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Identification of a novel class of selective Tpl2 kinase inhibitors: 4-Alkylamino-[1,7]naphthyridine-3-carbonitriles

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Abstract—We have previously reported the discovery and initial SAR of the [1,7]naphthyridine-3-carbonitriles and quinoline-3-carbonitriles as Tumor Progression Loci-2 (Tpl2) kinase inhibitors. In this paper, we report new SAR efforts which have led to the identification of 4-alkylamino-[1,7]naphthyridine-3-carbonitriles. These compounds show good in vitro and in vivo activity against Tpl2 and improved pharmacokinetic properties. In addition they are highly selective for Tpl2 kinase over other kinases, for example, EGFR, MEK, MK2, and p38. Lead compound 4-cycloheptylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (**30**) was efficacious in a rat model of LPS-induced TNF-α production.

1. Introduction

Tumor Progression Loci-2 (Tpl2) kinase has been shown to be involved in both production and signaling of TNF-α.[1] TNF-α is a pro-inflammatory cytokine and its role in several inflammatory diseases, most notably rheumatoid arthritis (RA), is well established.[2] Thus, Tpl2 is an attractive target for the treatment of RA, a chronic incapacitating disease that currently affects more than five million people worldwide. The use of kinase inhibitors in the treatment of chronic diseases like RA has not been clinically proven.[3] Many compounds have failed in phase 1 clinical trials due to adverse effects. The toxicity observed could be a result of poor selectivity against other kinases.

Several members of the mitogen-activated protein kinase (MAP kinase) family, particularly the serine/threonine kinase p38 (Fig. 1), have been investigated by several companies. Four compounds have progressed to the clinic,[3] AMG-548, BIRB-796, SCIO-469[4] (SCIO-323), and VX-702.[4] Two of these, AMG-548 and BIRB-796, have failed to advance further due to toxicity concerns.

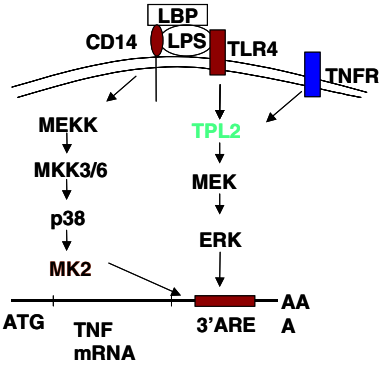

Figure 1. Tpl2 is essential for TNF-α production and TNF-α signaling.

Keywords: Tpl2 kinase; [1,7]Naphthyridine-3-carbonitriles; Quinoline-3-carbonitriles.
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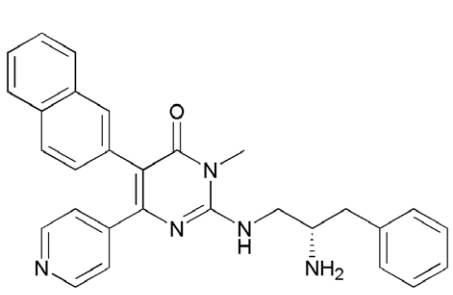
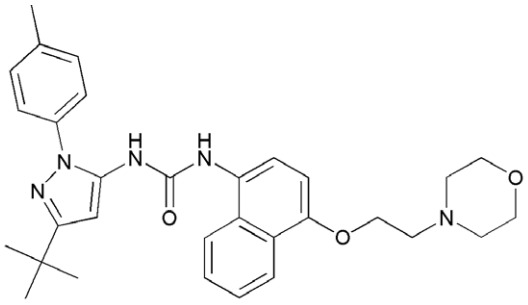
Tpl2 is a serine/threonine kinase in the MAP3K family that is upstream of MEK in the ERK pathway (Fig. 1).[1] Tpl2 is the only known human kinase that has a proline instead of a conserved glycine at the first glycine in the GXGXXG motif of the ATP binding loop.[5a] Tpl2 is also not inhibited by the pan kinase inhibitor staurosporine.[5b] Therefore, we believe that Tpl2 has some distinct structural features that will confer selectivity against other kinases and circumvent unwanted side effects.

In our previous work on Tpl2[6,7] we have reported the [1,7]naphthyridine-3-carbonitriles (**1**) and quinoline-3-carbonitriles (**2**) as potent, reversible, ATP-competitive Tpl2 inhibitors with selectivity against certain kinases. The naphthyridine carbonitriles **1** were identified via 'directed screening' of our kinase library.[6] Further SAR evaluation of **1** indicated that, unlike other kinases, the inhibitory activity of Tpl2 depended on substitution at the C-6 position (tailpiece). In the literature, the C-6 position of naphthyridine-3-carbonitriles[8] and 1,3-quinazolines[9] has been shown to be useful for optimization of solubility and permeability of kinase inhibitors, but to have minimal effect on isolated kinase activity. However, several potent tailpieces were identified for class **1** compounds (**1a–c**). Further investigation showed that the closely related quinoline-3-carbonitriles (**2**) had very similar SAR to carbonitriles **1**.[7] A detailed study of the C-6 substitution in carbonitriles **2**[7] led to the identification of a potent Tpl2 inhibitor, compound **2a**, with selectivity against several kinases (MEK, PKA, p38, Src, CAMKII, MK2, PKC and S6). In vivo, **2a** showed 70% inhibition of TNF-α production in the LPS-mouse model when dosed ip at 25 mg/kg. However, compound **2a** has poor selectivity against epidermal growth factor receptor (EGFR) tyrosine kinase. A pharmacokinetic (PK) study of **2a** revealed that it also showed poor bioavailability (*F*) and very high total clearance (Cl) in rats ($F = 1\%$; Cl = 97 mL/min/kg). In this article, we report the design and synthesis of carbonitriles that contain novel headpieces and show selectivity against EGFR, improved PK properties and activity in the LPS mouse model.



AMG-548



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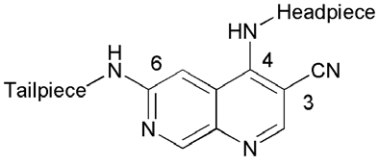
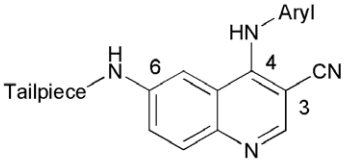


1



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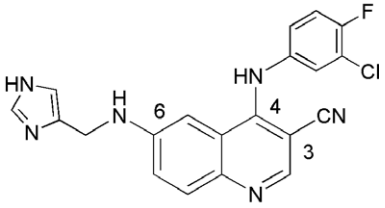
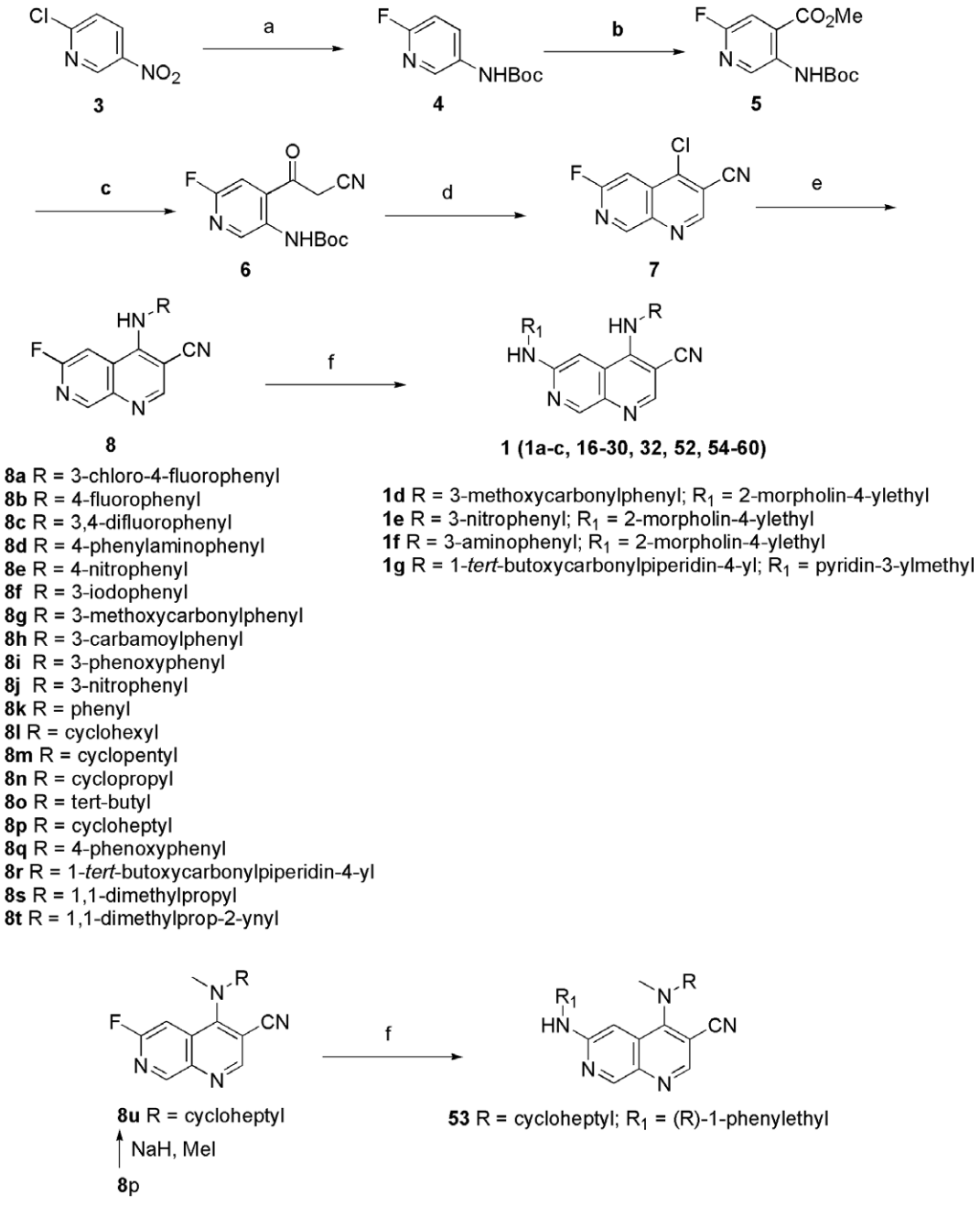


2a

Compound	Tailpiece	Headpiece
1a	morpholin-4-yl-ethyl	3-Chloro-4-fluorophenyl
1b	pyridin-3-yl-methyl	3-Chloro-4-fluorophenyl
1c	(1R)-1-phenylethyl	3-Chloro-4-fluorophenyl

2. Chemistry

The synthesis of compounds with the general structure **1** was accomplished in a manner reported by Wissner et al.[10] As shown in Scheme 1 the key intermediate **7** was prepared in seven steps from commercially available 2-chloro-5-nitropyridine **3**. Compound **7** and appropriate headpiece amines were either refluxed in ethanol or irradiated by microwave to give intermediates **8**. The 6-fluoro groups of these intermediates were displaced with a variety of tailpiece amines using microwave irradiation or heating in pyridine to give final products **1** (Tables 1–3, 5, 6). Compound **21** (Table 1) was prepared by addition–elimination reaction of intermediate **7** with methyl-3-amino benzoate to give **8g**, subsequent reaction with 4-(2-aminoethyl)morpholine to afford **1d**, and finally hydrolysis of the methyl ester to give **21**. Compound **24** (Table 1) was synthesized in three steps from intermediate **8j**. Displacement of the fluoro group

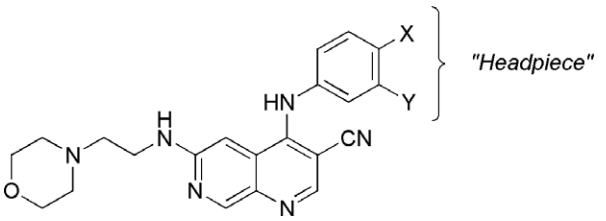


Scheme 1. Synthesis of [1,7]naphthyridine-3-carbonitriles. Reagents and conditions: (a) i—KF, DMSO, 70 °C, 18 h; ii—$SnCl_2$·$2H_2O$, EtOAc; iii—Boc_2O, *t*-BuOH 40 °C, 3 h, 53%, three steps; (b) i—*n*-BuLi, TMEDA, ether, −78 °C; then CO_2; ii—$TMSCH_2N_2$, $CHCl_3$/MeOH, 51%, two steps; (c) CH_3CN, *n*-BuLi, THF, −78 °C, 76%; (d) i—DMF–DMA, rt, 1 h; ii—oxalyl chloride, DMF, CH_2Cl_2, 59%, two steps; (e) RNH_2, 2-ethoxyethanol or EtOH, reflux; or RNH_2, DME, 140 °C microwave, 10 min; (f) R_1NH_2, THF or DMA or neat, 150–190 °C microwave; or R_1NH_2, pyridine, 80 °C.

with 4-(2-aminoethyl)morpholine generated **1e**. The latter upon reduction with $SnCl_2$ yielded **1f**, which upon treatment with methyl sulfonylchloride gave **24**. Compound **53** (Table 5) was prepared by N-methylation of **8p** followed by reaction of intermediate **8u** with (*R*)-1-phenylethylamine. Compound **58** (Table 6) was prepared by condensing intermediate **7** with *N*-Boc piperidine to give **8r**, subsequent reaction with pyridin-3-ylmethylamine to give **1g**, and removal of the Boc group using HCl in dioxane to give **58**.

Target compounds with the general structure **2** were made according to Scheme 2.[7] Synthesis of compound **33** (Table 3) started with commercially available *para*-nitroaniline **9**. The tailpiece was first installed by treating **9** with 4-(2-chloroethyl)morpholine, and the nitro compound (**10a**) obtained was then reduced to **10b**. The latter was alkylated by Michael addition–elimination to give the 2-cyano-3-ethoxyacrylate **11b**. Cyclization in Dowtherm followed by chlorination gave **13b**. Nucleophilic displacement of chloride **13b** with 4-phenoxyphenylamine yielded **33**. Since this method was not suitable for analoging at the 6 position it was not further pursued. An alternate route using common intermediate **13a** was used to synthesize all other such compounds. Cyanoquinoline **13a**[7] was obtained in three steps from

Table 1. Tpl2 activity of 6-(2-morpholin-4-ylethylamino)-4-arylamino-[1,7]napthyridine-3-carbonitriles



Compound	X	Y	Tpl2 IC_{50} (μM)
1a	F	Cl	1.5
16	F	H	10.7
17	F	F	3.6
18	NHPh	H	7.6
19	NO_2	H	7.0
20	H	I	7.1
21	H	COOH	>10
22	H	$CONH_2$	>10
23	H	OPh	11.5
24	H	$NHSO_2CH_3$	>10

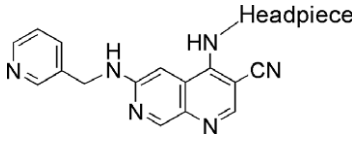
commercially available *para*-nitroaniline **9**.[8j] Refluxing intermediate **13a** with various amines in ethanol or irradiation of these mixtures by microwave gave intermediates **14(a–d)**. The C-6 nitro group was reduced to give intermediates **15(a–d)**. Tailpiece installation was carried out by reductive amination of **15** with a variety of aldehydes to give **2** (Tables 3–5). Two of the aldehydes used were not commercially available. Morpholin-4-ylacetaldehyde needed for the synthesis of **31** was prepared *in situ* from its dimethyl acetal[11] by heating with aqueous HCl overnight. Refluxing 2-picoline-*N*-oxide with selenium dioxide in pyridine gave the 1-oxypyridine-2-carbaldehyde[12] required for the synthesis of **44** and **36**.

3. Results and discussion

All compounds were studied for inhibition of isolated Tpl2 enzyme via quantification of MEK phosphorylation in an ELISA format. For selected compounds, EGFR selectivity was measured by looking at the inhibition of EGFR kinase autophosphorylation in A431 cells overexpressing this enzyme, via quantification of P-EGFR. Functional inhibition of TNF-α production was measured using LPS-stimulated monocytes and LPS-stimulated human blood. Compounds studied in vivo were first assessed for selectivity against other kinases (including p38α, CAMKII, PKC, PKA, MK2, Src, S6K, IKKβ, and Jnk-1).

A brief SAR study of headpieces in the naphthyridine-3-carbonitrile series (**1**)[6] had revealed the 4′-fluoro-3′-chloro aniline as the optimal arylamino substitution at the 4-position. However, the effect of polar functionality had not been explored. Thus, the headpiece moiety was varied starting with the lead compound (**1a**) in this series. As shown in Table 1, all these compounds showed

Table 2. Tpl2 activity and EGFR selectivity for 6-[(pyridine-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitriles



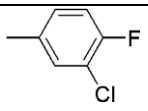
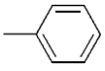
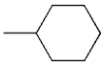
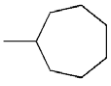
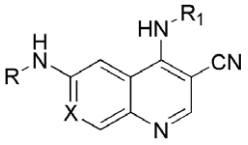
Compound	Headpiece	Tpl2 IC_{50} (μM)	LPS-induced TNF-α inhibition		EGFR(A431) IC_{50} (μM)
			Monocyte IC_{50} (μM)	Human blood IC_{50} (μM)	
1b		0.05	1.1	>20	<5
25		0.4	1.1	20	<5
26		0.47	0.5	4.7	>40
27		0.32	0.6	7.7	>40
28		2.8			>40
29		0.63	0.6	4.2	>40
30		0.16	0.5	4.5	>40

Table 3. Tpl2 activity of 4-arylamino-[1,7]naphthyridine-3-carbonitriles versus 4-arylaminoquinoline-3-carbonitriles



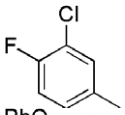
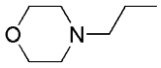
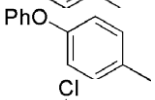
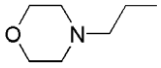
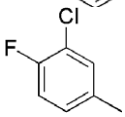
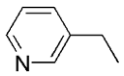
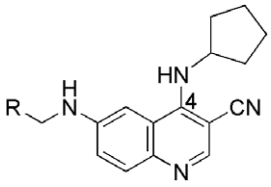
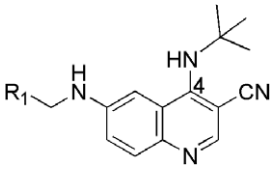
R_1	R	X = N	Tpl2 IC_{50} (μM)	X = CH	Tpl2 IC_{50} (μM)
		**1a**	1.5	**31**	1.0
		**32**	0.38	**33**	0.25
		**1c**	0.05	**34**	0.018

Table 4. Tpl2 activity of 4-cyclopentylaminoquinoline-3-carbonitrile and 4-*tert*-butylaminoquinoline-3-carbonitrile





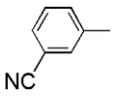
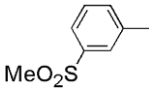
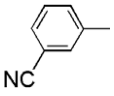
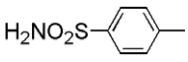
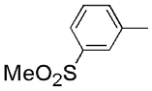
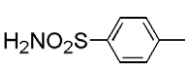
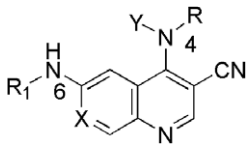
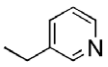
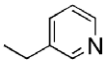
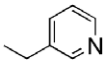
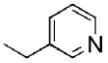
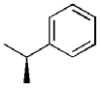
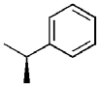
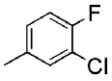
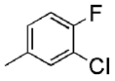
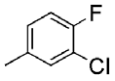
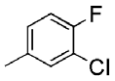
Compound	R	Tpl2 IC_{50} (μM)	Compound	R_1	Tpl2 IC_{50} (μM)
35		21	**44**		1.3
36		1.3	**45**		1.7
37		1.7	**46**		2.9
38		2.2	**47**		7.3
39		2.9	**48**		1.6
40		7.3	**49**		3
41		1.6			
42		3			
43		19.7			

Table 5. SAR mapping of 4-alkylamino-[1,7]naphthyridine-3-carbonitriles

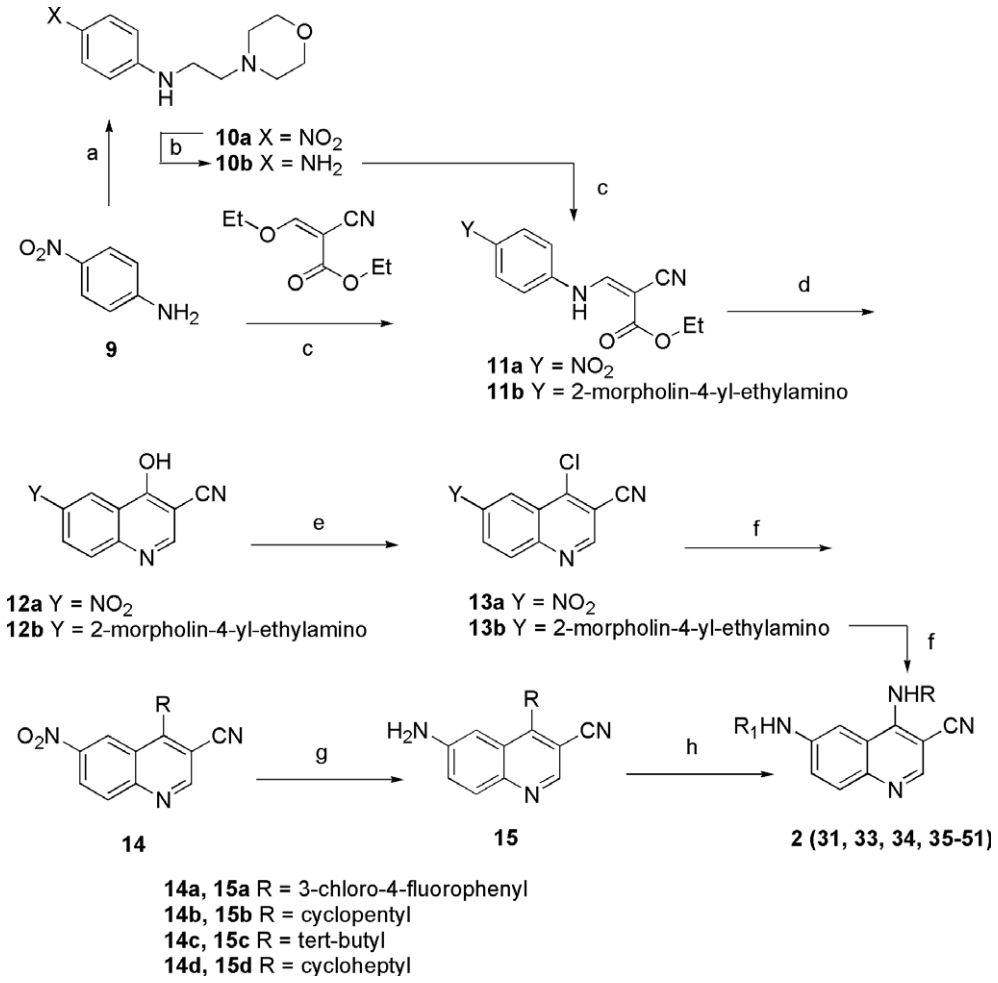


Compound	R1	R	X	Y	Tpl2 IC_{50} (μM)
30			N	H	0.16
50			C	H	2.6
27			N	H	0.32
51			C	H	2.4
52			N	H	0.92
53			N	CH_3	>40
54			N	H	8
55			N	H	7.6
56			N	H	>40
57			N	H	5.2

diminished activity. This result is not unexpected since a common feature seen in the ATP binding site for the naphthyridine-3-carbonitriles[8] and 1,3-quinazolines[9] is that the headpiece lies in a mostly lipophilic pocket buried deep within the active site with limited bulk tolerance. Next we looked at the cyclohexylamino headpiece (Table 2). Here the more potent 3-pyridylmethylamino group was used as a tailpiece. We found that compound **26** was equipotent to **25**. This result was a pleasant surprise since an alkyl headpiece usually leads to diminished activity in this core when applied to other kinase targets. We expected that this unusal SAR would grant selectivity over other kinases.

Further analoging of **26** and evaluation in other assays showed that the 4-alkylamino substituted [1,7]naphthyridine-3-carbonitriles had moderate activity in the Tpl2 enzymatic assay but improved activity in the monocyte and human blood assays compared to the 4-arylamino analogs (Table 2). Most importantly, these compounds show excellent selectivity against other kinases, particularly EGFR. In our earlier compounds, lack of selectivity against EGFR had been a concern.

In the past, we had found that the [1,7]naphthyridine-3-carbonitriles (**1**) and quinoline-3-carbonitriles (**2**) had very similar SAR for Tpl2 inhibition. For example, in Table 3, compounds **1a**, **32**, and **1c** of the [1,7]naphthyridine-3-carbonitrile series have similar Tpl2 activity to the corresponding compounds **31**, **33**, and **34** in the quinoline-3-carbonitriles series.[7] Since the SAR tracks between the two different cores, we made a library of quinoline carbonitrile compounds combining the *tert*-butyl and cyclopentylamino headpiece with our most potent tailpieces from the naphthyridine carbonitrile series.[6,7] (Table 4). Unfortunately all compounds showed diminished activity. These findings prompted us to do further SAR mapping (Table 5). First, the quinoline analogs of naphthyridines **27** and **30** were evaluated. Both compounds (**50** and **51**) showed 10-fold lower Tpl2 activity than their naphthyridine analogs. It appears that N7 is important for activity in the 4-alkylamino series. Next, the importance of the 4-NH group was tested. Methylating the C-4 nitrogen atom in compound **52** abolished activity (**53**). One hypothesis was that perhaps the 4-alkylamino analogs were binding in a flipped mode compared to the 4-arylamino analogs, with the key interaction made by the 3-cyano group



Scheme 2. Synthesis of quinoline-3-carbonitriles. Reagents and conditions: (a) 4-(2-chloroethyl)morpholine hydrochloride, 170 °C, 14 h; (b) 10% Pd/C, H_2, rt, 24 h; (c) Cs_2CO_3, DMF, rt, 2 h; (d) Dowtherm A, 260 °C, 4–5 h; (e) $(COCl)_2$, DMF (cat.), DCE; or $POCl_3$, reflux, 2-12 h; (f) RNH_2, EtOH, microwave or conventional heating; (g) $SnCl_2{\cdot}2H_2O$, EtOH, microwave (or conventional heating) or Fe, NH_4Cl, $MeOH/H_2O$, heat; (h) R_1CHO, $NaCNBH_3$, HOAc, EtOH.

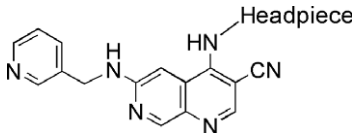
replaced by one with the N7 atom. The 4-alkylamino groups would then be behaving as the tailpieces. A few compounds were made to test the hypothesis. The 6-(3-chloro-4-fluorophenylamino)-4-cyclohexylamino-[1,7]naphthyridine-3-carbonitrile (**54**) and the 4-(3-chloro-4-fluorophenylamino)-6-(alkylamino)-[1,7]naphthyridine-3-carbonitriles (**55**–**57**) did not show any appreciable activity, indicating that our hypothesis was probably incorrect.

In an attempt to improve the activity of the alkylamino series a few additional analogs were prepared (Table 6). Insertion of heteroatoms into the alkyl headpiece was not accepted (**26** vs **58**). Introduction of an alkynyl group was tolerated (**60**). Although there was no significant improvement in activity, we were able to identify a second compound (**59**) with comparable whole blood activity to lead **30**. This compound was twofold less potent in the Tpl2 enzymatic and monocyte assay.

With this new alkylamino class of compounds we have identified a series that is selective against EGFR and demonstrates improved human blood activity (Tables 2 and 6). Selected compounds were next evaluated for IV PK in rats (Table 7). Compound **30** showed good exposure and moderate clearance and was taken forward for in vivo testing. It was examined in the LPS-induced mouse model at an ip dose of 10 mg/kg. Compound **30** caused an 86% inhibition of TNF-α production compared to vehicle in female C57Bl/6 mice (Fig. 2). Upon screening for selectivity over a panel of kinases in both in vitro and cellular assays (Table 8), it showed >200-fold selectivity against MK2 and p38, two other kinases involved in TNF-α production. It also had >200-fold selectivity against MEK and other serine-threonine kinases. Table 8 also shows a comparison of Tpl2 activity (in vitro and in vivo), kinase selectivity profiles, and PK parameters of lead candidate **30** versus our earlier lead **2a**. Compound **30** has an improved selectivity profile, lower clearance, and improved exposure and bioavailability in rats. In addition, **30** showed better in vivo activity at 10 mg/kg (ip) compared to **2a** at 25 mg/kg (ip) in the LPS mouse model.

In summary, we have identified a novel class of selective Tpl2 inhibitors, the 4-alkylamino-[1,7]naphthyridine-3-carbonitriles. These compounds are potent in cellular

Table 6. Tpl2 activity and EGFR selectivity for 6-[(pyridine-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitriles



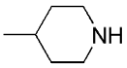
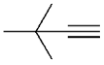
Compound	Headpiece	Tpl2 IC_{50} (μM)	LPS-induced TNF-α inhibition		EGFR(A431) IC_{50} (μM)
			Monocyte IC_{50} (μM)	Human blood IC_{50} (μM)	
58		>40			
59		0.36	0.8	4.5	>40
60		0.27	2	NA	

Table 7. Rat PK parameters for potent 4-alkylamino naphthyridine carbonitriles

Compound	Clearance (mL/min/kg)	AUC iv/dose (h kg ng/mL/mg)
29	50	328
27	28	732
30	30	1041

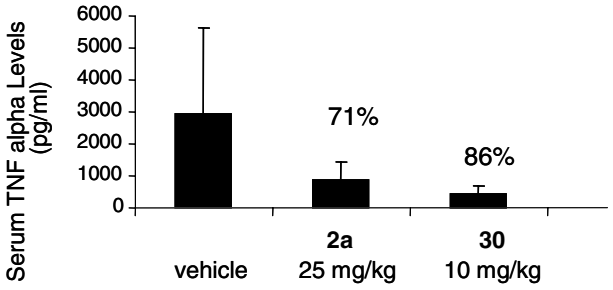


Figure 2. Effect of compounds **2a** (ip) and **30** (ip) on LPS/D-Gal-induced TNF-α release.

and human blood assays indicating their functional ability to inhibit release of TNF-α. In addition, they show good in vivo activity; a result, we believe, of their improved pharmacokinetic properties. On the basis of this data the alkylamino-naphthyridine-carbonitriles have been selected for further investigation.

4. Experimental

4.1. Chemistry

Reactions were run using commercially available starting materials and solvents, without further purification. Proton NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer using TMS (δ 0.0) as a reference. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson-Microlit. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4 of the theoretical values. Low resolution mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrupole mass spectrometer. High resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 Tesla superconducting magnet (Magnex Scientific Ltd, UK) and an external Bruker APOLLO electrospray ionization

Table 8. Kinase selectivity profile (IC_{50}, μM), Tpl2 activity and PK parameters for compounds **30** and **2a**

Compound	MEK[a]	p38[a]	Src[a]	CAMKII[a]	MK2[a]	PKA[a]	PKC[a]	S6[a]	P-p38[b]	EGFR[c]
30	>40	>40	38	NA	NA	>40	>40	>40	NA	>20
2a	2.4	30	NA	NA	NA	5.84	>10	>10	2.11	0.03

Compound	Tpl2 IC_{50} (μM)	LPS-induced TNF-α inhibition		PK parameters in rat		
		Monocyte IC_{50} (μM)	Human blood IC_{50} (μM)	Clearance (Cl/min/kg)	AUC iv/dose (h kg ng/mL/mg)	Bioavailability (%)
30	0.16	0.5	4.5	30	1041	14
2a	0.019	0.6	3.3	97	171	1

NA, not active at 40 μM.
[a] Enzymatic assays.
[b] In human monocytes.
[c] In A431 cells.

(ESI) source. The microwave procedures were carried out with a Biotage microwave. Preparative HPLC was run using a Waters reverse phase preperative HPLC with Xterra C18 5 μM, 30 × 100 mm column. The flow rate was 40 mL/min and mobile phase A was water; mobile phase B was CH_3CN; triethylamine or formic acid was used as a modifier.

4.1.1. General procedure for the synthesis of [1,7]naphthyridine-3-carbonitriles (1). Step 1 Method A. In a 250 mL round-bottomed flask fitted with a condenser, 4-chloro-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**7**,[10] 1.25 g, 6.02 mmol) and the appropriate aniline (1.32 mmol) were taken up in 100 mL of 2-ethoxyethanol or ethanol and heated at reflux for 1–10 h, until t.l.c. analysis showed complete disappearance of the 4-chloronaphthyridine. After cooling to room temperature, the reaction mixture was partitioned between EtOAc and 5% Na_2CO_3. The aqueous layer was extracted twice more with EtOAc, and the combined organic layers were washed three times with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated to give 4-substituted-6-fluoro-[1,7]naphthyridine-3-carbonitriles which were considered of sufficient purity to be used directly in the next step. Method B. In a microwave vial, 4-chloro-6-fluoro-[1,7]naphthyridine-3-carbonitrile (1.25 g, 6.02 mmol) and the appropriate aniline (6.6 mmol) were taken up in DME. The vial was crimp-sealed and heated in a microwave reactor at 140 °C for 10 min. The contents of the vials were transferred to a separatory funnel and worked up as described above for Method A, giving 4-substituted-6-fluoro-[1,7]naphthyridine-3-carbonitriles of sufficient purity to be used directly in the next step. Step 2 Method C. A solution of the appropriate amine (1.7 mmol) and 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) in pyridine (3.3 mL) was heated to 80 °C for 7 days. Pyridine was removed by evaporation and crude products purified. Method D. The 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) was mixed with the appropriate amine (20 mmol) in a microwave vial. The sealed vial was heated in a microwave reactor at 190 °C for 5–10 min (until t.l.c. analysis showed complete consumption of starting material). The contents of the vial were transferred into a separatory funnel and partitioned between 300 mL each EtOAc and brine, and the aqueous layer extracted twice more with EtOAc. The combined organic extracts were washed with brine (3×), dried over anhydrous $MgSO_4$, filtered, and evaporated. Method E. The 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) was taken up in a microwave vial in 5.6 mL THF or DMA, with the appropriate amine (20 mmol). The sealed vial was heated in a microwave reactor at 150 °C for 1 h, until t.l.c. analysis showed complete disappearance of the starting material. Then the THF was removed under reduced pressure. The crude products from Step 2 were purified by recrystallization, flash chromatography over silica gel or preparative HPLC (water/acetonitrile, triethylamine or formic acid modifier).

4.1.1.1. 4-(3-Chloro-4-fluorophenylamino)-6-(2-morpholin-4-yl-ethylamino)-[1,7]naphthyridine-3-carbonitrile (1a). Step 1 Method B. 4-(3-Chloro-4-fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8a**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32–7.49 (m, 1H) 7.53 (t, J = 9.0 Hz, 1H) 7.72 (dd, J = 6.3, 2.3 Hz, 1H) 8.16 (s, 1H) 8.69 (s, 1H) 9.08 (s, 1H) 10.14 (s, 1H). Step 2 Method C. 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.5 (m, 4H) 2.6 (d, J = 11.9 Hz, 2H) 3.2 (m, 2H) 3.7 (m, 4H) 5.5 (t, J = 5.1 Hz, 1H) 6.2 (s, 1H) 6.9 (s, 1H) 7.1 (m, 1H) 7.2 (t, J = 8.6 Hz, 1H) 7.3 (m, 1H) 8.5 (s, 1H) 9.1 (d, J = 0.5 Hz, 1H). Anal. ($C_{21}H_{20}ClFN_6O$ + 0.9H_2O) C, H, N.

4.1.1.2. 4-(3-Chloro-4-fluorophenylamino)-6-(pyridin-3-ylmethylamino)-[1,7]naphthyridine-3-carbonitrile (1b). Intermediate **8a** was treated with pyridin-3-ylmethylamine following the procedure for Step 2 Method D. 24% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.58 (d, J = 6.3 Hz, 2H) 7.10 (s, 1H) 7.26–7.39 (m, 2H) 7.46 (t, J = 9.0Hz, 1H) 7.54 (t, J = 6.3 Hz, 1H) 7.59 (dd, J = 6.6, 2.8 Hz, 1H) 7.75 (dt, J = 7.8, 1.6 Hz, 1H) 8.29 (s, 1H) 8.44 (dd, J = 4.7, 1.6 Hz, 1H) 8.60 (d, J = 1.8 Hz, 1H) 8.87 (s, 1H) 9.67 (s, 1 H). Anal. ($C_{21}H_{14}ClFN_6$) C, H, N.

4.1.1.3. 4-[(3-Chloro-4-fluorophenyl)amino]-6-{[(1*R*)-1-phenylethyl]amino}-[1,7]naphthyridine-3-carbonitrile (1c). Intermediate **8a** was treated with (1*R*)-1-phenylethylamine following the procedure for Step 2 Method E. 35% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.51 (d, J = 14.3 Hz, 3H), 3.46–3.61 (m, 1H), 4.34–4.56 (br s, 1H), 5.06 (br s, 1H), 6.73 (d, J = 15.9 Hz, 1H), 7.54–7.69 (m, 5H), 7.73–7.85 (m, 4H), 7.90 (d, J = 15.9 Hz, 1H). HRMS (ESI+) calcd for $C_{23}H_{17}ClFN_5$ (M+H) 418.1230, found 418.1231.

4.1.1.4. 4-(4-Fluorophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (16). Step 1 Method A. 4-(4-Fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8b**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27–7.36 (m, 2H) 7.41–7.49 (m, 2H) 8.19 (s, 1H) 8.63 (s, 1H) 9.05 (s, 1H) 10.09 (s, 1H). Step 2 Method E. 26% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (br s, 4H) 2.58 (br s, 2H) 3.38 (q, J = 6.4 Hz, 2H) 3.52–3.64 (m, 4H) 6.60 (t, J = 6.2 Hz, 1H) 7.06 (s, 1H) 7.27 (t, J = 8.8 Hz, 2H) 7.33–7.43 (m, 2H) 8.22 (s, 1H) 8.82 (s, 1H) 9.60 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{22}FN_6O$ (M+H) 393.1834, found 393.1833.

4.1.1.5. 4-(3,4-Difluorophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (17). Step 1 Method B. 6-Fluoro-4-(3,4-difluorophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8c**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18–7.36 (m, 1H) 7.43–7.65 (m, 2H) 8.17 (s, 1H) 8.69 (s, 1H) 9.08 (s, 1H) 10.16 (s, 1H). Step 2 Method E. 24% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (br s, 4H) 2.57 (t, J = 6.4 Hz, 2H) 3.34–3.42 (m, 2H) 3.51–3.64 (m, 4H) 6.66 (t, J = 5.7 Hz, 1H) 7.02 (s, 1H) 7.13–7.24 (m, 1H) 7.43–7.58 (m, 2H) 8.28 (s, 1H) 8.85 (s, 1H) 9.68 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{21}F_2N_6O$ (M+H) 411.1740, found 411.1737.

4.1.1.6. 6-(2-Morpholinoethylamino)-4-(4-(phenylamino)phenylamino)-[1,7]naphthyridine-3-carbonitrile (18). Step 1 Method A. 6-Fluoro-4-(4-(phenylamino)phenylamino)-[1,7]naphthyridine-3-carbonitrile (**8d**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (tt, J = 7.3, 1.0 Hz, 1H) 7.07–7.17 (m, 4H) 7.21–7.32 (m, 4H) 8.21 (s, 1H) 8.37 (s, 1H) 8.58 (s, 1H) 9.02 (s, 1H) 9.99 (s, 1H). Step 2 Method E. 13% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.45 (br s, 4H) 2.58 (t, J = 6.4 Hz, 2H) 3.37 (q, J = 6.7 Hz, 2H) 3.55–3.64 (m, 4H) 6.53 (t, J = 5.4 Hz, 1H) 6.85 (t, J = 7.2 Hz, 1H) 7.05–7.17 (m, 5H) 7.17–7.31 (m, 4H) 8.17 (s, 1H) 8.31 (s, 1H) 8.79 (s, 1H) 9.53 (s, 1H). HRMS (ESI+) calcd for $C_{27}H_{28}N_7O$ (M+H) 466.2350, found 466.2350.

4.1.1.7. 6-(2-Morpholin-4-yl-ethylamino)-4-(4-nitro phenylamino)-[1,7]naphthyridine-3-carbonitrile (19). Step 1 Method A gave crude **8e**, which was taken to the next step. Step 2 Method E. 18% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35–2.42 (m, J = 3.8 Hz, 4H) 2.46–2.50 (m, 2H) 3.34–3.39 (m, 2H) 3.52–3.57 (m, 4H) 6.78 (s, 1H) 6.89 (t, J = 5.6 Hz, 1H) 7.23 (d, J = 9.1 Hz, 2H) 8.18–8.23 (m, J = 9.7, 3.0, 2.7 Hz, 2H) 8.54 (s, 1H) 8.98 (s, 1H) 10.12 (s, 1H). HRMS calcd for $C_{21}H_{21}N_7O_3$ (M+H), 420.1779, found 420.1777.

4.1.1.8. 4-(3-Iodophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (20). Step 1 Method A. 6-Fluoro-4-(3-iodophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8f**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (t, J = 8.0 Hz, 1H) 7.40 (ddd, J = 8.1, 2.2, 0.9 Hz, 1H) 7.67 (dt, J = 7.8, 1.7 Hz, 1H) 7.75 (t, J = 1.8 Hz, 1H) 8.15 (s, 1H) 8.72 (s, 1H) 9.09 (s, 1H) 10.10 (s, 1H). Step 2 Method E. 17% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.43 (s, 4H) 2.55 (t, J = 6.4 Hz, 2H) 3.36 (q, J = 6.2 Hz, 2H) 3.53–3.61 (m, 4H) 6.66 (t, J = 5.4 Hz, 1H) 6.97 (s, 1H) 7.19 (t, J = 7.8 Hz, 1H) 7.25–7.31 (m, 1H) 7.57 (d, J = 8.1 Hz, 1H) 7.62–7.65 (m, 1H) 8.31 (s, 1H) 8.86 (s, 1H) 9.60 (s, 1H). Anal. ($C_{21}H_{21}IN_6O$) C, H, N.

4.1.1.9. 3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzoic acid (21). Compound **7** was treated with methyl 3-aminobenzoate following the procedure for Step 1 Method A to give methyl 3-(3-cyano-6-fluoro-[1,7]naphthyridine-4-ylamino)benzoate (**8g**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.88 (s, 3H) 7.62 (t, J = 7.7 Hz, 1H) 7.64–7.69 (m, J = 8.5, 1.9, 1.5 Hz, 1H) 7.88–7.93 (m, 2H) 8.19 (s, 1H) 8.72 (s, 1H) 9.10 (s, 1H) 10.22 (s, 1H). Above compound was treated with 4-(2-aminoethyl)morpholine following the procedure for Step 2 Method E to give methyl 3-(3-cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzoate (**1d**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40–2.46 (m, 4H) 2.55 (t, J = 6.4 Hz, 2H) 3.34–3.39 (m, 2H) 3.54–3.60 (m, 4H) 3.87 (s, 3H) 6.67 (t, J = 5.2 Hz, 1H) 7.01 (s, 1H) 7.56 (dt, J = 4.4, 1.2 Hz, 2H) 7.79–7.83 (m, 2H) 8.32 (s, 1H) 8.87 (s, 1H) 9.73 (s, 1H). To **1d** (2 mmol) in tetrahydrofuran (12 mL) were added methyl alcohol (4.5 mL) and lithium hydroxide (1 N, 4.5 mL). After 12 h the solvents were evaporated and the crude mixture was purified by preparative HPLC to give 3-(3-cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzoic acid (**21**) in 54% overall yield. ^{1}H NMR (400 MHz, MeOD) δ 3.13–3.23 (m, 6H) 3.72 (t, J = 6.1 Hz, 2H) 3.84–3.90 (m, 4H) 7.09 (s, 1H) 7.48–7.55 (m, 2H) 7.90–7.96 (m, 2H) 8.29 (s, 1H) 8.90 (s, 1H). HRMS (ESI+) calcd for $C_{22}H_{22}N_6O_3$ (M+H), 419.1826, found 419.1821.

4.1.1.10. 3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]-naphthyridine-4-ylamino)benzamide (22). Step 1 Method A. 3-(3-Cyano-6-fluoro-[1,7]naphthyridine-4-ylamino)benzamide (**8h**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.00–7.08 (m, 1H) 7.49–7.59 (m, 2H) 7.81–7.92 (m, 2H) 8.04 (s, 1H) 8.21 (s, 1H) 8.70 (s, 1H) 9.09 (s, 1H) 10.18 (s, 1H). Step 2 Method E. 37% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38–2.48 (m, 4H) 2.52–2.59 (m, 2H) 3.34–3.41 (m, 2H) 3.53–3.62 (m, 4H) 6.64 (s, 1H) 7.05 (s, 1H) 7.40–7.52 (m, 3H) 7.73–7.81 (m, 2H) 8.02 (s, 1H) 8.29 (s, 1H) 8.86 (s, 1H) 9.69 (s, 1H). HRMS (ESI+) calcd for $C_{22}H_{23}N_7O_2$ (M+H), 418.1986, found 419.1980.

4.1.1.11. 6-(2-Morpholinoethylamino)-4-(3-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (23). Step 1 Method A. 6-Fluoro-4-(3-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (**8i**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.94–7.18 (m, 6H) 7.33–7.51 (m, 3H) 8.15 (s, 1H) 8.68 (s, 1H) 9.05 (s, 1H) 10.12 (s, 1H). Step 2 Method E. 27% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (br s, 4H) 2.57 (t, J = 6.3 Hz, 2H) 3.34–3.43 (m, 2H) 3.49–3.64 (m, 4H) 6.64 (t, J = 5.8 Hz, 1H) 6.90 (dd, J = 3.8, 1.5 Hz, 2H) 7.00 (s, 1H) 7.03–7.11 (m, 3H) 7.14 (t, J = 7.5 Hz, 1H) 7.33–7.47 (m, 3H) 8.28 (s, 1H) 8.84 (s, 1H) 9.64 (s, 1H). HRMS (ESI+) calcd for $C_{27}H_{27}N_6O_2$ (M+H) 467.2190, found 467.2188.

4.1.1.12. *N*-(3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)phenyl)methanesulfonamide (24). Compound **7** was treated with 3-nitroaniline following the procedure for Step 1 Method A to give 6-fluoro-4-(3-nitrophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8j**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (t, J = 8.1 Hz, 1H) 7.80–7.85 (m, 1H) 8.12–8.16 (m, 1H) 8.17 (s, 1H) 8.21 (t, J = 2.0 Hz, 1H) 8.80 (s, 1H) 9.14 (s, 1H) 10.35 (s, 1H). The above compound was treated with 4-(2-aminoethyl)morpholine following the procedure for Step 2 Method E to give 6-(2-morpholinoethylamino)-4-(3-nitrophenylamino)-[1,7]naphthyridine-3-carbonitrile (**1e**). 19% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.37–2.48 (m, 4H) 2.52–2.59 (m, 2H) 3.35–3.41 (m, 2H) 3.54–3.61 (m, 4H) 6.71–6.80 (m, 1H) 6.96 (s, 1H) 7.66–7.71 (m, 2H) 8.00–8.05 (m, 1H) 8.06–8.08 (m, 1H) 8.40 (s, 1H) 8.92 (s, 1H) 9.89 (s, 1H). A mixture of **1e** (120 mg, 0.29 mmol) and $SnCl_2.2H_2O$ (348 mg, 1.54 mmol) in ethyl alcohol (12 mL) was heated to reflux for 2.5 h. After cooling to room temperature, water (10 mL) was added followed by sodium bicarbonate (500 mg). The mixture was stirred at room temperature for 1 h. Ethyl acetate extraction followed by column chromatography (3–5% methyl alcohol in dichloromethane) yielded 43 mg of 4-(3-aminophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile, (**1f**) 39% yield. ^{1}H

NMR (400 MHz, DMSO-d_6) 2.40–2.48 (m, 4H) 2.53–2.60 (m, 2H) 3.34–3.40 (m, 2H) 3.54–3.63 (m, 4H) 5.18–5.27 (m, 2H) 6.39–6.47 (m, 3H) 6.57 (s, 1H) 7.01–7.08 (m, 2H) 8.22 (s, 1H) 8.81 (s, 1H) 9.43 (s, 1H). Compound **1f** (111 mg, 0.29 mmol) was suspended in methylene chloride (10 mL). Triethylamine (44 μL, 0.32 mmol) was added and the mixture was cooled to 0 °C. Methylsulfonyl chloride (24 μL, 0.31 mmol) was added and the mixture was stirred at room temperature for 12 h. Another 44 μL triethylamine and 48 μL methylsulfonyl chloride were added and the reaction mixture was stirred for 12 h. Work-up and preparative HPLC yielded 45.5 mg of product **24** (34% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.39–2.47 (m, 4H) 2.56 (t, J = 6.8 Hz, 2H) 2.99–3.03 (m, 3H) 3.27–3.42 (m, 2H) 3.55–3.61 (m, 4H) 6.63 (t, J = 6.1 Hz, 1H) 7.00–7.04 (m, 1H) 7.04 (s, 1H) 7.08 (d, J = 8.6 Hz, 1H) 7.12–7.16 (m, 1H) 7.36 (t, J = 8.3 Hz, 1H) 8.26 (s, 1H) 8.84 (s, 1H) 9.69 (s, 2H). HRMS (ESI+) calcd for $C_{22}H_{25}N_7O_3S$ (M+H), 468.1812, found 468.1810.

4.1.1.13. 4-Phenylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (25). Step 1 Method B. 6-Fluoro-4-phenylamino-[1,7]naphthyridine-3-carbonitrile (**8k**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25–7.33 (m, 3H) 7.42 (t, J = 7.7 Hz, 2H) 8.13 (s, 1H) 8.52 (s, 1H) 8.96 (s, 1H) 10.15 (s, 1H). Step 2 Method D. 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.56 (d, J = 6.3 Hz, 2H) 7.10–7.16 (m, 1H) 7.22–7.30 (m, 3H) 7.33 (dd, J = 7.6, 4.8 Hz, 1H) 7.41 (t, J = 7.8 Hz, 2H) 7.44–7.54 (m, 1H) 7.75 (d, J = 7.8 Hz, 1H) 8.25 (s, 1H) 8.39–8.48 (m, 1H) 8.59 (s, 1H) 8.85 (s, 1H) 9.62 (s, 1H). Anal. ($C_{21}H_{16}N_6$ + 0.8 H_2O) C, H, N.

4.1.1.14. 4-Cyclohexylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (26). Step 1 Method B. 4-Cyclohexylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8l**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48–1.84 (m, 8H) 2.01–2.11 (m, 2H) 4.53 (d, J = 5.1 Hz, 1H) 7.86 (d, J = 8.8 Hz, 1H) 8.28 (s, 1H) 8.57 (s, 1H) 8.92 (s, 1H). Step 2 Method D. 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05–1.88 (m, 8H) 2.05 (s, 2H) 4.23 (s, 1H) 4.48–4.68 (m, 2H) 7.07–7.13 (m, 1H) 7.26–7.44 (m, 3H) 7.77 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.43 (d, J = 6.1 Hz, 1H) 8.61 (s, 1H) 8.71 (s, 1H). Anal. ($C_{21}H_{22}N_6$ + 1.8H_2O) C, H, N.

4.1.1.15. 4-Cyclopentylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (27). Step 1 Method B. 4-Cyclopentylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8m**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.59–1.66 (m, 2H) 1.74–1.88 (m, 4H) 2.03–2.12 (m, 2H) 4.69–4.74 (m, 1H) 7.88 (d, J = 7.3 Hz, 1H) 8.30 (s, 1H) 8.59 (s, 1H) 8.93 (s, 1H). Step 2 Method D. 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.71–1.84 (m, 2H) 2.02–2.12 (m, 1H) 3.32 (s, 6H) 4.58 (d, J = 6.3 Hz, 2H) 7.06–7.14 (m, 1H) 7.28–7.36 (m, 2H) 7.41 (d, J = 7.6 Hz, 1H) 7.77 (dd, J = 7.8, 1.8 Hz, 1H) 8.19 (s, 1H) 8.43 (dd, J = 4.7, 1.6 Hz, 1H) 8.61 (d, J = 2.0 Hz, 1H) 8.72 (s, 1H). Anal. ($C_{20}H_{20}N_6$ + 1.2H_2O) C, H, N.

4.1.1.16. 4-Cyclopropylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (28). Step 1 Method B. 4-Cyclopropylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8n**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89–1.01 (m, 4H) 3.09–3.18 (m, J = 6.2, 3.9 Hz, 1H) 8.11 (s, 1H) 8.54 (s, 1H) 8.65 (s, 1H) 8.95 (s, 1H). Step 2 Method D. 22% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80–0.89 (m, J = 3.5, 3.5 Hz, 1H) 0.95 (dd, J = 6.8, 1.8 Hz, 2H) 4.54 (d, J = 6.3 Hz, 2H) 7.02–7.08 (m, 1H) 7.26–7.40 (m, 2H) 7.76 (dd, J = 7.8, 1.5 Hz, 1H) 8.15 (d, J = 2.3 Hz, 1H) 8.23 (s, 1H) 8.43 (dd, J = 4.8, 1.5 Hz, 1H) 8.60 (d, J = 2.0 Hz, 1H) 8.73 (s, 1H). HRMS (ESI+) calcd for $C_{18}H_{16}N_6$ (M+H), 317.1509, found 317.1504.

4.1.1.17. 4-*tert*-Butylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (29). Step 1 Method B. 4-*tert*-Butylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8o**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (s, 9H) 7.26 (s, 1H) 8.17 (s, 1H) 8.66 (s, 1H) 8.96 (s, 1H). Step 2 Method D. 37% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42–1.62 (m, 9H) 4.58 (d, J = 5.8 Hz, 2H) 6.58 (s, 1H) 6.89 (s, 1H) 7.25–7.38 (dd, J = 7.7, 4.9 Hz, 1H) 7.45–7.53 (m, 1H) 7.71–7.81 (m, 1H) 8.15 (s, 1H) 8.26 (s, 1H) 8.43 (d, J = 4.8 Hz, 1H) 8.62 (s, 1H). HRMS (ESI+) calcd for $C_{19}H_{20}N_6$ (M+H), 333.1822, found 333.1824.

4.1.1.18. 4-Cycloheptylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (30). Step 1 Method B. 4-Cycloheptylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8p**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11–1.58 (m, 6H) 1.60–1.87 (m, 4H) 2.03–2.12 (m, 2H) 4.29 (s, 1H) 7.84–7.96 (m, 1H) 8.26 (s, 1H) 8.57 (s, 1H) 8.92 (s, 1H). Step 2 Method D. 33% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46–1.84 (m, 10H) 1.95–2.11 (m, 2H) 4.38–4.52 (m, 1H) 4.58 (d, J = 6.6 Hz, 2H) 7.11 (d, 1H) 7.22–7.45 (m, 3H) 7.77 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.43 (dd, J = 4.7, 1.4 Hz, 1H) 8.61 (s, 1H) 8.71 (s, 1H). Anal. ($C_{22}H_{24}N_6$ + 2.0 H_2O) C, H, N.

4.1.1.19. 6-[(2-Morpholin-4-ylethyl)amino]-4-[(4-phenoxyphenyl)amino]-[1,7]naphthyridine-3-carbonitrile (32). Step 1 Method A. 6-Fluoro-4-(4-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (**8q**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.1 (m, 2H) 7.1 (m, 3H) 7.4 (m, 4H) 8.2 (s, 1H) 8.6 (s, 1H) 9.1 (s, 1H) 10.1 (m, 1H) Step 2 Method C. 44% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.5 (m, 4H) 2.6 (t, J = 6.8 Hz, 2H) 3.4 (m, 2H) 3.6 (m, 4H) 6.6 (t, J = 5.6 Hz, 1H) 7.0 (m, 2H) 7.1 (m, 4H) 7.4 (m, 3H) 8.2 (s, 1H) 8.3 (s, 1H) 8.8 (s, 1H) 9.6 (s, 1H). HRMS (ESI+) calcd for $C_{27}H_{26}N_6O_2$ (M+H), 467.2190, found 467.2196.

4.1.1.20. 4-Cycloheptylamino-6-(*R*)-1-phenylethylamino-[1,7]naphthyridine-3-carbonitrile (52). Intermediate **8p** was treated with (*R*)-1-phenylethylamine following the procedure for Step 2 Method D. 7% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (d, J = 6.8 Hz, 3H) 1.46–1.89 (m, 12H) 4.32–4.52 (m, 1H) 4.89–5.10 (m, 1H) 7.10–7.21 (dd, J = 7.3, 7.3 Hz, 1H) 7.22–7.44 (m, 5H) 7.85 (s, 1H) 8.18 (s, 1H) 8.38 (s, 2H). HRMS (ESI+) calcd for $C_{24}H_{27}N_5$ (M+H), 386.2339, found 386.2335.

4.1.1.21. 4-(Cycloheptylmethylamino)-6-(*R*)-1-phenyl ethylamino-[1,7]naphthyridine-3-carbonitrile (53). To a solution of 4-(cycloheptylmethylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8p**, 0.2 g, 0.7 mmol) in DMF (3 mL) was added NaH (60% in mineral oil, 40 mg, 0.8 mmol) at room temperature. After stirring for 10 min 0.2 mL of MeI was added. The reaction mixture was stirred for 4 h, then quenched with water. The aqueous solution was filtered to give 4-(cycloheptylmethylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8u**) as a beige solid (150 mg, 71% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50–1.84 (m, 12H) 3.32 (s, 3H) 7.69 (d, J = 2.0 Hz, 1H) 8.14 (s, 1H) 8.47 (s, 1H). Intermediate **8u** was treated with (*R*)-1-phenylethylamine following the procedure for Step 2 Method D to give compound **53**. 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37–1.85 (m, 12H) 1.84–2.17 (m, 2H) 3.32 (s, 3H) 4.31–4.49 (m, J = 4.0 Hz, 1H) 4.96–5.08 (m, J = 7.8 Hz, 1H) 6.89–6.99 (m, 1H) 7.10–7.32 (m, 5H) 7.47 (d, J = 7.3 Hz, 2H) 8.13 (s, 1H) 8.66 (s, 1H). HRMS (ESI+) calcd for $C_{25}H_{29}N_5$ (M+H), 400.2496, found 400.2499.

4.1.1.22. 6-(3-Chloro-4-fluorophenylamino)-4-(cyclopentylamino)-[1,7]naphthyridine-3-carbonitrile (54). A mixture of 3-chloro-4-fluorobenzeneamine (550 mg, 3.78 mmol), 4-(cyclopentylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8m**, 28 mg, 0.1 mmol), and cesium carbonate (506 mg, 1.55 mmol, 15.5 equiv) in DMF (4 mL) was heated to 200 °C in a microwave tube for 1 h using a microwave reactor. Preparative HPLC purification yielded 15 mg product (36% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52–1.68 (m, 2H) 1.70–1.91 (m, 4H) 1.99–2.16 (m, 2H) 4.58–4.76 (m, 1H) 7.33 (t, J = 9.1 Hz, 1H) 7.45–7.56 (m, 1H) 7.76 (s, 1H) 7.80 (d, J = 7.1 Hz, 1H) 7.87–7.97 (m, 1H) 8.32 (s, 1H) 8.90 (s, 1H) 9.64 (s, 1H). HRMS (ESI+) calcd for $C_{20}H_{17}ClFN_5$ (M+H), 382.1229, found 382.1237.

4.1.1.23. 4-(3-Chloro-4-fluorophenylamino)-6-cyclopentylamino-[1,7]naphthyridine-3-carbonitrile (55). Step 1 Method A. 4-(3-Chloro-4-fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8a**). Step 2 Method D. 41% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43–1.63 (m, 4H) 1.65–1.78 (m, 2H) 1.91–2.07 (m, J = 9.4 Hz, 2H) 3.84–4.02 (m, 1H) 6.81–6.98 (m, 2H) 7.30–7.40 (m, 1H) 7.41–7.53 (m, 1H) 7.56–7.65 (m, 1H) 8.26 (s, 1H) 8.83 (s, 1H) 9.63 (s, 1H). Anal. ($C_{20}H_{17}ClFN_5$ + 1.1 H_2O) C, H, N.

4.1.1.24. 6-*tert*-Butylamino-4-(3-chloro-4-fluorophenylamino)-[1,7]naphthyridine-3-carbonitrile (56). Intermediate **8a** was treated with *tert*-butylamine following the procedure for Step 2 Method E. 21% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32–1.51 (m, 9H) 6.40–6.48 (m, 1H) 6.94 (s, 1H) 7.28 (s, 1H) 7.37–7.59 (m, 2H) 8.27 (s, 1H) 8.85 (s, 1H) 9.67 (s, 1H). HRMS (ESI+) calcd for $C_{19}H_{17}ClFN_5$ (M+H), 370.1229, found 370.1229.

4.1.1.25. 4-(3-Chloro-4-fluoro-phenylamino)-6-cycloheptylamino-[1,7]naphthyridine-3-carbonitrile (57). Intermediate **8a** was treated with cycloheptylamine following the procedure for Step 2 Method D. 19% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27–2.03 (m, 12H) 3.66–3.83 (m, 1H) 6.72–6.87 (m, 2H) 7.27–7.38 (m, 1H) 7.45 (t, J = 9.1 Hz, 1H) 7.52–7.62 (m, 1H) 8.26 (s, 1H) 8.83 (s, 1H) 9.61 (s, 1H). Anal. ($C_{22}H_{21}ClFN_5$ + 0.6CH_3OH) C, H, N.

4.1.1.26. 4-(Piperidin-4-ylamino)-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (58). Compound **7** was treated with 4-aminopiperidine-1-carboxylic acid *tert*-butyl ester following the procedure for Step 1 Method B to give 4-(3-cyano-6-fluoro-[1,7]naphthyridin-4-ylamino)piperidine-1-carboxylic acid *tert*-butyl ester (**8r**). Crude **8r** was treated with pyridin-3-ylmethylamine following the procedure for Step 2 Method D to give 4-{3-cyano-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-4-ylamino}-piperidine-1-carboxylic acid *tert*-butyl ester (**1g**). Crude **1g** (0.285 mg, 0.621 mmol) from above was treated with 4 M HCl in dioxane (21 mL) at 0 °C. The reaction mixture was stirred overnight at 0 °C and then purified using preparative HPLC to give compound **58**. 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.61–1.86 (m, 2H) 2.03 (d, J = 13.1 Hz, 2H) 2.71 (t, J = 12.4 Hz, 2H) 3.18 (d, J = 12.4 Hz, 3H) 4.33 (s, 1H) 4.58 (d, J = 6.3 Hz, 2H) 7.13–7.20 (m, 1H) 7.26–7.39 (m, 2H) 7.53 (d, J = 7.3 Hz, 1H) 7.77 (d, J = 7.8 Hz, 1H) 8.19 (s, 1H) 8.29 (s, 1H) 8.42 (d, J = 4.6 Hz, 1H) 8.61 (s, 1H) 8.73 (s, 1H). HRMS (ESI+) calcd for $C_{20}H_{21}N_7$ (M+H), 360.1931, found 360.1925.

4.1.1.27. 4-(1,1-Dimethylpropylamino)-6-[(pyridin-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitrile (59). Step 1 Method B. 4-(1,1-Dimethylpropylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8s**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, J = 7.5 Hz, 3H) 1.56 (s, 6H) 2.02 (q, J = 7.3 Hz, 2H) 7.12 (s, 1H) 8.18 (s, 1H) 8.65 (s, 1H) 8.97 (s, 1H). Step 2 Method D. The reaction mixture was heated at 140 °C for 10 min. 19% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82 (t, J = 7.5 Hz, 3H) 1.47 (s, 6H) 1.79–2.02 (m, 2H) 4.59 (d, J = 5.8 Hz, 2H) 6.44 (s, 1H) 6.90 (s, 1H) 7.32 (dd, J = 8.1, 4.8 Hz, 1H) 7.49 (t, J = 6.4 Hz, 1H) 7.76 (d, J = 7.8 Hz, 1H) 8.25 (s, 1H) 8.43 (d, J = 3.5 Hz, 1H) 8.61 (d, J = 1.3 Hz, 1H) 8.77 (s, 1H). Anal. ($C_{20}H_{22}N_6$ + 1.3H_2O) C, H, N.

4.1.1.28. 4-(1,1-Dimethylprop-2-ynylamino)-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (60). Step 1 Method B. The reaction mixture was heated at 140 °C for thirty minutes to give **8t**. Crude **8t** was taken forward to the next step. Step 2 Method D. The reaction mixture was heated at 140 °C for ten minutes. 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80 (s, 6H) 3.50–3.61 (m, 1H) 4.57 (d, J = 6.6 Hz, 2H) 6.95 (d, J = 14.4 Hz, 2H) 7.29–7.37 (m, 1H) 7.52 (t, J = 6.4 Hz, 1H) 7.77 (dd, J = 7.8, 2.0 Hz, 1H) 8.31 (s, 1H) 8.38–8.45 (dd, J = 4.7, 1.6 Hz, 1H) 8.62 (s, 1H) 8.80 (s, 1H). Anal. ($C_{20}H_{18}N_6$ + 0.4H_2O) C, H, N.

4.1.2. General procedure for the synthesis of quinoline-3-carbonitriles (2). Step 1 Method F. In a microwave vial, 4-chloro-6-nitro-quinoline-3-carbonitrile (**13a**,[8j] 0.4 g,

1.71 mmol) and the appropriate amine (2.05 mmol) were taken up in 2 mL EtOH. The vial was crimp-sealed and heated in a microwave reactor at 150 °C for 45 min. The solvent was removed and the residue was partitioned between ether and H_2O. This gave a suspension, which was filtered, washed with H_2O, and dried on a high vacuum pump overnight to give a solid, which was used in the next step without purification.

Method G. 4-Chloro-6-nitroquinoline-3-carbonitrile (**13a**,[8j] 2.33 g, 10 mmol) and the appropriate amine (1.74 g, 12 mmol) were suspended in ethanol (60 mL) and heated at reflux for 3 h or until t.l.c. analysis showed that the reaction was complete. After cooling, the solvent was removed in vacuo and the residue was stirred with ether/saturated aqueous $NaHCO_3$ (100 mL/75 mL) for 2.5 h to triturate. The solid was collected by suction filtration and used in the next step.

Step 2 Method H. In a microwave vial, 4-amino-6-nitroquinoline-3-carbonitrile (**14**, 0.97 mmol) and $SnCl_2{\cdot}2H_2O$ (1.09 g, 4.83 mmol) were taken up in 2 mL EtOH. The vial was sealed and heated in a microwave reactor at 110 °C for 10 min, until LC/MS analysis showed complete disappearance of the nitroquinoline. The reaction mixture was poured into ice water, neutralized with saturated $NaHCO_3$, and extracted with EtOAc (2× 150 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated. These products were used in the next step without purification.

Method I. 4-Amino-6-nitroquinoline-3-carbonitrile (**14**, 7.29 mmol) was suspended in ethanol (85 mL), then $SnCl_2{\cdot}2H_2O$ (8.3 g, 36.5 mmol) was added and the reaction mixture heated at reflux for 2.5 h or until complete by t.l.c. The reaction mixture was diluted with 100 mL of water, and then solid $NaHCO_3$ was added until the pH was basic (~11). The solution was extracted with chloroform, washed with brine, treated with activated charcoal, dried over $MgSO_4$, and solvent evaporated. These products were used in the next step without purification.

Step 3 Method J. In a 50 mL round-bottomed flask, the 6-aminoquinoline-3-carbonitrile (**15**, 0.29 mmol) was taken up in 3 mL EtOH, and the appropriate aldehyde (0.37 mmol) was added. Acetic acid was added to bring the pH of the solution to 4, and the mixture stirred for 15 min. $NaCNBH_3$ (12.5 mg, 0.20 mmol) was then added, and the reaction mixture allowed to stir from 4 to 24 h at 25–30 °C. Ethanol was then removed under reduced pressure and the crude product was purified by preparative HPLC.

4.1.2.1. 4-[(3-Chloro-4-fluorophenyl)amino]-6-[(2-morpholin-4-ylethyl)amino]quinoline-3-carbonitrile (31). Step 1 Method G was used to synthesize 4-(3-chloro-4-fluoro-phenylamino)-6-nitroquinoline-3-carbonitrile (**14a**) in 80% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28–7.35 (m, 1H) 7.47 (t, J = 9.0 Hz, 1H) 7.56 (dd, J = 6.7, 2.7 Hz, 1H) 7.99 (d, J = 9.1 Hz, 1H) 8.49 (dd, J = 9.4, 2.5 Hz, 1H) 8.64 (s, 1H) 9.47 (d, J = 2.3 Hz, 1H) 10.72 (s, 1H). Step 2 Method I was used to obtain 6-amino-4-(3-chloro-4-fluorophenylamino)quinoline-3-carbonitrile (**15a**) in 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.78 (s, 2H) 7.12–7.19 (m, 2H) 7.25 (dd, J = 8.8, 2.3 Hz, 1H) 7.34–7.42 (m, 2H) 7.70 (d, J = 9.1 Hz, 1H) 8.34 (s, 1H) 9.36 (s, 1H). 4-(2,2-Dimethoxyethyl)morpholine [11] was heated with 37% aqueous HCl (0.6 mL for 0.5 mmol) overnight at 70 °C. This reaction mixture was treated with intermediate **15a** following the procedure for Step 3 Method J. 60% yield. ^{1}H NMR (400 MHz, MeOD) δ 2.49 (m, 2H), 2.51–2.53 (m, 2H), 3.18–3.30 (m, 4H), 3.49–3.52 (m, 2H), 3.57 (m, 2H), 6.88 (s, 1H), 7.02–7.23 (m, 3H), 7.25–7.33 (m, 1H), 7.58 (d, J = 9.1 Hz, 1H), 8.18 (s, 1H). HRMS (ESI+) calcd for $C_{22}H_{21}ClFN_5O$ (M+H), 426.1491; found 426.1497.

4.1.2.2. 4-(3-Chloro-4-fluorophenylamino)-6-[(pyridin-3-ylmethyl)amino]quinoline-3-carbonitrile (34). Intermediate **15a** was treated with pyridine-3-carbaldehyde following the procedure for Step 3 Method J. 95% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.35 (dd, J = 3.3, 0.8 Hz, 1H) 6.39 (dd, J = 3.3, 1.8 Hz, 1H) 6.79 (t, J = 5.6 Hz, 1H) 7.21–7.27 (m, 3H) 7.35 (dd, J = 9.1, 2.5 Hz, 1H) 7.43 (t, J = 9.0 Hz, 1H) 7.48 (dd, J = 6.7, 2.7 Hz, 1H) 7.60 (dd, J = 1.8, 0.8 Hz, 1H) 7.70 (d, J = 9.1 Hz, 1H) 8.33 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{14}ClFN_4O$ (M+H) 393.0913, found 393.0917.

4.1.2.3. 6-Benzylamino-4-cyclopentylaminoquinoline-3-carbonitrile (35). Step 1 Method F was used to synthesize 4-cyclopentylamino-6-nitroquinoline-3-carbonitrile (**14b**) in 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.59–1.68 (m, 2H) 1.76–1.93 (m, 4H) 2.05–2.15 (m, 2H) 4.72–4.80 (m, 1H) 7.97 (d, J = 9.1 Hz, 1H) 8.40 (s, 1H) 8.46 (dd, J = 9.1, 2.3 Hz, 1H) 8.66 (s, 1H) 9.56 (d, J = 2.3 Hz, 1H). Step 2 Method H was used to obtain 6-amino-4-cyclopentylaminoquinoline-3-carbonitrile (**15b**) in 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60 (s, 2H) 1.77 (d, J = 3.0 Hz, 4H) 2.01–2.11 (m, 2H) 4.64 (d, J = 5.8 Hz, 1H) 5.47 (s, 2H) 7.00–7.06 (m, J = 7.6 Hz, 1H) 7.11–7.16 (m, 1H) 7.25 (d, J = 1.8 Hz, 1H) 7.54 (d, J = 8.6 Hz, 1H) 8.15 (s, 1H). Step 3 Method J gave product **35** in 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.57–1.67 (m, 2H) 1.73–1.83 (m, 4H) 2.02–2.11 (m, 2H) 4.42–4.47 (m, J = 5.8 Hz, 2H) 4.62–4.70 (m, 1H) 6.72 (t, J = 5.7 Hz, 1H) 7.05–7.13 (m, 2H) 7.22–7.28 (m, 2H) 7.33 (t, J = 7.5 Hz, 2H) 7.44 (d, J = 7.3 Hz, 2H) 7.55 (d, J = 8.8 Hz, 1H) 8.21 (s, 1H). HRMS (ESI+) calcd for $C_{18}H_{20}N_6$ (M+H), 321.1822; found 321.1822.

4.1.2.4. 4-Cyclopentylamino-6-[(1-oxypyridin-2-ylmethyl)amino]quinoline-3-carbonitrile (36). Intermediate **15b** was treated with 1-oxypyridine-2-carbaldehyde[12] following the procedure for Step 3 Method J. 28% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.59 (s, 2H) 1.69–1.78 (m, 4H) 2.00–2.08 (m, 2H) 4.59–4.68 (d, J = 6.6 Hz, 2H) 6.76 (t, J = 6.8 Hz, 1H) 7.03 (d, J = 7.8 Hz, 1H) 7.20–7.43 (m, 5H) 7.59 (d, J = 8.8 Hz, 1H) 8.19 (d, J = 7.3 Hz, 2H) 8.32 (d, J = 6.3 Hz, 1H). HRMS (ESI+) calcd for $C_{21}H_{21}N_5O$ (M+H), 360.1819; found 360.1819.

4.1.2.5. 6-(2-Cyanobenzylamino)-4-cyclopentylamino-quinoline-3-carbonitrile (37). Intermediate **15b** was treated with 2-cyanobenzaldehyde following the procedure for Step 3 Method J. 55% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.55–1.65 (m, 2H) 1.66–1.82 (m, 4H) 1.98–2.10 (m, 2H) 4.58–4.68 (m, 3H) 6.69–6.76 (dd, J = 5.8, 5.8 Hz, 1H) 6.97 (d, J = 7.8 Hz, 1H) 7.11 (d, J = 2.0 Hz, 1H) 7.24 (dd, J = 9.1, 2.5 Hz, 1H) 7.45–7.52 (m, 1H) 7.56–7.70 (m, 3H) 7.87 (d, J = 7.8 Hz, 1H) 8.19 (s, 1H). Anal. ($C_{23}H_{21}N_5$ + 1.0 H_2O) C, H, N.

4.1.2.6. 6-(3-Cyanobenzylamino)-4-cyclopentylamino-quinoline-3-carbonitrile (38). Intermediate **15b** was treated with 3-cyanobenzaldehyde following the procedure for Step 3 Method J. 95% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.54–1.64 (m, 2H) 1.68–1.79 (m, 4H) 2.00–2.11 (m, 2H) 4.49–4.54 (m, J = 6.1 Hz, 2H) 4.59–4.66 (m, 1H) 6.79–6.86 (dd, J = 5.8, 5.8 Hz, 1H) 6.92 (d, J = 6.3 Hz, 1H) 7.03 (d, J = 2.0 Hz, 1H) 7.21 (dd, J = 9.1, 2.3 Hz, 1H) 7.51–7.59 (m, 2H) 7.74 (dd, J = 18.8, 8.0 Hz, 2H) 7.90 (s, 1H) 8.17 (s, 1H). HRMS (ESI+) calcd for $C_{23}H_{21}N_5$ (M+H), 368.1870; found 368.1866.

4.1.2.7. 4-Cyclopentylamino-6-(3-methanesulfonyl-benzylamino)quinoline-3-carbonitrile (39). Intermediate **15b** was treated with 3-methanesulfonylbenzaldehyde following the procedure for Step 3 Method J. 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.55–1.63 (m, 2H) 1.69–1.81 (m, 4H) 2.01–2.11 (m, 2H) 3.18 (s, 3H) 4.58 (d, J = 5.8 Hz, 2H) 4.60–4.66 (m, 1H) 6.83 (t, J = 6.1 Hz, 1H) 6.96 (d, J = 7.3 Hz, 1H) 7.10 (d, J = 2.3 Hz, 1H) 7.23 (dd, J = 8.8, 2.3 Hz, 1H) 7.54–7.64 (m, 2H) 7.77–7.84 (m, 2H) 8.00 (t, J = 1.6 Hz, 1H) 8.17 (s, 1H). Anal. ($C_{23}H_{24}N_4O_2S$ + 1.3H_2O) C, H, N.

4.1.2.8. 4-[(3-Cyano-4-cyclopentylaminoquinolin-6-yl-amino)methyl]benzenesulfonamide (40). Intermediate **15b** was treated with 4-formyl-benzenesulfonamide following the procedure for Step 3 Method J. 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.61 (t, J = 4.8 Hz, 2H) 1.71–1.81 (m, 4H) 2.01–2.11 (m, 2H) 4.53 (d, J = 6.1 Hz, 2H) 4.61–4.67 (m, 1H) 6.79 (t, J = 5.9 Hz, 1H) 6.98 (d, J = 7.8 Hz, 1H) 7.09 (d, J = 2.5 Hz, 1H) 7.21 (dd, J = 9.1, 2.3 Hz, 1H) 7.29 (s, 2H) 7.56 (d, J = 9.1 Hz, 1H) 7.61 (d, J = 8.3 Hz, 2H) 7.75–7.80 (m, 2H) 8.17 (s, 1H). Anal. ($C_{22}H_{23}N_5O_2S$ + 1.0 CH_3OH) C, H, N.

4.1.2.9. 4-Cyclopentylamino-6-[(2H-pyrazol-3-ylmethyl)amino]quinoline-3-carbonitrile (41). Intermediate **15b** was treated with 2H-pyrazole-3-carbaldehyde following the procedure for Step 3 Method J. 28% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (s, 2H) 1.79 (s, 4H) 2.05–2.14 (m, 2H) 4.40 (d, J = 5.3 Hz, 2H) 4.64–4.72 (m, 1H) 6.27 (d, J = 1.8 Hz, 1H) 6.38 (t, J = 5.3 Hz, 1H) 7.03 (d, J = 7.8 Hz, 1H) 7.16 (s, 1H) 7.26 (dd, J = 9.0, 2.15 Hz, 1H) 7.55 (d, J = 8.8 Hz, 1H) 7.60 (s, 1H) 8.17 (s, 1H) 8.20 (s, 1H). HRMS (ESI+) calcd for $C_{19}H_{20}N_6$ (M+H), 333.1822; found 333.1818.

4.1.2.10. 4-Cyclopentylamino-6-[(1-methyl-1H-imidazol-2-ylmethyl)amino]quinoline-3-carbonitrile (42). Intermediate **15b** was treated with 1-methyl-1H-imidazole-2-carbaldehyde following the procedure for Step 3 Method J. 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60–1.66 (m, 2H) 1.74–1.84 (m, 4H) 2.08–2.16 (m, 2H) 3.76 (s, 3H) 4.68 (d, J = 5.1 Hz, 3H) 6.65 (t, J = 5.2 Hz, 1H) 6.98 (d, J = 7.8 Hz, 1H) 7.14 (d, J = 2.3 Hz, 1H) 7.22 (dd, J = 9.2, 2.2 Hz, 1H) 7.27 (d, J = 1.8 Hz, 1H) 7.55 (d, J = 1.8 Hz, 1H) 7.61 (d, J = 9.1 Hz, 1H) 8.23 (s, 1H) 8.30 (s, 1H). HRMS (ESI+) calcd for $C_{20}H_{22}N_6$ (M+H), 347.1979; found 347.1974.

4.1.2.11. 4-Cyclopentylamino-6-(2-morpholin-4-ylethylamino)quinoline-3-carbonitrile (43). 4-(2,2-Dimethoxyethyl)morpholine [11] was heated with 37% aqueous HCl (0.6 mL for 0.5 mmol) overnight at 70 °C. This reaction mixture was treated with intermediate **15b** following the procedure for Step 3 Method J. 22% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.59–1.65 (m, 2H) 1.75–1.83 (m, 4H) 2.05–2.14 (m, 2H) 2.43–2.48 (m, J = 8.1 Hz, 4H) 2.55–2.60 (m, 2H) 3.27–3.34 (m, 4H) 3.57–3.64 (m, 4H) 4.64–4.70 (m, 1H) 5.87 (t, J = 4.2 Hz, 1H) 7.03–7.09 (m, 2H) 7.21 (dd, J = 9.1, 2.3 Hz, 1H) 7.54 (d, J = 8.8 Hz, 1H) 8.16 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{27}N_5O$ (M+H), 366.2288; found 366.2291.

4.1.2.12. 4-(*tert*-Butylamino)-6-{[(1-oxidopyridin-2-yl)-methyl]amino}quinoline-3-carbonitrile (44). Step 1 Method F was used to synthesize 4-cyclopentylamino-6-nitroquinoline-3-carbonitrile (**14c**). The crude product was taken to the next step. Step 2. To a solution of **14c** (800 mg, 2.95 mmol) in methanol (5 mL) and water (2 mL) were added iron powder (935 mg, 16.7 mmol) and ammonium chloride (1.47 g, 27.7 mmol). The mixture was stirred vigorously and heated to 75 °C for 4 h. After allowing the mixture to cool to rt, EtOAc (25 mL) and saturated aqueous $NaHCO_3$ (10 mL) were added. The organic layer was dried over $MgSO_4$ and concentrated to afford 6-amino-4-(*tert*-butylamino)quinoline-3-carbonitrile (**15c**) as a tan solid in quantitative yield. The product was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52 (s, 9H) 5.67 (br s, 2H), 5.99 (br s, 1H), 7.12 (s, 1H), 7.69 (d, J = 9.1 Hz, 1H) 8.28 (s, 1H). Intermediate **15c** was treated with 1-oxypyridine-2-carbaldehyde[12] following the procedure for Step 3 Method J, giving product **44** in 60% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.61 (s, 9H), 3.15–3.26 (m, 2H), 7.57 (s, 1H), 7.74–7.96 (m, 4H), 8.11 (d, J = 9.1 Hz, 1H), 8.77 (d, J = 9.1 Hz, 1H) 8.99 (s, 1H). HRMS (ESI+) calcd for $C_{20}H_{21}N_5O$ (M+H), 348.1819; found 348.1821.

4.1.2.13. 4-(*tert*-Butylamino)-6-[(3-cyanobenzyl)-amino]quinoline-3-carbonitrile (45). Intermediate **15c** was treated with 3-cyanobenzaldehyde following the procedure for Step 3 Method J. 65% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.48 (s, 9H), 3.32–3.49 (m, 2H), 4.58 (s, 1H), 6.74 (d, J = 2.3 Hz, 1H), 7.29 (dd, J = 9.1, 2.3 Hz, 1H), 7.45 (m, 1H), 7.55–7.78 (m, 4H), 8.28 (s, 1H). HRMS (ESI+) calcd for $C_{22}H_{21}N_5$ (M+H), 356.1870; found 356.1872.

4.1.2.14. 4-(*tert*-Butylamino)-6-{[3-(methylsulfonyl)-benzyl]amino}quinoline-3-carbonitrile (46). Intermediate **15c** was treated with 3-methanesulfonylbenzaldehyde

following the procedure for Step 3 Method J. 42% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.61 (s, 9H), 3.03 (s, 3H), 3.16–3.24 (m, 2H), 4.58 (s, 1H) 6.90 (d, J = 2.3 Hz, 1H) 7.37 (dd, J = 9.1, 2.27 Hz, 1H) 7.52 (t, J = 7.7 Hz, 1H), 7.59 (d, J = 9.1 Hz, 1H) 7.68 (d, J = 8.3 Hz, 1H), 7.76 (dd, J = 7.8, 2.0 Hz, 1H), 7.90 (s, 1H) 8.55 (s, 1H). HRMS (ESI+) calcd for $C_{22}H_{24}N_4O_2S$ (M+H), 409.1693, found 409.1692.

4.1.2.15. 4-({[4-(*tert*-Butylamino)-3-cyanoquinolin-6-yl]amino}methyl)benzenesulfonamide (47). Intermediate **15c** was treated with 4-formylbenzenesulfonamide following the procedure for Step 3 Method J. 55% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.40 (s, 9H), 3.22–3.34 (m, 2H), 4.56 (s, 1H) 6.92 (s, 1H), 7.43–7.49 (m, 1H), 7.72–7.79 (m, 2H), 7.82 (d, J = 9.1 Hz, 1H), 8.02 (br s, 2H), 8.44 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{23}N_5O_2S$ (M+H), 410.1645; found 410.1640.

4.1.2.16. 4-(*tert*-Butylamino)-6-[(1*H*-pyrazol-5-ylmethyl)amino]quinoline-3-carbonitrile (48). Intermediate **15c** was treated with 2H-pyrazole-3-carbaldehyde following the procedure for Step 3 Method J. 70% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.68 (s, 9H), 3.46 (s, 2H), 4.57 (s, 1H), 6.42 (s, 1H), 7.07 (s, 1H), 7.38 (dd, J = 9.1, 2.3, 1H), 7.71 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 8.41 (s, 1H), 8.59 (s, 1H). HRMS (ESI+) calcd for $C_{18}H_{20}N_6$ (M+H), 321.1822, found 321.1822.

4.1.2.17. 4-(*tert*-Butylamino)-6-{[(1-methyl-1*H*-imidazol-2-yl)methyl]amino}quinoline-3-carbonitrile (49). Intermediate **15c** was treated with 1-methyl-1H-imidazole-2-carbaldehyde following the procedure for Step 3 Method J. 47% yield. ^{1}H NMR (400 MHz, MeOD) δ 1.58 (s, 9H), 3.26 (s, 2H), 3.85 (s, 3H), 7.20 (s, 1H), 7.32 (s, 1H), 7.38 (dd, J = 9.1, 2.3, 1H), 7.47 (s, 1H), 7.69 (d, J = 9.1 Hz, 1H), 8.67 (s, 1H). HRMS (ESI+) calcd for $C_{19}H_{22}N_6$ (M+H), 335.1979; found 335.1980.

4.1.2.18. 4-(Cycloheptylamino)-6-[(pyridin-3-ylmethyl)-amino]quinoline-3-carbonitrile (50). Step 1 Method F was used to synthesize 4-cycloheptylamino-6-nitroquinoline-3-carbonitrile (**14d**) in 70% crude yield. Step 2 Method H was used to prepare 6-amino-4-cycloheptylaminoquinoline-3-carbonitrile (**15d**, 83% crude yield). Step 3 Method J gave compound **50** in 69.5% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37–1.78 (m, 10H) 1.89–2.05 (m, 2H) 4.31–4.39 (m, 1H) 4.41 (d, J = 5.8 Hz, 2H) 6.65 (t, J = 6.0 Hz, 1H) 6.88 (d, J = 8.3 Hz, 1H) 7.05 (d, J = 2.3 Hz, 1H) 7.15 (dd, J = 9.0, 2.4 Hz, 1H) 7.29 (dd, J = 7.3, 4.3 Hz, 1H) 7.49 (d, J = 9.1 Hz, 1H) 7.71–7.80 (m, 1H) 8.10 (d, J = 6.3 Hz, 1H) 8.39 (dd, J = 4.8, 1.8 Hz, 1H) 8.60 (d, J = 1.8 Hz, 1H). HRMS (ESI+) calcd for $C_{23}H_{25}N_5$ (M+H) 372.2182, found 372.2186.

4.1.2.19. 4-Cyclopentylamino-6-[(pyridin-3-ylmethyl)-amino]quinoline-3-carbonitrile (51). Intermediate **15b** was treated with pyridine-3-carbaldehyde following the procedure for Step 3 Method J. 20% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58–1.66 (m, 2H) 1.73–1.81 (m, 4H) 2.01–2.11 (m, 2H) 4.48 (d, J = 5.8 Hz, 2H) 4.61–4.69 (m, 1H) 6.50 (s, 1H) 6.72 (t, J = 6.1 Hz, 1H) 6.98 (d, J = 8.3 Hz, 1H) 7.11 (s, 1H) 7.22 (dd, J = 9.1, 2.3 Hz, 1H) 7.36 (dd, J = 7.5, 5.2 Hz, 1H) 7.56 (d, J = 9.1 Hz, 1H) 7.82 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.46 (d, J = 3.0 Hz, 1H) 8.67 (s, 1H). HRMS (ESI+) calcd for $C_{21}H_{21}N_5$ (M+H), 344.1870; found 344.1872.

4.1.3. 6-[(2-Morpholin-4-ylethyl)amino]-4-[(4-phenoxyphenyl)amino]quinoline-3-carbonitrile (33). To a 250 mL Erlenmeyer flask equipped with a large stir bar were added 4-nitroaniline (27.6 g, 0.2 mol) and 4-(2-chloroethyl)morpholine hydrochloride (18.6 g, 0.1 mol). The mixture was heated at a sand bath temperature of 170 °C for 14 h. After allowing the reaction mixture to cool to 60 °C, EtOAc (100 mL) was added. The thick mixture was partitioned between EtOAc (600 mL) and water (600 mL). The aqueous phase was extracted with EtOAc (3× 200 mL), then adjusted to pH 9 with 1 N NaOH and further extracted with dichloromethane (3× 200 mL). The organic phase was washed with brine and dried over Na_2SO_4 to provide 10.1 g (40%) of *N*-(2-morpholinoethyl)-4-nitroaniline (**10a**) isolated as a yellow solid. The material was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29–2.58 (m, 4H), 3.15–3.40 (m, 4H), 3.48–3.70 (m, 4H), 6.58–6.76 (m, 2H), 7.18 (t, J = 5.3 Hz, 1H), 7.99 (d, J = 9.4 Hz, 2H).

To a solution of **10a** (8.5 g, 33.7 mmol) in methanol (200 mL) was added a slurry of 10% Pd/C (1.2 g) in EtOAc (100 mL). Hydrogen gas was bubbled through the solution for 1 min. The mixture was then stirred at rt under an atmosphere of hydrogen gas for 24 h, at which time N_2 gas was bubbled into the mixture for 15 min. The mixture was filtered through a pad of Celite and the filtrate concentrated to give 7.4 g (99%) of *N*1-(2-morpholinoethyl)benzene-1,4-diamine (**10b**) as a red syrup. The material was used without further purification. ^{1}H NMR (400 MHz, CD_2Cl_2-d_2) δ 2.26–2.41 (m, 2H), 2.43–2.56 (m, 2H), 2.89–3.04 (m, 4H), 3.54–3.63 (m, 4H), 6.33–6.44 (m, 2H), 6.44–6.53 (m, 2H)

To a solution of **10b** (5.0 g, 22 mmol) in toluene (100 mL) was added ethyl 2-cyano-3-ethoxyacrylate (3.8 g, 22 mmol). The solution was heated at 110 °C for 8 h, allowed to cool to rt, and stirred overnight. Hexanes (800 mL) were added with vigorous stirring. The resulting suspension was heated to 60 °C and EtOAc was added slowly until a copious precipitate formed. After allowing to cool to rt the solid was isolated by filtration to give 4.17 g of 2-cyano-3-(4-(2-morpholinoethylamino)phenylamino)acrylic acid ethyl ester (**11b**) as a yellow powder. After standing for 2 h, the mother liquor was filtered to give 3.47 g of a yellow solid for a combined yield of 7.54 g (99%), which was used directly in the next step. Dowtherm A (300 mL) was bubbled with Ar gas for 1h while being heated to 100 °C. The acrylate **11b** (3.76 g, 10.9 mmol) was added in three portions and the mixture was heated to 259 °C under a stream of Ar. After gently refluxing for 6h, the reaction mixture was allowed to cool below 50 °C and then poured into 1500 mL of hexanes. Filtration provided 4-hydroxy-6-(2-morpholin-4-yl-ethylamino)quinoline-3-carbonitrile (**12b**) as a tan powder (1.15 g, 37%). ^{1}H NMR

(400 MHz, DMSO-d_6) δ 2.33–2.59 (m, 4H), 3.13–3.27 (m, 4H), 3.51–3.65 (m, 4H), 6.06 (t, J = 5.3 Hz, 1H), 7.08 (d, J = 2.8 Hz, 1H), 7.17 (dd, J = 8.8, 2.8 Hz, 1H), 7.41 (d, J = 9.1 Hz, 1H), 8.47 (s, 1H).

A solution of the quinoline **12b** (1.0 g, 3.3 mmol) in phosphorus oxychloride (50 mL) was heated at 120 °C for 12 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was stirred in dichloromethane (200 mL) and cooled to 0 °C. Water (100 mL) was added followed by solid Na_2CO_3, added slowly until the aqueous phase reached pH 9. The organic layer was dried over Na_2SO_4 and concentrated to provide 4-chloro-6-(2-morpholinoethylamino)quinoline-3-carbonitrile (**13a**) (800 mg, 69%) as a light yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.37–2.54 (m, 4H), 2.55–2.67 (m, 4H), 3.33 (s, 4H), 3.61 (s, 4H), 6.82 (d, 1H), 6.87 (d, J = 2.5 Hz, 1H), 7.52 (dd, J = 9.2, 2.7 Hz, 1H), 7.87 (d, J = 9.1 Hz, 1H), 8.74 (s, 1H).

To a solution of **13b** (135 mg, 0.39 mmol) in 2-ethoxyethanol (5 mL) was added 4-phenoxyaniline (57 mg, 0.39 mmol). The mixture was heated at 135 °C for 36 h. After allowing to cool to rt the reaction mixture was diluted with ether and hexane (1:1, 20 mL), stirred for 4 h, and filtered. The solid was washed with ether and dried on a vacuum pump to give **33** (106 mg, 55%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.01–3.55 (m, 6H), 3.65–4.19 (m, 6H), 7.03–7.08 (m, 1H), 7.09–7.20 (m, 4H), 7.34–7.50 (m, 5H), 7.55 (m, 1H), 7.82 (d, J = 9.1 Hz, 1H), 8.65 (s, 1H). HRMS (ESI+) calcd for $C_{28}H_{27}N_5O_2$ (M+H), 466.2238; found 466.2234.

4.2. Pharmacokinetic studies

The animals used in the pharmacokinetic (PK) studies were male adult Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA). All the PK studies were performed at Wyeth Research Laboratories (Andover, MA) under the supervision of the Institutional Animal Care and Use Committee. The dose formulation for intravenous administration was 50% DMSO/50% polyethylene glycol 200 (v/v, 1 mL/kg). The oral dose formulation was an aqueous suspension containing 2% Polysorbate 80 (a.k.a. Tween 80), 0.5% methylcellulose, and 0.06% lactic acid. Blood samples of approximately 0.25 mL were collected into K_2 EDTA coated sampling tubes at 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 h post dose administration. Plasma samples were harvested and stored at −80 °C until analysis.

In a 0.5 mL 96-well plate, 50 μl of plasma sample was precipitated with 100 μl acetonitrile containing 500 ng/mL internal standard. The internal standard is a compound with a similar chemical structure as that of the test article. The samples were vortexed and centrifuged at 5700 rpm for 10 min. Supernatants were subjected to LC-MS/MS analysis. HPLC separation was performed on a Perkin-Elmer Series 200 HPLC system (Perkin-Elmer, Norwalk, CT) using an XTerra MS C18 column (2.1 × 20 mm, 2.5 μm; Waters, Milford, MA). The detection of test articles was performed on a PESCIEX API-3000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, L4K4V8) using TurboIon Spray source. Plasma standard curves were generated by plotting peak area ratio of test article and internal standard against nominal concentrations.

The pharmacokinetic parameters were determined using WinNonlin (version 4.1, Pharsight, Mountain View, CA). Calculations were performed using the non-compartmental analysis approach. The estimation of area under the plasma concentration versus time curve (AUC) was based upon the log trapezoidal rule. The terminal rate constant (λ) was derived from the slope of the terminal log-linear phase of plasma concentrations–time curves. The apparent terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda$. No statistical analysis other than descriptive statistics was conducted.

4.3. Biology

4.3.1. Tpl2 enzymatic assay. Tpl2/Cot activity was directly assayed using GST-MEK1 as a substrate. The phosphorylation on serine residues 217 and 221 of GST-MEK1 was detected by an ELISA. Briefly, 0.4 nM Tpl2 was incubated with 35 nM GST-MEK1 in a kinase reaction buffer of pH 7.2 containing 20 mM Mops, 50 μM ATP, 20 mM $MgCl_2$, 1 mM DTT, 25 mM β-glycerophosphate, 5 mM EGTA, and 1 mM sodium orthovanadate for 1 h at 30 °C. Compounds solubilized in 100% DMSO were pre-diluted in assay buffer so that the final concentration of DMSO in the reaction was 1%. The kinase reaction was carried out in 100 μl volume on 96-well plates. The kinase reaction was then stopped by the addition of 100 mM EDTA. The entire reaction mixture was then transferred to the detection plate, a 96-well Immunosorb plate that had been pre-coated with anti-GST antibody (Amersham). After 1 h incubation at room temperature, the detection plate was washed four times with TBST (TBS + 0.05% Tween 20) and then incubated for another hour at room temperature with anti-phospho-MEK1 antibody (Cell Signaling) 1:1000 in 10 mM Mops 7.5, 150 mM NaCl, 0.05% Tween 20, 0.1% gelatin, 0.02% NaN_3, and 1% BSA. The detection plate was washed again and incubated for 30 min with DELFIA Europium (Eu) labeled goat anti-rabbit IgG (Perkin-Elmer), 1:4000 in the same buffer used for the primary incubation. After a final wash, Eu detection solution was added to each well and the Eu signal was measured in a Wallac Victor Multilabel Counter. Data were imported into Excel and IC_{50} calculations were performed using the Xlfit (IDBS) software package.

4.3.2. Inhibition of TNF-α in primary human monocytes. Human blood buffy coat (containing 1 mM EDTA) was incubated with RosetteSep human monocyte enrichment antibody cocktail (Stem Cell Technologies #15068). This mixture was then diluted with an equal volume of PBS/2% FBS/1 mM EDTA, layered onto an equal volume of Histopaque-1077 (Sigma #H8889), and centrifuged for 20 min at 1500 rpm. The monocytes were then recovered from the interface, diluted, and

reisolated on a Histopaque-1077 cushion. After this second round of purification, the monocytes were washed twice in PBS/2% FBS/1 mM EDTA, resuspended in RPMI/0.5% FBS to 2×10^6 cells/mL, plated at 800,000 cells per well in 48-well formats, and incubated at 37 °C/5% CO_2. Thirty minutes prior to LPS stimulation, compounds (in 100% DMSO) were added. LPS was then added to 10 ng/mL and the cultures allowed to incubate for an additional ~3 h. Media supernatants were then harvested and analyzed for TNF-α by standard ELISA or electrochemiluminescence on a Sector6000 reader (Meso Scale Discovery).

4.3.3. Inhibition of TNF-α in human blood. Blood was drawn from healthy male volunteers into heparin tubes. At 37 °C, the blood was diluted 1–5 with pre-warmed RPMI/3% FCS, and then dispensed into 96-well-formatted 200 μL tube strips and capped. Thirty minutes prior to LPS stimulation compounds (in 100% DMSO) were added. Final DMSO concentrations equaled 1.0%. LPS was then added to 10 ng/mL and the cultures are allowed to incubate for an additional ~3 h. Samples were centrifuged in a strip rotor at 6500 rpm, and the plasma supernatants were analyzed by standard ELISA or electrochemiluminescence on a Sector6000 reader (Meso Scale Discovery).

4.3.4. LPS-induced acute TNF-α production in mice. LPS/D-Gal-induced acute TNF-α production in mouse sera: female C57Bl/6 mice, 8–10 weeks of age, were obtained from the Jackson Laboratory. The animals were fed food and water ad libitum and all procedures were approved by the Institutional Animal Care and Use Committee. Compound at 10 or 25 mg/kg, or vehicle, was administered to the mice by the intraperitoneal (ip) route. One hour after the compound, LPS plus D-galactosamine in PBS was administered ip. Final LPS and D-gal concentrations in each animal were 2 and 160 ng/kg, respectively. The mice were euthanized with carbon dioxide 1.5 hour after the LPS/D-Gal injection and bled by cardiac puncture. TNF-α levels were measured in the serum samples using a TNF-α ELISA.

References and notes

1. (a) Lin, X.; Cunningham, E. T., Jr.; Mu, Y.; Geleziunas, R.; Greene, W. C. *Immunity* **1999**, *10*, 271–280; (b) Chiariello, M.; Marinissen, M. J.; Gutkind, J. S. *Mol. Cell. Biol.* **2000**, *20*, 1747–1758; (c) Salmeron, A.; Ahmad, T. B.; Carlile, G. W.; Pappin, D.; Narsimhan, R. P.; Ley, S. C. *EMBO J.* **1996**, *15*, 817–826; (d) Sasai, H.; Higashi, T.; Nakamori, S.; Miyoshi, J.; Suzuki, F.; Nomura, T.; Kakunaga, T. *Br. J. Cancer* **1993**, *67*, 262–267; (e) Miyoshi, J.; Higashi, T.; Mukai, H.; Ohuchi, T.; Kakunaga, T. *Mol. Cell. Biol.* **1991**, *11*, 4088–4096; (f) Schlesinger, T. K.; Franger, G. R.; Yujiri, T.; Johnson, G. L. *Front. Biosci.* **1998**, *3*, D1181–D1186; (g) Eliopoulos, A. G.; Dumitru, C. D.; Wang, C. C.; Cho, J.; Tsichlis, P. N. *EMBO J.* **2002**, *21*, 4831–4840; (h) Dumitru, C. D.; Ceci, J. D.; Tsatsanis, C.; Kontoyiannis, D.; Stamatakis, K.; Lin, J. H.; Patriotis, C.; Jenkins, N. A.; Copeland, N. G.; Kollias, G.; Tsichlis, P. N. *Cell* **2000**, *103*, 1071–1083.
2. (a) Choy, E. H. S.; Panayi, G. S. *N. Eng. J. Med.* **2001**, *344*, 907–916; (b) Feldmann, M.; Brennan, F. M.; Foxwell, B. M.; Maini, R. N. *Curr. Dir. Autoimmun.* **2001**, *3*, 188–199; (c) Feldmann, M.; Maini, R. N. *Ann. Rev. Immuol.* **2001**, *19*, 163–196.
3. Dominguez, C.; Powers, D. A.; Tamayo, N. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 421–430.
4. Structure not disclosed.
5. (a) Patriotis, C.; Makris, A.; Bear, S. E.; Tsichlis, P. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2251–2255; (b) Luciano, B. S.; Hsu, S.; Channavajhala, P. L.; Lin, L.-L.; Cuozzo, J. W. *J. Biol. Chem.* **2004**, *279*, 52117–52123.
6. Gavrin, L. K.; Green, N.; Hu, Y.; Janz, K.; Kaila, N.; Li, H. Q.; Tam, S. Y.; Thomason, J. R.; Gopalsamy, A.; Ciszewski, G.; Cuozzo, J. W.; Hall, J. P.; Hsu, S.; Telliez, J. B.; Lin, L. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5288–5292.
7. Hu, Y.; Green, N.; Gavrin, L. K.; Janz, K.; Kaila, N.; Li, H. Q.; Thomason, J. R.; Cuozzo, J. W.; Hall, J. P.; Hsu, S.; Nickerson-Nutter, C.; Telliez, J. B.; Lin, L. L.; Tam, S. Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6067–6072.
8. (a) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, B. M.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakantan, R.; Reich, F. M.; Shen, R.; Tsou, H.; Upeslacis, E.; Wang, Y. F.; Wu, B.; Ye, F.; Zhang, N. *J. Med. Chem.* **2000**, *43*, 3244–3256; (b) Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B.; Floyd, B. M.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2477–2480; (c) Boschelli, D. H.; Wang, Y. D.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D. W.; Wissner, A.; Arndt, K.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 822–833; (d) Boschelli, D. H.; Ye, F.; Wang, Y. D.; Dutia, M.; Johnson, S. L.; Wu, B.; Miller, K.; Powell, D. W.; Yaczko, D.; Young, M.; Tischler, M.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 3965–3977; (e) Zhang, N.; Wu, B.; Wissner, A.; Powell, D. W.; Rabindran, S. K.; Kohler, C.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 423–425; (f) Berger, D.; Dutia, M.; Powell, D.; Wu, B.; Wissner, A.; Demorin, F.; Weber, J.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2761–2765; (g) Wissner, A.; Floyd, B. M.; Rabindran, S. K.; Nilakantan, R.; Greenberger, L. M.; Shen, R.; Wang, Y. F.; Tsou, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2893–2897; (h) Berger, D.; Dutia, M.; Powell, D.; Wissner, A.; Demorin, F.; Raifeld, Y.; Weber, J.; Boschelli, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2989–2992; (i) Berger, D.; Dutia, M.; Powell, D.; Wu, B.; Wissner, A.; Boschelli, D. H.; Floyd, B. M.; Zhang, N.; Torres, N.; Levin, J.; Du, X.; Wojciechowicz, D.; Discafani, C.; Kohler, C.; Kim, S. C.; Feldberg, L. R.; Collins, K.; Mallon, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3031–3034; (j) Wissner, A.; Overbeek, E.; Reich, M. F.; Floyd, B.; Johnson, B. D.; Mamuya, N.; Rosfjord, E. C.; Discafani, C.; Davis, R.; Shi, X.; Rabindran, S. K.; Gruber, B. C.; Ye, F.; Hallett, W. A.; Nilakantan, R.; Shen, R.; Wang, Y. F.; Greenberger, L. M.; Tsou, H. *J. Med. Chem.* **2003**, *46*, 49–63.
9. (a) Tsou, H.; Mamuya, N.; Johnson, B. D.; Reich, M. F.; Gruber, B. C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F. E.; Greenberger, L. M.; Wang, Y. F.; Wissner, A. *J. Med. Chem.* **2001**, *44*, 2719–2734; (b) Smaill, J. B.; Showalter, H. D. H.; Zhou, H.; Bridges, A. J.; McNamara, D. J.; Fry, D. W.; Nelson, J. M.; Sherwood, V.; Vincent, P. W.; Roberts, B. J.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2001**, *44*, 429–440; (c) Hennequin, L. F.; Stokes, E. S. E.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.;

Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300–1312; (d) Banerjee, R.; Rachid, Z.; McNamee, J.; Jean-Claude, B. J. *J. Med. Chem.* **2003**, *46*, 5546–5551; (e) Plé, P. A.; Green, T. P.; Hennequin, L. F.; Curwen, J.; Fennell, M.; Allen, J.; Lambert-van der Brempt, C.; Costello, G. *J. Med. Chem.* **2004**, *47*, 871–887.

10. Wissner, A.; Hamann, P. R.; Nilakantan, R.; Greenberger, L. M.; Ye, F.; Rapuano, T. A.; Loganzo, F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1411–1416.
11. Kaye, I. A.; Minsky, I. *J. Am. Chem. Soc.* **1949**, *71*, 2272–2273.
12. Jerchel, D.; Heider, J.; Wagner, H. *Justus Liebigs Ann. Chem.* **1958**, *613*, 153–170.