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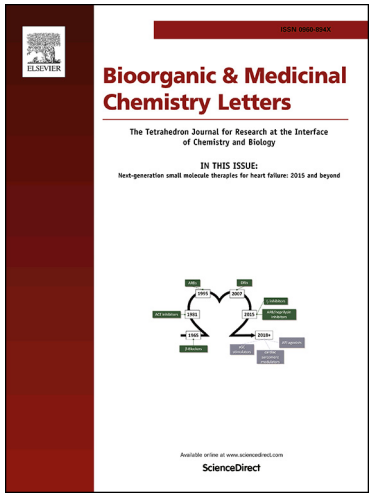

Synthesis and Evaluation of Tetrahydropyrazolopyridine Inhibitors of Anion Exchange Protein SLC26A4 (Pendrin)

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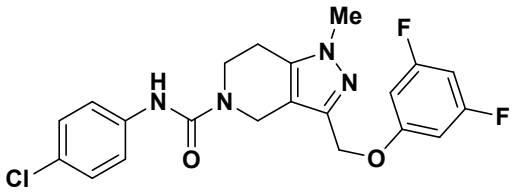

Regioselective pyrazole synthesis

Pendrin IC_{50} = 3.1 μM

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Synthesis and Evaluation of Tetrahydropyrazolopyridine Inhibitors of Anion Exchange Protein SLC26A4 (Pendrin)

Jie S. Zhu[a], Julia Y. Lu[a], Joseph-Anthony Tan[b], Amber A. Rivera[b], Puay-Wah Phuan[b], Marina E. Shatskikh[a], Jung-Ho Son[a], Peter M. Haggie[b], Alan S. Verkman[b], Mark J. Kurth[a*]

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ABSTRACT

Pendrin is a transmembrane chloride/anion antiporter that is strongly upregulated in the airways in rhinoviral infection, asthma, cystic fibrosis and chronic rhinosinusitis. Based on its role in the regulation of airway surface liquid depth, pendrin inhibitors have potential indications for treatment of inflammatory airways diseases. Here, a completely regioselective route to tetrahydropyrazolopyridine pendrin inhibitors based on 1,3-diketone and substituted hydrazine condensation was been developed. Structure-activity relationships at the tetrahydropyridyl nitrogen were investigated using a focused library, establishing the privileged nature of *N*-phenyl ureas and improving inhibitor potency by greater than 2-fold.

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Pendrin (PDS) is a 780 amino acid sodium-independent chloride/anion antiporter containing twelve putative transmembrane spanning domains and cytoplasmic amino and carboxy-termini.[1] The PDS gene (*SLC26A4*) was identified by positional cloning in subjects with the autosomal recessive disorder Pendred syndrome, which is characterized by hearing impairment and thyroid goiter.[2] Functional studies show that PDS mediates electroneutral exchange of Cl^- with various anions including I^-, HCO_3^-, OH^-, and SCN^- at the apical membrane of epithelial cells in the inner ear, thyroid, kidney, airways, and adrenal gland.[1, 3-8] PDS upregulation is observed in the airways of humans with rhinovirus infection, asthma, cystic fibrosis and chronic rhinosinusitis, in rodent models of inflammatory pulmonary disease including asthma, infection, and toxin exposure, and in airway epithelial cultures after exposure to inflammatory cytokines.[9-19] PDS knockout reduces pathology in various mouse models of inflammatory lung diseases.[15, 20] The mechanism of PDS involvement in pulmonary inflammation is thought to involve regulation of airway surface liquid (ASL) volume. Small molecule PDS inhibitors increase airway hydration in cytokine-stimulated human airway epithelial cultures.[21] Together, these studies support the therapeutic utility of PDS inhibitors for lung diseases including asthma and cystic fibrosis.[22, 23]

Prior screening of 36,000 synthetic, drug-like small molecules identified several chemical classes of PDS inhibitors containing a tetrahydro-1*H*-pyrazolo[4,3-c]pyridine (TPP) core, with the most potent compound having an IC_{50} of ~7 μM for both human and murine PDS (Fig 1A).[21, 24] Analysis of commercially available TPP pendrin inhibitor analogs revealed minimal opportunities for modification of the pyrazole nitrogen or ether oxygen.[21] While substitutions at the tetrahydropyridyl nitrogen were relatively underexplored in the commercial library, the sulfonamide moiety – one of the most privileged functional groups in drug discovery[25] – was prevalent in PDS and *SLC26A3* inhibitors discovered by high-throughput screening (Fig 1B).[21, 26] We reasoned that developing a versatile route to TPP compounds would be valuable because chemical synthesis affords us the opportunity to generate a focused library at the tetrahydropyridyl nitrogen. Herein, we report the development of a completely regioselective route to the TPP core and structural analogs to further investigate structure–activity relationships for this class of compounds.

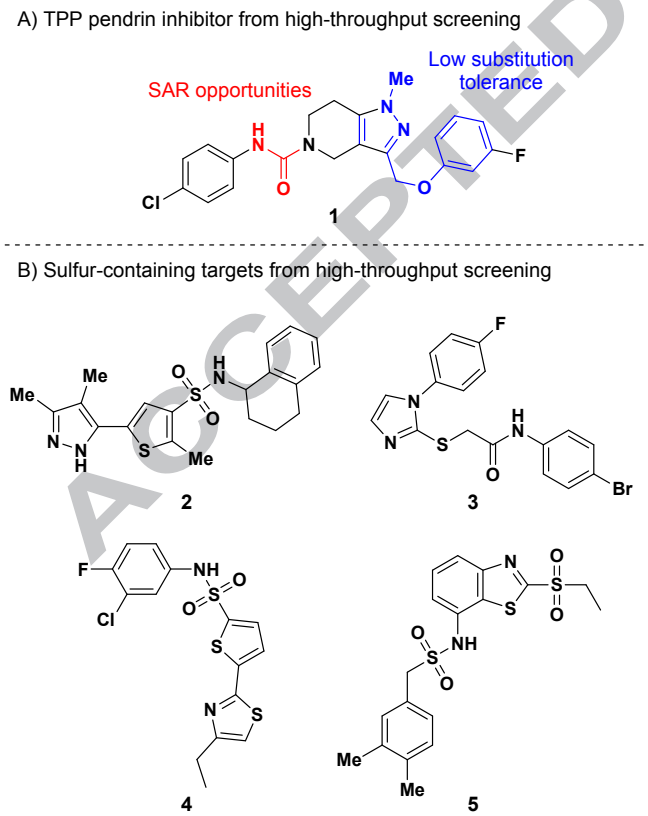
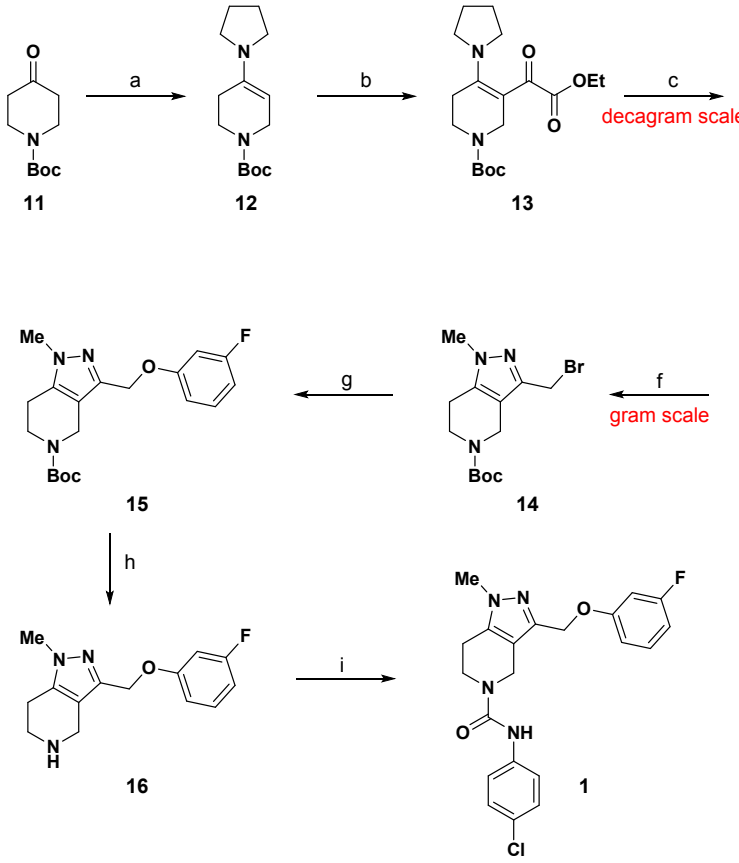


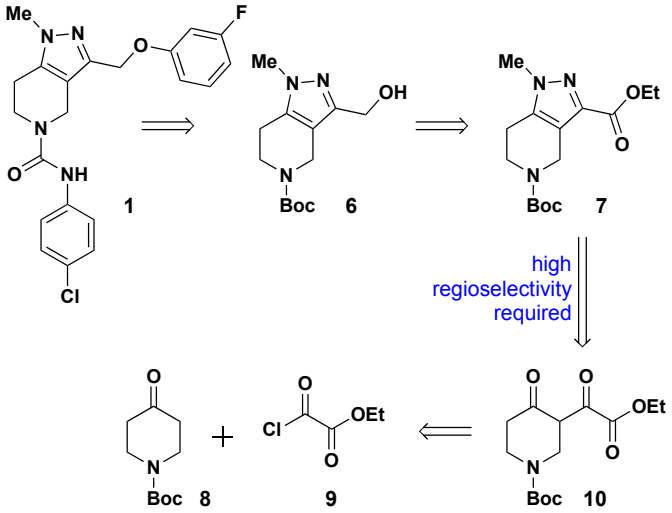
Fig 1. Previously identified inhibitors of *SLC26A4* and *SLC264A3*

Work began by resynthesizing the original active compound (**1**). However, controlling the position of the *N*-methyl group was a major challenge due to regioselectivity issues associated with pyrazole synthesis.[27] Indeed, it is known that pyrazoles do not undergo selective *N*-alkylations and the reaction of *N*-substituted hydrazine with unsymmetrical 1,3-diketones is also not regioselective. Despite these challenges, the Knorr pyrazole synthesis remains one of the most robust and reliable methods for accessing these nitrogen heterocycles.[27] In order to address this regioselectivity problem, we took advantage of the benzylic CH_2–O moiety in the lead compound (Scheme 1). This moiety allowed us to attempt the Knorr pyrazole synthesis using an 1,3-diketone with an electron withdrawing group attached (**10**). Theoretically, the presence of the electron withdrawing group should activate the α-carbonyl, allowing selective pyrazole formation to occur.

Scheme 2. Regioselective synthesis of the lead compound.



Scheme 2. Regioselective synthesis of the lead compound. Reagents and conditions: (a) pyrrolidine, toluene, Dean-Stark, reflux; (b) ethyl chlorooxoacetate, Et_3N, DCM, rt; (c) DCM, 1 M HCl, rt, 85% over 3 steps; (d) methylhydrazine, EtOH, reflux, 52%; (e) $LiBH_4$, Et_2O, reflux, 66%; (f) PPh_3, NBS, DCM, rt, 84%; (g) 3-fluorophenol, Cs_2CO_3, DMF, 90 °C, 90%; (h) TFA, DCM, rt; (i) 4-chlorophenyl isocyanate, DCM (anh), rt, 77% over 2 steps.



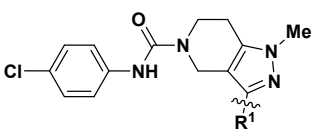
Scheme 1. Retrosynthetic analysis.

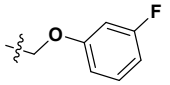
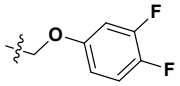
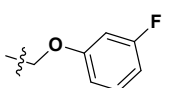
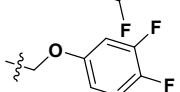
The synthesis of **1** (Scheme 2) began with a Stork enamine synthesis between *N*-Boc piperidin-4-one and ethyl 2-chloro-2-oxoacetate. Hydrolysis of **13** using a biphasic system of DCM and aqueous HCl gave the ester-containing 1,3-diketone **10** in 85% yield over 3 steps at 25 g scale. Next, methylhydrazine was refluxed with **10** to deliver pyrazole **7** as the only regioisomer in 52% yield. Note, the reaction can also be stopped after just one day of reflux, but the yield of **7** decreases to 33%. Increasing the reaction time to three days did not give a higher yield of **7**. Routine purification of pyrazole **7** was straightforward because the undesired regioisomer was not formed/detected throughout the course of the reaction. This reaction was repeated on large scale and the structure of **7** was confirmed using X-ray crystallography (Scheme 2). Subsequent reduction of **7** using $LiBH_4$ gave the corresponding alcohol in 66% yield. Alcohol **6** was converted to bromide **14** in 84% yield using an Appel reaction.[28] It should be noted that NBS could be substituted completely by Br_2 with little impact on the yield of the Appel reaction. Reaction of 3-fluorophenol with **14** under basic conditions gave the targeted ether in 90% yield. The Boc group of **15** was more resilient than expected, as deprotection using varying concentrations of HCl ranging from 1 to 6 M was ineffective in both methanol and dioxane. Boc deprotection was finally accomplished using TFA in DCM, followed by a basic work up to give amine **16**. Reaction of **16** with 4-chlorophenyl isocyanate gave the lead compound **1** in 77% yield over the last two steps. The synthesized **1** had a similar potency compared to commercial **1** (Figure 2).

As previously described, a functional cell-based assay of PDS-mediated Cl^-/I^- exchange was used to measure the PDS inhibition activity of TPP analogs.[21, 24] In brief, Fischer rat thyroid cells stably expressing murine PDS and a halide-sensitive fluorescent protein (EYFP-H148Q/I152L/F46L) were used. PDS activity was determined from the kinetics of fluorescence decrease in response to addition of an I^--containing solution to cells, with inhibitors reducing the rate of Cl^-/I^- exchange and hence the rate of reduction in fluorescence.

A small set of ether analogs were synthesized to address the effect of fluorine substitution (Table 1). These minor modifications improved PDS inhibition activity compared to compound **1**. Analogs **17** and **18** inhibited PDS with IC_{50} of 4.6 and 3.3 μM, while 3,5-difluoro-substituted **19** resulted in an IC_{50} of 3.1 μM. Ester analog **20** and carboxylic acid analog **21** both had minimal PDS inhibition activity.

Table 1. Synthesized TPP with varying substitution at benzylic position and activity against PDS.



Comp.	R^1	PDS IC_{50}
1		6.6 μM
17		4.6 μM
19		3.1 μM
18		3.3 μM
20		

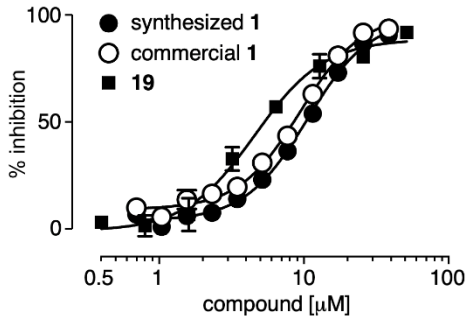
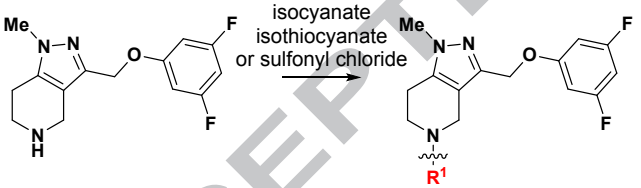
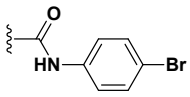
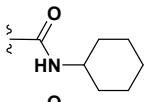
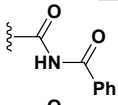
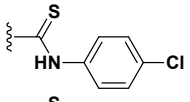
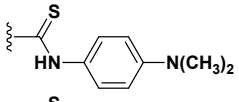
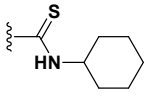
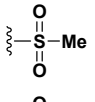


Fig 2. Concentration-dependence for inhibition of pendrin anion exchange by synthesized and commercial **1**, and by **19**.

Moving forward, the effect of substitution at the tetrahydropyridine nitrogen was investigated (see Fig 1A). Although previous high-throughput screening revealed that sulfonamides were common structural features among hit compounds, minimal inhibition activity was observed when sulfonamides were introduced into the TPP scaffold even when the 4-chlorophenyl moiety was preserved (Table 2). Similarly,

Table 2. TPP with different substitution patterns at tetrahydropyridyl nitrogen and activity against PDS.



Comp.	R^1	PDS IC_{50}
22		3.3 μM
23		>10 μM
24		>10 μM
25		>10 μM
26		>10 μM
27		>10 μM
28		>10 μM
29		>10 μM

attempts to incorporate sulfur into the molecule *via* thioureas were not successful at increasing PDS inhibition activity. This series of analogs gave insights into the privileged nature of the *N*-phenyl urea, as both alkyl and acylated ureas performed poorly.

In summary, we developed a completely regioselective route to tetrahydropyrazolopyridines and synthesized a focused library of analogs with substitution at the tetrahydropyridyl nitrogen. This chemistry allowed us to further investigate the structure-activity relationship of this class of PDS inhibitors. Introduction of an additional fluorine atom significantly improved the IC_{50} from ~7 μM to ~3 μM. Although the sulfur atom was well-represented in other classes of SLC26 inhibitors, sulfur analogs such as thioureas, sulfonamides, and sulfuric diamide were not useful for PDS inhibition activity. Indeed, throughout the course of these studies. *N*-phenyl ureas were found to be highly privileged.

Acknowledgments

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Supplementary Material

Supplementary data containing detailed synthesis procedures, ^{1}H, ^{13}C, and crystallography information (X-ray coordinates have been deposited with the Cambridge Crystallographic Data Centre under CCDC 1913463).

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