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Article

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Human Toll-like Receptor (TLR) 8-specific Agonistic Activity in Substituted Pyrimidine-2,4-diamines

Mallesh Beesu, Alex C. D. Salyer, Kathryn L. Trautman, Justin K. Hill, and Sunil A. David*

Department of Medicinal Chemistry,
University of Minnesota, Minneapolis, MN 55455, USA.

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Abstract

Activation of human toll-like receptor-8 (TLR8) evokes a distinct cytokine profile favoring the generation of Type 1 helper T cells. A multiplexed high-throughput screen had led to the identification of N^4-butyl-5-iodo-6-methylpyrimidine-2,4-diamine as a pure TLR8 agonist, and a detailed SAR study of this chemotype was undertaken. A butyl substituent at N^4 was optimal, and replacement of the 5-iodo group with chloro, bromo, or fluoro groups led to losses in potency, as did the introduction of aromatic bulk. Drawing from our previous structure-based design, several 5-alkylamino derivatives were evaluated. Significant enhancement of potency was achieved in 5-(4-aminobutyl)-N^4-butyl-6-methylpyrimidine-2,4-diamine. This compound potently induced Th1-biasing IFN-γ and IL-12 in human blood, but lower levels of the proinflammatory cytokines IL-1β, IL-6 and IL-8. These results suggest that the inflammatory and reactogenic propensities of this compound could be considerably more favorable than other TLR8 agonists under evaluation.

Introduction

Toll-like receptors (TLRs) constitute important sensors in the activation of innate immune cells including monocytes, macrophages and dendritic cells. These cells function as sentinels against foreign antigens and pathogens, recognizing pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs).[1-4] Activation of PRRs initiate an array of innate immune responses, which serve to augment subsequent adaptive immune responses.[5-10] The 10 functional TLRs in the human encode proteins with an extracellular domain having leucine-rich repeats (LRR) and a cytosolic domain called the Toll/IL-1 receptor (TIR) domain.[7] TLR1, -2, -4, -5, and -6 recognize extracellular stimuli, while TLR3, -7, -8 and -9 function within the endolysosomal compartment. The ligands for TLRs are highly conserved molecules such as lipopolysaccharides (LPS) and monophosphoryl lipid A (MPLA) (recognized by TLR4), lipopeptides (TLR2 in combination with TLR1 or TLR6), flagellin (TLR5), single stranded RNA (TLR7 and TLR8), double stranded RNA (TLR3), and CpG motif-containing DNA (recognized by TLR9).[7]

T lymphocytes bear antigen specific receptors on their cell surface to allow recognition of foreign pathogens, and their effector functions are determined in part by the production of key cytokines. The two main subsets of T lymphocytes are distinguished by cell surface markers Cluster of Differentiation 4 (CD4) and CD8. CD4-expressing helper T lymphocytes are prolific cytokine producers and can be further subdivided into Th1 and Th2 subsets, depending on specific cytokine signatures.[11, 12] In the context of Th1-biased adaptive immune responses, TLR8 is of particular significance. The engagement of TLR8, which is expressed predominantly in myeloid dendritic cells, monocytes, and monocyte-derived dendritic cells,[13, 14] potently enhances the production of

Th1-polarizing cytokines, tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), IL-18, and interferon-γ (IFN-γ).[13, 15-17] Our interest in Th1-polarizing small molecule agonists of TLR8 has led to the exploration of a variety of pure TLR8 agonists with no detectable activity at TLR7, including the 2,3-diamino-furo[2,3-*c*]pyridines (**1a**),[18] 4-amino-furo[2,3-*c*]quinolones (**1b**),[19] 3-alkyl-quinoline-2-amines (**1c**),[20] 1-alkyl-2-aminobenzimidazoles (**1d**),[21] 2-amino-3-pentyl-5-alkylaminoquinolines (**1e**),[22] and the 2-aminoimidazoles (**1f**) (Fig. 1).[23]

A recent multiplexed, reporter gene-based high-throughput screen led to the identification of N^4-butyl-5-iodo-6-methylpyrimidine-2,4-diamine as a pure TLR8 agonist,[24] and we report here a structure-activity relationship study of the 2,4-diaminopyrimidines. The protonatable C4-amine appears to serve as a H-bond donor, since substitutions of the amine abrogated activity, and a butyl substituent at this position was found to be optimal. A dramatic enhancement of potency (and preservation of pure TLR8 agonistic activity) was achieved in substituting the iodo group at C5 with an aminobutyl group. This compound potently induced Th1-biasing IFN-γ and IL-12 in human blood, but lower levels of the proinflammatory cytokines IL-1β, IL-6 and IL-8. These results suggest that the inflammatory and reactogenic propensities of this compound could be considerably more favorable than other TLR8 agonists under evaluation.

Results and Discussion

The discovery and development of safe and effective vaccine adjuvants has been a central research goal in our laboratory, which has led us to explore structure-activity relationships (SAR) in a variety of innate immune-stimulatory chemotypes.[20-31] We recently concluded a multiplexed, reporter gene-based high-throughput screen with a view to identifying novel immunostimulatory molecular classes; 552 provisional hits were identified from among 123,943 compounds.[24] Deconvolution and validation of hits led to the identification of N^4-butyl-5-iodo-6-methylpyrimidine-2,4-diamine (**4b**, Scheme 1, Table 1) as a pure TLR8 agonist.

It is to be noted that several aminopyrimidines have been reported in the patent literature[32-34] to possess mixed TLR7/8-agonistic activity, and it was of particular interest to examine analogues of **4b**. SAR studies on **4b** began with the re-synthesis of the hit, as well as a verification of optimal alkyl chain length which we previously have shown to be a crucial determinant of activity. Compound **4b** (N^4-butyl), its homologs **4c** (N^4-pentyl), **4d** (N^4-hexyl), as well as a shorter-chain analogue **4a** (N^4-propyl) were synthesized in a straightforward manner from commercially-available 4-chloro-6-methylpyrimidin-2-amine by iodination at C5 and aromatic nucleophilic substitution with alkylamines (Scheme 1). Primary screens in reporter gene assays verified that **4b** was a *bona fide* TLR8-specific agonist, and the N^4-butyl substituent in **4b** was optimal (EC_{50}: 1.64 μM, Table 1, Fig. 2). The C4-amine appears to serve as a H-bond donor, since substitutions of the amine (**4e**, **4f**) abrogated activity (Table 1). This was borne out in the bioisosteric 4-butoxy (**4g**) and 4-thiobutyl (**4h**) analogues which were also inactive. However, we had previously observed in the case of the 3-alkyl-quinoline-2-amines that the C-alkyl compound, exemplified by **1c**,[20] was significantly more

active than its heteroatom-linked congeners, we sought to synthesize the C-alkyl analogue with a chain length comparable to that of **4b**. The pentyl-substituted pyrimidine analogue **4k** was accessed via Sonogashira coupling of **2** with 1-pentyne, reduction of the alkyne, followed by iodination (Scheme 1). Compound **4k** as well as its precursors **4j** and **4i** were found to be inactive, indicating that a H-bond donor functionality was indispensable at C4.

We then systematically examined the influence of various halogens at C5. The 5-chloro and 5-bromo analogues (**6a** and **6b**, respectively) were obtained from **2** using conventional method (Scheme 1). We found it expedient to synthesize the 5-fluoro substituted **6c** by standard condensation-cyclization of 2-fluoroethylacetoacetate with guandine via the 4-hydroxy-2-amino-5-fluoro-6-methylpyrimidine intermediate (Scheme 1). Replacement of the 5-iodo group with chloro (**6a**), bromo (**6b**), or fluoro (**6c**) groups led to substantial losses in potency.

We next explored the consequences of replacing the 6-methyl group of **4b**, holding the N^4-butyl group invariant. These target compounds (**15**-**23**) were readily accessed from commercially-available substituted 2-amino-4-chloropyrimidines (Scheme 2). The EC_{50} of the *des*-iodo analogue **15** was 22 μM (ca. thirteen-fold less active than **4b**; Table 1, Fig. 2); the *des*-iodo, *des*-methyl compound **16** was feebly active (EC_{50}: 73.2 μM); and the *des*-methyl analogue **17** was entirely without TLR8-agonistic activity (Table 1). The retention of activity in **15** prompted the exploration of analogues with various electron-donating and -withdrawing groups at C6, in the presence or absence of iodo substitution at C5 (**18**-**23**); all of these compounds, however, were inactive, pointing strongly to electronic, rather than steric effects of substitutions at C6. Crystallographic studies are being planned to examine the structural basis of attenuation of activity in **15** and **16**, and

the complete loss of activity in **17**.

Mindful of the prominent π-π interactions that we had previously observed in the crystal structures of a variety of chemotypes with Phe405 in TLR8, [20, 22, 23, 25] we asked if functionalizing C5 with a bulky aromatic group would enhance potency. The C5-benzyl analogue **24**, synthesized from **4b** via conventional Suzuki coupling (Scheme 3), resulted in diminution of activity, exhibiting greatly attenuated area-under-the curve in dose-response assays. We have, in the course of exploring several chemotypes,[19-23] observed that such compounds are inactive in secondary screens.

In our previous efforts, crystallographic observations in two regioisomeric, dual TLR7/8-active imidazoquinolines had suggested that the formation of a strong ionic H-bond (salt bridge) with the side chain carboxylate of Asp545 not only resulted in enhancement of agonistic activity in primary screens, but also in higher proinflammatory cytokine induction in whole human blood assays;[22] we were successful in testing this hypothesis whereby strategically grafting an aminoalkyl group on an aminoquinoline scaffold resulted in a twenty-fold enhancement of potency.[22] Our attention therefore turned to 5-alkylamino derivatives of **15**. Sonogashira coupling of **4b** with amino-substituted alkynes (Scheme 3) afforded access to compounds **27a-c** (propyl-, butyl-, pentyl-amino derivatives). The potency of the propylamino derivative **27a** increased nearly ten-fold (2.31 μM) relative to that of **4b** (22 μM); the potency of the butylamino compound **27b** was ca. 73-fold higher (0.3 μM; Table 1, Fig. 2) and appeared to plateau with the pentylamine homologue, **27c (**0.3 μM). We were gratified that this approach resulted in enhanced potency.

In our earlier work on the exploration of SAR in the 2-aminoimidazoles, we had observed

that the introduction of a phenyl group at C4 allowed for aromatic stacking with near-perfect coplanarity between the C4-phenyl ring of Phe405 of TLR8; the C4-phenyl group was accomodated in a pocket tightly circumscribed and delimited by the hydrophobic residues Tyr353, Val378, Val520 and Gln519.[23] We were unsuccessful in enhancing potency in the C5-derivatized analogue **24**, and it remained for us to explore the functionalization of C6. The C6-phenyl analogue **28**, synthesized via Suzuki coupling (Scheme 4) resulted in a TLR8-selective agonist with an EC_{50} of 6.7 μM (Table 1, Fig. 2). We were, however, surprised to find that its 5-iodo derivative **29** was inactive. Compound **29** served as a convenient substrate for the syntheses of the 5-alkylamino derivatives **32a-c** (Scheme 4). Activity in TLR8-specific primary screen was reinstated in the 5-butylamino (**32b**; EC_{50}: 2.7 μM) and 5-pentylamino (**32c**; EC_{50}: 2.35 μM) analogues, similar to what we had observed in the **27a-c** series of compounds.

We screened the hit compound **4b**, alongside the 6-methyl analogue **15**, the 5-butylamino analogue **27b**, the 6-phenyl substituted **28**, and its 5-butylamine derivative **32b**, for cytokine and chemokine induction in human PBMCs using a 41-analyte multiplexed immunoassay platform. TLR8 agonists upregulate the production of proinflammatory cytokines such as TNF-α, IL-1β, IL-8, as well as a number of chemokines; in addition, TLR8 agonists appear particularly potent in inducing the Th1-biasing cytokines IFN-γ and IL-12.[19-23, 31] As depicted in Fig. 3, **27b** induces a cytokine/chemokine signature prototypical of TLR8 agonists in *ex vivo* human blood stimulation experiments, the potency (EC_{50}: 3 μg/mL; 9.26 μM for TNF-α) of which exceeds that of the benzimidazole **1d** (used as a comparator/reference compound). Compound **27b** was also found to be particularly potent in inducing Th1-biasing IFN-γ and IL-12, while the levels of the proinflammatory cytokines IL-1β, IL-6 and IL-8 appear considerably lower than those observed for

1d (Fig. 3). We have hitherto not observed a cytokine profile characterized by an apparent dissociation between strong IFN-γ, IL-12 and chemokine responses on the one hand, and considerably weaker IL-1β, IL-6 and IL-8 levels on the other. These results suggest that the inflammatory and reactogenic propensities of **27b** could be considerably more favorable than other TLR8 agonists that we are currently examining in detail in preclinical studies. Notably, the C6-phenyl substituted analogues **28** and **32b** are significantly weaker than both the hit compound **4b**, as well as the best-in-class aminopyrimidine **27b**. We had previously observed an apparent dissociation of activity in primary cell-based reporter assays and secondary screens in human PBMCs,[18, 23] and the basis for why the C6-phenyl substituted analogues **28** and **32b** are active in primary screens, and become highly attenuated in human PBMCs remains to be elucidated.

In an effort to understand the mechanistic basis of amplified adaptive immune functions resulting from TLR8 engagement, we had previously used multi-color flow cytometry to interrogate activation markers (CD40, CD80) in major cellular subsets (granulocytes, monocytes ($CD14^+$), T cells ($CD3^+$), B cells ($CD19^+$), NK cells ($CD3^-CD56^+$) and cytokine-induced killer cells ($CD3^+CD56^+$) in human whole blood; we had shown that TLR8 stimulation strongly induces CD80 expression in the monocytes, signifying a possible specific role for TLR8 agonists in enhancing antigen presentation.[22] We therefore also examined CD80 responses by the aminopyrimidine compounds (**4b**, **15**, **27b**, **28**, **32b;** Fig. 4). As depicted in Fig. 4, the hit compound **4b**, itself, was more active (1.5 μg/mL; 5 μM) than **1d** (3 μg/mL; 14 μM in upregulating CD80, and **27b** was very potent (0.5 μg/mL; 1.5 μM).

The activation of TLRs by cognate ligands culminates in the recruitment of cytosolic

Toll/interleukin-1 receptor (TIR) domain-containing adaptors coupling TLRs to downstream effector proteins. Five adaptors have thus far been identified in signaling: Myeloid differentiation primary response gene 88 (MyD88), MyD88-adapter-like (MAL), TIR-domain-containing adapter-inducing interferon-β (TRIF), TRIF-related adaptor molecule (TRAM) and Sterile-alpha and Armadillo motif containing protein (SARM).[35, 36] These adaptors are thought to converge on either MyD88- or TRIF-dependent pathways leading to the production of inflammatory cytokines (Myd88-dependent), or IFNα/β (TRIF-dependent).[35, 36]

The structural features in small-molecule ligands that govern the recruitment of downstream signaling pathways is not understood as yet, and our results indicating strong IFN-γ, IL-12, and chemokine responses and weaker proinflammatory responses by **27b** suggest that selective targeting of these signaling pathways may be a tractable goal in this chemotype, amenable to classic medicinal chemistry approaches. These finding have been instructive, and efforts are currently underway to further explore substitutions at C5 and C6.

Experimental Section

Chemistry. All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or air-sensitive reactions were conducted under nitrogen atmosphere in oven-dried (120 ºC) glass apparatus. Solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep Rf 'Gold' high performance silica columns on CombiFlash Rf instruments unless otherwise

mentioned; thin-layer chromatography was carried out on silica gel CCM pre-coated aluminum sheets. Purity for all final compounds was confirmed to be greater than 98% by LC-MS using a Zorbax Eclipse Plus 4.6 mm x 150 mm, 5 μm analytical reverse phase C_{18} column with H_2O-CH_3CN and H_2O-MeOH gradients and an Agilent 6520 ESI-QTOF Accurate Mass spectrometer (mass accuracy of 5 ppm) operating in the positive ion acquisition mode.

4-Chloro-5-iodo-6-methylpyrimidin-2-amine (**3**). To a solution of compound **2** (143.6 mg, 1 mmol) in anhydrous DMF (5 mL) was added NIS (225 mg, 1 mmol), and the reaction mixture was stirred for 12 h. The reaction mixture was diluted with water and extracted with EtOAc (3 x 30 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified by flash chromatography (40% EtOAc/hexanes) to obtain the compound **3** as a white solid (210 mg, 78 %). ^{1}H NMR (500 MHz, MeOD) δ 2.55 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 173.86, 165.51, 163.76, 79.58, 30.16. MS (ESI-TOF) for $C_5H_5ClIN_3$ $[M + H]^+$ calculated 269.9289, found 269.9350.

***N*^{4}-Butyl-5-iodo-6-methylpyrimidine-2,4-diamine** (**4b**). To a solution of compound **3** (53.8 mg, 0.2 mmol) in MeOH (3 mL), was added Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol). The reaction mixture was stirred for 12 h at 70 ºC. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The reaction mixture was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (70% EtOAc/hexanes) to afford the compound **4b** as a white solid (46 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24 (s, 1H), 4.76 (s, 2H), 3.42 – 3.34 (m, 2H), 2.40 (s, 3H), 1.63 –

1.53 (m, 2H), 1.44 – 1.34 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.52, 162.16, 161.04, 68.50, 41.59, 31.65, 28.84, 20.26, 13.99. MS (ESI-TOF) for $C_9H_{15}IN_4$ $[M + H]^+$ calculated 307.0414, found 307.0408.

Compounds **4a** and **4c-f** were synthesized similarly as compound **4b**.

5-Iodo-6-methyl-*N*^{4}-propylpyrimidine-2,4-diamine (**4a**). Compound **3** (53.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and propylamine (32.8 μL, 0.4 mmol) were used as reagents. White solid (40 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (s, 1H), 4.78 (s, 2H), 3.39 – 3.31 (m, 2H), 2.40 (s, 3H), 1.67 – 1.56 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.55, 162.18, 161.05, 68.47, 43.57, 28.84, 22.77, 11.56. MS (ESI-TOF) for $C_8H_{13}IN_4$ $[M + H]^+$ calculated 293.0258, found 293.0249.

5-Iodo-6-methyl-*N*^{4}-pentylpyrimidine-2,4-diamine (**4c**). Compound **3** (53.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and amylamine (46.4 μL, 0.4 mmol) were used as reagents. White solid (55 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24 (s, 1H), 4.67 (s, 2H), 3.42 – 3.34 (m, 2H), 2.41 (s, 3H), 1.65 – 1.55 (m, 2H), 1.41 – 1.31 (m, 4H), 0.92 (t, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.66, 162.21, 161.05, 68.58, 41.87, 29.26, 29.25, 28.91, 22.56, 14.18. MS (ESI-TOF) for $C_{10}H_{17}IN_4$ $[M + H]^+$ calculated 321.0571, found 321.0566.

***N*^{4}-Hexyl-5-iodo-6-methylpyrimidine-2,4-diamine** (**4d**). Compound **3** (53.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and hexylamine (52.6 μL, 0.4 mmol) were used as reagents. White solid (56 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.25 (s, 1H), 4.76 (s, 2H), 3.41 – 3.33 (m, 2H), 2.41 (s, 3H),

1.63 – 1.54 (m, 2H), 1.40 – 1.29 (m, 6H), 0.89 (t, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.51, 162.16, 161.03, 68.51, 41.89, 31.65, 29.49, 28.84, 26.74, 22.72, 14.18. MS (ESI-TOF) for $C_{11}H_{19}IN_4$ $[M + H]^+$ calculated 335.0727, found 335.0895.

***N*^{4}-Butyl-5-iodo-*N*^{4},6-dimethylpyrimidine-2,4-diamine** (**4e**). Compound **3** (53.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and *N*-methyl-1-butanamine (47.4 μL, 0.4 mmol) were used as reagents. White solid (46 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.73 (s, 2H), 3.38 (t, J = 7.5 Hz, 2H), 2.98 (s, 3H), 2.50 (s, 3H), 1.68 – 1.58 (m, 2H), 1.37 – 1.25 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.11, 167.82, 161.10, 68.63, 53.08, 39.65, 30.36, 29.78, 20.23, 14.09. MS (ESI-TOF) for $C_{10}H_{17}IN_4$ $[M + H]^+$ calculated 321.0571, found 321.0593.

***N*^{4}-Benzyl-*N*^{4}-butyl-5-iodo-6-methylpyrimidine-2,4-diamine** (**4f**). Compound **3** (53.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and *N*-benzyl-n-butylamine (71.7 μL, 0.4 mmol) were used as reagents. White solid (62 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.29 (m, 4H), 7.26 – 7.21 (m, 1H), 4.75 (s, 2H), 4.63 (s, 2H), 3.34 (t, J = 7.5 Hz, 2H), 2.53 (s, 3H), 1.62 – 1.51 (m, 2H), 1.30 – 1.19 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.42, 167.72, 161.14, 138.75, 128.40, 127.99, 127.03, 70.82, 54.22, 50.38, 30.38, 29.77, 20.27, 14.06. MS (ESI-TOF) for $C_{16}H_{21}IN_4$ $[M + H]^+$ calculated 397.0884, found 397.0888.

4-Butoxy-5-iodo-6-methylpyrimidin-2-amine (**4g**). To a solution of compound **3** (53.8 mg, 0.2 mmol) in 1-butanol (3 mL), was added K_2CO_3 (138 mg, 1 mmol). The reaction mixture was stirred for 24 h at 85 ºC. The reaction mixture was cooled to room temperature and the solvent was

removed under reduced pressure. The reaction mixture was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (30% EtOAc/hexanes) to afford the compound **4g** as a white solid (40 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.92 (s, 2H), 4.28 (t, *J* = 6.6 Hz, 2H), 2.49 (s, 3H), 1.79 – 1.70 (m, 2H), 1.54 – 1.43 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.68, 168.10, 162.01, 67.60, 67.54, 30.88, 28.29, 19.35, 13.96. MS (ESI-TOF) for $C_9H_{14}IN_3O$ $[M + H]^+$ calculated 308.0254, found 308.0250.

4-(Butylthio)-5-iodo-6-methylpyrimidin-2-amine (**4h**). To a solution of compound **3** (53.8 mg, 0.2 mmol) in MeOH (3 mL), was added Et_3N (56 μL, 0.4 mmol) and 1-butanethiol (43 μL, 0.4 mmol). The reaction mixture was stirred for 12 h at 70 ºC. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The reaction mixture was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (30% EtOAc/hexanes) to afford the compound **4h** as a white solid (50 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.92 (s, 2H), 3.05 (t, *J* = 7.4 Hz, 2H), 2.49 (s, 3H), 1.72 – 1.63 (m, 2H), 1.52 – 1.40 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.52, 167.19, 160.88, 81.55, 32.27, 30.95, 28.65, 22.26, 13.86. MS (ESI-TOF) for $C_9H_{14}IN_3S$ $[M + H]^+$ calculated 324.0026, found 324.0021.

4-Methyl-6-(pent-1-yn-1-yl)pyrimidin-2-amine (**4i**). To a solution of compound **2** (72 mg, 0.5 mmol) in 2:1 mixture of CH_3CN (6 mL) and Et_3N (3 mL) were added $Pd(PPh_3)_4$ (57.8 mg, 0.05

mmol) and CuI (19 mg, 0.1 mmol). The reaction mixture was degassed with dry nitrogen for 5 min., then 1-pentyne (98.5 µL, 1 mmol) was added. The resulting reaction mixture was stirred for 12 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was diluted with water and extracted with ethylacetate (3 x 30 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified by flash chromatography (70% EtOAc/hexanes) to obtain the compound **4i** as a pale yellow solid (65 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.57 (s, 1H), 5.06 (s, 2H), 2.40 (t, *J* = 7.1 Hz, 2H), 2.31 (s, 3H), 1.70 – 1.58 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.43, 162.92, 151.71, 113.30, 94.00, 79.32, 23.88, 21.77, 21.41, 13.70. MS (ESI-TOF) for $C_{10}H_{13}N_3$ $[M + H]^+$ calculated 176.1182, found 176.1172.

4-Methyl-6-pentylpyrimidin-2-amine (**4j**). To a solution of compound **4i** (35.4 mg, 0.2 mmol) in anhydrous EtOAc (20 mL) was added a catalytic amount of Pd/C, and the reaction mixture was subjected to hydrogenation at 50 psi for 5 h. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure. The crude material was purified using silica gel column chromatography (60 % EtOAc/hexanes) to obtain **4j** as white solid (30 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (s, 1H), 5.11 (s, 2H), 2.49 (t, *J* = 7.8 Hz, 2H), 2.29 (s, 3H), 1.69 – 1.59 (m, 2H), 1.37 – 1.28 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.04, 167.86, 162.97, 110.12, 37.84, 31.73, 28.68, 23.95, 22.62, 14.11. MS (ESI-TOF) for $C_{10}H_{17}N_3$ $[M + H]^+$ calculated 180.1495, found 180.1486.

5-Iodo-4-methyl-6-pentylpyrimidin-2-amine (**4k**). To a solution of compound **4j** (17.9 mg, 0.1 mmol) in anhydrous DMF (1 mL) was added NIS (22.5 mg, 0.1 mmol), and the reaction mixture

was stirred for 12 h. The reaction mixture was diluted with water and extracted with EtOAc (3 x 10 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified by flash chromatography (40% EtOAc/hexanes) to obtain the compound **4k** as a white solid (25 mg, 82 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.02 (s, 2H), 2.77 (t, *J* = 8.0 Hz, 2H), 2.54 (s, 3H), 1.68 – 1.61 (m, 2H), 1.42 – 1.32 (m, 4H), 0.91 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.78, 169.92, 162.02, 84.73, 41.50, 31.83, 29.70, 28.06, 22.61, 14.16. MS (ESI-TOF) for $C_{10}H_{16}IN_3$ $[M + H]^+$ calculated 306.0462, found 306.0459.

Compounds **5a** and **5b** were synthesized similarly as compound **3**.

4,5-Dichloro-6-methylpyrimidin-2-amine (**5a**). Compound **2** (143.6 mg, 1 mmol) and *N*-chlorosuccinimide (133.5 mg, 1 mmol) were used as reagents. White solid (126 mg, 71%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.17 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.74, 160.51, 156.68, 113.06, 22.68. MS (ESI-TOF) for $C_5H_5Cl_2N_3$ $[M + H]^+$ calculated 177.9933, found 177.9962

5-Bromo-4-chloro-6-methylpyrimidin-2-amine (**5b**). Compound **2** (143.6 mg, 1 mmol) and *N*-bromosuccinimide (178 mg, 1 mmol) were used as reagents. White solid (155 mg, 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.18 (s, 2H), 2.40 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.47, 161.12, 158.71, 103.25, 25.21. MS (ESI-TOF) for $C_5H_5BrClN_3$ $[M + H]^+$ calculated 221.9428, found 221.9470.

Compounds **6a** and **6b** were synthesized similarly as compound **4b**.

***N*^{4}-Butyl-5-chloro-6-methylpyrimidine-2,4-diamine** (**6a**). Compound **5a** (35.6 mg, 0.2 mmol), Et_3N (56 µL, 0.4 mmol) and butylamine (39.5 µL, 0.4 mmol) were used as reagents. White solid (34 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17 (s, 1H), 4.78 (s, 2H), 3.43 – 3.35 (m, 2H), 2.28 (s, 3H), 1.62 – 1.52 (m, 2H), 1.44 – 1.33 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.68, 160.30, 158.43, 103.19, 40.83, 31.76, 21.69, 20.20, 13.95. MS (ESI-TOF) for $C_9H_{15}ClN_4$ $[M + H]^+$ calculated 215.1058, found 215.1050.

5-Bromo-*N*^{4}-butyl-6-methylpyrimidine-2,4-diamine (**6b**). Compound **5b** (44.5 mg, 0.2 mmol)), Et_3N (56 µL, 0.4 mmol) and butylamine (39.5 µL, 0.4 mmol) were used as reagents. White solid (45 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.24 (s, 1H), 4.80 (s, 2H), 3.41 – 3.36 (m, 2H), 2.32 (s, 3H), 1.62 – 1.52 (m, 2H), 1.44 – 1.33 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.57, 160.97, 159.11, 93.92, 41.10, 31.70, 24.23, 20.22, 13.96. MS (ESI-TOF) for $C_9H_{15}BrN_4$ $[M + H]^+$ calculated 259.0553, found 259.0543.

***N*^{4}-Butyl-5-fluoro-6-methylpyrimidine-2,4-diamine** (**6c**). To a solution of guanidine hydrochloride (95.5 mg, 1 mmol) in MeOH (5 mL), were added compound **7** (125 µL, 1 mmol) and Et_3N (280 µL, 2 mmol). The reaction mixture was refluxed for 12 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was filtered and washed with methanol, to yield compound **8** as a white solid (100 mg, 69%). A suspension of compound **8** (71.5 mg, 0.5 mmol) in phosphorus(V) oxychloride was placed in a pressure vessel. The reaction mixture was stirred for 3 h at 85 ºC. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The reaction mixture was diluted with water and extracted with

EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (40% EtOAc/hexanes) to afford the compound **9** as a white solid (50 mg, 62%). MS (ESI-TOF) for $C_5H_5ClFN_3$ $[M + H]^+$ calculated 162.0229, found 162.0278. To a solution of **9** (32.3 mg, 0.2 mmol) in MeOH (3 mL), was added Et_3N (56 µL, 0.4 mmol) and butylamine (39.5 µL, 0.4 mmol). The reaction mixture was stirred for 12 h at 70 ºC. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The reaction mixture was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (80% EtOAc/hexanes) to afford the compound **6c** as a white solid (30 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.76 (s, 1H), 4.58 (s, 2H), 3.44 – 3.36 (m, 2H), 2.18 (d, J = 2.9 Hz, 3H), 1.62 – 1.52 (m, 2H), 1.45 – 1.34 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.14 (d, J = 5.1 Hz), 152.99 (d, J = 12.7 Hz), 147.58 (d, J = 13.7 Hz), 139.53 (d, J = 238.8 Hz). 40.31, 31.88, 20.18, 16.61, 13.95. MS (ESI-TOF) for $C_9H_{15}FN_4$ $[M + H]^+$ calculated 199.1354, found 199.1347.

Compound **11** was synthesized similarly as compound **3**.

4-Chloro-5-iodopyrimidin-2-amine (**11**). 4-Chloropyrimidin-2-amine (**10)** (129.5 mg, 1 mmol) and NIS (225 mg, 1 mmol) were used as reagents. White solid (170 mg, 67.5%). MS (ESI-TOF) for $C_4H_3ClIN_3$ $[M + H]^+$ calculated 255.9133, found 255.9205.

Compounds **15-20** were synthesized similarly as compound **4b**.

***N*^{4}-Butyl-6-methylpyrimidine-2,4-diamine** (**15**). 4-Chloro-6-methylpyrimidin-2-amine (**2**) (28.7 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. White solid (28 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.61 (s, 1H), 4.81 (s, 2H), 4.72 (s, 1H), 3.24 – 3.16 (m, 2H), 2.18 (s, 3H), 1.58 – 1.49 (m, 2H), 1.43 – 1.31 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.28, 164.06, 162.81, 93.09, 41.16, 31.67, 23.97, 20.18, 13.89. MS (ESI-TOF) for $C_9H_{16}N_4$ $[M + H]^+$ calculated 181.1448, found 181.1442.

***N*^{4}-Butylpyrimidine-2,4-diamine** (**16**). 4-Chloropyrimidin-2-amine (**10**) (25.9 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. White solid (25 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 5.9 Hz, 1H), 5.74 (d, *J* = 5.9 Hz, 1H), 4.84 (s, 3H), 3.26 – 3.18 (m, 2H), 1.60 – 1.50 (m, 2H), 1.44 – 1.32 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.55, 162.96, 156.60, 94.73, 41.13, 31.62, 20.18, 13.89. MS (ESI-TOF) for $C_8H_{14}N_4$ $[M + H]^+$ calculated 167.1291, found 167.1282.

***N*^{4}-Butyl-5-iodopyrimidine-2,4-diamine** (**17**). 4-Chloro-5-iodopyrimidin-2-amine (**11**) (51.1 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. White solid (42 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H), 5.07 (s, 1H), 4.78 (s, 2H), 3.44 – 3.36 (m, 2H), 1.63 – 1.53 (m, 2H), 1.46 – 1.34 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.41, 161.60, 160.26, 65.42, 41.21, 31.56, 20.24, 13.98. MS (ESI-TOF) for $C_8H_{13}IN_4$ $[M + H]^+$ calculated 293.0258, found 293.0251.

***N*^{4}-Butylpyrimidine-2,4,6-triamine** (**18**). 6-Chloropyrimidine-2,4-diamine (**12**) (28.9 mg, 0.2 mmol), K_2CO_3 (56 mg, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. Pale

yellow solid (25 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.95 (s, 1H), 4.57 (s, 3H), 4.44 (s, 2H), 3.15 – 3.08 (m, 2H), 1.59 – 1.49 (m, 2H), 1.44 – 1.32 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.50, 164.45, 162.68, 74.37, 41.53, 31.56, 20.24, 13.91. MS (ESI-TOF) for $C_8H_{15}N_5$ $[M + H]^+$ calculated 182.1400, found 182.1389.

***N*^{4}-Butyl-6-methoxypyrimidine-2,4-diamine** (**19**). 4-Chloro-6-methoxypyrimidin-2-amine (**13**) (31.9 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. White solid (32 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (s, 1H), 4.74 (s, 2H), 4.69 (s, 1H), 3.81 (s, 3H), 3.18 – 3.10 (m, 2H), 1.59 – 1.49 (m, 2H), 1.43 – 1.31 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.75, 165.40, 162.52, 75.70, 53.39, 41.55, 31.51, 20.20, 13.88. MS (ESI-TOF) for $C_9H_{16}N_4O$ $[M + H]^+$ calculated 197.1397, found 197.1392.

***N*^{4}-Butyl-6-chloropyrimidine-2,4-diamine** (**20**). 4,6-Dichloropyrimidin-2-amine (**14**) (32.8 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol) and butylamine (39.5 μL, 0.4 mmol) were used as reagents. White solid (34 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74 (s, 1H), 5.35 (s, 3H), 3.26 – 3.06 (m, 2H), 1.57 – 1.47 (m, 2H), 1.40 – 1.29 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.40, 162.57, 159.89, 91.77, 41.30, 31.43, 20.09, 13.81. MS (ESI-TOF) for $C_8H_{13}ClN_4$ $[M + H]^+$ calculated 201.0902, found 201.0895.

Compounds **21-23** were synthesized similarly as compound **4k**.

***N*^{4}-Butyl-5-iodopyrimidine-2,4,6-triamine** (**21**). Compound **18** (18.1 mg, 0.1 mmol) and NIS (22.5 mg, 0.1 mmol) were used as reagents. Pale yellow solid (15 mg, 49%). ^{1}H NMR (500 MHz,

$CDCl_3$) δ 4.92 (s, 1H), 4.81 (s, 2H), 4.60 (s, 2H), 3.44 – 3.35 (m, 2H), 1.62 – 1.52 (m, 2H), 1.45 – 1.34 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.12, 161.66, 161.49, 46.15, 41.67, 32.00, 20.25, 14.02. MS (ESI-TOF) for $C_8H_{14}IN_5$ $[M + H]^+$ calculated 308.0367, found 308.0336.

N^4-Butyl-5-iodo-6-methoxypyrimidine-2,4-diamine (**22**). Compound **19** (19.6 mg, 0.1 mmol) and NIS (22.5 mg, 0.1 mmol) were used as reagents. White solid (22 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.06 (s, 1H), 4.71 (s, 2H), 3.87 (s, 3H), 3.43 – 3.35 (m, 2H), 1.62 – 1.52 (m, 2H), 1.45 – 1.33 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.90, 162.50, 162.34, 54.44, 46.69, 41.67, 31.94, 20.23, 14.00. MS (ESI-TOF) for $C_9H_{15}IN_4O$ $[M + H]^+$ calculated 323.0363, found 323.0362.

N^4-Butyl-6-chloro-5-iodopyrimidine-2,4-diamine (**23**). Compound **20** (20 mg, 0.1 mmol) and NIS (22.5 mg, 0.1 mmol) were used as reagents. White solid (23 mg, 70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.54 (s, 2H), 6.45 (t, J = 5.8 Hz, 1H), 3.35 – 3.27 (m, 2H), 1.54 – 1.44 (m, 2H), 1.33 – 1.22 (m, 2H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.48, 161.89, 161.12, 61.91, 40.94, 30.92, 19.56, 13.78. MS (ESI-TOF) for $C_8H_{12}ClIN_4$ $[M + H]^+$ calculated 326.9868, found 326.9863.

5-Benzyl-N^4-butyl-6-methylpyrimidine-2,4-diamine (**24**). To a solution of compound **4b** (30.6 mg, 0.1 mmol), benzylboronic acid pinacol ester (44.4 μL, 0.2 mmol) and K_2CO_3 (55 mg, 0.4 mmol) in 4:1 mixture of 1,4-dioxane (2 mL) and water (0.5 mL) was added $Pd(dppf)Cl_2$ (7.3 mg, 0.01 mmol). The reaction mixture was degassed with dry nitrogen for 5 min, then stirred for 12 h at

85 ºC under nitrogen atmosphere. The reaction mixture was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified by flash chromatography (10% MeOH/CH_2Cl_2) to obtain the compound **24** as a pale yellow solid (20 mg, 74%). ^{1}H NMR (500 MHz, MeOD) δ 7.29 – 7.24 (m, 2H), 7.20 – 7.16 (m, 1H), 7.15 – 7.10 (m, 2H), 3.81 (s, 2H), 3.34 – 3.29 (m, 2H), 2.16 (s, 3H), 1.49 – 1.39 (m, 2H), 1.24 – 1.12 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.45, 162.37, 162.24, 140.45, 129.59, 128.74, 127.29, 105.17, 41.44, 32.55, 31.31, 20.93, 20.88, 14.15. MS (ESI-TOF) for $C_{16}H_{22}N_4$ $[M + H]^+$ calculated 271.1917, found 271.1917.

5-(3-Aminopropyl)-*N*^{4}-butyl-6-methylpyrimidine-2,4-diamine dihydrochloride (27a). To a solution of compound **4b** (92 mg, 0.3 mmol) in 2:1 mixture of DMF (6 mL) and DIPEA (3 mL) were added $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol) and CuI (11.4 mg, 0.06 mmol). The reaction mixture was degassed with dry nitrogen for 5 min, then *N*-boc-propargylamine (93 mg, 0.6 mmol) was added. The resulting reaction mixture was stirred for 12 h under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with water and extracted with ethylacetate (3 x 30 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, the crude material was purified by flash chromatography (90% EtOAc/hexanes) to obtain the compound **25a** as yellow oil (72 mg, 72%). MS (ESI-TOF) for $C_{17}H_{27}N_5O_2$ $[M + H]^+$ calculated 334.2238, found 334.2254. To a solution of compound **25a** (33.3 mg, 0.1 mmol) in anhydrous EtOAc (10 mL) was added a catalytic amount of Pd/C, and the reaction mixture was subjected to hydrogenation at 50 psi for 5 h. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure. The crude material was purified by flash chromatography (20%

MeOH/CH_2Cl_2) to obtain *N*-boc protected alkylamine **26a** as a pale oil (24 mg, 71%). MS (ESI-TOF) for $C_{17}H_{31}N_5O_2$ $[M + H]^+$ calculated 338.2551, found 338.2698. To a stirred solution of *N*-boc protected alkylamine **26a** (17 mg, 0.05 mmol) in 1,4-dioxane (1 mL) was added hydrogen chloride (1 mL, 4 M in dioxane), and the reaction mixture was stirred for 3 h at room temperature. Excess solvent was removed under reduced pressure and the resulted residue was thoroughly washed with diethyl ether to obtain the desired compound **27a** as a white solid (12 mg, 77%). ^{1}H NMR (500 MHz, , MeOD) δ 3.53 (t, *J* = 7.3 Hz, 2H), 3.05 (t, *J* = 7.6 Hz, 2H), 2.56 (t, *J* = 8.0 Hz, 2H), 2.32 (s, 3H), 1.84 – 1.73 (m, 2H), 1.68 – 1.58 (m, 2H), 1.43 – 1.32 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.44, 155.71, 149.22, 107.20, 42.26, 40.08, 32.19, 27.02, 22.19, 21.14, 16.50, 14.17. MS (ESI-TOF) for $C_{12}H_{23}N_5$ $[M + H]^+$ calculated 238.2026, found 238.2021.

5-(4-Aminobutyl)-*N*^{4}-butyl-6-methylpyrimidine-2,4-diamine dihydrochloride (**27b**). To a solution of compound **4b** (92 mg, 0.3 mmol) in 2:1 mixture of DMF (6 mL) and DIPEA (3 mL) were added $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol) and CuI (11.4 mg, 0.06 mmol). The reaction mixture was degassed with dry nitrogen for 5 min, then *tert*-butyl but-3-yn-1-ylcarbamate (101.4 mg, 0.6 mmol) was added. The resulting reaction mixture was stirred for 12 h under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with water and extracted with ethylacetate (3 x 30 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, the crude material was purified by flash chromatography (90% EtOAc/hexanes) to obtain the compound **25b** as yellow oil (75 mg, 72%). MS (ESI-TOF) for $C_{18}H_{29}N_5O_2$ $[M + H]^+$ calculated 348.2394, found 348.2407. To a solution of compound **25b** (34.7 mg, 0.1 mmol) in anhydrous EtOAc (10 mL) was added a catalytic amount of Pd/C, and the reaction mixture was subjected to

hydrogenation at 50 psi for 5 h. The reaction mixture was filtered, and the filtrate concentrated under reduced pressure. The crude material was purified by flash chromatography (20% MeOH/CH_2Cl_2) to obtain *N*-boc protected alkylamine **26b** as a pale oil (25 mg, 71%). MS (ESI-TOF) for $C_{18}H_{33}N_5O_2$ $[M + H]^+$ calculated 352.2707, found 352.2755. To a stirred solution of *N*-boc protected alkylamine **26b** (17.6 mg, 0.05 mmol) in 1,4-dioxane (1 mL) was added hydrogen chloride (1 mL, 4 M in dioxane), and the reaction mixture was stirred for 3 h at room temperature. Excess solvent was removed under reduced pressure and the resulted residue was thoroughly washed with diethyl ether to obtain the desired compound **27b** as a white solid (13 mg, 80%). ^{1}H NMR (500 MHz, MeOD) δ 3.52 (t, *J* = 7.3 Hz, 2H), 2.96 (t, *J* = 7.7 Hz, 2H), 2.51 (t, *J* = 8.0 Hz, 2H), 2.30 (s, 3H), 1.81 – 1.71 (m, 2H), 1.67 – 1.59 (m, 2H), 1.55 – 1.46 (m, 2H), 1.43 – 1.32 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.60, 155.67, 148.81, 108.22, 42.26, 40.60, 32.21, 28.20, 26.17, 24.58, 21.16, 16.56, 14.19. MS (ESI-TOF) for $C_{13}H_{25}N_5$ $[M + H]^+$ calculated 252.2183, found 252.2182.

Compounds **27c** was synthesized similarly as compound **27b**.

5-(5-Aminopentyl)-*N*^{4}-butyl-6-methylpyrimidine-2,4-diamine dihydrochloride (27c). Intermediate compound **25c**. Compound **4b** (92 mg, 0.3 mmol), $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol), CuI (11.4 mg, 0.06 mmol) and *tert*-butyl pent-4-yn-1-ylcarbamate (109.8 mg, 0.6 mmol) were used as reagents. Yellow oil (75 mg, 69%). MS (ESI-TOF) for $C_{19}H_{31}N_5O_2$ $[M + H]^+$ calculated 362.2551, found 362.2578.

Intermediate compound **26c**. Pale oil (28 mg, 77%). MS (ESI-TOF) for $C_{19}H_{35}N_5O_2$ $[M + H]^+$ calculated 366.2864, found 366.2897.

Compound **27c**. White solid (13 mg, 77%). ^{1}H NMR (500 MHz, MeOD) δ 3.52 (t, *J* = 7.3 Hz, 2H), 2.94 (t, *J* = 7.6 Hz, 2H), 2.49 (t, *J* = 7.0 Hz, 2H), 2.30 (s, 3H), 1.74 – 1.69 (m, 2H), 1.64 – 1.57 (m, 2H), 1.54 – 1.46 (m, 4H), 1.43 – 1.31 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.62, 155.63, 148.53, 108.60, 42.18, 40.59, 32.21, 28.76, 28.51, 27.05, 24.85, 21.14, 16.57, 14.19. MS (ESI-TOF) for $C_{14}H_{27}N_5$ $[M + H]^+$ calculated 266.2339, found 266.2335.

***N*^{4}-Butyl-6-phenylpyrimidine-2,4-diamine** (**28**). To a solution of compound **20** (400 mg, 2 mmol), phenylboronic acid (366 mg, 3 mmol) and K_2CO_3 (828 mg, 6 mmol) in 4:1 mixture of 1,4-dioxane (8 mL) and water (2 mL) was added Pd(dppf)Cl_2 (73.1 mg, 0. 1 mmol). The reaction mixture was degassed with dry nitrogen for 5 min, then stirred for 12 h at 85 ºC under nitrogen atmosphere. The reaction mixture was diluted with water and extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (80% EtOAc/hexanes) to obtain the compound **28** as a white solid (420 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.87 (m, 2H), 7.47 – 7.38 (m, 3H), 6.16 (s, 1H), 4.78 (s, 2H), 4.73 (s, 1H), 3.36 – 3.28 (m, 2H), 1.65 – 1.56 (m, 2H), 1.49 – 1.37 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.70, 164.47, 163.28, 138.70, 129.76, 128.62, 126.96, 90.86, 41.30, 31.70, 20.22, 13.93. MS (ESI-TOF) for $C_{14}H_{18}N_4$ $[M + H]^+$ calculated 243.1604, found 243.1596.

Compound **29** was synthesized similarly as compound **3**.

***N*^{4}-Butyl-5-iodo-6-phenylpyrimidine-2,4-diamine** (**29**). Compound **28** (242.3 mg, 1 mmol) and NIS (225 mg, 1 mmol) were used as reagents. White solid (300 mg, 82%). ^{1}H NMR (500 MHz,

$CDCl_3$) δ 7.46 – 7.45 (m, 2H), 7.43 – 7.37 (m, 3H), 5.48 (t, *J* = 5.5 Hz, 1H), 4.92 (s, 2H), 3.49 – 3.41 (m, 2H), 1.67 – 1.57 (m, 2H), 1.49 – 1.38 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.91, 162.20, 161.53, 142.01, 128.86, 128.55, 128.15, 67.28, 41.74, 31.58, 20.29, 14.01. MS (ESI-TOF) for $C_{14}H_{17}IN_4$ $[M + H]^+$ calculated 369.0571, found 369.0572.

Compounds **32a-c** were synthesized similarly as compound **27a**.

5-(3-Aminopropyl)-*N*^{4}-butyl-6-phenylpyrimidine-2,4-diamine dihydrochloride (32a). Intermediate compound **30a**. Compound **29** (110.4 mg, 0.3 mmol), $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol), CuI (11.4 mg, 0.06 mmol) and *N*-boc-propargylamine (93 mg, 0.6 mmol) were used as reagents. Yellow oil (90 mg, 76%). MS (ESI-TOF) for $C_{22}H_{29}N_5O_2$ $[M + H]^+$ calculated 396.2394, found 396.2447.

Intermediate compound **31a**. Pale oil (32 mg, 80%, MS (ESI-TOF) for $C_{22}H_{33}N_5O_2$ $[M + H]^+$ calculated 400.2707, found 400.2723).

Compound **32a**. White solid (15 mg, 81%). ^{1}H NMR (500 MHz, MeOD) δ 7.64 – 7.58 (m, 3H), 7.56 – 7.49 (m, 2H), 3.62 (t, *J* = 7.3 Hz, 2H), 2.81 (t, *J* = 7.9 Hz, 2H), 2.49 (t, *J* = 8.1 Hz, 2H), 1.83 – 1.74 (m, 2H), 1.73 – 1.65 (m, 2H), 1.47 – 1.38 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.69, 155.80, 150.93, 133.01, 131.88, 130.47, 129.63, 107.90, 42.48, 40.04, 32.16, 27.43, 22.94, 21.23, 14.23. MS (ESI-TOF) for $C_{17}H_{25}N_5$ $[M + H]^+$ calculated 300.2183, found 300.2177.

5-(4-Aminobutyl)-*N*^{4}-butyl-6-phenylpyrimidine-2,4-diamine dihydrochloride (32b). Intermediate compound **30b**. Compound **29** (110.4 mg, 0.3 mmol)**,** $Pd(PPh_3)_4$ (34.6 mg, 0.03

mmol), CuI (11.4 mg, 0.06 mmol) and *tert*-butyl but-3-yn-1-ylcarbamate (101.4 mg, 0.6 mmol) were used as reagents. Yellow oil (75 mg, 61%). MS (ESI-TOF) for $C_{23}H_{31}N_5O_2$ $[M + H]^+$ calculated 410.2551, found 410.2596.

Intermediate compound **31b**. Pale oil (28 mg, 68%). MS (ESI-TOF) for $C_{23}H_{35}N_5O_2$ $[M + H]^+$ calculated 414.2864, found 414.2882.

Compound **32b**. White solid (16 mg, 83%). ^{1}H NMR (500 MHz, MeOD) δ 7.63 – 7.57 (m, 3H), 7.55 – 7.48 (m, 2H), 3.60 (t, J = 7.3 Hz, 2H), 2.76 (t, J = 7.1 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 1.73 – 1.63 (m, 2H), 1.55 – 1.48 (m, 4H), 1.46 – 1.38 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.86, 155.75, 150.59, 133.28, 131.80, 130.37, 129.69, 108.87, 42.45, 40.31, 32.16, 28.05, 26.47, 25.13, 21.22, 14.21. MS (ESI-TOF) for $C_{18}H_{27}N_5$ $[M + H]^+$ calculated 314.2339, found 314.2335.

5-(5-Aminopentyl)-*N*^{4}-butyl-6-phenylpyrimidine-2,4-diamine dihydrochloride (32c).

Intermediate compound **30c**. Compound **29** (110.4 mg, 0.3 mmol), $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol), CuI (11.4 mg, 0.06 mmol) and *tert*-butyl pent-4-yn-1-ylcarbamate (109.8 mg, 0.6 mmol) were used as reagents. Yellow oil (80 mg, 63%). MS (ESI-TOF) for $C_{24}H_{33}N_5O_2$ $[M + H]^+$ calculated 424.2707, found 424.2770.

Intermediate compound **31c**. Pale oil (30 mg, 70%). MS (ESI-TOF) for $C_{24}H_{37}N_5O_2$ $[M + H]^+$ calculated 428.3020, found 428.3051.

Compound **32c**. White solid (16 mg, 80%). ^{1}H NMR (500 MHz, MeOD) δ 7.61 – 7.57 (m, 3H), 7.53 – 7.49 (m, 2H), 3.60 (t, J = 7.3 Hz, 2H), 2.81 (t, J = 7.7 Hz, 2H), 2.41 (t, J = 8.0 Hz, 2H), 1.73 – 1.63 (m, 2H), 1.57 – 1.36 (m, 6H), 1.34 – 1.23 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 163.89, 155.70, 150.27, 133.34, 131.71, 130.30, 129.67, 109.34, 42.36, 40.45,

32.15, 29.03, 28.13, 26.91, 25.34, 21.20, 14.21. MS (ESI-TOF) for $C_{19}H_{29}N_5$ $[M + H]^+$ calculated 328.2496, found 328.2493.

Human TLR8-specific reporter gene assays (NF-κB induction), and TLR-2/-3/-4/-5/-7/-9 counter-screens: The induction of NF-κB was quantified using human TLR-2/-3/-4/-5/-7/-8/-9-specific, rapid-throughput, liquid handler-assisted reporter gene assays as previously described by us.[31, 29, 37, 38] HEK293 cells stably co-transfected with the appropriate hTLR and secreted alkaline phosphatase (sAP) were maintained in HEK-Blue™ Selection medium. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by appropriate TLR agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. Reporter 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 in the presence of graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by InvivoGen) at 620 nm.

Immunoassays for cytokines. Fresh human peripheral blood mononuclear cells (hPBMC) were isolated from human blood obtained by venipuncture in Cell Preparation Tubes (CPT, Beckton-Dickinson) with informed consent and as per guidelines approved by the University of Minnesota Human Subjects Experimentation Committee. Aliquots of PBMCs (10^5 cells in 100 μL/well) were stimulated for 16 h with graded concentrations of test compounds. Supernatants were isolated by centrifugation, and were assayed in duplicates using analyte-specific multiplexed cytokine/chemokine bead array assays (HCYTMAG-60K-PX29 MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel, EMD Millipore, Billerica, MA) as reported by us

previously.[23] The following analytes were quantified: sCD40L, VEGF, TNF-β, TNF-α, TGF-α, RANTES, PDGF-AB/BB, PDGF-AA, MIP-1β, MIP-1α, MDC (CCL22), MCP-3, MCP-1, IP-10, IL-17A, IL-15, IL-13, IL-12 (p70), IL-12 (p40), IL-10, IL-9, IL-8, IL-7, IL-6, IL-5, IL-4, IL-3, IL-2, IL-1ra, IL-1β, IL-1α, IFN-γ, IFN-α2, GRO, GM-CSF, G-CSF, fractalkine, Flt-3 ligand, FGF-2, eotaxin, EGF.

Flow-cytometric immunostimulation experiments: Cell surface marker upregulation was determined by flow cytometry using protocols published by us previously.[23] Briefly, heparin-anticoagulated whole blood samples were obtained by venipuncture from healthy human volunteers with informed consent and as per guidelines approved by the University of Minnesota Human Subjects Experimentation Committee. Serial dilutions of selected compounds were performed using a Bio-Tek Precision 2000 XS liquid handler in sterile 96-well polypropylene plates, to which were added 100 µL aliquots of anticoagulated whole human blood. The plates were incubated at 37^{o}C for 16 h. Negative (DMSO) controls were included in each experiment. The following fluorochrome-conjugated antibodies were used: CD14-FITC, CD40-APC, CD80-PE-Cy7, CD86-V450 (Becton-Dickinson Biosciences, San Jose, CA). Following incubation, 2.5 µg of each antibody was added to wells with a liquid handler, and incubated at 4^{o}C in the dark for 60 min. Following staining, erythrocytes were lysed and leukocytes fixed by mixing 200 µL of the samples in 800 µL pre-warmed Whole Blood Lyse/Fix Buffer (Becton-Dickinson Biosciences, San Jose, CA) in 96 deep-well plates. After washing the cells twice at 300 g for 10 minutes in RPMI, the cells were transferred to a 96-well plate. Flow cytometry was performed using a BD FACSVerse instrument for acquisition on 200,000 gated events. Compensation for spillover was computed for each experiment on singly-stained UltraComp Beads (eBioscience, Inc., San Diego, CA).

Supporting Information: Characterization data (^{1}H, ^{13}C, mass spectra), LC-MS analyses of key precursors and final compounds.

Corresponding Author Address*:*

Sunil A. David,

Department of Medicinal Chemistry,

2-132, Cancer & Cardiovascular Research Building, 2231 6th St. SE.,

University of Minnesota, Minneapolis, MN 55455.

Tel: 612-625-3317

Email: sdavid@umn.edu

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Abbreviations: APCs, Antigen-presenting cells; CD, Cluster of differentiation; DIPEA, *N*,*N*-Diisopropylethylamine; EC_{50}, Half-maximal effective concentration; ESI-TOF, Electrospray ionization-time of flight; HEK, Human embryonic kidney; IFN, Interferon; IL, Interleukin; MAL, MyD88-adapter-like; MyD88, Myeloid differentiation primary response gene 88; MCP-1, Monocyte chemotactic protein 1; MCP-3, Monocyte chemotactic protein 3; MPLA, Multiplex ligation-dependent probe amplification; NBS, *N*-Bromosuccinimide; NCS, *N*-Chlorosuccinimide; NIS, *N*-Iodosuccinimide; NF-κB, Nuclear factor-κB; PRRs, pattern-recognition receptors; PBMCs, Peripheral blood mononuclear cells; sAP, Secreted alkaline phosphatase; Th1, Helper T lymphocyte, type 1; Th2, Helper T lymphocyte, type 2; TIR, Toll/interleukin-1 receptor; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-α; TRIF, TIR-domain-containing adapter-inducing interferon-β; TRAM, TRIF-related adaptor molecule.

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Legends to Figures

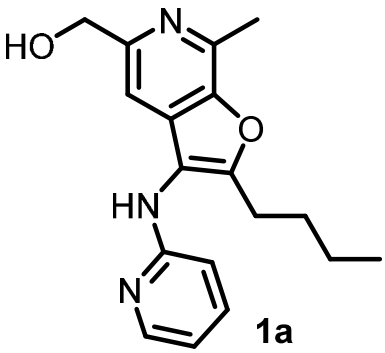
Figure 1. Structures of the TLR8-active compounds. Compound **1d** was used a comparator.

Figure 2. Agonistic activities of 2,4-diaminopyrimidine analogues in human TLR8 reporter gene assays. Means± SD on quadruplicates are shown. Also included is **1d**, used as a reference TLR8-active compound. The bimodal nature of agonistic responses resulting in apparent inhibition at high ligand concentrations was verified not to be due to cytotoxicity. None of these compounds were active in TLR-2/-3/-4/-5/-7/-9 counter-screens (Figs. S1 and S2).

Figure 3. Representative cytokine induction data (excerpted from a 41 cytokine panel) in human PBMCs. Means of duplicates are shown.

Figure 4. CD80 expression in monocytes. Monocytes were identified directly based on $CD14^+$ phenotype. Mean fluorescence intensity (MFI) of CD80 signals are shown.

Figure 1



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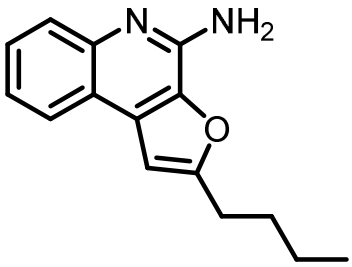

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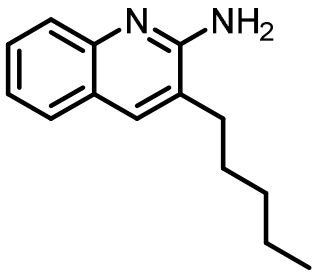

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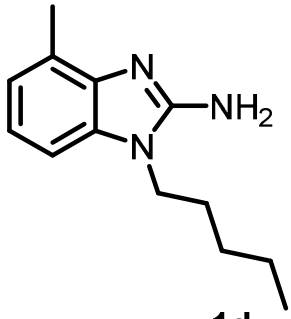

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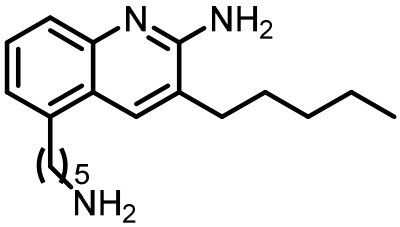

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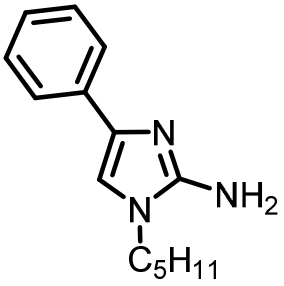

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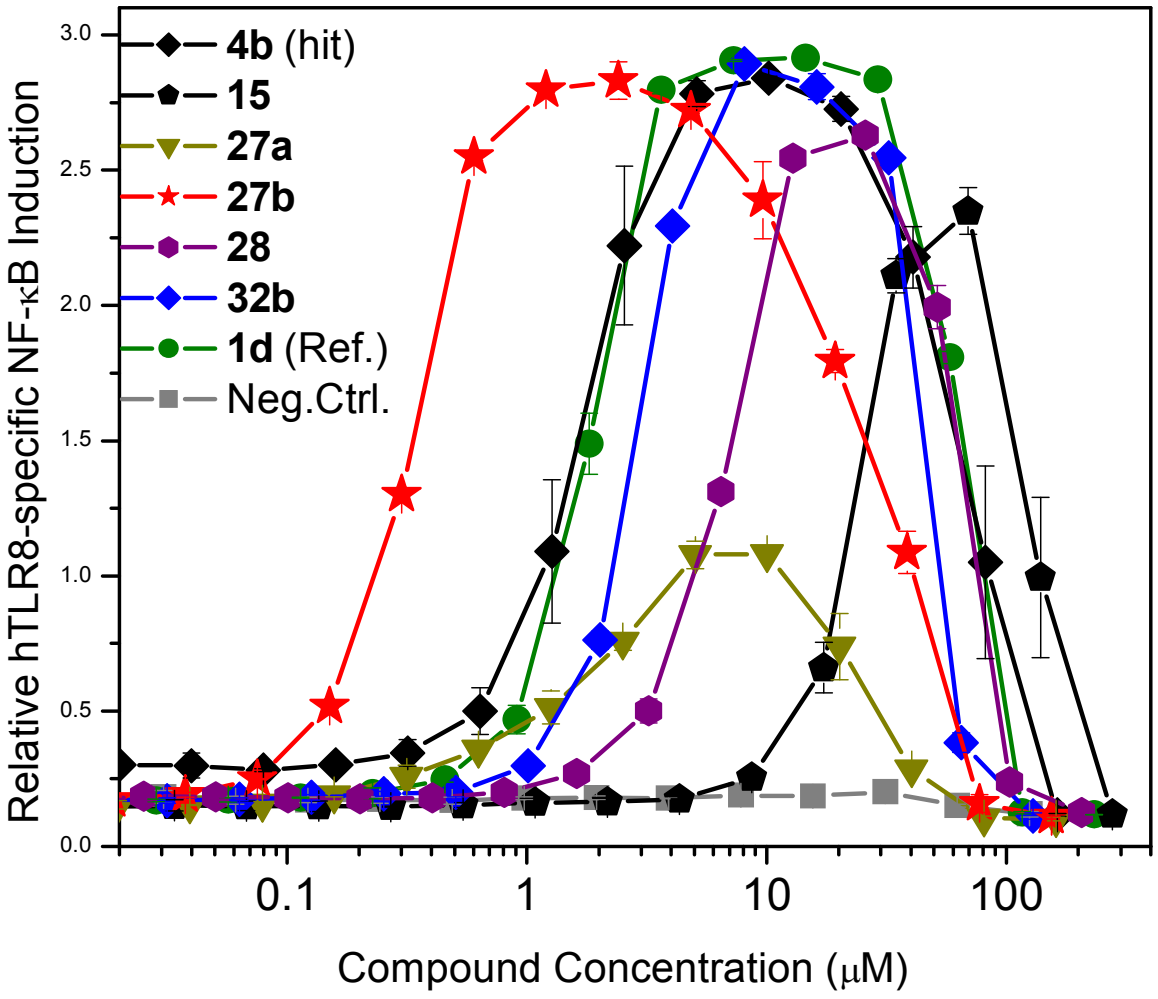
Figure 2


4b (hit)
15
27a
27b
28
32b
1d (Ref.)
Neg.Ctrl.
Relative hTLR8-specific NF-κB Induction
Compound Concentration (μM)
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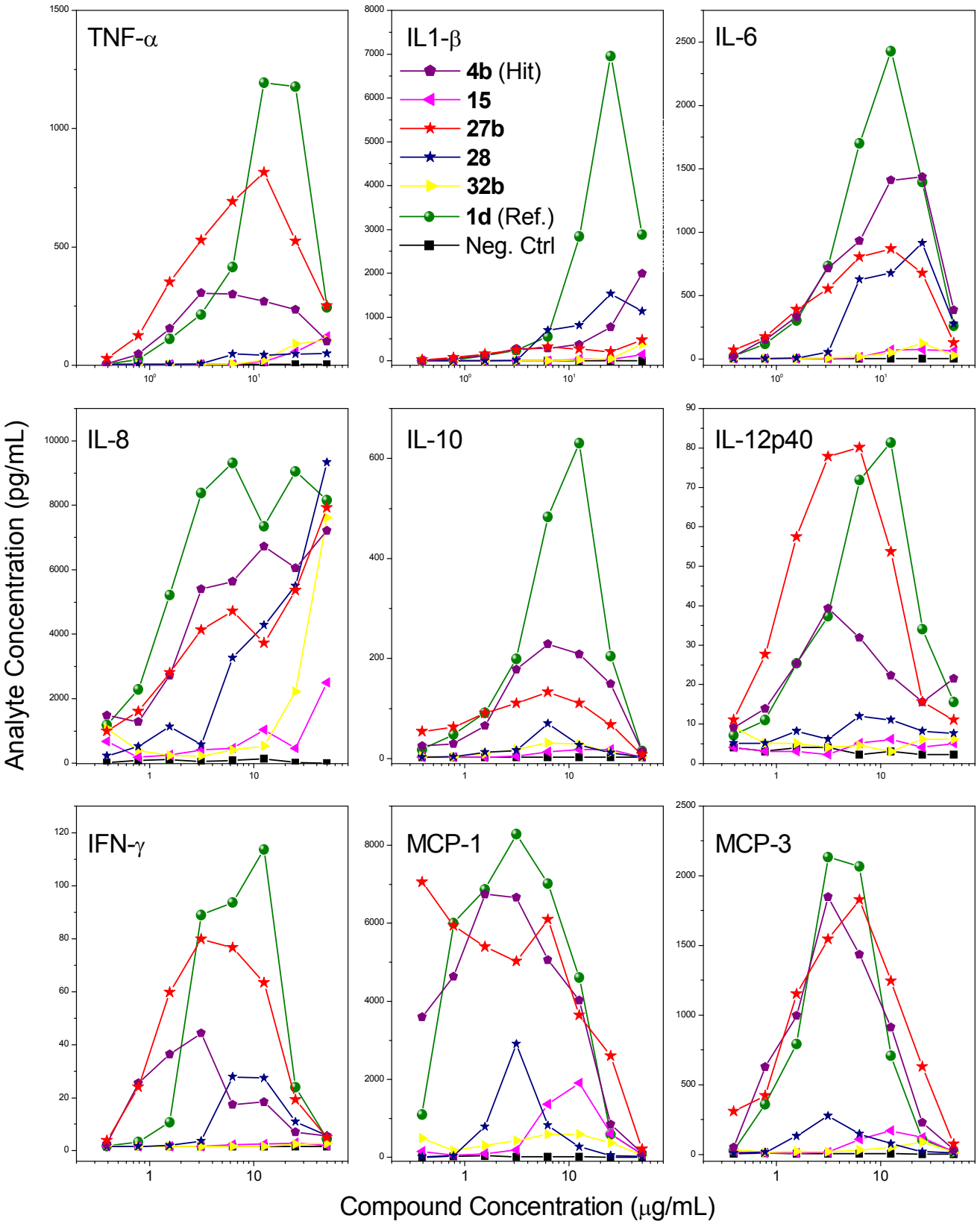
3.0
0.1
1
10
100

Figure 3


TNF-α
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IFN-γ
MCP-1
MCP-3
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27b
28
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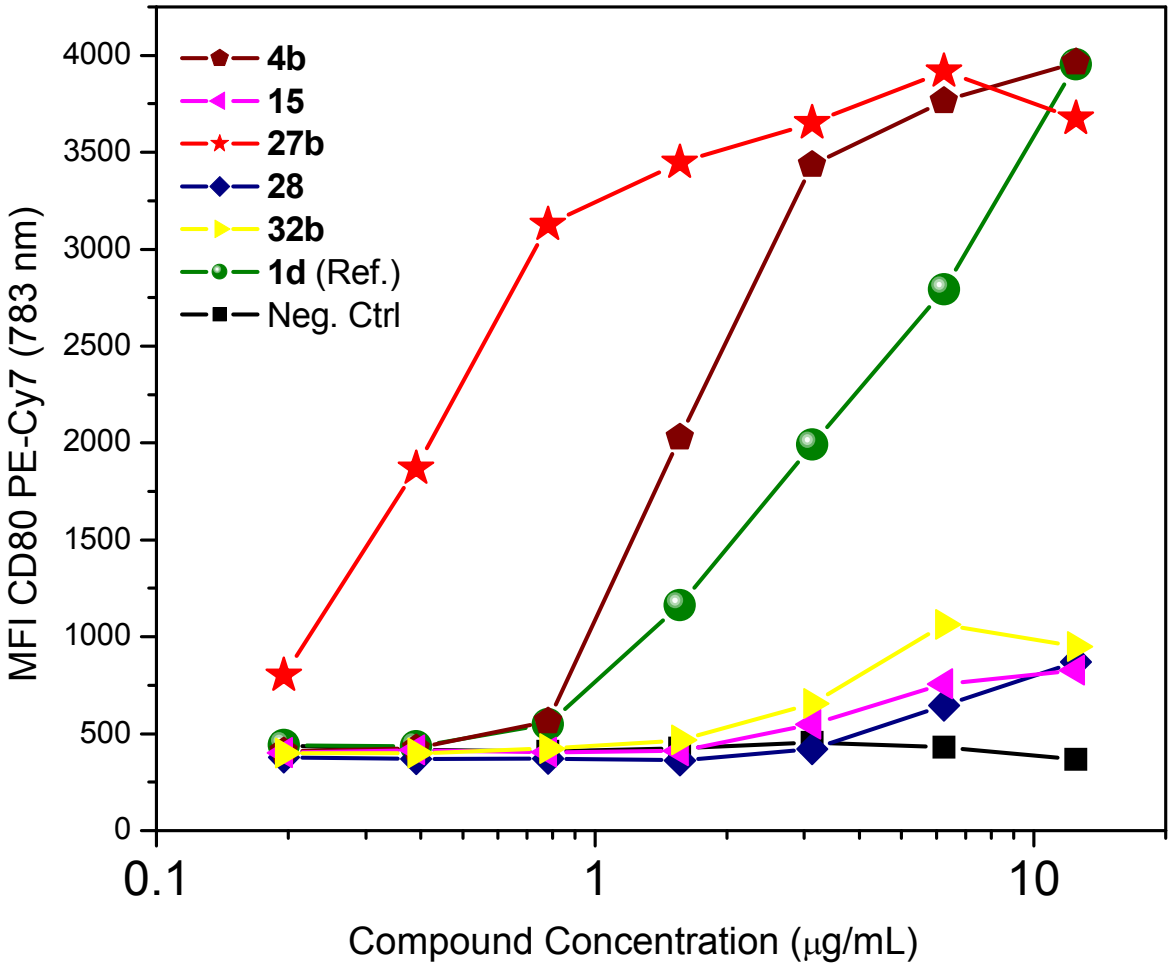
Neg. Ctrl
Analyte Concentration (pg/mL)
Compound Concentration (μg/mL)

Figure 4


4b
15
27b
28
32b
1d (Ref.)
Neg. Ctrl
MFI CD80 PE-Cy7 (783 nm)
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1000
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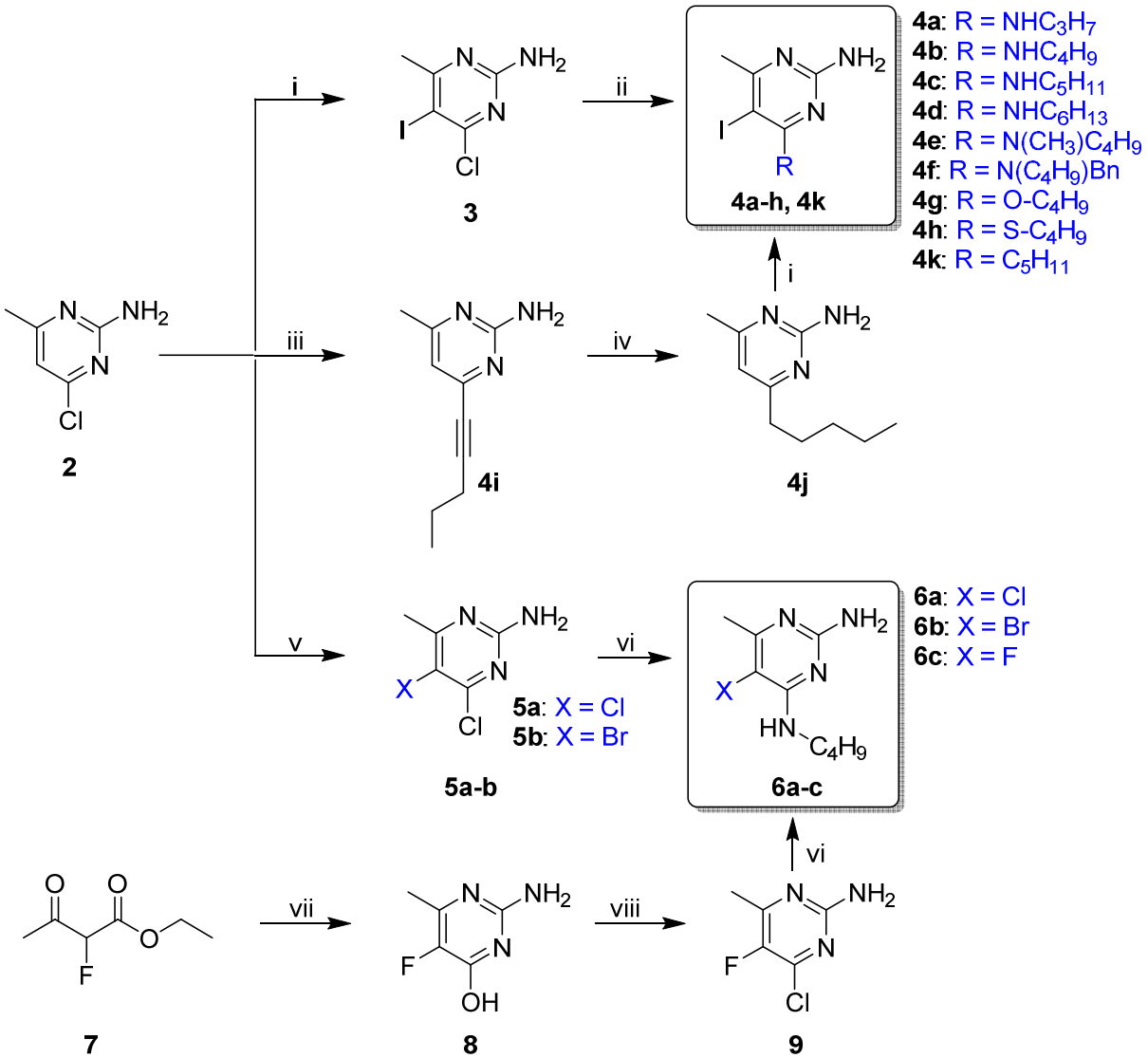
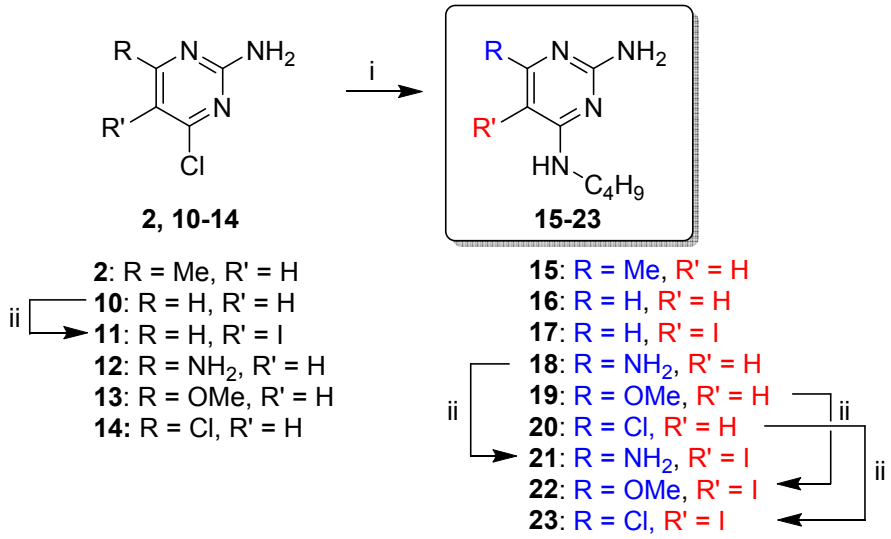
0
0.1
1
10
Compound Concentration (μg/mL)

Scheme 1.



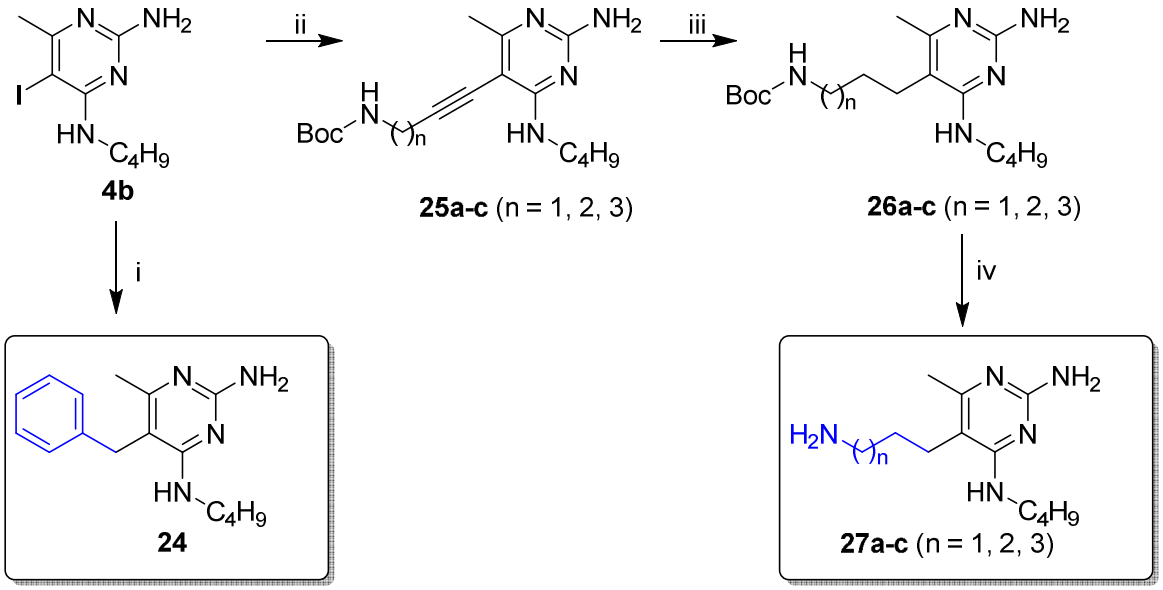
Reagents and conditions: (i) NIS, DMF, 12 h; (ii) alkylamine, Et_3N, MeOH, 70 ºC, 12 h (for **4a-f)** or n-butanol, K_2CO_3, 85 ºC, 24 h (for **4g**) or 1-butanethiol, Et_3N, MeOH, 70 ºC, 12 h (for **4h**); (iii) 1-pentyne, $Pd(pph_3)_4$, CuI, Et_3N/CH_3CN (1:2), 12 h; (iv) Pd/C, EtOAc, 50 psi, 5 h; (v) NCS (for **5a**) or NBS (for **5b**), DMF, 12 h; (vi) **5a** (for **6a**) or **5b** (for **6b**) or **9** (for **6c**), butylamine, Et_3N, MeOH, 70 ºC, 12 h; (vii) guanidine hydrochloride, Et_3N, MeOH, reflux,12 h; (viii) $POCl_3$, 85 ºC, 3 h.

Scheme 2.



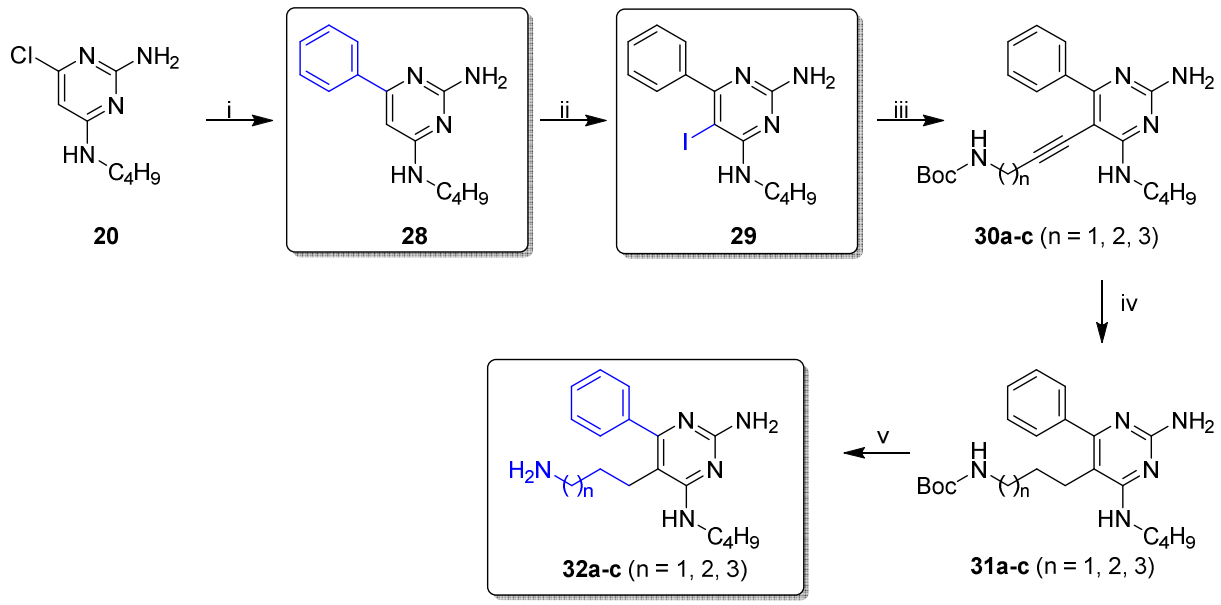
Reagents and conditions: (i) Butylamine, Et_3N, MeOH, 70 ºC, 12 h; (ii) NIS, DMF, 12 h.

Scheme 3.



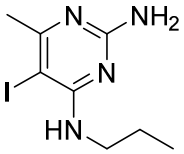
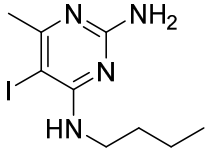
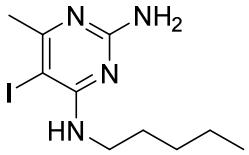
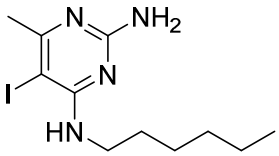
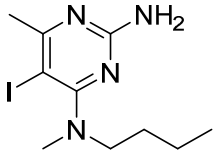
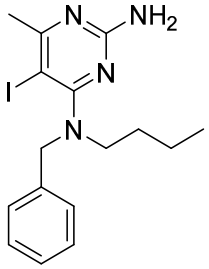
Reagents and conditions: (i) Benzylboronic acid pinacol ester, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/water (4:1), 85 ºC,12 h; (ii) *N*-boc-propargylamine (for **25a**) or *N*-boc-3-butyne-1-amine (for **25b)** or *N*-boc-4-pentyne-1-amine (for **25c)**, $Pd(pph_3)_4$, CuI, DIPEA/DMF (1:2), 12 h; (iii) Pd/C, EtOAc, 50 psi, 5 h; (iv) HCl, 4 M, 3 h.

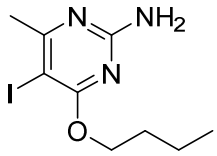
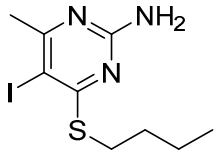
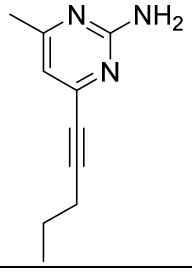
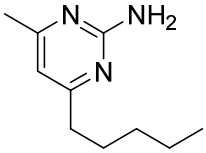
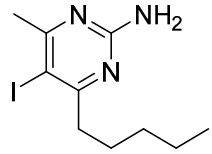
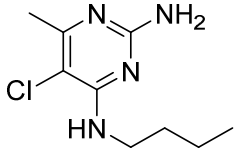
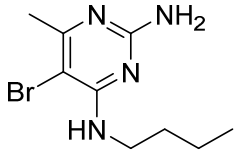
Scheme 4.

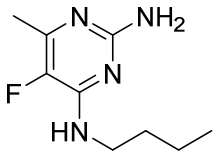
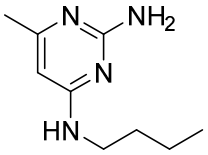
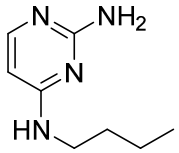
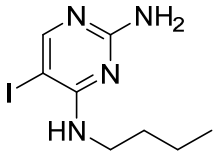
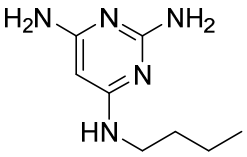
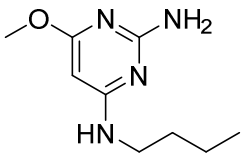
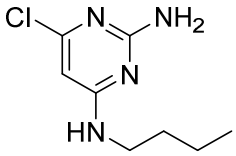


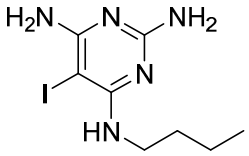
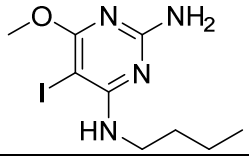
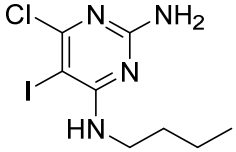
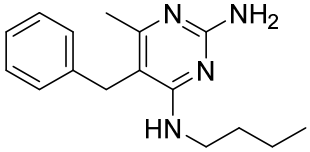
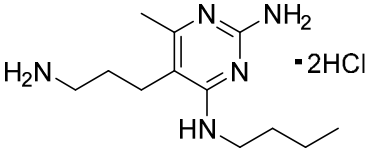
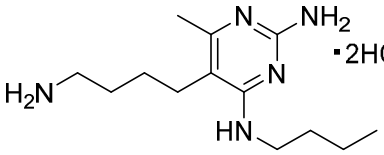
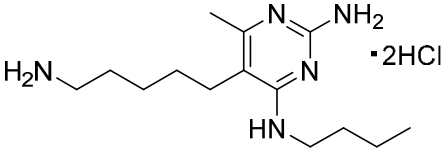
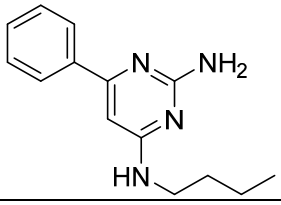
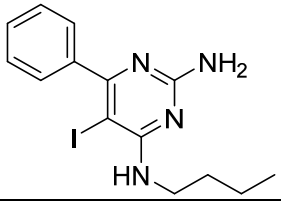
Reagents and conditions: (i) Phenylboronic acid, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/water (4:1), 85 ºC,12 h; (ii) NIS, DMF, 12 h; (iii) *N*-boc-propargylamine (for **30a**) or *N*-boc-3-butyne-1-amine (for **30b)** or *N*-boc-4-pentyne-1-amine (for **30c)**, $Pd(pph_3)_4$, CuI, DIPEA/DMF (1:2), 12 h; (iv) Pd/C, EtOAc, 50 psi, 5 h; (v) HCl, 4 M, 3 h.

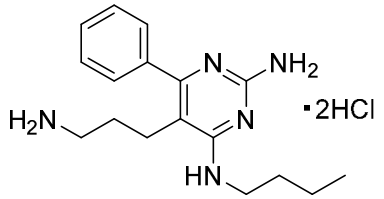
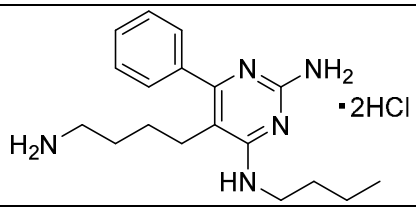
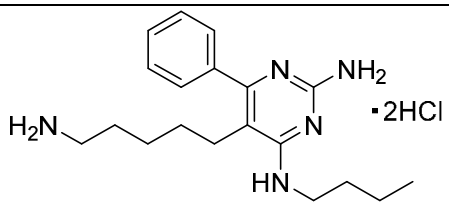
Table 1. EC_{50} values of compounds in human TLR 8-specific reporter gene assays

Compound Numbers	Structure	TLR8 Agonistic Activity (μM)[a]
4a		Inactive
4b		1.64
4c		3.70
4d		Inactive
4e		Inactive
4f		Inactive

4g		Inactive
4h		Inactive
4i		Inactive
4j		Inactive
4k		Inactive
6a		20.0 (Very low AUC)
6b		8.50 (Very low AUC)

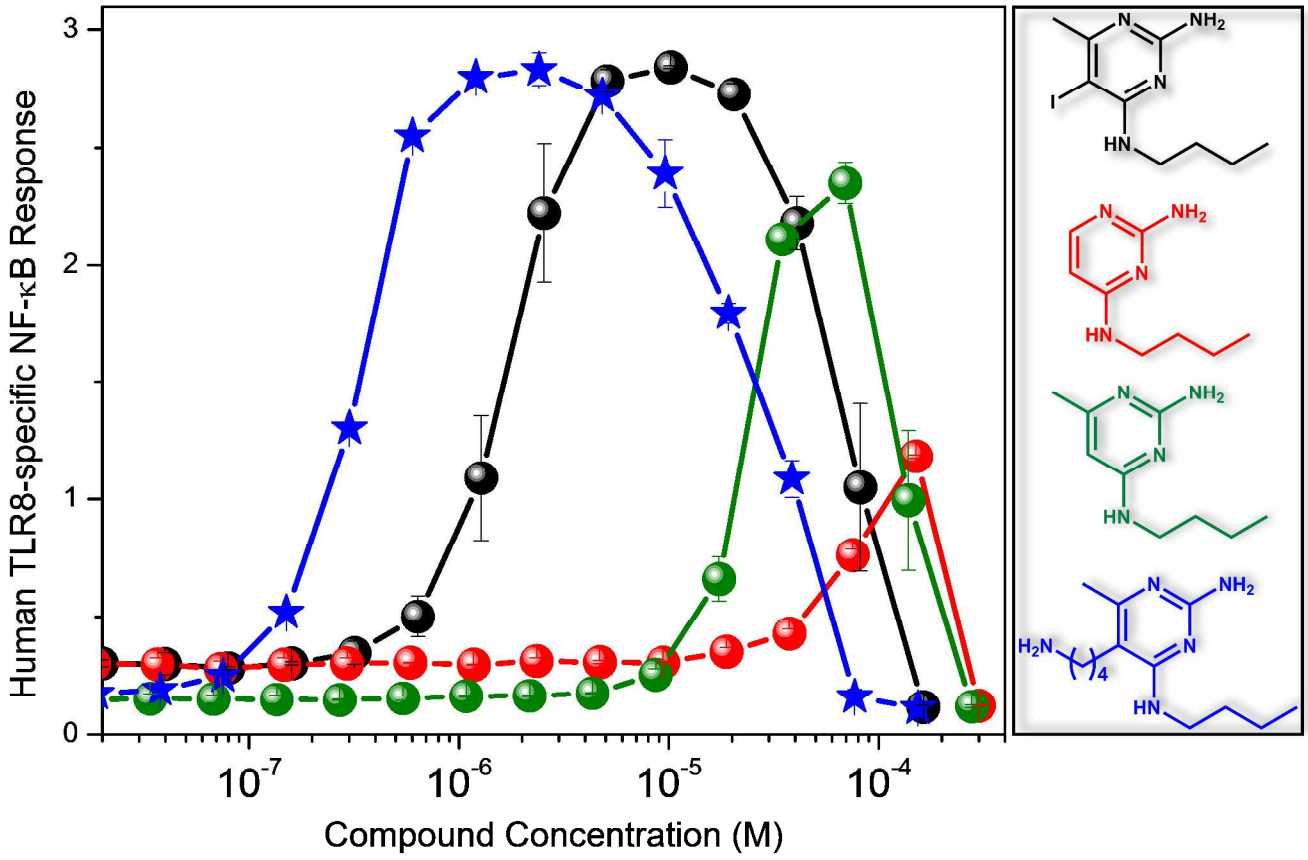
6c		34.0 (Very low AUC)
15		22.0
16		73.2 (Very low AUC)
17		Inactive
18		Inactive
19		Inactive
20		Inactive

21		Inactive
22		Inactive
23		Inactive
24		1.20 (Low AUC)
27a		2.31
27b		0.30
27c		0.30
28		6.70
29		Inactive

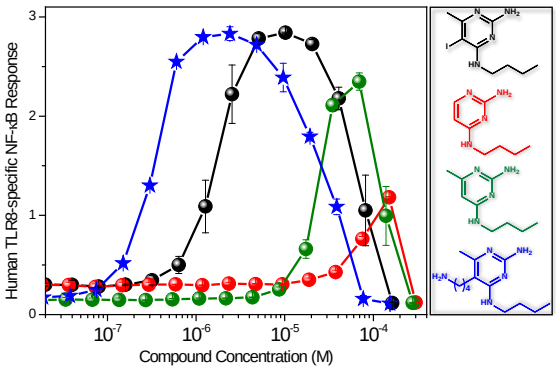
32a		Inactive
32b		2.70
32c		2.35

[a] EC_{50} values represent the arithmetic means obtained on quadruplicate samples. The EC_{50} of **1d**, used as a reference/comparator pure TLR8 agonist was 1.8 μM. Inactive denotes no activity detected at 100 μM.

TOC Graphic


Human TLR8-specific NF-κB Response
0
1
2
3
10-7
10-6
10-5
10-4
Compound Concentration (M)
N
NH2
I
HN
N
NH2
N
HN
N
NH2
N
HN
N
NH2
H2N
4
N
HN

TOC Graphic


Human TLR8-specific NF-κB Response
0
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2
3
10-7
10-6
10-5
10-4
Compound Concentration (M)