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Discovery and Optimization of A Potent and Selective Indazolamine Series of IRAK4 Inhibitors

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ABSTRACT

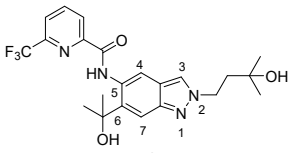
IRAK4 is a key mediator of innate immunity. There is a high interest in identifying novel IRAK4 inhibitors for the treatment of inflammatory autoimmune diseases. We describe here a highly potent and selective IRAK4 inhibitor (HS271) that exhibited superior enzymatic and cellular activities, as well as excellent pharmacokinetic properties. HS271 displayed robust *in vivo* anti-inflammatory efficacy as evaluated in rat models of LPS induced TNFα production collagen-induced arthritis.

Interleukin-1 receptor associated kinase 4 (IRAK4) is an intracellular serine-threonine kinase that belongs to the IRAK family.[1-2] IRAK4 is a key signaling mediator downstream of IL-1 family receptors (IL-1Rs) and Toll like receptors (TLRs), and plays an essential role in the initiation and control of innate immune responses.[3]

Multiple genetic evidences have demonstrated the associations of IRAK4 with inflammation. IRAK4-deficient or kinase dead knock-in mice showed severe defects in cytokine responses in arthritic models[4]. Additionally, cells derived from IRAK4-deficient individuals have impaired responses to ILR/TLR receptor stimulation.[5-6] Therefore, modulation of IRAK4 activity has emerged as a promising strategy to control inflammatory responses. IRAK4 has been recognized as an attractive target for the treatment of inflammatory autoimmune diseases such as rheumatoid arthritis[7-8] (RA) and other related diseases[9,10].

There has been considerable interest in developing orally available IRAK4 inhibitors. Although diverse molecules have been reported[11-19], there is still a need for potent and selective IRAK4 inhibitors with improved potency and pharmacokinetic (PK) profiles. Herein, we report a series of indazolamine IRAK4 inhibitors which showed potent anti-inflammatory activities in cell-based assays, excellent PK properties in preclinical models, and more importantly, robust *in vivo* efficacy, as reflected by suppressed LPS-induced TNFα production and alleviated collagen-induced arthritis (CIA) in rat models.

In the exploration of various scaffolds, the indazole compound **1**[20] was chosen as a starting point for medicinal chemistry efforts (Figure 1).



1

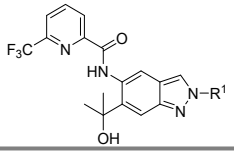
IRAK4 IC_{50} (nM): 8.6
THP-1 IC_{50} (nM): 128
IRAK1 IC_{50} (uM): 3.6
Solubility (PBS 7.4, uM): 6.7
Microsomal Clearance CL_{int} (mL/min/Kg, Human /Mouse /Rat): 1.28/ 3.91/ 3.11.
Rat PK: Rat PK @ 5mpk,po (Cmax/AUC/t1/2/F) :2313/18400/3.6/84%.

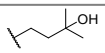
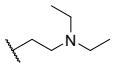
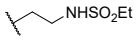
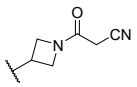
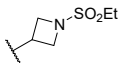
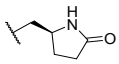
Figure 1. Key properties of compound **1**

Firstly, we screened various compounds with substitutes on the N2 position of compound **1**. To our delight, the replacement of tertiary alcohol with amines delivered improved biochemical and cellular activities. Thus, a series of indazolamine derivatives were further synthesized (Table 1). The dimethylamino compound **2** was first examined, and found that 15-fold enhancement in the biochemical assay, and 2-fold enhancement in the cell-based assay as compared with the compound **1**. As compared with compound **2**, the diethylamino analog **3** delivered loss in kinase potency and cellular potency. The azetidino derivatives **4** and **5** were explored with poor cellular potency. Additional SAR studies on the N2 position, including amide, sulfonamide and pyrazole derivatives

(cor…fore, than compound **2**.

Table 1. SAR of Indazolamines at the N2 Position

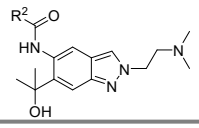
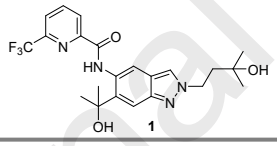


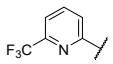
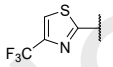
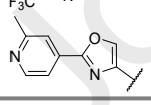
Cmpd	R^1	IRAK4 IC_{50} (nM)	THP-1 IC_{50} (nM)
1		8.6	128
2		0.55	55
3		16.7	514
4		10.6	1400
5		176	ND[a]
6		128	ND[a]
7		36.7	>2000
8		57	256
9		47	730
10		113	ND[a]

[a]ND: Not Detect

Secondly, the R^2 moiety was evaluated in the scaffold of compound **2**. As shown in Table 2, two derivatives **11** and **12** were designed and synthesized. The bi-aryl compound **12** was found with comparable activity as compound **2**.

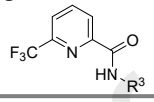
Table 2. SAR of R^2 substituents

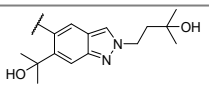
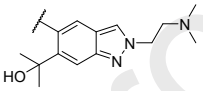
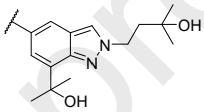
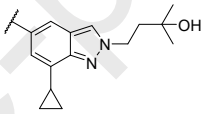
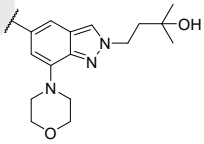
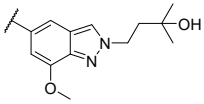
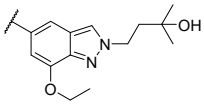
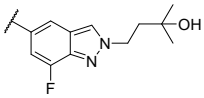
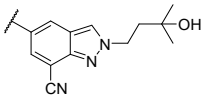
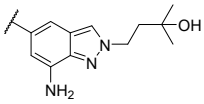
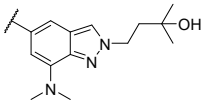
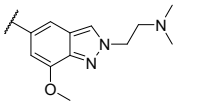
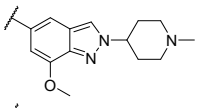
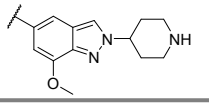



Cmpd	R^2	IRAK4 IC_{50} (nM)	THP-1 IC_{50} (nM)
1		8.6	128
2		0.55	55
11		34.8	198
12		7.7	58.7

Thirdly, the migration of C6 tertiary alcohol of compound **1** was explored (Table 3). Nevertheless, the IRAK4 potency of compound **13** was completely lost. While the cyclopropyl substituent (compound **14)** showed moderate potency on IRAK4 kinase and was subjected to further optimization. A large morpholine substituent (compound **15)** and a small methoxy one (compound **16**) were chosen to be synthesized. Gratifyingly, the introduction of a methoxy group (compound **16**) improved the IRAK4 -inhibitory activity as well as THP-1 potency significantly. Inspired by the discovery, further modification at the C7 position was undertaken. The introduction of an ethoxy group (compound **17**) led to a substantial loss in IRAK4 potency, which suggested that additional small substituents such as F, CN, NH_2 and NMe_2 were employed. However, none of these (compound **18-21**) could retain the potency well. Given the key dimethylamino group of compound **2**, compound **22** that combined the C7-methoxy and dimethylamino group was rationally designed. However, compound **22** was proved to be less potent than compound **2** and **16**. Furthermore, replacement of the dimethylamino fragment of the compound **22** with a piperidine ring, the analog **23**, showed an acceptable THP-1 activity. The demethylation of compound **23** was unable to retain the potency (compound **24** vs compound **23**).

Table 3. SAR for C-7 migration



Cmpd	R^3	IRAK4 IC_{50} (nM)	THP-1 IC_{50} (nM)
1		8.6	128
2		0.55	55
13		>2000	ND[a]
14		170	ND[a]
15		>2000	ND[a]
16		8.3	110
17		202	ND[a]
18		309	ND[a]
19		1600	ND[a]
20		>2000	ND[a]
21		>2000	ND[a]
22		96	223
23		5.7	125
24		41	375

As we know, the co-crystal structure of lead compound 1 and IRAK4 protein was not available. Three strategies including bio-isosteres replacement, C7-migration and aryl modification were conducted for the lead compound 1 (Figure 2). It has been found out that 1) N-7 position exploration revealed that the dimethylamine was the best one, the bulky group in N-7 was unfavorable; 2) bi-aryl derivative retained the potency well; 3) C7-migration developed a new series, the methoxy group were the best choice. Several functional groups were not tolerated well in the C-7 position, including the bulky alkyl group, basic amino derivatives, and electron-withdrawing group.

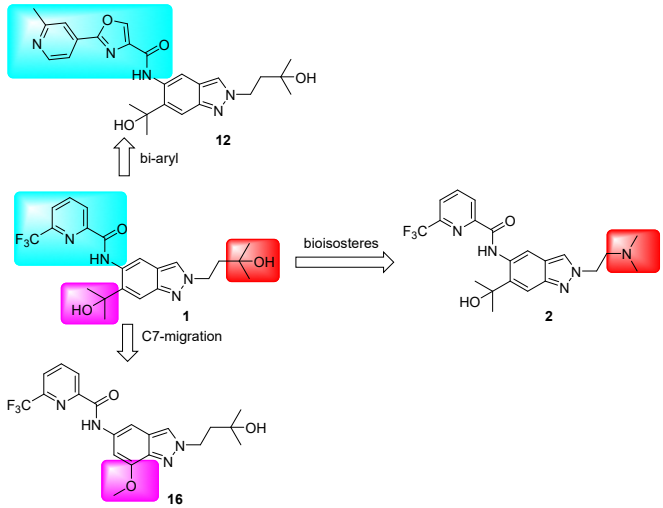


Figure 2. Three Strategies for lead optimization of compound **1**

With these promising biochemical and cellular potency data, compound **2**, **12**, **16** and **23** were selected for further studies. As shown in Table 4, compound **2**, **16** and **23** exhibited excellent selectivity against IRAK1. In a rat PK study, the bi-aryl compound **12** exhibited inferior exposure as compared with others. Moreover, compound **23** displayed a hERG inhibition risk (hERG IC_{50} = 6.9 μM). Taken together, compound **2** and **16** were advanced into *in vivo* efficacy studies.

Table 4. Enzymatic selectivity, PK exposure (rat), and hERG inhibition of compound **2**, **12**, **16** and **23**

Cmpd	**2**[a,e]	**12**[b,f]	**16**[a,e]	**23**[a,f]
IRAK1 IC_{50} (μM)	7.2	ND[d]	14.4	10.4
C_{max} (ng/mL)	2107	21	3323	344
$AUC_{0\text{-}24h}$ (ng/mL·h)	11920	104	34533	5080
$T_{1/2}$ (h)	3.3	3.1	3.5	6.5
Cl (mL/min/kg)[c]	8.3	ND[d]	5.5	19.7
hERG[e] IC_{50} (μM)	16.5	ND[d]	>30	6.9

[a] PO 10 mg/kg; PO: peroral administration; [b] PO 2 mg/kg; [c]Cl data was tested @ 1 mg/kg IV; IV: intravenous injection; [d]ND: Not Detect; [e]Formulation: DMA:30%Solutol HS-15:Saline = 5:5:90 (v/v/v); [f]Formulation: DMSO:PEG200 = 5:95 (v/v); [e]hERG: Human ether-a-go-go related gene.

The rat systemic LPS induced TNFα response model was conducted to assess compound **2** and **16** for their *in vivo* efficacy in blocking cytokine production (Figure 3). Male Sprague-Dawley rats were orally administered (PO) with either vehicle or compound at different dosages. At 1 h post-dosing, the animals were stimulated with LPS at 1 mg/kg intravenously, and blood was collected 3 h post-stimulation for TNFα assay. Compound **2** achieved up to 91% TNFα inhibition in a dose-dependent manner production with a maximum inhibition at 53% (Figure 3B). Therefore, compound **2** (hereafter named HS271) was chosen for further investigations.

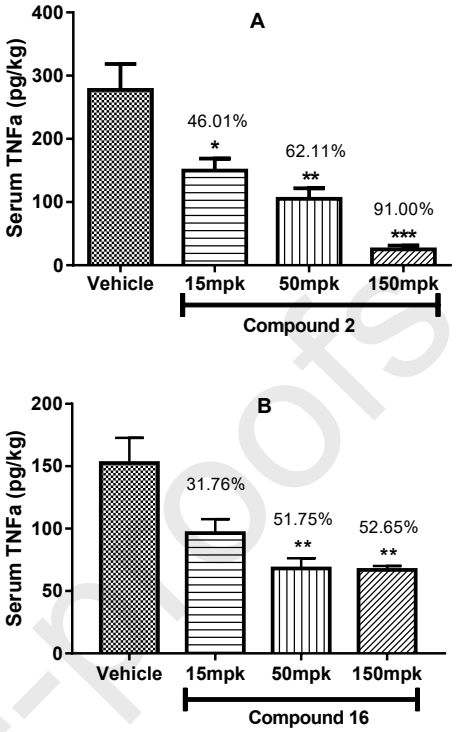
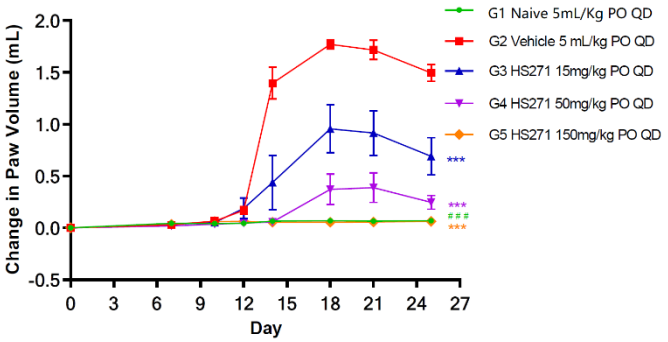


Figure 3. Evaluation of compound 2 and 16 in a rat model of LPS-induced TNF response. *P<0.05, **P<0.01，***P<0.001, vs vehicle

HS271 was evaluated using a rat model of collagen induced arthritis (CIA) (Figure 4). It led to a significant reduction in paw swelling as compared to vehicle control, with a minimum effective dose at 15 mg/kg QD. Notably, at 150 mg/kg QD, HS271 eliminated the paw swelling.

The HS271 was stable in liver microsome assays across other species, including rat, mouse, monkey, and human (Table 5) and a good correlation between metabolic stability and the *in vivo* PK profile (Table 6).



*p<0.05, **p<0.01, ***p<0.001, G3-G5 VS G2 (Repeat measurement ANOVA/Bonferroni's)
#p<0.05, ##p<0.01, ###p<0.001, G1 VS G2 (Repeat measurement ANOVA/Bonferroni's)

Figure 4. Evaluation of HS271 (compound **2**) in a rat model of collagen induced arthritis (CIA). PO: peroral administration; QD: quaque die.

Table 5. *In Vitro* ADME Properties of HS271

Micorsomal Clearance (mL/min/kg)	Cl_{int}	5.25 (human)	15 (rat)	8.82 (mouse)	116 (dog)	1.96 (monkey)

P_{app} (A-B) (10^{-6} cm/s)	27.27 (A-B), 22.70 (B-A)
P_{app} (B-A)/ P_{app} (A-B)	0.83 (efflux ratio)
Human hepatocyte stability $t_{1/2}$	583 min
Human PPB[a] (%)	98.6 (human)
Solubility (μM)	602 (PBS 7.4), 92.3 (FassIF 6.5)

[a] PPB: Plasma Protein Binding

Table 6. *In Vivo* Pharmacokinetic Profiles of HS271 in Mouse, Rat, Dog, and Monkey

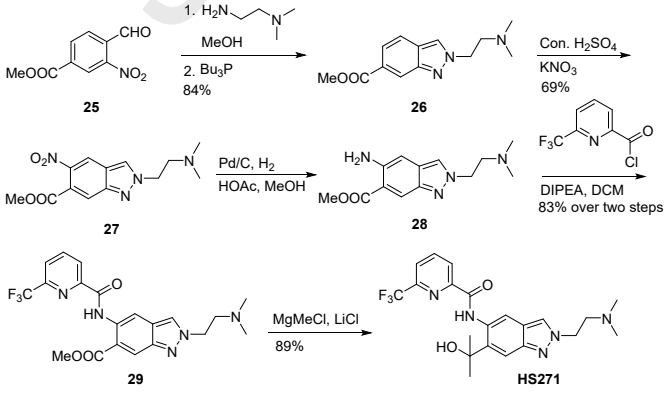
species	mouse		rat		dog		monkey	
	IV[d]	PO[e]	IV[d]	PO[e]	IV[d]	PO[e]	IV[d]	PO[e]
dose (mg/kg)	1	2	1	10	0.5	2	0.5	2
$t_{1/2}$ (h)	1.62	NA[c]	2.01	3.31	0.72	6.40	1.96	3.50
CL (mL/min/kg)	17.1	NA[c]	8.3	NA[c]	55.6	NA[c]	4.86	NA[c]
V_{ss}(L/kg)	2.43	NA[c]	1.30	NA[c]	2.63	NA[c]	0.67	NA[c]
$AUC_{0\text{-}24h}$ (ng/mL▪h)	966	1300	2023	11920	148	85.3	1870	3670
F (%)	NA[c]	67.3	NA[c]	58.2	NA[c]	14.4	NA[c]	49

[a]Formulation: DMA:30%Solutol HS-15:Saline = 5:5:90 (v/v/v); [b]Formulation: DMA:30%Solutol HS-15:Saline = 10:10:80 (v/v/v); [c]NA: Not Applicable; [d]IV: intravenous injection; [e]PO: peroral administration.

Furthermore, the cytochrome P450 inhibitions of HS271 were investigated to assess possible drug-drug interactions. No inhibition was observed (IC_{50} values > 30μM) in major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4t, CYP3A4m, CYP2B6 and CYP2C8).

The synthesis of HS271 was straightforward, as shown in Scheme 1. The indazolamine core **26** from commercially available ortho-nitrobenzaldehyde (compound **25**) via a mild, one-pot condensation−Cadogan reductive cyclization[21] was efficient synthesized. The intermediate **27** was prepared under KNO_3 and con H_2SO_4 conditions, and then reduced under 1 atm H_2 to furnish the amino intermediate **28**. Subsequently, the intermediate **28** was coupled with 6-(trifluoromethyl)picolinoyl chloride[22] to afford the amide product **29**. Finally, the Grignard reaction was conducted under MgMeCl and LiCl conditions to successfully deliver HS271. The overall yield was approximately 43%.

Scheme 1. Synthetic Route of HS271



In conclusion, we have identified a novel indazolamine series of IRAK4 Inhibitors. The pre-clinical candidate HS271 exhibited superior biochemical and cellular activities, as well as excellent ADME properties. HS271 displayed robust anti-inflammatory efficacy in rat models of acute inflammation and CIA. To date, the IRAK4 inhibitor HS271 has advanced into pre-clinical development.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

References and Notes

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22. The 6-(trifluoromethyl)picolinoyl chloride was synthesized from the commercial available material 6-(trifluoromethyl)picolinic acid (CAS No.: 131747-42-7). The detail was found in support information.

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Discovery and Optimization of A Potent and Selective Indazolamine Series of IRAK4 Inhibitors

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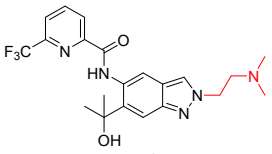

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IRAK4 IC_{50} (nM): 0.55
THP-1 IC_{50} (nM): 55
IRAK1 IC_{50} (uM): 7.2