Acta Pharmaceutica Sinica B 2021;11(1):30–54

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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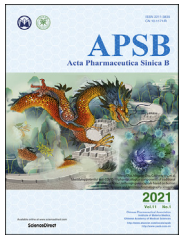


REVIEW

Selective inhibition of CDK4/6: A safe and effective strategy for developing anticancer drugs

Kai Yuan[a,b], Xiao Wang[a,b], Haojie Dong[a,b], Wenjian Min[a,b], Haiping Hao[a,b,*], Peng Yang[a,b,*]

[a]*State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China*
[b]*Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China*

Received 20 March 2020; received in revised form 27 April 2020; accepted 4 May 2020

KEY WORDS

CDK4/6;
Cell cycle;
Cancer;
Selectivity;
Drug resistance;
PROTAC

Abstract The sustained cell proliferation resulting from dysregulation of the cell cycle and activation of cyclin-dependent kinases (CDKs) is a hallmark of cancer. The inhibition of CDKs is a highly promising and attractive strategy for the development of anticancer drugs. In particular, third-generation CDK inhibitors can selectively inhibit CDK4/6 and regulate the cell cycle by suppressing the G1 to S phase transition, exhibiting a perfect balance between anticancer efficacy and general toxicity. To date, three selective CDK4/6 inhibitors have received approval from the U.S. Food and Drug Administration (FDA), and 15 CDK4/6 inhibitors are in clinical trials for the treatment of cancers. In this perspective, we discuss the crucial roles of CDK4/6 in regulating the cell cycle and cancer cells, analyze the rationale for selectively inhibiting CDK4/6 for cancer treatment, review the latest advances in highly selective CDK4/6 inhibitors with different chemical scaffolds, explain the mechanisms associated with CDK4/6

Abbreviations: AKT, protein kinase B; AML, acute myeloid leukemia; CDKs, cyclin-dependent kinases; CIP/KIP, cyclin-dependent kinase inhibitor 1/kinase inhibitory protein; CKIs, cyclin-dependent kinase inhibitors; CPU, China Pharmaceutical University; CRPC, castration-resistant prostate cancer; ER, estrogen receptor; ERK, extracellular regulated protein kinases; FDA, U.S. Food and Drug Administration; FLT, fms-like tyrosine kinase; HER2, human epidermal growth factor receptor 2; INK4, inhibitors of CDK4; JAK, janus kinase; MCL, mantle cell lymphoma; MM, multiple myeloma; NSCLC, non-small cell lung cancer; ORR, overall response rates; PDK1, 3-phosphoinositide-dependent protein kinase 1; PFS, progression-free survival; PI3K, phosphatidylinositol 3-hydroxy kinase; PR, progesterone receptor; PROTAC, proteolysis targeting chimera; RB, retinoblastoma protein; SPH, Shanghai Pharmaceuticals Holding Co., Ltd.; STATs, signal transducers and activators of transcription; UNISA, University of South Australia.

*Corresponding authors. Tel.: +86 13681986682.
E-mail addresses: hhp_770505@hotmail.com (Haiping Hao), pengyang@cpu.edu.cn (Peng Yang).

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2020.05.001

inhibitor resistance and describe solutions to overcome this issue, and briefly introduce proteolysis targeting chimera (PROTAC), a new and revolutionary technique used to degrade CDK4/6.

1. Introduction

The sustained cell proliferation caused by uncontrolled cell division is one of the key pathological manifestations of cancer transformation[1]. Therefore, inhibition of aberrant cell division and proliferation is a promising strategy in cancer therapies. In particular, cyclin-dependent kinases (CDKs) are crucially involved in the regulation of cell division and proliferation. The inhibition of CDKs prevents cell proliferation and plays an increasingly important role in the treatment of cancers[2,3]. Leland H. Hartwell, Paul M. Nurse and R Timothy Hunt were awarded the Nobel Prize in Physiology or Medicine for their discoveries of "key regulators of the cell cycle"[4,5], which has inspired new ideas for cancer treatment.

Due to the crucial function of CDKs in the regulation of cell division and proliferation, numerous drugs that target CDKs have been developed to treat cancers over the past 20 years[6,7]. Despite promising preclinical results, the first- and second-generations of CDK inhibitors were discontinued during clinical trials, as these nonselective pan-CDK inhibitors led to serious cytotoxic effects toward normal cells[1,8]. However, the third-generation of CDK inhibitors, **1** (palbociclib), **2** (ribociclib), and **3** (abemaciclib), which exhibit selectivity for CDK4/6 over other CDKs (Table 1), have received regulatory approval from the U.S. Food and Drug Administration (FDA) for the treatment of patients with breast cancer[9]. Research on CDK4/6 has consistently been a hot topic that has attracted significant attention (Fig. 1), and the number of CDK4-related journal papers and patents has significantly increased in the past ten years. Besides three approved selective CDK4/6 inhibitors, 15 CDK4/6 inhibitors are in different phases of clinical trials as anticancer drugs (Table 1).

In this perspective, we discuss the important roles of CDK4/6 in the regulation of cell cycle progression in normal cells and summarize the multiple mechanisms by which the dysregulation of the CDK4/6 pathway results in the uncontrolled proliferation of cancer cells. In particular, we discuss the rationale for selectively inhibiting CDK4/6 for cancer treatment and review the recent advances in the development of different chemical scaffolds for highly selective CDK4/6 inhibition. Although selective CDK4/6 inhibitors have demonstrated excellent effects in cancer treatment, drug resistance to CDK4/6 inhibitors cannot be ignored, which has emerged and gradually increased. Therefore, we explain the mechanisms of resistance toward CDK4/6 inhibitors, provide some potential solutions to delay or overcome this resistance, and introduce a novel technique, proteolysis targeting chimera (PROTAC).

2. CDK4/6 in the cell cycle and cancer treatment

2.1. *Important roles of CDK4/6 in regulating the G1 to S phase transition in normal cells*

The cell cycle is a highly conserved process that consists of four sequential phases: G1 (pre-DNA synthesis), S (DNA synthesis), G2 (pre-division), and M (cell division). The transition from one phase to the next phase is regulated by different CDKs with their partner cyclins to ensure the normal progression through the entire cell cycle.

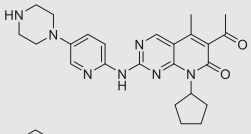
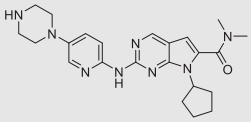
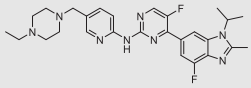
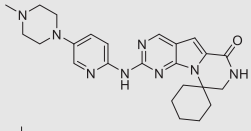
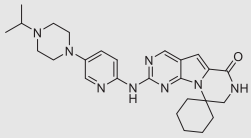
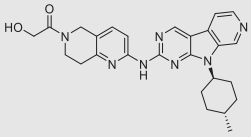
CDK4 and CDK6 share very similar biochemical and biological properties[10], and CDK4/6 can be activated by the crucial initiator of the transition from G1 to S phase, D-type cyclins[11,12]. The level of the D-type cyclins increases with the response to proliferative stimuli in the early G1 phase[13,14], after which these cyclins interact with and activate CDK4/6 (Fig. 2). The cyclin D−CDK4/6 complex subsequently phosphorylates retinoblastoma protein (RB), which binds to the transactivation domain of the E2F family of transcription factors[15,16]. The E2F transcription factor is released as a result of the phosphorylation of RB[17,18]. In addition, the expression of the E-type cyclins is induced by the E2F transcription factor, which then interacts with CDK2[19,20]. This cyclin E−CDK2 complex further accelerates RB phosphorylation, decreasing inhibition of E2F and facilitating the G1 to S phase transition[21]. Thus, CDK4/6 are key initiators of the G1 to S phase transition, and it is important to inhibit both CDK4 and CDK6 to effectively impair the G1/S transition. The third-generation of CDK inhibitors cannot selectively only target CDK4 or CDK6, they are stilled called selective CDK4/6 inhibitors. If the level of cyclin D or the activity of CDK4/6 increases, the cyclin D−CDK4/6 complex will be hyperactivated, then the progression of the G1 to S phase transition and the cell cycle will be accelerated. Furthermore, uncontrolled cell proliferation resulting from an accelerated cell cycle will lead to the development of cancer. Therefore, inhibition of CDK4/6 can cause G1 arrest of cell cycle and is a promising and effective strategy for cancer treatment.

Cyclin-dependent kinase inhibitors (CKIs), including inhibitors of CDK4 (INK4) and cyclin-dependent kinase inhibitor 1/kinase inhibitory protein (CIP/KIP), are involved in the regulation of CDK activity to ensure the smooth progression of the cell cycle[22−24]. The INK4 proteins ($P16^{INK4A}$, $P15^{INK4B}$, $P18^{INK4C}$ and $P19^{INK4D}$) restrain CDK4/6 activity by specifically disrupting the binding of cyclin D to CDK4/6 or by directly binding to CDK4/6 to suppress its catalytic activity[25]. Unlike the INK4 proteins, the CIP/KIP proteins ($P27^{KIP1}$, $P21^{CIP1}$ and $P57^{KIP2}$) interact with all of the CDKs that are crucially involved in the cell cycle and inhibit or activate the activity of CDKs depending on the cellular context[26,27]. Thus, the function of CKIs is crucial for proper CDK activity and normal cell proliferation, and cancer may arise as a result of the loss of their function.

2.2. *Crucial roles of CDK4/6 in cancer cells*

The vast majority of human cancers exhibit dysregulation of the CDK4/6−RB pathway through multiple mechanisms (Fig. 3) and the cyclin D−CDK4/6 complex is hyperactivated in many types of human cancers. Several common oncogenic signaling pathways, such as janus kinase (JAK)−signal transducers and activators of transcription (STATs)[28,29], phosphatidylinositol 3-hydroxy kinase

Table 1 Selective CDK4/6 inhibitors approved by the FDA or in clinical trials.

Name	Structure	Clinical status	Company
1 (Palbociclib)		Launched 2015	Pfizer
2 (Ribociclib)		Launched 2017	Novartis
3 (Abemaciclib)		Launched 2017	Lilly
4 (G1T28)		Phase II	G1 Therapeutics
5 (G1T38)		Phase I/II	G1 Therapeutics
6 (AMG 925)		Phase I	Amgen
SHR-6390	Structure not yet disclosed	Phase III	Hengrui
BPI-1178	Structure not yet disclosed	Phase I/II	Beta
BPI-16350	Structure not yet disclosed	Phase I/II	Betta
FCN 437	Structure not yet disclosed	Phase I	Fosun
Birociclib	Structure not yet disclosed	Phase I	Sihuan
BEBT-209	Structure not yet disclosed	Phase I	BeBetter
TY-302	Structure not yet disclosed	Phase I	TYK
TQB-3616	Structure not yet disclosed	Phase I	Chin Tai Tianqing
HS-10342	Structure not yet disclosed	Phase I	Hansoh
PF-06842874	Structure not yet disclosed	Phase I	Pfizer
CS-3002	Structure not yet disclosed	Phase I	Cstone
MM-D37K	Structure not yet disclosed	Phase I/II	MetaMax

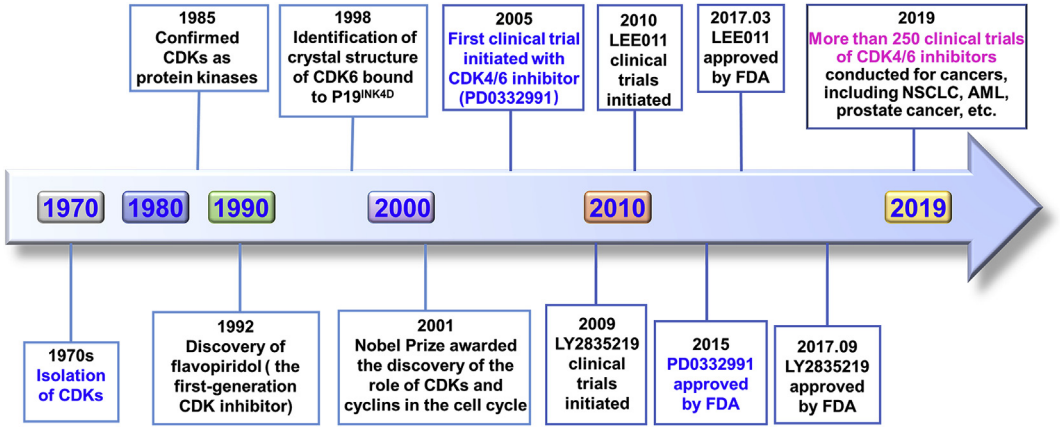


Figure 1 The history of the discovery and development of CDK4/6 inhibitors.

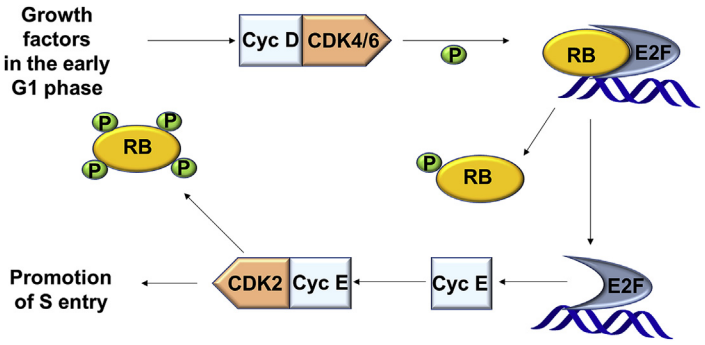


Figure 2 Role of CDK4/6 in the G1 to S phase transition. At the early stage of the G1 phase, the expression of D-type cyclins increases with the recognition of proliferative stimuli, and then these cyclins combine with and activate CDK4/6 to phosphorylate RB, thereby releasing the E2F transcription factor. As a result, the expression of E-type cyclins increases and these cyclins combine with and activate CDK2, which further phosphorylates RB and promotes S phase entry. Cyc represents for cyclin.

(PI3K)−protein kinase B (AKT)[29], and RAS−RAF−extracellular regulated protein kinases (ERK)[30,31], induce cyclin D overexpression and promote CDK4/6 activity, leading to uncontrolled cell proliferation. For example, the overexpression of cyclin D1 has been detected in breast cancer[32,33]. In addition, hyperactive CDK4 was reported in liposarcomas[34], and CDK6 activation was observed in esophageal squamous cell carcinoma[35]. In contrast, the inactivation of endogenous CDK inhibitors removes the primary inhibitory brake on the CDK4/6−RB pathway[36,37]. For instance, the loss of $P16^{INK4A}$ often appears in glioblastoma[38]. In addition, the CDK4/6−RB pathway is also associated with the P53 signaling pathway *via* the transcription of $P21^{CIP1}$, which can inhibit the cyclin D−CDK4/6 and cyclin E−CDK2 complexes[39,40]. Mutations in P53 result in G1 checkpoint abolishment and promote uncontrolled cell proliferation that frequently occurs in advanced ovarian cancer[41,42]. The dysregulation of CDK4/6 in multiple pathways results in the uncontrolled proliferation of cancer cells through different mechanisms. Thus, CDK4/6 are valuable and promising therapeutic targets in the development of anticancer drugs.

2.3. Rationale for selectively inhibiting CDK4/6 for cancer treatment

In addition to CDK4/6, other CDKs also play significant roles in regulating the cell cycle. CDK1 is vital for the proper progression

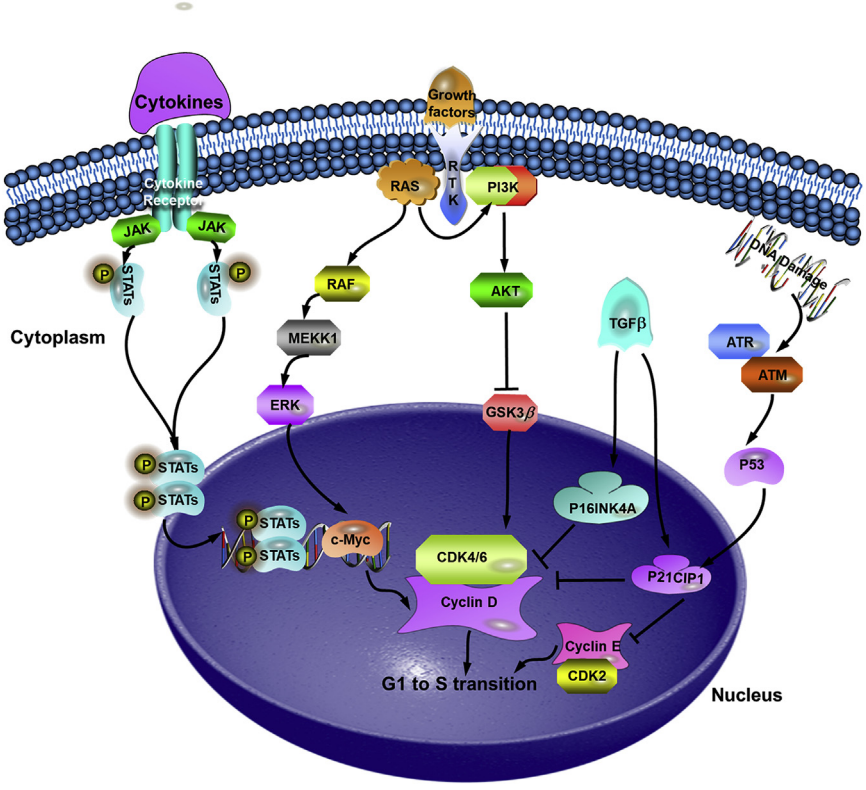


Figure 3 Regulation of the cyclin D−CDK4/6 complex. The cyclin D−CDK4/6 complex can be activated by the JAK−STATs, PI3K−AKT, and RAS−RAF−ERK signaling pathways and can be inhibited by $P16^{INK4A}$. Additionally, the inactivation of CDKs can be mediated by the P53 signaling pathway *via* $P21^{CIP1}$.

of cell mitosis[43,44], and mouse embryos cannot grow beyond the blastocyst stage in the absence of CDK1[45,46]. Similar to CDK4/6, CDK2 facilitates the G1 to S phase transition. Furthermore, the cyclin E−CDK2 complex regulates DNA replication, and the cyclin A−CDK2 complex regulates the progression of the cell cycle through S phase[47,48]. CDK3 regulates the G0−G1 transition by binding with cyclin C[49]. Beyond the role of CDKs in the cell cycle, CDK5 participates in regulating neuron activity by binding to P35 and P39[50]. CDKs 7, 8, 9, and 12 are involved in basal transcriptional regulation. In addition, the cyclin H−CDK7, cyclin T−CDK9, and cyclin K−CDK12 complexes phosphorylate RNA polymerase II to initiate RNA transcription longation[51−54]. The cyclin C−CDK8 complex also plays a key role in transcriptional regulation by inhibiting the activity of the cyclin H−CDK7 complex[55]. In addition to facilitating transcription elongation, CDK10 and CDK11 are also involved in pre-mRNA splicing[56,57]. Therefore, the use of pan-CDK inhibitors is likely to cause significant toxicity because several CDKs that are essential for maintaining the growth and function of normal cells are also inhibited[1,58].

CDK inhibitors are classified into first-, second- and third-generations. The first-generation CDK inhibitors, including flavopiridol and seliciclib, have almost no selectivity among the CDK family. **7** (flavopiridol, Fig. 4), discovered by Sanofi, is the most well-studied first-generation CDK inhibitor and inhibits CDKs 1, 2, 4, 6, 7, and 9[59,60]. Despite promising results *in vitro*, **7** did not display great activity *in vivo*[61], and clinical trials of **7** in many different types of solid tumors did not achieve the desired results. Some advances were made in the clinical trials of flavopiridol with respect to hematological malignancies[62,63], and the compound received orphan drug designation for the treatment of acute myeloid leukemia (AML) in the U.S. and chronic lymphocytic leukemia (CLL) in Europe[64]. **8** (seliciclib, Fig. 4), a first-generation CDK inhibitor that inhibits CDKs 2, 7, and 9, was discovered by Cyclacel[65]. In the phase I trial, common adverse events, such as nausea, vomiting, and asthenia, were resolved after the discontinuation of drug, but the hematological toxicity that was often caused by **7** did not occur[66]. Currently, two clinical trials of **8** for Cushing disease (NCT03774446) and advanced solid tumors (NCT00999401) are ongoing.

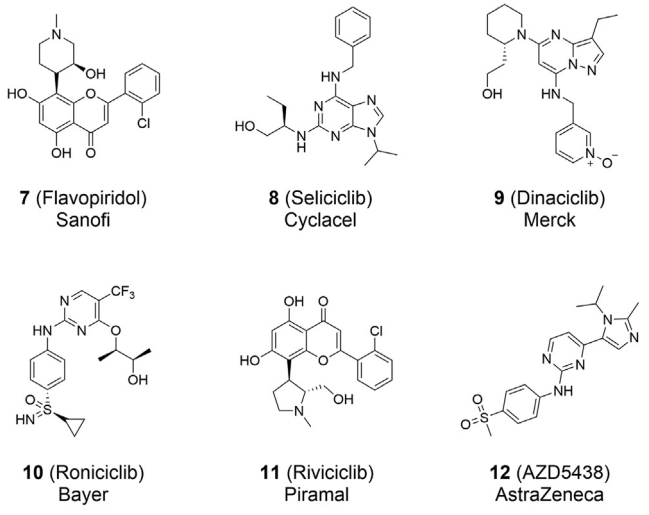


Figure 4 Representative first- and second-generation CDK inhibitors.

Based on first-generation CDK inhibitors, second-generation CDK inhibitors were developed with the aims of increasing selectivity against CDK1 and CDK2 and reducing off-target risks. **9** (dinaciclib, Fig. 4), developed by Merck[67], was shown to inhibit CDKs 1, 2, 5, and 9 with IC_{50} values of 3, 1, 1, and 4 nmol/L, respectively. Compared with **7**, **9** exhibited a better profile in a tumor xenograft model and was superior at inhibiting DNA synthesis and RB phosphorylation. In a phase I clinical trial (NCT00871663), **9**, which was administered once a week by intravenous infusion, demonstrated great safety and tolerability. The inhibition of lymphocyte proliferation and the stabilization of disease indicated the potential of **9** in the treatment of advanced malignancies[68]. However, the phase II clinical trials for acute leukemias[69], breast cancer[70], non-small cell lung cancer (NSCLC) did not display remarkable treatment effects[71]. Similar to **7**, **9** also received orphan drug designation for the treatment of CLL and displayed obvious therapeutic effects in a phase III clinical trial (NCT01580228)[72]. **10** (roniciclib, Fig. 4), developed by Bayer[73], was shown to inhibit CDK1−cyclin B, CDK2−cyclin E, CDK4−cyclin D, CDK7−cyclin H−MAT1, and CDK9−cyclin T1 with IC_{50} values of 7, 9, 11, 25, and 5 nmol/L, respectively. **10** showed antiproliferative activity toward various cancer cell lines, such as lung cancer and breast cancer, with observed IC_{50} values of less than 100 nmol/L, and strongly inhibited tumor growth in different types of tumor xenografts, including in the MX-1 breast cancer and NCI-ADR-Res ovarian cancer models. Moreover, the combination of **10** with cisplatin and etoposide exhibited much better treatment efficacy than individual drugs[73]. However, the phase II clinical trial (NCT02161419) of **10** in combination with cisplatin/etoposide in small cell lung cancer was prematurely terminated due to an unfavorable therapeutic effect and serious adverse events[74]. Currently, the development of **10** has been terminated. **11** (riviciclib, Fig. 4), developed by Piramal[75], was shown to selectively inhibit CDK1−cyclin B, CDK4−cyclin D1, and CDK9−cyclin T1 (IC_{50} = 79, 63, and 20 nmol/L, respectively) with almost no activity against CDK2−cyclin E, CDK7−cyclin H and non-CDK enzymes. **11** strongly inhibited the proliferation of 12 cancer cell lines with IC_{50} values ranging from 310 to 800 nmol/L, and few effects on normal fibroblast cells (WI-38 and MRC-5) were observed at IC_{50} values greater than 10 μmol/L **11** was shown to decrease the levels of CDK4 and cyclin D1, reduce the phosphorylation of RB, and induce apoptosis in HL-60 cells[75]. Furthermore, **11** inhibited tumor growth in murine lung carcinoma, human colon carcinoma, and NSCLC xenograft models[76]. A phase I clinical trial (NCT00407498) demonstrated the great tolerability and mild efficacy of **11** in refractory solid neoplasms, but a phase II study (NCT00843050) in mantle cell lymphoma (MCL) was terminated because no objective responses in patients were observed[77]. Phase II trials of **11** in squamous cell carcinoma of the head and neck, melanoma, multiple myeloma (MM), and pancreatic cancer were completed, but no results have been reported. **12** (AZD5438, Fig. 4), developed by AstraZeneca[78], was shown to inhibit CDK1−cyclin B1, CDK2−cyclin A, CDK2−cyclin E, CDK5−P25, CDK6−cyclin D3, and CDK9−cyclin T with IC_{50} values of 16, 45, 6, 14, 21, and 20 nmol/L, respectively. **12** can inhibit the proliferation of a broad range of cancer lines with IC_{50} values ranging from 0.20 to 1.70 μmol/L, suppressing the synthesis of DNA by arresting the cell cycle in the G1, M, and G2−S phase and reducing tumor volume in colorectal, prostate and ovarian cancer xenografts[78]. The phase I study of **12** (NCT00088790) was completed in 2005[79], but its subsequent clinical study was suspended due to the intolerable adverse effects at high doses[80]. These pan-CDK inhibitors demonstrated

unsatisfied efficacy or unacceptable toxicity in clinical trials, therefore, the development of CDK inhibitors was then shifted toward reducing the risk of toxicity while maintaining potent efficacy, and third-generation CDK inhibitors with better selectivity for CDK4/6 were developed.

Compared with nonspecific CDK inhibitors, selective CDK4/6 inhibitors do not inhibit the CDKs that regulate and control the cell cycle of normal cells, thus avoiding off-target toxicity and providing a definite therapeutic window[81]. Genetic knock-out experiments have also indicated that CDK4/6 are not absolutely necessary in normal fibroblast cells due to the compensatory effects of CDK1[45]. Furthermore, clinical studies have demonstrated that the cellular sensitivity to drugs will improve with the loss of $P16^{INK4A}$ or the overexpression of cyclin D[82]. Because the cyclin D−CDK4/6 complex is typically overactive when $P16^{INK4A}$ is inactive or cyclin D is overexpressed, cancer cells are more sensitive to CDK4/6 inhibitors than normal cells, and cytotoxic effects and off-target effects can be avoided to some extent. Therefore, selective inhibition of CDK4/6 has become a potentially safe and effective strategy for developing anticancer drugs with potent efficacy and tolerable side effects.

2.4. *Trends in the treatment strategies used for breast cancer*

Breast cancer is the most common tumor and the major cause of death among women worldwide[83]. Breast cancer can be classified into different subtypes according to the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)[84], and the treatment options depend on the subtype of breast cancer. Hormone receptor (HR)$^+$/HER2$^-$ (ER$^+$/HER2$^-$ or PR$^+$/HER2$^-$) breast cancer is the most common type of breast cancer, accounting for approximately 60%−70%. The treatment for breast cancer has always been paid close attention, and many treatment strategies have been developed to decrease breast cancer mortality rates. Among them, endocrine therapy played significant role in the treatment of breast cancer, which reduced estrogen levels or affected estrogen function to inhibit the proliferation of breast cancer cells and achieve the purpose of controlling the breast cancer.

Currently, novel CDK4/6 inhibitors plus endocrine therapy demonstrate greatly improved therapeutic effects in HR$^+$/HER2$^-$ breast cancer, and this is a standard therapy for HR$^+$/HER2$^-$ breast cancer[85]. Compared with letrozole alone, **1** plus letrozole prolongs the median progression-free survival (PFS) of HR$^+$/HER2$^-$ breast cancer patients from 14.5 to 24.8 months[86]. The combination of **1** and fulvestrant demonstrates better antitumor activity than fulvestrant alone, with median PFS values of 9.5 and 4.6 months, respectively[87]. As the first CDK4/6 inhibitor, **1** initiated a new era in the treatment of breast cancer. The sales of **1** reached 4.96 billion dollars in 2019 and are expected to reach 9 billion dollars by 2025.

3. Selective CDK4/6 inhibitors

Due to the intolerable toxic effects resulting from treatment with nonspecific CDK inhibitors, the development of selective CDK4/6 inhibitors has emerged as a promising direction for cancer treatment. The following section summarizes major CDK4/6 inhibitors that have been developed, and they are classified below based on their chemical scaffolds.

3.1. *Pyrido[2,3-d]pyrimidin-7(8H)-one scaffold*

Pfizer[88,89] advanced the first selective CDK4/6 inhibitor **1** to the market; this medication was approved on an accelerated path in 2015 for the treatment of postmenopausal women with HR$^+$/HER2$^-$ advanced metastatic breast cancer in combination with letrozole. The discovery of **1** is summarized in Fig. 5. The hit compound **1a** (Scheme 1) inhibited CDK4/cyclin D with an IC_{50} value of 0.62 μmol/L[90]. The introduction of a cyclopentyl group at the N′8 position (**1b**, Scheme 1) and the piperazine ring at the C′2-position (**1c**, Scheme 1) increased the potency on CDK4[91,92]. The introduction of a methyl group at the C′5 position (**1d**, Scheme 1) significantly improved the selectivity over CDK2 while retaining the inhibition of CDK4[92]. The subsequent introduction of an acetyl group at the C′6 position (**1e**, Scheme 1) and replacement of the phenyl group by a pyridinyl group at the C′2 position led to the identification of the selective CDK4/6 inhibitor **1**[92]. **1** displayed potent inhibitory activity against CDK4/6 (IC_{50} = 9 and 15 nmol/L, respectively) and showed less activity against CDKs 1, 2, and 5 with IC_{50} values all above 10 μmol/L[93,94]. The cocrystal structure of CDK6 and **1** (Fig. 5A) revealed that **1** had strong interactions with CDK6, including two hydrogen bonds with the conserved residue Val101 and one hydrogen bond with the conserved residue Asp163. In addition, its positively charged piperazine ring was stabilized by lying against a solvent-exposed ridge consisting of Asp104 and Thr107 residues[95,96].

Compound **1** can be administered orally, but patients must have a 7-day break after 21 days of continuous administration due to the neutropenia that occurs as an adverse event. In addition to breast cancer, a phase II trial of **1** (NCT01209598) in 48 liposarcoma patients demonstrated that PFS was 66% at 12 weeks and the median PFS was 18 weeks[97], and a phase I trial of **1** (NCT00420056) in 17 MCL patients demonstrated that five patients achieved PFS of more than 1 year[98]. Clinical studies for acute myeloid and lymphoblastic leukemias (NCT03472573 and NCT03472573), NSCLC (NCT03170206), liver cancer (NCT01356628), colorectal cancer (NCT03446157), chordomas (NCT03110744), and many other types of solid tumors are underway in an effort to extend the therapeutic range of **1**.

Given the success of **1**, many compounds with similar structures have been designed and developed as selective CDK4/6 inhibitors. Compound **13** (Fig. 6), developed by Jiangsu Hengrui Pharmaceutical Co., Ltd.[99], inhibits CDK4, CDK6, CDK1, CDK2, and CDK9 with IC_{50} values of 12, 12, >1000, >1000 and 4026 nmol/L, respectively. Hengrui also developed another CDK4/6 inhibitor, SHR-6390, but its structure has not been disclosed to date. SHR-6390 was shown to inhibit tumor growth in a panel of tumor xenografts with an efficacy that is equivalent to or better than that of **1**. Currently, SHR-6390 is in phase III clinical trials for the treatment of patients with HR$^+$/HER2$^-$ breast cancer[100,101].

When the piperazine ring of **1** is acylated with an amino acid that preserves the basicity, the resulting analog **14** (Fig. 6), discovered by China Pharmaceutical University (CPU)[102], retains a high selectively toward CDK4 and CDK6 with IC_{50} values of 13 and 18 nmol/L, respectively[102]. **14** exhibits excellent antiproliferative activity in different breast cancer cell lines and leads

to considerable control of tumor progression, with no significant body weight reductions in a 15-day rat xenograft MCF-7 model.

HEC Pharm[103,104] also developed many analogs of **1**, such as **15** and **16** (Fig. 6), by modifying the piperazine ring, and **15** and **16** retained inhibitory activity against CDK4/6. The synthesis of analogs is not limited to altering the piperazine ring. **17** (Fig. 6), discovered by Shanghai Pharmaceuticals Holding Co., Ltd. (SPH)[105], bearing a fused ring, retained potency on CDK4/6 (IC_{50} = 3.6 and 10.2 nmol/L, respectively) and inhibited the proliferation of MCF-7 breast cancer cells (IC_{50} = 57.8 nmol/L).

A multikinase inhibitor may have better anticancer effects because it can block more than one pathway at the same time. In 2014, Reddy et al.[106] reported the multikinase inhibitor **18** (ON-123300, Fig. 6), which was identified based on the antiproliferative activity in K562 and DU145 cell lines. **18a** (Scheme 2) inhibited proliferation of K562 and DU145 cell lines with IC_{50} values of 100 and 75 μmol/L, respectively. The change from benzylamine to phenylamine (**18b**, Scheme 2) increased antiproliferative activity in K562 and DU145 cell lines with same IC_{50} value of 30 μmol/L. The subsequent introduction of a morpholine ring (**18c**, Scheme 2) also slightly increased the antiproliferative activity. The replacement of morpholine group by piperazine group (**18**, Scheme 2) observably improved antiproliferative activity in K562 and DU145 cell lines with IC_{50} values of 0.05 and 0.025 μmol/L, respectively. **18** showed inhibition of CDK4, CDK6, ARK5, FGFR1, PDGFR-β, and PI3K-δ (IC_{50} = 3.87, 9.82, 4.95, 26.00, 26.00, and 144 nmol/L, respectively) and displayed selectivity over CDKs 1, 2, 5, 8, and 9[106]. All of the kinases suppressed by **18** were related to growth, survival, and metastasis in human tumor cells, resulting in synergistic effects. Cell apoptosis could be induced by **18**, although this effect was not observed in cells treated with **1**. In breast tumor xenografts, **18** was shown to strongly inhibit the growth of tumors without causing a loss of body weight[106].

3.2. *7H-Pyrrolo[2,3-d]pyrimidine scaffold*

In parallel to the development of **1**, the series leading to **2** was studied by Novartis[94,107,108], which was the second oral CDK4/6 inhibitor approved by the FDA. **2** inhibits CDK4/6 with IC_{50} values of 10 and 39 nmol/L, respectively. Similar to that of **1**, the cocrystal structure of CDK6 and **2** (Fig. 5B) reveals that the Val101, Asp163, Asp104, and Thr107 residues play significant roles in the interaction between the protein and the compound[96], and **2** and **1** were used to treat the same diseases. Moreover, patients also need to take 7 days off after continuous treatment with **2** for 21 days[107]. In clinical trials of 668 patients with $HR^+/HER2^-$ breast cancer, the overall response rates (ORR) and the PFS of the **2** plus letrozole group were 52.7% and 63%, respectively, which were higher than those of the placebo plus letrozole group (37.1% and 42.2%, respectively)[109,110]. To further explore the therapeutic utility of **2**, clinical studies for various types of diseases, such as myelofibrosis (NCT02370706), liposarcoma (NCT03096912), ovarian cancer (NCT03056833), and head and neck cancer (NCT03179956), are underway.

After the disclosure of **2** in 2010[111], many follow-up studies were carried out. Novartis itself also conducted more vigorous studies and synthesized many analogs. For example, **19** (Fig. 7), published by Novartis[112] in 2011, showed great selectivity for CDK4 (IC_{50} = 3 nmol/L) over CDK1 (IC_{50} = 5.32 μmol/L). HEC Pharm[113] also modified the piperazine ring of **2** and disclosed a new CDK4/6 inhibitor **20** (IC_{50} = 25 and 279 nmol/L, respectively, Fig. 7) in 2016.

The amide group in **2** was replaced by a methylsulfonyl group to generate the CDK4/6 inhibitor **21** (Fig. 7). Also, **21** demonstrated a potent inhibition of CDK4/6 (IC_{50} = 0.8 and 5.7 nmol/L, respectively) and inhibited cell proliferation of MCF7 breast cancer cells and Colo-205 colon cancer cells (IC_{50} = 114.4 and 270.8 nmol/L, respectively)[114].

3.3. *6-(Pyrimidin-4-yl)-1H-benzo[d]imidazole scaffold*

In addition to **1** and **2**, Eli Lilly[115,116] also developed the oral CDK4/6 inhibitor **3**, which represented an alternative chemical scaffold and was approved shortly after **2** in 2017. The optimization of the discovery of **3** is summarized in Scheme 2. The pyrimidine-benzimidazole scaffold was identified as a promising CDK4/6 inhibitor through virtual screening[117]. On account of *in silico* properties, ligand efficiency and potency, **3a** (Scheme 3) was chosen as the positive hit to base the construction of the novel CDK4/6 inhibitor pharmacophore[58]. The change from benzene to

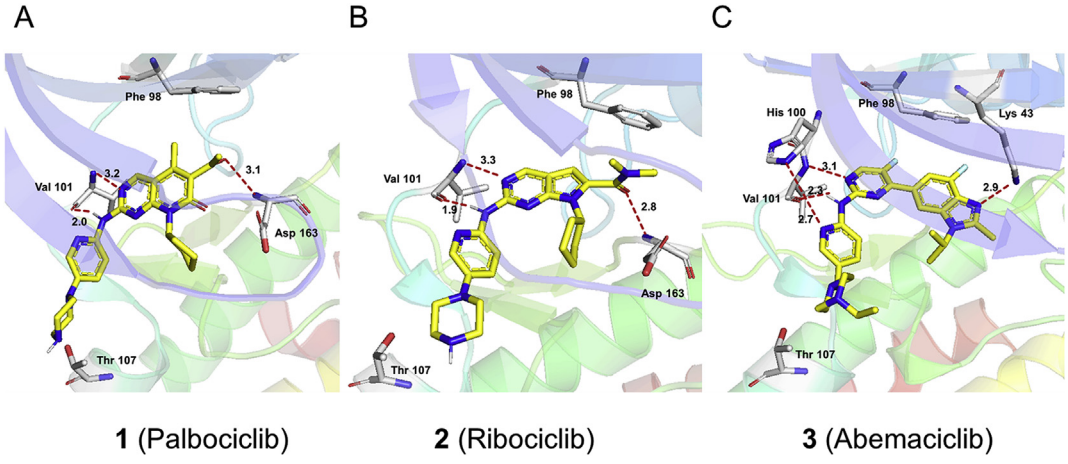
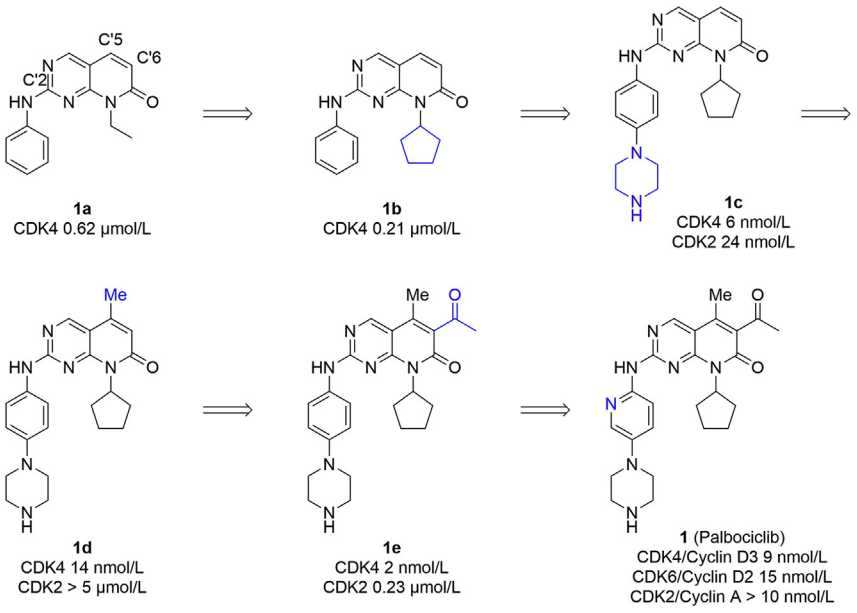


Figure 5 Interactions of CDK6 with three approved drugs, **1** (PDB code 5L2I), **2** (PDB code 5L2T), and **3** (PDB code 5L2S). The hydrogen bonds are shown as red dashed lines, and the number is the distance (Å). The figures were prepared using PyMOL (http://www.pymol.org/).



Scheme 1 Discovery of selective CDK4/6 inhibitor **1**.

pyridine and the introduction of a piperazine ring (**3b**, Scheme 3) decreased the inhibition of the CDK1. The methylene linker between pyridine and piperazine and the isopropyl substitution of the piperazine ring (**3c**, Scheme 3) further optimized selectivity over CDK1 while maintaining the potent inhibition of CDK4[58]. The following substitution of pyrimidine and benzimidazole rings by fluorine improved the specificity and pharmacokinetic properties and resulted in the selective CDK4/6 inhibitor **3** (IC_{50} = 2 and 10 nmol/L, respectively)[58,117]. The cocrystal structures of **1**, **2** and **3** with CDK6 (Fig. 7) displayed similar binding modes. The aminopyrimidine moiety of **1** and **2** formed three hydrogen bonds with Val101 and Asp163, and the aminopyrimidine moiety **3** formed three hydrogen bonds with Val101 and Lys43, but a water molecule was also observed to bridge the residue His100 and the pyridine nitrogen of **3**[96]. **3** is used in combination with fulvestrant to treat patients with $HR^+/HER2^-$ advanced or metastatic breast cancer, especially with disease progression following endocrine therapy[115].

Three approved CDK4/6 inhibitors are classified into two classes: one class includes **1** and **2**, with similar efficacy and toxicity, while **3** is in the other class. Beyond the inhibition of CDK4/6, **3** also shows inhibition of CDK9 with an IC_{50} value of 57 nmol/L, but **1** and **2** do not show inhibitory activity against CDK9[81,94]. Furthermore, **3** has been approved as a single-agent treatment for $HR^+/HER2^-$ breast cancer[115], while **1** and **2** need to be combined with endocrine therapy. In clinical trials, the single-agent **3** gave an objective response in $HR^+/HER2^-$ breast cancer, and the median PFS was 6 months. Compared with single endocrine therapy (fulvestrant), the combination of **3** and fulvestrant can prolong median PFS from 9.3 to 16.4 months[118,119]. **1** and **2** cannot be dosed continuously due to the decrease in neutrophil counts, but **3** can be used continuously without intermittent administration. Although **1** can inhibit cell proliferation only in the presence of RB, **3** can inhibit the cell cycle in both RB-dependent and RB-deficient cell lines[120]. In addition, **3** can be well-absorbed when crossing the blood−brain barrier[81], and

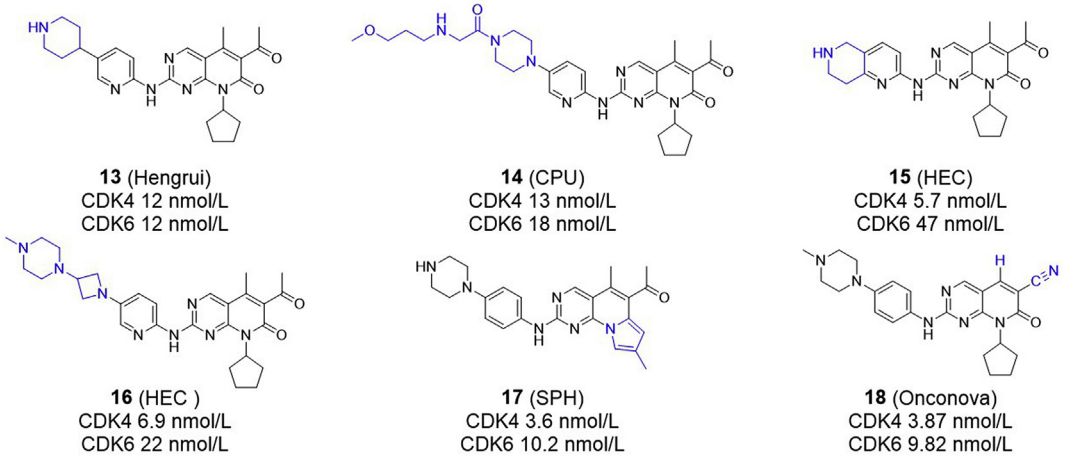
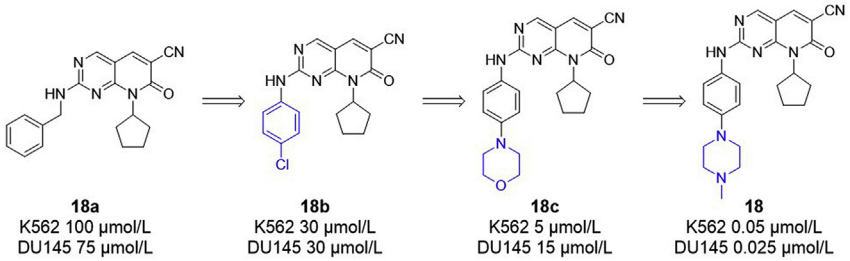


Figure 6 Representative pyrido[2,3-*d*]pyrimidin-7(8*H*)-one derivatives.



Scheme 2 Discovery of the multikinase inhibitor **18**.

clinical trials for the treatment of brain tumors (NCT03220646, NCT02308020) are underway. Furthermore, clinical trials for other types of diseases are also ongoing, including NSCLC (NCT02779751), head and neck cancer (NCT03356223), liposarcoma (NCT02846987), and MCL (NCT02745769).

Since the approval of **3**, many new analogs have been designed and developed. Gan & Lee Pharmaceuticals[121] developed **22** (Fig. 8) by modifying the imidazole ring. Also, **22** showed highly potent inhibition of CDK4−cyclin D3 and CDK6−cyclin D3 (IC_{50} = 7.4 and 0.9 nmol/L, respectively) with a significant selectivity over CDK1−cyclinA2 (IC_{50} = 2.67 μmol/L). Furthermore, **22** exhibited extraordinary potency in inhibiting the proliferation of the MDA-MB-231 cells (IC_{50} = 232 nmol/L)[121].

The modifications of the piperazine ring of **3** also generated many novel CDK4/6 inhibitors, including **23** and **24** (Fig. 8). Next, **23** was determined by HEC Pharm[122] to have potent inhibition of CDK4/6 (IC_{50} = 1.5 and 22 nmol/L, respectively). In 2018, Zha et al.[123] introduced the CDK4 and CDK6 inhibitor **24** (IC_{50} = 1.4 and 1.6 nmol/L, respectively, Fig. 8). Also, **24** showed the inhibition of CDK9 (IC_{50} = 66 nmol/L) and a weak inhibition of CDK1 (IC_{50} = 1.18 μmol/L), somewhat similar to **3**. Furthermore, **24** could reduce tumor volume in a Colo-205 xenograft model[123].

3.4. *Tricyclic lactam scaffold*

G1 Therapeutics is one of the companies that has made efforts to develop novel selective CDK4/6 inhibitors. G1 Therapeutics invented the tricyclic lactam scaffold[124], and two compounds (**4** and **5**, Table 1) from this exclusive scaffold are in clinical trials.

In this study, **4** (G1T28, Table 1) is a CDK4/6 inhibitor bearing a tricyclic lactam scaffold (IC_{50} = 1 and 4 nmol/L) that also shows activity against CDK9 (IC_{50} = 50 nmol/L)[124]. The docking of CDK6 with **4** (Fig. 9A) suggests that **4** directly forms two hydrogen bonds with Val101. Cytotoxic chemotherapy is often used in cancer treatment but causes dose-limiting damage to hematopoietic stem cells, and **4** can protect hematopoietic stem cells from the harm induced by cytotoxic chemotherapy[125]. Compared with carboplatin, the combination of **1** and carboplatin demonstrated better effects in inhibiting the apoptosis of hematopoietic stem cells[126]. **1** is an oral drug with a $t_{1/2}$ of 25.9 h[127], and **4** is administered by intravenous injection with a $t_{1/2}$ of approximately 5 h[124]. Therefore, between **4** and **1**, **4** is a more appropriate combination with short-acting chemotherapy. Furthermore, **4** can promote the activity of T cells by increasing the activity of nuclear factor of activated T cells (NFAT) proteins[128]. PD-1 protein is an important immunosuppressive molecule that can prevent the immune system from killing cancer cells. The combination of **4** and PD-1 blockade can lead to increased antitumor activity, and this effect is largely dependent on T cells[128]. A phase I study of **4** (NCT02243150) in healthy volunteers has been completed, and phase II trials for the extensive stage small-cell lung cancer (NCT03041311) and metastatic triple-negative breast cancer (NCT02978716) are underway.

In addition, G1 Therapeutics[129] is developing an oral CDK4/6 inhibitor **5** (G1T38, Table 1) that shows the potent inhibition of CDK4 and CDK6 (IC_{50} = 1 and 2 nmol/L, respectively) and displays the inhibition of CDK9 (IC_{50} = 28 nmol/L). Like **1**, **2** and **3**, **4** and **5** also shared the pyrimidine−NH−pyridine motif and the tailed piperazine ring. Similar to **3**, **5** can be administered continuously as well. Because **5** was as effective as taxanes in tumor models of treatment-resistant castration-resistant prostate cancer (CRPC) with less toxicity, it was regarded as a valid alternative to taxanes in CRPC[130]. The phase I study of G1T38 (NCT02821624) in healthy volunteers was completed, while phase I/II trials in patients with metastatic breast cancer (NCT02983071) and NSCLC (NCT03455829) are underway.

3.5. *Pyrido[4′,3′:4,5]pyrrolo[2,3-d]pyrimidine scaffold*

6 (AMG 925, Table 1), discovered by Amgen[131], is a dual inhibitor of CDK4 and fms-like tyrosine kinase (FLT) 3 (IC_{50} = 3 and 1 nmol/L, respectively) and shows weak inhibition of CDK1

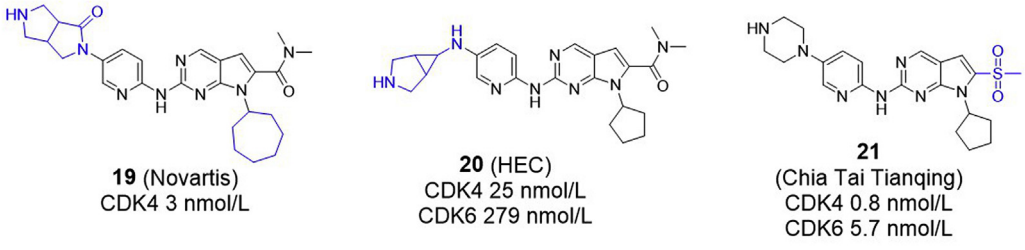
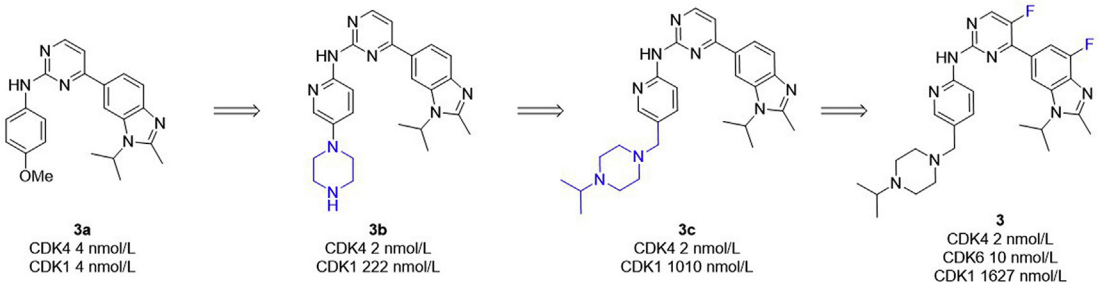


Figure 7 Representative 7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives.



Scheme 3 Discovery of selective CDK4/6 inhibitor **3**.

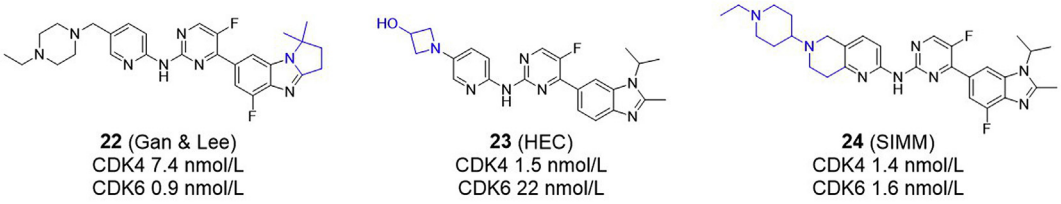


Figure 8 Representative 6-(pyrimidin-4-yl)-1*H*-benzo[*d*]imidazole derivatives.

(IC_{50} = 2.22 μmol/L). This compound now belongs to FLX Bio. The optimization of the discovery of **6** is summarized in Scheme 4. **6a** (Scheme 4) was discovered as a CDK4/6 inhibitor, which was then found to exhibit potent inhibition of FLT3 and selectivity over other kinases as well[131]. The optimization of **6a** focused on decreasing the inhibition of CYP3A4 (IC_{50} = 0.55 μmol/L), improving kinase selectivity and increasing oral bioavailability (F_{rat} = 9.4%)[131]. Although chlorine substitution of pyridine (**6b**, Scheme 4) led to CYP3A4 inhibition with an IC_{50} value of more than 10 μmol/L and a retained potency on CDK4 and FLT3, pyridine was left unsubstituted in the following optimization due to physicochemical and pharmacokinetic properties[131]. Replacement of the cyclopentyl with a polar group (**6c**, Scheme 4) resulted in a prominently decreased CYP3A4 inhibition, but the potency on CDK4 and FLT3 was significantly reduced. The bulky *trans*-4-methycyclohexyl substitution (**6d**, Scheme 4) significantly lowered the inhibition of CYP3A4 without reducing the potency on CDK4 and FLT3, which was selected for further optimization[131]. After reducing the CYP3A4 inhibition, modifications at the piperazine ring and heteroarene were conducted to improve oral bioavailability, which resulted in **6e** (Scheme 4) with an increased bioavailability but low drug exposure. Eventually, a nonbasic polar group was used to replace the basic amine and generated **6** (Scheme 4), with improved drug exposures and excellent oral bioavailability (F_{rat} = 75%)[131]. The docking of CDK6 with **6** (Fig. 9B) suggests that **6** directly forms two hydrogen bonds with Val101 and forms hydrogen bonds with Lys29 and Asp163.

The inhibition of pSTAT5 in MOLM13 cells (IC_{50} = 0.005 μmol/L) and pRb in Colo-205 cells (IC_{50} = 0.023 μmol/L) further demonstrated that **6** was capable of blocking FLT3 and CDK4[131]. Drug resistance has been a major problem when FLT3 inhibitors are used to treat AML, but **6** has the potential to overcome FLT resistance as a result of its CDK4-inhibiting activity[132]. **6** was shown to inhibit tumor growth in MOLM13 and MOLM13-Luc systemic xenograft tumor models[133], and a phase I/Ib study of AMG 925 in subjects with relapsed or refractory AML (NCT02335814) was completed in 2017.

3.6. *4-Thiazol-N-(pyridin-2-yl)pyrimidin-2-amine scaffold*

Wang et al.[134–136] at the university of South Australia (UNISA), who have a long history of investigating CDK inhibitors, previously reported **25a** (Scheme 5) and synthesized many analogs of CDK inhibitors, including moderately potent CDK4 inhibitors[134]. Based on previous studies of developing CDK inhibitors, a methyl group at the C′4 of the thiazole ring formed hydrophobic interactions with the gatekeeper residue of CDKs, which was not altered in the following optimization[137]. A C′2-amino substitution of the thiazole ring made a significant contribution to the inhibitory activity of CDK2 and CDK9, which was introduced into the developing CDK4/6 inhibitors, as well[137]. Replacement of the phenyl ring with a pyridine ring enhances the interaction with CDK4/6 and improves the selectivity for CDK4/6 over CDK2. Inspired by the structure of **1**, a six-membered heterocycle was introduced[137]. Based on all these factors (**25b**, Scheme 5), **25** (Scheme 5) was discovered, which displayed excellent activity against for CDK4/6 (K_i = 4 and 30 nmol/L, respectively) with selectivity over CDKs 1, 2, 7, and 9[137]. Moreover, **25** retained great selectivity for CDK4/6 in a test of inhibitory activity against a panel of 369 kinases. The docking of CDK6 with **25** (Fig. 10A) suggests that **25** forms hydrogen bonds with Glu99 and Asp104. **25** remarkably inhibited tumor growth and prolonged life span without weight loss in an MV4-11 AML mouse xenograft model[137].

When a C′2-amino substituent of the thiazole ring was changed to S, the corresponding product **26** (Scheme 5) retained potency on CDK4/6 (K_i = 7 and 42 nmol/L, respectively) and good selectivity over CDKs 1, 2, 7 and 9 (K_i > 5, = 2.70, >5, and >5 μmol/L, respectively)[138]. Also, **26** inhibited the growth of a panel of human cell lines, reduced the phosphorylation of RB at serine 780, and caused G1 arrest in M249 and M249R melanoma cell lines[138].

Structural modifications at the pyrimidine ring, such as a cyano substitution (**27**, Scheme 5) or fluorine substitution (**28**, Scheme 5), were also made. **27** and **28** remained as CDK4/6 inhibitors (K_i = 4/30 and 2/55 nmol/L, respectively) with selectivity over CDKs 1, 2, 7, and 9[139].

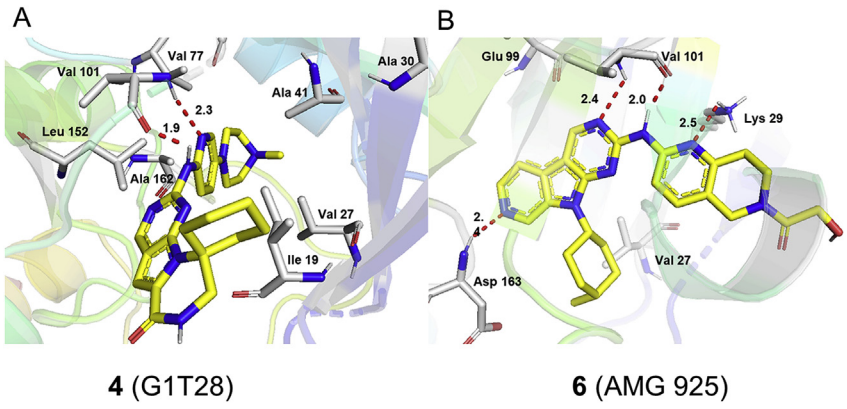


Figure 9 Docking modes of CDK6 (PDB code 4AUA) with **4** and CDK6 (PDB code 4TTH) with **6** were generated using the *Libdock protocol* in Discovery Studio 2019 from Biovia® (formerly Accelrys®) Software Inc. The hydrogen bonds are shown as red dashed lines, and the number is the distance (Å). Figures were prepared using PyMOL.

3.7. *7-Azabenzimidazoles scaffold*

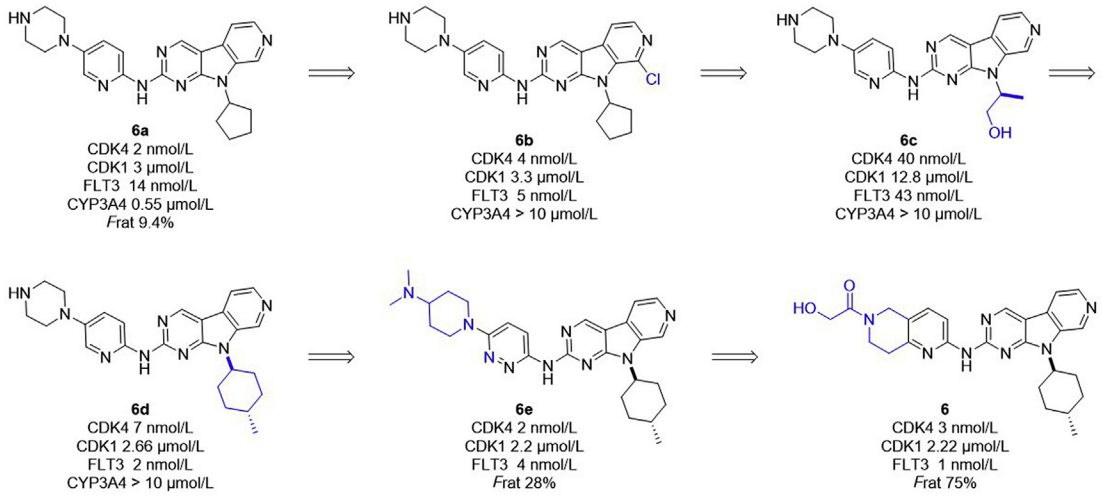
The hit **29a** (Scheme 6), discovered by CDK6 fragment-based screening, exhibited inhibition towards CDK6 with an IC_{50} value of 7.2 μmol/L[140]. Based on the X-ray crystal structure of **29a** bound to CDK6, the pyrrole ring was changed to a pyridine ring (**29b**, Scheme 6) with the intention of forming a hydrogen bond with Lys43[140]. A bulky piperidine ring (**29c**, Scheme 6) was then introduced with the aim of decreasing inhibitory activity against CDK1/2 and improving water solubility. Subsequent modification focused on the pyridine ring, and replacement of the pyridine with a trimethylpyrazole ring (**29d**, Scheme 6) not only increased inhibition against CDK4 but also significantly improved the selectivity for CDK4 over CDK1/2[140]. The introduction of azabenzimidazole and benzonitrile groups (**29**, Scheme 6) further improved the potency on CDK4/6 while maintaining selectivity over CDK1/2[140]. **29** displayed inhibition of CDKs 4, 6, 1, and 2 with IC_{50} values of 15 nmol/L, 120 nmol/L, > 15 μmol/L, and >15 μmol/L, respectively[140]. The docking of CDK6 with **29** (Fig. 10B) suggested that **29** formed two hydrogen bonds with Val101, one hydrogen bond with Lys29 and one hydrogen bond with Asp163. Moreover, **29** showed selectivity over a panel of 35 kinases with IC_{50} values ranging from 4.9 μmol/L to more than 10 μmol/L **29** inhibited the phosphorylation of RB in JeKo-1 cells, caused a G1 arrest of the cell cycle, and significantly reduced the tumor volume in a Jeko-1 xenograft model when orally administered at a dose of 250 mg/kg/day[140].

3.8. *4-(Pyrazol-4-yl)-pyrimidine scaffold*

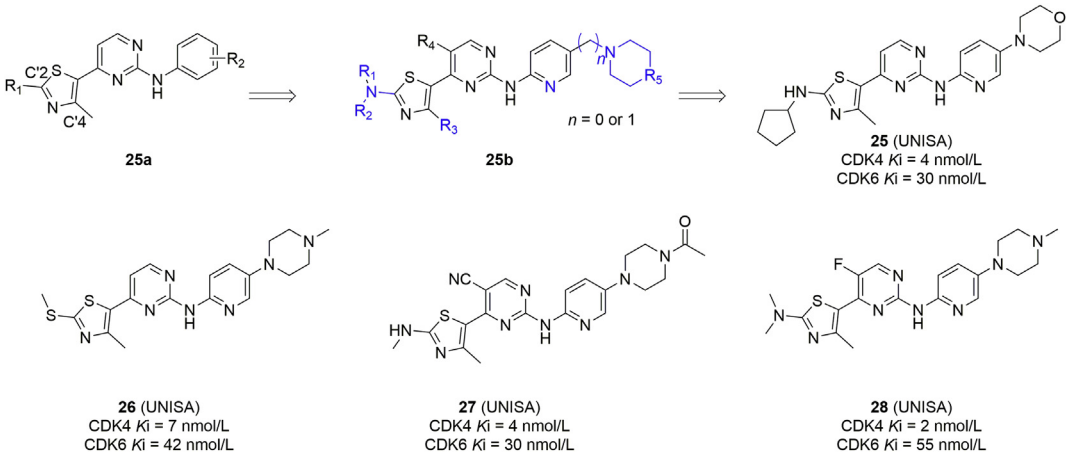
Novartis[141] discovered a promising CDK4 inhibitor **30a** (Scheme 7) by high-throughput screening of their company's compound collection. The early modifications focused on the existed substitutions of the pyrazole and pyrimidine ring. Replacement of the methyl and cyclopentyl with isopropyl and *N*-methylpiperidine (**30b**, Scheme 7) significantly improved inhibitory activity against CDK4 (IC_{50} = 12 nmol/L), but the selectivity over CDK1/2 remained unsatisfactory[141]. However, the introduction of a methyl substitution on the pyrazole ring (**30c**, Scheme 7) led to a large reduction in the inhibitory activity against CDK4 (IC_{50} = 1.979 μmol/L). Based on the analysis of X-ray structures of **30c** and **1** bound to CDK6, a piperazine-substituted pyridine group was used to take the place of the piperidine group (**30d**, Scheme 7). The pyridine ring was introduced with the aim of improving selectivity for CDK4/6 by forming interactions with His100. The introduction of the piperazine was intended for increasing selectivity over CDK1/2 by generating electrostatic repulsion for them. As a result, **30d** retained potency on CDK4 (IC_{50} = 25 nmol/L) and strongly improved selectivity over CDK1/2 (IC_{50} = 5.576 and 6.498 μmol/L, respectively). Then, a chlorine substitution on the pyrazole ring (**30e**, Scheme 7) increased the inhibition against CDK4 (IC_{50} = 11 nmol/L) and led to the improvement of metabolic stability in rat liver microsomes[141]. Finally, to further reduce inhibitory activity against CDK1/2, the piperazine ring was changed to different basic solubilizing groups, resulting in the identification of **30** (Scheme 7), which retained potency on CDK4 (IC_{50} = 12 nmol/L) and demonstrated excellent selectivity over CDK1/2 (IC_{50} > 15 and = 5.265 μmol/L, respectively). **30** also showed broad selectivity in a panel of 13 kinases, including ALK, JAK1, PKA, among others, with IC_{50} values ranging from 6.8 μmol/L to more than 10 μmol/L. The docking of CDK6 with **30** (Fig. 11A) suggested that **30** formed one hydrogen bond with Lys29 and two hydrogen bonds with Val101. Moreover, **30** inhibited the phosphorylation of RB and caused G1 arrest in Jeko-1 cells[141].

3.9. *Thieno[2,3-d]pyrimidin-4-yl hydrazone scaffold*

Daiichi Sankyo[142] has also been interested in developing selective CDK4 inhibitors, and they discovered a hit compound **31a** (Scheme 8) with inhibition activity on CDK4 and CDK2 (IC_{50} = 0.75 and 1.1 μg/mL, respectively) through in-house high-throughput screening. Replacement of an ethyl group with a *tert*-butyl group (**31b**, Scheme 8) enhanced the potency on CDK4 and selectivity over CDK2. When the pyridine ring (**31c**, Scheme 8) was introduced to take the place of the thiophene ring, the CDK4 inhibitory activity was substantially decreased, but selectivity over CDK2 was somewhat improved[142]. The subsequent modifications focused on improving the aqueous solubility, and an aminomethyl substituent (**31**, Scheme 8) of the pyridine ring was introduced. The solubility in water of **31** was indeed improved to 44 μg/mL, while **31** demonstrated potent inhibition against CDK4



Scheme 4 Discovery of the dual CDK4 and FLT3 inhibitor **6** (AMG 925).



Scheme 5 Representative 4-thiazol-*N*-(pyridin-2-yl)pyrimidin-2-amine derivatives.

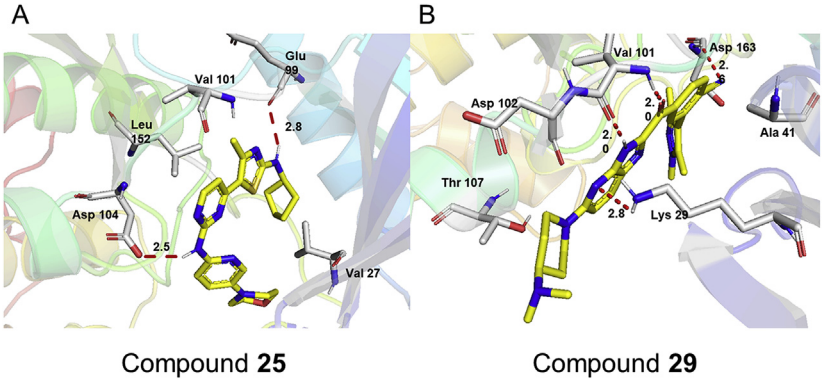


Figure 10 Docking modes of CDK6 (PDB code 4AUA) with **25** and CDK6 (PDB code 4TTH) with **29** were generated using the *Libdock protocol* in Discovery Studio 2019 from Biovia® (formerly Accelrys®) Software Inc. The hydrogen bonds are shown as red dashed lines, and the number is the distance (Å). Figures were prepared using PyMOL.

(IC_{50} = 56 ng/mL) and great selectivity over CDK2 (IC_{50} = 1.4 μg/mL)[142].

Through the analysis of the docking mode of **31** with the CDK4 homology protein, researchers speculated that the nitrogen in the pyridine ring had no effects in improving the inhibition against CDK4, and the phenyl ring was used to replace the pyridine ring[143]. **32a** (Scheme 8), bearing an unsubstituted phenyl ring, inhibited CDK4 and CDK2 with IC_{50} values of 0.61

and >20 μg/mL, respectively. The introduction of an aminomethyl substituent on the phenyl ring (**32**, Scheme 8) improved the inhibition against CDK4 (IC_{50} = 38 ng/mL) and the aqueous solubility (783 μg/mL). Also, **32** displayed potent antiproliferative activity against HCT-116 cells (IC_{50} = 56 ng/mL) and inhibited tumor growth in mice bearing HCT-116 xenografts[143]. However, subsequent investigations found that **32** was not stable under acidic conditions[144]. Therefore, **32** was not appropriate to be used as an oral drug, which could be easily degraded by gastric acid.

The following modifications focused on improving stability. Heteroaryl groups, including thiazoles, oxazoles, and imidazoles, were introduced to take the place of the phenyl ring[144]. Among these groups, thiazole substitution (**33a**, Scheme 8) retained the potency on CDK4 (IC_{50} = 41 ng/mL) and significantly increased chemical stability[144]. **32** remained 38% in pH 1.2 buffer for 1.5 h, and **33a** remained 79% in pH 1.2 buffer for 3 h. A substitution on the thiazole ring (**33b**, Scheme 8) further improved the inhibition against CDK4 and selectivity over CDK2, which inhibited the proliferation of HCT116 and PC-6 cells with the IC_{50} values of 0.343 and 0.214 μg/mL, respectively[144]. Then, the alkyl substitution was optimized to improve cytotoxic activity, and branched alky substitution **33** (Scheme 8) was discovered. The antiproliferative activity of **33** in HCT116 and PC-6 cells was improved (IC_{50} = 0.315 and 0.108 μg/mL, respectively), while inhibition against CDK4 (IC_{50} = 22 ng/mL), selectivity over CDK2 (IC_{50} = 0.88 μg/mL), and chemical stability (83% remaining rate in pH 1.2 buffer for 3 h) was maintained. **33** inhibited tumor growth by 52% (intravenous injection) or 45% (oral) at a dose of 300 mg/kg in mice bearing HCT-116 xenografts[144].

3.10. 5-Pyrimidinyl-2-aminothiazole scaffold

Banyu Pharmaceutical Co., Ltd.[145] discovered the 5-pyrimidinyl-2-aminothiazole scaffold with inhibition against all CDKs by screening the Merck sample repository. Structure modifications revealed that the cyclohexyloxy substitution on the pyrimidine ring significantly contributed to CDK4 inhibition and resulted in the identification of **34a** (Scheme 9)[145]. Next, **34a** demonstrated potent inhibitory activity against CDKs 1, 2, 4, 5, 7, and 9 with IC_{50} values of 24, 14, 4.2, 34, 20, and 2.5 nmol/L, respectively. Through the analysis of the docking of **34a** with CDK4, a methyl substitution on the pyrimidine ring (**34b**, Scheme 9), directed toward the gatekeeper residue, was introduced, and this modification effectively improved the selectivity for CDK4 over other CDKs. To increase solubility, a piperazine substitution on the pyridine ring was introduced and led to the identification of **34** (Scheme 9)[145]. The docking of **34** with CDK6 (Fig. 11B) suggested that **34** formed one hydrogen bond with Asp104 and two hydrogen bonds with Val101. Also, **34** displayed high selectivity for CDK4 and CDK6 (IC_{50} = 9.2 and 7.8 nmol/L, respectively) over CDK1, 2, 5, 7, and 9 (IC_{50} = 0.6, 1.7, 3.0, 0.53 and 2.5 μmol/L, respectively), and exhibited potent antiproliferative activities against EOL-1, KU812, and Jurkat cell lines (IC_{50} = 54, 150, and 230 nmol/L, respectively)[145].

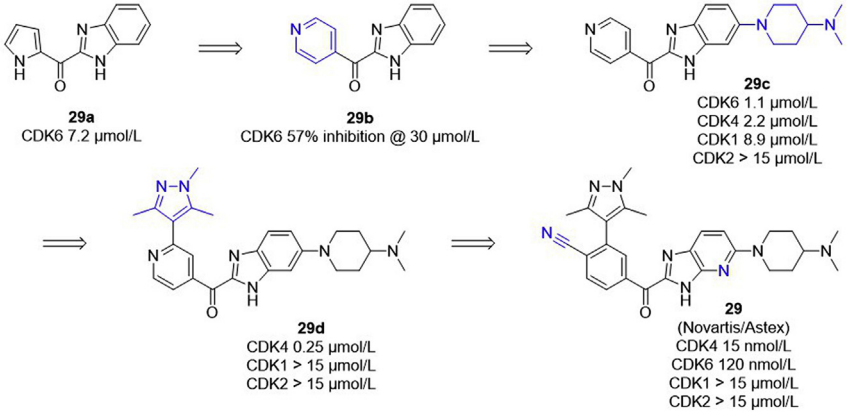
3.11. Pyrrolo[3,4-c]carbazole scaffold

Natural PKC inhibitor **35a** (arcyriaflavin A, Scheme 10) demonstrated inhibitory activity against CDK4 (IC_{50} = 0.14 μmol/L) and caused G1 arrest[146]. Therefore, scientists at Eli Lilly[146] made modifications of **35a** to generate a potent and selective CDK4 inhibitor. The researchers speculated that the CDK4 inhibitory activity could be increased by introducing a substitution on the nitrogen atom of the indole moiety. A methyl substitution of the indole moiety (**35**, Scheme 10) enhanced the inhibition against CDK4 (IC_{50} = 80 nmol/L) and selectivity for CDK2 (IC_{50} > 1 μmol/L)[146], and a fused ring (**36**, Scheme 10) also modulated inhibition against CDK4 and CDK2 (IC_{50} = 0.11 and >1 μmol/L, respectively)[147].

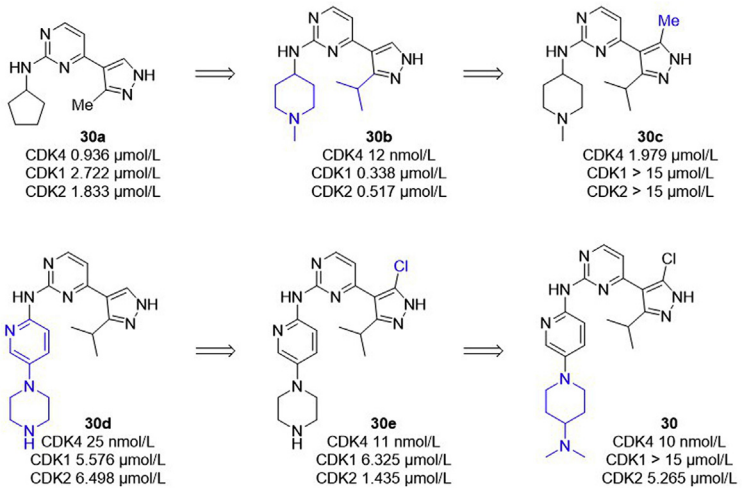
To explore new selective CDK4 inhibitors, modifications focused on replacing one indole moiety with another heteroaromatic ring. **37** (Scheme 10), bearing another type of indole, displayed inhibition against CDK4 and CDK2 with IC_{50} values of 36 and 64 nmol/L, respectively[148]. When the indole moiety was altered to an isoquinoline or naphthaline ring, the corresponding compounds **38** and **39** (Scheme 10) retained potency on CDK4 (IC_{50} = 62 and 45 nmol/L, respectively)[149,150].

3.12. Diarylurea scaffold

Based on a *de novo* design strategy, the commercially available hit **40a** (Scheme 11) was discovered to have inhibitory activity against CDK4 (IC_{50} = 44 μmol/L), and the diarylurea scaffold played a significant role in interacting with CDK4[151]. Modification first focused on the 5-chloro-2-methylphenyl group, and the



Scheme 6 Discovery of the selective CDK4/6 inhibitor **29**.



Scheme 7 Discovery of the selective CDK4 inhibitor **30**.

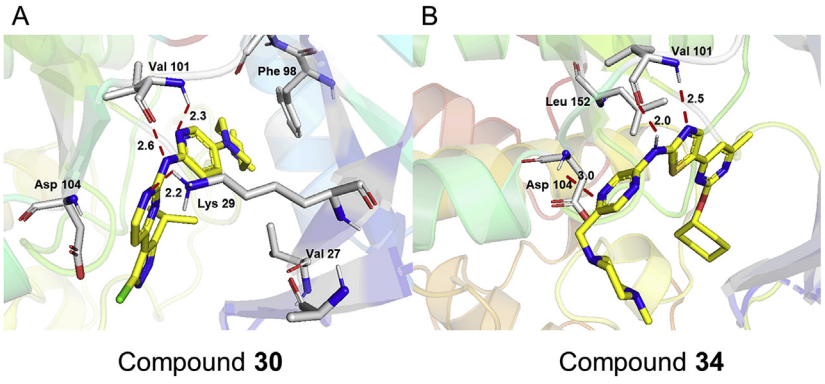
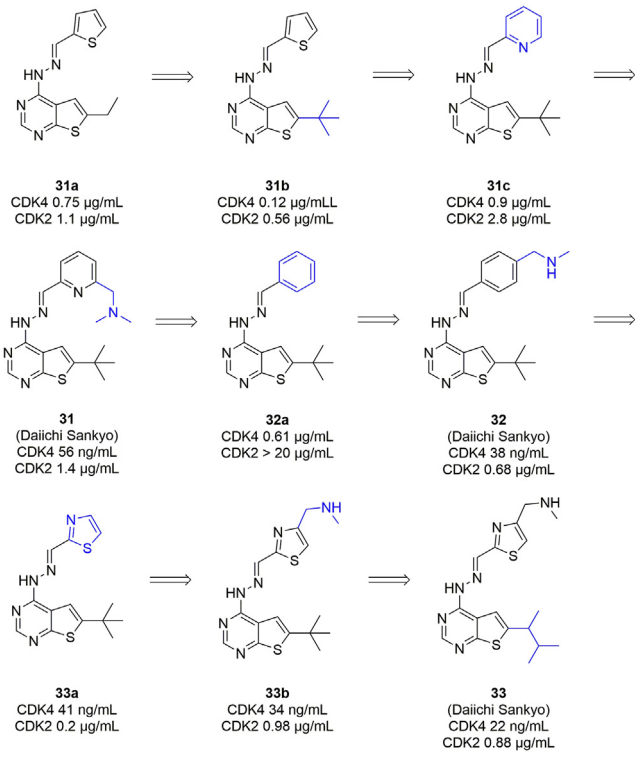


Figure 11 Docking modes of CDK6 (PDB code 4TTH) with **30** and CDK6 (PDB code 4AUA) with **34** were generated using the *Libdock protocol* in Discovery Studio 2019 from Biovia® (formerly Accelrys®) Software Inc. The hydrogen bonds are shown as red dashed lines, and the number is the distance (Å). Figures were prepared using PyMOL.

replacement of a pyridinyl group (**40b**, Scheme 11) increased the inhibition against CDK4 (IC_{50} = 44 μmol/L). The next modifications focused on the 7-hydroxynaphthyl group and rapidly synthesized more than 400 urea compounds through amines coupling with pyridine-2-carbonyl azide[151]. The aromatic substituted **40c** (Scheme 11), bearing a hydrogen-bonding acceptor, prominently improved inhibition against CDK4 (IC_{50} = 0.1 μmol/L). The docking model of **40c** with CDK4 suggested the existence of steric repulsion between the terminal benzene ring and the pyridine ring; therefore, the further modifications changed the terminal benzene ring to five-membered rings. As a result, **40** (Scheme 11) was discovered, with a potent inhibition against CDK4 (IC_{50} = 42 nmol/L)[151]. However, **40** also demonstrated inhibition against CDK1 and CDK2 (IC_{50} = 120 and 78 nmol/L, respectively)[151,152]. Therefore, **40** needs further modifications to improve selectivity for CDK4 over CDK1 and CDK2.

Specific amino acid residues around the ATP binding pocket of CDK4 were identified, and the subsequent enhancement of interactions with these specific residues helped to improve the selectivity for CDK4. Moreover, the docking model of **40** with CDK4 suggested that the pyridine ring of **40** was directed toward these residues[152]. Based on the docking model, replacement of the pyridine ring with a pyrazole ring (**41a**, Scheme 11) was predicted to be better for interacting with specific amino acid residues, including Asp99, Thr102, and Gln98. Based on the *de novo* design strategy, aminomethyl substituents and cyclized amino substituents on the pyrazole ring were predicted to further improve the interactions with specific amino acid residues[152]. Although **41b** (Scheme 11) showed reduced inhibition against CDK4, the selectivity of **41b** for CDK4 over CDK2 significantly improved when compared to **40**. A bulky cyclopentyl substituent (**41c**, Scheme 11), bearing a hydrophobic interaction with CDK4, increased both the inhibition against CDK4 and selectivity for CDK4 over CDK2. To further gain hydrophobic interaction, a 5-chloroindan-2-ylaminomethyl substation was introduced, leading to the identification of **41** (Scheme 11), which exhibited potent inhibitory against CDK4 (IC_{50} = 2.3 nmol/L), displayed great selectivity over CDK1 (780-fold) and CDK2 (190-fold), and caused G1 arrest of the cell cycle[152].

These 12 representative scaffolds for selective CDK4/6 inhibitors are summarized in Fig. 12. Besides these synthetic small molecule CDK4/6 inhibitors, some of natural components, such as Asparanin A[153], Icariside II[154,155], Licochalcone B[156], Juglone[157],



Scheme 8 Discovery of the selective CDK4 inhibitor **33**.

etc., also demonstrate the anticancer activity *via* decreasing the expression of CDK4/6.

4. Drug resistance and drug combination of CDK4/6 inhibitors

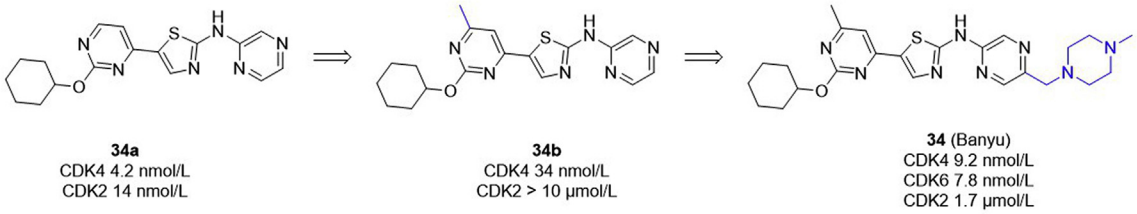
Selectively targeting one signaling pathway cannot prevent the proliferation of cancer cells due to the compensatory modulation of other related biochemical signaling pathways. Currently, cases of resistance to approved CDK4/6 inhibitors have emerged, the number of which has gradually increased[158–160]. Given the role of CDK4/6 inhibitors in cancer treatment, it is necessary to identify the origins of drug resistance and develop strategies to delay or overcome this resistance.

Different aspects of the origins of resistance to the CDK4/6 inhibitors have been identified, including the mutation of RB, the overexpression of cyclin E1, and the amplification of CDK6[160,161]. Breast cancer cells can adapt to **1** just 72 h after administration. The nonclassical activation of the cyclin D1−CDK2 complex plays an important role in the generation of the early adaptation response, and this adaptation can be prevented using a combination of **1** and the PI3K inhibitor GDC-0941. The acquired resistance to **1** occurs as a result of the overexpression of cyclin E1 or the loss of RB, and the combination of CDK4/6 and PI3K inhibitors cannot make the resistant cells sensitive to **1**. However, the combination of CDK2 silencing and **1** treatment can resensitize cells and increase cell cycle arrest[162].

Breast cancer cells with resistance to **2** have increased levels of 3-phosphoinositide-dependent protein kinase 1 (PDK1), an important kinase in the PI3K/AKT signaling pathway, and the cell cycle progression of the resistant cells is advanced by the cyclin E−CDK2 and A−CDK2 complexes. The combination of a PDK1 inhibitor or CDK2 inhibitor with **2** restores sensitivity to **2**[163].

MCF-7 cells can generate resistance to **3** through exposure to **3** over 21 weeks. The resistant cells increase the expression of CDK6. Reducing the level of CDK6 can resensitize the resistant cells to **3**, while the overexpression of CDK6 causes cells to develop resistance to **3**. Furthermore, the amplification of CDK6 also results in downregulation of the levels of ER and PR such that the responsiveness to ER antagonists is reduced. Therefore, the effects of endocrine therapy in patients may be reduced after resistance to the CDK4/6 inhibitors is established[164]. In addition to long-term exposure to CDK4/6 inhibitors, the loss of FAT1 also leads to the amplification of CDK6 and resistance to CDK4/6 inhibitors. FAT1 loss results in the suppression of the Hippo pathway and activation of the transcription factors YAP and TAZ, which accumulate at the CDK6 promoter and induce the overexpression of CDK6[165].

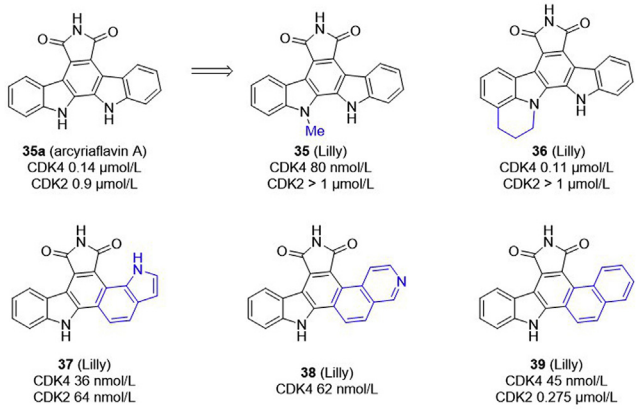
The models of the origins of resistance to the CDK4/6 inhibitors give a direction in determining how to delay or overcome drug resistance. CDK6 is more frequently associated with resistance to CDK4/6 inhibitors than CDK4. The overexpression of CDK4 cannot induce resistance, whereas a decrease in the level of CDK4 is often observed in resistant cells. Because amplification of CDK6 generates resistance to CDK4/6 inhibitors[164,165], potent and selective inhibition of CDK6 likely overcomes drug resistance. The activation of CDK2 plays a major role in cell cycle progression when resistance to CDK4/6 inhibitors occurs[161–163]. The dual inhibition of CDK2 and CDK4 by the BrkSH3 peptide, ALT, displays potent and prolonged efficacy in the arrest of the cell cycle[166]. Therefore, the CDK inhibitor targeting CDK2,



Scheme 9 Discovery of the selective CDK4/6 inhibitor **34**.

CDK4 and CDK6 may demonstrate potent treatment effects and overcome the resistance to the CDK4/6 inhibitor. Currently, PF-06873600, a CDK2, CDK4 and CDK6 inhibitor developed by Pfizer[167], has entered phase I/II clinical trials (NCT03519178). The combination of PF-06873600 and fulvestrant can strongly inhibit tumor growth and prolong PFS by more than 20 days in a mouse model with resistance to **1**. The combination of CDK4/6 and PI3K/AKT pathway inhibition prevents early adaptation or resistance to CDK4/6 inhibitors[162,163] and can be used in patients to guard against resistance to CDK4/6 inhibitors and achieve more potent effects[168].

Drug combination can not only overcome drug resistance, but also increase the clinical indications of the CDK4/6 inhibitor. Goel et al.[169] reported that CDK4/6 inhibition augmented antitumor immunity through enhancing tumor antigen presentation and promoting clearance of tumor cells mediated by T cells. Deng et al.[128] reported that CDK4/6 inhibition increased tumor infiltration and enhanced T cells activation to improve antitumor immunity. The findings provided biological basis for the combination of CDK4/6 inhibitors and immune checkpoint inhibitors and the clinal trials of this combination therapy (NCT03294694 and NCT03997448) are ongoing. In addition, the triplet combination of CDK4/6, BRAF, and MEK inhibitors exhibited synergistic anticancer efficacy in treating BRAF mutant melanoma[170]. The combination of CDK4/6 inhibitors with taxanes generated an excellent synergy in squamous cell lung cancer[171]. The combination of CDK4/6 and dual mTOR inhibitors demonstrated superior efficacy in ER^- breast cancer[172]. Currently, a large number of clinical trials involving the use of CDK4/6 inhibitors in combination therapy are in progress (Table 2) to identify their efficacy toward other cancers, including MCL, MM, squamous cell carcinoma of the head and neck, NSCLC, and prostate cancer. A phase I/II trial of the combination of **1**, bortezomib and dexamethasone (NCT00555906) in MM patients demonstrated that 5 (20%) patients achieved objective responses and 11 (44%) patients achieved stable disease[173].



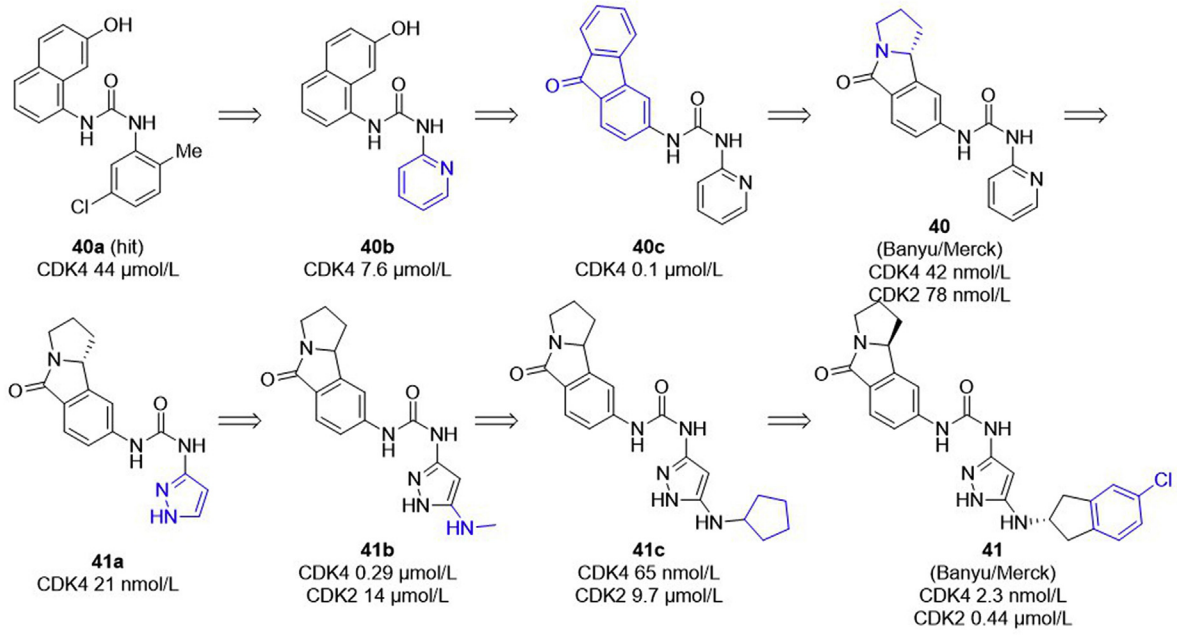
Scheme 10 Representative pyrrolo[3,4-*c*]carbazole derivatives.

5. Proteolysis targeting chimera (PROTAC)

CDK4/6 are very valuable targets because their inhibition has been well validated and shown to have effective therapeutic potential for cancer treatment. However, because drug resistance reduces the efficacy of CDK4/6 inhibitors for the treatment of cancer, inducing the degradation of CDK4/6 *via* PROTACs has become a promising choice in the anticancer battle[174,175]. As a type of protein degrader, PROTACs can induce the ubiquitin-proteasome system of cells to find, degrade and destroy disease-related proteins[176]. A PROTAC molecule consists of two functional molecular fragments and a linker between them[177]. As shown in Fig. 13, one moiety of the PROTAC molecule interacts with the target protein CDK6 that needs to be degraded, while the other moiety docks with an E3 ubiquitin ligase[178]. With the help of a PROTAC, CDK6 and E3 ubiquitin ligase forms a ternary complex with the PROTAC. Subsequently, the complex initiates ubiquitination of CDK6, and then CDK6 is degraded by the proteasome[179].

The cocrystal structures of **1**, **2** and **3** with CDK6 (Fig. 5) demonstrated that the aminopyrimidine moiety of **1**, **2** and **3** formed three hydrogen bonds with CDK6 and was crucial for the affinity to CDK4/6. Therefore, Jiang et al.[180] retained the aminopyrimidine moiety and attached linkers at the piperazine moiety to generate CDK4/6 degrader **42** and **43** (Fig. 14) in 2019. **42** and **43** consist of three parts: the blue moiety is the CDK4/6 inhibitor **1**, which interacts with the target protein CDK6; the purple moiety is the proteasome inhibitor pomalidomide, which specifically docks with an E3 ubiquitin ligase; and the green moiety is the linker connecting the two fragments to form the PROTAC. **42** and **43** could induce the degradation of both CDK4 and CDK6 and cause a G1 arrest of cell cycle in Granta-519 cells. Moreover, **42** also induced the degradation of IKZF1 and IKZF3, which were reported targets of imides and some imide-based degraders, while **43** had no effects on KZF1 and IKZF3. **42** demonstrated more potent antiproliferative activity in a panel of MCL cell lines when compared to both **1** and **43**, which indicated that the dual inhibition of CDK4/6 and KZF1/3 hold the promise for the treatment of MCL[180].

In the meantime, Zhao et al.[181] reported **44** (Fig. 14), which was capable of inducing the degradation of CDK4/6. In MDA-MB-231 cells, **44** induces the degradation of CDK4 and CDK6 with DC_{50} (the concentration for 50% protein degradation) values



Scheme 11 Discovery of the selective CDK4 inhibitor **41**.

of 13 and 34 nmol/L, respectively, and decreases the level of the RB phosphorylation in a dose-dependent manner[181].

Soon after, Brand et al.[182] reported **45** (Fig. 14), which is a PROTAC consisting of **1** and pomalidomide. However, unlike **44**, which shows greater effects on the degradation of CDK4 than CDK6, **45** selectively degrades CDK6 with no effects on CDK4. Therefore, **45** can distinguish CDK4 from CDK6 by forming different ternary complexes to selectively degrade CDK6[182]. In CDK4-dependent cancer cell lines, **45** showed no antiproliferative effect. In contrast, in CDK6-dependent AML cell lines, **45** suppressed the phosphorylation of RB and caused cell cycle arrest in the G1 phase to inhibit cell proliferation[182].

Rana et al.[183] reported **46** (Fig. 14), which also selectively induced the degradation of CDK6 while showing no effect on CDK4 in HPNE and MiaPaCa2 cell lines. Su et al.[184] reported **47** (Fig. 14), a CDK6 degrader (DC_{50} = 2.1 nmol/L) with good selectivity over CDK4 (DC_{50} > 100 nmol/L) in U251 cells. **47** also had no effect on CDK1/2/5/9, MEK1, and EGFR, which significantly reduced its off-target effects[184]. **47** exhibits stronger cell inhibition than **1** in MM.1S MM cell and Mino MCL cells[184].

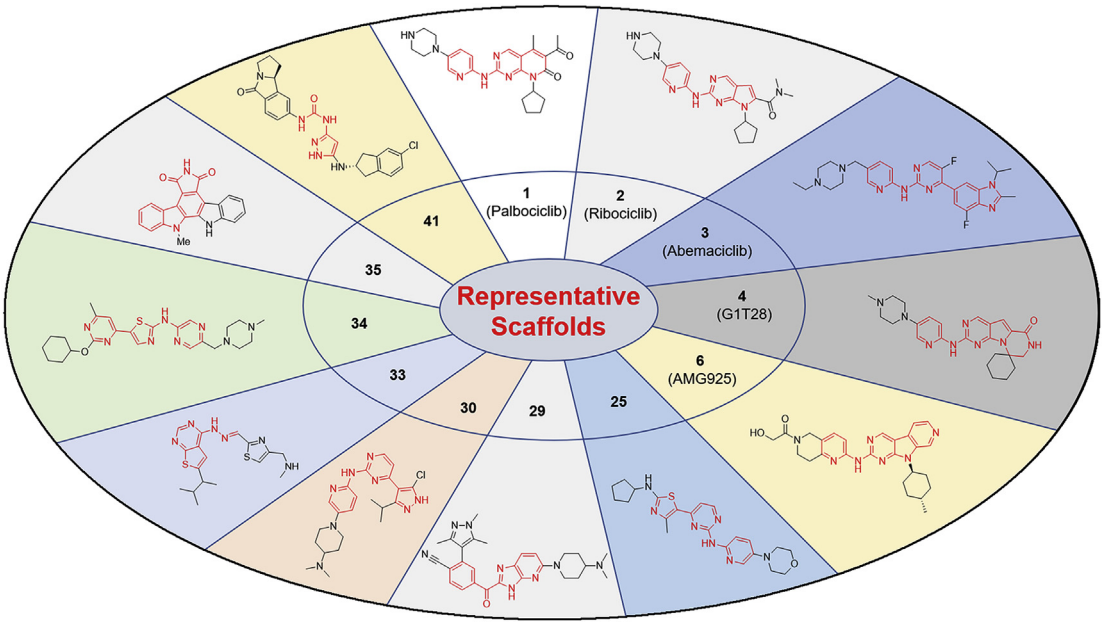


Figure 12 Representative chemical scaffolds of selective CDK4/6 inhibitors.

Table 2 Representative clinical trials using CDK4/6 inhibitors in combination therapy.

NCT number	Drug	Condition/disease
NCT03478514	Palbociclib; ibrutinib	Mantle cell lymphoma
NCT03446157	Palbociclib; cetuximab	Metastatic colorectal cancer
NCT00555906	Palbociclib; bortezomib; dexamethasone	Multiple myeloma
NCT04129151	Palbociclib; ganitumab	Ewing sarcoma
NCT03170206	Palbociclib; binimetinib	Advanced KRAS mutant non-small cell lung cancer
NCT03844997	Palcociclib; vyxeos	Acute myeloid leukemia
NCT03386929	Palbociclib; avelumab; axitinib	Non-small cell lung cancer
NCT03194373	Palbociclib; carboplatin	Squamous cell carcinoma of the head and neck
NCT03056833	Ribociclib; paclitaxel; carboplatin	Recurrent platinum sensitive ovarian cancer
NCT03070301	Ribociclib; everolimus	Neuroendocrine tumors
NCT02292550	Ribociclib; ceritinib	ALK-positive non-small cell lung cancer
NCT02343172	Ribociclib; siremadlin	Liposarcoma
NCT02985125	Ribociclib; everolimus	Metastatic pancreatic adenocarcinoma
NCT03008408	Ribociclib; everolimus; letrozole	Advanced or recurrent endometrial carcinoma
NCT03090165	Ribociclib; bicalutamide	Triple-negative breast cancer
NCT02429089	Ribociclib; cetuximab	Squamous cell carcinoma of the head and neck
NCT03740334	Ribociclib; everolimus; dexamethasone	Acute lymphoblastic leukemia
NCT03294694	Ribociclib; PDR001; fulvestrant	Breast cancer; ovarian cancer
NCT03114527	Ribociclib; everolimus	Soft tissue sarcoma
NCT01781572	Ribociclib; binimetinib	NRAS mutant melanoma
NCT02555189	Ribociclib; enzalutamide	Prostate cancer
NCT03673124	Ribociclib; letrozole	Ovarian cancer
NCT03834740	Ribociclib; everolimus	Glioblastoma multiforme; glioma of brain
NCT02370706	Ribociclib; ruxolitinib; PIM447	Myelofibrosis
NCT02703571	Ribociclib; trametinib	Solid tumors; pancreatic cancer; colorectal cancer
NCT01543698	Ribociclib; binimetinib; encorafenib	Solid tumors harboring a BRAF V600 mutation
NCT03905889	Abemaciclib; sunitinib	Renal cell carcinoma metastatic
NCT04074785	Abemaciclib; bevacizumab	Recurrent glioblastoma
NCT03781960	Abemaciclib; nivolumab	Hepatocellular carcinoma
NCT02411591	Abemaciclib; necitumumab	Non-small cell lung cancer
NCT03997448	Abemaciclib; pembrolizumab	Gastroesophageal cancer; adenocarcinoma
NCT02152631	Abemaciclib; erlotinib	Non-small cell lung cancer
NCT03994796	abemaciclib; GDC-0084; entrectinib	Brain metastases

The hyperactivated cyclin D−CDK4/6 complex accelerates the G1/S transition of cell cycle, which ultimately leads to uncontrolled cell proliferation and cancer. Therefore, inhibition of CDK4/6 can cause G1 arrest of cell cycle and is a promising and effective strategy for cancer treatment.

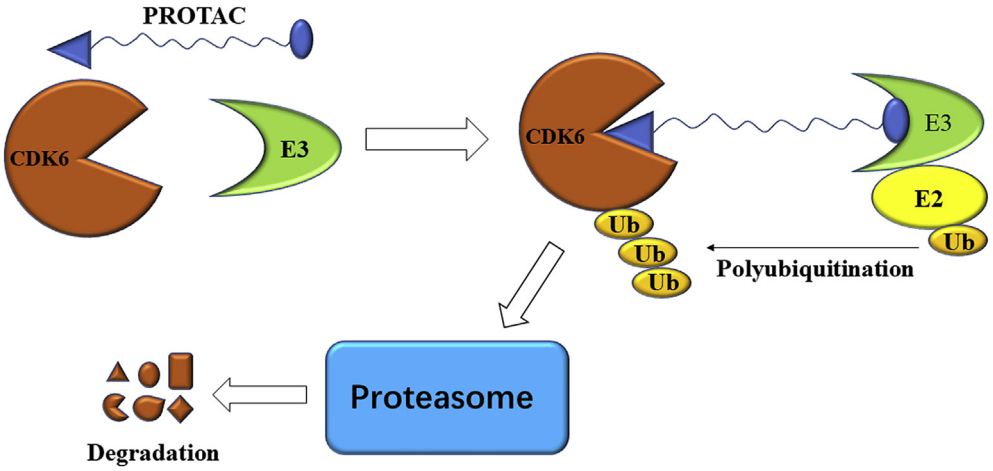


Figure 13 Degradation of CDK6 *via* PROTACs. A PROTAC forces the proximity of CDK6 and E3 ubiquitin ligase and then they form a ternary complex. Subsequently, CDK6 is polyubiquitinated by the ternary complex and degraded by the proteasome.

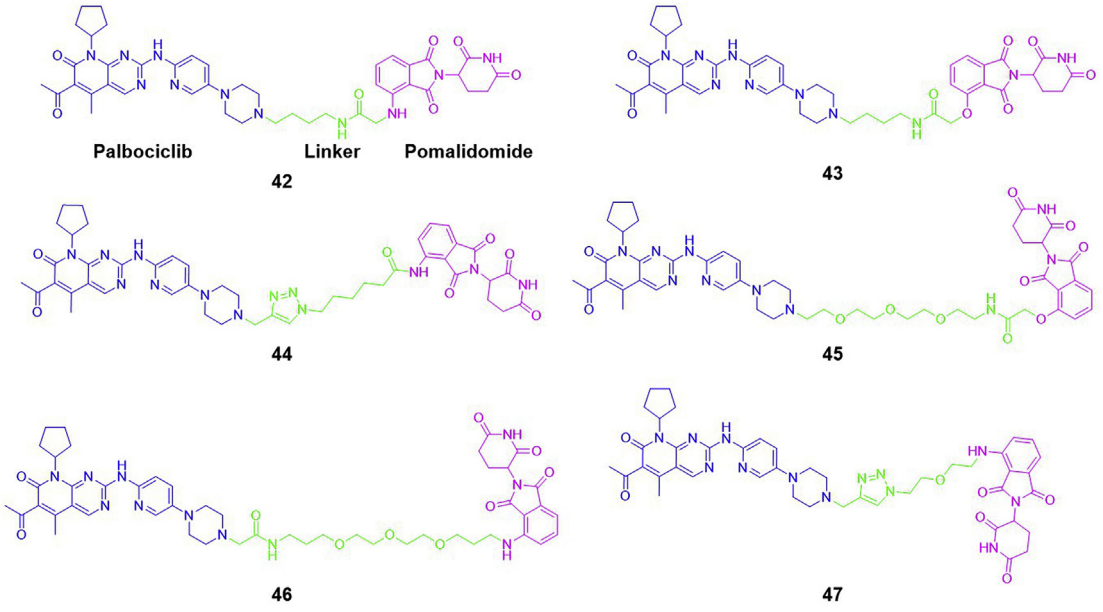


Figure 14 Representative CDK4/6 degraders.

47 can induce the degradation of CDK6 in palbociclib-resistant cancer cells to inhibit cell proliferation and degrade mutated forms of CDK6. Thus, PROTAC technology provides a promising means for addressing drug resistance.

6. Summary and perspectives

Due to their crucial roles in regulating the cell cycle, CDKs are promising targets for the development of anticancer drugs. Scientists are putting more and more effort into CDK-related research. Although the first- and second-generation CDK inhibitors with no selectivity for CDKs demonstrated promising efficacy in preclinical trials, no pan-CDK inhibitors were developed into CDK-targeted drugs due to disappointing efficacy or significant toxicity in clinical trials[1,185]. While, the third-generation CDK inhibitors with high selectivity on CDK4/6, **1**, **2**, and **3**, have been approved by the FDA for the treatment of breast cancer. For pan-CDK inhibitors, it is not clear which CDKs are actually being inhibited *in vivo*[1], making it difficult to thoroughly investigate the mechanism of action. The lack of clear target information further makes it hard to precisely select special patient cohorts, and the unsatisfied therapeutic effect is not beyond our expectations[1]. Besides low therapeutic effects, the toxicity of pan-CDK inhibitors is unlikely to be tolerable because they also target essential proteins of normal cells. Therefore, low selectivity of pan-CDK inhibitors results in the lack of clear mechanism of action, difficulty of appropriate patient selection and narrow range of therapeutic window, which are three potential principles leading to the failure of pan-CDK inhibitors[1]. In contrast, the pure inhibition of CDK4/6 causes G1 arrest of the cell cycle and suppresses cell proliferation, which is a specific effect in tumors. Compared with tumors with deregulation of other CDKs, it is more actionable to selectively inhibit CDK4/6 of tumors with deregulation of CDK4/6[1]. Due to the compensatory effects of other CDKs, the absence of CDK4/6 does not injure the development of normal tissues, which significantly decreases the toxicity of selective CDK4/6 inhibitors. Therefore, highly selective CDK4/6 inhibitors display potent efficacy and acceptable toxicity in the cancer treatment[186].

Although high selectivity of CDK4/6 inhibitors can greatly lower the toxicity, the compensatory modulation of other signaling pathways results in unsatisfied efficacy and drug resistance as time goes on. To delay or overcome the gradual increase of drug resistance to approved CDK4/6 inhibitors, drug combination has attracted more and more attention and become an effective strategy. Combination of inhibitors targeting upstream and downstream pathways have synergistic effects to overcome drug resistance. For example, the combination of CDK4/6 inhibitors with CDK2 inhibitors or PI3K/AKT pathway inhibitors can help to overcome the resistance. Besides great contribution in overcoming drug resistance, drug combination can also increase the clinical indications of the CDK4/6 inhibitor. We hope the numerous clinical trials of CDK4/6 inhibitors in combination therapy can afford us new drugs toward multiple cancers.

Besides drug combination, the revolutionary PROTAC technology is currently being used to induce the degradation of CDK4/6, which provides a novel strategy to overcome drug resistance. PROTACs can selectively induce the degradation of CDK6 with no effects on CDK4, demonstrating more selectivity than the approved CDK4/6 inhibitors. Due to the high selectivity of PROTACs, they are likely to exhibit lower off-target toxicity when compared to CDK4/6 inhibitors. Importantly, resistance toward kinase inhibitors cannot be avoided, and cases of resistance to CDK4/6 inhibitors have appeared. PROTACs may also exhibit an advantage in avoiding drug resistance that often occurs to traditional kinase inhibitors due to the mutations of the target kinases. The controversy of PROTACs is primarily focused on whether it is possible that they can actually be used as drugs for patients since PROTACs have difficulty passing through cell

membranes. Excitingly, new PROTAC technology can greatly improve the permeability, which is gradually moving PROTAC from basic research to clinical application. The androgen receptor protein degrader ARV-110 and the ER protein degrader ARV-471, discovered through PROTAC technology, came into clinical trials in 2019. Currently, PROTAC technology is entering an important development period, and more and more candidate drugs will come to clinical trials in the next three years. CDK4/6 protein degraders are likely to possess excellent efficacy, low side effects, and no resistance for cancer treatment and deserve increased attention in the future.

In addition to CDK4/6 inhibitors, the multi-target CDK inhibitor targeting CDK2, 4, and 6, and several CDK 7, 8, and 9 inhibitors that modulate transcription have also entered into clinical trials for cancer treatment[187], and the crucial roles of CDK inhibitors for cancer therapy will attract increasing attention from researchers in the future. With great efforts being made in CDK-related research, we expect that highly potent and selective CDK inhibitors will be discovered and ultimately translated into new anticancer drugs with great efficacy and low toxicity.

Acknowledgments

This study was supported by the Project Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (SKLNMZZRC07, China), 111 Project (B16046, China), "Double First-Class" University Project (CPU2018GF04, China), Jiangsu Key Laboratory of Drug Design and Optimization (DDORC201801, China), and 65th China Postdoctoral Science Foundation (2019M652030).

Author contributions

Kai Yuan, Xiao Wang and Haojie Dong wrote the manuscript. Kai Yuan, Xiao Wang and Wenjian Min drew the figures in the article. Haiping Hao and Peng Yang revised the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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