Journal of Medicinal Chemistry

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Drug Annotation

Discovery of 4-[(2R,4R)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-(difluoromethoxy)-3,4-dihydro-2H-chromen-2-yl]benzoic acid (ABBV/GLPG-2222), a Potent Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Corrector for the Treatment of Cystic Fibrosis

Xueqing Wang, Bo Liu, Xenia Searle, Clinton Yeung, Andrew R Bogdan, Stephen Nathaniel Greszler, Ashvani Singh, Yihong Fan, Andrew M. Swensen, Timothy Vortherms, Corina Balut, Ying Jia, Kelly E. Desino, Wenqing Gao, Hong Yong, Chris Tse, and Philip R. Kym

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Discovery of 4-[(2R,4R)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-(difluoromethoxy)-3,4-dihydro-2H-chromen-2-yl]benzoic acid (ABBV/GLPG-2222), a Potent Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Corrector for the Treatment of Cystic Fibrosis

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Abstract

Cystic fibrosis (CF) is a multi-organ disease of the lungs, sinuses, pancreas, and gastrointestinal tract that is caused by a dysfunction or deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an epithelial anion channel that regulates salt and water balance in the tissues in which it is expressed. To effectively treat the most prevalent patient population (F508del mutation), two biomolecular modulators are required, correctors to increase CFTR levels at the cell surface, and potentiators to allow the effective opening of the CFTR channel. Despite approved potentiator and potentiator/corrector combination therapies, there remains a high need to develop more potent and efficacious correctors. Herein, we disclose the discovery of a highly potent series of CFTR correctors and the structure activity relationship (SAR) studies that guided the discovery of ABBV/GLPG-2222 (**22**), which is currently in clinical trials in patients harboring the F508del CFTR mutation on at least one allele.

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Introduction

Cystic fibrosis (CF) is a multi-organ disease of the lungs, sinuses, pancreas, and gastrointestinal tract that is caused by a dysfunction or deficiency of the cystic fibrosis transmembrane conductance regulator protein (CFTR). CFTR is an epithelial anion channel that regulates salt and water homeostasis in the tissues in which it is expressed. The onset of lung disease as a result of defects in mucociliary clearance is the primary driver of morbidity in CF patients.

There are more than 2000 mutations to the CFTR protein, and among them 281 are disease-causing,[1] which can be categorized into six broad classes that result in defects on CFTR production, trafficking, function, and/or stability.[2-3] The two most severe mutations lead to gating or folding defects. Approximately 10% of patients have gating mutations (e.g., G551D), where wild type levels of CFTR channels on the cell surface are not functional. These patients can be treated with a potentiator such as Ivacaftor,[4] which enhances the channel opening and has demonstrated remarkable and sustained clinical efficacy represented by the significant improvements of forced expiratory volume in one second (FEV_1).[5] For about 50% patients who harbor a homozygous F508del mutation, where the phenylalanine 508 is omitted, the disease is characterized by both a gating defect and reduced quantity of matured CFTR channels on the cell surface.[2-3, 6] To improve life of these patients, two biomolecular modulators are required, correctors to increase CFTR levels at the cell surface, and potentiators to allow the effective opening of the CFTR channel.[7] Orkambi, a combination of Lumacaftor (VX-809)[8] and Ivacaftor, has been approved for the treatment of this patient population.[9] Despite the approval, compared to the transformational effects of Ivacaftor on patients with gating mutations, the effects of Orkambi on F508del homozygous patients are modest (2-4% improvement of FEV_1 for

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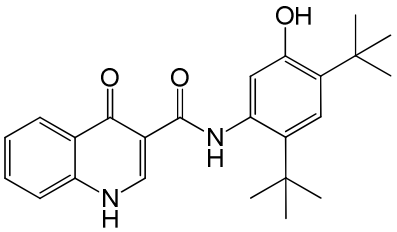
Orkambi vs 10% for Ivacaftor). In the clinic, bronchoconstriction has been reported for Lumacaftor treated individuals, likely due to off-target effects of Lumacaftor.[10-12] Additionally, Lumacaftor has been reported to be a cytochrome P450 3A4 (CYP3A4) inducer[13] and therefore a drug-drug interaction (DDI) perpetrator.[14] More recently, Phase 3 results with the combination of Tezacaftor (VX-661) and Ivacaftor have been reported that also demonstrated a modest improvement in FEV_1,[15] while the liabilities associated with Lumacaftor (i.e., bronchoconstriction and CYP3A4 induction) were not observed. The improvement in tolerability of Tezacaftor/Ivacaftor appears sufficient for an approval submission even though there is no statistically significant improvement in clinical efficacy. Preclinical characterization of Tezacaftor suggests that the improvement in safety may have come at the expense of efficacy given the reduced maximum current restored in F508del homozygous human bronchial epithelial (HBE) cells.[13]

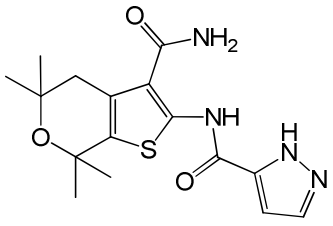
In summary, there is a clear need to continue to develop additional CF therapies to ensure effective options to the patients. In this regard, we report the discovery of **22** (ABBV/GLPG-2222), a CFTR corrector that is currently in clinical trials and serves as a foundation for combination therapies being investigated for CF patients harboring a F508del mutation on at least one allele.

Figure1. Structure of known CFTR potentiators and correctors

Potentiators



Ivacaftor (VX-770)



GLPG1837

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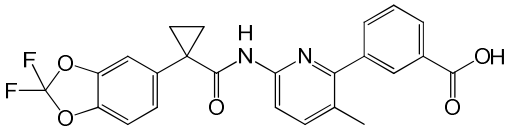
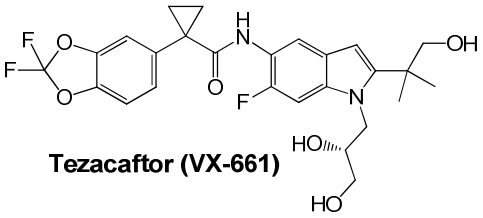
Correctors



Lumacaftor (VX-809)



Tezacaftor (VX-661)

Strategy

Among the correctors that have been disclosed, the most notable correctors are VX-809 (Lumacaftor) and VX-661 (Tezacaftor) (Figure 1). We sought to identify a corrector with differentiated structures that can provide improved potency, efficacy and selectivity with the rationale that this would lead to potential enhanced clinical characteristics. From a strategic perspective we planned to leverage AbbVie's large collection of acid and amine monomers to assemble novel lead compounds through a final amide bond coupling reaction (Figure 2). From a design perspective, we selected acid and amine monomers that contained optimal sp^3 character and physiochemical properties.

The overall process began with the selection of a large number of monomers, either amines or acids, based upon physiochemical parameters such as clogP, molecular weight, and sp^3 content (Figure 2). Virtual libraries were then enumerated using these monomers.

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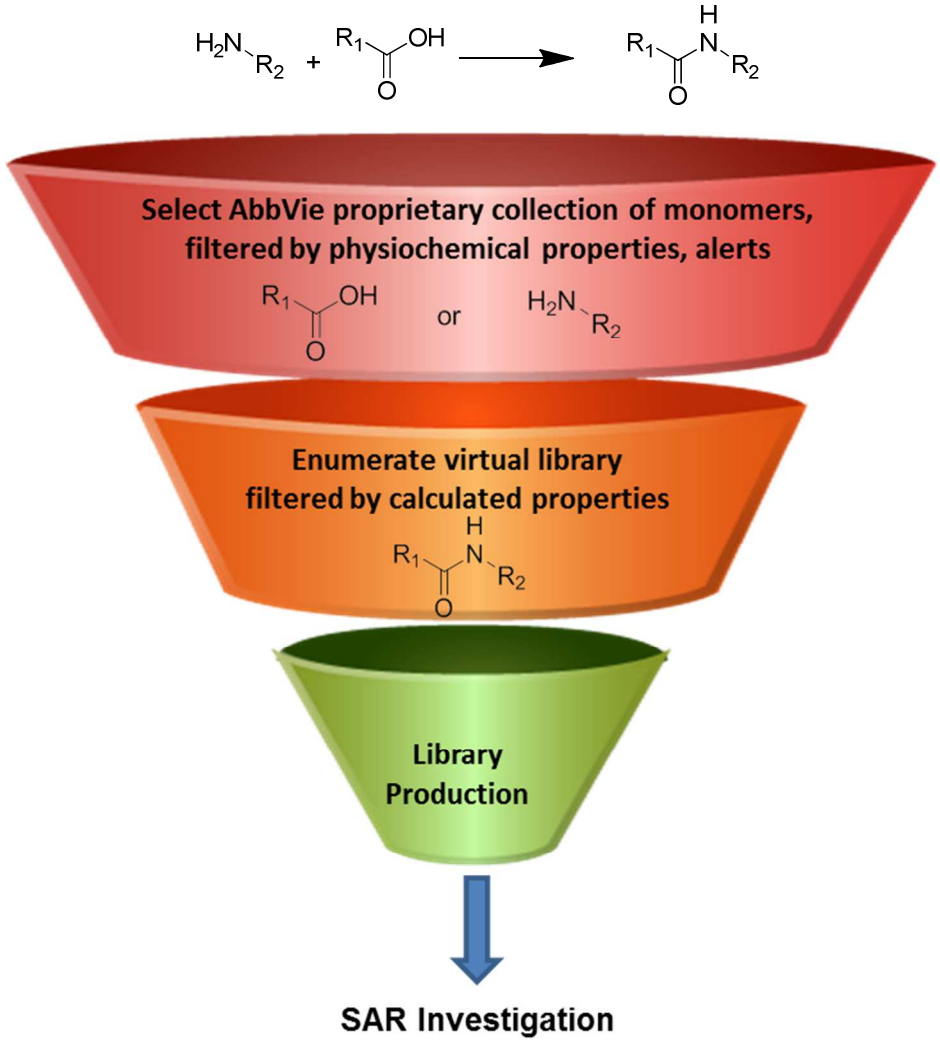
Figure 2. Lead identification approach



Physicochemical and ADME (absorption, distribution, metabolism, excretion) properties of the resulting compounds were calculated, from which compounds were selected for library production depending on the selected cut-off of molecular weight, cLogP, clearance. To ensure a broad lead set was generated, the property cutoffs for enumerated compounds slightly loosened (cLogP< 5.5, and molecular weight < 550). We rationalized that once active compounds were

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identified, an empirical based SAR effort would be devoted to optimizing potency, clearance, and other drug-like properties.

Assays

Different assays were utilized to evaluate correctors. By definition CFTR correctors rectify mis-folding of mutant CFTR, thereby restoring the channel maturation on the cell surface. A cell surface assay, which utilizes an immortalized bronchial epithelial cell (CFBE)[16] transduced with a F508del CFTR and fused with horseradish peroxidase (HRP) to detect the matured CFTR level at the cell surface, was established in-house and referred to as the cell surface expression (CSE-HRP) assay.[17] However, the ultimate goal is to improve the function of CFTR channels. To measure the function of the matured CFTR proteins, a semi-automated HBE-TECC (Human Bronchial Epithelial- Trans-Epithelial Current Clamp) assay was established.[18] In this assay, primary human bronchial epithelial (HBE) cells from F508del CF patients were cultured and electrophysiology (E-phys) measurement of the CFTR chloride ion current cross the apical membrane was taken. The magnitude of current measured in this assay is proposed to be a reasonable predictor of achieving efficacy on clinical endpoints such as FEV_1, and this correlation was validated in the clinical development of potentiators.[19]

Results and Discussion

Following the lead identification approach, various amine monomers were coupled with 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (Scheme 1). Among these amines, some chromanyl amines were included (Scheme 2). The success of this approach is illustrated in Table 1, where multiple active compounds were identified (**1-5**). These 2,2-disubstituted chromanamines could be readily synthesized as enantiomers,[20] and the availability

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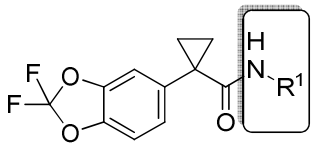
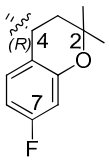
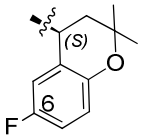
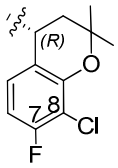
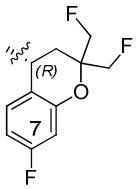
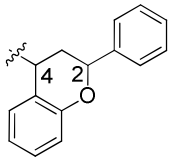
of these amine monomers in house allowed us to quickly expand understanding of the SAR and glean important information. For instance, the C4 position of the chromane ring prefers the (R)-configuration (compound **1** and **4** vs compound **2**). Substitution at the 8-position of the chromane phenyl ring resulted in diminished activity and efficacy (**3** vs **1**) due to likely unproductive interactions. Notably, the C-2 phenyl substituted compound (**5**) was also active. Most of these compounds have molecular weight below 500, and HPLC-measured LogD values (eLogD) around 5.

Having established that the chromane compounds are pharmacologically active, we decided to focus on exploring C-2 phenyl analogs. In addition to the efficacy that **5** displayed in CSE assay, we also reasoned that activity and properties would be readily tunable through modification of substituents on the phenyl ring, providing ready access points for optimization. An internal collection of some of these phenyl substituted amines enabled us to quickly prepare analogs (Table 2). The para-fluoro analog **6** maintained activity, and the para-chloro analog **9** was more active, while the activity of meta-chloro (**8**) and ortho-chloro (**7**) analogs were progressively weaker than **9**, indicating a substitution pattern and size preference. The C7-methoxy substitution on the chromane phenyl ring provided a significant potency improvement (**10** vs **5**). Also, substitution of the distal phenyl ring with two more methoxy groups (**11)** provided an additional boost in potency. Since **11** was a mixture of stereoisomers, the diastereomers would need to be separated to identify the active component.

Table 1. SAR of Chromane Analogs



Compd	R^1	CSE-HRP (EC_{50} (μM), Max. Act% **15**)[a]	MW/eLogD[b]
1		2.7, 98%	419/NT[c]
2		>20, 9%	419/5.1
3		2.3, 51%	454/5.4
4		4.3,102%	455/NT[c]
5		2.5, 108%	449/5.31

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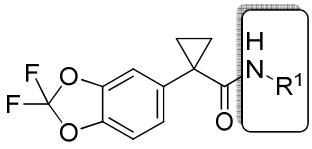
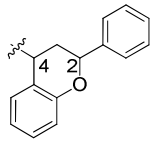
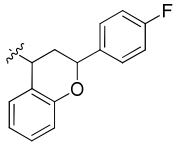
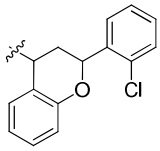
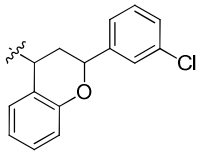
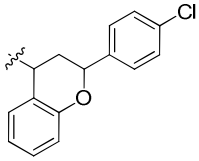
[a] Assay was run according to experimental description, using compound **15** (3 μM) as the positive control. All values are the geometric mean of at least two determinations. [b]HPLC measured LogD. [c]Not tested

Table 2. SAR of 2-Phenyl Substituted Chromane Analogs



Compd	R^1	CSE-HRP (EC_{50} (μM), Max. Act% **15**)[a]	MW/eLogD[b]
5		2.5, 108%	449/5.3
6		2.5, 95%	467/5.3
7		11, 31%	484/5.6
8		3.9, 83%	484/5.6
9		0.94, 94%	484/5.6

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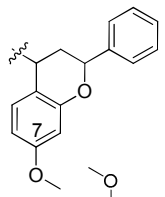
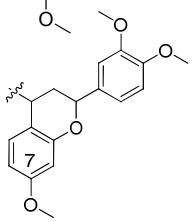
10		0.45, 112%	479/5.4
11		0.20, 118%	539/5.0

[a]Assay was run according to experimental description, using compound **15** (3 μM) as the positive control. All values are the geometric mean of at least two determinations. [b]HPLC measured LogD

Compound **11** was purified by chiral supercritical fluid chromatography (SFC), resulting in two cis-isomers as shown as **12** and **13**. X-ray crystallography confirmed the absolute stereo configuration (Figure 3) of **13** as having the (R,R)-configuration. Upon testing, **13** was confirmed to be the more active enantiomer, consistent with the early observation that the (R)-configuration at 2-position was preferred, with EC_{50} of 130 nM. The corresponding (S,S) isomer **12** was significantly weaker.

Figure 3. X-ray Structure of **13** indicating the active enantiomer is the (R,R)-form

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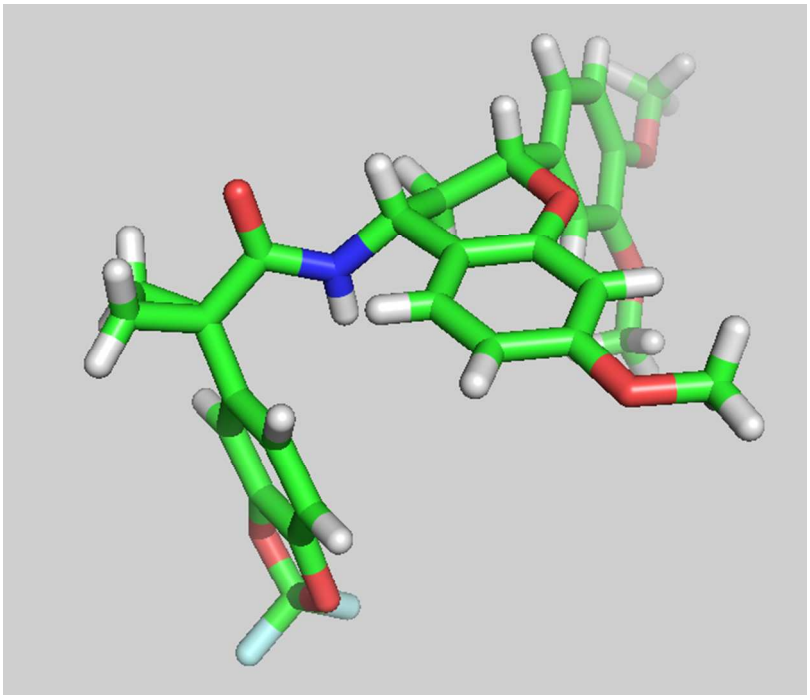

Now equipped with a potent compound in the CSE assay, compound **13** was tested in human bronchial epithelial (HBE) cells to define its functional potency and efficacy. Active enantiomer **13** exhibited high potency of 28 nM with efficacy comparable to that of Lumacaftor (data now shown). The corresponding (S,S)-isomer **12** was weaker in the assay, highlighting the concordance of CSE-HRP with HBE-TECC assay. Based on this data, compounds were automatically advanced into HBE-TECC assay if they were active in the CSE assay (EC_{50} < 1 μM, 50% Max. Act% of positive control). For the discussion of the subsequent compounds, only HBE-TECC data will be reported.

While **13** possesses high potency and efficacy in this HBE-TECC assay that assess the CFTR function, this lead resides in undesirable drug-like space with high molecular weight, and high HPLC LogD, indicating high lipophicity.[21] Not surprisingly it suffers from poor passive permeability measured by parallel artificial membrane permeability assay (PAMPA), and low *in*

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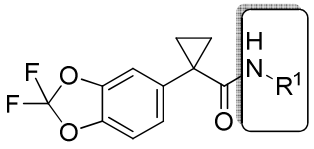
vitro hepatocyte stability (Table 3), which led to in vivo high clearance (>liver blood flow rate (Qh)), low half-life, and low oral bioavailability (data not shown) in a rat in vivo pharmacokinetic (PK) study.

Despite the high lipophilicity and undesirable ADME properties of lead **13**, the observation that dimethoxy substitution was beneficial for the activity suggested that more polar groups could be tolerated at these positions on the distal phenyl ring. With the objective of maintaining potency, minimizing molecular weight increase, and reducing unbound clearance,[22] we decided to build in polarity by replacing methoxy groups of **13** with an alcohol or acid group. The primary alcohol analog **14** had slightly improved HPLC LogD and permeability, but displayed weaker potency with high hepatocyte clearance. Removing both methoxy groups on the distal phenyl ring, and replacing them with a carboxylic acid group yielded compound **15,** which had comparable molecular weight to **13**, and retained high potency and efficacy. The reduced lipophilicity (HPLC LogD 3.8) resulted in higher permeability and reduced hepatocyte clearance. Compound **15** was characterized in rat PK studies and demonstrated reasonable clearance, half-life, and bioavailability (Table 5). Compound **15** also shows a favorable profile in the CYP3A4 induction assay (Table 4), exhibiting low induction compared with the positive control rifampin. In this assay (supplemental material), the compound is tested at 10 μM, and the response is compared to that of 10 μM rifampin, a CYP3A4 inducer. Compounds with a response of less than 20% of rifampin are considered to have low induction risk. The potency and efficacy of **15** in HBE-TECC assay are very comparable to that of Lumacaftor (data now shown)**,** hence **15** was used as the positive control for characterization of additional leads in both CSE-HRP and HBE-TECC assays.

Table 3. SAR of 2-Phenyl Substituted Chromane Analogs 13-16



Compd	R^1	CSE-HRP (EC_{50} (μM), Max. Act% **15**)[a]	HBE-TECC (EC_{50} (μM), Max. Act% **15**)[b]	MW eLogD[c]	PAMPA[d] (10^{-6}cm/s)	Hepatocyte $Cl_{int,u}$ (human/rat) (L/hr/kg)

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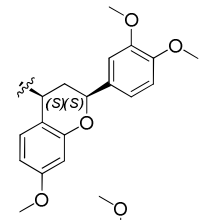
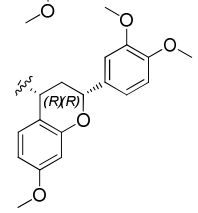
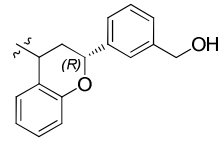
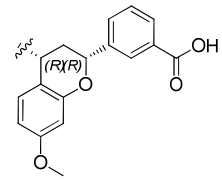
12		1.0, 69%	<30% @ 3 μM	540/4.96	0.30	1250/775
13		0.13, 123%	0.028, 117%	540/4.88	0.56	1540/1026
14		1.6, 93%	0.75, 103%	479/4.82	1.15	235/471
15		0.23, 101%	0.24, 95%	523/3.84	14.3	25/17

[a]Assay was run according to experimental description, using compound **15** (3 μM) as the positive control. All values are the geometric mean of at least two determinations. [b]Assay was run according to experimental description, using **23 (**GLPG1837, 1 μM)[23] as the potentiator, compound **15** (3 μM) as positive control. All values are the geometric mean of at least two determinations. [c]HPLC measured LogD. [d]Parallel artificial membrane permeability assay

In order to optimize the *in vivo* clearance of the lead compounds, a common practice was to assess if there is an *in vitro* and *in vivo* correlation (IVIVC) in clearance. A large number of amide compounds which have 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid linked to a wide range of amines, include chromanyl amines, demonstrated a reasonable correlation ($R^2 = 0.798$) between unbound hepatocyte clearance and unbound *in vivo* clearance in rat (Figure **4**). This analysis provided support to use *in vitro* hepatocyte clearance as a surrogate for *in vivo* clearance optimization.

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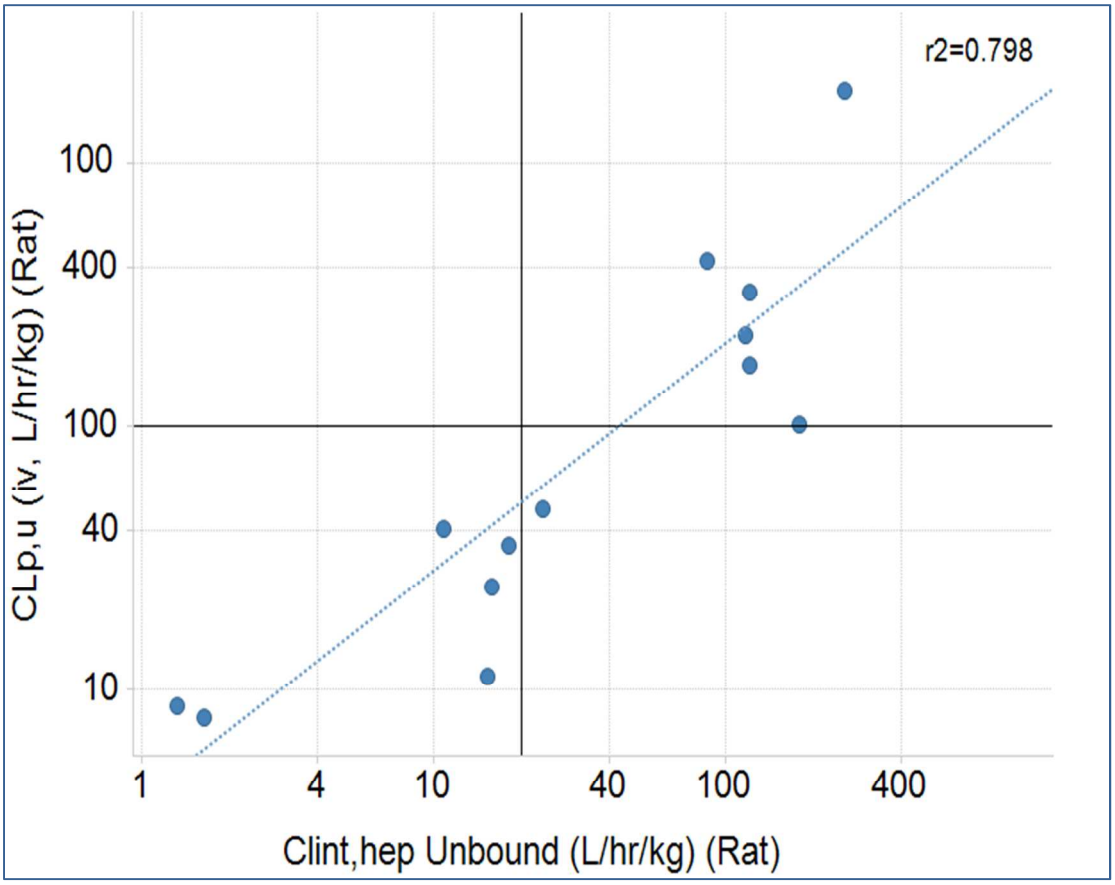
To understand if this series of compounds has potential systemic DDI liabilities, a subset of those compounds which were active in CSE assay (>30% efficacy) was also sampled in drug-drug interaction (DDI) assays (Figure **5**), including CYP3A4 induction and CYP inhibition assays. Many lead compounds, including chromane analogs, were shown to have low induction of CYP 3A4 (<20% of rifampin), and low inhibition against various CYP phenotypes, indicating there is no chemotype-dependent DDI perpetrator liability.

Given the combination of desired biological activity and properties of **15**, subsequent SAR studies were focused on exploring this carboxylic acid analog. To improve potency and reduce clearance, substituents on the chromane ring were surveyed. Substitutions such as 6-methoxy (**16**) and 7-fluoro (**17**) maintained potency, and provided improved human hepatocyte clearance. A methyl substitution at the 7- position resulted in reduced potency (**18**), but larger groups such as trifluoromethyl (**19**) and difluoromethoxy (**20**) led to improved potency and clearance when compared to the methoxy analog **15**. Between **19** and **20**, the eLogD of **20** is much smaller and comparable to that of methoxy analog **15**. The combination of low LogD, high potency, and low clearance indicates that difluoromethoxy group may provide optimal overall benefits. Among these compounds, methoxy substituted analogs (**15** and **16**) showed low induction; while fluoro- (**17**) and trifluoromethyl- (**19)** substituted analogs were found to induce CYP3A4 mRNA upregulation. These analogs indicated that various substituents on the chromane phenyl ring provided only modest impact on the potency, prompting us to look at other parts of the molecule.

Figure 4. *In vitro in vivo* correlation (IVIVC) of unbound *in vivo* plasma clearance and unbound *in vitro* hepatocyte clearance in rat


r2=0.798
100
400
100
40
10
CLp,u (iv, L/hr/kg) (Rat)
1
4
10
40
100
400
Clint,hep Unbound (L/hr/kg) (Rat)

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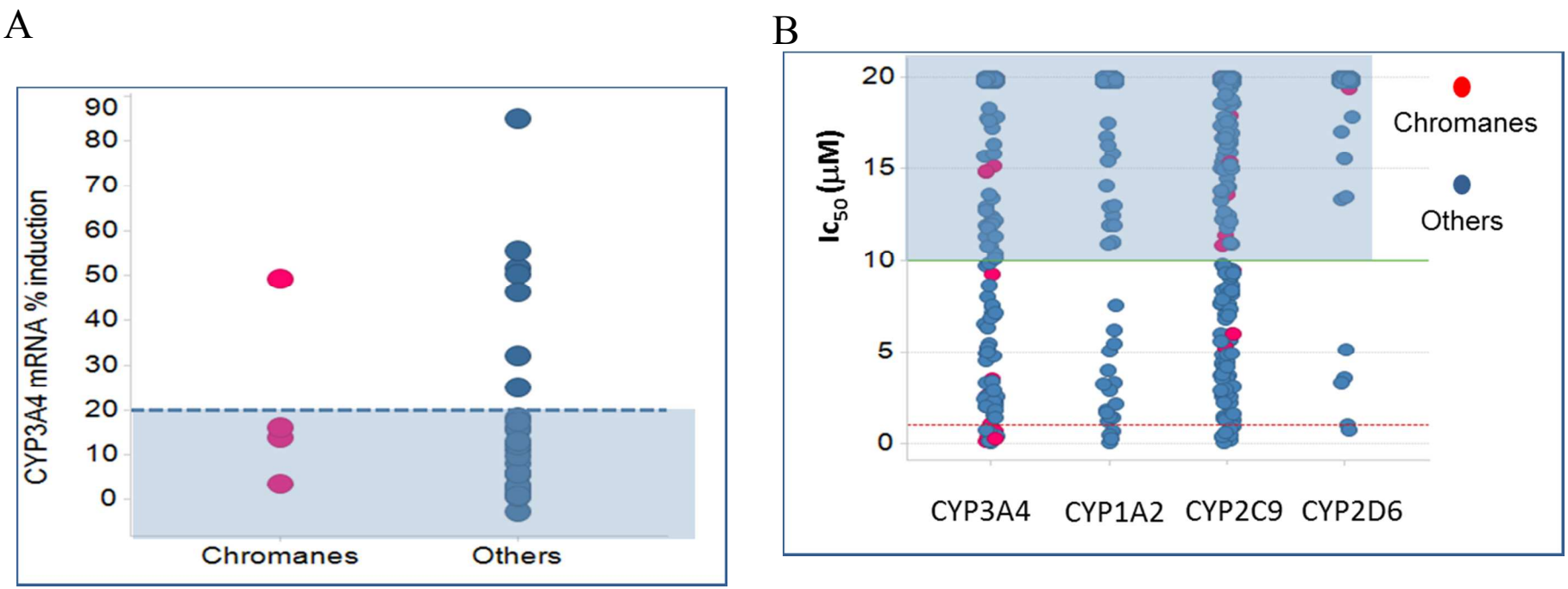

Figure 5. CYP perpetrator evaluation (A) CYP3A4 induction plot of compounds. (B) CYP3A4, 1A2, 2C9 and 2D6 inhibition of compounds.

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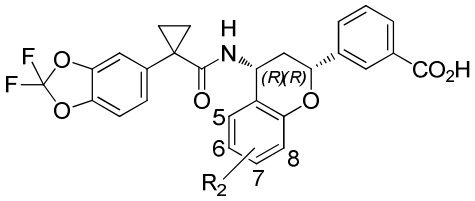
Table 4. SAR of Acid Analogs of 15



Compd	R^2	HBE (EC_{50} (μM), Max. Act% **15**)[a]	MW eLogD[c]	Hepatocyte $Cl_{int,u}$ (human/rat) (L/hr/kg)	CYP3A4 induction (%rifampin)[b]
15	7-OCH_3	0.24, 95%	523/3.84	25/17	14%
16	6-OCH_3	0.21, 111%	523/3.84	11/2	9.4%
17	7-F	0.11, 93%	511/4.03	26/4	40%
18	7-Me	< 60% @ 3 uM	507/4.01	21/8	NT[c]
19	7-CF_3	0.087, 97%	561/5.02	18/5	40%
20	7-$OCHF_2$	0.075, 117%	559/4.02	14/7	NT[c]

[a]Assay was run according to experimental description, using **23** (1 μM) as the potentiator, compound **15** (3 μM) as positive control. All values are the geometric mean of at least two determinations. [b]CYP3A4 induction was measured at 10 μM, using 10 μM rifampin as positive control. [c.]Not tested

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Upon inspection of the SAR trend from early analogs in this series, it was clear that the substitution pattern on the distal phenyl ring can be particularly important. For example, para-chloro analog **9** was 4-fold more potent than the meta-chloro analog **8** (Table 2)**.** Recognizing that the carboxylic acid is tolerated on the distal phenyl ring, the para carboxylic acid analog **21** was prepared and found to exhibit a potency improvement of more than 20-fold to 9 nM EC_{50} in HBE-TECC assay (Table 6)**.** Compound **21** was also found to have a clean CYP3A4 induction profile. Additionally, the reduced hepatocyte clearance of **21** translated into lower *in vivo* clearance (Table 5**)**.

Table 5. Rat Pharmacokinetic Parameters for Compounds 15 and 21

Compd	IV Dose			Oral Dose	
	Dose[a]	Cl[b]	$t_{1/2}$[c]	Dose[a]	F (%)
15[d]	1	1.24	2.8	1	24
21[e]	1	0.62	4.4	1	78

[a]Dose (mg/kg). [b]Clearance (L/hr/kg). [c]iv half-live(h).

Given improved potency of the difluoromethoxy analog over the methoxy analog (**20** over **15**), the para-carboxylic acid analog **22** was synthesized and characterized (Table 6). Compound **22** was highly potent (5 nM) and efficacious in cells from multiple CF patient donors who have F508del homozygous mutation. In comparison with Lumacaftor, **22** was significantly more potent (>25 fold), and exhibited comparable efficacy.[24] It demonstrated low clearance

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across multiple preclinical species, did not inhibit CYP enzymes, was not a CYP3A4 inducer, and therefore presented a low DDI perpetrator liability.

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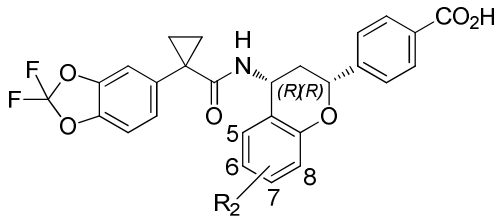
Table 6. SAR of Para-substituted Acid Analogs



Compd	R^2	HBE (EC_{50} (μM), Max. Act% **15**)	Hepatocyte $Cl_{int,u}$ (human/rat) (L/hr/kg)	CYP3A4 induction (%rifampin)[b]
21	7-OCH_3	0.009, 94%	15/3	0%
22	7-$OCHF_2$	0.005, 87%	7/3	6.4%

[a.] Assay was run according to experimental description, using **23** (1 μM) as the potentiator, compound **15** (3 μM) as positive control. All values are the geometric mean of at least two determinations. [b]CYP3A4 induction was measured at 10 μM, using 10 μM rifampin as positive control.

The pharmacokinetic profile of **22** is provided in Table 7. Compound **22** is primarily cleared via glucoronidation in human plasma, presenting a low DDI victim liability. Thorough preclinical characterization including DMPK, and safety pharmacology and toxicology of compound **22** in rat and dog support this compound as a candidate for clinical development. In a first-in-human phase I study, compound **22** was given to healthy volunteers at single doses up to 800 mg and multiple doses up to 600 mg daily for 14 days. The drug was well tolerated and had

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a favorable safety profile, with no early discontinuations of study drug and no severe adverse events.[25]

Table 7. Rat and Dog Pharmacokinetic Parameters for Compound 22

Species	IV Dose			Oral Dose	
	Dose[a]	Cl[b]	$t_{1/2}$[c]	Dose[a]	F (%)
rat	1	0.55	2.7	1	74
dog	2	0.83	5.0	5	40

[a]Dose (mg/kg). [b]Clearance (L/hr/kg). [c]iv half-live (h).

Conclusion

By utilizing an internal collection of proprietary monomers, we were able to quickly identify chromane substituted amides as CFTR correctors. The initial collection of enriched analogs greatly facilitated early SAR work of identifying active compounds. Introduction of a carboxylic acid group at the distal phenyl ring maintained the desired pharmacological activity, and optimized the ADME properties significantly, especially permeability and unbound clearance. Good *in vitro-in vivo* correlation in clearance helped guide the effort for *in vivo* pharmacokinetic evaluation. Through empirical and iterative SAR investigation the para-carboxylic acid analog,

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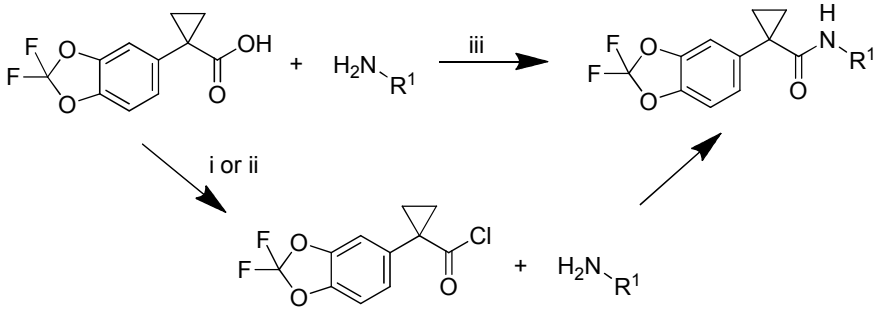
22, a highly potent and efficacious CFTR corrector, was identified and advanced into clinical trials.

Chemistry

Synthesis of amides were by coupling of acid and amine, either through use of coupling reagent, or by preparing the corresponding carbonyl chloride followed by reacting with the amines (Scheme 1).

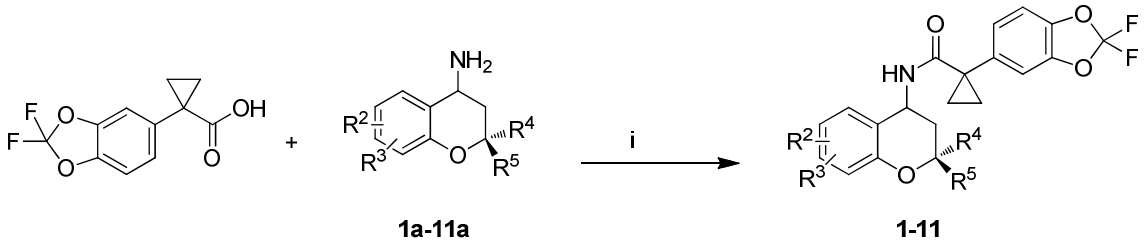
For analogs **1-11**, chiral chromanyl amines[20] and racemic 2-phenyl chromanyl amines were coupled to 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid. For compounds with chiral 2-phenyl substituted chromanyl amines (**14-22**), synthesis from the appropriated substituted chromenones (**14a**-**22a**) is described (Scheme 3). The corresponding chromenone were either prepared by condensing substituted hydroxyacetonphenone with DMF-DMA, or could be purchased. Palladium catalyzed conjugated addition of aromatic boronic acid was carried out using (S)-t-butyl Py-OX as chiral ligand, affording product **14b-22b** in modest yield and good enantioselectivity.[26] The resulting chromanones **14b-22b** were condensed with hydroxylamine or alkoxyamine to form the oxime, which underwent hydrogenation. Raney Nickel provided a mixture of trans:cis substituted compounds, while platinum yielded cis-selectivity, enriching desired diastereomers. The resulting amine can be coupled with acid or carbonyl chloride as shown for compounds **14e-22e**. For **15e-21e**, and **22f**, saponification provided the final acid analogs **15-22**.

Scheme 1. Amide coupling of early libraries[a]



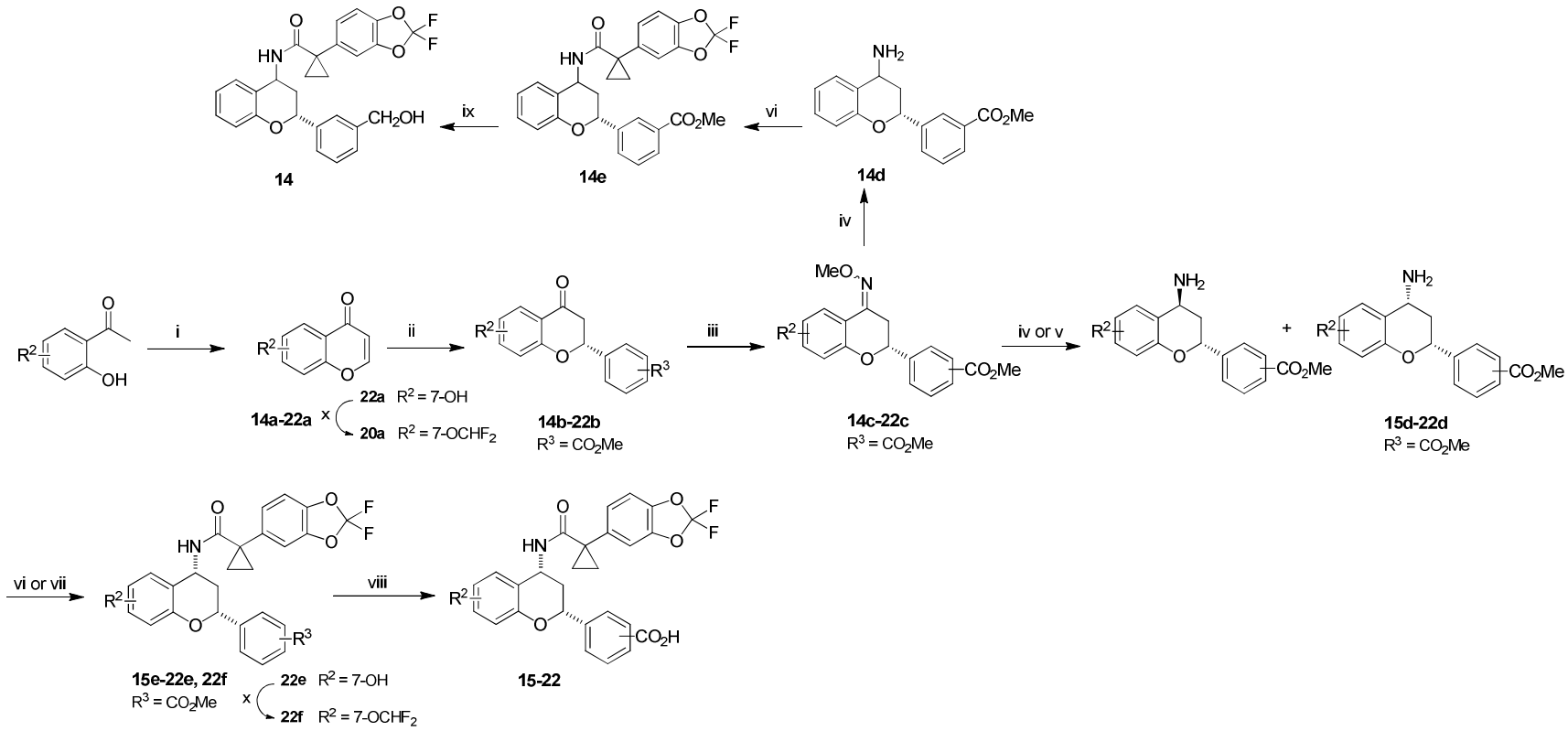
[a]Reagents and conditions: (i) oxalyl chloride, cat. DMF, DCM; (ii) thionyl chloride, reflux; (iii) HATU, DIPEA, DMA.

Scheme 2. Amide coupling of chromanylamines[a]



[a]Reagents and conditions: (i) HATU, DIPEA, DMA, 7-98%.

Scheme 3. Synthesis of amides with chiral 2-phenyl substituted chromanyl amines[a]



[a]Reagents and conditions: (i) DMF-DMA, 115-120 ^{o}C, 64-80%; (ii) $Pd(TFA)_2$, (S)-t-butyl PyOX, R^3-$PhB(OH)_2$, DCE, 34-83%; (iii) $MeONH_2$ HCl, pyridine or NaOAc, 77%-quantitative; (iv) Raney Ni, H_2, MeOH, 94-99%; (v) Pt/C, H_2, AcOH, 34-55%, 10-20:1 dr; (vi) 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride, Hunig's base, DCM, 30-85%; (vii) 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid, HATU, Hunig's base, DMF, 28-76%; (viii) NaOH, LiOH or KOTMS, 71-90%; (ix) $NaBH_4$, THF/MeOH, 85%; (x) diethyl (bromodifluoromethyl)phosphonate, KOH, CH_3CN/H_2O, 50-72%.

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Experimental section

Chemistry

Synthetic Materials and Methods. Unless otherwise specified, reactions were performed under an inert atmosphere of nitrogen and monitored by thin-layer chromatography (TLC) and/or LCMS. All reagents were purchased from commercial suppliers and used as provided. Flash column chromatography was carried out on pre-packed silica gel cartridges. Reverse phase chromatography samples were purified by preparative HPLC on a Phenomenex Luna C8(2) 5 um 100Å AXIA column (30mm × 75mm). A gradient of acetonitrile (A) and 0.1% trifluoroacetic acid in water (B) was used, at a flow rate of 50 mL/min (0-0.5 min 10% A, 0.5-7.0 min linear gradient 10-95% A, 7.0-10.0 min 95% A, 10.0-12.0 min linear gradient 95-10% A). Samples were injected in 1.5mL DMSO:methanol (1:1). A custom purification system was used, consisting of the following modules: Waters LC4000 preparative pump; Waters 996 diode-array detector; Waters 717+ autosampler; Waters SAT/IN module, Alltech Varex III evaporative light-scattering detector; Gilson 506C interface box; and two Gilson FC204 fraction collectors. The system was controlled using Waters Millennium32 software, automated using an AbbVie developed Visual Basic application for fraction collector control and fraction tracking. Fractions were collected based upon UV signal threshold and selected fractions subsequently analyzed by flow injection analysis mass spectrometry using positive APCI ionization on a Finnigan LCQ using 70:30 methanol:10 mM NH_4OH (aq) at a flow rate of 0.8 mL/min. Loop-injection mass spectra were acquired using a Finnigan LCQ running LCQ Navigator 1.2 software and a Gilson 215 liquid handler for fraction injection controlled by an AbbVie developed Visual Basic application. All NMR spectra were recorded on 300-500 MHz instruments as specified with chemical shifts given in ppm (δ) and are referenced to an internal standard of tetramethylsilane

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(δ 0.00). ^{1}H – ^{1}H couplings are assumed to be first-order and peak multiplicities are reported in the usual manner. HPLC purity determinations were performed on a Waters e2695 Separation Module / Waters 2489 UV/Visible Detector. Column types and elution methods are described in the Supporting Information section. The purity of the biologically evaluated compounds was determined by HPLC to be >95%. Solvents used for HPLC analysis and sample preparation were HPLC grade.

1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[(4*R*)-7-fluoro-2,2-dimethyl-3,4-dihydro-2H-chromen-4-yl]cyclopropanecarboxamide (1) A stock solution of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid and diisopropylethylamine (0.218 M and 0.654 M in dimethylacetamide, respectively), 284 µL, 0.061 mmol 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (1.0 eq) and 0.18 mmol diisopropylethylamine (3.0 eq)), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (0.26 M in dimethylacetamide, 284 µL, 0.074 mmol, 1.2 eq), and (R)-7-fluoro-2,2-dimethylchroman-4-amine (2S,3S)-2,3-dihydroxysuccinate (0.40 M in dimethylacetamide, 232 µL, 0.093 mmol, 1.5 eq) were aspirated from their respective source vials, mixed through a PFA mixing tube (0.2 mm inner diameter), and loaded into an injection loop. The reaction segment was injected into the flow reactor (Hastelloy coil, 0.75 mm inner diameter, 1.8 mL internal volume) set at 100 °C, and passed through the reactor at 180 µL min^{-1} (10 minute residence time). Upon exiting the reactor, the reaction was loaded directly into an injection loop and purified using prep LC method TFA1 to yield the title compound (4.1 mg, 16% yield). ^{1}H NMR (400 MHz, DMSO-d_6 :D_2O = 9:1 (v/v)) δ 7.39 (d, J = 1.7 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.22 (dd, J = 8.3, 1.7 Hz, 1H), 7.09 – 6.97 (m, 1H), 6.69 (td, J = 8.6, 2.7 Hz, 1H), 6.52 (dd, J = 10.6, 2.6 Hz, 1H), 5.07 (t, J = 8.8 Hz, 1H), 1.83 (d, J = 9.0 Hz, 2H), 1.56 –

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1.45 (m, 1H), 1.44 – 1.35 (m, 1H), 1.33 (s, 3H), 1.21 (s, 3H), 1.19 – 0.99 (m, 2H); MS (APCI+) m/z 420.1 $(M+H)^+$.

1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[(4*S*)-6-fluoro-2,2-dimethyl-3,4-dihydro-2H-chromen-4-yl]cyclopropanecarboxamide (2). A stock solution of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid and diisopropylethylamine (0.218 M and 0.654 M in dimethylacetamide, respectively, 284 μL, 0.061 mmol 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (1.0 eq) and 0.18 mmol diisopropylethylamine (3.0 eq)), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (0.26 M in dimethylacetamide, 284 μL, 0.074 mmol, 1.2 eq), and ((S)-6-Fluoro-2,2-dimethyl-chroman-4-ylamine with (2R,3R)-2,3-dihydroxy-succinic acid (0.40 M in dimethylacetamide, 232 μL, 0.093 mmol, 1.5 eq) were aspirated from their respective source vials, mixed through a PFA mixing tube (0.2 mm inner diameter), and loaded into an injection loop. The reaction segment was injected into the flow reactor (Hastelloy coil, 0.75 mm inner diameter, 1.8 mL internal volume) set at 100 °C, and passed through the reactor at 180 μL min^{-1} (10 minute residence time). Upon exiting the reactor, the reaction was loaded directly into an injection loop and purified using prep LC method TFA1 to yield the title compound (1.9 mg, 7% yield). ^{1}H NMR (400 MHz, DMSO-d_6 :D_2O = 9:1 (v/v)) δ 7.41 (d, J = 1.7 Hz, 1H), 7.32 (d, J = 8.3 Hz, 1H), 7.23 (dd, J = 8.3, 1.7 Hz, 1H), 6.94 (td, J = 8.6, 3.3 Hz, 1H), 6.78 – 6.66 (m, 2H), 5.14 – 5.01 (m, 1H), 1.87 – 1.73 (m, 2H), 1.56 – 1.44 (m, 1H), 1.43 – 1.36 (m, 1H), 1.32 (s, 3H), 1.20 (s, 3H), 1.14 – 1.03 (m, 2H); MS (APCI+) m/z 420.1 $(M+H)^+$.

1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-(7-methoxy-2-phenyl-3,4-dihydro-2H-chromen-4-yl)cyclopropanecarboxamide (10) In a 4 mL vial, 300 μL of a stock solution containing 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.25 M, 0.073

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mmol, 1.0 equivalent) and diispropylethylamine (0.74 M, 0.22 mmol, 3.0 equivalents) in dimethyl acetamide was added to a stock solution containing 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (0.30 M in dimethyl acetamide, 300 µL, 0.089 mmol, 1.2 equivalents). A stock solution of 7-methoxy-2-phenyl-chroman-4-ylamine hydrochloride (0.40 M in dimethyl acetamide, 278 µL, 0.111 mmol, 1.5 equivalents) was added and the reaction was stirred at 50 °C until complete as determined by LC. The material was loaded directly into a Gilson GX-271 autosampler and purified using preparative LC method TFA4 to provide the title compound (27.1mg, 76% yield). ^{1}H NMR (400 MHz, DMSO-d_6 :D_2O = 9:1 (v/v)) δ 7.44 – 7.25 (m, 7H), 7.25 – 7.12 (m, 2H), 6.95 (dd, J = 8.6, 1.1 Hz, 1H), 6.52 (dd, J = 8.5, 2.6 Hz, 1H), 6.36 (d, J = 2.5 Hz, 1H), 5.38 – 5.27 (m, 1H), 5.22 (dd, J = 11.3, 2.5 Hz, 1H), 3.69 (s, 3H), 2.19 – 1.93 (m, 2H), 1.50 (dt, J = 8.5, 3.0 Hz, 1H), 1.44 – 1.32 (m, 1H), 1.12 – 1.00 (m, 2H); MS (APCI+) m/z 480.4 $(M+H)^+$.

1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[2-(3,4-dimethoxyphenyl)-7-methoxy-3,4-dihydro-2H-chromen-4-yl]cyclopropanecarboxamide (11). Compound **11** (39.4 mg, 98% yield) was prepared according to the procedure similar to that as described in compound **7**, substituting 2-(3,4-dimethoxy-phenyl)-7-methoxy-chroman-4-ylamine for 7-methoxy-2-phenyl-chroman-4-ylamine. ^{1}H NMR (400 MHz, DMSO-d_6 :D_2O = 9:1 (v/v)) δ 7.38 (d, J = 1.7 Hz, 1H), 7.30 (d, J = 8.3 Hz, 1H), 7.22 (dd, J = 8.4, 1.7 Hz, 1H), 7.15 (d, J = 8.9 Hz, 1H), 7.07 – 6.90 (m, 4H), 6.51 (dd, J = 8.6, 2.6 Hz, 1H), 6.35 (d, J = 2.5 Hz, 1H), 5.36 – 5.22 (m, 1H), 5.19 – 5.04 (m, 1H), 3.77 – 3.75 (m, 6H), 3.68 (s, 3H), 2.20 – 1.95 (m, 2H), 1.57 – 1.46 (m, 1H), 1.46 – 1.32 (m, 1H), 1.21 – 0.98 (m, 2H); MS (APCI+) m/z 540.4 $(M+H)^+$.

1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[(2*S*,4*S*)-2-(3,4-dimethoxyphenyl)-7-methoxy-3,4-dihydro-2H-chromen-4-yl]cyclopropanecarboxamide (12) and 1-(2,2-difluoro-

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1,3-benzodioxol-5-yl)-N-[(2*R*,4*R*)-2-(3,4-dimethoxyphenyl)-7-methoxy-3,4-dihydro-2H-chromen-4-yl]cyclopropanecarboxamide (13). Compound **11** was subjected to preparative supercritical fluid chromatography set to maintain a backpressure at 100 bar using a CHIRALPAK IA®, 21 × 250 mm, 5 micron, with the sample at a concentration of 20 mg/mL in methanol using 16 % methanol in CO_2 at a flow rate of 70 mL/minute with a retention time of 4.5 minutes to give the compound **12** (106 mg, 0.196 mmol, 39.7 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, J = 1.6 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.20 (dd, J = 8.3, 1.7 Hz, 1H), 7.13 (d, J = 9.1 Hz, 1H), 6.98 - 6.91 (m, 4H), 6.50 (dd, J = 8.5, 2.6 Hz, 1H), 6.36 (d, J = 2.5 Hz, 1H), 5.36 - 5.24 (m, 1H), 5.15 (dd, J = 11.5, 1.9 Hz, 1H), 3.75 (d, J = 1.4 Hz, 6H), 3.68 (s, 3H), 2.10 (q, J = 11.8 Hz, 1H), 1.99 (ddd, J = 12.9, 6.2, 2.1 Hz, 1H), 1.53 - 1.46 (m, 1H), 1.37 (ddd, J = 8.4, 5.8, 2.8 Hz, 1H), 1.05 (dtdd, J = 12.7, 9.6, 6.4, 3.3 Hz, 2H); MS (ESI+) *m/z* 402 $(M+H)^+$. Compound **13** had retention time 7.2 minute and was obtained as a white solid (111 mg, 0.206 mmol, 41.5 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, J = 1.6 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.20 (dd, J = 8.3, 1.7 Hz, 1H), 7.13 (d, J = 9.1 Hz, 1H), 6.98 - 6.91 (m, 4H), 6.50 (dd, J = 8.5, 2.6 Hz, 1H), 6.36 (d, J = 2.5 Hz, 1H), 5.36 - 5.24 (m, 1H), 5.15 (dd, J = 11.5, 1.9 Hz, 1H), 3.75 (d, J = 1.4 Hz, 6H), 3.68 (s, 3H), 2.10 (q, J = 11.8 Hz, 1H), 1.99 (ddd, J = 12.9, 6.2, 2.1 Hz, 1H), 1.53 - 1.46 (m, 1H), 1.37 (ddd, J = 8.4, 5.8, 2.8 Hz, 1H), 1.05 (dtdd, J = 12.7, 9.6, 6.4, 3.3 Hz, 2H); MS (ESI+) *m/z* 402 $(M+H)^+$. Absolute stereochemistry was assigned by X-ray diffraction analysis.

7-methoxy-4H-chromen-4-one (15a). 1,1-Dimethoxy-N,N-dimethylmethanamine (1.0 mL, 7.53 mmol) and 1-(2-hydroxy-4-methoxyphenyl)ethanone (1.251 g, 7.53 mmol) were heated in the microwave at 115 °C for 15 seconds to give a red solution which solidified upon cooling. The solid was triturated with heptane to give the enamine intermediate as red crystals. ^{1}H NMR

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(400 MHz, DMSO-d_6) δ 14.96 (s, 1H), 7.82 (dd, J = 10.6, 1.6 Hz, 2H), 6.37 (dd, J = 8.8, 2.6 Hz, 1H), 6.32 (d, J = 2.5 Hz, 1H), 5.84 (d, J = 12.0 Hz, 1H), 3.75 (s, 3H), 3.17 (s, 3H), 2.95 (s, 3H). The enamine was dissolved in dichloromethane (40 mL) and treated with HCl (4 mL) at reflux for one hour. The aqueous layer was removed and extracted with 3 x 40mL of dichloromethane. The combined extracts were washed with saturated aqueous sodium bicarbonate and dried over sodium sulfate, then filtered and the solvent removed under reduced pressure to give title compound (0.854 g, 4.85 mmol, 64.4 % yield) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 6.0 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.06 (dd, J = 8.9, 2.4 Hz, 1H), 6.27 (d, J = 6.0 Hz, 1H), 3.90 (s, 3H); MS (ESI+) m/z 177 $(M+H)^+$.

(*R*)-methyl 3-(7-methoxy-4-oxochroman-2-yl)benzoate (15b). A 4 mL vial was charged with bis(2,2,2-trifluoroacetoxy)palladium (9.44 mg, 0.028 mmol), (*S*)-4-(tert-butyl)-2-(pyridin-2-yl)-4,5-dihydrooxazole (6.96 mg, 0.034 mmol), ammonium hexafluorophosphate(V) (27.8 mg, 0.170 mmol), and 3-methoxycarbonylphenylboronic acid (204 mg, 1.135 mmol) were stirred in dichloroethane (1.0 mL) for 5 minutes, and a pale yellow color was observed. To this suspension was added compound **15a** (100 mg, 0.568 mmol) and water (0.051 mL, 2.84 mmol) and the sides of the vial washed with more dichloroethane (1.0 mL). The vial was capped and the mixture stirred at 60 °C overnight. The mixture was filtered through a plug of silica gel and eluted with dichloromethane and then ethyl acetate. The solvent was removed and the crude material was chromatographed using a 12g silica gel cartridge with a gradient of 5-50 % ethyl acetate/heptanes over 20 minutes to give the title compound (133 mg, 0.426 mmol, 75 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (t, J = 1.8 Hz, 1H), 7.98 (dt, J = 7.8, 1.4 Hz, 1H), 7.84 (dt, J = 7.9, 1.5 Hz, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.61 (t, J = 7.8 Hz, 1H), 6.69 (d,

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J = 8.6 Hz, 2H), 5.77 (dd, J = 12.9, 2.9 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.17 (dd, J = 16.8, 13.0 Hz, 1H), 2.80 (dd, J = 16.8, 3.0 Hz, 1H); MS (ESI+) m/z 313 $(M+H)^+$.

(*R*)-methyl 3-(7-methoxy-4-(methoxyimino)chroman-2-yl)benzoate (15c). Compound 15B (100 mg, 0.320 mmol) and O-methylhydroxylamine hydrochloride (29.4 mg, 0.352 mmol) were stirred in pyridine (640 µL) at 60 °C overnight. Added an additional 0.3 equivalent (7 mg) of amine and heated at 60 °C for 12 hours. The mixture was concentrated and then diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and saturated aqueous ammonium chloride sequentially. The solvent was removed and the crude material purified using a 12 g silica gel cartridge eluting with 5-20 % ethyl acetate/heptanes over 20 minutes to give the title compound (107 mg, 0.313 mmol) as a light pink oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (t, *J* = 1.9 Hz, 1H), 8.03 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.77 - 7.63 (m, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 6.59 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 5.12 (dd, *J* = 12.5, 3.1 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.80 (s, 3H), 3.48 (dd, *J* = 17.2, 3.1 Hz, 1H), 2.65 (dd, *J* = 17.1, 12.5 Hz, 1H); MS (ESI+) m/z 342.0 $(M+H)^+$.

3-((2*R*)-4-amino-7-methoxychroman-2-yl)benzoate (15d). Compound **15c** (50 mg, 0.146 mmol) and methanol (10 mL) were added to Ra-Ni 2800, water slurry (150 mg, 1.150 mmol) in a 50 mL pressure bottle and stirred for 16 hours at 30 psi of hydrogen gas and at ambient temperature. The reaction was filtered and the solvent removed. The residue (44 mg) was dissolved in methyl-tert-butyl ether. HCl (4.0 M in dioxane, 0.3 mL) was added dropwise, and the resulting suspension was filtered to give the hydrochloride salt of the title compound as a mixture of two diastereomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 6H), 8.06 (dt, *J* = 6.1, 1.8 Hz, 2H), 7.97 (ddd, *J* = 9.2, 3.1, 1.4 Hz, 2H), 7.74 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.60 (t, *J* = 7.7 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 6.65 (ddd, *J* = 8.7, 6.3, 2.6 Hz, 2H),

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6.55 (d, *J* = 2.6 Hz, 1H), 6.52 (d, *J* = 2.6 Hz, 1H), 5.51 (dd, *J* = 11.9, 2.3 Hz, 1H), 5.33 (dd, *J* = 11.8, 1.8 Hz, 1H), 4.75 (dd, *J* = 11.0, 6.4 Hz, 1H), 4.45 (dd, *J* = 5.0, 2.4 Hz, 1H), 3.89 (s, 3H), 3.89 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 2.61 (ddd, *J* = 13.1, 6.5, 1.9 Hz, 1H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.31 (ddd, *J* = 15.0, 11.9, 5.0 Hz, 1H), 2.08 (dt, *J* = 13.0, 11.4 Hz, 1H); MS (ESI-) m/z 297.1 $(M\text{-}NH_3)^-$.

Methyl 3-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonylamino)-7-methoxy-3,4-dihydro-2H-chromen-2-yl]benzoate (15e).

To a suspension of the product from compound **15d** (90 mg, 0.257 mmol) in 1.3 mL of dichloromethane was added N,N-diisopropylethylamine (155 µL, 0.772 mmol). After a solution was achieved, a solution of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride (84 mg, 0.322mmol) in 1 mL of dichloromethane was added dropwise at ambient temperature and the reaction was stirred for 1 hour. The reaction mixture was diluted with 5 mL of methyl-*tert*-butyl ether and quenched with saturated aqueous sodium bicarbonate. After stirring for 10 minutes, the aqueous layer was removed and the organic layer was washed twice more with saturated aqueous sodium bicarbonate. The organics were dried over sodium sulfate then concentrated. The residue was chromatographed using a 40 g silica gel cartridge with 10-20 % methyl-*tert*-butyl ether/heptanes over 3 minutes then 20 % methyl-*tert*-butyl ether /heptanes for 17 minutes then a 20-30 % methyl-*tert*-butyl ether /heptanes gradient over 10 minutes to provide the title compound as the second eluting isomer. Relative stereochemistry confirmed by H NMR NOE analysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.58 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 7.12 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.08 (d, *J* = 1.7 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.96 (dd, *J* = 8.7, 1.0 Hz, 1H), 6.51 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 5.46 - 5.38 (m, 1H), 5.33 (d, *J* = 8.8 Hz, 1H), 5.21 (dd, *J* = 11.3,

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1.9 Hz, 1H), 3.92 (s, 3H), 3.75 (s, 3H), 2.51 (ddd, *J* = 13.3, 6.0, 2.0 Hz, 1H), 1.86 - 1.62 (m, 3H), 1.11 - 1.03 (m, 2H); MS (ESI-) m/z 536.1 $(M-H)^-$.

The first eluting isomer is methyl 3-[(2R,4S)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2H-chromen-2-yl]benzoate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (t, *J* = 1.8 Hz, 1H), 8.01 (d, *J* = 7.9 Hz, 1H), 7.59 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.15 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.12 (d, *J* = 1.7 Hz, 1H), 7.07 - 6.99 (m, 2H), 6.52 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 5.58 (d, *J* = 6.6 Hz, 1H), 5.06 - 4.96 (m, 1H), 4.81 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.94 (s, 3H), 3.75 (s, 3H), 2.31 (dt, *J* = 14.3, 2.5 Hz, 1H), 2.15 (ddd, *J* = 14.4, 11.5, 4.6 Hz, 1H), 1.71 - 1.66 (m, 2H), 1.10 - 1.05 (m, 2H); MS (ESI-) m/z 536.1 $(M-H)^-$.

3-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2H-chromen-2-yl]benzoic acid (15). To a solution of compound **15e** (25 mg, 0.047 mmol) in tetrahydrofuran (233 µL) was added lithium hydroxide hydrate (233 µL of a 0.8 M solution in water). The resulting biphasic mixture was stirred vigorously for 16 hours at room temperature, followed by addition of more lithium hydroxide hydrate (233 µL of a 0.8 M solution). The reaction mixture was stirred for an additional 5 hours at room temperature, acidified by the addition of 6 M HCl (0.040 mL) and the resulting biphasic mixture loaded directly onto a 4g silica gel cartridge and eluted with 30 % ethyl acetate/heptanes over 15 minutes to give the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.06 (dd, J = 7.9, 1.5 Hz, 1H), 7.71 - 7.61 (m, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.13 (dd, J = 8.2, 1.7 Hz, 1H), 7.09 (d, J = 1.7 Hz, 1H), 7.01 (d, J = 8.2 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.52 (dd, J = 8.6, 2.5 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 5.49 (td, J = 9.9, 6.0 Hz, 1H), 5.40 (d, J = 8.9 Hz, 1H),

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5.33 - 5.22 (m, 1H), 3.76 (s, 3H), 2.58 (ddd, J = 13.3, 5.9, 2.0 Hz, 1H), 1.82 - 1.72 (m, 2H), 1.69 - 1.63 (m, 1H), 1.09 (q, J = 2.8 Hz, 2H); MS (ESI-) m/z 522.1 $(M-H)^-$.

7-methoxy-4H-chromen-4-one (21a). 1,1-Dimethoxy-N,N-dimethylmethanamine (1.0 mL, 7.53 mmol) and 1-(2-hydroxy-4-methoxyphenyl)ethanone (1.251 g, 7.53 mmol) were heated in the microwave at 115 °C for 15 seconds to give a red solution which solidified upon cooling. The solid was triturated with heptane to give the enamine intermediate as red crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.96 (s, 1H), 7.82 (dd, J = 10.6, 1.6 Hz, 2H), 6.37 (dd, J = 8.8, 2.6 Hz, 1H), 6.32 (d, J = 2.5 Hz, 1H), 5.84 (d, J = 12.0 Hz, 1H), 3.75 (s, 3H), 3.17 (s, 3H), 2.95 (s, 3H). The enamine was dissolved in dichloromethane (40 mL) and treated with HCl (4 mL) at reflux for one hour. The aqueous layer was removed and extracted with 3 x 40mL of dichloromethane. The combined extracts were washed with saturated aqueous sodium bicarbonate and dried over sodium sulfate, then filtered and the solvent removed under reduced pressure to give title compound (0.854 g, 4.85 mmol, 64.4 % yield) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 6.0 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.06 (dd, J = 8.9, 2.4 Hz, 1H), 6.27 (d, J = 6.0 Hz, 1H), 3.90 (s, 3H); MS (ESI+) m/z 177 $(M+H)^+$.

(*R*)-methyl 4-(7-methoxy-4-oxochroman-2-yl)benzoate (21b). A 20 mL vial was charged with bis(2,2,2-trifluoroacetoxy)palladium (0.264 g, 0.795 mmol), (*S*)-4-(tert-butyl)-2-(pyridin-2-yl)-4,5-dihydrooxazole (0.195 g, 0.954 mmol), ammonium hexafluorophosphate(V) (0.777 g, 4.77 mmol) and (4-(methoxycarbonyl)phenyl)boronic acid (2.86 g, 15.89 mmol). The reaction was stirred in dichloroethane (5 mL) for 5 minutes, and a pale brown color suspension was observed. To this suspension was added compound **21a** (1.4 g, 7.95 mmol) and water (0.716 mL, 39.7 mmol) and the sides of the vial washed with more dichloroethane (5 mL). The vial was capped and the mixture stirred at 60 °C overnight. The mixture was filtered through a

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plug of silica gel and celite and eluted with ethyl acetate to give a red solution. The solvent was removed under reduced pressure and the crude material was chromatographed using a 24 g silica gel cartridge with a gradient of 5-60 % ethyl acetate/heptanes over 20 minutes, a white solid precipitated in the middle of fractions collection and clogged up the line into the IR detection unit. The output line was unclogged and the white solid was filtered, the filtrate was concentrated and chromatographed again using a 12 g cartridge eluting with 100 % dichloromethane to give a white solid which was combined to give the title compound (1.6 g, 5.12 mmol, 64.5 % yield)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (dd, J = 8.4, 2.1 Hz, 2H), 7.75 - 7.72 (m, 1H), 7.70 (d, J = 8.4 Hz, 2H), 6.72 - 6.66 (m, 2H), 5.77 (dd, J = 12.9, 3.1 Hz, 1H), 3.87 (s, 3H), 3.83 (d, J = 2.0 Hz, 3H), 3.14 (dd, J = 16.8, 12.9 Hz, 1H), 2.82 (dd, J = 16.7, 3.1 Hz, 1H); MS (ESI+) m/z 313 $(M+H)^+$.

(*R*)-methyl 4-(7-methoxy-4-(methoxyimino)chroman-2-yl)benzoate (21c). A solution of the product from compound **21b** (0.6 g, 1.921 mmol), O-methylhydroxylamine hydrochloride (0.241 g, 2.88 mmol) in pyridine (1.921 mL) in a 20mL vial was stirred at ambient temperature for 5 minutes and 65 °C for 1 hour. The solvent was removed under reduced pressure. The crude material was dissolved in 10 % methanol/dichloromethane and washed with water. The organic layer was separated, and concentrated in vacuo. The resulted white solid was rinsed with 10 % dichloromethane/hexane and collected by filtration to give title compounds as white solid (0.581 g, 1.702 mmol, 89 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 - 7.96 (m, 2H), 7.71 (d, J = 8.7 Hz, 1H), 7.69 - 7.63 (m, 2H), 6.62 (dd, J = 8.8, 2.5 Hz, 1H), 6.59 (d, J = 2.5 Hz, 1H), 5.32 (dd, J = 11.8, 3.2 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.76 (s, 3H), 3.36 (d, J = 3.4 Hz, 1H), 2.71 (dd, J = 17.1, 11.9 Hz, 1H); MS (ESI+) *m/z* 342 $(M+H)^+$.

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Methyl 4-((2*R*,4*R*)-4-amino-7-methoxychroman-2-yl)benzoate (21d). To a mixture of compound **21c** (580 mg, 1.69 mmol) and acetic acid (20 mL) was added platinum (180 mg, 0.923 mmol) in a 50 mL pressure bottle and stirred for 32 hours at 30 psi of hydrogen and at ambient temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a 24 g cartridge, and eluted with 5-70 % ethyl acetate/heptane over 20 minutes to provide the title compound (240 mg, 0.766 mmol, 45.1 % yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 - 7.97 (m, 2H), 7.64 - 7.57 (m, 2H), 7.47 (d, J = 8.5 Hz, 1H), 6.52 (dd, J = 8.6, 2.6 Hz, 1H), 6.38 (d, J = 2.5 Hz, 1H), 5.29 (dd, J = 11.9, 1.7 Hz, 1H), 4.07 (dd, J = 11.0, 5.7 Hz, 1H), 3.87 (s, 3H), 3.70 (s, 3H), 2.28 (ddd, J = 13.1, 5.7, 1.9 Hz, 1H), 1.72 (dt, J = 13.0, 11.4 Hz, 1H); MS (ESI+) m/z 314 $(M+H)^+$.

Methyl 4-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2H-chromen-2-yl]benzoate (21e). To 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (83 mg, 0.345 mmol) in DMF (1 mL) was added HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (183 mg, 0.483 mmol). The solution was stirred for 15 minutes at room temperature, followed by sequential addition of Example 110C (108 mg, 0.345 mmol) and triethylamine (0.144 mL, 1.034 mmol). The mixture was stirred at ambient temperature for 5 hours and water (10 mL) was added. The resulted white precipitate was filtered and purified by flash chromatography on a 12 g cartridge, eluted with 5-60 % ethyl acetate/heptane over 20 minutes to provide the title compound (126 mg, 0.234 mmol, 68.0 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 - 7.94 (m, 2H), 7.58 - 7.52 (m, 2H), 7.37 (d, J = 1.7 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.18 (dd, J = 8.4, 1.7 Hz, 1H), 7.15 (d, J =

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8.9 Hz, 1H), 6.93 (dd, J = 8.5, 1.1 Hz, 1H), 6.51 (dd, J = 8.6, 2.6 Hz, 1H), 6.39 (d, J = 2.5 Hz, 1H), 5.33 (q, J = 9.5, 8.4 Hz, 2H), 3.84 (s, 3H), 3.67 (s, 3H), 2.11 - 1.99 (m, 2H), 1.54 - 1.41 (m, 1H), 1.41 - 1.29 (m, 1H), 1.07 - 0.96 (m, 2H); MS (ESI-) m/z 536 $(M-H)^-$.

4-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2H-chromen-2-yl]benzoic acid (21). To a solution of the product from compound **21e** (81 mg, 0.151 mmol) in ethanol (1 mL) and tetrahydrofuran (0.4 mL) was added 3 N sodium hydroxide (0.201 mL, 0.603 mmol). The reaction was stirred at room temperature for 16 hours. The reaction was quenched with HCl (1 N, 1 mL), and water (2 mL) was added. The organics were removed under a stream of nitrogen to give an off-white precipitate. The precipitate was collected by filtration, washed with water, and then purified by flash chromatography on a 12 g silica gel cartridge, and eluted with a gradient of 5-90 %ethyl acetate/heptanes over 20 minutes to provide the title compound (65 mg, 0.124 mmol, 82 % yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, 1H), 7.95 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 1.7 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.21 - 7.12 (m, 2H), 6.93 (d, J = 8.5 Hz, 1H), 6.50 (dd, J = 8.6, 2.6 Hz, 1H), 6.39 (d, J = 2.5 Hz, 1H), 5.39 - 5.28 (m, 2H), 3.67 (s, 3H), 2.04 (td, J = 7.9, 2.3 Hz, 2H), 1.51 - 1.43 (m, 1H), 1.41 - 1.33 (m, 1H), 1.03 (q, J = 2.6 Hz, 2H); MS (ESI+) m/z 522 $(M-H)^-$.

(*R*)-methyl 4-(7-hydroxy-4-oxochroman-2-yl)benzoate (22b). A mixture of bis(2,2,2-trifluoroacetoxy)palladium (271 mg, 0.816 mmol), (*S*)-4-(tert-butyl)-2-(pyridin-2-yl)-4,5-dihydrooxazole (200 mg, 0.979 mmol), ammonium hexafluorophosphate(V) (798 mg, 4.90 mmol), (4-(methoxycarbonyl)phenyl)boronic acid (2203 mg, 12.24 mmol) and dichloroethane (8 mL) in a 20 mL vial was stirred for 5 minutes at room temperature, followed by the addition of 7-hydroxy-4H-chromen-4-one (**22a**, CAS 59887-89-7, MFCD00209371, 1323 mg, 8.16 mmol)

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and water (256 mg, 14.19 mmol). The vial was capped and the mixture was stirred at 60 °C overnight. The reaction gradually turned black, with Pd plated out on the sides of the vial. The mixture was filtered through a plug of celite and eluted with ethyl acetate to give a red solution which was washed with brine. The solvent was removed in vacuo and the crude material was chromatographed using a 100 g silica gel cartridge and eluted with a gradient of 5-40% ethyl acetate in heptane to provide the title compound (1.62g, 66.6 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 - 8.04 (m, 2H), 7.87 (d, J = 8.7 Hz, 1H), 7.60 - 7.49 (m, 2H), 6.62 - 6.45 (m, 2H), 5.87 (s, 1H), 5.53 (dd, J = 12.8, 3.2 Hz, 1H), 3.94 (s, 3H), 3.07 - 2.80 (m, 2H); MS (ESI+) m/z = 299 $(M+H)^+$.

(*R*)-methyl 4-(7-hydroxy-4-(methoxyimino)chroman-2-yl)benzoate (22c). The mixture of compound **22b** (960 mg, 3.22 mmol), sodium acetate (528 mg, 6.44 mmol) and O-methylhydroxylamine, hydrochloric acid (538 mg, 6.44 mmol) in methanol (10 mL) was stirred at 60 °C overnight. Solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with water. The organic layers was dried over $MgSO_4$, filtered, and concentrated. The residue was washed with ether to provide the title compound (810 mg, 2.475 mmol, 77 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 - 8.03 (m, 2H), 7.81 (d, J = 8.7 Hz, 1H), 7.58 - 7.43 (m, 2H), 6.50 (dd, J = 8.6, 2.5 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 5.21 (d, J = 3.0 Hz, 1H), 5.12 (dd, J = 12.2, 3.2 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.45 (dd, J = 17.2, 3.2 Hz, 1H), 2.63 (dd, J = 17.2, 12.2 Hz, 1H); MS (ESI+) m/z 328 $(M+H)^+$.

Methyl 4-((2*R*,4*R*)-4-amino-7-hydroxychroman-2-yl)benzoate (22d). A mixture of compound **22c** (570 mg, 1.741 mmol) was treated with 5% platinum (0.05 equivalent) on carbon in acetic acid (5 mL). The reaction was stirred at room temperature under hydrogen (1 atmosphere) for 24 hours, LC/MS showed conversion over 95%. The mixture was filtered

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through a celite pad and solvent removed under reduced pressure. The residue was purified by preparative LC method TFA2 to provide the trifluroroacetic acid salt of the title compound (300 mg, 44% yield). LC/MS m/z 283 $(M\text{-}NH_2)^+$.

Methyl 4-((2*R*,4*R*)-4-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-7-hydroxychroman-2-yl)benzoate (22e). A mixture of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (162 mg, 0.668 mmol) and HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, 380 mg, 1.0 mmol) in DMF (2 mL) was stirred for 5 minutes at room temperature, followed by the addition of compound **22d** (200 mg, 0.334 mmol) and N-ethyl-N-isopropylpropan-2-amine (0.466 ml, 2.67 mmol). The mixture was stirred at room temperature for 2 hours, LC/MS showed reaction complete. The mixture was loaded on to a 25 g silica gel cartridge eluting with 5-50% ethyl acetate in heptane provide the title compound (204 mg, 58.3 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 - 7.90 (m, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.16 - 7.02 (m, 2H), 6.94 (dd, J = 37.7, 8.3 Hz, 2H), 6.49 - 6.32 (m, 2H), 5.67 (s, 1H), 5.36 (dt, J = 15.3, 8.7 Hz, 2H), 5.18 (d, J = 10.7 Hz, 1H), 3.93 (s, 3H), 2.56 - 2.36 (m, 1H), 1.80 - 1.70 (m, 2H), 1.26 (d, J = 2.2 Hz, 1H), 1.10 - 1.04 (m, 2H); MS (ESI-) m/z = 521.9 $(M\text{-}H)^-$.

Methyl 4-((2*R*,4*R*)-4-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-7-(difluoromethoxy)chroman-2-yl)benzoate (22f). To Example 23e (190 mg, 0.363 mmol) and diethyl (bromodifluoromethyl)phosphonate (0.129 ml, 0.726 mmol) in a mixture of acetonitrile (2 mL) and water (1 mL) was added 50% aqueous potassium hydroxide (244 mg, 2.178 mmol) drop wise via syringe while stirring vigorously. After the addition was completed, LC/MS showed conversion was complete with a small by-product peak. Additional water was added to the mixture and the mixture was extracted with

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ethyl acetate (3 x 20 mL). The combined organic extracts were washed with 1 M HCl (5 mL) and water, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative LC method TFA2 to provide the title compound (150 mg, 72 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 - 8.00 (m, 2H), 7.49 - 7.41 (m, 2H), 7.15 - 6.99 (m, 4H), 6.75 - 6.66 (m, 2H), 5.50 - 5.40 (m, 1H), 5.33 (d, J = 8.9 Hz, 1H), 5.25 (dd, J = 11.3, 2.0 Hz, 1H), 3.93 (s, 3H), 2.50 (ddd, J = 13.4, 6.1, 2.1 Hz, 1H), 1.84 - 1.71 (m, 2H), 1.65 (d, J = 2.8 Hz, 1H), 1.11 - 1.06 (m, 2H); MS (ESI-) m/z = 572 $(M-H)^-$.

4-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-(difluoromethoxy)-3,4-dihydro-2H-chromen-2-yl]benzoic acid (22). To compound **22f** (130 mg, 0.227 mmol) in methanol (2 mL) and water (0.5 mL) was added lithium hydroxide (32.6 mg, 1.360 mmol). The mixture was stirred at 35 °C for 4 hours, LC/MS showed the conversion was complete. Solvent was removed under reduced pressure and water (2 mL) was added. The pH of the mixture was adjusted to pH 1~2 with the addition of 2 M HCl. The precipitated white solid was collected by filtration, and dried to provide the title compound (110 mg, 0.197 mmol, 87 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 - 8.03 (m, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.16 - 6.99 (m, 4H), 6.73 - 6.67 (m, 2H), 6.38 (d, J = 73.6 Hz, 1H), 5.48 (td, J = 10.4, 6.1 Hz, 1H), 5.36 (d, J = 8.8 Hz, 1H), 5.31 - 5.21 (m, 1H), 2.52 (ddd, J = 13.3, 6.0, 2.2 Hz, 1H), 1.86 - 1.71 (m, 2H), 1.68 - 1.60 (m, 1H), 1.10 (q, J = 3.7, 2.4 Hz, 2H); MS (ESI-) m/z = 558 $(M-H)^-$.

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Biology

Determination of Biological Activity

Cellular Assays

Cell Surface Expression-Horse Radish Peroxidase (CSE-HRP) Assay:

A cellular assay for measuring the F508del CFTR cell surface expression after correction with test compounds was developed in human lung derived epithelial cell line (CFBE41o-).[16] This was achieved by expressing the F508del CFTR mutation along with a horseradish peroxidase (HRP) in the fourth exofacial loop and then measuring the HRP activity using luminescence readout from these cells, CFBE41o-F508del CFTR-HRP, that were incubated overnight with the test corrector compounds.[17] Briefly, for this primary assay, the CFBE41o-F508del CFTR-HRP cells were plated in 384-well plates (Greiner Bio-one; Cat 781080) at 4,000 cells/well along with 0.5 μg/mL doxycycline to induce the F508del CFTR-HRP expression and further incubated at 37 ^{o}C, 5% CO_2 for 72 hours. The test compounds were then added at the required concentrations and further incubated for 18-24 hours at 33 °C. The highest concentration tested was 20 μM with an 8-point concentration response curve using a 3-fold dilution. Three replicate plates were run to determine one EC_{50}. All plates contained negative controls (dimethyl sulfoxide, DMSO) and positive controls (3 μM of 3-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2*H*-chromen-2-yl]benzoic acid) (Compound **15**) as well as on-plate concentration response of the positive control. Post incubation, the plates were washed 5× times with Dulbecco's phosphate buffered saline (DPBS), followed by the addition of the HRP substrate, luminol (50 μL), and measuring the HRP activity using luminescence readout on EnVision® Multilabel Plate Reader

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(Perkin Elmer; product number 2104-0010). The raw counts from the experiment are analyzed using Accelrys® Assay Explorer v3.3.

The % activity measured at each of the 8 test concentrations of the test compound was normalized to the on-plate positive control using the following formula:

% activity = [(test compound response – DMSO response) / (positive control response – DMSO response)]*100

The maximum % activity achieved for the test compound at any tested concentration is presented in Tables along with the EC_{50} calculated using the general sigmoidal curve with variable Hill slope equation.

Trans-epithelial Current Clamp on Human Bronchial Epithelial Cells Conductance Assay:

A cell based assay using the primary human bronchial epithelial cells (hBE) was used as a secondary assay to test novel F508del CFTR correctors for their activity on primary hBE cells with F508del/F508del CFTR mutation. Primary human bronchial epithelial (hBE) cells from F508del/F508del CFTR patients were expanded from 1×10^6 to 250×10^6 cells.[27] For this purpose, cells isolated from CF patients with the homozygous mutation were seeded onto 24 well Corning (Cat # 3378) filter plates that were coated with 3T3 conditioned media and grown at an air-liquid interface for 35 days using an Ultroser® G supplemented differentiation media. Apical surface mucus was removed 72 hours before the experiment by incubating the apical surface of the cells for 30 minutes with 3 mM dithiothreitol (DTT) prepared in the differentiation media, followed by aspiration of the mucus along with the media. The apical surface is washed again with phosphate buffered saline (PBS) incubated for 30 minutes followed with aspiration. The cells were then incubated with the desired dose of the corrector compounds 18-24 hours at 37 ^{o}C, 5%

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CO_2. The corrector compounds were prepared as 10 mM stocks and the desired concentrations were prepared in differentiation media and were always applied on the basolateral side of the epithelial cells.

On the day of measuring the corrector activity on the TECC, the cells were switched into a bicarbonate and serum free F-12 Coon's medium and allowed to equilibrate for 90 minutes in a CO_2 free incubator. At the time of measurement, the apical and basolateral sides of the filter were bathed with the F-12 Coon's modification media (with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4 (using 1 M tris(hydroxymethyl)aminomethane (Tris)), and the measurements were made at 36.5 ^{o}C. Current responses before and after the sequential addition of benzamil (apical 6 μM addition; for inhibiting epithelial ENaC channel), forskolin (apical and basolateral 10 μM addition; for activating the CFTR channel), control potentiator (*N*-(3-carbamoyl-5,5,7,7-tetramethyl-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-1*H*-pyrazole-5-carboxamide; apical and basolateral 1 μM addition; for potentiating the CFTR channel) and bumetanide (basolateral 20 μM addition; for inhibiting the Na:2Cl:K co-transporter, an indirect measure of inhibiting the Cl- secretion driven by CFTR channel) were measured. The assay uses a TECC-24 (Transepithelial Current Clamp for 24 wells) instrument that measures the functionality of the mutated channel by measuring the equivalent CFTR current (I_{EQ}) generated by the polarized primary epithelial cells. The instrument works by measuring the transepithelial potential difference (V_T) and transepithelial conductance (G_T) under current clamp conditions using a custom designed multi-channel current clamp and electrode manifold. Each measured V_T values are corrected for the electrode offset potential, and each measured G_T values are corrected for the combined solution series and empty filter resistances. The corrected V_T and G_T values were then used to calculate the equivalent current,

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I_{EQ} using Ohm's law ($I_{EQ} = V_T.G_T$). In addition to calculating the I_{EQ}, the area under the curve (AUC) for the time period between the forskolin peak I_{EQ} response and at the time of bumetanide addition was also calculated using a one-third trapezoid method. The assay was run in a 24-well format and all 24-wells were measured at the same time point giving a higher throughput for this assay.

All plates contained negative controls (dimethyl sulfoxide, DMSO) which coupled with the control potentiator (*N*-(3-carbamoyl-5,5,7,7-tetramethyl-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-1*H*-pyrazole-5-carboxamide) sets the null response and positive controls (3 μM of 3-[(2*R*,4*R*)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-methoxy-3,4-dihydro-2*H*-chromen-2-yl]benzoic acid) coupled with the control potentiator sets the 100% response to measure the correction of the mutated CFTR channel. The maximum percent activity is reported relative to the positive control value.

The % activity measured at each of the 6 test concentrations of the test compound was normalized to the on-plate positive control using the following formula:% activity = [(test compound response – DMSO response) / (positive control response – DMSO response)]*100 The I_{EQ} and AUC at different test concentrations were fit and an EC_{50} was calculated using the general sigmoidal curve with variable Hill slope equation included in the Prism *v5* software.

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Supporting Information

Purification conditions, syntheses and analytical information for the tabulated CFTR correctors compounds **3**, **4**, **5**, **6**, **7**, **8**, **9**, **14**, **16**, **17**, **18**, **19**, and **20**. CYP3A4 induction protocol.

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