Structure–activity relationships in Toll-like receptor 7 agonistic 1*H*-imidazo[4,5-*c*]pyridines†

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Engagement of TLR7 in plasmacytoid dendritic cells leads to the induction of IFN- α/β which plays essential functions in the control of adaptive immunity. We had previously examined structure–activity relationships (SAR) in TLR7/8-agonistic imidazoquinolines with a focus on substituents at the N^1 , C^2 , N^3 and N^4 positions, and we now report SAR on 1*H*-imidazo[4,5-*c*]pyridines. 1-Benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine was found to be a pure TLR7-agonist with negligible activity on TLR8. Increase in potency was observed in N^6 -substituted analogues, especially in those compounds with electron-rich substituents. Direct aryl–aryl connections at C6 abrogated activity, but TLR7 agonism was reinstated in 6-benzyl and 6-phenethyl analogues. Consistent with the pure TLR7-agonistic behavior, prominent IFN- α induction in human PBMCs was observed with minimal proinflammatory cytokine induction. A benzologue of imidazoquinoline was also synthesized which showed substantial improvements in potency over the parent imidazopyridine. Distinct differences in N^6 -substituted analogues were observed with respect to IFN- α induction in human PBMCs on the one hand, and CD69 upregulation in lymphocytic subsets, on the other.

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Introduction

Host responses to pathogens are mediated *via* highly coordinated mechanisms involving both the innate and adaptive limbs of the immune system. The innate immune system utilizes germline-encoded pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs) that are distinct and unique to the pathogen.^{1–3} PRRs encompass a broad range of molecules⁴ that are secreted into the extracellular environment (such as the collectins,⁵ ficolins,⁶ pentraxins,⁷ alarmins⁸), exist in the cytosol (examples of which include the retinoic acid-inducible gene I-like receptors,⁹ and the nucleotide-binding domain and leucine-rich repeat-containing receptors¹⁰), or are present on membranes.

Important among the transmembrane PRRs include the Toll-like receptors¹¹ (TLRs) which are either expressed on the plasma membrane or in the endolysosomal compartments.^{1,12} At least 10 functional TLRs are encoded in the human genome, each with an extracellular domain having leucine-rich repeats (LRR) and a cytosolic domain called the Toll/IL-1

receptor (TIR) domain.¹³ The ligands for these receptors are highly conserved microbial molecules such as lipopolysaccharides (LPS) (recognized by TLR4), lipopeptides (TLR2 in combination with TLR1 or TLR6), flagellin (TLR5), single stranded RNA (TLR7 and TLR8), double stranded RNA (TLR3), CpG motif-containing DNA (recognized by TLR9), and profilin present on uropathogenic bacteria (TLR11).^{14,15} TLR1, -2, -4, -5, and -6 recognize extracellular stimuli, while TLR3, -7, -8 and -9 function within the endolysosomal compartment.¹³ The activation of TLRs by their cognate ligands leads to production of inflammatory cytokines, and up-regulation of major histocompatibility complex (MHC) molecules and co-stimulatory signals in antigen-presenting cells as well as activating natural killer (NK) cells (innate immune response), which leads to the priming and amplification of T-, and B-cell effector functions (adaptive immune responses).^{16–19}

The Type I interferon (IFN) family in humans include approximately 20 IFN- α subtype genes in addition to individual genes encoding IFN- β , - κ , - ϵ and - ω ; these monomeric secreted proteins bind to a single IFN- α/β receptor, which is constitutively expressed in virtually all cell types.²⁰ Occupancy of TLR7^{21–23} or TLR9^{24,25} in professional antigen-presenting cells (APCs), particularly plasmacytoid dendritic cells (pDCs), leads to the induction of IFN- α/β . Although the Type I IFNs are best known historically for their antiviral activities,²⁶ recent studies show that they have many essential functions in the control of adaptive immunity.² First, Type I IFNs promote cross-priming

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through direct stimulation of DCs, leading to specific CD8⁺ lymphocytic responses to soluble antigens.²⁷ Second, Type I IFNs potentially enhance the primary antibody responses to soluble antigens, inducing sustained and durable humoral responses with appropriate isotype switching, as well as the induction of immunological memory.²⁸ B lymphocytes can differentiate into two distinct types of functionally polarized effectors: B-effector-1-cells (Be-1), producing a Th-1-like cytokine pattern, or Be-2, characterized by a Th-2-like profile.^{29,30} It is of particular interest that recent reports suggest that IFN- α may serve as an initial trigger for Be-1-biased differentiation pattern.³¹ Third, Type I interferons secondarily induce Type II IFN (IFN- γ) secretion, also driving Th-1-biased adaptive immune responses.³² Type I IFN-inducing TLR ligands may therefore hold promise as vaccine adjuvants.

In an effort to identify optimal immunostimulatory chemotypes, we have screened representative members of virtually the entire compendium of known TLR agonists in a series of hierarchical assays including primary TLR-reporter assays, secondary indices of immune activation such as IFN- $\alpha/\beta/\gamma$ and cytokine induction, activation of lymphocytic subsets in whole human blood, and tertiary screens characterizing transcriptomal activation patterns.³³ In these assays, small-molecule agonists of TLR7 were uniquely immunostimulatory; they were potent inducers of Type I IFN and, unlike TLR-4, -5, or -8 agonists,³³ did not evoke dominant proinflammatory cytokine responses, suggesting that they may be effective, yet safe vaccine adjuvants, a premise that we have been actively exploring.³⁴ Small molecule TLR7 agonists are also being investigated as orally bioavailable, endogenous Type I IFN inducers for the management of chronic viral diseases,³⁵ especially hepatitis C and hepatitis B. Current therapeutic regimens for the therapy of hepatitis C and hepatitis B include parenteral IFN- α .³⁶ Clinical trials of TLR7 agonists for hematological malignancies are also currently underway.³⁷

The currently known small molecule agonists of TLR7 occupy a very small chemical space, and are represented by the 1*H*-imidazo[4,5-*c*]quinolines,³⁸ 8-hydroxy-^{39–41} or 8-oxoadenines,⁴² and guanine nucleoside analogues.^{43,44} We had previously reported structure–activity relationships (SAR) in the imidazoquinolines with a focus on substituents at the *N*¹, *C*², *N*³ and *N*⁴ positions,^{45,46} and we had observed that relatively minor structural modifications at these positions yielded compounds with widely differing immunomodulatory properties.^{34,47–49} It was of interest, therefore, to extend our SAR studies to the quinoline ring system. We asked if a part-structure (imidazopyridine) or a benzologue (benzoimidazoquinoline) would alter the biological properties of the parent imidazoquinoline compound. Examination of the structures of 3M-003⁵⁰ and the 8-hydroxy- and 8-oxoadenines (Fig. 1) suggested that the quinoline system may be dispensable, and activity would be retained in imidazopyridines. Indeed, imidazopyridine derivatives with alkyl groups at *C*⁶ and *C*⁷ positions,⁵¹ hydroxyalkyl,⁵² oxime and hydroxylamine-bearing substituents⁵³ at *C*², and alkylsulfonamide substituents at the *N*¹ position⁵⁴ have been reported in the patent literature.

Fig. 1 Structures of small molecule agonists of TLR7 represented by the 8-oxoadenine (SM360320, ref. 42), 8-hydroxyadenines (CL264, ref. 39), 1*H*-imidazo[4,5-*c*]quinolines 3M-003 (ref. 49), 1-isobutyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (Imiquimod) and 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (IMDQ, ref. 45).

Detailed activity profiles of these compounds, however, are not available, perhaps owing to the fact that the investigations of such compounds precede the discovery of the TLRs.

Incorporating substituents that we had previously determined to be optimal in the imidazoquinolines (*N*¹-benzyl and *C*²-butyl; IMDQ, Fig. 1), we embarked on the syntheses and biological evaluation of novel 1*H*-imidazo[4,5-*c*]pyridine analogues with modifications at the *N*¹- and *C*⁶ positions. The parent imidazopyridine compound, 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine, exhibited moderate TLR7-agonistic activity. *N*¹-Acyl or -alkyl substitutions abrogated activity. The majority of *C*⁶ derivatives bearing aryl groups were also inactive, but analogues with *N*⁶-benzyl substituents gained TLR7-specific activity. Particular *N*⁶ substituents were found to augment TLR7-specific agonistic potency without compromising specificity at TLR7; consistent with their pure TLR7 activity (and undetectable TLR8 agonism), these compounds potentially induced IFN- α in human peripheral blood mononuclear cells (PBMCs), upregulated CD69 in lymphocytic subsets, and yet showed very weak proinflammatory cytokine-inducing activities. Strong Type I IFN inducers, especially in conjunction with attenuated proinflammatory profiles are expected to be potentially adjuvant without inducing prominent local or systemic inflammation.

Results and discussion

Our interest in exploring TLR7 agonists as vaccine adjuvants has been greatly reinforced by our observations that pure TLR7 agonists, unlike other TLR ligands, are potentially immunostimulatory without prominently activating inflammatory programs in human whole blood model systems.⁵⁵ As mentioned earlier, the structures of 3M-003⁵⁰ and the 8-hydroxy- and 8-oxoadenines (Fig. 1), as well as patent literature suggested that the quinoline system may be dispensable, and activity would be retained in imidazopyridines. Our previous SAR studies on the imidazoquinolines had established that *N*¹-benzyl and

Scheme 1 Reagents: (i) BnNH_2 , NEt_3 , CH_2Cl_2 ; (ii) Zn , HCOONH_4 , MeOH ; (iii) a. $\text{C}_4\text{H}_9\text{COCl}$, NEt_3 , THF ; b. NaOH , EtOH ; (iv) $m\text{CPBA}$, CHCl_3 ; (v) a. Benzoyl isocyanate, CH_2Cl_2 ; b. NaOMe , MeOH .

C^2 -butyl substituents were optimal;⁴⁶ our point of departure in examining structure–activity relationships in the imidazopyridines consequently began with the evaluation of 1-benzyl-2-butyl-1H-imidazo[4,5-c]pyridin-4-amine (5), following the synthetic strategy described earlier (Scheme 1).^{38,46} Compound 5, itself a novel and unprecedented structure, was found to possess TLR7-specific agonistic activity (EC_{50} : 1.57 μM , Fig. 2, Table 1), with negligible TLR8 activity. The potency of the lead TLR7-specific imidazoquinoline (1-benzyl-2-butyl-1H-imidazo[4,5-c]quinolin-4-amine, structure in Fig. 1) was 0.06 μM (Fig. 2).⁴⁶

Acylation (6a, 6b, Scheme 2) of the C^4 - NH_2 resulted in complete abrogation of activity (Table 1). C6-modified analogues were synthesized *via* an alternate route. Nitration of 4-amino-2-chloropyridine resulted, as expected, in a mixture of the 3- and 5-nitro intermediates 7a and 7b, which were taken forward to obtain the 4- and 6-chloroimidazopyridines 10a and 10b (Scheme 3). Excellent chromatographic separation of these advanced intermediates was possible. Pd-catalyzed C–N cross-coupling reactions using *n*-butylamine and benzylamine furnished the C^4 -*N*-alkylated analogues 11a and 11b, respectively (Scheme 3). A 4-butoxy analogue 11c was also obtained by *ipso*-chloro displacement with 1-butanol. Compounds 11a–c were, however, inactive (Table 1). We had envisaged utilizing the

Fig. 2 TLR7 agonistic activity of imidazopyridine compounds. Data points represent means and standard deviations on quadruplicates.

Table 1 EC_{50} values of compounds in human TLR7-specific reporter gene assay

Compound number	R^1	R^2	hTLR7-agonistic activity (μM)
5	H	H	1.57
6a	COCH_3	H	Inactive ^a
6b	COC_3H_7	H	Inactive
11a	C_4H_9	H	Inactive
11b	$\text{CH}_2\text{C}_6\text{H}_5$	H	Inactive
17	H	Cl	Inactive
19a	H	NH_2	1.25
19b	H	NHC_4H_9	0.34
19c	H	$\text{NHC}_7\text{H}_{15}$	0.76
19d	H		0.32
19e	H	NHC_6H_5	1.72
19f	H	$\text{NHCH}_2\text{C}_6\text{H}_5$	0.21
19g	H		0.075
19h	H		0.61
19i	H		1.04
19j	H		0.64
19k	H		0.075
19l	H		0.25
19m	H		0.25
19n	H		Inactive
19o	H		1.09
19p	H		0.26
19q	H		0.37
19r	H		1.08
19s	H		Inactive
23a	H	C_4H_9	0.28
23b	H	C_6H_5	Inactive
23c	H		Inactive
23d	H		Inactive
23e	H		Inactive

Table 1 (Contd.)

Compound number	R ¹	R ²	hTLR7-agonistic activity (μM)
23f	H		Inactive
23g	H		0.28
23h	H		1.80
23i	H		Inactive
23j	H		0.57
30	Benzologue (Scheme 7)		0.22

^a Inactive: no activity was detected up to a concentration of 500 μg mL⁻¹.

6-chloro imidazopyridine intermediate **10b** for synthesizing C6-functionalized analogues. However, this intermediate exhibited unexpectedly low reactivity to displacement with nucleophiles or to Buchwald–Hartwig coupling reactions. As outlined in Scheme 4, we utilized 4-amino-2,6-dichloropyridine as the

Scheme 2 Reagents: (i) RCOCl, NEt₃, CH₂Cl₂.

starting material and obtained **13** as a key intermediate which, upon reaction with *tert*-octylamine⁵⁶ provided exclusively the N²-alkylated intermediate **14**. The 6-chloro-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazopyridin-4-amine intermediate **16** was obtained without difficulty, and we were able to synthesize the N⁶-substituted analogues **19a–o** under conventional Buchwald–Hartwig conditions (Scheme 4). TLRs signal *via* ligand-induced dimerization, but since that the crystal structure of human TLR7 and of its ligand binding modes are as yet unknown, we utilized intermediate **16** in constructing ‘dimeric’ imidazopyridines (using *p*- and *m*-xylenediamine, Scheme 5) to ascertain if such pre-organized dimeric ligands could yield high-potency agonists. For the C6-substituted compounds **23a–j** (Scheme 6), we observed mediocre yields in pilot Suzuki coupling reactions with the advanced intermediate **16**. We therefore exploited the electron-withdrawing resonance effect of the 3-nitro group in **14**. As expected, the classical Suzuki reaction on intermediate **14** using various aliphatic and aromatic boronic acids/boronate esters resulted in the intermediates **20a–j** (Scheme 6), which were further derivatized to obtain the desired C⁶ alkyl/aryl substituted imidazopyridines **23a–j**.

The 6-chloro imidazopyridine, **17** (Scheme 4) was inactive (Table 1). Buchwald–Hartwig-derived N⁶-substituted analogues **19a–q**, however, showed a distinctive SAR. Compound **19a** with a free NH₂ at C6, obtained by coupling the *tert*-octylamine and subsequent *N*-dealkylation with HCl (Scheme 4, Table 1) displayed TLR7-specific agonism with a potency comparable to that of the parent C6-unsubstituted compound **5**.

Modest gains in potency were obtained in analogues with short aliphatic substituents with N⁶-butyl (**19b**) and N⁶-cyclohexylmethyl (**19d**), but potency diminished in the N⁶-heptyl analogue (**19c**). The N⁶-phenyl-substituted compound **19e** was marginally weaker than **5**; however, the potency of the N⁶-benzyl analogue **19f** was ~7.6 times that of **5** (Table 1, Fig. 2), triggering a detailed SAR investigation on various aryl substituents at N⁶. Both steric and electronic effects appear to play a role in governing TLR7-agonistic potency, since the biphenyl-methyl-substituted compound **19o** was active, whereas the

Scheme 3 Reagents: (i) a. H₂SO₄, HNO₃; b. H₂SO₄; (ii) BnBr, NaH, THF; (iii) Zn, HCOONH₄, MeOH; (iv) a. C₄H₉COCl, NEt₃, THF; b. NaOH, EtOH; (v) For **11a** and **11b**, amines (*n*-BuNH₂ and BnNH₂, respectively), Pd₂(dba)₃, DavePhos, KOtBu, dioxane; For **11c**, BuOH, NaH, THF.

Scheme 4 Reagents: (i) a. H_2SO_4 , HNO_3 ; b. H_2SO_4 ; (ii) BnBr , NaH , THF ; (iii) t -Octylamine, NEt_3 , CH_2Cl_2 ; (iv) Zn , HCOONH_4 , MeOH ; (v) a. $\text{C}_4\text{H}_9\text{COCl}$, NEt_3 , THF ; b. NaOH , EtOH ; (vi) RNH_2 , $\text{Pd}_2(\text{dba})_3$, DavePhos , KOtBu , dioxane; (vii) HCl .

Scheme 5 Reagents: (i) $\text{Pd}_2(\text{dba})_3$, DavePhos , KOtBu , dioxane; (ii) HCl .

naphthylmethyl analogue **19n** was quiescent; to a first approximation, electron-rich N^6 substituents appear to be preferred, with the methoxybenzyl derivatives (**19g** and **19h**) and the pyridinylmethyl compounds (**19l** and **19m**) being marginally more active than the trifluoromethyl-(**19i**) or chloro-(**19j**) substituted analogues. Compounds **19p** and **19q** were also active in primary screens, with EC_{50} values of 0.26 and 0.37 μM , respectively (Table 1). In the C^6 -alkyl or -aryl analogues (Scheme 6), the SAR appeared more stringent. Whereas the C^6 -butyl compound **23a** was more active than **5**, direct aryl-aryl connections at C^6 (**23b-f**) abrogated activity, but TLR7 agonistic properties were restored in the 6-benzyl (**23g**) and 6-phenethyl analogues (**23j**). Taken together with activity data of compounds of the **19** series, we surmised that rotational constraints about the C^6 -aryl groups may hinder TLR7 occupancy. Unlike TLR2, TLR3, TLR4,⁵⁷ and TLR5⁵⁸ for which crystal structures are available as complexes with their cognate ligands, a detailed structural characterization of the mode of binding of TLR7 ligands is not yet available to guide structure-based design of small molecule agonists of TLR7, necessitating classical SAR approaches to refine successive iterations of ligand design.

The benzologue **30** was synthesized as shown in Scheme 7. It showed substantial improvements in potency over the parent imidazopyridine **5** (Fig. 3, Table 1), but the two most potent compounds in the entire series as adjudged by primary screens were the N^6 -(4-methoxybenzyl) and N^6 -(furan-2-ylmethyl) analogues (**19g** and **19k**, respectively), both of which were approximately twenty-fold more potent than **5** (Fig. 2, Table 1).

We chose the nine most active compounds (**19b**, **19d**, **19f-g**, **19k-m**, **19p** and **23a**) for evaluation in secondary screens using IFN- α and cytokine release in human PBMCs. We used, as reference compounds, imiquimod, a known TLR7 agonist,^{59,60} as well as CL075^{61,62} (2-propylthiazolo[4,5-*c*]quinolin-4-amine), a predominantly TLR8-active agonist with an EC_{50} of 1.32 μM in hTLR8 assays.⁶³ Given that the imidazopyridine compounds are pure TLR7 agonists, we expected to find prominent IFN- α induction,³³ and this was indeed the case, with **19p**, **19m** and **19k** being the most potent (EC_{50} : 0.3 μM , 0.4 μM and 0.7 μM , respectively; Fig. 4). CL075 was among the least potent in IFN- α induction (EC_{50} : 2.6 μM ; Fig. 4), and as expected for a TLR8 agonist, CL075 was dramatically more active in inducing

Scheme 6 Reagents: (i) For **20a–f** and **20j**, R-boronic acid, Pd(dppf)Cl₂, Cs₂CO₃, dioxane; for **20c**, 4-cyanophenyl boronic acid, Pd(dppf)Cl₂, Cs₂CO₃, dioxane; for **20g–i**, R-boronic acid pinacol ester, Pd(dppf)Cl₂, Cs₂CO₃, dioxane; (ii) Zn, HCOONH₄, MeOH; (iii) a. C₄H₉COCl, NEt₃, THF; b. NaOH, EtOH; (iv) HCl.

Scheme 7 Reagents: (i) a. HCl, HON=CHCH₂NO₂, H₂O; b. (CH₃CO)₂O, CH₃COOK; (ii) POCl₃; (iii) BnNH₂, NEt₃, CH₂Cl₂; (iv) Zn, HCOONH₄, MeOH; (v) a. C₄H₉COCl, NEt₃, THF; b. NaOH, EtOH; (vi) mCPBA, CH₂Cl₂, CHCl₃, MeOH; (vii) a. Benzoyl isocyanate, CH₂Cl₂; b. NaOMe, MeOH.

proinflammatory cytokines such as TNF- α , IL-1 β , and IL-8 (Fig. 4). We do not yet understand the basis for the slight discrepancy between rank-order potency in primary screens (**19k** $\approx$ **19g** > **19f** > **19p**; Fig. 2, Table 1) *vis-à-vis* IFN- α -inducing potency in human PBMCs (**19p** $\approx$ **19m** > **19k**; Fig. 4), and we surmise that analogues with more basic C6 substituents may allow for higher endolysosomal partitioning. The dose-response profiles show characteristic biphasic responses (dose-dependent activation, followed by apparent suppression) as we had previously observed in several chemotypes. We verified

Fig. 3 Dose-response profiles of TLR7 agonistic activity of compounds **5** and **30**. Data points represent means and standard deviations on quadruplicates.

that the apparent suppression was not due to cytotoxicity using LDH release and mitochondrial redox-based assays.

We had previously shown that of all the various classes of innate immune stimuli, TLR7 agonists were extraordinarily immunostimulatory, stimulating virtually all subsets of lymphocytes (assessed by quantifying CD69 expression), and yet without inducing dominant proinflammatory cytokine responses,³³ and we wished to confirm the rank-order potency observed in IFN- α induction assays described above. We observed considerable dissociation between Type I IFN induction on the one hand (Fig. 4), and CD69 upregulation in lymphocytic subsets on the other (Fig. 5). Whereas the subset of active compounds induced IFN- α with similar potencies (EC₅₀ values between 0.3–2 μ M; Fig. 4), pronounced differences were observed in CD69 expression in natural killer, cytokine-induced killer and B lymphocytic subsets with **19p** being as active as the reference TLR7 agonist IMDQ, and **19d** showing virtually no activity (Fig. 5). Possible mechanisms underlying the differential activity in these two compounds are being investigated.

Conclusions

These findings raise the possibility of utilizing these compounds in selectively targeting Type I IFN induction *versus* lymphocytic activation, and are being explored in greater detail.

The potential advantages of strong Type I IFN inducers as candidate vaccine adjuvants have been discussed earlier. Such compounds, especially in conjunction with attenuated proinflammatory cytokines, are expected to be potently adjuvantive without inducing prominent local or systemic inflammation. As mentioned earlier, the prominent Type I IFN inducing abilities of the imidazopyridines may also find utility as an alternative therapeutic strategy to address disease states wherein systemic IFN- α is of proven benefit. A clear delineation of structural features that confer TLR specificity not only charts a rational course for the development of effective, yet

Fig. 4 Dose-response profiles of Type I interferon (IFN- α) and proinflammatory cytokine (IL-8, IL-1 β , and TNF- α) induction by selected imidazopyridine (and reference) compounds. Representative data from three independent experiments are presented.

safe vaccine adjuvants, but also provides tools to understand innate immune function in greater detail.

Experimental

Materials and equipment

All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or

Fig. 5 CD69 upregulation in human natural killer (NK), cytokine-induced killer (CIK) and nominal B lymphocytes by select imidazopyridine (and reference) compounds.

air-sensitive reactions were conducted under nitrogen atmosphere in oven-dried (120 °C) glass apparatus. The solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep R_f 'Gold' high performance silica columns on CombiFlash R_f instrument unless otherwise mentioned, while thin-layer chromatography was carried out on silica gel (200 μ m) CCM pre-coated aluminum sheets. Purity for all final compounds was confirmed to be greater than 97% by LC-MS using a Zorbax Eclipse Plus 4.6 mm $\times$ 150 mm, 5 μ m analytical reverse phase C_{18} column with H_2O -isopropanol or H_2O - CH_3CN gradients and an Agilent 6520 ESI-QTOF Accurate

Mass spectrometer (mass accuracy of 5 ppm) operating in the positive ion (or negative ion, as appropriate) acquisition mode.

Synthesis of compound 1: *N*-benzyl-3-nitropyridin-4-amine

To a solution of 4-chloro-3-nitropyridine (1.0 g, 6.31 mmol) in 25 mL of CH₂Cl₂ were added triethylamine (1.32 mL, 9.47 mmol) and benzyl amine (0.83 mL, 7.57 mmol). The reaction mixture was refluxed for 18 h. The solvent was then evaporated under vacuum and H₂O was added to the residue. The solution was extracted with CH₂Cl₂ (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was evaporated and the residue was purified using silica gel column chromatography (5% MeOH–CH₂Cl₂) to obtain compound 1 as a yellow solid (1.4 g, 94%). ¹H NMR (500 MHz, CDCl₃) δ 9.22 (s, 1H), 8.53 (s, 1H), 8.25 (dd, *J* = 6.1, 0.6 Hz, 1H), 7.41–7.35 (m, 2H), 7.32 (dd, *J* = 7.2, 5.3 Hz, 3H), 6.69 (d, *J* = 6.2 Hz, 1H), 4.56 (d, *J* = 5.7 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 153.38, 149.06, 148.68, 135.96, 130.04, 129.21, 128.22, 127.10, 108.25, 46.85. MS (ESI) calculated for C₁₂H₁₂N₃O₂, *m/z* 230.0924, found 230.0949 (M + H)⁺.

Synthesis of compound 2: *N*⁴-benzylpyridine-3,4-diamine

To a solution of compound 1 (1.0 g, 4.36 mmol) in 40 mL of MeOH were added zinc dust (1.4 g, 21.8 mmol) and ammonium formate (1.4 g, 21.8 mmol). The reaction mixture was stirred at room temperature for 10 min and filtered through celite. Then the solvent was evaporated and the residue was dissolved in water. This was extracted with EtOAc (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was evaporated under vacuum to obtain the compound 2 (0.8 g, 92%). ¹H NMR (500 MHz, CDCl₃) δ 7.81 (d, *J* = 5.4 Hz, 1H), 7.77 (s, 1H), 7.29–7.19 (m, 5H), 6.36 (d, *J* = 5.4 Hz, 1H), 4.87 (s, 1H), 4.28 (d, *J* = 5.4 Hz, 2H), 3.28 (s, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 144.52, 143.51, 138.22, 137.53, 128.96, 128.79, 127.55, 127.48, 105.29, 47.22. MS (ESI) calculated for C₁₂H₁₄N₃, *m/z* 200.1182, found 200.1242 (M + H)⁺.

Synthesis of compound 3: 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine

To a solution of compound 2 (400 mg, 2.00 mmol) in 20 mL of anhydrous THF were added triethylamine (0.29 mL, 2.10 mmol) and valeryl chloride (0.27 mL, 2.20 mmol). The reaction mixture was refluxed for 2 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The EtOAc fraction was dried using sodium sulfate and evaporated under vacuum to obtain the intermediate amide compound. This was dissolved in 20 mL of EtOH and NaOH (160 mg, 4.00 mmol) in 2 mL of H₂O was added. The reaction mixture was refluxed for 4 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The organic layer was dried using sodium sulfate and evaporated and purified using column chromatography (5% MeOH–CH₂Cl₂) to obtain the compound 3 (210 mg, 40%). ¹H NMR (500 MHz, CDCl₃) δ 9.04 (d, *J* = 0.6 Hz, 1H), 8.34 (d, *J* = 5.6 Hz, 1H), 7.34–7.28 (m, 3H), 7.14 (dd, *J* = 5.6, 1.0 Hz, 1H), 7.01 (dd, *J* = 7.7, 1.8 Hz, 2H),

5.33 (s, 2H), 2.86–2.81 (m, 2H), 1.82 (dt, *J* = 15.5, 7.7 Hz, 2H), 1.46–1.36 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 157.30, 142.14, 142.00, 140.48, 140.01, 135.26, 129.28, 128.37, 126.27, 105.12, 47.25, 29.44, 27.38, 22.61, 13.85. MS (ESI) calculated for C₁₇H₂₀N₃, *m/z* 266.1652, found 266.1715 (M + H)⁺.

Synthesis of compound 4: 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine 5-oxide

To a solution of compound 3 (210 mg, 0.79 mmol) in 15 mL of was added *m*-chloroperoxybenzoic acid (443 mg, 1.98 mmol), and the solution was refluxed at 45–50 °C for 1 h. The solvent was then removed and the residue was purified using column chromatography (10% MeOH–CH₂Cl₂) to obtain the *N*-oxide derivative (188 mg, 85%). ¹H NMR (500 MHz, CDCl₃) δ 8.71 (d, *J* = 1.3 Hz, 1H), 8.05 (dd, *J* = 7.0, 1.6 Hz, 1H), 7.36–7.30 (m, 3H), 7.01 (dd, *J* = 10.4, 4.3 Hz, 3H), 5.31 (s, 2H), 2.85–2.80 (m, 2H), 1.79 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.44–1.34 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 160.72, 140.89, 134.42, 134.32, 133.94, 131.52, 129.48, 128.75, 126.24, 106.63, 47.75, 29.25, 27.49, 22.52, 13.78. MS (ESI) calculated for C₁₇H₂₀N₃O, *m/z* 282.1601, found 282.1612 (M + H)⁺.

Synthesis of compound 5: 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine

To a solution of compound 4 (188 mg, 0.67 mol) in 15 mL of CH₂Cl₂ was added benzoyl isocyanate (197 mg, 1.34 mmol) and heated at 45 °C for 2 h. The solvent was then removed under vacuum, and the residue was dissolved in 15 mL of anhydrous MeOH, followed by the addition of excess sodium methoxide. The reaction mixture was then heated at 80 °C for 1 h. The solvent was removed under vacuum and the residue was purified using column chromatography (7% MeOH–CH₂Cl₂) to obtain the compound 5 (56 mg, 30%). ¹H NMR (500 MHz, CDCl₃) δ 7.78 (d, *J* = 5.8 Hz, 1H), 7.34–7.28 (m, 3H), 7.03 (d, *J* = 6.4 Hz, 2H), 6.59 (d, *J* = 5.8 Hz, 1H), 5.27 (s, 2H), 5.15 (s, 2H), 2.83–2.75 (m, 2H), 1.72 (ddd, *J* = 13.0, 9.0, 7.7 Hz, 2H), 1.44–1.34 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 154.06, 151.00, 140.61, 140.41, 135.76, 129.21, 128.23, 126.33, 97.74, 47.52, 30.10, 27.42, 22.68, 13.89. HRMS (ESI) calculated for C₁₇H₂₁N₄, *m/z* 281.1761, found 281.1795 (M + H)⁺.

Synthesis of compound 6a: *N*-(1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)acetamide

To a solution of compound 5 (30 mg, 0.11 mmol) in 2 mL of CH₂Cl₂ were added triethylamine (17 μL, 0.12 mmol) and acetyl chloride (8 μL, 0.11 mmol). The reaction mixture was stirred at room temperature for 3 h and purified using column chromatography (5% MeOH–CH₂Cl₂) to obtain the compound 6a as white solid (6 mg, 16%). ¹H NMR (500 MHz, CDCl₃) δ 11.60 (s, 1H), 8.20 (s, 1H), 7.37 (dd, *J* = 7.7, 5.5 Hz, 3H), 7.20 (d, *J* = 5.6 Hz, 1H), 7.05 (dd, *J* = 6.3, 2.5 Hz, 2H), 5.46 (s, 2H), 3.03–2.94 (m, 2H), 2.55 (s, 3H), 1.85 (dt, *J* = 15.0, 7.6 Hz, 2H), 1.43 (dq, *J* = 14.6, 7.3 Hz, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 141.48, 129.64, 129.40, 129.17, 126.30,

126.25, 48.47, 29.06, 24.87, 22.44, 13.60. HRMS (ESI) calculated for $C_{19}H_{23}N_4O$, m/z 323.1866, found 323.1911 ($M + H$)⁺.

Compounds **6b** was synthesized similarly as compound **6a**.

N-(1-Benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-yl)butyramide (**6b**)

Butyryl chloride was used as a reagent. (4 mg, 14%). ¹H NMR (500 MHz, CDCl₃) δ 11.31 (s, 1H), 8.30 (d, J = 4.8 Hz, 1H), 7.38–7.34 (m, 3H), 7.27 (s, 1H), 7.04 (dd, J = 6.4, 2.6 Hz, 2H), 5.50 (s, 2H), 2.94 (t, J = 7.5 Hz, 2H), 2.78 (t, J = 7.4 Hz, 2H), 1.80 (dp, J = 22.1, 7.5 Hz, 4H), 1.41 (dt, J = 14.7, 7.4 Hz, 2H), 1.01 (t, J = 7.4 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 174.32, 141.35, 133.50, 129.69, 129.16, 126.46, 103.38, 48.58, 39.27, 29.16, 27.45, 22.55, 18.38, 13.75. HRMS (ESI) calculated for $C_{21}H_{27}N_4O$, m/z 351.2179, found 351.2240 ($M + H$)⁺.

Synthesis of compound 10a: 1-benzyl-2-butyl-4-chloro-1*H*-imidazo[4,5-*c*]pyridine

4-Amino-2-chloropyridine (2.0 g, 15.6 mmol) was taken in 20 mL of conc. H₂SO₄ in an ice-bath to which was added 10 mL of conc. HNO₃ slowly. The reaction mixture was gradually brought to room temperature and stirred for 1 h. The reaction was quenched by pouring the reaction mixture on ice. Ammonium hydroxide solution was slowly added until a pH of 3.0 was reached. A white solid was obtained which was filtered, washed with water, and dried. This (*N*-nitro)aminopyridine intermediate was taken up in 10 mL of conc. H₂SO₄ and the reaction solution was heated at 90 °C for 30 min. It was cooled to room temperature and poured into ice. It was slowly neutralized with ammonium hydroxide solution until a pH of 7 and the formed yellow solid was filtered, washed with water and dried to obtain compound **7** as a mixture of 2-chloro-3-nitropyridin-4-amine and 2-chloro-5-nitropyridin-4-amine intermediates. Sodium hydride (275 mg, 6.90 mmol) was carefully added to 20 mL of THF under N₂ and compound **7** (1.0 g, 5.76 mmol) was slowly added to the solution at 0 °C. The reaction mixture was stirred for 1 h, followed by the addition of benzyl bromide (0.75 mL, 6.34 mmol). The reaction mixture was stirred at room temperature for 2 h and poured into ice water. Then it was extracted with EtOAc (3 × 20 mL), washed with water, dried over sodium sulfate. The solvent was removed and the crude residue was purified using column chromatography (20% EtOAc–hexane) to obtain the compound **8** as a mixture of regioisomeric *N*-benzyl-2-chloro-3-nitropyridin-4-amine and *N*-benzyl-2-chloro-5-nitropyridin-4-amine intermediates. To this regioisomeric mixture (1.0 g, 4.2 mmol) in 20 mL of MeOH were added zinc dust (1.4 g, 21.0 mmol) and ammonium formate (1.4 g, 21.0 mmol). The reaction mixture was stirred at room temperature for 10 min and filtered through celite. Then the solvent was evaporated and the residue was dissolved in water. This was extracted with EtOAc (3 × 20 mL), washed with water and dried over sodium sulfate. The filtrate evaporated under vacuum, and chromatographed (20% EtOAc–hexane) to obtain the required *N*⁴-benzyl-2-chloropyridine-3,4-diamine compound **9a**. Also obtained was

*N*⁴-benzyl-6-chloropyridine-3,4-diamine as a side-product. To a solution of compound **9a** (495 mg, 2.12 mmol) in 20 mL of anhydrous THF were added triethylamine (0.31 mL, 2.23 mmol) and valeryl chloride (0.28 mL, 2.33 mmol). The reaction mixture was refluxed for 1 h. The solvent was then removed under vacuum, and the residue was dissolved in 20 mL of EtOH and NaOH (170 mg, 4.24 mmol) in 2 mL of H₂O was added. The reaction mixture was refluxed for 2 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The EtOAc fraction was dried using sodium sulfate and evaporated and purified using column chromatography (5% MeOH–CH₂Cl₂) to obtain the compound **10a** (203 mg, 32%). ¹H NMR (500 MHz, CDCl₃) δ 8.10 (d, J = 5.6 Hz, 1H), 7.35–7.30 (m, 3H), 7.08 (d, J = 5.6 Hz, 1H), 7.01 (dd, J = 7.4, 2.0 Hz, 2H), 5.34 (s, 2H), 2.91–2.86 (m, 2H), 1.78 (dt, J = 15.7, 7.7 Hz, 2H), 1.45–1.36 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 158.03, 141.88, 141.75, 141.12, 137.03, 134.84, 129.39, 128.58, 126.25, 105.18, 47.90, 30.04, 27.63, 22.73, 13.82. MS (ESI) calculated for $C_{17}H_{19}ClN_3$, m/z 300.1262, found 300.1159 ($M + H$)⁺.

Synthesis of compound 11a: 1-benzyl-*N*,2-dibutyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine

To a solution of compound **10** (50 mg, 0.17 mmol) in 1 mL of dioxane were added potassium *tert*-butoxide (57 mg, 0.51 mmol), catalytic amount of 2-dicyclohexylphosphino-2'-(*N,N*-dimethylamino)biphenyl (DavePhos) and tris(dibenzylideneacetone)dipalladium(0) (Pd₂(dba)₃) and butyl amine (83 μ L, 0.83 mmol). The reaction mixture was then heated under microwave conditions (500 W, 100 °C) in a sealed vial for 1 h. It was cooled to room temperature and filtered through celite and washed with MeOH. The solvent was removed and the crude residue was purified using column chromatography (5% MeOH–CH₂Cl₂) to obtain the compound **11a** (21 mg, 36%). ¹H NMR (500 MHz, CDCl₃) δ 7.84 (d, J = 5.9 Hz, 1H), 7.33–7.27 (m, 3H), 7.03 (dd, J = 4.5, 3.6 Hz, 2H), 6.48 (d, J = 5.9 Hz, 1H), 5.41 (s, 1H), 5.25 (s, 2H), 3.60 (dt, J = 12.9, 6.5 Hz, 2H), 2.80–2.74 (m, 2H), 1.73–1.67 (m, 4H), 1.49 (dt, J = 15.0, 7.4 Hz, 3H), 1.38 (dd, J = 15.0, 7.5 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 153.15, 151.39, 135.95, 129.24, 129.15, 128.15, 127.25, 126.34, 126.18, 96.16, 47.43, 41.09, 32.22, 30.27, 27.41, 22.69, 20.47, 14.11, 13.89. HRMS (ESI) calculated for $C_{21}H_{29}N_4$, m/z 337.2387, found 337.2451 ($M + H$)⁺.

Compounds **11b** was synthesized similarly as compound **11a**.

N,1-Dibenzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine (**11b**)

Benzyl amine was used as a reagent. (33 mg, 52%). ¹H NMR (500 MHz, CDCl₃) δ 7.86 (d, J = 5.9 Hz, 1H), 7.45 (dd, J = 7.9, 0.9 Hz, 2H), 7.35–7.27 (m, 6H), 7.03 (d, J = 6.4 Hz, 2H), 6.54 (d, J = 5.9 Hz, 1H), 5.76 (s, 1H), 5.26 (s, 2H), 4.83 (d, J = 5.6 Hz, 2H), 2.79–2.74 (m, 2H), 1.72–1.67 (m, 2H), 1.37 (dq, J = 14.8, 7.4 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 153.39, 150.92, 140.51, 139.81, 139.78, 135.88, 129.18, 128.62, 128.23, 128.19, 127.24, 126.36, 126.24, 96.73, 47.46,

45.40, 30.14, 27.38, 22.67, 13.88. HRMS (ESI) calculated for $C_{24}H_{27}N_4$, m/z 371.2230, found 371.2303 ($M + H$)⁺.

Synthesis of compound 11c: 1-benzyl-4-butoxy-2-butyl-1*H*-imidazo[4,5-*c*]pyridine

To a suspension of sodium hydride (48 mg, 2.00 mmol) in 2 mL of anhydrous THF was added 1-butanol (0.18 mL, 2.00 mmol). It was stirred at room temperature for 1 h, followed by the addition of compound **10** (100 mg, 0.33 mmol). The reaction mixture was heated at 60 °C for 18 h and then solvent was evaporated under vacuum. The residue was extracted with EtOAc (3 × 10 mL), washed with water and dried over sodium sulfate. The solvent was removed and the crude residue was purified using column chromatography (5% MeOH-CH₂Cl₂) to obtain the compound **11c** (61 mg, 55%). ¹H NMR (500 MHz, CDCl₃) δ 7.85 (d, J = 5.8 Hz, 1H), 7.33–7.28 (m, 3H), 7.01 (dd, J = 7.7, 1.7 Hz, 2H), 6.77 (d, J = 5.8 Hz, 1H), 5.30 (s, 2H), 4.52 (t, J = 7.0 Hz, 2H), 2.87–2.82 (m, 2H), 1.90 (dd, J = 15.0, 7.1 Hz, 2H), 1.75 (dt, J = 15.7, 7.7 Hz, 2H), 1.58–1.48 (m, 2H), 1.38 (dq, J = 14.7, 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 156.04, 155.18, 142.02, 138.95, 135.59, 129.21, 128.27, 127.41, 126.27, 100.47, 66.25, 47.61, 31.34, 30.21, 27.44, 22.75, 19.45, 14.09, 13.85. HRMS (ESI) calculated for $C_{21}H_{28}N_3O$, m/z 338.2227, found 338.2307 ($M + H$)⁺.

Synthesis of compound 12: 2,6-dichloro-3-nitropyridin-4-amine

4-Amino-2,6-dichloropyridine (2.0 g, 12.27 mmol) was added to 20 mL of conc. H₂SO₄. The mixture was cooled to 0 °C and 10 mL of conc. HNO₃ was dropwise at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then poured into crushed ice. The white solid was filtered, washed with water and dried. This intermediate was dissolved in 10 mL of conc. H₂SO₄ and the reaction solution was heated at 90 °C for 30 min. It was cooled to room temperature and poured into ice. It was slowly neutralized with ammonium hydroxide solution until a pH of 9 and the formed yellow solid was filtered, washed with water and dried to obtain compound **12** as light yellow solid. ¹H NMR (500 MHz, MeOD) δ 6.84 (s, 1H). ¹³C NMR (126 MHz, MeOD) δ 152.53, 151.18, 144.57, 111.12.

Synthesis of compound 13: *N*-benzyl-2,6-dichloro-3-nitropyridin-4-amine

Sodium hydride (138 mg, 5.77 mmol) was carefully suspended in 10 mL of dry THF under N₂. The grey suspension was cooled to 0 °C and compound **12** (1.0 g, 4.81 mmol) was slowly added to the suspension at 0 °C. The reaction mixture was stirred for 1 h, followed by the addition of benzyl bromide (0.5 mL, 5.29 mmol). The reaction mixture was stirred at room temperature for 2 h and poured into ice water. Then it was extracted with EtOAc (3 × 20 mL), washed with water, dried over sodium sulfate. The solvent was removed and the crude residue was purified using column chromatography (20% EtOAc-hexane) to obtain the compound **13** as yellow solid. ¹H

NMR (500 MHz, CDCl₃) δ 7.44–7.34 (m, 3H), 7.32–7.27 (m, 2H), 6.99 (s, 1H), 6.67 (s, 1H), 4.47 (d, J = 5.4 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 152.07, 149.97, 145.68, 135.01, 129.48, 128.71, 127.32, 106.62, 47.74.

Synthesis of compound 14: *N*¹-benzyl-6-chloro-3-nitro-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine

To a solution of compound **13** (300 mg, 1.02 mmol) in 20 mL of CH₂Cl₂ were added triethylamine (0.21 mL, 1.53 mmol) and *tert*-octylamine (0.5 mL, 3.06 mmol). The reaction mixture was refluxed for 48 h and the solvent was removed under vacuum. The crude residue was purified using column chromatography (20% EtOAc-hexane) to obtain the compound **14** as yellow solid (360 mg, 91%). ¹H NMR (500 MHz, CDCl₃) δ 9.57 (s, 1H), 9.51 (s, 1H), 7.39 (ddd, J = 7.5, 4.4, 1.3 Hz, 2H), 7.36–7.30 (m, 3H), 5.92 (s, 1H), 4.44 (d, J = 5.4 Hz, 2H), 1.95 (s, 2H), 1.56 (s, 6H), 0.98 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 155.05, 154.25, 153.44, 136.10, 129.24, 128.24, 127.43, 115.80, 94.21, 57.32, 51.54, 47.64, 31.94, 31.66, 29.84. MS (ESI) calculated for $C_{20}H_{28}ClN_4O_2$, m/z 391.1895, found 391.1901 ($M + H$)⁺.

Synthesis of compound 16: 1-benzyl-2-butyl-6-chloro-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine

To a solution of compound **14** (260 mg, 0.66 mmol) in 20 mL of MeOH were added zinc dust (434 mg, 6.60 mmol) and ammonium formate (416 mg, 6.60 mmol). The reaction mixture was stirred at room temperature for 10 min and filtered through celite. Then the solvent was evaporated and the residue was dissolved in water. This was extracted with EtOAc (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was removed under vacuum to obtain compound **15**, brown oil (184 mg, 77%). To a solution of compound **15** (184 mg, 0.50 mmol) in 10 mL of anhydrous THF were added triethylamine (74 μL, 0.52 mmol) and valeryl chloride (62 μL, 0.50 mmol). The reaction mixture was refluxed for 1 h. The solvent was then removed under vacuum, and the residue was dissolved in 10 mL of EtOH and NaOH (40 mg, 1.00 mmol) in 1 mL of H₂O was added. The reaction mixture was refluxed for 5 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The EtOAc fraction was dried using sodium sulfate and evaporated and purified using column chromatography (20% EtOAc-hexane) to obtain the compound **16** as yellow solid (120 mg, 56%). ¹H NMR (500 MHz, CDCl₃) δ 7.34–7.28 (m, 3H), 7.02 (d, J = 6.5 Hz, 2H), 6.42 (s, 1H), 5.38 (s, 1H), 5.16 (s, 2H), 2.76–2.69 (m, 2H), 2.02 (s, 2H), 1.71–1.65 (m, 4H), 1.60 (s, 6H), 1.37 (dd, J = 15.0, 7.5 Hz, 2H), 1.01 (s, 9H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 153.37, 149.45, 141.67, 140.77, 135.64, 129.21, 128.22, 126.31, 125.50, 93.95, 55.74, 51.42, 47.42, 31.91, 31.73, 30.12, 29.96, 27.37, 22.66, 13.87. MS (ESI) calculated for $C_{25}H_{36}ClN_4$, m/z 427.2623, found 427.2635 ($M + H$)⁺.

Synthesis of compound 17: 1-benzyl-2-butyl-6-chloro-1*H*-imidazo[4,5-*c*]pyridin-4-amine

Compound **16** (34 mg, 0.082 mmol) was dissolved in 1 mL of HCl (4 M in dioxane) and stirred at room temperature for

30 min. Then the solvent was removed under vacuum to obtain compound **17** (11 mg, 42%). ^1H NMR (500 MHz, CDCl_3) δ 7.36–7.30 (m, 3H), 7.01 (d, J = 6.4 Hz, 2H), 6.58 (s, 1H), 5.23 (s, 2H), 5.21 (s, 2H), 2.80–2.73 (m, 2H), 1.72 (dt, J = 15.5, 7.7 Hz, 2H), 1.39 (dq, J = 14.8, 7.4 Hz, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 154.83, 149.94, 142.31, 141.73, 135.26, 129.32, 128.57, 128.41, 126.26, 96.29, 47.59, 29.92, 27.36, 22.63, 13.87. HRMS (ESI) calculated for $\text{C}_{17}\text{H}_{20}\text{ClN}_4$, m/z 315.1371, found 315.1422 ($\text{M} + \text{H}$) $^+$.

Synthesis of compound **19a**: 1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]-pyridine-4,6-diamine

To a solution of compound **16** (70 mg, 0.16 mmol) in 1 mL of dioxane were added potassium *tert*-butoxide (92 mg, 0.82 mmol), catalytic amount of DavePhos and $\text{Pd}_2(\text{dba})_3$ and *tert*-octylamine (83 μL , 0.83 mmol). The reaction mixture was then heated under microwave conditions (500 W, 100 $^\circ\text{C}$) in a sealed vial for 1 h. It was cooled to room temperature and filtered through celite and washed with MeOH. The solvent was removed and the crude residue was purified using column chromatography (20% EtOAc–hexane) to obtain the compound **18a**, brown solid (41 mg, 49%). Compound **18a** (33 mg, 0.063 mmol) was dissolved in 1 mL of HCl (4 M in dioxane) and stirred at room temperature for 30 min. Then the solvent was removed under vacuum to obtain compound **19a** as brown solid (11 mg, 58%). ^1H NMR (500 MHz, DMSO) δ 7.37–7.31 (m, 2H), 7.30–7.25 (m, 1H), 7.09–7.04 (m, 2H), 6.41 (s, 2H), 5.65 (s, 1H), 5.24 (s, 2H), 2.73–2.67 (m, 2H), 1.59 (dt, J = 15.3, 7.5 Hz, 2H), 1.31 (dq, J = 14.7, 7.4 Hz, 2H), 0.83 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 151.81, 147.80, 144.08, 136.91, 128.81, 128.77, 127.47, 126.29, 117.96, 76.19, 46.11, 29.27, 26.17, 21.80, 13.68. HRMS (ESI) calculated for $\text{C}_{17}\text{H}_{22}\text{N}_5$, m/z 296.1870, found 296.1906 ($\text{M} + \text{H}$) $^+$.

Compounds **19b–19o** were synthesized similarly as compound **19a**.

1-Benzyl-*N* 6,2 -dibutyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**19b**)

Butyl amine was used as a reagent. Brown solid (23 mg, 79%). ^1H NMR (500 MHz, CDCl_3) δ 7.31 (ddd, J = 8.6, 6.4, 3.4 Hz, 3H), 7.05 (d, J = 6.7 Hz, 2H), 5.46 (s, 1H), 5.26 (s, 1H), 5.16 (s, 2H), 3.03 (t, J = 7.1 Hz, 2H), 2.72–2.66 (m, 2H), 1.68 (dd, J = 15.5, 7.6 Hz, 2H), 1.55 (dd, J = 14.8, 7.2 Hz, 2H), 1.43–1.33 (m, 4H), 0.90 (dt, J = 16.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 152.42, 148.50, 144.42, 135.99, 129.14, 128.06, 126.33, 74.80, 47.20, 43.09, 31.41, 30.03, 27.37, 22.66, 20.40, 14.02, 13.89. HRMS (ESI) calculated for $\text{C}_{21}\text{H}_{30}\text{N}_5$, m/z 352.2496, found 352.2515 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-*N* 6 -heptyl-*N* 4 -(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**18c**)

Heptylamine was used as a reagent. Brown oil (43 mg, 65%). ^1H NMR (500 MHz, CDCl_3) δ 7.32–7.26 (m, 3H), 7.05 (d, J = 6.7 Hz, 2H), 5.39 (s, 1H), 5.13 (s, 1H), 5.11 (s, 2H), 3.13 (t, J = 7.2 Hz, 2H), 2.68–2.64 (m, 2H), 2.04 (s, 2H), 1.65–1.55 (m, 11H), 1.41–1.22 (m, 12H), 1.01 (s, 9H), 0.89–0.84 (m, 6H). ^{13}C NMR

(126 MHz, CDCl_3) δ 154.28, 150.38, 149.22, 142.39, 136.63, 128.97, 128.05, 127.76, 126.40, 120.44, 95.52, 73.57, 55.27, 51.72, 47.00, 43.63, 31.97, 31.94, 31.90, 31.79, 30.47, 30.29, 29.97, 29.32, 27.44, 27.39, 22.77, 22.71, 14.25, 13.90. MS (ESI) calculated for $\text{C}_{32}\text{H}_{52}\text{N}_5$, m/z 506.4217, found 506.4209 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-*N* 6 -heptyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**19c**)

Light brown solid (24 mg, 80%). ^1H NMR (500 MHz, DMSO) δ 8.03 (s, 2H), 7.38–7.33 (m, 2H), 7.32–7.28 (m, 1H), 7.12 (d, J = 7.2 Hz, 2H), 6.84 (s, 1H), 5.98 (s, 1H), 5.39 (s, 2H), 3.08 (t, J = 7.0 Hz, 2H), 2.73–2.68 (m, 2H), 1.59 (dt, J = 15.3, 8.2 Hz, 2H), 1.50 (dd, J = 14.3, 7.2 Hz, 2H), 1.33–1.21 (m, 12H), 0.85 (t, J = 7.0 Hz, 3H), 0.81 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 154.62, 145.66, 145.15, 136.32, 128.96, 128.86, 127.73, 126.64, 126.55, 74.21, 46.34, 42.24, 31.25, 28.78, 28.42, 28.09, 26.34, 26.18, 22.07, 21.71, 13.98, 13.62. HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{36}\text{N}_5$, m/z 394.2965, found 394.3001 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-*N* 6 -(cyclohexylmethyl)-*N* 4 -(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**18d**)

N-Cyclohexylmethylamine was used as a reagent. Yellow solid (50 mg, 62%). ^1H NMR (500 MHz, CDCl_3) δ 7.32–7.27 (m, 3H), 7.06 (d, J = 6.7 Hz, 2H), 5.37 (s, 1H), 5.10 (s, 2H), 2.99 (d, J = 6.7 Hz, 2H), 2.69–2.64 (m, 2H), 2.04 (s, 2H), 1.79 (d, J = 13.2 Hz, 2H), 1.73–1.60 (m, 9H), 1.58 (s, 6H), 1.35 (dd, J = 15.0, 7.5 Hz, 2H), 1.27–1.13 (m, 4H), 1.01 (s, 9H), 0.98–0.91 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 154.37, 150.36, 149.19, 142.41, 136.65, 128.97, 127.76, 126.44, 120.35, 73.62, 55.26, 51.67, 50.18, 47.02, 37.88, 31.91, 31.80, 31.48, 30.51, 30.31, 27.46, 26.80, 26.17, 22.72, 13.91. MS (ESI) calculated for $\text{C}_{32}\text{H}_{50}\text{N}_5$, m/z 504.4061, found 504.41 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-*N* 6 -(cyclohexylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**19d**)

Light brown (28 mg, 80%). ^1H NMR (500 MHz, DMSO) δ 7.35 (t, J = 7.3 Hz, 2H), 7.31–7.27 (m, 1H), 7.10 (d, J = 7.2 Hz, 2H), 6.34 (d, J = 3.1 Hz, 1H), 5.85 (s, 1H), 5.33 (s, 2H), 2.92 (t, J = 6.1 Hz, 2H), 2.71–2.66 (m, 2H), 1.75–1.64 (m, 4H), 1.57 (dt, J = 15.3, 7.6 Hz, 3H), 1.49 (dtd, J = 11.2, 7.4, 3.7 Hz, 1H), 1.30 (dt, J = 14.8, 7.4 Hz, 2H), 1.21–1.09 (m, 4H), 0.91 (dd, J = 22.3, 10.5 Hz, 2H), 0.81 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 153.32, 146.28, 145.05, 136.69, 128.79, 127.61, 126.53, 116.94, 74.21, 48.49, 46.22, 36.76, 30.47, 29.02, 26.21, 26.05, 25.43, 21.75, 13.65. HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{34}\text{N}_5$, m/z 392.2809, found 392.2806 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-*N* 6 -phenyl-*N* 4 -(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (**18e**)

Aniline was used as a reagent. Yellow solid (51 mg, 55%). ^1H NMR (500 MHz, CDCl_3) δ 7.31 (dt, J = 16.5, 5.5 Hz, 3H), 7.22 (t, J = 7.8 Hz, 2H), 7.16 (d, J = 7.6 Hz, 2H), 7.05 (d, J = 6.7 Hz, 2H), 6.90 (t, J = 6.9 Hz, 1H), 6.16 (s, 1H), 6.02 (s, 1H), 5.23 (s, 1H), 5.12 (s, 2H), 2.74–2.67 (m, 2H), 2.03 (s, 2H), 1.66 (dd,

$J = 15.8, 8.2$ Hz, 4H), 1.60 (s, 6H), 1.38 (dd, $J = 15.0, 7.5$ Hz, 2H), 1.02 (s, 9H), 0.89 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 151.49, 149.42, 149.11, 142.46, 141.51, 136.32, 129.16, 129.06, 127.93, 126.53, 121.98, 120.91, 118.83, 55.42, 51.80, 47.26, 31.93, 31.80, 30.44, 30.19, 27.46, 22.71, 13.91. MS (ESI) calculated for $\text{C}_{31}\text{H}_{42}\text{N}_5$, m/z 484.3435, found 484.3459 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -phenyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19e)

Light yellow solid (28 mg, 76%). ^1H NMR (500 MHz, DMSO) δ 9.10 (s, 1H), 8.08 (s, 1H), 7.39–7.35 (m, 2H), 7.33–7.28 (m, 3H), 7.11 (t, $J = 7.3$ Hz, 4H), 7.01 (t, $J = 7.3$ Hz, 1H), 6.41 (s, 1H), 5.44 (s, 2H), 2.82 (t, $J = 7.5$ Hz, 2H), 1.63 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.33 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.84 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 146.34, 140.41, 136.06, 129.52, 128.92, 127.84, 126.69, 122.44, 118.87, 46.68, 28.89, 26.15, 21.72, 13.63. HRMS (ESI) calculated for $\text{C}_{23}\text{H}_{26}\text{N}_5$, m/z 372.2183, found 372.2219 ($\text{M} + \text{H}$) $^+$.

$N^{6,1}$ -Dibenzyl-2-butyl- N^4 -(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (18f)

Benzyl amine was used as a reagent. Light brown solid (30 mg, 46%). ^1H NMR (500 MHz, CDCl_3) δ 7.35 (d, $J = 7.4$ Hz, 2H), 7.26 (dddd, $J = 9.4, 7.2, 6.1, 1.6$ Hz, 6H), 7.03 (d, $J = 6.8$ Hz, 2H), 5.44 (s, 1H), 5.17 (s, 1H), 5.07 (s, 2H), 4.42 (s, 2H), 2.70–2.64 (m, 2H), 1.67–1.60 (m, 2H), 1.55 (s, 6H), 1.35 (dq, $J = 14.7, 7.4$ Hz, 2H), 1.02–0.94 (m, 9H), 0.87 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 153.83, 150.58, 149.20, 142.27, 140.52, 136.49, 128.98, 128.56, 127.80, 127.68, 126.97, 126.46, 126.39, 74.17, 55.29, 51.62, 47.59, 47.07, 31.87, 31.76, 30.45, 30.28, 30.20, 27.42, 22.70, 13.90. MS (ESI) calculated for $\text{C}_{32}\text{H}_{44}\text{N}_5$, m/z 498.3591, found 498.3599 ($\text{M} + \text{H}$) $^+$.

$N^{6,1}$ -Dibenzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19f)

White solid (22 mg, 85%). ^1H NMR (500 MHz, DMSO) δ 8.07 (s, 2H), 7.38–7.35 (m, 2H), 7.34–7.29 (m, 5H), 7.29–7.25 (m, 1H), 7.07 (d, $J = 6.3$ Hz, 2H), 6.06 (s, 1H), 5.33 (s, 2H), 4.38 (s, 2H), 2.75–2.69 (m, 2H), 1.58 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.30 (dt, $J = 14.8, 7.4$ Hz, 2H), 0.81 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 154.68, 145.29, 137.99, 136.12, 128.85, 128.45, 127.78, 127.51, 127.23, 126.74, 75.08, 46.43, 45.67, 28.76, 26.16, 21.70, 13.62. HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{28}\text{N}_5$, m/z 386.2339, found 386.2389 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -(4-methoxybenzyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19g)

4-Methoxybenzyl amine was used as a reagent. Light yellow solid (22 mg, 69%). ^1H NMR (500 MHz, DMSO) δ 8.07 (s, 2H), 7.34–7.28 (m, 5H), 7.08 (d, $J = 6.4$ Hz, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 6.04 (s, 1H), 5.34 (s, 2H), 4.29 (s, 2H), 3.72 (s, 3H), 2.75–2.70 (m, 2H), 1.58 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.29 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.81 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 158.48, 154.67, 145.28, 145.27, 136.13, 129.73, 128.92, 128.84, 127.78, 126.74, 113.82, 75.11, 55.08, 46.43,

45.18, 28.76, 26.16, 21.71, 13.62. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{30}\text{N}_5\text{O}$, m/z 416.2445, found 416.2490 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -(3-methoxybenzyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19h)

3-Methoxybenzyl amine was used as a reagent. Brown solid (27 mg, 69%). ^1H NMR (500 MHz, DMSO) δ 7.32–7.27 (m, 3H), 7.23 (t, $J = 7.9$ Hz, 1H), 7.06 (d, $J = 6.1$ Hz, 2H), 6.94 (dd, $J = 12.3, 4.8$ Hz, 2H), 6.83 (dd, $J = 8.1, 2.2$ Hz, 1H), 6.03 (s, 1H), 5.31 (s, 2H), 4.33 (d, $J = 5.8$ Hz, 2H), 3.71 (s, 3H), 2.70 (t, $J = 7.6$ Hz, 2H), 1.57 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.29 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.81 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 159.37, 136.32, 129.52, 128.80, 127.72, 126.70, 119.55, 113.13, 112.50, 75.05, 55.00, 46.33, 45.63, 40.11, 40.02, 39.94, 28.89, 26.21, 21.72, 13.63. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{30}\text{N}_5\text{O}$, m/z 416.2445, found 416.2506 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -(4-(trifluoromethyl)benzyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19i)

4-(Trifluoromethyl)benzyl amine was used as a reagent. Brown solid (17 mg, 77%). ^1H NMR (500 MHz, DMSO) δ 7.60 (d, $J = 8.2$ Hz, 2H), 7.51 (d, $J = 8.1$ Hz, 2H), 7.29–7.21 (m, 3H), 7.02 (d, $J = 6.7$ Hz, 2H), 6.23 (s, 1H), 5.70 (s, 2H), 5.57 (s, 1H), 5.15 (s, 2H), 4.41 (d, $J = 6.3$ Hz, 2H), 2.69–2.63 (m, 2H), 1.56 (dt, $J = 15.3, 7.5$ Hz, 2H), 1.29 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.82 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 153.45, 150.32, 149.41, 146.48, 143.12, 137.24, 128.58, 127.82, 127.31, 126.51, 124.88, 124.85, 118.91, 75.32, 46.04, 44.94, 29.43, 26.19, 21.82, 13.69. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{27}\text{F}_3\text{N}_5$, m/z 454.2213, found 454.2284 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -(4-chlorobenzyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19j)

4-Chlorobenzylamine was used as a reagent. Yellow solid (15 mg, 63%). ^1H NMR (500 MHz, DMSO) δ 7.33–7.25 (m, 8H), 7.02 (d, $J = 6.6$ Hz, 2H), 6.18 (s, 1H), 5.83 (s, 2H), 5.59 (s, 1H), 5.16 (s, 2H), 4.30 (d, $J = 6.0$ Hz, 2H), 2.68–2.64 (m, 2H), 1.56 (dt, $J = 15.3, 7.5$ Hz, 2H), 1.29 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.82 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 150.57, 149.14, 143.25, 140.18, 137.18, 130.77, 129.08, 128.63, 127.96, 127.37, 126.56, 118.72, 75.34, 46.06, 44.71, 40.11, 40.02, 39.94, 29.40, 26.20, 21.82, 13.69. HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{27}\text{ClN}_5$, m/z 420.1950, found 420.1976 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl- N^6 -(furan-2-ylmethyl)- N^4 -(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (18k)

Furfuryl amine was used as a reagent. Yellow oil (53 mg, 54%). ^1H NMR (500 MHz, CDCl_3) δ 7.30 (ddd, $J = 10.9, 4.6, 1.1$ Hz, 4H), 7.04 (d, $J = 6.6$ Hz, 2H), 6.28 (dd, $J = 3.2, 1.9$ Hz, 1H), 6.15 (dd, $J = 3.2, 0.6$ Hz, 1H), 5.50 (s, 1H), 5.10 (s, 2H), 4.43 (s, 2H), 2.72–2.64 (m, 2H), 2.03 (s, 2H), 1.68–1.59 (m, 4H), 1.58 (s, 6H), 1.35 (dt, $J = 14.7, 7.4$ Hz, 2H), 1.00 (s, 9H), 0.87 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 153.92, 153.28, 150.77, 149.15, 142.19, 141.69, 136.47, 129.00, 127.83, 126.42, 110.36, 106.53, 74.85, 55.32, 51.68, 47.09, 40.78, 31.89, 31.77, 30.47,

30.31, 27.43, 22.71, 13.90. MS (ESI) calculated for $C_{30}H_{42}N_5O$, m/z 488.3384, found 488.3446 ($M + H$)⁺.

1-Benzyl-2-butyl-*N*⁶-(furan-2-ylmethyl)-1*H*-imidazo[4,5-*c*]-pyridine-4,6-diamine (19k)

Brown solid (21 mg, 55%). ¹H NMR (500 MHz, DMSO) δ 7.55 (s, 1H), 7.34 (t, $J = 7.3$ Hz, 2H), 7.31–7.27 (m, 1H), 7.10 (d, $J = 7.3$ Hz, 2H), 6.36 (dd, $J = 3.0, 1.9$ Hz, 1H), 6.29 (d, $J = 2.6$ Hz, 1H), 5.99 (s, 1H), 5.31 (s, 2H), 4.35 (d, $J = 5.8$ Hz, 2H), 2.73–2.67 (m, 2H), 1.58 (dt, $J = 15.2, 7.6$ Hz, 2H), 1.30 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.82 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 142.17, 136.66, 128.79, 127.62, 126.61, 110.39, 107.43, 75.34, 60.19, 46.26, 38.71, 29.07, 26.21, 21.76, 13.65. HRMS (ESI) calculated for $C_{22}H_{26}N_5O$, m/z 376.2132, found 376.2184 ($M + H$)⁺.

1-Benzyl-2-butyl-*N*⁶-(pyridin-4-ylmethyl)-*N*⁴-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (18l)

4-Picolylamine was used as a reagent. Yellow solid (25 mg, 31%). ¹H NMR (500 MHz, CDCl₃) δ 8.48 (dd, $J = 4.5, 1.6$ Hz, 2H), 7.29–7.25 (m, 3H), 7.24 (dd, $J = 4.5, 1.5$ Hz, 2H), 7.01 (d, $J = 6.1$ Hz, 2H), 5.40 (s, 1H), 5.06 (s, 2H), 4.49 (s, 2H), 2.70–2.64 (m, 2H), 1.92 (s, 2H), 1.63 (dt, $J = 15.5, 7.7$ Hz, 2H), 1.48 (s, 6H), 1.36 (dt, $J = 14.9, 7.4$ Hz, 2H), 0.95 (s, 9H), 0.87 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 149.83, 136.31, 129.01, 127.89, 126.40, 122.20, 74.67, 55.25, 51.56, 47.13, 46.09, 31.81, 31.75, 31.71, 30.44, 30.23, 27.41, 22.69, 13.89. MS (ESI) calculated for $C_{31}H_{43}N_6$, m/z 499.3544, found 499.3475 ($M + H$)⁺.

1-Benzyl-2-butyl-*N*⁶-(pyridin-4-ylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19l)

Brown solid (7 mg, 47%). ¹H NMR (500 MHz, DMSO) δ 8.43 (dd, $J = 4.5, 1.5$ Hz, 2H), 7.27 (ddd, $J = 9.7, 6.8, 4.4$ Hz, 5H), 7.03 (d, $J = 6.7$ Hz, 2H), 6.50–6.28 (m, 2H), 5.65 (s, 1H), 5.18 (s, 2H), 4.37 (d, $J = 6.3$ Hz, 2H), 2.69–2.65 (m, 2H), 1.57 (dt, $J = 15.3, 7.5$ Hz, 2H), 1.30 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.81 (d, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 149.28, 137.09, 130.51, 128.65, 127.43, 126.53, 122.35, 113.97, 75.37, 46.09, 44.38, 29.34, 26.19, 21.80, 13.68. HRMS (ESI) calculated for $C_{23}H_{27}N_6$, m/z 387.2292, found 387.2299 ($M + H$)⁺.

1-Benzyl-2-butyl-*N*⁶-(pyridin-3-ylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19m)

3-Picolylamine was used as a reagent. Light brown solid (33 mg, 63%). ¹H NMR (500 MHz, DMSO) δ 8.92 (s, 1H), 8.80 (d, $J = 5.3$ Hz, 1H), 8.46 (d, $J = 8.0$ Hz, 1H), 8.12 (s, 2H), 7.94 (dd, $J = 7.9, 5.7$ Hz, 1H), 7.69 (s, 1H), 7.33–7.25 (m, 3H), 7.04 (d, $J = 6.9$ Hz, 2H), 6.03 (s, 1H), 5.32 (s, 2H), 4.64 (s, 2H), 2.72 (t, $J = 7.6$ Hz, 2H), 1.56 (dt, $J = 15.2, 7.6$ Hz, 2H), 1.28 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.80 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 154.65, 145.80, 145.01, 143.55, 141.29, 141.12, 138.17, 136.10, 128.83, 127.76, 126.68, 126.52, 75.49, 46.45, 42.51, 28.77, 26.12, 21.68, 13.60. HRMS (ESI) calculated for $C_{23}H_{27}N_6$, m/z 387.2292, found 387.2296 ($M + H$)⁺.

1-Benzyl-2-butyl-*N*⁶-(naphthalen-1-ylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19n)

1-Naphthylmethylamine was used as a reagent. Light brown solid (28 mg, 70%). ¹H NMR (500 MHz, DMSO) δ 12.12 (s, 1H), 8.10 (d, $J = 8.0$ Hz, 1H), 8.04 (s, 2H), 7.99–7.96 (m, 1H), 7.88 (d, $J = 8.2$ Hz, 1H), 7.61–7.53 (m, 3H), 7.45 (dd, $J = 8.1, 7.1$ Hz, 1H), 7.33–7.28 (m, 3H), 7.08 (d, $J = 6.4$ Hz, 2H), 6.22 (s, 1H), 5.35 (s, 2H), 4.83 (s, 2H), 2.74–2.68 (m, 2H), 1.58 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.29 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.81 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 154.69, 145.51, 145.31, 136.23, 133.44, 132.90, 130.92, 128.83, 128.63, 127.99, 127.76, 126.67, 126.39, 125.97, 125.49, 125.43, 123.63, 74.91, 46.39, 43.99, 28.80, 26.20, 21.71, 13.62. HRMS (ESI) calculated for $C_{28}H_{30}N_5$, m/z 436.2496, found 436.2549 ($M + H$)⁺.

*N*⁶-([1,1'-Biphenyl]-4-ylmethyl)-1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19o)

[1,1'-biphenyl]-4-yl methanamine was used as a reagent. Light brown solid (21 mg, 51%). ¹H NMR (500 MHz, DMSO) δ 12.36 (s, 1H), 8.04 (s, 2H), 7.63 (dd, $J = 12.4, 7.8$ Hz, 4H), 7.50–7.42 (m, 4H), 7.40–7.33 (m, 1H), 7.28 (t, $J = 7.3$ Hz, 2H), 7.23 (t, $J = 7.2$ Hz, 1H), 7.07 (d, $J = 7.3$ Hz, 2H), 6.07 (s, 1H), 5.33 (s, 2H), 4.42 (s, 2H), 2.71 (t, $J = 7.6$ Hz, 2H), 1.57 (dt, $J = 15.2, 7.7$ Hz, 2H), 1.29 (dq, $J = 14.6, 7.3$ Hz, 2H), 0.81 (t, $J = 7.3$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 154.65, 145.40, 145.35, 139.80, 139.07, 137.24, 136.17, 128.95, 128.80, 128.06, 127.67, 127.43, 126.71, 126.69, 126.57, 75.11, 46.38, 45.31, 28.81, 26.18, 21.69, 13.61. HRMS (ESI) calculated for $C_{30}H_{32}N_5$, m/z 462.2652, found 462.2698 ($M + H$)⁺.

Synthesis of compound 19p and 19r: *N*⁶-(4-(aminomethyl)-benzyl)-1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine

To a solution of compound 16 (70 mg, 0.16 mmol) in 1 mL of dioxane were added potassium *tert*-butoxide (90 mg, 0.80 mmol), catalytic amount of DavePhos and Pd₂(dba)₃ and *p*-xylenediamine (109 mg, 0.80 mmol). The reaction mixture was then heated under microwave conditions (500 W, 100 °C) in a sealed vial for 1 h. It was cooled to room temperature and filtered through celite and washed with MeOH. The solvent was removed and the crude residue was purified using column chromatography (20% EtOAc–hexane) to obtain the compound 18p and 18r. Compound 18p (33 mg, 0.063 mmol) was dissolved in 1 mL of HCl (4 M in dioxane) and stirred at room temperature for 30 min. Then the solvent was removed under vacuum to obtain compound 19p as light yellow solid (5 mg, 63%). ¹H NMR (500 MHz, DMSO) δ 8.37 (s, 2H), 8.06 (s, 2H), 7.42 (q, $J = 8.5$ Hz, 4H), 7.36–7.30 (m, 3H), 7.08 (d, $J = 6.8$ Hz, 2H), 6.08 (s, 1H), 5.34 (s, 2H), 4.40 (s, 2H), 3.98 (q, $J = 5.8$ Hz, 2H), 2.75–2.67 (m, 2H), 1.57 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.28 (dd, $J = 14.9, 7.4$ Hz, 2H), 0.80 (t, $J = 7.4$ Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 154.71, 145.41, 145.34, 138.43, 136.19, 132.98, 129.02, 128.89, 127.79, 127.75, 126.65, 46.40, 45.31, 41.89, 28.78, 26.18, 21.70, 13.62. HRMS (ESI) calculated for $C_{25}H_{31}N_6$, m/z 415.2605, found 415.2606 ($M + H$)⁺.

***N*⁶,*N*^{6'}-(1,4-Phenylenebis(methylene))bis(1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine) (19r)**

Compound **18r** (35 mg, 0.038 mmol) was dissolved in 1 mL of HCl (4 M in dioxane) and stirred at room temperature for 30 min. Then the solvent was removed under vacuum to obtain compound **19r** as light yellow solid (12 mg, 45%). ¹H NMR (500 MHz, MeOD) δ 7.27 (s, 4H), 7.21–7.17 (m, 6H), 7.02–6.98 (m, 4H), 5.20 (s, 4H), 4.33 (s, 4H), 2.78–2.73 (m, 4H), 1.67 (dt, *J* = 15.3, 7.6 Hz, 4H), 1.37 (dd, *J* = 15.0, 7.5 Hz, 4H), 0.89 (t, *J* = 7.4 Hz, 6H). ¹³C NMR (126 MHz, MeOD) δ 155.94, 150.96, 148.16, 146.47, 138.77, 137.12, 130.00, 129.03, 128.70, 127.70, 118.63, 48.01, 47.26, 30.28, 27.76, 23.29, 14.05. HRMS (ESI) calculated for C₄₂H₄₉N₁₀, *m/z* 693.4136, found 693.4331 (*M* + *H*)⁺.

Compounds **19q** and **19s** were synthesized similarly as compounds **19p** and **19r**.

***N*⁶-(3-(Aminomethyl)benzyl)-1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine (19q)**

m-Xylylenediamine was used as a reagent. Light yellow solid. ¹H NMR (500 MHz, MeOD) δ 7.49 (s, 1H), 7.41 (dd, *J* = 3.9, 1.5 Hz, 2H), 7.39–7.35 (m, 1H), 7.32–7.28 (m, 3H), 7.05–7.02 (m, 2H), 5.31 (s, 2H), 4.44 (s, 2H), 4.07 (s, 2H), 2.79–2.75 (m, 2H), 1.70 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.38 (dq, *J* = 14.8, 7.4 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, MeOD) δ 157.36, 147.23, 146.79, 140.00, 136.86, 134.96, 130.63, 130.12, 129.26, 129.19, 129.14, 129.00, 127.62, 48.10, 47.15, 44.18, 30.00, 27.75, 23.24, 14.03. HRMS (ESI) calculated for C₂₅H₃₁N₆, *m/z* 415.2605, found 415.2610 (*M* + *H*)⁺.

***N*⁶,*N*^{6'}-(1,3-Phenylenebis(methylene))bis(1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridine-4,6-diamine) (19s)**

Light yellow solid. ¹H NMR (500 MHz, DMSO) δ 12.38 (s, 2H), 8.04 (s, 4H), 7.38 (s, 1H), 7.30–7.22 (m, 9H), 7.04 (d, *J* = 6.8 Hz, 4H), 6.01 (s, 2H), 5.28 (s, 4H), 4.34 (s, 4H), 2.73–2.68 (m, 4H), 1.56 (dd, *J* = 15.3, 7.7 Hz, 4H), 1.29 (dd, *J* = 14.9, 7.4 Hz, 4H), 0.81 (t, *J* = 7.4 Hz, 6H). ¹³C NMR (126 MHz, DMSO) δ 154.62, 145.36, 145.31, 138.22, 136.15, 128.80, 128.61, 127.72, 126.66, 126.56, 126.38, 46.36, 45.73, 28.80, 26.19, 21.71, 13.62. HRMS (ESI) calculated for C₄₂H₄₉N₁₀, *m/z* 693.4136, found 693.4131 (*M* + *H*)⁺.

Synthesis of compound 23a

To a solution of compound **14** (120 mg, 0.31 mmol) in 1 mL of dioxane were added cesium carbonate (303 mg, 0.93 mmol) in H₂O (0.5 mL), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl₂) (15 mg, 0.019 mmol) and *n*-butylboronic acid (98 μL, 0.46 mmol) under N₂. The reaction mixture was then heated at 90 °C in a sealed vial for 18 h. It was cooled to room temperature and filtered through celite and washed with MeOH. The solvent was removed and the crude residue was purified using column chromatography (15% EtOAc–hexane) to obtain the compound **20a** (97 mg, 76%). To a solution of compound **20a** (94 mg, 0.23 mmol) in 10 mL of MeOH were added zinc dust (149 mg, 2.30 mmol)

and ammonium formate (145 mg, 2.30 mmol). The reaction mixture was stirred at room temperature for 10 min and filtered through celite. Then the solvent was evaporated and the residue was dissolved in water. This was extracted with EtOAc (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was removed under vacuum to obtain compound **21a** (45 mg, 51%). To a solution of compound **21a** (42 mg, 0.11 mmol) in 7 mL of anhydrous THF were added triethylamine (16 μL, 0.12 mmol) and valeryl chloride (13 μL, 0.11 mmol). The reaction mixture was refluxed for 1 h. The solvent was then removed under vacuum, and the residue was dissolved in 5 mL of EtOH and NaOH (10 mg, 0.22 mmol) in 1 mL of H₂O was added. The reaction mixture was refluxed for 18 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The EtOAc fraction was dried using sodium sulfate and evaporated and purified using column chromatography (20% EtOAc–hexane) to obtain the compound **22a** (25 mg, 51%). Compound **22a** (22 mg, 0.049 mmol) was dissolved in 1 mL of HCl (4 M in dioxane) and stirred at room temperature for 30 min. Then the solvent was removed under vacuum to obtain compound **23a** (11 mg, 69%).

***N*⁴-Benzyl-6-butyl-3-nitro-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20a)**

Yellow oil (97 mg, 76%). ¹H NMR (500 MHz, CDCl₃) δ 9.51 (t, *J* = 4.4 Hz, 1H), 9.40 (s, 1H), 7.37 (ddd, *J* = 7.1, 4.4, 1.6 Hz, 2H), 7.34–7.30 (m, 3H), 5.74 (s, 1H), 4.45 (d, *J* = 5.4 Hz, 2H), 2.44 (t, *J* = 7.5 Hz, 2H), 2.01 (s, 2H), 1.67–1.61 (m, 2H), 1.30 (dd, *J* = 15.0, 7.4 Hz, 2H), 0.96 (s, 9H), 0.89 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 166.49, 154.69, 152.58, 136.94, 129.07, 127.92, 127.42, 115.70, 94.41, 56.59, 51.17, 47.44, 38.87, 31.95, 31.64, 30.61, 30.19, 22.43, 14.11. MS (ESI) calculated for C₂₄H₃₇N₄O₂, *m/z* 413.2911, found 413.3267 (*M* + *H*)⁺.

1-Benzyl-2,6-dibutyl-1*H*-imidazo[4,5-*c*]pyridine-4-amine (23a)

White solid (11 mg, 69%). ¹H NMR (500 MHz, CDCl₃) δ 7.37–7.31 (m, 3H), 7.10 (d, *J* = 6.9 Hz, 2H), 6.81 (s, 1H), 5.47 (s, 2H), 2.85 (t, 2H), 2.72 (t, 2H), 1.74 (dd, *J* = 15.3, 7.6 Hz, 2H), 1.67 (ddd, *J* = 15.3, 10.4, 7.0 Hz, 2H), 1.38 (ddq, *J* = 14.8, 10.2, 7.4 Hz, 4H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 157.75, 150.13, 143.74, 137.10, 130.16, 129.22, 127.59, 124.62, 97.63, 35.61, 32.84, 30.27, 27.85, 23.31, 23.22, 14.12, 14.04. HRMS (ESI) calculated for C₂₁H₂₉N₄, *m/z* 337.2387, found 337.2476 (*M* + *H*)⁺.

Compounds **23b–23j** were synthesized similarly as compound **23a**.

***N*⁴-Benzyl-3-nitro-6-phenyl-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20b)**

Phenylboronic acid was used as a reagent. Yellow solid (28 mg, 57%). ¹H NMR (500 MHz, CDCl₃) δ 9.68 (s, 1H), 9.54 (s, 1H), 7.92 (ddd, *J* = 4.4, 2.5, 1.4 Hz, 2H), 7.45–7.42 (m, 3H), 7.40–7.38 (m, 3H), 7.34–7.31 (m, 1H), 7.26 (s, 1H), 6.39 (s, 1H), 4.58 (d, *J* = 5.4 Hz, 2H), 2.09 (s, 2H), 1.64 (s, 6H), 0.99 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 159.01, 154.74, 153.13, 138.88,

136.87, 130.20, 129.19, 128.66, 128.06, 127.50, 127.44, 92.16, 56.72, 51.69, 47.62, 32.02, 31.74, 30.15. MS (ESI) calculated for $C_{26}H_{33}N_4O_2$, m/z 433.2598, found 433.2615 ($M + H$)⁺.

1-Benzyl-2-butyl-6-phenyl-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (22b)

Yellow solid (31 mg, 47%). ¹H NMR (500 MHz, CDCl₃) δ 8.02 (dd, J = 8.3, 1.2 Hz, 2H), 7.39 (t, J = 7.7 Hz, 2H), 7.34–7.27 (m, 4H), 7.07 (d, J = 6.7 Hz, 2H), 6.94 (s, 1H), 5.34 (s, 1H), 5.28 (s, 2H), 2.79–2.73 (m, 2H), 2.18 (s, 2H), 1.73–1.66 (m, 8H), 1.39 (dd, J = 15.0, 7.5 Hz, 2H), 1.03 (s, 9H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 153.22, 150.01, 148.43, 141.28, 140.32, 136.16, 129.61, 129.42, 129.15, 128.41, 128.07, 127.67, 127.54, 126.72, 126.36, 126.28, 126.22, 91.77, 55.45, 51.63, 47.28, 31.98, 31.81, 30.33, 30.25, 27.50, 22.72, 13.90. MS (ESI) calculated for $C_{31}H_{41}N_4$, m/z 469.3326, found 469.3350 ($M + H$)⁺.

1-Benzyl-2-butyl-6-phenyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine (23b)

White solid (13 mg, 73%). ¹H NMR (500 MHz, CDCl₃) δ 7.91 (d, J = 7.2 Hz, 2H), 7.54–7.44 (m, 3H), 7.42–7.34 (m, 3H), 7.05 (dd, J = 6.4, 1.1 Hz, 2H), 6.83 (s, 1H), 5.37 (s, 2H), 2.88–2.78 (m, 2H), 1.78 (dd, J = 15.2, 7.8 Hz, 2H), 1.42 (dq, J = 14.7, 7.4 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 157.54, 149.18, 142.11, 141.93, 134.08, 132.07, 130.82, 129.68, 129.66, 129.01, 127.38, 126.25, 95.25, 48.13, 29.31, 27.40, 22.52, 13.84. HRMS (ESI) calculated for $C_{23}H_{25}N_4$, m/z 357.2074, found 357.2155 ($M + H$)⁺.

4-(4-(Benzylamino)-5-nitro-6-((2,4,4-trimethylpentan-2-yl)-amino)pyridin-2-yl)benzonitrile (20c)

4-Cyanophenylboronic acid was used as a reagent. Yellow solid (30 mg, 55%). ¹H NMR (500 MHz, CDCl₃) δ 9.72 (s, 1H), 9.51 (s, 1H), 7.97 (d, J = 8.6 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.44–7.31 (m, 5H), 6.38 (s, 1H), 4.59 (d, J = 5.4 Hz, 2H), 2.06 (s, 2H), 1.63 (s, 6H), 0.99 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 156.75, 154.69, 153.26, 143.14, 136.53, 132.50, 129.28, 128.23, 127.93, 127.35, 118.79, 113.38, 93.05, 56.87, 51.72, 47.68, 32.01, 31.72, 30.10. MS (ESI) calculated for $C_{27}H_{32}N_5O_2$, m/z 458.2551, found 458.2571 ($M + H$)⁺.

4-(1-Benzyl-2-butyl-4-((2,4,4-trimethylpentan-2-yl)amino)-1*H*-imidazo[4,5-*c*]pyridin-6-yl)benzamide (22c)

Cyano group was converted into amide in the basic condition. Yellow solid (22 mg, 41%). ¹H NMR (500 MHz, CDCl₃) δ 8.10 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.5 Hz, 2H), 7.34–7.28 (m, 3H), 7.08–7.04 (m, 2H), 6.99 (s, 1H), 6.14 (s, 1H), 5.79 (s, 1H), 5.41 (s, 1H), 5.29 (s, 2H), 2.81–2.74 (m, 2H), 2.17 (s, 2H), 1.72–1.66 (m, 8H), 1.43–1.33 (m, 2H), 1.03 (s, 9H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 169.46, 153.74, 150.08, 147.00, 144.87, 140.11, 135.97, 131.83, 129.20, 128.15, 127.62, 126.76, 126.74, 126.31, 92.63, 55.50, 51.55, 47.31, 31.95, 31.79, 30.25, 30.17, 27.46, 22.69, 13.89. MS (ESI) calculated for $C_{32}H_{42}N_5O$, m/z 512.3384, found 512.3408 ($M + H$)⁺.

4-(4-Amino-1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-6-yl)-benzamide (23c)

White solid (11 mg, 79%). ¹H NMR (500 MHz, MeOD) δ 8.09–8.05 (m, 2H), 7.89–7.85 (m, 2H), 7.51 (s, 1H), 7.41–7.32 (m, 3H), 7.17 (d, J = 7.0 Hz, 2H), 5.62 (s, 2H), 3.76–3.72 (m, 1H), 3.68–3.64 (m, 1H), 3.58 (dd, J = 7.0, 2.7 Hz, 1H), 2.94–2.89 (m, 2H), 1.78 (dt, J = 15.3, 7.6 Hz, 2H), 1.42 (dd, J = 15.0, 7.5 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, MeOD) δ 171.03, 159.91, 149.79, 143.90, 141.45, 137.23, 136.75, 136.73, 130.29, 129.74, 129.43, 128.43, 127.76, 125.80, 98.94, 73.57, 72.45, 62.18, 43.73, 30.01, 27.99, 23.28, 14.04. HRMS (ESI) calculated for $C_{24}H_{26}N_5O$, m/z 400.2132, found 400.2177 ($M + H$)⁺.

*N*⁴-Benzyl-5-nitro-*N*⁶-(2,4,4-trimethylpentan-2-yl)-[2,3'-bipyridine]-4,6-diamine (20d)

4-Pyridinylboronic acid was used as a reagent. Orange solid (135 mg, 82%). ¹H NMR (500 MHz, CDCl₃) δ 9.74 (t, J = 5.1 Hz, 1H), 9.54 (s, 1H), 9.10 (dd, J = 2.2, 0.6 Hz, 1H), 8.65 (dd, J = 4.8, 1.6 Hz, 1H), 8.21–8.16 (m, 1H), 7.42–7.36 (m, 5H), 7.35–7.31 (m, 1H), 6.38 (s, 1H), 4.59 (d, J = 5.5 Hz, 2H), 2.07 (s, 2H), 1.63 (s, 6H), 0.99 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 156.52, 154.78, 153.22, 150.86, 148.93, 136.60, 134.75, 134.36, 129.26, 128.17, 127.35, 123.54, 116.37, 92.38, 56.86, 51.65, 47.65, 32.01, 31.72, 30.11. MS (ESI) calculated for $C_{25}H_{32}N_5O_2$, m/z 434.2551, found 434.2563 ($M + H$)⁺.

1-Benzyl-2-butyl-6-(pyridin-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (23d)

Light yellow solid (10 mg, 72%). ¹H NMR (500 MHz, MeOD) δ 9.28 (d, J = 1.9 Hz, 1H), 8.94 (dd, J = 5.5, 1.2 Hz, 1H), 8.83 (d, J = 8.2 Hz, 1H), 8.11 (ddd, J = 8.2, 5.6, 0.5 Hz, 1H), 7.72 (s, 1H), 7.40–7.32 (m, 3H), 7.18 (d, J = 6.9 Hz, 2H), 5.65 (s, 2H), 2.98–2.91 (m, 2H), 1.78 (dd, J = 15.3, 7.7 Hz, 2H), 1.42 (dd, J = 15.0, 7.5 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, MeOD) δ 150.19, 143.97, 143.57, 143.11, 136.48, 130.41, 130.32, 129.52, 128.03, 127.74, 127.73, 100.47, 48.55, 29.93, 27.90, 23.26, 14.02. HRMS (ESI) calculated for $C_{22}H_{24}N_5$, m/z 358.2026, found 358.2067 ($M + H$)⁺.

*N*⁴-Benzyl-6-(furan-3-yl)-3-nitro-*N*²-(2,4,4-trimethylpentan-2-yl)-pyridine-2,4-diamine (20e)

3-Furylboronic acid was used as a reagent. Yellow solid (95 mg, 70%). ¹H NMR (500 MHz, CDCl₃) δ 9.68 (t, J = 4.8 Hz, 1H), 9.49 (s, 1H), 7.95 (dd, J = 1.5, 0.8 Hz, 1H), 7.45 (t, J = 1.7 Hz, 1H), 7.41–7.34 (m, 4H), 7.34–7.30 (m, 1H), 6.69 (dd, J = 1.9, 0.8 Hz, 1H), 6.07 (s, 1H), 4.53 (d, J = 5.4 Hz, 2H), 2.05 (s, 2H), 1.60 (s, 6H), 0.98 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 154.88, 153.66, 153.09, 143.99, 143.68, 136.85, 129.19, 128.06, 127.39, 127.36, 108.83, 91.66, 56.68, 51.51, 47.61, 31.99, 31.71, 30.13. MS (ESI) calculated for $C_{24}H_{31}N_4O_3$, m/z 423.2391, found 423.2401 ($M + H$)⁺.

1-Benzyl-2-butyl-6-(furan-3-yl)-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (22e)

Brown oil (30 mg, 41%). ¹H NMR (500 MHz, CDCl₃) δ 7.94 (dd, J = 1.6, 0.7 Hz, 1H), 7.41 (t, J = 1.7 Hz, 1H), 7.34–7.27 (m, 3H),

7.05 (d, $J = 6.6$ Hz, 2H), 6.76 (dd, $J = 1.8, 0.8$ Hz, 1H), 6.61 (s, 1H), 5.30 (s, 1H), 5.24 (s, 2H), 2.76–2.71 (m, 2H), 2.13 (s, 2H), 1.70–1.65 (m, 2H), 1.64 (s, 6H), 1.36 (dt, $J = 14.7, 7.4$ Hz, 2H), 1.02 (s, 9H), 0.88 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 152.80, 150.15, 143.24, 142.70, 140.71, 140.01, 136.16, 129.15, 128.83, 128.06, 126.33, 125.88, 108.87, 91.20, 55.44, 51.45, 47.25, 31.93, 31.79, 30.29, 30.16, 27.45, 22.71, 13.90. MS (ESI) calculated for $\text{C}_{29}\text{H}_{39}\text{N}_4\text{O}$, m/z 459.3118, found 459.3168 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-(furan-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (23e)

Light yellow solid (12 mg, 64%). ^1H NMR (500 MHz, MeOD) δ 8.23–8.19 (m, 1H), 7.73–7.70 (m, 1H), 7.42 (s, 1H), 7.40–7.32 (m, 3H), 7.15 (d, $J = 6.9$ Hz, 2H), 6.97 (dd, $J = 2.0, 0.9$ Hz, 1H), 5.58 (s, 2H), 2.90–2.85 (m, 2H), 1.75 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.40 (dq, $J = 14.8, 7.4$ Hz, 2H), 0.91 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 159.50, 149.42, 146.46, 144.24, 142.92, 136.72, 135.38, 130.27, 129.41, 127.69, 124.89, 121.45, 109.21, 96.82, 48.27, 29.95, 27.91, 23.26, 14.03. HRMS (ESI) calculated for $\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}$, m/z 347.1866, found 347.1921 ($\text{M} + \text{H}$) $^+$.

*N*⁴-Benzyl-3-nitro-6-(thiophen-3-yl)-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20f)

3-Thienylboronic acid was used as a reagent. Yellow solid (107 mg, 76%). ^1H NMR (500 MHz, CDCl_3) δ 9.69 (s, 1H), 9.52 (s, 1H), 7.89 (dd, $J = 3.0, 1.2$ Hz, 1H), 7.49 (dd, $J = 5.1, 1.2$ Hz, 1H), 7.42–7.30 (m, 6H), 6.26 (s, 1H), 4.56 (d, $J = 5.4$ Hz, 2H), 2.08 (s, 2H), 1.63 (s, 6H), 0.99 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 154.88, 154.61, 153.25, 142.21, 136.90, 129.19, 128.05, 127.38, 126.59, 126.56, 126.28, 91.99, 56.68, 51.59, 47.61, 32.02, 31.72, 30.16. MS (ESI) calculated for $\text{C}_{24}\text{H}_{31}\text{N}_4\text{O}_2\text{S}$, m/z 439.2162, found 439.2183 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-(thiophen-3-yl)-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (22f)

Brown solid (25 mg, 35%). ^1H NMR (500 MHz, CDCl_3) δ 7.79 (dd, $J = 3.1, 1.2$ Hz, 1H), 7.58 (dd, $J = 5.0, 1.2$ Hz, 1H), 7.33–7.27 (m, 4H), 7.06 (d, $J = 6.6$ Hz, 2H), 6.79 (s, 1H), 5.35 (s, 1H), 5.25 (s, 2H), 2.78–2.70 (m, 2H), 2.17 (s, 2H), 1.70 (dd, $J = 8.7, 7.0$ Hz, 3H), 1.67 (s, 6H), 1.38 (dq, $J = 14.7, 7.4$ Hz, 2H), 1.26 (dd, $J = 8.7, 5.6$ Hz, 1H), 1.02 (s, 9H), 0.89 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 153.04, 150.02, 144.89, 144.32, 140.11, 136.13, 129.14, 128.07, 126.45, 126.34, 125.94, 125.41, 121.55, 91.54, 55.43, 51.49, 50.96, 47.26, 31.96, 31.80, 30.26, 30.21, 27.46, 22.71, 13.89. MS (ESI) calculated for $\text{C}_{29}\text{H}_{39}\text{N}_4\text{S}$, m/z 475.2890, found 475.2922 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-(thiophen-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (23f)

White solid (9 mg, 53%). ^1H NMR (500 MHz, MeOD) δ 8.04 (dd, $J = 2.9, 1.4$ Hz, 1H), 7.66 (dd, $J = 5.1, 2.9$ Hz, 1H), 7.58 (dd, $J = 5.1, 1.4$ Hz, 1H), 7.48 (s, 1H), 7.41–7.32 (m, 3H), 7.17 (d, $J = 6.9$ Hz, 2H), 5.59 (s, 2H), 2.94–2.86 (m, 2H), 1.76 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.41 (dq, $J = 14.8, 7.4$ Hz, 2H), 0.91 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 159.63, 149.38, 144.24,

137.90, 136.73, 135.24, 130.28, 129.42, 129.31, 127.74, 126.73, 126.03, 124.88, 97.21, 49.07, 29.96, 27.94, 23.26, 14.03. HRMS (ESI) calculated for $\text{C}_{21}\text{H}_{23}\text{N}_4\text{S}$, m/z 363.1638, found 363.1686 ($\text{M} + \text{H}$) $^+$.

N^{4,6}-Dibenzyl-3-nitro-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20g)

Benzylboronic acid pinacol ester was used as a reagent. Orange oil (135 mg, 91%). ^1H NMR (500 MHz, CDCl_3) δ 9.54 (t, $J = 5.0$ Hz, 1H), 9.37 (s, 1H), 7.36 (ddd, $J = 7.4, 4.5, 1.4$ Hz, 2H), 7.31 (dt, $J = 9.8, 4.4$ Hz, 1H), 7.27 (ddd, $J = 6.7, 3.3, 1.4$ Hz, 5H), 7.22–7.18 (m, 3H), 5.75 (s, 1H), 4.40 (d, $J = 5.4$ Hz, 2H), 3.75 (s, 2H), 1.89 (s, 2H), 1.49 (s, 6H), 0.90 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 164.70, 154.68, 152.84, 138.77, 136.80, 129.45, 129.07, 128.54, 127.91, 127.46, 126.50, 124.96, 115.72, 94.56, 56.63, 51.05, 47.44, 45.61, 31.87, 31.60, 30.09, 24.86. MS (ESI) calculated for $\text{C}_{27}\text{H}_{35}\text{N}_4\text{O}_2$, m/z 447.2755, found 447.2737 ($\text{M} + \text{H}$) $^+$.

1,6-Dibenzyl-2-butyl-*N*-(2,4,4-trimethylpentan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-4-amine (22g)

Light brown solid (55 mg, 60%). ^1H NMR (500 MHz, CDCl_3) δ 7.33–7.27 (m, 5H), 7.24 (t, $J = 7.6$ Hz, 2H), 7.14 (t, $J = 7.3$ Hz, 1H), 7.01 (d, $J = 6.4$ Hz, 2H), 6.31 (s, 1H), 5.22 (s, 1H), 5.16 (s, 2H), 3.96 (s, 2H), 2.74–2.67 (m, 2H), 2.02 (s, 2H), 1.66–1.61 (m, 2H), 1.56 (s, 6H), 1.35 (dd, $J = 15.0, 7.5$ Hz, 2H), 0.93 (s, 9H), 0.86 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 152.37, 151.90, 150.06, 141.57, 140.10, 136.28, 129.31, 129.06, 128.21, 127.95, 126.38, 125.78, 124.94, 93.59, 55.35, 50.98, 47.19, 45.39, 31.85, 31.68, 30.41, 30.27, 27.42, 22.67, 13.87. MS (ESI) calculated for $\text{C}_{32}\text{H}_{43}\text{N}_4$, m/z 483.3482, found 483.3526 ($\text{M} + \text{H}$) $^+$.

1,6-Dibenzyl-2-butyl-1*H*-imidazo[4,5-*c*]pyridin-4-amine (23g)

White solid (23 mg, 58%). ^1H NMR (500 MHz, DMSO) δ 13.63 (s, 1H), 8.27 (s, 2H), 7.37–7.29 (m, 7H), 7.27–7.23 (m, 1H), 7.15 (s, 1H), 7.11 (d, $J = 6.9$ Hz, 2H), 5.52 (s, 2H), 4.08 (s, 2H), 2.86–2.79 (m, 2H), 1.61 (dt, $J = 15.3, 7.6$ Hz, 2H), 1.31 (dt, $J = 14.9, 7.4$ Hz, 2H), 0.81 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 156.72, 148.03, 142.12, 141.72, 137.39, 135.93, 128.96, 128.72, 128.70, 127.92, 126.98, 126.63, 123.29, 97.60, 46.84, 40.11, 38.20, 28.89, 26.28, 21.69, 13.61. HRMS (ESI) calculated for $\text{C}_{24}\text{H}_{27}\text{N}_4$, m/z 371.2230, found 371.2285 ($\text{M} + \text{H}$) $^+$.

*N*⁴-Benzyl-6-(4-methylbenzyl)-3-nitro-*N*²-(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20h)

4-Methyl benzylboronic acid pinacol ester was used as a reagent. Yellow oil (130 mg, 91%). ^1H NMR (500 MHz, CDCl_3) δ 9.52 (t, $J = 5.0$ Hz, 1H), 9.37 (s, 1H), 7.37–7.30 (m, 3H), 7.28–7.24 (m, 3H), 7.10–7.03 (m, 7H), 5.74 (s, 1H), 4.39 (d, $J = 5.4$ Hz, 2H), 3.70 (s, 2H), 2.31 (s, 3H), 1.90 (s, 2H), 1.51 (s, 6H), 0.90 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 165.00, 154.68, 152.83, 136.82, 135.98, 135.70, 135.50, 134.26, 129.28, 129.23, 129.12, 129.05, 128.98, 127.89, 127.47, 115.70, 94.47, 83.50, 56.63, 51.03, 47.44, 45.22, 31.87, 31.57, 30.12, 24.87, 21.19, 21.11. MS (ESI) calculated for $\text{C}_{28}\text{H}_{37}\text{N}_4\text{O}_2$, m/z 461.2911, found 461.3003 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-(4-methylbenzyl)-1H-imidazo[4,5-c]pyridin-4-amine (23h)

White solid (19 mg, 66%). ^1H NMR (500 MHz, MeOD) δ 7.38–7.32 (m, 3H), 7.16 (d, J = 7.9 Hz, 2H), 7.13–7.08 (m, 4H), 6.89 (s, 1H), 5.49 (s, 2H), 4.08 (s, 2H), 2.92–2.87 (m, 2H), 2.32 (s, 3H), 1.76 (dt, J = 15.3, 7.6 Hz, 2H), 1.40 (dt, J = 14.8, 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 159.11, 149.35, 144.27, 143.93, 138.37, 136.53, 134.52, 130.68, 130.25, 129.85, 129.42, 127.74, 124.41, 99.41, 39.40, 30.01, 27.84, 23.26, 21.08, 14.03. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{29}\text{N}_4$, m/z 385.2387, found 385.2451 ($\text{M} + \text{H}$) $^+$.

 N^4 -Benzyl-3-nitro-6-(4-(trifluoromethoxy)benzyl)- N^2 -(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20i)

4-(Trifluoromethoxy)benzylboronic acid pinacol ester was used as a reagent. Yellow solid (115 mg, 70%). ^1H NMR (500 MHz, CDCl_3) δ 9.57 (t, J = 5.0 Hz, 1H), 9.37 (s, 1H), 7.38–7.29 (m, 3H), 7.28 (t, J = 1.8 Hz, 2H), 7.21–7.17 (m, 2H), 7.10 (d, J = 7.9 Hz, 2H), 5.74 (s, 1H), 4.43 (d, J = 5.5 Hz, 2H), 1.83 (s, 2H), 1.45 (s, 6H), 0.88 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 163.87, 154.70, 152.91, 137.54, 136.71, 130.77, 129.10, 127.95, 127.33, 121.09, 115.75, 94.57, 56.63, 50.96, 47.44, 44.69, 31.82, 31.55, 30.02. MS (ESI) calculated for $\text{C}_{28}\text{H}_{34}\text{F}_3\text{N}_4\text{O}_3$, m/z 531.2578, found 531.2738 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-(4-(trifluoromethoxy)benzyl)-1H-imidazo[4,5-c]pyridin-4-amine (23i)

White solid (21 mg, 47%). ^1H NMR (500 MHz, CDCl_3) δ 7.37–7.29 (m, 5H), 7.21 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 6.5 Hz, 2H), 6.79 (s, 1H), 5.45 (s, 2H), 4.09 (s, 2H), 2.87 (dd, J = 9.3, 6.1 Hz, 2H), 1.74 (dt, J = 15.3, 7.6 Hz, 2H), 1.41 (dt, J = 15.0, 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 157.96, 150.50, 149.43, 149.42, 143.48, 138.54, 136.96, 131.59, 130.16, 129.25, 127.66, 124.97, 122.37, 99.02, 40.76, 30.29, 27.86, 23.31, 14.05. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{26}\text{F}_3\text{N}_4\text{O}$, m/z 455.2053, found 455.2131 ($\text{M} + \text{H}$) $^+$.

 N^4 -Benzyl-3-nitro-6-phenethyl- N^2 -(2,4,4-trimethylpentan-2-yl)pyridine-2,4-diamine (20j)

2-Phenylethylboronic acid was used as a reagent. Yellow oil (85 mg, 59%). ^1H NMR (500 MHz, CDCl_3) δ 9.52 (t, J = 4.9 Hz, 1H), 9.43 (s, 1H), 7.36 (t, J = 7.2 Hz, 2H), 7.33–7.28 (m, 4H), 7.27–7.25 (m, 3H), 7.19 (d, J = 7.3 Hz, 1H), 7.17 (d, J = 7.0 Hz, 2H), 5.69 (s, 1H), 4.39 (d, J = 5.4 Hz, 2H), 2.99 (dd, J = 9.1, 6.8 Hz, 2H), 2.75 (dd, J = 9.1, 6.8 Hz, 2H), 2.04 (s, 2H), 1.58 (s, 6H), 0.98 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 165.06, 154.74, 152.65, 141.77, 136.85, 129.09, 128.52, 128.49, 127.95, 127.39, 126.10, 115.78, 94.52, 56.68, 51.35, 47.41, 40.87, 34.61, 31.97, 31.69, 30.20. MS (ESI) calculated for $\text{C}_{28}\text{H}_{37}\text{N}_4\text{O}_2$, m/z 461.2911, found 461.3096 ($\text{M} + \text{H}$) $^+$.

1-Benzyl-2-butyl-6-phenethyl-1H-imidazo[4,5-c]pyridin-4-amine (23j)

Yellow solid (11 mg, 42%). ^1H NMR (500 MHz, CDCl_3) δ 7.36–7.30 (m, 3H), 7.23–7.18 (m, 2H), 7.13 (ddd, J = 5.7, 3.7,

1.5 Hz, 3H), 7.02 (d, J = 6.5 Hz, 2H), 6.68 (s, 1H), 5.38 (s, 2H), 2.99 (s, 4H), 2.85–2.79 (m, 2H), 1.69 (ddd, J = 13.2, 8.5, 6.7 Hz, 2H), 1.37 (dq, J = 14.8, 7.4 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 157.35, 150.48, 148.64, 143.39, 141.89, 137.14, 130.14, 129.53, 129.43, 129.16, 127.59, 127.22, 124.68, 97.93, 48.17, 38.42, 36.84, 30.28, 27.83, 23.31, 14.03. HRMS (ESI) calculated for $\text{C}_{25}\text{H}_{29}\text{N}_4$, m/z 385.2387, found 385.2446 ($\text{M} + \text{H}$) $^+$.

Synthesis of compound 24: 3-nitrobenzo[g]quinolin-4-ol

Nitromethane (0.96 mL, 18 mmol) was added dropwise to a solution of NaOH (2.2 g, 54 mmol) in water (5 mL), at 0 °C. The mixture was then warmed to 40 °C and nitromethane (0.96 mL, 18 mmol) was again added slowly at 40–45 °C. The temperature was maintained until a clear solution was obtained. The reaction mixture was then heated to 55 °C for 2–5 minutes, cooled to 30 °C, poured onto crushed ice and acidified with conc. HCl (5 mL). The resultant solution of methazoic acid was added immediately to a filtered solution of 3-amino-2-naphtholic acid (3 g, 16 mmol) and conc. HCl (1 mL) in water (20 mL). The reaction mixture was allowed to stand at room temperature for 12 h. After filtration, the residue obtained was washed with water, and dried (1.1 g, 90%). A solution of intermediate (2 g, 7.75 mmol) in acetic anhydride (10 mL) was placed in a 2-neck flask fitted with a reflux condenser. It was stirred and heated to 105 °C until a clear solution was obtained. Heating was then discontinued and potassium acetate (0.77 g, 7.90 mmol) was added. The mixture was then refluxed for 15 min with vigorous stirring, until a solid started to precipitate. The reaction mixture was then slowly cooled to room temperature. The residue was filtered, washed with glacial acetic acid until the washings were colorless, then suspended in water, filtered, washed with water and dried at 110 °C to get compound 24 (0.93 g, 50%). ^1H NMR (500 MHz, DMSO) δ 13.12 (s, 1H), 9.28 (s, 1H), 8.95 (s, 1H), 8.27–8.21 (m, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.69 (t, J = 7.1 Hz, 1H), 7.61 (t, J = 7.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 168.61, 144.40, 135.05, 134.63, 130.14, 129.54, 128.94, 128.71, 127.46, 127.41, 126.65, 126.48, 116.78. MS (ESI) calculated for $\text{C}_{13}\text{H}_8\text{N}_2\text{O}_3$, m/z 240.05, found 263.04 ($\text{M} + \text{Na}$) $^+$.

Synthesis of compound 25: 4-chloro-3-nitrobenzo[g]quinoline

A suspension of compound 24 (2.0 g, 8.30 mmol) in phosphorus(v) oxychloride was placed in a pressure vessel and it was heated at 150 °C. After a clear solution was obtained, the reaction mixture was kept at 150 °C for 1 h. Then it was slowly cooled to room temperature and the solvent was evaporated under vacuum. The residue was poured over crushed ice while stirring and the formed solid was filtered, washed with water and dried to obtain compound 25 (1.95 g, 91%). ^1H NMR (500 MHz, DMSO) δ 13.34 (d, J = 6.9 Hz, 1H), 9.26 (d, J = 7.3 Hz, 1H), 8.95 (s, 1H), 8.25 (d, J = 9.4 Hz, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.72–7.67 (m, 1H), 7.61 (ddd, J = 8.1, 6.8, 1.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 168.64, 144.23, 134.96, 134.64, 130.16, 129.56, 128.98, 128.70, 127.48, 127.43, 126.69, 126.48, 116.74.

Synthesis of compound 26: *N*-benzyl-3-nitrobenzo[g]quinolin-4-amine

To a solution of compound 25 (1.0 g, 3.90 mol) in 20 mL of CH₂Cl₂ was added triethylamine (0.81 mL, 5.80 mmol) and benzylamine (0.50 mL, 4.60 mmol). The reaction mixture was refluxed for 2 h. The solvent was then evaporated under vacuum and H₂O was added to the residue. The solution was extracted with CH₂Cl₂ (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was evaporated and the residue was purified using silica gel column chromatography (5% MeOH–CH₂Cl₂) to obtain compound 26 as a yellow solid (1.1 g, 88%). ¹H NMR (500 MHz, CDCl₃) δ 10.57 (s, 1H), 9.41 (s, 1H), 8.87 (s, 1H), 8.50 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.65–7.60 (m, 1H), 7.55–7.51 (m, 1H), 7.50–7.47 (m, 4H), 7.42 (ddd, *J* = 11.0, 5.4, 3.0 Hz, 1H), 5.28 (d, *J* = 5.9 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 152.50, 147.73, 145.88, 136.85, 135.48, 130.53, 129.64, 129.12, 129.03, 128.98, 128.83, 128.11, 128.04, 127.29, 126.86, 124.21, 118.09, 53.19. MS (ESI) calculated for C₂₀H₁₆N₃O₂, *m/z* 330.1237, found 330.1304 (*M* + H)⁺.

Synthesis of compound 30: 1-benzyl-2-butyl-1*H*-benzo[g]-imidazo[4,5-*c*]quinolin-4-amine

To a solution of compound 26 (300 mg, 0.91 mmol) in 20 mL of MeOH were added zinc dust (594 mg, 9.10 mmol) and ammonium formate (574 mg, 9.10 mmol). The reaction mixture was stirred at room temperature for 30 min and filtered through celite. Then the solvent was evaporated and the residue was dissolved in water. This was extracted with EtOAc (3 × 20 mL), washed with water and dried over sodium sulfate. The solvent was removed under vacuum to obtain compound 27 (100 mg, 37%). To a solution of compound 27 (98 mg, 0.33 mmol) in 10 mL of anhydrous THF were added triethylamine (48 μL, 0.35 mmol) and valeryl chloride (40 μL, 0.33 mmol). The reaction mixture was refluxed for 2 h. The solvent was then removed under vacuum, and the residue was dissolved in 10 mL of EtOH and NaOH (26 mg, 0.66 mmol) in 1 mL of H₂O was added. The reaction mixture was refluxed for 2 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water. The EtOAc fraction was dried using sodium sulfate and evaporated and purified using column chromatography (10% MeOH–CH₂Cl₂) to obtain the compound 28 (76 mg, 63%). To a solution of compound 28 (76 mg, 0.21 mmol) in a solvent mixture of MeOH–CH₂Cl₂–CHCl₃ (1 : 10 : 10) was added 3-chloroperoxy benzoic acid (443 mg, 1.98 mmol), and the solution was refluxed at 45–50 °C for 1 h. The solvent was then removed and the residue was purified using column chromatography (10% MeOH–CH₂Cl₂) to obtain the *N*-oxide derivative 29 (64 mg, 80%). To a solution of compound 29 (64 mg, 0.17 mol) in 10 mL of CH₂Cl₂ was added benzoyl isocyanate (37 mg, 0.25 mmol) and heated at 45 °C for 18 h. The solvent was then removed under vacuum, and the residue was dissolved in 15 mL of anhydrous MeOH, followed by the addition of excess sodium methoxide. The reaction mixture was then heated at

80 °C for 2 h. The solvent was removed under vacuum and the residue was purified using column chromatography (10% MeOH–CH₂Cl₂) to obtain the compound 30 (20 mg, 30%). ¹H NMR (500 MHz, DMSO) δ 8.40 (s, 1H), 8.05 (s, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.33 (dd, *J* = 12.9, 5.2 Hz, 3H), 7.24 (d, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 7.4 Hz, 2H), 6.91 (s, 2H), 6.02 (s, 2H), 3.00–2.92 (m, 2H), 1.74 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.40 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 136.73, 131.80, 128.91, 127.99, 127.81, 127.46, 126.82, 125.81, 123.63, 118.72, 48.20, 29.71, 26.27, 21.85, 13.69. HRMS (ESI) calculated for C₂₅H₂₅N₄, *m/z* 381.2074, found 381.2089 (*M* + H)⁺.

Human TLR-7/8 reporter gene assays (NF-κB induction)

The induction of NF-κB was quantified using HEK-Blue-7 (hTLR7-specific) and HEK-Blue-8 (hTLR8-specific) cells as previously described by us.^{33,45,46} HEK293 cells stably co-transfected with human TLR7 or human TLR8, MD2, and secreted alkaline phosphatase (sAP), were maintained in HEK-Blue™ Selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by appropriate TLR agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue cells were incubated at a density of ~10⁵ cells per mL in a volume of 80 μL per well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently stimulated with graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.

Immunoassays for interferon (IFN)-α, and cytokines

Fresh human peripheral blood mononuclear cells (hPBMC) were isolated from human blood obtained by venipuncture with informed consent and as per institutional guidelines on Ficoll-Hypaque gradients as described elsewhere.⁶⁴ Aliquots of PBMCs (10⁵ cells in 100 μL per well) were stimulated for 12 h with graded concentrations of test compounds. Supernatants were isolated by centrifugation, and were assayed in triplicates using either high-sensitivity multi-subtype IFN-α ELISA kits (PBL Interferon Source, Piscataway, NJ and R&D Systems, Inc., Minneapolis, MN), or analyte-specific multiplexed cytokine/chemokine bead array assays as reported by us previously.⁶⁵

Flow-cytometric immunostimulation experiments

CD69 upregulation was determined by flow cytometry using protocols published by us previously,³³ and modified for rapid-throughput. Briefly, heparin-anticoagulated whole blood samples were obtained by venipuncture from healthy human volunteers with informed consent and as per guidelines approved by the University of Kansas Human Subjects Experimentation Committee. Serial dilutions of selected imidazopyridine compounds (and imiquimod, used as a reference compound) were performed using a Bio-Tek Precision 2000 XS liquid handler in sterile 96-well polypropylene plates, to which

were added 100 μ L aliquots of anticoagulated whole human blood. The plates were incubated at 37 °C for 16.5 h. Negative (endotoxin free water) controls were included in each experiment. Following incubation, fluorochrome-conjugated antibodies (CD3-PE, CD56-APC, CD69-PE-Cy7, 10 μ L of each antibody, Becton-Dickinson Biosciences, San Jose, CA) were added to each well with a liquid handler, and incubated at 37 °C in the dark for 30 min. Following staining, erythrocytes were lysed and leukocytes fixed by mixing 200 mL of the samples in 2 mL pre-warmed Whole Blood Lyse/Fix Buffer (Becton-Dickinson Biosciences, San Jose, CA) in 96 deep-well plates. After washing the cells twice at 200 g for 8 minutes in saline, the cells were transferred to a 96-well plate. Flow cytometry was performed using a BD FACSArray instrument in the tri-color mode (tri-color flow experiment) and two-color mode (two-color flow experiment) for acquisition on 100 000 gated events. Compensation for spillover was computed for each experiment on singly-stained samples. CD69 activation in the major lymphocytic populations, *viz.*, natural killer lymphocytes (NK cells: CD3[−]CD56⁺), cytokine-induced killer phenotype (CIK cells: CD3⁺CD56⁺), nominal B lymphocytes (CD3[−]CD56[−]), and nominal T lymphocytes (CD3⁺CD56[−]) were quantified using FlowJo v 7.0 software (Treestar, Ashland, OR).

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Notes and references

- 1 A. Iwasaki and R. Medzhitov, *Science*, 2010, **327**, 291.
- 2 A. Iwasaki and R. Medzhitov, *Nat. Immunol.*, 2004, **5**, 987.
- 3 C. A. Janeway Jr. and R. Medzhitov, *Annu. Rev. Immunol.*, 2002, **20**, 197.
- 4 M. S. Lee and Y. J. Kim, *Mol. Cells*, 2007, **23**, 1.
- 5 B. A. Seaton, E. C. Crouch, F. X. McCormack, J. F. Head, K. L. Hartshorn and R. Mendelsohn, *Innate Immun.*, 2010, **16**, 143.
- 6 V. L. Runza, W. Schwaeble and D. N. Mannel, *Immunobiology*, 2008, **213**, 297.
- 7 A. Agrawal, P. P. Singh, B. Bottazzi, C. Garlanda and A. Mantovani, *Adv. Exp. Med. Biol.*, 2009, **653**, 98.
- 8 M. E. Bianchi, *J. Leukocyte Biol.*, 2007, **81**, 1.
- 9 M. Yoneyama and T. Fujita, *J. Biol. Chem.*, 2007, **282**, 15315.
- 10 M. H. Shaw, T. Reimer, Y. G. Kim and G. Nunez, *Curr. Opin. Immunol.*, 2008, **20**, 377.
- 11 T. Kawai and S. Akira, *Nat. Immunol.*, 2010, **11**, 373.
- 12 A. Iwasaki and R. Medzhitov, *Nat. Immunol.*, 2004, **5**, 987.
- 13 Y. Kumagai, O. Takeuchi and S. Akira, *J. Infect. Chemother.*, 2008, **14**, 86.
- 14 Y. Kumagai, O. Takeuchi and S. Akira, *J. Infect. Chemother.*, 2008, **14**, 86.
- 15 K. Takeda and S. Akira, *Curr. Protoc. Immunol.*, 2007, DOI: 10.1002/0471142735.im1412s77.
- 16 S. Akira, *Adv. Immunol.*, 1902, **78**, 1.
- 17 S. Akira, K. Takeda and T. Kaisho, *Nat. Immunol.*, 2001, **2**, 675.
- 18 A. Cottalorda, C. Vershelde, A. Marcais, M. Tomkowiak, P. Musette, S. Uematsu, S. Akira, J. Marvel and N. Bonnefoy-Berard, *Eur. J. Immunol.*, 2006, **36**, 1684.
- 19 T. Kaisho and S. Akira, *Biochim. Biophys. Acta*, 2002, **1589**, 1.
- 20 G. R. Stark, I. M. Kerr, B. R. Williams, R. H. Silverman and R. D. Schreiber, *Annu. Rev. Biochem.*, 1998, **67**, 227.
- 21 S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira and C. Reis e Sousa, *Science*, 2004, **303**, 1529.
- 22 A. D. Edwards, S. S. Diebold, E. M. Slack, H. Tomizawa, H. Hemmi, T. Kaisho, S. Akira and C. Reis e Sousa, *Eur. J. Immunol.*, 2003, **33**, 827.
- 23 D. Rajagopal, C. Paturel, Y. Morel, S. Uematsu, S. Akira and S. S. Diebold, *Blood*, 2010, **115**, 1949.
- 24 A. Krug, G. D. Luker, W. Barchet, D. A. Leib, S. Akira and M. Colonna, *Blood*, 2004, **103**, 1433.
- 25 J. Lund, A. Sato, S. Akira, R. Medzhitov and A. Iwasaki, *J. Exp. Med.*, 2003, **198**, 513.
- 26 A. ISAACS and J. Lindemann, *Proc. R. Soc. London, Ser. B*, 1957, **147**, 258.
- 27 A. Le Bon, N. Etchart, C. Rossmann, M. Ashton, S. Hou, D. Gewert, P. Borrow and D. F. Tough, *Nat. Immunol.*, 2003, **4**, 1009.
- 28 A. Le Bon, G. Schiavoni, G. D'Agostino, I. Gresser, F. Belardelli and D. F. Tough, *Immunity*, 2001, **14**, 461.
- 29 D. Durali, M. G. de Goer de Herve, J. Giron-Michel, B. Azzarone, J. F. Delfraissy and Y. Taoufik, *Blood*, 2003, **102**, 4084.
- 30 F. E. Lund and T. D. Randall, *Nat. Rev. Immunol.*, 2010, **10**, 236.
- 31 M. G. de Goer de Herve, D. Durali, B. Dembele, M. Giuliani, T. A. Tran, B. Azzarone, P. Eid, M. Tardieu, J. F. Delfraissy and Y. Taoufik, *PLoS One*, 2011, **6**, e19366.
- 32 T. Sareneva, S. Matikainen, M. Kurimoto and I. Julkunen, *J. Immunol.*, 1998, **160**, 6032.
- 33 J. D. Hood, H. J. Warshakoon, M. R. Kimbrell, N. M. Shukla, S. Malladi, X. Wang and S. A. David, *Hum. Vaccin.*, 2010, **6**, 1.
- 34 N. M. Shukla, T. C. Lewis, T. P. Day, C. A. Mutz, R. Ukani, C. D. Hamilton, R. Balakrishna and S. A. David, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3232.
- 35 N. J. Horscroft, D. C. Pryde and H. Bright, *J. Antimicrob. Chemother.*, 2012, **67**, 789.
- 36 S. J. Bergman, M. C. Ferguson and C. Santanello, *Infect. Dis. Clin. North Am.*, 2011, **25**, 819.
- 37 B. J. Weigel, S. Cooley, T. Defor, D. J. Weisdorf, A. Panoskaltis-Mortari, W. Chen, B. R. Blazar and J. S. Miller, *Am. J. Hematol.*, 2012, 953.
- 38 J. F. Gerster, K. J. Lindstrom, R. L. Miller, M. A. Tomai, W. Birmachu, S. N. Bomersine, S. J. Gibson, L. M. Imbertson, J. R. Jacobson, R. T. Knafla, P. V. Maye,

- N. Nikolaidis, F. Y. Oneyemi, G. J. Parkhurst, S. E. Pecore, M. J. Reiter, L. S. Scribner, T. L. Testerman, N. J. Thompson, T. L. Wagner, C. E. Weeks, J. D. Andre, D. Lagain, Y. Bastard and M. Lupu, *J. Med. Chem.*, 2005, **48**, 3481.
- 39 K. Hirota, K. Kazaoka, I. Niimoto, H. Kumihara, H. Sajiki, Y. Isobe, H. Takaku, M. Tobe, H. Ogita, T. Ogino, S. Ichii, A. Kurimoto and H. Kawakami, *J. Med. Chem.*, 2002, **45**, 5419.
- 40 Y. Isobe, M. Tobe, H. Ogita, A. Kurimoto, T. Ogino, H. Kawakami, H. Takaku, H. Sajiki, K. Hirota and H. Hayashi, *Bioorg. Med. Chem.*, 2003, **11**, 3641.
- 41 A. Kurimoto, T. Ogino, S. Ichii, Y. Isobe, M. Tobe, H. Ogita, H. Takaku, H. Sajiki, K. Hirota and H. Kawakami, *Bioorg. Med. Chem.*, 2004, **12**, 1091.
- 42 A. Kurimoto, K. Hashimoto, T. Nakamura, K. Norimura, H. Ogita, H. Takaku, R. Bonnert, T. McNally, H. Wada and Y. Isobe, *J. Med. Chem.*, 2010, **53**, 2964.
- 43 J. Lee, T. H. Chuang, V. Redecke, L. She, P. M. Pitha, D. A. Carson, E. Raz and H. B. Cottam, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 6646.
- 44 A. X. Xiang, S. E. Webber, B. M. Kerr, E. J. Rueden, J. R. Lennox, G. J. Haley, T. Wang, J. S. Ng, M. R. Herbert, D. L. Clark, V. N. Banh, W. Li, S. P. Fletcher, K. R. Steffy, D. M. Bartkowski, L. I. Kirkovsky, L. A. Bauman and D. R. Averett, *Nucleosides Nucleotides Nucleic Acids*, 2007, **26**, 635.
- 45 N. M. Shukla, M. R. Kimbrell, S. S. Malladi and S. A. David, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2211.
- 46 N. M. Shukla, S. S. Malladi, C. A. Mutz, R. Balakrishna and S. A. David, *J. Med. Chem.*, 2010, **53**, 4450.
- 47 N. M. Shukla, S. S. Malladi, V. Day and S. A. David, *Bioorg. Med. Chem.*, 2011, **19**, 3801.
- 48 N. M. Shukla, C. A. Mutz, R. Ukani, H. J. Warshakoon, D. S. Moore and S. A. David, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6384.
- 49 N. M. Shukla, C. A. Mutz, S. S. Malladi, H. J. Warshakoon, R. Balakrishna and S. A. David, *J. Med. Chem.*, 2012, **55**, 1106.
- 50 E. Brummer, M. A. Antonysamy, L. Bythadka, G. W. Gullikson and D. A. Stevens, *FEMS Immunol. Med. Microbiol.*, 2010, **59**, 81.
- 51 K. J. Lindstrom and N. Nikolaidis, Imidazo[4,5-c]pyridin-4-amines, *World patent*, WO 95/02597, 1995.
- 52 T. A. Kshirsagar, B. A. Merrill, S. E. Langer, K. J. Lindstrom, S. C. Johannessen, G. J. Marszalek, J. R. Wurst, K. J. Manske, S. Niwas, G. D. Lundquist Jr., P. D. Heppner, G. W. Griesgraber and M. E. Danielson, Method of preferentially inducing the biosynthesis of interferon, *World patent*, WO 2006/091467 A2[1], 2006, 252.
- 53 T. A. Kshirsagar, G. D. Lundquist Jr. J. F. Dellaria Jr. M. R. Radmer and B. M. Zimmermann, Oxime and hydroxylamine substituted imidazo[4,5-c] ring compounds and methods, *World patent*, WO 2006/086634, 2006.
- 54 J. F. Dellaria, G. A. Haraldson, P. D. Heppner, K. J. Lindstrom and B. A. Merrill, Sulfonamido substituted imidazopyridines, *US* 6,525,064 B1, 2003, 1–35.
- 55 J. D. Hood, H. J. Warshakoon, M. R. Kimbrell, N. M. Shukla, S. Malladi, X. Wang and S. A. David, *Hum. Vaccin.*, 2010, **6**, 1.
- 56 A. T. Baviskar, C. Madaan, R. Preet, P. Mohapatra, V. Jain, A. Agarwal, S. K. Guchhait, C. N. Kundu, U. C. Banerjee and P. V. Bharatam, *J. Med. Chem.*, 2011, **54**, 5013.
- 57 M. S. Jin and J. O. Lee, *Curr. Opin. Immunol.*, 2008, **20**, 414.
- 58 S. I. Yoon, O. Kurnasov, V. Natarajan, M. Hong, A. V. Gudkov, A. L. Osterman and I. A. Wilson, *Science*, 2012, **335**, 859.
- 59 H. Hemmi, T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda and S. Akira, *Nat. Immunol.*, 2002, **3**, 196.
- 60 T. Kaisho and S. Akira, *J. Allergy Clin. Immunol.*, 2006, **117**, 979.
- 61 K. B. Gorden, K. S. Gorski, S. J. Gibson, R. M. Kedl, W. C. Kieper, X. Qiu, M. A. Tomai, S. S. Alkan and J. P. Vasilakos, *J. Immunol.*, 2005, **174**, 1259.
- 62 K. S. Gorski, E. L. Waller, J. Bjornton-Severson, J. A. Hanten, C. L. Riter, W. C. Kieper, K. B. Gorden, J. S. Miller, J. P. Vasilakos, M. A. Tomai and S. S. Alkan, *Int. Immunol.*, 2006, **18**, 1115.
- 63 H. P. Kokatla, E. Yoo, D. B. Salunke, D. Sil, C. F. Ng, R. Balakrishna, S. S. Malladi, L. M. Fox and S. A. David, *Org. Biomol. Chem.*, 2013, **11**, 1179.
- 64 S. A. David, M. S. Smith, G. Lopez, S. Mukherjee, S. Buch and O. Narayan, *AIDS Res. Hum. Retroviruses*, 2001, **17**, 59.
- 65 M. R. Kimbrell, H. Warshakoon, J. R. Cromer, S. Malladi, J. D. Hood, R. Balakrishna, T. A. Scholdberg and S. A. David, *Immunol. Lett.*, 2008, **118**, 132.