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Regioisomerism-dependent TLR7 agonism and antagonism in an imidazoquinoline

Nikunj M. Shukla, Matthew R. Kimbrell, Subbalakshmi S. Malladi, Sunil A. David *

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

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ABSTRACT

Chronic immune activation is a hallmark of progressive HIV infection. Recent reports point to the engagement of toll-like receptor 7 (TLR7) and -9 by viral RNA as contributing to the activation of innate immune responses, which drive viral replication leading to immune exhaustion. The only known class of TLR7 antagonists is single-stranded phosphorothioate oligonucleotides, which has been demonstrated to inhibit immune activation in human and *Rhesus* macaque in vitro models. The availability of a selective and potent small-molecule TLR7 antagonist should allow the evaluation of potential benefits of suppression of TLR7-mediated immune activation in HIV/AIDS. Gardiquimod is a known N^1-substituted 1*H*-imidazoquinoline TLR7 agonist, the synthesis of which has not been published. We show that the 3*H* regioisomer is completely inactive as a TLR7 agonist and is weakly antagonistic. A *des*-amino precursor of the 3*H* regioisomer is more potent as a TLR7 antagonist, with an IC_{50} value of 7.5 μM. This class of compound may serve as a starting point for the development of small-molecule inhibitors of TLR7.

Chronic immune activation is a hallmark of progressive HIV infection which is strongly associated with dysregulated cellular and humoral immune responses;[1,2] furthermore, the resulting accelerated turnover of $CD4^+$ lymphocytes provides a milieu for HIV replication.[3] The majority of the lymphocytic pool resides in the gastrointestinal tract and it has recently been demonstrated that during acute HIV infection, a tremendous depletion submucosal $CD4^+$ lymphocytes occurs as a result of massive viral replication.[4–6] The loss of mucosal integrity is due to the resultant enteropathy, which has been shown to contribute to sustained systemic immune activation as a consequence of translocation of bacterial products such as lipopolysaccharide,[7] an agonist of toll-like receptor 4 (TLR4).[8] The engagement of TLR7 and -9 by single-stranded viral RNA has recently been reported to play a central role in immune activation-driven HIV replication. For instance, progressive $CD4^+$ T lymphocyte depletion in non-human primates is highly correlated with TLR7-mediated interferon-α (IFN-α) by plasmacytoid dendritic cells, and antagonists of TLR7 inhibit immune activation.[9]

Certain 4-aminoquinolines such as chloroquine and hydroxychloroquine inhibit TLR7 indirectly via blocking endolysosomal maturation,[10,11] and the only known true TLR7 receptor antagonists are single-stranded phosphorothioate oligonucleotides.[12,13] The availability of a selective and potent small-molecule TLR7 antagonist should allow the formal testing of potential benefits of suppression of TLR7-mediated immune activation in HIV/AIDS. Gardiquimod is a commercially available N^1-substituted 1*H*-imidazoquinoline TLR7 agonist,[14] the synthesis of which is not available in the scientific literature, although closely related analogues have been disclosed in a patent.[15] The syntheses of both gardiquimod and its 3*H* regioisomer were undertaken in an effort to explore hitherto poorly-characterized structure–activity relationships of substituents on the imidazoline ring (Scheme 1); the 3*H* regioisomer was found to be completely inactive as a TLR7 agonist and was, in fact, weakly antagonistic. A *des*-amino precursor of the 3*H* regioisomer was more potent as a TLR7 antagonist, with an IC_{50} value of 7.5 μM. This class of compound may serve as a useful lead and a starting point for the development of small-molecule inhibitors of TLR7.

Following literature precedents,[16] anthranilic acid **1** was condensed with 2-nitroacetaldehyde oxime which was prepared in situ from nitromethane and sodium hydroxide to give 2-(2-nitroethylideneamino)benzoic acid, **2**; this was then cyclized to form the quinoline ring using acetic anhydride and potassium acetate to give 3-nitro-4-hydroxyquinoline (**3**). Treatment with phosphorous oxychloride afforded 3-nitro-4-chloroquinoline (**4**), which served as the common precursor for the synthesis of gardiquimod (**9**), as well as its regioisomer **15**. 1-amino-2-methylpropan-2-ol (**18**) was obtained by reacting NH_4OH with 2,2-dimethyloxirane at 0 °C; isolation and purification were facilitated by temporary *N*-Boc protection, which was eventually deprotected with CF_3CO_2H. The *N*-Boc-protected *N*-ethylglycine derivative (**22**), was synthesized from the methyl ester of glycine (**19**) via reductive

* Corresponding author. Tel.: +1 785 864 1610; fax: +1 785 864 1961.
E-mail address: sdavid@ku.edu (S.A. David).

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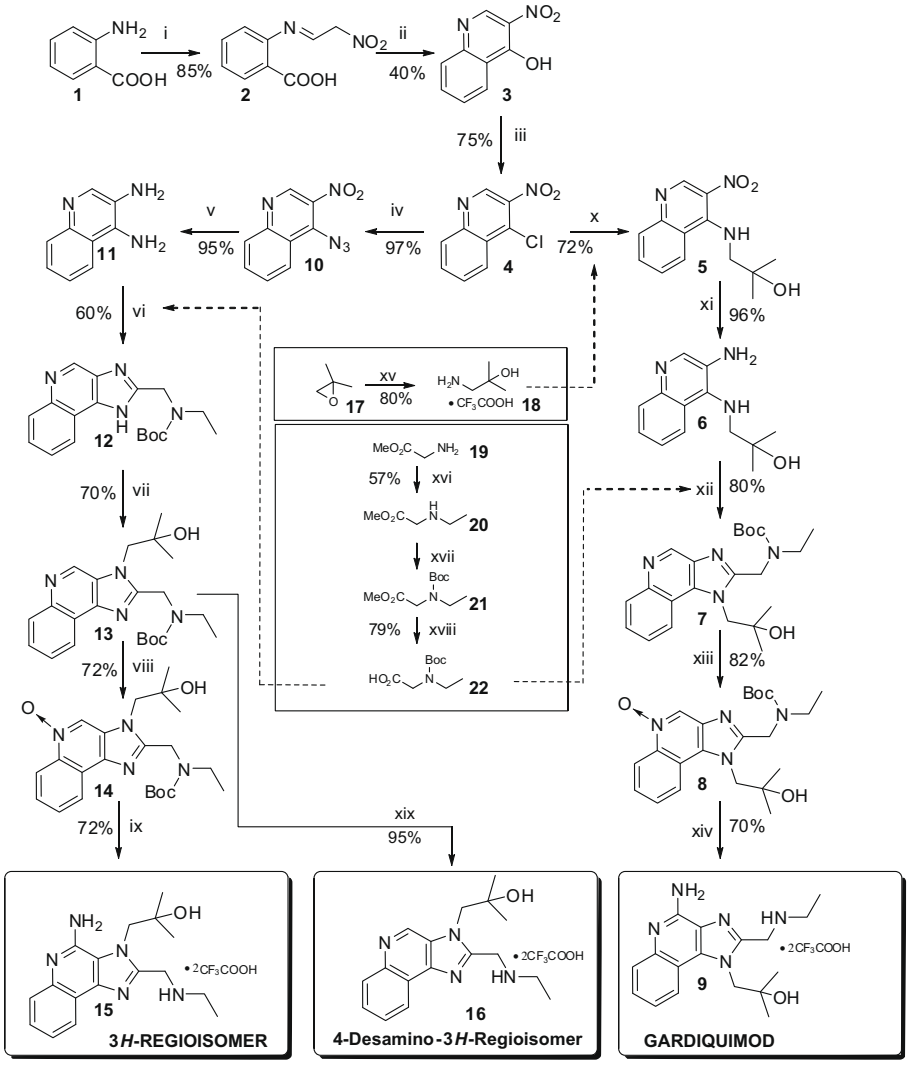

Scheme 1. Synthesis of gardiquimod, its 3*H*-regioisomer, and the 4-desamino-3*H*-regioisomer. Reagents and conditions: (i) HCl, HON=CH $CHCH_2NO_2$; (ii) $(CH_3CO)_2O$, CH_3COOK; (iii) $POCl_3$; (iv) NaN_3, DMF; (v) Pd/C, H_2, MeOH; (vi) 2-(*tert*-butoxycarbonyl(ethyl)amino)acetic acid, HATU, DMF; (b)$NaOH/H_2O$, EtOH; (vii) DBU, 2,2-dimethyloxirane; (viii) 3-chloroperoxybenzoic acid, CH_2Cl_2, $CHCl_3$, MeOH; (ix) (a) benzoyl isocyanate, CH_2Cl_2, (b) $NaOCH_3$, MeOH (c) CF_3CO_2H; (x) 1-amino-2-methylpropan-2-ol, CH_2Cl_2, Et_3N; (xi) Pd/C, H_2, MeOH; (xii) (a) 2-(*tert*-butoxycarbonyl(ethyl)amino)acetic acid, HATU, DMF; (b) $NaOH/H_2O$, EtOH; (xiii) 3-chloroperoxybenzoic acid, CH_2Cl_2, $CHCl_3$, MeOH; (xiv) (a) benzoyl isocyanate, CH_2Cl_2 (b) $NaOCH_3$, MeOH (c) CF_3CO_2H; (xv) (a) NH_4OH, 0 °C; (b) $(Boc)_2O$, MeOH; (c) CF_3CO_2H; (xvi) CH_3CHO, $NaCNBH_3$, MeOH; (xvii) $(Boc)_2O$, MeOH; (xviii) $LiOH/H_2O$, THF/MeOH; (xix) CF_3CO_2H.

amination using acetaldehyde and sodium cyanoborohydride, followed by *N*-Boc protection using $(Boc)_2O$, and subsequent ester hydrolysis using LiOH.

Synthesis of gardiquimod: An S_NAr reaction on **4** with 1-amino-2-methylpropan-2-ol (**18**) afforded the 3-nitro-4-aminoquinoline derivative (**5**), which was converted to the imidazoquinoline **7** via sequential Pd-catalyzed reduction of the nitro group of **6**, amidation with the *N*-Boc-protected amino acid (**22**), followed by cyclization with NaOH to afford the intermediate **7**.[17] *N*-oxidation of the quinoline nitrogen of **7** with *m*-CPBA yielded **8**, which was reacted with benzoyl isocyanate to provide a 4-*N*-benzoyl derivative. This amide was cleaved using NaOMe in MeOH as has been reported[17] to yield the *N*-Boc-protected precursor of gardiquimod. Final deprotection was performed with CF_3CO_2H to give the target molecule **9**, as the TFA salt, which was as active as the commercially-available reference compound (Fig. 1).

Synthesis of the 3H regioisomer of gardiquimod: An S_NAr displacement of **4** to the corresponding azido derivative **10** with NaN_3, followed by reduction of both the 3-nitro- and the 4-azido groups with Pd/C under hydrogenation conditions gave the 3,4-diamino-

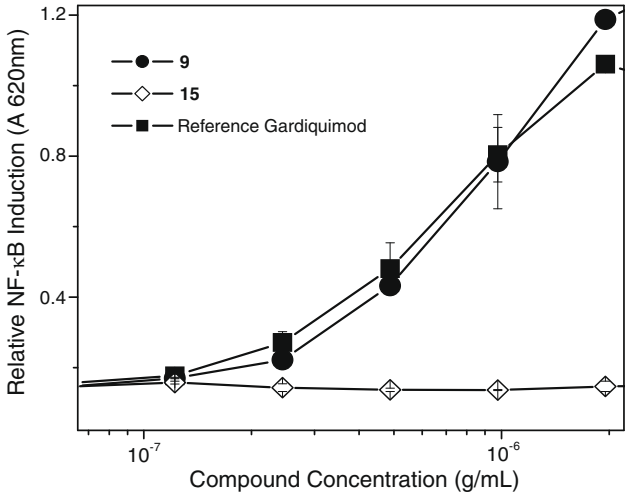

Figure 1. NF-κB induction activities of **9** and reference gardiquimod in a TLR7-specific reporter gene assay. IC_{50} values of both compounds is 0.65 μg/mL. **15** was inactive at the highest concentration test (25 μg/mL).

quinoline **11**. Amidation of **11** with **22**, followed by cyclization using NaOH afforded the *C*-2-substituted imidazoquinoline scaffold **12**. Reaction of **12** with excess of the 2,2-dimethyloxirane in the presence of DBU afforded the *N*-3 substituted imidazoquinoline **13**, which after deprotection yielded **16**. Subsequent steps, similar to the ones described above afforded the 3*H*-regioisomer **15**. Comparison of the ^{1}H NMR spectra of **9** and **15** showed, as expected, differences in the chemical shifts of the substituents on the imidazole ring; significant differences were also seen for the C9 protons, probably due to through-space effects of the substituents (Supplementary data).

TLR7 agonism and antagonism: A reporter gene assay using TLR7-dependent NF-κB induction was used. The induction of NF-κB was quantified using HEK-Blue-cells as previously described by us.[18] HEK293 cells were stably transfected with plasmids encoding TLR7 as well as an NF-κB reporter gene coupled to secreted alkaline phosphatase (sAP) (InvivoGen, San Diego, CA), 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 agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue-7 cells were incubated at a density of ~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 were added. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm. The TLR7 agonistic potency of **9** was indistinguishable from that of the commercially available gardiquimod (Fig. 1), while **15** was devoid of agonistic activity (Fig. 1).

None of the precursors of **9** displayed any significant agonistic activities (data not shown). Antagonistic activities were examined by incubating HEK-Blue-7 cells with graded concentrations of test compounds in the presence of 250 ng/mL of gardiquimod. **15** displayed weak TLR7 antagonism (IC_{50}: 25 μM, Fig. 2), which prompted us to carefully examine all its precursors. The *des*-amino compound **16** obtained after deprotecting **13** was found to be maximally antagonistic with an IC_{50} of 7.5 μM. Compound **16**, fortuitously, also crystallized with ease in MeOH, the structure of which is shown in Fig. 2.

Compound **16**, to our knowledge, is the first small molecule with TLR7-antagonistic activity, and may therefore be useful as a lead for the syntheses of focused libraries toward the generation of more potent small-molecule receptor antagonists of TLR7.

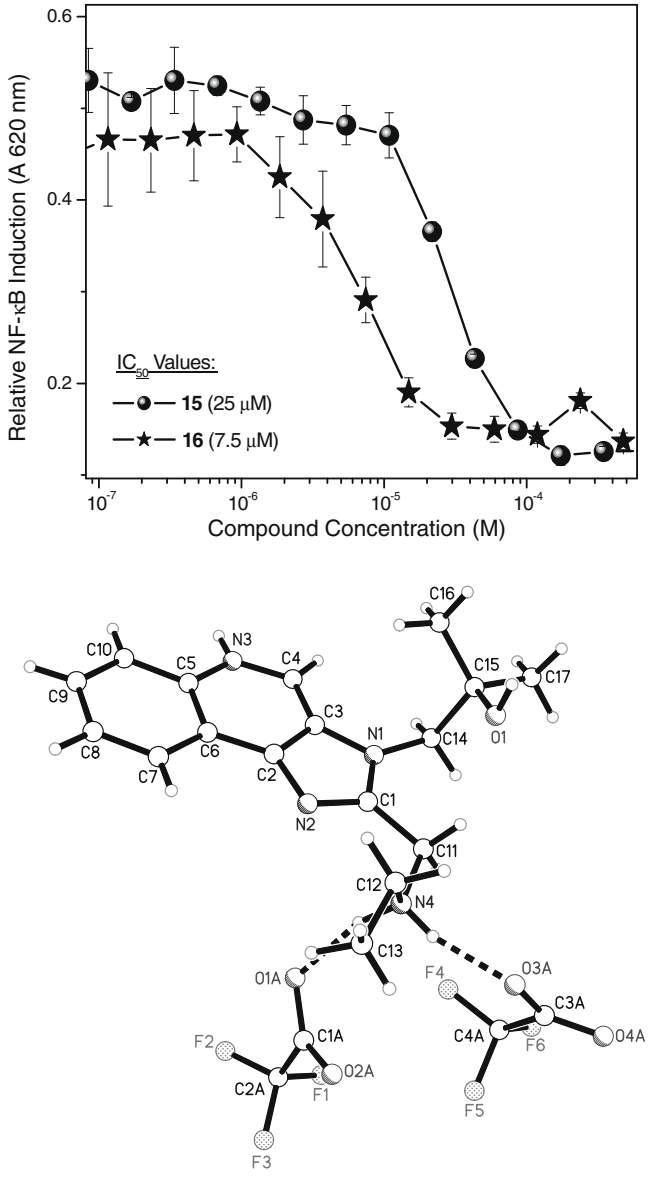

Figure 2. Top: TLR7-antagonistic activities of the 3*H* regioisomer **15**, and of its *des*-amino precursor **16**. Bottom: Crystal structure of **16** as the di-TFA salt. The locations of all the hydrogens were determined from a difference Fourier and refined as individual isotropic atoms.

Acknowledgements

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Supplementary data

The crystal structure of **16** has been deposited at the Cambridge Crystallographic Data Centre (Accession number: CDC 718787). Synthetic procedures, ^{1}H, ^{13}C NMR, mass-spectral data of target compounds and precursors, and ^{1}H NMR spectra overlays of **9** and **15**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.100.

References and notes

1. Boasso, A.; Shearer, G. M. *Clin. Immunol.* **2008**, *126*, 235.
2. Douek, D. C.; Roederer, M.; Koup, R. A. *Annu .Rev. Med.* **2009**, *60*, 471.
3. Pantaleo, G.; Graziosi, C.; Demarest, J. F.; Butini, L.; Montroni, M.; Fox, C. H.; Orenstein, J. M.; Kotler, D. P.; Fauci, A. S. *Nature* **1993**, *362*, 355.
4. Brenchley, J. M.; Schacker, T. W.; Ruff, L. E.; Price, D. A.; Taylor, J. H.; Beilman, G. J.; Nguyen, P. L.; Khoruts, A.; Larson, M.; Haase, A. T.; Douek, D. C. *J. Exp. Med.* **2004**, *200*, 749.
5. Brenchley, J. M.; Douek, D. C. *Mucosal. Immunol.* **2008**, *1*, 23.
6. Mehandru, S.; Poles, M. A.; Tenner-Racz, K.; Horowitz, A.; Hurley, A.; Hogan, C.; Boden, D.; Racz, P.; Markowitz, M. *J. Exp. Med.* **2004**, *200*, 761.
7. Douek, D. *Top. HIV Med.* **2007**, *15*, 114.
8. Akira, S. *Immunol. Rev.* **2009**, *227*, 5.
9. Mandl, J. N.; Barry, A. P.; Vanderford, T. H.; Kozyr, N.; Chavan, R.; Klucking, S.; Barrat, F. J.; Coffman, R. L.; Staprans, S. I.; Feinberg, M. B. *Nat. Med.* **2008**, *14*, 1077.
10. Lee, J.; Chuang, T. H.; Redecke, V.; She, L.; Pitha, P. M.; Carson, D. A.; Raz, E.; Cottam, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6646.
11. Sun, S.; Rao, N. L.; Venable, J.; Thurmond, R.; Karlsson, L. *Inflamm. Allergy Drug Targets* **2007**, *6*, 223.
12. Barrat, F. J.; Meeker, T.; Gregorio, J.; Chan, J. H.; Uematsu, S.; Akira, S.; Chang, B.; Duramad, O.; Coffman, R. L. *J. Exp. Med.* **2005**, *202*, 1131.
13. Wang, D.; Bhagat, L.; Yu, D.; Zhu, F. G.; Tang, J. X.; Kandimalla, E. R.; Agrawal, S. *J. Med. Chem.* **2009**, *52*, 551.
14. Hemmi, H.; Kaisho, T.; Takeuchi, O.; Sato, S.; Sanjo, H.; Hoshino, K.; Horiuchi, T.; Tomizawa, H.; Takeda, K.; Akira, S. *Nat. Immunol.* **2002**, *3*, 196.
15. Radmer, M. R.; Moser, W. H.; Moseman, J. T.; Dellaria, J. F., Jr. PCT/US2004/040383[WO/2005/076783], 2005.

16. Goblyos, A.; Gao, Z. G.; Brussee, J.; Connestari, R.; Santiago, S. N.; Ye, K.; Ijzerman, A. P.; Jacobson, K. A. *J. Med. Chem.* **2006**, *49*, 3354.
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. *J. Med. Chem.* **2005**, *48*, 3481.
18. Kimbrell, M. R.; Warshakoon, H.; Cromer, J. R.; Malladi, S.; Hood, J. D.; Balakrishna, R.; Scholdberg, T. A.; David, S. A. *Immunol. Lett.* **2008**, *118*, 132.