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2-Azidoalkoxy-7-hydro-8-oxoadenine derivatives as TLR7 agonists inducing dendritic cell maturation

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ABSTRACT

The synthesis of an array of 2-azidoalkoxy substituted 7-hydro-8-oxoadenines is described. The relation of the structure of these compounds and their ability to induce maturation of dendritic cells is evaluated.

Toll-like receptor 7 (TLR7), one of the thirteen mammalian TLRs[1] currently known, can be activated by specific imidazoquinolines.[2] As such, TLR7 is the first TLR for which small molecule modifiers were identified, a result that has spawned great interest worldwide in the search after specific agents to either enhance a specific immune response (through activating a given TLR) in battling cancer or infectious disease[3], or reduce an immune response (through inactivating a TLR) in autoimmune disease therapies.[4–6] TLRs appear to have evolved to recognize (partially degraded) biomolecules from microbial origin and upon recognition initiate a signaling cascade leading to a specific immune response against these microbial invaders.[7] The natural TLR7 ligands are single strand RNA (ssRNA) oligonucleotides[8] and the therapeutic potential of ssRNA molecules is thought to be limited due to their low enzymatic and chemical stability and high cost. It is therefore fortuitous that TLR7 also recognizes imidazoquinolines and 7-hydro-8-oxoadenines.[9] Whether TLR7 activation by these adenine analogues (see for representative examples structure 1–4, Fig. 1) occurs by interaction with the ssRNA binding site or by occupation of an altogether different site of the TLR 7 receptor remains to be established. The archetypal imidazoquinoline-based TLR agonist is imiquimod **1**[10], also known as R-837. Imiquimod is a component of the therapeutic ointment Aldara[11] that is currently in use as a treatment for several skin diseases[12,13] including superficial basal

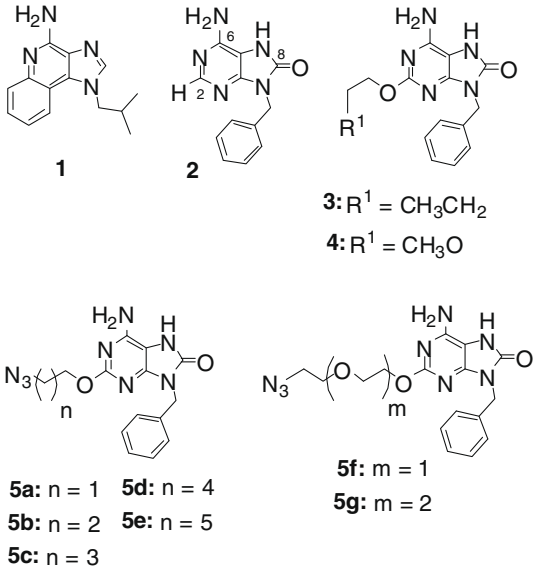

Figure 1. Adenine analogues known to be TLR-7 agonists.

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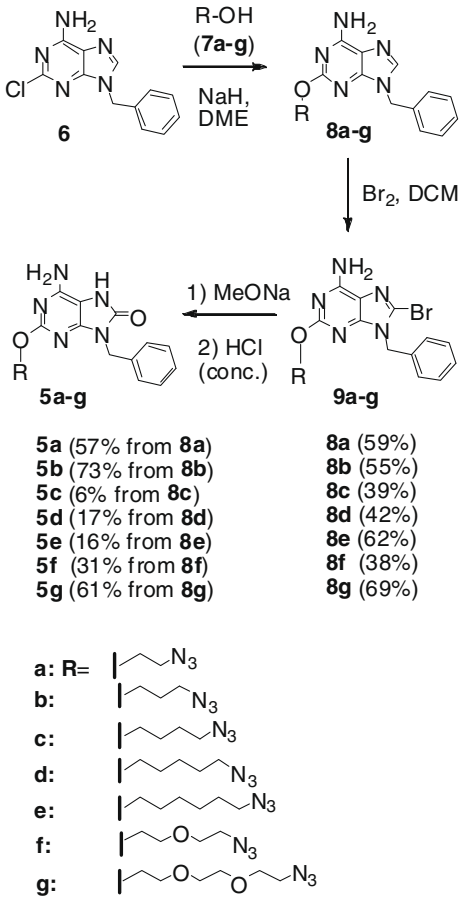

Scheme 1. Synthesis of 7-hydro-8-oxoadenines derivatives **5a**–**g**.

cell carcinoma. Imiquimod, however is not an optimal TLR7 ligand and some side effects that could be linked to imiquimod[14,15] spurned research towards alternative small molecule TLR7 agonists.

Hirota and co-workers[16] conducted a study in which a compound library assembled from modified purines and pyrimidines were assessed on their IFN-α inducing properties. From these studies compound **2** emerged as a new lead (Fig. 1). Ensuing optimization studies[17–24] led, amongst others to 7-hydro-8-oxoadenine derivative **3**[25] as the most potent IFN- α inducer for in vitro assays, and to the analogue compound **4**[16] with improved bioavailability for in vivo applications. Subsequent work suggested the TLR7 agonizing activity as the molecular basis of the biological action of 7-hydro-8-oxoadenine derivatives.[23] Towards our goal to discover TLR-ligands capable to induce dendritic cell (DC) maturation we set out to synthesize an array of analogues of compound **3** and evaluate their potency as DC maturating agents. The aim of the study described here is two-fold: a) is it possible to enhance TLR7 binding and agonistic activity by directed modification of the alkyl substituents at C-2 in **4**, and b) to introduce in derivatives of **3** a ligation handle for conjugation purposes, such as the TLR-ligand-peptide constructs as leads for vaccine development alluded to in the introduction.

Target compounds **5a–g** were prepared starting from known[25] 9-benzyl-2-chloro-6-aminopurine (**6**) in a straightforward fashion as outlined in Scheme 1. Nucleophilic aromatic substitution of the chlorine at C2 in **6** with a range of alkoxides (**7a–g**) proceeded uneventfully to give 2-azidoalkoxypurine derivatives **8a–g** in 38–69% yield. These intermediates were transformed into the target 8-oxopurines **5a–g** by means of a reported three step procedure[24]: (1) bromination, (2) displacement of Br with OMe and (3) acidic hydrolysis with aqueous HCl. Although the yields vary for the individual steps, (see the Scheme 1 and Supplementary data for specific yields) each target compound was prepared in sufficient quantities and purity for ensuing TLR-binding studies.

The ability of the new purine derivatives to stimulate TLR7 mediated dendritic cell activation was assessed in a comparative study in which the literature compounds **3** and **4** were included. Upon stimulation of TLR7 by its natural agonist (ssRNA) a series of events takes place resulting in, amongst others, the secretion of the cytokine IL12[2,26] and upregulation of surface markers associated with dendritic cell maturation.[27] Briefly, murine bone marrow derived dendritic cells (BMDCs) were incubated for 24 h with compounds **3**, **4** and **5a–g** at different concentration (see Fig. 2 legends). The supernatants were harvested in the next step and the amounts of IL12 in these were assessed using a standard sandwich ELISA assay[28] or phenotypic characterization of the dendritic cells was done after 48 h stimulation with the different TLR7 derivatives by staining for different surface markers associated with DC maturation.[28]

As shown in Figure 2A, all compounds were able to induce upregulation of surface markers CD86, MHC class II (Fig. 2A) and CD40 (data not shown) on dendritic cell at 1 μM. At this concentration minor distinction between the potencies of the different compounds can be made, except for dendritic cells incubated with compound **5-g** which showed less pronounced upregulation of the maturation markers. Next, the ability of the dendritic cells to produce IL-12 upon stimulation with the individual compounds was addressed (Fig. 2B). As is evident all compounds except for compound **5-g** induced comparable level of IL-12 at 5 μM. However, more subtle differences become apparent at lower concentrations (0.1 μM), with analogues **5a,** and **5b** expressing activities similar to that of reference compound **3**. At the lowest concentration used (10 nM) the superior activity of **3** compared to all others comes to light.

Returning to the dual aim referred to in the introduction the small panel of compounds generated here does not contain a more potent TLR7 agonist compared to the literature compound **3**, at least not in the assays employed here. However, introduction of an azide moiety does not necessarily impede TLR7 activating properties, as is evidenced by the rather potent IL12 stimulating activities of derivatives **5a** and **5b**. This holds promise for the further derivatization of these new lead TLR7-ligands, and two separate strategies can be envisaged.

In the first, the azide can be reduced to the amine which is then condensed with a range of acids to give a number of amides. In an alternative approach the azides in **5a–g** may be employed in a Huisgen [3+2] cycloaddition mediated derivatization approach. Here, a library of acetylenes should give access to a library of triazoles. We have previously shown that **5g** is a suitable substrate for Huisgen reaction.[29]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.095.

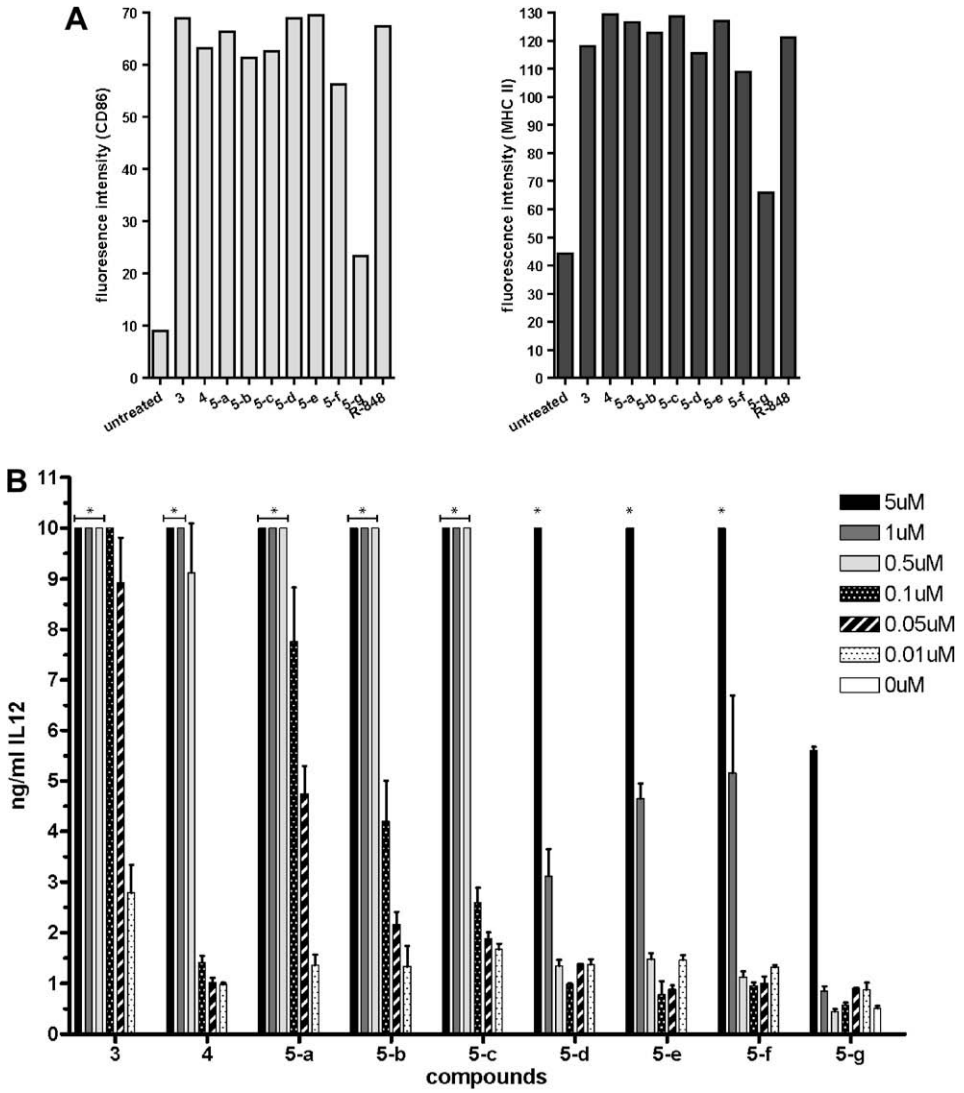

Figure 2. Activation of dendritic cells by compounds **3**, **4** and **5a–g**. (A) BMDCs were incubated with 1 μM of the indicated compounds for 48 h or left untreated. Cells were stained with CD86 or MHC II antibodies and subjected to flow cytometry analysis (Supplementary data), R848 (resiquimod) a known[2] derivative of **1** was used as positive control; (B) BMDC were incubated with the indicated compounds for 24 h supernatant was harvested and the concentration of IL-12p40 was determined.[28] * Indicate that the values were higher than the standard curve (>10 ng/ml IL-12). Results are means of triplicates ± SEM.

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