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Article

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1-Substituted Dibenzo[*b,d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-ones Endowed With Dual DNA-PK/PI3-K Inhibitory Activity

Céline Cano,[a] Kappusamy Saravanan ,[a] Chris Bailey,[b] Julia Bardos,[b] Nicola J. Curtin,[c] Mark Frigerio,[b] Bernard T. Golding,[a] Ian R. Hardcastle,[a] Marc G. Hummersone,[b] Keith A. Menear,[b] David R. Newell,[c] Caroline J. Richardson,[b] K. Shea,[b,d] Graeme C. M. Smith,[b,d] Pia Thommes,[b,d] Attilla Ting[d] and Roger J. Griffin.*[a]*

[a] Newcastle Cancer Centre, Northern Institute for Cancer Research, School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom. [b] KuDOS Pharmaceuticals, Ltd., 410 Cambridge Science Park, Milton Road, Cambridge CB4 0PE, United Kingdom. [c] Newcastle Cancer Centre, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom. [d] AstraZeneca, Oncology Innovative Medicines, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

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Abstract

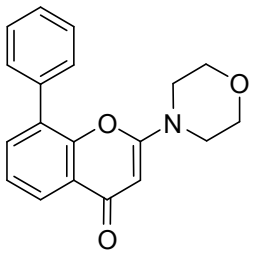
Analogues of (dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (NU7441), a potent inhibitor of DNA-dependent protein kinase (DNA-PK; IC_{50} = 42 ± 2 nM), have been synthesized in which water-solubilizing groups [$NHCO(CH_2)_nNR^1R^2$, where n = 1 or 2 and the moiety R^1R^2N was derived from a library of primary and secondary amines, e.g. morpholine] were placed at the 1-position. Several of the newly synthesized compounds exhibited high potency against DNA-PK and potentiated the cytotoxicity of ionizing radiation (IR) *in vitro* 10-fold or more (e.g. 2-(4-ethylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thio-phen-1-yl)acetamide, **39**; DNA-PK IC_{50} = 5.0

± 1 nM, IR Dose Modification Ratio = 13). Furthermore, **39** was shown to potentiate not only IR *in vitro* but also DNA -inducing cytotoxic anticancer agents, both *in vitro* and *in vivo*. Counter-screening against other members of the phosphatidylinositol 3-kinase (PI-3K) related kinase (PIKK) family unexpectedly revealed that some of the compounds were potent mixed DNA-PK and PI-3K inhibitors.

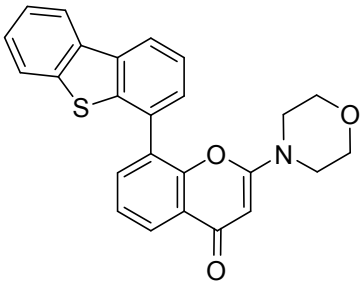
Introduction

Repair of potentially lethal DNA double-strand breaks (DSBs) within mammalian cells is a key component of the DNA damage-response (DDR) pathway and arises principally *via* the processes of homologous recombination (HR) and non-homologous end joining (NHEJ).[1-3] The detection, signaling and repair of DSBs by HR and NHEJ are coordinated by members of an atypical class of serine-threonine kinases, collectively termed the phosphatidylinositol 3-kinase (PI-3K) related kinase (PIKK) family.[4-6] Paradoxically, while essential for cell survival, DNA DSB repair may also constitute a mechanism of resistance to DNA-damaging cancer therapeutics. DNA-repair inhibitors are thus of increasing interest as chemo- and radio-sensitizing agents in cancer treatment.[7-9] Pharmacological modulation of DNA repair has also gained considerable impetus more recently as a consequence of the synthetic lethality of PARP-1 inhibitors in BRCA1 and BRCA2 deficient tumors, suggesting that DNA-repair inhibitors may have single agent anticancer activity in tumors harboring DDR defects.[10]

The DNA-dependent protein kinase (DNA-PK), a multicomponent PIKK family member comprising a catalytic subunit (DNA-PKcs) and two Ku subunits, plays an essential role in NHEJ-mediated DSB repair.[11,12] Importantly, tumor cell lines defective in DSB repair as a consequence of compromised DNA-PK activity are highly sensitive to topoisomerase II inhibitors and ionizing radiation (IR),[13,14] whereas over-expression of DNA-PKcs confers resistance to these agents by enhancing DSB repair.[15,16] Taken together, these data provide compelling evidence that DNA-PK is an attractive therapeutic target for the modulation of DNA DSB repair in cancer therapy, and preliminary results with inhibitors have proved encouraging.[17-20]



1



2

Our interest in the development of small-molecule DNA-PK modulators stemmed from the observation that the PI-3K inhibitor LY294002 (2-morpholino-8-phenyl-4*H*-chromen-4-one, **1**)[21] also exhibits albeit modest ATP-competitive inhibition of DNA-PK (K_i = 6 μM).[22] Extensive structure-activity relationship (SAR) studies conducted with **1** were directed towards the development of potent and selective DNA-PK inhibitors. For this chemotype, the 2-morpholino-4*H*-chromen-4-one moiety is connected at the 8-position to an aryl or heteroaryl ring, which may be substituted or unsubstituted, with a dibenzofuran-4-yl or dibenzothiophen-4-yl group proving especially favorable.[23-26] These studies culminated in the identification of NU7441 (**2**), which combined potent DNA-PK inhibition (IC_{50} = 42 ± 2 nM) with good selectivity over other PIKKs as well as PI-3K family members.[24] Sensitization of human tumor cell lines to IR and etoposide *in vitro* and *in vivo* was also demonstrated with **2**,[27] although that further biological studies were impeded by formulation problems arising from the poor aqueous solubility of the chromenone derivative.

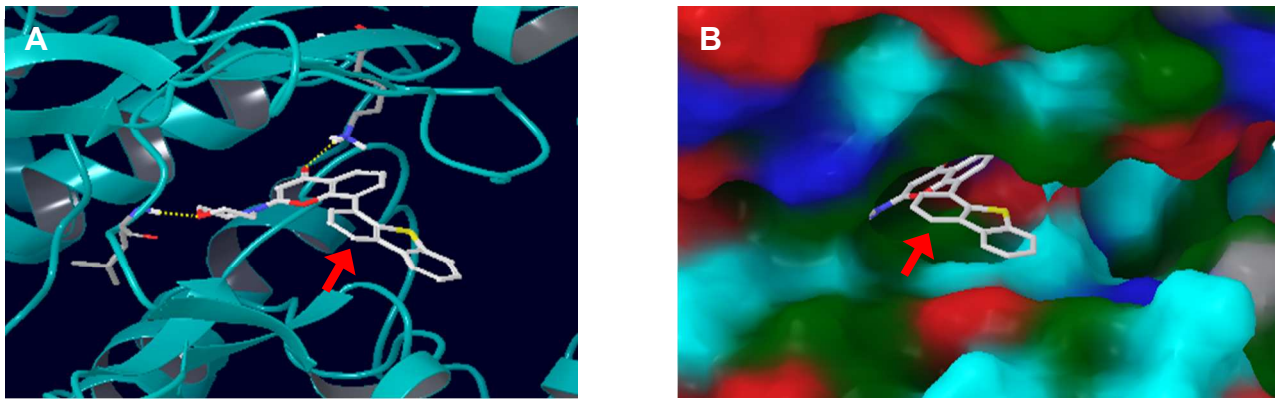
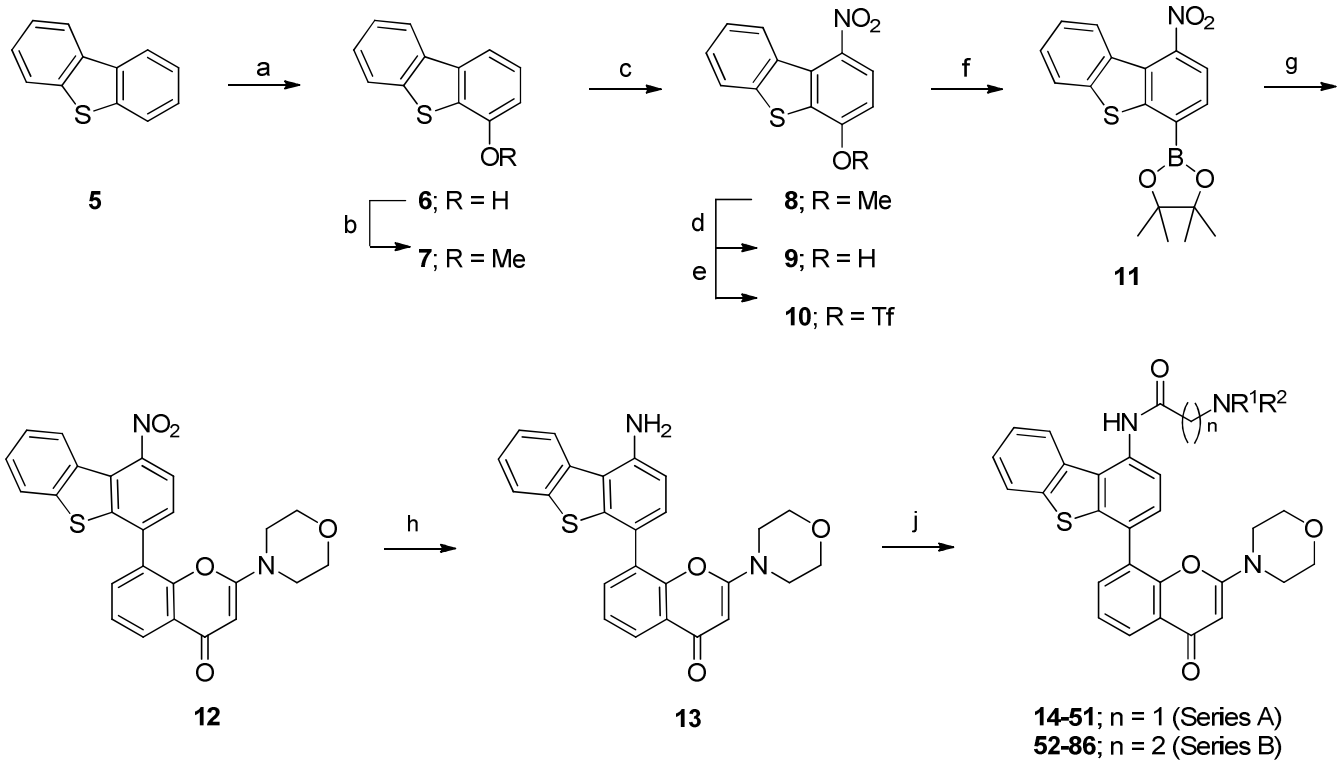


Figure 1. Homology model of the ATP-binding site of DNA-PK with NU7441 (**2**) docked and

minimized. **A**; Ball and stick model indicating putative H-bond interactions (dashed lines). **B**; Protein surface colored by residue properties (red – acidic, blue – basic, dark green – hydrophobic and cyan – polar). The homology model of DNA-PK was derived from the known X-ray crystal structure of PI3Kγ (RCSB protein databank: PDB ID: 1E7V), employing the DNA-PK sequence from Swiss-Port (ID: PRKDC_DICDI). The model of **2** was constructed using the Maestro molecular modeling program, minimized using the OPLS_2005 force field in Macromodel and docked using Glide. Macromodel, Glide and Maestro were licensed from Schrödinger, LGG (www.schrodinger.com).

In the absence of a high resolution crystal structure of DNA-PK to direct inhibitor design, we have utilized a homology model of the ATP-binding domain derived from the crystal structure of PI-3Kγ.[28] In accordance with this model (Figure 1A), the chromenone carbonyl and morpholine oxygens of **2** serve as hydrogen bond acceptors to anchor the inhibitor within the ATP-binding site of DNA-PK. Importantly, the model predicts that groups introduced at the dibenzothiophene 1-position of **2** will be directed out of the binding pocket into bulk solvent (Figure 1B, red arrow). In this paper we describe the synthesis and biological evaluation of a series of derivatives of **2** bearing polar substituents at the dibenzothiophene 1-position, in the expectation that this would improve the physicochemical properties of the DNA-PK inhibitors without compromising potency. Surprisingly, the potent DNA-PK inhibitory activity arising from this structural modification was accompanied by a concomitant increase in activity against other PI-3K family members, resulting in the identification of a new series of potent dual DNA-PK/PI-3K inhibitors.

Scheme 1[a] Synthesis of dibenzothiophene-chromenone derivatives bearing 2-aminoacetyl (Series A) or 3-aminopropionyl (Series B) substituents at the dibenzothiophene 1-position



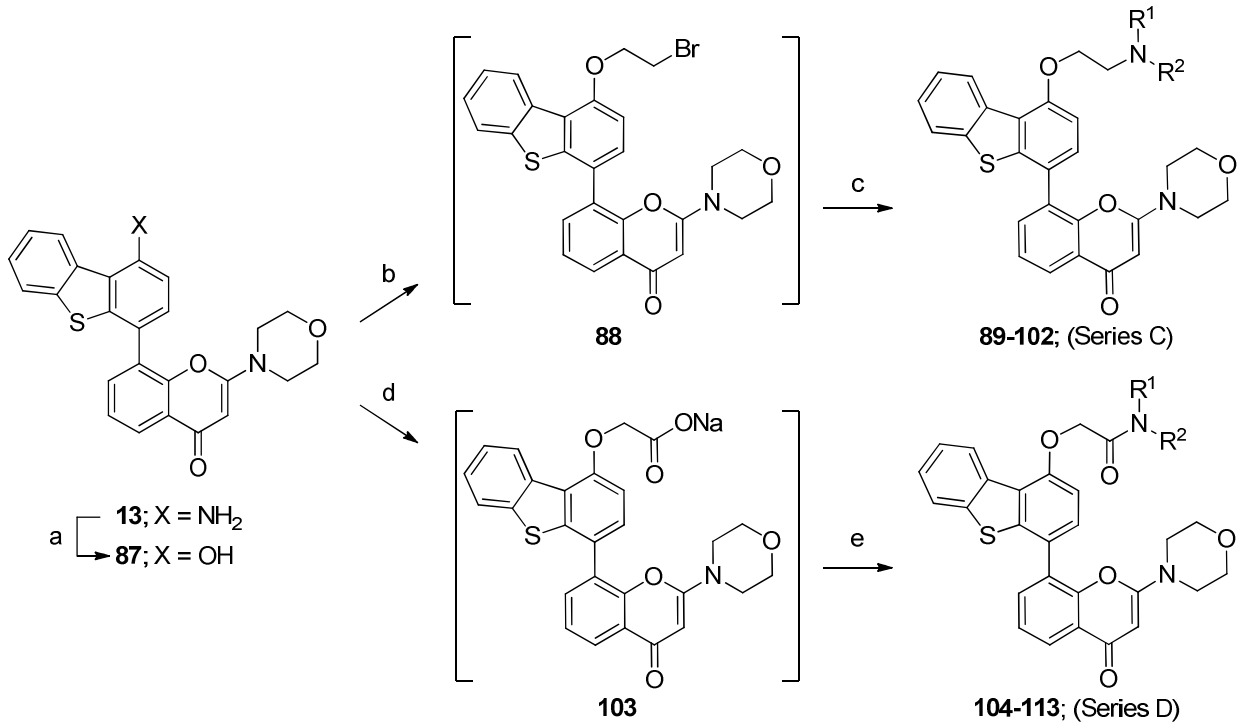
[a] Reagents and conditions: (a) i) *n*-BuLi, THF, reflux; ii) MeMgBr, O_2, 25 ^oC, 39%; (b) MeI, K_2CO_3, acetone, reflux, 96%; (c) HNO_3, AcOH, 25 ^oC, 99%; (d) pyridine hydrochloride, 150 °C, 41%; (e) triflic anhydride, NEt_3, DCM, 0 °C, 98%; (f) *bis*(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dppf, dioxane, 95 °C, 66%; (g) 2-morpholino-4-oxo-4*H*-chromen-8-yl trifluoromethanesulfonate, $PdCl_2$(dppf), Cs_2CO_3, THF, 85 °C, 46%; (h) Zn, AcOH, 25 ^oC, 95%; (j) i) chloroacetyl chloride or bromopropionyl chloride, NEt_3, DMA, 25 ^oC; ii) HNR^1R^2, DMA, 25 ^oC.

Chemistry

The structures of all compounds synthesized and evaluated for biological activity are recorded in Tables 1 and 2. Intermediates required for the synthesis of chromen-4-one DNA-PK inhibitors were prepared following standard procedures. The preparation of 4-hydroxydibenzothiophene **6** was achieved by reaction of 4-lithiodibenzothiophene with dioxygen in the presence of methylmagnesium bromide.[29] Nitration of **6** gave appreciable *ortho*-nitration; this was avoided by prior methylation of the hydroxyl

group of **6** to afford **7**, with subsequent nitration occurring exclusively at the 1-position to give **8**. *O*-Demethylation of **8** to furnish **9**, followed by reaction with triflic anhydride/triethylamine gave the required triflate **10**, which was converted into the corresponding boronate **11** by treatment with *bis*(pinacolato)diboron as described previously.[30] Nitroboronate ester **11** was coupled to 2-morpholino-4-oxo-4*H*-chromen-8-yl trifluoromethanesulfonate [26] *via* Suzuki-Miyaura methodology to give **12**, and reduction to the required amine **13** proceeded in excellent yield using zinc in acetic acid (Scheme 1).

Scheme 2[a] Synthesis of dibenzothiophene-chromenone derivatives bearing 2-aminoethoxy (Series C) or 2-oxoacetamido (Series D) substituents at the dibenzothiophene 1-position

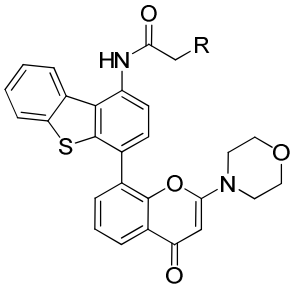


[a] Reagents and conditions: (a) i) HBF_4, *t*-butyl nitrite, 0 °C; ii) $Cu(NO_3)_2$, Cu_2O, H_2O, 25 °C, 76%; (b) 1,2-dibromoethane, K_2CO_3, DMF, 25 °C; (c) HNR^1R^2, DMF, 25 °C; (d) i) $BrCH_2CO_2Me$, K_2CO_3, DMF, 60 °C, 41%; ii) NaOH, MeOH, 60 °C; (e) HBTU, HOBt, HNR^1R^2, DMF, 25 °C.

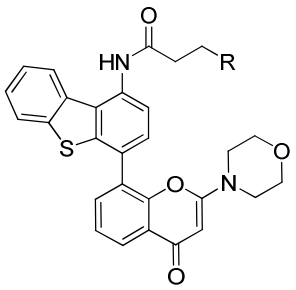
Library Syntheses: The effect of substitution at the 1-position of the dibenzothiophen-4-yl moiety was investigated through the preparation of focused libraries, employing a solution multiple-parallel

approach. Acylation of arylamine **13** with chloroacetyl chloride or 3-bromopropionyl chloride, followed by treatment with a range of amines (Scheme 1), enabled access to compound libraries with a methylene (Series A; **14**-**51**) or ethylene (Series B; **52**-**86)** spacer separating the amide from the side-chain amine group, following purification by semi-preparative HPLC (Table 1). The synthesis of small compound libraries where the side-chain group at the dibenzothiophene 1-position was attached *via* an ether linkage required the 1-hydroxydibenzothiophene-chromenone derivative **87**, which was readily accessible from **13** (Scheme 2). Subsequent alkylation or acylation of **87** with 1.2-dibromoethane or methyl bromoacetate, respectively, followed by treatment with the appropriate amines under standard conditions, gave the target 2-aminoethoxy (Series C; **89**-**102**) and 2-oxoacetamido (Series D; **104**-**113**, Scheme 2 and Table 2).

Table 1. Chemical structures and biological activities for inhibitors bearing a dibenzothiophen-1-yl acylamino motif (Series A and B).

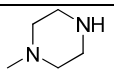
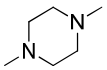
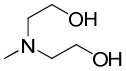
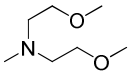
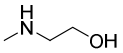
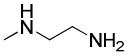
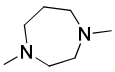
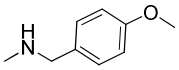
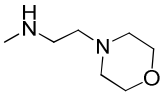
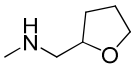
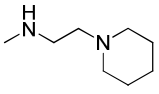
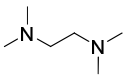
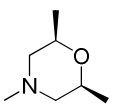
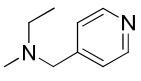


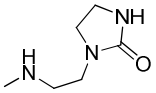
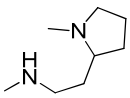
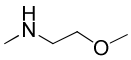
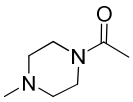
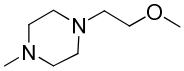
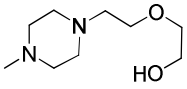
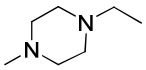
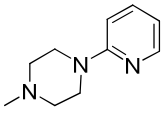
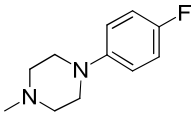
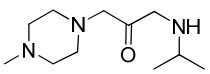
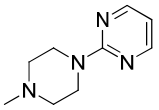
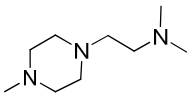
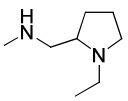
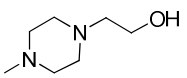
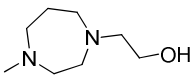
(A)

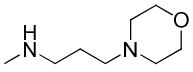
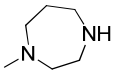


(B)

R	No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR		No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR	
				0.5 μM	0.1 μM				0.5 μM	0.1 μM
	**14**	**A**	11 ± 4	12.8	3.9	**52**	**B**	52, 41	6.1	2
	**15**	**A**	21 ± 4	10.4	1.2	**53**	**B**	37 ± 8	5	1.9

	**16**	**A**	24 ± 6	5	1.4	**54**	**B**	68, 37	1.5	1
	**17**	**A**	12 ± 4	7.4	2.4	**55**	**B**	28, 37	6.6	1
	**18**	**A**	7, 6	1.9	1.1	**56**	**B**	35, 26	0.8	1
	**19**	**A**	21, 18	7.9	1.5	-	**B**	-	-	-
	**20**	**A**	8, 25	6.2	1.6	**57**	**B**	16, 10	0.9	0.9
	**21**	**A**	16, 29	1.5	1.1	**58**	**B**	26, 22	1.4	1.2
	**22**	**A**	55, 111	7.6	1.7	**59**	**B**	59, 41	2.6	1.1
	**23**	**A**	114, 51	3.9	1.3	**60**	**B**	17, 11	8.4	3.1
	**24**	**A**	84, 38	6.8	1.4	**61**	**B**	11, 9	7.2	1.7
	**25**	**A**	84, 100	3.7	1.2	**62**	**B**	16, 12	5.7	1.8
	**26**	**A**	27, 12	9	2.5	**63**	**B**	10, 8	1.4	1.1
	**27**	**A**	31, 12	4.7	1.4	**64**	**B**	16, 6	1.9	1.3
	**28**	**A**	38, 14	7.7	1.7	**65**	**B**	7, 10	4.7	2
	**29**	**A**	97, 61	9.6	1.3	**66**	**B**	74, 33	3.3	1
	**30**	**A**	151, 57	5.7	1.1	**67**	**B**	78, 106	5.1	1.2
	**31**	**A**	4, 9	8.2	1.3	**68**	**B**	19, 9	9.3	2.4
	**32**	**A**	29, 8	14.1	1.5	**69**	**B**	31, 36	1.6	1

	**33**	**A**	21, 7	1.2	1.2	**70**	**B**	6, 12	0.9	1.1
	**34**	**A**	66, 43	2.4	1.1	**71**	**B**	13, 35	1.2	1.3
	**35**	**A**	21, 7	5.9	1.2	**72**	**B**	8, 10	5.9	2.3
	**36**	**A**	5, 7	4.8	1.4	**73**	**B**	23, 28	1.4	0.9
	**37**	**A**	10, 6	7.7	2.2	**74**	**B**	19, 34	3.5	1.3
	**38**	**A**	6, 7	3.2	1.6	**75**	**B**	29, 32	1	1.1
	**39**	**A**	5.0 ± 1	13.0	4.0	**76**	**B**	33, 69	4.1	1.4
	**40**	**A**	25, 7	9.9	1.8	**77**	**B**	44, 24	4.4	1.5
	**41**	**A**	59, 15	4.9	1	**78**	**B**	30, 170	3.8	1.5
	**42**	**A**	6, 10	8.8	2	**-**	**B**	-	-	-
	**-**	**A**	-	-	-	**79**	**B**	18, 31	7.1	1.6
	**43**	**A**	10, 11	5.9	1.7	**80**	**B**	10, 37	1.3	1.1
	**44**	**A**	60, 30	5.9	1.2	**81**	**B**	62, 49	1.7	1.2
	**45**	**A**	5 ± 2	8.4	2	**82**	**B**	29, 42	1.2	1
	**46**	**A**	30, 52	3.3	1.2	**83**	**B**	42, 54	1.1	1.2

	**47**	**A**	24, 13	7.4	2	**84**	**B**	9, 13	1.4	0.9
	**48**	**A**	22, 40	1.5	1.4	**-**	**B**	-	-	-
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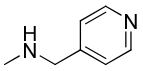
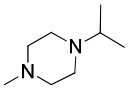
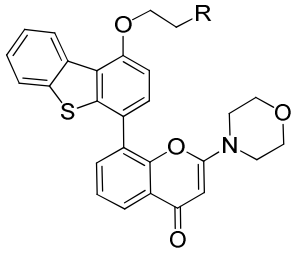
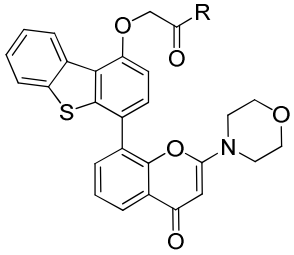
	**50**	**A**	8, 10	5.5	1.4	**85**	**B**	12, 15	2.6	1.1
	**51**	**A**	9, 19	12.9	1.7	**86**	**B**	37, 62	3.6	1.2

[a] IC_{50} values were determined at concentrations in the range 0.001-10 μM. Values are the mean ± the standard deviation or the values for at least two separate determinations.

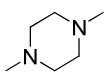
Table 2. Chemical structures and biological activities for inhibitors bearing a dibenzothiophen-1-yloxy motif (Series C and D).

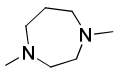
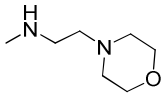
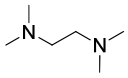
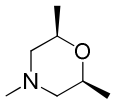
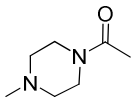
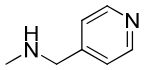
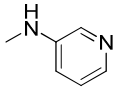
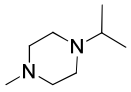
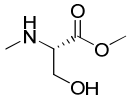
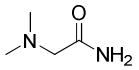


(C)



(D)

R	No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR		No	Structure	DNA-PK Inhibition (IC_{50} nM)[a]	DMR in HeLa cells at 2 Gy IR	
				0.5 μM	0.1 μM				0.5 μM	0.1 μM
	**89**	**C**	8, 33	6.4	1.5	**-**	**D**	-	-	-
	**90**	**C**	20, 47	3.5	1.4	**104**	**D**	21, 19	5.1	1.4
	**91**	**C**	7, 13	5.1	3.1	**105**	**D**	31, 29	1.8	1.1

	**92**	**C**	10, 10	3.6	1.5	**106**	**D**	47, 44	2	1.1
	**93**	**C**	27, 22	-	-	-	**D**	-	-	-
	**94**	**C**	9, 7	10.1	1.4	-	**D**	-	-	-
	**95**	**C**	5, 16	-	-	**107**	**D**	53, 76	2.1	1.4
	**96**	**C**	10, 29	3.2	1.1	**108**	**D**	244, 61	1	0.9
	**97**	**C**	2, 2	7.4	2.4	**109**	**D**	16, 26	1.6	1
	**98**	**C**	10, 8	3.3	1.8	-	**D**	-	-	-
	-	**C**	-	-	-	**110**	**D**	10, 20	1.1	1
	**99**	**C**	4, 2	1	0.9	-	**D**	-	-	-
	**100**	**C**	3, 4	6.3	1.6	**111**	**D**	16, 16	3.1	1.3
	**101**	**C**	2, 3	10.5	2.9	**112**	**D**	9, 6	6.1	1.5
	**102**	**C**	3, 3	7.7	1.4	**113**	**D**	16, 11	1.4	0.9

[a] IC_{50} values were determined at concentrations in the range 0.001-10 μM. Values are the mean ± the standard deviation or the values for at least two separate determinations.

Results and Discussion

The major overall objective of the studies described in this paper was to improve the physicochemical properties of **2** without compromising biological activity, with a view to facilitating a more extensive

biological evaluation of this interesting class of DNA-PK inhibitor. On the basis of homology modeling that predicted tolerance to substitution at the dibenzothiophene 1-position of **2** (Figure 1), focused compound libraries (A-D) were synthesized, where a polar functionality was linked to the dibenzothiophene *via* an amide or ether group. Members of the four compound libraries arising from this empirical approach, and deemed sufficiently pure by HPLC (> 95%), were initially screened for DNA-PK inhibitory activity (IC_{50}) utilizing an established biochemical assay (Tables 1 and 2).[30] Potency at least comparable with the parent chromenone **2** (DNA-PK, IC_{50} = 30 nM) was observed for the majority of derivatives across the four series A-D, supporting the proposal that the dibenzothiophene 1-position is tolerant to substitution. Notably, a number of compounds (Series A/B - **18**, **31**, **36**, **38**, **39, 45**; Series C/D - **97**, **99-102**) were at least 5-fold more potent than **2** as DNA-PK inhibitors. However, there were no obvious differences in activity between series A and the homologous series B with otherwise identical structures, or for the more limited number of compounds synthesized where the 1-acetamido linker was replaced by a 1-alkoxy (series C) or 1-acetyloxy (series D) linker.

Having established that modifications at the dibenzothiophene 1-position were generally tolerated without detriment to potency against DNA-PK compared with **2**, cellular activity was assessed in the HeLa cervical carcinoma cell line employing a radiosensitization survival assay.[31] Briefly, cells were exposed to 2 Gy of IR in the absence or presence of a defined concentration (0.1 or 0.5 μM) of DNA-PK inhibitor, and the number of surviving cells was determined by a colony-forming assay. The IR Dose Modification Ratio (DMR) thereby derived is defined as the ratio of the number of cells that survive a single 2 Gy dose of ionizing radiation, to those that survive a single dose of 2 Gy in combination with a given concentration of DNA-PK inhibitor. The DMR assay represents a rapid measure of the ability of the compound to modulate cell survival following the induction of DNA damage. Assuming that the potentiation of IR-induced cytotoxicity arises as a consequence of DNA-PK inhibition, the DMR values reflect both potency against DNA-PK and the cell permeability and cellular retention of a particular compound. Importantly, there was little intrinsic cytotoxicity observed at the

concentration of inhibitors employed, and the data obtained are thus a measure of radiosensitizsation rather than the combined cytotoxic effect of the DNA-PK inhibitor and IR.

As shown in Tables 1 and 2, at an inhibitor concentration of 0.5 μM, seven compounds from series A generated DMR values of ≥10 in combination with IR, namely **14**, **15**, **29**, **32**, **39**, **40**, and **51**, whereas none of the homologous chromenones in series B gave comparable DMR values. Moreover, only **94** and **101** from the ether-linked series C and D afforded DMR values of ≥10. This implied that an aminoacyl group at the dibenzothiophene 1-position was generally preferred over an ether substituent as the linker for attachment of the polar substituent, possibly as a consequence of favorable cellular uptake and/or retention of the DNA-PK inhibitors. Within series A, compound **39** emerged as of particular interest by exhibiting DMR values of 13.0 and 4.0 at 0.5 μM and 0.1 μM, respectively, in tandem with potent DNA-PK inhibitory activity (IC_{50} = 5 nM), and this chromenone was selected for further studies. Perhaps more importantly, **39** exhibited low intrinsic cytotoxicity, as reflected by the > 50-fold difference between the concentration of compound alone that inhibited HeLa cell survival by 50% (>25 μM) and that necessary to reduce survival of irradiated cells by 50% (0.5 μM).

Table 3. Biological and physicochemical properties of representative DNA-PK inhibitors.[a]

Assay	Compound	2	17	39	45	97
Enzyme	DNA-PK IC_{50} (nM)	42 ± 2	12 ± 4	5.0 ± 1	5.2 ± 1.8	2.2, 2.1
Cellular	pDNA-PK EC_{50} (nM)	212, 339	426 ± 97	136 ± 17	109 ± 22	166 ± 70
	DMR (0.1 μM DNA-PK inhibitor)	2.2 ± 0.2	2.4 ± 0.4	4.0 ± 0.4	2.1 ± 0.3	2.3, 2.4
	DMR (0.5 μM DNA-PK inhibitor)	2.8 ± 0.1	7.4 ± 0.7	13 ± 2	14 ± 11	9.6 ± 2.5
Other	Log*D* (pH = 7.4)	>4.3	3	3.05	2.7	-
	hERG IC_{50} (μM)	14, 19	>10	>20	>30	>33
	Solubility at pH 7.4 (μM)	<0.3, <0.2	5	161 ± 103[b]	154, 194	-
	Human plasma protein binding (% Free)	0.04, 0.17	3.6	6.2, 3.6	9.8, 15	0.85, 0.49
	CYP_{450} inhibition (μM)[c]	-	>10	> 10	>10	-

[a]Data are individual values or the mean ± the standard deviation.

[b]Amorphous material (crystalline solubility at pH7.4 buffer = 6.0 μM)

[c]Tested in CYP 3A4, 2D6, 2C9, 2C19 and 1A2

Table 3 summarizes the biological and physicochemical properties of **39** in comparison with **2** and representative library members **17**, **45**, and **97**. The promising biological activity of **39** was accompanied by better drug-like properties compared to **2**, and acceptable plasma protein binding, combined with weak activity against hERG and a panel of CYP450 enzymes (Table 3). To assess kinase selectivity, **39** was screened against a diverse panel of 131 kinases (MRC Protein Phosphorylation Unit, University of Dundee) at a single concentration of 1 μM (data not shown). Only one kinase (Aurora B –

44% inhibition) was inhibited by > 30% at this concentration and the selective inhibition profile of **39** was remarkably similar to that observed for the parent dibenzothiophene derivative **2** in the same screen.

To establish whether the potent DNA-PK inhibitory activity of **39** in the biochemical assay translated into cellular enzyme inhibition, the ability of the compound to inhibit DNA-PK autophosphorylation of S2056 was determined in HeLa cells as described previously, employing a polyclonal antibody specific for DNA-PK phospho-S2056.[31] The cellular potency of **39** as a DNA-PK inhibitor (EC_{50} = 136 nM) was approximately 25-fold lower than for the cell-free assay (IC_{50} = 5 nM), and comparable differences were observed for **2**, **17**, **45**, and **97** (Table 3). This disparity may either relate to concentration of ATP in the cellular (~ 5 mM) versus DNA-PK assay (50 μM) or reflect differences in cell permeability and/or sequestration of the compounds by intracellular proteins.

The relationship between cellular DNA-PK inhibition by **39** and radiopotentation was investigated further employing a human glioma cell line model. MO59J cells are DNA-PK deficient through a lack of functional DNA-PKcs, whereas their MO59J-Fus1 counterparts have been modified by chromosome transfer to re-express human DNA-PKcs.[32] Significant DNA-PK autophosphorylation at S2056 was observed in MO59J-Fus1 cells following exposure to 10 Gy IR, and this was completely abrogated by **39** (1.0 μM) (Figure 2). By contrast, the corresponding phosphoprotein was not detectable in the DNA-PK deficient MO59J cells, confirming that the cellular activity of DNA-PK following exposure to IR is attenuated by **39**.

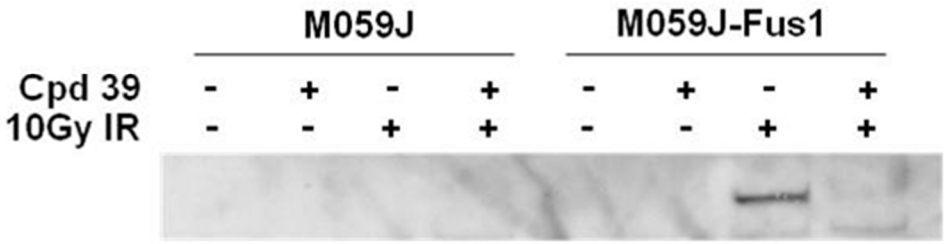
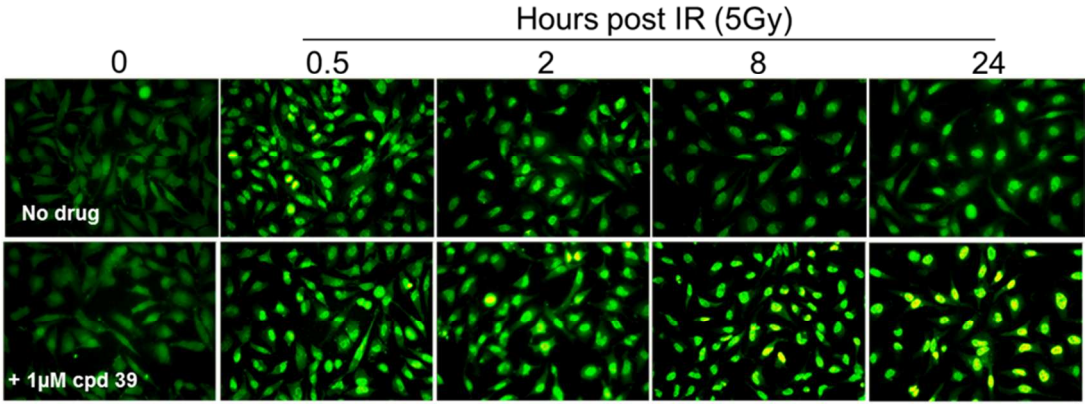


Figure 2. Inhibition of IR-induced DNA-PK autophosphorylation (p2056) by compound **39** in MO59J-Fus-1 cells. Data presented are from a single representative experiment.

Further evidence to support the mechanism of action of **39** was adduced using the H2AX phosphorylation assay. Generation of DNA DSBs following exposure of cells to IR manifests as nuclear γH2AX foci, which accumulate rapidly at sites of DSBs and stalled replication forks, and can be determined by immunofluorescence microscopy.[27] Within 30 minutes of a single exposure to 5 Gy IR, the nuclei of HeLa cells exhibited high levels of γH2AX foci, which subsequently declined as a result of DSB repair (Figure 3). However, cellular γH2AX foci were observed to persist and continue to accumulate in the presence of **39** (1.0 μM). These data are supportive of the proposed mechanism of action of **39**, namely that by inhibiting DNA-PK activity, repair of DSB will also be compromised, and are also consistent with data generated previously with the parent compound **2**.[27]

A.



B.

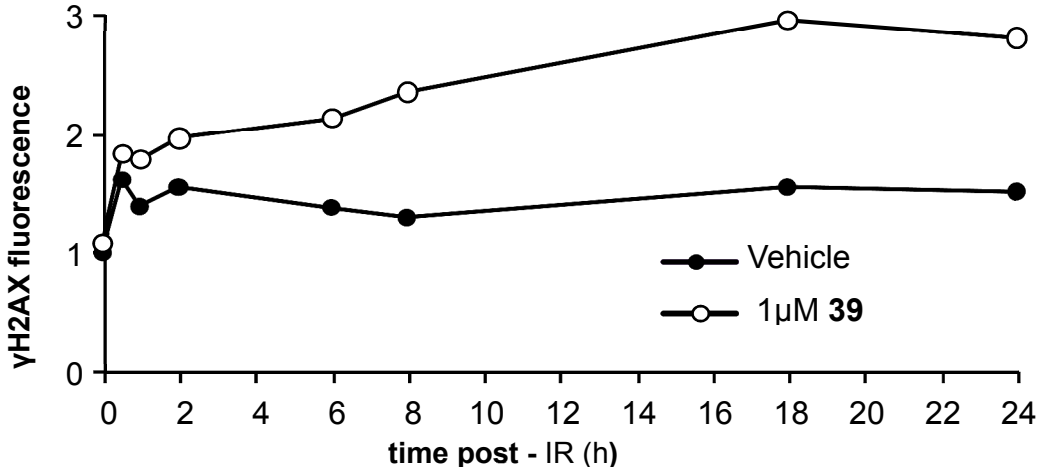


Figure 3. Repair of radiation induced DNA damage in Hela cells is inhibited by compound **39**. A) Immunostaining with antibodies against γH2Ax as a time course after treatment with 5 Gy of ionizing radiation; B) Quantification of immunostaining shown in panel A. Data presented are from a single representative experiment.

Preliminary *in vitro* radiosensitization studies with **39** were also very promising. Thus, exposure of FaDu head and neck squamous cell carcinoma (HNSCC) cells to IR in the presence of **39** (0.1 μM) enhanced cytotoxicity, with clonogenic survival being significantly reduced at all doses of IR studied, and an approximately 3-fold sensitization being observed at the therapeutically relevant dose of 2 Gy (Figure 4). The choice of the FaDU HNSCC tumor cell line for this study is noteworthy, as radiotherapy combined with chemotherapy is the standard treatment regimen for HNSCC.

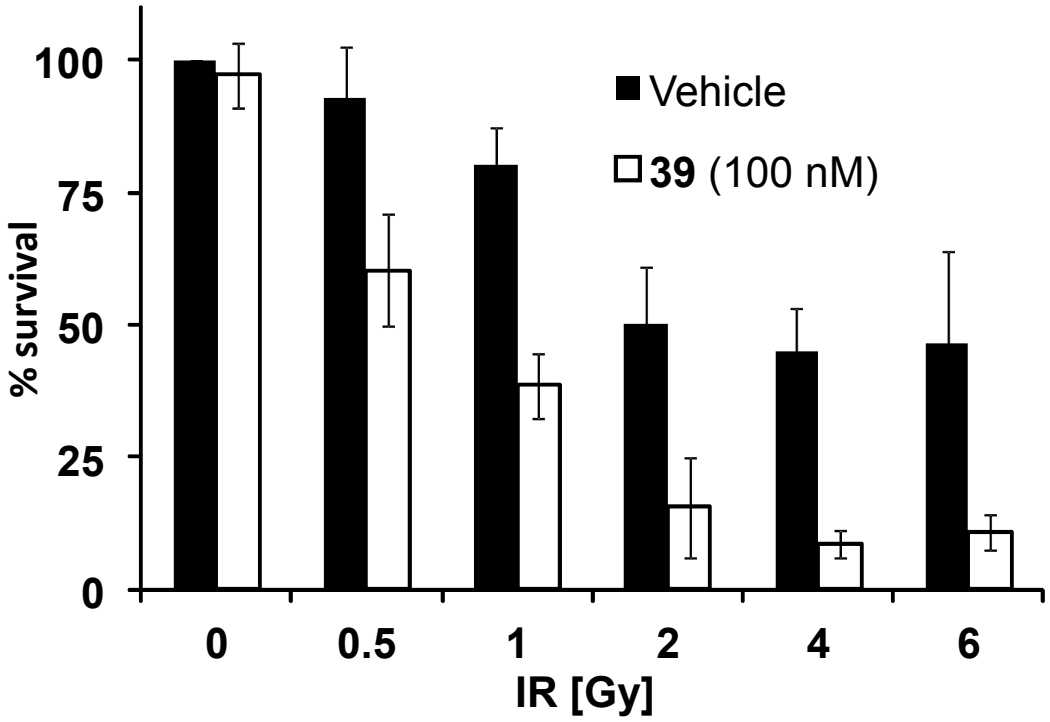


Figure 4. Compound **39** potentiates IR-induced cytotoxicity at 100 nM in a clonogenic survival assay with FaDu cells. Data are the mean ± the standard deviation.

Although **39** was found to show negligible inhibitory activity against a panel of 131 representative kinases, PIKK and PI3K family members were not included in this initial screen, and hence the chromenone was evaluated in-house against these kinases (Table 4). As expected, and in common with **1** and **2**, the 1-substituted chromenone derivative **39** was essentially inactive against ATM and ATR (IC_{50} > 10 μM) and exhibited only micromolar activity against mTOR. By contrast, and unexpectedly, **39** proved to be an extremely potent PI-3K inhibitor, with low to sub-nanomolar activity being observed against PI-3Kα, PI-3Kβ and PI-3Kδ. A comparison of the PI-3K inhibitory activity of **39** with the parent dibenzothiophene-chromenone derivative **2**, clearly implicates the 1-acylamino substituent on the dibenzothiophene of **39** as responsible for conferring activity against PI-3K family members through favorable interactions within the ATP-binding domain.

Table 4. Inhibitory activity of chromenones **1**, **2** and **39** against PIKK and PI3-K family members.[a]

Kinase	Inhibitory activity (IC_{50} μM)		
	1	**2**	**39**
DNA-PK	1.4	0.04	0.005
ATM	> 10[b]	> 10	> 10
ATR	> 10	> 10	> 10
mTOR	2.8	2.4	10
PI-3Kα	0.3	0.13	0.004
PI-3Kβ	0.27	0.016	0.0005
PI-3Kγ	3.02	0.22	0.59
PI-3Kδ	0.22	0.03	<0.0001

[a]Values are the means of at least two determinations, and were measured as described in reference 23.

[b]No inhibitory activity observed at 10 μM.

The precise nature of the additional binding interactions made by the 1-substituent of **39** has not been elucidated. However, docking and minimization of **39** within the homology model of the DNA-PK ATP-binding domain, indicates that the 3-(4-ethylpiperazin-1-yl)propanoylamino substituent on the dibenzothiophene group of **39** is readily accommodated, with the terminal *N*-ethylpiperazinyl group being located within a polar region (Figure 5A). Interestingly, an overlay of the best docking pose of **39** within the ATP-binding pockets of DNA-PK and PI-3Kγ, reveals that the 1-substituent is located within a region of structural similarity for both kinases as illustrated in Figure 5B. In this model, the 1-substituent is readily accommodated in a common solvent channel, and this may accounts for the high potency of **39** against both DNA-PK and PI-3K.

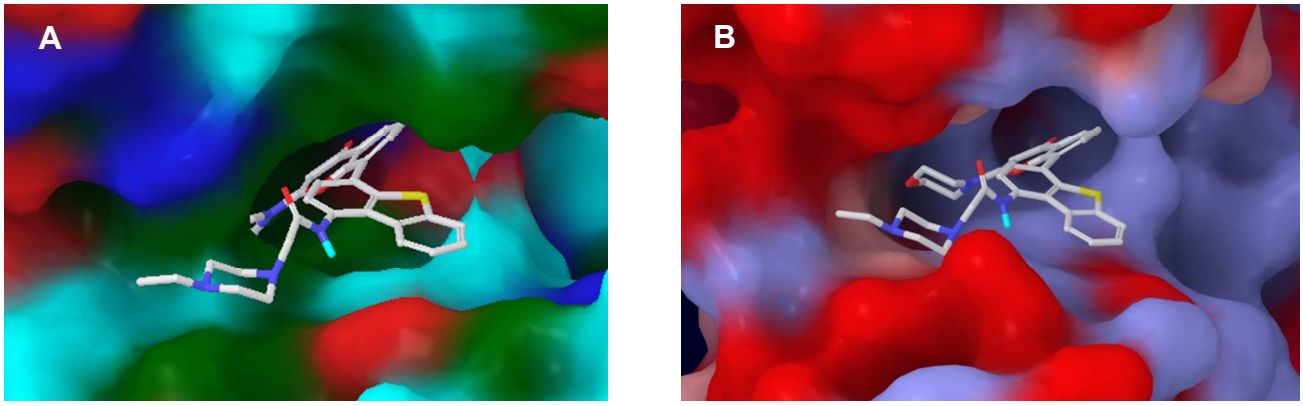


Figure 5. **A** Homology model of the ATP-binding site of DNA-PK with **39** docked and minimized. Protein surface colored by residue properties (red – acidic, blue – basic, dark green – hydrophobic and cyan – polar). **B** Crystal structure of PI-3Kα with the surface colored to indicate differences between DNA-PK and PI-3Kα (red-most dissimilar, and blue most similar). The homology model of DNA-PK was derived from the known X-ray crystal structure of PI3Kγ (RCSB protein databank: PDB ID: 1E7V), employing the DNA-PK sequence from Swiss-Port (ID: PRKDC_DICDI). The model of **39** was constructed using the Maestro molecular modeling program, minimized using the OPLS_2005 force field in Macromodel and docked using Glide. Macromodel, Glide and Maestro were licensed from Schrödinger, LGG (www.schrodinger.com).

Conclusions

The pivotal role of DNA-PK in DNA DSB repair renders this kinase of considerable interest in cancer chemotherapy, both in the context of tumor chemo- and radio-sensitization by modulation of DNA-PK activity, and potentially as a single-agent therapeutic target for tumors harboring appropriate DNA repair defects. Preliminary biological investigations with NU7441 (**2**), the prototypic chromenone-derived DNA-PK inhibitor, were encouraging, prompting a lead optimization campaign with the goal of improving physicochemical properties to facilitate *in vivo* studies. The introduction of polar substituents at the dibenzothiophene 1-position of **2**, guided by homology modeling of the DNA-PK ATP-binding domain, was evaluated employing an empirical compound library approach. Chromenone **39** emerged as a potent cell permeable DNA-PK inhibitor (IC_{50} = 5 nM), demonstrating significant *in vitro* tumor cell radiosensitization at sub-micromolar concentrations, and combining improved drug-like properties with excellent selectivity in a broad kinase screen. A major objective of improving aqueous amorphous solubility compared with **2** was also achieved with **39**, albeit that the lower solubility of the crystalline compound (6.0 μM) compared with the amorphous material (161 ± 103 μM) presented subsequent formulation challenges. More detailed validation studies with **39** established that the compound inhibits DNA-PK activity in intact cells, retards DNA DSB repair, and sensitizes human cervical and head and neck cancer cell lines to IR.

The discovery that **39** is also a highly potent inhibitor of several PI-3K family members was unexpected, although a retrospective comparison of the ATP-binding domain of PI-3Kγ with that of the homology model of DNA-PK indicates a putative common binding pocket. Screening of other compound library members has since confirmed that dual DNA-PK/PI-3K inhibition is shared by other library members, and appears to be a common facet of this structural class (data not shown). The role of PI-3K in promoting tumor growth and survival is very well established, and a number of small-molecule

PI-3K inhibitors are currently in advanced clinical trial for cancer as well as other diseases.[33] In this context, it is perhaps surprising that **39** was essentially devoid of intrinsic growth-inhibitory activity in the HeLa and FaDu tumor cell lines utilized for the radiosensitization studies. However, a more comprehensive assessment of the activity of **39** (designated the house code KU-0060648) against a panel of human cancer cell lines has since been conducted, and substantial single-agent growth inhibition has been observed, albeit in a cell line-specific manner. The results of these studies have been published elsewhere.[34] Encouragingly, the use of isogenically paired DNA-PKcs-proficient and –deficient tumor cell lines with comparable levels of PI-3K activity, confirmed that potentiation of etoposide and doxorubicin cytotoxicity arises predominantly *via* DNA-PK inhibition.[34]

In summary, our studies have resulted in the identification of a new chemotype exhibiting potent dual DNA-PK/PI-3K inhibition as exemplified by the 1-substituted dibenzothiophene-chromenone derivative **39**. Subsequent pharmacokinetic and efficacy studies have demonstrated that **39** is orally bioavailable, effectively inhibits DNA-PK and PI-3K in tumors, and potentiates the activity of DSB-inducing chemotherapeutic anticancer drugs both *in vitro* and *in vivo*.[34]

Experimental Section

Solvents were purified and stored according to standard procedures. Petrol refers to that fraction of hexanes boiling in the range 40-60 °C. Melting points were obtained on a Stuart Scientific SMP3 apparatus and are uncorrected. TLC was performed with Merck 60 F254 silica gel plates. 'Flash' column chromatography was conducted under medium pressure on silica (Merck silica gel 40-63 μm). HPLC purification were performed on Gilson LC instruments, with a 15 min gradient of 0.1% aqueous TFA and 10-97% acetonitrile, at a flow rate of 6 mL/min, using as the stationary phase a Jones Chromatography Genesis 4μ C18 column, 10 mm x 250 mm, and peak acquisition based on UV detection at 254 nm. Solution-phase palladium-mediated coupling reactions were conducted in Greenhouse reactors (Radley's Ltd., U.K.) under an argon atmosphere. ^{1}H NMR and ^{13}C NMRspectra were recorded either on a Bruker Spectrospin AC 300E spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C) or a Bruker AMX (500MHz for ^{1}H, 125 MHz for ^{13}C) using $CDCl_3$ as solvent, unless indicated otherwise. LCMS was carried out on either a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 x 4.6 mm C18 column (Supelco Discovery or Waters Symmetry) and a 15 min gradient elution of 0.05% formic acid and methanol (10-90%), or on a Finnegan LCQ instrument in positive ion mode with a Phenomenex 5μ Luna C18 column, 4.6 mm x 50 mm and an 8 min gradient of 0.1% aqueous formic acid and acetonitrile (5-98%), with a flow rate of 2 mL/min. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Analytical purity of compounds was determined using Waters XTerra RP18, 5 μm (4.6 × 150 mm) column at 1 mL/min using either 0.1% aqueous ammonia and acetonitrile or 0.1% aqueous formic acid and acetonitrile with a gradient of 5-100% over 15 minutes and all library compounds were found to be ≥ 95% pure. Accurate masses were measured using a Finnigan MAT 95 XP or a Finnigan MAT 900 XLT by the EPSRC National Mass Spectrometry Service Centre, Swansea, SA2 8PP.

Compounds **6** to **11** were synthesized as described in reference 19.

2-Morpholino-8-(1-nitrodibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (12). In a Schlenk tube, 4,4,5,5-tetramethyl-2-(1-nitrodibenzo[*b*,*d*]thiophen-4-yl)-1,3,2-dioxaborolane **11** (550 mg, 1.55 mmol) and cesium carbonate (1.52 g, 1.55 mmol) were mixed in THF (4 mL) and degassed. Concurrently, 2-morpholino-4-oxo-4*H*-chromen-8-yl trifluoromethanesulfonate[26] (645 mg; 1.70 mmol) and $PdCl_2$dppf (62 mg; 0.08 mmol) were suspended in THF (4 mL) and degassed. The solutions were mixed together in the Schlenk tube, stirred and heated at 80 °C for 18 h. Upon cooling, DCM (20 mL) was added. The solution was washed with water (20 mL), dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography using AcOEt/MeOH (9:1) as eluent. After evaporation, the product (327 mg, 46%) was obtained as a yellow solid: R_f = 0.57 (AcOEt/MeOH 9:1); mp: 179-183 °C; IR (cm^{-1}) 3095, 2980, 2858, 1709, 1681, 1619, 1559, 1404; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 2.99-3.02 (4H, m, 2 x CH_2N-morpholine), 3.41-3.47 (4H, m, 2 x CH_2O-morpholine), 5.43 (1H, s, H-3), 7.39-7.48 (3H, m, H-Ar), 7.75 (1H, d, J = 7.5 Hz, H-Ar), 7.82 (1H, d, J = 8.1 Hz, H-Ar), 8.05 (1H, d, J = 7.5 Hz, H-Ar), 8.25 (1H, dd, J = 1.8 and 8.1 Hz, H-Ar); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 45.1 (2 x CH_2N-morpholine), 66.2 (2 x CH_2O-morpholine), 87.6 (C-3), 120.7, 123.0, 124.4, 125.2, 127.1, 127.4, 127.5, 128.7, 128.9, 129.8, 131.7, 133.3, 138.9, 140.6, 143.4, 146.7, 150.9, 162.6, 176.7.

8-(1-Aminodibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (13). Zinc powder (317 mg, 4.84 mmol) was added to 2-morpholino-8-(1-nitrodibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one **12** (222 mg, 0.48 mmol) in AcOH (8 mL) and stirred at room temperature overnight. The reaction mixture was filtered through Celite and washed successively with methanol (4 x 35 mL) and DCM (4 x 35 mL). The combined organic layers were evaporated under reduced pressure and the residue was purified by flash chromatography using AcOEt/MeOH (9:1) as eluent. After evaporation, the product (197 mg, 95%) was obtained as a white solid: R_f = 0.51 (AcOEt/MeOH 9:1); mp: 103-105 °C; IR (cm^{-1}) 2925, 2857, 1616, 1558, 1507, 1408, 1435, 1359, 1246, 1119, 1020, 978, 852; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 3.08-3.11 (4H, m, 2 x CH_2N-morpholine), 3.31-3.38 (4H, m, 2 x CH_2O-morpholine), 5.67 (1H, s, H-3), 6.88 (1H, d, J = 7.8 Hz, H-Ar), 7.25 (1H, d, J = 7.8 Hz, H-Ar), 7.28-7.48 (3H, m, H-Ar),

7.71 (1H, dd, *J* = 1.8 and 7.5 Hz, H-Ar), 7.77 (1H, dd, *J* = 1.8 and 8.1 Hz, H-Ar), 8.24 (1H, td, *J* = 1.5 and 7.8 Hz, H-Ar); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 45.0 (2 x CH_2N-morpholine), 66.1 (2 x CH_2O-morpholine), 87.1 (C-3), 113.1, 121.9, 122.9, 123.7, 124.9, 125.1, 125.8, 125.9, 128.9, 129.3, 134.1, 135.9, 138.9, 141.7, 144.5, 151.3, 162.7, 177.8; LCMS *m/z* 427.2 ($[M+H]^+$).

Solution-Phase Library Synthesis of 2-amino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamides (14-51) : Method I. General Procedure. To 8-(1-aminodibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **13** (1 mol equiv.) in dry DMA (19 mL), were added triethylamine (2.2 mol equiv.) and chloroacetyl chloride (1 mol equiv.) and the reaction mixture was stirred at room temperature for 18 h. Aliquots (0. 5 mL) of the resulting solution were added to each of 38 tubes in a Greenhouse workstation, with each tube containing a different amine (3 equiv.). The reaction mixtures were stirred in parallel at room temperature for 18 h. After dilution with 1.5 mL of methanol, the product was purified by semi-preparative HPLC.

2-Morpholino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (14). Compound **14** was prepared from morpholine (16 mg, 0.19 mmol) according to Method I and was obtained in 97% purity: LCMS *m/z* 556.4 ($[M+H]^+$); mp: 165-167 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 3.18-3.20 (4H, m, 2 x CH_2N-morpholine), 3.25-3.27 (4H, m, 2 x CH_2N-morpholine), 3.52-3.54 (4H, m, 2 x CH_2O-morpholine), 3.82 (2H, s, NCH_2), 4.01-4.03 (4H, m, 2 x CH_2O-morpholine), 6.59 (1H, s, H-3), 7.54-7.58 (4H, m, H-Ar), 7.85-7.87 (2H, m, H-Ar), 8.04 (1H, d, *J* = 8.1 Hz, H-Ar), 8.29 (1H, d, *J* = 7.9 Hz, H-Ar), 8.44-8.46 (1H, m, H-Ar), 10.27 (1H, s, NH).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(piperidin-1-yl)acetamide (15).** Compound **15** was prepared from piperidine (16 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 554.5 ($[M+H]^+$); mp: 187-189 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 1.95-1.97 (6H, m, CH_2-piperidine), 3.09-3.42 (14H, m, CH_2-morpholine and CH_2-piperidine), 4.10 (2H, s, NCH_2), 6.15 (1H, s, H-3), 7.37-7.40 (3H, m, H-Ar), 7.47-7.50 (2H, m, H-Ar),

7.70-7.72 (2H, m, H-Ar), 8.19 (1H, dd, *J* = 8.1 and 1.6 Hz, H-Ar), 8.26-8.28 (1H, m, H-Ar), 10.80 (1H, s, NH).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(piperazin-1-yl)acetamide (16).** Compound **16** was prepared from piperazine (16 mg, 0.19 mmol) according to Method I and was obtained in 100% purity: LCMS *m/z* 555.4 ($[M+H]^+$).

2-(4-Methylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (17). Compound **17** was prepared from 1-methyl-piperazine (19 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 569.4 ($[M+H]^+$).

2-(*Bis*(2-hydroxyethyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (18). Compound **18** was prepared from diethanolamine (20 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 574.4 ($[M+H]^+$).

2-(*Bis*(2-methoxyethyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (19). Compound **19** was prepared from bis(2-methoxyethyl)amine (25 mg, 0.19 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 602.4 ($[M+H]^+$).

2-(2-Hydroxyethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (20). Compound **20** was prepared from ethanolamine (16 mg, 0.12 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 530.4 ($[M+H]^+$).

2-(2-Aminoethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (21). Compound **21** was prepared from ethylenediamine (11 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 529.4 ($[M+H]^+$).

2-(4-Methyl-1,4-diazepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (22). Compound **22** was prepared from 1-methylhomopiperazine (21 mg, 0.19 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 583.5 ($[M+H]^+$).

2-(Azepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (23). Compound **23** was prepared from hexamethyleneimine (19 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 568.5 ($[M+H]^+$).

2-(4-Methoxybenzylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (24). Compound **24** was prepared from 4-methoxybenzylamine (26 mg, 0.19 mmol) according to Method I and was obtained in 97% purity: LCMS *m/z* 606.4 ($[M+H]^+$).

2-(Diethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (25). Compound **25** was prepared from diethylamine (14 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 542.5 ($[M+H]^+$).

2-Amino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (26). Compound **26** was prepared from ammonia (7N solution in methanol) (3.2 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 486.4 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(2-morpholinoethylamino)acetamide (27).** Compound **27** was prepared from 4-(2-aminoethyl)morpholine (24 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 599.4 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-((tetrahydrofuran-2-yl)methylamino)acetamide (28).** Compound **28** was prepared from tetrahydrofurfurylamine (19 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 570.4 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(2-(piperidin-1-yl)ethylamino)acetamide (29).** Compound **29** was prepared from 1-(2-aminoethyl)piperidine (24 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 597.5 ($[M+H]^+$).

2-((2-(Dimethylamino)ethyl)(methyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (30). Compound **30** was prepared from *N*,*N*,*N*'-trimethylethylenediamine (19 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 571.4 ($[M+H]^+$).

2-((2*S*,6*R*)-2,6-dimethylmorpholino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (31). Compound **31** was prepared from *cis*-2,6-dimethylmorpholine (22 mg, 0.19 mmol) according to Method I and was obtained in 97% purity: LCMS *m/z* 584.5 ($[M+H]^+$); mp: 152-154 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 1.21 (6H, d, J = 6.2 Hz, 2 x CH_3), 2.50-2.52 (2H, m, CH_2N-*cis*-dimethylmorpholine), 3.15-3.17 (4H, m, 2 x CH_2N-morpholine), 3.27-3.29 (2H, m, CH_2N-*cis*-dimethylmorpholine), 3.44-3.45 (4H, m, 2 x CH_2O-morpholine), 3.77-3.78 (2H, m, CH-*cis*-dimethylmorpholine), 3.96 (2H, br s, NCH_2), 6.40 (1H, s, H-3), 7.47-7.51 (4H, m, H-Ar), 7.74-7.77 (2H, m, H-Ar), 7.90 (1H, d, J = 8.1 Hz, H-Ar), 8.20 (1H, d, J = 6.4 Hz, H-Ar), 8.32 (1H, d, J = 6.4 Hz, H-Ar), 10.26 (1H, s, NH).

2-(Ethyl(pyridin-4-ylmethyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (32). Compound **32** was prepared from *N*-(4-pyridylmethyl)ethylamine (26 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 605.5 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[b,d]thiophen-1-yl)-2-(2-(2-oxoimidazolidin-1-yl)ethylamino)acetamide (33).** Compound **33** was prepared from 1-(2-aminoethyl)-2-imidazolidinone (24 mg, 0.19 mmol) according to Method I and was obtained in 99% purity: LCMS *m/z* 598.5 ($[M+H]^+$).

2-(2-(1-Methylpyrrolidin-2-yl)ethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (34). Compound **34** was prepared from 2-(2-aminoethyl)-1-methylpyrrolidine (24 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 597.5 ($[M+H]^+$).

2-(2-Methoxyethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (35). Compound **35** was prepared from 2-methoxyethylamine (14 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 544.4 ($[M+H]^+$).

2-(4-Acetylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (36). Compound **36** was prepared from 1-acetylpiperazine (24 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 597.6 ($[M+H]^+$).

2-(4-(2-Methoxyethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (37). Compound **37** was prepared from 1-(2-methoxyethyl)-piperazine (27 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 613.6 ($[M+H]^+$).

2-(4-(2-(2-Hydroxyethoxy)ethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (38). Compound **38** was prepared from 1-hydroxyethylethoxypiperazine (33 mg, 0.19 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 643.6 ($[M+H]^+$).

2-(4-Ethylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (39). Compound **39** was prepared from 1-ethylpiperazine (21 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 583.6 ($[M+H]^+$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (3H, t, *J* = 7.2 Hz, CH_3), 2.53 (2H, m, CH_2CH_3), 2.70 (4H, br. s, 2 x CH_2N-piperidine), 2.90 (4H, br. s, 2 x CH_2N-morpholine), 3.08-3.13 (4H, m, 2 x CH_2N-piperidine), 3.49-3.54 (4H, m, 2 x CH_2O-morpholine), 3.41 (2H, s, CH_2), 5.50 (1H, s, H-3), 7.45-7.54 (4H, m, 4 x H-Ar), 7.74 (1H, dd, *J* = 1.7 and 7.4 Hz, H-Ar), 7.85-7.89 (1H, m, H-Ar), 8.27 (1H, dd, *J* = 1.7 and 7.9 Hz, H-Ar), 8.46 (1H, d, *J* = 8.2 Hz, H-Ar), 8.55-8.58 (1H, m, H-Ar), 10.17 (1H, s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.0 (CH_3), 44.5 (2 x CH_2N-morpholine), 52.5 (2 x CH_2N-piperidine), 52.8 (2 x CH_2N-piperidine), 54.0, 62.7, 65.8 (2 x CH_2O-morpholine), 87.0 (C-3), 118.2, 123.2, 123.6, 123.6, 124.8, 124.9, 125.7, 126.0, 126.6, 127.3, 128.2, 128.4, 133.6, 134.2, 139.4, 141.0, 150.7, 162.2, 168.9.

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(4-(pyridin-2-yl)piperazin-1-yl)acetamide (40).** Compound **40** was prepared from 1-pyridin-2-yl-piperazine (31 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 632.6 ($[M+H]^+$); mp: 171-174 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 3.02-3.05 (4H, m, 2 x CH_2N-piperazine), 3.15-3.17 (4H, m,

2 x CH_2N-morpholine), 3.45-3.47 (4H, m, 2 x CH_2N-piperazine), 3.50-3.52 (4H, m, 2 x CH_2O-morpholine), 3.89 (2H, br s, NCH_2), 6.32 (1H, s, H-3), 6.88-6.91 (2H, m, H-Ar), 7.44-7.51 (4H, m, H-Ar), 7.76-7.80 (3H, m, H-Ar), 8.22-8.25 (3H, m, H-Ar), 8.38-8.40 (1H, m, H-Ar), 9.90 (1H, s, NH).

2-(4-(4-Fluorophenyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (41). Compound **41** was prepared from 1-(4-fluorophenyl)piperazine (34 mg, 0.19 mmol) according to Method I and was obtained in 99% purity: LCMS *m/z* 649.6 ($[M+H]^+$).

2-(4-(3-(Isopropylamino)-2-oxopropyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (42). Compound **42** was prepared from *N*-isopropyl-1-piperazineacetamide (35 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 654.5 ($[M+H]^+$).

2-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (43). Compound **43** was prepared from 1-(2-dimethylaminoethyl)piperazine (30 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 626.4 ($[M+H]^+$).

2-((1-Ethylpyrrolidin-2-yl)methylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (44). Compound **44** was prepared from 2-(aminomethyl)-1-ethylpyrrolidine (24 mg, 0.19 mmol) according to Method I and was obtained in 97% purity: LCMS *m/z* 597.4 ($[M+H]^+$).

2-(4-(2-Hydroxyethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (45). Compound **45** was prepared from 1-(2-hydroxyethyl)piperazine (24 mg, 0.19 mmol) according to Method I and was obtained in 97% purity: LCMS *m/z* 599.4 ($[M+H]^+$).

2-(4-(2-Hydroxyethyl)-1,4-diazepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (46). Compound **46** was prepared from 2-(1,4-diazepan-1-

yl)ethanol (27 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 613.4 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(3-morpholinopropylamino)acetamide (47).** Compound **47** was prepared from 4-(3-aminopropyl)morpholine (27 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 613.4 ($[M+H]^+$).

2-(1,4-Diazepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (48). Compound **48** was prepared from homopiperazine (19 mg, 0.19 mmol) according to Method I and was obtained in 95% purity: LCMS *m/z* 569.4 ($[M+H]^+$).

2-(Dimethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (49). Compound **49** was prepared from dimethylamine (2M solution in THF) (8 mg, 0.19 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 514.4 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-2-(pyridin-4-ylmethylamino)acetamide (50).** Compound **50** was prepared from 4-(aminomethyl)pyridine (20 mg, 0.19 mmol) according to Method I and was obtained in 98% purity: LCMS *m/z* 577.4 ($[M+H]^+$).

2-(4-Isopropylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)acetamide (51). Compound **51** was prepared from 1-isopropylpiperazine (24 mg, 0.19 mmol) according to Method I and was obtained in 96% purity: LCMS *m/z* 597.4 ($[M+H]^+$).

Solution-Phase Library Synthesis of 3-amino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamides (52-86): Method II. General Procedure. To 8-(1-aminodibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **13** (1 mol equiv.) in dry DMA (17 mL) were added triethylamine (2.2 mol equiv.) and 3-bromopropionyl chloride (1.1 mol equiv.) and the reaction mixture stirred at room temperature for 4 h. Aliquots (0.5 mL) of the resulting solution were added to each of 34 tubes in a Greenhouse workstation, with each tube containing a different amine (3

equiv.). The reaction mixtures were stirred in parallel at room temperature for 12 h. After dilution with 1.5 mL of methanol, the product was purified by semi-preparative HPLC.

3-Morpholino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (52). Compound **52** was prepared from morpholine (16 mg, 0.19 mmol) according to Method II and was obtained in 97% purity: LCMS *m/z* 570.5 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(piperidin-1-yl)propanamide (53).** Compound **53** was prepared from piperidine (16 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 568.4 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(piperazin-1-yl)propanamide (54).** Compound **54** was prepared from piperazine (16 mg, 0.19 mmol) according to Method II and was obtained in 100% purity: LCMS *m/z* 569.4 ($[M+H]^+$).

3-(4-Methylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (55). Compound **55** was prepared from 1-methyl-piperazine (19 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 583.4 ($[M+H]^+$).

3-(Bis(2-hydroxyethyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (56). Compound **56** was prepared from diethanolamine (20 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 588.4 ($[M+H]^+$).

3-(2-Hydroxyethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (57). Compound **57** was prepared from ethanolamine (16 mg, 0.12 mmol) according to Method II and was obtained in 98% purity: LCMS *m/z* 544.4 ($[M+H]^+$).

3-(2-Aminoethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (58). Compound **58** was prepared from ethylenediamine (11 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 543.4 ($[M+H]^+$).

3-(4-Methyl-1,4-diazepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (59). Compound **59** was prepared from 1-

methylhomopiperazine (21 mg, 0.19 mmol) according to Method II and was obtained in 98% purity: LCMS *m/z* 597.4 ($[M+H]^+$).

3-(Azepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (60). Compound **60** was prepared from hexamethyleneimine (19 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 582.5 ($[M+H]^+$); ^{1}H NMR, (300 MHz, $CDCl_3$) δ 1.51-1.99 (8H, m, 4 x CH_2-homopiperazine), 2.90-3.22 (6H, m, 2 x CH_2N-homopiperazine and CH_2CO), 3.29-3.31 (2H, m, CH_2N), 3.44-3.47 (4H, m, 2 x CH_2N-morpholine), 3.52-3.56 (4H, m, 2 x CH_2O-morpholine), 6.12 (1H, s, H-3), 7.39-7.48 (4H, m, H-Ar), 7.58 (1H, d, J = 7.9 Hz, H-Ar), 7.72-7.75 (3H, m, H-Ar), 8.18-8.24 (2H, m, H-Ar), 9.32 (1H, s, NH).

3-(4-Methoxybenzylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (61). Compound **61** was prepared from 4-methoxybenzylamine (26 mg, 0.19 mmol) according to Method II and was obtained in 97% purity: LCMS *m/z* 620.5 ($[M+H]^+$).

3-(Diethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (62). Compound **62** was prepared from diethylamine (14 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 556.5 ($[M+H]^+$).

3-Amino-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (63). Compound **63** was prepared from ammonia (7N solution in methanol) (3.2 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 500.3 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(2-morpholinoethylamino)propanamide (64).** Compound **64** was prepared from 4-(2-aminoethyl)morpholine (24 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 613.5 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-((tetrahydrofuran-2-yl)methylamino)propanamide (65).** Compound **65** was prepared from tetrahydrofurfurylamine (19 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 584.4 ($[M+H]^+$); mp: 146-150 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 2.45-2.88 (7H, m, 2 x CH_2-tetrahydrofuran, CH_2CO

and NH), 3.14-3.20 (4H, m, 2 x CH_2N-morpholine), 3.45-3.556 (8H, m, 2 x CH_2O-morpholine, CH_2NH and C$H_2$$CH_2$CO), 3.66-3.69 (2H, m, CH_2O-tetrahydrofuran), 4.16-4.18 (1H, m, CH-tetrahydrofuran), 6.00 (1H, s, H-3), 7.40-7.42 (1H, m, H-Ar), 7.53-7.54 (1H, m, H-Ar), 7.67-7.70 (2H, m, H-Ar), 8.18-8.22 (2H, m, H-Ar), 9.19 (1H, s, NH).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(2-(piperidin-1-yl)ethylamino)propanamide (66).** Compound **66** was prepared from 1-(2-aminoethyl)piperidine (24 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 611.5 ($[M+H]^+$).

3-((2-(Dimethylamino)ethyl)(methyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (67). Compound **67** was prepared from *N*,*N*,*N*'-trimethylethylenediamine (19 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 585.4 ($[M+H]^+$).

3-((2*S*,6*R*)-2,6-dimethylmorpholino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (68). Compound **68** was prepared from *cis*-2,6-dimethylmorpholine (22 mg, 0.19 mmol) according to Method II and was obtained in 97% purity: LCMS *m/z* 598.4 ($[M+H]^+$).

3-(Ethyl(pyridin-4-ylmethyl)amino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (69). Compound **69** was prepared from *N*-(4-pyridylmethyl)ethylamine (26 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 619.4 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[b,d]thiophen-1-yl)-3-(2-(2-oxoimidazolidin-1-yl)ethylamino)propanamide (70).** Compound **70** was prepared from 1-(2-aminoethyl)-2-imidazolidinone (24 mg, 0.19 mmol) according to Method II and was obtained in 99% purity: LCMS *m/z* 612.4 ($[M+H]^+$).

3-(2-(1-Methylpyrrolidin-2-yl)ethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (71). Compound **71** was prepared from 2-(2-aminoethyl)-

1-methylpyrrolidine (24 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 611.4 ($[M+H]^+$).

3-(2-Methoxyethylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (72). Compound **72** was prepared from 2-methoxyethylamine (14 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 558.3 ($[M+H]^+$).

3-(4-Acetylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (73). Compound **73** was prepared from 1-acetylpiperazine (24 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 611.4 ($[M+H]^+$).

3-(4-(2-Methoxyethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (74). Compound **74** was prepared from 1-(2-methoxyethyl)-piperazine (27 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 627.4 ($[M+H]^+$).

3-(4-(2-(2-Hydroxyethoxy)ethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (75). Compound **75** was prepared from 1-hydroxyethylethoxypiperazine (33 mg, 0.19 mmol) according to Method II and was obtained in 98% purity: LCMS *m/z* 657.4 ($[M+H]^+$).

3-(4-Ethylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (76). Compound **76** was prepared from 1-ethylpiperazine (21 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 597.4 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(4-(pyridin-2-yl)piperazin-1-yl)propanamide (77).** Compound **77** was prepared from 1-pyridin-2-yl-piperazine (31 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 646.4 ($[M+H]^+$).

3-(4-(4-Fluorophenyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (78). Compound **78** was prepared from 1-(4-fluorophenyl)piperazine (34 mg, 0.19 mmol) according to Method II and was obtained in 99% purity: LCMS *m/z* 663.4 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(4-(pyrimidin-2-yl)piperazin-1-yl)propanamide (79).** Compound **79** was prepared from 2-(1-piperazinyl)pyrimidine (31 mg, 0.19 mmol) according to Method II and was obtained in 99% purity: LCMS *m/z* 647.5 ($[M+H]^+$).

3-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (80). Compound **80** was prepared from 1-(2-dimethylaminoethyl)piperazine (30 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 668.5 ($[M+H]^+$).

3-((1-Ethylpyrrolidin-2-yl)methylamino)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (81). Compound **81** was prepared from 2-(aminomethyl)-1-ethylpyrrolidine (24 mg, 0.19 mmol) according to Method II and was obtained in 97% purity: LCMS *m/z* 611.4 ($[M+H]^+$).

3-(4-(2-Hydroxyethyl)piperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (82). Compound **82** was prepared from 1-(2-hydroxyethyl)piperazine (24 mg, 0.19 mmol) according to Method II and was obtained in 97% purity: LCMS *m/z* 613.5 ($[M+H]^+$).

3-(4-(2-Hydroxyethyl)-1,4-diazepan-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (83). Compound **83** was prepared from 2-(1,4-diazepan-1-yl)ethanol (27 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 627.5 ($[M+H]^+$).

***N*-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(3-morpholinopropylamino)propanamide (84).** Compound **84** was prepared from 4-(3-aminopropyl)morpholine (27 mg, 0.19 mmol) according to Method II and was obtained in 95% purity: LCMS *m/z* 627.5 ($[M+H]^+$).

***N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)-3-(pyridin-4-ylmethylamino)propanamide (85).** Compound **85** was prepared from 4-(aminomethyl)pyridine (20 mg, 0.19 mmol) according to Method II and was obtained in 98% purity: LCMS *m/z* 591.3 ($[M+H]^+$).

3-(4-Isopropylpiperazin-1-yl)-*N*-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yl)propanamide (86). Compound **86** was prepared from 1-isopropylpiperazine (24 mg, 0.19 mmol) according to Method II and was obtained in 96% purity: LCMS *m/z* 611.3 ($[M+H]^+$).

8-(1-Hydroxydibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (87). HBF_4 (2.67 mL, 15.2 mmol) was added dropwise to 8-(1-aminodibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **13** (436 mg, 1.0 mmol) in ethanol (40 mL) at room temperature. After stirring for 15 min, the reaction mixture became a clear solution, which was cooled to 0 ^oC and *t*-butylnitrite (138 mg, 2.0 mmol) was added. After 30 min, the reaction mixture was diluted with ether (80 mL). The precipitated solid was filtered, washed with ether (2 x 20 mL) and dried. This solid was added to a solution of cupric nitrate (73.7 g, 300 mmol) in 1 L of water containing cuprous oxide (143 mg, 1 mmol) and stirred for 1 h at room temperature. The aqueous solution was filtered to afford the product as a brown solid, which was purified by flash chromatography using DCM/MeOH (95:5) as eluent (326 mg, 76%). R_f = 0.19 (DCM /MeOH 95:5); mp: 189-191 °C; IR (cm^{-1}) 3100, 2961, 2862, 1618, 1576, 1551, 1499, 1475, 1441, 1413, 1389, 1244, 1117, 779; ^1H NMR, (300 MHz, MeOD) δ 3.07-3.09 (4H, m, 2 x CH_2N-morpholine), 3.32-3.35 (4H, m, 2 x CH_2O-morpholine), 5.50 (1H, s, H-3), 6.92 (1H, d, *J* = 8.1 Hz, H-Ar), 7.25 (1H, d, *J* = 8.1 Hz, H-Ar), 7.29-7.45 (3H, m, H-Ar), 7.71 (2H, d, *J* = 7.4 Hz, H-Ar), 8.03 (1H, d, *J* = 7.9 Hz, H-Ar), 8.65 (1H, d, *J* = 8.4 Hz, H-Ar); ^{13}C NMR, (75 MHz, MeOD) δ 45.8 (2 x CH_2N-morpholine), 66.8 (2 x CH_2O-morpholine), 86.8 (C-3), 111.8, 118.1, 122.9, 123.9, 125.0, 125.5, 125.7, 126.2, 127.0, 127.1, 129.9, 130.6, 135.3, 136.9, 139.1, 142.4, 152.0, 156.7, 163.9, 179.2; LCMS *m/z* 430.1 ($[M+H]^+$).

Solution-Phase Library Synthesis of 8-(1-(2-aminoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (88-101). Method III. General Procedure. To a solution of 8-(1-

hydroxydibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **87** (1 mol equiv.) in dry DMF (7 mL) was added potassium carbonate (1.1 mol equiv.) and dibromoethane (1.1 mol equiv.) and the reaction mixture was stirred at room temperature for 12 h. Aliquots (0.5 mL) of the resulting solution of 8-(1-(2-bromoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **88** were added to each of the 14 vials in a Greenhouse workstation, with each tube containing a different amine (3 equiv.). The reaction mixtures were stirred in parallel at room temperature for 12 h. After dilution with 1.5 mL of methanol, the product was purified by semi-preparative HPLC.

2-Morpholino-8-(1-(2-morpholinoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (89). Compound **89**was prepared from morpholine (16 mg, 0.19 mmol) according to Method III and was obtained in 97% purity: LCMS *m/z* 543.6 ($[M+H]^+$).

2-Morpholino-8-(1-(2-(piperidin-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (90). Compound **90** was prepared from piperidine (16 mg, 0.19 mmol) according to Method III and was obtained in 96% purity: LCMS *m/z* 541.4 ($[M+H]^+$).

8-(1-(2-(4-Methylpiperazin-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (91). Compound **91** was prepared from 1-methyl-piperazine (19 mg, 0.19 mmol) according to Method III and was obtained in 95% purity: LCMS *m/z* 556.4 ($[M+H]^+$).

8-(1-(2-(4-Methyl-1,4-diazepan-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (92). Compound **92** was prepared from 1-methylhomopiperazine (21 mg, 0.19 mmol) according to Method III and was obtained in 98% purity: LCMS *m/z* 570.4 ($[M+H]^+$).

8-(1-(2-(Diethylamino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (93). Compound **93** was prepared from diethylamine (14 mg, 0.19 mmol) according to Method III and was obtained in 96% purity: LCMS *m/z* 529.4 ($[M+H]^+$).

2-Morpholino-8-(1-(2-(2-morpholinoethylamino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (94). Compound **94** was prepared from 4-(2-aminoethyl)morpholine (24 mg, 0.19 mmol) according to Method III and was obtained in 95% purity: LCMS *m/z* 586.4 ($[M+H]^+$).

8-(1-(2-((2-(Dimethylamino)ethyl)(methyl)amino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (95). Compound **95** was prepared from *N*,*N*,*N*'-trimethylethylenediamine (19 mg, 0.19 mmol) according to Method III and was obtained in 95% purity: LCMS *m/z* 558.3 ($[M+H]^+$).

8-(1-(2-((2*S*,6*R*)-2,6-Dimethylmorpholino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (96). Compound **96** was prepared from *cis*-2,6-dimethylmorpholine (22 mg, 0.19 mmol) according to Method III and was obtained in 97% purity: LCMS *m/z* 571.4 ($[M+H]^+$).

8-(1-(2-(4-Acetylpiperazin-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (97). Compound **97** was prepared from 1-acetylpiperazine (24 mg, 0.19 mmol) according to Method III and was obtained in 95% purity: LCMS *m/z* 584.5 ($[M+H]^+$).

8-(1-(2-(Dimethylamino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (98). Compound **98** was prepared from dimethylamine (2M solution in THF) (8 mg, 0.19 mmol) according to Method III and was obtained in 98% purity: LCMS *m/z* 501.4 ($[M+H]^+$).

2-Morpholino-8-(1-(2-(pyridin-3-ylamino)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (99). Compound **99** was prepared from 3-aminopyridine (18 mg, 0.19 mmol) according to Method III and was obtained in 98% purity: LCMS *m/z* 550.4 ($[M+H]^+$).

8-(1-(2-(4-Isopropylpiperazin-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (100). Compound **100** was prepared from 1-isopropylpiperazine (24 mg, 0.19 mmol) according to Method III and was obtained in 96% purity: LCMS *m/z* 584.5 ($[M+H]^+$).

(*S*)-Methyl 3-hydroxy-2-(2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)ethylamino)propanoate (101). Compound **101** was prepared from *L*-serine methyl ester hydrochloride (23 mg, 0.19 mmol) according to Method III and was obtained in 96% purity: LCMS *m/z* 575.4 ($[M+H]^+$).

2-(Methyl(2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)ethyl)amino)acetamide (102). Compound **102** was prepared from glycinamide hydrochloride (14 mg, 0.19 mmol) according to Method III and was obtained in 96% purity: LCMS *m/z* 530.4 ($[M+H]^+$).

Sodium 2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)acetate (103). To a mixture of 8-(1-hydroxydibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one **87** (300 mg, 0.70 mmol) and K_2CO_3 (115 mg, 0.84 mmol) in dry DMF (20 mL) was added methyl bromoacetate (79.3 μL, 0.84 mmol). The reaction mixture was stirred at 60 ^oC for 12 h and monitored by LCMS. Upon completion, the reaction mixture poured into water and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine, dried and concentrated *in vacuo* to give the methyl ester as a yellow solid (LCMS *m/z* 502.1 ($[M+H]^+$)). The product was dissolved in MeOH (10 mL) and a 1M aqueous solution of NaOH (10 mL) was added. The reaction mixture was stirred at 60 ^oC for 1 h and concentrated *in vacuo* to afford the sodium salt (LCMS showed the presence of the carboxylic acid at *m/z* 488.1 ($[M+H]^+$)). The title compound was used in the next step without any further purification.

Solution-Phase Library Synthesis of 8-(1-(2-aminoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (104-113). Method IV. General Procedure. Sodium salt **103** (1 mol equiv.) was divided into 10 portions and added to 10 different amines (3 equiv.) in a Greenhouse workstation. A solution of HOBt (1.5 equiv.) and HBTU (1.5 equiv.) in DMF (10 mL) was added to each of the 15 vials. The reaction mixtures were stirred in parallel at room temperature for 12 h. After dilution with 1.5 mL of methanol, the product was purified by semi-preparative HPLC.

2-Morpholino-8-(1-(2-oxo-2-(piperidin-1-yl)ethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (104). Compound **104** was prepared from piperidine (16 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 555.4 ($[M+H]^+$).

8-(1-(2-(4-Methylpiperazin-1-yl)-2-oxoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (105). Compound **105** was prepared from 1-methyl-piperazine (19 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 570.4 ($[M+H]^+$).

8-(1-(2-(4-Methyl-1,4-diazepan-1-yl)-2-oxoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (106). Compound **106** was prepared from 1-methylhomopiperazine (21 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 584.5 ($[M+H]^+$).

***N*-(2-(Dimethylamino)ethyl)-*N*-methyl-2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)acetamide (107).** Compound **107** was prepared from *N*,*N*,*N*'-trimethylethylenediamine (19 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 572.4 ($[M+H]^+$).

8-(1-(2-((2*R*,6*S*)-2,6-Dimethylmorpholino)-2-oxoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (108). Compound **108** was prepared from *cis*-2,6-dimethylmorpholine (22 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 585.4 ($[M+H]^+$); mp: 240-242 °C; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 1.12 (3H, d, J = 6.2 Hz, CH_3), 1.22 (3H, d, J = 6.2 Hz, CH_3), 3.14-3.18 (4H, m, 2 x CH_2O-morpholine), 3.48-3.52 (8H, m, 2 x CH_2N-morpholine and 2 x CH_2N-*cis*-dimethylmorpholine), 3.95-3.98 (1H, m, CH-*cis*-dimethylmorpholine), 4.45-4.49 (1H, m, CH-*cis*-dimethylmorpholine), 5.07 (2H, d, J = 9.5 Hz, -OCH_2), 5.95 (1H, s, H-3), 7.11 (1H, d, J = 8.2 Hz, H-Ar), 7.35 (1H, d, J = 8.2 Hz, H-Ar), 7.49-7.55 (3H, m, H-Ar), 7.76 (2H, m, H-Ar), 8.28 (1H, d, J = 7.9 Hz, H-Ar), 8.84 (1H, m, H-Ar).

8-(1-(2-(4-Acetylpiperazin-1-yl)-2-oxoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (109). Compound **109** was prepared from 1-acetylpiperazine (24 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 598.5 ($[M+H]^+$).

2-(4-(2-Morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)-*N*-(pyridin-4-ylmethyl)acetamide (110). Compound **110** was prepared from 4-(aminomethyl)pyridine (20 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 578.5 ($[M+H]^+$).

8-(1-(2-(4-Isopropylpiperazin-1-yl)-2-oxoethoxy)dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (111). Compound **111** was prepared from 1-isopropylpiperazine (24 mg, 0.19 mmol) according to Method IV and was obtained in 95% purity: LCMS *m/z* 598.5 ($[M+H]^+$).

(*S*)-Methyl 3-hydroxy-2-(2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)acetamido)propanoate (112). Compound **112** was prepared from *L*-serine methyl ester hydrochloride (23 mg, 0.19 mmol) according to Method IV and was obtained in 96% purity: LCMS *m/z* 589.4 ($[M+H]^+$).

***N*-(2-Amino-2-oxoethyl)-*N*-methyl-2-(4-(2-morpholino-4-oxo-4*H*-chromen-8-yl)dibenzo[*b*,*d*]thiophen-1-yloxy)acetamide (113).** Compound **113** was prepared from glycinamide hydrochloride (14 mg, 0.19 mmol) according to Method IV and was obtained in 96% purity: LCMS *m/z* 544.4 ($[M+H]^+$).

DNA-PK Enzyme 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 concentration of 0.5 ng/mL) to initiate the reaction. After 1 hour 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 hour at room temperature. To detect the phosphorylation event on the serine-15 residue of p53 elicited by DNA-PK, a rabbit 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). IC_{50} was derived from a sigmoidal plot using the graphic package Prism, in which the DNA-PK activity in the varying concentrations of compounds was plotted against the concentration of compound.

DMR clonogenic assay. Cells were seeded per well into a 6 well tissue culture treated dish and incubated overnight at 37°C/ 5% CO_2. Following a 1 hour pre-treatment with either vehicle or

compound the plates were exposed to 2 Gy ionizing radiation (IR) using a Faxitron 43855D x-ray source and incubated overnight at 37°C/ 5% CO_2. The media was replaced with fresh media in the absence of compound or vehicle and incubated for a further 6-8 days. The media was removed and the cell colonies were fixed and stained with Giemsa and scored with an automated colony counter (Oxford Optronics Ltd., Oxford, United Kingdom). The Dose Modification Ratio (DMR) at 2 Gy irradiation was calculated as follows:

$$\mathrm{DMR} = \frac{\%\ \mathrm{survival}_{\text{-compound/+2 Gy}}}{\%\ \mathrm{survival}_{\text{+compound/+2 Gy}}}$$

hERG Inhibition Assay. hERG currents from individual hERG expressing CHO cells were recorded using an IonWorks Quattro Automated Patch Clamp System (Molecular Devices).% inhibition was determined as a ratio of the pre- and post-compound hERG current metric with DMSO vehicle and 10μM Cisapride treated cells as 0 and 100% inhibition respectively. hERG inhibition was expressed as an IC_{50}.

PI3Kα Inhibition Assay. Purified PI3Kα was pre-incubated with compound in 50 mM Tris pH 7.4; 0.05% v/v CHAPS; 2.1 mM DTT; 10 mM $MgCl_2$ for 20 min at room temperature before addition of 80 μM PIP_2 and 8 μM ATP. ATP remaining after 80 min was measured using a Kinase-Glo® Luminescent Kinase Assay according to the manufacturer's instructions (Promega).

Cellular Inhibition of DNA-PK autophosphorylation Assay. HT29 cells were treated with compound 1 hour prior to exposure to an 8Gy dose of ionizing radiation (MDS Nordion gamma cell 3000 Elan instrument). 1 hour post irradiation the cells were lysed and dispensed into an ELISA plate coated with a total DNA-PK antibody (Thermo Scientific MS-370-PABX). Following washing phosphorylated DNA-PK was detected using a primary pDNA-PK Ser2056 (Abcam) and secondary HRP tagged antibody.

Immunofluorescence of DNA-PK phosphorylation. HeLa cells were seeded onto Poly-D-Lysine coated coverslips overnight before being pre-treated with 1μM of compound 39 for 1 hour. Cells were subsequently exposed to 5Gy ionizing radiation (Faxitron 43855D). At the appropriate time post irradiation the cells were fixed in 4% paraformaldehyde, blocked in 5% foetal calf serum and stained with a γH2AX (Ser139) antibody (Upstate). Coverslips were mounted with VectaShield and DAPI (Vector Laboratories, CA) and imaged on a BD Pathway bioimaging system. The nuclear region was identified using the DAPI stain and the γH2AX staining intensity within this measured using BD Pathway image analysis software.

Amino acid sequences for the homology model:

FISWISHMVALLDKDQAVAVQHSVEEITDNYPQAIVYPFIISSESYSFKDTSTGHKNKEFVARIKS
KLDQGGVIQDFINALDQLSNPELLFKDWSNDVRAELAKTPVNKKNIEKMYERMYAALGDPKA
PGLGAFRRKFIQTFGKEFDKHFGKGGSKLLRMKLSDFNDITNMLLLKMNKDSKPPGNLKECSP
WMSDFKVEFLRNELEIPGQYDGRGKPLPEYHVRIAGFDERVTVMASLRRPKRIIIRGHDEREHP
FLVKGGEDLRQDQRVEQLFQVMNGILAQDSACSQRALQLRTYSVVPMTSRLGLIEWLENTVTL
KDLLLNTMSQEGANRTETVTSFRKRESKVEAFLALRSHFASSHALICISHWILGIGDRHLNNFM
VAMETGGVIGIDFGHAFGSATQFLPVPELMPFRLTRQFINLMLPMKETGLMYSIMVHALRAFRS
DPGLLTNTMDVFVKEPSFDWKNFEQKMLKKGGSWIQEINVAEKNWYPRQKICYAKRKLAGA
NPAVITCX

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Abbreviations: DNA-PK, DNA-dependent protein kinase; IC_{50}, concentration of inhibitor leading to 50% inhibition; SAR, structure activity relationship; PI, phosphatidylinositol; PIKK, phosphatidylinositol 3-kinase related kinase; DSBs, double-strand breaks; DMR, dose modification ratio; IR, ionizing radiation; DMA, *N*,*N*-dimethylacetamide; HOBt, hydroxybenzotriazole; HBTU, *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compound **39**. This information is available free of charge via the Internet at http://pubs.acs.org.

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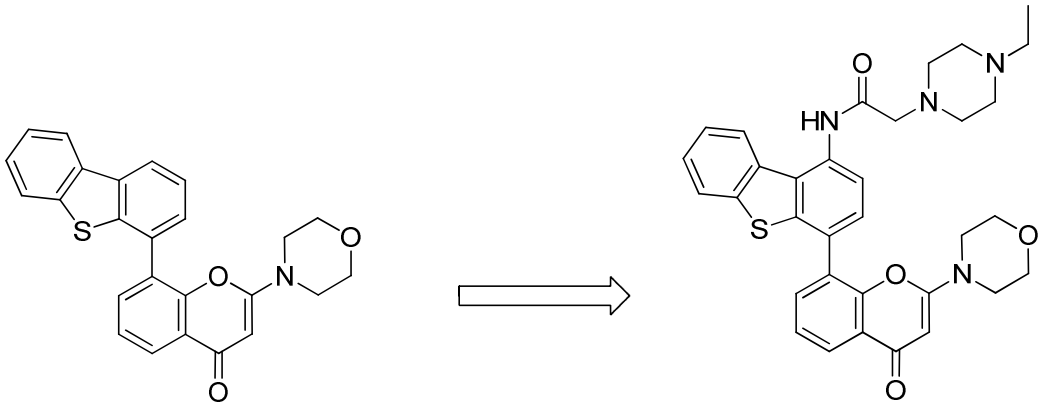
Table of Contents Graphic



2
DNA-PK; IC_{50} = 0.042 μM
PI-3Kα; IC_{50} = 0.13 μM

39
DNA-PK; IC_{50} = 0.005 μM
PI-3Kα; IC_{50} = 0.004 μM