

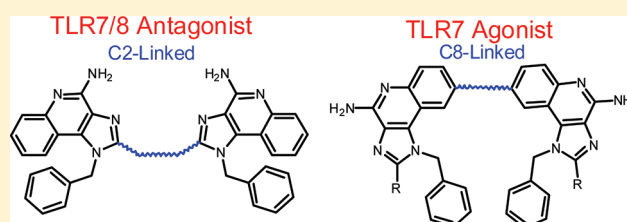
Toll-Like Receptor (TLR)-7 and -8 Modulatory Activities of Dimeric Imidazoquinolines

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S Supporting Information

ABSTRACT: Toll-like receptors (TLRs) are pattern recognition receptors that recognize specific molecular patterns present in molecules that are broadly shared by pathogens but are structurally distinct from host molecules. The TLR7-agonistic imidazoquinolines are of interest as vaccine adjuvants given their ability to induce pronounced Th1-skewed humoral responses. Minor modifications on the imidazoquinoline scaffold result in TLR7-antagonistic compounds which may be of value in addressing innate immune activation-driven immune exhaustion observed in HIV. We describe the syntheses and evaluation of TLR7 and TLR8 modulatory activities of dimeric constructs of imidazoquinoline linked at the C2, C4, C8, and N^1 -aryl positions. Dimers linked at the C4, C8, and N^1 -aryl positions were agonistic at TLR7; only the N^1 -aryl dimer with a 12-carbon linker was dual TLR7/8 agonistic. Dimers linked at C2 position showed antagonistic activities at TLR7 and TLR8; the C2 dimer with a propylene spacer was maximally antagonistic at both TLR7 and TLR8.



INTRODUCTION

Toll-like receptors (TLRs) are pattern recognition receptors that recognize specific molecular patterns present in molecules that are broadly shared by pathogens but are structurally distinct from host molecules.^{1,2} There are 10 TLRs in the human genome.² 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 (TLR 11).^{3,4} TLR1, -2, -4, -5, and -6 respond to extracellular stimuli, while TLR3, -7, -8, and -9 respond to intracytoplasmic pathogen-associated molecular patterns (PAMPs), being associated with the endolysosomal compartment.²

We have earlier explored structure–activity relationships in TLR2- and TLR7-active ligands^{5,6} toward exploiting them as potential vaccine adjuvants;^{7,8} the TLR7-agonistic imidazoquinolines are of particular interest in that they induce pronounced Th1-skewed humoral responses in animal models.⁹ Of equal interest is our finding that relatively minor modifications on the imidazoquinoline scaffold result in TLR7-antagonistic compounds,^{10,11} which may be of value in addressing the relentless innate immune activation-driven immune exhaustion observed in HIV.^{12–14}

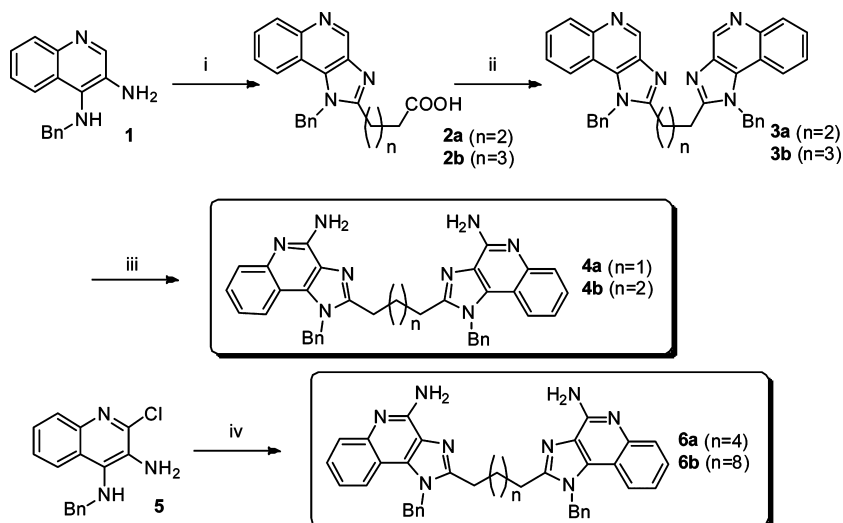
Several TLRs are thought to signal via ligand-induced dimerization,¹⁵ as evident in the crystal structures of TLR2^{16,17} and TLR3.¹⁸ It is not yet understood, however, how TLR7 and TLR8, whose endogenous ligands are single-

stranded viral RNA (ssRNA), recognize and transduce signals upon engagement by small, nonpolymeric molecules such as the imidazoquinolines^{19,20} and the oxadenines.^{21,22} We were not only desirous of examining the effect of variously configured, preorganized dimeric constructs of the imidazoquinolines on TLR7 and TLR8 in our continuing efforts to identify efficacious and safe vaccine adjuvants but also motivated in the hope that we may, by design or accident, discover small-molecule modulators of TLR3. It may be noted that although TLR3, like TLR7, is an attractive target for adjuvant design and development,^{23–28} polyriboinosinic:polyribocytidylic acid, poly(I:C), is presently the only available synthetic TLR3 agonist.²⁹ No small molecule agonists for TLR3 have been described to date; however, benzothioephene antagonists of TLR3 have been reported recently.³⁰

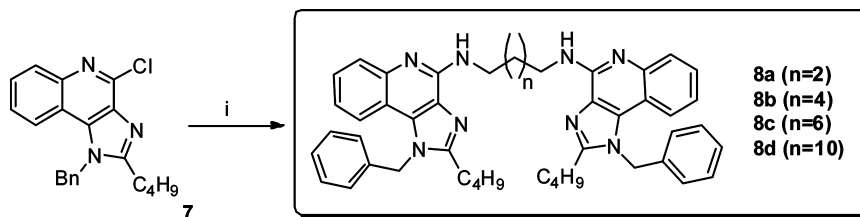
We describe in the paper the syntheses and evaluation of TLR7, -8, and -3 modulatory activities of dimeric constructs of imidazoquinoline linked at the C2, C4, C8, and N^1 -aryl positions. Dimers linked at the C4, C8, and N^1 -aryl positions were agonistic at TLR7; only the N^1 -aryl dimer with a 12-carbon linker was found to be dual TLR7/8 agonistic. The imidazoquinoline dimers linked at C2 position showed antagonistic activities at TLR7 and TLR8; the C2 dimer with a 3-carbon spacer was found to be maximally antagonistic at both TLR7 and TLR8, and its activities were preserved in secondary screens employing human blood.

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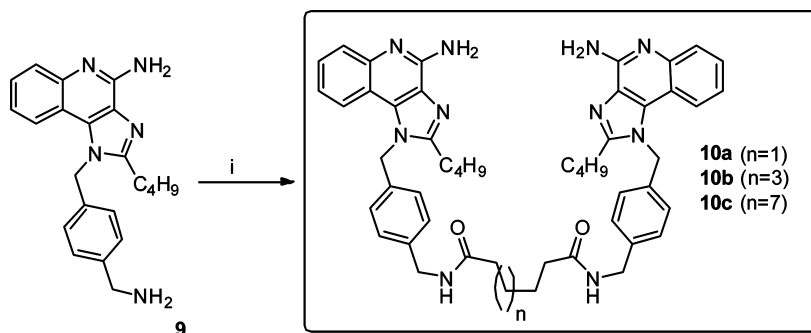
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Scheme 1. Syntheses of Imidazoquinoline Dimers Linked at C2^a

^aReagents: (i) glutaric anhydride ($n = 2$) or adipic anhydride ($n = 3$), Et₃N, THF, 110 °C; (ii) 1, HBTU, Et₃N, DMAP, DMF, 90 °C; (iii) (a) 3-chloroperoxybenzoic acid, CH₂Cl₂, CHCl₃, MeOH, 45 °C, (b) benzoyl isocyanate, CH₂Cl₂, 45 °C, (c) NaOCH₃, MeOH, 80 °C; (iv) (a) Suberoyl chloride ($n = 4$) or dodecanedioyl dichloride ($n = 8$), Et₃N, THF, (b) NH₃/MeOH, 150 °C.

Scheme 2. Syntheses of Imidazoquinoline Dimers Linked at C4-NH₂^a

^aReagents: (i) 1,4-diaminobutane ($n = 2$) or 1,8-diaminooctane ($n = 4$) or 1,10-diaminododecane ($n = 6$) or 1,12-diaminododecane ($n = 10$), MeOH, 140 °C.

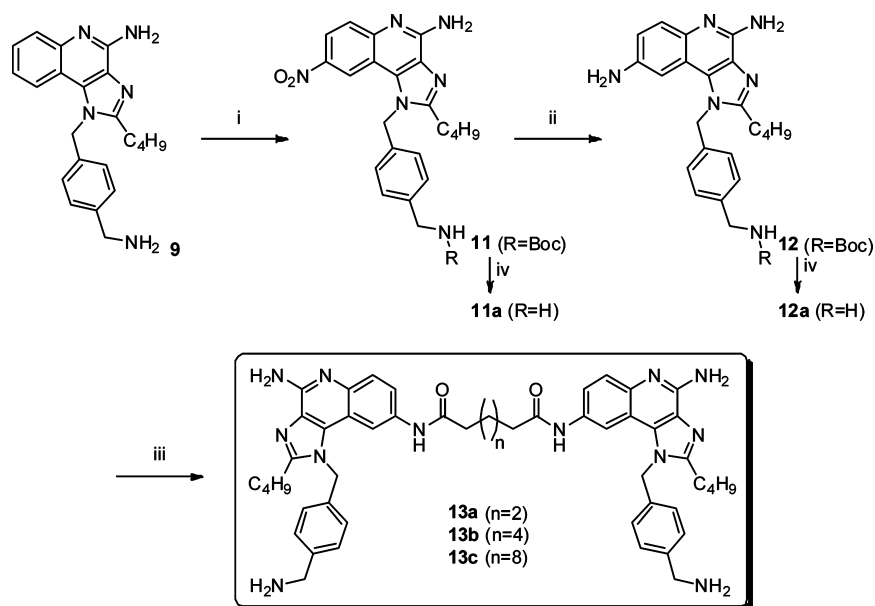
Scheme 3. Syntheses of Imidazoquinoline Dimers Linked at N¹-(4-Aminomethylene)benzyl^a

^aReagents: (i) adipoyl chloride ($n = 1$) or suberoyl chloride ($n = 3$) or dodecanedioyl dichloride ($n = 7$), Et₃N, THF.

RESULTS AND DISCUSSION

The majority of TLRs signal via homo- or heterodimerization,¹⁵ and it was therefore of interest to examine dimeric constructs with differing geometries. The first series of dimeric imidazoquinolines were linked at the C2 position, and their syntheses necessitated two different routes. Whereas the hexamethylene- and decamethylene-bridged compounds 6a and 6b could be conveniently obtained from 5 by a direct, one-step, bis-amidation using the corresponding dicarboxylic acid chlorides and cyclization (Scheme 1), the shorter chain analogues were not amenable to this method because of

intramolecular cyclization, giving rise to undesired quinolin-3-yl piperidinediones. This problem was circumvented by first reacting glutaric or adipic anhydride with 1, which yielded the monocarboxylic imidazoquinolines 2a and 2b, respectively; these intermediates were taken forward without purification and reacted again with 1 to afford the C4,C4'-des-amino precursors 3a and 3b (Scheme 1). Compounds 4a and 4b (with amines at C4 and C4', respectively) were obtained by sequential N-oxidation of the quinoline nitrogen, reaction with benzoyl isocyanate to afford the C4 and C4' N-benzoyl

Scheme 4. Syntheses of Imidazoquinoline Dimers Linked at C8-NH₂^a

^aReagents: (i) (a) HNO₃, H₂SO₄, (b) (Boc)₂O, Et₃N, MeOH; (ii) H₂, Pt/C, MeOH, 60 psi; (iii) (a) adipoyl chloride (*n* = 2) or suberoyl chloride (*n* = 4) or dodecanedioyl dichloride (*n* = 8), Et₃N, THF, (b) HCl/dioxane, (iv) HCl/dioxane.

intermediate, and finally, cleavage of the *N*-benzoyl group using sodium methoxide as described by us earlier.^{5,11}

Next, the dimers linked via the C4-NH₂ (**8a–d**) were synthesized by direct S_NAr on **7** using α,ω -bis-amino alkanes (Scheme 2). Similarly, dimers linked at the N¹ position on the 4-aminomethylene benzyl group (**10a–c**) were obtained using appropriate dicarboxylic acid chlorides (Scheme 3).

Linking the **13** series of dimers via the quinoline ring required the introduction of an additional amine at position C8 (Scheme 4). This was achieved via carefully controlled nitration of **9** using 1.2–1.3 equiv of HNO₃, followed by *N*-Boc protection of the amine on the N¹ substituent and subsequent reduction. Dimerization of the 4,8-diaminoimidazoquinoline **12** proceeded smoothly using dicarboxylic acid chlorides as described in the previous schemes. It is to be noted that the mononitro and monoamino precursors **11** and **12** were also *N*-Boc deprotected and tested for TLR-modulatory activities (Table 1).

All compounds were unfortunately found to be inactive in TLR3 reporter gene assays. However, with the exception of **4** and **6** series of compounds, all other dimers retained TLR7-agonistic properties, the **10** series being the most potent (Figure 1); interestingly, only **10c** displayed both TLR7 and TLR8 agonism (Figure 1 and Table 1), pointing to the necessity of a long linker for dual agonism. The C2-linked dimeric compounds **4a**, **4b**, **6a**, and **6b** unexpectedly showed potent antagonistic activity in both TLR7 and TLR8 assays, with **4a** being most potent (IC₅₀ values of 3.1 and 3.2 μ M in TLR7 and TLR8 assays, respectively; Figure 2, Table 1). A distinct relationship between linker length and inhibitory potency was not observed in this series. Given that the propylene linked **4a** was the most active compound, it appeared possible that a shorter homologue with an ethylene linker may further enhance antagonistic activity; however, the solubility of this compound was intractably poor despite our attempts at evaluating a variety of salt forms, and we therefore decided not to include it in our bioassay screens.

Both agonistic and antagonistic compounds were then tested in appropriate secondary screens employing ex vivo human blood-derived models. The ligation of TLR7 and TLR8 trigger inflammatory responses characterized by the elaboration of type I interferon (IFN- α/β) by virus-infected cells via activation of downstream NF- κ B and IFN- β promoters.^{31–36} IFN production is a hallmark response underlying cellular antiviral immune responses. It was desirable to verify that TLR7 agonism that we had observed (Figure 1) manifested in IFN production in secondary screens. Using an ex vivo stimulation model using human peripheral blood mononuclear cells (hPBMC), it was demonstrated that IFN- α was indeed induced in a dose-dependent, bimodal manner as expected for innate immune responses (Figure 3). Compound **10c** was found to be the most potent; we surmise that this is due to its dual TLR7/8 agonistic activity. The **4** and **6** series were quiescent (Figure 2), consistent with their apparent antagonistic behavior.

We elected to examine in detail the antagonistic properties of **4a** in inhibiting TLR7- and TLR8-mediated induction of proinflammatory cytokines (Figure 4) and chemokines (Figure 5) in ex vivo models using human blood because this compound was found to be the most potent antagonist in the series in primary screens (Table 1). We compared the potency of **4a** alongside chloroquine, which is known to selectively suppress intracellular TLR7 but not TLR8 signaling via inhibition of endolysosomal acidification.^{37,38} We found **4a** to be a potent inhibitor of both TLR7- and TLR8-induced cytokine and chemokine release with IC₅₀ values of about 0.05–0.3 μ M (Figures 4, 5). TLR8 signaling manifests predominantly in the induction of pro-inflammatory cytokines such as TNF- α and IL-1 β .^{39,40} Chloroquine, a TLR7 antagonist, is a feeble inhibitor of TNF- α and IL-1 β , while **4a**, as would be expected for a TLR8 antagonist, potently inhibits the production of these proinflammatory cytokines (Figure 4), as well as IL-6 and IL-8, which are typically induced secondarily, in an autocrine/paracrine manner.

Table 1. Agonistic and Antagonistic Activities of the Dimers in TLR7 and TLR8 Reporter Gene Assays^a

Compound number	Structure	TLR7		TLR8	
		Agonism (μM)	Antagonism (μM)	Agonism (μM)	Antagonism (μM)
4a		ND	3.1	ND	3.2
4b		ND	ND	ND	15.63
6a		ND	ND	ND	10.92
6b		ND	17.88	ND	4.65
8a		2.05	ND	ND	ND
8b		0.56	ND	ND	ND
8c		3.00	ND	ND	ND
8d		1.42	ND	ND	ND
10a		0.11	ND	ND	ND
10b		0.24	ND	ND	ND
10c		0.17	ND	4.78	ND

Table 1. continued

Compound number	Structure	TLR7		TLR8	
		Agonism (μM)	Antagonism (μM)	Agonism (μM)	Antagonism (μM)
11a		0.56	ND	ND	ND
12a		0.45	ND	ND	ND
13a		7.24	ND	ND	ND
13b		4.02	ND	ND	ND
13c		5.4	ND	ND	ND

^aND = not detected; NT = not tested.

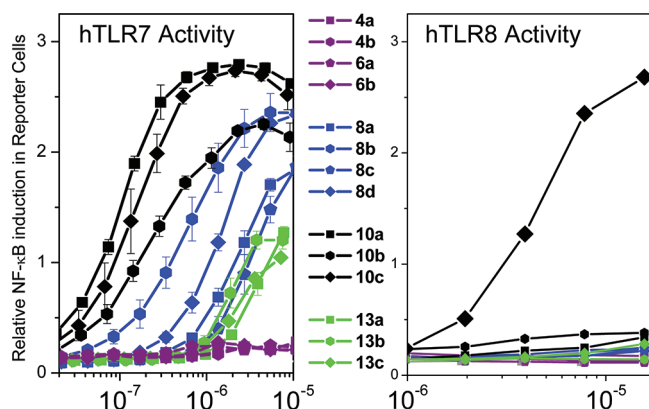


Figure 1. TLR7 and TLR8 agonistic activities of the imidazoquinoline dimers in human TLR-specific reporter gene assays.

The relative specificity of chloroquine in inhibiting TLR7 as well as the dual TLR7/8-inhibitory activities of **4a** are also

evident in Schild plots (Figure 6). Although the relationship between antagonist concentration and change in EC_{50} for TLR7 inhibition by **4a** is near-ideal (slope: 1.12, Figure 6), a distinct deviation from ideal competitive inhibition for TLR8 is observed (slope: 0.51), suggesting that additional mechanisms for TLR8 inhibition, possibly allosteric, may be operational. This is being investigated in greater detail.

CONCLUSION

In conclusion, we have observed that the C4, C8, and N^1 -aryl-linked dimers are agonists, with the last being most potent. The N^1 -aryl-linked dimers are of particular interest as potential vaccine adjuvants are currently being evaluated in animal models. The C2-linked dimers were found to be potentially antagonistic at both TLR7 and TLR8 and may be useful as small molecule probes for examining the effects of inhibiting endolysosomal TLR signaling in HIV.

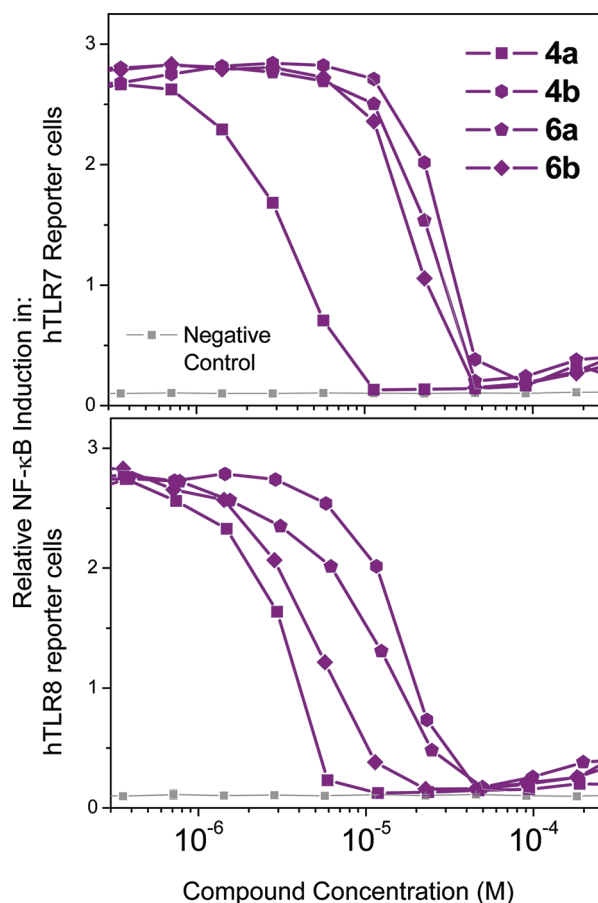


Figure 2. TLR7 (top) and TLR8 (bottom) antagonistic activities of the imidazoquinoline dimers 4a–b and 6a–b in human TLR-specific reporter gene assays.

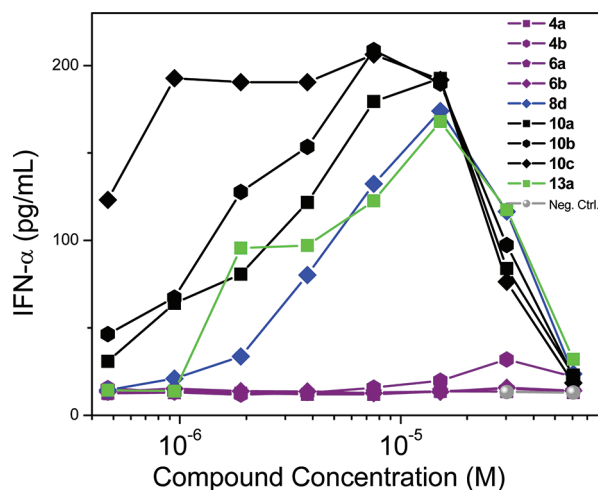


Figure 3. IFN- α induction by select dimers in human peripheral blood mononuclear cells. IFN- α was assayed by analyte specific ELISA after incubation of hPBMCs with graded concentrations of the test compound for 12 h. A representative experiment of three independent experiments is shown.

EXPERIMENTAL SECTION

All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or 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 CCM precoated 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 C₁₈ column with H₂O–isopropyl alcohol or H₂O–CH₃CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion acquisition mode. All the compounds synthesized were obtained as solids.

Synthesis of Compound 4a: 2,2'-(Propane-1,3-diyl)bis(1-benzyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine). To a solution of 1 (100 mg, 0.4 mmol) in anhydrous THF were added triethylamine (53 mg, 0.52 mmol) and glutaric anhydride (60 mg, 0.52 mmol), and the reaction vessel was heated in a microwave for 2 h at 110 °C. The solvent was then removed under vacuum to obtain the crude product 2, which was then dissolved in anhydrous DMF, and to this solution were added HBTU (167 mg, 0.44 mmol), triethylamine (53 mg, 0.52 mmol), 1 (100 mg, 0.4 mmol), and a catalytic amount of DMAP. The reaction mixture was stirred for 12 h at 90 °C. The solvent was then removed under vacuum, and the residue was purified using column chromatography (12% MeOH/dichloromethane) to obtain the intermediate the compound 3 (157 mg). To a solution of 3 in solvent mixture of MeOH/dichloromethane/chloroform (0.1:1:1) was added 3-chloroperoxybenzoic acid (242 mg, 1.4 mmol), and the reaction mixture was refluxed at 45 °C for 40 min. The solvent was then removed, and the residue was purified using column chromatography (35% MeOH/dichloromethane) to obtain the bis-*N*-oxide derivative (130 mg). Bis-*N*-oxide derivative (110 mg, 0.19 mmol) was then dissolved in anhydrous dichloromethane, followed by the addition of benzoyl isocyanate (96 mg, 0.67 mmol) and heated at 45 °C for 15 min. The solvent was then removed under vacuum, and the residue was dissolved in anhydrous MeOH, followed by addition of excess of sodium methoxide, and heated at 80 °C for 2 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (50% MeOH/dichloromethane) to obtain the compound 4a (25 mg, 11%). ¹H NMR (500 MHz, DMSO) δ 14.30 (s, 2H), 9.48–8.30 (bs, 4H), 7.93 (d, *J* = 8.3 Hz, 2H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.65–7.60 (m, 2H), 7.38–7.34 (m, 2H), 7.26 (t, *J* = 7.6 Hz, 4H), 7.17 (t, *J* = 7.4 Hz, 2H), 7.03 (d, *J* = 7.4 Hz, 4H), 5.94 (s, 4H), 3.16 (t, *J* = 7.2 Hz, 4H), 2.44–2.35 (m, 2H). ¹³C NMR (126 MHz, DMSO) δ 156.22, 148.86, 135.32, 135.30, 133.48, 129.51, 128.93, 127.57, 125.47, 124.72, 124.54, 121.49, 118.31, 112.16, 48.35, 25.21, 24.43. MS (ESI) calculated for C₃₇H₃₂N₈, *m/z* 588.2750; found 589.2860 (*M* + *H*)⁺.

Compound 4b Was Synthesized Similarly As Described for Compound 4a. 4b: ¹H NMR (500 MHz, DMSO) δ 13.86 (s, 2H), 8.88 (bs, 4H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.83–7.79 (m, 2H), 7.66–7.61 (m, 2H), 7.39–7.34 (m, 2H), 7.27 (t, *J* = 7.6 Hz, 4H), 7.18 (t, *J* = 7.4 Hz, 2H), 7.01 (d, *J* = 7.4 Hz, 4H), 5.94 (s, 4H), 2.99 (s, 4H), 1.83 (s, 4H). ¹³C NMR (126 MHz, DMSO) δ 156.55, 148.75, 135.38, 133.62, 129.50, 128.95, 127.60, 125.42, 124.74, 124.54, 121.54, 118.48, 112.28, 48.34, 26.49, 26.14. MS (ESI) calculated for C₃₈H₃₄N₈, *m/z* 602.2906; found 603.3272 (*M* + *H*)⁺ and 302.1705 (*M* + 2*H*)²⁺.

Synthesis of Compound 6a: 2,2'-(Hexane-1,6-diyl)bis(1-benzyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine). To a solution of 5 (60 mg, 0.21 mmol) in anhydrous THF were added triethylamine (54 mg, 0.53 mmol) and suberoyl chloride (23 mg, 0.11 mmol), and the reaction mixture was stirred for 6 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc and washed with water/brine. The EtOAc fraction was then dried using sodium sulfate and evaporated under vacuum to obtain the intermediate amide compound, which was then dissolved in 1 mL solution of 2 M ammonia in MeOH and heated at 150 °C for 15 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (20% MeOH/dichloromethane) to obtain the compound 6a (8 mg, 12%). ¹H NMR (500 MHz, MeOD) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.65 (dd, *J* = 11.5, 4.2 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 4H), 7.25 (t, *J* =

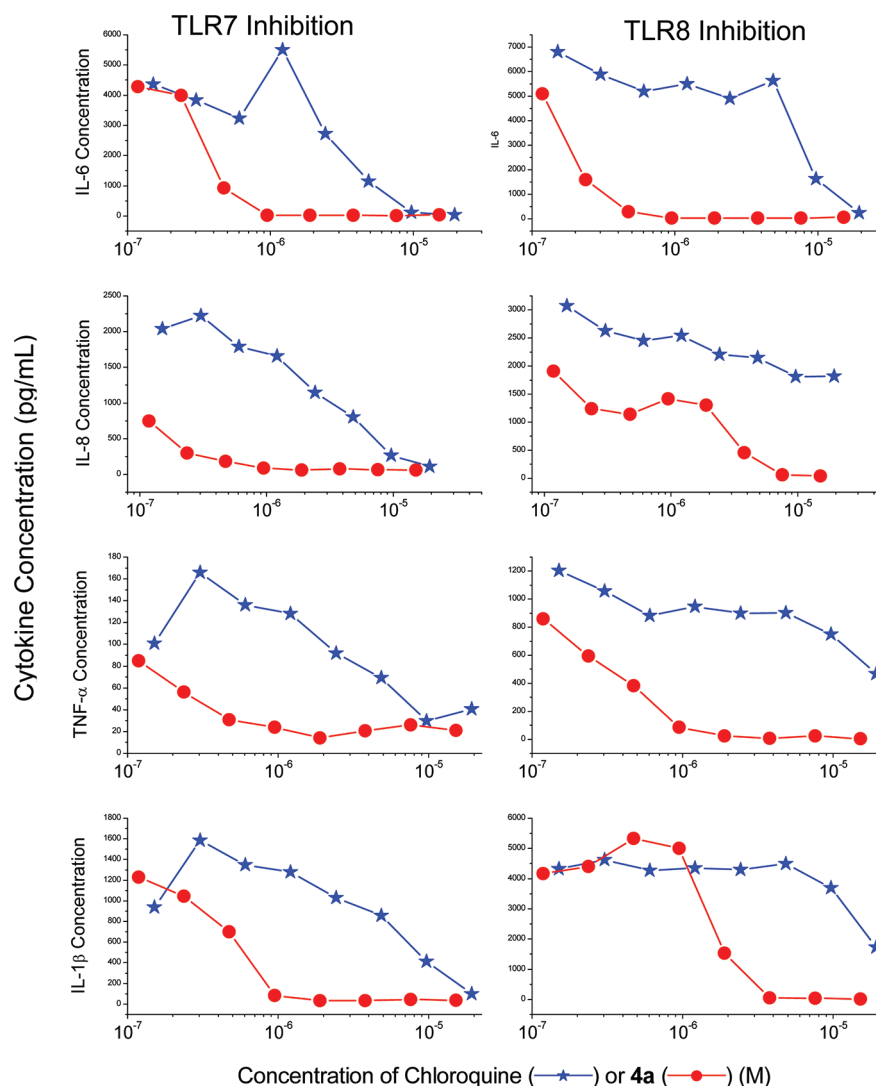


Figure 4. Inhibition of TLR7- and TLR8-mediated proinflammatory cytokine production in human peripheral blood mononuclear cells by chloroquine or **4a**. Proinflammatory cytokines were assayed by cytokine bead array methods after incubation of hPBMCs with graded concentrations of the test compound for 12 h in the presence of 10 $\mu\text{g/mL}$ of either CL075 (TLR8 agonist) or gardiquimod (TLR7 agonist). A representative experiment of three independent experiments is shown.

7.3 Hz, 2H), 7.06 (d, $J = 7.4$ Hz, 4H), 5.93 (s, 4H), 2.97 (t, $J = 7.5$ Hz, 4H), 1.85 (d, $J = 7.0$ Hz, 4H), 1.43 (s, 4H). ^{13}C NMR (126 MHz, MeOD) δ 158.93, 137.73, 136.20, 135.30, 131.11, 130.47, 129.31, 126.65, 126.57, 125.81, 122.94, 119.65, 114.20, 50.13, 29.70, 27.92. MS (ESI) calculated for $\text{C}_{40}\text{H}_{38}\text{N}_8$, m/z 630.3219; found 631.3415 ($\text{M} + \text{H}^+$).

Compound 6b Was Synthesized Similarly As Described for Compound 6a. **6b:** ^1H NMR (500 MHz, MeOD) δ 7.86 (d, $J = 8.3$ Hz, 2H), 7.69 (d, $J = 8.4$ Hz, 2H), 7.51 (t, $J = 7.7$ Hz, 2H), 7.32 (t, $J = 7.3$ Hz, 4H), 7.28 (d, $J = 7.2$ Hz, 2H), 7.23 (t, $J = 7.7$ Hz, 2H), 7.06 (d, $J = 7.4$ Hz, 4H), 5.88 (s, 4H), 2.96 (t, $J = 7.6$ Hz, 4H), 1.79 (dt, $J = 15.3, 7.7$ Hz, 4H), 1.37 (dd, $J = 14.9, 7.4$ Hz, 4H), 1.32–1.24 (m, $J = 11.6$ Hz, 4H), 1.23 (d, $J = 10.1$ Hz, 4H). ^{13}C NMR (126 MHz, MeOD) δ 157.44, 151.60, 136.61, 130.41, 129.74, 129.22, 126.69, 126.46, 124.90, 123.38, 122.15, 115.12, 50.05, 30.32, 30.25, 30.17, 28.49, 28.17. MS (ESI) calculated for $\text{C}_{44}\text{H}_{46}\text{N}_8$, m/z 686.3845; found 687.3749 ($\text{M} + \text{H}^+$) and 344.1949 ($\text{M} + 2\text{H}^+$) $^{2+}$.

Synthesis of Compound 8b: *N*¹,*N*⁸-Bis(1-benzyl-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-4-yl)octane-1,8-diamine. To a solution of **7** (50 mg, 0.14 mmol) in 1 mL of anhydrous MeOH was added 1,8-diaminooctane (10 mg, 0.07 mmol), and the reaction mixture was heated at 140 $^{\circ}\text{C}$ for 4 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography

(8% MeOH/dichloromethane) to obtain the compound **8b** (12 mg, 22%). ^1H NMR (400 MHz, CDCl_3) δ 7.87 (d, $J = 8.3$ Hz, 2H), 7.68 (d, $J = 8.2$ Hz, 2H), 7.41 (t, $J = 7.7$ Hz, 2H), 7.36–7.26 (m, 6H), 7.09–7.01 (m, 6H), 5.78 (s, 2H), 5.72 (s, 4H), 3.77 (dd, $J = 12.8, 6.6$ Hz, 4H), 2.92–2.83 (m, 4H), 1.87–1.74 (m, 8H), 1.58–1.50 (m, 4H), 1.45 (dt, $J = 15.1, 7.5$ Hz, 8H), 0.93 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 153.34, 150.78, 145.51, 135.62, 129.20, 127.93, 127.41, 127.07, 126.68, 125.59, 121.34, 119.52, 114.87, 48.81, 40.76, 30.18, 29.98, 29.50, 27.21, 22.56, 13.77. MS (ESI) calculated for $\text{C}_{50}\text{H}_{58}\text{N}_8$, m/z 770.4784; found 771.4963 ($\text{M} + \text{H}^+$) and 386.2570 ($\text{M} + 2\text{H}^+$) $^{2+}$.

Compounds 8a, 8c, and 8d Were Synthesized Similarly As Described for Compound 8b. **8a:** ^1H NMR (500 MHz, CDCl_3) δ 7.86 (d, $J = 8.2$ Hz, 2H), 7.65 (dd, $J = 8.2, 1.0$ Hz, 2H), 7.41–7.35 (m, 2H), 7.35–7.24 (m, 6H), 7.09–6.99 (m, 6H), 5.86 (s, 2H), 5.69 (s, 4H), 3.87 (s, 4H), 2.88–2.81 (m, 4H), 2.00 (s, 4H), 1.76 (ddd, $J = 13.0, 9.0, 7.7$ Hz, 4H), 1.46–1.37 (m, 4H), 0.90 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 151.27, 148.66, 143.44, 133.52, 130.85, 128.77, 127.28, 127.13, 125.85, 125.38, 124.95, 124.60, 123.50, 123.40, 119.29, 117.45, 112.81, 46.71, 38.40, 28.01, 25.51, 25.10, 20.47, 11.68. MS (ESI) calculated for $\text{C}_{46}\text{H}_{50}\text{N}_8$, m/z 714.4158; found 715.4333 ($\text{M} + \text{H}^+$) and 358.2263 ($\text{M} + 2\text{H}^+$) $^{2+}$.

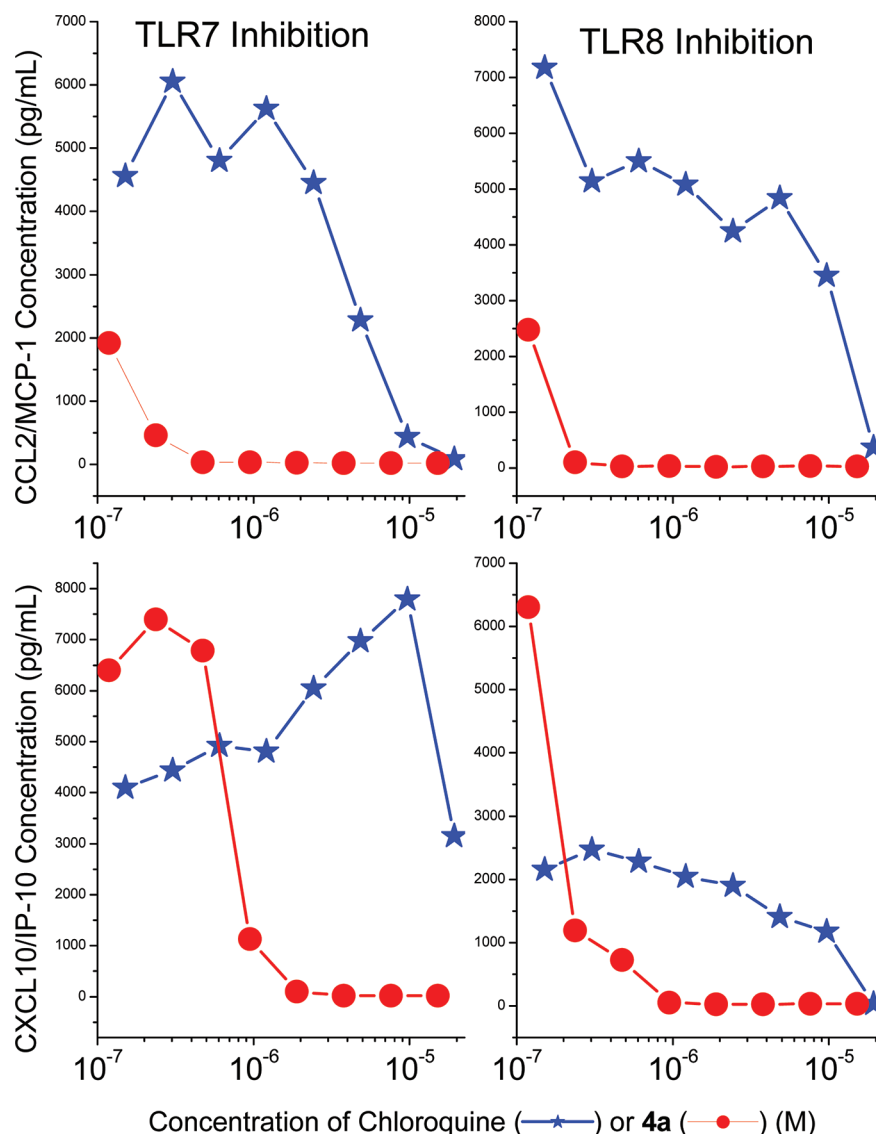


Figure 5. Inhibition of TLR7- and TLR8-mediated chemokine production in human peripheral blood mononuclear cells by chloroquine or **4a**. Chemokines were assayed by cytokine bead array methods after incubation of hPBMCs with graded concentrations of the test compound for 12 h in the presence of 10 $\mu\text{g/mL}$ of either CL075 (TLR8 agonist) or gardiquimod (TLR7 agonist). A representative experiment of three independent experiments is shown.

8c: ^1H NMR (500 MHz, CDCl_3) δ 7.84 (d, J = 8.2 Hz, 2H), 7.66 (dd, J = 8.2, 1.0 Hz, 2H), 7.40–7.36 (m, 2H), 7.33–7.26 (m, 6H), 7.07–7.01 (m, 6H), 5.73 (s, 2H), 5.70 (s, 4H), 3.74 (dd, J = 12.7, 6.5 Hz, 4H), 2.88–2.83 (m, 4H), 1.80–1.72 (m, 8H), 1.53–1.24 (m, 16H), 0.91 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 151.76, 149.23, 143.97, 134.05, 131.32, 127.81, 127.63, 126.36, 125.85, 125.50, 125.10, 124.01, 123.84, 119.75, 117.95, 113.30, 47.23, 39.17, 28.62, 28.39, 28.06, 27.95, 25.65, 20.98, 12.20. MS (ESI) calculated for $\text{C}_{52}\text{H}_{62}\text{N}_8$, m/z 798.5097; found 799.5416 ($\text{M} + \text{H}$) $^+$ and 400.2799 ($\text{M} + 2\text{H}$) $^{2+}$.

8d: ^1H NMR (500 MHz, CDCl_3) δ 7.84 (d, J = 8.2 Hz, 2H), 7.65 (dd, J = 8.2, 1.0 Hz, 2H), 7.38 (ddd, J = 8.3, 7.1, 1.3 Hz, 2H), 7.34–7.24 (m, 6H), 7.06–7.00 (m, 6H), 5.74 (s, 2H), 5.69 (s, 4H), 3.74 (dd, J = 12.6, 6.5 Hz, 4H), 2.86 (dd, J = 17.4, 9.6 Hz, 4H), 1.83–1.69 (m, 8H), 1.56–1.30 (m, 20H), 0.91 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 151.81, 149.27, 144.02, 134.08, 131.36, 127.68, 126.41, 125.87, 125.53, 125.16, 124.05, 119.81, 118.00, 113.34, 47.27, 39.25, 28.65, 28.44, 28.15, 28.13, 28.01, 25.70, 25.68, 21.03, 12.24. MS (ESI) calculated for $\text{C}_{54}\text{H}_{66}\text{N}_8$, m/z 826.5410; found 827.5796 ($\text{M} + \text{H}$) $^+$ and 414.2977 ($\text{M} + 2\text{H}$) $^{2+}$.

Synthesis of Compound 10b: *N*¹,*N*⁸-Bis(4-((4-amino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)methyl)benzyl)octanediamide.

To a solution of **9** (25 mg, 0.058 mmol) in anhydrous THF were added triethylamine (15 mg, 0.15 mmol) and suberoyl chloride (6 mg, 0.029 mmol). The reaction mixture was stirred for 1 h, and then the solvent was removed under vacuum. The residue was then purified using column chromatography (30% MeOH/dichloromethane) to obtain the compound **10b** (8 mg, 32%). ^1H NMR (500 MHz, MeOD) δ 7.65 (dd, J = 8.3, 0.9 Hz, 2H), 7.55–7.51 (m, 2H), 7.27 (ddd, J = 8.3, 7.1, 1.2 Hz, 2H), 7.12 (d, J = 8.2 Hz, 4H), 6.95 (ddd, J = 8.2, 7.2, 1.1 Hz, 2H), 6.88 (d, J = 8.2 Hz, 4H), 5.69 (s, 4H), 4.18 (s, 4H), 2.84–2.78 (m, 4H), 2.03 (t, J = 7.5 Hz, 4H), 1.64 (dt, J = 15.4, 7.6 Hz, 4H), 1.47–1.37 (m, 4H), 1.30 (dq, J = 14.8, 7.4 Hz, 4H), 1.16–1.10 (m, 4H), 0.80 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 176.03, 156.18, 152.62, 144.89, 140.08, 136.09, 135.55, 129.44, 128.54, 126.95, 126.86, 126.21, 123.42, 121.58, 115.75, 49.58, 43.58, 36.85, 30.85, 29.75, 27.82, 26.74, 23.43, 14.11. MS (ESI) calculated for $\text{C}_{52}\text{H}_{60}\text{N}_{10}\text{O}_2$, m/z 856.4901; found 879.4711 ($\text{M} + \text{Na}$) $^+$ and 429.2430 ($\text{M} + 2\text{H}$) $^{2+}$.

Compounds 10a and 10c Were Synthesized Similarly As Described for Compound 10b. **10a:** ^1H NMR (400 MHz, MeOD)

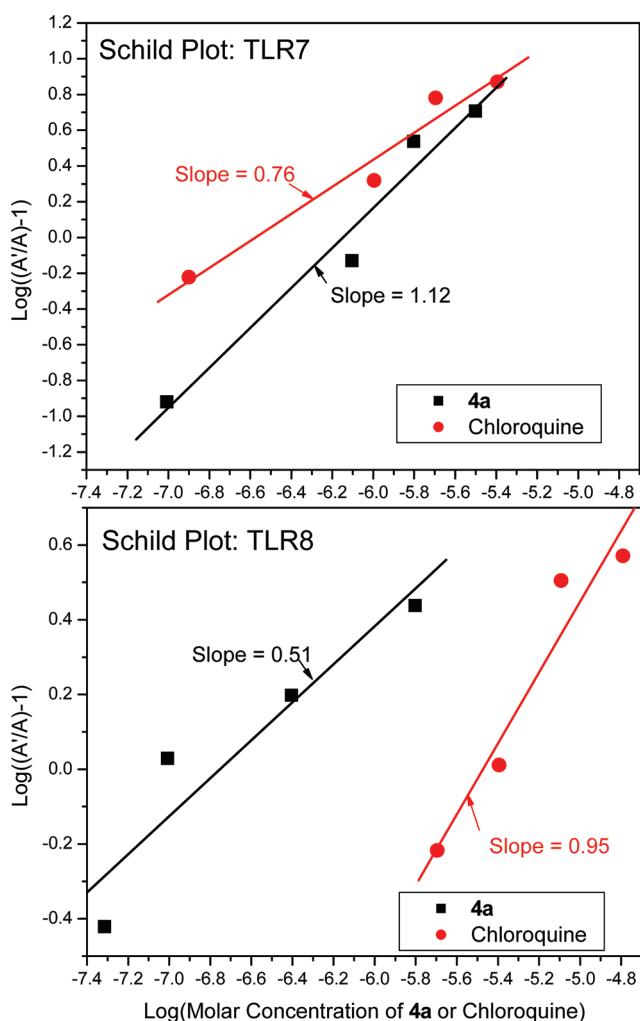


Figure 6. Schild plot analyses of inhibition of TLR7- and TLR8-induced activation. Experiments were performed in checkerboard format, using a liquid handler, in 384-well plates which permitted the concentrations of both agonist and antagonist to be varied simultaneously along the two axes of the plate. Either imidazoquinoline (TLR7-specific agonist) or CL075 (TLR8-specific agonist) was used at a starting concentration of 20 $\mu\text{g}/\text{mL}$ and were 2-fold diluted serially (along the rows). Next, **4a** or chloroquine were 2-fold diluted serially in HEK detection medium (along columns). Reporter cells were then added, incubated, and NF- κB activation measured as described in the text. A and A' (Y-axis) are defined respectively as the EC_{50} value in the absence of antagonist, and the EC_{50} values in the presence of varying concentrations of antagonist.

δ 7.85 (d, J = 7.6 Hz, 2H), 7.68 (d, J = 7.8 Hz, 2H), 7.52–7.45 (m, 2H), 7.26 (d, J = 8.2 Hz, 4H), 7.23–7.16 (m, 2H), 7.02 (d, J = 8.2 Hz, 4H), 5.85 (s, 4H), 4.30 (s, 4H), 2.99–2.93 (m, 4H), 2.19 (t, J = 6.0 Hz, 4H), 1.80 (dd, J = 15.3, 7.7 Hz, 4H), 1.58 (t, J = 3.1 Hz, 4H), 1.45 (dd, J = 15.0, 7.5 Hz, 4H), 0.94 (t, J = 7.4 Hz, 6H). ^{13}C NMR (101 MHz, MeOD) δ 174.28, 156.00, 150.28, 138.78, 135.01, 134.27, 128.13, 128.05, 125.44, 123.28, 121.97, 120.73, 113.67, 48.28, 42.15, 35.17, 29.21, 26.38, 25.03, 21.96, 12.68. MS (ESI) calculated for $\text{C}_{50}\text{H}_{56}\text{N}_{10}\text{O}_2$, m/z 828.4588; found 829.4440 ($\text{M} + \text{H}$) $^+$ and 415.2244 ($\text{M} + 2\text{H}$) $^{2+}$.

10c: ^1H NMR (500 MHz, MeOD) δ 7.69 (dd, J = 8.3, 0.8 Hz, 2H), 7.54 (dd, J = 8.4, 0.7 Hz, 2H), 7.31 (ddd, J = 8.4, 7.2, 1.2 Hz, 2H), 7.14 (d, J = 8.2 Hz, 4H), 7.00 (ddd, J = 8.2, 7.2, 1.1 Hz, 2H), 6.90 (d, J = 8.2 Hz, 4H), 5.73 (s, 4H), 4.20 (s, 4H), 2.85–2.81 (m, 4H), 2.06 (t, J = 7.4 Hz, 4H), 1.66 (dt, J = 15.4, 7.6 Hz, 4H), 1.44 (dt, J = 14.4, 7.3 Hz, 4H), 1.31 (dq, J = 14.8, 7.4 Hz, 4H), 1.10 (dd, J = 29.4, 26.2 Hz,

12H), 0.81 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 176.17, 156.64, 152.26, 143.34, 140.20, 135.93, 135.90, 129.44, 128.92, 126.85, 126.77, 125.13, 123.90, 121.81, 115.51, 49.64, 43.55, 36.99, 30.78, 30.36, 30.23, 30.11, 27.82, 26.96, 23.41, 14.11. MS (ESI) calculated for $\text{C}_{56}\text{H}_{68}\text{N}_{10}\text{O}_2$, m/z 912.5527; found 913.5886 ($\text{M} + \text{H}$) $^+$ and 457.2974 ($\text{M} + 2\text{H}$) $^{2+}$.

Synthesis of Compound 11: *tert*-Butyl 4-((4-Amino-2-butyl-8-nitro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)methyl)-benzylcarbamate. To a solution of **9** (500 mg, 1.16 mmol) in H_2SO_4 was added HNO_3 (95 mg, 1.511 mmol). The reaction mixture was stirred for 12 h, followed by neutralization of sulfuric acid by slow addition of sodium carbonate solution. EtOAc was added to this solution to extract the compound, followed by washing with water/brine. The EtOAc fraction was then dried using sodium sulfate and evaporated under vacuum to obtain the residue. The residue as dissolved in MeOH, and di-*tert*-butyl dicarbonate was added to it. The reaction was stirred for 30 min, followed by removal of the solvent under vacuum to obtain the residue, which was purified using column chromatography (7% MeOH/dichloromethane) to obtain the compound **11** (200 mg, 34%). ^1H NMR (400 MHz, CDCl_3) δ 8.67 (d, J = 2.5 Hz, 1H), 8.24–8.18 (m, 1H), 7.76 (d, J = 9.2 Hz, 1H), 7.28 (d, J = 7.0 Hz, 2H), 7.08 (d, J = 8.1 Hz, 2H), 5.95 (s, 2H), 5.76 (s, 2H), 4.87 (s, 1H), 4.29 (d, J = 5.5 Hz, 2H), 3.05–2.95 (m, 2H), 1.88 (dt, J = 15.5, 7.6 Hz, 2H), 1.51 (dd, J = 14.9, 7.3 Hz, 2H), 1.45 (s, 9H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 155.18, 153.33, 148.68, 141.64, 139.53, 133.91, 133.29, 128.41, 127.42, 127.25, 125.92, 121.27, 117.28, 113.88, 48.81, 44.09, 30.00, 28.35, 27.20, 22.54, 13.78. MS (ESI) calculated for $\text{C}_{27}\text{H}_{32}\text{N}_6\text{O}_4$, m/z 504.2485; found 505.2541 ($\text{M} + \text{H}$) $^+$.

Synthesis of Compound 11a: 1-(4-(Aminomethyl) benzyl)-2-butyl-8-nitro-1*H*-imidazo[4,5-*c*]quinolin-4-amine. Compound **11** (10 mg, 0.02 mmol) was dissolved in 1 mL solution of HCl/dioxane and stirred for 12 h. The solvent was then removed under vacuum, and the residue was washed with diethyl ether to afford the compound **11a** in quantitative yields. ^1H NMR (400 MHz, MeOD) δ 8.77 (d, J = 1.7 Hz, 1H), 8.47–8.41 (m, 1H), 7.98 (d, J = 9.1 Hz, 1H), 7.54 (d, J = 7.7 Hz, 2H), 7.30 (d, J = 7.7 Hz, 2H), 6.09 (s, 2H), 4.11 (s, 2H), 3.12 (t, J = 7.5 Hz, 2H), 1.98–1.88 (m, 2H), 1.59–1.49 (m, 2H), 1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 158.58, 150.14, 143.93, 137.46, 135.58, 135.36, 133.41, 129.86, 126.29, 125.89, 123.43, 119.40, 117.94, 112.44, 48.52, 42.36, 29.05, 26.44, 21.94, 12.71. MS (ESI) calculated for $\text{C}_{22}\text{H}_{24}\text{N}_6\text{O}_2$, m/z 404.1961; found 405.1993 ($\text{M} + \text{H}$) $^+$.

Synthesis of Compound 12: *tert*-Butyl 4-((4,8-Diamino-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)methyl)-benzylcarbamate. To a solution of **11** (190 mg, 0.377 mmol) in anhydrous MeOH were added a catalytic amount of Pd/C, and the reaction mixture was subjected to hydrogenation at 60 psi hydrogen pressure for 4 h. The reaction mixture was then filtered through Celite, and the filtrate was evaporated under vacuum to obtain the compound **12** (160 mg, 90%). ^1H NMR (400 MHz, MeOD) δ 7.49 (d, J = 8.8 Hz, 1H), 7.25 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 2.3 Hz, 1H), 7.02 (d, J = 8.1 Hz, 2H), 6.97 (dd, J = 8.9, 2.4 Hz, 1H), 5.75 (s, 2H), 4.19 (s, 2H), 2.92–2.86 (m, 2H), 1.73 (dt, J = 15.4, 7.6 Hz, 2H), 1.43 (s, 9H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 154.56, 148.78, 142.89, 139.42, 136.71, 134.64, 133.60, 127.53, 125.80, 125.57, 118.16, 115.16, 103.42, 78.81, 43.18, 29.41, 27.33, 26.41, 22.01, 12.67. MS (ESI) calculated for $\text{C}_{27}\text{H}_{34}\text{N}_6\text{O}_2$, m/z 474.2743; found 475.2733 ($\text{M} + \text{H}$) $^+$.

Synthesis of Compound 12a: 1-(4-(Aminomethyl)benzyl)-2-butyl-1*H*-imidazo[4,5-*c*]quinoline-4,8-diamine. Compound **12** (10 mg, 0.021 mmol) was dissolved in 1 mL of HCl/dioxane solution and stirred for 12 h. The solvent was then removed under vacuum, and the residue was washed with diethyl ether to obtain the compound **12a** in quantitative yields. ^1H NMR (500 MHz, MeOD) δ 8.10 (s, 1H), 7.97 (d, J = 8.9 Hz, 1H), 7.67 (dd, J = 8.9, 2.2 Hz, 1H), 7.51 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 8.1 Hz, 2H), 6.04 (s, 2H), 4.12 (s, 2H), 3.03 (t, J = 7.6 Hz, 2H), 1.92–1.84 (m, 2H), 1.53–1.43 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 156.84, 147.83, 135.56, 134.67, 133.58, 129.58, 129.54, 126.08, 125.63, 124.97, 119.52, 113.08,

48.08, 41.64, 29.09, 26.17, 21.75, 13.66. MS (ESI) calculated for $C_{22}H_{26}N_6$, m/z 374.2219; found 375.2508 ($M + H$)⁺.

Synthesis of Compound 13b: *N*¹,*N*⁸-Bis(4-amino-1-(4-(aminomethyl)benzyl)-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-8-yl)octanediamide. To a solution of 12 (74 mg, 0.156 mmol) in anhydrous THF were added triethylamine (39 mg, 0.39 mmol) and suberoyl chloride (15 mg, 0.07 mmol), and the reaction mixture was stirred for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (20% MeOH/dichloromethane) to obtain the bis-*N*-Boc protected compound, which was then dissolved in 1 mL of HCl/dioxane solution and stirred for 14 h. The solvent was then removed under vacuum, and the residue was washed with diethyl ether to afford the compound 13b (12 mg, 19%; low yields were due to partial acylation of the C4-NH₂ which was found to be unstable). ¹H NMR (500 MHz, MeOD) δ 8.62 (s, 2H), 7.70–7.65 (m, 2H), 7.61 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 7.9 Hz, 4H), 7.18 (d, J = 7.7 Hz, 4H), 5.93 (s, 4H), 4.07 (s, 4H), 2.98 (t, J = 7.5 Hz, 4H), 2.37 (dd, J = 16.6, 9.4 Hz, 4H), 1.85 (dt, J = 15.0, 7.6 Hz, 4H), 1.71 (s, 4H), 1.52–1.40 (m, 8H), 0.94 (t, J = 7.3 Hz, 6H). ¹³C NMR (126 MHz, MeOD) δ 174.75, 159.05, 149.75, 137.64, 137.34, 137.11, 134.51, 131.24, 130.93, 127.87, 126.22, 123.18, 119.97, 114.28, 111.99, 49.82, 43.82, 37.93, 30.27, 30.01, 27.81, 26.63, 23.36, 14.11. MS (ESI) calculated for $C_{52}H_{62}N_{12}O_2$, m/z 886.5119; found 909.5031 ($M + Na$)⁺ and 444.2632 ($M + 2H$)²⁺.

Compounds 13a and 13c Were Synthesized Similarly As Described for Compound 13b. 13a: ¹H NMR (500 MHz, MeOD) δ 8.57 (s, 2H), 7.62–7.54 (m, 4H), 7.35 (d, J = 8.1 Hz, 4H), 7.10 (d, J = 8.0 Hz, 4H), 5.85 (s, 4H), 3.98 (s, 4H), 2.90 (t, J = 7.6 Hz, 4H), 2.36 (s, 4H), 1.77 (dt, J = 15.2, 7.6 Hz, 4H), 1.73–1.61 (m, 4H), 1.45–1.30 (m, 4H), 0.86 (t, J = 7.4 Hz, 6H). ¹³C NMR (126 MHz, MeOD) δ 174.43, 159.11, 149.83, 137.66, 137.40, 137.16, 134.53, 131.33, 130.94, 127.89, 126.30, 123.18, 120.01, 114.38, 112.05, 49.82, 43.83, 37.65, 30.32, 27.81, 26.28, 23.37, 14.12. MS (ESI) calculated for $C_{50}H_{58}N_{12}O_2$, m/z 858.4806; found 859.4131 ($M + H$)⁺ and 430.2113 ($M + 2H$)²⁺.

13c: ¹H NMR (500 MHz, MeOD) δ 8.63 (s, 2H), 7.66 (d, J = 9.0 Hz, 2H), 7.58 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 7.9 Hz, 4H), 7.15 (d, J = 7.8 Hz, 4H), 5.91 (s, 4H), 4.04 (s, 4H), 2.96 (t, J = 7.5 Hz, 4H), 2.33 (t, J = 7.1 Hz, 4H), 1.83 (dt, J = 15.2, 7.7 Hz, 4H), 1.65 (s, 4H), 1.44 (dq, J = 14.7, 7.3 Hz, 4H), 1.38–1.21 (m, 12H), 0.92 (t, J = 7.3 Hz, 6H). ¹³C NMR (126 MHz, MeOD) δ 174.82, 159.01, 149.63, 137.68, 137.27, 137.10, 134.50, 131.24, 130.93, 129.84, 127.86, 126.00, 123.16, 120.01, 114.24, 111.92, 49.87, 43.82, 43.75, 38.04, 30.45, 30.34, 30.29, 30.23, 27.78, 26.78, 23.35, 14.11. MS (ESI) calculated for $C_{56}H_{70}N_{12}O_2$, m/z 942.5745; found 943.5746 ($M + H$)⁺ and 472.2987 ($M + 2H$)²⁺.

TLR3/7/8 Reporter Gene Assays (NF- κ B Induction). The induction of NF- κ B was quantified using HEK-Blue-3, HEK-Blue-7, and HEK-Blue-8 cells as previously described by us.^{5,7,11} HEK293 cells were stably transfected with human TLR3 (or human TLR7 or human TLR8), MD2, and secreted alkaline phosphatase (sAP), and were maintained in HEK-Blue selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF- κ B/AP-1 promoters is inducible by the TLR3 (or TLR7 or TLR8) agonists, and extracellular sAP in the supernatant is proportional to NF- κ B induction. HEK-Blue cells were incubated at a density of ~ 105 cells/mL in a volume of 80 μ L/well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently 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.

Antagonism assays were done as described by us earlier¹⁰ using the following agonists at a constant concentration: TLR3 Poly(I:C) (10 ng/mL); TLR7, gardiquimod (1 μ g/mL); TLR8, CL075 (1 μ g/mL) mixed with graded concentrations of the test compounds.

IFN- α Induction in Human PBMCs. Aliquots (10^6 cells in 100 μ L) of hPBMCs isolated from blood obtained from healthy human donors after informed consent by conventional Ficoll–Hypaque gradient centrifugation were stimulated for 12 h with graded

concentrations of test compounds. The supernatant was isolated by centrifugation, diluted 1:20, and IFN- α was assayed in triplicate using a high-sensitivity human IFN- α -specific ELISA kit (PBL Interferon Source, Piscataway, NJ).

Cytokine and Chemokine in Human PBMCs. Aliquots (10^6 cells in 100 μ L) of hPBMCs isolated from blood obtained from healthy human donors after informed consent by conventional Ficoll–Hypaque gradient centrifugation were stimulated for 12 h with graded concentrations of test compounds. The supernatant was isolated by centrifugation, diluted 1:20, and cytokines and chemokines were assayed in triplicate using analyte-specific cytokine/chemokine bead array assays as reported by us previously.⁴¹

■ ASSOCIATED CONTENT

Supporting Information

Characterization of intermediates and final compounds (¹H, ¹³C, mass spectra). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ABBREVIATIONS USED

DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; EC₅₀, half-maximal effective concentration; ELISA, enzyme linked immunosorbent assay; ESI-TOF, electrospray ionization-time-of-flight; HBTU, *O*-benzotriazole-*N,N,N',N'*-tetramethyl uronium hexafluorophosphate; HEK, human embryonic kidney; hPBMCs, human peripheral blood mononuclear cells; IFN, interferon; LPS, lipopolysaccharide; m-CPBA, *meta*-chloroperoxybenzoic acid; NF- κ B, nuclear factor-kappa B; PAMP, pathogen-associated molecular patterns; sAP, secreted alkaline phosphatase; ssRNA, single-stranded RNA; Th1, helper T-type 1; THF, tetrahydrofuran; TLR, Toll-like receptor

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