Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2610–2614
Bioorganic & Medicinal Chemistry Letters

4′-Methyl-4,5′-bithiazole-based correctors of defective ΔF508-CFTR cellular processing

Choong Leol Yoo,[a] Gui Jun Yu,[a] Baoxue Yang,[b] Lori I. Robins,[a] A. S. Verkman[b] and Mark J. Kurth[a,*]

[a]*Department of Chemistry, University of California, One Shields Avenue, Davis, CA 95616, USA*
[b]*Departments of Medicine and Physiology, University of California, San Francisco, CA 94143, USA*

Received 24 December 2007; accepted 11 March 2008
Available online 16 March 2008

Abstract—The synthesis and ΔF508-CFTR corrector activity of a 148-member methylbithiazole-based library are reported. Synthetic routes were devised and optimized to generate methylbithiazole analogs in four steps. Corrector potency and efficacy were assayed using epithelial cells expressing human ΔF508-CFTR. These structure–activity data establish that the bithiazole substructure plays a critical function; eight novel methylbithiazole correctors were identified with low micromolar potencies.

Cystic fibrosis (CF), a lethal genetic disease afflicting ~0.04% of white individuals,[1] results in chronic lung infections because mutant cystic fibrosis transmembrane conductance regulator (CFTR) protein fails to confer chloride permeability to epithelial cells in lung and other tissues.[2] ΔF508-CFTR, the most common CF mutation (present in at least 1 allele in ~90% of CF patients),[1] contains a single amino acid deletion of phenylalanine 508, which causes the nascent protein to be retained in the endoplasmic reticulum and rapidly degraded.[3] When allowed to reach the cell plasma membrane by low-temperature (27 °C) rescue, ΔF508-CFTR can function as a cAMP-activated chloride channel, but with significantly decreased activity compared with WT-CFTR.[4] While programs aimed at the discovery of small-molecule effectors of defective ΔF508-CFTR folding and cellular processing (e.g., correctors) and channel gating (e.g., potentiators) have identified a number of ΔF508-CFTR potentiators,[5] the discovery of effective correctors is a substantially greater challenge as protein folding and trafficking are complex processes involving multiple cellular targets, some of which may be cell type-specific. As previously reported, we identified several small-molecule ΔF508-CFTR correctors [aminoarylthiazoles (e.g., **1** and **2**), quinazolinylaminopyrimidinones (e.g., **3**), and 4′-methyl-4,5′-bithiazole (e.g., **4**); Fig. 1] by screening a structurally diverse set of 150,000 compounds.[6] Functional and biochemical analyses established methylbithiazoles as particularly promising for further development based on their efficacy in human ΔF508-CFTR airway epithelial cells and their CFTR-specificity. Herein, we report the preparation and screening of 148 new methylbithiazole analogs aimed at establishing initial SAR data for this lead class of correctors.

To verify our initial screening result as well as to develop an effective route to methylbithiazoles, we began with a resynthesis of corrector **4**. This work commenced by treating 3-chloropentane-2,4-dione with thiourea (Scheme 1) under reflux in absolute ethanol; 5-acetyl-2-amino-4-

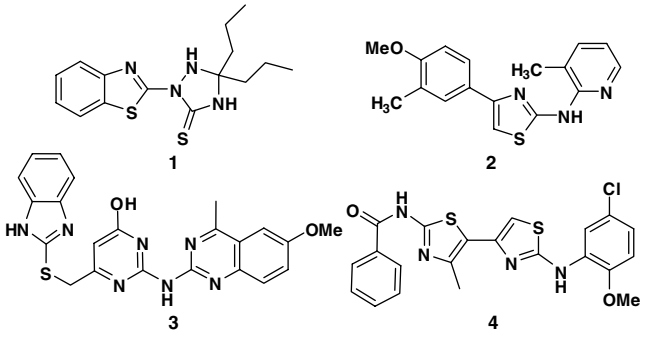
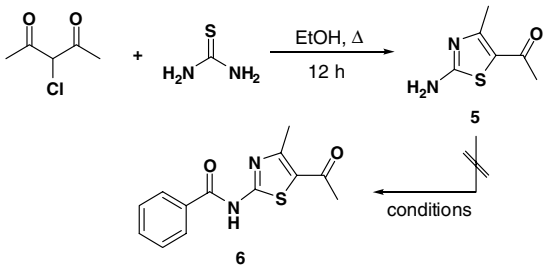


Figure 1. Correctors of defective human ΔF508-CFTR.

Keywords: ΔF508-CFTR correctors; Bithiazole; Structure–activity; Small-molecule library.
* Corresponding author. Tel.: +1 530 752 8192; fax: +1 530 752 8995; e-mail: mjkurth@ucdavis.edu

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.037



Scheme 1. Aminothiazole N-acylation problem. Reagents and conditions: (1) Et_3N (1.2 equiv), C_6H_5COCl, DCM, 0 °C to rt, overnight, **5** is recovered; (2) Et_3N (1.2 equiv), C_6H_5COCl, DCM, 0 °C to rt, overnight, except **5** was pre-washed with 10% aq NaOH, results in complex mixture; (3) Et_3N (1.2 equiv), C_6H_5COCl, toluene, reflux, overnight, **5** is recovered + trace of **6**; (4) *i*-Pr_2NEt (15 equiv), C_6H_5COCl (10 equiv), DCM, rt, overnight, **5** is consumed, mono- and bis-benzoylation products.

methylthiazole (**5**) was obtained in 90% yield. Surprisingly, attempts to *N*-acylate the C2-amino moiety in **5** with benzoyl chloride under various bases, solvents, and temperature conditions failed or delivered the targeted *N*-acyl aminothiazole **6** in low yield. While perhaps surmountable in any particular case, the malfunction of **5** → **6** is a consequence of the 5-acetyl moiety reducing the nucleophilicity of the C2-amino group of **5**. Since the plan was to ultimately diversify with a spectrum of acid chlorides, this problematic reaction caused us to evaluate the inverse of these two reactions—that is, use benzoyl isothiocyanate as the starting material in place of thiourea (Scheme 2). Passing ammonia gas through a dichloromethane solution of benzoyl isothiocyanate delivered *N*-carbamothioylbenzamide (**7**) which then reacted with 3-chloropentane-2,4-dione to afford **6** in good yield. α-Bromination of the acetyl group of **6** proved to be quite challenging with the recovery of unreacted **6** being the principle issue [Br_3 on Amberlite A-26 resin, $CHCl_3$, rt, 24 h/no reaction; Br_2 in AcOH, rt, 24 h/>50% recovered **6**; NBS, $CHCl_3$, reflux, 24 h/>50% recovered **6**]. Pyridinium tribromide in a 30% HBr in acetic acid solution resolved this problem, presumably because this strongly acidic medium promotes enolization. Finally, bromoacetyl thiazole **8** (obtained from **6**) was treated with thiourea **9**, in turn prepared by the reaction of 5-chloro-2-methoxyaniline with ammonium thiocyanate, in refluxing ethanol to deliver corrector **4** in excellent yield. Importantly, no chromatic purifications were required throughout the four-step process outlined in Scheme 2.

The concentration–activity data for resynthesized methylbithiazole **4** are shown in Figure 2 where the dashed line indicates the level of activity with low-temperature (27 °C) rescue which is used as a positive control and reference. Activation of ΔF508-CFTR was confirmed for each of the compounds by showing no activity on non-transfected FRT cells and near-complete inhibition of the increased iodide influx by the thiazolidinone $CFTR_{inh}$-172 at 10 μM (data not shown).[7]

To establish SAR information, it was decided to begin by making a small collection of analogs of **4** in which the *N*-(4′-methyl-4,5′-bithiazol-2′-yl)benzamide moiety was held constant while the aniline moiety was varied. As outlined in Scheme 3, each of these derivatives was

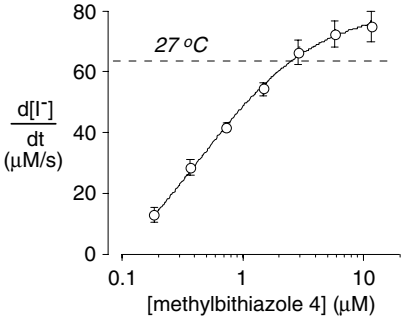
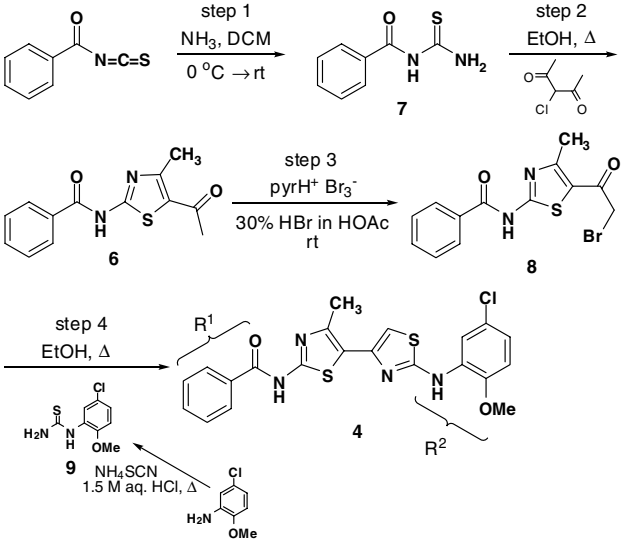
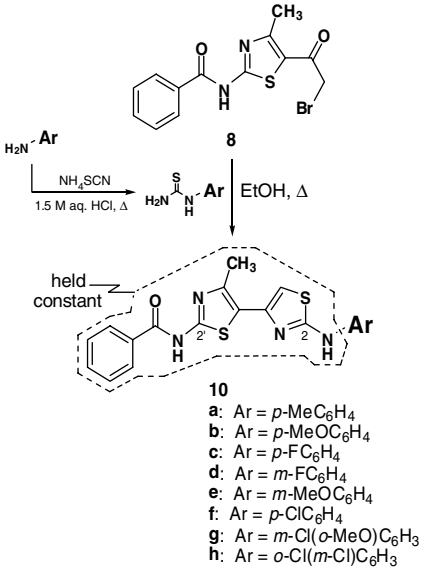


Figure 2. Concentration–activity analysis of methylbithiazole 4 (mean ± SE, $n = 4$).



Scheme 2. Synthesis of corrector **4**.



Scheme 3. Aniline derivatives of the methylbithiazole corrector.

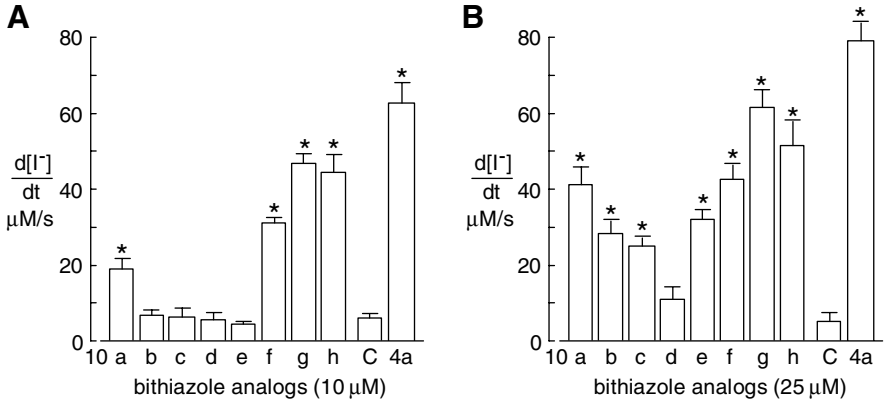


Figure 3. Maximal iodide influx in ΔF508-CFTR-expressing FRT cells incubated at 37 °C for aniline-based methylbithiazole correctors **10a**–**h** (see Scheme 3) compared with 4a (C, negative DMSO vehicle control) (mean ± SE, $n = 4$, $^{*}P < 0.05$ tested by Student's test comparing with C).

available in one step by condensation of the appropriate arylthiourea with **8**.

The corrector efficacies of methylbithiazoles **10a**–**h** are summarized in Figure 3. Although all eight of these methylbithiazoles were less effective correctors of ΔF508-CFTR than the initial hit compound **4**, we were encouraged to find that three (**10f**, **10g**, and**10h**) were moderately effective. Indeed, compounds **10g** and **10h** showed comparable corrector activity to **4** at both 10 and 25 μM concentrations. Additionally, this small set of compounds established that placing an electron withdrawing fluorine group on the aniline moiety (e.g., **10c** and **10d**) caused a significant decrease in activity and this decrease was independent of where the fluoride was located (**10c** and **10d**). This small initial set of compounds established that the peripheral modification of the methylbithiazole core modulates corrector activity and, as such, a broader study of the amide and aniline substructures of methylbithiazole **4** was undertaken.

With these data for bithiazoles **10a**–**h** in hand, a 40-member second library set (**11Aa**–**11Dj**; Fig. 4) was prepared by reagent-based modification of the protocol outlined in Scheme 2 (e.g., the isothiocyanate employed in step 1 incorporate aryl moieties **A**–**D** and the *N*-aryl thioureas used in step 4 incorporated aryl moieties **a**–**j**). Based on the results with **10c** and **10d**, fluorine was precluded from the R^2 aryls.

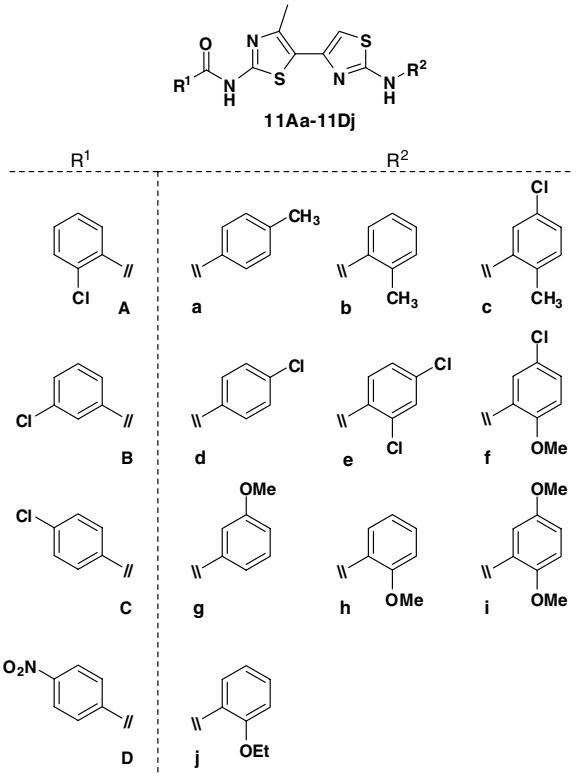
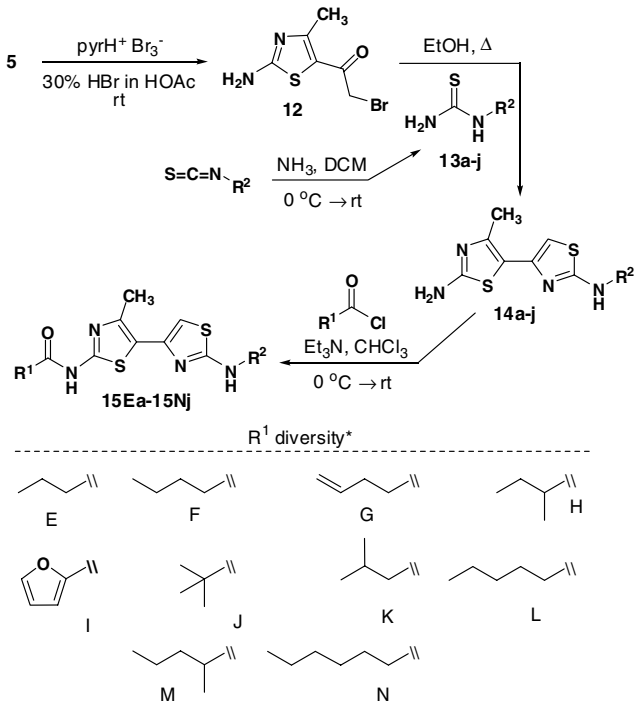


Figure 4. Library set two bithiazoles.

While library set two was under preparation, a third library set was targeted in which R^1 diversity (Scheme 2) was expanded to include non-aryl substituents. Scheme 2 chemistry afforded high yielding reactions and required no chromatographic purifications, but R^1 diversity in the bithiazoles accessible by this chemistry was limited in that few isothiocyanates are commercially available. Since previous attempts at benzoylation **5** → **6** (Scheme 1) were problematic, it was decided to construct library set three by assembly of the bithiazole first with subsequent N-acylation (Scheme 4). It was postulated that the C2′-amino group in the planned 4′-methyl-*N*-aryl-4,5′-bithiazole-2,2′-diamine intermediate (e.g., **14a**–**j**) would be considerably more nucleophilic than the corresponding amine in aminothiazole **5** with its C5 acyl moiety. In addition, adding the R^1 diversity input in the last step was expected to provide considerable time- and labor-saving benefits, particularly with regards to product purification.

Bromination of **5** using pyridinium tribromide plus 30% HBr in acetic acid afforded **12** in 50% yield. While the isolated product yield of this reaction was significantly lower than the nearly quantitative bromination **6** → **8**, the cause of this modest yield was the water solubility of the ammonium salt of **12**. Aromatic thioureas **13a**–**j** were prepared by bubbling ammonia gas through the commercially available aromatic isothiocyanates in dichloromethane. After simply evaporating the dichloromethane, pure aromatic thioureas **13a**–**j** were obtained



Scheme 4. Synthesis of library set three bithiazoles.

in high yield. Bromothiazole **12** was subsequently refluxed in ethanol with thioureas **13a**–**j** to afford C2′-aminobithiazoles **14a**–**j** in 60–80% yields. These compounds were contaminated by the modest quantities of impurities which proved difficult to remove by flash column chromatography as product and impurity R_f values were similar. Consequently, the crude C2′-aminobithiazoles were used in the next step without purification. As hoped, acylation of the C2′-amino moiety of **14a**–**j** with various acid chlorides in the presence of triethylamine in chloroform afforded library set three bithiazoles **15Ea**–**15Nj** (Scheme 4). LC–MS analysis of this last reaction generally showed 40–60% reaction completion. Additional reaction time or the addition of more acid chloride made product purification more difficult due to a corresponding increase in side products. Upon work-up, the products were purified by prep-HPLC and again analyzed by LC–MS. All showed 90% or better purity as well as the correct molecular ion peak.

The K_d/V_{max} data for the eight most active methylbithiazoles from **11Aa**–**11Dj** and **15Ea**–**15Nj** series are shown in Figure 5. It is interesting to note that six out of the eight best methylbithiazole share either a 4-chlorobenzamide (**11Ca**, **11Cd**, **11Ci**) or a pivalamide (**15Jf**, **15Jh**, **15Jj**) at the C2′-position and five out of these eight share either *o*-methoxy or *o*-ethoxy aromatic amines (**11Ci**, **15Jf**, **15Jh**, **15Jj**, **15Ni**) at the C2-position.

When there is a *p*-substituted aromatic amide at the C2′-position (**11Ca**, **11Cd**, **11Ci**, **11Dd**), *p*-substituted aromatic amines at the C2-position have elevated activity (**11Ca**, **11Cd**, **11Dd**). It is also interesting that bithiazole **15Ni**, which presents a relatively long alkyl amide chain, expressed comparable corrector activity. None of the methylbithiazoles incorporating a 2,4-dichloroaniline

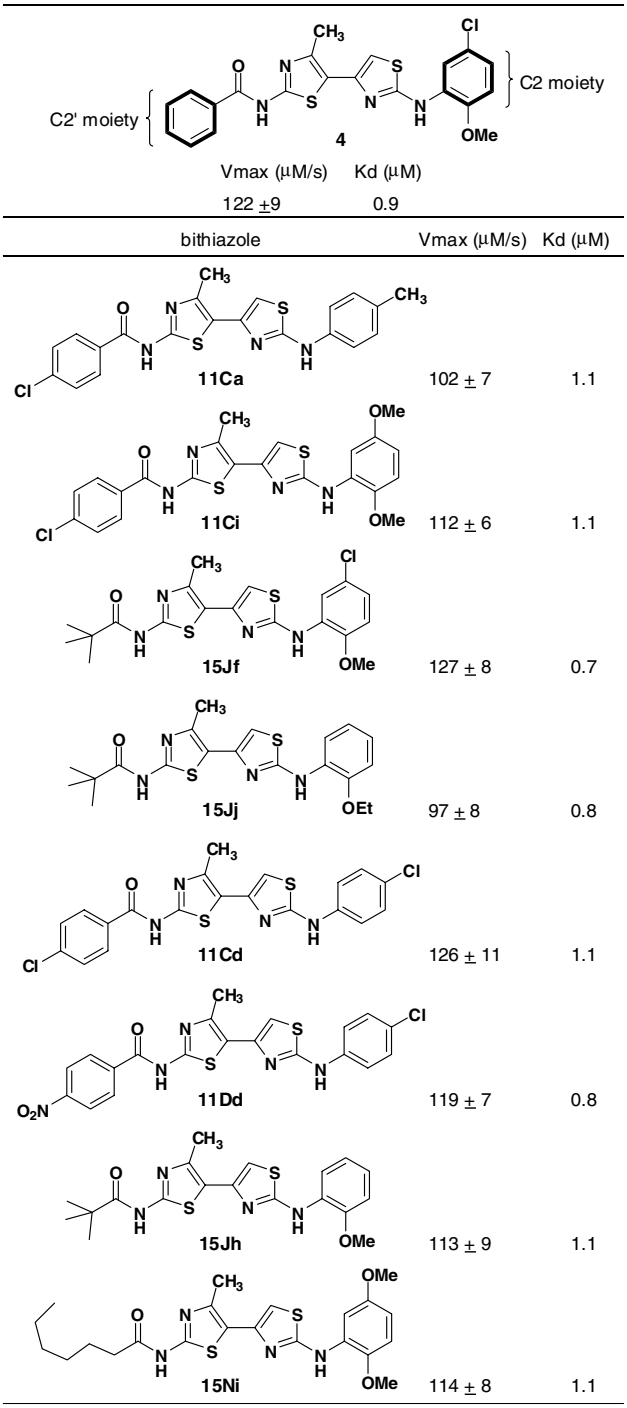


Figure 5. K_d/V_{max} data for the most active bithiazoles in library sets two and three.

substituent showed ΔF508-CFTR corrector activity. Nevertheless, among the bithiazoles screened (including the original hit **4**), new bithiazole **15Jf** is the most effective corrector as judged by both V_{max} and K_d data.

ΔF508-CFTR corrector activity measurements were made in this study as described previously[6] using FRT epithelial cells stably co-expressing human ΔF508-CFTR and the sensitive halide-sensing green fluorescent protein YFP-H148Q/I152L.[8] Cells were grown at 37 °C (90%

humidity; 5% CO_2) for 24 h and then incubated for 20 h with 50 μl of medium containing the test compounds. At the time of the assay, cells were washed with PBS and then incubated with PBS containing forskolin (20 μM) and genistein (50 μM) for 20 min. Measurements were carried out using FLUOstar fluorescence plate readers (Optima; BMG LABTECH GmbH), each equipped with 500 ± 10 nm excitation and 535 ± 15 nm emission filters (Chroma Technology Corp.). Each well was assayed individually for iodide influx by recording fluorescence continuously (200 ms per point) for 2 s (baseline) and then for 12 s after rapid (<1 s) addition of 165 μl PBS in which 137 mM chloride was replaced by iodide. The iodide influx rate was computed by fitting the final 11.5 s of the data to an exponential for the extrapolation of initial slope and normalized for the background-subtracted initial fluorescence. All experiments contained negative controls (DMSO vehicle) and positive controls (methylbithiazole **4**).

In conclusion, we have developed two versatile synthetic routes for the reliable preparation of bithiazole derivatives. Screening data for the 148 bithiazoles provides valuable structural activity information. In particular, the information gathered from bithiazoles **11Ca**, **11Cd**, **11Ci**, **11Dd**, **15Jf**, **15Jh**, **15Jj**, and **15Ni** is guiding our ongoing efforts to further refine and improve the ΔF508-CFTR corrector activity of bithiazoles.

Acknowledgments

The authors thank the National Institutes of Health (DK072517 and GM076151) and the National Science Foundation [CHE-0614756 as well as CHE-0443516 and CHE-9808183 (NMR spectrometers)] for their generous financial support.

References and notes

1. Bobadilla, J. L.; Macek, M., Jr.; Fine, J. P.; Farrell, P. M. *Hum. Mutat.* **2002**, *19*, 575.
2. Akabas, M. H. *J. Biol. Chem.* **2000**, *275*, 3729.
3. Sheppard, D. N.; Welsh, M. J. *Physiol. Rev.* **1999**, *79*, S23.
4. Benharouga, M.; Haardt, M.; Kartner, N.; Lukacs, G. L. *J. Cell Biol.* **2001**, *153*, 957.
5. (a) Al-Nakkash, L.; Hwang, T.-C. *Pflugers Archiv* **1999**, *437*, 553; (b) Drumm, M. L.; Wilkinson, D. J.; Smit, L. S.; Worrell, R. T.; Strong, T. V.; Frizzell, R. A.; Dawson, D. C.; Collins, F. S. *Science (Washington, DC, USA)* **1991**, *254*, 1797; (c) Hwang, T.-C.; Sheppard, D. N. *Trends Pharmacol. Sci.* **1999**, *20*, 448; (d) Hwang, T.-C.; Wang, F.; Yang, I. C. H.; Reenstra, W. W. *Am. J. Physiol.* **1997**, *273*, C988; (e) Pedemonte, N.; Sonawane, N. D.; Taddei, A.; Hu, J.; Zegarra-Moran, O.; Suen, Y. F.; Robins, L. I.; Dicus, C. W.; Willenbring, D.; Nantz, M. H.; Kurth, M. J.; Galietta, L. J. V.; Verkman, A. S. *Mol. Pharmacol.* **2005**, *67*, 1797; (f) Yang, H.; Shelat, A. A.; Guy, R. K.; Gopinath, V. S.; Ma, T.; Du, K.; Lukacs, G. L.; Taddei, A.; Folli, C.; Pedemonte, N.; Galietta, L. J. V.; Verkman, A. S. *J. Biol. Chem.* **2003**, *278*, 35079.
6. Pedemonte, N.; Lukacs, G. L.; Du, K.; Caci, E.; Zegarra-Moran, O.; Galietta, L. J. V.; Verkman, A. S. *J. Clin. Invest.* **2005**, *115*, 2564.
7. Ma, T.; Thiagarajah, J. R.; Yang, H.; Sonawane, N. D.; Folli, C.; Galietta, L. J.; Verkman, A. *J. Clin. Invest.* **2002**, *110*, 1651.
8. Galietta, L. J.; Haggie, P. M.; Verkman, A. S. *FEBS Lett.* **2001**, *499*, 220.