8498 J. Med. Chem. 2010, 53, 8498–8507
DOI: 10.1021/jm100608j

Journal of Medicinal Chemistry Article

DNA-Dependent Protein Kinase (DNA-PK) Inhibitors. Synthesis and Biological Activity of Quinolin-4-one and Pyridopyrimidin-4-one Surrogates for the Chromen-4-one Chemotype

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†*Newcastle Cancer Centre, Northern Institute for Cancer Research, School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom, and* ‡*KuDOS Pharmaceuticals, Ltd., 410 Cambridge Science Park, Milton Road, Cambridge, CB4 0PE, United Kingdom*

Received May 19, 2010

Following the discovery of dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (NU7441) (Leahy, J. J. J.; Golding, B. T.; Griffin, R. J.; Hardcastle, I. R.; Richardson, C.; Rigoreau, L.; Smith, G. C. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6083−6087) as a potent inhibitor (IC_{50} = 30 nM) of DNA-dependent protein kinase (DNA-PK), we have investigated analogues in which the chromen-4-one core template has been replaced by aza-heterocyclic systems: 9-substituted 2-morpholin-4-ylpyrido[1,2-*a*]-pyrimidin-4-ones and 8-substituted 2-morpholin-4-yl-1*H*-quinolin-4-ones. The 8- and 9-substituents were either dibenzothiophen-4-yl or dibenzofuran-4-yl, which were each further substituted at the 1-position with water-solubilizing groups [$NHCO(CH_2)_nNR^1R^2$, where n = 1 or 2 and the moiety R^1R^2N was derived from a library of primary and secondary amines (e.g., morpholine)]. The inhibitors were synthesized by employing a multiple-parallel approach in which the two heterocyclic components were assembled by Suzuki−Miyaura cross-coupling. Potent DNA-PK inhibitory activity was generally observed across the compound series, with structure−activity studies indicating that optimal potency resided in pyridopyrimidin-4-ones bearing a substituted dibenzothiophen-4-yl group. Several of the newly synthesized compounds (e.g., 2-morpholin-4-yl-*N*-[4-(2-morpholin-4-yl-4-oxo-4*H*-pyrido[1,2-*a*]-pyrimidin-9-yl)dibenzothiophen-1-yl]acetamide) combined high potency against the target enzyme (DNA-PK IC_{50} = 8 nM) with promising activity as potentiators of ionizing radiation-induced cytotoxicity in vitro.

Introduction

DNA-dependent protein kinase (DNA-PK[a]), a member of the phosphatidylinositol (PI) 3-kinase related kinase (PIKK) family, is a multicomponent serine/threonine protein kinase that plays a key role in the repair of mammalian DNA double-strand breaks (DSBs) via the nonhomologous end joining pathway of DNA repair.[1,2] Human cell lines defective in DNA-PK function are hypersensitive to agents that elicit DNA DSBs.[3,4] By impeding DNA DSB repair, selective DNA-PK inhibitors have potential application as radio- and chemopotentiators in the treatment of cancer.[5−9] By use of 2-morpholino-8-phenyl-4*H*-chromen-4-one (**1**, LY294002)[10] as a template for inhibitor design, a number of potent DNA-PK inhibitors have been developed and structure−activity relationships (SARs) have evolved for these structural classes.[11−15] In these inhibitors the 2-morpholino-4*H*-chromen-4-one moiety is connected at the 8-position to an aryl or heteroaryl ring, which can be substituted or unsubstituted.[12,13,15] Notably, the incorporation of a dibenzothiophen-4-yl (**2**, NU7441)[12] or dibenzofuran-4-yl substituent at the chromenone 8-position conferred high inhibitory activity against DNA-PK.[13] In addition, **2** exhibited good selectivity for DNA-PK (IC_{50} = 30 nM) over other PIKK family members and a panel of diverse kinases. Chromenone **2** has also been demonstrated to sensitize a human tumor cell line to both ionizing radiation and the topoisomerase II inhibitor etoposide in vitro and in vivo.[16]

With a view to improving the pharmaceutical properties of **2** and related compounds, the introduction of water-solubilizing substituents at the dibenzothiophene 1-position was investigated, leading to the identification of **3** as a highly potent (IC_{50} = 6 nM) DNA-PK inhibitor with promising biological activity, and further studies with this compound are in progress.[17] However, we have accrued some evidence to suggest that **3** does not entirely resemble **2** with regard to kinase selectivity, and potentially problematic off-target activity has been observed for **3**. This has prompted the investigation of alternative heterocycles to the chromen-4-one of **2** and **3**, as well as the introduction of water-solubilizing groups elsewhere on the molecule.

Early SARs conducted with benzo[*h*]chromen-4-ones (e.g., **4**, IC_{50} = 230 nM) and the isosteric pyrimido[1,2-*a*]isoquinolin-4-one (**5**, IC_{50} = 280 nM) indicated a comparable pattern of DNA-PK inhibitory activity for a series of derivatives,

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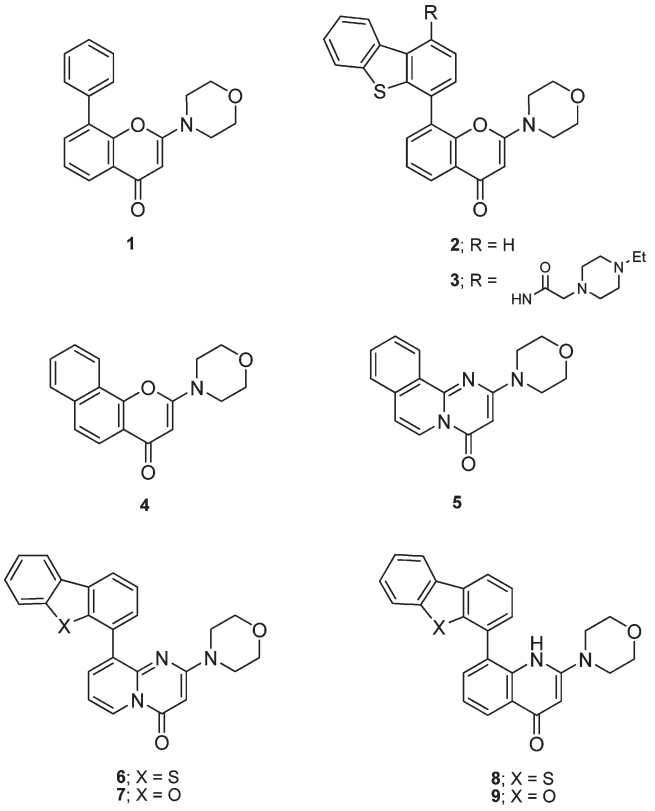
[a]Abbreviations: DNA-PK, DNA-dependent protein kinase; IC_{50}, concentration of inhibitor leading to 50% inhibition; SAR, structure−activity relationship; PI, phosphatidylinositol; PIKK, phosphatidylinositol 3-kinase related kinase; DSBs, double-strand breaks; DMR, dose-modification ratio; IR, ionizing radiation.

 Published on Web 11/16/2010

suggesting that these molecules make similar binding interactions within the ATP-binding domain of the kinase.[11]



We have subsequently reported the synthesis and DNA-PK-inhibitory activity of derivatives of the pyridopyrimidin-4-ones (**6**, **7**) and quinolin-4-ones (**8**, **9**).[18] Following previous studies with chromen-4-ones, indicating that the 1-position of the dibenzothiophen-4-yl moiety was tolerant to substitution, the introduction of water-solubilizing groups was investigated at this position on the pyridopyrimidin-4-one and quinolin-4-one series. In the present paper, we describe the synthesis and biological evaluation of focused libraries of pyridopyrimidin-4-one and quinolin-4-one DNA-PK inhibitors bearing water-solubilizing groups at the dibenzothiophene and dibenzofuran 1-position.

Chemistry

The structures of all compounds synthesized and evaluated for biological activity are recorded in Tables 1 and 2. Intermediates required for the synthesis of pyridopyrimidin-4-one and quinolin-4-one DNA-PK inhibitors were prepared following standard procedures. The preparation of 4-hydroxydibenzothiophene **12** and 4-hydroxydibenzofuran **13** was achieved by reaction of 4-lithiodibenzothiophene or 4-lithiodibenzofuran, respectively, with dioxygen in the presence of methylmagnesium bromide.[19] Nitration of these phenols gave appreciable ortho-nitration. This was avoided by methylation of the hydroxyl groups of **12** and **13** to afford **14** and **15**, respectively, with subsequent nitration occurring exclusively at the 1-position to afford **16** and **17**. Deprotection of the methyl ethers to the corresponding phenols followed by reaction with triflic anhydride/triethylamine gave the required triflates **20** and **21**. The 4-*O*-triflates were readily converted into the corresponding boronates **22** and **23** by treatment with bis(pinacolato)diboron (Scheme 1).

Nitroboronate ester **22** was coupled to 9-hydroxy-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one 9-*O*-triflate[18] and 8-bromo-2-morpholin-4-yl-1*H*-quinolin-4-one[18] via Suzuki−Miyaura reactions to give **24** and **25**. The nitro intermediates were reduced to the required amines **26** and **27** in excellent yields using zinc in acetic acid (Scheme 2).

A similar sequence was utilized with 1-nitrodibenzofuran-4-yl boronate **23**. The Suzuki−Miyaura reactions were performed in a microwave reactor, giving better yields of compounds **28** and **29** in shorter reaction times than under classical conditions. The nitro intermediates **28** and **29** were reduced to the required amines **30** and **31** in excellent yields employing zinc in acetic acid (Scheme 3).

Library Syntheses. The effect upon biological activity of substitution at the 1-position of the dibenzothiophen-4-yl and dibenzofuran-4-yl moieties was investigated through the preparation of focused libraries employing a solution multiple-parallel approach. Acylation of arylamines **26**, **27**, **30**, and **31** with a suitably functionalized acid chloride (chloroacetyl chloride or bromopropionyl chloride) enabled access to eight compound libraries, following reaction with a range of amines (Scheme 4 and Table 1).

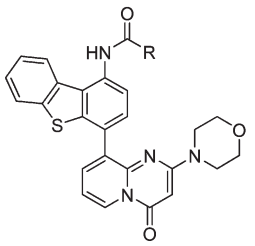
Results and Discussion

The principal objective of the studies described in this paper was to evaluate the biological activities of DNA-PK inhibitors incorporating a quinolin-4-one or pyridopyrimidin-4-one scaffold and bearing substituted acylamino groups at the 1-position on the pendent dibenzothiophen-4-yl or dibenzofuran-4-yl ring. We also wished to compare the biological activity of these two series with the isosteric chromen-4-ones from which **3** is derived. The DNA-PK inhibitory activity and dose-modification ratio (DMR) values for library compounds of suitable purity are shown in Tables 1 and 2.

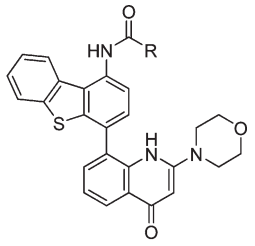
Structure−Activity Relationships for DNA-PK Inhibition. The introduction of amine-substituted acylamino groups at the dibenzothiophene 1-position on the pyridopyrimidin-4-one (**32**−**42**, **52**−**58**) and quinolin-4-one (**43**−**51**, **59**−**64**) systems was generally well tolerated, with most compounds exhibiting DNA-PK inhibitory activity in the nanomolar range (Table 1). This compared favorably with the activities of the parent pyridopyrimidin-4-one (**6**, IC_{50} = 13 nM) and quinolin-4-one (**8**, IC_{50} = 8 nM). Where comparisons were possible, inhibitors based on the pyridopyrimidin-4-one template were generally more potent than the corresponding quinolinones (compare **32** with **44**, and **52** with **59**), although some exceptions are evident (e.g., **57** and **62**, and **38** and **50**). The nature of the alkyl group between the acylamino substituent and the amine function did not appear to markedly influence potency against DNA-PK, with a 1-carbon (**32**−**51**) or 2-carbon (**52**−**64**) spacer conferring comparable activity across the library series in most cases. However, homologation of the spacer group for pyridopyrimidin-4-ones bearing a morpholin-4-yl or *cis*-2,6-dimethylmorpholin-4-yl group (compare **32** and **40** with **52** and **58**) compromised the high potency of the parent compounds **32** (IC_{50} = 8 nM) and **40** (IC_{50} = 7 nM) in both cases. Similar trends were also observed for the small compound library where an isosteric dibenzofuran-4-yl group replaced dibenzothiophen-4-yl on the pyridopyrimidinone (**65**−**81**) and quinolinone (**71**−**83**) templates (Table 2).

With a view to delineating SARs in more detail, relationships were analyzed as illustrated in Figure 1. With the exclusion of compounds **36** and **48**, where compound **36** had

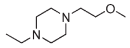
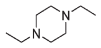
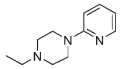
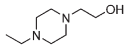
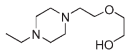
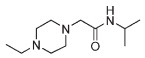
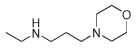
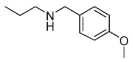
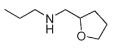
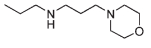
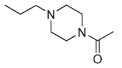
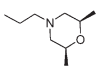
Table 1. Chemical Structures and Biological Activity of Dibenzothiophen-4-yl Substituted Quinolinones and Pyridopyrimidin-4-ones



(**A**)



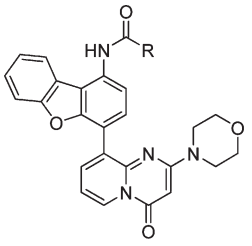
(**B**)

R	No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR		No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR	
				0.5 μM	0.1 μM				0.5 μM	0.1 μM
	-	A	-	-	-	**43**	B	198	2.6	-
	**32**	A	8	9.9	3.5	**44**	B	60	3.1	-
	**33**	A	25	6.2	1.5	**45**	B	133	2.2	-
	**34**	A	22	14.4	4	**46**	B	33	8.2	1.2
	**35**	A	18	7.7	1.4	**47**	B	61	3.4	-
	**36**	A	175	3.9	-	**48**	B	19	5.8	-
	**37**	A	24	9.2	2.4	**49**	B	43	2.5	-
	**38**	A	19	13.6	1.4	**50**	B	18	2.1	-
	**39**	A	29	3.8	1.1	-	B	-	-	-
	**40**	A	7	15.1	-	-	B	-	-	-
	**41**	A	51	8.3	1	-	B	-	-	-
	**42**	A	25	7.3	-	-	B	-	-	-
	-	A	-	-	-	**51**	B	64	4	-
	**52**	A	86	6.2	4	**59**	B	179	2.8	-
	**53**	A	28	14.3	1.5	**60**	B	51	2.9	-
	**54**	A	12	6.7	1.3	-	B	-	-	-
	**55**	A	77	4.3	0.7	**61**	B	102	2.1	-
	**56**	A	29	1.7	-	-	B	-	-	-
	**57**	A	32	2.1	-	**62**	B	21	2.6	-
	-	A	-	-	-	**63**	B	141	2.2	-
	**58**	A	27	4.6	1	**64**	B	14	2.2	-

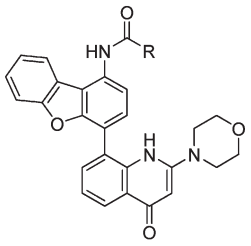
[a] IC_{50} values were determined over a range of different concentrations, usually from 0.01 to 10 μM. Values are the mean of at least three separate determinations.

unexpectedly poor activity (IC_{50} = 175 nM), there was a linear correlation between the DNA-PK inhibitory activities of the dibenzothiophen-4-yl pyridopyrimidin-4-ones and quinolin-4-ones (r = 0.69, r^2 = 0.48, p = 0.02, Figure 1A). Furthermore, overall, the dibenzothiophen-4-ylpyridopyrimidin-4-one DNA-PK inhibitors were more potent than the

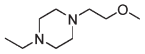
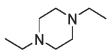
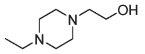
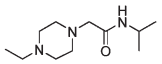
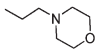
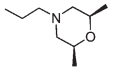
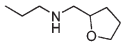
Table 2. Chemical Structures and Biological Activity of Dibenzothiofuran-4-yl Substituted Quinolinones and Pyridopyrimidin-4-ones



(**C**)

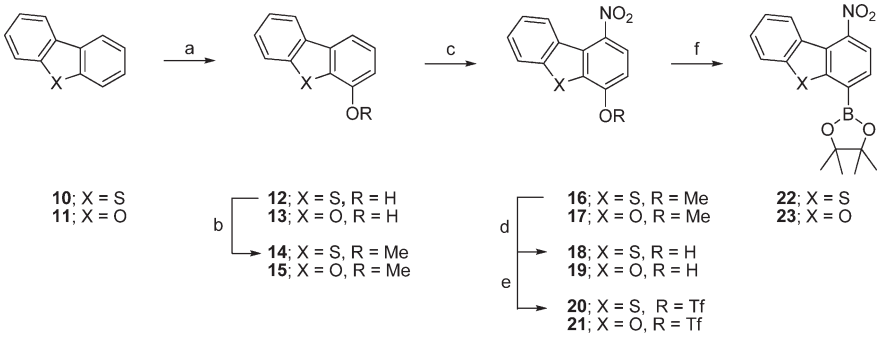


(**D**)

R	No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR		No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR	
				0.5 μM	0.1 μM				0.5 μM	0.1 μM
	**65**	**C**	59	2.3	1.4	**71**	**D**	112	1.6	1.1
	-	**C**	-	-	-	**72**	**D**	68	1.4	1.1
	**66**	**C**	30	4.3	1.1	**73**	**D**	116	1.6	1.2
	**67**	**C**	15	3	1	**74**	**D**	37	1.6	1.1
	**68**	**C**	29	6.4	1.1	**75**	**D**	77	3.6	1.2
	**69**	**C**	17	5.8	1.3	**76**	**D**	39	1.5	1.3
	**70**	**C**	36	13	1.7	**77**	**D**	55	1.6	1.4
	**78**	**C**	77	6.3	1.3	-	**D**	-	-	-
	**79**	**C**	21	1	2.5	**82**	**D**	314	1.1	1
	**80**	**C**	70	4.1	0.9	**83**	**D**	151	0.9	0.8
	**81**	**C**	75	7.4	1.3	-	**D**	-	-	-

[a] IC_{50} values were determined over a range of different concentrations, usually from 0.01 to 10 μM. Values are the mean of at least three separate determinations.

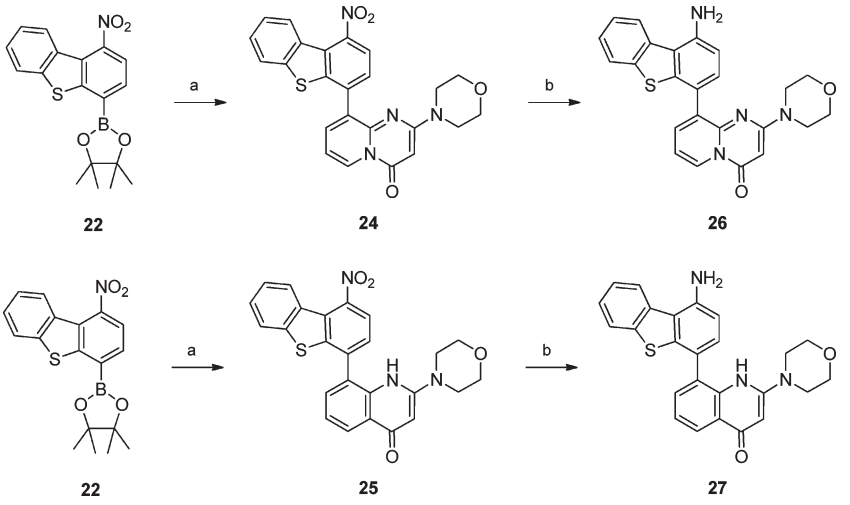
Scheme 1[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, THF, reflux; (ii) MeMgBr, O_2, 25 °C, 39% for **12** and 36% for **13**; (b) MeI, K_2CO_3, acetone, reflux, 96% for **14** and 99% for **15**; (c) HNO_3, AcOH, 25 °C, 99% for **16** and 48% for **17**; (d) pyridine hydrochloride, 150 °C, 41% for **18** and 99% for **19**; (e) triflic anhydride, NEt_3, DCM, 0 °C, 98% for **20** and 94% for **21**; (f) bis(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dppf, dioxane, 95 °C, 66% for **22** and 87% for **23**.

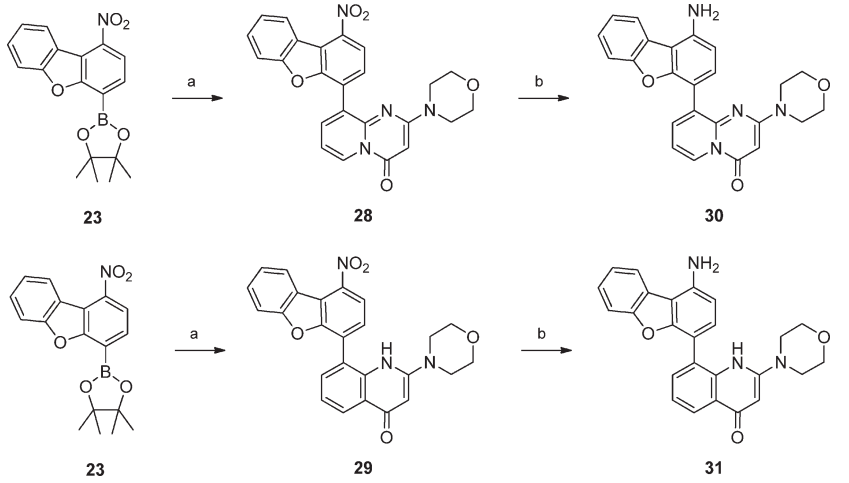
corresponding quinolin-4-one inhibitors (paired *t* test, $p = 0.024$). In an analogous manner to the dibenzothiophen-4-yl quinolinone and pyridopyrimidin-4-one inhibitors and with the exclusion of compounds **79** and **82** where compound **82** had

Scheme 2[a]



[a] Reagents and conditions: (a) pyridopyrimidinone triflate[18] or bromoquinolinone,[18] $PdCl_2$(dppf), Cs_2CO_3, THF, 85 °C, 92% for **24** and 20% for **25**; (b) Zn, AcOH, 25 °C, 99% for **26** and 85% for **27**.

Scheme 3[a]



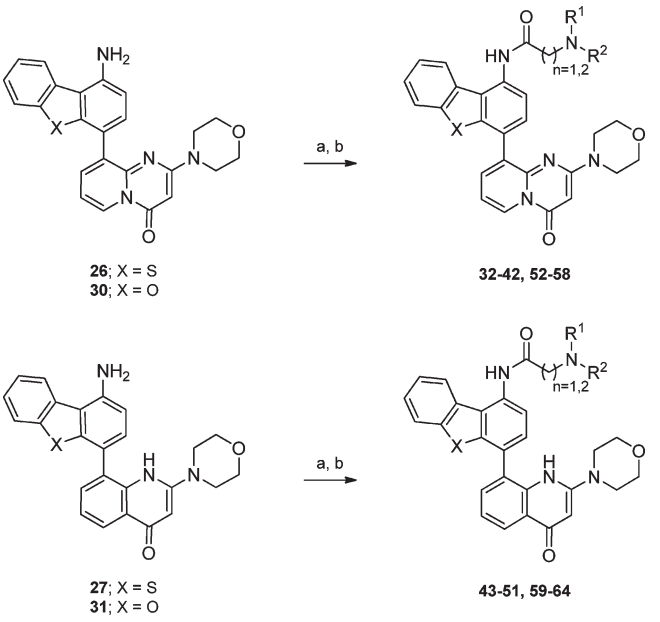
[a] Reagents and conditions: (a) pyridopyrimidin-4-one triflate, $Pd(PPh_3)_4$, K_2CO_3, dioxane, 180 °C, microwave, 30 min, 69% for **28** and 60% for **29**; (b) Zn, AcOH, 25 °C, 96% for **30** and 91% for **31**.

unexpectedly poor activity (IC_{50} = 314 nM), there was also a linear correlation between the DNA-PK inhibitory activities of the dibenzofuran-4-ylquinolinones and the pyridopyrimidin-4-ones (r = 0.85, r^2 = 0.73, p = 0.01, Figure 1B). As with the dibenzothiophen-4-yl DNA-PK inhibitors, the dibenzofuran-4-ylpyridopyrimidin-4-one DNA-PK inhibitors were more potent than the corresponding quinolinone inhibitors (paired t test, p = 0.004), confirming that in both series pyridopyrimidin-4-one is the preferred core pharmacophore for DNA-PK inhibitor potency. Lastly, the relationship between the DNA-PK inhibitory potency of the dibenzothiophen-4-yl and dibenzofuran-4-yl substituents on the quinolin-4-one and pyridopyrimidin-4-one scaffolds was studied, data for 17 pairs of compounds being available, i.e., 10 pyridopyrimidin-4-ones and 7 quinolinones (Tables 1 and 2). For the pyridopyrimidin-4-ones there was a significant linear correlation between the DNA-PK inhibitory potency of the dibenzothiophen-4-yl and dibenzofuran-4-yl inhibitors (r = 0.70, r^2 = 0.49, p = 0.02, Figure 1C), with the dibenzothiophen-4-yl inhibitors being in general more potent that the dibenzofuran-4-yl derivatives. By contrast, there was no relationship between the inhibitory potencies of the dibenzothiophen-4-yl and dibenzofuran-4-yl derivatives in the quinolin-4-one series, reflecting the lack of significant SAR for the dibenzofuran-4-yl compounds, although this analysis was necessarily restricted to only seven pairs of compounds (Figure 1C).

Cell-Based Studies. The dose modification ratio (DMR) is defined as the ratio of the number of cells that survive a single 2 Gy dose of ionizing radiation (IR) to that of the number of cells that survive the same dose of (IR) in combination with a given concentration of DNA-PK inhibitor. This value provides an indirect measure of the ability of a particular compound to potentiate the DNA damage elicited by IR and also indicates whether or not the compound in question is cell permeable. IR alone at the dose used (2 Gy) caused 40−60% survival. The DNA-PK inhibitors alone, at the concentrations used (0.5 and 0.1 μM), were not cytotoxic, and hence, the dose modification observed represents radiopotentiation and not additive toxicity.

In cell-based studies (HeLa cells), the DNA-PK inhibitors potentiated IR-induced cell killing at 0.5 μM, with DMRs of over 10 being observed for selected compounds (i.e., > 10-fold

Scheme 4[a]



[a] Reagents and conditions: (a) chloroacetyl chloride or bromopropionyl chloride, NEt_3, DMA, 25 °C; (b) HNR^1R^2, DMA, 25 °C.

enhanced cell killing at a dose of 2 Gy radiation). However, the dibenzothiophen-4-yl-substituted pyridopyrimidin-4-one inhibitors demonstrated superior activity in the cell-based assay and hence are the preferred chemotype for further investigation. To investigate the impact of DNA-PK inhibitory potency on the potentiation of IR-mediated cytotoxicity, the relationship between DNA-PK inhibition (IC_{50}) and the DMR was studied. When the dibenzothiophen-4-yl-substituted pyridopyrimidin-4-one inhibitors were used, there was a trend toward greater potentiation (i.e., higher DMR values) with the more potent DNA-PK inhibitors. Thus, compounds **34**, **38**, **40**, and **53** produced a greater than 10-fold potentiation of IR-induced cytotoxicity (Table 1, Figure 2A). However, although there was a trend toward higher DMR values for the more potent dibenzothiophen-4-yl-substituted pyridopyrimidin-4-one DNA-PK inhibitors, this trend was not significant at the 5% level ($r = 0.37$, $p = 0.14$), indicating that there are additional determinants of the cellular activity, compound solubility and cell permeability most probably being key factors. A similar analysis was performed for the dibenzothiophen-4-yl-substituted quinolin-4-one inhibitors (Figure 2B). Notably, for this series there was no relationship between DNA-PK inhibition and potentiation of IR-induced cytotoxicity, the majority of compounds generating DMR values of only 2−4 (Table 1). Two dibenzothiophen-4-yl-substituted quinolinone DNA-PK inhibitors, namely, **46** and **48**, produced notable IR potentiation with DMR values of 8.2 and 5.8, respectively. However, as a class, none of these DNA-PK inhibitors generated DMR values of >10, and the lack of even a tentative relationship between potency of DNA-PK inhibition and potentiation of IR-induced cytotoxicity further limits interest in this class of inhibitors. The promising results observed for DNA-PK inhibitors in the IR potentiation studies at 0.5 μM (Table 1) prompted the evaluation of selected compounds at 0.1 μM. Encouragingly, **32**, **34**, and **52** also displayed pronounced potentiation (DMR $>$ 3) at the lower concentration, identifying these pyridopyrimidin-4-ones as potential candidates for further investigations.

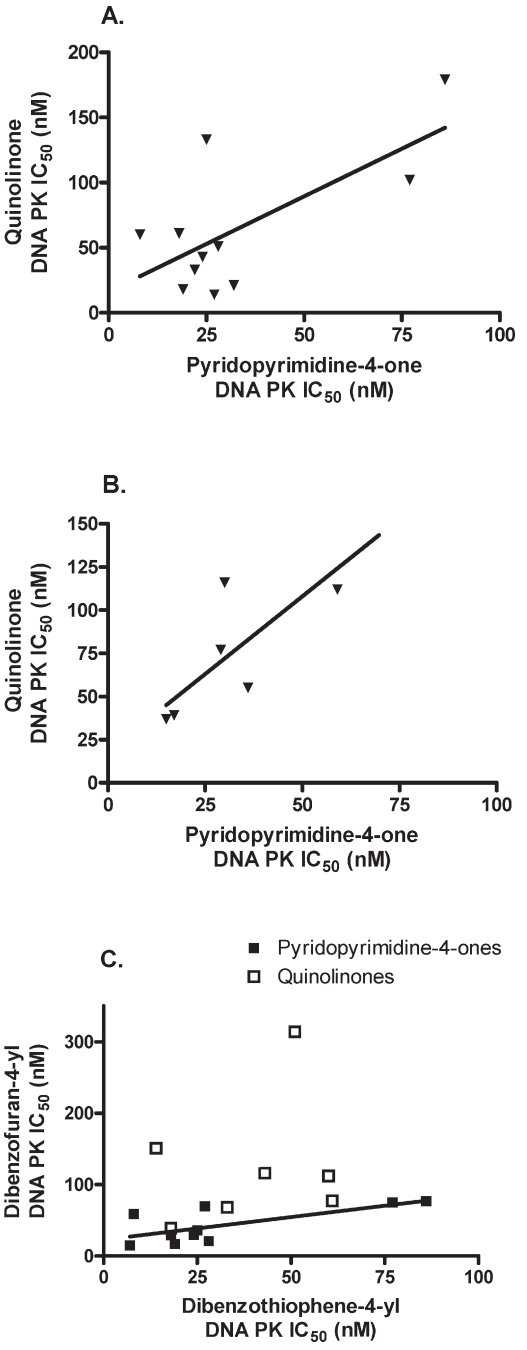


Figure 1. (A) Correlation between DNA-PK inhibition for dibenzothiophen-4-ylpyridopyrimidin-4-ones and quinolinones. Each point represents data for a pair of compounds with otherwise identical structures. Data are from Table 1, and the line is that given by unweighted linear regression analysis. (B) Correlation between the DNA-PK inhibition for dibenzofuran-4-ylpyridopyrimidin-4-ones and quinolinones. Each point represents data for a pair of compounds with otherwise identical structures. Data are from Table 2, and the line is that given by unweighted linear regression analysis. (C) Relationship between the DNA-PK inhibition for dibenzothiophen-4-yl- and dibenzofuran-4-ylquinolinone and pyridopyrimidin-4-one DNA-PK inhibitors. Each point represents data for a pair of compounds with otherwise identical structures. Data are from Tables 1 and 2, and the line is that given by unweighted linear regression analysis of the relationship between the DNA-PK inhibitory activity of dibenzothiophen-4-yl- and dibenzofuran-4-ylpyridopyrimidin-4-ones.

Again, with regard to cellular activity, quinolinone and the pyridopyrimidin-4-one DNA-PK inhibitors bearing a

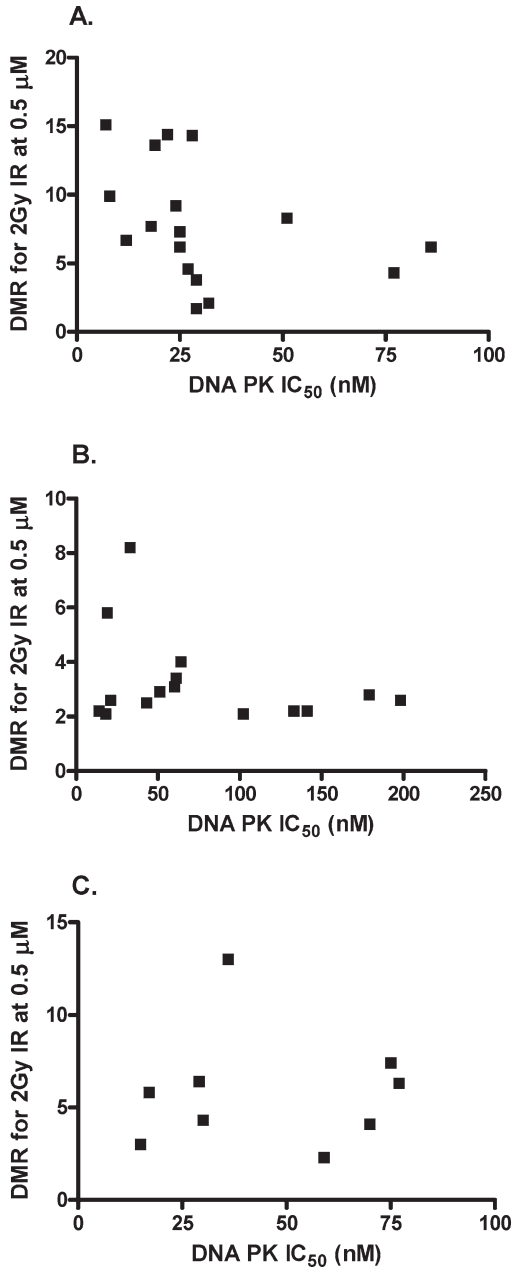


Figure 2. Relationship between potentiation of IR-induced cytotoxicity (DMR) and DNA-PK inhibitory potency (IC_{50}). (A) Relationship between DNA-PK inhibition and potentiation of IR-induced cytotoxicity by dibenzothiophen-4-ylpyridopyrimidin-4-one DNA-PK inhibitors. Each point represents data for an individual compound and are from Table 1. (B) Relationship between DNA-PK inhibition and potentiation of IR-induced cytotoxicity by dibenzothiophen-4-ylquinolinone DNA-PK inhibitors. Each point represents data for an individual compound and are from Table 1. (C) Relationship between DNA-PK inhibition and potentiation of IR-induced cytotoxicity by dibenzofuran-4-ylpyridopyrimidin-4-one DNA-PK inhibitors. Each point represents data for an individual compound and are from Table 2.

dibenzofuran-4-yl substituent generally produced lower levels of potentiation of IR (Table 2) than the analogous dibenzothiophen-4-yl series (Table 1). Indeed the only occasion when DMR values of over 2 were consistently observed for the dibenzofuran-4-yl substituted pyridopyrimidin-4-ones arose at the higher inhibitor concentration of 0.5 μM (Table 2). Importantly, for this series of compounds there was no relationship between potency of DNA-PK inhibition and the DMR value (Figure 2C).

Conclusions

In this paper, we have investigated the possibility of replacing the chromen-4-one scaffold of previously identified DNA-PK inhibitors, with isosteric pyridopyrimidin-4-one and quinolin-4-one heterocycles, and have elucidated the effects of introducing prospective water-solubilizing groups on the pendent dibenzothiophen-4-yl and dibenzufuran-4-yl substituents. Substitution at the dibenzothiophene and dibenzofuran 1-positions by a range of acylamino groups was generally tolerated without detriment to DNA-PK inhibitory activity. Delineation of SARs for DNA-PK inhibition indicated a preference for the pyridopyrimidin-4-one core heterocycle over the corresponding quinolin-4-one, with 1-substituted dibenzothiophen-4-yl proving superior to the dibenzofuran-4-yl isostere. This trend also extended to cell-based radiopotentiation studies in vitro, where pyridopyrimidin-4-ones bearing 1-substituted dibenzothiophen-4-yl groups exhibited excellent potentiation of IR-induced cytotoxicity in the HeLa tumor cell line [e.g., **32**, DNA-PK IC_{50} = 8 nM, DMR (0.1 μM) = 3.5 and **34**, DNA-PK IC_{50} = 22 nM, DMR (0.1 μM) = 4]. Encouragingly, these values are comparable with those obtained for the analogous chromen-4-one DNA-PK inhibitor **3** [IC_{50} = 6 nM, DMR (0.1 μM) = 3.2],[17] and further studies are in progress.

Experimental Section

Solvents were purified and stored according to standard procedures. Petrol refers to that fraction of hexanes boiling in the range 40−60 °C. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. TLC was performed with Merck 60 F254 silica gel plates. "Flash" column chromatography was conducted under medium pressure on silica (Merck silica gel 40−63 μm). HPLC purification were performed on Gilson LC instruments, with a 15 min gradient of 0.1% aqueous TFA and 10−97% acetonitrile, at a flow rate of 6 mL/min, using as the stationary phase a Jones Chromatography Genesis 4 μm C18 column, 10 mm × 250 mm, and peak acquisition based on UV detection at 254 nm. Solution-phase palladium-mediated coupling reactions were conducted in greenhouse reactors (Radley's Ltd., U.K.) under an argon atmosphere. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin AC 300E spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C) or a Bruker AMX (500 MHz for ^{1}H, 126 MHz for ^{13}C) using $CDCl_3$ as solvent, unless indicated otherwise. LCMS was carried out either on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 mm × 4.6 mm C18 column (Supelco Discovery or Waters Symmetry) and a 15 min gradient elution of 0.05% formic acid and methanol (10−90%), or on a Finnegan LCQ instrument in positive ion mode with a Phenomenex 5 μm Luna C18 column, 4.6 mm × 50 mm, and an 8 min gradient of 0.1% aqueous formic acid and acetonitrile (5−98%) with a flow rate of 2 mL/min. Library compounds were found to be >95% pure by HPLC and NMR analyses. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Accurate masses were measured using a Finnigan MAT 95 XP or a Finnigan MAT 900 XLT by the EPSRC National Mass Spectrometry Service Centre, Swansea, SA2 8PP, U.K.

9-(1-Nitrodibenzothiophen-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]-pyrimidin-4-one (24). In a Schlenk tube, 1-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)dibenzothiophene **22** (0.98 g, 2.77 mmol) and cesium carbonate (2.71 g, 8.30 mmol) were mixed in THF (8 mL) and degassed. Concurrently, 9-hydroxy-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one 9-*O*-triflate[16] (1.15 g, 3.05 mmol) and $PdCl_2$dppf (0.11 g, 0.14 mmol) were suspended in THF (8 mL) and degassed. The solutions were mixed together in the Schlenk tube, stirred, and heated at 80 °C for 18 h. When the mixture was cooled, DCM (20 mL) was added. The solution

was washed with water (20 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography using AcOEt/DCM (1:1) as eluent. After evaporation, the product (1.17 g, 92%) was obtained as a yellow solid: R_f = 0.37 (AcOEt/DCM 1:1); mp 259−260 °C; IR (cm^{-1}) 3095, 2980, 2858, 1709, 1628, 1580, 1539, 1516, 1493, 1427, 1356, 1329, 1298, 1236, 1175, 1119, 1070, 995; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.33−3.35 (4H, m, 2 × CH_2N-morpholine), 3.61−3.63 (4H, m, 2 × CH_2O-morpholine), 5.68 (1H, s, H-3), 7.08 (1H, dd, J = 7.4 and 7.3 Hz, H-7), 7.48−7.65 (3H, m, H-Ar), 7.83−7.90 (3H, m, H-Ar), 8.16 (1H, d, J = 7.8 Hz, H-Ar), 9.10 (1H, d, J=8.1 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.8 (2 × CH_2N-morpholine), 66.8 (2 × CH_2O-morpholine), 81.6 (C-3), 112.3 (C-7), 120.5, 123.0, 125.4, 125.7, 127.1, 128.0, 128.9, 129.3, 131.7, 132.9, 136.5, 138.1, 140.4, 143.1, 146.4, 148.6, 159.0, 160.6; HRMS calcd for $C_{24}H_{18}N_4O_4S$ [M + H]$^+$ 459.1122, found 459.1121.

2-Morpholin-4-yl-8-(1-nitrodibenzothiophen-4-yl)-1*H*-quinolin-4-one (25). In the manner described for **24**, 2-morpholin-4-yl-8-(1-nitrodibenzothiophen-4-yl)-1*H*-quinolin-4-one (**25**) was prepared from 1-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)dibenzothiophene **22** (983 mg, 2.77 mmol), cesium carbonate (2.71 g, 8.30 mmol), 8-bromo-2-morpholin-4-yl-1*H*-quinolin-4-one[16] (941 mg, 3.05 mmol), and $PdCl_2$dppf (113 mg, 0.14 mmol) and was obtained as a yellow solid (256 mg, 20%): R_f = 0.24 (AcOEt/DCM 1:1); mp 77−78 °C; IR (cm^{-1}) 2848, 2364, 2338, 1670, 1615, 1584, 1516, 1414, 1348, 1302, 1230, 1187, 1112, 998, 932; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.19−3.21 (4H, m, 2 × CH_2N-morpholine), 3.67−3.69 (4H, m, 2 × CH_2O-morpholine), 6.21 (1H, s, H-3), 7.43−7.57 (3H, m, H-Ar), 7.60-7.72 (2H, m, H-Ar), 7.83 (1H, d, J = 7.8 Hz, H-Ar), 7.94 (1H, d, J = 8.0 Hz, H-Ar), 8.30 (1H, d, J = 7.7 Hz, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.8 (2 × CH_2N-morpholine), 66.4 (2 × CH_2O-morpholine), 92.8, 121.4, 123.2, 123.4, 125.5, 125.8, 126.5, 127.6, 127.7, 129.0, 131.8, 132.9, 140.7, 143.8, 146.6, 171.2; HRMS calcd for $C_{25}H_{19}N_3O_4S$ [M + H]$^+$ 458.1169, found 458.1168.

9-(1-Aminodibenzothiophen-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one (26). Zinc powder (883 mg, 13.51 mmol) was added to 9-(1-nitrodibenzothiophen-4-yl)-2-morpholin-4-ylpyrido [1,2-*a*]pyrimidin-4-one **24** (619 mg, 1.35 mmol) in AcOH (10 mL) and stirred at room temperature overnight. The reaction mixture was filtered through Celite and washed successively with methanol (4 × 50 mL) and DCM (4 × 50 mL). The combined organic layers were evaporated under reduced pressure, and the residue was diluted with water (100 mL). Aqueous ammonia (25 mL) was added to the solution, and the resultant precipitate was collected by filtration. The product was dried under reduced pressure and was obtained as a yellow solid (575 mg, 99%): R_f = 0.36 (AcOEt/DCM 1:1); mp 208−209 °C; IR (cm^{-1}) 3435, 3358, 2916, 2856, 1693, 1618, 1582, 1539, 1489, 1431, 1366, 1333, 1296, 1231, 1176, 1113, 1072, 1022, 999, 928, 899; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41−3.43 (4H, m, 2 × CH_2N-morpholine), 3.59−3.61 (4H, m, 2 × CH_2O-morpholine), 5.68 (1H, s, H-3), 6.90 (1H, d, J = 7.8 Hz, H-Ar), 7.02 (1H, dd, J = 7.9 and 7.8 Hz, H-7), 7.43−7.53 (3H, m, H-Ar), 7.82 (1H, d, J = 8.2 Hz, H-Ar), 7.89 (1H, d, J = 7.8 Hz, H-8), 8.28 (1H, d, J = 8.2 Hz, H-Ar), 9.01 (1H, d, J = 8.1 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.8 (2 × CH_2N-morpholine), 66.9 (2 × CH_2O-morpholine), 81.4 (C-3), 112.7, 112.9, 122.9, 123.0, 123.6, 124.9, 125.9, 127.6, 129.6, 134.9, 135.9, 137.4, 138.9, 141.5, 144.3, 149.1, 159.7, 160.5, 163.19; HRMS calcd for $C_{24}H_{20}N_4O_2S$ [M + H]$^+$ 429.1380, found 429.1381.

8-(1-Aminodibenzothiophen-4-yl)-2-morpholin-4-yl-1*H*-quinolin-4-one (27). In the manner described for **26**, 8-(1-aminodibenzothiophen-4-yl)-2-morpholin-4-yl-1*H*-quinolin-4-one (**27**) was prepared from 2-morpholin-4-yl-8-(1-nitrodibenzothiophen-4-yl)-1*H*-quinolin-4-one **25** (365 mg, 0.80 mmol) and zinc powder (522 mg, 7.98 mmol) and was obtained as a brown oil (291 mg, 85%): R_f = 0.35 (AcOEt/DCM 1:1); IR (cm^{-1}) 3365, 2870, 1666, 1624, 1589, 1493, 1406, 1363, 1247, 1180, 1116, 797, 739, 713; ^{1}H NMR (300 MHz, MeOD) δ 3.32−3.35 (4H, m, 2 × CH_2N-morpholine), 3.66−3.68 (4H, m, 2 × CH_2O-morpholine), 7.01 (1H, d, J = 8.0 Hz, H-Ar), 7.29 (1H, d, J = 7.8 Hz, H-Ar), 7.48-7.58 (4H, m, H-Ar), 7.78 (1H, d, J = 7.5 Hz, H-Ar), 7.84 (1H, d, J = 6.9 Hz, H-Ar), 8.26-8.28 (2H, m, H-Ar); ^{13}C NMR (75 MHz, MeOD) δ 47.7 (2 × CH_2N-morpholine), 67.3 (2 × CH_2-O-morpholine), 115.2, 124.5, 125.3, 126.1, 127.9, 130.1, 136.3, 137.2; HRMS calcd for $C_{25}H_{21}N_3O_2S$ [M + H]$^+$ 428.1427, found 428.1431.

9-(1-Nitrodibenzofuran-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one (28). In a Schlenk tube, 1-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)dibenzofuran **23** (500 mg, 1.47 mmol) and potassium carbonate (480 mg, 3.7 mmol) were mixed in dioxane (10 mL) and degassed. Concurrently, 9-hydroxy-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one 9-*O*-triflate[18] (466 mg, 1.23 mmol) and $Pd(PPh_3)_4$ (71 mg, 0.06 mmol) were suspended in dioxane (10 mL) and degassed. The solutions were mixed together in a microwave vessel and heated with the microwave reactor at 180 °C for 30 min. When the mixture was cooled, DCM (20 mL) was added. The solution was washed with water (20 mL), dried over magnesium sulfate, and concentrated under reduced pressure. The residue was triturated with hot methanol, and the product (375 mg, 69%) was filtered off as a brown solid: R_f = 0.51 (AcOEt); mp 262−265 °C; IR (cm^{-1}) 1706, 1673, 1631, 1599, 1541, 1511, 1430, 1338, 1306, 1230, 1194, 1116, 1070, 1028, 999, 975, 860; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26−3.29 (4H, m, 2 × CH_2N-morpholine), 3.47−3.49 (4H, m, 2 × CH_2O-morpholine), 5.57 (1H, s, H-3), 7.03 (1H, dd, J = 7.8 and 7.6 Hz, H-7), 7.44 (1H, dd, J = 7.6 and 7.5 Hz, H-Ar), 7.58−7.61 (2H, m, H-Ar), 7.74 (1H, d, J = 7.6 Hz, H-Ar), 7.86 (1H, d, J = 7.5 Hz, H-Ar), 8.24 (1H, d, J = 8.0 Hz, H-Ar), 8.66 (1H, d, J = 8.0 Hz, H-Ar), 9.10 (1H, d, J = 7.8 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 44.4 (2 × CH_2N-morpholine), 66.3 (2 × CH_2O-morpholine), 81.1 (C-3), 111.6, 112.1, 119.3, 120.7, 123.9, 126.3, 127.6, 128.3, 128.8, 128.9, 130.0, 138.4, 142.9, 143.9, 148.5, 154.7, 157.0, 158.7, 160.3; HRMS calcd for $C_{24}H_{19}N_4O_5$ [M + H]$^+$ 443.1350, found 443.1352.

2-Morpholin-4-yl-8-(1-nitrodibenzofuran-4-yl)-1*H*-quinolin-4-one (29). In the manner described for **28**, 2-morpholin-4-yl-8-(1-nitrodibenzofuran-4-yl)-1*H*-quinolin-4-one (**29**) was prepared from 1-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) dibenzofuran **23** (500 mg, 1.47 mmol), potassium carbonate (480 mg, 3.69 mmol), 8-bromo-2-morpholin-4-yl-1*H*-quinolin-4-one[18] (380 mg, 1.23 mmol), and $Pd(PPh_3)_4$ (71 mg, 0.06 mmol) and was obtained as a yellow solid (303 mg, 60%): R_f = 0.67 (AcOEt); mp 247−249 °C; IR (cm^{-1}) 3421, 2852, 2360, 2333, 1614, 1573, 1500, 1435, 1429, 1348, 1309, 1233, 1199, 1152, 1124, 1039, 991, 917, 866, 823; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04−3.06 (4H, m, 2 × CH_2N-morpholine), 3.54−3.60 (4H, m, 2 × CH_2O-morpholine), 5.92 (1H, s, H-3), 7.19−7.45 (2H, m, H-Ar), 7.51−7.58 (2H, m, H-Ar), 7.65−7.70 (2H, m, H-Ar), 8.31 (2H, m, H-Ar), 8.67 (1H, d, J = 7.5 Hz, H-Ar); HRMS calcd for $C_{25}H_{20}N_3O_5$ [M + H]$^+$ 442.1397, found 442.1398.

9-(1-Aminodibenzofuran-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]-pyrimidin-4-one (30). In the manner described for **26**, 9-(1-aminodibenzofuran-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one (**30**) was prepared from 9-(1-nitrodibenzofuran-4-yl)-2-morpholin-4-ylpyrido[1,2-*a*]pyrimidin-4-one **28** (300 mg, 0.68 mmol) and zinc powder (445 mg, 6.8 mmol) and was obtained as a white solid (269 mg, 96%): R_f = 0.32 (AcOEt); mp 294−295 °C; IR (cm^{-1}) 3340, 3224, 2937, 2872, 2258, 1697, 1637, 1623, 1543, 1493, 1440, 1373, 1309, 1258, 1234, 1191, 1150, 1109, 1073, 999, 909, 856, 776; ^{1}H NMR (300 MHz, MeOD) δ 3.36−3.38 (4H, m, 2 × CH_2N-morpholine), 3.52−3.55 (4H, m, 2 × CH_2O-morpholine), 6.72 (1H, d, J = 7.8 Hz, H-Ar), 6.88 (1H, dd, J = 7.6 and 7.5 Hz, H-Ar), 7.31−7.47 (5H, m, H-Ar), 7.90−7.92 (2H, m, H-Ar), 8.90 (1H, d, J = 7.5 Hz, H-Ar); ^{13}C NMR (75 MHz, MeOD) δ 46.2 (2 × CH_2N-morpholine), 68.0 (2 × CH_2O-morpholine), 110.4, 112.8, 114.6, 122.5, 124.5, 125.6, 127.5, 128.0, 131.8, 133.6, 138.9, 145.1, 150.8, 156.7, 156.9, 161.7, 162.1; LCMS m/z

413.19 ([M + H]$^+$). Anal. Calcd for 0.86 mol of $C_{24}H_{20}N_4O_3$ + 0.14 mol of MeOH: C, 69.48, H, 4.99, N, 13.41. Found: C, 69.22, H, 4.79, N, 13.38. HRMS calcd for $C_{24}H_{21}N_4O_3$ [M + H]$^+$ 413.1608, found 413.1609.

8-(1-Aminodibenzofuran-4-yl)-2-morpholin-4-yl-1*H*-quinolin-4-one (31). In the manner described for **26**, 8-(1-aminodibenzofuran-4-yl)-2-morpholin-4-yl-1*H*-quinolin-4-one (**31**) was prepared from 2-morpholin-4-yl-8-(1-nitrodibenzothiophen-4-yl)-1*H*-quinolin-4-one **29** (290 mg, 0.66 mmol) and zinc powder (430 mg, 6.58 mmol) and was obtained as a brown oil (246 mg, 91%): R_f = 0.44 (AcOEt); IR (cm^{-1}) 1708, 1572, 1374, 1278, 1190, 1116, 1049, 1010, 931, 880, 827, 748, 722, 688, 688; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11−3.14 (4H, m, 2 × CH_2N-morpholine), 3.58−3.60 (4H, m, 2 × CH_2O-morpholine), 5.98 (1H, s, H-3), 6.85 (1H, d, J = 8.1 Hz, H-Ar), 7.25−7.51 (3H, m, H-Ar), 7.52−7.68 (3H, m, H-Ar), 8.05 (1H, t, J = 8.1 Hz, H-Ar), 8.67 (1H, d, J = 7.8 Hz, H-Ar); ^{13}C NMR (75 MHz, MeOD) δ 48.0 (2 × CH_2N-morpholine), 67.4 (2 × CH_2O-morpholine), 111.9, 112.6, 122.9, 125.1, 125.6, 127.6, 127.9, 131.6, 132.7, 135.3, 146.2, 156.8; HRMS calcd for $C_{25}H_{22}N_3O_3$ [M + H]$^+$ 412.1656, found 412.1654.

Enzyme Inhibition Assay. Mammalian DNA-PK (500 ng/μL) was isolated from HeLa cell nuclear extract by Q-Sepharose, followed by S-Sepharose chromatography and a final step of heparin-agarose chromatography. DNA-PK (250 ng) activity was measured at 30 °C, in a final volume of 40 μL, in buffer containing 25 mM Hepes, pH 7.4, 12.5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 10% glycerol, 0.1% NP-40, and 1 μg of the substrate GST-p53N66 (the amino-terminal 66 amino acid residues of human wild-type p53 fused to glutathione *S*-transferase) in polypropylene 96-well plates. To the assay mix were added varying concentrations of inhibitor (in DMSO at a final concentration of 1%). After 10 min of incubation, ATP was added to give a final concentration of 50 μM along with a 30mer double-stranded DNA oligonucleotide (final concentration of 0.5 ng/mL) to initiate the reaction. After 1 h with shaking, 150 μL of phosphate-buffered saline (PBS) was added to the reaction and 5 μL was then transferred to a 96-well opaque white plate containing 45 μL of PBS per well, where the GST-p53N66 substrate was allowed to bind to the wells for 1 h at room temperature. To detect the phosphorylation event on the serine-15 residue of p53 elicited by DNA-PK, a rabbit phosphoserine-15 antibody (Cell Signaling Technology) was used in a basic ELISA procedure. An anti-rabbit HRP conjugated secondary antibody (Pierce) was then employed in the ELISA before the addition of chemiluminescence reagent (NEN Renaissance) to detect the signal as measured by chemiluminescent counting via a TopCount NXT (Packard). IC_{50} was derived from a sigmoidal plot using the graphic package Prism, in which the DNA-PK activity in the varying concentrations of compounds was plotted against the concentration of compound.

DMR Clonogenic Assay. Cells were seeded per well into a six-well tissue culture treated dish and incubated overnight at 37 °C/5% CO_2. Following a 1 h pretreatment with either vehicle or compound the plates were exposed to 2 Gy ionizing radiation using a Faxitron 43855D X-ray source and incubated overnight at 37 °C/5% CO_2. The medium was replaced with fresh medium in the absence of compound or vehicle and incubated for a further 6−8 days. The medium was removed, and the cell colonies were fixed and stained with Giemsa and scored with an automated colony counter (Oxford Optronics Ltd., Oxford, U.K.). The dose modification ratio (DMR) at 2 Gy irradiation was calculated as follows:

$$\mathrm{DMR} = \frac{\%\ \mathrm{survival}_{-\ \mathrm{compound}/+\ 2\ \mathrm{Gy}}}{\%\ \mathrm{survival}_{+\ \mathrm{compound}/+\ 2\ \mathrm{Gy}}}$$

Acknowledgment. We thank Adrian Moore for help with the purification of the libraries, and we thank Cancer Research UK for generous support. The EPSRC Mass Spectrometry Service at the University of Wales (Swansea, U.K.) is also gratefully acknowledged.

Supporting Information Available: Synthesis and analytical details for compounds **12**−**23** and solution-phase library synthesis and analytical details for inhibitors **32**−**83**. This material is available free of charge via the Internet at http://pubs.acs.org.

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