

# Structure-Based Design of Novel Human Toll-like Receptor 8 Agonists

Hari Prasad Kokatla,<sup>[a]</sup> Diptesh Sil,<sup>[a]</sup> Hiromi Tanji,<sup>[b]</sup> Umeharu Ohto,<sup>[b]</sup>  
Subbalakshmi S. Malladi,<sup>[a]</sup> Lauren M. Fox,<sup>[a]</sup> Toshiyoki Shimizu,<sup>\*,[b]</sup> and Sunil A. David<sup>\*,[a]</sup>

Toll-like receptor (TLR)-8 agonists activate adaptive immune responses by inducing robust production of T helper 1-polarizing cytokines, suggesting that TLR8-active compounds might be promising candidate vaccine adjuvants. Recently, a C2-butyl furo[2,3-c]quinoline was reported with purely TLR8 agonistic activity. This compound was successfully co-crystallized with the human TLR8 ectodomain, and the co-crystal structure revealed ligand-induced reorganization of the binding pocket of TLR8. The loss of a key hydrogen bond between the oxygen atom of the furanyl ring of the agonist and Thr574 in TLR8 suggested that the furan ring is dispensable. Employing a disconnection strategy, 3- and 4-substituted aminoquinolines were investigated. Focused structure-based ligand design studies led to the identification of 3-pentyl-quinoline-2-amine as a novel, structurally simple, and highly potent human TLR8-specific agonist ( $EC_{50}=0.2\text{ }\mu\text{M}$ ). Preliminary evaluation of this compound in ex vivo human blood assay systems revealed that it retains prominent cytokine-inducing activity. Together, these results indicate the suitability of this compound as a novel vaccine adjuvant, warranting further investigation.

The immune system protects the host from infectious agents by first recognizing the presence of the infectious organism and then responding rapidly and appropriately to contain and eliminate the threat. The mobilization of adaptive immune responses involving T- and B-lymphocytic effector functions are exquisitely pathogen-specific but relatively slow, requiring days or weeks.<sup>[1]</sup> The enormous diversity of infectious organisms and their short generation times led Charles Janeway to postulate that *"the immune system has evolved specifically to recognize and respond to infectious microorganisms, and that this in-*

*volves recognition not only of specific antigenic determinants, but also of certain characteristics or patterns common on infectious agents but absent from the host."*<sup>[2]</sup> Janeway's seminal ideas of nonclonal recognition of nonself by pattern recognition receptors (PRRs) served as the foundation for the discovery of the sensors of the innate immune system.<sup>[3]</sup> Among the well-studied of such PRRs initiating innate immune afferent signals are the Toll-like receptors (TLRs).<sup>[4]</sup> There are ten functional TLRs encoded in the human genome. TLRs -3, -7, -8 and -9 function within the endolysosomal compartment.<sup>[5]</sup> The activation of TLRs by their cognate ligands leads to production of inflammatory cytokines, and upregulation of major histocompatibility complex molecules and co-stimulatory signals in antigen-presenting cells, as well as activating natural killer cells (innate immune response).<sup>[6]</sup> These events lead to the priming of naïve lymphocytes and subsequent induction and amplification of antigen-specific T- and B-cell effector functions (adaptive immune responses).<sup>[7]</sup>

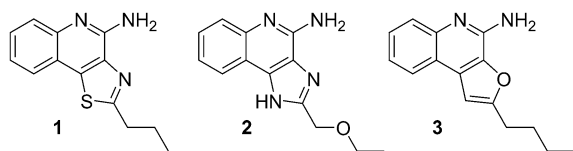
Our recent efforts on evaluating small-molecule agonists of TLR8<sup>[8]</sup> are primarily aimed at examining such compounds as potential vaccine adjuvants. TLR8 is expressed in myeloid dendritic cells, monocytes, and monocyte-derived dendritic cells. Engagement by TLR8 agonists evokes a dominant proinflammatory cytokine profile, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-12, and IL-18,<sup>[9]</sup> and appear unique in markedly upregulating the production of Th1-polarizing cytokines TNF- $\alpha$  and IL-12 in neonatal antigen-presenting cells.<sup>[10]</sup> These data, taken together, suggest that TLR8 agonists could be useful as adjuvants for enhancing immune responses in newborns.<sup>[11]</sup>

A prerequisite for the careful evaluation of TLR8 agonists as potential vaccine adjuvants is the characterization of pure TLR8 agonists with negligible TLR7 activity, for almost all known agonists of TLR8, typified by certain imidazoquinolines and thiazoloquinolines, such as CL097 (**2**)<sup>[12]</sup> and CL075 (**1**),<sup>[8b, 13]</sup> respectively (Figure 1), and the 2-aminobenzazepine VTX-2337,<sup>[14]</sup> display mixed TLR7/TLR8 agonism. TLR8-biased agonistic properties have been described for a novel 2-aminobenzazepine derivative (VTX-294),<sup>[15]</sup> whose complete structure has not been disclosed. We recently reported pure TLR8 agonistic activity in a C2-butyl furo[2,3-c]quinoline (**3**) with IL-12 and IL-18 induction profiles, and yet without IFN- $\alpha$  inducing properties, confirming its selectivity for human TLR8.<sup>[8a]</sup> Crystal structures of the ectodomain of human TLR8 in complex with mixed TLR7/TLR8-agonistic thiazoloquinolines and imidazoquinolines (including **1** and **2**)<sup>[16]</sup> allowed a rationalization of our

[a] Dr. H. P. Kokatla, Dr. D. Sil, S. S. Malladi, L. M. Fox, Prof. S. A. David  
Department of Medicinal Chemistry, University of Kansas  
Multidisciplinary Research Building, Room 320D  
2030 Becker Drive, Lawrence KS 66047 (USA)  
E-mail: sdavid@ku.edu

[b] Dr. H. Tanji, Dr. U. Ohto, Prof. T. Shimizu  
Graduate School of Pharmaceutical Sciences  
Faculty of Pharmaceutical Sciences, University of Tokyo  
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)  
and  
Core Research for Evolutional Science & Technology (CREST)  
Japan Science & Technology Agency (JST)  
K's Gobancho 6F, 7, Gobancho, Chiyoda-ku, Tokyo 102-0076 (Japan)  
E-mail: shimizu@mol.f.u-tokyo.ac.jp

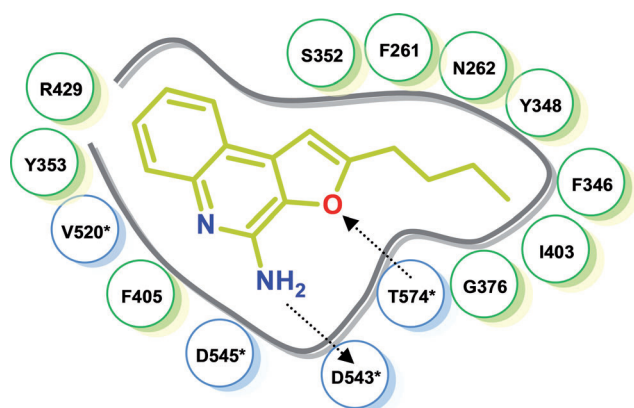
Supporting information for this article is available on the WWW under  
<http://dx.doi.org/10.1002/cmdc.201300573>.



**Figure 1.** Representative heterocyclic small molecules with TLR8 agonistic activity.

experimentally determined structure–activity relationship (SAR) data via induced-fit docking techniques.<sup>[8a]</sup>

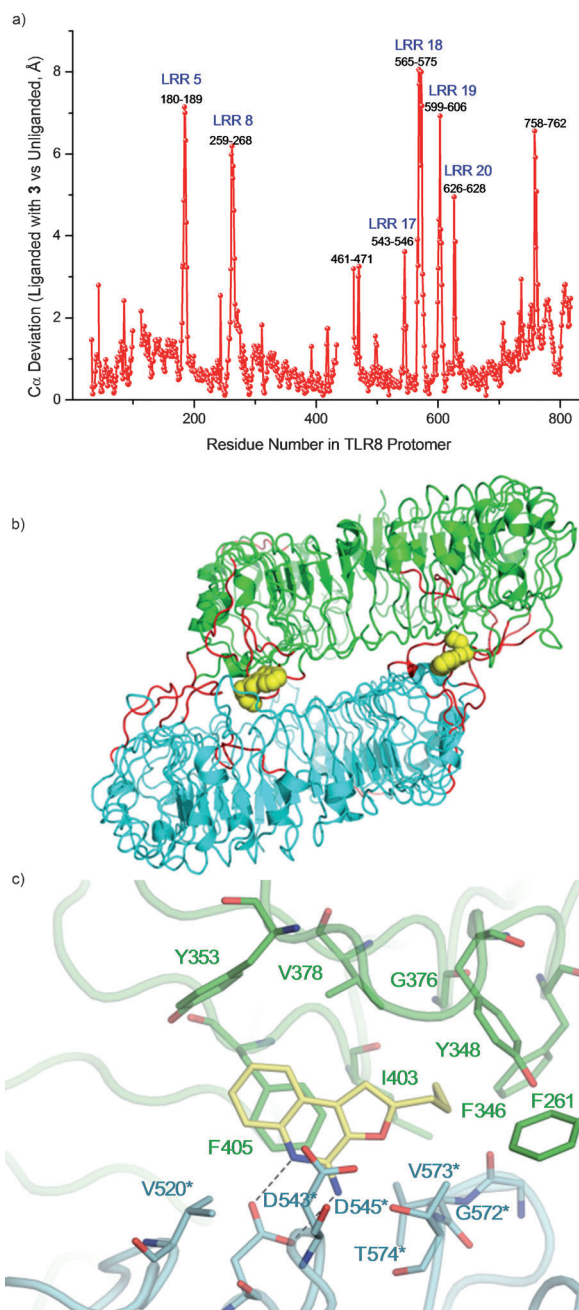
Thiazoloquinoline **1** and furoquinoline **3** were predicted to occupy the same binding pocket formed by both the TLR8 protomers with the binding geometry of the ligands and interacting residues being virtually identical; ionic hydrogen bonds were observed between the C4 amine of both **1** and **3** with the side chain carboxylate of Asp543 of TLR8, with additional stabilization derived from a hydrogen bond between the  $\beta$ -OH group of Thr574 and either the N<sup>2</sup> atom of the thiazole ring of **1** or the oxygen atom of the furanyl ring of **3**. Key  $\pi$ – $\pi$  interactions of the quinoline moieties of **1** and **3** (Phe405/Tyr353), as well as hydrophobic interactions of the C2 alkyl group (Phe346/Ile403/Tyr348), were also predicted to occur (Figure 2).



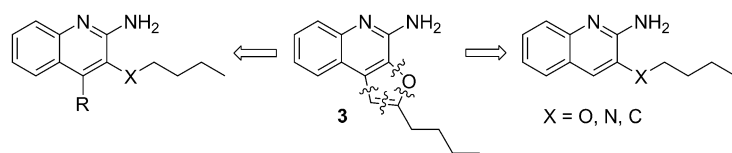
**Figure 2.** Induced-fit docking<sup>[8a]</sup> of compound **3** in the crystal structure of human TLR8 (PDB ID: 3W3K<sup>[16]</sup>) showing a salt bridge between the C4 amine and Asp543\*, and a hydrogen bond between the furanyl oxygen atom and Thr574. Interacting residues in protomers A and B (\*) are highlighted in green and cyan, respectively.

Recognizing limitations inherent in docking methods,<sup>[17]</sup> and cognizant of the crystallographically determined observation of large structural excursions (15 Å) of the top lateral face of TLR8 upon ligand binding,<sup>[16]</sup> we sought to directly verify and validate our docking results. We were delighted to obtain a high-resolution (1.8 Å) structure of human TLR8 co-crystallized with **3** (Figure S1 and Table S1 in the Supporting Information; The atomic coordinates and experimental data have been deposited in the Protein Data Bank under ascension code 3WN4). An examination of the complex confirmed similar binding geometries of **2** and **3** (Figure S2 in the Supporting Information). The occupancy of TLR8 with **3** induced, as expected,

a significant reorganization to form the binding pocket, reflected in significant C $\alpha$  deviations (Figure 3a) corresponding primarily to loops of leucine-rich repeats (LRRs) (Figure 3b). However, the occupancy of **3** in TLR8 is associated with greater excursions of LRR8 and, particularly, of residues 572–574 in LRR18 such that the predicted hydrogen bond between Thr574 and the oxygen atom of the furanyl ring of **3** is not observed in the crystal structure of the complex (Figure 3c). This led us to hypothesize that the furan ring in **3** was dispensable,

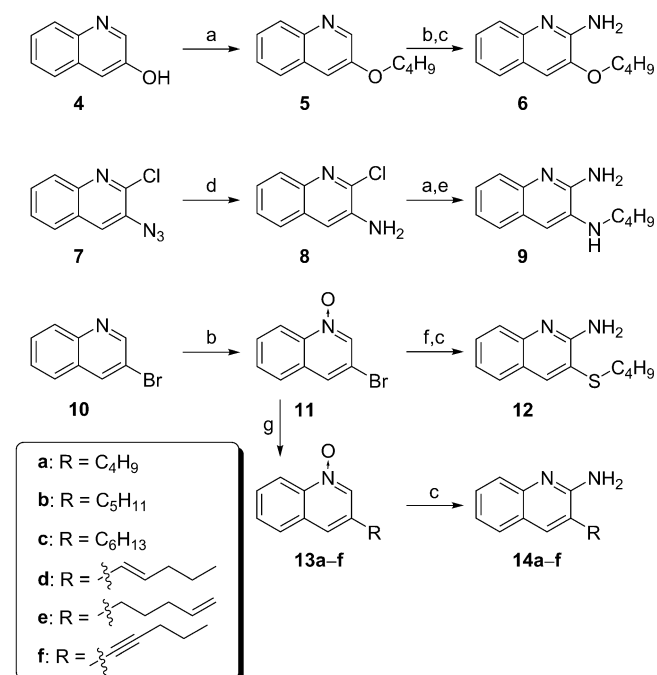


**Figure 3.** a) C $\alpha$  deviation in TLR8 bound to **3** versus unliganded TLR8. b) Regions undergoing ligand-induced C $\alpha$  movements of more than 2.5 Å are shown in red for the TLR8 protomers. c) TLR8 (protomers A and B represented in green and cyan, respectively) in complex with compound **3** showing the loss of a hydrogen bond of the furanyl oxygen atom due to reorganization of residues in the binding pocket (PDB code: 3WN4).



**Figure 4.** Disconnection strategy of compound **3** leading to substituted aminoquinolines.

and we envisaged simpler 3- and 4-substituted aminoquinolines via classic disconnection strategies<sup>[18]</sup> as shown schematically in Figure 4. We first targeted the 3-alkoxy-2-aminoquinoline series derived by disconnection at C1 in **3**. 3-Butoxy analogue **6** was synthesized from commercially available 3-hydroxyquinoline via O-alkylation and installation of the amine at C2 using reported methods (Scheme 1).<sup>[8a,b,19]</sup> A homologous



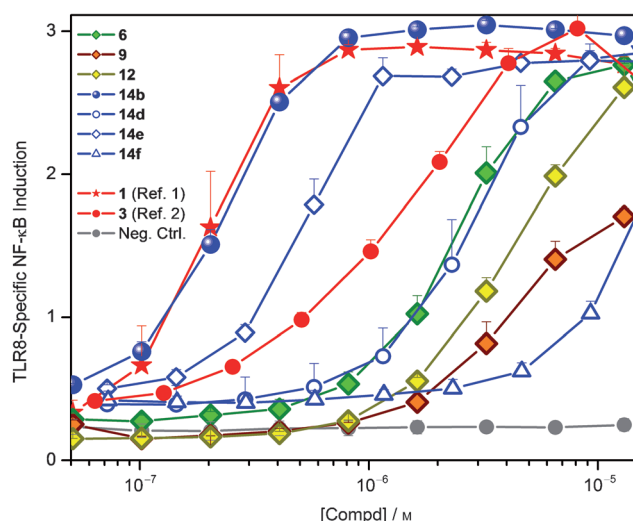
**Scheme 1.** Syntheses of 3-substituted quinolin-2-amine analogues. *Reagents and conditions:* a) butyl iodide,  $K_2CO_3$ , DMSO, 80 °C, 4 h; b) *m*-CPBA,  $CHCl_3$ , RT, 4 h; c) 1. benzoyl isocyanate,  $CH_2Cl_2$ , 55 °C, 1 h; 2. NaOMe, MeOH, reflux, 2 h; d)  $H_2$  (50 psi), Pt/C, EtOH, RT, 1 h; e)  $NH_3$ , MeOH, 100 °C, 24 h; f) butylSH, NaH, DMSO, RT, 2 h; g)  $Pd(PPh_3)_4$ ,  $RB(OH)_2$ ,  $K_2CO_3$ , 1,4-dioxane, 90 °C, 12 h; for **14 f**:  $Pd(PPh_3)_4$ , CuI, 1-pentyne,  $Et_3N/CH_3CN$  (1:3), 90 °C, 12 h.

series of compounds were also synthesized (Scheme S1 in the Supporting Information). As in other chemotypes that we had previously explored,<sup>[8a,b,19b,20]</sup> and consistent with the dimensions of the binding pocket in TLR8,<sup>[16]</sup> we observed in this homologous series a clear dependence of substituent chain length at C3 with the optimal analogue being **6**, which showed maximal agonistic potency in a cell-based TLR8-specific NF- $\kappa$ B transactivation assay ( $EC_{50}$  = 2.2  $\mu$ M; Figure 5 and Table 1; see also Figure S3 in the Supporting Information).

We reasoned that the electronegativity of the heteroatom at C3 might differentially modulate electron density of the quinoline ring and thus affect both the strength of the salt bridge of the C2 amine with Asp543, as well as  $\pi$ - $\pi$  interactions with

Phe405. We therefore synthesized *N*<sup>3</sup>-butylquinoline and 3-(butylthio)quinoline analogues (**9** and **12**, respectively), as well as 3-alkylquinolin-2-amines **14a-f** (Scheme 1). Accessing *N*<sup>3</sup>-butylquinoline **9** by conventional strategies via N-oxidation of the commercially available 3-aminoquinoline was problematic, and we found it expedient to utilize 2-chloro-3-azidoquinoline (**7**)—derived from commercially available 2-chloro-3-quinolineboronic acid (see Supporting Information)—as the starting material. S-Alkylation of 3-bromoquinoline 1-oxide obviated the problem of overoxidation to the sulfone derivative (which was found to be completely inactive) in the synthesis of 3-(butylthio)quinoline **12**.

A comparison of the activities of these analogues in TLR8 primary screens yielded a clear structure–activity relationship. The TLR8-agonistic potency of 3-pentyl quinoline **14b** was 0.2  $\mu$ M, i.e., tenfold greater than that of 3-butoxy analogue **6b**, eight-times greater than that of parent compound **3** ( $EC_{50}$  =



**Figure 5.** Dose–response profiles of human TLR8 agonistic activities of 3-substituted 2-aminoquinolines. Error bars represent standard deviations obtained from quadruplicates. Compounds **1** and **3** were used as comparators.

**Table 1.** Human TLR8-specific agonistic activities ( $EC_{50}$ ) of the investigated analogues.

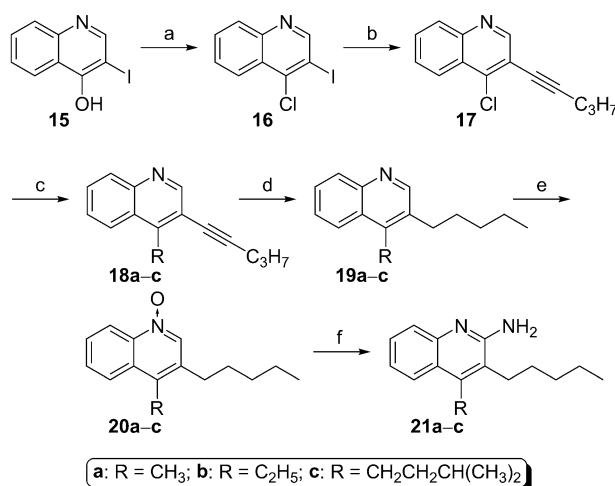
Compd	IUPAC name	$EC_{50}$ [ $\mu$ M] <sup>[a]</sup>
<b>1</b>	2-propylthiazolo[4,5-c]quinolin-4-amine	0.2 <sup>[b]</sup>
<b>3</b>	2-butylfuro[2,3-c]quinolin-4-amine	1.6 <sup>[c]</sup>
<b>6</b>	3-butoxyquinolin-2-amine	2.18
<b>9</b>	<i>N</i> <sup>3</sup> -butylquinoline-2,3-diamine	4.28
<b>12</b>	3-(butylthio)quinolin-2-amine	4.16
<b>14a</b>	3-butylquinolin-2-amine	0.41
<b>14b</b>	3-pentylquinolin-2-amine	0.2
<b>14c</b>	3-hexylquinolin-2-amine	inactive
<b>14d</b>	( <i>E</i> )-3-(pent-1-en-1-yl)quinolin-2-amine	2.67
<b>14e</b>	3-(pent-4-en-1-yl)quinolin-2-amine	0.49
<b>14f</b>	3-(pent-1-yn-1-yl)quinolin-2-amine	12.96

[a]  $EC_{50}$  values were obtained using hTLR8-specific reporter gene assays. Data are representative of three experiments. Inactive compounds did not show appreciable activity at 500  $\mu$ M. [b] Data taken from Ref. [1]. [c] Data taken from Ref. [2].

1.6  $\mu\text{M}$ ), and rivaling that of reference compound **1** (Figure 5 and Table 1), while the 3-(butylthio)quinoline and *N*<sup>3</sup>-butylquinoline analogues were weaker ( $\text{EC}_{50}$  = 4.2  $\mu\text{M}$  and 4.3  $\mu\text{M}$  for **12** and **9**, respectively; Figure 5 and Table 1).

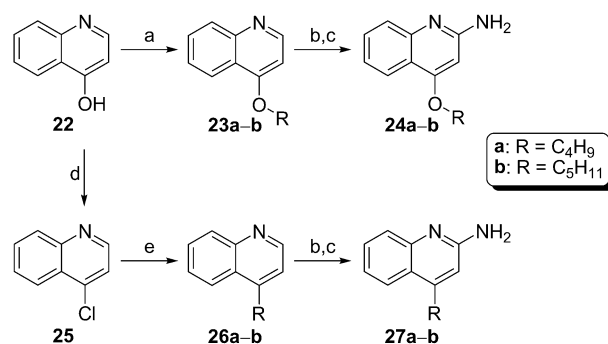
An examination of the dihedral angles indicated a quasi-*gauche* conformation of the proximal methylene units of the C2 butyl substituent in the crystal structure of **3** bound to TLR8, and, as expected, the introduction of unfavorable geometrical constraints in the (*E*)-3-(pent-1-en-1-yl)quinoline and 3-(pent-1-yn-1-yl)quinoline analogues **14d** and **14f**, respectively, diminishes activity while potency is largely spared in compound **14e** with a terminal alkene (Figure 5 and Table 1).

Additionally, in examining the interfacial surface topology of the binding site formed by the protomers of TLR8<sup>[16]</sup> using Voronoi polyhedral modeling,<sup>[21]</sup> we noticed an accessory hydrophobic groove bounded by Phe346 and Tyr348, which is contiguous with the hydrophobic pocket accommodating the C2 butyl substituent in **3**. We were therefore interested in examining whether additional substituents at C4 would further augment the potency of **14b**. As shown in Scheme 2, analogues **21a–c** were synthesized starting from 3-iodoquinolin-4-ol (**15**).<sup>[8a]</sup>



**Scheme 2.** 4-Alkyl-3-pentylquinolin-2-amines. *Reagents and conditions:* a)  $\text{POCl}_3$ ,  $100^\circ\text{C}$ , 2 h; b)  $\text{Pd(PPh}_3)_4$ ,  $\text{CuI}$ , 1-pentyne,  $\text{Et}_3\text{N/CH}_3\text{CN}$  (1:3),  $70^\circ\text{C}$ , 12 h; c)  $\text{Pd(PPh}_3)_4$ ,  $\text{RB(OH)}_2$ ,  $\text{K}_2\text{CO}_3$ , 1,4-dioxane,  $90^\circ\text{C}$ , 12 h; d)  $\text{H}_2$  (50 psi),  $\text{Pt/C}$ ,  $\text{EtOH}$ , RT, 1 h; e) *m*-CPBA,  $\text{CHCl}_3$ , RT, 4 h; f) 1. benzoyl isocyanate,  $\text{CH}_2\text{Cl}_2$ ,  $55^\circ\text{C}$ , 1 h; 2.  $\text{NaOMe}$ ,  $\text{MeOH}$ , reflux, 2 h.

We had initially envisaged a step-wise Suzuki coupling of appropriate alkylboronic acids with the 3-iodo-4-chloroquinoline intermediate (**16**); however, this approach was not optimal because of the formation of a mixture of isomers. We therefore first installed the 3-pentyne substituent by Sonogashira coupling to obtain **17**, which proved to be an excellent substrate for subsequent Suzuki coupling, leading to required analogues **21a–c** (Scheme 2). These compounds were feeble in their TLR8-agonistic activity (data not shown), suggesting poor tolerance of steric bulk at C4. In order to confirm that substitutions at C4 are not tolerated, we synthesized congeners of both 4-alkoxy (**24a,b**) and 4-alkyl quinolin-2-amines (**27a,b**;



**Scheme 3.** 4-Substituted quinolin-2-amines. *Reagents and conditions:* a) butyl iodide,  $\text{NaH}$ ,  $\text{DMSO}$ ,  $80^\circ\text{C}$ , 4 h; b) *m*-CPBA,  $\text{CHCl}_3$ , RT, 4 h; c) 1. benzoyl isocyanate,  $\text{CH}_2\text{Cl}_2$ ,  $55^\circ\text{C}$ , 1 h; 2.  $\text{NaOMe}$ ,  $\text{MeOH}$ , reflux, 2 h; d)  $\text{POCl}_3$ ; e)  $\text{Pd(PPh}_3)_4$ ,  $\text{RB(OH)}_2$ ,  $\text{K}_2\text{CO}_3$ , 1,4-dioxane,  $90^\circ\text{C}$ , 12 h.

Scheme 3) and, as expected, all of these analogues were found to be inactive (Table S2 in the Supporting Information).

All analogues were counter-screened<sup>[8c,19b,22]</sup> in reporter cell lines specific for human TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, TLR10, Nod1 and Nod2, and compounds **6**, **9**, **12**, and **14a–f** were confirmed to be specific for human TLR8. The most potent analogue (**14b**) was characterized further by determining its cytokine/chemokine induction profile in a panel of secondary screens employing human peripheral blood mononuclear cells,<sup>[8a]</sup> as well as whole human blood.<sup>[22c]</sup> Consistent with its specificity for TLR8, we observed the induction of a specific set of chemokines and proinflammatory cytokines, including IL-12 and -18 (Figure 6).

In summary, we have effectively utilized the structure of TLR8 in complex with ligands in the rational design of a novel TLR8-specific chemotype that retains prominent cytokine-inducing activity profiles in ex vivo human blood assay systems, paving the way for evaluation of this compound as a candidate vaccine adjuvant in appropriate animal models.

## Experimental Section

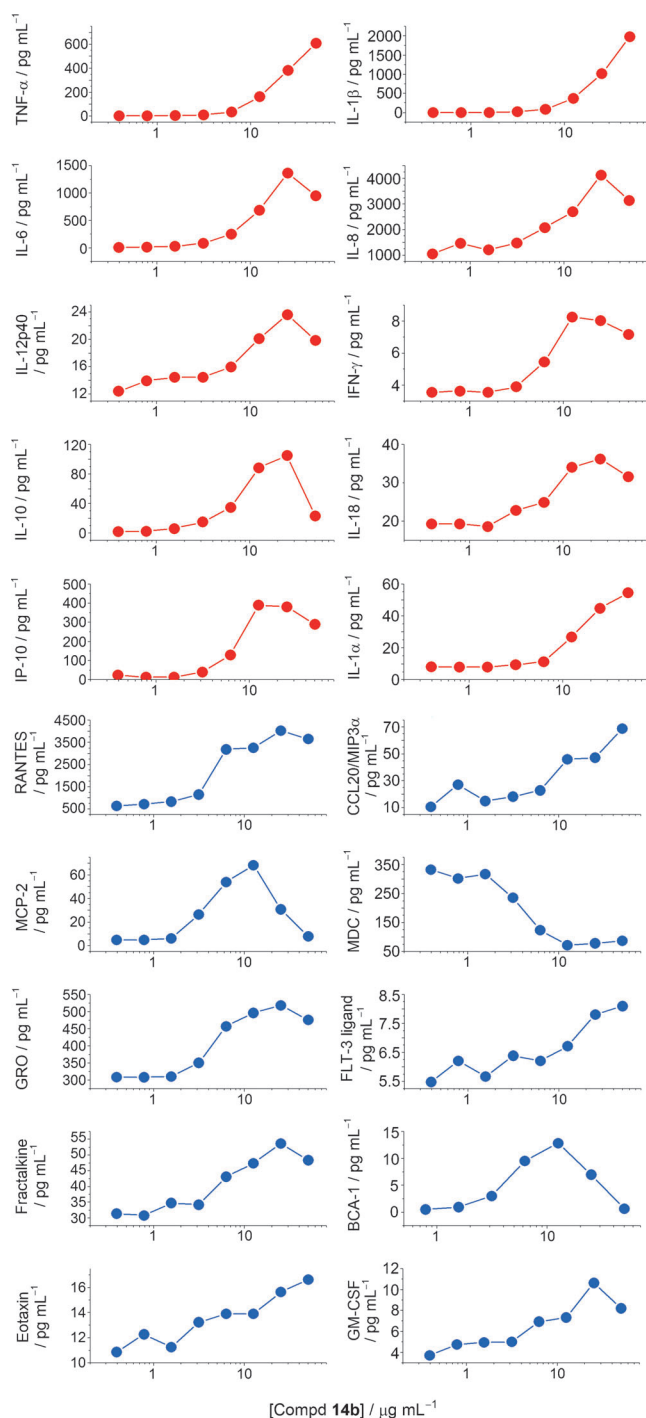
X-ray diffraction data and all synthetic and immunological experimental methods are provided in the Supporting Information.

## Acknowledgements

This work was supported by the US National Institutes of Health (NIH)/US National Institute of Allergy and Infectious Diseases (NIAID) contract HSN272200900033C (S.A.D.), a Grant-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (U.O., K.M., and T.S.), the Takeda Science Foundation (U.O. and T.S.), the Mochida Memorial Foundation for Medical and Pharmaceutical Research (U.O.), and Core Research for Evolutional Science and Technology (CREST), Japan Science & Technology Agency (JST) (T.S.).

**Keywords:** aminoquinolines • innate immunity • structure-based drug design • toll-like receptor-8 (TLR8) • vaccine adjuvants





**Figure 6.** Induction of cytokines (red) and chemokines (blue) in human peripheral blood mononuclear cells by lead compound **14b**. Data represent the mean of triplicate determinations.

- [1] a) S. A. Plotkin, *J. Infect. Dis.* **2003**, *187*, 1349–1359; b) S. A. Plotkin, *Clin. Vaccine Immunol.* **2009**, *16*, 1709–1719.  
[2] C. A. Janeway, Jr., *Cold Spring Harbor Symp. Quant. Biol.* **1989**, *54*, 1–13.  
[3] a) A. J. Ciechanover, J. I. Sznajder, *Am. J. Respir. Crit. Care Med.* **2011**, *184*, i–ii; b) R. Medzhitov, *J. Immunol.* **2013**, *191*, 4473–4474.  
[4] a) S. Akira, *Proc. Jpn. Acad. Ser. B* **2009**, *85*, 143–156; b) S. Akira, S. Uematsu, O. Takeuchi, *Cell* **2006**, *124*, 783–801; c) T. Kawai, S. Akira, *Semin. Immunol.* **2007**, *19*, 24–32.

- [5] a) T. Kawai, S. Akira, *Int. Immunol.* **2009**, *21*, 317–337; b) T. Kawai, S. Akira, *Nat. Immunol.* **2010**, *11*, 373–384; c) J. Hoffmann, S. Akira, *Curr. Opin. Immunol.* **2013**, *25*, 1–3.  
[6] a) L. A. O'Neill, D. Golenbock, A. G. Bowie, *Nat. Rev. Immunol.* **2013**, *13*, 453–460; b) R. C. Coll, L. A. O'Neill, *J. Innate Immun.* **2010**, *2*, 406–421.  
[7] a) A. Iwasaki, R. Medzhitov, *Nat. Immunol.* **2004**, *5*, 987–995; b) C. Pasare, R. Medzhitov, *Semin. Immunol.* **2004**, *16*, 23–26; c) C. Pasare, R. Medzhitov, *Immunity* **2004**, *21*, 733–741; d) C. Pasare, R. Medzhitov, *Nature* **2005**, *438*, 364–368; e) D. Nemazee, A. Gavin, K. Hoebe, B. Beutler, *Nature* **2006**, *441*, E4; f) A. Iwasaki, R. Medzhitov, *Science* **2010**, *327*, 291–295.  
[8] a) H. P. Kokatla, D. Sil, S. S. Malladi, R. Balakrishna, A. R. Hermanson, L. M. Fox, X. Wang, A. Dixit, S. A. David, *J. Med. Chem.* **2013**, *56*, 6871–6885; b) H. P. Kokatla, E. Yoo, D. B. Salunke, D. Sil, C. F. Ng, R. Balakrishna, S. S. Malladi, L. M. Fox, S. A. David, *Org. Biomol. Chem.* **2013**, *11*, 1179–1198; c) D. B. Salunke, E. Yoo, N. M. Shukla, R. Balakrishna, S. S. Malladi, K. J. Serafin, V. W. Day, X. Wang, S. A. David, *J. Med. Chem.* **2012**, *55*, 8137–8151.  
[9] K. B. Gorden, K. S. Gorski, S. J. Gibson, R. M. Kedl, W. C. Kieper, X. Qiu, M. A. Tomai, S. S. Alkan, J. P. Vasilakos, *J. Immunol.* **2005**, *174*, 1259–1268.  
[10] V. J. Philbin, D. J. Dowling, L. C. Gallington, G. Cortes, Z. Tan, E. E. Suter, K. W. Chi, A. Shuckett, L. Stoler-Barak, M. Tomai, R. L. Miller, K. Mansfield, O. Levy, *J. Allergy Clin. Immunol.* **2012**, *130*, 195–204.  
[11] O. Levy, *Nat. Rev. Immunol.* **2007**, *7*, 379–390.  
[12] a) B. Petricevic, B. Wessner, M. Sachet, D. Vrbanec, A. Spittler, M. Bergmann, *Shock* **2009**, *32*, 484–490; b) T. Tajuddin, E. J. Ryan, S. Norris, J. E. Hegarty, C. O'Farrelly, *J. Gastroenterol. Hepatol.* **2010**, *25*, 1883–1890; c) K. Makni-Maalej, T. Boussetta, M. Hurtado-Nedelec, S. A. Belambri, M. A. Gougerot-Pocidallo, J. El-Benna, *J. Immunol.* **2012**, *189*, 4657–4665.  
[13] S. Spranger, M. Javorovic, M. Burdek, S. Wilde, B. Mosetter, S. Tippmer, I. Bigalke, C. Geiger, D. J. Schendel, B. Frankenberger, *J. Immunol.* **2010**, *185*, 738–747.  
[14] H. Lu, G. N. Dietsch, M. A. Matthews, Y. Yang, S. Ghanekar, M. Inokuma, M. Suni, V. C. Maino, K. E. Henderson, J. J. Howbert, M. L. Disis, R. M. Hershberg, *Clin. Cancer Res.* **2012**, *18*, 499–509.  
[15] D. J. Dowling, Z. Tan, Z. M. Prokopowicz, C. D. Palmer, M. A. Matthews, G. N. Dietsch, R. M. Hershberg, O. Levy, *PLoS One* **2013**, *8*, e58164.  
[16] H. Tanji, U. Ohto, T. Shibata, K. Miyake, T. Shimizu, *Science* **2013**, *339*, 1426–1429.  
[17] A. M. Davis, S. J. Teague, G. J. Kleywegt, *Angew. Chem.* **2003**, *115*, 2822–2841; *Angew. Chem. Int. Ed.* **2003**, *42*, 2718–2736.  
[18] S. Warren, P. Wyatt, *Organic Synthesis: The Disconnection Approach*, 2nd ed., Wiley, Chichester, **2008**.  
[19] a) J. F. Gerster, K. J. Lindstrom, R. L. Miller, M. A. Tomai, W. Birmachou, S. N. Bomersine, S. J. Gibson, L. M. Imbertson, J. R. Jacobson, R. T. Knaffa, P. V. Maye, N. Nikolaides, F. Y. Oneyemi, G. J. Parkhurst, S. E. Pecore, M. J. Reiter, L. S. Scribner, T. L. Testerman, N. J. Thompson, T. L. Wagner, C. E. Weeks, J. D. Andre, D. Lagain, Y. Bastard, M. Lupu, *J. Med. Chem.* **2005**, *48*, 3481–3491; b) N. M. Shukla, S. S. Malladi, C. A. Mutz, R. Balakrishna, S. A. David, *J. Med. Chem.* **2010**, *53*, 4450–4465.  
[20] E. Yoo, B. M. Crall, R. Balakrishna, S. S. Malladi, L. M. Fox, A. R. Hermanson, S. A. David, *Org. Biomol. Chem.* **2013**, *11*, 6526–6545.  
[21] a) F. M. Richards, *J. Mol. Biol.* **1974**, *82*, 1–14; b) M. Gerstein, C. Chothia, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10167–10172.  
[22] a) D. B. Salunke, S. W. Connelly, N. M. Shukla, A. R. Hermanson, L. M. Fox, S. A. David, *J. Med. Chem.* **2013**, *56*, 5885–5900; b) N. M. Shukla, D. B. Salunke, R. Balakrishna, C. A. Mutz, S. S. Malladi, S. A. David, *PLoS One* **2012**, *7*, e43612; c) J. D. Hood, H. J. Warshakoon, M. R. Kimbrell, N. M. Shukla, S. Malladi, X. Wang, S. A. David, *Hum. Vaccines* **2010**, *6*, 322–335; d) R. Ukani, T. C. Lewis, T. P. Day, W. Wu, S. S. Malladi, H. J. Warshakoon, S. A. David, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 293–295; e) G. Agnihotri, R. Ukani, S. S. Malladi, H. J. Warshakoon, R. Balakrishna, X. Wang, S. A. David, *J. Med. Chem.* **2011**, *54*, 1490–1510.

Received: December 31, 2013

Published online on January 28, 2014