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Mapping the ATP-binding domain of DNA-dependent protein kinase (DNA-PK) with coumarin- and isocoumarin-derived inhibitors

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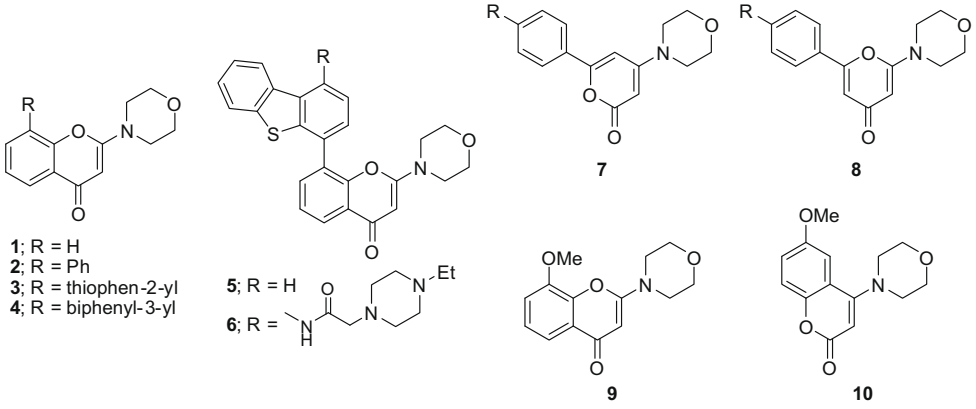
ABSTRACT

Replacement of the core heterocycle of a defined series of chromen-4-one DNA-PK inhibitors by the isomeric chromen-2-one (coumarin) and isochromen-1-one (isocoumarin) scaffolds was investigated. Structure–activity relationships for DNA-PK inhibition were broadly consistent, albeit with a reduction of potency compared with the parent chromenone.



Detection and repair of the numerous, and potentially lethal, DNA lesions arising in human cells daily is largely mediated by an efficient system collectively termed the DNA-damage response (DDR).[1] Paradoxically, DNA-repair processes within cancer cells also constitute a mechanism of resistance to DNA-damaging anticancer therapies, and agents that impede DNA repair are thus of potential therapeutic interest as chemo- and radio-sensitising agents in the treatment of cancer.[2,3] Perhaps more interestingly, DDR defects are a common feature of cancer and the 'mutator-phenotype' associated with the resultant genomic instability may offer a survival advantage over normal tissues, albeit that tumour dependency on a remaining DNA-repair pathway can result. This offers the exciting opportunity of achieving tumour selectivity through pharmacological inhibition of an essential DNA repair pathway, as elegantly demonstrated recently by the synthetic lethality achieved with poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors in BRCA1 and BRCA2 deficient tumours (reviewed in Refs. 1 and 3).

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The phosphatidylinositol-3-kinase related kinase (PIKK) family member DNA-dependent protein kinase (DNA-PK) plays a key role in the DDR, via the non-homologous end-joining pathway of DNA double-strand break (DSB) repair.[4] Importantly, inhibition of this kinase has been demonstrated to potentiate the cytotoxicity of DNA DSB-inducing anticancer therapies,[5] and there is evidence that DNA-PK is over-expressed in a number of tumours. The development of clinically useful ATP-competitive DNA-PK inhibitors is a major goal of our research. In the absence of suitable structural information for DNA-PK, we have conducted structure-activity relationship (SAR) studies around the 2-morpholino-4*H*-chromen-4-one pharmacophore (**1**) from which the non-selective PIKK inhibitor LY294002 (**2**) was derived.[6] These studies resulted in the identification of a number of interesting DNA-PK inhibitors, including the thiophen-2-yl (**3**)[7] and 8-biphenyl derivatives (**4**),[8] as well as NU7441 (**5**; IC_{50} = 30 nM),[7,9] elaboration of which has led to the highly potent water-soluble DNA-PK inhibitor KU-0060648 (**6**; IC_{50} = 5 nM).[10] Importantly, preclinical proof-of-principle studies with **5** and **6** have demonstrated activity in vitro as chemo- and radio-potentiators in a range of human tumour cell lines, and initial in vivo investigations with these agents have been promising.[9,10]

With a view to identifying new DNA-PK inhibitors, we have investigated alternative heterocyclic scaffolds, and have previously demonstrated that sub-micromolar DNA-PK inhibitory activity also resides in suitably substituted pyran-2-ones (**7**) and pyran-4-ones (**8**).[11,12] These studies imply that the ring oxygen of chromenone- and pyranone-based DNA-PK inhibitors does not contribute directly to inhibitor binding. By contrast, the morpholin-4-yl and carbonyl oxygen functions are thought to make key hydrogen bond interactions within the ATP-binding domain, with the pendant aryl substituent occupying a putative hydrophobic pocket. This '3-point binding' interaction is thought to determine the overall orientation and positioning of the inhibitor. Accordingly, a superimposition of the chromenone of **2** with the isomeric coumarin and isocoumarin heterocyclic systems provided an opportunity to investigate a scaffold-hopping strategy, through the introduction of aryl substituents at the coumarin 6- or 7-positions and the isocoumarin 5-position (Figure 1). This was supported by a previous observation that 6-methoxycoumarin (**10**) is approximately equipotent with the isomeric 8-methoxychromenone (**9**) (DNA-PK, IC_{50} values of 1.8 μM and 1.2 μM, respectively).[7] Given the high potency of **5** and **6**, it was also of interest to examine the impact on activity of the subtle structural changes imposed by moving the ring oxygen atoms.

In this Letter, we describe the synthesis and DNA-PK-inhibitory activity of a defined series of coumarins and isocoumarins, and demonstrate SARs that generally parallel those observed for the corresponding 8-substituted 2-morpholin-4-yl-chromen-4-ones. The surprising loss of activity for the coumarins and isocoumarins is discussed in terms of conformational effects.

The target 6- and 7-substituted coumarins **16**–**27** were readily prepared as summarised in Scheme 1. Thus, thermal condensation of commercially available 5-substituted-2-hydroxyacetophenones (**11a–11c**) with diethyl carbonate afforded the corresponding 6-substituted-4-hydroxycoumarins (**13a**–**13c**) in high yield. 4-Bromo-2-hydroxyacetophenone (**11d**) was prepared in good overall yield from 3-bromophenol by sequential O-acetylation–Fries

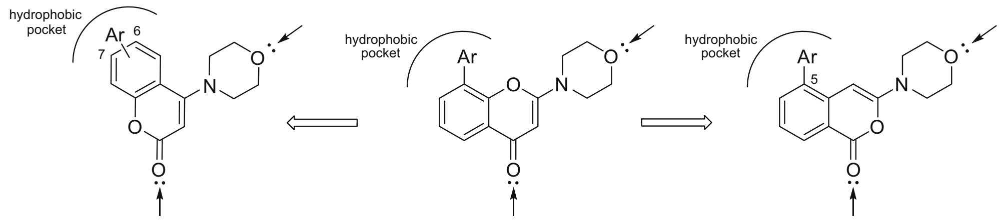
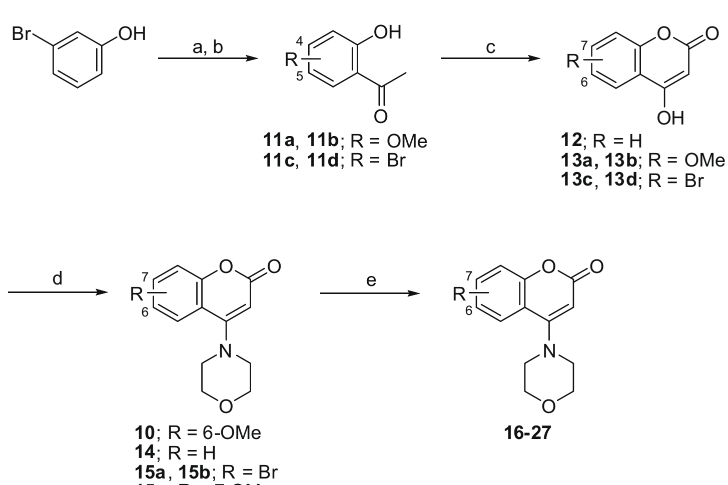


Figure 1. Comparison of the putative relative binding orientations of the coumarin, chromenone and isocoumarin core templates within the ATP-binding domain of DNA-PK (→ = hydrogen bond interaction).



Scheme 1. Reagents and conditions: (a) Ac_2O, H_2SO_4, 25 °C, 1 h; (b) $AlCl_3$, 170 °C, 3 h, 78–80%; (c) Na, $CO(OEt)_2$; Dowtherm A, 160 °C, 2 h, 50–60%; (d) triisopropylbenzenesulfonyl chloride, Et_3N, THF, 120 °C, 10 min, MW, followed by morpholine, 25 °C, 18 h, 50–70%; (e) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, reflux, 18 h, 40–70%.

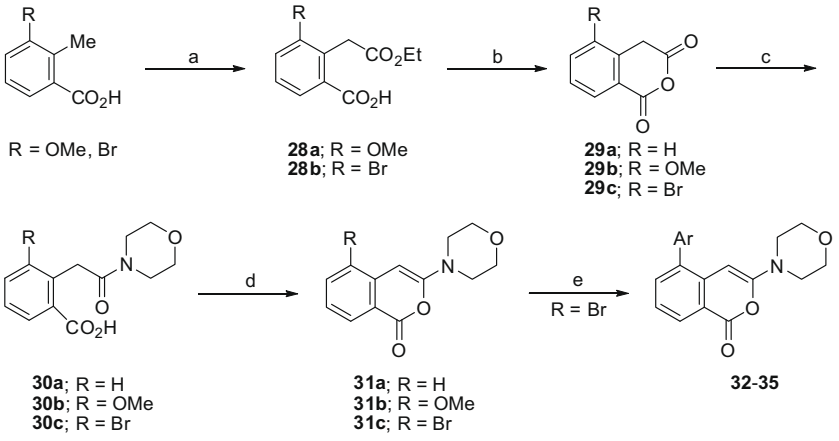
rearrangement,[13] with subsequent conversion into the require 7-bromocoumarin (**13d**) proceeding smoothly. Introduction of the 4-morpholinyl function was achieved by a previously optimised one-pot procedure entailing activation of the phenol as the tri-isopropylbenzenesulfonyl ester, followed by displacement with morpholine, to give coumarins **10**, and **15a**–**15c** in excellent yields.[7] The parent 4-morpholinylcoumarin (**14**) was similarly prepared from 4-hydroxycoumarin (**12**). Final Suzuki–Miyaura coupling of bromocoumarins **15a** and **15b** with the appropriate arylboronic acids under standard conditions,[14] gave the target 6- and 7-arylcoumarins (**16–27**).

The synthetic route employed for the preparation of the isocoumarins is shown in Scheme 2. Commercially available 3-methoxy- and 3-bromo-2-methylbenzoic acids afforded the corresponding ethyl homophthalates (**28a** and **28b**), respectively, on treatment with LDA-diethyl carbonate, and subsequent acid-catalysed cyclisation to the desired 5-bromohomophthalic anhydrides (**29b** and **29c**) proceeded in high yield.[15] The use of a hindered base (LDA) at −50 °C avoided unwanted halogen-metal exchange reactions involving the bromo group.[16] Reaction with morpholine, and conversion of the resulting 2-(2-morpholin-4-yl-2-oxoethyl)benzoic acids (**30b** and **30c**) into the key 5-substituted-3-morpholin-4-yl-isochromen-1-one (**31b** and **31c**), proceeded smoothly in good overall yield. The parent isocoumarin (**31a**) was readily prepared from commercially available homophthalic anhydride (**29a**) under essentially identical conditions. Final palladium-catalysed cross-coupling of **31c** with arylboronic acids was achieved under standard conditions ($Pd[PPh_3]_4$, Cs_2CO_3, dioxane), to afford the target 5-aryl-3-morpholin-4-yl-isochromen-1-ones (**32**–**35**) in good overall yields (Scheme 2). Although these reactions proved sluggish under conventional reflux conditions, the use of microwave (MW) heating dramatically shortened reaction times, with complete consumption of **31c** occurring within 40–90 min.

The overall objective of this study was to investigate the effect upon DNA-PK inhibitory activity of replacing the core chromenone heterocycle by coumarin and isocoumarin templates, whilst retaining the relative positioning of key groups, notably a ring carbonyl and a morpholinyl substituent. Our previous SAR studies have also highlighted the importance of an appropriate 8-aryl substituent for chromenone-based inhibitors, and the nature and position of aryl groups on the coumarins and isocoumarins was directed by these observations. Methoxy or phenyl group substitution on the coumarin and isocoumarin rings, at positions analogous to the chromenone 8-position, afforded compounds with comparable DNA-PK inhibitory activity (compare **9** with **10**, **15c**, and **31b** and **2** with **16**, **22** and **32**), consistent with similar binding orientations. Interestingly, this was not the case for the parent heterocycles, and while the coumarin (**14**) exhibited low micromolar potency, the corresponding chromenone (**1**) and isochromenone (**31a**) derivatives were essentially devoid of activity.

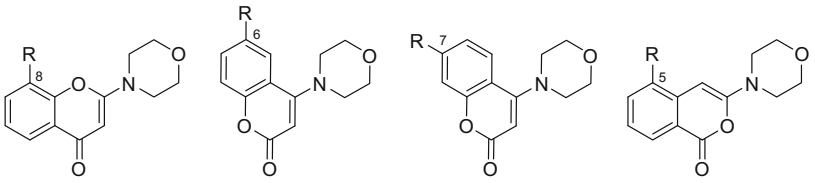
Introduction of a 2-thienyl group at the coumarin 7-position (**23**) conferred sub-micromolar potency comparable with that of the chromenone (**3**), whereas the corresponding 6-substituted coumarin (**17**) was slightly less potent. However, the most dramatic differences in potency were observed for larger aryl substituents. Thus, the 6-(3-phenylphenyl)coumarin (**18**) proved some 10-fold less potent than the analogous chromen-4-one (**4**), with the 7-substituted coumarin (**24**) proving inactive. These difference in potency were even more pronounced for the benzothiophen-2-yl, dibenzothiophen-1-yl and dibenzofuran-1-yl analogues, and while the 7-substituted coumarins **26** and **27** were 70-fold less potent than **5**, the other derivatives (**19–21**, **25**) were inactive. The proposed binding mode for the 6-substituted coumarin scaffold (B) positions the fused phenyl ring within the putative hydrophobic pocket occupied by substituents at the chromenone 8-position (A) and the isocoumarin 5-position (D). As such, the loss of activity observed for larger groups at the coumarin 6-position (e.g., **20** and **21**) may reflect steric constraints within the hydrophobic pocket. Interestingly, the isocoumarin series proved more tolerant to substitution at the 5-position, with only a fourfold loss of activity being observed for the 5-(3-phenylphenyl)isocoumarin (**33**) compared with **4**. Similarly, isocoumarins bearing a dibenzothiophen-1-yl or dibenzofuran-1-yl group at the 5-position (**34** and **35**) retained very reasonable DNA-PK inhibitory activity (IC_{50} ~300 nM) compared with the parent chromenone (**5**).

The accentuation of the difference in potency between the chromenones (e.g., **5**) and isocoumarins (e.g., **34**) with a larger substituent at the 8-position (in **5**) or 5-position (in **34**) could also be due to the effect of the 4-H atom in the isochromenones (vs O in the chromenones) on the preferred conformation about the σ-bond connecting the substituent to the other heteroaromatic system. This suggestion is consistent with the relative poor activity of structural classes B and C (Table 1), which are conformationally constrained at the C–C bond corresponding to that connecting the two (hetero)aromatic systems of A and D. Current studies are exploring the influence of such conformational factors on the stereochemistry and activities of chromen-4-ones and isochromen-1-ones.



Scheme 2. Reagents and conditions: (a) (i) LDA, THF, −50 °C, 63%; (ii) $ClCO_2Et$; (b) CSA, toluene, reflux, 85%; (c) morpholine, toluene, 80 °C, 70–90%; (d) $ClCO_2Et$, Et_3N, toluene, −78 °C, 65–80%; (e) K_2CO_3, $Pd(PPh_3)_4$, dioxane, $ArB(OH)_2$, MW 175 °C, 40–90 min, 35–80%.

Table 1
DNA-PK inhibitory activity of chromenones, coumarins, and isocoumarins



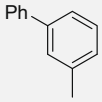
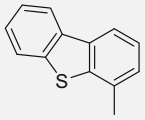
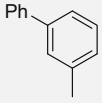
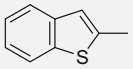
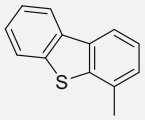
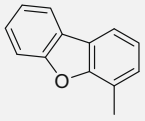
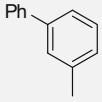
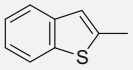
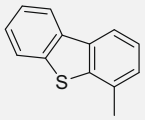
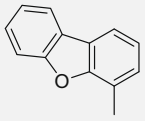
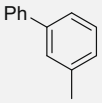
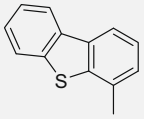
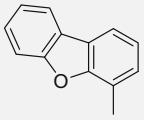
Compd	Structure	R	IC_{50}[a] (μM)
1	A	H	>10
2	A	Ph	1.6[b]
3	A		0.7[c]
4	A		0.18[b]
5	A		0.03
9	A	MeO	1.8
14	B/C	H	4.8
10	B	MeO	1.7
16	B	Ph	1.8
17	B		1.2
18	B		1.6
19	B		>10
20	B		>10
21	B		>10
15c	C	OMe	5.4
22	C	Ph	1.7
23	C		0.6
24	C		>10
25	C		>10
26	C		2.1
27	C		2.1

Table 1 (*continued*)

Compd	Structure	R	IC_{50}[a] (μM)
31a	D	H	>10
31b	D	MeO	3.2
32	D	Ph	2.6
33	D		0.68
34	D		0.34
35	D		0.33

[a] IC_{50} values were determined in accordance with Ref. 7.
[b] Ref. 7.
[c] Ref. 8.

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