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Amidine derived inhibitors of acid-sensing ion channel-3 (ASIC3)

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ABSTRACT

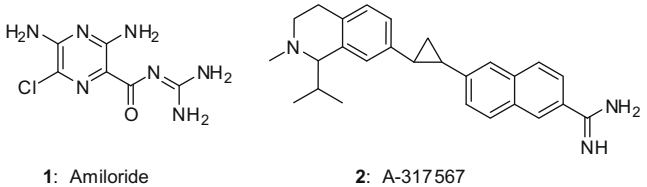
A series of indole amidines modified at the 2-position of the indole ring were evaluated as inhibitors of Acid-Sensing Ion Channel-3 (ASIC3), a novel target for the treatment of chronic pain.

Despite the presence of a multitude of analgesic medications on the market, chronic pain is a condition that continues to afflict a significant portion of the population. Chronic pain can result from different pathways including inflammation associated with osteoarthritis and direct damage to the nervous system in the form of neuropathic pain. Because of this, it is imperative that the search for new analgesics to treat chronic pain continues.[1]

The neurons in the central nervous system contain a number of ion channels and receptors that are sensitive to acidosis.[2] Prolonged illness or trauma often leads to a drop in pH, which activate these signaling pathways.[3] It is widely accepted that one of these classes of ion channels is the vanilloid receptor subtype-1 (VR1), which is sensitive to a variety of stimuli,[3] and is activated under tissue acidification to pH of less than 6.0.[4] Consequently, a number of VR1 antagonists are currently being examined in clinic trials for the treatment of pain.[5,6]

A second class of pH sensitive ion channels is the acid-sensing ion channels (ASICs), which are members of the proton-gated epithelial Na^+/degenerin superfamily.[7] There are currently seven known ASICs subunits (ASIC1a/b, ASIC1b2, ASIC2a/b, ASIC3, and ASIC4) that are susceptible to activation by very modest changes in pH. In some cases, the ASICs can even detect subtle decreases in extracellular pH from 7.4 to 7.0.[8] Studies have demonstrated that increased ASIC activity and expression can result from a number of inflammatory mediators.[9] Thus, similar to the VR1 class of ion channels, the ASICs are also believed to act as messengers of pain resulting from acidosis.[10,11] The ASIC3 channel, in particular, is highly expressed in nociceptors[12,13] and in vivo studies in ASIC3 knock-out mice[14] have suggested that the ASIC3 channel may be a viable target for the blocking of chronic inflammatory pain.

Few small molecule inhibitors of ASIC channels have been reported, hindering the further understanding of the physiological role of ASIC3. The potassium sparing diuretic agent amiloride **1** has been shown to be a non-selective inhibitor of ASIC channels[15] and exhibits a modest effect in rat pain models at very high plasma concentrations.[16] We recently reported an SAR study for a series of modified amiloride derivatives that exhibited enhanced activity for ASIC3, but suffered from poor pharmacokinetic properties.[17] A group from Abbott has previously shown that amidine A-317567 **2** is a much more potent blocker of ASIC3 than amiloride (Fig. 1).[18] In addition, A-317567 showed full efficacy in the rat Complete Freud's Adjuvant (CFA)-induced inflammatory thermal



1: Amiloride **2**: A-317567

Figure 1. Known ASIC channel blockers.

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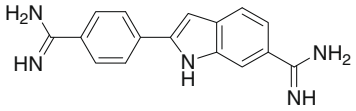


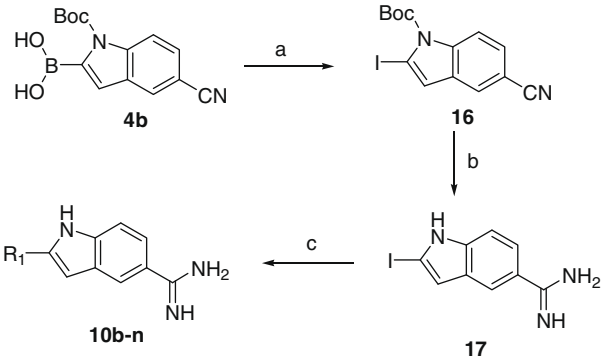
3: MW = 277
ASIC3 Inhibition: 33% @ 1 μM

Figure 2. Lead compound **3**.

hyperalgesia model and a skin incision model of post-operative pain.

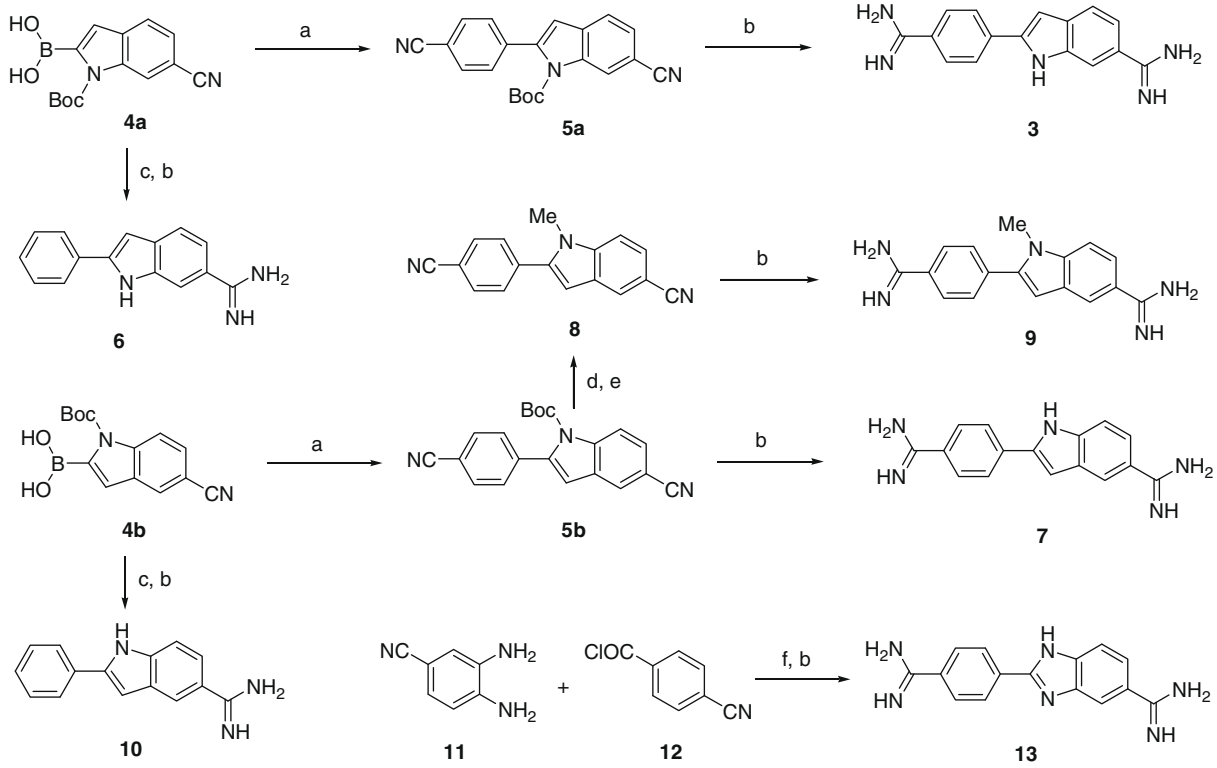
Identifying new small molecule leads as inhibitors of ASIC3 has been impaired by the lack of an effective high-throughput assay to screen for hits. All biochemical evaluation of ASIC3 inhibition has been carried out using labor intensive electrophysiology studies, thus precluding a large HTS campaign as a means of identifying lead structures. Upon further analysis of compound **2**, we proposed the amidine moiety as a critical requisite for ASIC3 activity. Accordingly, the MRL sample repository was mined for aryl amidines that were subsequently evaluated in the ASIC3 electrophysiology assay. Indole bis-amidine **3** was identified and proved to be a relatively potent, low MW lead compared to compound **2**, and this communication will describe the preliminary SAR for this compound class (Fig. 2).

The synthesis of **3** and related analogs is shown in Schemes 1 and 2. Commercially available indole boronic acids **4a** and **4b** served as the starting points. Suzuki cross-coupling of **4a, b** with 4-cyanophenyl iodide affords **5a, b**. These may be directly converted to the corresponding bis amidines **3** and **7** via generation of the *O*-ethyl imidates under acidic conditions followed by reaction with ammonia. The mono amidine products **6** and **10** can be accessed via the same route by using iodobenzene in the Suzuki step with subsequent amidine formation. The Boc group in indole **5b** can be removed and then undergo a base mediated methylation to provide **8**, which can then proceed to bis amidine **9**. Finally, the benzimidazole bis-amidine **13** is prepared via condensation of diamine **11** with acid chloride **12** followed by amidine formation under standard conditions.



Scheme 2. Reagents and conditions: (a) NIS, CH_3CN. (b) (i) HCl, EtOH, 80 °C; (ii) NH_3–EtOH, rt to 45 °C. (c) $Pd(dppf)Cl_2$, K_2CO_3, DMF, $R_1B(OH)_2$, 80 °C.

In order to facilitate a higher throughput synthesis of substituted analogs of **10**, an alternative approach was taken as shown in Scheme 2. Iodide **16** was sought as a useful intermediate, but attempts to prepare it via direct metallation of the 2-position of *tert*-butyl-5-cyano-indole-1-carboxylate followed by quenching with an iodide source were largely unsuccessful. However, conversion of indole boronic acid **4b** directly to iodide **16** could be effected using *N*-iodosuccinimide (NIS) in CH_3CN in near quantitative yield.[19] Amidine formation with concomitant removal of the Boc group afforded iodide **17**. Subsequent Suzuki couplings with the appropriate boronic acids afforded analogs **10b–n** in a single step.



Scheme 1. Reagents and conditions: (a) $Pd(dppf)Cl_2$, K_2CO_3, DMF, 4-iodobenzonitrile, 80 °C; (b) (i) HCl, EtOH, 80 °C; (ii) NH_3–EtOH, rt to 45 °C; (c) $Pd(dppf)Cl_2$, K_2CO_3, DMF, iodobenzene, 80 °C; (d) $HCl_{(g)}$, EtOAc, 0 °C; (e) NaHMDS, MeI, DMF; (f) BF_3–OEt_2, dioxane, 0–120 °C.

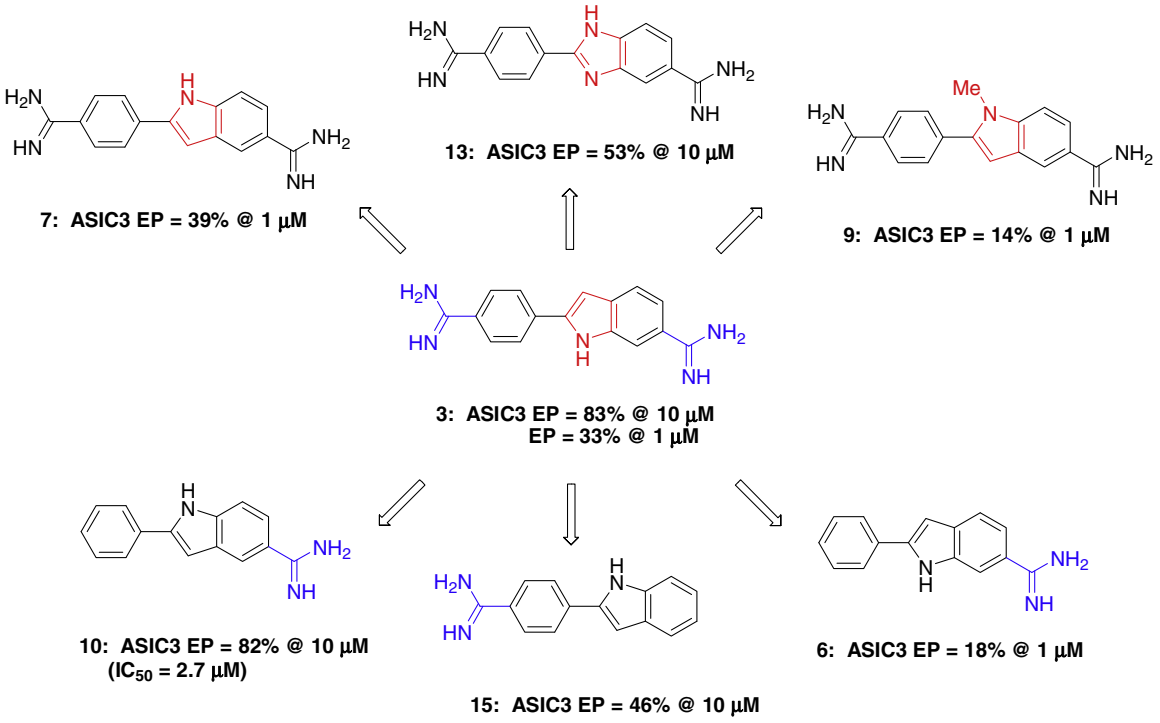
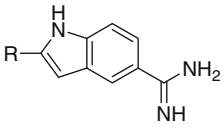


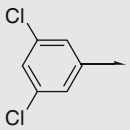
Figure 3. SAR of amidines.

The initial SAR exploration for select amidine structures is shown in Figure 3. For this initial analysis, compounds were screened in the electrophysiology assay at 10 and/or 1 μM concentrations.[20] The indole isomer of lead **3** that has the amidine at the 6-position of the indole ring, bis-amidine **7**, gave slightly greater channel block at 1 μM. The *N*-methyl analog **9** and benzimidazole **13** were both markedly less potent compared to **7**. The evaluation of having a single amidine group present was also examined. Removal of the amidine on the indole (**15**) significantly reduced channel block inhibition relative to the amidine on the phenyl ring (**6** and **10**). In these latter cases, having the amidine at the 6-position of the indole (**10**) gave the compound with better potency, consistent with the preference observed with bis amidine **7**. In fact, **10** was the most potent mono-amidine ASIC channel blocker identified with an ASIC3 IC_{50} = 2.7 μM. As a result, all further SAR investigation was carried out using **10** as the core scaffold for exploration. It should also be noted that the primary amidine was a requirement. Attempts to add substituents onto the amidine moiety present in compound **10** via alkylation, acylation, and hydroxylation all had deleterious effects on ASIC3 inhibition (data not shown).

A library approach was employed using the chemistry described in Scheme 2 to investigate SAR of the aryl group at the 2-position of the indole. It was quickly found that a phenyl ring was required (data not shown) with a strong preference for 3,5-substituted aryl rings. The SAR analysis with this substitution pattern is shown in Table 1. Compounds were screened initially in the electrophysiology assay at 10 and/or 1 μM, followed by a five point titration.

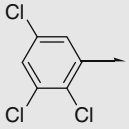
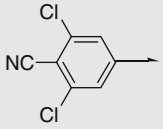
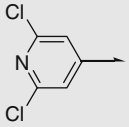
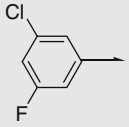
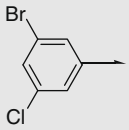
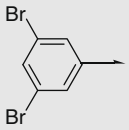
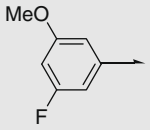
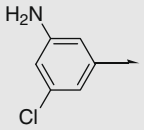
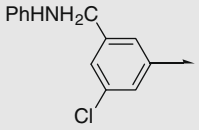
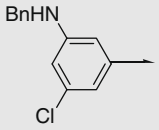
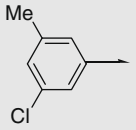
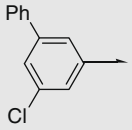
Table 1
Inhibition of ASIC3 by select amidine derivatives **10a–k**



Compd	R	% Inh. @ 1 μM	% Inh. @ 10 μM	IC_{50} (nM)[a]
a		nd	88	2765
b		92	98	133

(*continued on next page*)

Table 1 (*continued*)

Compd	R	% Inh. @ 1 μM	% Inh. @ 10 μM	IC_{50} (nM)[a]
c		91	nd	135
d		83	nd	272
e		84	nd	291
f		82	99	278
g		93	96	59
h		92	92	59
i		77	98	374
j		78	nd	95
k		83	nd	114
l		91	nd	77
m		87	nd	51
n		94	nd	nd

[a] Electrophysiology recording, inhibition of current was expressed as percent inhibition of peak current vs. baseline peak current. $N = 3$.

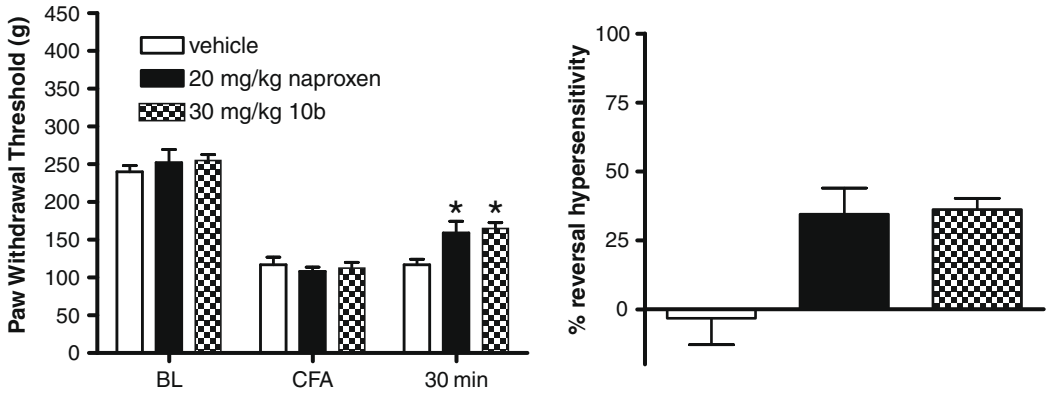


Figure 4. Effect of **10b** in the rat CFA inflammatory pain model (left); data represented as % reversal of mechanical hypersensitivity (right); p <0.05 vs vehicle.

Highlighting the preference for meta-substitution, the 3,5-dichloro analog **10b** was ~20-fold more potent (ASIC3 IC_{50} = 133 nM) than the phenyl comparator **10a**. Placement of additional groups on **10b** at the ortho (**10c**) or para-positions (**10d**) had no effect or led to a decrease in channel inhibition. Similarly, insertion of a pyridine (**10e**) led to a modest twofold drop in activity. The lipophilicity of the substituents also appears to have effects on activity. For example, exchanging one of the chlorines with a fluorine (**10f**), led to a decrease in activity, while replacement with a bromine (**10g**) led to one of the most potent compounds observed (IC_{50} = 59 nM). Addition of a second bromine (**10h**) did not provide any further improvements. Interestingly, as shown with compounds **10i–n**, the meta position is remarkably tolerant of substitution so long as the other meta group has a halogen (ideally chlorine) in this position.

In order to more fully characterize this indole amidine class of ASIC3 channel blockers, 3,5-dichloro analog **10b** was examined in vivo in the rat Complete Freud's Adjuvant (CFA) model of inflammatory pain.[21] In this study, **10b** showed a robust reversal of mechanical hypersensitivity 30 min post-dosing in male Sprague–Dawley rats at 30 mg/kg ip that was comparable to the NSAID naproxen (dosed at 20 mg/kg po). Interestingly, when plasma[22] and brain were analyzed for compound levels of **10b** at 30 min, all results were below the level of quantification (Fig. 4).

To follow up on this result, a rat pharmacokinetic study was carried out. Compound **10b** was not orally bioavailable, which was not necessarily unexpected due to the presence of the amidine moiety.[23] The iv plasma pharmacokinetics showed very high clearance (Cl = 52 ml/min/kg) with a short half-life ($t_{1/2}$ = 2.0 h). However, analysis of the blood samples showed lower clearance (Cl = 8.5 ml/min/kg) with a more moderate half-life ($t_{1/2}$ = 4.4 h) indicating that **10b** was partitioning into red blood cells.

In summary, screening a sub-set of the MRL sample repository for aryl amidines led to the discovery of indole bis-amidine **3** as a low MW, modest ASIC channel blocker. SAR analysis quickly led to the identification of mono-amidine **10b** as a potent (IC_{50} = 133 nM) ASIC3 channel blocker that exhibited efficacy similar to that of naproxen in the rat CFA model of mechanical hyperalgesia. This indole amidine class of compounds does not possess any oral bioavailability in rat and also exhibits high partitioning into red blood cells. Accordingly, efforts have been initiated to replace the amidine moiety to improve upon these latter two issues, and results will be reported in due course.

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20. Acid-evoked (pH5.5 for 3 s) current was recorded at −60 mV using an automated patch clamp instrument (PatchXpress, MDS, Inc.). The intracellular solution contained (mM): 119 K-gluconate, 15 KCl, 3.2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 K2ATP, pH 7.3. The extracellular solution contained (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 12 Dextrose, and 10 HEPES (pH 7.4) or 10 MES (pH5.5). Compounds were applied 120 s prior to acid application. Peak current following compound incubation was expressed as a fraction of the control (vehicle) peak current. IC_{50} values were determined by fitting data to the Hill equation.
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