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An Overview of 3rd & 4th Generation EGFR Tyrosine Kinase Inhibitors for NSCLC - Emphasizing on Cell Lines Activities, SAR & Synthesis

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Shilpa Patel*, Gurubasavarajaswamy P.M

Department of Pharmaceutical Chemistry, Acharya & BM Reddy College of Pharmacy, Bangalore, India

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ABSTRACT

All the First-, Second- and Third-generation EGFR TKIs (Epidermal Growth Factor Receptor *Tyrosine Kinase* Inhibitors) developed to treat NSCLC (Non-Small Cell Lung Cancer) are ATP-competitive inhibitors and bind in the ATP binding site of mutant EGFR. T790M, an acquired mutation, is the major cause of First Generation EGFR TKIs ineffectiveness and it does so by increasing ATP binding affinity to EGFR and by decreasing the binding of inhibitors due to steric hindrance. Acrylamide group of Third-generation EGFR TKIs restores binding affinity of compounds and also improves selectivity over WT EGFR by forming an irreversible covalent bond with gatekeeper residue Met790 via Michael addition reaction. Recently, a tertiary mutation C797S has been reported to be the dominant cause of the Third-Generation TKIs resistance mechanism. EAI045 is so far the only Fourth-Generation TKI reported and it is an allosteric non-ATP competitive inhibitor. This article summarizes - strategies for Drug Development for NSCLC due to mutant EGFR, 3rd & 4th Generation EGFR TKIs, their SARs and synthesis, 4th Generation allosteric inhibitors to target C797S mutant EGFR, C797S mutation *in cis* and *in trans* with T790M & U-shaped configuration of 3rd Generation TKIs to Y-shaped configuration of 4th Generation TKI.

INTRODUCTION:

Cancer is the second leading cause of death worldwide, exceeded only by heart diseases, of which Lung cancer accounts for more than one-quarter of cancer-related death worldwide and is the most common and deadly cancer around the world. Lung cancer is the uncontrolled growth of abnormal cells in one or both lungs which do not carry out the function of normal lung cells and do not develop into healthy lung tissue. Lung cancer begins with a mutation or a series of mutations in a lung cell's DNA. DNA mutations can be caused by the normal aging process or through environmental factors, such as cigarette smoke, breathing in asbestos fibers, exposure to radiation, chemicals, and environmental carcinogen. [1]

There are two major types of lung cancer:

(1) Non-small cell lung cancer (NSCLC): Non-small cell lung cancer is the most common subtype of lung cancer, which accounts for about 80-85%. NSCLC includes Adenocarcinoma (the most common form of lung cancer), Squamous cell carcinoma (account for 25% of all lung cancer), and Large cell carcinoma (account for about 10% of NSCLC tumors) that originate from lung tissue. It occurs in both smokers and non-smokers.

(2) Small cell lung cancer (SCLC): It always begins in the bronchi and is rarely seen in non-smokers. SCLC rapidly metastasizes to other organs much faster than NSCLC types and can be fatal in a few weeks if untreated, in contrast to most cases of NSCLC metastases. SCLC accounts for 15% to 20% of lung cancer.

Also, the EGFR/ErbB1/HER1 (170-kd transmembrane protein), belonging to a family of receptor tyrosine kinases (RTKs) can trigger a vast array of signaling pathways leading to cell growth, proliferation, survival, migration, adhesion, and differentiation and is considered one of the most valuable drug target for the treatment of NSCLC. [2]

Moreover, studies reveal that overexpression of EGFR is closely linked to tumorigenesis while activating mutations in the EGFR tyrosine kinase domain (L858R mutation and exon-19 deletion) have been identified as oncogenic drivers for NSCLC.

First-generation TKIs (Tyrosine Kinase Inhibitors), Gefitinib, Erlotinib are effective for the treatment of EGFR activating mutations. However, patients typically develop secondary "T790M gatekeeper" drug resistance after about 12 months of treatment. Also, the clinical

application of Second-generation irreversible inhibitor Afatinib is limited due to its poor therapeutic window and its off-target affinity (WT- EGFR).

The third-generation EGFR inhibitors such as Osimertinib and Olmutinib have been developed for effective management of T790M associated resistance while sparing WT-EGFR (wild-type EGFR). However, the selectivity of Osimertinib to mutant kinases has not achieved the desired effect. It's binding to non-target EGFR receptors *in vivo* has caused serious side effects (diarrhoea, rash, and cardiotoxicity), which severely limits the clinical application of Osimertinib. Also, a tertiary C797S point mutation leading to drug resistance has been reported. [3,4]

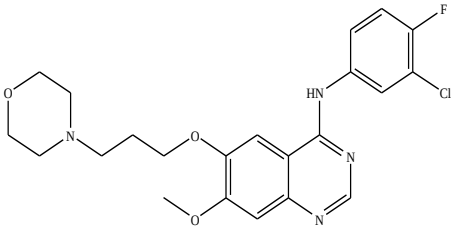
EAI045 (a Fourth-Generation EGFR inhibitor to overcome C797S mutation) is so far the first allosteric TKI designed to overcome T790M & C797S mutations. However, EAI045 is ineffective alone due to receptor dimerization. Combined with Cetuximab, EAI045 turns fully active against T790M & C797S. [5]

This article summarizes - strategies for Drug Development for NSCLC due to mutant EGFR, 3rd & 4th Generation EGFR TKIs, their SARs and synthesis, 4th Generation allosteric inhibitors to target C797S mutant EGFR, C797S mutation *in cis* and *in trans* with T790M & U-shaped configuration of 3rd Generation TKIs to Y-shaped configuration of 4th Generation TKI.

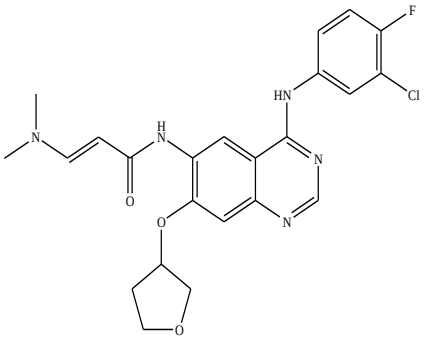
EGFR TKIs (Figure 1) developed for the treatment of NSCLC and the problems associated with them are summarized in Table 1.

Table No. 1:

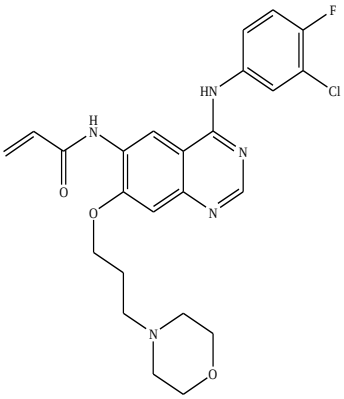
EGFR TKIs	Examples	Problems associated
First generation	Gefitinib, Erlotinib, Icotinib	Drug resistance due to secondary mutation T790M
Second generation	Afatinib, Canertinib, Dacomitinib	Off-target affinity
Third generation	Osimertinib, Rociletinib, Olmutinib	Reduced binding affinity due to tertiary mutation C797S and increased toxicity
Fourth generation	EAI045	Ineffective alone



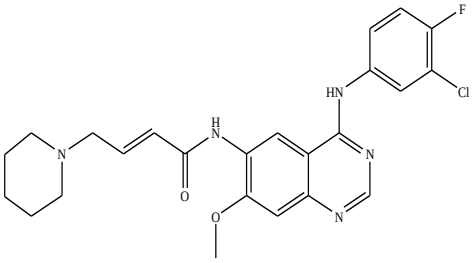
Gefitinib



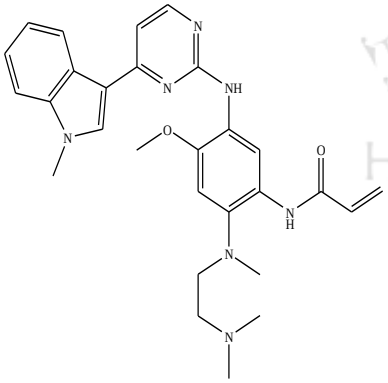
Afatinib



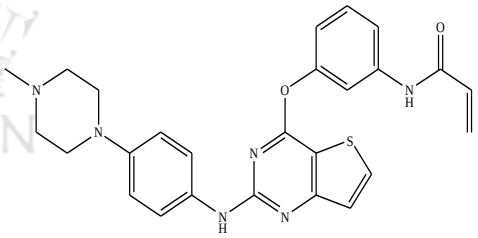
Canertinib



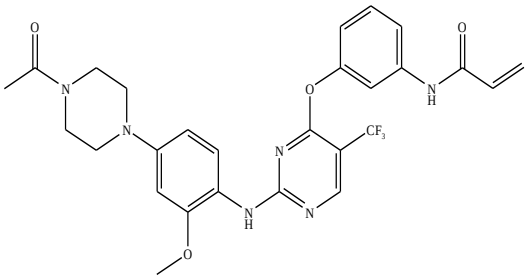
Dacomitinib



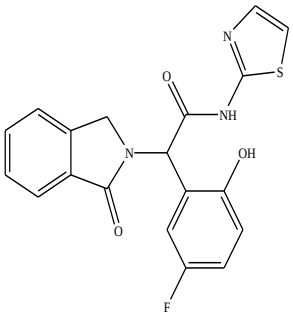
Osimertinib



Olmutinib



Rociletinib



EAI045

Figure No. 1: EGFR TKIs.

EGFR MUTATIONS AND RESISTANCE [6]

Activating mutations (AMs) in the EGFR gene are located in the first four exons 18-21 of the tyrosine kinase domain as shown in Figure 2. These activating mutations can be categorized into 3 main classes.

Class I

In-frame deletions in exon 19, E.g. exon 19 del (E746-A750).

Class II

Point mutations in exon 21 (E.g. L858R) & in exon 18 (E.g. G719S/A/C)

Class III

In-frame duplication and/or insertions in exon 20.

Various mutations leading to NSCLC and their locations are displayed in Table 2.

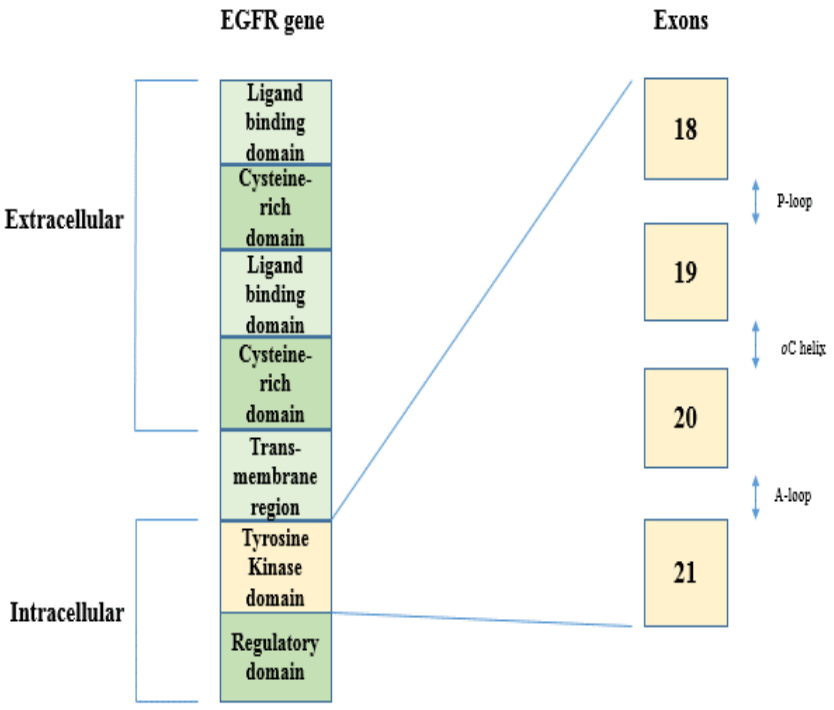


Figure 2: EGFR TK

Table No. 2.

Mutation	Location	Resistance
Primary mutations (activating mutations) [L858R, G719S/A/C, E746-A750 del]	L858R : exon 21 G719S/A/C: exon 18 E746-A750 del : exon 19	Intrinsic resistance
Secondary mutation [T790M]	T790M: exon 20	Acquired resistance
Tertiary mutation [C797S]	C797S : exon 20	Acquired resistance

STRATEGIES FOR DRUG DEVELOPMENT TARGETING NSCLC [7]

STRATEGY 1: Covalent/irreversible inhibition

Examples: Second- and Third-Generation EGFR TKIs (Afatinib, WZ4002, AZD9291, CO1686, Olmutinib, etc.) carry acrylamide (Michael acceptor) group to irreversibly alkylate residue Cys797 and thus eliminate the problem of enhanced ATP binding.

STRATEGY 2: Super-high-potency non-covalent/reversible inhibition

Examples: First-Generation EGFR TKIs (Gefitinib, Erlotinib) binds non-covalently to ATP-binding sites in the L858R mutant EGFR.

STRATEGY 3: Non-ATP-competitive inhibition

Example: EAI045, an allosteric non-ATP competitive inhibitor.

3rd & 4th GENERATION EGFR TKIs

1) WZ4002

WZ4002 (Figure 3), an Anilinopyrimidine scaffold containing compound, is a selective mutant-EGFR inhibitor with IC_{50} values of 2, 8, 3, and 2 nM for L858R EGFR, L858R/T790M EGFR, E746-A750 EGFR and E746-A750/T790M EGFR, respectively. WZ4002 increases cellular potency correlated with inhibition of EGFR, AKT, and ERK1/2 phosphorylation in NSCLC cell lines and EGFR phosphorylation in NIH-3T3 cells expressing different $EGFR^{T790M}$ mutant alleles. WZ4002 inhibits EGFR kinase activity of recombinant L858R/T790M protein more potently than of WT EGFR. [8]

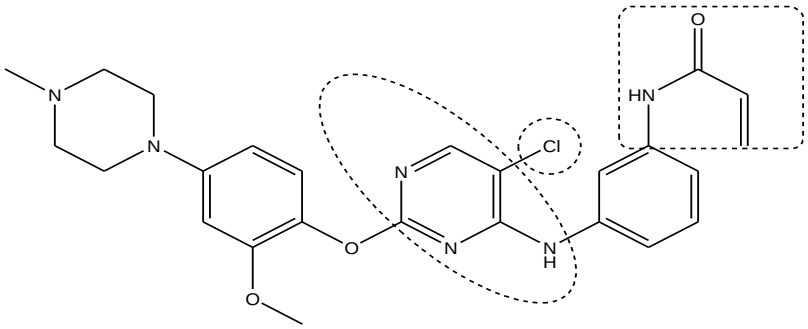


Figure No. 3: WZ4002

SAR of WZ4002

- Anilinopyrimidine core forms two H-bonds with the residue Met793 in the hinge region.
- Acrylamide binds with the residue Cys797 through a covalent bond.
- The chlorine group interacts with the gatekeeper Met790, improving selectivity for L858R/T790M EGFR over WT EGFR.

2) CHMFL-EGFR-202

CHMFL-EGFR-202 (Figure 4), pyrazolopyrimidine scaffold, is a potent, irreversible inhibitor of EGFR mutant kinase, with IC_{50} of 5.3 nM and 8.3 nM for drug-resistant mutant EGFR T790M and WT EGFR kinases, respectively. CHMFL-EGFR-202 exhibit ~10-fold selectivity for EGFR L858R/T790M against the EGFR wild-type in cells. CHMFL-EGFR-202 adopts a covalent "DFG-in-C-helix-out" inactive binding conformation with EGFR, with strong antiproliferative effects against EGFR mutant-driven NSCLC cell lines.

Wang A *et al*., reported the synthesis of CHMFL-EGFR-202 as depicted in Scheme 1. [9]

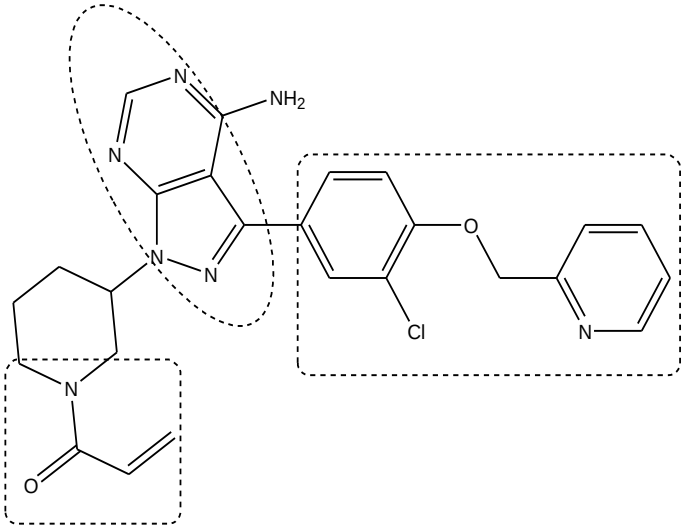


Figure No. 4: CHMFL-EGFR-202

SAR of CHMFL-EGFR-202

- Pyrazolopyrimidine forms two hydrogen bonds with Met793 and Gln791 residues in the hinge binding area.
- The acrylamide group forms a covalent bond with Cys797 to achieve irreversible binding.
- 3-chloro-4-(pyridine-2-ylmethoxy)phenyl group is flexible and large enough to accommodate hydrophobic pocket generated by the C-helix-out conformation, thus improving binding affinity and selectivity against EGFR T790M mutant. This also improves compound potency. [9]

3) EGFR mutant-IN-1

EGFR mutant-IN-1 (Figure 5), a 5-methylpyrimidopyridone derivative, is a potent and selective $EGFR^{L858R/T790M/C797S}$ mutant inhibitor with an IC_{50} of 27.5 nM while being significantly less potent for $EGFR^{WT}$ (IC_{50} >1.0 µM).

Shen J *et al*., reported the synthesis of EGFR mutant-IN-1 as depicted in Scheme 2. [10]

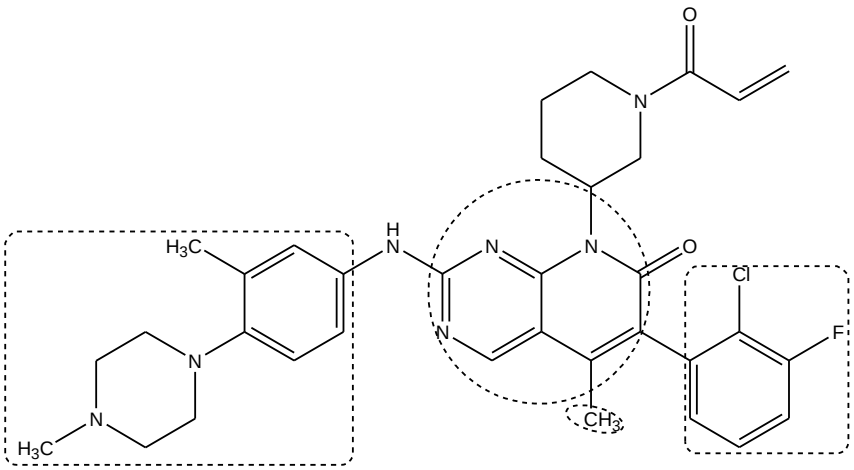


Figure No. 5: EGFR mutant-IN-1

SAR of EGFR mutant-IN-1

➢ 5-methylpyrimidopyridone forms the classical H-bond with the residue Met793, in the hinge region.

➢ 6-(2'-chloro-3'-fluoro)phenyl group dwells in a hydrophobic pocket formed by the residues Lys745, Glu762, Leu788, Met766, and Met790.

➢ 4-methylpiperazinylphenyl and propionylpiperidine are directly approachable to the solvent surface and influence the physicochemical properties of the compounds.

➢ 5-methyl group contacts Met790 within a distance of 3.5A° in mutant EGFR T790M/C797S to form a favorable hydrophobic interaction.

➢ The hydrophilic property of Thr790 weakens the hydrophobic interactions of the 6-(2'-chloro-3'-fluoro)phenyl group with WT EGFR, thus increasing its selectivity for mutant EGFR. [10]

4) TAS6417

TAS6417 (Figure 6) is an EGFR inhibitor and an efficacious drug candidate for patients with NSCLC, with IC_{50} values ranging from 1.1-8.0 nM. TAS6417 causes persistent tumor regression *in vivo* in EGFR exon 20 insertion-driven tumor models. TAS6417 inhibits mutant EGFR in tumors but not WT EGFR in skin tissues. TAS6417 prolongs survival of animals bearing lung cancer. [11]

The synthesis of TAS6417 has been depicted in Scheme 3. [23]

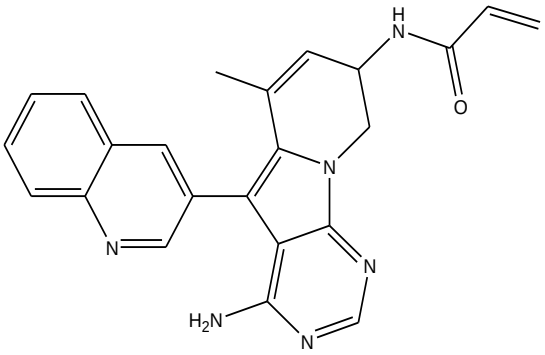


Figure No. 6: TAS6417

5) Nazartinib [EGF816]

Nazartinib [EGF816] (Figure 7), 2-aminobenzimidazole scaffold, is an irreversible, mutant-selective EGFR TKI, with K_i and K_{inact} of 31 nM and 0.222 min^{-1} on L858R/T790M EGFR, respectively. Nazartinib potentially & selectively inhibits mutant-EGFR cell lines with IC_{50} values of 4 nM, 6 nM, and 2 nM in H1975, H3255 and HCC827 respectively, and exhibits improved PK and ADME properties. Nazartinib exhibits potent inhibition of phosphorylated EGFR levels in H3255, HCC827, and H1975 cell lines with EC_{50} values of 5 nM, 1 nM, and 3 nM respectively. Nazartinib prevents cell growth with EC_{50} values of 9 nM, 11 nM, and 25 nM in H3255, HCC827, and H1975, respectively. Nazartinib has an OC_{50} (compound concentration at 50% occupancy) value of 2 nM and 5 nM on HCC827 and H1975 respectively. [12,13]

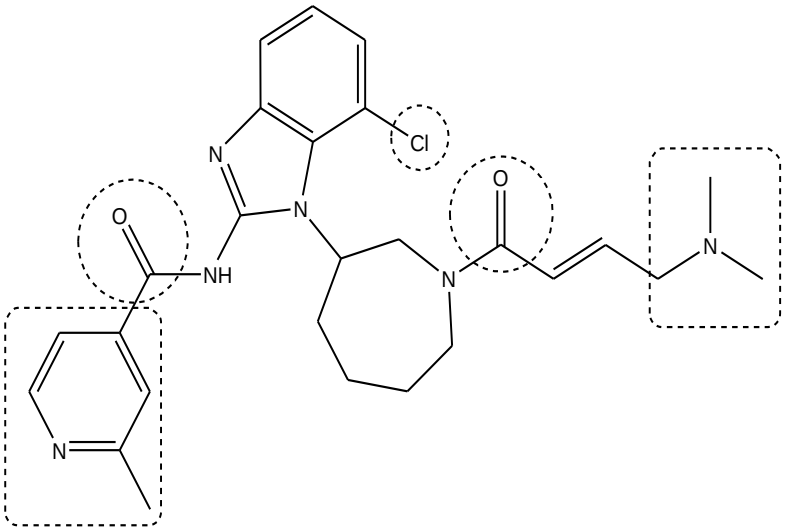


Figure No. 7: Nazartinib

SAR of Nazartinib

- The carbonyl group of amide linker binds with the residue Met793 in the hinge region by forming an H-bond.

- The carbonyl group of acrylamide moiety forms an additional H-bond with the backbone amide of Cys797.

- 7-chloro group, dimethyl methylamino group, and methyl pyridyl group improve PK and ADME properties.

6) Rociletinib [(CO-1686]

Rociletinib [CO-1686] (Figure 8), 2, 4-diamino-5-trifluoromethyl pyrimidine scaffold, is an orally administered TKI that selectively targets the activating mutation L858R mutant EGFR along with secondary mutation T790M mutant EGFR, and the K_i values for EGFR (L858R/T790M) and EGFR (WT) are 21.5 nM and 303.3 nM respectively. Rociletinib (0.1 μM) selectively and potently inhibits more than 50% of 23 targets. Rociletinib prevents the proliferation of NSCLC cells harboring mutant-EGFR, both active and acquired, and induces apoptosis. [14]

Baevsky MF *et al*., reported the synthesis of Rociletinib as depicted in Scheme 4. [24]

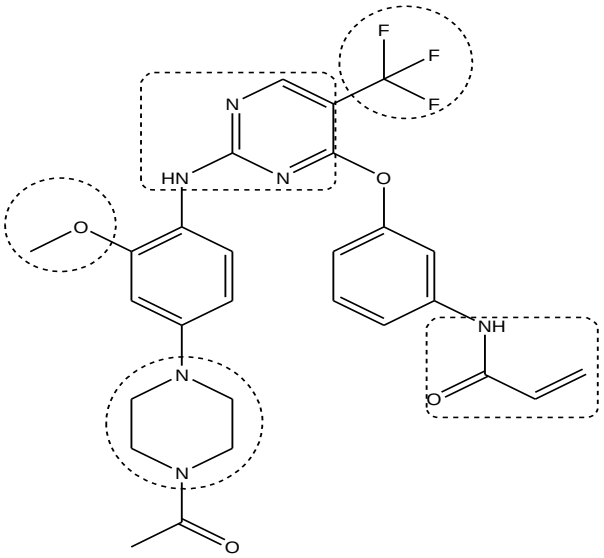


Figure No. 8: Rociletinib

SAR of Rociletinib

➢ Trifluoromethyl group at 5th position of pyrimidine reaches the hydrophobic gatekeeper Met790 residue and thus contributes to improving Rociletinib potency.

➢ Anilinopyrimidine binds to the ATP-binding site of mutant EGFR by two H-bonds, in the hinge region.

➢ Acrylamide group binds covalently and irreversibly at the edge of the hydrophobic pocket to the cysteine residue Cys797.

➢ Piperazine, and a methoxy group located ortho to the aniline ring, has been observed to increase tyrosine kinase selectivity.

7) Osimertinib [AZD9291]

Osimertinib [AZD9291] (Figure 9), an amino pyrimidine scaffold, is an irreversible and mutant-selective EGFR TKI with an IC_{50} value of 12 nM and 1 nM against L858R EGFR and L858R/T790M EGFR, respectively. Osimertinib exhibits similar potency to 1st & 2nd Generation TKIs in inhibiting EGFR phosphorylation in mutant-EGFR cell lines expressing activating mutations such as PC-9 (exon-19 del), H3255 (L858R) and H1650 (exon-19 del), with IC_{50} values ranging from 13 nM to 54 nM. Osimertinib also potently inhibits EGFR phosphorylation in T790M-mutant cell lines such as H1975 (L858R/T790M), PC-9VanR (exon-19 del/T790M), with IC_{50} values less than 15 nM. [15,16]

Butterworth S *et al.*, reported the synthesis of Osimertinib as depicted in Scheme 5. [25]

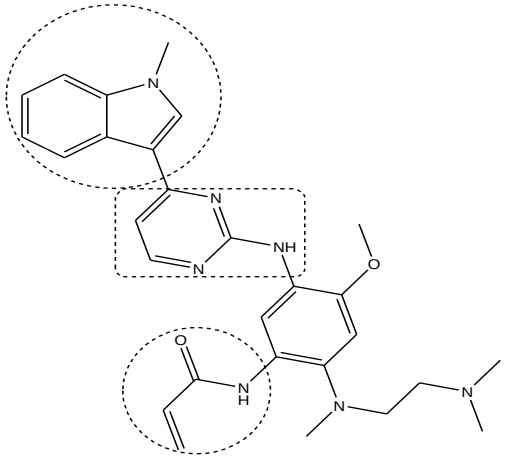


Figure No. 9: Osimertinib (AZD9291)

SAR of Osimertinib

➢ The acrylamide group binds covalently and irreversibly at the edge of the hydrophobic pocket to the cysteine residue Cys797.

➢ Methylated indole group, observed adjacent to the hydrophobic gatekeeper Met790 in molecular modeling studies of AZD9291 is responsible for increased selectivity for L858R/T790M EGFR over WT EGFR.

➢ Anilinopyrimidine binds to the ATP-binding site of mutant-EGFR, in the hinge region.

8) Olmutinib [HM61713; BI-1482694]

Olmutinib (Figure 10), thieno[3,2-d]pyrimidine, is an irreversible EGFR TKI that binds near the kinase domain to a cysteine residue Cys797. Olmutinib potentially inhibits mutant EGFR in cell lines - HCC827 [del19 EGFR (IC_{50}=9.2 nM)] and H1975 [L858R/T790M EGFR (IC_{50}=10 nM)]. Unlikely, the IC_{50} value of Olmutinib against cell lines expressing WT EGFR is 2225 nM. [17]

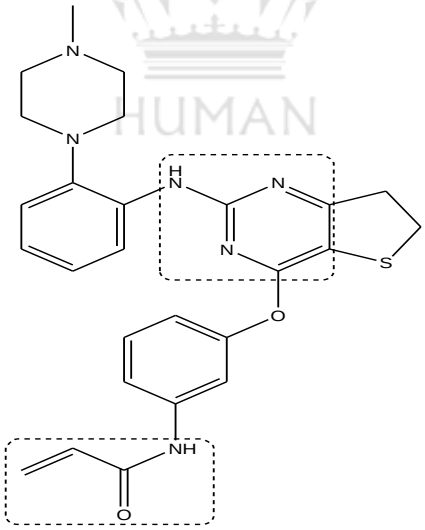


Figure No. 10: Olmutinib

SAR of Olmutinib

➢ Anilinopyrimidine binds to the ATP-binding site of mutant EGFR by two H-bonds, in the hinge region.

➢ The acrylamide group binds covalently and irreversibly at the edge of the hydrophobic pocket with the cysteine residue Cys797.

9) PF-06459988

PF-06459988 (Figure 11), a pyrrolopyrimidine scaffold containing compound, is an irreversible inhibitor of T790M EGFR with IC_{50} values of 13 nM and 5.1 µM against H1975 (EGFRT790M/L858R) and A549 (WT EGFR) cell lines, respectively. [18]

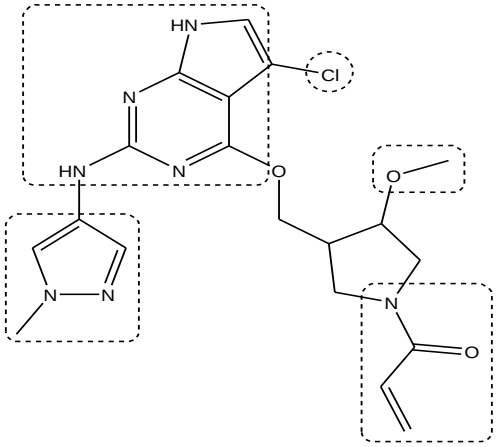


Figure No. 11: PF-06459988

SAR of PF-06459988

➢ PF-06459988 binds at the ATP pocket with a U-shaped binding conformation.

➢ Aminopyrrolopyrimidine scaffold forms three H-bonds with the residues Gln791 and Met793 in the hinge region.

➢ Acrylamide binds with the residue Cys797 through a covalent bond.

➢ The chlorine group interacts with the gatekeeper Met790 and residue Phe856 through hydrophobic interactions.

➢ Methoxy group of the moiety pyrrolidine occupies a hydrophobic pocket formed by the residues Phe856 and Phe723.

➢ Methylpyrazole points to the solvent-accessible region.

10) Mavelertinib [PF-06747775]

Mavelertinib (Figure 12), an orally administered EGFR TKI, possesses potential anti-neoplastic activity. It binds specifically to mutant EGFR T790M and effectively inhibits the growth of NSCLC cells by preventing EGFR-mediated signaling in EGFR T790M-expressing tumor cells. Compared to some other EGFR TKIs, Mavelertinib has therapeutic benefits in tumors with T790M-mediated drug resistance. It exhibits minimal activity against WT EGFR and does not cause dose-limiting toxicities. [19, 20]

Behenna DC *et al*., reported the synthesis of Mavelertinib as depicted in Scheme 6. [26, 27]

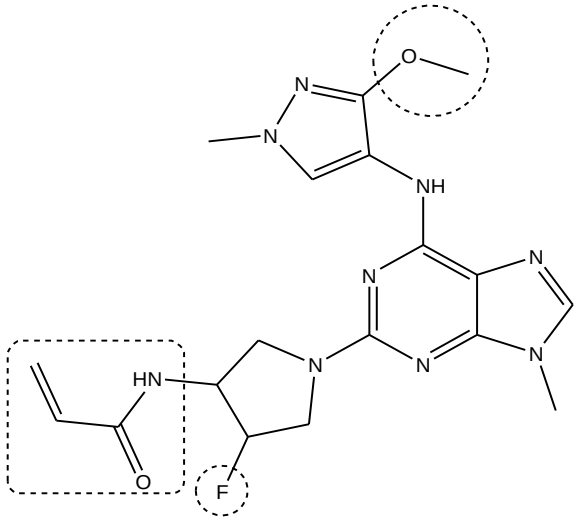


Figure No. 12: Mavelertinib (PF-06747775)

SAR of Mavelertinib

➢ Pyrrolidine-linked acrylamide increases compound potency by forming a covalent bond with residue Cys797.

➢ Fluorine atom at the 4th position of the pyrrolidine group causes a gauche orientation and improves binding affinity.

➢ Methoxy group at 2nd position of N1-methyl pyrazole targets hinge residue Leu792 and maintains potency and selectivity.

11) EAI045

EAI045 (Figure 13) is a 4th generation allosteric inhibitor of mutant-EGFR with IC_{50} values of 1.9 μM, 0.019 μM, 0.19 μM and 0.002 μM for EGFR, L858R EGFR, T790M EGFR and L858R/T790M EGFR respectively, at 10 μM ATP.

EAI045 potentially inhibits EGFR phosphorylation in the H1975 cell line (EC_{50} = 2 nM), it inhibits L858R/T790M mutant EGFR with 1000-fold selectivity over WT EGFR at 1 mM ATP. In H1975 [L858R/T790M] cell line, EAI045 activity decreases due to its inability to completely abolishing the EGFR phosphorylation. In the H3255 [L858R] cell line, EAI045 shows moderate activity.

EAI045 (a Fourth-Generation EGFR inhibitor to overcome C797S mutation) is so far the first allosteric TKI designed to overcome T790M & C797S mutations. However, EAI045 is ineffective alone due to receptor dimerization. Combined with Cetuximab EAI045 turns fully active against T790M & C797S.

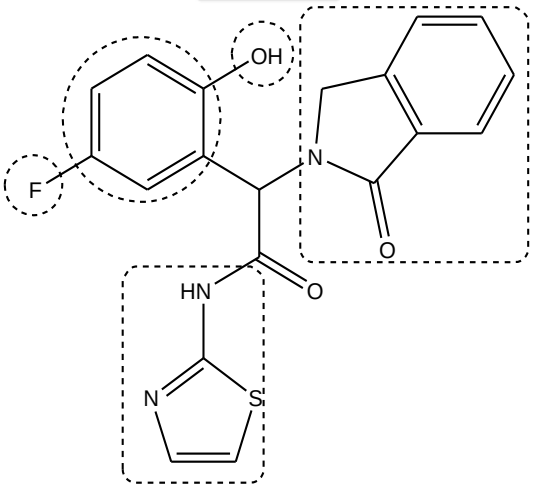
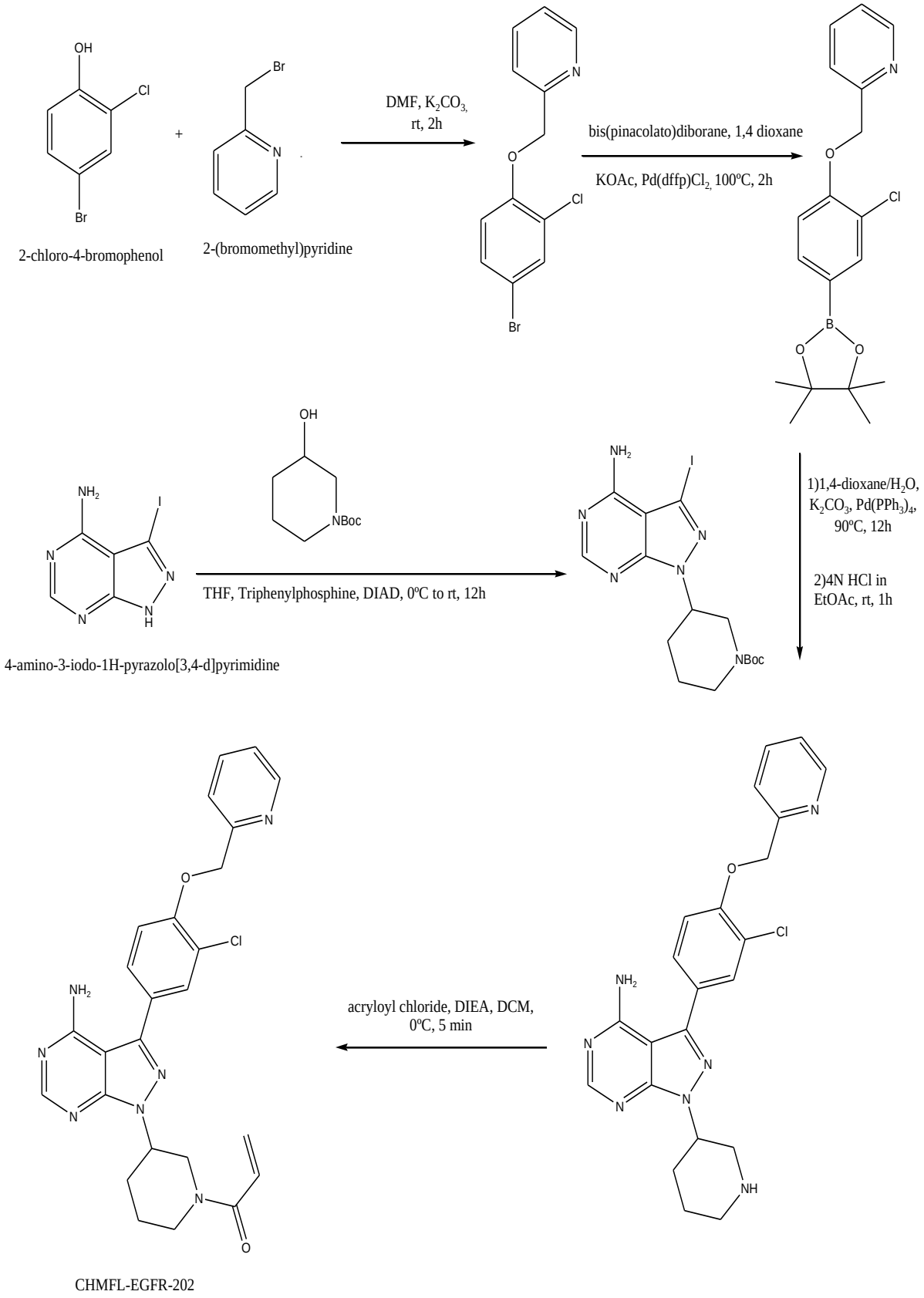


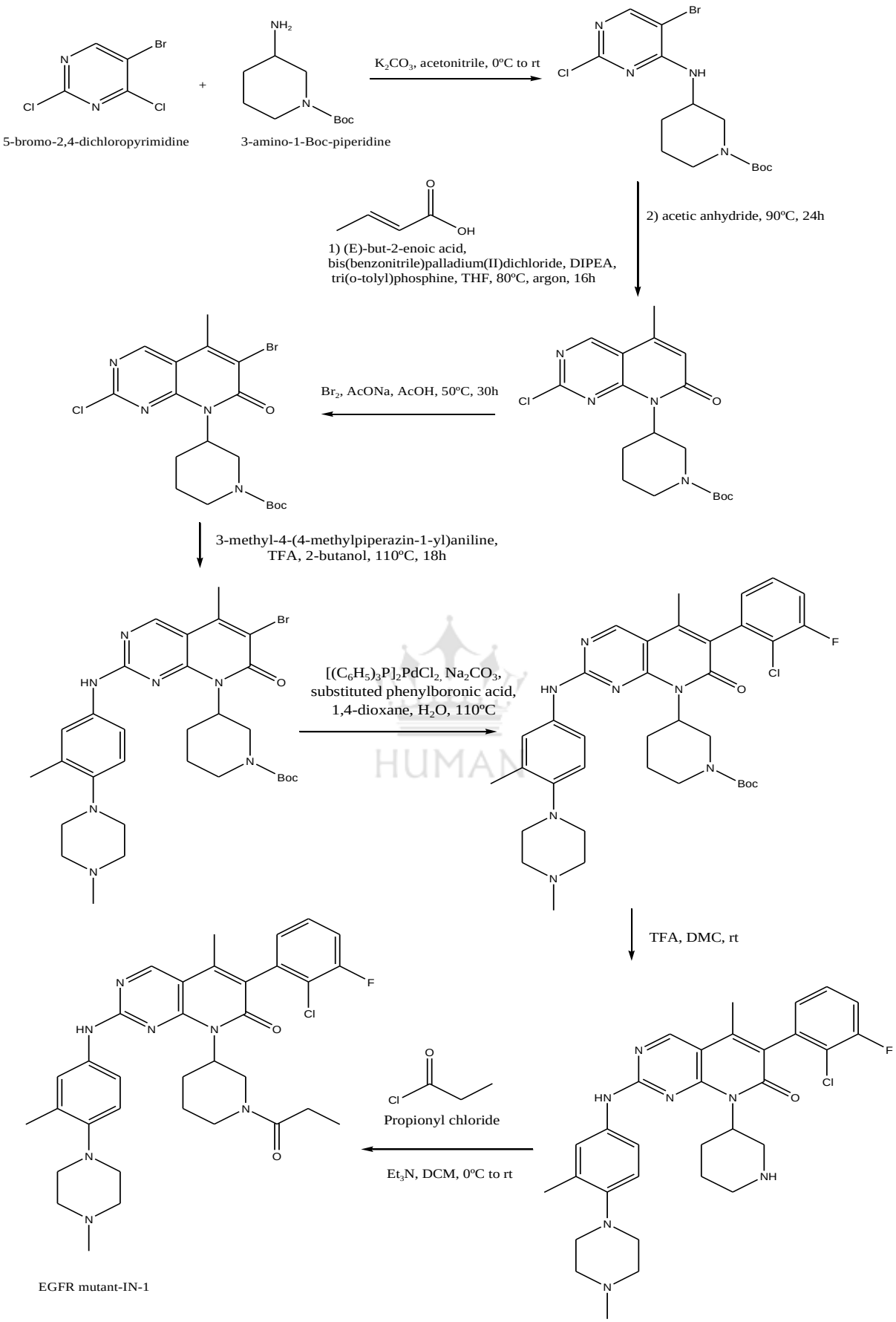
Figure No. 13: EAI045

SAR of EAI045

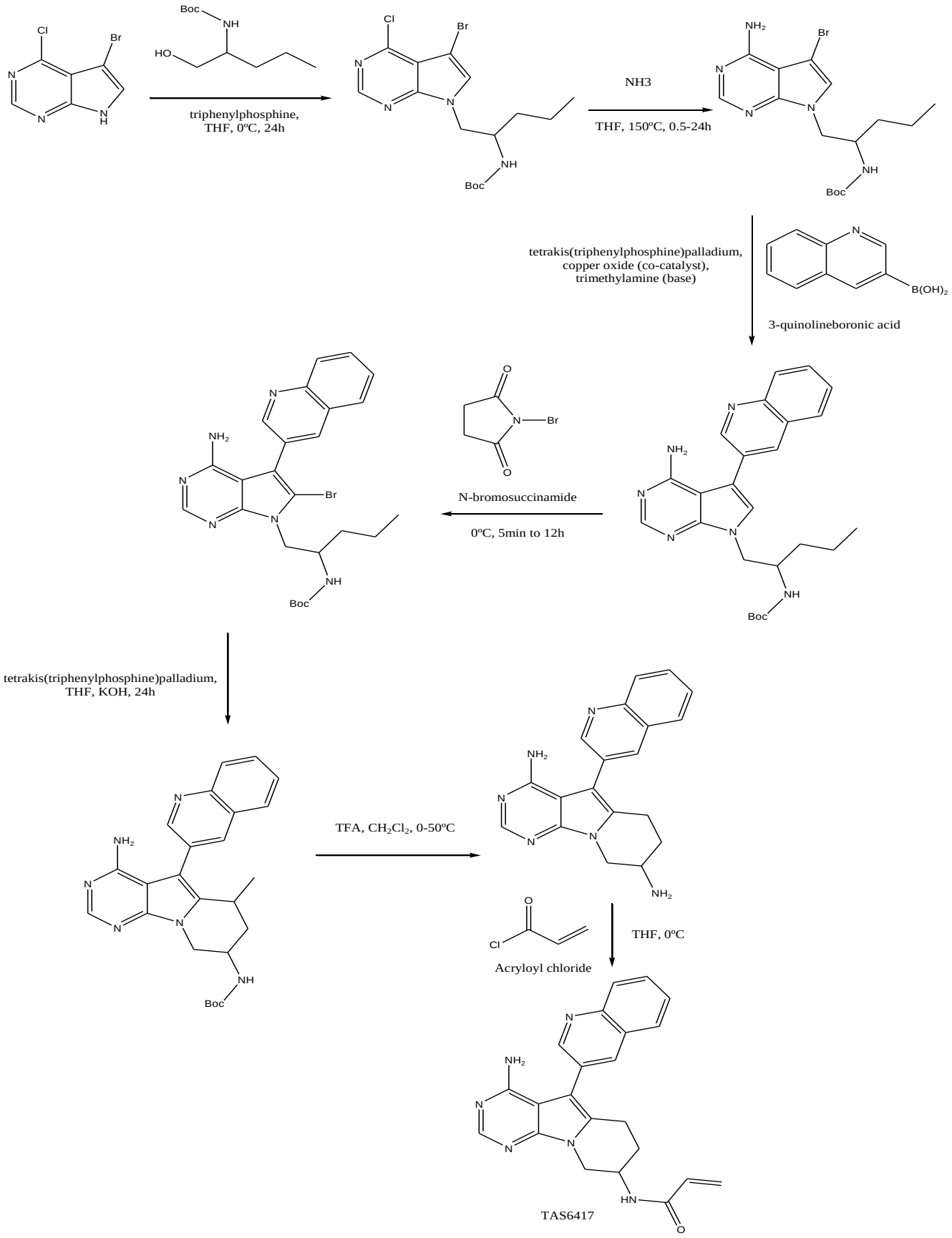
- The Aminothiazole group of EAI045 makes direct proximity to the lipophilic Gatekeeper Met790 residue.
- 1-oxoisoindolinyl reaches to the solvent-accessible region.
- The pendent phenyl group dwells in a small hydrophobic pocket formed by the residues Met766, Leu777, and Phe856.
- 3'-fluoro group and 5'-hydroxy group increase mutant inhibitory potency and selectivity. [21, 22]



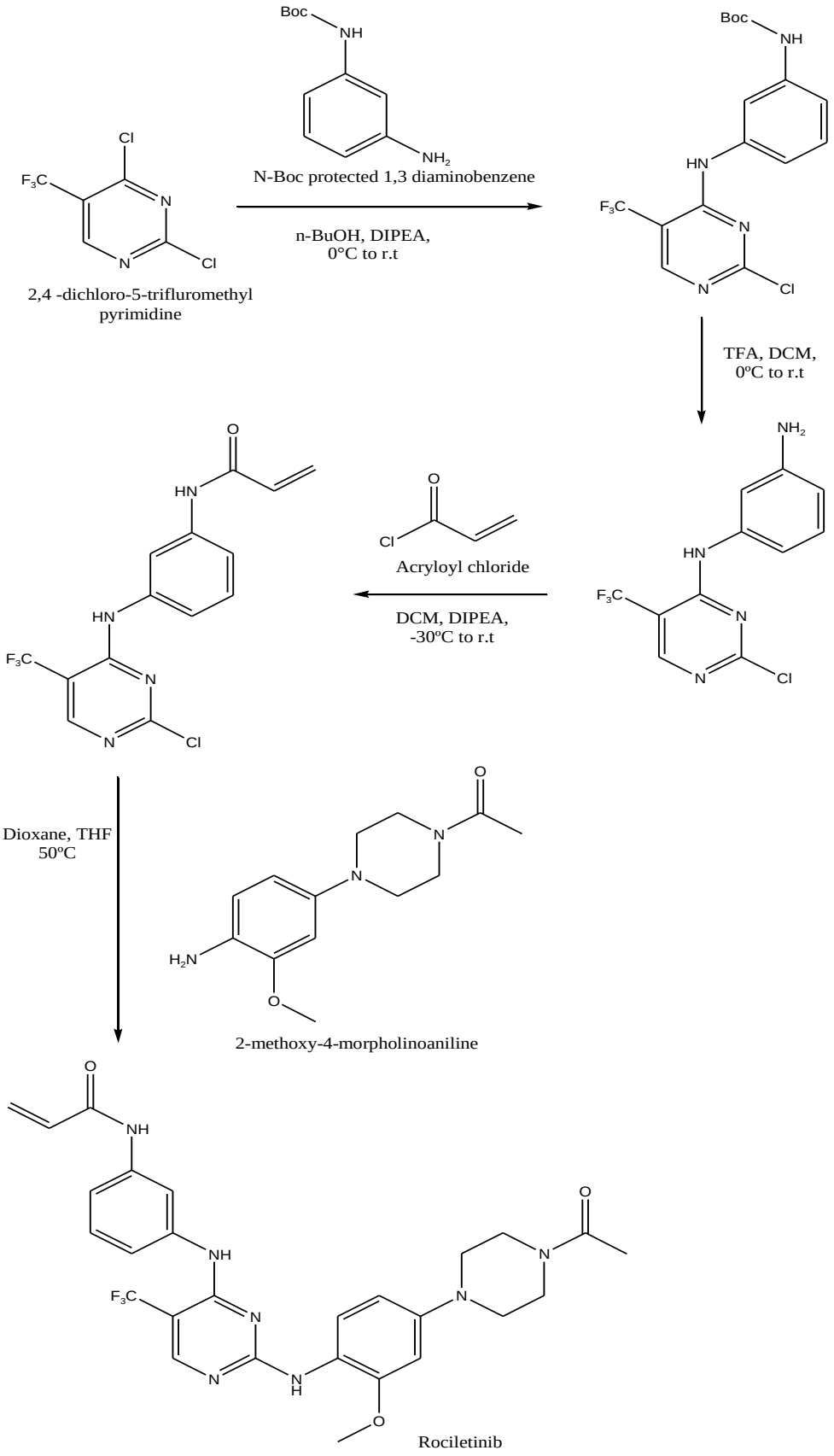
Scheme 1: Synthesis of CHMFL-EGFR-202 [9]



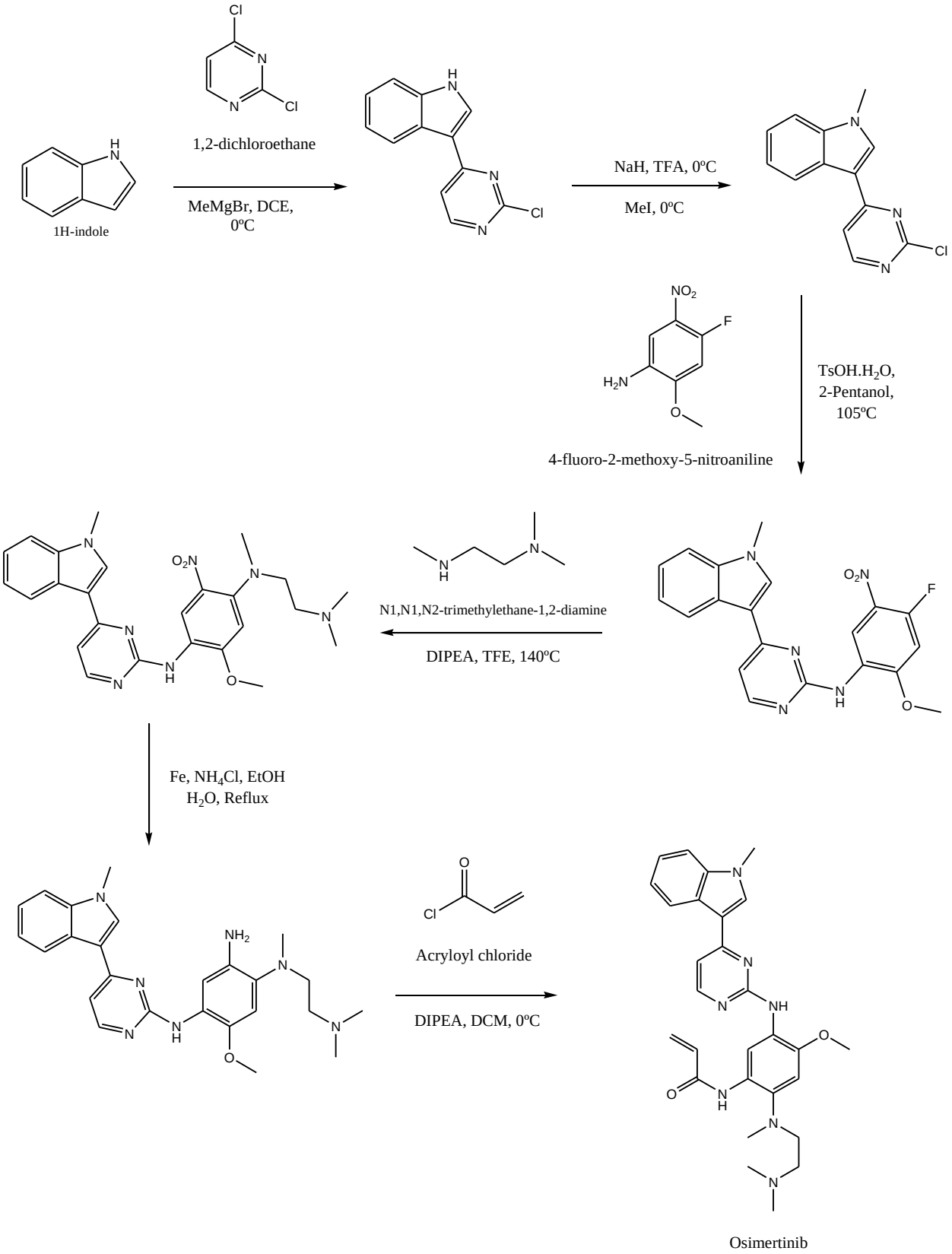
Scheme 2: Synthesis of EGFR mutant-IN-1 [10]



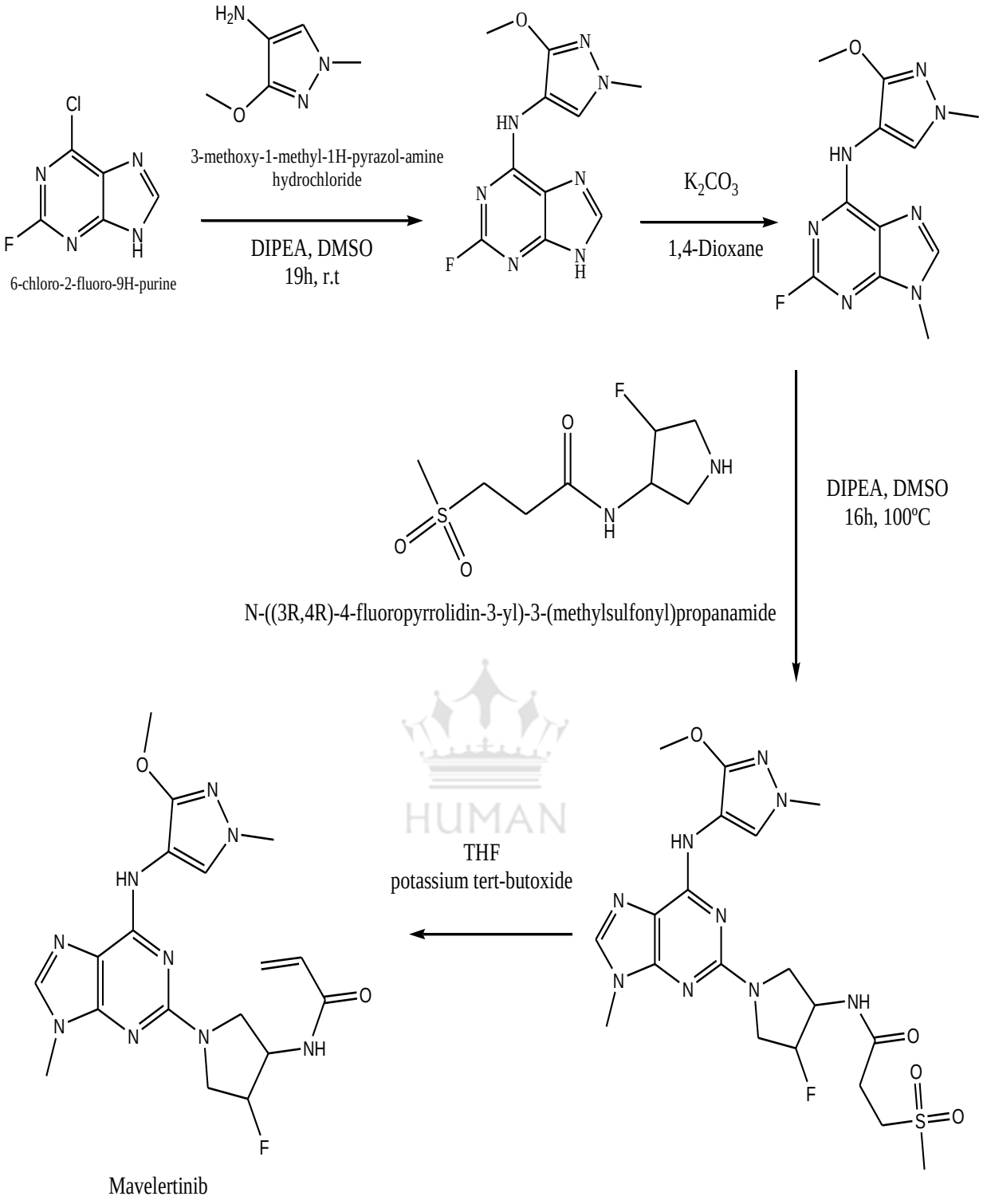
Scheme 3: Synthesis of TAS6417 [23]



Scheme 4: Synthesis of Rociletinib [24]



Scheme 5: Synthesis of Osimertinib [25]



Scheme 6: Synthesis of Mavelertinib [26, 27]

C797S mutation *in cis* and *trans* with T790M

Tertiary acquired mutation C797S in EGFR is a novel mechanism of drug resistance to 3rd generation EGFR TKIs such as Olmutinib, Rociletinib, Nazartinib and Osimertinib. Piotrowska *et al*. evaluated the Guardant Health database of plasma samples of lung adenocarcinoma patients with C797S mutation. The patients had acquired T790M mutation and were treated with Osimertinib. The study findings showed C797S/T790M *in cis* in 82% patients; C797S/T790M *in trans* in 10% patients; C797S alone without T790M in 6% patients & two co-existed C797S clones (one *in cis* with T790M and one *in trans*) in 2% patients. Also, 84% of patients had at least one bonafide resistance mechanism co-occurring with C797S, namely EGFR amplification (48%), MET amplification (16%), BRAF V600E (5%) and PIK3CA mutation (15%). [28]

The context in which the C797S develops concerning the other EGFR alleles impacts the efficacy of subsequent treatments. Niederst *et al*. studied the mechanism in which the allelic context of the C797S mutation acquired upon treatment with 3rd Generation EGFR TKIs impacts sensitivity to subsequent treatment strategies and the study results show that the C797S and T790M mutations *in trans* renders 3rd generation EGFR TKIs ineffective, but responds to a combination of 1st & 3rd generation TKIs. The mutations *in cis* are unresponsive to any EGFR TKIs alone or in combination. If C797S develops in WT EGFR (when third-generation TKIs are administered in the first-line setting), the cells become resistant to 3rd generation EGFR TKIs but show the response to 1st generation EGFR TKIs. [29]

U-shaped configuration of 3rd Generation TKIs to combat T790M EGFR resistance (to) Y-shaped configuration of 4th Generation TKIs to combat C797S EGFR resistance

Studies on X-ray crystallographic co-crystal structures of AZD9291 (PDB code: 3IKA) [30] & CO-1686 (PDB code: 5UWD) [31] in complex with mutant EGFR (T790M) reveals that 3rd generation EGFR TKIs bind to mutant EGFR with a U-shaped configuration. Common in all these TKIs, aminopyrimidine scaffold interacts with residue Met793 by two hydrogen bonds in the hinge region in the ATP-binding site in mutant EGFR and acrylamide group forms an irreversible covalent bond with residue Cys797 via Michael addition reaction.

EAI045, the only 4th generation TK reported to date, binds to an allosteric site in a mutant EGFR with a Y-shaped configuration as revealed by studies of the co-crystal structure of EAI045 (PDB code: 5D41) [32] in complex with T790M EGFR kinase domain.

SUMMARY

Irreversible **covalent binding** of **Michael acceptor (acrylamide group)** of 3rd Generation TKIs with **CYS797** has resulted in **acquired mutation C797S** wherein Cysteine at position 797 is substituted by Serine. Michael acceptor group plays a significant role in 3rd Generation TKIs development, its covalent binding to receptor restores compound affinity which is lost in the case of 1st Generation TKIs due to T790M mutation and thus improves potency.

Hydrogen bondings are observed in the **hinge region** between residue **MET793** and anilinopyrimidine (in AZD9291, CO-1686, HM61713, WZ4002), pyrazolopyrimidine (in CHMFL-EGFR-202). Aminopyrrolopyrimidine scaffold in PF-06459988 forms three H-bonds with the hinge residues **MET793** & **GLN791**. Methoxy group at 2nd position of N1-methyl pyrazole in Mavelertinib targets hinge residue **LEU792**.

The hydrophobic pocket formed by **residues LYS745, GLU762, LEU788, MET766, and MET790** is occupied by 6-(2'-chloro-3'-fluoro)phenyl group in EGFR mutant-IN-1.

The solvent-accessible region is targeted by Methylpyrazole in PF-06459988 and by the 1-oxoisoindolinyl group in EAI045.

Gatekeeper residue MET790 targeted by aminothiazole in EAI045, methyl on 5th position of pyrimidopyridone scaffold in EGFR mutant-IN-1, methylated indole in Osimertinib, trifluoromethyl at 5th position of the pyrimidine ring in Rociletinib and Cl group in PF-06459988 improves selectivity over WT EGFR.

EAI045 (a Fourth-Generation EGFR inhibitor to overcome C797S mutation) is so far the first **allosteric TKI** designed to overcome T790M & C797S mutations. However, EAI045 is ineffective alone due to receptor dimerization. Combined with **Cetuximab** EAI045 turns fully active against T790M & C797S.

The C797S and T790M mutations *in trans* renders 3rd generation EGFR TKIs ineffective but responds to a combination of 1st & 3rd generation TKIs. The mutations *in cis* are unresponsive to any EGFR TKIs alone or in combination.

3rd generation EGFR TKIs bind to mutant EGFR with a U-shaped configuration, whereas EAI045, the only 4th Generation TK reported to date, binds to an allosteric site in a mutant EGFR with a Y-shaped configuration.

REFERENCES

1. Chen L, Fu W, Zheng L, Liu Z, Liang G. Recent progress of small-molecule epidermal growth factor receptor (EGFR) inhibitors against C797S resistance in non-small cell lung cancer. J Med Chem. 2018;61:4290-300.
2. Lu X, Yu L, Zhang Z, Ren X, Smaill JB, Ding K. Targeting $EGFR^{L858R/T790M}$ and $EGFR^{L858R/T790M/C797S}$ resistance mutations in NSCLC: Current developments in medicinal chemistry. Med Res Rev. 2018;38:1550-81.
3. Karachaliou N, Fernandez-Bruno M, Bracht JWP, Rosell R. EGFR first- and second-generation TKIs- there is still place for them in EGFR-mutant NSCLC patients. Trasl Cancer Res. 2018;8:S23-S47.
4. Patel H, Pawara R, Ansari A, Surana S. Recent updates on third generation EGFR inhibitors and emergence of fourth generation EGFR inhibitors to combat C797S resistance. Eur J Med Chem. 2017;142:32-47.
5. Wang S, Song Y, Liu D. EAI045: The fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. Cancer Lett. 2017;385:51-4.
6. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene. 2019;28:S24-S31.
7. Zhu S, Zhao P, Yang J, Ma R, Yan X, Yang X, *et al*. Structural insights into drug development strategy targeting EGFR T790M/C797S. Oncotarget. 2018;9:13652-65.
8. Sakuma Y, Yamazaki Y, Nakamura Y, Yoshihara M, Matsukuma S, Nakayama H, *et al*.WZ4002, a third-generation EGFR inhibitor, can overcome anoikis resistance in EGFR-mutant lung adenocarcinomas more efficiently than Src inhibitors. LaboratoryInvestigation. 2012;92:371–83. doi: 10.1038/labinvest.2011.187
9. Wang A, Li X, Wu H, Zou F, Yan X, Chen C, *et al.* Discovery of (R)-1-(3-(4-Amino-3-(3-chloro-4-(pyridine-2-ylmethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)prop-2-en-1-one(CHMFL-EGFR-202) as a Novel Irreversible EGFR Mutant Kinase Inhibitor with a Distinct Binding Mode. JMed Chem. 2017;60:2944-62.
10. Shen J, Zhang T, Zhu S, Sun M, Tong L, Lai M, et al. Structure-Based Design of 5-Methylpyrimidopyridone Derivatives as New Wild-Type Sparing Inhibitors of the Epidermal Growth Factor Receptor Triple Mutant (EGFRL858R/T790M/C797S). J Med Chem. 2019;62:7302-08.
11. Hasako S, Terasaka M, Abe N, Uno T, Ohsawa H, Hashimoto A, *et al*. TAS6417, A Novel EGFR Inhibitor Targeting Exon 20 Insertion Mutations. Mol Cancer Ther. 2018;17:1648-58.
12. Sakagami H, *et al*. ASP8273, a novel mutant-selective irreversible EGFR inhibitor, inhibits growth of non-small cell lung cancer (NSCLC) cells with EGFR activating and T790M resistance mutations. [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5-9; San Diego, CA. Philadelphia (PA): AACR; Cancer Res. 2014;74(19 Suppl): Abstract nr 1728. doi:10.1158/1538-7445.AM2014-1728
13. Konagai S, *et al*. ASP8273 selectively inhibits mutant EGFR signal pathway and induces tumor shrinkage in EGFR mutated tumor models. [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; Cancer Res. 2015;75:Abstract nr 2586. doi:10.1158/1538-7445.AM2015-2586
14. Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer Discov. 2013. doi: 10.1158/2159-8290.CD-13-0314
15. Cross DAE, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov. 2014;4:1046-61.
16. Finlay MRV, Anderton M, Ashton S, Ballard P, Bethel PA, Box MR, et al. Discovery of a potent and selective EGFR inhibitor (AZD9291) of both sensitizing and T790M resistance mutations that spares the wild type form of the receptor. J Med Chem. 2014;57:8249-67.
17. Kim ES. Olmutinib: First Global Approval. 2016;76:1153-7.
18. Cheng H,Nair SK, Murray BW, Almaden C, Bailey S, Baxi S, *et al*. Discovery of 1-{(3R,4R)-3-[({5-Chloro-2-[(1-methyl-1H-pyrazol-4-yl)amino]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}oxy)methyl]-4-methoxypyrrolidin-1-yl}prop-2-en-1-one (PF-06459988), a Potent, WT Sparing, Irreversible Inhibitor of T790M-Containing EGFR Mutants. J Med Chem. 2016;59: 2005-24. doi: 10.1021/acs.jmedchem.5b01633.

19. Cheng H, Nair SK, Murray BW. Recent progress on third generation covalent EGFR inhibitors. Biorg Med Chem Lett. 2016;26:1861-8.
20. Wang S, Liu SCD. Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. J Hematol Oncol. 2016;9:34.
21. Jia Y, Yun C, Park E, Ercan D, Manuia M, Juarez J, et al. Overcoming EGFR (T790M) and EGFR (C797S) resistance with mutant-selective allosteric inhibitors. Nature. 2016;534:129-32.
22. Wang S, Song Y, Liu D. EAI045: The fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. Cancer Lett. 2017;385:51-4.
23. Novel quinoline-substituted compound. WO2015025936 A1.
24. Baevsky MF, Lee K, Niu D, Petter RC, Singh J. Heterocyclic Compounds and Uses Thereof. WO2012061299 A1.
25. Butterworth S, Finlay MRV, Ward RA, Kadambar VK, Chintakuntla CR, Murugan A, *et al*. 2-(2,4,5-substituted-anilino)pyrimidine Derivatives as EGFR Modulators Useful for Treating Cancer. WO2013014448 A1.
26. Behenna DC, Cheng H, Schultz SC, Johnson TO, Kath JC, Nagata A, Nair SK, Planken SP, Purine Derivatives. CAS No.1776112-90-3, US 20150141402 A1.
27. www.apisynthesisint.in.
28. Piotrowska Z, Niederst MJ, Wakelee CA, Neal JW, Mino-Kenudson M, Fulton L, *et al*. Heterogeneity Underlies the Emergence of EGFR T790 Wild-Type Clones Following Treatment of T790M-Positive Cancers with a Third Generation EGFR Inhibitor. Cancer discovery. 2015;5:713-22. doi: 10.1158/2159-8290.CD-15-0399
29. Niederst MJ, Hu H, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z, *et al*. The allelic context of the C797S mutation acquired upon treatment with third generation EGFR inhibitors impacts sensitivity to subsequent treatment strategies. Clin Cancer Res. 2015;21: 3924-33.
30. http://www.rcbs.org/pdb/explore/explore.do?structureId=3IKA
31. http://www.rcbs.org/pdb/explore/explore.do?structureId=5UWD
32. http://www.rcbs.org/pdb/explore/explore.do?structureId=5D41