Abstract

II

Type II kinase inhibitors act by targeting the inactive conformation of kinases and interact with the catalytic site of the unphosphorylated inactive conformation of kinase. Type II kinase inhibitors exploit new interactions inside the lipophilic pocket derived from the change of con rmation of the phenylalanine residue of the "Asp-Phe-Gly (DFG)" N-terminal loop conformation of kinases [9]. ese inhibitors interact reversibly with the target kinase which leads to the formation of single or multiple hydrogen bonds with the protein in the 'hinge region' and also causes extra interactions in the open DFG-out conformation.

ese lipophilic interactions have a high degree of selectivity towards unwanted kinases a ecting an increase in the safety pro le of Type II kinase inhibitors. Type II inhibitors also display a high conservation of distinctive H-bond pattern between the inhibitor and the glutamic and aspartic acids of the kinase. Due to the exclusivity of inactive protein kinase conformations, it was theorized than type II kinase inhibitors would be more selective. However, there is considerable overlap of selectivity between type I and type II inhibitors. e discovery of Type II kinase inhibitors such as imatinib and sorafenib was serendipitous, and it wasn't until much later that their mode of action was discovered.

e role of imatinib in the consequent development of small molecule protein kinase inhibitors cannot be overstated. All Type II inhibitors share a similar pharmacophore and hydrogen bonds that interact with DFG-out kinase conformational structure as revealed by the discovery of the Type II kinase inhibitor co-crystal structure. Since canonical ATP-binding sites of activated kinases, the target sites of Type I inhibitors, do not share these features, this pocket is conserved to a lesser extent across the kinome, and hence promises better prospects for the rational design of selective inhibitors. Overall, Type II kinase inhibitors display high selectivity towards kinase inhibition as compared to Type I kinase inhibitors along with the profound impact on cellular activity.

III

e third class of kinase inhibitors bind outside the catalytic domain/ATP-binding site and modulates kinase activity in an allosteric manner. Some authors have divided the allosteric inhibitors into two subtypes where type A inhibitors bind to an allosteric site next to the adenine-binding pocket whereas the type B inhibitors bind elsewhere. Overall, Allosteric or Type III inhibitors exhibit the highest degree of target kinase selectivity as they exploit binding sites and physiological mechanisms that are exclusive to a particular kinase [10]. With respect to ATP, these drugs are steady-state noncompetitive or uncompetitive inhibitors because ATP cannot prevent their interaction with the target kinase. One of the earliest allosteric inhibitors was CI-1040, an orally active, highly speci c, small-molecule inhibitor of the MEK1/MEK2 pathway. A recent chemical proteomics study con rms the allosteric activity of type III inhibitors as they showed a higher selectivity, but also stated that these are special cases as most of them are designated MEK1/2 inhibitors that bind to a particular cavity adjacent to the ATP-binding site. Another allosteric kinase inhibitor GnF2 binds to the myristate binding site of BCR–ABL1. GnF2 also displays sound IL-3 reversible anti-proliferative and apoptotic e ect on two mutants identi ed as E255V and Y253H. Likewise, TAK-733 binds to the MEK1-ATP complex in the gate area and the back cle adjacent to the ATP-binding pocket; however, it cannot bind to the adenine pocket owing to its occupation by ATP. Other examples include RO0281675 and analogs thereof. Overall, targeting kinases using allosteric inhibitors is thought to be a crucial approach for overcoming hurdles in kinase inhibitor research, such as limited selectivity, o -target side e ects, and drug resistance. In future, more active and target speci c allosteric inhibitors will be discovered as larger stress is placed on cell-based assays in which kinases are explored in their native cellular context.

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ese are also called Type IV kinase inhibitors and undergo a reversible interaction outside the ATP pocket, located in the kinase substrate-binding site. ese inhibitors don't compete with ATP and o er a higher degree of selectivity against targeted kinases. Substrate-directed inhibitors include ATP-noncompetitive inhibitors such as ON012380 which are targeted against Philadelphia chromosome-positive leukemias. More importantly, ON012380 was found to override imatinib resistance at physiologically relevant concentrations of < 10 nM.

e covalent kinase inhibitors form an irreversible covalent bond with the kinase active site and target a catalytic nucleophile cysteine within the active site of the enzyme. e chemical rationale for developing Type V inhibitors is based on exposed cysteine side chain in the ATP site which can be targeted for covalent reaction with a drug candidate with an electrophilic Michael acceptor in the right position.

is type of kinase inhibition takes place via trapping of a solvent-exposed cysteine residue either by SN2 displacement of a leaving group or by reacting with a Michael acceptor incorporated within the kinase inhibitor. Covalent inhibitors target respective kinase by formation of a rapidly reversible collision complex followed by an irreversible enzyme-inhibitor complex. Afatinib (targets EGFR (ErbB1), ErbB2, and ErbB4) and ibrutinib are currently FDA-approved drugs that form a covalent bond with their target kinase. Afatinib, unlike the rst-generation EGFR-TKIs such as ge tinib and erlotinib, is a mutant-selective EGFR inhibitor with low toxicity pro le despite its irreversible mechanism. Similar to Afatinib, ibrutinib also targets mutant-EGFR kinase with a distinct binding conformation . Both of these kinase inhibitors initiate Michael reaction with the addition of a nucleophile (the -SH of

cysteine) to an α, β unsaturated carbonyl compound . C481 within hinge region of the Bruton tyrosine-protein kinase is hypothesized to form a covalent link with ibrutinib. A recently approved kinase inhibitor, neratinib (HKI-272), inhibits Herceptin-2 (HER-2), and prevents recurrence in patients with early-stage HER2-positive breast cancer . Overexpression of HER-2 is seen in 25–30% of breast cancer patients and predicts a poor outcome in patients with primary disease. Likewise, CL-387785, a covalent inhibitor, overcomes resistance caused by T790 M mutation of the epidermal growth factor receptor (EGFR). ese kinase inhibitors also display an extended dissociation half-life which minimizes o -target side e ects. Other advantages include prolonged pharmacodynamics, suitability for rational design, high potency, and ability to validate pharmacological speci city through mutation of the reactive cysteine residue. e approved covalent kinase inhibitors (Ibrutinib, Afatinib, and Neratinib) have shown that small molecules containing weak reactive electrophiles can be mutant speci c in action with low toxicity. ese kinase inhibitors have initiated resurgence of interest in covalent inhibitors, and feature an acrylamide functionality to speci cally target the cysteine side chains of kinases. Example include a recent study showing nine irreversible EGFR and two BTK inhibitors with higher kinase inhibitory selectivity than reversible compounds. e Type V or covalent kinase inhibitors have substantial potential for exploration as 200 di erent kinases have a cysteine chain located near the ATP pocket.

B M

Biochemically, kinase inhibitors are classi ed according to the activation state of the protein kinase target including the nature of DFG-Asp (active in, inactive out), the C-helix (active in, inactive out), and the regulatory spine (active linear, inactive distorted). Apart from type III or allosteric inhibitors, all the FDA-approved kinase inhibitors

kinases have been approved for clinical use. With over 500 members, the kinase family has received a high degree of attention from academic researchers as well as pharmaceutical industries . A er the clearance of possible hindrances, owing to the high degree of active site similarities and possible o -target activity, kinase inhibitors have gained scienti c limelight. In a 13-year summary of targeted therapies including kinase inhibitors, the clinical success rate of kinase inhibitors was superior to other cancer therapies. Nevertheless, this clinical success does come with exceptions; attempts to control cytotoxicity during treatment, particularly with sunitinib and EGFR/VEGF-system targeting drugs have yielded disappointing results. Overall, during the last 5 years, Aurora kinases, casein kinase II, cyclin-dependent kinases, focal adhesion kinase, protein kinase B, phosphatidylinositol 4,5-bisphosphate 3-kinase delta and gamma, polo-like kinase I, tyrosine-protein kinase SYK, high a nity nerve growth factor receptor family and Wee1-like protein kinase have been targeted in Phase I clinical trials. Although recent developments have shown Aurora kinases as major new targets in kinase inhibitor development. A er initial hurdles, two compounds palbociclib and ribociclib have passed the phase III clinical trials and are in clinical use.

Recent kinase developments include precision therapy based on tumor genomic data. e ability to perform genetic studies of tumors and follow-up treatment decisions based on the identi cation of tumorigenesis drivers has resulted in signi cant bene ts for patients in need of e ective systemic therapy. e detailed information regarding all the clinical trials is out of the scope of this mini-review; however, a few important developments are highlighted. A small number of small molecule tyrosine kinase inhibitors have recently received FDA approval for treatment of non-small cell lung cancer (NSCLC) with EGFR mutations or ALK translocations. Afatinib, a second-generation, non-competitive kinase inhibitor targeting all members of the ErbB family of receptors (also known as Her-2/neu) was approved in 2013 as frontline therapy for NSCLC patients with EGFR-deletion 19 and L858R mutations. Despite several challenges that need to be overcome, reviewed in, precision medicine has yielded important dividends for patients with advanced cancers. In order to counter currently undruggable targets and acquired resistance, immunotherapy has gained widespread recognition in recent years. Additionally, kinase targeted antibody therapy for hematological malignancies, and solid tumors have become established over the past 20 years. Key examples of antibody constructs targeting kinases include Trastuzumab and T-DM1 (targeting ERBB2/HER2) in breast and bladder cancer, Bevacizumab (targeting VEGF) in ovarian, metastatic colon cancer and glioblastoma , Cetuximab, Panitumumab and necitumumab (targeting EGFR) in colorectal cancer and NSCLC . Other experimental candidates include scFv, a body and minibody (ERBB2/HER2 and FGFR1), Protein–Fc (VEGFR1 and VEGFR2) and Intact IgG (EGFR, ERBB2, and VEGF) in breast and lung cancer studies. Also, there is an increased development of PI3K and mTOR inhibiting compounds. Dual PI3K/mTOR inhibitors in advanced clinical trials include NVP-BEZ235 (glioblastomas), XL765 (breast cancer), GDC0980 (mRCC), PF04691502 (breast cancer), GSK2126458 (colorectal, breast, non-small cell lung, and pancreatic cancers), Quinacrine (various leukemias) and PKI587 (advanced solid malignancies). Also, buparlisib and idelalisib, both PI3K inhibitors, have entered phase III clinical trials. In line with PI3K/mTOR inhibitors, various kinase inhibitors have entered into clinical trials for gastrointestinal cancers, thyroid carcinoma , breast cancer, and endocrine tumors . Many previously approved kinase inhibitors are being tested in clinical trials against BRAF and cyclin-dependent kinases 4/6 mutations. BRAF somatic mutation, particularly BRAF V600E/K, drive tumorigenesis through constitutive activation of the downstream MAPK pathway. Multiple drugs including vemurafenib, dabrafenib, PLX3603, ARQ736, CEP-32496, BMS-908662, BGB283, encorafenib in combination with other chemotherapies are being targeted for BRAF-mutated cancers. It is now suggested that dabrafenib, a selective BRAF inhibitor may target other kinases indicating polypharmacology (that is, drugs that act on more than one target) . A paper published by Klaeger and colleagues explains the potential of 243 clinically evaluated kinase drugs . Although multiple new kinases have been targeted during the last 5 years, a large share of the cancer kinome is still untargeted. Furthermore, use of these targeted therapies is not without limitations. Reservations on the use of kinase inhibitors include the development of resistance and the lack of tumor response in the general population and these constraints still need to be resolved.

N B K I

Overexpression of kinases is observed in multiple carcinomas. In recent years, there has been a major paradigm shi in discovery and screening of natural compounds as potential kinase inhibitors. Emerging data has revealed numerous mechanisms by which natural compounds mitigate kinase mutations. Classically, many of the biological actions of small molecule compounds, especially polyphenols, have been credited with their antioxidant properties, either through their reducing capacities or their possible in uence on intracellular redox states. ese small molecule bioactives can directly bind receptor tyrosine kinases and alter their phosphorylation state to regulate multiple cell signaling pathways . Elevated levels of the EGFR and HER-2 have been identi ed as common components of multiple cancer types and appear to promote solid tumor growth EGFR inhibition is exhibited by multiple polyphenols including resveratrol, quercetin , curcumin , and green tea extracts . HER-2 overexpression in tumor cells is also attenuated by these bioactives. Fibroblast growth factors are involved in a variety of cellular processes, such as tumor cell proliferation, drug resistance, and angiogenesis. Oncogenic alterations of RTK kinases including FGFR1, FGFR3, and FGFR4 are inhibited by natural compounds .Similarly, curcumin and chrysin block expression of receptor d'origine nantais (RON) in tumor cells e product of the human SRC gene, c-Src, is found to be over-expressed and highly activated in a wide variety of human cancers . It is also accompanied by elevated levels of Abl and JAK-2 kinases . Interestingly, the overexpression and translocation of oncogenic cytoplasmic tyrosine kinases such as c-SRC , Abl, c-Met and JAK-2 are tempered by natural compounds. Serine/threonine kinases, within the kinase family, play vital roles regarding their involvement in human cancers. Akt, a crucial kinase modulates diverse cellular processes involved in the regulation of cell survival, cell cycle progression and cellular growth. Up to date, more than 50 proteins have been identi ed as the phosphorylation substrates of Akt. Resveratrol modulates expression of Akt in breast, uterine, prostate skin and glioma cells. It targets the kinases at ATP-binding site competitively and reversibly.

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