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Targeting DNA-PK for Cancer Therapy

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Abstract

The catalytic activity of DNA-dependent protein kinase (DNA-PK) is critical to its ability to repair lethal DNA-double strand breaks (DSBs). This includes repair of DSB lesions resulting from oxidative stress, oncogene-induced transcription, or following therapeutic treatment of cancer cells. Armed with this knowledge, many attempts have been made to identify small molecule inhibitors of DNA-PK activity as an approach to induce tumour chemo- and radio-sensitisation. This review examines the structures of known reversible and irreversible inhibitors, including those based upon chromen-4-one, arylmorpholine, and benzaldehyde scaffolds. DNA-PK catalytic inhibitors, such as VX-984 and M3814, have now progressed into clinical development, which should help to further advance our understanding of whether this approach represents a promising therapeutic strategy for the treatment of cancer.

Introduction

DNA-dependent protein kinase (DNA-PK) is a key protein involved in the repair of DNA double-strand breaks (DSBs) that arise from oxidative damage and exogenous

stimuli such as ionizing radiation treatment. The active DNA-PK complex is composed of a catalytic serine/threonine protein kinase (DNA-PKcs) and two heterodimeric subunits (Ku70 and Ku80) which bind to the DSB to direct the catalytic subunit to the site requiring repair.[1,2]

The potential lethality of even a single DSB has driven the evolution of two repair pathways in mammalian cells. Homologous recombination (HR) takes place during the S and G2 phases of the cell cycle when sister chromatids are available to act as a template, allowing accurate repair of the genome.[3] By contrast, a canonical non-homologous end joining (NHEJ) pathway[4-6] can occur throughout the cell cycle, allowing cells to mount a rapid repair response to maintain genome integrity. DNA-PK predominantly mediates DSB repair through canonical NHEJ, by regulatory mechanisms that have been shown to be critically dependent upon its kinase activity. The recruitment of DNA-PKcs to a DSB by the heterodimeric subunits shifts the Ku heterodimer so that DNA-PK is able to structurally tether the broken DNA-double strands together while simultaneously stimulating DNA-PKcs activity. [7,8]

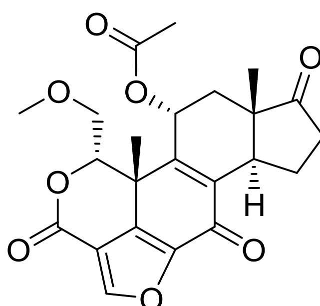
DNA-PKcs has been shown to have altered activity and expression in several tumour types. Early studies in chronic lymphocytic leukaemia (CLL) showed that increased DNA-PKcs activity correlated with resistance to chemotherapy and poor outcome.[9,10] Elevated DNA-PKcs expression has also been reported in gastric cancer[11] and found to correlate with poor clinical outcome in patients with ovarian[12] and hepatocellular cancer (HCC).[13] The identification of the role of DNA-PK in resistance to chemo and radiotherapy has led to multiple attempts to identify effective inhibitors of the catalytic subunit. DNA-PK has so far proven an intractable target for structural biology, due to the multi-component structure of the active holoenzyme so the identification of inhibitors has been largely approached via

classical screening methods and medicinal chemistry optimisation. To date, these approaches have focused on Type 1 inhibitors, those that occupy the ATP-binding site of the kinase domain, but vary widely in potency, selectivity, and with differences in the reversibility of their inhibition. DNA-PKcs is a member of the phosphatidylinositol 3-kinase (PI-3K) related kinase (PIKK) family, which includes proteins also of interest in anti-cancer drug discovery such as ATM (Ataxia telangiectasia mutated kinase), ATR (AT and Rad3-related kinase) and mTOR (Mammalian Target of Rapamycin).[4-6] It is therefore crucial to ensure sufficient selectivity over these other enzymes, as well as the wider PI-3K family, before a tool compound can be used to accurately assess the clinical relevance of DNA-PK inhibition.

Small Molecule Inhibitors of DNA-PK Catalytic activity

Wortmannin

Wortmannin (**1**) is a naturally occurring compound, first derived from *Penicillium wortmannii K*, which was originally identified as having antifungal and anti-inflammatory properties and was subsequently found to inhibit PI-3K activity with an IC_{50} of 4.2 nM.[14]

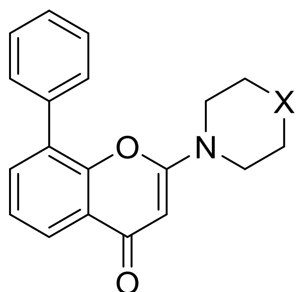


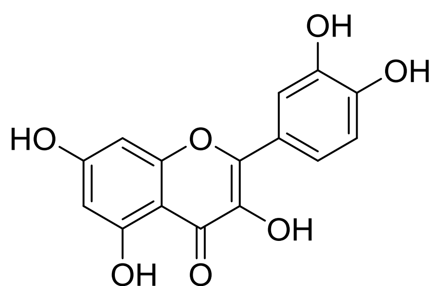
1; Wortmannin

Kinetic analysis of the PI-3K inhibition by wortmannin indicated a non-competitive irreversible inhibition which was further verified by Walker *et al*[15] with co-crystallographic studies of the resulting covalent complex in the ATP-binding pocket of PI3Kγ.[15,16] Additional work demonstrated that wortmannin also inhibits DNA-PK at higher concentrations (Ki = 120 nM) by an irreversible mechanism, forming covalent adducts with DNA-PKcs lysine 3751, located in the ATP binding site of the kinase domain.[17] Although an interesting early tool compound, the structural complexity of wortmannin and its poor selectively for DNA-PK over other related kinases limit its utility. Despite these limitations, wortmannin was used to demonstrate a 3- to 5-fold enhancement of IR-induced cytotoxicity and an inhibition of IR-induced DSB repair in chinese hamster ovary cells.[18]

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

The chromen-4-one structure LY294002 (**2**) was reported by Lilly Pharmaceuticals in 1994 as an inhibitor of PI-3K.[19] It was identified through a screen of compounds derived from quercetin (**3**) with the objective of developing more PI-3K-specific inhibitors.





2; LY294002; X = O
4; X = S
5; X = NH

3

Subsequent evaluation of **2**, showed that the compound exhibited similar inhibitory activity against DNA-PK to that demonstrated against PI-3K and mTOR (Table 1).[19,20]

Table 1: Reported inhibitory activity of LY294002 (**2**) against different PIKK family members. Values taken from references 19[a] and 20[b].

PIKK	DNA-PK	PI-3K (p110$_{\alpha}$)	ATM	ATR	mTOR
IC_{50} (μM)	1.5±0.2[a]	2.3±0.8[a] (1.4)[b]	>100[a]	>100[a]	2.5±0.2[a]

X-ray crystallography verified the key role of the morpholine substituent of **2**. When in complex with human PI3Kγ, the morpholine oxygen of **2** makes a hydrogen bond interaction with the backbone amide group of Val-882 within the ATP-binding domain of the kinase (Figure 1).[15] The importance of the oxygen of the morpholine substituent of **2** was also reported, and while replacement by a thiomorpholine (**4**) reduced potency, the piperizine derivative (**5**) proved essentially devoid of activity, indicative of a crucial hydrogen bond acceptor function for the morpholine oxygen.

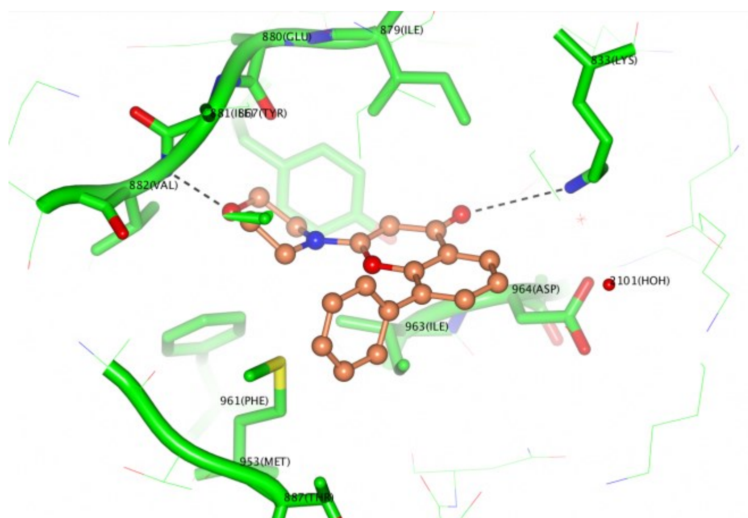
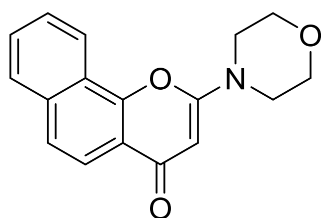


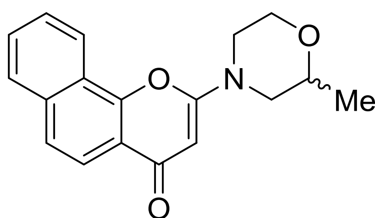
Figure 1. Crystal structure of LY294002 (**2**) in complex with the ATP-binding domain of PI3Kγ.[15] The figure was prepared from PDB file 1E90 using CCP4MG.

Despite suffering from high clearance and *in vivo* toxicity, LY294002 (**2**) aided in the development of more potent and selective DNA-PK lignds with improved physicochemical properties.

Studies undertaken by the Northern Institute for Cancer Research in Newcastle in collaboration with KuDOS Pharmaceuticals investigated a series of benzopyranones.[20] Fusing the chromenone biaryl system gave a 5-fold increase in potency against DNA-PK (NU7026 (**6**); IC_{50} = 0.23 μM) (Table 2) and placement of a methyl group on the morpholine ring was equipotent (**7**; IC_{50} = 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.[20,21]



6; NU7026



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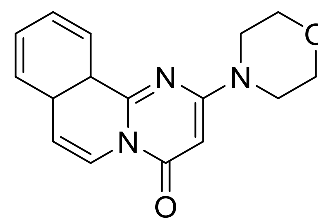


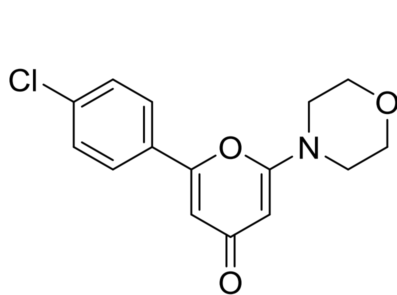
8

These early derivatives showed a higher selectivity for DNA-PK over other PIKK family members, for example NU7026 (**6**) is 60-fold more potent against DNA-PK than PI-3K (p110α) (Table 2). Replacement of the chromen-4-one scaffold by the isosteric pyrimidoisoquinolinone structure gave equipotent compounds (**8**; IC_{50} = 0.28 μM).[22] Interestingly, **8** retained selectivity for DNA-PK. NU7026 was shown to act *in vitro* as a radiosensitiser, providing a 2-fold dose enhancement in mouse embryonic fibroblast cells[23] and was also found to increase sensitivity to TOP2 poisons by 2-19 fold.[24] NU7026 has been used widely as a tool compound to explore novel DNA-PK biology, appearing in over 75 publications.

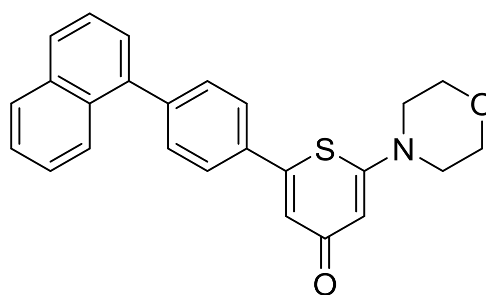
Table 2: Inhibitory activity (IC_{50} μM) against different PIKK family members (adapted from reference 20).

PIKK	DNA-PK	PI-3K (p110α)	ATM	ATR	mTOR
6	0.23	13	>100	>100	6.4
7	0.19	2.4	>100	>100	4.8
8	0.28	>100	>100	>100	5.3

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.[25] The pyran-4-one system and substitution at the pyranone 3- or 5-positions gave a loss of activity. However, extended library work on 6-substituted-2-morpholino-pyran-4-one and 6-substituted-2-morpholinothiopyran-4-one, resulted in the identification of NU7059 (**9**; IC_{50} = 0.18 μM) and NU7279 (**10**; IC_{50} = 0.19 μM), both 10-fold more potent against DNA-PK than the parent **2**.[25,26]

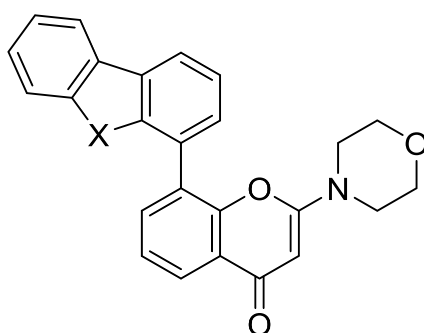


9; NU7059



10; NU7279

The most significant improvement in activity and selectivity against DNA-PK, was achieved as a result of extensive library work carried out through the preparation of 6-, 7-, and 8-aryl substituted chromen-4-ones.[27] Incorporation of a dibenzofuranyl group gave the first sub-micromolar inhibitor of DNA-PK, NU7427 (**11**) (IC_{50} = 0.04 μM) and subsequent derivatisation of this scaffold, by introduction of the dibenzylthiophenyl moiety, afforded the highly potent and selective compound 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (NU7441; **12**; (IC_{50} = 0.02 μM).



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

NU7441 (**12**) is of special interest, not only due to its potency against DNA-PK, but also for its selectivity over other kinases (Table 3).[27]

Table 3: NU7441 (**12**) inhibitory activity against different kinases of the PIKK family.[27] PI4Kβ data supplied by KuDOS Pharmaceuticals.

PIKK	DNA-PK	PI-3K	PI4K$_\beta$	ATM	ATR	mTOR
IC_{50} (μM)	0.014	5	40	>100	>100	1.7

Compound **12** has been used in over 50 studies in the literature. Charactrisation of **12** using the SW620 and LoVo cell lines, found that 1uM NU7441 enhanced the cytotoxicity of DSBs induced by topoisomers II inhibitors etoposide (2-12 fold), and doxorubicin (2-10 fold) as well as IR (2-4 fold).[28] Importantly, *in vivo* studies showed that despite its relatively poor solubility, NU7441 increased etoposide-induced tumour growth delay in the SW620 tumour xenograft model.[28]

In collaboration with AstraZeneca, a homology model of the ATP-binding site of DNA-PK, derived from the crystal structure of PI3Kγ was used to guide further inhibitor design.[29] Significantly, the model predicted that groups introduced at the dibenzothiophene 1-position of **12** would be directed out of the binding pocket into bulk solvent (Figure 2A, red arrow).

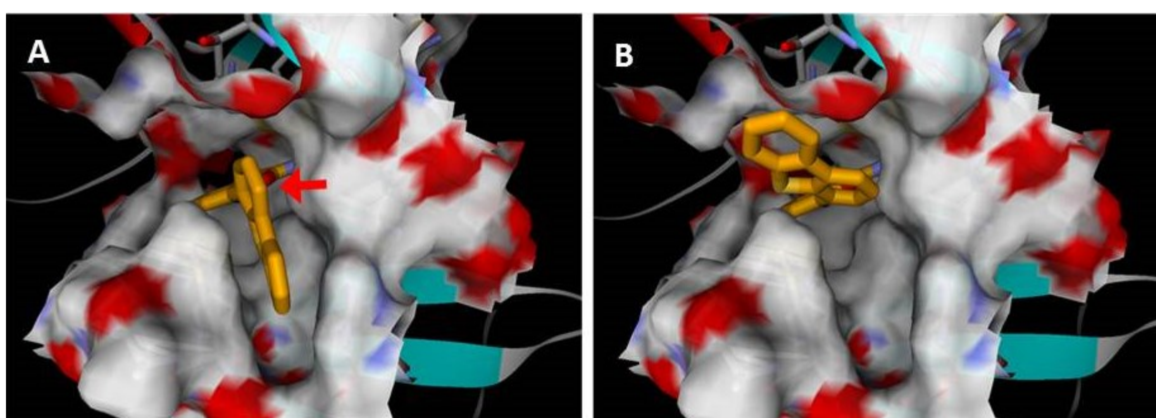
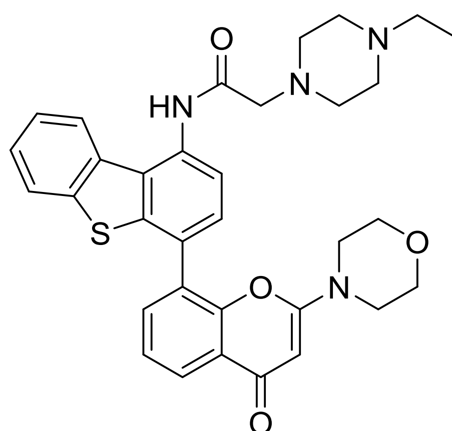


Figure 2: Homology model of the ATP-binding site of the DNA-Dependent Protein Kinase (DNA-PK) used to guide inhibitor design.[29] The 3D model was constructed on the basis of the known X-ray crystal structure of PI3Kγ from RCSB protein data

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 (**12**) is represented in (**A**) an orthogonal, and (**B**) 'in plane' pose.

Synthesis of a focused library allowed the effect of substitution at the 1-position of the dibenzothiophen-4-yl moiety on both potency and physochemical properties to be studied.



13; KU-0060648

A number of the newly synthesized compounds exhibited high potency against DNA-PK and potentiated the cytotoxicity of ionizing radiation (IR) *in vitro* 10-fold or more (e.g. KU-0060648 (**13**); DNA-PK IC_{50} = 5.0 ± 1 nM, IR dose modification ratio = 13). In addition, **13** was shown to potentiate not only IR *in vitro*, but also DNA-damage inducing TOP2 poisons (doxorubicin, etoposide) both *in vitro* and *in vivo*.[41] In a counter-screen against other members of the phosphatidylinositol 3-kinase (PI-3K) related kinase (PIKK) family, several compounds were found to be potent mixed DNA-PK and PI-3K inhibitors, including **13**.[30,31] The encouraging biological activity of **13** was accompanied by improved drug-like properties compared to NU7441 (**12**),

and 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 (Table 4).

Table 4. Properties of NU7441 (**12**) and KU-0060648 (**13**) (Data are the mean ± the standard deviation or individual values; adapted from reference 30).

Assay		**12**	**13**
Enzyme	DNA-PK IC_{50} (nM)	42 ± 2	5.0 ± 1
Cellular (HeLa)	pDNA-PK EC_{50} (nM)	212, 339	136 ± 17
	DMR (0.1 μM DNA-PK inhibitor)	2.2 ± 0.2	4.0 ± 0.4
	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

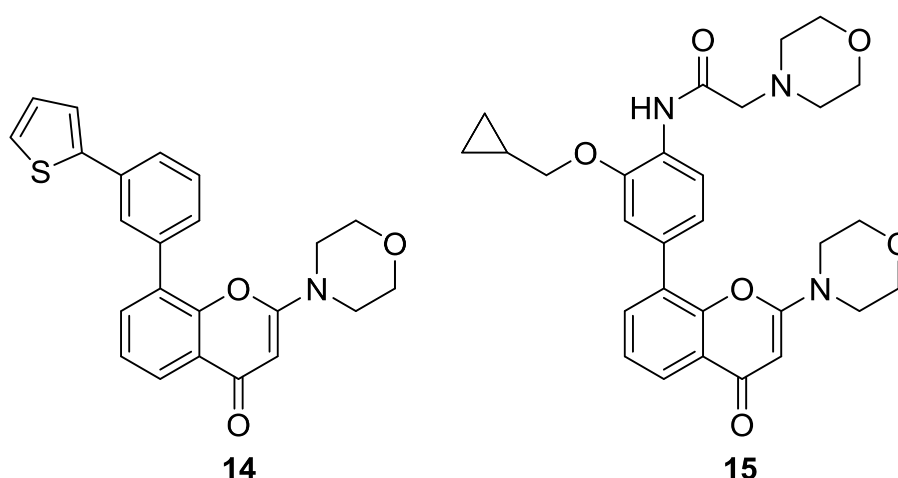
[a] Amorphous material (crystalline solubility at pH7.4 buffer = 6.0 μM)

[b] Tested in CYP 3A4, 2D6, 2C9, 2C19 and 1A2

(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

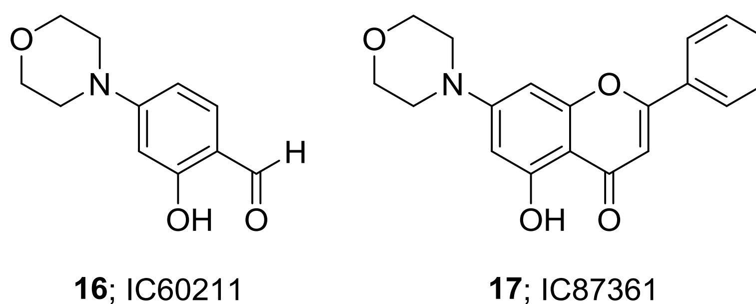
compound in 1-octanol *versus* that in water at equilibrium; hERG, the human ether-a-go-go-related gene).

Further derivatives of LY294002 (**2**) and NU7441 (**12**) have been reported to have improved DNA-PK inhibitory activity (i.e. 8-biarylchromenon-4-one (**14**), IC_{50} = 18 nM and *O*-alkoxyphenylchromen-4-one (**15**), IC_{50} = 8 nM).[32,33]



Phenol Related IC Series

Reported by the ICOS Corporation and Array Biopharma, 2-hydroxy-4-morpholin-4-yl-benzaldehyde (IC60211, IC_{50} = 400 nM) (**16**) is an example of phenolic DNA-PK inhibitors possessing the morpholine pharmacophore.[34]



16; IC60211 **17**; IC87361

Optimisation of **16** resulted in numerous DNA-PK selective inhibitors including IC87361 (**17**), a flavonoid compound found to be 50-fold more selective for DNA-PK than for p110β.[34,35] It should be noted that the morpholine substituent is categorically required to maintain kinase inhibitory activity.

Table 5: Inhibitory activity (IC_{50} nM) of representative DNA-PK inhibitors against various PI-3Ks (adapted from references 34 and 35).

	DNA-PK	**p110α**	**p110β**	**p110δ**	**p110γ**
16	400	10000	2800	5100	37000
17	34	3800	1700	2800	7900

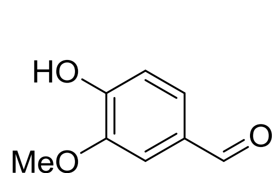
In cell culture, **17** directly inhibits the repair of DNA DSBs and enhances the cytotoxicity of chemical and physical agents that induce DSB formation. Furthermore, compounds in this series are relatively non-toxic in animal models and have improved pharmacokinetic profiles over other specific DNA-PK inhibitors, while enhancing the efficacy of ionizing radiation *in vitro* and *in vivo*.[35,36]

Vanillins

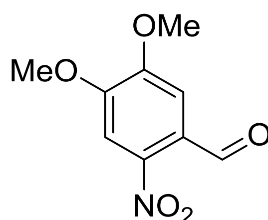
It has been shown that certain members of the vanillin family are irreversible inhibitors of DNA-PK. Like wortmannin, vanillin (**18**) interacts preferentially with protein lysine residues in the catalytic centre of PI-3K, in this case via Schiff base formation.[37,38]

A screen for structurally related benzaldehyde derivatives was undertaken of in excess of 53,000 drug-like compounds, which identified two low micromolar

inhibitors of DNA-PK. 4,5-Dimethoxy-2-nitrobenzaldehyde (**19**) (IC_{50} = 15 μM) is 100-fold more potent than vanillin, while 2-bromo-4,5-dimethoxybenzaldehyde (**20**) (IC_{50} = 30 μM) is 50-fold more potent.[37]



18; Vanillin



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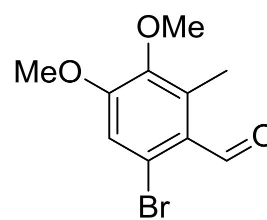

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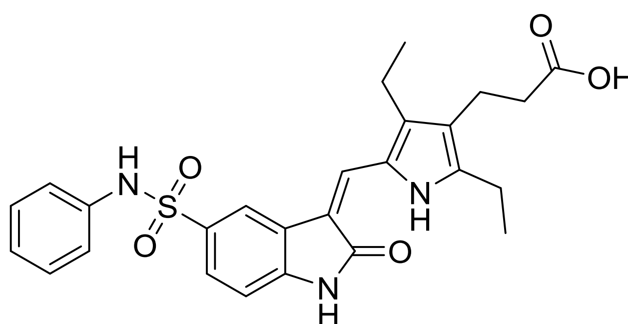
Vanillin itself has fragment-like properties (IC_{50} = 1.5 mM, MW = 152.15) and so the opportunity exists for further elaboration of this scaffold.[37]

SU11752

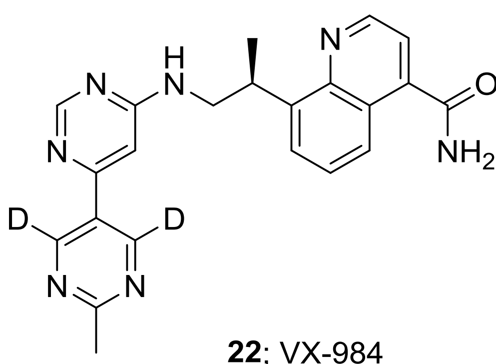
In efforts towards developing a specific DNA-PK inhibitor, Sugen identified the ATP-competititive DNA-PK inhibitor, SU11752 (**21**) through library screening of three-substituted indolin-2-ones. Compound **21** showed good potency against DNA-PK(IC_{50} = 0.13 ± 0.028 μM), comparable potency to wortmannin (IC_{50} = 0.10 μM),[17] but with selectivity for DNA-PK over PI-3K and ATM.[39]



21; SU11752

VX-984

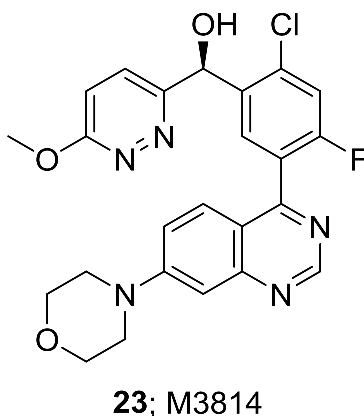
VX-984 (**22**) is a DNA-PK inhibitor designed by Vertex Pharmaceuticals which is currently undergoing a phase I clinical study, in combination with pegylated liposomal doxorubicin in patients with advanced solid tumours or lymphomas. Compound **22** is reported to have an IC_{50} of 88 ± 64nM for inhibition of DNA-PKcs autophosphorylation (Ser2056) in A549 lung cancer cells, with good selectivity versus other PI3K family members.[40]



22; VX-984

M3814

Merck KGaA have developed M3814 (**23**) (also described as MSC2490484A), an orally-administered, highly potent and selective inhibitor of DNA-PK.[41]



23; M3814

Information relating to the structure of the compound were released publically in April 2017.[42] M3814 entered phase I clinical development in December 2014

(NCT02316197) for use in patients with solid tumours who had DNA repair deficiencies, and in patients with Chronic Lymphocytic LeukaemiaM3814 subsequently entered phase I trials in July 2015 (NCT02516813) in combination with DNA damaging modalities such as radio-chemotherapy and radiation. The trial incorporates a proof-of-principle study to examine the pharmacodynamic and mechanistic consequences of drug treatment. Disclosure of results by Merck in 2016, indicated that M3814 is active in a preclinical setting, exhibiting efficacy in all mouse models of human cancer, in combination with IR.[41,43]

Summary

DNA-PK remains an interesting therapeutic target. Previous investment made by academia and pharmaceutical companies to identify inhibitors of DNA-PKcs activity has resulted in the identification of candidate drugs that are now being tested clinically in solid tumours and haematological malignancies both as a monotherapy and in combination with chemotherapy or radiotherapy. This progress may enable us to shortly realise the potential of exploiting DNA-PK inhibition for the benefit of cancer patients.

Keywords: CHEMOPOTENTIATION, DNA DAMAGE RESPONSE, DNA-PK, INHIBITOR, KINASE.

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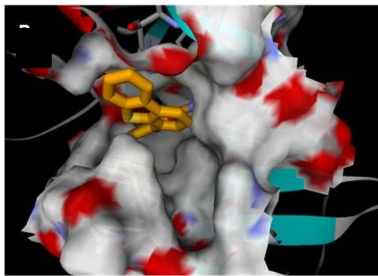

The catalytic activity of DNA-dependent protein kinase (DNA-PK) is critical to its ability to repair lethal DNA-double strand breaks and many attempts have been made to identify small molecule inhibitors of DNA-PK activity as an approach to induce tumour chemo- and radio-sensitisation. DNA-PK catalytic inhibitors have now progressed into clinical development, which should help to further advance our understanding of whether this approach represents a promising therapeutic strategy for the treatment of cancer.