A Comprehensive Review on Ivacaftor

Radheshyam Sonar*, Rutwik Talokar, Ved Yawalikar,Aditya Ubhale

Department of Pharmacology, P.R Pote Patil College of Pharmacy, Amravati-444602

ABSTRACT: Cystic fibrosis (CF) is multisystemic disorder presenting in new born period to adulthood, predominantly affecting respiratory system. It is caused by mutation in CF transmembrane conductance regulator gene. ΔF508 is the most common mutation seen worldwide. Supportive management with bronchodilators, anti-inflammatory, mucolytics, antibiotics are the corner stone of therapy. Mutation specific drug, Ivacaftor, was recently approved USFDA in January 2012 for patients carrying G551D mutation. It is approved in patients who are six years and older in 150 mg twice daily dosing schedule with fat containing meals. It improves the lung function and other aspects of disease including weight gain. The side effects like upper respiratory infection, headache, rash, diarrhoea, stomach ache and dizziness are mild and self-limiting. This is excellent example of promise of personalised medicine – targeted drug that treat patients with specific genetic makeup. Ivacaftor is First drug that treats an underlying cause of cystic fibrosis to be licensed for use Increases the open probability (i.e. gating) of cystic fibrosis transmembrane conductance regulator channels with the G551D mutation, thus augmenting chloride transport Convenient oral administration Improves lung function and bodyweight parameters when used in combination with standard care in adults, adolescents and children (aged C6 years) with cystic fibrosis and the G551D mutation Generally well tolerated.

KEY WORDS: Ivacaftor, cystic fibrosis, treatment of cystic fibrosis, cystic fibrosis transmembrane conductance regulator (CFTR) potentiators, synthesis of ivacaftor.

INTRODUCTION:

Cystic fibrosis is a complex, life-limiting, genetic disorder that can affect multiple organs throughout the body, leading to pulmonary disease (most cystic fibrosis-related deaths are due to pulmonary insufficiency), reproductive, hepatic, pancreatic and gastrointestinal dysfunction and malnutrition [1, 2]. Cystic fibrosis results from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a glycoprotein present in the apical membrane of epithelial cells, where it functions as a chloride channel predominantly, but also regulates transport of sodium (via epithelial sodium channels) as well as various other processes [3, 4]. It is largely accepted that defective ion transport caused by CFTR dysfunction leads to depletion of airway surface liquid in the lungs of patients with cystic fibrosis, which impairs ciliary function, resulting in mucus obstruction of the airways and consequently infection and inflammation [5] .Cystic fibrosis is incurable at present [6]. Therapy can involve a variety of different medications, including antibiotics (e.g. tobramycin) to treat lung infections, the DNase dornase alfa to clear lung mucus, hypertonic saline to improve muco ciliary clearance, as well as anti-inflammatory agents, bronchodilators and pancreatic enzymes [7, 8].These therapies treat the downstream consequences of CFTR dysfunction, rather than the underlying abnormality, and although their aggressive use has helped to increase the life expectancy of patients with cystic fibrosis [3], most deaths still occur in early adulthood [9]. Understanding of the molecular biology of the CFTR protein has increased over the last two decades and [1,500 CFTR mutations have now been identified [4]. Mutations can be classified on the basis of their functional consequences, which include CFTR protein that is truncated and fails to reach the cell surface (class I) [e.g. R1162X]; is misfolded, improperly processed and defective, little of which reaches the cell surface (class II) [e.g. F508del]; is unable to open and transport chloride properly (class III) [e.g. G551D] or has reduced chloride conductance due to channel narrowing (class IV) [e.g. R117H] but reaches the cell surface; or transports chloride effectively but reaches the cell surface in reduced amounts due to defective transcript splicing (class V) [e.g. 3120?1G?A] [2, 4]. As a result of this knowledge, therapies specifically targeting known CFTR defects have been a focus of drug development for cystic fibrosis in recent years. Ivacaftor (Kalydeco ™) is the first drug to treat an underlying cause of cystic fibrosis to be licensed for use in the EU [10] and USA [11]. The drug potentiates the open probability (i.e. gating) of the CFTR channel, thus enhancing its transport of chloride, and is indicated for the treatment of patients with cystic fibrosis aged C6 years who carry the CFTR gating mutation G551D [10, 11]. This narrative review focuses on pharmacological, clinical efficacy and tolerability data relevant to the use of ivacaftor in this indication.

Synthesis:

Retrosynthetic analysis In generating PAL probe 2, it was important to ensure that the installation of the photoreactive group would not significantly alter the binding affinity and bioactivity compared to ivacaftor. It has been shown that modifications to either of the two t-butyl groups on the scaffold could be tolerated with retention of CFTR potentiating activity.[13] Modification of the t-butyl group ortho to the phenolic moiety affected the potentiation EC50 to a greater degree than did changes to the para-t-buty[12], suggesting that the ortho group might have a closer interaction with the surface of the binding site on CFTR[13]Considering the similar size and lipophilicity of a diazirine moiety compared to a t-butyl group, we envisaged a target PAL probe where a diazirine motif replaces the t-butyl group ortho to the phenolic moiety, and thus the molecule 2 was chosen as our target PAL probe. Retrosynthetic analysis of compound 2 suggested two possible synthetic routes (Figure 1). The first involved forming an amide bond between carboxylic acid A (potentially substituted with a reporter tag), and an aniline B containing the diazirine moiety. B could be prepared from D where the diazirine group would be installed via manipulation of a synthetic handle such as X = Br or I. The second route reversed this process, forming the amide bond between aniline D and acid A to give compound C, followed by installation of the diazirine group (Figure 1).

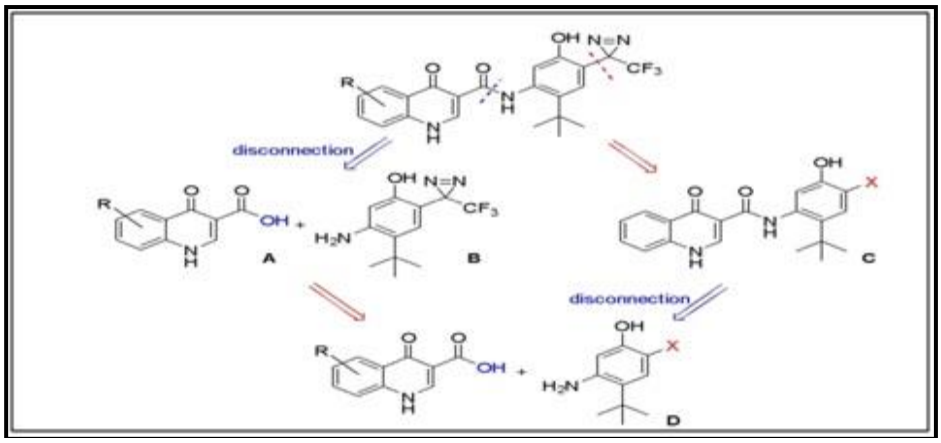


Figure 1: Retrosynthetic Analysis of Routes of Synthesis of the Diazirine-Containing PAL Probe(R Indicates Potential Attachment of A Reporter Tag)

Synthesis:

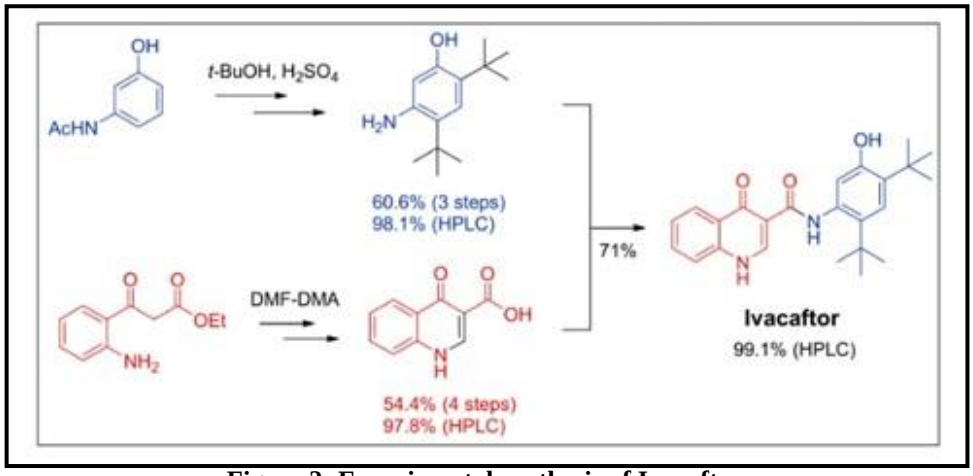


Figure 2: Experimental synthesis of Ivacaftor

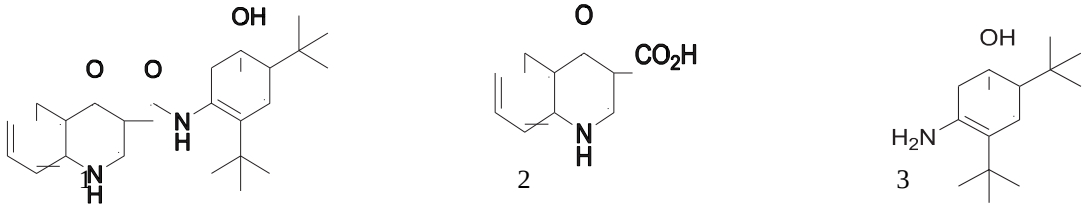
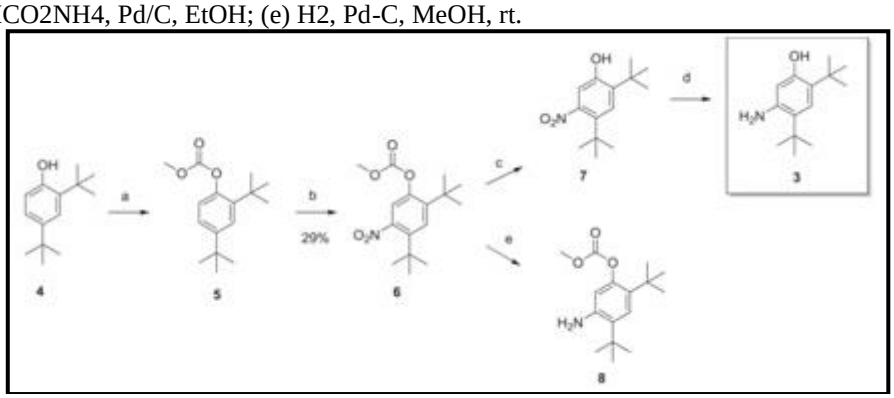
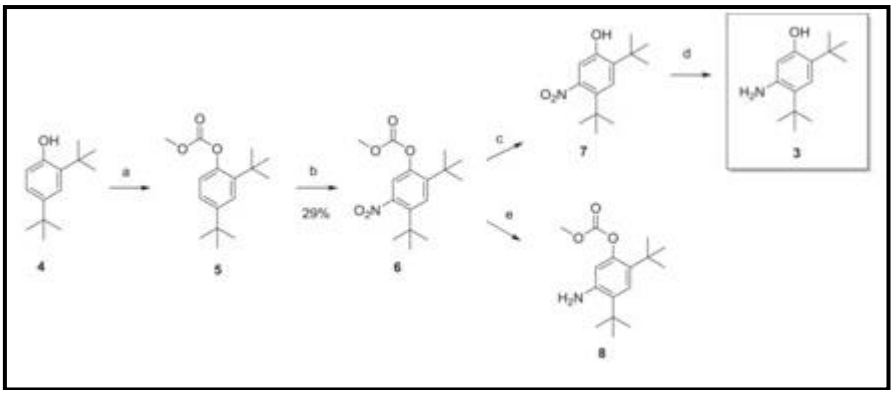


Figure 3: Chemical Structures of Ivacaftor (1), (2) And (3).

Scheme1: Reagents and conditions: (a) CH3CO2Cl, Et3N, DMAP, CH2Cl2; (b) HNO3, H2SO4, 29% via column chromatography; (c) KOH, MeOH; (d) HCO2NH4, Pd/C, EtOH; (e) H2, Pd-C, MeOH, rt.

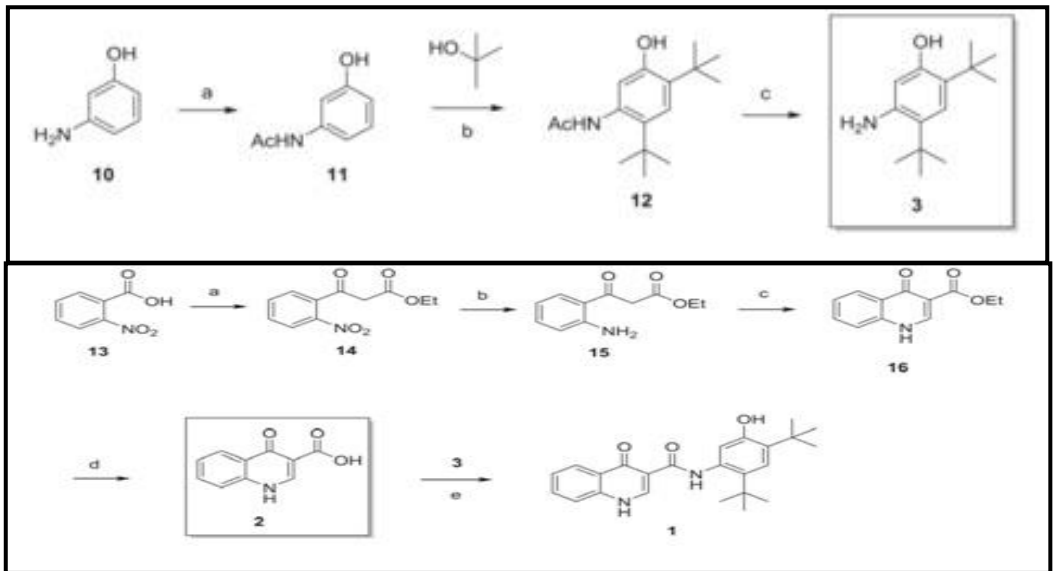


Scheme 2. Reagents and conditions: (a) (i) PhOPh, 230–240°C, 40–60%; (ii) NaOH, EtOH, reflux; (b) (i) 1,3,5,2,4,6-trioxatriphosphorinane (T3P), pyridine, 2-MeTHF, 50°C, 8 h, 70%; (ii) NaOMe, rt; (c) HBTU, Et3N, DMF, 71%.



Scheme 3. Reagents and conditions: (a) Ac2O, HOAc, 50°C, 2 h, 92%; (b) H2SO4, CH2Cl2, rt, 48 h, 74%; (c) HCl, EtOH-H2O, 89%

Scheme 4. Reagents and conditions: (a) (i) CDI, MeCN; (ii) ethyl potassium malonate, MgCl2, Et3N, rt - 35°C; (iii) 3 N HCl, 82%; (b) H2, Raney-Ni, THF, rt; (c) DMF-DMA, toluene, 100°C, 6 h, 73%; (d) NaOH, EtOH-H2O, reflux, 2 h, 91%; (e) EDCI, HOBt, Et3N, DMF, rt, 71%.



Experimental:

All commercially available chemicals and solvents were used as received without any further purification.[14] H NMR and [15]C NMR spectra were recorded on a Bruker UltraShield 400 Plus spectrometer using tetramethylslane or trimethylsilyl as an internal standard. Mass spectra were obtained from a Finnigan MAT-95/711 spectrometer. Melting points were measured on a Shenguang WRS-1B melting point apparatus and are uncorrected. The HPLC results were generated using a Waters 2487 UV/Visible Detector and Waters 515 Binary HPLC Pump.

N-(3-Hydroxyphenyl)acetamide (11):

A mixture of 3-aminophenol (109.1 g, 1.0 mol) and HOAc (700 mL) was stirred and heated to 50°C. Ac2O (112.3 g, 1.1 mol) was added to the reaction solution over 1 h and stirred at the temperature for another 1 h. It was cooled to room temperature, and poured slowly into ice-water (3 L) while stirring constantly. The solid formed was filtered off and washed with cold water (300 mL × 2), dried at 45°C for 6 h to afford 11 (139.0 g, 92%) as a white solid, mp 146–148°C.[15]H NMR [dimethyl sulfoxide (DMSO)-d6, δ]: 2.01 (s, 3H), 6.42 (m, 1H), 6.92 (m, 1H), 7.04 (m,1H), 7.18 (m, 1H), 9.33 (s, 1H), 9.78 (s, 1H); electrospray ionization-mass spectrometry (ESI-MS) (m/z) 152.2 (M + H)+.

N-(2,4-di-tert-butyl-5-hydroxyphenyl)acetamide(12):

Concentrated H2SO4 (176.4 g, 1.8 mol) was added slowly to the stirred solution of 11 (120.0 g, 0.79 mol), t-butanol (205.0 g, 2.77 mol) and CH2Cl2 (1.5 L) below 20°C. White sticky solid was formed immediately, and the suspension was stirred quickly at room temperature for 48 h to give a white homogeneous suspension. The resulting white solid was filtered, washed with H2O (400 mL × 2) and then mixed with 300 mL H2O and stirred quickly. Saturated aqueous NaHCO3 was added slowly to the mixture to adjust the pH ~ 6. The resulting white solid was filtered, washed with H2O (400 mL × 2) and dried at 50°C for 5 h to give the crude product 12 (187 g), which was stirred and heated with 1:1 (v/v) hexane/EtOAc (500 mL) at reflux for 2 h then cooled to room

temperature overnight, the resulting solid was filtered off and washed with 1:1 (v/v) hexane/EtOAc (200 mL × 2), dried at 40°C for 3 h to afford 12 (154.0 g, 74%) as a white solid, mp 91–94°C. [15]H NMR (DMSO-d6, δ): 1.25 (s, 9H), 1.33 (s, 9H), 1.99 (s, 3H), 6.45 (s, 1H), 7.11 (s, 1H), 9.00 (s, 1H), 9.16 (s, 1H). 13C NMR (100 MHz, DMSO-d6): δ = 24.0, 30.9, 31.2, 35.4, 36.8, 110.3, 126.0, 131.5, 132.2, 132.6, 149.0, 169.3. ESI-MS (m/z) 264.3 (M + H)+, 527.4 (2 M + H)+, 549.4 (2 M + Na**)+.**

5-amino-2,4-di-tert-butylphenol(3):

Concentrated HCl (167.0 mL, 2.0 mol) was added to a suspension of 12 (135.0 g, 0.51 mol), EtOH (1.4 L) and H2O (200 mL).The reaction mixture was stirred and heated to reflux for 6 h to give a clear solution. It was cooled to room temperature, and around 1 L solvent was recovered under reduced pressure. The residue was stirred at room temperature for 2 h. The resulting white solid was filtered, washed with cold water (200 mL × 2) and then mixed with 200 mL water and stirred quickly. Saturated aqueous NaHCO3 was added slowly to the mixture to adjust the pH ~ 6 and the mixture were stirred for another 2 h. The resulting white solid was filtered, washed with cold water (200 mL × 2) and dried at 45°C for 5 h to afford the crude product (110 g), which was stirred and heated with 1:1 (v/v) hexane/EtOAc (350 mL) at reflux for 2 h then cooled to room temperature overnight, the resulting solid was filtered off and washed with 1:1 (v/v) hexane/EtOAc (100 mL × 2), dried at 40°C for 3 h to afford 3 (100.5 g, 89%) as a gray solid, mp 86–89°C. 1H NMR (DMSO-d6, δ): 1.32 (s, 9H), 1.34 (s, 9H), 6.79 (s, 1H), 7.19 (s, 1H), 9.78 (brs, 2H). ESI-MS (m/z) 222.3 (M + H) +. HPLC Conditions: Column: InertSustain C18 (250 mm × 4.6 mm × 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30°C; Injection load: 1 μL; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 15 min; Mobile phase A: water; Mobile phase B: MeOH/AcOH = 100:0.1; Gradient program: Mobile phase A/Mobile phase B = 10/90: tR = 5.404 min, purity: 98.1%.

Ethyl3-(2-nitrophenyl)-3-oxopropanoate (14):

Flask A: To a suspension of 2-nitrobenzoic acid (167.1 g, 1.0 mol) in CH3CN (2 L) was added CDI (178.4 g, 1.1 mol) portion-wise, gas evolution was observed. This solution was stirred for 4 h at the ambient temperature. Flask B: To a solution of ethyl potassium malonate (510.0 g, 3.0 mol) in CH3CN (4 L) was added MgCl2 (103.0 g, 1.08 mol) in portions over 15 min. The mixture was stirred at 35°C for 30 min and then cooled to 25°C and triethylamine (253.0 g, 2.5 mol) was added. The reaction mixture became very thick, and the slurry was stirred for 30 min. The solution in flask A was then transferred to the slurry in flask B over 15 min. The reaction temperature rose to 35°C, and gas evolution was observed. The reaction mixture was stirred for 1.5 h, cooled to 5°C, and quenched with 3 N HCl (3 L), while maintaining the reaction temperature < 20°C. The resulting solution was distilled to remove CH3CN and the resulting concentrate extracted with EtOAc (4 L). The organic phase was washed with water (2 L), saturated NaHCO3 (2 L), and brine (2 L). The organic solution was concentrated under reduced pressure to give light yellow oil, which was solidified at room temperature to afford 14 (194.5 g, 82%) as a light yellow solid, mp 34–36°C. 1H NMR (CDCl3, δ): 1.33 (t, J = 7.2 Hz, 3H), 3.48 (s, 2H), 4.27 (q, J = 7.2 Hz, 2H), 7.71 (m, 2H), 7.90 (m, 1H). 13C NMR (100 MHz, DMSO-d6): δ = 13.9, 49.0, 61.8, 124.1, 128.0, 130.9, 132.8, 134.7, 144.6, 166.5, 194.5. HRMS (ESI): Calcd for C11H11NO5: 237.2110, Found 237.2105.

Ethyl3-(2-aminophenyl)-3-oxopropanoate(15):

Compound 14 (130.0 g, 0.55 mol) and Raney Ni (wet, 30 g) were added to THF (2 L), and stirred for 6 h at room temperature under hydrogen bag at atmospheric pressure to form a clear brown solution. The reaction mixture was then filtered through a celite pad, the filter cake was washed by THF (200 g × 2). The combined filtrate was concentrated to give the product 15 (114.0 g) as a light brown oil, which was used directly at the next step. 1H NMR (CDCl3, δ): 1.31 (t, J = 7.2 Hz, 3H), 3.44 (s, 2H), 4.24 (q, J = 7.2 Hz, 2H), 6.68 (m, 2H), 7.30 (m, 1H), 7.86 (m, 1H), 8.77 (brs, 2H). ESI-MS (m/z) 207.0 (M – H)-.

Ethyl4-oxo-1,4-dihydroquinoline-3-carboxylate(16):

To a stirred solution of 15 (50.0 g, 0.24 mol) in toluene (600 mL) was added DMF-DMA (57.2 g, 0.43 mol). The mixture was stirred and heated to 100°C for 6 h and then cooled to room temperature. A gray suspension was formed and the resulting solid was collected by suction filtration, washed with 50% EtOH/H2O (80 mL × 2), and dried at 60°C to give a gray solid. The crude product was stirred and heated with 1:1 (v/v) EtOH/EtOAc (160 mL) at reflux for 2 h then cooled to room temperature overnight, the resulting solid was filtered off and washed with 1:1 (v/v) EtOH/EtOAc (50 mL × 2), dried at 60°C for 4 h to afford 16 (38.0 g, 73%) as an light yellow solid, mp >250°C. 1H NMR (DMSO-d6, δ): 1.29 (t, J = 7.2 Hz, 3H), 4.22 (q, J = 7.2 Hz, 2H), 7.42 (m, 1H), 7.62 (m, 1H), 7.71 (m, 1H), 8.16 (m, 1H), 8.55 (s, 1H), 12.31 (s, 1H). 13C NMR (100 MHz, DMSO-d6): δ = 14.5, 59.5, 110.0, 118.8, 124.6, 125.6, 127.4, 132.4, 139.1, 144.9, 164.9, 173.5. MS (ESI): m/z = 218.2 (M + H)+. HPLC Conditions: Column: InertSustain C18 (250 mm × 4.6 mm × 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30°C; Injection load: 1 μL; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 15 min; Mobile phase A: water; Mobile phase B: MeOH; Gradient program: Mobile phase A/Mobile phase B = 10/90: tR = 3.436 min, purity: 98.6%.

4-Oxo-1,4-dihydroquinoline-3-carboxylicacid(2):

NaOH (13.8 g, 0.34 mol) was disolved in water (150 mL) and ethanol (250 mL) solution, and compound 16 (30.0 g, 0.13 mol) was added, and the resulting suspention was stirred and heated to reflux for 2 h to give a clear solution. Most of ethanol was removed under vacuum and the residue was adjusted with 6 N HCl to pH ~ 3, the resulting solid was filtered, washed withwater (50 g × 2), and dried at 60°C for 5 h to give product 2 (22.3 g, 91%) as a white solid, mp >250°C. 1H NMR (DMSO-d6, δ): 7.60 (m, 1H), 7.82

(m, 2H), 8.29(m, 1H), 8.88 (s, 1H), 13.45 (brs, 1H), 15.35 (s, 1H). 13C NMR (100 MHz, DMSO-d6): δ = 107.8, 119.6, 124.5, 125.1, 126.3, 144.0, 145.2, 166.6, 178.4. ESI-MS (m/z) 190.1 (M + H)+. HPLC Conditions: Column: Inert Sustain C18 (250 × 4.6 mm × 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30°C; Injection load: 1 μL; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 15 min; Mobile phase A: water; Mobile phase B: MeOH/HCO2H = 100:0.1; Gradient program: Mobile phase A/Mobile phase B = 10/90: tR = 4.075 min, purity: 97.8%.

Ivacaftor(1):

To a suspension of compound 2 (9.8 g, 0.052 mol) and 3 (11.5 g, 0.052 mol) in DMF (100 mL) was added triethylamine (22.0 mL, 0.16 mol). The mixture was stirred at room temperature, HOBt (10.8 g, 0.08 mol) and EDCI (15.3 g, 0.08 mol) were added successively. The reaction mixture was stirred at the ambient temperature overnight. The suspension was diluted with water (500 mL) to give a light yellow solid, which was filtered, washed by water (50 mL × 3), dried at 50°C for 4 h to afford the crude product 1 (19.0 g). The crude product was stirred and heated with 1:2 (v/v) MeOH/EtOAc (120 mL) at reflux for 1 h then cooled to room temperature overnight, the resulting solid was filtered off and washed with 1:2 (v/v) MeOH/EtOAc (20 mL × 2), dried at 50°C for 3 h to afford 1 (14.5 g, 71%) as an off-white solid, mp 124–128°C. [15]H NMR (DMSO-d6, δ): 1.36 (s, 9H), 1.38 (s, 9H), 7.11 (s, 1H), 7.17 (s, 1H), 7.51 (m, 1H), 7.80 (m, 2H), 8.33 (m, 1H), 8.85 (m, 1H), 9.23 (s, 1H), 11.81 (s, 1H), 13.12 (brs, 1H). [16]C NMR (100 MHz, DMSO-d6): δ = 29.9, 31.1, 34.4, 34.8, 111.3, 116.4, 119.5, 124.2, 125.6, 126.0, 126.5, 132.0, 132.8, 133.3, 134.0, 139.6, 144.5, 153.7, 163.3, 176.9. HRMS (m/z): Calcd for C24H28N2O3: 392.4990, Found 392.4944 HPLC Conditions: Column: InertSustain C18 (250 mm × 4.6 mm × 5 μm); Detection: 220 nm; Flow rate: 0.8 mL/min; Temperature: 30°C; Injection load: 1 μL; Solvent: MeOH; Concentration: 0.2 mg/mL; Run time: 15 min; Mobile phase A: water; Mobile phase B: MeOH/HCO2H = 100:0.1; Gradient program: Mobile phase A/Mobile phase B = 10/90: tR = 4.942 min, purity: 99.1%.

Pharmacokinetics:

Administered as an oral dose, ivacaftor absorbs readily from the gut, but has low solubility in water (< 0.05 ug/mL) [17]. Taking a 150 mg dose of ivacaftor with a high fat meal improves absorption, increases AUC by 2.5 times, and delays T_{max} from 3 to 5 hours [18]. Dose/time pharmacokinetics follows a linear profile to a dose of 250 mg, though C_{max} plateaus for doses 375 mg and higher [17]. Ivacaftor is transported in the plasma highly bound (99%), preferentially to alpha-1-acid glycoprotein but also to albumin, to its site of action, which is the apical membrane of epithelial cells [18, 19]. However, no drug-drug interactions related to protein binding competition are expected [17]. As shown in figure, ivacaftor is metabolized in the liver by cytochrome P450 3A (CYP3A), including both CYP3A4 and CYP3A5, into a metabolite hydroxymethyl-ivacaftor (M1), which is considered to be active with a potency 1/6th that of ivacaftor itself, and the inactive metabolite ivacaftor-carboxylate (M6), which not considered active and has an activity level 1/50th that of ivacaftor [20]. Elimination of the parent drug and metabolites occurs predominantly (87%) through the bile, with 22% being the M1 metabolite and 43% being the M6 metabolite [19]. The M6 metabolite is excreted in the bile through the solute carrier organic anion transporter 1B1 (SLCO1B1) transporter, but the mechanism for the elimination of M1 is unknown [21].Ivacaftor has a half-life of 12–14 hours [21] Ivacaftor and M1 are thought to be weak inhibitors of CYP3A4, increasing midazolam area under the curve of drug concentration in the plasma over time (AUC) by 54% [20]. They are also weak inhibitors of P-glycoprotein (*ABCB1*), increasing digoxin AUC by 32% [20]. CYP3A inducers, such as rifampin, carbamazepine and phenytoin, reduce exposure to ivacaftor and CYP3A inhibitors increase exposure to ivacaftor [20, 22]. When administered with known CYP3A inhibitors, it is recommended that the frequency of ivacaftor administration be reduced from twice to once daily [6].

Pharmacodynamics:

Ivacaftor is indicated for use in CF patients who carry at least one of 11 genetic variants, most of which are class III variants, which affect the activation of the chloride channel of CFTR and thereby inhibit normal chloride movement [23, 24]. These variants, called "gating" variants, are listed in Table. Tab The functional consequence of class III variants means that CFTR localizes to the apical cell membrane as normal, but cannot undergo cAMP-mediated activation and so is non-functional [24, 25]. Ivacaftor is a selective potentiator of CFTR and is believed to stabilize the open state of the channel, enabling chloride transport (figure) [26]. The exact mechanism of how this potentiation works is unknown, though it may be through decoupling the gating cycle and ATP hydrolysis cycle, or by increasing the ATP-dependent opening rate and slowing the closing rate [26, 27]. It is believed that by binding to CFTR in the epithelial cell membrane, ivacaftor improves the function of both CFTR with gating mutations and CFTR with normal function [19, 26].

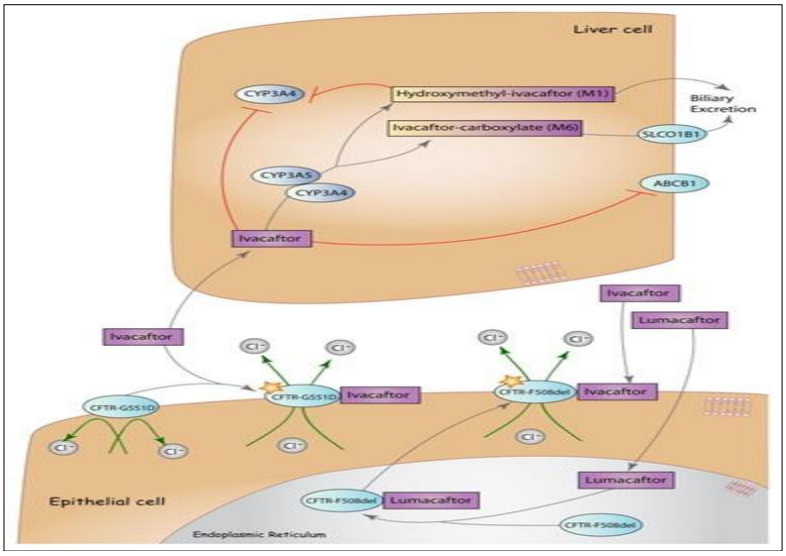


Figure: - Stylized cells depicting the metabolism and mechanism of action of ivacaftor

Amino Acid Change	ers number	cDNA change
R117H	rs78655421	350G>A/C/T
G178R	rs80282562	532G-A
$549N	rs121908755	1646G-A/T
$549R	rs121908757;	1645AC 1647T>G
G551S	rs121909013	1651G>A
G551D	rs75527207	1652G-A
G1244E	rs267606723	3731G-A/T
$1251N	rs74503330	3752G>A
$1255P	rs121909041	3763T C
G1349D	rs193922525	4046G A

Table 1.1 Variants in CFTR approved for treatment with ivacaftor, with their u nique rs numbers, cDNA changes, and resulting amino acid changes

Ivacaftor potentiates CFTR to restore chloride gating function in CFTR with class III gating defects, such as with the G551D variant. The star symbols on the CFTR-G551D protein icon and the CFTR-F508del protein icon indicate that they are active. The metabolism of ivacaftor and the elimination of its metabolites in the liver is shown. The figure also contains the mechanism of action of lumacaftor, which chaperones the protein folding of the CFTR-F508del and thereby restores correct localization of the protein, where ivacaftor can improve gating function. Initial validation studies were conducted in patients with CF and at least one G551D *CFTR* gating variant (c.1652G>A, rs75527207); treatment was reported to improve lung function by >10%, enough to see a marked improvement in symptoms of CF [28]. Ivacaftor treatment was reported to result in improved pulmonary function tests as measured by the Forced Expiratory Volume in 1 second (FEV-1) by 10.4 – 17.5% over the course of 24 weeks, and this difference was maintained to the trial end at 48 weeks [29, 30]. Ivacaftor treatment was reported to improve weight gain (3.7 kg on ivacaftor compared with 1.8 kg on placebo over 24 weeks) and to decrease chloride concentration compared to baseline (−55.5 mmol/L ivacaftor compared to −1.8 mmol/L on placebo) in clinical trials with 6–11 year olds [29]. Changes in sweat chloride concentrations are often used as a biomarker of drug bioactivity for cystic fibrosis, though there is debate as to the validity of this measure [31–33]. Improvement in chloride transport was reported in patients 12 years and older [30]. Respiratory improvements were reported after 2 weeks of treatment with ivacaftor compared to placebo [30].The folding malfunction caused in patients homozygous for the F508del variant (c.1521_1523delCTT; rs113993960 or rs199826652), which is not a gating variant and instead prevents CFTR protein from exiting the endoplasmic reticulum and localizing to the cell membrane (figure), showed no improvement with ivacaftor treatment alone [34]. However, a combination drug of ivacaftor with lumacaftor (VX-809) was approved by the FDA and European Medicines Agency (EMA) in 2015 for use in patients homozygous for the F508del allele [35]. Lumacaftor is reported to restore CFTR function Trials to about 15% of normal in lower airway epithelial cells derived from patients homozygous for the F508del allele by chaperoning protein folding [22]. By ameliorating by that allele, lumacaftor is thought to enable the localization of F508del CFTR to the cell membrane [22]. When lumacaftor is combined with ivacaftor, the F508del CFTR folding repair from lumacaftor

combined with increased potentiation from ivacaftor is reported to result in a 30% improvement of CFTR function in lower airway epithelial cells derived from individuals homozygous for the F508del allele [22]. Phase 2 and 3 trials have also shown clinically meaningful efficacy in patients homozygous for the F508del allele, with results measured by change in chloride concentration and FEV-1 [22].

Pharmacogenetics:

CFTR has two nucleotide binding domains and two membrane spanning domains, and variants can occur throughout all regions [36]. Shuttled from the Golgi to the apical membrane in secretory vesicles, CFTR turns over at a rate of 10% per minute and has a half -life of 12–24 hours [36]. More than 2000 variants have been identified in the *CFTR* gene, though only 127 have been confirmed to be pathogenic [37]. The American College of Medical Genetics guidelines include 23 of these variants in their recommended panel for determining carrier status for cystic fibrosis [38]. Pathogenic variants in *CFTR* are categorized into 5 classes based on their effect on CFTR function. Class I variants affect biosynthesis of CFTR, producing truncated or unstable protein that is quickly degraded. Class II variants affect protein folding, which can reduce protein stability and appropriate localization. The F508del variant, which is a class II variant and prevents localization to the cell membrane, is identified in over 90% of CF cases [13]. Class III variants, those that are targeted by ivacaftor, fold and localize appropriately, but cannot be regulated by ATP or phosphorylation as needed for normal gating function. Class IV variants also affect ion flow, but through decreased permeability of chloride ions, and class V variants usually result in splicing changes that reduce the amount of CFTR expressed in the cell membrane [36]. The most important genetic variants to consider in ivacaftor treatment are the class III variants in *CFTR* that cause problems with gating. The G551D variant is the most common gating variant and that allele is thought to be present in about 4–5% of patients with CF [23]. The FDA and EMA originally approved the use of ivacaftor in the United States and in Europe in 2012 to treat cystic fibrosis caused by the G551D gating variant [19]. Since then, approval has been expanded to include other gating variants. The list of variants approved for treatment with ivacaftor is found in Table and includes G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, and S549R [19, 18]. These variants each occur in less than 1% of cystic fibrosis patients [16, 28]. In addition to these other class III variants, the FDA has approved ivacaftor for treatment of patients with the R117H variant, a class IV variant that is primarily a conductance variant, but also has defective gating activity [39].

Therapeutic Efficacy:

Three randomized controlled trials have been identified investigating the efficacy and safety of ivacaftor. The first study conducted by Accurso and colleagues [40] evaluated the safety and adverse-event profile of ivacaftor in patients age 18 years or older with at least 1 G551D mutation. Doses in 25, 75, or 150 mg were studied for 14 days followed by a dose of 150 or 250 mg for 28 days. It was concluded that ivacaftor was associated with few severe side effects. Although efficacy was not a primary end point, an 8.7% (range, 2.3–31.3) change in forced expiratory volume in 1 second (FEV_1) from baseline was observed, and median sweat chloride decreased 59.5 mmol/L. A second study conducted by Ramsey and colleagues [41] sought to evaluate the efficacy and safety of ivacaftor 150 mg twice daily for 48 weeks in 161 patients age 12 years or older with at least 1 G551D mutation. At 24 weeks, ivacaftor administered at this dose resulted in a 10.6% improvement in predicted FEV_1 from baseline versus placebo. A 55% reduction in the risk of pulmonary exacerbations was observed. Mean values for sweat chloride at 24 weeks were 47.8 mmol/L in the ivacaftor group versus 100.0 mmol/L in the placebo group.[41] Cystic Fibrosis Questionnaire-Revised respiratory domain scores increased by 5.9 points versus 2.7 points in the placebo group by week 48 signifying improved quality of life. Patients in the ivacaftor group also gained 3.1 kg, which was also statistically significant when compared to placebo. In regards to safety, elevated hepatic enzyme levels led to discontinuation of ivacaftor in 1 subject. The studies in children 6 to 12 years of age are currently unpublished, but a 48-week study is reported to have included 52 patients and resulted in a 12.5% improvement in FEV_1 and 2.8 kg weight gain compared to placebo.[42] Additionally, decrease in mean sweat chloride of 54 mmol/L was observed. Flume and colleagues [43] evaluated the safety of ivacaftor 150 mg twice daily for 96 weeks in patient's age 12 years or older with homozygous delta F508 CFTR mutations. Similar adverse events were reported between ivacaftor and placebo. However, lack of clinical efficacy supports the idea that ivacaftor alone is not sufficient in patients without a gating defect. A phase II randomized, double-blind, placebo-controlled study including patients 18 years or older with 2 copies of delta F508 combined Vx-809 (a F508del corrector) and ivacaftor (250 mg every 12 hours). From Day 28 to 56, a mean absolute improvement in lung function of 6.1% within the group was observed. A mean absolute improvement in lung function of 8.6% was observed when compared to placebo.[44] At this time, ivacaftor has only been studied as adjunct therapy to standard of care. In clinical trials, patients remained on their pre-study medications. Use of CYP 3A4 inhibitors, inducers, and inhaled hypertonic saline were prohibited.

Dosing:

Ivacaftor is available as 150 mg tablets and approved for patients with CF age 6 and older who possess at least 1 G551D mutation in the CFTR gene [42] The initial dose is 150 mg orally every 12 hours for both the adult and paediatric population [42] Dosage adjustments are necessary if ivacaftor is administered with moderate or strong CYP3A inhibitors, 150 mg once daily and 150 mg twice weekly, respectively.[42]

Carcinogenicity, Teratogenicity and Excretion:

In studies using rats and mice, no evidence of tumorigenicity was observed. Impaired fertility and reproductive indices were found in male and female rats at doses of 200 mg/kg/day. Also noted was an increased number of female rats with nonviable embryos as well as decreased corpora lutea, viable embryos, and implantations at the aforementioned dose. These impairments are attributed to severe toxicity as none were observed at doses less than or equal to 100 mg/kg/day.[42] Ivacaftor is FDA pregnancy risk category B as there are no well-controlled or adequate studies in pregnant women. Thus, ivacaftor should only be used during pregnancy if

benefits outweigh risks and on a highly individualized patient-specific case-by-case basis [42] Studies in lactating rats show that ivacaftor is excreted into their milk. Currently there is no data available on human breast milk excretion and caution should be exercised if administered to nursing women.[42]

Side Effect:

The most common adverse effects in clinical trials included headache, upper respiratory tract infection, nasal congestion, rash, and dizziness.[40,41] Incidence of adverse effects were similar to placebo with fewer serious adverse effects leading to discontinuation occurring in the ivacaftor group than with placebo.[41] The only adverse event experienced by 1 patient leading to discontinuation in the ivacaftor group was increased hepatic enzyme levels,[41] hence the recommendation to monitor liver function tests every 3 months for the first year of therapy and subsequently once a year. Interruption of therapy is warranted in patients with an aspartate aminotransferase or alanine transaminase greater than 5 times the upper limit of normal. Clinicians should consider the risks versus benefits prior to resuming ivacaftor once hepatic enzymes return to baseline.[42]

Drug Interaction:

Ivacaftor exhibits multiple mechanisms by which drug interactions occur, one of which is cytochrome P450 isoenzymes. Ivacaftor is a sensitive CPY3A substrate.[42] Therefore, co-administration with CYP3A inhibitors or inducers warrants caution.[42] With strong CYP3A inhibitors such as ketoconazole, ivacaftor's exposure is increased by approximately ninefold, whereas moderate inhibitors of CYP3A increases ivacaftor's exposure by threefold. Conversely, when co-administered with a strong CYP3A inducer, ivacaftor's exposure is decreased by approximately ninefold.[42] Inducers such as St. John's Wort or rifampin are not recommended for use if a patient is currently being treated with ivacaftor.[42] Co-administered medications or ivacaftor may require dose adjustments.

Co administered medication	CYP Interaction	Recommendation
Midazolam, alprazolam, diazepam, triazolam	3A substrate	Use with caution and monitor benzodiazepine-related side effects
Rosiglitazone	2C substrate	No dose adjustments necessary
Warfarin	2C9 substrate	Monitor INR
Desipramine	2D6 substrate	No dose adjustments necessary
Ketoconazole,itraconazole, posaconazole,voriconazole, clarithromycin, telithromycin	Strong 3A inhibitors	Dose adjust to ivacaftor 150 mg twice weekly
Rifampin, rifabutin, phenobarbital, phenytoin, carbamazepine, St. John's Wort	Moderate 3A inhibitors	Concomitant use not recommended
Digoxin, cyclosporine, tacrolimus	P- glycoprotein substrate	Monitor serum concentrations of co- administered medications

Table2.1: Drug interactions and Dose management

Potential inhibition of CYP3A and P-glycoprotein may be seen with ivacaftor and its pharmacologically active metabolite, M1. [42] Increased systemic exposure of medications that are substrates of CYP3A or P-glycoprotein may increase therapeutic effects and likelihood of adverse events. Caution is recommended when substrates such as digoxin are co-administered with ivacaftor. [42] Several food-drug interactions exist as well. Foods containing 1 or more components that moderately inhibit CYP3A such as Seville oranges or grapefruit should be avoided as they may increase exposure of ivacaftor.[42]

Conclusion:

Ivacaftor (Kalydeco™) is a potentiator of the cystic fibrosis transmembrane conductance regulator (CFTR) and is the first drug that treats an underlying cause of cystic fibrosis to be licensed for use. Ivacaftor increases the open probability (i.e., gating) of CFTR channels with the G551D mutation, thus enhancing chloride transport, and is indicated in a number of countries for the treatment of cystic fibrosis in patients aged ≥6 years who carry this mutation. First drug that treats an underlying cause of cystic fibrosis to be licensed for use Increases the open probability (i.e. gating) of cystic fibrosis transmembrane conductance regulator channels with the G551D mutation, thus augmenting chloride transport Convenient oral administration Improves lung function and bodyweight parameters when used in combination with standard care in adults, adolescents and children (aged C6 years) with cystic fibrosis and the G551D mutation Generally well tolerated. Improves lung function by &3 % in patients with cystic fibrosis (aged C12 years) homozygous for theF508del-CFTR mutation. Reduces pulmonary exacerbations to a clinically meaningful extent, and increases BMI in some instances. In order to develop practical method for preparation of ivacaftor, 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2) and 5-amino-2,4-di-*t*-butylphenol (3) are prepared in new route, as shown in Schemes 3 and 4.

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