Aurora-A kinase inhibitor scaffolds and binding modes

Aixia Yan[1], Liyu Wang[1], Shuyu Xu[1] and Jun Xu[2]

[1] State Key Laboratory of Chemical Resource Engineering, Department of Pharmaceutical Engineering, PO Box 53, Beijing University of Chemical Technology, 15 BeiSanHuan East Road, Beijing 100029, China
[2] School of Pharmaceutical Sciences, Sun Yat-Sen University, 132 East Circle at University City, Guangzhou 510006, China

Aurora kinases (A–C) belong to the serine/threonine protein kinase family. In recent years, the constitutive or elevated expression of Aurora kinases has been found in cancer cells and oncogene transfected cells. In this review, we summarize the common binding modes of Aurora-A kinase inhibitors, the hot spot residues in the binding sites and the privileged inhibitor structures. Our review of the reported chemical scaffolds of Aurora-A kinase inhibitors and their binding modes could provide a useful framework from which new design strategies for inhibitors might be assessed or developed.

Introduction

Every year, more than 10 million people worldwide are diagnosed with cancer, with more than 6.2 million deaths in 2000 alone as a result of the disease [1]. Thus, there is an urgent need to discover new drugs against refractory tumors to cure or alleviate the illness and suffering of patients with cancer.

Correct cell division is crucial for the health and survival of cells and organisms. The process of cell division or mitosis is complex and tightly regulated. It involves a full intracellular reorganization and the formation of a microtubule-based apparatus (the bipolar spindle), which segregates the two identical copies of the chromosomes between daughter cells [2]. Errors in this process result in the formation of cells with abnormal chromosome content, that is, more or less than two copies of each chromosome (aneuploidy). This can either lead to cell death or contribute actively to, or even drive, tumor development. These phenotypes are hallmarks of most human cancers [3]. Human cancer cells are generally characterized by changes in the amount or organization of DNA owing to errors in mitosis, resulting in chromosome instability and aneuploidy. Targeting components of the mitotic machinery to block tumor progression has thus been an area of intense research [2–4].

Corresponding authors:. Yan, A. (aixia_yan@yahoo.com, yanax@mail.buct.edu.cn), Xu, J. (xujun9@mail.sysu.edu.cn)

Progression through mitosis depends on three main regulatory mechanisms [(i) protein localization; (ii) proteolysis; and (iii) phosphorylation], each involving several serine/threonine (Ser/Thr) kinases, known as mitotic kinases [4]. Recent evidence indicates that mitotic kinases have a key role in regulating the cell cycle. Proper chromosome segregation is tightly controlled by mitotic kinases such as cyclin-dependent kinase 1 (Cdk1), Polo-like kinase (Plk1), NIMA-related kinase 2 (Nek2) and Aurora kinases [4–6].

In this review, we summarize the roles that each of the Aurora kinases has in mitosis and cancer biology, and discuss the binding modes of Aurora-A kinase inhibitors, the hot spot residues in the binding sites and the privileged inhibitor structures. Our review of the reported chemical scaffolds of Aurora-A kinase inhibitors and their binding modes could provide a useful framework from which new design strategies for inhibitors might be assessed or developed. In addition, we review the main preclinical and clinical studies concerning Aurora kinase inhibitors currently under investigation. The extensive preclinical information supports the development of targeted Aurora kinase inhibitors in specific tumor types.

Aurora kinases

The Aurora kinases are a family of three highly homologous Ser/Thr protein kinases, including Aurora-A, -B and -C. Since their discovery in 1995 and the first observation of their expression in human cancer tissue in 1998, these kinases have been the subject

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.12.003

of intense research in both the academic and industrial oncology communities [3]. The three homologous Aurora kinases have a conserved catalytic domain and an N-terminal domain that varies in sequence and length, but are distinct in function [7,8]. However, all three are overexpressed in many types of cancer, in which polyploid cells containing multiple centrosomes are observed [8].

Aurora-A

Aurora-A (alternatively known as Aurora-2) localizes to the centrosomes, where it is required for their maturation and separation, thereby promoting mitotic entry and spindle assembly. In mitosis, Aurora-A associates with the spindle poles and is involved in both centrosomal assembly and acentrosomal spindle assembly [9]. The gene encoding Aurora-A lies within a region of chromosome 20q13, which is amplified in many epithelial malignant tumors, including breast, gastric, colon, ovarian and pancreatic cancers. Furthermore, overexpression of an active mutant of Aurora-A in rat1 cells induced neoplastic transformation, indicating that Aurora-A is an oncogene [4]. Overexpression of Aurora-A contributes to genetic instability and tumorigenesis by disrupting the proper assembly of the mitotic checkpoint complex. Ectopic overexpression of Aurora-A can transform rodent cells (NIH3T3 and rat1) and the resulting cells can induce tumor formation in nude mice. Further support for its oncogenic role(s) was provided when *Xenopus* Aurora-A transformed NIH3T3 fibroblasts led to the development of tumors in mice [10]. In addition, this kinase is a key regulatory component of the p53 pathway and its overexpression leads to an increase in p53 degradation, which again facilitates oncogenic transformation [4,9,11].

In the absence of Aurora-A, centrosomes fail to assemble bipolar spindles, which can result in mitotic arrest. Research also indicates that a decrease in Aurora-A protein levels induced by RNA interference leads to G2–M arrest, many spindle defects, the appearance of tetraploid cells and apoptosis [8]. At the molecular level, the roles that Aurora-A has in many of the mitotic processes remain to be fully elucidated; however, there have been some crucial discoveries that help define the profile for Aurora-A inhibition and that help identify Aurora-A specific biomarkers [3]. Therefore, Aurora-A kinase represents an attractive target for anticancer drug discovery.

Aurora-B

Aurora-B (alternatively known as Aurora-1) is a component of the chromosome passenger complex and localizes to the centromeres in prometaphase, relocating to the spindle midzone at anaphase. It has functions associated with phosphorylation of histone H3 on Ser10 and chromatin condensation in prophase, chromosome alignment and segregation, and the regulation of a mitotic checkpoint at metaphase; it also has a role in cytokinesis [4,9,12]. The gene encoding Aurora-B is located at chromosome 17p13.1, which has not been associated with significant amplification in cancer cells. Despite reports of overexpression in certain cancers, the increased expression might reflect hyperproliferation rather than carcinogenesis [13]. The mechanism by which Aurora-B controls biorientation was recently identified to be its interaction with the microtubule-destabilizing mitotic centromere-associated kinesin (MCAK). As a result, inhibition of Aurora-B function results in abolishing a crucial spindle checkpoint and in premature exit from mitosis without undergoing chromosome segregation and cytokinesis. This series of events leads to polyploidization and potentially to continued re-entry into the cell cycle, resulting in multinucleated cells [4,6]. Although Aurora-B overexpression alone did not transform rodent fibroblast cells, it did potentiate H-Ras-induced transformation. In endometrial carcinomas, in particular, the expression of Aurora-B was significantly increased in high-grade tumors. Finally, Aurora-B overexpression correlates with the level of genomic instability within a tumor, suggesting that Aurora-B contributes to the acquisition of genetic alterations that are crucial for neoplastic transformation [7].

Aurora-C

Aurora-C (alternatively known as Aurora-3) has functions similar to those of Aurora-B. The gene encoding Aurora-C lies within a region of chromosome 19q13. It is highly expressed in the testis and is also present at a low level in other tissues [9]. Aurora-C was first thought to be involved in meiotic spindle formation and its localization was restricted to centrosomes from anaphase through to cytokinesis [4]. However, the centrosome localization of Aurora-C is not definitive, and other researchers have yet to observe this localization. Instead, Aurora-C has been reported by Li *et al.* to be a chromosome passenger protein strictly localizing in a manner similar to, or even with, Aurora-B. In their study, Aurora-C was found to co-immunoprecipitate with Aurora-B [14]. A recent study has shown that Aurora-C can rescue Aurora-B-depleted cells [15]. Nevertheless, Aurora-C expression has not yet been found in somatic cells, and its expression is restricted to testis.

Among the three human Aurora kinases, Aurora-A has been the family member most consistently associated with cancer. The X-ray crystal structures of Aurora-A kinase and its inhibitors are useful for anticancer drug design. Therefore, it is anticipated that Aurora-A inhibitors will have a significant antimitotic effect. The cell profile following Aurora-A inhibition is expected to include a delay in mitotic entry followed by defects in chromosome segregation as a result of aberrant spindle formation that can lead to aneuploidy.

The active site of Aurora-A kinase

Each member of the Aurora family consists of approximately 400 residues and has a conserved C-terminal catalytic domain and a short N-terminal domain that vary in size. Both Aurora-A and -B contain specific sequences (the A-box in the N-terminal region and the D-box in the C-terminal region) that mediate degradation at the end of the M-phase. By contrast, Aurora-C has a putative D-box, but lacks an A-box and is not targeted to proteolysis during the exit from M-phase [10,16–18]. The activation loop domain of the three members is conserved, with a consensus sequence DFGWSxxxxxxxRxTxCGTxDYLPPE. Numerous protein kinases are activated by phosphorylation in this sequence. In the consensus sequence, the conserved threonine (Thr288) residue is the target for an activating kinase [16,19].

The activity of Aurora-A depends on phosphorylation by other kinases. In *Xenopus* eggs, three sites for phosphorylation were identified, Ser53, Thr295, Ser349 (which are equivalent to Ser53, Thr288 and Ser342 in humans, respectively). Thr295 in the activation loop of the kinase is a key residue for phosphorylation. Ser349 has an important role for either the proper protein folding or

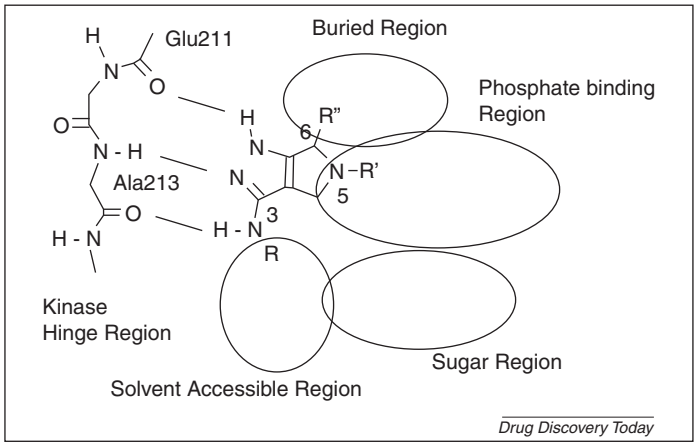


FIGURE 1

Schematic representation of the 1,4,5,6-tetrahydro-pyrrolo[3,4-c]pyrazole scaffold in the kinase ATP binding pocket. Reproduced, with permission, from [22].

regulation of Aurora-A [20]. In addition, the extensive rearrangements that the protein undergoes during activation highlight a high degree of flexibility. This appears to be particularly the case for the activation loop and the ATP-binding pocket [3].

It has been recognized that highly specific ATP-competitive inhibitors can be obtained against several different kinases with clinical uses as cancer therapeutic agents. Understanding the molecular constraints of the ATP-binding site of Aurora-A kinase and the structural basis for its interactions with ATP and ATP-competitive inhibitors is an essential step in designing inhibitors for this subfamily of kinases that are both selective and potent [21].

Fancelli *et al.* identified the ATP-binding pocket (Fig. 1) of the Aurora-A kinase [22]. The synergic nitrogen atoms in the NH_2-C-N-NH pattern of the 3-aminopyrazole moiety (which is stereochemically well suited to form hydrogen-bonding interactions with the kinase hinge region of the ATP pocket) are embedded within the 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole to give an original scaffold endowed with additional positions for increasing diversity [22]. The pocket can be divided into five areas: (i) the kinase hinge region; (ii) the solvent accessible region; (iii) the sugar region; (iv) the phosphate-binding region; and (v) the buried region. The buried region is small, because it is close to the main chain of the kinase and cannot accommodate a big group. Therefore, the R2 should also be a small group, such as -H, $-CH_3$ or $-OCH_3$. The phosphate-binding region is where the ATP tail is placed. The solvent accessible region is partly touched by the solvent. The hinge region (residues 210–216) has an important role in forming the catalytic active site. In the hinge region (Fig. 1), the scaffold has direct H-bonding network interactions with the main chain of the Aurora-A kinase, especially through the amino acids Glu211 and Ala213. In addition, we superimposed 25 crystal structures of Aurora-A kinase in complex with inhibitors, and then examined the frequency of the residues interacting with the inhibitors. The result indicates that the most significant residues are Glu211, Ala213, Lys162, Leu139 and Leu263 (with interacting number of times >30%), in that they contribute the most to direct binding interactions with the ligands.

The important interactions between the inhibitor scaffold and the Aurora-A kinase are located at the hinge region (Fig. 1). It is important to change the R1 group in the phosphate-binding region to design new inhibitors. As the phosphate-binding region of the Aurora-A kinase has enough space to accept a large group, its structural diversity is high. Compared with an R group in the solvent accessible region, the R1 group in the phosphate-binding region always has stronger interactions with Aurora-A kinase.

Figure 2 shows the superposition of the two crystal structures of Aurora-A kinases (PDB code: 2C6D [23] and 2C6E [23]) through the α-carbon of the backbones of the two kinases. The figure shows that the binding pocket of the Aurora-A kinase is not fixed and is slightly flexible. The binding pocket for inhibitors of Aurora-A kinase is formed by the following key interacting residues: Leu210, Glu211, Tyr212, Ala213, Leu139, Val147 and Leu263. Therefore, the ATP-binding pocket of Aurora-A kinase is hydrophobic, a feature that should be considered when designing Aurora-A kinase inhibitors.

Figure 3a details one of the crystal structures of Aurora-kinase in complex with ligand MPY (PDB code: 2BMC [22]), and shows the hydrophobic pocket. From the figure, one can see that the binding pocket of Aurora-A kinase can accommodate a large ligand. There is a deep hydrophobic fluorophenyl pocket adjacent to the ATP binding site formed by the flexible glycine-rich loop in the hinge region of the Aurora-A. This makes this form of the enzyme an attractive target, particularly to gain selectivity over other kinases.

Figure 3b shows the ligand MPY (compound 4 in Table 1) binding to the binding pocket of Aurora-A through two H-bond interactions between the scaffold 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole of the ligand MPY and the residues Ala213 and Glu211 of Aurora-A in its hinge region. The =N- and -NH of the pyrazole group form H bonds with the backbone of Ala213 and Glu211, respectively. The 3-amino group of the tetrahydropyrrolo[3,4-c]pyrazole forms a hydrogen bond with the backbone of

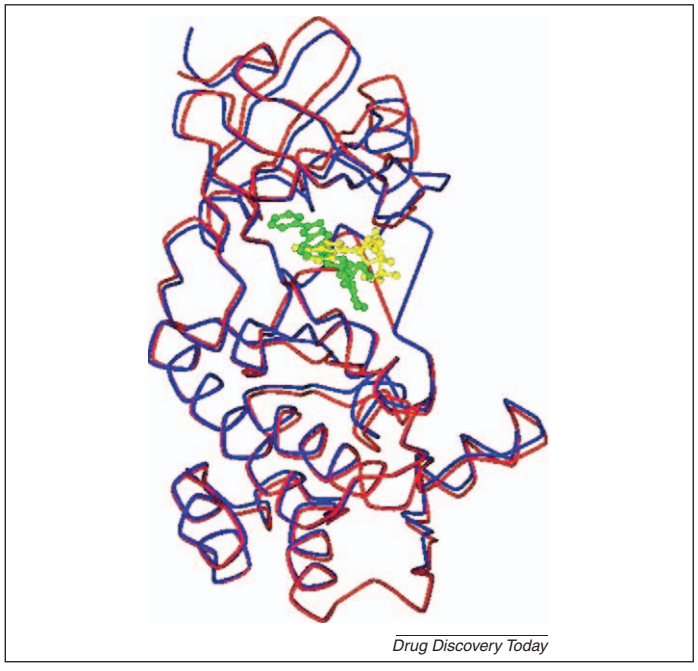


FIGURE 2

Superimposing of the backbones (through the α-carbon) of the two crystal structures of Aurora-A 2C6D (in blue) with its ligand ANP (in yellow), and Aurora-A 2C6E (in red) with its ligand HPM (in green).

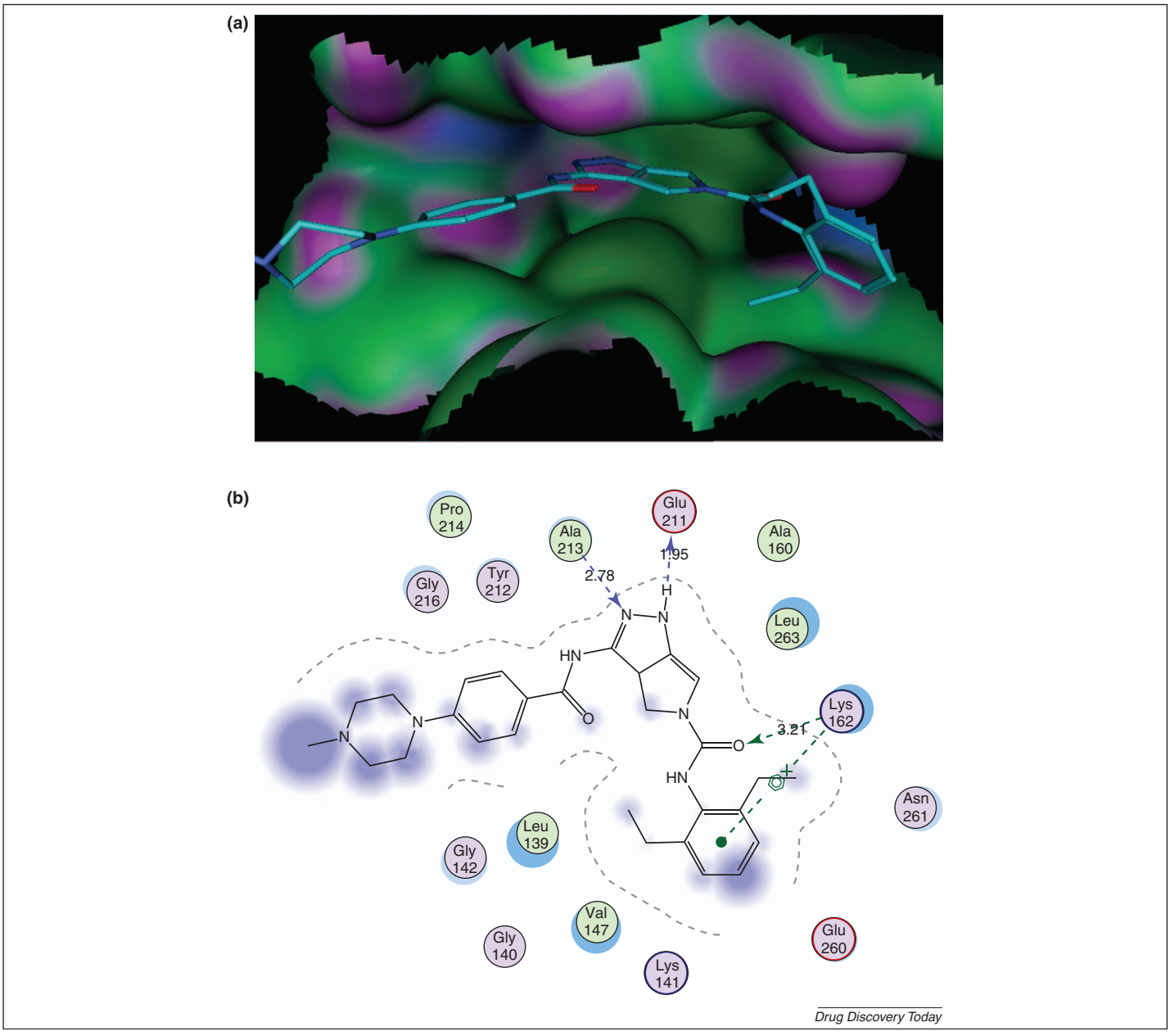


FIGURE 3

The co-complex crystal structure of ligand MPY (compound 4 in Table 1) bound to the hydrophobic binding pocket of Aurora-A kinase and the interactions between them (PDB code: 2BMC). **(a)** The co-complex crystal structure of ligand MPY bound to Aurora-A (PDB code: 2BMC). This shows the hydrophobic binding pocket of Aurora-A. Purple indicates the H-bonding area; green the hydrophobic area and blue the mild polar area. **(b)** Interactions between ligand MPY (compound 4 in Table 1) and Aurora-A (PDB code: 2BMC). Two important H-bonds are formed between the pyrazole group of the ligand MPY and the backbone Ala213 and Glu211 in the hinge region of Aurora-A kinase. The phenyl group on the tail of the inhibitor forms a π-bond with the Lys162 side chain. The Lys162 residue also forms a H-bond with the carboxide in the R1 group. This figure was produced using the program MOE.

Ala213. Thus, a strong H-bonding network is formed. An π-bond also forms between Lys162 and the phenyl group at the tail of the ligand MPY. The other side tail (4-methylpiperazin-1-yl) of the ligand MPY is partly exposed to the solvent, and does not form strong interactions with Aurora-A.

Scaffolds from known inhibitors

Most Aurora-A kinase inhibitors contain adenine-like scaffolds, and have similar binding modes, forming an H-bonding network between the inhibitor and the kinase. The scaffolds of the known inhibitors can be divided into four main groups labeled A–D, as shown in Fig. 4a: (A) contains a core of 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole; (B) contains a core of pyrrolo[2,3-b]pyrimidine; (C) contains a core of quinoline; and (D) contains a core of 2-anilino-diaminopyrimidine.

These scaffolds can form H bonds with the backbone Glu211 and Ala213 in the hinge region of the kinase. Other interactions, such as π–π stacking and p–π conjugation between the R1-group (Fig. 1) of the ligand and Aurora-A kinase in the phosphate-binding region, can also have important roles.

The adenosine-like inhibitors of Aurora-A kinase and their inhibitory activity (IC_{50}) values [3,22,24–38] are summarized in

TABLE 1

Inhibitors of Aurora-A kinase with their IC_{50} values[a].

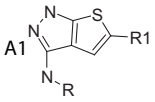
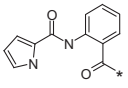
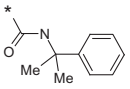
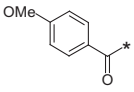
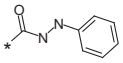
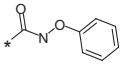
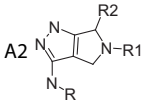
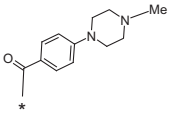
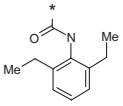
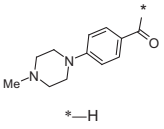
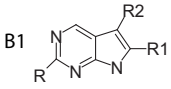
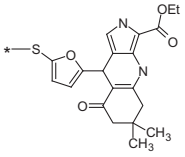
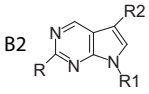
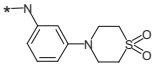
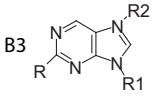
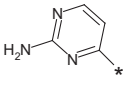
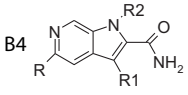
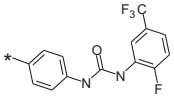
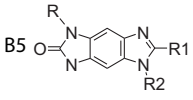
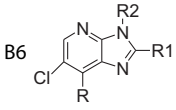
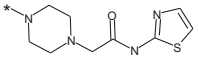
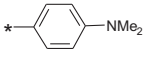
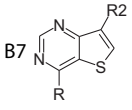
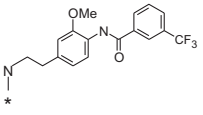
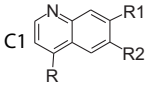
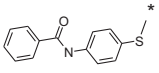
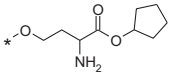
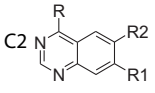
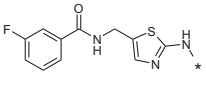
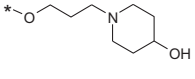
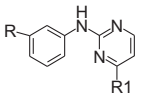
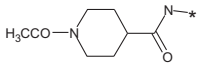
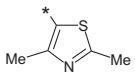
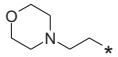
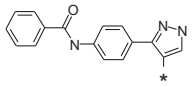
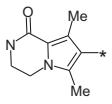
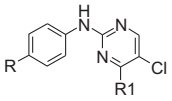
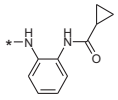
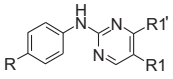
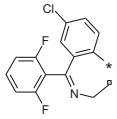
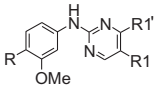
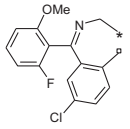
Number	Scaffold	R	R1	R2	IC_{50} (nM)	Reference
1					6	[24]
2					8	[25]
3					<50	[26]
4				*–H	27	[22]
5				*–H	13	[3]
6		*–H		*–H	8	[27]
7				*–H	0.8	[28]
8		*–H		*–H	270	[29]
9		*–H		*–H	172	[30]
10				*–H	158	[31]
11				*–H	<500	[32]
12				*–H	>1000	[33]
13				*–OMe	<2000	[34]
14				*–OMe	<1	[3]

TABLE 1 (*Continued*)

Number	Scaffold	R	R1	R2	IC_{50} (nM)	Reference
15	D1 				4	[35]
16					<10,000	[36]
17					<1000	[37]
18	D2 	*–CN			1.3	[38]
19[b]	D3 				4	[3]
20[b]	D4 				1	[3]

[a] Asterisk (*) indicates a link position.
[b] The substitution group at * is the R1 group, at □ is the R1′ group in scaffold D3 and D4.

Table 1. We only list the representative inhibitors in this article. Several other interesting and unique Aurora-A inhibitors have also recently been reported by Howard [39] and Zhong [40], the scaffolds of inhibitors in Howard's work [39] are similar to the scaffolds B5 and B6 (Table 1), the scaffold of inhibitors in Zhong's work [40] is similar to the scaffold B7 (Table 1). In Table 1, most of the scaffolds contain two rings, although the size of the ring and the way in which the two rings are combined are different. Group A has two fused five-member rings, group B typically has a six-member-ring fused with a five-member-ring (B5 has two five-member-rings fused with a six-member-ring), group C has two six-member-rings fused together and group D has two six-member-rings connected by a secondary amine.

As mentioned above, the R2 group points to the buried region of Aurora-A kinase, where there is only a small space. Therefore, the R2 groups always are small, such as –H, $–CH_3$ or $–OCH_3$ (Table 1). However, the atom (at the five-member-ring of the scaffold) connected with the R2 group is variable; it could be C, S, N or O. (When it is an S or O atom, the R2 group will not exist.) These variations can cause changes in the electrostatic nature of the scaffold. Some patent compounds with the scaffolds of group A have been published [24–26] and all show inhibitory activity. Therefore, it is reasonable to consider them as screening candidates for Aurora-A kinase.

Scaffolds B can be considered as mimics of either adenine or guanine. Group B can be further divided into seven subtypes: B1–B7. B1 and B2 both contain a pyrimidine combined with a pyrrole, and the only difference between B1 and B2 lies in the connection position of the R1 group; while the R1 group is located at the phosphate binding region. The change at R1 group does not significantly affect the inhibitory activity. B3 is a ring system of pyrimidine fused with imidazole, a scaffold that is most similar to adenine or guanine. This scaffold has been well investigated, and most of it has anti-neoplastic activities. B4–B7 are also mimics of adenine or guanine; however, the two synergic N atoms forming the H bonds are absent. Therefore, it is expected that their inhibitory activity will be significantly reduced.

C1 and C2 are also mimics of adenine or guanine but with modification of the five-member-ring and changes in hetero-degree at the six-member-ring. Given that C1 is less similar to the adenine or guanine scaffold, C2 scaffold's inhibition will be stronger than C1 scaffold's inhibition. D1–4 are considered to be derivates with a guanidine core. This core maintains the two synergic N atoms that can form H bonds (one is the H bond donor, the other is the acceptor). Therefore, compounds with this kind of scaffold should have potent inhibitory activity. However, compounds 16 and 17 (Table 1) have weak inhibitory activity because the R groups are hydrophobic and exposed to the solvent. By contrast, the R groups at D2–4 are hydrophilic, which make compounds 18–20 highly potent. This can be proven by the use of an inhibitor MLN8237 (compound 20 in Table 1), the inhibitory activity of which (i.e. its IC_{50} value) is only 1 nM; this compound is currently in Phase I/II clinical trials ([3,41]; http://clinicaltrials.gov/ct2/show/study/NCT00651664).

In short, Aurora-A kinase inhibitors can contain an adenine- or guanine-mimic scaffold, or a guanidine core (the 2–3 synergic N atoms that can form an H-bonding network with Aurora-A kinase). At these scaffolds, three substitutes (R, R1 and R2) point to the

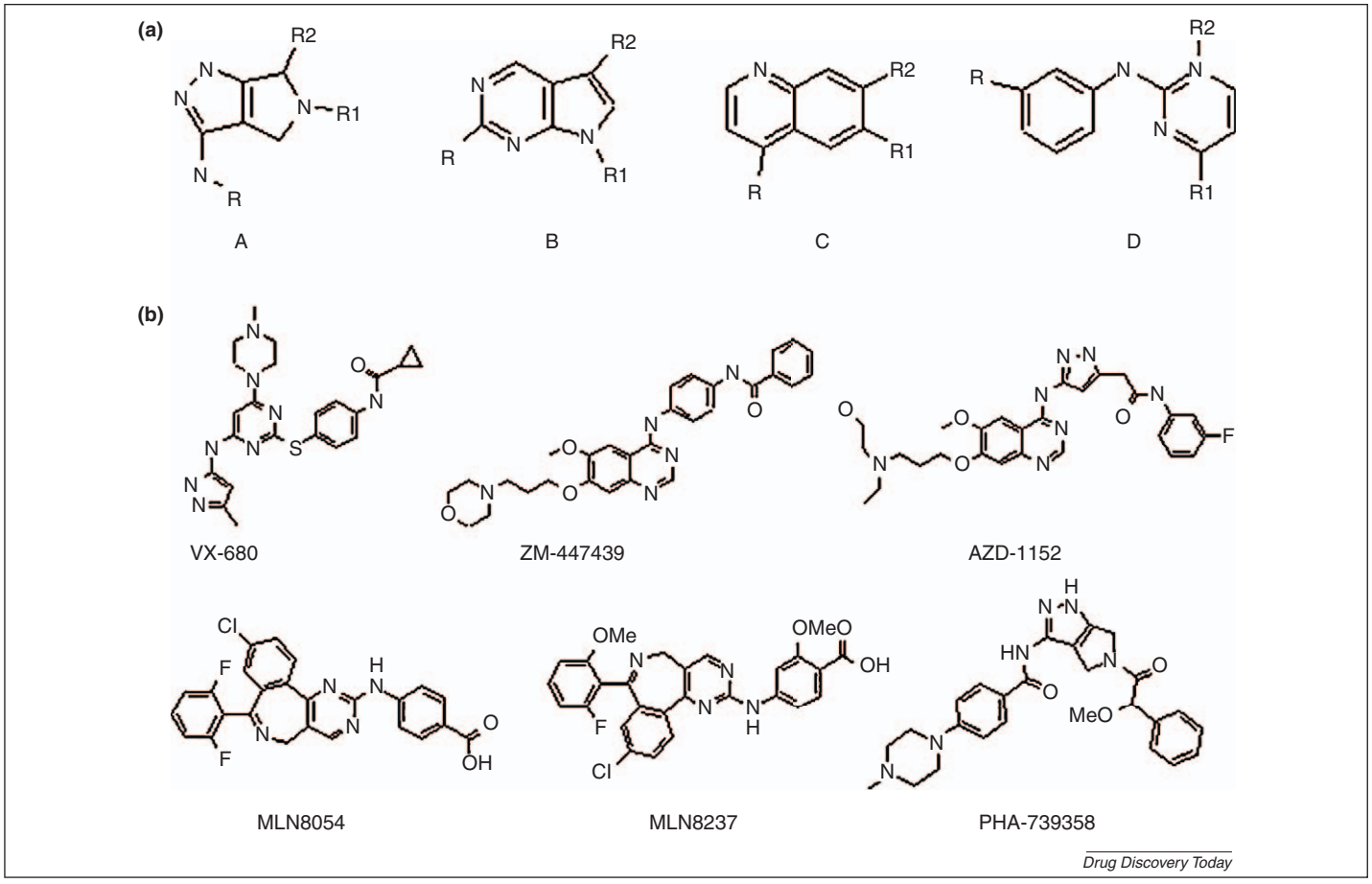


FIGURE 4

Four main types of scaffold found in reported ATP competitive inhibitors of Aurora-A kinase and the potent Aurora kinase inhibitors under clinical study. **(a)** Four main types of scaffold found in reported ATP competitive inhibitors of Aurora-A kinase. (A) 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole scaffold; (B) pyrrolo[2,3-b]pyrimidine scaffold; (C) quinoline scaffold; and (D) 2-anilino-diaminopyrimidine scaffold. Here, the groups R, R1 and R2 are variable. **(b)** Examples of potent Aurora kinase inhibitors current under clinical study.

corresponding solvent accessible, phosphate binding and buried regions of the binding site, respectively. The diverse structures of Aurora-A inhibitors are generated by the various R and R1 groups. The R groups are either polar or hydrophilic, and the R1 groups can vary in size or their electrostatic properties.

Binding modes of Aurora-A kinase inhibitors

Most scaffolds of the Aurora-A kinase inhibitors (Table 1) contain a bicyclic (fused or connected by an atom) system. They bind to the hinge region of the kinase via H bonds with the backbone Glu211 and Ala213 (Figs 1,3b). The ligand forms at least one H bond with the backbone Glu211 or Ala213, or both (PDB code: 1OL6 [19], 2BMC [22], 2J4Z [42]). Some inhibitors (PDB code: 2J50 [42], 2NP8 [43]) form one H bond with the backbone Glu211 and two H bonds with the backbone Ala213 when the scaffold and the R group connected by an N atom (Fig. 1). The interactions between the small-molecule ligand and the residues within the hinge region contribute significantly to the binding affinity of the compound. The phenyl group on the tail of the inhibitor (e.g. MPY in PDB code 2BMC [22]; Fig. 3b) can form a π-bond with the Lys162 side chain located in the upper lobe of the solvent-exposed phosphate-binding site of Aurora-A kinase. R1 groups can also form an H bond with the Lys162 side chain (e.g. MPY in PDB code 2BMC and 627 in PDB code 2J50). Exposure to the solvent offers a way of improving the pharmacokinetic profile through chemical modification. In addition to the main interaction of the ligand with the backbone Glu211 and Ala213, further stabilization of the binding can be mediated by the contact of the R and R1 groups with the hydrophobic surface formed by the Leu139, Val147, Leu210, Tyr212, Pro214, Leu215, Thr217 and Leu263 amino acid side chains, according to the analysis of the interactions formed by 25 crystal structures of Aurora-A kinase with the inhibitors discussed above.

In addition, a series of biochemical and structural studies has shown that Aurora kinases require other cofactors for activation. For Aurora-A, the most recognized cofactor is TPX2, which was initially described as a microtubule-associated protein with involvement in bipolar spindle assembly. It was then found to bind and direct Aurora-A to the mitotic spindle [13]. Autophosphorylation on Thr288 in the activation loop and binding to TPX-2 are required for full activation of Aurora-A [3]. By comparing the co-crystal structures of Aurora-A–TPX2–VX-680 and Aurora-A–VX-680 and analyzing the interactions between VX-680 and Aurora-A, it was found that TPX2 can alter the binding mode of VX-680 with Aurora-A [44]. Further investigation of the co-crystal structures of Aurora-A–TPX2 and their inhibitors could be beneficial to the discovery and optimization of enzyme inhibitors as therapeutic agents.

Strategy to design new leads against Aurora-A kinases

Given the effect of the cofactor TPX2 on Aurora-A, one can keep at least one direct H-bond interaction with the backbone of the Aurora-A in the hinge region when designing an Aurora-A kinase ATP-competitive inhibitor. Glu211 and Ala213 are considered to be hot spots because they contribute significantly to the binding interactions with the inhibitors. The phosphate-binding region of the Aurora-A has enough space to dock large entities with structurally diverse R1 groups. Compared with the R group in the solvent accessible region, the R1 group in the phosphate-binding region always has stronger interactions with Aurora-A. Thus, it is possible to design new inhibitors of Aurora-A with the scaffolds detailed in Table 1 and with different R and R1 groups. However, it is important to maintain the main interactions between the inhibitor and the kinase to ensure potent inhibitory activity.

Currently, most Aurora-A kinase inhibitors identified through an Aurora-A kinase inhibitory activity-based screen were also found to have potent activities on Aurora-B kinase [11]. In recent research, the Aurora kinase inhibitors can be subdivided into three general classes: (i) selectivity for Aurora-A over -B; (ii) selectivity for Aurora-B over -A; and (iii) potent inhibitors of both Aurora-A and -B. Several selective and nonselective Aurora kinase inhibitors are currently being tested in preclinical and clinical trials as anti-tumor agents [11]. The first reported kinase inhibitor with selectivity for Aurora-A was MLN8054 (Fig. 4b; compound 19 in Table 1). This compound has a 40-fold selectivity for Aurora-A over Aurora-B in enzyme assays (IC_{50} of 4 nM and 172 nM, respectively) and shows a greater apparent selectivity for Aurora-A over Aurora-B in cells (IC_{50} for the inhibition of Aurora-A autophosphorylation of 34 nM, with an IC_{50} for the inhibition of histone H3 phosphorylation of 4 μM) [45]. The first reported Aurora kinase inhibitor with selectivity for Aurora-B (AZD-1152; Fig. 4b) has entered clinical trials. This compound has a 1400-fold selectivity for Aurora-B over Aurora-A in enzyme assays (IC_{50} of 1 nM and 1400 nM, respectively). ZM447439, hesperadin and VX680 (MK-0457) inhibit both Aurora-A and -B *in vitro* with various efficiencies, but they induce cellular phenotypes that are more compatible with the inhibition of Aurora-B *in vivo* [2]. Preclinical work using these compounds as tools and the application of biological techniques, such as siRNA depletion, has provided insight into the differential effects of inhibiting each of the Aurora kinases [3]. However, it is still unclear whether this alternative selectivity profile will confer differences in the clinic [3]. According to siRNA depletion experiments, depletion of Aurora-A can result in delayed entry into mitosis and marked disruption of the spindle with monopolar spindles being frequently observed. Knockdown of Aurora-A and TPX2 in U2OS cells also resulted in mitotic arrest [11]. Thus, it should be of interest to researchers to design new Aurora-A inhibitors as potential anti-tumor agents for use by patients with cancer.

Aurora kinase inhibitors in clinic trials

To date, more than ten small-molecule inhibitors of Aurora-A kinases have entered clinical studies as potential anticancer agents. These small-molecule inhibitors all interact with the catalytic domain and locate at the ATP-binding pocket of the enzymes. Some potent inhibitors against Aurora kinase are listed in Fig. 4b ([3,4,41, 46–50] see also http://clinicaltrials.gov/ct2/show/NCT00335868; http://www.clinicaltrials.gov/ct2/results?term=NCT00249301; http://www.clinicaltrials.gov/ct2/results?term=NCT00652158; http://www.clinicaltrials.gov/ct2/results?term=NCT01091428; http://www.clinicaltrials.gov/ct2/results?term=NCT01045421; http://www.clinicaltrials.gov/ct2/results?term=NCT00500903; http://www.clinicaltrials.gov/ct2/results?term=NCT00697346; and http://www.clinicaltrials.gov/ct2/results?term=NCT00766324; all accessed 6 December 2010).

ZM-447439

ZM-447439 was the first Aurora kinase inhibitor to be developed and inhibits both Aurora-A and Aurora-B *in vitro* with an IC_{50} of 110 nM and 130 nM, respectively. However, recent studies indicate that the phenotypic events that occur following exposure to the drug *in vivo* are the result of inhibition of Aurora-B, rather than of Aurora-A [4]. It was reported that ZM-447439 had no effect on either the kinetics or amplitude in the oscillations of activity of several key cell cycle regulators [46]. The chromosome could condensate in the presence of ZM-447439, but fail to mature [46].

ZM-447439 profoundly inhibited the proliferation of Philadelphia chromosome (Ph+)-positive PALL-1 and PALL-2 ALL cells. It was also active in primary Ph+ALL cells that relapsed after conventional chemotherapy with imatinib. In general, prognosis of patients with Ph+ALL is poor, and future clinical studies with the Aurora kinase inhibitor should be considered for those patients with this lethal disease [47]. ZM-447439 has also been used to study the biology of Aurora kinase in the initial stages of Aurora drug-target validation.

VX-680 (MK-0457)

In 2004, Vertex (http://www.vrtx.com) published details of the activity of VX-680, validating Aurora kinase as a drug target for cancer in preclinical animal models [41]. VX-680 is a potent inhibitor of the Aurora kinases, with inhibition constant values [$K_{i(app)}$] of 0.6 nM, 18 nM and 4.6 nM for Aurora-A, Aurora-B and Aurora-C, respectively *in vitro* [4]. In various human tumor cell lines, VX-680 reduced cell proliferation with IC_{50} values ranging from 15 to 13 nM [41].

Studies show that VX-680 is a selective inhibitor of Aurora-A kinase, blocking cell proliferation but inducing cell death by reducing multiple tumor types, both *in vitro* and *in vivo*. Tumor inhibition was achieved with VX-680 at a tolerated dose, and no sign of toxicity was observed [48].

Despite this positive note, in 2007, Merck (http://www.merck.-com) suspended enrollment in Phase I/II clinical trials of MK-0457, pending a full analysis of all available safety and efficacy data on the compound. The decision was based on preliminary safety data, in which a clinical safety finding of QTc prolongation was observed in one patient [42].

AZD-1152

AZD-1152 is the derivative of pyrazoloquinazoline-dihydrogen phosphate [49] (Foote, K.M. (2008) Readily activated and highly soluble pyrazoloquinazoline phosphate derivatives with potent and durable anti-tumor activity. Department of Medicinal Chemistry. Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, USA, April 6–10 2008), which was developed by AstraZeneca (http://www.astrazeneca.co.uk). AZD-1152 is readily converted to the active species (AZD-1152HQPA), which is a

highly selective inhibitor of Aurora-B kinase, with $IC_{50} < 1$ nM. In human cancer xenograft models, AZD-1152 causes pharmacodynamic changes that result in durable anti-tumor growth inhibition at well-tolerated doses. AZD-1152 has the potential for inhibitory activity in a range of human tumors and is currently in Phase II clinical trials (http://clinicaltrials.gov/ct2/show/NCT00335868). Clinical investigation into AZD-1152 was performed in patients with advanced solid malignancies, such as colon cancer. Dose-limiting toxicity was CTC grade 4 neutropenia in three patients at 450 mg on the given schedule [41].

MLN8054

MLN8054 was developed by Millennium (http://www.millennium.com) [3,45,50], which is a selective Aurora-A kinase inhibitor that entered Phase I clinical trials for advanced solid tumors in 2005. MLN8054 inhibits recombinant Aurora-A kinase activity *in vitro* and is selective for Aurora-A over the family member Aurora-B in cultured cells, with IC_{50} of 4 nM and 172 nM, respectively.

In Phase I clinical trials, orally administrated MLN8054 was shown to be rapidly absorbed and displayed dose-proportionate exposure. However, somnolence as a dose-limiting toxicity was observed in patients treated with MLN8054 [50]. Recently, two Phase I clinical trials of the study of MLN8054 in patients with advanced solid tumors and extended MLN8054 dosing in patients with advanced malignancies have been terminated by Millennium without a clear explanation (http://www.clinicaltrials.gov/ct2/results?term=NCT00249301; http://www.clinicaltrials.gov/ct2/results?term=NCT00652158).

MLN8237

MLN8237 is a novel highly selective inhibitor of Aurora-A kinase, with an IC_{50} of 1 nM in biochemical assays and it has 200-fold selectivity for Aurora-A over Aurora-B in cell assays [3]. It inhibits growth of various cancer cell lines, such as HCT-116, PC3, SK-OV-3 and LY-3, with growth inhibition (GI_{50}) values ranging from 16 to 469 nM *in vitro*. Studies of MLN8237 have entered Phase I/II clinical trials [41]. Randomized Phase II study of MLN8237 plus weekly paclitaxel or weekly paclitaxel alone is ongoing in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer, preceded by a Phase I portion in patients with ovarian or breast cancer (http://www.clinicaltrials.gov/ct2/results?term=NCT01091428). A Phase I dose escalation study of MLN8237 is ongoing in adult patients with nonhematological malignancies, followed by a Phase II of MLN8237 in patients with lung, breast, head and neck, or gastroesophageal malignancies (http://www.clinicaltrials.gov/ct2/results?term=NCT01045421). Phase I clinical trials are ongoing in patients with advanced solid tumors and advanced hematological malignancies (http://www.clinicaltrials.gov/ct2/results?term=NCT00500903; http://www.clinicaltrials.gov/ct2/results?term=NCT00697346).

PHA-739358

PHA-739358 is an inhibitor of both Aurora-A and Aurora-B, with IC_{50} of 13 nM and 79 nM, respectively [3]. It was designed based on the X-ray co-crystal structure of a preclinical candidate, PHA-680632, in complex with Aurora-A. PHA-739358 shows higher cellular potency and Aurora inhibitory activity than does PHA-680632 [41]. PHA-739358 is now undergoing Phase II clinical trials to treat patients either with chronic myelogenous leukemia who relapsed after imatinib mesylate or c-Abl-targeted therapy or with metastatic hormone refractory prostate cancer (http://www.clinicaltrials.gov/ct2/results?term=NCT00335868; http://www.clinicaltrials.gov/ct2/results?term=NCT00766324).

Extensive preclinical information supports the development of specific Aurora kinase inhibitors in specific tumor types [7]. This information can provide a comprehensive overview of the development of Aurora kinases as molecular targets for anticancer therapy by focusing on their physiological role in mitosis, their implication in oncogenesis and the potential ways of inhibiting their activity.

Clinical inhibitors (e.g. VX-680, MLN8054, MLN8237 and PHA-739358) also bind to Aurora-A kinase in a similar binding mode (i.e. forming the H-bonding network in the hinge region of the binding site). The ligands VX-680 and PHA-739358 bind to Aurora-A through direct H bonding with the residues Glu211 and Ala213 in the hinge region of binding site. The ligands MLN8054 and MLN8237 bind to Aurora-A through direct H bonding with the residues Ala213 in the hinge region of binding site. They all bind to the Aurora-A through the direct H-bond interactions with the main chain in the hinge region. Although most of these clinic inhibitors show high inhibitory activity against Aurora kinase, their toxicity and safety for use need to be studied further.

Concluding remarks

The Aurora kinase family has emerged as an attractive target family for cancer therapies. Future drug designs based on Aurora-A kinase can begin with the known crystal structure of Aurora-A, the feature of the ATP-binding pocket, the common characteristics of the known inhibitors and their main binding modes with Aurora-A. The adenosine mimic scaffolds described in this review could be used for further study to design additional potential inhibitors of Aurora-A with high inhibitory activity. The selectivity and the different effects of the inhibitor against Aurora-A and Aurora-B in a clinic setting could also be studied further.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20605003 and 20975011), and the Chinese Universities Scientific Fund (ZZ0911) of the Beijing University of Chemical Technology.

References

1 Brown, J.R. and DuBois, R.N. (2005) COX-2: a molecular target for colorectal cancer prevention. *J. Clin. Oncol.* 4, 2840–2855

2 Sardon, T. *et al.* (2009) Development and biological evaluation of a novel Aurora A kinase inhibitor. *ChemBioChem* 10, 464–478

3 Pollard, J.R. and Mortimore, M. (2009) Discovery and development of Aurora kinase inhibitors as anticancer agents. *J. Med. Chem.* 52, 2629–2651

4 Kitzen, J.J.E.M. *et al.* (2010) Aurora kinase inhibitors. *Crit. Rev. Oncol. Hematol.* 73, 99–110

5 Malumbres, M. and Barbacid, M. (2007) Cell cycle kinases in cancer. *Curr. Opin. Genet. Dev.* 17, 60–65
6 Nigg, E.A. (2001) Mitotic kinases as regulators of cell division and its checkpoints. *Nat. Rev. Mol. Cell Biol.* 2, 21–32
7 Mountzios, G. *et al.* (2008) Aurora kinases as targets for cancer therapy. *Cancer Treat. Rev.* 34, 175–182
8 Giet, R. *et al.* (2005) Aurora kinases, aneuploidy and cancer, a coincidence or a real link? *Trends Cell Biol.* 15, 241–250
9 Wang, S. *et al.* (2010) Discovery of N-phenyl-4-(thiazol-5-yl)pyrimidin-2-amine Aurora kinase inhibitors. *J. Med. Chem.* 53, 4367–4378
10 Mahadevan, D. and Beeck, S. (2007) Aurora kinase targeted therapeutics in oncology: past, present and future. *Expert Opin. Drug Discov.* 2, 1011–1026
11 Yang, H. *et al.* (2005) Mitotic requirement for Aurora A kinase is bypassed in the absence of Aurora B kinase. *FEBS Lett.* 579, 3385–3391
12 Aliagas-Martin, I. *et al.* (2009) A class of 2,4-bisanilinopyrimidine Aurora A inhibitors with unusually high selectivity against Aurora B. *J. Med. Chem.* 52, 3300–3307
13 Lok, W. (2010) Aurora kinase inhibitors as anti-cancer therapy. *Anticancer Drugs* 21, 339–350
14 Li, X. *et al.* (2004) Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora-C. *J. Biol. Chem.* 279, 47201–47211
15 Sasai, K. *et al.* (2004) Aurora-C kinase is a novel chromosomal passenger protein that can complement Aurora-B kinase function in mitotic cells. *Cell Motil. Cytoskeleton* 59, 249–263
16 Giet, R. and Prigent, C. (1999) Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases. *J. Cell Sci.* 112, 3591–3601
17 Crane, R. *et al.* (2004) Requirements for the destruction of human Aurora-A. *J. Cell Sci.* 117, 5975–5983
18 Walter, A.O. *et al.* (2000) The mitotic serine/threonine kinase Aurora2/AIK is regulated by phosphorylation and degradation. *Oncogene* 19, 4906–4916
19 Bayliss, R. *et al.* (2003) Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol. Cell* 12, 851–862
20 Littlepage, L.E. *et al.* (2002) Identification of phosphorylated residues that affect the activity of the mitotic kinase aurora-A. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15440–15445
21 Talele, T.T. and McLaughlin, M.L. (2008) Molecular docking/dynamics studies of Aurora A kinase inhibitors. *J. Mol. Graph. Model.* 26, 1213–1222
22 Fancelli, D. *et al.* (2005) Potent and selective aurora inhibitors identified by the expansion of a novel scaffold for protein kinase inhibition. *J. Med. Chem.* 48, 3080–3084
23 Heron, N.M. *et al.* (2006) SAR and inhibitor complex structure determination of a novel class of potent and specific Aurora kinase inhibitors. *Bioorg. Med. Chem. Lett.* 16, 1320–1323
24 Fancelli, D. *et al.* (2007) [Nerviano Medical Sciences] Preparation of 1*H*-thieno[2,3-c]pyrazoles as kinase, particularly Aurora kinases and IGF-1R inhibitors for treating cancer. PIXXD2 WO 2007009898 A1 20070125
25 Barberis, C. *et al.* (2006) [Aventis Pharma] Hydrazinocarbonyl-thieno[2,3-c]pyrazoles, their preparation, compositions containing them and their use as inhibitors of protein kinases. PIXXD2 WO 2006092510 A1 20060908
26 Carry, J.C. *et al.* (2006) [Aventis Pharma] Substituted 1*H*-thieno[2,3-c]pyrazoles, their preparation, compositions containing them and their use as inhibitors of protein kinases for treating cancer. PIXXD2 WO 2006056697 A1 20060601
27 Mauger, J. *et al.* (2007) [Aventis Pharma] Preparation of 1,4-dihydropyridine-fused heterocycles as inhibitors of Aurora kinases. EPXXDW EP 1746097 A1 20070124
28 Moriarty, K.J. *et al.* (2006) The synthesis and SAR of 2-amino-pyrrolo [2,3-d]pyrimidines: a new class of Aurora-A kinase inhibitors. *Bioorg. Med. Chem. Lett.* 16, 5778–5783
29 Fischer, P.M. *et al.* (2006)[Cyclacel Ltd.] Preparation of 4-(1*H*-indol-3-yl)-pyrimidin-2-ylamine derivatives as protein kinase inhibitors and their use in therapy. PIXXD2 WO 2006075152 A1 20060720
30 Tabart, M. *et al.* (2006) [Aventis Pharma] Preparation of substituted pyrrolopyridines as kinase inhibitors, and their compositions and use for treatment of cancer. FRXXBL FR 2884821 A1 20061027
31 Georges, G. *et al.* (2006) [F. Hoffmann-La Roche] Preparation of pyrazolylbenzimidazole derivatives as Aurora A kinase inhibitors for treatment of cancer. PIXXD2 WO 2006032519 A1 20060330
32 Bavetsias, V. *et al.* (2007) [Chroma Therapeutics Ltd.] Preparation of imidazopyridines as enzyme inhibitors, especially Aurora kinase inhibitors, for treating cell proliferative diseases. PIXXD2 WO 2007072017 A2 20070628
33 Lew, W. *et al.* (2006) [Sunesis Pharmaceuticals] Thienopyrimidines useful as Aurora kinase inhibitors and their preparation, pharmaceutical compositions, and their use for treatment of Aurora kinase-mediated diseases. USXXCO US 2006035908 A1 20060216
34 Davidson, A.H. *et al.* (2006) [Chroma Therapeutics Ltd.] Preparation of quinoline and quinazoline amino acid derivatives as inhibitors of kinase enzymatic activity. PIXXD2 WO 2006117552 A1 20061109
35 Tomozane, H. *et al.* (2005) [Mitsubishi Pharma Corporation] Preparation of aminopyrimidine derivatives as aurora 2 kinase inhibitors for treatment of cancer. PIXXD2 WO 2005113550 A1 20051201
36 Adams, J.L. *et al.* (2007) [Smithkline Beecham Corporation] Pyrimidinyl-pyrazole inhibitors of Aurora kinases and their preparation, pharmaceutical compositions and use in the treatment of cell proliferative diseases. PIXXD2 WO 2007024843 A2 20070301
37 Jones, S. *et al.* (2007) [Cyclacel Ltd.] Preparation of pyrimidinyl substituted pyrrolopyrazinones as protein kinase inhibitors useful in treatment of diseases. PIXXD2 WO 2007042784 A2 20070419
38 Krueger, E.B. *et al.* (2008) [Genentech, Inc.] Preparation of pyrimidines as Aurora kinase inhibitors. PIXXD2 WO 2008079719 A1 20080703
39 Howard, S. *et al.* (2009) Fragment-based discovery of the pyrazol-4-yl urea (AT9283). A multitargeted kinase inhibitor with potent Aurora kinase activity. *J. Med. Chem.* 52, 379–388
40 Zhong, M. *et al.* (2009) 2-Aminobenzimidazoles as potent Aurora kinase inhibitors. *Bioorg. Med. Chem. Lett.* 19, 5158–5161
41 Cheung, C.H.A. *et al.* (2009) Aurora kinase inhibitors in preclinical and testing. *Expert Opin. Investig. Drugs* 18, 379–398
42 Fancelli, D. *et al.* (2006) 1,4,5,6-Tetrahydropyrrolo[3,4-c]pyrazoles: identification of a potent Aurora kinase inhibitor with a favorable antitumor kinase inhibition profile. *J. Med. Chem.* 49, 7247–7251
43 Tari, L.W. *et al.* (2007) Structural basis for the inhibition of Aurora A kinase by a novel class of high affinity disubstituted pyrimidine inhibitors. *Bioorg. Med. Chem. Lett.* 17, 688–691
44 Zhao, B. *et al.* (2008) Modulation of kinase-inhibitor interactions by auxiliary protein binding: crystallography studies on Aurora A interactions with VX-680 and with TPX2. *Protein Sci.* 17, 1791–1797
45 Manfredi, M.G. *et al.* (2007) Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. *Proc. Natl. Acad. Sci. U. S. A.* 104, 4106–4111
46 Gadea, B.B. and Ruderman, J.V. (2005) Aurora kinase inhibitor ZM447439 blocks chromosome-induced spindle assembly, the completion of chromosome condensation, and the establishment of the spindle integrity checkpoint in *Xenopus* egg extracts. *Mol. Biol. Cell* 16, 1305–1318
47 Ikezoe, T. *et al.* (2007) A novel treatment strategy targeting Aurora kinases in acute myelogenous leukemia. *Mol. Cancer Ther.* 6, 1851–1857
48 Harrington, E.A. *et al.* (2004) VX-680, a potent and selective small molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. *Nat. Med.* 10, 262–267
49 Montembault, E. and Prigent, C. (2005) Aurora kinases: therapeutic potential. *Drug Fut.* 30, 1–9
50 Jones, S.F. *et al.* (2007) Phase I clinical trial of MLN8054, a selective inhibitor of Aurora A kinase. *J. Clin. Oncol.*, 2007 ASCO Annual Meeting Proceedings Part I. Vol. 25, No. 18S (June 20 Supplement), 2007: 3577. (http://www.asco.org/ascov2/Meetings/Abstracts?&vmview=abst_detail_view&confID=47&abstractID=34708)