Atropisomeric 8-arylchromen-4-ones exhibit enantioselective inhibition of the DNA-dependent protein kinase (DNA-PK)

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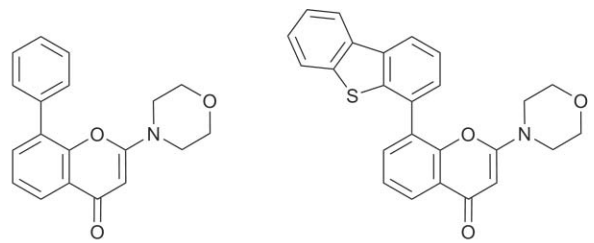
Substitution at the 3-position of the dibenzothiophen-4-yl ring of 8-(dibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one NU7441, a potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor, with propyl, allyl or methyl enabled the separation by chiral HPLC of atropisomers. This is a consequence of restricted rotation about the dibenzothiophene–chromenone bond. Biological evaluation against DNA-PK of the pairs of atropisomers showed a marked difference in potency, with only one enantiomer being biologically active.

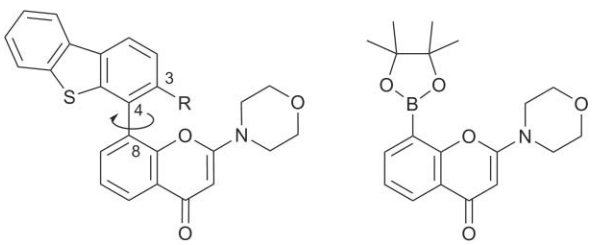
Introduction

DNA-dependent protein kinase (DNA-PK), a member of the phosphatidylinositol (PI[a]) 3-kinase related kinase (PIKK) family, is a multi-component serine/threonine protein kinase that participates in the repair of mammalian DNA double-strand breaks (DSBs).[1,2] Human cell lines defective in DNA-PK function are hypersensitive to agents that elicit DNA DSBs.[3,4] DNA-PK inhibitors could be used to gain a better understanding of the role of DNA-PK. In addition, by impeding DNA DSB repair, selective DNA-PK inhibitors have potential application as radio- and chemo-potentiators in the treatment of cancer.[5–9] Using 2-morpholino-8-phenyl-4*H*-chromen-4-one ('LY294002') as a template for inhibitor design, we have previously identified potent DNA-PK inhibitors for which structure–activity relationships (SARs) have been determined.[10–14] Notably, the incorporation of a dibenzothiophen-4-yl substituent at the 8-position of 2-morpholino-4*H*-chromen-4-one conferred inhibitory activity against DNA-PK (NU7441, IC_{50} = 30 nM) that was 40-fold greater than the 8-phenyl derivative. Furthermore, there was good selectivity over other PIKK family members. NU7441 has also been demonstrated to sensitise a human tumour cell line to both ionising radiation and the topoisomerase II inhibitor etoposide *in vitro*, and to potentiate the *in vivo* antitumour activity of etoposide.[15]

With a view to optimising the biological and pharmaceutical properties of NU7441, and to expand SARs, both the core chromenone scaffold and the pendant dibenzothiophen-4-yl group have been systematically modified. Thus, replacement of the chromenone by a quinolin-4-one or pyridopyrimidin-4-one heterocycle is tolerated.[16] Substitution of the dibenzothiophen-4-yl group by the isosteric dibenzofuran-4-yl or the 3-phenyl-phen-1-yl functionality does not compromise potency. In the absence of structural biology for DNA-PK of sufficient resolution to assist inhibitor design, an homology model of the ATP-binding site has been constructed, based on the crystal structure of PI3K γ.[17] The addition of substituents to the chromenone and dibenzothiophene rings of NU7441 has provided valuable SAR information and enabled further refinement of the homology model.

We now report studies of the effect upon biological activity of alkylation at the 3-position of the dibenzothiophen-4-yl group of NU7441. It was recognised that in the preferred conformation of NU7441, the chromen-4-one and dibenzothiophene rings would not be coplanar owing to interactions between these rings. Hence, the molecule is chiral by virtue of a stereogenic axis between the two heterocyclic systems. With appropriate substituents at the 3-position of the dibenzothiophene ring, restricted rotation between the chromen-4-one and dibenzothiophene rings was expected to be sufficient to afford resolvable atropisomers. The possibility of differential DNA-PK inhibitory activity between the two atropisomers offered an opportunity to probe the stereochemistry of interaction of this inhibitor class within the ATP-binding domain of DNA-PK.





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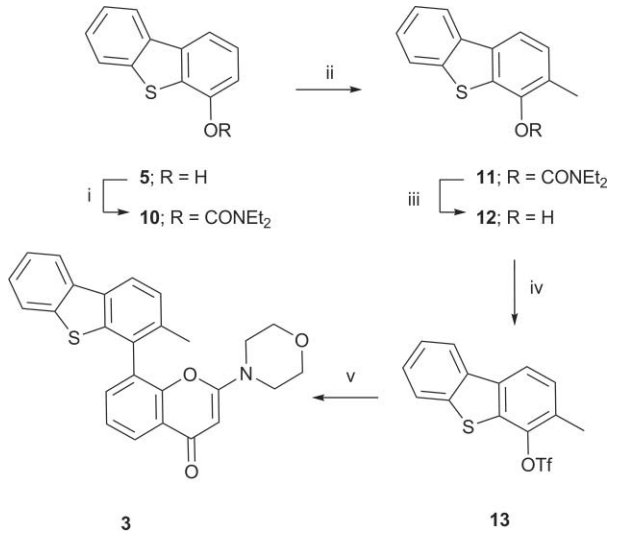
Results and discussion

Synthesis of 3-substituted dibenzothiophen-4-yl chromenones

Our previous studies have utilised the chromenone boronate **4** as a building block for the preparation of 8-substituted chromenones, employing Suzuki–Miyaura Pd-catalysed cross-coupling reactions.[10–12,14] This strategy was also amenable for the synthesis of the 3-substituted NU7441 analogues (Scheme 1). Introduction of 3-substituents was initially achieved by alkylation of phenol **5** with allyl bromide, followed by a Claisen rearrangement to furnish the intermediate **7**.[18] Catalytic hydrogenation of **7** followed by triflation afforded the *n*-propyl derivative **8** in 91% overall yield, while direct triflation of **7** gave the corresponding allyl derivative **9** in 83% yield. Suzuki–Miyaura coupling of dibenzothiophenes **8** and **9** with 2-morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (**10**) under microwave conditions furnished the 3-substituted NU7441 analogues **1** and **2**, respectively. Introduction of a 3-methyl substituent on the pendant dibenzothiophene of NU7441 was achieved *via* a directed *ortho*-metallation (D*o*M) reaction analogous to that reported by Snieckus *et al.* (Scheme 2).[19,20] Thus, carbamoylation of **5**, followed by sequential treatment of carbamate **10** with *sec*-BuLi/TMEDA and iodomethane, gave 3-methyldibenzothio-phen-4-yl diethylcarbamate **11** in 50% yield. Subsequent removal of the carbamoyl group with base followed by triflation gave intermediate **13**, which was cross-coupled with **9** using Suzuki–Miyaura conditions.[21]



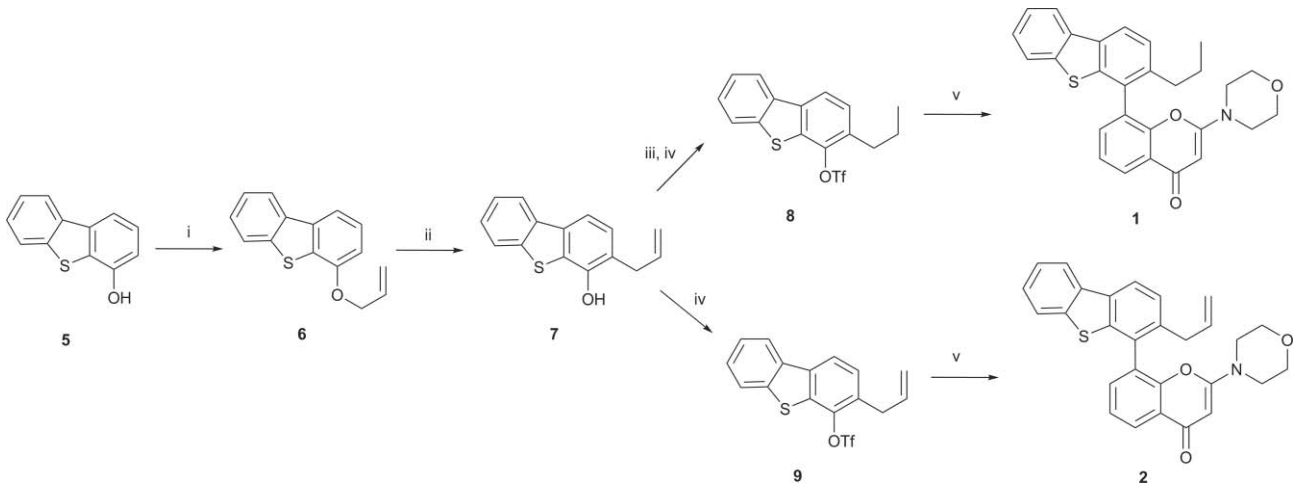
Scheme 2 *Reagents and conditions*: (i) *N*,*N*-diethylcarbamoyl chloride, Et_3N, DCM, 0 °C, 96%; (ii) *sec*-BuLi, TMEDA, THF, −78 °C, 1 h then MeI, −78 °C to r.t., 51%; (iii) NaOH, EtOH, reflux, 62%; (iv) Tf_2O, Et_3N, DCM, 0 °C, 71%; (v) **4**, Cs_2CO_3, $PdCl_2$(dppf), THF, MW, 100 °C, 73%.

Atropisomer resolution by chiral HPLC

The separation of atropisomers of chromenones **1–3** was confirmed by analytical chiral HPLC studies, where two distinct peaks were observed. By contrast, NU7441 gave only a single peak under these conditions (Fig. 1). The excellent separation subsequently achieved by semi-preparative chiral HPLC enabled the resolution of each pair of atropisomers of **1–3** in milligram quantities. The integrity of each pair was established by polarimetry, with optical activity values confirming the resolution of the dextro- and laevorotatory stereoisomers in each case. The atropisomers proved remarkably stable to thermal racemisation. Thus, only chemical decomposition occurred after heating atropisomer **2-(+)** in mesitylene up to 170 °C for 10 h, with no evidence of racemisation by chiral HPLC analysis.

Biological evaluation

DNA-PK inhibitory activity was determined for the racemates **1–3**, together with the corresponding pairs of atropisomers, following a published procedure,[10] and the results are summarised in Table 1. The activity of NU7441 is included for comparative purposes. The introduction of a *n*-propyl (**1**), allyl (**2**) or methyl (**3**) substituent at the 3-position of the dibenzothiophen-4-yl group of NU7441, resulted in an approximately 80-fold reduction in potency of the racemic compounds against DNA-PK in all cases compared with the parent compound. This may have arisen



Scheme 1 *Reagents and conditions*: (i) allyl bromide, K_2CO_3, CH_3CN, reflux, 96%; (ii) DMF, 180 °C, 98%; (iii) H_2, Pd/C, MeOH, r.t., 94%; (iv) Tf_2O, Et_3N, DCM, 0 °C, 96% for **8** and 83% for **9**; (v) 2-morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (**4**), Cs_2CO_3, $PdCl_2$(dppf), THF, MW, 90 °C, 46% for **1** and 31% for **2**.

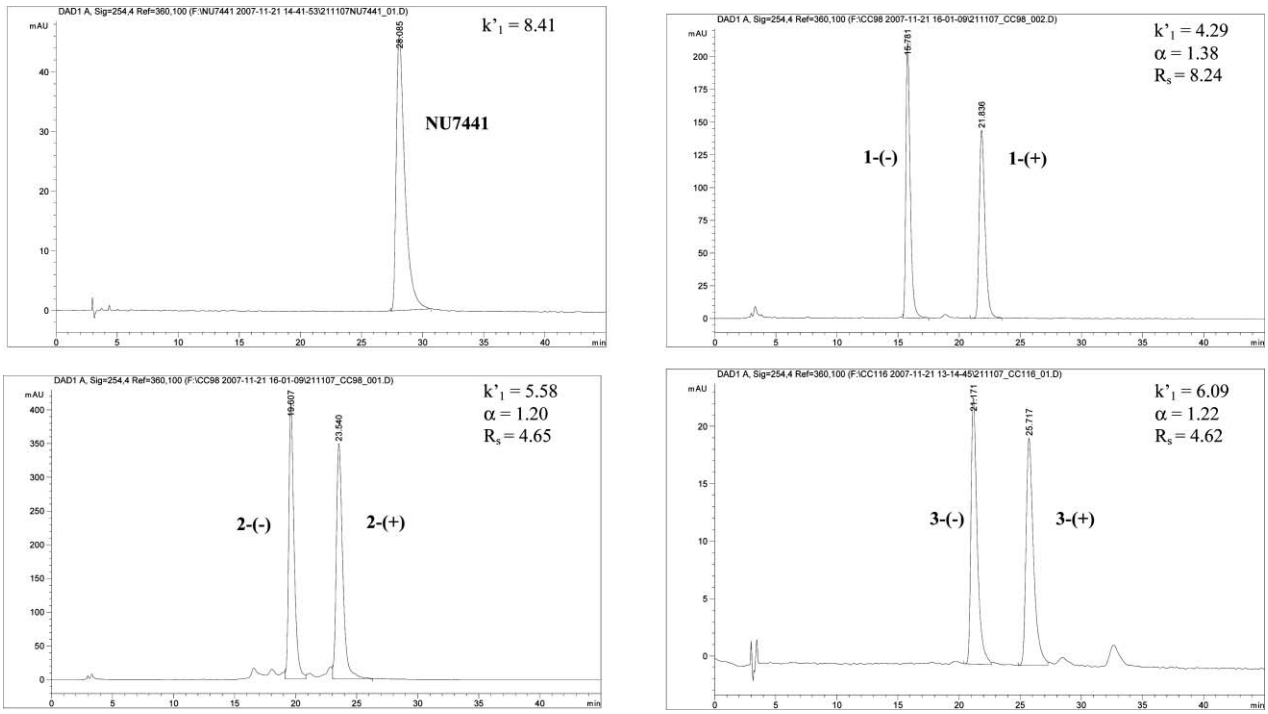
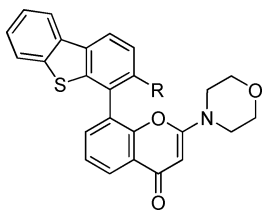


Fig. 1 Resolution of racemates **1**, **2** and **3** by chiral HPLC.

Table 1 Inhibition of DNA-PK by 3-substituted dibenzothiophen-4-yl derivatives (**1–3**)



R	Compound number	DNA-PK inhibition (IC_{50}/μM)[a]	Specific Rotation
H	**NU7441**	0.03	0
n-Propyl	**1**	2.7	0
n-Propyl	**1-(–)**	2.1	$[\alpha_D]^{16.7} = -95$
n-Propyl	**1-(+)**	100	$[\alpha_D]^{17.6} = +96$
Allyl	**2**	1.96	0
Allyl	**2-(–)**	3.1	$[\alpha_D]^{19.5} = -114$
Allyl	**2-(+)**	100	$[\alpha_D]^{19.6} = +114$
Me	**3**	1.7	0
Me	**3-(–)**	1.2	$[\alpha_D]^{18.5} = -127$
Me	**3-(+)**	100	$[\alpha_D]^{18.9} = +127$

[a] DNA-PK inhibitory activity was determined as described in ref. 10.

as a consequence of a steric 'clash' between the 3-substituent and amino-acid residues within the ATP-binding site of the kinase. Alternatively, substitution at the 3-position may confer unfavorable conformational constraints, resulting in a sub-optimal binding mode. Previous SAR studies have demonstrated the importance of the 2-morpholin-4-yl substituent of NU7441 and related DNA-PK inhibitors, with the morpholine oxygen making a critical hydrogen bond interaction within the ATP-binding domain of the kinase. It is conceivable that substitution at the 3-position of the dibenzothiophen-4-yl ring may adversely influence this important interaction.

Comparison of the DNA-PK inhibitory activity of each enantiomeric pair of chromenones **1–3** revealed that biological activity resided exclusively in the (–)-atropisomer ('eutomer') in each case, with the antipodal (+)-atropisomer ('distomer') proving inactive at 100 μM. Enantioselective binding of chiral drugs to enzymes is a well-established phenomenon with important implications for both pharmacological activity and toxicity.[22–26] Indeed, the resolution and biological characterisation of each stereoisomer of a chiral drug candidate is a prerequisite of the approval process for a new pharmaceutical entity. Although there are currently no marketed examples of a single atropisomeric drug, a recent comprehensive review has highlighted the importance of this aspect of drug stereochemistry.[27] In the present study, the identification of resolvable atropisomers, and the observation that only the (–)-enantiomer exhibits activity against DNA-PK, has important implications for future inhibitor design, not least the prospect of further refining the homology model. To date, all attempts to obtain suitable crystals to enable assignment of the absolute configuration of atropisomers of **1–3** by X-ray crystallography have proven unsuccessful, but experiments are ongoing.

Conclusions

By impeding DNA DSB repair, DNA-PK inhibitors have considerable therapeutic potential as chemo- and radio-potentiating agents in the therapy of cancer. The development of small molecule kinase inhibitors that combine potency with kinase-selectivity has proven a major challenge to the drug discovery community. In this paper, we report the results of ongoing studies designed to

optimise the biological activity of the potent chromenone-based inhibitor NU7441, through substitution on the dibenzothiophen-4-yl ring. Notably, alkyl substitution at the 3-position of the dibenzothiophene moiety has facilitated the resolution of relatively stable atropisomers, which exhibit differential DNA-PK inhibitory activity, albeit with a loss of potency compared with the parent inhibitor. Studies are in progress to elucidate the absolute configuration of the biologically active (–)-atropisomers, prior to conducting studies with the DNA-PK homology model. Alternative methods for the preparation of resolvable atropisomers are currently being investigated.

Experimental

Materials and methods

Solvents were either dried by standard techniques or purchased as anhydrous. Triethylamine was dried by distillation from calcium hydride and stored over potassium hydroxide, under nitrogen. Reactions needing microwave irradiation were carried out in an Initiator™ Sixty Biotage apparatus. Chiral HPLC analysis was performed on an Agilent (Agilent Technologies, Palo Alto, CA, USA) 1200 HPLC system equipped with a binary pump, autosampler, column oven and diode array detector and controlled by Agilent Chemstation software. Analyses were performed isocratically using a Daicel (Chiral Technologies Europe, Illkirch, France) Chiralpak IA column, 250 × 4.6 mm, 5 µm, with a mobile phase consisting of dichloromethane + 2.5% v/v ethanol–hexane (40 : 60) at a flow rate of 1.0 ml min^{-1} with detection at 254 nm. Semi-preparative chiral resolution was carried out using a Varian (Varian Inc., Walnut Creek, CA, USA) ProStar HPLC system equipped with 2 ProStar 210 solvent delivery modules, a ProStar 320 UV-Vis detector and a ProStar 701 fraction collector and controlled by Varian Star Chromatography Workstation. Separations were performed using a Daicel Chiralpak IA column, 250 × 10 mm, 5 µm, with a mobile phase consisting of dichloromethane + 2.5% v/v ethanol–hexane (40 : 60) at a flow rate of 3.8 ml min^{-1} with detection at 254 nm. Petrol refers to petroleum ether (bp 40–60 °C, reagent grade, Aldrich). NMR spectra were recorded on a Bruker Spectrospin AC 300E (300 MHz) NMR Spectrometer. IR spectra were recorded on a Bio-Rad FTS 3000MX diamond ATR. Optical rotations were measured on a PolAAR3001 instrument. LCMS was carried out on a Micromass Platform instrument operating in positive and negative ion electrospray mode, employing a 50 × 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%). HRMS were measured using a Finnigan MAT 95 XP or a Finnigan MAT 900 XLT by the EPSRC National Mass Spectrometry Service Centre (Swansea).

2-Morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (4)

To a solution of chromenone triflate[28] (1.25 g, 3.30 mmol) in anhydrous 1,4-dioxane (20 mL) was added potassium acetate (0.97 g, 9.9 mmol) and bis(pinacolato)diboron (1.01 g, 3.96 mmol). The reaction mixture was sonicated for 15 min. To the mixture was added $PdCl_2$(dppf) (135 mg, 0.165 mmol) and dppf (9.1 mg, 0.165 mmol). The reaction was heated under microwave irradiation for 60 min at 150 °C. The crude mixture was diluted with DCM (20 mL) and washed with saturated brine (3 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a dark brown oil. The crude product was purified by medium pressure liquid chromatography (MLPC) (DCM–MeOH 9 : 1) yielding a brown solid (0.88 g, 77%): R_f 0.26 (DCM–MeOH 9 : 1); mp: 150-152 °C; IR (cm^{-1}) 3400-3200, 2973, 2859, 1164, 1556, 1409, 1333, 1245, 1143, 1113, 948, 861; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (12H, s, CH_3), 3.51-3.56 (4H, m, CH_2-morpholine), 3.86-3.84 (4H m, CH_2-morpholine), 5.32 (1H, s, H-3), 7.63-7.55, (1H, m, H-Ar), 8.02 (1H, d, J = 7.2 Hz, H-Ar), 8.29 (1H, d, J = 7.8 Hz, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3 (CH_3), 45.2 (CH_2-*N*-morpholine), 66.2 (CH_2-*O*-morpholine), 87.7 (C-2), 99.9 (C-4′ and C-5′), 116.2 (C-8), 124.9 (C-4a), 125.1 (C-6), 126.1 (C-5), 137.0 (C-7), 145.9 (C-9a), 162.2 (C-3), 175.2 (C-4); MS (ES+) m/z 358.2 $[M+H]^+$.

4-(Allyloxy)dibenzo[*b*,*d*]thiophene (6)

To dibenzo[*b*,*d*]thiophen-4-ol (**5**) (10 mg, 0.050 mmol) in acetonitrile (5 mL) containing suspended potassium carbonate (104 mg, 0.075 mmol) was added dropwise allyl bromide (6 µL, 0.075 mmol). The mixture was heated at reflux for 9 h, cooled, diluted with DCM (15 mL) and washed with water (2 × 10 mL) and brine (1 × 10 mL). The organic layer was dried ($MgSO_4$) and the solvent removed to give a light yellow oil (115 mg, 96%) that was used directly in the subsequent reaction: R_f 0.79 (DCM–petrol 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.68 (2H, d, J = 5.1 Hz, CH_2-CH=CH_2), 5.26 (1H, dd, J = 1.5 and 10.5 Hz, CH_2-CH=CH_2*cis*), 5.42 (1H, dd, J = 1.5 and 17.1 Hz, CH_2-CH=CH_2*trans*), 6.05 (4H, ddt, J = 5.1, 10.5 and 17.1 Hz, CH_2-C*H*=CH_2), 6.82 (1H, d, J = 7.8 Hz, H-Ar), 7.28-7.38 (3H, m, H-Ar), 7.68-7.81 (2H, m, H-Ar), 8.03-8.06 (1H, m, H-Ar).

3-Allyldibenzo[*b*,*d*]thiophen-4-ol (7)

4-(Allyloxy)dibenzo[*b*,*d*]thiophene (**6**) (115 mg, 0.05 mmol) in DMF (8 mL) was heated at 160 °C overnight. After cooling, water (15 mL) was added and the resulting solution was extracted with DCM (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed to give a brown oil. The crude product was purified by MLPC using DCM–petrol 6 : 4, to furnish the title compound as a light yellow oil (113 mg, 98%): R_f 0.37 (DCM–petrol 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42 (2H, d, J = 6.0 Hz, CH_2-CH=CH_2), 5.18-5.26 (2H, m, CH_2-CH=CH_2), 5.49 (1H, s, OH), 6.05 (1H, ddt, J = 16.2, 10.2 and 6.0 Hz, CH_2-C*H*=CH_2), 7.15 (1H, d, J = 8.1 Hz, H-Ar), 7.36-7.41 (2H, m, H-Ar), 7.65 (1H, d, J = 7.8 Hz, H-Ar), 7.78-7.82 (1H, m, H-Ar), 8.02-8.05 (1H, m, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.7 (*C*H_2-CH=CH_2), 114.7 (CH_2-CH=*C*H_2), 117.3 (C-1), 122.0 (C-3), 122.3 (C-6), 123.4 (C-9), 124.7 (C-9a), 126.9 (C-8), 127.8 (C-7), 132.4 (C-2), 136.5 (C-5a), 136.6 (C-4a), 137.1 (CH_2-*C*H=CH_2), 140.1 (C-9b), 149.5 (C-4).

3-Propyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (8)

To 3-allyldibenzo[*b*,*d*]thiophen-4-ol (**7**) (100 mg, 0.416 mmol) in ethanol (10 mL) cooled in an ice-bath was added 10% Pd/C (30 mg). The reaction mixture was stirred at room temperature for 18 h under a H_2 atmosphere. The mixture was filtered through a pad of Celite and concentrated *in vacuo* to give

3-propyldibenzo[*b*,*d*]thiophen-4-ol (95 mg, 94%) that was used in the subsequent reaction. 3-Propyldibenzo[*b*,*d*]thiophen-4-ol (95 mg, 0.392 mmol) and triethylamine (0.16 mL, 1.176 mmol) in DCM (4 mL) was cooled to −5 °C. Triflic anhydride (0.11 mL, 0.585 mmol) was added and the resulting solution was kept at 0 °C for 1 h. The reaction mixture was diluted with water (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by MPLC (DCM–petrol 5 : 5) to yield a colorless oil (141 mg, 96%) : R_f 0.80 (DCM–petrol 5 : 5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (3H, t, J = 7.2 Hz, CH_2-CH_2-CH_3), 1.74-1.82 (2H, m, CH_2-CH_2-CH_3), 2.90 (2H, t, J = 7.8 Hz, CH_2-CH_2-CH_3), 7.39 (1H, d, J = 8.1 Hz, H-Ar), 7.47-7.53 (1H, m, H-Ar), 7.39 (1H, d, J = 8.1 Hz, H-Ar), 7.85-7.88 (1H, m, H-Ar), 8.04 (1H, d, J = 7.8 Hz, H-Ar), 8.09-8.12 (1H, m, H-Ar).

3-Allyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (9)

A solution of 3-allyldibenzo[*b*,*d*]thiophen-4-ol (**7**) (35 mg, 0.146 mmol) and triethylamine (0.06 mL, 0.437 mmol) in DCM (5 mL) was cooled to −5 °C. Triflic anhydride (0.04 mL, 0.218 mmol) was added and the resulting solution kept at 0 °C for 1 h. The reaction mixture was diluted with water (10 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by MPLC (DCM–petrol 5 : 5) yielding a colorless oil (45 mg, 83%) : R_f 0.80 (DCM–petrol 5 : 5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.59 (2H, d, J = 6.5 Hz, CH_2-CH=CH_2), 5.18-5.26 (2H, m, CH_2-CH=CH_2), 5.90 (1H, ddt, J = 17.1, 10.5 and 6.5 Hz, CH_2-CH=CH_2), 7.30 (1H, d, J = 8.1 Hz, H-Ar), 7.39-7.42 (2H, m, H-Ar), 7.75-7.78 (1H, m, H-Ar), 7.98 (1H, d, J = 8.1 Hz, H-Ar), 7.80-8.04 (1H, m, H-Ar);^{13}C NMR (75 MHz, $CDCl_3$) δ 34.7 (CH_2-CH=CH_2), 117.8 (CH_2-CH=CH_2), 121.6 (C-1), 122.2 (C-3), 123.2 (C-6), 125.3 (C-9), 127.8 (C-9a), 128.1 (C-8), 132.3 (C-7), 133.9 (C-2), 135.2 (C-4a), 135.3 (CH_2-CH=CH_2), 137.9 (C-9b), 139.7 (C-4), 141.9 (CF_3).

2-Morpholino-8-(3-propyldibenzo[*b*,*d*]thiophen-4-yl)-4*H*-chromen-4-one (1)

In a microwave vial, 2-morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (**4**) (147 mg, 0.411 mmol) and 3-propyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (**8**) (140 mg, 0.374 mmol) were solubilised in degassed THF (10 mL). Caesium carbonate (365 mg, 1.12 mmol) and $PdCl_2$dppf (15.5 mg, 0.019 mmol) were added and the resulting reaction heated at 90 °C for 1.5 h under microwave irradiation. Upon cooling, DCM (10 mL) was added and the solution was washed with water (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a dark brown oil. The crude product was purified by MPLC (EtOAc–MeOH 9 : 1) yielding a light beige solid (0.079 g, 46%): R_f 0.55 (EtOAc–MeOH 9 : 1); mp: 214-216 °C; IR (cm^{-1}) 2953, 2864, 1622, 1570, 1442, 1408, 1356, 1244, 1236, 1066, 1034, 982, 850, 737, 674; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (3H, t, J = 7.2 Hz, CH_2-CH_2-CH_3), 1.52 (2H, br sextet, J = 7.2 and 7.5 Hz, CH_2-CH_2-CH_3), 2.48 (1H, dt, J = 13.8 and 7.5 Hz, CH_2-CH_2-CH_3), 2.61 (1H, dt, J = 13.8 and 7.5 Hz, CH_2-CH_2-CH_3), 3.00-3.04 (4H, m, CH_2-morpholine), 3.47-3.50 (4H, m, CH_2-morpholine), 5.53 (1H, s, H-3), 7.43-7.55 (4H, m, H-Ar), 7.64 (1H, dd, J = 1.8 and 7.5 Hz, H-Ar), 7.74-7.77 (1H, m, H-Ar), 8.14-8.19 (2H, m, H-Ar), 7.31 (1H, dd, J = 1.8 and 7.8 Hz, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (CH_3), 24.6 (CH_2-CH_2-CH_3), 35.8(CH_2-CH_2-CH_3), 44.9 (CH_2-*N*-morpholine), 66.0 (CH_2-*O*-morpholine), 87.5 (C-3), 121.5 (C-Ar), 121.7 (C-Ar), 123.0 (C-Ar), 124.2 (C-Ar), 124.8 (C-Ar), 125.2 (C-Ar), 126.4 (C-Ar), 126.5 (C-Ar), 126.9 (C-Ar), 128.1 (C-Ar), 130.3 (C-Ar), 133.9 (C-Ar), 136.2 (C-Ar), 139.9 (C-Ar), 140.2 (C-Ar), 141.9 (C-Ar), 151.3 (C-9a), 162.6 (C-2), 177.4 (C-4); MS(ES+) m/z 456.2 $[M+H]^+$; HRMS calcd for $C_{28}H_{26}NO_3S$ $[M+H]^+$ 456.1628, found 456.1625.

8-(3-Allyldibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (2)

In a microwave vial, 2-morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (**4**) (40 mg, 0.112 mmol) and 3-allyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (**9**) (40 mg, 0.107 mmol) were solubilised in degassed THF (3 mL). Caesium carbonate (104 mg, 0.321 mmol) and $PdCl_2$dppf (4 mg, 0.005 mmol) were added and the resulting reaction heated at 90 °C for 1.5 h under microwave irradiation. Upon cooling, DCM (10 mL) was added and the solution was washed with water (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a dark brown oil. The crude product was purified by MPLC (EtOAc–MeOH 9 : 1) yielding a light beige solid (0.151 g, 31%): R_f 0.43 (EtOAc–MeOH 9 : 1); mp: 203-205 °C; IR (cm^{-1}) 2913, 2868, 1622, 1570, 1441, 1408, 1244, 1111, 1065, 1037, 905, 776, 741, 686; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.99-3.01 (4H, m, CH_2-morpholine), 3.25-3.39 (2H, m, CH_2-CH=CH_2), 3.46-3.48 (4H, m, CH_2-morpholine), 4.82 (1H, dd, J = 0.8 and 16.2 Hz, CH_2-CH=CH_2*trans*), 4.97 (1H, dd, J = 0.8 and 9.9 Hz, CH_2-CH=CH_2*cis*), 5.49 (1H, s, H-3), 4.82 (1H, ddt, J = 6.3, 9.9 and 16.2 Hz, CH_2-CH=CH_2), 7.41-7.55 (4H, m, H-Ar), 7.64 (1H, dd, J = 1.8 and 7.5 Hz, H-Ar), 7.74-7.77 (1H, m, H-Ar), 8.14-8.19 (2H, m, H-Ar), 7.31 (1H, dd, J = 1.8 and 7.5 Hz, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.1 (CH_2-CH=CH_2), 44.9 (CH_2-*N*-morpholine), 66.0 (CH_2-*O*-morpholine), 87.6 (C-3), 116.3 (CH_2-CH=CH_2), 121.7 (C-Ar), 121.9 (C-Ar), 123.1 (C-Ar), 124.4 (C-Ar), 124.9 (C-Ar), 125.2 (C-Ar), 126.5 (C-Ar), 126.7 (C-Ar), 127.1 (C-Ar), 127.7 (C-Ar), 130.5 (C-Ar), 133.9 (C-Ar), 134.4 (C-Ar), 136.1 (CH_2-CH=CH_2), 136.9 (C-Ar), 137.6 (C-Ar), 139.9 (C-Ar), 141.9 (C-Ar), 151.3 (C-8a), 162.5 (C-2), 177.5 (C-4); MS(ES+) m/z 454.4 $[M+H]^+$; HRMS calcd for $C_{28}H_{24}NO_3S$ $[M+H]^+$ 454.1471, found 454.1470.

Dibenzo[*b*,*d*]thiophen-4-yl diethylcarbamate (10)

To a mixture of dibenzo[*b*,*d*]thiophen-4-ol (**5**) (200 mg, 1.0 mmol) and K_2CO_3 (207 mg, 1.5 mmol) in acetone (10 mL) at 40 °C, was added slowly diethylcarbamoylchloride (1.27 mL, 10.0 mmol). The suspension was heated to reflux for 24 h, cooled down to room temperature, filtered through a pad of Celite and concentrated *in vacuo*. The residue was then dissolved in DCM (10 mL) and washed with water (10 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and the crude product purified by MPLC (DCM–petrol 6 : 4) yielding the title compound as a colorless oil (0.287 g, 96%): R_f 0.50 (DCM–petrol 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (3H, t, J = 7.1 Hz, CH_2-CH_3), 1.30 (3H,

t, $J = 7.1$ Hz, CH_2-CH_3), 3.35 (2H, q, $J = 7.1$ Hz, CH_2-CH_3), 3.47 (2H, q, $J = 7.1$ Hz, CH_2-CH_3), 7.30 (1H, d, $J = 7.8$ Hz, H-Ar), 7.33-7.41 (3H, m, H-Ar), 7.73-7.76 (1H, m, H-Ar), 7.90 (1H, d, $J = 7.5$ Hz, H-Ar), 8.03-8.06 (1H, m, H-Ar);^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2 (CH_3), 42.8 (CH_2), 118.4 (C-3), 119.7 (C-1), 122.2 (C-6), 123.1 (C-9), 124.8 (C-9a), 125.7 (C-8), 127.3 (C-7), 132.4 (C-2), 136.2 (C-5a), 138.2 (C-4a), 139.9 (C-9b), 147.1 (C-4), 153.4 (C=O); MS(ES+) m/z 300.2 $[M+H]^+$.

3-Methyldibenzo[*b*,*d*]thiophen-4-yl diethylcarbamate (11)

To a solution of dibenzo[*b*,*d*]thiophen-4-yl diethylcarbamate (**10**) (100 mg, 0.334 mmol) in THF (10 mL) and TMEDA (0.06 mL, 0.37 mmol) at −78 °C under N_2 atmosphere, was slowly added *sec*-BuLi (1.4 M in cyclohexane, 0.36 mL, 0.37 mmol). The reaction was left at −78 °C for 1 h and quenched with MeI (0.03 mL, 0.501 mmol). The reaction was allowed to warm up to room temperature and stirred for a further 12 h. A saturated aqueous NH_4Cl solution (10 mL) was added and the resulting solution extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was purified by MPLC using DCM–petrol 6 : 4, to furnish the title compound as a colorless oil (53 mg, 51%): R_f 0.41 (DCM–petrol 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, $J = 7.1$ Hz, CH_2-CH_3), 1.43 (3H, t, $J = 7.1$ Hz, CH_2-CH_3), 2.41 (3H, s, CH_3), 3.45 (2H, q, $J = 7.1$ Hz, CH_2-CH_3), 3.59 (2H, q, $J = 7.1$ Hz, CH_2-CH_3), 7.28-7.53 (3H, m, H-Ar), 7.82-7.86 (1H, m, H-Ar), 7.89-8.02 (1H, m, H-Ar), 7.10-8.17 (1H, m, H-Ar).^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (CH_3), 15.6 (CH_2-CH_3), 42.7 (CH_2-CH_3), 119.5 (C-1), 122.2 (C-6), 123.1 (C-9), 124.8 (C-9a), 125.7 (C-8), 126.8 (C-3), 127.3 (C-7), 132.3 (C-2), 136.1 (C-5a), 138.0 (C-4a), 139.9 (C-9b), 147.2 (C-4), 153.2 (C=O).

3-Methyldibenzo[*b*,*d*]thiophen-4-ol (12)

To a solution of 3-methyldibenzo[*b*,*d*]thiophen-4-yl diethylcarbamate (**11**) (50 mg, 0.159 mmol) in EtOH (5 mL) was added a 10% NaOH aqueous solution (1.5 mL). The reaction mixture was refluxed overnight, cooled down to room temperature and neutralised to pH 7 with an 1 M HCl aqueous solution. The resulting solution was extracted with DCM (3 × 15 mL), the combined organic layers dried ($MgSO_4$) and the solvent removed to give a light yellow oil. The crude product was purified by MPLC using DCM to furnish the title compound as a white solid (21 mg, 62%): R_f 0.63 (DCM); mp: 103-105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (3H, s, CH_3), 4.96 (1H, br s, OH), 7.15 (1H, d, $J = 8.1$ Hz, H-Ar), 7.35-7.38 (2H, m, H-Ar), 7.60 (1H, d, $J = 8.1$ Hz, H-Ar), 7.77-7.79 (1H, m, H-Ar), 8.00-8.03 (1H, m, H-Ar).^{13}C NMR (75 MHz, $CDCl_3$) δ 15.5 (CH_3), 114.6 (C-1), 120.8 (C-6), 121.9 (C-9), 123.3 (C-9a), 124.7 (C-8), 126.7 (C-3), 127.5 (C-7), 128.2 (C-2), 136.5 (C-5a), 136.7 (C-4a), 139.8 (C-9b), 148.7 (C-4); MS(ES+) m/z 214.0 $[M]^+$.

3-Methyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (13)

A solution of 3-methyldibenzo[*b*,*d*]thiophen-4-ol (**12**) (15 mg, 0.07 mmol) and triethylamine (0.03 mL, 0.21 mmol) in DCM (10 mL) was cooled to −5 °C. Triflic anhydride (0.02 mL, 0.105 mmol) was added and the resulting solution kept at 0 °C for 1 h. The reaction mixture was diluted with water (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by MPLC (DCM–petrol 6 : 4) yielding a colorless oil (17.2 mg, 71%): R_f 0.84 (DCM–petrol 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (3H, s, CH_3), 7.30 (1H, d, $J = 8.1$ Hz, H-Ar), 7.39-7.45 (2H, m, H-Ar), 7.77-7.82 (1H, m, H-Ar), 7.96 (1H, d, $J = 8.1$ Hz, H-Ar), 8.03-8.06 (1H, m, H-Ar).

8-(3-Methyldibenzo[*b*,*d*]thiophen-4-yl)-2-morpholino-4*H*-chromen-4-one (3)

In a microwave vial, 2-morpholino-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4*H*-chromen-4-one (**4**) (11.3 mg, 0.032 mmol) and 3-methyldibenzo[*b*,*d*]thiophen-4-yl trifluoromethanesulfonate (**13**) (10 mg, 0.029 mmol) were dissolved in degassed THF (2 mL). Caesium carbonate (28.3 mg, 0.087 mmol) and $PdCl_2$dppf (1.2 mg, 0.0015 mmol) were added and the resulting reaction heated at 100 °C for 1.5 h under microwave irradiation. Upon cooling, DCM (10 mL) was added and the solution was washed with water (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a dark brown oil. The crude product was purified by MPLC (EtOAc–MeOH 9 : 1) yielding a white solid (9 mg, 73%): R_f 0.56 (EtOAc–MeOH 9 : 1); mp: 194-197 °C; IR (cm^{-1}) 2934, 2857, 1622, 1551, 1470, 1442, 1406, 1247, 1150, 978, 852, 745; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.94-2.99 (4H, m, CH_2-morpholine), 3.41-3.44 (4H, m, CH_2-morpholine), 5.45 (1H, s, H-3), 7.33-7.47 (4H, m, H-Ar), 7.58 (1H, dd, $J = 1.8$ and 7.5 Hz, H-Ar), 7.68 (1H, dd, $J = 1.8$ and 7.5 Hz, H-Ar), 8.01-8.10 (2H, m, H-Ar), 8.23 (1H, dd, $J = 1.8$ and 7.5 Hz, H-Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1 (CH_3), 45.0 (CH_2-*N*-morpholine), 66.1 (CH_2-*O*-morpholine), 87.6 (C-3), 121.5 (C-Ar), 121.7 (C-Ar), 123.1 (C-Ar), 124.9 (C-Ar), 125.4 (C-Ar), 126.4 (C-Ar), 126.9 (C-Ar), 127.2 (C-Ar), 130.5 (C-Ar), 133.8 (C-Ar), 134.2 (C-Ar), 135.5 (C-Ar), 136.2 (C-Ar), 139.9 (C-Ar), 141.8 (C-Ar), 151.3 (C-8a), 162.5 (C-2), 178.1 (C-4); MS(ES+) m/z 428.3 $[M+H]^+$; HRMS calcd for $C_{28}H_{24}NO_3S$ $[M+H]^+$ 428.1315, found 428.1316.

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