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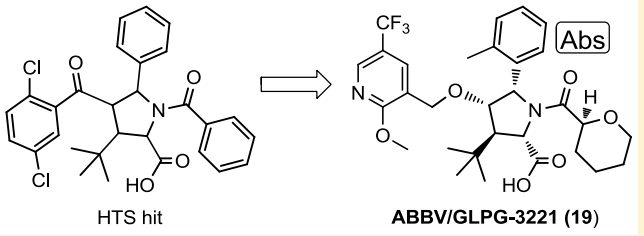
Discovery of ABBV/GLPG-3221, a Potent Corrector of CFTR for the Treatment of Cystic Fibrosis

Marc J. C. Scanio,* Xenia B. Searle, Bo Liu, John R. Koenig, Robert Altenbach, Gregory A. Gfesser, Andrew Bogdan, Stephen Greszler, Gang Zhao, Ashvani Singh, Yihong Fan, Andrew M. Swensen, Timothy Vortherms, Arlene Manelli, Corina Balut, Ying Jia, Wenqing Gao, Hong Yong, Michael Schrimpf, Chris Tse, Philip Kym, and Xueqing Wang

Research and Development, AbbVie, Inc., 1 North Waukegan Road, North Chicago, Illinois 60064, United States

Supporting Information

ABSTRACT: Cystic fibrosis (CF) is a genetic disorder that affects multiple tissues and organs. CF is caused by mutations in the *CFTR* gene, resulting in insufficient or impaired cystic fibrosis transmembrane conductance regulator (CFTR) protein. The deletion of phenylalanine at position 508 of the protein (F508del-CFTR) is the most common mutation observed in CF patients. The most effective treatments of these patients employ two CFTR modulator classes, correctors and potentiators. CFTR correctors increase protein levels at the cell surface; CFTR potentiators enable the functional opening of CFTR channels at the cell surface. Triple-combination therapies utilize two distinct corrector molecules (C1 and C2) to further improve the overall efficacy. We identified the need to develop a C2 corrector series that had the potential to be used in conjunction with our existing C1 corrector series and provide robust clinical efficacy for CF patients. The identification of a pyrrolidine series of CFTR C2 correctors and the structure−activity relationship of this series is described. This work resulted in the discovery and selection of (2*S*,3*R*,4*S*,5*S*)-3-(*tert*-butyl)-4-((2-methoxy-5-(trifluoromethyl)pyridin-3-yl)methoxy)-1-((*S*)-tetrahydro-2*H*-pyran-2-carbonyl)-5-(*o*-tolyl)pyrrolidine-2-carboxylic acid (ABBV/GLPG-3221), which was advanced to clinical trials.



KEYWORDS: *Cystic fibrosis, ABBV/GLPG-3221, cystic fibrosis transmembrane conductance regulator protein (CFTR)*

Cystic fibrosis (CF) is a genetic disorder that affects multiple tissues and organs. CF impacts more than 70 000 people worldwide.[1] It is caused by mutations in the *CFTR* gene, resulting in insufficient or impaired cystic fibrosis transmembrane conductance regulator (CFTR) protein.[2] CFTR is an anion channel that is capable of transporting both Cl^- and HCO_3^- and is expressed in many epithelial cells. It is also responsible for regulating the balance of water and salt in multiple tissues. The impairment of the normal CFTR channel function in the lungs results in the buildup of a thick mucus that leads to bacterial colonization, resulting in the loss of lung function in CF patients. The loss of this lung function is the leading cause of death in these CF patients. These patients are also affected by potentially life-threatening pancreatic insufficiency and gastrointestinal issues.

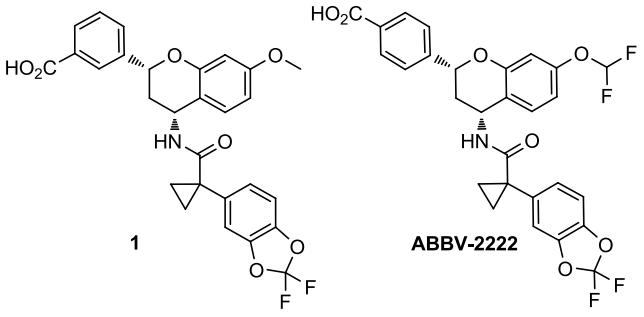


Figure 1. Structures of previously reported CFTR C1 corrector compounds.

Over 2000 single nucleotide polymorphisms (SNPs) have been identified in the CFTR protein of CF patients, 336 of which are disease-causing.[3] The complications that result from these mutations fall into six classes affecting the stability, function, trafficking, or production of CFTR.[4] The deletion of phenylalanine at position 508 of the protein (F508del-CFTR) is the most common mutation observed in CF patients. Approximately 85% of CF patients have at least one copy of the F508del-CFTR allele, and the homozygous F508del-CFTR mutation is observed in ∼50% of CF cases. This deletion prevents the nascent protein from folding correctly and translocating to the plasma membrane for normal anion transport.[5,6] As a result, far fewer F508del-CFTR channels are expressed on the cell membrane compared with wild-type

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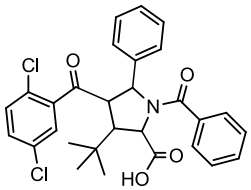
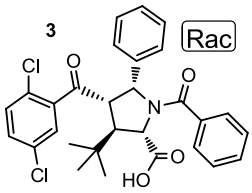
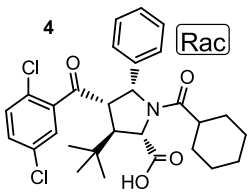
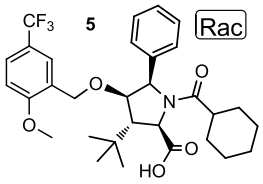
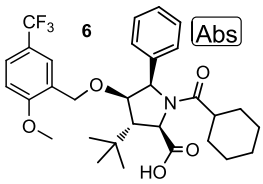
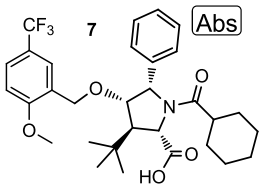
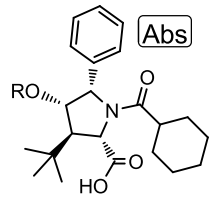
Table 1. CSE-HRP SAR for the Initial Series Hits

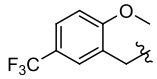
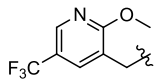
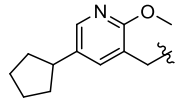
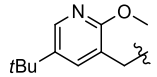
Structure/Compound	CSE-HRP[a] EC_{50} (Range), Max Act% 1	CSE-HRP With 1[b] EC_{50} (Range), Max Act% 1
 **2**, HTS hit Mixture of diastereomers	2.72 μM (2.3-3.2 μM), 186%	2.11 μM (1.7-2.7 μM), 681%
 **3** Rac	2.66 μM (2.2-3.2 μM), 151% N = 326	1.97 μM (1.5-2.5 μM), 527% N = 326
 **4** Rac	2.52 μM (2.3-2.7 μM), 464%	2.01 μM (1.7-2.3 μM), 1420%
 **5** Rac	1.32 μM (0.96-1.8 μM), 139%	1.13 μM (0.85-1.5 μM), 590%
 **6** Abs	>20 μM, 10%	1.29 μM, 148%
 **7** Abs	0.82 μM (0.81-0.83 μM), 217%	0.58 μM (0.54-0.62 μM), 730%

[a]CSE-HPR assay was run using compound **1** (3 μM) as the positive control. All values are the geometric means of at least two determinations. [b]This format of the CSE-HPR assay was run with compound **1** (3 μM) as a C1 corrector and as the positive control. All values are the geometric means of at least two determinations.

CFTR (WT-CFTR)-expressing cells.[7] Furthermore, F508del-CFTR channels are less effective at channel gating compared with WT-CFTR.[8] The most effective treatments of these patients employ two CFTR modulator classes.[9] CFTR correctors increase protein levels at the cell surface;[10] CFTR

Table 2. HBE-TECC SAR for Ether Analogs 7−10



Compound	R	HBE-TECC[a] EC_{50}, Max Act% ABBV-2222 + GLPG1837
7		173 nM, 590%
8		150 nM, 650%
9		247 nM, 203%
10		132 nM, 455%

[a]HBE-TECC assay was run using ABBV-2222 (0.15 μM) and GLPG1837 (0.75 μM) as the positive controls. All values are the geometric means of at least two determinations.

potentiators enable the functional opening of CFTR channels at the cell surface. Dual-combination therapies such as Orkambi and Symdeko have been approved,[11−14] restoring lung function to a modest degree in F508del homozygous patients. Triple combinations utilizing two distinct corrector molecules and a potentiator molecule have demonstrated transformational clinical benefit in patients with a F508del-CFTR mutation on at least one allele.[15−18] A New Drug Application has recently been filed to the FDA for one such triple combination.[19,14] However, how universal this will be across the multitude of genotypes remains to be assessed.

Herein we report the identification of a pyrrolidine series of CFTR C2 correctors and the structure−activity relationship (SAR) of this series, which leads to the identification of ABBV-3221 (also known as GLPG-3221). This CFTR C2 corrector has been advanced to clinical trials.

We have previously reported a novel series of CFTR C1 corrector compounds, including ABBV-2222 (Figure 1),[20] which are currently in clinical trials in CF patients. These C1 correctors restore the early folding process of the protein to increase the number of CFTR channels expressed on the cell membrane. C2 correctors work by any complementary mechanism to further increase CFTR at the cell surface.[15] In an effort to develop a CF triple therapy, we initiated efforts to develop a CFTR C2 corrector.

Two cell-based assays were employed to determine the activity of CFTR modulators. CFTR correctors are a class of modulators that correct the misfolding mutant CFTR protein, increasing the CFTR channel expression on the cell surface. The CFTR expression was quantified using immortalized bronchial epithelial cells (CFBE41o)[21] transduced with

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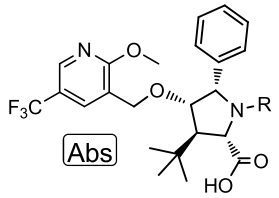
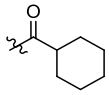
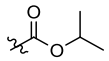
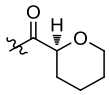
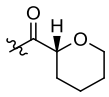
Table 3. HBE-TECC and CYP3A4 Induction SAR for N1 Analogs 8 and 11−13



Compound	R	HBE-TECC[a] EC_{50}, Max Act% ABBV-2222 + GLPG1837	CYP3A4 induction[b] (% rifampin)
8		150 nM, 650%	74% @ 10 μM
11		137 nM, 438%	1% @ 10 μM
12		213 nM, 720%	0% @ 10 μM
13		346 nM, 351%	NT

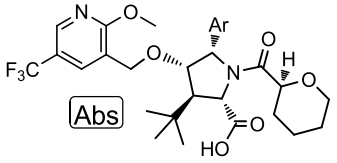
[a]HBE-TECC assay was using ABBV-2222 (0.15 μM) and GLPG1837 (0.75 μM) as the positive controls. All values are the geometric means of at least two determinations. [b]All values are the means of at least three determinations.

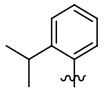
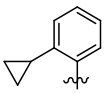
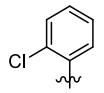
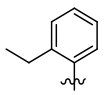
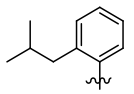
F508del-CFTR fused with horseradish peroxidase (HRP). See the Supporting Information for experimental details. Test corrector compounds were incubated with or without a cocorrector, 2 μM of compound **1**, overnight, followed by the measurement of the HRP activity to detect matured F508del-CFTR levels.[22] This CSE-HRP (cell surface expression−HRP) assay was used for primary high-throughput screens (HTSs) and as a primary assay for driving the medicinal chemistry efforts.

The objective of this research is to increase the membrane expression and improve the stability and function of the corrected F508del-CFTR channels. We measured the function of the channels using a HBE-TECC (human bronchial epithelial−transepithelial current clamp) assay. Primary human bronchial epithelial (HBE) cells from F508del CF patients were used, and an electrophysiology (E-Phys) measurement of the CFTR chloride ion current across the apical membrane was taken. See the Supporting Information for experimental details. In brief, the assay uses a transepithelial current clamp (TECC)[23] instrument to measure the function of the F508del-CFTR channel after the overnight incubation of the test corrector compounds in the presence of a potentiator[24] and cocorrector and activated with forskolin. The assay uses negative and positive controls (0.15 μM of ABBV-2222) to measure the correction of the mutated CFTR channel.[25]

An HTS was conducted, with the objective of identifying compounds that could serve as hits for the development of a CFTR C2 corrector series using the CSE-HRP assay. The

Table 4. HBE-TECC and CYP3A4 Induction SAR for Aromatic Analogs 10 and 12−17



Compound	Ar	HBE-TECC[a] EC_{50}, Max Act% ABBV-2222 + GLPG1837	CYP3A4 induction[b] (% rifampin)
12		213 nM, 720%	0% @ 10 μM
14		35 nM, 570%	96% @ 1 μM
15		66 nM, 610%	60% @ 1 μM
16		222 nM, 694%	8% @ 1 μM
17		48 nM, 490%	78% @ 10 μM
18		52 nM, 472%	118% @ 10 μM
19		105 nM, 585%	5% @ 1 μM

[a]HBE-TECC assay using ABBV-2222 (0.15 μM) and GLPG1837 (0.75 μM) as the positive controls. All values are the geometric means of at least two determinations. [b]All values are the means of at least three determinations.

desired compound profile would improve the cell surface expression with and without cocorrector **1** present. Compound **2**, a pentasubstituted pyrrolidine, was identified as a potential hit. It had an average EC_{50} of 2.72 μM, with a maximum response of 186% relative to compound **1** alone (Table 1). In the presence of **1**, compound **2** had an average EC_{50} of 2.11 μM, with a maximum response of 681% relative to compound **1** (Table 1). To confirm the potency of this hit, the CSE-HRP assay was repeated four times with and without compound **1** as a C1 corrector. This resulted in an EC_{50} range of 2.3 to 3.2 μM alone and an EC_{50} range of 1.7 to 2.7 μM in the presence of compound **1**.

The exact stereochemistry of **2** was not specified at the time of registration in 1982, and the compound was clearly a mixture of diastereomers. One of the diastereomers of **2** was synthesized and isolated as a racemic mixture, compound **3**. It had an average EC_{50} of 2.66 μM (151%) and an average EC_{50} of 1.97 μM (527%) in the presence of compound **1** (Table 1). This key hit compound was used as a positive control in the

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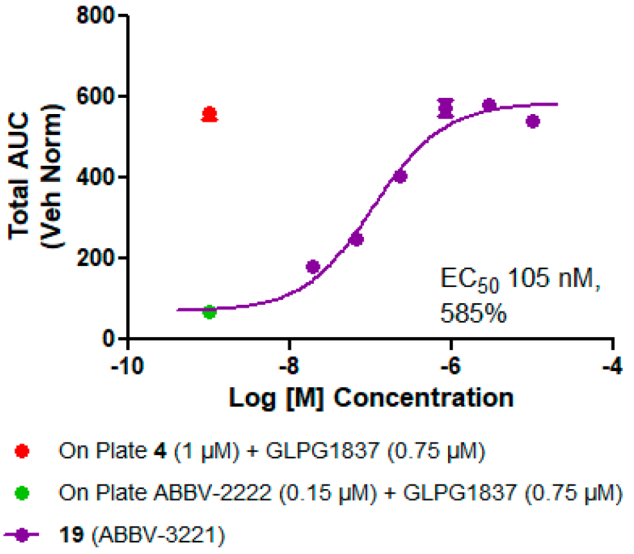
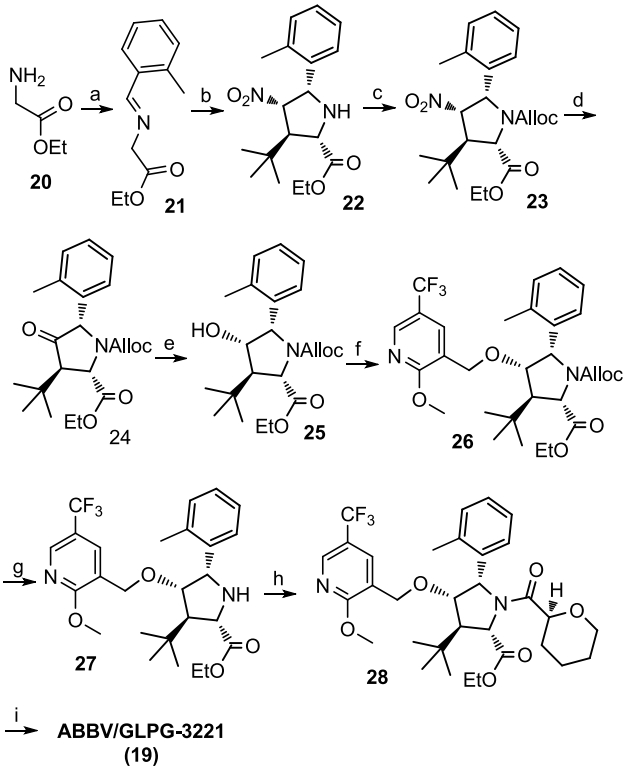


Figure 2. Dose−response graph for **19** in triple combination format. The data are a combination from two separate experiments run on different days with cells from one CF donor with homozygous F508delCFTR mutation, with $n = 3$ replicates at each concentration.

Scheme 1. Discovery Synthesis of ABBV/GLPG-3221 (19)[a]



[a]Reagents and conditions: (a) $MgSO_4$, TEA, 2-methylbenzaldehyde, DCM; (b) (2-(bis(3,5-bis(trifluoromethyl)phenyl)phosphino)-3-((*S*)-4-isopropyl-4,5-dihydrooxazol-2-yl)cyclopenta-2,4-dien-1-yl)-(cyclopenta-2,4-dien-1-yl)iron, $Cu(OTf)_2$, KOtBu, (*E*)-3,3-dimethyl-1-nitrobut-1-ene, THF; (c) allyl chloroformate, toluene; (d) PDC, 6 N HCl, Zn dust; (e) $NaBH_4$, EtOH; (f) KOtBu, 3-(bromomethyl)-2-methoxy-5-(trifluoromethyl)pyridine, DMF; (g) 1,3-dimethlbarbituric acid, $Pd(PPh_3)_4$, EtOAc/DCM; (h) tetrahydropyran-2-carboxylic acid, oxalyl chloride, DMF, DCM; and (i) LiOH, MeOH/THF.

Table 5. Pharmacokinetic Properties of 19 in Rat and Dog[a]

	IV dose		oral dose
	Cl[b]	$t_{1/2}$[c]	*F* (%)
rat PK	0.13	3.0	53
dog PK	0.64	6.4	78

[a]Parameters were determined using a 1 mg/kg dose. [b]Clearance (L/h/kg). [c]Half life (h).

CSE-HRP assay and was tested over 300 times with and without cocorrector **1**. In an effort to improve the properties of compound **3**, by increasing the fraction of sp^3-hybridized carbons (Fsp3), the phenyl group of the *N*-benzoyl moiety was replaced with a cyclohexane, resulting in compound **4**. This compound had comparable potency, with greatly improved efficacy in the CSE assay. The ketone moiety of compounds **2**−**4** was flagged for replacement. Moving from the ketone in **4** to the benzyl ether in **5** increased the Fsp3 and removed any issues of reactivity associated with the acetophenone group.[26] Compound **5** maintained reasonable potency. The individual enantiomers of **5** were isolated by chiral preparative supercritical fluid chromatograpy (SFC) to afford enantiomers **6** and **7**. The majority of the potency and the efficacy of **5** clearly resides in the (2*S*,3*R*,4*S*,5*S*)-isomer, **7** (Table 1). Running the CSE-HRP assay in the presence of C1 corrector, **1**, made the EC_{50} slightly more potent while greatly improving the efficacy. On the basis of the data presented in Table 1, we decided to focus our efforts on benzyl ether analogs with the (2*S*,3*R*,4*S*,5*S*) stereochemistry, analogs to compound **7**.

Compound **7** was thus identified as submicromolar in the CSE-HRP assay and was then advanced to testing in human bronchial epithelial (HBE) cells and evaluated in the TECC assay. It showed good potency (173 nM) and efficacy (590%) relative to the positive control, ABBV-2222 (0.15 μM) in the presence of potentiator GLPG1837 (0.75 μM) (Table 2). Initially, we chose to evaluate the SAR of the benzyl ether portion of this series (Table 2). The cLogP of compound **7** is 6.01, and we were interested in introducing a heteroatom into the aromatic ring of **7**, such as that found in pyridine **8**. The cLogP of compound **8** was reduced to 5.27, and the compound had improved potency in the HBE-TECC assay (150 nM, 650%) (Table 2). Replacing the trifluoromethyl group of compound **8** with a cyclopentane or a *tert*-butyl group led to compounds that were both less potent and had higher cLogP values (5.95, **9**; 6.12, **10**).

The further interrogation of compound **8** indicated that it is a CYP3A4 inducer in an induction assay compared with the positive control rifampin (Table 3). In this assay (Supporting Information), the compound is tested at 10 or 1 μM in triplicate, and the response is compared with that of 10 μM rifampin, a CYP3A4 inducer. Compounds with a response of <20% of rifampin at 10 μM are considered to have a low induction risk. Compound **8** had a response of 74% at 10 μM.

Analogs of compound **8** were synthesized that differed in the substitution off of the pyrrolidine nitrogen. Switching from the cyclohexanecarboxamide of **8**, to the isopropyl carbamate found in **11** led to a compound that was slightly more potent but less efficacious and, very importantly, not a CYP3A4 inducer (Table 3). The diastereomeric pyran compounds **12** and **13** were prepared as direct analogs of compound **8**. The (*S*)-pyran analog **12** was more potent and much more efficacious than the (*R*)-pyran analog **13**. Compound **12** was advanced to the CYP3A4 induction assay, where it did not

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induce CYP3A4 (Table 3). Compared with cyclohexane **8**, pyran **12** was somewhat less potent (213 vs 150 nM), but more efficacious (720 vs 650%).

Because of the improvement in CYP3A4 induction, we focused on optimizing compounds with the (*S*)-pyran substitution. These analogs varied the aromatic group off the C5 position of the pyrrolidine ring (Table 4). The introduction of large alkyl groups at the ortho position of the aromatic ring (compounds **14**, **15**, **17**, and **18**) led to a dramatic improvement in potency (35−66 vs 213 nM, compound **12**) while maintaining reasonable efficacy. All of these compounds were, however, potent CYP3A4 inducers whether they were tested at 1 or 10 μM. Chloro substitution at the ortho position (**16**) afforded a compound that did not show induction at 1 μM but did not have the desired potency (Table 4). Finally, the substitution of a methyl group at the ortho position resulted in a compound with good balance of characteristics (Compound **19**, Table 4). Whereas compound **19** was somewhat less potent than compounds with a larger alkyl substitution, it still had an EC_{50} = 105 nM and, importantly, was not found to have a high CYP3A4 induction risk (Table 4).

The detailed dose response HBE-TECC data for compound **19** are shown in Figure 2. The 100% response is set by the combination of ABBV-2222 (0.15 μM) and GLPG1837 (0.75 μM). The addition of compound **19** to the combination of ABBV-2222 and GLPG1837 increased the CFTR current with EC_{50} = 105 nM and a maximum response of 585% compared with the double combination alone.

Compound **19** was selected for advancement into additional characterization. An optimized discovery scale synthesis is illustrated in Scheme 1. Commercially available glycine ethyl ester hydrochloride (**20**) was condensed with 2-methylbenzaldehyde to afford imine **21**. An enantioselective [3 + 2] cycloaddition was an ideal method to forge the stereogenic pyrrolidine core (**22**) from imine **21** and (*E*)-3,3-dimethyl-1-nitrobut-1-ene.[27] This was achieved using a Cu-catalyzed enantioselective cycloaddition catalyzed by a Cu(I)/(2-(bis-(3,5-bis(trifluoromethyl)phenyl)phosphino)-3-((*S*)-4-isopropyl-4,5-dihydrooxazol-2-yl)cyclopenta-2,4-dien-1-yl)-(cyclopenta-2,4-dien-1-yl)iron complex. Reacting pyrrolidine **22** with allyl chloroformate gave the Alloc-protected intermediate **23**. A Nef reaction converted the nitro moiety of compound **23** into the ketone of **24**. Sodium borohydride in ethanol gave the selective reduction of the ketone to alcohol **25**. The alkylation of alcohol **25** with 3-(bromomethyl)-2-methoxy-5-(trifluoromethyl)pyridine using potassium *tert*-butoxide as the base in dimethylformamide (DMF) generated ether **26**. The Alloc group of **26** was removed with catalytic Pd to afford the NH pyrrolidine **27** that was readily acylated with the acid chloride generated in situ from tetrahydropyran-2-carboxylic acid to yield ester **28**. The ester was saponified to the acid using lithium hydroxide in MeOH/THF to afford compound **19**, ABBV-3221. Optimizations required for a multikilogram scale process will be reported.[28]

Compound **19** was also found to have a good pharmacokinetic (PK) profile in both rats and dogs, which is summarized in Table 5.

On the basis of the data presented here, along with additional preclinical safety evaluations, compound **19** (ABBV-3221) was identified as a novel, potent, and efficacious corrector of CFTR with a reasonable safety and PK profile. Compound **19** provides substantial CFTR functional activity improvement over a dual combination of GLPG-1837 and ABBV-2222 when used as a triple combination in in vitro studies; therefore, it was selected to advance into clinical trials to evaluate its potential for treating cystic fibrosis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00377.

Synthetic procedures and compound characterization data for ether compounds **5**−**19** and intermediates as well as assay procedures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1-847-935-1064. E-mail: marc.scanio@abbvie.com.

ORCID

Marc J. C. Scanio: 0000-0002-3503-8020
Andrew Bogdan: 0000-0003-0913-0779
Stephen Greszler: 0000-0003-1993-2417

Author Contributions

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■ ABBREVIATIONS

Alloc, allyloxycarbonyl; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator protein; CSE-HRP, cell surface expression−horseradish peroxidase; CYP3A4, cytochrome P450 3A4; DCM, dichloromethane; DDI, drug−drug interaction; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; E-Phys, electrophysiology; Emax, maximum percent activity; ER, endoplasmic reticulum; FEV_1, forced expiratory volume in 1 s; Fsp3, sp^3-hybridized carbons; HBE, human bronchial epithelial; HBE-TECC, human bronchial epithelial−transepithelial current clamp; HRP, horseradish peroxidase; HTS, high-throughput screen; I_{EQ}, equivalent CFTR current; PDC, potassium dichromate; PK, pharmacokinetic; SAR, structure−activity relationship; SNPs, single nucleotide polymorphisms; SFC, supercritical fluid chromatography; TEA, triethylamine; TECC, transepithelial current clamp; THF, tetrahydrofuran; WT-CFTR, wild-type CFTR

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