Advances in Mitotic Inhibitors for Cancer Treatment

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Abstract: Based on their mechanism of action, anti-tumor drugs that target the cell cycle can be generally divided into three categories, namely, blocking DNA synthesis, causing DNA damage, and disrupting mitotic processes. In terms of mitotic inhibitors, most compounds used in the clinic impair the normal function of mitotic spindles by targeting tubulins, basic building blocks of microtubules. *In vivo*, these compounds often exhibit significant side effects, thus limiting their efficacy. Mitotic processes are under tight control through surveillance mechanisms commonly termed checkpoints. Defects in the regulation of these checkpoints often result in genomic instability, which predisposes the cell to malignant transformation. As cancer is the consequence of uncontrolled cell division, great efforts have been devoted to discover drugs that target mitosis, thereby halting cell division and inducing mitotic catastrophe with minimal cytotoxicity to non-dividing or normally dividing cells. This review primarily focuses on mitotic proteins that have been explored as new targets for anti-cancer drug development during the past decade.

Keywords: Mitosis, microtubules, kinesins, Aurora kinases, Polo-like kinases, small compounds, cell cycle checkpoint, mitotic catastrophe.

INTRODUCTION

There are more than fifty chemotherapeutical drugs on the market for cancer treatment [1]. Most of them kill cancer cells through alteration of DNA structures or inhibition of DNA replication. However, many of these drugs have moderate to severe side effects simply because of the fact that they also kill dividing normal cells [2]. Therefore, special emphases have been placed on identifying alternative targets from which selective anti-cancer drugs can be discovered and developed. During the past decade, many new molecules that regulate mitotic checkpoints or mitotic progression have been described. Given that deregulation of mitotic processes often leads to mitotic catastrophe, a specialized case of apoptosis, many mitotic proteins have been considered as new classes of targets for cancer drug discovery.

MICROTUBULE INHIBITORS

1. Taxanes and Vincas

The microtubule spindles were the only mitotic target for therapeutic intervention for decades. Taxanes (Fig. **1**) and vinca alkaloids (Fig. **2**) are two groups of the oldest mitotic inhibitors targeting microtubules [3]. The two taxanes in clinical uses are paclitaxel (Taxol by Bristol-Myers-Squibb) **(1)** [4] and docetaxel (Taxotere by Aventis) **(2)** [5]. Both compounds, obtained via semi-synthetic processes from yew plants [4.5], prevent the cell from completion of mitosis through binding to β-subunit of tubulin, resulting in the formation of stable, non-functional microtubule bundles. Paclitaxel arrests cells at G_2/M whereas docetaxel primarily functions during S phase. The taxanes also exhibit pro-apoptosis and anti-angiogenic properties [4]. In fact, these compounds have been used for the treatment of a panel of cancers either alone or in combination with other therapeutic drugs or approaches [6].

Vindesine **(3)** [7] and vinorelbine **(4)** [7] are two vinca alkaloid derivatives approved for clinical uses as anti-cancer drugs. Both are semi-synthesized from vinblastine **(5)** [7], a natural vinca alkaloid from leaves of *Vinca rosea* plant. It has been shown that vinca alkaloids halt cell division by binding to microtubules, thus preventing formation of mitotic spindles. Vinflunine **(6)** [7] is a novel Vinca alkaloid currently in clinical trials. This compound is synthesized through introduction of changes in the backbone of Vinca alkaloid molecules by the traditional chemical approach. Although a relatively high dose of Vinflunine is required during clinic trials due to its weak affinity for tubulin, this compound is superior to other vinca alkaloids in terms of neurotoxicity, *in vitro* anti-tumor efficacy, drug resistance, and reversibility [7].

2. Epothilones

During the past several years, many companies and research institutes have developed new microtubule-targeting compounds for cancer treatment including epothilones, discodermolide, eleutherobins dolastatins, cryptophycins, indanocine, halicondrin B, and peloruside A, among which epothilones are most promising because they exhibit broad-spectrum anti-tumor activities in cell culture and in xenograft animal models. These compounds are initially identified from fermentation products for their anti-fungal activity. Subsequently, they are found to be capable of stabilizing microtubules in a manner similar to taxane [8]. In a variety of cells tested, epothilones are more cytotoxic than paclitaxel with IC_{50} values in the sub or low nanomolar (nM) ranges. The cytotoxicity of epothilones is minimally affected by over-expression of P-glycoprotein or by certain

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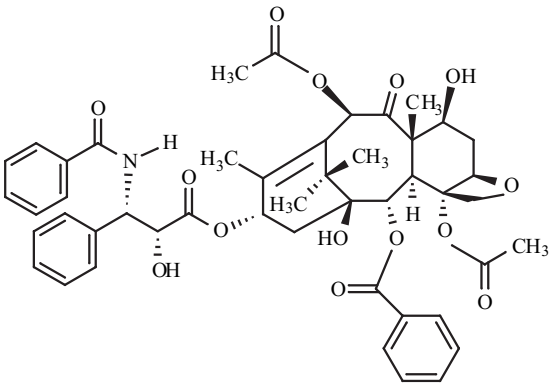
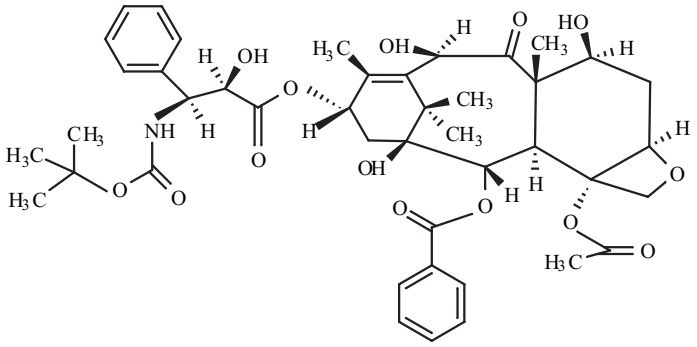
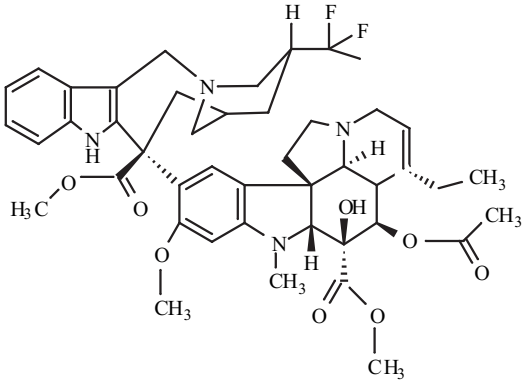


1 Paclitaxel

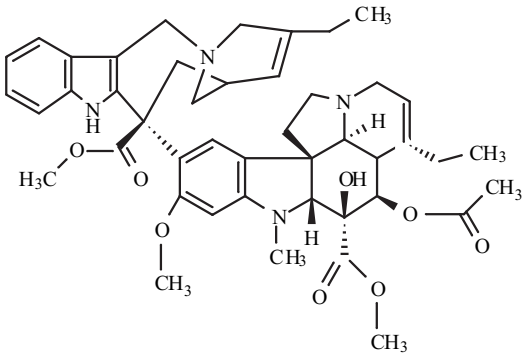


2 Docetaxel

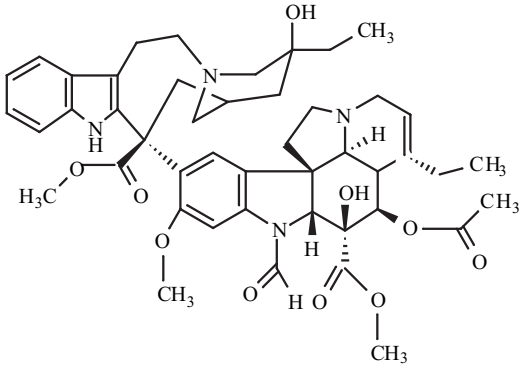
Fig. (1). Chemical structure of taxanes.



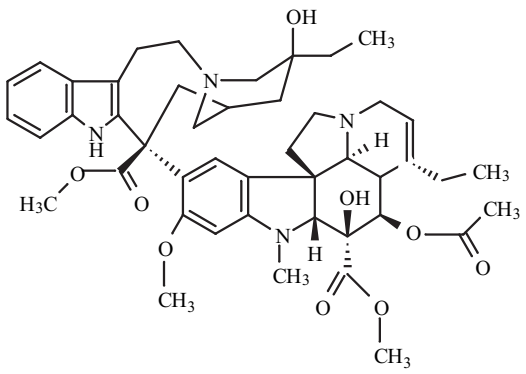
3 Vindesine



4 Vinorelbine

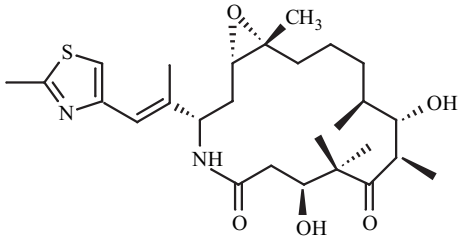


5 Vinblastine

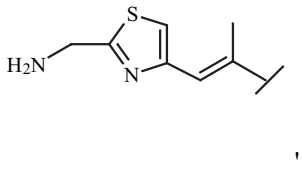


6 Vinflunine

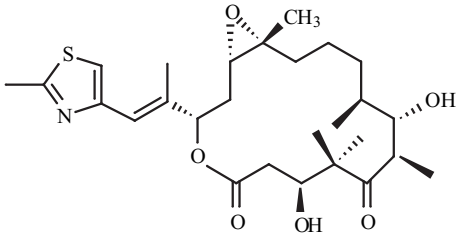
Fig. (2). Chemical structure of vincas.



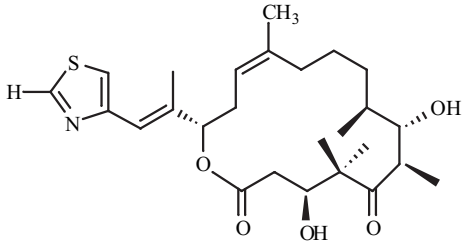
7 Aza-epothilone B (BMS-247550)



8 BMS-310705



9 Eepothilone B (EPO906)



10 EpothiloneD (KOS-862)

Fig. (3). Chemical structure of epothilones.

mutations in β-tubulin, the latter being the major mechanism of taxane resistance. Thus, epothilones may be effective in those cancers that fail to respond to taxane-based chemotherapies [8,9,10]. Four epothilone analogs are currently in clinical trials, including epothilone B (EPO906) **(9)**, aza-epothilone B (BMS-247550) **(7)**, a water-soluble semi-synthetic analog of epothilone B (BMS-310705) **(8)**, and epothilone D (KOS-862) **(10)** [11-13] (also refer to Fig. **3**).

However, one major problem of these microtubule-targeting drugs is that they often cause severe neuropathies because microtubules are crucial for molecular transport within neurons [3]. Therefore, it is highly desirable to discover new agents with different modes of action so that the efficacy is coupled with a minimal side effect.

KINESIN MOTOR INHIBITORS

1. Biological Function of Kinesins

Kinesins represent a family of motor proteins important for intracellular trafficking along microtubules. They all share a very conserved, ~350 amino acid long, motor domain at the N-terminus containing binding sites for ATP and microtubules [14]. The energy released by ATP hydrolysis can be translated into motile force through the motor domain [15]. In addition to their essential role in membrane transport, kinesins participate in the processes of mitosis and meiosis. There are 14 mitotic kinesins that have been identified [16]. These proteins are highly expressed in proliferating tissues and absent from terminal differentiated neurons [17]. Real time quantitative PCR assays demonstrate that expression of kinesins of known mitotic functions is increased in tumor tissues relative to normal adjacent tissues and decreased upon neuronal differentiation or senescence, thus, positively correlated with the proliferative index of the cells [17]. Kinesin spindle protein (known as KSP or Eg5), one of the best studied members, functions at the earliest stage of mitosis mediating separation of centrosomes and formation of bipolar mitotic spindles; defects in KSP function induce mitotic arrest, resulting in monopolar mitotic spindles [14]. These unique features make mitotic kinesins attractive targets for development of specific inhibitors for cancer intervention.

Table 1. Inhibitors of kinesin spindle protein

Name	*In Vitro* Enzyme Assays (IC_{50} or Ki)	Cell Based Assays (IC_{50})	Anti-tumor Activities in Animal Models	Specificity for KSP Inhibition
Monastrol	15 μM	50 μM	N/A	Yes
CK0106023	12 nM	364 nM	Ovarian tumor xenografts	Yes
HR22C16	800 nM	0.8-2.3 μM	N/A	Yes
S-trityl-L-cysteine	0.14-1.0 μM	700 nM	N/A	N/A
SB-715992	N/A	N/A	A broad range of tumor xenografts	Yes
SB-743921	100 pM	N/A	A broad range of tumor xenografts.	Yes
CRx-026	N/A	N/A	Nude mouse tumor xenografts	N/A

N/A, Not Available; IC_{50}, Inhibitory Concentration 50; Ki, Inhibitory Constant

2. Kinesin Inhibitors

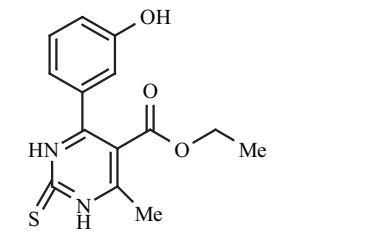
During the past few years, several small molecular weight compounds have been reported for their ability to inhibit KSP (Table **1**). They include Monastrol **(11)** [18,19], CK0106023 (a quinazolinone-based compound) **(12)** [14], HR22C16-A1 **(13)** [20], S-trityl-L-cysteine **(14)** [21], SB-715992 (Ispinesib) **(15)** [22], CRx-026 (in combination of chlorpromazine **(16)** [23], and pentamidine **(17)** [24] (also see Fig. **4**).

Monastrol

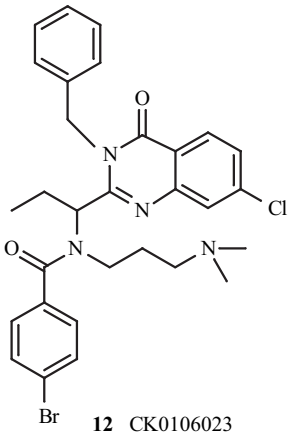
Monastrol is the first KSP inhibitor identified by a phenotype-based screening approach [18]. It binds to the Eg5-ADP complex and impairs ADP release, resulting in inhibition of productive microtubule-binding [25,26]. Monastrol inhibits KSP-driven microtubule motility with a half maximal inhibitory concentration (IC_{50}) about 14 μM. In the cell culture, monastrol causes formation of monoastral spindles followed by mitotic arrest with an IC_{50} value of about 50 μM [19]. Due to its weak binding and inhibitory activity with KSP, monastrol can only serve as a useful tool for cell cycle study rather than for clinical use as a drug. Nevertheless, its highly selective binding to KSP as well as its inhibitory activity towards this motor protein *in vitro* provide a useful prototype for rational designs of more effective compounds for cancer treatment [19,25-29].

CK0106023

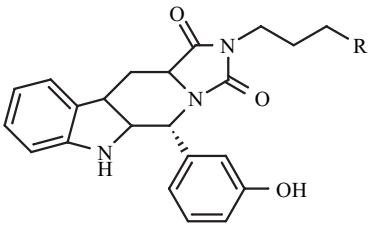
CK0106023 is identified through a combination of screening small synthetic organic compounds and chemical optimization. Like monastrol, CK0106023 is an allosteric inhibitor of KSP motor domain that binds KSP with high specificity. It inhibits the KSP ATPase activity with a *K*i of 12 nM and causes mitotic arrest and growth inhibition in



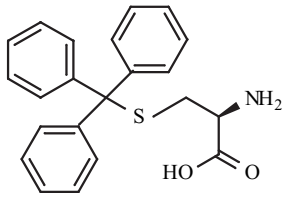
11 Monastrol



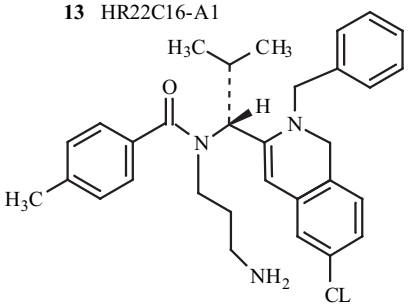
12 CK0106023



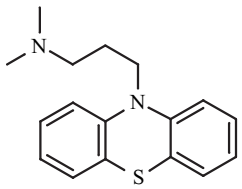
13 HR22C16-A1



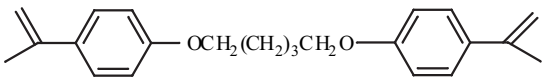
14 S-trityl-L-cysteine



15 SB-715992 (Ispinesib)



16 chlorpromazine



17 pentamidine

Fig. (4). Examples of chemical structures of kinesin spindle protein inhibitors.

several human cancer cell lines including three multidrug-resistant lines at a relatively low concentration (GI_{50} =364 nM). In ovarian tumor-bearing mice, CK0106023 exhibits anti-tumor activities comparable to or exceeding that of paclitaxel due to its inhibition of KSP, leading to the formation of monoastral spindles. Given the observed *in vitro* activities, CK0106023 is expected to be a useful agent for cancer treatment in the clinic [14].

HR22C16

HR22C16, also a KSP inhibitor, was discovered during screening of 16,000 compounds using a microscopy-based forward chemical genetic approach. This compound has anti-mitotic activity and inhibits the Eg5 motor function *in vitro* with an IC_{50} of about 800 nM [20]. Moreover, a variety of analogs of HR22C16 with a stronger potency have also been developed. HR22C16 and its analogs inhibit cell survival in both Taxol-sensitive and taxol-resistant ovarian cancer cells with at least 30-fold greater efficacy than monastrol [20]. Taken together, HR22C16 has a promising anticancer activity that can be explored for the treatment of taxol-resistant cancer cells [20].

S-trityl-L-cysteine

S-trityl-L-cysteine is identified as a very potent KSP inhibitor by French scientists through screening small-molecule libraries. The IC_{50} value of this compound that inhibits the basal ATPase activity and microtubule-activated ATPase activity is 1.0 μM and 140 nM, respectively. This compound induces mitotic arrest in HeLa cells with characteristic monoastral spindles at IC_{50} value of 700 nM [21]. Further studies show that both S-trityl-l-cysteine and monastrol bind Eg5 at the same region; both inhibitors trigger similar local conformational changes within the interaction site [30].

SB-715992 (Ispinesib) and SB-743921

SB-715992 (Ispinesib) [31] and SB-743921 [32] are two novel KSP inhibitors developed by Cytokinetics and GlaxoSmithKline. SB-715992 has been studied in a large clinical trial program that consists of nine Phase II trials and five Phase I/Ib trials [33]. This compound may have the potential for low toxicities to non-dividing cells compared to other anti-cancer drugs targeting microtubule dynamics. In preclinical efficacy studies, doses of SB-715992 substantially below the maximum tolerated dose (MTD) produce prominent inhibition of tumor growth, leading to tumor regression and cures. The safety profile and the efficacy of SB-715992 used alone or in combination with other chemotherapy drugs are being evaluated in a broad range of tumors including ovarian cancer, non-small cell lung carcinoma, breast cancer, colorectal cancer, prostate cancer, renal cancer, cell hepatocellular cancer, head and neck cancer, melanoma, and leukemia [34].

To date, there is no information available from public databases about the chemical structure of SB-743921. Like SB-715992/Ispinesib, SB-743921 is a potent and selective inhibitor of KSP with a K_i of about 100 pM, causing mitotic arrest and growth inhibition of tumor cells. In pre-clinical studies, SB-743921 causes mitotic arrest and potent inhibition of tumor cell proliferation. SB-743921 is active in a wide range of human tumor xenograft models. In some models, doses well below MTD induce complete tumor regressions. A clinical trial to determine tolerability and pharmacokinetics is also in progress [31].

CRx-026

CRx-026 is an excellent case in syncretic drugs discovery. It was discovered through a high-throughput combinatorial screening of tens of thousands of existing compounds for compounds that selectively kill human tumor cell lines [35]. CRx-026 is a combination of chlorpromazine, a phenothiazine sedative, and pentamidine, an anti-infective agent [35]. Although neither compound was approved for its anti-tumor activity before, chlorpromazine is confirmed to be a KSP inhibitor while pentamidine is hypothesized to be an inhibitor of PRL phosphatases, which play an important role in regulating mitotic progression and chromosomal separation [36]. CRx-026 effectively inhibits tumor formation in a nude mouse xenograft model whereas the individual compounds alone are less effective [36].

AURORA KINASE INHIBITORS

1. Biological Function of Aurora Kinases

Aurora kinases are protein serine/threonine kinases essential to mitotic progression [37]. In mammalian cells, the Aurora kinase family consists of three members, namely, Aurora A, Aurora B, and Aurora C. These kinases share a very conserved catalytic domain and participate in regulating mitotic processes although there exist some differences in their subcellular localization and mitotic functions [37,38]. Aurora A is localized to duplicated centrosomes and spindle poles during mitosis. Functional studies show that this protein is required for centrosome maturation, separation, and mitotic spindle formation. Suppression of Aurora A expression by RNA interference (RNAi) delays mitotic entry in human cells and over-expression of this kinase compromises spindle-checkpoint function and inhibits cytokinesis [37]. Different from Aurora A, Aurora B is a chromosome passenger protein, which is localized to the centromeric region of chromosomes in early stages of mitosis; it translocates to the spindle equator and the spindle midzone during anaphase A, and to the midbody between anaphase B and cytokinesis [37]. It is believed that this protein is actively involved in regulating chromosome alignment and segregation, spindle-checkpoint function, and cytokinesis. Over-expression of kinase dead Aurora-B protein blocks the attachment of chromosomes to mitotic spindles, strongly suggestive of defective kinetochores [37]. In addition, impaired functions of Aurora B as the result of RNAi or antibody injection result in spindle checkpoint failure because the cells are unable to undergo mitotic arrest in response to exposure to nocodazole and paclitaxel [37]. To date, little is known about the function of Aurora C although recent studies show that it is a centrosome-associated kinase that may also play a role in the development and progression of cancer [37].

Deregulated expression of Aurora kinases is closely associated with tumorigenesis. Indeed, many studies show that Aurora-A [37,39,40] and Aurora B [40] genes are either over-expressed or amplified in a broad range of tumors. The Aurora A gene is localized to 20q13, an amplicon associated with poor prognosis in patients with breast and colon

Table 2. Aurora Kinase Inhibitors

Name	*In Vitro* Enzyme Assays (IC_{50} or Ki)	Cell Based Assays (IC_{50})	Anti-tumor Activity in Animal Studies	Specificity for Aurora Kinase Inhibition
VX-680	Aurora A, 0.6 nm Aurora B, 18 nm Aurora C, 4.6 nm	15-304 nM	AML, pancreatic, and colon cancer xenografts	Aurora kinases, Fms-related tyrosine kinase (Ki=30 nM)
ZM447439	Aurora A, 110 nM Aurora B, 130 nM	~2 μM	N/A	Aurora A, Aurora B
Hesperadin	Aurora B immuno-precipitates, 250 nM	20 nM	N/A	Aurora B, AMPK, Lck, MKK1, MAPK, AP-K1, Chk1, and PHK (IC_{50}=1 μM)
Compound 18	Aurora A, 27 nM	50-500 nM	N/A	Aurora A
Compound 667	N/A	$p53^{+/+}$, 4.0 nM $p53^{-/-}$, 2.0 nM $p21^{-/-}$, 1.5 nM	N/A	Aurora B
Inhibitor from cyclacel	Aurora A, Aurora B, nM ranges	N/A	Leukemia and non-small cell lung cancer	Aurora A, Aurora B
R763	Aurora kinases, nM ranges	5-106 nM	N/A	Aurora kinases

N/A, Not Available; Ki, Inhibitory Constant; IC_{50}, Inhibition concentration 50.

cancers [37,39]. Recently, a systematic analysis using the gene profiling technology shows that Aurora A and Aurora B mRNAs are significantly over-expressed in primary tumor specimens of diverse origins and various tumor stages when they are compared with normal control samples [37], indicating a coordinated regulation of expression of these two Aurora proteins.

2. Aurora Kinase Inhibitors

Due to the important role of Aurora kinases in the mitosis as well as tumorigenesis, great efforts have been directed to the development of compounds targeting these molecules. In fact, many Aurora kinase inhibitors have been developed, which include VX-680 **(18)** [41], ZM447439 **(19)** [42], and Hesperadin **(20)** [43]. Preclinical studies show that these compounds exhibit potent anti-proliferation activities (see Fig. **5**).

VX-680

VX-680 is a 4,6-di-amino pyrimidine, suggesting its potential function as an ATP competitive inhibitor. This compound is highly selective in blocking the activity of Aurora A, B, and C with inhibitory constants (Ki) of 0.6, 18.0, and 4.6 nM, respectively. Except for Fms-related tyrosine kinase 3 (Ki=30 nM), it does not inhibit any other members of a panel consisting of 60 protein kinases [41]. Functional assays show that VX-680 significantly blocks cell growth and induces cell cycle arrest in a wide variety of human tumor cells with IC_{50} values ranging from 15 to 113 nM, which is accompanied by accumulation of cells with 4N DNA content; the cell cycle arrest is followed by rapid apoptosis, which is especially effective towards leukemia, lymphoma and colorectal cancer cell lines [41]. In addition, this compound also inhibits the colony formation of primary acute myelogenous leukemic (AML) cells from patients who have failed in standard therapies. It is interesting to note that about half of these patients harbor a mutation in *Flt3* (coding for Fms-related tyrosine kinase), resulting in its activation. In fact, this mutation is a poor prognostic marker for those patients with AML. In the nude mouse models bearing human AML, pancreatic cancer, or colon cancer xenografts, VX-680 regresses the tumor size in a dose-dependent manner. Given the evidence, VX-680 is considered a promising compound for AML patients, especially when they fail to respond to standard treatments [41].

ZM447439

ZM447439 is a quinazoline derivative, which works as an ATP competitive inhibitor as well. The crystal structure of Aurora A reveals that ZM447439 occupies the ATP binding pocket and an adjacent cleft [42]. This compound is highly selective for inhibiting Aurora A and B with IC_{50} values of 110 and 130 nM, respectively; it does not inhibit most mitotic kinases including Cdk1 and Plk1 [42]. During *in vivo* assays using a panel of human cell lines, ZM447439 inhibits cell division, resulting in accumulation of a DNA content of 4N or more. It appears that p53-deficient cells are more likely to override ZM447439-induced cell cycle arrest by continuing cell cycle progression, which eventually leads to loss of viability, suggesting the possibility that ZM447439 can selectively kill p53-deficient tumor cells [42,44].

Hesperadin

Hesperadin is a novel indolinone. The crystal structure of the ternary complex (Aurora B60–361/INCENP790–847/Hesperadin) at 1.8 Å resolution shows that this compound also competes with ATP for binding to the kinase [45]. *In vitro* kinase assays reveal that Hesperadin inhibits immunoprecipitated Aurora-B with an IC_{50} value of 250 nM; however, this compound markedly reduces the activities of six other kinases (AMPK, Lck, MKK1, MAPK-

APK1, Chk1, and PHK) [43]. *In vivo* assays show that 20 nM Hesperadin is sufficient to cause polyploidy in HeLa cells due to induction of defects in mitosis and cytokinesis [43]. In addition, Hesperadin overrides the spindle checkpoint in HeLa cells treated with taxol and monastrol but not with nocodazole [43].

Although the mechanism of anti-proliferative activities for Hesperadin, VX-680, and ZM447439 remains unknown, it is clear that they inhibit phosphorylation of histone H3 on serine 10 [41,42,43]. Interestingly, although these three compounds block the activity of both Aurora A and Aurora B, the mild phenotypes such as assembly of bipolar spindles and formation of polyploid cells caused by treatment with ZM447439 and Hesperadin are reminiscent of those due to Aurora B deficiency [46]. One possible explanation is that mitotic requirement for Aurora A kinase is bypassed in the absence of Aurora B [46].

Derivative of 1,4,5,6-Tetrahydropyrrolo[3,4-c]Pyrazole Bi-cycle

The ATP binding pocket in protein kinases is a logical target for designing small molecular inhibitors due to their important catalytic function. Ideal compounds should bind to this region by competing with ATP, thereby inactivating the kinase function of these proteins. The ATP binding pocket as well as divergent residues lining the ATP binding cavity of different kinases can provide the molecular basis for rational designing of selective kinase inhibitors. One example of utilizing this strategy is the identification of Aurora kinase inhibitor through combinatorial expansion based on the template [47]. Compound 18 **(21)** [47] (see Fig. **5**) exhibits a potent inhibitory activity for Aurora A with an IC_{50} value at 27 nM. At a concentration range from 50 nM to 500 nM, this compound causes accumulation of cells with a 4N DNA content in various cell lines and inhibits phosphorylation of histone H3 on serine-10 in HCT-116 cells [47]. Although additional studies are required for understanding its specificity and efficacy this method does provide a convenient approach to the development of kinase inhibitors [47].

Other Novel Aurora Kinase Inhibitors

A few new Aurora kinase inhibitors have been developed recently although the information about their biochemical and biological activities remains fragmentary. These compounds include Compound 677 (Memorial Sloan-Kettering Cancer Center) [50], AZD1152 (AstraZeneca) [48], MLN8054 (Millennium Pharmaceuticals) [49], CYC116 (Cyclacel) [51], and R783 (Rigel Pharmaceuticals) [52]. However, the chemical structures of those compounds remain proprietary and no information is available in public databases. (i) Compound 677 is a novel, selective inhibitor of Aurora B that targets the division of tumor cells. Colony formation analyses reveal that it has an IC_{50} value of 4 nM for $p53^{+/+}$ cells; both $p53^{-/-}$ and $p21^{-/-}$ cells display an increased sensitivity to Compound 677 with an IC_{50} 2.0 and 1.5 nM, respectively. Cells treated with this compound become polyploid, which is accompanied by inhibition of phospho-histone H3; normal bipolar spindles are formed but chromosome segregation is inhibited [50]. In addition, apoptosis induced by this compound is not significant until 6 days post treatment [50]. Colony formation assays reveal that Compound 677 exhibits a synergistic effect with SN-38 (the active metabolite of CPT-11), docetaxel, vinorelbine, gemcitabine, oxaliplatin, or 5-fluorouracil [50]. Interestingly, SN-38 or gemcitabine administered prior to the treatment with Compound 677 antagonizes the effect exhibited by either compounds alone [50]. (ii) CYC116 (2-anilino-4-heteroaryl-pyrimidines) selectively and potently

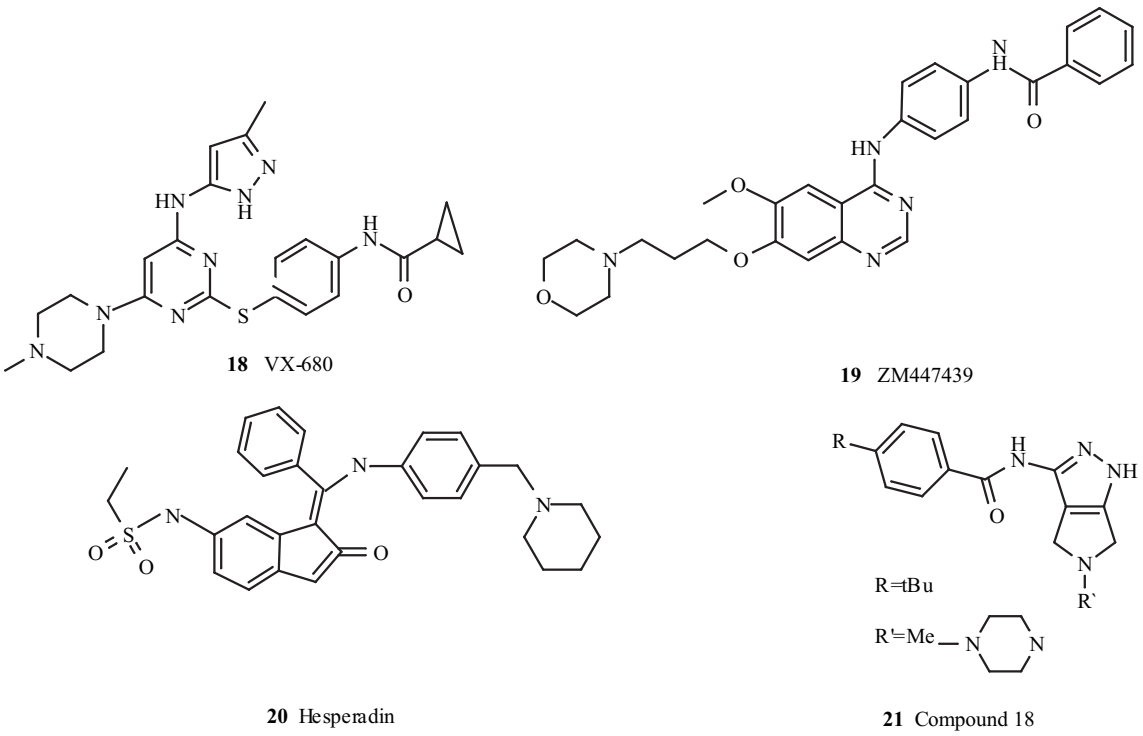
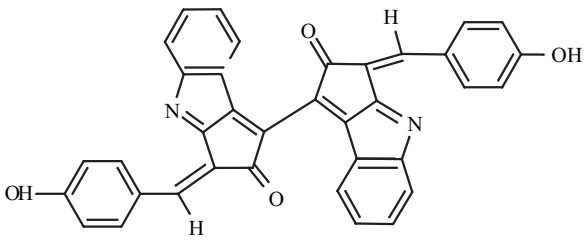
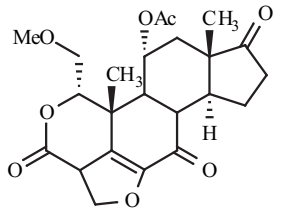


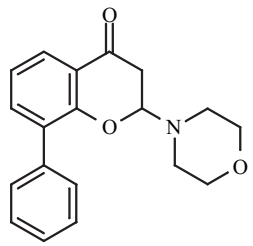
Fig. (5). Examples of chemical structures of prominent Aurora kinase inhibitors.



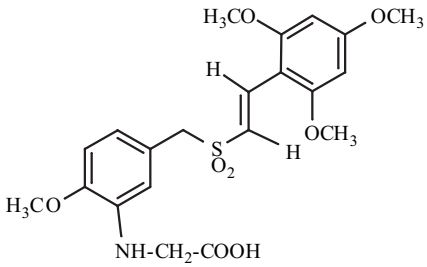
22 Scytonemin



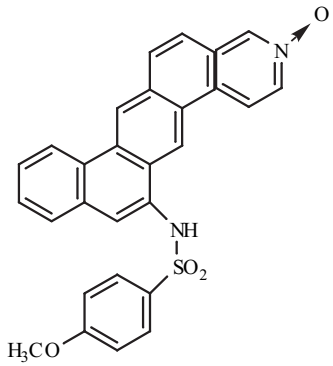
23 Wortmannin



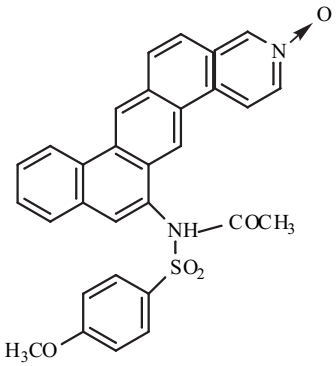
24 LY294002



25 ON01910



26a HMN-214



26b HMN-176

Fig. (6). Examples of chemical structures of Plk inhibitors.

inhibits Aurora kinases A and B at low nM concentrations, killing a wide range of tumor cells [51]. Cells treated with this compound lack histone H3 phosphorylation, enter mitosis slowly, exhibit defects in cytokinesis, and become polyploid [51]. Moreover, it significantly inhibits tumor growth in a non-small cell lung cancer *in vivo* and prolongs the survival of mice with leukemia [51]. (iii) R763 is also a highly selective inhibitor towards Aurora kinases. It exhibits an activity more potent than VP-680 in various tumor cell assays [52].

POLO-LIKE KINASE INHIBITORS

1. Biological Function of Polo-like Kinases

Polo-like kinases (Plks) are a family of protein serine/threonine kinases highly conserved among eukaryotes. In mammals, The Polo kinase family consists of four members, namely, Plk1, Plk2, Plk3, and Plk4. In addition to the conserved kinase domain, Plks share unique motifs termed Polo box domains (PBDs) that are present in the C-terminus of this group of proteins [53]. Several studies show that PBDs play a critical role in regulation of the subcellular localization probably through interaction with certain phosphorylated proteins critical for cell proliferation [54].

Plks have multiple functions in regulating the cell cycle, especially during mitosis [53]. Plks are able to activate the Cdk1/cyclinB complex, which is the key molecule to initiation of mitosis entry. Plk1 also phosphorylates components of the anaphase-promoting complex such as Cdc16 and Cdc27, suggesting that Plk1 is an important regulator of metaphase and anaphase transition. This notion is confirmed by multiple studies that depletion of Plk1 via RNAi arrests cells primarily at the metaphase/anaphase transition [54,55]. Plks are also required for completion of mitosis since point mutations or N-terminal truncation cause cytokinesis failure [54,55]. Numerous studies show that Plk1 is a proliferation marker [56]. Over-expression of Plk1 is detected in a majority of human tumor cells and tumor cell lines [56,57]. In addition, elevated Plk1 expression is closely associated with poor prognosis in cancer patients as

Table 3. Plk Inhibitors

Name	*In Vitro* Enzyme Assays (IC_{50})	Cell Based Assays (IC_{50})	Anti-tumor Activities in Animal Models	Specificity
Scytonemin	2 μM	1.5-7.8 μM	N/A	Plk1, Myt1, Chk1, CDK1/cyclin B, PKC2 (IC_{50}=2 μM)
Wortmannin	5.8-24 nM	50-500 nM	Tumor xenografts	Plk1 (IC_{50}=24 nM) p110 (IC_{50}=2-5 nM) PI3K (IC50=400 nM) PI4K, ATM, DNA-PK, mTOR, ATR (IC_{50}=70-3,500 nM)
LY294002	24 nM	50μM	Colon cancer, NSCLC, and bladder cancer xenografts	Plk1 (IC_{50}=24 nM) p110 (IC_{50}~1.0 μM) PI3K (IC_{50}=_20 μM) PI4Kβ, mTOR, DNA-PK (IC_{50}=1-100 μM)
ON01910	~10 nM	50-200 nM	Liver, breast, and pancreatic cancer xenografts	Plk1, PDGFR, Abl, Flt-1 (IC_{50}=18-42 nM) CDK1, Plk2, Src, Fyn (IC_{50}=182-260 nM)
HMN-176 & HMN-214	N/A	nM ranges	Mouse xenografts	Plks and CDKs
CYC 800	low nM ranges	N/A	N/A	Plk1
R763	nM ranges	5-106 nM	N/A	Plk1 Aurora kinases

N/A, Not Available; IC_{50}, Inhibition Concentration 50

well as the metastatic potential of certain tumors [57]. Thus, it is believed that Plk1 plays a causative role in oncogenic transformation [56,57].

2. Plk Inhibitors

Many Plk inhibitors have been developed and they include Scytonemin **(22)** [58], Wortmannin **(23),** LY294002 **(24)** [59-61], ON01910 **(25)** [62], HMN-214 and HMN-176 **(26)** [63], and CYC 800.

Scytonemin

Scytonemin, originally isolated from cyanobacteria, is the first Plk1 inhibitor discovered. Scytonemin blocks Plk1 activity with a mixed mechanism functioning both as a competitive and noncompetitive inhibitor [58]. This compound also exhibits inhibitory activity towards Myt1, Chk1, CDK1/cyclin B, and PKC2 with an IC_{50} value around 2 μM [58]. Scytonemin suppresses the growth of a variety of normal or transformed cells stimulated by cytokines [58]. Despite of its inhibitory activities to some other cellular kinases, Scytonemin can at least serve as an important prototype from which new compounds with a higher specificity towards tumor cells and better efficacy can be discovered [58].

Wortmannin and LY294002

Wortmannin and LY294002 are initially considered as inhibitors of phosphatidylinositol 3-kinase (PI3K) [59-61]. Recent studies indicate that both compounds significantly inhibit Plk1 activity with an IC_{50} value at about 24 nM in *in vitro* kinase assays [59]. When Jurket cell lysates are used, Wortmannin and LY294002 can also inhibit Plk1 activity with IC_{50} values at 5.8 nM and 2.1 μM, respectively. Further studies indicate that the concentrations (50–500 nM) that inhibit PI3K *in vivo* can also inhibit the Plk1 activity [59].

ON01910

ON01910 is the most promising compound developed specifically for inhibiting Plk1. Instead of targeting the ATP binding pocket like most other inhibitors, this compound targets the substrate binding region of Plk1. Given frequently observed mutations in the ATP binding pocket, it is more rationale to design drugs that target the substrate binding region as it is subjected to less selection pressure, thus more stable. In addition, this type of compound can avoid direct competition with a high concentration of endogenous ATP. Therefore, it is predicted that slow drug resistance, if any, would be developed to this type of compound [62].

During *in vitro* kinase assays, ON01910 efficiently inhibits Plk1 at an IC_{50} value as low as 10 nM; at higher concentrations, inhibition of other protein kinases has also been observed [62]. In addition, this compound induces apoptosis of 94 different tumor cell lines including multi-drug resistant (MDR) positive cell lines with GI_{50} values ranging between 50 to 200 nM [62]. ON01910-induced cell death appears to result from spindle abnormalities and prolonged G_2/M arrest. In xenograft mouse models, ON01910 inhibits growth of a wide variety of human tumors including those derived from liver, breast, and pancreatic cancers; it synergizes with several chemotherapeutic agents such as oxaliplatin and doxorubicin, leading to complete regression of tumors; animals also tolerate this compound very well, exhibiting no apparent side effects such as myelotoxicity, neuropathy, and cardiotoxicity [62].

HMN-176 and HMN-214

HMN-176, an active metabolite of HMN-214 ((*E*)-4-{2-[2-(*N*-acetyl-*N*-[4-methoxybenzenesulfonyl] amino) stilbazole]}1-oxide), shows a potent cytotoxic effect towards various human tumor cell lines [63,64,65]. It causes mitotic arrest through destruction of spindle polar bodies leading to apoptosis. Gene profiling reveals that HMN-176 can potentially overcome tumor drug resistance [65]. In mouse xenograft models, HMN-214 has a potent antitumor activity [64]. In phase I clinical trials, HMN-214 also causes neutropenia; in some patients, minor sensory neuropathy and ileus are also observed [63]. Several studies show that HMN-176 alters subcellular localization of Plk1, presumably negatively affecting the cellular function of this kinase [63].

CYC 800

CYC 800 is a highly selective and potent Plk1 inhibitor developed by Cyclacel Inc. This compound competes with the ATP binding site and specifically inhibits Plk1 at low nM ranges. It induces mitotic arrest and exhibits favorable anti-tumor effects when tumor cell lines are used for *in vitro* assays [51]. The chemical structure of this compound is not available from public databases.

CONCLUSIONS AND PERSPECTIVE

During the past decade, several categories of new compounds that target mitotic proteins have been developed, many of which are currently under clinical trials. These compounds appear to be superior to those existing compounds used as chemotherapeutical drugs. Collectively, these compounds exhibit several salient features such as potent activities towards inhibition of target molecules, selectively killing p53-deficient cells, less cytotoxicity to normal cells, no or low myelotoxicity and neurotoxicity, and/or less potential to develop drug resistance. It is particularly interesting to note that some of these compounds induce complete regression of tumors in animal models.

Mitotic catastrophe is a special type of programmed cell death due partly to the abnormal activation/inactivation or the maintenance of mitotic kinases. Recent studies have identified two subtypes of mitotic catastrophe. Mitotic catastrophe occurs (i) during or close to metaphase, and (ii) after failed attempt to undergo successful mitosis. In both conditions, cells die of apoptosis as a result of caspase activation and mitochondrial membrane permeabilization. Many types of tumor cells are defective in mitotic checkpoints, which would make them more susceptible or sensitive to stresses by induction of apoptosis/mitotic catastrophe. Thus, further studies of mitotic control would lead to identification of additional targets for rationale drug design for cancer intervention.

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