

Review

Cyclin-Dependent Kinase Inhibitors as Marketed Anticancer Drugs: Where Are We Now? A Short Survey

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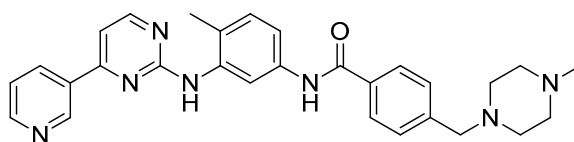
Abstract: In the early 2000s, the anticancer drug imatinib (Glivec[®]) appeared on the market, exhibiting a new mode of action by selective kinase inhibition. Consequently, kinases became a validated therapeutic target, paving the way for further developments. Although these kinases have been thoroughly studied, none of the compounds commercialized since then target cyclin-dependent kinases (CDKs). Following a recent and detailed review on the subject by Galons *et al.*, we concentrate our attention on an updated list of compounds under clinical evaluation (phase I/II/III) and discuss their mode of action as ATP-competitive inhibitors. CDK inhibition profiles and clinical development stages are reported for the 14 compounds under clinical evaluation. Also, tentative progress for forthcoming potential ATP non-competitive inhibitors and allosteric inhibitors are briefly described, along with their limitations.

Keywords: CDK; kinase; inhibitor; anticancer; heterocycle; clinical evaluation; ATP-competitive; allosteric site; ATP non-competitive

1. Introduction

At the beginning of the 21st century, a breakthrough arose with the development of a tyrosine kinase inhibitor, imatinib (generic name stem-*ib* standing for inhibitor, Figure 1), as a new type of cancer drug. As a result, protein kinases became clearly validated drug targets for cancer therapy [1,2].

Figure 1. Imatinib (Glivec[®]), the first kinase inhibitor.



Protein kinases, through phosphorylation reactions, are implicated in the regulation of various cellular activities since they belong to the cellular signaling molecules and therefore regulate all the processes that are essential for growth, development and homeostasis of eukaryotic cells [1,3].

Although the key role of phosphorous in cellular metabolism has been known for more than a century, it was only in the 1950 that Burnett and Kennedy discovered the phenomenon of protein phosphorylation [1]. Since the 1980s the role of protein kinases in oncogenesis and tumor growth was clearly demonstrated, and the profound understanding of the regulation mechanism led to the Nobel Prize award to Krebs and Fischer in 1992 [1].

The human protein kinases set (kinome), is constituted of 518 identified proteins, divided in seven families [4]. Cyclin-Dependent Kinases (CDKs) are part of the CMGC family named after the members: Cyclin-dependent kinases (CDKs), Mitogen-activated protein kinases (MAPKs), Glycogen synthase kinases (GSKs) and CDK-like kinases (CLKs). The CDK sub-family comprises thirteen members (CDK1 to CDK13). For their discovery, Hartwell, Nurse and Hunt received the Nobel Prize in 2001 [5].

CDKs can be separated roughly in two groups, those that mediate cell progression (CDK1, CDK2, CDK3, CDK4, CDK6), and those that regulate transcription (CDK7-CDK9 and CDK11-CDK13). CDKs' regulation is due to the association with a protein, a cyclin (25 members are known). It is noteworthy that the expression of CDKs remains quite constant, while cyclin levels vary throughout cell cycle. Abnormal expression of cyclins has a direct impact on cell deregulation that may lead to tumor development. Moreover, CDK11 and CDK12 are involved in the regulation of RNA splicing, and CDK5 and CDK10 in neuronal functions [6–8].

Overall, the regulation mechanism for CDKs is mediated by the association with cyclins which leads to several conformational modifications, for instance the 90° shift of CDKs PSTAIRE α -helix (see also Section 4) [9]. The PSTAIRE helix is an α -helix in the amino-terminal lobe of CDKs (also known as the $\alpha 1$ helix), which is moved inward upon cyclin binding, resulting in reorientation of key active-site residues (the name of this helix comes from its amino-acid sequence, which is conserved among all major CDKs) [9,10]. Some other regulation mechanisms are the activation/inhibition through phosphorylation [10–12] and also the action of inhibitory proteins (Cyclin-dependent Kinase Inhibitors or CKIs) due to their association with CDKs (e.g., p15, p16, p18, p19) or CDK/cyclin complexes (e.g., p21, p27, p57) [13,14].

This review will focus at first on ATP-competitive CDK inhibitors studied in the clinic, which still remain the most successful approach. In order to keep on with recent results we have cited when appropriate data reported for clinical trials on the National Institute of Health website. Then, we report briefly on ATP non-competitive CDK inhibitors (small molecules or small peptides) and finally we shed light on allosteric CDK inhibitors which is a burgeoning field of investigation.

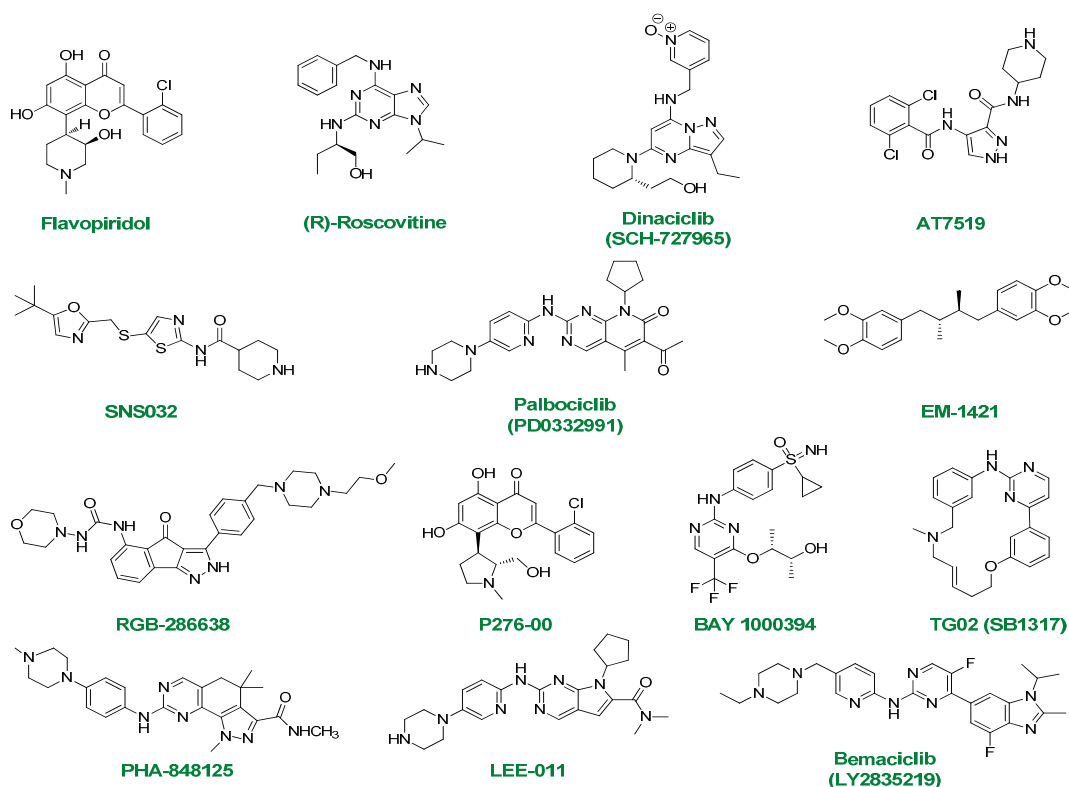
2. ATP-Competitive Inhibitors

The great majority of known CDK inhibitors are ATP-competitive, interacting with cyclin-dependent kinases within their catalytic ATP-site. Until now, this strategy has been the most successful one in order to develop powerful inhibition of CDKs implicated in the cell cycle [1,15,16].

Many compounds have been patented due to their high inhibition profiles against CDKs, and they have been thoroughly reviewed [17–21], particularly those nowadays under clinical development [15,22,23]. The structures of these molecules are quite diverse and they are generally constituted or derived from various heterocyclic families such as purines, pyrimidines, indoles, pyrazoles, thiazoles, or derived from natural products such as staurosporine or flavones [16].

We have chosen to present only the molecules which are currently under clinical evaluation. To our knowledge, they are 14 compounds (Figure 2) at present at this stage (in comparison to a list of 11 reported recently) [22]. For some compounds (R-547, ZK-304709, AZD-5438, AG-24322) the clinical trials have been discontinued [21,22] and some were not treated in this review (e.g., UCN-01, a 7-hydroxystaurosporine derivative), due to several drawbacks such as suboptimal human pharmacokinetics (low solubility, high doses to be administered) or the lack of selectivity towards CDKs [1,20–22].

Figure 2. Cyclin-dependent kinase inhibitors (14 compounds) under clinical evaluation.



Among the CDKs inhibitors presented in Figure 2, we find flavopiridol [24,25] and roscovitine [26–28] which were the first generation CDK inhibitors to enter clinical trials for use in anticancer therapy. Clearly, their extensive study paved the way for the development of the second generation compounds.

A brief description for each compound presented in Figure 2 will be given below. Further, readers will also find a table summarizing the data for these drug candidates (Table 1).

Table 1. CDKs inhibitor in clinics: An overview.

Drug Candidate	Company	Administration Mode	CDK Inhibition Profile: IC ₅₀	Clinical Trial Stage
Flavopiridol	Sanofi-Aventis	intravenous	CDK1: 30 nM, CDK2: 100 nM CDK4: 20 nM, CDK6: 60 nM CDK7: 10 nM, CDK9: 10 nM	II
Roscovitine	Cyclacel	oral	CDK1: 2.7 μM, CDK2: 0.1 μM CDK7: 0.5 μM, CDK9: 0.8 μM	II
Dinaciclib	Merck	intravenous	CDK1: 3 nM, CDK2: 1 nM CDK5: 1 nM, CDK9: 4 nM	III
SNS032	Sunesis	intravenous	CDK2: 38 nM, CDK7: 62 nM CDK9: 4 nM	I
AT7519	Astex/Novartis	intravenous	CDK1: 190 nM, CDK2: 44 nM CDK4: 67 nM, CDK5: 18 nM CDK9: <10 nM	I/II
PD0332991	Pfizer	oral	CDK4: 11 nM, CDK6: 16 nM	III
EM-1421	Erimos	intravenous	CDK1: N/A	I/II
RGB-286638	Agennix	intravenous	CDK1: 2 nM, CDK2: 3 nM CDK3: 5 nM, CDK4: 4 nM CDK9: 1 nM	I
P276-00	Nicholas Piramal	intravenous	CDK9: 20 nM, CDK1: 79 nM CDK2: 224 nM, CDK4: 63 nM	II
BAY-1000394	Bayer	oral	CDK1-4, 7, 9: 5-25 nM;	I
TG02/SG1317	S*BIO/Tragara	oral	CDK9: 3nM, CDK5: 4 nM, CDK2: 5 nM, CDK3: 8 nM, CDK1: 9nM	I
PHA-848125 AC	Nerviano	oral	CDK1: 2 nM, CDK2: 3 nM CDK4: 5 nM, CDK5: 4 nM	II
LEE-011	Novartis/Astex	oral	CDK4, 6: N/A	III
LY2835219	Eli Lilly	oral	CDK4, 6: N/A	I/(III)

2.1. Flavopiridol

Flavopiridol, also called alvocidib (generic name stem *-cidib* standing for cyclin-dependent kinase inhibitor), is a flavonoid derived from an Indian plant, rohitukine. This ATP-competitive derivative was jointly developed by Sanofi-Aventis and the US National Cancer Institute (NCI) [1]. Flavopiridol has the potential to inhibit several CDKs having a key role in the cell cycle at submicromolar concentrations: CDK1 (IC₅₀: 30 nM), CDK2 (IC₅₀: 100 nM), CDK4 (IC₅₀: 20 nM), CDK6 (IC₅₀: 60 nM) and CDK7 (IC₅₀: 10 nM). It also inhibits CDK9 (IC₅₀: 10 nM), which plays a role in the regulation of the transcription of mRNA via the phosphorylation of RNA polymerase II. This compound is capable

of stopping the cell cycle at two levels: either during phase G1, or during the switch between G2 phase to M phase [29].

Flavopiridol underwent phase II clinical trial for the treatment of acute myeloid leukemia along with chronic lymphoid leukemia. It was shown that flavopiridol also possesses a synergistic action with other antitumor agents such as docetaxel, irinotecan or cisplatin. The co-administration with another cytotoxic agent proved to be a useful tactic in order to reduce the amount of flavopiridol, thus limiting its side-effects [20,30].

However, Sanofi-Aventis seems to have abandoned the development of flavopiridol around 2010, or at least put it on stand-by [31], although a positive phase II trial for patients with acute myeloid leukemia just came out in 2014 with Tolero Pharmaceuticals, and was reported at the American Society of Clinical Oncology annual meeting [32]. Indeed, several studies have already demonstrated that induction therapy with flavopiridol (50 mg/m² days 1–3), followed by cytarabine (667 mg/m²/days 6–8) and mitoxantrone (40 mg/m² day 9) yields complete remission rates of nearly 70% in patients with newly diagnosed, poor-risk acute myeloid leukemia. This randomized phase II trial was performed in order to study how alvociclib, cytarabine, and mitoxantrone work compared to cytarabine and daunorubicin in treating patients with newly diagnosed acute myeloid leukemia. The results showed that mitoxantrone induction results in significantly higher complete remission rates compared with the other treatments [32].

2.2. (R)-Roscovitine

(R)-roscovitine, also called seliciclib (generic name stem *-ciclib* standing for cyclin-dependent kinase inhibitor as for *-cidib* above) or CYC202, was entered into clinical trials in 2001 by Cyclacel Pharmaceuticals, Inc. This derivative inhibits several CDKs with various IC₅₀ values: CDK1 (2.7 μM), CDK2 (0.1 μM), CDK7 (0.5 μM) and CDK9 (0.8 μM) [1,33], but the inhibition activity of this compound is poor towards CDK4 and CDK6 (IC₅₀ > 100 μM). (R)-roscovitine inhibits both the phosphorylation of retinoblastoma protein (pRb protein) and RNA polymerase II [1,34].

(R)-roscovitine is in phase II clinical trials and has been tested on lung cancer (non-small cell lung cancer, NSCLC). In other phase II studies seliciclib was administered in combination with gemcitabine and cisplatin as first-line treatment and with docetaxel as second-line treatment in NSCLC [35]. This compound is administered *per os* and demonstrated good bioavailability in phase I studies. Seliciclib is also being evaluated in a phase II study as a single agent in patients with nasopharyngeal cancer and displayed noticeable tumor shrinkage [35].

2.3. Dinaciclib

Dinaciclib, also called SCH-727965, is under study at Merck. It is a particularly potent inhibitor of CDK1 (IC₅₀: 3 nM), CDK2 and CDK5 (IC₅₀: 1 nM), and also of CDK9 (IC₅₀: 4 nM). This derivative is in fact far more efficient than flavopiridol, because even if there is a poor selectivity within the CDKs, it exhibits an excellent selectivity towards other kinases, thus showing a better toxicity profile.

Dinaciclib has entered phase III clinical development for the treatment of chronic lymphocytic leukemia in 2012 [36], therefore no data are available yet, but this study is being conducted to

demonstrate the superiority of dinaciclib compared to ofatumumab in chronic lymphocytic leukemia participants who are refractory to either fludarabine treatment or chemoimmunotherapy [36].

2.4. AT7519

AT7519 was advanced to the clinic by Astex Pharmaceuticals. It inhibits several CDKs, thus affecting cell cycle regulation, and is also a potent inhibitor of RNA polymerase II-dependent transcription. The IC_{50} values against CDKs are: CDK1 (IC_{50} : 190 nM), CDK2 (IC_{50} : 44 nM), CDK4 (IC_{50} : 67 nM), CDK5 (IC_{50} : 18 nM) and CDK9 (IC_{50} : < 10 nM).

Novartis has an option to develop and commercialize AT7519, which is in phase I of clinical development for treating refractory solid tumors and is given intravenously [37]. In this phase I pharmacokinetic and pharmacodynamic study of AT7519, among the twenty-eight patients treated, one developed hypotension, and one died, but they were able to determine the dose-limiting toxic effects [37]. Two separate phase II trials have also been underway since 2012 for mantle cell lymphoma and chronic lymphocytic leukemia [38], but no further data are available.

2.5. SNS032

SNS032, previously called BMS-387032, has been developed by Sunesis. This compound, which contains a thiazole unit, selectively inhibits CDK2 (IC_{50} : 38 nM), CDK7 (IC_{50} : 62 nM) and CDK9 (IC_{50} : 4 nM) [39]. Preclinical studies demonstrated that SNS032 was able to inhibit cell cycle activity along with transcription [20].

SNS032 is in phase I clinical trials for the treatment of chronic lymphoid leukemia along with multiple myeloma, and the mode of administration is intravenous [39]. The purpose is to evaluate the dose-escalation of SNS-032 along with its safety, pharmacokinetics, pharmacodynamic activity and clinical efficacy. Biomarker analyses demonstrated mechanism-based pharmacodynamic activity with inhibition of CDK7 and CDK9, although limited clinical activity in heavily pretreated patients was observed [39].

2.6. PD0332991

PD0332991, also called palbociclib, is the property of Pfizer. PD0332991 shows a strong inhibition selectivity against CDK4 (IC_{50} : 11 nM) and CDK6 (IC_{50} : 16 nM) [20]. This compound inhibits the phosphorylation of pRb protein which results in the cell cycle arrest at the G1 phase.

Several phase II clinical studies are ongoing or terminated on solid tumors. Indeed, Pfizer has already reported encouraging phase II data suggesting that its breast cancer candidate can extend progression-free survival by 18 months compared to standard therapy, therefore encouraging a planned phase III clinical trial for the treatment of breast cancer [31]. The blockbuster dreams seem very close [40].

2.7. EM-1421

EM-1421, often called terameprocol, is the property of the pharmaceutical company Erimos. It inhibits CDK1 [41]. EM-1421 seems to be still in phase I/II clinical trials for the treatment of patients with refractory solid tumors [22]. A phase I study is also conducted in order to determine the safety,

maximum tolerated dose, dose limiting toxicity, pharmacokinetics (clearance from the blood) of EM-1421 given to patients with leukemia, as intravenous infusion three times a week [42].

2.8. RGB-286638

RGB-286638, developed by Agennix, also inhibits several CDKs in the low nanomolar IC_{50} range: CDK1 (IC_{50} : 2 nM), CDK2 (IC_{50} : 3 nM), CDK3 (IC_{50} : 5 nM), CDK4 (IC_{50} : 4 nM) and CDK9 (IC_{50} : 1 nM).

RGB-286638 has completed phase I clinical development for the treatment of multiple myeloma and solid tumors [43], but a planned phase I clinical trial for relapsed or refractory hematological malignancies was withdrawn in 2012 prior to enrollment [44]. Moreover, Agennix announced on May 2013 its dissolution and that the liquidation should take place in 2014, therefore we have no clear vision for the future development of RGB-286638 [45].

2.9. P276-00

P276-00 is an analog of flavopiridol where the piperidine moiety has been changed for a pyrrolidine and resulted from an intense structure-activity and co-crystallization effort [24,46,47]. P276-00 inhibits efficiently CDK9 (IC_{50} : 20 nM), but also other CDKs such as CDK1 (IC_{50} : 79 nM), CDK2 (IC_{50} : 224 nM) and CDK4 (IC_{50} : 63 nM) [24,47].

The development is being performed by Nicholas Piramal Company and phase II clinical trials for various types of tumours have been announced (e.g., recurrent and/or locally advanced head and neck cancer, relapsed and/or refractory mantle cell lymphoma) [48]. Although several phase II trials ended in 2012/2013 no results could be found.

2.10. BAY-1000394

BAY-1000394, developed by Bayer, inhibits CDKs involved in the cell cycle (CDK1, CDK2, CDK3 and CDK4) along with the one implicated in the regulation of transcription (CDK7 and CDK9), with IC_{50} values ranging from 5 to 25 nM.

This compound is in phase I clinical development for various types of tumours. BAY-1000394 studies also shown that it could be efficient in combination with cisplatin [49]. BAY 1000394 clearly overcomes many limitations of other drugs since it displays high solubility in water, even at neutral pH, and low efficacious oral doses. Furthermore, BAY 1000394 has proven antitumor activity in xenograft models resistant to standard drugs such as doxorubicin, cisplatin, or paclitaxel, and has shown its potential for combination treatment with drugs on the market [49]. The full story about this nanomolar pan-CDK inhibitor BAY 1000394, which contains an unusual sulfoximide group, has been recently disclosed [50].

2.11. TG02

TG02/SG1317, is a pyrimidine-based derivative that inhibits CDKs together with JAK2 and FLT3 [51]. TG02 induces G1 cell cycle arrest and apoptosis in a broad range of tumor cell lines. TG02, originally designated SB1317, was discovered by S*Bio and licensed to Tragara

Pharmaceuticals in 2008. The *in vitro* kinase spectrum of TG02 is CDK9 (IC₅₀: 3 nM), CDK5 (IC₅₀: 4 nM), CDK2 (IC₅₀: 5 nM), CDK3 (IC₅₀: 8 nM) and CDK1 (IC₅₀: 9 nM) [52].

TG02 was selected for phase I clinical trials [53] and the results are expected in 2014 for the phase I study in patients with chronic lymphocytic leukemia and small lymphocytic lymphoma [54] and in 2015 for phase I study in patients with advanced hematological malignancies [55].

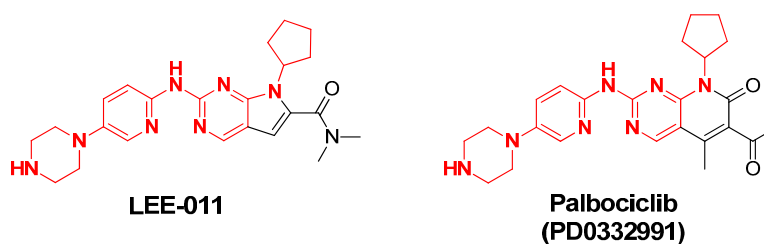
2.12. PHA-848125

PHA-848125 AC, also called milciclib, developed by Nerviano, is selective towards several CDKs: CDK1 (IC₅₀: 2 nM), CDK2 (IC₅₀: 3 nM), CDK4 (IC₅₀: 5 nM), CDK5 (IC₅₀: 4 nM), and is in phase II clinical trials for treating thymic carcinoma [56]. The results of phase II for milciclib in patients with thymic carcinoma have been reported at the 2014 American Society of Clinical Oncology (ASCO) annual meeting [57]. Indeed, 43 patients have been treated and the second stage of the trial is still ongoing. Out of 30 patients whose data are available, 14 are successes with a progression free survival rate at 3 months of 46.7%, the toxicity being generally moderate [57]. Recently, a patent was filed for the treatment of mesothelioma [58].

2.13. LEE-011

LEE-011 is one of the most selective inhibitors for CDK4 and CDK6 [59] and is being developed by Astex Pharmaceuticals™ and Novartis. In January 2014 this inhibitor entered phase III clinical trials for the treatment of breast cancer [60]. Due to encouraging results LEE-011 has now become the main competing drug-candidate with Pfizer's PD0332991 (palbociclib), see Figure 3 [59].

Figure 3. Comparison of Astex/Novartis' LEE-011 and Pfizer's PD0332991 structures.



Upon comparison of the chemical structure of Novartis' LEE-011 and Pfizer's PD0332991, the similarity is evident. The major difference lies in the bicyclic core since LEE-011 possesses a pyrrolo-pyrimidine and PD0332991 a pyridopyrimidine. The "east" part of the structure is also modified. The structural similarities make their analogous CDKs inhibition profiles (high selectivity for CDK4 and CDK6) quite obvious. Moreover, both derivatives are orally administered which is pretty advantageous compared with dinaciclib, which is also in phase III clinical trials but is administered intravenously.

2.14. LY2835219

LY2835219, also called abemaciclib, is also a selective CDK4/CDK6 inhibitor, blocking cells at G1 phase due to the inhibition of the phosphorylation of pRb protein. Abemaciclib has been developed by

Eli Lilly and is in phase I clinical trials for treating non-small-cell lung carcinoma. LY2835219 already demonstrated very good results for patients suffering from breast cancer and a phase I trial in combination with hormone therapies for breast cancer will finish in 2016 [61]. A phase III study of LY2835219 combined with fulvestrant for women with HER2-negative metastatic breast cancer is planned in 2014 [62].

2.15. An Overview

Table 1 represents an overview of the data detailed above. Indeed, for every drug candidate, the company involved is listed along with the administration mode, the CDKs targeted and finally the actual clinical trials status. In general, all drug candidates have planar structures constituted by heterocycles or hydrophobic aromatic rings. Usually, they are poorly selective between the different CDKs and even against other kinases. This low selectivity induces difficulties for drug dosing during the clinical studies, and the use of higher doses results in toxicity. On the contrary, if lower doses are used they become inefficient. Therefore, one way to avoid side effects is to combine these drug candidates with classical cytotoxic agents. This process proved to be very efficient in the case of some inhibitors such as flavopiridol or BAY-1000394. This poor selectivity is also the sign that there is a high degree of homology between all the CDKs, especially within the ATP-binding site.

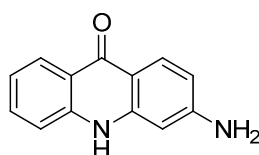
3. ATP Non-Competitive Inhibitors

Compounds belonging to this family are a new inhibitor generation which does not enter in competition with ATP, therefore exhibiting an action mode different from the other type of inhibitors. In fact, finding inhibitors following this strategy is a recent tactic, so very few molecules have been identified and to date none of them have entered clinical stage [30]. Two classes of ATP non-competitive CDKs inhibitors have been identified:

3.1. Small Molecules Inhibitors

These small molecules inhibitors have been essentially identified by their IC_{50} values on CDK/cyclin complexes in the presence of high ATP concentration. Therefore, these small molecules have the ability to inhibit the CDK/cyclin complex without interacting with the ATP-binding site. One typical example of such small molecule inhibitors is 3-aminothioacridinone (3-ATA), discovered by Kelley's group [63], see Figure 4.

Figure 4. 3-Aminothioacridinone (3-ATA).



Kelley's team demonstrated that 3-ATA was able to inhibit CDK4/cyclinD complex by interaction with a site different from the ATP-binding site. Furthermore, this represents an interesting therapeutic strategy for patients presenting a tumor p16 deficit. Indeed, when p16 protein is in deficit, the CDK

cannot be inactivated, therefore inducing a deregulation in the cell cycle leading to tumor genesis [64]. Moreover, 3-ATA exhibited very interesting *in vitro* inhibition towards CDK4/cyclinD (3.1 μM). Additionally, it was shown that this inhibitor did not affect CDK4/p16 interaction, suggesting that it is not a p16-mimic. Remarkably, *in vitro* tests revealed that 3-ATA had a greater inhibitory activity on tumor cells compared to normal cells. Among the compounds screened with 3-ATA, two derivatives (BTD and NSC 625987) were shown to inhibit *in vitro* cyclin D1 binding to CDK4 at nearly 1000-fold higher concentration than their IC_{50} on CDK4 kinase, indicating that for these compounds direct inhibition of cyclin D1 binding to CDK4 was not the central mechanism for CDK4 kinase activity inhibition [63]. This study is of great interest since a lead compound, 3-ATA, was identified, with specific CDK4/cyclinD complex inhibitory properties. It is also noteworthy that the deregulation in cancer of cyclins D and their ability to activate CDK4 and CDK6, also makes them an attractive therapeutic target [65].

3.2. Small Peptide Inhibitors

The second class of ATP non-competitive CDKs inhibitors includes small peptides designed based on their similarity with endogenous CDKs inhibitors, such as p21, p27 and p57 [30].

Seminal work of Kaelin'group [66] led the way for the conception of small peptides inhibitors. Indeed, they studied the interaction of the CDK2/cyclin complex with several cell cycle regulators and they were able to identify a common recognition motif for these CDKs regulators (including the endogenous CDKs inhibitors: p21, p27 and p57) [67]. This eight-residue peptide motif displays a highly conserved amino-acid sequence and the peptides containing this sequence are able to inhibit the activity of CDK2/cyclin complex. Since this seminal work several peptides having this motif have been developed in order to mimic the endogenous CDKs inhibitors [30,68–70].

In addition, to create a more drug-like and less peptidic compound, development of the REplacement with Partial Ligand Alternatives through Computational Enrichment (REPLACE) strategy was used in order to generate inhibitors of CDKs/cyclin interactions [71]. Thanks to this method, fragment optimization could be successfully obtained, in particular for compounds targeting CDK2 substrate recruitment site on the cyclin regulatory subunit. Indeed, inhibition of CDKs through the cyclin provides an approach to obtain selectivity against other protein kinases and to specifically block the activity of the G1- and S-phase CDKs, since they are the only ones containing a functional cyclin binding groove. The most potent compound identified so far exhibits an IC_{50} of 18.1 μM against CDK2/cyclinA [71].

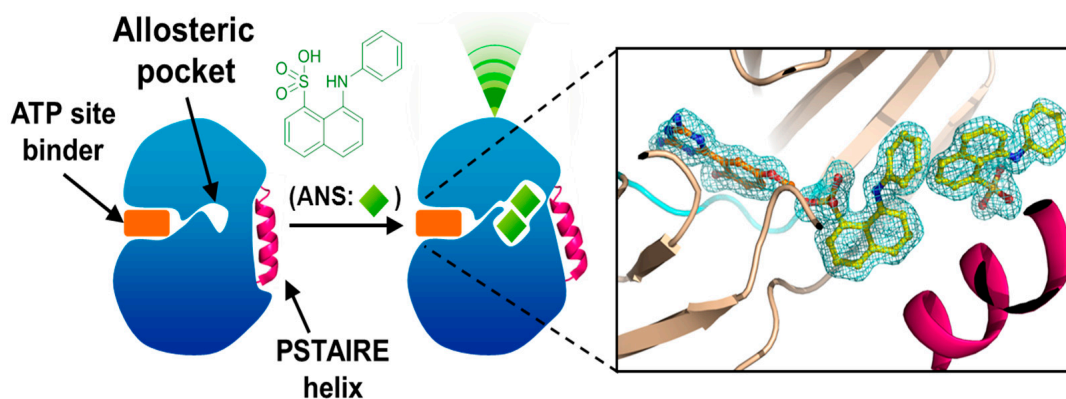
4. Allosteric Inhibitors

The inhibitors of this family have the specificity to link CDKs away from their ATP-binding site. The fixation on the allosteric sites will induce a conformational change to the CDKs, thus modifying their activity. These inhibitors have the advantage to be more selective since they exploit a specific environment for each CDK. This seems a promising strategy for CDKs since it has already been successful for other protein kinases such as Abl, p38 and MEK1 [72,73].

To our knowledge, no CDK inhibitors of this type have been created yet. However, it is noteworthy that the team of Schönbrunn has discovered a potential allosteric site for CDK2 protein [74]. For this

purpose of identifying an allosteric site which could therefore alter the interaction between the CDK and the cyclin, they have explored CDK2 protein with a fluorophore 1-AnilinoNaphthalene-8-Sulfonic acid (1,8-ANS). Gratifyingly, thanks to spectroscopy fluorescence analysis, an area within the CDK2 itself has been identified (Figure 5).

Figure 5. CDK2 allosteric site [74] (reproduction/modification by courtesy of E. Schönbrunn).



Furthermore, using co-crystallization data, Schönbrunn's group observed that two ANS molecules were able to interact with CDK2 in a different site from the ATP-binding site. More importantly, once the ANS molecules (in green, Figure 5) are linked to CDK2, a change of conformation of the PSTAIRE helix (in magenta, Figure 5) occurred, thus rendering impossible the association with the cyclin.

5. Conclusions

ATP non-competitive inhibitors and allosteric inhibitors strategies are of great interest since they should allow access to more selective inhibitors. However, the main drawback is still to know which CDK or CDK/cyclin complex should be selectively targeted. There are still many details to understand about the implication of CDKs in the cell cycle and their precise implication in tumor growth. The lack of selectivity of CDKs inhibitors tested up to now is a disadvantage since we still cannot draw clear conclusions for the inhibition of a specific CDK. Moreover, Schönbrunn's group has shown recently that the acetyllysine binding site of bromodomain-containing protein 4 (BRD4) could interact with diverse kinase inhibitors (e.g., dinaciclib and flavopiridol) [75,76]. This observation suggests that this protein family could be an off-target of kinase inhibitors; therefore this dual inhibition could be profitable for the design of a new generation of inhibitors.

Nevertheless, designing ATP-competitive inhibitors is still the most studied approach. Drug candidates such as dinaciclib, PD0332991 and LEE-011 are leading the way since they are in phase III clinical trials. These three compounds are the most advanced in the study of CDK inhibition as a treatment for cancers, but there are still no drugs on the market for which the therapeutic target is proven to be CDKs. Therefore this field of research remains an intriguing and exciting one!

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Author Contributions

All authors have participated in the preparation of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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