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Structure–Activity Relationship Analysis of Imidazoquinolines with Toll-like Receptors 7 and 8 Selectivity and Enhanced Cytokine Induction

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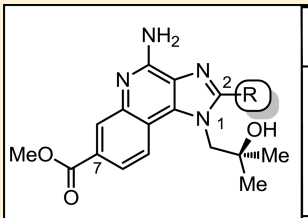

R	TLR-7 EC_{50} (μM)	TLR-8 EC_{50} (μM)	IL-1β (pg/mL)	IL-12 (pg/mL)
H	n/a[a]	n/a	6 ± 3	8 ± 2
methyl	49.2 ± 17.3	n/a	13 ± 1	73 ± 4
ethyl	29.1 ± 4.3	n/a	11 ± 4	78 ± 17
n-propyl	23.8 ± 4.3	n/a	33 ± 5	365 ± 38
n-butyl	1.5 ± 0.1	49.6 ± 1.0	108 ± 11	393 ± 39
n-pentyl	2.6 ± 0.2	7.2 ± 0.3	1006 ± 28	312 ± 27

[a]no activity

ABSTRACT: Toll-like receptors 7 and 8 (TLRs) have emerged as key targets in the design of small molecule adjuvants and stimulants for use in immunotherapies. This study examines the structure–activity relationship of a series of C2- and N1-substituted C7-methoxycarbonylimidazoquinolines to gain insight to the structural basis to TLR-7 and -8 selective activity. The analysis is further applied to evaluate the induction of multiple cytokines, including IL-10, IL-12, IL-1β, TNF-α, IFN-α, and IFN-γ, using murine BMDCs and human PBMCs. The results show TLR-7/8 activity is correlated to the C2-alkyl chain length, with peak activity occurring for the butyl (TLR-7) and pentyl (TLR-8) derivatives. A similar SAR is identified in the production of IL-1β, IL-12, and IFN-γ, which are shown to depend on both the C2-alkyl chain length and substitution to the N1-position. The compounds were also potent stimulators of IFN-α and IL-10 production but with less pronounced structure-based correlations.

INTRODUCTION

Toll-like receptors (TLRs) have emerged as key targets in the design and development of immunomodulating agents for use as vaccine adjuvants.[1,2] These receptors recognize pathogen associated molecular patterns and signal the release of proinflammatory cytokines and chemokines. Although at least 10 human TLRs are known to exist, TLR-7 and -8 have become popular targets for drug discovery, since they recognize small synthetic molecules.[3] Both receptors are located on the endosomal walls of monocytes, macrophages, and dendritic cells; TLR-7 and TLR-8 are also expressed on the endosomal walls of B cells and mast cells, respectively. In all cell types, both receptors respond to the presence of viral single stranded RNA (ssRNA) within the cytoplasm.[4] Their activation induces the NF-κB mediated transcription of cytokines and chemokines through a myeloid differentiation protein 88 (MyD88) dependent pathway.[5,6] The first FDA approved drug that took advantage of this signaling pathway was topical imiquimod (**1**), an imidazoquinoline analogue with potent activity in stimulating interferon production. Imiquimod first appeared in the patent literature in 1985 and was approved for the treatment of basal cell carcinoma in 1997.

Since that time, significant progress has been made in understanding the molecular basis of the function of these nucleoside analogues. In 2005, Gerster et al. reported the IFN-α inductive effects of a large collection of imidazoquinolines (Figure 1), including imiquimod.[7] A structure–activity relationship (SAR) analysis was presented that indicated that increased IFN-α production could be obtained by addition of short alkyl substituents to the C2 position of the imidazole ring. The second generation congener resiquimod (**2**) contains this design element and is an extremely potent, dual TLR-7/8 agonist. More detailed studies of receptor activation have shown that substitution to the C2 position may also have an impact on receptor selectivity. Shukla et al. reported an SAR study that evaluated the TLR-7 activity of a variety of C2-substituted imidazoquinolines, including the highly TLR-7 selective agonist gardiquimod (**3**).[8] A correlation between the C2-alkyl chain length and activity was reported that identified the butyl substitution to be optimal for activity. Heteroatom substitutions to the C2-alkyl chain, such as that found in

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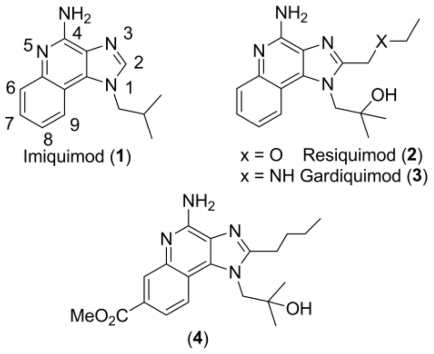


Figure 1. Imidazoquinolines.

gardiquimod, did not improve the activity, which is somewhat surprising given the potency of resiquimod at TLR-8. Resiquimod and gardiquimod differ by a single heteroatom substitution in the C2 alkyl chain. Although not elaborated upon, the data also show that potency may depend on the substituent on the N1 position with the most potent compound containing an N1-2-methyl-2-hydroxypropyl group identical to that of resiquimod.

A limiting factor to the design of SAR studies involving imidazoquinolines is the synthesis. The traditional route employs a modified Traube reaction to cyclize the imidazo ring system from a substituted *o*-diaminoquinoline. The approach is outlined in Figure 2 and requires the C4-amino

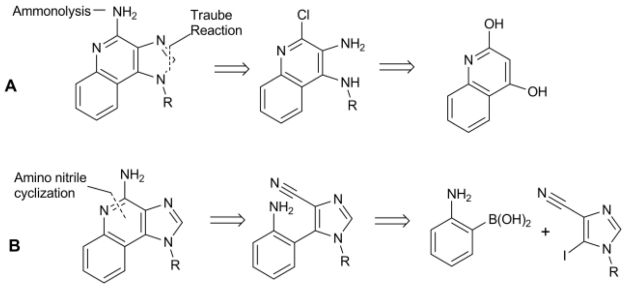


Figure 2. Retrosynthetic analysis showing the key differences in construction of the imidazoquinoline structure. The traditional route (A) is limited by the use of quinoline building blocks that must be functionalized early in the reaction sequence. This not only adds additional steps to the synthesis but limits the complexity of the substituents to those that can tolerate fairly harsh reaction conditions. The route reported by Shi et al. (B) applies a biaryl cross-coupling reaction to construct highly substituted biaryl precursors that are cyclized under mild conditions, allowing complex functionalities to be built into the imidazoquinoline structure.

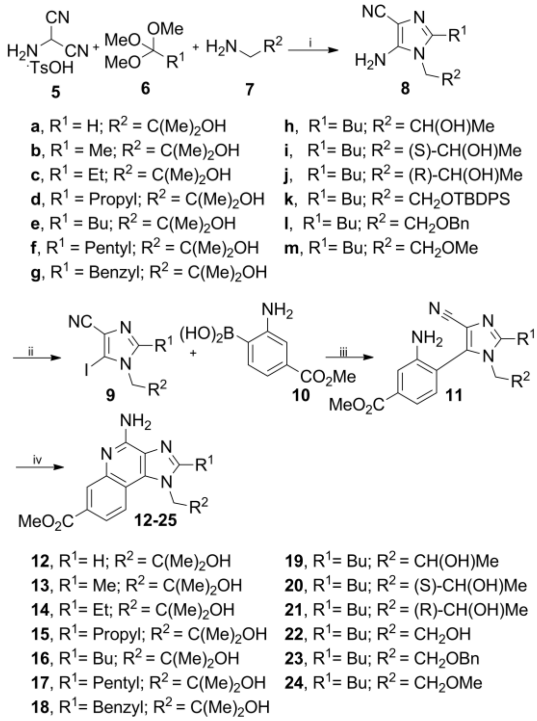
group to be installed as the last step in the sequence by either rearrangement of an N-oxide or ammonolysis.[7] While this scheme is amenable to modifications to the N1 and C2 positions, the reaction conditions limit substitutions to the C6−C9 aryl positions to end stage bromination or nitration. The quinoline precursor can also be modified, but once again, this approach is limited to those substitutents that can tolerate the reaction sequence. Gerster et al. examined the IFN inductive activity of a collection of C6−C9 analogues that were mainly composed of halo, nitro, hydroxy, methoxy, and methyl substitutions. Only the C6 hydroxy and C7 methyl, methoxy, and hydroxyl modifications retained activity. The SAR analysis presented, however, did not examine the effect of combining N1 or C2 substitutions with C6−C9 aryl modified analogues.

More recently, Shi et al reported an alternative strategy to obtain highly substituted imidazoquinolines in approximately four steps.[9] A retrosynthetic analysis of the methods is compared in Figure 2. The approach exploits a Suzuki coupling reaction to construct a substituted biaryl precursor that is readily cyclized to the final product under mild conditions. Shi et al. applied this scheme to functionalize the aryl ring to include a C7 methyl ester. The lead compound that emerged, **4**, was found to stimulate enhanced levels of cytokine production using bone marrow derived dendritic cells (BMDCs) as the model system.[9] Moreover, data were presented that directly linked the C2-alkyl chain substitution to increased TLR-7 and -8 function for this C7-acylated imidazoqinoline congener. In this report, we evaluate the TLR-7 and -8 activity and cytokine induction of a series of C2- and N1-substituted 7-methoxycarbonylimidazoquinolines. An SAR analysis is presented to gain insight to the structural features that confer TLR-7 and -8 binding and selectivity as well as the requirements for stimulating cytokine production.

RESULTS AND DISCUSSION

Chemistry. The synthetic route to obtaining highly substituted imidazoquinolines is shown in Scheme 1. The 4-

Scheme 1[a]



a, R^1 = H; R^2 = $C(Me)_2OH$
b, R^1 = Me; R^2 = $C(Me)_2OH$
c, R^1 = Et; R^2 = $C(Me)_2OH$
d, R^1 = Propyl; R^2 = $C(Me)_2OH$
e, R^1 = Bu; R^2 = $C(Me)_2OH$
f, R^1 = Pentyl; R^2 = $C(Me)_2OH$
g, R^1 = Benzyl; R^2 = $C(Me)_2OH$
h, R^1= Bu; R^2 = CH(OH)Me
i, R^1= Bu; R^2 = (S)-CH(OH)Me
j, R^1= Bu; R^2 = (R)-CH(OH)Me
k, R^1= Bu; R^2 = $CH_2OTBDPS$
l, R^1= Bu; R^2 = CH_2OBn
m, R^1= Bu; R^2 = CH_2OMe

12, R^1= H; R^2 = $C(Me)_2OH$
13, R^1= Me; R^2 = $C(Me)_2OH$
14, R^1= Et; R^2 = $C(Me)_2OH$
15, R^1= Propyl; R^2 = $C(Me)_2OH$
16, R^1= Bu; R^2 = $C(Me)_2OH$
17, R^1= Pentyl; R^2 = $C(Me)_2OH$
18, R^1= Benzyl; R^2 = $C(Me)_2OH$
19, R^1= Bu; R^2 = CH(OH)Me
20, R^1= Bu; R^2 = (S)-CH(OH)Me
21, R^1= Bu; R^2 = (R)-CH(OH)Me
22, R^1= Bu; R^2 = CH_2OH
23, R^1= Bu; R^2 = CH_2OBn
24, R^1= Bu; R^2 = CH_2OMe

[a]Reagents and conditions: (i) NEt_3, THF, reflux to rt, 15 h, 40− 88%; (ii) CH_2I_2, isopentyl nitrite, $CHCl_3$, 80 °C, 30 min, 30−90%; (iii) $Pd(PPh_3)_3Cl_2$, K_2CO_3, THF−H_2O; (iv) 4 N HCl in dioxane (excess), 100 °C, 20−70% yield over two steps.

cyano-5-iodoimidazole precursors (**8a−m**) are synthesized through the multicomponent condensation of aminomalononitrile (**5**), orthoester (**6**), and *β*-amino alcohol (**7**).[10,11] In general, the reaction proceeded in good yield and without complications. The exception to this was the synthesis of **8g**. Prior work has shown the benzyl orthoester to be remarkably stable to pyrolysis.[12] This problem was overcome by the

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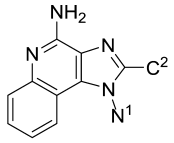
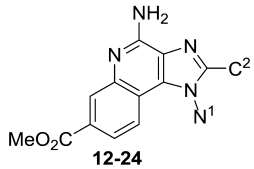
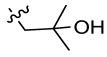
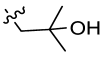
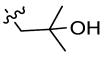
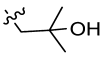
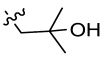
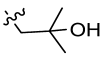
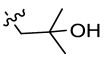
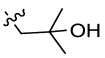
Table 1. TLR-7 and -8 Activities and Cytokine Induction of Substituted Imidazoquinolines[a,c]



1-2



12-24

			Cytokine (pg/mL)				EC_{50} (μM)[b]	
Compound	N^1	C^2	TNF[a]	IL–1β[a]	IL–12p40[a]	IL–10[a]	TLR–7	TLR–8
1 imiquimod		H	308 ± 8	19 ± 5	133 ± 14	17 ± 4	10.7 ± 1.6	n/a
2 resiquimod		CH_2OEt	1738 ± 42	16 ± 2	236 ± 13	49 ± 2	1.5 ± 0.3	4.5 ± 3.2
12		H	40 ± 3	6 ± 3	8 ± 2	9 ± 8	n/a	n/a
13		Methyl	819 ± 27	13 ± 1	73 ± 4	27 ± 6	49.2 ± 17.3	n/a
14		Ethyl	1174 ± 151	11 ± 4	78 ± 17	24 ± 4	29 .1 ± 4.3	n/a
15		Propyl	1171 ± 91	33 ± 5	365 ± 38	23 ± 4	23.8 ± 4.3	n/a
16		Butyl	1100 ± 58	108 ± 11	393 ± 39	24 ± 2	1.5 ± 0.07	49.6 ± 1
17		Pentyl	1810 ± 256	1006 ± 28	312 ± 27	37 ± 4	2.6 ± 0.2	7.2 ± 0.3
18		Benzyl	971 ± 21	60 ± 4	500 ± 36	15 ± 1	4.8 ± 0.5	n/a
19		Butyl	1201 ± 46	275 ± 32	533 ± 102	22 ± 3	3.3 ± 0.6	4.9 ± 0.3
20		Butyl	1193 ± 86	301 ± 35	597 ± 135	25 ± 3	2.9 ± 0.6	2.7 ± 0.3
21		Butyl	1160 ± 102	157 ± 42	416 ± 43	20 ± 2	4.4 ± 0.39	1.5 ± 0.2
22		Butyl	1206 ± 82	109 ± 5	290 ± 18	18 ± 4	23.0 ± 5.3	4.4 ± 0.5
23		Butyl	2235 ± 125	104 ± 17	136 ± 12	25 ± 2	2.42 ± 0.19	31.0 ± 10.6
24		Butyl	1370 ± 254	484 ± 162	267 ± 26	22 ± 3	5.7 ± 0.8	n/a
Control	-	-	38 ± 16	2 ± 2	261 ± 12	16.2 ± 4.7	n/a	n/a

[a]Multiplexed cytokine production was measured in triplicate using analogues at 30 μM. [b]HEK SEAP reporter assay. [c]n/a indicates no activation.

addition of another equivalent of the benzyl orthoester and extending the initial reaction time to 6 h. The conversion of **8a–m** to **9a–m** is accomplished using a modified Sandmeyer reaction employing isoamyl nitrite and diiodomethane. Lower yields (~30%) were noted when R2 contained a secondary alcohol which most likely acted as a proton source.[13] The highest yields (~90%) were obtained when R1 was unsubstituted. Suzuki–Miyaura coupling of **9a–m** with 2-amino-4-methoxycarbonylphenylboronic acid (**10**) provided intermediates **11a–m**, which are directly carried onto the next step without purification. It is interesting to note that these heterobiaryl intermediates are axially chiral because of the hindered rotation about the ortho substituted biaryl bond. The diasterotopic protons of the methylene unit of **11a** are readily identified in the ^{1}H NMR as a doublet of doublets with a geminal coupling constant of 14–15 Hz.[9] Cyclization of **11a–m** employing anhydrous HCl in dioxane affords imidazoquinolines **12–24** in 20–70% yield over two steps as white to yellow amorphous solids. The variable yields can be directly attributed to the efficiency of the biaryl coupling step. Although the variability in the Suzuki yields was not studied in any systematic matter, the results suggest that the efficiency may be related to solubility. The coupling was performed in a mixed solvent aqueous environment. More hydrophilic iodide **9a** proceeded in higher yield, 67%, while hydrophobic **9l** resulted in the lowest yield, 23%, of all the compounds. The cyclization was determined to occur in near quantitative yield.

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Biological Activity. The analogues (**12**–**25**) were screened for TLR-7 and -8 activity in vitro using HEK-293 cells that were transfected with either the human TLR-7 or -8 gene along with the NF-κB inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. This assay measures NF-κB mediated SEAP production spectrophotometrically following TLR-7 or -8 specific activation. An SAR analysis of the data given in Table 1 shows some general trends in TLR selective activity. TLR-7 is clearly more tolerant to substitution than TLR-8, and a general trend in increased activity is apparent when the C2 alkyl chain is lengthened from methyl to butyl. A slight decrease is noted in activity when the alkyl chain length reaches pentyl and beyond (to benzyl), suggesting that the butyl is optimal for TLR-7 activity. This general trend is followed for TLR-8 as well, albeit TLR-8 binding is less tolerant of substitution, with only the butyl and pentyl retaining activity. The failure of the C2-unsubstituted 7-ester analogue **12** to activate TLR-7 as well as TLR-8 is quite interesting and has been discussed in a previous publication.[9] From a comparison of the structure of **12** with that of imiquimod, the compounds differ by the addition of the C7-ester and the tertiary alcohol at N1. The C7 unsubstituted congener of **12**, however, selectively activates TLR-7, similar to that of imiquimod.[9] While the data are limited, the results indicate the C7 ester substitution decreases binding affinity at TLR-7 and -8 while the addition of C2-alkyl group restores activity.

A limited series of N1-modified analogues containing the C2-butyl group and C7-ester functionality are also reported in Table 1. The results indicate that the 2-hydroxypropyl stereoisomers (**19**–**21**) show the greatest potencies as mixed TLR-7/8 agonists, with EC_{50} values similar to that of resiquimod (which contains an N1-2-methy-2-hydroxypropyl group). A comparison of the racemate (**19**) and the *S* (**20**) and *R* (**21**) enantiomers reveals no significant differences in TLR-7 and -8 activity, suggesting that receptor binding is not enantioselective. The des-methyl primary alcohol congener (**22**), however, shows a significant reduction in TLR-7 function. Although the function can be restored by the addition of a methyl or benzyl ether, the loss of the hydroxyl group at the N1 position has a significant impact on TLR-8 activity. The data suggest that high affinity binding to TLR-8 may depend on the presence of a hydrogen bonding donor on the alkyl chain of N1. TLR-7 activity, on the other hand, correlates well with increased substitution to the N1-hydroxyalkyl side chain, including the addition hydrophobic groups (e.g., the benzyl ether).

The imidazoquinolines (**12**–**25**) were further screened for cytokine production in BMDCs using a flow cytometry reporter assay. Dendritic cells are antigen presenting cells (APCs) that play a crucial role in activating the immune response through the presentation of antigen to T cells (both CD4+ and CD8+), expression of ligands for co-stimulatory receptors on T cells, and release of stimulatory cytokines. It is important to point out, however, that the function of TLR-8 in triggering an NF-κB mediated response in the murine system is not fully understood. While the original reports by Hemmi et al. and Jurk et al. strongly suggested that the TLR-8 is not functional in recognizing small molecule agonists, more recent work has suggested that the receptor plays a role in regulating TLR-7 activation.[14−17] Nevertheless, murine BMDCs provide a direct measure of an immune cell specific response to TLR agonists and provide a well established model for assaying cytokine induction. Table 1 reports the production of four key cytokines including TNF-α, IL-1β, IL-12, and IL-10. IL-1β plays an important role in activating the adaptive immune system and in CD4+ T cell health and has been implicated in T cell memory.[18] In general, the results show that IL-1β production increases as a function of the C2-alkyl chain length and reaches a maximum for the pentyl derivative. The potency of the C2-butyl derivatives, however, is shown to improve when the N1 is modified to a 2-hydroxypropyl or methoxyethyl side chain. Similar results are observed for IL-12 production. This cytokine is produced by dendritic cells and leads to the differentiation of immature CD4+ T cells, the activation and memory formation of $CD8^+$ T cells, and the activation of NK cells.[19] Once again, the C7-ester analogues are the most potent with peak induction occurring for the *S*-configured enantiomer of the C2-2-hydroxypropyl analogue (**20**). A further evaluation of the related stereoisomers (**19**–**21**) shows only a slight preference for the *S*-isomer, indicating that the induction is not enantioselective (similar to the TLR-7/8 results). The activity is reduced, however, when the N1 position is substituted with the bulky benzoyloxyethyl moiety. IL-12 production also shows a correlation with C2-alkyl chain length, which reaches a maximum for the butyl substitution in this case.

The final two cytokines, TNF-α and IL-10, show much weaker correlations with the N1 and C2 substitutions. IL-10 is an anti-inflammatory cytokine that stimulates the humoral immune response including B cell maturation and antibody production.[20] High production of this cytokine, however, down-regulates the cytotoxic T-cell response to APCs, which can be detrimental to the efficacy of some types of immunotherapies (such as cancer vaccines). TNF-α, on the other hand, is a proinflammatory cytokine and potent immunostimulant.[21] While TNF-α is considered beneficial to many immunotherapies, high systemic levels can produce severe toxicity. The results indicate that IL-10 and TNF-α induction both fail to display the structure-based trends noted above for IL-1, IL-12, and TLR-7/8 activity. In fact, the most active IL-10 and TNF-α stimulants are resiquimod and the N1-benzyloxyethyl derivative (**23**), respectively, which are both weak stimulants of IL-1β and IL-12.

The compounds were also evaluated using human peripheral blood mononuclear cells (PBMCs). This assay captures the stimulation of cytokine production from multiple cell types, including dendritic cells, T cells, B cells, monocytes, and natural killer (NK) cells. The population of these cell types, however, varies greatly among donors, producing significant variance in the assay. Figure 3 shows the TNF-α, IFN-α, and IFN-γ induction derived from three human blood donors. The more active compounds identified using BMDCs are potent stimulants of PBMCs as well (e.g., the C2-pentyl (**17**) and the secondary alcohols **19**–**21**). The IFN-α levels show the least variance across the series, which is not surprising, since this cytokine is secreted by multiple cells types. IFN-α primarily functions in antiviral responses and activates a cohort of genes in producing an immune response. IFN-γ, on the other hand, is immune cell specific and is primarily secreted by NK and T cells. It functions by up-regulating antigen specific responses in the immune system (such as antigen presentation) and by activating macrophages. The IFN-γ data are more consistent with the structure-based trends established for IL-1β and IL-12 using BMDCs and once again identify the C2-butyl, C2-pentyl, and N1-2-hydroxypropyl derivatives as potent stimulants. The TNF-α values show similar structure-based trends with the exception of resiquimod, which is significantly more potent in

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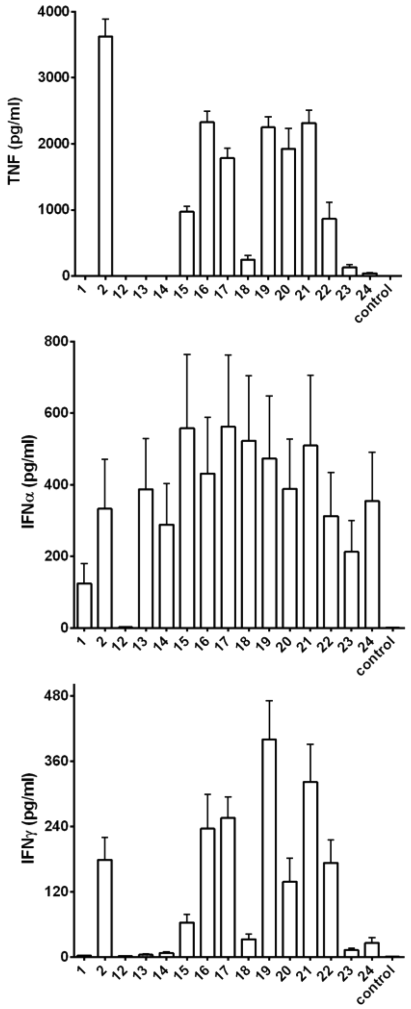

Figure 3. Human PBMC cytokine induction derived from three donors. Average values are reported.

producing TNF-α in PBMCs than the other compounds. Although resiquimod is also potent in producing TNF-α in BMDCs (as noted above), the trends observed in the remaining compounds (shown in Figure 3) are not as pronounced using the murine cell line. This may be due in part to differences in the function of murine and human TLR-7 and -8 receptors or to inherent differences in the response of TNF-α to immune modifying agents in the human and mouse.

CONCLUSION

An efficient synthesis for obtaining highly substituted imidazoquinolines has been described and applied to evaluate the SAR of a series of N1- and C2-modifed C7-methoxycarbonyl analogues with selective TLR-7 and -8 agonist activity and potent cytokine induction. The results show that TLR-7 and -8 activity directly correlates with C2-alkyl chain length, with peak activity occurring for the butyl (TLR-7) and pentyl (TLR-8) derivatives. Only the C2-butyl and -pentyl analogues are TLR-8 active, however, indicating that this receptor has much more stringent requirements on binding. TLR-8 activity is also sensitive to N1-modifications and dramatically decreases with benzylation or methylation of the hydroxyl group of the alkyl chain. This suggests that TLR-8 may be specific for a proton donating group at this position. Once again, TLR-7 activity is more tolerant of substitution at this position, suggesting that TLR-7 may be the "default" receptor for imidazoquinoline recognition. The idea that certain receptors within families contain additional selectivity elements is not new and is known to be important to G-protein-coupled receptor function and recognition (e.g., the μ, δ, and κ opioid receptors).[22] While this may simply mean that it is more difficult to selectively activate TLR-8, the finding may have larger ramifications to the function TLR-7 versus TLR-8 plays in mediating the innate immune response to viral RNA. The availability of crystallographic data for the TLRs will have a great impact on understanding the role of ligand binding in mediating receptor function.[23]

This study has also reported the production of several cytokines using both murine BMDCs and human PBMCs. The results indicate that the production of IL-12, IL-1β, and IFN-γ follows similar structural correlations to those reported for TLR-7 and -8 activation. Structural correlations are also noted in the enhanced production of individual cytokines (e.g., **17** and IL-1β and **19** and IFN-γ), suggesting it may be possible to design compounds with selectivity for particular cytokines. This is not a new idea. Using human PBMCs, Gorden et al. showed that TLR-8 agonists are more potent stimulators of proinflammatory cytokines, such as TNF-α, than TLR-7 agonists (which were more potent IFN-α stimulators).[24] Our PBMC data support this hypothesis and show a similar correlation between TLR-8 activity and TNF-α production. Moreover, this correlation is not apparent in the IFN-α values. None of the compounds reported here, however, are TLR-8 selective, so a more detailed analysis of this structure−function relationship is not possible. Nevertheless, the data reported here suggest that the addition of TLR-8 activity generally enhances the cytokine production of mixed agonists in both murine BMDCs and human PBMCs. Given the prior work on murine TLR-8 function,[14−17] the ability of murine BMDCs to register a TLR-8 dependent response in cytokine production is noteworthy. Although beyond the scope of this study, the results suggest that a more complete evaluation of the role TLR-7 and -8 play in mediating the production of cytokines in mouse models is warranted. This is especially true if mouse models continue to be used in the design and development of immune modifying agents and as models for evaluating immunotherapies.

EXPERIMENTALS

General Experimental Conditions. All solvents were used as received from commercial vendors, with the exception of THF, which was purified by passage through two alumina columns in a solvent purification system (J. C. Meyer). All chemicals were purchased from Sigma-Aldrich, TCI America, Combi-Blocks (boronic acid), and Strem or were synthesized using the cited literature protocol. Compounds **1**,[7] **2**,[7] **12**,[9] **16**,[9] 1-amino-2-methylpropan-2-ol,[25] 1,1,1-trimethoxyhexane,[26] (2,2,2-trimethoxyethyl)benzene,[27] 2-(*tert*-butyldiphenylsilanyloxy)ethylamine,[28] and 2-(phenylmethoxy)-ethanamine[29] were prepared according to the respective literature procedure. Purity (≥95%) of all final compounds was confirmed by reverse-phase HPLC using a mobile phase of 40% MeCN/60% 25 mM NH_4OAc buffer adjusted to pH 4−5. All reactions were conducted under an atmosphere of N_2 or Ar. Thin layer chromatography (TLC) was performed on 0.25 mm hard-layer silica G plates. Developed plates were visualized with a hand-held UV lamp or iodine chamber, 10% sulfuric acid or 10% PMA solution. ^1H and ^{13}C NMR spectra were recorded on a Varian 600 MHz or a Varian 400 MHz spectrometer in the noted solvent. Peaks are reported as chemical shift (multiplicity, J couplings in Hz, number of protons). High resolution mass spectra

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were obtained on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface.

General Procedure for the Synthesis of Imidazole 8. To a suspension of aminomalononitrile *p*-toluenesulfonate (4.0 mmol, 1.0 equiv) in 30 mL of THF at 25 °C was added NEt_3 (4.8 mmol, 1.2 equiv) in one portion. The mixture was stirred for 30 min, by which time the mixture turned into a homogeneous solution. To this solution was added orthoester **6** (4.8 mmol, 1.2 equiv), and the solution was heated at reflux for 3 h before being cooled to 25 °C. NEt_3 (4.8 mmol, 1.2 equiv) and primary amine **7** (4.8 mmol, 1.2 equiv) were added sequentially, and the mixture was stirred at 25 °C for 15 h. Solvent was removed in vacuo and the crude residue was redissolved in CH_2Cl_2 (100 mL) and washed with saturated Na_2CO_3 solution (25 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic fractions were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel.

General Procedure for the Synthesis of Imidazole 9. To a solution of **8** (2.5 mmol, 1 equiv) in CH_2I_2 (5 mL) at 80 °C was added a solution of isoamylnitrite (10.2 mmol, 4 equiv) in $CHCl_3$ (5 mL) over 20 min. The mixture was heated for an additional 30 min and allowed to cool to 25 °C. The mixture was concentrated in vacuo, and the crude residue was purified by silica gel column chromatography.

General Procedure for the Synthesis of Imidazoquinolines 13–24. $Pd(OAc)_2$ (0.05 mmol, 0.05 equiv) and PPh_3 (0.1 mmol, 0.1 equiv) were placed in a round-bottom flask and purged with argon for 10 min before DME (4 mL) was added. The resulting suspension was stirred at 25 °C for 5 min, and **9** (1.0 mmol, 1.0 equiv), 2-amino-4-methoxycarbonylphenylboronic acid hydrochloride salt (1.5 mmol, 1.5 equiv), and 1.5 M Na_2CO_3 aqueous solution (3 mmol, 3.0 equiv) were added sequentially. The mixture was heated to 100 °C under argon for 3 h. TLC and MS analysis indicated complete conversion. The mixture was then cooled to 25 °C and diluted with EtOAc (50 mL) and H_2O (10 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layer was washed with saturated NaCl aqueous solution (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting brown oil was loaded onto a short pad of silica gel, followed by flashing with CH_2Cl_2 (30 mL). The filtrate was concentrated in vacuo to afford the crude **11** which was used without further purification in the next step.

To a round-bottom flask containing **11** (0.5 mmol, 1.0 equiv) was added 4 N HCl in dioxane (8 mmol, 16 equiv). The mixture was heated at reflux for 4 h and then cooled to 25 °C. The mixture was concentrated in vacuo and partitioned between 10% MeOH in EtOAc (50 mL) and saturated $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with 10% MeOH in EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel.

5-Amino-1-(2-hydroxy-2-methylpropyl)-2-methyl-1*H*-imidazole-4-carbonitrile (8b). The title compound was prepared according to the general procedure using 1,1,1-trimethoxyethane and 1-amino-2-methylpropan-2-ol as an off white solid in 69% yield: R_f = 0.25 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD), 600 MHz) δ 3.78 (s, 2H), 2.27 (s, 3H), 1.24 (s, 6H); ^{13}C NMR (CD_3OD), 150 MHz) δ 150.9, 143.2, 117.7, 90.6, 72.8, 55.0, 27.5, 14.0. HRMS (ESI+): calcd $C_9H_{15}N_4O$ $[M + H]^+$ 195.1240, found 195.1237 (error 1.5 ppm).

5-Amino-2-ethyl-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazole-4-carbonitrile (8c). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypropane and 1-amino-2-methylpropan-2-ol as an off white solid in 75% yield: R_f = 0.30 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 400 MHz) δ 3.79 (s, 2H), 2.35 (q, J = 7.6 Hz, 2H), 1.27–1.23 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.9, 147.8, 117.8, 91.0, 72.8, 54.5, 27.5, 21.5, 11.9. HRMS (ESI+): calcd $C_{10}H_{17}N_4O$ $[M + H]^+$ 209.1397, found 209.1405 (error 3.8 ppm).

5-Amino-1-(2-hydroxy-2-methylpropyl)-2-propyl-1*H*-imidazole-4-carbonitrile (8d). The title compound was prepared according to the general procedure using 1,1,1-trimethoxybutane and 1-amino-2-methylpropan-2-ol and was carried on directly to the next step without further purification: R_f = 0.35 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 3.78 (s, 2H), 2.57 (t, J = 7.8 Hz, 2H), 1.47 (hex, J = 6.6 Hz, 2H), 1.23 (s, 6H), 0.97 (t, J = 7.8 Hz, 3H). LRMS (ESI+): calcd $C_{11}H_{19}N_4O$ $[M + H]^+$ 223.1, found 223.1.

5-Amino-1-(2-hydroxy-2-methylpropyl)-2-pentyl-1*H*-imidazole-4-carbonitrile (8f). The title compound was prepared according to the general procedure using 1,1,1-trimethoxyhexane and 1-amino-2-methylpropan-2-ol as an off white solid in 85% yield: R_f = 0.3 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 3.79 (s, 2H), 3.00 (t, J = 7.8 Hz, 2H), 1.69 (p, J = 7.2 Hz, 2H), 1.38–1.35 (m, 4H), 1.23 (s, 6H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 150 MHz) δ 150.8, 145.8, 117.8, 91.7, 72.8, 54.5, 32.7, 28.3, 27.6, 23.6, 14.5. HRMS (ESI+): calcd $C_{13}H_{23}N_4O$ $[M + H]^+$ 251.1872, found 251.1872 (error 0 ppm).

5-Amino-2-benzyl-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazole-4-carbonitrile (8g). The title compound was prepared according to the general procedure using (2,2,2-trimethoxyethyl)-benzene and 1-amino-2-methylpropan-2-ol as a light yellow solid in 65% yield: R_f = 0.35 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.31 (t, J = 7.2 Hz, 2H), 7.24 (t, J = 7.2 Hz, 1H), 7.12 (d, J = 7.2 Hz, 2H), 4.82 (br s, 2H), 3.59, (s, 2H), 3.39 (s, 2H), 2.52 (br s, 1H), 1.22 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 148.5, 142.5, 136.0, 128.9, 128.1, 127.2, 116.4, 92.4, 72.9, 53.2, 34.3, 27.7. HRMS (ESI+): calcd $C_{15}H_{19}N_4O$ $[M + H]^+$ 271.1559, found 271.1563 (error 1.5 ppm).

5-Amino-2-butyl-1-(2-hydroxypropyl)-1*H*-imidazole-4-carbonitrile (8h). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and 1-amino-2-methylpropanol as a beige solid in 68% yield: R_f = 0.8 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 4.45 (s, 2H), 4.23–4.14 (m, 1H), 3.78 (dd, J = 15.0, 2.54 Hz, 1H), 3.67 (dd, J = 15.0, 9.1 Hz, 1H), 3.45 (s, 1H), 2.50 (dt, J = 7.2, 2.7 Hz, 2H), 1.73–1.63 (m, 2H), 1.40 (hex, J = 7.4 Hz, 2H), 1.33 (d, J = 6.3 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.3, 143.3, 118.0, 88.4, 65.1, 49.2, 28.5, 25.9, 21.8, 20.9, 13.8. HRMS (APCI+): calcd $C_{11}H_{19}N_4O$ $[M + H]^+$ 223.1553, found 223.1557 (error 1.5 ppm).

(*S*)-5-Amino-2-butyl-1-(2-hydroxypropyl)-1*H*-imidazole-4-carbonitrile (8i). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and (*S*)-1-amino-2-methylpropanol as a beige solid in 80% yield. The analytical data matched those of racemic **8h**.

(*R*)-5-Amino-2-butyl-1-(2-hydroxypropyl)-1*H*-imidazole-4-carbonitrile (8j). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and (*R*)-1-amino-2-methylpropanol as a beige solid in 73% yield. The analytical data matched those of racemic **8h**.

5-Amino-2-butyl-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-1*H*-imidazole-4-carbonitrile (8k). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and 2-(*tert*-butyldiphenylsilanyloxy)ethylamine and was carried on directly to the next step.

5-Amino-1-(2-(benzyloxy)ethyl)-2-butyl-1*H*-imidazole-4-carbonitrile (8l). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and 2-(phenylmethoxy)ethanamine as a beige solid in 72% yield: R_f = 0.25 (20:80, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 7.39–7.29 (m, 3H), 7.24–7.19 (m, 2H), 4.51 (s, 2H), 4.38 (s, 2H), 3.95 (t, J = 4.7 Hz, 2H), 3.70 (t, J = 4.7 Hz, 2H), 2.49 (t, J = 7.6 Hz, 2H), 1.86 (p, J = 7.8 Hz, 2H), 1.37 (hex, J = 7.43 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 147.2, 143.7, 136.6, 128.7, 128.3, 127.7, 116.4, 93.1, 73.8, 69.7, 43.4, 29.2, 26.8, 22.4, 13.7. HRMS (APCI+): calcd $C_{17}H_{23}N_4O$ $[M + H]^+$ 299.1886, found 299.1874 (error 2.5 ppm).

5-Amino-2-butyl-1-(2-methoxyethyl)-1*H*-imidazole-4-carbonitrile (8m). The title compound was prepared according to the general procedure using 1,1,1-trimethoxypentane and 2-methoxyethanamine as a beige solid in 72% yield: R_f = 0.24 (80:20, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 4.41 (s, 2H), 3.93 (t, J = 4.8 Hz, 2H), 3.61 (t, J = 4.8 Hz, 2H), 3.37 (s, 3H), 2.53 (t, J = 7.7 Hz, 2H), 1.70 (p, J = 7.6 Hz, 2H), 1.41 (hex, J = 7.4 Hz, 2H), 0.95 (t, J = 7.43 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.4, 143.7, 116.4,

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93.1, 72.5, 59.3, 43.4, 29.3, 26.9, 22.4, 13.8. HRMS (APCI+): calcd $C_{11}H_{19}N_4O$ [M + H]$^+$ 223.1553, found 223.1582 (error 3.9 ppm).

1-(2-Hydroxy-2-methylpropyl)-5-iodo-2-methyl-1*H*-imidazole-4-carbonitrile (9b). The title compound was prepared according to the general procedure using **8b** as a yellow foam in 56% yield: R_f = 0.50 (50:50, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.94 (s, 2H), 2.57 (s, 3H), 1.33 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.7, 120.5, 114.9, 83.5, 71.8, 57.1, 28.4, 15.6. HRMS (APCI+): calcd $C_9H_{13}IN_3O$ [M + H]$^+$ 306.0098, found 306.0090 (error 2.6 ppm).

2-Ethyl-1-(2-hydroxy-2-methylpropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9c). The title compound was prepared according to the general procedure using **8c** as a yellow foam in 56% yield: R_f = 0.55 (50:50, EtOAc/hexanes); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.05 (s, 2H), 2.98 (q, J = 7.6 Hz, 2H), 1.25−1.30 (m, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 158.1, 121.2, 116.3, 87.7, 72.1, 58.3, 28.5, 23.0, 12.1. HRMS (APCI+): calcd $C_{10}H_{15}IN_3O$ [M + H]$^+$ 320.0254, found 320.0268 (error 4.4 ppm).

1-(2-Hydroxy-2-methylpropyl)-5-iodo-2-propyl-1*H*-imidazole-4-carbonitrile (9d). The title compound was prepared according to the general procedure using **8d** as a yellow foam in 56% yield: R_f = 0.55 (50:50, EtOAc/hexanes); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.85 (s, 2H), 2.93 (t, J = 7.6 Hz, 2H), 1.74 (hex, J = 7.6 Hz, 2H), 1.25 (s, 6H), 0.98 (t, J = 7.6 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 157.0, 121.2, 116.3, 87.6, 72.1, 58.3, 31.5, 28.5, 22.1, 14.2. HRMS (APCI+): calcd $C_{11}H_{17}IN_3O$ [M + H]$^+$ 334.0411, found 334.0408 (error 0.9 ppm).

1-(2-Hydroxy-2-methylpropyl)-5-iodo-2-pentyl-1*H*-imidazole-4-carbonitrile (9f). The title compound was prepared according to the general procedure using **8f** as a yellow foam in 66% yield: R_f = 0.45 (50:50, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.96 (s, 2H), 2.89 (t, J = 7.2 Hz, 2H), 2.00 (br s, 1H), 1.71 (p, J = 7.8 Hz, 2H), 1.31 (s, 6H), 1.20−1.35 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.4, 120.9, 115.1, 83.3, 71.7, 56.6, 31.4, 28.6, 28.4, 27.3, 22.3, 13.9. HRMS (ESI+): calcd $C_{13}H_{21}IN_3O$ [M + H]$^+$ 362.0729, found 362.0731 (error 0.5 ppm).

2-Benzyl-1-(2-hydroxy-2-methylpropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9g). The title compound was prepared according to the general procedure using **8g** as a light yellow foam in 70% yield: R_f = 0.55 (50:50, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.31 (t, J = 7.2 Hz, 2H), 7.26 (t, J = 6.6 Hz, 1H), 7.16 (d, J = 7.2 Hz, 2H), 4.46 (s, 2H), 3.84 (s, 2H), 1.62 (br s, 1H), 1.32 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 153.5, 135.9, 128.9, 128.6, 127.1, 114.9, 84.2, 72.1, 56.5, 35.0, 28.7. HRMS (ESI+): calcd $C_{15}H_{17}IN_3O$ [M + H]$^+$ 382.0416, found 382.0409 (error 1.8 ppm).

2-Butyl-1-(2-hydroxypropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9h). The title compound was prepared according to the general procedure using **8h** as an amber solid in 31% yield: R_f = 0.33 (50:50, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 4.24−4.14 (m, 1H), 3.94−3.82 (m, 2H), 2.88−2.72 (m, 2H), 2.19 (d, J = 3.7 Hz, 1H), 1.73 (p, J = 7.43 Hz, 2H), 1.41 (hex, J = 7.4 Hz, 2H), 1.35 (d, J = 6.2 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H); ^{13} C NMR (400 MHz, $CDCl_3$) δ 154.5, 120.6, 114.8, 81.6, 66.8, 54.4, 29.3, 27.7, 22.3, 21.0, 13.7. HRMS (APCI+): calcd $C_{11}H_{17}IN_3O$ [M + H]$^+$ 334.0411, found 334.0406 (error 1.45 ppm).

(*S*)-2-Butyl-1-(2-hydroxypropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9i). The title compound was prepared according to the general procedure using **8i** as an amber solid in 21% yield. The analytical data matched those of racemic **8h**.

(*R*)-2-Butyl-1-(2-hydroxypropyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9j). The title compound was prepared according to the general procedure using **8j** as an amber solid in 33% yield. The analytical data matched those of racemic **8h**.

2-Butyl-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-5-iodo-1*H*-imidazole-4-carbonitrile (9k). The title compound was prepared according to the general procedure using **8k** as a black solid in 21% yield over two steps. R_f = 0.48 (30:70, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50−7.35 (m, 10H), 4.09 (t, J = 5.4 Hz, 2H), 3.85 (t, J = 5.4 Hz, 2H), 2.81−2.74 (m, 2H), 1.70 (p, J = 7.4 Hz, 2H), 1.38 (hex, J = 7.43 Hz, 2H), 1.01 (s, 9H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.1, 135.4, 132.1, 130.1, 128.0, 120.9, 115.0, 81.2, 62.0, 49.0, 29.1, 27.7, 26.7, 22.4, 19.0, 13.8. HRMS (APCI+): calcd $C_{26}H_{33}ISiN_3O$ [M + H]$^+$ 558.1432, found 558.1432 (error 0.02 ppm).

1-(2-(Benzyloxy)ethyl)-2-butyl-5-iodo-1*H*-imidazole-4-carbonitrile (9l). The title compound was prepared according to the general procedure using **8l** as a black solid in 30% yield: R_f = 0.35 (30:70, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.12 (m, 5H), 4.46 (t, 2H), 4.13 (t, J = 5.2 Hz, 2H), 3.68 (t, J = 5.2 Hz, 2H), 2.78 (t, J = 7.6 Hz, 2H), 1.72 (p, J = 7.8 Hz, 2H), 1.37 (hex, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.3, 140.0, 128.5, 128.1, 127.6, 120.9, 114.9, 80.9, 73.4, 67.7, 47.5, 29.2, 27.5, 22.3, 13.7. HRMS (APCI+): calcd $C_{17}H_{21}IN_3O$ [M + H]$^+$ 410.0724, found 410.0723 (error 0.2 ppm).

2-Butyl-5-iodo-1-(2-methoxyethyl)-1*H*-imidazole-4-carbonitrile (9m). The title compound was prepared according to the general procedure using **8m** as an amber solid in 29% yield (two steps): R_f = 0.52 (50:50, EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.12 (t, J = 5.3 Hz, 2H), 3.60 (t, J = 5.3 Hz, 2H), 3.31 (s, 3H), 2.79 (t, J = 2.8 Hz, 2H), 1.74 (p, J = 7.6 Hz, 2H), 1.42 (hex, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.3, 120.9, 114.9, 81.0, 70.7, 59.2, 47.5, 29.2, 27.5, 22.3, 13.8. HRMS (APCI+): calcd $C_{11}H_{17}IN_3O$ [M + H]$^+$ 334.0411, found 334.0416 (error 1.6 ppm).

4-Amino-1-(2-hydroxyl-2-methylpropyl)-7-methoxycarbonyl-2-methyl-1*H*-imidazo[4,5-*c*]quinoline (13). The title compound was prepared according to the general procedure using **9b** as a off white solid in 75% yield over two steps: R_f = 0.50 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.37 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.8, 2.0 Hz, 1H), 4.65 (br s, 2H), 3.96 (s, 3H), 2.80 (s, 3H), 1.30 (br s, 6H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 166.6, 152.8, 152.4, 144.2, 133.1, 127.5, 127.4, 126.6, 121.6, 119.7, 118.7, 70.9, 55.0, 52.0, 27.6, 14.7. HRMS (ESI+): calcd $C_{17}H_{21}N_4O_3$ [M + H]$^+$ 329.1608, found 329.1602 (error 1.8 ppm).

4-Amino-2-ethyl-1-(2-hydroxyl-2-methylpropyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (14). The title compound was prepared according to the general procedure using **9c** as a white solid in 40% yield over two steps: R_f = 0.50 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 8.38 (d, J = 9.0 Hz, 1H), 8.15 (d, J = 1.8 Hz, 1H), 7.70 (dd, J = 9.0, 1.8 Hz, 1H), 6.62 (br s, 2H), 4.79 (br s, 2H), 3.88 (s, 3H), 3.05 (q, J = 7.8 Hz, 2H), 1.35 (t, J = 7.8 Hz, 3H), 1.23 (br s, 6H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 166.6, 157.2, 152.4, 144.2, 133.1, 121.7, 119.8, 118.8, 70.8, 54.6, 52.1, 27.0, 20.6, 12.1. HRMS (ESI+): calcd $C_{18}H_{23}N_4O_3$ [M + H]$^+$ 343.1765, found 343.1764 (error 0.3 ppm).

4-Amino-1-(2-hydroxyl-2-methylpropyl)-7-methoxycarbonyl-2-propyl-1*H*-imidazo[4,5-*c*]quinoline (15). The title compound was prepared according to the general procedure using **9d** as a white solid in 34% yield over two steps: R_f = 0.50 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 8.34 (d, J = 8.4 Hz, 1H), 8.32 (s, 1H), 7.84 (d, J = 8.4 Hz, 1H), 4.59 (br s, 2H), 3.95 (s, 3H), 3.10 (t, J = 7.8 Hz, 2H), 1.94 (hex, J = 7.8 Hz, 2H), 1.28 (br s, 6H), 1.09 (t, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.9, 158.7, 153.7, 145.1, 135.6, 129.5, 128.7, 128.5, 122,9, 122.6, 120.1, 72.7, 56.4, 52.9, 30.9, 28.0, 22.4, 14.4. HRMS (ESI+): calcd $C_{19}H_{25}N_4O_3$ [M + H]$^+$ 357.1921, found 357.1908 (error 3.6 ppm).

4-Amino-1-(2-hydroxyl-2-methylpropyl)-7-methoxy carbonyl-2-pentyl-1*H*-imidazo[4,5-*c*]quinoline (17). The title compound was prepared according to the general procedure using **9f** as a white solid in 42% yield over two steps: R_f = 0.45 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (DMF-d_7, 600 MHz) δ 8.53 (d, J = 9.0 Hz, 1H), 8.31 (d, J = 1.8 Hz, 1H), 7.78 (dd, J = 8.4, 1.8 Hz, 1H), 6.69 (br s, 2H), 5.01 (s, 1H), 4.67 (br s, 2H), 3.96 (s, 3H), 3.14 (t, J = 7.8 Hz, 2H), 1.91 (p, J = 7.2 Hz, 2H), 1.42−1.46 (m, 4H), 1.31 (br s, 6H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (DMF-d_7, 150 MHz) δ 167.8, 157.7, 153.6, 145.5, 134.4, 129.0, 128.8, 128.0, 122.8, 121.0, 120.1, 72.0, 55.8, 52.6, 32.4, 28.4, 28.3, 28.2, 23.2, 14.5. HRMS (ESI+): calcd $C_{21}H_{29}N_4O_3$ [M+H]$^+$ 385.2234, found 385.2234 (error 0 ppm).

4-Amino-2-benzyl-1-(2-hydroxyl-2-methylpropyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (18). The title compound was prepared according to the general procedure using **9g** as an off white solid in 45% yield over two steps: R_f = 0.50 (10:90, MeOH/

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CH_2Cl_2); ^{1}H NMR (DMF-d_7, 600 MHz) δ 8.49 (d, J = 9.0 Hz, 1H), 8.30 (s, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.33−7.35 (m, 4H), 7.25−7.27 (m, 1H), 6.78 (br s, 2H), 5.28 (br s, 1H), 4.65 (br s, 4H), 3.95 (s, 3H), 1.33 (br s, 6H); ^{13}C NMR (DMF-d_7, 150 MHz) δ 167.8, 155.8, 153.8, 145.8, 138.7, 134.7, 129.7, 129.5, 129.2, 129.1, 128.3, 127.5, 122.7, 121.1, 120.1, 72.1, 56.2, 52.6, 34.9, 28.1. HRMS (ESI+): calcd $C_{23}H_{25}N_4O_3$ [M + H]$^+$ 405.1927, found 405.1935 (error 2.0 ppm).

(±)-4-Amino-2-butyl-1-(2-hydroxypropyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (19). The title compound was prepared according to the general procedure using **9h** as a white solid in 51% yield over two steps: R_f = 0.4 (10:90, MeOH/EtOAc); ^{1}H NMR (DMF-d_7, 400 MHz) δ 8.31 (d, J = 1.61 Hz, 1H), 8.24 (d, J = 8.7 Hz, 1H), 7.80 (dd, J = 8.5, 1.84 Hz, 1H), 6.68 (s, 2H), 5.29 (s, 1H), 4.70 (dd, J = 15.2, 3.22 Hz, 1H), 4.46 (dd, J = 14.94, 9.49 Hz, 1H), 4.32−4.21 (m, 1H), 3.95 (s, 3H), 3.12−3.02 (m, 2H), 1.90 (p, J = 7.8 Hz, 2H), 1.52 (hex, J = 7.3 Hz, 2H), 1.39 (d, J = 6.2 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR (DMF-d_7, 100 MHz) δ 167.1, 156.1, 153.1, 144.5, 132.8, 128.4, 128.3, 127.6, 121.2, 121.1, 119.0, 66.3, 52.8, 52.0, 29.7, 27.3, 22.6, 20.8, 13.8. HRMS (APCI+): calcd $C_{19}H_{25}N_4O_3$ [M + H]$^+$ 357.1921, found 357.1926 (error 1.4 ppm).

(*S*)-4-Amino-2-butyl-1-(2-hydroxypropyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (20). The title compound was prepared according to the general procedure using **9i** as a white solid in 64% yield over two steps. The analytical data matched those of racemic **19**. The absolute stereochemistry was confirmed using Mosher ester analysis following the Hoye protocol.[30] The use of (*R*)-MTPA resulted in a ^{19}F NMR peak at −74.5.

(*R*)-4-Amino-2-butyl-1-(2-hydroxypropyl)-7-methoxycarbonyl-1*H*-imidazo [4,5-*c*]quinoline (21). The title compound was prepared according to the general procedure using **9j** as a white solid in 50% yield over two steps. The analytical data matched those of racemic **19**. The absolute stereochemistry was confirmed using Mosher ester analysis following the Hoye protocol.[29] The use of (*R*)-MTPA resulted in a ^{19}F NMR peak at −74.2.

4-Amino-2-butyl-1-(2-hydroxyethyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (22). The title compound was prepared according to the general procedure using **9k** as a white solid in 23% yield over two steps: R_f = 0.2 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR (DMF-d_7, 400 MHz) δ 8.37−8.27 (m, 2H), 7.82 (dd, J = 8.61, 1.76 Hz, 1H), 6.95 (s, 2H), 5.28 (t, J = 5.2 Hz, 1H), 4.77 (t, J = 5.2 Hz, 2H), 4.10−4.05 (m, 2H), 3.96 (s, 3H), 3.07 (t, J = 7.8 Hz, 2H), 1.90 (p, J = 7.63 Hz, 2H), 1.52 (hex, J = 7.6 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); ^{13}C NMR (DMF-d_7, 100 MHz) δ 167.8, 157.0, 153.7, 133.7, 129.2, 128.6, 122.1, 122.0, 119.5, 61.6, 52.8, 48.9, 27.9, 23.4, 14.5 (peak missing at ∼144). HRMS (APCI+): calcd $C_{18}H_{23}N_4O_3$ [M + H]$^+$ 343.1765, found 343.1754 (error 3.1 ppm).

4-Amino-1-(2-(benzyloxy)ethyl)-2-butyl-7-methoxycarbonyl 1*H*-imidazo[4,5-*c*]quinoline (23). The title compound was prepared according to the general procedure using **9l** as a white solid in 27% yield over two steps: R_f = 0.50 (10:90, MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 8.53 (s, 1H), 7.95−7.85 (m, 2H), 7.26−7.09 (m, 5H), 5.48 (s, 2H), 4.70 (t, J = 5.6 Hz, 2H), 4.49 (s, 2H), 3.96 (s, 3H), 3.94 (t, J = 5.6 Hz, 2H), 2.97 (t, J = 7.8 Hz, 2H), 1.86 (p, J = 7.83 Hz, 2H), 1.49 (hex, J = 7.43 Hz, 2H), 0.98 (t, J = 7.43 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.3, 155.4, 151.6, 144.0, 137.0, 132.7, 129.3, 128.4, 127.9, 127.6, 122.1, 119.3, 118.5, 73.5, 67.9, 52.2, 45.6, 29.9, 27.2, 22.6, 13.8. HRMS (APCI+): calcd $C_{25}H_{29}N_4O_3$ [M + H]$^+$ 433.2234, found 433.2235 (error 0.14 ppm).

4-Amino-2-butyl-1-(2-methoxyethyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-*c*]quinoline (24). The title compound was prepared according to the general procedure using **9m** as a white solid in 43% yield over two steps: R_f = 0.3 (10:90, MeOH/EtOAc); ^{1}H NMR (DMF-d_7, 400 MHz) δ 8.31 (d, J = 1.76 Hz, 1H), 8.27 (d, J = 8.61 Hz, 1H), 7.80 (dd, J = 8.6, 1.7 Hz, 1H), 6.69 (s, 2H), 4.86 (t, J = 5.2 Hz, 2H), 3.96 (s, 3H), 3.91 (t, J = 5.2 Hz, 2H), 3.26 (s, 3H), 3.03 (t, J = 7.6 Hz, 2H), 1.89 (p, J = 7.6 Hz, 2H), 1.52 (hex, J = 7.6 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); ^{13}C NMR (DMF-d_7, 100 MHz) δ 168.0, 156.5, 154.0, 145.8, 133.4, 129.5, 129.4, 128.5, 121.7, 119.7, 72.2, 59.5, 52.8, 46.4, 27.7, 23.4, 14.5. HRMS (APCI+): calcd $C_{19}H_{25}N_4O_3$ [M + H]$^+$ 357.1921, found 357.1920 (error 0.3 ppm).

TLR7/8-NF-κB Reporter Assay. Human embryonic kidney (HEK) cells that were stably transfected with human TLR-7 or TLR-8 and an NF-κB-responsive secreted embryonic alkaline phosphatase (SEAP) gene (HEK-TLR-7 and -8) were purchased from InvivoGen (San Diego, CA). The procedure used to measure TLR7 or TLR8 agonist activity was conducted as described by Hood et al.[1] HEK-TLR7/8 cells were stimulated with 30 μM compound in a 96-well plate in DMEM containing 10% FBS and 0.01% Normocin (InvivoGen) for 24 h. Then 20 μL of the supernatant from each well was incubated with Quanti-blue substrate solution (InvivoGen) at 37 °C for 1 h and absorbance was read at 650 nm using a Synergy plate reader (Biotek, Winooski, VT).

Measurement of Proimflammatory Cytokines. Bone marrow derived dendritic cells (BMDC) were generated by isolating a single cell suspension of marrow from the femur of C57BL/6 mice (6−8 weeks of age). Red blood cells were lysed with 0.83% NH_4Cl, 0.1% $KHCO_3$, and 0.009% EDTA. Five million cells were seeded in each of well of a six-well plate in complete RPMI medium (Invitrogen,Grand Island, NY) and supplemented with mouse 20 ng/mL granulocyte-macrophage colony stimulating factor (PeproTech, Rocky Hill, NJ). Six days after culture, BMDCs were stimulated with 30 μM compound for 3 days. An amount of 25 μL of supernatant was then removed and assayed for TNF-α, IL-12p40, IL-1β, and IL-10 using a flow cytometric bead array according to the manufacturers' instructions (BD Bioscience, San Jose,CA). Peripheral blood mononuclear cells (PBMCs) were isolated from three independent blood donor samples using the Ficoll method and plated in triplicate at a concentration of 5 × 10^5 cells in a 24-well plate. The cells were stimulated at a compound concentration of 22.5 μM and incubated for 24 h. An amount of 50 μL of medium was isolated and analyzed for TNF-α and IFN-γ by bead array (as described above) or IFN-α by ELISA (eBioscience, San Diego, CA). Flow cytometry was performed on a FACSCanto II (BD Bioscience), and data were analyzed using Flowjo software (Tree Star, Inc., Ashland, OR).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; NF-κB, nuclear factor κB; MyD88, myeloid differentiation protein 88; PBMC, peripheral blood mononuclear cell; BMDC, bone marrow derived dendritic cell; TLR, Toll-like receptor; CD8, cluster of differentiation 8; CD4, cluster of differentiation 4; APC, antigen presenting cell

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