Small Molecule Kinase Inhibitors as Anti-Cancer Therapeutics

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Abstract: Protein kinases have emerged as the most important class of targets in oncology drug discovery because of their major roles in regulating cellular growth and survival. At least, 11 kinase inhibitors have received FDA approval to be used as cancer treatments, and there are continuous efforts to bring more candidates from laboratory benches to the clinic. Although many protein kinase inhibitors directly interact with the ATP binding site, other can alter the kinase conformation to prevent productive ATP binding. Herein we discuss the different mechanisms of action of kinase inhibitors and provide classification of the inhibitors according to their binding sites. Some of these are allosteric inhibitors, ATP competitive inhibitors, protein substrate competitive inhibitors, and covalent bond forming inhibitors. This review provides a broad overview of the relation between mechanism of action and the issues of target selectivity and resistance. Special attention was given to the kinase inhibitors currently in clinical trials.

Keywords: Allosteric inhibitors, anti-cancer agents, antiproliferative activity, ATP competitive inhibitors, non-ATP competitive inhibitors, protein kinase, target specificity.

INTRODUCTION

More than 500 human genes encode proteins that serve as kinases. 478 of these belong to a single group whose catalytic domains are related in sequence and are classified into a number of subsets or families based mainly on sequence and structure similarities [1-3]. Protein kinases catalyse the transfer of the terminal phosphoryl group of ATP to specific hydroxyl groups of serine, threonine or tyrosine amino acid residues of their protein substrates. Therefore, they can be grouped broadly as serine/threonine or tyrosine kinases. In some cases dual specificity kinases, such as mitogen-activated protein kinase kinases (MEKs) [4], phosphorylate both serine/threonine and tyrosine hydroxyl residues. Protein phosphorylation regulates a diverse range of cellular processes including proliferation and apoptosis, so even mild changes in kinase activity may lead to a variety of diseases such as cancer. This crucial role has made kinases an extremely important and tractable therapeutic class for anti tumour drug discovery [5-7].

KINASES AS DRUG TARGETS IN CANCER

Kinases, which are validated as targets for anti cancer agents development, fall into one of three main classes [5]. First, there are kinases which have become unresponsive to normal regulatory cascades after a mutation or translocation. These have transforming capability and are considered oncogenic. Many cancers that contain multiple genetic, epigenetic, and chromosomal abnormalities are dependent on or addicted to one or few of this class of kinases for maintenance of proliferation, survival and malignant phenotype [8, 9]. The so-called oncogene addiction makes the malignant cell dramatically sensitive to the respective kinase inhibitor [10]. The MET tyrosine kinase gene deregulation with consequent kinase activation has been reported in MET-addicted cancers such as gastric, oesophageal and non small cell lung carcinomas and medulloblastomas [11]. Treatment of these cancers with MET inhibitors results in dramatic response such as massive apoptosis or proliferative block [12]. The importance of mutated kinases, to which cancers are addicted, has motivated an intensive effort to screen the kinome across a wide range of tumour types for mutations, amplifications and overexpression [13, 14]. The discovery that many tumours are addicted to Phosphatidylinositol 3 kinases (PI3Ks) mutations was one of the most notable successes [15-17]. Similarly, the discovery that many malignancies are addicted to the activating mutations of Janus kinase 2 (JAK-2) has stimulated the development of several JAK-2 inhibitors into Phase I trials [18-21]. Addiction to activating mutations in anaplastic lymphoma kinase (ALK) have also been found in neuroblastoma [22-24], non-small cell lung cancer and anaplastic large cell lymphomas [25].

The second group of kinases that can be targeted by anti-cancers consists in synthetic lethal kinases. Two genes are considered synthetic lethal if mutation of either gene alone does not affect cell viability but simultaneous mutation of both causes apoptosis [26, 27]. Targeting a kinase that is synthetic lethal to a tumour related mutated gene should affect only malignant and spare normal cells. Thus, synthetic lethality provides a basis for the discovery and development of cancer specific cytotoxic drugs [28-30]. Inhibition of the second class of kinase targets results in a synthetic lethal

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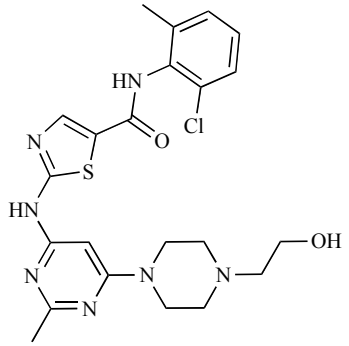
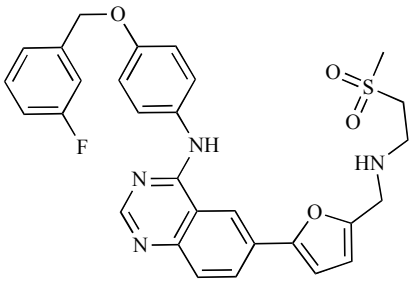
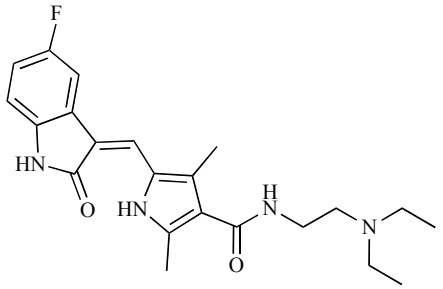
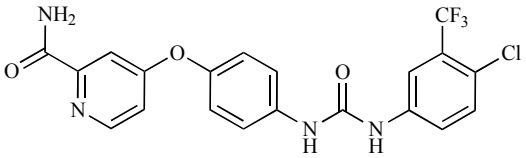
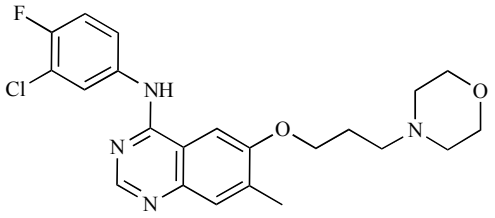
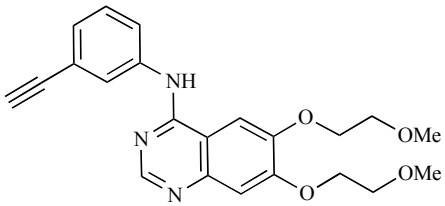
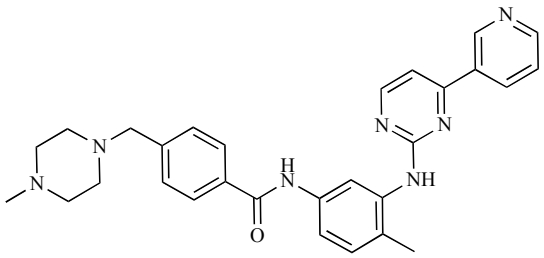
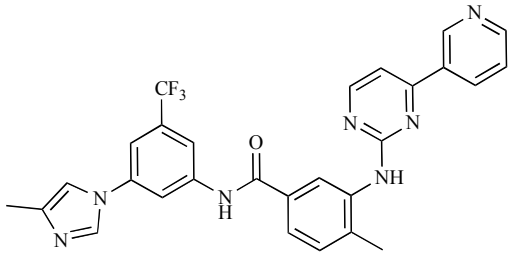
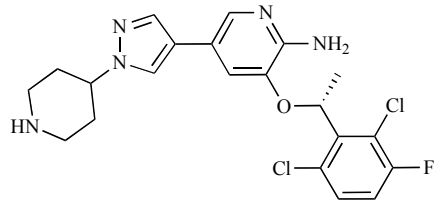
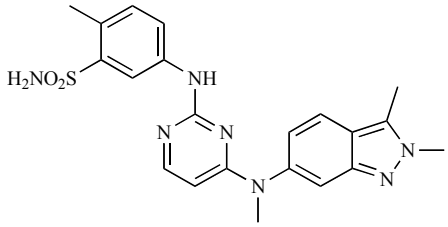
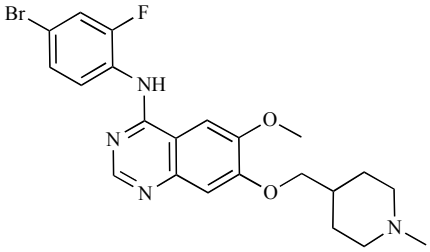
1875-5607/12 $58.00+.00

Table 1. FDA-Approved Kinase Inhibitors

Structure	Name	Targets	Indications	Ref
	Dasatinib (Sprycel®; BMS)	Bcr-Abl, SRC family, TEC family, c-Kit	Imatinib-resistant CML	[5, 48, 49]
	Lapatinib (Tykerb®; GSK)	ERBB2, EGFR/HER2	Breast cancer	[48, 50, 51]
	Sunitinib (Sutent®; Pfizer)	c-KIT, PDGR, VEGFR	Renal cancer, imatinib-refractory GIST, Pancreatic neuroendocrine tumors	[48]
	Sorafenib (Nexavar®; Bayer/ Onyx)	c-KIT, PDGFR, VEGFR	Renal cancer, advanced primary liver cancer	[48, 52, 53]
	Gefitinib (Iressa®; AstraZeneca)	EGFR, ERBB2, HER4	NSCLC	[48, 54, 55]
	Erlotinib (Tarceva®; OSI/ Genentech/ Roche)	EGFR, ERBB2	NSCLC, pancreas cancer	[48, 56, 57]

(Table 1). Contd…..

Structure	Name	Targets	Indications	Ref
	Imatinib (Gleevec®; Novartis)	Bcr-Abl, c-Abl, PDGFR, c-KIT, DDR1	CML, GIST, HES	[48]
	Nilotinib (Tasigna®; Novartis)	Bcr-Abl, c-Abl, PDGFR, c-KIT, DDR1	Imatinib-resistant CML	[5, 48]
	Crizotinib (Xalkori®; Pfizer)	ALK	NSCLC with ALK mutation	[58-60]
	Pazopanib (Votrient®; GSK)	c-KIT, PDGR, VEGFR	Renal cell carcinoma	[61-63]
	Vandetanib (Caprelsa®; AstraZeneca)	VEGFR, EGFR, RET	Medullary thyroid cancer	[64-66]

CML (chronic myelogenous leukemia), NSCLC (non-small cell lung cancer), GIST (gastrointestinal stromal tumours), HES (hypereosinophilic syndrome).

phenotype when it is coupled with the mutation in the particular pathology of the cancer cell. Although these kinase targets are neither oncogenic nor frequently mutated in cancer, they are required for the survival and proliferation of tumour cells and may be key players in vital signalling pathways. Examples include the mammalian target of rapamycin (mTOR), which is an important player in the Phosphatidylinositol 3 kinase-protein kinase B (PI3K–PKB) signalling pathway and has a synthetic sick relationship with the phosphatase and tensin homolog (PTEN) frequently mutated in cancer [31-33], and mitogen-activated protein kinase kinase 1 and 2 (MEK 1 and 2) which are synthetic lethal to activating mutation in B-Raf kinase [9, 34, 35]. Other important examples of this class of kinase targets include kinases required to maintain cell survival and proliferation, such as Aurora kinases, which are critical during mitosis for the maintenance of genomic stability [36, 37]; the cyclin-dependent kinases (CDKs), which control cell cycle progression [38, 39]; and the polo-like kinases, which are crucial for mitosis and cytokinesis [40, 41].

The third group of targets are expressed in the tumour or surrounding tissues and are required for tumour formation and maintenance in the human host. For instance,

Mammalian LIM kinase 1 (LIMK1) is reported to play an important role in cell motility, angiogenesis and invasion [42, 43] and the neurotrophic growth factor receptor tyrosine kinase (nTRK2) is essential for cells to survive detachment and is required for tumour cell metastasis [5]. Examples also include the vascular endothelial growth factor receptor (VEGFR) and the FGFR kinases, which are crucial in developing and maintaining tumour blood supply [5, 44]. Pyruvate kinase and protein kinase B, essential for the switch to aerobic glycolysis (Warburg effect) in tumour cells, represent another example [45-47].

Molecular technologies facilitated the discovery of the recurrent kinases aberrations in cancer. The comprehensive analysis of the full spectrum of kinome changes in each human cancer will be essential for improved cancer diagnosis and treatment and will promote our fundamental understanding of the kinase pathways and networks perturbed by genomic aberrations. The full understanding of the kinase aberrations can improve cancer diagnosis through more developed molecular classification, enhance the selection of cellular targets for drug development and facilitate the design of faster and more efficient clinical trials using agents targeted to specific kinomic abnormalities. A comprehensive review of kinase aberrations implicated in different human cancer types was recently published [5]. Kinase inhibitors that have received FDA approval to be used as cancer treatments are summarised in Table **1**.

Kinase inhibitors according to their binding site

The popularity of kinases as oncology drug targets was driven by the high profile success, 80% response rates in chronic myelogenous leukemia (CML) patients [67], of the first FDA approved small molecule protein kinase inhibitor imatinib [68, 69]. Although many kinase inhibitors interact directly with the ATP binding pocket (type I inhibitors), alternative approaches targeting sites other than the ATP cleft are increasingly being pursued. Type II inhibitors also bind to the ATP binding pocket, but they also exploit unique interactions with a hydrophobic pocket directly adjacent to the ATP binding site [70].Type III kinase inhibitors, sometimes called simply allosteric inhibitors, bind to a hydrophobic pocket remote from the ATP binding site. They do not perturb the binding of ATP, but they rather induce conformational changes that make the kinase catalytically inert. Other types of kinase inhibitors, such as covalent bonding and protein substrate competitive will be also briefly discussed in this review.

TYPE I KINASE INHIBITORS

Kinases share a bi-lobed catalytic structure with the ATP binding site in a deep cleft located between the lobes [1, 71]. ATP binds to the cleft through hydrogen bonds formed between the ATP adenine ring and the kinase hinge region, which connects the amino- and carboxy-terminal domains. ATP ribose and triphosphate groups bind to the hydrophilic channel near the substrate binding site that features conserved residues essential for catalysis. Kinases have a conserved activation loop that regulates the enzyme activity and is tagged by conserved DFG and APE motifs at the start and the end of the loop, respectively. The activation loop has different conformations which may be catalytically active and often phosphorylated or inactive in which case the loop blocks the substrate binding site. In the active conformation (also called DFG-in conformation), the aspartic acid of the DFG motif points into the ATP binding site and the phenylalanine points away from the ATP site. Type I inhibitors recognize the active conformation of the kinase in which transfer of phosphate from ATP to the substrate is facilitated. They bind to the hinge segment of the target kinase with up to three hydrogen bonds in a way mimicking the hydrogen bonds normally formed by the ATP adenine ring. The majority of ATP competitive kinase inhibitors do not exploit the ribose or the triphosphate binding site of ATP (one exception is the novel Src and Abl dual family kinase inhibitor AZD0530 [72-74]). This group includes the majority of ATP competitive inhibitors. The large number of members in this group may be a consequence of many inhibitor compounds having been discovered by means of *in vitro* enzymatic assays in which kinases were used in the active conformation with low ATP concentration and because many kinase inhibitors have been designed and synthesised to mimic ATP. Molecules in this group typically consist of a heterocyclic ring system that fills the Adenine binding site and serves as a scaffold for the side chains which occupy one or both of the neighbouring hydrophobic regions I and II (Fig. **1**) [75, 76].

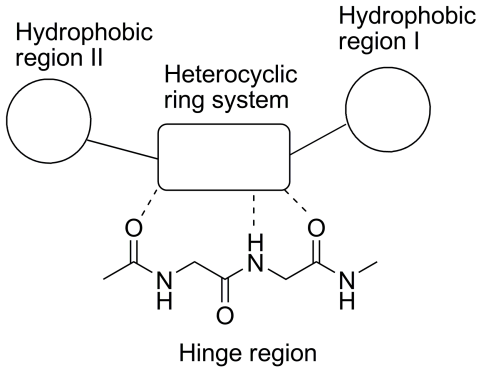


Fig. (1). ATP competitive inhibitors pharmacophore model. Hydrogen bonds are shown as dashed lines.

One recent example of this group is the MET tyrosine kinase inhibitor MK-2461 (Fig. **2**) [77] which was designed around 5H benzo[4,5]cyclohepta[1,2-b]pyridin-5-one scaffold and was efficacious in preclinical animal models. X-ray analysis has proved that MK-2461 binds preferentially to the activated (phosphorylated) form of the kinase [78]. Protein kinase CK2 is considered a prime cancer drug target because of the key roles of deregulated CK2 in cancer anti-apoptotic pathways. The suitability of CK2's small ATP binding site for the design of relatively selective inhibitors, led to the identification of the novel, first in class, orally bioavailable, ATP competitive inhibitor CX-4945 (Fig. **2**) [79] with an IC_{50} of 1nM in enzymatic assay and 100 nM in cellular assay [80]. The selective CK2 inhibitor CX-4945 is now progressing through phase I clinical trials. Another example of this type of inhibitor is the potent and relatively selective Polo-like kinase (PLK) family inhibitor BI-2536 [81] (Fig. **2**). Because PLK1 is the primary target, this

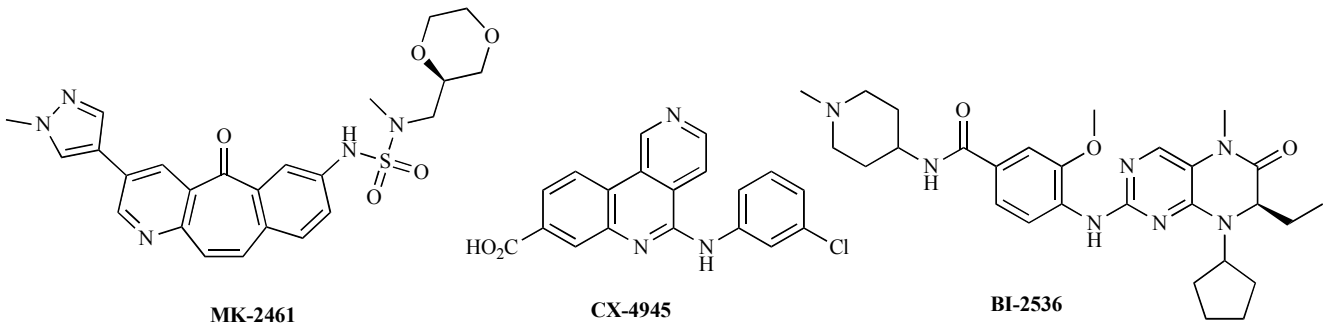


Fig. (2). Chemical structure of type I kinase inhibitors.

compound provided insights into the phenotypic outcome of PLK1 inhibition and showed that cells treated with BI-2536 are delayed in prophase and enter prometaphase after import of Cdk1-cyclin B into the nucleus. Moreover, treated cells lack prophase microtubule asters and form monopolar spindles that are unstably attached to kinetochores. BI-2536 prevents PLK1 localization at kinetochores and centrosomes and, when added to metaphase cells, leads to spindle collapse [81, 82]. BI-2536 is now progressing through phase II clinical trials for treating patients with recurrent or metastatic solid tumours.

TYPE II KINASE INHIBITORS

Type II inhibitors cause allosteric kinase inhibition through altered ATP binding and recognise the inactive conformation of the kinase. This is sometimes referred to as 'DFG-out' conformation where aspartic acid of the DFG motif points away from the ATP binding site and the phenylalanine points to the ATP site. Movement of the activation loop to the DFG out conformation exposes an additional hydrophobic allosteric binding pocket directly adjacent to the ATP binding site (Fig. **3**) [83-85]. Type II inhibitors use the ATP binding site and also bind to the available allosteric site. Because the amino acids in the allosteric pocket are less conserved than those in the ATP binding site, it may be easier to achieve better selectivity profiles with type II inhibitors. Many type II inhibitors form one or two hydrogen bonds (using amide, urea or heteroatom linkers such as nitrogen, sulphur or oxygen) with residues near the allosteric site: one bond with the side chain of a conserved glutamic acid and the other with the backbone amide of aspartic acid in the DFG-out motif. All type II inhibitors also have a hydrophobic moiety that occupies the allosteric site and forms Van der Waal's interactions with it [70].

The discovery that inhibitors like imatinib (Gleevec®) and sorafenib (Nexavar®) (Fig. **4**) bind to the inactive conformation was serendipitous, but subsequent analysis of similar kinase inhibitor co-crystal structures has shown that all share a common pharmacophore and have a conserved set of hydrogen bonds [86]. The novel MET receptor tyrosine kinase SGX-523 (Fig. **4**) is a notable example of type II inhibitors because it stabilizes MET in a unique inactive conformation that is inaccessible to other protein kinases, suggesting an explanation for the selectivity [87]. AAL-993 (Fig. **4**) inhibits angiogenesis through the inhibition of VEGF-R1, VEGF-R2 and VEGF-R3 and represents one of the latest examples of second type inhibitors. AAL-993 targets the inactive DFG out conformation of these VEGF-Rs and exhibits good biopharmaceutical properties and oral availability in animal models, with very good anti-tumour efficacy in an orthotopic animal model [75, 88]. VEGF-R inhibitors were recently reviewed in this journal [89]. The first reported case of a type II PLK1 inhibitor was SBE13 (Fig. **4**), which had sub-nanomolar (IC_{50} = 0.2 nM) inhibitory activity against PLK1. In addition to lack of activity on other mitotic kinases, such as Aurora A, SBE13 was selective for PLK1 over other tested PLK isoforms, including PLK2 (IC_{50} > 66 μM) or PLK3 (IC_{50} = 875 nM). Treatment of tumour cells with SBE13 resulted in decreased cellular proliferation, a G_2/M arrest followed by apoptosis, and formation of an abnormal mitotic phenotype, therefore justifying further preclinical investigation of this compound [90, 91].

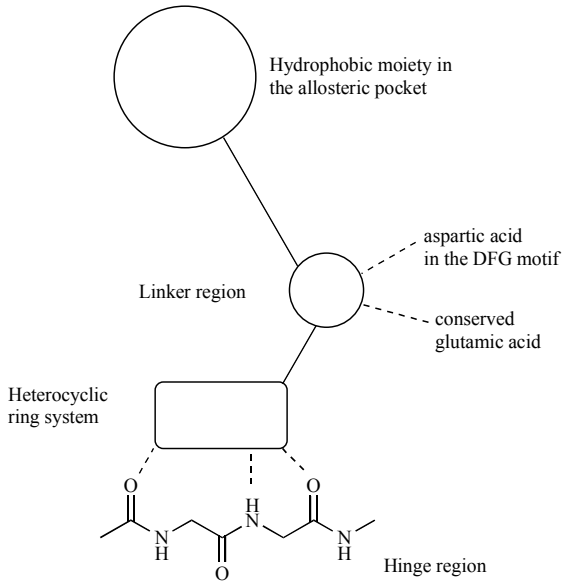


Fig. (3). Type II inhibitors pharmacophore model. Hydrogen bonds are shown as dashed lines.

The ATP competitive kinase inhibitors (type I and II) must have high affinity to be able to compete with intracellular ATP concentrations as high as 10 mM. Development of inhibitors that posses high target affinity and at the same time are selective enough to discriminate

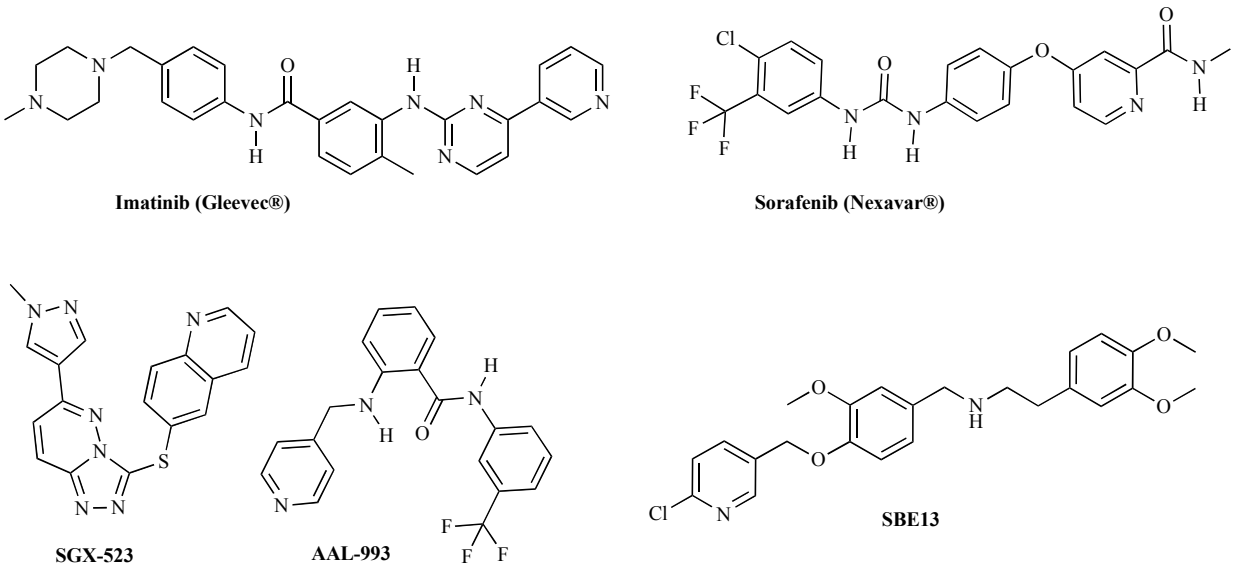


Fig. (4). Chemical structure of type II kinase inhibitors.

between the 518 human kinases is complicated by the highly conserved nature of the ATP binding pocket across the protein kinase family [91, 92]. The lower specificity for their planned target may restrict the clinical use of this class of inhibitors, especially in the case of strong off-target toxicities or side effects. Another problem with ATP competitive protein kinase inhibitors is drug resistance as a result of mutations in the ATP binding site which has been observed in patients undergoing treatment with imatinib. Consequently, there is now considerable interest in discovering new classes of inhibitors that do not directly compete with ATP. The new inhibitors may enable the selective regulation of certain protein kinases associated with cancer without affecting other kinases involved in normal physiological functions [75, 93].

However after the emerge of resistance of some cancers to kinase inhibitors, some researchers argued that the development of "clean" drug that selectively targets one protein in the aberrant pathway was not a great idea, and "dirty", or sometimes called "promiscuous", drugs that had been discovered few decades ago are still the most effective in treating cancer. The reason for this is that cancer is a complex disorder results from multiple molecular abnormalities, not from a single defect, e.g. the average number of mutant CAN-genes is 12 in breast cancers and 9 in colorectal cancers. So, a drug with a single target is unlikely to help because cells can often find ways to compensate for a protein whose activity is affected by a drug. "Dirty" drugs, though more toxic, target several kinases and bind to them less precisely than "clean" inhibitors. This is why it is considerably more difficult to dislodge "dirty" inhibitors as the mutation required to prevent them binding is then so severe thus of no value to the cell in its struggle to survive [94, 95].

TYPE III KINASE INHIBITORS

Type III inhibitors, sometimes simply called allosteric inhibitors, are the most selective type of kinase inhibitors. They bind to a pocket remote from the ATP binding site. They induce conformational changes that make the kinase catalytically inactive. Two recent examples of type III inhibitors will be discussed here.

MEK Inhibitors

The Ras-Raf-MEK-ERK signalling pathway, a crucial regulator of cell growth, differentiation and proliferation, continues to draw massive efforts to develop specific inhibitors to its components as potential treatment for cancer [96]. In early 1990s, researchers at Parke-Davis (now Pfizer Inc.) identified, by mass screening, PD0098059 and PD0045443 (Fig. **5**) as weak MEK inhibitors [97, 98] Interestingly, they were found to be not ATP or ERK competitive. However, the role of PD0098059 was limited to the *in-vitro* studies due to its low inhibitory activity (IC_{50} = 1.3 μM) and poor bioavailability. PD0045443, which exhibited a similar IC_{50} to PD0098059, was weakly active in cells at 30 μM. In attempts to increase the potency of this derivative, the 4-chloro substituent on the anthranilic acid was replaced by the H bond acceptor fluorine atom, resulting in PD0169373 with a resulting 70-fold increase of potency, however it also retained no cellular activity. This problem was partially resolved by masking the carboxylate function of PD0169373. The hydroxamic acid PD0170611 (Fig. **5**) showed much improved potency compared to PD0169373 attested by an IC_{50} of 7 nM against purified MEK and a cellular IC_{50} of 50 nM. However, the biological half-life of PD0170611 was too short at only 2h. This was attributed to the rapid metabolism of PD0170611 into a mixture of a glucuronide and the parent carboxylic acid. To overcome this rapid metabolism, the Parke-Davis medicinal chemists designed a focused array of hydroxamate esters. PD0184352 turned out to be the most active compound in this array and it exhibited the best combination of potency and stability. PD0184352 was the first MEK inhibitor to reach clinical trials, but failed in phase II due to insufficient bioavailability. This low bioavailability can be explained by the poor water-solubility of PD0184352 (less than1 μM/mL).

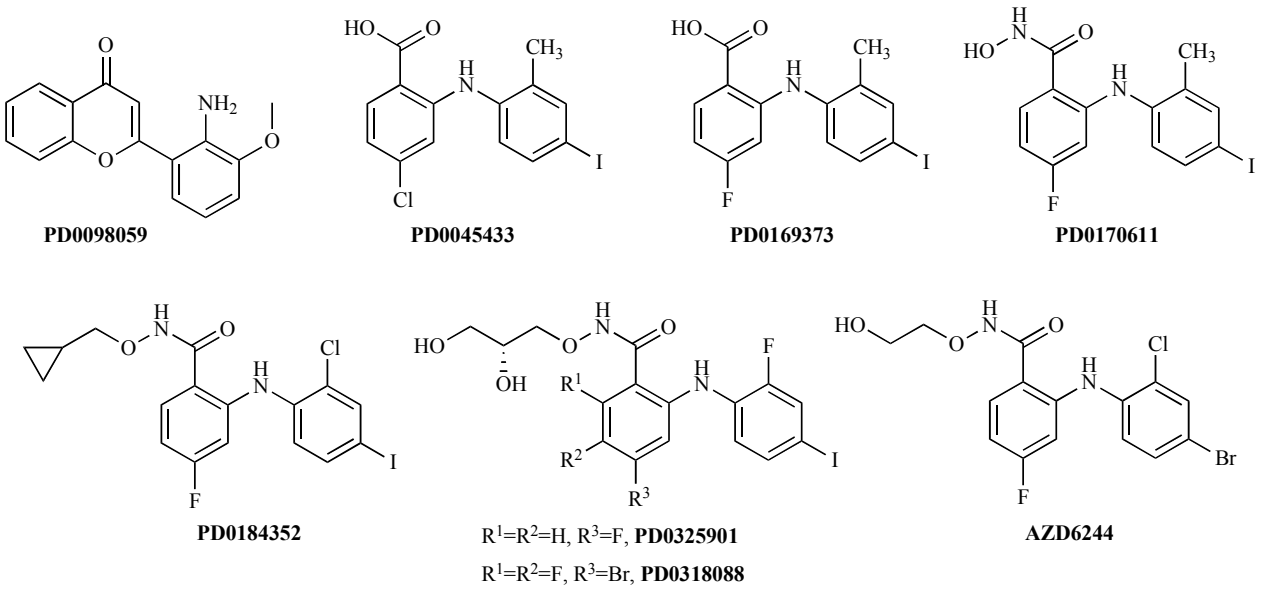


Fig. (5). Chemical structures of allosteric MEK inhibitors.

A dramatic increase of water-solubility (190 µM/mL) was achieved by the concomitant replacement of the chloro substituent of PD0184352 by a fluorine atom and the cyclopentyl moiety by a dihydroxypropyl chain. The resultant PD0325901 was characterised by higher potency, better stability and improved bioavailability than PD0184352 [99]. Unfortunately, the clinical trials of PD0325901 were terminated in phase II due to its unexpected ocular toxicity [100]. A structurally related analogue of PD0325901, AZD6244 (Fig. **5**) was developed by Array BioPharma and later licensed to AstraZeneca. AZD6244 is currently progressing through phase II of the clinical trials [101].

The 3D structure of MEK bound to a variant of PD0184352 was resolved in 2004 [102]. It showed that this type of inhibitor binds to a unique inhibitor-binding pocket within the catalytic cleft, but adjacent to the Mg-ATP binding site (Fig. **6**). The binding of inhibitor to this site not only does not prevent the binding of ATP, but may well stabilize it. This explains why some allosteric inhibitors like AZD6244 are more active in inhibiting MEK in cellular assay than in enzymatic assay, as the ATP concentration in the former is considerably higher than in the later. The crystal structure showed that the binding of the inhibitor causes MEK to undergo important perturbations in the conformation of the kinase activation loop and N-terminal loop ensuring that this new conformation is catalytically inactive. The crystal structure also revealed that the sequence of this inhibitor-binding pocket has low homology with other kinases, explaining the high selectivity of these inhibitors.

CHK1 Inhibitors

The cell cycle is controlled by several checkpoints to insure the genome stability. The checkpoint kinase CHK1 protects cells that have suffered DNA damage by arresting the cell cycle at a G2/M checkpoint to allow DNA repair [103]. DNA damaging approaches such as chemotherapy and radiation are still one of the most relied upon cancer treatments. Since cancerous cells use checkpoints to facilitate DNA repair; the inhibition of CHK1 will abrogate the G2/M checkpoint in these tumour cells and allow premature mitotic entry in the presence of DNA damage, leading ultimately to cell death [103]. Several Chk1 ATP competitive inhibitors are already known, including natural products such as staurosporine. Some of them (e.g. UCN-01, XL-884 and PF-00477736) reached clinical trials phase I or II [104, 105]. The major disadvantages of this type of inhibitors are their lack of selectivity and their difference in

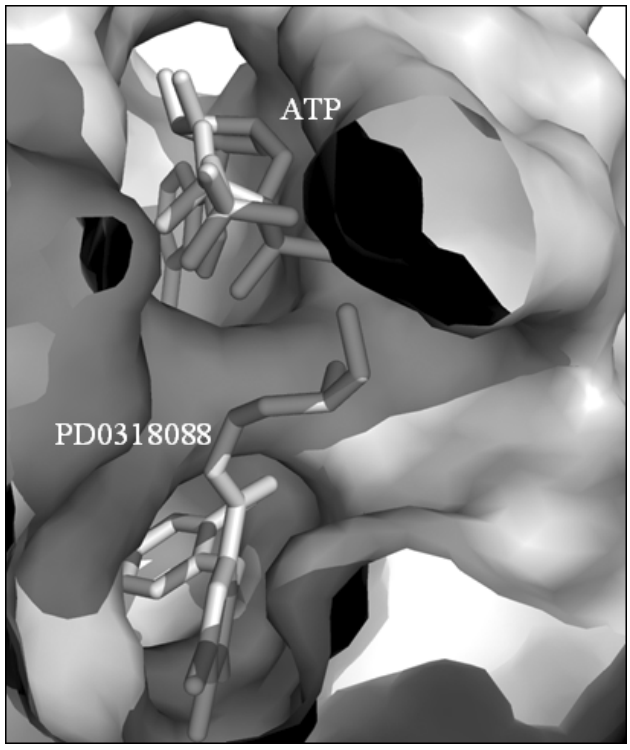


Fig. (6). ATP and PD0318088 occupy distinct and adjacent binding sites within the catalytic cleft of MEK kinase. PDBid 1S9J.

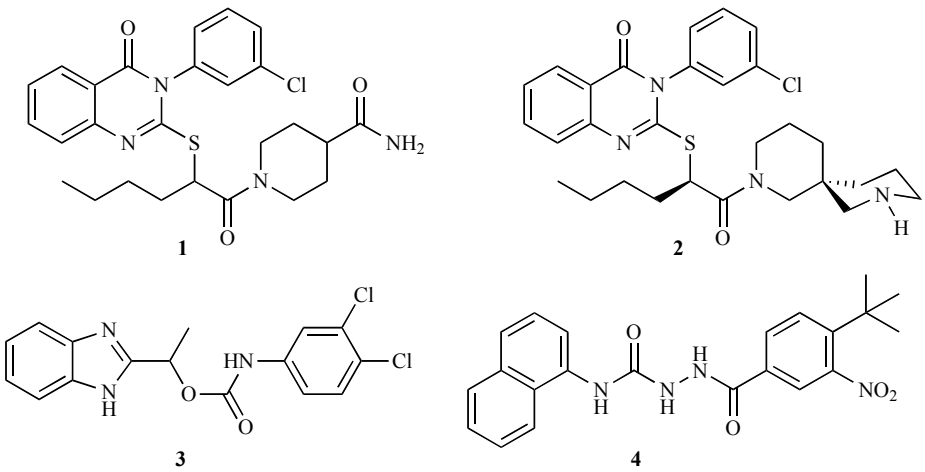


Fig. (7). Chemical structures of CHK1 allosteric inhibitors.

potency when tested in cell and biochemical assays. This is the reason why a lot of effort is now concentrated in developing CHK1 non-ATP competitive inhibitors. A high throughput screening project was started at Merck & Co to identify CHK1 allosteric inhibitors [106]. Hits were evaluated in the presence of physiological ATP concentrations (0.1 and 2.0 mM) in order to eliminate ATP competitive compounds, as the potency of allosteric inhibitors should be insensitive to ATP concentration. The thioquinazolinone derivative **1** (Fig. **7**) was isolated with an IC_{50} of 17 and 24 μM at anATP concentration of 0.1 and 2.0 mM, respectively. The optimisation of this hit led the team at Merck & Co to compound **2** (Fig. **7**) with an IC_{50} of 1.3 μM in presence of 0.1 mM ATP [106]. The crystal structure of **2** bound to CHK1 confirmed **2** as an allosteric inhibitor. It binds to a hydrophobic pocket 13 Å away from ATP binding site [106]. More recently, scientists in Pfizer described two potent CHK1 allosteric inhibitors **3** and **4** (Fig. **7**) with K_i of 1.89 and 0.146 μM respectively [107]. Crystallographic studies showed that these compounds bind to an allosteric site that shares a pocket with the previously reported site [107]. The identification of this allosteric site provides an opportunity to develop more selective and potent CHK1 inhbitors for the treatment of cancer.

OTHER TYPES OF KINASE INHIBITORS

Covalent Inhibitors

The inhibitors in this family are capable of forming an irreversible covalent bond to the kinase active site by reacting with a nucleophilic thiol of a cysteine residue [108]. The irreversible kinase inhibitors of the epidermal growth factor receptor (EGFR), CL-387785 [109-111] and Neratinib (HKI-272) [112] (Fig. **8**), were designed to target the cysteine residue (C773) located at the lip of the ATP binding pocket [5]. These compounds were rationally developed by attaching an electrophile, which is reactive with the electron rich sulfur in cysteine, to the well established EGFR selective 4-anilinoquinazoline and 4-anilinoquinoline-3-carbonitrile scaffolds. The structure of 4-anilinoquinazolines complexes with EGFR was used to anticipate the optimal position for electrophile attachment. The designed inhibitors undergo Michael addition reaction, the cysteine residue in EGFR (C773) forming a covalent bond with the inhibitor. Consequently, the inhibitor, with infinite affinity due to the covalent bond, irreversibly blocks binding of ATP to the kinase and inactivates the enzyme. Neratinib, which inhabits HER2 and HER4 kinases in addition to EGFR, is now progressing in phase III clinical trials.

Analysis of the human kinome showed 46 kinases that have this particular cysteine and around 200 different kinases that have a cysteine residue located in the vicinity of the ATP binding site suggesting that many kinases could be targeted by irreversible inhibitors. However, many natural toxic compounds have also evolved to exhibit their toxicity by irreversibly reacting with kinase cysteine residues. Thus, many drug developers are concerned about the possible toxicity of covalent inhibitors as a result of reacting with unpredicted targets [5, 113-115].

Protein Substrate Competitive Inhibitors

Protein kinases normally phosphorylate different protein substrates. Thus, the specificity in targeting particular protein kinases can be achieved more easily by targeting remote sites like the substrate binding regions with substrate competitive inhibitors. One example is the tyrosine kinase inhibitor AG538 (Fig. **9**) that has been identified as a protein substrate competitive inhibitor of the insulin-like growth factor receptor (IGF-R1) and has been further chemically modified by introducing the catechol moiety instead of benzoxazolone groups on either side of the molecule to increase its stability in cells. The ability of AG538 and its derivatives to stop growth of prostate and breast cancer cells *in vitro* suggested that these substrate competitive inhibitors may be useful as anticancer agents [5, 75, 76, 116-118].

A further example of a small molecule protein kinase inhibitor is ON 01910.Na [119] (Fig. **9**), a substrate competitive inhibitor of polo-like kinase 1 (PLK1). However, 20 to 40 fold higher concentrations of ON 01910.Na inhibit other kinases including PDGFR, FLT-1, Abl, PI3K, Fyn and polo-like kinase-2 as well as the serine/threonine cyclin-dependent kinase-1. Whether the efficacy of ON 01910.Na in inhibiting tumour growth is the

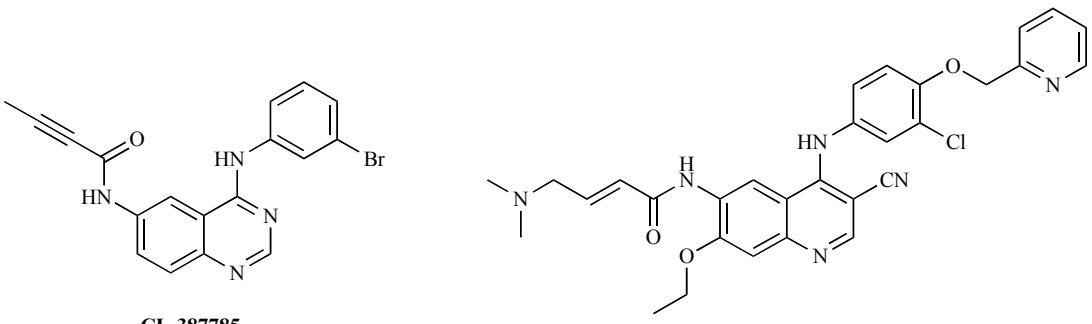


Fig. (8). Chemical structure of kinase covalent inhibitors.

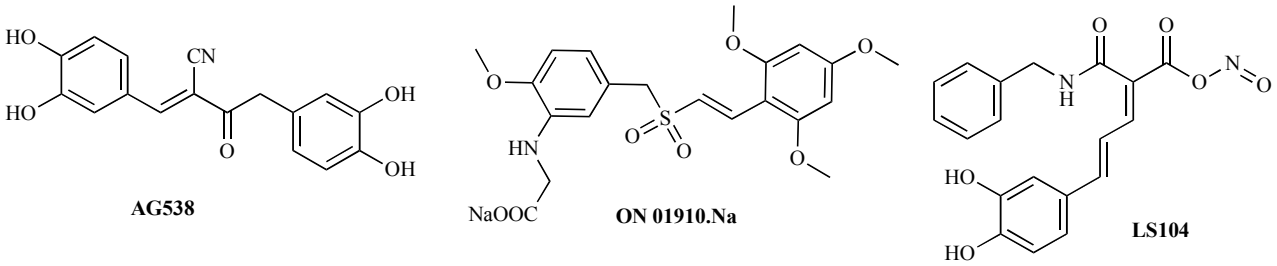


Fig. (9). Chemical structure of substrate-competitive kinase inhibitors.

direct result of inhibiting polo-like kinase-1 or other protein kinases remains a controversial issue that requires further investigation [119-122]. Recent phase I studies in human B-cell chronic lymphocytic leukemia (CLL) demonstrated that ON 01910.Na selectively induced apoptosis in all CLL samples tested and reduced PLK1 activity in the leukemic cells [123, 124]. ON 01910.Na is currently also being tested in phase I and II combination therapy in patients with solid tumours. FDA granted ON 01910.Na orphan drug designation for the treatment of myelodysplastic syndromes (MDS), and agreed special protocol assessment (SPA) for phase III trial as monotherapy in patients with MDS.

LS-104 (Fig. **9**) is a kinase inhibitor that inhibits the growth of leukemia cell lines *in vitro* by targeting JAK2. It works in ATP independent and substrate competitive manner. The activating JAK2 V617F mutation is described in the majority of patients with BCR-ABL negative myeloproliferative disorders (MPD). Treatment of V617F-positive cells with LS104 resulted in dose dependent induction of apoptosis and inhibition of JAK2. This effect was not altered using elevated ATP concentrations, whereas variation of the substrate peptide resulted in modulation of the IC_{50} value. LS104 is currently being tested in a Phase 1 clinical trial [125, 126].

CONCLUSION

The selective inhibition of protein kinases is an important approach for the treatment of cancer. The discovery of allosteric non-ATP competitive kinase inhibitors has had a major impact on the development of promising new strategies to tackle human cancers. Since this type of inhibitor does not interact with the ATP binding site, and consequently does not have to compete with high physiological ATP concentrations, they are much more selective and potent than their ATP competitive analogues. Some of these allosteric non-ATP competitive kinase inhibitors are now in advanced stages of clinical trials.

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