.375 Å, and the grid center is 1.5, 4.687, 50. During docking, the number of individuals in population, maximum number of energy evaluation, maximum number of generations and GA-LS runs are rectified to 150, 2 500 000, 27 000 and 100, respectively. Other parameters set as the default values implemented by the program.

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