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Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries

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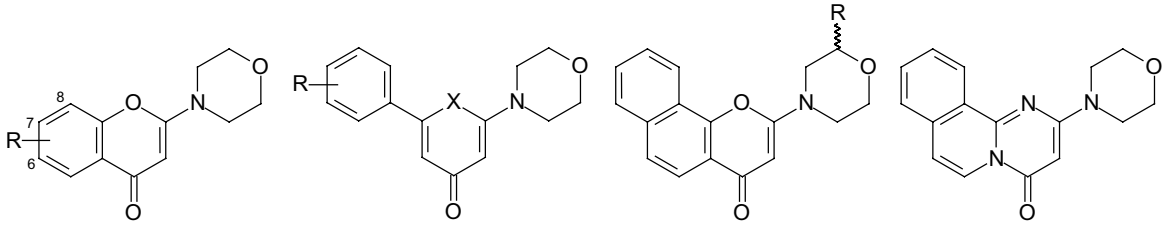
Abstract—A solution-phase multiple-parallel synthesis approach was employed for the preparation of 6-, 7- and 8-aryl-substituted chromenone libraries, which were screened as inhibitors of the DNA repair enzyme DNA-dependent protein kinase (DNA-PK). These studies resulted in the identification of 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (NU7441) as a highly potent and selective DNA-PK inhibitor (IC_{50} = 14 nM), exhibiting ATP-competitive inhibition kinetics.

1. Introduction

The ability of cancer cells to repair DNA damage is an important determinant of their susceptibility to chemo- or radio-therapy in the treatment of cancer.[1] The DNA-dependent protein kinase (DNA-PK) plays an important role in the detection and repair of DNA double-strand breaks (DSBs) via the non-homologous end-joining (NHEJ) pathway.[2] DNA-PK is a member of the phosphatidylinositol (PI) 3-kinase related kinase (PIKK) family involved in the signalling of cellular stress responses, which also includes the mammalian proteins ATM, ATR, mTOR and hSMG1.[3] The intact enzyme apparatus comprises a catalytic unit (DNA-PKcs) and a heterodimeric regulatory factor (Ku70/Ku80). Detection and binding of Ku to DNA DSBs is thought to precede recruitment of DNA-PKcs to generate the active serine/threonine kinase. Once bound to the site of a DNA DSB, DNA-PK is believed to act as a scaffold for the other components of the NHEJ pathway. The kinase activity of DNA-PK appears essential for DNA DSB repair, as DNA-PK defective cell lines



1; R = 8-Ph
7; R = H

2; X = O
3; X = S

4; R = H
5; R = Me

6

Keywords: Chromenones; DNA repair; DNA-PK; Inhibitors.
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are hypersensitive to DNA-damaging agents.[2,4] Importantly, inhibition of DNA-PK has been demonstrated to elicit chemo- and radio-sensitization, and to potentiate the cytotoxicity of ionizing radiation and a number of DSB-inducing agents in vitro.[5,6]

Our studies have centred on the development of potent and selective DNA-PK inhibitors, suitable for clinical evaluation as agents to enhance the cytotoxicity of DNA-damaging anticancer therapies. We have previously utilized the chromen-4-one LY294002 (**1**), a non-specific ATP-competitive PIKK inhibitor (IC_{50} for DNA-PK = 1.2 μM),[7,10] as a template for DNA-PK inhibitor design, and have identified novel pyranone (**2**), thiopyranone (**3**), chromenone (**4**, **5**) and pyrimidoisoquinolone (**6**) pharmacophores.[8–10] A number of these inhibitors, including NU7026 (**4**; IC_{50} = 0.23 μM) and NU7163 (**5**; IC_{50} = 0.19 μM), exhibit good selectivity for DNA-PK over other PIKK family members, and have been demonstrated to act as radio- and chemo-sensitizers in several human tumour cell lines in vitro.[11,12]

With the objective of improving activity further, we wished to investigate the introduction of functionality at the 6-, 7-, and 8-positions on the chromenone template (**7**), as the ATP-binding domain of DNA-PK had previously been found[10] to tolerate substitution at these positions. In this paper we describe the use of a solution-phase multiple-parallel synthesis approach for the preparation of 6-, 7-, and 8-aryl-substituted chromenone libraries, and the identification of NU7441 as a highly potent and selective DNA-PK inhibitor.

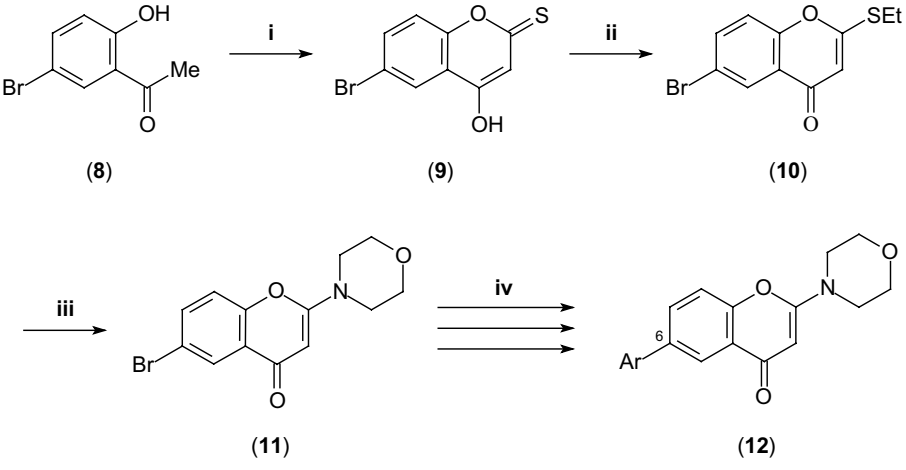
2. Chemical synthesis

The required chromen-4-one libraries were prepared by palladium catalyzed cross-coupling (Suzuki–Miyaura) reactions[13,14] with suitably functionalized chromen-4-one derivatives. 6-Bromochromen-4-one (**11**) was readily prepared from commercially available 5-bromo-2-hydroxyacetophenone (**8**) by a modification of the method of Vlahos et al. (Scheme 1).[15] Thus, cyclocondensation of **8** with CS_2 gave the chromene-2-thione (**9**), with subsequent conversion into the *S*-ethyl derivative (**10**) enabling direct introduction of the 2-morpholine substituent to furnish **11** in good yield. The corresponding 7- and 8-bromo-2-hydroxyacetophenenones were not readily accessible, and an alternative approach utilizing the 7- and 8-chromenone triflates (**19**, **20**) was employed, as shown in Scheme 2. Dihydroxybenzoate esters (**13**, **14**), prepared from the corresponding phenols by standard carboxylation–esterification methods, were converted into the monotriflate esters (**15**, **16**) on treatment with triflic anhydride under basic conditions. The regioselectivity of the sulfonation of esters (**13**, **14**) is presumably due to deactivation of the 2-hydroxyl function by the neighbouring carbomethoxy group.

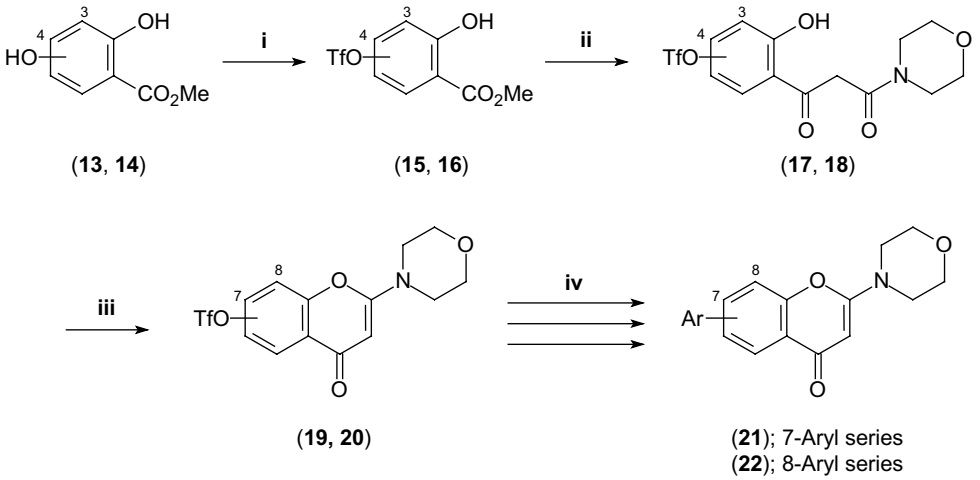
Reaction with *N*-acetylmorpholine gave the β-ketoamides (**17**, **18**), and ring closure to the required 7-, and 8-chromen-4-one triflate esters (**19**, **20**) occurred smoothly on treatment with triflic anhydride.[16] The 6-, 7-, and 8-aryl-substituted chromen-4-one libraries (**12**, **21**, **22**) were synthesized by Suzuki–Miyaura reactions of the 6-bromochromenone (**11**) and the 7- and 8-chromenone triflates (**19**, **20**), with 64 commercially available arylboronic acids of diverse structure.[17] Reactions were conducted in a solution-phase multiple-parallel format using a Greenhouse™ reactor station (Radleys). The reaction progress was monitored by LC–MS analysis, and product purification achieved by semi-preparative HPLC.

3. Results and discussion

Our previous studies have demonstrated that the chromen-4-one template (**7**) can serve as a versatile platform for the design of selective DNA-PK inhibitors, and structure–activity relationships are beginning to emerge for this pharmacophore (Fig. 1).[8–10] A core six-membered heterocyclic ring bearing a 4-carbonyl or thiocar-



Scheme 1. Reagents and conditions: (i) CS_2, KO^{t}Bu, toluene, 25 °C, 40–50%; (ii) EtI, K_2CO_3, acetone, reflux, 64%; (iii) morpholine, $HOCH_2CH_2OH$, 120 °C, 63%; (iv) cat. $Pd(PPh_3)_4$, K_2CO_3, $ArB(OH)_2$, dioxane, 90 °C, 10–30%.



Scheme 2. Reagents and conditions: (i) pyridine, cat. DMAP, Tf_2O, DCM, 0 °C, 37–40%; (ii) *N*-acetylmorpholine, LDA, THF, −78 → 25 °C, 35–40%; (iii) Tf_2O, DCM, 0 °C, 16 h, 41%; (iv) cat. $Pd(PPh_3)_4$, K_2CO_3, $ArB(OH)_2$, dioxane, 90 °C, 10–30%.

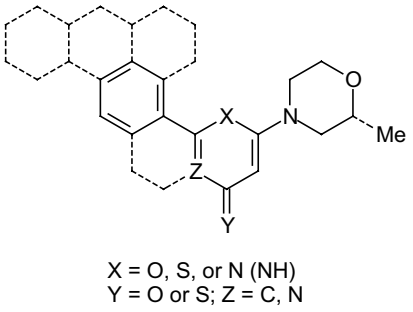


Figure 1. Putative structure–activity relationships for inhibition of DNA-PK by chromen-4-one derivatives. The minimum pharmacophore is shown in bold, with dotted lines indicating regions tolerant to structural modification. For a detailed discussion see Ref. 10.

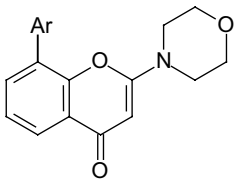
bonyl group and a 2-morpholinyl substituent, appear to be a prerequisite for significant inhibitory activity. Elaboration to a chromen-4-one or pyrimidoisoquinolone is clearly favourable, as witnessed by the activities of **4** and **6**, and alkyl group substitution at the morpholine 2-position as for **5** is tolerated. Notably, the 'north–west' position of the pharmacophore was found to be very tolerant to aromatic group substitution, and we wished to probe this region of the ATP-binding pocket further, with the objective of improving potency while retaining selectivity for DNA-PK over other PIKK family enzymes.

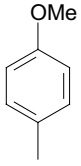
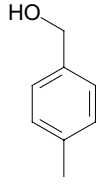
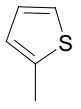
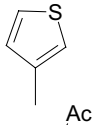
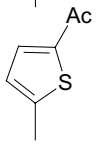
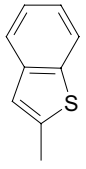
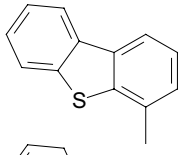
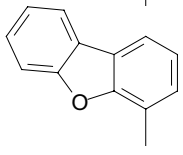
Three focused libraries (**12**, **21** and **22**) totalling 152 aryl-substituted chromen-4-ones (48, 54 and 50 compounds, respectively) were successfully synthesized from **11**, **19** and **20**. Reactions were not optimized, and products were generally isolated in modest yields. However, with the exception of a small number of compounds that proved inseparable from palladium- and triphenylphosphine-derived contaminants, product isolation was readily achieved by semi-preparative HPLC. Thus, 136 compounds satisfied the minimum purity criteria required ($\geqslant$85%) for biological evaluation, and were pre-screened for DNA-PK inhibitory activity at an initial concentration of 0.5 μM.[18] Interestingly, only modest inhibitory activity (~40–60% inhibition) was observed at this concentration for members of the 6- and 7-aryl-substituted chromen-4-one libraries (**12** and **21**), with no compounds proving more active than the parent 2-morpholinylchromen-4-one (**7**, IC_{50} = 1.3 μM). By contrast, eight members of the 8-aryl-substituted chromen-4-one library (**22a**–**h**) were found to exhibit promising activity at 0.5 μM, and IC_{50} values were determined for these hits (Table 1).

Four of the library members (**22a**–**c**,**f**) were of comparable potency with IC_{50} values of 0.6–0.9 μM, while the 2-thienyl derivative (**22d**) was some twofold more potent (IC_{50} = 0.3 μM). However, the structurally similar dibenzothiophene-1-yl and dibenzofuran-1-yl derivatives (**22g** and **22h**) were found to be extremely potent DNA-PK inhibitors, giving IC_{50} values of approximately 20 and 40 nM, respectively. Confirmation of this activity was achieved following the independent synthesis, purification and full characterization of each compound,[19] with respective IC_{50} values of 14±1 and 41±9 nM being observed against DNA-PK for **22g** and **22h**. ATP-competitive inhibition kinetics were established for the more potent dibenzothiophen-1-yl derivative **22g**, which was assigned the house number NU7441. Crucially, NU7441 (**22g**) retained excellent selectivity for DNA-PK over other PIKK family members compared with NU7026 (**4**), with only a modest increase in potency being observed against PI 3-K and mTOR, and no significant activity against ATM or ATR (Table 2). The high DNA-PK selectivity of **22g** was also confirmed against a commercial panel of 60 diverse kinases, with no inhibitory activity being observed at an inhibitor concentration of 10 μM (data not shown).

In summary, the synthesis and screening of focused chromen-4-one libraries has resulted in the identification of NU7441 (**22g**) as a highly potent and selective inhibitor of DNA-PK. In addition to the potential therapeutic value of NU7741 as an adjunct to the chemo- and radio-therapy of cancer, a potent and selective small

Table 1. 8-Aryl-substituted chromen-4-one library members (**22**) selected from the DNA-PK inhibition pre-screen[a]



Compound number	Ar	IC_{50} (μM)[b]
22a		0.9
22b		0.6
22c		0.7
22d		0.3
22e		0.8
22f		0.8
22g		0.02
22h		0.04

[a] Library compounds were pre-screened against DNA-PK at 0.5 μM concentration.
[b] IC_{50} values were determined in accordance with Ref. 18.

molecule DNA-PK inhibitor will represent a useful tool for elucidating the role of DNA-PK in the signalling and modulation of DNA DSBs. Preliminary studies have demonstrated that NU7441 effectively sensitizes a human tumour cell line (HeLa) to the cytotoxic effects of both ionizing radiation and the topoisomerase II inhibitor etoposide at submicromolar concentrations,[20]

Table 2. Inhibition of PIKK family kinases by NU7441 (**22g**), NU7026 (**4**) and LY294002 (**1**)[a]

Compound	Kinase inhibition (IC_{50} μM)				
	DNA-PK[a]	PI 3-K[b]	ATM[c]	ATR[d]	mTOR[e]
NU7441 (**22g**)	0.014	5.0	>100	>100	1.7
NU7026 (**4**)	0.23	13	>100	>100	6.4
LY294002 (**1**)	1.2	2.3	>100	>100	2.5

[a] IC_{50} values were determined in accordance with Ref. 18. See Ref. 11 for details of the other assays procedures.
[b] P110α.
[c] Mutated in ataxia telangiectasia protein.
[d] Ataxia telangiectasia related protein.
[e] Mammalian target of rapamycin.

and the results of these studies will be published subsequently.

Acknowledgements

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17. A general procedure for the MPS of aryl-substituted chromen-4-ones is as follows: a mixture of the bromochromenone (**11**) or chromenone triflate (**19**, **20**) (20 mg, 0.053 mmol), the appropriate arylboronic acid (0.06 mmol), and powdered potassium carbonate (14.6 mg, 0.106 mmol) was purged with nitrogen, and dioxane (0.5 mL, degassed by sonication–purging with nitrogen) was added. A solution of tetrakis(triphenylphosphine)palladium(0) (3.1 mg) in degassed dioxane (0.3 mL) was added, and the mixture was stirred under nitrogen for 18 h at 90 °C. The reaction mixture was cooled, and filtered through silica (isolute Si 0.5 g cartridge) eluting with DCM–MeOH (7:3 v/v, 8 mL). Confirmation of product formation and approximate purity was determined by LC–MS/UV analysis, and further purification was achieved by semi-preparative HPLC, furnishing the required chromenones in 10–30% yields.
18. The DNA-PK used for in vitro assays was purified from HeLa cell nuclear extract. The known ability of DNA-PK to phosphorylate the serine-15 residue of a p53 peptide in vitro was exploited in a classic ELISA style assay using an antibody that only recognises the p53 serine-15 site when phosphorylated (Cell Signalling Technology). The primary antibody to p53 phosphoserine-15 was detected using an HRP conjugated goat anti-rabbit antibody (Pierce) with ECL reagent (NEN) being used for the readout. The ability of compounds to inhibit this phosphorylation event was monitored over a concentration range with IC_{50} values generated from these results. See Ref. 10 for full details of the assay.
19. The independent synthesis of compound **22g** was conducted as follows: to a solution of **20** (0.15 g, 0.40 mmol) in dioxane (5 mL) was added K_2CO_3 (0.11 g, 0.79 mmol), dibenzothiophene-4-boronic acid (0.11 g, 0.48 mmol) and tetrakis(triphenylphosphine)palladium(0) (23 mg, 0.02 mmol), and the mixture was stirred at 90 °C for 16 h. The reaction mixture was cooled to 25 °C, diluted with DCM (15 mL), washed with water (10 mL) and the organic fraction was evaporated to dryness in vacuo. Purification by column chromatography employing DCM–MeOH (10:1) as eluent, gave **22g** as an off-white solid (59 mg, 0.14 mmol, 35%), mp 220–221 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.02–3.05 (4H, m, CH_2NCH_2); 3.43–3.48 (4H, m, CH_2OCH_2); 5.45 (1H, s, 3-H); 7.40–7.46 (4H, m, Ar-*H*); 7.50–7.52 (1H, m, Ar-*H*); 7.70–7.74 (2H, m, Ar-*H*); 8.15–8.19 (2H, m, Ar-*H*); 8.21–8.23 (1H, m, Ar-*H*); MS (ES+) *m*/*z* 414 $[M+H^+]^+$; IR (film) 3055, 2948, 2855, 1622, 1562 cm^{-1}; found: C, 72.51; H, 4.69; N, 3.35; $C_{25}H_{19}NO_3S$ requires: C, 72.62; H, 4.63; N, 3.39. Compound **22h** was prepared in a similar manner, and exhibited spectral (^{1}H NMR, IR, UV) and analytical (elemental analysis and LC–MS) data fully consistent with the assigned structure.
20. Smith, G. C. M., et al., unpublished results.