Potent and Selective Amidopyrazole Inhibitors of IRAK4 That Are Efficacious in a Rodent Model of Inflammation

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Ⓢ Supporting Information

ABSTRACT: IRAK4 is a critical upstream kinase in the IL-1R/TLR signaling pathway. Inhibition of IRAK4 is hypothesized to be beneficial in the treatment of autoimmune related disorders. A screening campaign identified a pyrazole class of IRAK4 inhibitors that were determined by X-ray crystallography to exhibit an unusual binding mode. SAR efforts focused on the identification of a potent and selective inhibitor with good aqueous solubility and rodent pharmacokinetics. Pyrazole C-3 piperidines were well tolerated, with N-sulfonyl analogues generally having good rodent oral exposure but poor solubility. N-Alkyl piperidines exhibited excellent solubility and reduced exposure. Pyrazoles possessing N-1 pyridine and fluorophenyl substituents were among the most active. Piperazine **32** was a potent enzyme inhibitor with good cellular activity. Compound **32** reduced the *in vivo* production of proinflammatory cytokines and was orally efficacious in a mouse antibody induced arthritis disease model of inflammation.

IRAK4 IC$_{50}$ = 690 nM

IRAK4 IC$_{50}$ = 5 nM

KEYWORDS: Interleukin-1 receptor-associated kinase 4, inflammation, drug discovery, SAR, structure-based drug design
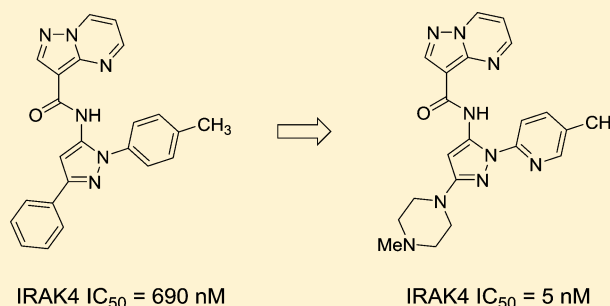
Inflammation, the response by an organism to nonself molecules, pathogens, cellular damage, or cellular debris, is highly controlled in mammalian biology. The dysregulation of this process is the underlying cause of autoimmune disorders, including rheumatoid arthritis and inflammatory bowel disease, which afflict millions worldwide.[1,2] Chronic inflammation furthermore plays a role in numerous other disease states including cancer[3] and cardiovascular disease.[4] While a number of orally administered anti-inflammatory and analgesic agents are available to address the symptoms of inflammation, most of the current medicines that modify the autoimmune disease state itself are biologics.[5] These therapies are generally efficacious, but are expensive and require IV administration. The development of an orally available agent to treat autoimmune disorders remains an area of intense investigation.[6]

The interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) inflammation signaling pathway is an attractive target for therapeutic intervention in autoimmune disease.[7] Members of the IL-1R/TLR superfamily of proteins recognize foreign pathogens and endogenous inflammation signaling agents, and are key upstream drivers of inflammation. These proteins all possess a conserved intracellular Toll/interleukin-1R (TIR) domain. Upon ligand binding to the receptor, the TIR domain recruits the scaffolding protein myeloid differentiation primary response gene 88 (MYD88). The resulting complex activates interleukin-1 receptor-associated kinase 4 (IRAK4).[8] IRAK4 initiates a signaling cascade terminating in activation of the transcription factors activator protein-1 (AP-1) and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), leading to the production of proinflammatory signaling agents including tumor necrosis factor α (TNFα) and interleukin-1 (IL-1).[9]

The role of IRAK4 in innate immunity is supported by mouse knockout studies.[10] Importantly, cells derived from a small human population that lack the IRAK4 protein do not respond to ligands known to activate the TIR pathway.[11] The genetic association of IRAK4 with inflammation and the fact that it is the most proximal kinase to the IL-1R/TLR family render it an attractive target for drug discovery. A number of organizations have described IRAK4 inhibitor programs, although the poor kinase selectivity associated with many of these chemotypes limits a full understanding of their *in vivo* efficacies.[12−15] We report herein the discovery of a novel, highly selective IRAK4 inhibitor chemotype and the identification of a compound, which reduces the *in vivo* production of TLR2-induced cytokines and is orally efficacious in a rodent model of inflammation.

A screening campaign identified pyrazoles **1** and **2** as modest IRAK4 kinase inhibitors (IC$_{50}$ values = 2.2 and 0.69 μM, respectively, Figure 1). Interest in this series was initially based
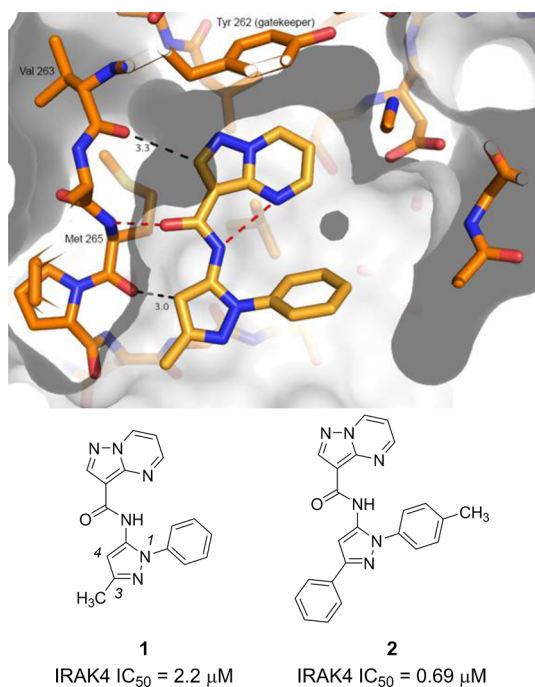

Figure 1. Pyrazoles **1** and **2** and X-ray cocrystal structure of **1** with IRAK4. Not shown on this picture is the interaction with the peptide bond between Met192 and Gly193, which lies above the top clipping plane.

on the unusual binding mode of **1** as determined by X-ray crystallography. Three H-bonds between **1** and the kinase hinge were observed, with one ring proton from each pyrazole ring functioning as hydrogen bond donors and the amide carbonyl of **1** acting as a hydrogen bond acceptor. An intramolecular hydrogen bond between the inhibitor amide N−H and pyrimidine nitrogen stabilizes the pyrazolopyrimidine moiety into a planar conformation that allows for a favorable slanted face-to-edge π−π interaction with the gatekeeper Tyr262 phenol. The pyrazole phenyl substituent is positioned against the peptide bond between Met192 and Gly193, at the N-terminal end of a glycine rich loop of the protein, and is oriented at about 55° relative to the pyrazole ring. Finally, the pyrazole C-3 methyl group points toward the solvent exposed region.

The observation that inhibitor **1** bound to the IRAK4 hinge without a typical kinase binding motif[16] such as 2-aminopyrimidine or benzimidazole led us to suspect that analogues in this series may exhibit high selectivity for IRAK4, although a more potent inhibitor would be required in order to accurately interpret selectivity data. With this assumption, an SAR program was undertaken with the goal of identifying an analogue that could be used in a rodent model of inflammation proof-of-concept study. Such a compound should be a potent (<10 nM) enzyme inhibitor with excellent kinase selectivity. Additionally, the analogue sought should possess both oral rodent exposure and good aqueous solubility, which would allow for dosing using various routes of administration.

The general synthesis depicted in Scheme 1, using analogue **6** as an example, was employed for most of the pyrazoles in this

Scheme 1^a
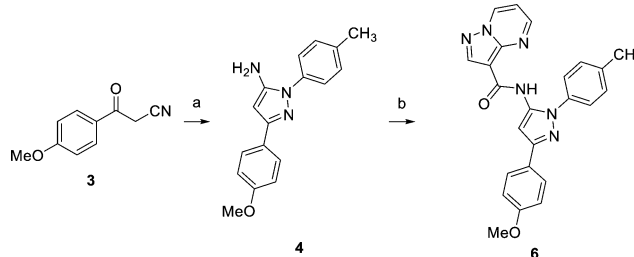
^{a}Reagents and conditions: (a) *p*-tolylhydrazine, montmorillonite K-10, *i*-PrOH, Δ, 75%; (b) pyrazolo[1,5-*a*]pyrimidine-3-carbonyl chloride (**5**), DIPEA, CH$_2$Cl$_2$, 28%.

Letter.[17] α-Ketonitrile **3** was condensed with 4-methylphenylhydrazine to form **4**.[18] Aminopyrazole **4** was allowed to react with acid chloride **5** to give **6**. The analogous reaction between **4** and a carboxylic acid using peptide coupling agents was unsuccessful as only starting materials were recovered even under forcing conditions. In instances where the starting ketonitriles were not commercially available, they were prepared from their corresponding esters and the anion derived from acetonitrile.
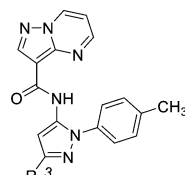
Initial SAR studies focused on the pyrazole C-3 position (Table 1). In addition to IRAK4 inhibition, kinetic aqueous solubility at pH 7 was measured for most analogues. Rat oral exposure was determined in cassette mode for select inhibitors. In these studies,[19] Sprague−Dawley rats were dosed p.o. at 10 mg/kg, in 20% HPbCD, with drug plasma concentrations measured at multiple time points and AUC values from 0 to 6 h calculated. The pyrazole C-3 phenyl analogue **2** was chosen as a starting point for SAR development. Since the C-3 substituent points toward solvent in the X-ray structure, it was hypothesized that the incorporation of polar groups on the phenyl ring may improve enzyme activity through desolvation. This was generally found to be true. The addition of a *para*-methoxyphenyl substituent (**6**) resulted in an approximately 4-fold increase in potency relative to **2**. Inhibitor **6** was also assessed for rat pharmacokinetics (PK) and kinase selectivity. Although **6** exhibited no oral exposure, it was found to be very selective: against a panel of 108 kinases, >80% inhibition was observed for only 2 kinases (IRAK4 and MINK1) at 10 μM. This confirmed the expectation that analogues in this series are highly selective.

The dimethoxyphenyl analogue **7** exhibited excellent IRAK4 inhibition (IC$_{50}$ = 44 nM). As with **6**, compound **7** had no measurable plasma exposure in rat. The poor PK was investigated using metabolite identification studies, whereby analogue **7** was incubated with rat hepatocytes. Monooxidation was found to be the dominant route of metabolism, with the oxidation occurring on the tolyl, pyrazole, or dimethoxyphenyl ring. Although this study did not conclusively identify the site of metabolism, it was suspected that the oxidation may be occurring at the electron rich dimethoxyphenyl ring.

Replacement of the methoxyphenyl ring with more electron deficient aromatic systems was explored next. The 4-pyridyl analogue **8** maintained good IRAK4 activity and selectivity (6/108 kinases, >80% inhibition at 10 μM). However, no improvement in plasma exposure was observed.

Analogue **9** was designed with the expectation that the addition of the methoxy group would both improve IRAK4 activity, in a similar manner as seen with the phenyl analogues,

Table 1. SAR Studies at Pyrazole C-3 Substituent

R, compound		IRAK-4 IC$_{50}$ (nM)	solubility (pH 7, μM)	rat PK AUC (0-6 h, μM.h)
(phenyl)	2	690	< 3	N/A
MeO (phenyl)	6	170	< 2	0
MeO OMe (phenyl)	7	44	< 2	0
(pyridyl N)	8	61	< 2	0
N OMe (pyridyl)	9	390	< 2	1.39
N (pyridyl)	10	99	< 2	0.44
MeO N (pyridyl)	11	310	3	N/A
(cyclopropyl)	12	370	99	2.37
(cyclohexyl)	13	2400	< 2	N/A
O (THP)	14	410	24	0.45
HN (piperidine)	15	80	172	0
MeN (piperidine)	16	150	165	N/A
i-PrN (piperidine)	17	47	170	0.28
AcN (piperidine)	18	180	163	0.71
O N NH$_2$ (urea piperidine)	19	220	N/A	N/A
MeO$_2$SN (piperidine)	20	67	< 2	19.27

and reduce any oxidative metabolism of the pyridine nitrogen and increase rat PK. Although some loss in activity was observed, it was significant that **9** became the first compound in this chemotype to exhibit any rat exposure. The regioisomeric pyridines **10** and **11** displayed similar IRAK4 IC$_{50}$ values relative to their 4-pyridyl counterparts. It is noteworthy that the unsubstituted 3-pyridine **10** also exhibited measurable but poor rodent exposure.

Collectively the above findings demonstrated that modification of the pyrazole C-3 substituent was a viable strategy to improve both IRAK4 potency and rodent PK in this series. It was hypothesized that the poor solubilities of **2** and **6−11** were due to the expected planar nature of the compounds.[20] In order to address this, alicyclic C-3 substituents were explored next. Replacement of the methyl group in the original hit **1** (IRAK4 IC$_{50}$ = 2.2 μM) with a cyclopropyl ring (**12**) gave an approximately 6-fold improvement in IRAK4 potency. It was encouraging that **12** also exhibited better solubility and oral exposure than analogues in the (hetero)aryl series. However, the more lipophilic cyclohexyl analogue **13** was only equipotent to **1** and was poorly soluble.
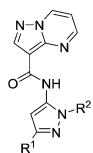
In a similar manner as described above, heteroatom containing substituents were prepared with the goals of improving IRAK4 potency and decreasing lipophilicity and thus improving solubility relative to **13**. The THP species **14** was a modest inhibitor of IRAK4, but showed improved solubility and measurable exposure. Encouraged by this result, piperidine analogues were explored next. The unsubstituted 4-piperidine **15** was a relatively potent enzyme inhibitor with excellent solubility, likely due to the charged nature of this species at pH 7. N-Alkylated analogues **16** and **17** displayed similar activities and maintained excellent solubilities relative to N-H piperidine **15**. Disappointingly, exposure was below the limit of detection for **15** and minimal for N-iso-propyl analogue **17**.

Replacement of the piperidine N-alkyl substituents with other functional groups was explored with the hope that this may improve rat PK. N-Acetamide **18** had a similar profile to the parent piperidine **15**, although improved pharmacokinetics. This result led to the design of piperidines with other nitrogen functional groups. The urea **19** was somewhat less potent than **15**. Importantly, methanesulfonamide **20** was identified as having reasonable IRAK4 potency and high plasma exposure in rat. Furthermore, the excellent kinase selectivity observed with earlier analogues was preserved, as **20** inhibits only 2/108 kinases with >80% inhibition at 10 μM (IRAK4, TRKB).

Although the enzyme potency of **20** was below that of our desired criteria, the excellent selectivity and rat oral exposure made this compound suitable for the development of an *in vivo* biomarker assay. In this experiment, female Lewis rats (n = 9) were dosed by oral gavage at 100 mg/kg with **20**. One hour later, the rats were injected intraperitoneally with the TLR2 agonist PAM2CSK4.[21] Three hours postinjection, plasma was collected and levels of TLR2-derived cytokines were measured relative to the control group that was dosed with only the vehicle. Modest but not statistically significant reductions of TNFα (21%) and IL-1β (28%) were observed, with no decrease seen of either IFNγ or IL-6. The average exposure of **20** in this study was 2.66 μM at 3 h postinjection.

The lack of a robust decrease in cytokine levels observed with **20** was attributed to its modest IRAK4 enzyme activity. Although permeability[22] for **20** was good (P_{app} = 31 × 10^{-6} cm/s), we were concerned that the poor solubility of **20** (<2 μM) conflated an understanding of its cellular activity (IC$_{50}$ = 2.4 μM). We thus undertook SAR investigations into the pyrazole N-1 position. For these studies, the C-3 substituents were restricted to either the piperidine sulfonamide (due to the high exposure of **20**) or N-iso-propyl piperidine (given the excellent potency and solubility of **17**). The findings are summarized in Table 2. Rat AUC values were measured over 6 or 8 h as indicated.

Table 2. SAR Studies at Pyrazole C-3 and N-1 Substituents

compound	R^1	R^2	IRAK-4 IC$_{50}$ (nM)	solubility (pH 7, µM)	rat PK AUC (10 mpk, po), µM·h
20	(piperidine, H$_3$CO$_2$S)	(phenyl-CH$_3$)	67	< 2	19.27 (0-6 h)
21	(piperidine, H$_3$CO$_2$S)	(phenyl-CH$_3$, meta)	2100	N/A	N/A
22	(piperidine, H$_3$CO$_2$S)	(phenyl)	280	N/A	N/A
23	(piperidine, H$_3$CO$_2$S)	(phenyl-OMe)	71	< 3	0.43 (0-6 h)
24	(piperidine, H$_3$CO$_2$S)	(phenyl-cyclopropyl)	59	< 3	5.26 (0-6 h)
25	(piperidine, H$_3$CO$_2$S)	(phenyl-CH$_3$, F)	40	5	11.82 (0-6 h)
26	(piperidine, H$_3$CO$_2$S)	(phenyl-cyclopropyl, F)	85	<2	N/A
27	(piperidine, H$_3$CO$_2$S)	(pyridine-CH$_3$)	27	< 5	0.045 (0-8 h)
28	(piperidine, H$_3$CO$_2$S)	(pyridine-cyclopropyl)	20	< 4	1.90 (0-8 h)
29	(piperidine, H$_3$CO$_2$S)	(pyrimidine-CH$_3$)	180	< 2	N/A
30	(piperidine, isopropyl N)	(phenyl-cyclopropyl, F)	13	167	1.11 (0-8 h)
31	(piperidine, isopropyl N)	(pyridine-CH$_3$)	7	N/A	N/A
32	(piperazine, H$_3$CN)	(pyridine-CH$_3$)	5	158	1.70 (0-8 h)
33	(piperazine, H$_3$CO$_2$S)	(pyridine-CH$_3$)	2	< 2	8.90 (0-8 h)

Movement of the *para*-methyl group to the *meta* position (**21**) led to marked decrease in IRAK-4 inhibition compared to **20**, while the removal of this substituent altogether (**22**) gave a 4-fold loss in activity. Subsequent studies thus focused on analogues with para-substituted aromatic rings at the N-1 position. Replacement of the methyl group with a methoxy functionality had no effect on potency but did result in a dramatic loss in rat exposure, possibly due to metabolism of **23** via demethylation of the ether. Replacement of the methyl group with a cyclopropyl substituent gave analogue **24** that had similar enzyme potency to **20** and approximately 4-fold less plasma exposure. This was curious since it was expected that the cyclopropyl group would confer improved metabolic stability relative to its methyl counterpart.

None of Table 2 analogues thus far (**21**−**24**) exhibited improved solubility or potency relative to **20**. A strategy involving the addition of an *ortho* substituent was designed. This was expected to improve solubility by forcing the pyrazole and phenyl rings to adopt a noncoplanar arrangement in solution. It was also known from the X-ray structure of **1** that these groups are oriented at a 55° angle relative to one another in the enzyme active site. It was thus believed that the ortho substituent would have the additional benefit of preorganizing the inhibitor into its biologically active conformation, as the predicted dihedral angle is 5° larger for an analogue with an *ortho*-fluoro substituent.

ortho-Fluorophenyl analogue **25** had IRAK4 IC$_{50}$ = 40 nM. Although solubility remained poor, **25** was the first compound in the sulfonamide series for which a measurable value (5 µM) could be obtained. Replacement of the *para*-methyl substituent with a cyclopropyl group (**26**) resulted in a slight decrease in potency and a loss of whatever solubility had been gained with **25**.

It was believed that replacing the fluorophenyl rings of **25** and **26** with their bioisosteric pyridines may lead to an improvement in solubility. Although this was not observed with pyridines **27** and **28**, these compounds were slightly more potent than their phenyl or fluorophenyl counterparts. Further, it was found that **27** was highly selective: in a panel of 108 kinases, the only kinase with >80% inhibition at 10 µM was IRAK4. We attempted to further capitalize on this finding by using pyrimidine **29**, but this led to a loss in IRAK4 activity relative to **27**.

Having been unsuccessful in the quest to use N-1 substituents to improve solubility, C-3 *N*-alkyl piperidines were revisited. Analogue **30** was designed with a fully basic nitrogen for solubility and an *ortho*-fluorophenyl pyrazole for improved potency. Compound **30** was a potent enzyme inhibitor (IC$_{50}$ = 13 nM) with excellent solubility and modest exposure in rat. An X-ray cocrystal structure shows that **30** retained the same interactions with the protein as compound **1** (Figure 2). The pyrazolopyrimidine and Tyr262 are oriented at approximately 40° to one another (slanted face-to-edge interaction). The pyrazole and fluorophenyl rings are as expected perpendicular to one another. The fluorine atom engages in a hydrogen bond with the N−H of Ser269. Replacement of the fluorophenyl with a pyridine gave analogue **31**, which was the first inhibitor in this series to possess IRAK4 IC$_{50}$ < 10 nM. We suspect that the additional interactions between the fluorine atom and, by extended reasoning, the pyridine nitrogen are responsible for the improved potencies of **30** and **31**.

A key finding was the discovery that replacement of the pyrazole C-3 piperidine ring with a piperazine provided analogues displaying small but significant improvements in both enzyme inhibition and exposure. Thus, *N*-methyl piperazine **32** had IRAK4 IC$_{50}$ = 5 nM, excellent aqueous solubility, and rat AUC = 1.7 µM·h (0−8 h). As expected, replacement of the *N*-methyl group with a methanesulfonamide (**33**) improved plasma exposure but decreased solubility.

Piperazine **32** was selected for further profiling (Figure 3). In order to determine its cellular activity, THP1-XBlue cells were preincubated with inhibitor **32**. Inflammatory signaling was then stimulated with either the TLR4 agonist LPS, which signals through IRAK4, or TNFα, which acts through IRAK4 independent pathways. Activation of the downstream transcription factor NF-κB was then determined. Analogue **32** exhibits an IC$_{50}$ = 83 nM in cells stimulated by LPS, and no measurable inhibition at concentrations up to 31.6 µM in cells
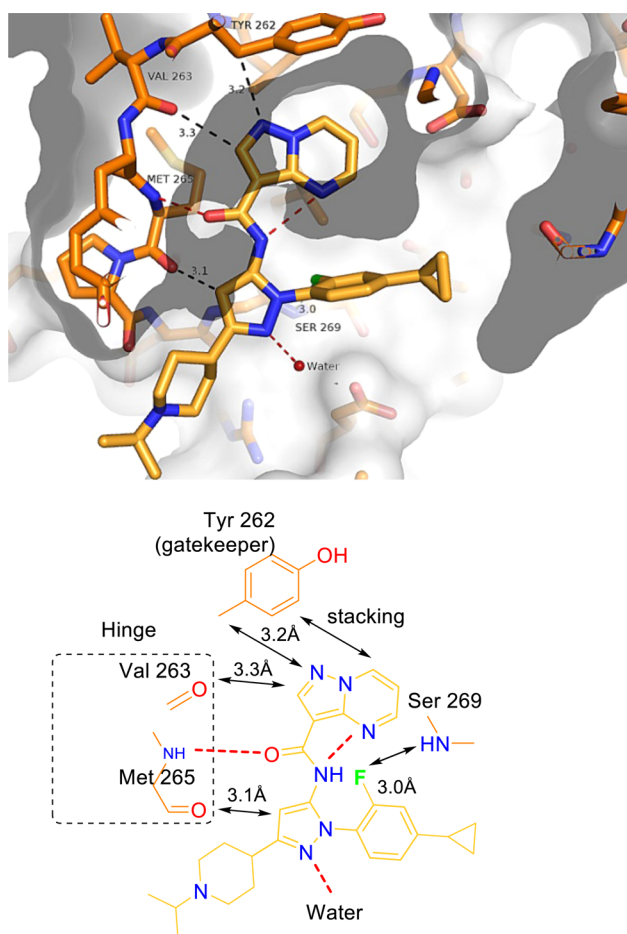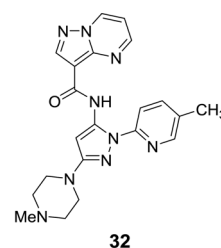
Figure 2. X-ray cocrystal of **30** with IRAK4.

32

IRAK4 IC$_{50}$	5 nM
THP-1 X Blue IC$_{50}$	83 nM

1/108 kinases > 80% inhibition @ 1 μM (IRAK4)
rat PK (po, 0-8 h, 10 mpk, 0.4% MC vehicle)
    AUC 1.71 μM.h
    F = 28%
rat PK (iv, 0-8 h, 2mpk, 20% HPbCD vehicle)
    AUC 1.29 μM
rat ppb 21% unbound
rat P_{app} = 32 x 10^{-6} cm / s
mouse PK AUC (po, 0-24 h, 5mpk)
    AUC 2.30 μM.h
solubility pH 7 = 158 μM
in vivo cytokine reduction (100 mpk i.p., 3h post PAM2CSK4)
    TNFα 32%
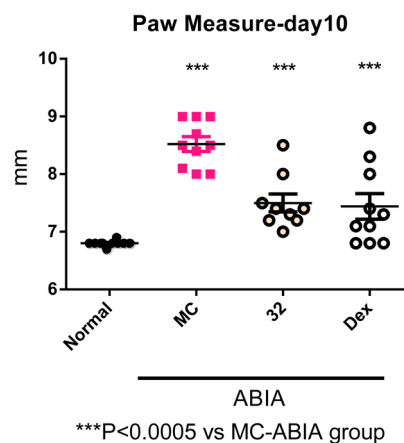   IL-1β 28%
    IL-6 85%
    IL-8 34%
    INF-γ 30%

***P<0.0005 vs MC-ABIA group

Figure 3. Profile of analogue **32** and paw size reduction in mouse ABIA study.

stimulated by TNFα. Compound **32** was highly selective, as the only kinase with >80% inhibition at 1 μM in a panel of 108 is IRAK4. Bioavailability when dosed at 10 mg/kg was 28%, with 21% unbound in rat plasma. This analogue was assessed for its ability to reduce proinflammatory cytokine production *in vivo*, using a similar experiment as performed with **20** (*vide supra*). Good reduction in levels of TNFα (32%), IL-1β (28%, $p < 0.05$), IL-6 (85%, $p < 0.005$), IL-8 (34%), and IFNγ (30%, $p < 0.005$) were observed upon dosing at 100 mg/kg.

Analogue **32** was assessed in the antibody induced arthritis model of inflammation in mice.[23] In this study, BALB/c female mice were immunized with an arthritogenic monoclonal antibody (CIA-MAB-50, 2.5 mg) on day 0. On day 3, the mice were given LPS (25 μg i.p.) as a booster. On days 3−10, mice were dosed q.d. by oral gavage with 100 mg/kg of compound **32** or dexamethasone (2 mg/kg) as a positive control. Paw size was measured on days 0, 3, 6, 8, and 10. On day 10 the study was terminated. The results of the paw measure on day 10 are shown in Figure 3. Compound **32** gave a robust decrease in paw size relative to the control vehicle group (dosed with methylcellulose, MC) and displayed similar efficacy to dexamethasone.

In summary, a series of potent, selective, and orally bioavailable pyrazole IRAK4 inhibitors have been described. A screening campaign identified pyrazoles **1** and **2** as promising hits, and an X-ray structure of **1** indicated that the pyrazole C-3 substituent, pointing toward the solvent exposed portion of the protein, and N-1 substituent offered opportunities to improve

IRAK4 activity. SAR studies at the C-3 position indicated that piperidine and piperazine substituents were well tolerated, and the identity of the nitrogen substituent could be used to modulate potency, solubility, and rat oral exposure. Although sulfonamides conferred excellent rat PK, they were universally poorly soluble. In contrast, alkyl substituted piperidines and a piperazine were highly soluble and very potent. Limited SAR studies at the pyrazole N-1 position indicated that *ortho*-fluoro and pyridine substituents provided the best levels of potency. These studies ultimately led to the discovery of analogue **32**, which reduced the production of TLR4-mediated cytokines and was efficacious in a mouse antibody induced arthritis model.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Compound synthesis and characterization, and assay protocols. The Supporting Information is available free of charge on the

ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00106.

Accession Codes

X-ray coordinates for compounds **1** and **30** in the Protein Data Bank are 4YO6 and 4YP8, respectively.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ ABBREVIATIONS

IRAK4, interleukin-1 receptor-associated kinase 4; SAR, structure−activity relationship; IL-1R/TLR, interleukin-1 receptor/Toll-like receptor; TIR, Toll/interleukin-1R; MYD88, myeloid differentiation primary response gene 88; AP-1, activator protein-1; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; TNFα, tumor necrosis factor α; IL-1, interleukin-1; TLR2, Toll-like receptor 2; i-PrOH, isopropanol; DIPEA, diisopropylethylamine; N/A, not available; HPbCD, (2-hydroxypropyl)-β-cyclodextrin; AUC, area under curve; PK, pharmacokinetic; MINK1, misshapen-like kinase 1; THP, tetrahydropyran; TRKB, tyrosine receptor kinase B; PAM2CSK4, palmitoyl-2-cysteine-serine-lysine; IL-1β, interleukin-1 β; IL-6, interleukin-6; IFNγ, Interferon γ; TLR4, Toll-like receptor 4; LPS, lipopolysaccharide; IL-8, interleukin-8; ABIA, antibody induced arthritis; MC, methylcellulose

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