Current development of CFTR potentiators in the last decade.

Virginia Spanò[a,§], Arianna Venturini[b,§], Michele Genovese[b], Marilia Barreca[a], Maria Valeria Raimondi [a], Alessandra Montalbano[a*], Luis J.V. Galietta[b,c], Paola Barraja[a]

[a]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 32, 90123, Palermo, Italy

[b]Telethon Institute of Genetics and Medicine (TIGEM), Campi Flegrei 34, 80078, Pozzuoli, NA, Italy

[c]Department of Translational Medical Sciences (DISMET), University of Naples, "Federico II", Via Sergio Pansini 5, 80131, Naples, Italy

[§] The authors contributed equally to this work.

[*]Corresponding author: Alessandra Montalbano Email address: alessandra.montalbano@unipa.it

Tel: +39-091 6161606; +39-091 23896822

ABSTRACT

Cystic fibrosis (CF) is a genetic disorder produced by the loss of function of CFTR, a main chloride channel involved in transepithelial salt and water transport. CFTR function can be rescued by small molecules called "potentiators" which increase gating activity of CFTR on epithelial surfaces. High throughput screening (HTS) assays allowed the identification of new chemical entities endowed with potentiator properties, further improved through medicinal chemistry optimization. In this review, the most relevant classes of CFTR potentiators developed in the last decade were explored, focusing on structure-activity relationships (SAR) of the different chemical entities, as a useful tool for the improvement of their pharmacological activity.

KEYWORDS: Cystic fibrosis, Cystic fibrosis transmembrane conductance regulator, CFTR, CFTR potentiators, VX-770

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations affecting the CF transmembrane conductance regulator (*CFTR*) gene.[1] The protein coded by the *CFTR* gene works as a cAMP-regulated Cl^- channel expressed in many types of epithelial cells and involved in transepithelial salt and water transport. Loss of function of CFTR protein causes a multi-organ disease with a main involvement of respiratory system. In the airways, defective anion transport impairs mucociliary transport. Consequently, there is accumulation of very viscous mucus that

becomes the favorable substrate for survival and proliferation of bacteria. Bacterial infection causes a progressive damage with loss of respiratory function. Besides the lungs, pancreas, liver, and intestine are also seriously affected. More than 2,000 *CFTR* mutations have been identified, but a careful analysis involving genotype-phenotype correlation and *in vitro* functional/biochemical studies is revealing that real CF-causing mutation are a few hundreds, the remaining being polymorphisms.[2] Among mutations, deletion of phenylalanine at position 508 (Phe508del) is the most frequent one, 50-90% depending on the geographic area: high in North America and Northern Europe, low in the Mediterranean area.[3] The other mutations have all much lower frequency.[4] CF mutations impair CFTR expression and function with a variety of mechanisms. At least six classes of mutations have been proposed based on the mechanism of action.[1] Class 1 includes stop codon mutations (e.g. Gly542X, Trp1282X) that cause arrest of protein synthesis. Class 2, which includes Phe508del, is characterized by mutations that cause CFTR protein misfolding and instability with consequent mistrafficking and premature degradation.[5] Class 3 comprises several types of missense mutations (e.g. amino acid substitutions Gly178Arg, Gly551Asp, Gly1349Asp) that impair the process of CFTR channel opening. Class 4 mutations cause a mild defect in the ability of CFTR to transport Cl^- anions. Class 5 typically includes mutations that reduce CFTR protein synthesis by altering the process of RNA splicing. Finally, class 6 mutations cause reduced half-time of CFTR protein at the plasma membrane.[1] Importantly, there is no strict separation among classes since many mutations have multiple defects.[6] As a classical example, Phe508del remains trapped in the endoplasmic reticulum because of misfolding and instability (class 2 defect). However, a small fraction of the protein reaches the plasma membrane where it shows reduced channel opening (class 3 defect) and accelerated internalization (class 6 defect).

Importantly, molecular defects caused by CF mutations are in many cases druggable. Indeed, just a few years after CFTR gene discovery,[7] it was already demonstrated that CFTR expression/function can be restored by small molecules or other types of treatments. In particular, it was found that defective channel opening can be overcome with genistein, xanthines, or benzimidazolones.[8–10] These molecules received the generic name “potentiators” to indicate pharmacological agents able to potentiate the physiological mechanism leading to CFTR activation. Regarding the mistrafficking defect caused by Phe508del, it was found that the protein could be rescued from degradation by incubating the cells at low temperature or with chemical chaperones.[11,12] This was an important proof of principle that stimulated the search of small molecules, named “correctors”, having the same type of activity. In the subsequent years, both academies and pharmaceutical companies have been involved in searching for correctors and potentiators able to rescue CFTR activity. In the absence of CFTR structure information that could

guide a rational design of drugs, it was believed that functional assays could be used to screen chemical libraries in order to find active compounds. Initial small scale screenings demonstrated the feasibility of this approach, with the identification of benzo[c]quinolizinium compounds and 7,8-benzoflavones as novel potentiators.[13,14] With the development of methods for high-throughput screening of very large chemical libraries, in the order of hundreds of thousands compounds, many other potentiators were subsequently found.[15–18] The high-throughput approach was also successful in discovering Phe508del correctors.[19,20] This type of research has brought a real benefit to CF patients with the development of potentiators and correctors that have been approved by FDA and EMA for the treatment of patients with responsive mutations. In particular, the potentiator VX-770 (**3**),[21] also named ivacaftor (Figure 1), was initially approved (drug name: Kalydeco) for patients with Gly551Asp, given its high efficacy in improving respiratory function and other clinically-relevant parameters.[22] Later, ivacaftor was also approved for patients with many other types of class 3 mutations (e.g. Gly178Arg, and Gly1349Asp) and, in combination with correctors, for Phe508del patients.[23,24]

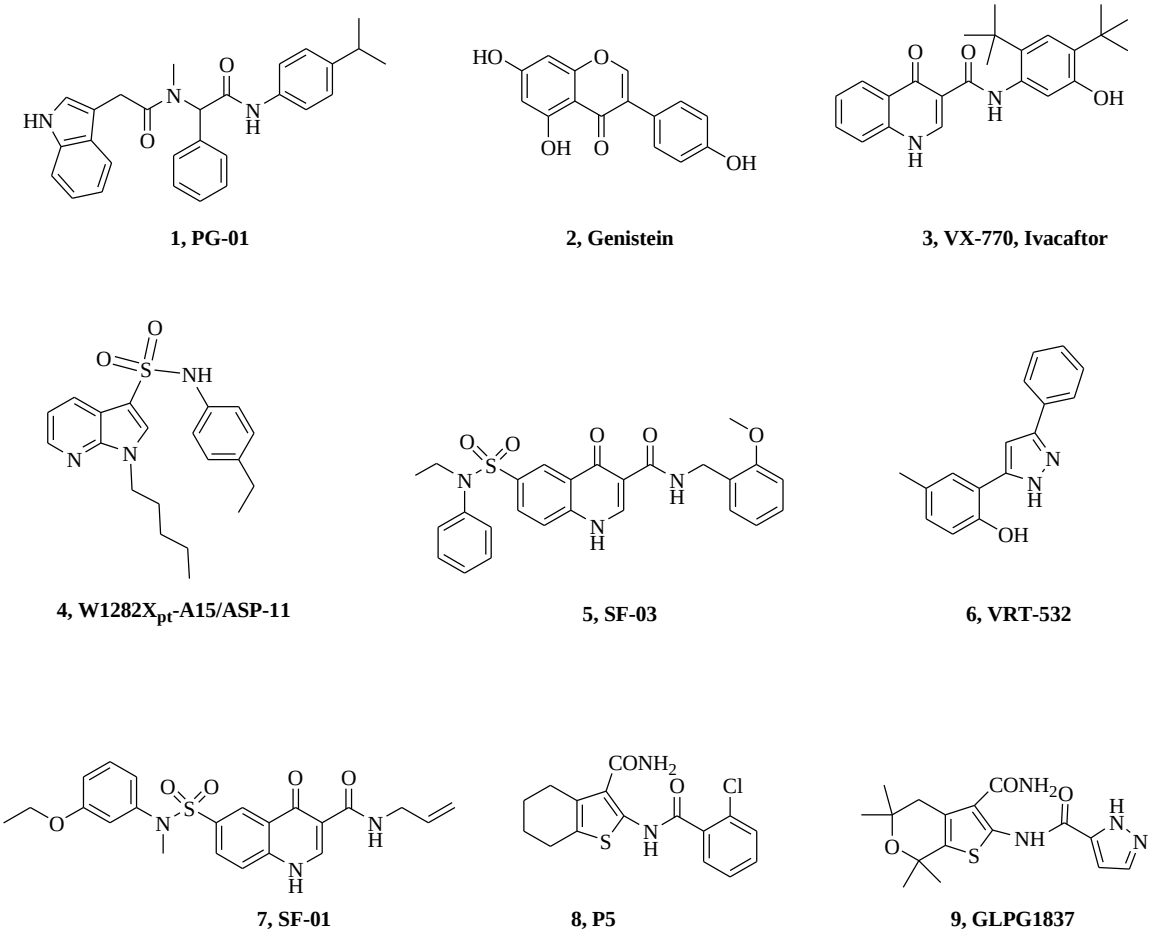
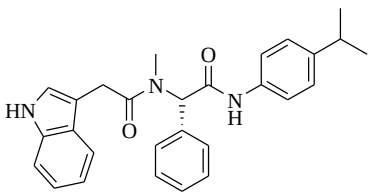


Figure 1. Structures of most relevant potentiators

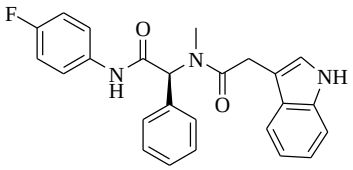
In this review, we will present the most relevant classes of CFTR potentiators developed in the last years, briefly mentioning those discovered in the early years of CFTR drug discovery. Structure-activity relationships (SAR) of the different chemical entities were also considered in order to give a useful basis for further advancement in identifying small molecules with increased pharmacological activity.

2. Phenylglicines

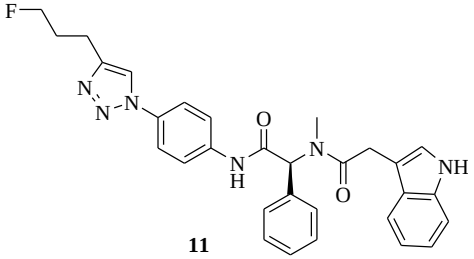
Phenylglicines (PGs) belong to a valuable class of compounds that emerged from a high-throughput screening (HTS) of 50,000 diverse small molecules aiming at the identification of potent and selective CFTR potentiators.[17] The search was done using Fisher rat thyroid (FRT) cells co-expressing the Phe508del-CFTR mutant and a special halide-sensitive yellow fluorescent protein, HS-YFP. [25] FRT cells, seeded in 96-well microplates, were kept at low temperature for 24h to enhance the amount of Phe508del-CFTR in the plasma membrane and then briefly exposed to test compounds plus forskolin as a cAMP-elevating agent to induce mutant CFTR phosphorylation. The detection of potentiators, i.e. compounds enhancing Phe508del-CFTR activity above the level achieved with forskolin alone, was detected as an accelerated rate of fluorescence quenching caused by iodide influx. [25] The screening detected active compounds with various chemical scaffolds, in particular phenylglycines (PGs). A secondary screening on 1,000 structural analogues identified PG-01 (**1**) (Figure 2) as a potentiator endowed with high efficacy and potency. In particular, PG-01 (**1**) had a half effective concentration (EC_{50}) of ~70 nM, a nearly 100-fold increase in potency compared to the reference potentiator genistein. Importantly, PG-01 (**1**) was also effective on CFTR mutants with pure channel gating defect, namely Gly551Asp and Gly1349Asp. The mechanism of action of PGs as direct modulators of mutant CFTR was supported by single-channel recordings that demonstrated an increase in open channel probability in isolated patches of membrane.[17]



1, PG-01



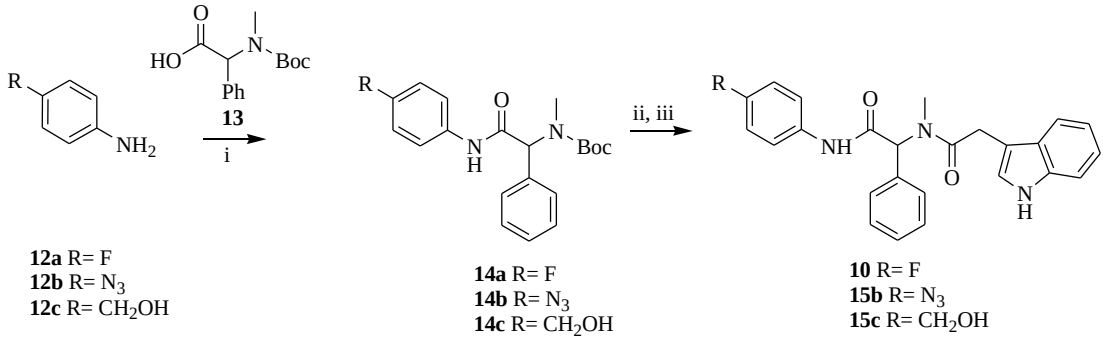
10



11

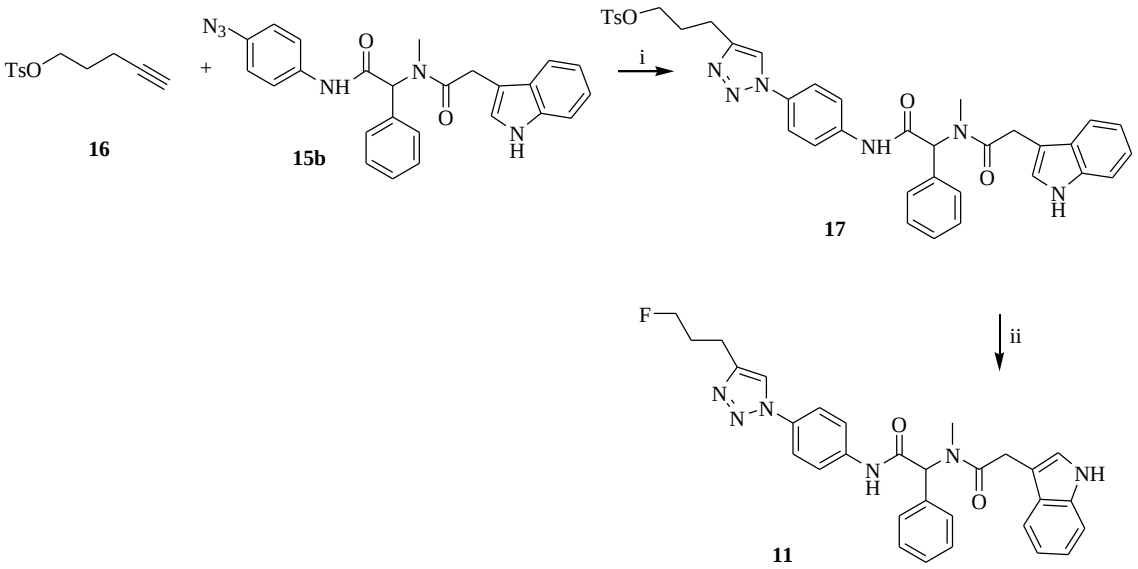
Figure 2. Structure of phenylglicine **PG-01** (1) and ^{19}F fluorinated potentiators.

In a subsequent study, these authors generated fluorinated PGs as potentially useful tools for *in vivo* PET (Positron Emission Tomography) biodistribution imaging.[26] The synthesis of two fluorinated ^{19}F-potentiators **10** and **11** was reported. The synthesis of phenylglicines starts from the proper aniline derivatives **12a,b** that were coupled with *N*-methyl-Boc-phenylglycine **13** (Scheme 1). Deprotection of the amino group with TFA and subsequent coupling with indole-3-acetic acid allowed the isolation of fluorinated potentiator **10** and **15b.**



Scheme 1. Synthesis of PGs. Reagents and conditions: (i) EDC, DMF/ CH_2Cl_2; (ii) TFA, CH_2Cl_2; (iii) indole-3-acetic acid, EDC, DMF/ CH_2Cl_2.

Huisgen copper catalized 1,3-dipolar cycloaddiction with tosylate **16** and the azido intermediate **15b**, afforded compound **17**, which was further treated with $Bu_4N^+F^-(t\text{-BuOH})_4$ to give the desired derivative **11** (Scheme 2).



Scheme 2. Synthesis of the fluorinated potentiator **11**. Reagents and conditions: (i) Na ascorbate, $CuSO_4$, CH_2Cl_2, *t*BuOH, H_2O; (ii) $Bu_4N^+F^-(t\text{-BuOH})_4$, CH_3CN.

Phe508del-CFTR potentiator activity of fluorinated **10** and **11** was comparable to that of PG-01 (**1**) having EC_{50}=0.09 µM and 1.1 µM, respectively, again better than the benchmark potentiator genistein (EC_{50}=7.0 µM). This can be ascribed to the fluorine atom that generally enhances physicochemical properties and binding interactions of drugs.[27]

In another study, PGs were considered to generate hybrid molecules consisting of a fusion between a potentiator and a corrector molecule.[28] The goal was to generate a single drug targeting the two defects caused by Phe508del mutation. Kurth and coll. designed and synthetized an hybrid molecule **18** containing both the corrector fragment corr-4a [19] and the potentiator PG-01 linked together with an ethylene glycol spacer through an ester bond (Figure 3).

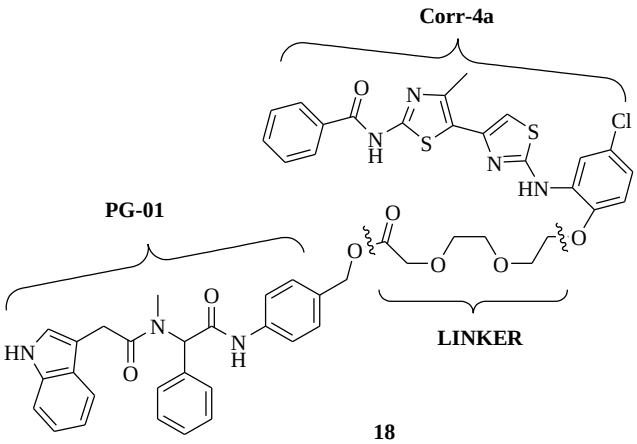
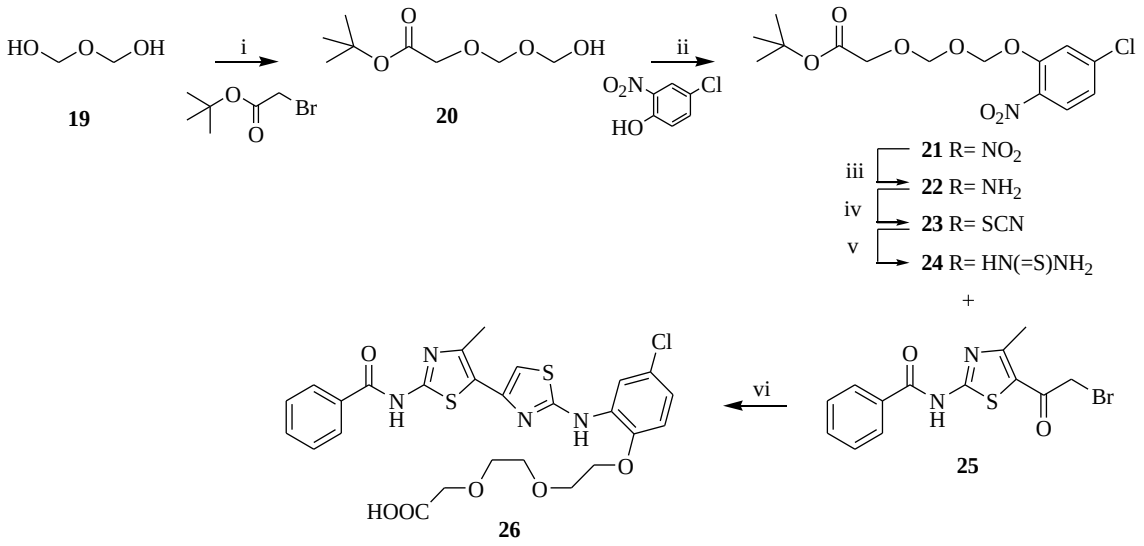


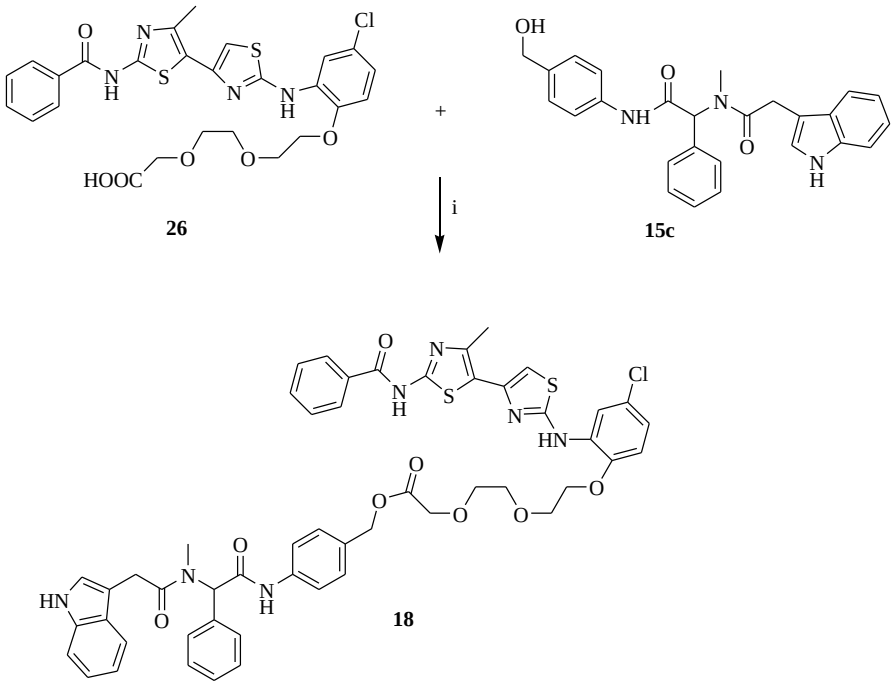
Figure 3. Structure of hybrid potentiator-corrector **18**.

Thus the 4-isopropylaniline of PG-01 (**1**) [17] was replaced with a benzyl moiety, and linked to the 2-methoxy group of the corrector corr-4a.[29] Initially, the corrector-linker fragment **26** was prepared through the multistep synthetic pathway described in Scheme 3.



Scheme 3. Synthesis of corrector-linker fragment **26**. Reagents and conditions: (i) NaHMDS, THF; (ii) PPh_3, DIAD, THF; (iii) $SnCl_2$, EtOH; (iv) $S{=}CCl_2$, Et_3N, H_2O/CH_2Cl_2; (v) NH_3, CH_2Cl_2; (vi) EtOH, then TFA, CH_2Cl_2.

Finally, EDC coupling of the two fragments **26** and **15c**, the latter in turn prepared from the corresponding alcohol **12c** (Scheme 1), allowed the isolation of the hybrid derivative **18** in 11% yield (Scheme 4).



Scheme 4. Synthesis of the hybrid **18**. Reagents and conditions: (i) EDC, DMF/CH_2Cl_2.

Disappointingly, hybrid compound **18** was devoid of activity, as potentiator and corrector, in contrast to the separated compounds PG-01 **(1)** and corr-4a. [28] This negative outcome was

explained as a low cell membrane permeability of compound **18**. However, when compound **18** was subjected to hydrolysis under physiological condition in the presence of intestinal enzymes, it released the corrector and potentiator fragments which retained strong activity. These results provided proof-of-concept that a potentiator and a corrector can be administered as a single hybrid that is then cleaved in the intestine and then possibly absorbed to reach the target tissues expressing mutant CFTR.[28]

3. Quinolines

3.1 Ivacaftor and deuterated analogues

Ivacaftor (VX-770, **3**) (Figure 5) is a potent, selective and orally bioavailable CFTR potentiator developed by Vertex Pharmaceuticals. It is the active principle of Kalydeco, the drug that has been approved for the treatment of CF patients with Gly551Asp [22] and many other CFTR gating mutations. Ivacaftor is also present in drug combinations designed to rescue Phe508del-CFTR trafficking and function.[23,24] Ivacaftor (**3**) was the result of a large scale high-throughput screening [21] followed by extensive chemical optimization.[30] The screening was done using a cell-based assay designed to detect CFTR activity using a membrane potential-sensitive fluorescent probe. By screening the library, containing 228,000 structurally diverse small molecules, the authors identified four scaffolds (**27-30**) with potentiator activity (Figure 4). Compound **30** was confirmed with secondary assays on cell lines and primary airway epithelial cells expressing Phe508del- and Gly551Asp-CFTR. In particular, compound **30** displayed an EC_{50} of 2.1 ± 1.4 μM as potentiator of Phe508del-CFTR, a 4-fold improvement over genistein. These properties, in combination with a relatively low molecular weight of 368 and a cLogP of 2.9, made **30** an attractive, validated starting point to develop new CFTR potentiators. Several rounds of chemical synthesis and functional evaluation led, in the end, to the quinolinone-3-carboxamide derivative **3** [30] which was the first CFTR potentiator evaluated in clinical trials.

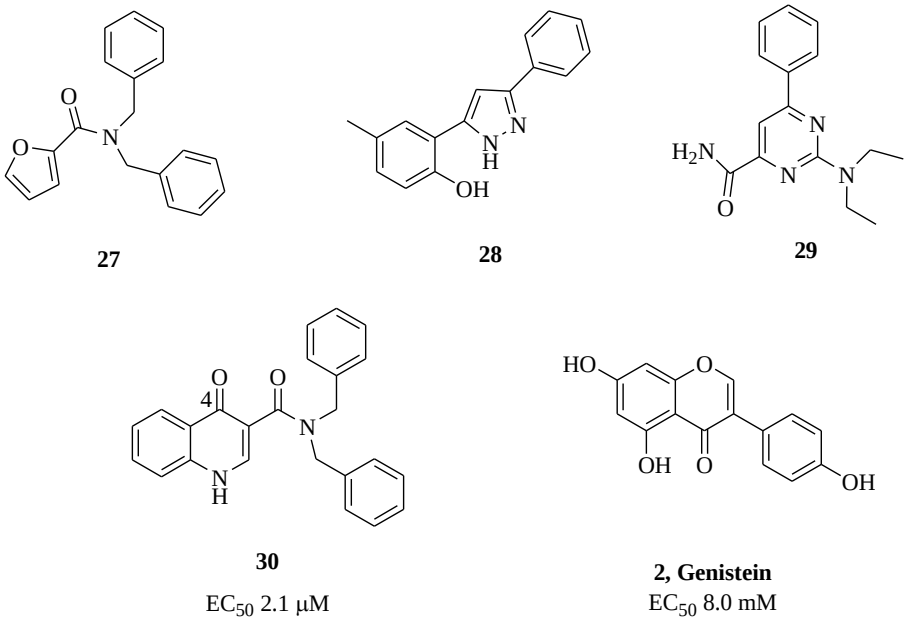


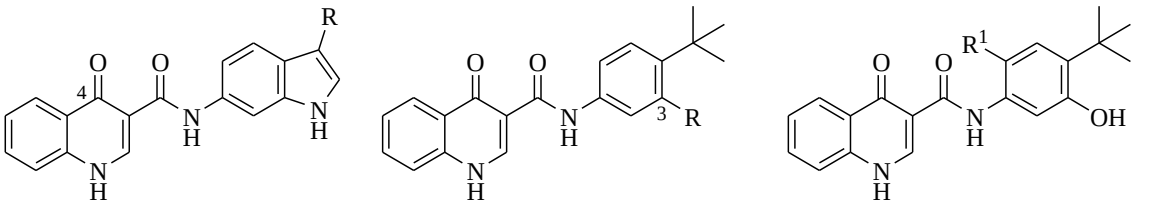
Figure 4. Hit scaffolds identified by Vertex Pharmaceuticals.

A range of distinct analogs were synthesized to examine the structural requirements:

- removal of the quinolinone 4-oxo group
- truncation of the fused phenyl with pyridone
- alkylation of the quinolinone nitrogen
- replacement of the quinolinone ring with pyridopyrimidone.

However, all these modifications led to reduced potencies whereas quinolinone replacement with naphthol derivative retained activity suggesting that fundamental chemical features are both the hydrophobic phenyl ring and the quinolinol tautomer which is stabilized by intramolecular hydrogen bonding with the lone pair on the carbonyl oxygen of the amide.

A set of ~70 amines including primary, secondary, aliphatic, aromatic, and heterocyclic amines were synthesized, allowing the identification of 6-indolyl derivative **31** (Figure 5) with a 20-fold improvement in potency over compound **30** (EC_{50} = 0.1 μM).



31

R= H EC$_{50}$ 0.1 µM
R= *t*-Butyl

32

R= NH_2 IC$_{50}$ 0.1 µM
R= H IC$_{50}$ 0.1 µM
R=CH_2OH IC$_{50}$ 0.1 µM
R= F IC$_{50}$ 0.1 µM

33 R^1= H IC$_{50}$ 0.003 µM
34 R^1= F IC$_{50}$ 0.002 µM
35 R^1= CF_3 IC$_{50}$ 0.002 µM
3 R^1= *t*-Butyl IC$_{50}$ 0.002 µM

Figure 5. Lead optimization of ivacaftor **3**.

Methylation of the NH indole led to a 60-fold decrease in activity, thus suggesting its involvement in an important interaction, possibly a hydrogen bond. Further attempts to increase the polarity of the molecule by replacing the indole ring with other heterocycles, while retaining the orientation of the NH (azaindole, indazole, benzimidazole, and oxyindole) or interchanging alkyl groups with polar moieties, resulted in reduction of potency.

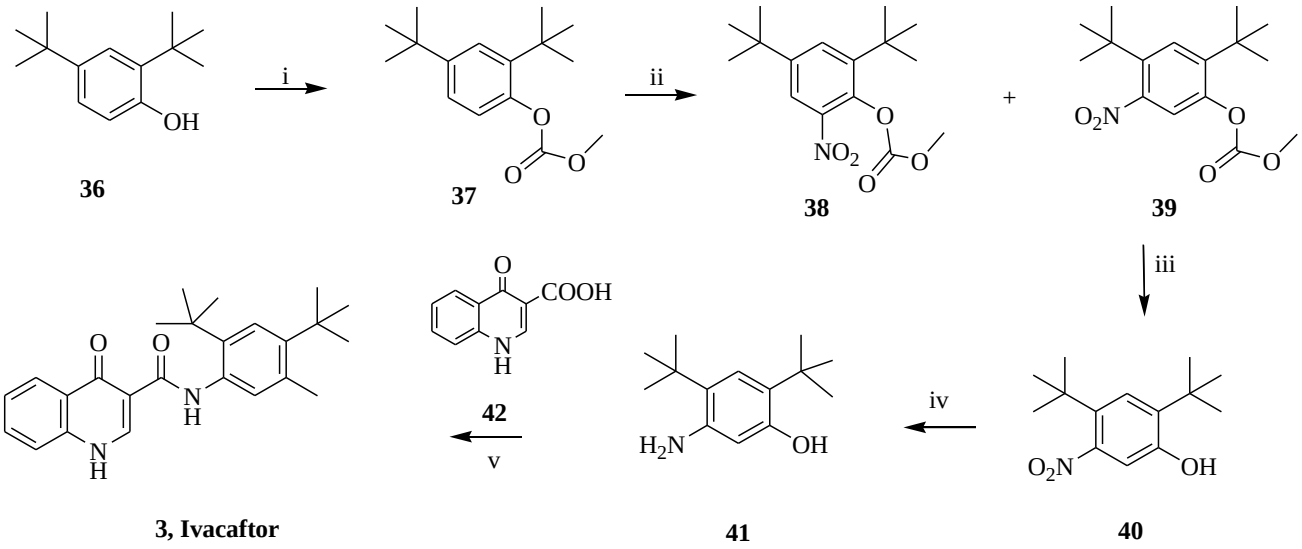
Due to its planarity and an intramolecular hydrogen bonding between the 4-carbonyl group and the amide hydrogen, derivative **31** had a tightly packed crystal lattice, resulting in a low aqueous and organic solubility. In addition, it displayed low oral bioavailability in rats (11%), a short half-life in dogs (0.9 h), and a significant activity (IC$_{50}$ of ~0.1 µM) against the GABA A benzodiazepine receptor, a ligand-gated chloride channel. In order to disrupt the planarity of compound **31** and improve solubility, changes to the amide linker were investigated. Ester, sulfonamide reduced analogues, and reverse amide were synthesized but they displayed lower potencies.

When the indole moiety was replaced with a 3-anilino group substituted with alkyl groups of different size at the 4-position, an improvement of potency with increasing size and branching, was observed. From these modifications compounds **32** were found equipotent with the parent indole derivative **31** (Figure 5). The role of the aniline moiety at the 3 position was further explored. Its removal retained activity (IC$_{50}$ = 0.1 µM in NIH-3T3 cells), while its acylation or carbamate derivatization had a detrimental effect on activity. Similarly, substitution of the aniline moiety with a benzylamine, a carboxylic acid, or a sulfonamide group significantly reduced activities. Substitution of the 3-amino group with a hydroxymethyl group (IC$_{50}$= 0.1 µM) or a fluorine atom (IC$_{50}$ = 0.1 µM) was tolerated, while replacement with a phenolic group (**33**) (Figure 5) considerably increased the activity (IC$_{50}$= 0.003 µM).

With the aim of introducing a substituent of a different size and lipophilic character at the para position of phenol **33**, 2-fluoro (**34**), 2-trifluoromethyl (**35**), and 2-*tert*-butyl (**3-ivacaftor**) derivatives were synthesized. All three compounds were equipotent with **33** (IC$_{50}$ ~ 2-3 nM), but

derivative **3** displayed the best pharmacokinetic profile with low clearance and long half-life. Moreover it had an improved organic solubility, no major CYP450 activity, did not inhibit the cardiac potassium channel hERG (IC_{50} > 10 μM) and showed no significant activity against 160 targets tested including the GABA A benzodiazepine receptor.[30]

The synthetic pathway used for the isolation of ivacaftor is shown in Scheme 5. Commercially available 2,4-di-*tert*-butylphenol **36** was protected as a carbonate **37**. Nitration with nitric acid in sulfuric acid afforded a mixture of two isomers **38** and **39**. The latter was deprotected under basic conditions, followed by reduction and coupling with 4-oxo-1,4-dihydroquinoline-3carboxylic acid (**42**) affording compound **3** in moderate yield.



Scheme 5. Synthesis of ivacaftor. Reagents and conditions: (i) CH_3CO_2Cl, Et_3N, DMAP, CH_2Cl_2; (ii) HNO_3, H_2SO_4; (iii) KOH, MeOH; (iv) ammonium formate, Pd/c, EtOH; (v) HBTU, Et_3N, DMF.

Ivacaftor (**3**) showed potentiator activity against mutant CFTR in human bronchial epithelial cells [21] with greater potency than genistein (70-fold in Gly551Asp/Phe508del, 200-fold in Phe508del/Phe508del). Moreover, considering the favourable pharmacokinetic properties in rodents and non-rodents, it was selected for clinical evaluation for the treatment of CF patients.[22]

There is a growing interest on deuterated agents as pharmaceuticals. Indeed, deuterium has minimal impact on the structure or on the physical properties of the drug but it can affect the pharmacokinetics (PK) profile due to the deuterium isotope effect (DIE), which can lower the rate of carbon–deuterium versus carbon–hydrogen bond cleavage.[31–33] For these reasons, the deuterated ivacaftor analogues **43**, CTP-656 (d_9-ivacaftor) and **44**, d_{18} ivacaftor (Figure 6) were synthesized as reported by Morgan and coll.[34,35]

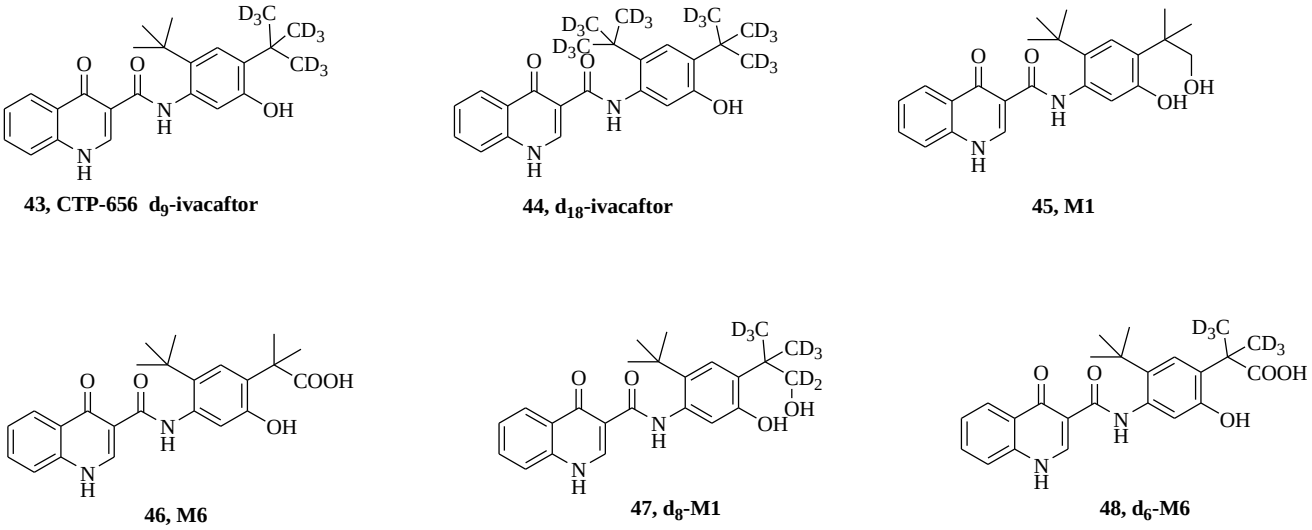
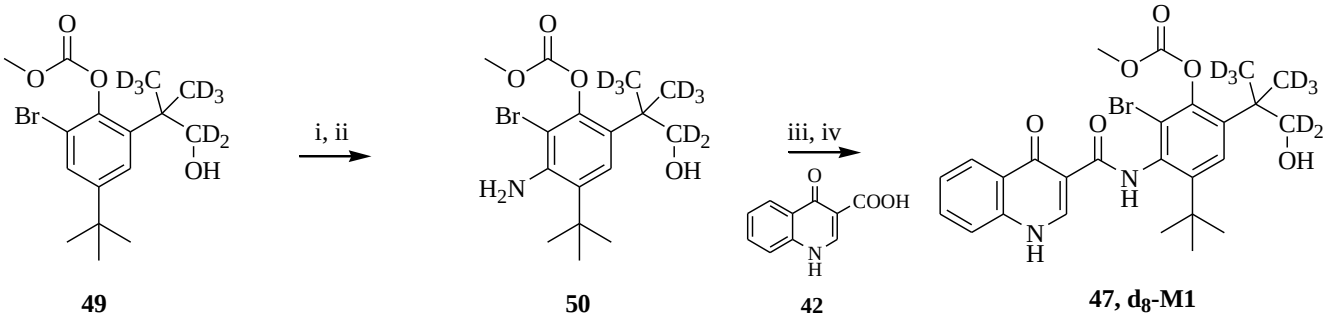
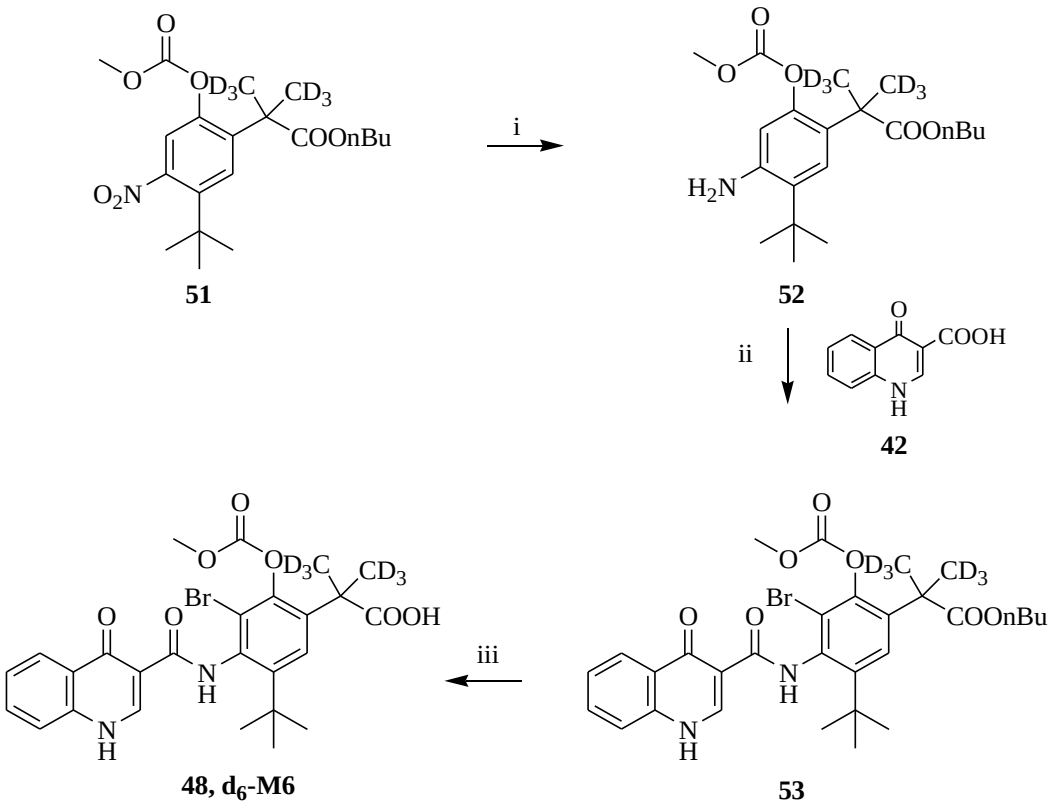


Figure 6. Deuterated analogues of ivacaftor **3** and its metabolites M1 and M6.

Since ivacaftor is extensively metabolized in humans into two major circulating metabolites, M1 (**45**) and M6 (**46**), the latter and the corresponding deuterated metabolites d_8-M1(**47**) and d_6-M6 (**48**) (Figure 6) were also prepared as described in Schemes 6 and 7 respectively from properly prepared key intermediates.[36]



Scheme 6. Synthesis of d_8-M1 (**47**). Reagents and conditions: (i) HNO_3; (ii) H_2, Pd/C; (iii) T3P; (iv) MeONa



Scheme 7. Synthesis of d_6-M6 (**48**). Reagents and conditions: (i) H_2; Pd/C; (ii) T3P; (iii) NaOH

The oxidative metabolite M1 showed approximately one-sixth the potency of ivacaftor and is considered pharmacologically active, differently from the carboxylic acid M6 which has less than one-fifty the potency of ivacaftor.[34]

Deuterated derivatives **43**,**44** and **47**,**48** were further investigated: *in vitro* metabolism, and *in vivo* PK studies were performed. The deuterated M1 and M6 metabolites **47**,**48** showed a pharmacological profile equivalent to that of the corresponding metabolites of ivacaftor.

CTP-656 (**43**) and d_{18}-ivacaftor (**44**) demonstrated equivalent potency to ivacaftor (**3**) in multiple *in vitro* studies, but they showed increased metabolic stability versus ivacaftor. *In vitro* metabolic stabilization did produce an *in vivo* exposure parameters when the compounds were orally dosed in rats and dogs. However, Harbeson and coll. stated that the PK studies were particularly challenging since rats and dogs did not metabolize ivacaftor as extensively as humans.[34] The observed increase in exposure and half-life (t1/2) compared with that reported for ivacaftor indicated the possibility for CTP-656 (**43**) to be dosed once daily. In the first half of 2019, a phase 2 randomized, double-blind study was initiated to explore the efficacy and safety of d_9-ivacaftor versus ivacaftor monotherapy or placebo in CF patients bearing 1 of the following 9 CFTR mutations on at least 1 allele: Gly551Asp, Gly178Arg, Ser549Asn, Ser549Arg, Gly551Ser, Gly1244Glu, Ser1251Asn,

Ser1255Pro, or Gly1349Asp (ClinicalTrials.gov Identifier: NCT03911713). Results are expected in mid 2020.

3.2 Triazole-quinolines

Hadida and co-workers suggested the tautomer **54** of ivacaftor (VX-770, **3**), in which the amide carbonyl oxygen forms an intramolecular H-bond with the quinolinol hydroxyl group, as the bioactive structural isomer.[30] Due to the widespread success of the 1,2,3-triazole as an amide bioisostere, the triazole analogue of ivacaftor (**55**) was synthesized and evaluated for CFTR modulation on the basis of its ability to form a similar intramolecular H-bonding interaction.[37]

Within the class of triazole analogues, compounds **56,57** were also synthesized (Figure 7), as analogues of highly effective compounds emerged from the previous study on ivacaftor chemical structure.[30]

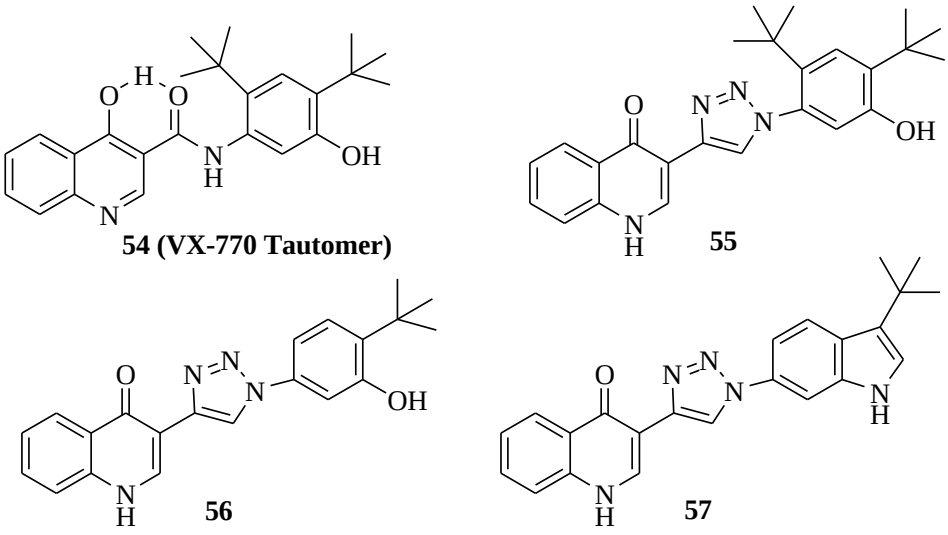
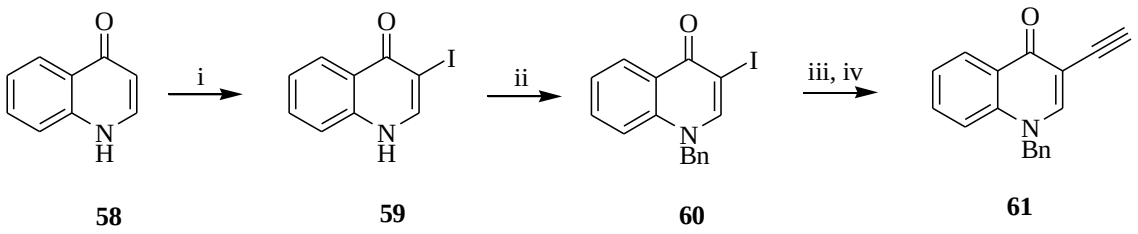


Figure 7. Triazole analogues of ivacaftor (**3**).

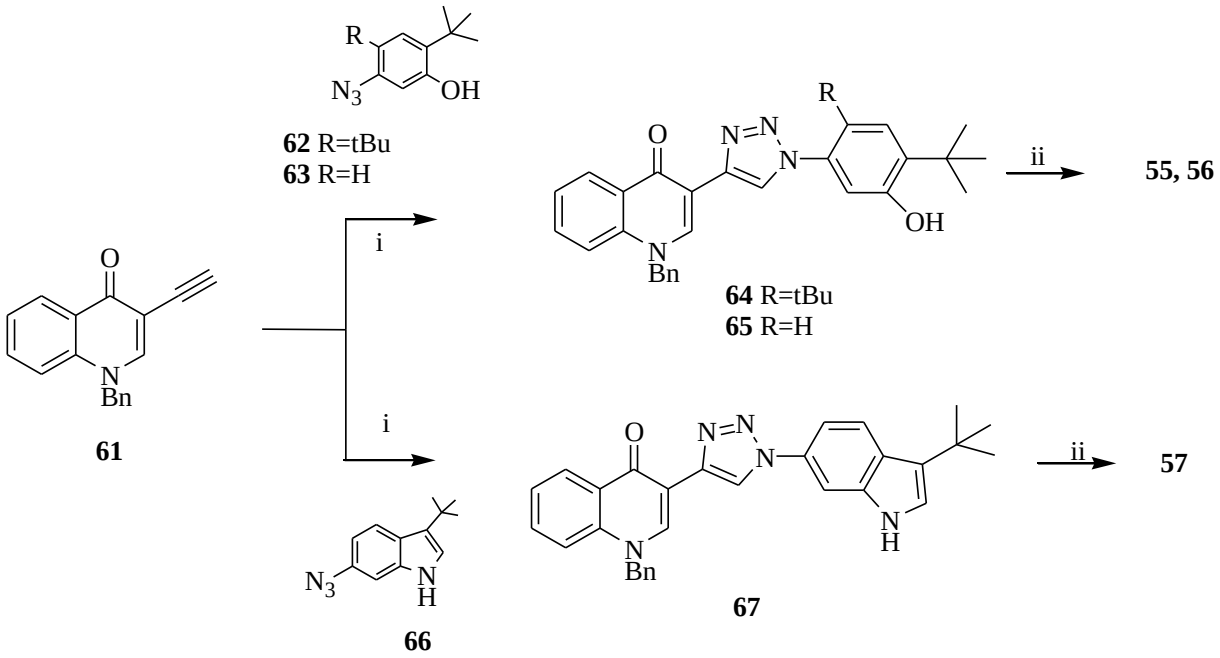
Triazole analogues **55-57** were prepared through copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction starting from alkyne **61**. This latter was obtained from *N*-benzyl-3-iodo quinolin-4(1*H*)-one **60** through Sonogashira coupling with trimethylsilyl acetylene, and TMS deprotection using K_2CO_3 in MeOH as shown in Scheme 8.[37]



58 **59** **60** **61**

Scheme 8. Synthesis of **61**. Reagents and conditions: (i) I_2, Na_2CO_3, THF; (ii) NaH, benzyl bromide, THF; (iii) trimethylsylylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, DIPEA, THF; (iv) K_2CO_3, MeOH.

The alkyne **61** was subsequently reacted with azides **62**, **63**, **66** (Scheme 9) producing the desired click product **64**,**65**,**67**. After removing benzyl group by refluxing with ammonium formate and Pearlman's catalyst in DMF the desired triazole analogues **55-57** were obtained.[37]



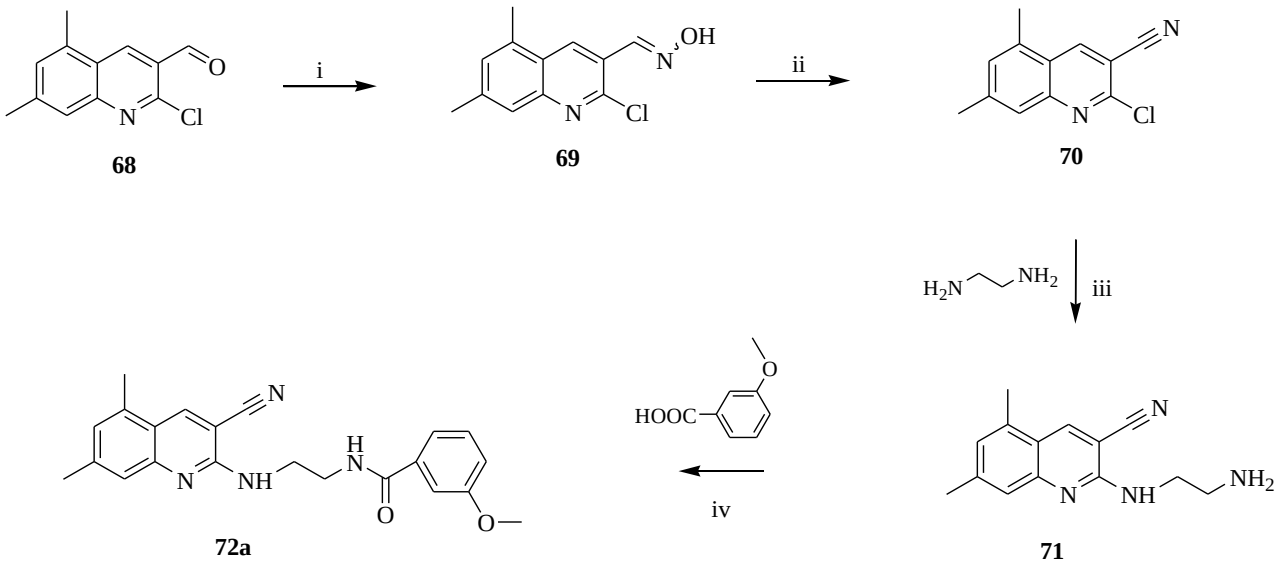
Scheme 9. Synthesis of VX-770 triazole analogues **55-57**. Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$ sodium ascorbate, CH_2Cl_2/tBuOH/H_2O (1:2:2); (ii) $Pd(OH)_2$, ammonium formate, DMF.

Disappointingly, triazole analogues of ivacaftor showed no potentiation of Phe508del, Gly551Asp, or wild type CFTR in functional assays.[37] It was hypothesized that VX-770 triazole derivatives did not reach the CFTR binding site under cellular conditions, due either to poor membrane permeability or active transport out of the cell. Moreover triazoles displayed decreased metabolic stability in human hepatic microsomes compared to the analogous amide, so overall this structural modification was not successful in CFTR potentiation activity.

3.3 Cyanoquinolines (CoPo)

Usually, potentiators and correctors of mutant CFTR are molecules with separate mechanism of action. However, a study in 2011 revealed the possibility to identify compounds with dual activity.[38] A screening of 110,000 small molecules identified cyanoquinolines as "CoPo" compounds, i.e. acting as potentiators and correctors. In particular, the compound **72a** (Scheme 10, Table 1) had efficacy comparable to that of corr-4a, as corrector, and to that of genistein, as potentiator. Cyanoquinoline **72a** was synthesized in six steps in 52% overall yield as reported in Scheme 10.

.



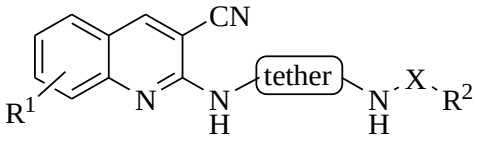
Scheme 10. Synthesis of **72a**. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, Et_3N, EtOH; (ii) $SOCl_2$; (iii) Dioxane; (iv) EDC, Et_3N, CH_2Cl_2/DMF

The quinoline ring was achieved starting from the commercially available 3,5-dimethylaniline which was subjected to acetylation with acetic anhydride and subsequent cyclization through reaction with phosphorous oxychloride. Dehydration of the 2-chloroquinoline carbaldehyde thus obtained, allowed the isolation of cyanoquinoline **70**. Nucleophilic substitution with 1,2-diaminoethane was followed by coupling reaction with *m*-anisic acid to give **72a** in 73% yield.

In order to explore structure-activity relationship and to identify more potent **72a** analogues, 180 commercially available quinolines were screened (Table 1). None of the derivatives tested showed improved potentiator activity with respect to **72a**,[38] but it was possible to assess that:

- best activity was achieved when the quinoline ring was 6-methoxy substituted; 6-methyl or 5,7-dimethyl derivatives, still showed good activity;
- replacement of methoxybenzene with 3-ethoxybenzene or 2-halobenzene or thiophene-2-yl, led to derivatives still endowed with good activities;
- replacement of methoxybenzene with benzosulfonamide moiety still mantained activity while sulfonamide group led to inactive compounds;
- replacement of the tether ethylene bridge with a costrained six or seven membered ring (piperazine or 1,4-diazepane ring) led to similar activity.

Table 1. CoPo analogues activities of a selection of most potent compounds.



					Corrector		Potentiator	
Cpd	R^1	Tether	X	R^2	EC_{50} (μM)	V_{max} (μM/s)	EC_{50} (μM)	V_{max} (μM/s)
72a	5,7-dimethyl	$(CH_2)_2$	CO	3-methoxy-phenyl	2.2	300	14	306
72b	8-methyl	$(CH_2)_2$	CO	3,4-dimethoxy-phenyl	3.8	223	15	250
72c	6-methyl	$(CH_2)_2$	CO	3,4-dimethoxy-phenyl	3.9	281	15	297
72d	6-methyl	piperazine	SO_2	4-methoxy-phenyl	5.0	102	3.8	72
72e	6-methoxy	piperazine	CO	2-fluoro-phenyl	4.2	140	11	195

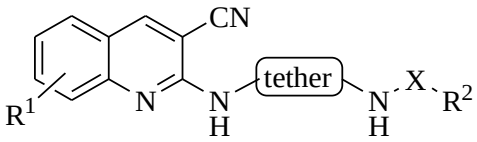
Among compounds showing both corrector and potentiator activities, **72a** was the most potent corrector. Compounds **72b** and **72c** are structurally related to **72a** and showed comparable corrector and potentiator activities. The benzosulfonamide derivative **72e**, also showed dual activities, albeit of lower potency and/or efficacy. Moreover, replacing the flexible ethylene tether with a constrained six- or seven-membered ring reduced or abolished corrector activity. Overall, SAR data suggested that the dual corrector-potentiator activity is not a general feature of all cyanoquinolines but is dependent on the particular scaffold and substituents. In other words, it appeared that the two activities could be dissociated depending on the type of substituents. Indeed, some compounds had dual activity whereas others only acted as potentiators.[38]

The potentialities of this class of compounds were further explored in a subsequent study [39] by examining how the tethering moiety and, secondarily, the arylamide subunits were able to influence CoPo activities. Twenty-four cyanoquinolines were synthesized starting from the key intermediate

70 obtained according to the synthetic pathway depicted in Scheme 10. Aromatic substitution of **70** with various diamines (1,2-diaminoethane, 1,3-diaminopropane and 1,4-piperazine) allowed the isolation of different amino-tethered intermediates further coupled with pyridyl acids, arylmethoxy acids, and one methoxypyridyl acid in the presence of EDC as activating agent.

Corrector and potentiator activities for the newly synthesized cyanoquinolines were assayed separately in FRT cells expressing Phe508del-CFTR, reported in Table 2 as EC_{50} values (a measure of compound potency) and Vmax (a measure of compound efficacy in increasing CFTR-dependent anion transport).

Table 2. Corrector and potentiator activities of selected CoPo derivatives.



				Corrector		Potentiator	
Cpd	Tether	X	R^2	EC_{50} (μM)	V_{max} (μM/s)	EC_{50} (μM)	V_{max} (μM/s)
72a	$(CH_2)_2$	CO	3-methoxy-phenyl	2.2	300	5.9	216
72f	$(CH_2)_2$	CO	2,4-dimethoxy-phenyl	4.2	108	55.0	426
72g	$(CH_2)_2$	CO	4-methoxy-phenyl	3.0	172	4.1	181
72h	$(CH_2)_2$	CO	pyridin-3-yl	2.7	151	13.2	242
72i	$(CH_2)_2$	CO	3,4-dimethoxy-phenyl	1.5	380	48.0	216
72j	$(CH_2)_2$	CO	5- methoxy-pyridin-2-yl	2.7	88	3.4	70
72k	$(CH_2)_2$	CO	2,6-dimethoxy-phenyl	3.7	111	11.5	119
72l	$(CH_2)_3$	CO	3-methoxy-phenyl	4.6	134	4.6	147
72m	$(CH_2)_3$	CO	4-methoxy-phenyl	4.3	112	5.0	231
72n	$(CH_2)_3$	CO	pyridin-3-yl	3.0	140	16.0	115

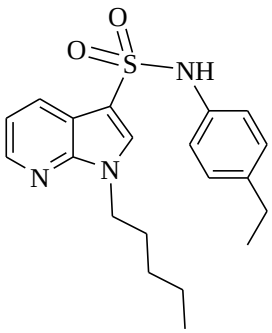
Data collected suggested that the diamino tether in a piperazine ring resulted in excellent potentiator activity abolishing corrector activity. Conversely, the increase in flexibility of the tether by chain lengthening resulted in CoPo derivatives with comparable or lower corrector and potentiator activity compared to that of **72a**. Additionally, variations at the arylamide moiety were able to tune the corrector and/or potentiator activities: ortho substituted arylamide generally gave more efficient potentiators.

Even if the target site(s) of CoPo's remain unknown, the authors postulated that **72a** adopts two active conformations that are responsible for corrector and potentiator activities by binding to a

single site of Phe508del-CFTR protein. This site would undergo conformational changes during protein transport from the endoplasmic reticulum to the cell plasma membrane. Accordingly, the dual activity of cyanoquinolines would depend on how a specific compound fits equally well to the binding site in the two conditions.

4. Pyridines and CFTR co-potentiators

Novel potentiator scaffolds were identified by Haggie and coll. [40] in 2017 while they were testing correctors and potentiators to achieve a functional rescue of the truncated translation product resulting from Trp1282X (W1282X) mutation. The authors of this study reasoned that this mutation, leaving 1281 of the total 1480 amino acid residues composing CFTR, could lead to a protein with a potential ability to work as a channel if properly stimulated with pharmacological agents. Therefore, using the HS-YFP assay, they performed a screening of 30,000 synthetic small molecules and drugs/nutraceuticals on FRT cells expressing $CFTR_{1281}$. To improve the trafficking of the truncated protein to the plasma membrane, the cells were previously treated with the VX-809 corrector.[40]. The screening revealed five active compounds, W1282Xpot-A15 (**4**, Figure 8), -C01 (**79**, Figure 9), -D01, -E01 and -B01 (chemical structures not reported), each one chemically distinct from VX-770. Given the relatively low activity of $CFTR_{1281}$ in response to the new potentiators, the authors postulated that combinations of compounds could show additive or perhaps synergistic effects. Indeed, they found that combination of VX-770 with W1282Xpot-A15, -C01, -D01, or -E01 strongly increased $CFTR_{1281}$ activity. In particular, W1282Xpot-A15 (**4**) acted synergistically with VX-770 (**3**) to increase $CFTR_{1281}$ function, ~ 8-fold over that of VX-770 alone. Obviously, these results revealed that VX-770 (**3**) and the new compounds have a different mechanism of action leading to the concept of "co-potentiator", i.e. molecules that in combination with a classical potentiator further enhance CFTR channel activity. Further studies were performed on 120 commercially available analogs of W1282Xpot-A15 (**4**) to investigate the structure-activity relationship (SAR) of the pyrrolo[2,3-*b*]pyridine chemical family.



4, W1282X$_{pot}$-A15/ASP-11

Figure 8. Chemical structure of W1282Xpot-A15/ASP-11 (**4**).

SAR analysis higlighted the following features to get the optimal activity:

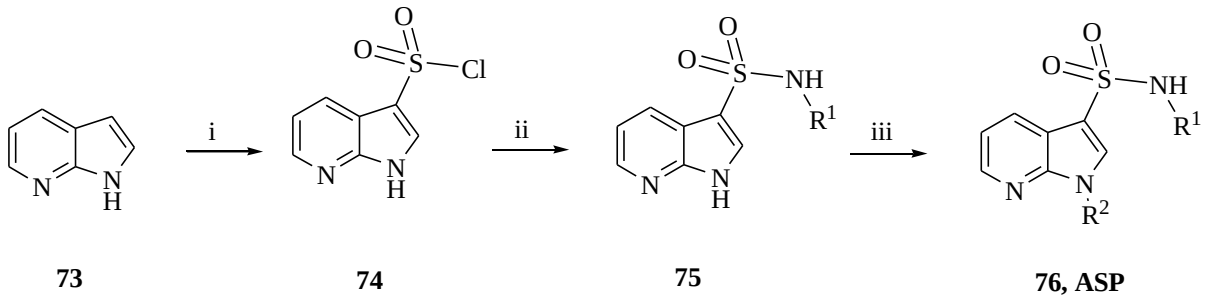
- In R^1 butyl, *n*-butyl, isobutyl groups are preferred. Shorter alkyl groups reduce the activity.
- Electron donating groups in R^2 improve activity (i.e. ethyl, methyl, methoxy). In this case *para* and *ortho* substitutions are preferred with respect to *meta* substitution.
- Electron withdrawing groups in R^2 reduce activity (i.e. chloro, fluoro)

Despite the good results of W1282Xpot-A15 (**4**) in cell lines, this compound lacked activity in nasal epithelial cells from a patient homozygous for the Trp1282X mutation.[40]

To look for further compounds with co-potentiator activity, the same research group carried out a dedicated "synergy screen" in which new compounds (16,000 small molecules) were tested in combination with VX-770 (**3**).[41] In this case, the screen was done on FRT cells expressing Asn1303Lys, a mutation characterized by severe trafficking and gating defects. The screening revealed compounds belonging to oxadiazolebenzamide, phenoxy-benzimidazole, and triazolocarboxamide classes as possible co-potentiators. However, none of these molecules had the potency and efficacy of W1282Xpot-A15 (**4**), which was renamed ASP-11. Indeed, ASP-11 (**4**) combined with VX-770 (**3**), increased Asn1303Lys-CFTR chloride currents 7-fold more than VX-770 (**3**) alone. Furthermore, ASP-11 (**4**) was also effective as co-potentiator on other CF mutations such as Gly551Asp and Phe508del. Importantly, ASP-11 (**4**) efficacy was confirmed in primary airway epithelial cells from patients with Asn1303Lys.[41] Structure-activity relationship studies on 11 synthesized ASP-11 analogs produced compounds with EC_{50} down to 0.5 μM.

To further investigate the co-potentiator structure-activity requirements, a synthetic pathway was optimized [41] to obtain diversification of the arylsulfonamide pyrrolopyridine (ASP) scaffold (Scheme 11). Starting from commercially available 1*H*-pyrrolo[2,3-*b*]pyridine **73** which was

converted into the corresponding 3-sulfonyl chloride and further reacted with several amines, sulfonamides ASP were obtained. The latter were subjected to N-alkylation of the pyrrole nitrogen giving the arylsulfonamide-pyrrolopyridines **ASP** (76).



73 **74** **75** **76, ASP**

Scheme 11. Reagents and conditions (i) Chlorosulfonic acid; (ii) R^1NH_2, TEA; (iii) R^2-halide, K_2CO_3, DMF

From a structure-activity relationship it was observed that substituents on the pyrrole ring strongly influence co-potentiator activity. In particular,

- The presence of a nitrogen atom in the bicyclic core is crucial for the activity. Its removal produces the loss of activity.
- Aryl substituted moieties bearing a *para* alkyl-substitution in R^1 appears to be best for activity (**76a** R^1= 4-Et Ph, R^2= 2-methylpyridine). The activity is lost in the presence of heterocyclic, aralkyl or aliphatic substituents.
- Benzyl, pyridine rings and short alkyl chain on the pyrrole nitrogen (R^2) are preferred to obtain the best activity **76b** (R^1= 4-Et Ph, R^2= 2-cyano benzyl EC_{50} 0.7 µM), **76a** (EC_{50} 0.6 µM), ASP-11(4) (R^1= 4-Et Ph, R^2= buthyl EC_{50} 1 µM). Longer alkyl chain reduced activity or heterocycle moieties produce inactive compounds.

Based on their encouraging previous data, Phuan and coll. [42] evaluated the activity of co-potentiators on other CFTR mutations that are still undruggable. The synergic activity of ASP-11 (4) with VX-770 (3) was studied in several minimal function CFTR mutants in transfected FRT cells. The analysis of short-circuit current response to modulators suggested that different missense, deletion, and truncation mutations located in the second nucleotide binding domain (NBD2) of CFTR generate proteins that are responsive to a co-potentiator therapy. The authors postulated that this combined action of potentiators and co-potentiators could be explained by a distinct binding site on the CFTR mutants, thus proposing a novel classification of potentiators to predict synergy: VX-770 (3), having a quinoline core structure, was defined as Class I potentiator, while the ASP-11 (4), an arysulfonamide-pyrrolopyridine compound, as a Class II potentiator. Based on this classification, different combinations of compounds were tested in cells expressing Asn1303Lys-

CFTR or Gln1313X-CFTR.[42] Presence/absence of synergistic effects when combined with VX-770 (**3**) or ASP-11 (**4**) allowed to categorize previously known potentiators (e.g. GLPG1837 and apigenin) as Class I or Class II compounds (Figure 9).

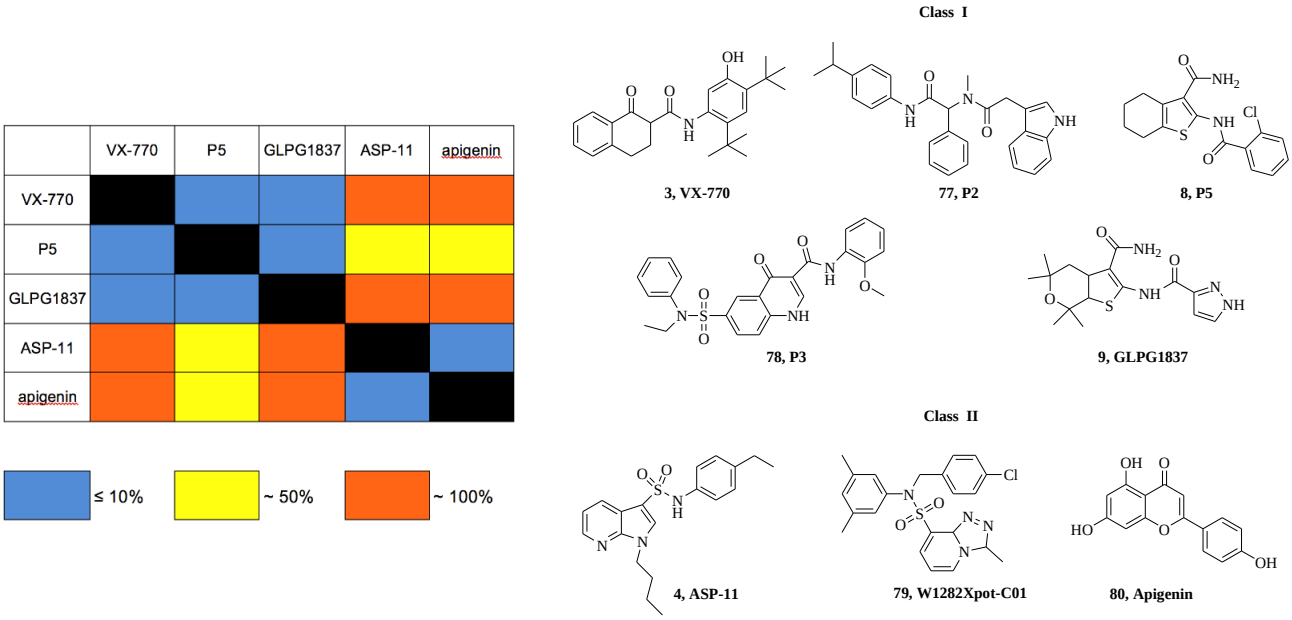
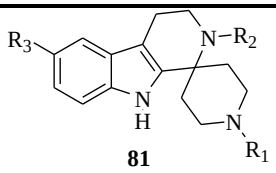
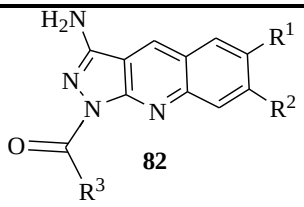


Figure 9. Class I and Class II potentiators.

To find novel co-potentiators scaffolds active on minimal function CFTR mutants, a new screening of 120,000 drug-like small molecules was performed using FRT cells stably expressing Trp1282X-CFTR. The screening identified 212 compounds giving channel activity >50% of that produced by the cocktail composed by forskolin, VX-770 (**3**) and 20 μM ASP-11 (**4**).[42] Subsequent secondary assays allowed selection of the 21 most active compounds, belonging to four different chemical classes: pyrazoloquinoline, piperidine-pyridoindole, tetrahydroquinoline and phenylazepine. These compounds showed EC_{50} values down to ~300 nM and worked in synergy with VX-770 (**3**) and other Class I potentiators to enhance CFTR function. To investigate SAR, 240 piperidine-pyridoindoles and 160 pyrazoloquinolines were purchased and evaluated on FRT cells expressing Trp1228X-CFTR. Three points of variability were considered for each scaffold R^1, R^2, R^3. As indicated in Table 3 some structural requirements appeared crucial for activity.

Table 3. SAR analysis of piperidine-pyridoindoles and pyrazoloquinolines. Activity was evaluated on FRT cells expressing Trp1282X-CFTR

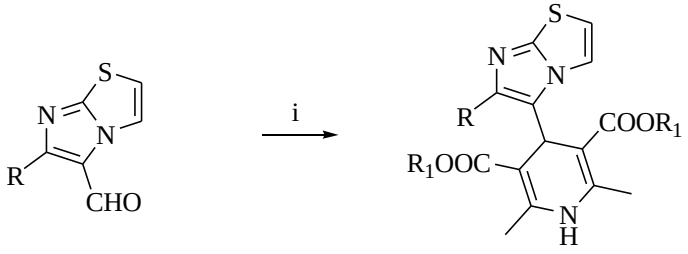
	 **81**	Effect on activity	 **82**	Effect on activity
R^1	**Bn-bisubstituted**	**optimal**	**OMe**	**optimal**
	Bn-monosubstituted	reduction	Me	reduction
	thiazole	reduction	H	reduction
	COPh	loss		
	SO_2NHR	loss		
	$CONH_2$	loss		
	Alkyl	loss		
	Cyclohexyl	loss		
R^2	**H**	**optimal**	**H**	**optimal**
	Me	loss	OMe	reduction
R^3	**OMe**	**optimal**	**2-thiophene quinoline**	optimal
	H	reduction	Ph 2,4-disubstituted	reduction
			Ph-monosubstituted	loss
			Bn	Loss
			pyridine	Loss

Overall, among piperidine-pyridoindoles **81** the best activity was obtained when R^1= 2,4-difluorobenzil or 3,4-difluorobenzil, R^2 = H and R^3 = OMe (EC_{50} 2.0-2.6 µM respectively). For the pyrazoloquinoline class of compounds **82** the most potent potentiator activity was obtained when R^1= OMe, R^2 = H and R^3= 2-thiophene quinoline (EC_{50} 0.3 µM) being one order of magnitude higher than derivatives with R^3 = 2-chloro-4-NO_2 benzyl or 2-chloro-4-F benzyl (EC_{50} 1.7-2.4 µM). In both cases the presence of an halo disubstituted aromatic moiety, in conjunction with an hydrogen and a methoxy group are required to achieve the best activity.

The activity of these new co-potentiators was also tested in 16HBE14o$^-$ human airway epithelial cells, in which the Asn1303Lys mutation was introduced in the endogenous CFTR locus by gene

editing, and in primary cultures of human bronchial epithelial cells from a Asn1303Lys homozygous CF subject. The addition of CP-A061 (**81** R^1= 2,4-difluoro-benzyl; R^2=H, R^3=OMe) or CP-D123 (**82** R^1= OMe, R^2=H, R^3=2-thiophene quinoline) enhanced CFTR channel activity by ~6 fold if compared to VX-770 alone. Although the putative binding site of Class II potentiators is still unknown, early *in silico* docking studies [43] showed that genistein, which is structurally similar to the Class II compound apigenin, binds at the NBD dimer interface. For this reason, Phuan and coll. reflected that this class of compounds is able to bind CFTR in a manner that stabilizes misfolded NBD2 structurally or thermodynamically.[42]

The 1,4-Dihydropyridines (DHPs, **84**) represent another class of CFTR potentiators. A DHP active on mutant CFTR was initially discovered in the same high-throughput screening that led to the identification of other potentiators.[16] Later, a screening done on a collection of approved drugs discovered that many DHPs used as anti-hypertensive agents, including felodipine, nitrendipine, nifedipine (structures not reported), were strong CFTR potentiators,[44] although at concentrations much higher than those active on their primary target, i.e. voltage dependent Ca^{2+} channels (VDCCs). In a subsequent study, a panel of 333 felodipine analogs was studied using functional assays detecting mutant CFTR and VDCC activity.[45] The goal was to improve the selectivity for CFTR. The results showed that alkyl substitutions at the para position of the 4-phenyl ring led to compounds with low activity on VDCCs and strong effect as potentiators on the Phe508del-, Gly551Asp-, and Gly1349Asp-CFTR. Further efforts were subsequently done to rescind CFTR potentiator activity from the Ca^{2+} channel blocking ability. The class of 4-imidazo[2,1-*b*]thiazole was explored for its potentiatior effect on Phe508del-CFTR and other mutations.[46] Starting from a previous study of the class of imidazo[2,1-*b*]thiazole derivatives, Budriesi and coll. investigated the structural features required for CFTR activation, screening a small set of thirty derivatives bearing the 1,4-dihydropyridine moiety at C-4. The most powerful compounds were obtained by the synthetic scheme summarized below (Scheme 12), by Hantzsch reaction starting from the proper aldehyde **83**.



83 **84, DHPs**

Scheme 12. Reagents and condition: (i) isopropanol, 30% NH_4OH, substituted acetoacetates

Structure activity relationship indicated some crucial structural requirements of the imidazo[2,1-*b*]core. In particular,

- The presence of 3- or 4-trifluoromethoxy(or methyl)-phenyl groups in the 6-position (R) produced the most effective compounds. The 2-substituted analogs in the same position are inactive.
- Replacement of the trifluoromethoxy-phenyl group with a methoxy produces loss of activity
- Aromaticity of the core system must be kept
- The presence of a 2-methyl substituent in the thiazole system increases the activity
- R^1= Me are optimal CFTR potentiator activity. More bulky groups decrease potency.

5. Thiophenes

Thiophenes represent an interesting class of molecules with the ability to potentiate mutant CFTR. An initial active compound was the tetrahydrobenzothiophene ΔF508$_{act}$-02, later named P5 (**8**, Figure 10), which was discovered by high-throughput screening of a highly diverse chemical library containing 100,000 compounds.[16] P5 (**8**) had a good potency and efficacy on Phe508del-CFTR but was poorly effective on the Gly551Asp mutant. The interest on P5 (**8**) was renewed by a study published in 2014.[47] In this study, it was found that P5 (**8**) had a positive feature that was advantageous for corrector/potentiator combinations. Indeed, while ivacaftor appeared to decrease the efficacy of correctors,[47,48] P5 (**8**) did not had this interfering effect.[47]

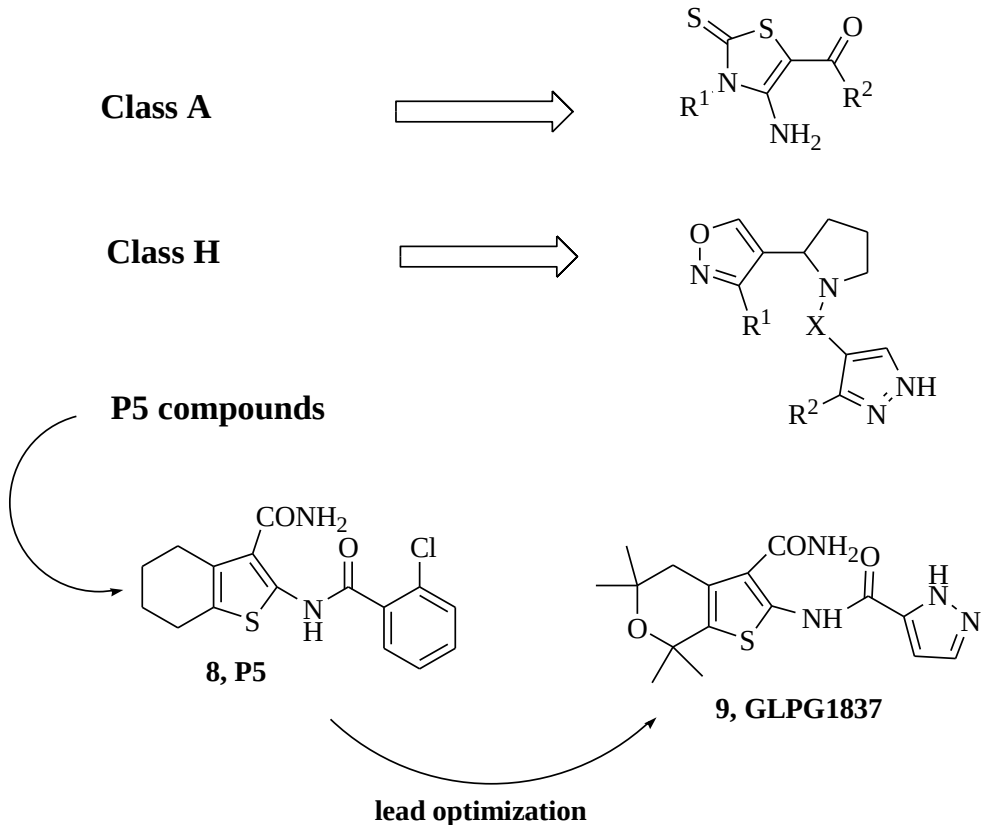
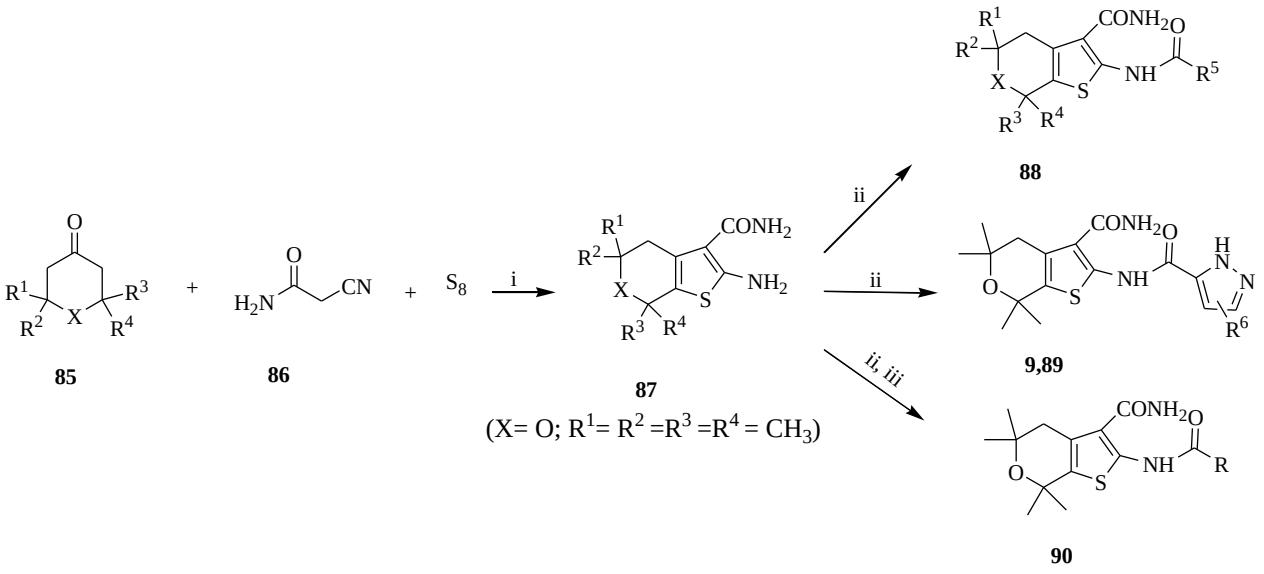


Figure 10. Identification of GLPG1837 (**9**).

The positive features of P5 (**8**) inspired a subsequent study that looked for potentiators particularly suited for combinations with correctors.[49] The study was based on the screening of a wide library of 60,000 small molecules and of a selected panel of 273 analogs of P5. The 50 most active compounds were counter-screened using an assay measuring Phe508del-CFTR at the cell surface in order to find potentiators devoid of interference with correctors. Three classes of compounds were identified: 2-thioxo-4-aminothiazoles (class A), pyrazole-pyrrole-isoxazoles (class H), and P5 analogs (Figure 10). In the latter class, compound G01 bearing a ciclohexyl amido moiety in α-position to the sulphur structurally related to P5 (**8**) was one of the most active compounds.[49] Overall benzothiophene structural determinants for the activity were:

- The presence of a six-membered ring rather than a five- or seven-membered which were less active
- The primary carboxyamide group ($CONH_2$) was crucial for the activity. Its replacement with cyano, ester groups or substituted anilines produced loss of activity
- The presence of a substituent on the tetrahydrobenzo ring was dendrimental for activity
- Replacement of 2-chlorobenzene with 2-fluorobenzene or chloro-, methoxy- and nitro-phenyl groups were tolerated, giving EC_{50} values similar or better than P5 (**8**).

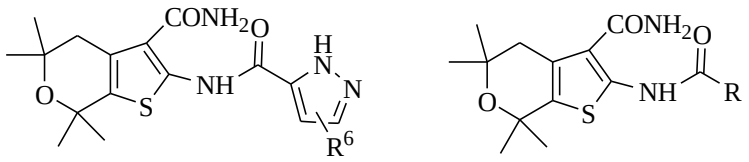
In another study, P5 **(8)** was also the starting point for the discovery of GLPG1837 (N-(3-carbamoyl-5,5,7,7-tetramethyl-4H-thieno[2,3-c]pyran-2-yl)-1H-pyrazole-5-carboxamide) **(9)**, one of the most potent and effective potentiators available so far.[50] This study initially evaluated 589 commercial compounds structurally related to existing potentiators: genistein, sulfonamide SF-01 **(7)**, ivacaftor **(3)**, PG-01 **(1)**, VRT-532 **(6)** and P5 **(8)** (Figure 1). The most active compounds arising from the screening all belonged to the class of P5 analogs. The authors of the study considered that the original P5 **(8)** had a good potency on Phe508-CFTR (326 nM) but it was largely ineffective on Gly551Asp-CFTR. Furthermore, due to its lipohilic nature (XLogP3 > 4), P5 suffered of high unbound clearance in rat liver microsomal stability assay. Therefore, Van der Plas and coll. worked at the optimization of P5 **(8)** to improve its potentiator and MedChem properties.[50] Considering that the thiophene core can be easily accessed through Gewald reaction, chemical modification of the hit scaffold was planned by changing either the starting ketone and/or the side amido chain (Scheme 13).



Scheme 13. Synthesis of thiophene derivatives **9**,**88-90**. Reagents and conditions: (i) Diethylamine, EtOH; (ii) R^5COOH (for derivatives **88**), 2*H*-pyrazole-3-carboxylic acid (for derivatives **9,89**), RCOOH (for derivatives **90**), 2-chloro-1-methylpyridinium iodide, DMAP, TEA, CH_2Cl_2; (iii) $LiBH_4$, THF.

In order to improve polarity, heteroatoms were introduced into the cyclohexyl ring. In particular, when it was replaced with a cyclic amine (X=NH), a loss in the activity was observed. On the contrary, introduction of an oxygen atom (X=O), led to an increase in efficacy not only on Phe508del-CFTR but also on Gly551Asp-CFTR, particularly in the presence of bulky groups next to the oxygen. Therefore, the tetramethyl-tetrahydropyran (THP) (X=O, R^1=R^2=R^3=R^4=CH_3) core

was further explored, modifying the amide moiety. From a broad amide SAR exploration it was pointed out that groups containing a proton donor were consistently more active and two subseries containing the tetramethyl pyrano moiety, emerged as very promising, namely the pyrazoles **9**,**89** and alcohols **90** (Figure 11).



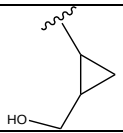
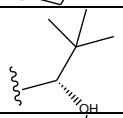
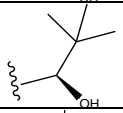
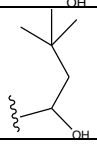
9,89 **90**

Figure 11. Tetramethylpyrano pyrazole and alcohol subseries **9**,**89** and **90**.

Pyrazole derivatives **9,89** were synthesized according to the general procedure reported in Scheme 13, starting from the proper 2*H*-pyrazole-3-carboxylic acid which was coupled with the amino intermediate **87** (X=O, R^1=R^2=R^3=R^4=CH_3) in the presence of Mukaiyama reagent.

For the synthesis of compounds **90**, the alcohol function needed to be masked to guarantee a successful amide coupling. Thus the amino intermediate **87** (X=O, R^1=R^2=R^3=R^4=CH_3) was coupled with the corresponding ketone or ester, followed by reduction with $LiBH_4$. Alternatively, the alcohol had to be protected before the amide coupling.

Table 4. Pyrazoles and alcohols structure-activity relationship.

CPD	R^6	F580del EC_{50} % activity	G551D EC_{50} % activity	CPD	R	F580del EC_{50} % activity	G551D EC_{50} % activity
9	H	3 nM 105	339 nM 103	**90a**		15 nM 105	1,320 nM 57
89a	4-Me	2 nM 96	123 nM 83	**90b**		2.7 nM 95	421 nM 50
89b	3-CF_3	3 nM 107	130 nM 49	**90c**		127 nM 95	7,777 nM 31
89c	3-Me	13 nM 81	590 nM 73	**90d**		0.54 nM 102	981 nM 58
89d	3-Me,4-Cl	11 nM 92	199 nM 68				
89e	4-Cl	2 nM 96	81 nM 77				

An overview on the structure activity relationship around the pyrazole and alcohol derivatives **9**,**89**,**90** is reported in Table 4. According to the percent activation, a measure for the extent or efficacy of channel opening, the alcohol subseries was less effective in opening Gly551Asp-CFTR with respect to Phe508del mutant. In fact, the best compound, **90d**, had EC_{50} value of 981 nM on Gly551Asp, but it was 3 orders of magnitude more potent on Phe508del (EC_{50}= 0.54 nM). The potency was also influenced by the chirality, since the *S* enantiomer **90b** was 47 and 18 times more potent on Phe508del- and GlyG551Asp-CFTR respectively, than its *R* isomer **90c**.

Concerning the pyrazole series, substitution on the pyrazole moiety either on the 3 or 4 position improved the potency for Gly551Asp-CFTR (2-4-fold), but resulted in a loss of efficacy. No benefit was obtained on Phe508del-CFTR. A double substitution, as in the case of **89d**, resulted in a similar profile as the mono-substituted pyrazoles.

The most interesting derivative proved to be **9**, with EC_{50} of 3 and 339 nM on Phe508del- and Gly551Asp-CFTR, respectively and being twice as efficacious as ivacaftor in opening the Gly551Asp-CFTR in the YFP halide assay in HEK293 cells.

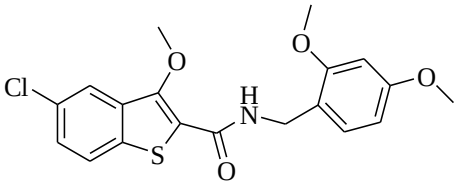
In comparison to ivacaftor (**3**), **9** showed an improved potency and efficacy on human airway epithelial cells from CF patients with various class mutations. Besides the classical Gly551Asp, **9** was also effective on Gly178Arg (158%), Ser549Asn (143%), and Arg117His (119%).

Even if derivative **9** had an attractive in vitro ADME profile, it suffered of low solubility, which could not be attributed to lack of polarity and poor solvation, since its calculated lipophilicity was 1.8. The crystal lattice seemed to be stabilized by strong intermolecular hydrogen bonds originating from the carboxamide and the pyrazole ring since both groups are a donor-acceptor pair in terms of hydrogen bonding. Moreover, the X-ray analysis showed extensive intramolecular bonding. In order to improve the solubility, **9** was converted into a phosphate prodrug (R^6 = $CH_2PO_4.Na_2$). It was selectively cleaved in the gut by alkaline phosphatases, at the brush border of the epithelial cells efficiently releasing the parent drug.

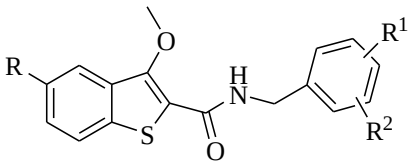
In a parallel effort, various solid state formulations were explored and, in the end, similar results as those obtained with the phosphate prodrug were obtained with a nano suspension of **9** which was nominated as **GLPG1837** and selected for preclinical safety evaluation. It was then progressed into a phase I study to evaluate its PK and safety in humans. **GLPG1837** (**9**) was generally safe and well tolerated in healthy volunteers, with single doses up to 2000 mg and 14-day dosing up to 800 mg b.i.d. The efficacy was explored in two phase II studies the SAPHIRA I and SAPHIRA II.

Thiophenes moiety emerged again in a study that was specifically focused on the NBD1 domain of CFTR.[51] Instead of using the whole CFTR protein, the authors designed a special construct

consisting of NBD1, carrying the Phe508del mutation, fused to CD4 transmembrane domain and an extracellular horseradish peroxidase (HRP) reporter. Using luminescence, a library of 20,000 compounds was screened to find novel correctors. Unexpectedly, the active compounds arising from the screening were inactive as correctors of the full length Phe508del-CFTR but acted as potentiators. In particular, the benzocondensed thiophene derivative A-01 (**91**) emerged as interesting lead (Figure 10). This compound increased chloride current at low micromolar concentration with a maximal effect (reached at 30 μM) that was ~50% of that produced by VX-770 (**3**). Moreover it selectively potentiated full-length Phe508del-CFTR but not wild-type or Gly551Asp-CFTR, thus indicating a specific interaction with the Phe508del-NBD1 topological and electrostatic surfaces and not with those of wild-type or Gly551Asp-CFTR.



A-01, 91



92a R^1= 2-Br R^2= H
92b R^1= 2-Cl R^2= 4-F
92c R^1= 3-OCH_3 R^2= 5-OCH_3

Figure 12. Carboxamide-benzothiophenes **91**,**92**.

Three additional carboxamide-benzothiophenes **92a-c** were identified from a further screening of 120 commercially available A-01 analogs (Figure 12). When tested in cells expressing Phe508del-NBD1-CD4-HRP reported, they showed efficacy, with 2.4-, 2.0-, and 1.7-fold increased luminescence signal, respectively, over the control vehicle-treated cells. These compounds were confirmed as potentiators in short-circuit current recordings on full-length Phe508del-CFTR potentiator assay. The three analogs **92a-c** showed activity at micromolar levels.[51]

6. Conclusions

From the initial evidence that mutant CFTR activity could be stimulated with drug-like small molecules, an extensive work by industry and academic institutions has led to the development of highly potent and effective potentiators. Despite the large number of active compounds discovered in academic laboratories, none of them has been advanced to clinical trial stage. Actually, the only potentiator that has been approved so far for clinical use, ivacaftor (VX-770), was developed by

Vertex Pharmaceuticals, a pharmaceutical industry. A second promising potentiator, GLPG1837, has also been developed by a pharmaceutical industry (Galapagos). but it is still being evaluated in clinical studies.

Ivacaftor, the active principle of Kalydeco, a drug that has been approved for a large list of CF mutations causing channel-gating defect, is also one of the ingredients of the triple drug combination that is highly effective on patients with Phe508del mutation. Interestingly, the discovery of ivacaftor has been achieved without knowing the CFTR 3D structure and the site/mechanism of action of potentiators. Without this knowledge, it has not been possible to proceed with a rational design of drugs. Therefore, the optimization of potentiator activity has been guided by the results of functional assays done on cells expressing mutant CFTR. Amazingly, it is now possible to do the reverse process, i.e. to use the most potent potentiators as molecular probes to identify their site of action on CFTR protein. In this respect, a recent study reported the binding site of ivacaftor and of GLPG1837.[52] Using cryo-EM technique, the structure of the complex formed by CFTR with these two potentiators was determined. Interestingly, this approach identified a common binding site for the two molecules, localized at the lipid-protein interface, within a cleft formed by transmembrane helices 4, 5, and 8. Also the type and strength of interactions of ivacaftor and its analogs with the binding site at the CFTR protein, revealed by molecular docking, explained well the structure-activity relationship obtained with functional data.[52] To explain the mechanism of action of potentiators, it was postulated that these molecules interact at the binding site with a hinge that it is important for CFTR gating. This interaction stabilizes CFTR in the open channel conformation. The identification of the potentiator binding site will give the opportunity to explore the possibility to design molecules endowed with even higher potency and efficacy. We expect that this site will be different from that of other CFTR modulators such as co-potentiators and correctors. Therefore, additional studies are needed to discover how these molecules bind to CFTR and the basis for synergy or antagonism.

Acknowledgments

We thank Fondazione Italiana per la Ricerca sulla Fibrosi Cistica for supporting our work (grant FFC #4/2018)

List of abbreviations

EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
DMF	N,N-Dimethylformamide

TFA	Trifluoroacetic acid
NaHMDS	Sodium bis(trimethylsilyl)amide
THF	Tetrahydrofuran
DIAD	Diisopropyl azodicarboxylate
DMAP	4-(Dimethylamino)pyridine
HBTU	N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
T3P	1-Propanephosphonic anhydride
DIPEA	N,N-Diisopropylethylamine
TEA	Triethylamine

REFERENCES

[1] J.S. Elborn, Cystic fibrosis, Lancet. 388 (2016) 2519–2531. https://doi.org/10.1016/S0140-6736(16)00576-6.

[2] www.cftr2.org.

[3] J.L. Bobadilla, M. Macek, J.P. Fine, P.M. Farrell, Cystic fibrosis: A worldwide analysis of CFTR mutations - Correlation with incidence data and application to screening, Hum. Mutat. 19 (2002) 575–606. https://doi.org/10.1002/humu.10041.

[4] www.ecfs.eu/projects/ecfs-patient-registry/annual-reports.

[5] G.L. Lukacs, A.S. Verkman, CFTR: Folding, misfolding and correcting the ΔF508 conformational defect, Trends Mol. Med. 18 (2012) 81–91. https://doi.org/10.1016/j.molmed.2011.10.003.

[6] G. Veit, R.G. Avramescu, A.N. Chiang, S.A. Houck, Z. Cai, K.W. Peters, J.S. Hong, H.B. Pollard, W.B. Guggino, W.E. Balch, W.R. Skach, G.R. Cutting, R.A. Frizzell, D.N. Sheppard, D.M. Cyr, E.J. Sorscher, J.L. Brodsky, G.L. Lukacs, From CFTR biology toward combinatorial pharmacotherapy: Expanded classification of cystic fibrosis mutations, Mol. Biol. Cell. 27 (2016) 424–433. https://doi.org/10.1091/mbc.E14-04-0935.

[7] B.S. Kerem, J.M. Rommens, J.A. Buchanan, D. Markiewicz, T.K. Cox, A. Chakravarti, M. Buchwald, L.C. Tsui, Identification of the cystic fibrosis gene: genetic analysis, Science (80). 245 (1989) 1073–1080. https://doi.org/10.1126/science.2570460.

[8] B. Illek, L. Zhang, N.C. Lewis, R.B. Moss, J. Dong, H. Fischer, Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein, Am. J. Physiol. 277 (1999) C833–C839. https://doi.org/10.1152/ajpcell.1999.277.4.C833.

[9] M.L. Drumm, D.J. Wilkinson, L.S. Smit, R.T. Worrell, T. V. Strong, R.A. Frizzell, D.C. Dawson, F.S. Collins, Chloride conductance expressed by ΔF508 and other mutant CFTRs in Xenopus oocytes, Sci. (Washington, D.C.). 254 (1991) 1797–1799. https://doi.org/10.1126/science.1722350.

[10] V.K. Gribkoff, G. Champigny, P. Barbry, S.I. Dworetzky, N.A. Meanwell, M. Lazdunski, The substituted benzimidazolone NS004 is an opener of the cystic fibrosis chloride channel,

J. Biol. Chem. 269 (1994) 10983–10986.

[11] G.M. Denning, M.P. Anderson, J.F. Amara, J. Marshall, A.E. Smith, M.J. Welsh, Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive, Nat. (London, United Kingdom). 358 (1992) 761–764. https://doi.org/10.1038/358761a0.

[12] S. Sato, C.L. Ward, M.E. Krouse, J.J. Wine, R.R. Kopito, Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation, J. Biol. Chem. 271 (1996) 635–638. https://doi.org/10.1074/jbc.271.2.635.

[13] F. Becq, Y. Mettey, M.A. Gray, L.J.V. Galietta, R.L. Dormer, M. Merten, T. Métayé, V. Chappe, C. Marvingt-Mounir, O. Zegarra-Moran, R. Tarran, L. Bulteau, R. Dérand, M.M.C. Pereira, M.A. McPherson, C. Rogier, M. Joffre, B.E. Argent, D. Sarrouilhe, W. Kammouni, C. Figarella, B. Verrier, M. Gola, J.M. Vierfond, Development of substituted benzo[c]quinolizinium compounds as novel activators of the cystic fibrosis chloride channel, J. Biol. Chem. 274 (1999) 27415–27425. https://doi.org/10.1074/jbc.274.39.27415.

[14] L.J.V. Galietta, M.F. Springsteel, M. Eda, E.J. Niedzinski, K. By, M.J. Haddadin, M.J. Kurth, M.H. Nantz, A.S. Verkman, Novel CFTR Chloride Channel Activators Identified by Screening of Combinatorial Libraries Based on Flavone and Benzoquinolizinium Lead Compounds, J. Biol. Chem. 276 (2001) 19723–19728. https://doi.org/10.1074/jbc.M101892200.

[15] M. Tonghui, L. Vetrivel, H. Yang, N. Pedemonte, O. Zegarra-Moran, L.J.V. Galietta, A.S. Verkman, High-affinity activators of cystic fibrosis transmembrane conductance regulator (CFTR) chloride conductance identified by high-throughput screening, J. Biol. Chem. 277 (2002) 37235–37241. https://doi.org/10.1074/jbc.M205932200.

[16] H. Yang, A.A. Shelat, R.K. Guy, V.S. Gopinath, T. Ma, K. Du, G.L. Lukacs, A. Taddei, C. Folli, N. Pedemonte, L.J.V. Galietta, A.S. Verkman, Nanomolar affinity small molecule correctors of defective DF508-CFTR chloride channel gating, J. Biol. Chem. 278 (2003) 35079–35085. https://doi.org/10.1074/jbc.M303098200.

[17] N. Pedemonte, N.D. Sonawane, A. Taddei, J. Hu, O. Zegarra-Moran, Y.F. Suen, L.I. Robins, C.W. Dicus, D. Willenbring, M.H. Nantz, M.J. Kurth, L.J.V. Galietta, A.S. Verkman, Phenylglycine and Sulfonamide Correctors of Defective DeltaF508 and G551D Cystic Fibrosis Transmembrane Conductance, Mol. Pharmacol. 67 (2005) 1797–1807. https://doi.org/10.1124/mol.105.010959.membrane.

[18] F. Van Goor, K.S. Straley, D. Cao, J. González, S. Hadida, A. Hazlewood, J. Joubran, T. Knapp, L.R. Makings, M. Miller, T. Neuberger, E. Olson, V. Panchenko, J. Rader, A. Singh, J.H. Stack, R. Tung, P.D.J. Grootenhuis, P. Negulescu, Rescue of ΔF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules, Am. J. Physiol. - Lung Cell. Mol. Physiol. 290 (2006) L1117–L1130. https://doi.org/10.1152/ajplung.00169.2005.

[19] N. Pedemonte, L.J.V. Galietta, A.S. Verkman, N. Pedemonte, G.L. Lukacs, K. Du, E. Caci, Small-molecule correctors of defective ΔF508- CFTR cellular processing identified by high-throughput screening, J Clin Invest. 115 (2005) 2564–2571. https://doi.org/10.1172/JCI24898.2564.

[20] F. Van Goor, S. Hadida, P.D.J. Grootenhuis, B. Burton, J.H. Stack, K.S. Straley, C.J. Decker, M. Miller, J. McCartney, E.R. Olson, J.J. Wine, R.A. Frizzell, M. Ashlock, P.A. Negulescu, Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18843–18848.

https://doi.org/10.1073/pnas.1105787108.

[21] F. Van Goor, S. Hadida, P.D.J. Grootenhuis, B. Burton, D. Cao, T. Neuberger, A. Turnbull, A. Singh, J. Joubran, A. Hazlewood, J. Zhou, J. McCartney, V. Arumugam, C. Decker, J. Yang, C. Young, E.R. Olson, J.J. Wine, R.A. Frizzell, M. Ashlock, P. Negulescu, Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 18825–18830. https://doi.org/10.1073/pnas.0904709106.

[22] B.W. Ramsey, J. Davis, G. McElvaney, E. Tullis, S.C. Bell, P. Dievinek, M. Griese, E.F. McKone, C.E. Wainwright, M.W. Konstan, R. Moss, F. Ratjen, I. Sermet-Gaudelus, S.M. Rowe, Q. Dong, S. Rodriguez, K. Yen, C. Ordonez, J.S. Elborn, A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation, N. Engl. J. Med. 365 (2011) 1663–1672. https://doi.org/10.1056/NEJMoa1105185.

[23] C.E. Wainwright, J.S. Elborn, B.W. Ramsey, G. Marigowda, X. Huang, M. Cipolli, C. Colombo, J.C. Davies, K. De Boeck, P.A. Flume, M.W. Konstan, S.A. McColley, K. McCoy, E.F. McKone, A. Munck, F. Ratjen, S.M. Rowe, D. Waltz, M.P. Boyle, Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for phe508del CFTR, N. Engl. J. Med. 373 (2015) 220–231. https://doi.org/10.1056/NEJMoa1409547.

[24] D. Keating, G. Marigowda, L. Burr, C. Daines, M.A. Mall, E.F. McKone, B.W. Ramsey, S.M. Rowe, L.A. Sass, E. Tullis, C.M. McKee, S.M. Moskowitz, S. Robertson, J. Savage, C. Simard, F. Van Goor, D. Waltz, F. Xuan, T. Young, J.L. Taylor-Cousar, VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles, N. Engl. J. Med. 379 (2018) 1612–1620. https://doi.org/10.1056/NEJMoa1807120.

[25] L.J.V. Galietta, P.M. Haggie, A.S. Verkman, Green fluorescent protein-based halide indicators with improved chloride and iodide affinities, FEBS Lett. 499 (2001) 220–224. https://doi.org/10.1016/S0014-5793(01)02561-3.

[26] H.R. Davison, D.M. Solano, P.W. Phuan, A.S. Verkman, M.J. Kurth, Fluorinated DF508-CFTR correctors and potentiators for PET imaging, Bioorg. Med. Chem. Lett. 22 (2012) 1602–1605. https://doi.org/10.1016/j.bmcl.2011.12.128.

[27] W.K. Hagmann, The many roles for fluorine in medicinal chemistry, J. Med. Chem. 51 (2008) 4359–4369. https://doi.org/10.1021/jm800219f.

[28] A.D. Mills, C. Yoo, J.D. Butler, B. Yang, A.S. Verkman, M.J. Kurth, Design and synthesis of a hybrid potentiator-corrector agonist of the cystic fibrosis mutant protein ΔF508-CFTR, Bioorg. Med. Chem. Lett. 20 (2010) 87–91. https://doi.org/10.1016/j.bmcl.2009.11.020.

[29] J.Y. Gui, C.L. Yoo, B. Yang, M.W. Lodewyk, L. Meng, T.T. El-Idreesy, J.C. Fettinger, D.J. Tantillo, A.S. Verkman, M.J. Kurth, Potent s-cis-locked bithiazole correctors of ΔF508 cystic fibrosis transmembrane conductance regulator cellular processing for cystic fibrosis therapy, J. Med. Chem. 51 (2008) 6044–6054. https://doi.org/10.1021/jm800533c.

[30] S. Hadida, F. Van Goor, J. Zhou, V. Arumugam, J. McCartney, A. Hazlewood, C. Decker, P. Negulescu, P.D.J. Grootenhuis, Discovery of N-(2,4-Di- tert -butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770, Ivacaftor), a potent and orally bioavailable CFTR potentiator, J. Med. Chem. 57 (2014) 9776–9795. https://doi.org/10.1021/jm5012808.

[31] D.J. Kushner, A. Baker, T.G. Dunstall, Pharmacological uses and perspectives of heavy water and deuterated compounds, Can. J. Physiol. Pharmacol. 77 (1999) 79–88. https://doi.org/10.1139/y99-005.

[32] S.D. Nelson, W.F. Trager, The use of deuterium isotope effects to probe the active site

properties, mechanism of cytochrome P450-catalyzed reactions, and mechanisms of metabolically dependent toxicity, Drug Metab. Dispos. 31 (2003) 1481–1498. https://doi.org/10.1124/dmd.31.12.1481.

[33] S.L. Harbeson, R.D. Tung, Deuterium in Drug Discovery and Development, 1st ed., Elsevier Inc., 2011. https://doi.org/10.1016/B978-0-12-386009-5.00003-5.

[34] S.L. Harbeson, A.J. Morgan, J.F. Liu, A.M. Aslanian, S. Nguyen, G.W. Bridson, C.L. Brummel, L. Wu, R.D. Tung, L. Pilja, V. Braman, V. Uttamsingh, Altering metabolic profiles of drugs by precision deuteration 2: Discovery of a deuterated analog of ivacaftor with differentiated pharmacokinetics for clinical development, J. Pharmacol. Exp. Ther. 362 (2017) 359–367. https://doi.org/10.1124/jpet.117.241497.

[35] A.J. Morgan, Deuterated CFTR Potentiators, US 9,181,192 B2, 2015.

[36] J. Zha, J. Zhang, C. Ordonez, Pharmacokinetics (PK), Metabolism and Excretion of [14C] VX-770, a Potentiator of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), in Healthy Subjects, in: J. Clin. Pharmacol., 2011: pp. 1358–1359. https://doi.org/10.1177/0091270010418046.

[37] J.E. Doiron, C.A. Le, B.K. Ody, J.B. Brace, S.J. Post, N.L. Thacker, H.M. Hill, G.W. Breton, M.J. Mulder, S. Chang, T.M. Bridges, L. Tang, W. Wang, S.M. Rowe, S.G. Aller, M. Turlington, Evaluation of 1,2,3-Triazoles as Amide Bioisosteres In Cystic Fibrosis Transmembrane Conductance Regulator Modulators VX-770 and VX-809, Chem. - A Eur. J. 25 (2019) 3662–3674. https://doi.org/10.1002/chem.201805919.

[38] P.W. Phuan, B. Yang, J.M. Knapp, A.B. Wood, G.L. Lukacs, M.J. Kurth, A.S. Verkman, Cyanoquinolines with independent corrector and potentiator activities restore ΔPhe508-cystic fibrosis transmembrane conductance regulator chloride channel function in cystic fibrosis, Mol. Pharmacol. 80 (2011) 683–693. https://doi.org/10.1124/mol.111.073056.

[39] J.M. Knapp, A.B. Wood, P.W. Phuan, M.W. Lodewyk, D.J. Tantillo, A.S. Verkman, M.J. Kurth, Structure-activity relationships of cyanoquinolines with corrector-potentiator activity in Δf508 cystic fibrosis transmembrane conductance regulator protein, J. Med. Chem. 55 (2012) 1242–1251. https://doi.org/10.1021/jm201372q.

[40] P.M. Haggie, P.W. Phuan, J.A. Tan, H. Xu, R.G. Avramescu, D. Perdomo, L. Zlock, D.W. Nielson, W.E. Finkbeiner, G.L. Lukacs, A.S. Verkman, Correctors and potentiators rescue function of the truncated W1282X-Cystic Fibrosis Transmembrane Regulator (CFTR) translation product, J. Biol. Chem. 292 (2017) 771–785. https://doi.org/10.1074/jbc.M116.764720.

[41] P.W. Phuan, J.H. Son, J.A. Tan, C. Li, I. Musante, L. Zlock, D.W. Nielson, W.E. Finkbeiner, M.J. Kurth, L.J. Galietta, P.M. Haggie, A.S. Verkman, Combination potentiator (‘co-potentiator’) therapy for CF caused by CFTR mutants, including N1303K, that are poorly responsive to single potentiators, J. Cyst. Fibros. 17 (2018) 595–606. https://doi.org/10.1016/j.jcf.2018.05.010.

[42] P.W. Phuan, J.A. Tan, A.A. Rivera, L. Zlock, D.W. Nielson, W.E. Finkbeiner, P.M. Haggie, A.S. Verkman, Nanomolar-potency ‘co-potentiator’ therapy for cystic fibrosis caused by a defined subset of minimal function CFTR mutants, Sci. Rep. 9 (2019) 1–12. https://doi.org/10.1038/s41598-019-54158-2.

[43] O. Moran, L.J.V. Galietta, O. Zegarra-Moran, Binding site of activators of the cystic fibrosis transmembrane conductance regulator in the nucleotide binding domains, Cell. Mol. Life Sci. 62 (2005) 446–460. https://doi.org/10.1007/s00018-004-4422-3.

[44] N. Pedemonte, T. Diena, E. Caci, E. Nieddu, M. Mazzei, R. Ravazzolo, O. Zegarra-Moran, L.J.V. Galietta, Antihypertensive 1,4-dihydropyridines as correctors of the cystic fibrosis transmembrane conductance regulator channel gating defect caused by cystic fibrosis mutations, Mol. Pharmacol. 68 (2005) 1736–1746. https://doi.org/10.1124/mol.105.015149.

[45] N. Pedemonte, D. Boido, O. Moran, M. Giampieri, M. Mazzei, R. Ravazzolo, L.J.V. Galietta, Structure-activity relationship of 1,4-dihydropyridines as potentiators of the cystic fibrosis transmembrane conductance regulator chloride channel, Mol. Pharmacol. 72 (2007) 197–207. https://doi.org/10.1124/mol.107.034702.

[46] R. Budriesi, P. Ioan, A. Leoni, N. Pedemonte, A. Locatelli, M. Micucci, A. Chiarini, L.J.V. Galietta, Cystic fibrosis: A new target for 4-imidazo[2,1-b]thiazole-1, 4-dihydropyridines, J. Med. Chem. 54 (2011) 3885–3894. https://doi.org/10.1021/jm200199r.

[47] G. Veit, R.G. Avramescu, D. Perdomo, P.W. Phuan, M. Bagdany, P.M. Apaja, F. Borot, D. Szollosi, Y.S. Wu, W.E. Finkbeiner, T. Hegedus, A.S. Verkman, G.L. Lukacs, Cystic fibrosis: Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression, Sci. Transl. Med. 6 (2014) 246ra97. https://doi.org/10.1126/scitranslmed.3008889.

[48] D.M. Cholon, N.L. Quinney, M.L. Fulcher, C.R. Esther, J. Das, N.V. Dokholyan, S.H. Randell, R.C. Boucher, M. Gentzsch, Cystic fibrosis: Potentiator ivacaftor abrogates pharmacological correction of ΔF508 CFTR in cystic fibrosis, Sci. Transl. Med. 6 (2014) 246ra96. https://doi.org/10.1126/scitranslmed.3008680.

[49] P.W. Phuan, G. Veit, J.A. Tan, W.E. Finkbeiner, G.L. Lukacs, A.S. Verkman, Potentiators of defective DF508-CFTR gating that do not interfere with corrector action, Mol. Pharmacol. 88 (2015) 791–799. https://doi.org/10.1124/mol.115.099689.

[50] S.E. Van Der Plas, H. Kelgtermans, T. De Munck, S.L.X. Martina, S. Dropsit, E. Quinton, A. De Blieck, C. Joannesse, L. Tomaskovic, M. Jans, T. Christophe, E. Van Der Aar, M. Borgonovi, L. Nelles, M. Gees, P. Stouten, J. Van Der Schueren, O. Mammoliti, K. Conrath, M. Andrews, Discovery of N-(3-Carbamoyl-5,5,7,7-tetramethyl-5,7-dihydro-4H-thieno[2,3-c]pyran-2-yl)-lH-pyrazole-5-carboxamide (GLPG1837), a Novel Potentiator Which Can Open Class III Mutant Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Channels to a High Extent, J. Med. Chem. 61 (2018) 1425–1435. https://doi.org/10.1021/acs.jmedchem.7b01288.

[51] P.W. Phuan, G. Veit, J.A. Tan, A. Roldan, W.E. Finkbeiner, P.M. Haggie, G.L. Lukacs, A.S. Verkman, ΔF508-CFTR Modulator Screen Based on Cell Surface Targeting of a Chimeric Nucleotide Binding Domain 1 Reporter, SLAS Discov. 23 (2018) 823–831. https://doi.org/10.1177/2472555218763310.

[52] F. Liu, Z. Zhang, A. Levit, J. Levring, K.K. Touhara, B.K. Shoichet, J. Chen, Structural identification of a hotspot on CFTR for potentiation, Science (80). 364 (2019) 1184–1188. https://doi.org/10.1126/science.aaw7611.