Bioorganic & Medicinal Chemistry Letters 25 (2015) 1318–1323

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural requirements for TLR7-selective signaling by 9-(4-piperidinylalkyl)-8-oxoadenine derivatives

Hélène G. Bazin *, Yufeng Li †, Juhienah K. Khalaf ‡, Sandra Mwakwari, Mark T. Livesay, Jay T. Evans, David A. Johnson

GlaxoSmithKline Vaccines, 553 Old Corvallis Road, Hamilton, MT 59840, USA

ARTICLE INFO

Article history:
Received 24 November 2014
Revised 14 January 2015
Accepted 19 January 2015
Available online 31 January 2015

Keywords:
TLR7/8 agonist
Oxoadenine
Immunostimulants

ABSTRACT

We report the synthesis and biological evaluation of a new series of 8-oxoadenines substituted at the 9-position with a 4-piperidinylalkyl moiety. In vitro evaluation of the piperidinyl-substituted oxoadenines **3a**–**g** in human TLR7- or TLR8-transfected HEK293 cells and in human PBMCs indicated that TLR7/8 selectivity/potency and cytokine induction can be modulated by varying the length of the alkyl linker. Oxoadenine **3f** containing a 5-carbon linker was found to be the most potent TLR7 agonist and IFNα inducer in the series whereas **3b** possessing a 1-carbon linker was the most potent TLR8 agonist.

Toll-like receptors (TLR) are a family of structurally related receptors that detect highly conserved microbial components common to large classes of pathogens. These receptors are expressed on immune cells and upon activation mobilize defense mechanisms aimed at eliminating the invading pathogens. Of the more than 10 known TLRs that have been identified in humans, five are associated with the recognition of bacterial components (TLRs 1, 2, 4, 5, 6) and four others (TLRs 3, 7, 8, 9) appear to be restricted to cytoplasmic compartments and are involved in the detection of viral RNA (TLRs 3, 7, 8) and unmethylated DNA (TLR9).[1,2] Activation of TLRs regulates intracellular signaling pathways leading to the expression of inflammatory cytokines/chemokines and type I interferons (IFNα/β), which can lead to the preferential enhancement of innate anti-microbial responses and antigen-specific humoral and cell-mediated immune responses.

In the case of TLR7 and TLR8 activation, a few different classes of small molecule mimetics of the natural uridine- and/or guanosine-rich viral ssRNA ligands have been identified,[3–5] including 1*H*-imidazo[4,5-*c*]quinolines[6] and 8-hydroxyadenines.[7] Through screening of a library of purine derivatives, Hirota[7] discovered that 9-benzyl-8-hydroxyadenine **1** (Fig. 1; shown as favored keto/oxo tautomer) possessed IFN-inducing activity in vitro. Further evaluation of structure-activity relationships (SAR) in the 9-benzyl hydroxyadenine or 'oxoadenine' series revealed that a hydroxy group at the 8-position is essential for IFN-inducing activity and that an alkylated heteroatom at the 2-position dramatically increases IFN activity (e.g., compound **2**, Fig. 1).

While an extensive evaluation of SAR in the 9-arylmethyl and heteroarylmethyl oxoadenine series has been carried out over the past several years,[8–16] to our knowledge no systematic studies have been performed on the corresponding saturated derivatives (i.e., cycloalkyl or heterocycloalkyl-substituted oxoadenines). The few reported oxoadenines with cycloalkyl groups attached to the nitrogen at the 9-position (directly or via a methylene unit) have demonstrated weak or diminished IFN induction.[7,11] Since alkyl linker length on the corresponding N1 of 1-phenylalkylimidazoquinolines is known to profoundly effect IFN-inducing activity,[6] we were particularly interested in the synthesis and biological evaluation of a series of 4-piperidinylalkyl derivatives of **2** in which the length of the N9 alkyl linker was systematically varied (compounds **3a**–**g**, Fig. 1). In addition to the ability to form water-soluble salts, the 4-piperidinyl moiety of oxoadenines **3a**–**g** also provides a suitable handle for potential N-derivatization and conjugation as substitution of the aromatic amino group in both the oxoadenine and imidazoquinoline series abolishes IFN-inducing activity.[6,17] Conjugation of TLR7/8 agonists to lipids, proteins, and other molecules is known to enhance immune responses and decrease toxic effects.[6,18,19]

The piperidinylalkyl oxoadenines **3a**–**g** were synthesized from 2-*n*-butoxy-8-methoxyadenine (**6**)[20] and *N*-*t*-butoxycarbonyl (Boc)-4-piperidinyl derivatives **10** or **11** in a convergent manner as shown in Scheme 1. Adenine **6** was prepared in 6 steps and in

* Corresponding author.
† Present address: GlaxoSmithKline Oncology R&D, 1250 S. Collegeville Road, Collegeville, PA 19426, USA.
‡ Present address: Omeros Corporation, 201 Elliot Avenue W., Seattle, WA 98199, USA.

http://dx.doi.org/10.1016/j.bmcl.2015.01.037
0960-894X/

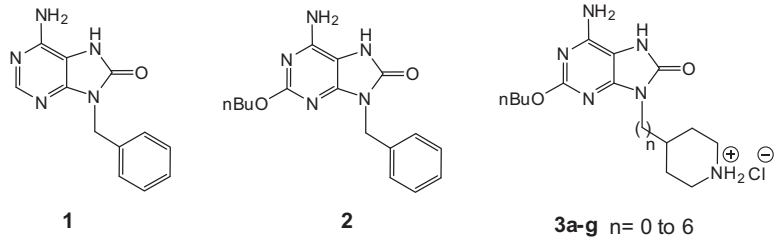
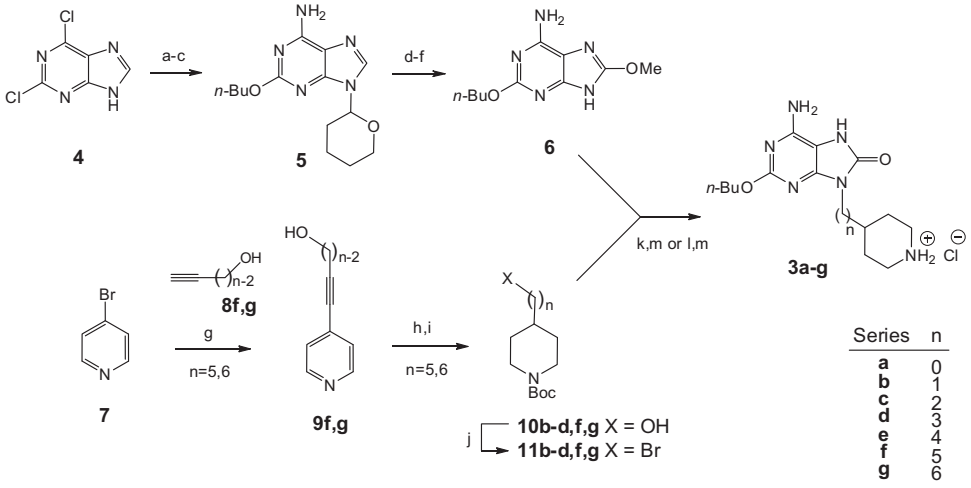


Figure 1. Structures of prototypical oxoadenines **1** and **2** and piperidinyl-substituted oxoadenines **3a**–**g**.

56% overall yield from commercially available 2,6-dichloropurine (**4**).[21] N-protection of **4** as the tetrahydropyranyl (THP) aminal, followed by sequential displacement of the 6- and 2-chloro groups with ammonia and *n*-butoxide gave the THP-protected adenine **5**[20] in 73% overall yield from **4**. Adenine **5** was then converted to **6** in 3 steps and in 77% overall yield by 8-bromination, bromide displacement with methoxide, and THP-deprotection with trifluoroacetic acid (TFA). The requisite Boc-protected 4-piperidinylalkyl bromides were either purchased commercially (**11a**,**e**) or prepared from the corresponding alcohols using Appel conditions (Ph_3P/CBr_4). The two piperidinyl alcohols not commercially available (**10f**,**g**) were prepared in 3 steps from 4-bromopyridine (**7**) by Sonogashira coupling of **7** with acetylenic alcohols **8f**,**g**, followed by hydrogenation of the alkynyl pyridines **9f**,**g** and *N*-Boc protection of the resulting piperidinyl alkanols.[22] Subsequent N-alkylation of adenine **6** with the Boc-protected piperidinylalkyl bromides **11b**–**g**[23] in the presence of potassium carbonate in dimethylformamide (DMF) followed by cleavage of the Boc and methoxy groups with 4 N HCl in dioxane afforded the desired oxoadenine hydrochloride salts **3b**–**g**[24] in 41–84% overall yield from **6**. Since N-alkylation of **6** with 1-Boc-4-bromopiperidine (**11a**) failed to give any product under these conditions (K_2CO_3, DMF), oxoadenine **3a**[25] was conveniently prepared according to a modification of a literature method[26] by Mitsunobu reaction of **6** with 1-Boc-4-hydroxypiperidine (**10a**) in presence of diisopropyl azodicarboxylate (DIAD) and PPh_3 followed by acidic deprotection.

The human (h) TLR7/8 activity of new oxoadenines **3a**–**g** was assessed by a reporter gene assay using HEK293 cells stably transfected with either hTLR7 or hTLR8 and the NFκB SEAP (secreted embryonic alkaline phosphatase) reporter (Fig. 2).[27] This assay measures NFκB mediated SEAP production following TLR7- or TLR8-specific activation. It should be noted that the HEK reporter assay only measures the NFκB pathway so additional assay systems are necessary to evaluate IRF7 pathway activation. The hTLR7 and hTLR8 specificity and potency (EC_{50}) of oxoadenines **3a**–**g** are shown in Figure 2. Oxoadenine **3a** was not active on hTLR7 or hTLR8 but the other oxoadenines **3b**–**g** were all active. While increasing the linker length beyond one carbon dramatically increased hTLR7 potency, no linear correlation between linker length and hTLR7 potency was observed in this assay. The 5-carbon linker oxoadenine **3f** was the most potent hTLR7 agonist of the series while the 1-carbon linker oxoadenine **3b** was the most potent hTLR8 agonist of the series, with hTLR8 potency significantly decreasing with longer linkers.

The loss of hTLR8 activity observed after stimulation with higher doses of oxoadenines **3e**–**g** suggested possible toxicity or activation induced cell death in HEK293-hTLR8 cells. Live/dead® fixable Aqua staining was used to evaluate potential cell death following HEK293-hTLR8 stimulation with oxoadenines **3e**–**g**. Aqua staining (cell death) at 24 h following stimulation correlated with both oxoadenine linker length and dose (Fig. 3). Cell toxicity was not observed after 24 h stimulation with the shorter linker oxoadenines **3b**–**d** (data not shown). The oxoadenine dose triggering HEK293-hTLR8 cell toxicity was the lowest with the 6-carbon linker (**3g**) and increased with decreased linker length (**3e**,**f**). However, the longer linker length oxoadenines (**3e**–**g**) induced modest NFκB activation in the HEK293-hTLR8 cells suggesting that the toxicity observed in HEK293-hTLR8 cells is dissociated from NFκB activity and possibly associated with activation of another intracellular signaling pathway via TLR8.



Scheme 1. Reagents and conditions: (a) 3,4-dihydropyran, *p*-TsOH, AcOEt, 50 °C; (b) 2 M NH_3 in *i*-PrOH, 60 °C, 86% (2 steps); (c) *t*-BuONa, *n*-BuOH, 100 °C, 85%; (d) *N*-bromosucccinimide, $CHCl_3$, rt, 88%; (e) NaOMe, MeOH, reflux; (f) TFA, MeOH, rt, 87% (2 steps); (g) $(PPh_3)PdCl_2$, CuI, Et_3N, Δ, 82% (**9f**), 21% (**9g**); (h) 10% $Pd(OH)_2$/C, H_2, AcOH, 90 °C; (i) Boc_2O, Et_3N, CH_2Cl_2, rt, 80% (2 steps); (j) CBr_4, PPh_3, CH_2Cl_2, rt, 92–99%; (k) Et_3N, PPh_3, DIAD, DMF, 70 °C, 63%; (l) K_2CO_3, DMF, 50 °C; (m) 4 N HCl/dioxane, MeOH, rt, 59% (**3a**), 75% (**3b**), 83% (**3c**), 41% (**3d**), 63% (**3e**), 64% (**3f**), 84% (**3g**).