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Modulation of the DNA damage-response by inhibitors of the phosphatidylinositol 3-kinase related kinase (PIKK) family

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Abstract - The ability of cancer cells to repair DNA damage is an important determinant of their susceptibility to DNA-damaging anticancer therapies, and inhibition of DNA repair processes can thus lead to the potential therapeutic endpoints of radio- and chemosensitisation. As such, a number of agents that target DNA-repair enzymes are currently the subject of clinical trials. The phosphatidylinositol 3-kinase-related kinase (PIKK) family of enzymes, which includes DNA-dependent protein kinase (DNA-PK), ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia mutated related (ATR), mammalian target of rapamycin (mTOR) and hSMG1, are thought to act as nucleic acid surveillance proteins. DNA-PK and ATM, the normal function of which is lost in ataxia-telangiectasia, both play an important role in the detection and repair of DNA double-strand breaks (DSBs). DNA-PK is an essential component of the non-homologous end-joining pathway of DNA DSB-repair, while ATM signals to cell cycle and DNA repair components by phosphorylating multiple downstream targets, including p53, checkpoint kinase 2 (CHK2), NBS1 and BRCA1. Crucially, loss of DNA-PK or ATM activity results in an increased sensitivity to ionising radiation and certain chemotherapeutic agents that elicit DNA DSBs. These kinases therefore represent attractive targets for the development of radio- and chemo-sensitising agents.
1. DNA Damage

DNA within cells is constantly exposed to damage, which requires repair in order for the integrity of DNA to be maintained. Damage to DNA can lead to harmful mutations, which affect normal cellular processes and can cause cancer if left unrepaired. DNA is intrinsically susceptible to modification by reactive species and estimates suggest that there are in the region of $10^{16}$-$10^{18}$ repair events which take place in a human on a daily basis.\(^1\)

Fortunately, cells have evolved efficient processes for the repair of DNA damage, collectively known as the DNA-damage response (DDR) (Figure 1).\(^2\) Examples of endogenous damaging agents are ROS from cellular metabolism and endogenous methylating agents, whereas damage can be induced from external sources such as ultraviolet (UV) light and xenobiotics and in response to established cancer treatment such as DNA damaging chemotherapy and ionising radiation.\(^3,4\)

![Figure 1: DNA damage and response. Adapted from Hoeijmakers.\(^2\)](image-url)
1.1 DNA Inter- and Intra-Strand Crosslinks

Well characterised forms of DNA damage include inter- and intra-strand crosslinks. A covalent bond is formed between two bases on opposite strands of DNA in the case of an interstrand crosslink and within same strand with intrastrand crosslinks.\(^5\) Interstrand crosslinks are extremely toxic to the cell as they prevent the separation of the different strands, an important part of both DNA replication and transcription.\(^5\) Intrastrand crosslinks are less toxic given that they can be bypassed by certain enzymes. The nitrogen mustards are known to elicit this type of damage.\(^6\)

1.2 DNA Backbone Damage

Deoxyribose moieties within the DNA backbone are particularly susceptible to attack by ROS generated intracellularly.\(^6\) Hydroxyl radicals, for example, can abstract a hydrogen atom from the sugar to give a radical species which, after degradation, can result in a break to the DNA backbone. The structural units 1, 2 and 3 are all formed in this manner.\(^7\) These single strand breaks (SSBs) can be repaired by base excision repair (BER) but when numerous SSBs occur in close proximity to one another, a DNA double strand break (DSB) can be formed.\(^6,8\) Ionising radiation is capable of inducing DNA DSBs and these lesions are the most cytotoxic to the cell.
1.3 DNA Base Damage

Abasic sites can be produced when the glycosidic bond in DNA is hydrolysed (Scheme 1), which is a very common occurrence. It has been estimated that this process can occur 10,000 times per cell per day.\(^9\) Loss of a base is harmful to the cell for two reasons. Firstly, this can lead to mutations when the DNA is replicated and, secondly, SSBs can also occur if they are left untreated.\(^6,10\)

**Scheme 1:** Mechanism by which abasic sites are formed on DNA.\(^{10}\)

Deamination is another process which may impair the DNA sequence and this occurs on amino groups of bases, most commonly on cytosine (8). Compound 8 can undergo nucleophilic attack by water and, following liberation of ammonia, uracil (9) is produced (Scheme 2), resulting in a change in the genetic code.
Scheme 2: Deamination of cytosine (8) to yield uracil (9).

External agents such as UV radiation can cause damage to DNA bases as Figure 9 shows. UV light leads to the formation of photoadducts such as the cyclobutane dimer (10). Xenobiotics such as polycyclic hydrocarbons can, upon metabolic activation, react with DNA, leading to the formation of bulky adducts such as 11. Due to the steric bulk added by such modifications, they interfere with the secondary structure of DNA and this can affect cellular processes such as transcription.
2. The DNA Damage Response (DDR)

The wide diversity of DNA-lesion types necessitates multiple, largely distinct DNA-repair mechanisms.

2.1 Base Excision Repair (BER)

BER, discovered by Lindhal, is a DDR process employed when, for example, oxygen radicals and alkylating agents act on genetic material (Figure 1). The BER pathway relies on proteins recognising the damage. The downstream effect of this impairment is often mutations, which is clearly disadvantageous. Interestingly, this error free repair mechanism can occur via one of two mechanisms, short-patch repair (in which a single nucleotide is replaced) or long-patch repair (where at least two nucleotides are replaced). However, it is poorly understood as to what determines which of these options is undertaken. Short-patch repair does, nevertheless, constitute the main pathway. Essentially, a damaged base is replaced in order to prevent potentially dangerous mutations.

2.2 Nucleotide Excision Repair (NER)

NER acts on the products of damage induced in DNA by UV light or with any covalent adducts (Figure 1) and occurs during G1 phase of the cell cycle. Altogether, a broad range of damage is repaired via this mechanism and it can be subdivided into three subcategories: global genome repair (GGR), transcription-coupled repair (TCR) and transcription domain-associated repair (DAR). TCR, as the name suggests, is coupled to transcription, DAR works on both strands of active genes and GGR is any other NER that occurs. Unlike BER,
NER does not rely on having specific enzymes each recognising a different lesion; instead, inaccuracies in the structure of genetic material are enough to trigger a response. Following identification of the damage, the site of impairment is removed, leaving a break which DNA polymerases act upon to liberate the repaired DNA.

### 2.3 Mismatch Repair (MMR)

DNA has to be duplicated many times in order to pass vital genetic information on from one generation to the next, a process which is not without error. Fortunately, there is a repair mechanism that can recognise faults in the daughter strand post-replication. MMR maintains genetic integrity by correcting insertions, deletions and discrepancies in base pairings. Figure 2 shows the key phases involved in MMR. In Stage I (upper strand), hMSH2/6 recognises non-complimentary bases whereas hMSH2/3 recognises insertion/deletion errors (Stage II, lower strand). Following recognition, other essential proteins, for example mutator S (MutS), are recruited, exonucleases excise the errors and, finally, resynthesis can occur (Stage III).
Figure 2: The process by which DNA is repaired by MMR. In Stage I, heterodimers of hMSH2/6 focus on mismatches and single-base loops, whereas in Stage II, hMSH2/3 dimers recognise insertion/deletion loops. In Stages II and III, a number of proteins, including exonuclease 1, are associated in the excision of the new strand past the mismatch and resynthesis steps.

2.4 Double Strand Break (DSB) Repair

Although less prevalent than other DNA lesions, the DSB is considered to be the most cytotoxic and difficult to repair and there is evidence to suggest that a single DSB may prove lethal to a cell. Two principal mechanisms are responsible for the repair of DSBs, namely the error free homologous recombination (HR) process, and non-homologous end joining.
(NHEJ), which is an error prone ligation mechanism (Figure 3). Whereas HR repair is, by necessity, restricted to S and G2, NHEJ may operate at any phase of the mitotic cell cycle, although this repair process occurs predominantly in G1 and G0.²,¹⁷ Importantly, initiation of the DDR as a consequence of DSB detection has far reaching implications within a cell, resulting in the activation of a complex signalling network, which modulates cell cycle progression, gene expression and protein synthesis.¹,¹⁸ Members of an atypical class of protein kinases termed the phosphatidylinositol (PI) 3-kinase related kinase (PIKK) family are major players in the detection, signalling and repair of DNA DSBs.

The process of HR is initiated, following DSB detection, by the Rad50/Mre11/NBS1 (MRN) complex (Figure 3A). This complex performs a nucleolytic resection to expose single stranded 3’ overhangs of double-stranded DNA, which facilitates binding of a RAD51 nucleoprotein filament.¹⁹ This nucleoprotein filament has the capability to exchange the single strand of DNA with the identical sequence in a double stranded DNA counterpart. The intact double stranded copy is then used as a template to repair the DSB by the action of DNA ligases.²⁰ The DNA strand invasion results in the formation of Holliday junctions. These are mobile junctions between four strands of DNA that are repaired by resolvase enzymes thus completing the overall repair process.²¹

NHEJ can operate at any stage of the cell cycle as it does not require a DNA template for the restoration process but it predominantly acts at G1 and G0 (Figure 3B). NHEJ functions by directly ligating the end of DNA DSBs.²²,²³ NHEJ is a rapid process, compared to HR, but can sometimes result in a loss/gain of nucleotides.²
Figure 3: Recombinational repair processes. A) Mechanism of homologous recombination (HR). Nucleolytic resection of DSB in direction 5' → 3' by the MRE11-Rad50-NBS1 complex forms a 3' single-stranded DNA fragment. Rad52 binds, interacts with Rad51 to elicit a DNA strand exchange with undamaged, homologous DNA molecule. The resulting structure is resolved after DNA synthesis, ligation and branch migration. B) Mechanism of non-homologous end joining (NHEJ). Ku70-Ku80 complex binds to damaged DNA, then the Ku heterodimer binds to catalytic sub-unit of the DNA-dependent protein kinase (DNA-PKcs) to form the DNA-PK holoenzyme. The X-Ray repair cross complementing 4 (XRCC4)-ligase IV is activated by DNA-PK, which links the broken DNA ends.
3. The Phosphatidylinositol 3-Kinase Related Kinase (PIKK) Family

The PIKK family is a group of six atypical serine/threonine protein kinases (Figure 4). They are so named due to their similarities with the phosphoinositide 3-kinase (PI3K) family which are a series of lipid kinases possessing a variety of biological activities.

Comprising the PIKK family are DNA-PK, ATM, ataxia-telangiectasia mutated related kinase (ATR), mammalian target of rapamycin (mTOR), transformation/transcription domain associated protein (TRRAP) and suppressor of morphogenesis in genitalia 1 (SMG-1). ATM, ATR and DNA-PK are all involved in DNA repair and are discussed further later. mTOR is involved in organismal growth and homeostasis whilst SMG-1 is involved in nonsense-mediated RNA decay.25,26

**Figure 4**: The PIKK family of atypical protein kinases. Adapted from reference.27

The PIKK proteins consist of a PI3K domain and, also, FRAP-ATM-TRAPP (FAT) and FAT carboxy-terminal (FATC) regions surrounding the catalytic region, with FAT being located on the N-terminal side. Although some of the functions of these domains remain unclear, it
has been reported that the FAT domain of ATM contains a serine residue which is key for the activation of the kinase.27

3.1 DNA-Dependent Protein Kinase (DNA-PK)

3.1.1 Structural Features of DNA-PK

The DNA-PK catalytic subunit is the largest of the PIKK family members, consisting of a polypeptide of 4128 amino acids. Owing to the large size of the peptide, crystal structure determination has proven challenging. However, in January 2010 Sibonda et al. published the first known crystal structure of DNA-PKcs in complex with C-terminal fragments of Ku80 (the 80 kDa protein subunit of the Ku complex, also known as ATP-dependent DNA helicase II), albeit at a resolution of 6.6Å.4 Whilst this resolution cannot provide useful information on the structure of the ATP-binding domain, it gives a general overview of the structural features of the protein (Figure 5).
Figure 5: Crystal structure of DNA-PKcs. Yellow: kinase domain, green: ring structure, light green: forehead region that is part of the ring structure, blue: putative DNA-binding domain, magenta: C-terminal region carrying the FAT and FATC domains.

The major role of the Ku70/Ku80 heterodimer within the non-homologous end joining pathway is recognition and binding of DNA ends to facilitate correct alignment for subsequent processing and ligation. The proteins themselves are known to consist of three distinct functional regions. An amino terminal Willebrand A-domain (vWA) is thought to be a protein-protein interacting domain which may play a key role in heterodimerisation. A central core domain is then linked to a carboxy-terminal region, which in Ku80 is elongated and contains the regions to which the DNA-PKcs binds (Figure 6).
3.1.2 DNA-PK and the NHEJ DNA Repair Pathway

As previously mentioned, the major pathway for DSB repair in non-replicating mammalian cells is non-homologous end joining, and DNA-PK plays a key role in this process (Figure 3). The Ku70/Ku80 heterodimer is responsible for binding to the broken DNA ends. Ku binds to both of the broken strands and forms a bridging complex to lock the damaged ends in place. The catalytic sub-unit of the DNA-PKcs is recruited to the site of damage to form a ‘synaptic complex’. Formation of the holoenzyme greatly enhances the serine/threonine protein kinase activity of DNA-PKcs resulting in autophosphorylation of multiple sites on the protein. A small cluster of seven phosphorylation sites between residues 2609 and 2647 are thought to be key for a change in the tertiary structure of the DNA-PK complex, which makes the DNA termini accessible for further processing. DNA docks in the DNA binding site within DNA-PKcs, which is in the N-terminal domain. Should Ku be absent, then another process can anneal DNA DSBs via alternative NHEJ that involves poly(ADP-ribose) polymerase 1 (PARP1) and XRCC1. Once the DNA is bound, processing enzymes, such as Artemis and DNA polymerases, locate to the site of damage. Artemis is a nuclease that is phosphorylated by DNA-PKcs following
formation of the DNA-PK holoenzyme. It is responsible for the cleavage of non-complementary bases from the DNA termini.\textsuperscript{33} Whilst the exact types of lesions requiring Artemis-mediated repair have not been identified, it is thought that 15-20% of DSBs resulting from the action of ionising radiation remain unrepaired in cells deficient in the Artemis protein.\textsuperscript{34}

The complexity of the DSBs formed by ionising radiation dictates that, whilst some may require DNA base removal, a number will conversely require base insertion. This role is fulfilled by a number of DNA-polymerases, most notably DNA polymerase μ and λ, both of which are recruited to the break site through their interaction with Ku.\textsuperscript{35} It has been demonstrated that cells deficient in either DNA polymerase μ or λ are not highly sensitised to ionising radiation, suggesting that these processing factors are only required in a small subset of strand breaks.\textsuperscript{34}

Current literature reports implicate a number of different processing factors in the NHEJ pathway including PNF, APLF and the WRN complex. The increasing number implicated would seem to suggest that the specific enzymes recruited for end processing may be relatively flexible, depending upon the nature of the break.\textsuperscript{35}

Upon completion of end processing, the remainder of the pathway is directed towards end ligation. This is performed following the recruitment of a DNA ligase IV/XRCC4 complex to the break site. XRCC4 has no known enzymatic activity, but instead acts as a scaffolding protein for the interaction of DNA ligase IV with Ku, and thus the DSB. Binding of XRCC4 to DNA ligase IV promotes its enzymatic activity, which subsequently instigates the ligation and repair of the break site.\textsuperscript{36}
3.1.3 DNA-PK Small Molecule Inhibitors

Following the discovery that DNA-PK inhibition can potentiate \textit{in vitro} cytotoxicity of ionising radiation and a number of anticancer drugs, a number of small-molecules with DNA-PK inhibitory activity have been developed. To date, the most successful approach to DNA-PK inhibition has been with small molecule targeting the ATP-binding site of the kinase.

\textbf{Wortmannin}

The first example within this specific group of compounds is wortmannin (12), a steroidal derivative reported as a potent PI3K inhibitor in 1994.\textsuperscript{37}

\begin{center}
\includegraphics[width=0.2\textwidth]{wortmannin.png}
\end{center}

Wortmannin is a natural fungal metabolite, derived from \textit{Penicillium wortmannii} \textit{K} and first observed to have antifungal and anti-inflammatory properties. This sterol-like compound is a potent and selective inhibitor of PI3K family kinases without activity against other intracellular signalling enzyme targets, inhibiting PI3K activity with an IC\textsubscript{50} of 4.2 nM.\textsuperscript{37} Kinetic analysis of the PI3K inhibition by wortmannin indicated a non-competitive irreversible inhibition, which was further verified by Williams \textit{et al.} with co-crystallographic studies of the resulting covalent complex in the ATP-binding pocket of PI3K\textit{\gamma}.\textsuperscript{38, 39} Further studies demonstrated that wortmannin also inhibits DNA-PK (IC\textsubscript{50} = 16 nM) by a non-competitive mechanism, forming covalent adducts with DNA-PKcs lysine 802 in the region
of the molecule harbouring its kinase domain.\textsuperscript{40} Although the activity of this sterol-like structure and its mode of interaction make it very attractive, the relative structural complexity of wortmannin, together with its irreversible inhibition and poor selectivity, limit its potential as a drug molecule.

**Chromen-4-ones and Surrogates: LY2094002 and Derivatives**

The chromen-4-one structure LY294002 (13) was reported by Lilly pharmaceuticals in 1994 as an inhibitor of PI3K.\textsuperscript{41} It was identified through a screen of compounds derived from quercetin (14) with the objective of developing PI3K-specific inhibitors.

\begin{center}
\includegraphics[width=\textwidth]{ly294002.png}
\end{center}

Subsequent evaluation of 13 as a DNA-PK inhibitor, showed that the compound exhibited similar DNA-PK inhibitory activity to that against PI3K, highlighting the non-selective profile of the compound (Table 1).\textsuperscript{41, 42}

**Table 1:** Reported inhibitory activity of LY294002 (13) against different PIKK family members.\textsuperscript{a} Values from ref 42. \textsuperscript{b}Literature value from ref 41.

<table>
<thead>
<tr>
<th></th>
<th>DNA-PK</th>
<th>PI3K (p110\textsubscript{α})</th>
<th>ATM</th>
<th>ATR</th>
<th>mTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC\textsubscript{50}(\textmu M)</td>
<td>1.5±0.2\textsuperscript{a}</td>
<td>2.3±0.8\textsuperscript{a} (1.4)\textsuperscript{b}</td>
<td>&gt;100\textsuperscript{a}</td>
<td>&gt;100\textsuperscript{a}</td>
<td>2.5±0.2\textsuperscript{a}</td>
</tr>
</tbody>
</table>
Research conducted at the Northern Institute for Cancer Research at Newcastle University and KuDOS Pharmaceuticals (now part of AstraZeneca) revealed the importance of the oxygen of the morpholine substituent of 13, and while replacement by thiomorpholine (15) dramatically reduced potency, the piperidine derivative 16 proved essentially devoid of DNA-PK inhibitory activity. The key role of the morpholine substituent of 13 was later confirmed by X-ray crystallography when the structure of 13 in complex with human PI3Kγ revealed that the morpholine oxygen makes a hydrogen bond interaction with the backbone amide group of Val-882 within the ATP-binding domain of the kinase (Figure 7).  

![Figure 7. Crystal structure of LY294002 (13) in complex with the ATP-binding domain of PI3Kγ.](image)

Although 13 suffered from rapid metabolic clearance (1h), in vivo toxicity and lack of specificity, the compound helped the design of derivatives with improved potency against DNA-PK, selectivity and better physicochemical properties.
The first generation of analogues were benzopyranone and pyrimidoisoquinolinone derivatives. Incorporation of a fused ring on the chromenone, gave a 5-fold improvement in potency against DNA-PK (17; DNA-PK IC₅₀ = 0.23 µM) (Table 2). Introduction of a methyl group on the morpholine ring gave no improvement in activity against DNA-PK (18; DNA-PK IC₅₀ = 0.19 µM). However, additional methyl groups, at the 2 or 6-position of morpholine, or replacement of the morpholine ring (e.g. piperidine, piperazine) resulted in a loss of activity.⁴², ⁴³ Encouragingly, all early derivatives showed better selectivity for DNA-PK over other PIKK family members than the parent compound, e.g. 17 is 60-fold more potent against DNA-PK than PI3K (p110α) (Table 2).

![Chemical structures]( attachment:image)

Replacement of the chromen-4-one scaffold by the isosteric pyrimidoisoquinolinone structure gave equipotent compounds (19; IC₅₀ = 0.28 µM).⁴⁴ Several studies have demonstrated that 17 acts in vitro as a radiosensitiser,⁴⁵ and as a chemo-potentiator of topoisomerase II poisons in human leukaemia cell lines.⁴⁶

**Table 2:** Inhibitory activity (IC₅₀ µM) against different PIKK family members.⁴²

<table>
<thead>
<tr>
<th></th>
<th>DNA-PK</th>
<th>PI3K (p110α)</th>
<th>ATM</th>
<th>ATR</th>
<th>mTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>0.23</td>
<td>13</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6.2</td>
</tr>
<tr>
<td>18</td>
<td>0.19</td>
<td>2.4</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>4.8</td>
</tr>
<tr>
<td>19</td>
<td>0.28</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>5.3</td>
</tr>
</tbody>
</table>
A simplification of the chromenone core was attempted with the synthesis of a series of substituted monocyclic pyran-2-one, pyran-4-one, thiopyran-4-one and pyridin-4-one derivatives. The pyran-4-one system and substitution at the pyranone 3- or 5-positions gave a loss of activity. However, library work on 6-substituted-2-morpholino-pyran-4-one and 6-substituted-2-morpholinothiopyran-4-one, led to the identification of 20 (DNA-PK; IC$_{50}$ = 0.18 µM) and 21 (DNA-PK IC$_{50}$ = 0.19 µM), both 10-fold more potent against DNA-PK than the parent 13.$^{47,48}$

A library of 6-, 7- and 8-substituted chromenones were synthesised using a multi-parallel approach.$^{49}$ Interestingly, compound 22 (DNA-PK IC$_{50}$ = 0.11 µM) showed a 10-fold increase in potency compared to 13, whereas the dibenzofuranyl derivative (23) (DNA-PK IC$_{50}$ = 0.04 µM) displayed impressive potency against DNA-PK. The incorporation of a dibenzothiophenyl group led to NU7441 (24), a compound with a 100-fold increase in potency compared with the parent phenyl derivative 13 (DNA-PK IC$_{50}$ = 0.02 µM), along with excellent selectivity over the other PIKK family members (Table 3).
Table 3: NU7441 (24) inhibitory activity against different kinases of the PIKK family.

<table>
<thead>
<tr>
<th>IC₅₀(µM)</th>
<th>DNA-PK</th>
<th>PI3K</th>
<th>PI4Kβ</th>
<th>ATM</th>
<th>ATR</th>
<th>mTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>5</td>
<td>40</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

Compound 24 has been reported to sensitize tumour cells to ionizing radiations (Figure 8A) and increased the effect of etoposide (Figure 8B) in vitro, indicating a good cellular permeability with a low inherent growth inhibitory activity.⁴⁵, ⁵⁰ The compound has also been reported to cause doxorubicin-induced DNA DSBs (measured by γ-H2AX foci) to persist and also slightly decrease homologous recombination activity (assessed by Rad51 foci).⁵¹

A

![Figure 8: (A) Radio-sensitisation of HeLa cells by NU7441 (24). (B) Enhancement of etoposide cytotoxicity by NU7441 (24) in HeLa cells.⁴⁵, ⁵⁰](image)

An homology model of the ATP-binding site of DNA-PK, derived from the crystal structure of PI3Kγ was used to guide further inhibitor design.⁵² The newly synthesised compounds all possessed polar substituents at the dibenzothiophene 1-position, with an aim to improve physicochemical properties.⁵³ Several exhibited high potency against DNA-PK and
potentiating the cytotoxicity of ionising radiation (IR) in vitro 10-fold or more (e.g., 25; DNA-PK IC₅₀ = 5.0 ± 1 nM, IR dose modification ratio = 13). Furthermore, 25 was shown to potentiate not only IR in vitro but also DNA-inducing cytotoxic anticancer agents, both in vitro and in vivo. Counter-screening against other members of the PIKK family unexpectedly revealed that some of the compounds were potent mixed DNA-PK and PI3K inhibitors. The promising biological activity of 25 was accompanied by better drug-like properties compared to NU7441 (24), and acceptable plasma protein binding, combined with weak activity against hERG and a panel of CYP450 enzymes.

In efforts to optimise the biological and pharmaceutical properties of 24, and to expand structure-activity relationships (SARs), the core chromenone scaffold as well as the dibenzothiophen-4-yl moiety have been systematically modified. As 22 is approximately 10-fold more potent than the parent 8-phenylchromenone (13) this strongly indicated that the 3-phenyl substituent of 22 made additional binding interactions within the ATP-binding domain of DNA-PK. By probing this presumed binding interaction further, replacement of the 3-phenyl group of 22 by an isosteric thiophen-2-yl substituent (26) improved DNA-PK inhibitory activity approximately 10-fold (DNA PK; IC₅₀ = 18 nM). Subsequent homology modelling studies suggested that the heteroaryl substituent may occupy a putative hydrophobic pocket that could be further exploited, with further SAR resulting in the discovery of O-alkoxyphenylchromen-4-one (27) (DNA PK; IC₅₀ = 8 nM).

Resolvable atropisomeric derivatives of 13 have also been described. Introduction of a methyl substituent, as a representative example, at the dibenzothiophene 3-position (28) (DNA PK; IC₅₀ = 1.7 μM) or at the chromenone 7-position (29) (DNA PK; IC₅₀ = 0.005 μM) of NU7441, generated stable pairs of atropisomers due to restricted rotation between the chromen-4-one and dibenzothiophene rings. Substitution at the dibenzothiophene 3-position (28) resulted in an approximately 60-fold reduction in potency of the racemic
compound against DNA-PK compared with the parent compound (24). Interestingly, 29 showed a 6-fold improvement in potency compared with the parent compound (Table 4).

Following resolution by chiral high-performance liquid chromatography (HPLC), biological evaluation against DNA-PK of each pair of atropisomers showed that DNA-PK inhibitory activity resided exclusively in the (−)-atropisomer (‘eutomer’) enantiomer, with the antipodal (+)-atropisomer (‘distomer’) proving inactive at 100 μM.\textsuperscript{58,59}

![Chemical structures](image)

**Table 4:** Inhibition of DNA-PK (IC\textsubscript{50} μM) by 3-substituted dibenzothiophen-4-yl derivatives and 7-substituted chromen-4-one derivatives

<table>
<thead>
<tr>
<th></th>
<th>R\textsuperscript{1}</th>
<th>R\textsuperscript{2}</th>
<th>DNA-PK inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>H</td>
<td>H</td>
<td>0.03</td>
</tr>
<tr>
<td>28</td>
<td>Me</td>
<td>H</td>
<td>1.7</td>
</tr>
<tr>
<td>28(−−)</td>
<td>Me</td>
<td>H</td>
<td>1.2</td>
</tr>
<tr>
<td>28(+)</td>
<td>Me</td>
<td>H</td>
<td>100</td>
</tr>
<tr>
<td>29</td>
<td>H</td>
<td>Me</td>
<td>0.005</td>
</tr>
<tr>
<td>29(−−)</td>
<td>H</td>
<td>Me</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Pyridone OK-1035

3-Cyano-5-(4-pyridyl)-6-hyrazonomethyl-2-pyridone, OK-1035 (30) was reported to inhibit DNA-PK activity with an IC_{50} of 8 µM and excellent selectivity (Table 5), appearing at first to be a good candidate for optimisation. However this proved not to be the case and subsequent structural modifications provided only compounds with inferior potency against DNA-PK.\textsuperscript{60}

\[ \text{\includegraphics[width=0.3\textwidth]{pyridone_OK-1035.png}} \]

Table 5: Inhibition of DNA-PK and various protein kinases by OK-1035 (30).

<table>
<thead>
<tr>
<th>DNA-PK</th>
<th>PKA</th>
<th>PKC</th>
<th>CDK2</th>
<th>CKI</th>
<th>CKII</th>
<th>MAPK</th>
<th>EGFRK</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC_{50}(µM)</td>
<td>8</td>
<td>390</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>420</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Phenol Related IC Series

Reported by the ICOS Corporation and Array Biopharma as part of a new series of DNA-PK inhibitors, 2-hydroxy-4-morpholin-4-yl-benzaldehyde (IC60211, IC_{50} = 400 nM) (31) is a representative example of DNA-PK inhibitors possessing a morpholine motif. Optimisation
of 31 led to the identification of a number of selective inhibitors (32-36; Table 6), all of which maintain the arylmorpholine substructure.  

![Chemical structures of compounds 31-36](image)

Table 6: Inhibitory activity (IC_{50} nM) of representative DNA-PK inhibitors against various PI3Ks.  

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA-PK</th>
<th>p110α</th>
<th>p110β</th>
<th>p110δ</th>
<th>p110γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>400</td>
<td>10000</td>
<td>2800</td>
<td>5100</td>
<td>37000</td>
</tr>
<tr>
<td>32</td>
<td>120</td>
<td>1400</td>
<td>135</td>
<td>880</td>
<td>1000</td>
</tr>
<tr>
<td>33</td>
<td>44</td>
<td>890</td>
<td>42</td>
<td>490</td>
<td>180</td>
</tr>
<tr>
<td>34</td>
<td>35</td>
<td>2700</td>
<td>400</td>
<td>1800</td>
<td>5000</td>
</tr>
<tr>
<td>35</td>
<td>34</td>
<td>3800</td>
<td>1700</td>
<td>2800</td>
<td>7900</td>
</tr>
</tbody>
</table>

Compounds 32-34 have been reported to inhibit DNA DSB repair in cells not only as single agents but also in combination with chemotherapy and radiotherapy.  In vivo efficacy has also been demonstrated in combination studies using ionising radiation.  The small-molecule
DNA-PK inhibitor 36 was found to synergise with 7-ethyl-10-hydroxy-camptothecin (SN38), to enhance killing of colon cancer cells in vitro. Compound 36 is relatively non-toxic, with an IC$_{50}$ of 29 µM in both HCT-116 and HT-29 cell lines. Significant reductions in the IC$_{50}$ values of SN38 were detected at 5 and 10 µM of inhibitor.$^{51}$ Additionally, 36 can synergistically sensitise three genetically diverse breast cancer cell lines to doxorubicin and cisplatin.$^{64}$ Improved cytotoxicity and significant synergy were observed with the topoisomerase II inhibitor, doxorubicin or cisplatin in the presence of non-toxic concentrations of 36. Furthermore, 36 was shown to decrease doxorubicin-induced DNA-PKcs autophosphorylation on Ser2056 and increase doxorubicin-induced DNA fragmentation.$^{65}$

**SU11752**

In an effort to develop a specific DNA-PK inhibitor, Sugen undertook a screening campaign of 3-substituted indolin-2-ones and successfully identified the ATP-competitive DNA-PK inhibitor SU11752 (37). Interestingly, the compound showed good potency against DNA-PK (IC$_{50} = 0.13 \pm 0.028$ µM) combined with selectivity over PI3K (p110γ; IC$_{50} = 1.10$ µM).$^{66,67}$ The compound was also reported as a poor inhibitor of ATM kinase activity in cells.$^{67}$
Vertex

In a patent published by Vertex Pharmaceuticals, 251 of the compounds described had a $K_i$ of less than 0.1 µM for the inhibition of DNA-PK. More recently, it has been disclosed that VX-984 (38) is a DNA-PK inhibitor currently undergoing a phase I clinical study, in combination with pegylated liposomal doxorubicin in patients with advanced solid tumours or lymphomas.

![Chemical structure of compound 38](image)

Compound 38 is reported to have an IC$_{50}$ of $88 \pm 64$ nM for inhibition of DNA-PKcs autophosphorylation (Ser2056) in A549 lung cancer cells, with good selectivity versus other PI3K family members.

3.2 Ataxia Telangiectasia Mutated (ATM) Kinase

3.2.1 Structural Features of ATM

The ATM gene codes for a 350 kDa protein, consisting of 3056 amino acids. Characteristic for ATM are five domains: from the N-Terminus to C-Terminus these are the HEAT repeat domain, the FRAP-ATM-TRRAP (FAT) domain, the kinase domain (KD), the PIKK-regulatory domain (PRD) and the FAT-C-terminal (FATC) domain. The FAT domain
interacts with the kinase domain of ATM to stabilize the C-terminal region. The KD effects kinase activity, while the PRD and the FATC domain regulate it. Although no structure for ATM has been solved, the overall shape of ATM is envisaged to be very similar to DNA-PKcs, comprised of a head and a long arm, which wraps around double-stranded DNA after a conformational change. The entire N-terminal domain, together with the FAT domain, are predicted to adopt an α-helical structure. This α-helical structure is believed to form a tertiary structure, which has a curved, tubular shape. FATC is the C-terminal domain with a length of about 30 amino acids. It is highly conserved and consists of an α-helix followed by a sharp turn, which is stabilized by a disulfide bond.

### 3.2.2 ATM and the Repair Pathway

ATM is one of the best known PIKK members. The absence of this protein is characteristic of a neurodegenerative disorder termed ataxia-telangiectasia, which involves a high sensitivity to ionising radiation, neurodegeneration and immunodeficiency. ATM has the main function of regulating the G1/S and G2/M cell cycle checkpoints in response to double strand breaks (DSBs), preventing progression of the cell cycle when DNA is damaged. The cascade mechanism involves activation of tumour suppressor protein p53 via phosphorylation, which stimulates the transcription of p21 protein and interacts with CDK2/Cyclin E halting progression of the cycle from G1 to S phase and allowing DNA repair. Another pathway of interaction between ATM and p53 involves activation of CHK2 protein kinase by ATM. CHK2 obstructs the p53-MDM2 interaction by phosphorylation of p53, leading to arrest of the cell cycle. In addition, ATM also directly phosphorylates MDM2 (Ser395), altering its interaction with p53. These pathways act via ATM to halt cell cycle progression in response to DNA-damage. Thus, the main role of ATM is the detection of
DNA damage, being itself an important component of the non-homologous end-joining pathway (NHEJ).\textsuperscript{76}

### 3.2.3 ATM Inhibitors

Although ATM has attracted considerable interest as a therapeutic target, very few useful inhibitors with potent activity have been reported. Many of the known inhibitors of ATM, including wortmannin (12) (IC\textsubscript{50} = 150 nM) and caffeine (39) (IC\textsubscript{50} = 200 μM), lack specificity, hence inhibiting a wide range of PIKKs.\textsuperscript{77}

![Molecule 12](image1.png)

![Molecule 39](image2.png)

**KU-55933, KU-60019 and KU-59403**

Important inhibitors were developed by screening of combinatorial libraries around the structure of LY294002 (13). The KuDOS Pharmaceuticals-Newcastle group identified weak ATM-inhibitory activity within a pyran-4-one series (exemplified by 40), which prompted further chemical library generation based on this scaffold.\textsuperscript{47} Replacing the 4-methoxyphenyl group of 40 resulted in the identification of 41, a highly selective inhibitor of ATM. Compound 41 has an IC\textsubscript{50} of 13 nM and Ki of 2.2 nM, with marked sensitisation of cancer cells to IR and topoisomerase II inhibitors \textit{in vitro}.\textsuperscript{47,78}
Modification of the thianthrene substituent of 41 was investigated, but minor structural changes were commonly detrimental to activity. An improved water-soluble analogue 42, included a 2,6-dimethylmorpholin-4-yl group at position 2 on thioxanthene, leading to potent ATM-inhibitory activity (IC₅₀ = 6.3 nM), a 2-fold improvement in ATM inhibition over 41. Combining potency, cellular activity (enhancement ratios of 2.1-2.9 at 0.6 μM) and pharmacokinetics, 42 was proven to be highly selective with limited toxicity towards healthy cells. It was shown to inhibit the DNA damage response, reduce AKT phosphorylation and prosurvival signalling. Inhibition of migration and invasion was also reported, along with effective radiosensitisation of human glioma cells. A further analogue KU-59403 (43) showed improved potency over 41 (Table 7), as well as improved solubility and bio-availability, allowing for in vivo studies and advanced preclinical evaluation. Compound 41 is without intrinsic cytotoxicity but is a potent enhancer of topoisomerase I and II poison cytotoxicity in vitro and was shown to increase the efficacy of topoisomerase I and II poisons in vivo without intrinsic toxicity despite normal tissue exposure.
Table 7: Inhibition of ATM and related kinase activity by LY294002 (13), KU55933 (41), and KU59403 (43)

<table>
<thead>
<tr>
<th>Enzyme IC\textsubscript{50}, \textmu mol/L</th>
<th>ATM</th>
<th>DNA-PK</th>
<th>PI3K</th>
<th>ATR</th>
<th>PI4K</th>
<th>mTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>&gt;100</td>
<td>1.5</td>
<td>2</td>
<td>100</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>41</td>
<td>0.013</td>
<td>2.5</td>
<td>1.7</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>9.3</td>
</tr>
<tr>
<td>43</td>
<td>0.003</td>
<td>9.1</td>
<td>10</td>
<td>&gt;100</td>
<td>&gt;10</td>
<td>14</td>
</tr>
</tbody>
</table>

CP466722

Identified by Pfizer from a targeted library screening campaign, CP466722 (44) is a highly selective and rapidly reversible ATM inhibitor, that does not inhibit other PIKK family members or PI3K in a cellular setting.

It has been shown in clonogenic survival assays to be sufficient to sensitise cells to IR and suggests that therapeutic radiosensitisation may only require ATM inhibition for short periods of time. Transient exposure of tumour cells to 44 inhibits ATM adequately to enhance the cell’s sensitivity to irradiation. After compound washout, the inhibition was rapidly and completely reversed, suggesting that a treatment with a minimal dose of 44 for brief periods may achieve a maximum therapeutic effect.
3-Quinoline Carboxamides

A novel series of 3-quinoline carboxamides was recently identified at AstraZeneca through a direct screening approach. Initial screening hit 45 was a submicromolar inhibitor of ATM (IC$_{50}$ = 0.008 μM), displaying promising selectivity over other PIKKs and PI3K isoforms (>10-fold against DNA-PK and >400-fold against mTOR). Compound 46 exhibited a 10-fold improvement in potency (IC$_{50}$ = 0.073 μM) over 45, along with increased selectivity over ATR.

![Chemical structures](image)

Subsequent investigation at the C4 position did not provide any significant improvement, but rather gave vital insight into the how the nature of substituents could influence pharmacokinetic profiles and selectivity. Investigation at C6 was carried out in parallel, whereby it was discovered that p-substituted analogue, 47 exhibited improved potency (IC$_{50}$ = 0.045 μM) and kinase selectivity. Extensive SAR at each position on the quinoline core allowed the rapid discovery of optimum combinations and resulted in the finding of 48 (IC$_{50}$ = 0.046 μM) and 49 (IC$_{50}$ = 0.033 μM) as potent and highly selective ATM inhibitors, with
overall absorption, distribution, metabolism, and excretion (ADME) properties suitable for oral dosing. In combination with the DSB-inducing agent irinotecan, significant tumour volume reductions were observed in a SW620 colorectal cancer xenograft model.82

3.3 Ataxia Telangiectasia and Rad3-related (ATR) Kinase

3.3.1 Structural Features of ATR

ATR (human AT and Rad3 related) is a large kinase comprising 2644 amino acids, with a molecular weight of 300 kDa. ATR-interacting protein (ATRIP) is the 85 kDa binding partner of ATR, which binds to the N-terminus to promote localization to sites of replication stress.83 There are no recognised differences in the phenotypes that result from the loss of ATR or ATRIP, which suggests that ATRIP should be considered a subunit of ATR holoenzyme.84

3.3.2 ATR and the Repair Pathway

ATR is a member of the PIKK family closer in size and function to ATM, and sharing around 60% of the kinase domain. This similarity is reflected by a degree of overlapping function between the two kinases (Figure 9).75 ATR has shown ability to phosphorylate p53 on Ser15 in vitro85 and in vivo86 similar to ATM. However, ATR phosphorylation responds mainly to DNA damage induced by UV radiation and has a faster response to DNA damage. It is believed that ATR is responsible for maintaining the phosphorylated state of the targets p53, murine double minute 2 (MDM2) and CHK2.86 ATR is also involved in a pathway initiated by phosphorylation of checkpoint kinase 1 (CHK1) kinase (Ser345).87
3.2.3 ATR Small Molecule Inhibitors

Research on inhibitors of ATR (ATRi’s) has been on-going for more than a decade, with the first small-molecule inhibitors identified in the late 1990’s. Despite this, novel patented data has only been disclosed from 2009 onwards, with the first report on ATR-selective small molecule inhibitors published in 2009. In 2006, Knight et al conducted a study in which a chemically diverse set of PI3K inhibitors in pre-clinical development were screened against several lipid kinases. Compounds 50-55 were identified as moderate ATRi’s in an enzymatic assay (ATR IC_{50} 0.85 to 21 µM), but with no selectivity over DNA-PK or ATM. In the same study, LY294002 (13), originally developed as a PI3K inhibitor, but later recognised as a pan-inhibitor of the PIKK family, was determined to be inactive (ATR IC_{50} = >100 µM).
Wortmannin and Caffeine

The fungal metabolite wortmannin (12) is an irreversible pan-inhibitor of the PIKK family but has relatively weak ATR activity (ATR IC$_{50}$ = 1.8 µM, ATM IC$_{50}$ = 150 nM, DNA-PK IC$_{50}$ = 16 nM). It has been proposed that the radio-sensitising effect is perhaps due to the inhibition of DNA-PK kinase activity rather than ATR inhibition.$^{92}$

Similarly, caffeine (39) has been recognised as a weak pan-PIKK inhibitor (ATR IC$_{50}$ = 1.1 mM, ATM IC$_{50}$ = 0.2 mM, DNA-PK IC$_{50}$ = 10 mM), with the radiosensitising effect attributed to both ATR and ATM kinases.$^{93}$
Schisandrin B

In 2007, Nishida et al filed a patent which revealed the dibenzocyclooctadiene lignans schisandrin B and gomisin A as the first ATR-selective small-molecule inhibitors. One of the compounds of interest, Schisandrin B (56), is a naturally occurring dibenzocyclooctadiene lignin found in Scirandra chinensis, a medicinal herb. It was demonstrated that Schisandrin B was able to abolish UV-induced intra-S-phase and G2/M cell cycle checkpoints and increase the cytotoxicity of UV radiation in human lung cancer cells (A549 adenocarcinoma). Furthermore, Schisandrin B was found to inhibit p53 and CHK1 ATR-dependent phosphorylation but did not prevent ATR-activating association to the ATRIP. It is selective for ATR kinase activity in vitro (IC₅₀ = 7.25 µM), but due to the weak inhibitory potency against ATR, high drug concentrations (30 µM for cellular assays) were necessary.

Torin 2

Torin 2 (57) was described in 2013 as a potent ATP-competitive inhibitor of ATM and ATR (ATM IC₅₀ = <0.01 µM, ATR IC₅₀ = <0.01 µM).
Torin 2 inhibited the cellular activity of the ATR kinase, as assessed by phosphorylation status of CHK1 following exposure of HCT116 colon cancer cells to UV-induced DNA damage.96,97

**ETP-46464**

Researchers at the Spanish National Cancer Research Centre screened a collection of 623 PI3K inhibitors, aiming to find hits against the PI3KK family, specifically ATR.98 Following a high-throughput microscopy screen, ETP-46464 (58), was identified as an encouraging hit, which potently inhibited ATR in vitro (IC$_{50}$ = 14 nM) and showed some selectivity over ATM. ETP-46464 suffers from poor pharmacological properties in mice, halting development.
NU6027

In 2001, a more potent ATR inhibitor, NU6027 (59) (IC$_{50}$ = 0.1 μM), was reported to sensitize several breast and ovarian cancer cell lines to IR and several chemotherapeutic agents.

As 59 was originally developed as a CDK2 inhibitor (IC$_{50}$ = 2.2 μM), it was found to be a non-selective inhibitor. Compound 59 was synergistic with the PARP inhibitor rucaparib, in MCF7 cells and synthetically lethal in XRCC1-defective EM9 cells, confirming that impaired DNA single-strand break repair is synthetically lethal with ATR inhibition.

NVP-BEZ235

In 2011, Toledo et al. reported the results of a cell-based compound library screening approach for the identification of potent ATR inhibitors. One of the compounds identified to possess significant inhibitory against ATR kinase was NVP-BEZ235 (60) (ATR, ATM, DNA-PK IC$_{50}$ <21 nM), a drug originally revealed by Novartis as a highly potent PI3K/mTOR inhibitor.
NVP-BEZ235 inhibited ionising radiation-induced ATM, CHK1, CHK2 and DNA-PK phosphorylation and affected ionising radiation-induced γH2AX formation. NVP-BEZ235 was, at the time of writing, undergoing clinical trials for solid tumours.

**WYC02 and WYC0209**

WYC02 (61) is a flavonoid isolated and identified from the whole plant extract of *Thelypteris torresiana*, a fern species native to Taiwan.

The natural compound 61, and its more potent synthetic analogue WYC0209 (62), have been shown to inhibit ATR-mediated CHK1 phosphorylation, impair the G2/M checkpoint and improve cancer sensitivity to cisplatin. Further studies showed that 62 inhibits ATR kinase activity at least 4 times greater than 61.
Another ATR inhibitor with excellent drug metabolism and pharmacokinetic (DMPK) properties currently in clinical development is the AstraZeneca inhibitor AZD6738 (63). Initial hit (64) was identified as a potent ATRi from a screening campaign (cellular IC$_{50}$ = 1.1 nM), with high selectivity against other PIKK and PI3K kinases.

Optimisation from the superior screening hit 64 within tight SAR space, enabled the discovery of 65, a potent and selective ATR inhibitor which has been shown to possess substantial in vivo single agent activity in MRE11A-deficient cancer cells at well tolerated doses.$^{103,104}$ Compound 65 had poor aqueous solubility but displayed high Caco-2 permeability and good stability in rat hepatocytes, leading to satisfactory bioavailability in a rat PK study. In contrast to 65, 63 incorporates a structural change, whereby a sulfoximine replaced the methyl sulfone as a rational attempt to improve solubility. As a result, 63 has notably improved solubility, bioavailability, pharmacokinetics, pharmacodynamics and is suitable for oral dosing.$^{105}$ It acts to inhibit phosphorylation of the ATR downstream target CHK1, while increasing phosphorylation of the DNA DSB marker $\gamma$H2AX in vitro. It significantly increases anti-tumour activity of IR or carboplatin in vivo.$^{105,106}$ A phase I clinical trial to assess the safety of AZD6738 alone and in combination with radiotherapy in patients with solid tumours was underway at the time of writing.
A high-throughput screening campaign led to the discovery of the first series of both potent and selective ATR kinase inhibitors by Vertex Pharmaceuticals. The compounds generally featured a central 2-aminopyrazine core. Compounds that lacked the 2-amino group resulted in higher enzymatic ATR $K_i$ values.\(^{107}\)

Pyrazine derivative (66) was a promising ATP-competitive hit (ATR; IC\(_{50}\) = 0.62 \(\mu\)M), with excellent selectivity over DNA-PK and ATM.\(^{108}\) The sub-optimal potency and cellular activity was addressed by further structure-activity relationships (SAR), guided by homology modelling of the ATP binding-domain of ATR. The pyrazine N1 serves as the hydrogen bond acceptor group for the interaction with the backbone NH of Val2378, and the adjacent 2-amino group donates a hydrogen bond to the carbonyl of Glu2380.\(^{109}\)

The introduction of a 4-sulfonyl group (67) (IC\(_{50}\) = 26 nM) increased the potency and selectivity, attributed to a favourable interaction between the sulphone oxygen and the NH of the ATR-specific residue Gly2385. Compound 67 was shown to be a potent ATP-competitive inhibitor of ATR ($K_i$ = 13 nM) with good selectivity over a panel of the related PIKKs, ATM, DNA-PKcs and mTOR.\(^{108}\) It inhibited phosphorylation of the ATR downstream target CHK1 at Ser345 and showed strong synergy with genotoxic agents from multiple classes in the
colorectal cancer cell line HCT116. The observed chemosensitisation was most pronounced with DNA cross-linking agents such as cisplatin and carboplatin, and was further enhanced by knockdown of p53 expression, in ATM-deficient cells or in combination with the specific ATM inhibitor KU-55933 (41) (see Section 3.2.3). Significantly, the cytotoxicity of 67 in normal cells appeared minimal, causing only a reversible growth arrest without significant induction of cell death.\textsuperscript{108}

Further optimisation of 67 led to compound 68, which was selected for in depth biological evaluation. Compound 68 combines potent ATP-competitive ATR-inhibitory activity ($K_i = 6 \text{ nM}$) and excellent cellular activity. It also possesses good physicochemical properties, including aqueous solubility, cell permeability and minimal efflux.

**VE-822/VX-970**

VE-822 (69) is an analogue of 67, which possesses increased potency and selectivity against ATR. It also benefits from increased solubility and good pharmacokinetic properties.

![Chemical structure of VE-822 (69)](attachment:image.png)

It was shown to potently radiosensitise pancreatic cancer cell lines \textit{in vitro}. Additionally, treatment with 69 profoundly radiosensitised xenograft models of human pancreatic cancer and further increased the growth delay induced by IR combined with gemcitabine. Crucially,
was well tolerated in mice and did not enhance toxicity in normal cells and tissues.\textsuperscript{10} The compound is now known as VX-970 and was the first selective ATR inhibitor to enter clinical trial development. In 2014, Vertex Pharmaceuticals published a study in which it was shown to markedly sensitise a panel of non-small cell lung cancer cell lines, but not normal cells, to multiple DNA damaging drugs, namely cisplatin, oxaliplatin, gemcitabine and etoposide.\textsuperscript{11} The data suggested that VX-970 may have the potential to increase the efficacy of DNA damaging therapy in patients with lung cancer. The safety, tolerability and pharmacokinetics were, at the time of writing, being assessed in a phase I clinical trial. The strategy was to evaluate VX-970 in early-stage trials in selected tumour types and patient subtypes that are expected to be responsive to ATR inhibition based on biomarker data. It is expected to be evaluated as monotherapy and in combination with other cancer therapies, including PARP inhibitors and other targeted agents, chemotherapy and radiotherapy.

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