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Discovery of a novel chemotype of potent human ENaC blockers using a bioisostere approach. Part 1: Quaternary amines

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ABSTRACT

We report the identification of a novel series of human epithelial sodium channel (ENaC) blockers that are structurally distinct from the pyrazinoyl guanidine chemotype found in prototypical ENaC blockers such as amiloride. Following a rational design hypothesis a series of quaternary amines were prepared and evaluated for their ability to block ion transport via ENaC in human bronchial epithelial cells (HBECs). Compound **11** has an IC_{50} of 200 nM and is efficacious in the Guinea-pig tracheal potential difference (TPD) model of ENaC blockade with an ED_{50} of 44 μg kg^{-1} at 1 h. As such, pyrazinoyl quaternary amines represent the first examples of a promising new class of human ENaC blockers.

The epithelial sodium channel (ENaC) is a highly selective sodium ion channel that is involved in the reabsorption of sodium ions across the apical membrane of a number of epithelia including the lung, kidney and colon.[1] ENaC is a heteromeric channel comprising α, β and γ subunits in a 1:1:1 or 2:1:1 stoichiometry.[2] ENaC and the cystic fibrosis transmembrane conductance regulator (CFTR) are believed to be two of the key ion channels involved in maintaining appropriate hydration of the airway surface liquid (ASL) that lines the epithelia of the lungs. In cystic fibrosis (CF), where CFTR function is impaired, the net action of ENaC is postulated to lead to reduced mucosal hydration via absorption of sodium ions, creating an osmotic gradient that drives water out of the ASL.[3] This results in the formation of a thick, sticky layer of mucus within the lungs that is difficult to remove via mucociliary clearance (MCC) leading to chronic infection and inflammation.[4]

Blockade of ENaC is anticipated to increase hydration of the ASL, enhance MCC and so help protect the lungs of CF patients from decline in pulmonary function.[5] Aerosolized amiloride (**1**, see Fig. 1), a potassium-sparing diuretic that blocks ENaC has been shown to improve MCC,[6] but its effectiveness is limited by poor potency and pharmacokinetic profile.[7]

To date, the most potent ENaC blockers all contain the same pyrazinoyl guanidine chemotype based on amiloride and SAR has

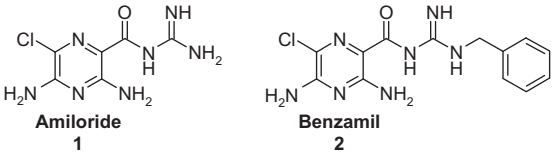

Figure 1. Examples of pyrazinoyl guanidine ENaC blockers.

demonstrated that the 3,5-diamino-6-chloro-pyrazinoyl guanidine is a privileged structure for ENaC blockade.[8] Improvements in potency have been obtained by derivatization of the guanidine with lipophilic tail groups as in the case of benzamil (**2**, see Fig. 1).[9] Although no crystal structure of ENaC exists modeling suggests that the relative spatial arrangement of the pyrazine ring with respect to the positively charged acylguanidinium functional group is essential for blockade of ENaC.[10] There have been a number of isolated reports where the acylguanidine group has been replaced with an appropriately positioned amino group. For example, Li et al. demonstrated that compound **3** (see Fig. 2) was up to 40-fold more potent than amiloride against a range of amphibian epithelial sodium channels.[11] A similar result was reported by Reid and co-workers who demonstrated that tertiary amine **4** or alkyl guanidine **5** were effective diuretic, natiuretic and antikaliuretic agents when dosed iv to rats, but both were less effective than amiloride.[12]

In this Letter we wish to communicate our approach to identifying novel and potent human ENaC blockers with a chemotype

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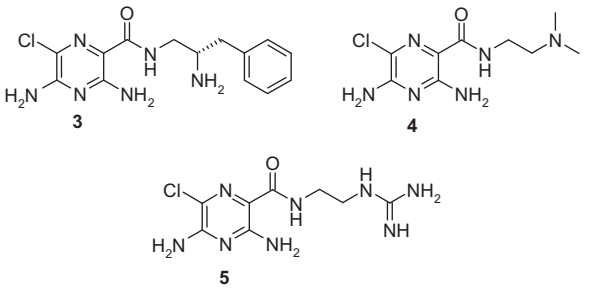


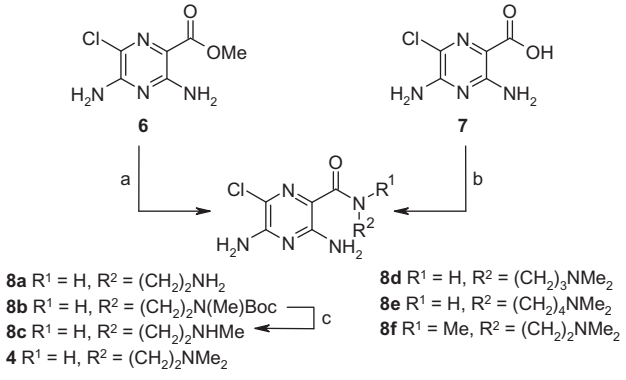
Figure 2. Examples of non-pyrazinoyl guanidine ENaC blockers.

distinct from the prototypical pyrazinoyl guanidines that will be suitable for the treatment of CF via inhaled delivery (dry powder or nebuliser).[13] We speculated that a group that is significantly ionized at physiological pH should function as a bioisostere for the acylguanidinium cation. Our investigations began by exploring amines as bioisosteres for the acyl guanidine present in amiloride; although less basic than an acylguanidine an amine should be significantly protonated at physiological pH.[14] A small range of previously reported amines (**4** and **8a–f**),[8b,12,15] were rapidly prepared by either reaction of methyl ester **6** with neat amine under microwave irradiation, or a *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)-mediated amide coupling reaction of carboxylic acid **7** (Scheme 1). Compound **8c** was synthesized via Boc-deprotection of compound **8b** with trifluroacetic acid.

Amine **4** demonstrates for the first time that blockade of human ENaC is possible with a chemotype different to the prototypical pyrazinoyl guanidines, albeit with a drop in potency (>10-fold) compared to amiloride (Table 1). It was observed that a two-carbon linker between the amide and the amine was optimal (compounds **4**, **8a**, **8c**) with a large drop in potency observed as the linker length increased to 3 or 4 carbons (compounds **8d,e**).

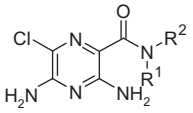
Substitution of the distal amine with small alkyl groups was well tolerated (compound **4**) but a large drop in potency was observed when the amide N–H was methylated (compound **8f**) indicating that the N–H is potentially providing a key binding interaction with the ion channel.

We speculated that generating a permanent cation via quaternization of the amine would improve ENaC potency and increase solubility.[13,17] Increased solubility would enable either dry powder or nebuliser formulations to be considered. Quaternization would also reduce cellular permeability and this could potentially reduce systemic absorption from the lung and provide a longer duration of action for an inhaled ENaC blocker.



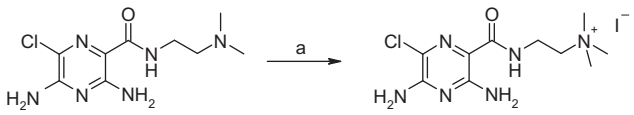
Scheme 1. Reagents and conditions: (a) R^1R^2NH, neat, μwave, 130 °C, 31–58%; (b) HATU, 4-methylmorpholine, DMF, rt, 40%; (c) CF_3CO_2H, CH_2Cl_2, rt, 34%.

Table 1
Blockade of ENaC by amines **4**, **8a** and **8c–f** in HBECs



Compd	R^1	R^2	HBEC IC_{50}[a,b] (μM)
Amiloride[b]	H	C(N=H)NH_2	0.22 (93)
Benzamil[b]	H	C(N=H)$NHCH_2Ph$	0.020 (9)
4	H	$(CH_2)_2NMe_2$	4.78 (2)
8a	H	$(CH_2)_2NH_2$	3.97 (4)
8c	H	$(CH_2)_2NHMe$	4.57 (2)
8d	H	$(CH_2)_3NMe_2$	17.35 (2)
8e	H	$(CH_2)_4NMe_2$	Inactive[c]
8f	Me	$(CH_2)_2NMe_2$	>30 (2)

[a] Mean IC_{50} data, number in parentheses refers to the number of repetitions.
[b] A description of the assay conditions used and previously reported IC_{50} data for amiloride and benzamil can be found in Ref. 16.
[c] <60% inhibition at 30 μM.



Scheme 2. Reagents and conditions: (a) MeI, CH_2Cl_2, rt, 97%.

Quaternization of amine **4** with methyl iodide afforded quaternary amine **9** (Scheme 2) that showed comparable potency to amine **4** illustrating that this strategy is tolerated but the anticipated increase in ENaC potency was not observed (Table 2). Previous work has demonstrated that the binding site in ENaC possesses a hydrophobic region that is consistent with the increased potency observed between amiloride and benzamil.[9,16]

To explore this hypothesis amines **4**, **8a** and **8c** were quaternized by mono-, di- or tri-alkylation with excess bromide **10** in refluxing acetone for 1–3 days to generate quaternary amines **11–13**, respectively (Scheme 3). Compound **13** could only be isolated in low yield (0.3%); the major product isolated was the tertiary amine resulting from dialkylation of amine **8a** with bromide **10**.

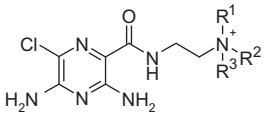
Pleasingly compound **11** showed improved potency with an IC_{50} of 0.27 μM (Table 2). Unlike the pyrazinoyl guanidine series, further lipophilic groups can be incorporated around the quaternary centre which dramatically improves ENaC blockade (compound **12** is approximately 100-fold more potent that amiloride).

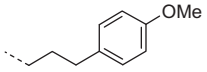
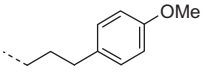
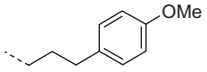
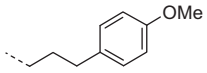
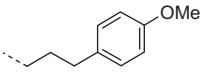
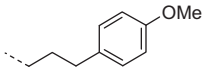
The thermodynamic aqueous solubility of compounds **11** and **12** was assessed in a range of pH buffers and is summarized in Table 3. Compared to amiloride quaternary amine **11** shows significantly improved solubility in a range of pH buffers. Although the greater lipophilicity of compound **12** affords a 10-fold improvement in potency versus compound **11**, this is to the detriment of solubility, which decreases 5- to 10-fold.

A parallel synthetic approach was adopted to generate a library of 67 aryl and alkylaryl amides and sulfonamides to explore the SAR around the hydrophobic pocket as outlined in Scheme 4. Reaction of amine **4** with the corresponding *N*-phthalimido protected alkyl bromides generated the series of quaternary ammonium bromides **14** that were subsequently deprotected with hydrazine monohydrate in ethanol to give amines **15**.

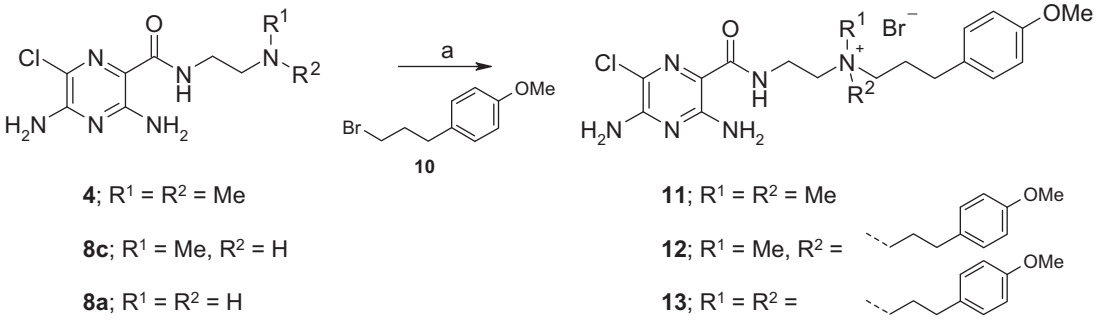
The amines provided the necessary handle for the library and the opportunity to introduce some polarity into the linker between the quaternary amine and the terminal group. Guided by previously

Table 2
Blockade of ENaC by quaternary amines **9** and **11–13** in HBECs



Compd	R^1	R^2	R^3	HBEC IC_{50}[a,b] (μM)
9	Me	Me	Me	5.81 (3)
11	Me	Me		0.27 (11)
12	Me			0.013 (24)
13				0.037 (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 can be found in Ref. 16.



Scheme 3. Reagents and conditions: (a) Na_2CO_3, acetone, reflux, 0.3–90%.

Table 3
Thermodynamic solubility

Compd	Salt	Thermodynamic solubility[a] (mg/mL)			
		pH 1	pH 4	pH 6.8	pH 9
Amiloride	HCl	0.4	1.0	<0.0005	0.8
11	Bromide	4.8	4.9	5	4.7
12	Bromide	0.6	0.5	0.9	1.0

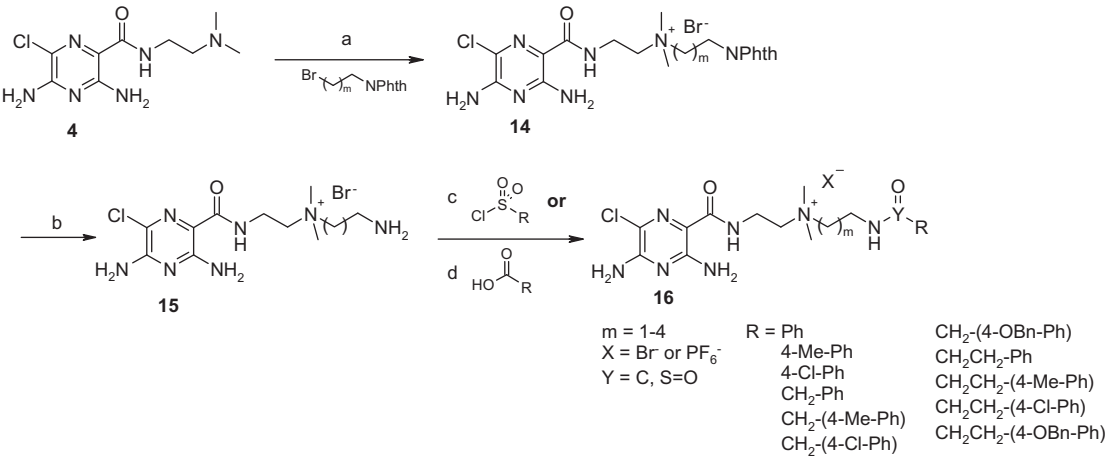
[a] Thermodynamic solubility was determined by the shake-flask methodology after 24 h equilibriation.

reported SAR,[18] amines **15** were derivatized with a focused set of sulfonyl chlorides and carboxylic acids to generate amides and sulfonamides of the general structure **16**.

Selected results from this library are shown in Table 4. In the series of amides where the terminal R group is 4-chlorophenyl (**16a–d**) the optimal linker length, *m*, is 3 suggesting that there is a preference for the position of the aromatic group. This trend was confirmed with the analogous series of sulfonamides (**16e–h**) that also demonstrated that a sulfonamide linker is equally well tolerated. Variation of the terminal aryl group indicated a preference for 4-benzyloxy phenyl group (**16n** and **16r**) suggesting that an electron rich first aromatic ring is preferred with sufficient space for a further large lipophilic group consistent with a large hydrophobic pocket.

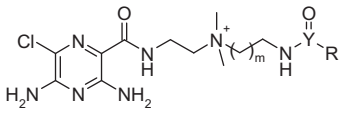
Compounds **11** and **16n** were profiled further to establish if quaternization led to reduced cellular permeability that we anticipated. Pleasingly, both compounds do show poor cellular permeability with Caco-2 ratios B–A/A–B of 1.67/0.93 and 2.55/3.33 cm/s × 10^{-6}, respectively). This was demonstrated further by the activity of compound **16n** on the hERG channel which had high in vitro binding affinity (IC_{50} = 0.33 μM), but in patch clamp studies inhibition was reduced (IC_{50} = 10.8 μM), presumably due to reduced cell permeation. As ENaC is expressed on the surface of lung epithelia we anticipated that low cellular permeability would not affect the in vivo efficacy of an inhaled therapeutic.

Cross-reactivity to Guinea-pig ENaC was assessed using Fischer Rat Thyroid (FRT) cells transiently infected with Guinea-pig ENaC[16] and in vivo efficacy was determined using the previously reported Guinea-pig TPD model.[16] Data for amiloride in these assays has been previously reported and is shown in Table 5.[16] Compound **11** shows excellent cross reactivity to Guinea-pig ENaC with an IC_{50} of 180 nM. Intratracheal (i.t.) dosing of compound **11** in the Guinea-pig TPD model showed compound **11** is efficacious with an ED_{50} at 1 h of 44 μg kg^{-1} which compares favorably with the ED_{50} for amiloride at 1 h of 16 μg kg^{-1} (Table 5).



Scheme 4. Reagents and conditions: (a) 2-butanone, reflux, 75–98%; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux; (c) 4-methylmorpholine, CH_2Cl_2, rt, 14–54% (over two steps); (d) HATU, 4-methylmorpholine, DMF, rt, 10–89% (over two steps).

Table 4
ENaC inhibition data for a series of quaternary amines **16a–r** in HBECs



Compd	*m*	Y	R	HBEC IC_{50}[a] (μM)
16a	1	C	4-Cl-Ph	1.66
16b	2	C	4-Cl-Ph	0.74
16c	3	C	4-Cl-Ph	0.51
16d	4	C	4-Cl-Ph	0.75
16e	1	S=O	4-Cl-Ph	1.12
16f	2	S=O	4-Cl-Ph	0.29
16g	3	S=O	4-Cl-Ph	0.25
16h	4	S=O	4-Cl-Ph	0.96
16i	3	C	Ph	1.23
16j	3	C	4-Me-Ph	0.88
16k	3	C	CH_2-Ph	0.95
16l	3	C	CH_2-(4-Me-Ph)	0.75
16m	3	C	CH_2-(4-Cl-Ph)	0.72
16n	3	C	CH_2-(4-OBn-Ph)	0.06
16o	3	C	CH_2CH_2-Ph	0.91
16p	3	C	CH_2CH_2-(4-Me-Ph)	0.59
16q	3	C	CH_2CH_2-(4-Cl-Ph)	0.16
16r	3	C	CH_2CH_2-(4-OBn-Ph)	0.08

[a] Mean IC_{50} data from at least two repetitions.

Table 5
ENaC Guinea-pig cross reactivity and in vivo Guinea-pig TPD efficacy for amiloride and compound **11**

Compd	HBEC IC_{50}[a] (μM)	Guinea-pig FRT IC_{50}[a] (μM)	Guinea-pig TPD 1 h ED_{50} (μg kg^{-1})
Amiloride[b]	0.22 (93)	0.54 (37)	16
11	0.27 (11)	0.18 (3)	44

[a] Mean IC_{50} data, number in parentheses refers to the number of repetitions.
[b] Data for amiloride has been previously reported using these assay conditions in Ref. 16.

In summary, we have identified the first potent human ENaC blockers which have an alternative core to the prototypical pyrazinoyl guanidine. Compound **11** represents a series of compounds that potently inhibit sodium ion transport via ENaC in HBECs and Guinea-pig FRTs with activity comparable to amiloride resulting in in vivo efficacy in the Guinea-pig TPD model. In addition, quaternary amines provide a feasible route to enhancing the solubility of ENaC blockers whilst reducing cellular permeability making them suitable for nebuliser, as well as dry powder delivery. As such, the discovery of the pyrazinoyl quaternary amines through a bioisostere approach represents a novel class of ENaC blockers that are worthy of further investigation as potential inhaled therapeutics for the improvement of mucociliary clearance in diseases such as cystic fibrosis.

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