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Discovery of a novel chemotype of potent human ENaC blockers using a bioisostere approach. Part 2: α-Branched quaternary amines

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ABSTRACT

We report the synthesis and biological evaluation of a series of novel α-branched pyrazinoyl quaternary amines for their ability to block ion transport via the epithelial sodium channel (ENaC) in human bronchial epithelial cells (HBECs). Compound **12g** has an IC_{50} of 30 nM and is highly efficacious in the Guinea-pig tracheal potential difference (TPD) model of ENaC blockade with an ED_{50} of 1 μg kg^{-1} at 1 h. In addition the SAR results demonstrate for the first time the chiral nature of the binding site of human ENaC. As such, pyrazinoyl quaternary amines represent a promising new class of ENaC blockers for the treatment of cystic fibrosis that are structurally distinct from the pyrazinoyl guanidine chemotype found in prototypical ENaC blockers such as amiloride.

Cystic fibrosis (CF) is an autosomal recessive disease that affects approximately 70,000 people worldwide. The leading cause of death in CF patients is respiratory failure resulting from chronic bacterial infection of the lung. Treatment with physiotherapy and antibacterial agents has improved the management of CF, but the median life expectancy in the US is still less than 40 years.[1] Current marketed therapeutics only target the infection and inflammation symptoms of CF, as such there is an unmet medical need for novel treatments that address the underlying cause of CF.[2] The 'low volume' hypothesis suggests that dehydration of the airway surface liquid in the lung leads to mucostasis in the airway lumen providing an environment for bacterial colonization leading to the chronic infections seen in CF patients.[3]

Inhibition of the epithelial sodium channel (ENaC) is widely believed to be one method of rehydrating the airway lumen and improving mucociliary clearance (MCC).[4] Supporting evidence comes from patients with pseudohypoaldosteronism type I, where loss-of-function mutations in ENaC result in pulmonary edema and increased rates of MCC.[5] Aerosolized amiloride (**1**, see Fig. 1), is a potassium-sparing diuretic that blocks ENaC and has been shown to improve MCC,[6] but its effectiveness is limited by poor potency and pharmacokinetic profile.[7] Parion Sciences developed amiloride analogue 552-02 (**2**, see Fig. 1), which completed Phase II trials as a nebulized therapy for CF. Compound **2** is reported to have improved potency at ENaC in HBECs (IC_{50} 8 nM) and improved in vivo efficacy and duration of action in a sheep model of MCC when compared to amiloride.[8]

As part of our work to identify novel blockers of ENaC for the treatment of CF, we recently reported the identification of a novel chemotype of pyrazinoyl quaternary amines, exemplified by compound **3** (see Fig. 2), that blocks ENaC function with comparable activity to amiloride in vitro and in vivo.[9] The SAR indicated that improved potency could be achieved by addition of large lipophilic tail groups. Herein, we report our efforts to optimize the potency and in vivo efficacy of this series.

To achieve an order of magnitude improvement in potency we sought to identify a specific binding interaction rather than relying on increasing lipophilicity to improve potency. Li et al. have reported a stereochemical preference between enantiomers of compound **4** (see Fig. 2) for ENaC blockade in amphibian ENaC with a ratio of 4:1 in favor of the (*S*)-enantiomer.[10]

We began our investigations to establish if human ENaC possessed a chiral binding site similar to amphibian ENaC by preparing a series of α-branched quaternary amines as illustrated in Scheme 1. Chiral 1,2-amino alcohols **5a–k** (purchased commercially with >97% ee) were protected as their Boc derivatives, **6a–k** were then converted into **7a–k** a Mitsunobu reaction with phthalimide and diethyl azodicarboxylate (DEAD). Removal of the phthalimide group provided primary amines **8a–k** which underwent an *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)-mediated amide coupling reaction with carboxylic

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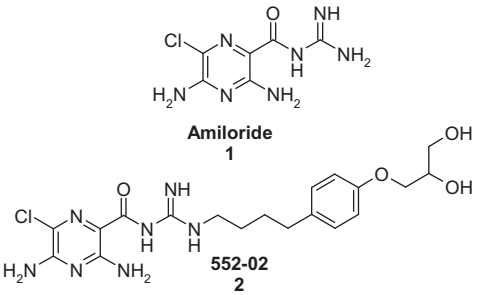


Figure 1. Examples of pyrazinoyl guanidine ENaC blockers.

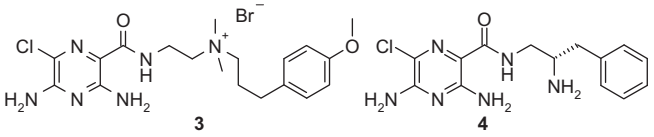
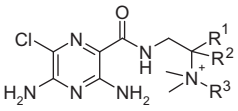


Figure 2. Examples of non-pyrazinoyl guanidine ENaC blockers.

acid **9** to give Boc-protected amides **10a–k**. The resulting amides were deprotected under acidic conditions to generate the α-branched amines **11a–k** that were exhaustively methylated with methyl iodide to generate the series of α-branched quaternary amines **12a–k**. This series of α-branched quaternary amines were tested in HBECs to investigate the stereochemical SAR with respect to ENaC blockade, Table 1 summarizes these data. The introduction of a single methyl group alpha to the quaternary amine afforded only weakly active compounds (**12a** and **12b**, Table 1). Introducing a second methyl group was not tolerated in this series of compounds (**12c**, Table 1). The ethyl analogues (**12d** and **12e**) do give a significant improvement in the potency of ENaC blockade but displayed no stereochemical preference. The ^{n}propyl and ^{i}propyl analogues (**12f–i**) provide the first evidence that the binding site in human ENaC has a stereochemical preference. Consistent with Li's observations,[10] the (*S*)-enantiomers (**12g** and **12i**) were more potent than the (*R*)-enantiomers with a ratio of up to 7:1. In this small data set an optimal inhibition of ENaC is achieved with compound **12g** (R^2 is (*S*)-^{n}propyl), which is approximately 10-fold more potent than amiloride. As the size of the alpha substituent increases or decreases from ^{n}propyl, or if branching is introduced, there is a trend for decreasing potency (compounds **12b, e, g** and **i–k**).

Table 1
Blockade of ENaC by α-branched quaternary amines **3**, **12a–k** and **16** in HBECs



Compd	R^1	R^2	R^3	HBEC IC_{50}[a,b] (μM)
Amiloride[b]	—	—	—	0.22 (93)
3[c]	H	H	$(CH_2)_3$-4-OMe-Ph	0.27 (11)
12a	H	(*R*)-Me	Me	>10 (2)
12b	H	(*S*)-Me	Me	8.09 (2)
12c	Me	Me	Me	>30 (2)
12d	H	(*R*)-Et	Me	0.51 (2)
12e	H	(*S*)-Et	Me	0.75 (2)
12f	H	(*R*)-^{n}Pr	Me	0.29 (6)
12g	H	(*S*)-^{n}Pr	Me	0.030 (14)
12h	H	(*R*)-^{i}Pr	Me	19.51 (2)
12i	H	(*S*)-^{i}Pr	Me	2.39 (3)
12j	H	(*S*)-^{n}Bu	Me	0.059 (2)
12k	H	(*S*)-Bn	Me	0.17 (6)
16	H	(*S*)-^{n}Pr	$(CH_2)_3$-4-OMe-Ph	0.004 (4)

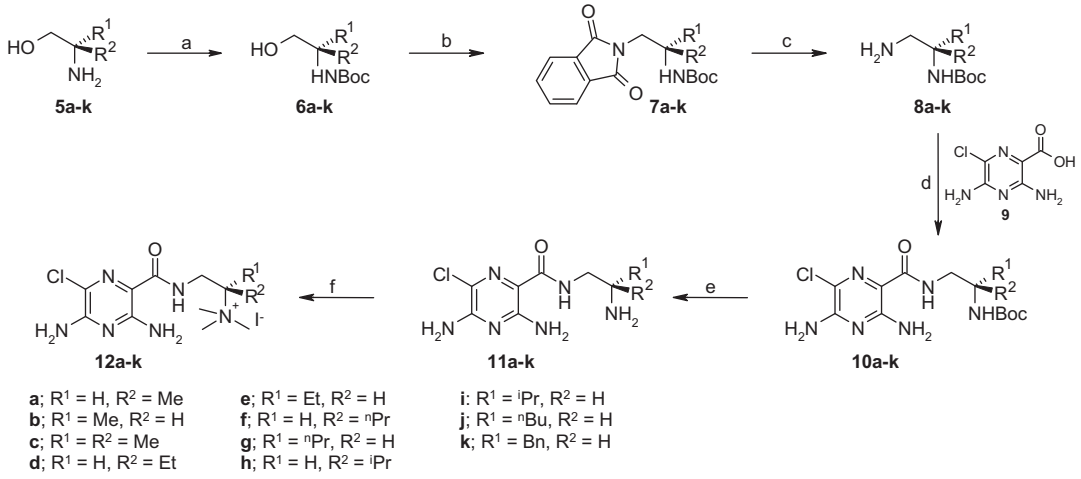
[a] Mean IC_{50} data, number in parentheses refers to the number of repetitions.
[b] A description of the assay conditions used to determine IC_{50}'s and the previously reported data for amiloride can be found in Ref. 13.
[c] Data for compound **3** has been previously reported using these assay conditions in Ref. 9b.

Compound **12g** was docked into an ENaC homology model based on the crystal structure of the related chicken acid-sensing ion channel shown in Figure 3.[11,12]

Compounds **12a–k** were aligned such that the pyrazine moieties overlaid and the more active enantiomer (compound **12g**) was minimized within the transmembrane (TM) environment.

Figure 3 shows that compound **12g** contacts the TM domain (Van der Waals mesh surface) whilst maintaining the ionic and hydrogen bonding interactions seen with amiloride whereas the other epimer would direct the hydrophobic substituent more parallel to the helical axis and towards the solvent exposed exterior. It suggests that the ^{n}propyl α-substituent has a key interaction with a hydrophobic region of one of the pore-forming α-helix residues. This model suggests that smaller or larger α-substituents would interact less favorably with the pore-forming α-helix residue consistent with the observed SAR in Table 1.

In vivo efficacy was assessed using the Guinea-pig TPD model using intratracheal (it) dosing.[13] Amiloride and compound **12g**



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt (99%); (b) phthalimide, PPh_3, DEAD, CH_2Cl_2, rt (39–92%); (c) $N_2H_4{\cdot}H_2O$, $EtOH/CH_2Cl_2$, rt; (d) HATU, 4-methylmorpholine, DMF, rt (34–74% over two steps); (e) 4 M HCl in dioxane, rt (65–92%); (f) MeI, K_2CO_3, MeCN, rt (31–99%).

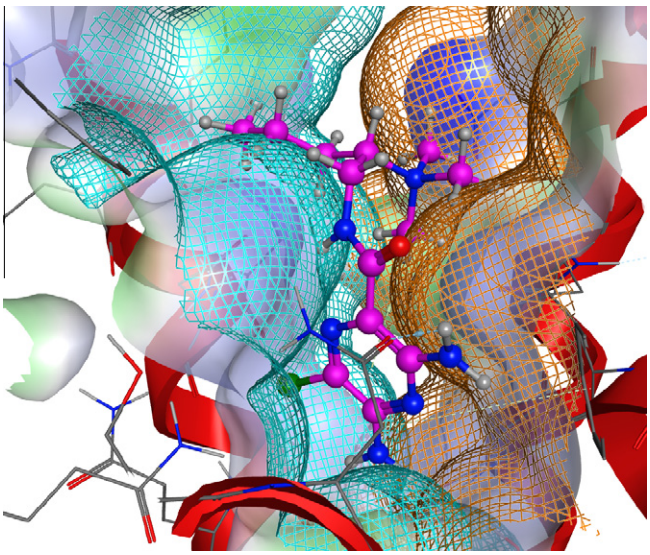
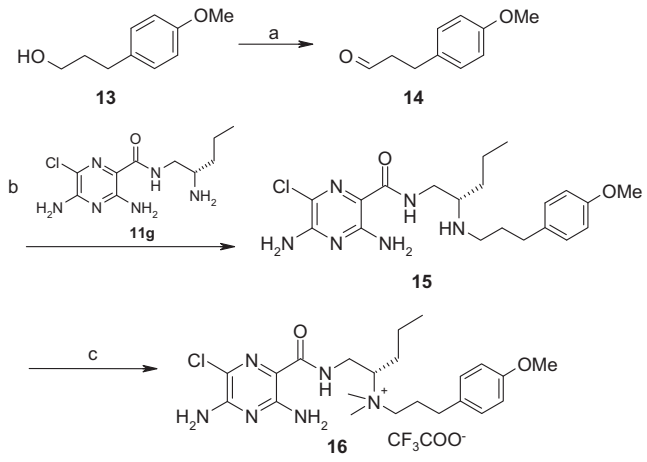


Figure 3. Compound **12g** docked into an ENaC homology model.

Table 2
ENaC Guinea-pig cross-reactivity and in vivo Guinea-pig TPD efficacy for amiloride and compounds **2** and **12g**

Compd	HBEC IC_{50}[a] (μM)	Guinea-pig FRT IC_{50}[a] (μM)	Guinea-pig TPD 1 h ED_{50}[b] (μg kg^{-1})
Amiloride[b]	0.22 (93)	0.54 (37)	16
2[b]	0.002 (12)	0.004 (32)	0.2
12g	0.030 (14)	0.030 (4)	1.0

[a] Mean IC_{50} data, number in parentheses refers to the number of repetitions.
[b] A description of these assay conditions and the previously reported data for amiloride and compound **2** can be found in Ref. 13.



Scheme 2. Reagents and conditions: (a) Dess–Martin Periodane, CH_2Cl_2, rt (90%); (b) **11g**, $NaB(OAc)_3H$, CH_2Cl_2, reflux (69%); (c) MeI, K_2CO_3, MeCN, 80 °C (99%).

both show excellent cross-reactivity to Guinea-pig ENaC (using Fischer Rat Thyroid (FRT) cells transiently infected with Guinea-pig ENaC).[13] Compound **3** was the first example of a quaternary amine that was tested in this model and gave promising results with an ED_{50} of 44 μg kg^{-1}.[9b] Pleasingly, when the more potent ENaC blocker, compound **12g**, was dosed it showed a significant improvement in efficacy and was 16-fold more efficacious than amiloride with an ED_{50} of 1 μg kg^{-1} and is comparable to compound **2** (Table 2).

We have previously demonstrated that adding large lipophilic groups around the quaternary amine provides up to a 20-fold improvement in potency.[9] The modeling of compound **12g** suggests that this may also be tolerated in the α-branched quaternary amine series. To test this hypothesis compound **16** was synthesized as illustrated in Scheme 2. Reductive amination of amine **11g** with aldehyde **14**[14] (formed by oxidation of alcohol **13** with Dess–Martin Periodane) provides amine **15**. As before, quaternization is accomplished by exhaustive methylation with methyl iodide to give quaternary amine **16**.

The addition of this tail group provides an approximate 10-fold enhancement in in vitro ENaC potency (Table 1, compound **16** vs **12g**). This demonstrates that our initial bioisostere hypothesis can deliver compounds equipotent with the leading amiloride-based analogues such as compound **2**. The importance of the alpha substituent can be seen by comparing compounds **3** and **16** with an approximate 50-fold improvement in ENaC blockade suggesting a key interaction within the binding site has been utilized. It is important to note that, due to the size and shape differences between a quaternary amine and a guanidine, the chiral SAR developed can not be readily transferred on to the pyrazinoyl guanidine chemotype.

In summary, we have demonstrated that the binding site of human ENaC displays a stereochemical preference that can be successfully exploited in the quaternary amine series. This led to the identification of a series of α-branched quaternary amines such as compounds **12g** and **16** which potently inhibit sodium ion transport via ENaC in HBECs. In compound **12g** the (*S*)-^{n}propyl α-substituent greatly improves potency and in vivo efficacy demonstrating that a bioisostere approach can deliver a novel class of human ENaC blockers that are comparable to the leading amiloride-based analogues in vitro and in vivo.

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12. The homology model for ENaC was created using the modified chicken ASIC crystal structure (PDB code 2QTS) as a template. The sequences of Human α, β and γ subunits were aligned with the 2QTS sequence using the alignment methodology within MolSoft's ICM Pro software with some manual alterations in the regions where the homology was low due to the presence of large amino acid insertions in the ENaC sequences. The SAR around the amiloride pyrazine ring is known to be tight and so this was positioned into the transmembrane region of the model in such a way that hydrogen bonding could be maximized, the chloro substituent was directed towards a gap in the TM bundle (to account for the ability to replace this group by hydrophobic replacements such as a phenyl ring) and this directed the basic guanidine towards the exterior of the TM bundle (which was likely considering the Parion derivatives such as compound **2**) and also close to GLU68 on the α subunit which could act as a counterion to the guanidine.
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