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IRAK-4 inhibitors. Part 1: A series of amides

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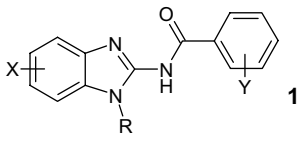
Dedicated to the memory of our friend and colleague David Rainey.

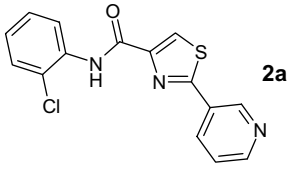
Abstract—The synthesis and profile of a series of amides are described. Some of these compounds were potent IRAK-4 inhibitors and two examples were evaluated in vivo.

The interleukin-1 receptor associated kinases (IRAKs) are a family of serine/threonine kinases involved in mediating cellular signalling downstream of IL-1, IL-18 and a number of Toll-like receptors.[1] IRAK-4 is critical for the activation of intracellular signalling cascades, such as the NFκB and MAPK pathways, which are essential for the production of inflammatory cytokines.[2] Mice lacking IRAK-4 are viable and show complete abrogation of inflammatory cytokine production in response to IL-1, IL-18 or LPS.[3] Similarly, human patients lacking IRAK-4 are severely immunocompromised and are not responsive to these cytokines.[4,5] The role of IRAK-4 in innate immunity makes it an interesting target for inhibition by small molecules.[6] The SAR of some *N*-acyl 2-aminobenzimidazole IRAK-4 inhibitors[7] **1** and the crystal structure of one of these complexed with the kinase domain of human IRAK-4[8] have recently been reported in the literature.



1

Through screening a library of small molecules against IRAK-4, we identified the thiazole amide **2a** as a promising starting point (IRAK-4 $IC_{50} = 2.8\ \mu M$) for a medicinal chemistry programme.



2a

Compound **2a** was docked, using Gold,[9] into a set of homology models generated *in-house* with Modeller software,[10] using known structures of kinases possessing similarities to IRAK-4 (Fig. 1).[11] This study suggested that the amide linker formed a key interaction with the hinge region of IRAK-4, the terminal pyridine had the potential for a π-stacking interaction with the tyrosine gatekeeper, and that space was available for substitution around the aniline.

To investigate SAR around the aniline ring, compounds with the general structure **2** were prepared (Scheme 1) from 2-(3-pyridyl)-1,3-thiazole-4-carboxylic acid and a diverse set of commercially available anilines selected on the basis of electronics, lipophilicity, weight and shape. Mono-substituted and multi-substituted anilines were included, selecting such that the molecular weight of the final compounds would not exceed 420. Single point determinations of IRAK-4 by each compound at

Keywords: IRAK; IRAK-4 inhibitor; Inflammation; Kinase; SAR; Thiazole amide; Pyridine amide; Suzuki reaction; Docking; Microsomes; Solubility; In vivo exposure.
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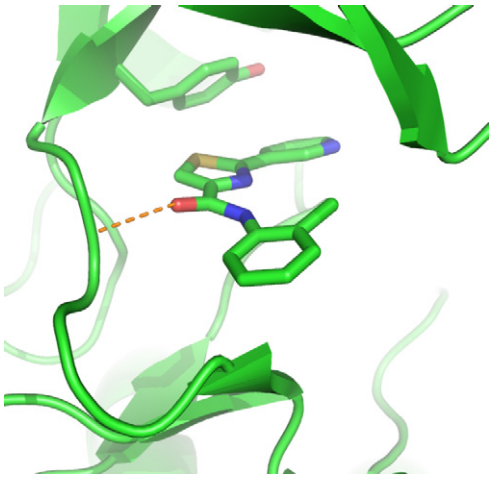
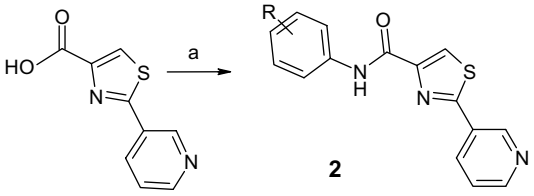


Figure 1. Compound **2a** docked into IRAK-4 homology model. (Figure generated with the program PyMOL).[12]



Scheme 1. Reagents: (a) $(COCl)_2$, DMF, DCM; then appropriate aniline, base, NMP, 18–60%.

10 μM were obtained on the whole library, giving a range of activities from 0% to 98%. IRAK-4 IC_{50} values and rates of in vitro clearance by human microsomes were then determined for selected compounds **2b**–**2r** (Table 1).

Compared with the unsubstituted phenyl amide **2b**, *ortho*-substitution with chloro, methoxy and difluoromethoxy (**2a**, **2e** and **2f**, respectively) significantly improved potency against IRAK-4, as did *para*-substitution with electron-donating substituents such as methoxy and nitrogen-linked heterocycles (**2i**, **2k**, **2l** and **2m**). These potency effects were additive, with the most active examples in the set being the disubstituted compounds **2q** and **2r**. The presence of nitrogen-linked substituents at the *para* position also had a beneficial effect on the rate of turnover by human microsomes.

Turning our attention to the other end of the molecule, we next sought an alternative to the exposed terminal pyridine ring to avoid potential complications associated with pyridine/pyridine-*N*-oxide cycling in vivo. The pyridine *N*-oxide of **2a** (readily formed by reacting **2a** with urea hydroperoxide and trifluoroacetic anhydride in DCM) was significantly less active (6.9 μM) against IRAK-4 than the parent compound.

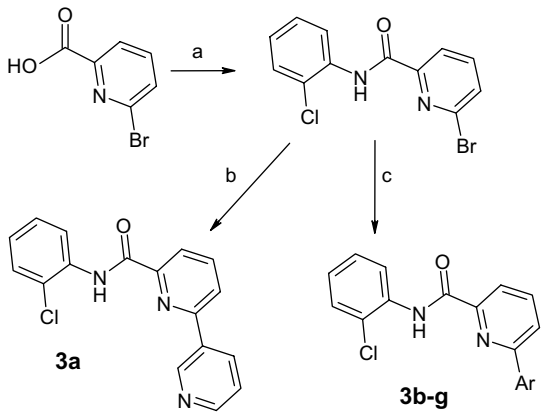
To enable us to quickly investigate SAR around the terminal aromatic, we next changed the thiazole core to pyridine with the pyridine nitrogen between the two points of substitution. We found no evidence that a 2,6-disubstituted pyridine of this type would be prone

Table 1. IRAK-4 inhibition and rate of human microsomal clearance by compounds **2a**–**2r**

Compound	R	IRAK-4 IC_{50} (μM)[13]	Human Cl_{int} (μL/min/mg protein)[14]
2a	2-Cl	2.8	185
2b	H	7.2	209
2c	2-Me	7.3	205
2d	2-^{i}Pr	>10	Not done
2e	2-OMe	0.8	300
2f	2-$OCHF_2$	1.0	103
2g	3-Me	4.8	197
2h	3-OMe	8.9	149
2i	4-OMe	3.7	62
2j	4-Oxazole	6.4	59
2k	4-Piperidine	2.4	40
2l	4-(*N*-Methyl) piperazine	0.7	24
2m	4-Morpholine	2.8	36
2n	2,6-Di-Cl	>10	Not done
2o	2,6-Di-Me	>10	Not done
2p	2,4-Di-OMe	1.2	111
2q	2-OMe, 4-Morpholine	0.16	20
2r	2-OMe, 4-(*N*-Methyl) piperazine	0.35	14

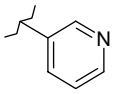
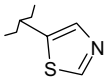
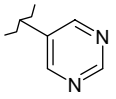
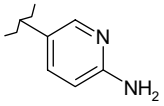
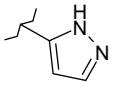
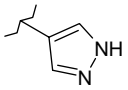
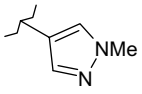
Values are means of at least two experiments.

to in vivo oxidation. 6-Bromo-2-pyridinecarboxylic acid was coupled with 2-chloroaniline to give a versatile bromoaryl intermediate, which was reacted with boronate esters under Suzuki conditions to give **3a**–**3g** (Scheme 2). The IRAK-4 IC_{50} values for these compounds were determined (Table 2). The similar potency of the pyridine **3a** to its thiazole analogue **2a** gave us confidence that SAR would track between the two sub-series. The inactivity of the thiazole, pyrimidine and amino pyridine compounds **3b**, **3c** and **3d** suggested that this part of the molecule was involved in something more than a simple π-stacking interaction and that the orientation and basicity of the nitrogen atom was key for modulating activity against IRAK-4. The pyrazoles **3f** and **3g** were more successful, having improved potency against IRAK-4 compared with **3a**.



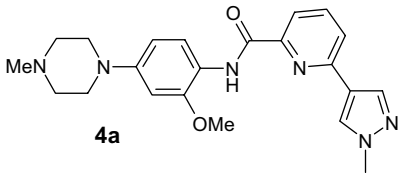
Scheme 2. Reagents and conditions: (a) 2-chloroaniline, HBTU, DIPEA, DMF, 60%; (b) pyridyl boronate ester, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, μW, 150 °C, 5 min, 10%; (c) appropriate aryl boronate ester, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, μW, 150 °C, 5 min, 24–80%.

Table 2. Inhibition of IRAK-4 by compounds **3a–3g**

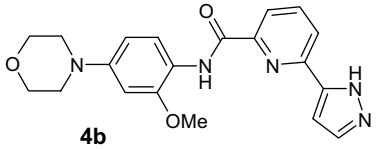
Compound	Ar	IRAK-4 IC_{50} (μM)[13]
3a		3.4
3b		>10
3c		>10
3d		>10
3e		1.6
3f		0.66
3g		0.71

Values are means of at least two experiments.

Table 3. IRAK-4 inhibition and rate of rat and human microsomal clearance by compounds **4a** and **4b**



4a



4b

Compound	IRAK-4 IC_{50} (μM)[13]	Rat Cl_{int}[14] (μL/min/mg protein)	Human Cl_{int}[14] (μL/min/mg protein)
4a	0.42	9	5
4b	0.02	51	14

Values are means of at least two experiments.

Following the synthetic route outlined in Scheme 2, but using 2-methoxy-4-piperidin-1-yl aniline and 2-methoxy-4-morpholin-4-yl aniline in place of 2-chloroaniline in the first step, we combined some of the most promising anilines from the thiazole series with some of our best terminal aromatics from the pyridine series to give compounds such as **4a** and **4b**. Both of these were submicromolar inhibitors of IRAK-4 with acceptable rates of clearance in both rat and human microsomes (Table 3).

Table 4. In vivo blood exposure of **4a** and **4b**

Compound	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-7} (ng h/mL)
4a	652	7	7176
4b	266	1	402

Compounds **4a** and **4b** were progressed into an in vivo PK study[15] in Lewis rats dosed orally at 10 mpk (Table 4). Compound **4a** was found to achieve higher levels of exposure than **4b**, as expected from the in vitro clearance. **4a** had the additional advantage of excellent aqueous solubility (3009 μM at pH 7.4).

In conclusion, a set of novel potent IRAK-4 inhibitors has been prepared, and the rapid rate of microsomal clearance associated with the original screening hit significantly reduced. A selected example (**4a**) had suitable properties, excellent solubility and good levels of in vivo exposure.

Acknowledgment

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References and notes

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13. The IRAK-4 in vitro enzyme assay determined the effect of test compounds on the phosphorylation of a fluorescein

labelled peptide detected using IMAP. It was performed in black 384-well plates in a volume of 20 μL, comprising of reaction buffer (10 mM Tris–HCl, 10 mM $MgCl_2$, 0.1% BSA, 1 mM DTT, 0.05% NaN_3, pH 7.2), 30 μM ATP, 100 nM F-peptide substrate (5FAM-ERMRPRKRQ GSVRRRV-NH2 Molecular Devices #RP7048), 2 nM Baculo His-IRAK-4 (*in-house*) and test compound in 2% DMSO. The kinase reaction was incubated for 1 h at room temperature then terminated by addition of 60 μL Progressive Buffer A (Molecular Devices #R8124) containing 1/400 IMAP binding reagent (Molecular Devices #R8124). The binding of IMAP to the phosphorylated F-peptide substrate required 1 h of incubation at room temperature. The fluorescence polarisation was subsequently measured using a Tecan Ultra plate reader.

14. To investigate rates of clearance by human and rat liver microsomes, test compound (0.5 μM) and microsomal protein (final concentration 1 mg/mL) were pre-incubated at 37 °C for 10 min. Reactions were started by the addition of NADPH and subsequent time points were stopped by the addition of acetonitrile containing an internal standard (ketoconazole). Samples were centrifuged 10,000 rpm for 10 min before analysis by LCMS. Disappearance of compound was measured over 40 min.
15. Test compound was administered orally in a vehicle of 5% DMSO/95% methylcellulose (0.5% w/v, 400 Cps) to a male Lewis rat (Charles River). Serial blood samples were collected by tail vein sampling and frozen before analysis. Samples were analysed and concentrations quantified by LCMS/MS. PK Parameters were calculated using WinNonLin (Pharsight).