Ivacaftor (VX-770)

Subjects: Medicine, Research & Experimental

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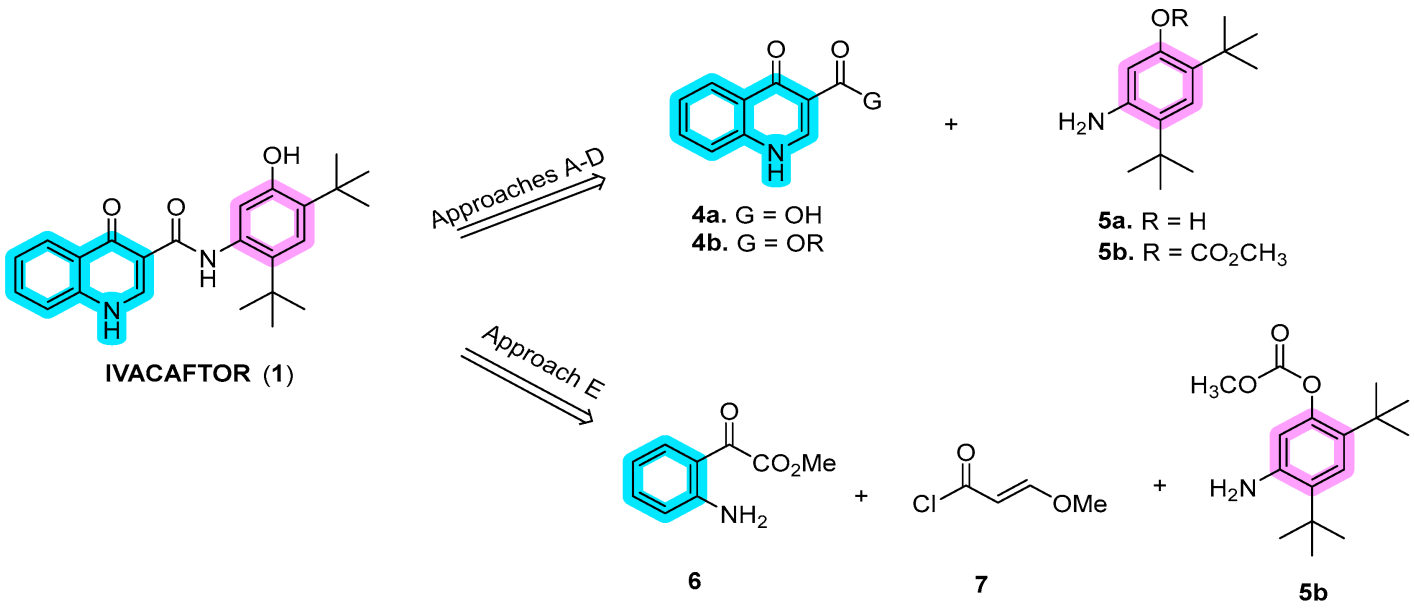
Ivacaftor possesses a molecular structure characterized by the presence of an *N*-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydro-quinoline-3-carboxamide moiety. The quinolone scaffold within its composition is a crucial pharmacophore, significantly influencing drug discovery. This scaffold holds prominence as one of the primary classes of nitrogen-containing heterocycles found in various biologically active compounds and blockbuster drugs, as highlighted in the literature. The amide group serves as a crucial link between the “privileged building block” and the di-tert-butylphenol in ivacaftor’s structure. This linkage is of considerable importance in medicinal chemistry due to its multifaceted role.

CFTR potentiator cystic fibrosis

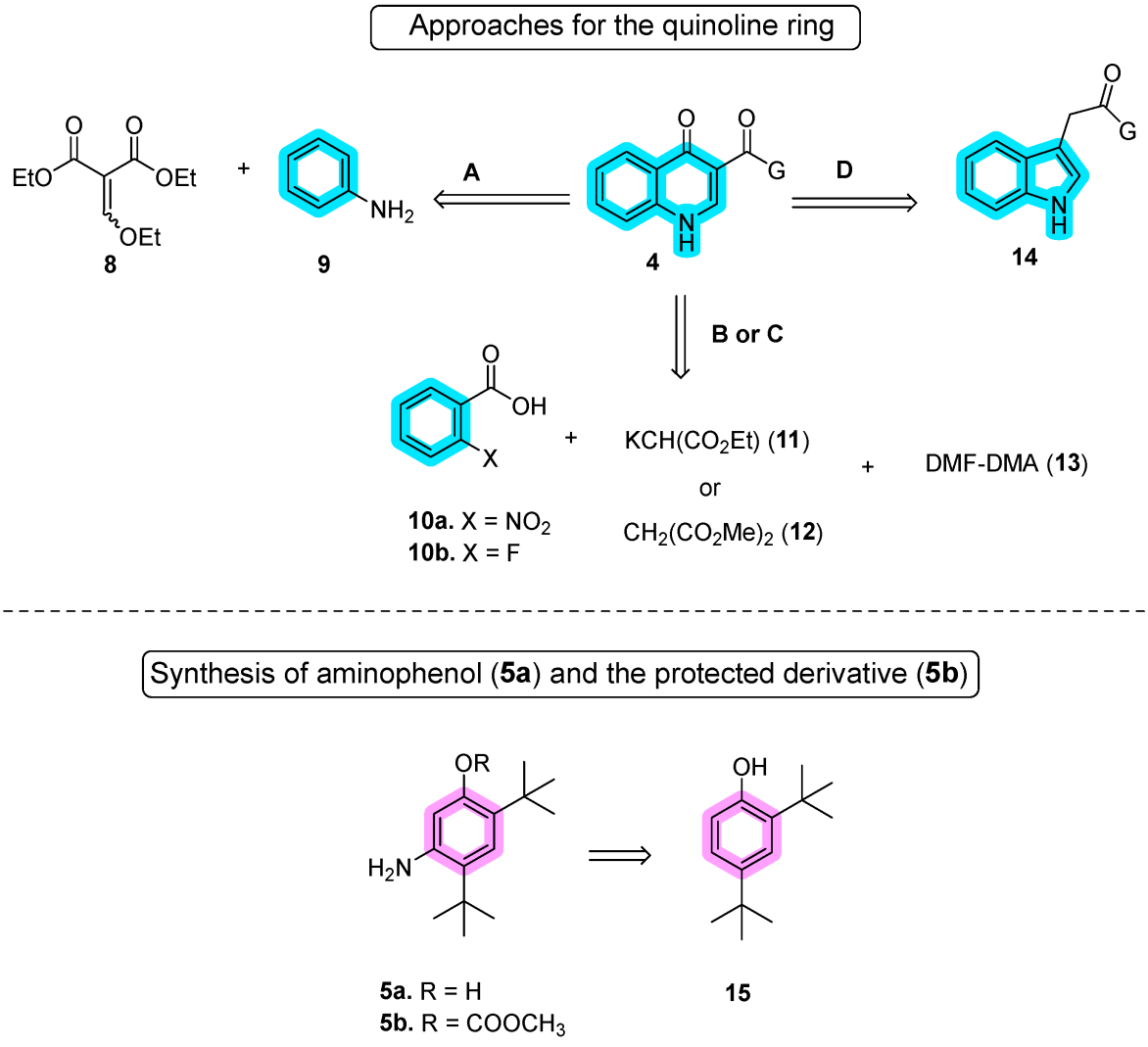
1. Synthetic Routes

In recent years, several synthetic routes have been explored for ivacaftor, primarily focusing on the synthesis of dihydroquinoline fragments **4a** or **4b** (**Scheme 1**). These fragments are subsequently connected to 5-amino-2,4-di-tert-butyl-phenol (**5a**) or its protected derivative **5b** through amide bond coupling, as outlined in approaches A to D. An alternative method involves the synthesis of the quinoline moiety in the final stages, commencing with methyl anthranilate, (*E*)-3-methoxyacryloyl chloride, and 5-amino-2,4-di-tert-butylphenyl methyl carbonate (**5b**) (approach E) [1].



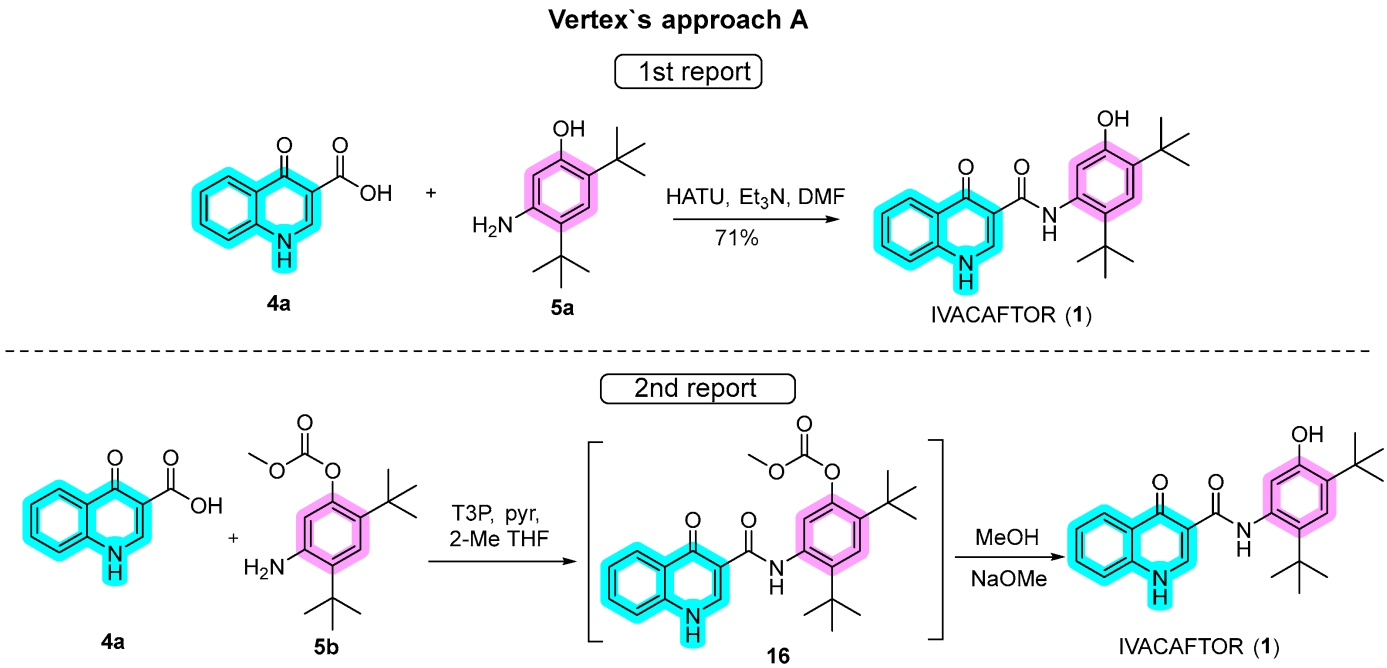
Scheme 1. Disconnection approaches for the synthesis of ivacaftor (**1**).

In the first stage of synthesizing quinoline moiety **4** (approaches A–D), Vertex introduced the Gould–Jacobs reaction using diethyl ethoxymethylene malonate (**8**) and aniline (**9**), as documented in a pivotal work [2]. Vertex has adapted and modified this route over the years to meet the demands of large-scale production [3][4]. Approaches B and C share similarities and were independently developed by the Shanghai University of Engineering Science [5] and Laurus Pharma [6]. These results are outlined in **Scheme 2**. Yang's approach started from *o*-nitrobenzoyl acid (**10a**), while Laurus Pharma initiated the synthesis with *o*-fluoro-benzoyl acid (**10b**). In approach D, Vasudevan and co-authors reported the synthesis of ivacaftor using the Witkop–Winterfeldt oxidation of indolyl group **14** to form the quinoline through ozone oxidation [7][8]. For amide bond coupling with quinoline, Vertex employed aniline **5a** in the initial route or 5-amino-2,4-di-tert-butylphenyl methyl carbonate (**5b**) in the second improved route, both derived from di-*tert*-butyl-phenol (**15**). In approach B, only aniline **5a** was utilized [9], whereas in approach E, **5b** was used instead. This intricate synthesis strategy showcases the diverse approaches taken by various researchers in the development of ivacaftor.



Scheme 2. Different approaches for the synthesis of quinoline and aniline moieties.

Approach A, as employed by Vertex in the synthesis of ivacaftor (**Scheme 3**), is noteworthy for its efficiency. In the initial report of this approach, quinoline **4a** was synthesized through a three-step convergent route utilizing the Gould–Jacobs reaction, while aniline **5a** was obtained in four steps, as detailed below. The pivotal connection of these building blocks occurred through amide bonding coupling using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), in dimethylformamide (DMF). The reaction typically involves the activation of a carboxylic acid group to form an active intermediate, which then reacts with an amine to produce the amide bond with a high coupling efficiency in relatively mild reaction conditions. Subsequent purification by column chromatography resulted in ivacaftor with a notable 71% yield [10][11][12][13].

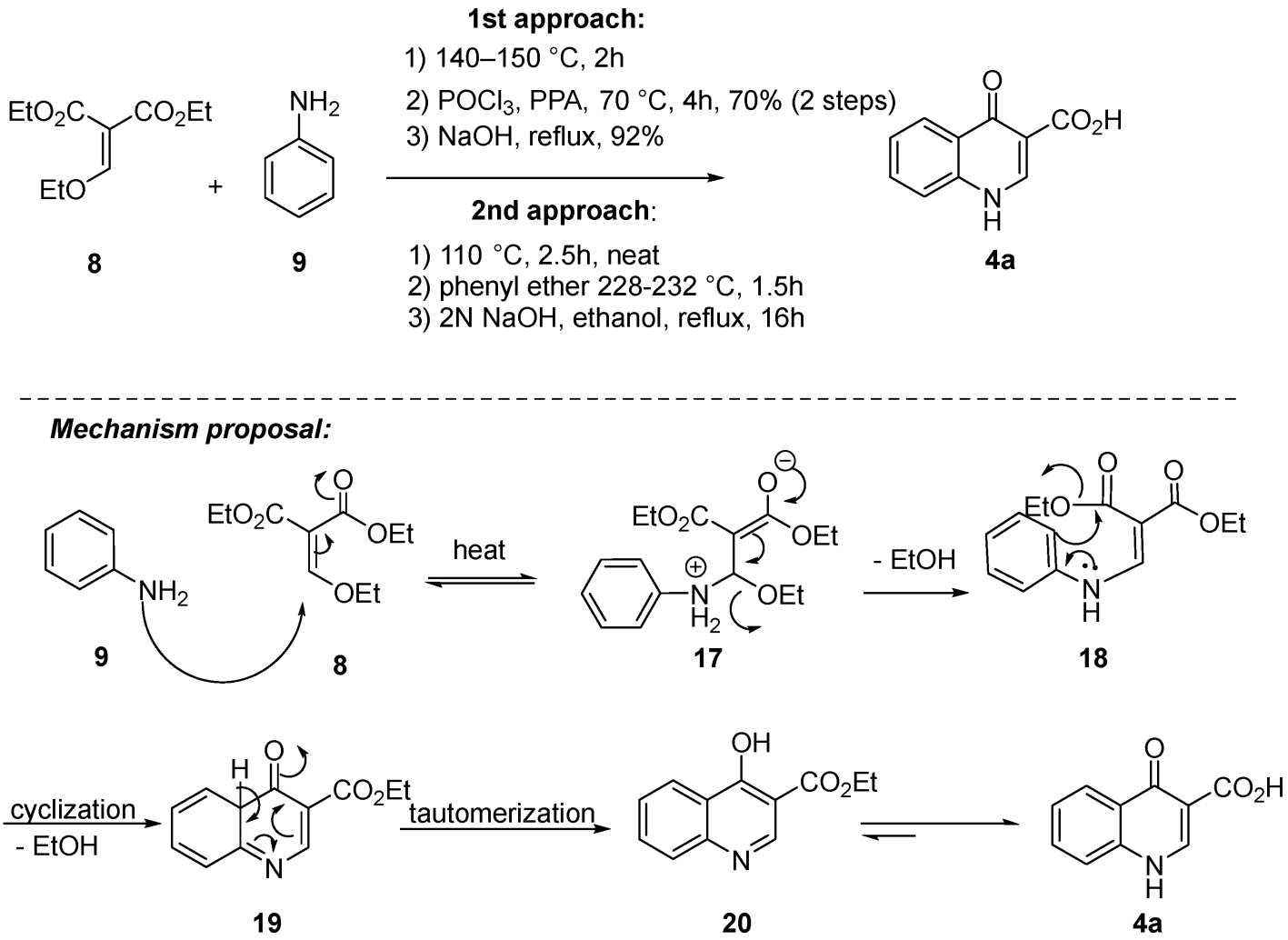


Scheme 3. Approaches for the synthesis of ivacaftor used by Vertex.

In a subsequent report, refinements to the synthetic route were implemented. Protected aniline **5b** replaced **5a**, and amide coupling was facilitated by propanephosphonic acid anhydride (T3P) with pyridine in 2-methyltetrahydrofuran (2-MeTHF), an alternative to HATU. While the reason for the switch to T3P as a coupling agent is not evident, it exhibits effectiveness under relatively mild conditions, reducing the risk of side reactions or unwanted byproducts. Additionally, it is compatible with a wide range of functional groups, rendering it suitable for use with a variety of substrates. Furthermore, T3P is a crystalline solid, which generally makes it easy to handle and store. Despite an additional protection step, intermediate **16** was conveniently hydrolyzed to ivacaftor in the same pot by the addition of MeOH and MeONa. A notable advantage of this modified approach was the elimination of chromatography steps, although specific yields for each step were not provided.

Beyond the choice of amide coupling and/or the use of protected or deprotected aniline in the final step, notable advancements have been made in the synthesis of quinoline **4b** and aniline **5a** or its derivative **5b** in recent years (**Scheme 4**). For quinoline **4b**, the Gould–Jacobs reaction played a crucial role in generating quinolone **4a**. This involved a Claisen condensation between aniline **9** and diethyl ethoxymethylene malonate **8**, followed by

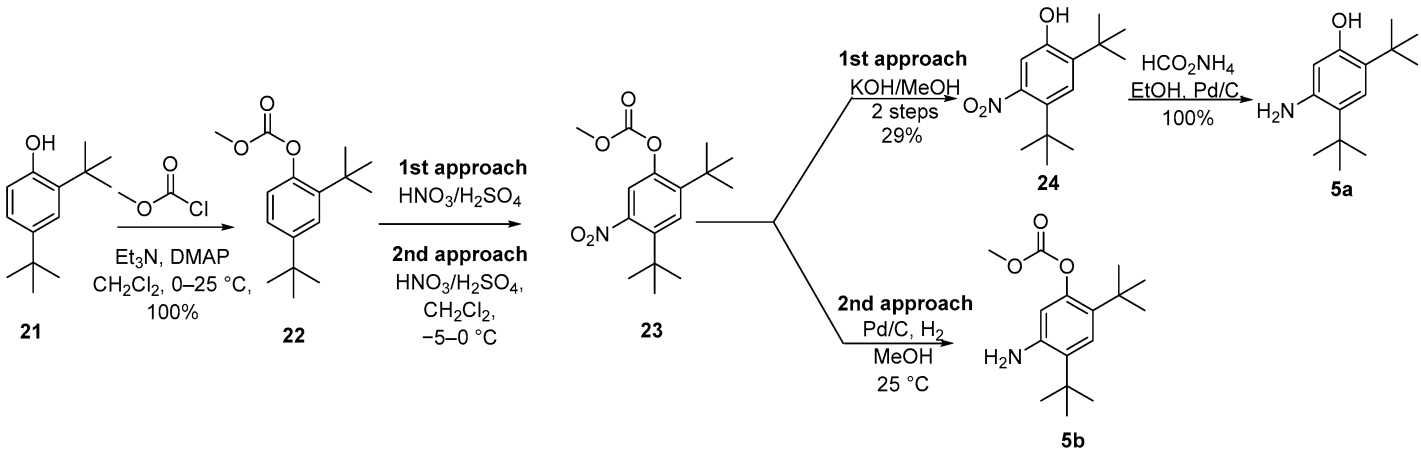
Friedel−Crafts cycloacylation. The methodologies differed in some aspects. In the initial approach, diethyl ethoxymethylene malonate **8** reacted with aniline **9** under neat conditions for 2 h at 140–150 °C to yield enamine **18**. Subsequently, a $POCl_3$/polyphosphoric acid (PPA) mixture was employed for the Friedel–Crafts cycloacylation through intermediates **19** and **20** [14], resulting in a 64% yield over the three steps. The mixture of $POCl_3$/PPA is important to facilitate the cyclization process. $POCl_3$ may potentially convert carboxylic acid, originating from the hydrolysis of the ester in the reaction medium, into acyl chloride. Simultaneously, dehydrating agent PPA aids in the removal of water molecules from the reaction mixture. In the second approach, enamine **18** was generated under neat conditions for 2.5 h at 110 °C, followed by the addition of diphenyl ether and heating to 228–232 °C for 1.5 h. Unfortunately, specific yields were not provided in this case [1][15][16].



Scheme 4. Gould–Jacobs reaction as a key step for the synthesis of quinoline **4a**.

Since the coupling step with quinoline carboxylic acid **4a** may occur with aniline **5a** or **5b**, two reports were described for them. In the first report, a four-step route involved the protection of di-tert-butylphenol **21** to produce the methyl carbonate derivative **22** followed by nitration of **22** to afford **23** as a mixture of 8:1 of the desired 5-nitro regioisomer **23** and the undesired 6-nitro regioisomer. After hydrolysis with KOH in MeOH and purification by column chromatography, nitrophenol **24** was isolated in a 29% yield. Reduction of the nitro group employing

transfer hydrogenation with ammonium formate led to **5a** in quantitative yield. In the second approach, nitration of carbonate **22** was performed in dichloromethane at −5 to 0 °C, and carbonate **23** was isolated by crystallization from hexane without the need for chromatography. The step of reduction of the nitro group occurred without deprotection of **23** by Pd catalyzed hydrogenation with a 2 bar hydrogen gas in MeOH, and product **5b** was purified by crystallization from MeOH/water (**Scheme 5**) [15][16]. The electron-withdrawing carbonate group may allow for nitration to occur primarily ortho/para to the tert-butyl groups since it minimizes the ortho/para-directing effect of the oxygen substituent. Data suggest that the second approach is the manufacturing of one European Public Assessment Report: Symkevi® (26 July 2018) [17].



Scheme 5. Synthesis of 5-amino-2,4-di-tert-butylphenol (**5a**) and 5-amino-2,4-di-*tert*-butylphenyl methyl carbonate (**5b**).

2. Mechanisms of Action

Although the identification of ivacaftor by high-throughput (HT) screenings occurred in 2006, the first publications refer to 2009 as its discovery date. While ivacaftor was still in clinical development, Van Goor and colleagues [18] described the initial pharmacological properties of this potentiator in vitro, reporting its ability to partially restore CFTR activity. In this study, ivacaftor was found to increase the CFTR-mediated transepithelial current by increasing the CFTR Po, specifically after its activation by protein kinase A (PKA), in both cell lines and primary bronchial epithelial cells carrying variants p.PheF508del and/or p.Gly551Asp (legacy name *G551D*). The authors suggested that ivacaftor likely increases CFTR gating activity by directly binding to the protein [18], although at that time it was not yet elucidated whether it could instead act on an associated kinase or phosphatase.

The clinical approval of ivacaftor by both the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) came in 2012 initially for PwCF carrying at least one p.Gly551Asp [19] and extended in the following years for several gating/conductance variants [20][21][22]. In the same year of its approval, Eckford and collaborators [23] provided further insight into the MoA of ivacaftor by using a reconstitution system for purified CFTR protein. In this study, ivacaftor was found to bind directly to wild-type (WT) and mutant CFTR and stimulate the channel activity in an ATP-independent PKA phosphorylation-dependent process. This evidence suggested that ivacaftor

binds to an allosteric site, distinct from the canonical, catalytic site, thus mediating CFTR channel potentiation by a nonconventional, ATP-independent mechanism of gating [23]. In a subsequent study, Jih and Hwang [24] performed electrophysiological recordings and used these results to propose a unified theory for the MoA of ivacaftor. Based on a CFTR gating model, in the presence of ATP, ATP-independent gating is an inherent component of the gating transitions observed for WT-CFTR. Additionally, this model includes a flexible coupling between ATP hydrolysis and gating events. These findings indicated that ivacaftor potentiates CFTR by promoting decoupling between gating cycles and ATP hydrolysis. The authors also suggested that the binding site of ivacaftor in CFTR is unlikely to be on the RD but instead may be located on the TMDs [24].

Two publications from independent research groups investigated whether ivacaftor was involved in the limited improvement of p.Phe508del-CFTR when co-administered with correctors, namely lumacaftor and tezacaftor [25] [26]. Cholon and colleagues observed that, in primary cells, chronic exposure to ivacaftor promotes inhibition of lumacaftor-rescued p.Phe508del-CFTR by decreasing the stability of the corrected protein in a dose-dependent manner [25]. In parallel, Veit and collaborators also demonstrated that exposure to ivacaftor decreases the rescue efficacy of lumacaftor and tezacaftor in p.Phe508del-CFTR-expressing immortalized and primary cells [26]. Prolonged exposure (24 h) to ivacaftor reduced not only the folding efficiency but also the biochemical stability of p.Phe508del-CFTR. These findings were further confirmed by subsequent publications, demonstrating an inhibitory effect of ivacaftor on lumacaftor- or tezacaftor-rescued p.Phe508del-CFTR PM expression [27]. Similar effects were also observed in a later study in which long-term exposure to ivacaftor decreased elexacaftor-rescued p.Phe508del-CFTR [28]. In dynamics simulation and molecular docking studies, a putative binding site was proposed for ivacaftor in a region localized in the NBD1:NBD2 interface and the coupling helix of the intracellular loop (ICL) 1 [26].

In 2015, Yeh and co-authors [29] delved into the understanding of the modulation of CFTR channel gating by permeant ions, namely nitrate and ivacaftor. In this study, similarly to ivacaftor, nitrate increased the Po of the WT-CFTR channel, indicating that it has potentiator activity. Additive effects on CFTR gating were observed when both molecules were used together, suggesting that they work independently, through different binding sites to stimulate CFTR activity. Furthermore, this study proposed a putative binding site for ivacaftor on the TMDs of CFTR, at the interface between the membrane lipids and the protein channel [29]. These authors performed subsequent studies using electrophysiological recordings to further investigate ivacaftor and ABBV-974 (formerly GLPG1837) [30]. Interestingly, both potentiators were found to share a common mechanism to stimulate CFTR gating by competing for the same binding site, notwithstanding their variations in chemical structure, affinity, potency, and efficiency. This study also served as a basis to propose a four-state kinetic model based on a classic allosteric modulation model to explain the MoA and energetic coupling of potentiator binding and the opening of the CFTR channel [30]. In the following assessments, in silico molecular docking and electrophysiological assays were combined to provide further evidence of binding sites for ivacaftor and ABBV-974 [31]. Data from this study allowed for the identification of two potential binding sites located at the TMD1/2 interface, reinforcing the previous findings indicating that ivacaftor and ABBV-974 share the same binding site.

Another putative binding site for ivacaftor was proposed in the study of Byrnes and colleagues [32] using hydrogen/deuterium exchange (HDX) mass spectrometry to characterize CFTR conformational dynamics and its binding interactions with ligands. Using this approach, ivacaftor was suggested to bind to a region of amino acids in ICL4 of the “ball and socket” joint at the TMD2:NBD1 interface—a region close to where the p.Phe508 residue is present. This implies that HDX protection by ivacaftor at ICL4 may extend to the region of this residue as well. Using another approach, Csanády and Töröcsik [33] explored the solubility profile and potency of ivacaftor by stimulating WT and mutant CFTR channels in cell-free membrane patches. It was found that the aqueous solubility of ivacaftor is two orders of magnitude lesser than previously suggested [34]. Furthermore, CFTR stimulation by ivacaftor in cell-free patches was shown to be fully reversible, contrary to what was previously reported [24][29][30][35][36][37]. Based on these observations, the authors mentioned that up to that point, ivacaftor effects were assessed only at high supersaturated concentrations. This study also proposed a kinetic model for the MoA of ivacaftor in CFTR potentiation consisting of two independent binding sites with identical affinity, which thus requires that two molecules bind to the channel to potentiate CFTR [33].

Using cryo-EM, Liu and collaborators [38] were able to determine the structure of CFTR in complex with ivacaftor (PDB: 6O2P; **Figure 1**) and separately with ABBV-974 (PDB: 6O1V), allowing for more direct evidence of these potentiators’ binding site. These results validated previous reports that suggested a common binding site for both ivacaftor and ABBV-974 located in a region inside the lipid bilayer, within the protein–lipid interface of TMD1/2. This hotspot coincides with a hinge region involved in the gating of the channel. Such evidence allowed the authors to suggest that the open configuration of the CFTR channel is stabilized (instead of the closed) when a drug is present in that binding pocket [38]. A subsequent study by Righetti and collaborators [39] used this recently determined structure of human CFTR in complex with ivacaftor [38] to investigate the binding of potentiator to p.Phe508del-CFTR by combining molecular docking, pharmacophore mapping, and quantitative structure–activity relationship (SAR) analysis. By exploiting the most relevant amino acids involved in the ivacaftor binding site, the authors found key residues such as p.Phe931 and p.Arg933 that act via Van der Waals interactions, *H*-bonds, cation-π contacts and involve additional polar interactions in other residues to stabilize the binding of potentiators like ivacaftor [39].

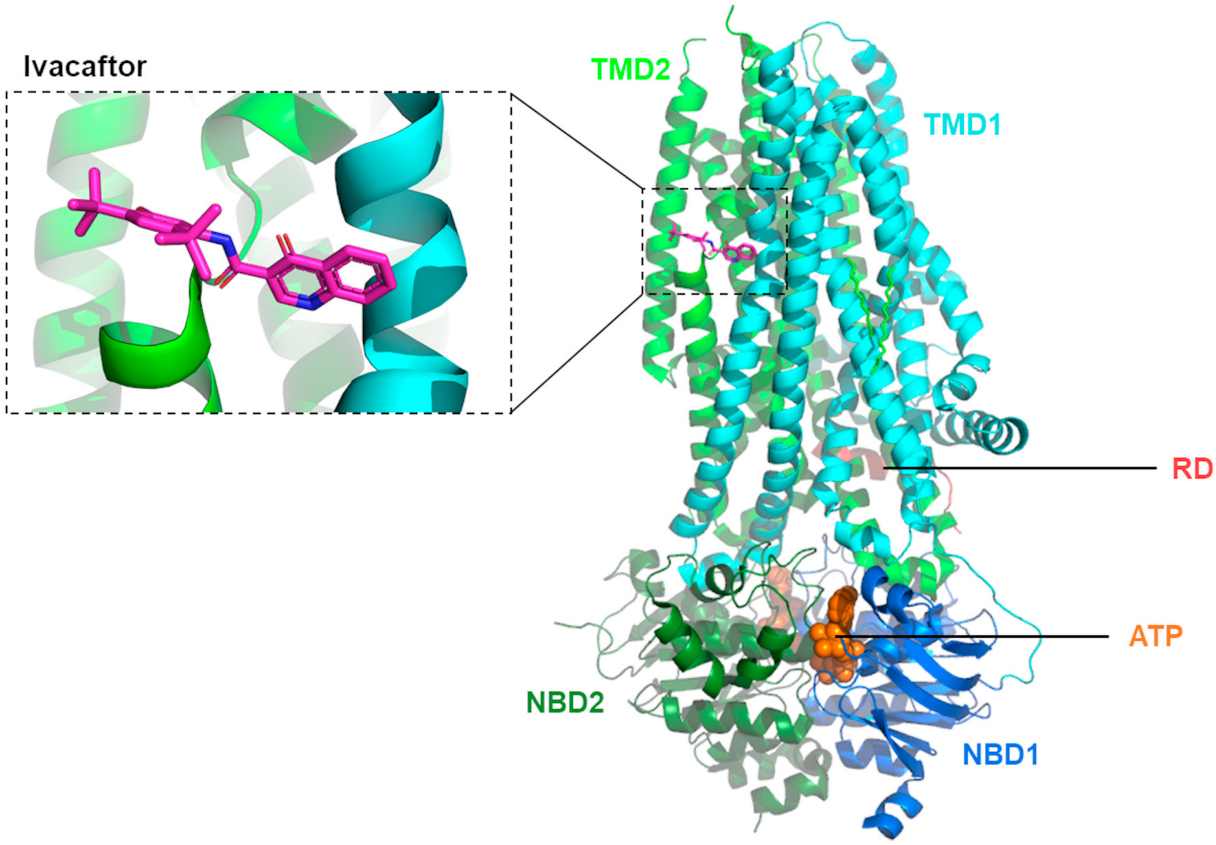


Figure 1. Molecular structure of phosphorylated, ATP-bound human CFTR in complex with ivacaftor. Ribbon diagram adapted from PDB: 6O2P [38] using PyMol Version 2.5.7.

Observing such contradictory results regarding the MoA of CFTR potentiation by ivacaftor, Laselva and collaborators [40] decided to re-evaluate these putative binding sites in the natural context of this channel protein in the lipid bilayer by using photoactivatable ivacaftor probe analogs. With the evidence obtained in this study, the authors proposed a model with two specific binding sites for ivacaftor: one in ICL4 at the NBD1:TMD2 interface and the other in the region of TMD1/2 and membrane lipid interface, as identified by cryo-EM [38]. Whether ivacaftor stabilizes CFTR open channel configuration by binding to ICL4 independently or in conjunction with its binding in the other region remains to be further elucidated [40].

Recently, Levring and collaborators [41] explored SAR in human WT-CFTR at a single-molecule resolution, combining ensemble ATPase activity measurements, single-molecule fluorescence resonance energy transfer (FRET), imaging, electrophysiology, and kinetic simulations. Using this integrative approach, the authors showed the occurrence of dimerization of the two NBDs before the channel opening, revealing an allosteric gating mechanism involving the channel pore and the catalytical binding site. Furthermore, it was observed that potentiators ivacaftor and ABBV-974 act on CFTR and enhance channel activity by increasing pore opening while the NBDs are dimerized, thus influencing the coupling efficiency between ion permeation and NBD dimerization [41]. More recent insights into the mechanisms underlying the action of ivacaftor were described by Ersoy and colleagues [42] who investigated allosteric communications in the CFTR protein by using computational analysis. The authors observed that the binding site for ivacaftor comprises some residues that are main allosteric sources,

suggesting a role for this compound as an allosteric modulator. Furthermore, it was found that ivacaftor's binding site shares similarities with the ATP binding site as both send information to an almost identical set of residues, suggesting that ivacaftor indirectly increases the Po by replicating the combined allosteric signaling triggered by the binding of ATP and by gating residues [42].

It should be noted that, despite the therapeutic accomplishments of ivacaftor, it only attains a partial restoration of the CFTR gating activity [18][24][43]. Accordingly, novel potentiators have been investigated, and their combination with complementary mechanisms (i.e., co-potentiators) has emerged as a strategy to further enhance CFTR gating activity [43][44][45][46][47][48].

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