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LETTER

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# Identification of Inhibitors of NOD1-Induced Nuclear Factor-κB Activation

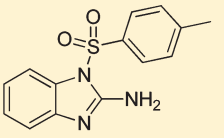
Pasha M. Khan,[†] Ricardo G. Correa,[‡] Daniela B. Divlianska,[†] Satyamaheshwar Peddibhotla,[†] E. Hampton Sessions,[†] Gavin Magnuson,[‡] Brock Brown,[‡] Eigo Suyama,[†] Hongbin Yuan,[‡] Arianna Mangravita-Novo,[†] Michael Vicchiarelli,[†] Ying Su,[‡] Stefan Vasile,[†] Layton H. Smith,[†] Paul W. Diaz,[‡] John C. Reed,[‡] and Gregory P. Roth*[,†]

[†]Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, Orlando, Florida 32827

[‡]Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, La Jolla, California 92037

Supporting Information

**ABSTRACT:** NOD1 (nucleotide-binding oligomerization domain 1) protein is a member of the NLR (NACHT and leucine rich repeat domain containing proteins) protein family, which plays a key role in innate immunity as a sensor of specific microbial components derived from bacterial peptidoglycans and induction of inflammatory responses. Mutations in NOD proteins have been associated with various inflammatory diseases that affect NF-κB (nuclear factor κB) activity, a major signaling pathway involved in apoptosis, inflammation, and immune response. A luciferase-based reporter gene assay was utilized in a high-throughput screening program conducted under the NIH-sponsored Molecular Libraries Probe Production Center Network program to identify the active scaffolds. Herein, we report the chemical synthesis, structure−activity relationship studies, downstream counterscreens, secondary assay data, and pharmacological profiling of the 2-aminobenzimidazole lead (compound **1c**, ML130) as a potent and selective inhibitor of NOD1-induced NF-κB activation.



**ML130**

NOD1 $IC_{50}$ = 0.56±0.04 μM
NOD2 $IC_{50}$ = >20μM
TNF-α $IC_{50}$ = >20μM
NOD1 to NOD2 fold Selectivity >36

**KEYWORDS:** NOD1, NF-κB activation, 2-aminobenzimidazole, hit-to-probe, ML130, MLPCN

The mammalian innate immune system serves as a first line of defense and relies on the detection of specific microbial ligands or "pathogen-associated molecular patterns" (PAMPs) by pattern recognition molecules (PRMs), acting as microbial sensors.[1−7] In this context, among the major classes of PRMs is the NLR (NACHT and leucine rich repeat domain containing proteins) family, which has emerged as a key player in innate immunity responses. The NLR family comprises a large number of proteins from both vertebrate and invertebrate animal species, with >20 human proteins recognized.[8−15] It has been shown that some NLR proteins detect bacterial cell wall components such as lipopolysaccharides and/or peptidoglycan (NOD1 or -2) as well as bacterial flagellin (IPAF, NAIP).[16−19] Indeed, NOD2 is a general bacterial sensor that participates in the innate immunity against Gram-positive bacteria (*Streptococcus pneumoniae* and *Listeria monocytogenes*), Gram-negative bacteria (*Salmonella typhimurium*), and mycobacteria (*Mycobacterium tuberculosis*), while NOD1 recognizes primarily Gram-negative bacteria (*Escherichia coli*, *Chlamydia*, and *Helicobacter pylori*). NOD1 and 2 have also been involved in the induction of NF-κB activation, caspase-1 activation, and apoptosis.[20−22] Mutations in NOD genes are also associated with a number of human inflammatory diseases such as Crohn's disease (CD) and Blau syndrome.[23−28] Hence, NLR proteins present an interesting avenue for the discovery and development of novel therapeutics for autoimmune and inflammatory diseases.[29,30] Our efforts toward the discovery and optimization of a 2-aminobenzimidazole scaffold-based inhibitor that specifically inhibits NOD1 induced NF-κB activation are described.

A library of ∼290,000 compounds from the NIH Molecular Libraries Small Molecule Repository compound collection (MLSMR)[31] was evaluated using a cell-based NF-κB driven luciferase reporter gene activity as a measure of NOD1 modulation. The goal was to identify compounds (chemical probes) that inhibited NOD1-mediated NF-κB activation with an $IC_{50}$ of ≤1 μM and with dose−response Hill slopes between 0.5 and 1.4. The hit validation criteria also included 10-fold inhibition selectivity over NOD2 and a 5-fold target-based selectivity over TNF-α (tumor necrosis factor α)-mediated NF-κB activation. The compounds satisfying the above-mentioned criteria were further subjected to a series of counterscreen assays including an Alamar blue cytotoxicity filter (AID1849).[32] Because multiple cellular stimuli, acting through various pathways, can lead to NF-κB activation, the TNF-α assay was designed to identify hits specific to TNF-α modulated pathways (hence, non-NOD modulated). Secondary assays were also performed to confirm that these compounds (a) inhibit interleukin-8 (IL-8) secretion, which is a biologically relevant downstream target of NOD1-stimulated signaling pathway (AID2250), and (b) selectively inhibit NOD1-dependent NF-κB activation in other cell lines (AID2264).[32] Ultimately, the high-throughput screening (HTS) campaign resulted in the

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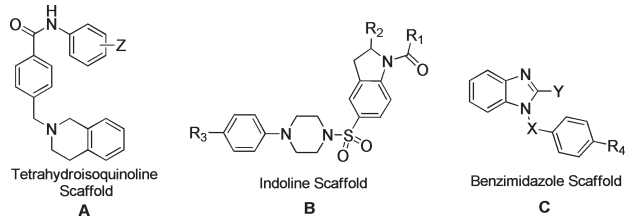


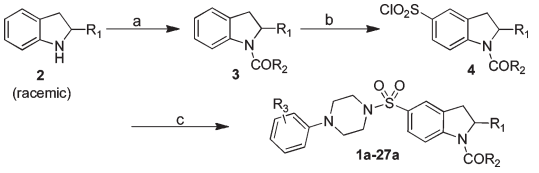

**Figure 1.** Three scaffolds ($IC_{50} \leq 10\ \mu M$) identified in the HTS campaign.

identification of three different scaffolds, namely, tetrahydroisoquinoline (A), indoline (B), and benzimidazole (C) scaffolds that met the initial confirmatory screening criteria of inhibition of NOD1-mediated NF-κB activation with $IC_{50}$ of ≤10 μM (Figure 1). Of these three compound classes, only compounds represented by scaffold C fulfilled the final probe criteria of activity (<1 μM) and selective inhibition of NOD1-induced NF-κB activation. Details of the comprehensive screening platform and pathway specific selectivity analysis are described in a separate publication.[33]

After confirmation of the initial results, structure−activity relationship (SAR) development was initiated for the indoline scaffold using both an "analogue-by-catalog" approach and synthetic efforts. This class of compounds was prepared via acylation of the starting indoline (**2**) with select acid chlorides in the presence of pyridine (Table 1). Generation of the sulfonyl chloride (**4**) was achieved by a sequential treatment of the corresponding indoline derivative with chlorosulfuric acid in the presence of phosphorus pentachloride.[34,35] The resulting indoline sulfonyl chloride (**4**) was reacted with various substituted *N*-phenylpiperazines, under basic conditions, to furnish the final products (**1a**−**27a**, Table 1) in high yields (85−95%).

The indoline scaffold was modified at three key positions annotated as $R_1$−$R_3$ (Table 1). In the first set of compounds ($R_1$ = Me, **1a**−**7a**, **9a**-**16a**, Table 1), analogues were synthesized to test the effect of the size of the aliphatic group at $R_2$ (Me vs Et vs cyclopropyl) and the stereoelectronic influence of the different groups at $R_3$—electron-deficient [Cl, $NO_2$, C(O)Me, and CN] vs electron-rich or neutral groups (OMe and Me). Subsequently, similar analogues with $R_1$ being H were prepared to study the effect of the indoline substitution. It was observed that the bioactivity of these compounds (**1a**−**27a**) is dependent on the nature of the substituent $R_1$. Compound **4a** with $R_1$ = Me exhibited an $IC_{50}$ = 1.87 ± 0.41 μM, but replacing the methyl group in compound **4a** with hydrogen (**23a**, Table 1) resulted in complete loss of activity. A similar trend was observed with another set of compounds (**5a** vs **18a**, **7a** vs **20a**, and **12a** vs **21a**, Table 1). The effect of substituent $R_2$ on the activity of these compounds depends on the electronic nature of $R_3$. For example, if $R_3$ = OMe (electron-donating group), the cyclopropyl analogue (**2a**, Table 1) shows superior activity as compared to the corresponding methyl analogue (**15a**, Table 1). On the other hand, if $R_3$ = C(O)Me (electron-withdrawing group), the cyclopropyl analogue (**7a**, Table 1) is less active than the corresponding methyl analogue (**6a**, Table 1). The nature of substituent $R_3$ also influenced the activity of these compounds. Having $R_3$ as an electron-withdrawing group such as $NO_2$ (**9a**, Table 1) and C(O)Me (**6a**, Table 1) improved the activity of these compounds. Replacing $R_3$ with neutral or electron-donating substituents decreases the NOD1 inhibitory activity (**9a** vs **14a** and **15a**, Table 1). The data shown in Table 1 also indicate that the compounds belonging to indoline scaffold are generally

**Table 1. SAR Analysis of the Indoline Scaffold**[a]




| | | | | | $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|---|
| entry[b] | $R_1$ | $R_2$ | $R_3$ | *n* | NOD1 | NOD2 | TNF-α |
| **1a** | Me | Me | Cl | 3 | 2.57 ± 0.40 | 1.75 ± 0.07 | 5.76 ± 1.50 |
| **2a** | Me | cyclo-propyl | OMe | 2 | 0.51 ± 0.15 | 0.39 ± 0.01 | 3.85 ± 1.50 |
| **3a** | Me | cyclo-propyl | H | 2 | >20 | 17.4 ± 1.83 | 15.3 ± 0.46 |
| **4a** | Me | Et | $NO_2$ | 3 | 1.87 ± 0.41 | 1.55 ± 0.28 | 4.92 ± 1.52 |
| **5a** | Me | cyclo-propyl | Cl | 3 | 5.6 ± 1.12 | 3.63 ± 0.58 | 7.26 ± 1.6 |
| **6a** | Me | Me | COMe | 3 | 4.42 ± 0.48 | 3.72 ± 0.21 | 6.0 ± 0.91 |
| **7a** | Me | cyclo-propyl | COMe | 5 | 10.26 ± 3.51 | 7.90 ± 1.6 | >20 |
| **8a** | H | Et | Cl | 2 | >20 | >20 | >20 |
| **9a** | Me | Me | $NO_2$ | 3 | 1.7 ± 0.28 | 1.41 ± 0.16 | 3.27 ± 0.58 |
| **10a** | Me | Et | Cl | 2 | 1.01 ± 0.23 | 0.6 ± 0.12 | 0.58 ± 0.03 |
| **11a** | Me | Et | COMe | 2 | 2.0 ± 0.04 | 1.58 ± 0.11 | 1.63 ± 0.23 |
| **12a** | Me | cyclo-propyl | $NO_2$ | 2 | 0.26 ± 0.05 | 0.20 ± 0.02 | 0.24 ± 0.01 |
| **13a** | Me | Me | H | 2 | 11.15 ± 0.39 | 8.27 ± 0.06 | 14.1 ± 1.41 |
| **14a** | Me | Me | Me | 2 | 5.76 ± 0.57 | 3.45 ± 0.06 | 4.32 ± 0.05 |
| **15a** | Me | Me | OMe | 2 | 3.63 ± 0.11 | 3.29 ± 0.25 | 4.3 ± 0.27 |
| **16a** | Me | cyclo-propyl | Me | 2 | >20 | >20 | >20 |
| **17a** | H | Et | CN | 2 | >20 | >20 | >20 |
| **18a** | H | cyclo-propyl | Cl | 2 | >20 | >20 | >20 |
| **19a** | H | cyclo-propyl | CN | 2 | >20 | >20 | >20 |
| **20a** | H | cyclo-propyl | COMe | 2 | >20 | >20 | >20 |
| **21a** | H | cyclo-propyl | $NO_2$ | 2 | 6.5 ± 0.27 | 8.45 ± 0.14 | >20 |
| **22a** | H | Et | COMe | 2 | >20 | >20 | >20 |
| **23a** | H | Et | $NO_2$ | 2 | >20 | >20 | >20 |
| **24a** | H | Me | CN | 2 | 5.87 ± 0.14 | 9.24 ± 0.23 | 8.79 ± 0.55 |
| **25a** | H | Me | COMe | 2 | 17.4 ± 1.69 | >20 | >20 |
| **26a** | H | Me | $NO_2$ | 2 | >20 | >20 | >20 |
| **27a** | H | Me | Cl | 2 | >20 | >20 | >20 |

[a] All structures were inactive in an Alamar blue cytoxicity assay (0% activity at 20 μM). (a) $R_2COCl$, pyridine, room temperature, 12 h, ∼80−90%. (b) $ClSO_3H$, $PCl_5$, 0 °C, 75−95%. (c) N-phenylpiperidine, $Et_3N$, room temperature, 6 h, 85−95%. [b] The racemic mixture of compounds (not the individual enantiomers) was subjected to the biological assays.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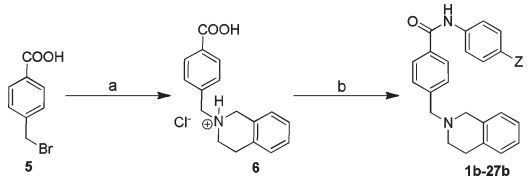
**Scheme 1. Synthesis of the Tetrahydroisoquinoline Scaffold and Its Analogues 1b−26b**[a]



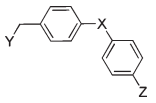

[a] (a) (i) Two equivalents of tetrahydroisoquinoline, THF, reflux, 12 h, 80−85%; (ii) aqueous NaOH; (iii) 10% HCl. (b) Anilines, HBTU, DIPEA, DMF, room temperature, 12 h, 55−75%.

nonselective toward NOD1 and NOD2 and also have an inhibitory activity in the TNF-α assay, which suggests that these analogues act via a nonselective mechanism. These observations, along with the poor pH 7.4 buffer solubility of the series (~0.1 μg/mL), guided our decision to cease pursuit of this scaffold for probe generation.

The tetrahydroisoquinoline scaffold can be obtained via alkylation of the 4-bromomethyl benzoic acid **5** using various secondary amines.[36] The corresponding amine was isolated as a hydrochloride salt **6**, which was coupled with various anilines using standard coupling conditions (HBTU/DIPEA) to yield the final products **1b**−**27b** (Scheme 1). It was observed that the bioactivity of compounds belonging to tetrahydroisoquinoline series is highly dependent on the nature of the substituent Y. Having Y as a tetrahydroisoquinoline moiety (**1b**−**5b**, Table 2) is pertinent for the activity. Replacement of Y with other *N*-heterocycles such as piperidine (**6b**, **7b**, **10b**, **12b**, and **15b**, Table 2) or indoline (**8b**, **9b**, **11b**, **14b**, and **17b**, Table 2) resulted in complete loss of activity. Also, increasing the carbon chain length resulted in loss of activity (**4b** vs **19b** and **3b** vs **20b**, Table 2). Substituent "X" as the −C(O)NH− group is imperative as reversal of the amide group [replacement by −NHC(O)−] results in loss of the NOD1/2 and TNF-α inhibitory activity (**2b** vs **26b** and **4b** vs **22b**). Varying the type of substituent Z shows that the electron-donating substituents provided the more active compounds from the series (**1b**, **4b**, **13b**, and **16b** vs **5b**, **21b**, Table 2). The data shown in Table 2 also indicate the nonselective nature of the tetrahydroisoquinoline scaffold toward inhibition of NOD1- and NOD2-mediated NF-κB activation. This series also exhibit inhibitory activity in the TNF-α assay, which indicates that these analogues may act via a nonselective mechanism, similar to the indoline scaffold (Table 1).

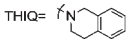
The 2-aminobenzimidazole scaffold (C) was also explored for optimization and SAR development (Table 3). Commercially available 2-aminobenzimidazole **7** was treated with various sulfonyl chlorides, in the presence of pyridine, to obtain the corresponding sulfonamides.[37] The nature of substituent Y and linker X also influenced the activity of the 2-aminobenzimidazole analogues (Table 3). The presence of substituent Y as the $NH_2$ group on the benzimidazole ring is imperative as replacement renders the compound inactive (**6**−**8c**, Table 3). For example, the absence of the $NH_2$ group in compound **6c** decreases NOD1 inhibitory activity by 10-fold. Replacement of $NH_2$ group with a methyl (**7c**) or SH group (**8c**) results in loss of activity ($IC_{50}$ > 20 μM). Replacing the substitutions $R_1$ and $R_2$ with methyl groups instead of hydrogen also jeopardizes the bioactivity (**8**−**9c**, Table 3). Varying the type of substituent X shows that the sulfonyl group provides the most active compound from the series (**1c** vs **10**−**15c**, Table 3). Compound **10c** with a methylene linker or compound **13c** with an ethoxy linker exhibited a

**Table 2. SAR Analysis of the Tetrahydroisoquinoline Scaffold**[a]




| Entry | Z | X | Y | n | NOD1 $IC_{50}$ (μM) | NOD 2 $IC_{50}$ (μM) | TNF-α $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| **1b** | Me | CONH | THIQ | 2 | 3.44±1.10 | >20 | >20 |
| **2b** | Cl | CONH | THIQ | 2 | 0.83±0.09 | 3.54±0.41 | 0.49±0.21 |
| **3b** | COOEt | CONH | THIQ | 2 | 0.15±0.01 | 0.40±0.02 | 0.14±0.01 |
| **4b** | OEt | CONH | THIQ | 3 | 1.13±0.26 | 0.57±0.17 | 2.05±0.39 |
| **5b** | F | CONH | THIQ | 3 | 3.11±0.65 | 1.85±0.32 | 2.88±0.75 |
| **6b** | F | CONH | Piperidinyl | 3 | >20 | >20 | >20 |
| **7b** | OEt | CONH | Piperidinyl | 3 | >20 | >20 | >20 |
| **8b** | OEt | CONH | Indolinyl | 3 | >20 | >20 | >20 |
| **9b** | F | CONH | Indolinyl | 3 | >20 | >20 | >20 |
| **10b** | OMe | CONH | Piperidinyl | 2 | >20 | >20 | >20 |
| **11b** | OMe | CONH | Indolinyl | 2 | >20 | >20 | >20 |
| **12b** | SMe | CONH | Piperidinyl | 3 | >20 | >20 | >20 |
| **13b** | SMe | CONH | THIQ | 3 | 0.24±0.08 | 0.26±0.02 | 0.37±0.16 |
| **14b** | SMe | CONH | Indolinyl | 2 | >20 | >20 | >20 |
| **15b** | $NMe_2$ | CONH | Piperidinyl | 2 | >20 | >20 | >20 |
| **16b** | $NMe_2$ | CONH | THIQ | 2 | 1.61±0.26 | 3.58±0.13 | 1.83±0.66 |
| **17b** | $NMe_2$ | CONH | Indolinyl | 3 | >20 | >20 | >20 |
| **18b** | $OCF_3$ | CONH | THIQ | 4 | 0.08±0.01 | 0.03±0.01 | 0.03±0.01 |
| **19b** | OEt | CONH |  | 2 | >20 | >20 | >20 |
| **20b** | COOEt | CONH | | 2 | 10.6±0.97 | >20 | 10.77±1.15 |
| **21b** | $SO_3H$ | CONH | THIQ | 2 | >20 | >20 | >20 |
| **22b** | OEt | NHCO | THIQ | 2 | >20 | >20 | >20 |
| **23b** | OMe | CONH | THIQ | 2 | 0.51±0.01 | 0.78±0.02 | 0.65±0.15 |
| **24b** | Br | CONH | THIQ | 2 | 1.03±0.14 | 1.62 | 1.50±0.17 |
| **25b** | Cl | CONH |  | 2 | >20 | >20 | >20 |
| **26b** | Cl | NHCO | THIQ | 2 | >20 | >20 | >20 |

[a] All structures were inactive in an Alamar blue cytoxicity assay (0% activity at 20 μM).

THIQ= 

reduction in activity (15-fold) or loss of activity. Compound **11c** with a carbonyl linker results in loss of selectivity, showing similar activity in NOD1 (2.8 μM) and NOD2 (3.8 μM) assays. Also, changing the linker to $-CH_2CO$ (**14c**) or $-COCH_2$ (**15c**) resulted in loss of activity. It was observed that the bioactivity of **1c** and its analogues is highly dependent on the nature of the substituent $R_3$ (Table 3). The absence of the 4-substituent (**4c**) or presence of other substituents such as 4-OMe (**3c**) or 4-$NO_2$ (**5c**) reduces the compound potency by 4−20-fold.

In summary, compound **1c** selectively (>36-fold) inhibits NOD1-dependent activation of NF-κB as ascertained through γ-tri-DAP-stimulated luciferase signaling in a NF-κB-linked reporter assay (AID2333) in HEK293T cells containing endogenous NOD1 levels with submicromolar potency ($IC_{50}$ = 0.56 ± 0.04 μM), while not inhibiting either MDP-stimulated (NOD2-dependent, AID2334) signaling in reporter cell lines containing both endogenous and overexpressed NOD2 proteins or TNF-α-induced NF-κB activation (AID2337) (Table 3). Furthermore, compound **1c** also shows selective inhibition of NOD1-(γ-tri-DAP)-dependent IL-8 secretion but neither NOD2-dependent nor TNF-α-dependent IL-8 secretion in biologically relevant MCF-7 cells (Figure 1 and Table 1 in the Supporting Information). Compound **1c** also showed selective NOD1-dependent inhibition of NF-κB activation via NOD1 (DAP, AID2264) modulation but did not inhibit NF-κB activation via PMA/ionomycin (AID2261) and doxorubicin


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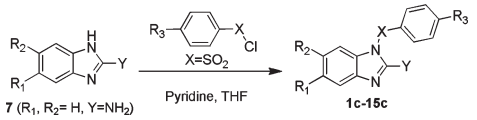
**Table 3. SAR Analysis of the 2-Amino Benzimidazole Scaffold[a]**




| | | | | | | | $IC_{50}$ ($\mu$M) | | |
|---|---|---|---|---|---|---|---|---|---|
| entry | $R_1$ | $R_2$ | $R_3$ | X | Y | *n* | NOD1 | NOD2 | TNF-α (NOD1-dependent) |
| **1c** | H | H | Me | $SO_2$ | $NH_2$ | 6 | 0.56 ± 0.04 | >20 | >20 |
| **2c** | H | H | Cl | $SO_2$ | $NH_2$ | 2 | 0.09 ± 0.01 | 19.9 ± 0.15 | >20 |
| **3c** | H | H | OMe | $SO_2$ | $NH_2$ | 4 | 2.7 ± 0.69 | >20 | >20 |
| **4c** | H | H | H | $SO_2$ | $NH_2$ | 4 | 2.2 ± 0.21 | >20 | >20 |
| **5c** | H | H | $NO_2$ | $SO_2$ | $NH_2$ | 6 | 14.0 ± 1.8 | >20 | >20 |
| **6c** | H | H | Me | $SO_2$ | H | 2 | 6.3 ± 0.81 | >20 | >20 |
| **7c** | H | H | Me | $SO_2$ | Me | 2 | >20 | >20 | >20 |
| **8c** | Me | H | Me | $SO_2$ | SH | 2 | >20 | >20 | >20 |
| **9c** | Me | Me | Me | $SO_2$ | $NH_2$ | 4 | >20 | >20 | >20 |
| **10c** | H | H | Cl | $CH_2$ | $NH_2$ | 2 | 7.7 ± 0.82 | 11.9 ± 0.5 | >20 |
| **11c** | H | H | 2,4-*di*Cl | CO | $NH_2$ | 2 | 2.8 + 0.57 | 3.8 ± 1.5 | 3.2 |
| **12c** | H | H | F | CO | $NH_2$ | 2 | 18.0 ± 2.0 | >20 | >20 |
| **13c** | H | H | Cl | $(CH_2)_2CO$ | $NH_2$ | 2 | 16.3 ± 3.7 | >20 | >20 |
| **14c** | H | H | OMe | $CH_2CO$ | $NH_2$ | 2 | >20 | >20 | >20 |
| **15c** | H | H | H | $COCH_2$ | $NH_2$ | 2 | >20 | >20 | >20 |

[a] All compounds were inactive in an Alamar blue cytoxicity assay (0% activity at 20 $\mu$M).

**Table 4. In Vitro ADME Data for Compound 1c (ML130)**

| solubility ($\mu$g/mL) | permeability[a,b] Pe ($\times 10^{-6}$ cm/s) | plasma protein binding (% bound) | | plasma stability[c] (% remaining) | microsome stability[d] (% remaining) | |
|---|---|---|---|---|---|---|
| pH 5.0/6.2/7.4 | pH 5.0/6.2/7.4 | human 10 $\mu$M/1 $\mu$M | mouse 10 $\mu$M/1 $\mu$M | human/mouse | human | mouse |
| 2/2/2 | 491/562/382 | 97.7/97.5 | 95.5/95.0 | 100/100 | 41.8 | 0.8 |

[a] Compound at 50 $\mu$M. [b] PAMPA Pe: low, $5 \times 10^{-6}$; moderate, $250 \times 10^{-6}$; high, $1000 \times 10^{-6}$. [c] Plasma/PBS; compound at 1 $\mu$M, 3 h. [d] Percent remaining at 1 h.

(AID2255)-induced pathways (Figure 2 in the Supporting Information).

In vitro ADME data for probe compound **1c** indicate low solubility and moderate to high "effective" cell permeability at the three pH levels tested (Table 4). Compound **1c** also exhibits moderate plasma protein binding with excellent stability in both human and mouse plasma. It shows low stability in the presence of mouse microsomes but moderate stability in human microsomes and exhibits $LD_{50}$ >50 $\mu$M toward Fa2N-4 immortalized human hepatocytes. Preliminary in vivo dose−exposure data (using a rapid assessment of compound exposure method, "RACE") were also obtained for compound **1c** in conjunction with quantitative bioanalytical analysis to understand its pharmacokinetics in mice. Mice dosed with compound **1c** (30 and 15 mg/kg, ip) exhibited significant compound exposure at $t$ = 20 min, which rapidly decreased by $t$ = 120 min. Mice dosed with compound **1c** at 30 mg/kg, ip, exhibited a higher initial compound exposure than mice dosed at 15 mg/kg, ip (5.5 vs 1.2 $\mu$g/mL); however, plasma levels at $t$ = 120 min were not improved (<0.5 $\mu$g/mL).

Compound **1c** showed moderate activity (>50% inhibition) when tested at a single concentration (10 $\mu$M) across PDSP panel (NIMH Psychoactive Drug Screening Program) that included 89% H1 (histamine), 92% 5HT6 (serotonin), and 69% 5HT2B receptors (see the Supporting Information, section D). Compound **1c** also exhibited excellent selectivity in Ambit KinomeScreen showing little activity against 443 kinases at a 10 $\mu$M concentration. Inhibitory activity (reported as % control) was observed only for the following kinases: AURK (Aurora kinase) B and C (not A), 21 and 23%, respectively; CFLT3 (Fms-like tyrosine kinase), 27% C; and RIOK2 (right open reading frame), 6% C. Compound **1c** was also found to be noncytotoxic in NCI 60 human tumor cell line anticancer drug screen.

In summary, a new class of 2-aminobenzimidazoles has been identified as potent and selective inhibitors of NOD1-induced NF-kB activation. Compound **1c** has shown selective inhibition of NOD1-induced NF-κB activation in HEK293 cells with no cytotoxicity and was selected as a probe candidate molecule (notated in PubChem as ML130).[38] Compound **1c** was also confirmed in secondary assays by selectively inhibiting NOD1-dependent IL-8 secretion and also selectively inhibiting the NOD1-dependent pathway to NF-κB activation. Thus, ML130 and related analogues provide new chemical tools for development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of pathway selective inhibitors of NF-κB activation. Further work focused on improvement of potency and pharmacological profile of the probe molecule **1c** is underway.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Synthetic procedures, characterization of final products, biological assay protocols and data, and pharmacology profile. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Tel: 407-745-2062. E-mail: groth@sanfordburnham.org.

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## ■ REFERENCES

(1) Akira, S.; Uemastu, S.; Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **2006**, *124*, 783–801.

(2) Dangl, J. L.; Jones, J. D. Plant pathogens and integrated defence responses to infection. *Nature* **2001**, *411*, 826–833.

(3) Medzhitov, R. Toll-like receptors and innate immunity. *Nature Rev. Immunol.* **2001**, *1*, 135–145.

(4) Jones, D. A.; Takemoto, D. Plant innate immunity-direct and indirect recognition of general and specific pathogen-associated molecules. *Curr. Opin. Immunol.* **2004**, *16*, 48–62.

(5) Kumagai, Y.; Takeuchi, O.; Akira, S. Pathogen recognition by innate receptors. *J. Infect. Chemother.* **2008**, *14*, 86–92.

(6) Akira, S. Pathogen recognition by innate immunity and its signaling. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **2009**, *85*, 143–156.

(7) Meylan, E.; Tschopp, J.; Karin, M. Intracellular pattern recognition receptors in the host response. *Nature* **2006**, *442*, 39–44.

(8) Chamaillard, M.; Girardin, S. E.; Viala, J.; Philpott, D. J. Nods, Nalps and Naip: Intracellular regulators of bacterial-induced inflammation. *Cell. Microbiol.* **2003**, *5*, 581–592.

(9) Inohara, N.; Nuñez, G. NODs: Intracellular proteins involved in inflammation and apoptosis. *Nature Rev. Immunol.* **2003**, *3*, 371–382.

(10) Strober, W.; Murray, P. J.; Kitani, A.; Watanabe, T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nature Rev. Immunol.* **2006**, *6*, 9–20.

(11) Inohara, N.; Nuñez, G. The NOD: A signaling module that regulates apoptosis and host defense against pathogens. *Oncogene* **2001**, *20*, 6473–6481.

(12) Inohara, N.; Ogura, Y.; Nuñez, G. Nods: A family of cytosolic proteins that regulate the host response to pathogens. *Curr. Opin. Immunol.* **2002**, *5*, 76–80.

(13) Kawai, T.; Akira, S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int. Immunol.* **2009**, *21*, 317–337.

(14) Rietdijk, S. T.; Burwell, T.; Bertin, J.; Coyle, A. J. Sensing intracellular pathogens-NOD-like receptors. *Curr. Opin. Pharmacol.* **2008**, *8*, 261–266.

(15) Murray, P. J. NOD proteins: An intracellular pathogen-recognition system or signal transduction modifiers? *Curr Opin. Immunol.* **2005**, *17*, 352–358.

(16) Girardin, S. E.; Boneca, I. G.; Viala, J.; Chamaillard, M.; Labigne, A.; Thomas, G.; Philpott, D. J.; Sansonetti, P. J. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J. Biol. Chem.* **2003**, *278*, 8869–8872.

(17) Chamaillard, M.; Hashimoto, M.; Horie, Y.; Masumoto, J.; Qiu, S.; Saab, L.; Ogura, Y.; Kawasaki, A.; Fukase, K.; Kusumoto, S.; Valvano, M. A.; Foster, S. J.; Mak, T. W.; Nuñez, G.; Inohara, N. An essential role for Nod1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat. Immunol.* **2003**, *4*, 702–707.

(18) Uehara, A.; Fujimoto, Y.; Kawasaki, A.; Kusumoto, S.; Fukase, K.; Takada, H. Meso-Diaminopimelic Acid and Meso-Lanthionine, Amino Acids Specific to Bacterial Peptidoglycans, Activate Human Epithelial Cells through NOD1. *J. Immunol.* **2006**, *177*, 1796–1804.

(19) Girardin, S. E.; Travassos, L. H.; Herve, M.; Blanot, D.; Boneca, I. G.; Philpott, D. J.; Sansonetti, P. J.; Mengin-Lecreulx, D. Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J. Biol. Chem.* **2003**, *278*, 41702–41708.

(20) Franchi, L.; Amer, A.; Body-Malapel, M.; Kanneganti, T. D.; Ozoren, N.; Jagirdar, R.; Inohara, N.; Vandenabeele, P.; Bertin, J.; Coyle, A.; Grant, E. P.; Nuñez, G. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat. Immunol.* **2006**, *7*, 576–582.

(21) Miao, E. A.; Alpuche-Aranda, C. M.; Dors, M.; Clark, A. E.; Bader, M. W. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin-1 beta via Ipaf. *Nat. Immunol.* **2006**, *7*, 569–575.

(22) Kanazawa, N.; Okafuji, I.; Kambe, N.; Nishikomori, R.; Nakata-Hizume, M.; Nagai, S.; Fuji, A.; Yuasa, T.; Manki, A.; Sakurai, Y.; Nakajima, M.; Kobayashi, H.; Fujiwara, I.; Tsutsumi, H.; Utani, A.; Nishigori, C.; Heike, T.; Nakahata, T.; Miyachi, Y. Early-onset sarcoidosis and CARD15 mutations with constitutive nuclear factor-κB activation: common genetic etiology with Blau syndrome. *Blood* **2005**, *105*, 1195–1197.

(23) Carneiro, L. A.; Travassos, L. H.; Girardin, S. E. Nod-like receptors in innate immunity and inflammatory diseases. *Ann. Med.* **2007**, *39*, 581–593.

(24) Inohara, N.; Ogura, Y.; Fontalba, A.; Gutierrez, O.; Pons, F.; Crespo, J.; Fukase, K.; Inamura, S.; Kusumoto, S.; Hashimoto, M.; Foster, S. J.; Moran, A. P.; Fernandez-Luna, J. L.; Nuñez, G. Host recognition of bacterial muramyl dipeptide mediated through NOD2: Implications for Crohn's disease. *J. Biol. Chem.* **2003**, *278*, 5509–5512.

(25) Hugot, J. P.; Chamaillard, M.; Zouali, H.; Lesage, S.; Cezard, J. P.; Belaiche, J.; Almer, S.; Tysk, C.; O'Morain, C. A.; Gassull, M.; Binder, V.; Finkel, Y.; Cortot, A.; Modigliani, R.; Laurent-Puig, P.; Gower-Rousseau, C.; Macry, J.; Colombel, J. F.; Sahbatou, M.; Thomas, G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **2001**, *411*, 599–603.

(26) Ogura, Y.; Bonen, D. K.; Inohara, N.; Nicolae, D. L.; Chen, F. F.; Ramos, R.; Britton, H.; Moran, T.; Karaliuskas, R.; Duerr, R, H.; Achkar, J.-P.; Brant, S. R.; Bayless, T. M.; Kirschner, B. S.; Hanauer, S. B.; Nuñez, G.; Cho, J. H. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **2001**, *411*, 603–606.

(27) Miceli-Richard, C.; Lesage, S.; Rybojad, M.; Prieur, A. M.; Manouvrier-Hanu, S.; Hafner, R.; Chamaillard, M.; Zouali, H.; Thomas, G.; Hugot, J. P. CARD15 mutations in Blau syndrome. *Nat. Genet.* **2001**, *29*, 19–20.

(28) Maeda, S.; Hsu, L. C.; Liu, H.; Bankston, L. A.; Iimura, M.; Kagnoff, M. F.; Eckmann, L.; Karin, M. Nod2 mutation in Crohn's disease potentiates NF-kappa B activity and IL-1beta processing. *Science* **2005**, *307*, 734–738.

(29) Ulevitch, R. J. Therapeutics targeting the innate immune system. *Nat Rev. Immunol.* **2004**, *4*, 512–520.

(30) Pfeifer, A.; Verma, I. M. Gene therapy: promises and problems. *Annu. Rev. Genomics Hum. Genet.* **2001**, *2*, 177–211.

(31) http://mlsmr.glpg.com/MLSMR_HomePage.

(32) For assay details, see the Supporting Information and PubChem link to AIDs.

(33) Correa, R. G.; Khan, P. M.; Askari, N.; Zhai, D.; Gerlic, M.; Brown, B.; Magnuson, G.; Spreafico, R.; Albani, S.; Sergienko, E.; Diaz, P. W.; Roth, G. P.; Reed, J. C. Discovery and characterization of 2-aminobenzimidazole derivatives as selective NOD1 inhibitors. *Chem. Biol.* **2011**, *18*, 825–832.

(34) Shalygina, E. E.; Kobylinskii, D. V.; Ivanovskii, S. A.; Balakin, K. V.; Dorogov, M. V.; Toporova, T. A. Synthesis and properties of

1-acylindolinesulfonamides. *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.* **2004**, *47*, 91–96.

(35) Guan, H.; Laird, A. D.; Blake, R. A.; Tang, C.; Liang, C. Design and synthesis of aminopropyl tetrahydroindole-based indolin-2-ones as selective and potent inhibitors of Src and Yes tyrosine kinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 187–190.

(36) Lombardino, J. G. 4-(4-Substituted benzoyloxy)-N-substituted-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxides having antiarthritic activity. U.S. Patent 514,212,000, May 27, 1986.

(37) Benvenuti, S.; Severi, F.; Vampa, G.; Malmusi, L. Synthesis, characterization, and crystallographic analysis of some benzimidazole derivatives. *J. Heterocycl. Chem.* **1995**, *32*, 1613–1619.

(38) Magnuson, G.; Khan, P.; Yuan, H.; Brown, B.; Divlianska, D. B.; Stonich, D.; Peddibhotla, S.; Su, Y.; Dad, S.; Sergienko, E.; Chung, T. D. Y.; Roth, G. P.; Wimer, C.; Diaz, P.; Correa, R. G.; Reed, J. C. High Throughput Screening Assays for NOD1 Inhibitors. 2010 Feb 28 [updated 2010 Oct 4]. Probe Reports from the NIH Molecular Libraries Program [Internet]; National Center for Biotechnology Information (US): Bethesda, MD, 2010. Available from http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mlprobe&part=ml130; PubMed PMID: 21433376.