0026-895X/11/8004-683–693$25.00
MOLECULAR PHARMACOLOGY
Copyright © 2011 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 80:683–693, 2011

Vol. 80, No. 4
73056/3715523
Printed in U.S.A.

Cyanoquinolines with Independent Corrector and Potentiator Activities Restore ΔPhe508-Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channel Function in Cystic Fibrosis

Puay-Wah Phuan, Baoxue Yang, John M. Knapp, Alex B. Wood, Gergely L. Lukacs, Mark J. Kurth, and A. S. Verkman

Departments of Medicine and Physiology, University of California, San Francisco, California (P.-W.P., B.Y., A.S.V.); Department of Chemistry, University of California, Davis, California (J.M.K., A.B.W., M.J.K.); and Department of Physiology and Groupe de Recherche Axé sur la Structure des Protéine (GRASP), McGill University, Montreal, Quebec, Canada (G.L.L.)

Received April 16, 2011; accepted July 5, 2011

ABSTRACT

The ΔPhe508 mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) protein impairs its folding, stability, and chloride channel gating. Although small molecules that separately correct defective ΔPhe508-CFTR folding/cellular processing ("correctors") or chloride channel gating ("potentiators") have been discovered and are in clinical trials, single compounds with bona fide dual corrector and potentiator activities have not been identified. Here, screening of ~110,000 small molecules not tested previously revealed a cyanoquinoline class of compounds with independent corrector and potentiator activities (termed CoPo). Analysis of 180 CoPo analogs revealed 6 compounds with dual corrector and potentiator activities and 13 compounds with only potentiator activity. *N*-(2-((3-Cyano-5,7-dimethylquinolin-2-yl)amino)ethyl)-3-methoxybenzamide (CoPo-22), which was synthesized in six steps in 52% overall yield, had low micromolar EC_{50} for ΔPhe508-CFTR corrector and potentiator activities by short-circuit current assay. Maximal corrector and potentiator activities were comparable with those conferred by the bithiazole Corr-4a and the flavone genistein, respectively. CoPo-22 also activated wild-type and G551D CFTR chloride conductance within minutes in a forskolin-dependent manner. Compounds with dual corrector and potentiator activities may be useful for single-drug treatment of cystic fibrosis caused by ΔPhe508 mutation.

Introduction

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel expressed in airway, intestinal, pancreatic, and other epithelia. Deletion of phenylalanine at residue 508 (ΔPhe508) in CFTR, which is by far the most common CF-causing CFTR mutation, is present in at least one allele in ~90% of patients with CF. Whereas the wild-type CFTR protein functions as a chloride channel at the apical plasma membrane of target cells, the ΔPhe508-CFTR protein is misfolded and largely retained at the endoplasmic reticulum (ER) for degradation by the ubiquitin proteasome system. A small fraction of newly synthesized ΔPhe508-CFTR, however, can reach the cell surface in a tissue-specific manner but exhibits impaired channel gating and metabolic stability (Cheng et al., 1990; Dalemans et al., 1991; Lukacs et al., 1994). Multiple structural defects have been identified in ΔPhe508-CFTR, affecting four of its five domains to various extents, including the first nucleotide binding domain, in which the ΔPhe508 mutation is located, the nucleotide binding domain 2, and membrane-spanning domains 1 and 2 (Du and Lukacs, 2009; Thibodeau et al., 2010).

This work was supported by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grants DK72517, DK075302, DK86125, DK35124]; the National Institutes of Health National Heart, Lung, and Blood Institute [Grant HL73856]; the National Institute of Health National Institute of Biomedical Imaging and Bioengineering [Grant EB00415]; the National Institute of Health National Eye Institute [Grant EY135740]; the Cystic Fibrosis Foundation; the Canadian Cystic Fibrosis Foundation; and a Canada Research Chair (to G.L.L.).

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

doi:10.1124/mol.111.073056.

ABBREVIATIONS: CF, cystic fibrosis; AAT, arylaminothiazole; CFTR, cystic fibrosis transmembrane conductance regulator; CoPo, corrector-potentiator; DCM, dichloromethane; ER, endoplasmic reticulum; FRT, Fisher rat thyroid; YFP, yellow fluorescence protein; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; VX-770, *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide; VX-809, 3-[6-[[[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl]amino]-3-methyl-2-pyridinyl]-benzoic acid; VRT-532, pyrazole 4-methyl-2-(5-phenyl-1*H*-pyrazol-3-yl)-phenol.

There is great interest in the development of drugs that restore chloride permeability to CF cells (Riordan, 2008; Verkman and Galietta, 2009). An ideal drug for treatment of CF caused by the ΔPhe508 mutation would be a single, nontoxic small molecule that normalizes defective ΔPhe508-CFTR folding and cellular processing, fully restoring cell chloride permeability. The drug discovery strategy used to date, as first introduced by our laboratory, uses separate assays for the identification of ΔPhe508-CFTR "potentiators," which normalize defective ΔPhe508-CFTR chloride channel gating, and "correctors," which correct defective ΔPhe508-CFTR protein processing and promote ER-to-plasma membrane targeting. Using cell-based high-throughout screens, we reported small-molecule ΔPhe508-CFTR potentiators (Yang et al., 2003; Pedemonte et al., 2005b; benzothiophenes, phenylglycines, and sulfonamides) and correctors (Pedemonte et al., 2005a; Yu et al., 2008; Ye et al., 2010; aminoarylthiazoles and bithiazoles). Subsequent small-scale screening by several groups identified additional candidate potentiators (Van Goor et al., 2006; Pedemonte et al., 2007) and correctors (Noël et al., 2008; Robert et al., 2008, 2010; Kalid et al., 2010; Sampson et al., 2011). A potentiator, *N*-(2,4-di-*tert*-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770) (Van Goor et al., 2009), and a corrector, VX-809 (Van Goor et al., 2010), identified by Vertex Pharmaceuticals are in clinical trials (Accurso et al., 2010). In a recent proof-of-concept study, we synthesized a hybrid bithiazole-phenylglycine corrector-potentiator which, when cleaved by intestinal enzymes, yielded an active bithiazole corrector and phenylglycine potentiator (Mills et al., 2010). Pedemonte et al. (2011) have reported that aminoarylthiazoles, which were identified in our original corrector screen (Pedemonte et al., 2005a), had corrector activity, as reported previously, and the ability to correct defective ΔPhe508-CFTR gating when incubated with cells over many hours. However, a bona fide potentiator should exert its effect within minutes (see below).

The goal of this study was to identify dual-acting compounds with independent ΔPhe508-CFTR potentiator and corrector activities. Potentiator activity is defined as compound efficacy in increasing ΔPhe508-CFTR chloride conductance at the cell plasma membrane. Operationally, potentiator activity is assayed in low-temperature rescued ΔPhe508-CFTR-expressing cells in which ΔPhe508-CFTR is targeted to the plasma membrane by >12-h incubation at reduced temperature, and test compound (together with cAMP agonist) is added just before or at the time of fluorescence or electrophysiological assay (Yang et al., 2003). Corrector activity is defined as compound efficacy in increasing ΔPhe508-CFTR cell surface expression. Corrector activity is assayed in ΔPhe508-CFTR-expressing cells by >12 h incubation with test compound at 37°C, followed by washout and incubation with a potentiator such as genistein (together with cAMP agonist) just before or at the time of assay (Pedemonte et al., 2005a). We report here the identification a cyanoquinoline class of compounds having independent corrector and potentiator activities, termed CoPo. Although the corrector and potentiator activities of CoPos depended on their chemical structure and cell type, the cyanoquinolines are the first class of compounds with bona fide corrector and potentiator activities, providing rationale for their further chemical optimization and for additional screening to identify other compound classes with dual corrector and potentiator activities.

Materials and Methods

Cell Lines

Fisher rat thyroid (FRT) epithelial cells were stably transfected with ΔPhe508, G551D, or wild-type CFTR as reported previously (Pedemonte et al., 2005b). A549 cells stably expressing ΔPhe508-CFTR (Pedemonte et al., 2010) were provided by Dr. Luis Galietta (Genoa, Italy). Each of the CFTR-expressing cell lines (and the nontransfected parental cells) was also transfected with halide-sensitive green fluorescent protein YFP-H148Q/I152L/F46L. FRT cells were cultured in Coon's modified Ham's F-12 medium and A549 cells in Dulbecco's modified Eagle's medium/Ham's F-12 (1:1). All media were supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For primary screening, ΔPhe508-CFTR-expressing FRT cells were plated in black, 96-well microplates (Corning Life Sciences, Lowell, MA) at 50,000 cells/well. For short-circuit current measurements, cells were cultured on Snapwell permeable supports (Corning Life Sciences) at 500,000 cells/insert.

Compounds

A total of 110,000 diverse drug-like synthetic compounds (>90% with molecular masses of 250–500 Da; ChemDiv Inc., San Diego, CA, and Asinex Inc., Moscow, Russia) were used for initial screening. For optimization, ~180 commercially available cyanoquinoline analogs were tested. Structures of active compounds were confirmed by ^{1}H NMR and liquid chromatography/mass spectrometry.

Screening Procedures

Screening was carried out using a Beckman Coulter platform equipped with FLUOstar fluorescence plate readers (Optima; BMG Labtech, Durham, NC) with dual syringe pumps and 500 ± 10 nm excitation and 535 ± 15 nm emission filters (Chroma Corporation, McHenry, IL). For corrector assay, FRT cells were grown at 37°C/5% CO_2 for 18 to 24 h and then incubated for 18 to 24 h with 100 μl of medium containing test compounds (25 μM final concentration). At the time of the assay, cells were washed with PBS and then incubated for 10 min with PBS containing forskolin (20 μM) and genistein (50 μM). For potentiator assay, FRT cells were grown at 37°C/5% CO_2 for 18 to 24 h and then for 18 to 24 h at 27°C. At the time of the assay, cells were washed with PBS and then incubated for 10 min with PBS (50 μl) containing forskolin (20 μM) and test compound (0–25 μM final concentration). For both of the corrector and potentiator assays, each well was assayed individually for I^- influx by recording fluorescence continuously (200 ms per point) for 2 s (baseline) and then for 12 s after rapid addition of 165 μl of PBS, in which 137 mM Cl^- was replaced by I^-. Initial I^- influx rate was computed by fitting the final 11.5 s of the data to an exponential for extrapolation of initial slope, which was normalized for background-subtracted initial fluorescence. All compound plates contained negative controls (DMSO vehicle) and positive controls (10 μM Corr-4a for corrector assay; 50 μM genistein for potentiator assay).

Short-Circuit Current Measurements

ΔPhe508-CFTR-expressing FRT cells were cultured on Snapwell inserts for 7 to 9 days. For corrector testing, test compounds were incubated with FRT cells for 18 to 24 h at 37°C before measurements. For potentiator testing, the FRT cells were incubated for 18 to 24 h at 27°C before measurements. The basolateral solution contained 130 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM glucose, and 10 mM sodium-HEPES, pH 7.3. In the apical bathing solution, 65 mM NaCl was replaced by sodium gluconate, and $CaCl_2$ was increased to 2 mM. Solutions were bubbled with air and maintained at 37°C. The basolateral membrane was permeabilized with 250 μg/ml amphotericin B. Hemichambers were connected to a DVC-1000 voltage clamp (World Precision Instruments, Inc., Sarasota, FL) via

Ag/AgCl electrodes and 1 M KCl agar bridges for recording of short-circuit current.

CFTR Immunoblot

ΔPhe508-CFTR-expressing FRT cells grown on six-well plates were treated with Corr-4a (10 μM), *N*-(2-((3-Cyano-5,7-dimethylquinolin-2-yl)amino)ethyl)-3-methoxybenzamide (CoPo-22; 20 μM), or vehicle (DMSO) at 37°C for 24 h. After treatment, cells were washed with PBS and lysed in 20 mM HEPES, pH 7, 150 mM NaCl, 1 mM EGTA, and 1% Igepal containing complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). After preclearing, lysates were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by immunoblot. Proteins were immunodetected using a mouse monoclonal anti-CFTR antibody (M3A7; Millipore Corporation, Billerica, MA) followed by horseradish peroxidase-conjugated anti-mouse IgG, and visualized by chemiluminescence (ECL Plus; GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK).

Cyclic Nucleotide Assay

FRT cells expressing ΔPhe508-CFTR were grown in six-well plates and incubated with CoPo-22 (0, 5, and 10 μM) for 10 min at 37°C in the absence or presence of forskolin (20 μM) or forskolin + isobutyl methylxanthine (500 μM). Cells were lysed by freeze-thaw, centrifuged to remove cell debris (600*g*, 10 min, 4°C), and assayed for cAMP using a parameter cAMP immunoassay kit (R&D Systems, Minneapolis, MN).

CoPo-22 Synthesis

***N*-(3,5-Dimethylphenyl)acetamide (1).** Acetic anhydride (2.84 ml, 30 mmol) was dissolved in dry tetrahydrofuran (10 ml), purged with N_2, and brought to 0°C. 3,5-Dimethylaniline (1.25 ml, 10 mmol) was then added drop-wise. After the addition of the aniline, the reaction was allowed to warm to room temperature and stirred for an additional hour. The solution was then poured over ice and 1 M NaOH (aqueous) was added until the pH was between 12 and 14. The precipitate was filtered, dissolved in dichloromethane (DCM), and dried over Na_2SO_4. The drying agent was filtered and solvent was removed under reduced pressure to afford pure product in 99% yield as a white solid (Moussaoui et al., 2002).

2-Chloro-5,7-dimethylquinoline-3-carbaldehyde (2). Phosphorous oxychloride (6.52 ml, 70 mmol) and dry dimethylformamide

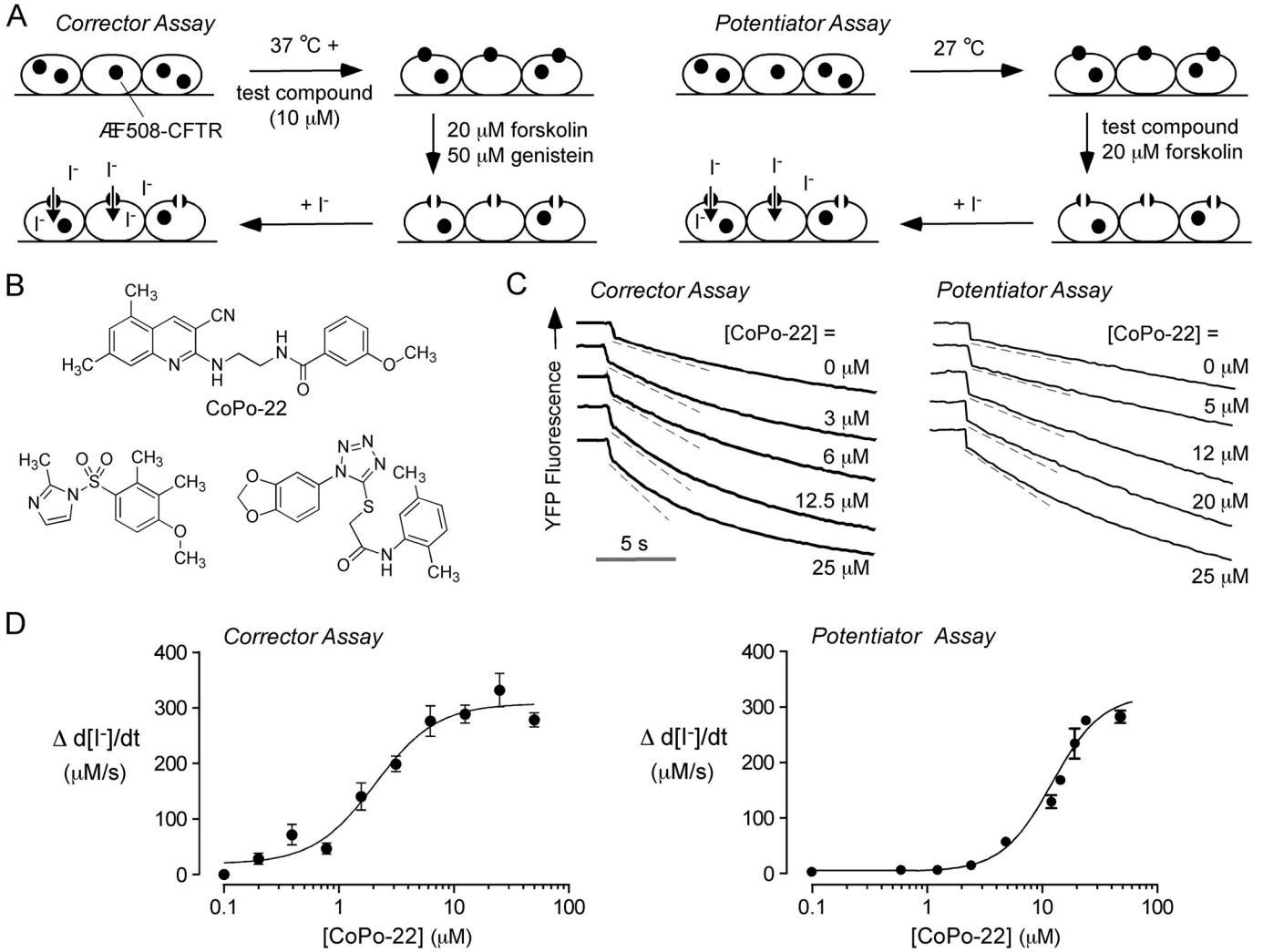

Fig. 1. Dual-acting ΔPhe508-CFTR CoPo identified by high-throughput screening. A, screening procedure. Left, corrector assay: FRT cells coexpressing human ΔPhe508-CFTR and a halide-sensing YFP were incubated with test compounds at 37°C for 24 h. ΔPhe508-CFTR function was assayed in a plate reader from YFP fluorescence quenching in response to iodide addition in the presence of forskolin (20 μM) plus genistein (50 μM). Right, potentiator assay: cells were incubated at 27°C for 24 h before assay (temperature rescue) to target ΔPhe508-CFTR to the plasma membrane. Test compounds were added for 10 min at room temperature in presence of forskolin (20 μM) before iodide addition. B, structures of novel ΔPhe508-CFTR correctors identified in the primary screen. C, representative traces showing iodide influx at different [CoPo-22] for corrector assay (left) and potentiator assay (right). D, dose-response data of CoPo-22 in corrector (left) and potentiator (right) assays (S.E.M., $n = 3$). Fits to single-site activation model shown.

(1.94 ml, 25 mmol) were refluxed for 2 h under N_2. Acetamide **1** (1.632 g, 10 mmol) was added to the reaction solution as a solid and stirred at room temperature for an additional 3 h. The solution was poured slowly over ice, diluted with water (200 ml), and neutralized with solid K_2CO_3. The precipitate was then filtered, dissolved in chloroform, and dried over Na_2SO_4. The drying agent was filtered and solvent was removed under reduced pressure to afford pure product as an orange solid in 95% yield (Moussaoui et al., 2002).

2-Chloro-5,7-dimethylquinoline-3-carbonitrile (3). Aldehyde **2** (1.095 g, 5 mmol), hydroxylamine hydrochloride (0.365 g, 5.25 mmol), and triethylamine (1.00 ml, 7 mmol) were combined in ethanol (50 ml). The solution was refluxed for 3 h, and then the ethanol was removed under reduced pressure. HCl (1 M aqueous, 100 ml)

TABLE 1
Corrector and potentiator activities of selected CoPo analogs

Compound	Corrector		Potentiator	
	EC_{50}	V_{max}	EC_{50}	V_{max}
	μM	$\mu M/s$	μM	$\mu M/s$
CoPo-22	2.2	300	14	306
CoPo-01	3.8	223	15	250
CoPo-02	3.9	281	15	297
CoPo-03	14	288	14	289
CoPo-05	5.0	102	3.8	72
CoPo-08	4.2	140	11	195
CoPo-20	Inactive		13	261
CoPo-09	Inactive		4.6	280
CoPo-10	Inactive		5.0	235
CoPo-14	Inactive		6.0	275

was added to the crude material, and product was extracted with DCM (100 ml). The organic layer was separated and dried over Na_2SO_4, the drying agent was removed by filtration, and the solvent was removed under reduced pressure. The crude product was then dissolved in 50 ml of dry benzene. Thionyl chloride (0.73 ml, 10 mmol) was added drop-wise to the solution, and the reaction was refluxed for 4 h under N_2. The solution was allowed to cool to room temperature, then the benzene and excess thionyl chloride were removed under reduced pressure to afford the known product **3** in 93% yield as a light brown solid [^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.62 (s, 1H), 7.33 (s, 1H), 2.66 (s, 3H), 2.54 (s, 3H)], which was used directly in the next reaction.

2-((2-Aminoethyl)amino)-5,7-dimethylquinoline-3-carbonitrile (4). Carbonitrile **3** (1.083 g, 5 mmol) and 1,2-aminoethane (1.00 ml, 15 mmol) were refluxed in dioxane (50 ml). The reaction was allowed to cool to room temperature, and dioxane was removed under reduced pressure. The crude product was suspended in saturated NH_4Cl (aqueous) and filtered. The solids were washed with diethyl ether and allowed to dry on filter paper under vacuum to give the known product **4** in 80% yield [^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.32 (s, 1H), 6.92 (s, 1H), 5.64 (t, J = 4.8, 1H), 3.65 (q, J = 5.7, 2H), 3.01 (t, J = 6.0, 2H), 2.52 (s, 3H), 2.42 (s, 3H)], which was used directly in the next reaction.

CoPo-22. EDC hydrochloride (0.192 g, 1 mmol), *m*-anisic acid (0.152 g, 1 mmol), and triethylamine (0.14 ml, 2.5 mmol) were dissolved in dry DCM (10 ml). The reaction was stirred at room temperature for 30 min. Carbonitrile **4** (0.201g, 1 mmol) dissolved in 5 ml of dry DCM was added drop-wise to the solution, and the reaction was stirred for 18 h. The reaction was diluted with DCM (50 ml) and washed with 1 M $NaHSO_4$ (aqueous, 2 × 100 ml). Organics were dried over Na_2SO_4, filtered, and solvent was removed under reduced pressure. The crude product was then purified by flash chromatography (4:1 hexane/ethyl acetate mobile phase) to produce a light yellow solid (CoPo-22) in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.13 (s, 1H), 7.32 (s, 1H), 7.28 (s, 1H), 7.20 (d, J = 7.5, 1H), 7.13 (t, J = 7.8, 1H), 6.98 (s, 1H), 6.95 (d, J = 8.1, 1H), 5.82 (s, 1H), 3.91 (q, J = 5.3, 2H), 3.79 to 3.68 (m, 5H), 2.55 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.10, 159.87, 155.10, 144.27, 140.97, 136.27, 135.41, 129.42, 127.13, 124.48, 119.35, 119.23, 117.72, 116.87, 112.46, 94.30, 55.49, 43.39, 41.25, 29.92, 22.21, 18.57. IR (neat): 3380, 3357, 2957, 2925, 2217, 1605, 1583, 1535, 1258. Electrospray ionization-liquid chromatography/mass spectrometry, m/z $[M + H]^+$ = 375.18.

Results

Identification of Cyanoquinoline Correctors/Potentiators of ΔPhe508-CFTR. Screening of ~110,000 small

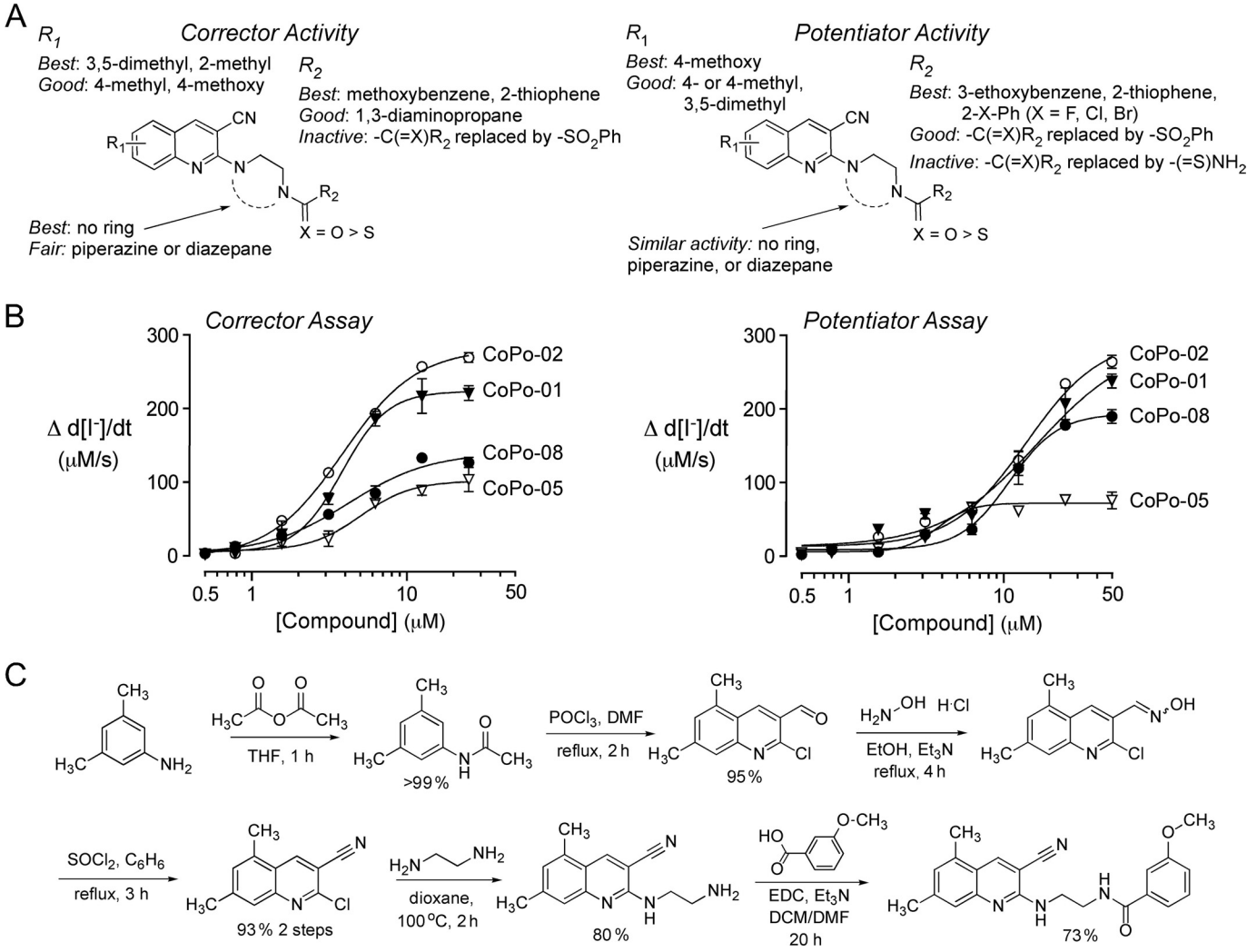

Fig. 2. Structure-activity relationship analysis of CoPo analogs. A, structural determinants of CoPo corrector (left) and potentiator (right) activities. (see Table 1 for activity data of CoPo analogs). B, concentration-dependence of corrector (left) and potentiator (right) activities of CoPo-01, CoPo-02, CoPo-05, and CoPo-08. C, CoPo-22 synthesis scheme.

molecules was done to identify new ΔPhe508-CFTR corrector scaffolds having high correction efficiency or having independent potentiator activity. As diagrammed in Fig. 1A (left), primary screening for corrector activity was done using a cell-based fluorescence assay of iodide influx in which FRT cells expressing ΔPhe508-CFTR and an iodide-sensitive YFP were incubated with test compounds at 10 μM for 18 to 24 h before assay. Iodide influx was measured by the addition of extracellular iodide in the presence of maximal concentrations of a potentiator (50 μM genistein) and cAMP agonist (20 μM forskolin). Compound efficacy and potency (from concentration-dependence studies) in the corrector assay were compared with reference bithiazole Corr-4a (10 μM) and to low-temperature-rescued cells. Active compounds were counter-screened for potentiator activity (Fig. 1A, right), in which iodide influx was measured in the ΔPhe508-CFTR expressing FRT cells after low-temperature rescue (to target ΔPhe508-CFTR to the plasma membrane) and in the presence of 20 μM forskolin.

Screening yielded three novel scaffolds with corrector activity (Fig. 1B), which was verified by $CFTR_{inh}$-172 inhibition of corrector-dependent iodide influx and inability to increase iodide influx in FRT null cells (data not shown). Although none of the three compounds had greater corrector potency than Corr-4a, the cyanoquinoline CoPo-22 showed independent potentiator activity. Representative iodide influx data from the corrector (left) and potentiator (right) fluorescence plate reader assays of CoPo-22 are shown in Fig. 1C. We have not observed potentiator activity previously by a ΔPhe508-CFTR corrector, in which compound is added only a few minutes before assay. Concentration-dependence data are shown in Fig. 1D. As shown by electrophysiological analysis below, the maximal efficacy of CoPo-22 corrector and potentiator actions were comparable with those of the bithiazole Corr-4a and the flavone genistein, respectively.

Synthesis and Structure-Activity Relationship Analysis of CoPo-22. In an attempt to identify CoPo analogs with improved potency as well as to establish initial structure-activity relationship data of the core cyanoquinoline scaffold, we screened 180 commercially available analogs of CoPo-22. Table 1 summarizes corrector and potentiator activities (EC_{50} and V_{max} from concentration-dependence studies) of active compounds, and Fig. 2A summarizes the structural requirements for corrector and potentiator activities. Figure 2B shows representative concentration-dependence data for four analogs. The majority of the 180 cyanoquinoline analogs were inactive. Six compounds showed both corrector and potentiator activities, with CoPo-22 being the most potent corrector. CoPo-01 and CoPo-02 are structurally similar to CoPo-22 and have corrector and potentiator activities comparable with those of CoPo-22. Compounds containing heterocycles, such as the thiophene CoPo-03 and the benzosulfonamide CoPo-05, also show dual activities, albeit of lower potency and/or maximum efficacy. Interestingly, several compounds, such as CoPo-14, showed potentiator-only activity. Replacing the ethylene bridge with a piperazine or 1,4-diazepane ring diminished or abolished corrector activity (comparing CoPo-03 and CoPo-20). No compounds were identified with corrector-only activity. These data suggest that the dual corrector-potentiator activity is not a general feature of cyanoquinolines but is dependent on the particular scaffold and substituents.

For further characterization studies, we resynthesized CoPo-22 in >98% purity in six steps with an overall yield of

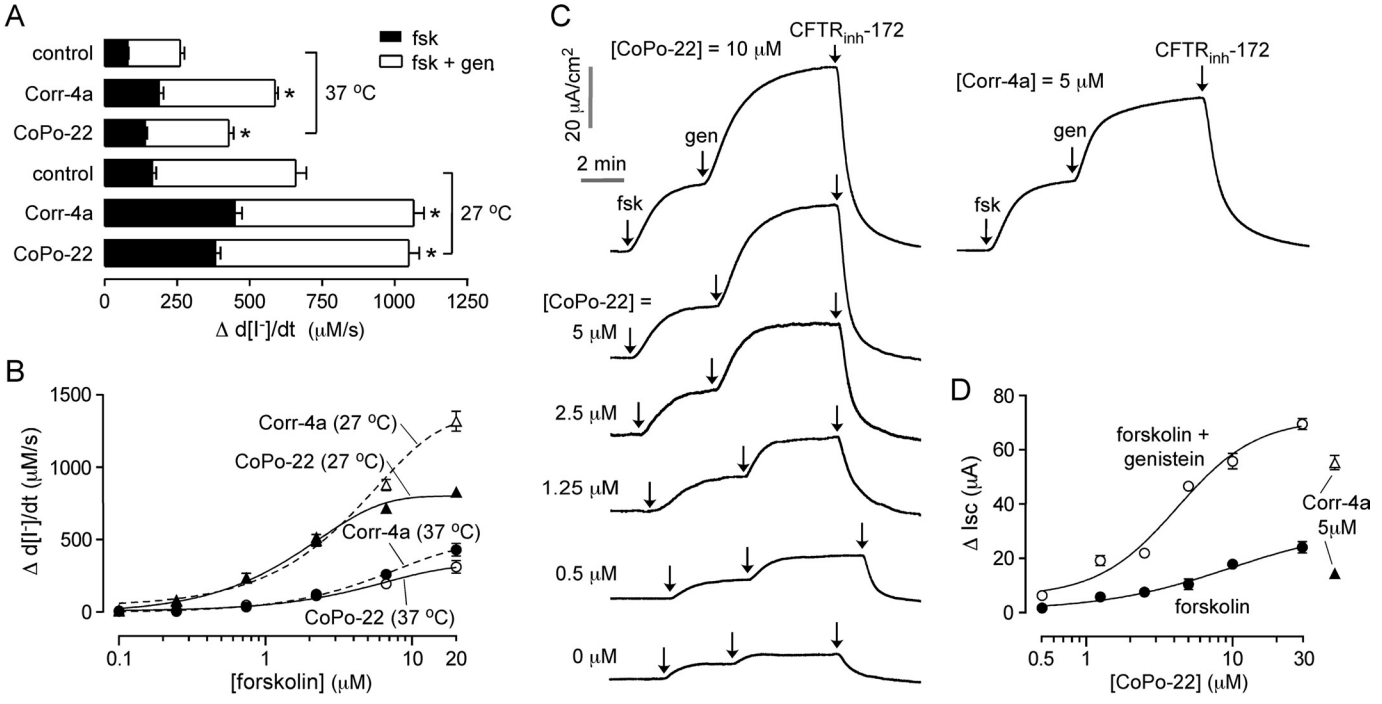

Fig. 3. Characterization of CoPo-22 corrector activity. A, ΔPhe508-CFTR expressing FRT cells were incubated at 37 or 27°C with or without CoPo-22 (20 μM) or Corr-4a (10 μM). Iodide influx (S.E.M., $n = 4$) shown in the presence of forskolin (20 μM) or forskolin (20 μM) plus genistein (50 μM). *, $P < 0.01$ compared with control. B, forskolin dose-response for experiments as in A, measured in the presence of genistein (50 μM). C, representative short-circuit measurements showing apical membrane chloride current after incubation for 24 h at 37°C with indicated [CoPo-22]. Incubation with Corr-4a (5 μM) shown as reference (right). Forskolin (20 μM), genistein (50 μM), and $CFTR_{inh}$-172 (10 μM) added where indicated. D, CoPo-22 concentration-dependence deduced from experiments as in C (S.E.M., $n = 3$–4). Fits to single-site binding model shown.

52% (Fig. 2C). Acetylation of commercially available 3,5-dimethylaniline produced the corresponding acetamide in near quantitative yield. Formation of the quinoline ring was achieved through reaction with phosphorous oxychloride, giving 2-chloroquinoline carbaldehyde. Condensation of the carbaldehyde with hydroxylamine followed by dehydration using thionyl chloride gave the cyanoquinoline core in 93% yield. Displacement of the chloride by 1,2-di-aminoethane gave the aminocyanoquinoline, which was coupled with *m*-anisic acid using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide to give CoPo-22 in 73% yield after purification.

Characterization of CoPo-22 Corrector Activity. To investigate whether CoPo-22 corrector activity is additive with low-temperature rescue, as found previously for Corr-4a (Pedemonte et al., 2005a), ΔPhe508-CFTR expressing FRT cells were incubated with CoPo-22 (20 μM) or Corr-4a (10 μM) for 24 h at 27 or 37°C. As shown in Fig. 3A, CoPo-22 and Corr-4a each increased iodide influx at 37°C, with the need for inclusion of genistein. Both compounds substantially increased iodide influx at 27°C in an approximately additive manner with low-temperature rescue (control, 27°C), suggesting different mechanisms for low-temperature rescue and corrector action. To further investigate the forskolin requirement to increase ΔPhe508-CFTR conductance in the corrector assay, a forskolin concentration-dependence was done in the ΔPhe508-CFTR-expressing FRT cells after corrector incubation and/or low-temperature rescue. Figure 3B shows that substantial increase in iodide influx by each of the rescue/corrector maneuvers required relatively high concentrations of forskolin compared with that of <1 μM needed for activation of wild-type CFTR.

Short-circuit current was measured as a definitive electrophysiological assay to verify CoPo-22 corrector action. Apical membrane chloride current was measured in ΔPhe508-CFTR-expressing FRT cells after basolateral membrane permeabilization and in the presence of a transepithelial chloride gradient (apical, 65 mM; basolateral, 130 mM). Figure 3C shows increased apical membrane current when cells were incubated for 18 to 24 h with increasing [CoPo-22] before short-circuit current assay. The increased apical membrane current was fully inhibited by $CFTR_{inh}$-172. The increase in apical membrane current conferred by 5 and 10 μM CoPo-22 was comparable with that conferred by 5 μM Corr-4a (Fig. 3C, right). Figure 3D summarizes CoPo-22 concentration dependence data from short-circuit current studies.

To investigate possible synergy between CoPo-22 and Corr-4a in corrector efficacy, a Corr-4a concentration-dependence was done at submaximal [CoPo-22] of 0.3 and 1 μM, which did not by itself increase iodide influx significantly. Figure 4A shows a small but significant increase in iodide influx at relatively high [Corr-4a] for 1 versus 0 μM CoPo-22. We further investigated additivity from measurements of iodide influx done after incubation with maximal concentrations of CoPo-22 and Corr-4a, alone or in combination. Figure 4B shows significant additivity of CoPo-22 and Corr-4a action, supporting the possibility of independent actions of these correctors.

The action of CoPo-22 as a corrector of defective ΔPhe508-CFTR cellular processing was verified by CFTR immunoblot analysis. Wild-type CFTR was detected as a strong band at 170 kDa (band C), corresponding to complex glycosylated CFTR. Little or no band C for ΔPhe508-CFTR was detected in

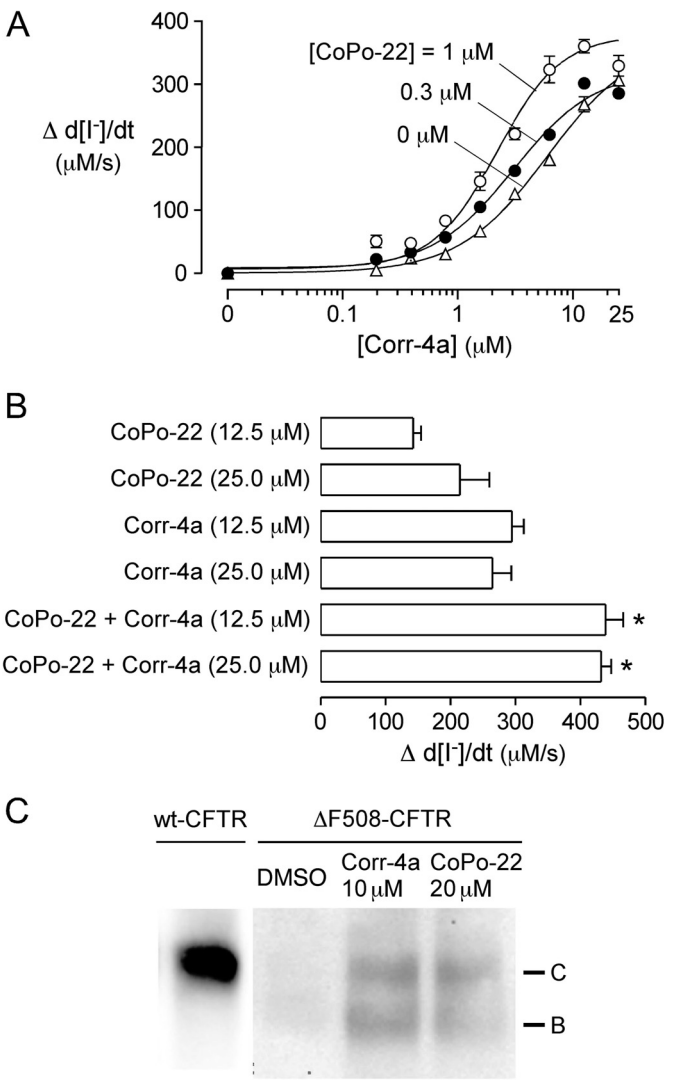

Fig. 4. Additive corrector efficacy of CoPo-22 and Corr-4a. A, Corr-4a concentration-dependence of iodide influx (measured with 20 μM forskolin + 50 μM genistein) in the presence of indicated (submaximal) [CoPo-22] (S.E.M., $n = 4$). B, additivity studies showing iodide influx after incubation with maximal CoPo-22 and Corr-4a (S.E.M., $n = 4$, *, $P < 0.01$ compared with CoPo-22 or Corr-4a alone). C, immunoblot (anti-CFTR antibody) after 24-h incubation at 37°C of ΔPhe508-CFTR expressing FRT cells with Corr-4a or CoPo-22 (or DMSO vehicle). Bands B (core-glycosylated) and C (complex-glycosylated) indicated. For comparison, data shown for (untreated) FRT cells expressing wild-type CFTR.

the absence of corrector, but band C was visualized after 24-h incubation at 37°C with CoPo-22 or Corr-4a. Band B, which corresponds to core-glycosylated ΔPhe508-CFTR, was also seen.

Characterization of CoPo-22 Potentiator Activity. Short-circuit current measurements were done to characterize CoPo-22 potentiator activity, in which apical membrane chloride current was measured in ΔPhe508-CFTR-expressing FRT cells, after low-temperature rescue, in response to CoPo-22 additions. Figure 5A shows CoPo-22 concentration-dependent increases in apical membrane current seen in the presence of forskolin. The lack of CoPo-22 effect in the absence of forskolin indicates the need for ΔPhe508-CFTR phosphorylation, as has been found for other potentiators. Genistein produced a small increase in chloride current after maximal CoPo-22. $CFTR_{inh}$-172 abolished all chloride cur-

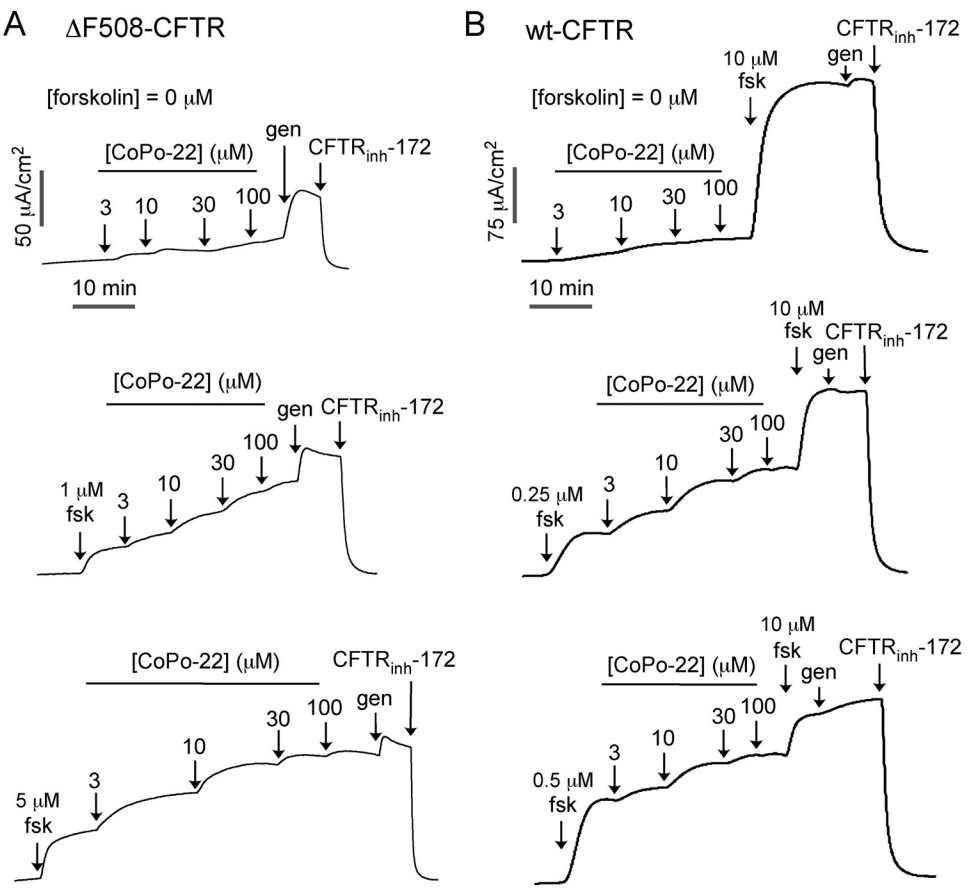

Fig. 5. Characterization of CoPo-22 potentiator activity. Short-circuit current measured in FRT cells expressing ΔPhe-CFTR (A) and wild-type (B), showing responses to indicated forskolin and CoPo-22 concentrations. ΔPhe508-CFTR expressing cells were incubated at 27°C for 24 h before measurement. Where indicated, genistein (50 μM) and $CFTR_{inh}$-172 (10 μM) were added. Representative of two to four sets of measurements.

rent, as expected. Apparent EC_{50} for CoPo-22 potentiator activity as measured by short-circuit current was ~10 μM.

To further investigate CoPo-22 potentiator action, short-circuit current was measured in FRT cells expressing wild-type CFTR (Fig. 5B). Studies were done as in Fig. 5A, except that low concentrations of forskolin (0–0.5 μM) were used because higher concentrations fully activate wild-type CFTR and thus would mask CoPo-22 potentiator action. As found for ΔPhe508-CFTR, there was little effect of CoPo-22 in the absence of forskolin. In each experiment, after CoPo-22 additions, 10 μM forskolin was added to fully activate wild-type CFTR, followed by 50 μM genistein, which had little effect, followed by 10 μM $CFTR_{inh}$-172, which inhibited all chloride current. CoPo-22 partially activated wild-type CFTR when added after 0.25 or 0.5 μM forskolin, with EC_{50} ~10 μM.

Potentiator studies were also done in FRT cells expressing G551D-CFTR, a CF-causing CFTR mutation with defective channel gating but not plasma membrane trafficking. Figure 6A shows that CoPo-22 functioned as a weak potentiator of G551D-CFTR, producing a smaller increase in chloride current than that produced by genistein. Fluorescence plate reader assays in Fig. 6B confirmed that CoPo-22 activated G551D-CFTR in the presence of forskolin, albeit with lower maximal efficacy than genistein. Apparent EC_{50} for CoPo-22 activation of G551-CFTR was ~5 μM (Fig. 6A, bottom), with maximum efficacy much lower than that of genistein.

CoPo-22 did not affect cellular cAMP levels in assays done on the FRT cells after 10-min incubation with CoPo-22 alone or in the presence of 20 μM forskolin. cAMP levels were 4.1 ± 0.4, 4.2 ± 0.5 and 4.3 ± 0.4 pmol/ml (S.E., $n = 4$) for 0, 5, and 10 μM CoPo-22 alone, respectively, and 26 ± 1, 22 ± 2, and 23 ± 2 pmol/ml for CoPo-22 plus forskolin. Addition of the phosphodiesterase inhibitor isobutyl methylxanthine (500 μM) together with forskolin increased cAMP levels to 192 ± 10, 214 ± 18, and 207 ± 26 pmol/ml.

Characterization of CoPo-22 Activity in Human A549 Cells. To test whether CoPo-22 is active in a different cell background, potentiator and corrector assays were done in ΔPhe508-CFTR-transfected A549 cells, which are of human lung epithelial origin. Figure 6B (top) shows that CoPo-22 had potentiator activity in the A549 cells comparable with that in FRT cells. Apparent EC_{50} for CoPo-22 potentiator activity was ~8 μM (Fig. 6B). However, CoPo-22 showed little corrector activity compared with Corr-4a when incubated for 24 h at 37°C (Fig. 6C, top). We further tested CoPo-22 for corrector activity in A549 cells under the low-temperature synergy condition; however, we found that ΔPhe508-CFTR was fully activated by forskolin (20 μM) and genistein (50 μM) in this cell model, precluding analysis of possible synergy (Fig. 6C, bottom). Cell-specific corrector activity is well described (Pedemonte et al., 2010); however, the mechanism(s) responsible for cell type-specificity are not clear.

Discussion

We report here the identification of a novel class of cyanoquinolines, some of which have independent corrector and potentiator activities for normalization of defective ΔPhe508-CFTR folding/ER retention and chloride channel gating, respectively. The dual corrector-potentiator activity of CoPo-22 is consistent with the possibility of binding to site(s) on the CFTR protein. The rapid normalization of defective

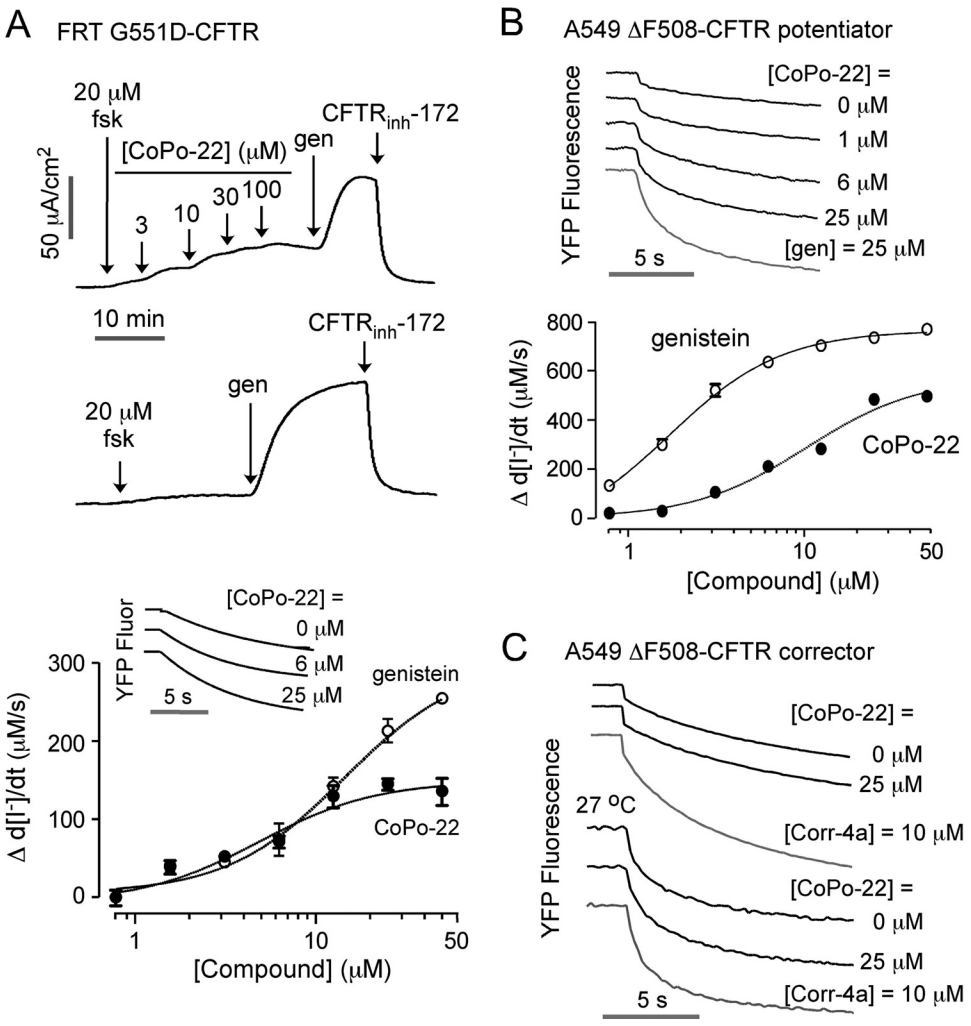

Fig. 6. CoPo-22 activity in G551D-CFTR expressing FRT cells and ΔPhe508-CFTR-expressing A459 cells. A, top, short-circuit current measured in FRT cells expressing G551D-CFTR, showing responses to indicated forskolin and CoPo-22 concentrations. Where indicated, genistein (100 μM) and $CFTR_{inh}$-172 (10 μM) were added. Representative of three sets of measurements. Bottom, plate reader assay of G551D-CFTR chloride conductance showing representative fluorescence quenching curves (inset) and deduced concentration dependence of CoPo-22 and genistein potentiator action (S.E.M., $n = 4$). Measurements were made in the presence of 20 μM forskolin. B, potentiator assay done in ΔPhe508-CFTR expressing A549 cells by YFP/iodide fluorescence quenching as in Fig. 1. Representative fluorescence quenching curves (top) shown with deduced CoPo-22 and genistein concentration-dependence (bottom S.E.M., $n = 4$). C, corrector assay done in ΔPhe508-CFTR expressing A549 cells by YFP/iodide fluorescence quenching, in which cells were incubated with vehicle or indicated correctors at 37°C (top) or 27°C (bottom) for 24 h before iodide influx measurement. Representative of three sets of measurements.

ΔPhe508-CFTR channel gating by CoPo-22 is probably a consequence of direct binding, as is its efficacy in the rapid activation of chloride conductance in wild-type and G551D-CFTR. The mechanism of CoPo-22 correction action is less clear.

The molecular mechanisms remain largely unknown by which ΔPhe508-CFTR correctors partially promote ΔPhe508-CFTR escape from the ER, allowing Golgi and plasma membrane targeting. The general possibilities include corrector action as a pharmacological chaperone by direct ΔPhe508-CFTR binding to improve its folding efficiency and stability at the endoplasmic reticulum and plasma membrane and/or by influencing activity of the proteostasis network (Powers et al., 2009). The latter may entail transcriptional, translational, and/or posttranslational modulations to enhance ΔPhe508-CFTR biogenesis and/or to impede degradation. For example, ΔPhe508-CFTR escape from the ER could be facilitated by extending its folding time and delaying degradation by inhibiting Rma1 and C terminus of Hsc70-interacting protein (CHIP), E3 ubiquitin ligases (Grove et al., 2009) and the 90-kDa heat shock protein cochaperone Aha1 (Wang et al., 2006a). Initial studies of Corr-4a mechanism support the possibility of direct bithiazole-ΔPhe508-CFTR interaction (Pedemonte et al., 2005a), because Corr-4a was ineffective in correcting other mutant membrane proteins for which pharmacological or low-temperature rescue is possible, including a non-ΔPhe508 CFTR mutant. Mutagenesis studies using a double-cysteine CFTR mutant, in which cross-linking occurs only when protein folds into native structure, suggested that Corr-4a interacts with ΔPhe508-CFTR in the ER to promote its folding (Loo et al., 2008), although this could be also an indirect consequence of Corr-4a effect via proteostasis. Although the peripheral stabilization of ΔPhe508-CFTR in the presence of Corr-4a could be explained by a more native-like structure, recent evidence suggests that Corr-4a may interfere with the ubiquitination machinery (Jurkuvenaite et al., 2010) that is involved in disposal of rescued ΔPhe508-CFTR from the cell surface (Okiyoneda et al., 2010). Direct compelling evidence is lacking for the mechanisms by which correctors promote ΔPhe508-CFTR plasma membrane targeting.

An interesting observation was the apparent dissociation of corrector and potentiator activities of the cyanoquinolines, in which some compounds have dual activities whereas others have only potentiator activity. SAR analysis indicated that replacing the flexible ethylene bridge, as found in CoPo-22 or CoPo-03, with a constrained six- or seven-membered ring diminished or abolished corrector activity. Several mechanisms could account for dissociation between corrector and potentiator activities. One possibility is distinct compound binding sites on ΔPhe508-CFTR, or other secondary targets, for corrector and potentiator activities. Alternatively, the differential sensitivity of the protein quality control mech-

anism and CFTR functional responsiveness may account for their discordant potentiator and corrector activities.

Although there are no prior published data on CoPo-22 or the cyanoquinoline analogs tested here, there are several reports in the patent literature on biological data for other compounds containing the cyanoquinoline core. Cyanoquinolines containing a six- or seven-membered ring with structure similar to CoPo-09 and CoPo-20 have been reported to inhibit neuronal degeneration and stimulate neurogenesis (Kelleher, 2007). Cyanoquinolines have also been reported to have B-raf kinase inhibition activity for the potential treatment of cancer (Gahman et al., 2006). Compounds containing only the cyanoquinoline core but with different bridging substituents have been described as orexin antagonists (Branch et al., 2002) and adenosine A2A receptor antagonists (Kosakata et al., 2005). The patent literature thus suggests little cyanoquinoline toxicity. To our knowledge, ion channel-modulating effects of cyanoquinolines have not been reported.

Two classes of compounds had been described to have corrector and potentiator-like activities, the pyrazole 4-methyl-2-(5-phenyl-1*H*-pyrazol-3-yl)-phenol (VRT-532) (Wang et al., 2006a) and Corr-2b-related arylaminothiazoles (AAT) (Pedemonte et al., 2011). VRT-532 was found to decrease ATP turnover rate by purified and reconstituted ΔPhe508-CFTR and reduce ΔPhe508-CFTR susceptibility to trypsin digestion (Wellhauser et al., 2009). It was proposed that VRT-532 might bind directly to ΔPhe508-CFTR and induce and/or stabilize a structure that promotes the channel open state. The mechanism of action of AATs is also unclear, although it was speculated that AATs bind directly to a different site from that of other potentiators, as evidenced by their similar efficacy for activation of G551D and other CFTR mutants.

In conclusion, the cyanoquinolines identified here have bona fide, dual ΔPhe508-CFTR potentiator and corrector activities. Although their rapid, cell type-independent potentiator action on ΔPhe508, G551D, and wild-type CF suggests direct CFTR binding, their corrector mechanism is less clear. The apparent dissociation of cyanoquinoline potentiator and corrector activities, which depended on chemical structure and cell type, suggests the possibility of a coincidental second site of action on ΔPhe508-CFTR or other target(s) involved in cellular protein homeostasis. Further structural modifications of the cyanoquinoline scaffold are needed to improve compound potency and to identify analogs that are effective in human cell lines and primary bronchial cell cultures. Such compounds might be of use as a single-drug therapy of CF caused by the ΔPhe508-CFTR mutation. Single-drug therapy has a priori advantages over multidrug therapy in terms of development costs and the probability of success. The alternative single-drug therapy for CF caused by the ΔPhe508 mutation, which remains an unrealized possibility, is the identification of a highly effective corrector that normalizes ΔPhe508-CFTR folding so as to obviate the need for potentiator activity.

Acknowledgments

We thank Dr. Luis Galietta (Genoa, Italy) for providing transfected A549 cells and for continued advice.

Authorship Contributions

Participated in research design: Phuan, Yang, and Verkman.
Conducted experiments: Phuan and Yang.
Contributed new reagents or analytic tools: Knapp, Wood, and Kurth.
Wrote or contributed to the writing of the manuscript: Phuan, Lukacs, Knapp, Kurth, and Verkman.

References

Accurso FJ, Rowe SM, Clancy JP, Boyle MP, Dunitz JM, Durie PR, Sagel SD, Hornick DB, Konstan MW, Donaldson SH, Moss RB, Pilewski JM, Rubenstein RC, Uluer AZ, Aitken ML, Freedman SD, Rose LM, Mayer-Hamblett N, Dong Q, Zha J, Stone AJ, Olson ER, Ordoñez CL, Campbell PW, Ashlock MA, and Ramsey BW (2010) Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N Engl J Med* **363:**1991–2003.

Branch CL, Coulton S, Johns A, Johnson CN, Porter RA, Stemp G, Thewlis K (2002), inventors; SmithKline Beecham PLC, Branch CL, Coulton S, Johns A, Johnson CN, Porter RA, Stemp G, and Thewlis K, assignees. Preparation of N-aroyl cyclic amines as orexin antagonists. World patent WO2002090355A1. 2002 Nov 14.

Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, and Smith AE. (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* **63:**827–834.

Dalemans W, Barbry P, Champigny G, Jallat S, Dott K, Dreyer D, Crystal RG, Pavirani A, Lecocq JP, and Lazdunski M (1991) Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. *Nature* **354:**526–528.

Du K and Lukacs GL (2009) Cooperative assembly and misfolding of CFTR domains in vivo. *Mol Biol Cell* **20:**1903–1915.

Gahman TC, Lang H, Davis RL, Scranton SA (2006), inventors; Kalypsys Inc., Gahman TC, Lang H, Davis RL, and Scranton SA, assignees. Nitrogen-containing heterocyclic compounds as inhibitors of B-Raf kinase. World patent WO2006124874A2. 2006 Nov 23.

Grove DE, Rosser MF, Ren HY, Naren AP, and Cyr DM (2009) Mechanisms for rescue of correctable folding defects in CFTR ΔF508. *Mol Biol Cell* **20:**4059–4069.

Jurkuvenaite A, Chen L, Bartoszewski R, Goldstein R, Bebok Z, Matalon S, and Collawn JF (2010) Functional stability of rescued ΔF508 cystic fibrosis transmembrane conductance regulator in airway epithelial cells. *Am J Respir Cell Mol Biol* **42:**363–372.

Kalid O, Mense M, Fischman S, Shitrit A, Bihler H, Ben-Zeev E, Schutz N, Pedemonte N, Thomas PJ, Bridges RJ, Wetmore DR, Marantz Y, and Senderowitz H (2010) Small molecule correctors of F508del-CFTR discovered by structure-based virtual screening. *J Comput Aided Mol Des* **24:**971–991.

Kelleher JA (2007), inventor; Neuronascent Inc. and Kelleher JA, assignees. Methods and compositions using heterocyclic compounds for stimulating neurogenesis and inhibiting neuronal degeneration, and therapeutic use. World patent WO2007035722A2. 2007 Mar 29.

Kim Chiaw P, Wellhauser L, Huan LJ, Ramjeesingh M, and Bear CE (2010) A chemical corrector modifies the channel function of F508del-CFTR. *Mol Pharmacol* **78:**411–418.

Kosakata N, Takizawa T, Uchida S, Ono T, and Nakazato Y (2005) inventors; Kyowa Hakko Kogyo Co Ltd., assignee. Preparation of quinoline derivatives, pharmaceuticals and adenosine A2A receptor antagonists containing them, and their use. Japan patent JP2005132834. 2005 May 26.

Loo TW, Bartlett MC, and Clarke DM (2008) Correctors promote folding of the CFTR in the endoplasmic reticulum. *Biochem J* **413:**29–36.

Lukacs GL, Mohamed A, Kartner N, Chang XB, Riordan JR, and Grinstein S (1994) Conformational maturation of CFTR but not its mutant counterpart (ΔF508) occurs in the endoplasmic reticulum and requires ATP. *EMBO J* **13:**6076–6086.

Mills AD, Yoo C, Butler JD, Yang B, Verkman AS, and Kurth MJ (2010) Design and synthesis of a hybrid potentiator-corrector agonist of the cystic fibrosis mutant protein ΔF508-CFTR. *Bioorg Med Chem Lett* **20:**87–91.

Moussaoui F, Belfaitah A, Debache A, and Rhouati S (2002) Synthesis and characterization of some new aryl quinolyl α,β-unsaturated ketones. *J Soc Alger Chim* **12:**71–75.

Noël S, Wilke M, Bot AG, De Jonge HR, and Becq F (2008) Parallel improvement of sodium and chloride transport defects by miglustat (n-butyldeoxynojyrimicin) in cystic fibrosis epithelial cells. *J Pharmacol Exp Ther* **325:**1016–1023.

Okiyoneda T, Barrière H, Bagdány M, Rabeh WM, Du K, Höhfeld J, Young JC, and Lukacs GL (2010) Peripheral protein quality control removes unfolded CFTR from the plasma membrane. *Science* **329:**805–810.

Pedemonte N, Boido D, Moran O, Giampieri M, Mazzei M, Ravazzolo R, and Galietta LJ (2007) Structure-activity relationship of 1,4-dihydropyridines as potentiators of the cystic fibrosis transmembrane conductance regulator chloride channel. *Mol Pharmacol* **72:**197–207.

Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galietta LJ, and Verkman AS (2005a) Small-molecule correctors of defective ΔF508-CFTR cellular processing identified by high-throughput screening. *J Clin Invest* **115:**2564–2571.

Pedemonte N, Sonawane ND, Taddei A, Hu J, Zegarra-Moran O, Suen YF, Robins LI, Dicus CW, Willenbring D, Nantz MH, Kurth MJ, Galietta LJ, and Verkman AS (2005b) Phenylglycine and sulfonamide correctors of defective ΔF508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. *Mol Pharmacol* **67:**1797–1807.

Pedemonte N, Tomati V, Sondo E, Caci E, Millo E, Armirotti A, Damonte G, Zegarra-Moran O, and Galietta LJ (2011) Dual activity of aminoarylthiazoles on the trafficking and gating defects of the cystic fibrosis transmembrane conductance regulator chloride channel caused by cystic fibrosis mutations. *J Biol Chem* **286:**15215–15226.

Pedemonte N, Tomati V, Sondo E, and Galietta LJ (2010) Influence of cell background on pharmacological rescue of mutant CFTR. *Am J Physiol Cell Physiol* **298:**C866–C874.
Powers ET, Morimoto RI, Dillin A, Kelly JW, and Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* **78:**959–991.
Riordan JR (2008) CFTR function and prospects for therapy. *Annu Rev Biochem* **77:**701–726.
Robert R, Carlile GW, Liao J, Balghi H, Lesimple P, Liu N, Kus B, Rotin D, Wilke M, de Jonge HR, Scholte BJ, Thomas DY, and Hanrahan JW (2010) Correction of the Delta phe508 cystic fibrosis transmembrane conductance regulator trafficking defect by the bioavailable compound glafenine. *Mol Pharmacol* **77:**922–930.
Robert R, Carlile GW, Pavel C, Liu N, Anjos SM, Liao J, Luo Y, Zhang D, Thomas DY, and Hanrahan JW (2008) Structural analog of sildenafil identified as a novel corrector of the F508del-CFTR trafficking defect. *Mol Pharmacol* **73:**478–489.
Sampson HM, Robert R, Liao J, Matthes E, Carlile GW, Hanrahan JW, and Thomas DY (2011) Identification of a NBD1-binding pharmacological chaperone that corrects the trafficking defect of F508del-CFTR. *Chem Biol* **18:**231–242.
Thibodeau PH, Richardson JM 3rd, Wang W, Millen L, Watson J, Mendoza JL, Du K, Fischman S, Senderowitz H, Lukacs GL, Kirk K, and Thomas PJ (2010) The cystic fibrosis-causing mutation ΔF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. *J Biol Chem* **285:**35825–35835.
Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, Zhou J, McCartney J, Arumugam V, Decker C, Yang J, Young C, Olson ER, Wine JJ, Frizzell RA, Ashlock M, and Negulescu P (2009) Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc Natl Acad Sci USA* **106:**18825–18830.
Van Goor F, Hadida S, Grootenhuis PD, Stack JH, Burton B, Olson E, Wine J, Frizzell RA, Ashlock M, and Negulescu P (2010) Rescue of the protein folding defect in cystic fibrosis in vitro by the investigational small molecule, VX-809. *J Cyst Fibros* **9 (Suppl 1):**S14.
Van Goor F, Straley KS, Cao D, González J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuis PD, and Negulescu P (2006) Rescue of ΔF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* **290:**L1117–L1130.
Verkman AS and Galietta LJ (2009) Chloride channels as drug targets. *Nat Rev Drug Discov* **8:**153–171.
Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, Gurkan C, Kellner W, Matteson J, Plutner H, Riordan JR, Kelly JW, Yates JR 3rd, and Balch WE (2006a) Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. *Cell* **127:**803–815.
Wang Y, Bartlett MC, Loo TW, and Clarke DM (2006b) Specific rescue of cystic fibrosis transmembrane conductance regulator processing mutants using pharmacological chaperones. *Mol Pharmacol* **70:**297–302.
Wellhauser L, Kim Chiaw P, Pasyk S, Li C, Ramjeesingh M, and Bear CE (2009) A small-molecule modulator interacts directly with deltaPhe508-CFTR to modify its ATPase activity and conformational stability. *Mol Pharmacol* **75:**1430–1438.
Yang H, Shelat AA, Guy RK, Gopinath VS, Ma T, Du K, Lukacs GL, Taddei A, Folli C, Pedemonte N, Galietta LJ, and Verkman AS (2003) Nanomolar affinity small molecule correctors of defective ΔF508-CFTR chloride channel gating. *J Biol Chem* **278:**35079–35085.
Ye L, Knapp JM, Sangwung P, Fettinger JC, Verkman AS, and Kurth MJ (2010) Pyrazolylthiazole as ΔF508-cystic fibrosis transmembrane conductance regulator correctors with improved hydrophilicity compared to bithiazoles. *J Med Chem* **53:**3772–3781.
Yu GJ, Yoo CL, Yang B, Lodewyk MW, Meng L, El-Idreesy TT, Fettinger JC, Tantillo DJ, Verkman AS, and Kurth MJ (2008) Potent s-cis-locked bithiazole correctors of ΔF508 cystic fibrosis transmembrane conductance regulator cellular processing for cystic fibrosis therapy. *J Med Chem* **51:**6044–6054.

Address correspondence to: Dr. Alan S. Verkman, 1246 Health Sciences East Tower, University of California, San Francisco, CA 94143-0521. E-mail: alan.verkman@ucsf.edu