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

Roger J. Griffin,*,† Gabriele Fontana,† Bernard T. Golding,† Sophie Guiard,† Ian R. Hardcastle,† Justin J. J. Leahy,† Niall Martin,‡ Caroline Richardson,‡ Laurent Rigoreau,†,‡ Martin Stockley,† and Graeme C. M. Smith*,‡

Northern Institute for Cancer Research, School of Natural Sciences—Chemistry, Bedson Building, The University, Newcastle upon Tyne NE1 7RU, U.K., and KuDOS Pharmaceuticals Ltd., 327 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, U.K.

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A diverse range of chromen-2-one, chromen-4-one and pyrimidoisoquinolin-4-one derivatives was synthesized and evaluated for inhibitory activity against the DNA repair enzyme DNA-dependent protein kinase (DNA-PK), with a view to elucidating structure−activity relationships for potency and kinase selectivity. DNA-PK inhibitory activity varied widely over the series of compounds evaluated (IC_{50} values ranged from 0.19 to >10 μM), with excellent activity being observed for the 7,8-benzochromen-4-one and pyrimido[2,1-*a*]isoquinolin-4-one templates. By contrast, inhibitors based on the benzochromen-2-one (coumarin) or 2-aryl-7,8-benzochromen-4-one (flavone) scaffolds were less potent. Crucially, these studies revealed a very constrained structure−activity relationship at the 2-position of the benzopyranone and pyrimido[2,1-*a*]-isoquinolin-4-one pharmacophore, with only a 2-morpholino or 2-(2′-methylmorpholino) group being tolerated at this position. More detailed biological studies conducted with the most potent inhibitor NU7163 (**48**; IC_{50} = 0.19 μM) demonstrated ATP-competitive DNA-PK inhibition, with a K_i value of 24 nM, and **48** exhibited selectivity for DNA-PK compared with the related enzymes ATM, ATR, mTOR, and PI 3-K (p110alpha). Compound **48** sensitized the HeLa human tumor cell line to the cytotoxic effects of ionizing radiation in vitro, a dose modification factor of 2.3 at 10% survival being observed with an inhibitor concentration of 5 μM. This study identified these structural classes as novel DNA-PK inhibitors and delineated initial structure−activity relationships against DNA-PK.

Introduction

The ability of cancer cells to survive radio- or chemotherapy is often due to their efficiency in repairing the DNA damage that these treatments elicit.[1−3] A key enzyme involved in the repair of DNA double strand breaks (DSBs) is the serine/threonine protein kinase DNA-dependent protein kinase (DNA-PK).[4] DNA-PK comprises a large (465 kDa.) catalytic subunit, DNA-PKcs, and a heterodimeric DNA targeting subunit termed Ku, with subunits of ∼70 kDa (Ku70) and ∼83 kDa (Ku80). DNA-PK plays a key role in the non-homologous end-joining (NHEJ) component of the DNA DSB repair pathway and in the site-specific end joining process of V(D)J recombination.[3,4] A wealth of genetic data has revealed that cells that are ablated for any of the components of DNA-PK are sensitive to ionizing radiation and to chemotherapeutics that induce DNA DSBs, including bleomycin and etoposide. Although DNA-PKcs has been shown to bind to DNA alone, it appears that its activation and stabilization at a DNA break is greatly dependent on the high-affinity DNA-end binding properties of Ku.[5−7] Once bound to the site of a DNA-DSB, DNA-PK is believed to act as a scaffold for other components of the NHEJ pathway including Artemis[8] and the DNA-ligase IV/XRCC4 complex.[9] The kinase activity of DNA-PK appears essential for the cellular ability to repair DNA DSBs, as a DNA-PK deficient cell line was found to retain a radiosensitive phenotype following introduction of a "kinase dead" version of DNA-PK.[10]

One of the few clearly identifiable motifs in the 4,128 amino acid polypeptide of DNA-PKcs is a kinase domain of some 400 amino acid residues in the extreme COOH-terminal of the protein.[11] Notably, this region shows very little homology to classical serine/threonine protein kinases, but it does show extensive sequence homology to the phosphatidylinositol (PI) 3-kinase related kinase (PIKK) family, a group of large (∼250 kDa) protein kinases involved in the signaling of cellular stress responses, which includes the mammalian proteins ATM, ATR, mTOR, and hSMG1.[12,13] Despite their kinase domain homology to the lipid kinases,[14] PIKK family members have not been demonstrated to phosphorylate lipid substrates.[15]

Consistent with the homology found within the kinase domain of the PIKK family, the benchmark PI 3-K inhibitors wortmannin[16] (**1**) and LY294002[17] (**2**) have also been shown to inhibit other family members, including DNA-PK (K_i values of 20−120 nM and 6 μM, respectively).[11,18−20] Both **1** and **2** have been shown to

* Corresponding authors. Inquiries concerning chemistry should be addressed to R.J.G., and those concerning biology should be addressed to G.C.M.S. For R.J.G.: telephone, (44) 191 222 8591; fax, (44) 191 222 8591; e-mail, r.j.griffin@ncl.ac.uk. For G.C.M.S.: telephone, (44) 122 343 4731; fax, (44) 122 371 9720; e-mail, gcmsmith@kudospharma.co.uk.

† The University, Newcastle upon Tyne.

‡ KuDOS Pharmaceuticals Ltd.

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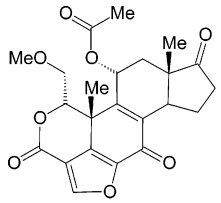

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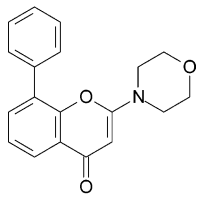


2

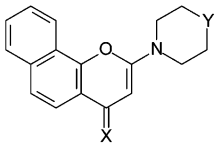
sensitize tumor cells to ionizing radiation and DSB-inducing chemotherapeutics, as well as inhibiting DNA-DSB repair,[21−23] most probably as a result of inhibition of DNA-PK and ATM. It is thus evident that **1** and **2** are able to inhibit multiple kinases and down-regulate a variety of stress response pathways. Importantly, these studies have clearly revealed that inhibition of PIKKs (e.g. DNA-PK or ATM) can lead to the potential therapeutic end-points of radio- and chemo-sensitization. Kinase-specific small-molecule inhibitors would also aid in elucidating the roles played by PIKKs (and PI 3-Ks) in the cellular response to DNA damage.

Our studies have centered on the development of potent and selective inhibitors of DNA-PK as agents to enhance the cytotoxicity of DNA-damaging therapies used in the treatment of cancer. In the absence of a suitable crystal structure of DNA-PK, we have utilized **2** as a core pharmacophore template for inhibitor design and have conducted structural modifications with a view to elucidating structure−activity relationships (SARs) for DNA-PK inhibition. In this paper we describe the synthesis and biological evaluation of a range of benzopyranone and pyrimidoisoquinolin-4-one DNA-PK inhibitors and the identification of compounds that combine excellent DNA-PK inhibitory activity with selectivity over other PIKK family members. One of the most potent compounds identified, 2-(2-methylmorpholin-4-yl)benzo[*h*]chromen-4-one (**48**), was shown to exhibit ATP-competitive inhibition kinetics against DNA-PK and to sensitize a human tumor cell line to ionizing radiation in vitro. A preliminary account of some of these studies has been published previously.[24]

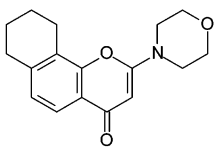
Chemistry

The structures and properties of all compounds synthesized and evaluated for DNA-PK-inhibitory activity are recorded in Tables 1 and 2. Unless indicated, compounds with a chiral center were prepared as a racemic mixture and evaluated as such. Although utilized for the synthesis of the known 7,8-benzochromone **8**, the literature procedure[17] offered limited scope for structural diversity, and the alternative method developed by Morris et al.[25] was found to be more versatile. Reaction of the appropriate 2-hydroxyarylcarboxylate esters, prepared by carboxylation−esterification of the corresponding phenols by standard methods, with *N*-acetylmorpholine, *N*-acetylthiomorpholine, or *N*-acetylpiperidine afforded the *β*-ketoamides (**14**−**26**), and ring closure to the required chromones (**2**, **3**, **5**−**7**, **30**−**36**, **38**) was readily effected with triflic anhydride (Scheme 1). The 4-thiochromone (**4**) was prepared from the chromone (**3**), on treatment with Lawesson's reagent, while treatment of the 2-thiomorpholinylchromone (**36**) with *m*-CPBA gave the corresponding oxothiomorpholine derivative (**37**).

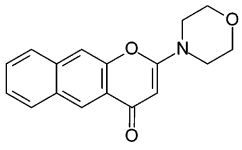
Table 1. Physical Data and Biological Activity of the Core Templates



3; X = Y = O
4; X = S; Y = O
5; X = O; Y = S



6



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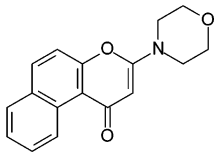

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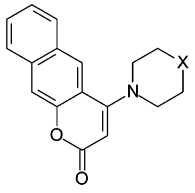
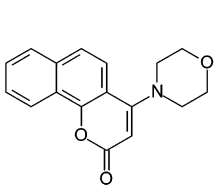


9; X = O
10; X = S



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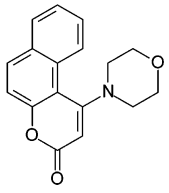

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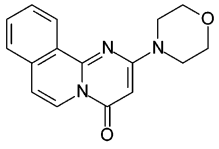
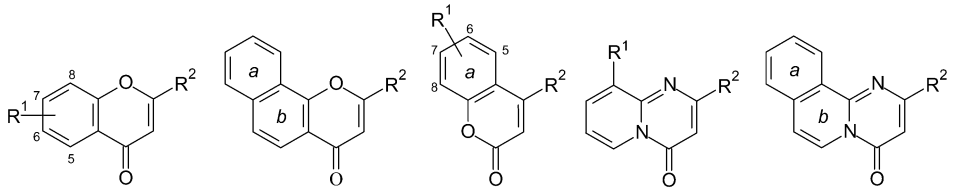


13

compd	method[a]	solvent[b]	yield (%)	mp (°C)	formula	DNA-PK inhibn, IC_{50} (μM)
2	I	A	74	183−5	$C_{19}H_{17}NO_3$	1.47
3	I	A	63	267−9	$C_{17}H_{15}NO_3$	0.23
4	−	C	53	271−2	$C_{17}H_{15}NO_2S$	0.26
5	I	A	46	171−3	$C_{17}H_{15}NO_2S$	1.38
6	I	A	66	220−2	$C_{17}H_{19}NO_3$	0.36
7	I	D	66	219−20	$C_{17}H_{15}NO_3$	0.39
8[c]	−	−	−	−	$C_{17}H_{15}NO_3$	1.25
9	−	E	12	244−5	$C_{17}H_{15}NO_3$	3.24
10	VI	E	32	218−20	$C_{17}H_{15}NO_2S$	5.15
11	V	E	3	207−8	$C_{17}H_{15}NO_3$	6.66
12	V	E	7	206−7	$C_{17}H_{15}NO_3$	1.9
13	VII	B	83	184−6	$C_{16}H_{15}N_3O_2$	0.28

[a] See the Experimental Section. [b] Recrystallization solvents: *A*, MeOH−H_2O; *B*, EtOH (trituration). Chromatography solvents: *C*, petrol:EtOAc (4:1); *D*, DCM:MeOH (95:5); *E*, EtOAc. [c] Prepared as described in ref 17.

Table 2. Physical Data and Biological Activity for Benzopyran-4-ones, Benzopyran-2-ones, and Pyrimidoisoquinolinones



A **B** **C** **D** **E**

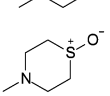
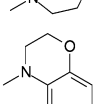
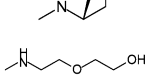
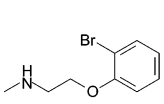
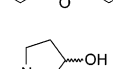
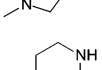
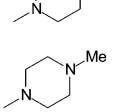
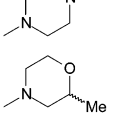
Compound No.	general structure	R^1	R^2	Method[a]	*Solvent system*[b]	yield (%)	mp (°C)	formula	DNA-PK Inhibition IC_{50} (μM)
30	A	H		I	*F*	49	143-144	$C_{13}H_{13}NO_3$	1.26
31	A	8-Me		I	*F*	32	147-148	$C_{14}H_{15}NO_3$	1.65
32	A	7-MeO		I	*F*	72	174-175	$C_{14}H_{15}NO_4$	0.95
33	A	8-MeO		I	*F*	32	182-184	$C_{14}H_{15}NO_4$	1.20
34	A	6-Ph		I	*F*	72	218-220	$C_{19}H_{17}NO_3$	2.24
35	A	8-Ph		I	*F*	67	150-151	$C_{20}H_{19}NO_2$	4.67
36	A	8-Ph		I	*F*	44	191-192	$C_{19}H_{17}NO_2S$	1.61
37	A	8-Ph		–	*F*	34	243-244	$C_{19}H_{17}NO_3S$	5.71
38	B	–		I	*B*	32	205-207	$C_{18}H_{17}NO_2$	3.81
39	B	–		–	*B*	19	234-236	$C_{17}H_{15}NO_2$	6.17
40	B	–		II	*B*	57	138-140	$C_{18}H_{17}NO_3$	2.01
41	B	–		–	*B*	10	233-234	$C_{21}H_{15}NO_3$	> 10
42	B	–		II	*F*	14	oil	$C_{18}H_{15}NO_3$	3.19
43	B	–		II	*F*	86	106-107	$C_{17}H_{17}NO_4$	> 10
44	B	–		II	*F*	43	207-208	$C_{21}H_{16}BrNO_3$	> 10
45	B	–		III	*A*	72	256-257	$C_{17}H_{15}NO_3$	9.27
46	B	–		III	*B*	28	208-209	$C_{17}H_{16}N_2O_2$	> 10
47	B	–		III	*C*	67	184-185	$C_{18}H_{18}N_2O_2$	> 10
48	B	–		III	*F*	21	181-183	$C_{18}H_{17}NO_3$	0.19

Table 2. (Continued)

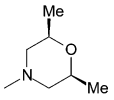
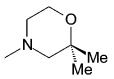
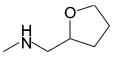
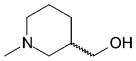
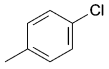
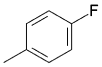
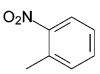
Compound No.	general structure	R^1	R^2	Method[a]	*Solvent system*[b]	yield (%)	mp (°C)	formula	DNA-PK Inhibition IC_{50} (μM)
49	B	–		III	*F*	44	211-213	$C_{19}H_{19}NO_3$	4.14
50	B	–		III	*F*	31	182-184	$C_{19}H_{19}NO_3$	3.52
51	B	–		III	*A*	37	139-140	$C_{18}H_{17}NO_3$	> 10
52	B	–		III	*A*	43	209-210	$C_{19}H_{19}NO_3$	> 10
59	C	H		V	*G*	62	143-144	$C_{13}H_{13}NO_3$	4.82
60	C	H		V	*E*	51	106-107	$C_{14}H_{15}NO_2$	8.81
61	C	6-Me		V	*E*	65	171-172	$C_{14}H_{15}NO_3$	5.35
62	C	8-Me		V	*E*	72	132-133	$C_{14}H_{15}NO_3$	8.05
63	C	5-MeO		V	*E*	42	182-183	$C_{14}H_{15}NO_4$	> 10
64	C	6-MeO		V	*E*	34	153-154	$C_{14}H_{15}NO_4$	1.75
65	C	7-MeO		V	*E*	17	147-148	$C_{14}H_{15}NO_4$	11.31
66	C	8-MeO		-	*G*	6	125-126	$C_{14}H_{15}NO_4$	10.94
67	C	6-Cl		V	*E*	75	163-164	$C_{13}H_{12}ClNO_3$	3.27
68	A	H	Ph	–	–	–	–	–	> 10
69	B	–	Ph	VI	*B*	45	159-161	$C_{19}H_{12}O_2$	4.98
70	B	–		VI	*B*	55	180-181	$C_{19}H_{11}ClO_2$	> 10
71	B	–		VI	*B*	55	195-197	$C_{19}H_{11}ClO_2$	> 10
72	B	–		VI	*B*	88	241-243	$C_{19}H_{11}ClO_2$	> 10
73	B	–		VI	*B*	38	172-173	$C_{19}H_{11}FO_2$	8.59
74	B	–		VI	*B*	55	196-197	$C_{19}H_{11}FO_2$	5.71
75	B	–		VI	*B*	71	205-206	$C_{19}H_{11}FO_2$	> 10
76	B	–		VI	*B*	77	223-224	$C_{19}H_{11}NO_4$	> 10

Table 2. (Continued)

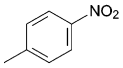
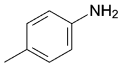
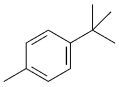
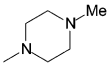
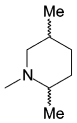
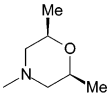
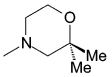
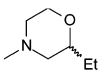
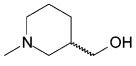
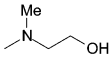
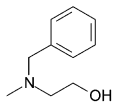
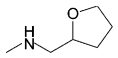
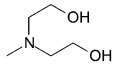
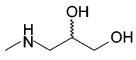
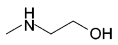
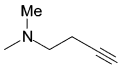
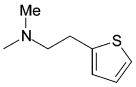
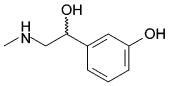
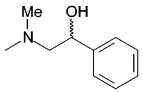
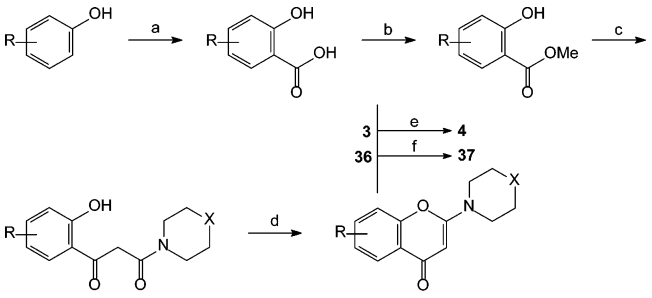
Compound No.	general structure	R^1	R^2	Method[a]	*Solvent system*[b]	yield (%)	mp (°C)	formula	DNA-PK Inhibition IC_{50} (μM)
77	B	–		VI	*B*	51	263-264	$C_{19}H_{11}NO_4$	> 10
78	B	–		VI	*B*	57	290-292	$C_{19}H_{11}NO_4$	> 10
79	B	–		–	*B*	45	257-259	$C_{19}H_{13}NO_2$	7.70
80	B	–		–	*B*	79	191-192	$C_{19}H_{13}NO_2$	> 10
81	B	–		–	*B*	81	186-188	$C_{19}H_{13}NO_2$	> 10
82	B	–		VI	*B*	34	232-233	$C_{18}H_{11}NO_2$	1.92
83	B			VI	*B*	52	208-209	$C_{18}H_{11}NO_2$	> 10
84	B			VI	*B*	71	137-138	$C_{23}H_{20}O_2$	> 10
87	D	H		VII	-	-	-	$C_{12}H_{13}N_3O_2$	2.0
88	E	–		VII	*D*	86	240-242	$C_{16}H_{15}N_3OS$	1.69
89	E	–		VII	*D*	55	178-180	$C_{16}H_{15}N_3O$	6.68
90	E	–		VII	*D*	69	190-192	$C_{16}H_{13}N_3O$	2.81
91	E	–		VII	*D*	75	240-241	$C_{16}H_{15}N_3O_2$	6.49
92	E	–		VII	*D*	32	> 285[c]	$C_{17}H_{18}N_4O$	> 10
93	E	–		VII	*D*	41	214-216	$C_{19}H_{21}N_3O$	> 10
94	E	–		VII	*D*	56	208-209	$C_{18}H_{19}N_3O_2$	3.25
95	E	–		VII	*D*	35	184-185	$C_{18}H_{19}N_3O_2$	6.71
96	E			VII	*D*	14	183-185	$C_{18}H_{19}N_3O_2$	> 10
97	E	–		VII	*D*	50	165-166	$C_{16}H_{19}N_3O_2$	> 10
98	E	–		VII	*D*	58	178-179	$C_{15}H_{15}N_3O_2$	> 10

Table 2. (Continued)

Compound No.	general structure	R^1	R^2	Method[a]	*Solvent system*[b]	yield (%)	mp (°C)	formula	DNA-PK Inhibition IC_{50} (μM)
99	E	–		VII	*D*	44	218-219	$C_{21}H_{19}N_3O_2$	> 10
100	E	–		VII	*D*	58	174-175	$C_{17}H_{17}N_3O_2$	> 10
101	E	–		VII	*D*	26	211-212	$C_{16}H_{17}N_3O_3$	> 10
102	E	–		VII	*D*	32	215-216	$C_{15}H_{15}N_3O_3$	8.46
103	E	–		VII	*D*	54	199-200	$C_{15}H_{15}N_3O_2$	> 10
104	E	–		VII	*D*	72	281-221	$C_{14}H_{13}N_3O_2$	> 10
105	E	–		VII	*D*	48	166-167	$C_{16}H_{14}N_4O$	> 10
106	E	–		VII	*D*	72	162-163	$C_{18}H_{15}N_3OS$	> 10
107	E	–		VII	*D*	68	159-161	$C_{20}H_{17}N_3O_3$	> 10
108	E	–		VII	*D*	66	195-196	$C_{21}H_{18}N_3O_2$	10.47

[a] See the Experimental Section. [b] Recrystallization solvents: *A*, MeOH:H_2O; *B*, EtOAc:petrol; *C*, Et_2O; *D*, EtOH (trituration). Chromatography solvents: *E*, petrol:EtOAc (4:1); *F*, DCM:MeOH (95:5); *G*, EtOAc. [c] Sublimes.

Scheme 1[a]

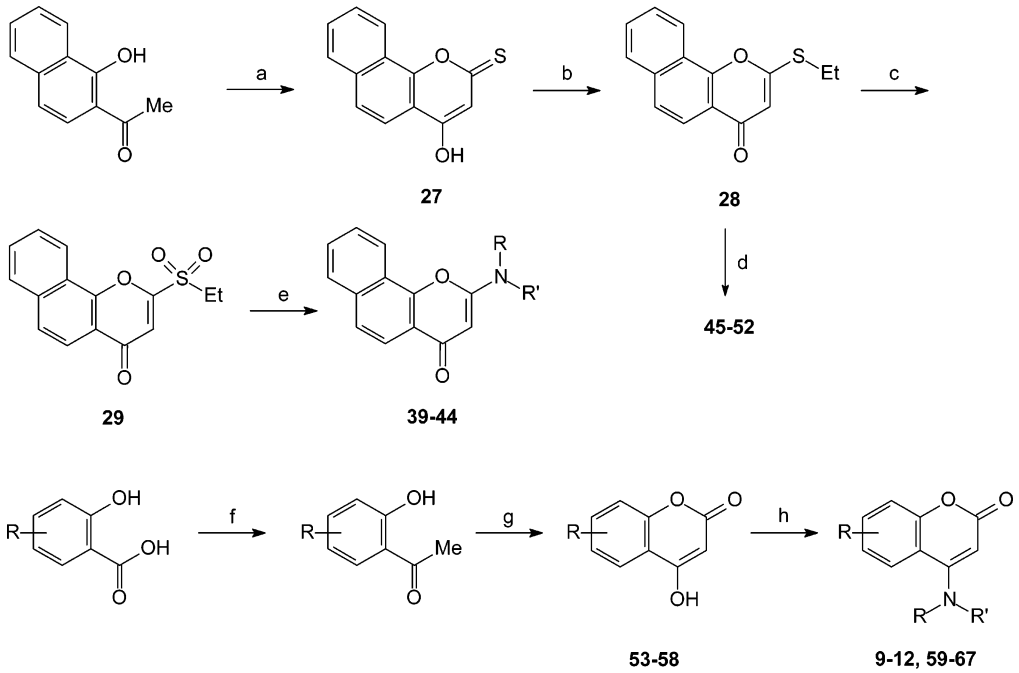


[a] Reagents and conditions: (a) K_2CO_3, CO_2, 40−60 bar, 150 °C or Na, xylene, CO_2, 130 °C; (b) MeOH, H_2SO_4, reflux; (c) *N*-acetylamine, LDA, THF, −78 to 25 °C; (d) Tf_2O, DCM, 0 °C, 16 h; (e) Lawesson's reagent, MeC_6H_5, reflux; (f) *m*-CPBA, DCM, 25 °C. R = substituent or additional fused six-membered ring, X = O, S, or CH_2.

A modification of the literature procedures described for the original synthesis of **2** and related compounds[17,18] proved useful for the introduction of a range of amines other than morpholine at the chromone 2-position (Scheme 2). Treatment of 1-hydroxy-2-acetonaphthone, prepared from methyl 1-hydroxy-2-naphthoate and methyllithium, with CS_2 under basic conditions gave the 4-hydroxybenzo[*h*]chromen-2-thione (**27**). *S*-Alkylation of **27** with EtI and oxidation of the resultant ethylthiochromone (**28**) to the sulfone (**29**) with *m*-CPBA enabled introduction of the requisite amine at the 2-position (**39**−**44**) in good yields under ambient conditions. However, direct treatment of **28** with the appropriate amine in ethane-1,2-diol at 160 °C also gave the target amine-substituted benzochromones (**45**−**52**) in reasonable yields.

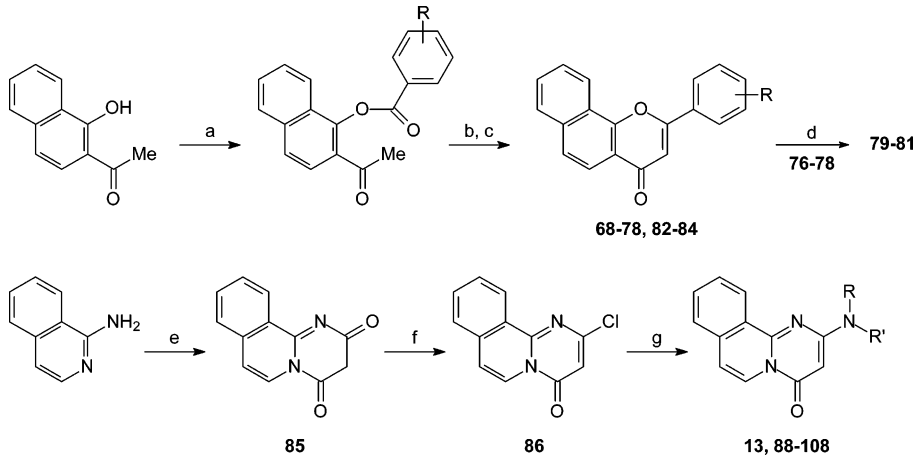
A literature method[26,27] was also adapted for the synthesis of the target 4-amine-substituted chromen-2-ones (coumarins) (**9**−**12**, **59**−**67**). The precursor 4-hydroxychromen-2-one derivatives (**53**−**58**) were either available commercially or prepared in good yields by the base-catalyzed condensation of 2-hydroxyacetophenones with diethyl carbonate as described previously.[28] Subsequent introduction of the 4-amine substituent was achieved in a "one-pot" reaction, by conversion into the 2,4,6-triisopropylbenzenesulfonyloxy derivative and reacting with the appropriate amine (Scheme 2). This differs from the reported procedure that involves preparation of the 4-chlorochromen-2-one employing PPh_3/CCl_4, isolation of the 4-chloro derivative, and reaction with the required amine. The use of the bulky triisopropylbenzenesulfonyl ester proved necessary, as analogous reactions conducted with mesylates or tosylates gave only the corresponding sulfonamides arising through

Scheme 2[a]



[a] Reagents and conditions: (a) KO^{t}Bu, CS_2, toluene, 16 h, H_2SO_4; (b) EtI, K_2CO_3, acetone, reflux; (c) *m*-CPBA, DCM; (d) RR′NH, $(CH_2OH)_2$, 160 °C; (e) RR′NH, MeCN 25 °C; (f) MeLi, dioxane; (g) $CO(OEt)_2$, Na, 100 °C; (h) 2,4,6-triisopropylbenzenesulfonyl chloride, NEt_3, DCM, 25 °C, RR′NH. R = substituent or additional fused six-membered ring.

Scheme 3[a]



[a] Reagents and conditions: (a) RC_6H_4COCl, pyridine, 25 °C; (b) pyridine, KOH, 50 °C; (c) AcOH, H_2SO_4 reflux; (d) Pd/C, H_2, AcOH; (e) $CH_2(CO_2Et)_2$, EtOH, 170 °C; (f) $POCl_3$, reflux; (g) RR′NH, EtOH, reflux.

amine attack at the sulfonyl center. Our preliminary studies have also demonstrated that the entire sequence of reactions required for the preparation of 4-amine-substituted chromen-2-ones from 2-hydroxyacetophenones may be conducted in a single-pot, enabling the multiple-parallel synthesis of chromen-2-one libraries. The results of these studies will be published elsewhere.

Benzo[*h*]chromen-4-ones (**68**−**78**, **82**−**84**) were either commercially available or prepared by the classical Baker−Venkataraman rearrangement of 1-aroyloxy-2-acetonaphthone derivatives,[29,30] available by treatment of 1-hydroxy-2-acetonaphthone with the appropriate aroyl chloride (Scheme 3). The 2-arylaminobenzo[*h*]chromen-4-ones (**79**−**81**) were obtained by reduction of the corresponding nitro derivatives (**76**−**78**). The pyrimido-[2,1-*a*]isoquinoline template (**85**) was readily synthesized from the reaction of 2-aminoisoquinoline with diethyl malonate,[31,32] with subsequent conversion into the 2-chloro derivative (**86**) enabling the introduction of a range of amine substituents at the 2-position (**13**, **88**−**108**) as shown in Scheme 3. The pyrido[1,2-*a*]-pyrimidin-4-one derivative (**87**) was similarly prepared for comparative purposes.

Results and Discussion

We initially investigated alternatives to the 8-phenylchromone system of **2**, with a view to identifying templates exhibiting improved potency and selectivity as DNA-PK inhibitors (Table 1). The 7,8- and 6,7-fused-ring chromones (**3** and **7**) exhibited excellent DNA-PK inhibitory activity (IC_{50} = 0.23 and 0.39 μM, respectively), whereas the 5,6-benzochromone (**8**; IC_{50} = 1.25 μM) proved approximately equipotent with **2**. Saturation of the 7,8-fused ring was tolerated, with **6** proving

Table 3. Inhibitory Activity IC_{50} (μM) of **2**, **3**, **13**, and **48** against PIKK Family Members[a]

kinase	2	3	13	48
DNA-PK	1.5 ± 0.2[b]	0.23 ± 0.01	0.28 ± 0.02	0.19 ± 0.01
PI 3-K (p110α)	2.3 ± 0.8 (1.4)[c]	13 ± 3.0 (6)[c]	>100	2.4 ± 0.7
ATM	>100[d]	>100[d]	>100[d]	>100[d]
ATR	>100[d]	>100[d]	>100[d]	>100[d]
mTOR	2.5 ± 0.2	6.4 ± 0.1	5.3 ± 0.8	4.8 ± 1.4

[a] See Experimental Section. [b] Values are the means of at least three independent determinations ± SE. [c] Literature values (ref 17). [d] No inhibitory activity observed at 100 μM.

essentially equipotent with **3**. Interestingly, the 4-thiochromone derivative (**4**) exhibited DNA-PK inhibitory activity comparable with **3**, suggesting that the 4-carbonyl function does not make a critical hydrogen-bond interaction within the ATP-binding domain. Although active as DNA-PK inhibitors, the corresponding fused-ring coumarin templates (**9**−**12**) did not exhibit superior potency to **2**.

The possibility that the linear tricyclic ring system of **7** might confer undesirable DNA intercalating properties mitigated against the choice of this template, and the 7,8-benzochromone **3** was instead selected for more detailed studies. The promising activity of **3** also prompted the synthesis of the isosteric pyrimido[2,1-*a*]-isoquinolin-4-one (**13**), which was found to exhibit DNA-PK inhibitory activity comparable with that of **3**. Consistent with this observation, the approximately 6-fold greater potency of the 7,8-fused-ring chromone **3** compared with **2** was also observed for the pyrimidoisoquinolone system (compare **13** with **87**). Compounds **3** and **13** were counterscreened against PI 3-K and related PIKK family members and found to exhibit high selectivity for DNA-PK compared with **2** (Table 3). This correlates with previously reported SARs indicating that fused-ring derivatives of **2** are poor PI 3-K inhibitors,[17] although in our hands **3** was found to be a slightly weaker PI 3-K inhibitor.

On the basis of these preliminary data, more detailed SAR studies were conducted with the chromone scaffold (**2**), and a series of analogous coumarin derivatives was also evaluated for comparative purposes (Table 2). The very promising activity exhibited by the 7,8-fused-ring chromone (**3**) and pyrimido[2,1-*a*]isoquinolin-4-one (**13**) core templates also warranted further investigation. In particular, we wished to identify alternatives to the morpholino substituent, as preliminary studies with **2** have identified this functionality as vulnerable to metabolism.[33] Although reportedly important for PI 3-K inhibitory activity,[17] the 8-phenyl substituent of **2** does not appear to be necessary for inhibition of DNA-PK, with the unsubstituted derivative **30** exhibiting comparable activity to **2**. Replacing the 8-phenyl group of **2** by Me (**31**) or MeO (**33**) also had a negligible effect upon potency. Transposing the phenyl group to the chromone 6-position (**34**) resulted in a modest reduction in potency, whereas a methoxy group at the 7-position was tolerated, with **32** being at least equipotent with **2**.

Elucidation of the crystal structure of **2** in complex with porcine PI 3-K (p110γ) has revealed that the 2-morpholino oxygen makes a key hydrogen-bond interaction with a backbone Val882 in the ATP-binding pocket.[34] Accordingly, replacement of the morpholino group by piperidino (**35**) or thiomorpholino (**36**) is reported to dramatically reduce PI 3-K inhibitory activity (IC_{50} > 100 μM).[17] Given the likely close homology between PI 3-K and DNA-PK within the ATP-binding pocket, analogous modifications would also be predicted to be detrimental to DNA-PK inhibitory activity. However, a less pronounced effect was observed, and while removal of the morpholino oxygen of **2** reduced potency approximately 3-fold (**35**, IC_{50} = 4.67 μM), replacement by sulfur was tolerated (**36**, IC_{50} = 1.61 μM). Perhaps surprisingly, oxidation to the sulfoxide (**37**) reduced activity to a level similar to that of the piperidine derivative (**35**).

Studies with the equipotent 7,8-fused-ring chromone and pyrimido[2,1-*a*]isoquinolin-4-one leads **3** and **13** centered on replacing the metabolically vulnerable 2-morpholino group with a more robust alterative. To this end, a diverse range of amine substituents was investigated (Table 2). As expected, the 2-thiomorpholino derivatives (**5** and **88**) were 4−5-fold less potent than the respective parent inhibitors **3** and **13**, while the 2-piperidinochromone (**38**) was 10-fold less active than **3**, and the piperazinyl and *N*-methylpiperazinyl analogues (**46**, **47**, **92**) were at least 30-fold less active. The effect of a 2-pyrrolidinyl (**39**, **89**) or 2-(3′-hydroxy)-pyrrolidinyl group (**45**, **91**) was less pronounced, but with the exception of the 2,5-dihydropyrrolyl derivative (**90**, IC_{50} = 2.81 μM), potency was still reduced 20−30-fold compared with **3** or **13**.

More dramatic modifications at the 2-position of **3** and **13** were also detrimental, as witnessed by the absence of significant DNA-PK inhibitory activity at 10 μM for derivatives **43**, **44**, **51**, **52**, **93**, **97**−**108**. The lack of inhibitory activity observed for several possible metabolites (**43**, **98**, **101**−**104**) of the 2-morpholino function of **3** and **13** reaffirmed the importance of identifying viable alternatives to this group. 7,8-Fused ring chromones bearing a 2-aryl substituent (**68**−**84**) were generally very poor DNA-PK inhibitors, with no compounds exhibiting sub-micromolar potency. Only the 3-pyridyl derivative (**82**) gave activity comparable with **2**, and presumably the 3-pyridyl nitrogen is able to serve as a hydrogen-bond acceptor analogously to the 2-morpholine substituent. The activity of the 3-fluorophenyl derivative **74** (IC_{50} = 5.7 μM), was also notable, suggesting that a fluoro substitutent in this position may also serve as a hydrogen-bond acceptor. However, the comparable potency of the parent chromone **69** (IC_{50} = 4.98 μM) is difficult to reconcile.

The very constrained SARs observed at the 2-position of the chromone (**3**) and pyrimidoisoquinolinone (**13**) templates prompted an investigation of more subtle changes to the morpholino function. Encouragingly, the introduction of a 2-methyl substituent onto the morpholine ring was not detrimental, and the racemic chromone **48** exhibited potent DNA-PK inhibitory activity. Equally as importantly, **48** retained the desired selectivity for DNA-PK over related PIKK family members (Table 3). An additional methyl group substituent at the 6-position was not tolerated, with the 2,6-dimethylmorpholino derivatives **49** and **94** proving an order of magnitude less potent than **3** and **13**, and a similar effect was observed for the *gem*-dimethylmorpholinylchromones **50** and **95**. These results suggest that the morpholino group is located within a sterically constrained region of the ATP-binding domain and that

additional methyl group substitution positions the morpholine oxygen into an unfavorable orientation for hydrogen bonding. This is consistent with the very poor activity exhibited by the 2-ethylmorpholinyl (**96**) and benzoxazinyl (**41**) derivatives. The reduced activity of the homomorpholine analogue (**40**) and the bridged morpholine (**42**) may be attributable to steric effects, or arise as a consequence of the suboptimal positioning of the oxygen with respect to the donor amino acid residue.

Detailed interpretation of the results obtained in the coumarin series (**59**−**67**) is difficult in the absence of knowledge about the binding mode of the inhibitors within the ATP-binding site of DNA-PK. An assumption that the morpholine and carbonyl groups make identical binding interactions to those of the other templates enables an overlay of the *a*-ring of the coumarin with those of the 7,8-fused-ring chromones, and pyrimido-[2,1-*a*]isoquinolin-4-ones (Table 2, general structures **B**, **C**, and **E**). The reduced potency of the piperidino derivative (**60**) compared with the parent coumarin **59** supports this model. The poor activity of **59** compared with **3** and **13** may then be attributed to the unfavorable positioning of the coumarin ring oxygen in a region of the ATP pocket occupied by the *b*-rings of the 7,8-fused-ring chromone and pyrimido[2,1-*a*]isoquinolin-4-one systems. This is also consistent with the overall lower potency of the fused-ring coumarins **9**−**12** compared with the chromones **3**−**7** (Table 1). Tentative SARs based on the results for coumarins **61**−**67** suggest that the 6-position is more tolerant to substitution than the 7- or 8-positions, with the 6-methoxy and 6-chloro derivatives (**64** and **67**) proving the most potent inhibitors. Consistent with the putative binding mode of these compounds, the 5-methoxycoumarin (**63**) is markedly less potent, presumably as a consequence of adverse steric effects on the morpholino substituent.

Chromone **48**, the most potent and selective DNA-PK inhibitor emerging from these studies (Table 3), was subjected to a more detailed biological evaluation. Lineweaver−Burk plots for DNA-PK inhibition demonstrated that **48** exhibits competitive inhibition kinetics with respect to ATP (Figure 1a), and a plot of the slopes from the primary graph (Figure 1a) versus inhibitor concentration (Figure 1b) gave a K_i value of 24 nM for **48**. The ATP-competitive mode of inhibition by **48** against DNA-PK is in keeping with the previously demonstrated competitive activity of **2** against this enzyme.[19] To the best of our knowledge, **48** is the most potent and kinase-selective DNA-PK inhibitor described to date.

The cellular activity of **48** as a DNA-PK inhibitor and radiosensitizer was assessed in a human tumor cell line in vitro. Figure 2 shows that at concentrations of 5 and 10 μM, **48** was able to enhance the cytotoxicity of ionizing radiation against HeLa B tumor cells, whereas these concentrations of inhibitor alone had no effect on the survival of the cells. Thus, at 10% survival, doses of 5 and 10 μM of **48** gave dose modification factors of 2.3 and 3.7, respectively. In a recent related study, NU7026 (**3**) has also been demonstrated to act as both a radio- and chemo-sensitizer in vitro.[35,36] The data presented here are not only in line with these studies but consistent with the enhancement of ionizing radiation cytotoxicity observed with **2**[22] and the nonspecific DNA-PK inhibitor wortmannin.[22,23]

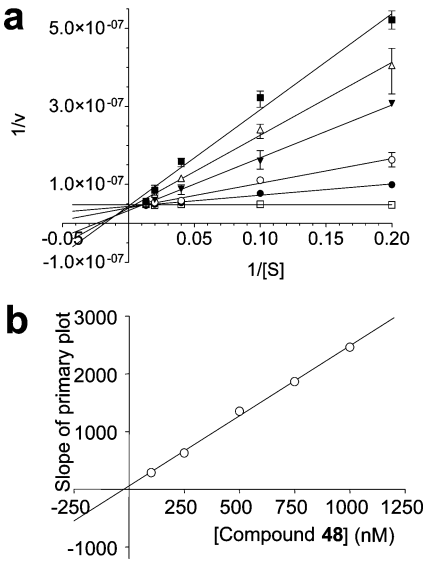


Figure 1. Characterization of the inhibition of DNA-PK activity by compound **48**. (a) Lineweaver−Burk plot of the inhibition of DNA-PK activity by **48**. Inhibition kinetics were performed with 0 μM (□), 0.1 μM (●), 0.25 μM (○), 0.5 μM (▼), 0.75 μM (△), or 1 μM (■) of **48** in varying concentration of ATP. Data shown are the mean ± SEM from three independent experiments. (b) K_i determination for compound **48**. Slopes from the Lineweaver−Burk plots (a) were plotted against the concentration of **48**. Intersection of the plot on the compound concentration axis gave a K_i value of 24 nM.

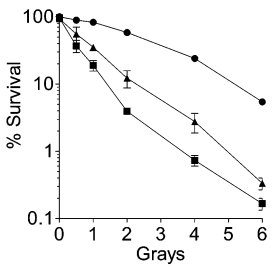


Figure 2. Effects of increasing doses of ionizing radiation (Gy), in the absence (●) or presence of 5 μM (▲) or 10 μM (■) of **48**, on the clonogenic survival of HeLa B cells. Cells were preincubated with **48** for 1 h before exposure to ionizing radiation and incubated for a further 16 h prior to fresh media being added, and colony formation was determined after 8 days. Data are the mean ± SEM of at least three independent experiments.

Conclusions

In this paper we have delineated structure−activity relationships for the ATP-competitive inhibition of DNA-PK by benzopyranones and pyrimidoisoquinolones and have established the importance of a 2-morpholino substituent. Several potent and selective DNA-PK inhibitors have been identified, and enhancement of the cytotoxicity of ionizing radiation by one such inhibitor (**48**) has been demonstrated in a tumor cell line in vitro. Subsequent studies will utilize the information obtained from these studies to further optimize potency and selectivity, with a view to identifying a clinical candidate.

Experimental Section

Reagents were purchased from any of the major 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 Merck 1.05554 aluminum sheets precoated with Kieselgel $60F_{254}$ (0.2 mm) as the adsorbent, with visualization by potassium permanganate or UV light at 254 and 365 nm. Column chromatography was conducted under medium pressure on silica (Kieselgel 60, 240−400 mesh). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 300 and 75.5 MHz, respectively, on a Bruker Avance 300 spectrometer, employing deuterated 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. Chemical shift values are quoted in parts per million (ppm) and coupling constants (J) in hertz (Hz). Key: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, m = multiplet. LC−MS analysis was conducted on a Waters/Micromass Platform LC instrument, with LC chromatography employing a Waters Symmetry column (50 × 4.6 mm) with a 10 min methanol/0.05% formic acid gradient. UV detection was achieved with a Waters 996 detector scanning from 240 to 400 nm, and MS was measured in positive and negative ion electrospray mode. Electron impact (EI) mode mass spectra were determined on a Kratos MS80 spectrometer. IR spectra were recorded either on a Nicolet 20 PC Fourier Transform spectrometer as KBr disks or neat on an Excalibur series spectrophotometer. Elemental analyses were performed either in house on a Carlo-Erba Instrumentazione 1106 analyzer or by Butterworth Laboratories, Middlesex, UK, and are within ±0.4% of theory unless otherwise specified.

2-Aminochromones (2, 3, 5−7, 30−36, 38): Method I. General Procedure. Triflic anhydride (3.6 equiv) in DCM was added to the appropriate β-ketoamide (1.0 equiv) in DCM, and the solution was stirred at room temperature for 20 h at 0 °C under N_2. The solvent was evaporated in vacuo and the residue was redissolved in methanol and stirred for 4 h, and an equal volume of water was added. After stirring the solution for 1 h, the MeOH was evaporated and the residual aqueous solution was adjusted to pH 8 with saturated aqueous $NaHCO_3$ solution. The mixture was extracted three times with DCM, and the combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The product was triturated with ether and further purified by recrystallization from suitable solvents.

4-Hydroxybenzo[*h*]chromen-2-thione (27). To a stirred solution of potassium *tert*-butoxide (3.72 g, 60.0 mmol) in toluene (50 mL) was added a solution of 1-hydroxy-2-acetonaphthone (3.72 g, 20 mmol) and carbon disulfide (1.52 g, 20 mmol) in toluene (50 mL) at 15−20 °C. The reaction mixture was stirred for 16 h at room temperature, water (500 mL) was added, and the orange reaction mixture was extracted with ether (4 × 50 mL). The aqueous portion was acidified with 10% aqueous H_2SO_4 and the reaction mixture was stirred for 16 h. A yellow solid was precipitated and collected by filtration under vacuum, washed with petroleum ether, and recrystallized from hot THF to provide the title compound as an orange crystalline solid (1.09 g, 29%): ^{1}H NMR δ 4.21 (1H, br s, OH), 6.89 (1H, s, 3-*H*), 7.89−7.95 (2H, m, Ar-*H*), 7.97−8.08 (2H, m, Ar-*H*), 8.16−8.19 (1H, m, Ar-*H*), 8.52−8.57 (1H, m, Ar-*H*); MS (ESI+) *m/z* 229 [M + 1].

2-Ethylsulfanylbenzo[*h*]chromen-4-one (28). A mixture of **27** (0.80 g, 4.15 mmol), potassium carbonate (0.57 g, 4.15 mmol), and iodoethane (0.70 g, 4.15 mmol) in acetone (25 mL) was heated at reflux for 2 h. After cooling, the solvent was removed by evaporation in vacuo and the residue partitioned between water (100 mL) and DCM (100 mL). The aqueous layer was extracted into DCM (3 × 50 mL), the DCM extracts were combined and dried (Na_2SO_4), and the solvent was removed by evaporation in vacuo to yield a brown solid, which was recrystallized from EtOAc−petroleum ether to provide the title compound as pale brown crystals (0.42 g, 62%): mp 155−157 °C; ^{1}H NMR δ 1.45 (3H, t, J = 7.4 Hz, CH_3), 3.13 (2H, q, J = 7.4 Hz, CH_2), 6.36 (1H, s, 3-*H*), 7.55−7.73 (4H, m, Ar-*H*), 8.03−8.07 (1H, m, Ar-*H*), 8.32−8.37 (1H, m, Ar-*H*); MS (ESI+) *m/z* 257 [M + 1].

2-Ethanesulfinylbenzo[*h*]chromen-4-one (29). To a solution of **28** (2.0 g, 7.81 mmol) in dry DCM (50 mL) at 0 °C was added *m*-CPBA (75%, 4.83 g, 19.53 mmol), and the solution was stirred for 3 h at 25 °C. Water (40 mL) was added and the reaction mixture was extracted with DCM (3 × 50 mL). The combined organic layers were washed successively with saturated aqueous $NaHCO_3$ (3 × 30 mL) and NH_4Cl (3 × 30 mL) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residual solid was triturated with diethyl ether, to furnish the product as a yellow solid (1.80 g, 80%): mp 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, CH_3, J = 7.5 Hz), 3.35 (2H, q, CH_2S, J = 7.5 Hz), 7.05 (1H, s, Ar-*H*), 7.70 (3H, m, Ar-*H*), 8.30 (1H, d, Ar-*H*) 8.45 (1H, d, Ar-*H*).

2-Aminochromones (39−40, 42−44): Method II. General Procedure. The appropriate amine (10.0 mol equiv) was added to a suspension of **29** (1.0 mol equiv) in MeCN or DCM (10−20 mL), and the mixture was stirred at room temperature for 16 h. Solvents were evaporated in vacuo, and the residual solid was redissolved in EtOAc (100 mL), washed with 50% aqueous $NaHCO_3$ (2 × 100 mL), and dried (Na_2SO_4). Removal of solvents furnished the product chromone, which was purified by chromatography on silica or by recrystallization.

2-Aminobenzo[*h*]chromen-4-ones (45−52): Method III. General Procedure. A mixture of **28**, the appropriate amine (10 mol equiv), and ethane-1,2-diol (10 mL) was heated to 160 °C, with stirring, for 3 h. Upon cooling to room temperature the reaction mixture was poured onto ice water (100 mL) and extracted into DCM. The organic extracts were collected and dried (Na_2SO_4), and the solvent was removed by evaporation in vacuo to yield the product as a pale solid. The product was purified by recrystallization from a suitable solvent.

2-(Morpholin-4-yl)-8-phenylchromen-4-one (2). The title compound was prepared from **16** (1.10 g, 3.38 mmol) according to method I: IR (KBr) 3419, 1621, 1563, 1414 cm^{-1}; ^{1}H NMR δ 3.45 (4H, m), 3.74 (4H, m), 5.66 (1H, s), 7.57 (4H, m), 7.73 (3H, m), 8.06 (1H, m); MS (EI) *m/z* = 307 (M^+).

7,8-Benzo-2-(morpholin-4-yl)chromen-4-one (3). The title compound was prepared from **17** (2.40 g, 8.00 mmol) according to method I: IR 1641, 1626, 1605, 1509, 1562 cm^{-1}; ^{1}H NMR δ 3.74 (4H, m), 3.91 (4H, m), 5.79 (2H, s), 7.88 (1H, m), 8.02 (2H, m), 8.16 (1H, m), 8.56 (1H, m); MS (EI) *m/z* = 281 (M^+).

2-(Morpholin-4-yl)benzo[*h*]chromene-4-thione (4). A solution of **3** (0.10 g, 0.35 mmol) and Lawesson's reagent (0.173 g, 0.35 mmol) in dry toluene (10 mL) was refluxed under N_2 for 24 h. The solvent was removed in vacuo, and the residual solid purified by chromatography on silica to give the title compound as a yellow solid: IR 3058, 2960, 2859, 1589, 1548 cm^{-1}; ^{1}H NMR δ 3.78 (8H, d, CH_2N + CH_2O), 6.94 (1H, s, H-3), 7.68−8.03 (4H, m, Ar-*H*), 8.42−8.51 (2H, m, Ar-*H*); MS (ESI+) *m/z* 298 (M + 1).

7,8-Benzo-2-(thiomorpholin-4-yl)chromen-4-one (5). The title compound was prepared from **19** (1.00 g, 3.17 mmol) according to method I, affording the title compound as an orange solid: IR 3087, 2963, 1642, 1604, 1562 cm^{-1}; ^{1}H NMR δ 2.79−2.88 (4H, m, CH_2S), 3.99−4.10 (4H, m, CH_2N), 5.80 (1H, s, 3-*H*), 7.82−7.84 (2H, m, Ar-*H*), 7.96−8.04 (2H, m, Ar-*H*), 8.12−8.17 (1H, m, Ar-*H*), 8.43−8.47 (1H, m, Ar-*H*); MS (EI) *m/z* 297 (M^+).

7,8,9,10-Tetrahydrobenzo[*h*]-2-(morpholin-4-yl)-chromen-4-one (6). The title compound was prepared from **23** (0.65 g, 2.14 mmol) according to method I, affording the title compound as an off-white powder: IR (KBr) 1628, 1592, 1561 cm^{-1}; ^{1}H NMR δ 1.87 (4H, m), 2.90 (4H, m), 3.59 (4H, m), 3.82 (4H, m), 5.56 (1H, s), 7.17 (1H, m), 7.72 (1H, m); MS (EI) *m/z* 285 (M^+).

2-(Morpholin-4-yl)benzo[*g*]chromen-4-one. (7). The title compound was prepared from **18** (0.60 g, 2 mmol) according to method I. Purification by chromatography on silica afforded a pale brown solid: IR (KBr) 3048, 2906, 2869, 1598, 1569 cm^{-1}; ^{1}H NMR δ 3.60 (4H, t, J = 4.5 Hz, CH_2N), 3.88 (4H, t, J = 4.5 Hz, CH_2O), 5.54 (1H, s, H-4), 7.55 (1H, m, Ar-*H*), 7.74

(1H, m, Ar-*H*), 8.04 (1H, m, Ar-*H*), 8.74 (1H, m, Ar-*H*); HRMS (EI) M^+ 281.1048 ($C_{17}H_{15}NO_3$ calcd as 281.1052).

2-(Morpholin-4-yl)chromen-4-one (30) was prepared from **14** (1.25 g, 5 mmol) by method I and purified by chromatography on silica to yield a white solid: IR (KBr) 3067, 3035, 2960, 1620, 1555 cm^{-1}; ^1H NMR δ 3.19 (4H, t, J = 4.5 Hz, CH_2N), 3.87 (4H, t, J = 4.5 Hz, CH_2O), 5.67 (1H, s, H-4), 7.26 (2H, m, Ar-*H*), 7.49 (2H, m, Ar-*H*); HRMS (EI) M^+ 231.0890 ($C_{13}H_{13}NO_3$ calcd as 231.0895).

8-Methyl-2-(morpholin-4-yl)chromen-4-one (31) was prepared from **15** (1.05 g, 4 mmol) according to method I and purified by chromatography on silica to yield an orange solid: IR (KBr) 3069, 2963, 2860, 1629, 1570 cm^{-1}; ^1H NMR δ 2.51 (3H, s, Me), 3.60 (4H, t, J = 5 Hz, CH_2N), 3.85 (4H, t, J = 5 Hz, CH_2O), 5.62 (1H, s, H-4), 7.37 (1H, m, Ar-*H*), 7.61 (1H, m, Ar-*H*), 7.86 (1H, m, Ar-*H*); HRMS (EI) M^+ 245.1052 ($C_{14}H_{15}NO_3$ calcd as 245.1052).

7-Methoxy-2-(morpholin-4-yl)chromen-4-one (32). The title compound was prepared from **24** (1.12 g, 4.0 mmol) according to method I: IR (KBr) 3440, 1631, 1601, 1566 cm^{-1}; ^1H NMR δ 3.57 (4H, m), 3.81 (4H, m), 3.94 (3H, s), 5.50 (1H, s), 7.03 (1H, dd, J = 2.3 Hz, 8.7 Hz), 7.16 (1H, d, J = 2.3 Hz), 7.90 (1H, d, J = 8.7 Hz); HRMS (EI) M^+ 261.0996 ($C_{14}H_{15}NO_4$ calcd as 261.1001).

8-Methoxy-2-(morpholin-4-yl)chromen-4-one (33). The title compound was prepared from **25** (1.12 g, 4.0 mmol) according to method I: IR 3412, 1616, 1563 cm^{-1}; ^1H NMR δ 3.60 (4H, m), 3.80 (4H, m), 4.01 (3H, s), 5.62 (1H, s), 7.39 (2H, m), 7.55 (1H, m); HRMS (EI) M^+ 261.0992 ($C_{14}H_{15}NO_4$ calcd as 261.1001).

6-Phenyl-2-(morpholin-4-yl)chromen-4-one (34). The title compound was prepared from **26** (0.20 g, 0.61 mmol) according to method I: IR (KBr) 1611, 1558 cm^{-1}; ^1H NMR δ 3.66 (4H, m), 3.85 (4H, m), 5.68 (1H, s), 7.57 (3H, m), 7.72 (1H, m), 7.83 (2H, m), 8.08 (1H, m), 8.24 (1H, m); MS (EI) *m/z* 307 (M^+).

8-Phenyl-2-(piperidin-4-yl)chromen-4-one (35). The title compound was prepared from **21** (0.14 g, 0.43 mmol) according to method I: IR (KBr) 3441, 1625, 1564 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (6H, m), 3.27 (4H, m), 5.46 (1H, s), 7.37 (7H, m), 8.09 (1H, m); MS (EI) *m/z* 305 (M^+).

8-Phenyl-2-(thiomorpholin-4-yl)chromen-4-one (36). The title compound was prepared from **22** (0.79 g, 0.23 mmol) according to method I: IR (KBr) 3425, 1619, 1588, 1563 cm^{-1}; ^1H NMR δ 2.77−2.80 (4H, m, CH_2SCH_2), 3.76−3.80 (4H, m, CH_2NCH_2), 5.70 (1H, s, 3-H), 7.55−7.78 (7H, m, Ar-*H*), 8.05 (1H, dd, J = 1.6 and 6.0 Hz, Ar-*H*); MS (EI) *m/z* 323 (M^+).

2-(Oxothiomorpholin-4-yl)-8-phenylchromen-2-one (37). To a solution of **36** (0.10 g, 0.29 mmol) in DCM (5 mL) was added *m*-CPBA (0.06 g, 0.30 mmol), and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was washed with saturated $NaHCO_3$ (2 × 50 mL), the organic layer was dried (Na_2SO_4), and solvents were evaporated in vacuo. The residual solid was purified by chromatography on silica to provide the title compound as a white solid: IR (KBr) 1630, 1571, 1049 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.69−2.71 (4H, m,), 3.76−4.06 (4H, m,), 5.54 (1H, s,), 7.32−7.54 (8H, m), 8.11(1H, dd); MS (EI) *m/z* 339 (M^+).

2-(Piperidin-1-yl)benzo[*h*]chromen-4-one (38). The title compound was prepared according to method I from **20** (0.11 g, 0.37 mmol) as a pale brown solid: ^1H NMR δ 1.61−1.75 (6H, m, piperidine CH_2), 3.52−3.60 (4H, m, CH_2NCH_2), 5.59 (1H, s, 3-*H*), 7.53−7.56 (2H, m, Ar-*H*), 7.81−7.96 (2H, m, Ar-*H*), 8.21 (1H, d, Ar-*H*), 8.20−8.25 (1H, m, Ar-*H*); MS (ESI+) *m/z* 279 (M^+).

2-(Pyrrolidin-1-yl)benzo[*h*]chromen-4-one (39). To a solution of **28** (0.40 g, 1.56 mmol) in DCM (10 mL) was added *m*-CPBA (0.80 g, 2.34 mmol). The reaction mixture was stirred for 3 h at ambient temperature, cooled to −15 °C, and filtered. The filtrate was evaporated to dryness in vacuo to yield an off-white solid, which was redissolved in MeCN (25 mL), and pyrrolidine (1.00 mL, 12.03 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, the solvent was removed by evaporation in vacuo, and the residual solid was dissolved in DCM (50 mL), washed sequentially with water (4 × 50 mL) and saturated NaCl solution (50 mL), and dried (Na_2SO_4). Evaporation of the solvent in vacuo gave the title compound: ^1H NMR ($CDCl_3$) δ 2.02−2.09 (4H, m, CH_2), 3.39−3.56 (4H, m, CH_2NCH_2), 5.36 (1H, s, 3-*H*), 7.49−7.56 (2H, m Ar-*H*), 7.65 (1H, d, J = 8.6 Hz Ar-*H*), 7.80−7.85 (1H, m, Ar-*H*), 8.10 (1H, d, J = 8.6 Hz, Ar-*H*), 8.15−8.22 (1H, m, Ar-*H*); MS (EI) *m/z* 265 (M^+).

2-(1,4-Oxazepan-4-yl)benzo[*h*]chromen-4-one (40). The title compound was prepared from **29** (0.10 g, 0.37 mmol), homomorpholine hydrochloride (0.20 g, 1.48 mmol), and NEt_3 (0.23 mL, 1.62 mmol) according to method II, to yield the product as an off-white solid: ^1H NMR ($CDCl_3$) δ 2.09 (2H, m), 3.79 (6H, m), 3.89 (2H, m), 5.55 (1H, s), 7.59 (2H, m), 7.68 (1H, d), 7.87 (1H, m), 8.11 (1H, d), 8.17 (1H, m); MS (ESI+) *m/z* 296 (M + 1).

2-(2,3-dihydrobenzo[1,4]oxazin-4-yl)benzo[*h*]chromen-4-one (41). To a solution of 3,4-dihydro-*2H*-benzo[1,4]oxazine (0.32 g, 1.6 mmol) in THF (5 mL) was added *n*-BuLi (2.5 M in THF, 3.12 mmol, 1.24 mL) dropwise, and the mixture was stirred for 30 min at 0 °C. A solution of **29** (0.44 g, 1.6 mmol) in THF (10 mL) was added, and the reaction mixture was stirred for a further 20 h at 25 °C, poured into aqueous HCl solution (2.0 M, 10 mL), and extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by chromatography on silica, to yield the title compound as a yellow solid: ^1H NMR ($CDCl_3$) δ 4.01 (2H, m, CH_2N), 4.35 (2H, m, CH_2O), 6.06 (1H, s), 6.85−7.05 (4H, m, Ar-*H*), 7.44−8.28 (6H, m, Ar-*H*); MS (ESI+) *m/z* 330 (M + 1).

2-(2-Oxa-5-azabicyclo[2.2.1]hept-5-yl)benzo[*h*]chromen-4-one (42). The title compound was prepared from **29** (0.14 g, 0.48 mmol), 2-oxa-5-azabicyclo[2.2.1]heptane (0.01 g, 0.96 mmol), and NEt_3 (0.20 g, 2.0 mmol) according to method II. The reaction was conducted in MeCN under reflux. The crude product was purified by chromatography on silica to yield the title compound as a red solid: IR 1639, 1591, 1551, 795 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39−8.27 (m, 6H), 5.52 (s, 1H), 4.84 (d, J = 9.1 Hz, 2H), 4.02 (m, 2H), 3.64 (m, 2H), 2.13 (m, 2H); MS (ESI+) *m/z* 294 (M + 1).

2-[2-(2-Hydroxyethoxy)ethylamino]benzo[*h*]chromen-4-one (43). The title compound was prepared from **29** (0.30 g, 1.1 mmol) and 2-(2-aminoethoxy)ethanol (1.1 mL, 11 mmol) according to method II, to yield a pale yellow solid: ^1H NMR δ 3.70 (8H, m, 4 × CH_2), 4.90 (1H, t, OH), 5.55 (1H, s, CH), 7.70 (2H, m, 7-*H*, 8-*H*), 7.80 (1H, m, 9-*H*), 7.90 (1H, m, 10-*H*), 8.05 (1H, m, 6-*H*), 8.40 (1H, m, 5-*H*); MS (ESI+) *m/z* 300 (M + 1).

2-[2-(2-Bromophenoxy)ethylamino]benzo[*h*]chromen-4-one (44). The title compound was prepared from **29** (0.08 g, 0.27 mmol), 2-(2-bromophenoxy)ethylamine (0.23 g, 0.11 mmol), and NEt_3 (0.11 g, 1.1 mmol) according to method II. The reaction was conducted in MeCN under reflux. The crude product was purified by chromatography on silica gel to yield the title compound as a yellow solid: IR: 3201, 1643, 1600, 796 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.70−3.73 (4H, m), 4.23−4.28 (4H, m), 5.54 (1H, s), 6.85−6.91 (4H, m), 7.49−8.11 (6H, m); MS (ESI+) *m/z* 410 (M + 1).

2-(3-Hydroxypyrrolidin-1-yl)benzo[*h*]chromen-4-one (45). The title compound was prepared as a white solid as outlined in method III, from **28** (0.25 g, 0.98 mmol) and 3-pyrrolidinol (0.84 g, 9.88 mmol): ^1H NMR δ 2.12−2.19 (2H, m, *CH*$_2$CHOH), 3.44−3.47 (4H, m, NCH_2), 4.56−4.59 (1H, m, *CH*OH), 5.32 (1H, br s, OH), 5.41 (1H, s, 3-*H*), 7.80−7.85 (2H, m, Ar-*H*), 7.93 (1H, d, J = 8.5 Hz, Ar-*H*), 8.05 (1H, d, J = 8.5 Hz, Ar-*H*), 8.13−8.17 (1H, m, Ar-*H*), 8.42−8.46 (1H, m, Ar-*H*); MS (EI) *m/z* 281 (M^+).

2-(Piperazin-1-yl)benzo[*h*]chromen-4-one (46). The title compound was prepared by method III, from **28** (0.38 g, 1.5 mmol) and piperazine (1.29 g, 15 mmol). The crude product was recrystallized from EtOAc to provide the title compound as an off white solid: ^1H NMR ($CDCl_3$) δ 2.97−3.03 (4H, m, NCH_2), 3.52−3.57 (4H, m, $HNCH_2$), 5.57 (1H, s, 3-*H*), 7.54−7.57 (2H, m, Ar-*H*), 7.66 (1H, d, J = 8.6 Hz, Ar-*H*), 7.82−7.87

(1H, m, Ar-*H*), 8.08 (1H, d, J = 8.6 Hz, Ar-*H*), 8.19−8.23 (1H, m, Ar-*H*); MS (EI) *m/z* 280 (M^+).

2-(4-Methylpiperazin-1-yl)benzo[*h*]chromen-4-one (47). The title compound was prepared as described in method III, from **28** (0.25 g, 0.98 mmol) and *N*-methylpiperazine (1.08 mL, 10 mmol), as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.32 (3H, s, CH_3), 2.54 (4H, t, J = 5.3 Hz, $CH_3N(CH_2)_2$), 3.60 (4H, t J = 5.3 Hz, NCH_2), 5.59 (1H, s, 3-*H*), 7.54−7.59 (2H, m, Ar-*H*), 7.67 (1H, d, J = 8.7 Hz, Ar-*H*), 7.81−7.86 (1H, m, Ar-*H*), 8.08 (1H, d, J = 8.7 Hz, Ar-*H*), 8.19−8.24 (1H,,m, Ar-*H*); MS (EI) *m/z* 294 (M^+).

2-(2-Methylmorpholin-4-yl)benzo[*h*]chromen-4-one (48). The title compound was prepared from **28** (0.38 g, 1.41 mmol) and 2-methylmorpholine (0.57 g, 5.10 mmol) according to the method III to yield a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, d), 2.81 (1H, t), 3.16 (1H, m), 3.71 (2H, m), 3.83 (2H, t), 4.02 (1H, m), 5.55 (1H, s), 7.55 (2H, m), 7.66 (1H, d), 7.83 (1H, d), 8.06 (1H, d), 8.17 (1H, d); MS (ESI+) *m/z* 296 (M + 1).

2-(2,6-*cis*-Dimethylmorpholin-4-yl)benzo[*h*]chromen-4-one (49) was prepared from **28** (0.38 g, 1.5 mmol) and 2,6-*cis*-dimethylmorpholine (1.14 g, 10.0 mmol) according to method III to yield a white solid: ^{1}H NMR ($CDCl_3$) δ 1.27 (6H, d, J = 5.8 Hz, CH_3), 2.68−2.74 (2H, m, NCH_2), 3.69−3.78 (2H, m, CH), 3.78−3.88 (2H, d, NCH_2), 5.56 (1H, s, 3-*H*), 7.55−7.60 (2H, m, Ar-*H*), 7.67 (1H, d, J = 8.7 Hz, Ar-*H*), 7.83−7.89 (1H, m, Ar-*H*), 8.08 (1H, d, J = 8.7 Hz, Ar-*H*), 8.16−8.21 (1H, m, Ar-*H*); MS (ESI+) *m/z* 310 (M + 1).

2-(2,2-Dimethylmorpholin-4-yl)benzo[*h*]chromen-4-one (**50**) was prepared according to method III from **28** (0.20 g, 0.78 mmol) and 2,2-dimethylmorpholine (1.14 g, 10.0 mmol), to afford off-white crystals: IR 3018, 2974, 1596, 1555 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, s, CH_3), 3.37 (2H, s, $NCH_2C(CH_3)_2$), 3.55 (2H, t, NCH_2), 3.87 (2H, t, OCH_2), 5.56 (1H, s, 3-*H*), 7.57 (2H, m, Ar-*H*), 7.68 (1H, d, Ar-*H*), 7.85 (1H, m, Ar-*H*), 8.09 (1H, d, Ar-*H*), 8.18 (1H, m, Ar-*H*); MS (ESI+) *m/z* 310 (M + 1).

2-[(Tetrahydrofuran-2-ylmethyl)amino]benzo[*h*]-chromen-4-one (51). The title compound was prepared from **28** (0.25 g, 0.98 mmol) and tetrahydrofurfurylamine (1.01 mL, 9.80 mmol) according to method III to yield an off-white crystalline solid: ^{1}H NMR ($CDCl_3$) δ 1.56−1.63 (1H, m, CH_2), 1.89−2.02 (3H, m, CH_2), 3.13−3.41 (2H, m, CH_2N), 3.81−3.92 (2H, m, CH_2O), 4.07−4.15 (1H, m, CH), 5.47 (1H, s, 3-*H*), 7.50−7.55 (2H, m, Ar-*H*), 7.65 (1H, d. J = 8.5 Hz, Ar-*H*), 7.80−7.85 (1H, m, Ar-*H*), 8.08 (1H, d, J = 8.5 Hz, Ar-*H*), 8.23−8.27 (1H, m, Ar-*H*); MS (EI) *m/z* 295 (M^+).

2-(3-Hydroxymethylpiperidin-1-yl)benzo[*h*]chromen-4-one (52). The title compound was prepared as an off-white solid as detailed in method III, from **28** (0.25 g, 0.98 mmol) and piperidine-3-methanol (1.13 g, 9.77 mmol): IR 3300, 2924, 2854, 1640, 1609, 1559 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (1H, m, CH), 1.77−1.85 (4H, m, CH_2), 3.00−3.17 (2H, m, NCH_2), 3.46−3.63 (2H, m, NCH_2), 3.95−4.02 (1H, m, CH_2OH), 4.10−4.17 (1H, m,.CH_2OH), 5.64 (1H, br s, OH), 7.47−7.51 (2H, m, Ar-*H*), 7.60 (1H, d, J = 8.7 Hz, Ar-*H*), 7.75−7.79 (1H, m, Ar-*H*), 8.02 (1H, d, J = 8.7 Hz Ar-*H*), 8.15−8.20 (1H, m, Ar-*H*); MS (EI) *m/z* 309 (M^+).

4-Hydroxymethoxychromen-2-ones (56-58): Method IV. General Procedure. To a solution of the appropriate 2-hydroxyacetophenone (1.0 mol equiv) in diethyl carbonate (80 mL) under N_2 was added sodium wire (1.0 mol equiv), and the mixture was stirred for 30 min at 0 °C. The resultant solution was heated at 100 °C for 3 h, cooled to 0 °C, and 50% aqueous MeOH (20 mL) was cautiously added. After extraction with ether (3 × 100 mL), the reaction mixture was acidified to pH 1−2 with concentrated hydrochloric acid, and the precipitated solid was collected and washed with petroleum ether to give the 4-hydroxychromen-2-one derivative.

4-Hydroxy-5-methoxychromen-2-one (56). Treatment of 2-hydroxy-6-methoxyacetophenone (3.32 g, 20 mmol) according to method IV gave **56** as a pale brown solid (1.71 g, 45%): ^{1}H NMR δ 3.90 (3H, s MeO), 5.87 (1H, s, H-3), 7.38 (3H, m, Ar-*H*), 13 (1H, br s, OH).

4-Hydroxy-6-methoxychromen-2-one (57) was prepared from 2-hydroxy-5-methoxyacetophenone (3.32 g, 0.02 mmol) according to method IV to furnish **57** as a pale brown solid (3.02 g, 79%): ^{1}H NMR δ 3.90 (3H, s MeO), 5.87 (1H, s, H-3), 7.38 (3H, m, Ar-*H*), 13 (1H, br s, OH).

4-Hydroxy-7-methoxychromen-2-one (58). Treatment of 2-hydroxy-4-methoxyacetophenone (3.32 g, 0.02 mmol) according to method IV, gave the title compound as a white solid (2.18 g, 57%): ^{1}H NMR δ 3.94 (3H, s MeO), 5.55 (1H, s, H-3), 7.08 (2H, m, Ar-*H*), 7.80 (1H, m, Ar-*H*), 12.5 (1H, br s, OH).

4-Amine-Substituted Chromen-2-ones (9−12, 59−67): Method V. General Procedure. To a solution of the appropriate 4-hydroxychromen-2-one (1 mol equiv) and triisopropylbenzenesulfonyl chloride (1 mol equiv) in DCM (5 mL), was added NEt_3 (1 mol equiv) dropwise over 15 min. The reaction mixture was stirred for 12 h, and the appropriate amine (2 mol equiv) was added dropwise. The reaction mixture was stirred for a further 12 h, solvents were evaporated in vacuo, and the product was isolated by chromatography on silica.

4-(Morpholin-4-yl)benzo[*g*]chromen-2-one (9). To a stirred solution of 3-hydroxy-2-naphthoic acid (0.94 g, 5 mmol) in dry dioxane (20 mL) at 0 °C was added methyllithium (1.6 M, 16.6 mL, 15 mmol) dropwise over 15 min. After warming the reaction mixture to room temperature, diethyl carbonate (40 mL) was added, and the reaction mixture was stirred under reflux for 48 h. Water (100 mL) was added, and the reaction mixture was extracted with diethyl ether (3 × 50 mL). The aqueous layer was acidified to pH 2−3 with concentrated aqueous HCl and extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The residual brown solid was redissolved in DCM (5 mL), and triisopropylbenzenesulfonyl chloride (0.30 g, 1 mmol) and NEt_3 (0.13 mL, 1 mmol) were added. The reaction mixture was stirred at 25 °C for 12 h, morpholine (0.17 mL, 2 mmol) was added, and stirring was continued for a further 12 h. The reaction mixture was filtered through a short column of silica to give **9** as a white solid: IR (KBr) 3022, 2852, 1706, 1608 cm^{-1}; ^{1}H NMR δ 3.41 (4H, m, CH_2N), 3.96 (4H, m, CH_2O), 5.90 (1H, s, H-3), 7.64 (2H, m, Ar-*H*), 7.96 (1H, m, Ar-*H*), 8.05 (1H, m, Ar-*H*), 8.24 (1H, m, Ar-*H*), 8.42 (1H, m, Ar-*H*); HRMS (EI) M^+ 281.1053 ($C_{17}H_{15}NO_3$ calcd as 281.1052).

4-(Thiomorpholin-4-yl)benzo[*g*]chromen-2-one (10). Reaction of 4-hydroxybenzo[*g*]chromen-2-one **53** (1.06 g, 5.0 mmol) and thiomorpholine (1.03 g 10.0 mmol) according to method V gave the title compound as a pale brown powder: ^{1}H NMR δ 3.05 (4H, m), 3.65 (4H, m), 5.94 (1H, s), 7.68 (2H, m), 7.98 (1H, s), 8.07 (2H, d), 8.37 (1H, s); MS (EI) *m/z* 297 (M^+).

4-(Morpholin-4-yl)benzo[*f*]chromen-2-one (11) was prepared from 4-hydroxybenzo(*f*)chromen-2-one **54** according to method V: IR (KBr) 2852, 1718, 1619, 1542 cm^{-1}; ^{1}H NMR δ 2.97 (2H, m, CH_2N), 3.40 (2H, m, CH_2N), 3.95 (4H, m, CH_2O), 6.00 (1H, s, H-3), 7.65 (2H, m, Ar-*H*), 7.83 (1H, m, Ar-*H*), 8.13 (1H, m, Ar-*H*), 8.25 (1H, m, Ar-*H*), 9.15 (1H, m, Ar-*H*); HRMS (EI) M^+ 281.1054 ($C_{14}H_{15}NO_4$ calcd as 281.1052).

4-(Morpholin-4-yl)benzo(*h*)chromen-2-one (12) was obtained as a white solid from 4-hydroxybenzo[*g*]chromen-2-one **55** (0.64 g, 3.0 mmol) according to method V and purified by chromatography on silica to yield a white solid: IR (KBr) 3041, 2992, 2822, 1714, 1596, 1468 cm^{-1}; ^{1}H NMR δ 3.38 (4H, br s, CH_2N), 3.95 (4H, br s, CH_2O), 5.93 (1H, s, H-3), 7.83 (4H, m, Ar-*H*), 8.14 (1H, m, Ar-*H*), 8.46 (1H, m, Ar-*H*); HRMS (EI) M^+ 281.1046 ($C_{17}H_{15}NO_3$ calcd as 281.1052).

4-(Morpholin-4-yl)chromen-2-one (59) was obtained as a white solid from 4-hydroxycoumarin (0.49 g, 3.0 mmol) as described in method V: IR (KBr) 3032, 2957, 2862, 1706, 1600, 1552 cm^{-1}; ^{1}H NMR δ 3.19 (4H, t, J = 4.5 Hz, CH_2N), 3.87 (4H, t, J = 4.5 Hz, CH_2O), 5.67 (1H, s, H-3), 7.26 (2H, m, Ar-*H*), 7.49 (2H, m, Ar-*H*); HRMS (EI) M^+ 231.0890 ($C_{13}H_{13}NO_3$ calcd as 231.0895)

4-(Piperidin-1-yl)chromen-2-one (60) was obtained as a white solid from 4-hydroxycoumarin (0.49 g, 3.0 mmol) according to method V: IR (KBr) 3064, 2942, 2814, 1708, 1604

cm^{-1}; ^{1}H NMR δ 1.76 (6H, m, $CH_2CH_2CH_2N + CH_2CH_2CH_2N$), 3.20 (4H, m, CH_2N), 5.73 (1H, s, H-3), 7.42 (2H, m, Ar-*H*), 7.72 (2H, m, Ar-*H*); HRMS (EI) M$^+$ 229.1101 ($C_{14}H_{15}NO_2$ calcd as 229.1103).

6-Methyl-4-(morpholin-4-yl)chromen-2-one (61) was prepared as a white solid according to method V, from 4-hydroxy-6-methylcoumarin (0.53 g, 3.0 mmol): IR (KBr) 3080, 2973, 1695, 1562 cm^{-1}; ^{1}H NMR δ 2.48 (3H, s, Me), 3.32 (4H, t, J = 4.5 Hz, CH_2N), 3.92 (4H, t, J = 4.5 Hz, CH_2O), 5.80 (1H, s, H-3), 7.37 (2H, m, Ar-*H*), 7.53 (2H, m, Ar-*H*); HRMS (EI) M$^+$ 245.1048 ($C_{14}H_{15}NO_3$ calcd as 245.1052).

8-Methyl-4-(morpholin-4-yl)chromen-2-one (62) was obtained as a yellow powder from 4-hydroxy-8-methylcoumarin (0.53 g, 3.0 mmol) following method V: ^{1}H NMR δ 2.43 (3H, s, 8-Me), 3.22 (4H, t, J = 4.5 Hz, CH_2N), 3.92 (4H, t, J = 4.5 Hz, CH_2O), 5.73 (1H, s, H-3), 7.13 (1H, m, H-6), 7.34 (1H, m, H-7), 7.43 (1H, m, H-5); MS (EI) *m/z* 245 (M$^+$).

5-Methoxy-4-(morpholin-4-yl)chromen-2-one (63) was prepared from **56** (0.58 g, 3.0 mmol) according to method V as a white solid: IR (KBr) 3078, 2976, 1695, 1592 cm^{-1}; ^{1}H NMR δ 3.18 (4H, br s, CH_2N), 3.85 (4H, br s, CH_2O), 3.99 (3H, s, OMe), 5.60 (1H, s, H-3), 7.04 (2H, m, Ar-*H*), 7.66 (1H, m, Ar-*H*); HRMS (EI) M$^+$ 261.0994 ($C_{14}H_{15}NO_4$ calcd as 261.1001).

6-Methoxy-4-(morpholin-4-yl)chromen-2-one (64) was synthesized following method V, from **57** (0.58 g, 3.0 mmol): IR (KBr) 3073, 2952, 1697, 1563 cm^{-1}; ^{1}H NMR δ 3.35 (4H, t, J = 4.5 Hz, CH_2N), 3.92 (7H, br s, CH_2O + OMe), 5.84 (1H, s, H-3), 7.14 (1H, m, Ar-*H*), 7.30 (1H, m, Ar-*H*), 7.44 (1H, m, Ar-*H*); HRMS (EI) M$^+$ 261.0991 ($C_{14}H_{15}NO_4$ calcd as 261.1001).

7-Methoxy-4-(morpholin-4-yl)chromen-2-one (65) was obtained as a white powder from **57** (0.58 g, 3.0 mmol), according to method V: IR (KBr) 3087, 2901, 1696, 1606 cm^{-1}; ^{1}H NMR δ 3.31 (4H, br s, CH_2N), 3.90 (4H, br s, CH_2O), 3.94 (3H, s, OMe), 5.67 (1H, s, H-3), 7.02 (2H, m, Ar-*H*), 7.74 (1H, m, Ar-*H*); HRMS (EI) M$^+$ 261.0990 ($C_{14}H_{15}NO_4$ calcd as 261.100).

8-Methoxy-4-(morpholin-4-yl)chromen-2-one (66). To a stirred solution of 3-methoxysalicylic acid (0.84 g, 5.0 mmol) in dry dioxane (20 mL) at 0 °C was added methyllithium (1.6 M in THF, 16.6 mL, 15 mmol) dropwise over 15 min. After warming to room temperature, diethyl carbonate (40 mL) was added, and the reaction mixture was stirred under reflux for 48 h. Water (100 mL) was added, and the reaction mixture was extracted with diethyl ether (3 × 50 mL). The aqueous layer was acidified to pH 2−3 with concentrated aqueous HCl and extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The residual brown solid was redissolved in DCM (15 mL), and triisopropylbenzenesulfonyl chloride (0.82 g, 2.71 mmol) and NEt_3 (0.36 mL, 2.71 mmol) were added. The reaction mixture was stirred at 25 °C for 12 h, morpholine (0.47 mL, 5.4 mmol) was added, and stirring was continued for a further 12 h. The product was purified by chromatography on silica, to give the title compound as a white solid: IR (KBr) 3086, 2971, 2848, 1712, 1608, 1566 cm^{-1}; ^{1}H NMR δ 3.32 (4H, t, J = 4 Hz, CH_2N), 3.91 (4H, t, J = 4 Hz, CH_2O), 3.99 (3H, s, OMe), 5.83 (1H, s, H-3), 7.37 (3H, m, Ar-*H*); HRMS (EI) M$^+$ 261.1016 ($C_{14}H_{15}NO_4$ calcd as 261.1001).

6-Chloro-4-(morpholin-4-yl)chromen-2-one (67) was obtained as a white powder from 6-chloro-4-hydroxycoumarin (0.59 g, 3.0 mmol) in accordance with method V: IR (KBr) 3082, 2974, 2849, 1729, 1552 cm^{-1}; ^{1}H NMR δ 3.32 (4H, t, J = 4 Hz, CH_2N), 3.91 (4H, t, J = 4 Hz, CH_2O), 5.91 (1H, s, H-3), 7.53 (1H, m, Ar-*H*), 7.77 (2H, m, Ar-*H*); HRMS (EI) M$^+$ 265.0499 ($C_{13}H_{12}ClNO_3$ calcd as 265.0506).

2-Arylbenzo[*h*]chromen-4-ones (68−84): General Method VI. To a solution of 1-hydroxy-2-acetonaphthone (0.02 mol) in dry pyridine (15−20 mL) was added the appropriate acid chloride (0.03 mol), and the reaction mixture was stirred for 30 min at 25 °C. Aqueous HCl (1 M, 120 mL) was added, and the solid that deposited was collected, washed sequentially with cold MeOH (20 mL) and water (50 mL), and recrystallized from MeOH−EtOAc. The 1-acyloxy-2-acetonaphthone (1 mol equiv) was dissolved in pyridine (15−20 mL), and KOH (2 mol equiv) was added. The mixture was stirred for 15 min at 50 °C and cooled, and aqueous AcOH (20%, 75 mL) was added. The resultant solid was collected and redissolved in glacial AcOH (20−30 mL), and concentrated H_2SO_4 (1 mL) was added. After stirring under reflux for 1 h, the mixture was poured onto ice−water (100−200 mL), and the solid that deposited was collected, washed with water, and dried (Na_2SO_4). Recrystallization from EtOAc−petrol furnished the required 2-arylbenzo[*h*]chromen-4-one.

2-Phenylbenzo[*h*]chromen-4-one (69) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and benzoyl chloride: ^{1}H NMR δ 7.31 (1H, s), 7.74 (3H, m), 7.92 (2H, m), 8.07 (2H, m), 8.20 (1H, m), 8.34 (2H, m), 8.78 (1H, m); MS (EI) *m/z* 272 (M$^+$).

2-(2-Chlorophenyl)benzo[*h*]chromen-4-one (70) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 2-chlorobenzoyl chloride: ^{1}H NMR δ 6.97(1H, s), 7.68−8.27 (9H, m), 8.59 (1H, dd); MS (EI) *m/z* 306 (M$^+$).

2-(3-Chlorophenyl)benzo[*h*]chromen-4-one (71) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 3-chlorobenzoyl chloride: ^{1}H NMR δ 7.41(1H, s), 7.80 (2H, m), 7.95 (2H, m), 8.09 (2H, m), 8.22 (1H, m), 8.36 (2H, m), 8.79 (1H, m); MS (EI) *m/z* 306 (M$^+$).

2-(4-Chlorophenyl)benzo[*h*]chromen-4-one (72) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 4-chlorobenzoyl chloride: ^{1}H NMR δ 7.35 (1H, s), 7.75 (2H, d), 7.90 (2H, m), 8.10 (2H, s), 8.20 (1H, m), 8.35 (2H, d), 8.80 (1H, m); MS (EI) *m/z* 306 (M$^+$).

2-(2-Fluorophenyl)benzo[*h*]chromen-4-one (73) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 2-fluorobenzoyl chloride: ^{1}H NMR δ 7.35 (1H, s, H-3), 7.47−7.59 (2H, m, Ar-*H*), 7.70−8.32 (7H, m, Ar-*H*), 8.65 (1H, m, Ar-*H*); MS (EI) *m/z* 290 (M$^+$).

2-(3-Fluorophenyl)benzo[*h*]chromen-4-one (74) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 3-fluorobenzoyl chloride: IR (KBr) 3094, 3080, 3064, 1644, 1609, 1570 cm^{-1}; ^{1}H NMR δ 7.33 (1H, s, 3-H), 7.52 (1H, m, Ar-*H*), 7.69−8.18 (8H, m, Ar-*H*), 8.73−8.81 (1H, m, Ar-*H*); MS (EI) *m/z* 290 (M$^+$).

2-(4-Fluorophenyl)benzo[*h*]chromen-4-one (75) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 4-fluorobenzoyl chloride: IR (KBr) 3081, 3070, 3053, 1659, 1602, 1513 cm^{-1}; ^{1}H NMR δ 7.25 (1H, s, H-3), 7.52 (2H, m, Ar-*H*), 7.84−8.21 (5H, m, Ar-*H*), 8.33−8.41 (2H, m, Ar-*H*), 8.71−8.78 (1H, m, Ar-*H*); MS (EI) *m/z* 290 (M$^+$).

2-(2-Nitrophenyl)benzo[*h*]chromen-4-one (76) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 2-nitrobenzoyl chloride: IR (KBr) 3079, 3057, 3034, 2864, 1649, 1523,1393 cm^{-1}; ^{1}H NMR δ 7.11 (1H, s, H-3), 7.79−8.37 (10H, m, Ar-*H*); MS (EI) *m/z* 317 (M$^+$).

2-(3-Nitrophenyl)benzo[*h*]chromen-4-one (77) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 3-nitrobenzoyl chloride: IR (KBr) 3092, 3078, 3067, 3037, 1639, 1574, 1531, 1509,1344 cm^{-1}; ^{1}H NMR δ 7.52 (1H, s, H-3), 7.97−9.04 (10H, m, Ar-*H*); MS (EI) *m/z* 317 (M$^+$).

2-(4-Nitrophenyl)benzo[*h*]chromen-4-one (78) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 4-nitrobenzoyl chloride: IR (KBr) 3074, 3053, 3009, 1653, 1526, 1345 cm^{-1}; ^{1}H NMR δ 7.51 (s, 1H, H-3), 7.95−8.83 (m, 10H, 10 × Ar-*H*); MS (EI) 317 (M$^+$).

2-(4-Aminophenyl)benzo[*h*]chromen-4-one (79). A solution of **78** (0.16 g, 0.50 mmol) in glacial AcOH (10 mL), containing 10% Pd on carbon (0.03 g) was hydrogenated at atmospheric pressure for 16 h, the catalyst was removed by filtration, and the solvent was removed under reduced pressure. The residual solid was washed with saturated aqueous $NaHCO_3$ solution and extracted with DCM (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The resultant solid was redissolved in EtOAc (50 mL) and extracted with aqueous HCl (1 M, 2 × 50 mL).

The combined aqueous layer was neutralized with saturated aqueous $NaHCO_3$, and the solid which was deposited was collected and washed with water to give **79** as a pale yellow solid: ^{1}H NMR δ 6.17 (2H, br s, NH_2), 6.93 (2H, d, Ar-*H*), 7.00 (1H, s, H-3), 7.87−8.81 (8H, m, Ar-*H*); MS (ESI+) *m/z* 288 (M + 1).

2-(3-Aminophenyl)benzo[*h*]chromen-4-one (80). The title compound was prepared from **77** (0.32 g, 1.0 mmol) as for **79**: ^{1}H NMR δ 5.61 (2H, br s, NH_2), 6.93 (1H, m, Ar-*H*), 7.11 (1H, s, H-3), 7.32−7.46 (2H, m, Ar-*H*), 7.55 (1H, m, Ar-*H*), 7.89−8.26 (5H, m, Ar-*H*), 8.75−8.82 (1H, m, Ar-*H*); MS (ESI+) *m/z* 288 (M + 1).

2-(2-Aminophenyl)benzo[*h*]chromen-4-one (81). The title compound was prepared from **76** (1.25 g, 3.94 mmol) analogously to **79**: ^{1}H NMR δ 7.16 (1H, s, H-3), 7.81−8.37 (12H, m, Ar-*H* + NH_2); MS (ESI+) *m/z* 288 (M + 1).

2-(3-Pyridyl)benzo[*h*]chromen-4-one (82) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and pyridine-3-carbonyl chloride: IR (KBr) 3089, 3068, 3059, 3025, 2961, 2900, 1652, 1600, 1593 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.58 (1H, s, H-3), 7.00−8.86 (10H, m, Ar-*H*); MS (EI) *m/z* 273 (M^+).

2-(4-Pyridyl)benzo[*h*]chromen-4-one (83) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and pyridine-4-carbonyl chloride: IR (KBr) 3082, 3058, 3021, 3004, 1652, 1585, 1571 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.95 (1H, s, H-3), 7.48−9.27 (10H, m, Ar-*H*); MS (EI) *m/z* 273 (M^+).

2-(4-*tert*-Butylphenyl)benzo[*h*]chromen-4-one (84) was prepared following the procedure described in method VI, from 1-hydroxy-2-acetonaphthone and 4-*tert*-butylbenzoyl chloride: IR 3059, 2962, 2903, 2868, 1645, 1631, 1613, 1571 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, $C(CH_3)_3$), 7.27 (1H, s, H-3), 7.72−8.29 (9H, m, Ar-*H*), 8.76−8.81 (1H, m, Ar-*H*); MS (EI) *m/z* 328 (M^+).

Pyrimido[2,1-*a*]isoquinoline-2,4-dione (85). 2-Aminoisoquinoline (5.16 g, 35.79 mmol) was dissolved in diethyl malonate (5.43 mL, 35.79 mmol). EtOH (20 mL) was added, and the solution was heated to 170 °C for 4 h. EtOH was removed by distillation and, after cooling, the dark residue was triturated with EtOAc (10 mL). The residual pale solid was collected by filtration and washed with EtOAc to furnish the title compound as a pale brown solid (4.43 g, 24.89 mmol, 70%): mp 294−296 °C; ^{1}H NMR ($CDCl_3$) δ 3.39 (2H, s, CH_2), 6.10−6.14 (1H, m, Ar-*H*), 7.21−7.42 (5H, m, Ar-*H*); MS (ESI+) *m/z* 213 (M^+).

2-Chloropyrimido[2,1-*a*]isoquinolin-4-one (86). A solution of **85** (4.43 g, 24.89 mmol) in $POCl_3$ (20 mL) was heated under reflux for 5 h, cooled, and poured carefully into ice water (∼250 mL). The aqueous solution was adjusted to pH 7 with saturated Na_2CO_3 solution, and the solid that deposited was collected and washed thoroughly with water. The crude product was purifed by chromatography on silica, eluting with DCM, to provide the title compound as pale yellow crystals. (5.21 g 91%) mp 197−199 °C; ^{1}H NMR ($CDCl_3$) δ 6.12−6.17 (1H, m, Ar-*H*), 6.81 (1H, s, CH), 7.24−7.45 (5H, m, Ar-*H*); MS (ESI+) *m/z* 231.5 (M^+).

2-Amine-Substituted Pyrimido[2,1-*a*]isoquinolin-4-ones (13, 88−108): General Method VII. To a solution of **86** (1 mol equiv) in boiling EtOH (20 mL) was added the appropriate amine (4 mol equiv), and the solution was stirred vigorously under reflux for 16 h. The solid that crystallized upon cooling of the reaction mixture was collected and washed with cold EtOH (30 mL) to furnish the title compound.

2-(Morpholin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (13) was prepared according to method VII from **86** (0.23 g, 1 mmol) and morpholine (0.35 mL, 4 mmol). White crystals: IR (KBr) 3070, 2983, 2945, 2911, 2864, 1701, 1641, 1574, 1546, 1522 cm^{-1}; ^{1}H NMR δ 3.82 (8H, m, morpholine), 5.73 (1H, s, H-3), 7.37 (1H, d, Ar-*H*), 7.75 (1H, m, Ar-*H*), 7.77 (1H, d Ar-*H*), 7.91 (1H, d Ar-*H*), 8.62 (1H, d, Ar-*H*), 8.88 (1H, d, Ar-*H*); MS (EI) *m/z* 281 (M^+).

2-(Thiomorpholin-4-yl)pyrimido[2,1-*a*]isoquinolin-4-one (88) was prepared as outlined in method VII from **86** (0.23 g, 1 mmol) and thiomorpholine (0.38 mL, 4 mmol) as pale yellow crystals: ^{1}H NMR ($CDCl_3$) δ 2.63−2.68 (4H, m, CH_2S), 4.03−4.08 (4H, m, CH_2N), 5.62 (1H, s, 3-*H*), 7.01 (1H, d, J = 7.8 Hz, 7-*H*), 7.51−7.72 (3H, m, Ar-*H*), 8.60 (1H, d, J = 7.8 Hz, 6-*H*), 8.75 (1H, d, J = 8.5 Hz, 11-*H*); MS (EI) *m/z* 298 (M + 1).

2-(Pyrrolidin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (89) was synthesized from **86** (0.23 g, 1 mmol) and pyrrolidine (0.34 mL, 4 mmol) in accordance with method VII, to give white crystals: ^{1}H NMR ($CDCl_3$) δ 1.98−2.11 (4H, m, CH_2CH_2), 3.51−3.61 (4H, m, NCH_2), 5.49 (1H, s, 3-*H*), 7.04 (1H, d, J = 7.6 Hz, 7-*H*), 7.54−7.69 (3H, m, Ar-*H*), 8.65 (1H, d, J = 7.6 Hz, 6-*H*), 8.85−8.88 (1H, m, Ar-*H*); MS (ESI+) *m/z* 266 (M + 1).

2-(2,5-Dihydropyrrol-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (90). Reaction of **86** (0.35 g, 1.5 mmol), 3-pyrroline (0.14 mL, 1.8 mmol), and NEt_3 (0.25 mL, 1.8 mmol) following method VII gave the title compound as beige crystals: ^{1}H NMR ($CDCl_3$) δ 4.11 (2H, br s, NCH_2), 4.53 (2H, br s, NCH_2), 5.35 (1H, s, 3-*H*), 5.90 (2H, br s, CH=CH), 6.98 (1H, d, J = 7.6 Hz, 7-*H*), 7.52−7.76 (3H, m, Ar-*H*), 8.64 (1H, d, J = 7.6 Hz, 6-*H*), 8.80−8.85 (1H, m, Ar-*H*); MS (ESI+) *m/z* 264 (M + 1).

2-(3-(*R*)-Hydroxypyrrolidin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (91). The title compound was prepared by method VII, from **86** (0.23 g, 1 mmol) and 3-(*R*)-pyrrolidinol (0.36 mL, 4 mmol), as a cream solid: ^{1}H NMR δ 1.98−2.19 (2H, m, CH_2CH(OH)), 3.41−3.55 (2H, m, NCH_2), 3.81−3.96 (2H, m, NCH_2), 4.48−4.55 (1H, m, *CH*(OH)), 5.17 (1H, s, 3-*H*), 7.36 (1H, d, J = 7.6 Hz, 7-*H*), 7.77−7.83 (1H, m, Ar-*H*), 7.91−7.99 (2H, m, Ar-*H*), 8.63 (1H, d, J = 7.6 Hz, 6-*H*), 8.86 (1H, d, J = 8.0 Hz, 11-*H*); MS (ESI+) *m/z* = 282 (M + 1).

2-(4-Methylpiperazin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (92) was prepared by method VII, from **86** (0.23 g, 1.00 mmol) and *N*-methylpiperazine (0.44 mL, 4.00 mmol), as a white solid: ^{1}H NMR δ 2.91 (3H, s, NCH_3), 3.39−3.47 (8H, m, piperazine CH_2), 5.95 (1H, s, 3-*H*), 7.49 (1H, d, J = 7.6 Hz, 7-*H*), 7.84−7.99 (1H, m, Ar-*H*), 8.00−8.04 (2H, m, Ar-*H*), 8.67 (1H, d, J = 7.6 Hz, 6-*H*), 8.99 (1H, d, J = 8.0 Hz, 11-*H*); MS (ESI+) *m/z* 295 (M + 1).

2-(2,5-Dimethylpiperidin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (93). The title compound was synthesized by method VII, from **86** (0.23 g, 1.00 mmol) and 2,5-dimethylpiperidine (0.52 mL, 4.00 mmol) as white crystals: ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, s), 0.93 (3H, s), 1.65 (4H, m), 4.42 (2H, s), 5.62 (1H, s), 6.96 (1H, d), 7.61 (3H, m), 8.59 (1H, d), 8.76 (1H, m); MS (ESI+) *m/z* 308 (M + 1).

2-(*cis*-2,6-Dimethylmorpholin-4yl)pyrimido[2,1-*a*]isoquinolin-4-one (94). The title compound was prepared as detailed in method VII from **86** (0.12 g, 0.50 mmol) and *cis*-2,6-dimethylmorpholine (0.25 mL, 2 mmol) as white crystals: ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, d), 2.68 (2H, dd), 3.70 (2H, m), 4.30 (2H, m), 5.63 (1H, s), 7.06 (1H, d), 7.67 (3H, m), 8.65 (1H, d), 8.81 (1H, d); MS (ESI+) *m/z* 310 (M + 1).

2-(2,2-Dimethylmorpholin-4-yl)-pyrimido[2,1-*a*]isoquinolin-4-one (95) was prepared from **86** (0.12 g, 1.00 mmol), 2,2-dimethylmorpholine (0.12 mL, 1.04 mmol), and NEt_3 (0.17 mL, 1.22 mmol) according to method VII, to give a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, s, Me), 3.48 (2H, s, $NCH_2C(Me_2)$), 3.65−3.82 (4H, m, NCH_2CH_2O), 5.58 (1H, s, 3-*H*), 7.03 (1H, d, J = 7.7 Hz, 7-*H*), 7.51−7.74 (3H, m, Ar-*H*), 8.61 (1H, d, J = 7.7 Hz, 6-*H*), 8.77 (1H, d, J = 7.8 Hz, 11-*H*); MS (ESI+) *m/z* 310 (M + 1).

2-(2-Ethylmorpholin-4-yl)pyrimido[2,1-*a*]isoquinolin-4-one (96). Reaction of **86** (0.12 g, 1.00 mmol) with 2-ethylmorpholine (0.12 mL, 1.04 mmol) and NEt_3 (0.17 mL, 1.22 mmol) according to method VII gave the title compound as a pale brown solid: ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t, J = 7.5 Hz, CH_3), 1.49−1.63 (2H, m, CH_2), 2.72−2.77 (1H, m), 3.06−3.11 (1H, m), 3.35−3.40 (1H, m), 3.59−3.65 (1H, m), 3.98−4.01 (1H, m), 4.21−4.26 (1H, m), 5.62 (1H, s, 3-*H*), 7.06 (1H, d, J = 7.6 Hz, 7-*H*), 7.63−7.74 (3H, m, Ar-*H*), 8.61 (1H, d, J = 7.6 Hz, 6-*H*), 8.83 (1H, d, J = 8.2 Hz, 11-*H*); MS (ESI+) *m/z* 310 (M + 1).

2-(3-Hydroxymethylpiperidin-1-yl)pyrimido[2,1-*a*]isoquinolin-4-one (97). The title compound was prepared by method VII from **86** (0.23 g, 1.00 mmol) and 3-piperidinemethanol (0.46 g, 4.00 mmol) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.64−1.89 (5H, m), 2.31−2.42 (1H, m), 3.29−3.33 (1H, m), 3.49−3.65 (3H, m), 4.01−4.14 (2H, m), 5.64 (1H, s), 7.01 (1H, d, J = 7.6 Hz), 7.58−7.77 (3H, m), 8.63 (1H, d, J = 7.6 Hz), 8.77 (1H, d, J = 7.8 Hz); MS (ESI+) *m/z* 310 (M + 1).

2[*N*-(2-Hydroxyethyl)-*N*-methylamino]pyrimido[2,1-*a*]-isoquinolin-4-one (98). Reaction of **86** (0.12 g, 0.5 mmol) and 2-(*N*-methylamino)ethanol (0.16 mL, 2 mmol) according to method VII gave the title compound as white crystals: ^{1}H NMR ($CDCl_3$) δ 3.15 (3H, s, NCH_3), 3.91−3.99 (4H, m, NCH_2CH_2OH), 5.55 (1H, s, 3-*H*), 7.00 (1H, d, J = 7.6 Hz, 7-*H*), 7.57−7.72 (3H, m, Ar-*H*), 8.62 (1H, d, J = 7.6 Hz, 6-*H*), 8.73-(1H, d, J = 7.8 Hz, 11-*H*); MS (ESI+) *m/z* 270 (M + 1).

2-[*N*-Benzyl-*N*-(2-hydroxyethyl)amino]pyrimido[2,1-*a*]-isoquinolin-4-one (99). The title compound was prepared by method VII, from **86** (0.12 g, 0.50 mmol) and *N*-benzylethanolamine (0.28 mL, 2.00 mmol) as white crystals: ^{1}H NMR δ 3.67−3.82 (4H, m, NCH_2), 4.95−5.01 (2H, m, CH_2OH), 5.63 (1H, s, 3-*H*), 7.31−7.43 (6H, m, Ar-*H*), 7.71−7.95 (3H, m, Ar-*H*), 8.63 (1H, d, J = 7.6 Hz, 6-*H*), 8.82−8.85 (1H, m, Ar-*H*); MS (ESI+) *m/z* 346 (M + 1).

2-[(Tetrahydrofuran-2-ylmethyl)amino]pyrimido[2,1-*a*]isoquinolin-4-one (100). The title compound was synthesized by following method VII, from **86** (0.23 g, 1.00 mmol) and tetrahydrofurfurylamine (0.42 mL, 4 mmol) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.56−1.88 (4H, m, CH_2CH_2), 3.42−3.64 (2H, m, CH_2O), 3.71−3.77 (1H, m, NCH_2), 3.82−3.89 (1H, m, NCH_2), 4.01−4.12 (1H, m, CH), 5.29 (1H, s, NH), 5.43 (1H, s, 3-*H*), 6.96 (1H, d, J = 7.7 Hz, 7-*H*), 7.49−7.70 (3H, m, Ar-*H*), 8.57 (1H, d, J = 7.7 Hz, 6-*H*), 8.76 (1H, d, J = 7.9 Hz, 11-*H*); MS (ESI+) *m/z* 296 (M + 1).

2-[Bis(2-hydroxyethyl)amino]pyrimido[2,1-*a*]isoquinolin-4-one (101) was prepared by method VII from **86** (0.23 g, 1.0 mmol) and diethanolamine (0.42 g, 4.0 mmol) as a white solid: ^{1}H NMR δ 3.59−3.91 (8H, m, $N[CH_2CH_2OH]_2$), 5.62 (1H, s, 3-*H*), 7.38 (1H, d, J = 7.7 Hz, 7-*H*), 7.78−7.87 (2H, m, Ar-*H*), 7.93−8.05 (1H, m, Ar-*H*), 8.58 (1H, d, J = 7.7 Hz, 6-*H*), 8.87−8.90 (1H, m, Ar-*H*); MS (ESI+) *m/z* 300 (M + 1).

2-(2,3-Dihydroxypropylamino)pyrimido[2,1-*a*]isoquinolin-4-one (102). Reaction of **86** (0.12 g, 0.5 mmol) with 2,3-dihydroxypropylamine (0.30 g, 4.00 mmol) as described in method VII afforded the title compound as an off-white crystalline solid: ^{1}H NMR δ 3.74−3.78 (2H, m), 4.47 (1H, t, J = 5.2 Hz), 4.77 (1H, m), 5.01 (1H, m), 5.51 (1H, s), 7.36 (1H, d, J = 7.6 Hz), 7.78−7.83 (1H, m), 7.92−7.99 (2H, m), 8.63 (1H, d, J = 7.6 Hz), 8.82−8.87 (1H, m); MS (ESI+) *m/z* 286 (M + 1)

2-(2-Hydroxypropylamino)pyrimido[2,1-*a*]isoquinolin-4-one (103) was prepared according to method VII from **86** (0.12 g, 0.50 mmol) and 2-hydroxypropylamine (0.30 g, 4.00 mmol), to give the title compound as an off-white crystalline solid: ^{1}H NMR δ 1.23 (3H, d, J = 6.2 Hz CH_3), NCH_2), 3.92−4.01 (2H, m, CH_2), 4.93 (1H, d, J = 4.6 Hz, CH), 5.50 (1H, s, 3-*H*), 7.36 (1H, d, J = 7.7 Hz, 7-*H*), 7.0−7.85 (1H, m, Ar-*H*), 7.93−8.01 (2H, m, Ar-*H*), 8.63 (1H, d, J = 7.7 Hz, 6-*H*), 8.87−8.90 (1H, m, Ar-*H)*; MS (ESI+) *m/z* 270 (M + 1).

2-(2-Hydroxyethylamino)pyrimido[2,1-*a*]isoquinolin-4-one (104). The title compound was synthesized as an off-white crystalline solid, from **86** (0.12 g, 0.50 mmol) and 2-aminoethanol (0.24 mL, 4.0 mmol), following method VII: ^{1}H NMR ($CDCl_3$) δ 3.44−3.51 (2H, m, NH + CH), 3.70−3.75 (2H, m, CH_2OH), 4.92−4.94 (1H, br s, NH), 5.49 (1H, s, 3-*H*), 7.39 (1H, d, J = 7.6 Hz, 7-*H*), 7.81−7.85 (1H, m, Ar-*H*), 7.95−8.02 (2H, m, Ar-*H*), 8.64 (1H, d, J = 7.6 Hz, 6-*H*), 8.87−8.91 (1H, m, Ar-*H*); MS (ESI+) *m/z* 256 (M + 1).

3-[Methyl(4-oxo-4*H*-pyrimido[2,1-*a*]isoquinolin-2-yl)-amino]propionitrile (105) was prepared as an off-white crystalline solid, by reaction of **86** (0.12 g, 0.50 mmol) with *N*-methyl-β-alanine nitrile (0.38 mL, 4.0 mmol) in accordance with method VII: ^{1}H NMR ($CDCl_3$) δ 2.80 (2H, t, J = 6.5 Hz, CH_2CH_2CN), 3.18 (3H, s, CH_3), 4.08 (2H, t, J = 6.5 Hz, CH_2N), 5.58 (1H, s, 3-*H*), 7.01 (1H, d, J = 7.6 Hz, 7-*H*), 7.58−7.79 (3H, m, Ar-*H*), 8.68 (1H, d, J = 7.6 Hz, 6-*H*), 8.73−8.76 (1H, m, Ar-*H*); MS (ESI+) *m/z* 279 (M + 1).

2-(2-[Thiophen-2-yl]ethylamino)pyrimido[2,1-*a*]isoquinolin-4-one (106) was synthesized by method VII, from **86** (0.12 g, 0.5 mmol) and 2-(2-thienyl)ethylamine (0.48 mL, 4 mmol), to provide the title compound as an off-white crystalline solid: ^{1}H NMR ($CDCl_3$) δ 3.08−3.17 (2H, m, NCH_2), 3.49−3.68 (2H, m, CH_2CHS), 5.07 (1H, s, NH), 5.47 (1H, s, 3-*H*), 6.70−6.97 (2H, m, C(S)CH−CH), 7.05−7.19 (2H, m, Ar-*H*, SCH), 7.54−7.66 (3H, m, Ar-*H*), 8.60 (1H, d, J = 7.6 Hz, 6-*H*), 8.73−8.77 (1H, m, Ar-*H*); MS (ESI+) *m/z* 322 (M + 1).

2-[2-Hydroxy-2-(3-hydroxyphenyl)ethylamino]pyrimido[2,1-*a*]isoquinolin-4-one (107) was prepared by method VII from **86** (0.12 g, 0.50 mmol) and α-aminomethyl-3-hydroxybenzyl alcohol (0.75 g, 4.0 mmol), to afford the title compound as an off-white crystalline solid: ^{1}H NMR δ 4.80−4.84 (2H, m, CH_2NH), 5.52 (1H, s, 3-*H*), 5.64 (1H, d, J = 4.1 Hz, *CH*(OH)), 6.73−6.79 (1H, m, Ar-*H*), 6.92−6.99 (2H, m, Ar-*H*), 7.24 (1H, t, J = 7.5 Hz, Ar-*H*), 7.39 (1H, d, J = 7.4 Hz, 7-*H*), 7.79−7.83 (1H, m, Ar-*H*), 7.91−7.98 (2H, m, Ar-*H*), 8.65 (1H, d, J = 7.6 Hz, 6-*H*), 8.88−8.92 (1H, m, Ar-*H*), 9.48 (1H, br s, OH); MS (ESI+) *m/z* 348 (M + 1).

2-[*N*-(2-Hydroxy-2-phenylethyl)-*N*-methyl]pyrimido-[2,1-*a*]isoquinolin-4-one (108) was prepared by method VII from **86** (0.12 g, 0.50 mmol) and 2-hydroxy-2-(phenylethyl)-methylamine (0.30 g, 4.0 mmol), as an off-white crystalline solid: ^{1}H NMR ($CDCl_3$) δ 3.96−4.02 (2H, m, NCH_2), 4.60 (1H, s, OH), 5.09−5.15 (1H, m, *CH*(OH)), 5.54 (1H, s, 3-*H*), 7.04 (1H, d, J = 7.6 Hz, 7-*H*), 7.31−7.48 (5H, m, Ar-*H*), 7.68−7.74 (3H, m, Ar-*H*), 8.65 (1H, d, J = 7.6 Hz, 6-H), 8.80−8.84 (1H, m, Ar-*H*); MS (ESI+) *m/z* 346 (M + 1).

DNA-PK Inhibition Assay. Mammalian DNA-PK (500 ng/μL) was isolated from HeLa cell nuclear extract by Q-sepharose, followed by S-sepharose chromatography, and a final step of heparin-agarose chromatography. DNA-PK (250 ng) activity was measured at 30 °C, in a final volume of 40 μL, in buffer containing 25 mM Hepes, pH 7.4, 12.5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 10% glycerol, 0.1% NP-40, and 1 μg of the substrate GST−p53N66 (the amino-terminal 66 amino acid residues of human wild-type p53 fused to glutathione *S*-transferase) in polypropylene 96-well plates. To the assay mix were added varying concentrations of inhibitor (in DMSO at a final concentration of 1%). After 10 min of incubation, ATP was added to give a final concentration of 50 μM along with a 30mer double stranded DNA oligonucleotide (final concentraion of 0.5 ng/mL) to initiate the reaction. After 1 h with shaking, 150 μL of phosphate-buffered saline (PBS) was added to the reaction and 5 μL was then transferred to a 96-well opaque white plate containing 45 μL of PBS per well, where the GST−p53N66 substrate was allowed to bind to the wells for 1 h at room temperature.

To detect the phosphorylation event on the serine-15 residue of p53 elicited by DNA-PK, a phosphoserine-15 antibody (Cell Signaling Technology) was used in a basic ELISA procedure. An anti-rabbit HRP conjugated secondary antibody (Pierce) was then employed in the ELISA before the addition of chemiluminescence reagent (NEN Renaissance) to detect the signal as measured by chemiluminescent counting via a TopCount NXT (Packard). The IC_{50} for compounds was then derived from a sigmoidal plot using the graphic package Prism, in which the DNA-PK activity in the varying concentration of compounds was plotted against the concentration of compound. For kinetic analysis of compound **48**, the same assay format was employed but in the presence of varying ATP concentrations. For rates of reaction, it is assumed that the chemiluminescent signal derived from the assay is proportional to the amount of phosphorylation that has taken place at the serine-15 residue.

Other PIKK Kinase Assays. The determination of ATM kinase and ATR kinase activities was performed essentially according to methodologies described previously[37,38] using rabbit polyclonal antisera raised to the COOH-terminal 400 amino acids of ATM, and antisera raised to amino acids 400−

480 of ATR. The PI 3-kinase assay was performed essentially as described previously[17] using baculoviral derived recombinant human p110α and p85α (a kind gift from Prof. Mike Waterfield, Ludwig Institute, London). mTOR protein was isolated from HeLa cell cytoplasmic extract by immunoprecipitation, and activity determined essentially as described previously using recombinant PHAS-1 as substrate.[39]

Clonogenic Assays. Clonogenic survival assays were performed using HeLa cells. HeLa cells were seeded ~100 colonies per well for each data point, in a 6-well dish 4−6 h prior to the addition of 5 or 10 μM of **48**. Stock solutions of **48** were prepared at 10 mM in 100% DMSO, with a final DMSO concentration of 0.1% on the cells. After 1 h of incubation with **48**, cells were irradiated at 1 Gy/min using a Faxitron 43855D X-ray cabinet. Drug-containing media was removed after a further 16 h, and fresh media was added prior to a further incubation of 8 days before staining of the colonies with Giemsa. Resulting colonies containing >50 cells were counted as positives.

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Supporting Information Available: Experimental details for the synthesis of compounds **14**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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