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

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ABSTRACT

A series of amiloride derivatives modified at the 5-position of the pyrazine ring were evaluated as inhibitors of acid-sensing ion channel-3 (ASIC3), a novel target for the treatment of chronic pain.

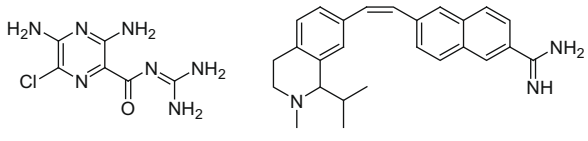
Chronic pain is the hallmark of a number of diseases that can arise from direct insult to the nervous system as seen in neuropathic pain, or by a number of inflammatory processes as is the case with osteoarthritis. Consequently, there remains significant medical need for new analgesics[1] to treat the ever increasing number of patients suffering from chronic pain and the co-morbidities associated with it.

Under conditions of acidosis, tissue damage can often result leading to acute or chronic pain.[2] A number of receptors and ion channels expressed in neurons have been shown to be modulated by protons.[3] The vanilloid receptor subtype-1 (VR1) represents one class of channel that is widely viewed as a major detector of multiple pain-causing stimuli,[4] and a number of highly potent VR1 antagonists are currently undergoing clinical evaluation.

The acid-sensing ion channels (ASICs) represent a proton-gated subgroup of degenerin/epithelial Na^+ cation channel family.[5] There is substantial evidence that the ASICs serve as pH sensors and play an important role in conveying the pain sensation resulting from tissue acidosis.[6,7] Unlike the VR1 channel which requires extreme acidification to a pH less than 6.0 for activation,[8] the ASICs are extremely sensitive, and depending on the exact composition of the subunits, can detect extracellular pH drops from 7.4 to 7.0.[9] In addition, a number of inflammatory mediators have been shown to lead to an enhancement of ASIC activity and expression.[10]

To date, seven subunits (ASIC1a/b, ASIC1b2, ASIC2a/b, ASIC3, and ASIC4) have been identified that are encoded by four genes.[11] Amongst these, the ASIC3 channel has received considerable interest as a target for blocking chronic inflammatory pain. For example, mice lacking the ASIC3 channel do not develop chronic muscle pain from repeated administration of acid, while ASIC1 knock-out mice were similar to wild-type.[12] Additionally, ASIC3 is highly abundant in nociceptors where it may act as a sensor for pain from tissue acidosis.[13,14]

The paucity of small molecule inhibitors of ASIC channels has hindered elucidation of the physiological role of ASIC3. The potassium sparing diuretic agent amiloride, **1**, is a non-selective inhibitor of ASIC channels[15] and exhibits a modest effect in rat pain models at high concentrations[16] (Fig. 1). Abbott more recently reported that amidine A-317567 (**2**), was a more potent blocker of ASIC3 than amiloride.[17] This compound is effective in the rat



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

Figure 1. Known ASIC channel blockers.

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Complete Freud's Adjuvant (CFA)-induced inflammatory thermal hyperalgesia model and skin incision model of post-operative pain.

The lack of an effective high-throughput assay to screen for ASIC inhibition has impaired identification of new small molecule ASIC3 inhibitors. To minimize the use of labor intensive electrophysiological assays, we developed an alternative approach by preparing more potent amiloride derivatives. As a first step, the MRL sample repository was examined for analogs of amiloride. These derivatives were subsequently evaluated on ASIC3 channels at a single dose using an automated patch clamp assay.[18] Compounds of interest were followed up at additional doses. An overview of the key findings is shown in Figure 2.

The 2-acylguandine and 3-amino groups on the pyrazine ring were required for inhibition of ASIC3. Aryl or alkyl groups extending off of the guanidine led to decreases in efficacy. The 6-chloro group was only slightly favored over just having hydrogen present at this position. The 5-position seemed quite tolerant of change and as a result became the primary region to explore SAR.[19]

The chemistry utilized to prepared amiloride derivatives is shown in Scheme 1. Methyl ester **3**[20] was treated with guanidine in isopropanol to afford acylguanidine **4**. Displacement of the 5-chloro group in **4** with the appropriate amines, thiols, or phenols affords amiloride analogs **5a–i′, 6a–l**.

Palladium catalyzed cross couplings were investigated on **4** to prepare 5-carbon-linked analogs, but proved to be unselective for the chlorines at the 5- and 6-positions leading to a mixture of products. Accordingly, the SAR for 5-carbon-linked analogs was investigated using the 6-des-chloro starting material since only a very modest (less than twofold) drop-off in activity was noted without this halogen present. When chloropyrazine **8**, which was prepared in two steps starting from commercially available **7**, was treated with guanidine, the major product resulted from a displacement of the 5-chlorine and not from reaction with the methyl ester to form the acyl guanidine. This was surprising since this transformation of the dichloro variant **3** to **4** favored attack at the ester position. To circumvent this issue, Suzuki coupling was first carried out on **8**, followed by treatment with guanidine to produce 6-des-chloro amiloride derivatives **9a–k**.

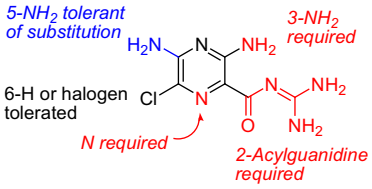


Figure 2. Amiloride ASIC3 SAR.

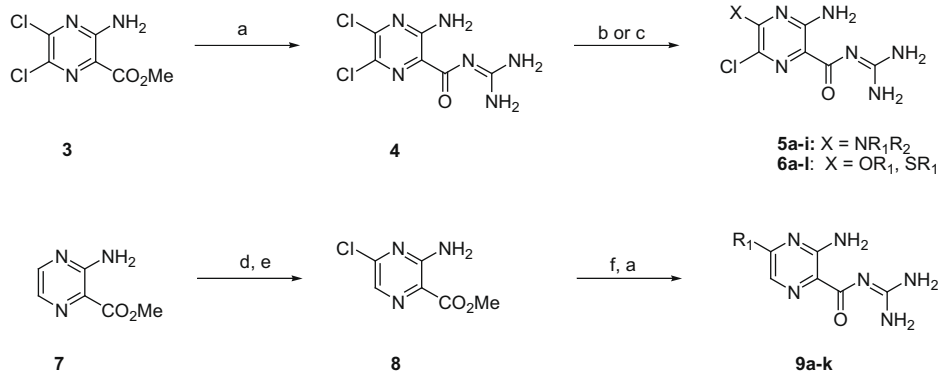
A variety of amines (>80) were employed in a library approach to evaluate the SAR at the 5-position of the pyrazine, and results for selected amiloride analogs are shown in Table 1. As noted previously, due to the lack of a high-throughput biochemical assay, analogs were evaluated for percent inhibition at 1 or 20 μM using patch clamp electrophysiology.[21] In general, aliphatic groups had enhanced inhibition relative to amiloride **1**, shown for comparison purposes. Primary (**5a–g**) or secondary amines (**5h–o**) were similar in efficacy, while too much steric bulk had a deleterious effect (**5c**).

Aromatic amines had moderately higher inhibition than their aliphatic counterparts as exemplified by aniline **5r**. The SAR for substituent groups placed on the aniline ring was generally flat, although reduced lipophilicity was an important factor with pyridines **5u–v** showing less activity. The most potent compound was naphthalene **5w** with 62% inhibition at 1 μM, with related loss of potency with the more polar quinolines (**5x–y**). A number of benzylamines (**5z–5i′**) were also of interest, but again, flat SAR was observed for more functionalized benzylamines across the series.

Guided by the results obtained with the amine derivatives in Table 1, selected amiloride analogs with ether functionality linked at the 5-position to the pyrazine were evaluated as shown in Table 2. Generally, these derivatives were not as effective inhibitors as their amine congeners. Aromatic ethers (**6f–l**) were modestly favored over their alkyl counterparts, while thioethers were significantly less active. Consistent with the amine series, naphthyloxy analog **6h** was the most effective amongst the series, while certain quinoline or isoquinoline derivatives could be tolerated depending upon the position of the nitrogen.

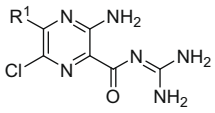
Selected amiloride analogs that are carbon linked at the 5-position to the pyrazine are shown in Table 3. While placement of a phenyl group (**9a**) provided 35% inhibition @ 1 μM, consistent with trends observed with anilines and phenol from Tables 1 and 2, the corresponding naphthalenes **9c–d** and benzyl **9e** derivatives were very weak ASIC3 channel blockers. SAR around the *ortho*, *meta*, or *para* positions on the phenyl ring was generally flat with only xylene **9f** showing similar inhibition. One notable exception was from the biaryl series in which meta-biphenyl **9g** gave the strongest block (72% @ 1 μM) of all amiloride analogs examined, while *ortho*- and *para*-biaryls (**9h–k**) were much less active.

A very limited number of the highly potent amiloride analogs were selected for 5-point titration in electrophysiology. Results are shown in Table 4. Amiloride provided an IC_{50} of 4.4 μM, while



Scheme 1. (a) Na(s), *i*-PrOH, guanidine-HCl. (b) R_1R_2NH, DMSO, TEA, 80–120 °C. (c) R_1SH or R_1OH, NaH, DMF. (d) *meta*chloroperoxybenzoic acid. (e) $POCl_3$, DMF, rt. (f) $Pd(dppf)Cl_2$, K_2CO_3, DMF, $R_1B(OH)_2$.

Table 1
Inhibition of ASIC3 by select amiloride derivatives **5a**–**5i′**



5a-i'

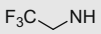
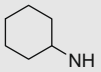
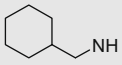
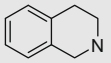
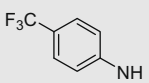
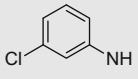
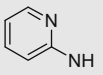
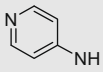
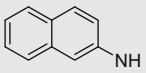
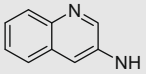
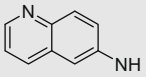
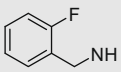
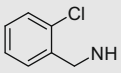
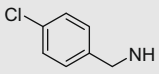
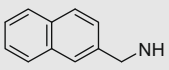
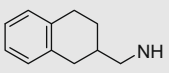
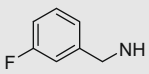
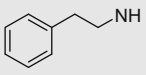
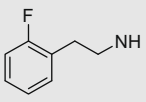
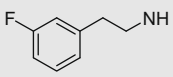
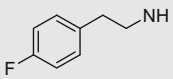
Compds	R^1	% Inh. @ 1 μM [a]	% Inh. @ 20 μM [a]
1	NH_2	22	65
5a		16	78
5b		24	91
5c		nd	48
5d		nd	74
5e		nd	78
5f		21	92
5g		28	96
5h		37	91
5i		32	91
5j		nd	57
5k		12	84
5l		nd	85
5m		nd	51
5n		nd	51
5o		nd	60
5p		46	89
5q		52	96
5r		44	95
5s		50	nd
5t		47	94

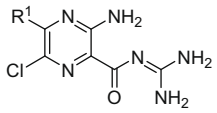
Table 1 (*continued*)

Compds	R^1	% Inh. @ 1 μM [a]	% Inh. @ 20 μM [a]
5u		nd	25
5v		14	nd
5w		62	98
5x		28	nd
5y		41	nd
5z		26	92
5a′		31	90
5b′		46	97
5c′		27	93
5d′		44	nd
5e′		29	90
5f′		46	94
5g′		34	nd
5h′		46	nd
5i′		32	nd

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

5-aminonaphthalene **5w** gave an $IC_{50} = 0.51$ μM. Biaryl derivatives **9g** ($IC_{50} = 0.49$ μM) and **9h** ($IC_{50} = 0.64$ μM) were similarly potent.

Table 2
Inhibition of ASIC3 by select amiloride derivatives **6a–l**



6a-l

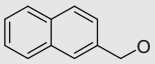
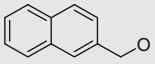
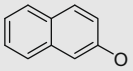
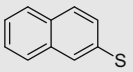
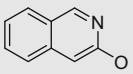
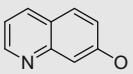
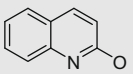
Compds	R^1	% Inh. @ 1 μM [a]	% Inh. @ 20 μM [a]
6a	HO	nd	9
6b	MeO	21	nd
6c		9	nd
6d	MeS	12	nd
6e		11	nd
6f		23	90
6g		9	nd
6h		33	91
6i		12	nd
6j		11	nd
6k		32	nd
6l		34	nd

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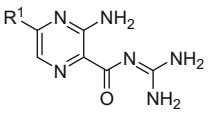
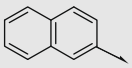
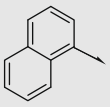
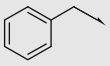
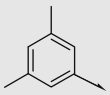
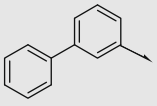
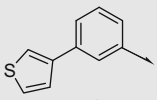
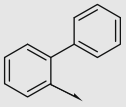
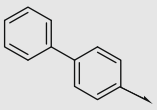
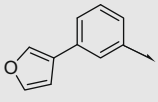
Table 3
Inhibition of ASIC3 by select amiloride derivatives **9a–k**



9a-k

Compds	R^1	% Inh. @ 1 μM[a]	% Inh. @ 20 μM[a]
9a		35	nd
9b		23	nd
9c		6	nd
9d		21	nd
9e		4	nd
9f		39	95
9g		72	nd
9h		58	90
9i		8	nd
9j		7	nd
9k		30	82

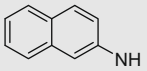
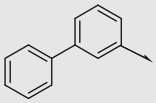
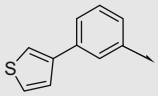
[a] Electrophysiology recording, inhibition of current was expressed as percent inhibition of peak current versus baseline peak current. $N = 3$.

In addition, biaryl **9g** was shown to inhibit rat ASIC3 with an $IC_{50} = 0.95$ μM.

Biphenyl derivative **9g** was examined in vivo in the rat Complete Freud's Adjuvant (CFA) model of inflammatory pain[22] (Fig. 3). In this experiment, **9g** showed a robust, dose-dependent reversal of mechanical hypersensitivity in male Sprauge Dawley rats at 30 min post-dosing, with a maximal effect at 30 mg/kg ip that was comparable to the NSAID naproxen. The average plasma concentration at 30 min was 2.9 μM[23] with very low levels in brain (~0.079 μM) suggesting that this compound is acting in a peripheral manner. It should be noted that the pharmacokinetic properties of **9g** in rat were poor, with no oral bioavailability and clearance in excess of hepatic blood flow.

In summary, derivatives of the potassium sparing diuretic amiloride were prepared and evaluated as inhibitors of the ASIC3 channel. Evaluation of amiloride analogs from the MRL sample collection guided the SAR strategy leading to the incorporation of potency enhancing lipophilic groups at the 5-position of the pyrazine nucleus. One compound in particular, *meta*-biphenyl **9g**, was a sub-micromolar inhibitor of ASIC3, and was efficacious in the CFA mechanical hypersensitivity model of inflammatory pain. Although biaryl **9g** possesses sub-standard pharmacokinetic properties, it might serve as a useful starting point for more drug-like, amiloride inspired ASIC3 inhibitors.

Table 4
ASIC3 titration of select amiloride derivatives

Compds	R^1	IC_{50}[a] (μM)
1	NH_2	4.4
5w		0.51
9g		0.49
9h		0.64

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

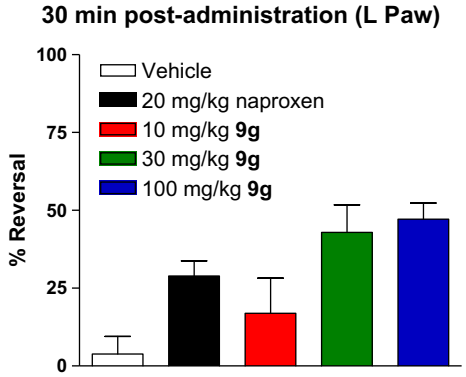


Figure 3. CFA mechanical hyperalgesia data with compound **9g** and naproxen.

References and notes

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18. Acid-evoked (pH 5.5 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 (pH 5.5). Compounds were applied 120s 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.
19. For an overview of the amiloride structure–activity relationships, see: Cragoe, E. J. In *Historical perspective of amiloride and its analogs, unique cation transport inhibitors*; Cragoe, E. J., Jr., Kleyman, T. R., Simchowitz, L., Eds.; VCH: New York, 1992; pp 3–8.
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21. Screening was generally done at a single concentration (20 μM) initially and followed up at 1 μM if warranted. Alternatively, compounds thought to be more potent initially were tested at 1 μM, thus some were not determined at 20 μM.
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23. Compound **9g** was found to have no measurable binding to rat plasma proteins.