Letter

Design and Synthesis of N1-Modified Imidazoquinoline Agonists for Selective Activation of Toll-like Receptor 7 and 8

Peter Larson, Tamara A. Kucaba, Zhengming Xiong, Michael Olin, Thomas S. Griffith, and David M. Ferguson

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00256 • Publication Date (Web): 16 Oct 2017

Design and Synthesis of N1-Modified Imidazoquinoline Agonists for Selective Activation of Toll-like Receptor 7 and 8

Peter Larson,[1] Tamara A. Kucaba[2], Zhengming Xiong,[3] Michael Olin,[3,4] Thomas S. Griffith[2,4,5,6], and David M. Ferguson[1,7]*

[1]Department of Medicinal Chemistry, [2]Department of Urology, [3]Department of Pediatrics, [4]Masonic Cancer Center, [5]Center for Immunology, [6]Microbiology, Immunology, and Cancer Biology Graduate Program, [7]Center for Drug Design, University of Minnesota, Minneapolis, MN 55455

ABSTRACT: A series of N1-modified imidazoquinolines were synthesized and screened for Toll-like receptor (TLR) 7 and 8 activity to identify recognition elements that confer high affinity binding and selectivity. These receptors are key targets in the development of immunomodulatory agents that signal the NF-κB mediated transcription of pro-inflammatory chemokines and cytokines. Results are presented showing both TLR7/8 activation is highly correlated to N1-substitution, with TLR8 selectivity achieved through inclusion of an ethyl-, propyl-, or butylamino group at this position. While the SAR analysis indicates TLR7 activity is less sensitive to N1-modification, extension of the aminoalkyl chain length to pentyl and *p*-methylbenzyl elicited high affinity TLR7 binding. Cytokine profiles are also reported that show the pure TLR8 agonist [4-amino-2-butyl-1-(2-aminoethyl)-7-methoxycarbonyl-1*H*-imidazo[4,5-c]quinoline] induces higher levels of IL-1β, IL-12, and IFNγ when compared with TLR7 selective or mixed TLR7/8 agonists. The results are consistent with previous work suggesting TLR8 agonists are Th1 polarizing and may help promote cell-mediated immunity.

Keywords: Toll-like receptor, TLR7, TLR8, imidazoquinoline, SAR, cytokine

The connection between Toll-like receptor (TLR) 7 and TLR8 activation and cytokine stimulation is well documented. These endosomal transmembrane receptors recognize and bind pathogen-associated single stranded RNA and signal (through the adapter protein MyD88) the NF-κB-mediated transcription of proinflammatory cytokines. This process is critical for mounting a robust immune response to pathogens by promoting dendritic cell (DC) maturation, T cell differentiation, B cell differentiation and class switching, antibody production, and other antigen specific responses. These receptors were also found to recognize small molecule nucleoside base analogs. In seminal work on TLR7/8 function, Hemmi et al. directly linked the cytokine inductive effects of imiquimod (**1**), an imidazoquinoline, with TLR7 activation.[1] In subsequent work by Jurk et al., another imidazoquinoline, resiquimod (**2**) was shown to be a mixed agonist, activating both TLR7 and TLR8.[2] Imiquimod, the prototypical TLR7 selective agonist, was commercialized in 1997 (prior to elucidating its mechanism of action) as a single agent topical ointment for treating basal and squamous cell carcinoma and genital warts, demonstrating the potential utility of small molecule immunostimulatory agents in treating disease.

Although TLR7 and TLR8 recognize similar molecular patterns and share common epitopes in binding small molecules, evidence has emerged in recent years suggesting they are functionally different. TLR7 and TLR8 agonists induce different cytokine profiles and target different cell types. TLR8, for example, is functional on myeloid dendritic cells and produces Th1-polarizing effects which may have significant benefits for the development of cancer vaccines.[3] TLR8 activation also correlates with enhanced cytokine production when compared to TLR7 selective agents and to the induction of higher levels of TNFα and IFNγ, consistent with the role of TLR8 in Th1 differentiation.[4,5] The first TLR8 selective agonist was described

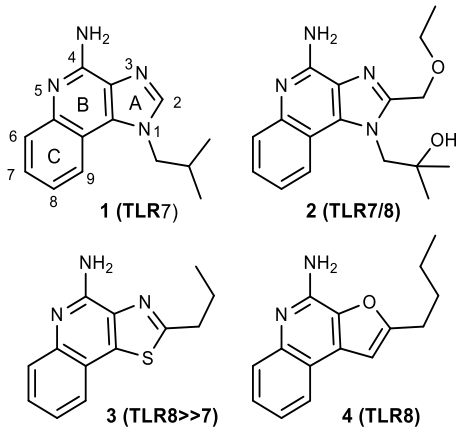


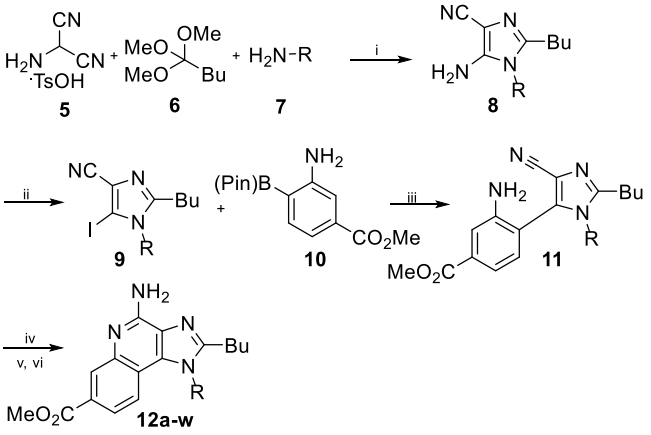
Figure 1. Heterocycles showing selective and mixed TLR7 and TLR8 activity.

by Gorden et al. in 2005 to study TLR7/8 functional differences.[3] The compound, 3M-002 (**3**), contains a monoalkyl substitution to the C2-position of the thiozoloquinoline ring. Although no details regarding the design of 3M-002 were given, it is reasonable to conclude the diminished TLR7 activity displayed by this compound was due to the absence of the N1-isobutyl group (typical of TLR7 active agents). In 2013, Kokatla et al. reported the design of a monoalkyl furoquinoline (**4**) with high selectivity for TLR8 (and no measurable TLR7 activity).[6] Using rational design strategies and X-ray crystallographic data, this tricyclic scaffold was subsequently refined to yield three additional classes of TLR8 selective agents, including bicyclic aminoquinolines and benzamidazoles, and substituted aminoimidazoles.[7-9]

A common feature shared by many TLR8 selective imidazoquinolines is an alkyl substitution to the A-ring. An SAR study of imidazoquinolines found activation of both TLR7 and TLR8 increases with C2 alkyl chain length and peaks with a butyl at TLR7 and a pentyl at TLR8.[4,5] These results are partially explained by the X-ray crystal structure of resiquimod bound to the TLR8 homodimer complex that shows the C2 substituent projects into a hydrophobic pocket formed by aliphatic residues in the receptor.[6] The X-ray structure also revealed specific contacts between the N3-nitrogen and 4-amino group with aspartate and threonine residues of the receptor.[10] These data support the general hypothesis that a hydrogen bond acceptor at position 3 is important to maintain high affinity binding at both TLR7 and TLR8 (as homology modeling and sequence analysis indicates these contacts may be conserved).[11] Our previous work indicated a hydrogen-bonding donor at the N1 position may also favor TLR8 activation.[4] The SAR data showed a correlation between TLR8 potency and the presence of an N1-alkylhydroxyl group. Since the emphasis of that study was on the C2-position and alkyl chain length, the N1 substitutions were limited to short hydroxyalkyl groups.

In this study, we expand our original SAR analysis to include a more diverse set of N1-substitutions (including N1 thiols, amines, alcohols, esters, and carboxylic acids) to further explore the structural epitopes that confer TLR7/8 activation and selectivity. The series design starts with the N1 hydroxyalkyl analogs identified in our previous work bearing both C2-butyl and C7-methylester functionalities. The synthetic strategy is shown in scheme 1 and begins with a multicomponent pyrolysis reaction to obtain the appropriately N1-substituted imidazoles (**8a-w**).[12,13] This reaction produced reasonably good yields (~50-80%) for most N1 substituents, but proved much less efficient when the primary amine was poorly soluble in tetrahydrofuran (especially **7k-o**, **u**, **v**). In most cases, yields approaching 30% were afforded by changes to the solvent conditions or the use of HCl salts. In some cases, the addition of excess TEA proved beneficial in further improving yields. However, a detailed analysis of all problematic substrates was not performed so it is unclear if this effect was from proton scavenging or simply due to increased reaction temperatures. The boc-protecting group of the amines (**7p-t**) was ultimately removed *in situ* during the final acid catalyzed cyclization step. Conversion of the 5-amino group of the imidazole (**8a-w**) to the desired iodo product was accomplished using a modified Sandmeyer reaction.[14] As expected from our earlier work using this pathway,

Scheme 1. Imidazoquinoline synthesis.[a]



a, R = $CH_2C(Me)_2OH$
b, R = $CH_2CH(Me)OH$
c, R = $CH_2CH(Me)OC(O)Me$
d, R = $CH_2CH(Me)OC(O)C_7H_{15}$
e, R = $(CH_2)_2OH$
f, R = $(CH_2)_3OH$
g, R = $(CH_2)_4OH$
h, R = $(CH_2)_2SMe$
i, R = $(CH_2)_3SMe$
j, R = $(CH_2)_2SEt$
k, R = CH_2CO_2Me
l, R = $(CH_2)_2CO_2Me$
m, R = $(CH_2)_3CO_2Me$
n, R = $(CH_2)_4CO_2Me$
o, R = $(CH_2)_5CO_2Me$
p, R = $(CH_2)_2NHR'$
q, R = $(CH_2)_3NHR'$
r, R = $(CH_2)_4NHR'$
s, R = $(CH_2)_5NHR'$
t, R = $CH_2(p\text{-Phe})CH_2NHR'$
u, R = $CH_2(p\text{-Phe})CO_2R''$
v, R = $(CH_2)_2(p\text{-Phe})CO_2R''$
w, R = $(CH_2)_4COOR''$

where R' = Boc or H
R'' = Me or H

[a]Reagents and conditions: (i) NEt_3, THF, reflux to rt, 18 h, 20–79%; (ii) CH_2I_2, isopentyl nitrite, $CHCl_3$, 80 °C, 1 h, 8–48%; (iii) $Pd(PPh_3)_2Cl_2$, K_2CO_3, THF-H_2O; (iv) 4N HCl in dioxane (excess), 100 °C. trace–40% yield over two steps. For **12c**: (v) **12b**, DCC, DMAP, CH_3COOH, CH_2Cl_2, rt, 1 h, 32%. For **12d**: (vi) **12b**, DCC, DMAP, $CH_3(CH_2)_6COOH$, CH_2Cl_2, rt, 1h, 52%.

the reaction produced lower yields (<20%) in the presence of a proton donating group at the N1 position. The methylesters **9u-w** were strategically saponified using potassium hydroxide in THF-H_2O (rt) to liberate the carboxylic acids prior to installation of the arylester. The Iodo-imidazoles **9a-w** were subsequently cross coupled with 2-amino-4-methoxycarbonylphenylboronic acid (**10**) using the Suzuki-Miyaura reaction. Inefficiencies were noted in the cross coupling where solubility or steric effects played a significant role. The synthesis of **11k** proved especially problematic with the standard triphenylphosphine ligand. Yields suggested there was less than one successful turnover of the catalytic cycle even while all the iodo-imidazole **9k** was depleted from the reaction mixture. Changing the ligand to 2-dicyclohexylphosphino-2′,6′- dimethoxybiphenyl (SPhos) and the base to cesium carbonate provided substantially better yields. Impure intermediates **11a-w** were taken directly on to cyclization using anhydrous HCl in dioxane affording imidazoquinolines **12a-w** in trace-40% yields. The cyclization was also hindered by competing intermolecular cross coupling reactions in the presence of N1 primary amines.

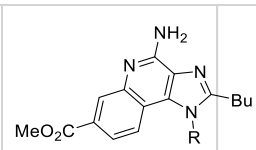
The compounds were evaluated for TLR7/8 activity using HEK cells expressing TLR7 or TLR8 and the NF-κB/SEAP (secreted embryonic alkaline phosphatase) gene reporter system.

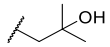
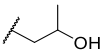
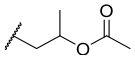
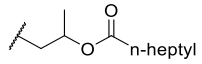
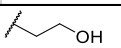
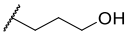
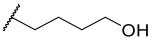
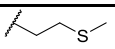
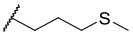
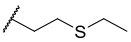
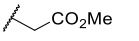
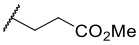
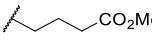
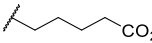
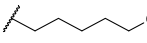
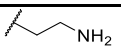
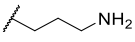
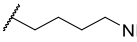
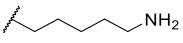
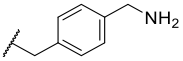
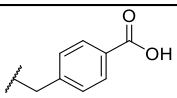
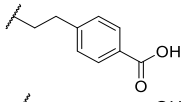
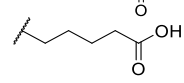
This assay measures agonist potency in triggering the downstream expression of SEAP mediated by NF-κB, which is subsequently quantified using colorimetry. It is important to point out this assay is not a direct measure of receptor activation or ligand binding affinity. In addition, some compounds are cytotoxic, yielding low optical densities and incomplete dose response curves. The results, given in Table 1, show several substitution-specific trends in TLR7 and TLR8 activity. Prior work by our lab has shown the presence of an alkyl hydroxyl group at the N1 position enhanced TLR8 activation. The importance of this proton donating group at this position is further validated here by esterification of the 2-hydroxypropyl group of **12b**, yielding TLR8 inactive analogs **12c** and **12d**. It is noteworthy to add that the less bulky acetate derivative **12c** retained TLR7 activity while the bulkier octanoate derivative **12d** was completely inactive. The results presented here indicate the alkyl chain length and position of hydroxyl group may be critical as well given the failure of the hydroxypropyl and hydroxybutyl derivatives to trigger TLR8.

In contrast to the hydroxyl derivatives, the aminoalkyls (**12p-t**) show a positive correlation with increasing alkyl chain length, with the maximum occurring for the aminopentyl substitution. This SAR may be in part explained by an X-ray crystallographic structure reported by Beesu et al. that shows the terminal amino group of an N1-*p*-aminomethylbenzyl-substituted imdizoquinoline (similar to **12t**) extends into a pocket within the TLR8 ectodomain and formed favorable hydrogen bonding interactions with the mainchain carbonyl group of glycine 351.[7] It is reasonable to conclude that the correlation in chain length and TLR8 activity reported here for the aminoalkyl-substituted analogs may also be due to this interaction. Although X-ray crystallographic data has indicated another TLR8 residue, Asp545, may also play a role in stabilizing binding through salt link formation with terminal amino groups of alkyl substituted ligands,[7] this interaction may not be a contributing factor to the trend displayed here. The Asp545 residue is replaced by Leu in the TLR7 sequence. The potent activity of **12s** and **12t** in activating TLR7 suggests an alternate site exists for recognition of the amino group. The finding that the carboxylates (**12u**-**12w**) are devoid of appreciable TLR7 activity further supports the argument that an alternative cationic binding site may exist within the pocket of TLR7 (and perhaps TLR8) for recognition of the amino group.

The remaining compounds in the series show similar activity in activating TLR7 with little correlation to N1 substitution, supporting the general conclusion that the structural requirements to activate TLR7 are less stringent than TLR8. The activity of the bulky methyl esters (**12n** and **12o**) and sulfides (**12i** and **12j**) suggests the binding site in TLR7 is more tolerant to aliphatic substitutions. However, the compounds showing the highest affinity to both TLR7 and TLR8 contain a proton donating group at the N1 position, suggesting this is a primary anchor point in the design of next generation analogs with improved potency and selectivity. Given the location of these receptors within the endosome, it may also be important to include cell permeability as a screening tool. An analysis of calculated logP values (reported in the SI) shows that the majority of compounds fall in the range of from 2 to 4. The outliers are

Table 1. TLR7 and TLR8 agonist activities.[a]



Compound	R	TLR-7 (μM)	TLR-8 (μM)
1		11.4 ± 4.5	N/A
2		1.34 ± 0.26	6.13 ± 0.26
12a[b]		1.69 ± 0.11	20.86 ± 2.28
12b[b]		2.22 ± 0.08	9.88 ± 2.13
12c		3.06 ± 0.34	N/A
12d		N/A	N/A
12e[b]		14.37 ± 0.63	37.00 ± 2.51
12f		1.72 ± 0.23	N/A[c]
12g		3.21 ± 0.38	N/A
12h		2.89 ± 0.28	N/A[c]
12i		3.84 ± 0.38	N/A
12j		6.67 ± 1.71	N/A
12k		3.33 ± 0.46	N/A
12l		3.64 ± 0.74	N/A
12m		4.53 ± 1.06	N/A
12n		1.74 ± 0.88	N/A
12o		2.89 ± 0.61	N/A
12p		N/A	49.8 ± 11.0
12q		N/A	26.7 ± 13.9
12r		N/A	3.85 ± 0.85
12s		0.60 ± .13	2.21 ± 0.31
12t		0.18 ± 0.01	5.34 ± 0.83
12u		190 ± 44	N/A
12v		N/A	N/A
12w		N/A	N/A

[a]Calculated average EC_{50} based on a minimum of two independent measurements performed in triplicate using the HEK-NF-kB-SEAP-hTLR7 or –hTLR8 reporter cell lines. N/A = no activity at 180μM. [b]Ref. 4. [c]Low optical density.

the highly lipophilic octanoate derivative **12d** (logP = 5.41) and the short, polar aminoethyl substituted analog **12p** (logP = 1.54).

We next tested the ability of the TLR7 selective compound **12h**, the TLR8 selective compound **12p**, the TLR7/8 mixed agonist **12t**, and the prototypical TLR7 selective agent imiquimod (**1**) to stimulate cytokine production from human peripheral blood mononuclear cells (PBMC). This was accomplished by measuring the amount of IL-1β, IL-12p70, IFNγ, and TNFα by ELISA after adding titrated doses (50-0.39 μM) of each agonist to 5 x 10^5 PBMC. The data (shown in Figure 2) indicate the TLR8 active compounds produce higher peak cytokine levels when compared to TLR7 selective compounds. The TLR8 selective agonist **12p** was the most potent inducer of IL-1β, IL-12p70, and IFNγ, while the TLR7/8 mixed agonist **12t** stimulated the most TNFα production in terms of the peak amount production for each cytokine. Both compounds reached maximum responses at approximately 12.5μM. In comparison, the TLR7 selective agonist **12h** showed moderate activity with one exception, IL-12p70. This compound reached peak activity at approximately 1 μM and was close in magnitude to **12t** in stimulating this cytokine. The prototypical TLR7 agonist imiquimod showed no significant cytokine-inducing activity at the concentrations tested. The cytokine induction profiles for each agonist were also quite distinct. The most potent compounds induced sharper peaks. The significance of this result is not clear but may be due to cell toxicity or downregulation of TLR signaling at higher ligand concentrations.

The results also suggest TLR7 and TLR8 activity may not be the only factor involved in triggering cytokine production. While the data shows TLR8 activity correlates with high cytokine induction, the most potent inducer of IL-1β, **12p**, is approximately 6-fold less active than **12t** as a TLR8 agonist as determined by the TLR7/8-HEK reporter system. IL-1β is a key cytokine in ramping up the adaptive immune response to antigens and is critical to the development of long term immunity. Production of this cytokine is mediated by the NLRP3 inflammasome (a multi-protein complex also activated by pathogen or danger associated molecular patterns). Although it is not clear to what extent **12p** directly activates the inflammasome, the results presented here and in previous work reported by our laboratory suggest this pathway may be involved in the production of high IL-1β levels produced by some TLR ligands.[5] The activity of the TLR7 selective agent **12h** in producing IL-12p70 in the low μM range is also noteworthy. IL-12 is Th1 polarizing and critical to the generation of NK cells, cytotoxic T cells, and release of IFNγ and TNFα from T cells. The result suggests some TLR7 selective agents may also be functional in promoting Th1 differentiation and the generation of antigen specific cellular responses. However, it is important to point out that we have not evaluated the production of cytokines associated with Th2 differentiation (e.g. IL-4), so it is not possible to conclude that this effect is selective.

In summary, this data presented herein show TLR7 and TLR8 selectivity is sensitive to substitutions to the N1 position of the imidazoquinoline scaffold. Consistent with prior SAR work by

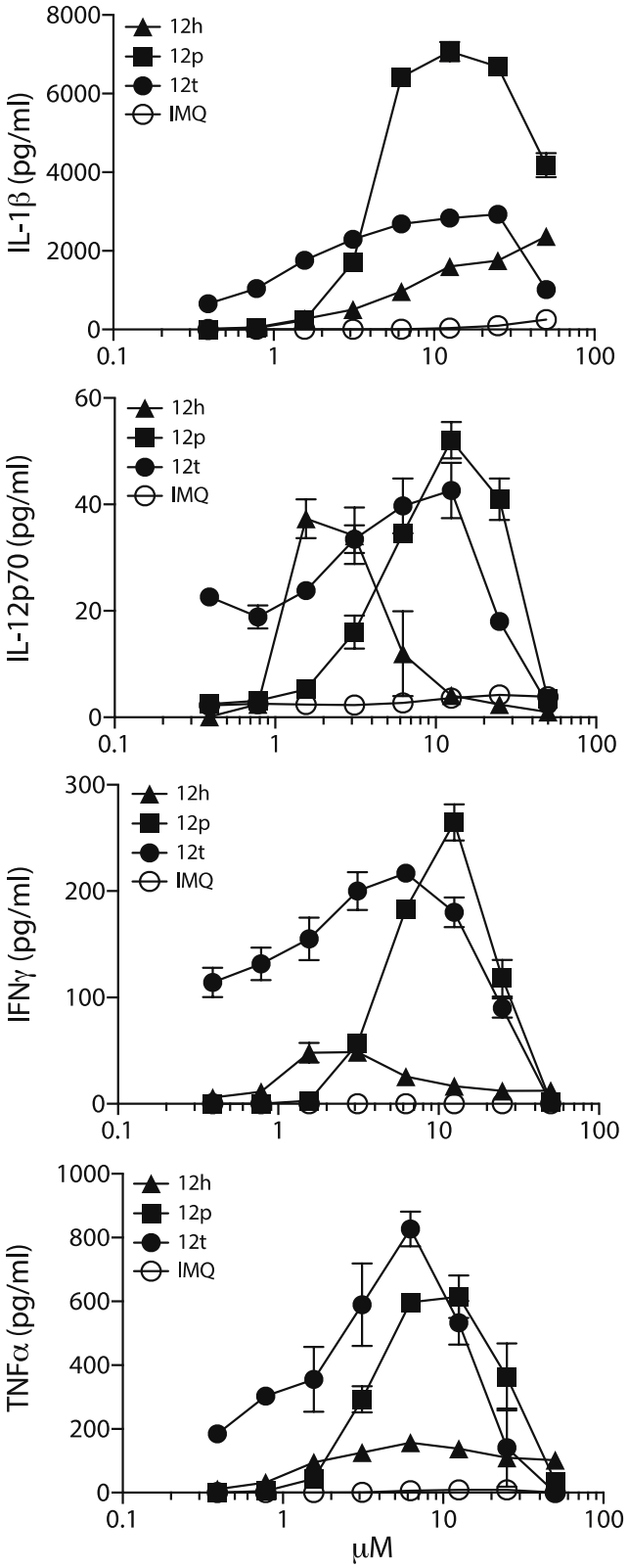


Figure 2. hPBMC cytokine production measured in triplicate following treatment with selective and mixed TLR7/8 agonists.

our group and others, TLR8 activity was found to correlate with a hydrogen bonding donor at the N1 position, with the most potent and selective compounds containing a terminal amino group. Our results also show TLR7 affinity significantly increases with longer N1 chain lengths containing a terminal amine. The TLR7 crystallographic structure.[15] however, provided no insight to the structural basis for the high TLR7 potency of **12s** and **12t**. An analysis of the residues within 6 Å of the N1 position of resiquimod within the X-ray structure revealed no docking points for a charged amine, suggesting the agonists may induce changes in the receptor binding site or may adopt a different orientation. The potent activity of the TLR8 active compounds in producing IL-1β, IL-12p70, TNFα, and IFNγ is also consistent with prior work suggesting TLR8 activation is Th1 polarizing. These four cytokines are critical for the production of cell-mediated immunity and Th1 differentiation. The data further suggest pure TLR8 agonists are more potent stimulators of cytokine production than TLR7 or mixed TLR7/8 agonists. The potent activity of some agonists in promoting the release of specific cytokines raises additional questions regarding the existence of alternative pathways that may be activated either directly or indirectly by some ligands. In particular, the high levels of IL-1β induced by **12p** may point to the recruitment of the NLRP3 inflammasome as a second danger message sent by the agonist. It is interesting to note that another study has shown that inert TLR7 ligands are also capable of enhancing cytokine production when coupled to peptide antigens.[16,17] The mechanism by which these inactive analogs function, however, is not yet known. More work is therefore required to understand the role of alternative pathways in the production of cytokines and to what extent these pathways or mechanisms can be exploited in the design of cytokine-selective inductors that avoid the wholesale expression of pro-inflammatory cytokines triggered by TLR-activation. Given that one of the main limitations to the use TLR-based immunotherapies is cytokine-associated toxicity, the results presented here are noteworthy and deserving of additional investigation.

ASSOCIATED CONTENT

Supporting Information. Full experimental details and compound characterization data as well as biological methods and procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 612-626-2601. E-mail: ferguson@umn.edu.

Funding

Support for this study, in part, was provided by the Masonic Cancer Center, University of Minnesota (to TSG) and the Randy Shaver Cancer Research & Community Fund (to TSG).

ABBREVIATIONS

TLR, toll-like receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IFN, interferon; PBMC, peripheral blood mononuclear cell; IL, interleukin; TNF, tumor necrosis factor.

REFERENCES

(1) 1. 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.

(2) 2. Jurk, M.; Heil, F.; Vollmer, J.; Schetter, C.; Krieg, A. M. Wagner, H.; Lipford, G.; Bauer, S. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat. Immunol*. **2002**, *3*, 499.

(3) 3. Gorden, K. B.; Gorski, K. S.; Gibson, S. J.; Kedl, R. M.; Keiper, 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.

(4) 4. Schiaffo, C. E.; Shi, C.; Xiong, Z.; Olin, M.; Ohlfest, J. R.; Aldrich, C.A.; Ferguson, D. M. Structure–Activity Relationship Analysis of Imidazoquinolines with Toll-like Receptors 7 and 8 Selectivity and Enhanced Cytokine Induction. *J. Med. Chem*. **2014**, *57*, 339-47.

(5) 5. Shi, C. X., Z.; Chittepu, P.; Aldrich, C.C.; Ohlfest, J.R.; and Ferguson, D.F. Discovery of human toll-like receptor 7/8 ligand analogs with novel cytokine induction. *ACS Med. Chem. Lett.* **2012,** *3*, 501-504.

(6) 6. Kokatla, H. P.; Sil, D.; Malladi, S. S.; Balakrishna, R.; Hermanson, A. R.; Fox, L. M.; Wang, X.; Dixit, A.; David, S. A. Exquisite Selectivity for Human Toll-Like Receptor 8 in Substituted Furo[2,3-c]quinolones. *J. Med. Chem*. **2013**, *56*, 6871-6885.

(7) 7. Beesu, M.; Caruso, G.; Salyer, A. C. D.; Khetani, K. K.; Sil, D.; Weerasinghe, M.; Tanji, H.; Ohto, U.; Shimizu, T.; David, S. A. Structure-Based Design of Human TLR8-Specific Agonists with Augmented Potency and Adjuvanticity. *J. Med. Chem*. **2015**, *58*, 7833-7849

(8) 8. Beesu,M.; Malladi,S. S.; Fox, L. M.; Jones, C. D.; Dixit, A.; David, S. A. Human Toll-Like Receptor 8-Selective Agonistic Activities in 1-Alkyl-1H-benzimidazol-2-amines *J. Med. Chem*. **2014**, *57*, 7325-7341.

(9) 9. Beesu, M.; Caruso, G,; Salyer, A. C. D.; Shukla, N. M.; Khetani, K. K.; Smith, L. J.; Fox, L. M.; Tanji, H.; Ohto, U.; Shimizu, T.; David, S. A. Identification of a Human Toll-Like Receptor (TLR) 8-Specific Agonist and a Functional Pan-TLR Inhibitor in 2-Aminoimidazoles. *J. Med. Chem*. **2016**, *59*, 3311-3330.

(10) 10. Tanji, H.; Ohto, U.; Shibata, T.; Miyake, K.; Shimizu, T. Structural reorganization of the Toll-like receptor 8 dimer induced by agonistic ligands. *Science*, **2013**, *339*, 1426-1429.

(11) 11. Gentile , F.; Deriu, M.A.; Licandro, G.; Prunotto, A.; Danani, A.; Tuszynski, J. A. Structure Based Modeling of Small Molecules Binding to the TLR7 by Atomistic Level Simulations. *Molecules*, ***2015***, *20*, 8316-8340.

(12) Ruske, W.; Ruske, E. Nitromalonsäure-dinitril. *Chem. Ber*. **1958**, *91*, 2505–2512.

(13) Peinador, C.; Quintela, J. M.; Moreira, M. J. A short and facile synthesis of Heteromine A. *Tetrahedron*, **1997**, *53*, 8269–8272.

(14) Minakawa, N.; Kojima, N.; Matsuda, A. Nucleosides and nucleotides. 184. Synthesis and conformational investigation of anti-fixed 3-deaza-3-halopurine ribonucleosides. *J. Org. Chem.* **1999**, *64*, 7158–7172.

(15) Zhang, Z.; Ohto, U.; Shibata, T.; Krayukhina, E.; Taoka, M.; Yamauchi, Y.; Tanji, H.; Isobe, T.; Uchiyama,

S.; Miyake, K.; Shimizu, T. Structural Analysis Reveals that Toll-like Receptor 7 Is a Dual Receptor for Guanosine and Single-Stranded RNA. *Immunity* **2016**, *45*, 737-748.

(16) Gao, D.; Liu, Y.; Diao, Y.; Gao, N.; Wang, Z.; Jiang, W.; Jin, G. Synthesis and Evaluation of Conjugates of Novel TLR7 Inert Ligands as Self-Adjuvanting Immunopotentiators. *ACS Med. Chem. Lett*., **2015**, *6*, 249−253.

(17) Gao, D.; Diao, Y.; Li, W.; Gao, N.; Liu, Y.; Wang, Z.; Jiang, W.; Jin, G. Toll-Like Receptor 7 Inactive Ligands Enhanced Cytokine Induction by Conjugation to Weak Antigens. *ChemMedChem*. **2015**, *10*, 977-980.

Design and Synthesis of N1-Modified Imidazoquinoline Agonists for Selective Activation of Toll-like Receptor 7 and 8

Peter Larson,[1] Tamara A. Kucaba[2], Zhengming Xiong,[3] Michael Olin,[3,4] Thomas S. Griffith[2,4,5,6], and David M. Ferguson[1,7]*

[1]Department of Medicinal Chemistry, [2]Department of Urology, [3]Department of Pediatrics, [4]Masonic Cancer Center, [5]Center for Immunology, [6]Microbiology, Immunology, and Cancer Biology Graduate Program, [7]Center for Drug Design, University of Minnesota, Minneapolis, MN 55455

TOC Graphic