Discovery of Potent Chromen-4-one Inhibitors of the DNA-Dependent Protein Kinase (DNA-PK) Using a Small-Molecule Library Approach

Ian R. Hardcastle,*,† Xiaoling Cockcroft,‡ Nicola J. Curtin,# Marine Desage El-Murr,† Justin J. J. Leahy,† Martin Stockley,† Bernard T. Golding,† Laurent Rigoreau,‡ Caroline Richardson,§ Graeme C. M. Smith,§ and Roger J. Griffin†

Northern Institute for Cancer Research, School of Natural Sciences - Chemistry, Bedson Building, Newcastle upon Tyne NE1 7RU, U.K., KuDOS Pharmaceuticals Ltd., 327 Cambridge Science Park, Milton Road, Cambridge CB4 4WG, U.K., Northern Institute for Cancer Research, Medical School, Paul O'Gorman Building, Framlington Place, University of Newcastle upon Tyne, NE2 4HH, U.K., and KuDOS Horsham Ltd., Foundry Lane, Horsham, West Sussex RH13 5PX, U.K.

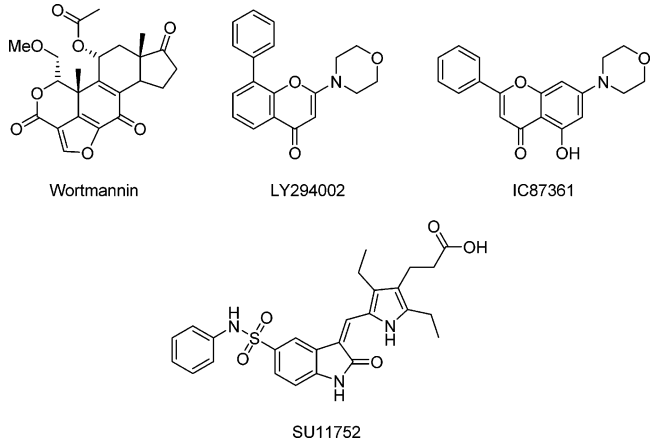
Received May 10, 2005

Structure−activity relationships for inhibition of DNA-dependent protein kinase (DNA-PK) have been defined for substituted chromen-4-ones. For the 2-amino-substituted benzo[*h*]-chromen-4-ones, a morpholine substituent at this position was essential for activity. Small libraries of 6- and 7-alkoxy-substituted chromen-4-ones showed that a number of 7-alkoxy-substituted chromenones displayed improved activity. Focused libraries incorporating 6-, 7-, and 8-aryl and heteroaryl substituents were prepared. In these cases, 6- and 7-substitution was disfavored, whereas 8-substitution was largely tolerated. Surprisingly, two compounds, 2-*N*-morpholino-8-dibenzofuranyl-chromen-4-one (NU7427, **32**{*38*}) and the 2-*N*-morpholino-8-dibenzothiophenyl-chromen-4-one (NU7441, **32**{*26*}) were excellent inhibitors (IC_{50} vs DNA-PK = 40 and 13 nM, respectively). The ring-saturated analogue 2-*N*-morpholino-8-(6′,7′,8′,9′-tetrahydrodibenzothiophene)chromen-4-one, **36**, retained potent activity (IC_{50} vs DNA-PK = 23 nM). The dibenzothiophene **32**{*38*} sensitized HeLa cells to ionizing radiation in vitro, with dose modification factors of 2.5 at 10% survival being observed at 0.5 μM. The cytotoxicity of the topoisomerase II inhibitor etoposide was also potentiated.

Introduction

The DNA-dependent protein kinase (DNA-PK) is a nuclear serine/threonine kinase member of the phosphatidylinositol (PI) 3-kinase-like (PIKK) family.[1] The DNA-PK complex comprises a large 465 kDa catalytic subunit (DNA-PKcs), and a heterodimeric DNA-targeting regulatory factor Ku made up of Ku70 and Ku80 subunits. DNA-PKcs shares significant homology in the kinase domain with the other PIKK family members: ATR, ATM, FRAP, mTOR, hSMG-1, and to a lesser extent the PI 3-kinase catalytic subunits p110, and PI4K.[2] DNA-PK becomes catalytically active on binding to DNA double-strand breaks (DSBs) and may phosphorylate a number of downstream targets including itself and the variant histone H2AX.[3−7] Cells that are defective in either DNA-PKcs or either of the regulatory Ku subunits are unable to effectively repair DNA DSBs produced from either exogenous sources (e.g., ionizing radiation) or endogenous processes (e.g., V(D)J recombination). These cell lines are highly radiosensitive and display increased sensitivity to cytotoxic drugs that induce DNA DSBs.[8,9] For this reason, we and others have proposed that specific DNA-PK inhibitors could be used to potentiate radiotherapy and chemotherapy in cancer treatment.

Chart 1. Structures of DNA-PK Inhibitors

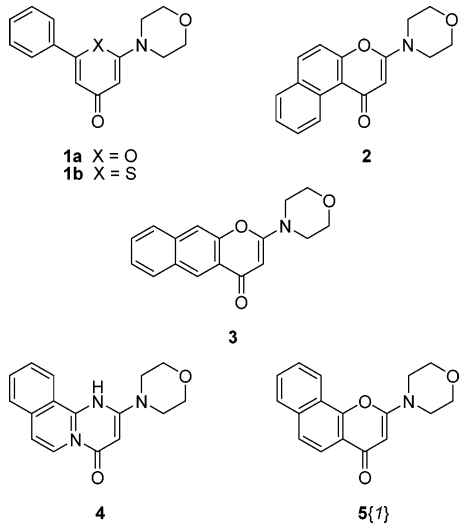


A number of small-molecule inhibitors of DNA-PK have been identified, including the irreversible inhibitor wortmannin[10,11] and the ATP-competitive inhibitors SU11752,[12] IC87361,[13] and LY294002[11,14] (Chart 1). Cells treated with these compounds become radiosensitized and also chemosensitive to DNA DSB-causing therapeutics, such as etoposide and bleomycin.[15,16] Previously, we employed the chromenone LY294002, originally designed as an ATP-competitive phosphatidylinositol 3-kinase (PI3K) inhibitor, as a structural lead, enabling the identification of a number of potent, selective ATP-competitive DNA-PK inhibitors, for example pyranone (**1a**, IC_{50} = 1.1 μM), thiopyranone (**1b**, IC_{50} = 0.72 μM), benzo[*f*]chromen-4-one (**2**, IC_{50} = 4 μM),

* Corresponding author. Tel: +44 191 222 6645. Fax: +44 191 8591. E-mail: I.R.Hardcastle@ncl.ac.uk.
† Northern Institute for Cancer Research, School of Natural Sciences.
‡ KuDOS Horsham Ltd.
# Northern Institute for Cancer Research, Medical School.
§ KuDOS Pharmaceuticals Ltd.

10.1021/jm050444b CCC: $30.25
Published on Web 10/29/2005

benzo[*g*]chromen-4-one (**3**, $IC_{50} = 0.4\ \mu M$), pyrimidoisoquinolinone (**4**, $IC_{50} = 0.25\ \mu M$), and the benzo[*h*]chromen-4-one NU7026 (**5**{*1*}, $IC_{50} = 0.23\ \mu M$).[17−19]



Selected compounds from these series have been demonstrated to sensitize tumor cell lines in vitro to ionizing radiation and DNA DSB-inducing chemotherapeutic agents.[19,20]

In the absence of detailed structural information for DNA-PKcs, we have employed a pharmacophore mapping approach, using small focused libraries to define more extensive structure−activity relationships (SARs) for ATP-competitive DNA-PK inhibition by chromenone-based inhibitors. We have previously detailed the effect on DNA-PK-inhibitory activity of modifying or replacing the 2-morpholinyl substituent of **4** and **5**.[19] In this paper, we present the results of further variations to the 2-morpholinyl function of the benzo[*h*]chromenone pharmacophore. We also report the synthesis, using solution- and solid-phase multiple-parallel approaches, and biological evaluation of chromen-4-one libraries bearing a variety of alkoxy- and aryl-substituents at the 6-, 7-, and 8-positions. These studies have resulted in the discovery of 8-aryl-chromen-4-one derivatives exhibiting potent and kinase-selective DNA-PK-inhibitory activity. A preliminary account of selected results from this study has been communicated.[18]

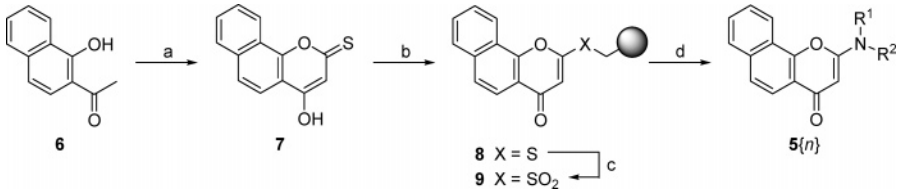
Chemistry

2-Aminobenzo[*h*]chromenones. A small library of 2-aminobenzo[*h*]chromenones (**5**{*4*−*22*}, Table 1) was prepared using the published procedure in a modified format.[14,19] The synthesis was adapted to a solid-phase capture and release strategy to simplify workup procedures and trap the sulfur-containing byproducts on the solid phase (Scheme 1). The chromenethione (**7**) was prepared, as previously described, from naphthyl ketone (**6**) and CS_2 with *tert*-butoxide as base. Thione (**7**) was readily alkylated with chloromethylpolystyrene resin (Merrifield, 1% divinylbenzene (DVB)) with diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF) at 60 °C (∼96% loading). The resin-bound alkylsulfanyl compound (**8**) was oxidized to the sulfone (**9**) with meta-chloroperbenzoic acid (mCPBA) in dichloromethane (DCM) and reacted with the chosen amine in DCM to liberate the products (**5**{*4*−*22*}) into solution. Excess amine was removed from the product by evaporation in vacuo, or for involatile amines, an acidic scavenger resin (Amberlite IR120+) was used. Despite the presence of a basic group, products **5**{*6*} and **5**{*9*} did not bind appreciably to the scavenger resin in the time required for complete sequestration of the remaining amine. Products were obtained in high purity and submitted for assay without further purification.

7-Alkoxy-chromenones by Solution-Phase Synthesis. The 7-hydroxychromone precursor (**13b**, Scheme 2) was prepared according to the method of Morris et al.[21] Direct conversion of methyl 2,4-dihydroxybenzoate (**10**) into the required β-ketoamide failed due to precipitation of the insoluble dilithium phenolate. Protection of the 4-hydroxyl group as the 2,6-dichlorobenzyl ether (**11**) allowed the reaction sequence to proceed smoothly via the β-ketoamide (**12**) giving protected chromenone (**13a**). Removal of the protecting group to furnish (**13b**) was readily achieved by hydrogenolysis. Reaction of **13b** with alkyl halides in the presence of Triton B gave the required 7-alkoxy-2-morpholinyl-chromenones (**13**{*1*−*10*}) in modest yields, while treatment of **13b** with benzoyl chloride afforded the corresponding 7-benzoyl ester (**13**{*11*}; Table 2, method A).

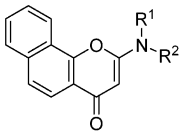
6- and 7-Alkoxy-chromenones by Solid-Phase Synthesis. The solid-phase capture and release strategy was also employed for the synthesis of 6- and 7-alkoxy-chromenones. The 7-*p*-methoxybenzyloxy-chromene-2-thione precursor (**15**) was prepared from 2,4-dihydroxyacetophenone by reaction with freshly prepared *p*-methoxybenzyl chloride in THF to give the ether (**14a**), which upon reaction with CS_2 in toluene with potassium *t*-butoxide as base, followed by a careful acidic workup, gave **15** (Scheme 3). The chromenone was attached to the resin with DBU in DMF to give the chromenone resin with good loading. Deprotection of the *p*-methoxybenzyl group was effected with 75% TFA in DCM at 0 °C giving the 7-hydroxy-chromene thio-resin (**16**). Cleavage of the chromenone from the resin was not observed under these conditions. Alkylation of **16** to give resin-bound 7-alkoxy-chromenone (**17**) was achieved by treatment of the preswelled resin in DMF with a variety of benzylic and alkyl halides using DBU. Subsequent oxidation of the sulfide linker to the sulfoxide (**18**) with mCPBA in DCM, followed by cleavage with morpholine, gave the 7-alkoxy-chromenones (**13**{*3*−

Scheme 1. Solid-Phase Synthesis of 2-Aminobenzo[*h*]chromen-4-ones (**5**{*4*−*22*})[a]

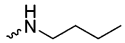
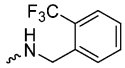
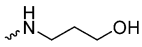
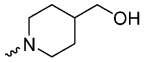
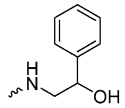
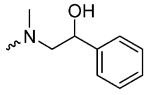
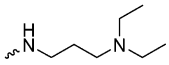
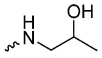
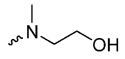
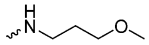
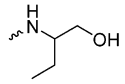
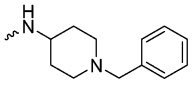
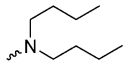
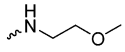
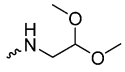


[a] Reagents and conditions: (a) CS_2, KO^{t}Bu, PhMe; (b) Merrifield resin, DBU, DMF, 60 °C; (c) mCPBA, DCM; (d) HNR^1R^2, DCM, rt.

Table 1. Chemical Structures, Synthetic Yields and Purities, and Biological Activities for the Benzo[*h*]chromenone Library

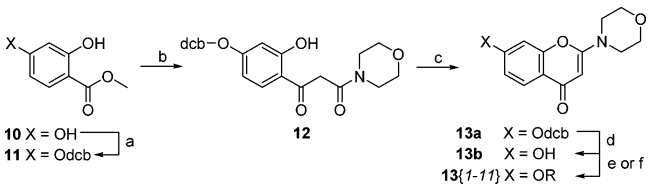


5{*n*}

Compound **5**{*n*}	~NR^{1}R^2	% Purity[a]	DNA-PK Inhibition IC_{50} (μM)	Compound **5**{*n*}	~NR^{1}R^2	% Purity[a]	DNA-PK Inhibition IC_{50} (μM)
1		Ref 19	0.23	*12*		90	>10
2		Ref 19	1.38	*13*		90	>10
3		Ref 19	3.81	*14*		90	>10
4		89	>10	*15*		90	>10
5		92	>10	*16*		90	>10
6		98	>10	*17*		72	>10
7		90	>10	*18*		82	~10
8		90	>10	*19*		85	>10
9		90	>10	*20*		85	>10
10		90	>10	*21*		85	>10
11		90	>10	*22*		89	>10

[a] Estimated by LCMS.

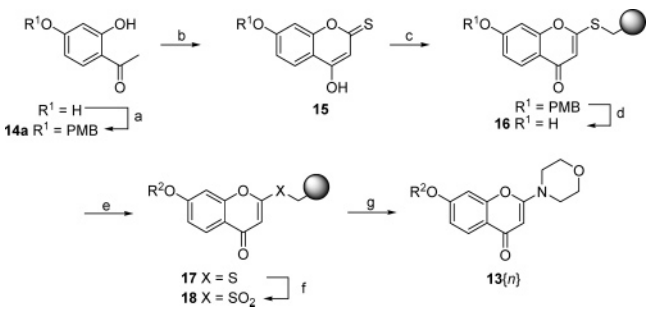
Scheme 2. Synthesis of 7-Alkoxy-chromen-4-ones (**13**{*1*−*11*})[a]



[a] Reagents and conditions, Method A: (a) 2,6-dichlorobenzyl chloride (dcbCl), K_2CO_3, NaI, acetone, reflux; (b) *N*-acetylamine, LDA, THF, −78−25 °C; (c) Tf_2O, DCM, 0 °C, 16 h; (d) 10% Pd/C, H_2, MeOH; (e) RBr, Triton B, DMF, 90 °C or (f) PhCOCl, pyridine, DMF, 0 °C.

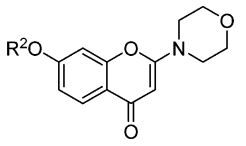
18}) in 30−90% purity as estimated by liquid chromatography−mass spectrometry, LCMS (Table 2, method B). An additional series of alkylations was performed

Scheme 3. Solid-Phase Synthesis of 7-Alkoxy-chromen-4-ones (**13**{*3*−*49*})[a]



[a] Reagents and conditions: (a) PMBCl, THF; (b) (i) CS_2, KO^{t}Bu, PhMe, (ii) aq H_2SO_4; (c) Merrifield resin, DBU, DMF; (d) 75% TFA, DCM, 0 °C; (e) method B, R^{2}Br, DBU, DMF, 65 °C; method C, R^{2}OH, DIAD, PPh_3, Et_3N, THF; (f) mCPBA, DCM; (g) morpholine, DCM.

Table 2. Chemical Structures, Synthetic Yields and Purities, and Biological Activities for 7-Alkoxy-chromenone Libraries



13{*n*}

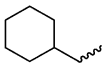
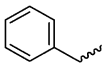
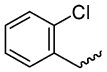
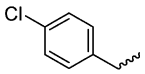
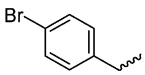
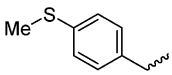
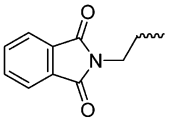
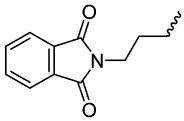
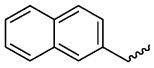
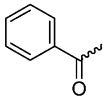
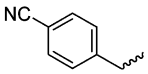
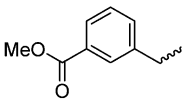
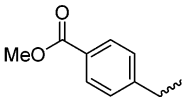
Compound **13**{*n*}	~OR^2	Method A[a]	Method B[b]	Method C[b]	DNA-PK Inhibition
		% Yield	% Purity[c]	% Purity[c]	IC_{50} (μM)
1	*n*Pr	27			1.32[e]
2		32			0.94[e]
3		48	90[d]	92	0.38[e]
4		28			0.79[e]
5		46	88	88	0.5[e]
6		36	77		0.56[e]
7		50	30		0.71[e]
8		3	45		4.33[e]
9		16	94		0.35[e]
10		30	90		0.44[e]
11		55			1.68[e]
12			88		0.69
13			82		0.84
14			74		3.94

Table 2 (Continued)

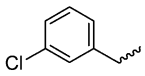
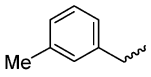
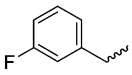
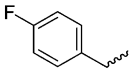
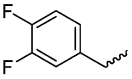
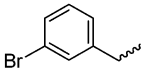
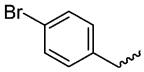
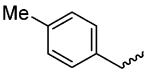
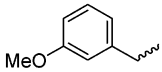
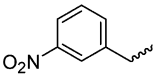
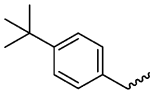
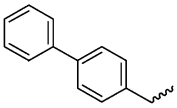
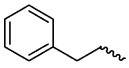
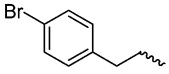
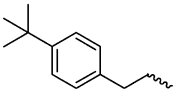
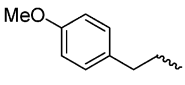
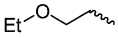
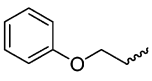
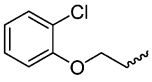
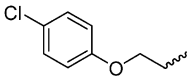
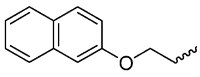
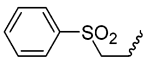
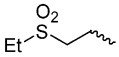
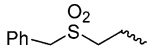
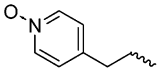
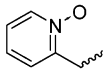
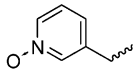
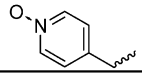
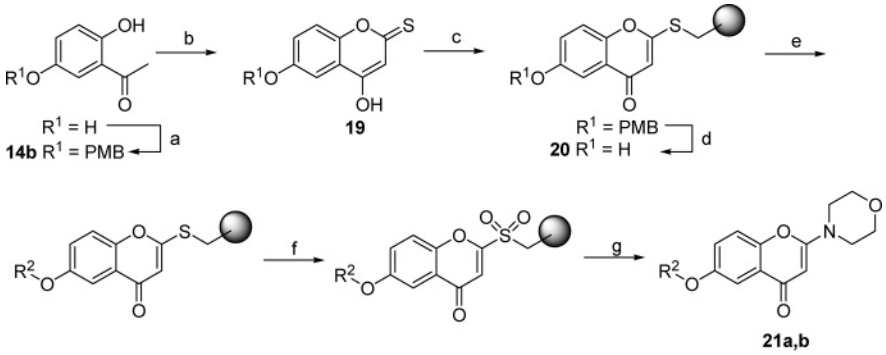
Compound **13**{*n*}	~OR^2	Method A[a]	Method B[b]	Method C[b]	DNA-PK Inhibition
		% Yield	% Purity[c]	% Purity[c]	IC_{50} (μM)
15			90	90	0.49
16			86	95	0.8
17				90	3
18		5		92	0.44[e]
19				92	2.53
20				87	1.92
21				93	2.02
22				93	3.94
23				95	3.76
24				91	>10
25				88	9.44
26				90	>10
27				87	2.99
28				82	4.13
29				95	>10
30				95	3.1
31				84	8.4
32				74	4.59
33				69	2.3

Table 2 (Continued)

Compound **13**{*n*}	~OR^2	Method A[a] % Yield	Method B[b] % Purity[c]	Method C[b] % Purity[c]	DNA-PK Inhibition IC_{50} (μM)
34				92	8.49
35				68	8.15
36				63	0.47
37				100	8.9
38				73	5.58
39				100	>10
40				97	9.32
41				97	>10
42				91	8.18

[a] Scheme 2. [b] Scheme 3. [c] Estimated by LCMS. [d] Alkylation repeated 3 times. [e] Values determined from compound prepared by method A.

Scheme 4. Solid-Phase Synthesis of 6-Alkoxy-chromen-4-ones (**21a**−**t**)[a]

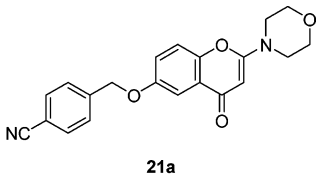


[a] Reagents and conditions: (a) PMBCl, THF; (b) (i) CS_2, KO^{t}Bu, PhMe, (ii) aq H_2SO_4; (c) Merrifield resin, DBU, DMF, 60 °C; (d) 75% TFA, DCM, 0 °C; (e) R^2Br, DBU, DMF, 65 °C; (f) mCPBA, DCM; (g) morpholine, DCM.

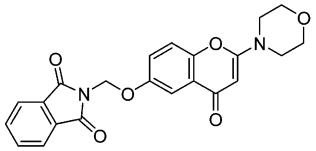
under Mitsunobu conditions with a set of 36 alcohols, which included 21 benzyl alcohols. Oxidation with mCPBA, followed by cleavage with morpholine, gave the products **13**{*3*,*5*,*12*,*16*,*17*,*19*−*49*} in 28−100% purity (Table 2, method C). Concomitant N-oxidation was observed in the cases where the alcohol contained a pyridine group (**13**{*46*−*49*}).

The resin-bound 6-hydroxy-chromenone (**20**) was prepared using a similar sequence of reactions starting from 2,5-dihydroxyacetophenone via thione (**19**) (Scheme 4). Alkylation of **20** was achieved by treatment of the preswelled resin in DMF with a variety of benzylic and alkyl halides using DBU as base. Subsequent oxidation with mCPBA in DCM followed by cleavage with morpholine gave the 6-alkoxy-chromenones (**21a**,**b**) in good purity as determined by LCMS. Reactions with other substituents failed to give the product in greater than 80% purity and were not submitted for assay. This result suggests that the 6-hydroxyl position is substantially less reactive in the alkylation reactions than the 7-position of the alkylthiochromenones.

6-, 7-, and 8-Aryl and Vinyl Chromenones: The 6-bromochromenone intermediate for the Suzuki−Miyura coupling library was prepared by condensation



21a



21b

of 5-bromo-2-hydroxyacetophenone (**22**) with CS_2 in toluene, with potassium *t*-butoxide as base, followed by treatment with aqueous sulfuric acid giving the 6-bromothione (**23**) (Scheme 5). Alkylation with ethyl iodide gave the thioether (**24**). Finally treatment of **24** with morpholine in ethylene glycol at 140 °C gave the desired 6-bromochromenone derivative (**25**).

Selective monotriflation at the 3- and 4-hydroxyl groups of the diphenols (**26a** and **26b**, respectively) was possible using triflic anhydride under standard conditions due to the reduced reactivity of the 2-hydroxyl group, which is believed to be due to intramolecular hydrogen bonding to the ketone (Scheme 6). The triflates **27a** and **27b** were reacted with the lithium enolate of *N*-acetamidomorpholine to give the respective diketones (**28a** and **28b**). Cyclization to the corresponding morpholinochromenones (**29a** and **29b**) was effected with triflic anhydride.

The 6-bromo- and 7- and 8-triflyl-chromenones (**25**, **29a**, and **29b**) were reacted with a range of aryl and vinyl boronic acids (**B1**−**53**) under standard conditions (dioxane, $Pd(PPh_3)_4$, K_2CO_3, 80 °C) in a parallel format to give the 6-, 7-, and 8-aryl and vinyl chromenones (**30**, **31**, and **32**), respectively (Scheme 7, Table 3).

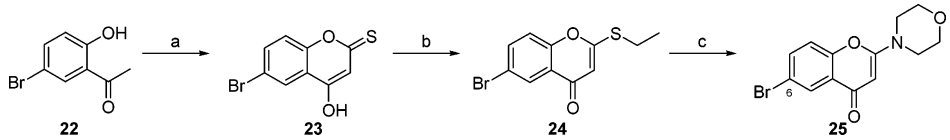
The tetrahydrodibenzothiophene boronate (**35**) was prepared in three steps from 2-chlorocyclohexanone (Scheme 8). Nucleophilic substitution with 2-bromothiophenolate gave the thioether (**33**), which was cyclized under forcing conditions (P_4O_{10}, polyphosphoric acid, 180 °C) to give the 6-bromotetrahydrodibenzothiophene derivative (**34**) in good yield.[22,23] Coupling of the 6-bromo compound with bispinacolatodiboron under standard conditions gave a moderate yield of the boronate (**35**).[24] A second coupling with the chromenone triflate **29b** gave the desired 8-substituted chromenone (**36**) in acceptable yield.[25]

Results and Discussion

Structure−Activity Relationships for DNA-PK Inhibition. 2-Aminobenzo[*h*]chromenones. Previous studies in the benzochromenone and related pyrimidoisoquinolinone series suggested that the 2-morpholino substituent was optimal for DNA-PK inhibition and that this position was intolerant to substitution.[19] To validate this observation, compounds **5**{*4*−*22*} were prepared. The amine substituents included a set chosen using a 2D-similarity search with morpholine as the "seed" compound. It was anticipated that compounds able to accept the hydrogen bond postulated to form between DNA-PK and the oxygen of the morpholino substituent would retain some activity. In fact, as proposed previously,[19] none of the compounds showed any inhibitory activity, thus confirming morpholine as the optimal substituent at this position. The morpholino oxygen of LY294002 makes important interactions with the backbone NH of Val882 of PI3Kγ by accepting a hydrogen bond, as observed in the X-ray structure of LY294002 bound to PI3Kγ.[26] It is likely that analogous important interactions arise between our inhibitors and the homologous region of the DNA-PKcs ATP-binding site.

6- and 7-Alkoxy-chromenones: A small series of 7-alkoxy-chromenones, **13**{*1*−*11*}, prepared via the solution-phase route, was evaluated (Table 2, Scheme 2−method A). In comparison with the parent **13b** (IC_{50} vs DNA-PK = 0.45 μM),[19] the introduction of alkyl substituents, for example, **13**{*1*}, resulted in a loss of activity; however, compounds with benzylic substituents displayed comparable activity in the majority of examples. The series of compounds was expanded by the synthesis of further analogues using the solid-phase route (Table 2, Scheme 3−methods B and C). None of the compounds evaluated displayed improved activity over **13b**, benzylic substitution generally resulting in a modest loss of potency. In the case of bulky substituents, such as 4-phenylbenzyl, **13**{*30*}, and *tert*-butylbenzyl, **13**{*33*}, a significant loss of activity was observed, indicating a limit to the amount of steric bulk able to be accommodated by the enzyme. Compounds bearing the polar pyridine *N*-oxides, **13**{*46*−*49*}, were markedly less potent than the parent **13b**.

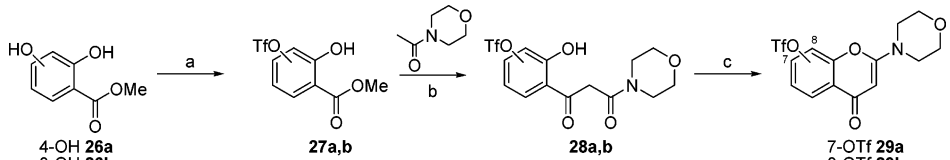
Scheme 5. Synthesis of 6-Bromo-2-*N*-morpholinochromen-4-one (**25**)[a]



22 **23** **24** **25**

[a] Reagents and conditions: (a) (i) CS_2, KO^{t}Bu, PhMe, (ii) aq H_2SO_4; (b) EtI, K_2CO_3, acetone; (c) morpholine, ethylene glycol, 140 °C.

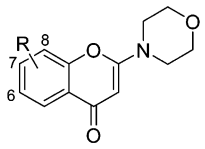
Scheme 6. Synthesis of 7- and 8-Triflyl-2-*N*-morpholinochromen-4-ones (**29a**,**b**)[a]



4-OH **26a**
3-OH **26b**
27a,b **28a,b**
7-OTf **29a**
8-OTf **29b**

[a] Reagents and conditions: (a) Tf_2O; (b) LDA, THF, 0 °C; (c) Tf_2O, DCM.

Table 3. Chemical Structures, Synthetic Yields, and Biological Activities for 6-Bromochromenone (**25**), 7-Triflyl Chromenone (**29a**), and 8-Triflyl Chromenone (**29b**) Libraries



30 6-subsn
31 7-subsn
32 8-subsn

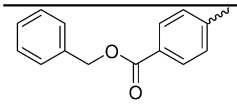
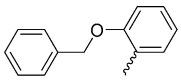
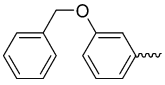
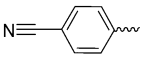
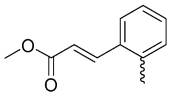
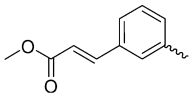
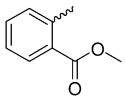
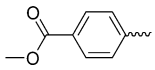
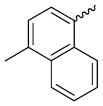
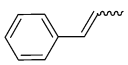
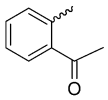
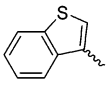
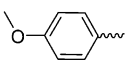
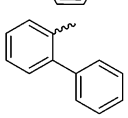
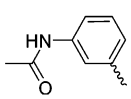
R	{*n*}	Compound 30{*n*}		Compound 31{*n*}		Compound 32{*n*}	
		Yield (%)	%inh at 0.5 μM	Yield (%)	%inh at 0.5 μM	Yield (%)	IC_{50} (μM)
	*1*	5	31	10	24	10	**3.5**
	*2*	26	40	21	30	4	**26.6**
	*3*	26	35	23	29	22	**5.9**
	*4*	13	37	3	29	24	**4.9**
	*5*	42	63	27		38	**>30**
	*6*	20	76	24	34	17	**>30**
	*7*	16	30	21	30	8	**>30**
	*8*	21	16				
	*9*	27	25	18		22	**8.7**
	*10*	7	6			33	**1.5**
	*11*	30	61	31	26	18	**14.9**
	*12*	20	50	15		7	**3.1**
	*13*	18	57	14	22	14	**0.9**
	*14*	15	56	25	25	34	**10.6**
	*15*	5	37	34	28	13	**14.7**

Table 3 (Continued)

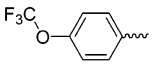
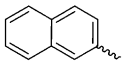
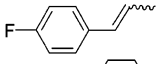
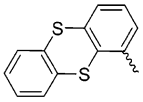
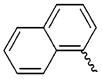
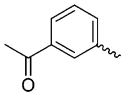
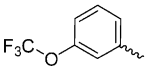
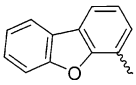
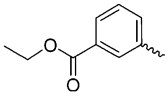
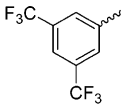
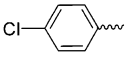
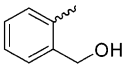
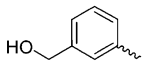
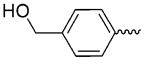
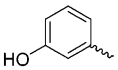
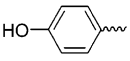
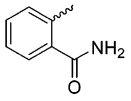
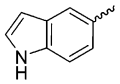
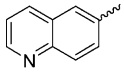
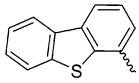
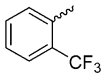
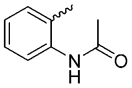
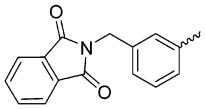
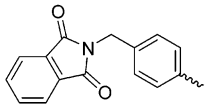
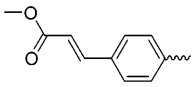
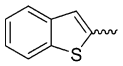
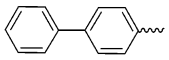
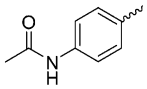
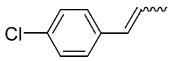
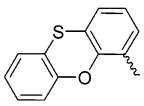
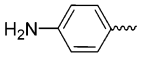
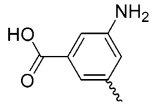
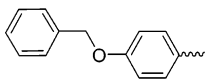
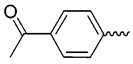
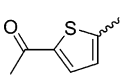
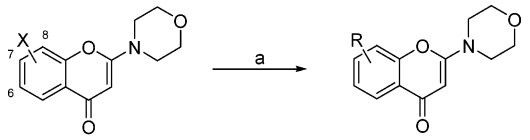
R	{*n*}	Compound 30{*n*}		Compound 31{*n*}		Compound 32{*n*}	
		Yield (%)	%inh at 0.5 μM	Yield (%)	%inh at 0.5 μM	Yield (%)	IC_{50} (μM)
	*16*	6	42	13		16	**0.3**
	*17*	1	43	7		18	**0.7**
	*18*	9	19	30	39	19	**1.4**
	*19*	11	4	39		12	**3.0**
	*20*	18	14	38		8	**2.8**
	*21*	14	57	43		16	**1.1**
	*22*	11	55	14			
	*23*	34	52	18		27	**>30**
	*24*	4	46	28	6		
	*25*	19	47	33	20	35	**4.3**
	*26*	8	37	23		28	**0.04**
	*27*	31	13	27	26	28	**2.7**
	*28*	4	13	28		11	**>30**
	*29*	17	6	14	4	17	**2.4**
	*30*	20	48	29	15	22	**2.9**
	*31*	22	51	25	16	27	**4.0**
	*32*	17	58	21	35	31	**0.6**
	*33*	9	56	29	33	34	**1.2**
	*34*	30	57	14	22	7	**2.6**
	*35*	17	43				

Table 3 (Continued)

R	{*n*}	Compound 30{*n*}		Compound 31{*n*}		Compound 32{*n*}	
		Yield (%)	%inh at 0.5 μM	Yield (%)	%inh at 0.5 μM	Yield (%)	IC_{50} (μM)
	*36*	12	17	33		15	**2.6**
	*37*	32	13	24		50	**>30**
	*38*	11	14	29		19	**0.02**
	*39*	23	7	27	20	31	**6.2**
	*40*	21	60	24	21	14	**>30**
	*41*	19	55	8		6	**9.7**
	*42*	9	49	7			
	*43*			10	14		
	*44*			17		9	**0.8**
	*45*			25	35		
	*46*			13	20	23	**1.2**
	*47*			17			
	*48*			18		34	**5.8**
	*49*			20	31		
	*50*			7			
	*51*			17		29	**2.0**
	*52*					15	**1.0**
	*53*					39	**0.8**

Scheme 7. Synthesis of 6-, 7-, and 8-Substituted 2-*N*-Morpholinochromen-4-one Libraries (**30**, **31**, and **32**)[a]

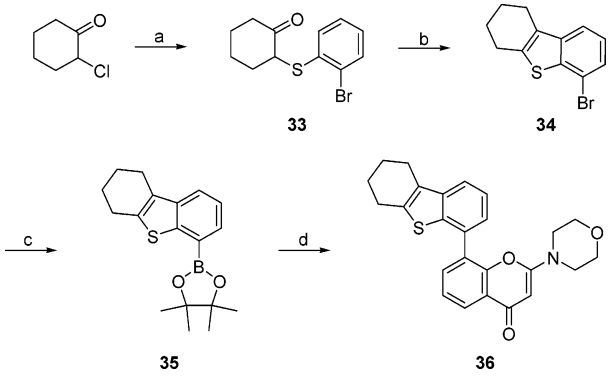


25 X = 6-Br
29a X = 7-OTf
29b X = 8-OTf

30 6-subsn
31 7-subsn
32 8-subsn

[a] Reagents and conditions: $RB(OH)_3$ (**B1**−**53**), K_2CO_3, $Pd(PPh_3)_4$, dioxane, 90 °C, 18 h.

Scheme 8. Synthesis of 8-(6′,7′,8′,9′-Tetrahydrodibenzothiophen-4′-yl)-2-*N*-morpholinochromen-4-one (**36**)[a]



[a] Reagents and conditions: (a) 2-bromobenzenethiol, NaOH, EtOH, reflux; (b) PO_5, polyphosphoric acid, 180 °C; (c) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, THF, reflux; (d) **29**, $PdCl_2$(dppf), $CsCO_3$, THF, reflux.

The results from the 6-alkoxy-chromenone library were disappointing as the purity of the majority of the compounds was below the 85% standard required for biological evaluation. Two compounds were assayed, namely, the benzonitrile **21a** (IC_{50} = 4.0 μM) and the phthalimide **21b** (IC_{50} = 4.3 μM), both of which were an order of magnitude less potent than the corresponding 7-substituted analogues, **13**{*13*} and **13**{*8*}, respectively.

6-, 7-, and 8-Substituted Chromenones. The 6- and 7-substituted products from the Suzuki libraries (**30**{*1*−*42*} and **31**{*1*−*51*}, respectively) were assayed against DNA-PK at a single concentration of 0.5 μM. None of the compounds displayed significant inhibition at this concentration. Consequently, IC_{50} determinations were not performed on this library.

The 8-substituted products from the Suzuki libraries, **32**{*1*−*55*}, were initially assayed at a single concentration of 0.5 μM. In this case, a number of compounds showed improved activity over the benchmark compound LY294002 (IC_{50} = 1.47 μM). Consequently, IC_{50} determinations were carried out on the entire library. Eleven compounds showed a significant loss of activity, in particular, those bearing an ortho-substituted phenyl group, for example, **32**{*2*,*7*,*11*,*14*,*40*}, and the bis-trifluoromethyl compound **32**{*28*}, indicating a lack of steric tolerance within the active site at this position. The 2- and 3-thienyl isosteres of LY294002, **32**{*16*} and **32**{*17*}, displayed a modest improvement in activity over the parent compound, as did the 2-benzothiophene compound **32**{*44*}, the 4-methoxyphenyl derivative **32**{*13*}, and the 4-hydroxymethyl analogue **32**{*32*}.

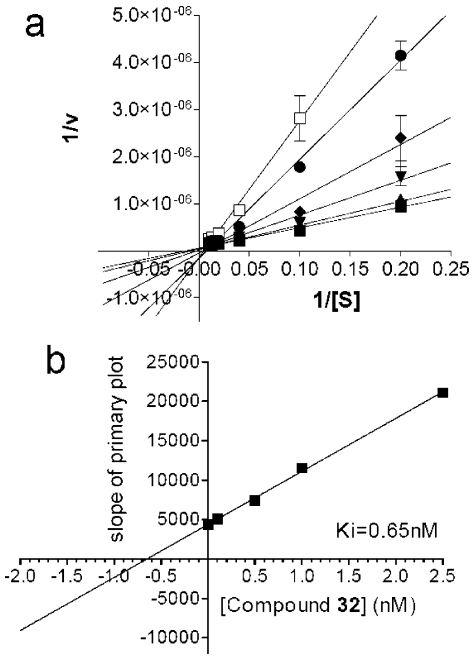


Figure 1. Characterization of the inhibition of DNA-PK activity by compound **32**{*38*}: (a) Lineweaver−Burk analysis of the inhibition of DNA-PK by **32**{*38*}. Inhibition kinetics were performed with 0 (■), 0.1 (▲), 0.5 (▼), 1 (◆), 2.5 (●) or 5 nM (□) of **32** in a range of ATP concentrations (5−100 μM). Data shown are the mean ± sem from at least three independent experiments. (b) K_i determination for compound **32**. Slopes from the Lineweaver−Burk plots were plotted against the range of concentration of **32** used. Intersection of the plot on the compound concentration axis gave a K_i value of 0.65 nM.

Interestingly, two compounds, the dibenzofuran **32**{*26*} and the dibenzothiophene **32**{*38*} (NU7441), exhibited a marked improvement in potency. Compound **32**{*38*} was resynthesized to analytical purity and reassayed, giving an IC_{50} of 13 nM against DNA-PK. This inhibitor was assigned the house number NU7441.

To explore further the importance of the terminal ring of the dibenzothiophene in **32**{*38*} and its interaction with DNA-PKcs, the 6′,7′,8′,9′-tetrahydrodibenzothiophene derivative **36** was synthesized. Interestingly, **36** was essentially equipotent with the parent **32**{*38*} (IC_{50} = 23 nM), suggesting a degree of steric tolerance in this region of the DNA-PKcs active site. The potent activity of the chromenones bearing dibenzofuran or dibenzothiophene substituents at the 8-position (**32**{*26*}, **32**{*38*}, and **36**) is remarkable in the light of the relatively flat SARs for other aromatic and heteroaromatic substitutions in this series.

Characterization of DNA-PK Inhibition by (32-{*38*}). Lineweaver−Burk plots for DNA-PK inhibition by **32**{*38*} revealed that this inhibitor acted in an ATP-competitive fashion (Figure 1a), as seen previously for LY294002 and NU7163.[11,19] Notably, when the slopes of the plots shown in Figure 1a were plotted against the inhibitor concentration (Figure 1b), it was found that **32**{*38*} exhibited a K_i value of 0.65 nM, confirming this chromenone as the most potent DNA-PK inhibitor reported to date.

Characterization of Kinase Selectivity of (32-{*38*}). NU7441 was further tested for its ability to inhibit the DNA-PK-related enzymes ATM (ataxia-telangiectasia mutated), ATR (ATM and Rad-3 related),

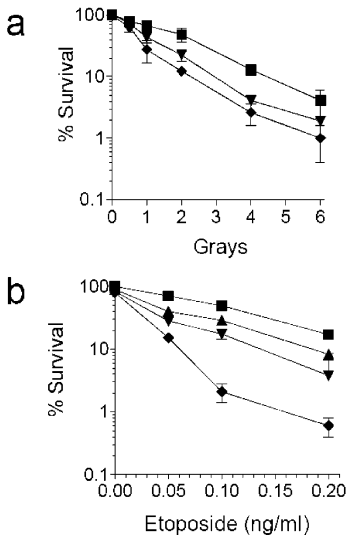


Figure 2. In vitro radio- and chemosensitization of HeLa cells by the DNA-PK inhibitor **32**{*38*}: (a) Effects of increasing doses of ionizing radiation (Gy) in the absence (■) or presence of 0.2 (▼) or 0.5 μM (◆) **32**{*38*}, on the clonogenic survival of HeLa B cells. Cells were preincubated with **32**{*38*} for 1 h before exposure to ionizing radiation and incubated for a further 16 h prior to fresh media being added, and colony formation was determined after 8 days. Data are the mean ± sem of at least three independent experiments. (b) Effects of increasing doses of etoposide in the absence (■) or presence of 0.2 (▲), 0.5 (▼), or 1 μM (◆) **32**{*38*} on the clonogenic survival of HeLa B cells. Cells were preincubated with **32**{*38*} for 1 h before exposure to etoposide and incubated for a further 16 h prior to fresh media being added, and colony formation was determined after 8 days. Data are the mean ± sem of at least three independent experiments.

mTOR (mammalian target of rapamycin), and PI 3-kinase p110α (phosphatidylinositol 3-kinase). At concentrations up to 100 μM, **32**{*38*} did not inhibit the ATM or ATR protein kinases but did exhibit activity against mTOR and PI 3-kinase, with IC_{50} values of 1.7 and 5.0 μM, respectively. This represents a selectivity window of at least 100-fold against the kinases most closely related to DNA-PK. This dramatic selectivity was further highlighted when the compound was found to have no significant inhibitory activity against the Upstate panel of 60 kinases at a concentration of 10 μM.

Radiosensitization and Enhancement of Etoposide Cytotoxicity by 32{*38*} in HeLa Cells. Previous work has revealed that inhibition of DNA-PK by a small molecule approach leads to radio- and chemopotentiation through the inhibition of DNA DSB break repair.[27] We therefore evaluated the potential of **32**{*38*} to potentiate the cytotoxicity of both ionizing radiation (IR) and etoposide in a cell-based assay. As shown in Figure 2a, sub-micromolar concentrations of **32**{*38*} were able to radiosensitize HeLa cells over a wide range of IR doses. Relative to the controls, **32**{*38*} was found to have dose modification factors at 10% survival of 1.3 and 2.5 at doses of 0.2 and 0.5 μM, respectively. When HeLa cells were treated with the DNA-PK inhibitor in combination with etoposide, significant potentiation of cell kill was seen at inhibitor concentrations as low as 0.1 μM (Figure 2b). Coupled with the in vitro IC_{50} data, these cell-based data further highlight **32**{*38*} as a potent and specific DNA-PK inhibitor. It was also observed that at concentrations up to 10 μM the DNA-PK inhibitor showed no inherent toxicity in this cell-based assay system.

Conclusions

We have successfully employed a pharmacophore mapping approach to establish structure−activity relationships for chromen-4-one-based DNA-PK inhibitors. Consistent with our previous results,[19] replacement of the 2-morpholinyl substituent in the benzo[*h*]-chromenone series **5**{*4*−*22*} was not tolerated, providing further evidence for a crucial interaction between this group and the ATP-binding site. The introduction of alkoxy substituents at the chromenone 6-position was detrimental to DNA-PK inhibitory activity, whereas 7-alkoxy substitution was generally tolerated and in some examples produced a modest improvement in potency. Aryl and heteroaryl substitutions were achieved at the 6-, 7-, and 8-positions by palladium-mediated coupling with the appropriate chromenone (**25**, **29a**, and **29b**). Aryl substituents at the chromenone 6- or 7-positions dramatically reduced or abolished activity, whereas analogous groups at the 8-position were generally well tolerated. Strikingly, the 8-dibenzofuran, 8-dibenzothiophene, and 8-tetrahydrodibenzothiophene derivatives (**32**{*26*}, **32**{*38*}, and **36**) were found to be nanomolar inhibitors of DNA-PK.

Compound **32**{*38*} exhibits excellent selectivity for DNA-PK over other PIKK family members and shows good cellular radio- and chemopotentiation properties at pharmacologically relevant concentrations. Preliminary studies to explore additional SARs in this series have revealed a degree of tolerance to modification of the dibenzothiophene ring of **32**{*38*}. Studies are in progress to optimize cellular and in vivo activities with a view to identifying a clinical candidate.

Experimental Section

Reagents were purchased from fine chemicals vendors, and used as received unless otherwise stated. Solvents were purified and stored according to standard procedures. Petroleum ether refers to that fraction in the boiling range 40−60 °C. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. Thin-layer chromatography was performed using silica gel plates (Kieselgel $60F_{254}$; 0.2 mm) and visualization with UV light or potassium permanganate. Chromatography was conducted under medium pressure on silica (BDH silica gel 40−63 μm). HPLC refers to purification 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 4μ C18 column, 10 mm × 250 mm, and peak acquisition based on UV detection at 254 nm. Solution-phase Suzuki reactions (general procedure L) were conducted in "Greenhouse" reactors (Radley's Ltd., U.K.) in batches of 24 reactions under an argon atmosphere. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin AC 200E spectrometer at 200 and 50 MHz, respectively, or on a Bruker Avance 300 spectrometer at 300 or 75 MHz, repectively, employing tetramethylsilane (TMS) or the solvent as internal standard. Unless indicated otherwise, spectra were recorded in [$^2H_6$]-DMSO as solvent. NH signals appeared as broad singlets (br s) exchangeable with D_2O. Mass spectra were determined on a Micromass Autospec M spectrometer in electron impact (EI) mode. Liquid chromatography−mass spectrometry (LCMS) was carried out on either 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 5μ 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. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Elemental analyses were performed by Butterworth Laboratories, Middlesex, U.K., and are within ±0.4% of theory unless otherwise specified.

General Procedure A: Alkylation of Dihydroxy Benzoates and Acetophenones. A solution of the appropriate phenol (1.0 equiv) in acetonitrile was treated with potassium carbonate (2.2 equiv), followed by the appropriate alkylating agent (1.0 equiv) under nitrogen. After being heated to reflux for 16 h, the mixture was cooled and treated with 1 M hydrochloric acid. The mixture was extracted into ethyl acetate, washed with 1 M hydrochloric acid and brine, dried over sodium sulfate, and evaporated in vacuo. The product was purified by recrystallization from suitable solvents.

4-(4-Methoxybenzyloxy)-2-hydroxyacetophenone (14a). General procedure A was followed with 2,4-dihydroxyacetophenone (7.30 g, 53 mmol) and 4-methoxybenzyl chloride, prepared by stirring 4-methoxybenzyl alcohol (6.0 mL, 48 mmol) in concentrated hydrochloric acid (40 mL) for 4 h and extracting into DCM. Beige powder (6.36 g, 49%). Mp 82−83 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.67 (3H, s, $COCH_3$); 3.87 (3H, s, OCH_3); 5.21 (2H, s, OCH_2); 6.66 (1H, d, J = 2.4 Hz, 3-H); 6.69 (1H, dd, J = 2.4, 8.6 Hz, 5-H); 7.06 (2H, d, J = 8.6 Hz); 7.49 (2H, d, J = 8.6 Hz); 7.95 (1H, d, J = 8.6 Hz, 6-H), 12.7 (1H, bs, OH). LCMS (ESI+) m/z = 273 $[M + H]^+$. HRMS (EI) for $C_{16}H_{16}O_4$ calcd 272.1049, obsd 272.1054.

5-(4-Methoxybenzyloxy)-2-hydroxyacetophenone (14b). General procedure A was followed with 2,5-dihydroxyacetophenone (3.65 g, 24.0 mmol) and 4-methoxybenzyl chloride (prepared by stirring 4-methoxybenzyl alcohol (3.0 mL, 24 mmol) in concentrated hydrochloric acid (20 mL) for 4 h and extracting into DCM). Off-white powder (4.03 g, 62%). Mp 86−87 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.73 (3H, s, $COCH_3$); 3.86 (3H, s, OCH_3); 5.12 (2H, s, OCH_2); 7.00 (1H, d, J = 9.1 Hz, 3-H); 7.05 (2H, d, J = 8.7 Hz); 7.33 (1H, dd, J = 3.0, 9.1 Hz, 5-H); 7.49 (2H, d, J = 8.7 Hz); 7.53 (1H, d, J = 3.0 Hz, 6-H), 11.6 (1H, bs, OH). LCMS (ESI+) m/z = 273 $[M + H]^+$. HRMS (EI) for $C_{16}H_{16}O_4$ calcd 272.1049, obsd 272.1051.

Methyl 4-(2,6-dichlorobenzyloxy)-2-hydroxybenzoate (11). General procedure A was followed with methyl 2,4-dihydroxybenzoate (**10**)(1.68 g, 10.0 mmol), 2,6-dichlorobenzyl chloride (1.95, 10.0 mmol), and sodium iodide (1.50 g, 10.0 mmol). Off-white powder (2.07 g, 63%). Mp 127−129 °C. IR (Diamond ATR) υ_{max} (cm^{-1}): 2951, 1671, 1616, 1580, 1435, 1344, 1248, 1215, 1184, 1134, 1092, 773. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.97 (3H, s, CH_3); 5.38 (2H, s, OCH_2Ar); 6.73 (1H, dd, J = 2.4, 8.8 Hz, 5-H); 6.82 (1H, d, J = 2.4 Hz, 3-H); 7.62 (3H, m, $C_6H_3Cl_2$); 7.84 (1H, d, J = 8.8 Hz, 6-H); 10.88 (1H, br, OH). MS (EI+) m/z = 330, 328, 326 $[M]^+$. HRMS (EI) for $C_{15}H_{12}Cl_2O_4$ calcd 326.0113, obsd 326.0122.

General Procedure B: Synthesis of 4-Hydroxythiocoumarins. A solution of potassium *tert*-butoxide (3.2 equiv) in THF was slowly treated with a mixture of the appropriate 2-hydroxyacetophenone (1.0 equiv) and carbon disulfide (1.0 equiv) in THF at 10 °C under nitrogen. After the reaction mixture was stirred for 16 h at room temperature, the thick mixture was treated with water and washed twice with ether. The aqueous solution was adjusted to pH 4 by treatment with 10% sulfuric acid and stirred for 16 h, passing nitrogen through the flask, with an aqueous bleach trap fitted to trap the hydrogen sulfide generated (CAUTION: hydrogen sulfide is a highly toxic colorless gas). The precipitate was allowed to settle, and the supernatant liquid was decanted. The yellow solid residue was stirred vigorously in petrol until a fine precipitate had formed, which was collected by filtration and washed with copious amounts of cold petrol. The crude product was purified by recrystallization from the appropriate solvent.

7-(4-Methoxybenzyloxy)-4-hydroxy-chromen-2-thione (15). General procedure B was followed with 4-(4-methoxybenzyloxy)-2-hydroxyacetophenone (5.44 g, 20.0 mmol). Recrystallization (ethyl acetate) gave **15** as a yellow powder (2.04 g, 32%). Mp 150−152 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.87 (3H, s, OCH_3); 5.28 (2H, s, OCH_2); 6.68 (1H, s, 3-H); 7.07 (2H, d, J = 8.5 Hz); 7.18 (1H, dd, J = 2.1, 8.8 Hz, 6-H); 7.34 (1H, d, J = 2.1 Hz, 8-H); 7.53 (2H, d, J = 8.5 Hz); 7.89 (1H, d, J = 8.8 Hz, 5-H). LCMS (ESI+) m/z = 315 $[M + H]^+$. HRMS (EI) for $C_{17}H_{14}O_4S$ calcd 314.0613, obsd 314.0606.

6-(4-Methoxybenzyloxy)-4-hydroxy-chromen-2-thione (19). General procedure B was followed with 5-(4-methoxybenzyloxy)-2-hydroxyacetophenone (4.00 g, 14.7 mmol). Recrystallization (ethyl acetate) gave (**19**) as a yellow powder (2.06 g, 44%). Mp 157−158 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.99 (3H, s, OCH_3); 5.33 (2H, s, CH_2Ar); 6.90 (1H, s, 3-H); 7.18 (2H, m); 7.62 (4H, m); 7.77 (1H, m). LCMS (ESI+) m/z = 315 $[M + H]^+$. HRMS (EI) for $C_{17}H_{14}O_4S$ calcd 314.0613, obsd 314.0608.

6-Bromo-4-hydroxy-chromene-2-thione (23). General procedure B was followed with 5-bromo-2-hydroxyacetophenone (4.30 g, 20 mmol). Recrystallization (THF) gave **23** as a pale orange crystalline solid (1.85 g, 36%). Mp 122−123 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.89 (1H, s, 3-H); 7.45 (1H, d, J = 8.8 Hz, 8-H); 7.86 (1H, dd, J = 2.4, 8.8 Hz, 7-H); 8.02 (1H, d, J = 2.4 Hz, 5-H); MS ESI+ m/z = 258 $[M + H]^+$.

General Procedure C: Resin Loading Procedure. Merrifield resin (1% cross-linked, 1.2 mmol/g; 0.70 g, 0.84 mmol) was swelled in anhydrous DMF (4 mL). The mixture was shaken gently for 15 min and then treated with a solution of the appropriate chromen-2-thione (2.2 mmol) in DMF (3 mL). After shaking for a further 15 min, the mixture was treated with DBU (0.4 mL, 2.7 mmol). The reaction mixture was heated to 70 °C and gently shaken for 24 h. The resin was collected by filtration, washed with DMF (5 mL), methanol (5 mL), and DCM (2 × 5 mL), and dried in vacuo.

(Benzo[*h*]-4*H*-chromen-2-yl)thiomethylpolystyrene resin (8). General procedure C was followed with 4-hydroxybenzo[*h*]chromen-2-thione (0.50 g, 2.2 mmol) in DMF (3 mL) yielding a pale yellow resin.

***S*-(7-Hydroxy-4-oxo-4*H*-chromen-2-yl)thiomethylpolystyrene resin (16).** General procedure C was followed with 7-(4-methoxybenzyloxy)chromen-2-thione (**15**) (1.20 g, 3.82 mmol) giving a pale yellow resin. The resin was swelled in DCM (2.5 mL) and gently agitated for 15 min. The mixture was cooled (ice-acetone), TFA (7.5 mL) was added, and the mixture was agitated for 4 h, then filtered, washed (DCM, methanol and DCM) and dried yielding a pale yellow resin, 1.37 g (~1.38 mmol, ~96%, based on mass).

***S*-(6-Hydroxy-4-oxo-4*H*-chromen-2-yl)thiomethylpolystyrene resin (20).** General procedure C was followed with 6-(4-methoxybenzyloxy)-4-hydroxy-chromen-2-thione (0.70 g, 2.2 mmol). The resin was swelled in DCM (1.5 mL) and gently agitated for 15 min. The mixture was cooled (ice-acetone), TFA (4.5 mL) was added, and then the mixture was agitated for 4 h, filtered, and washed (DCM, methanol, DCM) yielding a pale yellow resin, 0.74 g (~0.74 mmol, ~89%, based on mass).

General Procedure D: Synthesis of a Benzo[*h*]chromen-4-ones Library. Benzo[*h*]-4-oxo-4*H*-chromen-2-yl-thiomethylpolystyrene resin (maximum 0.036 mmol) was swelled in anhydrous DCM (1 mL) and gently shaken for 15 min. The reaction mixture was treated with a solution of the appropriate amine (0.36 mmol) in DCM (0.2 mL). The mixture was vigorously shaken at room temperature for 16 h, followed by addition of Amberlite IR120+ resin and shaking for a further 1 h. The reaction mixture was filtered, and the resin was washed successively with DCM (2 × 5 mL), MeOH (2 × 5 mL), and DCM (5 mL). The filtrate was evaporated in vacuo to provide the title compounds. The products were submitted for analysis by LC-MS without further purification.

1-(4-(2,6-Dichlorobenzyloxy)-2-hydroxyphenyl)-3-(morpholin-4-yl)-propan-1,3-dione (12). To a solution of diisopropylamine (3.2 mol equiv) in THF at −78 °C was added *n*-BuLi (2.5 M in hexanes, 3.0 mol equiv). The resulting

mixture was allowed to warm to 0 °C over 15 min under N_2 and cooled to −10 °C, acetyl morpholine (0.92 mL, 8.0 mmol) was added, and stirring was continued for 90 min. Methyl 4-(2,6-dichlorobenzyloxy)-2-hydroxybenzoate (**11**; 1.64 g, 5.00 mmol) was added and stirring was continued for 16 h at room temperature. Water (5 mL) was added, and the solution was adjusted to pH 1.0 with hydrochloric acid (2 M) and extracted (DCM, 3 × 80 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to give the crude product. Trituration with ether gave **12** as an off-white powder (1.68 g, 78%). Mp 117−121 °C. IR (Diamond ATR) υ_{max} (cm^{-1}): 3071, 2968, 2853, 1627, 1599, 1439, 1360, 1220, 1196, 1114, 994. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.57 (4H, m); 3.67 (4H, m); 4.31 (2H, s, COCH_2); 5.42 (2H, s, OCH_2Ar); 6.76 (1H, dd, J = 2.3, 8.8 Hz, 5-*H*); 6.79 (1H, d, J = 2.3 Hz, 3-*H*); 7.64 (3H, m, $C_6H_3Cl_2$); 7.94 (1H, d, J = 8.8 Hz, 6-*H*); 12.2 (1H, br, O*H*). MS (ESI+) m/z = 426, 424 $[M + H]^+$. HRMS (EI) for $C_{20}H_{19}Cl_2NO_5$ calcd 423.0640, obsd 423.0649.

7-(2,6-Dichlorobenzyloxy)-2-(morpholin-4-yl)-chromen-4-one (13a). A solution of 1-(morpholin-4-yl)-3-(4-(2,6-dichlorobenzyloxy)-2-hydroxyphenyl)-propan-1,3-dione (8.60 g, 20.3 mmol) in DCM was treated with a solution of trifluoromethanesulfonic anhydride (3.6 equiv) in DCM under nitrogen at 0 °C. After stirring at room temperature for 20 h, the reaction mixture was evaporated in vacuo. The residue was redissolved in methanol and stirred for 4 h before treatment with an equal volume of water. The mixture was stirred for 1 h, evaporated in vacuo to provide an aqueous mixture, and adjusted to pH 8 by treatment with saturated sodium hydrogen carbonate solution. The mixture was extracted three times into DCM, dried (Na_2SO_4), and evaporated in vacuo. The crude product was vigorously stirred in ether for 16 h, and the precipitate was collected by filtration. Recrystallization gave **13a** as tan crystals (6.68 g, 81%). Mp 194−195 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.62 (4H, m); 3.82 (4H, m); 5.44 (2H, s, CH2Ar); 5.55 (1H, s, 3-H); 7.13 (1H, dd, J = 2.4, 8.8 Hz, 6-H); 7.43 (1H, d, J = 2.4 Hz, 8-H); 7.57−7.73 (3H, m, C6H3Cl2); 7.94 (1H, d, J = 8.8 Hz, 5-H). LCMS (ESI+) m/z = 410, 408, 406 $[M + H]^+$. HRMS (EI) for $C_{20}H_{17}Cl_2NO_4$ calcd 405.0535, obsd 405.0542.

7-Hydroxy-2-(morpholin-4-yl)-chromen-4-one (13b). A suspension of **13a** (6.60 g, 16.2 mmol) and 10% Pd/C (0.15 g) in methanol (150 mL) was stirred under an atmosphere of hydrogen for 40 h. The catalyst was removed by filtration through Celite, washing with methanol. The filtrate was concentrated in vacuo to provide an off-white solid. This was treated with fresh catalyst, resuspended in methanol, and stirred under hydrogen for a further 72 h. The catalyst was removed by filtration through Celite, washing with methanol. The filtrate was evaporated in vacuo, and the crude product was recrystallized from methanol to provide **13b** as a white solid (2.26 g, 57%). Mp 250 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.78 (4H, m); 3.86 (4H, m); 6.15 (1H, s, 3-*H*); 7.05−7.13 (2H, m, 6,8-*H*); 7.93 (1H, d, 5-*H*); 11.3 (1H, br, O*H*). MS (EI) m/z = 247 (M^+), 190, 105. HRMS (EI) for $C_{13}H_{13}NO_4$ calcd 247.0845, obsd 247.0837.

General Procedure E: Solution-Phase Alkylation. A solution of 7-hydroxy-2-(morpholin-4-yl)-chromen-4-one **13b** (124 mg, 0.5 mmol) in DMF (5 mL) was treated with a methanolic solution of benzyltrimethylammonium hydroxide (0.54 mL, 1.2 mmol), followed by a solution of the appropriate alkylating agent (2.0 mmol) in DMF (1.0 mL). The reaction mixture was heated in an aluminum block at 90 °C for 16 h. The solution was diluted with ethyl acetate (25 mL) and washed with water (10 mL). The ethyl acetate extract was evaporated in vacuo to dryness, and the residue was stirred vigorously in ether for 16 h. The solid product was collected by filtration and purified by recrystallization from methanol.

7-Propoxy-2-(morpholin-4-yl)-chromen-4-one (13{*1*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 1-bromopropane (0.18 mL, 2.0 mmol) gave **13**{*1*} as white crystals (39 mg, 27%). Mp 115 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.08 (3H, t, $CH_2CH_2CH_3$); 1.86 (2H, m, $CH_2CH_2CH_3$); 3.60 (4H, m); 3.81 (4H, m); 4.12 (2H, t, $CH_2CH_2CH_3$); 5.51 (1H, s, 3-*H*); 7.04 (1H, dd, J = 2.0, 8.7 Hz, 6-*H*); 7.18 (1H, d, J = 2.0 Hz, 8-*H*); 7.89 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 290 $[M + H]^+$. HRMS (EI) for $C_{16}H_{19}NO_4$ calcd 289.1314, obsd 289.1311.

7-Cyclohexylmethoxy-2-(morpholin-4-yl)-chromen-4-one (13{*2*}). General procedure E with **13b** (124 mg, 0.5 mmol) and cyclohexylmethyl bromide (0.28 mL, 2.0 mmol) gave **13**{*2*} as white crystals (55 mg, 32%). Mp 187−188 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.16 (5H, m, cyclohexyl); 1.87 (6H, m, cyclohexyl); 3.60 (4H, m, morpholine); 3.80 (4H, m, morpholine); 3.97 (2H, s, CH_2); 5.50 (1H, s, 3-*H*); 7.12 (1H, dd, J = 2.1, 8.7 Hz, 6-*H*); 7.18 (1H, d, J = 2.1 Hz, 8-*H*); 7.88 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 344 $[M + H]^+$. HRMS (EI) for $C_{20}H_{25}NO_4$ calcd 343.1784, obsd 343.1793.

7-Benzyloxy-2-(morpholin-4-yl)-chromen-4-one (13{*3*}). General procedure E with **13b** (124 mg, 0.5 mmol) and benzyl bromide (0.50 mL, 2.0 mmol) gave **13**{*3*} as white crystals (98 mg, 48%). Mp 170−172 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.59 (4H, m); 3.82 (4H, m); 5.31 (2H, s, CH_2); 5.52 (1H, s, 3-*H*); 7.13 (1H, dd, J = 2.3, 8.7 Hz, 6-*H*); 7.28 (1H, d, J = 2.3 Hz, 8-*H*); 7.45−7.60 (5H, m, C_6H_5); 7.91 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 338 $[M + H]^+$, 179. C, H, N.

7-(2-Chlorobenzyloxy)-2-morpholin-4-yl-chromen-4-one (13{*4*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 2-chlorobenzyl bromide (0.26 mL, 2.0 mmol) gave **13**{*4*} as white crystals (52 mg, 28%). Mp 167−168 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.61 (4H, m); 3.81 (4H, m); 5.36 (2H, s, CH_2); 5.54 (1H, s, 3-*H*); 7.15 (1H, dd, J = 2.3, 8.7 Hz, 6-*H*); 7.35 (1H, d, J = 2.3 Hz, 8-*H*); 7.50−7.76 (4H, m); 7.93 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 374, 372 $[M + H]^+$. HRMS (EI) for $C_{20}H_{18}ClNO_4$ calcd 371.0924, obsd 371.0929.

7-(4-Chlorobenzyloxy)-2-morpholin-4-yl-chromen-4-one (13{*5*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 4-chlorobenzyl bromide (0.41 g, 2.0 mmol) gave **13**{*5*} as white crystals (840 mg, 46%). Mp 185 °C (dec).^{1}H NMR (200 MHz, DMSO-d_6) δ 3.60 (4H, m); 3.82 (4H, m); 5.31 (2H, s, CH_2); 5.52 (1H, s, 3-*H*); 7.13 (1H, dd, J = 2.2, 8.7 Hz, 6-*H*); 7.28 (1H, d, J = 2.2 Hz, 8-*H*); 7.59−7.71 (4H, m, C_6H_4Cl); 7.92 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 374, 372 $[M + H]^+$. HRMS (EI) for $C_{20}H_{18}ClNO_4$ calcd 371.0924, obsd 371.0931.

7-(4-Bromobenzyloxy)-2-morpholin-4-yl-chromen-4-one (13{*6*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 4-bromobenzyl bromide (0.50 g, 2.0 mmol) gave **13**{*6*} as white crystals (75 mg, 36%). Mp 221−222 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.60 (4H, m); 3.82 (4H, m); 5.30 (2H, s, CH_2); 5.52 (1H, s, 3-*H*); 7.13 (1H, dd, J = 2.0, 8.7 Hz, 6-*H*); 7.27 (1H, d, J = 2.0 Hz, 8-*H*); 7.53 (2H, d, J = 8.3 Hz, 2′,6′-*H*); 7.72 (2H, d, J = 8.3 Hz, 3′,5′-*H*); 7.92 (1H, d, J = 8.7 Hz, 5-*H*). LCMS (ESI+) m/z = 418, 416 $[M + H]^+$. HRMS (EI) for $C_{20}H_{18}BrNO_4$ calcd 415.0419, obsd 415.0424.

7-(4-Methylsulfanylbenzyloxy)-2-(morpholin-4-yl)-chromen-4-one (13{*7*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 4-thiomethylbenzyl chloride (0.48 g, 2.0 mmol) gave **13**{*7*} as an off-white powder (96 mg, 50%). Mp 157−159 °C. IR (Diamond ATR) υ_{max} (cm^{-1}): 1589, 1550, 1410, 1219, 771, 490. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.76 (3H, s, SCH_3); 3.76 (4H, m); 3.99 (4H, m); 5.43 (2H, s, CH_2Ar); 5.70 (1H, s, 3-*H*); 7.29 (1H, m, 6-*H*); 7.44 (1H, m, 8-*H*); 7.55 (2H, m, 2′,6′-*H*); 7.69 (2H, m, 3′,5′-*H*); 8.08 (1H, m, 5-*H*). LCMS (ESI+) m/z = 384 $[M + H]^+$. HRMS (EI) for $C_{21}H_{21}NO_4S$ calcd 383.1191, obsd 383.1186. C, H, N.

***N*-[2-(2-(Morpholin-4-yl)-4-oxo-4*H*-chromen-7-yloxy)-ethyl]phthalimide (13{*8*}).** General procedure E with **13b** (124 mg, 0.5 mmol) and *N*-(2-bromoethyl)-phthalimide (0.406 g, 2.0 mmol) gave **13**{*8*} as white crystals (6 mg, 3%). Mp 230 °C (dec). LCMS (ESI+) m/z = 421 $[M + H]^+$. HRMS (EI) for $C_{23}H_{20}N_2O_6$ calcd 420.1321, obsd 420.1328.

***N*-[3-(2-(Morpholin-4-yl)-4-oxo-4*H*-chromen-7-yloxy)-propyl]phthalimide (13{*9*}).** General procedure E with **13b** (124 mg, 0.5 mmol) and *N*-(3-bromopropyl)phthalimide (0.538 g, 2.0 mmol) gave **13**{*9*} as white crystals (35 mg, 16%). Mp 210−211 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.60 (2H, m, $NCH_2CH_2CH_2O$); 3.58 (4H, m, morpholine); 3.81 (4H, m, morpholine); 3.89 (2H, m, $NCH_2CH_2CH_2O$); 4.22 (2H, m, NCH_2-

CH_2CH_2O); 5.50 (1H, s, 3-*H*); 6.86 (1H, dd, J = 2.0, 8.6 Hz, 6-*H*); 7.03 (1H, d, J = 2.0 Hz, 8-*H*); 7.83 (1H, d, J = 8.6 Hz, 5-*H*); 7.95 (4H, m, phth-H_4). LCMS (ESI+) m/z = 435 $[M + H]^+$. HRMS (EI) for $C_{24}H_{22}N_2O_6$ calcd 434.1478, obsd 434.1475.

7-(Naphthalen-2-ylmethoxy)-2-morpholin-4-yl-chromen-4-one (13{*10*}). General procedure E with **13b** (124 mg, 0.5 mmol) and 2-(bromomethyl)-naphthalene (442 mg, 2.0 mmol) gave **13**{*10*} as white crystals (58 mg, 30%). Mp 263−264 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.60 (4H, m); 3.81 (4H, m); 5.49 (2H, s, CH_2); 5.53 (1H, s, 3-*H*); 7.19 (1H, dd, J = 2.2, 8.7 Hz, 6-*H*); 7.34 (1H, d, J = 2.2 Hz, 8-*H*); 7.62−7.73 (3H, m); 7.92 (1H, d, J = 8.7 Hz, 5-*H*); 8.02−8.11 (4H, m). LCMS (ESI+) m/z = 388 $[M + H]^+$. HRMS (EI) for $C_{24}H_{21}NO_4$ calcd 387.1471, obsd 387.1476.

7-Benzoyloxy-2-(morpholin-4-yl)-chromen-4-one (13{*11*}). A solution of 7-hydroxy-2-(morpholin-4-yl)-chromen-4-one (**13b**) (0.25 g, 1.0 mmol) in DMF (10 mL) was treated with benzoyl chloride (0.13 mL, 1.1 mL), followed by pyridine (0.10 mL, 1.2 mmol) at 0 °C. The solution was warmed to room temperature, stirred 16 h, then diluted with ethyl acetate (100 mL), and washed with hydrochloric acid (0.5 M; 50 mL), water (50 mL), and brine (50 mL). The organic phase was dried (Na_2SO_4) and evaporated in vacuo. Recrystallization (EtOAc) gave **13**{*11*} as white crystals (0.19 g, 55%). Mp 204−206 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.64 (4H, m); 3.83 (4H, m); 5.65 (1H, s, 3-*H*); 7.45 (1H, m); 7.74 (3H, m); 8.87 (1H, m); 8.09 (1H, m); 8.26 (2H, m). MS (ES) m/z = 352 $[M + H]^+$; 179. C, H, N.

General Procedure F: Solid-Phase Alkylation. The appropriate resin-bound hydroxy-chromenone (0.030 g, <0.036 mmol) was swelled in anhydrous DMF (5 mL) in a polytetrafluoroethylene (PTFE) fritted vessel and gently shaken for 15 min. The mixture was treated with DBU (0.2 mL, 1.3 mmol). After shaking for a further 15 min, the mixture was treated with the appropriate alkylating agent (0.7 mmol). The reaction was heated to 65 °C and shaken for 20 h. The resin was collected by filtration and washed in order with DMF × 1, methanol × 1, and DCM × 2. The resin was resuspended in DCM (2 mL), and the desired compound was cleaved from the resin according to general procedure G.

General Procedure G: Cleavage Procedure. The resin-bound chromenone (maximum 0.036 mmol) was suspended in DCM (2 mL), and after shaking for 10 min, the mixture was treated with mCPBA (0.2 g, 1.1 mmol). The mixture was shaken at room temperature for 3 h and then filtered. The resin was washed in order with DCM × 2, methanol × 2, and DCM × 2 and re-suspended in DCM (2 mL). After shaking for 15 min, the mixture was treated with a solution of morpholine (0.005 mL, 0.05 mmol) in DCM (2 mL). The mixture was shaken at room temperature for 16 h and filtered and the resin was washed with methanol (2 × 2 mL). The filtrate was evaporated in vacuo to provide the title compound. The product was submitted for analysis by LCMS without further purification.

General Procedure H: Mitsunobu Alkylation. Compound **13b** (20 mg, < 0.024 mmol) was swelled in THF (1 mL) in a PTFE fritted vessel and gently shaken for 15 min. With gentle agitation for 10 min between the addition of each reagent, the vessel was sequentially treated with TEA (0.05 mL), a solution of triphenylphosphine (0.063 g) in THF (0.5 mL), and a solution of the appropriate alcohol (0.25 mmol) in THF (0.5 mL). After a further 10 min, the vessel was treated with a solution of diisopropyl azodicarboxylate (DIAD) (0.047 mL) in THF (0.5 mL) chilled in a dry ice/acetone bath prior to addition. The reaction vessels were gently agitated for 20 h and drained, the resin was washed with DCM × 2, DMF × 1, methanol × 1, and DCM × 2, and the desired compound was cleaved from the resin according to General procedure G.

6-Bromo-2-ethylsulfanyl-chromen-4-one (24). A mixture of **23** (1.01 g, 4.43 mmol), potassium carbonate (0.70 g, 5.0 mmol), and iodoethane (1.3 mL, 16 mmol) in acetone (50 mL) was heated to reflux for 2 h. The mixture was concentrated in vacuo, and the residue was partitioned between water (100 mL) and DCM (100 mL). The aqueous layer was extracted into DCM (3 × 50 mL), and the organic extracts were combined, dried (Na_2SO_4), and evaporated in vacuo to yield a brown solid. Recrystallization (EtOAc, petrol) gave **24** as off-white crystals (0.820 g, 64%). Mp 155−157 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.38 (3H, t, J = 7.4 Hz, CH_3); 3.02 (2H, q, J = 7.4 Hz, CH_2); 6.19 (1H, s, 3-*H*); 7.45 (1H, d, J = 7 Hz, 8-*H*); 7.65 (1H, dd, J = 2.4, 7 Hz, 7-*H*); 8.23 (1H, d, J = 2.4 Hz, 5-*H*). MS (ESI+) m/z = 288 $[M + H]^+$.

6-Bromo-2-morpholin-4-yl-chromen-4-one (25). A mixture of **24** (0.45 g, 1.58 mmol) and *m*-chloroperbenzoic acid (2.0 mol equiv) in DCM (10 mL) was stirred for 3 h at room temperature, then cooled to −15 °C and filtered, and the filtrate was collected and concentrated in vacuo. The residues were dissolved in warm acetonitrile (25 mL), and morpholine (5 mol equiv) was added. The mixture was stirred at room temperature for 16 h, then concentrated in vacuo. The residues were dissolved in DCM and washed with 2 N HCl, water, and saturated sodium chloride and then dried (Na_2SO_4) and concentrated in vacuo. Recrystallization (MeOH) gave **25** as an off white solid (0.31 g, 63%). Mp 147−149 °C. λ(nm/MeOH) = 217.5 (λ_{max}), 234.0, 301.5; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.44 (4H, m, CH_2N); 3.77 (4H, m, CH_2O); 5.42 (1H, s, 3-*H*); 7.11 (1H, d, 8-*H*); 7.57 (1H, dd, 7-*H*); 8.20 (1H, d, 5-*H*); MS (ESI+) m/z = 310.24 $[M + H]^+$; IR (Diamond ATR) υ_{max} (cm^{-1}) 2968, 2909, 2868, 1639, 1604, 1556, 1408, 1246, 985, 788.

General Procedure I: 6-Position Suzuki Library. To a mixture of the appropriate boronic acid or ester (0.071 mmol), **25** (20 mg, 0.064 mmol), and powdered potassium carbonate (18 mg, 0.13 mmol) in degassed 1,4-dioxane (0.5 mL) was added a solution of tetrakis(triphenylphosphine)palladium(0) (3 mg) in degassed dioxane (0.3 mL), and the resulting mixture was heated to 90 °C for 18 h under nitrogen. The mixture was cooled and passed through a silica plug (isolute Si 500 mg cartridge) and eluted with 30% methanol/DCM (8 mL). The eluant was analyzed by LCMS and purified by preparative HPLC to >95% purity, as estimated by LCMS.

6-(4-Methoxyphenyl)-2-morpholin-4-yl-chromen-4-one (30{*13*}). To a solution of **23** (60 mg, 0.194 mmol) in DME (1 mL) was added aq Na_2CO_3 (1 M, 0.46 mL), 4-methoxyphenylboronic acid (32 mg, 0.21 mmol), and tetrakis(triphenylphosphine)palladium(0) (7 mg, 0.006 mmol). The reaction mixture was heated to reflux for 16 h, cooled, diluted with DCM (10 mL), and washed with water (10 mL), and the organic fraction was evaporated in vacuo. Chromatography (10% MeOH/DCM) gave **30**{*13*} as a white solid (32 mg, 49%). Mp 220−223 °C. IR (KBr) υ_{max} (cm^{-1}) 3072, 2860, 1604, 1554, 1519, 1439, 1352, 1245, 1112, 812. MS (ESI+) m/z = 338.28 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46 (4H, m); 3.78 (7H, m); 5.45 (1H, s); 7.92 (2H, d); 7.26 (1H, d); 7.52 (2H, d); 7.68 (1H, dd); 8.26 (1H, d).

***N*-[2-(2-Morpholin-4-yl-4-oxo-4*H*-chromen-6-yl)phenyl]-acetamide (30{*40*}).** General procedure I with (2-acetylaminophenyl)boronic acid pinacol ester (0.019 g, 0.071 mmol) gave **30**{*40*}, 4.8 mg (21%). MS (ESI+) m/z = 365 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93 (3H, s, CH_3); 2.25 (1H, br s, NH); 3.51 (4H, t, J = 4.8 Hz, NCH_2); 3.78 (4H, t, J = 4.8 Hz, OCH_2); 5.59 (1H, s, 3-*H*); 7.25−7.51 (4H, m, ArH); 7.53−7.58 (1H, m, ArH); 7.93 (1H, d, J = 8.1 Hz, ArH); 8.01 (1H, d, J = 2.8 Hz, ArH). MS (EI), m/z (%): 364 (M^+) (25). HRMS (EI) ($C_{21}H_{20}N_2O_4$) calcd 364.142, obsd 364.141.

2-Hydroxy-4-trifluoromethanesulfonyloxybenzoic acid methyl ester (27a). Trifluromethanesulfonic anhydride (1.31 mL, 7.81 mmol) was added dropwise to a mixture of **26a** (5.0 g, 29.8 mmol), DCM (30 mL), pyridine (1.2 mL, 14.9 mmol), and dimethylaminopyridine (DMAP) (0.07 g, 0.58 mmol) at 0 °C. The mixture was allowed to warm to room temperature, stirred 16 h, then washed with HCl (1 M; 50 mL), dried (Na_2SO_4) and concentrated in vacuo. Recrystallization (EtOAc) gave **27a** as a white solid (4.10 g, 46% yield). Mp 104−105 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00 (3H, s, CH_3); 6.84 (1H, dd, J = 2.5, 6.3 Hz,6-*H*); 6.94 (1H, d, J = 2.5 Hz, 8-*H*); 7.95 (1H, d, J = 6.3 Hz, 5-*H*); MS (ESI+) m/z = 300 $[M + H]^+$.

2-Hydroxy-3-trifluoromethanesulfonyloxybenzoic Acid Methyl Ester (27b). To a mixture of **26b** (4.00 g, 24 mmol), pyridine (0.96 mL, 12 mmol), and DMAP (0.07 g, 0.6 mmol) in

DCM (25 mL) was added trifluoromethanesulfonic anhydride (1.05 mL, 6.25 mmol) dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 60 h, then washed with 1 M HCl (40 mL), dried (Na_2SO_4), and concentrated in vacuo. Recrystallization (EtOAc) gave **27b** as white crystals (2.62 g, 37%). Mp 91.5−92 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (1H, d, Ar4), 7.45 (1H, d, Ar6), 6.95 (1H, t, Ar5), 4.00 (3H, d, CH_3). MS (ESI+) m/z = 300.0 $[M + H]^+$.

General Procedure J: *β*-Keto Amide Formation. To a solution of diisopropylamine (3.28 mol equiv) in THF (15 mL) cooled to −78 °C under nitrogen was added 2.5 M *n*-butyllithium (2.5 M in hexanes, 3.28 mol equiv), and the mixture was stirred for 30 min at 0 °C; a solution of the appropriate of *N*-acetylamine (1.7 mol equiv) in THF (20 mL) was added and stirring continued for 1 h at 0 °C, and then a solution of the appropriate hydroxybenzoic acid methyl ester in THF (20 mL) was added and stirring continued for 5 h. The mixture was adjusted to pH 7 by addition of 1 M hydrochloric acid and extracted into DCM. The combined DCM fractions were dried (Na_2SO_4) and concentrated in vacuo. The residues were triturated with ether to provide the title compound.

Trifluoromethanesulfonic Acid 3-Hydroxy-4-(3-morpholin-4-yl-3-oxopropionyl)phenyl Ester (28a). General procedure J with **27a** (1.65 g, 7 mmol) and *N*-acetylmorpholine (1.09 mL, 9.35 mmol) afforded a brown solid (0.535 g, 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (2H, s, CH_2); 3.64 (8H, m, morpholine); 6.70 (1H, dd, J = 2.4, 8.9 Hz, Ar-*6H*); 7.85 (1H, d, J = 2.4 Hz Ar-*2H*); 7.35 (1H, d, J = 8.9 Hz Ar-*5H*). MS (ESI+) m/z = 398.

Trifluoromethanesulfonic Acid 2-Hydroxy-3-(3-morpholin-4-yl-3-oxopropionyl)phenyl Ester (28b). General procedure J with **27b** (2.10 g, 7 mmol) gave **28b** as brown solid (1.10 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50 (8H, m, morpholine); 4.05 (2H, s, CH_2); 6.90 (1H, dd, Ar−H); 7.85 (1H, d, Ar−H); 7.35 (1H, d, Ar−H). MS (ESI+) m/z = 398.25.

General Procedure K: Synthesis of 2-Amino Chromen-4-ones. To a solution of the appropriate 1-(1-hydroxyaryl)-3-amino propan-1,3-dione in DCM (5 mL) was added triflic anhydride (3.0 mol equiv) under N_2 at 0 °C, and the mixture was stirred at room temperature for 72 h, then concentrated in vacuo. The residues were dissolved in methanol (20 mL) and stirred for 5 h. An equal volume of water (20 mL) was added, and the mixture was stirred for 16 h, then concentrated in vacuo. The aqueous concentrate was adjusted to pH 8 with $NaHCO_3$ (sat.) and extracted with DCM × 3. The DCM extracts were combined, dried (Na_2SO_4), and concentrated in vacuo. Triturated in ether followed by column chromatography gave the desired product.

Trifluoromethanesulfonic Acid 2-Morpholin-4-yl-4-oxo-4*H*-chromen-7-yl Ester (29a). General procedure K with **28a** (0.32 g, 0.81 mmol) gave **29a** as an off-white solid (0.127 g, 41%). Mp 143−145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ3.45 (4H, m, CH_2N); 3.77 (4H, m, CH_2O); 5.36 (1H, s,3-*H*); 7.32 (2H, m, Ar−H); 8.01 (1H, m, Ar−H). MS ESI+ m/z = 380.21 $[M + H]^+$. IR (Diamond ATR) υ_{max} (cm^{-1}) 3084, 1639, 1599, 1571, 1409, 1282, 1132, 781 cm^{-1}.

Trifluoromethanesulfonic Acid 2-Morpholin-4-yl-4-oxo-4*H*-chromen-8-yl Ester (29b). General procedure K with **28b** (0.91 g, 2.3 mmol) gave **29b** as a white solid (0.25 g, 29%). Mp 178−179 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50 (4H, m, CH_2N); 3.78 (4H, m, CH_2O); 5.46 (1H, s, 3-*H*); 7.40 (2H, m, Ar-*H*6, 7); 8.09 (1H, m, Ar-*H*5). MS ESI+ m/z = 380 $[M + H]^+$. λ(nm/MeOH) = 208.5(λ_{max}), 314.0. IR (Diamond ATR) υ_{max} (cm^{-1}) 2870, 1606, 1558, 1207, 1138, 785. C, H, N.

General Procedure L: 7- and 8-Position Suzuki Library. To a mixture of the appropriate boronic acid (0.058 mmol), **29a** or **29b** (20 mg, 0.053 mmol), and powdered potassium carbonate (14.6 mg, 0.106 mmol) in degassed 1,4-dioxane (0.5 mL) was added a solution of tetrakis(triphenylphosphine)palladium(0) (3.1 mg) in degassed dioxane (0.3 mL), and the reaction mixture was heated to 90 °C for 18 h under nitrogen. The mixture was cooled and passed through a silica plug (isolute Si 500 mg cartridge) and eluted with 30% methanol/DCM (8 mL). The solution was analyzed by LCMS and purified by preparative HPLC to >95% purity, as estimated by LCMS.

7-(2-Benzyloxyphenyl)-2-morpholin-4-yl-chromen-4-one (31{*2*}). General procedure L was followed with **29a** and (2-benzyloxyphenyl)boronic acid (0.0132 g, 0.058 mmol) yielding **31**{*2*}, 4.7 mg (21%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45 (4H, t, J = 4.8 Hz, NCH_2); 3.76 (4H, t, J = 4.8 Hz, OCH_2); 5.05 (2H, s, Ar−CH_2); 5.57 (1H, s, 3-*H*); 6.98−7.04 (2H, m, ArH); 7.28−7.32 (4H, m, ArH); 7.49−7.55 (2H, m, ArH); 8.10 (1H, d, J = 8.1 Hz, ArH). MS (ESI+) m/z = 414 $[M + H]^+$. HRMS ($C_{26}H_{23}NO_4$) calcd 413.163, obsd 413.161.

7-(3-Benzyloxyphenyl)-2-morpholin-4-yl-chromen-4-one (31{*3*}). General procedure L was followed with **29a** and (3-benzyloxyphenyl)boronic acid (0.0132 g, 0.058 mmol) yielding **31**{*3*}, 5.0 mg (23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (4H, t, J = 4.8 Hz, NCH_2); 3.78 (4H, t, J = 4.8 Hz, OCH_2); 5.07 (2H, s, Ar−CH_2); 5.49 (1H, s, 3-*H*); 6.94 (1H, d, J = 9.5 Hz, ArH); 7.25−7.49 (7H, m, ArH); 8.13 (1H, d, J = 8.1 Hz). MS (ESI+) m/z = 414 $[M + H]^+$. HRMS (EI) ($C_{26}H_{23}NO_4$) calcd 413.162, obsd 413.161.

3-[3-(2-Morpholin-4-yl-4-oxo-4*H*-chromen-7-yl)phenyl]-acrylic Acid Methyl Ester (31{*6*}). General procedure L with **29a** and [3-(*E*-3-methoxy-3-oxo-1-propen-1-yl)phenyl]-boronic acid (0.0119 g, 0.058 mmol) gave **31**{*6*}, 4.9 mg (24%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (4H, t, J = 4.8 Hz, NCH_2); 3.76 (3H, s, OCH_3); 3.79 (4H, t, J = 4.8 Hz, OCH_2); 5.57 (1H, s, 3-*H*); 6.47 (1H, d, J = 16 Hz, *CH*=CH); 7.30−7.62 (6H, m, ArH); 7.71 (1H, d, J = 16 Hz, CH=*CH*); 8.17 (1H, d, J = 8.2 Hz, ArH). MS (ESI+) m/z = 392 $[M + H]^+$. MS (EI), m/z (%): 391 (M^+) (100). HRMS (EI) ($C_{23}H_{21}NO_5$) calcd 391.063, obsd 391.067.

7-Biphenyl-2-yl-2-morpholin-4-yl-chromen-4-one (31{*14*}). General procedure L with **29a** and 2-biphenylboronic acid (0.0115 g, 0.058 mmol) gave **31**{*14*}, 5.0 mg (25%). MS (ESI+) m/z = 384 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46 (4H, t, J = 4.9 Hz, NCH_2); 3.75 (4H, t, J = 4.9 Hz, OCH_2); 5.69 (1H, s, 3-*H*); 6.98−7.21 (6H, m, ArH); 7.34−7.49 (4H, m, ArH); 7.87 (1H, d, J = 8.1 Hz, ArH). MS (EI), m/z (%): 383 (M^+) (100). HRMS (EI) ($C_{25}H_{21}NO_3$) calcd 383.152, obsd 383.152.

2-Morpholin-4-yl-7-thiophen-2-yl-chromen-4-one (31{*17*}). General procedure L with **29a** and 2-thiophene boronic acid (0.0074 g, 0.058 mmol) gave **31**{*17*}, 1.1 mg (7%). MS (ESI+) m/z = 314 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (4H, t, J = 4.8 Hz, NCH_2); 3.81 (4H, t, J = 4.8 Hz, OCH_2); 6.20 (1H, s, 3-*H*); 7.08 (1H, dd, J = 1.4 and 3.7 Hz, CH−*CH*−CH−S); 7.29−7.55 (3H, m, ArH,); 8.10 (1H, d, J = 8.3 Hz). MS (EI), m/z (%): 313 (M^+) (100). HRMS (EI) ($C_{17}H_{15}NO_3S$) calcd 313.077, obsd 313.077.

2-Morpholin-4-yl-7-(4-trifluoromethoxyphenyl)-chromen-4-one (31{*18*}). General procedure L with **29a** and 4-(trifluoromethoxy)phenyl boronic acid (0.0119 g, 0.058 mmol) gave **31**{*18*}, 6.3 mg (30%). MS (ESI+) m/z = 392 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.56 (4H, t, J = 4.8 Hz, NCH_2); 3.80 (4H, t, J = 4.8 Hz, OCH_2); 5.95 (1H, s, 3-*H*); 7.28 (2H, d, J = 9.8 Hz, ArH); 7.51−7.58(4H, m); 8.17 (1H, d, J = 7.6 Hz, ArH). MS (EI), m/z (%): 391 (M^+) (100). HRMS (EI) ($C_{20}H_{16}NO_4F_3$) calcd 391.103, obsd 391.103.

7-Furan-2-yl-2-morpholin-4-yl-chromen-4-one (31{*20*}). General procedure L with **29a** and 2-furan boronic acid (0.0065 g, 0.058 mmol) gave **31**{*20*}, 6.0 mg (38%). MS (ESI+) m/z = 298 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.59 (4H, t, J = 4.8 Hz, NCH_2); 3.80 (4H, t, J = 4.8 Hz, OCH_2); 6.11 (1H, s, 3-*H*); 6.48 (1H, dd, J = 1.6 and 1.8 Hz); 6.79 (1H, d, J = 2.9 Hz); 7.48 (1H, d, J = 1.3 Hz); 7.58−7.62 (2H, m, ArH); 8.08 (1H, d, J = 8.3 Hz, ArH). MS (EI), m/z (%): 297 (M^+) (100). HRMS (EI) ($C_{17}H_{15}NO_4$) calcd 297.100, obsd 297.099.

7-(3-Acetylphenyl)-2-morpholin-4-yl-chromen-4-one (31{*24*}). General procedure L with **29a** and 3-acetylphenylboronic acid (0.0095 g, 0.058 mmol) gave **31**{*24*}, 5.1 mg (28%). MS (ESI+) m/z = 350 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (3H, s, CH_3); 3.52 (4H, t, J = 4.8 Hz, NCH_2); 3.80 (4H, t, J = 4.8 Hz, OCH_2); 5.61 (1H, s, 3-*H*); 7.49−7.60 (3H, m, ArH); 7.76−7.79 (1H, m, ArH); 7.90−7.94 (1H, m, ArH); 8.15−8.19

(2H, m, ArH). MS (EI), *m/z* (%): 349 (M^+) (100). HRMS (EI) ($C_{21}H_{19}NO_4$) calcd 349.131, obsd 349.131.

2-Morpholin-4-yl-7-(3-trifluoromethoxyphenyl)-chromen-4-one (31{*25*}). General procedure L with **29a** and 3-(trifluoromethoxy)phenylboronic acid (0.0119 g, 0.058 mmol) gave **31**{*25*}, 6.8 mg (33%). MS (ESI+) *m/z* = 392 $[M + H]^+$. ^{1}H NMR (300 MHz, CDCl3) δ 3.53 (4H, t, J = 4.8 Hz, NCH2); 3.79 (4H, t, J = 4.8 Hz, OCH2); 5.70 (1H, s, 3-H); 7.20−7.25 (1H, m, ArH); 7.41−7.54 (5H, m, ArH); 8.17 (1H, d, J = 8.2 Hz, ArH). MS (EI), *m/z* (%): 391 (M^+) (79). HRMS (EI) ($C_{20}H_{16}NO_4F$) calcd 391.103, obsd 391.104.

7-(4-Hydroxymethylphenyl)-2-morpholin-4-yl-chromen-4-one (31{*32*}). General procedure L with **29a** and 4-(hydroxymethyl)phenyl boronic acid (0.0088 g, 0.058 mmol) gave **31**{*32*}, 3.7 mg (21%). MS (ESI+) *m/z* = 338 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (4H, t, J = 4.6 Hz, NCH_2); 3.78 (4H, t, J = 4.6 Hz, OCH_2); 4.71 (2H, s, CH_2OH); 5.54 (1H, s, 3-*H*); 7.41−7.45 (3H, m, ArH); 7.51−7.59 (3H, m, ArH); 8.12 (1H, d, J = 8.2 Hz, ArH). MS (EI), *m/z* (%): 337 (M^+) (100). HRMS (EI) ($C_{20}H_{19}NO_4$) calcd 337.131, obsd 337.133.

2-Morpholin-4-yl-7-(2-trifluoromethylphenyl)chromen-4-one (31{*39*}). General procedure L with **29a** and 2-(trifluoromethyl)phenylboronic acid (0.0110 g, 0.058 mmol) gave **31**{*39*}, 5.3 mg (27%). MS (ESI+) *m/z* = 376 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.46 (4H, t, J = 4.7 Hz, NCH_2); 3.77 (4H, t, J = 4.7 Hz, OCH_2); 5.51 (1H, s, 3-*H*); 7.23−7.29 (3H, m, ArH); 7.44−7.56 (2H, m, ArH); 7.72 (1H, d, J = 7.1 Hz, ArH); 8.11 (1H, d, J = 8.2 Hz, ArH). MS (EI), *m/z* (%): 375 (M^+) (100). HRMS ($C_{20}H_{16}NO_3F_3$) calcd 375.108, obsd 375.109.

8-(4-Methoxyphenyl)-2-morpholin-4-yl-chromen-4-one (32{*13*}). General procedure L with **29b** and 4-methoxyphenyl boronic acid (8.8 mg, 0.058 mmol) gave **32**{*13*}, 2.5 mg (14%). MS (ESI+) *m/z* = 338 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30 (4H, t, J = 4.8 Hz, NCH_2); 3.68 (4H, t, J = 4.8 Hz, OCH_2); 3.81 (3H, s, CH_3); 5.48 (1H, s, 3-*H*); 6.90−6.93 (2H, m, ArH); 7.29−7.37 (3H, m, ArH); 7.41−7.49 (1H, m, ArH); 8.07 (1H, d, J = 7.8 Hz, ArH). MS (EI), *m/z* (%): 337 (M^+) (100). HRMS (EI) ($C_{20}H_{19}NO_4$) calcd 337.131, obsd 337.130.

2-Morpholin-4-yl-8-(4-trifluoromethoxyphenyl)-chromen-4-one (32{*18*}). General procedure L with **29b** and 4-(trifluoromethoxy)phenyl boronic acid (11.9 mg, 0.058 mmol) gave **32**{*18*}, 3.9 mg (19%). MS (ESI+) *m/z* = 392 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.34 (4H, t, J = 4.8 Hz, NCH_2); 3.75 (4H, t, J = 4.8 Hz, OCH_2); 5.56 (1H, s, 3-*H*); 7.33−7.36 (2H, m, ArH); 7.41−7.46 (1H, m, ArH); 7.54−7.57 (3H, m, ArH); 8.21 (1H, d, J = 7.8 Hz, ArH). MS (EI), *m/z* (%): 383 (M^+) (65). HRMS (EI) ($C_{25}H_{21}NO_3$) calcd 383.152, obsd 383.153.

2-Morpholin-4-yl-8-(3-trifluoromethoxyphenyl)-chromen-4-one (32{*25*}). General procedure L with **29b** and 3-(trifluoromethoxy)phenyl boronic acid (11.9 mg, 0.058 mmol) gave **32**{*25*}, 7.2 mg (35%). MS (ESI+) *m/z* = 392 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.35 (4H, t, J = 4.8 Hz, NCH_2); 3.75 (4H, t, J = 4.8 Hz, OCH_2); 5.57 (1H, s, 3-*H*); 7.44−7.47 (4H, m, ArH); 7.51−7.59 (2H, m, ArH); 8.23 (1H, d, J = 7.8 Hz, ArH). MS (EI), *m/z* (%): 391 (M^+) (100). HRMS (EI) ($C_{20}H_{16}NO_4F_3$) calcd 391.103, obsd 391.105.

8-Dibenzothiophen-1-yl-2-morpholin-4-yl-chromen-4-one (32{*38*}). To a solution of **29b** (150 mg, 0.396 mmol) in dioxane (5 mL) was added K_2CO_3 (109 mg, 0792 mmol), dibenzothiophene-4-boronic acid (108 mg, 0.475 mmol), and tetrakis(triphenylphosphine)palladium(0) (23 mg, 0.020 mmol). The reaction mixture was heated to reflux for 16 h, cooled, diluted with DCM (15 mL), and washed with water (10 mL), and the organic fraction was evaporated in vacuo. Chromatography (10% MeOH/DCM) gave **32**{*38*} as an off-white solid (59 mg, 35%). Mp 219.5−221 °C. UV (nm/EtOH) λ_{max} = 233.0. IR (Diamond ATR) υ_{max} (cm^{-1}): 3055, 2948, 2855, 1622, 1562, 1408, 1242, 1114, 991, 867, 787, 740. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (4H, m); 3.43 (4H, m); 5.45 (1H, s); 7.41 (4H, m); 7.51 (1H, t); 7.72 (2H, m); 8.17 (2H, m); 8.21 (1H, m); MS (ESI+) *m/z* = 414 $[M + H]^+$; Anal. Calcd for $C_{25}H_{19}NO_3S$; C, 72.62, H, N; Found: C, 71.51.

2-(2-Bromophenylsulfanyl)cyclohexanone (33).[22] To a solution sodium hydroxide (156 mg, 3.8 mmol) in ethanol (5 mL) and water (5 mL) was added dropwise 2-bromobenzenethiol (0.46 mL, 3.8 mmol), followed by 2-chlorocyclohexanone (560 mg, 42 mmol) in ethanol (3 mL). The mixture was stirred 30 min at room temperature then heated to reflux 16 h. When the mixture cooled, water (10 mL) was added, the organic layer was removed, and the aqueous phase was extracted with chloroform (30 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated in vacuo. Chromatography (neutral alumina; 40% toluene, hexane) gave **33** as a translucent oil (841 mg, 78% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53−1.98 (m, 4H, CH_2); 2.02−2.26 (m, 3H, CH_2); 2.82−2.98 (m, 1H, CH_2); 3.84 (t, J = 5.9 Hz, 1H, CHS); 6.98 (t, J = 7.2 Hz, 1H, Ph); 7.16 (t, J = 7.2 Hz, 1H, Ph); 7.33 (d, J = 7.8 Hz, 1H, Ph); 7.47 (d, J = 7.8 Hz, 1H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7; 27.5; 33.8; 39.1; 54.9; 125.9; 128.0; 128.3; 132.1; 133.3; 135.4; 207.5.

6-Bromo-1,2,3,4-tetrahydrodibenzothiophene (34).[23] A mixture of polyphosphoric acid (1.12 g, 6.5 mmol) and phosphorus pentoxide (250 mg, 1.8 mmol) was slowly heated to 180 °C with continuous stirring. Compound **33** (203 mg, 0.71 mmol) was added, and the reaction mixture was stirred for 30 min at 180 °C. When the mixture cooled, water (30 mL) was added, and the mixture was extracted with ether (90 mL). The organic extract was dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography (silica gel; petroleum ether) gave **34** as an oil (153 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79−1.85 (m, 4H, CH_2); 2.58−2.61 (m, 2H, CH_2); 2.73−2.76 (m, 2H, CH_2); 7.08 (t, J = 7.8 Hz, 1H, Ph); 7.29 (d, J = 7.7 Hz, 1H, Ph); 7.38 (d, J = 7.9 Hz, 1H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.5; 23.9; 24.3; 26.0; 116.2; 119.7; 125.6; 126.8; 130.9; 138.7; 141.3.

4,4,5,5-Tetramethyl-2-(6,7,8,9-tetrahydrodibenzothiophen-4-yl)-1,3,2]dioxaborolane (35). A mixture of **34** (500 mg, 1.87 mmol), $PdCl_2$(1,1′-Bis(diphenylphosphino)ferrocene(dppf)) (150 mg, 0.19 mmol), potassium acetate (1.10 g, 11.23 mmol), and bis(pinacolato)diboron (713 mg, 2.81 mmol) in THF (25 mL) was heated to reflux 16 h. Ethyl acetate (20 mL) was added, and the organic layer was washed with water (20 mL) and brine (20 mL), then dried ($MgSO_4$), and concentrated in vacuo. Chromatography on (silica; 5% ethyl acetate, petroleum ether) gave **35a** (282 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 12H, $C(CH_3)_2$); 1.78−1.80 (m, 4H, CH_2); 2.59−2.61 (m, 2H, CH_2); 2.74−2.76 (m, 4H, CH_2); 7.22 (t, J = 7.5 Hz, 1H, Ph); 7.52 (d, J = 7.9 Hz, 1H, Ph); 7.65 (d, J = 7.0 Hz, 1H, Ph).

2-Morpholin-4-yl-8-(6′,7′,8′,9′-tetrahydrodibenzothiophen-4′-yl)chromen-4-one (36). A mixture of **35** (48 mg, 0.75 mmol), **29b** (63 mg, 0.83 mmol), $PdCl_2$(dppf) (4 mg, 0.023 mmol), and Cs_2CO_3 (147 mg, 2.25 mmol) in degassed THF (5 mL) was heated to reflux for 16 h. Water (10 mL) was added, and the mixture was extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. HPLC (Genesis C18; methanol, water) gave **36** (23 mg, 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80−1.90 (m, 4H, CH_2); 2.69−2.75 (m, 4H, CH_2); 3.05−3.08 (m, 4H, CH_2N); 3.52−3.55 (m, 4H, CH_2O); 5.44 (s, 1H, CH); 7.22 (dd, J = 1.0 and 7.3, 1H, Ph); 7.34−7.40 (m, 2H, Ph); 7.56 (d, J = 7.9 Hz, 1H, Ph); 7.69 (dd, J = 1.7 and 7.8 Hz, 1H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7; 24.0; 24.3; 26.0; 44.9; 66.3; 87.2; 120.7; 124.4; 125.1; 125.4; 126.0; 129.3; 130.2; 133.7; 138.0; 140.6; 143.7; 147.9; 162.5; 177.6; 200.5. IR (Diamond ATR) υ_{max} (cm^{-1}) 2918, 2853, 1619, 1563, 1386, 1238, 1104, 1032, 840, 776, 728. LCMS (ESI+) *m/z* = 419 $[M + H]^+$. C, H, N.

Enzyme Inhibition and Clonogenic Survival Assays. In vitro assays for DNA-PK, ATM, ATR, mTOR, and PI3-K (p110α) were conducted as previously described.[19] Cell-based clonogenic assays were performed as previously described.[19]

Acknowledgment. The authors thank Adrian Moore for purification of the Suzuki-coupling library compounds and Cancer Research U.K. and EPSRC for funding.

Supporting Information Available: Full experimental details for 2-alkylaminobenzo[*h*]chromenone library, full experimental details for the 6- and 7- alkoxy-chromenone librar-

ies, a list of kinases included in the 60 kinase panel, and combustion analyses for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Smith, G. C. M.; Jackson, S. P. The DNA-dependent protein kinase. *Genes Dev.* **1999**, *13*, 916−934.

(2) Shiloh, Y. ATM and related protein kinases: Safeguarding genome integrity. *Nat. Rev. Cancer* **2003**, *3*, 155−168.

(3) Gottlieb, T. M.; Jackson, S. P. The DNA-Dependent Protein-Kinase − Requirement for DNA Ends and Association with Ku Antigen. *Cell* **1993**, *72*, 131−142.

(4) Chan, D. W.; Chen, B. P.-C.; Prithivirajsingh, S.; Kurimasa, A.; Story, M. D.; Qin, J.; Chen, D. J. Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. *Genes Dev.* **2002**, *16*, 2333−2338.

(5) Ding, Q.; Reddy, Y. V. R.; Wang, C. G.; Woods, T.; Douglas, P.; Ramsden, D. A.; Lees-Miller, S. P.; Meek, K. Autophosphorylation of the Catalytic Subunit of the DNA-Dependent Protein Kinase Is Required for Efficient End Processing during DNA Double-Strand Break Repair. *Mol. Cell. Biol.* **2003**, *23*, 5836−5848.

(6) Stiff, T.; O'Driscoll, M.; Rief, N.; Iwabuchi, K.; Lobrich, M.; Jeggo, P. A. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res.* **2004**, *64*, 2390−2396.

(7) Jack, M. T.; Woo, R. A.; Motoyama, N.; Takai, H.; Lee, P. W. K. DNA-dependent protein kinase and checkpoint kinase 2 synergistically activate a latent population of p53 upon DNA damage. *J. Biol. Chem.* **2004**, *279*, 15269−15273.

(8) Jackson, S. P. DNA damage detection by DNA dependent protein kinase and related enzymes. *Cancer Surv.* **1996**, *28*, 261−279.

(9) Jackson, S. P.; Jeggo, P. A. DNA double-strand break repair and V(D)J recombination; involvement of DNA-PK. *Trends Biochem. Sci.* **1995**, *20*, 412−415.

(10) Powis, G.; Bonjouklian, R.; Berggren, M. M.; Gallegos, A.; Abraham, R.; Ashendel, C.; Zalkow, L.; Matter, W. F.; Dodge, J.; Grindey, G.; Vlahos, C. J. Wortmannin, a Potent and Selective Inhibitor of Phosphatidylinositol-3-Kinase. *Cancer Res.* **1994**, *54*, 2419−2423.

(11) Izzard, R. A.; Jackson, S. P.; Smith, G. C. M. Competitive and noncompetitive inhibition of the DNA-dependent protein kinase. *Cancer Res.* **1999**, *59*, 2581−2586.

(12) Ismail, I. H.; Martensson, S.; Moshinsky, D.; Rice, A.; Tang, C.; Howlett, A.; McMahon, G.; Hammarsten, O. SU11752 inhibits the DNA-dependent protein kinase and DNA double-strand break repair resulting in ionizing radiation sensitization. *Oncogene* **2004**, *23*, 873−882.

(13) Kashishian, A.; Douangpanya, H.; Clark, D.; Schlachter, S. T.; Eary, C. T.; Schiro, J. G.; Huang, H. M.; Burgess, L. E.; Kesicki, E. A.; Halbrook, J. DNA-dependent protein kinase inhibitors as drug candidates for the treatment of cancer. *Mol. Cancer Ther.* **2003**, *2*, 1257−1264.

(14) Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F. A Specific Inhibitor of Phosphatidylinositol 3-Kinase, 2-(4- Morpholinyl)-8-Phenyl-4h-1-Benzopyran-4-One (LY294002). *J. Biol. Chem.* **1994**, *269*, 5241−5248.

(15) Boulton, S.; Kyle, S.; Yalcintepe, L.; Durkacz, B. W. Wortmannin is a potent inhibitor of DNA double strand break but not single strand break repair in Chinese hamster ovary cells. *Carcinogenesis* **1996**, *17*, 2285−2290.

(16) Rosenzweig, K. E.; Youmell, M. B.; Palayoor, S. T.; Price, B. D. Radiosensitization of human tumor cells by the phosphatidylinositol 3-kinase inhibitors Wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G(2)-M delay. *Clin. Cancer Res.* **1997**, *3*, 11149−1156.

(17) Hollick, J. J.; Golding, B. T.; Hardcastle, I. R.; Martin, N.; Richardson, C.; Rigoreau, L. J. M.; Smith, G. C. M.; Griffin, R. J. 2,6-Disubstituted pyran-4-one and thiopyran-4-one inhibitors of DNA-dependent protein kinase (DNA-PK). *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3083−3086.

(18) Leahy, J. J. J.; Golding, B. T.; Griffin, R. J.; Hardcastle, I. R.; Richardson, C.; Rigoreau, L.; Smith, G. C. M. Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6083−6087.

(19) Griffin, R. J.; Fontana, G.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Leahy, J. J. J.; Martin, N.; Richardson, C.; Rigoreau, L.; Stockley, M.; Smith, G. C. M. Selective benzopyranone and pyrimido[2,1-*a*]isoquinolin-4-one inhibitors of DNA-dependent protein kinase: Synthesis, structure−activity studies, and radiosensitization of a human tumor cell line in vitro. *J. Med. Chem.* **2005**, *48*, 569−585.

(20) Veuger, S. J.; Curtin, N. J.; Richardson, C. J.; Smith, G. C.; Durkacz, B. W. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. *Cancer Res.* **2003**, *63*, 6008−6015.

(21) Morris, J.; Wishka, D. G. Synthesis of 2-amino-6-phenyl-4H-pyran-4-ones. *Synthesis* **1994**, 43−46.

(22) Tedjamulia, M. L.; Tominaga, Y.; Castle, R. N.; Lee, M. L. The Synthesis of All of the Dimethyldibenzothiophenes and Monoethyldibenzothiophenes. *J. Heterocyclic Chem.* **1983**, *20*, 1485−1495.

(23) Ong, H. H.; Caldwell, S. R.; Profitt, J. A.; Tegeler, J. J.; Agnew, M. N.; Wilker, J. C.; Ress, R. J.; Kitzen, J. M. [(6,7-Dichlorobenzo-[*b*]thien-5-yl)oxy]acetic acids and 1,1-dioxides. 1. A structurally novel class of diuretics with hypotensive activity. *J. Med. Chem.* **1987**, *30*, 2295−2303.

(24) Murata, M.; Oyama, T.; Watanabe, S.; Masuda, Y. Palladium-catalyzed borylation of aryl halides or triflates with dialkoxyborane: A novel and facile synthetic route to arylboronates. *J. Org. Chem.* **2000**, *65*, 164−168.

(25) Colandrea, V. J.; Naylor, E. M. Synthesis and regioselective alkylation of 1,6-and 1,7- naphthyridines. *Tetrahedron Lett.* **2000**, *41*, 8053−8057.

(26) Walker, E.; Pacold, M.; Perisic, O.; Stephens, L.; Hawkins, P.; Wymann, M.; Williams, R. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol. Cell* **2000**, *6*, 909−919.

(27) Veuger, S.; Durkacz, B. W.; Golding, B. T.; Griffin, R. J.; Martin, N.; Newell, D. R.; Rigoreau, L.; Stockley, M.; Webber, S. E.; Hostomsky, Z.; Curtin, N. J. Radiosensitization by novel inhibitors of PARP, PI, and DNA-PK, NU7026, in vitro. *Br. J. Cancer* **2001**, *85*, 37−37.

JM050444B