Structure−Activity Relationships in Human Toll-Like Receptor 7-Active Imidazoquinoline Analogues

Nikunj M. Shukla, Subbalakshmi S. Malladi, Cole A. Mutz, Rajalakshmi Balakrishna, and Sunil A. David*

Department of Medicinal Chemistry, University of Kansas, Multidisciplinary Research Building, Room 320D, 2030 Becker Drive, Lawrence Kansas 66047

Engagement of toll-like receptors serve to link innate immune responses with adaptive immunity and can be exploited as powerful vaccine adjuvants for eliciting both primary and anamnestic immune responses. TLR7 agonists are highly immunostimulatory without inducing dominant proinflammatory cytokine responses. A structure−activity study was conducted on the TLR7-agonistic imidazoquinolines, starting with 1-(4-amino-2-((ethylamino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-2-methylpropan-2-ol as a lead. Modifications of the secondary amine of the C2 ethylaminomethylene side chain are poorly tolerated. The 4-amino group must be retained for activity. Replacement of the imidazole ring of the scaffold with triazole or cyclic urea led to complete loss of activity. A systematic exploration of N^1-benzyl-C2-alkyl substituents showed a very distinct relationship between alkyl length and TLR7-agonistic potency with the optimal compound bearing a C2-*n*-butyl group. Transposition of the N^1 and C2 substituents led to the identification of an extremely active TLR7-agonistic compound with an EC_{50} value of 8.6 nM. The relative potencies in human TLR7-based primary reporter gene assays were paralleled by interferon-α induction activities in whole human blood models.

Introduction

Toll-like receptors (TLRs[a]) 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 (TLR11).[3,4] TLR1, -2, -4, -5, and -6 respond to extracellular stimuli, while TLR3, -7, -8, and -9 respond to intracytoplasmic PAMPs, being associated with the endolysosomal compartment.[2]

The activation of TLRs by their cognate ligands leads to activation of innate immune effector mechanisms, including the production of pro-inflammatory cytokines and up-regulation of MHC molecules and costimulatory signals in antigen-presenting cells as well as activating natural killer (NK) cells. The consequence of activation of the innate immune system mobilizes and amplifies specific adaptive immune responses involving both T- and B-cell effector functions.[5−7] Thus, TLR stimuli serve to link innate and adaptive immunity[5] and can therefore be exploited as powerful adjuvants in eliciting both primary and anamnestic immune responses.

We have recently examined representative members of virtually the entire compendium of known TLR agonists in a series of hierarchical assays including primary TLR-reporter assays, secondary indices of immune activation such as cytokine induction and activation of lymphocytic subsets in whole human blood, and tertiary screens characterizing transcriptomal activation patterns with a view to identifying optimal immunostimulatory chemotypes.[8] Of all the innate immune stimuli examined, we found that TLR7 agonists were extraordinarily immunostimulatory, stimulating virtually all subsets of lymphocytes, and yet without inducing dominant proinflammatory cytokine responses (unlike TLR4, -5 or -8 agonists, which were proinflammatory and therefore may exert systemic toxicity).[8] We therefore became especially desirous of evaluating TLR7-active compounds as potential vaccine adjuvants.

Long before endosomal TLR7 was discovered to serve as the primary sensor for short, single-stranded, GU-rich RNA sequences (ssRNA), mainly of viral origin,[9−11] a number of small molecules were synthesized and evaluated in the 1970s and 1980s for antiviral activities owing to their pronounced type I interferon (IFN-α and -β) inducing properties.[12−16] Although the mechanisms of innate immune stimulation of several of these compounds (such as tilorone[14] and bromopirone[16]) remain yet to be formally elucidated, members of the

---

*To whom correspondence should be addressed. Phone: 785-864-1610. Fax: 785-864-1961. E-mail: sdavid@ku.edu.

[a] Abbreviations: AP-1, activator protein-1; CD, cluster of differentiation; DC, dendritic cells; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; EC_{50}, half-maximal effective concentration; EDCI·HCl, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; ELISA, enzyme linked immunosorbent assay; ESI-TOF, electrospray ionization-time-of-flight; HATU, 2-(1*H*-7-azabenzo-triazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; HBTU, *O*-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl uronium hexafluorophosphate; HEK, human embryonic kidney; IFN, interferon; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NF-*κ*B, nuclear factor-kappa B; NK, natural killer; PAMP, pathogen-associated molecular patterns; sAP, secreted alkaline phosphatase; SAR, structure−activity relationship; ssRNA-single stranded RNA; Th1, helper T−type 1; THF, tetrahydrofuran; TLR, Toll-like receptor.

1*H*-imidazo[4,5-*c*]quinolines were found to be good Type I IFN inducers in human cell-derived assays,[17] and FDA approval was obtained in 1997 for Imiquimod (**1**) for the treatment of basal cell carcinoma and actinic keratosis.[18] It was not until 2002, however, that the mechanistic basis of IFN induction by the imidazoquinolines was found to be a consequence of TLR7 engagement and activation.[19] Other than the original landmark studies performed by investigators at 3M Pharmaceuticals,[17] structure−activity relationships of the imidazoquinoline chemotype remains poorly explored, perhaps attributable in part to recent interest in the 8-hydroxyadenine compounds as alternate TLR7-agonists,[20−23] which appear to lack emetic side effects observed in ferrets upon oral administration.[22]

An aspect of our recent work in this area[8,24,25] focuses on developing adjuvants for transcutaneous vaccines (needle-free vaccine patches).[26−28] Given that Imiquimod is already approved for topical use, it was of particular interest not only to explore chemical space around the imidazoquinolines but also to carefully "immunophenotype" such compounds in human TLR7-based assays to verify that they did not have any TLR8-driven proinflammatory properties. One of our goals was also to learn from such structure−activity studies optimal positions on the scaffold that would tolerate the introduction of electrophilic or photoactivable labels for purposes of developing self-adjuvanting vaccine constructs[29,30] by covalently modifying protein antigens. We report here the synthesis and evaluation of a focused library of variously substituted 1*H*-imidazo[4,5-*c*]quinolines, and the identification of a novel TLR7 agonist whose potency is 250 times that of Imiquimod.

Results and Discussion

As alluded to earlier, long before the fact that imidazoquinolines specifically engaged and activated TLR7 was known, it was recognized that these compounds were potently adjuvantic[31−34] in addition to displaying antiviral effects via the induction of IFN-α/β.[35,36] In human blood stimulated ex vivo with TLR7 agonists, upregulation of the surface expression of costimulatory molecules such as CD40, CD80, and CD86 occurred in both CD11c⁻ plasmacytoid DCs and CD11c⁺ myeloid DCs; furthermore, the Th1 stimulatory ability of both DC subsets was enhanced in response to TLR7 ligands.[37] Because TLR7 agonists transmit a T-helper-like signal to antibody-producing B cells, it is a highly effective adjuvant even for synthetic peptides that lack T-cell epitopes, possibly even replacing the function of T-helper cells, and providing a potential T-cell-independent vaccination strategy.[33]

Our initial interest in exploring TLR7 agonists as vaccine adjuvants has been greatly reinforced by our recent observations that pure TLR7 agonists, unlike other TLR ligands, are potently immunostimulatory without activating inflammatory programs in human whole blood model systems.[8] We elected to choose 1-(4-amino-2-((ethylamino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-2-methylpropan-2-ol, **2**, as our point of departure in examining structure−activity relationships in the imidazoquinolines rather than Imiquimod because we had earlier established that this analogue was more active than the parent compound and was also verified to be a pure TLR7 agonist in human primary cells (Figure 1).[8,24] We have recently reported the synthesis of **2**.[24] Furthermore, the only report on a detailed examination of SAR on the imidazoquinolines by the 3M group[17] had used indirect bioassays (indirect assay for IFN-α using inhibition of virus-induced
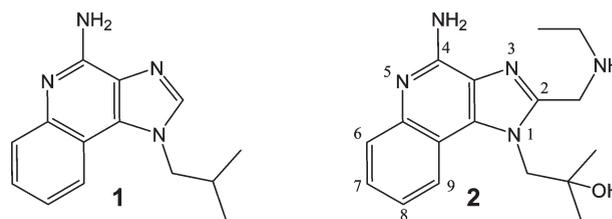

Figure 1. Structures of Imiquimod and **2**.

cytopathic effect) which predated the discovery and development of TLR7-specific assays. In the studies reported below, all compounds have been evaluated in human TLR7 and TLR8 reporter gene assays; highly active compounds were further examined for IFN-α induction in whole human blood using analyte-specific immunoassays.

Recent studies show that the TLR7 agonistic imidazoquinolines are indeed potently adjuvantic in vaccine constructs;[38,39] however, the route of administration appears to be crucial because intradermal but not subcutaneous administration of the vaccine adjuvanted with **2** is immunogenic and protective.[38] Epidermal Langerhans cells as well as dermal dendritic cells (myeloid and plasmacytoid subsets)[40] function as specialized professional antigen presenting cells in the skin.[41,42] The plasmacytoid dendritic cell subset appears to be the target cell-type for TLR7 agonists.[43] We hypothesized that the C2 ethylaminomethylene substituent of **2** renders the molecule sufficiently polar as to hinder transcutaneous penetration to effectively access and stimulate plasmacytoid dendritic cells. It was therefore desirable to evaluate more hydrophobic analogues of **2** as potential candidate transcutaneous vaccine adjuvants.

Unlike TLR2, TLR3, and TLR4 for which crystal structures are available as complexes with their cognate ligands,[44] a detailed structural characterization of the mode of binding of the imidazoquinolines to TLR7 is not yet available. Therefore, our strategy in examining structure−activity relationships was focused rather on exploring chemical space systematically around the validated lead compound **2** toward realizing the two major objectives mentioned earlier. We began with derivatization of the secondary amine of the C2 ethylaminomethylene side chain in an effort to examine whether this could be a potential site for introducing photoactivable or electrophilic functionalities. We found that modifications on this substituent are poorly tolerated, with only the *N*-ethyl analogue **3** (Scheme 1) retaining partial activity and acyl (**4**, **5**) and guanidine[45] (**6**) derivatives being bereft of activity (Table 1). The loss of activity appeared not to be a consequence of altered basicity of the amine; surmising that it may, in part, be due either to the hydrophobicity of the substituent, or the conformal constraint imposed by the amide or guanidine, we asked if a polar spacer group such as triethyleneglycol (**11**) may mitigate loss of activity, but we were proven wrong because **11** was also found to be completely inactive. Concurrent with the syntheses of the above compounds, we also synthesized the *N*-propyleneisothiocyanate derivative (**14**) via the *N*-propyleneamino (**13**) and *N*-ethylenenitrile (**12**) precursors. Compound **14** was not tested in reporter gene assays because we anticipated spurious results on account of its reactivity; the reaction of **14** with the spermine derivative (used as a model amine) afforded the expected thiourea adduct (**16**) which, disappointingly, was also inactive (Scheme 2). These results conclusively showed that the secondary amine on the C2 substituent was not amenable to modifications. Replacement of the terminal ethyl group on the C2 substituent with an

Scheme 1. Syntheses of 2-Imidazolyl Side Chain Modified Analogues of **2** [a]
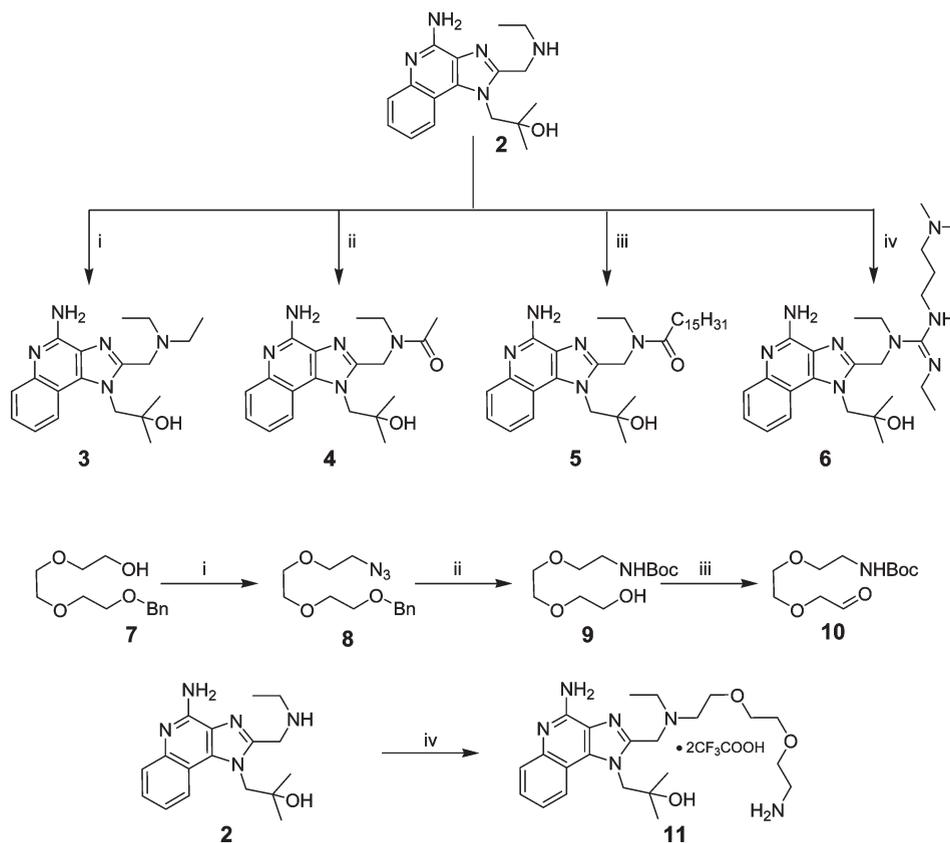
[a] Reagents for **2**–**6**: (i) CH_3CHO, $NaCNBH_3$, MeOH; (ii) CH_3COCl, Et_3N, THF; (iii) $C_{15}H_{31}COCl$, Et_3N, THF; (iv) EDCI, Et_3N, DMF. Reagents for **7**–**11**: (i) (a) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, (b) NaN_3, DMF, 70 °C; (ii) $(Boc)_2O$, $Pd(OH)_2/C$, H_2, MeOH; (iii) Dess–Martin periodinane, CH_2Cl_2; (iv) (a) **10**, $NaCNBH_3$, MeOH (b) CF_3CO_2H.

n-undecanyl group in **22** resulted in complete loss of activity (Table 1), signaling that the length of the C2 substituent was crucial; this was to become dramatically apparent when **32**, with a pentyl substituent on C2 was evaluated later (see below).

Desiring more lipophilic analogues which are expected to permeate the dermal barrier better, we sought to examine N^1-benzyl analogues (Scheme 3), noting that similar substitutions have resulted in augmented activity in the 8-hydroxy-adenine series.[20–23] Compound **32** was the first to be synthesized in this series which, to our pleasant surprise, showed an activity 20-fold greater than **2**. Coupled with our observation that **22** was completely inactive, we sought to systematically explore SAR of the N^1-benzyl C2 alkyl substituents. As depicted in Figure 2, a very distinct relationship between alkyl length and TLR7-agonistic potency was observed, with the optimal compound being **31** (C2-*n*-butyl), with an EC_{50} of 59 nM (Table 1). *n*-Butylene (**38**) and *n*-butylyne (**43**) analogues showed decrease in potency. It is noteworthy that a C2-*n*-butyl, N^1-phenyl analogue synthesized by the 3M group[17] was not the most active; our results indicate that an N^1-benzyl substituent is preferred.

In recognition that bioactivity readouts using cell-culture systems may not always reflect with fidelity in vivo behavior owing to differential plasma protein binding (that we ourselves have observed and characterized),[46] it was important to verify that the activity profiles observed in TLR7-specific reporter gene assays (Table 1) was also seen in whole blood with its full complement of plasma proteins. It was gratifying that we observed the expected **31** ≫ **2** ∼ **34** activity profile in whole blood IFN-α induction experiments (Figure 3).

Bioisosteric replacements of the C4-NH$_2$ group with −NMe, −OH, and −SMe groups on the Imiquimod scaffold have been explored earlier,[17] and it was of interest to examine if the previously described SAR would hold true in our analogues and also to test whether the introduction of phenyl, −NHNH$_2$, and −NHOH groups would alter TLR7 activity (Scheme 4). The 4-Cl precursor (**44**) and the *des*-amino analogue **50**, as well as the 4-OH (**46**) and 4-Ph (**47**) analogues, were completely inactive, while the 4-NHOH (**48**) and 4-NHNH$_2$ (**49**) compounds were substantially weaker (Table 1) than their 4-NH$_2$ counterparts (**31**, **32**). These results indicate the importance of the preservation of the NH$_2$ group on C4 for maximal activity.

Up to this point in our SAR studies, the most active and chemically distinct species were compound **2** and the N^1-benzyl-C2-*n*-butyl analogue **31**. We were curious to examine whether transposing the N^1 and C2 substituents on these molecules would result in enhanced activity. Fortuitously, both the "hybrids" (**52**, **54**; Scheme 5) were very active. **54** was found to be extraordinarily potent, with an EC_{50} of 8.6 nM (Figure 4, Table 1).

Finally, we attempted to modify the imidazole ring of the scaffold. The triazole compounds **55** and **56**, as well as the cyclic urea **57**, were completely inactive, which emphasizes the role of the recognition of the imidazole ring system by TLR7 (Scheme 6). Mention must also be made that these studies have been useful in delineating possible sites for introducing labeling functionalities. Preliminary studies (data not shown) show that the N^1-benzyl substituent may be appropriate for introducing arylazido functional groups, while the quinoline ring may be amenable to introducing electrophilic groups. These results will be communicated in a future publication.

Table 1. EC$_{50}$ Values (μM) of Compounds in Human TLR7-Specific Reporter Gene Assay[a]

Structure	Compound Number	TLR7-Agonistic Activity (μM)
	1	2.12
	2	2.0
	3	7.71
	4	ND
	5	ND
	6	ND
	11	ND
	12	8.39
	13	ND
	16	ND
	22	ND
	28	0.955

Table 1. Continued

Structure	Compound Number	TLR7-Agonistic Activity (µM)
	29	0.472
	30	0.647
	31	0.0591
	32	0.0915
	33	0.479
	34	0.822
	35	3.35
	36	ND
	37	ND
	38	0.262
	43	0.209
	44	ND
	46	ND
	47	ND

Table 1. Continued

| Structure	Compound Number	TLR7-Agonistic Activity (µM)
	48	0.723
	49	0.873
	50	ND
	52	0.385
	54	0.00858
	55	ND
	56	ND
	57	ND

[a] ND denotes no activity detected at 100 µM.

All of the C2-alkyl compounds reported herein were tested for TLR8 activity using the thiazoloquinoline CL075 (3M002) as a reference TLR8 agonist,[47,48] and none had any appreciable activity in these assays. It should also be pointed out that while potencies of compounds are conventionally represented as EC_{50} (or IC_{50}) values, that metric alone appears to be inadequate in rank-ordering the activities of the active compounds tested. An inspection of Figure 4 indicates, for instance, that while the EC_{50} values of both Imiquimod and **2** are comparable (∼2 µM), compound **2** induces a more robust response than Imiquimod. Similarly, the area-under-curve estimates of **54** (analogous to combined "avidity" and affinity measures of antigen−antibody interactions)[49,50] indicate that consideration of the EC_{50} values alone may underestimate its potency.

Given that TLR7 is sequestered in an acidic endolysosomal compartment, and these compounds are weak bases and are therefore expected to accumulate in that acidic organelle, we wondered if the observed SAR could simply be a consequence of differential hydrophobicity of the compounds, modulating uptake into the cell and entry into the endolysosomal compartment activity, rather than governing specific molecular interactions with TLR7. A comparison of observed retention times on C_{18}-reverse-phase mass-chromatograms (a measure of hydrophobicity)[51,52] with the potencies of the active compounds indicate a distinct deviation from the trend of **54**, suggesting specific and idiosyncratic effects rather than bulk effects (Figure 5).

These structure−activity studies have been instructive and rewarding in that they not only complement the seminal work at 3M but also have led to the discovery of highly potent, lipophilic, human TLR7 agonist, **54**. We note, not without irony, that this highly potent compound differs from its parent molecule by only a replacement of a secondary amine by a methylene unit. The oral bioavailability of **54** is predicted to be good, and it is possible that the systemic IFN-α inducing effects of this imidazoquinoline may find additional uses in the treatment of conditions such as hepatitis[53] and chronic myelogenous[54] and hairy cell leukemias.[55] However, for purposes of developing dermal vaccine adjuvants, **31** may be more efficacious for reasons discussed earlier. Compounds **54** and **31** are being exhaustively immuno-profiled and characterized in a variety of assays.

Scheme 2. Syntheses of 2-Imidazolyl Side Chain Modified Analogues of **2**[a]
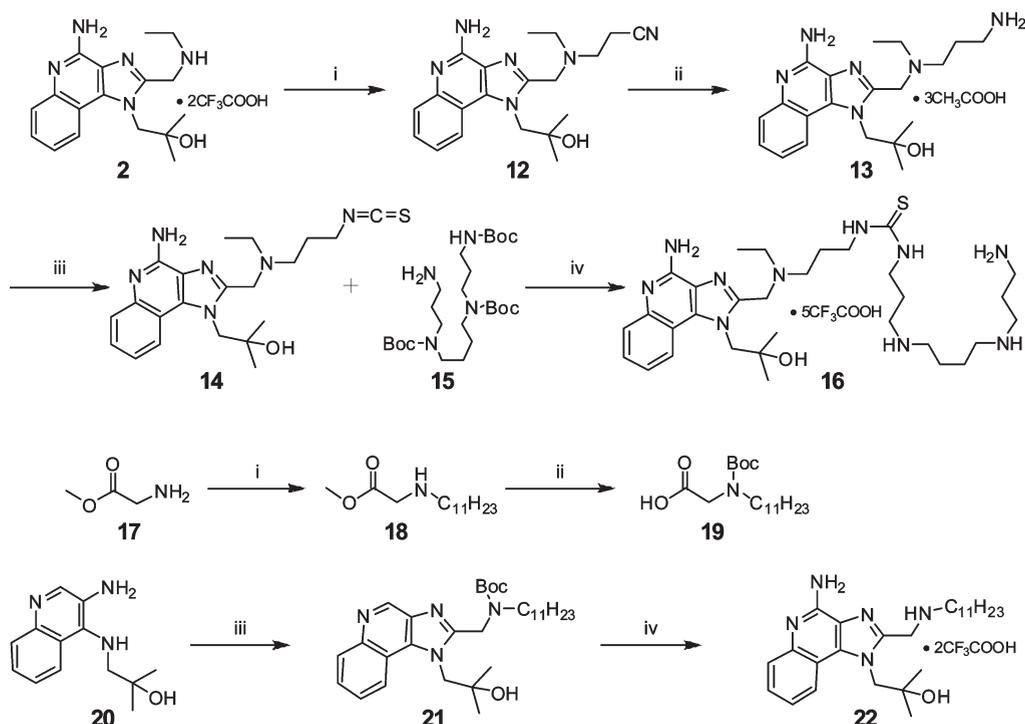
[a] Reagents for **2**–**16**: (i) CH$_2$=CHCN, Et$_3$N, MeOH; (ii) Pd(OH)$_2$/C, H$_2$, CH$_3$CO$_2$H; (iii) CS$_2$, Et$_3$N, (Boc)$_2$O, EtOH; (iv) CH$_2$Cl$_2$. Reagents for **17**–**22**: (i) C$_{10}$H$_{21}$CHO, NaCNBH$_3$, MeOH; (ii) (a) (Boc)$_2$O, Et$_3$N, MeOH, (b) LiOH, THF/MeOH; (iii) (a) **19**, HATU, Et$_3$N, DMAP, DMF, (b) NaOH, EtOH, reflux; (iv) (a) 3-chloroperoxybenzoic acid, CH$_2$Cl$_2$, CHCl$_3$, MeOH, (b) benzoyl isocyanate, CH$_2$Cl$_2$ (c) NaOCH$_3$, MeOH, (d) CF$_3$CO$_2$H.

Scheme 3. Syntheses of 1-Benzyl-2-(alkyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amines[a]
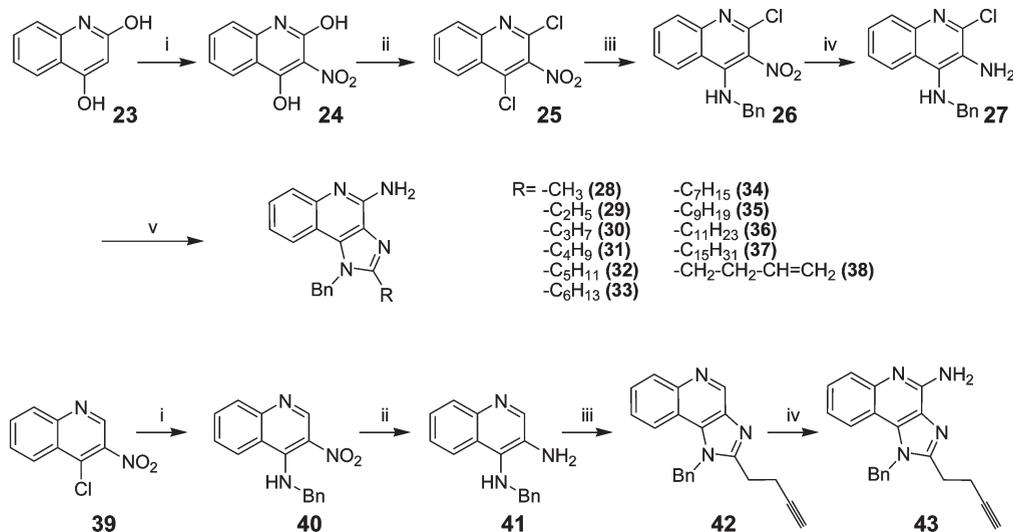
[a] Reagents for **23**–**38**: (i) HNO$_3$, 75 °C; (ii) PhPOCl$_2$, 135 °C; (iii) benzylamine, Et$_3$N, CH$_2$Cl$_2$; (iv) Pt/C, H$_2$, Na$_2$SO$_4$, EtOAc; (v) (a) RCOCl, Et$_3$N, THF, (b) NH$_3$/MeOH, 150 °C. Reagents for **39**–**43**: (i) benzylamine, Et$_3$N, CH$_2$Cl$_2$; (ii) Pt/C, H$_2$, Na$_2$SO$_4$, EtOAc; (iii) (a) 4-pentynoic acid, HBTU, Et$_3$N, DMAP, DMF, 90 °C; (iv) (a) 3-chloroperoxybenzoic acid, CH$_2$Cl$_2$, CHCl$_3$, MeOH, (b) benzoyl isocyanate, CH$_2$Cl$_2$, (c) NaOCH$_3$, MeOH.

Experimental Section

Chemistry. 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 Rf "Gold" high performance silica columns on CombiFlash Rf 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 × 150 mm, 5 *μ*m analytical reverse phase C$_{18}$ column with H$_2$O–2-propanol or H$_2$O–CH$_3$CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion (or negative ion, as appropriate) acquisition mode.
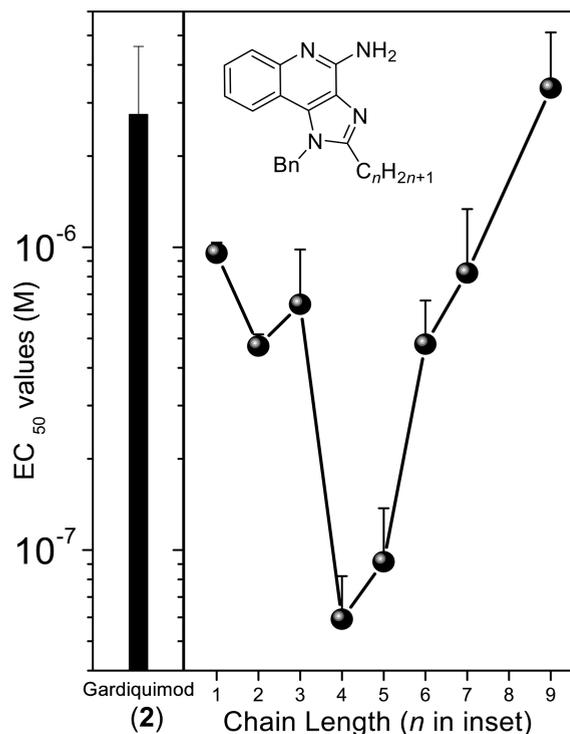
Figure 2. Alkyl chain length dependence of TLR7-agonistic activity profiles of the N^1-benzyl-C2-alkyl compounds (right panel). The activity of **2** (reference compound) is shown on the left. Error bars represent standard deviations obtained on quadruplicate samples.

Figure 3. Dose-dependent induction of IFN-α in whole human blood by **2**, **31**, and **34**. Plasma IFN-α was assayed in triplicate by ELISA.

Synthesis of Compound 3: 1-(4-Amino-2-((diethylamino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-2-methylpropan-2-ol. To a solution of trifluoroacetate salt of **2** (75 mg, 0.14 mmol) in anhydrous MeOH were added acetaldehyde (6.6 mg, 0.15 mmol), 12−14 drops of acetic acid, and sodium cyanoborohydride (10 mg, 0.15 mmol). After 4 h, the solvent was removed using vacuum, and the residue was purified using column chromatography (7% MeOH/dichloromethane) to obtain the compound **3** (16 mg, 34%). ^{1}H NMR (500 MHz, MeOD) δ 8.36 (d, J = 7.8 Hz, 1H), 7.73 (dd, J = 8.4, 1.0 Hz, 1H), 7.69−7.64 (m, 1H), 7.52 (ddd, J = 8.4, 7.2, 1.2 Hz, 1H), 4.83 (s, 2H), 4.75−4.63 (m, 2H), 3.42 (dd, J = 14.2, 7.0 Hz, 4H), 1.39−1.29 (m, 6H), 1.21 (dd, J = 13.5, 6.2 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 150.85, 150.16, 138.41, 136.09, 131.52, 126.42, 126.18, 123.69, 120.11, 114.65, 72.54, 56.68, 49.70, 49.56, 27.65, 9.45. MS (ESI) calcd for $C_{19}H_{27}N_5O$, m/z 341.22, found 342.24 (M + H)$^+$.

Synthesis of Compound 4: *N*-((4-Amino-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)-*N*-ethylacetamide. To a solution of trifluoroacetate salt of **2** (40 mg, 0.074 mmol) in anhydrous THF were added triethylamine (23 mg, 0.22 mmol) and acetyl chloride (6 mg, 0.08 mmol). After 30 min, the solvent was removed under vacuum. The residue was dissolved in EtOAc, washed with water, and dried over Na$_2$SO$_4$. The EtOAc fraction was then concentrated under vacuum, and the residue was purified using column chromatography (6% MeOH/dichloromethane) to obtain the compound **4** (22 mg, 85%). ^{1}H NMR (400 MHz, MeOD) δ 8.34 (d, J = 8.3 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H), 5.23 (s, 2H), 4.81 (s, 2H), 3.63 (q, J = 7.1 Hz, 2H), 2.23 (s, 3H), 1.34−1.13 (m, 9H). ^{13}C NMR (101 MHz, MeOD) δ 172.18, 154.62, 136.88, 134.07, 129.68, 129.48, 124.60, 122.64, 122.31, 118.21, 113.67, 70.85, 65.49, 55.12, 43.70, 41.79, 19.68, 14.03, 12.30. MS (ESI) calcd for $C_{19}H_{25}N_5O_2$, m/z 355.20, found 356.21 (M + H)$^+$.

Synthesis of Compound 5: *N*-((4-Amino-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)-*N*-ethylpalmitamide. To a solution of trifluoroacetate salt of **2** (20 mg, 0.05 mmol) in anhydrous THF were added triethylamine (15 mg,
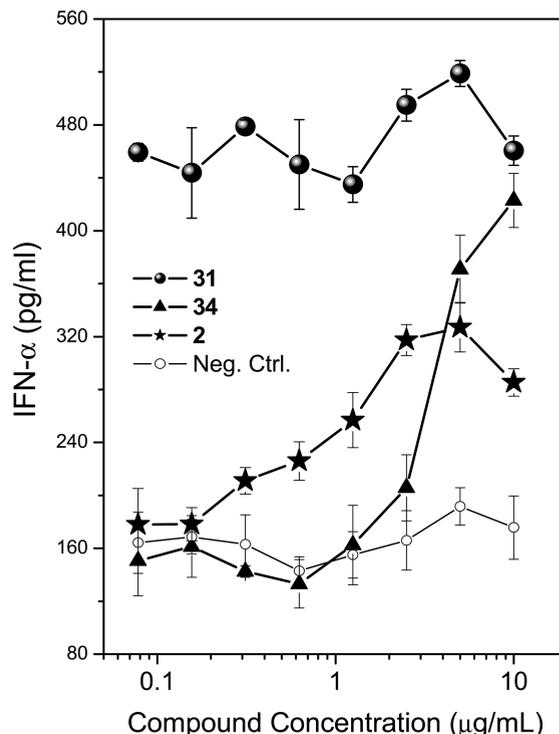
0.15 mmol) and palmitoyl chloride (16 mg, 0.06 mmol). After 30 min, the solvent was removed under vacuum, and residue was dissolved in EtOAc and washed with water. The EtOAc fraction was then dried under vacuum, and the residue was purified using column chromatography (5% MeOH/dichloromethane) to obtain the compound **5** (10 mg, 30%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.12 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.38 (t, J = 7.8 Hz, 1H), 4.75 (s, 2H), 3.56 (q, J = 6.8 Hz, 2H), 2.45−2.34 (m, 2H), 1.76−1.57 (m, 2H), 1.58−1.11 (m, 35H), 0.90 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 192.82, 173.95, 152.03, 150.36, 135.45, 130.90, 128.29, 124.10, 123.24, 120.62, 114.81, 71.96, 55.58, 42.81, 42.30, 33.06, 31.92, 29.68, 29.65, 29.52, 29.47, 29.42, 29.35, 25.37, 22.69, 14.12, 13.70, 5.61. MS (ESI) calcd for $C_{33}H_{53}N_5O_2$, m/z 551.42, found 552.41 (M + H)$^+$.

Synthesis of Compound 6: 1-((4-Amino-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)-3-(3-(dimethylamino)propyl)-1,2-diethylguanidine. To a solution of trifluoroacetate salt of **2** (50 mg, 0.092 mmol) in anhydrous DMF were added triethylamine (51 mg, 0.51 mmol), EDCI·HCl (53 mg, 0.28 mmol), and a catalytic amount of DMAP. The reaction was stirred for 24−30 h. The solvent was then removed under vacuum, and the residue was purified using C$_{18}$ reverse-phase column chromatography to obtain the compound **6** (9 mg, 24%). ^{1}H NMR (400 MHz, MeOD) δ 8.48 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 7.4 Hz, 1H), 7.76 (t, J = 7.7 Hz, 1H), 7.63 (t, J = 7.2 Hz, 1H), 5.18 (s, 2H), 4.85−4.71 (m, 2H), 3.58 (q, J = 7.3 Hz, 2H), 3.54−3.41 (m, 4H), 3.27−3.18 (m, 2H), 2.90 (d, J = 3.7 Hz, 6H), 2.27−2.00 (m, 2H), 1.30 (dt, J = 14.4, 7.2 Hz, 12H). ^{13}C NMR (101 MHz, MeOD) δ 160.10, 153.79, 137.08, 134.45, 129.86, 124.93, 122.19, 118.63, 113.38, 71.07, 55.30, 54.69, 46.25, 45.16, 42.06, 41.41, 39.78, 24.50, 13.79, 11.77. MS (ESI) calcd for $C_{25}H_{40}N_8O$, m/z 468.33, found 469.33 (M + H)$^+$ and 235.17 (M + 2H)$^{2+}$.

Synthesis of Compound 9: *tert*-Butyl 2-(2-(2-hydroxyethoxy)ethoxy)ethylcarbamate. To a solution of **7** (500 mg, 2.1 mmol) in anhydrous dichloromethane, cooled to 0 °C, were added triethylamine (420 mg, 4.2 mmol), tosyl chloride (609 mg, 3.54 mmol),

Scheme 4. Syntheses of 4-Substituted-1-benzyl-2-(alkyl)-1*H*-imidazo[4,5-*c*]quinolines[a]

R=-C₅H₁₁ (**44**)
or -C₄H₉ (**45**)
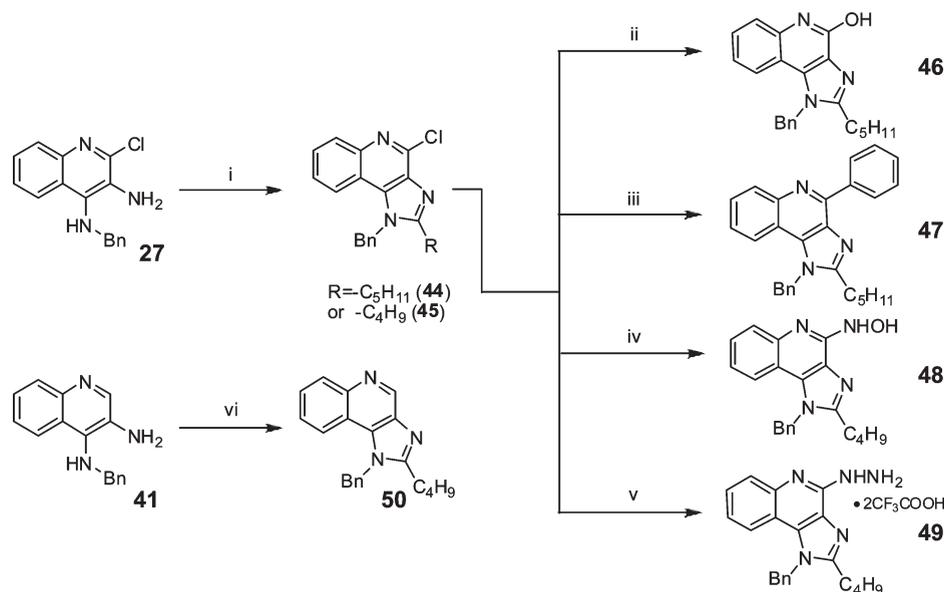
[a] Reagents: (i) (a) RCOCl, Et₃N, THF, (b) CaO, MeOH, 110 °C; (ii) 6N aq. HCl, 110 °C; (iii) polystyrene-bound PPh₃-Pd, phenyl boronic acid, K₂CO₃, THF/H₂O; (iv) NH₂OH·HCl, Et₃N, MeOH, 50 °C; (v) (a) *t*-butyl carbazate, 80 °C, MeOH, (b) CF₃CO₂H; (vi) (a) RCOCl, Et₃N, THF, (b) CaO, MeOH, 110 °C.

Scheme 5. Syntheses of C-2/N-1 Substituent-Swapped **31/2** Hybrids[a]
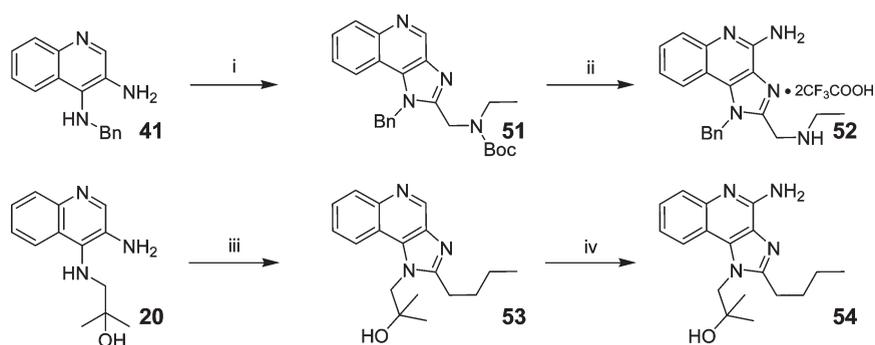
[a] Reagents: (i) HBTU, 2-(*tert*-butoxycarbonyl(ethyl)amino)acetic acid, Et₃N, DMAP, DMF, 70 °C; (ii) (a) 3-chloroperoxybenzoic acid, CH₂Cl₂, CHCl₃, MeOH, (b) benzoyl isocyanate, CH₂Cl₂, (c) NaOCH₃, MeOH, (d) CF₃CO₂H; (iii) (a) C₄H₉COCl, Et₃N, THF, (b) CaO, MeOH, 110 °C; (iv) (a) 3-chloroperoxybenzoic acid, CH₂Cl₂, CHCl₃, MeOH, (b) benzoyl isocyanate, CH₂Cl₂ (c) NaOCH₃, MeOH.

and a catalytic amount of DMAP. The reaction was stirred for 16 h, and then the mixture was washed with water and dried over Na₂SO₄. The solvent was then removed under vacuum. This residue *O*-tosylated compound was dissolved in anhydrous DMF, to which sodium azide (273 mg, 4.2 mmol) was added and the reaction was heated at 70 °C for 1 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc, washed with water, and dried over Na₂SO₄. The EtOAc fraction was concentrated under vacuum to obtain the compound **8** (600 mg). This **8** was then dissolved in MeOH, and di-*tert*-butyl dicarbonate (687 mg, 3.5 mmol) was added to it. The solution was subjected to catalytic hydrogenolysis using Pd(OH)₂/C at 60 psi hydrogen pressure for 4 h. The solution was then filtered through celite, and filtrate was evaporated under reduced pressure to afford the residue, which was purified using column chromatography (3% MeOH/dichloromethane) to obtain the compound **9** (320 mg, 62%). ¹H NMR (400 MHz, CDCl₃) δ 5.13 (s, 1H), 3.77 (d, *J* = 3.5 Hz, 2H), 3.70−3.61 (m, 6H), 3.58 (t, *J* = 5.2 Hz, 2H), 3.34 (d, *J* = 4.6 Hz, 2H), 2.50 (s, 1H), 1.46 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 159.62, 79.34, 72.55, 70.43, 70.29, 61.78, 40.34, 28.41. MS (ESI) calcd for C₁₁H₂₃NO₅, *m/z* 249.16, found 272.15 (M + Na⁺).

Synthesis of Compound 11: 1-(4-Amino-2-(((2-(2-(2-amino-ethoxy)ethoxy)ethyl) (ethyl)amino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-2-methylpropan-2-ol. To a solution of **9** (200 mg, 0.8 mmol) in 4 mL of 0.3 M Dess−Martin periodinane solution in dichloromethane, 10−12 drops of acetic acid were added and the reaction was stirred for 4−5 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (40−50% EtOAc/dichloromethane) to obtain the intermediate aldehyde derivative **10** (71 mg). To a solution of trifluoroacetate salt of **2** (150 mg, 0.29 mmol) in anhydrous MeOH were added **10** (71 mg, 0.29 mmol), 6−8 drops of acetic acid, and sodium cyanoborohydride (22 mg, 0.35 mmol). The solution was stirred for 14−15 h, and the solvent was removed under vacuum to obtain the residue, which was purified using column chromatography (10% MeOH/dichloromethane) to obtain the intermediate *N*-Boc derivative. This was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of the compound **11** (110 mg, 60%). ¹H NMR (400 MHz, MeOD) δ 8.49 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.76 (t, *J* = 7.3 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 4.82 (s, 4H), 3.88 (t, *J* = 5.1 Hz, 2H), 3.70 (d, *J* = 6.5 Hz, 6H), 3.50 (s, 2H), 3.38 (s, 2H), 3.17−3.09 (m, 2H), 1.55−1.10 (m, 9H). ¹³C NMR (101 MHz,

MeOD) δ 149.53, 148.21, 136.86, 134.61, 130.12, 122.23, 118.64, 113.15, 71.15, 69.99, 69.93, 66.50, 64.83, 55.36, 52.60, 49.74, 48.69, 46.46, 39.10, 26.24, 7.96. MS (ESI) calcd for $C_{23}H_{36}N_6O_3$, m/z 444.29, found 467.27 (M + Na$^+$) and 223.15 (M + 2H)$^{2+}$.

Synthesis of Compound 12: 3-(((4-Amino-1-(2-hydroxy-2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)(ethyl)amino)-propanenitrile. To a solution of **2** (250 mg, 0.46 mmol) in anhydrous MeOH were added triethylamine (117 mg, 1.16 mmol) and acrylo-nitrile (61 mg, 1.16 mmol). The reaction was stirred for 30 h, followed by removal of the solvent under vacuum. The residue was dissolved in EtOAc, washed with water, and dried over Na_2SO_4. The EtOAc fraction was evaporated under vacuum, and the residue was purified using column chromatography (11% MeOH/dichloro-methane) to obtain the compound **12** (145 mg, 86%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.06 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 6.04 (s, 2H), 4.88 (s, 2H), 4.12 (s, 2H), 2.94 (t, J = 6.7 Hz, 2H), 2.75 (q, J = 7.0 Hz, 2H), 2.54 (t, J = 6.7 Hz, 2H), 1.35 (s, 6H), 1.19 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 150.98, 150.41, 135.44, 127.81, 126.16, 122.48, 122.40, 122.18, 120.20, 118.64, 115.30, 71.49, 56.48, 50.85, 47.92, 47.64, 27.99, 15.33, 11.12. MS (ESI) calcd for $C_{20}H_{26}N_6O$, m/z 366.22, found 367.22 (M + H)$^+$.

Synthesis of Compound 13: 1-(4-Amino-2-(((3-aminopropyl)-(ethyl)amino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-2-methyl-propan-2-ol. To a solution of **12** (160 mg, 0.44 mmol) in glacial acetic acid, a catalytic amount of Pd(OH)$_2$/C was added and the reaction mixture was subjected to hydrogenation at 55 psi hydrogen pressure for 4 h. The solution was then filtered. The filtrate was concentrated to obtain the acetate salt of the compound **13** (55 mg). This residue (20 mg, 0.05 mmol) was then dissolved in MeOH and di-*tert*-butyl dicarbonate (18 mg, 0.08 mmol) was added. After 30 min, the solvent was removed under vacuum and the residue was purified using column chromatography (15% MeOH/dichloromethane) to obtain the intermediate *N*-Boc derivative. This was then dissolved in 2 mL of HCl solution in dioxane and stirred for 2 h. The solvent was then removed under vacuum to obtain the hydrochloride salt of the compound **13** (15 mg, 74%). ^{1}H NMR (400 MHz, MeOD) δ 8.51 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.65 (t, J = 7.7 Hz, 1H), 5.09 (s, 2H), 3.74−3.50 (m, 6H), 3.12 (d, J = 7.0 Hz, 2H), 2.32 (s, 2H), 1.51 (t, J = 6.9 Hz, 3H), 1.35 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 156.18, 151.90, 140.30, 137.56, 129.79, 126.47, 124.63, 123.13, 123.00, 72.46, 56.49, 53.02, 52.27, 48.77, 40.46, 27.84, 24.89,
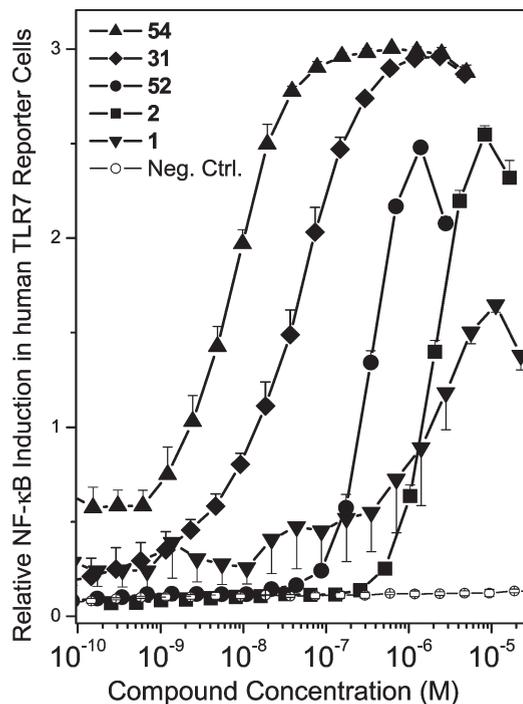

Figure 4. TLR7-agonistic activities of active compounds in a human TLR7-specific reporter gene assay. Error bars represent standard deviations obtained on quadruplicate samples.
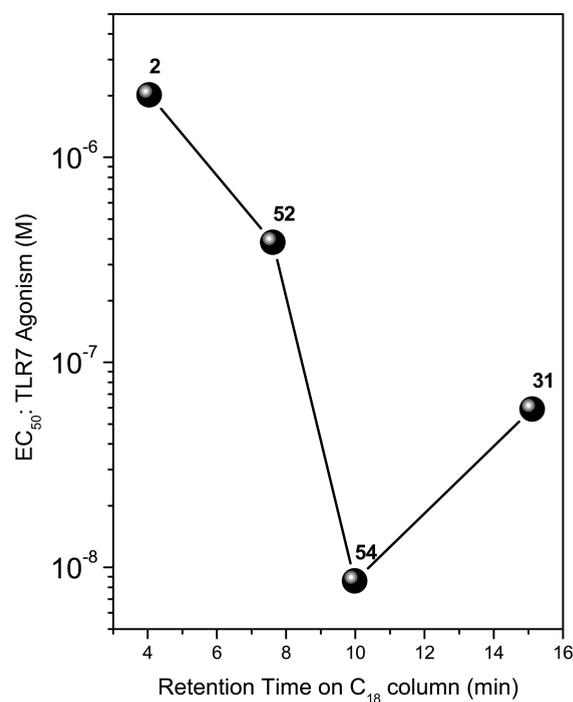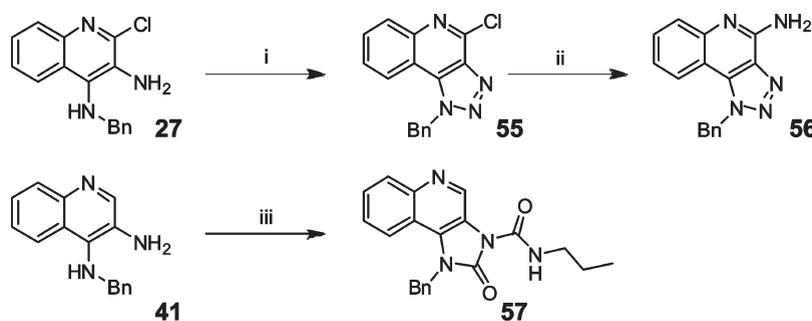

Figure 5. Correlation of TLR7-agonistic potency and hydrophobicity (measured as retention time on C$_{18}$ reverse-phase chromatography).

Scheme 6. Syntheses of Imidazole-Modified Analogues^a

^{a} Reagents: (i) NaNO$_2$, CH$_3$CO$_2$H, H$_2$O, 0 °C; (ii) NH$_3$/MeOH, 150 °C; (iii) *n*-propyl isocyanate, Et$_3$N, 100 °C, THF.

11.74. MS (ESI) calcd for $C_{20}H_{30}N_6O$, m/z 370.25, found 371.26 $(M + H)^+$.

Synthesis of Compound 16: 1-(3-(((4-amino-1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-2-yl)methyl)(ethyl)amino)-propyl)-3-(3-(4-(3-aminopropylamino)butylamino)propyl) thiourea. To a solution of **13** (35 mg, 0.07 mmol) in anhydrous ethanol were added triethylamine (22 mg, 0.21 mmol) and CS_2 (54 mg, 0.71 mmol). The reaction mixture was stirred for 30 min and then cooled to 0 °C. Di-*tert*-butyl dicarbonate (15 mg, 0.07 mmol) and a catalytic amount of DMAP dissolved in ethanol were added to the reaction mixture. After 30 min, the solvent was removed under vacuum, and the residue was purified using column chromatography to obtain the compound **15**. To a solution of **15** (10 mg, 0.02 mmol) in anhydrous dichloromethane N^1,N^5,N^{10}-*tris* boc spermine (37 mg, 0.073 mmol) was added. The reaction mixture was stirred for 3 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (6% MeOH/dichloromethane) to obtain the intermediate N-Boc derivative. This was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of the compound **16** (5 mg, 20%). ^{1}H NMR (500 MHz, MeOD) δ 8.45 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.67–7.56 (m, 1H), 4.96 (d, J = 6.9 Hz, 2H), 4.81–4.70 (m, 2H), 3.77–3.38 (m, 8H), 3.13 (dd, J = 18.0, 10.2 Hz, 2H), 3.10–2.96 (m, 8H), 2.16–2.04 (m, 4H), 1.97–1.88 (m, 2H), 1.84–1.74 (m, 4H), 1.44 (t, J = 7.2 Hz, 3H), 1.29 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 150.81, 138.54, 138.41, 135.99, 131.60, 126.50, 123.71, 120.06, 114.65, 72.55, 56.76, 52.24, 50.71, 49.86, 49.68, 48.26, 48.06, 46.25, 45.87, 37.80, 27.48, 25.39, 24.29, 9.47. MS (ESI) calcd for $C_{31}H_{54}N_{10}OS$, m/z 614.42, found 615.43 $(M + H)^+$ and 308.22 $(M + 2H)^{2+}$.

Synthesis of Compound 19: 2-(*tert*-Butoxycarbonyl(undecyl)-amino)acetic acid.[24] To a solution of **17** (200 mg, 1.6 mmol) in anhydrous MeOH were added undecanal (218 mg, 1.28 mmol), 5–6 drops of acetic acid, and sodium cyanoborohydride (111 mg, 1.76 mmol). The reaction was stirred for 3 h, and then the solvent was removed under vacuum. The residue was dissolved in dichloromethane and washed with water, brine, and dried over Na_2SO_4. The dichloromethane fraction was then concentrated to obtain the residue **18**, which was dissolved in MeOH, followed by the addition of triethylamine (202 mg, 2 mmol) and di-*tert*-butyl dicarbonate (436 mg, 2 mmol). The reaction was stirred for 4 h, and the solvent was removed under vacuum. The residue was then dissolved in a solvent mixture of THF:MeOH (3:1) and lithium hydroxide that was dissolved in water was added until the pH went above 12. The reaction was stirred for 24 h, followed by removal of the solvent under vacuum. The residue was dissolved in water, and the solution was acidified using 10% HCl until pH was less than 2. The water fraction was then extracted with EtOAc, followed by washing the EtOAc fraction with water and drying over Na_2SO_4. The EtOAc fraction was then concentrated under vacuum to obtain the compound **19** (320 mg, 61%). MS (ESI) calcd for $C_{18}H_{35}NO_4$, m/z 329.26, found 328.25 $(M - H)^-$.

Synthesis of Compound 22: 1-(4-Amino-2-((undecylamino)-methyl)-1H-imidazo[4,5-c]quinolin-1-yl)-2-methylpropan-2-ol. To a solution of **19** (171 mg, 0.52 mmol) in anhydrous DMF were added HATU (217 mg, 0.57 mmol), triethylamine (58 mg, 0.57 mmol), a catalytic amount of DMAP, and **20** (120 mg, 0.52 mmol). The reaction mixture was stirred for 3–4 h. The solvent was evaporated under vacuum. The residue was dissolved in EtOAc, washed with water, and dried over Na_2SO_4 to obtain the residue. This was dissolved in 10 mL of ethanol, and a solution of NaOH (105 mg, 2.62 mmol) dissolved in 1 mL of water was added to it. The reaction mixture was refluxed for 5–6 h, followed by evaporation under reduced pressure. The residue was purified by column chromatography (5% MeOH/dichloromethane) to obtain the compound **21** (165 mg). To a solution of **21** in a solvent mixture of MeOH:dichloromethane:chloroform (0.1:1:1), 3-chloro-peroxybenzoic acid (135 mg, 0.79 mmol) was added and the

solution was refluxed at 45–50 °C for 40 min. The solvent was then removed and the residue was purified using column chromatography (7% MeOH/dichloromethane) to obtain the N-oxide derivative (75 mg). This was then dissolved in anhydrous dichloromethane, followed by the addition of benzoyl isocyanate (37 mg, 0.25 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 the addition of excess sodium methoxide and heating at 80 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (8% MeOH/dichloromethane) to obtain the intermediate N-Boc derivative (27 mg). This was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of the compound **22** (33 mg, 12%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 14.65 (s, 1H), 10.11 (s, 2H), 8.06 (s, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 4.76 (s, 2H), 4.61 (s, 2H), 3.23 (s, 2H), 1.93 (s, 2H), 1.50–1.11 (m, 22H), 0.91 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 149.04, 148.91, 136.14, 134.18, 129.98, 124.99, 124.25, 120.76, 119.24, 112.18, 72.07, 49.07, 44.01, 31.89, 29.55, 29.49, 29.34, 29.31, 28.98, 26.58, 25.46, 22.67, 14.10, 5.61. MS (ESI) calcd for $C_{26}H_{41}N_5O$, m/z 439.33, found 440.33 $(M + H)^+$ and 220.67 $(M + 2H)^{2+}$.

Synthesis of Compound 24: 3-Nitroquinoline-2,4-diol. Compound **23** (5 g, 31 mmol) was dissolved in 30 mL of nitric acid and stirred at room temperature for 10 min, followed by heating the reaction mixture at 75 °C for another 15 min. The reaction mixture was then allowed to cool down to room temperature and was added to ice–water mixture to precipitate the product. The solid, yellow precipitate was filtered and dried to obtain the compound **24** (5.9 g, 92%). ^{1}H NMR (400 MHz, DMSO) δ 12.00 (s, 1H), 8.04 (d, J = 7.9 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 156.69, 156.23, 138.56, 133.61, 127.71, 124.92, 122.81, 116.32, 114.47. MS (ESI) calcd for $C_9H_6N_2O_4$, m/z 206.03, found 205.04 $(M - H)^-$.

Synthesis of Compound 25: 2,4-Dichloro-3-nitroquinoline. Compound **24** (2 g, 9.7 mmol) was dissolved in 20 mL of phenylphosphonyl dichloride and heated at 135 °C for 3 h. The reaction mixture was poured into ice–water and stirred vigorously to obtain the precipitate, which was filtered and dried to afford the compound **25** (2.24 g, 74%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.30 (ddd, J = 8.4, 1.4, 0.6 Hz, 1H), 8.14 (ddd, J = 8.5, 1.2, 0.6 Hz, 1H), 7.97 (ddd, J = 8.5, 7.0, 1.4 Hz, 1H), 7.84 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 146.64, 139.73, 135.62, 133.44, 129.76, 129.37, 125.22, 124.46.

Synthesis of Compound 26: N-Benzyl-2-chloro-3-nitroquinolin-4-amine. To a solution of **25** (2.24 g, 9.26 mmol) in 30 mL of anhydrous dichloromethane were added triethylamine (1.4 g, 13.9 mmol) and benzylamine (1.19 g, 11.1 mmol). The reaction mixture was refluxed at 45 °C for 30 min. The solvent was then evaporated under vacuum, and water was added to the residue to obtain the precipitate. This was filtered, washed several times with water, and dried to obtain the compound **26** (2.1 g, 73%). ^{1}H NMR (400 MHz, DMSO) δ 8.54 (dd, J = 12.9, 7.3 Hz, 2H), 7.89–7.81 (m, 2H), 7.69 (td, J = 8.6, 5.0 Hz, 1H), 7.39–7.21 (m, 5H), 4.46 (d, J = 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 145.79, 144.82, 141.51, 138.27, 132.70, 129.08, 128.94, 127.89, 127.40, 127.15, 126.96, 123.52, 120.12, 47.24. MS (ESI) calcd for $C_{16}H_{12}ClN_3O_2$, m/z 313.06, found 312.06 $(M - H)^-$.

Synthesis of Compound 27: N^4-Benzyl-2-chloroquinoline-3,4-diamine. To a solution of **26** (2.21 g, 7.06 mmol) in 20 mL of EtOAc were added a catalytic amount of Pt/C and Na_2SO_4. The reaction mixture was subjected to hydrogenation at 55 psi hydrogen pressure for 3 h. The reaction mixture was then filtered through celite, and the filtrate was evaporated under vacuum to obtain the compound **27** (1.8 g, 90%). ^{1}H NMR (400 MHz, MeOD) δ 7.90 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.4 Hz,

1H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.38−7.31 (m, 3H), 7.25 (t, *J* = 7.5 Hz, 2H), 7.20 (d, *J* = 7.0 Hz, 1H), 4.51 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 141.54, 141.28, 139.93, 137.86, 128.45, 128.04, 127.44, 126.96, 126.80, 126.22, 125.31, 123.22, 121.40, 49.56. MS (ESI) calcd for $C_{16}H_{14}ClN_3$, *m/z* 283.09, found 284.10 (M + H)$^+$.

General Procedure for Synthesis of C-2 alkyl imidazoquioline compounds (28−38). Synthesis of Compound 32: 1-Benzyl-2-pentyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. To a solution of **27** (50 mg, 0.18 mmol) in anhydrous THF were added triethyl-amine (26 mg, 0.19 mmol) and hexanoyl chloride. The reaction mixture was stirred at room temperature for 6 h. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate and washed with water. The ethyl acetate fraction was dried using Na_2SO_4 and evaporated under vacuum to obtain the intermediate amide compound. This was dissolved in 0.1 mL of 2 M solution of ammonia in MeOH. The sealed reaction vessel was heated in microwave at 150 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (7% MeOH/dichloro-methane) to obtain the compound **32** (8 mg; unoptimized yields). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (d, *J* = 8.3 Hz, 1H), 7.72 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.44 (qd, *J* = 7.0, 3.5 Hz, 1H), 7.38−7.29 (m, 3H), 7.18−7.10 (m, 1H), 7.06 (d, *J* = 6.9 Hz, 2H), 5.74 (s, 2H), 5.60 (s, 2H), 2.95−2.79 (m, 2H), 1.80 (dt, *J* = 15.6, 7.6 Hz, 2H), 1.42−1.27 (m, 4H), 0.87 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 153.50, 150.28, 134.61, 133.41, 128.61, 127.40, 126.51, 125.94, 125.85, 124.85, 121.69, 119.07, 114.42, 48.20, 30.85, 26.97, 26.77, 21.65, 13.23. MS (ESI) calcd for $C_{22}H_{24}N_4$, *m/z* 344.20, found 345.21 (M + H)$^+$. 2D ^{1}H COSY and NOESY experiments (included in Supporting Information) provided unambiguous assignment of ^{1}H resonances.

28: 1-Benzyl-2-methyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.75 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.45 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 7.38−7.28 (m, 3H), 7.15 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.10−7.05 (m, 2H), 5.73 (s, 2H), 5.64 (s, 2H), 2.63 (s, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 149.17, 148.91, 142.26, 133.27, 132.64, 127.74, 126.56, 125.74, 124.90, 124.82, 123.96, 120.90, 117.99, 113.37, 47.59, 12.20. MS (ESI) calcd for $C_{18}H_{16}N_4$, *m/z* 288.14, found 289.15 (M + H)$^+$.

29: 1-Benzyl-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.80 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.44 (qd, *J* = 7.2, 3.7 Hz, 1H), 7.37−7.28 (m, 3H), 7.13 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.09−7.01 (m, 2H), 5.73 (s, 2H), 5.59 (s, 2H), 2.97−2.86 (m, 2H), 1.41 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 153.83, 149.91, 143.24, 134.14, 133.05, 128.19, 126.96, 126.04, 125.64, 125.52, 124.43, 121.20, 118.62, 114.05, 47.69, 19.72, 10.98. MS (ESI) calcd for $C_{19}H_{18}N_4$, *m/z* 302.15, found 303.16 (M + H)$^+$.

30: 1-Benzyl-2-propyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.80 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.71 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.49−7.42 (m, 1H), 7.40−7.30 (m, 3H), 7.18−7.10 (m, 1H), 7.10−7.05 (m, 2H), 5.74 (s, 2H), 5.66 (s, 2H), 2.92−2.80 (m, 2H), 1.91−1.85 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 153.50, 150.46, 143.54, 134.77, 133.58, 128.82, 127.60, 126.73, 126.12, 126.05, 125.01, 121.90, 119.30, 114.59, 48.36, 28.88, 20.92, 13.52. MS (ESI) calcd for $C_{20}H_{20}N_4$, *m/z* 316.17, found 317.17 (M + H)$^+$.

31: 1-Benzyl-2-butyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.80 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.72 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.43 (ddd, *J* = 8.4, 7.0, 1.4 Hz, 1H), 7.36−7.28 (m, 3H), 7.12 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.08−7.04 (m, 2H), 5.73 (s, 2H), 5.56 (s, 2H), 2.91−2.83 (m, 2H), 1.84−1.74 (m, 2H), 1.43 (dq, *J* = 14.8, 7.4 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 152.56, 149.54, 142.97, 133.87, 132.54, 127.81, 126.58, 125.61, 125.34, 125.21, 124.06, 120.78, 118.25, 113.71, 47.38, 28.54, 25.71, 21.04, 12.28. MS (ESI) calcd for $C_{21}H_{22}N_4$, *m/z* 330.18, found 331.19 (M + H)$^+$.

33: 1-Benzyl-2-hexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.82 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.38−7.29 (m, 3H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 7.4 Hz, 2H), 5.73 (s, 2H), 5.63 (s, 2H), 2.93−2.83 (m, 2H), 1.87−1.73 (m, 2H), 1.40 (dd, *J* = 14.3, 6.7 Hz, 2H), 1.34−1.24 (m, 4H), 0.88 (t, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 154.01, 151.10, 144.59, 135.38, 133.98, 129.25, 128.02, 127.02, 126.87, 126.69, 125.54, 122.17, 119.70, 115.20, 48.84, 31.44, 29.05, 27.90, 27.46, 22.46, 14.01. MS (ESI) calcd for $C_{23}H_{26}N_4$, *m/z* 358.22, found 359.22 (M + H)$^+$.

34: 1-Benzyl-2-heptyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.71 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.44 (ddd, *J* = 8.4, 7.1, 1.3 Hz, 1H), 7.35−7.29 (m, 3H), 7.12 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 5.72 (s, 4H), 2.86 (dd, *J* = 18.2, 10.2 Hz, 2H), 1.79 (dt, *J* = 15.5, 7.7 Hz, 2H), 1.39 (dt, *J* = 14.9, 7.0 Hz, 2H), 1.34−1.18 (m, 6H), 0.86 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 152.52, 149.22, 142.11, 133.55, 132.39, 127.57, 126.36, 125.49, 124.86, 124.69, 123.80, 120.69, 118.05, 113.31, 47.15, 29.89, 27.60, 27.19, 26.21, 25.75, 20.85, 12.34. MS (ESI) calcd for $C_{24}H_{28}N_4$, *m/z* 372.23, found 373.24 (M + H)$^+$.

35: 1-Benzyl-2-nonyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.85−7.79 (m, 1H), 7.71 (dd, *J* = 8.3, 0.9 Hz, 1H), 7.44 (qd, *J* = 6.9, 3.4 Hz, 1H), 7.38−7.29 (m, 3H), 7.13 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.05 (d, *J* = 6.9 Hz, 2H), 5.85 (s, 2H), 5.72 (s, 2H), 2.90−2.84 (m, 2H), 1.87−1.71 (m, 2H), 1.44−1.33 (m, 2H), 1.33−1.18 (m, 10H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 152.31, 148.72, 141.13, 133.07, 132.10, 127.20, 126.00, 125.25, 124.35, 123.92, 123.40, 120.47, 117.71, 112.77, 46.79, 29.72, 27.59, 27.26, 27.24, 27.13, 27.12, 25.78, 25.35, 20.53, 12.00. MS (ESI) calcd for $C_{26}H_{32}N_4$, *m/z* 400.26, found 401.27 (M + H)$^+$.

36: 1-Benzyl-2-undecyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (d, *J* = 8.2 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.47−7.42 (m, 1H), 7.36−7.28 (m, 3H), 7.15−7.11 (m, 1H), 7.06 (d, *J* = 7.0 Hz, 2H), 5.73 (s, 2H), 5.70 (s, 2H), 3.02−2.71 (m, 2H), 1.79 (dt, *J* = 15.5, 7.8 Hz, 2H), 1.44−1.34 (m, 2H), 1.34−1.18 (m, 14H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 152.56, 149.23, 142.11, 133.58, 132.43, 127.60, 126.39, 125.53, 124.89, 124.73, 123.83, 120.73, 118.08, 113.34, 47.19, 30.20, 27.88, 27.73, 27.67, 27.62, 27.56, 26.24, 25.78, 20.98, 12.43. MS (ESI) calcd for $C_{28}H_{36}N_4$, *m/z* 428.29, found 429.30 (M + H)$^+$.

37: 1-Benzyl-2-pentadecyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.72 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.44 (ddd, *J* = 8.4, 7.1, 1.3 Hz, 1H), 7.36−7.27 (m, 3H), 7.13 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.09−7.02 (m, 2H), 5.73 (s, 2H), 5.69 (s, 2H), 2.91−2.83 (m, 2H), 1.79 (dt, *J* = 15.5, 7.7 Hz, 2H), 1.59−1.33 (m, 2H), 1.33−1.16 (m, 22H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 151.99, 148.66, 141.59, 133.01, 131.85, 127.03, 125.82, 124.96, 124.32, 124.17, 123.26, 120.16, 117.50, 112.78, 46.62, 29.66, 27.43, 27.40, 27.39, 27.37, 27.32, 27.17, 27.11, 27.10, 26.99, 25.68, 25.21, 20.43, 11.86. MS (ESI) calcd for $C_{32}H_{44}N_4$, *m/z* 484.36, found 485.36 (M + H)$^+$.

38: 1-Benzyl-2-(but-3-enyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.80 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.46−7.42 (m, 1H), 7.36−7.29 (m, 3H), 7.13 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.08−7.05 (m, 2H), 5.96−5.84 (m, 1H), 5.74 (s, 2H), 5.58 (s, 2H), 5.11−4.97 (m, 2H), 3.03−2.93 (m, 2H), 2.64−2.54 (m, 2H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 150.99, 148.87, 134.50, 133.06, 131.93, 127.16, 125.97, 125.02, 124.64, 124.58, 123.43, 120.17, 117.57, 113.92, 46.72, 29.59, 24.77. MS (ESI) calcd for $C_{21}H_{20}N_4$, *m/z* 328.17, found 329.18 (M + H)$^+$.

Synthesis of Compound 40: *N*-Benzyl-3-nitroquinolin-4-amine. To a solution of **39** (2.03 mg, 9.76 mmol) in 20 mL of anhydrous dichloromethane were added triethylamine (1.48 g, 14.64 mmol) and benzylamine (1.36 g, 12.69 mmol). The reaction mixture was refluxed at 45 °C for 30 min. The solvent was then evaporated

under vacuum, and water was added to the residue to obtain the precipitate This was filtered, washed several times with water, and dried to obtain the compound **40** (2.6 g, 95%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.89 (s, 1H), 9.42 (s, 1H), 8.31 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.79 (t, J = 7.6 Hz, 1H), 7.56–7.35 (m, 6H), 5.13 (d, J = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 151.01, 150.64, 147.37, 136.84, 132.73, 130.51, 129.36, 128.54, 127.16, 126.84, 126.33, 125.58, 119.14, 53.06. MS (ESI) calcd for C$_{16}$H$_{13}$N$_3$O$_2$, m/z 279.10, found 280.11 (M + H)$^+$.

Synthesis of Compound 41: N^4-Benzylquinoline-3,4-diamine. To a solution of **40** (2.6 g, 7.06 mmol) in 10 mL of EtOAc were added a catalytic amount of Pt/C and Na$_2$SO$_4$. The reaction mixture was subjected to hydrogenation at 55 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 **41** (2.3 g, 96%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.53 (s, 1H), 8.00 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.56–7.29 (m, 7H), 4.43 (s, 2H), 4.05 (s, 1H), 3.78 (s, 2H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 143.59, 143.48, 141.10, 139.77, 129.89, 129.84, 128.81, 127.77, 127.60, 126.05, 125.76, 120.06, 99.98, 50.98. MS (ESI) calcd for C$_{16}$H$_{15}$N$_3$, m/z 249.13, found 250.13 (M + H)$^+$.

Synthesis of Compound 43: 1-Benzyl-2-(but-3-ynyl)-1H-imidazo-[4,5-c]quinolin-4-amine. To a solution of pentynoic acid (37 mg, 0.38 mmol) in anhydrous DMF were added HBTU (142 mg, 0.38 mmol), triethylamine (52 mg, 0.51 mmol), a catalytic amount of DMAP, and **41** (85 mg, 0.34 mmol). The reaction mixture was then heated to 90 °C for 1 h. The solvent was then removed under vacuum. The residue was dissolved in EtOAc, washed with water, dried over Na$_2$SO$_4$, and then concentrated to obtain the residue. This was purified using column chromatography (7% MeOH/ dichloromethane) to obtain the compound **42** (60 mg). To a solution of **42** in a solvent mixture of MeOH:dichloromethane: chloroform (0.1:1:1) was added 3-chloroperoxybenzoic acid (83 mg, 0.48 mmol), and the solution was refluxed at 45–50 °C for 40 min. The solvent was then removed under vacuum, and the residue was purified using column chromatography (8% MeOH/ dichloromethane) to obtain the *N*-oxide derivative (30 mg). This was then dissolved in anhydrous dichloromethane, followed by the addition of benzoyl isocyanate (20 mg, 0.14 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 the addition of excess sodium methoxide. The reaction mixture was then heated at 80 °C for 1 h. The solvent was removed under vacuum, and the residue was purified using column chromatography (6% MeOH/dichloromethane) to obtain the compound **43** (15 mg, 12%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.81 (dd, J = 8.4, 0.8 Hz, 1H), 7.74 (dd, J = 8.3, 1.0 Hz, 1H), 7.46 (ddd, J = 8.4, 5.7, 1.3 Hz, 1H), 7.37–7.30 (m, 3H), 7.15 (ddd, J = 8.3, 7.1, 1.2 Hz, 1H), 7.09–7.05 (m, 2H), 5.79 (s, 2H), 5.68 (s, 2H), 3.11 (dd, J = 8.8, 6.4 Hz, 2H), 2.87–2.71 (m, 2H), 1.99 (t, J = 2.6 Hz, 1H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 150.49, 149.42, 133.45, 132.82, 127.91, 126.75, 126.01, 125.19, 124.98, 124.08, 121.13, 118.33, 113.52, 81.16, 68.15, 47.46, 25.06, 15.63. MS (ESI) calcd for C$_{21}$H$_{18}$N$_4$, m/z 326.15, found 327.16 (M + H)$^+$.

Synthesis of Compound 44: 1-Benzyl-4-chloro-2-pentyl-1H-imidazo[4,5-c]quinoline. To a solution **27** (200 mg, 0.71 mmol) in anhydrous THF were added triethylamine (179 mg, 1.77 mmol) and hexanoyl chloride (143 mg, 1.06 mmol). The reaction mixture was then stirred for 6–8 h, followed by removal of the solvent under vacuum. The residue was dissolved in EtOAc, washed with water and brine and then dried over Na$_2$SO$_4$ to obtain the intermediate amide compound. This was dissolved in MeOH, followed by the addition of calcium oxide. The reaction mixture was then heated in microwave at 110 °C for 1 h. The solvent was removed under vacuum, and the residue was purified using column chromatography (3% MeOH/dichloro- methane) to obtain the compound **44** (170 mg, 66%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.16 (d, J = 8.4 Hz, 1H), 7.88

(d, J = 8.4 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.38–7.30 (m, 3H), 7.05 (d, J = 7.0 Hz, 2H), 5.81 (s, 2H), 3.05–2.95 (m, 2H), 1.84 (dt, J = 15.6, 7.6 Hz, 2H), 1.48–1.24 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.40, 143.96, 143.60, 135.40, 134.74, 133.74, 129.93, 129.42, 128.30, 127.56, 126.58, 125.40, 119.91, 117.29, 49.19, 31.63, 27.91, 27.70, 22.29, 13.90. MS (ESI) calcd for C$_{22}$H$_{22}$ClN$_3$, m/z 363.15, found 364.15 (M + H)$^+$.

Synthesis of Compound 46: 1-Benzyl-2-pentyl-1H-imidazo-[4,5-c]quinolin-4-ol. The compound **44** (50 mg, 0.14 mmol) was dissolved in 6 M HCl, and the solution was heated in a micro- wave at 110 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromato- graphy (7% MeOH/dichloromethane) to obtain the compound **46** (38 mg, 80%). ^{1}H NMR (400 MHz, DMSO) δ 11.59 (s, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 8.2 Hz, 1H), 7.39–7.30 (m, J = 8.3 Hz, 3H), 7.30–7.23 (m, J = 7.2 Hz, 1H), 7.08–7.00 (m, J = 7.5 Hz, 3H), 5.83 (s, 2H), 2.83 (t, J = 7.5 Hz, 2H), 1.79–1.65 (m, 2H), 1.40–1.16 (m, 4H), 0.82 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 157.78, 154.36, 137.04, 136.73, 134.44, 131.09, 129.43, 128.08, 127.95, 125.94, 121.98, 121.27, 116.69, 112.04, 48.44, 31.22, 27.11, 26.69, 22.30, 14.26. MS (ESI) calcd for C$_{22}$H$_{23}$N$_3$, m/z 345.18, found 346.19 (M + H)$^+$.

Synthesis of Compound 47: 1-Benzyl-2-pentyl-4-phenyl-1H-imidazo[4,5-c]quinoline. Suzuki coupling of the compound **44** with phenyl boronic acid was performed. To a solution of **44** (70 mg, 0.19 mmol) in THF were added polystyrene-bound PPh$_3$-Pd (9.5 mg, 0.00095 mmol), K$_2$CO$_3$ (40 mg, 0.29 mmol) dissolved in water, and phenyl boronic acid (28 mg, 0.23 mmol). The reaction was heated at 80 °C for 10–12 h. The solution was filtered to remove the solid resin, and the filtrate was evaporated under vacuum to obtain the residue. This was purified using column chromatography (30% EtOAc/hexane) to obtain the compound **47** (25 mg, 33%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.81–8.77 (m, 1H), 8.33 (dd, J = 8.5, 0.8 Hz, 1H), 7.93 (dd, J = 8.4, 0.8 Hz, 1H), 7.64–7.58 (m, 3H), 7.55–7.49 (m, 1H), 7.36 (dddd, J = 9.8, 6.9, 6.1, 2.2 Hz, 4H), 7.12–7.07 (m, 2H), 5.84 (s, 2H), 3.03–2.85 (m, 2H), 1.91 (dt, J = 15.5, 7.6 Hz, 2H), 1.42 (ddt, J = 36.4, 21.9, 7.4 Hz, 4H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 155.17, 151.02, 144.50, 138.18, 135.43, 135.10, 135.01, 130.96, 130.01, 129.31, 129.27, 128.37, 128.05, 126.74, 125.76, 125.52, 119.61, 117.17, 48.92, 31.58, 27.56, 27.48, 22.37, 13.97. MS (ESI) calcd for C$_{28}$H$_{27}$N$_3$, m/z 405.22, found 406.23 (M + H)$^+$.

Synthesis of Compound 48: N-(1-Benzyl-2-butyl-1H-imidazo-[4,5-c]quinolin-4-yl)hydroxylamine. To a solution of **27** (100 mg, 0.35 mmol) in anhydrous THF were added triethylamine (72 mg, 0.71 mmol) and valeryl chloride (51 mg, 0.42 mmol). The reaction mixture was then stirred for 6–8 h, followed by removal of the solvent under vacuum. The residue was dissolved in EtOAc, washed with water and brine, and then dried over Na$_2$SO$_4$ to obtain the residue. This was dissolved in MeOH, followed by the addition of calcium oxide. The reaction mixture was then heated in microwave at 110 °C for 1 h. The solvent was removed under vacuum, and the residue was purified using column chromatography (3% MeOH/dichloromethane) to ob- tain **45** (75 mg, 62%). To a solution of **45** (25 mg, 0.072 mmol) in anhydrous MeOH were added triethylamine (72 mg, 0.72 mmol) and hydroxylamine hydrochloride (76 mg, 1.08 mmol). The reaction mixture was heated at 50 °C for 10–12 h. The solvent was then removed under vacuum, and the residue was dissolved in EtOAc, washed with water, and dried over Na$_2$SO$_4$. The EtOAc fraction was then evaporated under vacuum, and the residue was purified using basic-alumina (pH > 7) column chromatography (30% MeOH/dichloromethane) to obtain the compound **48** (10 mg, 40%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.41 (d, J = 8.1 Hz, 1H), 7.32 (dq, J = 25.0, 7.2 Hz, 4H), 7.21 (s, 1H), 7.06 (d, J = 7.1 Hz, 2H), 6.88 (dd, J = 10.6, 5.7 Hz, 1H), 5.61 (s, 2H), 2.84–2.77 (m, 2H), 1.78 (dt, J = 15.5, 7.7 Hz, 2H), 1.40 (dt, J = 15.0, 7.4 Hz, 2H), 0.90–0.86 (m, 3H). ^{13}C NMR (126 MHz,

CDCl$_3$) δ 151.78, 133.18, 127.13, 125.87, 125.13, 123.30, 119.10, 117.94, 46.50, 27.68, 24.81, 20.34, 11.58. MS (ESI) calcd for C$_{21}$H$_{22}$N$_4$O, *m/z* 346.18, found 347.19 (M + H)$^+$.

Synthesis of Compound 49: 1-Benzyl-2-butyl-4-hydrazinyl-1*H*-imidazo[4,5-*c*]quinoline. To a solution of **45** (60 mg, 0.17 mmol) in anhydrous MeOH was added *tert*-butyl carbazate (57 mg, 0.43 mmol). After 15 h, the solvent was removed under vacuum and the residue was purified using column chromatography (4% MeOH/dichloromethane) to obtain the intermediate *N*-Boc derivative. This was then dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of the compound **49** (55 mg, 57%). ^{1}H NMR (500 MHz, MeOD) δ 7.82 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 7.45−7.40 (m, 1H), 7.28 (dt, *J* = 25.5, 7.2 Hz, 3H), 7.15−7.10 (m, 2H), 7.03 (d, *J* = 7.4 Hz, 2H), 5.82 (s, 2H), 2.94−2.90 (m, 2H), 1.79−1.69 (m, 2H), 1.40 (dq, *J* = 14.8, 7.4 Hz, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 154.65, 151.25, 143.19, 135.64, 133.54, 128.81, 128.68, 127.41, 126.96, 125.31, 125.10, 124.68, 122.23, 120.02, 114.47, 48.23, 29.28, 26.26, 21.84, 12.51. MS (ESI) calcd for C$_{21}$H$_{23}$N$_5$, *m/z* 345.20, found 346.20 (M + H)$^+$.

Synthesis of Compound 50: 1-Benzyl-2-butyl-1*H*-imidazo[4,5-*c*]-quinoline. To a solution of **41** (300 mg, 1.221 mmol) in anhydrous THF were added triethylamine (183 mg, 1.82 mmol) and valeryl chloride (174 mg, 1.45 mmol). The reaction mixture was then stirred for 6−8 h, followed by removal of the solvent under vacuum. The residue was dissolved in EtOAc, washed with water and brine, and then dried over Na$_2$SO$_4$ to obtain the intermediate amide compound. This was dissolved in MeOH, followed by the addition of calcium oxide. The reaction mixture was then heated in microwave at 110 °C for an hour. The solvent was removed under vacuum, and the residue was purified using column chromatography (9% MeOH/dichloromethane) to obtain the compound **50** (360 mg, 94%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.38 (s, 1H), 8.25 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.66−7.57 (m, 1H), 7.42 (dd, *J* = 11.2, 4.1 Hz, 1H), 7.40−7.31 (m, 3H), 7.06 (d, *J* = 6.6 Hz, 2H), 5.80 (s, 2H), 3.02−2.85 (m, 2H), 1.90 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.57−1.39 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 155.81, 144.98, 144.75, 136.53, 135.14, 134.10, 130.83, 129.35, 128.15, 126.72, 126.29, 125.47, 119.88, 117.58, 48.94, 29.57, 27.18, 22.53, 13.77. MS (ESI) calcd for C$_{21}$H$_{21}$N$_3$, *m/z* 315.17, found 316.18 (M + H)$^+$.

Synthesis of Compound 52: 1-Benzyl-2-((ethylamino)methyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine. To a solution of 2-(*tert*-butoxycarbonyl(ethyl)amino)acetic acid (90 mg, 0.44 mmol) in anhydrous DMF were added HBTU (167 mg, 0.44 mmol), triethylamine (53 mg, 0.52 mmol), a catalytic amount of DMAP, and **41** (100 mg, 0.4 mmol). The reaction mixture was then heated to 70 °C for 18 h. The solvent was then removed under vacuum. The residue was dissolved in EtOAc, washed with water, dried using Na$_2$SO$_4$, and then concentrated to obtain the residue, which was purified using column chromatography (6% MeOH/dichloromethane) to obtain the compound **51** (133 mg). To a solution of **51** in a solvent mixture of MeOH:dichloromethane:chloroform (0.1:1:1), 3-chloroperoxybenzoic acid (138 mg, 0.8 mmol) was added and the solution was refluxed at 45−50 °C for 40 min. The solvent was then removed under vacuum, and the residue was purified using column chromatography (10% MeOH/dichloromethane) to obtain the *N*-oxide derivative (31 mg). This was then dissolved in anhydrous dichloromethane, followed by the addition of benzoyl isocyanate (16 mg, 0.11 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 the addition of excess sodium methoxide and heated at 80 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (8% MeOH/dichloromethane) to obtain the intermediate *N*-Boc derivative. This

was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min, followed by removal of the solvent by purging nitrogen and drying under vacuum to obtain the trifluoroacetate salt of the compound **52** (26 mg, 20%). ^{1}H NMR (400 MHz, MeOD) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.49−7.32 (m, 4H), 7.15 (d, *J* = 7.6 Hz, 2H), 6.00 (s, 2H), 4.71 (s, 2H), 3.38 (q, *J* = 7.3 Hz, 2H), 1.45 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 149.52, 148.45, 136.86, 134.46, 134.03, 130.18, 129.15, 128.12, 125.29, 125.15, 124.97, 121.76, 118.34, 112.47, 48.94, 43.17, 42.36, 10.06. MS (ESI) calcd for C$_{20}$H$_{21}$N$_5$, *m/z* 331.18, found 332.19 (M + H)$^+$.

Synthesis of Compound 54: 1-(4-Amino-2-butyl-1*H*-imidazo-[4,5-*c*]quinolin-1-yl)-2-methylpropan-2-ol. To a solution of **20** (100 mg, 0.43 mmol) in anhydrous THF were added triethylamine (66 mg, 0.65 mmol) and valeryl chloride (62 mg, 0.52 mmol). The reaction mixture was then stirred for 6−8 h, followed by removal of the solvent under vacuum. The residue was dissolved in EtOAc, washed with water and brine, and then dried over Na$_2$SO$_4$ to obtain the intermediate amide compound. This was dissolved in MeOH, followed by the addition of calcium oxide, and was heated in microwave at 110 °C for 1 h. The solvent was then removed and the residue was purified using column chromatography (9% MeOH/dichloromethane) to obtain the compound **53** (58 mg). To a solution of **53** in a solvent mixture of MeOH:dichloromethane:chloroform (0.1:1:1) was added 3-chloroperoxybenzoic acid (84 mg, 0.49 mmol), and the solution was refluxed at 45−50 °C for 40 min. The solvent was then removed and the residue was purified using column chromatography (20% MeOH/dichloromethane) to obtain the *N*-oxide derivative (55 mg). This was then dissolved in anhydrous dichloromethane, followed by the addition of benzoyl isocyanate (39 mg, 0.26 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 the addition of excess sodium methoxide. The reaction mixture was then heated at 80 °C for an hour. The solvent was removed under vacuum, and the residue was purified using column chromatography (11% MeOH/dichloromethane) to obtain the compound **54** (40 mg, 30%). ^{1}H NMR (400 MHz, MeOD) δ 8.26 (d, *J* = 8.3 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 4.63 (s, 2H), 3.20−3.02 (m, 2H), 1.98−1.80 (m, 2H), 1.62−1.45 (m, 2H), 1.27 (s, 6H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 156.33, 151.22, 143.80, 134.53, 126.75, 125.41, 125.12, 121.49, 120.93, 115.31, 71.16, 54.80, 29.72, 27.12, 26.52, 22.24, 12.86. MS (ESI) calcd for C$_{18}$H$_{24}$N$_4$O, *m/z* 312.20, found 313.20 (M + H)$^+$.

Synthesis of Compound 55: 1-Benzyl-4-chloro-1*H*-[1,2,3]triazolo-[4,5-*c*]quinoline. To a solution of **27** (60 mg, 0.21 mmol) in 1.5 mL of 1:1 acetic acid/water at 0 °C was added 0.5N aqueous solution of sodium nitrite (0.56 mL, 0.28 mmol) and the reaction was stirred for 2 h. The solvent was then removed under vacuum. The residue was dissolved in EtOAc, washed with water, and dried over Na$_2$SO$_4$. The EtOAc fraction was then concentrated under vacuum to obtain the compound **55** (58 mg, 94%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.12 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.96 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.70 (ddd, *J* = 8.4, 7.2, 1.4 Hz, 1H), 7.52 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H), 7.30−7.21 (m, 3H), 7.12−7.09 (m, 2H), 6.19 (s, 2H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 142.94, 141.16, 136.74, 132.71, 131.61, 128.30, 127.80, 127.24, 126.61, 125.80, 124.24, 124.20, 120.01, 112.71, 52.00. MS (ESI) calcd for C$_{16}$H$_{11}$ClN$_4$, *m/z* 294.07, found 317.06 (M + Na$^+$) and 612.11 (M + M + Na$^+$).

Synthesis of Compound 56: 1-Benzyl-1*H*-[1,2,3]triazolo[4,5-*c*]quinolin-4-amine. Compound **55** (46 mg, 0.16 mmol) was dissolved in 0.1 mL of 2 M ammonia solution in MeOH, and the reaction was heated in a microwave at 150 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (65% EtOAc/hexane) to obtain the compound **56** (35 mg, 82%). ^{1}H NMR (500 MHz, TFA) δ 8.25−8.13 (m, 1H), 7.87 (d, *J* = 7.2 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 6.1 Hz, 1H), 7.39 (s, 3H), 7.24 (s, 2H),

6.38 (d, J = 23.6 Hz, 2H). ^{13}C NMR (126 MHz, TFA) δ 134.33, 133.27, 131.52, 129.18, 129.06, 127.08, 125.83, 123.58, 118.48, 117.54, 115.29, 113.04, 110.78, 54.87. MS (ESI) calcd for $C_{16}H_{11}N_5$, m/z 275.12, found 276.12 (M + H)$^+$.

Synthesis of Compound 57: 1-Benzyl-2-oxo-N-propyl-1H-imidazo[4,5-c]quinoline-3($2H$)-carboxamide. To a solution of **41** (30 mg, 0.12 mmol) in anhydrous THF were added triethylamine (15 mg, 0.14 mmol) and n-propyl isocyanate (33 mg, 0.39 mmol); the n-propylamine released as a consequence of cyclization en route to the cyclic urea was found to consume n-propyl isocyanate, necessitating the use of additional equivalents of the reagent. The reaction was heated in microwave at 100 °C for 1 h. The solvent was then removed under vacuum, and the residue was purified using column chromatography (6% MeOH/dichloromethane) to obtain the compound **57** (26 mg, 61%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.90 (s, 1H), 8.79 (s, J = 5.4 Hz, 1H), 8.15 (d, J = 8.5, 0.6 Hz, 1H), 7.91 (d, J = 8.6, 0.6 Hz, 1H), 7.60 (t, J = 8.4, 6.9, 1.3 Hz, 1H), 7.40 (t, J = 8.3, 6.9, 1.2 Hz, 1H), 7.37−7.32 (m, 2H), 7.31−7.28 (m, 1H), 7.26 (d, J = 6.7, 5.4 Hz, 2H), 5.60 (s, 2H), 3.49−3.45 (m, J = 7.1, 5.8 Hz, 2H), 1.78−1.68 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 151.08, 148.67, 143.44, 136.25, 132.69, 128.54, 127.08, 127.02, 125.87, 125.74, 124.61, 123.68, 118.43, 117.87, 112.78, 44.45, 39.68, 20.57, 9.22. MS (ESI) calcd for $C_{21}H_{20}N_4O_2$, m/z 360.16, found 361.17 (M + H)$^+$.

NF-κB Induction. The induction of NF-κB was quantified using HEK-Blue-7 cells and HEK-Blue-8 cells as previously described by us.[8,24] HEK293 cells were stably transfected with 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-κB/AP-1 promoters is inducible by the TLR7 (or TLR8) agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue cells were incubated at a density of $\sim$10^5 cells/mL in a volume of 80 μL/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.

IFN-α Induction in Whole Human Blood. Aliquots (3 mL) of heparin-anticoagulated blood obtained from healthy human donors after informed consent and stimulated for 12 h with graded concentrations of test compounds. The plasma 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).

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

References

(1) Kawai, T.; Akira, S. TLR signaling. *Semin. Immunol.* **2007**, *19*, 24–32.

(2) Kumagai, Y.; Takeuchi, O.; Akira, S. Pathogen recognition by innate receptors. *J. Infect. Chemother.* **2008**, *14*, 86–92.

(3) Akira, S. Innate immunity to pathogens: diversity in receptors for microbial recognition. *Immunol. Rev.* **2009**, *227*, 5–8.

(4) Akira, S.; Uematsu, S.; Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **2006**, *124*, 783–801.

(5) Akira, S.; Takeda, K.; Kaisho, T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nature Immunol.* **2001**, *2*, 675–680.

(6) Cottalorda, A.; Verschelde, C.; Marcais, A.; Tomkowiak, M.; Musette, P.; Uematsu, S.; Akira, S.; Marvel, J.; Bonnefoy-Berard, N. TLR2 engagement on CD8 T cells lowers the threshold for optimal antigen-induced T cell activation. *Eur. J. Immunol.* **2006**, *36*, 1684–1693.

(7) Kaisho, T.; Akira, S. Toll-like receptors as adjuvant receptors. *Biochim. Biophys. Acta* **2002**, *1589*, 1–13.

(8) Hood, J. D.; Warshakoon, H. J.; Kimbrell, M. R.; Shukla, N. M.; Malladi, S.; Wang, X.; David, S. A. Immunoprofiling toll-like receptor ligands: comparison of immunostimulatory and proinflammatory profiles in ex vivo human blood models. *Hum. Vaccines* **2010**, *6*, 1–14.

(9) Lee, J.; Chuang, T.-H.; Redecke, V.; She, L.; Pitha, P. M.; Carson, D. A.; Raz, E.; Cottam, H. B. Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6646–6651.

(10) Crozat, K.; Beutler, B. TLR7: A new sensor of viral infection. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 6835–6836.

(11) Diebold, S. S.; Kaisho, T.; Hemmi, H.; Akira, S.; Reis e Sousa, C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* **2004**, *303*, 1529–1531.

(12) Wierenga, W.; Skulnick, H. I.; Stringfellow, D. A.; Weed, S. D.; Renis, H. E.; Eidson, E. E. 5-Substituted 2-amino-6-phenyl-4(3H)-pyrimidinones. Antiviral- and interferon-inducing agents. *J. Med. Chem.* **1980**, *23*, 237–239.

(13) Li, L. H.; Wallace, T. L.; Wierenga, W.; Skulnick, H. I.; DeKoning, T. F. Antitumor activity of pyrimidinones, a class of small-molecule biological response modifiers. *J. Biol. Response Mod.* **1987**, *6*, 44–55.

(14) Stringfellow, D. A.; Glasgow, L. A. Tilorone hydrochloride: an oral interferon-inducing agent. *Antimicrob. Agents Chemother.* **1972**, *2*, 73–78.

(15) Stringfellow, D. A. Comparation interferon- inducing and antiviral properties of 2-amino-5-bromo-6-methyl-4-pyrimidinol (U-25,166), tilorone hydrochloride, and polyinosinic-polycytidylic acid. *Antimicrob. Agents Chemother.* **1977**, *11*, 984–992.

(16) Hamilton, R. D.; Wynalda, M. A.; Fitzpatrick, F. A.; Teagarden, D. L.; Hamdy, A. H.; Snider, B. G.; Weed, S. D.; Stringfellow, D. A. Comparison between circulating interferon and drug levels following administration of 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP) to different animal species. *J. Interferon Res.* **1982**, *2*, 317–327.

(17) Gerster, J. F.; Lindstrom, K. J.; Miller, R. L.; Tomai, M. A.; Birmachu, W.; Bomersine, S. N.; Gibson, S. J.; Imbertson, L. M.; Jacobson, J. R.; Knafla, R. T.; Maye, P. V.; Nikolaides, N.; Oneyemi, F. Y.; Parkhurst, G. J.; Pecore, S. E.; Reiter, M. J.; Scribner, L. S.; Testerman, T. L.; Thompson, N. J.; Wagner, T. L.; Weeks, C. E.; Andre, J. D.; Lagain, D.; Bastard, Y.; Lupu, M. Synthesis and structure−activity relationships of 1H-imidazo[4,5-c]quinolines that induce interferon production. *J. Med. Chem.* **2005**, *48*, 3481–3491.

(18) Miller, R. L.; Gerster, J. F.; Owens, M. L.; Slade, H. B.; Tomai, M. A. Imiquimod applied topically: a novel immune response modifier and new class of drug. *Int. J. Immunopharmacol.* **1999**, *21*, 1–14.

(19) Hemmi, H.; Kaisho, T.; Takeuchi, O.; Sato, S.; Sanjo, H.; Hoshino, K.; Horiuchi, T.; Tomizawa, H.; Takeda, K.; Akira, S. Small antiviral compounds activate immune cells via the TLR7MyD88-dependent signaling pathway. *Nat. Immunol.* **2002**, *3*, 196–200.

(20) Weterings, J. J.; Khan, S.; van der Heden van Noort, G. J.; Melief, C. J.; Overkleeft, H. S.; van der Burg, S. H.; Ossendorp, F.; Van der Marel, G. A.; Filippov, D. V. 2-Azidoalkoxy-7-hydro-8-oxoadenine derivatives as TLR7 agonists inducing dendritic cell maturation. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2249–2251.

(21) Hirota, K.; Kazaoka, K.; Niimoto, I.; Kumihara, H.; Sajiki, H.; Isobe, Y.; Takaku, H.; Tobe, M.; Ogita, H.; Ogino, T.; Ichii, S.; Kurimoto, A.; Kawakami, H. Discovery of 8-hydroxyadenines as a novel type of interferon inducer. *J. Med. Chem.* **2002**, *45*, 5419–5422.

(22) Isobe, Y.; Tobe, M.; Ogita, H.; Kurimoto, A.; Ogino, T.; Kawakami, H.; Takaku, H.; Sajiki, H.; Hirota, K.; Hayashi, H. Synthesis and structure−activity relationships of 2-substituted-8-hydroxyadenine derivatives as orally available interferon inducers without emetic side effects. *Bioorg. Med. Chem.* **2003**, *11*, 3641–3647.

(23) Kurimoto, A.; Ogino, T.; Ichii, S.; Isobe, Y.; Tobe, M.; Ogita, H.; Takaku, H.; Sajiki, H.; Hirota, K.; Kawakami, H. Synthesis and evaluation of 2-substituted 8-hydroxyadenines as potent interferon inducers with improved oral bioavailabilities. *Bioorg. Med. Chem.* **2004**, *12*, 1091–1099.

(24) Shukla, N. M.; Kimbrell, M. R.; Malladi, S. S.; David, S. A. Regioisomerism-dependent TLR7 agonism and antagonism in an imidazoquinoline. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2211–2214.

(25) Warshakoon, H. J.; Hood, J. D.; Kimbrell, M. R.; Malladi, S.; Wu, W. Y.; Shukla, N. M.; Agnihotri, G.; Sil, D.; David, S. A. Potential adjuvantic properties of innate immune stimuli. *Hum. Vaccines* **2009**, *5*, 381–394.

(26) Nicolas, J. F.; Guy, B. Intradermal, epidermal and transcutaneous vaccination: from immunology to clinical practice. *Expert Rev. Vaccines* **2008**, *7*, 1201–1214.

(27) Skountzou, I.; Kang, S. M. Transcutaneous immunization with influenza vaccines. *Curr. Top. Microbiol. Immunol.* **2009**, *333:347−68*, 347–368.

(28) Giudice, E. L.; Campbell, J. D. Needle-free vaccine delivery. *Adv. Drug Delivery Rev.* **2006**, *58*, 68–89.

(29) Goldstein, G.; Chicca, J. J. A universal anti-HIV-1 Tat epitope vaccine that is fully synthetic and self-adjuvanting. *Vaccine* **2010**, *28*, 1008–1014.

(30) Fujita, Y.; Abdel-Aal, A. B.; Wimmer, N.; Batzloff, M. R.; Good, M. F.; Toth, I. Synthesis and immunological evaluation of self-adjuvanting glycolipopeptide vaccine candidates. *Bioorg. Med. Chem.* **2008**, *16*, 8907–8913.

(31) Gupta, S.; Vayuvegula, B.; Gollapudi, S. Substituted guanine ribonucleosides as B cell activators. *Clin. Immunol. Immunopathol.* **1991**, *61*, S21–S27.

(32) Pope, B. L.; Chourmouzis, E.; Sigindere, J.; MacIntyre, J. P.; Capetola, R. J.; Lau, C. Y. In vivo activation of natural killer cells and priming of IL-2 responsive cytolytic cells by loxoribine (7-allyl-8-oxoguanosine). *Cell Immunol.* **1993**, *147*, 302–312.

(33) Goodman, M. G. A new approach to vaccine adjuvants. Immuno-potentiation by intracellular T-helper-like signals transmitted by loxoribine. *Pharm. Biotechnol.* **1995**, *6*, 581–609.

(34) Reitz, A. B.; Goodman, M. G.; Pope, B. L.; Argentieri, D. C.; Bell, S. C.; Burr, L. E.; Chourmouzis, E.; Come, J.; Goodman, J. H.; Klaubert, D. H.; Maryanoff, B. D.; McDonnell, M. E.; Rampulla, M. S.; Schott, M. R.; Chen, R. Small-molecule immunostimulants. Synthesis and activity of 7,8-disubstituted guanosines and structurally related compounds. *J. Med. Chem.* **1994**, *37*, 3561–3578.

(35) Durand, V.; Wong, S. Y.; Tough, D. F.; Le Bon, A. Shaping of adaptive immune responses to soluble proteins by TLR agonists: a role for IFN-alpha/beta. *Immunol. Cell Biol.* **2004**, *82*, 596–602.

(36) Heil, F.; Ahmad-Nejad, P.; Hemmi, H.; Hochrein, H.; Ampenberger, F.; Gellert, T.; Dietrich, H.; Lipford, G.; Takeda, K.; Akira, S.; Wagner, H.; Bauer, S. The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur. J. Immunol.* **2003**, *33*, 2987–2997.

(37) Ito, T.; Amakawa, R.; Kaisho, T.; Hemmi, H.; Tajima, K.; Uehira, K.; Ozaki, Y.; Tomizawa, H.; Akira, S.; Fukuhara, S. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J. Exp. Med.* **2002**, *195*, 1507–1512.

(38) Baldwin, S. L.; Bertholet, S.; Kahn, M.; Zharkikh, I.; Ireton, G. C.; Vedvick, T. S.; Reed, S. G.; Coler, R. N. Intradermal immunization improves protective efficacy of a novel TB vaccine candidate. *Vaccine* **2009**, *27*, 3063–3071.

(39) Zhang, W. W.; Matlashewski, G. Immunization with a Toll-like receptor 7 and/or 8 agonist vaccine adjuvant increases protective immunity against *Leishmania major* in BALB/c mice. *Infect. Immun.* **2008**, *76*, 3777–3783.

(40) Zaba, L. C.; Krueger, J. G.; Lowes, M. A. Resident and "inflammatory" dendritic cells in human skin. *J. Invest. Dermatol.* **2009**, *129*, 302–308.

(41) Miller, L. S.; Modlin, R. L. Toll-like receptors in the skin. *Semin. Immunopathol.* **2007**, *29*, 15–26.

(42) Mathers, A. R.; Larregina, A. T. Professional antigen-presenting cells of the skin. *Immunol. Res.* **2006**, *36*, 127–136.

(43) Rajagopal, D.; Paturel, C.; Morel, Y.; Uematsu, S.; Akira, S.; Diebold, S. S. Plasmacytoid dendritic cell-derived type I interferon is crucial for the adjuvant activity of Toll-like receptor 7 agonists. *Blood* **2010**, *115*, 1949–1957.

(44) Jin, M. S.; Lee, J. O. Structures of TLR−ligand complexes. *Curr. Opin. Immunol.* **2008**, *20*, 414–419.

(45) Adediran, S. A.; Day, T. P.; Sil, D.; Kimbrell, M. R.; Warshakoon, H. J.; Malladi, S. S.; David, S. A. Synthesis of a highly water-soluble derivative of amphotericin B with attenuated proinflammatory activity. *Mol. Pharmaceutics* **2009**, *6*, 1582–1590.

(46) Nguyen, T. B.; Kumar, E. V.; Sil, D.; Wood, S. J.; Miller, K. A.; Warshakoon, H. J.; Datta, A.; David, S. A. Controlling plasma protein binding: structural correlates of interactions of hydrophobic polyamine endotoxin sequestrants with human serum albumin. *Mol. Pharmaceutics* **2008**, *5*, 1131–1137.

(47) Gorden, K. B.; Gorski, K. S.; Gibson, S. J.; Kedl, R. M.; Kieper, W. C.; Qiu, X.; Tomai, M. A.; Alkan, S. S.; Vasilakos, J. P. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. *J. Immunol.* **2005**, *174*, 1259–1268.

(48) Gorski, K. S.; Waller, E. L.; Bjornton-Severson, J.; Hanten, J. A.; Riter, C. L.; Kieper, W. C.; Gorden, K. B.; Miller, J. S.; Vasilakos, J. P.; Tomai, M. A.; Alkan, S. S. Distinct indirect pathways govern human NK-cell activation by TLR-7 and TLR-8 agonists. *Int. Immunol.* **2006**, *18*, 1115–1126.

(49) Middlebrooks, B. L.; Voss, P. G.; Douglas, W. L.; Toom, P. M. Procedure for selecting monoclonal antibodies for use in a ligand displacement assay of serum antibody levels. *J. Immunoassay* **1991**, *12*, 125–144.

(50) Simon, F.; Rahimy, C.; Krivine, A.; Levine, M.; Pepin, J. M.; Lapierre, D.; Denamur, E.; Vernoux, L.; De Crepy, A.; Blot, P. and . Antibody avidity measurement and immune complex dissociation for serological diagnosis of vertically acquired HIV-1 infection. *JAIDS, J. Acquired Immune Defic. Syndr.* **1993**, *6*, 201–207.

(51) Liu, X.; Hefesha, H.; Tanaka, H.; Scriba, G.; Fahr, A. Lipophilicity measurement of drugs by reversed phase HPLC over wide pH range using an alkaline-resistant silica-based stationary phase, XBridge Shield RP(18). *Chem. Pharm. Bull.* (*Tokyo*). **2008**, *56*, 1417–1422.

(52) Fuguet, E.; Rafols, C.; Bosch, E.; Roses, M. Determination of the chromatographic hydrophobicity index for ionisable solutes. *J. Chromatogr., A* **2007**, *1173*, 110–119.

(53) Aghemo, A.; Rumi, M. G.; Colombo, M. Pegylated IFN-α2a and ribavirin in the treatment of hepatitis C. *Expert Rev. Anti-Infect. Ther.* **2009**, *7*, 925–935.

(54) Guilhot, F.; Roy, L.; Saulnier, P. J.; Guilhot, J. Interferon in chronic myeloid leukaemia: past and future. *Best Pract. Res. Clin. Haematol.* **2009**, *22*, 315–329.

(55) Cannon, T.; Mobarek, D.; Wegge, J.; Tabbara, I. A. Hairy cell leukemia: current concepts. *Cancer Invest.* **2008**, *26*, 860–865.