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8-Biarylchromen-4-one inhibitors of the DNA-dependent protein kinase (DNA-PK)

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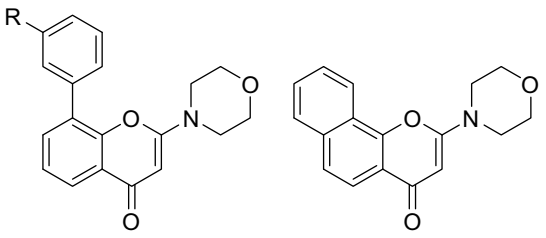
ABSTRACT

The synthesis and biological evaluation of libraries of 8-biarylchromen-4-ones enabled the elucidation of structure–activity relationships for inhibition of the DNA-dependent protein kinase (DNA-PK), with 8-(3-(thiophen-2-yl)phenyl)chromen-4-one and 8-(3-(thiophen-3-yl)phenyl)chromen-4-one being especially potent inhibitors.

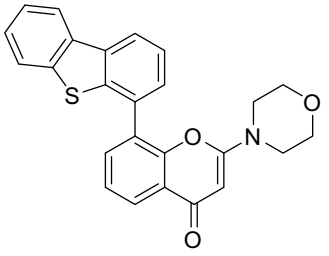
The cellular response to DNA double-strand break (DSB) formation is an essential component of normal cell survival, following exposure to DNA-damaging chemicals and ionising radiation.[1] The DNA-dependent protein kinase (DNA-PK), a member of the phosphatidylinositol (PI) 3-kinase-related kinase (PIKK) family, plays an important role in DNA DSB repair via the non-homologous end-joining (NHEJ) pathway.[2–4] The ability of DNA-PK to detect and signal the repair of DNA damage may also protect cancer cells from the cytotoxic effects of DNA-damaging cancer therapies. Accordingly, inhibition of DNA-PK has been demonstrated to potentiate the cytotoxicity of ionising radiation and a number of DSB-inducing antitumour agents in vitro.[5,6] A major objective of our research is the development of potent and selective DNA-PK inhibitors, suitable for clinical evaluation as chemo- and radio-sensitisers in the treatment of cancer.

In the absence of suitable structural biology information for DNA-PK, inhibitor design has been guided by a combination of homology modelling, utilising the known crystal structure of PI 3-kinase,[7] and pharmacophore mapping based on the non-selective DNA-PK inhibitor LY294002 (**1**).[8] These initial studies enabled the elucidation of structure–activity relationships (SARs) for DNA-PK inhibition, and the discovery of potent and selective chromenone inhibitors, exemplified by NU7026 (**2**).[9] Encouraged by the potency and kinase-selectivity of **2** and related compounds, a systematic variation of the substitution pattern on the chromenone



1; R = H
4; R = Ph

2



3

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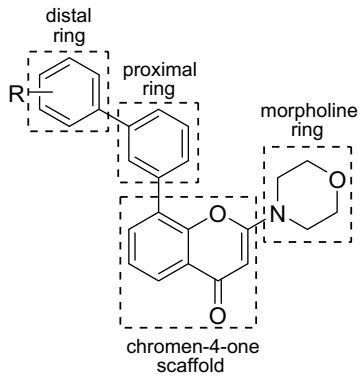


Figure 1. Structural components of 8-(biaryl-3-yl)chromen-4-one inhibitors of DNA-PK.

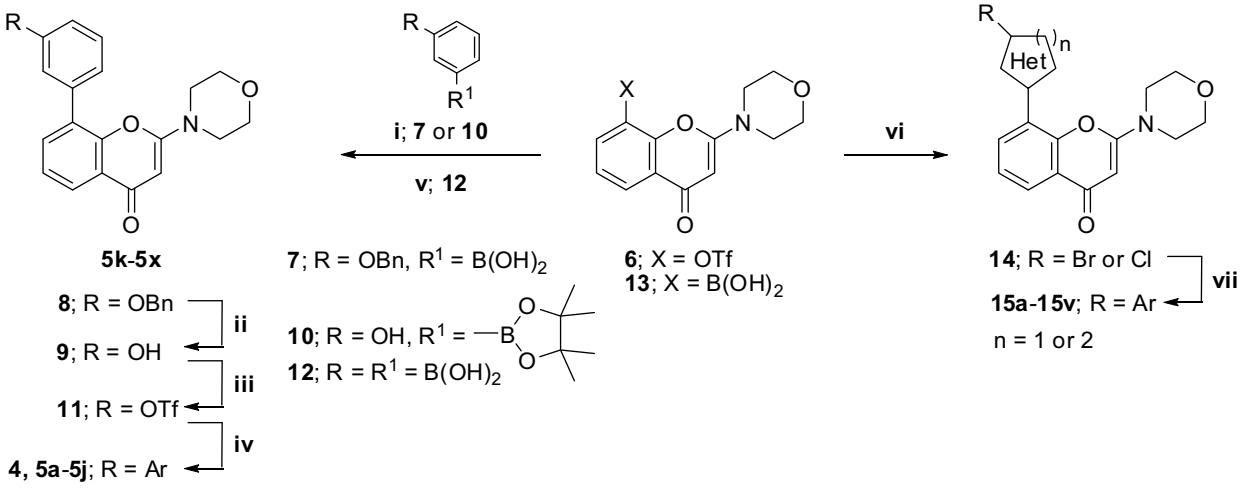
pharmacophore was undertaken, employing a solution-phase multiple-parallel synthesis approach for the preparation of focused compound libraries.[10,11] NU7441 (**3**) was prominent amongst several hits emanating from the library screen, with an independent resynthesis confirming the high potency (IC_{50} = 12 nM) and DNA-PK selectivity of this chromenone. Preclinical antitumour studies with **3** have demonstrated that this DNA-PK inhibitor potentiates the in vivo cytotoxicity of topoisomerase inhibitors in a human tumour model.[12]

Although less potent than NU7441 as a DNA-PK inhibitor, the 8-(biphenyl-3-yl)chromen-4-one derivative (**4**; IC_{50} = 180 nM) was also identified from the library as a potentially interesting structural lead. In particular, the non-planar biphenyl motif of **4** offered the opportunity to probe alternative regions of the ATP-binding domain of the kinase. In this letter, we report the results of studies designed to elucidate preliminary SARs for the 8-biarylchromenone pharmacophore varied with respect to three parameters as shown in Figure 1, namely the substituent R on the 3′-phenyl group, and the nature of the 'proximal' and 'distal' aryl groups. For this purpose, the core chromen-4-one scaffold and the 2-morpholin-4-yl substituent were retained. A solution-phase multiple-parallel synthesis approach was employed for the preparation of libraries of the required 8-biarylchromenones, several members of which were found to exhibit potency comparable with the benchmark DNA-PK inhibitor NU7441 (**3**).

Our previous studies have utilised the chromenone triflate derivative **6** as a key reagent for the preparation of 8-substituted chromenone libraries, employing Suzuki–Miyaura palladium-catalysed cross-coupling reactions.[9–11,13] This strategy was also amenable for the synthesis of the target substituted 8-biaryl-3-ylchromenones (**5**) through analogous reactions on the triflate building block **11**, which was readily accessible from **6** (Scheme 1). For ease of manipulation, and with a view to preparing **11** on a convenient scale, initial studies utilised 3-benzyloxyphenylboronic acid (**7**), which was available from 3-bromophenol by standard methods. Coupling of **7** with **6** gave the chromenone **8** in high yield, and subsequent removal of the benzyl protecting group by hydrogenation afforded the required phenol **9**.

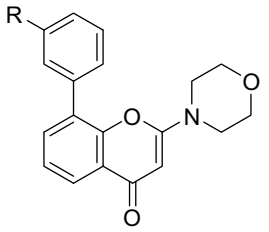
Consistent with the known tolerance of the Suzuki–Miyaura reaction to a wide range of aryl substituents,[14] we subsequently found that the commercially available 3-hydroxyphenylboronic acid pinacol ester (**10**) could be coupled to **6**, to give **9** directly and in comparable yield. Smooth conversion of **9** into **11** was readily achieved with the mild triflating agent *N*-phenyltriflimide. Prior to undertaking library synthesis, a coupling reaction was conducted between triflate (**11**) and phenylboronic acid under previously optimised reaction conditions ($Pd(PPh_3)_4$, K_2CO_3, dioxane, reflux). The resulting 8-biphenylchromenone (**4**) proved identical to that prepared previously by an alternative method.[11] Analogous cross-coupling reactions were conducted with **11** and 10 commercially available arylboronic acids in a GreenHouse™ reactor (Radleys) to furnish the target arylchromenones (**5a**–**5j**) (Scheme 1).

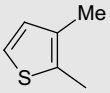
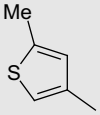
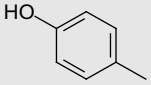
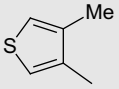
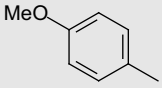
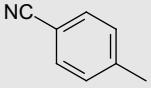
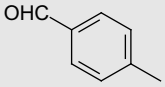
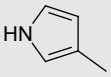
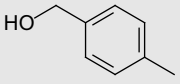
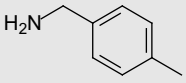
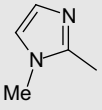
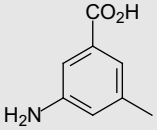
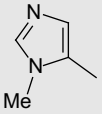
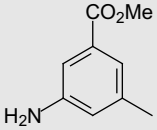
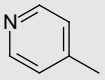
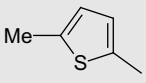
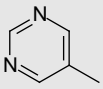
Given the commercial availability of 1,3-phenylene-bis-boronic acid (**12**), the possibility of undertaking a 'one-pot' double Suzuki–Miyaura coupling reaction with chromenone triflate (**6**) and the appropriate bromoheterocycle (ArBr) was also investigated.[15] Again, a model reaction conducted with **12**, the triflate **6** and bromobenzene under microwave conditions (5 min at 150 °C) confirmed the viability of the reaction, with **4** being obtained in good yield (60%). This approach was utilised for the preparation of compounds (**5k–5x**) as shown in Scheme 1. Replacement of the 8-phenyl substituent of **4** by a substituted thienyl, thiazolyl or pyridyl group was achieved by coupling either the chromenone triflate (**6**) or the chromenone-8-boronate (**13**) with the appropriate heterocyclic boronic acid or dihaloheterocycle, respectively, as



Scheme 1. Reagents and conditions: (i) (**6** and **7**) cat. $Pd(PPh_3)_4$, K_2CO_3, dioxane, reflux, 88%, (**6** and **10**) cat. $PdCl_2(dppf)$, Cs_2CO_3, THF, 90%; (ii) H_2, Pd/C, MeOH, 93%; (iii) $PhNTf_2$, Et_3N, THF, 77%; (iv) cat. $Pd(PPh_3)_4$, K_2CO_3, $ArB(OH)_2$, dioxane, reflux, 10–30%; (v) (**6** and **12**) ArBr, cat. $PdCl(PPh_3)_2$, Na_2CO_3, DME/H_2O/EtOH (7:3:2), microwave, 150 °C, 5 min, 50–70%; (vi) cat. $Pd(PPh_3)_4$, K_2CO_3, $ArB(OH)_2$ or aryl halide, DMF, 150 °C, 10 min, 40–90%; (vii) cat. $Pd(P^tBu_3)_2$, K_2CO_3, $ArB(OH)_2$, DMF, 150 °C, 5 min, 50–60%. [See Tables 1 and 2 for the nature of R in **5a**–**5x** and **15a**–**15v**, respectively.]

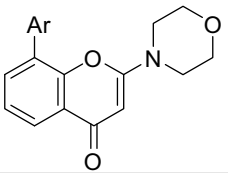
Table 1
Chemical structures and DNA-PK inhibitory activity of 2-morpholin-4-yl-8-phenyl-chromen-4-ones bearing a 3′-aryl substituent



Compound	R	IC_{50}[a] (μM)	Compound	R	IC_{50}[a] (μM)
1 (LY294002)	H	1.6	**5l**		0.36
4		0.18	**5m**		1.13
5a		0.14	**5n**		0.64
5b		0.83	**5o**		0.38
5c		1.48	**5p**		0.27
5d		1.10	**5q**		1.82
5e		1.0	**5r**		0.49
5f		1.9	**5s**		3.01
5g		0.51	**5t**		1.23
5h		0.26	**5u**		0.88
5i		0.018	**5v**		0.51
5j		0.02	**5w**		3.05
5k		1.07	**5x**		1.57

[a] IC_{50} values were determined in accordance with Ref. 9.

Table 2
Chemical structures and DNA-PK inhibitory activity of 2-morpholin-4-yl-chromen-4-ones bearing 8-heteroaryl substituents



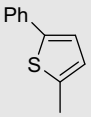
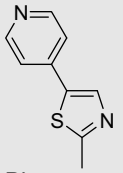
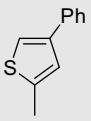
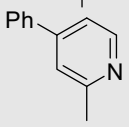
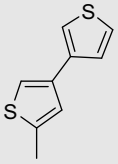
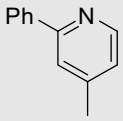
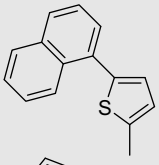
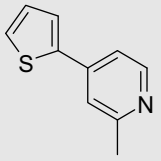
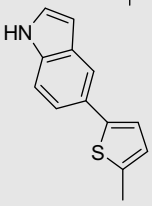
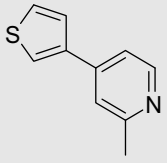
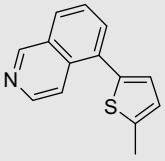
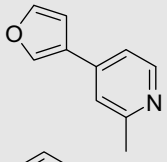
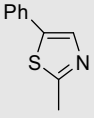
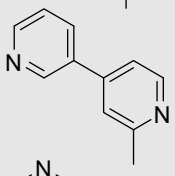
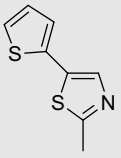
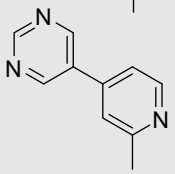
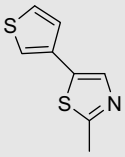
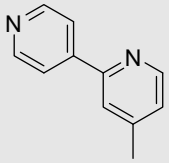
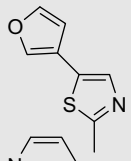
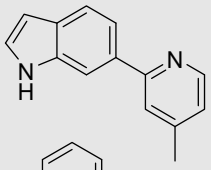
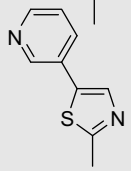
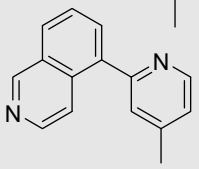
Compound	Ar	IC_{50}[a] (μM)	Compound	Ar	IC_{50}[a] (μM)
15a		0.40	**15l**		0.17
15b		0.09	**15m**		1.55
15c		0.09	**15n**		0.67
15d		2.46	**15o**		1.26
15e		0.34	**15p**		0.30
15f		0.71	**15q**		1.24
15g		0.37	**15r**		2.37
15h		0.48	**15s**		1.41
15i		0.24	**15t**		1.75

Table 2 (*continued*)

Compound	Ar	IC_{50}[a] (μM)	Compound	Ar	IC_{50}[a] (μM)
15j		0.24	**15u**		0.61
15k		0.51	**15v**		4.45

[a] IC_{50} values were determined in accordance with Ref. 9.

shown in Scheme 1. Subsequent Suzuki–Miyaura arylation of the intermediate **14** with a range of arylboronic acids afforded the target heterobiaryl-3-ylchromenones (**15a**–**15v**). In all cases, reaction progress was monitored by LC–MS analysis and the products were purified by semi-preparative HPLC. The DNA-PK inhibitory activity of both compound libraries is summarised in Tables 1 and 2.

Results and discussion. Our ongoing programme to develop inhibitors of DNA damage-activated kinases as radio- and chemopotentiators in cancer therapy has resulted in the identification of a number of potent and kinase-selective inhibitors of DNA-PK, most notably NU7441 (**3**). That significant inhibitory activity also resides in the simple 8-biphenylchromenone (**4**) was surprising, in light of our previous structure–activity studies indicating that an extended planar aromatic system at the chromenone 8-position is a prerequisite for potent DNA-PK inhibition. Previous studies had also demonstrated that chromenones bearing a biphenyl-3-yl group (e.g., **4**) were more active than the corresponding biphenyl-2-yl and biphenyl-4-yl isomers.[11] This is consistent with the likely disposition of the 3-phenyl group of **4** relative to the terminal aryl ring of the dibenzothiophen-4-yl substituent in **3** within the ATP-binding pocket. The fact that **4** is approximately 10-fold more potent than the parent 8-phenylchromenone LY294002 (**1**, IC_{50} = 1.6 μM) strongly suggests that the 3-phenyl substituent of **4** is making additional binding interactions within the ATP-binding domain of DNA-PK. The overall objective of this study was thus to probe this putative binding interaction further, with a view to delineating SARs and improving potency.

For the small series of 4-substituted biphenyl-3-ylchromenones evaluated (**5a**–**5f**), it is evident that substitution did not improve activity, and with the exception of the 4-hydroxy derivative (**5a**), which proved to be equipotent with the biphenyl-3-ylchromenone (**4**), a 5- to 10-fold reduction in potency was observed (Table 1). A modest improvement in activity over compounds **5b**–**5f** was observed for the 3,5-disubstituted derivatives (**5g** and **5h**), but both compounds were less potent than **4**. By contrast, replacement of the 3-phenyl group of **4** by an isosteric thiophen-2-yl (**5i**) or a thiophen-3-yl (**5j**) substituent improved DNA-PK inhibitory activity approximately 10-fold, with **5i** and **5j** exhibiting IC_{50} values of 18 nM and 20 nM, respectively. Interestingly, the introduction of a methyl group onto the thiophene ring (**5k**–**5n**) proved detrimental to inhibitory activity, with a 50-fold reduction in potency being observed for derivatives bearing a methyl group *ortho* to the heteroatom (**5k** and **5m**). This is consistent with the evidence adduced previously indicating limited steric tolerance at this position. The high potency exhibited by the thiophene derivatives **5i** and **5j** implies that a small electron-rich aryl ring is a prerequisite for DNA-PK inhibitory activity, and this is supported by the activity of phenol **5a**. However, although the reduced potency of the corresponding 3-furanylchromenones (**5o** and **5p**), the thiazole (**5r**) and the imidazole derivatives (**5s** and **5t**) is consistent with this proposal, the weak activity of the analogous pyrrole derivative (**5q**) was unexpected. Perhaps not surprisingly, replacement of the pendant phenyl ring of **4** by a pyridyl or pyrimidinyl heterocycle (**5u**–**5x**) resulted in a loss of potency.

The activity of chromenones bearing heteroaryl groups at the 8-position is summarised in Table 2. Replacement of the 8-phenyl substituent of **4** by a thiophen-2-yl group (**15a** and **15b**) did not improve DNA-PK inhibitory activity, although the 4-phenylthiophen-2-yl derivative (**15b**), together with the bithiophene analogue (**15c**), proved the most potent member of this series (IC_{50} = 90 nM). With the exception of the indolyl derivative (**15e**), larger heterocyclic groups on the thiophene ring were detrimental to potency, and where direct comparisons were possible (**15a** with **15g**, and **15c** with **15i**), replacement of thiophen-2-yl by thiazol-2-yl was not beneficial. Substitution of the 8-phenyl ring of **4** by a 2- or 4-pyridyl group (**15m** and **15n**) resulted in a reduction in potency, and derivatives bearing other heterocycles on the pyridyl ring (**15o–15v**) were all also less active.

In summary, we have identified a novel series of 8-biarylchromen-4-ones as inhibitors of DNA-PK that exhibit a range of potencies against the isolated enzyme. Notably, 8-(3-(thiophen-2-yl)phenyl)chromenone (**5i**), the most potent of these inhibitors, was also found to potentiate the cell killing of 2 Gy of ionising radiation by a factor of 1.6 when used at concentration of 500 nM in a Hela cervical carcinoma cell-based assay.[12] This demonstrates that **5i** is cell permeable, and that cellular inhibition of DNA-PK is achievable with this compound at pharmacologically relevant concentrations. Overall, the studies described in this letter have further elucidated an understanding of SARs for DNA-PK inhibition, and will provide a platform for ongoing efforts to optimise potency and in vitro/in vivo activity for this chemotype.

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