

## Part II

### Drug Class Studies

## 3

### Kinase Inhibitor Drugs

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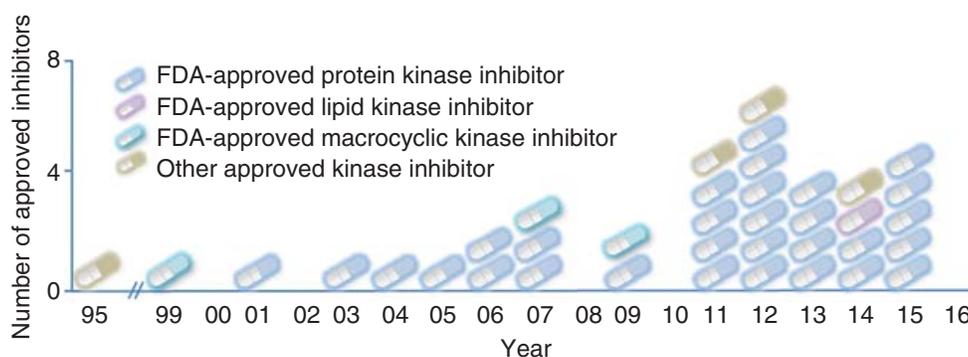
#### 3.1 Introduction

Kinases are enzymes that catalyze the transfer of a phosphoryl group to specific substrates from ATP [1, 2]. They are key nodes in the complex cellular signaling network that regulate a large range of cellular activities, which include growth, proliferation, survival, apoptosis, metabolism, motility, transcription, differentiation, angiogenesis, and response to DNA damage [3–10]. Kinase dysfunction has been linked to different human diseases with kinase mutations often contributing to the disease pathology [11]. Kinases are currently under intense scrutiny as drug targets in the treatment of multiple types of cancer, inflammatory diseases, developmental disorders, metabolic disorders, and neurodegenerative diseases [4, 5, 12–18].

Rapid development of kinase-based therapeutics was observed in the past decades [19–23], which have ushered in an era of targeted therapies. This has been particularly evident in treatments of different forms of cancer [24–29]. This chapter will focus on the history and the clinical landscape of kinase inhibitors that include 38 drugs approved for use in humans as of December 2016 (Figure 3.1 and Table 3.1) [19–21].

Human kinases share a high degree of structural similarity, particularly in the kinase domain with N-terminal and C-terminal lobes forming a cleft where ATP binding pocket is located (Figure 3.2a) [30]. Most reported kinase inhibitors have been designed to interact with this site. A flexible activation loop, which starts with the conserved amino acid sequence Asp-Phe-Gly (DFG), controls access to the ATP binding site [31].

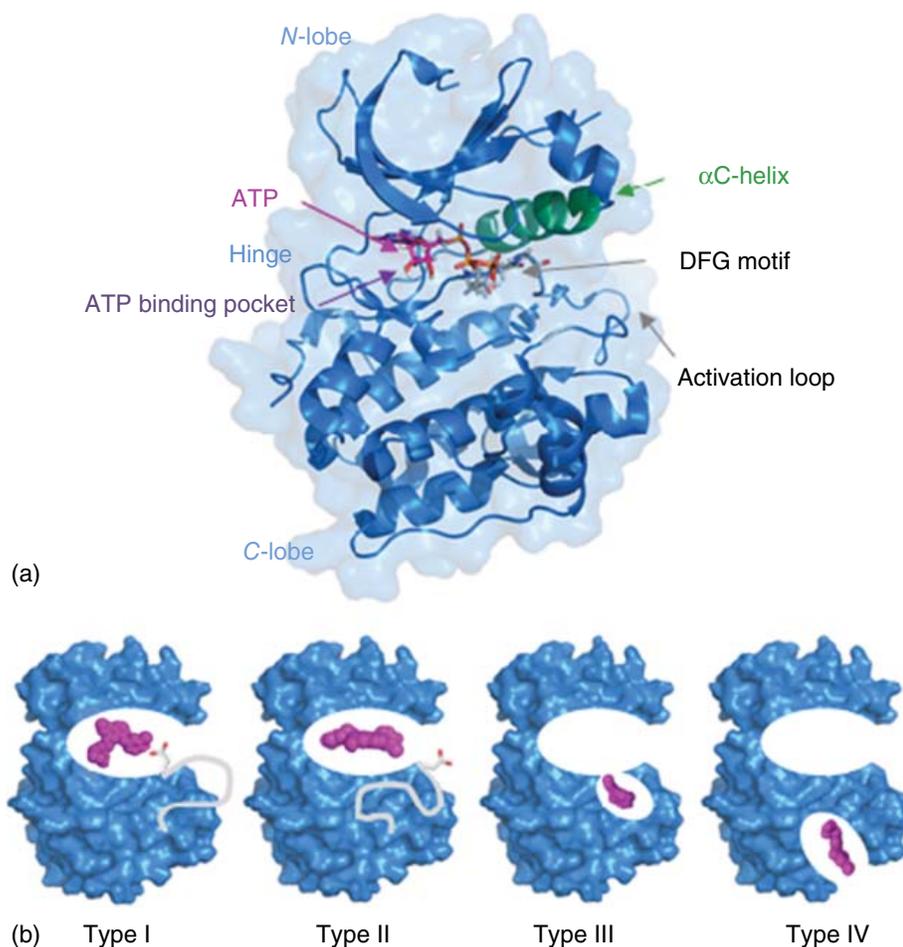
Kinase inhibitors can be classified into two categories based on their size: rapalogs and small-molecule kinase inhibitors (SMKIs), which can be grouped into covalent and non-covalent [32]. Non-covalent SMKIs can be classified into type I–V inhibitors. Key conformational features to define different binding modes of SMKIs include the DFG motif, the activation loop, and the  $\alpha$ C-helix



**Figure 3.1** The number of approved kinase inhibitor drugs over the last 2 decades. Adapted with permission from Elsevier, based on Figure 2 of [21].

**Table 3.1** Targets of approved kinase inhibitor drugs.

FDA-approved protein kinase inhibitors		FDA-approved protein kinase inhibitors	
Imatinib	Bcr–Abl, PDGFR, Kit, Ret, Src	Afatinib	EGFR, ErbB2, ErbB4
Gefitinib	EGFR, Gak	Ibrutinib	BTK
Erlotinib	EGFR, Slk, ILK	Ceritinib	ALK
Sorafenib	VEGFR, EGFR, PDGFR, Raf, Kit, Ret	Nintedanib	VEGFR, EGFR, PDGFR
Sunitinib	VEGFR, PDGFR, Kit, Flt, Ret	Palbociclib	CDK4, CDK6
Dasatinib	Bcr–Abl, Src	Lenvatinib	VEGFR2, VEGFR3
Lapatinib	EGFR, ErbB2	Cobimetinib	MEK1, MEK2
Nilotinib	Bcr–Abl, PDGFR, Kit, Src	Osimertinib	MEK1, MEK2
Pazopanib	VEGFR, PDGFR, Kit, EGFR	Alectinib	ALK
Vandetanib	VEGFR, EGFR	<b>FDA-approved lipid kinase inhibitor</b>	
Vemurafenib	B-Raf	Idelalisib	PI3K $\delta$
Crizotinib	ALK, c-Met	<b>FDA-approved macrocyclic kinase inhibitors</b>	
Ruxolitinib	JAK1, JAK2	Sirolimus	mTOR
Axitinib	VEGFR, KIT, PDGFR	Temsirolimus	mTOR
Bosutinib	Bcr–Abl, Src, Lyn, Hck, CDK, MEK	Everlimus	mTORC1
Regorafenib	VEGFR, PDGFR, FGFR, Raf, Kit, Ret	<b>Other approved kinase inhibitors</b>	
Tofacitinib	JAK1, JAK2, JAK3	Fasudil	ROCK
Cabozantinib	VEGFR2, Met, Ret, Flt, Axl, TIE	Ripasudil	ROCK
Ponatinib	Bcr–Abl, FGFR, Src, VEGFR, PDGFR	Icotinib	EGFR, ErbB2
Dabrafenib	B-Raf	Radotinib	Bcr–Abl



**Figure 3.2** Structural features of a typical protein kinase and classification of inhibitor binding modes. (a) General structural features of kinases (PDB ID: 4RRV). (Adapted with permission from Elsevier, based on Figure 1 of [21].) (b) Four types of non-covalent kinase inhibitors. Activation loop is indicated as a gray curve. (Reproduced with permission from Elsevier, based on Figure 1 of [21].) (Note: ATP binding is required in several type III inhibitors that bind adjacent to the ATP binding pocket).

located at the C-terminal lobe of the kinase (Figure 3.2b) [33–36]. Type I and type II inhibitors are ATP competitive, with the type I inhibitors binding to the active kinase conformation that is characterized by three features: an open activation loop, the aspartate residue of the DFG motif facing toward the active site (“DFG-in”), and the  $\alpha$ C-helix adopting an “in” conformation. Comparatively, type II inhibitors bind to the inactive kinase conformation with closed activation loop and the aspartate residue protruding from the ATP binding site (“DFG-out”) [37]. Type I $\frac{1}{2}$  inhibitors bind to the inactive kinase conformation with a DFG-in and  $\alpha$ C-helix “out” conformation, thereby exhibiting binding features of both type I and II inhibitors [35]. Additionally, type II inhibitors can bind at the allosteric pocket created by rotation of the DFG motif in the vicinity of the ATP binding site. Type III and IV inhibitors bind exclusively in an allosteric pocket and do not engage in any interactions with the ATP binding site. Type III inhibitors bind adjacent to the ATP binding site, while type IV inhibitors bind at a remote pocket [38–40]. Type V bivalent inhibitors bind to two different kinase portions [41, 42].

## 3.2 Historical Overview

A chronological summary of major discoveries of kinase inhibitors and related key events is illustrated in Figure 3.3.

### 3.2.1 Before 1980

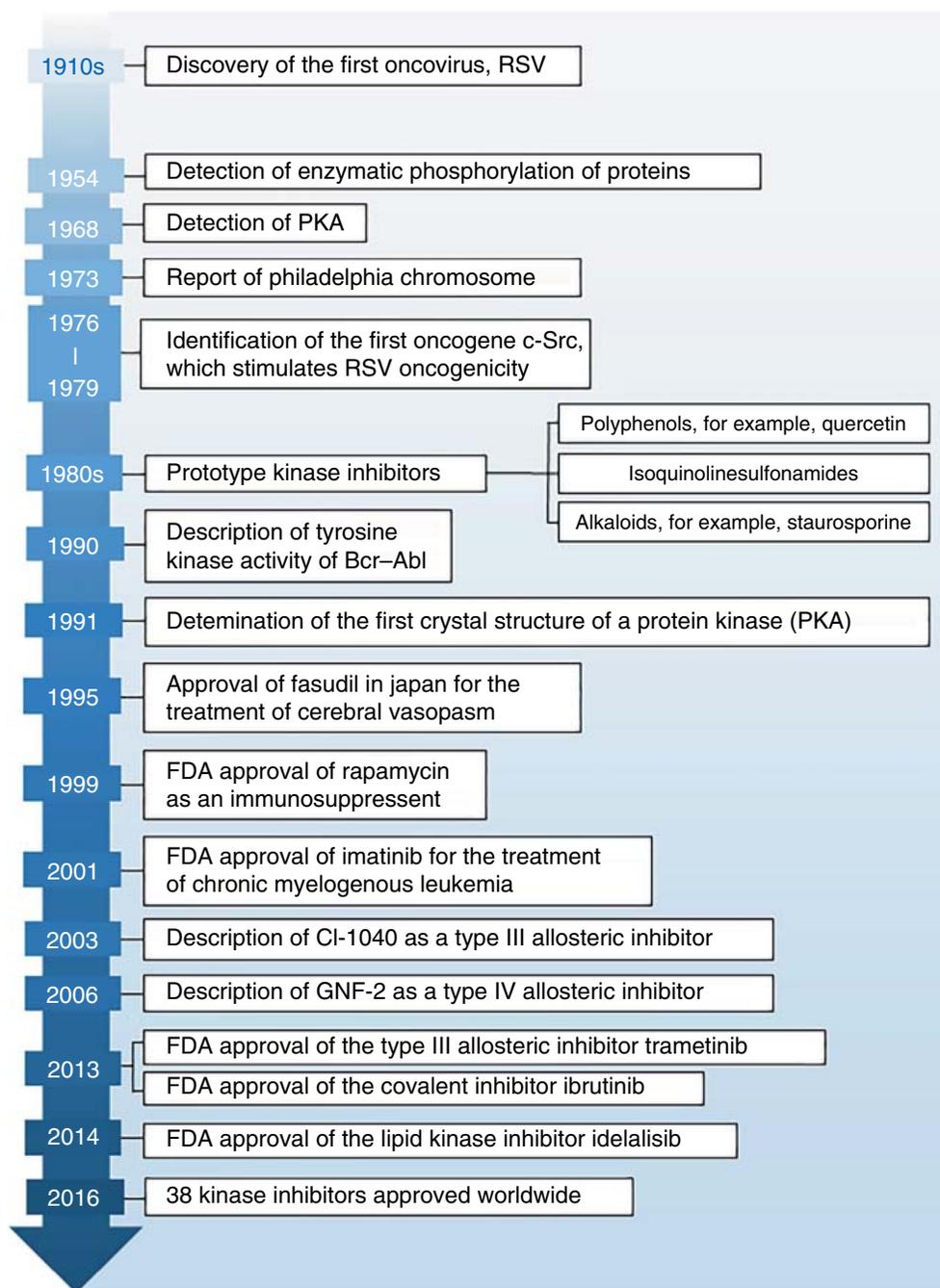
Discovery of kinase inhibitor dates back in the early 1910s when Francis Peyton Rous made the seminal observation that cancer can be transmitted by Rous sarcoma virus (RSV) after injecting cell-free extract of the tumor from a sick chicken into healthy fowls [43, 44]. In the 1970s, John M. Bishop and Harold E. Varmus identified cellular Src (c-Src), which stimulates RSV [45]. c-Src encodes a non-receptor tyrosine kinase that belongs to the Src kinase family, [46, 47], which also includes Fyn, Lyn, Blk, Hck, Lck, Fgr, Yrs, and Yes kinases [10]. In the 1950s, phosphorylase kinase was characterized by George Burnett and Eugene P. Kennedy, while in the 1960s protein kinase A (PKA)-mediated signaling pathway was determined by Walsh, Perkins, Krebs, and Fischer [48–52]. In 1973, Janet D. Rowley reported that the presence of abnormality in the Philadelphia chromosome in chronic myelogenous leukemia (CML) patients was caused by a translocation between the long arms of chromosomes 9 and 22 [53]. This translocation was later identified to produce a fusion protein tyrosine kinase encoded by the Abelson murine leukemia viral oncogene homologue 1 (Abl) on chromosome 9 juxtaposing to a part of the breakpoint cluster region (Bcr) on chromosome 22 [54, 55].

### 3.2.2 1980s

Following the identification of the first human oncogene and kinase signaling cascade, polyphenols were the first prototype small-molecule kinase inhibitors reported in 1980s [56–58], such as the naturally occurring bioflavonoid, quercetin (Figure 3.4), which is a nonselective kinase inhibitor targeting several tyrosine, serine/threonine kinases, and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), with low micromolar potency [58]. Isoquinolinesulfonamides were also reported with inhibitory potency against cyclic nucleotide-dependent protein kinase and protein kinase C (PKC) [59] and alkaloids (e.g., staurosporine (Figure 3.4)) with potent inhibitory activities of kinases [60, 61]. Staurosporine is an antifungal natural product originally isolated in the 1970s [62] and, being an ATP-competitive inhibitor, has a poor selectivity but a high affinity with members of many kinase families [63]. Its bis-indole scaffold is displayed in many natural alkaloids and in many SMKIs. Staurosporine has precluded clinical applications although it is used extensively as a chemical probe [61, 64].

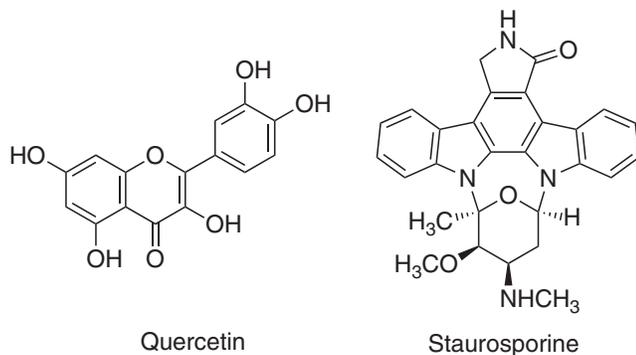
### 3.2.3 1990s

In the twentieth century, several important kinase signaling cascades were identified: Ras–Raf–mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) pathway [65, 66], the Janus kinase (JAK) pathway



**Figure 3.3** Chronological summary of the discovery history of kinase inhibitors and related key events.

[67–69], and the PI3K pathway [70–73]. In 1991, the first crystal structure of a kinase domain was determined by Knighton and coworkers, who described the two-lobe structure of the catalytic domain of cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) [30]. These findings laid the foundation for the structure-based design of kinase inhibitors. Fasudil (Eri<sup>l</sup>, Asahi Kasei Pharma) was approved in Japan in 1995 for the treatment of cerebral vasospasm,



**Figure 3.4** Prototype kinase inhibitors.

although its Rho-associated protein kinase (ROCK) inhibitory activity was not described until 1997 [74–76]. The first US Food and Drug Administration (FDA) approval of a kinase inhibitor was given in 1999, when rapamycin was approved as an immunosuppressive agent for kidney transplant under the trade name Rapamune<sup>®</sup> (Wyeth/Pfizer) [77].

### 3.2.4 After 2000

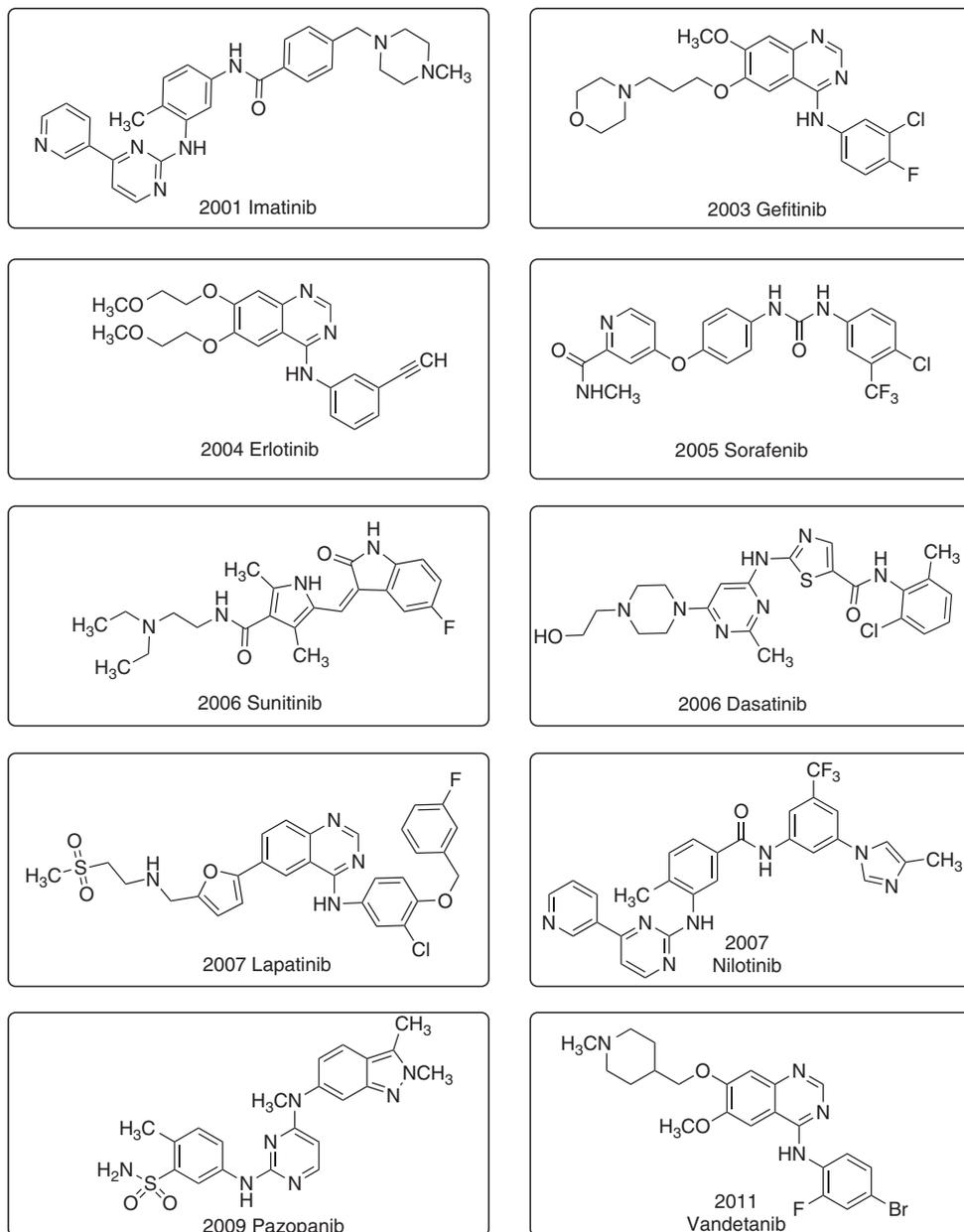
Bcr–Abl inhibitor imatinib (Gleevec<sup>®</sup>, Novartis) was approved in 2001 by the FDA. Although fasudil was approved in 1995, imatinib is widely perceived as the first approved SMKI mainly owing to the fact that fasudil's kinase inhibitory mechanism was unknown at the time of approval, and efforts to gain approval of fasudil have been unsuccessful in the United States and Europe.

The field of kinase inhibitor development has evolved rapidly since the approval of imatinib [4, 19–21]. Some of the key discoveries and events include (i) the discovery of MAPK/ERK inhibitors, for example, CI-1040 (PD184352), as the first series of type III inhibitors in 2003 [78]; (ii) the approval of first dual tyrosine kinase and serine/threonine kinase inhibitor sorafenib in 2005; (iii) the description of the first series of allosteric type IV kinase inhibitor, that is, GNF-2 and analogues that inhibit Bcr–Abl through an allosteric non-ATP-competitive mode, by Gray and coworkers in 2006 [79]; (iv) the approval of the first type III inhibitor trametinib in 2013; (v) the approval of the first covalent kinase inhibitors, afatinib and ibrutinib, in 2013; and (vi) the approval of the first lipid kinase inhibitor, that is, the PI3K inhibitor idelalisib, in 2014 [80].

By December 2016, kinase inhibitor drug discovery can leverage the structures of over 200 human kinases and 5000 kinases of all types, over 1 million publications, clinical data from more than 200 molecules currently in phase I–III trials, and post-marketing results from the approved 38 drugs [4, 19–21].

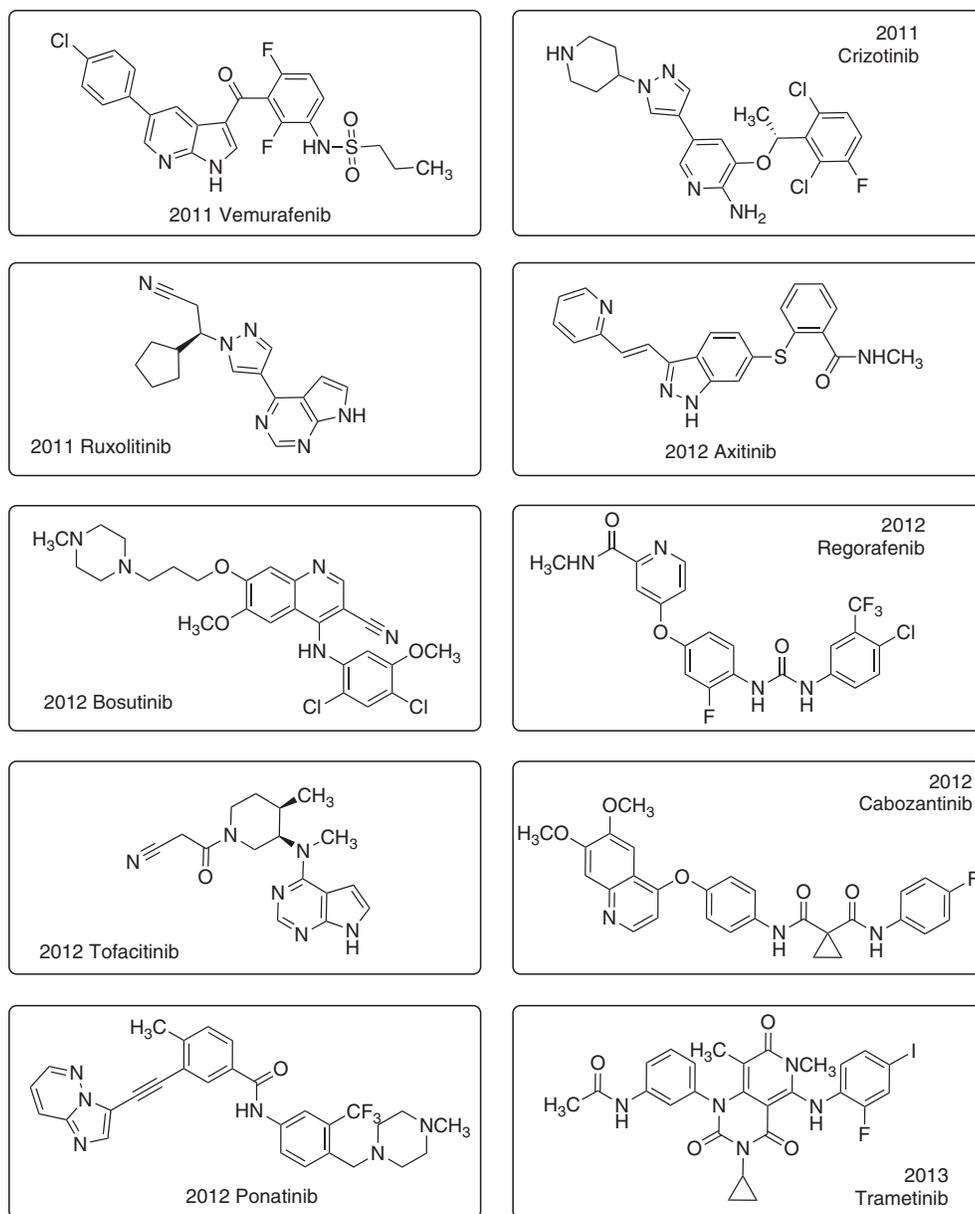
## 3.3 Approved Kinase Inhibitors

Among the 38 kinase inhibitors that are approved in the world, the US FDA has approved 34 kinase inhibitor drugs, which include 31 SMKIs (Figures 3.5–3.8)



**Figure 3.5** FDA-approved non-covalent small molecule kinase inhibitors (Part I).

and 3 macrocyclic inhibitors (Figure 3.9). In addition, two ROCK inhibitors were firstly approved in Japan (Figure 3.10), one epidermal growth factor receptor (EGFR) inhibitor in China (Figure 3.11), and one Bcr–Abl inhibitor in South Korea (Figure 3.11). Several hundred kinase inhibitors are currently in different clinical, preclinical, and discovery phases. The 31 FDA-approved SMKIs include 28 non-covalent inhibitors and three covalent ones. The 28 FDA-approved non-covalent SMKIs are grouped based on their kinase target(s) in the following discussion.



**Figure 3.6** FDA-approved non-covalent small molecule kinase inhibitors (Part II).

### 3.3.1 FDA-Approved Non-Covalent Small-Molecule Kinase Inhibitors

#### 3.3.1.1 Bcr–Abl Inhibitors

A total of five FDA-approved Bcr–Abl inhibitors are imatinib, dasatinib (Sprycel<sup>®</sup>, Bristol–Myers–Squibb), nilotinib (Tasigna<sup>®</sup>, Novartis), bosutinib (Bosulif<sup>®</sup>, Wyeth), and ponatinib (Iclusig<sup>®</sup>, Ariad Pharm.). Imatinib was a widely celebrated success at the time of its approval in 2001, when only cytotoxic drugs were available for the treatment of CML. Further, imatinib ushered in the era of target-oriented therapeutic strategy. The emergence of imatinib resistance has promoted the development of second-generation Bcr–Abl inhibitors [81–84], leading to the approval of dasatinib in 2006 and nilotinib in 2007 [85]. The high

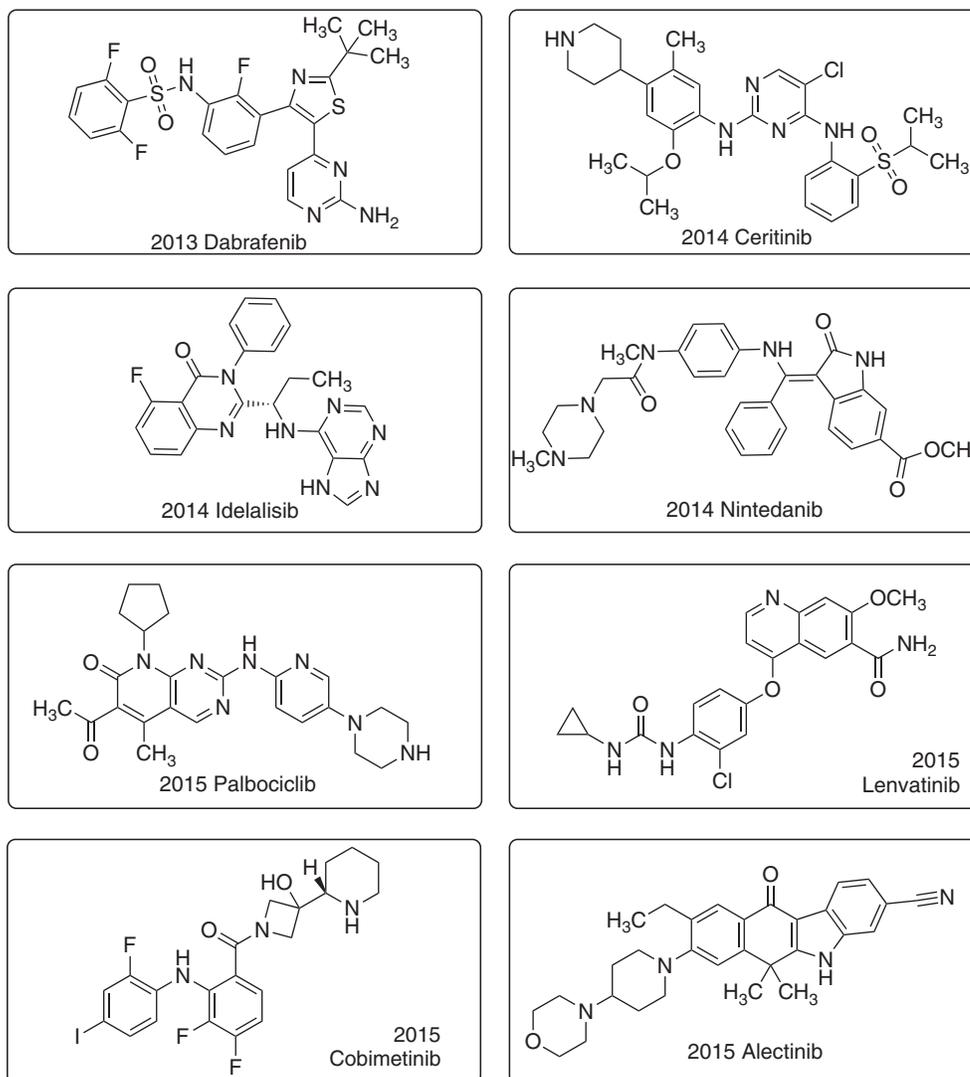


Figure 3.7 FDA-approved non-covalent small molecule kinase inhibitors (Part III).

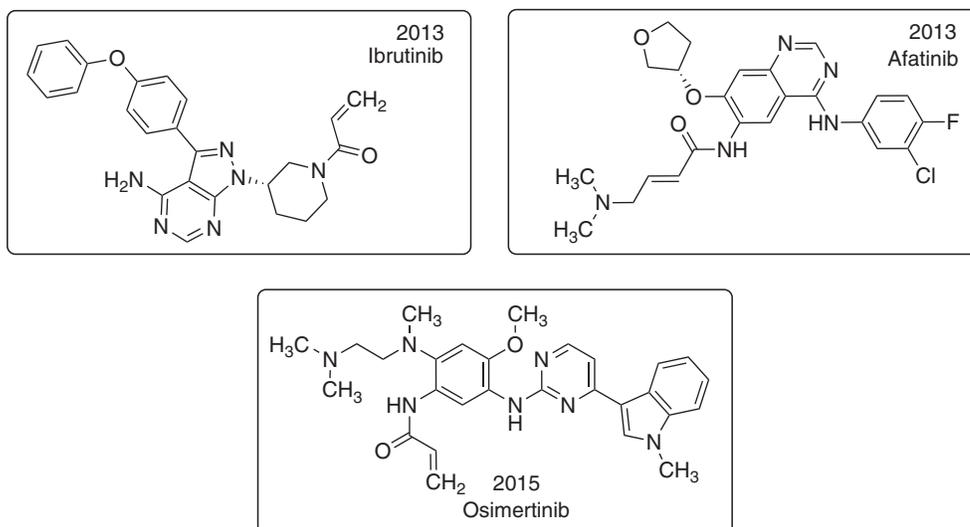
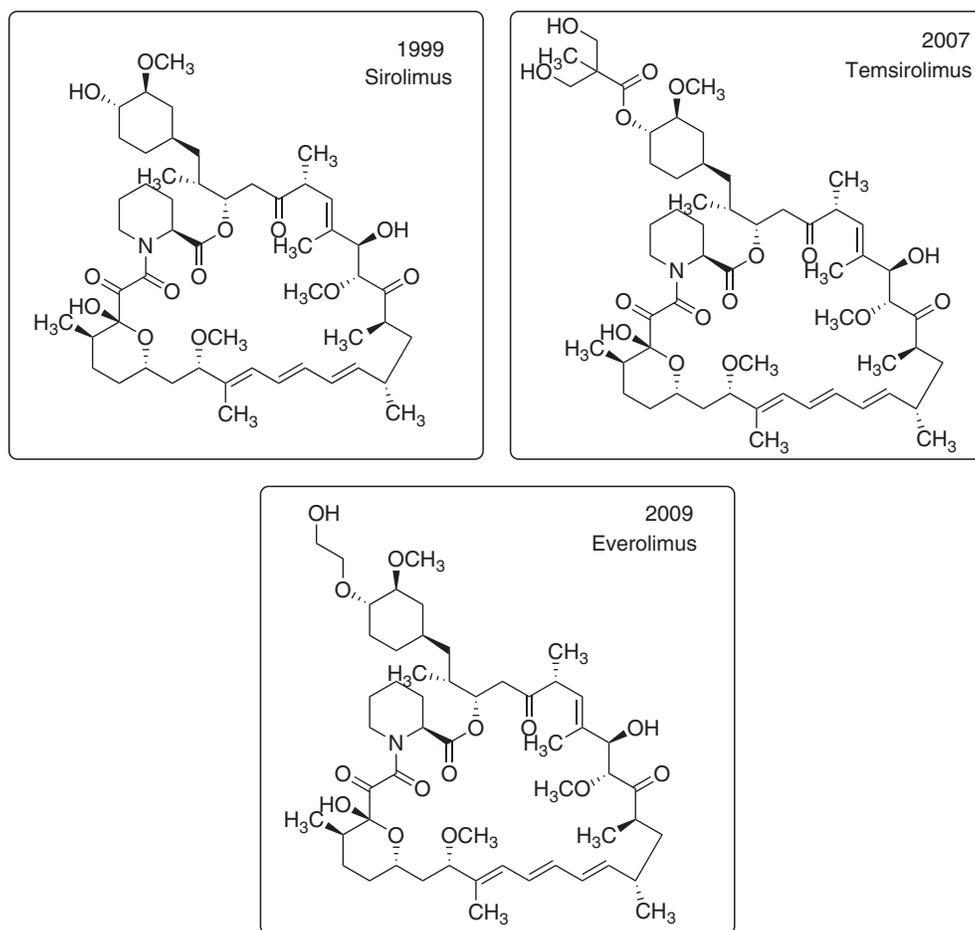
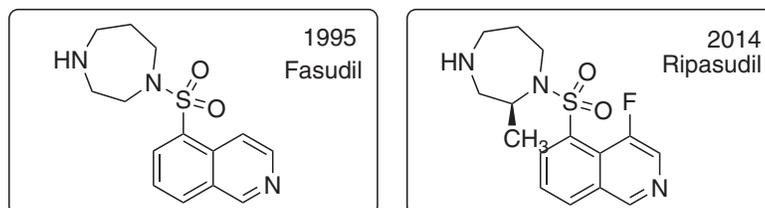


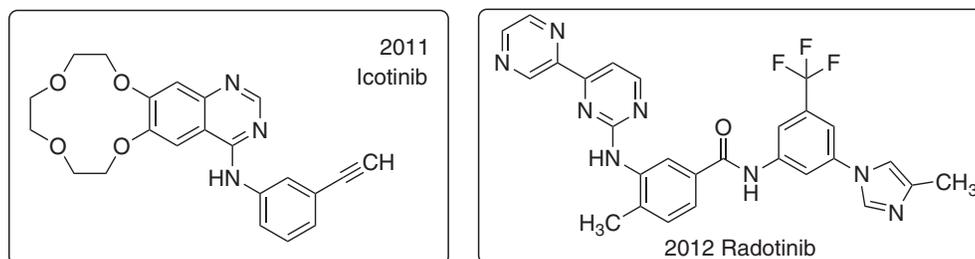
Figure 3.8 FDA-approved covalent small molecule kinase inhibitors.



**Figure 3.9** FDA-approved rapalogs.



**Figure 3.10** Approved ROCK kinase inhibitors by the Japanese Ministry of Health.



**Figure 3.11** Approved kinase inhibitors in China (icotinib) and South Korea (radotinib).

degree of structural similarity between imatinib and nilotinib results in a common binding mode, which is a type II binding with the DFG motif adopting an “out” conformation [37]. In contrast, dasatinib binds at the ATP binding pocket of Bcr–Abl with the DFG motif adopting an “in” conformation, reminiscent of type “I 1/2” binding mode [35]. Dasatinib and nilotinib are effective against most of the imatinib resistant mutations with the exception of the gatekeeper T315I mutation [81]. Bosutinib is a second-generation Bcr–Abl inhibitor, which bears a tetra-substituted quinoline scaffold that is widely present in approved EGFR inhibitors [86, 87]. All three second-generation Bcr–Abl inhibitors interact with the gatekeeper residue Thr315 via hydrogen bond and hydrophobic interaction and are ineffective against Thr315 mutation. Ponatinib was developed as a third-generation inhibitor that has shown nanomolar potency against both wild-type and T315I variant of Bcr–Abl [34, 88].

### 3.3.1.2 ErbB Family Inhibitors

The EGFR or ErbB1 inhibitor gefitinib (Iressa<sup>®</sup>, AstraZeneca) was originally approved by the US FDA in 2003 under accelerated regulations for the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) after progression on docetaxel- and platinum-based chemotherapy. AstraZeneca voluntarily withdrew gefitinib from the market in 2005, owing to failed verification of clinical benefit during post-approval studies. In July 2015, FDA reinstated the approval of gefitinib for a different group of patients (i.e., NSCLC patients with EGFR mutations).

Other approved kinase inhibitors targeting the ErbB family, which includes ErbB1/EGFR, ErbB2/human epidermal growth factor receptor 2 (Her2), ErbB3/Her3, and ErbB4/Her4 [89–91], are erlotinib (Tarceva<sup>®</sup>, OSI Pharm.), lapatinib (Tykerb<sup>®</sup>, GlaxoSmithKline), vandetanib (Caprelsa<sup>®</sup>, AstraZeneca), afatinib (Gilotrif<sup>®</sup>, Boehringer Ingelheim) [92], and osimertinib (Tagrisso<sup>®</sup>, AstraZeneca) [93, 94]. All approved EGFR family inhibitors share a common quinazoline scaffold with the exception of osimertinib, which has a pyrimidinylphenylamine scaffold that resembles that of imatinib and nilotinib [94]. Gefitinib and vandetanib adopt the type I binding mode with “DFG-in” and  $\alpha$ C-helix “in” conformation, while erlotinib and lapatinib bind to “DFG-in” with the  $\alpha$ C-helix adopting an “out” conformation. Afatinib and osimertinib are covalent inhibitors with an electrophilic enone moiety [21].

### 3.3.1.3 VEGFR Family Inhibitors

Sorafenib (Nexavar<sup>®</sup>, Bayer) was the first approved inhibitor targeting the vascular endothelial growth factor (VEGF) family kinases, which include VEGFR1, VEGFR2, and VEGFR3 [95, 96]. Sorafenib was originally approved for the treatment of renal cell carcinoma (RCC) in 2005, hepatocellular carcinoma in 2007, and locally recurrent or metastatic thyroid carcinoma refractory to radioactive iodine treatment in 2013. Six other approved inhibitors with VEGFRs as the main targets are sunitinib (Sutent<sup>®</sup>, Pfizer) for RCC, soft tissue sarcoma, thyroid cancer, metastatic pancreatic tumors, gastrointestinal stromal tumor, and several other types of carcinomas; pazopanib (Votrient<sup>®</sup>, GlaxoSmithKline) for RCC, soft tissue sarcoma, and thyroid cancer; axitinib (Inlyta<sup>®</sup>, Pfizer) for RCC,

thyroid cancer, and aplastic anemia, as well as T315I-mutant Bcr–Abl1-driven leukemia [97]; regorafenib (Stivarga<sup>®</sup>, Bayer) for gastrointestinal stromal tumors and colorectal cancer; nintedanib (Ofev<sup>®</sup>, Boehringer Ingelheim) for the non-oncological indication of idiopathic pulmonary fibrosis; and lenvatinib (Lenvima<sup>®</sup>, Eisai Inc.) for RCC and different types of thyroid cancers [98]. Sunitinib, pazopanib, and lenvatinib bind to the “DFG-in” conformation of VEGFRs, while axitinib, regorafenib, and nintedanib bind to inactive VEGFRs adopting the “DFG-out” conformation [21, 35].

#### 3.3.1.4 JAK Family Inhibitors

The JAK family includes four isoforms, JAK1, JAK2, JAK3, and tyrosine kinase (TYK2) [99]. Ruxolitinib (Jakafi<sup>®</sup>, Incyte Corp.) was the first approved JAK inhibitor, which inhibits both JAK1 and JAK2, used for the treatment of different types of myelofibrosis. Tofacitinib (Xeljanz<sup>®</sup>, Pfizer) was approved by FDA as a JAK3-selective inhibitor for the treatment of rheumatoid arthritis and is one of the only two FDA-approved kinase inhibitors for non-oncological indications.

#### 3.3.1.5 ALK Inhibitors

Crizotinib (Xalkori<sup>®</sup>, Pfizer) [100], approved in 2011, was the first approved inhibitor targeting anaplastic lymphoma kinase (ALK) [101]. ROS proto-oncogene 1-encoded kinase (ROS1) of the tyrosine kinase insulin receptor class and MET proto-oncogene-encoded kinase of the hepatocyte growth factor receptor (HGFR) class are other kinases targeted by crizotinib [100, 102]. When approved in 2011, crizotinib was the first drug specifically targeting NSCLC patients. However, resistance to crizotinib was usually observed in approximately 8 months after initial application and more than half of crizotinib-treated patients experienced gastrointestinal side effects [102, 103]. In 2016, crizotinib was additionally approved for ROS1-positive NSCLC by FDA.

Ceritinib (Zykadia<sup>®</sup>, Novartis), approved in 2014, was developed as a second-generation ALK inhibitor for patients with NSCLC who have developed crizotinib resistance [104]. Ceritinib addresses two of the most common ALK mutants that lead to crizotinib resistance, L1196M and G1269A, but is ineffective for G1202R and F1174C variants of ALK [105, 106].

Alectinib (Alecensa<sup>®</sup>, Roche) was approved first in Japan in 2014 and then by US FDA in 2015 as a second-generation ALK inhibitor for NSCLC treatment on patients who have progressed or do not tolerate crizotinib [107]. Developed through a structure-based drug design approach [108], alectinib is a benzocarbazolone derivative that has shown potent inhibitory activity against ALK (IC<sub>50</sub> value of 1.9 nM) and gatekeeper mutant L1196M ALK (IC<sub>50</sub> value of 1.6 nM). Alectinib is effective with crizotinib-resistant ALK mutations on L1196M, F1174L, R1275Q, and C1156Y [109]. In addition, an array of small-molecule inhibitors are currently being evaluated by several clinical trials for ALK-driven tumors [110].

#### 3.3.1.6 MET Inhibitors

Crizotinib and cabozantinib (Cometriq<sup>®</sup>, Exelixis) are the two currently approved MET inhibitors [100, 111]. Cabozantinib was approved for metastatic

medullary thyroid cancer in 2012 and advanced RCC in 2016 [112, 113]. Like crizotinib and cabozantinib, most reported MET inhibitors are multiple-kinase inhibitors except for a small group of selective MET inhibitors [63, 114].

#### 3.3.1.7 B-Raf Inhibitors

Gain-of-function mutations of B-Raf stimulate ERK-dependent signaling that drives cancer. The two approved B-Raf inhibitors are vemurafenib (Zelboraf<sup>®</sup>, Roche), approved in 2011 for the treatment of metastatic melanoma and thyroid tumors [115], and dabrafenib (Tafinlar<sup>®</sup>, GlaxoSmithKline), approved in 2013 for melanoma [116]. Both vemurafenib and dabrafenib inhibit V600E mutant monomers of B-Raf, but are ineffective in tumors driven by non-V600E BRAF mutants. Resistance to vemurafenib and dabrafenib usually emerge in approximately 7 months, and combination strategy using dabrafenib and the mitogen/extracellular signal-regulated kinase (MEK) inhibitor trametinib has been resorted to combat B-Raf inhibitor resistance associated with reactivation of the MEK pathway [117, 118]. It is noteworthy that a type II ATP-competitive RAF inhibitor, BGB659, was recently reported to bind Raf dimers and inhibit tumor growth in mice for all types of RAF mutants, including those driven by non-V600E BRAF mutants that function as constitutive dimers [119].

#### 3.3.1.8 MEK Inhibitors

MEK, also known as MAPK, is a dual specificity threonine/tyrosine kinase that is a key node in the Raf–Ras–MEK signaling pathway. Small-molecule MEK inhibitors represent the largest group of type III allosteric inhibitors that do not bind to the ATP binding pocket [40]. As of December 2016, besides the FDA-approved MEK1/2 inhibitors trametinib (Mekinist<sup>®</sup>, GlaxoSmithKline) and cobimetinib (Cotellic<sup>®</sup>, Roche), over 10 MEK inhibitors are currently in clinical trials. Trametinib was approved by FDA in 2013 for the treatment of patients with either B-Raf V600E or V600K mutated metastatic melanoma. Considering the fact that MEK and Raf are different kinases along the same pathway of Ras–Raf–MEK/ERK signaling cascade, combination strategies using both MEK and B-Raf inhibitors were utilized to overcome the observed progression using single-agent trametinib, which usually occurs within 7 months [118, 120]. FDA approved the combination of trametinib and dabrafenib for the treatment of B-Raf V600E/K mutated metastatic melanoma in January 2014 and the combination of cobimetinib and vemurafenib for the same type of indication [121]. Although significant improvement in progression-free survival was observed using MEK/B-Raf combination strategy, the incidence of some common adverse effects, such as vomiting, diarrhea, nausea, rash, and pyrexia, also increased [118, 120–122].

#### 3.3.1.9 PI3K Inhibitor

Among the large groups of structural diverse lipid kinase inhibitors, especially against PI3Ks [123–126], idelalisib (Zydelig<sup>®</sup>, Gilead Sciences) is the only inhibitor approved by FDA [127, 128] for the treatment of patients with relapsed chronic lymphocytic leukemia in combination with rituximab and patients

with relapsed follicular B-cell non-Hodgkin lymphoma or small lymphocytic lymphoma [129, 130].

#### 3.3.1.10 CDK Inhibitor

Palbociclib (Ibrance<sup>®</sup>, Pfizer) [131], a selective CDK4 and CDK6 inhibitor [132], received accelerated approval from FDA in 2015 for women with estrogen receptor-positive and HER2-negative breast cancer in combination with letrozole [133, 134].

### 3.3.2 FDA Approved Covalent Small Molecule Kinase Inhibitors

The collection of ibrutinib (Imbruvica<sup>®</sup>, Pharmacyclics Inc.) [135], afatinib [136], and osimertinib [137] represents the small, yet expanding, group of covalent SMKIs. Ibrutinib is a non-receptor Bruton's tyrosine kinase inhibitor approved for the treatment of relapsed chronic lymphocytic leukemia [138]. Afatinib, approved for NSCLC in 2013 and squamous NSCLC in 2016, is a second-generation irreversible EGFR inhibitor that targets wild-type EGFR, the mutant T790M EGFR, and HER2. Osimertinib (AZD9291), which was approved by FDA in November 2015, is a third-generation irreversible EGFR inhibitor that selectively targets the mutant T790M EGFR [139]. Rociletinib, which shares a high degree of structural similarity with that of osimertinib, is a promising covalent EGFR inhibitor developed by Clovis Oncology aimed for the treatment of patients with EGFR T790M-mutated NSCLC, until the company terminated its development in May 2016 following a negative vote from the FDA's Oncologic Drugs Advisory Committee [140, 141].

### 3.3.3 FDA-Approved Rapalogs

Mechanistic target of rapamycin (mTOR) is a serine/threonine-specific protein kinase in the PI3/PI4-kinase family [142]. mTOR was named after the natural macrolide rapamycin, also known as sirolimus, which was isolated from a soil sample from Easter Island in the 1970s [143] and later evaluated as an immunosuppressive agent. The anticancer activity of rapamycin was discovered in the 1980s, although the mechanism of action and the identification of the rapamycin target, mTOR, were not elucidated until the 1990s [144, 145]. Rapamycin and its macrocyclic analogues, such as temsirolimus (Torisel<sup>®</sup>, Wyeth/Pfizer) and everolimus (Afinitor<sup>®</sup>, Novartis), are grouped as "rapalogs" that constitute the first-generation mTOR inhibitors [146–148].

Rapamycin was approved by the US FDA in 1999 as an immunosuppressive agent to prevent organ rejection in patients receiving kidney transplants [77]. Although a large number of clinical studies have been performed to evaluate the anticancer activities of sirolimus in different types of cancers, such as invasive bladder cancer, breast cancer, and leukemia, most studies show limited efficacy [147]. Outside oncological indications, sirolimus was approved by FDA for the treatment of a rare progressive lung disease lymphangiomyomatosis in 2015. Temsirolimus was approved for the treatment of advanced RCC. Everolimus was approved in the EU for the prevention of organ rejection in heart and

kidney transplant recipients before FDA approved it in 2009 for the treatment of advanced RCC resistant to sunitinib or sorafenib and for the treatment of advanced or metastatic gastrointestinal and lung tumors in 2016 [147]. Additionally, rapamycin and rapalogs are being investigated as antiaging therapeutics or for the treatment of age-related diseases [148]. Studies have revealed that mTOR activity can be retained under hypoxic conditions via mutations in the PI3K pathway, leading to increased translation and hypoxic gene expression and tumor progression [149].

### 3.3.4 Other Approved Kinase Inhibitors

Originally approved for the treatment of cerebral vasospasm in Japan in 1995, fasudil has been evaluated as a ROCK inhibitor and vasodilator since 1997 [150]. ROCK is a serine/threonine kinase belonging to the protein kinase A, G, and C families. ROCK inhibitors were tested as potential therapeutic agents to treat many diseases including neurodegenerative diseases, diabetes, cancer, and pulmonary hypertension [150, 151], due to the roles of ROCK in organizing cytoskeleton, mediating vasoconstriction, cell mobility, migration, and contractility [152]. Thus, Asahi in collaboration with CoTherix pursued to obtain US and European approval for the use of fasudil to treat cardiovascular diseases, such as pulmonary arterial hypertension. The development of fasudil was terminated in 2007 when CoTherix was acquired by Actelion. As such, fasudil is yet to be approved by the US FDA or by the European Medicine Agency.

Ripasudil (Glanatec™, Kowa Pharmaceutical), a close derivative of fasudil, is another Rho kinase inhibitor approved in Japan at the end of 2014 for the treatment of glaucoma and ocular hypertension when other therapeutic agents are not effective or cannot be administered [153]. Additionally, ripasudil has been tested in diabetic retinopathy clinical trials and shown to promote corneal endothelial cell proliferation, endothelium regeneration, and wound healing [154].

Icotinib (Conmana<sup>®</sup>, BetaPharma), an EGFR inhibitor, was approved by the China State FDA in 2011 for the treatment of NSCLC. Icotinib resembles erlotinib in possessing the *N*-(3-ethylphenyl) quinazolin-4-amine core scaffold of erlotinib that binds to the EGFR ATP pocket. An adjacent hydrophobic group was also retained while the solvent exposed two 2-methoxyethoxy-substituents at the 6- and 7-positions of the quinazoline core were cyclized to afford the tetraoxacyclododecene moiety of icotinib. Efficacy and safety of icotinib as first-line therapy in patients has been evaluated for advanced NSCLC in recent clinical studies [155, 156].

Radotinib (Supect<sup>®</sup>, Il-Yang Pharmaceutical) is a Bcr–Abl inhibitor that was approved in South Korea in 2012 for the treatment of imatinib-resistant CML [157]. Radotinib, which has a terminal 4-(pyridine-2-yl) pyrimidine moiety, was developed based on the previously approved Bcr–Abl inhibitors nilotinib. Radotinib has equivalent efficacy with that of other second-generation Bcr–Abl inhibitors and is well tolerated in chronic-phase CML patients [158]. The lower cost of radotinib compared with other FDA-approved Bcr–Abl inhibitors makes it an attractive alternative for the treatment of CML in developing nations [159].

### 3.4 New Directions

The history of the development of kinase inhibitor drugs has seen many challenges and limitations. One of the most daunting challenges is the frequent emergence of drug resistance of tumor cells, which reduces the therapeutic response duration for most anticancer kinase inhibitor drugs from 6 to 12 months [160]. Thus, a key task in the kinase-targeted therapy is to better understand the resistance mechanism and develop strategies to overcome drug resistance.

Another avenue is exploration of the remaining kinases [161]. In addition to the aforementioned 38 approved kinase inhibitor drugs, over 200 other kinase inhibitors are being evaluated in various clinical trials and many more are in different preclinical and discovery phases [12, 20]. Yet, the majority of human kinome has not been thoroughly explored [162], with the development of allosteric modulators as another new trend [40]. The high degree of structural similarity of the active site makes selective targeting of kinase isoforms a challenging task, while allosteric targeting strategy may lead to the development of more selective kinase inhibitors with fewer off-target toxicities and application in chronic diseases.

There is rekindled interest in covalent SMKIs [163–165], especially for the treatment of different types of cancer. However, due to the possibility of forming irreversible covalent bonds with cysteines of off-target proteins, irreversible covalent inhibitors are less likely to meet the long-term safety margins required for the treatment of chronic disorders. Conversely, reversible covalent binding allows slow dissociation kinetics, resulting in enhanced potency and improved selectivity. The reversible covalent binding can be achieved by targeting non-catalytic cysteines using reactive electrophile attached to SMKIs. In addition to affinity, drug-target residence time contributes significantly to pharmacodynamics activity and efficacy *in vivo* [166]. Reversible covalent inhibition has the potential to be used as a general strategy to prolong on-target residence time for kinase inhibitors and may open new therapeutic areas for kinase inhibitors beyond cancers. One such molecule is the Bruton's tyrosine kinase inhibitor, PRN1008, which incorporates a cyanoacrylamide moiety that forms a covalent interaction with a noncatalytic cysteine residue Cys481. PRN1008 is being evaluated in clinical studies as a potential therapeutic for autoimmune and inflammatory disorders [167].

Selectivity is and will still be an ongoing critical topic in the development of kinase inhibitor drugs [168]. Most of the approved SMKIs that bind to the highly conserved ATP binding pocket of kinases target multiple kinases or kinase families. Large-scale analysis of kinase inhibitor selectivity has been performed to quantify varied grades of selectivity among reported kinase inhibitors [63, 114, 162, 169, 170]. High selectivity is most likely to be achieved against kinase of unique structural features or ones that have only few closely related homologues. A few approaches are being reported to develop inhibitors with improved selectivity, such as a recently revealed strategy of combining covalent targeting and allosteric inhibition for the development of protein kinase B inhibitors [171] and structure-based design aided by new crystallographic data and other biophysical techniques [172].

### 3.5 Conclusion

The development of kinase inhibitors as therapeutics is one of the most successful stories in drug discovery. Since the approval of the first kinase inhibitor drugs, a total of 38 kinase inhibitor drugs have been approved to date, mainly for the treatment of different types of cancers and a few non-cancer indications. The abundant data and results collected for these successful kinase inhibitor drugs have provided valuable chemical, biological, pharmaceutical, and clinical information for the development of next-generation kinase inhibitor drugs.

### List of Abbreviations

Abl	Abelson murine leukemia viral oncogene homologue 1
ALK	anaplastic lymphoma kinase
Bcr	breakpoint cluster region
cAMP	cyclic adenosine monophosphate
CML	chronic myelogenous leukemia
DFG	amino acid sequence Asp-Phe-Gly
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
HER2	human epidermal growth factor receptor 2
JAK	Janus kinase
MAPK	mitogen-activated protein kinase
MEK	MAPK/extracellular signal-regulated kinase
mTOR	mechanistic target of rapamycin
NSCLC	non-small cell lung cancer
PKA	protein kinase A
PKC	protein kinase C
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
RCC	renal cell carcinoma
ROCK	Rho-associated protein kinase
ROS1	ROS proto-oncogene 1
RSV	Rous sarcoma virus
SMKI	small-molecule kinase inhibitor
VEGF	vascular endothelial growth factor

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