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5-Aminomethylbenzimdazoles as potent ITK antagonists

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ABSTRACT

Benzamide **1** demonstrated good potency as a selective ITK inhibitor, however the amide moiety was found to be hydrolytically labile in vivo, resulting in low oral exposure and the generation of mutagenic aromatic amine metabolites. Replacing the benzamide with a benzylamine linker not only addressed the toxicity issue, but also improved the cellular and functional potency as well as the drug-like properties. SAR studies around the benzylamines and the identification of **10n** and **10o** as excellent tools for proof-of-concept studies are described.

Interleukin-2 inducible T-cell kinase (ITK) is a tyrosine kinase from the Tec kinase family[1] that is expressed in T-cells, NK cells and mast cells. Itk deficient murine $CD4^+$ T-cells demonstrate reduced IL-2, IL-4, IL-5 and IL-13 production upon T-cell receptor stimulation.[2–4] This suggests that a selective ITK inhibitor would reduce the production of these pro-inflammatory cytokines and hence ITK is considered an attractive target for the potential treatment of inflammatory diseases such as psoriasis and allergic asthma.[5]

Recently, we have reported the discovery of 5-aminobenzimidazoles as potent inhibitors of ITK.[6] Compounds such as **1** (Scheme 1) showed significant inhibitory activity in an ITK DELFIA-based molecular assay[7] (IC_{50} = 3 nM) and good cellular potency (IC_{50} = 110 nM), as measured in a stably transfected, ITK-expressing DT40 cell line assay.[8] However, in human hepatocytes compounds such as **1** suffered from amide bond cleavage, generating reactive aniline metabolites **2a** and **2b**. Upon subsequent testing, it was observed that, upon further metabolic activation in vitro, these metabolites formed adducts with glutathione (GSH) and demonstrated mutagenicity in an Ames test. These results were indicative of the formation of one or more reactive metabolites. Metabolites **2a** and **2b** were also generated in vivo in rat and the amide bond hydrolysis contributed to the very low plasma levels observed in mice after oral administration. Therefore, we embarked in a synthetic effort to identify potent, orally bioavailable ITK inhibitors that are not metabolized to a toxicophoric aniline.

The X-ray crystal structure of ITK[9] with a related compound bearing a cyclohexyl amide demonstrated that the cyclohexyl group forms a lipophilic interaction with a shallow pocket on the enzyme surface. Assuming that **1** binds in a similar fashion, a molecular modeling study suggested a number of opportunities for replacing the benzamide linker. Previously, we have reported a few examples of linkers such as benzylamine, ketone and ether linkers that were tolerated in this position.[10] These alternative linkers had been assessed at an early stage of the lead optimization effort, prior to the identification of the more preferred biaryl right hand side groups, and so their impact had not been fully determined. Therefore, upon achieving a significant increase in intrinsic potency with the biaryl right hand piece of the aminobenzimidazole based ITK inhibitors,[6] we decided to re-visit the more polar linker systems, such as benzylamines, to improve the overall physical properties as well as the potency in the functional assay of our ITK inhibitor series. In this Letter, we discuss an SAR of a new class of very potent ITK inhibitors having a benzylamine linker between the core and the left hand side functional groups.

To systematically explore the SAR, a variety of benzylamine analogs were prepared, as illustrated in Scheme 2. Compound

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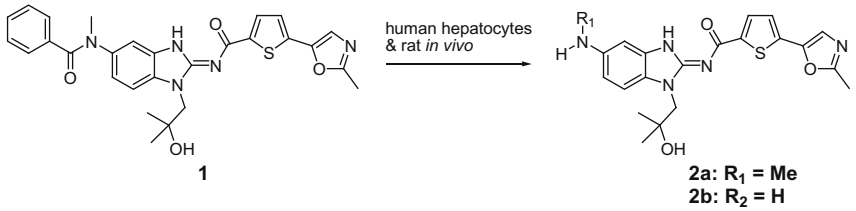
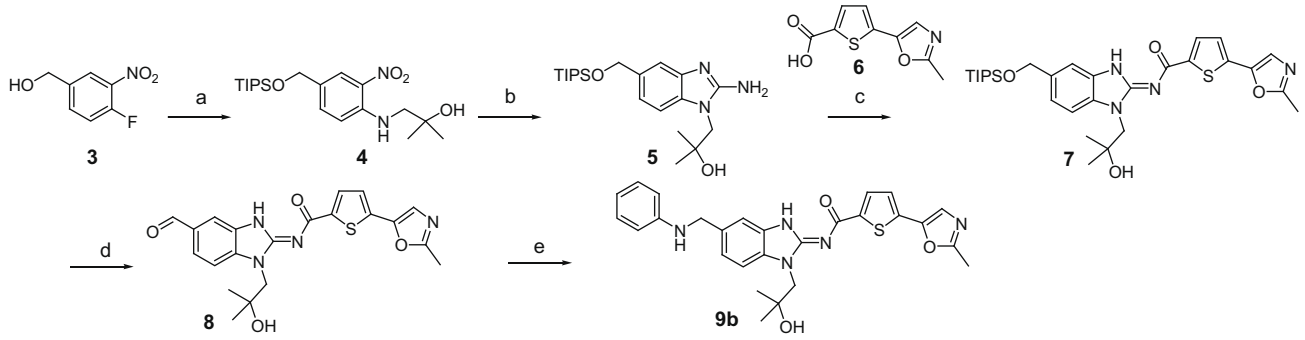


Scheme 1. Benzamide **1**: Cleavage to aniline metabolites **2a** and **b**.



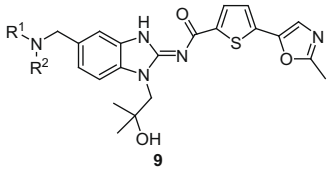
Scheme 2. Reagents and conditions: (a) (i) TIPS triflate, 2,6-lutidine, DCM; (ii) 2,2-dimethyl ethanolamine, CH_3CN, 61% over 2 steps; (b) (i) 10% Pd/C, $NH_4^+HCOO^-$, EtOH, 91%; (ii) cyanogen bromide, EtOH, rt, 88%; (c) EDC, HOBt, DIEA, 75%; (e) (i) 0.01 N HCl, dioxane, 95 °C, 92%; (ii) MnO_2, acetone, 84%; (f) amine, $NaBH(OAc)_3$, DCM, 75–95%.

9b was used as the starting point. In order to rapidly explore the SAR, we resorted to a parallel synthesis approach. Aldehyde precursor **8** was prepared as the divergent point for analog syntheses (Scheme 2).

Protection of commercially available benzyl alcohol **3** as the TIPS ether, followed by displacement of the fluorine with 2,2-dimethyl ethanolamine (SnAr), gave amine **4** in good yields. After reduction of the nitro group of **4**, the formation of benzimidazole **5** was accomplished uneventfully with cyanogen bromide. Standard amide coupling with thiophene acid **6**[11] yielded **7**. Acidic deprotection of the benzyl alcohol, followed by MnO_2 oxidation, gave aldehyde intermediate **8**. The reductive aminations were performed using polymer-supported reagents such as MP–$BH(OAc)_3$ or MP–BH_3CN. Scavenging of the remaining starting materials with PS-Benzaldehyde, PS-Isocyanate and PS-Trisamine, allowed efficient access to a wide variety of benzylamines such as **9b**. All of the analogs prepared were tested in an ITK DELFIA assay to determine their intrinsic binding affinities. Since the predicted assay range does not extend below 2 nM, based on the enzyme concentration used, we developed a complementary assay using a higher concentration of ATP (1 mM) in order to extend the dynamic range of the assay, thereby allowing comparisons between the most potent inhibitors.[7] The compounds were further tested for selectivity against insulin receptor kinase (IRK) due to their previously observed occasional cross-reactivity with this enzyme. The cellular potency of the compounds was determined first in a stably transfected ITK-expressing DT40 cell line assay.[8] The most potent compounds from the series were tested thereafter in a functional assay measuring inhibition of IL-2 secretion using human $CD4^+$ T-cells stimulated with anti-CD3 and anti-CD28 mAbs. The results are shown in Table 1.

Benzylamine inhibitor **9a** showed a significant loss in ITK binding affinity and a complete loss in cellular potency when compared to benzamide **1**. In contrast to the tertiary amine **9a**, secondary amine **9b** was fourfold less potent than **1** in the ITK binding assay, twofold less potent in the cellular assay, and equipotent to **1** in the functional assay. A similar trend was observed for cyclohexyl analogs **9c** and **9d** which were potent inhibitors in the functional assay

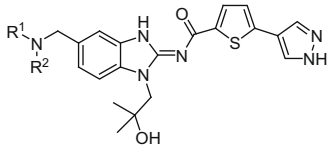
Table 1
Results of selected benzylamine analogs **9** with different R^1 and R^2 in comparison with the corresponding benzamide **1**



Compds	R^1	R^2	ITK IC_{50}[a] (nM)	ITK[b] IC_{50}[a] (nM)	IRK IC_{50}[a] (nM)	DT40 Cell IC_{50}[a] (nM)	IL-2 inhibition IC_{50}[a] (nM)
1	–	–	3	200	940	110	440
9a	Ph	Me	100	na	na	na	
9b	Ph	H	11	na	na	180	460
9c	Cyclohexyl	Me	8	670	350	180	230
9d	Cyclohexyl	H	8	940	700	210	180

[a] Values are means of at least three separate experiments, (na = not active: $IC_{50} > 5$ μM).
[b] High ATP (1 mM) concentration ITK molecular assay.

Table 2
Results of selected lipophilic benzylamine analogs **10**

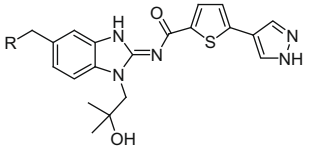


10

Compds	R^1	R^2	ITK IC_{50}[a] (nM)	ITK[b] IC_{50}[a] (nM)	IRK IC_{50}[a] (nM)	DT40 Cell IC_{50}[a] (nM)	IL-2 inhibition IC_{50}[a] (nM)
10a	Ph	Me	8	780	5500	120	
10b	Ph	H	2	75	1700	21	150
10c	Cyclohexyl	Me	2	160	480	13	59
10d	Cyclohexyl	H	1	99	400	20	93
10e	H	H	4	900	1200	na	
10f	Me	H	2	420	500	na	
10g	Me	Me	3	490	570	26	130
10h	Benzyl	H	1	140	480	19	37
10i	3-Pyridyl-methyl	Me	1	100	310	6	91
10j	1-Methyl-propyl	H	2	390	600	25	23
10k	2-Methyl-propyl	H	1	140	360	12	86
10l	(*1R*)-1,2,2-Trimethylpropyl	H	1	20	93	3	50
10m	(*1R*)-1,2,2-Trimethylpropyl	Me	1	20	230	2	64
10n	(*1S*)-1,2,2-Trimethylpropyl	H	1	13	160	4	20
10o	(*1S*)-1,2,2-Trimethylpropyl	Me	1	8	400	3	18
10p	2-*N*,*N*-Dimethylaminoethyl	Me	2	180	390	62	150

[a] Values are means of at least three separate experiments, (na = not active: IC_{50} > 5 μM).
[b] High ATP (1 mM) concentration ITK molecular assay.

Table 3
Results of selected cyclic benzylamine analogs **11**



11

Compds	R	ITK IC_{50}[a] (nM)	ITK[b] IC_{50}[a] (nM)	IRK IC_{50}[a] (nM)	DT40 Cell IC_{50}[a] (nM)	IL-2 inhibition IC_{50}[a] (nM)
11b	Piperidine	3	250	550	11	120
11c	2-Hydroxymethylpiperidine	3	660	1100	58	
11d	Morpholine	2	220	320	13	230
11e	3,5-Dimethylmorpholine	1	98	320	3	46
11f	3-Hydroxypyrrolidine	2	310	420	130	

[a] Values are means of at least three separate experiments, (na = not active: IC_{50} > 5 μM).
[b] High ATP (1 mM) concentration ITK molecular assay.

with IC_{50}s of 230 nM and 180 nM, respectively. This data suggested that with the benzylamine class of inhibitors, the loss of potency observed between the assays (ITK binding vs cellular assay using DT40/ITK cells vs functional assay) is significantly smaller than with the benzamide series. This phenomenon can possibly be explained by differences in aqueous solubility and protein plasma binding between benzamides and benzylamines. While benzamide **1** has a low solubility of 2 μg/mL in pH 7 buffer and is 98.7% protein bound in a dialysis plasma protein binding assay, benzylamine **9d** displays moderate solubility of 27 μg/mL and a substantially higher unbound fraction (93.3% protein bound).

Further optimization and SAR exploration was performed within the pyrazole RHS class of inhibitors **10** which we had found to be more potent when compared to the methyl-oxazole class **9**.[6] To access the pyrazole derivatives **10**, the same synthetic route as described in Scheme 2 was applied using the appropriate thiophene carboxylic acid in the amide coupling step.

Pyrazoles **10a–d** (Table 2) were prepared for direct comparison to the corresponding methyl-oxazoles **9a–d** and displayed comparable SAR trends. Removing all substituents to yield unsubstituted amine **10e** led to an inactive compound in the cellular assay, despite good binding potency. Introduction of one methyl group as in **10f** was not sufficient to introduce any cellular potency. In contrast the *N*,*N*-dimethyl amine **10g** demonstrated moderate potency in the cellular assay, as well as in the functional assay despite sim-

Table 4
Pharmacokinetic parameters of **10n** in Sprague–Dawley rats[a] administered in PEG/H_2O = 70:30

Administered iv (1 mg/kg)	
$t_{1/2}$[b]	2.5 h
V_{dss}	11.6 L/kg
CL	53.1 mL min^{-1} kg^{-1}
$AUC_{0-\infty}$	314 ng h/mL
Administered po (10 mg/kg)	
T_{max}	1.2 h
C_{max}	92 ng/mL
$AUC_{0-\infty}$	659 ng h/mL
F (%)	21%

[a] Values represent the means only for *n* = 3.
[b] Terminal half-life.

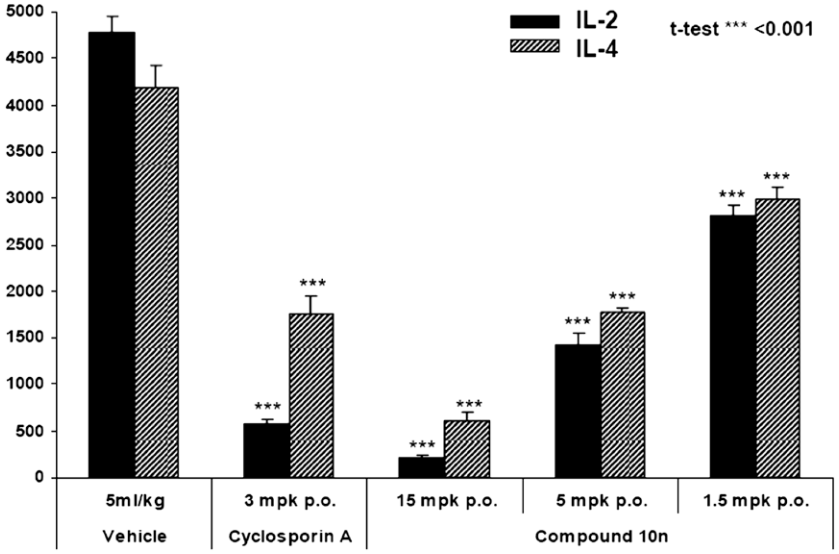


Figure 1. Effect of ITK inhibitor **10n** on in vivo cytokine production in anti-CD3 stimulated Balb/c mice.

ilar binding potency as **10f**. Increasing the lipophilicity of R^1 generally led to an increase in potency in both the cellular and functional assays. Both aromatic substituents such as benzyl **10h** and 3-pyridylmethyl **10i**, as well as aliphatic substituents were well tolerated. Among the most potent substituents within the benzylamine series we identified 1,2,2-trimethylpropyl as in **10l–o**. The stereochemistry of the 1,2,2-trimethylpropyl group had only a marginal effect on the functional potency. In the functional assay the (*S*)-enantiomers **10n** and **10o** achieved potencies of 20 nM and 18 nM, respectively, while the (*R*)-enantiomers **10l** and **10m** demonstrated potencies of 50 nM and 64 nM, respectively. Compounds **10n** and **10o** were profiled further and the additional data is discussed below.

The SAR further demonstrated that secondary (R^2 = H) and tertiary (R^2 = Me) amines are generally very comparable. Further exploration of R^1 showed that polar substituents such as hydroxyl and methoxy (not shown), or basic amines such as dimethylamino substituents as in **10p** led to a loss in potency. In comparison to acyclic amines, cyclic amines, as shown in Table 3, are slightly less potent and polar substituents such as in **11c** and **11f** again decreased potency.

Selected compounds were further profiled in a human whole blood assay (HWB) in which IL-2 secretion is measured after stimulation using staphylococcal entertoxin B. While benzamide **1** demonstrated an inhibitory potency of only 10 μM in the HWB assay, benzylamines **10n** and **10o** were found to be much more potent, with IC_{50}s of 240 nM and 690 nM, respectively. The pharmacokinetic profile of **10n** in Sprague–Dawley rats is shown in Table 4. The compound demonstrated a high volume of distribution at steady-state (11.6 L/kg) and a rapid plasma clearance (53.1 mL min/kg), resulting in a half-life of 2.5 h and a 21% bioavailability at 10 mg/kg.

Compound **10n** was also tested for its in vivo activity in a mouse model of T-cell activation. Female Balb/c mice were dosed orally with **10n**, followed 5 h later with an i.v. injection of anti-CD3. The ITK inhibitor **10n** dose-dependently (15, 5, and 1.5 mg/kg) inhibited IL-2 and IL-4 production (measured 3 h post anti-CD3 injection) (Fig. 1). The terminal plasma concentrations (8 h post dose) of **10n** were 1.3 μM, 0.4 μM and 0.1 μM, respectively, demonstrating that the in vivo EC_{50} was comparable to the HWB IC_{50}.

In summary, we have demonstrated that the amide moiety of benzamide ITK inhibitors such as **1** can be replaced with a benzylamine linker to address the liability of forming potentially toxic metabolites. The new benzylamines generally demonstrated increased potency in assays using primary human cells and are among the most potent orally bioavailable ITK inhibitors to date.

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