Universidade de Lisboa
Faculdade de Farmácia

Small-Molecule Kinase Inhibitors in Cancer Treatment

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Monografia orientada pela Professora Doutora Ana Paula Gameiro Francisco, Professora Auxiliar

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Declaração de cumprimento do Código de Conduta e de Boas Práticas da Universidade de Lisboa

Declaro ter desenvolvido e elaborado o presente trabalho em consonância com o Código de Conduta e de Boas Práticas da Universidade de Lisboa. Mais concretamente, afirmo não ter incorrido em qualquer das variedades de fraude académica, que aqui declaro conhecer, e que atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, assumindo na íntegra as responsabilidades da autoria.

Resumo

O cancro é uma das principais causas de mortalidade a nível mundial, o que impulsiona a necessidade contínua de estratégias terapêuticas mais eficazes. As cinases, enquanto reguladoras da proliferação celular, sobrevivência e apoptose, tornaram-se alvos importantes em diversos tipos de cancro. A síntese de pequenas moléculas inibidoras de cinases transformou significativamente o tratamento oncológico, particularmente no âmbito da terapêutica dirigida. Estes agentes oferecem maior especificidade e menor toxicidade sistémica em comparação com a quimioterapia tradicional. No entanto, apesar das suas vantagens, os inibidores de cinases continuam a apresentar limitações. Podem ocorrer efeitos adversos e o aparecimento de resistência adquirida aos fármacos constitui um desafio clínico significativo. Assim, o desenvolvimento contínuo de novos inibidores é essencial para superar os mecanismos de resistência e melhorar os resultados terapêuticos a longo prazo.

A atividade desregulada das cinases está implicada em diversos tipos de cancro, incluindo cancro do pulmão de não pequenas células, cancro da mama, leucemia mieloide crónica, cancro colorretal, carcinoma de células renais, entre outros. Esta desregulação pode resultar de vários fatores. Por exemplo, translocações cromossómicas estão presentes na fusão génica BCR-ABL na leucemia mieloide crónica e no cancro do pulmão de não pequenas células ALK-positivo. As mutações pontuais também contribuem significativamente, como é o caso da mutação BRAF V600E, frequentemente observada no melanoma e no cancro colorretal, e da mutação EGFR T790M, comum no cancro do pulmão de não pequenas células. Para enfrentar estes problemas, estão a ser desenvolvidos inibidores de nova geração, muitos dos quais demonstraram resultados promissores em estudos pré-clínicos e ensaios clínicos.

Neste trabalho, é apresentada uma revisão abrangente de diversos inibidores de cinases, com foco principal nos inibidores de proteínas cinases — nomeadamente, os inibidores de tirosina cinase e de serina/treonina cinase — incluindo também exemplos representativos de inibidores de cinases lipídicas, como os dirigidos à PI3K. Esta revisão destaca aspetos fundamentais como os mecanismos de ligação destes inibidores aos seus alvos, características estruturais essenciais à sua atividade, efeitos adversos associados e as suas aplicações clínicas em diferentes tipos de cancro.

Palavras-chave: Pequenas moléculas inibidoras de cinases, Terapêutica dirigida, Quimioterapia, Resistência aos fármacos, Proteínas cinases

Abstract

Cancer is one of the leading causes of mortality worldwide, driving the continuous need for more effective therapeutic strategies. Kinases, as regulators of cell proliferation, survival and apoptosis, have become important targets in various cancer types. The synthesis of small-molecule kinase inhibitors has significantly transformed cancer treatment, particularly in the realm of targeted therapy. These agents offer improved specificity and reduced systemic toxicity compared to traditional chemotherapy. However, despite their advantages, kinase inhibitors still have limitations. Toxic effects may still occur, and the emergence of acquired drug resistance is a major clinical challenge. As a result, the ongoing development of novel inhibitors is essential to overcome resistance mechanisms and improve long-term treatment outcomes.

Dysregulated kinase activity is implicated in numerous cancers, including non-small cell lung cancer, breast cancer, chronic myeloid leukemia, colorectal cancer, renal cell carcinoma, among others. This dysregulation can be caused by various factors. For instance, chromosomal translocations are present in BCR-ABL fusion gene in chronic myeloid leukemia and in ALK-positive non-small cell lung cancer. Point mutations also contribute significantly, including the BRAF V600E mutation frequently observed in melanoma and colorectal cancer and the EGFR T790M mutation, commonly found in non-small cell lung cancer. To address these problems, next-generation inhibitors are being developed, many of which have demonstrated promising results in preclinical studies and clinical trials.

In this work, we present a comprehensive review of various kinase inhibitors, focusing primarily on protein kinase inhibitors—specifically tyrosine kinase and serine/threonine kinase inhibitors—while also including examples of lipid kinase inhibitors, such as those targeting PI3K. The review highlights key aspects such as the binding mechanisms of these inhibitors to their targets, structural characteristics essential for their activity, associated adverse effects and their clinical applications across different cancer types.

Keywords: Small-molecule kinase inhibitors, Targeted therapy, Chemotherapy, Drug resistance, Protein kinases

Abbreviations

ALK – Anaplastic Lymphoma Kinase

APE – Alanine–Proline–Glutamate motif

ATP – Adenosine Triphosphate

BC – Breast Cancer

BCR-ABL – Breakpoint Cluster Region-Abelson

BRAF – B-Raf Proto-Oncogene

CDK-4/6 – Cyclin-Dependent Kinases 4 and 6

CML – Chronic Myeloid Leukemia

CRC – Colorectal Cancer

CSF1R – Colony Stimulating Factor 1 Receptor

DFG – Aspartate–Phenylalanine–Glycine motif

DSKs – Dual-Specificity Kinases

DTC – Differentiated Thyroid Carcinoma

EGFR – Epidermal Growth Factor Receptor

FLT3 – FMS-Like Tyrosine Kinase 3

GIST – Gastrointestinal Stromal Tumor

GPCR – G-Protein Coupled Receptor

HCC – Hepatocellular Carcinoma

HER-2 – Human Epidermal Growth Factor Receptor 2

HR – Hormone Receptor

LVSD – Left Ventricular Systolic Dysfunction

MAPK – Mitogen-Activated Protein Kinase

MEK-1/2 – Mitogen-Activated Protein Kinase 1 and 2

mAb – Monoclonal Antibody

mTOR – Mammalian Target of Rapamycin

NRTK – Non-Receptor Tyrosine Kinase

NSCLC – Non-Small Cell Lung Cancer

PDGFR – Platelet-Derived Growth Factor Receptor

PI3K – Phosphatidylinositol 3-kinase

PIK3CA – Phosphatidylinositol-3-Kinase Catalytic Subunit Alpha

PIK3R1 - Phosphatidylinositol -3-kinase regulatory subunit 1

PIP2 – Phosphatidylinositol 4,5-Bisphosphate

PIP3 – Phosphatidylinositol (3,4,5)-Trisphosphate

PPI – Proton Pump Inhibitor

PTC – Papillary Thyroid Carcinoma

PTEN – Phosphatase and Tensin Homolog

RET – Rearranged During Transfection (Proto-Oncogene)

RCC – Renal Cell Carcinoma

ROS-1 – Proto-Oncogene Tyrosine-Protein Kinase 1

RSTK – Receptor Serine/Threonine Kinase

RTK – Receptor Tyrosine Kinase

SAR – Structure–Activity Relationship

SMKI – Small-Molecule Kinase Inhibitor

TGF-βR – Transforming Growth Factor-Beta Receptor

TKI – Tyrosine Kinase Inhibitor

VEGFR – Vascular Endothelial Growth Factor Receptor

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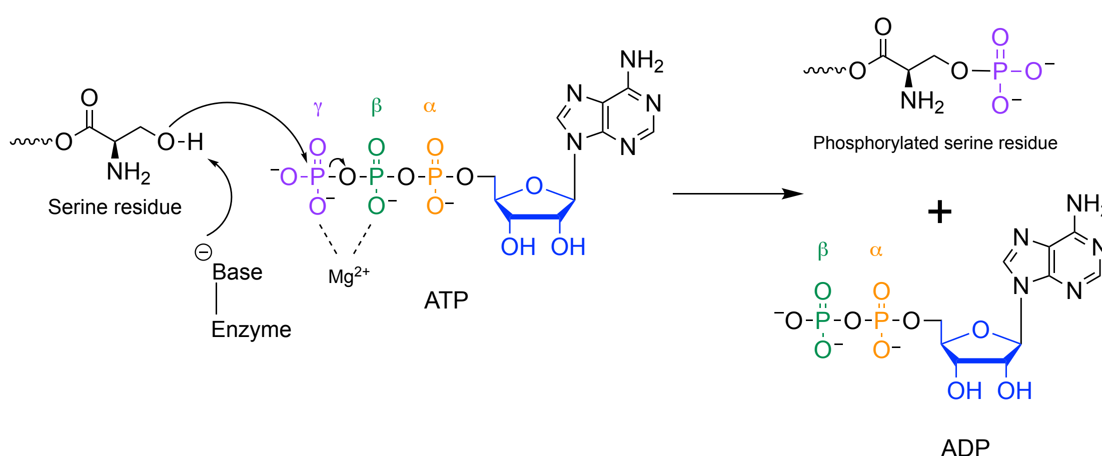
1 Introduction

1.1 Role of kinases in cell function (structure and function)

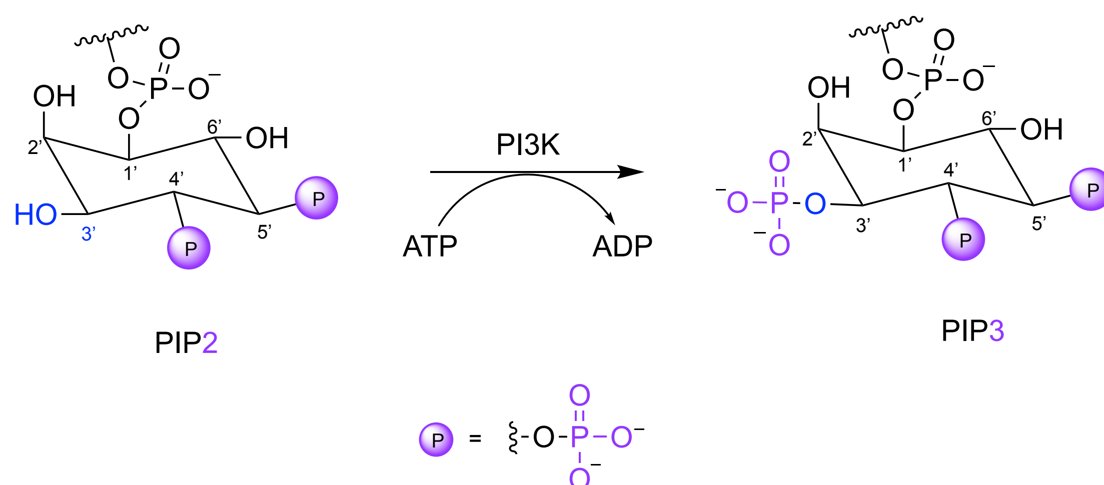
Kinases are regulatory signalling proteins that play a fundamental role in various cellular functions, including cell division, movement, transcription, metabolism, apoptosis and processes like immune response and nervous system regulation. These enzymes catalyze phosphorylation reactions, transferring the γ-phosphate group from Adenosine Triphosphate (ATP) to specific substrates, inducing conformational changes that modulate their activity. Depending on the chemical properties of the substrate, kinases can be classified into protein kinases, lipid kinases, carbohydrate kinases, nucleoside-phosphate kinases or nucleoside-diphosphate kinases [1,2]. Among these, protein kinases are the most relevant in cancer.

The total set of genes in the human genome that encode for all protein kinases is called kinome [3]. In eucaryotic cells, protein kinases catalyze the transfer of phosphoryl group from ATP ($-PO_3^{2-}$) to the hydroxyl group (-OH) of Serine/Threonine and Tyrosine residues of their substrate proteins [4]. ATP is typically bound to a divalent ion in the active site of the enzyme, usually Mg^{2+} to aid in the phosphoryl transfer reaction, coordinating with the beta and gamma phosphates (**Scheme 1**) [5,6].



Scheme 1: Example of a protein kinase simplified catalysis phosphorylation mechanism.

Lipid kinases like Phosphoinositide 3-kinase (PI3K) phosphorylate the inositol moiety of phospholipids at the 3'position [7]. **Scheme 2** represents the phosphorylation of the 3' position of the inositol group of phosphatidylinositol 4,5-biphosphate (PIP2) giving rise to phosphatidylinositol 3,4,5-triphosphate (PIP3).



Scheme 2: Simplified phosphorylation mechanism of PI3K kinase. Based on [7]

Eucaryotic protein kinases share a conserved structural core that is divided into two distinct lobes. The smaller N-terminal lobe is composed primarily of beta sheets and one alpha helix, known as αC-helix. The C-terminal lobe is predominantly helical and contains the activation segment. These two lobes are connected by the hinge region that recognizes the adenine base of the ATP molecule trough hydrogen bonds. The activation segment consists of the activation loop (A-loop), the aspartate-phenylalanine-glycine (DFG) motif and the alanine-proline-glutamate (APE) motif. The A-loop is a common phosphorylation site that regulates kinase activity and is also involved in the recognition of protein substrates (**Figure 1**) [8–10]. Additionally, the Glycine-rich loop (G-loop) participates in the catalyzes of the phosphoryl transfer reactions and mutations within the G-loop have an impact in the rate of these reactions [11].

The catalytic domain of protein kinases transitions between active and inactive states. This conformational switch is regulated by the movement of two highly conserved structural elements: the DFG motif and the αC-helix. In the active conformation, known as the DFG-in state, the aspartate residue of the DFG motif projects into the ATP-binding site, to coordinate with the magnesium ions essential for nucleotide binding

and catalysis. This positioning facilitates the alignment of ATP for efficient phosphoryl transfer to the substrate [12]. In the DFG-out state, the asparagine residue is oriented away from the ATP binding site, while the phenylalanine occupies the ATP binding site, opening an allosteric pocket [13].

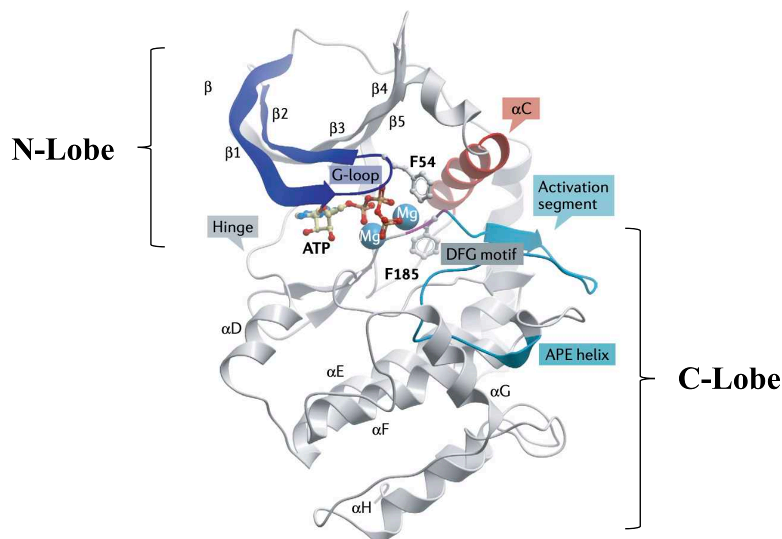


Figure 1: Protein kinase structure.

Adapted from [10]

The αC-helix in the active conformation is positioned inwards (αC-in) forming a salt bridge between Lysine 72 (K72) and Glutamate 91 (E91), as shown in **Figure 2**. This interaction is crucial for correctly positioning the lysine to stabilize the α-phosphate of ATP. In the inactive conformation, the αC-helix shifts outwards (αC-out) disrupting the salt bridge formation and destabilizing the catalytic site [14]. Kinases in the DFG-in and αC-in conformations are the only considered active [15].

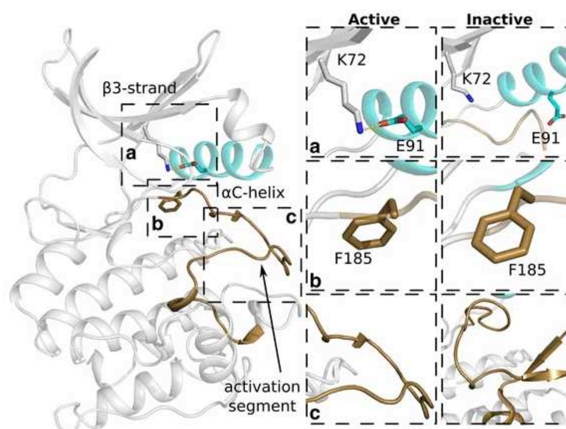


Figure 2: **αC-helix active and inactive conformation.**

Adapted from [14]

As mentioned earlier, protein kinases are classified by the residue that they phosphorylate.

Tyrosine kinases phosphorylate tyrosine residues within the substrate and can be divided into two categories: receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs) (**Table 1**). RTKs are a category of transmembrane receptors composed of conserved structural domains: the extracellular ligand-binding-domain, the transmembrane helix and the intracellular tyrosine kinase domain. These cell surface receptors respond to different ligand such as growth factors, hormones and cytokines [16]. When the ligand binds to the receptors, activation usually occurs by ligand-induced dimerization. This process leads to the autophosphorylation of tyrosine residues that serve as recruitment sites for downstream signaling proteins, thereby initiating a signal transduction pathway (**Figure 3**) [17]. The NRTKs are cytoplasmatic and nuclear proteins, lacking both the extracellular and transmembrane regions [18].

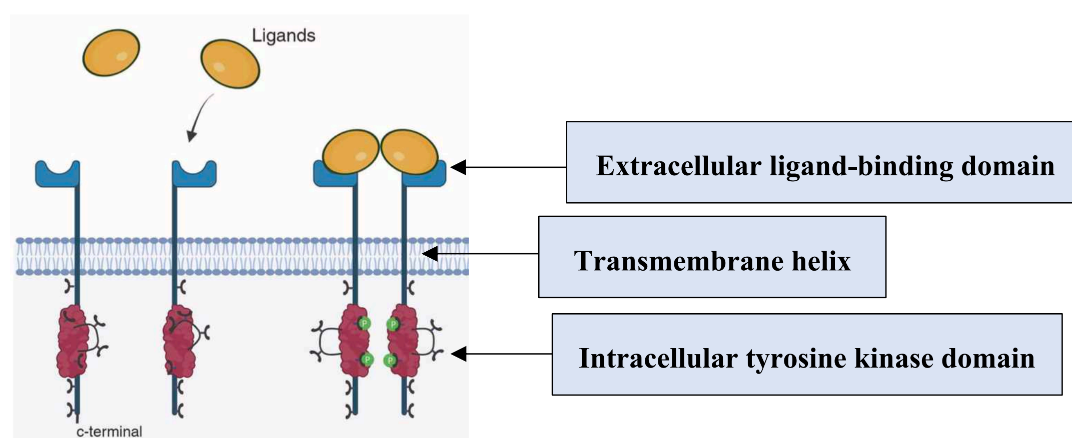


Figure 3: **RTK structure, ligand biding and dimerization.**

Adapted from [16]

Serine/Threonine kinases phosphorylate serine or threonine residues in their substrates. They can also be classified into two types: receptor serine/threonine kinases (RSTKs) and those who are cytoplasmatic proteins that represent most kinases in this class [19,20].

A third group of protein kinases called dual-specificity kinases (DSKs) was defined by Tony Hunter [21]. DSKs phosphorylate both tyrosine and serine/threonine residues [22].

Table 1: Summary of protein kinase types.

Protein Kinase Type	Examples
Receptor Tyrosine Kinases (RTKs)	EGFR, VEGFR, ALK
Non-Receptor Tyrosine Kinases (NRTKs)	BCR-ABL
Receptor Serine/Threonine Kinases (RSTKs)	TGF-βR
Cytoplasmatic Serine/Threonine Kinases	BRAF, mTOR, CDK-4/6
Dual-Specificity Kinases (DSKs)	MEK-1/2

EGFR = Epidermal Growth Factor Receptor; VEGFR = Vascular Endothelial Growth Factor Receptor; ALK = Anaplastic Lymphoma Kinase; BCR-ABL = Breakpoint Cluster Region-Abelson; TGF-βR = Transforming Growth Factor-Beta Receptor; BRAF = B-Raf proto-oncogene; mTOR = Mammalian Target of Rapamycin; CDK-4/6 = Cyclin-Dependent Kinases 4 and 6; MEK-1/2 = Mitogen-Activated Protein Kinase 1 and 2.

1.2 Targeting kinases in cancer (Targeted Therapy)

Chemotherapy is a highly toxic and nonspecific treatment for cancer. Supportive care has become very important in managing the side effects of these aggressive treatments. By specifically targeting kinases involved in cancer, it was expected that these effects would be reduce, providing patients with better treatment options specifically to their cancer type. Targeted therapy is less toxic than standard chemotherapy, however it has still numerous limitations [23], which will be discussed later in this work.

There are three main types of targeted therapy in cancer: monoclonal antibodies (mAbs), small molecular kinase inhibitors (SMKIs) and immunotoxins [24].

In cancer treatment, monoclonal antibodies can act by two different mechanisms. Using receptor tyrosine kinase (RTK) as an example, the mAb can either block the receptor making the dimerization impossible or directly block the binding of the ligand to its receptor. The SMKI, in this case, a tyrosine kinase inhibitor (TKI), blocks the ATP-

binding site in the catalytic domain of the RTK, inhibiting the phosphorylation reaction and thus the signal transduction pathway (**Figure 4**) [25].

Immunotoxin targeted therapy consists in a toxin connected to an antibody or growth factor. The immunotoxin is internalized by the target cells and the enzymatic fragment of the toxin is translocated to the cytosol [26].

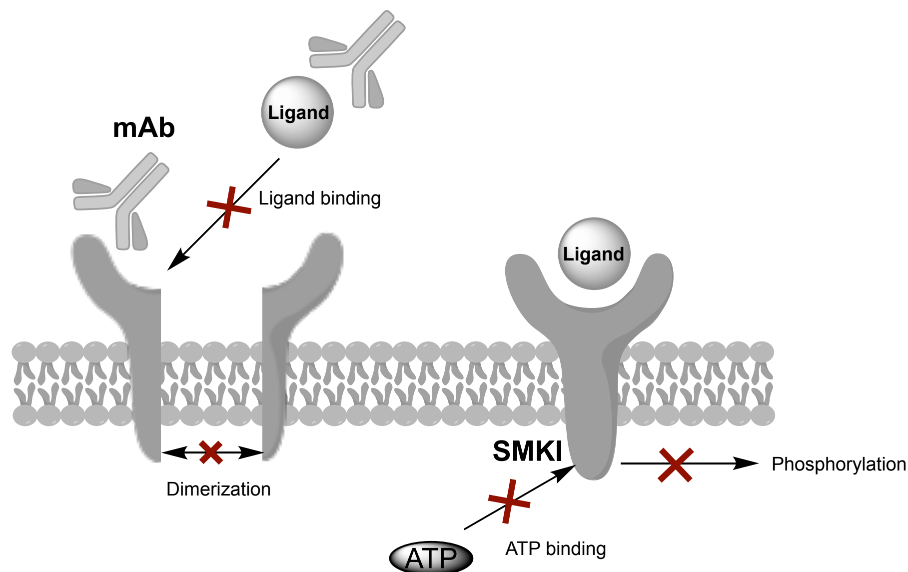


Figure 4: **Mode of action of mAbs and SMKIs on a RTK. Based on [25]**

1.3 Limitations of small-molecule kinase inhibitors

SMKIs offer the convenience of oral administration, allowing patients to take their medication at home and avoid hospital visits, thereby improving their quality of life. However, the fact that they are administered orally and at home, it is not possible to monitor patients as closely as with intravenous treatments [27]. Furthermore, the absorption can be affected by food and other concomitant medications. Proton pump inhibitors (PPIs), histamine 2 (H2) receptor blockers and antacids increase the stomach pH, decreasing the solubility of some kinase inhibitors, particularly those who are weak bases, as the non-ionised form of the drug becomes predominant [27,28]. Most kinase inhibitors (KIs) are metabolized by the liver, by cytochrome P450 enzyme family [27], being the isoform CYP3A4 the most important in drug metabolism and also the most abundant in the liver [29]. Grapefruit juice is a well-known example of an inhibitor of CYP3A4, and as a consequence increases the KIs plasma concentrations, which can lead to toxicity [27]. In contrast, CYP3A4 inducers, like phenytoin [30] will speed the metabolism of KIs making them less effective. Additionally, KIs can enhance the toxicity of other medications the patient may be taking. For example, if a patient is taking a KI that inhibits P-glycoprotein (P-gp), such as sorafenib or cabozantinib, along with another drug that is a P-gp substrate (for example fexofenadine, digoxin and colchicine), the reduced drug efflux can result in increased of these drug levels and a higher risk of toxicity [27,31].

As previously mentioned, targeted therapy is less toxic than traditional chemotherapy. However, KIs can still cause significant adverse effects. Cardiovascular toxicity is greatly associated with this therapy. For instance, vascular endothelial growth factor Receptor (VEGFR) inhibitors used for example in colon cancer and hepatocellular cancer, have been linked with hypertension, heart failure, asymptomatic left ventricular systolic dysfunction (LVSD), QTc prolongation, myocardial ischaemia and infarction [32]. On the other hand, epidermal growth factor receptor (EGFR) inhibitors are specifically associated with dermatological adverse effects, such as xerosis and papulopustular eruption [33].

Mutations in protein kinases lead to resistance to protein KIs. Consequently, the development of new drugs is crucial to overcome this problem [34]. Particularly, mutations in the gatekeeper residue of kinases are a way through which cancer cells

develop resistance to treatment [35]. This gatekeeper residue occur in a specific amino acid in the ATP binding pocket [36].

1.4 Types of cancers associated with kinase overexpression

The phosphorylation of target proteins is a regulated process, and any dysregulation can lead to a diseased state. There are several kinases that are dysregulated in various types of cancer (**Table 2**) [37].

Non-small cell lung cancer (NSCLC) represents approximately 75% of all lung cancer cases. One of the most common alterations is the EGFR overexpression which plays a key role in its pathogenesis [38]. In addition, ALK gene rearrangements, first identified in 2007 are also present in NSCLC [39]. Another important genetic alteration is the rearrangement of the proto-oncogene tyrosine-protein kinase 1 (ROS1) which is present in approximately 0.9-2.6% of NSCLC patients [40].

In colorectal cancer (CRC), epidermal growth factor receptor (EGFR) is frequently overexpressed and has been associated with more aggressive tumour behavior [41,42]. The vascular endothelial growth factor (VEGF) family also plays a crucial role in CRC progression. A study by D'Haene *et al.* suggested that high expression of VEGFR-1 and low expression of VEGFR-2 in endothelial cells contribute to CRC progression [43]. Additionally, BRAF mutations, particularly BRAF V600E, are commonly observed in CRC. The RAS/RAF/MEK/ERK signalling cascade, commonly known as the mitogen-activated protein kinase (MAPK) pathway, plays a fundamental role in cellular proliferation, differentiation, survival, and apoptosis [44].

In breast cancer (BC), human epidermal growth factor receptor 2 (HER2) is overexpressed in approximately 25–30% of cases and is associated with poor prognosis [45]. Hyperactivation of the PI3K/AKT/mTOR pathway has also been implicated in BC tumorigenesis [46]. Furthermore, cyclin-dependent kinases (CDKs) interact with cyclins to regulate cell cycle progression, thereby controlling cellular growth and division. CDKs can be overexpressed in BC, particularly in hormone receptor-positive breast cancer [47,48].

In renal cell carcinoma (RCC), VEGFR and platelet derived growth factor receptor (PDGFR) are specifically overexpressed. Approximately 75% of RCCs are clear cell carcinomas (ccRCCs) and current treatments are mostly anti-angiogenic tyrosine kinase inhibitors targeting VEGFR [48,49].

Table 2: Summary of some types of cancer associated with kinase overexpression

Cancer	Commonly overexpressed kinases
Lung	EGFR, ALK, ROS1
Colorectal	EGFR, VEGFR, BRAF
Breast	HER2, PI3K, AKT, mTOR, CDKs
Renal	VEGFR, PDGFR

EGFR = Epidermal Growth Factor Receptor; VEGFR = Vascular Endothelial Growth Factor Receptor; ALK = Anaplastic Lymphoma Kinase; ROS-1 = proto-oncogene tyrosine-protein kinase 1; HER-2 = human epidermal growth factor receptor 2; BRAF = B-Raf proto-oncogene; mTOR = Mammalian Target of Rapamycin; CDKs = Cyclin-Dependent Kinases; PDGFR = platelet derived growth factor receptor.

2 Small-Molecule Kinase Inhibitors

2.1 Types of kinase inhibitors based on their mechanism of action

Small-molecule kinase inhibitors can be classified by their mechanism of action mainly into six types: types I to VI, as explained bellow. The majority of approved kinase inhibitors fall into types I and II [50].

Type I inhibitors target the ATP-binding pocket of active kinases, with DFG-in and αC-in. They mimic the ATP. Type II inhibitors also mimic the ATP; however, they target the ATP-binding pocket when kinases are in the inactive conformation, with DGF-out and αC-out. Type III bind to an allosteric site near the ATP-binding pocket in contrast to type IV who bind in an allosteric site distant from ATP-binding pocket [51]. Type V are bivalent inhibitors, targeting both the ATP-binding-pocket and a secondary site [52]. Type VI are covalent inhibitors who bind irreversibly to the ATP-binding pocket or in an allosteric site (**Figure 5**) [51].

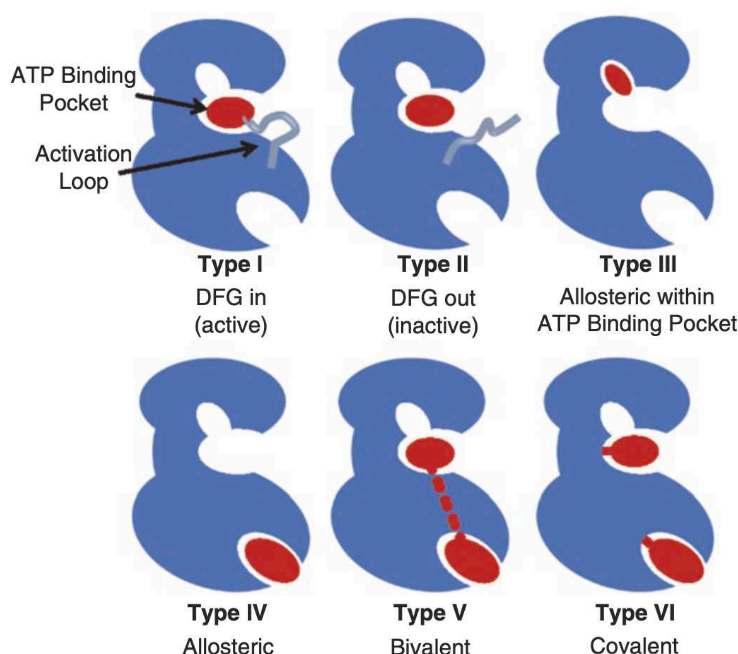


Figure 5: **Types of kinase inhibitors (I to VI)**

Adapted from [51]

2.2 Tyrosine kinase inhibitors

2.2.1 BCR-ABL inhibitors

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome. The Ph chromosome results from a reciprocal translocation between chromosomes 9 and 22, [t(9;22)(q34;q11)], which fuses the Abelson (ABL) tyrosine kinase gene from chromosome 9 (q34) with the break-point cluster (BCR) gene from chromosome 22 (q11). The resulting BCR-ABL fusion gene encodes a protein with abnormal tyrosine kinase activity, known as the BCR-ABL tyrosine kinase [53]. More than 90% of CML patients are positive for the Philadelphia chromosome (Ph+) (**Figure 6**) [54].

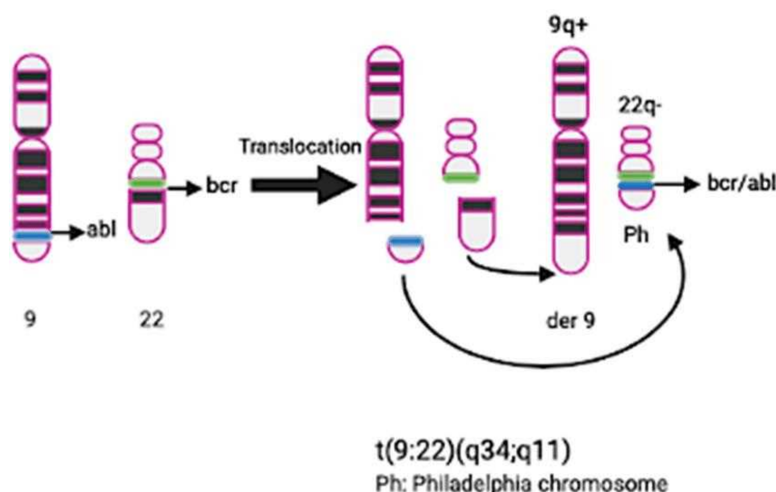


Figure 6: **Formation mechanism of Philadelphia chromosome. Adapted from [53]**

Imatinib (**Figure 7**), a first-generation TKI, was approved by the Food and Drug Administration (FDA) in 2001 and it transformed the treatment of CML patients, being the first TKI approved for the treatment of advanced stage Ph+ CML [54,55]. Imatinib's structure is based on a 2-phenylaminopyrimidine core, consisting in a pyrimidine ring linked to a phenyl group via an amine. This group is fundamental for the binding into the ATP-binding pocket. A pyridyl group was added at the 3'- position of the pyrimidine ring to enhance cellular activity. A methyl group in the *ortho*-position to the amino group increases selectivity for the BCR-ABL kinase. The amide group

attached to the phenyl ring confers inhibitory activity against tyrosine kinases. To improve solubility, an N-methylpiperazine group was introduced, improving also oral bioavailability. Additionally, a benzene ring between the amide and N-methylpiperazine groups acts as a spacer, eliminating the compound's mutagenic potential (**Figure 8**) [56]. Imatinib is a type II TKI inhibitor, binding to the inactive conformation of the BCR-ABL kinase [54].

Nilotinib (**Figure 7**), a second-generation TKI, was developed to overcome resistance mutations [55]. It is structurally similar to imatinib but features some key modifications. The amide linking group was inverted, the piperazine ring was removed, and a 3-methylimidazole group along with a trifluoromethyl group was added that increases van der Waals interactions [56]. Like imatinib, contains a 2-phenylaminopyrimidine core and functions as a type II TKI. Nilotinib has greater potency than imatinib, making it effective against certain imatinib-resistant point mutations. However, both drugs remain ineffective against the T315I gatekeeper mutation in the BCR-ABL kinase, which involves the substitution of threonine at position 315 by isoleucine [57]. This mutation inhibits the drug from binding due to a conformational change from the inactive conformation of the kinase to the active conformation [55].

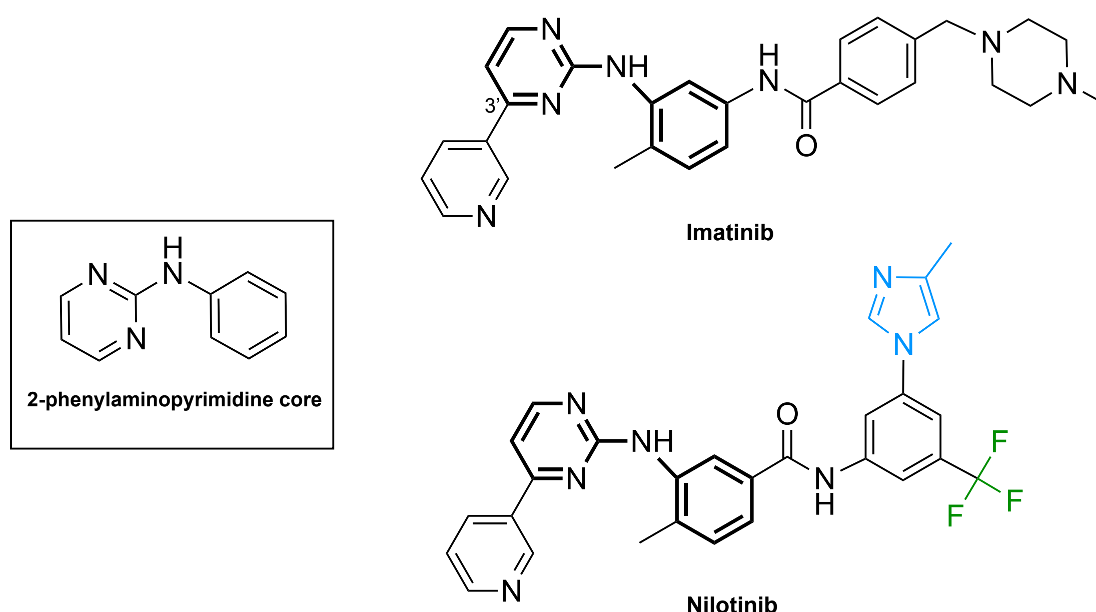


Figure 7: **Imatinib and Nilotinib structures.**

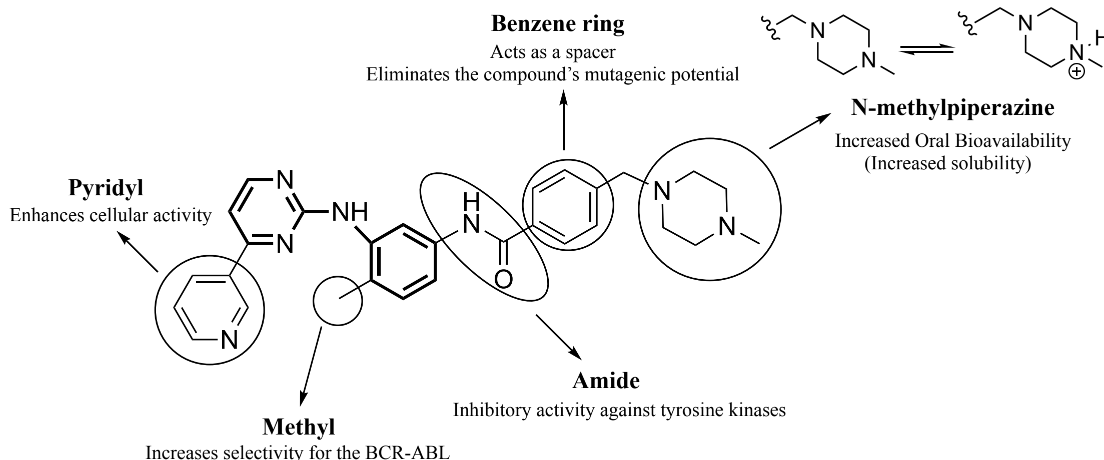


Figure 8: Imatinib's structure-activity relationship.

Dasatinib and bosutinib (**Figure 9**) are second-generation tyrosine kinase inhibitors (TKIs) that target BCR-ABL as well as Src family kinases. However, like imatinib and nilotinib, they are ineffective against the T315I mutation. Unlike imatinib and nilotinib, which bind to the inactive (DFG-out) conformation of BCR-ABL, dasatinib binds to the active (DFG-in) conformation, while bosutinib exhibits dual binding capability, interacting with both active and inactive conformations of the kinase [57].

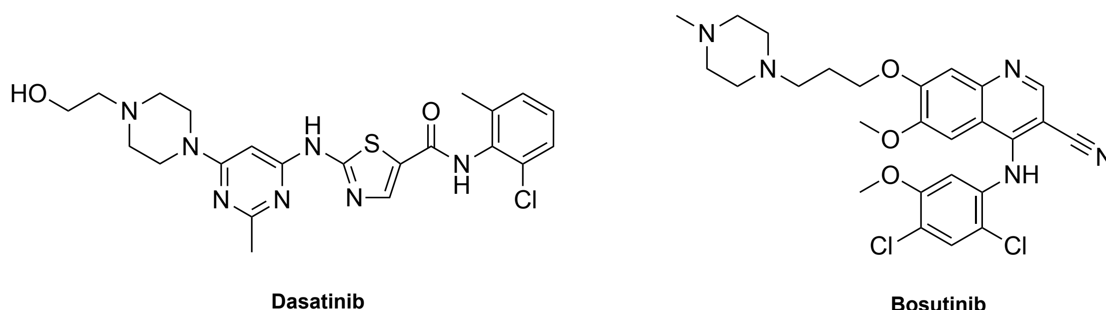


Figure 9: **Dasatinib and Bosutinib structures.**

Ponatinib (**Figure 10**), a third-generation TKI, was specifically developed to be effective against the T315I mutation. Structurally, it is similar to nilotinib. The methyl imidazole group in nilotinib is replaced by a methyl piperazine group, similar to imatinib. Additionally, instead of a pyridine-pyrimidine moiety, ponatinib features a

terminal imidazo[1,2-b]pyridazine group linked to a phenyl ring via a triple bond. This rigid and extended structure enhances binding affinity and allows ponatinib to bypass the steric hindrance caused by the isoleucine substitution at position 315 [56,57].

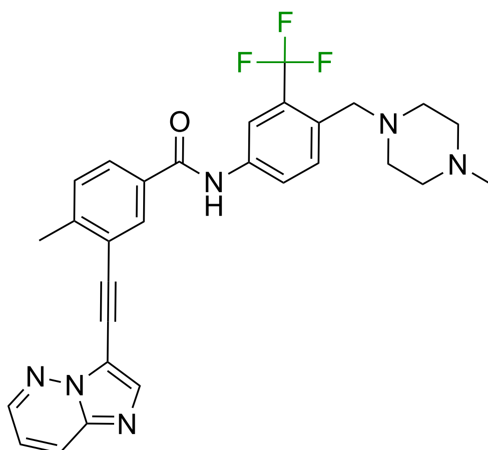


Figure 10: Ponatinib structure.

Asciminib (**Figure 11**), was the first allosteric BCR-ABL kinase inhibitor to reach clinical evaluation. It is active against several point mutations, including T315I. It binds to the myristoyl pocket which is a hydrophobic pocket located in the C-terminal lobe of the kinase [57].

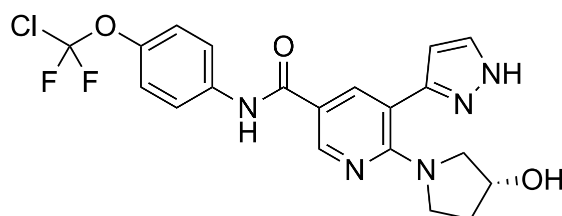


Figure 11: Asciminib structure.

BCR-ABL inhibitors are generally well tolerated, however, they can be associated with several adverse effects. Common reactions include cytopenias—likely due to the antileukemic activity of these TKIs—fluid retention, gastrointestinal disturbances, dermatologic issues, musculoskeletal pain, fatigue, headache, and, less frequently, cardiac toxicity [58].

2.2.2 HER/ERBB family inhibitors: EGFR and HER2 inhibitors

The HER/ERBB family is composed of four members of RTKs: **EGFR** (also known as ERBB1/HER1), ERBB2 (**HER2**), ERBB3 (HER3) and ERBB4 (HER4) [59].

EGFR inhibitors

4-Anilinoquinazolines are a class of compounds identified as inhibitors of the ATP-binding site of EGFR, as their molecular structure mimics adenine of ATP, allowing them to competitively block kinase activity [60].

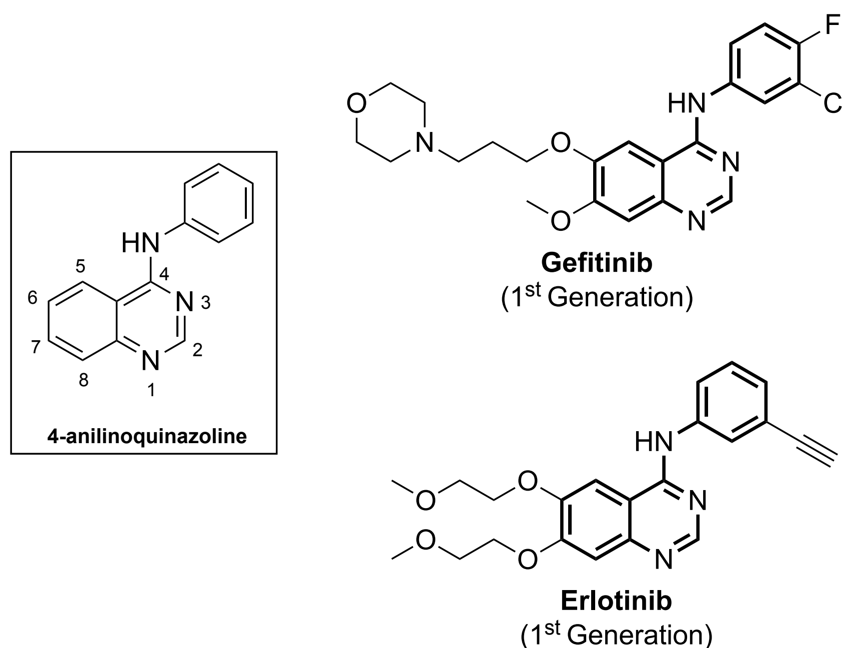


Figure 12: Gefitinib and Erlotinib structures.

Gefitinib (approved in 2003) and erlotinib (approved in 2004) are first-generation EGFR inhibitors (**Figure 12)** that share a 4-anilinoquinazoline core and are used in the treatment of non-small cell lung cancer (NSCLC). In gefitinib, the quinazoline group occupies the ATP-binding site of EGFR and is stabilized by a hydrogen bond between the N1 nitrogen and the backbone N-H of Met793 in the hinge region. The *meta*-chlorine and *para*-fluorine substituents on the aniline ring at the 4-position extend into the hydrophobic pocket at the back of the ATP-binding cleft, enhancing binding affinity through hydrophobic interactions. Substituents at positions 6 and 7 of the quinazoline

ring are directed toward the solvent-exposed region, contributing to solubility and pharmacokinetic properties. Furthermore, gefitinib interacts with the active conformation of EGFR with αC-in and DFG-in, therefore it is classified as a type I inhibitor [61]. Erlotinib binds to the EGFR active site in a similar manner, forming a hydrogen bond between the N1 nitrogen of the quinazoline ring and Met793 in the hinge region. Additionally, it establishes an extra hydrogen bond via a water-mediated bridge involving Gln767, Thr766, and Thr830, which further stabilizes its interaction with the receptor [60,62]. Erlotinib is classified as a type $I^{1/2}$ inhibitor because it binds to EGFR with DFG-in and αC-out conformation [61].

Patients treated with gefitinib or erlotinib may develop resistance over time. A common mechanism of resistance is the T790M mutation, which involves the substitution of threonine by methionine at position 790 in the kinase domain of EGFR [63]. Other mutations include deletions in exon 19 and substitution of L858R (leucine is replaced by arginine) in exon 21 [61]. These mutations significantly reduce the effectiveness of first-generation inhibitors, prompting the development of second-generation EGFR inhibitors designed to overcome this problem. Afatinib (approved in 2013) and dacomitinib (approved in 2018) are second-generation EGFR inhibitors (**Figure 13**) classified as covalent inhibitors (Type VI inhibitors) [61].

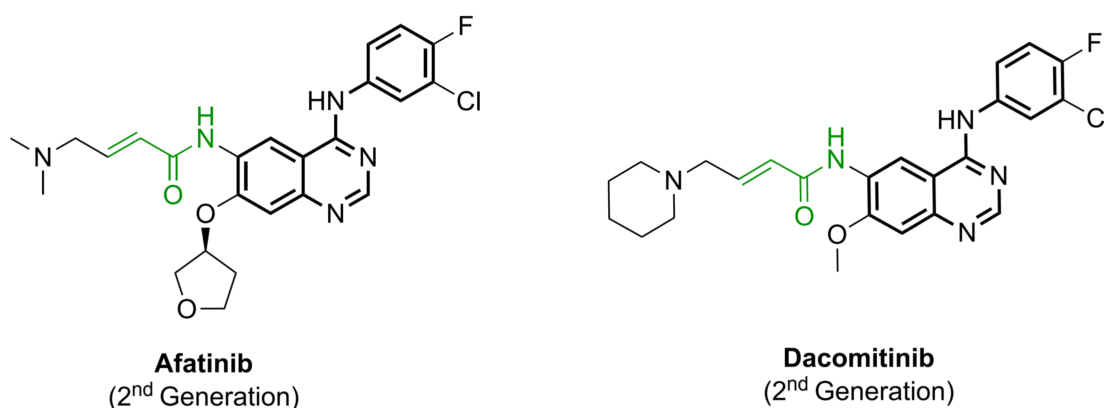
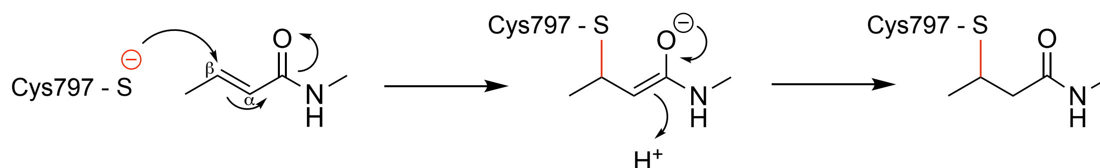


Figure 13: Afatinib and Dacomitinib structures.

Both compounds contain an acrylamide functional group that acts as a Michael acceptor, enabling them to form an irreversible covalent bond with the nucleophilic cysteine residue Cys797 in the EGFR active site. This bond is formed through a Michael addition reaction, where the nucleophilic cysteine thiol attacks the β-carbon of the

acrylamide group, functioning as a Michael acceptor (**Scheme 3**), leading to permanent inhibition of EGFR kinase activity [60,62].



Scheme 3: Simplified mechanism of covalent bond formation between Cys797 and an acrylamide group via Michael addition.

Improved affinity for the T790M mutation was achieved by replacing the quinazoline core with a more flexible pyrimidine scaffold, leading to the development of third-generation EGFR inhibitors (**Figure 14**) containing a 2-phenylaminopyrimidine core [62]. Osimertinib was the first third-generation TKI with a non-quinazoline core approved by the FDA. The nitrogen atom in the pyrimidine ring forms a hydrogen bond with the backbone N-H of Met793 in the EGFR hinge region. This interaction allows the pyrimidine core to mimic the binding mode of the quinazoline ring found in the first and second-generation inhibitors. Furthermore, like second-generation inhibitors, osimertinib features an acrylamide group that undergoes a Michael addition reaction with the nucleophilic cysteine residue Cys797, resulting in covalent bond formation [61]. Similarly, rociletinib contains a 2-phenylaminopyrimidine core and an acrylamide group, enabling it to form a covalent bond with Cys797 via the same mechanism. A study by Xiao-E Yan *et al*. analysed the crystal structures of EGFR T790M and L858R in complex with rociletinib. The anilinopyrimidine group forms two hydrogen bonds with the amide and carbonyl backbone of Met793 in the hinge region. The trifluoromethyl group (-CF3) engages in a hydrophobic interaction with the gatekeeper residue, Met790. This hydrophobic interaction is particularly beneficial for targeting the T790M mutation, as it enhances the drug's effectiveness. In contrast, the wild-type Thr790 residue does not support this interaction, making rociletinib significantly more effective against the T790M mutation [64].

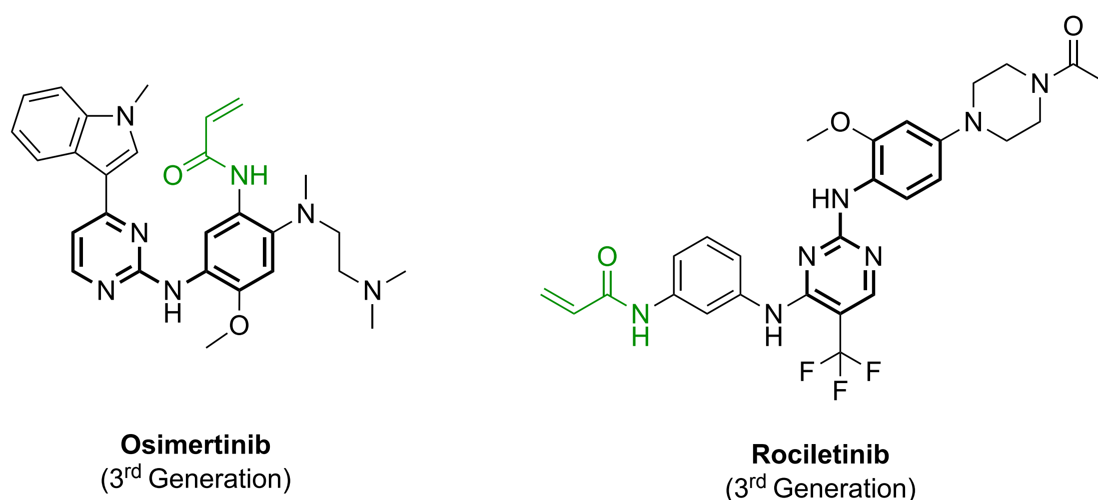


Figure 14: Osimertinib and Rociletinib structures.

Third-generation inhibitors are impacted by the C797S mutation, in which the cysteine residue at position 797 is replaced by serine. Cysteine contains a nucleophilic thiol group, whereas serine has a hydroxyl group, which is a weaker nucleophile (**Figure 15**), making the Michael addition reaction not possible [61].

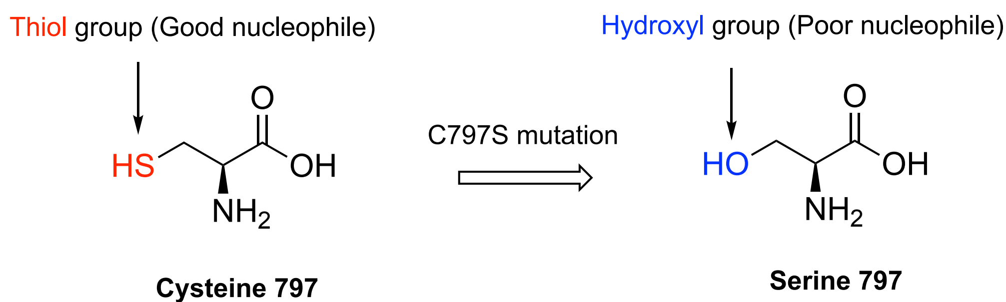


Figure 15: C797S mutation.

HER2 inhibitors

Lapatinib (**Figure 16**), approved in 2007, is a reversible small-molecule tyrosine kinase inhibitor that targets both HER2 and EGFR. It was the second anti-HER2 therapy developed, following the monoclonal antibody trastuzumab. Lapatinib is approved for use in combination with capecitabine (an antimetabolite chemotherapy agent) for the treatment of patients with HER2-positive advanced or metastatic breast cancer who have previously received therapy with an anthracycline, a taxane, and trastuzumab. [65].

Neratinib (**Figure 16**) is an irreversible inhibitor of HER2, EGFR and HER4 [66]. It forms a covalent bond to Cys805 in HER2 kinase [67]. In Europe, neratinib is approved for the treatment of early-stage breast cancer in patients with hormone receptor-positive/HER2-positive disease who have completed adjuvant trastuzumab-based therapy less than one year ago [65].

Tucatinib (**Figure 16**), on the other hand, is a highly selective HER2 reversible inhibitor, thereby decreasing EGFR related toxicities [66]. In Europe, tucatinib is approved for use in combination with trastuzumab and capecitabine for the treatment of patients with HER2-positive advanced unresectable or metastatic breast cancer (including patients with brain metastases) who have previously received at least two prior anti-HER2 regimens in any setting [65]. Both lapatinib and tucatinib share a 4-anilinoquinazoline core. In contrast, neratinib features a 4-anilinoquinoline core.

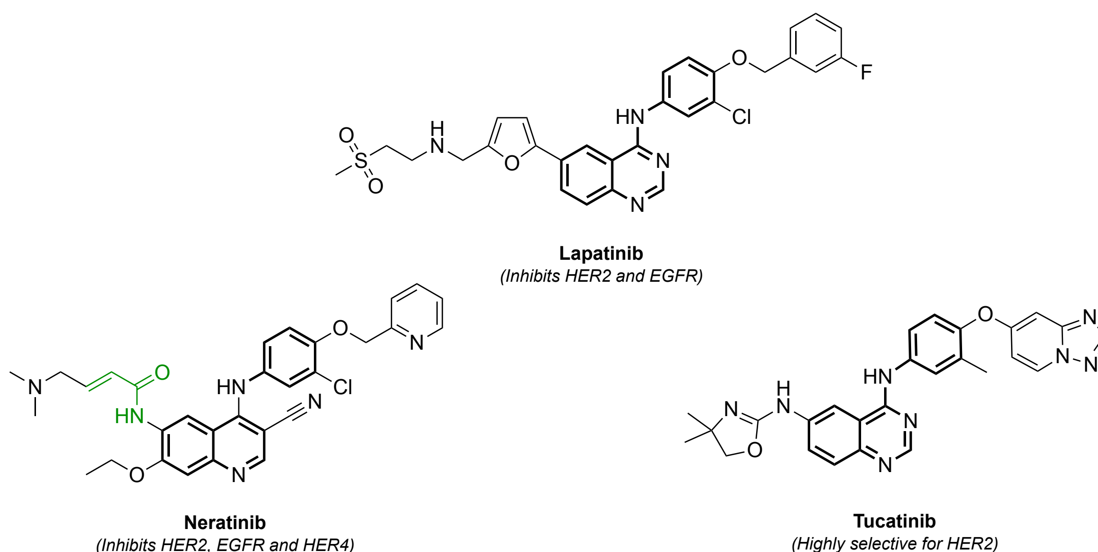


Figure 16: Lapatinib, Neratinib and Tucatinib structures.

2.2.3 VEGFR inhibitors

Vascular endothelial growth factor receptors (VEGFRs) play a crucial role in regulating tumor-induced angiogenesis. They are classified into three subtypes: VEGFR1, which is essential for hematopoietic cells development; VEGFR2, which supports vascular endothelial cells development; and VEGFR3, which is responsible for lymphatic endothelial cells development [68]. Most VEGFR inhibitors are multi-kinase inhibitors, meaning they also target other kinases in addition to VEGFR.

In the pharmaceutical field, the VEGF/VEGFR2 signalling cascade is an important target and a major research focus in the development of new VEGFR2 inhibitors [68,69]. The active site of VEGFR2 can be divided into four main regions: hydrophobic regions I and II, the hinge region, and the DFG motif region. Hydrophobic region I serves as the primary binding site for type I inhibitors, which interact selectively with amino acid residues such as Cys919 and Glu917. In contrast, hydrophobic region II is the typical binding site for most type II inhibitors (**Figure 17**) [69].

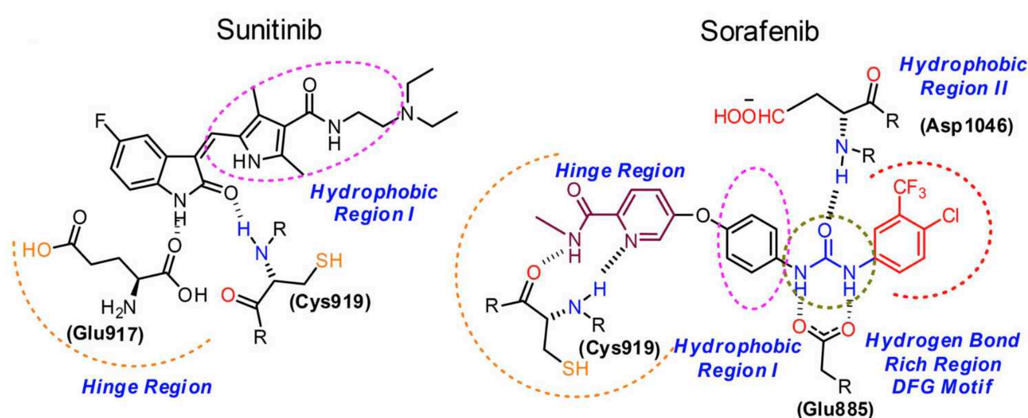


Figure 17: Sunitinib and Sorafenib binding to VEGFR2.

Adapted from [69]

Sorafenib was approved in 2005 for advanced renal cell carcinoma (RCC) and targets VEGFR-1/2/3, in addition to other RTKs including the stem cell-factor receptor (c-Kit), FLT3, PDGFR-β, RET and the papillary thyroid carcinomas (PTC) [70]. Later it was approved for hepatocellular carcinoma (HCC) in 2007, for differentiated thyroid carcinoma (DTC) in 2013 and for thyroid cancer in 2014 [71]. It binds to the kinases in the DFG-out conformation, making it a type II inhibitor [69].

Sunitinib was approved in 2006 also for advanced RCC, gastrointestinal stromal tumor (GIST) and inhibits VEGFR-1/2/3, as well as PDGFR-α/β, CSF1R, c-Kit, RET, and FLT3 [71,72]. Later, in 2011 was approved for pancreas neuroendocrine tumor [71]. It binds to kinases in the DFG-in conformation, classifying it as a type I inhibitor [69].

Axitinib (**Figure 18**) is a potent and selective inhibitor of VEGFR1/2/3 [73]. Similar to sunitinib, it binds kinases in the DFG-in conformation, classifying it as a type I inhibitor [69]. Compared to other VEGFR inhibitors, it is weakly active against other receptors such as c-Kit and PDGFR. It was approved in 2012 for advanced RCC as a second-line therapy. As with other VEGFR inhibitors, cardiovascular adverse events are common with Axitinib, with hypertension being particularly frequent. Additional adverse effects include gastrointestinal disturbances such as diarrhea and nausea, as well as fatigue and dysphonia [74].

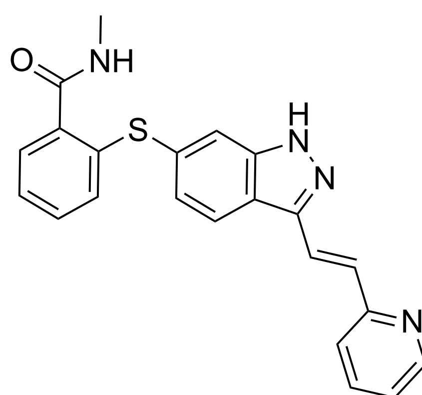


Figure 18: Axitinib structure.

2.2.4 ALK inhibitors

Anaplastic lymphoma kinase (ALK) gene rearrangements are oncogenic drivers in several malignancies, particularly in non-small cell lung cancer (NSCLC) [75]. The wild-type ALK gene encodes for RTK that undergoes autophosphorylation upon ligand binding. A chromosomal translocation results in the formation of an ALK fusion gene, leading to the translation of a chimeric oncoprotein composed of the C-terminal kinase domain of ALK fused to various N-terminal, non-kinase partner proteins. The most common fusion in NSCLC is EML4-ALK, with variant 1 (E13;A20) and variant 3 (E6;A20) being the most prevalent (**Figure 19**) [76].

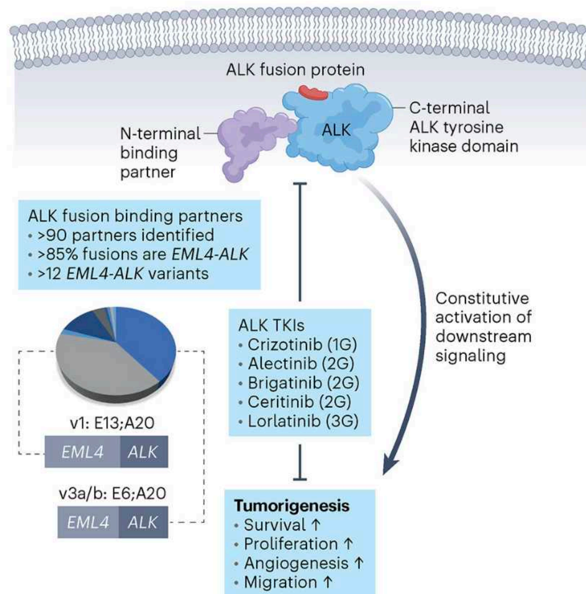


Figure 19: Oncogenic ALK signalling.

Adapted from [76]

Crizotinib (**Figure 20**) is a first-generation inhibitor of ALK, as well as ROS1 and MET, and was approved by the FDA in 2011 for the treatment of advanced ALK-positive NSCLC [75,76]. Due to the emergence of drug resistance to crizotinib, several second-generation inhibitors have been developed and subsequently received FDA approval, including ceritinib, alectinib and brigatinib [76]. A major limitation of crizotinib is its poor penetration of the blood–brain barrier, making it less effective in patients with brain metastases [77].

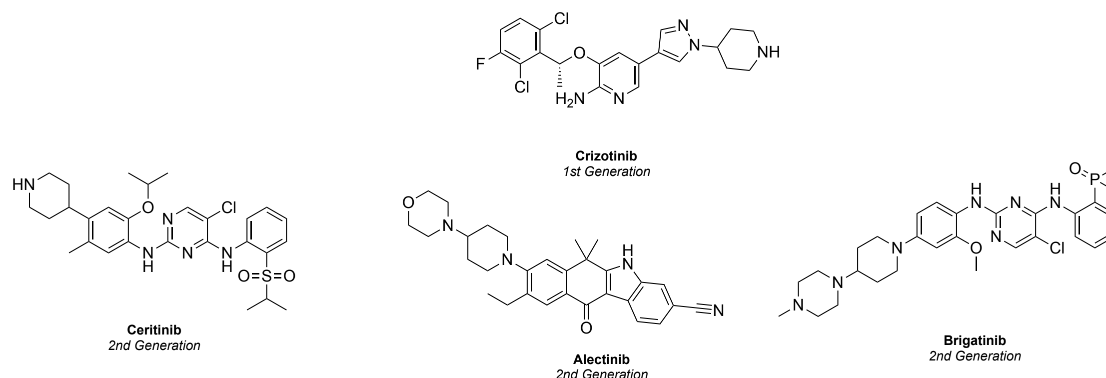


Figure 20: First and second-generation ALK inhibitors structure.

Most patients relapse on ALK-TKI therapy due to acquired resistance. Mutations such as L1196M in the gatekeeper residue and the solvent-front G1202R—one of the most common causes of resistance to first and second-generation ALK inhibitors—are frequently implicated. Lorlatinib, a third-generation inhibitor was developed to overcome resistant ALK mutations including G1202R and to penetrate the blood-brain barrier. Lorlatinib is characterized by its macrocyclic structure and it was developed from crizotinib (**Figure 21**) [77].

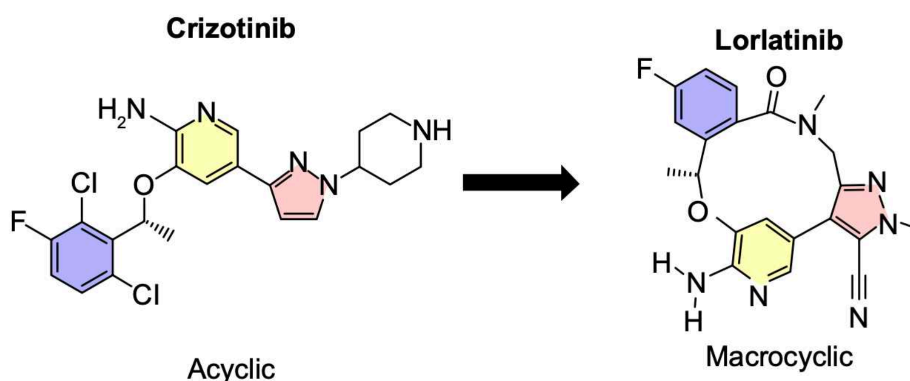


Figure 21: Crizotinib and Lorlatinib structures.

Adapted from [77]

2.3 Serine/Threonine kinase inhibitors

2.3.1 BRAF inhibitors

The B-raf proto-oncogene (*BRAF*) gene encodes a serine/threonine kinase that is a component of the MAPK/ERK signalling pathway, which regulates cell growth, differentiation, and survival. The most common mutation, V600E, results in constitutive activation of BRAF, leading to uncontrolled cell proliferation. This mutation is prevalent in various cancers, including melanoma, colorectal cancer, and multiple myeloma [78].

BRAF inhibitor monotherapy has been associated with the development of resistance and the paradoxical activation of the MAPK pathway. To address these limitations, the combination of a BRAF and MEK inhibitor has been explored and adopted clinically. This dual inhibition strategy provides a more prolonged and durable therapeutic response, delays the emergence of resistance, and significantly reduces the risk of secondary malignancies (**Figure 22**) [79].

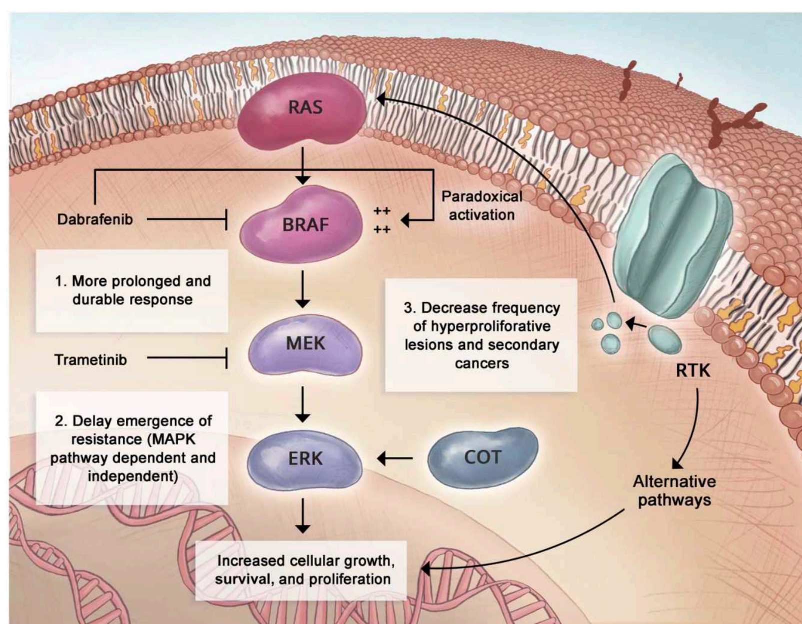


Figure 22: MAPK/ERK pathway and mechanism of action of combination therapy with Dabrafenib and Trametinib.

Adapted from [79]

Vemurafenib (**Figure 23**), approved by the FDA in 2011, specifically targets the mutated BRAF V600E kinase and is commonly used in combination with the MEK inhibitor cobimetinib. Dabrafenib (**Figure 23**), approved in 2013 for the same mutation,

is typically administered alongside the MEK inhibitor trametinib. More recently, encorafenib (**Figure 23**) has been approved for use in patients with BRAF V600E-mutant cancers, in combination with the MEK inhibitor binimetinib [79,80]. These inhibitors are classified as type $I^{1/2}$ since they bind to BRAF with DFG-in and αC-out conformation [81].

MEK kinases are dual-specificity enzymes and are also part of the MAPK/ERK signalling pathway. Trametinib (approved in 2013), Cobimetinib (approved in 2015) and Binimetinib (approved in 2018) are MEK inhibitors and are commonly used in combination with BRAF inhibitors, as previously mentioned. Trametinib was the first MEK inhibitor approved in 2013 [82].

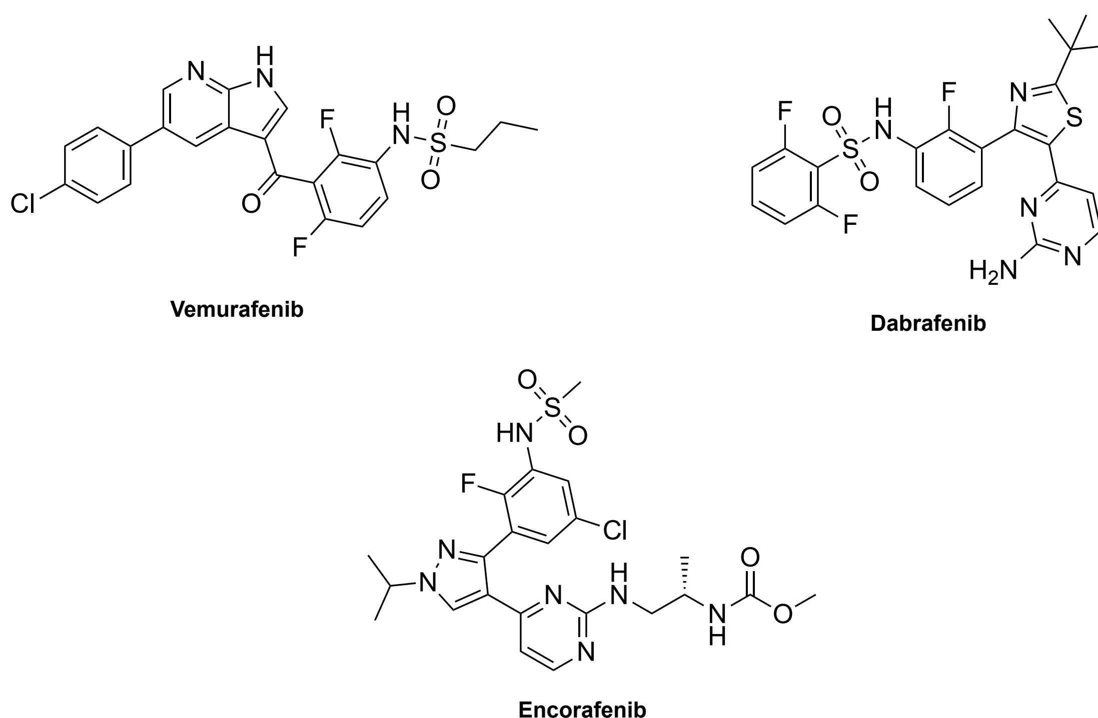


Figure 23: Second-generation BRAF inhibitors structures.

2.3.2 mTOR inhibitors

The mammalian target of rapamycin (mTOR) is part of the PI3K-Akt-mTOR signalling pathway and functions as part of two distinct complexes, mTORC1 and mTORC2 [83].

Allosteric mTOR inhibitors, such as rapamycin (also known as sirolimus) and its analogues (known as rapalogs) like everolimus, temsirolimus and ridaforolimus bind to the intracellular FK506-binding protein of 12 kD (FKBP12) and then form a complex with the rapamycin binding (FRB) domain of mTOR that allosterically inhibits its activity [83,84]. In **Figure 24** these inhibitors are represented, the regions responsible for FKBP12 binding are highlighted in blue, while the portions interacting with the FRB domain of mTOR are shown in red.

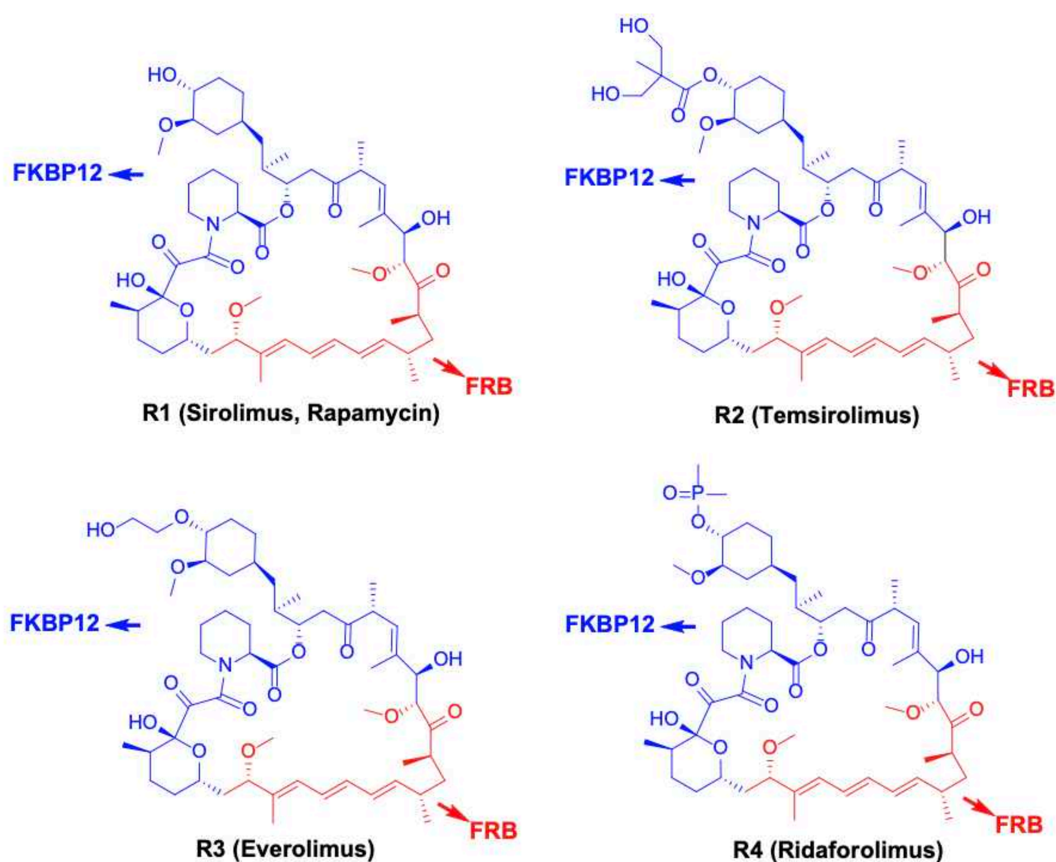


Figure 24: mTOR allosteric inhibitors structures.

Adapted from [83]

While traditional rapalogs have been applied in the treatment of several tumors, their effectiveness is limited by their incomplete suppression of mTORC1 and their inability

to inhibit mTORC2. In contrast, ATP-competitive mTOR inhibitors target the ATP-binding site within the catalytic domain of mTOR kinase, allowing them to block the activity of both mTORC1 and mTORC2, which may offer enhanced therapeutic benefits, although they are currently in investigation [83]. For example, sapanisertib (**Figure 25**) is an experimental dual mTORC1/2 inhibitor [85].

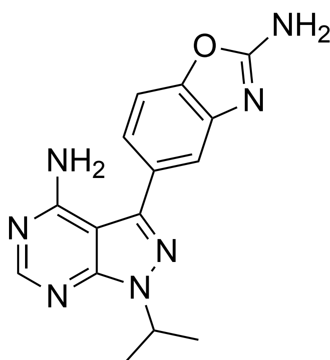


Figure 25: Sapanisertib structure.

2.3.3 CDK 4/6 inhibitors

Cyclin-dependent kinases (CDKs) participate in the mitotic cell cycle, a tightly controlled process essential for cell proliferation. Cyclin D, a member of the cyclin family, forms a complex with CDK4/6 kinases that are part of the cell cycle progression from the transition of the first growth phase (G1 phase) to the DNA synthesis phase (S phase). The complex Cyclin D – CDK4/6 gets activated in the nucleus and phosphorylates the retinoblastoma protein (Rb), a tumor suppressor. When Rb protein is phosphorylated inhibits transcription factors such as E2F and initiates the cell into the S phase. In cancer cells, Rb is often hyperphosphorylated and it can lead to the loss of its tumor suppressive function. Therefore, CDK4/6 inhibitors will decrease Rb phosphorylation to prevent the proliferation of tumor cells, arresting the cell cycle in the G1 phase (**Figure 26**) [86].

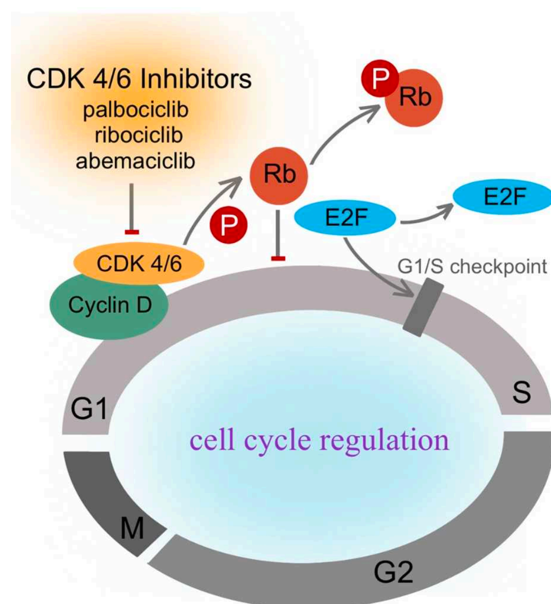


Figure 26: The role of CDK 4/6 inhibitors in cell cycle regulation.

Adapted from [86]

CDK4/6 inhibitors have significantly contributed to the evolution of targeted therapies in breast cancer management. Their combination with endocrine therapy (ET) has become a standard treatment strategy for patients with hormone receptor-positive (HR+)/HER2-negative (HER2−) breast cancer [87].

Palbociclib (**Figure 27 a)**), received approval in 2015 by the FDA for postmenopausal women with ER+/HER2- advanced breast cancer. Its structure is based on a pyridopyrimidine moiety, with a methyl, acetyl, and cyclopentyl substituents at the C5, C6, and C8 positions, respectively [88]. It also contains a 1-(pyridin-3-yl)piperazine side chain [89].

The structure‑activity relationship (SAR) of palbociclib is illustrated in **Figure 27 b)**. In the solvent-exposed region, the optimal substituent at the R5' position is a tertiary amine, which enhances solubility and, consequently, improves bioavailability. The nitrogen atom at the X position is essential for achieving selectivity toward CDK4. A methyl group at the R5 position is critical when an acetyl group is present at the R6 position within the lipophilic back pocket. Additionally, a cycloalkyl group at the R8 position, located in the lipophilic subpocket, is required for maintaining biological activity [89].

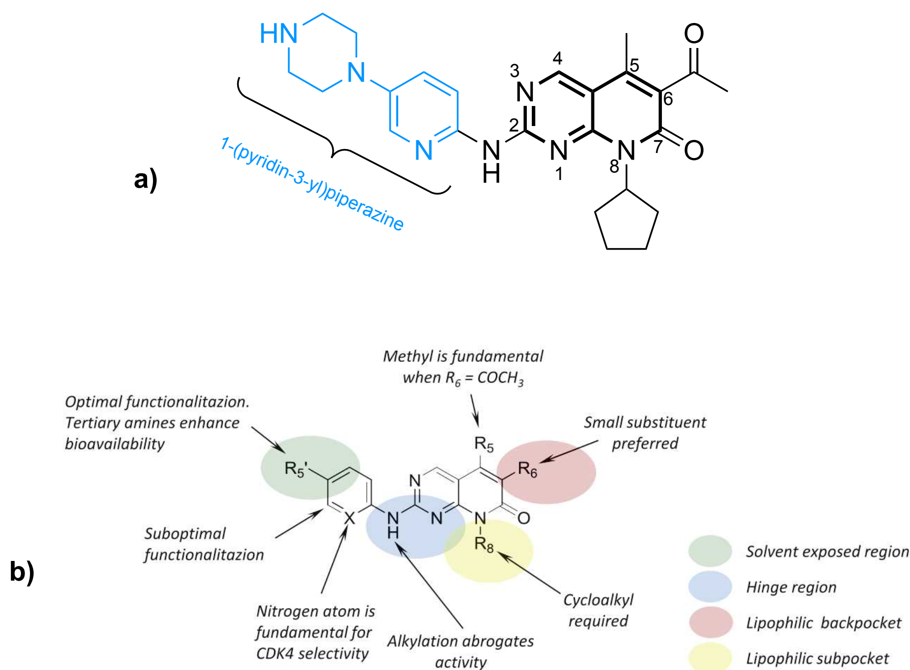


Figure 27: a) Palbociclib structure; b) Palbociclib structure-activity relationship (SAR) - adapted from [89]

Ribociclib (**Figure 28**) is a pyrrolopyrimidine containing the same 1-(pyridin-3-yl)piperazine side chain as palbociclib. It was approved in 2017 by the FDA for the combination treatment of pre/perimenopausal or postmenopausal women with HR+/HER2- metastatic breast cancer [89].

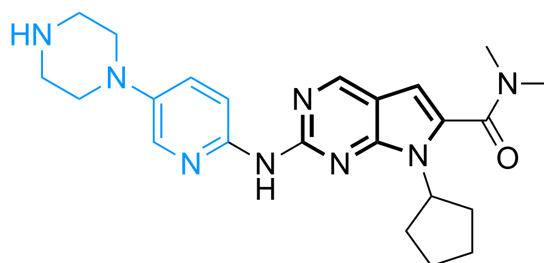


Figure 28: Ribociclib structure.

Abemaciclib (**Figure 29**) was approved by the FDA in 2017 and, in 2018 by the European Medicines Agency (EMA) for the treatment of postmenopausal women with HR+/ HER2− advanced breast cancer [90].

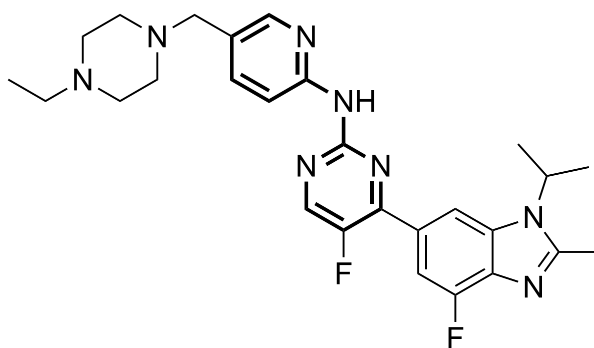


Figure 29: Abemaciclib structure.

The three molecules share common structural features: they bind to the inactive conformation of the kinase, form a hydrogen bond between the 3-position nitrogen of the pyrimidine ring and the NH group of His100, and establish another hydrogen bond between the carbonyl group of Val101 and the exocyclic NH group of their side chain. Additionally, Palbociclib and Ribociclib form a hydrogen bond between their carbonyl group and the DFG motif [89].

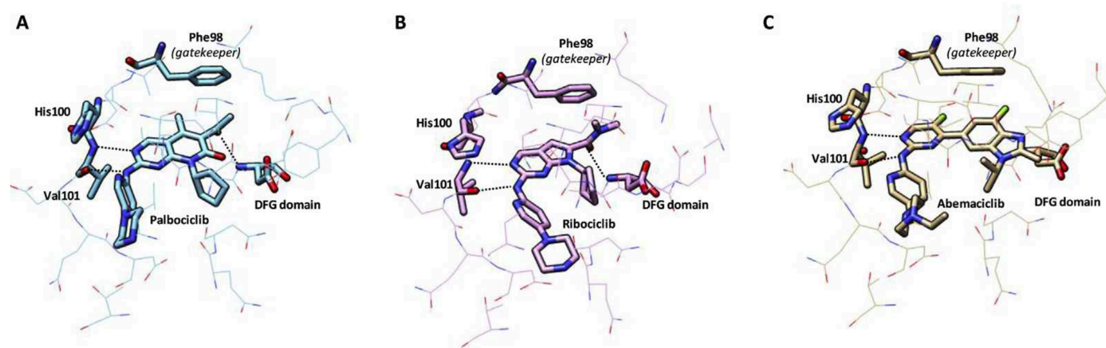


Figure 30: Binding modes of Palbociclib, Ribociclib and Abemaciclib with CDK6.

Adapted from [89]

2.4 Lipid kinase inhibitors

2.4.1 PI3K inhibitors

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases classified into three groups: Class I, Class II, and Class III. Among them, Class I PI3Ks are the most extensively studied and are particularly relevant to cancer. The PI3K signalling pathway can become abnormally activated through various genomic alterations, including mutations or amplifications in oncogenes such as phosphatidylinositol-3-Kinase catalytic subunit alpha (PIK3CA) and phosphatidylinositol -3-kinase regulatory subunit 1 (PIK3R1), as well as the tumor suppressor gene phosphatase and tensin homolog (PTEN). This pathway can be activated by a RTK or a G-protein coupled receptor (GPCR) [91].

The four isoforms of Class I PI3Ks are PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ. Isoform selectivity has been important in drug design and development. Furthermore, Class I PI3Ks can be subdivided into Class IA and Class IB. Class IA includes the catalytic subunits p110α, p110β, and p110δ, which form heterodimers with regulatory subunits from the p85 family. In contrast, Class IB consists of the catalytic subunit p110γ, which forms a heterodimer with either the p87 or p101 regulatory subunits [92]. Class I PI3Ks catalyse the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2), resulting in the production of phosphatidylinositol 3,4,5-trisphosphate (PIP3) and PTEN can dephosphorylate PIP3 back to PIP2 (**Figure 31**) [91].

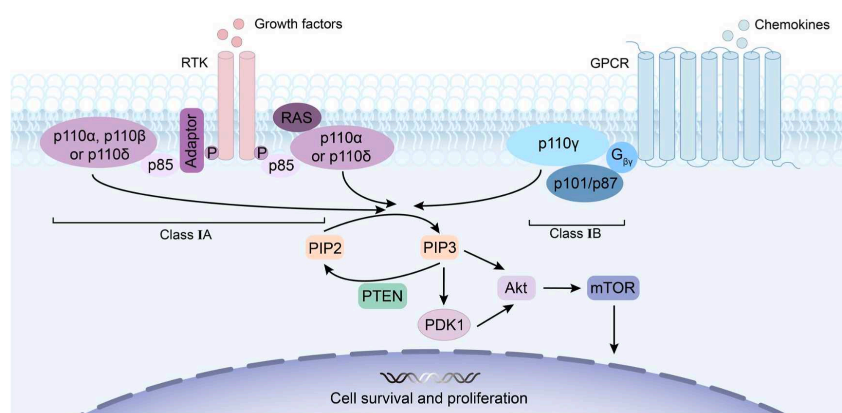
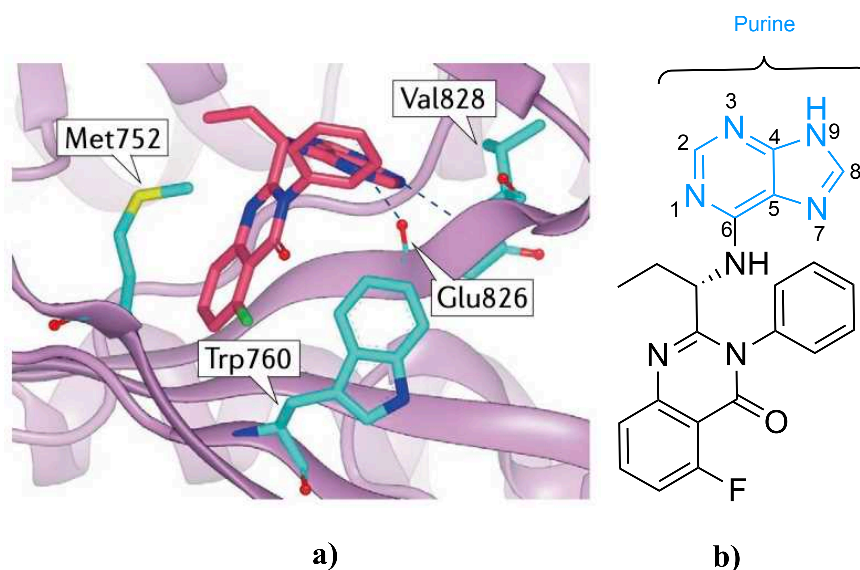


Figure 31: PI3K signalling pathway.

Adapted from [91]

Idelalisib became the first PI3K inhibitor approved by the FDA in 2014 for the treatment of patients with relapsed follicular B-cell non-Hodgkin lymphoma or small lymphocytic lymphoma (SLL) who had previously undergone at least two lines of systemic therapy and as a combination therapy with Rituximab in relapsed chronic lymphocytic lymphoma (CLL) [93,94]. Idelalisib is a selective inhibitor of PI3Kδ and acts as a competitive antagonist at the ATP-binding site of the p110δ catalytic subunit [93]. The purine moiety interacts with the hinge region by forming hydrogen bonds: the N3 atom forms a bond with the backbone carbonyl of Glu826, while the N9 atom binds to the backbone nitrogen of Val828 (**Figure 32 a)**) [92,95].



a) **b)**

Figure 32: Idelalisib binding to p110δ and structure.

a) Idelalisib binding to the catalytic subunit p110δ – adapted from [95]

b) Idelalisib structure

Alpelisib was approved in 2019 by the FDA in combination with fulvestrant for postmenopausal women, and men, with hormone receptor (HR)-positive, HER2-negative, with PIK3CA mutated, advanced or metastatic breast cancer [96]. Alpelisib is a selective inhibitor of PI3Kα (**Figure 33**) [95].

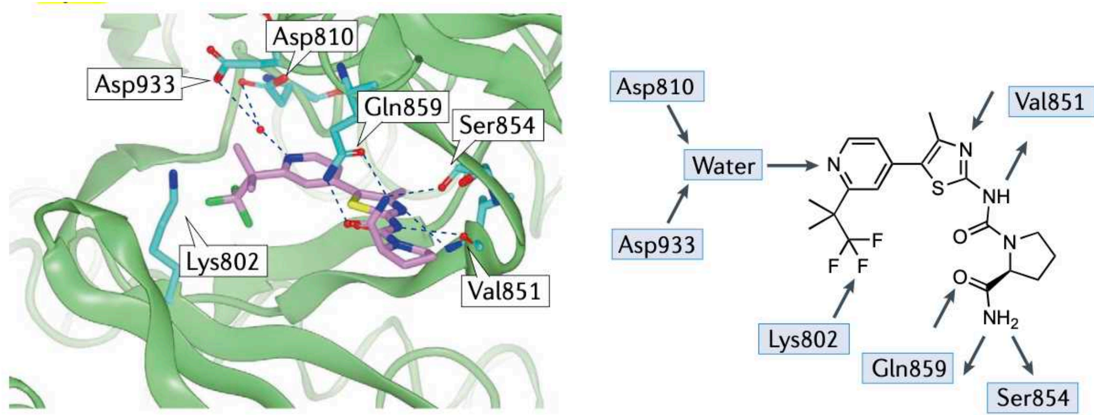


Figure 33: Alpelisib binding to PI3Kα and structure.

Adapted from [95]

3 Future perspectives

The continued development of SMKIs is essential, as resistance to existing therapies remains a major challenge in cancer treatment. Despite the clinical success of many kinase inhibitors, their long-term effectiveness is often limited by the emergence of resistance mechanisms and the occurrence of adverse effects. Therefore, future research must focus on designing next-generation inhibitors that retain potency against resistant mutations while minimizing off-target toxicity. The ultimate goal is to develop safer, more selective agents capable of achieving durable therapeutic responses across a broader range of patients and tumour types.

EGFR third-generation inhibitors, such as osimertinib and rociletinib, target the EGFR L858R/T790M mutations and have shown efficacy in overcoming resistance to earlier treatments. However, the emergence of the C797S mutation prevents covalent binding at the active site, leading to resistance against these drugs. To address this challenge, a library of compounds was screened against the EGFR L858R/T790M variant, with the goal of identifying fourth-generation inhibitors that act through an allosteric, non-covalent mechanism. Two compounds were obtained as the fourth-generation inhibitors, EAI001 and EAI045, but are still not approved (**Figure 34 a)**) [61].

EAI001 selectively inhibits mutant EGFR by binding allosterically and forms a hydrogen bond with Asp855 in the allosteric site of EGFR. The T790M mutation enables the thiazole group of EAI001 to form a π-sulfur interaction with the mutated gatekeeper residue. Additionally, its aromatic rings participate in hydrophobic interactions with Met766, Leu777, Leu788, and Phe856, that have a potential role in the allosteric activity. EAI045 was synthesized through the optimization of EAI001and it is an highly selective inhibitor of EGFR L858R/T790M [61]. The amide nitrogen interacts with Asp855 and the *para*-fluorophenol to Phe856 via hydrogen bonds in the allosteric binding pocket, as shown in **Figure 34 b)** [61,97]. A study in mice demonstrated that combining EAI045 with cetuximab, an anti-EGFR antibody, led to a significant reduction in tumor growth. However, EAI045 is not yet considered an optimal therapeutic agent [98].

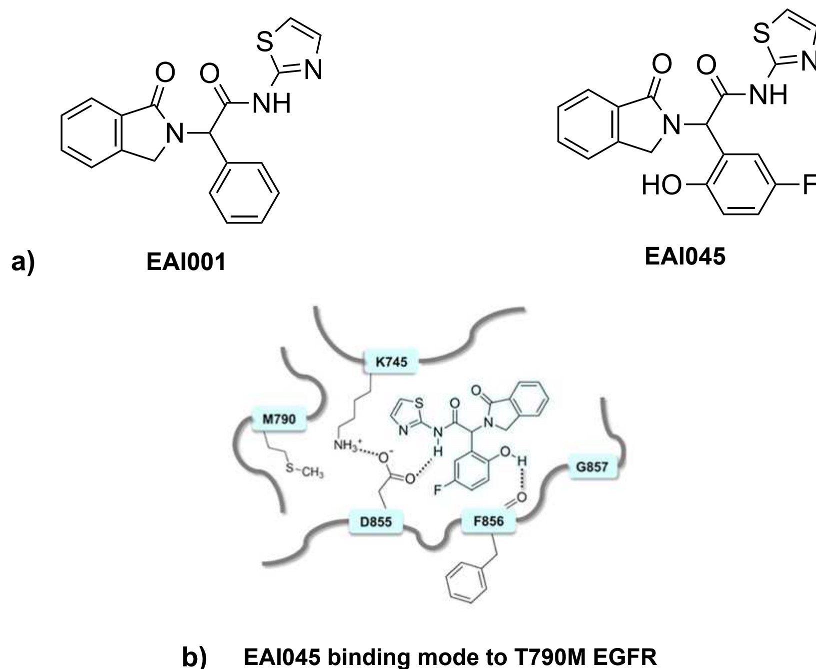


Figure 34: a) EAI001 and EAI045 structures; b) EAI045 biding mode to T790M EGFR – adapted from [97]

In the case of ALK inhibitors, fourth-generation inhibitors are also being developed. For example, TPX-0131 (**Figure 35**) is an ALK TKI developed for the advanced ALK--positive or metastatic NSCLC treatment. It has a macrocyclic structure like lorlatinib and an improved central nervous system penetration. It has been shown to inhibit both wild-type ALK and compound mutant G1202R/L1196M [99].

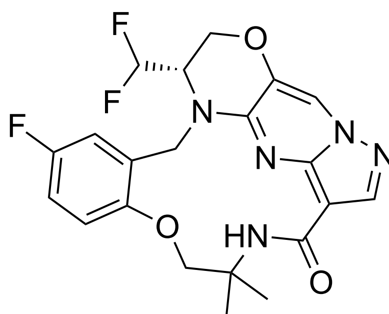


Figure 35: TPX-0131 structure.

4 Conclusion

Cancer remains one of the most prevalent and challenging diseases worldwide, affecting millions of people each year. Despite significant progress in diagnosis and treatment, there is a constant need for new and more effective therapeutic strategies. The introduction of small-molecule kinase inhibitors (SMKIs) has marked a major advancement in oncology, particularly within the field of targeted therapy. These agents allow for the precise inhibition of dysregulated kinases that drive tumor growth and progression, offering a more individualized approach to treatment compared to traditional chemotherapy.

One of the main advantages of SMKIs is their oral bioavailability, which enables many patients to undergo treatment at home, reducing the burden of hospital visits and improving overall quality of life. Their specificity not only enhances therapeutic efficacy but also helps limit systemic toxicity, making them a more tolerable option for many cancer patients. Furthermore, SMKIs have expanded treatment options for many tumours that previously had few effective therapies, demonstrating notable success in conditions such as chronic myeloid leukemia, non-small cell lung cancer and breast cancer.

However, several limitations continue to hinder their long-term effectiveness. The emergence of acquired resistance, particularly due to point mutations in the kinase domain, such as those occurring at gatekeeper residues, remains a significant obstacle. These mutations can impair drug binding, affecting treatment over time. In addition, while SMKIs generally have a more favourable toxicity profile than conventional therapies, adverse effects such as cardiotoxicity, dermatological reactions and gastrointestinal disturbances still pose clinical challenges.

As a result, the development of next-generation SMKIs is essential to address these limitations. Future efforts must focus on designing inhibitors capable of overcoming resistance mutations, improving target selectivity and reducing adverse effects. In the end, although SMKIs have transformed cancer treatment, continued research and innovation are essential to enhance their therapeutic efficacy and improve patients' quality of life.

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