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Identification of non-amidine inhibitors of acid-sensing ion channel-3 (ASIC3)

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ABSTRACT

A series of benzothiophene methyl amines were examined in an effort to identify non-amidine chemotypes with reduced polypharmacology from existing leads with the goal of finding potent ASIC3 channel blockers to advance the therapeutic evaluation of ASIC3 inhibition.

A significant medical need remains for new analgesics[1] to treat those who suffer from chronic pain due to damage or persistent inflammation of neuronal or somatic tissue, and the co-morbidities associated with it. Under conditions of tissue damage, acidosis often occurs and contributes to pain.[2] Highly sensitive proton-activated ion channels are present in sensory neurons and are activated by tissue acidification.[3] Acid-sensing ion channels (ASICs) represent a proton-activated subgroup of the degenerin/epithelial Na^+ channel family.[4] Evidence indicates that ASICs serve as proton sensors and play an important role in conveying the pain from tissue acidosis.[5,6] The ASICs are very sensitive, and depending on the exact composition of the subunits, can detect an extracellular pH decrease from 7.4 to 7.0.[7] A number of inflammatory mediators enhance ASIC activity and expression and further implicate neuronal ASICs as key players in pain from tissue acidosis.[8]

Several ASIC subunits (ASIC1a/b, ASIC1b2, ASIC2a/b, ASIC3, and ASIC4) have been identified that are encoded by four genes.[9] Channels containing the ASIC3 subunit have received particular interest as a target for blocking chronic inflammatory pain as they are highly abundant in nociceptors where they may act as sensors for pain from tissue acidosis.[10,11] In support of this, mice lacking the ASIC3 channel do not develop chronic muscle pain from repeated administration of acid, while ASIC1 knock-out mice are similar to wild-type.[12]

The dearth of potent and selective small molecule inhibitors of ASIC channels has hampered elucidation of the physiological role of ASIC3. Amiloride **1** is a potassium sparing diuretic agent that is also a weak, non-selective inhibitor of ASIC channels,[13] for which we have previously examined the ASIC3 SAR.[14] In addition, we reported on the identification of amidine derived ASIC3 inhibitors such as indole **2**[15] and naphthalene **3**, which is an analog of A-317567[16] (Fig. 1).

While ASIC3 has generated significant interest in the pain therapeutic field, ASIC1a activity has been associated with anxiety, a

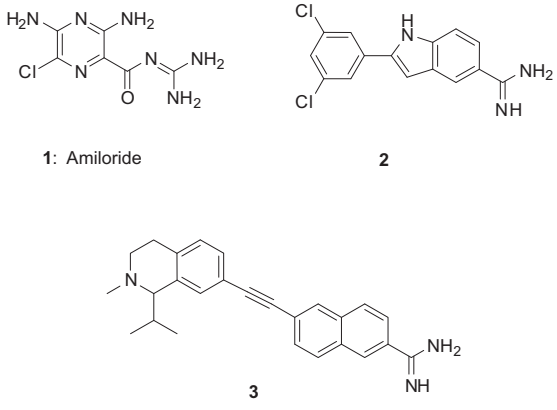

Figure 1. Known ASIC channel blockers.

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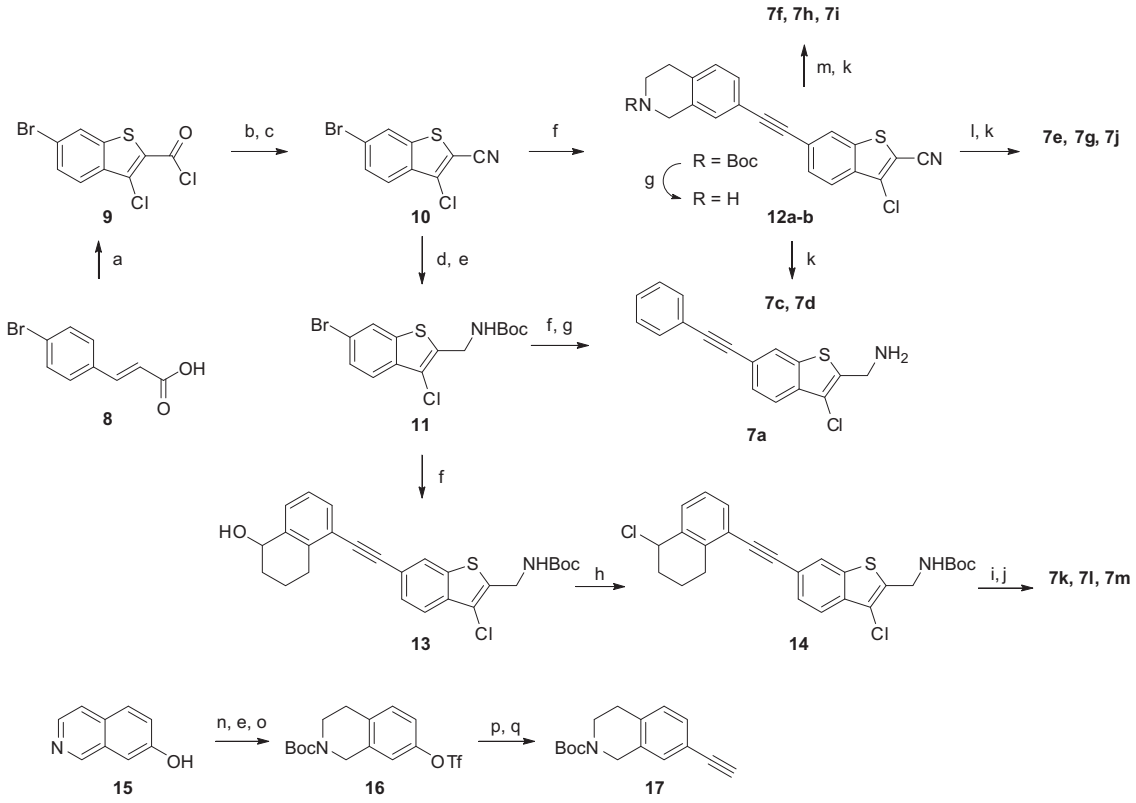
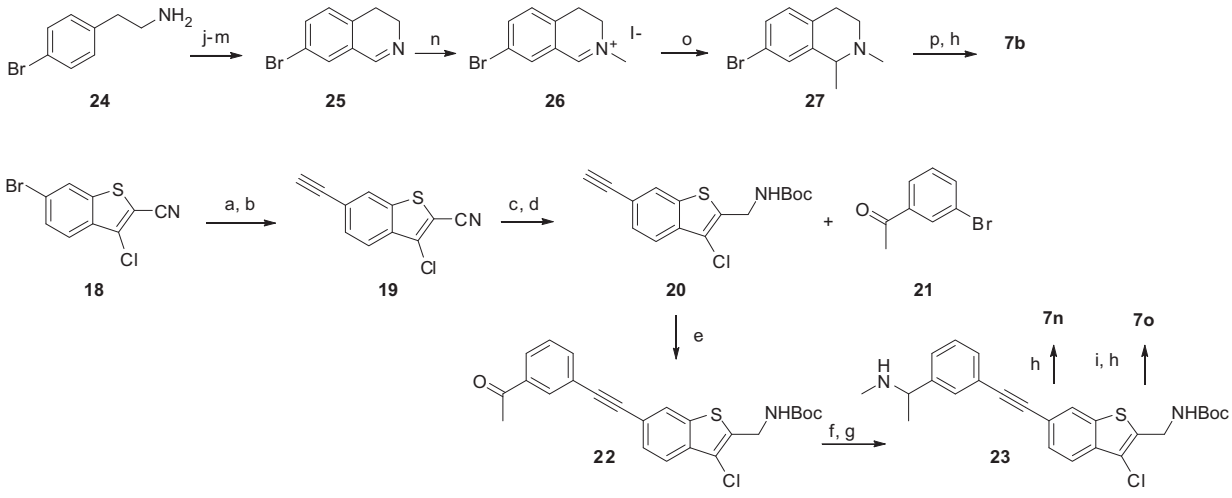


Scheme 1. Reagents and conditions: (a) $SOCl_2$, pyridine, chlorobenzene, 50→115 °C; (b) NH_3, MeOH; (c) TEA, TFAA, CH_2Cl_2; (d) BH_3–THF; (e) $(Boc)_2O$, CH_2Cl_2; (f) alkyne, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF; (g) 4 N HCl in dioxane, CH_2Cl_2; (h) $SOCl_2$, CH_2Cl_2; (i) NHR_1R_2, K_2CO_3, CH_3CN; (j) HCl, EtOAc; (k) LAH, THF, −40 °C; (l) RCHO, AcOH, TEA, $NaBH(OAc)_3$; (m) RX, TEA, CH_2Cl_2; (n) PtO_2, H_2, AcOH; (o) Tf_2O, CH_2Cl_2; (p) trimethylsilylacetylene, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, 80 °C; (q) TBAF, THF.



Scheme 2. Reagents and conditions: (a) Trimethylsilylacetylene, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, 80 °C; (b) TBAF, THF; (c) LAH, THF, −40 °C; (d) $(Boc)_2O$, CH_2Cl_2; (e) $Pd(Ph_3P)_4$, CuI, Et_3N, DMF; (f) $Ti(OiPr)_4$, NH_2Me, MeOH; (g) $NaBH_4$, MeOH; (h) 4 N HCl in dioxane, CH_2Cl_2; (i) RCHO, AcOH, TEA, $NaBH(OAc)_3$; (j) ethyl formate, reflux; (k) oxalyl chloride, CH_2Cl_2; (l) $FeCl_3$, −20 °C; (m) concd H_2SO_4, MeOH, reflux; (n) MeI, CH_3CN; (o) MeMgCl, THF, −78 °C; (p) alkyne **20**, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF.

symptom that can often accompany depression-like behaviors.[17,18] Wemmie and co-workers disclosed that A-317567 also blocks ASIC1a and produced antidepressant effects in mice when dosed intracerebroventricularly.[19] We recently reported the pharmacological characterization of **3**, a close molecular analog of A-317567, and questioned whether the observed antinociceptive effects attributed to ASIC3 may be due to off-target effects, potentially including, but not limited to other ASIC channel subtypes.[20] A common feature of the reported ASIC inhibitors in Figure 1 is the presence of an acylguanidine or amidine moiety, which may contribute to potential off-target effects. In addition, both series had poor pharmacokinetic properties. Accordingly, we wanted to identify non-amidine ASIC3 inhibitors that could serve as better tool compounds to explore the pharmacology of ASIC3.

The chemistry utilized to prepare requisite target molecules is highlighted in Schemes 1 and 2. Benzothiophene **9** was produced via thionyl chloride and subsequently can be converted in two steps to nitrile **10**. Sonogashira coupling of the bromide with acet-

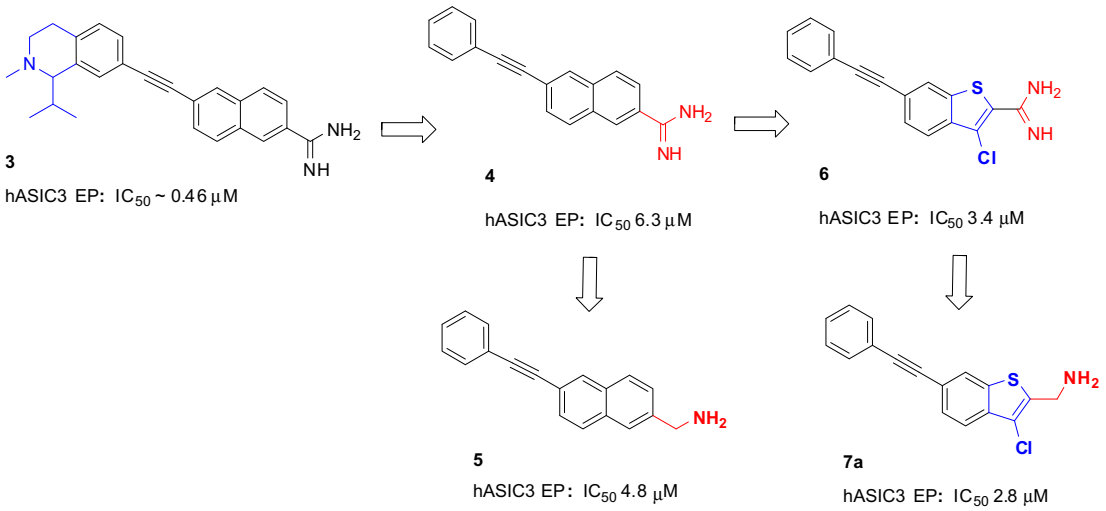


Figure 2. Derivation of amine and thiophene structures.

ylene **17** afforded Boc protected intermediate **12a**, which was readily converted to analogs **7c–j** as described. Alternatively, **10** was first reduced with borane followed by Boc protection to produce **11**, which underwent Sonogashira with the appropriate alkynes followed by deprotection to produce **7a** and **13**. Alcohol **13** was subsequently functionalized as described to afford **7k–m**.

In Scheme 2, bromide **27** was obtained via a four-step sequence with the key step involving addition of methyl Grignard to dihydroisoquinolinium salt **26**. Sonogashira coupling of **27** with alkyne **20** was followed by deprotection to afford **7b**. Finally, alkyne **20** was similarly utilized with bromide **21**, and after subsequent functionalization, **7n**,**o** were obtained.

The initial SAR exploration from amidine **3** began with truncated phenyl acetylene **4**, which lost ~14-fold potency relative to **3** (Fig. 2). The primary amine analog (**5**) of amidine **4** showed a slight improvement representing the first potent non-amidine structure. The chlorothiophene-amidine **6** represented a non-naphthalene template with ~2-fold improvement in ASIC3 inhibition. The hybrid of these two provided chlorothiophene primary amine **7a** with an ASIC3 IC_{50} = 2.8 μM, an improvement relative to naphthalene **5**.

With **7a** identified as a non-naphthyl, non-amidine starting point, SAR analysis was investigated, with a pictorial summary for the thiophene region shown in Figure 3. The primary amine was required as substitution directly on, or alpha to the nitrogen, led to a severe loss (>20×) in activity. The thiophene was required as indoles lost some activity, whereas furan derivatives were inactive. Lastly, the chlorine was required as polar or smaller groups were substantially weaker inhibitors. Based upon this analysis the right hand primary amine and chlorobenzothiophene of **7a** was maintained for all subsequent SAR analysis of the alkynyl region.

An SAR study was next conducted on the phenyl acetylene region of **7a** as shown in Table 1. Incorporation of the tetrahydroisoquinoline ring found in **3** provided a ~2-fold more potent

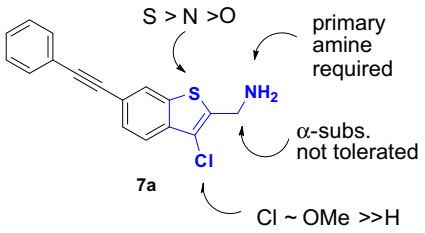


Figure 3. Thiophene SAR summary.

compound in the form of amine **7b**. For simplicity, SAR was conducted without the methyl group on the tetrahydroisoquinoline, which led to a very modest reduction in ASIC inhibition (**7e**, IC_{50} = 0.68 μM). Much of this could be recovered by placing a larger ethyl (**7f**; IC_{50} = 0.23 μM) or *i*Pr (**7g**; IC_{50} = 0.31 μM) on the nitrogen in lieu of the methyl. Having a basic amine proved critical as analogs **7c**, **7h–i** were considerably less active. Of note, the unsubstituted analog **7d** proved most potent with the additional benefit of high solubility.

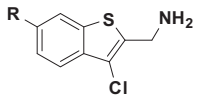
Alternative constraints were also studied and found to be tolerated (**7j–o**). The tetrahydronaphthalenes provided good inhibition, with the dimethyl analog (**7k**; IC_{50} = 0.23 μM) yielding similar activity to **7d**. The ring was critical as un-constrained dimethylamine **7n** lost greater than 16-fold relative to tetrahydronaphthalene **7k** and indene **7o**.

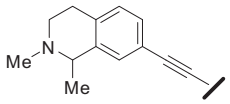
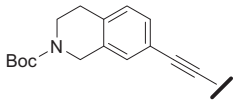
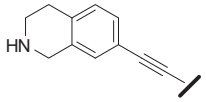
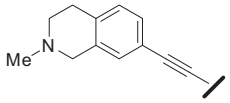
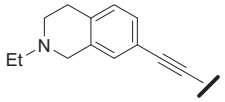
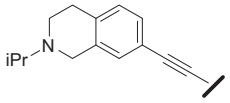
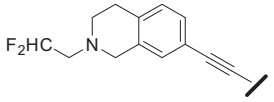
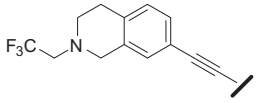
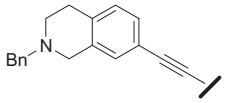
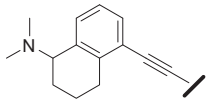
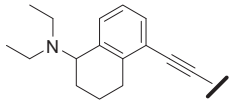
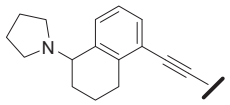
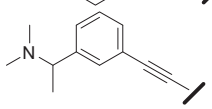
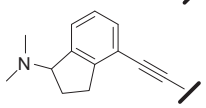
Based upon potency and good solubility properties, amine **7d** was evaluated further in the rat CFA model of inflammatory pain. In this study (Fig. 4), **7d** showed a robust reversal of mechanical hypersensitivity in the hind paw and decreased weight bearing 30 min post-dosing (ip) in male Sprague–Dawley rats. The magnitude of reversal was similar to that from the NSAID naproxen (dosed at 20 mg/kg po) at the 100 mpk dose of **7d**. The average plasma concentration[21] at 30 min was 3.6 μM at 100 mg/kg. Of note, very high levels in brain (14 μM) were observed at this dose, which unlike amidines **2** and **3** distinguishes amine **7d** as the only CNS penetrant ASIC3 antagonist we have identified and examined to date. However, rats treated with **7d** appeared sedated following administration, but were easily aroused and normal when handled, a phenomenon that has been observed previously in this model at an efficacious dose of amidine **3**.

Amine **7d** and related analogs also had exceptionally poor pharmacokinetic properties exhibiting super-hepatic clearance, particularly in dog. Upon further interrogation, it was realized that these compounds exhibited very high blood to plasma ratios, which complicates their analysis unlike their amidine counterparts. For example, amine **7d** had a blood to plasma ratio of 15.4 in dog, while amidine **3** gave a ratio of 2.6.

One of the complicating issues with amidine **3** as a tool compound was off-target activity. In addition to ASIC1a activity, **3** was evaluated against a number of receptors and enzymes (MDS Pharma Service, Taipei, Taiwan) and found to be highly promiscuous with binding affinity IC_{50}'s <10 μM against 39 targets that could contribute to the behavioral effects such as muscarinic, adrenergic, dopamine, norepinephrine, and serotonin receptors. Similar to amidine **3**, amine **7d** exhibited no selectivity for ASIC3 with an ASIC1a IC_{50} = 224 nM. In addition, **7d** was evaluated in the same

Table 1
ASIC3 titration of select amiloride derivatives



Compd	Structure	ASIC3 IC_{50}[a]
7b		0.27
7c		>50
7d		0.22
7e		0.68
7f		0.23
7g		0.31
7h		10.2
7i		>50
7j		17.1
7k		0.23
7l		0.51
7m		2.5
7n		4.3
7o		0.19

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

aforementioned MDS Pharma Service panel, and found to have binding affinities with IC_{50}'s <10 μM against 35 targets, with a substantial overlap with amidine **3**. Accordingly, the analgesic efficacy of non-selective ASIC3 antagonists as represented by these compounds, is further complicated by their CNS-related effects through ASIC1 or

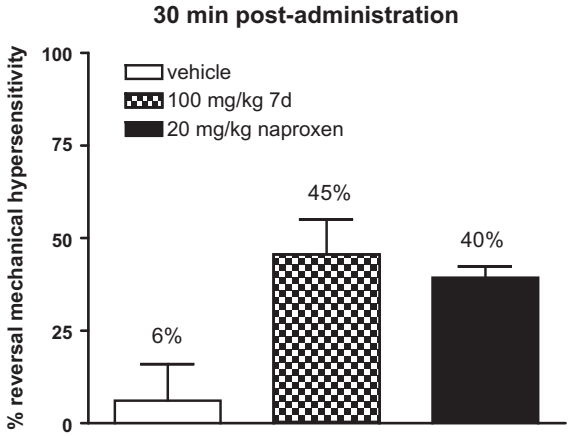


Figure 4. Evaluation of **7d** and naproxen in the rat CFA model of inflammatory pain at 30 min post-dosing. $^{*}p$ <0.05 (ANOVA).

by another mechanism altogether. This is particularly an issue for **7d** which is brain penetrant, unlike previously investigated ASIC3 antagonists.

In summary, amine **7d** was identified as a potent, non-amidine ASIC3 inhibitor with good brain penetration. However, **7d** also caused sedative or lethargic effects in the rat CFA model. In addition, poly-pharmacology for a number of targets including ASIC1a prevents this class from being pursued further. Accordingly the identification of an ASIC3 subtype-selective inhibitor lacking other off-target effects remains elusive as a tool to study the relationship between efficacy in pain models and the apparent peripheral sedating effects observed with non-selective compounds.

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21. Amine **7d** (brain to plasma ratio ~6) was found to be 100% bound to rat plasma proteins.