Chapter 13
Targeting DNA-PK as a Therapeutic Approach in Oncology

Celine Cano, Suzannah J. Harnor, Elaine Willmore, and Stephen R. Wedge

Abstract DNA-dependent protein kinase (DNA-PK) is a nuclear serine/threonine protein kinase member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family of enzymes and, once activated, is a key participant in the repair of DNA-double strand breaks (DSBs), playing a central role in non-homologous end joining (NHEJ).

There have been significant efforts to identify small molecule catalytic inhibitors of DNA-PK, predominantly as an approach to induce chemo- and radio-sensitisation. The catalytic inhibitors described to date, differ in their potency, selectivity and the reversibility of inhibition. These inhibitors have been established from varied chemical structures that includes use of arylmorpholine, benzaldehde, chromen-4-one and indolin-2-one scaffolds. Clinical exploitation of DNA-PK inhibition in combination with DNA-damaging therapies may require strategies to maximize the likelihood of attaining an increased therapeutic index, such as the use of appropriate biomarker strategies to guide inhibitor dose and schedule, localisation of genotoxin treatment, or the elucidation of additional determinants of tumour sensitivity. M-3814 and VX-984 (M-9831) are examples of DNA-PK catalytic inhibitors that have advanced into clinical development, and which may help to determine whether such an approach represents a plausible therapeutic strategy for cancer therapy.

Keywords DNA-PK · DNA-repair · NHEJ · Inhibitor · Kinase · Chemopotentiation · Radiopotentiation

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13.1 Introduction

DNA-dependent protein kinase (DNA-PK) plays a major role in the cellular response to DNA damage, mediating the rapid repair of double strand breaks (DSB). DNA-PK is activated by both endogenous DSB (such as that arising from oxidative damage occurring during metabolic processes) and by exogenous agents that induce DSB, including clinically-used chemotherapeutics and radiotherapy. DNA-PK comprises a catalytic subunit (DNA-PKcs) and a dimeric complex of the Ku70 and Ku80 subunits, which have high affinity for DSB ends and thereby recruit the catalytic subunit to the site of the DNA lesion (Smith and Jackson 1999; Hill and Lee 2010).

Mammalian cells have evolved two main pathways to resolve DSBs, which prove highly toxic if unrepaired (Jackson and Bartek 2009). Homologous recombination (HR) promotes highly accurate repair by using homologous templates on sister chromatids and therefore occurs during S and G2 phases of the cell cycle (West 2003). In contrast, non-homologous end joining (NHEJ) (Jackson and Bartek 2009; Khanna and Jackson 2001; Collis et al. 2005) has evolved primarily to allow cells to rapidly repair DSB during any phase of the cell cycle (though preferentially during G1) (Chapman et al. 2012). A third repair pathway, referred to as alternative non-homologous end-joining (Alt-NHEJ) or microhomology-mediated endjoining (MMEJ), can also be engaged as a backup to canonical NHEJ but is used less frequently, being particularly error-prone and leading to the induction of deletion mutations and gene translocations (Simsek and Jasin 2010).

Both the kinase activity of the catalytic subunit and the DNA binding activity of Ku70 and Ku80 are required for NHEJ. Recent evidence, guided by structural analysis of DNA-PKcs in complex with a Ku80 peptide, suggests that an allosteric mechanism is involved in kinase activation (Sibanda et al. 2017). Importantly, these data also reveal a putative mechanism responsible for directing the cell to either HR or NHEJ, resulting from competition between Ku80 and BRCA1 DNA binding. This conformational change also stimulates DNA-PKcs enzyme activity (Hammarsten and Chu 1998; West et al. 1998). Although kinase activity is known to be essential for repair by NHEJ, the exact sequence of critical phosphorylation events is unclear. Activated DNA-PKcs is itself heavily phosphorylated (via *trans*-autophosphorylation and by the activity of other proteins), with two particular clusters of phosphorylated residues at Ser2056 and Thr2609, thought to be involved in the restriction of DNA processing and the dissociation of DNA-PKcs from the Ku heterodimer respectively (Uematsu et al. 2007; Cui et al. 2005). DNA-PKcs activity can also promote phosphorylation of proteins responsible for DNA ligation (e.g., XRCC4 and DNA ligase IV) (Leber et al. 1998; Wang et al. 2004), and of other signaling proteins such as Akt (Bozulic et al. 2008).
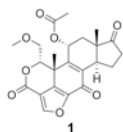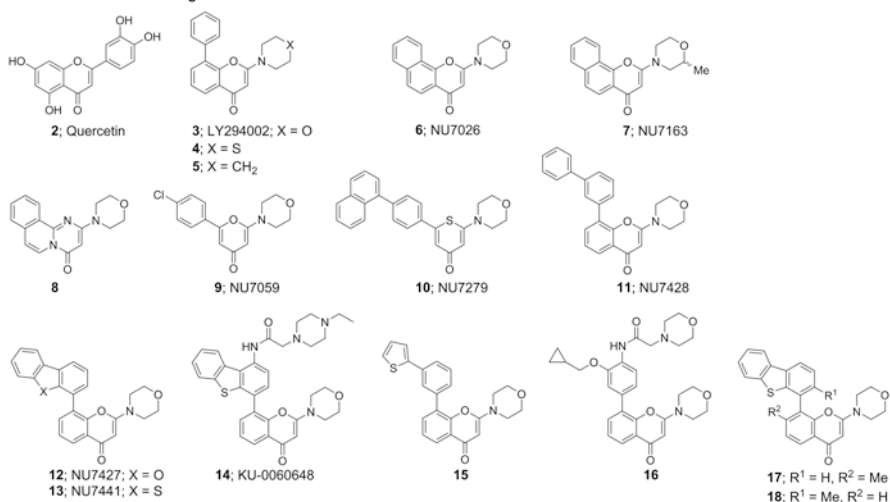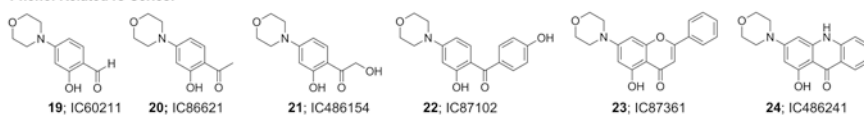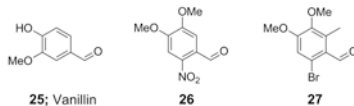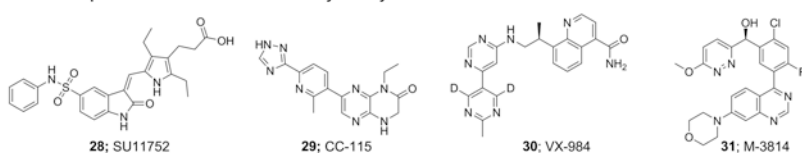
The catalytic activity of DNA-PKcs is also required for its function in V(D)J recombination, which is central to adaptive immunity, with loss of DNA-PK being

known to result in a severely immunocompromised phenotype (Blunt et al. 1995). Other biological processes reportedly influenced by DNA-PKcs activity, include a role in metabolic control where it can influence the activity of AMPK, a key energy sensor involved in the regulation of glucose uptake (Amatya et al. 2012; Park et al. 2017), and an ability to regulate a number of transcriptional responses, such as those induced by particular nuclear hormone receptors (Goodwin et al. 2015).

DNA-PKcs expression at both the protein and mRNA level varies among tumour types. In chronic lymphocytic leukaemia (CLL), high DNA-PKcs correlated with DNA-PK activity, and is associated with chemoresistance and a reduced treatment-free interval in patients (Muller et al. 1998; Willmore et al. 2008). Increased expression of DNA-PKcs has also been reported in gastric cancer (Li et al. 2013) and correlates with poor clinical outcome in ovarian cancer (Abdel-Fatah et al. 2014) and hepatocellular cancer (HCC) (Cornell et al. 2015). Whilst a prognostic association between DNA-PKcs and poor disease outcome does not necessarily confirm that the tumour has a dependency on the protein's kinase activity, this has been demonstrated experimentally in castrate-resistant prostate cancer, where DNA-PK catalytic function is found to be a driver of metastatic disease (Goodwin et al. 2015). Given the role of DNA-PK in chemo- and radio-resistance, and its potential role as a driver of other tumorigenic processes, there has been significant interest in identifying inhibitors of the catalytic subunit. However, DNA-PK has proven to be a significantly challenging target for structural biology, the holoenzyme structure only recently being resolved to 5.8 Å with the development of cryo-electron microscopy (Sharif et al. 2017). Consequently, the discovery of inhibitors has been driven by screening approaches with subsequent optimisation using medicinal chemistry expertise. The majority of inhibitors have been designed to occupy the ATP-binding site, however, given the homology between the kinase domains of the phosphatidylinositol 3-kinase (PI-3K) related kinase (PIKK) family members (including ATM (Ataxia telangiectasia mutated kinase), ATR (ATM and Rad3-related kinase) and mTOR (Mammalian Target of Rapamycin)) (Khanna and Jackson 2001; Collis et al. 2005), development of selective inhibitors has been challenging. The design of inhibitors with confirmed target specificity and potency, is essential to enable the potential of DNA-PK inhibition to be evaluated accurately in preclinical studies and clinical applications.

13.2   Small Molecule Inhibitors of DNA-PK Catalytic Activity

The development of small molecule DNA-PK inhibitors has been studied extensively over the past two decades, transitioning from early non-specific compounds to highly selective, potent inhibitors that have entered into clinical trials (Fig. 13.1).

Wortmannin:

Chromen-4-ones and Surrogates:

2; Quercetin

3; LY294002; X = O
4; X = S
5; X = CH₂

6; NU7026

7; NU7163

8

9; NU7059

10; NU7279

11; NU7428

12; NU7427; X = O
13; NU7441; X = S

14; KU-0060648

15

16

17; R¹ = H, R² = Me
18; R¹ = Me, R² = H

Phenol Related IC Series:

19; IC60211

20; IC86621

21; IC486154

22; IC87102

23; IC87361

24; IC486241

Vanillins:

25; Vanillin

26

27

Further Compounds With Demonstrated Inhibitory Activity:

28; SU11752

29; CC-115

30; VX-984

31; M-3814

Fig. 13.1 Small molecule inhibitors of DNA-PK catalytic activity

13.3 Wortmannin

Originally found to have antifungal and anti-inflammatory properties, wortmannin
(**1**), is a metabolite of the fungi *Penicillium wortmannii K*. This sterol-like compound
was subsequently found to be a potent and selective inhibitor of PI-3K family

kinases, including inhibition of purified bovine brain PI-3K activity with an IC_{50} of 4.2 nM (Powis et al. 1994). Walker *et al.* verified non-competitive irreversible inhibition with co-crystallographic studies of the resulting covalent complex in the ATP-binding pocket of PI3Kγ (Wymann et al. 1996; Walker et al. 2000). By forming covalent adducts with DNA-PKcs lysine 3751 in the region of the molecule harbouring its kinase domain, wortmannin inhibits DNA-PK at higher concentrations (Ki = 120 nM) via a non-competitive mechanism (Izzard et al. 1999). Despite being an interesting early tool compound, the relative structural complexity of wortmannin presents some challenges for synthetic chemistry and its poor selectively limit its potential. Despite these caveats, a three to five-fold enhancement of IR-induced cytotoxicity and an inhibition of IR-induced DSB repair in Chinese hamster ovary cells was determined using wortmannin (Boulton et al. 1996).

13.4   Chromen-4-ones and Surrogates: LY2094002, NU7441 and KU-0060648

With the aim of developing PI3K-specific inhibitors, Lilly pharmaceuticals undertook a screen of compounds derived from the polyphenol compound quercetin (**2**). In 1994, the chromen-4-one structure LY294002 (**3**) was reported as an inhibitor of PI3K (Vlahos et al. 1994). Further profiling of **3** showed that the compound was an equipotent inhibitor of DNA-PK, mTOR and PI3K (Table 13.1) (Vlahos et al. 1994; Griffin et al. 2005).

The importance of the oxygen of the morpholine of LY294002 was assessed by replacing the oxygen with either a sulfur or a carbon. Interestingly, replacement by sulphur, to provide thio derivative **4** (IC_{50} = 1.61 μM), was tolerated, whilst piperidine analogue **5** exhibited reduced potency (IC_{50} = 4.67 μM). When the structure of LY294002 is in complex with human PI-3Kγ, X-ray crystallography verifies that within the ATP-binding domain of the kinase, the morpholine oxygen makes a hydrogen bond interaction with the backbone amide group of Val-882. This further substantiates the key role of the morpholine substituent (Fig. 13.2) (Walker et al. 2000).

Table 13.1 Inhibitory activity (IC_{50} μM) of DNA-PK inhibitors against different PIKK family members

	DNA-PK	PI-3K (p110α)	ATM	ATR	mTOR
LY294002	1.5±0.2[a]	2.3±0.8[a] (1.4)[b]	>100[a]	>100[a]	2.5±0.2[a]
NU7026	0.23[b]	13[b]	>100[b]	>100[b]	6.4[b]
NU7163	0.19[b]	2.4[b]	>100[b]	>100[b]	4.8[b]
8	0.28[b]	>100[b]	>100[b]	>100[b]	5.3[b]
NU7441	0.014[c]	5[c]	>100[c]	<100[c]	1.7[c]

Values taken from references Vlahos et al. 1994[a], Griffin et al. 2005[b] and Leahy et al. 2004[c]
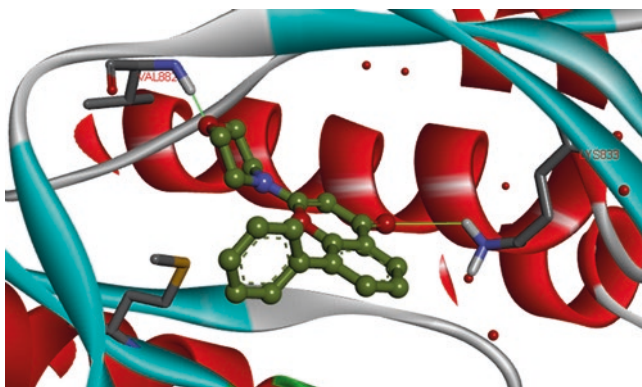
Fig. 13.2 Crystal structure of LY294002 (**3**) in complex with the ATP-binding domain of PI-3Kγ. The figure was prepared from PDB file 1E7V using Discovery Studio 4.1

Although LY294002 suffered from rapid metabolic clearance and induced toxicity *in vivo*, the compound guided the design of derivatives with improved potency and selectivity against DNA-PK. The first analogues investigated were benzopyranone and pyrimidoisoquinolinone derivatives, in studies undertaken by the Newcastle University Drug Discovery Group in collaboration with KuDOS Pharmaceuticals (Griffin et al. 2005). Incorporation of a fused ring on the chromen-4-one, led to an increase in potency against DNA-PK (NU7026 (**6**); IC_{50} = 0.23 μM) (Table 13.1). Variation of the fused phenyl ring around the chromenon-4-one structure gave slightly less active compounds than NU7026. In contrast, substituting the morpholine moiety with a methyl group improved potency (NU7163 (**7**); IC_{50} = 0.19 μM) (Table 13.1). It is worth noting that adding an additional methyl group at the morpholine 2 or 6-position resulted in reduced inhibitory activity (Griffin et al. 2005; Hardcastle et al. 2005). Interestingly, these early LY294002 derivatives demonstrated a better selectivity profile for DNA-PK over other PIKK family members, as exemplified with NU7026, reported to be 60-fold more potent against DNA-PK than PI-3K (p110α) (Table 13.1). Introducing a pyrimidoisoquinolinone scaffold as an isosteric replacement of the chromen-4-one also led to an equipotent compound (**8**; IC_{50} = 0.28 μM) (Table 13.1) (Cano et al. 2010a).

In vitro experiments demonstrated that NU7026 acts as a radiosensitiser, giving a 2-fold dose enhancement in mouse embryonic fibroblast cells (Veuger et al. 2003). Because of the known ability of topoisomerase II (TOP2) inhibitor-induced DSBs to activate DNA-PK, chemosensitisation by NU7026 was explored using a panel of TOP2 poisons in K562 and ML-1 human leukaemia cell lines. NU7026 (10 μM) was found to increase sensitivity to TOP2 poisons by 2–19 fold, by retarding DNA DSB repair and exacerbating the G2 cell cycle block (Willmore et al. 2004).

With a view to truncating the chromenone core, synthesis of substituted monocyclic pyran-2-one, pyran-4-one, thiopyran-4-one and pyridin-4-one derivatives was undertaken (Hollick et al. 2007). Structure-activity relationship (SAR) studies around

6-substituted-2-morpholino-pyran-4-ones and 6-substituted-2-morpholinothiopyran-4-ones led to the identification of NU7059 (**9**; DNA-PK; IC_{50} = 0.18 μM) and NU7279 (**10**; DNA-PK IC_{50} = 0.19 μM), both exhibiting a tenfold increase in potency against DNA-PK (Hollick et al. 2007; Hollick et al. 2003). A multi-parallel library approach was also conducted to synthesise 6-, 7-, and 8-aryl substituted chromen-4-ones. Encouragingly, substitution at the 8-position provided a group of inhibitors with activity comparable to NU7026. NU7428 (**11**; IC_{50} = 0.11 μM) showed a ten fold increase in potency compared with LY294002, and the dibenzofuranyl derivative NU7427 (**12**) demonstrated further improved inhibitory activity (IC_{50} = 0.04 μM). The incorporation of a dibenzothiophenyl group increased the potency by 100-fold when compared with LY294002 (NU7441 (**13**); IC_{50} = 0.02 μM), along with excellent selectivity over other PIKK family members (Table 13.1) (Leahy et al. 2004).

NU7441 (8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one) has been used in over 70 studies in the literature to date. NU7441 was characterised using the SW620 and LoVo cell lines and it was found that 1μM NU7441 enhanced the cytotoxicity of etoposide (2–12 fold), doxorubicin (2–10 fold) and IR (2–4 fold). (Zhao et al. 2006) Importantly, *in vivo* studies showed that despite its relatively poor solubility, which can limit bioavailability following oral administration, NU7441 increased etoposide-induced tumour growth delay in an SW620 human colon carcinoma xenograft model (Zhao et al. 2006). As well as the effects on cell growth and cytotoxicity, the ability of NU7441 to inhibit repair of DSBs was analysed. In breast cancer (Cowell et al. 2005) and HCC cell lines (Cornell et al. 2015). NU7441 delayed the disappearance of IR- and TOP2 poison-induced γH2AX foci, indicating that the mechanism by which NU7441 enhanced cytotoxicity of these agents was due at least in part to inhibition of DNA-PK-mediated DSB repair.

The potency and selectivity of NU7441 provided an opportunity to explore the translational potential of inhibiting DNA-PK activity. In a panel of 54 patient-derived CLL tumours, 1 μM NU7441 potentiated the cytotoxicity of cholorambucil and fludarabine (drugs used to treat CLL) from 2 to 20-fold, increased drug-induced DSB and γH2AX foci and inhibited the drug-stimulated autophosphorylation of DNA-PK (Ser2056) (Willmore et al. 2008). This study illustrates the concept of using a DNA-PK inhibitor to enhance the cytotoxicity of DNA damaging agents in a defined clinical indication. Additional data denoted that NU7441 was particularly effective at enhancement of TOP2 poison-induced cytotoxicity in multidrug resistant (MDR) cell lines (Mould et al. 2014), and since TOP2 poisons are good substrates for the MDR1 drug efflux pump, the effect of NU7441 on drug efflux was examined. Studies conducted in 4 paired cell lines, each comprising a sensitive (parental) and drug-resistant (MDR1-expressing) counterpart, showed that as well as inhibiting DNA-PK, NU7441 resulted in a small but significant increase in intracellular accumulation of doxorubicin and vincristine in MDR1 expressing cells. For example, in CCRF-CEM VCR/R cells, NU7441 (1 μM for 8 h) increased levels of vincristine by 2.1-fold, which was similar to the increase achieved following exposure to verapamil, a known MDR1 modulator. These data indicate that in MDR1 over-expressing cells, NU7441 can act as a dual DNA-PK and MDR1 inhibitor
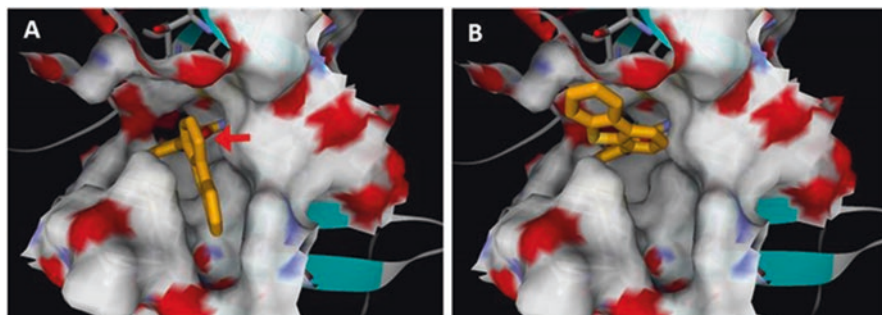
Fig. 13.3 Homology model of the ATP-binding site of the DNA-Dependent Protein Kinase (DNA-PK) used to guide inhibitor design (Clapham et al. 2012). The 3D model was constructed on the basis of the known X-ray crystal structure of PI3Kγ from RCSB protein data bank (PDB ID: 1E7V) as a template, and with DNA-PK sequence from Swiss-Port (ID: PRKDC_DICDI) using Prime in Maestro molecular modelling program (licensed from Schrödinger, LGG). NU7441 (**13**) is represented in (**a**) an orthogonal, and (**b**) "in plane" pose

(Mould et al. 2014). However, further studies to fully assess the potential of NU7441 as an inhibitor of the MDR1 drug efflux pump is merited since in a second study using a panel of HCC cell lines verapamil increased the nuclear accumulation of doxorubicin but NU7441 did not (Cornell et al. 2015).

A PI-3Kγ-derived homology model of the ATP-binding site of DNA-PK was used to guide further inhibitor design (Fig. 13.3) (Clapham et al. 2012).

According to this model, the dibenzothiophene 1-position of NU7441 is pointing towards solvent and therefore predicted to accommodate water-soluble side chains that can be used to increase compound solubility (Fig. 13.3a, arrow). A multi-parallel library approach was undertaken to investigate improvements in compound potency and physicochemical properties based on this hypothesis. The newly synthesised inhibitors all possessed polar substituents at the dibenzothiophene 1-position (Cano et al. 2013). Several compounds were highly potent against DNA-PK and potentiated the cytotoxicity of IR *in vitro* tenfold or more (e.g., KU-0060648 (**14**); DNA-PK IC_{50} = 5.0 ± 1 nM, IR dose modification ratio = 13). In addition, KU-0060648 was shown to potentiate not only IR *in vitro*, but also DNA-damage inducing TOP2 poisons (doxorubicin, etoposide) both *in vitro* and *in vivo (*Cano et al. 2013*)*. In addition to the promising biological activity and improved drug-like properties of KU-0060648 compared to NU7441, acceptable plasma protein binding, combined with weak activity against the hERG ion channel (involved in cardiac repolarisation) and a panel of CYP450 drug metabolizing enzymes was now evident (Table 13.2). However, a number of these compounds, including KU-0060648, were discovered in a counter screen against other PIKK family members, to be potent mixed DNA-PK and PI-3K inhibitors (Cano et al. 2013; Munck et al. 2012). Nonetheless, KU-0060648 demonstrated chemosensitisation effects that were dependent upon DNA-PK expression: the compound enhanced doxorubicin cytotoxicity significantly (up to 32-fold) in MO59-Fus-1 DNA-PK proficient cells but did not affect the cytotoxicity in MO59J DNA-PK deficient cells which were intrinsically more sensitive to doxorubicin

Table 13.2 Properties of NU7441 (**13**) and KU-0060648 (**14**) (Data are the mean ± the standard deviation or individual values; adapted from reference Cano et al. 2013)

Assay		NU7441	KU-0060648
Enzyme	DNA-PK IC_{50} (nM)	42 ± 2	5.0 ± 1
Cellular (HeLa)	pDNA-PK EC_{50} (nM)	212, 339	136 ± 17
	IR-DMR (0.1 µM DNA-PK inhibitor)	2.2 ± 0.2	4.0 ± 0.4
	IR-DMR (0.5 µM DNA-PK inhibitor)	2.8 ± 0.1	13 ± 2
Other	Log$D$ (pH = 7.4)	>4.3	3.05
	hERG IC_{50} (µM)	14, 19	>20
	Solubility at pH 7.4 (µM)	<0.3, <0.2	161 ± 103[a]
	Human plasma protein binding (% Free)	0.04, 0.17	6.2, 3.6
	CYP_{450} inhibition (µM)[b]	-	> 10

[a]Amorphous material (crystalline solubility at pH7.4 buffer = 6.0 µM)
[b]Tested in CYP 3A4, 2D6, 2C9, 2C19 and 1A2 (IR-DMR, the dose modification ratio, defined as the percentage of cell survival in the absence of compound with 2 Gy treatment divided by that in the presence of compound plus 2Gy treatment as determined in 6 – 8 day clonogenic assays; logD, the distribution coefficient calculated as the ratio for the sum of all species of a compound in 1-octanol *versus* that in water at equilibrium; hERG, the human ether-a-go-go-related gene)

(Munck et al. 2012). Importantly, this study also quantified potent cellular inhibition of DNA-PK activity by KU-0060648 (inhibition of IR-induced DNA-PKcs autophosphorylation at Ser2056) demonstrating an IC_{50} of 0.02 µM in MCF-7 breast tumour cells and 0.136 µM in HeLa cells (Table 13.2) (Cano et al. 2013; Munck et al. 2012).

Additional SAR studies around LY294002 and NU7441 have been conducted and have led to the identification of 8-biarylchromenon-4-one derivatives (e.g. **15**; IC_{50} = 18 nM) and *O*-alkoxyphenylchromen-4-one analogues (e.g. **16**; IC_{50} = 8 nM) (Desage-El Murr et al. 2008; Clapham et al. 2011).

Stable pairs of resolvable atropisomers, due to restricted rotation between the chromen-4-one and dibenzothiophene rings, were also generated via substitution of a methyl group at either the chromenone 7-position (**17**) (DNA PK; IC_{50} = 0.005 µM) or dibenzothiophene 3-position (**18**) (DNA PK; IC_{50} = 1.7 µM) of parent compound NU7441 (Clapham et al. 2012; Cano et al. 2010b). Interestingly, in comparison with NU7441, substitution at the chromenone 7-position gave an improvement in potency against DNA-PK, whereas substitution at the dibenzothiophene 3-position resulted in a reduction in potency. Following chiral resolution, each pair of atropisomers was evaluated and the conclusion made that DNA-PK inhibitory activity resided exclusively in the (−)-atropisomer enantiomer, with the antipodal (+)-atropisomer proving inactive (Mould et al. 2014; Clapham et al. 2012).

13.5 Phenol Related IC Series

Benzaldehyde derivative, 2-hydroxy-4-morpholin-4-yl-benzaldehyde (IC60211, IC_{50} = 400 nM) (**19**), is a representative example of a new series of morpholine containing DNA-PK inhibitors reported by the ICOS Corporation and Array

Table 13.3 Inhibitory activity (IC$_{50}$ nM) of representative DNA-PK inhibitors against various PI-3Ks (adapted from references Knight et al. 2004 and Kashishian et al. 2003)

	DNA-PK	p110α	p110β	p110δ	p110γ
IC60211	400	10000	2800	5100	37000
IC86621	120	1400	135	880	1000
IC486154	44	890	42	490	180
IC87102	35	2700	400	1800	5000
IC87361	34	3800	1700	2800	7900

Biopharma. Optimisation of IC60211 led to DNA-PK selective inhibitors (**20–24**), all of which maintained the arylmorpholine substructure, which was found to be critical for kinase inhibitory activity. IC86621 (**20**) is chemically stable and despite not being the most potent compound in the series (IC$_{50}$ = 120 nM), was found to act as a selective and reversible ATP-competitive inhibitor, exhibiting high selectivity against other kinases such as PI-3 K family members (Table 13.3) (Knight et al. 2004; Kashishian et al. 2003). IC86621 (**20**) and IC486154 (**21**) are selective with respect to PI-3K subunit (p110) α, γ and δ, but are equipotent with p110β. The more highly developed morpholino-flavanoid, IC87361 (**23**) is 50-fold more selective for DNA-PK than for p110β (Kashishian et al. 2003).

In vitro, the arylmorpholine compounds IC86621, IC87102 (**22**) and IC87361 are radio- and chemosensitisers and delay repair of DNA DSBs (Kashishian et al. 2003). These compounds also radiosensitise *in vivo* and display superior pharmacokinetic profiles in comparison to other specific DNA-PK inhibitors (Shinohara et al. 2005).

The small-molecule DNA-PK inhibitor IC486241 (**24**) (IC$_{50}$ < 100 μM) differs structurally by way of possessing an acridinone core and is relatively non-toxic as monotherapy (IC$_{50}$ ≥ 29 μM in both HCT-116 and HT-29 cell lines) and found to synergize with 7-ethyl-10-hydroxy-camptothecin (irinotecan, SN38) to enhance killing of colon cancer cells *in vitro (*Davidson et al. 2012a*)*. Additionally, IC486241 was shown to sensitise three genetically diverse breast cancer cell lines to the TOP2 inhibitor doxorubicin (Davidson et al. 2012b). Furthermore, IC486241 decreased doxorubicin-induced DNA-PKcs autophosphorylation on Ser2056 and increased doxorubicin-induced DNA fragmentation (Davidson et al. 2012b).

13.6 Vanillins

It has been demonstrated that members of the vanillin family are simple and relatively specific inhibitors of DNA-PK (Durant and Karran 2003). Even though the activity of vanillin (**25**) (IC$_{50}$ = 1.5 mM) was estimated to be 1000-fold lower than wortmannin, the simple low molecular weight structure presented the opportunity to synthesise further derivatives. A screen of approximately 53,000 organic drug-like

compounds was carried out, which aimed to discover structurally related benzaldehyde derivatives. Two compounds that exhibited DNA-PK inhibition at a concentration of 100 μM were 4,5-dimethoxy-2-nitrobenzaldehyde (**26**) (IC$_{50}$ = 15 μM) and 2-bromo-4,5-dimethoxybenzaldehyde (**27**) (IC$_{50}$ = 30 μM), 100-fold and 50-fold more potent than vanillin respectively. Interestingly, non-aldehyde analogues were ineffectual, indicating that the aldehyde group is essential for inhibitory activity (Durant and Karran 2003). Vanillin interacts preferentially with protein lysine residues in the catalytic centre of PI-3K, via Schiff base formation (Chobpattana et al. 2000). The simplicity of vanillin-based molecules makes them attractive initial compounds for structure-based chemistry optimisation, but to enable them to be useful tools for assessing the biochemical mechanism of DNA-PK and the contribution of pathways to DSB repair, the solubility and selectivity would have to be significantly enhanced (Durant and Karran 2003).

13.7 SU11752 (Sugen Incorporated)

SU11752 (**28**) was identified by library screening of three-substituted indolin-2-ones, as an ATP-competitive DNA-PK inhibitor (IC$_{50}$ = 0.13 ± 0.028 μM) with comparable potency to wortmannin (IC$_{50}$ = 0.10 μM) (Izzard et al. 1999), but with selectivity for DNA-PK over PI-3K (p110γ; IC$_{50}$ = 1.10 μM) (Ismail et al. 2004). SU11752 also does not inhibit ATM kinase activity in cells, at concentrations that result in inhibition of DSB repair (12 μM). SU11752 sensitised cells to ionising radiation but lacked sufficient potency for *in vivo* studies (Ismail et al. 2004).

13.8 CC-115 (Celgene)

CC-115 (**29**) is an orally bioavailable equipotent inhibitor of the kinase activities of both DNA-PK (IC$_{50}$ = 0.013 μM) and mammalian target of rapamycin (mTOR) (IC$_{50}$ = 0.021 μM) (Mortensen et al. 2015). By virtue of the latter activity, CC-115 will inhibit both the raptor-mTOR (TORC1) and rictor-mTOR (TORC2) complexes that transduce responses downstream of the PI-3K and Akt signalling pathway. Selective inhibition of mTOR has been pursued by others as a therapeutic strategy in oncology, and the entry of CC-115 into patient trials in 2011 (NCT01353625) as a monotherapy treatment in a range of tumour settings (prostate cancer, multiple myeloma, Ewing's osteosarcoma, lymphoma, CLL) suggests that these clinical studies have been developed to examine the compound's unique dual pharmacology. Whether having additional activity against mTOR will limit the dose of CC-115 that can be administered and hence limit the magnitude of DNA-PK inhibition that can be achieved clinically, remains to be determined, however early results from the trial have shown partial responses in 8 CLL patients, including those with ATM loss (Thijssen et al. 2016).

13.9 VX-984 (M-9831, Merck KGaA and Vertex Pharmaceuticals Incorporated)

Vertex Pharmaceuticals discovered VX-984 also known as M-9831 (**30**) as a DNA-PK inhibitor for clinical development and initiated a phase I combination study with pegylated liposomal doxorubicin in the USA in December 2015 (NCT02644278), in patients with advanced solid tumours or lymphomas. In isolated enzyme studies VX-984 (M-9831) is reported to demonstrate selectivity for DNA-PK in comparison to all Class I PI-3 K isoforms, ranging from 80-fold selectivity *versus* PI-3Kα to 1300-fold for PI-3Kβ (Boucher et al. 2016). The compound was also reported to have an IC$_{50}$ of 88 ± 64 nM for inhibition of DNA-PKcs autophosphorylation (Ser2056) in A549 lung cancer cells (Boucher et al. 2016). VX-984 (M-9831) is administered orally and augments the efficacy of radiotherapy in lung tumour models *in vivo* when administered at 50–100 mg/kg twice-daily (Boucher et al. 2016). As part of a licensing deal, Merck KGaA acquired the rights to VX-984 in January 2017 and are continuing to develop it for the treatment of solid tumours.

13.10 M-3814 (Merck KGaA)

Merck KGaA are known to be developing an orally-administered inhibitor of DNA-PK called M-3814 (**31**) (Zenke et al. 2016; Fuchss et al. 2017). In December 2014, M-3814 entered phase I clinical development in Germany (NCT02316197) in patients with solid tumours who had DNA repair deficiencies, such as loss of ATM or BRCA function, or in patients with CLL. In this study the compound was administered orally as monotherapy, chronically in continuous 21 day cycles (once- or twice-daily). M3814 was tolerated up to 400 mg twice-daily, with further dose-escalation being prohibited by an impurity issue. This dose resulted in stable disease in 6 patients (20% of those examined) for 18 weeks, but there was no evidence of any partial responses. Plasma pharmacokinetic analyses also revealed highly variable exposure (Van Bussel et al. 2017).

In July 2015, M-3814 entered a phase I trial in the USA and Germany (NCT02516813) in combination with radiotherapy. This included a Phase 1a in patients with solid tumours receiving palliative radiotherapy, involving compound administration 1.5 h prior to each 3Gy dose of radiotherapy, with a total of 10 fractions of radiotherapy (up to five fractions per week). In January 2017, seven patients had been treated and two of these had local tumour control, however, plasma pharmacokinetic analyses again demonstrated significant variability (Van Triest et al. 2017). In the Phase 1b, M-3814 is being combined with a more conventional 60 Gy total dose of radiotherapy, given as 2Gy fractions (5 fractions per week) in patients with advanced non-small cell lung cancer or head and neck squamous cell carcinoma. The trial also incorporates a proof-of-principle study to examine the pharmacodynamic and mechanistic consequences of drug treatment, whereby patients with

at least two solid tumour lesions, will have one lesion irradiated with a 10–25 Gy single dose of radiotherapy and samples taken for comparison with those from a second lesion treated the following day with an equivalent dose of radiotherapy but 1.5 h after the patient has received a single dose of M-3814.

13.11 Clinical Application of a DNA-PK Inhibitor

DNA-PK has all the potential hallmarks of an attractive target for cancer therapy, given its role in chemo- and radio-resistance and implication in tumourigenesis, that the protein possesses a druggable kinase domain that is critical for DSB repair, that there are potential markers for assessing the pharmacodynamic activity of an inhibitor such as the Ser2056 autophosphorylation site, and that additional markers of DSB repair (e.g., γH2AX) are available to provide direct proof-of-mechanism. The clinical use of a DNA-PK catalytic activity inhibitor for cancer treatment however, still requires further definition. The profound synthetic lethality observed between PARP inhibitors and a tumour cell HR deficiency such as loss of BRCA-1 or BRCA-2 (Bryant et al. 2005; Farmer et al. 2005), has created an expectation that similar genetically defined vulnerabilities will be identified for inhibitors of other DNA-repair processes. ATM, another DSB-activated signaling kinase, is one candidate gene whose loss may confer sensitivity to DNA-PK inhibitors, particularly since ATM and DNA-PK participate in complementary DSB repair pathways. ATM-deficient cancer cells have been found to rely on DNA-PK for survival after DNA damage, and inhibition or knock-down of DNA-PK can re-sensitise drug-resistant ATM-null tumors to the TOP2 poison, doxorubicin (Jiang et al. 2009). It has been proposed that this strategy would be effective where loss of ATM occurs with high frequency in drug-resistant tumours, such as in CLL (Knittel et al. 2015). Whilst there is some experimental evidence to support this concept (Riabinska et al. 2013), these data (including data with the dual PI3K and DNA-PK inhibitor, KU60648) are somewhat limited to date. Additional preclinical work and ongoing clinical studies, which aim to examine the activity of M-3814 in patients with ATM loss or CC-115 treatment in CLL, may help to clarify whether this is an attractive therapeutic strategy.

In addition to trying to identify a genetic susceptibility for monotherapy treatment based upon a deficiency in DNA repair, a DNA-PK inhibitor could also conceivably be used as a therapy in prostate cancer through its ability to suppress defined transcriptional responses. Activation of the androgen receptor (AR) which is known to drive prostate cancer progression, simulates transcription of *PRKDC*, which encodes DNA-PKcs, and DNA-PKcs is also able to bind directly to AR and act as a transcriptional co-activator (Goodwin et al. 2015). Treatment with NU7441 has been shown to perturb this regulatory signaling loop and reduce activated AR-gene transcription (Goodwin et al. 2015). DNA-PKcs has also been found to interact directly with the AR splice variant AR-V7, which can be expressed in late-stage disease and contributes to the development of castrate-resistant prostate

cancer (Goodwin et al. 2015). TMPRSS2-ERG gene rearrangements are also found in approximately 50% of prostate cancers (Tomlins et al. 2005) and known to drive cancer progression, in part by inducing the transcription of of a subset of genes involved invasion and metastasis (Tian et al. 2014). DNA-PKcs has been reported to interact directly with TMPRSS2-ERG and inhibition of its enzyme activity by treatment with NU7026 claimed to inhibit prostate cancer cell invasion (Brenner et al. 2011). Collectively these findings indicate that there may be a rationale for inhibition of DNA-PKcs activity in prostate cancer which is independent of an interaction with free DNA ends. Although sustained inhibition of the target would be required in this setting which may to lead to immunosuppression and have consequences on the maintenance of genomic fidelity, these side-effects may be permissible in the treatment of advanced cancer patients.

As a combination therapy, the ability of a DNA-PK inhibitor to augment the tumour cell killing of DNA DSB inducing TOP2 chemotherapy or radiotherapy is clear. However, such a strategy will require careful consideration of how to minimize normal tissue toxicities. At present, a rationale for being able to achieve an improved therapeutic index is not entirely obvious, although differences in tumour cell *versus* normal tissue responses may feasibly exist. Encouragingly, strong augmentation of the antitumour response to radiotherapy has been observed in mice (Fuchss et al. 2017) and clinically this approach may benefit further from advances in radiotherapy technologies that aim to limit broader effects upon the host. Well-characterised selective inhibitors of DNA-PK will be invaluable in assessing the translational potential of this approach.

13.12   Summary

DNA-PK is an exciting therapeutic target and catalytic inhibitors are effective at sensitising tumour cells to DSB-inducing agents. Current challenges in the development of these inhbitors include the development of strategies to maximize the therapeutic index when used in combination with genotoxic agents and the identification of patient populations that will particularly benefit from treatment. Ongoing clinical trials with novel DNA-PK catalytic inhibitors, as monotherapy and in combination with chemotherapy or radiotherapy, may begin to inform on whether this approach represents a promising strategy for cancer treatment.

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