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Inhibition of Protein Kinase C-Driven Nuclear Factor-κB Activation: Synthesis, Structure−Activity Relationship, and Pharmacological Profiling of Pathway Specific Benzimidazole Probe Molecules

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A unique series of biologically active chemical probes that selectively inhibit NF-κB activation induced by protein kinase C (PKC) pathway activators have been identified through a cell-based phenotypic reporter gene assay. These 2-aminobenzimidazoles represent initial chemical tools to be used in gaining further understanding on the cellular mechanisms driven by B and T cell antigen receptors. Starting from the founding member of this chemical series **1a** (notated in PubChem as CID-2858522), we report the chemical synthesis, SAR studies, and pharmacological profiling of this pathway-selective inhibitor of NF-κB activation.

Introduction

Members of the nuclear factor-kappa B (NF-κB[a]) family of transcription factors control many crucial physiological and pathological processes including host-defense, immune response, inflammation, and cancer.[1,2] A number of intracellular signaling pathways leading to NF-κB activation have been elucidated and have been the subject of several excellent reviews.[3,4] Most NF-κB activation pathways converge on IκB-α kinase (IKK) activation, resulting in phosphorylation of IκB-α, and thereby targeting this protein for ubiquitination with proteasome-dependent destruction. This cascade releases NF-κB heterodimers from the IκB-α complex in the cytosol and allows for translocation into nucleus where transcription of various pro-inflammatory genes is initiated.[5] The various pathways upstream of IKK activation are diverse, with most of them linked to cell surface and intracellular receptors that sense cell damage and pathogens as well as activation in response to cytokines. Accordingly, small molecule pathway selective inhibitors can serve as powerful chemical tools to dissect these molecular networks which are crucial for normal cellular survival but are dysregulated in specific disease states.

The NF-κB pathway activated by antigen receptors is critical for adaptive immunity contributing to T and B lymphocyte activation, proliferation, cell survival, and effector functions. Dysregulated NF-κB activation in lymphocytes contributes to development of a variety of autoimmune-based disease states, chronic inflammation, and lymphoid malignancy.[6,7] The NF-κB activation pathway linked to antigen receptors involves a cascade of adapter and signal transduction proteins that at minimum include a CARMA family protein, Bcl-10, MALT (paracaspase), TRAF6, Ubc13, caspase-8, and *c*-Flip. Formation of this complex is initiated by protein kinase C (PKC)-mediated phosphorylation of CARMA proteins. In T and B cells, this pathway is initiated by PKC-θ and PKC-β, respectively, leading ultimately to IKK activation through a mechanism potentially involving lysine 63-linked polyubiquitination of IKK-γ.[8] Thus, the antigen receptor pathway for NF-κB activation is both initiated and concluded by activation of protein kinases, namely the PKCs and IKKs, respectively.

Although the IKK complex and its individual subunits represent logical targets for potential drug discovery, small-molecule inhibitors of IKKs suppress all known NF-κB activation pathways. This approach lacks the selectivity required to inhibit lymphocyte responses without simultaneously interfering with overall innate immune responses and leads to general immunosuppression attended by risk of pathogenic infection as well as other severe side effects.[9,10]

A library of approximately 110,000 compounds from the NIH Molecular Libraries Small Molecule Repository (MLSMR) compound collection was screened using a HEK293 cell-based NF-κB luciferase reporter gene strategy.[11,12] Primary hit compounds with an $IC_{50} \leq 10\ \mu M$ were further characterized by 14 additional cell-based counter-screens that helped determine pathway selectivity in the HEK293 cell line and other lymphocytes. Ultimately, three hit series emerged and were confirmed through an extensive counter-screen assay platform to be selective for the antigen receptor activation pathway (Figure 1A). Of the three primary hit classes identified in the HTS campaign, only the 2-aminobenzimidazole series represented by 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(2-((3-hydroxypropyl)amino)-5,6-dimethyl-1*H*-benzo[*d*]-imidazol-1-yl)ethanone, **1a** (CID-2858522), selectively inhibited the NF-κB pathway induced by PKC activators and

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[a] Abbreviations: PKC, protein kinase C, NF-κB, nuclear factor-kappa B; IκB, inhibitor of NF-κB; IKK, IκB-α kinase; CARMA, caspase recruitment domain (CARD) carrying member of the membrane associated guanylate kinase (MAGUK) family proteins; Bcl-10, B-cell lymphoma/leukemia 10 protein; MALT, mucosa associated lymphoid tissue translocation protein; TNF-α, tumor necrosis factor alpha; TRAF, TNF receptor associated factor; Ubc13, ubiquitin conjugating enzyme 13; *c*-FLIP, cellular Fas-associated death domain-like IL-1-converting enzyme inhibitory protein; HTS, high throughput screening; MLSMR, NIH molecular libraries small molecule repository; HEK293, human embryonic kidney 293 cells; IL-8, interleukin-8; CD, cluster of differentiation; PMA, phorbol-12-myristate-13-acetate; CID, compound ID; SAR, structure−activity relationship; ADME/T, absorption, distribution, metabolism, excretion, and toxicology; PAMPA, parallel artificial membrane permeability assay; CYP3A4, cytochrome P450 3A4; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; MLPCN, The Molecular Libraries Probe Centers Network.

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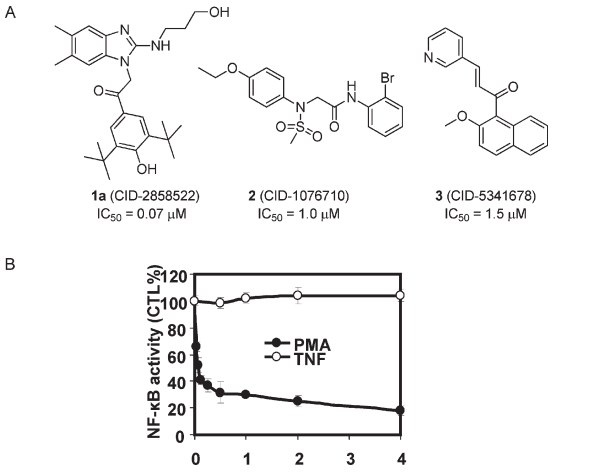


Figure 1. (A) Three scaffold classes (with $IC_{50} < 10\ \mu M$, most active members shown) identified in the high throughput screening (HTS) campaign. (B) Selectivity of **1a** for inhibition of the NF-κB antigen receptor pathway induced by PMA (a PKC activator) vs TNF-α activation.

antigen receptors. Compound **1a** did not inhibit activation of NF-κB through TNF-α stimulation (Figure 1B). Details of the comprehensive screening platform and pathway specific cell-based selectivity assessment, as outlined above, are described in our accompanying paper.[13]

On the basis of the above information and selectivity profile, the 2-aminobenzimidazole series was selected for chemistry follow-up as it met our initial potency criteria ($<1\ \mu M$) and selective inhibition of antigen receptor-mediated NF-κB activation. We discuss the development of an efficient synthetic route to the 2-aminobenzimidazole scaffold, the design and synthesis of SAR analogues, as well as in vitro and in vivo pharmacological profiling of **1a**. These structure−activity relationship studies provide molecular insight for further optimization and synthesis of proteomic probes, which include analogues to be used for the identification of the precise cellular target protein and subsequent mode of action studies.

Results and Discussion

The initial HTS hit compounds of interest (Figure 1) were resynthesized so that identity and cellular activity could be confirmed. Additional commercially available dry powder samples for closely related analogues were ordered (ChemBridge Corporation, San Diego, CA), and the quality, purity, and identity for each compound was confirmed. In the case where the commercial analogues were not homogeneous, they were purified by preparative HPLC to ≥98%. Each compound was assessed for bioactivity using a cell-based assay employing HEK293 cells containing a stably integrated NF-κB-luciferase reporter gene, which was stimulated with phorbol-12-myristate-13-acetate (PMA) and ionomycin.

The "analogue by catalogue" approach ultimately provided an initial set of 2-aminobenzimidazoles having NF-κB-luciferase IC_{50} values in the micromolar to nanomolar range as shown in Figure 1S (see Supporting Information). Analysis of the structure−activity relationships (SAR) pointed to features responsible for optimal activity. Truncation of 2-aminopropanol substituent and removal of both 5- and 6-methyl groups resulted in equipotent inhibitors (compounds **4** and **5**, Figure 1S, see Supporting Information). Most importantly, the di-*t*-butyl phenol moiety present in the N_1-acetophenone substituent of the 2-aminobenzimidazole scaffold seemed to be essential for activity (compounds **1a** and **6**−**8**, Figure 1S, see Supporting Information). 3,5-Di-*t*-butyl-4-methylphenol and 6,6′-methylenebis-(2-*tert*-butyl-4-methylphenol), commercial antioxidants were screened and found to be inactive, thus ruling out any connection between a potential antioxidant pharmacophore (possibly affecting nitric oxide signaling) and observed NF-κB inhibitory activity of **1a** (compounds **9** and **10**, Figure 1S, see Supporting Information).[14] The general synthetic route toward the targeted series is illustrated in Scheme 1. The desired scaffold was accessed from the corresponding 2-bromobenzimidazole intermediate (Scheme 1).

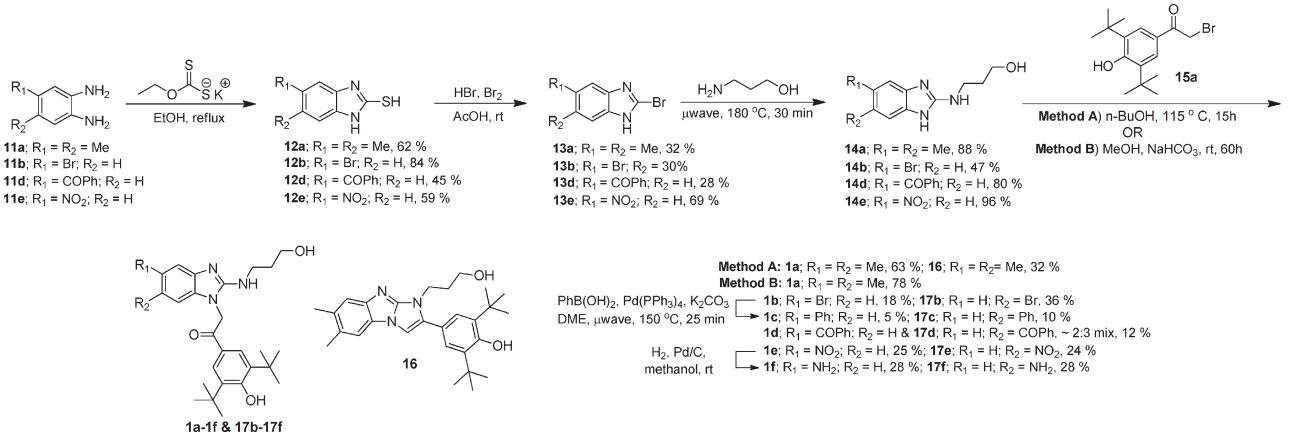
Accordingly, the aryl diamine **11a** was condensed with potassium ethyl xanthate in ethanol at reflux to afford 1**2a**, which on bromination furnished the key 2-bromo-5,6-dimethylbenzimidazole intermediate **13a**.[15−17] Amination of **13a** with 3-aminopropanol under neat conditions at high temperature afforded the highly polar intermediate **14a**, which required extensive chromatography for purification.[18] It was found that conducting the amination reaction under solvent-free microwave heating conditions at 180 °C/25 min afforded **14a** in high yield and purity after simple extraction.

The final alkylation of intermediate **14a** with α-bromo-3,5-di-*tert*-butyl-4-hydroxyacetophenone (**15a**) was conducted in *n*-butanol at reflux to furnish **1a** in moderate yield (Scheme 1, method A). The low isolated yield was attributed to the formation of tricyclic side product **16** via an intramolecular Schiff-base formation.[19] Purified **16** was found to be inactive ($IC_{50} > 25\ \mu M$) under the assay conditions. This competing intramolecular cyclization was completely eliminated by conducting the reaction at 23 °C in methanol using sodium bicarbonate as a mild base. Although alkylation under these conditions was incomplete even after 3 days, the formation of **16** was attenuated and compound **1a** was obtained in 78% yield and in >98% purity (Scheme 1, method B). The optimized route was utilized for the synthesis of an analogue library designed to explore, (a) monosubstitution at 5- and 6-positions of 2-aminobenzimidazole core, (b) substitution at the 2-position with diverse amines, (c) substitution of the aryl ring of the N_1-acetophenone moiety, and (d) replacement of the core 2-aminobenzimidazole with related 2*H*-benzimidazole and benzimidazolone ring systems. Commercially available 4-monosubstituted-phenyl-1,2-diamines were converted to the corresponding 2-aminobenzimidazoles (**14b**,**d**,**e**, Scheme 1) via the corresponding 2-bromo intermediates (**13b**,**d**,**e**).

Subsequent alkylation with **15a** under previously optimized conditions furnished the regioisomeric 5- and 6-substituted benzimidazole analogues (**1b**,**d**,**e**; **17b**,**d**,**e**), which could be separated by chromatography and characterized. The 5- and 6-bromo analogues (**1b** and **17b**), when subjected to the Suzuki coupling protocol with phenyl boronic acid, afforded 5- and 6-phenyl substituted analogues **1c** and **17c**, respectively. The 5- and 6-nitro analogues (**1e** and **17e**) were reduced to the corresponding amino compounds **1f** and **17f**, respectively, via hydrogenation. A variety of amines were then reacted with **3a** under the previously optimized amination conditions. Subsequent alkylation with **15a** generated the corresponding 2-aminoalkyl substituted analogues (**1g**−**1k**, Scheme 2A). The analogue library was then evaluated for inhibition of NF-κB activation using the cell-based HEK293 NF-κB luciferase reporter gene assay.[12,13]

To study the effect of variation of substitution on the aryl ring at the N_1-position of **1a**, suitably substituted α-bromoacetophenones were synthesized (**15l**−**15n**) from commercially available

Scheme 1. Synthetic Route to **1a** and Monosubstituted Benzimidazole Analogues **1b**−**f** and **17b**−**f**



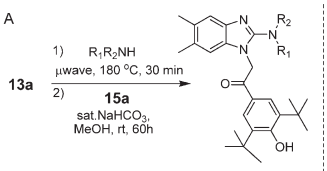
starting materials and then treated with intermediate **14a** to access analogues **1l**−**1n** (Scheme 2B; see Supporting Information for details). To replace the 2-aminobenzimidazole core of **1a** with related heterocycles, commercially available 5,6-dimethyl-1*H*-benzimidazole and 5,6-dimethyl-1*H*-benzimidazol-2-one were alkylated with **15a** in the presence of sodium hydride to furnish **18** and **19** in 63 and 55% yield, respectively (Scheme 3).

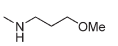
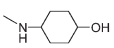
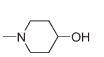
Through a systematic substitution process, we probed the individual contribution of the substituents at the 5- and 6-positions of the benzimidazole aryl moiety. It was found that substituents at the 5-position were better tolerated than at 6-position and activity of analogues was sensitive to both the electronic and steric nature of the substituents (**1b**−**f** and **17b**−**f**, Table 1A). The results indicate that the 6-position is more sensitive to the steric bulk of the substituent than the 5-position because replacement of either methyl group with a bromine led to a 40−80-fold drop in activity (**1a**, **1b**, and **17b**, Table 1A).

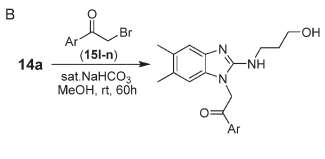
Similarly, the 5-nitro analogue was 20-fold less active than **1a** while the 6-nitro analogue was found to be inactive (**1e** and **17e**, Table 1A). Installation of a planar phenyl group resulted in analogues with activity approaching that of **1a**, suggesting a positive hydrophobic interaction at the 5-position (**1c** and **17c**, Table 1A). An unexpected result was that the 5- and 6-amino analogues were the most active analogues in this substitution series, suggesting access to H-bonding interactions (**1f** and **17f**, Table 1A). It was found that while *O*-methylation of the 2-aminopropanol side chain retained most of the activity (**1g**, Table 1B), removal of the hydroxyl substituent in the 2-butylamino analogue led to a moderate loss of activity (**1h**, Table 1B), suggesting that the electronegative oxygen atom is not essential for activity. Truncation of the butyl group in the 2-methylamino analogue led to improved activity and was found to be as potent as **1g** (**1i**, Table 1B). Replacement of 2-aminopropanol side chain with a conformationally restricted 4-hydroxycyclohexylamine did not result in any improvement over **1a** (**1j**, Table 1B). The presence of a tertiary amine at the benzimidazole 2-position was found to abolish activity (**1k**, Table 1B). These results, taken together, suggest that the entire 2-aminopropanol substituent is likely solvent exposed and may not be completely required (except the NH moiety) for binding to the cellular target protein.

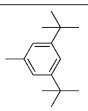
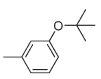
Replacement of the N^1-3,5-dimethyl-4-hydroxyacetophenone with either simple acetophenones or 3,5-dimethyl-4-hydroxyacetophenone resulted in complete loss of activity (**6**−**10**, Figure 1S, see Supporting Information). It was found that the des-4-phenoxy analogue retained complete activity (**1l**, Table 1C), and hence the 4-phenoxy substituent is not

Scheme 2. Synthesis of (A) 2-Aminoalkyl Benzimidazoles **1g**−**1k**; (B) Analogues **1l**−**1n** with Substitution on the Aryl N_1 Position

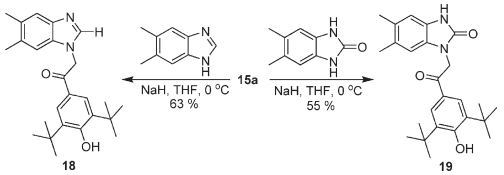


Entry	R_1R_2NH	% yield
1g		43
1h		59
1i		38
1j		45
1k		42



Entry	Ar	% yield
1l		32
1m		55
1n		62

Scheme 3. Synthesis of 2-*H*-Benzimidazole and 2-Benzimidazolone Analogues



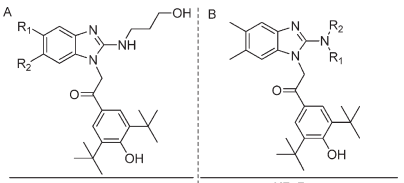
essential for activity, further confirming that NF-κB inhibition is not due to antioxidant activity. The SAR for aryl substitution at this position is steep. Elimination or replacement of one of the *tert*-butyl groups with a *tert*-butoxy group resulted in completely inactive analogues, suggesting that the di-*tert*-butyl phenyl group might represent a conformational lock and is extremely important for binding/activity (**1m** and **1n**, Table 1). Replacement of the 2-alkylamino moiety with hydrogen led to a significant loss of activity, however, the 2-benzimidazolone analogue was essentially equipotent to **1a** (**18** and **19**, Figure 2). These results indicate that a polar heteroatom is needed at the 2-position for activity, yet the 2-amino group is completely replaceable. This observation has important bearing on future probe SAR because it allows further derivatization at the N^3 position allowing exploration of additional chemical space via alkylation or acylation.

The SAR indicates the entire 2-aminopropyl chain does not contribute significantly to bioactivity. Because the precise cellular protein target is unknown, we capitalized on this information to synthesize two analogues that have an azide and a terminal alkyne incorporated in the 2-aminoalkyl side chain (**1o**, **1p**, Figure 2). The objective was to utilize these as building blocks for the synthesis of pull down probes for protein target identification. We were gratified that both analogues retain sufficient bioactivity (within 6-fold) and are currently being utilized in ongoing studies involving bioconjugation via "click chemistry" to furnish biotinylated derivatives for affinity studies.

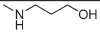
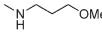
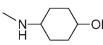
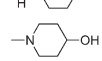
In Vitro and in Vivo Pharmacological Profiling. Compound **1a** was subjected to in vitro ADME/T assays to profile its general pharmacological properties (Table 2). Compound **1a** exhibits good to moderate solubility across pH ranges and moderate to high "effective" cell permeability (PAMPA). Although plasma stability is good, the probe exhibits high affinity toward plasma protein binding, possibly limiting available in vivo free fraction concentration. The compound was found to be nontoxic to human hepatocytes (Fa2N-4 cells; LD_{50} = 47 μM) and in the HEK293 cell line (LD_{50} > 50 μM) used in the primary activity assay. Compound **1a** is moderately stable to human hepatic microsomes at 1 and 10 μM but was rapidly metabolized by murine microsomes. **1a** is a weak inhibitor of CYP 3A4 (IC_{50} = 3 μM).

In vivo dose-exposure profiling of **1a** was conducted using a small cohort of three male mice was subjected to single ip doses at 10, 30, and 50 mg/kg. Blood was drawn at 0.5 and 3 h, and subsequent LC/MS analysis of pooled samples was performed to determine the overall blood levels of compound (Table 3). Probe compound **1a** exhibited nonlinear pharmacokinetics, showing higher serum levels at the 0.5 h measurement time for the 30 mg/kg dose compared to 50 mg/kg but displaying typical dose-dependent behavior when measured at t = 3 h. The increasing accumulation seen at a dose of 50 mg/kg may be due to a depot effect created by CYP3A4 inhibition. The cohort exhibited clear signs of morbidity at t = 3 h at the 50 mg/kg dose. On the basis of the above data, blood levels can be expected to reach maximum concentrations approximately 10-fold higher than IC_{50} at a dose of 30 mg/kg, thus defining a promising dose for future applications in short-term, acute biomarker studies such as in vivo measurements of cytokine production.

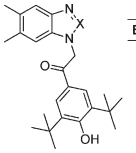
Table 1. Analogue SAR (A) 5 and 6-Monosubstitution of the Benzimidazole Core, (B) Substitution at the 2-Position, and (C) Substitution on the Aryl N^1-Acetophenone Moiety



Entry	R_1	R_2	IC_{50} (μM)
1a	Me	Me	0.07
1b	Br	H	3
1c	Ph	H	0.6
1d	-COPh	H	1.8
1e	NO_2	H	2
1f	NH_2	H	0.8
17b	H	Br	6
17c	H	Ph	0.9
17d	H	-COPh	N. A.
17e	H	NO_2	> 8
17f	H	NH_2	0.4

Entry	-NR_1R_2	IC_{50} (μM)
1		0.07
1g		0.3
1h		0.8
1i		0.3
1j		0.1
1k		13

1l, R = 3,5-di-tert-butylphenyl (IC_{50} = 0.1 μM)
1m, R = 3-tert-butylphenyl (IC_{50} = 11 μM)
1n, R = 3-tert-butoxyphenyl (IC_{50} = 16 μM)



Entry	X	IC_{50} (μM)
18	C-H	8.0
19	C-OH (urea)	0.1
1o	C-$NH(CH_2)_3N_3$	0.4
1p	C-$NH(CH_2)_3OC(=O)(CH_2)_4C{\equiv}CH$	0.6

Figure 2. Bioactivity of 2*H*-benzimidazole **18**, 2-benzimidazolone **19**, azide **1o**, and alkynyl ester **1p**.

Table 3. In Vivo Exposure Data for **1a**

dose (mg/kg)	concentration (μM) 0.5 h	concentration (μM) 3 h
10	1.81 ± 0.41	0.50 ± 0.04
30	8.28 ± 0.20	3.64 ± 0.76
50	5.93 ± 1.30	6.96 ± 0.76

General Target Inhibition Profile. Protein kinases play critical roles in NF-κB activation. PKCs are proximal kinases in the NF-κB pathways activated by PMA/ionomycin and by T-cell and B-cell antigen receptors, while the IKKs are distal kinases operating in the terminal segments of these and other NF-κB activation pathways.[2] At concentrations up to 8 μM, **1a** failed to suppress PKC-β and PKC-θ (the PKC family members implicated in TCR/BCR signaling), and IKK-β (a component of the IKK complex), while known PKC and IKK inhibitors and the broad-spectrum kinase inhibitor staurosporine afforded potent inhibition.[12,13] In addition, **1a** did not inhibit endogenous PKC activity or phosphorylation of downstream substrates.[13] Probe **1a** did not directly inhibit PKC-β, PKC-θ, or IKK-β. A broad kinome screen was then conducted using KINOMEscan (Ambit Biosciences) platform. Of 353 protein kinases surveyed, **1a** inhibited only three by more than 50% at 10 μM; Raf (57% inhibition), TLK1 (70% inhibition), and JAK2 (53% inhibition), none of which are clearly implicated in NF-κB regulation.

Compound **1a** was screened for general functional activity to determine effects on second messenger systems using a subset of cloned human or rodent receptors, ion channels, and transporters (NIMH-PDSP, University of North Carolina Chapel Hill). Of the 92 targets screened, **1a** showed greater than 50%

Table 2. In Vitro ADME Data for **1a**

solubility (μg/mL) pH 5.0/6.2/7.4	permeability[a,b] P_e ($\times 10^{-6}$ cm/s) pH 5.0/6.2/7.4	plasma protein binding (% bound) human 10 μM/1 μM	plasma protein binding (% bound) mouse 10 μM/1 μM	plasma stability[c] (% remaining) human/mouse	microsome stability[d] (% remaining) human 10 μM/1 μM	microsome stability[d] (% remaining) mouse 10 μM/1 μM
42/0.5/0.7	299/710/441	99.8/99.9	99.9/99.8	100/100	55/26	19/0.5

[a] Compound at 50 μM. [b] PAMPA P_e: low 5×10^{-6}, moderate 250×10^{-6}, high 1000×10^{-6}. [c] Plasma/PBS; compound at 40 μM, 3 h. [d] % remaining at 1 h.

inhibition at 10 μM for only eight targets (see Supporting Information for details). For all active targets assessed, the subsequent IC_{50} values were greater than 5 μM, highlighting that **1a** does not exhibit significant off-target activity.[20]

Conclusions

A series of substituted 2-aminobenzimidazoles has been identified that serve as promising research tools as pathway-selective chemical inhibitors of NF-κB activation induced by activators of PKC signaling through B and T cell antigen receptors. Interestingly, the MLPCN probe candidate (**1a**) was identified directly from the screening hit set and only lesser or equipotent analogues have been prepared, indicating a limited SAR for the series. Compound **1a** was found to be noncytotoxic and inhibited IL-8 production induced by PKC activators in HEK293 cells ($IC_{50} < 0.1$ μM), while failing to inhibit NF-κB reporter gene activation by agonists of the other NF-κB activation pathways. Compound **1a** attenuated CD3/CD28 and PMA/ionomycin-induced production of IL-2 by Jurkat T-cells ($IC_{50} < 5$ μM) and anti-IgM-stimulated proliferation of murine B-lymphocytes ($IC_{50} \sim 2$ μM). We have used the SAR data to generate the azide and alkynyl containing derivatives **1o** and **1p**. These have been utilized in the synthesis of biotinylated conjugates directed toward affinity-based target identification and future mode of action studies that will be reported separately. In addition, the equipotent 2-benzimidazolone analogues (**19**) provide a modified scaffold with an additional N_3-site for future exploration. Compound **1a** and its analogues represent new chemical tools that may be useful toward further development of therapeutically useful pathway selective modulators of NF-κB activity that may find application in disease models of inflammation, cancer and other autoimmune diseases.

Experimental Section

General Procedure (Method B, Scheme 1) of N_1-Alkylation of 2-Aminobenzimidazoles for the Synthesis 1a, 1b, 1d, 1 e, 1g−n, 17a, 17b, 17d, and 17e as Illustrated by Synthesis of 1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(2-(3-hydroxypropylamino)-5,6-dimethyl-1*H*-benzo[*d*]imidazol-1-yl)ethanone, 1a. To a 20 dram vial equipped with a stir bar was added 2-aminobenzimidazole **14a** (0.37 g, 1.6 mmol) followed by corresponding 2-bromoacetophenone **15a** (0.62 g, 1.9 mmol). To the mixture was added 10 mL of methanol and solid $NaHCO_3$ (3 equiv). The mixture was stirred at 23 °C for 3 days, after which the solvent was evaporated to a suitable volume and subsequent purification by silica gel flash column chromatography (hexanes to 1:4 hexanes/ethyl acetate) resulted in a yellow film, which on lyophilization from a 2:8 acetone/water mixture afforded **1a** as white solid (0.61 g, 78%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.87 (s, 2H), 6.95 (s, 1H), 6.77 (s, 1H), 5.94 (s, 1H), 5.52 (s, 2H), 3.55−3.47 (m, 2H), 3.42 (t, J = 5.6 Hz, 2H), 2.14 (s, 2H), 2.12 (s, 3H), 1.56 (p, J = 6.0 Hz, 2H), 1.40 (s, 18H). ^{13}C NMR (100 MHz, acetone-d_6) δ 191.58, 159.66, 156.73, 141.17, 137.58, 134.43, 128.85, 127.29, 127.19, 126.16, 116.74, 108.72, 57.65, 48.47, 39.19, 34.90, 34.67, 19.77, 19.74. LRMS (ESI): calcd for $C_{28}H_{39}N_3O_3$ [M + H] = 466.3, obsd [M + H] = 466.1. HRMS (ESI): calcd for $C_{28}H_{39}N_3O_3$ [M + H] = 466.3070, obsd [M + H] = 466.3091.

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Supporting Information Available: Detailed experimental procedures for the biology, pharmacology, and chemistry sections and spectroscopic characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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