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Identification of Novel and Potent Isoquinoline Aminooxazole-Based IMPDH Inhibitors

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Abstract—Screening of our in-house compound collection led to the discovery of 5-bromo-6-amino-2-isoquinoline **1** as a weak inhibitor of IMPDH. Subsequent optimization of **1** afforded a series of novel 2-isoquinolinoaminooxazole-based inhibitors, represented by **17**, with single-digit nanomolar potency against the enzyme.

As a rate-limiting key enzyme in the de novo biosynthetic pathway of guanine nucleotides, inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the irreversible NAD-dependent oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-mono-phosphate (XMP). This ultimately leads to the formation of guanosine phosphate precursors of the nucleic acid pool.[1] Studies have shown that there is a strong correlation between IMPDH activity and cell proliferation, a reflection of the dependence of growing cells on de novo purine synthesis.[2] Due to its intimate involvement in the DNA synthesis, IMPDH has long been recognized as a possible oncology target.[1,3] In addition, it was found that inhibition of this enzyme displayed a profound anti-proliferative effect on B and T lymphocytes owing to their dependence on the de novo pathway for the production of guanine nucleotides. Consequently, IMPDH has recently emerged as an immunology target for treatment of transplant rejection and other autoimmune disorders[4] in addition to its role as an oncology target for human leukemia and several colon and ovarian cell lines.[5–8]

We have been interested in developing small molecule IMPDH inhibitors for the treatment of B and T lymphocyte-mediated immunological diseases, such as rheumatoid arthritis (RA) and psoriasis. We report herein the identification of aminoisoquinoline oxazoles, represented by **17**, as a new class of IMPDH inhibitors that display excellent in vitro potency against the enzyme.

Screening of our in-house compound collection identified **1**, 5-bromo-6-aminoisoquinoline, as a relatively small molecule possessing modest activity ($IC_{50} = 0.55$ μM) against IMPDH-II (Fig. 1). Our initial focus was to establish the structural elements of **1** required for potency and to identify sites for further chemical modification.

The synthetic route to analogues **1**–**14** is shown in Scheme 1. Briefly, compounds **1** and **3** were obtained from the treatment of **2** (prepared from 5-bromoiso-quinoline[9]) with *N*-bromosuccinimide and *N*-chloro-succinimide, respectively. Alkylation of **1** with tetra-alkyltin in the presence of bis(triphenylphosphine) palladium dichloride afforded compounds **4**–**6**. Acetyllation of **1**–**3**, respectively, gave the corresponding analogues **9**–**11**. Reduction of **6** under hydrogenation conditions generated **7**. Compound **8** was obtained from 5-aminoquinoline in a five-step sequence via **12**.[10] Finally, compound

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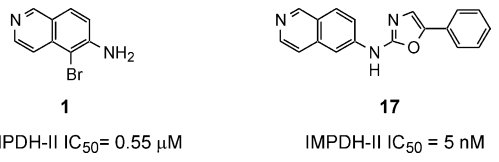


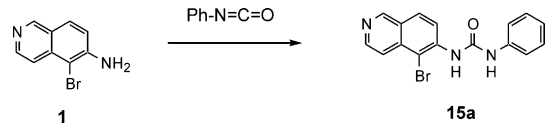
Figure 1. IMPDH inhibitors.

14 was obtained from **13**, which in turn was prepared from *p*-anisaldehyde.[11]

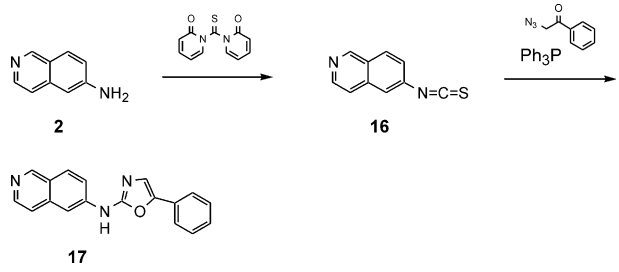
Further investigation of the SAR of the aminoquinoline lead was extended to analogues containing urea and amino oxazole linkages, two known scaffolds found in other IMPDH inhibitors.[12] The synthetic route to the diaryl urea analogues **15a**–**k** is straightforward, as exemplified by the preparation of analogue **15a** in Scheme 2. Thus, the reaction of 5-bromo-6-amino-isoquinoline **1** with commercially available phenyl isocyanate afforded the desired compound **15a** in quantitative yield.

Synthesis of oxazole analogues **17** and **18** were achieved by a two-step sequence, as exemplified by preparation of **17**. Reaction of 6-aminoisoquinoline **2** with 1,1′-thiocarbonyldi-2(1H)-pyridone provided thio-isocyanate intermediate **16**. Subsequent treatment with α-azidoacetophenone (prepared from commercially available acetophenone) in the presence of triphenyl phosphine gave desired **17** in good overall yield. (Scheme 3).

The analogues prepared in this study were evaluated for their inhibitory activity against IMPDH type II enzyme and are expressed as IC_{50} values.[13] As shown in Table 1, deletion of the 5-bromo-substituent from **1** was detrimental to the potency, resulting in a > 10-fold loss in activity (**2** vs **1**). Exchanging the 2-bromo substituent of **1** with 2-chloro group led to a 2-fold loss in activity (**3** vs **1**). In addition, replacement of the 5-bromo group of **1** with small alkyl groups, such as methyl (**5**), was not tolerated and resulted in a compound with > 5-fold reduction in activity. This trend is continued with other alkyl or amino analogues (**6**–**8**), with the exception of the vinyl analogue **5** (IC_{50} = 0.82 μM). Other efforts to improve the potency by simple replacement of the 6-amino group of **1** with acetoamino or methoxy substituents again resulted in large losses (> 10-fold) in activity (**9**–**11** and **14**).

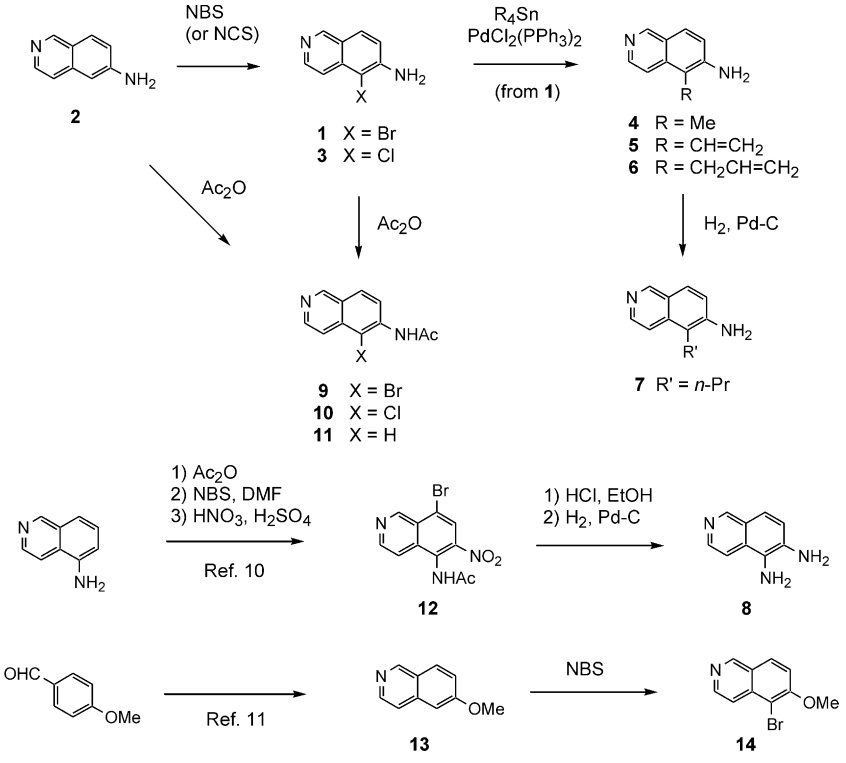


Scheme 2.



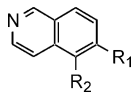
Scheme 3.

We next turned our attention to modifications around the 6-amino functionality of **1**. Previous work[16] in our lab as well as in others[17] has demonstrated that a biaryl urea isostere possess potent IMPDH inhibitory activity.



Scheme 1.

Table 1. IMPDH Inhibition (IC_{50} values) for compounds **1**–**11** and **14**



Compd	R_1	R_2	IC_{50} (μM)[13]
1	NH_2	Br	0.55
2	NH_2	H	>5
3	NH_2	Cl	1.2
4	NH_2	Me	>5
5	NH_2	$CH{=}CH_2$	0.82
6	NH_2	$CH_2CH{=}CH_2$	3.0
7	NH_2	$CH_2CH_2CH_3$	>5
8	NH_2	NH_2	>5
9	NHAc	Br	>5
10	NHAc	Cl	>5
11	NHAc	H	>5
14	OMe	Br	>5

It was reported and confirmed by X-ray crystallographic studies that biaryl ureas can form a key hydrogen bond interaction between one of the urea NH and the carboxylate of Asp 274 in the active site of the IMPDH enzyme.[18] In addition, the second aryl moiety of the biaryl urea occupies space in a large hydrophobic pocket. We envisioned that the extension of **1** through a rigid urea linkage would place the second aryl group in the proximity of the same hydrophobic pocket, thus allowing for further improvements in the binding affinity of **1**.

As shown in Table 2, biaryl urea **16a** displayed comparable activity to **1**. Replacement of the 5-bromo group of **16a** with a 5-chloro group (**16b**) resulted in a 2-fold loss in potency. However, deletion of the 5-chlorine regained activity (**16c**).

Interestingly, the most drastic change in activity observed in this series was derived from the substitution pattern of the phenyl ring. 3-Methylphenyl urea **16e** showed an IC_{50} of 18 nM, a >40-fold increase in potency from its parent compound **16c**!

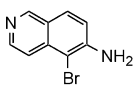
The significant differences in potency displayed by the regioisomers of **16d**, **16e** and **16f** are noteworthy: the 4-methylphenyl urea analogue **16f** is >13-fold less potent than **16e**, whereas the 2-methylphenyl urea **16d** is >200-fold less potent than **16e**, indicating a strong preference for the *meta*-substitution. In addition, the benzyl urea **16g** was roughly 2-fold more potent than the phenyl urea **16c**.

We also examined the SAR of other bicycloheteroaryl ureas in order to elucidate the effect of the isoquinoline nitrogen on the binding affinity. The 3-isoquinoline phenyl urea **16h**, a regioisomer of **16c**, was completely devoid of activity (IC_{50} >5 μM), implying a disruption of a possible hydrogen-bond interaction between the isoquinoline nitrogen and the backbone of the enzyme. In addition, the 1,2-cinnoline urea **16i** was significantly less active whereas its regioisomer, the 2,3-phthalazine urea **16j** was inactive (IC_{50} >5 μM). Interestingly, the 2,4-quinazoline urea **16k** displayed enhanced enzyme activity (IC_{50} = 150 nM), although it is still 8-fold less potent than the 2-isoquinoline **16e**. We speculated that the improved activity of **16k** may reflect a partially restored hydrogen-bond interaction with the enzyme, although the basicity of the N_2-nitrogen of **16k** may not be optimal.

Encouraged by the significant improvement in binding affinity of biaryl urea **16e** relative to the original lead **1** (30-fold), we set out to examine various conformationally constrained urea linkages. Initial efforts to mimic the conformation of the urea led to the preparation of 6-aminoisoquinoline oxazole **17** and 7-aminoquinazoline oxazole **18**.[12c] To our delight, both compounds displayed further enhanced potency against the enzyme (IC_{50}'s = 5 and 60 nM, respectively) over their corresponding urea counterparts **16e** and **16k** (Fig. 2).

In summary, the isoquinoline aminooxazole scaffold, represented by **17**, was identified as a novel structural array of highly potent IMPDH type II inhibitors from our initial screen lead, 6-amino-2-isoquinoline **1**, with over a 100-fold improvement in enzyme inhibition over **1**. The results of follow up SAR studies as well as the

Table 2. IMPDH Inhibition (IC_{50} values) for urea analogues **16a**–**k**



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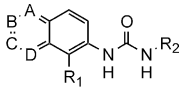

16a-k

Compd	A, B, C, D	R_1	R_2	IC_{50} (μM)[13]
1				0.55
16a	A = C = D = CH, B = N	Br	Ph	0.74
16b	A = C = D = CH, B = N	Cl	Ph	1.66
16c	A = C = D = CH, B = N	H	Ph	0.74
16d	A = C = D = CH, B = N	H	2-Me-Ph	3.8
16e	A = C = D = CH, B = N	H	3-Me-Ph	**0.018**
16f	A = C = D = CH, B = N	H	4-Me-Ph	0.247
16g	A = C = D = CH, B = N	H	CH_2Ph	0.4
16h	A = B = D = CH, C = N	H	Ph	>5
16i	A = B = N, C = D = CH	H	3-Me-Ph	>1.7
16j	A = D = CH, B = C = N	H	3-Me-Ph	>5
16k	A = C = CH, B = D = N	H	3-Me-Ph	0.15

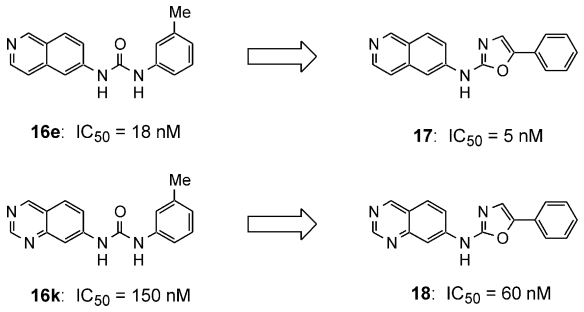

Figure 2.

optimization of the physical and chemical properties of **17** and **18** through various substitutions around the phenyl ring will be reported in due course.

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