Structure-based design, discovery and development of checkpoint kinase inhibitors as potential anti-cancer therapies

Thomas P Matthews, **Alan M Jones**, and **Ian Collins**[*]
Institute of Cancer Research, Cancer Research UK Cancer Therapeutics Unit, London, UK

Abstract

Introduction—Checkpoint kinase inhibitors offer the promise of enhancing the effectiveness of widely prescribed cancer chemotherapies and radiotherapy by inhibiting the DNA damage response, as well as the potential for single agent efficacy.

Areas covered—This article surveys structural insights into the checkpoint kinases CHK1 and CHK2 that have been exploited to enhance the selectivity and potency of small molecule inhibitors. The use of mechanistic cellular assays to guide the optimisation of inhibitors is reviewed. The status of the current clinical candidates and emerging new clinical contexts for CHK1 and CHK2 inhibitors are discussed, including the prospects for single agent efficacy.

Expert opinion—Protein bound water molecules play key roles in structural features that can be targeted to gain high selectivity for either enzyme. The results of early phase clinical trials of checkpoint inhibitors have been mixed, but significant progress has been made in testing the combination of CHK1 inhibitors with genotoxic chemotherapy. Second generation CHK1 inhibitors are likely to benefit from increased selectivity and oral bioavailability. While the optimum therapeutic context for CHK2 inhibition remains unclear, the emergence of single agent preclinical efficacy for CHK1 inhibitors in specific tumour types exhibiting constitutive replication stress represents exciting progress in exploring the therapeutic potential of these agents.

Keywords

Cancer; DNA damage; cell-cycle checkpoint; kinase inhibitor; structure-based drug design

1. Introduction

The research field of checkpoint kinase (CHK) inhibitors has seen several recent developments, including publications of early phase clinical trial data for inhibitors used in

combination with classical cancer chemotherapies, and also the first preclinical demonstrations of single agent efficacy for checkpoint kinase 1 (CHK1) inhibitors in specific genetic backgrounds. Previous reviews have covered the clinical development of CHK inhibitors [1-4] and the new structural classes of small molecules that have emerged from preclinical discovery [5-8]. In this article we concentrate on recently published structure-based drug design (SBDD) strategies that have enabled hit compounds against CHK1 and CHK2 to be developed to potent, selective late stage lead compounds and clinical candidates, and on advances made in understanding the determinants of inhibitor selectivity. We also focus on the cellular pharmacodynamic assays that have been used to drive optimisation of inhibitors to give mechanistically well-defined effects in relevant cancer models. We survey the development status of current clinical candidates and new potential contexts for CHK1 or CHK2 inhibition.

CHK1 and CHK2 are intracellular serine/threonine kinases that play pivotal roles in maintaining the integrity of cellular DNA. In response to intrinsic or genotoxic agent-induced DNA damage, e.g. single or double strand breaks or stalled replication forks, a network of sensors serves to activate multiple checkpoints to suspend progression through the cell cycle and simultaneously activate DNA repair mechanisms [9]. If repair is successful, checkpoints are lifted and cell duplication continues, otherwise the cell is directed to apoptosis. The DNA damage response and repair in cancer cells thus serves as a resistance mechanism to therapy with DNA-damaging cytotoxic agents or radiotherapy.

The CHK enzymes convey the signals received from upstream DNA-damage sensing proteins, principally ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR), to downstream effectors of cell cycle arrest and DNA repair [10]. There is substantial overlap in the activation and substrates of CHK1 and CHK2. Although CHK2 activation can contribute to S- and G2/M-phase checkpoints, CHK2 is particularly important in the response to double strand DNA breaks signalled through activation of ATM and controls the p53-dependent early phase G1/S checkpoint [2, 11]. CHK2 stimulates repair of double strand DNA breaks through BRCA1 mediated processes. In contrast, CHK1 signalling is more important in response to single strand DNA breaks and stalled DNA replication signalled by activation through ATR, and resulting in later phase S and G2/M checkpoint arrest [12].

Many cancer cells harbour defects in the early phase, p53-dependent G1/S checkpoint, particularly resulting from mutation or inactivation of p53 [13-15], and as a result are more dependent on the later checkpoints, including those in S and G2/M-phases controlled by CHK1. This leads to the opportunity to selectively target cancer cells with defects in the p53-dependent checkpoint through the combination of inhibition of CHK1 with classical DNA-damaging cytotoxic drugs. Preclinical proof-of-concept of this strategy has been achieved with several CHK1 inhibitors [1-3, 16-21]. The role of CHK1 in maintaining replication fork stability has more recently been recognised as a key vulnerability for cancer cells enduring high intrinsic replication stress, providing potential contexts for single agent inhibition of CHK1 as an anticancer strategy [22-25]. In comparison to the potentiation of DNA-damaging therapies by CHK1 inhibition, where agents have reached clinical trials, the therapeutic context for selective CHK2 inhibition has not been as well defined and remains

controversial. For example, inhibition of CHK2 has shown distinct and sometimes opposing effects to CHK1 inhibition when combined with DNA-damaging agents [2, 26].

2. Structure-based design applied to CHK inhibitors

2.1 CHK1 inhibitors

The majority of CHK1 inhibitors are ATP competitive and bind directly to the hinge peptide region found between the N- and C-terminal lobes of the kinase domain. The exceptions are recently described allosteric CHK1 inhibitors [27, 28]. Within the large selection of Type I inhibitors for which crystal structures have been published, similar interactions are regularly observed. With the inhibitors anchored by hydrogen bonds to one or more of Glu85, Tyr86 or Cys87 in the hinge region, they typically project polar substituents towards the ribose pocket and lipophilic groups to the selectivity surface. Beyond this surface the cleft opens up to solvent and hydrophilic groups may often be added here to balance the compound physicochemical properties. CHK1 has proven highly amenable to protein crystallography since the determination of the first apo-structure [29], thus a number of inhibitor series have been progressed using SBDD.

2.1.1 Thiophene carboxamide ureas—AZD7762 (**1**) is a dual CHK1/CHK2 inhibitor from AstraZeneca, for which details of the preclinical discovery using SBDD have been recently disclosed [30]. AZD7762 evolved from a thiophene carboxamide urea high-throughput screening (HTS) hit **2** [31] (Scheme 1A). Initial structure-activity studies showed the basic amine to be important for CHK1 *in vitro* potency, but that the series lacked activity in cellular assays quantifying abrogation of a camptothecin-induced G2/M checkpoint. Similar urea cores had been previously described as inhibiting a range of kinases [31] and prospects for gaining selectivity were based on the observation of a markedly different binding mode in CHK1.

An X-ray structure of **1** (Figure 1A) represents the binding mode found for this series in CHK1, with the urea carbonyl and terminal amino functionality contacting Cys87 and Glu85 at the hinge and the amide pointing towards the ribose pocket. An alternative binding mode for this scaffold was exemplified by a crystal structure in JNK1 which showed a molecule similar to **2** binding to the hinge region in a tridentate manner through the primary amide NH and carbonyl groups as well as the urea terminal amine [30]. A set of analogues containing substituted amides to discourage the tridentate binding mode increased selectivity for CHK1 and validated the design hypothesis [30]. Cyclic amine substituents conferred increased potency due to new polar interactions between the amine and Asp148, along with dipole-dipole interactions with the backbone carbonyl of Glu134 and the amide side chain of Asn135. Removal of the original ether-linked ethylamine of **2** gave the lead compound **3** with much improved cellular activity while retaining *in vitro* potency.

The regioisomeric thiophene seen in **1** could replace the thiophene ring of **3**, and optimisation of the terminal phenyl ring was focussed on increasing selectivity for CHK1, increasing oral bioavailability and improving efficacy. A hollow fibre *in vivo* pharmacodynamic model was used to differentiate compounds [16], wherein polyvinylidene difluoride fibres filled with topotecan-treated HCT116 colon cancer cells were implanted

into mice prior to drug treatment. After 30 h the fibres were recovered and the HCT116 cells were analysed by flow cytometry to determine the G1 and G2 cell cycle populations and assess checkpoint abrogation. 3-Fluorophenyl analogue **1** (AZD7762) was found to give the best balance of properties and was selected as a clinical candidate.

Merck have also developed CHK1 inhibitors starting from thiophene carboxamide ureas [32]. Ring formation to replace the pseudo-cycle formed by intramolecular hydrogen bonding between the amide and one of the urea amino groups gave scaffolds based around thienopyridines, thiazolopyridines and thienopyridazine cores, leading to potent CHK1 inhibitors *in vitro*.

2.1.2 Triazolones—In addition to the thiophene carboxamide urea **2**, the HTS screen by AstraZeneca also identified triazolone **4** [31] (Scheme 1B). An X-ray structure revealed that two nitrogens of the triazolone formed a donor-acceptor interaction with the backbone Cys87 and Glu85, and the carbonyl interacted through a bridging water to Ser147 (Figure 1B) [33]. Attempts to establish polar interactions in the ribose pocket with 7-substituted phenyl triazolones led to **5** which possessed good *in vitro* potency but failed to abrogate a G2/M checkpoint in cells (Scheme 1B). Only with heterocycles at the 7-position, e.g. **6**, designed to interact with Lys38 or the P-loop, was cellular activity observed. Crystal structures, e.g. **7** (Figure 1B), showed these compounds bound differently to **4**, with the carbonyl and neighbouring NH interacting with Cys87 and Glu85, respectively [34]. This projected the pendent heterocycle towards the hinge region, resulting in an additional H-bond between Cys87 and the pyrrole NH. Superposition of the X-ray structures of these triazolones and the thiophene carboxamide urea **3** suggested appending a basic piperidine or similar group to the methyl substituent should be beneficial [34]. However the structure-activity relationships for substituents in the ribose pocket did not translate between these series and ultimately the hydroxymethyl derivative **7** gave an acceptable balance of *in vitro*, cellular and PK properties. Modest chemo- and radiopotentiation by **7** was observed [34]. Challenges remained with optimising the physicochemical properties and cellular potency of the triazolones.

2.1.3 Indazoles—AZD7762 (**1**) is a potent inhibitor of both CHK1 and CHK2. Other series of inhibitors have exploited an additional unique structural feature of the CHK1 kinase to generate selectivity for CHK1 over CHK2 and other kinases. The interior pocket of CHK1, beyond the Leu84 gatekeeper residue, contains an unusual polar residue at Asn59 instead of a hydrophobic amino acid as is more commonly found at this position. For example, the equivalent residue in CHK2 is a non-polar leucine [35]. Combined with contributions from Glu55 and the Lys38-Asp148 salt bridge, Asn59 defines a buried hydrophilic pocket [36-38]. Furthermore, crystal structures of CHK1 typically show between 1 – 3 protein bound water molecules within this pocket. Specific polar interactions from ligands to this feature can therefore provide potency and selectivity gains.

In contrast to **1** (Figure 1A), interactions to the hydrophilic features of CHK1 are exemplified in crystal structures of other inhibitors [36-38] (Figure 2). An indazole inhibitor **8** from Merck contained a pendant hydroxymethyl triazole group, where the hydroxyl replaced one of the water molecules in the hydrogen-bonding network (Figure 2A). This was

associated with increased selectivity over CDK7. Indazoles such as **9** were reported by Vernalis and incorporated an *ortho*-methoxyphenol substituent [36]. This group displaced all three water molecules, and directly contacted Asn59 and Glu55 along with C-H···O and N-H···π-electron interactions to Val68 and Ser147, respectively (Figure 2B). This gave an increase in potency though the selectivity over other key kinases remained low. A preclinical candidate, VER-158411(structure not disclosed), has been nominated [39, 40].

2.1.4 Pyrazines—The urea inhibitor **10** from Abbot contained a cyanopyrazine group where one of the ring nitrogens interacted with the water network and the nitrile group hydrogen bonded to the nearby Lys38 (Figure 2C) [38]. The macrocyclic scaffold of **10** was a product of structure-based design starting from the crystal structure of an acyclic bisaryl urea bound to CHK1 [41]. Macrocyclisation reinforced the intramolecular hydrogen bond observed in **10** (and the CHK1-bound acyclic progenitor) between the urea NH and pyrazine nitrogen, which contributes to locking the acyclic urea in the bioactive cis-trans conformation. Three macrocyclic analogues similar to **10** displayed high selectivity against a panel of 70 kinases [38]. The majority of kinases gave Ki > 10μM while there was still 170-fold selectivity for CHK1 over the most potent kinase inhibited, PLK1. Notably, substituted pyrazines have been used in other series of CHK1 inhibitors to increase potency and gain selectivity over other kinases [5-7], and a pyrazine urea also appears in the currently most advanced clinical candidate CHK1 inhibitor, LY2603618 (Table 1, **11**) [6].

A collaboration between the Institute of Cancer Research, London and Sareum Ltd generated highly selective CHK1 inhibitors starting with virtual and high concentration biochemical screening to identify fragment hits [42]. Several fragments were pursued further using SBDD [43]. The morpholino-purine **12** (Scheme 1C) was advanced by fragment growing and scaffold morphing to the pyrazolopyridine **13** with increased potency resulting from interactions with the specificity surface of CHK1 and Glu91 in the ribose pocket [42]. Compounds were assessed for abrogation of an etoposide-induced G2/M checkpoint arrest in p53-deficient HT29 colon cells, and for single agent cytotoxicity in the same cell line. Analogues such as **13** showed encouraging 2-3 fold selectivity for the CHK1-mediated cellular effect over non-specific cytotoxicity and enhancing this differential was important in the subsequent optimisation of the compounds to ensure a selective mechanism of action.

The fusion of an additional pyridine ring to generate pyrimido[2,3-*b*]azaindoles, e.g. **14**, was used to contact the protein bound waters in the CHK1 interior pocket [44]. A further scaffold modification to give pyridoaminopyrazines, such as **15**, increased the opportunities to optimise the basic substituent and selectivity surface contacts. The pendant cyanopyrazine group of **15** interacted with Lys38 and protein bound water molecules in CHK1 with associated benefits in selectivity, exemplified by the 330-fold difference between CHK1 and CHK2 activity (Scheme 1C). The pyridine ester of **15** was exchanged for an isoquinoline in another scaffold modification, while the basic amine side chain was translocated to the pyrazine as had been demonstrated for urea-based CHK1 inhibitors [38], leading to SAR-020106 (**16**). The crystal structure of SAR-020106 bound to CHK1 showed extensive contacts of the cyanopyrazine substituent with Lys38 and the protein-bound water network (Figure 1C). As a result, SAR-020106 was a potent and highly selective CHK1 inhibitor.

The systemic inhibitor **16** potentiated the efficacies of irinotecan and gemcitabine in SW620 human colon cancer cells *in vitro* and when grown as xenografts in nude mice [18]. SAR-020106 was also a potent radiosensitiser in tumour cell lines defective in p53 function [45].

To produce oral inhibitors, the metabolically stable pyridine core of **15** was hybridised with the substituted cyanopyrazine **16** to generate a new core scaffold [46]. This led to CCT244747 (**17**) which showed substantial oral bioavailability (F = 61%) and is the first oral CHK1 inhibitor to be fully described in the literature. CCT244747 maintained the high selectivity of SAR-020106 (at a concentration of 1 μM only 13 out of 140 representative kinases were inhibited by >50%) and significantly enhanced gemcitibine and irinotecan efficacy in human tumour xenografts in nude mice.

2.1.5 Pyrazolo[1,5-*a*]pyrimidines—Discovery of the Merck (previously Schering Plough) CHK1 clinical candidate SCH900776 **20** started with the identification of the CDK2 inhibitor **18** from a compound library screen [47] (Scheme 1D). Medicinal chemistry exploration along two substituent vectors led to compound **19** with much improved CHK1 activity and selectivity against CDK2. A crystal structure confirmed that the lead compound bound to the hinge region of CHK1 through N1 and the C7-NH of the pyrazolo[1,5-*a*]pyrimidine core. The 1-methyl pyrazole at C3 contacted the protein bound waters in the interior pocket, while the piperidine interacted in the ribose pocket with Glu91 and the amide carbonyl of Glu134. These features were retained in the clinical candidate (Figure 1D). Attempts to access the specificity surface by substitution on the C7 amine proved challenging but halogen substituents in the C6 position gave a 20-fold improvement in CHK1 activity relative to the parent compound, leading ultimately to the clinical candidate SCH900776 **20** [48]. A cell based assay for γ-H2AX induction, a marker for the formation of double-strand DNA breaks, was used to define the optimal cellular phenotype of compounds exhibiting varying degrees of CHK1, CHK2 and CDK selectivity during lead optimization [17].

2.1.6 Thiazole-4-carboxamides and 2-aminothiazoles—The SBDD of two new classes of CHK1 inhibitors with high *in vitro* potency have been disclosed by Merck [49-51]. The thiazole-4-carboxamide **21** was identified using AS-MS ALIS (Affinity Selection-Mass Spectrometry-based Automated Ligand Identification System) (Scheme 1E) [49]. A CHK1 crystal structure showed **21** bound to the ATP site through untypical CH…O interactions, whereby the thiazole C5-H and 2,3-dihydrobenzofuran C6-H interacted with the carbonyls of Glu85 and Cys87, respectively (Figure 1E). Intramolecular hydrogen bonding conferred a "U-shaped" topology to **21** which may significantly reduce the entropic penalty to binding. The amide interacted with the water network in the interior pocket. Replacement of the 2,3-dihydrobenzofuran with indole **22** conferred enhanced CHK1 potency and selectivity over CDK2.

Another series from Merck originated from the observation that the VEGFR2 (KDR) inhibitor **23** [50] was also a CHK1 inhibitor (Scheme 1F). However, compound **23** showed no activity in a cellular checkpoint escape assay measuring the release of H1299 tumour cells from DNA-damage induced cell-cycle arrest and progression into mitosis following

CHK1 inhibition [37, 51]. This was attributed to the concomitant inhibition of CDK7 which, as with inhibition of other CDKs, may result in DNA-damage independent cell cycle arrest, nullifying the checkpoint abrogation activity. A crystal structure of **23** in CHK1 showed the scaffold bound to Cys87 in the hinge region via the aminothiazole (Figure 1F). This positioned the piperazine subsitutents towards solvent while the pyridine at C5 of the thiazole interacted with the water network in the interior pocket. Substituting from the meta position of this pyridine gave gains in CHK1 potency and selectivity through targeting the Glu55 and Asp148 residues. Analogues such as **24** were inhibitors of exceptional potency with very slow dissociation kinetics from the enzyme [50] (Scheme 1F). Reduction of the polar surface area of the compounds was sought to improve cell permeability and activity, leading to the difluoropiperidine **25** [51].

2.1.7 Allosteric inhibitors—Merck also developed a screening strategy to find non-ATP competitive CHK1 inhibitors [27], identifying a thioquinazolinone lead with an IC_{50} of 17 and 24 μM at 0.1 and 2.0 mM ATP concentrations, respectively. Medicinal chemistry optimisation gave **26** (Figure 3), with enhanced stability and potency against CHK1. The crystal structure of **26** in CHK1 showed it bound to the surface of the enzyme distant from the ATP site (Figure 3). The carbonyl from the quinazolinone ring formed a water mediated hydrogen bond to Glu134, while the piperidine amine and amide carbonyl interacted directly with Glu205 and the backbone of Leu206, respectively. The 3-chlorophenyl group fitted securely into a narrow hydrophobic cleft. Independently, Pfizer reported the discovery of potent allosteric inhibitors **27** and **28** (Figure 3) which utilised the same hydrophobic cleft but extended out in the opposite direction into a shallow groove [28].

2.2 CHK2 inhibitors

The five main CHK2 inhibitor classes published to date are all ATP-competitive and bind to the hinge region of the kinase through interactions with one or more of Glu301, Glu302, Leu303 and Met304. Unlike CHK1 inhibitors, the interactions of CHK2 ligands fall into two distinct classes; direct hydrogen bonding [52-54] or atypical water-mediated contacts [55-59]. Several recently reported inhibitors also interact with Asp368 in the DFG motif [52, 57].

2.2.1 2-Arylbenzimidazoles—The first selective ATP-competitive CHK2 inhibitors were based on 2-arylbenzimidazoles identified from HTS [60]. The carboxylic acid hit **29** was evolved to the more potent primary amide **30** (Scheme 2A). Docking to a homology model of the ATP binding site of CHK2 was used to predict the binding of **30**. One binding pose suggested the 5-amide to hydrogen bond to the hinge region. Loss of activity upon methylation of the amide nitrogen in **30** confirmed that hydrogen bond donating functionality was essential. The biaryl linker required a heteroatom, and the model suggested this was necessary to achieve a 90° twist allowing the terminal phenyl ring to maintain hydrophobic contacts in the ATP cleft. The 4-chlorophenyl analogue **31** had increased potency and it was suggested that the chlorine was partially solvent exposed. Replacement of the terminal aryl ring with alkyl-linked alcohols and amines improved solubility in selected examples [61], and the benzimidazole core was exchanged for other

[6,5]-heterocycles that retained the relative spatial arrangement of the amide and terminal phenyl substituents to further refine the proposed binding mode [62].

More recently, crystal structures of these benzimidazole CHK2 inhibitors have been reported and show a dramatically different binding pose than the original model [55] (Figure 4A). For **31** in CHK2, a water-mediated hydrogen bond was observed between the benzimidazole N1 and the carbonyl of Glu302 and amide NH of Met304 in the hinge. Instead of binding to the hinge region, the carboxamide substituent interacted with a network of amino acids deeper in the pocket, including Asp368. The 4-chlorophenyl substituent of **31** interacted loosely with hydrophobic residues at the entrance to the CHK2 ATP-pocket defined by Leu226, Leu236, Lys245, Leu303, and Glu305. The reported structure-activity relationships for the benzimidazoles [60-62] were better rationalised when this new binding pose involving water-mediated hydrogen bonding to the hinge was considered [55].

2.2.2 Guanidylhydrazones—Water-mediated hydrogen-bonding to the CHK2 hinge region is also a feature of a series of highly CHK2 selective guanidylhydrazone inhibitors derived from the symmetric inhibitor NSC109555 (**32**), the sole hit from HTS of 100,000 compounds [56, 58] (Scheme 2B). Although highly selective, **32** was not cell penetrant [58]. A crystal structure of **32** with the catalytic domain of CHK2 showed the central urea carbonyl to interact with Glu302 and Met304 in the hinge region through a mediating water molecule [56]. Again, molecular modelling had not predicted this novel binding pose. The ligand interacted with Glu273 on the C-α helix and other residues through one of the guanidylhydrazone termini. Replacement of the second guanidylhydrazone group, which had minimal contacts with the enzyme, and replacement of one side of the aryl urea with the less polar 7-nitroindole motif linked through an amide gave a cell penetrant compound, PV1019 (**33**) [59]. The crystal structure of **33** in CHK2 (Figure 4B) showed the nitro group facilitating the water mediated interaction with the hinge, as well as introducing a new direct interaction from the nitro group. Extensive interactions of the buried guanidylhydrazone were maintained and PV1019 showed excellent selectivity for CHK2 over other kinases.

Cyclisation of the guanidylhydrazone in **33** to give PV1115 (**34**) was pursued to maintain the interaction with Glu273 while removing two hydrogen bond donors to increase cell membrane permeability [57] (Scheme 2B). This also significantly enhanced CHK2 potency while maintaining high selectivity over CHK1. The crystal structure of **34** in CHK2 was similar to that of PV1019, with the difference that Lys249 was observed to move approx. 3.9 Å away from Glu273, breaking the salt bridge between these residues, to accommodate the larger cyclic guanidine group.

A superimposition of the coordinates of apo-CHK1 onto CHK2-PV1115 highlighted the presence of the bulky Tyr86 residue in the CHK1 hinge region compared to the smaller Leu303 corresponding residue in CHK2, which may render the 7-nitroindole group sterically disfavoured in CHK1. The region where the guanidine bound to Glu273 in CHK2 (Glu55 in CHK1), had an adjacent bulky residue (Tyr20) in the P-loop of CHK1 as opposed to the smaller Cys231 in CHK2. A hydrophobic pocket contacted by the methyl substituents

of PV1115 was bounded by the small Leu301 gatekeeper and Leu277 in CHK2, while in CHK1 the polar Asn59 residue replaces Leu277.

Modification of the guanidylhydrazone to the *N*-hydroxy guanylhydrazone PV1533 (**35**) was carried out to lower the pKa of the ligand and improve cell permeability while maintaining the interaction to Glu273. The crystal structure of **35** in CHK2 showed the addition of the *N*-oxime allowed the oxygen atom to participate in water-mediated hydrogen bonding with the carboxylate side chain of Asp368 in the DFG motif. This is the first time an interaction with the DFG motif was observed in this series.

2.2.3 2-Aminopyridines—3,5-Diaryl-2-aminopyridines such as **36** (Scheme 2C) were identified by the Institute of Cancer Research, London as CHK2 inhibitors following HTS of a 7,000 member kinase-focussed library [52]. Medicinal chemistry optimization of the pyridine 5-substituent gave the 1,3-benzodioxole **37** with improved CHK2 potency and 40-fold selectivity over CHK1. Incorporation of a fused dioxole moiety onto a thienyl group gave compound **38** for which the crystal structure in CHK2 showed the heteroaromatic scaffold sandwiched between Leu309 and Leu354 in the ATP cleft, with two direct hydrogen bonds to the hinge region from the 2-aminopyridine functionality (represented in Figure 4C with **39**). The terminal carboxamide of **38** accepted a hydrogen bond from Lys249, while also interacting with Glu273 in the αC-helix and Asp368 in the DFG-motif. The dioxacycle entered the solvent exposed but hydrophobic surface defined by Leu303 and Met304.

Deletion of the pyridine 3-aryl substituent and replacement with a 3-carboxamide containing a flexible alkylamine resulted in an improvement in CHK2 potency and 89-fold selectivity over CHK1 (**39**). A crystal structure of **39** in CHK2 revealed a water-mediated hydrogen bond between the amide and Asp368 as well as an *intra*-molecular hydrogen bond in the ligand between the 2-aminopyridine and the 3-carboxamide. The terminal unsubstituted amine was shown to make an additional interaction with Asp368 and through a water-mediated hydrogen bond the carboxamide interacted with Glu308. It was speculated that these new polar interactions compensated for the loss in rigidity of **39** compared to **38**, leading to improved potency. Wider kinome profiling showed the 3-carboxamides represented by **39** to be more CHK2 selective than compounds such as **38**.

2.2.4 2-(Quinazolin-2-yl)phenols—A distinct series of selective 2-(quinazolin-2-yl)phenol CHK2 inhibitors was discovered at the Institute of Cancer Research, London following kinome profiling of compounds from an unrelated drug discovery project (Scheme 2D) [54]. A crystal structure of the early lead **40** bound to CHK2 showed an interesting binding mode, with an intramolecular hydrogen bond between the phenol and the quinazoline N1 forming a planar pseudotetracyclic system. Furthermore, a hydrogen bond was formed between the phenolic oxygen and the amide NH of Met304 in the hinge region. The 6- and 7-positions of the quinazoline were directed out towards the solvent exposed region and the 3-aminopyrrolidine group occupied the ribose pocket, with the protonated pyrrolidine forming a charge-assisted hydrogen bond with the side chain of Asn352. These features were retained in the structure of the more optimised compound **42** bound to CHK2 (Figure 4D).

Fluorination of the phenol ring at the 5′-position increased CHK2 potency in this series. Based on the crystal structure of **40**, the pyrrolidine 4- and 5-positions were substituted to add interactions to the P-loop of the kinase. The 4-(1,1-dimethyl)methyl alcohol **41** was beneficial for reducing off-target hERG inhibition and also conferred increased CHK2 selectivity over CHK1. Methoxy substitution at the solvent exposed 6- and 7-positions of the quinazoline core increased CHK2 potency and maintained low hERG activity, leading to CCT241533 (**42**). Although less selective for CHK2 over CHK1 compared to **41**, CCT241533 showed good selectivity for CHK2 in a panel of 85 kinases. Additionally, CCT241533 had high passive permeability and inhibited CHK2 signalling in cancer cells [54]. Comparison of the binding mode of the 2-(quinazolin-2-yl)phenols with PV1019 and the benzimidazole CHK2 inhibitors shows that the hinge-binding phenolic OH of **42** occupies the same space as the mediating water molecule in the other two series.

2.2.5 Debromohymenialdisine analogues—Structure-based design was used to direct improvements to the CHK2 inhibitor debromohymeniadisine [53, 63]. Replacement of the pyrrole of the hymenialdisine core with an indole led to an indoloazepine (**43**, Table 2) [64]. Compound **43** showed improved CHK2 potency and selectivity for CHK2 over debromohymeniadisine. Substitution of the 2-pyrrole group in the hymenialdisine series with phenyl rings was found to reduce potency but increase selectivity over CHK1 in most examples [65].

2.2.6 Isothiazole-4-carboxamidines—In addition to the CHK2 inhibitors developed using SBDD, Valeant Pharmaceuticals International have reported VRX0466617 (**44**, Table 2), as a potent and selective inhibitor of CHK2 [66]. VRX0466617 was discovered starting from an isothiazole carboxamide identified from screening. Molecular docking in CHK2 was used to guide the optimisation to give **44** [67].

3. Therapeutic contexts and clinical experience with CHK inhibitors

3.1 CHK1 inhibitor clinical development

A summary of the clinical development of selected CHK1 inhibitors is shown in Table 1. The majority of early clinical trials have investigated CHK1 inhibition in combination with DNA-damaging chemotherapies. The first generation of inhibitors to reach clinical trial were intravenous agents, and often showed low or only moderate selectivity for inhibition of CHK1 over CHK2. Despite reaching multiple Phase II trials, the development of UCN-01 (7-hydroxystaurosporine, **45**) has been hindered by the low free drug levels resulting from the compound's high avidity for human α-acid glycoprotein [68, 69]. In a recently reported Phase II trial of UCN-01 in combination with irinotecan in triple negative breast cancer patients, inconsistent inhibition of CHK1 signalling at the trial dose was demonstrated using immunochemical assessment of downstream biomarkers [99].

The development of AZD7762 (**1**) [30], PF-00477736 (**46**) [19] and XL-844 (**47**) [70] has been reported as discontinued by the originating organisations. While AZD7762 (**1**) and SCH900776 (**20**) were discovered using SBDD as described above, details of the medicinal chemistry strategies used to identify PF-00477736 (**46**), LY2603618 (**11**) and LY2606368 (**47**) have not yet been reported. Published preclinical data on PF-0047736 (**46**) showed it to

be a potent inhibitor of CHK1 *in vitro* with some selectivity against CHK2 (100-fold) and CDK1 (20,000-fold) [19].

Other than UCN-01, LY2603618 (**11**) is apparently the most advanced CHK1 inhibitor from the first generation of compounds remaining in clinical trial (Table 1). Preclinical data on LY2603618 (**11**) and LY2606368 (**48**) have been reported in conference presentations and posters [71-73]. A phase I dose escalation study in patients with advanced solid tumours concluded that LY2603618 administered approximately 24 hours after pemetrexed showed acceptable safety and pharmacokinetic profiles [74]. Interestingly, the less progressed compound LY2606368 (**48**) shows no selectivity between CHK1 and CHK2 despite the presence of a pyrazine motif in the structure that is often associated with high CHK1 selectivity.

A published Phase I trial demonstrated that a combination of SCH900776 (**20**) with cytarabine was feasible and tolerated in adults with relapsed and/or refractory acute leukemias [75]. In addition there was preliminary evidence of some clinical activity. A dose-limiting asymptomatic prolongation of the QTcF interval was observed at doses of $80mg/m^2$.

GDC-0575 (previously ARRY-575) and GDC-0425 are the first orally bioavailable CHK1 inhibitors to enter Phase I clinical trials.

3.2 New potential contexts for CHK1 inhibition

While the majority of published preclinical data on the efficacy of CHK1 inhibitors has concentrated on combinations with DNA-damaging chemotherapy, it has long been recognised that abrogation of DNA damage response checkpoints and DNA repair by CHK1 inhibition also synergises with ionizing radiation (IR) [2, 76, 77]. In a recent example, the dual CHK1/CHK2 inhibitor AZD7762 (**1**) sensitised p53-deficient cell lines and xenografts to IR [78]. AZD7762 also sensitised MiaPaCa-2 pancreatic cancer cells to radiation *in vitro*, with a triple therapy of IR, gemcitabine and AZD7762 showing antitumour activity in MiaPaCa-2 and patient-derived pancreatic cancer xenografts [79]. AZD7762 has also been used recently to show that CHK1 inhibition radiosensitises xenografts of lung cancer brain metastases [80], and expression of activated (phosphorylated) CHK1 protein has been found to be elevated in radioresistant lung cancer patient-derived cell lines compared to radiosensitive cells [81]. These data suggest CHK1 inhibition may have potential in combination with radiotherapy in pancreatic and metastatic lung cancers. The more selective CHK1 inhibitor SAR-020106 (**16**) was shown to be a potent radiosensitiser in p53-deficient head-and-neck cancer cell lines and xenografts [45].

Combinations of CHK1 inhibition with other molecular targeted anticancer agents have been proposed as potential therapeutic contexts. Thus, inhibition of CHK1 with AZD7762 (**1**) or UCN-01 (**45**) combined with various MEK1/2 inhibitors was cytotoxic to several primary human glioma cell isolates and the CHK1 + MEK1/2 inhibitor treatment enhanced the sensitivity of glioma cells to IR [82]. The combination of PARP inhibitors and CHK1 inhibition was shown to act synergystically to suppress the growth of mammary carcinoma cells with several different genetic backgrounds, both *in vitro* and in xenograft models [83].

The combination of various CHK1 inhibitors and an inhibitor of the DNA damage response kinase WEE1 (MK-1775) synergistically inhibited growth and enhanced apoptosis in a variety of cancer cell lines and human tumour xenografts [84, 85, 86, 87]. Double strand breaks in DNA were observed in response to the combination of inhibitors without the application of an external genotoxic agent, which may suggest that the dual inhibition removes a survival mechanism for coping with high endogenous replication stress in cancer cells.

Perhaps the most exciting aspect of CHK1 inhibitor biology to emerge from recent work has been the discovery of defined contexts where single agent inhibition of CHK1 shows promising antitumour activity [2]. CHK1 signalling is critical in response to DNA damage resulting from defects in replication fork initiation and elongation during S-phase [24], and this key role provides a rationale for the observed high synergy seen when CHK1 inhibitors are combined with antimetabolites that generate DNA damage primarily during S-phase [88]. It has also led to the understanding that cancer cells with high intrinsic replication stress, i.e. a high level of endogenous DNA damage resulting from the highly replicative state, may come to depend on the DNA damage response and CHK1 function as a survival pathway. Cells with a complex karyotype isolated from patients with acute myeloid leukaemia (AML) were found to have elevated levels of constitutive DNA damage and CHK1 activation and to be more sensitive to CHK1 depletion by RNAi or CHK1 inhibition by UCN-01 **45** than AML cells with a normal cytogenetic profile or normal granulomonocytic progenitor cells [23]. The sensitivity of melanoma cell lines to single agent CHK1 inhibition also positively correlated to the level of endogenous DNA damage [22]. Single agent activity was also observed for an oral CHK1 inhibitor (structure undisclosed) in certain cancer cell lines, with *in vivo* activity demonstrated in HEL92.1.7 erythroleukamia cells grown as xenografts [89], although no marker for sensitivity was described.

Importantly, there is a growing body of evidence that overexpression of the MYC transcription factors leads to intrinsic replication stress and confers sensitivity to specific inhibition of CHK1 [25, 90]. CHK1 was identified from an RNAi screen as a potential therapeutic target in *MYC-N* amplified neuroblastoma, with constitutive activation of signalling through CHK1 observed in cells sensitive to RNAi or CHK1 inhibition [91]. The sensitivity of *MYC-N* driven neuroblastoma to single agent CHK1 inhibition was also demonstrated with the oral, selective CHK1 inhibitor CCT244747 (**17**) which showed efficacy in a transgenic mouse model of the disease [20]. Dual inhibition of CHK1 and WEE1 kinase is effective in models of MYCN-driven neuroblastoma [87]. Deregulated expression of the oncogene *c-MYC* in Eµ-myc lymphoma cells was also associated with sensitivity to CHK1 inhibition [92].

3.3 The therapeutic potential of CHK2 inhibitors

No selective CHK2 inhibitor has been progressed to clinical trials to date. In part this has reflected changing views over the most appropriate therapeutic context for CHK2 inhibition [2, 11]. CHK2 inhibition was originally perceived as a means to potentiate DNA-damaging anticancer chemotherapies, in a similar fashion to CHK1 inhibitors. However, some

pharmacological and siRNA studies in cancer cells have reported that dual inhibition of CHK2 and CHK1 offered no benefit over selective CHK1 inhibition, and that CHK2 inhibition may be antagonistic to CHK1-mediated potentiation of genotoxic efficacy [93-96]. Other studies have shown that the potentiation of the cytotoxicity of DNA-damaging agents by CHK2 inhibition is possible in certain cell lines [59]. Nevertheless, the selective CHK2 inhibitors from multiple chemotypes described above have proved useful chemical tools for investigating the therapeutic potential of CHK2 inhibition in cellular studies, and some patterns have emerged (Table 2).

Several selective CHK2 inhibitors have been shown to have protective effects against ionising radiation (IR) in p53 wild type cells (Table 2). Thus, benzimidazole **31** protected isolated peripheral human CD4$^+$ and CD8$^+$ T-cells from γ-irradiation in a concentration-dependent fashion [60]. The 2-(2-quinazolinyl)phenol CCT241533 (**42**) was shown to confer a radioprotective effect in isolated mouse thymocytes, with ablation of apoptosis being observed [54]. The inhibitor VRX0466617 (**44**) also suppressed IR-induced apoptosis in BJ-hTERT cells [66]. The negative regulator of p53, HDMX, is phosphorylated at Ser342 and Ser367 by CHK2 on exposure to IR, which results in its degradation. The extent of IR-induced HDMX degradation was diminished in a concentration-dependent fashion by **44**, while responses upstream to CHK2 induced by IR were not affected by **44**. PV1019 (**33**) was shown to ablate IR-mediated apoptosis in mouse thymocytes [59]. This reduction in apoptosis copied the behaviour of *chk2*(−/−) cells when exposed to IR, providing evidence of the mechanism of action of **33** through inhibition of CHK2. Cellular inhibition of CHK2 by PV1019 at relevant concentrations was demonstrated against three known substrates of the CHK2 kinase function; CHK2 autophosphorylation (IC$_{50}$ = 5 μM), HDMX, and Cdc25c. Non-malignant cells were also protected from radiation-induced apoptosis by the debromohymenialdisine derivative **43** [93].

While there is clear agreement on the radioprotective effect of selective CHK2 inhibition in cells with functional p53, there are still conflicting reports on the effects of combining CHK2 inhibitors with DNA-damaging cytotoxic drugs in cancer cells. Two studies have found no potentiation despite positive evidence of inhibition of CHK2 signalling in cells through assessment of pharmacodynamic biomarkers. Thus VRX0466617 (**44**) did not potentiate the cytotoxicity of doxorubicin or cisplatin in MCF7 cells, nor taxol in BJ-hTERT cells [66]. More comprehensively, CCT241533 (**42**) was found to give no potentiation of the cytotoxicity of the DNA-damaging agents SN38, gemcitabine, etoposide, mitomycin C or bleomycin in either HT29 colon cancer or HeLa cervical cancer cell lines, both of which are deficient in p53 function [26]. A similar lack of effect of CHK2 siRNA has been reported in cancer cells [94-96].

However, it has been shown that the selective CHK2 inhibitor PV1019 (**33**) potentiates the activity of the topoisomerase I inhibitors topotecan and camptothecin, as well as ionising radiation, in certain human tumour cells [59]. Treatment of the OVCAR-5 cell line with PV1019 (**33**) and topotecan increased the growth inhibitory effect of the cytotoxic agent. Treatment of the human brain tumour cell line U251 with PV1019 and IR resulted in a dose enhancement factor of 1.4, demonstrating the only reported radiosensitisation of a tumour cell line by a CHK2 inhibitor. Depletion of CHK2 by siRNA in two ovarian tumour cell

lines, OVCAR-4 and OVCAR-8, that expressed high levels of CHK2, led to increased growth inhibition compared to the control and provided evidence that CHK2 inhibition may lead to antiproliferative effects in tumour cell lines that over express CHK2. Thus, while the potentiation of DNA-damaging agents is a firmly established therapeutic context for CHK1 inhibitors, the case for CHK2 inhibition remains unclear and may depend more critically on the cell genetic background and cytotoxic agent employed.

In contrast to the lack of synergy with DNA-damaging chemotherapies exhibited by CCT241533 (**42**), this compound has been shown to potentiate the efficacy of two structurally distinct PARP inhibitors [26]. Both HeLa and HT29 cells displayed enhanced sensitivity to the PARP inhibitors AG14447 and olaparib in the presence of CCT241533 (**42**), with a sharp decrease in growth of the HeLa cells compared to the control in short and longer term colony-forming experiments. The combination of CCT241533 (**42**) and olaparib was shown to enhance apoptosis in HeLa cells and affect PARP inhibitor cytotoxicity through a CHK2-dependent mechanism. It is proposed that the cytotoxicity of the combination of CCT241533 and olaparib arises from inhibition of CHK2 leading to inhibition of BRCA1 phosphorylation and impairment of the homologous recombination DNA repair pathway. As PARP inhibitors prevent DNA repair through the alternative base excision repair pathway, the dual treatment produces unrepairable and lethal DNA double strand breaks.

4. Expert Opinion

There have clearly been substantial advances in the basic science and clinical progress of checkpoint kinase inhibitors, particularly in the past five years. To date, the majority of opened clinical trials have been designed to examine the preclinically well established concept of combination of CHK1 inhibition with DNA-damaging chemotherapies (Table 1). However, proof-of-concept clinical data with these agents has still to be achieved. Of the several first generation inhibitors that have entered Phase I clinical trials, the development of UCN-01 has been hindered by poor pharmacokinetic properties, while a number of other compounds have been reported not to be under further development by their originating organisations. These observations notwithstanding, at least one other first generation intravenous CHK1 inhibitor (LY2603618) has progressed to Phase II trials in the combination setting. The dose-limiting toxicities in Phase I clinical data for the different compounds reported to date vary considerably, suggesting that off-target effects differing between the chemical scaffolds tested may be major contributing factors [74, 75, 97, 98]. As yet no CHK2-specific inhibitor has been reported to enter clinical trials.

The potential importance of high selectivity for CHK1 over CHK2 and other kinases in determining the efficacy of checkpoint inhibition in combination with genotoxic agents has been highlighted through basic research with inhibitors and siRNA [2, 17, 95, 99]. Specific CHK1 inhibition in combinations with multiple DNA-damaging agents is effective in a range of tumour cell *in vitro* and *in vivo*. While certain tumour cells do appear susceptible to the potentiation of DNA-damaging agents by CHK2 inhibition, the phenomenon is less widely observed than for CHK1 inhibitors, and may be restricted to specific tumour genetic backgrounds.

Many of the first generation checkpoint inhibitors were characterised by no or modest selectivity for CHK1 over CHK2. Second generation CHK1 inhibitors are likely to be substantially more selective for CHK1 than previous agents. The need for selectivity over CDK enzymes, particularly CDK1, CDK2 and CDK7, to avoid confounding mechanistic effects is also important, and it is notable that recent preclinical research has emphasised the use of mechanistic cellular assays to drive optimization of a selective mechanism-of-action, in addition to biochemical kinome profiling [17, 46].

Structure based design has played an important role in the development of selective CHK1 and CHK2 inhibitors. Most interestingly, studies on both of these structurally distinct enzymes have called attention to the potential benefit to selectivity of incorporating protein-bound water molecules into ligand design. For CHK1, targeting the network of water molecules in the interior pocket of the kinase leads to very high selectivity across several chemical scaffolds. A set of functional groups enabling interaction with these waters have been identified, based around hydrogen-bond accepting nitrogen heterocycles such as pyridine, pyrazine, pyrazole and triazole [5, 6, 37, 38, 41, 44, 46, 47, 49, 50]. The structural element promoting the assembly of the water network in CHK1 can be traced to a single amino acid (Asn59). It is interesting to speculate that the accumulation of bound water molecules associated with this residue effectively magnifies the scale of the structural difference in the binding site between CHK1 and other enzymes without this polar substituent, which may account for the high selectivities that can be achieved through targeting this feature.

In CHK2 the discovery of water-mediated binding of inhibitors to the hinge peptide of the kinase has not been attributed to the presence of a particular amino acid. It is, however, reproduced across several inhibitor scaffolds, and the intervening water molecule can be mimicked to good effect with a phenolic group (a substitution also achieved with respect to the protein-bound water molecules in CHK1 [36]). Very high selectivity for CHK2 over CHK1 and other kinases is associated with the water-mediated hinge-binding mode. The selectivity data on CHK2 inhibitors adopting the water-mediated binding mode supports the idea that a lower reliance on strong interactions to the hinge region of kinases could be beneficial for the selectivity of type I ATP-competitive kinase inhibitors in general.

The first generation CHK1 inhibitors are intravenous agents. This would not necessarily be limiting in the clinic for combination with classical DNA-damaging chemotherapies, since the cytotoxics are typically administered intravenously over short periods in any given treatment cycle. Recent preclinical data have shown that prolonged inhibition of CHK1 after DNA-damage may be beneficial to maximise the potentiation of the anti-tumour effect of the chemotherapy [6, 20]. In this regard, the development of oral compounds [20, 46, 89] may offer advantages in the flexibility of treatment scheduling to the second generation of CHK1 inhibitors. The recent *in vivo* demonstrations of the expected potentiation of radiotherapy by CHK1 inhibitors [78-80] also argues for oral agents if the combination of CHK1 inhibitors with long-term fractionated radiotherapy schedules is to be optimally translated to the clinic. It is not yet clear what the most effective treatment schedules for the emerging single agent therapeutic contexts for CHK1 inhibition will be, but there is a

possibility that sustained exposure would be required, for which oral inhibitors could again offer greater flexibility in the clinic.

An exciting and important development in the checkpoint kinase field has been the preclinical demonstration of single agent efficacy for inhibitors of CHK1 in specific cancer types, which could significantly enhance the clinical benefit of future drugs in this class. Intrinsic DNA damage and the activation of CHK1 signalling resulting from high endogenous replication stress appear to underlie the effectiveness of the inhibitors in many cases, mirroring the potentiation of extrinsic DNA-damaging agents observed with CHK1 inhibitors. The association of overexpression of MYC transcription factors with constitutive activation of the DNA damage response and sensitivity to CHK1 inhibition is one of the potential strategies for patient stratification [25, 90]. Preclinical data reported to date suggests that this is most strongly evidenced for paediatric MYC-N driven neuroblastoma and some B-cell lymphomas [20, 91, 92]. However, it is also notable that sensitivity to CHK1 inhibition has been observed in other cancer cell types where overexpression of MYC may not be a dominant effect [22, 23, 89]. It may still be possible to stratify cancers that are likely to be sensitive to CHK1 inhibition alone based on quantifying high intrinsic activation of the DNA damage response through CHK1, and a range of pathway biomarkers have been investigated to enable this. While progress on defining contexts where selective CHK2 inhibition may have a single agent effect is less advanced, appropriate small molecule tool compounds to address this are now available.

Acknowledgments

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* Articles of interest

** Articles of high interest

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Article Highlights

- Structure-based design has been successfully applied to generate potent and selective inhibitors of the DNA-damage response effector kinases CHK1 and CHK2.

- Highly selective CHK1 inhibition is most commonly associated with binding to a unique network of buried, protein bound water molecules in the enzyme

- An unusual water-mediated hinge-binding mode for CHK2 inhibitors leads to very high selectivity for ATP-competitive kinase inhibitors.

- The therapeutic potential for CHK1 inhibitors in combination with DNA-damaging chemo- and radiotherapy is well established preclinically. There have been mixed outcomes in the clinical development of CHK1 inhibitors to date, but progress has been made and second generation inhibitors are entering clinical trials.

- Exciting therapeutic contexts for single agent selective CHK1 inhibition have emerged based on targeting cancer genotypes that lead to replication stress and constitutive activation of the DNA-damage response.
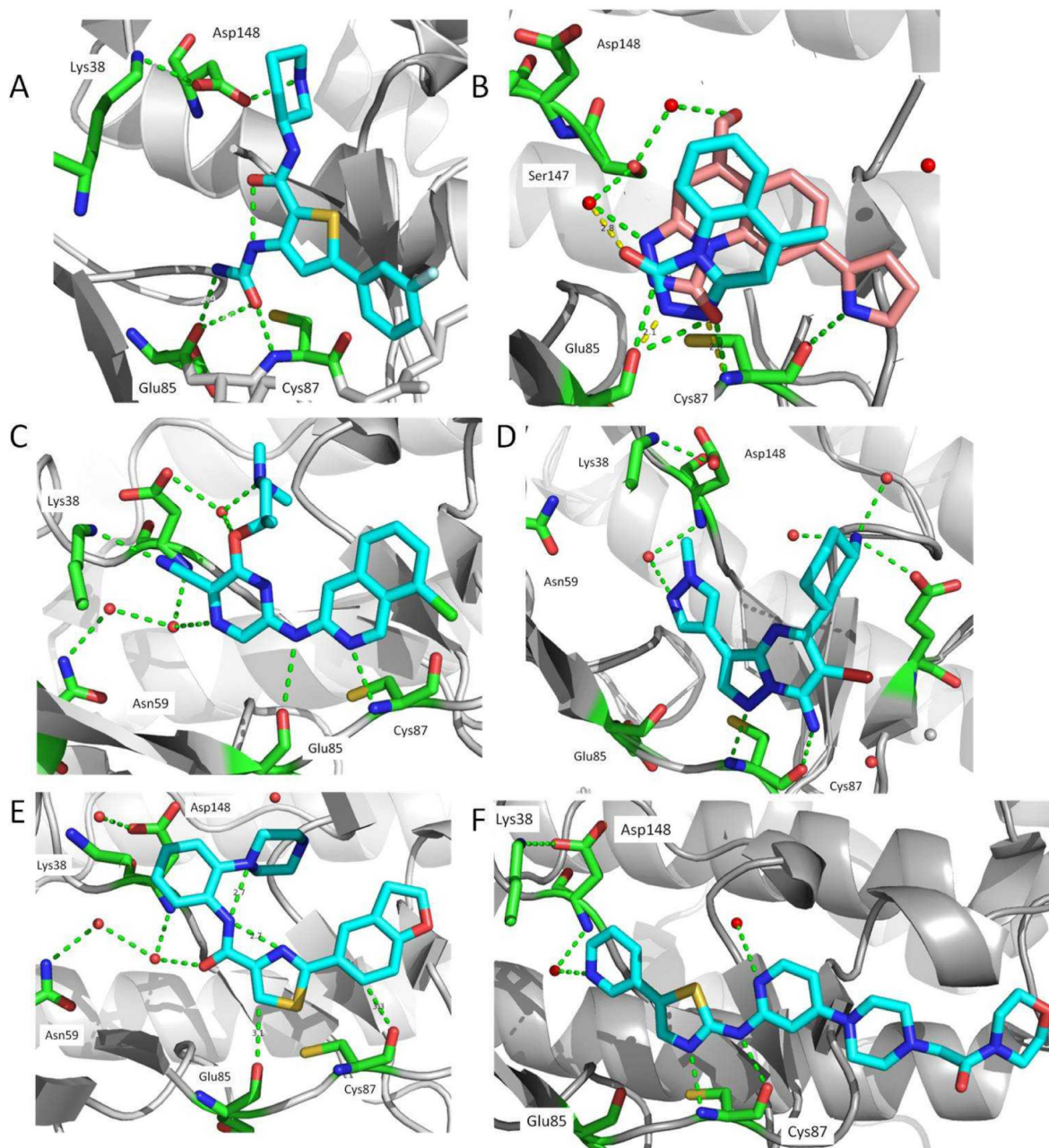
Figure 1.
Crystal structures of CHK1 in complex with inhibitors. A) **1** (PDB 2ydj); B) Overlay of **4** (blue, PDB 2x8d), **7** (pink, PDB 2yer); C) **16** (PDB 2ym8); D) **20** (PDB 3ot3); E) **21** (PDB 3u9n); F) **23** (PDB 3tkh); Hydrogen bonds are indicated as dashed lines.
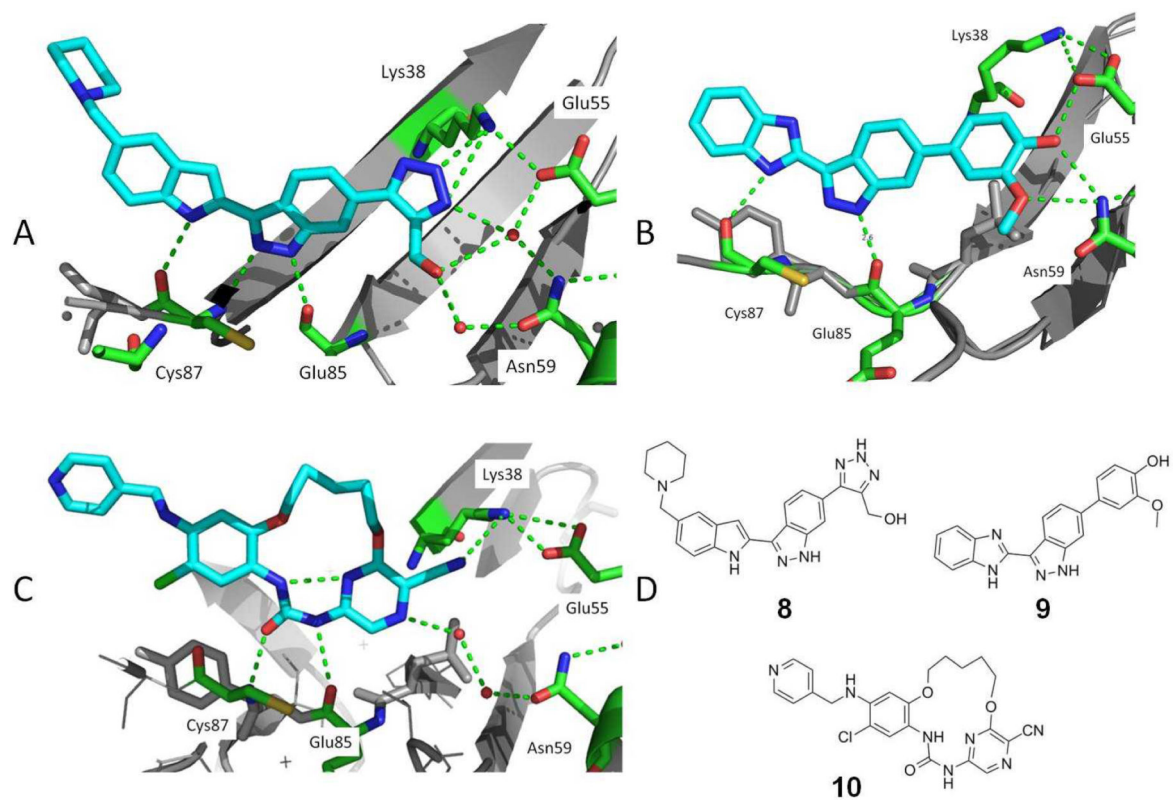
Figure 2.
Crystal structures showing small molecule inhibitors interacting with the hydrophilic buried pocket in the CHK1 ATP site. A) **8** [37], (PDB 2hog); B) **9** [36], (PDB 2c3k); C) **10** [38], (PDB 2e9v); D) Chemical structures of **8**, **9** and **10**. Hydrogen bonds are indicated as dashed lines.
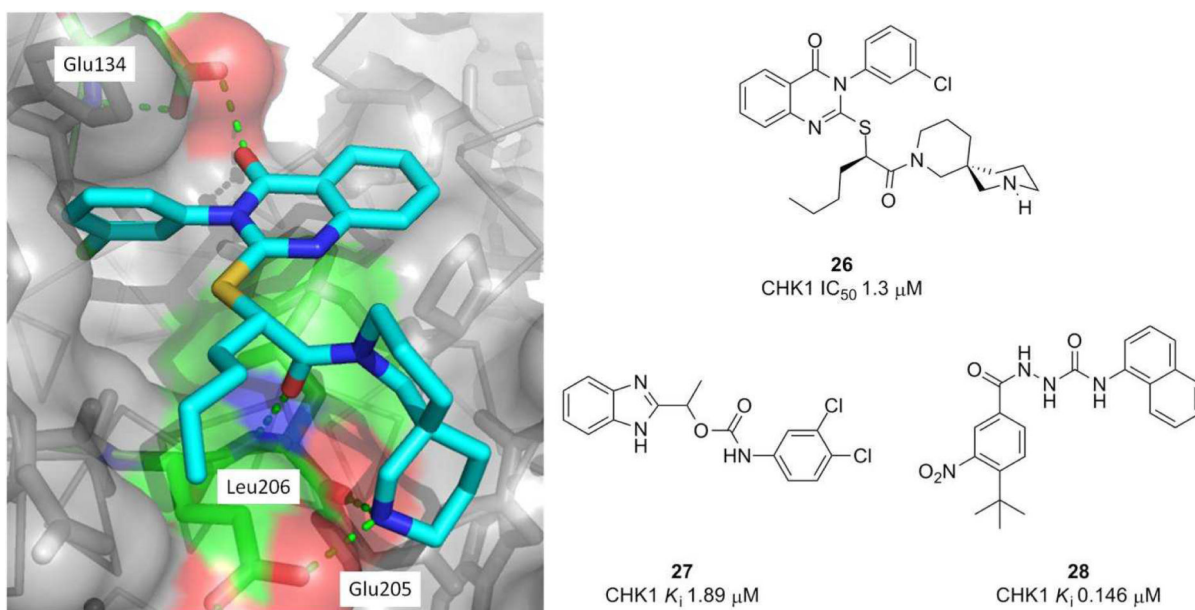
Figure 3.
Structures of the allosteric CHK1 inhibitors **26**-**28** and the crystal structure of **26** bound to CHK1 (PDB 3f9n).
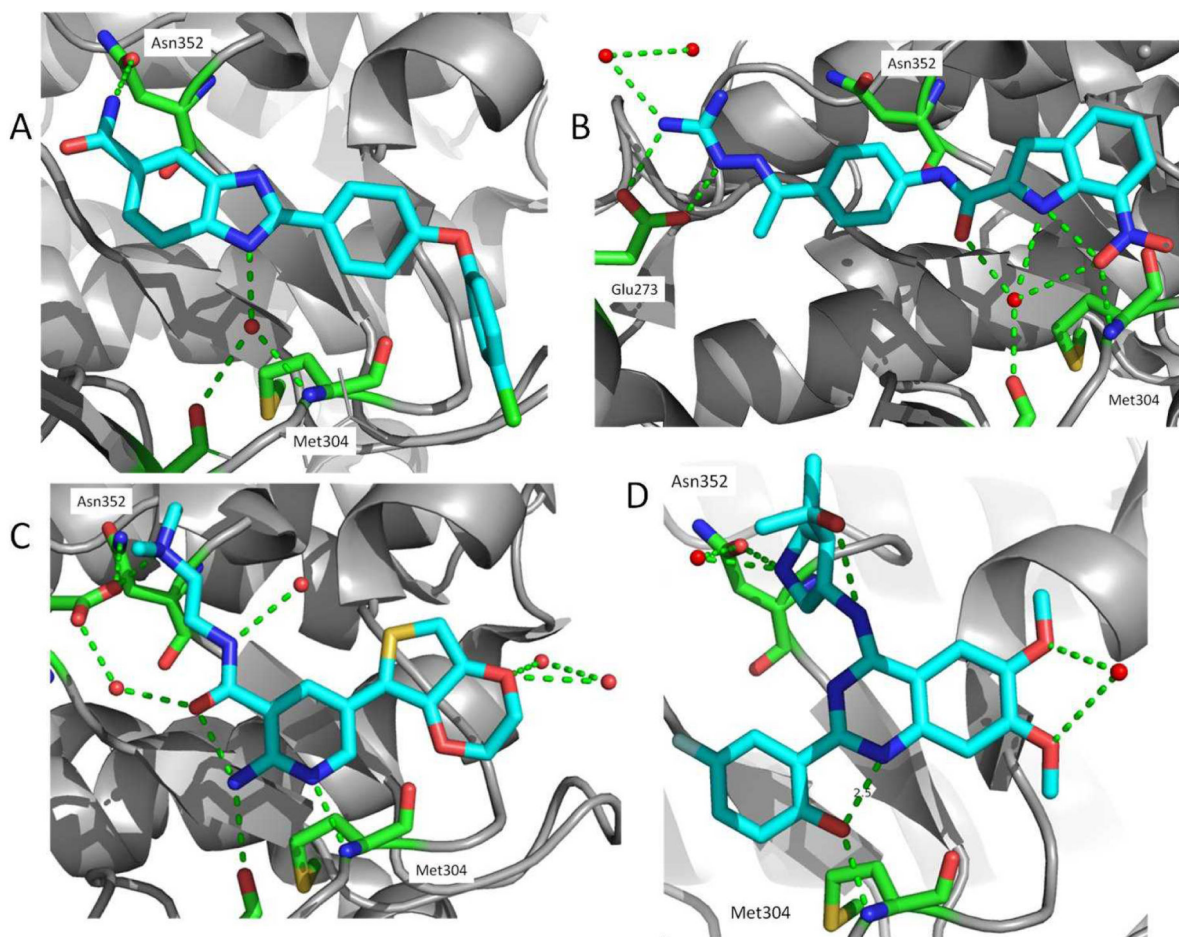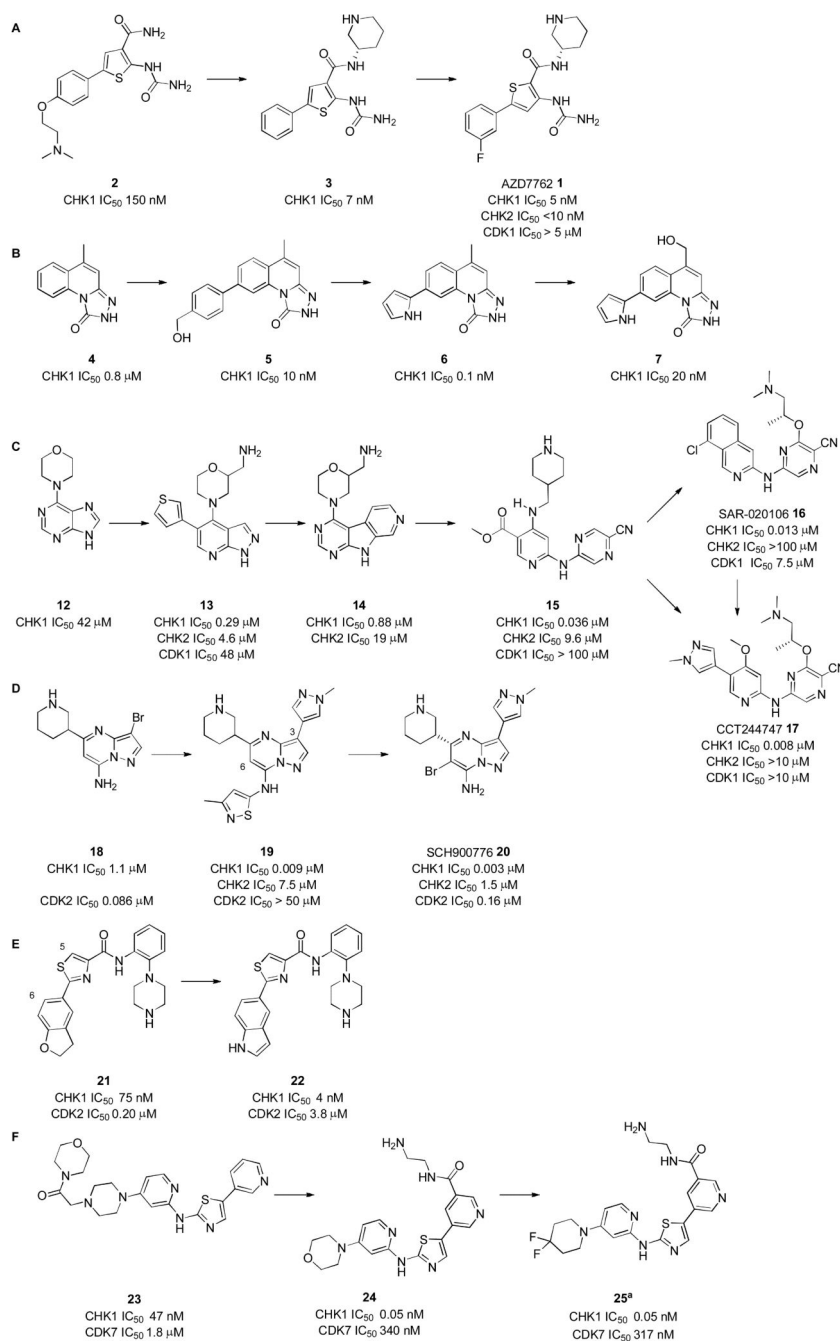
Figure 4.
Crystal structures of inhibitors in complex with CHK2 showing the different modes of interaction with the hinge region. A) **31** (PDB 4a9r), water-mediated hinge interaction; B) **33** (PDB 2w7x), water-mediated hinge interaction; C) **39** (PDB 2wtj), direct hinge interaction; D) **42** (PDB 2xbj), direct hinge interaction. Hydrogen bonds are indicated as dashed lines.
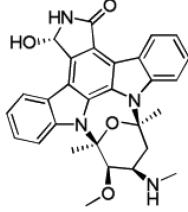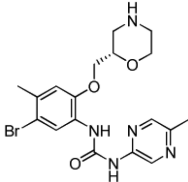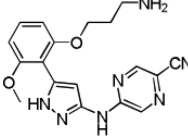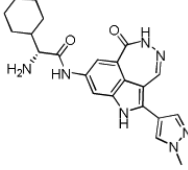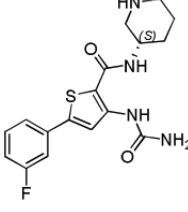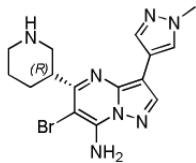
Scheme 1.

Examples of CHK1 inhibitors generated using SBDD from initial hit to late stage leads or clinical candidates. [a] The structure of 25 has been drawn as it appears in the graphical abstract of the reference [51] which differs from the representation in the body of the text.
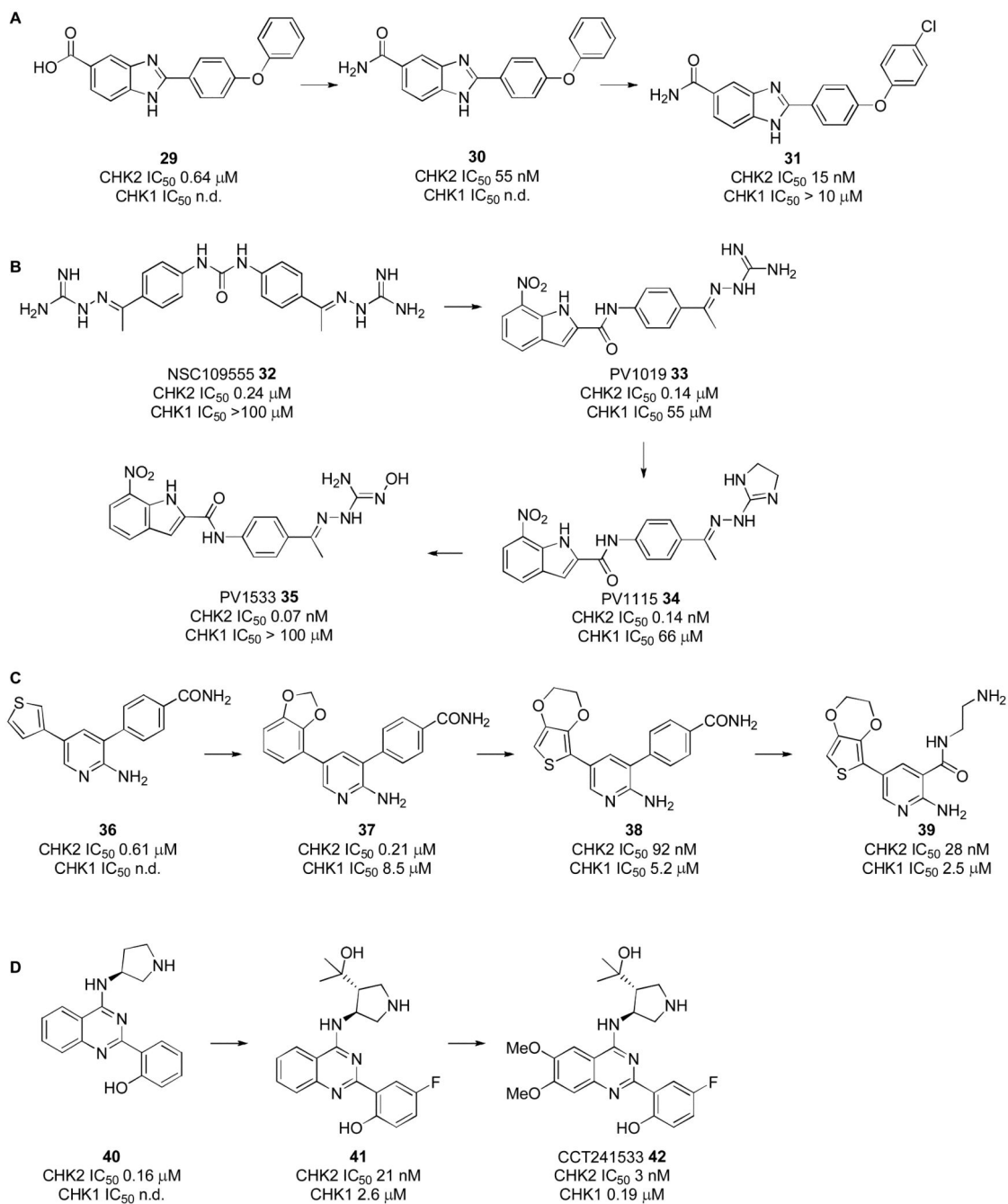
Scheme 2.
Examples of CHK2 inhibitors generated using SBDD.

Table 1

Selected clinical trial data for CHK1 clinical candidates

Inhibitor	Structure	Inhibitory Activity	Status of Clinical Development[a]	Ref
UCN-01 (**45**)		CHK1 IC$_{50}$ 11 nM	Phase II completed as single agent in relapsed T-cell lymphomas Phase II completed in combination with fluorouracil in pancreatic cancer Phase II completed in combination with topotecan for ovarian, fallopian tube and peritoneal cancers Phase II completed as single agent in metastatic melanoma Phase II completed in combination with topotecan in small cell lung cancer Multiple Phase I trials completed	[100-103]
XL-844 (**47**) (Previously EXEL-9844)	Not disclosed	CHK1 K$_i$ 2.2 nM CHK2 K$_i$ 0.07 nM	Phase I in combination with gemcitabine in advanced tumours and single agent in CLL terminated	-
LY2603618 (**11**)		CHK1 IC$_{50}$ 7 nM	Phase I completed in combination with pemetrexed. Phase I radiolabelled drug metabolism and CYP2D6 interaction studies completed Phase II active in combination with pemetrexed or pemetrexed + cisplatin in non-small cell lung cancer Phase II active in combination with gemcitabine in pancreatic and other solid tumours active	[74]
LY2606368 (**48**)		CHK1 IC$_{50}$ <1 nM CHK2 IC$_{50}$ 4.7 nM	Phase I recruiting for single agent in advanced cancers, squamous cell and head and neck cancers	-
PF-00477736 (**46**)		CHK1 K$_i$ 0.5 nM CHK2 K$_i$ 47 nM	Phase I in solid tumours in combination with gemcitabine terminated.	[104]
AZD7762 (**1**)		CHK1 IC$_{50}$ 5 nM CHK2 IC$_{50}$ 9.6 nM	Phase I completed in solid tumours alone and in combination with gemcitabine Two additional Phase I trials terminated	[97, 98]

Inhibitor	Structure	Inhibitory Activity	Status of Clinical Development[a]	Ref
SCH900776 (**20**)		CHK1 IC_{50} 3 nM CDK2 IC_{50} 160 nM CHK2 IC_{50} 1500 nM	Phase I completed in combination with gemcitabine in solid tumours and lymphoma Phase 1 in combination with cytarabine in acute leukaemias terminated	[75]
GDC-0575 (previously ARRY-575)	Not disclosed[b]	Not disclosed[b]	Phase I recruiting in combination with gemcitabine and as single agent in lymphoma and solid tumours	-
GDC-0425	Not disclosed	Not disclosed	Phase I recruiting in combination with gemcitabine and as a single agent in lymphoma and solid tumours	-

[a] www.clinicatrials.gov [last accessed on 23 January 2013]

[b] The structure has been disclosed of a compound which has *in vivo* efficacy data [105] matching that reported for ARRY-575 (GDC-0575) (**50**) [6, 106]

Table 2
Structures, selectivities and cellular activities of selected CHK2 inhibitors

Compound	Structure	Inhibitory activity	Cellular Studies	Ref
31		CHK2 IC$_{50}$ 15 nM CHK1 IC$_{50}$ >10 μM	Radioprotective in CD4$^+$ and CD8$^+$ T-cells exposed to IR. Isolated cells showed decreased apoptosis.	[60]
PV1019 (**33**)		CHK2 IC$_{50}$ 138 nM CHK1 IC$_{50}$ 50 μM	Abrogation of IR-induced apoptosis in mouse thymocytes. Radiosensitised U251 cells to IR. Potentiation of a topoisomerase I inhibitor in OVCAR-4 and OVCAR-5 cells. Antiproliferative effect on its own	[59]
CCT241533 (**42**)		CHK2 IC$_{50}$ 3 nM CHK1 IC$_{50}$ 190 nM	Radioprotective to isolated mouse thymocytes. No potentiation of the cytotoxicity of SN38, gemcitabine, etoposide, mitomycin C or bleomycin in HT29 or HeLa cancer cells Potentiation of the cytotoxicity of PARP inhibitors in BRCA-proficient HeLa and HT29 cells.	[54]
(**43**)		CHK2 IC$_{50}$ 8 nM CHK1 IC$_{50}$ 237 nM	ATM-dependent, CHK2-mediated radioprotective effect in 184B5 p53 wild-type cells but not p53 mutant MDA-MB-231 cells	[93]
VRX0466617 (**44**)		CHK2 IC$_{50}$140 nM CHK1 IC$_{50}$ >10 μM	Radioprotective in LCL-N cells, BJ-hTERT fibroblasts, HCT116 cells and mouse thymocytes No potentiation of doxorubicin and cisplatin cytoxicity in MCF7 or taxol in BJ-hTERT cells	[66]