Kinase Targeted Anticancer Agents: A Perspective

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ABSTRACT

The phosphate group present in the ATP (adenosine triphosphate) is transferred to the hydroxy group-containing tyrosine, serine, or threonine residue by the protein kinases encoded in the human genome. Till now, a large number of these kinases have been reported to be associated with the initiation and progression of human cancers. In clinical trials, it has been demonstrated that small-molecule kinase inhibitors can effectively cure a wide range of cancers. The FDA approved more than 40 kinase inhibitors for cancer treatment since the early 1980s when the first protein kinase inhibitor was developed. In this review, the authors explained the relevancies of the kinase with cancer. In addition, several FDA-approved drug candidates have been classified according to their binding with kinases.

Keywords: Anticancer, Kinase inhibitors, FDA approved drugs, kinase binding, types of kinase inhibitors.

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INTRODUCTION

Kinases are known to catalyse the transfer of phosphate group from the high-energy molecule ATP to a specific substrate, and this process is known as phosphorylation. Every signal transduction pathway involves phosphor transfer, indicating that kinases can target numerous dysregulated biological processes (Manning et al., 2002). Kinase dysfunction has been linked to immunological, cardiovascular, degenerative, inflammatory, metabolic, and viral infections

(Levitzki et al., 2003; Müller et al., 2015). The ongoing research to understand the kinase mechanism in disease processes provided insight into developing kinase-specific drug treatments. The constitutively active kinase activity known to present in the proliferative disorder like cancer has been targeted by the researcher as a treatment. The cancer initiation, growth, progression, and recurrence are all linked to genetically inherited variations of particular kinases (Kittler et al., 2018). Approximately 5,000 protein kinases are present in the public domain, actively explored by researchers for structure-based drug design. It is worth mentioning that the pharmaceutical industry uses many proprietary structures in the development of potent kinase inhibitors. Till now, about 180 orally active protein kinase inhibitors are in clinical studies (Carles et al., 2018).

The first report of the structure of protein kinase A (PKA) in a complex with ATP–Mg^{2+} and an inhibitory peptide was published by Knighton et al. in 1991(Knighton et al., 1991; Ten Eyck and SS, 1991). The protein kinases' activity can be generalized by the following reaction:

$MgATP^{1-}$ + Protein–OH ➔ Protein–OPO_3^{2-} + MgADP + H^+

The hydroxy group-containing protein residue (serine, threonine, and tyrosine) is used to segregate or classify these kinases (Roskoski et al., 2016). There are 518 protein kinase enzyme families are known, out of which 385 are protein-serine/threonine kinases, 90 are protein-tyrosine kinases and the rest 43 are protein-tyrosine kinase-like enzymes. The protein-tyrosine kinase group consists the 58 transmembrane receptors and 32 intracellular non-receptors (Manning et al., 2002). The The MEK (mitogen-activated protein kinase kinase) 1/2 are unique in the protein kinase superfamily because of their dual-specificity towards the phosphorylation of the activation segment of their target proteins with both tyrosine and threonine residues. The US FDA has approved the 68 therapeutic small molecule protein kinase inhibitors (SMPKIs) till 2021 (Ayala-Aguilera et al., 2021) Out of the 68 FDA-approved SMPKIs, 39 are known for their activity against the receptor protein-tyrosine kinases, 13 against the nonreceptor protein-tyrosine kinases, 12 against protein-serine/threonine protein kinases, and four are against dual-specificity protein kinases (MEK1/2). At least 25 of the drugs that have been authorized are multi-kinase antagonists. The multi-kinase inhibitors have some pros and cons. Pros: The efficacy of multi-kinase inhibitors is high, maybe because of their ability to interact with two or more targets simultaneously. Cons: due to its interaction with other targets may cause innumerable side effects. The eight FDA-approved SMPKIs molecules form a covalent bond with kinase and are classed as TCIs (targeted covalent inhibitors) (Roskoski et al., 2021). Most of these targeted covalent inhibitors target the ATP-binding site (Karaman et al., 2008; Shukla et al., 2009), except for a small number of non-ATP competitive kinase inhibitors that target specific allosteric sites (Kirkland et al., 2009). As a result, the suppression of kinase activity in cancer patients results in the activation of many anti-proliferative pathways, ultimately resulting in cancer's clinical remission.

The function of kinases in cancer

Cell transformation, tumor growth, survival, and proliferation are all dependent on several kinds of kinases. We tried our best to summarize the most crucial kinase responsible for oncogenesis. Several oncogenic diseases are associated with mutations in cytoplasmic tyrosine kinases, essential extracellular signal transporters. The most frequently mutated kinases linked to 30–50 percent of human malignancies are PI3K (Phosphoinositide 3-kinases) family of dual specific protein/lipid kinases (Samuels et al., 2004). The PI3KCA is the most significant member of the PI3K family known to involve in human cancer and has been hypothesized since 1995 (Samuels et al., 2004). The PIK3CA mutations were most common in endometrial (21%), colon (17%), ovarian (17%), breast (14%), cervical (13%), and head and neck squamous cell carcinomas (9%) (Janku et al., 2011). The PI3KCA, upon activation, catalyses the synthesis of PIP3 that attach to the lipid-binding domains of downstream targets to the cell membrane.

Many signaling proteins, including the kinases PDK1 and AKT, can bind to PI3K's lipid products and localize to the cell membrane, activating cell development and survival pathways (Manning and Cantley, 2007). Likewise, the activated form of the protein kinase Akt/PKB plays an essential role in cell oncogenic changes. Inhibition of Akt/PKB signaling in tumor cells causes anchorage-independent growth to be inhibited and apoptosis to be induced in a number of tumor cell lines (Cheng et al., 2005). The mutation in BRAF kinase (V599E and V600E) was found to be associated with various kinds of carcinomas (Davies et al., 2002). The oncogenic mutations in JAK2 (Val617 Phe or exon 12) have been associated with essential thrombocythemia, polycythemia vera, and myelofibrosis, as well as other myeloproliferative disorders (Kralovics et al., 2005).

Insulin-like growth factor 1 (IGF-1R), Anaplastic lymphoma kinase (ALK), tyrosine-protein kinase Met (c-Met), proto-oncogene tyrosine-protein kinase (c-SRC), ret proto-oncogene (c-Ret), Fibroblast growth factor 1 to 4 (FGFR 1-4) and RAF kinase are the few examples which regale the cell cycle, gene transcription, motility, cell death, and metabolism (Shawver et al., 2002). As a result of this, the genetic mutation led to the development and growth of tumour cell (Blume-Jensen et al., 2001). Along with their role in tumour initiation, kinases are also play an important role in the proliferation and survival of tumour cells, and they found as the downstream members of oncogenic kinase pathways.

The other receptor kinase type is the epidermal growth factor receptor (EGFR). The EGFR represents the ErbB family of receptor tyrosine kinases (RTKs). It is commonly mutated and or overexpressed in several human malignancies and is presently the focus of multiple cancer treatments (Yarden et al., 2012). The physical interaction of EGFR with sodium/glucose cotransporter 1 (SGLT1) led to an increase in glucose inflow by stabilizing the sodium-glucose cotransporter at the cell surface. When cells are grown in low glucose concentrations, this kinase-independent activity provides survival benefits, allowing them to avoid autophagic cell death (Weihua et al., 2008).

The most prevalent EGFR point mutation in the kinase domain is L858R, accounting for around 45 percent of all tyrosine kinase domain mutations (Paez et al., 2004). As a result, the inhibitory regulatory domains for dimerization are lost, leading to cancer cell overactive-proliferation via G1/S cell cycle progression (Sahin et al., 2009).

The other important member of the kinase family is the Aurora kinase (Aurora A-C) which are abbreviated as AURKA, AURKB and AURKC respectivley. These are are serine/threonine kinases necessary for mitosis and meiosis, the AURKA and AURKB are reponsible for regulation of mitosis and AURKC regulate the meiosis. The Aurora (A-C) kinases effect is well seen in the various human cancers like breast cancer, ovarian cancer, gastric/gastrointestinal cancer, and several other tumors because significant overexpression or amplification has been observed in these kinases during human malignancies (Goldenson et al., 2015; Honma et al., 2014). The p53, a well-known tumor suppressor known to regulate by the AURKA, because it regulates the p53 through phosphorylation on both Ser 215 and Ser 315 residues, these resiudues inhibits p53 transcriptional activity and improves the Mdm2-intermediated p53 degradation, respectively (Liu et al., 2004). AURKA is also responsible for the activation of NF-κb; the inhibition of NF-κb leads to the reduced expression of pro-survival genes e.g., Bcl-XL and Bcl-2 (Sun et al., 2007).

The MEK1 and MEK2 are members of the Mitogen Activated Protein (MAP) kinase kinase (MAPKK) (also recognized as MEKs or MKKs) family of enzymes, which are dual-specificity enzymes responsible for the phosphorylation of tyrosine and threonine residues in the activation loop of their MAP kinase substrates (Pearson et al., 2001). The ERK1/ERK2 activation is required for G1-to-S-phase progression in normal cells, and it is linked to the induction of cell cycle positive regulators and the inactivation of anti-proliferative genes (Meloche et al., 2007). The phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian target of rapamycin (mTOR) signaling pathways are both essential for cell growth and survival in many ways. The PI3K/Akt/mTOR pathway's activation causes a significant disruption in cell development and survival control, causing a competitive growth improvement, angiogenesis, metastatic competence, and therapeutic resistance (Porta et al., 2014).

The ribosomal protein S6 kinase 1 (S6K1) is involved in mRNA processing, cell development, protein synthesis, and homeostasis, among other important biological processes. As a result, it was discovered that S6K1 dysregulation was associated with breast, ovarian and lung cancers (Bostner et al., 2015; Choi et al., 2016; Poomakkoth et al., 2016). The p38 mitogen-activated protein kinases and c-Jun N-terminal kinases (JNKs) are additionally important targets for developing kinase inhibitors. Another category of oncogenesis-related kinases is those that are overexpressed in cancerous tumors and their surrounding tissues. They are crucial for tumor survival in the host. These comprise the neurotrophic factors (NTFs), which are the group of macromolecules that assist the development, survival, and differentiation

of both developing and adult neurons (Sahay et al., 2017). The Vascular Endothelial Growth Factor (VEGFRs), fibroblast growth factor receptor (FGFR) kinases, the protein kinase CK2, and Tropomyosin receptor kinase B (TrkB) are the other examples of this category (Davies et al., 2000; Futreal et al., 2004). Overall, oncogenic kinases are accountable for a range of characteristics of cancer, with fast proliferation, survival, growth, and metastasis. As a result, many kinase inhibitors have been developed to combat these characteristics.

This category of kinase includes the Receptor tyrosine kinases (RTKs). There are roughly 20 unique RTK classes that have been identified (Ségaliny et al., 2015). These kinases retain a very similar molecular framework, and mutation and unusual activation have been linked to cancer and angiogenesis. The gain-of-function mutations, chromosomal translocations, RTK overexpression, and autocrine activation all play a critical role in the unusual RTK activation in human carcinogenesis (Bhullar et al., 2018). Most RTKs are single-subunit receptors, but some exist in multimeric forms, such as the insulin dimeric receptor, which exists as multimeric complexes connected by a disulfide bond. RTKs can also exist in the formation of oligomers, even in the deficiency of an activating ligand (Ullrich et al., 1990; Ward et al., 2007). The epidermal growth factor receptor (EGFR) is a well-studied kinase that has been interconnected to numerous human cancers e.g., lung cancer, anal cancer, glioblastoma, epithelial tumors of the head and neck, and breast cancer (Libermann et al., 1985; Sharma et al., 2007; Slamon et al., 1989; Walker et al., 2009).

The kinase catalytic domain's structural characteristics

Knighton et al. published the first description of the kinase fold's architecture in 1991(Knighton et al., 1991). The amino-(N) and carboxy-(C) terminal lobes of the protein kinase have a conserved bilobed 3-D shape that is coordinated in its movement in respect to each other depending on the activity of the kinase. The structure of protein kinase A (PKA) in association with ATP–Mg^{2+} and an inhibitory peptide was described by Knighton et al. The five-stranded antiparallel β-sheet (β1–β5) is found in the smaller N-terminal lobe. In contrast, the C-terminal lobe contains alpha-helices (Figure 1). The ATP cofactor was found to be located between the adenine ring and hinge region, stabilized by establishing two hydrogen bonds between them, providing a backbone that connects the lobes. The hydrogen bonds between the adenine ring and hinge region were found to be essential for designing the ATP mimetic inhibitors, and they are also characterized as the crucial kinase inhibitory scaffolds. The glycine-rich loop (G-loop, also known as the phosphate-binding loop (P-loop)) also coordinates with the ATP. This G loop is very flexible in nature and exists between the β-sheet structures β1 and β2 (Figure 1). A salt bridge was found in the middle of the lysine residue in the β3 and conserved glutamate presented in αC positioning αC ('αC-in' conformation) is a structural requirement of active kinases.

The β3 salt bridge helps coordinate the ATP through the phosphate groups; hence the αC helix plays a crucial regulatory function. In the inactive state of kinase, the αC helix turns outwards (C-out), displacing itself

from its active state position and rotating the conserved glutamate away from the ATP-binding site. Additional stabilization of the αC helix C terminus in the 'αC -in' state is provided by association with the conserved Asp-Phe-Gly motif DFG, which also attaches to the activation segment N terminus.

The DFG aspartate residue is located towards the phosphate group of ATP and coordinates with catalytically essential Mg^{2+} ion in its active kinase configuration, known as the 'DFG-in' configuration. The dynamic nature of the DGF in inactive kinase is responsible for displacing the phenylalanine residue (Schindler et al., 2000). The 'DFG-out' conformation led to the deep, primarily hydrophobic binding pocket. The activation segment (A-loop) is a structural element of the variable sequence. The length that protects the DFG motif and the 'DFG-out' movement also led to the transformation in the structure of the A-loop. The kinase activation of kinases is also controlled by phosphorylation sites in the activation segment (A-loop) through autophosphorylation or transphosphorylation. A well-conserved Tyr/His-Arg-Asp motif is found in the catalytic loop with maintained aspartate, which is essential for catalysis.

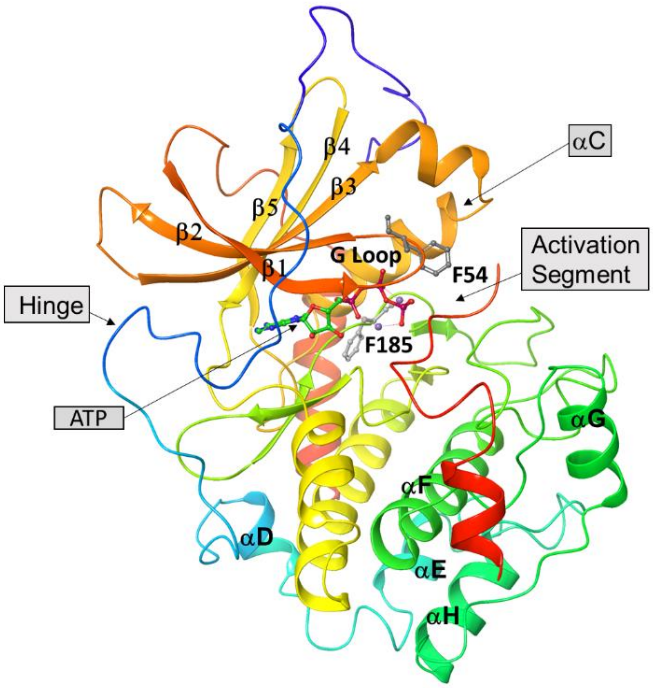

Figure 1: The structural overview of the kinase catalytic domain, ribbon representation (pdb: 3FJQ)

The eukaryotic kinases have a highly conserved 3D structure, including a network of hydrophobic residues that are structurally very crucial

(Kornev et al., 2006). These residues comprise two spines, one is the catalytic spine (C-spine), and the other one is the regulatory spine (R-spine) (Figure 2) (Kornev et al., 2008). These residues connect and align both kinase lobes in the active states and position key sequence motifs. The adenine ring part of the cofactor ATP completes the catalytic spine, which joins structural elements on the kinase domain's hinge side. When in the active state, the regulatory spine detects the appropriate alignment of the β4 sheet, αC,DFG motif, and lower lobe αE helix. In inactive states, the displacement of R-spine residues led to a break in the R-spine. Mutagenesis investigations confirmed the functional relevance of spine residues, demonstrating that hydrophobic interaction is necessary for effective catalytic activity (Meharena et al., 2013). Several kinase inhibitors are found to interact with the spine residue; in some cases, they intercalate or change the spine interaction due to their closeness to the ATP-binding site. At the N end of the hinge region, a residue known as the gatekeeper connects the C-spine with the R-spine (M120) (Figure 2). The significance of the gatekeeper residue in drug development has long been acknowledged (Fox et al., 1998). Small gatekeeper residue, for example, threonine, makes the back pocket available to small molecules; as a result, the inhibitors bound to this pocket can't bind to other kinases with large hydrophobic residues in the gatekeeper position, which is useful while designing the selective inhibitors (Blencke et al., 2004). In the human kinases, the glycine residues do not appear in the gatekeeper position, which results in the design of a bulky ATP analog that mainly targets kinase gatekeeper mutants. Nevertheless, gatekeeper mutations that swing to bulkier residues that sterically omit the inhibitors are a general reason for kinase drug resistance109 (Gorre et al., 2001)

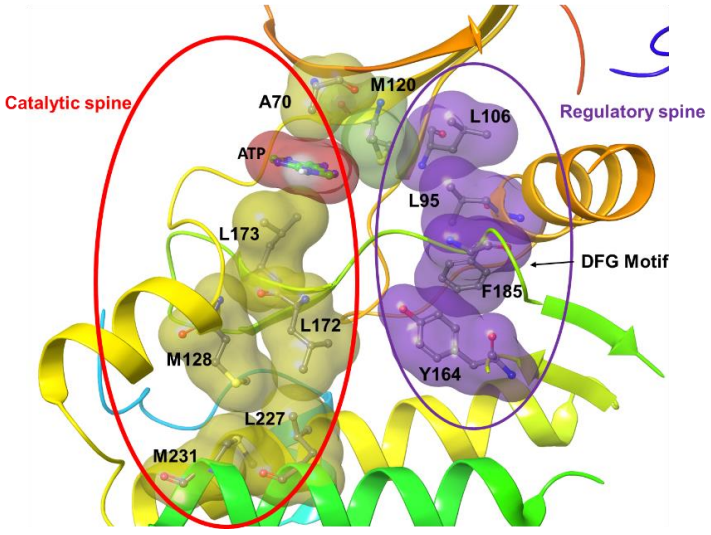

Figure 2: The aligned catalytic (yellow) and regulatory (violet) spine residues in the kinases active state. The gatekeeper residue (M120, grey) linked the two spines

Types of kinase inhibitors

Kinase inhibitors are quite successful in treating cancer, especially when it comes to targeting specific mutations that are the fundamental cause of cancer. They are categorized by their potential to catalyse the transfer of ATP's terminal phosphate to substrates via serine, threonine, or tyrosine residues. The protein kinases' extremely dynamic nature enables the development of inhibitors that detect active or inactive conformations. In the drug development, the inactive state of kinases has been extensively studied due to its structural diversity. The kinase catalytic domain has over 80 potential ligand-binding sites, which have been classified and characterized (Kanev et al., 2021). Figure **3** schematically illustrates all classified kinases based on their binding.

Types I of kinase inhibitors

Type I kinase inhibitors target the kinase active state by mimicking the purine ring of the adenine scaffold of ATP. The main region where the type I inhibitors bind in the area where the ATP adenine scaffold interacts except this these inhibitors also interact with the nearby region as well, for example, front pocket region, the hydrophobic pocket region, the DFG motif (‘in’ position), and the P-loop region. In the kinase structure, the G-loop is not found in the active conformation, leading to the aromatic π- π stacking with aromatic purine ring and tyrosine-253 (Y253) (Figure 4). The aromatic amino acids at the corner of the G-loop were found to be positioned away from the ATP site, in the ATP-bound active state. A favourable inhibitor selectivity has been linked to the presence of 'folded P-loop conformations' in many kinases’ inhibitor complexes. The marketed drugs can be further classified into several categories depending upon their binding mode into the active site of kinases. The figure 5 illustrates the market drugs bind with the front pocket region, the adenine-binding area, and the hydrophobic pocket region. Figure 6 shows the drug candidates' binds to the front pocket region, the adenine-binding area, and the DFG-motif region. Figure 7 represents the drug molecules that binds with the front-pocket region, adenine-binding area, DFG-motif region, and hydrophobic pocket region. Figure 8 illustrates the drug molecules that bind with the hinge region and P-loop region.

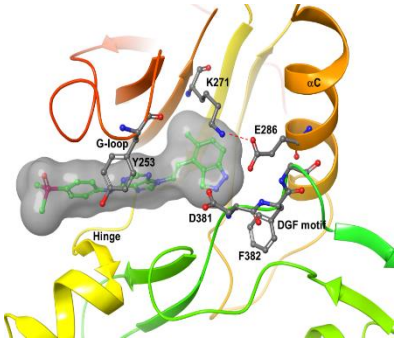

Figure 3: Schematic representation of kinase inhibitor binding modes

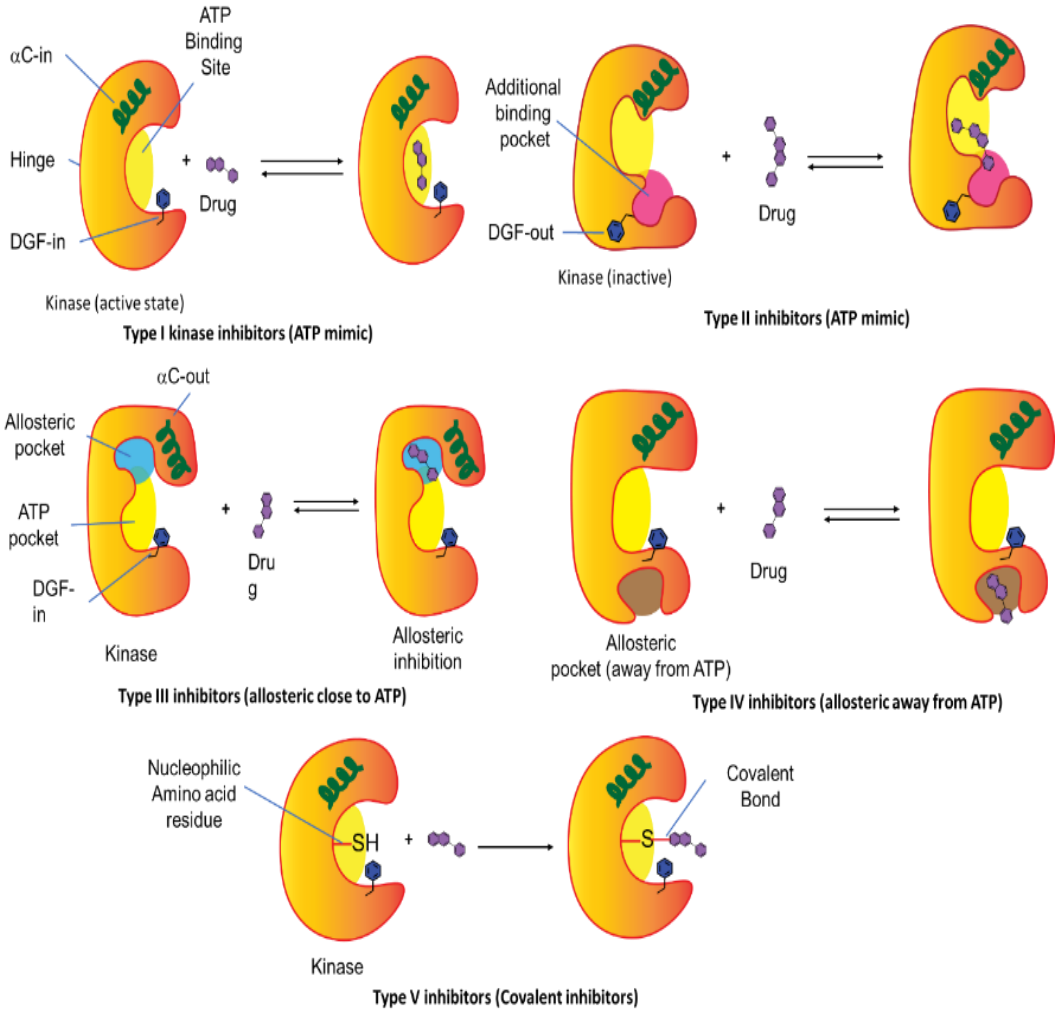

Figure 4: Details of the Types I of kinase inhibitors in ABL kinase. (pdb ID 3kf4)

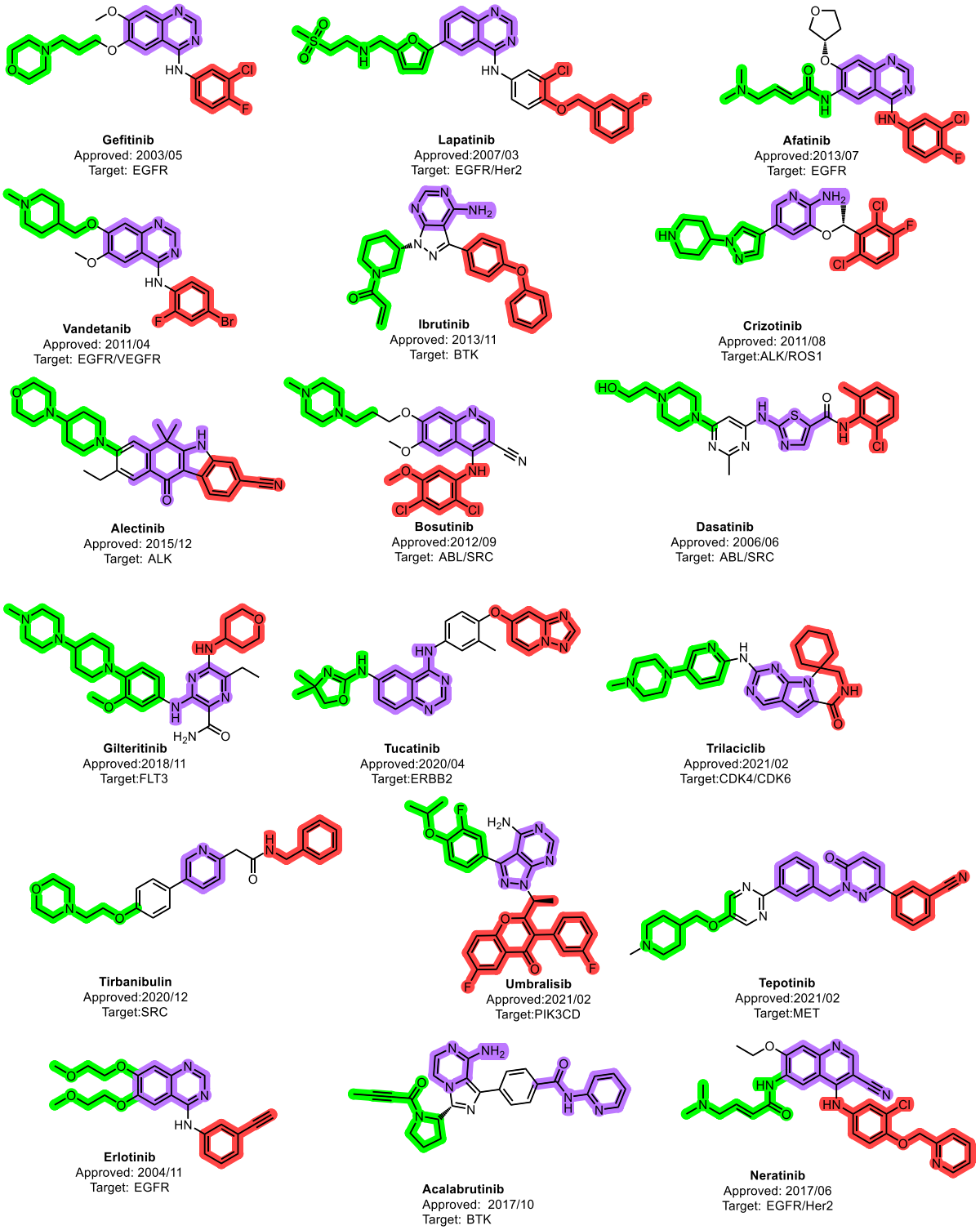

Figure 5: Type I inhibitors drug molecules (a): The green highlighted part binds with the front pocket region, the violet highlighted part bind in the adenine binding region, and red highlighted part binds with the hydrophobic pocket region

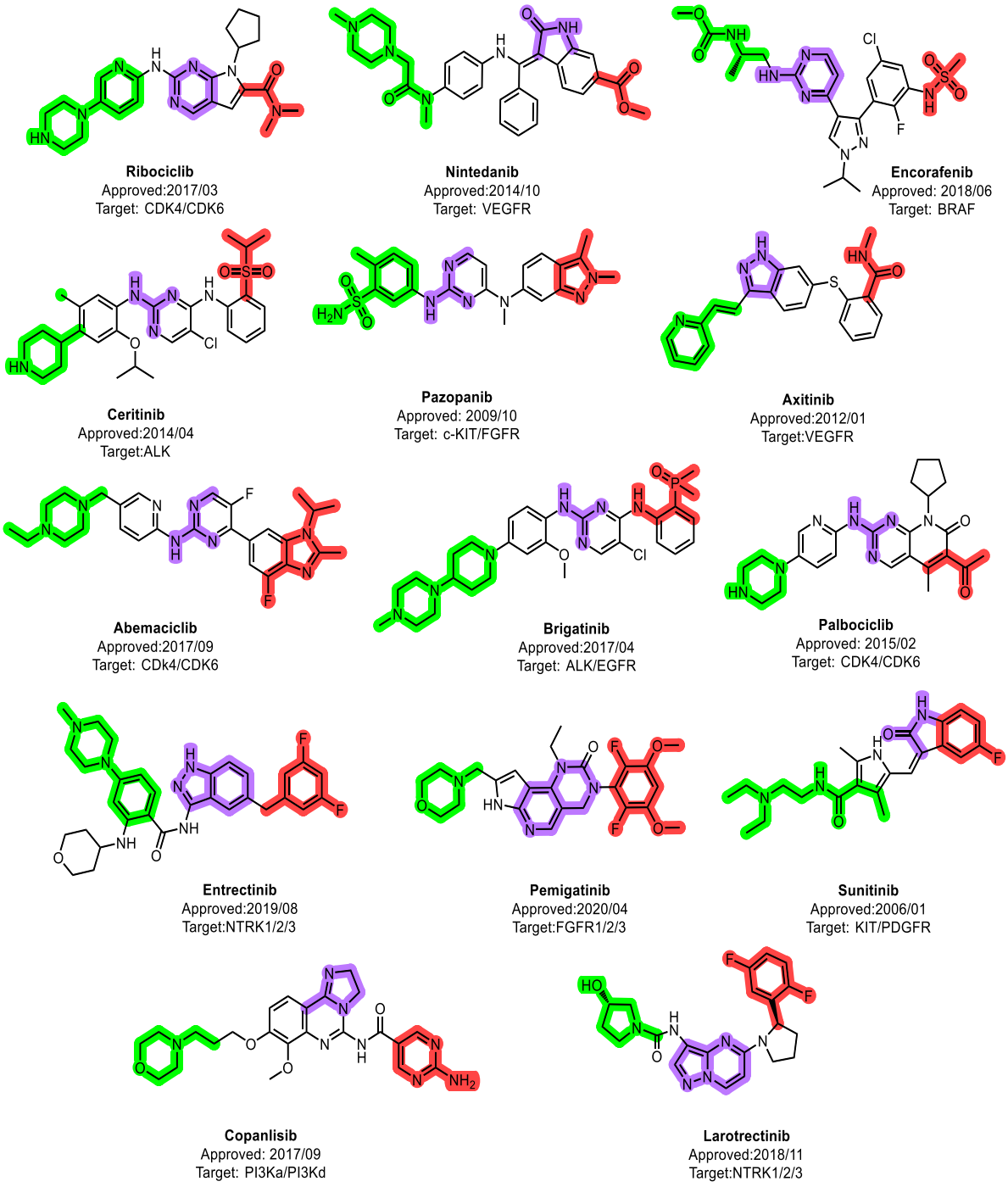

Figure 6: Type I inhibitors drug molecules (b): The green highlighted part binds with the front pocket region, the violet highlighted part bind in the adenine binding region, and the red highlighted part binds with the DFG-motif region

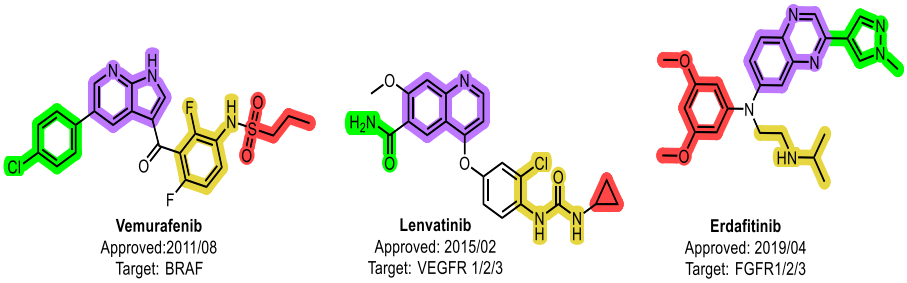

Figure 7: Type I inhibitors drug molecules (c): The green highlighted part binds with the front pocket region, the violet highlighted part bind in the adenine binding region, the yellow highlighted part binds with the hydrophobic pocket region, and the red highlight part binds with DFG-motif region

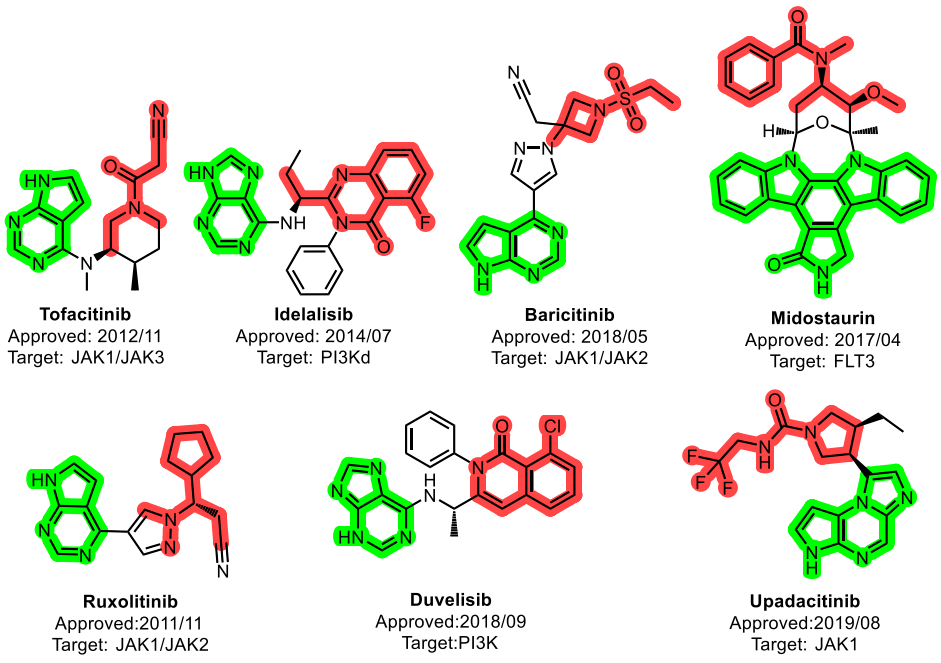

Figure 8: Type I inhibitors drug molecules (d): The green highlighted part binds in the adenine binding region, and the red part binds with the P-loop region

The drugs shown in figure 9 displayed different binding modes. Dabrafenib binds to the front pocket region, the adenine-binding area, and the P-loop region.

In the EGFR binding pocket, Osimertinib binds to the P-loop, the adenine-binding region, and the front pocket region. The Fostamatinib has a 3,4,5-trimethoxyphenyl group which binds to the front pocket region, a pyrimidine group binds with the adenine-binding region, a pyridine derivative interacts with the P-loop region, and a phosphate group binds within the DFG-motif region.

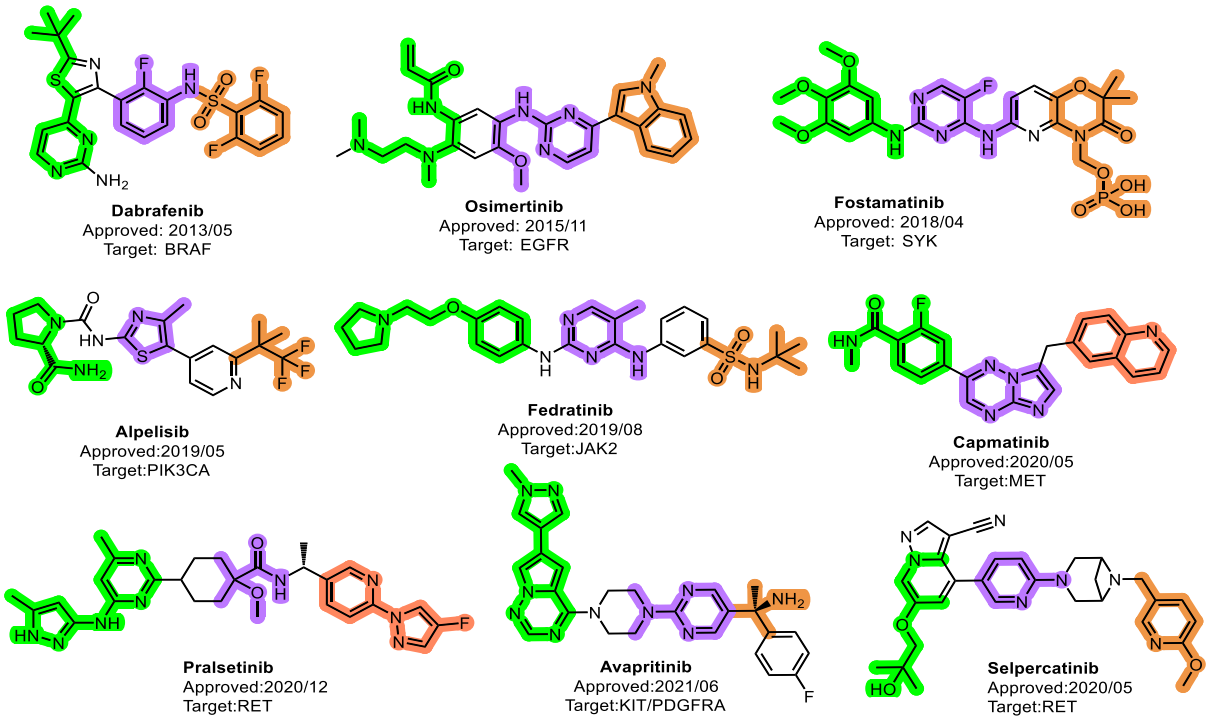

Figure 9: Type I inhibitors drug molecules (e): The green highlighted part represents the front pocket region, violet highlighted part represents the adenine-binding area and orange part represent the P-loop region

Types II of kinase inhibitors

Type II kinase inhibitors target the inactive conformation of kinases by interacting with the catalytic site of the unphosphorylated inactive conformation (Kufareva et al., 2008). These inhibitors reversibly bind to the target kinase, forming hydrogen bonds (single or multiple) with the protein in the 'hinge region' and causing further interactions in the ‘DFG-out’ conformation (Kufareva et al., 2008; Liu et al., 2006). The

exclusivity of inactive protein kinase conformations was hypothesized to make the type II kinase inhibitors more selective.

Because the target sites of Type I inhibitors, the classic ATP-binding sites of active kinases, do not possess these unique characteristics, this pocket is maintained to

a lower extent across the kinome, promising more significant opportunities for the rational design of selective kinase inhibitors (Davis et al., 2011). Their shared binding modes established the usual Type II binding pattern, which occupied the adenine-binding area, the DFG-out motif region, and the allosteric pocket region (Figure 10).

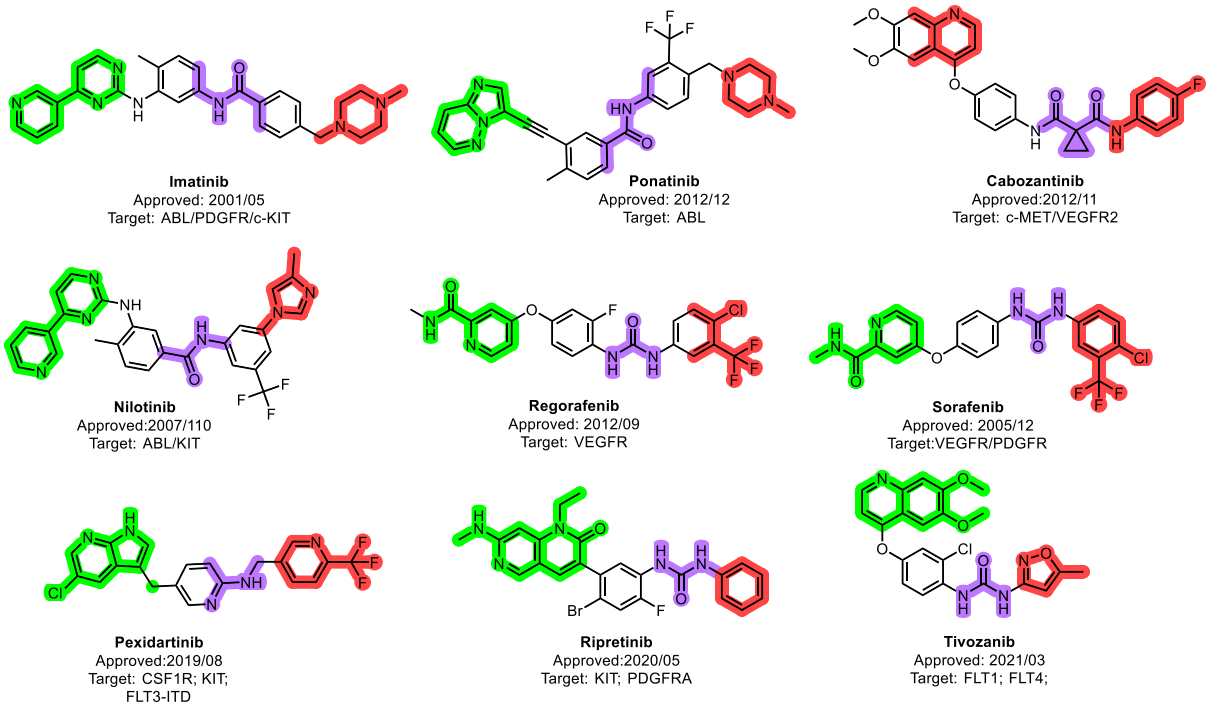

Figure 10: Type II inhibitors drug molecules: The green highlighted part binds in the adenine binding region, violet highlighted part binds with DFG out motif, and red highlighted part binds with the allosteric pocket region

Type III or allosteric kinase inhibitors

The third type of kinase inhibitor primarily binds outside the catalytic domain/ATP-binding site and affects kinase activity allosterically.

Type III inhibitors have the highest target kinase selectivity because they take advantage of binding sites and physiological processes unique to each kinase. The ' αC -out ' conformation allows these inhibitors to bind to the ATP-binding site in a pocket and, in certain instances, the inactive A-loop conformation. All four MEK1/MEK2 inhibitors approved are type III inhibitors binding to the allosteric back pocket (Figure 11). The DFG usually remains in an active 'in' conformation, permitting the type III inhibitor and ATP to bind simultaneously. Specific type III kinase inhibitors establish polar interactions with ATP's phosphate backbone, causing an ATP- uncompetitive mechanism of action. Using allosteric inhibitors to target kinases is a crucial

method to overcome obstacles in kinase inhibitor research, such as low selectivity, off-target adverse effects, and drug tolerance.

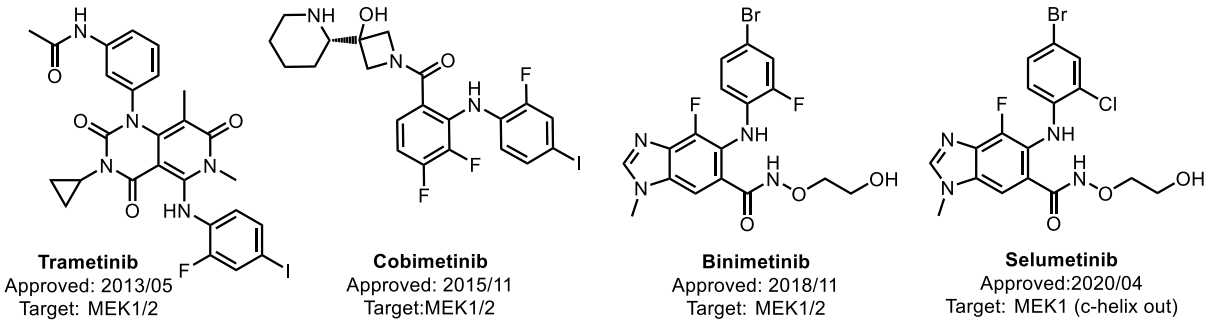

Figure 11. Approved Type III kinase inhibitors

Type IV or Substrate-directed inhibitors

Type IV kinase inhibitors interact with allosteric sites away from the ATP-binding site reversibly. These inhibitors are more selective against selected kinases since they don't compete with ATP (Blanc et al., 2013). GNF-2, an ABL inhibitor that binds to an induced pocket in the C-terminal kinase lobe that also serves as a binding site for lipids such as myristate, is an excellent example of a type IV kinase inhibitor (Zhang et al., 2010). This type IV inhibitor works by activating ABL in a particular way. In inactive ABL kinases, a myristoylated residue at the N terminus of ABL kinases binds to the C-lobe binding pocket resulting in the stability of a closed inactive conformation (Nagar et al., 2003). Due to the occurrence of the N-terminal fusion partner BCR in the oncogenic fusion protein BCR–ABL, this process of inactivation is dropped, resulting in constitutively active ABL. GNF-2, GNF-5, and Asciminib imitate myristate binding, causing an inactive state comparable to that of the inactive wild-type protein. Currently, three molecules got the approval as substrate directed kinase inhibitors (Figure 12).

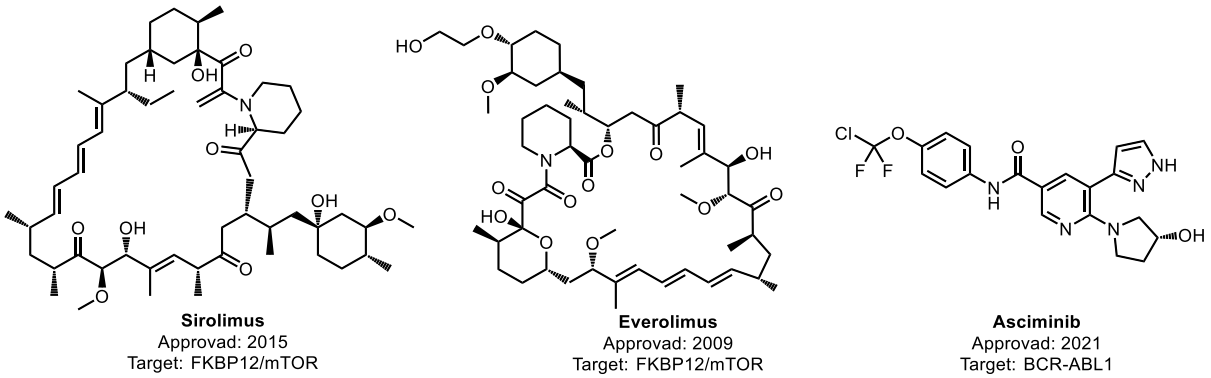

Figure 12. Approved Type IV kinase inhibitors

Type V or covalent inhibitors

The inhibitors of covalent kinases or type V form an irreversible covalent bond with the kinase's active site and target a catalytic nucleophile (-SH group) cysteine inside the enzyme's active site (Cohen et al., 2005; Kwak et al., 2005). Since FDA approved the afatinib (targets EGFR (ErbB1), ErbB2, and ErbB4) and BTK inhibitor ibrutinib, several covalent kinases or type V inhibitors have been approved (Figure 13).

The chemical rationality for developing this kind is based on an open and exposed cysteine side chain at the ATP site that may be a suitable target for a drug molecule with an electrophilic Michael acceptor in the proper orientation (Leproult et al., 2011).

These molecules form the irreversible enzyme-inhibitor complex after the reversible collision between the drug and binding site (Zhao et al., 2017). The afatinib and ibrutinib bind with the kinase through Michael's reaction through bond formation between a nucleophilic -SH of cysteine and an α, β unsaturated carbonyl counter port of drug molecule (Schwartz and Murray, 2011).

The cysteine-481 (C481) in the hinge region of the Bruton tyrosine-protein kinase is thought to establish a covalent bond with ibrutinib (Roskoski Jr, 2016b). The other approved example for this class of kinase is neratinib (HKI-272) which inhibits the Herceptin-2 (HER-2) (Figure 11) (Rabindran et al., 2004). The approved covalent kinase inhibitors have demonstrated that compounds containing weak reactive electrophiles may be highly selective and have low toxicity.

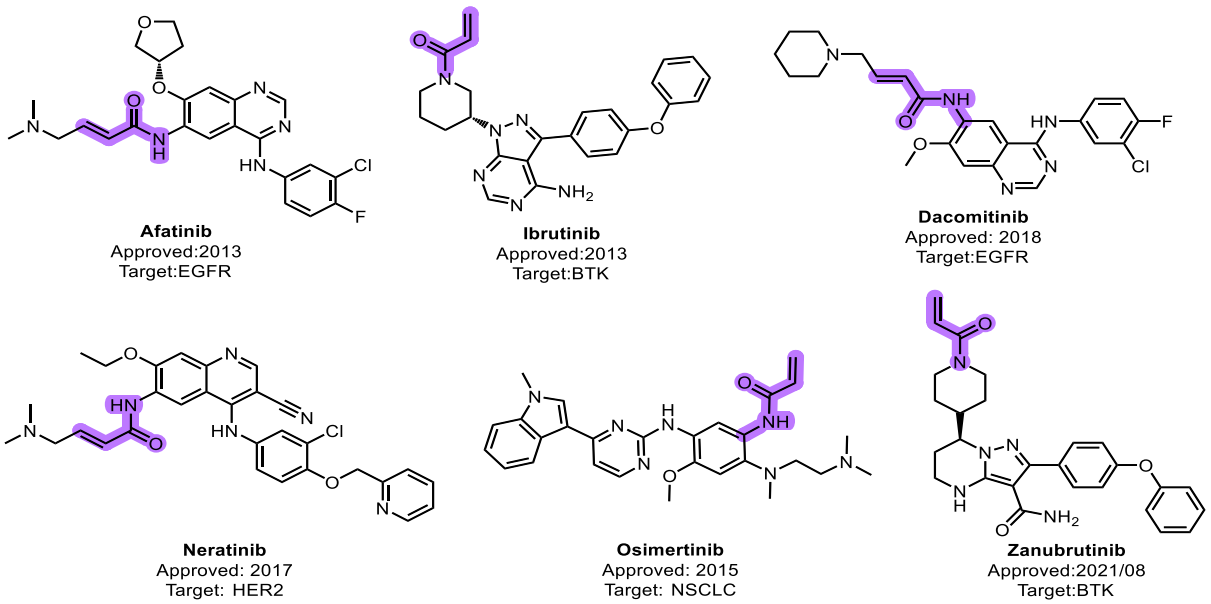

Figure 13. Approved Type V kinase inhibitors: The highlighted violet part binds with the cysteine inside the enzyme's active site

Macrocycles

Macrocyclization is a relatively new approach for improving the potency and selectivity of inhibitors and their pharmacokinetic features. The Lorlatinib has been devolved as the cyclic analog of crizotinib (Johnson et al., 2014). Macrocyclization stabilized crizotinib's bioactive structure, resulting in significantly increased efficacy against ROS and ALK, as well as superior central nervous system penetration (Figure 14) (Bauer et al., 2020). The other approved example of the macrocycle is repotrectinib, which is a next-generation ROS1-TKI with improved potency and selectivity.

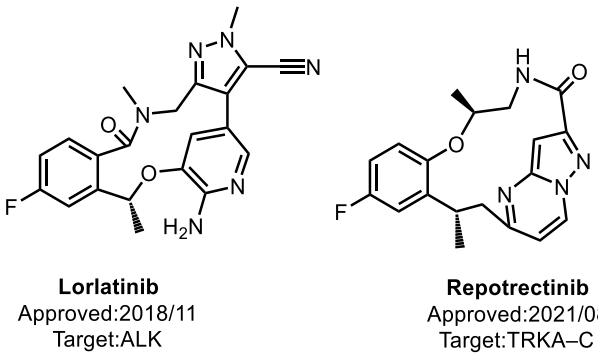

Figure 14. Approved type macrocyclic kinase inhibitors

CONCLUSION

Kinase drug research has made tremendous improvement over the past 30 years, changing kinase targets from "undruggable" to very tractable. Because of this, kinase-targeted medications have changed the treatment of many human malignancies, including non-small-cell lung cancer (NSCLC), thyroid cancer, melanoma, lymphomas, breast cancer, and leukemia. Characterizing genetic abnormalities in the cancer kinome and identifying genuine mechanisms accountable for tumor formation are major functional problems. According to a recent survey of kinase research, the well-known knowledge of kinases, with extensive structural coverage and a large number of chemical scaffolds for further study, pushes the boundaries in designing new, more effective kinase inhibitors. To continue expanding the kinase drug research, improved screening and profiling methodologies for small molecules and natural products are required. Additionally, attaining target selectivity to limit off-target toxicity is still a big challenge. Developing inhibitors with various binding modes, such as allosteric and covalent inhibitors, could have a crucial role in kinase drug discovery, and further expansion in this direction is needed.

Note: The protein structure visualizes in Schrödinger (2021).

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